UNIVERZITA PALACKÉHO V OLOMOUCI

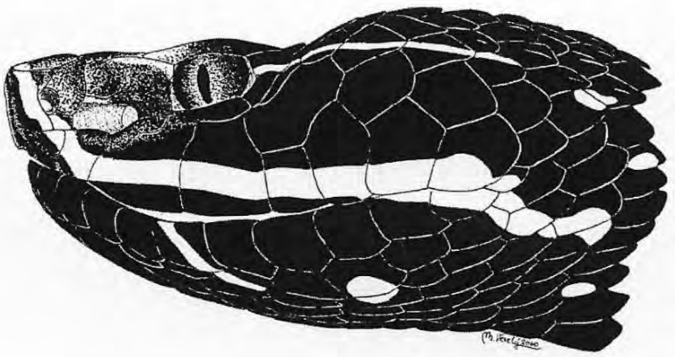
Přírodovědecká fakulta

Vybrané aspekty funkční morfologie, fylogeografie a systematiky obojživelníků a plazů

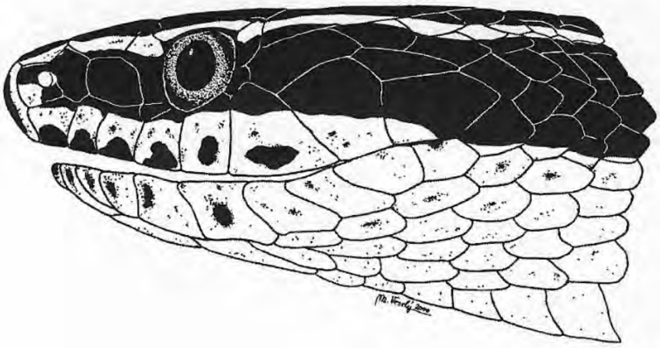
Habilitační práce v oboru zoologie

RNDr. Milan Veselý, Ph.D.

Olomouc 2021



Agkistrodon bilineatus (Günther, 1863); SMF 42218



Coniophanes piceivittis Cope, 1870; SMF 60745

Obsah

1. Úvod

Od útlého věku jsem byl fascinován biologií. K mým vůbec nejrannějším vzpomínkám patří to, jak jsem se pokoušel vysvětlit nechápajícím „tetám“ v mateřské školce, že přece nemůžu jít po obědě spát s ostatními, když jsem před chvílí viděl v bazénku na zahradě topící se samičku nosorožíka kapucínka, kterou je třeba neprodleně zachránit. Nakonec mi ji pomohli vytáhnout. Ve stejném věku jsem také nakreslil v rámci kreslířské soutěže leporelo „Brouci“, které bylo řadu let vystaveno v přírodovědné expozici Muzea Jihovýchodní Moravy na zámku v Malenovicích. Během následujících let jsem se pak účastnil všech myslitelných biologických aktivit (kroužek mladých přírodovědců při ODDM ve Zlíně, biologické olympiády, letní biologické expedice pořádané ODDM a další akce). Kromě sbírání brouků a přírodnin se u nás doma i přes odpor rodičů, ale s podporou starších bratrů, objevovalo čím dál více živého hmyzu (afrických zlatohlávků, kudlanek, strašilek atd.), pavoukovců (bičovců, sklípkanů, štírů) a posléze i plazů a obojživelníků. Tento stále se prohlubující zájem o přírodu pak nezbytně vyústil ve studium oboru Systematická biologie a ekologie na Přírodovědecké fakultě UP.

Po úspěšném ukončení studia jsem nastoupil jako asistent na katedru zoologie PřF UP, primárně s úkolem zajistit výuku mnoha hodin cvičení z obecné zoologie, která mi díky vysokým počtům studentů v kombinovaném studiu s biologií zabírala 18–24 hodin týdně (podle semestru). Postupem času se mi i díky nástupu nových kolegů, kteří se začali na výuce tohoto předmětu také podílet, podařilo rozšířit portfolio mnou vyučovaných předmětů nejprve o Herpetologii a později, po odchodu doc. Evžena Opatrného (nestora české herpetologie) do důchodu i o Batrachologii, kterou původně přednášel. Zároveň jsem se zásadně podílel na zajišťování a postupném rozšíření terénních praxí jakožto nezastupitelné formy výuky systému a ekologie živočichů a jejich vazby na konkrétní životní prostředí, včetně zavedení předmětu Terénní cvičení z mořské biologie, o který je tradičně velký zájem. Studenti zde mohou pozorovat v přirozeném prostředí život a ekologii i těch zástupců živočišných kmenů, které znají ze systému živočichů pouze teoreticky nebo z lihových preparátů.

V devadesátých letech jsem také nastoupil do interního doktorandského studia. Protože mě kromě herpetologie stále zajímala i entomologie, zapojil jsem se do mezinárodního projektu výzkumu zaměřeného na sledování vlivů ekologického zemědělství na kvalitu životního prostředí. Ekologické (alternativní) způsoby hospodaření jsou charakteristické menším zatěžováním životního prostředí syntetickými látkami či přímo jedy (pesticidy).

V České republice nepatřilo ekologické zemědělství mezi tradiční formy hospodaření, se zaváděním tohoto systému se začalo až po roce 1989. Principy ekologického hospodaření vycházejí ze zásad organizace IFOAM (International Federation of Organic Agriculture Movement), která v mezinárodním měřítku podporuje a organizuje rozvoj dlouhodobě stabilních zemědělských systémů (viz Nařízení rady EHS č. 2092/91, Metodický pokyn pro ekologické zemědělství čj. 655/93-340 ze dne 22. června 1993 a zákon o ekologickém zemědělství z r. 2000).

V rámci tohoto projektu jsem studoval společenstva střevlíkovitých v polích s rozdílným typem hospodaření. Uvedená skupina je jedním z ekologicky nejdůležitějších faktorů epigeonu, kde hrají ve své potravní hladině významnou roli jako primární, a především sekundární konzumenti. Jde o systematicky dobře zpracovanou skupinu s množstvím informací o geografickém rozšíření, ekologických nárocích a způsobu života jednotlivých druhů, z nichž mnohé jsou úzce vázány na své životní prostředí a jsou velmi citlivé na jeho změny, navíc lze početný materiál střevlíkovitých získat jednoduchými standardními metodami (Thiele 1977). V neposlední řadě jde o skupinu, s jejíž determinací jsem měl spoustu zkušeností.

Práce vycházela z předpokladu, že střevlíkovití, jako terminální predátoři v epigeonu, budou dobře indikovat změny dané přechodem na odlišný způsob obhospodařování. V systému alternativního zemědělství by mělo docházet k vyrovnávání poměrů živin v půdě, ke zvýšení obsahu organických zbytků při organickém hnojení, snížení vstupů syntetických látek (hnojení, pesticidy) a zvýšení heterogenity prostředí díky výraznějšímu zastoupení doprovodných rostlin (plevelů) v porostu. Očekával jsem, že dojde k detekovatelnému vzrůstu abundance a druhové pestrosti střevlíkovitých a k průkazným změnám charakteristik popisujících složení společenstva, jako je diverzita a ekvitabilita, jejichž hodnoty by se měly zvyšovat, případně dominance, jež by měla klesat. S předchozím souvisela otázka, nakolik jsou zmíněné cenologické indexy schopné odrážet případné změny ve společenstvu. Ukázalo se, že na sledovaných alternativně obhospodařovaných polích došlo k jednoznačně detekovatelné změně ve struktuře společenstva střevlíkovitých brouků v porovnání se situací na polích obhospodařovaných konvenčními metodami. I když byl tento posun relativně malý, ze statistického hlediska byl vysoce průkazný. Nicméně, v porovnání s metodami mnohorozměrné statistiky se použité cenologické indexy (Simpsonův index dominance, Shannon-Weaverův index diverzity a ekvitabilita podle Sheldona) jevily jako méně vhodné pro detekci jemných změn ve společenstvech, neboť se signifikantně nelišily mezi alternativně a konvenčně obhospodařovanými plochami ani nevykazovaly žádné jednoznačné trendy ke změnám v čase.

Během výzkumu jsem publikoval několik článků vztahujících se ke studované problematice (Veselý 1993; Veselý et al. 1997a,b; Veselý & Kostkan 2000). Výsledky výzkumu jsou pak podrobně zpracovány v mé disertační práci „Vliv typu hospodaření na společenstva střevlíkovitých brouků (Carabidae) v polních ekosystémech v oblasti Hané“, kterou jsem obhájil v roce 2001, a posléze byly publikovány v impaktovém časopise (Veselý & Šarapatka 2008).

V dalších letech jsem se věnoval se (už i svými diplomanty) také dalším aspektům biologie a ekologie bezobratlých, kromě střevlíkovitých brouků (Tuf et al. 2003, 2004; Holec et al. 2006; Gabriš et al. 2010, 2011; Veselý et al. 2010, 2011, 2012; Tuf et al. 2012; Růžičková et al. 2015; Konvička et al. 2018) a dalších skupin hmyzu (Dolný et al. 2001; Dolný et al. 2007; Veselý & Cabáková 2018) také např. pavoukům (Gogolka et al. 2008, Lukeš 2013; Javůrek 2017). Zpracování diplomových prací týkajících se střevlíků usnadnilo také to, že jsem na katedře s přispěním kolegů z řad amatérských entomologů postupně vybudoval téměř kompletní srovnávací sbírku střevlíkovitých brouků vyskytujících se v ČR, která studentům ulehčuje determinaci nasbíraného materiálu.

Na základě dosavadních prací s agroenvironmentální problematikou jsem byl také přizván do autorského týmu metastudie, zabývající se globální analýzou vlivů různých systémů hospodaření na diverzitu členovců v polních ekosystémech (Lichtenberg et al. 2017). Z důvodu lepšího pochopení složení a významu biocenóz střevlíkovitých v polních ekosystémech jsem zkoumal také pohyblivost velkých střevlíků, konkrétně habitatové preference a migrační vzdálenosti, které u bezkřídlých druhů hrají velkou roli při pronikání do polí. Velcí střevlíci totiž hrají významnou úlohu v ochraně polních ekosystémů před bezobratlými škůdci. V pilotní studii jsme se s mojí doktorandkou zaměřili zejména na použitelnost radiotelemetrie při výzkumu pohyblivosti střevlíka Ullrichova (Růžičková & Veselý 2016). Dalším, neméně důležitým cílem bylo zjistit, jak se brouci pohybují v konkrétním biotopu a jakou vzdálenost v něm dovedou překonat. Zjistili jsme, že existují rozdílné preference pro typ habitatu mezi samci a samicemi a že rychlost migrace v lesním prostředí dosahovala hodnot 20.1 m/ 3 h u samců a 19.6 m/ 3 h u samic. Na louce byla nejvyšší rychlost pohybu pouhých 7.5 m/ 3 h u samců a 17.6 m/ 3 h u samic (Růžičková & Veselý 2018). Abychom prozkoumali míru objektivity výzkumů využívajících při telemetrii střevlíků rodu *Carabus* aktivní tagy dosahující váhy přibližně 40% hmotnosti jedince, v laboratoři jsme testovali, zda existuje rozdíl v pohyblivosti mezi broukem se zátěží (nalepeným transmiterem) a bez zátěže. Výsledky ukázaly, že u námi použitých transmiterů není signifikantní rozdíl v uběhnuté vzdálenosti mezi zatíženým a nezatíženým jedincem (Veselý & Růžičková in prep.).

Biologie stála také v pozadí mé záliby v cestování. Poznávání fauny a flóry jiných zemí je asi snem každého biologa, u mě byla tato touha navíc ještě umocněna zájmem o plazy a obojživelníky, jejichž diverzita není v České republice v porovnání s teplejšími oblastmi zrovna vysoká. Proto jsem hned po sametové revoluci vyrazil do světa. První z tehdejšího pohledu „velkou“ cestu jsem podnikl ještě před ukončením vysokoškolského studia. Hned v lednu 1990 jsem obdržel nabídku zúčastnit se jako herpetolog pětitýdenní expedice Slovenského zväzu ochráncov prírody a krajiny do Sýrie. Přesto, že mě v květnu čekaly státnice, neváhal jsem ani vteřinu a nabídku jsem přijal. I když jsem se vrátil pouhé dva týdny před státnicemi, zkoušky jsem úspěšně složil – pravděpodobně i díky tomu, že jsem během cesty na vlastní oči viděl a pochopil spoustu věcí, které jsem znal jen z teorie. Během dalších cest jsem pak poznával nejen faunu pouští, ale v od r. 1992 i vysněných tropů. Zejména terénní zoologické zkušenosti z Mexika mi později otevřely cestu k herpetologickému výzkumu ve Střední Americe. V roce 1994 jsem se při návštěvě Senckenberg muzea ve Frankfurtu nad Mohanem seznámil s dr. Guntherem Köhlerem, vedoucím zdejšího oddělení, a navázal s ním spolupráci. Díky tomu jsem v muzeu absolvoval řadu výzkumných stáží a začal publikovat články s herpetologickou problematikou v zahraničních recenzovaných časopisech (Veselý et al. 1995; Köhler & Veselý 1996; Veselý 1998; Gvoždík & Veselý 1998; Veselý 1999). V r. 1997 jsem také vedl dvouměsíční muzejní expedici do Salvádoru, která nám poskytla zásadní část materiálu pro zpracování monografie The Amphibians and Reptiles of El Salvador, vydané ve Spojených Státech v roce 2006. Kromě práce na tomto velkém projektu a na dalších publikacích týkajících se středoamerické herpetofauny (podrobněji viz níže) jsem se ovšem věnoval i herpetologickým výzkumům na domácí půdě. Byl jsem spoluatorem atlasu naší herpetofauny (Veselý & Zavadil 1997), vydaného u příležitosti konání Světového Herpetologického Kongresu v Praze (1997), na jehož organizaci jsem se jako člen České herpetologické společnosti spolupodílel. Později už jako viceprezident ČHS jsem se věnoval dalšímu rozvoji a popularizaci herpetologie doma i ve světě.

Se svým dlouholetým kamarádem a kolegou Davidem Modrým z parazitologického ústavu VFU v Brně jsme koncem devadesátých let zkoumali parazitofaunu exotických plazů importovaných do zájmových chovů v ČR a z tohoto materiálu jsme popsali několik nových druhů parazitických sporozoí (Modrý et al. 1999, 2001, 2004). Později jsme se zaměřili i na parazity obojživelníků a do výzkumu jsem zapojil také svého diplomanta Miloše Jirků. Studovali jsme především prevalenci a virulenci eimerií z čeledí Eimeriidae a Barrouxiidae u našich „hnědých skokanů“ rodu *Rana* (Jirků et al. 2001, 2002).

Při pozorování plazů chovaných v zajetí v rámci výzkumu jejich parazitofauny mě zaujalo zvláštní chování některých pouštních agam při rosení vodou. Začal jsem se tedy zabývat výzkumem fenoménu tzv. „rain-harvestingu“, popsaného od australských molochů a amerických ropušníků, a podařilo se mi tento typ chování objevit u několika druhů agam z Blízkého východu (Veselý & Modrý 2002). Podrobněji je tento výzkum popsán níže.

Po roce 2000 jsem začal jako první v republice systematicky studovat tehdy jedinou známou populaci ještěrky zední na Štramberku. Postupem času jsem publikoval řadu poznatků o parazitofauně, ekologii, morfologii a nových nálezech ještěrky zední (Veselý et al. 2003; Urban & Veselý 2004; Veselý & Urban 2006; Veselý et al. 2007, 2009; Mačát & Veselý 2009, Moravec & Veselý 2015). Do výzkumu jsem zapojil také řadu svých studentů, kteří na dílčích datech z těchto výzkumů postavili a úspěšně obhájili bakalářské či diplomové práce (viz seznam obhájených BP a DP v doplňujících materiálech). Problematika původu českých populací je detailněji přiblížena v další kapitole.

Ve výzkumu tropické herpetofauny jsem se až na výjimky (Gvoždík & Veselý 1998; Veselý 1999) soustředil na oblast neotropů, ponejvíce pak Střední Ameriky. Zpočátku jsem řadu prací publikoval v zahraničních recenzovaných časopisech, které v té době ještě nebyly indexovány na WoS (Senckenbergiana Biologica, Salamandra), postupem času jsem se ale soustředil na publikace v impaktových zoologických žurnálech. Protože počet herpetologických prací v mém vědeckém portfoliu nyní výrazně převažuje nad počtem entomologických titulů, rozhodl jsem se v následujícím komentovaném přehledu představit pouze tuto část mé vědecké činnosti, neboť reprezentuje semknutější útvar a její objem a struktura jsou dostačující z hlediska požadavků na habilitační práci překládanou na přírodovědecké fakultě Univerzity Palackého. Zahrnutí entomologické sekce by neúměrně navýšilo rozsah této habilitace, což by nebylo účelné z hlediska šetření času jak oponentů, tak i případných čtenářů. Kdokoliv bude mít zájem o mé vědecké výsledky, může je snadno dohledat na webu (WoS, Scopus, Google Scholar) a podívat se přímo na originály publikací.

Ve třech následujících kapitolách bych tedy rád představil deset prací, přibližujících tři okruhy mé vědecké činnosti v herpetologii. U těchto publikací jsem vždy jedním z hlavních autorů (první, poslední nebo korespondenční). V doprovodném textu vycházejícím z některých mých starších komentářů k výzkumu (Veselý & Modrý 2002; Veselý & Jablonski 2018) je stručně shrnuta problematika a širší kontext, do něhož tyto práce zapadají a v některých případech i okolnosti, za nichž vznikaly. V textu zmiňuji i některé své další publikace, které se dotýkají daného tématu, i když nejsou přímou součástí této habilitační práce.

2. Funkční morfologie epidermis plazů – získávání vody v pouštních ekosystémech

Voda je jedním ze základních mezních faktorů pouštních a polopouštních ekosystémů, a proto je schopnost ji získávat a následně udržet v těle pro živočichy aridních oblastí klíčová. S potřebou vody se tito živočichové vyrovnávají různě. Některým, zejména větším masožravým druhům, stačí k životu voda obsažená v potravě. Typickým příkladem takového predátora je varan pustinný (*Varanus griseus*), který obývá rozsáhlý areál pouští a polopouští od Maroka až po střední Asii (Vernet et al. 1988). Velké býložravé druhy plazů často využívají jako zásobní zdroje tukovou tkáň a mají fyziologické mechanismy, které jim umožňují hospodárně využívat vodu a omezit její ztráty, např. solné žlázy nebo urikotelii (Schmidt-Nielsen 1988). Při získávání vody tito živočichové dokážou maximálně využít občasných vodních srážek, a to jak vertikálních, tak i horizontálních (ve formě mlhy). Kromě obvyklého olizování mokrých předmětů a substrátu je mnoho živočichů schopno zachycovat vodní srážky přímo povrchem těla, přičemž podobné vzorce chování nacházíme napříč spektrem nejrůznějších vzájemně nepříbuzných skupin bezobratlých i obratlovců. Jihoafričtí potemníci r. *Onymacris* z písečných dun pouště Namib žijí v prostředí, kde jediným zdrojem vláhy jsou po většinu roku mlhy přicházející od atlantského pobřeží. Potemníci zaujímají zvláštní postoj zadečkem vzhůru a voda, která kondenzuje na jejich tělech, stéká přímo k ústnímu ústrojí (Seely 1979). Podobně využívá povrchu těla k zachycování mlhy i jihoafrický chameleon *Chameleo namaquensis*, stejně jako zmije *Bitis peringuei* vyskytující se ve stejné oblasti (Cloudsley-Thompson 1991).

Využití vodních srážek dopadajících na povrch těla však není zdaleka typické jen pro plazy ze suchých oblastí. Šikmý postoj těla s hlavou v nejnižším bodě zaujímá např. floridská užovka páskovaná (*Nerodia fasciata*), stejně jako bičovky r. *Ahaetulla* z jihovýchodní Asie (Cundall 2000), přesto, že v prostředí, které obývají, je vody dostatek.

Chování spojené s příjmem vody má dvě základní formy: 1) prosté olizování kapek vody z povrchu vlastního těla, často spojené i s olizováním okolních předmětů a 2) tzv. rain–harvesting, tento termín označuje příjem vody s různým stupněm využití plochy těla k zachycení vodních srážek, doplněný o typické chování či postoje usnadňující tento proces.

Již od 20. let minulého století je známo, že kůže australského agamy molocha trnitého (*Moloch horridus*) má schopnost absorbovat vodu podobně jako savý papír. Původní teorii o permeabilitě kůže molocha pro vodu poprvé experimentálně vyvrátili Bentley a Blumer (1962), kteří prokázali pasivní transport vody z povrchu těla ke koutkům tlamy a její následný příjem. Přechod vody z povrchu kůže do ústní dutiny je kromě pohybu čelistí usnadněn vylučováním hygroskopických slin. Mechanismus proudění vody byl nejprve studován s využitím barviva a sledováním jeho pohybu po povrchu těla plaza. Proudění tekutiny vysvětlovali tito autoři mikrokapilární sítí ve vnější keratinové vrstvě povrchu šupin. Tuto hypotézu následně vyvrátili Gans et al. (1982), kteří vysvětlili transport vody kapilárními silami v hlubokých zářezech mezi jednotlivými šupinami. Práce Witherse (1993) a Sherbrooka (1993) detailně vysvětlují fyzikální podstatu tohoto jevu, doplněnou o popis chování spojeného s tímto způsobem příjmu tekutin. Moloch může díky kapilárním silám přijímat srážkovou vodu nejen z hřbetní plochy těla, ale tisknutím břišních partií k substrátu i z mokrého povrchu. Pozorování in situ dokázala, že během deště a po něm tyto agamy vyhledávají prohlubně, v nichž je písek vlhčí než v okolí a kde uvedeným způsobem získávají vodu. Autoři předpokládají, že mološi dokážou takto přijímat nejen vodu dešťovou, ale i mlhu kondenzující na jejich trnitých šupinách. Kromě molocha bylo podobné chování popsáno u celé řady ještěrů. Schwenk a Greene (1987) například pozorovali drobné středoasijské agamy *Phrynocephalus helioscopus*, u nichž je rain-harvesting doplněn o zvláštní postoj, kdy při maximálním natažení zadních končetin a pokrčení končetin předních je čenich (rostrum) nejnižším bodem těla. Při silných srážkách stéká dešťová voda účinkem gravitace k rostru, kde je přijímána pohyby čelistí a jazyka. V takovém případě je působení kapilárních sil nevýznamné. Autoři však předpokládají, že se situace může výrazně změnit při nedostatku vertikálních srážek, za přítomnosti mlhy nebo proudů vzduchu s vysokou relativní vlhkostí. Tehdy může orientace těla (při popsaném postoji) proti proudění vzduchu zvětšit plochu kůže, na níž probíhá kondenzace vody. Role kapilárních sil při transportu takto vzniklého vodního filmu může být primární. Sherbrooke (1990) popsal podobné chování u ropušníků *Phrynosoma cornutum*.

1) **Veselý, M.,** Modrý, D. (2002): Rain-harvesting behavior in Agamid lizards (*Trapelus*). *Journal of Herpetology* 36 (2): 311-314. IF – 0.649, **Q3**

Společně s Dr. Modrým jsme studovali chování typu rain-harvesting u pěti druhů agam r. *Trapelus* obývající aridní a semiaridní biotopy Severní Afriky, Blízkého východu a Střední Asie. Zkoumali jsme tři základní parametry tohoto chování: kvantitativní a kvalitativní charakteristiky zaujímaných postojů, schopnost epidermis vést vodu prostřednictvím kapilárních kanálů (s využitím potravinářského barviva) a mikrostrukturu šupin v dorzální části folidózy (za pomoci skenovacího elektronového mikroskopu). Z pěti studovaných druhů jsme rain-harvesting prokázali u *T. pallidus*, *T. flavimaculatus* a *T. mutabilis* (Veselý & Modrý 2002). První pozorování rain-harvestingu u agam pochází od Abela (1952), který podobné chování popsal u druhů *Trapelus agilis* a *T. ruderatus*. Jeho interpretace pozorovaného chování je však z dnešního pohledu kuriózní. Polohu těla typickou pro rain-harvesting vysvětluje jako reakci na zvířatům nepříjemný dotyk s deštěm smáčeným substrátem. Pokud šlo opravdu o *T. ruderatus* (Abelova zvířata pocházela údajně z východního Íránu, což je mimo známý areál rozšíření tohoto druhu), je překvapivé, že námi testovaní jedinci *T. ruderatus* během opakovaných experimentů tento postoj nezaujímali, podobně jako jedinci dalšího testovaného druhu *T. sanquinolentus* ze Střední Asie. Abel, stejně jako později Sherbrooke (1990), poukazuje na nápadnou podobnost pozorovaného postoje s postoji obrannými (stejně tomu je i u zmíněných potemníků r. *Onymacris*). Obranné chování tedy mohlo být preadaptací ke vzniku rain-harvestingu.

Srovnáme-li typ postoje, můžeme u šupinatých (Squamata) definovat tři kategorie rain-harvestingu lišící se různou rolí gravitace a kapilárních sil při vedení vody k tlamě.

- u molocha (*Moloch horridus*) proudí voda kapilárními kanály nezávisle na gravitaci, tudíž u něj není vyvinut typický rain-harvesting postoj s hlavou v nejnižším bodě.
- u ropušníka *Phrynosoma cornutum* jde o kombinaci využití kapilarity a gravitace, kdy se kapilární síly uplatňují při slabých srážkách a rain-harvesting postoj při stékání většího množství vody.

- u agam r. *Phrynocephalus* převažuje složka gravitační, kapilární síla zde dosud nebyla prokázána. Tomu odpovídá i extrémní úhel osy těla vzhledem k podkladu, který umožňuje stékání vody k tlamě gravitačním spádem. U r. *Trapelus* je postoj méně strmý a často kombinovaný s olizováním vlhkých předmětů nebo substrátu zadržujícího vodu. U všech sledovaných druhů r. *Trapelus* bylo (na rozdíl od r. *Phrynocephalus*) pozorováno i pití z povrchových zdrojů (Petriho misky), pokud byly dostupné.

Porovnání SEM snímků odhalilo přítomnost plástvovité mikroornamentace na povrchu šupin jak u rain-drinking pozitivních, tak i negativních druhů.

2) Yenmis, M., Ayaz, D., Sherbrooke, W.C., **Veselý, M**. 2016: A comparative behavioural and structural study of rain-harvesting and non-rain-harvesting agamid lizards of Anatolia (Turkey). *Zoomorphology* 135 (1): 137-148. IF – 1.038; **Q3**

V roce 2013 mě kontaktovala studentka Melodi Yenmis z Eger University v tureckém Izmiru kvůli programu Erasmus. Zaujal ji fenomén rain-harvestingu a měla zájem o spolupráci. Po příjezdu na půlroční stáž jsme oživili projekt výzkumu rain-harvestingu u agam a zaměřili se na druhy vyskytující se v Turecku. Studentku jsem seznámil s technikami práce v terénu a v laboratoři a připravili jsme projekt výzkumu tak, aby v něm mohla pokračovat na své mateřské univerzitě. Do studie byly zahrnuty čtyři druhy agam vyskytující se v Turecku a cílem studie bylo prozkoumat jejich chování spojené s příjmem vody. Protože se v předchozích studiích ukázalo, že povrchová ultrastruktura šupin se neliší mezi rain-harvesting pozitivními a negativními druhy (Veselý & Modrý 2002; Sherbrooke et al. 2007, Commans et al. 2011), zařadili jsme do studie také histologické řezy pokožkou v oblastech záhybů mezi šupinami. Ačkoliv jsme prostřednictvím pokusů s barvivem prokázali schopnost pokožky absorbovat vodu do prostorů mezi šupinami u všech zkoumaných druhů, směrový kapilární transport byl potvrzen pouze u druhu *Phrynocephalus horvathi*. U tohoto druhu jsme také na histologických řezech štěrbin mezi šupinami objevili ostrůvkovité struktury, které byly výraznější v dorzální části folidózy a slaběji patrné mezi šupinami ventrálními (Yenmis et al. 2016). Podobné struktury jsou známy pouze u dvou druhů šupinatých - *Moloch horridus* a *Phrynosoma cornutum* (Sherbrooke et al. 2007). Hypotézu navrhující fungování takového systému na principu biomimetického modelu „kapalinové diody“ publikovali Commans et al. (2015). V pokračujícím výzkumu se tedy zaměříme na histologickou analýza kůže u dalších rain-harvesting druhů plazů, kde chceme opět využít SEM pro prostorové zobrazení všech vrstev v zahybech mezi šupinami a zjistit, zda praktické uspořádání struktur odpovídá publikované hypotéze.

3. Fylogeografie populací ještěrky zední v České republice

Kolem přelomu tisíciletí jsem se začal systematičtěji věnovat v ČR poměrně čerstvě objevené populaci ještěrky zední (*Podarcis muralis*) na Štramberku. Samotný objev tohoto druhu na našem území byl překvapením, nezdálo se možné, že by na území České republiky mohla perzistovat bez povšimnutí populace tak výrazného druhu ještěrky. Když jsem začal pátrat po příčině, ukázalo se, že o utajení výskytu ještěrky zední ve Štramberku se nevědomky postaral už před více než půl stoletím český zoolog Otakar Štěpánek, který ve své krátké zprávě z terénního výzkumu štramberských lokalit (Štěpánek 1955) mylně identifikoval pozorované ještěrky jako ještěrky živorodé (*Zootoca vivipara*). Z popisu chování je však jasné, že muselo jít o ještěrku zední. A tak zřejmě řada pozdějších herpetologů nevěnovala této lokalitě pozornost, neboť občasné tradované zprávy o nálezech ještěrky zední se přičítaly špatné determinaci a záměně s ještěrkou živorodou — nikoho totiž nenapadlo podezřívat z omylu právě O. Štěpánka, jednoho z nejzkušenějších terénních herpetologů v poválečném Československu. Přesto, že současný majitel lomu Dolní Kamenárka ve Štramberku (dnes Botanická zahrada a arboretum), pan Petr Pavlík, zde ještěrku zední zdařile vyfotografoval už v r. 1971 (Pavlík in litt.), štramberská populace zůstala stranou zájmu odborné veřejnosti až do 90. let, kdy se objevila řada prací dokládajících existenci této jediné populace na území ČR (Hudeček & Šuhaj 1994, Zavadil 1998, 1999; Holuša & Holuša 1999, Šapovaliv 1999, a další). Zajímavou pointu celé historii dodala i práce Moravce a Beneše (2000), kteří revidovali materiál z r. 1952 sbíraný Štěpánkem na Štramberku a jednoznačně v něm identifikovali právě ještěrku zední.

V počátcích svého výzkumu ve Štramberku jsem studoval parazitofaunu, morfologické charakteristiky a početnost populace ještěrky zední v opuštěných lomech Horní a Dolní Kamenárka. Do výzkumu jsem zapojil také řadu svých BP a DP studentů (Veselý et al. 2003; Urban & Veselý 2004; Veselý & Urban 2006; Veselý et al. 2007, 2009; Mačát & Veselý 2009, Moravec & Veselý 2015). Nicméně, v pozadí všech prováděných výzkumů se stále vznášela otázka, zda je tato populace původní či vysazená. Dobře známý je ze zmíněné oblasti případ jasoně červenookého, jehož původní poddruh *Parnassius apollo strambergensis* Skala, 1912 na Štramberku vyhynul ve 30. letech minulého století, ale v dnešní době se zde vyskytuje dobře prosperující populace vzniklá relativně nedávnou introdukcí poddruhu *P. a. anticuus* Eisner & Zelný, 1977 z oblasti Manína ve Strážovských vrších na Slovensku (Lukášek 2000). Běžný návštěvník to samozřejmě nepozná, ale z hlediska historické biogeografie se vlastně jedná o výskyt allochtonní populace, i když genetika a postglaciální šíření tohoto motýla nejsou ve střední Evropě hlouběji studovány. Recentní fragmentovaný výskyt tohoto druhu ve vyšších nadmořských výškách naznačuje, že se jedná o glaciální relikt, v současné době stažený do tzv. interglaciálního refugia (Slabý 1952). Podobná refugia byla zaznamenána i u jiných druhů fauny Evropy, jež mají fragmentovaný, převážně na horské oblasti omezený areál rozšíření (např. Mizsei et al. 2016, 2017). To však není případ ještěrky zední. Ta sice na severní hranici svého přirozeného výskytu má izolovaný charakter výskytu, zhruba na jih od Karpatské kotliny je ale její rozšíření kontinuální a nejedná se o žádného vzácného plaza. V západní Evropě je tato ještěrka dokonce místně považována za rychle se šířící, až invazní druh, který byl uměle introdukován např. i na Britských ostrovech nebo v USA a rychle se tam adaptoval. V současnosti je fylogeograficky jedním z nejstudovanějších plazů Evropy s více jak dvaceti známými mitochondriálními liniemi, které však úplně neodpovídají zavedené poddruhové taxonomii (Schulte et al. 2012a, 2012b; Gassert et al. 2013; Salvi et al. 2013). Proto byla odpověď na otázku, zda je ještěrka zední biogeograficky původním či nepůvodním druhem na území ČR, velmi podstatná. Tato odpověď může sehrát roli ve stanovení ochranářských priorit, pohledu na historické šíření druhu nebo celkové biogeografické významnosti území České republiky pro jiné teplomilné druhy fauny.

V poslední dekádě byly objeveny také prosperující populace ve Strejčkově lomu v Grygově u Olomouce a v lomu Hády na severním okraji Brna. Většina recentních nálezů je podrobně shrnuta v monografii Fauna ČR - Plazi (Moravec a kol. 2015), zejména v kapitolách, věnovaných tomuto druhu (Moravec & Veselý 2015; Zavadil et al. 2015).

To, že štramberská populace nebyla založena recentní introdukcí v horizontu posledních 30 let, bylo spolehlivě doloženo prostřednictvím literárních pramenů a dokladového muzejního materiálu (viz výše) a v neposlední řadě osídlením velkého množství relativně vzdálených lokalit v katastru města Štramberk. Hudeček (2006) však upozornil na to, že pokusy o introdukce plazů na našem území nebyly vázány pouze na vzrůst aktivity teraristických zájmových spolků v posledních několika dekádách, ale že tyto snahy mohou být podstatně staršího data. To by totiž vysvětlovalo i naprostou absenci údajů o výskytu tohoto druhu v okolí Štramberku v prvorepublikové a starší literatuře, přestože lokalita byla koncem 19. a počátkem 20. století opakovaně navštěvována a zkoumána významnými osobnostmi tehdejší československé zoologie (Hudeček 2006). Z pozdějších let však byly doloženy snahy různých přírodozpytných spolků či přátel herpetologie a teraristiky o vysazování některých jihoevropských plazů, jako např. gekona tureckého (*Hemidactylus turcicus*), zmije skvrnité (*Vipera aspis*), zmije růžkaté (*V. ammodytes*) nebo štíhlovky kaspické (*Dolichophis caspius*) na území Moravy a Slezska (Adolph 1922, Pax 1925, Krátký & Wenig 1930). Populace ještěrky zední introdukovaná ve stejné době by tak měla dost času se rozšířit na území obývané dnes a populační charakteristiky schopné odhalit relativně mladou prudce rostoucí populaci by tak ztratily výpovědní schopnost. Ve světle těchto skutečností se jako jediná spolehlivá metoda k vystopování původu dané populace jevila molekulární analýza a porovnání sekvencí DNA našich ještěrek s DNA ještěrek z nejbližších populací na Slovensku a se vzdálenějšími populacemi balkánskými, jakožto potenciálním zdrojem introdukovaných exemplářů. Dalším zdrojem informací byly již publikované sekvence druhu ze západní části areálu dostupné v genových bankách.

3) Jablonski, D., Gvoždík, V., Choleva, L., Jandzik, D., Moravec, J., Mačát, Z. & **Veselý, M.**, 2019: Tracing the maternal origin of the common wall lizard (*Podarcis muralis*) on the northern range margin in Central Europe. *Mitochondrion* 46: 149-157. IF – 3.992; **Q2**

Pro rekonstrukci fylogeografie jsme využili *cytochrome b* (cyt b), jež byl v minulosti použit pro analýzu fylogeneze a fylogeografie nejen ještěrek zedních, ale všeobecně plazů a je v tomto ohledu velmi informativní (např. Kindler et al. 2013). Zejména z Německa je známo, že současný areál druhu je složen z populací, které mnohdy vznikly na základě introdukce z velmi vzdálených lokalit (Schulte et al., 2012a, 2012b). Pro severovýchodní okraj areálu druhu však podobná analýza doposud chyběla. Proto jsem se s kolegy z několika výzkumných institucí (Ústav biologie obratlovců Brno, Univerzita Palackého v Olomouci, Ostravská Univerzita, Univerzita Komenského v Bratislavě a Národní Muzeum Praha) zaměřil na výzkum populací nejen z Česka, ale i Slovenska, kde je výskyt také poměrně fragmentovaný (Lác 1968, 1970), a dále z Balkánu. Analyzovali jsme vztahy v tzv. Balkánské linii druhu, definované předchozími studiemi (např. Salvi et al. 2013). Jak ukázala naše data (Jablonski et al. 2019), tato linie tvoří pět geograficky ohraničených haploskupin. Námi analyzovaný materiál z českých a slovenských populací pak odhalil, že tyto populace náležejí do jedné haploskupiny tvořící 12 haplotypů a jsou blízce příbuzné populacím ze severovýchodního Německa, severního Maďarska, o něco vzdáleněji pak populacím ze severní Bosny a severovýchodního Chorvatska. Pravděpodobný je tedy model předpokládající, že recentní rozšíření ve střední Evropě je výsledkem postglaciální expanze druhu z balkánských refugií, která probíhala z oblasti severovýchodních Dinarid podél Panonské nížiny, západně od toku Dunaje. Na základě získaných poznatků pak můžeme předpokládat, že současný fragmentovaný výskyt této linie ve střední Evropě je výsledkem fluktuací okraje areálu druhu v průběhu holocénu, zapříčiněných změnami klimatu a vegetačního krytu v této oblasti (např. souvislý lesní porost v atlantiku). Následná změna charakteru krajiny způsobená člověkem mohla vytvořit vhodné podmínky pro šíření druhu, jak to v současnosti pozorujeme třeba na Balkáně, kde se ještěrky zední často vyskytují v antropogenních biotopech, ve městech apod. Pozdější ústup od extenzivního pastevního hospodaření a rozšiřování lesů se mohly podepsat na současném izolované rozšíření ve střední Evropě.

Další výsledky ukázaly, že všechny tři známé moravské populace jsou nejblíže příbuzné populacím vyskytujícím se podél Váhu a na středním Slovensku. Populace z Hádů je s těmito populacemi naprosto identická, v populacích z Grygova a Štramberka se vyskytuje kromě společného i unikátní haplotyp, který jsme nikde jinde nezaznamenali. To by mohlo naznačovat delší samostatný vývoj populací a tedy i jejich autochtonost. Zjištěný vzorec však může být také důsledkem nedostatečného provzorkování studovaných populací. Objevené „unikátní“ haplotypy se mohou vyskytovat i u jiných populací dané haploskupiny (např. na Slovensku), pouze nemusely být dosud odhaleny. Je možné si představit, že v případě populace z Grygova, jejíž autochtonní původ je značně pochybný (viz Moravec et al. 2015), byly ještěrky nachytány na nějaké populačně bohatší lokalitě např. na Slovensku nebo třeba i Štramberku a různé haplotypy se tedy na lokalitu dostaly uměle. Grygovská populace byla totiž objevena relativně nedávno (Mačát & Veselý 2009), což je poněkud v rozporu s tím, že lokalita byla od 60. let minulého století oblíbeným cílem zoologických exkurzí studentů i pedagogů blízké Univerzity Palackého v Olomouci, kde mimo jiné působil i nestor československé herpetologie a zakladatel Herpetologické sekce České zoologické společnosti doc. Evžen Opatrný. Je těžké si představit, že by dnes docela hojné ještěrky na stěnách lomu a kamenných polích unikly pozornosti, obzvláště když je lokalita pravidelně navštěvována v květnu, kdy jejich aktivita vrcholí. Stejně tak existují pochyby u Štramberské populace. Zvýšená frekvence anomálií ve folidóze hlavy, zahrnující rozdělení (často i několikanásobné) štítků pilea (Veselý et al. 2007, Moravec & Veselý 2015) totiž může být důsledkem demografického bottlenecku a tedy snížené alelické diverzity vyplývající z malého počtu zakladatelských jedinců, což by ukazovalo na dávnou introdukci (viz Gautschi et al. 2002).

Vzhledem k relativní obtížnosti získávání většího množství srovnávacího materiálu je momentální závěr stále nejasný, nicméně výzkum stále pokračuje. V roce 2019 byl publikován nález nové populace na zřícenině Šelenburk u Krnova (Vlček & Zavadil 2019). Haplotypová struktura této populace zatím není známa, neboť analýzu materiálu z této lokality zabrzdila epidemie Covid-19. V loňském roce se mi pak podařilo objevit ještě další dobře etablovanou populaci ještěrky zední přímo v intravilánu Opavy, vzorky z této populace také čekají na zpracování.

Může se stát, že některé haplotypy uniknou odhalení. Stejně tak není možné zatím vyloučit zavlečení zakladatelů našich populací z blízkých slovenských lokalit. Důležité však je, že naše ještěrky zapadají do fylogeografické hypotézy, že jsou součástí širší Balkánské linie, která je rozšířena ve střední a části jihovýchodní Evropy a že jsou součástí středoevropské haplotypové skupiny, kde v zásadě klastrují s běžně rozšířenými haplotypy této skupiny. Nemáme zde tedy ještěrky, které by pocházely z Itálie či jiných středomořských oblastí (diskutovalo se o možných snahách introdukce druhu v ČR z Turecka), jako je tomu na řadě míst v Rakousku, Německu, Švýcarsku, Francii či Velké Británii. Dokonce i pokud by naše ještěrky byly introdukovány z blízkých lokalit Slovenska, nejedná se o problematický přenos cizího genotypu na nové území a pouze by to korespondovalo s historickými fluktuacemi populací druhu, které zde koncem pleistocénu a holocénu probíhaly. Jinak řečeno, i v případě, že ještěrky z našeho území kdysi opravdu zmizely a po nedávné introdukci z blízkých lokalit se znovu uchytily (což by se s posunem severního okraje rozšíření v důsledku měnícího se klimatu u druhů s dobrou migrační schopností stalo pravděpodobně tak či onak), zapadají tyto populace do kontextu středoevropské přírody a měly by být proto chráněny zákonem.

4. Systematika a taxonomie herpetofauny Střední Ameriky

Střední Amerika tvoří pevninský most mezi Severní Amerikou a Jižní Amerikou. Z biogeografického hlediska je velmi významná, neboť její fauna obsahuje nejen prvky faun obou velkých kontinentů, ale také velké množství druhů endemických. Na příkladu herpetofauny lze demonstrovat, že tento podíl dosahuje téměř 60% z celkového druhového bohatství, které v současnosti představuje více než 1100 druhů obojživelníků a plazů (Johnson et al. 2015; Mata-Silva et al. 2019). Tato vysoká druhová bohatost je výsledkem jednak výjimečné pestrosti habitatů koncentrovaných na relativně malém území a jednak bouřlivé geologické historie oblasti.

Historie herpetologického výzkumu Střední Ameriky sahá do 19. století a je spojena s významnými evropskými herpetology té doby. Vůbec první obsáhlejší prací byla "Herpetologia Mexicana" (1834) od Arenda F. A. Wiegmanna působícího na Humboltově univerzitě v Berlíně. V letech 1885-1902 spatřila světlo světa "Biologia Centrali-Americana: Reptilia and Batrachia" od Alberta C. L. G. Günthera z British Museum of Natural History v Londýně. Téměř ve stejnou dobu (1870-1909) vydávali francouzští herpetologové A. H. A. Duméril, M. F. Bocourt a F. Mocquard dílo "Mission scientifique au Mexique et dans l'Amérique Centrale" a vycházely také díly katalogu obojživelníků a plazů ve sbírkách Britského muzea v Londýně z pera G. A. Boulengera. Řada druhů popsaných těmito autory je dodnes validní a jejich díla položila solidní základ modernímu herpetologickému výzkumu Střední Ameriky. Od té doby vznikly stovky prací, zabývajících se systematikou, ekologií a biogeografií herpetofauny této oblasti, jejichž výčet by byl naprosto mimo možnosti tohoto krátkého úvodu do problematiky. Bohužel, jak vědecký výzkum, tak i snaha o ochranu zdejší enormní biodiverzity jsou závod s časem, neboť tempo destrukce místních unikátních habitatů se stále zrychluje.

4) Köhler, G., **Veselý, M.** & Greenbaum, E., 2006: *The Amphibians and Reptiles of El Salvador*. Krieger Publishing Company, Malabar, Florida, 238pp. ISBN 1-57524-252-4

Nápad na souhrnné zpracování herpetofauny Salvádoru do podoby monografie byl výsledkem šťastné souhry okolností. Depozitář výzkumného institutu a muzea Senckenberg, kde jsem od r. 1996 pracoval jako hostující výzkumník během pravidelných stáží, totiž obsahuje největší kolekci salvádorské herpetofauny na světě. Tuto sbírku přivezl ze své předválečné expedice zdejší bývalý kurátor herpetologie a jeden z nejvýznamnějších moderních světových herpetologů, prof. Robert Mertens. Devadesátá léta byla ve Střední Americe dobou ukončení vlády zdejších diktatur a zároveň ukončením občanských válek, trvajících dlouhá desetiletí. Země se začaly otevírat světu (kromě Salvádoru také okolní státy Nikaragua, Guatemala a Honduras). Salvádorská junta, nechvalně proslulá brutálními vraždami civilistů i duchovních, byla podporována ze strany USA, které se prostřednictvím CIA přímo podílely na boji s partyzány z Fronty národního osvobození Farabunda Martího a výcviku eskader smrti. Proto se po ukončení války a demokratických volbách nová vláda v zájmu uklidnění situace ve společnosti orientovala spíše na spolupráci s Evropskou unií, což nám v této tradiční sféře vlivu USA poskytlo konkurenční výhodu a náskok před kolegy z amerických univerzit. Důležité pro nás také bylo, že tato nejmenší středoamerická země byla v regionu posledním státem, jehož herpetofauna nebyla souborně zpracována formou monografie. Rozhodli jsme se tedy s dr. Köhlerem společnými silami obohatit Mertensovu předválečnou kolekci recentním materiálem, analyzovat další muzejní sbírky v Salvádoru a USA a monografii sepsat.

Přípravu expedice však provázely komplikace. Nejprve jsme museli termín posunout o půl roku kvůli zpoždění ve vyřízení všech povolení ke sběru zoologického materiálu a pohybu v bývalých „partyzánských“ regionech. Také jsem byl přinucen vyřídit si druhý pas, abych mohl odjet do Frankfurtu, zanechat tam jeden z pasů za účelem vyřízení vstupních víz a letenek a pomocí druhého se vrátit zpět (tenkrát byl pro nás Schengen ještě v nedohlednu). Kvůli posunutému termínu expedice jsem také musel převzít celou organizaci na sebe, neboť kolegovi Köhlerovi nový termín kolidoval s již dříve připraveným projektem v Kostarice a další z plánovaných účastníků měl v té době závěrečné zkoušky. Do Salvádoru jsem tedy odletěl pouze se studentem zoologie na univerzitě J.W. Goethe ve Frankfurtu Jorgem Kreutzem. Odletem však potíže neskončily. V USA mě odmítli vpustit do letadla do Salvádoru, takže jsem musel znovu na salvadorskou ambasádu – tentokrát v Miami – pro další vízum. Bohužel, i tak mě po příletu zadrželi na letišti, protože Česká republika v té době u imigračních úředníků stále figurovala na seznamu teroristických zemí vedle Iránu či Izraele. Až po intervenci z ministerstva životního prostředí, které mělo nad naší expedicí záštitu, mě vpustili do země. I přes další komplikace však byla expedice nakonec úspěšná a zásadně obohatila materiál na přípravu monografie.

Přesto, že práce na monografii byla zdlouhavá a náročná, věnoval jsem se souběžně i dalším projektům souvisejících s problematikou středoamerické herpetofauny (Veselý 1998; Veselý & Köhler 2001; Köhler & Veselý 2003; Köhler et al. 2005). Po dokončení první verze rukopisu knihy dostala text k revizi Dr. Linda Trueb z katedry ekologie a evoluční biologie na University of Kansas. Ta nám doporučila svého PhD studenta Eliho Greenbauma, který v Salvádoru pracoval na své diplomové práci v době, kdy jsme rukopis dokončovali. Proto jsme ho přizvali do autorského týmu, zapracovali nejnovější poznatky a knihu rozšířili o další druhy, jejichž výskyt byl v Salvádoru prokázán po dokončení první verze. Monografie vyšla v roce 2006 v USA v prestižním vydavatelství Krieger Publishing Company, které se specializuje na odborné a vědecké tituly z oblasti přírodních věd, kosmických technologií, vzdělávání dospělých a historie. Kniha získala ocenění rektora UP za zahraniční monografii v r. 2006.

5) Köhler, G., **Veselý, M.,** 2010: A revision of the *Anolis sericeus* complex with the resurrection of *A. wellbornae* and the description of a new species (Squamata: Polychrotidae). *Herpetologica* 66 (2): 186-207. IF – 1.667; **Q1**

Norops sericeus (Hallowell, 1856) je hojným druhem štíhlého travního středoamerického anolise vyskytujícího se v široké škále habitatů od suchého opadavého lesa, savan a pastvin až po vegetaci podél cest a v okolí lidských sídel. který se od ostatních druhů na první pohled liší žlutě zbarveným hrdélkem s modrou skvrnou uprostřed. Areál druhu zasahuje od severního Mexika (Tamaulipas) až do Kostariky, kde obývá většinou nížinné biotopy, ale je doložen i z nadmořské výšky 1 040 m. Přesto, že tak rozlehlý areál naznačuje, že by se v rámci tohoto taxonu mohly skrývat kryptické druhy, dosavadní snahy o revizi skončily neúspěchem kvůli poměrně uniformní morfologii a zároveň značné vnitropopulační variabilitě v některých znacích. Řada autorů však upozornila na určité mezipopulační rozdíly mezi horskými a nížinnými populacemi, popř. mezi populacemi z karibského a pacifického pobřeží (Stuart 1955, Duellman 1965, Lee 1980).

Na výzkumu jsem začal pracovat už v r. 1996. Během práce na dalších herpetologických projektech a návštěvách světových muzejí jsem se vždy zajímal i o tento druh a časem se mi podařilo shromáždit úctyhodný dataset externích morfologických dat od 1614 exemplářů. Přesto že variabilita v datech byla značná, ani při využití technik multidimenzionální analýzy výsledky neposkytovaly smysluplný pattern, který by bylo možné prezentovat nikoliv jako pouhou klinální variabilitu. Naštěstí po letech bezvýsledných pokusů kolegu Köhlera napadlo zaměřit se na morfologii hemipenisů, které sice také patří k morfologickým znakům, ale podléhají jinému typu selekce než externí morfologické znaky a znaky na folidóze a jsou tedy z tohoto hlediska nezávislým datasetem. Hemipenisy šupinatých jsou vychlípitelné váčkovité orgány, které lze ale studovat pouze v evertovaném stavu, narazili jsme tedy na problém objevit v muzejním materiálu takové množství samců s vypreparovanými pářícími orgány, aby to umožnilo definování areálů operačních taxonomických jednotek. Je totiž prakticky nemožné zjistit tvar hemipenisu u konzervovaných exemplářů, u kterých nebyly pářící orgány nafouknuty před fixací. Vlivem etanolu a formalínu tkáně ztvrdnou a ztratí pružnost. Proto jsme se na druh zaměřili i během práce v terénu a zásadní část materiálu poskytly naše expedice do Mexika, Guatemaly, Hondurasu a Nikaragui v letech 2005 až 2008. Na základě studia nových exemplářů se nám podařilo komplex *Anolis sericeus* rozdělit na tři druhy: původní druh *A. sericeus* reprezentovaný Hallowellovým holotypem je rozšířen podél pobřeží Mexického zálivu a Karibského moře až do jižního Belize; druh *A. wellbornae* popsaný původně Ahlem jako poddruh *A. ustus wellbornae* v roce 1940 (*A. ustus* byl v r. 1955 synonymizován s *A. sericeus* Stuartem) jsme vyňali ze synonymie a znovu povýšili na druh, který je rozšířen podél pacifického pobřeží od jižní Guatemaly až po Nikaraguu; a konečně třetí druh, pro který nebyl k dispozici žádný starší typový exemplář (a tedy ani název) a popsali jsme ho jako nový druh *Anolis unilobatus.* Tento druh má malé, unilobátní hemipenisy a je rozšířen mezi areály dříve zmíněných dvou druhů, které mají mnohem větší bilobátní hemipenisy. Dosud není známá oblast, kde by se některé z těchto druhů vyskytovaly sympatricky, i když v jižní Guatemale jsou areály *A. unilobatus* a *A. wellbornae* ve velmi těsném kontaktu (lokality těchto druhů jsou méně než 20 km vzdálené).

6) Köhler, G., **Veselý, M.,** 2011: A new species of Thecadactylus from Sint Maarten, Lesser Antilles (Reptilia, Squamata, Gekkonidae). *ZooKeys* 118: 97-107. IF – 0.879; Q3

Areál rodu *Thecadactylus* zasahuje od jižního Mexika přes celou Střední Ameriku až do severní části Jižní Ameriky a zahrnuje také Malé Antily. Tito gekoni zde obývají nejrůznější typy tropických habitatů a se svou velikostí dosahující téměř 20 cm jsou to největší američtí gekoni. Tento rod byl tradičně vnímán jako monotypický s jediným zástupcem *T. rapicauda* (Peters & Donoso-Barros 1970; Hoogmoed 1973; Avila-Pires 1995), teprve v roce 2007 byl od tohoto druhu oddělen další taxon *T. solimoensis*, který se od původního druhu liší některými morfologickými znaky (Bergmann and Russell 2007). Do tohoto druhu nyní spadají populace vyskytující se v jižní části areálu původního druhu (tedy populace ze střední části Jižní Ameriky). Všechny populace z Malých Antil byly vždy pokládány za konspecifické s *T. rapicauda* (např. Powell et al. 1996, Censky & Kaiser 1999, Malhotra & Thorphe 1999 a další). Na podzim 2010 se v zájmových chovech v Německu začali objevovat zvláštně zbarvené formy tohoto gekona. Na rozdíl od krypticky zbarvených zástupců *T. rapicauda*, kteří mají na těle lichenózní vzor s nepravidelně roztroušenými tmavými a světlými skvrnami, měly tyto exempláře uniformně žlutohnědé, okrové nebo béžové zbarvení s více či méně početnými černými skvrnkami. Všechna zvířata pocházela od stejného dovozce, firmy Import Export Peter Hoch GmbH, na kterou jsme se obrátili s dotazem na původ dovezených exemplářů. Protože tyto firmy ale mají obvykle ještě řetězec místních dodavatelů, kteří si svá naleziště žárlivě střeží, zjistili jsme pouze to, že gekoni pocházejí z ostrova Sint Maarten v Malých Antilách. Nicméně se nám u firmy podařilo alespoň získat několik uhynulých exemplářů za účelem dalšího zkoumání. Získali jsme jistotu, že se tito jedinci liší od *T. rapicauda* nejen zbarvením, ale také morfologickými a morfometrickými znaky. Měli jsme tedy nový druh, ale chyběla nám typová lokalita, informace o rozšíření a ekologii. Protože zprávy o naší práci se rychle rozkřikly v rámci DGHT (Deutsche Gesellschaft für Herpetologie und Terrarienkunde), ozvali se nám Maciej Oskroba a Stefan Prein, kteří se na Holanské Antily právě chystali na dovolenou a souhlasili, že zkusí o gekonech na ostrově vypátrat více informací. To se podařilo, takže jsme do našeho popisu mohli zahrnout kromě klasických deskriptivních dat také poznatky z biologie a ekologie druhu a druh jsme pojmenovali na počest obou herpetologů, kteří se do našeho výzkumu zapojili.

7) Köhler, G., Diethert, H. H., **Veselý, M.,** 2012: A Contribution to the knowledge of the lizard genus *Alopoglossus* (Squamata: Gymnophthalmidae). *Herpetological Monographs* 26 (1): 173-188. IF – 1.818; **Q1**

Během práce na determinačním klíči neotropické čeledi Gymnophtalmidae jsme s kolegy stále častěji naráželi na inkonzistence ohledně druhového zařazení materiálu, patřícího do rodu *Alopoglossus*. Jedná se o drobné ještěry žijící v opadu nížinných deštných lesů Jižní Ameriky, kteří jsou pro svůj skrytý způsob života jen řídce zastoupeni v muzejních kolekcích. Nicméně jeden z druhů, *A. angulatus* byl popsán už Linném (1758) jako *Lacerta angulata*. Od ostatních gymnophtalmidů (s výjimkou r. *Ptychoglossus*) se odlišují přítomností šikmých záhybů na horní straně jazyka (versus šupinkovité papily u ostatních rodů) a téměř přímou linií zadního okraje parietálních a interparietálních šupin (Boulenger 1885). Druhy jsou si navzájem velmi podobné a zkoumané exempláře byly často chybně determinovány. Podobně jako u komplexu *Anolis sericeus* se nám během let podařilo postupně nashromáždit poměrně početný materiál ze světových muzeí. Revizí tohoto materiálu čítajícího 278 jedinců a jejich lokalitních dat se nám podařilo zpřesnit jak diagnózy jednotlivých druhů, tak i jejich geografické a altitudinální rozšíření. Tři druhy (*A. angulatus*, *A. atriventris* a *A. buckleyi*) mají výrazně větší areál, než se původně předpokládalo. Většina nálezů dokládá výskyt v nadmořských výškách 100-800 m, výjimečně nad touto hranicí až do 1500 m. Nález exempláře *A. festae* (Escuela Politecnica Nacional, Quito, Ecuador [EPN] 4212) pocházející z Pallatangy, vesnice na svazích And ve výšce 2250 m je z tohoto hlediska pravděpodobně chybně lokalizován. Kromě podrobného popisu obsahuje studie také kresby folidózy hlavy jednotlivých druhů, určovací klíč a mapky nálezů. Druh *Alopoglossus andeanus* Ruibal, 1952 jsme na základě revize synonymizovali s druhem *Alopoglossus angulatus* (Linnaeus, 1758).

8) Batista, A., Hertz, A., Mebert, K., Köhler, G., Lotzkat, S., Ponce, M., **Veselý, M.,** 2014: Two new fringe-limbed frogs of the genus Ecnomiohyla (Anura: Hylidae) from Panama *Zootaxa* 3826 (3): 449–474. IF – 0.906; Q3

Od roku 2011 jsem se ve výzkumu zaměřil na herpetofaunu Panamy – nejjihovýchodnějšího státu Střední Ameriky. Během uplynulých 10 let jsem se svým panamských kolegou Abelem Batistou podnikl řadu expedic do nejrůznějších částí Panamy, včetně legendárního Dariénu -– nedostupné a neprobádané oblasti horských hřbetů na hranicích Panamy a Kolumbie. Toto území patří v rámci neotropů mezi nejvýznamější „biodiversity hotspots“. Během těchto výprav jsme nasbírali velké množství materiálu, jehož dílčí části postupně zpracováváme. Jednou z lehce odlišitelných skupin neotropických Anura jsou rosničky rodu *Ecnomiohyla* Faivovich, Haddad, Garcia, Frost, Campbell, & Wheeler 2005. Jsou to velké žáby s neobvyklým vzhledem – mají zvláštní lichenózní kryptické zbarvení a výrazné kožní lemy na končetinách, které ještě umocňují maskovací efekt hřbetního zbarvení. Jsou to obyvatelé korunového patra deštných lesů od nížinných vlhkých lesů, přes deštné lesy podhorské až po horské mlžné lesy do nadmořských výšek okolo 2000 m. Rozmnožují se ve fytotelmách (Savage 2002; Mendelson et al. 2008; Savage & Kubicki 2010), takže koruny stromů neopouštějí ani v době rozmnožování. Kryptické zbarvení a zvláštní způsob života jsou pravděpodobně příčinou jejich řídkého zastoupení v kolekcích světových muzeí. Je možné, že tato vzácnost může být pouze důsledkem nesprávných herpetologických technik sběru, což jen podtrhuje význam jakýchkoliv informací o takových vzácně pozorovaných a zkoumaných taxonech.

Rod *Ecnomiohyla* je rozšířen od jižního Mexika až po severozápad Jižní Ameriky a v době před publikací našeho výzkumu obsahoval 12 druhů. U řady druhů byl holotyp po dlouhou dobu jediným známým exemplářem, naštěstí intenzivní herpetologický výzkum a nálezy dalších exemplářů v posledních třech dekádách podstatně přispěly k rozšíření znalostí o ekologii, příbuzenských vztazích uvnitř rodu a k objasnění jeho pozice v rámci čeledi Hylidae.

Rod *Ecnomiohyla* lze od všech ostatních rodů čeledi Hylidae odlišit pomocí následujících znaků: zástupci mají v poměru k tělu mohutné končetiny, dále mají zubovité kožní lemy na vnějším okraji předloktí a zadních končetinách, mají velké prstové disky a konečně i zvětšené prepollikální hrboly (Firschein & Smith 1956; Savage & Heyer 1969; Duellman 1970; Mendelson et al. 2008). Tyto hrboly jsou více vyvinuty u samců, kde obvykle mají ještě terminální trn (vyčnívající, jako u *E. miliaria*) nebo lopatkovitý výrůstek (např. u *E. valancifer*). U řady druhů druhů nesou tyto hrboly u samců také keratinizované černé trny, zatímco u samic jsou vždy bez keratinizovaných výrůstků a jsou také výrazně štíhlejší.

Na základě molekulárních (16S) a morfologických dat v této práci popisujeme dva nové druhy rodu *Ecnomiohyla*.

Ecnomiohyla bailarina pochází z východní Panamy a typový exemplář samčího pohlaví jsme nalezli během naší první Dariénské expedice do odlehlé horské oblasti Serranía de Jingurudó. Od ostatních druhů se liší externí morfologií i zbarvením a od sesterského druhu *E. rabborum* Mendelson, Savage, Griffith, Ross, Kubicki, & Gagliardo, 2008 ho dělí genetická vzdálenost p-distance >12%. V druhovém epiteton "bailarina" je skryto více významů. Jednak připomíná oblast, kde jsme typový exemplář nalezli (na svazích nejvyššího vrcholu Serranía de Jingurudó, které domorodí indiáni kmene Emberá nazývají Cerro Bailarin) a jednak v přeneseném významu výrazné kožní lemy na končetinách poněkud připomínají nadýchanou "tutu" sukýnku tanečnice (ve španělštine "bailarina").

Ecnomiohyla veraguensis pochází ze západní Panamy, typový exemplář samčího pohlaví byl nalezen na svazích Cerro Negro v národním parku Santa Fé v departamentu Veraguas. Od ostatních druhů se opět liší externí morfologií a od sesterského druhu *E. sukia* Savage & Kubicki, 2010 ho dělí genetická vzdálenost p-distance >7%. Druhové epiteton zde připomíná department Veraguas, který jako jediný z panamských departmentů zahrnuje obě pobřeží (jak tichomořské, tak i karibské, tedy atlantické) a na hornatém území mezi dvěma oceány se nachází velké množství habitatů, obývaných desítkami druhů obojživelníků.

Oba druhy jsou dodnes známy pouze z jediného exempláře i přes obohacení našich výzkumných metod o techniky průzkumu korunového patra deštných lesů na některých následných expedicích (Cordillera Talamanca, Reserva forestal Fortuna, 2014, Cerro Hoya 2016, Darién 2018).

9) Batista, A., Köhler, G., Mebert, K., Hertz, A., **Veselý, M.,** 2016: An integrative approach to reveal speciation and species richness in the genus *Diasporus* (Amphibia: Anura: Eleutherodactylidae) in eastern Panama. *Zoological Journal of the Linnean Society* 178 (10): 267–311. IF – 2.711; **D1**

Problematika kryptických druhů byla vždy výzvou pro taxonomy zabývajícími se nejrůznějšími skupinami živočichů. Zjednodušeně můžeme kryptické druhy definovat jako skupinu dvou nebo vice taxonů chybně zařazeným pod jedno druhové jméno (Bickford et al. 2007). Mezi neotropickými žábami byla vždy vyhlášená kryptickou diverzitou celá skupina Terrarana, zahrnující čeledi v minulosti řazené do čeledi Eleuterodactylidae, která se v současnosti rozpadla na čtyři čeledi žab: Eleuterodactylidae, Brachycephalidae, Craugastoridae a Strabomantidae, Tuto skupinu mimo jiné charakterizuje také jev u obojživelníků poměrně vzácný – přímý vývoj (a tedy nezávislost na vodním prostředí). V rámci první jmenované čeledi je příkladem kryptické diverzity rod *Diasporus*, kam patří drobné žabky (velikost okolo 20 mm) uniformního vzhledu, které je obtížné od sebe rozlišit jen na základě morfologických znaků. Nástrojem, který umožnil rozkrýt a pochopit rozdíly mezi jednotlivými druhy a jejich příbuzenské vztahy se stala integrativní taxonomie, kombinující znaky morfologické s jinými sadami nezávislých znaků – v tomto případě s molekulárně genetickými znaky a bioakustickou analýzou.

Všichni dosud známí zástupci rodu Diasporus mají krátké končetiny, jejichž prsty jsou opatřeny rozšířenými disky. Konce disků mohou mít kopinaté nebo prstíkovité výběžky. Jsou to typičtí obyvatelé deštných lesů od východního Hondurasu až po severozápad Jižní Ameriky, kde se často vyskytují ve vysokých denzitách. Samci mají subgulární rezonátor a vydávají charakteristické volání, podobné vysokému tikání či krátkým hvizdům, které spoluvytváří typický sound nočního deštného lesa. Vzhledem ke svému nenápadnému vzhledu jsou ale i volající samci velmi těžko k nalezení.

Do vydání naší studie rod obsahoval pouze 11 druhů, z nichž 7 je endemických pro Střední Ameriku, jeden zasahuje od východní Panamy až do Kolumbie a 3 druhy se vyskytují podél pacifického pobřeží Kolumbie a Ekvádoru. Během posledního desetiletí jsme podnikli několik expedic do východní Panamy a nasbírali řadu ceného materiálu. Předběžná barkodová analýza vylišila v materiálu r. *Diasporus* čtyři výrazné větve odlišné od z regionu jediného původně známého druhu *Diasporus quidditus* (Lynch 2001). Společně s poznatky z terénního výzkumu (nahrávky hlasů, ekologická data), morfologickými znaky a podprobnějším srovnání sekvencí dvou mtRNA markerů používaných v systematice obojživelníků (16S, COI) nám tyto údaje poskytly robustní dataset pro popis čtyř nových druhů, jejichž areály jsou navzájem allopatrické a omezené na čtyři horské celky v rámci hor východní Panamy.

Diasporus darienensis se vyskytuje v oblasti Cerro Pirre a přilehlém horském pásmu Serranía de Jingurudó v jihovýchodním Dariénu, *D. majeensis* obývá izolovanou vysočinu Majé u pacifického pobřeží středního Dariénu, areál *D. pequeno* zabírá hřeben Serranía de Darién u karibského pobřeží Dariénu a *D. sapo* je endemitem stejnojmenného vrcholu Cerro Sapo na severovýchodním výběžku nejjižnějšího dariénského pohoří. Ačkoliv naše studie výrazně zvýšila počet druhů tohoto rodu v regionu (ze 7 na 11), je jasné, že ani toto číslo není konečné. Dá se předpokládat, že aplikace principů integrativní taxonomie vyústí v obdobné rozkrytí kryptické diverzity i v ostatních oblastech výskytu tohoto rodu v rámci Střední a Jižní Ameriky.

10) **Veselý, M** & Batista, A., 2021: A New Species of *Atelopus* (Amphibia: Bufonidae) from eastern Panama. *Zoological Research*. DOI: 10.24272/j.issn.2095-8137.2020.319; **D1**

Ropuchy rodu *Atelopus* patří mezi vůbec nejvýraznější a nejpozoruhodnější skupiny neotropických žab. Zároveň je to jedna z nejvíce ohrožených skupin obojživelníků na světě, neboť v posledních 20 letech byl zaznamenán masivní úbytek či často úplné zmizení u řady populací (Ron *et al.* 2003; La Marca et al. 2005; McCaffery et al. 2015). V současné době je 97% známých druhů řazeno podle červeného seznamu IUCN do kategorie “ohrožený” (EN), “kriticky ohrožený” (CR) nebo “vyhynulý” (EX) (Stuart et al. 2008; Catenazzi 2015). Toto plošné vymírání bylo ve velké většině zjištěných případu způsobeno chytridiomykózou - nemocí způsobenou parazitickou houbou *Batrachochytrium dendrobatidis*, která byla objevena právě ve Střední Americe a v dnešní době je jednou z příčin tzv. globální krize obojživelníků. Z rodu *Atelopus* jsou touto nemocí decimovány zejména populace druhů žijících ve vyšších nadmořských výškách, z panamských např. *Atelopus zeteki*, *A. chiriquiensis* nebo *A. varius* (Brehm & Lips 2008; Woodhams et al. 2008; Crawford et al. 2010).

Areál rodu *Atelopus* sahá od Kostariky po Bolívii a recentně do něj patří 100 uznávaných druhů (AmphibiaWeb 2021). Pouze 6 druhů se vyskytuje v Panamě, přičemž poslední z nich byl popsán před více než čtvrt stoletím (Ibáñez *et al.* 1995). Vzhledem k pokračující deforestaci, ničení původních biotopů a všudypřítomné chytridiomykóze by se tedy nezdálo pravděpodobné, že by bylo možné nalézt dosud nepopsaný druh těchto pestrých a výrazných ropuch, který by dosud zůstal neobjeven herpetology pracujícími v Panamě. Přesto se nám během expedic do nepřístupných oblastí Dariénu podařilo objevit několik populací, které se už na pohled poněkud lišily od všech popsaných druhů. Následná molekulární analýza odebraných vzorků tkáně ukázala, že se opravdu jedná o samostatný klád reprezentující novou taxonomickou jednotku. Následné studium komparativního materiálu ze světových muzeí pak ukázalo další zajímavou věc – tento druh byl poprvé nalezen už v r. 1911 poblíž osady Puerto Obaldía na panamsko – kolumbijské hranici přírodovědcem Henrym Pitierem (Heackadon-Moreno 1996). Tento nejstarší známý exemplář uložený v United States National Museum (pod číslem USNM 48594), byl později herpetologem Dunnem (1931) zvolen jako paratypus druhu *Atelopus glyphus*. Už několik let před tím (v r. 1924) však uspořádal Smithonian Institut první biologickou expedici do Dariénu, které se zůčastnil také Charles M. Breder. Tento herpetolog nasbíral 29 jedinců podobné žáby na lokalitě Chalichimans Creek, malém přítoku, vlévajícím se do Río Sucubtí z jižních svahů pohoří Serrania San Blas v povodí řeky Chucunaque. Několik poznámek k biologii těchto žab Breder později publikoval ve zprávě z expedice zabývající se herpetofaunou Dariénu (Breder 1946). Téměř půl století od expedice (1971) revidoval Brederův materiál uskladněný ve sbírkách American Museum of Natural History Jay M. Savage při práci na monografii zabývající se středoamerickými zástupci rodu *Atelopus*. Sklenici s nálezy však bez dalších taxonomických konsekvencí označil pouze štítkem "*Atelopus* sp.". V roce 1975 podnikl výzkumnou expedici do Dariénu herpetolog Charles W. Myers, kterému se podařilo nalézt 14 jedinců těchto ropuch na svazích Cerro Malí a další 4 u Río Púcuro. Část tohoto materiálu byla opět uložena ve sbírkách AMNH, druhá část pak v zoologické sbírce Dr. Eustorgia Méndeze v Instituto Conmemorativo Gorgas De Estudios De La Salud (ICGES) v Panamě. V obou depozitářích byly sběry opět označeny jen jako *Atelopus* sp. Nicméně, v článku publikovaném Cocroftem et al. (1990) je zmínka o třech samčích exemplářích a záznamu jejich hlasu (Kansas University, pásek 815), pocházejících ze staršího nálezu C. W. Myerse (1967), z osady Sasardí v provincii San Blas. Na základě bioakustické analýzy hlasu autoři předpokládají, že tyto exempláře "mohou představovat dosud nepopsaný druh v druhovém komplexu *Atelopus varius*". Takovou interpretaci ostatně zmiňoval už Savage ve své klasické práci "The Harlequin Frogs, Genus *Atelopus*, of Costa Rica and Western Panama" (1972). V poslední dekádě někteří autoři uváděli tento taxon jako *Atelopus* cf. *limosus* (Lewis *et al*. 2019) nebo *Atelopus* sp. "Puerto Obaldía-Capurganá" (Ramírez *et al*. 2020).

Odpověď na otázku “jak může tak výrazná a barevná žába ležet bez povšimnutí v depozitářích významných muzeí více než století?” je celkem jednoduchá. Vzhledem k velké variabilitě barevných vzorů a morfometrických znaků je velmi obtížné charakterizovat druhy některých druhových komplexů v rodu *Atelopus* pouze na základě morfometrie (De la Riva *et al.*, 2011). Zřejmě proto zůstal tento taxon tak dlouho nepopsán, ač se jím zabývali přední světoví herpetologové. Velké pokroky v technologiích, využívaných v současné době v integrativní taxonomii nám dnes však umožňují vyřešení i takto obtížných taxonomických záhad. Díky tomu se nám podařilo druh přesvědčivě definovat nejen molekulárně, ale také bioakusticky a morfologicky. Nový druh jsme nazvali *Atelopus fronterizo*, přičemž druhové epiteton odráží název, kterým místní obyvatelé označují někoho, kdo žije poblíž hranice. Zároveň však používají toto označení i pro příslušníky panamské pohraniční vojenské policie (SENAFRONT – Servicio nacional de las fronteras), kteří zajišťují bezpečnost v této neklidné části Panamy. Bez logistické podpory této organizace by byl náš výzkum v některých částech Dariénu nemožný.

5. Poděkování

Během svého profesního života jsem měl možnost spolupracovat se spoustou skvělých kolegů a kolegyň z celého světa, jejichž seznam by vydal na několik stránek a stejně by určitě nebyl úplný. Z řady z nich se stali mí osobní přátelé, na které se mohu kdykoliv obrátit i s jinými než vědeckými problémy, bez ohledu na to, zda sedí ve vedlejší kanceláři nebo žijí Panamě, Indii, Austrálii či jinde na světě. V terénu mi nezištně pomáhala řada lidí, jejichž jméno jsem často ani neznal. Těm všem chci poděkovat, i když se to pravděpodobně nikdy nedozví. Děkuji také svým studentům. Těm, kteří se podíleli na našich společných projektech i těm, které jsem učil. Spoustě věcí mě naučili i oni. Nejvíc ze všeho ale děkuji svým nejbližším – manželce Lence a dětem Michaele a Ondrovi – za jejich celoživotní podporu a lásku.

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7. Přílohy

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Poznámka: Předložených deset publikací představuje výběr z mého výzkumu v oblasti herpetologie. Jsou zde prezentovány pouze práce, u kterých jsem jedním ze dvou hlavních autorů a můj přínos je zásadní (30% a více). Konkrétní vyjádření mého podílu je uvedeno u každé z příloh a je doloženo korespondenčním autorem v materiálech předložených s habilitační prací. U každé z příloh je také uveden ranking časopisu v dané kategorii a počet citací k datu 10. dubna 2021.

Příloha 1.

Veselý, M., Modrý, D. (2002): Rain-harvesting behavior in Agamid Lizards (*Trapelus*). *Journal of Herpetology* 36(2): 311-314.

The contribution in words:
MV and DM conceived and designed the study and carried out experiments, MV performed the microscopic skin transections and SEM. MV and DM wrote the text. Both authors approved the final version of the manuscript.

Ranking: IF_{2002} – 0.649; Q3 (Zoology)
Citace na WoS (Core collection; bez autocitací všech spoluautorů): 12
Citace celkem (Google Scholar): 19

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Accepted 24 August 2001.

Journal of Herpetology, Vol. 36, No. 2, pp. 311–314, 2002

Rain-Harvesting Behavior in Agamid Lizards (*Trapelus*)

MILAN VESELÝ [1], *Department of Zoology, Faculty of Natural Sciences, Palacky University, Svobody 26, 771 46 Olomouc, Czech Republic; E-mail: veselym@prfnw.upol.cz*

DAVID MODRÝ [2], *Department of Parasitology, University of Veterinary and Pharmaceutical Sciences, Palackého 1-3, 612 42 Brno, Czech Republic; E-mail: modry@dior.ics.muni.cz*

The ability of arid region reptiles to use the body surface as a collector of water has been known for many years, although it has been described only in a small number of species. Bentley and Blumer (1962) demonstrated that *Moloch horridus* (Agamidae) drink the water film passively transported by capillary action of the skin to the mouth, thus contradicting a theory that water is absorbed transcutaneously in this species (Buxton, 1923). Gans et al. (1982) reexamined *Moloch* skin with the aid of scanning electron microscope (SEM) photographs and attributed the water flow to capillary forces generated by grooves between scales. Schwenk and Greene (1987) described a similar system in another agamid, *Phrynocephalus helioscopus*, and reported that capillary forces pull water through interscalar channels. In addition, they described a stereotyped posture that was exhibited when this species was sprayed with water. This posture involved lowering the head, raising the splayed hindquarters, and protruding the tongue. Comparable behavior has not been observed in *Moloch* (Sherbrooke, 1993). In the iguanid lizard *Phrynosoma cornutum* a similar behavior was described by Sherbrooke (1990). His term ''rain-harvesting'' is used for a complex of behavioral and morphological characters associated with this form of water collection (Sherbrooke, 1990, 1993; Withers, 1993). More recently, such behavior has been described in *Phrynosoma platyrhinos* (Peterson, 1998).

Herein, we report rain-harvesting in three agamids (*Trapelus pallidus, Trapelus flavimaculatus* , and *Trapelus mutabilis*). Examination of two additional species of the same genus (*Trapelus ruderatus* and *Trapelus sanguinolentus*) did not reveal the presence of this behavior. All the animals examined have relatively similar biology, inhabiting more or less open arid to semiarid areas of Northern Africa, the Near East, and the Middle East.

During 1998, 41 specimens of *Trapelus* were examined for the presence or absence of rain-harvesting behavior. Numbers of specimens and their origin are as follows: *T. pallidus* (Reuss, 1834), 16 specimens from the Eastern Desert, Jordan; *T. flavimaculatus* (Rüppell, 1835), three specimens obtained from a commercial dealer; *T. mutabilis* (Merrem, 1820), 10 specimens from northern Egypt; *Trapelus ruderatus ruderatus* (Olivier, 1804), nine specimens, from the Amman region, western Jordan; *T. sanguinolentus* (Pallas, 1827), three specimens obtained from a private reptile keeper. Lizards were housed in glass terraria with sand or gravel substrate. Terraria were heated by incandescent lamps and illuminated by fluorescent tubes. Lizards were fed crickets, wormsmealworms, and *Zophobas morio* larvae, with vitamin-mineral supplement every two to three days. Water dishes were placed in each terrarium, and the enclosures were sprayed twice per week.

The drinking behavior of each animal was observed and recorded. Water was not available for three days prior to each trial. During trial observations, the subject was sprayed with water using a hand pump sprayer. Spraying lasted 3 min, and time to the beginning of drinking (movement of the jaw and tongue) was recorded. Additionally, the type and duration of rain-harvesting posture, and the presence or absence of wet stone licking was recorded. Minimum and maximum duration of these behavioral characters were measured in seconds. Animals exhibiting typical rain-harvesting posture were photographed using Nikon F90 camera with Sigma 90 mm macro-lens and Nikon SB 27 speed light. Each subject was observed in three separate trials with an interval of two weeks between trials.

The ability of skin to carry water by capillary action in interscalar channels was tested in two individuals of each species. The experiments were similar to those of Schwenk and Greene (1987). The animals were wetted by light spraying, then blotted dry with a paper tissue and placed back into the enclosure. Next, water

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[1] Corresponding Author.

[2] Institute of Parasitology, Academy of Sciences of the Czech Republic, Branisovská 31, 370 05 Ceské Budejovice, Czech Republic; E-mail: modry@dior.ics.muni.cz

FIG. 1. Rain-harvesting posture of female of *Trapelus pallidus*, 2 min after beginning of spraying.

dyed with blue food coloring (E 576, Ovo, Prague) was dropped on the dorsum to visualize the movement of water on the integument. Speed and direction of water movement were noted.

For morphological comparison of skin surface microstructure, scanning electron microscopy (SEM) photographs were used. A small piece of skin was taken from the scapular region of a specimen of *T. pallidus* and *T. ruderatus*, freshly killed by overdosing with barbiturates (Thiopental, Spofa). Samples for SEM were fixed in 4% buffered paraformaldehyde, dehydrated in ethanol and acetone series, and desiccated by critical point drying in carbon dioxide. Samples were then gold-coated and examined and photographed with a JEOL JMS 6300 scanning microscope using various magnifications (50X, 100X, and 350X).

Behavioral rain-harvesting was recorded in *T. pallidus, T. mutabilis*, and *T. flavimaculatus*. In contrast, *T. ruderatus* and *T. sanguinolentus* did not exhibit any indication of this behavior.

Typical rain-harvesting posture in *T. pallidus* was assumed 5–50 sec after the animal was sprayed. The head was depressed to within 2–9 mm above the substrate, and the hindquarters were elevated so that the sacral region, or base of the tail, was the highest point on the body. The limbs were splayed, particularly the hind limbs (Fig. 1). The posture varied little among individuals or among trials with the same individual. The rain-harvesting posture of *T. mutabilis* and *T. flavimaculatus* did not differ substantively from that observed in *T. pallidus*. However, in some trials the animals exhibited a modified rain-harvesting posture, in which head was slightly depressed, and the lizard protruded the tongue, but the angle of body axis remained unchanged.

In *T. ruderatus* and *T. sanguinolentus*, the reaction to spraying involved only lowering the head and licking drops from the surface of wet stones. Stone licking was observed in 23% of trials with *T. pallidus*, 10% of trials with *T. mutabilis*, and 11% of trials with *T. flavimaculatus*. During observations with dyed water, no obvious differences in speed and direction of water flow in interscalar channels were observed among individuals and species. Movement of dye was dependent on angle of body and the amount of water dropped on the body surface.

SEM photographs of integument from the dorsolateral body surface revealed similar-shaped scales and interscalar channels (Fig. 2A–B) in both *T. pallidus* (rain-harvesting) and *T. ruderatus* (non–rain-harvesting). In both species, typical honeycomb microstructures were present on the upper surfaces of scales in the scapular region, as well as in deep interscalar grooves (Fig. 2C–D).

Three species of *Trapelus* displayed rain-harvesting behavior. This behavior has not been reported in this genus before. Abel (1952) observed a similar posture in *T. ruderatus*, and suggested that this behavior occurred because of ''unpleasant contact of venter with wet substrate.'' We question both this interpretation and whether Abel's specimens were *T. ruderatus*, because he gave no description, and none of the nine *T. ruderatus* we examined exhibited this behavior.

Trapelus pallidus and *T. ruderatus* are strictly parapatric in Jordan, occupying similar niches in habitats differing primarily by altitude. *Trapelus ruderatus* occurs in a dry part of the Mediterranean ecozone and in a mesic part of the Irano-Turanian ecozone with annual rainfall varying between 150 and 300 mm. In contrast, *T. pallidus* inhabits semidesert and desert habitats of the Syrian Desert, where mean annual rainfall does not exceed 100 mm (Disi, 1996; Disi and Amr, 1998; Disi et al., 2001). Hence, the presence of rain-harvesting behavior in *T. pallidus* may represent a behavioral adaptation for inhabiting habitats more arid than those inhabited by *T. ruderatus*.

Honeycomb microstructures observed in SEM microphotographs of the integument of *T. pallidus* are similar to those of other rain-harvesting lizards (Sherbrooke, 1990; Schwenk and Greene, 1987). However, honeycomb structures are widely distributed among Iguania (Steward and Daniel, 1975; Ananjeva et al., 1991) and thus seem not directly correlated with rain-harvesting behavior. The nearly identical integumental micromorphology of *T. pallidus* (rain-harvesting) and *T. ruderatus* (non–rain-harvesting) also indicate that these structures are not necessarily adaptations for rain-harvesting. The steep angle of the longitudinal body axis associated with the rain-harvesting posture suggests that gravity may be sufficient for water transport, and capillary action is not necessary for transport of water collected on the dorsal surface in *Trapelus*.

Based on comparison with other reptile species in which rain-harvesting behavior has been reported, we propose three forms of this behavior, defined by body posture and role of capillary action. First, *Moloch horridus* is an Australian agamid lizard with the ability to drink water delivered to the mouth by capillary action in interscalar channels. These channels may be filled by water from rain, dew, droplets of water collected by body surfaces on vegetation or by absorption of water from damp sand. Although the ability to extract water from a wet substrate is evident in *Moloch*, the only stereotyped behaviors accompanying this process are rubbing of the ventral surface on the substrate and kicking sand onto the back (Gans et al., 1982; Sherbrooke, 1993; Withers, 1993).

Second, in *Phrynosoma cornutum* and *Phrynosoma platyrhinos*, stereotyped behavior accompanying collection of rainwater has been reported. In these species, the stereotyped posture includes flattening the body and spreading the dorsal surface to maximize

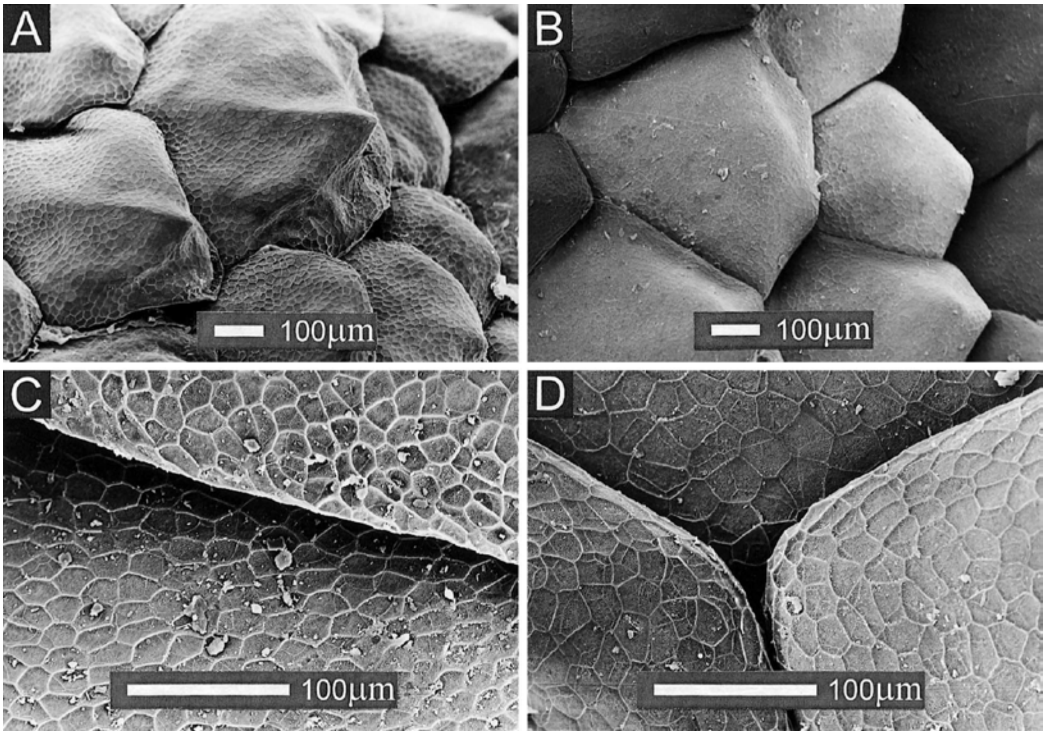

FIG. 2. Scanning electron photomicrograph of epidermal surface of *Trapelus pallidus* (A) and *Trapelus ruderatus* (B). Detail of interscalar space showing honeycomb microornamentation of scale surface in *T. pallidus* (C) and *T. ruderatus* (D).

interception of raindrops. The head is depressed to within several millimeters of the substrate, and the limbs are splayed (particularly the hind limbs). The posture is elicited primarily when the body surface is wetted; however, a similar posture termed ''dorsal shield'' has been reported by Sherbrooke (1991) as a defensive posture. Drinking of collected water is not accompanied by licking the substrate in *P. cornutum*, but this behavior has been documented in *P. platyrhinos* (Sherbrooke, 1981). Capillary transport of water is reported to be more significant during light precipitation, when the water amount collected by the dorsal surface is not large enough to flow by gravity alone. Presence of integumentary microstructures, as are reported in *Moloch*, was confirmed, but capillary flow seems to be less effective (Sherbrooke, 1990). Whether these animals are able to extract water from a wet substrate remains unclear (Sherbrooke, 1993) but seems rather improbable.

Third, in some species of the genus *Phrynocephalus* (Schwenk and Greene, 1987) and *Trapelus* (this study), stereotyped postures similar to those mentioned above have been observed. The postures involved head depression and elevation of the hindquarters, so that the caudal part of the dorsum was the highest part of the body. The limbs are splayed, particularly the hind limbs. The head is depressed, sometimes touching the substrate or stones, and the tongue is protruded repeatedly. This posture is usually assumed usually several seconds after the animal has been sprayed by water. In contrast to previous behavioral form, the body axis is steep, and water collected by the dorsum accumulates on the rostrum forming a drop, which is ingested by regular opening of mouth and protruding of the tongue. In this case, capillary action seems to play no role in water transport. Agamids of the genus *Trapelus* occasionally lick or touch the rostrum to water in depressions or on stone surfaces.

Within the Agamidae, *Phrynocephalus* and *Trapelus* represent rather distant evolutionary groups (Joger, 1991); presumably rain-harvesting behavior exhibited in these genera evolved independently. In both *Phrynocephalus* and *Trapelus*, the rain-harvesting posture resembles a threat posture and may have evolved by modification of this preexisting trait. It is probable that similar rain-harvesting behavior occurs in other agamid species inhabiting arid regions.

Acknowledgments.—We thank D. Hegner from providing experimental animals and B. Koudela (University of Veterinary and Pharmaceutical Sciences, Brno) for kind assistance with SEM microscopy. We are highly indebted to W. C. Sherbrooke (American Museum of Natural History) for language assistance and comments on manuscript. This study was supported by internal research grant (3210-3006) of Faculty of Natural Sciences of Palacky University, Olomouc.

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Accepted: 8 September 2001.

Journal of Herpetology, Vol. 36, No. 2, pp. 314–318, 2002

Environmental Factors Affecting Calling Activity of a Tropical Diurnal Frog (*Hylodes phyllodes*: Leptodactylidae)

FABIO H. HATANO, CARLOS F. D. ROCHA,[1] AND MONIQUE VAN SLUYS, *Setor de Ecologia, Departamento de Biologia Animal e Vegetal, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier 524, 20550-013, Rio de Janeiro, Rio de Janeiro, Brazil*

Calling activity of frogs is influenced by local environmental factors such as relative humidity (e.g., Cree, 1989), temperature (e.g., Licht, 1969; Pough et al., 1983; Navas, 1996), photoperiod (e.g., Jaeger et al., 1976; Hutchison and Maness, 1979; Whittier and Crews, 1987), and rainfall (e.g., Whittier and Crews, 1987; Duellman, 1995; Donnelly and Guyer, 1994). Most studies regarding calling activity have focused on nocturnally active species, whereas information on diurnally active species is scarce. This limits our knowledge of the extent to which diurnal activity is affected by a different range of environmental factors, particularly light intensity.

Hylodes phyllodes is a recently described leptodactylid inhabiting the Atlantic rain forest region of southeastern Brazil (Heyer and Cocroft, 1986; Heyer et al., 1990; Rocha et al., 1997). At the Atlantic rain forest of Ilha Grande, an island located in the south of Rio de Janeiro State, Brazil, *H. phyllodes* is commonly found calling during the day in small streams in the forest (Rocha et al., 1997). We investigated the extent to which calling activity of the frog *Hylodes phyllodes* correlates with light intensity, temperature, relative humidity and photoperiod.

The study was carried out from April 1997 to April 1999 in the Atlantic rain forest at Ilha Grande (23°11'S, 44°12'W), an island in southern Rio de Janeiro State, located approximately 150 km south of Rio de Janeiro City, southeastern Brazil. The forest exhibits different levels of regeneration because of disturbances caused by human activities, which ceased with the transformation of the area in to a state park (Araújo and Oliveira, 1988). Some remnants of primary forest (where apparently only selective wood cutting has occurred) can still be found in the most inaccessible areas of the island. Annual rainfall is about 2200 mm and mean annual temperature is about 23° C (Central Nuclear de Angra, NUCLEN). The study area is characterized by a 50-year-old patch of regenerating forest located about 180 m above sea level.

Calling activity of frogs was estimated monthly by counting the number of calling frogs and the number of calls within each sampling period. We established three observation points to record frog activity at the side of three small streams that were > 100 m apart. At each point we recorded the activity of *H. phyllodes* for 5 min at hourly intervals from 0500–1900 h. During each 5-min observation we recorded number of

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[1] Corresponding Author. E-mail: cfdrocha@uerj.br

Příloha 2.

Yenmis, M., Ayaz, D., Sherbrooke, W.C., **Veselý, M.** 2016: A comparative behavioural and structural study of rain-harvesting and non-rain-harvesting agamid lizards of Anatolia (Turkey). *Zoomorphology* 135 (1): 137-148.

The contribution in words:

MV and MY conceived and designed the study. MY and DA carried out the fieldwork, MY performed the microscopic skin transections and SEM. MY, MV and WS wrote the text. All authors approved the final version of the manuscript. MV supervised the work.

Ranking: IF_{2016} – 1.038; Q2 (Zoology), Q3 (Anatomy & Morphology)
Citace na WoS (Core collection; bez autocitací všech spoluautorů): 8
Citace celkem (Google Scholar): 13

DOI 10.1007/s00435-015-0285-4

ORIGINAL PAPER

A comparative behavioural and structural study of rain-harvesting and non-rain-harvesting agamid lizards of Anatolia (Turkey)

Melodi Yenmiş[1] · Dinçer Ayaz[1] · Wade C. Sherbrooke[2] · Milan Veselý[3]

Received: 19 June 2015 / Revised: 26 September 2015 / Accepted: 30 September 2015 / Published online: 12 October 2015

Abstract Some lizards living in arid or semi-arid habitats have developed a specific adaptation to capture and drink water. This adaptation, rain-harvesting, occurs when water is carried through interscalar channels between integumental scales to the mouth, the point where water is ingested. We investigated whether this special water-drinking adaptation is utilized in four species of agamid lizards living in Turkey *Stellagama stellio* (Linnaeus, 1758), *Paralaudakia caucasica* (Eichwald, 1831), *Trapelus lessonae* (De Filippi, 1865) and *Phrynocephalus horvathi* (Mehely, 1894). Using histological and scanning electron microscopic methods, we found that *P. horvathi* has the integumental adaptations to rain-harvest water; however, *S. stellio* and *P. caucasica* do not. Determination of the rain-harvesting condition in *T. lessonae* will require further research. In all four species, honeycomb-shaped microstructures cover dorsal scale surfaces, and in *P. horvathi,* a complex capillary system involving scale hinges was documented. In experiments with captive animals, which included putting dyed water drops onto the integument, we found that even the species that do not have ability to rain-harvest can collect water on their integuments. But in these species the water does not move into their mouth. Our new findings of rain-harvesting in *P. horvathi* are compared with published data to show that this specific adaptation has evolved convergently with other agamids and with iguanids. Also water flow within the scale hinge system of *P. horvathi* is directional, towards the head.

Keywords Rain-harvesting behaviour · Agamidae · Scale hinge · Hinge joint · Convergent evolution · Drinking behaviour · Capillary flow

Introduction

Rain-harvesting behaviour was first reported in the Australian agamid *Moloch horridus* Gray, 1841, by Buxton (1923) and Davey (1923). The description Buxton made was that: ‘‘a repulsive animal that bears tubercles and circles has the ability to absorb water through its skin after raining’’. Its skin was likened to a ‘‘blotting paper’’ (Davey 1923). Later observations (Bentley and Blumer 1962) reported movements of water across the skin surface of *Moloch* and jaw movements that were initiated when water reached the mouth where it was ingested. Thus, Bentley and Blumer (1962) demonstrated that water on the outer surface of skin did not pass through the integumental wall but was ingested. Gans et al. (1982) experimentally demonstrated the potential that the sharp spines of *M. horridus* may serve as ‘‘condensation foci’’ and take water from the air as dew in arid environments, but this has not been observed in the field. Bently and Blumer's (1962) observation of water movement across the skin between scales to the jaws for drinking was also discovered in the agamid *Phrynocephalus helioscopus* (Pallas, 1771) (Schwenk and Greene 1987) and in the iguanid *Phrynosoma cornutum* Harlan, 1824, by Sherbrooke (1990). Sherbrooke

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Communicated by A. Schmidt-Rhaesa.

& Melodi Yenmiş
yenmismelodi@gmail.com

[1] Department of Biology, Ege University, 35040 Bornova, İzmir, Turkey

[2] Southwestern Research Station, American Museum of Natural History, Portal, AZ 85632, USA

[3] Department of Zoology, Faculty of Science, Palacky University, 77146 Olomouc, Czech Republic

(1990, 1993) named the behaviour as "rain-harvesting". Rain-harvesting behaviour has been identified in some other members of the genus *Phrynosoma* as well: *Phrynosoma platyrhynos* Girard, 1852 (Peterson 1998) and *Phrynosoma modestum* Girard, 1852 (Sherbrooke 2002).

Schwenk and Greene (1987) discovered that when sprayed with water, *P. helioscopus* utilizes a stereotyped body posture during rain-harvesting behaviour. They divided the behaviour into a series of six movements: abdomen is lifted, lower extremities are splayed, dorso-ventral flattening of abdomen takes place, head and tail are lowered towards the ground, and jaws are opened and closed as water that accumulates between scales on the dorsal body surface is ingested, apparently facilitated by tongue protrusions. Sherbrooke (1990) reported a similar stereotyped behavioural stance in *Phrynosoma cornutum* during rain-harvesting, but he reported a lack of such a stance during rain-harvest drinking in *M. horridus* following field studies in Australia (Sherbrooke 1993). Veselý and Modrý (2002) studied rain-harvesting in five agamids of the genus *Trapelus*. They reported that *Trapelus pallidus* (Reuss, 1834), *Trapelus flavimaculatus* Rüppell, 1835 and *Trapelus mutabilis* (Merrem, 1820) exhibit a rain-harvesting behavioural stance, while *Trapelus ruderatus* (Oliver, 1804) and *Trapelus sanguinolentus* (Pallas, 1814) do not.

Utilizing histological and scanning electron microscopy (SEM) techniques, Sherbrooke et al. (2007) compared structures of the skin of *M. horridus* and *Phrynosoma cornutum* both of which exhibit rain-harvesting behaviour. Their study revealed unique, micro-architectural structures in the scale hinge water-transport systems of these lizards from different families. The uniqueness of these scale hinge joint structures and their absence in closely related species lead the authors to suggest that the micro-architectural features were convergently evolved for rain-harvesting.

Scale micro-ornamentation occurs in both species with or without rain-harvesting behaviour. Sherbrooke et al. (2007) stated that this scale micro-ornamentation was observed in all agamid and iguanid lizards that were examined within the scope of their study that included non-rain-harvesting species closely related to the two targeted rain-harvesting species. They stated doubts about an actively selected role of micro-ornamentation in the evolution of rain-harvesting behaviour. But they noted that its presence in scale hinges may facilitate water flow to hinge joint capillary channels from scale surfaces.

Similarly, Veselý and Modrý (2002) stated that honeycomb-shaped micro-structures do not have a role in rain-harvesting behaviour in *Trapelus*, occurring in rain-harvesting and non-rain-harvesting species. However, Comanns et al. (2011) investigated the morphology and hydrophilic characteristics of the integument in detail and reached a conclusion that micro-ornamentation contributed to a super hydrophilic attribute of the skin. Micro-ornamentation can exist in species that show rain-harvesting behaviour and in species that do not show this behaviour. Therefore, it appears that if a species shows rain-harvesting behaviour the micro-ornamentation on its integument may enhance water retention and spread as demonstrated by Comanns et al. (2011).

Comanns et al. (2011) studied the flow dynamics of skin-water movements in *M. horridus*, *Phrynosoma cornutum* and *Phrynocephalus arabicus* Anderson, 1894, in terms of integument morphology. They reported directional movement towards the head in *Phrynosoma cornutum*, but not in the other species studied. Rain-harvesting behaviour has been observed in other reptiles apart from lizards. Alibardi and Maderson (2003) described such behaviour in the tortoise *Psammobates tentorius trimeni* Boulenger, 1886, and Glaudas (2009) and Mata-Silva et al. (2014) in rattlesnake genus *Crotalus*. However, these reptiles drink from pooled water on substratum or skin surfaces, not from water channelled between scales to the mouth.

The aim of our study is to report an additional example of rain-harvesting behaviour with micro-architectural scale hinge structures (only previously known in *Phrynosoma cornutum* and *M. horridus*) and associated behaviours and note convergence of these characters among lizards and within the family Agamidae. We report on the histological and morphological structure of interscalar channels on the skin of agamid lizards in detail. We tested for the existence and non-existence of rain-harvesting behaviour in these species living in Turkey and report on a new rain-harvesting agamid lizard *P. horvathi*, which has convergently evolved rain-harvesting.

Materials and methods

Lizards were captured by hand throughout their distributions in Turkey. For *Stellagama stellio,* the localities were in Central, West and Southern Anatolia ($n = 12$); for *Trapelus lessonae,* they were in Central and South-eastern Anatolia ($n = 9$), and for *Phrynocephalus horvathi* and for *Paralaudakia caucasica,* they were in Eastern Anatolia ($n = 14$ and $n = 10$, respectively). A lacertid lizard *Eremias strauchi* Kessler, 1878 ($n = 2$) was collected from East Anatolia for comparison with the agamids.

Terraria were constructed of wood and glass and were filled with soil or sand suitable to species habitats and with rock, gravel and plants taken from the habitat of each species. UV-A and UV-B fluorescent lamps and additional incandescent light bulbs (15 and 20 watt) were provided. Their ambient effects were monitored by hygrometer and thermometer allowing regulation of suitable conditions.

Mealworms (*Tenebrio molitor*) and superworms (*Zophobas morio*) were supplied as food. Water dishes were placed in each terrarium but were removed three days prior to all trials.

Each species was placed into a separate terrarium. Captive observations were started when individuals were placed into the terraria. The drinking behaviour of each animal was observed and recorded during trials. The subject was sprayed with water using a hand-pump sprayer. Spraying lasted 3 min, and time to the beginning of drinking (movement of the jaw and tongue) was recorded. Additionally, the type and duration of rain-harvesting posture, and the presence or absence of licking wet stones was recorded. Minimum and maximum duration of these behavioural characters was measured in seconds. Animals showing typical rain-harvesting posture were photographed using Canon EOS 650D. Each subject was observed in three separate trials with two-week intervals between each trial.

Skin sample fixation was with 2.5 % glutaraldehyde followed by washing in buffer solution before skin samples were gold-plated by dehydration for SEM examinations. Preparations were examined with a FEI Quanta FeG 250 Scanning Electron Microscope. Standard paraffin histological preparations were also made using Bouin's fixative. Samples were cut into 5, 7, and 10 lm thick sections according to the rigidity of skin. Sections were dyed with Ehrlich's haematoxylin, examined with an Olympus CX31 microscope, and were photographed using an Olympus U-CMAD3-LC20 MODEL camera.

The process of following water movement across skin surfaces after dropping the single water drop from a syringe (5 ml, needle gage 0.8 9 38 mm) onto the skin was aided by mixing water with red food colouring dye (Idacol Ponceau 4r CI. NO: 16255) in a ratio of 99 % water to 1 % dye. Each lizard was tested once for water flow movements across the skin. For terminology describing integumental structure, we used Irish et al. (1988) and Alibardi and Toni (2006), and for terminology of rain-harvesting behaviour Sherbrooke et al. (2007) and Comanns et al. (2011).

Results

Macroscopic scale morphology

In *S. stellio* and *P. caucasica* adult snout-vent length (SVL) is 25–30 cm, whereas *T. lessonae* and *P. horvathi* are somewhat smaller having SVL, 7–14 cm (Fig. 1). All four species have sharp dorsal scales in imbricate lines. Tails of *P. caucasica* and *S. stellio* are covered with roundly organized thorny scales with keels. In all species, ventral scales lack keels and are not overlapping. In *P. horvathi*, there are imbricate dorsal scales which create water-transport channels that ensure rain-harvesting behaviour, between these scales.

Fig. 1 Four agamid lizard species studied during drinking trials in captivity. a *Stellagama stellio* with circular arranged tail scales. b *Phrynocephalus horvathi* c *Trapelus lessonae* d *Paralaudakia caucasica*

Reactions in the presence of sprayed water

Within the species examined, only *P. horvathi* showed rain-harvesting behaviour. During the behaviour, the lizard's head is bent downward until within a few millimetres from the ground and the body is curved upward in a way that it arches. The tail base is raised to become the highest point of the body (Fig. 2). Rhythmic mouth movements started simultaneously with adjusting to this posture. These movements consist of a series of transactions of opening the mouth slowly, protruding the tip of the tongue, retracting the tongue by rolling and closing the mouth (Fig. 3).

All individuals of *P. horvathi* exhibited rain-harvesting behaviour within 23 s of being sprayed with water. The assumed posture and mouth movements continued for 3 min, the duration of spraying, and continued after spraying was over for as long as 75 s. The timing of rhythmic opening and closing of jaws coordinated with tongue protrusions was approximately every 2 s After the posture was over, individuals resumed normal movements. An individual *P. horvathi* entering into the water dish outside of the experimental period of spraying showed rain-harvesting behaviour and jaw movements. Drinking from the ground was not observed.

Individuals of *S. stellio* and *P. caucasica* did not show rain-harvesting behaviour when sprayed. Individuals of these species ingested water from the ground by following water flowing from sprayed water, licking walls of the terrarium for accumulated water droplets, and drinking water collected in dorsal folds and protrusions on each other's skin (Fig. 4). No specific water-collecting behavioural postures were observed. Water drinking in both species was similar. They drank water by pressing their jaws to the ground, without protruding their tongue. Nevertheless, differences appeared in their reactions to flowing water within the terraria. *P. caucasica* individuals reached out into the flowing water and tried to drink by protruding their tongue, a behaviour not noted in *S. stellio*. In this study, *S. stellio* individuals ran away from sprayed and flowing water and waited to drink from the water that accumulated and formed a puddle in the terrarium. During non-trial periods, they drank water from a petri dish in the terrarium.

Fig. 2 Rain-harvesting posture in *Phrynocephalus horvathi* 60 s after the beginning of spraying of water. The head is depressed to 5 mm above the substrate, and the sacral region is elevated

None of the individuals of *T. lessonae* moved during spraying. Any human movement around the terrarium made them stop and stay in place, in some cases for hours. Thus, spraying by an experimenter, a simple, mechanic process using a hand sprayer apparently increased stress and/or elicited an anti-predator freeze or immobility response. Individuals simply closed their eyes when sprayed with water. Individuals were observed 12 h daily during day time for a week, between trials. During these observations, individuals were observed to enter a petri dish filled with water and one juvenile drank water from a rock surface.

Spread of coloured-water droplets on skin

In dyed water experiments, the speed of integumental flow of water, the pathway of water circulation and its direction among the scales were observed (Fig. 5). In *P. horvathi*, a water droplet was first absorbed by being taken into the scale hinge openings that lead to deeper channels. After absorption, water flowed anteriorly and laterally across body surfaces; lateral water flow then changed its course, turning towards the anterior within 20 s, and after 45 s, two streams came together and continued to move forward towards the head. Initial absorption occurred within 10 s in *T. lessonae,* and the drop was absorbed completely within 60 s. However, direction was different and ambiguous in each trial. In *S. stellio*, the drop was absorbed completely within 20 s. Drop spreading was towards anterior, posterior and lateral scales and thus non-directional. After the absorption of the water droplet was completed, further expansion of water fronts ended. The skin behaved like a sponge when water was dropped onto the integument of *Paralaudakia caucasia* and all water was transmitted into the channels within 2 s. Expansion of the spreading water front stopped the moment absorption of additional water from the droplet ended. Directional movement was not noted.

These experiments were repeated with a lacertid lizard *E. strauchi,* and a difference of absorbing water between this lizard and the agamids was observed. Scales on the dorsal of *E. strauchi* are not imbricated nor lined up. Scales are round, their size is similar and they are arranged side by side. The dyed water drop that was dropped onto the dorsal skin of *E. strauchi* stayed unspread after 60 s.

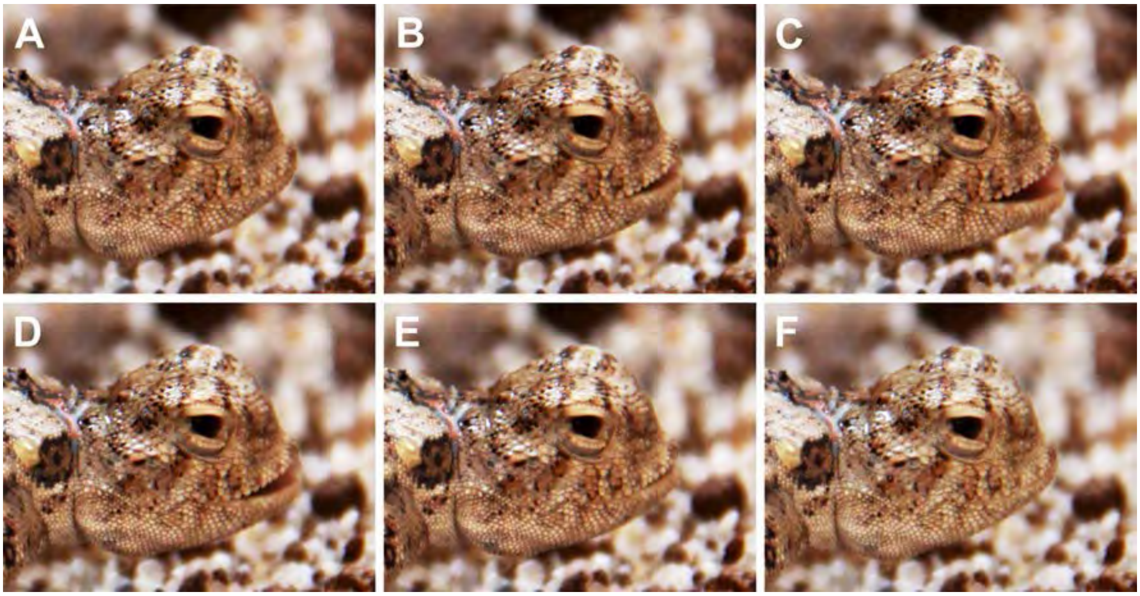

Fig. 3 Jaw and tongue movements that continue throughout rain-harvesting behaviour in *Phrynocephalus horvathi*. a–c *First part* of continuous jaw-opening/closing movements during ingestion of water obtained from the integument. Tongue is protruded. d–f *Second part* of the continuous movements. Ingestion occurs as the tongue is retracted

Fig. 4 a Three *Stellagama stellio* individuals drinking water from a petri dish. b Three *Trapelus lessonae* individuals one on top of the other during spraying of water. Reaction to the spraying consists of blinking eyes only. c *Paralaudakia caucasica* individual drinking water from petri dish. d *Paralaudakia caucasica* individual drinking from flowing water

Microscopic scale morphology

Skin sense organs were observed on endpoints of some scales in SEM examinations of *P. horvathi*. These sense organs constitute points of contact with the outer environment (Maclean 1980; Sherbrooke and Nagle 1996) (Fig. 6d, e). Other structures noted on surface examination of scales were Oberhäutchen and micro-ornamentation (Sherbrooke et al. 2007). This Oberhäutchen is made of honeycomb-like structures covering scale surfaces

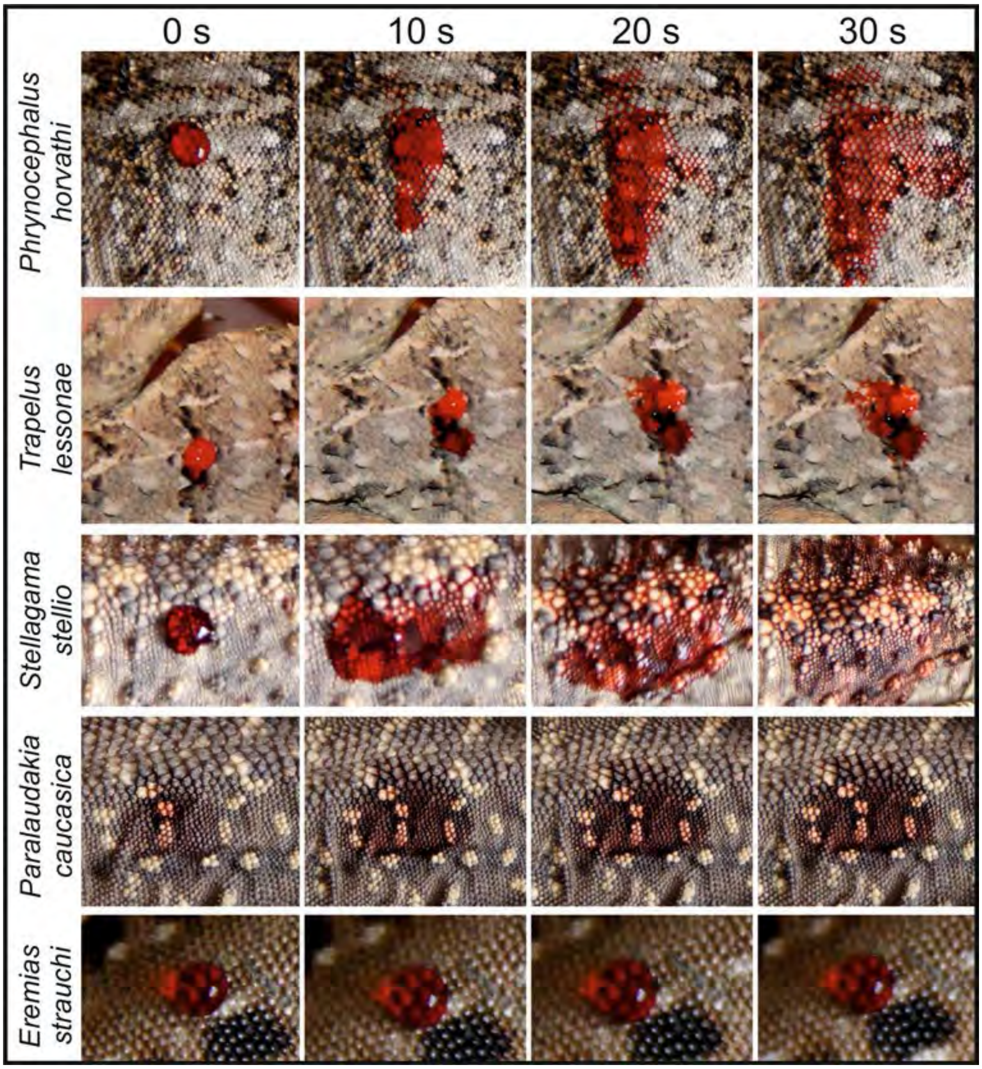

Fig. 5 Water droplet tests for transport through potential interscalar capillary systems of various lizards. Anterior is always on the right. The velocity of water flow through systems was highest in *Paralaudakia caucasica*, lowest in *Trapelus lessonae*. Water did not spread on the skin of *Eremias strauchi*. In *Phrynocephalus horvathi,* water spread towards the mouth. In *Paralaudakia caucasica* and *Stellagama stellio,* the skin spread water and moved it through channels, but movement was not directional. In *Trapelus lessonae,* multiple trials showed different and inconclusive results

(Figs. 6f, 7e, f). When examined under higher magnification, it can be seen that similar but tiny honeycomb structures are lined up inside each hexagon honeycomb.

In cross-sectional examinations of *P. horvathi*, the structural means of water transmission that are the basis of rain-harvesting behaviour were identified (Fig. 6a–c). These are semi-tubular channels (width about 55–60 lm) that cover the body horizontally and longitudinally like a web. Oberhäutchen (width about 35–40 lm), wrapping the body like a cover and being the very first layer in contacting with the outer environment, is clearly seen convolutedly in the channel walls as well as on exposed scale surfaces. These channels can exist because of the imbricate alignment of scales. On the point where one scale covers the other, water transmission and its filling into these channels are ensured; then, water is transmitted to the mouth from these channels. When semi-tubular channels are cross-sectioned, some structures that shaped the canal in the joint of two scales can be seen. The b-layer keratin under the Oberhäutchen extends over the scale surface with an average thickness, but it becomes thinner when it comes to form semi-tubular channels. This thinning could be seen on both sides of the *U*-shaped channel. The channel area that looks like a chamber in a cross section is called the hinge joint. On the walls of the hinge joint are found island-like protruding structures covered by thin b-layer keratin. These enhance surface area.

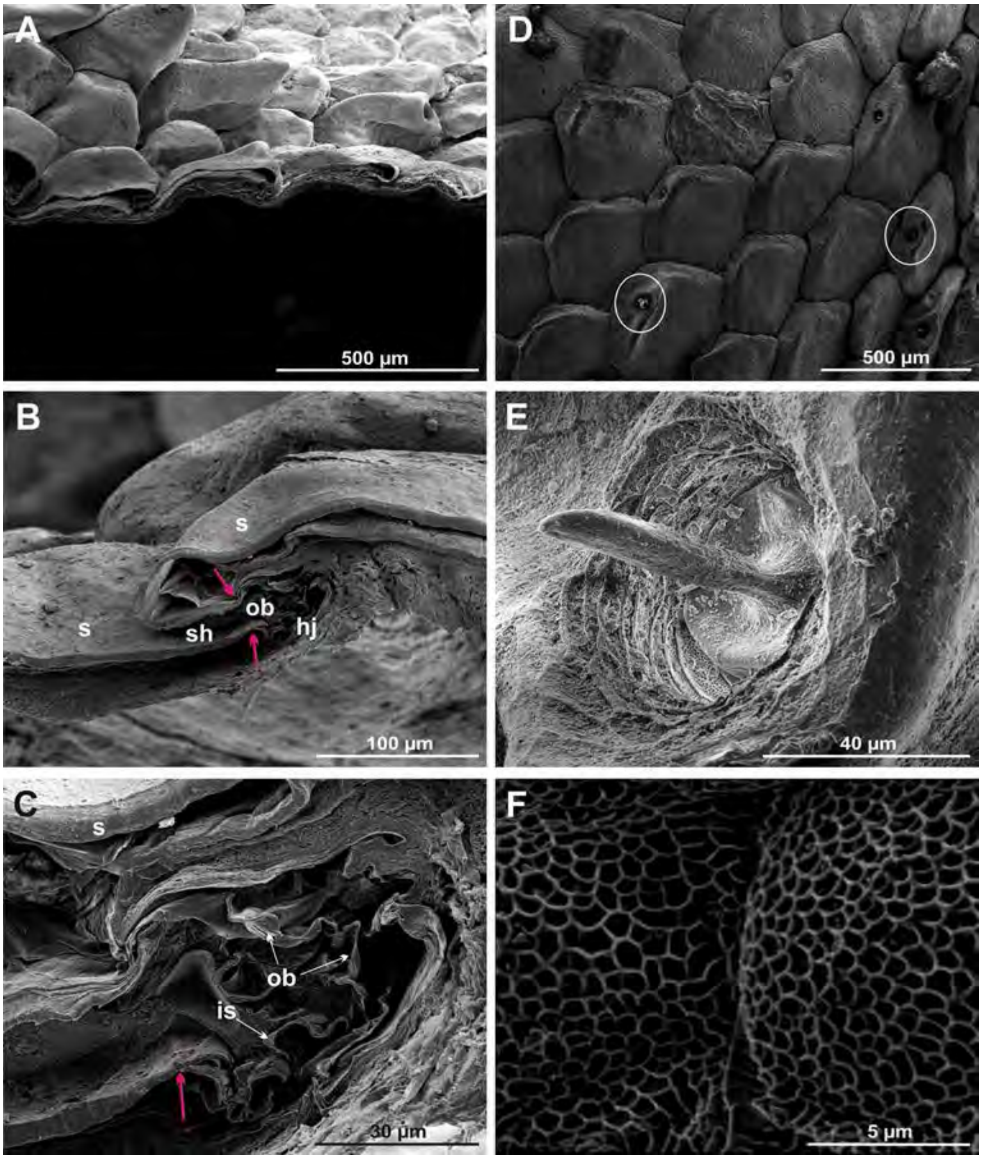

Fig. 6 SEM cross sections of integument of *Phrynocephalus horvathi*. a The arrangement of scales and channels between scales in cross sections. b Divided regions of channel system where water is collected in cross sections. c Detailed image of hinge joint. SEM examinations of surface integument of *Phrynocephalus horvathi*. d Imbricate arrangement of scales of the integument of *Phrynocephalus horvathi*. *White circle* points out a skin sense organ. e A skin sense organ situated on the tip of a scale. f Honeycomb-like micro-structures covering scale surfaces. *s* scale, *ob* oberhäutchen, *sh* scale hinge, *hj* hinge joint, *is* island-like structures. *Arrows* indicate thinning b-layer keratin points where hinge joint begins

In *Stellagama stellio,* keeled scales and skin sense organs were observed by SEM examination on both dorsal and ventral skins. Scales across dorsal surfaces show great morphological differences (Fig. 7a). While the diameter of square-like shaped tiny scales vary 0.2 mm to 0.3 mm, the diameter of medium-sized scales can be 0.4–0.5 mm and the larger round-shaped scales can be 0.7 mm. Extensive borders of big plate-like scales are covered by many adjacent small scales (Figs. 7a, 9b). This situation creates disarrangement or breaks in imbricate flow of scale arrangement. Skin sense organs are placed on the edge of small-, medium- and large-sized plates on dorsal scales and can number 1–3 on each scale. Skin sense organs on ventral scales are placed not only on the edges but also near the lateral borders of scales. Many sense organs could be seen on ventral scales.

Skin sense organs in *P. caucasica* are placed close to the carinal keeled centre of scale surfaces (Fig. 7b). In comparison with *P. horvathi,* the imbricate arrangement of scales is more irregular and the shapes of scales are round. Micro-ornamentation covering scale surfaces can be seen on all dorsal scales. Scales with keels and pointed tips are

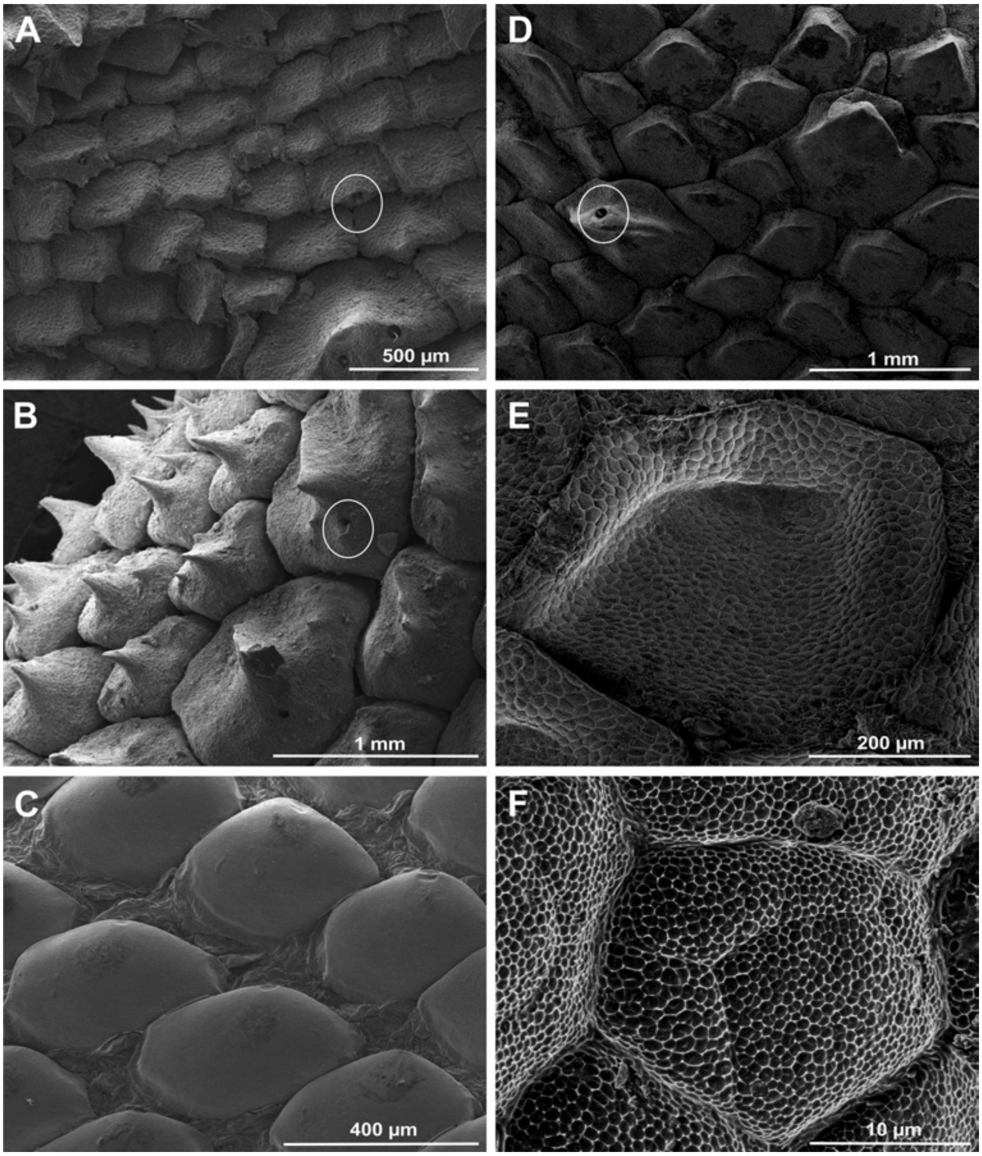

Fig. 7 SEM sections of skin surfaces from four lizard species investigated. a Imbricate scales of dorsal integument in *Stellagama stellio*. b Dorsal scales of *Paralaudakia caucasica*. c Dorsal scales of the lacertid lizard *Eremias strauchi*. Note lack of scale overlapping. Therefore, interscalar channels do not develop. d-f Dorsal scales of *Trapelus lessonae*. d Imbricate order of the scales. e Micro-ornamentation on scales. f One of the honeycomb-shaped micro-structures that consist of many small honeycombs. *White circles* point out skin sense organs

partially transparent. On the ventral surface, scales are square-like with round edges and the imbricate arrangement of overlapping scales is quite regular.

In comparison with the other three species examined, the number of skin sense organs in *T. lessonae* is lower (Fig. 7d). Those observed were more likely to be seen on the edges of large dorsal scales, while on ventral scales they occur more sparsely on the edges. Ventral scales are square-shaped, in regular imbricate arrangement, and have micro-ornamentation as do dorsal scales (Fig. 7e, f).

Skin sense organs and micro-ornamentation are lacking on dorsal scales of *E. strauchi* (Fig. 7c).

Histology

Histological examinations were made to show semi-tubular channels in cross sections in *P. horvathi* (Fig. 8). Skin samples taken from dorsal and ventral surfaces of the body trunk and legs were examined. Layers of skin, a- and b-layer keratin, and island-like structures forming in the channels were distinguished, and Oberhäutchen which is part of the b-layer keratin was clearly shown. Differences were seen in the arrangement and morphology of scales on dorsal and ventral surfaces. Because on the ventral surface, one scale's anterior does not cover the second scale's

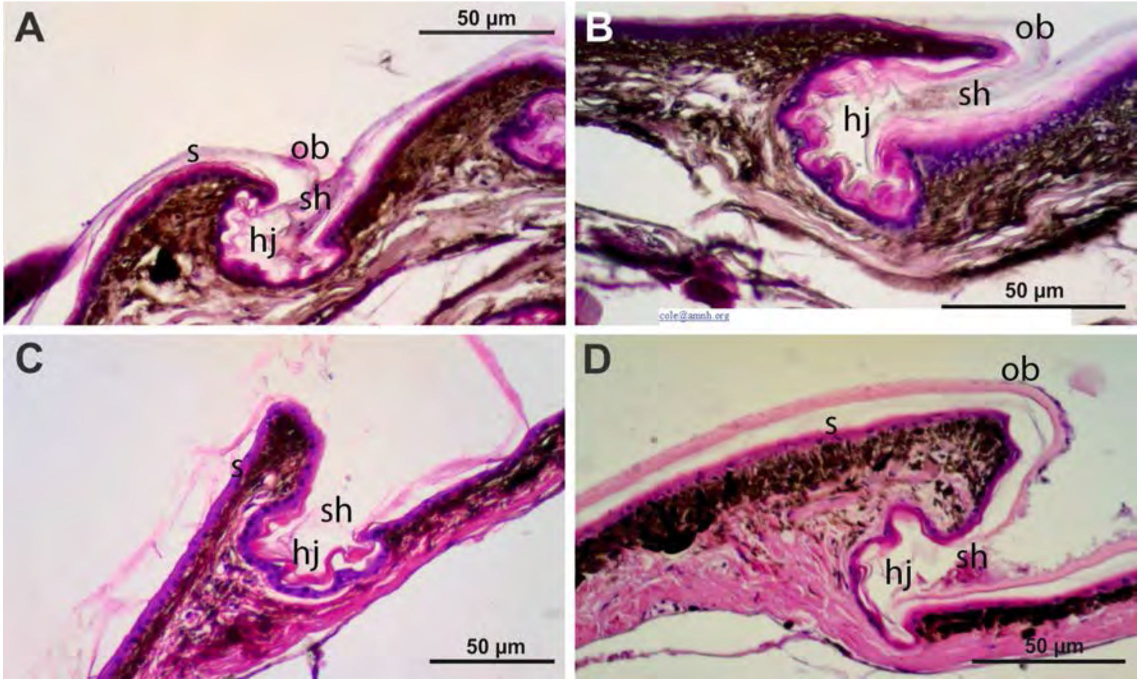

Fig. 8 Histological structure of hinge joints in *Phrynocephalus horvathi*. a Hinge joints between dorsal scales. b Dorsal scale hinge joint with island-like structures that have greater development than the ones in hinge joints on the ventral body and extremity scales. c Ventral scale with a hinge joint. Ventral scales are smoother and more orderly than dorsal scales, and the scale hinges are wider opened at the surface. d Hinge joint of lower extremity. Extremity scales are closer to each other than ventral scales, and thus, the scale hinges are narrower than ventral scale hinges

posterior edge, as is the case with dorsal scales, and the gap between scales is much wider and scale hinge gaps are wider.

An individual *P. horvathi* entered into a petri dish filled with water and assumed a rain-harvesting posture while standing in the water when there was no spraying of water. Therefore, we hypothesized that semi-tubular channels also exist between scales on the inside of the legs. Histological examinations of leg scale hinges revealed semi-tubular channels that verified this hypothesis (Fig. 8d).

Histological examinations made in *S. stellio* and *P. caucasica* showed that semi-tubular channels also exist in agamid species that did not show rain-harvesting behaviour (Fig. 9a–c). But these channels are more irregular, are lacking in island-like structure which enhance the channel surface area and are wider in internal surface spaces because of the arrangement of plates.

T. lessonae is reported as a species that does not clearly exhibit rain-harvesting behaviour based on observing its immobility in captivity and its minimum reactions in response to sprayed water. However, histological examinations do not add support to this conclusion. Thin sections of skin samples of this species showed b-layer keratin getting thinner in the scale hinges forming semicircular tubes that bear island-like structures which enhance surface area. When compared to histological sections of *P. horvathi* (Fig. 8a, b) that show rain-harvesting behaviour, it was found that channels on the dorsal surface of *T. lessonae* are much wider diameter and hinge joint surface openings are closer together as the scales are smaller (Fig. 9d).

Discussion

Of the four species examined, rain-harvesting exists only in *P. horvathi*. Sherbrooke et al. (2007) defined island-like structures that form in hinge joints as structures that are apart from each other and facilitate capillary action that pulls water through the semicircular channels formed by the hinge joints in rain-harvesting species. Hinge joints with such island-like structures were shown in the skin of *M. horridus* and *Phrynosoma cornutum*. Our observation of similar island-like structures in hinge joints that form semi-tubular channels in *P. horvathi* is supportive of their role as convergently evolved adaptations that lead to rain-harvesting behaviour in these three species. Rain water falling on the integument of *P. horvathi* enters into interscalar channels via scale hinges, thus filling hinge joints that have the function to collect water and transport it through semicircular channels around each scale and on to the mouth as in *Phrynosoma cornutum* and *M. horridus*

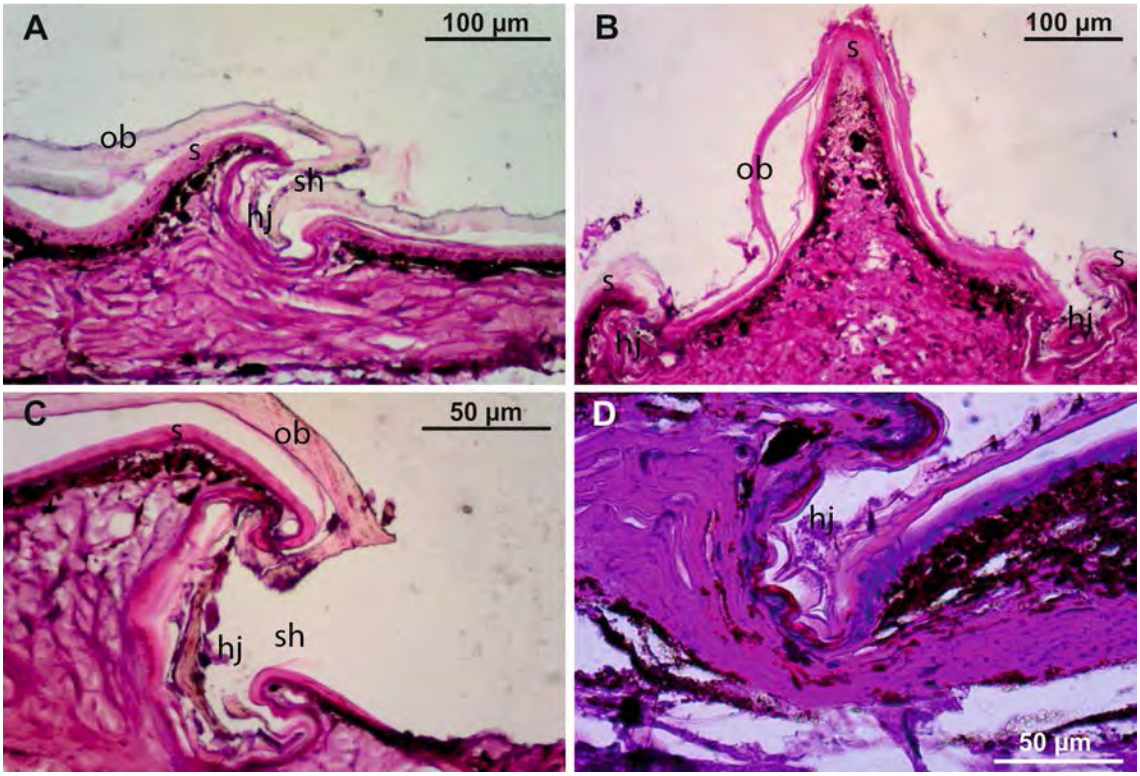

Fig. 9 Histological structures of dorsal scales in three lizards a,b *Stellagama stellio,* c *Paralaudakia caucasica and* d *Trapelus lessonae* a Dorsal hinge joint of *Stellagama stellio* does not have visible island-like structures. b Larger dorsal scales are surrounded by small scales. Therefore, interscalar channels can appear on both sides of one large scale. c *Paralaudakia caucasica* does not carry visible island-like structures on hinge joints, thus resembling *Stellagama stellio*. d *Trapelus lessonae* has a hinge joint structure similar to *Phrynocaephalus horvathi* with island-like structures

(Sherbrooke 2004; Sherbrooke et al. 2007). In *P. horvathi,* drinking water transmitted to the mouth is ingested as the protruded tongue is retracted, similar to rain-harvest drinking involving tongue protrusion in *P. helioscopus* (Schwenk and Greene 1987) and *Trapelus* spp. (Veselý and Modrý 2002).

Rain-harvesting behaviour was not observed in *S. stellio* and *P. caucasica*. In this study, SEM examinations of *S. stellio* showed that scale arrangement is irregular on the dorsal scales but regular on the ventral scales and sense organs on both dorsal and ventral scales are more frequent than in the other species examined. Histological examinations showed that there are no island-like structures in hinge joints and suggests that the skin is not adapted for rain-harvesting. *P. caucasica* that is distributed in the same climate zone as *P. horvathi* is a non-rain-harvesting species. It is similar to *S. stellio* in dorsal and ventral scale arrangement and water-drinking behaviours. Both *S. stellio* and *P. caucasica* individuals drink from standing water, from walls of terraria and from each other's dorsal surfaces.

Although *P. caucasica* is not a rain-harvesting species, speed of water transport is highest in this species. Water moves into the channels so fast because of the relatively larger scales and wider scale hinges between them. Water spreads but does not go through channels directionally to the anterior of the body. Water flow is similar in *S. stellio* in that water is not transmitted to the mouth. Yet, in this species, the expansion of a waterfront is slower and wider than in *P. caucasica*. These two species do not show rain-harvesting behaviour, but they do have interscalar channels, scale hinges, covering the integument. Therefore, the convergent evolution of rain-harvesting behaviour appears to depend on the development of interscalar channels, but the occurence of such channels is not always associated with rain-harvesting behaviour.

All four agamids studied exhibited Oberhäutchen and micro-ornamentation on scales, both rain-harvesting and non-rain-harvesting species, as has been noted in other agamids and iguanids (Veselý and Modrý 2002; Sherbrooke et al. 2007). This and the diversity of forms of micro-ornamentation on scale surfaces (Peterson 1984) and its relation to skin-shedding cycles (Sherbrooke et al. 2007) suggest that its evolutionary development was not specifically a selected adaptation for water retention and spreading during rain-harvesting. Rather, its role in facilitating aspects of rain-harvesting (Sherbrooke et al. 2007; Comanns et al. 2011) may be an exaptation. Further detailed studies of micro-architectural details of lizard skin in rain-harvesting and non-rain-harvesting species of diverse phylogenetic lineages may help to clarify this issue.

Veselý and Modrý (2002) demonstrated the existence of rain-harvesting behaviour in *T. pallidus*, *T. flavimaculatus* and *T. mutabilis* and the absence of this behaviour in *T. ruderatus* and *T. sanguinolentus*. *T. pallidus* which showed rain-harvesting behaviour and *T. ruderatus* which did not show this behaviour were compared in terms of geographical distribution and skin morphology. Both are scattered in Jordan parapatrically (Veselý and Modrý 2002). However, while *T. ruderatus* lives in dry areas in a Mediterranean ecozone, in a region that receives 150–300 mm rain per year, *T. pallidus* lives in drier arid or semi-arid regions of Syrian deserts where annual rainfall does not reach 100 mm. They suggested that these arid climate conditions played a primary role in *T. pallidus* developing rain-harvesting behaviour (Veselý and Modrý 2002).

One species (of undetermined rain-harvesting status) in our study, *T. lessonae,* is distributed in Central and South-eastern Anatolia where the total annual precipitation was 170–420 mm, and the one confirmed rain-harvesting species, *P. horvathi,* is distributed in North-eastern Anatolia where the total annual precipitation was 115–380 mm, data between 1970 and 2014 (from Turkish State Meteorological Service). The annual rainfall values of the regions where a non-rain-harvesting species, *T. ruderatus,* and a rain-harvesting species, *P. horvathi*, live are approximately the same. This shows that climatic data alone are inadequate to identify potential rain-harvesting species. Our non-rain-harvesting species, *P. caucasica* (annual precipitation was 115–380 mm) lives in areas near and similar to our rain-harvesting species, *P. horvathi*, whereas *S. stellio* lives both in dry and in humid areas (in Central Anatolia, annual precipitation was 170–420 mm; in West Anatolia, annual precipitation was 355–1045 mm) (data between 1970 and 2014 from Turkish State Meteorological Service). Based on the approximate precipitation values of Central and Eastern Anatolia, one could hypothesize that *T. lessonae* is a rain-harvesting species, but our behavioural experimental data have not confirmed behaviours associated with rain-harvesting, although our morphological data are suggestive, having structural similarities to *P. horvathi*.

Trapelus lessonae has difficulty adapting to captivity and a terrarium environment (stress). This made study of behaviour difficult and observing clear behavioural results impossible. Though many alternative captive environment changes were tried, none were successful. Individuals entered into a petri dish filled with water and stayed there for 1–3 h, and one juvenile was observed to drink water from a rock surface. However, these observations cannot be taken as an indication of lack of rain-harvesting behaviour, especially in as much as histological studies of hinge joints showed island-like structures with a resemblance to *P. horvathi* hinge joints. Further behavioural studies are needed, perhaps under natural rainfall conditions, to resolve the rain-harvesting status of *T. lessonae*.

Our observations of directional flow of water towards the mouth in *P. horvathi* are significant in light of a recent report by Commans et al. (2015) that scale hinge transport of water for rain-harvesting in *Phrynosoma cornutum* involves a directional, passive liquid transport system. They demonstrate that the micro-architecture of this capillary system is based on geometric principles, a periodic pattern of interconnected, half-open capillary channels that narrow and widen. The pattern was used to construct a biomimetic model resembling a ‘‘liquid diode’’. Our behavioural and coloured-water flow observations suggest the possibility of a similar directional, passive liquid transport system in *P. horvathi*, but further histological and fluid-dynamics studies are needed. *P. horvathi* is the only lizard, other than *Phrynosoma cornutum* in which directional flow has been reported, although Schwenk and Greene (1987) mentioned the influence of scale morphology on water flow pattern in *P. helioscopus*.

Acknowledgments We thank our funders: Financial support was provided by Ege University Accounting Office for Funds (Project No: 12-fen-063) and by The Scientific and Technological Research Council of Turkey (Project No: 114Z055).

Compliance with ethical standards

Human and animal rights This study gained acceptance from Ege University Animal Experimentations Local Ethics Board. (Document number: EÜHADYEK 2013-043). All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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Příloha 3.

Jablonski, D., Gvoždík, V., Choleva, L., Jandzik, D., Moravec, J., Mačát, Z. & **Veselý, M.**, 2019: Tracing the maternal origin of the common wall lizard (*Podarcis muralis*) on the northern range margin in Central Europe. *Mitochondrion* 46: 149-157.

The contribution in words:

MV and DJ conceived and designed the study. All authors carried out the fieldwork, DJ, VG and LC performed the molecular analyses. MV, DJ and LC wrote the manuscript. All authors contributed to manuscript revision. All authors approved the final version of the manuscript. MV supervised the work.

Ranking: IF_{2019} – 3.992; Q2 (Genetics & Heredity; Cell Biology)
Citace na WoS (Core collection; bez autocitací všech spoluautorů): 3
Citace celkem (Google Scholar): 7

Mitochondrion 46 (2019) 149–157

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Tracing the maternal origin of the common wall lizard (*Podarcis muralis*) on the northern range margin in Central Europe

Daniel Jablonski[a], Václav Gvoždík[b,c], Lukáš Choleva[d,e], David Jandzik[a,f], Jiří Moravec[c], Zdeněk Mačát[g,h], Milan Veselý[i,*]

[a] Department of Zoology, Comenius University in Bratislava, Mlynská dolina, Ilkovičova 6, 842 15 Bratislava, Slovakia
[b] The Czech Academy of Sciences, Institute of Vertebrate Biology, 603 65 Brno, Czech Republic
[c] Department of Zoology, National Museum, Cirkusová 1740, 193 00 Prague, Czech Republic
[d] Department of Biology and Ecology, Faculty of Science, University of Ostrava, Chittussiho 10, Ostrava 710 00, Czech Republic
[e] The Czech Academy of Sciences, Institute of Animal Physiology and Genetics, Laboratory of Fish Genetics, Rumburská 89, 277 21 Liběchov, Czech Republic
[f] Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7, Prague, 128 44 Prague, Czech Republic
[g] Department of Ecology and Environmental Sciences, Faculty of Science, Palacký University, Šlechtitelů 27, 783 71 Olomouc, Czech Republic
[h] Podyjí National Park Administration, Na Vyhlídce 5, 669 02 Znojmo, Czech Republic
[i] Department of Zoology, Faculty of Science, Palacký University, 17. listopadu 50, 771 46 Olomouc, Czech Republic

ARTICLE INFO

Keywords:
Isolated populations
Genetic diversity
Human-mediated introduction
Climate
Biogeography
Conservation

ABSTRACT

The maternal origin of isolated populations of the common wall lizard (*Podracis muralis*) in the Czech Republic, representing the north-eastern range border of the species, was addressed. We compared mitochondrial DNA sequences of the cytochrome *b* gene of samples from these populations with those from within the continuous range in Slovakia, the northern Balkan region, and those available from previous studies. We recorded five main haplogroups in the studied region, with all available Central European samples belonging to the same haplogroup. The star-like structure of this haplogroup suggests a scenario of relatively recent, post-glacial population expansion, which is further supported by a coalescent-based demographic analysis. The presence of unique haplotypes in two of the three isolated Czech populations together with close phylogenetic relationships to adjacent Slovak populations suggests either autochthonous origin or human-mediated introductions from geographically and genetically closest populations. We therefore support conservation programs for all three isolated Czech populations.

1. Introduction

Reptiles are ectothermic vertebrates and their spatial and temporal distribution is highly dependent on temperature (Markwick, 1998). In temperate zones of the western Palearctic region they are physiologically adapted to seasonal climate changes, where lower and higher temperature limits determine their distribution (Böhme, 2003; Sommer et al., 2009). Historically, climatic oscillations of glacial and interglacial cycles during the Quaternary caused significant range shifts in the European biota (Davies and Shaw, 2001; Sommer and Zachos, 2009). During the glacials, distributions of many species were restricted to refugia, typically at lower latitudes that later served as sources for colonization of higher latitudes as the climate warmed (Hewitt, 2000; Bennett and Provan, 2008). Given the current global warming trends causing growing temperatures in areas home to many temperate ectotherms, reptiles are interesting group to study distribution dynamics and historical origins of their populations, particularly at the higher latitude boundaries of the present-day ranges. Populations from these areas are good candidates for further expansions northward in the future (Parmesan, 2006).

The region of Central Europe was historically crossed by many expanding vertebrate taxa (Hewitt, 2004). In most cases it is thermophilic ectothermic vertebrates that find their climatic optima in this region since at least the end of Miocene (Böhme, 2003). Among the well-documented examples of vertebrates with wider ranges during the Holocene climatic optimum are e. g. turtle *Emys orbicularis* (Sommer et al., 2009), lizard *Lacerta viridis* (Böhme et al., 2007), and snake *Zamenis longissimus* (Musilová et al., 2010). Numerous records of subfossil specimens showed that these species have become extinct from much of their northern Holocene distribution, which spread as far north as Southern Scandinavia (Sommer et al., 2007; Musilová et al., 2010). Within the recent European ranges, the Central-European populations

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[*] Corresponding author.
E-mail address: veselym@prfnw.upol.cz (M. Veselý).

https://doi.org/10.1016/j.mito.2018.04.006
Received 24 August 2017; Received in revised form 6 March 2018; Accepted 20 April 2018

Table 1
Tissue samples and sequences of the Central Balkan clade of *Podarcis muralis* used in this study.

Code	Country	Locality	Haplogroup	Coordinates		Source	GenBank accession number
				N	E		
Pbh07_BA	Bosnia and Hercegovina	Kozara Mts.	I	45.04	16.97	This study	MG851979
P7_L1_BG, P8_L2_BG, P9_L3_BG	Bulgaria	Zgorigrad	V	43.19	23.53	This study	MG851950 MG851951 MG851952
P29_BG	Bulgaria	Vraca	V	43.20	23.53	This study	MG851931
P41_BG	Bulgaria	Bjala	V	42.88	27.88	This study	MG851942
P42_BG	Bulgaria	Ruse	V	43.83	25.97	This study	MG851943
478_BG	Bulgaria	Kralovo	V	42.65	24.81	This study	MG851961
594_BG	Bulgaria	Shumen	V	43.26	26.89	This study	MG851962
mur5	Croatia	Nova Gradiška, Strmac	I	45.25	17.38	Podnar et al. (2007)	DQ001025
P1_CZ, P2_CZ, P3_CZ, P4_L14_CZ, P5_L15_CZ, P6_CZ, P11_CZ, P12_CZ, P13_CZ, P14_CZ, P28_CZ	Czech Republic	Štramberk	I	49.59	18.12	This study	MG851924 MG851932 MG851940 MG851947 MG851948 MG851949 MG851915 MG851916 MG851917 MG851918 MG851930
3547_CZ, 3548_CZ	Czech Republic	Krčmaň	I	49.52	17.33	This study	MG851974 MG851975
3549_CZ	Czech Republic	Brno	I	49.22	16.67	This study	MG851976
HAN1	Germany	Halle an der Saale	I	51.74	11.97	Schulte et al. (2012a)	HQ652958
UU89, 90	Germany	Altenhain	I	51.29	12.70	Schulte et al. (2008)	HQ652886 HQ652887
UU34	Germany	Ammelshain	I	51.29	12.63	Schulte et al. (2008)	HQ652885
P33_HU, P34_HU, P35_HU, P45_HU	Hungary	Esztergom	I	47.80	18.75	This study	MG851935 MG851936 MG851937 MG851944
1961_RO	Romania	Sf. Elena	II	44.67	21.71	This study	MG851969
2415_RO	Romania	Soimos	V	46.10	21.72	This study	MG851970
3420_RO	Romania	Svinita	III	44.50	22.08	This study	MG851973
P30_RS	Serbia	Podujevace	IV	42.98	21.39	This study	MG851933
P32_RS	Serbia	Sokolac	II	44.26	19.43	This study	MG851934
1736_RS	Serbia	Sopotnica	V	43.15	22.13	This study	MG851968
2655_RS	Serbia	Maglič	IV	43.61	20.54	This study	MG851971
3579_RS	Serbia	Bukovi	II	44.15	19.91	This study	MG851977
djz_655_SK, djz_656_SK, djz_657_SK, djz_658_SK	Slovakia	Vlachy	I	49.10	19.48	This study	MG851953 MG851954 MG851955 MG851956
djz_1728_SK, djz_1729_SK, djz_1730_SK, djz_1731_SK	Slovakia	Ihráč	I	48.63	18.95	This study	MG851957 MG851958 MG851959 MG851960
P15_L4_SK	Slovakia	Temešská skala	I	48.87	18.37	This study	MG851919
P16_L5_SK	Slovakia	Bojnice	I	48.78	18.58	This study	MG851920
P17_L6_SK, P18_L7_SK, P19_SK	Slovakia	Bratislava	I	48.15	17.07	This study	MG851921 MG851922 MG851923
P22_L8_SK, P23_L9_SK, P24_SK	Slovakia	Bratislava II	I	48.14	17.07	This study	MG851925 MG851926 MG851927
P26_L10_SK, P27_L11_SK	Slovakia	Trubárka	I	48.88	18.15	This study	MG851928 MG851929
P38_SK	Slovakia	Zádiel	I	48.62	20.83	This study	MG851938
P39_SK	Slovakia	Plešivec	I	48.55	20.47	This study	MG851939
P40_SK	Slovakia	Kečovo	I	48.48	20.50	This study	MG851941
P46_SK	Slovakia	Višňové	I	49.17	18.78	This study	MG851945
P48_SK	Slovakia	Nižná Kamenica	I	48.77	21.53	This study	MG851946
1460_SK, 1462_SK	Slovakia	Šášovské Podhradie	I	48.57	18.90	This study	MG851965 MG851966
1726_SK	Slovakia	Plavecké Podhradie	I	48.49	17.26	This study	MG851967
3052_SK	Slovakia	Kováčov	I	47.82	18.77	This study	MG851972

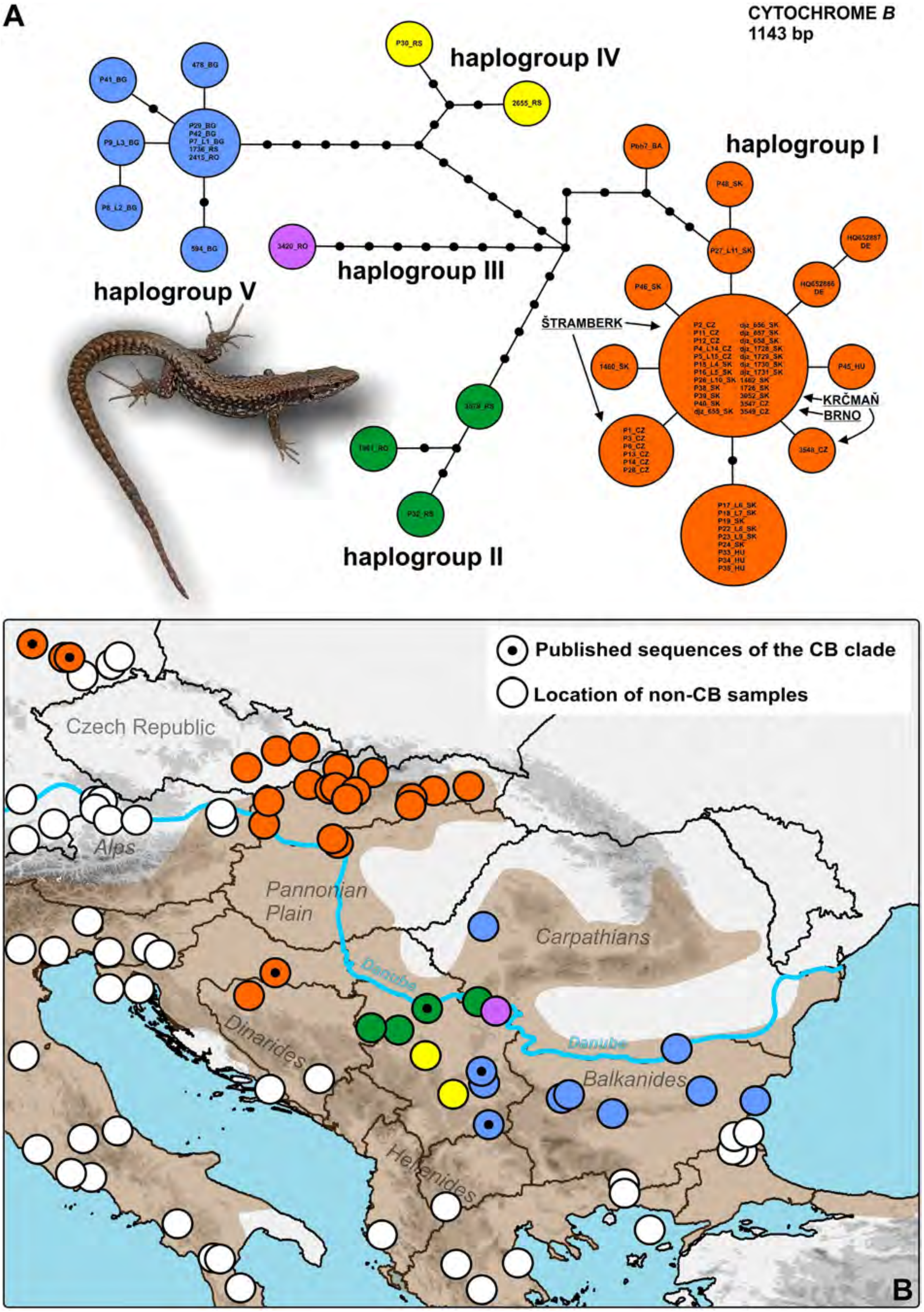

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Fig. 1. (A) Haplotype network of the Central Balkan (CB) clade of *Podarcis muralis* based on complete cytochrome *b* sequences, and (B) geographical distributions of the main haplogroups of the CB clade in the studied area according to results of the network- and tree-based approaches. Approximate species distribution is given in brown shading (Sillero et al., 2014). The three isolated populations from the Czech Republic are underlined, and positions of their haplotypes are indicated by arrows. Standard country codes are used in the sample coding. Inset: an adult male from Štramberk, Czech Republic. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

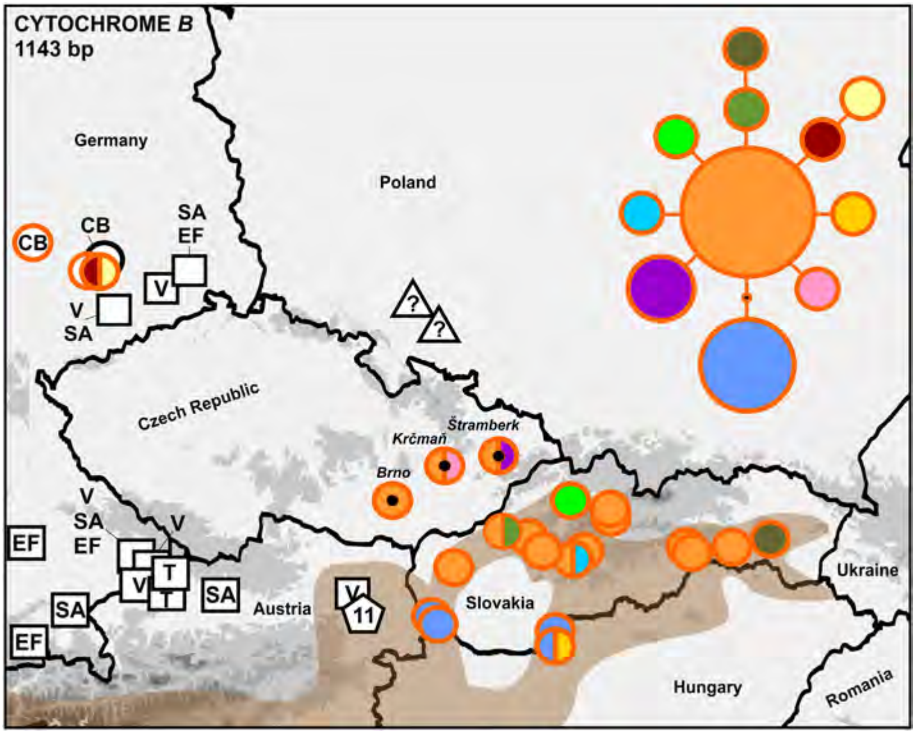

Fig. 2. Haplotype network and distribution of individual haplotypes of the haplogroup I of the Central Balkan clade (CB) in Central Europe (in circles). Approximate species distribution is given in brown shading. Populations of the Central Balkan clade in Germany: circle with orange border and colour haplotype affiliations is locality Altenhain (Saxony, HQ652886-7; Schulte et al., 2008); white circles with orange borders are localities Halle an der Saale (Saxony-Anhalt, HQ652958) and Ammelshain (Saxony, HQ652885) not included in the network analysis due to short sequence lengths; white circle with black border is locality Böhlitz (Saxony; Schulte et al., 2011) without available genetic data. Genetically investigated exotic introduced populations from Germany and Austria (Schulte et al., 2008; Schulte et al., 2011; Schulte et al., 2012a) are given in squares; abbreviations for their phylogenetic origin (*sensu* Schulte et al., 2011; Salvi et al., 2013): EF – East France, SA – South Alps, T – Tuscany, V – Venetian. The presumably autochthonous population belonging to the "clade 11" (*sensu* Salvi et al., 2013; AY185096, DQ001020) in Austria is in pentagon. Recently discovered isolated populations in Poland are in triangles with question marks (genetic data are not yet available; Wirga and Majtyka, 2013). The Czech isolated populations are marked by black dots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

show low haplotype diversity of mitochondrial DNA (mtDNA). Furthermore, in each of the mentioned species the post-glacial colonization of Central Europe was derived from two lineages originating from the Balkan refugia (Fritz et al., 2007; Sommer et al., 2009; Musilová et al., 2010).

In the present study, we analyzed the mtDNA phylogeography of another xerothermic reptile species, the Common Wall Lizard, *Podarcis muralis* (Laurenti, 1768), whose actual distribution in Central Europe resembles that of another xerothermic lizard *Lacerta viridis*, or snakes *Natrix tessellata* and *Z. longissimus* (Sillero et al., 2014). The species inhabits almost the entire southern part of the European landmass as it has successfully colonized regions in North-Western and Central Europe far outside its core range in the Mediterranean region (Gassert et al., 2013; Salvi et al., 2013; Michaelides et al., 2015a). Currently, more than twenty geographically restricted mtDNA evolutionary lineages of *P. muralis* are known (Schulte et al., 2012a, 2012b; Gassert et al., 2013; Salvi et al., 2013). More or less isolated populations from the northern range margins raise questions whether they are native and represent autochthonous populations or if they were introduced by humans (Schulte et al., 2008; Schulte et al., 2012a, 2012b). While the status of most populations from the north-western regions has been evaluated recently using a comparative phylogeographic approach (Schulte et al., 2012a, 2012b; Gassert et al., 2013; Michaelides et al., 2015a, 2015b), the relationships and origin of populations at the northern range margin in Central and Eastern Europe remain unknown. Understanding the history of populations in this region becomes particularly important given the fact that the common wall lizard is prone to establishing thriving and self-sustaining populations following human-mediated introductions. To date, > 150 cases of such introductions have been reported, and were the result of either deliberate releases of captive animals, arrival *via* timber trade or rail freight transport (Schulte et al., 2012c; Michaelides et al., 2015b). High phenotypic variability of *P. muralis* makes it merely impossible to distinguish between native and introduced populations based solely on morphological characters (Bellati et al., 2011; Schulte et al., 2012c).

The aim of this study is to ascertain the origin and population structure of *P. muralis* in the north-eastern range margin of Central Europe, where the common wall lizard as a xerothermic species is considered a reptile rarity. This could be illustrated by an anecdote of its first discovery in a close proximity of the north-western Carpathians in limestone quarries in Štramberk, Czech Republic. The species was first misidentified as the viviparous lizard (*Zootoca vivipara*) by Štěpánek (1955), and correctly re-identified as *P. muralis* not earlier than almost a half century later (Zavadil, 1999; Moravec and Beneš, 2000; Pavlík and Šuhaj, 2000). Currently, three isolated populations of *P. muralis* are known from the Czech Republic. Beside the one from Štramberk, two more were discovered in Brno and Krčmaň in the last decade. However, there are still doubts whether the species is native or was introduced in the Czech Republic. The reasoning behind the latter possibility is that keeping reptiles as pets, including wall lizards, was a popular hobby in the area during the 20th century. Many reptile species were known to be imported to captivity by Central Europeans travelling to the Balkans for holidays or business (see Steinicke, 2000; Schulte et al., 2008). We therefore compared mtDNA sequences of the cytochrome *b* gene (*cytb*) of the three isolated populations from the Czech Republic with those from the continuous range in Slovakia, the northern Balkans (Central Balkan clade *sensu* Schulte et al., 2011, 2012a), and ones available from previous studies to test, whether the Czech populations are (i) native populations at the north-eastern range margin, (ii) introduced populations stemming from one or more source regions, or (iii) mixed populations between native and introduced *P. muralis*. In addition, we present details of the genetic structure of the

Central Balkan clade, and briefly discuss potential conservation measures for the studied populations.

2. Materials and methods

We investigated the three known isolated northernmost populations of *P. muralis* from the Czech Republic (three abandoned quarries with xerothermic vegetation and areas ranging from 0.8 to 15 ha) together with samples from Slovakia, southeastern Europe, and the Balkans (Table 1, Figs. 1, 2). Blood samples or small tail tips were collected from living specimens, muscle tissues from dead individuals (road-kills). Tissue samples were preserved in 96% ethanol or frozen and stored at −25 to −80 °C. No lizards were killed for this study and no voucher specimens were deposited in museum collections.

We used standard phenol-chloroform extraction methods or various DNA extraction kits for DNA isolation. The complete cytochrome *b* gene (*cytb*) was amplified with primers L14910 and H16064 (Burbrink et al., 2000) and the same primers were used for sequencing. The sequencing was performed by Macrogen Inc. (Seoul, South Korea or Amsterdam, Netherlands; http://www.macrogen.com) and new sequences have been deposited in GenBank under the accession numbers MG851915–MG851983 (Table 1 and Additional file 1: Table S1). The final alignment was based on nucleotide sequences of complete *cytb* (1143 bp), which served for the construction of haplotype networks. For phylogenetic analyses we used trimmed sequences of *cytb* (411 bp-long fragment) to facilitate utilization of sequences available in GenBank (see Additional files 1,2: Table S1, Fig. S1).

DNA sequences were checked for their quality and mitochondrial origin by translating them into amino acids. No stop codons were detected when the sequences were translated using the vertebrate mitochondrial genetic code in the program DnaSP 5.10 (Librado and Rozas, 2009). The same program was used to calculate uncorrected *p*-distances among the main haplogroups within the lineage containing the Czech and Slovak populations, and to estimate the haplotype diversity (*Hd*), number of segregating sites (*S*) and nucleotide diversity (π) for selected haplogroups/populations. In order to reconstruct phylogenetic relationships of individuals from the Czech Republic and Slovakia, we applied phylogenetic analyses using the all-individuals dataset supplemented by distinct published haplotypes from the species range. Phylogenetic trees were inferred using the Bayesian approach (BA) and maximum likelihood (ML) by MrBayes 3.2 (Ronquist et al., 2012) and RAxML 8.0. (Stamatakis, 2014), respectively. The best-fit codon-partitioning schemes and the best-fit substitution models were selected for BA and ML analysis separately using PartitionFinder v1.1.1 (Lanfear et al., 2012), and according to the Bayesian information criterion (BIC): K80 + I + G (1st and 2nd codon position), GTR + G (3rd codon position) for BA; and GTR + G in each codon position in ML. The ML clade support was assessed by 1000 bootstrap pseudoreplicates. The MrBayes analysis was set as follows: two separate runs with four chains for each run, 10 million generations with samples saved every 100th generation. The convergence of the two runs was confirmed by the convergence diagnostics (average standard deviation of split frequencies, potential scale reduction factor). First 20% of trees were discarded as the burn-in after inspection for stationarity of log-likelihood scores of sampled trees in Tracer 1.6 (Rambaut et al., 2013) (all parameters had effective sample size > 200). A majority-rule consensus tree was drawn from the post-burn-in samples and posterior probabilities were calculated as the frequency of samples recovering any particular clade.

A haplotype-network approach can be more effective for presentation of intraspecific evolution than the tree-based phylogenetic approaches (Posada and Crandall, 2001). Therefore, we constructed a haplotype network using the 95% limit of parsimony as implemented in TCS 1.21 (Clement et al., 2000) for the Central Balkan clade, in which the populations from Central Europe clustered together (including two GenBank individuals from German Saxony: HQ652886-7; Schulte et al., 2008).

The past population dynamics was inferred using the Bayesian coalescent-based approach of the Bayeasian skyline plot (BSP; Drummond et al., 2005) as implemented in BEAST 2.1 (Bouckaert et al., 2014). This method computes the effective population size through the time directly from sampled sequences and does not require a specific *a priori* assumed demographic model. This method was applied for the haplogroup I (without geographically isolated samples from Bosnia and Hercegovina, and Croatia) of the Central Balkan clade. A uniform prior for the average substitution rate with the initial value 0.013 mutations/site/million years was applied according to Podnar et al. (2014). Preliminary analyses were run using both strict molecular clock and uncorrelated lognormal relaxed molecular clock. Since the parameter of the standard deviation of the uncorrelated lognormal relaxed clock was close to zero, the final analyses were run enforcing the strict molecular clock model. Using PartitionFinder v1.1.1. (Lanfear et al., 2012), all codon positions treated together as one partition and the HKY substitution model were selected as the best-fit partitioning scheme and the best-fit model, respectively. The final BSP analysis was run in duplicates to check for consistency between runs, each run for 10 million generations and sampled every 1000 generations. Convergence, effective sample size (ESS > 200), stationarity, and the appropriate number of generations to be discarded as burn-in (10%) were assessed using Tracer 1.6 (Rambaut et al., 2013). The resulting BSP was also summarized in Tracer 1.6 with the maximum time as the median of the root height parameter. In addition to the BSP, a signature of a possible expansion was assessed also by the neutrality-test statistics of the Fu's F_S (Fu, 1997) and Ramos-Onsins and Rozas's R_2 (Ramos-Onsins and Rozas, 2002) calculated in DnaSP 5.10 with the estimation of the statistical significance using 10,000 coalescent simulations.

3. Results

We obtained 69 complete *cytb* sequences with no signal of contamination or sequences of nuclear genomic origin. The BA/ML analyses resulted in phylogenetic trees with many distinct lineages but unresolved topology (Fig. S1), which is in a general concordance with previous studies (*e.g.* Salvi et al., 2013). Populations from the Czech Republic and Slovakia clustered with the Central Balkan clade (*sensu* Schulte et al., 2011, 2012a; or “clade 4” *sensu* Salvi et al., 2013), which was divided into four lineages with unresolved mutual relationships (Fig. S1). All samples from the Czech Republic and Slovakia form one lineage together with individuals from northern Bosnia and Herzegovina, north-eastern Croatia (DQ001025), Hungary, and eastern Germany (haplogroup I).

According to the haplotype-network analysis of 1143 bp-long sequence alignment (Fig. 1), 24 haplotypes were detected within the Central Balkan clade structured into five main haplogroups: haplogroup I found in the area from eastern Germany, Czech Republic, Slovakia, Hungary and northern Bosnia and Herzegovina; and four Balkan haplogroups (haplogroups II, III, IV, V) from western Romania, Serbia, and northern Bulgaria. These haplogroups are separated from each other by 0.2–1.2% of uncorrected *p*-distance in their *cytb* sequences. Twelve haplotypes were detected in the haplogroup I, including one slightly distant haplotype from northern Bosnia and Herzegovina (47 sequenced individuals from 21 localities and two GenBank sequences from one German locality). Overall, the Balkan haplogroups (II-V) included 12 haplotypes in 16 sequenced individuals, and the highest haplotype diversity was found in the Central Balkan mountains and neighboring areas (southern Carpathians, eastern Dinarides, Stara Planina Mts.). Three haplotypes were detected in haplogroup II (Serbia and western Romania), one haplotype in haplogroup III (near the Danube River in Romania), two haplotypes in haplogroup IV (two Serbian samples), and six haplotypes in haplogroup V (western Romania, eastern Serbia, and northern Bulgaria). All remaining sequenced individuals analyzed in this study from Albania, southern Bulgaria, southern Bosnia and

Herzegovina, and Italy corresponded to other phylogenetic clades of *P. muralis* (see Additional files 1,2: Table S1, Fig. S1). These clades are out of the focus of this study, therefore their structure and distribution are not discussed (see *e.g.* Salvi et al., 2013).

Focusing on populations from Central Europe, eleven different haplotypes were detected in the area, all within the haplogroup I ($S = 15$; $Hd = 0.71$; $\pi = 0.128$; Fig. 2). The most common haplotype of the haplogroup I is widely distributed across the Czech Republic and western, central, and eastern Slovakia (13 localities, 24 individuals). The second most common haplotype separated by two mutation steps from the most common haplotype was found along the Danube River (4 localities, 9 individuals). Other haplotypes are geographically restricted and some of them supposedly linked to discrete localities: Esztergom (Hungary), Krčmaň, Štramberk (Czech Republic), Nižná Kamenica, Šášovské Podhradie, Trubárka, and Višňové (Slovakia). All three isolated Czech populations share the most common haplotype, with Štramberk ($S = 1$; $Hd = 0.55$; $\pi = 0.048$) and Krčmaň ($S = 1$; $Hd = 1.00$, $\pi = 0.087$) also possessing haplotypes unique to each locality and separated from the most common haplotype by a single mutation step. Two unique haplotypes (one and two mutation steps distinct from the most common haplotype) were also detected in German locality Altenhain (UU89, UU90, HQ652886–7; Figs. 1 and 2). The haplogroup I is also present in other German localities from Saxony and Saxony-Anhalt (Ammelshain, UU34, HQ652885; Halle an der Saale, HAN1, HQ652958), but the sequences were not included in the haplotype networks due to their short lengths. The demographic analysis using the Bayesian skyline plot method (Fig. 3) gave evidence of population growth of the haplogroup I, which commenced at approximately 20 Kya. The neutrality tests significantly rejected the null hypothesis of selective neutrality and constant population size ($R_2 = 0.0557$, $P = 0.037$; $F_S = -5.3962$, $P = 0.003$).

4. Discussion

4.1. The Central Balkan clade: phylogeography and colonization of the northern range margin

Our results confirm previous reports on complex phylogeographic structure among *P. muralis* populations inside the detected mtDNA clades (*e.g.* Salvi et al., 2013). This is also the case of the Central Balkan clade (CB), which was sampled less thoroughly in previous studies (Schulte et al., 2008; Schulte et al., 2012a; Salvi et al., 2013). We uncovered five distinct haplogroups occurring throughout the northern Balkans, southern and northern Carpathians, and a part of the Pannonian Plain. The haplotype diversity markedly increases south of the Danube River, while the populations on the north-western edge of the CB distributional range are relatively uniform. Similar patterns of high haplotype diversity in the south have been found in several other Balkan-Carpathian reptile species that colonized northern areas of Europe and are in concordance with the refugia-within-refugia model (Gómez and Lunt, 2007). Based on the observed pattern, we predict existence of several smaller and isolated refugia in southern Europe. These refugia, which harbored populations of the Central Balkan clade during the Pleistocene climatic oscillations, could be located in the Dinarides, Carpathians, and Balkanides (Stara-Planina Mts.; see distributions of haplogroups in Fig. 1). Populations of *P. muralis* presumably colonized northern parts of Europe by expansion of the haplogroup I, which is supported by a typical star-like structure (Figs. 1, 2) of the haplotype network. Other haplogroups seem to be restricted to the southern Carpathians and eastern Dinarides, regions known as refugia for its specific topography (Jablonski et al., 2016). Areas between main topographic units of the Balkans (Dinarides/Hellenides and Balkanides/Macedonian-Thracian Massif) probably form borders between the Central Balkan clade and other endemic clades found in the southern parts of the Balkans (see Salvi et al., 2013). Similar pattern has also been observed in other reptiles of the peninsula (Jablonski et al., 2016; Marzahn et al., 2016).

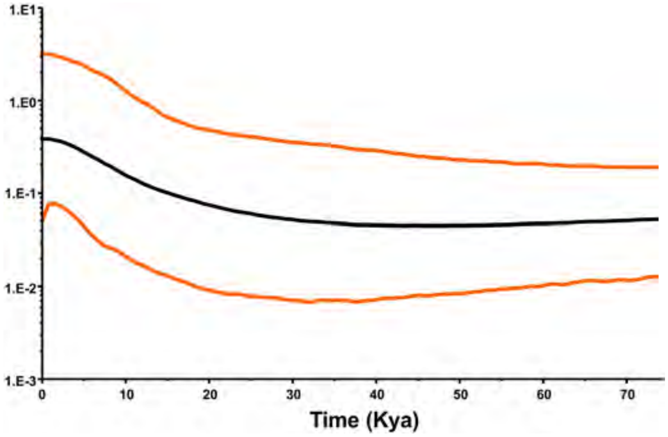

Fig. 3. Demographic history (effective population size through the time) of the Central European population of the haplogroup I of the Central Balkan clade of *Podarcis muralis* as estimated by the Bayesian skyline plot.

Our data provide evidence that common wall lizard populations in the Czech Republic and Slovakia belong to the same *cytb* haplogroup (Figs. 1 and 2) and they are closely related to populations from eastern Germany (Saxony and Saxony-Anhalt) and northern Hungary, and a bit more distantly to populations from northern Bosnia and Herzegovina and north-eastern Croatia (Fig. S1). As our sampling detected the genetically nearest population to Central Europe in the north-eastern Dinarides, we can expect that the population now distributed in Central Europe originated from this area and not from the southern Carpathian refugia, as would be more likely given the phylogeographic patterns of several other reptile species (*e.g.* Ursenbacher et al., 2006; Jablonski et al., 2016). However, due to a sampling gap in the Romanian Carpathians and most of Hungary, these conclusions can only be considered preliminary.

Looking at genetic structure of the haplogroup I, there is usually separation by one mutation step from the most common haplotype, except of some southern Slovak populations (from near the Danube River) possessing a haplotype separated by two steps (Fig. 2). This could suggest historical separation of the Danubian and Carpathian populations. The star-like pattern of the haplotype network of the haplogroup I supports a hypothesis of population expansion, likely post-glacial, which is further supported by our demographic analysis (Fig. 3) and neutrality tests. One possible explanation of the phylogeographic pattern in Central Europe is that dispersal in this region occurred primarily along the mountain chain of the northern Carpathians. As all mutations forming the star-like pattern of the haplotype network are synonymous, we do not expect that positive selection played a role in forming this pattern. Furthermore, since the studied populations are located on the range margin, we do not suspect the selective sweep was responsible for the observed genetic structure. However, to understand the colonization history of Central Europe by *P. muralis*, we need a better sampling from the western part of the Pannonian Plain and from the eastern and southern Carpathians.

4.2. Populations in the Czech Republic: origin and conservation implications

Due to the special position of the low number (three) mutually separated *P. muralis* populations within the herpetofauna of the Czech Republic (classified there among the critically endangered species), the question of their origin has been in the conservationist spotlight for a long time. Currently there are three isolated populations, all associated with old limestone quarries: two in northern/central Moravia (Štramberk, Krčmaň) and one in southern Moravia (Brno; all in the eastern part of the Czech Republic). During the early decades of the

20th century, several anecdotal reports were made on the occurrence of Mediterranean reptiles (*Hemidactylus turcicus*, *Dolichophis caspius*, *Vipera ammodytes*, *V. aspis*) in the area of the present-day north-eastern Czech Republic. These reports were probably results of the activities of enthusiastic herpetofauna fans attempting to establish populations of non-native species by introduction (Adolph, 1922; Pax, 1925; Krátký and Wenig, 1930; Moravec, 2015). Although we do not have clear evidence that *P. muralis* was included in such activities, it is easy to imagine that the species could be a target of similar attempts, especially when a similar problem was recorded in eastern Germany. As presented by Grosse et al. (2015), the first record of *P. muralis* in eastern Germany comes from roughly the same time of the early decades of the 20th century, when Mertens (1917), found a specimen of presumably Italian origin in the city park of Leipzig. Schulte et al. (2008) and Grosse et al. (2015) reported that the populations from Saxony and Saxony-Anhalt carry haplotypes of the Central Balkan clade, and these populations are considered introduced (Steinicke, 2000; Schulte et al., 2008, 2011).

Combining with our sampling of the Central Balkan clade we found out that three populations from eastern Germany belong to the haplogroup I, with two haplotypes found within the two individuals sampled. Both these haplotypes were unique and found in the population from Altenhain (which is probably a population derived from the neighboring Ammelshain population; Schulte et al., 2011). Similarly, the presence of two unique haplotypes has been detected in two Czech populations (Krčmaň, Štramberk; Fig. 2). This rather unexpected finding suggests two possible scenarios: i) the Czech populations (or at least the oldest known from Štramberk) are autochthonous remnants from the past Holocene climatic optimum, which would be similar to the situation found in other reptile species such as *Emys orbicularis*, *Lacerta viridis*, *Natrix tessellata*, or *Zamenis longissimus*; or ii) these populations are allochthonous, and were introduced either from Slovakia or from another yet uncovered geographically nearby region. The latter hypothesis of the allochthonous origin of the Czech population in Štramberk is supported by a high frequency of scale anomalies found in individuals of this population (Veselý et al., 2007; Moravec and Veselý, 2015). These anomalies may be a result of demographic bottlenecks (*i.e.* reduced levels of allelic diversity) resulting from a small founder population, frequently observed in introduced populations of reptiles (Gautschi et al., 2002). These authors demonstrated a significantly bigger loss of allelic diversity in a serially bottlenecked population than in a population that was bottlenecked just once.

On the other hand, our sampling throughout the nearest populations of *P. muralis* in Slovakia revealed close phylogenetic relationships to the Czech populations, which supports the autochthonous hypothesis. In this case, small haplotype differences may suggest long-term isolation of the Czech populations with an occurrence of historical bottlenecks. This hypothesis is further supported by relatively short geographical distance (about 60 km) between Štramberk and the nearest Slovak populations (Trubárka, Višňové; Table 1, Fig. 2). However, these localities are recently separated by a densely forested mountain chain with rather unfavorable habitat and microclimatic conditions. Therefore, a possible explanation is that *P. muralis* populations expanded and colonized suitable localities in the territory of the present-day Czech Republic during the Holocene climatic optimum (*i.e.* around 9000–5000 years ago) as is known for populations of two other ecologically similar reptile species, *Lacerta viridis* (Böhme et al., 2006) and *Zamenis longissimus* (Musilová et al., 2010). However, it is also possible that these “insular” populations might have persisted under more favorable microclimatic conditions during the glacials, and therefore may be older than populations established by later (re)colonization events. All Czech localities are situated in limestone areas and are characterized by Mediterranean-like climate conditions unlike other parts of the Czech Republic, and the regions might have acted as ideal spots for retraction during moderately cold periods, as is also supported by the distribution model based on paleoclimatic data presented by Gassert et al. (2013). To investigate this scenario in detail, employment of additional genetic markers and dense sampling in the Pannonian-Carpathian region will be necessary.

The localities in the Czech Republic represent isolated populations at the north-eastern range margin of the species distribution adjacent to the presently known indigenous range. Due to special habitat preferences, the species almost exclusively inhabits abandoned limestone quarries and their surroundings as such sites best fit Mediterranean conditions in microclimate, vertical orography, rocky substrate, and xerothermic vegetation. Despite its known genetic nature, we find it difficult to distinguish between alternative hypotheses when tracing mitochondrial origin in these cases. In the light of genetic evidence, we suggest the following scenario: (1) the populations from Štramberk and Krčmaň may represent autochthonous relict populations due to possession of unique haplotypes found nowhere else throughout the sampled range. These findings remain surprising due to the fact that despite Krčmaň has often been visited by zoologists from the nearby Palacký University in Olomouc in the last four decades, *P. muralis* records have appeared much later (Mačát and Veselý, 2009; Zavadil et al., 2015). Even if recent introduction would be the case, maternal lineages still come from the geographically and genetically closest populations; (2) the population from Brno is most likely the result of recent introduction from neighboring Slovak populations or even from Štramberk or Krčmaň.

Even if allochthonous transfers in the near past are considered as the plausible explanation, Czech populations do not represent a serious alien threat to genetic integrity of the geographically closest native populations in Slovakia. This contrasts to the situation well known from the north-western range margin of the species distribution, where often substantively genetically distant forms were introduced (Schulte et al., 2012a, 2012c; Michaelides et al., 2015b). The Czech populations would represent either a new category or a kind of assisted colonization within conservation translocations *sensu* Guidelines for Reintroductions and Other Conservation Translocations (IUCN/SSC, 2013). Although none of the three localities is seriously threatened by habitat destruction, they all are sensitive to vegetation overgrowth. All three localities are partially protected but only one population (Štramberk) is being regularly monitored for population size. Potential loss of these populations could mean the loss of adaptive genetic variation, which could be important for the survival of the species in the area and further expansion northward in the future (Nielsen et al., 2001; Parmesan, 2006). Considering the unique DNA variation, a conservation program is warranted for all three populations of the common wall lizard in the Czech Republic. To be successful, such program should include cleaning measures focused on control of bushes and trees serving as shelters for young individuals, but later leading to overshading of the habitat. As wall lizards prefer open exposed rocky habitats on limestone substrate, the overshaded places become unsuitable for population survival. On the other hand, it seems that human presence does not pose a serious threat to the populations (Urban et al., 2006). Two of the three localities are often visited by people with no visible impact on wall lizard abundance.

5. Conclusion

Central Europe was historically crossed by many naturally expanding species, including taxa that originated from the Mediterranean. The studied example of the common wall lizard, *Podarcis muralis*, on its north-eastern range margin in Central Europe, in the Czech Republic, demonstrate importance to understand origins of marginal populations. Considering possible human-mediated introductions of the common wall lizard from southern Europe, commonly establishing viable populations in Western Europe, it is important to give evidence that all so far studied Central European isolated populations belong to the same haplogroup as the one widespread within the continuous range in the region. The presence of unique haplotypes in two of the three isolated Czech populations, together with close phylogenetic relationships to adjacent Slovak populations suggests either an autochthonous origin of these populations or human-mediated introductions from the geographically and genetically closest populations. We are therefore in support of conservation programs for all three known Czech populations.

Acknowledgements

We would like to thank all colleagues and friends who donated samples and/or helped us in the field: M. Baláž, A. Bednařík, Z. Lajbner, P. Meduna, M. Minařík, M. Rindoš, R. Smolinský, and D. Zerzán. Special thanks belong to Petr Pavlík, who kindly permitted access to his private area in the old limestone quarry "Dolní Kamenárka", Štramberk. We also thank Peter Mikulíček for valuable comments on a draft of this manuscript. Tyler Square edited the language and style. We also thank two anonymous rewievers for valuable comments on the ms. The research was supported by the Slovak Research and Development Agency under the contract No. APVV-15-0147, and by the institutional support of the Palacký University and the internal grant UP IGA_PrF_2010_027 (MV). Work of JM and VG was financially supported by the Ministry of Culture of the Czech Republic (projects: DKRVO 2014/14, 2015/15, 2016/15, 2017/15, and 2018/14, National Museum Prague, 00023272), and work of VG also by the IVB CAS institutional support (RVO: 68081766). Work of LC was financially supported by the Czech Acad Sci, Inst Anim Physiol & Genet, institutional support (RVO: 67985904).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mito.2018.04.006.

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Additional file 1: Table S1. The list of sequences with GenBank accession numbers and their localities used for phylogenetic analyses (411 bp). For our sequences of the Central Balkan clade used in this study see Table 1.

GenBank Accession Number	Locality	Country	Authors	Reference
AY234155	Benasque	SPAIN	Busack et al.	Amphibia-Reptilia 26: 239-256 (2005)
FJ867366	Trieste	ITALY	Giovannotti et al.	Ital. J. Zool. 77: 277-288 (2010)
FJ867389	Mt. Alburni	ITALY	Giovannotti et al.	Ital. J. Zool. 77: 277-288 (2010)
FJ867393	Pollino	ITALY	Giovannotti et al.	Ital. J. Zool. 77: 277-288 (2010)
FJ867365	Trento	ITALY	Giovannotti et al.	Ital. J. Zool. 77: 277-288 (2010)
JX856985	Abbotsbury, Birdbook, Corfe Castle, Eastbourne, Folkestone, Holmsley, Newton Ferrers, Poole, Seacombe, Shoreham, Shorwell, Ventnor Botanical Garden, Winspit	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
JX856989	Boscombe, Corfe Castle, Dancing Ledge, Holmsley, Poole, Seacombe, Shorwell, Winspit	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
JX856990	Winspit, West Worthing	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
KP972516	Cento, Castelarrano, Nonantola, Olina, Pian Di Venola, Vignola, Motta di Livenza	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972517	Olina, Vignola, Nonantola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972518	Nonantola, Vignola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972519	Nonantola, Pian Di Venola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972520	Pian Di Venola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)

KP972521	Pian Di Venola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972523	Castelarrano	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972524	Olina	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972525	Olina	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972526	Olina	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972527	Nonantola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972528	Motta di Livenza	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972529	Cento	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972530	Pian Di Venola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972531	Nonantola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972532	Motta di Livenza	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972533	Nonantola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972534	Nonantola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972535	Cento	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972536	Cento	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972537	Vignola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972538	Olina	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972539	Pian Di Venola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
JX856991	Abbotsbury, Bristol, Boscombe, Corfe Castle, Dancing Ledge, Folkestone, Holmsley, Poole, Ventnor Botanical Garden, Winspit	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
KP972522	Castelarrano	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
JX856987	East Portland, Cheyne Weare, Holmsley, Wembdon, Wellington	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
JX856988	Wellington	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
JX856993	East Portland, Cheyne Weare	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
KP118978	Joselin	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118979	Jersey Is.	UK	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118980	Iles de Chausey, Jersey Is.	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118981	Jersey Is.	UK	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118982	Iles de Chausey	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118983	Nebias, Frontier Cabardes	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118984	Iles de Chausey, Chateau du Guildo, Dinan, Sees, Vitre, Josselin, Pontchateau, Puybelliard, Pouzagues, St. Gervais, St.	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)

	Michel, St. Lizier, St. Girons Frontier Cabardes			
KP118985	Cap Frehel	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118990	Cap Frehel	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118986	Bastide	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118987	Cap Frehel	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118988	St. Lizier, Nebias, Frontiers Cabardes	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP118989	St. Girons, Bastide	FRANCE	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
JX856983	Bury	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
JX856984	Bury	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
JX856982	Ventnor Town, Holmsley, Ventnor Botanical Garden, Shorwhel	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
KP972490	Prato, Vignola	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972492	Viareggio	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972495	Calci	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972496	Prato	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972497	Prato	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972498	Prato	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
JX856992	Bristol, Boscombe	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
KP972482	Buti, Travale, Colle di Val'Elsa, Greve in Chianti, Montemassi, Chianni, Crespina	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972484	Chianni	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972499	Montemassi	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972500	Travale	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972501	Travale	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972502	Greve in Chianti	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972503	Crespina	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972504	Montemassi, Travale, Colle di Val'Elsa	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972505	Crespina	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972506	Colle di Val'Elsa	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972507	Calci	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972512	Calci	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972508	Chianni, Crespina	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972509	Viareggio	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972510	Buti	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)

KP972511	Chianni	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972513	Montemassi	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972514	Chianni	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972515	Colle di Val'Elsa	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972481	Montemassi	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972483	Greve in Chianti	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972485	Viareggio	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972486	Greve in Chianti	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
KP972487	Greve in Chianti	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
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JX856986	Birdbook, Eastbourne	UK	Michaelides et al.	Biol. Inv. 15: 1101–1112 (2013)
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KP972474	Buti	ITALY	Michaelides et al.	Mol. Ecol. 24: 2702–2714 (2015)
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AY185096	Bade	AUSTRIA	Podnar et al.	Org. Div. Evol. 4: 307–317 (2004)
DQ001020	Gumpolds-Kirchen	AUSTRIA	Podnar et al.	J. Mol. Evol. 64: 308-20 (2007)
DQ001025	Nova Gradiska, Strmac	CROATIA	Podnar et al.	J. Mol. Evol. 64: 308-20 (2007)
AY185097	Vis isl.	CROATIA	Podnar et al.	Org. Divers. Evol. 4: 307–317 (2004)
AY896135	Kotili, Xanthi	GREECE	Poulakakis et al.	Mol. Phylogenet. Evol. 37: 845–857 (2005)
AY896136	Xanthi	GREECE	Poulakakis et al.	Mol. Phylogenet. Evol. 37: 845–857 (2005)
AY896126	Makedonia (Ag. Germanos)	GREECE	Poulakakis et al.	Mol. Phylogenet. Evol. 37: 845–857 (2005)
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KF372244	Fago del Soldato	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
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KF372237	Fondi	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
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KF372249	Zuce	SERBIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372250	Zuce	SERBIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
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KF372252	Sinevo	SERBIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372253	Road to Crnovska River	SERBIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372254	Road to Crnovska River	SERBIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372255	Road to Crnovska River	SERBIA	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
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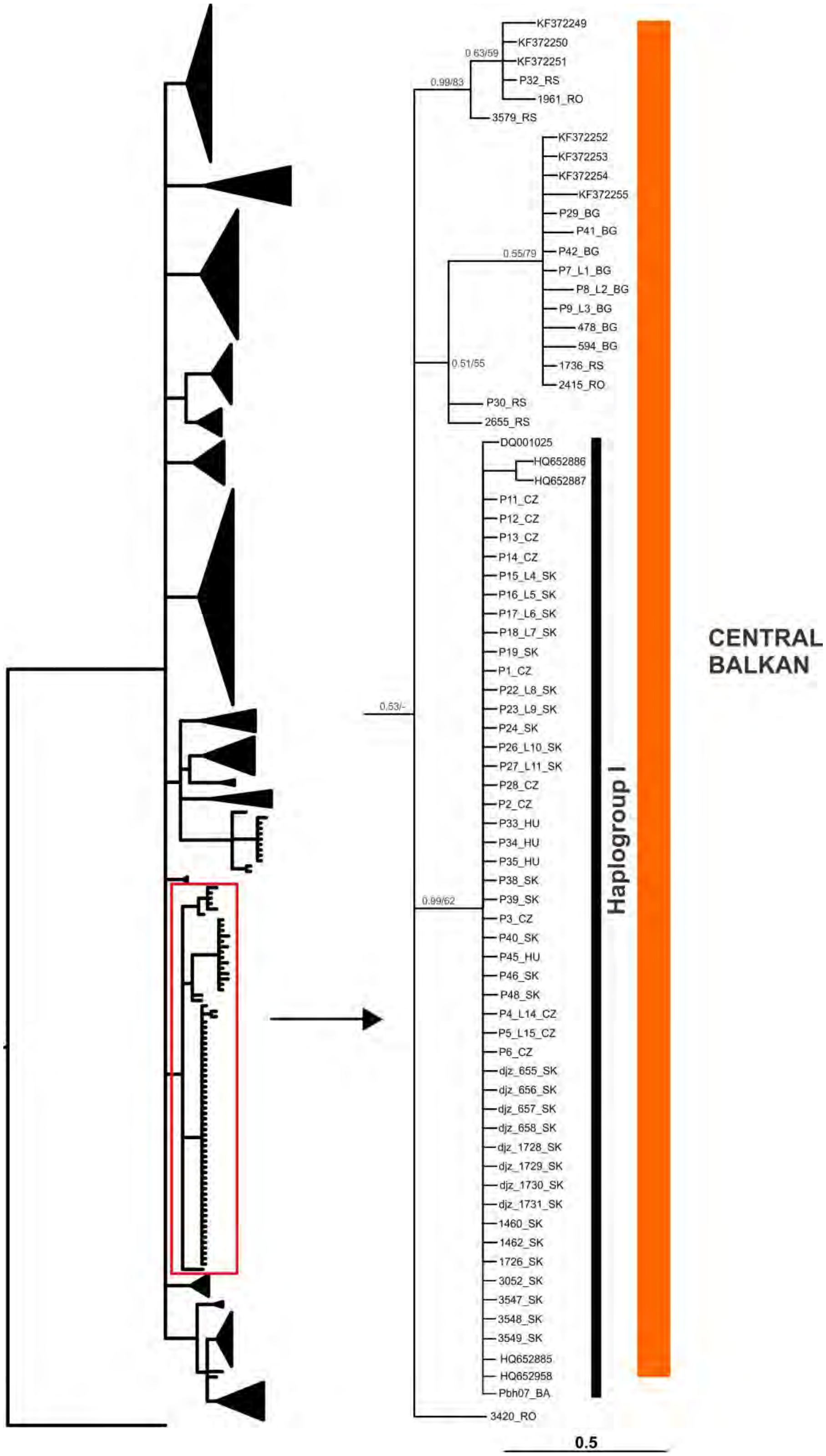
KF372226	Bianzano	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372227	Peschiera del Garda	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372228	Peschiera del Garda	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
KF372229	Peschiera del Garda	ITALY	Salvi et al.	BMC Evol. Biol. doi:10.1186/1471-2148-13-147 (2013)
HQ652932	Aschaffenburg	GERMANY	Schulte et al.	Z. Feldherpetol. 15: 139-156 (2008)
HQ652892	Darmstadt	GERMANY	Schulte et al.	Z. Feldherpetol. 15: 139-156 (2008)
HQ652908	Stuttgart-Wangen	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
HQ652876	Schloß Holte-Stukenbrock	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
HQ652880	Duisburg-Hüttenheim	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
HQ652886	Ammelshain	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
HQ652887	Ammelshain	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–

				211 (2012)
HQ652921	Lörrach	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
HQ652920	Lörrach	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
HQ652958	Halle an der Saale	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
HQ652874	Bramsche, Ueffeln	GERMANY	Schulte et al.	Z. Feldherpetol. 15: 139-156 (2008)
HQ652917	-	GERMANY	Schulte et al.	Z. Feldherpetol. 15: 139-156 (2008)
HQ652919	Inzlingen	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
HQ652875	Bielefeld	GERMANY	Schulte et al.	Z. Feldherpetol. 15: 139-156 (2008)
HQ652918	Inzlingen	GERMANY	Schulte et al.	Global Ecol. Biogeogr. 21: 198–211 (2012)
AF248007	Souprosse	FRANCE	Surget-Groba et al.	Mol. Phylogenet. Evol. 18: 449-459 (2001)
MG851983	Manarola	ITALY	P02_IT	This study
MG851982	Riomaggiore	ITALY	P01_IT	This study
MG851981	Ceresole d'Alba, Torino	ITALY	P47_IT	This study
MG851978	Šume	BOSNIA AND HERZEGOVINA	P36_BA	This study
MG851980	NP Llogare	ALBANIA	P31_AL	This study
MG851963	Ropotamo	BULGARIA	612_BG	This study
MG851964	Bjala Voda	BULGARIA	838_BG	This study

Additional file 2: Figure S1. Bayesian phylogeny of Podarcis muralis based on a fragment (411 bp-long) of mtDNA (cytb) showing schematically overal genetic diversity of the species and details of the Central Balkan clade. Numbers at nodes show Bayesian posterior probabilities and ML bootstrap support values.



Příloha 4.

Köhler, G., **Veselý, M.** & Greenbaum, E., 2006: *The Amphibians and Reptiles of El Salvador*. Krieger Publishing Company, Malabar, 238pp. ISBN 1-57524-252-4

The contribution in words:

GK and MV conceived the study and designed the book. GK and MV carried out the fieldwork, analysed specimens in museum collections and wrote substantial part of the book. EG added and updated some species accounts and provided language correcture. All authors contributed critically to the final draft of the book.

Citace na WoS (Biosis collection; bez autocitací všech spoluautorů): 27
Citace celkem (Google Scholar): 53

THE AMPHIBIANS AND REPTILES OF EL SALVADOR

Gunther Köhler, Milan Veselý, and Eli Greenbaum

About the Book

As presently understood, the herpetofauna of El Salvador consists of 130 species representing 88 genera and 30 families. For each of these species the following information is provided: (1) a partial synonymy, including reference, the current name, and references to the species in El Salvador; (2) the total geographic distribution; (3) ecological distribution in El Salvador; (4) a short description of the morphology; (5) natural history and taxonomic comments; (6) conservation status of evaluated species; and (7) a list of Salvadoran specimens examined and their locality data. Distribution maps and color photographs are provided for each species. Dichotomous keys for the identification of the orders, genera, and species of Salvadoran amphibians (including tadpoles) and reptiles are provided in English and Spanish.

About the Authors

Gunther Köhler, PhD, is the head of the Department of Herpetology at the Research Institute Senckenberg in Frankfurt am Main, Germany, and director of the Conservation Project Utila Iguana, Honduras. To date, he has produced hundreds of popular articles, scientific articles, and books on herpetology, most of which pertain to Central American amphibians and reptiles.

Milan Veselý, PhD, RNDr, is an assistant professor in the Department of Zoology at the Palacky University in Olomouc, Czech Republic, and vice president of the Czech Herpetological Society. He has published several scientific papers on reptile ecology, taxonomy, and parasites.

Eli Greenbaum is a PhD candidate in the Department of Ecology and Evolutionary Biology at the University of Kansas. He received degrees from Binghamton University (BS) and University of Louisiana at Monroe (MS). He has published several scientific papers on turtle development, viper behavior and venom biochemistry, and taxonomy of amphibians and reptiles from Central America and West Africa.

KRIEGER PUBLISHING COMPANY
Malabar, Florida

ISBN 1-57524-252-4
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Cover Photos:
Background—Cloud forest at Volcán de San Vicente (2000 m), Depto. San Vicente, El Salvador. (Photo by M. Veselý)
Top Center—*Bufo marinus* (KU 289750) at Parque National El Imposible (720 m), Depto. Ahuachapán, El Salvador. (Photo by E. Greenbaum)
Bottom Center—*Ninia sebae* (KU 289803) at Colegio Bautista, San Salvador, Depto. San Salvador, El Salvador. Gravid female. (Photo by E. Greenbaum)

Original Edition 2006

Printed and Published by
KRIEGER PUBLISHING COMPANY
KRIEGER DRIVE
MALABAR, FLORIDA 32950

Printed in China.

Library of Congress Cataloging-in Publication Data

Köhler, Gunther.
The amphibians and reptiles of El Salvador / Gunther Köhler, Milan Veselý, Eli Greenbaum.
p. cm.
Includes bibliographical references (p.) and index.
ISBN 1-57524-252-4 (alk. paper)
1. Reptiles—El Salvador. 2. Amphibians—El Salvador. I. Köhler, Gunther. II. Veselý, Milan. III. Greenbaum, Eli. IV. Title.

QL656.S2K64 2006
597.9!097284—dc22

2005045137

10 9 8 7 6 5 4 3 2

Contents

Abstract

As presently understood, the herpetofauna of El Salvador consists of 130 species representing 88 genera and 30 families. For each of these species the following information is provided: (1) a partial synonymy, including reference, the current name, and references to the species in El Salvador; (2) the total geographic distribution; (3) ecological distribution in El Salvador; (4) a short description of the morphology; (5) natural history and taxonomic comments; (6) conservation status of evaluated species; and (7) a list of Salvadoran specimens examined and their locality data. Distribution maps and color photographs are provided for each species. Dichotomous keys for the identification of the orders, genera, and species of Salvadoran amphibians (including tadpoles) and reptiles are provided in English and Spanish.

Resumen

Actualmente, se considera que la herpetofauna de El Salvador consta de 130 especies, incluídas en 88 géneros y 30 familias. Para cada una de esas especies se proporciona la siguiente información: (1) sinonimia parcial, incluyendo el nombre común, referencias de las especies en El Salvador y citas bibliográficas; (2) la distribución geográfica total; (3) distribución ecológica en El Salvador; (4) una breve descripción morfológica; (5) comentarios taxónomicos y sobre historia natural; (6) estado de conservación de las especies evaluadas y (7) una lista de los ejemplares examinados con su respectiva localidad de colección en El Salvador. Además, el libro contiene fotografías a color y mapas de distribucion para cada especie y claves dicotómicas en inglés y español para la identificación de géneros y especies de anfibios (renacuajos y adultos) y reptiles.

Chapter 5
Class Amphibia

Amphibians are tetrapods with glandular skin, which is not covered with scales as it is in reptiles. Although each of the three orders of Lissamphibia (Gymnophiona [caecilians], Caudata [salamanders], and Anura [frogs and toads]) is ancient (up to 350 mya), the phylogenetic relationships of each group to the others remains contentious (Duellman 2003a, D. Wake 2003a). Amphibians lack embryonic membranes and must reproduce in moist or aquatic habitats; these vertebrates can give live birth, lay eggs that hatch into free-swimming larvae, or lay eggs that contain direct-developing young. This group is distributed throughout the world except for the Arctic and Antarctic regions (Duellman 2003a). Adults range in size from tiny frogs (10.2 mm) to giant salamanders (150 cm). Many new species of amphibians are still being described, but the most recent count lists approximately 5800 species of caecilians, frogs, and salamanders in 44 families (Biju & Bossuyt 2003, Duellman 2003b, D. Wake 2003a, M. Wake 2003a, AmphibiaWeb 2005).

Key to Adults of the Orders of Amphibians

1 a. Limbs absent .... **Gymnophiona**
b. Limbs present **2**
2 a. Tail absent .... **Anura**
b. Tail present .... **Caudata**

Clave para Determinar el Orden de Anfibios Adultos

1 a. Extremidades ausentes .... **Gymnophiona**
b. Extremidades presentes **2**
2 a. Cola ausente .... **Anura**
b. Cola presente .... **Caudata**

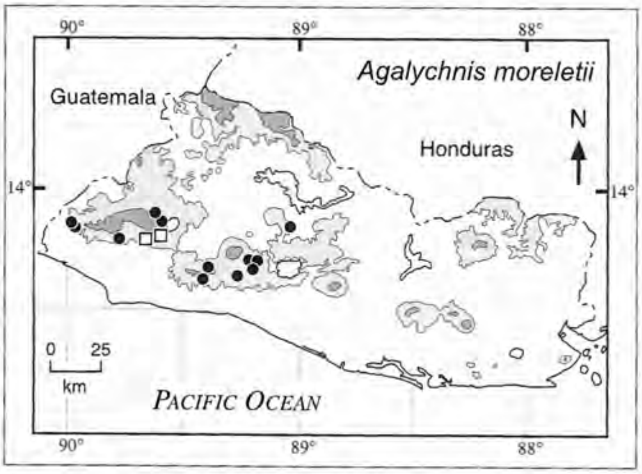
Order Gymnophiona (Caecilians)

The five families of caecilians (ca. 165 species in 33 genera) are distributed in the tropics throughout the world except for Madagascar and the Australo-Papuan region (M. Wake 2003a). Resembling earthworms, these fossorial and aquatic creatures use a tentacle between the eye and nostril to assist in locating invertebrate and small vertebrate prey (Zug et al. 2001). Adult caecilians range in size from 115 mm to 160 cm long. Although most structures of the eye have been lost, most caecilians can distinguish between light and dark. This group can exude a toxic substance through their skin to discourage predators. Depending on the family, caecilians can lay eggs or give live birth (M. Wake 2003a). One family is represented in El Salvador.

Family Caeciliidae

According to M. Wake (2003b) this family includes 26 genera in 107 species, but the family Typhlonectidae (*sensu* Wilkinson 1996), is not recognized as a separate group. Distributed from Mexico to Argentina in the New World, islands off the coast of West and East Africa, India, Sri Lanka, Bangladesh, southern China through the Malay Peninsula, and East Indies to the southern Philippines (McCranie & Wilson 2002, M. Wake 2003b). These caecilians are egg layers with free-swimming larvae that have small gills and tail fins (M. Wake 2003a). Only one genus occurs in El Salvador.

Plate 22: *Agalychnis moreletii* (KU 289773–75) EL SALVADOR: Santa Ana: Finca El Milagro (1300 m). Pair in amplexus. Photo: E. Greenbaum.



Map 14: Distribution of *Agalychnis moreletii* in El Salvador.

Closed circles represent examined-specimen locality records; open squares are published locality records. Light shading indicates 600–1200 m elevation; dark shading indicates elevations ≥ 1200 m; large bodies of freshwater are outlined in bold, black lines.

Agalychnis moreletii: Mertens 1952d, Duellman 1970, 2001, Villa et al. 1988, Campbell 1998a, Dueñas et al. 2001, D. Frost 2002, 2004, McCranie & Wilson 2002, Leenders 2003, Leenders & Watkins-Colwell 2004.

Geographic distribution: From northeastern Puebla and south-central Veracruz, Mexico, to northwestern Honduras on the Atlantic versant; from south-central Guerrero, Mexico, to central El Salvador on the Pacific versant (McCranie & Wilson 2002).

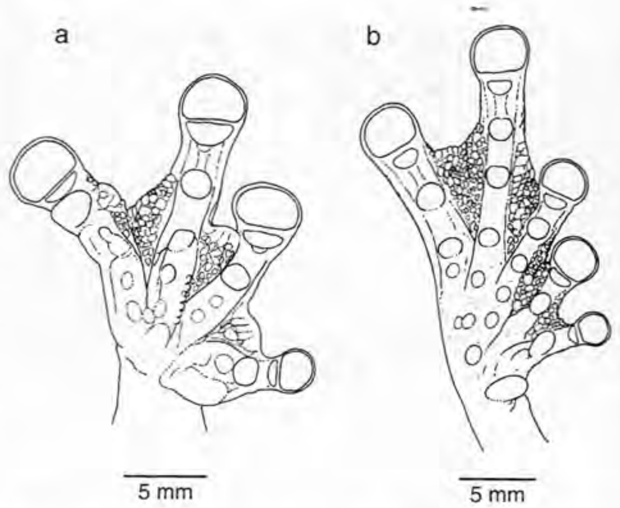


Figure 5: *Agalychnis moreletii* (SMF 78932): a. ventral view of right hand, b. ventral view of right foot.

Ecological distribution: Dry forest, premontane evergreen forest, and subtropical humid forest, usually near streams, ponds, and lakes at elevations of 200–1795 m.

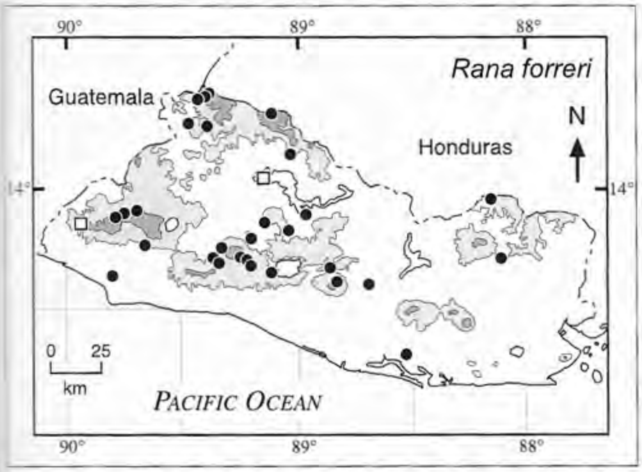
Description: Largest Salvadoran specimen is 75.0 mm SVL (Leenders & Watkins-Colwell 2004); HW/SVL 0.31–0.37; HL/SVL 0.33–0.37; shank length/SVL 0.43–0.51; foot length/SVL 0.36–0.39; tympanum diameter/SVL 0.05–0.06. Snout acuminate in dorsal aspect, sloping in profile; top of head flat or slightly concave, nostrils directed laterally, situated about two thirds of the distance between eyes and tip of snout; canthus rostralis rounded; loreal area concave; supratympanic fold present, extending from the posterior edge of eye around the upper and posterior margin of ear, almost touching angle of jaw; tympanum visible. Forelimbs robust, large; rounded disc on all fingers, equal or slightly larger than tympanum (except digit I is smaller); subarticular tubercles medium-sized and rounded, the distal ones on digits III and IV bifid; no distinct palmar tubercle; thumb pad in sexually active males bearing brown nuptial excrescences dorsally; relative length of fingers I < II < IV < III, finger webbing formula I $2^- - 2$ II $1\frac{1}{2} - 2^+$ III $2^- - 2^-$ IV. Hind limbs slender; weak dermal ridge extending along posterior ventrolateral edge of tarsus; inner tarsal fold generally present, but sometimes barely visible; relative length of toes I < II < III < V < IV; terminal disc slightly increasing in size from toe I to toe V; subarticular tubercles moderately large, rounded; inner metatarsal tubercle distinctly keeled; modal webbing formula of toes I 2^{-} – 2 II 1^{+} – 3 III 3^{-} – 3 IV 3^{-} – 1 V; skin on dorsum, head and dorsal surfaces of limbs smooth, skin on belly and ventral surface of thighs granular; 10–16 vomerine teeth on small, widely separated processes between choanae; vocal sac single, median.

In life, dorsum bright green with or without several white flecks; venter creamy yellow; flanks, anterior and posterior surfaces of thighs, inner surface of shanks, tarsi and forearms, upper arms, first three fingers and first four toes orange; stripes on the outer edges of forearm and tarsi white; iris dull red or maroon; palpebral membrane reticulated with gold. In preserved specimens, dorsum dark to pale blue; the rest creamy white. White flecks on dorsum remain without color changes.
Call: The call of this species resembles "woorp" which may be repeated at intervals of one to several minutes. The notes last for 0.09–0.27 second, and have a pulse rate of 55–61 pulses per second; each note consists of 3–6 pulses. The frequency spectrum is 1000–7500 Hz (Duellman 1970, 2001).
Natural history: E. Greenbaum (field notes) recorded great numbers of this species calling and in amplexus in man-made water basins (i.e., "pilas") in coffee plantations at Finca El Milagro, Depto. Santa Ana at night in the rainy season; *Physalaemus pustulosus* were breeding in these same pools. Mertens (1952d) described how these frogs sleep while attached to green leaves during the day. According to his observations, the calls of male *Agalychnis moreletii* are heard only during the rainy season. On 7 September, he found the egg masses (each the size of a human thumb and containing 60–200 eggs) between the leaves of *Gliricidia sepium* overhanging water. About eight days after egg deposition, the larvae hatched and dropped or jumped into the water beneath the leaves (Mertens 1952d). Campbell (1998a) noted breeding from May–August; females lay 25–75 greenish and cream eggs per clutch. McCranie & Wilson (2002) recorded adults on vegetation overhanging a lake in Honduras. Leenders (2003) commented that this species is often found at night near man-made water basins and natural pools at Parque Nacional El Imposible, Depto. Ahuachapán. Leenders & Watkins-Colwell (2004) noted calling males in January, February, October, and November; gravid females were noted in January and February in El Salvador. Metamorphs were found in January and November suggesting this species breeds year-round in El Salvador.
Conservation status: GAA: Critically endangered (CR A3e).
Specimens examined: Ahuachapán: Finca Concepción Miramar, 13°48.46′N, 89°48.03′W, 920 m: KU 289920 (tadpoles); El Refugio, vicinity of Mariposario of Francisco Serrano, 13°49.46′N, 89°59.98′W, 225 m: SMF 78932; Parque Nacional El Imposible, La Fincona, 13°50.8′N, 89°58.8′W, 720 m: SMF 81328–29. Cuscatlán: Tenancingo, Cantón El Tablón, 1500 m: KU 184724. La Libertad: near Finca Los Angeles, La Cumbre, 1500 m: SMF 42851–63 (tadpoles), 42931 (tadpole), 43122–30 (juv.); Finca El Paraíso near Nuevo San Salvador [= Santa Tecla]: SMF 42204, 42668–71, 42673, 42682–99 (tadpoles), 42700–01, 42703, 42714, 42716, 42717–91 (tadpoles), 42818–42 (tadpoles), 42935, 43121; Finca La Giralda, 13°39.34′N, 89°22.47′W, 1080 m: KU 289955, 289963, 289964 (tadpoles). San Salvador: San Antonio near San Salvador: SMF 43162; San Salvador, Instituto Tropical de Investigaciones Científicas: SMF 49624; San Salvador: ZMB 35723. Santa Ana: Volcán de Santa Ana, Finca Los Andes, 13°52.1′N, 89°37.2′W: MUHNES 1215, SMF 81330, VH 75; Finca El Milagro, 13°53.29′N, 89°37.17′W, 1300 m: KU 289773–78, 289782 (tadpoles), 289785, 290050, MUHNES C–30–1422–28.
Published locality records: Ahuachapán: Parque Nacional El Imposible: YPM 6414, 6422–23, 6457, 7222, 7286–89 (Leenders & Watkins-Colwell 2004); Munícipio San Pedro Puxtla, Cantón La Concepción, Cooperativa Concepción Miramar (13.810°N, 89.807°W), 950 m (Leenders & Watkins-Colwell 2004). Santa Ana: Munícipio Santa Ana, Cantón Los Flores, Parque Nacional Los Andes and Volcán Santa Ana (13.869°N, 89.620°W), 1795 m: YPM 7249–64, 7291 (Leenders & Watkins-Colwell 2004). Sonsonate: Munícipio Izalco, Cantón Cruz Grande, Finca Nuevos Horizontes (13.821°N, 89.653°W), 1250 m: YPM 6458 (Leenders & Watkins-Colwell 2004).

Genus *Hyla*

This very large (ca. 334 species) genus of treefrogs is distributed from Canada to Argentina in the New World, the Greater Antilles, extreme northwestern Africa, eastern Asia, and southern and central Europe (D. Frost 2004). Only one species is known from El Salvador.

Plate 40: *Rana forreri* (KU 289994) EL SALVADOR: Cabañas: Bosque Cinquera (400 m). Photo: E. Greenbaum.



Map 32: Distribution of *Rana forreri* in El Salvador. Closed circles represent examined-specimen locality records; open squares are published locality records. Light shading indicates 600–1200 m elevation; dark shading indicates elevations ≥ 1200 m; large bodies of freshwater are outlined in bold, black lines.

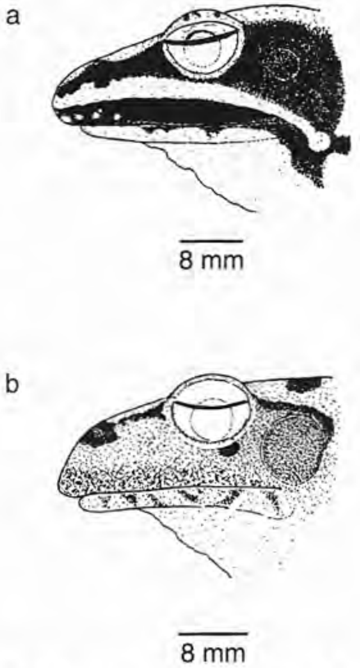


Figure 14: Lateral view of head of a. *Rana maculata* (SMF 81280); b. *Rana forreri* (SMF 81269).

Rana forreri: Villa et al. 1988, Dueñas et al. 2001, Leenders 2003.

Geographic distribution: From southern Sonora, Mexico, south along the Pacific coast to northwestern Costa Rica (McCranie & Wilson 2002).

Ecological distribution: Dry forest, premontane evergreen forest, subtropical humid forest, and pine-oak forest between sea level and 1960 m.

Description: Largest Salvadoran specimen examined is 94.4 mm SVL; HW/SVL 0.29–0.47; HL/SVL 0.28–0.52; shank length/SVL 0.49–0.66; foot length/SVL 0.49–0.95; tympanum diameter/SVL 0.06–0.11. Snout acutely round, tip of snout blunt in dorsal aspect, sloping to rounded in profile; top of the head flat, nostrils directed dorsolaterally, situated about 3/5 of distance from orbit to tip of snout; canthus rostralis rounded, indistinct, loreal region slightly concave, more or less distinct skin fold extending along the upper jaw to a point above the insertion of arms, in adult males interrupted by vocal sacs; tympanum large, distinct; forelimbs moderately short, robust, fingers with small pads on tips, relative length of fingers II < I < IV < III, webbing vestigial, subarticular tubercles round, subconical, palmar tubercle flat, pollical tubercle ovoid, in breeding males enlarged pollex bearing nuptial excrescence; hind limbs long and robust, distinct transverse dermal fold on the heels; toes slender, long, with small pads on the tips, relative length of toes I < II < V < III < IV, modal webbing formula of toes I 1^{+} – 2^{+} II 1^{+} – 1½ III 1^{+} – 2^{+} IV 2 – 1 V; subarticular tubercles large, rounded, inner metatarsal tubercle large, elongated, spade-like; outer metatarsal tubercle rounded and subconical, equal in size to subarticular tubercles; distinct inner tarsal fold present; skin on dorsum smooth with two dorsolateral folds that are continuous, posterior portion not inset medially; irregular shorter parallel folds scattered between them; ventral skin

Chapter 6
Class Reptilia

Reptiles are tetrapods that have adapted to life on land by evolving cornified scales, plates, or scutes on their bodies to prevent water loss. In contrast to amphibians that must live and breed in or near water, reptiles have amniotic eggs that allow them to reproduce far from sources of water (Chiszar & Smith 2003). Although the oldest-known reptiles (up to 270 mya) are not quite as ancient as the amphibians (up to 350 mya), the reptiles have evolved more species and occupy every corner of the globe except for the Arctic and Antarctic regions. Most reptiles are oviparous, but some groups are viviparous or ovoviviparous. In contrast to the amphibians, reptiles have invaded the marine environment, evolved venom-conducting fangs, and in some cases, are the top predators in their ecosystems (e.g., *Varanus komodoensis).* Major groups of reptiles include turtles, archosaurs (crocodilians and birds), and lepidosaurs (tuataras, amphisbaenians, lizards, and snakes). Although the phylogenetic positions of some reptile groups are well supported (e.g., *Sphenodon* is a basal lepidosaur), others remain contentious (e.g., phylogenetic position of turtles). Adults range in size from tiny geckos (< 30 mm) (Hedges & Thomas 2001) to long pythons and anacondas (ca. 10 m). Many new species of reptiles are still being described, but the most recent count lists approximately 7518 species of reptiles in 60 families (Zug et al. 2001, Baker 2003a, Britton 2003, Chiszar & Smith 2003, Pianka 2003a, Towns 2003).

Key to the Major Groups of Reptiles in El Salvador

1 a. Limbs absent .... **Serpentes**
b. Limbs present **2**
2 a. Most of body enclosed in a bony shell .... **Testudines**
b. Bony shell absent **3**
3 a. Thick, rectangular, upward pointing scales on the tail; massive jaws with socketed teeth .... **Crocodylia**
b. Tails lacking thick, rectangular, upward pointing scales; jaws lacking socketed teeth .... **Sauria**

Clave para los Grupos Mayores de Reptiles en El Salvador

1 a. Extremidades ausentes .... **Serpentes**
b. Extremidades presentes **2**
2 a. Mayor parte del cuerpo dentro de un caparazón óseo .... **Testudines**
b. Caparazón óseo ausente **3**
3 a. Cola con escamas gruesas, rectangulares, con puntas dirigidas hacia arriba formando serie de cretas; dientes incrustados en huecos de la maxila y mandíbula; maxila y mandíbula robustas .... **Crocodylia**
b. Cola sin escamas gruesas, rectangulares, que forman crestas; maxila y mandíbula sin huecos para la incrustación de dientes .... **Sauria**

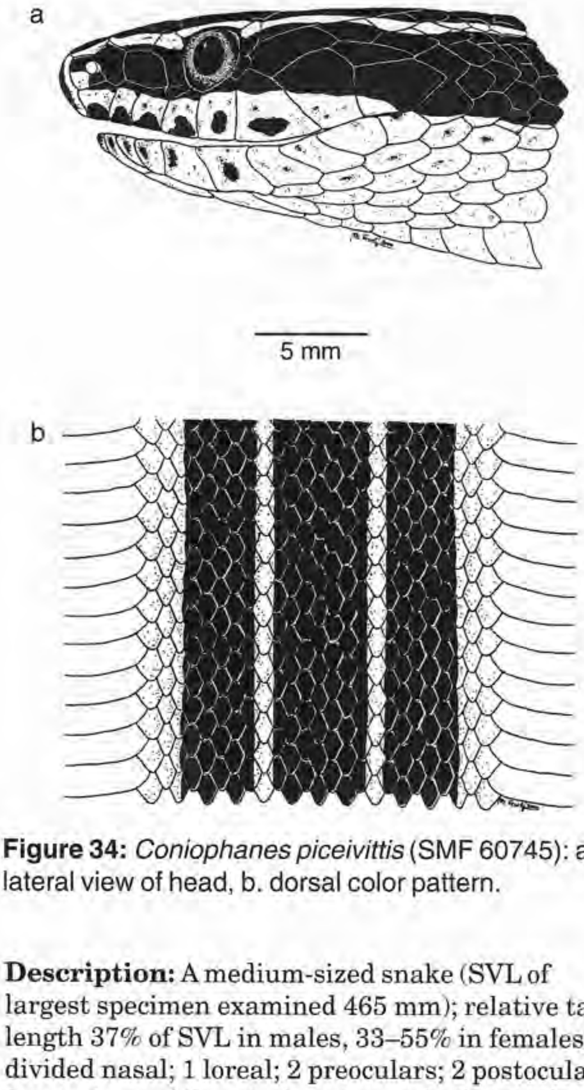


Figure 34: *Coniophanes piceivittis* (SMF 60745): a. lateral view of head, b. dorsal color pattern.

Description: A medium-sized snake (SVL of largest specimen examined 465 mm); relative tail length 37% of SVL in males, 33–55% in females; divided nasal; 1 loreal; 2 preoculars; 2 postoculars; 1 anterior and 2 posterior temporals; 8 supralabials, with the 4th and 5th entering orbit; dorsal scales smooth, without apical pits, in 25 rows at midbody, and 19–21 rows one head length anterior to vent; ventrals 165 in males, 162–170 in females; subcaudals 91 in males, 82–88 in females; anal plate divided.

Head dark brown dorsally and laterally, except lower portions of supralabials; infralabials and chin region are cream with some brown mottling; a narrow yellow stripe extends from rostral along canthus rostralis and above eye to upper temporal region (may be discontinuous); dorsal ground color dark brown with a pair of dorsolateral yellow (cream in preservative) longitudinal stripes (on all of dorsal scale row 9 and 1/2 to 2/3 of adjacent scale rows) beginning 3–4 scales behind parietals; dark brown lateral coloration reaches to 3rd dorsal scale row where it covers about half of the scales; all scale rows 1 and 2 and adjacent part of the 3rd dorsal scale row yellowish to cream as are the ventrals; venter immaculate or with few scatte brown dots.

Natural history: O. Komar (field notes) colle an adult female with eggs among rubbish at a plantation at Volcán de San Miguel, Depto. San Miguel in May. Mertens (1952d) reported a spe men found under a rock in El Salvador.

Specimens examined: Cuscatlán: Tenancingo, Copalchan, El Sitio de los Sánchez: KU 183859; km E Tenancingo, Río Quezalapa, 450 m: KU 183860. San Miguel: Volcán de San Miguel, 740 KU 291285. San Salvador: Doble vía near Parq Cuscatlán: SMF 60745; San Salvador, Ciudad Universitaria, 700 m: KU 183861; San Salvador, CAMRS: USNM 167215; Planes de Renderos: U 5394. Sonsonate: Finca La Joya, 587 m: km 48 San Salvador–Sonsonate: SMF 43187.

Genus *Conophis*

This genus includes two species of mildly venomous colubrids that are distributed from Veracruz, Mexico, to eastern Honduras and the Yucatán Peninsula on the Atlantic versant, and from Nayarit, Mexico, to Costa Rica on the Pac versant (Savage 2002, Köhler 2003b).

Conophis lineatus (Duméril, Bibron & Dum 1854)

(Lagartijera Rayada)
Plate 86, Map 78, Figure 35ab

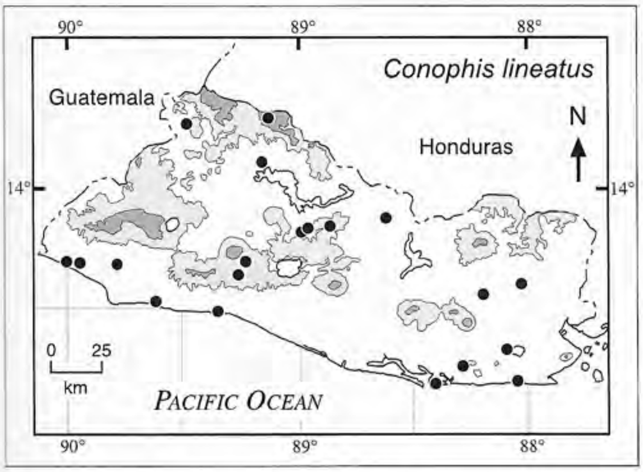
1854 *Tomodon lineatum* Duméril, Bibron & Duméril, Erp. Gén. 7: 936; type locality: Mexic
Conophis lineatus: K. Schmidt 1928, Peters & Orejas-Miranda 1970, Villa et al. 1988, Köhler 1 Dueñas et al. 2001, Köhler 2001a, 2003b, Leend 2003.
Conophis pulcher plagosus: Mertens 1952d.
Conophis lineatus dunni: Wellman 1963.

Geographic distribution: From Veracruz, Mexico, to eastern Honduras on the Atlantic versant, and from Oaxaca, Mexico, to southwest Costa Rica (possibly) on the Pacific versant (Sav 2002).

Ecological distribution: Dry forest and sava between sea level and 1170 m.

Description: A medium-sized snake (SVL of largest specimen examined 818 mm); relative

Plate 86: *Conophis lineatus* (KU 289911) EL SALVADOR: Usulután: Isla San Sebastián (20 m). Photo: E. Greenbaum.



Map 78: Distribution of *Conophis lineatus* in El Salvador.

Closed circles represent examined-specimen locality records; open squares are published locality records. Light shading indicates 600–1200 m elevation; dark shading indicates elevations ≥ 1200 m; large bodies of freshwater are outlined in bold, black lines.

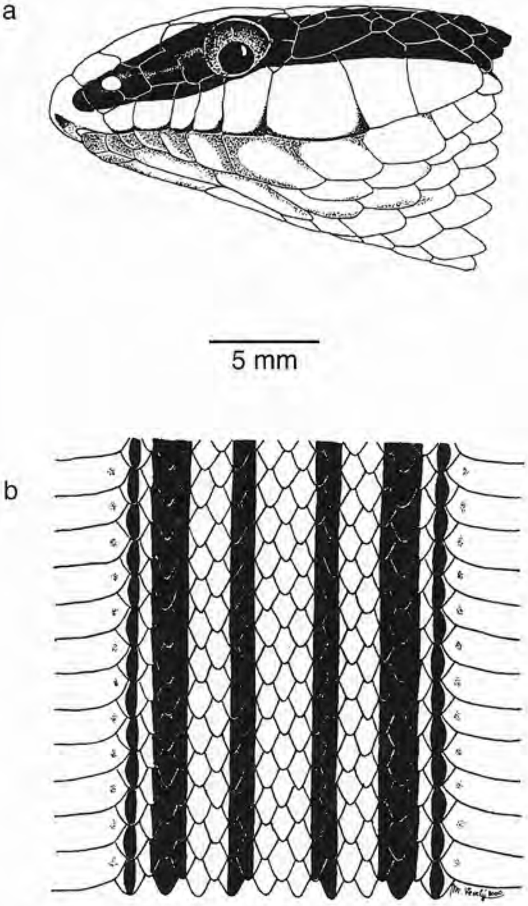


Figure 35: *Conophis lineatus* (SMF 42019): a. lateral view of head, b. dorsal color pattern.

length 26–49% of SVL in males, 21–30% in females; divided nasal; 1 loreal; 1 to 2 preoculars; 2 to 3 postoculars; 1–3 anterior and 2–4 posterior temporals; 8 supralabials, with the 4th and 5th entering orbit; 9–10 infralabials; dorsal scales smooth, without apical pits, in 19 rows at midbody, and 17 rows one head length anterior to vent; ventrals 161–171 in males, 165–178 in females; 58–83 subcaudals in males, 54–74 in females; anal plate divided.

Dorsum pale brown to gray with six dark brown or black longitudinal stripes extending the length of the body and well onto the tail. The first stripe is very narrow and runs through the first dorsal scale row, sometimes it is fragmented. The second stripe involves the 3rd and 4th, or 2nd, 3rd, and 4th dorsal scale rows, and the third (paramedian) stripe involves the 7th and 8th, or 7th, 8th, and 9th dorsal scale rows. The venter is immaculate. Head with a dark brown or black lateral stripe from temporal region to nostril, reaching the edge of the rostral. The paramedian stripes extend across the parietals and along the supraocular-frontal suture to the frontal-prefrontal border.

Natural history: E. Greenbaum (field notes) recorded an adult of this species on a beach near a

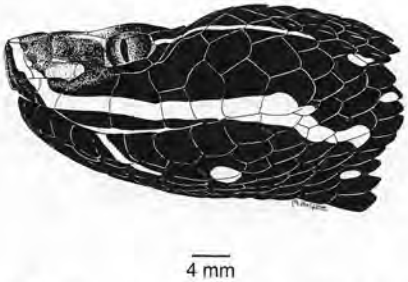


Figure 49. *Agkistrodon bilineatus* (SMF 42218): Lateral view of head.

species feeds upon a wide array of vertebrate and invertebrate prey; females give birth to 5–20 young from June–July. Juveniles use their brightly colored tails to lure anuran prey.
Conservation status: CITES: Deleted 15 December 2002.
Specimens examined: Cuscatlán: 5.2 km ESE Tenancingo, Cantón Ajuluco: KU 183982; Municipio de Colima: KU 291394. Sonsonate: Hacienda San Antonio near Sonsonate, 220 m: SMF 42218.

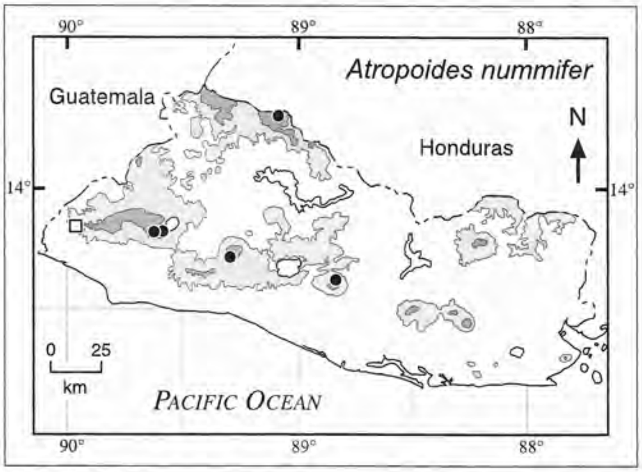
Genus *Atropoides*

The three species of Jumping Vipers are distributed from the highlands of eastern Mexico to Panama (McDiarmid et al. 1999). Castoe et al. (2003) discussed the possible paraphyly of this genus with respect to *Cerrophidion* and *Porthidium.* These authors also recognized that the species diversity within *Atropoides* is probably underestimated at the current time, but they argued against taxonomic changes without additional morphological and molecular data.

Atropoides nummifer **(Rüppell 1845)**
(Timbo)
Plate 137, Map 129

1845 *Atropos nummifer* Rüppell, Verh. Mus. Senckenberg 3: 313; type locality: Mexico.
Bothrops nummifer nummifer: Mertens 1952d.
Bothrops nummifera: Wilson & Meyer 1985, Villa et al. 1988.
Porthidium nummifer: Campbell & Lamar 1989.
Atropoides nummifer: Werman 1992, Campbell 1998a, McDiarmid et al. 1999, Dueñas et al. 2001, Köhler 2001a, Castoe et al. 2003, Köhler 2003b, Leenders 2003, Leenders & Watkins-Colwell 2004.
Atropoides occiduus: Campbell & Lamar 2004.

Plate 137: *Atropoides nummifer* EL SALVADOR: San Vicente: Finca El Carmen (1240 m). Photo: M. Veselý.



Map 129: Distribution of *Atropoides nummifer* in El Salvador.
Closed circles represent examined-specimen locality records; open squares are published locality records. Light shading indicates 600–1200 m elevation; dark shading indicates elevations ≥ 1200 m; large bodies of freshwater are outlined in bold, black lines.

Geographic distribution: San Luis Potosí and Oaxaca, Mexico, to central Panama (McDiarmid et al. 1999).
Ecological distribution: Cloud forest and pine-oak forest between 920 and 1700 m.
Description: A medium-sized snake (SVL of largest specimen 650 mm [Leenders & Watkins-Colwell 2004]); relative tail length 12–14% of SVL in males, 10–12% in females; 8–10 supralabials; 9–12 infralabials; 0–1 nasorostral; 10–12 intersupraoculars; 3 canthals; 9–10 postfoveals; 3–4 subfoveals; 5 prefoveals; 6–8 intercanthals; dorsal scales keeled, tuberculate, in 25–27 rows at mid-

Příloha 5.

Köhler, G., **Veselý, M.,** 2010: A revision of the *Anolis sericeus* complex with the resurrection of *A. wellbornae* and the description of a new species (Squamata: Polychrotidae). *Herpetologica* 66 (2): 186-207.

The contribution in words:
Both authors participated equally on all aspects of the study and contributed critically to the final draft of the manuscript.

Ranking: IF_{2010} – 1.667; **Q1** (Zoology)
Citace na WoS (Core collection; bez autocitací všech spoluautorů): 12
Citace celkem (Google Scholar): 33

Herpetologica, 66(2), 2010, 207–228

A REVISION OF THE *ANOLIS SERICEUS* COMPLEX WITH THE RESURRECTION OF *A. WELLBORNAE* AND THE DESCRIPTION OF A NEW SPECIES (SQUAMATA: POLYCHROTIDAE)

Gunther Köhler[1,3] and Milan Vesely[2]

[1]*Forschungsinstitut und Naturmuseum Senckenberg, Senckenberganlage 25, 60325 Frankfurt a.M., Germany*
[2]*Department of Zoology, Faculty of Natural Sciences, Palacký University, tř. Svobody 26, 77146 Olomouc, Czech Republic*

Abstract: We describe a new species of anole (genus *Anolis*) from Honduras formerly referred to as *Anolis* (or *Norops*) *sericeus*. The new species differs from *A. sericeus* by having a small unilobate hemipenis (large and bilobate in *A. sericeus*) and by having large male and no female dewlaps (small dewlaps present in both sexes in *A. sericeus*). Furthermore, we resurrect *Anolis wellbornae* for the populations of anoles on the Pacific versant of Nuclear Central America formerly referred to as *Anolis* (or *Norops*) *sericeus*. *Anolis wellbornae* differs from *A. sericeus* in the mean values of several scalation and morphometric characters and by having large male and no female dewlaps (small dewlaps present in both sexes in *A. sericeus*). *Anolis wellbornae* differs from the new species described here by having a large bilobate hemipenis (small and unilobate in the new species). We provide an identification key and standardized descriptions of *A. sericeus*, *A. wellbornae*, and the new species described here.

Resumen: Se describe una nueva especie de anolis (género *Anolis*) de Honduras anteriormente referida como *Anolis* (o *Norops*) *sericeus*. La nueva especie difiere de *A. sericeus* en tener un hemipene pequeño y unilobulado (grande y bilobulado en *A. sericeus*) y por tener una papera gular grande en machos adultos y ninguna papera gular en hembras (ambos sexos de *A. sericeus* tienen una papera gular pequeña). Adicionalmente se revalida *Anolis wellbornae* para las poblaciones de anolis de la vertiente del Pacífico de Centroamérica Nuclear anteriormente referidas como *Anolis* (o *Norops*) *sericeus*. *Anolis wellbornae* difiere de *A. sericeus* en los valores medios de ciertas características de escamación y morfometría, además de por tener una papera gular grande en machos adultos y ninguna papera gular en hembras (ambos sexos de *A. sericeus* tienen una papera gular pequeña). *Anolis wellbornae* difiere de la nueva especie aquí descrita en tener un hemipene grande y bilobulado (pequeño y unilobulado en la nueva especie). Se proporciona una clave dicotómica de identificación y descripciones estándar de *A. sericeus*, *A. wellbornae*, y de la nueva especie aquí descrita.

Key words: *Anolis*; New species; Polychrotidae; Reptilia; Squamata

Barbour (1932) confessed that it seems to be "a wicked and sinful affection to describe more anoles." However, the comparative study of hemipenial variation in anoles continues to reveal substantial cryptic diversity (Köhler and Kreutz, 1999; Köhler et al., 2003, 2007). Here, we report our results of a study on the geographic variation in hemipenis morphology as well as on pholidosis and morphometrics in the widespread anole species *Anolis sericeus*.

In 1856, Hallowell described the new species *A. sericeus* based on a specimen in the Academy of Natural Sciences, Philadelphia (ANSP) collection from "El Euceros le Jalapa, Mexico." Barbour (1934: 149) corrected the type locality to "El Encero de Jalapa, Vera Cruz, Mexico." According to Barbour (1934) and Stuart (1963), the holotype of *A. sericeus* is lost. Günther (1859) named *A. sallaei* based on an adult female specimen (now The Natural History Museum, London [BMNH] 1946.8.5.69) and gave the type locality as "Central America." However, Stuart (1955: 25), based on "correspondence with Dr. Parker of the British Museum," stated that the holotype of *sallaei* "almost certainly originated from Veracruz, probably the Jalapa region." In 1861, Hallowell described his new species *A. longicauda* based on a single specimen from "Nicaragua." The taxonomic identity of *A. longicauda* has been uncertain since its original description. By the early 1930s, the holotype of *A. longicauda* (ANSP 7884) was "dried and now unrecognizable" (Barbour, 1934: 150). Cope (1864)

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[3] Correspondence: e-mail, gkoehler@senckenberg.de

added another nominal species, *A. heliactin*, based on a specimen (now ANSP 7914) from "Mexico." Cope (1874) described the new species *A. ustus* based upon two specimens (now BMNH 1946.8.5.60-61) from "Belize." In 1873, Bocourt described *A. jacobi* based on an adult male specimen (now MNHN 2406) from "Veracruz." Boulenger (1885) placed *A. jacobi* in the synonymy of *A. sallaei* but retained *heliactin* and *ustus* as valid species. Ruthven (1933) described *A. kidderi* based on an adult male specimen (MCZ 72851) from "quinta at Merida, Yucatan." In 1940, Ahl described the new subspecies *A. ustus wellbornae* based on an adult male (ZMB 35710) from "San Salvador, El Salvador." Dunn (1930: 18) erroneously stated that *A. sallei* "is the same as *A. palpebrosus*" and referred specimens from western Panama to the former taxon. However, *A. palpebrosus* is a synonym of *A. lemurinus* (see Peters and Donoso-Barros, 1970), and no anoles related to *A. sericeus* are known to occur south of Costa Rica (Lee, 1980). Barbour (1934) regarded *sallaei*, *longicauda*, and *jacobi* as synonyms of *sericeus* but listed *heliactin* and *ustus* as valid species. Smith and Taylor (1950) listed *sallaei* and *jacobi* as synonyms of *sericeus* but listed *heliactin*, *ustus*, and *kidderi* as valid species. Taylor (1952) suggested that *sallaei* is a species distinct from *sericeus*. Stuart (1955) considered *sallaei* to be a synonym of *sericeus* but recognized *kidderi*, *ustus*, and *sericeus* as valid species, the latter divided into two subspecies, *A. s. sericeus* (Caribbean versant from Veracruz to Nicaragua) and *A. s. wellbornae* (Pacific versant of Guatemala). The names *sallaei*, *heliactin*, *jacobi*, and *ustus wellbornae* have remained in the synonymy of *A. sericeus*, whereas *ustus* has been retained as a valid subspecies of *sericeus* until relatively recently (Peters and Donoso-Barros, 1970). Lee (1980) analyzed the geographic variation of anoles allied to *sericeus* and concluded that the "recognition of subspecies cannot be justified on morphological grounds." Thus, he synonymized the nominal species *A. kidderi* and *A. ustus* with *A. sericeus*. Most subsequent authors followed his taxonomic conclusions and recognized the single widespread species *A. sericeus* for the small anoles with strongly keeled ventrals and an orange-yellow male dewlap with a blue or purple central blotch (Köhler, 2003; Savage, 2002; Villa et al., 1988).

Here, we report upon the results of our study of the variation in hemipenial and scalation morphology as well as morphometrics of the anoles commonly referred to as *A.* (or *Norops*) *sericeus*.

Materials and Methods

In evaluating whether multiple species exist within the *A. sericeus* complex, we follow the evolutionary species concept (Simpson, 1961; Wiley, 1978) and operationalize this concept by identifying species based on consistent differences between populations (Frost and Kluge, 1994). For this study, we examined 1614 specimens of *A. sericeus*. A list of these specimens is provided in Appendix I. Abbreviations for museum collections follow those of Leviton et al. (1985), except for ITAH (Instituto Tecnologico Agropecuario de Hidalgo, Huejutla de Reyes, Hidalgo, Mexico), MUHNES (Museo de Historia Natural de El Salvador, San Salvador, El Salvador), MZ-CIQRO (Museo de Zoología, El Colegio de la Frontera Sur, Chetumal, Quintana Roo, Mexico), MZ-UNICACH (Museo Zoológico de la Universidad de Ciencias y Artes del Estrado de Chiapas, Tuxtla Gutiérrez, Chiapas, Mexico), USAC (Universidad de San Carlos de Guatemala, Guatemala City, Guatemala), and JS field numbers, which refer to specimens that will be deposited in the Museo de Ciencias Naturales de la Universidad Centroamericana (UCA), Managua, Nicaragua. GK had the privilege of examining all extant primary types of nominal species regarded as synonyms of *A. sericeus* by previous authors (Lee, 1980; Peters and Donoso-Barros, 1970), except the types of *A. kidderi* and *A. jacobi*, respectively. For the synonymy lists, only the work that cites actual specimens has been included. Nomenclature of scale characters follows that of Köhler (2003). Terminology for hemipenial morphology follows that of Myers et al. (1993) and Savage (1997).

Scale sizes were measured using the ocular micrometer of a stereomicroscope (MZ 12, Leica, Wetzlar, Germany) and were rounded to the nearest 0.01 mm. All other measurements were made using precision calipers and were rounded to the nearest 0.1 mm. Head length was measured from the tip of the snout to the anterior margin of the ear opening. Snout length was measured from the tip of the snout to the anterior border of the orbit. Head width was determined as the distance between the oral ricti. Dorsal and ventral scales were counted at midbody along the midline. Tail height and width were measured at the point reached by the heel of the extended hind leg. Subdigital lamellae were counted on phalanges ii to iv of the fourth toe. We considered the scale directly anterior to the circumnasal to be a prenasal. The capitalized colors and color codes (the latter in parentheses) are those of Smithe (1975–1981). Abbreviations used are 2Canths (number of scales between second canthals), DHL (number of medial dorsal scales in one head length), HL (head length), HW (head width), INL (infralabials), IP (interparietal plate), SAM (scales around midbody), SO (subocular scales), SPL (supralabial scales), SS (supraorbital semicircles), SVL (snout–vent length), TNLS (total number of loreal scales on one side), and VHL (number of medial ventral scales in one head length).

To measure dewlap area, we took photographs of males in life with their dewlaps artificially extended using small forceps. The head portion was magnified and printed and then superimposed on millimetric paper; the total number of millimeter squares contained in the extended dewlap was counted. A straight line was drawn between both the anterior and posterior insertions of the dewlap. The HL on the printout was also determined. We used the following equation to convert the magnified dewlap area to the real size: $X=\left[\left(\sqrt{Y/A}\right)B\right]^2$, where X is the real area of the dewlap in square millimeters, Y is the total area (square millimeters) of the dewlap at a magnified scale, A is the HL measure (millimeters) of the anole at a magnified scale, and B is the HL measure (millimeters) of the anole at the real size.

The three groups defined by the morphology of hemipenis and dewlap were compared using standard univariate and multivariate statistical techniques. The analysis was performed separately for males and females; juveniles (SVL $<$ 35 mm) were excluded. For each of the characteristics of interest, the mean, standard deviation, range, and sample size were determined. The null hypothesis of identical distribution was tested for lengths and quotients of lengths using one-way analysis of variance and for scalation variables using the Kruskal-Wallis test. The Bonferroni criterion for significance was applied: to be considered significant at level α, a P value had to be less than alpha divided by the total number of tests made. Principal component analysis was applied to determine whether differences between the groups made a major contribution to the differences (morphometric and scalational) among the individuals of the pooled samples (Table 2). All variables were standardized to have unit variance. Results were displayed as scatter plots of the first two principal components. All statistical computations were performed with the statistical program package R, version 2.7.1 (R Foundation for Statistical Computing, 2008).

Results

Three distinctly different hemipenial morphotypes are evident in the specimens we examined. In Type A (n = 20 adult males with everted hemipenes), the hemipenis is a relatively large bilobated organ with strongly calyculate surface of lobes. Sulcus spermaticus bifurcates at the base of the apex, and shortly after the bifurcation, the branches open into broad concave areas, one bifurcation on each lobe. Hemipenial truncus is subequal to shorter than the lobes; there is no asulcate ridge and no ridge that reaches across tip of apex. In Type B (n = 25 adult males with everted hemipenes), the hemipenis is also a relatively large bilobated organ with lobes having a strongly calyculate surface. Sulcus spermaticus bifurcates at the base of the apex, and shortly after the bifurcation, the branches open into broad concave areas, one on each lobe. Hemipenial truncus is subequal to longer than the lobes; there is a distinct asulcate ridge but no ridge that reaches across tip of apex. In Type C (n = 95 adult males with everted hemipenes), the hemipenis is much smaller relative to body size compared with Type A and B hemipenes. Also, it is unilobate, and there is only a small calyculate area on the asulcate surface of the lower portion of apex. There is a distinct ridge-like structure emerging from the bifurcation of the sulcus spermaticus and reaching across the tip of apex to the asulcate side. On the sulcate side, this ridge has a median pocket. Sulcus spermaticus bordered by well developed sulcal lips and bifurcating at base of apex; shortly after the bifurcation, the branches open into broad, slightly convex areas on the sides of the medial ridge. Although these three hemipenial types show a parapatric geographical distribution pattern (Fig. 1), variation in hemipenial morphology within these discrete types is negligible both within populations and in a geographical context. Individuals of hemipenis Type A populations also differ from those of Type B and Type C in relative size of the dewlap in both sexes. Type A males have a small dewlap (smaller than 50 mm^2) as have the females of these populations. Type B males have a large dewlap (larger than 100 mm^2), whereas the females of these populations have no dewlap at all. All Type C males have a large dewlap (larger than 100 mm^2), and the females have no dewlap.

The results of the univariate comparison of the three groups are shown in Table 1. Because 23 variables were tested, each in two sexes, the Bonferroni limit for significance at the 0.05 level is $P < 0.05/46 = 0.00109$. The difference between the groups was significant at this level for several variables (Table 1). However, even for the most significant of these variables, there remains a very large overlap in the three groups. The situation with the principal components is similar. The values of principal component 1 are significantly different in the three groups, both for males and for females, but these two principal components in no way enable one to separate the three groups from each other (Fig. 2).

Based on our findings in hemipenis and dewlap morphology, we recognize three species of this complex, named Species A, B, and C as described below.

Species A: Type A hemipenis; male dewlap small (smaller than 50 mm^2), female dewlap approximately same size as male dewlap; distributed on the Atlantic versant of Mexico and on the Yucatán Peninsula (Fig. 1).

Species B: Type B hemipenis; male dewlap large (larger than 100 mm^2), no dewlap in females; distributed on the Pacific versant of Nuclear Central America (northwestern Nicaragua, El Salvador, extreme southern Honduras, and Pacific versant of Guatemala to approximately Mazatenango; Fig. 1).

Species C: Type C hemipenis; male dewlap large (larger than 100 mm^2), no dewlap in females; distributed from Costa Rica across most of Nicaragua (except northwestern corner of the country), Honduras, (except southern portion of the country), along the Caribbean versant of Guatemala and Chiapas, Mexico, to the Isthmus of Tehuantepec where it crosses to the Pacific versant and continues along the Pacific versant of Chiapas and Guatemala to approximately Mazatenango (Fig. 1).

Unfortunately, none of the type specimens mentioned in the introduction are males with their hemipenes everted. However, the type locality data of most of these nominal species allow for a reliable allocation to one of the three species that we distinguish. Thus, on geographic reasons the following taxa (respective type localities in parentheses) are clearly referable to our Species A: *A. sericeus* Hallowell (Jalapa, Veracruz, Mexico); *A. sallaei* Günther (given as "Central America" but "almost certainly originiated from Veracruz, probably the Jalapa region" according to Stuart, 1955: 25); *A. ustus* Cope ("Belize"); *A. jacobi* Bocourt ("Veracruz"); and *A. kidderi* Ruthven ("Quinta at Merida, Yucatan"). Because *A. sericeus* is the oldest available name for this species, our Species A has to be referred to that name and the other nominal species remain in the synonymy of *A. sericeus*. For our Species B, only one name seems to be available: *A. ustus wellbornae* Ahl (type locality: San Salvador, El Salvador). Therefore, we resurrect this name for our Species B. Two names cannot be assigned with certainty to one of the three species recognized by us: *A. heliactin* Cope and *A. longicauda* Hallowell. All that is left of the holotype of *A. heliactin* (ANSP 7914; examined by GK) is a skull with skin and two pieces of trunk of 10- and 12-mm length, respectively. The holotype

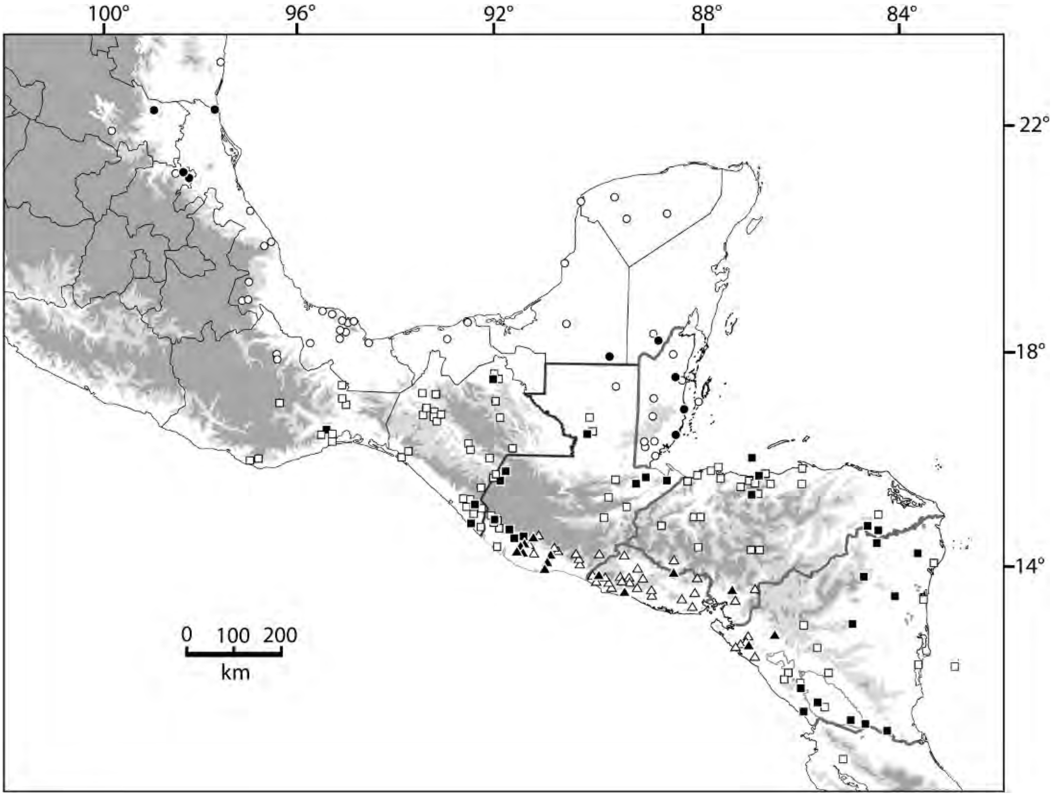

FIG. 1.—Map indicating known collecting sites mentioned in text of Central American anoles formerly referred to as *Anolis sericeus*. Each symbol can represent one or more nearby localities. Areas above 500 and 1000 m are shaded gray. Open symbols: specimens with no everted hemipenis; solid symbols: specimens with everted hemipenis. Circles: Hemipenis Type A; triangles: Hemipenis Type B; squares: Hemipenis Type C.

of *A. longicauda* (ANSP 7884; examined by GK) is a headless skeleton with remains of poorly preserved skin. Because of their poor conditions and unspecific locality data ("Mexico" and "Nicaragua," respectively), the taxonomic identities of these two names cannot be determined. Therefore, we consider *A. heliactin* and *A. longicauda* to be nomina dubia. Interestingly, there is no available scientific name for our Species C. We therefore describe it as a new species below.

Anolis sericeus *Hallowell*

Anolis sericeus Hallowell, 1856: 227. Holotype (now lost; Barbour, 1934) from "El Euceros le Jalapa, Mexico." Taylor (1949), Smith and Taylor (1950; in part), Martin (1958), Neill and Allen (1959), Stuart (1963; in part), Lee (1980; in part), Lee (1996; in part).

Anolis ustus Cope, 1864: 172. Syntypes males (BMNH 1946.8.5.60-61) from "Belize." Boulenger (1885; in part), Schmidt (1941), Smith and Taylor (1950), Stuart (1963).

Anolis jacobi Bocourt, 1873: 73. Holotype male (MNHN 2406) from "Veracruz." Brygoo (1989).

Anolis sallaei Günther, 1859: 421. Holotype female BMNH (1946.8.5.69) from "Central America." Boulenger (1885; in part), Taylor (1952, 1953).

Anolis kidderi Ruthven, 1933: 1. Holotype male (MCZ 72851) from "quinta at Merida, Yucatan."

Anolis sericeus sericeus: Stuart (1955; in part).

Anolis sericeus ustus: Duellman (1965).

Diagnosis.—A medium-sized species (SVL in largest specimen 50.0 mm) of the genus *Anolis* (sensu Poe, 2004) that differs from all

TABLE 1.—Selected characteristics of *Anolis sericeus*, *A. wellbornae*, and *A. unilobatus* (specimens listed in Appendix I): range, mean ± SD, sample size, results from analysis of variance or Kruskal-Wallis tests. Significance at the 0.05, 0.01, and 0.001 levels after Bonferroni correction for multiple testing are indicated by one, two, or three asterisks (*, **, ***). N.S. indicates no significant differences exist. For abbreviations, see text.

Character	Sex	*A. sericeus*	*A. wellbornae*	*A. unilobatus*	Analysis of variance/ Kruskal-Wallis
Maximum SVL	Male	46.5	49.5	49.0	
	Female	50.0	44.5	50.5	
Tail length/SVL	Male	1.94–2.54 (2.24 ± 0.17); 18	1.95–2.44 (2.19 ± 0.14); 35	1.95–2.72 (2.32 ± 0.17); 65	***
	Female	1.57–2.50 (2.06 ± 0.37); 13	1.57–2.24 (1.93 ± 0.28); 16	1.70–2.68 (2.20 ± 0.38); 37	***
Tail diameter vertical/horizontal	Male	1.00–1.50 (1.19 ± 0.12); 30	1.00–1.43 (1.26 ± 0.10); 51	0.77–1.53 (1.23 ± 0.10); 101	*
	Female	0.91–1.27 (1.10 ± 0.14); 16	0.74–1.44 (1.19 ± 0.27); 28	0.80–1.46 (1.21 ± 0.25); 63	**
HL/SVL	Male	0.25–0.30 (0.27 ± 0.01); 30	0.25–0.29 (0.26 ± 0.01); 61	0.23–0.29 (0.26 ± 0.01); 109	N.S.
	Female	0.24–0.29 (0.25 ± 0.02); 16	0.23–0.28 (0.26 ± 0.02); 29	0.24–0.29 (0.26 ± 0.01); 66	N.S.
HL/HW	Male	1.56–1.85 (1.70 ± 0.07); 30	1.51–1.82 (1.69 ± 0.07); 61	1.52–1.96 (1.70 ± 0.07); 109	N.S.
	Female	1.49–1.82 (1.68 ± 0.13); 16	1.47–1.83 (1.63 ± 0.14); 29	1.33–1.91 (1.69 ± 0.22); 66	**
Ear/IP	Male	0.18–0.49 (0.37 ± 0.09); 29	0.27–0.66 (0.45 ± 0.10); 53	0.18–0.83 (0.44 ± 0.13); 90	*
	Female	0.26–0.53 (0.38 ± 0.12); 15	0.18–0.68 (0.43 ± 0.20); 28	0.19–0.56 (0.42 ± 0.15); 53	N.S.
Shank length/SVL	Male	0.20–0.25 (0.22 ± 0.02); 31	0.21–0.25 (0.23 ± 0.01); 61	0.19–0.25 (0.22 ± 0.01); 106	*
	Female	0.19–0.24 (0.21 ± 0.02); 16	0.20–0.26 (0.22 ± 0.03); 29	0.11–0.24 (0.21 ± 0.05); 66	N.S.
Axilla–groin distance/SVL	Male	0.37–0.48 (0.42 ± 0.03); 31	0.27–0.49 (0.41 ± 0.03); 61	0.37–0.47 (0.42 ± 0.02); 106	N.S.
	Female	0.36–0.48 (0.44 ± 0.05); 16	0.41–0.49 (0.44 ± 0.03); 29	0.36–0.50 (0.44 ± 0.05); 66	N.S.
Subdigital lamellae of fourth toe		22–25 (23.54 ± 1.54); 47	20–27 (23.49 ± 1.35); 90	19–28 (23.56 ± 1.28); 174	N.S.
No. of scales between SS		0–2 (0.62 ± 0.57); 47	0–2 (0.57 ± 0.56); 90	0–2 (0.62 ± 0.61); 174	N.S.
No. of scales between IP and SS		0–2 (1.29 ± 0.62); 47	1–4 (1.98 ± 0.51); 90	0–4 (1.90 ± 0.59); 174	***
No. of scales between SO and SPL		0	0	0	N.S.
No. of SPL to level below center of eye		5–8 (6.79 ± 0.76); 47	6–9 (6.70 ± 0.64); 90	5–9 (6.77 ± 0.68); 174	N.S.
No. of INL to level below center of eye		6–8 (6.92 ± 0.49); 47	5–8 (6.69 ± 0.61); 90	5–8 (6.70 ± 0.61); 174	N.S.
Total no. of loreals		18–27 (22.61 ± 2.45); 47	18–39 (26.60 ± 4.60); 90	12–39 (25.64 ± 4.75); 174	*
No. of horizontal loreal scale rows		4–7 (5.10 ± 0.67); 47	4–7 (5.47 ± 0.63); 90	4–7 (5.35 ± 0.59); 174	N.S.
No. of postrostrals		5–6 (5.22 ± 0.43); 47	5–7 (5.72 ± 0.51); 90	4–7 (5.72 ± 0.61); 174	*
No. of postmentals		4–6 (4.68 ± 0.86); 47	4–7 (5.83 ± 0.53); 90	4–8 (5.69 ± 0.72); 174	***
No. of scales between nasals		5–8 (6.45 ± 0.75); 47	4–9 (6.81 ± 1.00); 90	5–8 (6.77 ± 0.91); 174	N.S.
No. of scales between second canthals		5–9 (6.66 ± 1.01); 47	5–10 (7.40 ± 0.90); 90	5–11 (7.76 ± 1.12); 174	*
No. of scales between posterior canthals		5–10 (8.11 ± 1.23); 47	7–12 (9.19 ± 1.14); 90	7–12 (9.16 ± 1.15); 174	*
DHL		20–40 (30.62 ± 5.03); 46	25–38 (30.02 ± 2.85); 88	23–44 (30.74 ± 3.54); 173	N.S.
VHL		14–34 (24.30 ± 4.71); 46	16–29 (23.11 ± 2.86); 88	16–32 (23.31 ± 3.03); 173	N.S.
SAM		93–114 (103.90 ± 5.96); 20	97–133 (114.26 ± 8.70); 29	80–137 (107.43 ± 12.66); 117	*

Table 2.—Principle Component Analysis variable loadings for the first and second principal components. For abbreviations, see text.

	PC1		PC2	
	Males	Females	Males	Females
SVL	0.325	0.402	−0.375	−0.062
HL/SVL	0.253	−0.407	0.081	0.224
Shank length/SVL	−0.098	−0.311	0.215	0.001
HL.HW	−0.277	0.155	−0.348	0.204
Tail diameter vertical/ horizontal	0.224	−0.067	−0.091	−0.132
HW/SVL	0.048	−0.417	0.400	0.015
Axilla–groin distance/SVL	0.129	0.274	−0.016	−0.187
Snout length/SVL	0.043	0.031	−0.176	0.092
VHL	−0.061	−0.286	−0.337	0.387
DHL	0.045	−0.292	−0.346	0.092
Subdigital lamellae of 4th toe	0.149	−0.091	−0.237	0.027
Number of horizontal loreal scale rows	0.423	−0.122	0.064	−0.320
Number of SPL to level below center of eye	0.142	−0.028	−0.314	0.130
Number of scales between SS	0.055	−0.128	0.080	−0.313
Number of scales between IP and SS	0.415	−0.199	0.208	−0.369
Number of scales between 2nd canthals	0.364	−0.085	0.002	−0.367
Number of postmentals	0.323	−0.160	0.172	−0.385
Number of scales between nasals	0.185	−0.132	−0.115	−0.218

Central American species of this genus except *A. wellbornae* and the new species described here by the following combination of characters: male dewlap yellowish orange with large blue-to-purple blotch; short legged (longest toe of adpressed hindlimb reaches to a point between shoulder and tympanum, rarely beyond tympanum); tympanum very small (less than one fourth the size of interparietal plate); anterior superciliary conspicuously large and elongate; and ventral scales strongly keeled and mucronate. *Anolis sericeus* differs from *A. wellbornae* by having a male dewlap that is smaller than 50 mm^2 (larger than 100 mm^2 in male *A. wellbornae*) and a female dewlap of approximately the same size as the male dewlap (no dewlap in females of *A. wellbornae*). In addition, the hemipenis of *A. sericeus* has no asulcate ridge (such a ridge present in *A. wellbornae*). For differences between *A. sericeus* and the new species described here, see the Diagnosis section for the new species.

Description (Fig. 3).—Maximum SVL 46.5 mm in males, 50.0 mm in females; ratio tail length/SVL 1.57–2.56 (2.16 ± 0.21); tail slightly compressed in cross section, ratio tail height/tail width 0.91–1.50 (1.15 ± 0.12); ratio axilla to groin distance/SVL 0.36–0.48 (0.43 ± 0.03); ratio head length/SVL 0.24–0.30 (0.26 ± 0.1); ratio snout length/head length 0.43–0.49 (0.49 ± 0.02); ratio head length/head width 1.49–1.87 (1.69 ± 0.08); longest toe of adpressed hindlimb reaches to a point between shoulder and tympanum, rarely beyond tympanum; ratio shank length/SVL 0.19–0.25 (0.22 ± 0.02); ratio shank length/head length 0.71–0.98 (0.83 ± 0.07). Scales on snout varying from faintly to strongly keeled; 5–6 (5.2 ± 0.4) postrostrals; 5–8 (6.4 ± 0.7) scales between nasals; one prenasal scale in contact with both rostral and first supralabial, occasionally only in contact with rostral; most scales in distinct prefrontal depression smooth, some of them keeled; supraorbital semicircles well developed, separated by zero to two (0.6 ± 0.6) scales; supraorbital disc composed of 1–10 (4.9 ± 1.9) distinctly enlarged keeled scales; circumorbital row of small scales often incomplete, if so, one to four enlarged supraorbitals in contact with supraorbital semicircles; a single large elongated superciliary; two to four rows of small-keeled scales extending between enlarged supraorbitals and superciliaries; a very shallow parietal depression present in most specimens; interparietal scale well developed, usually surrounded by scales of moderate size anteriorly and by small- to moderate-sized scales posteriorly; zero to two (1.3 ± 0.6) scales present between interparietal and supraorbital semicircles; canthal ridge dis-

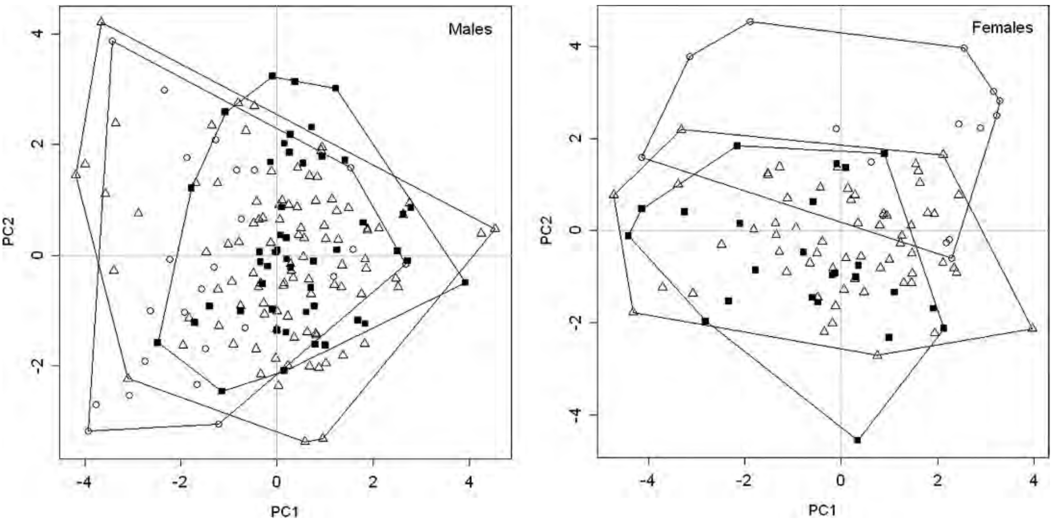

FIG. 2.—Principal component scatterplots for males and females of the three species of Central American anoles formerly referred to as *Anolis sericeus*. Open circles = Species A; black squares = Species B; open triangles = Species C. See text for details.

tinct, composed of six to eight (6.2 ± 0.9) canthal scales, with three (rarely four) larger posterior scales; five to nine (6.6 ± 1.0) scales present between second canthals; 5–10 (8.1 ± 1.2) scales present between posterior canthals; 18–27 (22.6 ± 2.5) loreal scales in a maximum of four to seven (5.1 ± 0.7) horizontal rows, with the scales mostly keeled; five to six keeled subocular scales arranged in a single row; five to eight (6.8 ± 0.5) supralabials to level below center of eye; two to four suboculars broadly in contact with supralabials; ear opening small, ratio tympanum height/parietal scale length 0.18–0.53 (0.37 ± 0.09); mental distinctly wider than long, completely divided medially, bordered posteriorly by four to six (4.7 ± 0.9) postmentals; six to eight (6.9 ± 0.5) infralabials to level below center of eye; sublabials undifferentiated; small elongate keeled scales present on chin and throat; dewlap small, extending from level below oral ricti to a point 2–3 mm anterior to level of axilla; dorsum of body with weakly keeled scales with rounded posterior margins, which are granular on the neck and becoming more flattened posteriorly, 8–26 (18.2 ± 3.3) medial rows slightly enlarged, ratio dorsal scale length/ventral scale length 0.6–1.0 (0.8 ±

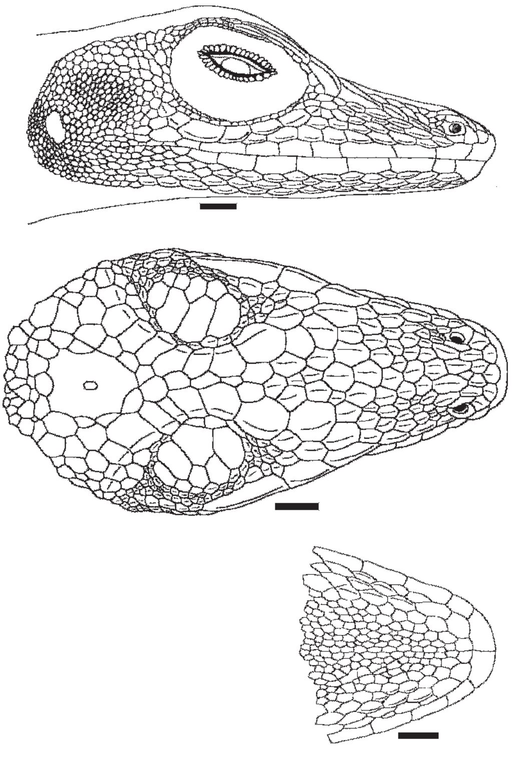

FIG. 3.—Head of *Anolis sericeus* (SMF 86350). Scale bars = 1.0 mm.

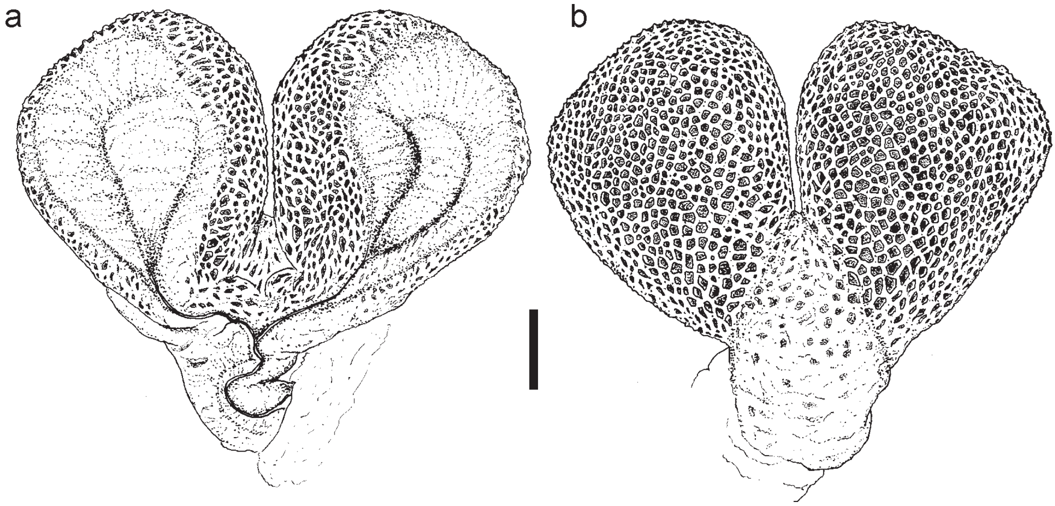

FIG. 4.—Hemipenis of *Anolis sericeus* (SMF 86349). (a) sulcate view; (b) asulcate view. Scale bar = 1.0 mm.

0.1), 20–41 (30.6 ± 5.0) medial dorsal scales in one head length; 35–68 (49.9 ± 7.7) medial dorsal scales between levels of axilla and groin; lateral scales homogeneous; ventrals at midbody keeled, mucronate, imbricate, 14–34 (24.3 ± 4.7) ventral scales in one head length, 26–54 (39.6 ± 7.0) ventral scales between levels of axilla and groin; 93–114 (103.9 ± 6.0) scales around midbody; caudal scales strongly keeled, without whorls of enlarged scales, although an indistinct division in segments is discernible; postanal scales not enlarged; no tube-like axillary pocket present; scales on dorsal surface of forelimb keeled, imbricate; digital pads dilated; distal phalanx narrower than and raised from dilated pad; 19–26 (23.5 ± 1.5) lamellae under phalanges ii–iv of fourth toe; five to seven (6.0 ± 0.3) scales under distal phalanx of fourth toe.

The completely everted hemipenis (SMF 86349; Fig. 4) is a large bilobate organ; sulcus spermaticus bordered by well developed sulcal lips and bifurcating at base of apex; shortly after the bifurcation, the branches open into broad concave areas, one on each lobe; truncus relatively short, shorter than or equal to length of each lobe; asulcate surface of apex and distal truncus strongly calyculate, base of truncus without transverse folds; no asulcate processes.

Distribution.—Atlantic versant of Mexico (Estados de Tamaulipas, Hidalgo, San Luis Potosí, Veracruz, Tabasco, Campeche, Quintana Roo, and Yucatán as well as the extreme northern portion of Oaxaca); from sea level to approximately 1200-m elevation.

Anolis wellbornae *Ahl*

Anolis ustus wellbornae Ahl, 1940: 246. Holotype male (ZMB 35710) from "San Salvador, El Salvador."
Anolis sallaei: Schmidt (1928).
Anolis sericeus wellbornae: Stuart (1955).
Anolis sericeus: Mertens (1952), Brongersma (1954), Rand (1957), Lee (1980; in part).
Norops sericeus: Köhler and Acevedo (2004), Köhler et al. (2006).

Diagnosis.—A medium-sized species (SVL in largest specimen 49.7 mm) of the genus *Anolis* (sensu Poe, 2004) that differs from all Central American species of this genus except *A. sericeus* and the new species described herein by the following combination of characters: male dewlap yellowish orange with large blue-to-purple blotch; short legged (longest toe of adpressed hindlimb reaches to a point between shoulder and tympanum, rarely beyond tympanum); tympanum very small (less than one fourth the size of interparietal plate); anterior superciliary conspicuously large and elongate; ventral scales strongly keeled and mucronate. *Anolis wellbornae* differs from *A. sericeus* by having a

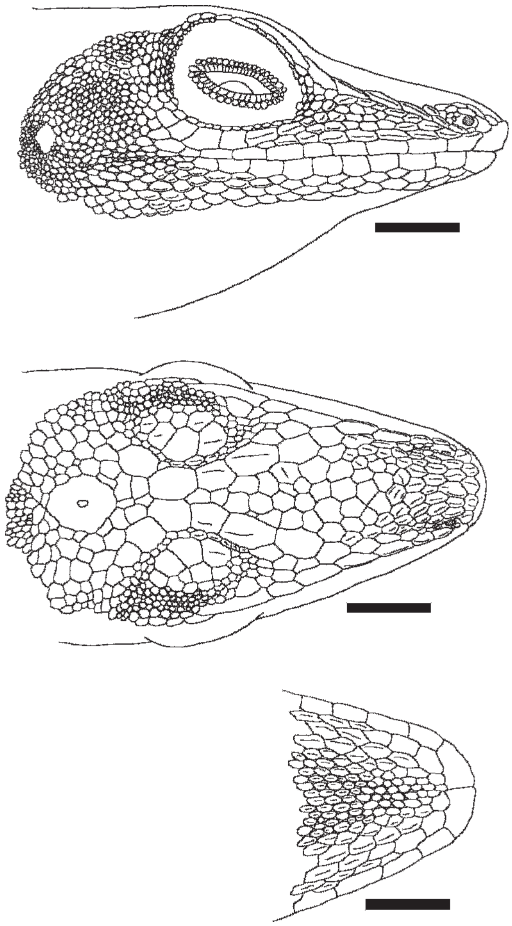

FIG. 5.—Head of *Anolis wellbornae* (SMF 82667). Scale bars = 1.0 mm.

male dewlap that is larger than 100 mm^2 (smaller than 50 mm^2 in male *A. sericeus*) and no dewlap in females (a female dewlap of approximately the same size as the male dewlap in *A. sericeus*). Additionally, the hemipenis of *A. wellbornae* has a distinct asulcate ridge (no such ridge in *A. sericeus*). For differences between *A. wellbornae* and the new species described herein, see the Diagnosis section of the new species.

Description (Fig. 5).—Maximum SVL 49.7 mm in males, 44.5 mm in females; ratio tail length/SVL 1.57–2.43 (2.11 ± 0.20); tail slightly compressed in cross section, ratio tail height/tail width 0.74–1.43 (1.23 ± 0.12); ratio axilla to groin distance/SVL 0.27–0.49 (0.42 ± 0.03); ratio head length/SVL 0.23–0.29 (0.25 ± 0.1); ratio snout length/head length 0.41–0.51 (0.45 ± 0.02); ratio head length/head width 1.47–1.83 (1.67 ± 0.08); longest toe of adpressed hindlimb reaches to a point between shoulder and tympanum, rarely beyond tympanum; ratio shank length/SVL 0.20–0.26 (0.22 ± 0.01); ratio shank length/head length 0.76–1.03 (0.86 ± 0.05). Scales on snout varying from faintly to strongly keeled; five to seven (5.7 ± 0.5) postrostrals; four to nine (6.8 ± 1.0) scales between nasals; one larger prenasal scale in contact with both rostral and first supralabial, occasionally only in contact with rostral; scales in distinct prefrontal depression generally flat, some of them keeled; supraorbital semicircles well developed, separated by zero to two (0.6 ± 0.6) scales; supraorbital disc composed of 2–10 (6.3 ± 1.5) distinctly enlarged keeled scales; circumorbital row of small scales rarely incomplete, if so, one to two enlarged supraorbitals in contact with supraorbital semicircles; a single large elongated superciliary; one to four rows of small keeled scales extending between enlarged supraorbitals and superciliaries; a very shallow parietal depression present in most specimens; interparietal scale well developed, usually surrounded by scales of moderate size anteriorly and by small- to moderate-sized scales posteriorly; one to four (2.0 ± 0.5) scales present between interparietal and supraorbital semicircles; canthal ridge distinct, composed of four to seven (6.8 ± 1.2) canthal scales, with three larger posterior scales; 5–10 (7.4 ± 0.1) scales present between second canthals; 7–12 (9.2 ± 1.1) scales present between posterior canthals; 18–39 (26.6 ± 4.6) loreal scales in a maximum of 4–7 (5.5 ± 0.6) horizontal rows, with the scales mostly keeled; four to seven keeled subocular scales arranged in a single row; six to nine (6.7 ± 0.6) supralabials to level below center of eye; one to four suboculars broadly in contact with supralabials; ear opening small, ratio tympanum height/parietal scale length 0.18–0.68 (0.44 ± 0.11); mental distinctly wider than long, completely divided medially, bordered posteriorly by four to seven (5.8 ± 0.5) postmentals; five to eight (6.7 ± 0.6) infralabials to level below center of eye; sublabials undifferentiated; small elongate keeled scales present on chin and throat;

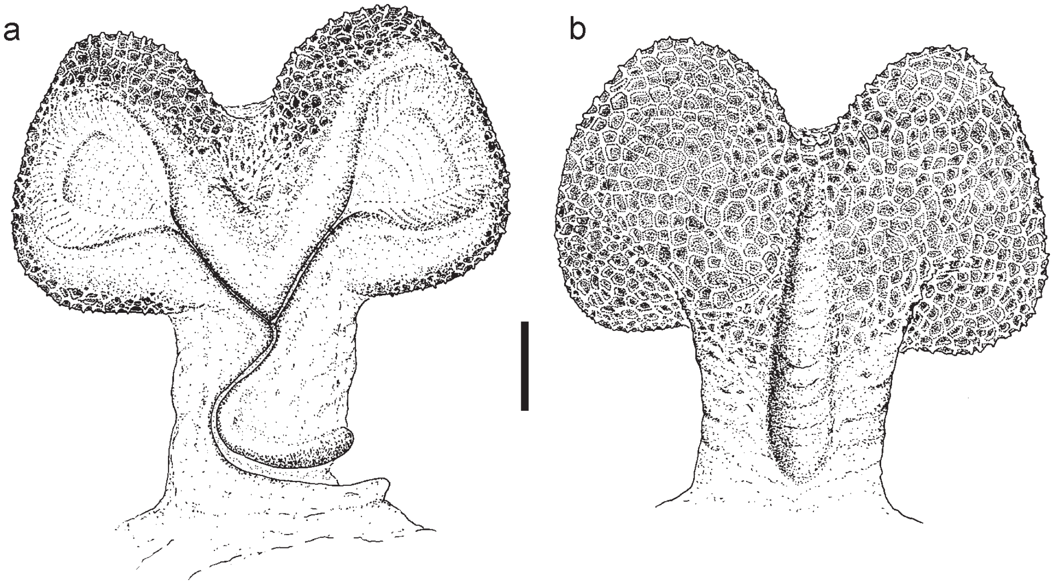

FIG. 6.—Hemipenis of *Anolis wellbornae* (SMF 79368). (a) Sulcate view. (b) Asulcate view. Scale bar = 1.0 mm.

dewlap extending from level below oral ricti to to a point 2–4 mm anterior to level of axilla; dorsum of body with weakly keeled scales with rounded posterior margins, which are granular on the neck and becoming more flattened posteriorly, 10–24 (18.7 ± 2.8) medial rows slightly enlarged, ratio dorsal scale length/ventral scale length 0.58–0.96 (0.77 ± 0.9); 24–38 (30.0 ± 2.8) medial dorsal scales in one head length; 39–63 (48.7 ± 5.6) medial dorsal scales between levels of axilla and groin; lateral scales homogeneous; ventrals at midbody keeled, mucronate, imbricate, 16–30 (23.1 ± 2.9) ventral scales in one head length, 25–47 (37.2 ± 3.9) ventral scales between levels of axilla and groin; 97–133 (114.3 ± 8.7) scales around midbody; caudal scales strongly keeled, without whorls of enlarged scales, although an indistinct division in segments is discernible; postanal scales not enlarged; no tube-like axillary pocket present; scales on dorsal surface of forelimb keeled, imbricate; digital pads dilated; distal phalanx narrower than and raised from dilated pad; 19–27 (23.5 ± 1.4) lamellae under phalanges ii–iv of fourth toe; four to seven (5.5 ± 0.6) scales under distal phalanx of fourth toe.

The completely everted hemipenis (SMF 82667; Fig. 6) is a large bilobate organ; sulcus spermaticus bordered by well developed sulcal lips and bifurcating at base of apex; shortly after the bifurcation, the branches open into broad concave areas, one on each lobe; truncus relatively long, longer than or equal to length of each lobe; asulcate surface of apex strongly calyculate, base of truncus without transverse folds; no asulcate processus present, although a distinct ridge is present on the asulcate side.

Coloration in life.—Adult male (SMF 79235) from western El Salvador: dewlap Orange-Yellow (18) with Brick Red (132A) stripes between rows of gorgetals and with a Spectrum Blue (69) central blotch.

Distribution.—Pacific versant of Nuclear Central America (northwestern Nicaragua, El Salvador, extreme southern Honduras and Pacific versant of Guatemala to approximately Mazatenango); from sea level to approximately 1050-m elevation.

Anolis **unilobatus** *sp. nov.*

Anolis sallaei: Boulenger (1885; in part), Barbour and Loveridge (1929).

Anolis sallaei wellbornae: Smith and Kerster (1955).

Anolis sericeus: Stuart (1935), Hartweg and Oliver (1940), Stuart (1948), Smith and Taylor (1950; in part), Stuart (1963; in part), Campbell and Howell (1965), Meyer and

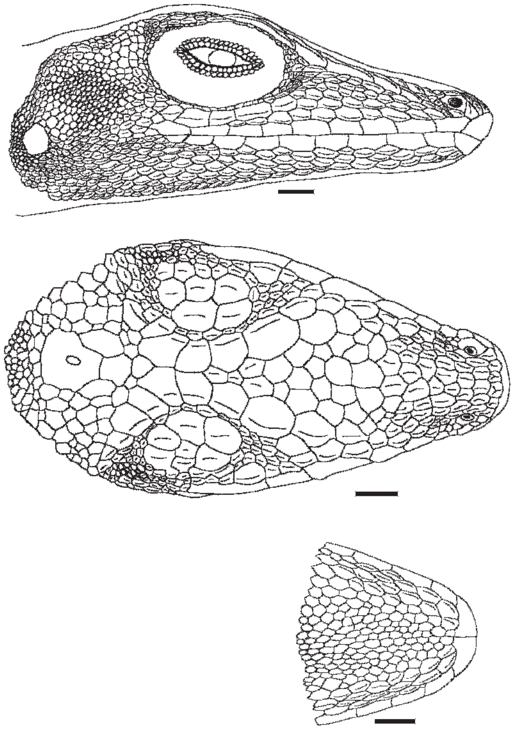

FIG. 7.—Head of holotype of *Anolis unilobatus* (SMF 87133). Scale bars = 1.0 mm.

Wilson (1973), Lee (1980; in part), Fitch and Seigel (1984), Lee (1996; in part), McCranie and Castañeda (2005).
Anolis sericeus sericeus: Stuart (1955; in part), Duellman (1963).
Anolis ustus: Boulenger (1885; in part).
Anolis ustus ustus: Stuart (1935).
Norops sericeus: Köhler (1999, 2001), McCranie et al. (2006).

Holotype.—SMF 87133, an adult male from Awasbila, a village along Río Coco, 14° 47′ N, 84° 45′ W, 60 m, Departamento Gracias a Dios, Honduras. Collected 29 July 2002 by James Randy McCranie and Larry David Wilson. Field tag no. LDW 12909.

Paratypes.—All from Honduras, Departamento Gracias a Dios: SMF 87136, same locality and collector data as holotype, collected 26 July 2001. SMF 87125-26, 87131, Rus Rus, 60 m, collected 8 October 2001 by James Randy McCranie. SMF 87127, 87144, Rus Rus, 60 m, collected 22 May 2004 by James Randy McCranie. SMF 87129, Rus Rus, 60 m, collected 24 August 2003 by Tomás Manzanares. SMF 87137, Rus Rus, 60 m, collected 8 October 2001 by James Randy McCranie. SMF 87143, Rus Rus, 60 m, collected 13 July 2001 by James Randy McCranie and Larry David Wilson. SMF 87147, Karasangkan, 70 m, collected 12 April 2002 by Tomás Manzanares. SMF 87151, Rus Rus, 60 m, collected 16 November 2001 by James Randy McCranie. SMF 87126-27, 87129, 87131, 87136, 87143 are adult males; SMF 87125, 87137, 87144, 87147, 87151 are adult females.

Diagnosis.—A medium-sized species (SVL in largest specimen 47.0 mm) of the genus *Anolis* (sensu Poe, 2004) that differs from all Central American species of this genus except *A. sericeus* and *A. wellbornae* by the following combination of characters: male dewlap yellowish orange with large blue-to-purple blotch; short legged (longest toe of adpressed hindlimb reaches to a point between shoulder and tympanum, rarely beyond tympanum); tympanum very small (less than one fourth the size of interparietal plate); anterior superciliary conspicuously large and elongate; ventral scales strongly keeled and mucronate. *Anolis unilobatus* differs from *A. sericeus* and *A. wellbornae* by having a unilobate hemipenis (bilobate in *A. sericeus* and *A. wellbornae*). Furthermore, *A. unilobatus* can be distinguished from *A. sericeus* in dewlap size (*A. unilobatus*: male dewlap larger than 100 mm^2, no female dewlap; *A. sericeus*: male dewlap smaller than 50 mm^2, females with a dewlap of approximately the size of the male dewlap). Also, *A. unilobatus* differs from *A. sericeus* and *A. wellbornae* in the mean values of several scalation and morphometric characters (Table 1).

Description of the holotype (Fig. 7).—Adult male as indicated by everted hemipenes; SVL 43.0 mm; tail length 108.0 mm, tail complete, ratio tail length/SVL 2.51; tail slightly compressed in cross section, tail height 2.30 mm, tail width 1.80 mm, ratio tail height/tail width 1.28; axilla to groin distance 19.0 mm, ratio axilla to groin distance/SVL 0.44; head length 11.3 mm, head length/SVL ratio 0.26; snout length 5.3 mm, ratio snout length/head length 0.47; head width 6.7 mm, ratio head length/head width 1.67; longest toe of adpressed hindlimb reaching to tympanum; shank length

9.2 mm, ratio shank length/SVL 0.21, ratio shank length/head length 0.82; longest finger of extended forelimb reaching to a point slightly beyond nostrils; longest finger of adpressed forelimb does not reach anterior insertion of hindlimbs. Most scales on snout keeled; six postrostrals; five scales between nasals; one large elongated prenasal scale in contact with both rostral and first supralabial; most scales in distinct prefrontal depression smooth, some weakly keeled; supraorbital semicircles well developed, in contact with each other; supraorbital discs composed of five to seven distinctly enlarged, weakly keeled scales; circumorbital row on the left side incomplete; therefore, one enlarged supraorbital in contact with left supraorbital semicircle; a single large elongated superciliary; approximately two to three rows of smaller keeled scales extending between enlarged supraorbitals and superciliaries; a very shallow parietal depression present; interparietal scale well developed, 1.3 × 1.3 mm (length × width), surrounded by scales of moderate size; two scales present between interparietal and supraorbital semicircles; canthal ridge distinct, composed of four large (posterior two largest) and two to three small anterior canthal scales; eight scales present between second canthals; eight scales present between posterior canthals; 23 (right)–21 (left) loreal scales in a maximum of six horizontal rows, with the scales mostly keeled; five keeled subocular scales arranged in a single row; eight (right) or seven (left) supralabials to level below center of eye; three suboculars broadly in contact with two supralabials; ear opening 0.75 × 0.75 mm (length × height); mental distinctly wider than long, completely divided medially, bordered posteriorly by six postmentals (outer pair much larger than the inner ones); seven infralabials to level below center of eye; sublabials undifferentiated; keeled granular scales present on chin and throat; dewlap extending from level below the posterior border of orbit to a point 2 mm beyond level of axilla; dorsum of body with weakly keeled scales with rounded posterior margins, which are granular on the neck and becoming more flattened posteriorly, 19 rows of medial dorsal scales enlarged, largest dorsal scales approximately 0.50 × 0.45 mm (length × width); approximately 30 medial dorsal scales in one head length; approximately 55 medial dorsal scales between levels of axilla and groin; lateral scales homogeneous, average size 0.20 mm in diameter; ventrals at midbody keeled, mucronate, imbricate, approximately 0.60 × 0.63 mm (length × width); approximately 25 ventral scales in one head length; approximately 44 ventral scales between levels of axilla and groin; 114 scales around midbody; caudal scales strongly keeled, equal in size; no enlarged postanal scales; no tube-like axillary pocket; scales on dorsal surface of forelimb keeled, imbricate, approximately 0.45 × 0.55 mm (length × width); digital pads dilated; distal phalanx narrower than and raised from dilated pad; 24 lamellae under phalanges ii–iv of fourth toe; six scales under distal phalanx of fourth toe.

The completely everted hemipenis (Fig. 8) is a small unilobate organ; there is a distinct ridge-like structure emerging from the bifurcation of the sulcus spermaticus and reaching across the tip of apex to the asulcate side; on the sulcate side, this ridge has a median pocket; sulcus spermaticus bordered by well developed sulcal lips and bifurcating at base of apex; shortly after the bifurcation, the branches open into broad, slightly convex areas on the sides of the medial ridge; a small calyculate area on the asulcate surface of the lower portion of apex; base of truncus without transverse folds; no asulcate processus present.

Coloration in preservative.—Dorsal ground color and flanks grayish brown; dorsal surface of head grayish brown, grading into dark brown in parietal and temporal regions; venter dirty white, grading into pale yellowish on ventral sides of limbs and gular region; ventral side of tail dirty white, grading into light brown posteriorly; dorsal surface of limbs and posterior part of tail light brown without any discernable pattern; dewlap dirty white with black central blotch.

Variation.—The paratypes agrees well with the holotype in general appearance, morphometrics, and scalation (Table 1). Color in life of an adult male paratype (SMF 87127) was recorded as follows: dorsal surface of body Cinnamon (123A) with dark brown vertebral small spots; dorsal surface of head Cinnamon

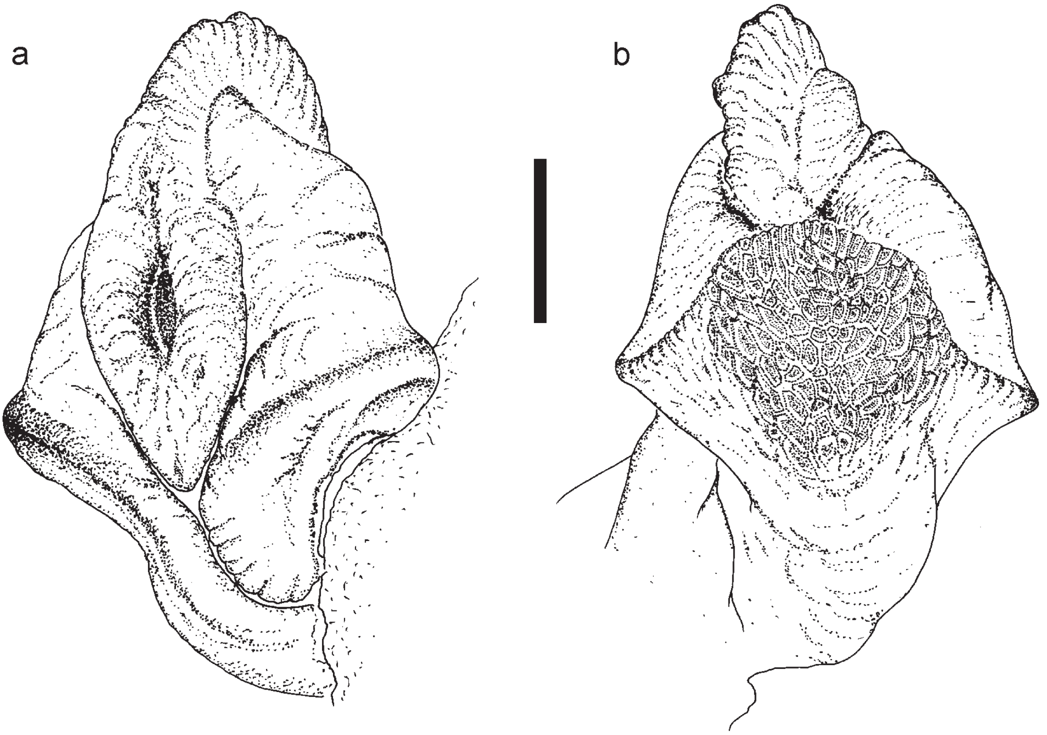

FIG. 8.—Hemipenis of *Anolis unilobatus* (SMF 80964). (a) Sulcate view. (b) Asulcate view. Scale bar = 1.0 mm.

(123A); dorsal surface of limbs Cinnamon (123A) with dark brown mottling; belly pale yellowish brown; dewlap Trogon Yellow (153) with Ultramarine (270) central blotch; iris brown with gold rim. Color in life of an adult male (SMF 79366) from Isla de Utila, Honduras, was recorded as follows: dorsal and lateral surfaces of body Straw Yellow (56) with paler dorsolateral and lateral longitudinal lines; dewlap Orange-Yellow (18) with Scarlet (14) stripes between rows of gorgetals and with a Smalt Blue (70) central blotch. Color in life of an adult male (SMF 83142) from Río San Juan, Nicaragua, was recorded as follows: dorsal and lateral surfaces of body Tawny Olive (223D) grading into Raw Umber (223) in vertebral region; venter Cream Color (54) but paler; dewlap Orange-Yellow (18) with Chestnut (32) stripes between rows of gorgetals and with a Cyanine Blue (74) central blotch.

Etymology.—The name *unilobatus* is formed from the Latin words unus (one) and lobus (lobe) and is used as an adjective; it refers to the unilobate hemipenis in this species which sets it apart from *A. sericeus* and *A. wellbornae* with which it has been confused in the past.

Natural history notes.—*Anolis unilobatus* is an extremely common anole that reaches high population densities at some localities. At most sites where we collected this species it was the most abundant species of anole. During daytime, most individuals were observed on branches and twigs of bushes and small trees, 80–200 cm above the ground. These anoles can also commonly be found on fences where they are usually perching head down on the posts, especially near bushes. Males are territorial and will show aggressive display behavior including head bobbing with extended dewlap towards a conspecific male that approaches it. A summary of what is known about the natural history of *A. unilobatus* (under the name *sericeus*) is found in Savage (2002) and McCranie et al. (2005, 2006).

Distribution.—From Costa Rica across most of Nicaragua (except northwestern

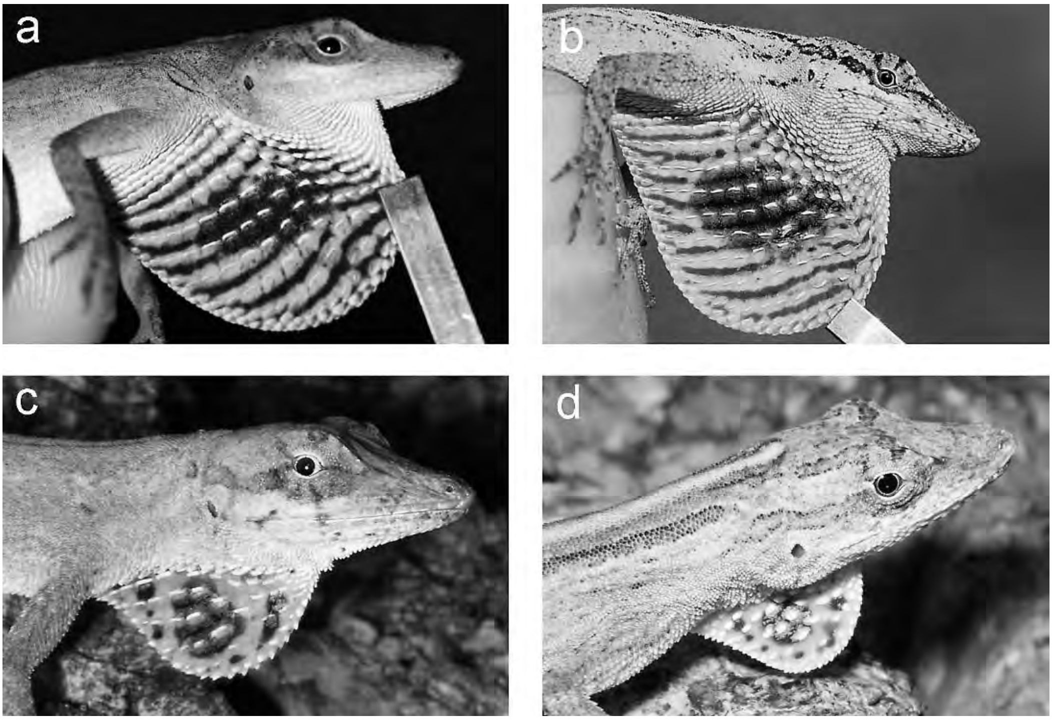

FIG. 9.—Specimens in life with extended dewlaps (a) *Anolis unilobatus*. Male (SMF 84471). (b) *Anolis wellbornae*. Male (SMF 84473). (c) *Anolis sericeus*. Male (Burrell Boom. Belize. Not preserved). (d) *Anolis sericeus*. Female (Burrell Boom. Belize. Not preserved).

corner of the country), Honduras, (except southern portion of the country), along the Caribbean versant of Guatemala and Chiapas, Mexico, to the Isthmus of Tehuantepec where it crosses to the Pacific versant and continues along the Pacific versant of Chiapas and Guatemala to approximately Mazatenango; from sea level to approximately 1200-m elevation.

KEY TO MALES OF SPECIES FORMERLY REFERRED TO AS *A. SERICEUS*

1.a. Hemipenis unilobate; males with large dewlaps (larger than 100 mm^2; Fig. 9a); no dewlap in females .... *Anolis unilobatus*
1.b. Hemipenis bilobate; male dewlap size large or small; dewlap in females present or absent 2
2.a. Male dewlap large (Fig. 9b), larger than 100 mm^2, no dewlap in females; hemipenis with an asulcate ridge .... *Anolis wellbornae*
2.b. Male dewlap small (Fig. 9c), smaller than 50 mm^2; females with a small dewlap (Fig. 9d), approximately the size of the male dewlap; hemipenis without an asulcate ridge.................................... *Anolis sericeus*

DISCUSSION

All three cryptic species discussed in this article are small, slender grass anoles occasionally also occupying lower parts of tree trunks or branches of low bushes. They are very common in all kinds of open habitats from forest edges and grasslands to urban areas. However during our extensive field observations of these anoles we did not encounter any differences in life history or behavior that could be congruent with certain species distribution. Although we have not documented a case of actual sympatry of the species formerly referred to as *A. sericeus*, they do approach each other very closely in some areas. In southwestern Guatemala, we sampled the road CA2 between Mazatenango and Santa Lucia Cotzumalguapa; from Mazatenango to the vicinity of San Antonio Suchitepéquez, we collected *A. unilobatus* and some 20 km down the road toward Santa Lucia Cotzumalguapa, we found only *A. wellbornae*. East of the latter locality we only collected *A. wellbornae*. Other possible contact zones are suspected in northwestern Nicaragua (*A. unilobatus* and *A. wellbornae*); southern Belize (*A. unilobatus* and *A. sericeus*); central El Petén, Guatemala (*A. unilobatus* and *A. sericeus*); and southern Veracruz, Mexico (*A. unilobatus* and *A. sericeus*). It seems that these species replace each other, and we are not aware of any locality where they occur syntopically. In the regions where two species approach each other, we searched for indications of hybridization but did not encounter any intermediate forms. With respect to hemipenial morphology, we found exclusively the three types described above, and variation within these types was negligible. This is particularly remarkable given the huge geographic distribution of *A. unilobatus*. This suggests the presence of barriers to hybridization between these very similar species. More research is needed to study the distribution and possible interactions of these species in their various potential contact zones.

The three species treated in this article were previously confused as one species, and they seem to be each others closest relatives. The unusual male dewlap coloration could be interpreted as a potential synapomorphy of these three species. Also, the single, extremely long superciliary and the conspicuously small ear opening are characteristics that can be judged as support for their close relationship. The fact that we were unable to separate these three species based on morphometric and pholidotic characters qualifies them as cryptic species that can be diagnosed only based on striking differences in hemipenial morphology and dewlap size. Examples for such cryptic species are known for several species pairs and complexes in Central American anoles (e.g., Köhler et al., 2007; Köhler and Sunyer, 2008). However, research is needed to evaluate the underlying mechanisms that trigger hemipenial differentiation in these species that seem conservative in external morphology. We assume that the *A. sericeus* complex has a history of allopatric speciation with subsequent fluctuations of the geographic distributions of the species, and the formation of secondary contact zones. Climatic fluctuations during the recent geological history (i.e., Pleistocene; see Savage, 1982) may have caused fragmentation of the former continuous range of the ancestral species in this species complex.

Acknowledgments.—Collecting and exportation permits were provided by A. N. Perez, Consejo Nacional de Areas Protegidas (CONAP), Guatemala City, Guatemala; A. M. Majano, P. Quintana vda. de López, C. C. Dueñas, and A. Sánchez, Ministerio de Agricultura y Ganadería, Dirección General de Recursos Naturales Renovables, Servicio de Parques Nacionales y Vida Silvestre, San Salvador, El Salvador; A. Barahona, T. García, A. P. Martinez, E. Muñoz Galeano, and C. Romero, Corporación Hondureña de Desarollo Forestal (COHDEFOR), Tegucigalpa, Honduras; M. Fonseca Cuevas, S. Tijerino, M. G. Camacho, and C. Peres-Román, Ministerio del Ambiente y los Recursos Naturales (MARENA), Managua, Nicaragua. For field assistance, we thank M. Acevedo, E. Köhler, J. Soto, and P. Soto. We thank Francisco Serrano, San Salvador, for the generous logistic support and valued cooperation. H. Bahena B., R. R. Calderón M., J. R. Cedeño, M. Dehling, J. Ferrari, G. Paiz, J. R. McCranie, C. Pozo, and J. Sunyer generously donated specimens for this study. We thank L. Czupalla for providing some of the drawings. We thank J. Ferebee for help with the statistical methods. For the loan of and/or access to specimens, we thank L. Ford, C. J. Raxworthy, and D. R. Frost, American Museum of Natural History (AMNH), New York; T. Daeschler and A. Gilmore (ANSP); C. J. McCarthy (BMNH); J. Vindum, California Academy of Sciences (CAS), San Francisco; S. P. Rogers, Carnegie Museum of Natural History (CM), Pittsburgh; N. M. Ferrer, J. C. López Vidal, and S. A. Murillo Jiménez, Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas (ENCB), Mexico City; A. Resetar, Field Museum of Natural History (FMNH), Chicago; V. H. Reynoso, Universidad Nacional Autónoma de México, Instituto de Biología (IBH), Mexico City; F. Mendoza Q., Instituto Tecnologico Agropecuario de Hidalgo (ITAH), Huejutla de Reyes, Hidalgo, Mexico; W. E. Duellman and J. E. Simmons, University of Kansas, Natural History Museum (KU), Lawrence; J. Hanken and J. P. Rosado, Museum of Comparative Zoology, Harvard University (MCZ), Cambridge; I. Ineich and A. Ohler, Museum National d'Histoire Naturelle (MNHN), Paris; E. E. Echeverría, (MUHNES); D. B. Wake and C. Cicero, Museum of Vertebrate Zoology (MVZ), Berkeley; H. Bahena B., R. R. Calderón M., J. R. Cedeño, and C. Pozo (MZ-CIQRO); N. Jiménez (MZ-UNICACH); K. L. Krysko and F. W. King, Florida Museum of Natural History (UF), Gainesville; C. A. Phillips and J. Petzing, Illinois Natural History Survey, Center for Biodiversity (UIMNH), Champaign; R. A. Nussbaum and G. Schneider, University of Michigan Museum of Zoology (UMMZ), Ann Arbor; S. Perez and L. Prado (USAC); R. W. McDiarmid and W. R. Heyer, National Museum of Natural History (USNM), Washington, DC; J. Campbell and C. Franklin, The University of Texas at Arlington (UTA), Arlington; M. Dix, M. Maldonado, and M. J. Illescas, Universidad del Valle de Guatemala (UVG), Guatemala City; W. Böhme, Zoologisches Forschungsin-

stitut und Museum A. Koenig (ZFMK), Bonn; and R. Günther, Museum für Naturkunde der Humboldt–Universität zu Berlin (ZMB), Berlin.

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Accepted: 22 December 2009
Associate Editor: Frank Burbrink

Appendix I

Specimens Examined

(^{H} = males with everted hemipenes)

Norops sericeus.—"Central America": BMNH 1946.8.5.69 (holotype of *Anolis sallaei* Günther); BELIZE: "Belize": USNM 58476; *Belize*: North River: USNM 58477–78; 1.5 mi. S, 13 mi. W Belize City: AMNH 126043; *Cayo*: 0.5 mi. S Georgeville on road to San Antonio: CM 105696, 105756; Mountain Pine Ridge, Augustine: CM 105945; W of Burrell Boom, 10 m: SMF 83233–34^{H}; W of Burrell Boom, Scotland Halfmoon, 15 m: SMF 83250–51$^{\text{H both}}$, 83252–55, 85871, 86349–50$^{\text{H both}}$; *Orange Walk*: 1 km S Carmelita, 17° 59′ 43″ N, 88° 31′ 59″ W, 25 m: SMF 83232; *Stann Creek*: 2 km W Dangriga, 5 m: SMF 83244^{H}, 83245–46, 83235, 83236–37$^{\text{H both}}$; Independence (=Mango Creek), 16° 31′ 60″ N, 88° 25′ 00″ W, 15 m: SMF 83247^{H}, 83248–49; *Toledo*: Blue Creek Village: ZFMK 41141; Blue Creek Village, vicinity of Slattery Field Station: UTA R11056–57; Punta Gorda: AMNH 125840, 125842–44; Big Falls, ca. 0.5 mi. SE of, Maya Mountain Archaeological Project Base Camp, 16° 15′ 57″ N, 88° 52′ 12″ W, 100 m: USNM 496668; Colombia Branch Camp: AMNH 126040–41. MEXICO: *Campeche*: 15.2 mi. S Ceiba Playa [=Seybaplaya or Seyba Playa]: UTA R3089; 24 km (by road) E Escarcega and then 20 km S on a dirt road: USNM 224573; Zona Arqueológica de Calakmul: MZ–CIQRO 1283^{H}; *Hidalgo*: Municipio Huahutla: ITAH 139, 140, 214, 502^{H}, 1150^{H}, 3914-16$^{\text{H all}}$; Huejutla: ITAH 1142, 1143-44$^{\text{H both}}$; Cerro Mantecotl: ITAH 510; Barrio Honda, Municipio Huahutla: ITAH 212; Calnali Atempa: ITAH 702; La Capilla: Municipio Jaltocan: ITAH 221, 258; *Oaxaca*: 6.8 km S Valle Nacional bridge on Mex Hwy 175: UTA R14546; Valle Nacional [near San José Chiltepec], Cerro Pelón: CM 41255; *Quintana Roo*: Zona Arqueológica Konhulich, municipio de Othón P. Blanco: MZ–CIQRO 1260; Colonia Alvaro Obregón, municipio de Othón P. Blanco: MZ–CIQRO 1557$^{\text{H}}$; *San Luis Potosí*: "San Luis Potosí": AMNH 149631$^{\text{H}}$; Alvarez Mts at km 42, Potosí=Río Verde: ANSP 20044; *Tabasco*: Frontera: USNM 3774840; 40 mi. N Villahermosa: USNM 192546; *Tamaulipas*: Tampico: SMF 28862$^{\text{H}}$; NE of Aldama, Rancho Nuevo, 9 mi. N Barra del Tordo: USNM 209827–28; *Veracruz*: "Veracruz": MNHN 2406; vicinity of Cuautlapan: USNM 224813; Carrizal, 17° 56′ N, 95° 42′ W: USNM 47646; 1.8 mi. S Juan Diaz Covarrubias: UTA R10078, R9794, R9824–30, R9928; 1.9 mi. N of Juan Diaz Covarrubias: UTA R2652; 2.0–6.0 km N of Juan Diaz Covarrubias: UTA R9822; 7.7–7.8 mi. (by road) NW Sontecomapan: UTA R9823, R9926, R9696, R10198–99; ca. Lerdo de Tejada: UTA R3152; La Victoria 3.1 mi. S Lago Catemaco: UTA R9898; Los Tuxtlas 2.5 mi. SSW Sontecomapan: UTA R3105, R3153; Los Tuxtlas, Catemaco: UTA R3146; Los Tuxtlas, El Encinal: UTA R3129; Los Tuxtlas, Eyipantla Falls: UTA R3143; Los Tuxtlas, Sontecomapan: UTA R3130; Los Tuxtlas, N side Lago Catemaco: UTA R3106; Sierra de Los Tuxtlas, Volcán San Martín, Rancho Primero de Mayo: UTA R51892; Tuxtlas Region, Altepec Island in Lake Catemaco: UTA R3000–01; Coatzacoalcos: USNM 47512; Jicaltepec, 20° 10′ N, 96° 50′ W: USNM 32145–47; E of Lago Catemaco, on road to Colonia Bostonal (12.7 mi. E and S (by road) of Catemaco): USNM 224808; ca. 2 km N Nautla: USNM 224812; Orizaba, 1200 m: USNM 84279–80; ca. 2 mi. S Tenochitlan, Rio Chiquito at San Lorenzo, 19° 44′ N, 96° 58′ W: USNM 123499–504; Mirador: ANSP 7886; Lake Catemaco, near Coyame: CM 75966–70; Huatusco: ZMB 6751; *Yucatán*: "Yucatán": USNM 24860–62; Chichen Itzá: AMNH 38861; Chichen Itzá, near ruins: UTA R3092; Ruinas de Mayapán: SMF 77168; 10 km N Celestún: MZ–CIQRO 1725; Merida: USNM 6571.

Norops unilobatus.—COSTA RICA: *Guanacaste*: 13.6 km SW of Canas, Estacion Experimental Enrique Jimenez Nunez, 10° 20′ N, 85° 09′ W, 10 m: USNM 219838. GUATEMALA: "Guatemala": ZMH 4595; Soc. Econom.: MNHN 5244, 1994.891; *Alta Verapaz*: N base Sierra de las Minas, Finca Pueblo Viejo, along Río Pueblo Viejo: UTA R26976; N slope Sierra de las Minas, Finca Pueblo Viejo, 2.5 air km SE Pueblo Viejo: UTA R R26974–78; Hacienda Chimoxán, ca. 1500 ft., MCZ 32325, 32330; *Huehuetenango*: 1.9 road km N Nentón on road to Gracias a Dios: UTA R45976; 4.3 km S Nentón: UTA R41483–85; 6.8 km N Nentón on road to La Trinidad: UTA R52082; 7.4 km NE Nentón on road to Barillas: UTA R41486–87$^{\text{H both}}$, R41488; 9.2 km N Cuatro Caminos at Río Azul: UTA R41491; 9.2 km NNW Nentón on road to Chacaj: UTA R52086; Cuilco, cerca Rio Mojubal: UTA R39951; Cuilco, cerca Rio Cuilco (y Caserio Mojubal): UTA R39952–53; Guatemala–Mexico border on CA–1: UTA R9069; Hacienda La Fortuna: UTA R45692$^{\text{H}}$, R45973$^{\text{H}}$, R45974, 45975$^{\text{H}}$; Hacienda Miramar: UTA R41492–93; Hotel El Reposo along Hwy CA–1 between La Democracia and La Mesilla: UTA R45979–80$^{\text{H both}}$; Intersección Carretera Camoj Grande–Nentón y Río Selequa: UTA R37624; La Democracia, Carretera Camoj Grande–Nentón, Km 336: UTA R37625–27; La Democracia, Km 332 carretera Interamericana, Gasolinera El Reposa: UTA R41481; Nentón (near Hotel Las Peñas): UTA R52083–85; Nentón Hacienda Miramar Finca Los Cimientos: UTA R41495; Nentón, Hacienda Miramar La Fortuna: UTA R41496–98; Nentón, near cemetary above town: UTA R45977–78; Nentón: UTA R45469$^{\text{H}}$, R45470, R45471$^{\text{H}}$, R45472; Sierra de Los Cuchumatanes, Finca Chiblac Buena Vista (now Aldea Buenos Aires): UTA R41494; *Izabal*: El Estor, El Chupón: UTA R29803–04; Las Dantas, 1.7 mi. W El Estor: UTA R26984, R9070–71; Livingston, Aldea La Libertad, Km 285 a Petén: UTA R39929–31; Livingston, Aldea La Libertad, Quebrada El Branchi: UTA R39936; Puerto Barrios, Finca El Jabalí: UTA R39932–35, R39938, R52108; Puerto Barrios, Hotel La Caribeña: UTA R29533$^{\text{H}}$; Sierra de Caral, Morales, Carretera Quebradas–San Miguelito: UTA R37623; Río Dulce, km 274 on Carretera al Petén; hotel Morimonte: AMNH 140271$^{\text{H}}$; El Paraiso, ca. 25 km by road E El Estor, 15° 34′ 17.3″ N, 89° 11′ 49.8″ W, 80 m: SMF 83970–72, 83973$^{\text{H}}$; *Petén*: "Peten": USNM 71917–19, 71930; Parque Nacional Tikal: UF 13777–78, 13802; Seibal [=El Ceibal], Río de Pasion, village Sta. Amelia: AMNH 72611, 72617; Sayaxché, SW of Flores: SMF 77186$^{\text{H}}$; Uaxactum: AMNH 68522–24; Libertad: USNM 71413; *Progreso*: Puente Hato, 0.4 km W Magdalena, 14° 55′ 13.9″ N, 89° 57′ 58.2″ W, 270 m: SMF 83974; *Quezaltenango*: El Palmar, Palajunoj, Finca Santa Anita: SMF 82622; Finca El Carmen, Km 199 on Guat Hwy CA–2 (near Coatepeque): UTA R20290–307, R26979–80; S slope Volcán Santa María, Finca El Faro, ca. 4.0 km N El Palmar: UTA R20252–56, R20257$^{\text{H}}$, R20258, R20259$^{\text{H}}$, R20260–89, R24077–81, R24082$^{\text{H}}$, R24083–91, R24092$^{\text{H}}$, R24093–127, R24129–33, R24134$^{\text{H}}$, R240135–160, R24162–253, R24254$^{\text{H}}$, R24255–349; S slope Volcán Santa María, Finca La Florida, 4.0 km N El Palmar: UTA R24350; *Retalhuleu*: Champerico: CAS 68038–41; Hacienda Casa Blanca, 1–2 km SW main house: UMMZ 107583; Hacienda Casa Blanca, 3 km W main house: UMMZ 107584; Sitio Archeologico Abaj Takalik, near El Asintal, 14° 38′ 03″ N, 91° 43′ 58″ W, 570 m: SMF 84451; *San Marcos*: 2 km W San Rafael Pie de la Cuesta: ANSP: 30587–88; ECA El Porvenir, camino de ingreso: USAC 277; SW slope of Volcán Tajumulco, El Porvenir, Río Camarón: USAC 278–79; Finca La Paz, 2 km NW La Reforma: UMMZ 98188–89; Finca El Porvenir, Finca Colima, 887 m: UMMZ 107128; El Amparo: SMF 78440; Malacatan, Finca Barranca Honda: UTA R39905; Malacatán, Finca San Ignacio: UTA R45740, R45741$^{\text{H}}$, R45742, R45743–45$^{\text{H all}}$, R45746, R45747–48$^{\text{H both}}$, R45749–53, R45754–56$^{\text{H all}}$, R45757–60, R45761–64$^{\text{H all}}$, R45765–67, R45768–69$^{\text{H both}}$, R45770, R45771$^{\text{H}}$, R45772–73, R45774–76$^{\text{H all}}$, R45777–78, R45779$^{\text{H}}$, R45780–82, R45783–84$^{\text{H both}}$, R45785–87, R45788$^{\text{H}}$, R45789, R45790$^{\text{H}}$, R45791–96, R45797$^{\text{H}}$, R45798; Municipio San Rafael Pie de la Cuesta, road from San Rafael Pie de la Cuesta to Aldea Patí: UTA R39902–04; 2 km SE of detour from Malacatán–San Marcos road toward El Tumbador, 14° 55′ 17.8″ N, 91° 58′ 09.9″ W, 630 m: SMF 84471–72$^{\text{H both}}$ *Suchitepéquez*: between San Miguel Panán and Chicacao, 14° 32′ 23.0″

N, 91° 21′ 38.4″ W, 440 m: SMF 84458^{H}, 84459^{H}, 84460–61; 3 km NW San Antonio, 14° 31′ 58.4″ N, 91° 23′ 43.9″ W, 350 m: SMF 84462–63$^{H\ \mathrm{both}}$, 84464; 1.5 km W Mazatenango, Puente Río Negro, 14° 32′ 03.8″ N, 91° 32′ 10.1″ W, 370 m: SMF 84450^{H}, 84465^{H}, 84466–69, 84470^{H}; Volcán Zunil: CAS 67163; Finca El Cipres [=near Samayac, 610 m]: MCZ 22965–67; *Zacapa*: near Arenal, 15° 07′ 51.3″ N, 89° 22′ 48.9″ W, 200 m: SMF 83969; Cabañas, Aldea El Arenal: UTA R41512; Cabañas, El Arenal, Laguna de Pinal: UTA R41511; Cabañas, Finca Jesús: UTA R29526; Cabañas, Quebrada Honda, El Vertiente: UTA R29529–31; Cabañas, Quebrada Honda: UTA R29527–28, R29802; San Vicente, Aldea "El Arenal": UTA R52137; Santa Cruz, 2.0 km N of Rio Pasabien: UTA R33445; Sierra del Merendón, Km 5.5 on road Gualán to Finca San Enrique: UTA R33448; Teculután, ca. 1.0 km N of central park of Teculután: UTA R41505–10. HONDURAS: *Atlántida*: La Ceiba: USNM 117607, 68059–60, 71721; Cordillera Nombre de Dios, Aldea Río Viejo: UTA R41234–36; 8 km W Sambo Creek: UF 90204; Barra de Río Ulúa (Punta Sal): SMF 81495, 81496^{H}; Laguna de Río Tinto, Tela (Punta Sal): SMF 81497, 81498^{H}, 81499; Los Bajos, Tela (Punta Sal): SMF 81500; Lancetilla, 10 m: AMNH 70422–29, 70431; SMF 77200–01; Parque Nacional Pico Bonito, Santiago, 110 m: SMF 77199; Parque Nacional Pico Bonito, La Muralla, 80 m: SMF 77198; CURLA forestry station, 10 km W La Ceiba, 120 m: SMF 87142; San Marcos: SMF 87128, 87134, 87140; *Colón*: Salama, 15° 00′ N, 86° 50′ W: USNM 242057–443; Trujillo: CM 59121, 64613; *Copán*: 1 km W Copán, 580 m: SMF 87150; *Cortés*: San Pedro Sula: USNM 24374; Peña Blanca, Plowdens's Finca, 14° 53′ N, 88° 05′ W: USNM 243382–86;5 mi. (by road) N Agua Azul, Bagope, Lago de Yojoa, 14° 51′ N, 87° 59′ W: USNM 243278–307; Amapa Res. Station, 300 ft: AMNH 70503; Agua Azul: AMNH 70400–01; 17 mi. W Puerto Cortés: SMF 79367; ca. 1 km SSE Tegucigalpita, 15° 37.17′ N, 88° 14.96′ W, 40 m: SMF 79137–38; 1 km SSE Tegucigalpita, 20 m: SMF 87139; *El Paraíso*: Carretera El Paraíso–Las Manos, Km 118: UTA R41264; *Francisco Morazán*: Reserva Biologica El Chile: SMF 80880^{H}, 80881; Madre Vieja: AMNH 70382–83; Bodega Wall: AMNH 70384; road from San Juan de Flores to Talanga, 14° 21′ 04″ N, 87° 02′ 29″ W, 928 m: UF 90205; Cantarranas: ANSP 26080–81; *Gracias a Dios*: Awasbila, 14° 47′ N, 84° 45′ W, 60 m: SMF 87133^{H}, 87136^{H}; Rus Rus, 60 m: SMF 87125–26, 87127, 87129^{H}, 87131, 87137, 87143^{H}, 87144, 87151; Karasangkan, 70 m: SMF 87147–48; Rio Coco (=Segovia River): USNM 24525–26; Patuca: USNM 20305; Mocorón: UTA R43564–65; *Intibucá*: Valle de Otoro, 14° 27.69′ N, 87° 57.87′ W, 690 m: SMF 77745; *Islas de la Bahía*: Isla de Utila, at town limit on trail to Rock Harbour, 16° 06.10′ N, 86° 53.77′ W: SMF 77763–64; Isla de Utila, Iguana Station, 16° 06.19′ N, 86° 53.93′ W, 30 m: SMF 78142^{H}, 79366^{H}, 79863; Isla de Utila, Jake's Bight: SMF 77112; Isla de Utila, 1 km N Utila town, trail to Pumpkin Hill: SMF 77113; Isla de Utila, 2 km N Utila town, trail to Iron Bound: SMF 77114–17; Utila (town): SMF 87132, 87141; *La Paz*: La Paz: SMF 77263^{H}, 77264; *Lempira*: Erandique: CM 64604; *Olancho*: San Esteban: UTA R41265; *Santa Barbara*: Quimistán: USNM 128090; *Yoro*: San Patricio, 280 m: SMF 77192, 77197^{H}; 4,7 km ESE San Lorenzo Arriba, 220 m: SMF 87130, 87138. MEXICO: *Chiapas*: Finca El Rancho, ca. 20 km (by road) NNW of Tapachula, on road to Nueva Alemania, 370–600 m: USNM 266269, 266278–79; Puerto Aristo: USNM 148539–40; Tonalá: USNM 47809; Huehuetan: USNM 47757; Piedra Parada, 10 mi. N Ocozocoautla: USNM 121403–14; Yaxoquintela [=Yaxoquintelz], 16° 58′ N, 91° 47′ W, 560 m: CM 88774–78; 18 km SE Raudales: ENCB 1931; 18 km SE Venustiano Carranza, 480 m: ENCB 7036; 35 km S, 15 km E Venustiano Carranza, 500 m: ENCB 8750; 35 km S, 4 km W Comitán: ENCB 8751–52; Finca Cuatimoc, 360 m: ENCB 384; Tapachula: AMNH 80014, ENCB 1804–05; Tuxtla Gutiérrez: ENCB 10224, UTA R11505–08; 9.3 km N, 3 km W Tuxtla Gutiérrez: ENCB 11086–89; 4 km N, 5.3 km W Tuxtla Gutiérrez: ENCB 11094, 11097; 16.3 km S, 1.6 km E Tuxtla Gutiérrez: ENCB 11098–102, 11104, 11106–07, 11109–14; Infronarit "Los Laguitos", Tuxtla Gutiérrez: MZ–UNICACH 658–59; Coapilla: MZ–UNICACH 189; Tuxtla Gutiérrez, El Zapotal: MZ–UNICACH 13A–B; Río Chiquito, 0.5 km NE Chiapa de Corzo: MZ–UNICACH 237; Río Chiquito, 1 km E Chiapa de Corzo: MZ–UNICACH 238; Colonia Francisco Sanabia, Chiapa de Corzo: MZ–UNICACH 243; Ciudad Hidalgo, near Guatemalan–Mexican border: SMF 77185; Finca Irlanda [40 km NE Tapachula]: SMF 81543; Finca Irlanda, near coffee plantation, 15° 09′ 36.9″ N, 92° 21′ 03.2″ W, 650 m: SMF 84456^{H}; Playa Linda, near Puerto Madero, 14° 41′ 05.1″ N, 92° 23′ 02.0″ W, 8 m: SMF 84457^{H}; Montocintla [=Motozintla de Mendoza, 15° 22′ N, 92° 14′ W], La Victoria: AMNH 66435; El Real: AMNH 66448–49; Huixtla: AMNH 101051–53; 40 mi. E Comitán: AMNH 79941; Ruinas de Palenque: AMNH 114820, SMF 78443–59^{H}; Palenque village: AMNH 114821; Hotel Chonkah, 4 km from Ruinas de Palenque: AMNH 114819; Río Nututun al W de Palenque: IBH 7460; 1 mi. S of Hwy 190, 6 mi. W Ocozocuautla: CM 64599; *Oaxaca*: "Oaxaca": USNM 47345; Chimalapa Cofradia: UTA R51891; N slope Sierra Juárez Metates: UTA R11509–10, R18249; vicinity slightly N of Palomares: UTA R51889–90; 5 km W Mixtequilla: SMF 84066^{H}; 24 km S Jesus Carranza: AMNH 79940; 30 km S Jesus Carranza, Hacienda La Oaxaceña: AMNH 62340; Mitla: AMNH 90827–28; Juquila Mixes: AMNH 89653–55, 102743; Quiengolani: AMNH 64998; Oscuranos, near Tehuantepec: AMNH 68147–48; Cafetal Mirador, San Miguel del Puerto: AMNH 17955, 17961; Mixtequilla: AMNH 19349; Sta. Lucia: AMNH 64975; Cerro San Pedro: AMNH 65814; San Pedro, near Tehuantepec: AMNH 66935; Matias Romero: AMNH 101055; Guichicovi [=San Juan Guichicovi, 16° 58′ N, 95° 06′ W]: USNM 47346; 10 mi. N Palomares, 213 m: USNM 148560; Tehuantepec (Isthmus of): USNM 30142–60; N Juchitán: SMF 78460–73^{H}; Tehuantepec (city): AMNH 58037, USNM 30481. NICARAGUA: "Nicaragua": ANSP 7884, FMNH 2063; Isla San Fernando: ZFMK 51872; *Atlántico Norte*: Wounta Haulover: ANSP 20044; 3–4 km W Puerto Cabezas (on road to Waspan), 30 m: KU 101410; Alamikamba, 13° 30.08′ N, 84° 13.64′ W: SMF 77343–47, 77348^{H}, 77349–50, 77351^{H}, 77352–55, 77356–57$^{H\ \mathrm{both}}$, 77359, 77360^{H}, 77361–63; Moss: JS 1094–96$^{H\ \mathrm{all}}$, JS 1097; cross section of roads from Puerto Cabezas to Waspam with road to Moss: JS 1146^{H}; Krin Krin: JS 1162^{H}, 1163, 1181; Saslaya, 13° 44′ 13.7″ N, 84° 53′ 90.8″ W, 110 m: JS 303^{H}; *Atlántico Sur*: Isla del Maíz Grande: AMNH 97034, KU 159642–43, 159645, 159680; Kukra: AMNH 17117–23, 17128; *Boaco*: 1 km N Camoapa: KU 195079; *Chontales*: Juigalpa: SMF 77590; *Granada*: 5 km W and 7 km S Granada, 280 m: KU 85719; Reserva Privada "Domitila", 11° 42′ N, 85° 55′ W (E Nandaime, S Volcan Mombacho): SMF 86936–39, 86940$^{\mathrm{H}}$, 86941; *Jinotega*: Bosawas: JS 917–18$^{\mathrm{H\ both}}$; *Managua*: Bologna: CM 67190; 3 mi. SW Managua: KU 42270–71; Las Nubes: JS 851; *Matagalpa*: Comarca Peñas Blancas Finca San Sebastián: UTA R44853–55$^{\mathrm{H\ all}}$; El Carmen: UTA R44852$^{\mathrm{H}}$; between Matagalpa and Jinotega: SMF 77333; Cerro Musún, village of Palán, District Bilampí, 13° 01′ 32.6″ N, 85° 14′ 20.7″ W, 458 m: JS 665, SMF 86681$^{\mathrm{H}}$; Cerro Musún, southern portion, 13° 00′ 41.0″ N, 85° 14′ 11.6″ W, 622 m: JS 684$^{\mathrm{H}}$, 685, SMF 86682; Cerro Musún, 12° 56′ 58.0″ N, 85° 14′ 01.6″ W, 450 m: SMF 86683; *Río San Juan*: Río San Juan, Bartola, 10° 58.37′ N, 84° 20.35′ W, 30 m: SMF 79819$^{\mathrm{H}}$, 79820, 80964$^{\mathrm{H}}$; Islas de Solentiname, Isla Mancarron, Hotel "Mancarron": SMF 82076$^{\mathrm{H}}$; Sabalos: SMF 86680; Fundeverde, Río Frío, 11° 04′ 37.0″ N, 84° 44′ 55.7″ W, 45 m: SMF 86685$^{\mathrm{H}}$, JS 326; *Rivas*: Isla de Ometepe: JS 1028$^{\mathrm{H}}$, SMF 82242–43, ZFMK 51871; Isla de Ometepe, within 2 km S Moyogalpa, 40 m: KU 85718; Isla de Ometepe, Volcán Maderas, N slope above Finca Magdalena, cacao plantation, 450 m: SMF 80277; Morgan Rock, 11° 19′ 36.9″ N, 85° 54′ 50.0″ W, 37 m: JS 818$^{\mathrm{H}}$, 823, SMF 86684.

Norops wellbornae.—EL SALVADOR: *Ahuachapán*: Finca Concepción Miramar, 13° 48.46′ N, 89° 48.03′ W, 920 m: KU 290054; El Refugio, vicinity of Mariposario of Francisco Serrano, 13° 49.46′ N, 89° 59.98′ W, 225 m: SMF 79235$^{\mathrm{H}}$, 79236–38; Parque Nacional El Imposible: MUHNES 912; Finca San Benito: MUHNES 1046, Río Guajapa: MUHNES 1063; Barra de Santiago: KU 184130; *Cuscatlán*: Finca near San Martín, ca. 700 m: SMF 42512–13; El Playón Cujiapa: MUHNES 603; Colima Dry Forest, 14° 03′ N, 89° 08′ W, 420 m: MUHNES C–30–1392; *La Libertad*: Laguna de Chanmico, 480 m: SMF 42443–45, 42481, 42625; Quetzaltepeque: CAS 94796; Finca Mirasol, 900 m: SMF 42702; Los Chorros, between Nuevo San Salvador [=Santa Tecla] and Colón: SMF 42448; Finca El Paraíso, Santa Tecla: SMF 42619, 42712, 42800, 42868; Nuevo San Salvador [=Santa Tecla]: SMF 43140–42; W of La Libertad (E side Río Chilama): SMF 42405–06$^{\mathrm{H}}$; Río San Antonio, 2 km E La Libertad: SMF 43093–99, 43185, 44391; Río San Antonio: SMF 45140; La Libertad: MUHNES 786; Laguna de Zapotitán, 1500 ft: MVZ 40047; Volcán San Salvador, "1917 Lava," 500 m: FMNH 65016; 1.8 km NE Quetzaltepeque: KU 184131; *La Paz*: Zacatecoluca: MUHNES 722; La Zunganera: KU 184132–33; *La Unión*: Punta Gorda near La Unión: SMF 43139; *Morazán*: Loma Tendida: MUHNES 605–09, 631–32, 634–36, 638, 640, 642, 646; 3 km W of Divisadero: SMF 42994; Montecristo Mine, 700 ft.: MVZ 39939–45, 39950–51, 39975–80, 39982–84; 1 mi. SE Divisadero, 850 ft.: MVZ 40030; Divisadero: FMNH 10982, 10950–55; Perquín: KU 291351; *San Miguel*: San Miguel: SMF 42948; Laguna Aramuaca: SMF 44329–30; Cantón El Colorado: MUHNES 604; Laguna de Olomega, 200 ft.: MVZ 39946–49, 39986–91, 39993–94, 40031; Volcán de San Miguel: MCZ 57062; San Pedro: MCZ 57079; *San Salvador*: San Salvador: ZMB 35710 (holotype of *A. ustus wellbornae* Ahl); San Salvador, 65 Avenida Sur, Casa Reich: SMF 42216; San Salvador, Colónia America Nr. 3, Villa Margarita: SMF 42306; San Salvador, vicinity of Instituto Tropical de Investigaciones Científicas: KU 62035–36, SMF 44323–28, 51980–81 (700-m elevation); "environs from San Salvador": SMF 51748–49; Colónia Altos del Cerro, near slope of San Jacinto Mountain: MUHNES C–30–1393; near Ateos, N of San Salvador: MCZ 54964; San Salvador, near airport: CAS 144032–35; 1 mi. NW San Salvador: KU 42263; *San Vicente*: crater of Volcán de San Vicente: SMF 46813; *Santa Ana*: San Juan Mine, 7.5 mi. SE Metapán, 1600 ft.: MVZ 40001–06, 40032; Hacienda San José: KU 67102, 6 km S Metapán: KU 184137; 6 km S Metapán, Cantón Las Piedras: KU 184134; Metapán, San Diego: MUHNES 1191–92; *Sonsonate*: Hacienda San Antonio, near Sonsonate: SMF 42490–93; Hacienda Chilata, 610 m: MVZ 40034–35, 40039; Bosque Las Lajas, 13° 50.04′ N, 89° 33.56′ W, 920 m: KU 289885; *Usulután*: Isla San Sebastián, 13° 10.01′ N, 88° 24.49′ W, 20 m: KU 289917. GUATEMALA: *Chimaltenango*: Finca Payacal, near Pochuta: MCZ 32496; *Escuintla*: Finca El Zapote: UF 33506; Finca Tecolote, Guanagazapa, 14° 16′ 10″ N, 90° 37′ 58″ W, 790 m: SMF 82623$^{\mathrm{H}}$, 82664$^{\mathrm{H}}$; Finca Medio Monte, 14° 21′ 02″ N, 90° 44′ 07″ W, 685 m: SMF 82665, UTA R33447; Río Coyolate: USNM 12598; Puerto San José: ZMH 4596; 1 km N Puerto San José: SMF 79368–69$^{\mathrm{H\ both}}$, 79370–71; 18.2 km on road from Puerto San José to Escuintla, 14° 04.35′ N, 90° 46.59′ W, 20 m: SMF 79372$^{\mathrm{H}}$, 79373; Masagua: UVG 2114; in and around town of Finca El Salto: AMNH 90366; Finca El Caobanal, ca. 26 air Km SE Escuintla, Km 86 on CA–2 (road to El Salvador): UTA R24356, R26982, R45956–57; ca. 6.0 km NNE Escuintla, Finca San Fernando: UTA R26983; Masagua, Cuyuta, Finca Nacional Icta (Km 84 on Carretera Puerto San José): UTA R29532$^{\mathrm{H}}$; S slope Volcán de Agua, Finca Rosario Vista Hermosa: UTA R22075–76, R22078, R26981; 5 km S Tiquisate, 14° 13′ 20.1″ N, 91° 19′ 44.3″ W, 54 m: SMF 84452$^{\mathrm{H}}$; *Jutiapa*: 7–8 km E Jutiapa along Río Amayita, 950 m: UMMZ 106950 (1–2); Finca La Trinidad, 1–4 km E main house: AMNH 80067, UMMZ 107619; Finca La Trinidad: UMMZ 107620; *Santa Rosa*: Finca La Gloria, 1–2 km NW main house, 1040 m: UMMZ 107591; Finca La Gloria, 800–1010 m: MCZ 56498, UMMZ 107592; Finca La Gloria, 7 km SE main house, 750 m: UMMZ 107594; Las Lisas: UMMZ 107596; Finca Los Positos: UVG 1546; Barberena, Laguna de Pino: UTA R37629; Cuilapa, Zona Militar Number 6: UTA R39785; El Oratorio 3.0 km E CA–8: UTA R33446; Km 26 on Hwy 16 between Cuilapa and Chiquimulilla (on lower slope of Volcán Tecuamburro): UTA R37628; Parque Nacional Laguna El Pino: UTA R24351; *Sololá*: Finca Olas de Moca: AMNH 38014; San Lucas Taliman: UTA R52134–36; *Suchitepéquez*: 5 mi. SE of Patulul, Rio Coyolate: USNM 125298; Reserva Natural Los Tarrales, 14° 31′ 57″ N, 91° 09′ 05″ W, 950 m: SMF 82624–25$^{\mathrm{H\ both}}$, 82670; Finca San Julian, 14° 28′ 01″ N, 91° 08′ 09″ W, 480 m: SMF 82666$^{\mathrm{H}}$, 82669$^{\mathrm{H}}$; road from Hacienda San Cayetano to San José El Idolo, 14° 15′ 47.3″ N, 91° 28′ 12.2″ W: SMF 84473$^{\mathrm{H}}$; Pueblo Talpushate, 2 km after detour from road Hacienda San Cayetano–San José El Idolo, 14° 23′ 54.5″ N, 91° 27′ 25.6″ W: SMF 84474$^{\mathrm{H}}$; 8.3 km W detour to Tiquisate, 14° 25′ 34.0″ N, 91° 22′ 43.5″ W, 127 m: SMF 84453, 84454–55$^{\mathrm{H\ both}}$. HONDURAS: *Choluteca*: between San Lorenzo and Choluteca: AMNH 70404–06; El Madrenal, 160 m: SMF 87124$^{\mathrm{H}}$, 87145–46, 87149; *Intibucá*: near Santa

Lucia, 13° 55.17′ N, 88° 23.43′ W, 375 m: SMF 78904^{H}, 79140. NICARAGUA: *Chinandega*: Corinto: AMNH 62778; Volcán Casita, Pikin Guerro (=Versalles), 600 m: SMF 82998; 4 km N, 2 km W Chichigalpa: KU 85678; Finca San Isidro, 10 km S Chinandega, 10 m: KU 85707–16; San Antonio, 15 m: KU 85679–706; *León*: Finca de Don O. Sampson: SMF 86698^{H}, JS 638^{H}; Palvon (=El Polvon): USNM 120757, 120759; km 16.5 on road León–Poneloya, 15 m: SMF 82996^{H}, 82997; El Jicaral: JS 868^{H}, 869, 879–80$^{H\ \mathrm{both}}$, 881–82.

Příloha 6.

Köhler, G., **Veselý, M.,** 2011: A new species of Thecadactylus from Sint Maarten, Lesser Antilles (Reptilia, Squamata, Gekkonidae). *ZooKeys* 118: 97-107.

The contribution in words:

Both authors participated equally on all aspects of the study and contributed critically to the final draft of the manuscript.

Ranking: IF_{2011} – 0.879; Q3 (Zoology)
Citace na WoS (Core collection; bez autocitací všech spoluautorů): 8
Citace celkem (Google Scholar): 16

ZooKeys 118: 97–107 (2011)
doi: 10.3897/zookeys.118.1476
www.zookeys.org

RESEARCH ARTICLE

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A new species of *Thecadactylus* from Sint Maarten, Lesser Antilles (Reptilia, Squamata, Gekkonidae)

Gunther Köhler[1,†], Milan Vesely[2,‡]

1 *Forschungsinstitut und Naturmuseum Senckenberg, Senckenberganlage 25, 60325 Frankfurt a.M., Germany* 2 *Department of Zoology, Faculty of Natural Sciences, Palacký University, tř. Svobody 26, 77146 Olomouc, Czech Republic*

† *urn:lsid:zoobank.org:author:26AFE6BA-9ABC-4EAE-93E8-CEFA92CCEC42*
‡ *urn:lsid:zoobank.org:author:2A36A593-38D1-4687-A38D-A35794B174B0*

Corresponding author: *Gunther Köhler* (gkoehler@senckenberg.de)

Academic editor: *Hans-Dieter Sues* | Received 04 May 2011 | Accepted 24 June 2011 | Published 13 July 2011

urn:lsid:zoobank.org:pub:D2CB2B84-A73E-47C4-AB88-F2DA1D2095A2

Citation: Köhler G, Vesely M (2011) A new species of *Thecadactylus* from Sint Maarten, Lesser Antilles (Reptilia, Squamata, Gekkonidae). ZooKeys 118: 97–107. doi: 10.3897/zookeys.118.1476

Abstract

We describe a new species of *Thecadactylus* from the Caribbean island of Sint Maarten. The new species differs from all other species in the genus by having a distinct dorsal pattern of numerous irregular but sharply deliminated black spots and blotches on an otherwise almost patternless background.

Keywords

Gekkonidae; Lesser Antilles; new species; Reptilia; Sint Maarten; Squamata; *Thecadactylus*.

Introduction

Turnip-tailed geckos (genus *Thecadactylus*) are moderate-sized to large geckos distributed from southeastern Mexico across most of Central America and mesic tropical South America, and also occupy the Lesser Antilles (Russell and Bauer 2002). Whereas these geckos have traditionally been understood as a monotypic genus (e.g., Peters and Donoso-Barros 1970; Hoogmoed 1973; Avila-Pires 1995), currently two species of

Thecadactylus are recognized (Bergmann and Russell 2007): *T. rapicauda* (Houttuyn, 1782) and *T. solimoensis* Bergmann & Russell, 2007. All *Thecadactylus* populations from the Lesser Antilles have always been considered as conspecific with *T. rapicauda* (Powell et al. 1996, Censky and Kaiser 1999, Malhotra and Thorphe 1999).

In 2010, several specimens of a distinctly spotted *Thecadactylus* were collected on the island of Sint Maarten (also known as Saint Martin), Lesser Antilles, and imported to Germany by a pet trade dealer. In order to verify the geographic origin of these individuals, Stephan Prein and Maciej Oskroba visited Sint Maarten in April 2011. They indeed encountered *Thecadactylus* there and found that all specimens from this island had a distinctly spotted but otherwise patternless dorsum. A research of the pertinent literature revealed that this peculiar form had already been reported and illustrated from Sint Maarten (Breuil 2002, 2003). A comparison with the *Thecadactylus* from many localities across the wide geographic range of these geckos demonstrated that the Sint Maarten population represents an undescribed species and therefore, we describe it as a new species below.

Materials and methods

A list of the comparative specimens examined is provided in the Appendix. Abbreviations for museum collections follow those of Leviton et al. (1985). Furthermore, we have studied and analysed photographic material published in Powell (1996), Breuil (2002), Russell and Bauer (2002), and Powell et al. (2005). Nomenclature of scale characters follows that of Avila-Pires (1995). Subdigital lamellae were counted as suggested by Bergmann and Russell (2003). Scale sizes were measured using the ocular micrometer of a stereo microscope (Leica MZ 12) and rounded to the nearest 0.01 mm. All other measurements were made using precision calipers and were rounded to the nearest 0.1 mm. Head length was measured from the tip of the snout to the anterior margin of the ear opening. Snout length was measured from the tip of the snout to the anterior border of the orbit. Head width was determined as the distance between the oral ricti. Tail height and width were measured at the point reached by the heel of the extended hind leg. Dorsal and ventral scales were counted at midbody along the midline. Abbreviations used are DHL (number of medial dorsal scales in one head length), HL (head length), HW (head width), INL (infralabials), SAM (scales around midbody), SPL (supralabial scales), SVL (snout–vent length), and VHL (number of medial ventral scales in one head length). For the synonymy list, only those works have been included that cite actual specimens from Sint Maarten. Temperature was recorded at the type locality (see below) in the time from 11 – 16 April 2011 using an automatic temperature data logger (HOBO Pendant temp) placed on an upstanding tree trunk about 3 m above the ground in the shade recording at intervals of 2 min.

Results

Thecadactylus oskrobapreinorum **sp. n.**

urn:lsid:zoobank.org:act:0F9770AC-C296-462A-B23F-F3356ECC4BE5
http://species-id.net/wiki/Thecadactylus_oskrobapreinorum
Figs 1–3

Thecadactylus rapicauda: Breuil (2002, 2003; in part.), Bergmann and Russell (2007; in part.).

Holotype. SMF 92120, an adult male from Sint Maarten, near the southern edge of the village of Dawn Beach, 18.042°N, 63.023°W, 45 m elevation; collected 12 April 2011 by Stephan Prein and Maciej Oskroba.

Paratypes. SMF 92194, 92721–29, same collecting data as holotype.

Diagnosis. A species (SVL in largest specimen examined 99 mm) of the genus *Thecadactylus* (sensu Russell and Bauer 2002) that differs from all other species in the genus by having a distinct dorsal pattern of numerous irregular but sharply deliminated black spots and blotches on an otherwise almost patternless background. *Thecadactylus oskrobapreinorum* lacks a dorsally directed postocular stripe (such stripe present in most specimens of *T. solimoensis*; see Bergmann and Russell 2007). *Thecadactylus oskrobapreinorum* differs further from *T. rapicauda* in the mean values of several morphometric and pholidotic characteristics, most pronounced in the number of subdigital lamellae and supralabial scales (see Table 1).

Description of the holotype. Adult male as indicated by partially everted hemipenes; SVL 95.5 mm; tail length 75.0 mm, tail complete; tail almost round in cross section, tail height 6.9 mm, width 7.6 mm; axilla to groin distance 37.0 mm; head length 24.5 mm; snout length 12.2 mm; head width 20.5 mm; shank length 16.2 mm. Rostral large, rectangular, about twice as wide as deep, visible from above, and with a long median cleft extending from posterior margin; 2 relatively large, rectangular postrostrals; nostril bordered by rostral, first supralabial, 3 small postnasals and one postrostral; scales on snout and on loreal region granular, mostly keeled; 22 loreal scales in a longitudinal line between rostral and orbit; scales on upper and posterior portions of head slightly smaller than on snout; scales in supraorbital region not differentiated from those on upper part of head; supraciliary flap bordered by a double row of scales, 18 in outer row between anterior border of flap and a point above center of eye, with 7 small spines posteriorly; pupil four-lobed, vertically elongate; 8 supralabials to level below center of eye, total number 10, anterior supralabials subequal in size, below eyes decreasing in size; ear opening obliquely oval, 3.0 x 1.5 mm (length x height) distinctly smaller than eye (eye length 6.1 mm); mental larger than adjacent scales, pentagonal; 2 relatively large postmentals, at each side followed by a row of smooth, polygonal scales, decreasing in size posteriorly, and in contact

Table 1. Selected measurements, proportions and scale characters of *Thecadactylus oskrobapreinorum* and *T. rapicauda*. Range is followed by mean value and standard deviation in parentheses. For abbreviations see text. For tail length, only complete original tails were measured.

		T. oskrobapreinorum ♂ 4 ♀ 4	***T. rapicauda*** ♂ 11 ♀ 9
SVL	♂	86.0–96.5 (90.6 ± 3.83)	74.0–95.0 (85.5 ± 6.87)
	♀	87.0–99.0 (94.0 ± 4.85)	79.0–119.0 (93.9 ± 11.65)
Tail length	♂	75.0–76.0 (75.3 ± 0.47)	65.0–72.0 (68.5 ± 3.50)
	♀	71.0–83.0 (77.0 ± 6.00)	75.0–76.0 (75.3 ± 0.47)
HL	♂	22.6–24.5 (23.5 ± 0.67)	18.2–24.7 (21.5 ± 1.75)
	♀	23.0–25.0 (24.3 ± 0.79)	18.8–28.0 (23.2 ± 2.56)
HW	♂	17.3–20.5 (18.4 ± 1.25)	14.7–19.9 (17.3 ± 1.95)
	♀	17.6–19.1 (18.2 ± 0.58)	13.2–22.9 (17.9 ± 2.74)
Shank length	♂	13.2–16.2 (14.2 ± 1.21)	10.0–14.5 (12.7 ± 1.29)
	♀	14.0–15.9 (14.5 ± 0.81)	10.6–17.2 (14.3 ± 1.95)
Axilla–groin distance	♂	37.0–38.7 (37.6 ± 0.68)	33.0–45.7 (39.1 ± 4.54)
	♀	32.5–43.0 (39.2 ± 4.14)	37.7–49.5 (42.5 ± 3.83)
Tail length / SVL	♂	0.78–0.87 (0.83 ± 0.04)	0.82–0.87 (0.85 ± 0.02)
	♀	0.72–0.90 (0.81 ± 0.09)	0.78–0.87 (0.83 ± 0.04)
HL / SVL	♂	0.25–0.27 (0.26 ± 0.01)	0.24–0.26 (0.25 ± 0.01)
	♀	0.25–0.27 (0.26 ± 0.01)	0.24–0.27 (0.25 ± 0.01)
Shank length / SVL	♂	0.15–0.17 (0.16 ± 0.01)	0.13–0.17 (0.15 ± 0.01)
	♀	0.14–0.16 (0.15 ± 0.01)	0.13–0.17 (0.15 ± 0.01)
Axilla–groin distance / SVL	♂	0.38–0.43 (0.42 ± 0.02)	0.41–0.53 (0.46 ± 0.03)
	♀	0.37–0.43 (0.42 ± 0.02)	0.42–0.49 (0.46 ± 0.02)
Subdigital lamellae of 4th toe		16–20 (18.13 ± 1.17)	18–23 (20.17 ± 1.62)
Subdigital lamellae of 4th finger		16–19 (17.88 ± 1.05)	17–23 (19.67 ± 2.13)
Number of SPL to level below center of eye		6–8 (6.63 ± 0.70)	8–10 (8.62 ± 0.62)
Number of INL to level below center of eye		8–10 (8.63 ± 0.70)	7–8 (7.62 ± 0.49)
Number of postrostrals		2	2
Number of postmentals		2	2
Number of medial dorsal scales in one head length		72–92 (81.25 ± 7.60)	64–88 (75.15 ± 8.24)
Number of medial ventral scales in one head length		44–56 (49.00 ± 4.24)	34–52 (40.62 ± 4.68)

with anterior infralabials; scales on chin granular, mostly pointed; scales on throat small, round, convex, juxtaposed; 8 (right)–7 (left) infralabials to level below center of eye, total number 10; infralabials mostly large, smooth, quadrangular to pentagonal, posterior ones smaller; dorsum of body with convex, juxtaposed to subimbricate scales with rounded posterior margins, about twice as large as scales on snout, largest dorsal scales about 0.35 x 0.29 mm (length x width); about 72 median dorsal scales in one head length; ventral scales at midbody smooth, juxtaposed to subimbricate with rounded posterior margins, forming oblique rows, about 0.42 x 0.39 mm (length x width); about 45 ventral scales in one head length; a gradual transition between dorsal and ventral scales; 218 scales around midbody; caudal scales smooth, imbricate, with rounded posterior margins, slightly larger ventrally; scales on limbs mostly smooth, subimbricate, with rounded posterior margins, equal to, to larger than dorsals; scales on posterior surfaces of forelimbs and on posterior and upper surfaces of hind limbs small, granular; fingers and toes depressed with a middorsal elevation, connected by a basal web; subdigital lamellae forming two transversely enlarged rows, divided by a median sulcus, 20 under fourth toe, 19 under fourth finger; claw on distal extremity of distal sulcus.

Coloration after one month in preservative (70% ethanol) was recorded as follows: Dorsal surfaces of head, body, limbs, and tail grayish brown with numerous irregular but sharply deliminated, black spots and blotches; ventral surfaces of head, body, and

Figure 1. Holotype of *Thecadactylus oskrobapreinorum* (SMF 92120). SVL = 95.5 mm.

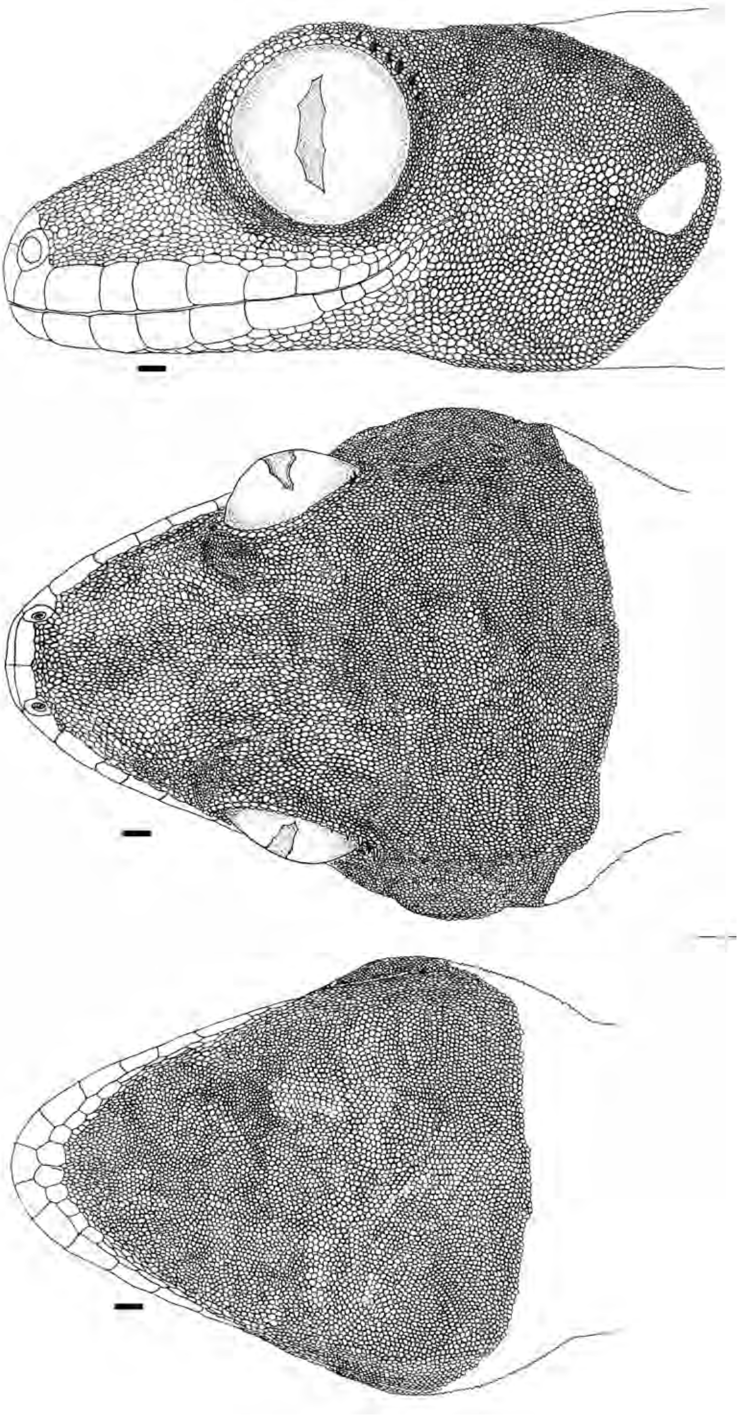

Figure 2. Head of holotype of *Thecadactylus oskrobapreinorum* (SMF 92120). Scale bar equals 1.0 mm.

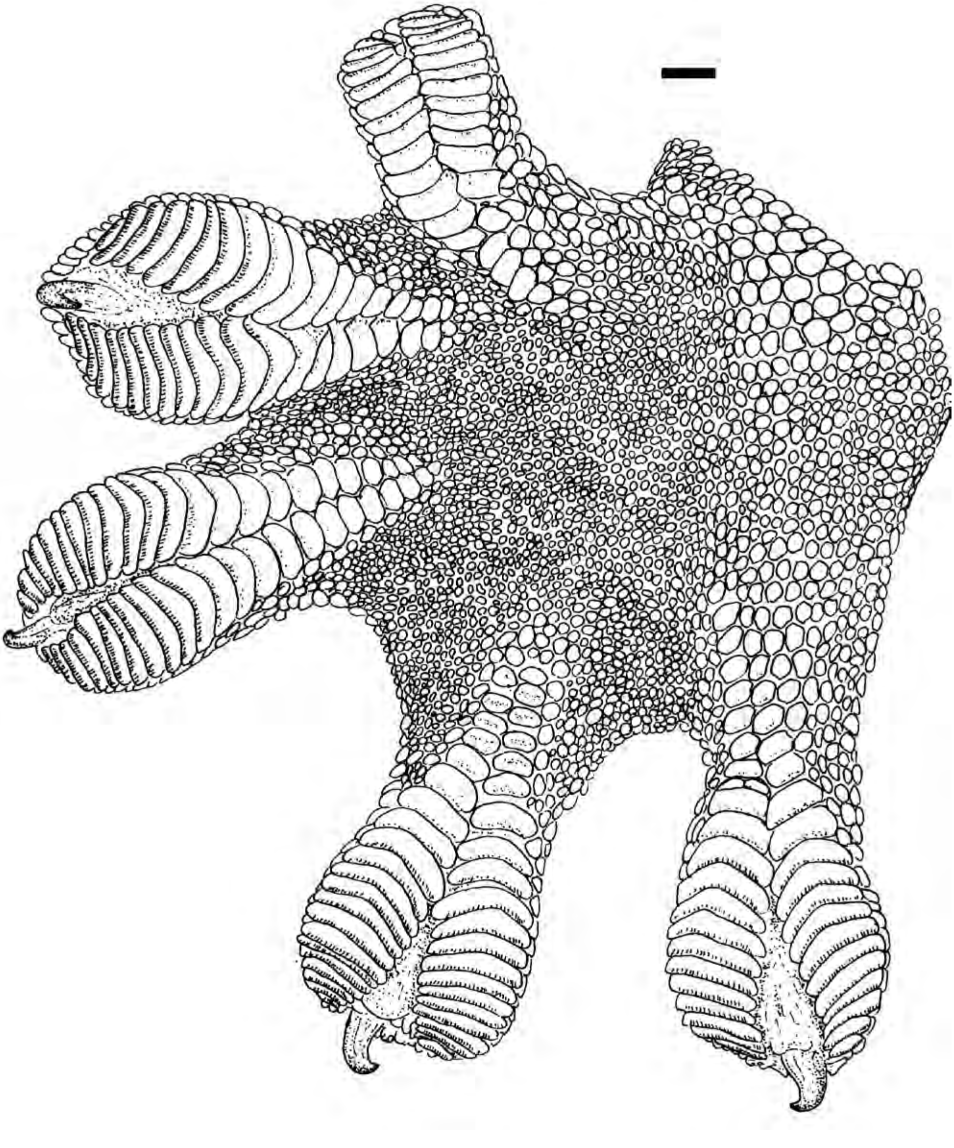

Figure 3. Right hind foot of holotype of *Thecadactylus oskrobapreinorum* (SMF 92120). Scale bar equals 1.0 mm.

limbs cream with gray shading and faint gray reticulations; widened lamellae of fingers and toes gray; ventral surface of tail brown with dark grayish brown reticulations.

Variation. The paratypes agree well with the holotype in general appearance, morphometrics and scalation (see Table 1). Variation of coloration in life is illustrated in Fig. 4. As can be seen, the number and distribution of the dark spots and blotches varies between individuals as does the background color which ranges from pale pearl gray over pale grayish yellow to grayish olive. Scale size differences in certain areas of gular

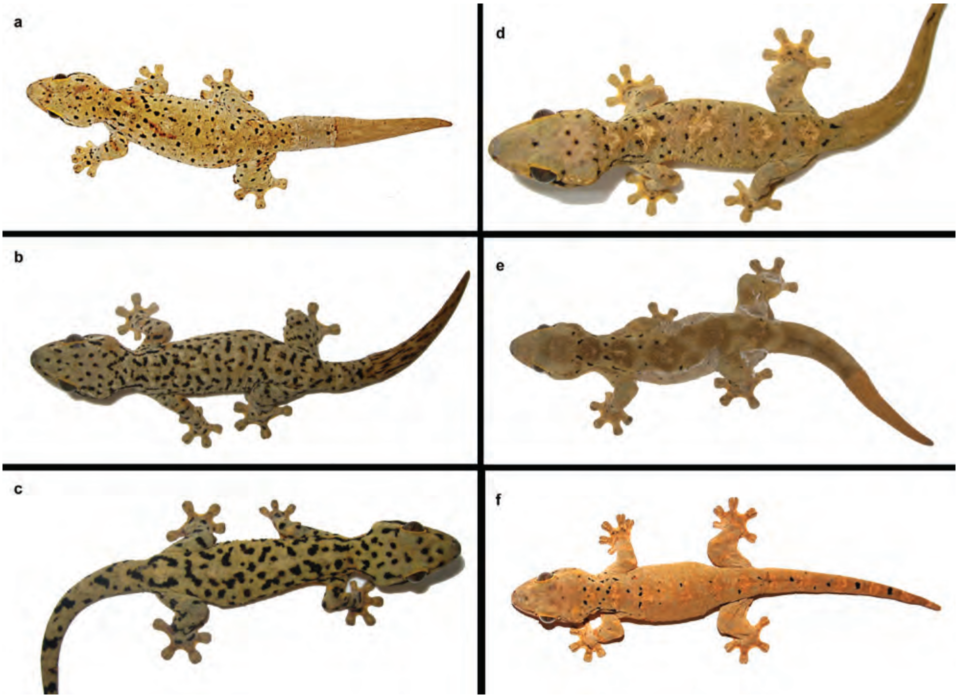

Figure 4. *Thecadactylus oskrobapreinorum* in life (specimens not preserved; all from Sint Maarten, Lesser Antilles). Photos a,e by Gunther Köhler; b,c,d by Stephan Prein, f by Maciej Oskroba

Figure 5. Habitat at the type locality of *Thecadactylus oskrobapreinorum* on Sint Maarten, Lesser Antilles. Photo by Maciej Oskroba

and temporal region of male holotype are probably the result of bites from other males in territorial fights. The damaged parts of the skin are covered by smaller granular scales as it is typical for scar tissue.

Etymology. The name *oskrobapreinorum* is a construction in the genitive plural honoring Maciej Oskroba and Stephan Prein, two German herpetoculturists who directed our attention to this new species and made field observations on this gecko on the island of Sint Maarten.

Natural history notes. All type specimens were collected at night while the lizards were active on the lower parts of the trunks of large living trees within or at the edge of forested areas (see also Fig. 5). From 11–16 April 2011, the air temperature (measured in the shade) varied at the type locality from 21.1–23.7°C (mean 22.7°C) in the morning hours and 24.6–28.2°C (mean 26.6°C) in the afternoon.

Geographic Distribution. As currently known, *Thecadactylus oskrobapreinorum* is restricted to the island of Sint Maarten, Lesser Antilles. Although the type locality is in the Dutch portion of the island, the species is also known from several localities in the French portion (see records in Breuil 2002).

Discussion

The distinctive dorsal pattern in *Thecadactylus oskrobapreinorum* appears to be a fixed character in this species since no individuals without dark spots on a otherwise patternless dorsum have been documented (see also a photo of a specimen of this species in Breuil 2002, 2003). As described and illustrated, individuals of the *Thecadactylus* populations from the nearby islands of St. Eustatius, St. Barthelemy, and Saba show the "normal" dorsal pattern of *T. rapicauda* (Breuil 2002, Powell et al. 2005). However, Breuil (2002) pictures a specimen from the island of La Désiderade which exhibits a strikingly aberrant coloration with a almost white head contrasting with the mostly dark grayish body, limbs, and tail. A more comprehensive survey and analysis of variation, both of molecular genetic and of morphological traits, is needed in order to shed light on the actual species diversity of *Thecadactylus* on the Lesser Antilles.

We have not examined the types of the nominal species placed in the synonymy of *Thecadactylus rapicauda* (following e.g., Peters and Donoso-Barros 1970, Russell and Bauer 2002). However, based on the respective type localities, even given the vague nature of most of them, none of them came from near Sint Maarten, not even from the Lesser Antilles: *Gekko laevis* Daudin, 1802 (type locality: "Amérique méridionale"); *Gekko surinamensis* Daudin, 1802 (type locality: "Surinam"); and *Pachydactylus tristis* Hallowell, 1854 (type locality: "Liberia, west coast of Africa", in error fide Russell and Bauer 2002). Therefore, none of the aforementioned names can be applied to the species described herein. In the cases of *Gekko laevis* and *Pachydactylus tristis*, the types of both of which are lost and no accurate type locality given, the synonymy assignment to either *T. rapicauda* or *T. solimoensis* remains unsettled. Also, as pointed out by Bergmann and Russell (2007), additional molecular genetic work with more intensive sampling is needed in order to clarify the geographic boundaries between *T. rapicauda* and *T. solimoensis*.

Acknowledgments

Exportation permits were provided by Mervyn Butcher, Government of St. Maarten, Sint Maarten. For the loan of and/or access to specimens we thank William E. Duellman and John E. Simmons, University of Kansas, Natural History Museum (KU), Lawrence. Sebastian Lotzkat, Frankfurt, provided unpublished morphological data pertaining to Panamanian *Thecadactylus*. We thank Aaron M. Bauer, Villanova, for comments on an early draft of the manuscript.

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Appendix

Comparative material examined

Thecadactylus rapicauda – **Brazil:** Maranhão: SMF 8378. **Colombia:** Melgar, Tolima: SMF 70233. **Guatemala:** Sitio Arqueologico Quirigua, 76 m: SMF 84018. **Honduras:** Isla de Utila, near Iguana Station: SMF 79871; Isla de Utila, 1 km S Rock Harbour: SMF 77098; west end of Isla de Utila: SMF 77099; Río Platano Biosphere Reserve, vicinity of Río Cuyamel, 15°34.43'N, 85°0.248'W, 170 m: SMF 85940–41; Parque Nacional Patuca, Matamoros, 150 m: SMF 80824–25. **Nicaragua:** Bartola at Río San Juan, 10°58.37'N, 84°20.35'W, 30 m: SMF 82103; Parque Nacional Saslaya, 13°42.84'N, 84°58.66'W, 400 m: SMF 82875–77; Biosphere Reserve Bosawas, near Wiso, 13°59.67'N, 85°19.70'W, 246 m: SMF 78555; El Recreo, S side Río Mico, 25 m: KU 113016. **Panama:** Volante: SMF 89601; Cerro Tebata, Bocas del Toro Province: SMF 83638. **Surinam:** no further data: SMF 8374. **Trinidad:** Hotel Robinson Crusoe, Scarborough: SMF 65848; Alefounder, Grafton Estate: SMF 65849, 66185, 66204; Prospect Estate: SMF 66829. **Trinidad:** no further data: SMF 8376. **Venezuela:** Puerto Caballo: SMF 8375; between Guaramaco and San Fernando: SMF 8377.

Thecadactylus solimoensis – **Ecuador**: Pastaza Province, Arutam Field Station, 700 m east from the Camp (coordinates of the Camp): 1°47.28'S 77°48.31'W, 790 m: SMF 91034.

Příloha 7.

Köhler, G., Diethert, H. H., **Veselý, M.,** 2012: A Contribution To the Knowledge of the Lizard Genus Alopoglossus (Squamata: Gymnophthalmidae). *Herpetological Monographs* 26(1): 173-188.

<u>The contribution in words:</u>
GK, HD and MV participated equally on all aspects of the study and contributed critically to the final draft of the manuscript.

Ranking: IF_{2012} – 1.818; **Q1** (Zoology)
Citace na WoS (Core collection; bez autocitací všech spoluautorů): 10
Citace celkem (Google Scholar): 12

A Contribution To the Knowledge of the Lizard Genus *Alopoglossus* (Squamata: Gymnophthalmidae)

Author(s): Gunther Köhler , Hans-Helmut Diethert , and Milan Veselý
Source: Herpetological Monographs, 26(1):173-188. 2012.
Published By: The Herpetologists' League
DOI: http://dx.doi.org/10.1655/HERPMONOGRAPHS-D-10-00011.1
URL: http://www.bioone.org/doi/full/10.1655/HERPMONOGRAPHS-D-10-00011.1

Herpetological Monographs, 26, 2012, 173–188

A CONTRIBUTION TO THE KNOWLEDGE OF THE LIZARD GENUS *ALOPOGLOSSUS* (SQUAMATA: GYMNOPHTHALMIDAE)

Gunther Köhler[1,3], Hans-Helmut Diethert[1], and Milan Veselý[2]

[1]*Forschungsinstitut und Naturmuseum Senckenberg, Senckenberganlage 25, 60325 Frankfurt am Main, Germany*
[2]*Department of Zoology, Faculty of Science, Palacký University, Třída Svobody 26, 77146 Olomouc, Czech Republic*

Abstract: We studied scutellation and morphometrics in the gymnophthalmid lizards of the genus *Alopoglossus*. Six species are recognized: *Alopoglossus angulatus*, *A. atriventris*, *A. buckleyi*, *A. copii, A. festae,* and *A. lehmanni*. We place the nominal species *Alopoglossus andeanus* Ruibal in the synonymy of *A. angulatus*. We provide detailed descriptions and head scalation illustrations of each species, an identification key, and a distribution map.

Resumen: Se estudia la folidosis y morfometría de las lagartijas gymnophthalmidas del género *Alopoglossus*. Se reconocen un total de seis especies: *Alopoglossus angulatus*, *A. atriventris*, *A. buckleyi*, *A. copii*, *A. festae* y *A. lehmanni*. La especie nominal *Alopoglossus andeanus* Ruibal es considerada como sinónimo de *A. angulatus*. Se provee ilustraciones de la escamación de las cabezas, claves de identificación y un mapa de distribución.

Key words: Alopoglossus; Geographic distribution; Gymnophthalmidae; Morphological variation; Reptilia; Squamata

Lizards of the genus *Alopoglossus* are small leaf-litter inhabiting gymnophthalmids widely distributed across tropical South America. They can be distinguished from all other gymnophthalmid genera, except *Ptychoglossus*, by having oblique rows of folds, or plicae, covering almost the entire surface of the tongue; the tongue is covered by scale-like papillae in the other genera (Ayala and Harris, 1984).

In 1885, Boulenger erected the genus *Alopoglossus* to accommodate his new species, *Alopoglossus copii*, as well as *A. carinicaudatus* and *A. buckleyi*, two species that previously had been allocated to the genus *Leposoma* (e.g., O'Shaughnessy, 1881). A few years later, based on a single female, Peracca (1904) described *A. festae* from Vinces, Ecuador. In 1924, Ruthven described his new species, *A. amazonius*, on the basis of a single specimen from "Villa Murtinho, Matto [sic!] Grosso, Brasil." Brongersma (1946) described *A. copii surinamensis* from the "forest on the Lucie River, Suriname," a taxon placed in the synonymy of *A. carinicaudatus* by Ruibal (1952). The latter author described a new species, *A. andeanus*, based on a single specimen from "La Pampa, Dept. of Puno, Peru," 760 m. This taxon is still known only from the holotype. Duellman (1973) added *A. atriventris*, a species described from "Lago Agrio, Provincia Napo, Ecuador." Hoogmoed (1973) resurrected Linné's *Lacerta angulata* (=*Alopoglossus angulatus*) and placed *A. carinicaudatus* in its synonymy. By neotype selection, Hoogmoed (1973) restricted the type locality of *A. angulatus* to "Brown's Mountain, Suriname." In 1984, Ayala and Harris described *A. lehmanni* based on a single male from "km 22 on the road from Buenaventura to San Isidro on the lower Calima River, Department Valle de Cauca, Colombia." Vanzolini (1986: 1) erroneously stated: "*A. copii* Boulenger = *Leposoma southi*, fide Uzzell and Barry, Postilla 154." However, Uzzell and Barry (1971) only corrected the identification of two misidentified ordinary specimens that Boulenger (1913: 1033) had referred to as *A. copii*. There is no indication in the work of Uzzell and Barry (1971) that they intended to synomymize *A. copii* with *L. southi*.

Avila-Pires (1995) published a detailed description of the Brazilian species of *Alopoglossus*. However, little data are available on the morphological variation of the *Alopoglossus* populations outside of Brazil. Therefore,

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[3] Correspondence: e-mail, gkoehler@senckenberg.de

here we present the results of our study on the variation in scutellation and morphometrics from populations from throughout the geographic range of the genus with an emphasis on the *Alopoglossus* species occurring in Ecuador and Peru.

Materials and Methods

The species descriptions are based on 278 specimens of *Alopoglossus* from Ecuador, Peru, Brazil, Colombia, French Guiana, and Suriname examined by the authors (Appendix). The format of the description (i.e., the sequence of characters described) follows Avila-Pires (1995). The distribution maps are based exclusively on specimens examined by the authors. Abbreviations for museum collections follow those of Leviton et al. (1985), except those of Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru (MUSM)—“MUSM USNM field series” and “MUSM WED field series” both refer to specimens in MUSM for which no permanent museum numbers are available—and Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador (QCAZ). We have converted data for specimens whose collecting site was recorded using the UK and US systems of linear measure to metric equivalents. All georeferences are in geographical coordinate system and WGS 1984 datum. Nomenclature of scale characters follows that of Avila-Pires (1995) and Köhler (2008). All measurements were made using precision calipers and were rounded to the nearest 0.1 mm. We measured head length from the tip of the snout to the anterior margin of the ear opening. We determined head width as the distance between oral ricti. We describe the scales on side of neck as “leaf-like” when these are somewhat expanded with a pointed end oriented posteriorly (as opposed to conical or granular scales that are not expanded and have their pointed end oriented off the axis of the animal). Abbreviations are used for snout–vent length (SVL), head length (HL), head width (HW), tail length (TL), shank length (ShL), axilla–groin distance (AGD), and lateral neck scale size (ANS). The synonymy lists contain exclusively synonyms, no cresonyms.

Results

The status of Alopoglossus andeanus *Ruibal*

Ruibal (1952) described a new species, *Alopoglossus andeanus*, based on a single specimen from “La Pampa, Dept. of Puno, Peru,” (Fig. 1). The holotype of *A. andeanus* (Museum of Comparative Zoology, Harvard University [MCZ] 45590; Fig. 2) is an adult male as indicated by the high number of femoral pores and the slightly swollen base of the tail. It resembles *A. angulatus* (Fig. 3) by the shape of the lateral neck scales (keeled, imbricate, and mucronate). It is said to differ from *A. angulatus* (1) by its dark-brown pigmentation of ventrals, gulars, and mental region (vs. not pigmented in *A. angulatus*) and (2) by having smooth, posteriorly rounded median gulars (vs. keeled or smooth, pointed in *A. angulatus*; Ruibal, 1952; Peters and Donoso-Barros, 1970). However, we found that the two features mentioned above show considerable individual variation in *A. angulatus*. About 20% of the specimens of *A. angulatus* have moderately pigmented ventral surfaces. Particularly, large males tend to have a more intense dark-brown pigmentation of the ventrals, gulars, and mental region. The condition of gular scales also varies considerably among specimens of *A. angulatus*: 10.5% of the specimens examined have smooth, posteriorly rounded median gulars; 63.2% have smooth, pointed gulars; and 26.3% have keeled, sharply pointed gulars. The distinction between these three types of gular scale conditions is somewhat artificial because there are individuals that fall in between these categories (e.g., individuals having smooth, only very slightly pointed gulars). We did not find any geographic pattern in the observed variation in gular scale condition. We have examined the holotype of *A. andeanus* (MCZ 45590) and found that in external morphology and coloration it falls well into the variation documented for *A. angulatus*. Therefore, we consider *A. andeanus* Ruibal a junior synonym of *A. angulatus* (Linnaeus).

Alopoglossus *Boulenger*

Diagnosis.—Gymnophthalmids having (1) the dorsal surface of tongue completely covered with anteromedially converging plicae rather than scale-like papillae; (2) the posterior borders of the parietal and interparietal plates

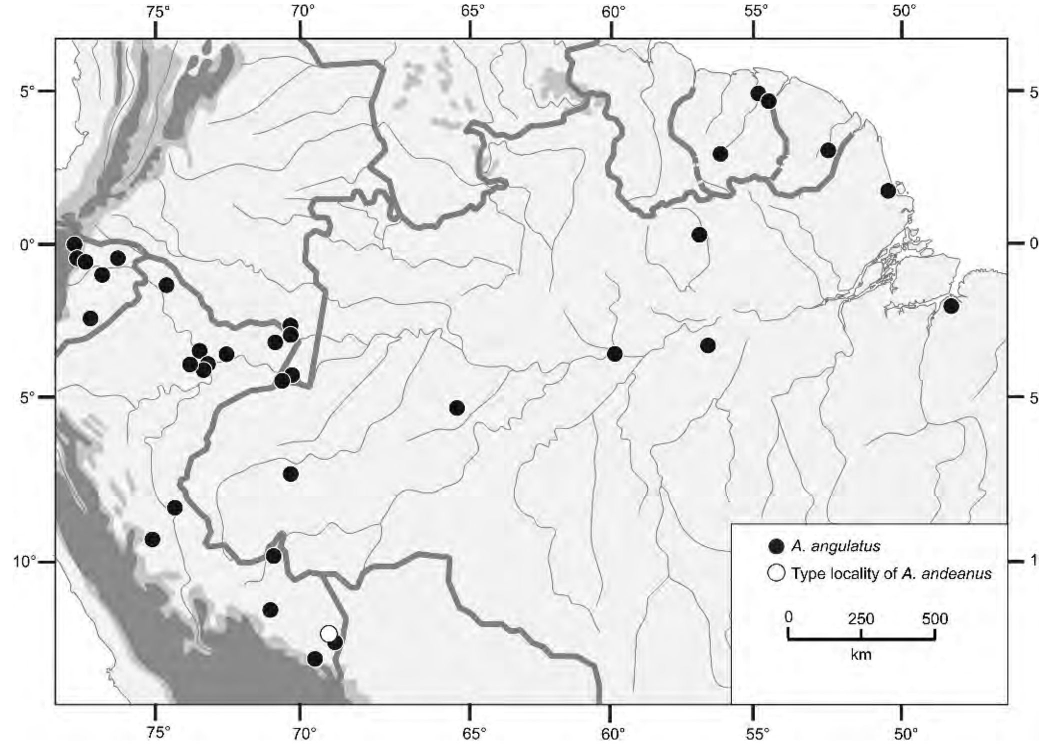

Fig. 1.—Map indicating collecting sites of *Alopoglossus angulatus* specimens. A single symbol can represent one or more nearby localities. Areas above 1000 and 2000 m are shaded gray.

forming a nearly straight rather than convex suture across the back of the head; (3) rhomboidal, lanceolate, or hexagonal strongly keeled dorsal scales, in oblique and/or transverse rows; (4) the dorsal scales covered with minute rounded to flattened projections; (5) the lower eyelid with semitransparent disc; (6) well-developed limbs, pentadactyl, with all digits clawed.

Synopsis of Species

We recognize six species in the genus *Alopoglossus*: *A. angulatus*, *A. atriventris, A. buckleyi*, *A. copii*, *A. festae,* and *A. lehmanni*. Their respective distributions are mapped in Figs. 1 and 4. Selected characters of scutellation and morphometrics are summarized in Table 1. We provide an identification key and standardized descriptions for these six species.

Key to the Species of Alopoglossus

1a. A double longitudinal row of widened gular scales; dorsal scales lanceolate in transverse rows only .... *Alopoglossus festae*

1b. No double longitudinal row of widened gular scales; dorsal scales hexagonal in transverse rows only or rhomboidal in oblique and transverse rows 2

2a. Dorsal scales hexagonal with parallel lateral edges, in transverse rows only; transverse ventral count 10 .... *Alopoglossus lehmanni*

2b. Dorsal scales rhomboidal or lanceolate, in oblique and transverse rows; transverse ventral count 4–8 3

3a. Keels on posterior part of dorsum form longitudinal ridges; scales on side of neck large and conical with apparent bare skin between conical scales; longitudinal dorsal count 23–24 .... *Alopoglossus copii*

3b. Keels on posterior part of dorsum do not form longitudinal ridges; scales on side of neck small and granular or keeled and somewhat imbricate without apparent bare skin between scales; longitudinal dorsal count 24–34 4

4a. Scales on side of neck leaf-like (similar in shape to dorsal scales, nongranular) and somewhat imbricate; longitudinal dorsal count 24–28 .... *Alopoglossus angulatus*

4b. Scales on side of neck small and granular; longitudinal dorsal count 29–34 5

5a. Ventral scales distinctly keeled. . . . . *Alopoglossus atriventris*

5b. Ventral scales smooth .... *Alopoglossus buckleyi*

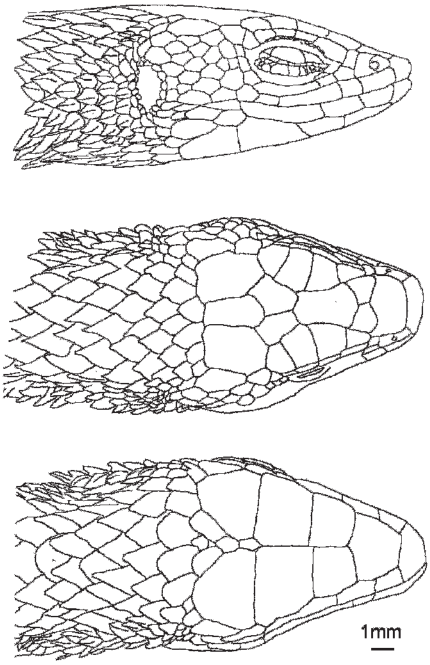

FIG. 2.—Head of the holotype of *Alopoglossus andeanus* (MCZ 45590).

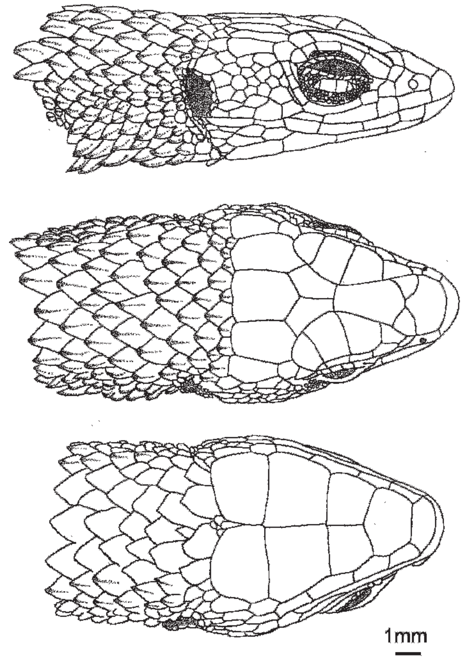

FIG. 3.—Head of *Alopoglossus angulatus* (USNM 288897).

Alopoglossus angulatus *(Linnaeus)*

Lacerta angulata Linnaeus, 1758: 204; type locality: America; restricted by Hoogmoed (1973) by neotype selection, to Brown's Mountain, Suriname; neotype: National Museum of Natural History, Leiden, The Netherlands (RMNH) 15200 (not examined).

Leposoma carinicaudatum Cope, 1876: 160; type locality: Valle of Río Marañon, Peru; holotype: Academy of Natural Sciences, Philadelphia (ANSP) 11371 (examined by the senior author).

Alopoglossus amazonius Ruthven, 1924: 1; type locality: Villa Murtinho, Mato Grosso (=Rondônia), Brazil; holotype: University of Michigan Museum of Zoology (UMMZ) 56853 (not examined).

Alopoglossus copii surinamensis Brongersma, 1946: 231; type locality: forest on the Lucie River, Suriname; holotype: RMNH 4858 (not examined).

Alopoglossus andeanus Ruibal, 1952: 510; type locality: La Pampa, Departamento Puno, Peru, 760 m; holotype: MCZ 45590 (examined by the authors).

Geographic distribution.—Amazonian region in Brazil, French Guiana, Suriname, Guyana, Colombia, Ecuador, and Peru; 100–760 m above sea level.

Diagnosis.—Scales on sides of neck keeled, at least posterior ones leaf-like; gulars in oblique irregular rows (Fig. 3); scales on dorsum and flanks rhomboidal, strongly keeled and mucronate; ventrals usually smooth (rarely broadly keeled), posterior margin blunt or pointed.

Description.—Largest specimen examined 60.0 mm SVL (males $n = 23$) and 61.6 mm (females $n = 39$); TL / SVL 1.41–1.92 (1.69 ± 0.10); HL / SVL 0.19–0.26 (0.22 ± 0.01) vs. 0.20–0.27 according to Avila-Pires (1995); HW / SVL 0.13–0.18 (0.15 ± 0.01); ShL / SVL 0.11–0.18 (0.15 ± 0.01); AGD / SVL 0.37–0.51 (0.44 ± 0.03); ANS / HL 0.02–0.08 (0.06 ± 0.01).

Rostral hexagonal, about three times as wide as high, visible from above, in broad contact with frontonasal (Fig. 3). Frontonasal irregularly pentagonal, usually wider than long, laterally in contact with nasal, occasionally touching loreal. Prefrontals irregularly triangular to pentagonal, wider than long, with a relatively short medial suture or

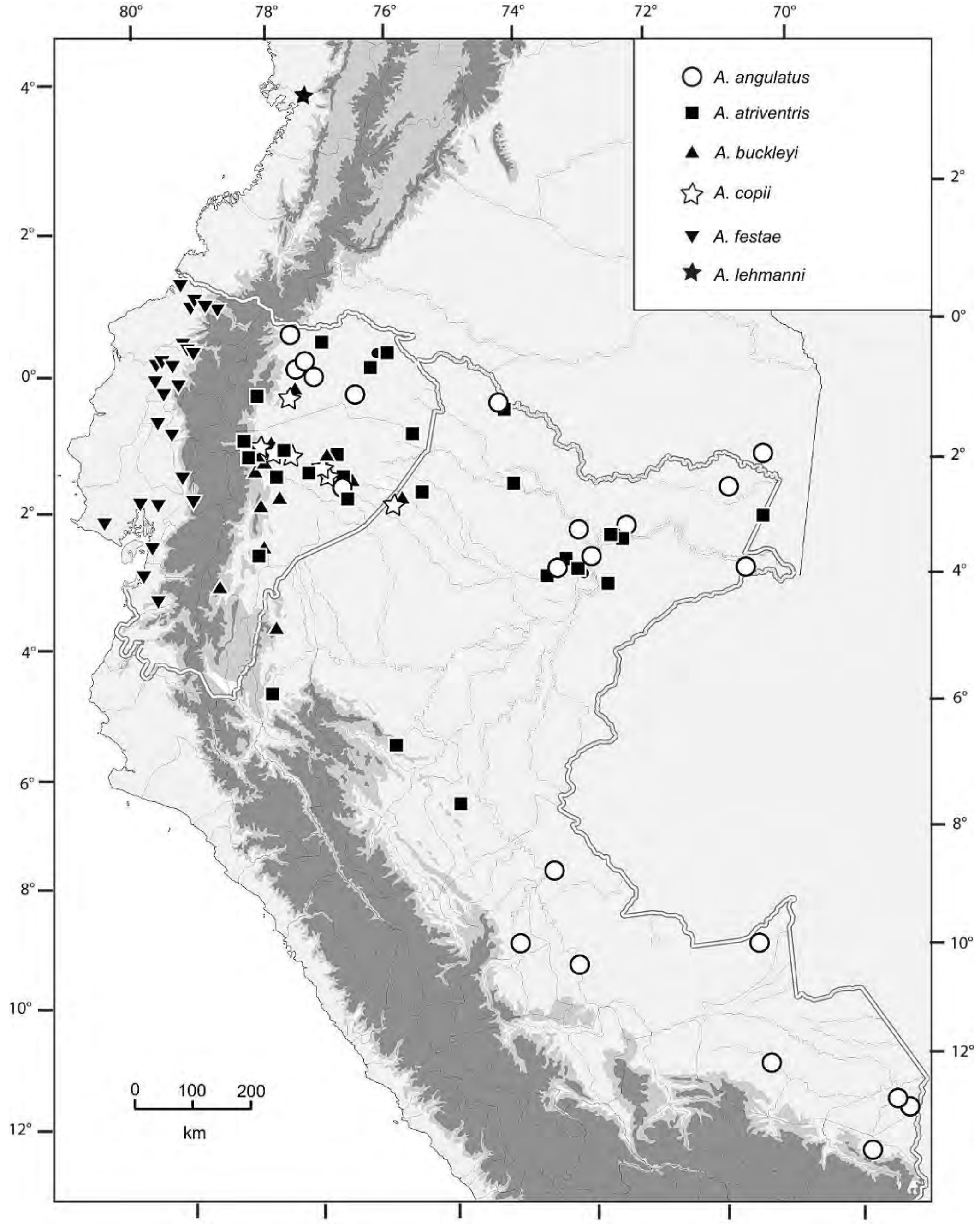

FIG. 4.—Map indicating collecting sites of *Alopoglossus* specimens. A single symbol can represent one or more nearby localities. Areas above 1000 and 2000 m are shaded gray.

Table 1.—Selected measurements, proportions, and scale characters for the species of *Alopoglossus*. Range is followed by mean value and one standard deviation in parentheses.

	A. angulatus	*A. atriventris*	*A. buckleyi*	*A. copii*	*A. festae*	*A. lehmanni*
Maximum SVL[a] males	60.0 mm	55.0 mm	62.3 mm	66.6 mm	60.0 mm	33.3 mm
Maximum SVL females	61.6 mm	52.0 mm	51.0 mm	80.0 mm	64.5 mm	
Longitudinal dorsal count	24–28 (26.65 ± 1.37)	29–34 (31.16 ± 1.14)	29–32 (30.36 ± 1.07)	23–24 (23.40 ± 0.49)	29–31 (30.14 ± 0.64)	33
Transversal dorsal count	13–19 (16.85 ± 1.48)	16–22 (18.38 ± 1.35)	16–25 (20.11 ± 2.72)	11–17 (14.50 ± 1.94)	16–24 (19.14 ± 2.25)	19
Longitudinal ventral count	16–19 (16.94 ± 1.03)	18–21 (19.29 ± 1.08)	15–21 (18.18 ± 1.90)	16–20 (18.00 ± 1.41)	16–19 (17.29 ± 1.03)	21
Transversal ventral count	4–6 (4.12 ± 0.49)	4–8 (6.70 ± 1.27)	6–8 (6.15 ± 0.53)	4 (4.00 ± 0.00)	6 (6.00 ± 0.00)	10
Gulars rows	5–9 (7.89 ± 0.93)	7–10 (9.20 ± 0.80)	7–9 (7.96 ± 0.89)	7–9 (7.87 ± 0.50)	6–8 (7.25 ± 0.68)	9
Frontonasals	1 (1.04 ± 0.20)	1–2 (1.04 ± 0.20)	1 (1.00 ± 0.00)	1 (1.00 ± 0.00)	1 (1.00 ± 0.00)	1
Supraoculars	4 (4.00 ± 0.00)	3–4 (3.99 ± 0.07)	3–4 (3.96 ± 0.20)	4 (4.00 ± 0.00)	3–4 (3.97 ± 0.18)	4
Anterior supralabials	3–4 (3.05 ± 0.22)	3 (3.00 ± 0.00)	3–4 (3.05 ± 0.22)	3 (3.00 ± 0.00)	3 (3.00 ± 0.00)	3
Posterior supralabials	3–4 (3.40 ± 0.20)	3–5 (3.95 ± 0.30)	4 (4.00 ± 0.00)	4 (4.00 ± 0.00)	3–4 (3.95 ± 0.21)	3
Infralabials	4–5 (4.33 ± 0.42)	4 (4.00 ± 0.00)	4–5 (4.31 ± 0.44)	4 (4.00 ± 0.00)	4–5 (4.82 ± 0.38)	4
Scales between third chin shields	1–3 (2.16 ± 0.92)	1–3 (2.65 ± 0.63)	1–3 (2.86 ± 0.52)	1–3 (2.65 ± 0.76)	1–2 (1.08 ± 0.26)	1
Transparent eye disk fragments	3–5 (4.09 ± 0.55)	3–6 (4.35 ± 0.61)	3–7 (4.76 ± 0.86)	4–5 (4.27 ± 0.44)	4–6 (4.90 ± 0.64)	3–4
Lamellae fourth toe	15–24 (20.83 ± 1.87)	16–26 (21.47 ± 1.58)	18–27 (20.58 ± 2.15)	23–27 (25.47 ± 1.29)	17–24 (18.77 ± 1.52)	20–22
Femoral pores	10–14 (12.60 ± 1.64)	11–17 (13.65 ± 2.88)	6–16 (11.53 ± 3.10)	10–19 (16.00 ± 2.50)	3–8 (5.67 ± 1.15)	9
Tail length / SVL (%)	141–184 (162.84 ± 13.11)	142.9–171.5 (157.77 ± 7.84)	130.0–174.1 (152.68 ± 12.05)	154–181 (167.60 ± 9.10)	134.1–222.5 (183.66 ± 22.54)	
Head length / SVL (%)	19.4–24.2 (22.10 ± 1.19)	20.8–26.6 (23.99 ± 1.18)	21.9–27.0 (23.29 ± 0.95)	20.5–24.9 (22.32 ± 1.12)	20.4–25.5 (22.73 ± 1.36)	20.2
Head width / SVL (%)	13.4–17.3 (15.36 ± 1.19)	13.8–18.5 (16.32 ± 0.98)	14.0–17.2 (15.66 ± 0.78)	14–17.8 (15.78 ± 1.13)	13.5–19.3 (15.90 ± 1.34)	13.3
Shank length / SVL (%)	13.1–17.6 (14.96 ± 1.20)	13.7–17.3 (15.69 ± 0.85)	12.9–18.9 (15.25 ± 1.15)	15–18.2 (16.38 ± 0.75)	13.0–18.1 (15.66 ± 1.22)	
Axilla–groin distance / SVL (%)	37.6–50.8 (44.53 ± 2.66)	36.3–52.4 (42.58 ± 3.17)	40.5–50.4 (44.69 ± 2.58)	37.6–52.5 (43.74 ± 3.53)	37.5–50.0 (44.23 ± 2.90)	44.7
Lateral neck scale size / head length (%)	2.2–7.8 (5.21 ± 1.27)	1.2–4.2 (2.56 ± 0.76)	1.39–4.6 (3.07 ± 0.87)	3.6–7.2 (5.38 ± 1.23)	1.3–5.5 (3.08 ± 0.95)	

[a] SVL = snout–vent length.

completely separated; laterally in contact with nasal (usually), loreal, and supraocular I. Frontal irregularly hexagonal, longer than wide, distinctly wider anteriorly; at each side in contact with supraoculars I, II, and (mostly) III. Frontoparietals irregularly pentagonal or hexagonal, longer than wide, with a wide medial suture; each in contact with supraoculars II (occasionally), III, and IV. Interparietal pentagonal, lateral borders parallel to each other. A pair of irregularly hexagonal parietals, slightly narrower and slightly longer than interparietal. Interparietal and parietals form a straight (or slightly undulating) posterior head margin. Occipitals absent. Four supraoculars, anteriormost smallest. Four elongate superciliaries, anteriormost widest, followed by a shorter and wider scale, also in contact with fourth supraocular. Nasal semidivided or divided, irregularly pentagonal, longer than wide. Nostril directed lateroposteriorly. Loreal small, rectangular. A small, irregularly pentagonal frenocular in contact with nasal separating loreal from supralabials. Usually three suboculars, anterior and posterior ones short, medial one elongated. Posterior subocular followed by two postoculars of similar size or a single postocular, twice as high as wide. Semitransparent disc with vertical sections in lower eyelid. Five, exceptionally six, supralabials, the third longest and below center of eye. Two postsupralabials. Temporals irregularly polygonal, subimbricate, keeled or smooth. Two large, smooth or keeled supratemporal scales. Ear opening vertically oval, anterior margin denticulate, posterior margin smooth. Tympanum recessed into a short auditory meatus. Except for temporals, all dorsal and lateral head scales juxtaposed. Frontal, frontoparietal, interparietal, and parietals either smooth or with lateral ridges, other head scales smooth.

Mental trapezoidal, with convex anterior margin. Postmental irregularly heptagonal, wider than long. Three pairs of chinshields, first two in contact medially and with infralabials; third either in contact medially, partly or completely separated by a row of small scales, and separated from infralabials. Third pair of chin shields may be in direct contact with gulars, separated from them by a row of granules, or by a row of larger scales (pregulars). Infralabials 4–5, suture between third and fourth below center of eye. Postinfralabials 1–3. Gulars imbricate, smooth or keeled, posterior margin from rounded to distinctly pointed; in 7–9 transverse rows (5 in one specimen). Posterior row (collar) with 5–10 scales, not differentiated from preceding rows.

Scales on nape similar to dorsals, except that anterior ones are shorter. Scales on sides of neck distinctly smaller than dorsals, posterior ones leaf-like, distinctly keeled, imbricate, anterior ones similar but shorter, or tuberculate. Dorsals and scales on flanks rhomboidal, strongly keeled and mucronate, imbricate, in oblique rows; number of scales along a middorsal line from nape to base of tail 23–30 (26.72 ± 1.88); transversal dorsal count 13–19 (16.50 ± 1.44). Ventrals imbricate, 16–21 (18.00 ± 1.35) in a longitudinal count, 4–6 (4.46 ± 0.86) in a transversal count. Scales in the four medial longitudinal rows either smooth or each with a low, broad keel, the keels forming longitudinal ridges, and with pointed or blunt posterior margins. Lateral rows keeled, mucronate, forming a transition to scales on flanks. Pores usually absent in females, exceptionally one or two small femoral pores present. In males a series of pores 10–14 (12.30 ± 1.22) on each side, series on one side well separated from that on the other side; in each series, one pore in preanal position.

Scales on tail keeled, slightly mucronate, imbricate; in transverse and longitudinal rows, proximally wider than long in the two paravertebral rows, longer than wide in other rows. Keels mostly sharp, except on ventral rows where they are lower and broader, forming distinct longitudinal ridges, of which four are dorsal, two lateral on each side, and four ventral.

Scales on limbs mostly rhomboidal, imbricate, sharply keeled, and mucronate; feebly keeled to smooth on ventral aspect of hind limbs, tuberculate on ventral aspect of upper arms and posterior aspect of thighs. Subdigital lamellae of fingers single, transversely enlarged, and smooth. Some subdigital lamellae of toes longitudinally divided; 15–25 (20.74 ± 1.74) under fourth toe.

Alopoglossus atriventris *Duellman*

Alopoglossus atriventris Duellman, 1973: 228; type locality: Lago Agrio, Provincia Napo, Ecuador, 330 m; holotype: University of Kansas, Natural History Museum (KU) 126783 (examined by the senior author).

Geographic distribution.—Western Amazonia in Brazil, Peru, Ecuador, and Colombia; 230–1500 m above sea level.

Diagnosis.—Scales on sides of neck very small and granular; gulars in oblique irregular rows; dorsals and scales on flanks rhomboidal, strongly keeled and mucronate; ventrals distinctly keeled, posterior margin pointed.

Description.—Largest specimen examined 55.0 mm SVL (males $n = 34$) and 52.0 mm (females $n = 46$); TL / SVL 1.42–1.72 (1.57 ± 0.08); HL / SVL 0.21–0.27 (0.24 ± 0.01); HW / SVL 0.14–0.19 (0.16 ± 0.01); ShL / SVL 0.13–0.17 (0.15 ± 0.01); AGD / SVL 0.36–0.52 (0.43 ± 0.03); ANS / HL 0.01–0.05 (0.03 ± 0.01).

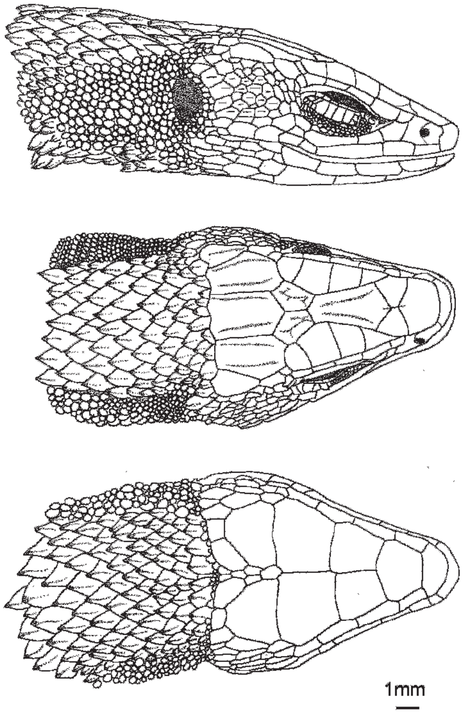

FIG. 5.—Head of *Alopoglossus atriventris* (USNM 321089).

Rostral hexagonal, about 2.5 times as wide as high, visible from above, in broad contact with frontonasal (Fig. 5). Frontonasal irregularly pentagonal, wider than long, laterally in contact with nasal, occasionally touching loreal; rarely (five specimens out of 80) two frontonasals. Prefrontals irregularly triangular to pentagonal, wider than long, with medial suture; laterally in contact with nasal, loreal, and first supraocular. Frontal irregularly hexagonal, much longer than wide, distinctly wider anteriorly; at each side in contact with supraoculars I–III. Frontoparietals irregularly pentagonal or hexagonal, longer than wide, with a wide medial suture; each in contact with supraoculars I–III. Interparietal pentagonal, lateral borders parallel to each other. A pair of irregularly hexagonal parietals, posteriorly narrower than and as long as or slightly longer than interparietal. Interparietal and parietals forming a straight or slightly undulating posterior head margin. Occipitals absent. Four supraoculars, first one smallest. A small postsupraocular scale between supraocular IV, parietal, anterior supratemporal, and postsuperciliary. Four elongate superciliaries, first one widest, followed by a smaller postsuperciliary scale, which is also in contact with the postsupraocular and the anterior supratemporal. Nasal semidivided or divided, irregularly pentagonal, longer than wide. Nostril directed lateroposteriorly. Loreal small, rectangular or pentagonal, frenocular in contact with nasal and thus separating the loreal from supralabials. Usually three, rarely two or four suboculars, the one below eye very elongated (2–3 times the size of adjacent suboculars). Posterior subocular continuous with two or three postoculars. Semitransparent disc with vertical sections in lower eyelid. Five, exceptionally six, supralabials, third longest and below center of eye. Two postsupralabials. Temporals small, irregularly polygonal, subimbricate, keeled, anterior ones partly smooth in some individuals. Two large, keeled supratemporal scales. Ear opening vertically oval, anterior margin finely denticulate, posterior margin smooth. Tympanum recessed into a short auditory meatus. Except for temporals, all dorsal and lateral head scales juxtaposed. Frontal, frontoparietal, interparietal and parietals with lateral ridges, other head scales smooth.

Mental trapezoidal, with convex anterior margin. Postmental irregularly heptagonal, slightly wider than long. Three pairs of chin shields, first two in contact medially and with infralabials; third one separated by a row of small scales, and separated from infralabials. Third pair of chin shields not in direct contact with gulars, separated from them by a pair of tiny scales and by a row of larger scales (pregulars). Four infralabials, suture between third and fourth below center of eye. One to three postinfralabials. Gulars imbricate, keeled, posterior margin distinctly pointed; in 9–10, rarely 7 or 8, transverse rows. Posterior row (collar) with 5–10 scales (vs. 8–13 according to Avila-Pires, 1995), not differentiated from preceding rows.

Scales on nape similar to dorsals, the anterior ones slightly shorter. Scales on sides of neck small and granular. Dorsals and scales on flanks rhomboidal, strongly keeled and mucronate, imbricate, in oblique rows; number of scales along a middorsal line from nape to base of tail 29–34 (31.07 ± 1.16); transversal dorsal count 16–22 (18.56 ± 1.34). Ventrals keeled, imbricate, with more or less pointed posterior margin; 16–21 (19.29 ± 1.15) in a longitudinal count; 6–8, rarely 5 (4 in one specimen), in a transversal count. Scales on flanks similar to ventrals and dorsals. Pores usually absent in females, exceptionally one or two small femoral pores present. Series of 11–17 (14.12 ± 1.40) pores on each side in males, series on one side well separated from that on the opposite side; in each series, one pore in preanal position.

Scales on tail keeled, slightly mucronate, imbricate, in transverse and longitudinal rows; keels sharp; forming four dorsal, four ventral and two or three lateral longitudinal ridges on each side; lateral caudal scales arranged somewhat irregularly.

Scales on limbs mostly rhomboidal, imbricate, sharply keeled, and mucronate; feebly keeled to smooth on ventral aspect of hind limbs, tuberculate on ventral aspect of upper arms and posterior aspect of thighs. Subdigital lamellae of fingers single, transversely enlarged, and smooth. Subdigital lamellae of toes partly divided; 16–27 (21.34 ± 1.58) under fourth toe.

Alopoglossus buckleyi *(O'Shaughnessy)*

Leposoma buckleyi O'Shaughnessy, 1881: 233; type locality: Canelos, Ecuador; holotype: Natural History Museum, London, (BMNH) 1946.8.31.66 (examined by the senior author).

Geographic distribution.—Amazonian Ecuador, Colombia, Peru, and Brazil; 210–1830 m above sea level.

Diagnosis.—Scales on sides of neck very small and granular; gulars in oblique irregular rows; dorsals rhomboidal, strongly keeled and mucronate; scales on flanks smaller than dorsals and irregular; ventrals smooth, posterior margins round or obtusely pointed.

Description.—Largest specimen examined 62.3 mm SVL (males $n = 15$) and 51.0 mm (females $n = 17$); TL / SVL 1.30–1.74 (1.52 ± 0.13); HL / SVL 0.22–0.27 (0.23 ± 0.01); HW / SVL 0.14–0.17 (0.16 ± 0.01); ShL / SVL 0.13–0.19 (0.15 ± 0.01); AGD / SVL 0.41–0.50 (0.45 ± 0.03); ANS / HL 0.01–0.05 (0.03 ± 0.01).

Rostral quadrangular, about 2.5 times as wide as high, visible from above, in broad contact with frontonasal (Fig. 6). Frontonasal irregularly pentagonal, wider than long, laterally in contact with nasal. Prefrontals irregularly quadrangular or pentagonal, wider than long, with medial suture; laterally in contact with nasal, loreal, and first supraocular. Frontal irregularly pentagonal, much longer than wide, distinctly wider anteriorly; in contact with supraoculars I–III on each side. Frontoparietals irregularly pentagonal, longer than wide, with a wide medial suture; each in contact with supraoculars III–IV. Interparietal pentagonal, posteriorly wider than or as wide as anteriorly. A pair of irregularly hexagonal parietals, longer and posteriorly narrower than interparietal. Interparietal and parietals forming a straight or slightly undulating posterior head margin. Occipitals absent. Four supraoculars (three in one specimen), first one smallest. A small postsupraocular scale present between fourth supraocular, parietal (in some individuals), anterior supratemporal and postsuperciliary. Four elongate superciliaries, first one widest, followed by a small postsuperciliary scale, which is also in contact with the postsupraocular and the anterior supratemporal. Nasal semidivided or divided, irregularly quadrangular, longer than wide. Loreal small, quadrangular. An irregular pentagonal frenocular in contact with nasal, separating loreal from supralabials. Three suboculars, the one below eye very elongated (3–4 times the

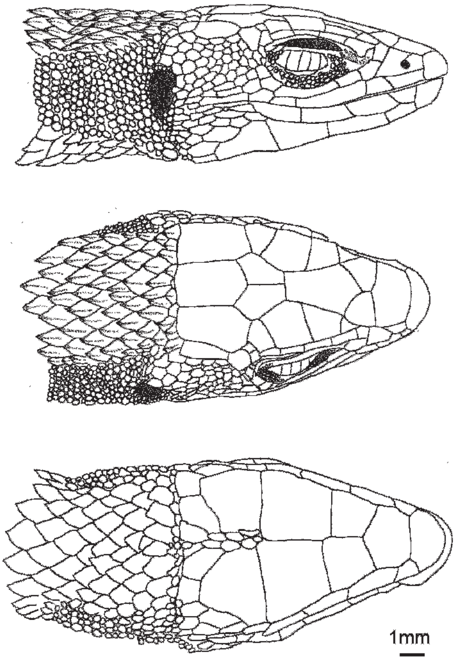

FIG. 6.—Head of *Alopoglossus buckleyi* (USNM 196059).

size of adjacent suboculars). Posterior subocular continuous with one or two postoculars. Semitransparent disc with vertical sections in lower eyelid. Five supralabials, third longest and below center of eye. Two postsupralabials. Temporals small, irregularly polygonal, subimbricate, smooth or weakly keeled. Two large, weakly keeled supratemporal scales. Ear opening round or vertically oval, anterior and posterior margins finely denticulate. Tympanum recessed into a short auditory meatus. All dorsal and lateral head scales juxtaposed. Interparietal and parietals with lateral ridges, other dorsal head scales smooth.

Mental trapezoidal, anterior margin nearly forming a semicircle. Postmental irregularly heptagonal, slightly wider than long. Three pairs of chin shields, first two in contact medially and with infralabials; third pair separated by several small pregulars, and separated from infralabials. Third pair of chin shields not in direct contact with gulars, separated from them by a pair of satellites and by a row of scales (pregulars). Four infralabials, suture between third and fourth below center of eye. One or two postinfralabials. Gulars imbricate, smooth or weakly keeled, with posterior margin more or less pointed, in 7–9 transverse rows. Posterior row of gulars (collar) with 8–9 scales (7–10 according to Avila-Pires, 1995), longer than in preceding rows.

Scales on nape similar to dorsals. Scales on sides of neck small and granular, mostly pointed. Dorsals rhomboidal, strongly keeled and mucronate; 29–32 (30.33 ± 1.07) scales along a middorsal line from nape to base of tail (26–32 according to Avila-Pires, 1995); transversal dorsal count 16–25 (20.17 ± 2.73). Ventrals smooth, imbricate, with round or obtusely pointed posterior margin; 15–21 (18.33 ± 1.97) in a longitudinal count; six (eight in one specimen) in a transversal count. Scales on flanks smaller than dorsals. Femoral pores usually absent in females (three pores per side in two specimens present). In males, a series of 6–16 (12.64 ± 2.47) pores on each side, series on one side well separated from that on other side; in each series, one pore in preanal position.

Scales on tail keeled, slightly mucronate, imbricate; in transverse and longitudinal rows; keels sharp, forming distinct longitudinal ridges, four dorsal ones, four ventral ones and two or three lateral ones on each side; lateral caudal scales arranged somewhat irregularly.

Scales on limbs mostly rhomboidal, imbricate, sharply keeled, and mucronate; smooth on ventral aspect of hind limbs, tuberculate on ventral aspect of upper arms and posterior aspect of thighs. Subdigital lamellae of fingers single, transversely enlarged, and smooth. Subdigital lamellae of toes divided; 17–27 (20.47 ± 2.24) under fourth toe.

Alopoglossus copii *Boulenger*

Alopoglossus copii Boulenger, 1885: 383; type locality: Pallatanga and Canelos, Ecuador; syntypes: BMNH 1946.8.31.58–59, 1946.9.8.17–18 (examined by the senior author).

Geographic distribution.—Amazonian slopes of Colombia, Ecuador, and adjacent regions in Peru; 310–1390 m above sea level.

Diagnosis.—Scales on sides of neck large and conical with free skin between scales; gulars in oblique irregular rows; keels of dorsals on posterior dorsum forming longitudinal ridges; scales on flanks similar to dorsals;

ventrals smooth or weakly keeled, posterior margins round or obtusely pointed.

Description.—Largest specimen examined 66.6 mm SVL (males $n = 8$) and 80.0 mm (females $n = 9$); TL / SVL 1.54–1.81 (1.67 ± 0.10); HL / SVL 0.21–0.25 (0.22 ± 0.01); HW / SVL 0.14–0.18 (0.16 ± 0.01); ShL / SVL 0.15–0.18 (0.16 ± 0.01); AGD / SVL 0.38–0.53 (0.44 ± 0.04); ANS / HL 0.04–0.07 (0.05 ± 0.01).

Rostral quadrangular, about two times as wide as high, visible from above, in broad contact with frontonasal (Fig. 7). Frontonasal irregularly pentagonal, wider than long, laterally in contact with nasal, occasionally touching loreal. Prefrontals irregularly pentagonal, as wide as long, with medial suture; laterally in contact with nasal, loreal, and first supraocular. Frontal irregularly hexagonal, much longer than wide, distinctly wider anteriorly; in contact with supraoculars I–III or II–III. Frontoparietals irregularly pentagonal, longer than wide, with a wide medial suture; each in contact with supraoculars III and (in most individuals) IV. Interparietal pentagonal, posteriorly wider than or as wide as anteriorly. A pair of irregularly pentagonal or hexagonal parietals, longer and posteriorly narrower than interparietal. Interparietal and parietals forming a straight or slightly undulating posterior head margin. Occipitals absent. Four supraoculars, first one smallest. Three or four elongate superciliaries, first one widest, followed by a small postsuperciliary scale, which is also in contact with supraocular IV and anterior supratemporal. Nasal irregularly pentagonal, longer than wide. Nostril in lower part of nasal, directed lateroposteriorly. Loreal relatively large, quadrangular. Frenocular pentagonal, in contact with nasal, separating loreal from supralabials. Usually two or three suboculars, the one below eye very elongated (3–4 times the size of adjacent suboculars). Posterior subocular continuous with two postoculars. Semitransparent disc with vertical sections in lower eyelid. Five supralabials, third one longest and below center of eye. Two postsupralabials. Temporals small, irregularly polygonal, subimbricate, keeled. Two large, weakly keeled supratemporal scales. Ear opening vertically oval, anterior margin denticulate, posterior margin smooth. Tympanum recessed into a short auditory meatus. Except for temporals, all dorsal and lateral head scales juxtaposed and smooth.

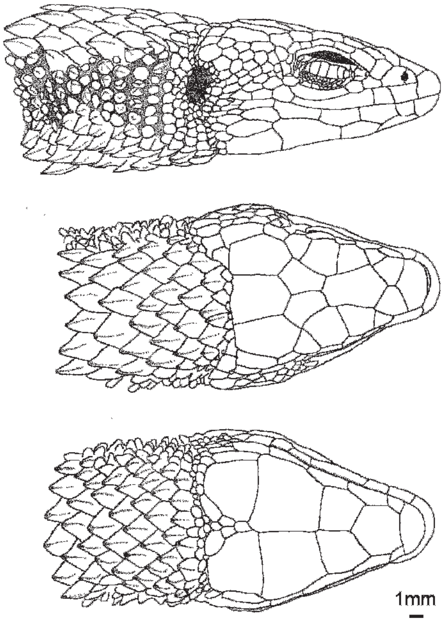

Fig. 7.—Head of *Alopoglossus copii* (USNM 196067).

Mental trapezoidal, with convex anterior margin. Postmental irregularly heptagonal, as wide as or slightly wider than long. Three pairs of chin shields, first two in contact medially and with infralabials; third one separated medially from infralabials by several small scales. Third pair of chin shields not in direct contact with gulars, separated from them by a pair of tiny scales and by a row of small scales. Four infralabials, suture between third and fourth below center of eye. One or two postinfralabials. Gulars imbricate, keeled, posterior margin distinctly pointed, in 6–7 transverse rows. Posterior row of gulars (collar) with 6–7 scales, not differentiated from preceeding rows.

Scales on nape similar to dorsals, except that anterior ones are shorter. Scales on sides of neck large and conical with free skin between scales. Dorsals and scales on flanks hexagonal, strongly keeled and mucronate, imbricate; keels of dorsals on posterior dorsum forming longitudinal rows (Fig. 8); number of scales along a middorsal line from nape to base of tail 23–24 (23.40 ± 0.55); transversal dorsal count 11–17 (14.50 ± 2.07).

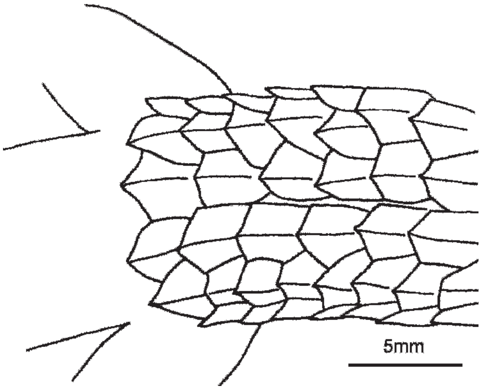

FIG. 8.—Posterior part of dorsum of *Alopoglossus copii* (ZMB 9986).

Ventrals weakly keeled, imbricate, with obtusely pointed posterior margin; 16–20 (18.00 ± 1.58) in a longitudinal count; four in a transversal count. Scales on flanks similar to dorsals. Femoral pores absent in females. In males a series of 10–19 (16.00 ± 2.67) pores on each side, series on one side well separated from that on other side; in each series, one pore in preanal position.

Scales on tail keeled, slightly mucronate, imbricate; in transverse and longitudinal rows; keels sharp, forming distinct longitudinal ridges, four dorsal ones, four ventral ones and two or three lateral ones on each side; lateral scales arranged somewhat irregularly.

Scales on limbs mostly rhomboidal, imbricate, sharply keeled, and mucronate; feebly keeled to smooth on ventral aspect of hind limbs, tuberculate on ventral aspect of upper arms and posterior aspect of thighs. Subdigital lamellae of fingers single, transversely enlarged, and smooth. Subdigital lamellae of toes divided; 23–27 (25.47 ± 1.33) under fourth toe.

Alopoglossus festae *Peracca*

Alopoglossus festae Peracca, 1904: 7; type locality: Vinces, Ecuador; holotype: MZUT R229 (not examined).

Geographic distribution.—Pacific slopes of Ecuador and southwestern Colombia; 10–770 m above sea level.

Diagnosis.—Scales on sides of neck small, keeled, mostly granular; gular scales in four longitudinal rows, with median pair distinctly widened; dorsals lanceolate in transverse rows; scales on flanks similar to dorsals; ventrals smooth, posterior margins round.

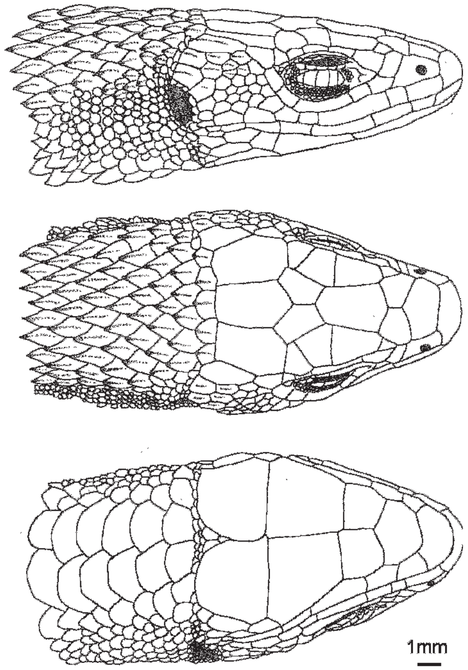

FIG. 9.—Head of *Alopoglossus festae* (USNM 286279).

Description.—Largest specimen examined 60.0 mm SVL (males $n = 18$) and 64.5 mm (females $n = 26$); TL / SVL 1.34–2.23 (1.90 ± 0.21); HL / SVL 0.20–0.26 (0.22 ± 0.01); HW / SVL 0.14–0.19 (0.16 ± 0.01); ShL / SVL 0.13–0.18 (0.16 ± 0.01); AGD / SVL 0.38–0.50 (0.44 ± 0.03); ANS / HL 0.01–0.06 (0.03 ± 0.01).

Rostral hexagonal, about two times as wide as high, visible from above, in broad contact with frontonasal (Fig. 9). Frontonasal irregularly pentagonal, wider than long, laterally in contact with nasal. Prefrontals irregularly pentagonal, wider than long, with medial suture; laterally in contact with nasal, loreal, and first and second supraocular. Frontal irregularly hexagonal, much longer than wide, distinctly wider anteriorly; at each side in contact with supraoculars II–III. Frontoparietals irregularly pentagonal, longer than wide, with a wide medial suture; each in contact with supraocular III. Interparietal pentagonal, lateral borders parallel to each other. A pair of irregularly pentagonal or hexagonal parietals, approximately as wide and as long as interparietal. Interparietal and parietals forming straight or slightly undulating posterior head margin. Occipitals absent. Four supraoculars (three in one specimen), first one smallest. Four elongate superciliaries, first one widest, followed by a postsuperciliary scale, which is also in contact with supraocular IV and anterior supratemporal. Nasal irregularly quadrangular, longer than wide. Nostril in lower part of nasal, directed lateroposteriorly. Loreal small, pentagonal. Frenocular in contacts with nasal, separating loreal from supralabials. Three suboculars, the one below eye very elongated (three times the size of adjacent suboculars). Posterior subocular continuous with two or three postoculars. Semitransparent disc with vertical sections in lower eyelid. Six supralabials, third one longest and below center of eye. One postsupralabial. Temporals small, irregularly polygonal, juxtaposed, keeled. Two large supratemporal scales, posterior one keeled. Ear opening vertically oval, anterior and posterior margin denticulate. Tympanum recessed into a short auditory meatus. All dorsal and lateral head scales juxtaposed. Interparietal and parietals with lateral ridges, other dorsal head scales smooth.

Mental trapezoidal, anterior margin nearly forming a semicircle. Postmental irregularly heptagonal, wider than long. Three pairs of chin shields, first two in contact medially and with infralabials; third one in contact medially but separated from infralabials. Third pair of chin shields in contact with gulars or separated from them by a row of small scales. Three or four infralabials, third one longest and below center of eye. One postinfralabial. Gulars imbricate, smooth, in four transverse rows, the medial double row distinctly widened. Posterior row (collar) with five scales, the medial three ones distinctly widened.

Scales on nape similar to dorsals, except that anterior ones are shorter. Scales on sides of neck small, keeled and mostly granular. Dorsals and scales on flanks lanceolate, strongly keeled and mucronate, imbricate, in transverse rows; number of scales along a middorsal line from nape to base of tail 29–31 (30.14 ± 0.66); transversal dorsal count 16–24 (19.14 ± 2.29). Ventrals smooth, imbricate, with round posterior margin; 16–19 (17.29 ± 1.07) in a longitudinal count; six in a transversal count. Scales on flanks similar to dorsals. Femoral pores absent in females. In males a series of 3–8 (5.71 ± 1.21) pores on each side, series on one side well separated from that on other side; in each series, one pore in preanal position.

Scales on tail keeled, slightly mucronate, imbricate; in transverse and longitudinal rows; keels sharp, forming distinct longitudinal ridges, four dorsal ones, four ventral ones and two or three lateral ones on each side.

Scales on limbs mostly rhomboidal, imbricate, sharply keeled, and mucronate; feebly keeled to smooth on ventral aspect of hind limbs, tuberculate on ventral aspect of upper arms and posterior aspect of thighs. Subdigital lamellae of fingers and toes single, transversely enlarged and smooth; 17–24 (18.76 ± 1.53) under fourth toe.

Alopoglossus lehmanni *Ayala and Harris*

Alopoglossus lehmanni Ayala and Harris, 1984: 154; type locality: km 22 on the road from Buenaventura to San Isidro on the lower Calima River, Department Cauca, Colombia; holotype: Field Museum of Natural History (FMNH) 165199, a male (examined by the authors).

Geographic distribution.—Known only from type locality; 30 m above sea level.

Diagnosis.—Scales on sides of neck large, nearly conical; gulars in transverse irregular rows; dorsals hexagonal with parallel lateral edges, in transverse rows; scales on flanks smaller than dorsals and irregular; ventrals keeled and hexagonal.

Description.—The only specimen examined 33.0 mm SVL; tail incomplete; HL / SVL 0.20; HW / SVL 0.13; AGD / SVL 0.45.

Rostral hexagonal, about two times as wide as high, visible from above, in broad contact with frontonasal (Fig. 10). Frontonasal pentagonal, wider than long, laterally in contact with nasal. Prefrontals irregularly pentagonal, wider than long, with medial suture; laterally in contact with nasal, loreal, and first supraocular. Frontal irregularly hexagonal, about 1.6 times longer than wide, distinctly wider anteriorly; at each side in contact with supraoculars I–III. Frontoparietals irregularly pentagonal, longer than wide, with a wide medial suture; each in contact with supraoculars III–IV. Interparietal pentagonal, slightly narrower posteriorly. A

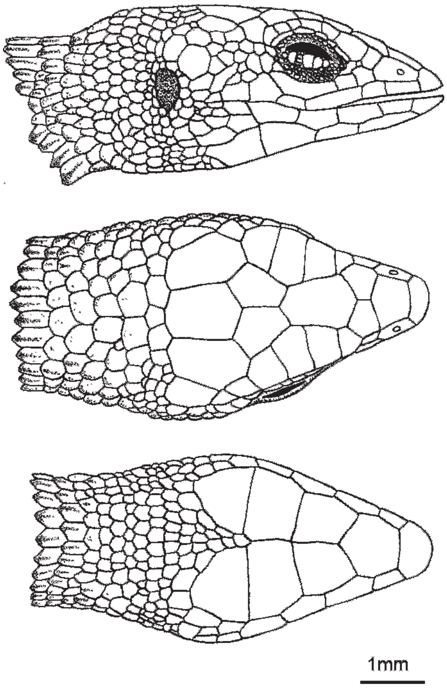

FIG. 10.—Head of *Alopoglossus lehmanni* (FMNH 165199).

pair of irregularly pentagonal or hexagonal parietals, approximately as wide and as long as interparietal. Interparietal and parietals forming a straight or slightly undulating posterior head margin. Occipitals absent. Four supraoculars, first one smallest. Five elongate superciliaries, first one longest. Nasal irregularly pentagular, longer than high. Nostril in lower part of nasal, directed lateroposteriorly. Loreal small, quadrangular. Frenocular irregularly pentagonal, in contacts with nasal and separating loreal from supralabials. Three suboculars, the one below eye very elongated (three times the size of adjacent suboculars). Posterior subocular continuous with two or three postoculars. Semitransparent disc with vertical sections in lower eyelid. Six supralabials, third one longest. One postsupralabial. Temporals small, irregularly polygonal, juxtaposed, keeled. Two large supratemporal scales, posterior one keeled. Ear opening vertically oval, anterior and posterior margin not denticulate. Tympanum recessed into a short auditory meatus. All dorsal and lateral head scales juxtaposed. Interparietal and parietals without lateral ridges, other dorsal head scales smooth.

Mental trapezoidal, anterior margin nearly forming a semicircle. Postmental irregularly heptagonal, about as wide as long. Three pairs of chin shields, first two ones in contact medially and with infralabials; third in weak contact with infralabials but separated medially by small pregulars. Third pair of chinshields separated from them by one or two rows of small scales. Four infralabials, third one below center of eye. Two postinfralabials. Gulars imbricate, smooth, in nine transverse rows. Posterior row of gulars (collar) with seven scales.

Scales on nape similar to dorsals, except that anterior ones are shorter. Scales on sides of neck small, keeled and mostly granular. Dorsals and scales on flanks lanceolate, strongly keeled and mucronate, imbricate, in transverse rows; number of scales along a middorsal line from nape to base of tail 33; transversal dorsal count 19. Ventrals smooth, imbricate, with round posterior margin; 21 in a longitudinal count; six in a transversal count. On flanks two lateral scales for each dorsal and ventral row. Series of nine pores on each side, series on one side well separated from that on other side; in each series, one pore in preanal position.

Scales on tail keeled, slightly mucronate, imbricate; in transverse rows; keels sharp; in first third of tail in alternating position.

Scales on limbs mostly rhomboidal, imbricate, sharply keeled, and mucronate. Subdigital lamellae of fingers and toes single, transversely enlarged and smooth; 20–22 under fourth toe.

DISCUSSION

Species of the genus *Alopoglossus* inhabit areas mostly below 1500 m above sea level. Most specimens were collected in the 100–800 m range. One specimen of *A. festae* (Escuela Politecnica Nacional, Quito, Ecuador [EPN] 4212) has supposedly been collected at “Pallatanga,” a village 2250 m above sea level. This appears to be out of the altitudinal range of any *Alopoglossus* species and might be a case of erroneous collecting data. Three of the six species have considerably large geographic distributions, whereas the geographic ranges of *A. copii* and *A. festae* are somewhat restricted, and *A. lehmanni* is still known only from the holotype. The four cis-Andean species show broadly overlapping geographic ranges and several cases of actual sympatry are evident among the specimens examined by us (e.g., *Alopoglossus buckleyi* and *A. copii* at Canelos, Ecuador, and *A. angulatus* and *A. atriventris* at Moropon, Peru; see also Fig. 4).

Acknowledgments.—For the loan of and/or access to specimens we thank T. Daeschler and A. Gilmore, Academy of Natural Sciences (ANSP), Philadelphia; C. J. McCarthy, The Natural History Museum (BMNH), London; J. Vindum, California Academy of Sciences (CAS), San Francisco; A. Almendáriz, Escuela Politecnica Nacional (EPN), Quito, Ecuador; A. Resetar, Field Museum of Natural History (FMNH), Chicago; W. E. Duellman and J. E. Simmons, University of Kansas, Natural History Museum (KU), Lawrence; D. Rossman, Museum of Natural Science, Louisiana State University (LSUMZ), Baton Rouge; J. Hanken and J. P. Rosado, Museum of Comparative Zoology, Harvard University (MCZ), Cambridge; I. Ineich and A. Ohler, Museum National d'Histoire Naturelle (MNHN), Paris; J. Cordova, Museo de Historia Natural, Universidad Nacional Mayor de San Marcos (MUSM), Lima, Peru; L. Coloma, Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ), Quito, Ecuador; R. de Ruiter, National Museum of Natural History (RMNH), Leiden, The Netherlands; R. K. Vaughan, Texas Cooperative Wildlife Collection, Texas A & M University (TCWC), College Station; F. W. King and K. L. Krysko, Florida Museum of Natural History (UF), Gainesville; C. A. Phillips and J. Petzing, Illinois Natural History Survey, Center for Biodiversity (UIMNH), Champaign; R. A. Nussbaum and G. Schneider, University of Michigan Museum of Zoology (UMMZ), Ann Arbor; R. W. McDiarmid and W. R. Heyer, National Museum of Natural History (USNM), Washington, D.C.; W. Böhme, Zoologisches Forschungsinstitut und Museum A. Koenig (ZFMK), Bonn; and R. Günther, Museum für Naturkunde der Humboldt–Universität zu Berlin (ZMB), Berlin. A. Resetar and K. Havener (FMNH), and R. de Ruiter (RMNH) provided digital images of specimens under their care.

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Accepted: 30 July 2012

APPENDIX I

Alopoglossus angulatus.—**BRAZIL: Amapa:** Serra de Navio, T.F. do Amapa: RMNH 25312–18; **Amazonas:** Tabatinga, Río Solimoes: RMNH 25303–04; 15 km NE Manacapuru, 72 km W of Manaus, road to N: RMNH 25307–08; E Porto Urucu, Río Urucu, near Petrobras RVC-2: RMNH 25321; **Pará:** Cruz Alta, 8 km S Río Trombetas, mun. De Oriximiná: RMNH 25319; Rio Tapajos, ca. 66 km SW Itaituba, Parque Nacional da Amazonia: USNM 288896–97; Belem: KU 127238, USNM 158083, 162209. **COLOMBIA:**

Amazonas: Leticia: MCZ 141227. **ECUADOR: Napo:** Estrellayacu: QCAZ 1715; San Francisco, Río Napo: UMMZ 84737; Río Yasuni, ca. 200 km upstream from Río Napo: UF 42503; **Pastaza:** Pica Montalvo-Chichiro: EPN 709–11; **Sucumbíos:** Comuna Chari: EPN 4973; Comuna Duvuno: EPN 4974; Reserva de Producción Faunistica Cuyabeno, 250 m: QCAZ 1523, 2355–57. **FRENCH GUYANA**: Mission Aubert de la Rue, Bienvenue, valley of Canopi River: MNHN 1950.22. **PERU**: **Huánuco:** Finca Panguana, Río Llullapichis, 4–5 km upstream from Río Pachitea, 100 m: KU 172197, ZFMK 41840–41, 41359; **Loreto:** Balta, Río Curanja: LSUMZ 14511–13; Colonia, Bora village, right bank of Río Zumun, left-hand tributary of Yahuasyaca: MNHN 1978.2055–59; Estiron, Río Ampiyacu: CAS 102967–68; Isla Pasto, Río Amazonas, opposite Aysana, ca. 80 km NE Iquitos: LSUMZ 44527, MUSM 8876; Moropon, Río Nanay: TCWC 36708, 44292; Río Yuvineto, right-hand tributary of Río Putumasso: MNHN 1978.2345; Iquitos: TCWC 36723, 39025, 42832, 43340–42, 47139; Santa Maria, 6.4 km NE Iquitos along Amazon River: UF 127223–24; **Madre de Dios:** Estacion Biologica Cocha Cashu, Parque Nacional Manu, 11°55′S, 71°18′W, 350 m: MUSM 8568, 8572, MUSM USNM field series 173168; Refugio Juliaca, Santuario Nacional "Pampas del Heath," 12°57.4′S, 68°52.9′W, 200 m: MUSM WED field series 58540; Explorer's Inn, ca. 30 km (airline) SSW Puerto Maldonado, Tambopata Reserve: USNM 247489; **Puno:** La Pampa, 760 m: MCZ 45590; **Ucayali:** Campamento Bolognesi, 10°6′13″S, 73°49′2″W, 230 m: SMF 80100; Río Shesha, junction with Río Abujao, 65 km ENE Pucallpa, Prov. Coronel Portillo, 8°20.4′S, 73°57.8′W: MUSM 13307. **SURINAME**: **Brokopondo:** Brownsberg National Park: RMNH 15200; Nassau Mountains, km 9.3: RMNH 13446; NE Airstrip Kayser Mountains: RMNH 15379; Lucie River: RMNH 4858.

Alopoglossus atriventris.—**BRAZIL: Amazonas:** Tabatinga, N bank Río Solimoes: RMNH 25302; Benjamin Constant, Río Solimoes: RMNH 25305–06; 15 km NE of Manacapuru, 72 km W of Manaus, road to N: RMHN 25309–10; E Porto Urucu, Río Urucu, near Petrobras RVC-2: RMNH 25322–23, 25325; E Benjamin Constant (Santo Antonio): RMNH 25326; W Benjamin Constant: RMNH 25327–31. **COLOMBIA: Amazonas:** Puerto Narino: MCZ 154004, 154010, 154531, 154533; ca. 50 km NW Puerto Narino: MCZ 154009; Río Amaca Yacu: MCZ 154534. **ECUADOR: Napo:** Cordillera de Guacamayos, 1500 m: QCAZ 3110; Lago Agrio, 330 m: KU 126783–84; Pozo petrolero Daimi 1, Bloque 16: EPN 3710; Río Conambo: USNM 196060; Yuturi, 230 m: QCAZ 3607; **Morona-Santiago:** Los Tayos: BMNH 1976.1852; **Pastaza:** Alpayacu Napo Pastaza: UMMZ 90774; Locatión Petrolera Garza 1, NE Montalvo: QCAZ 1554; Río Pastaza, 500 m: UMMZ 90775; Nuevo Golondrina, 130 km S Coca, 1°7′S, 76°57′W, 300 m: USNM 321089; UNOCAL Base Camp, Tiguino, 130 km S Coca, 1°7′S, 76°57′W, 300 m: USNM 321088; **Sucumbíos:** Pozo petrolero Zafiro, Sucumbios, Lago Agrio: EPN 5553; Reserva de Producción Faunistica Cuyabeno: QCAZ 2355–56. **PERU**: **Loreto:** Colonia, Bora village, right bank of Río Zumun, left-hand tributary of Yahuasyaca: MNHN 1978.2130–36, 1978.2060–74; Estiron, Río Ampiyacu: CAS 102969, 102971, 102973; Río Tahuayo-Punga, 7°47.6′S, 75°6.8′W: MUSM 13872–73; Centro Union: TCWC 41336, 41779, 41847; Explornapo camp, on Río Sucusari near confluence with Río Napo, 150 km (by river) NW Iquitos: USNM 313958; Quebrada Orán, ca. 5 km N Río Amazonas, 85 km NE Iquitos: LSUMZ 44524–26; 60 km W Iquitos: ZFMK 49113; Moropon: TCWC 41331–32, 41757, 41848–49, 41851; Río Yuvineto, right-hand tributary of Río Putumasso: MNHN 1978.2343–44, 1978.2432; Yanamono: TCWC 42540; **San Martín:** 33 km NE Tarapoto on road to Yurimaguas, Río Cainarache: KU 209518–19.

Alopoglossus buckleyi.—**ECUADOR: Napo:** El Chaco, El Salado, Alto Coca: EPN 5252; Loreto: USNM 196059; S slope Cordillera del Due above Río Coca, 1150 m: KU 122129–33; **Morona-Santiago:** Destacamento Militar Coangos: EPN 4104; Los Tayos: BMNH 1976.1851; Río Piuntza, 1830 m: KU 147185; Trail Lagrona to Yampi, Cordillera de Cutucú, 1700 m: ANSP 29112, 29114–15; **Pastaza:** Arutam, 1°47.28′S, 77°48.31′W, 820–830 m: SMF 83005–06, 83011; Canelos, 630 m: BMNH 1946.8.31.66; Santana, 1°36.36′S, 77°45.31′W, 890 m: SMF 83007–10; Pozo Petrolero Misión, Parroquia Montalvo, 02°13′S, 76°40′50″W: EPN 723–24, 750. **PERU: Amazonas:** Vicinity of Pais, on lower Río Alto Cenepa, tributary of Río Cenepa, 4°25′S, 78°12′W: USNM 316768; Vicinity of Kagka, on lower Río Alto Cenepa, tributary of Río Cenepa, 4°22′S, 78°13′W: USNM 316769; Vicinity of Huampami, on Río Cenepa, 4°28′S, 78°10′W, 210 m: USNM 316770; **Cajamarca:** Santuario Nacional Tabaconas Namballe, 1400–1800 m: ZFMK 89143; **Loreto:** 1.5 km N Teniente Lopez, 310 m: KU 222168.

Alopoglossus copii.—**ECUADOR: Napo:** Loreto: USNM 196065; Sarayacu, 1390 m: USNM 518295, ZMB 9987; Sarayacu, Bobonaza: USNM 196067; S Venecia, between Río Bununo and Río Nachiyacu: UMMZ 177888; Río Cotopino, 400 m: UMMZ 90776; **Pastaza:** Canelos, 630 m: BMNH 1912.11.1.32, 1946.8.31.58–59, 1946.9.8.17–18, ZMB 9986. PERU: Loreto: 1.5 km N Teniente Lopez, 310 m: KU 222169.

Alopoglossus festae.—**ECUADOR:** no further locality data: MNHN 1906.235; **Bolivar:** Balzapampa, 800 m: KU 134783–85; **Chimborazo:** Pallatanga: EPN 4212; **Cotopaxi:** 0.7 km N Las Juntas: USNM 286071; **El Oro:** 7 km ESE Machala near Santa Rosa road, 10 m: KU 154663–64, USNM 196091–92; 13 km W Pinas, on old road, 800 m: USNM 286279; **Esmeraldas:** El Placer, 670 m: ANSP 32721; 1 km W El Placer, 400 m: USNM 196090, 248255; Santo Domingo de los Colorados: ZFMK 42747; Hacienda Equinox, 38 km NW of Santo Domingo de los Colorados: USNM 196088; San Lorenzo: QCAZ 1404, UMMZ 18219; **Guayas:** Cooperativa Nueva Unión Campesina, Naranjal, 15 min. via Naranjal–Pasaje, 45 m: QCAZ 3325–26; Guayaquil, 45 m: ZFMK 40457; Hacienda San Miguel, 4.8 km E Milagro, 61 m: USNM 196089; **Imbabura:** Charguayacu, region of Intac: USNM 248254; Palma Real, 570 m: USNM 248251–52; Paramba, 0°49′N, 78°21′W, 770 m: BMNH 1901.6.27.6; **Los Ríos:** Centro Cientifico Río Palenque, km 48 via Santo Domingo–Quevedo: QCAZ 2180, 2222; 4 km N Quevedo, 140 m: KU 134782; Finca Playa Grande, 1 km N of Quevedo road: UIMNH 92152–56; Quevedo: 248253; **Pichincha:** near Puerto Quito (Reserva Forestal Endesa): QCAZ 1425; Estación Biologica Río Palenque, 56 km N Quevedo, 220 m: KU 147555; Río Baba, 10 km S, 4 km E Santo Domingo de los Colorados, 400 m: KU 142746; Río Baba, 19 km S, 5 km E Santo Domingo de los Colorados: UIMNH 92148–49; 4 km E of Río Baba Bridge, 24 km S of Santo Domingo de los Colorados: UIMNH 92157–58; Sector de Largartera, Region del Río Caoni: UIMNH 54330–32; Rancho Santa Teresita, Santo Domingo de los Colorados, km 25 on route to Chone: USNM 283526.

Alopoglossus lehmanni.—**COLOMBIA: Cauca:** km 22 on the road from Buenaventura to San Isidro on the lower Calima River: FMNH 165199.

Příloha 8.

Batista, A., Hertz, A., Mebert, K., Köhler, G., Lotzkat, S., Ponce, M., **Veselý, M.,** 2014: Two new fringe-limbed frogs of the genus *Ecnomiohyla* (Anura: Hylidae) from Panama. *Zootaxa* 3826(3): 449–474. IF_{2014} – 0.906; Q3

The contribution in words:
AB and MV conceived and designed the study. AB, MV, AH, GK, SL, MP and KM carried out the fieldwork, AB and MV analysed morphological data and performed the molecular analyses. AB and AH performed the analysis of bioacoustics. AB and MV wrote the manuscript. All authors contributed to manuscript revision. All authors approved the final version of the manuscript. MV supervised the work.

Ranking: IF_{2014} – 0.906; Q3 (Zoology)
Citace na WoS (Core collection; bez autocitací všech spoluautorů): 9
Citace celkem (Google Scholar): 15

Zootaxa 3826 (3): 449–474
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3826.3.2
http://zoobank.org/urn:lsid:zoobank.org:pub:4BEDE115-8C6E-46C6-AF6A-1F96209079ED

Two new fringe-limbed frogs of the genus *Ecnomiohyla* (Anura: Hylidae) from Panama

ABEL BATISTA[1,2,5,6], ANDREAS HERTZ[1,2], KONRAD MEBERT[3], GUNTHER KÖHLER[1], SEBASTIAN LOTZKAT[1,2], MARCOS PONCE[5] & MILAN VESELY[4]

[1] *Senckenberg Forschungsinstitut und Naturmuseum Frankfurt, Senckenberganlage 25, 60325 Frankfurt a. M., Germany*
[2] *Johann Wolfgang Goethe-University, Institute for Ecology, Evolution & Diversity, BioCampus-Westend, Siesmayerstr. 70, 60323 Frankfurt am Main, Germany*
[3] *Section of Conservation Biology, Department of Environmental Sciences, University of Basel, St. Johanns-Vorstadt 10, CH-4056 Basel, Switzerland*
[4] *Department of Zoology, Faculty of Natural Sciences, Palacký University, 17. Listopadu 50, 77146 Olomouc, Czech Republic*
[5] *Universidad Autónoma de Chiriquí, David, Panama*
[6] *Corresponding author. abelbatista@hotmail.com*

Abstract

Forest canopy-dwelling frogs are usually among the rarest anuran species observed in the neotropical forest, mainly because they fall outside of the scope of the standard search methods used by herpetologists. During field explorations undertaken in western and eastern Panama in recent years, we discovered two species belonging to the genus *Ecnomiohyla*, which showed significant differences in genetic distances (16S mtDNA gene) and morphological characteristics different from any known *Ecnomiohyla* species. The first specimen originates from the Serranía de Jingurudó, Darién province, southeastern Panamá, and is described herein as *E. bailarina* **sp. nov.**, and the second specimen was found at Santa Fe National Park, Veraguas province, central-western Panama, and is described as *E. veraguensis* **sp. nov.** We provide a detailed description of both new species, including comparisons of morphological and molecular characters of almost all members of the genus in lower Central America, as well as an identification key for the entire genus.

Key words: Fringe-limbed frogs, *Ecnomiohyla,* rare species, DNA barcoding, lower Central America, Panama

Introduction

After the description of a new species, subsequent sampling usually provides additional comparative specimens, which thus increases our knowledge about the biology and distribution of that species with time (Vrcibradic *et al.* 2008; Hertz *et al.* 2012a). Nonetheless, there are some apparently rare species, whose existence we know for decades based only on the type specimen(s) or material from the type locality (Pimenta *et al.* 2005; Frost 2013, Wickramasinghe *et al.* 2013). Burrowing caecilians, salamanders (*Oedipina* spp.) and forest canopy-dwelling frogs (e.g. *Pseudophilautus stellatus*) are examples of such infrequently encountered species, which are then perceived as very rare (García-París & Wake 2000; Hanken *et al.* 2005; Wilkinson *et al.* 2007; Kamei *et al*. 2009; Wickramasinghe *et al.* 2013), because the habitat of these amphibians usually falls outside of the scope of the standard search methods used by herpetologists. Thus, the perception of rarity might be only an artifact of limited or inappropriate search techniques. In this context, any information about such seldom-seen (or "rare") taxa can be relevant.

Among such rare species are most members of the fringe-limbed frogs of the genus *Ecnomiohyla* Faivovich, Haddad, Garcia, Frost, Campbell, & Wheeler 2005, which spend all their life phases in the canopy and only rarely climb down and become visible to us. Fringe-limbed frogs are large, morphologically unusual hylid frogs with a cryptic moss-like color pattern and dermal fringes on portions of the body, rendering them well camouflaged. They breed in phytotelmata (e.g. Savage 2002; Mendelson *et al.* 2008; Savage & Kubicki 2010), and most of them occur in wet lowland, premontane tropical, and cloud forests between 20–2000 m elevation (Wilson *et al*. 1985;

Accepted by V. Orrico: 9 Jun. 2014; published: 1 Jul. 2014

Duellman 2001; Frost 2013). The genus *Ecnomiohyla* is distributed from southern Mexico through Central America and into north-western South America, containing 12 species to date (Frost 2013), which are: *Ecnomiohyla echinata* (Duellman, 1961), *E. fimbrimembra* (Taylor, 1948), *E. miliaria* (Cope, 1886), *E. minera* (Wilson, McCranie, & Williams, 1985), *E. miotympanum* (Cope, 1863), *E. phantasmagoria* (Dunn, 1943), *E. rabborum* Mendelson, Savage, Griffith, Ross, Kubicki, & Gagliardo, 2008, *E. salvaje* (Wilson, McCranie, & Williams, 1985), *E. sukia* Savage & Kubicki, 2010, *E. thysanota* (Duellman, 1966), *E. tuberculosa* (Boulenger, 1882), and *E. valancifer* (Firschein & Smith, 1956). The holotypes of *Ecnomiohyla* species were often the only specimens known for an extended period of time (Taylor 1948; Duellman 1966). However, intensified sampling during the past three decades has contributed increasingly to our knowledge about the ecology of some species, while recent genetic studies helped to understand better the species relationships inside the genus *Ecnomiohyla* and its position in the amphibian tree of life (Wilson *et al*. 1985; Faivovich *et al*. 2005; Mendelson *et al*. 2008; Crawford *et al.* 2013).

The genus *Ecnomiohyla* can be differentiated from all other genera of Hylinae by the combination of the following characters: having immense hands and feet, scalloped dermal fringes on the outer margin of the forearm and foot, large digital disks, and enlarged prepollices (Firschein & Smith 1956; Savage & Heyer 1969; Duellman 1970; Mendelson *et al*. 2008). The prepollices are more developed in males and usually modified with a projecting terminal spine (protruding as in *E. miliaria*), or a spade-like plate (as in *E. valancifer*). In many species, male prepollices bear also keratinized black spines (Duellman 2001), whereas in females the prepollex is slender, straight and without spines.

Some uncertainty remains in unifying all currently recognized species within *Ecnomiohyla* based on the morphological characters mentioned above (Faivovich *et al.* 2005). *Ecnomiohyla miotympanum* and *E. tuberculosa* have been catalogued as problematic species due to substantial differences in adult and larval morphology and shared behavioral ecological traits in comparison to the other members of the genus (Faivovich *et al.* 2005; Mendelson *et al*. 2008). This problem is not solved yet, partly because of the lack of fresh material for genetic approaches in many species that prevents the construction of a well-resolved phylogeny of the genus. Recent phylogenetic studies lack most species of *Ecnomiohyla* (there are no sequences available for *E. tuberculosa* yet) thus some of its species (*E. miotympanum* and *E. tuberculosa* specially) are assigned to the genus only tentatively (Faivovich *et al*. 2005; Wiens *et al.* 2010; Pyron & Wiens 2011). The issues regarding exclusion of *E. miotympanum* and *E. tuberculosa* from the genus *Ecnomiohyla* (see Savage & Kubicki 2010) are not relevant in the context of this paper, but we include *E. miotympanum* to our phylogenetic analysis to discuss its relationship with other *Ecnomiohyla* from lower Central America.

Herein, we describe two new species of *Ecnomiohyla* from Panama, based on comparative morphology of the twelve known species of *Ecnomiohyla* and a genetic analysis of the species from lower Central America (except *E. thysanota*). The new species from eastern Panama can be distinguished from its congeners by the presence of cranial and dorsal osteoderms, and two clusters of nuptial spines, one at the distal end of prepollex and one at the end of the first phalanx of the thumb in males. The new species from western Panama has scattered minute keratin tipped tubercles on the dorsal skin, and 6–8 widely spaced keratinized black spines along the outer side of the thumb.

Material and methods

Fieldwork was carried out in eastern Panama in 2011 and 2012 (Fig. 1) and in central-western Panama during two field trips in 2009. Specimens were euthanized with a euthanasia solution (T61), fixed with a mixture of 5 ml formalin (5%) in 1L ethanol (94%), and then stored in ethanol (70%). Morphological nomenclature and diagnoses usually follow the methodology of Duellman (2001), except for standards of dorsal and lateral profiles of the snout that follow Savage (2002). Coding for webbing formulae follows Savage & Kubicki (2010): considerable (C) = not extending to base of disk on one margin of any digit; substantial (S) = extending to base of disk on one margin of one digit; extensive (EX) = extending to base of disk on one margin of two to four digits; full (F) = extending to base of disk on margins of all digits.

All measurements are given in millimeters, were rounded to the nearest 0.1 mm and follow Duellman & Lehr (2009). The following measurements were taken (with abbreviations indicated): length from snout to vent (SVL); head length (HL), measured diagonally from angle of jaw to tip of snout; head width (HW) between angles of jaws;

interorbital distance (IOD); eye diameter (ED); eye length (EL) from anterior to posterior edge; eye to nostril distance (END) from anterior edge of eye to posterior corner of nostril; internarial distance (IND) between centers of nostrils; forearm length (FAL) from proximal edge of palmar tubercle to outer edge of flexed elbow; hand length (HAL) from proximal edge of palmar tubercle to tip of third finger; tibia length (TL), distance from knee to distal end of the tibia; foot length (FL) from proximal edge of outer metatarsal tubercle to tip of fourth toe; width of third finger (3FW) at penultimate phalanx just anterior to disk; width of disk of third finger (3FD) at greatest width; width of third toe (3TW) at penultimate phalanx just anterior to disk; width of disk of third toe (3TD) at greatest width; width of fourth toe (4TW) at penultimate phalanx just anterior to disk; width of disk of fourth toe (4TD) at greatest width; body width (BW) at greatest width of body; tympanum diameter (TD), horizontal distance, based on an estimated circular tympanum. SVL, HL, HW, TL, and FL were measured with vernier calipers; all other variables were measured with an ocular micrometer in a Zeiss stereomicroscope.

Capitalized colors and color codes (the latter in parentheses) used in the color descriptions are those of Smithe (1975–1981), except those in the color description of the holotype of *Ecnomiohyla bailarina*, which are those of Köhler (2012). Specimens were deposited in the herpetological collection of the Senckenberg Forschungsinstitut and Naturmuseum Frankfurt (SMF) in Germany. Comparisons among similar species are based on data provided in the respective original descriptions. Geographic coordinates and altitude were taken with a Garmin GPSmap 60CSx given in decimal degrees and rounded to the fourth decimal place. Elevations are rounded up to the next tenth. All georeferences were recorded in WGS 1984 datum. The map was downloaded from the server of the Smithsonian Tropical Research Institute (http://mapserver.stri.si.edu/), and created using ArcGIS 10 (ESRI 2009). Detailed information about the specimens examined is given in Table 1.

Molecular Genetics

We took tissue samples from the two new species plus a newly collected specimen of *Ecnomiohyla fimbrimembra* (SMF89857, Hertz et al. 2012b) and a newly collected specimen of *E. sukia* (SMF94578, Köhler *et al.* 2013). Tissue for DNA was extracted by excision on finger-tips of preserved specimens, except for *Ecnomiohyla bailarina*, where the tissue was extracted from a fresh liver sample. A fragment of the mitochondrial 16S mtDNA gene was extracted following the protocol of Ivanova *et al.* (2006), and amplified using a Mastercycler pro S (Eppendorf, Hamburg, Germany) performing an initial denaturation for 60 sec at 94° C followed by 35 steps with denaturation for 15 sec at 94° C, hybridization for 45 sec at 45° C, elongation for 1.5 min at 72° C, final extension at 72° C for 7 min; reaction mix contained 1 µL DNA template, 2.5 µL Reaction Buffer x10 (PeqGold), 4 µL 2.5 mM dNTPs, 0.4 µL (containing 2.5 units) Taq Polymerase (PeqLab), 14.1 µL H2O, 1 µL 25 mM MgCl2, and 1 µL (containing 10 pmol) (forward: L2510, 5'-CGCCTGTTTATCAAAAACAT-3'; reverse: H3056, 5'-CCGGTCTGAACTCAGATCACGT-3'; eurofins MWG Operon).

To compare the 16S mtDNA data of our specimens with published sequences, we conducted a *BLAST* search in GenBank and took the sequences with the highest scores for comparison. Additionally, we used *Bromeliohyla bromeliacia* (Schmidt, 1933), *Duellmanohyla rufioculis* (Taylor, 1952), and *D. soralia* (Wilson & McCranie, 1985) as outgroups (the phylogenetically most closely related species according to Faivovich *et al.* 2005). All sequences were aligned and manually refined using Genious (Drummond *et al.* 2010). In MEGA5 (Tamura *et al.* 2011), we computed uncorrected pairwise genetic distances prior to the maximum likelihood and Bayesian analyses. We used JModeltest 0.1.1 (Posada 2008) with likelihood settings to find the best-fitting substitution model according the Akaike Information Criterion (AICc). The Bayesian phylogenetic analysis (MrBayes 3.1.2, Huelsenbeck & Ronquist 2001) was run under the model TPM3uf+G, for 2,000,000 generations with four Metropolis-coupled Markov Chain Monte Carlo (MCMC) sampled every 100 generations. The first 5% were discarded as burn-in (burn-in= 1000). The ML analysis was assessed via 1000 bootstrap replicates, using PAUP v4.0b10 (Swofford 1998). The Automatic Barcode Gap Discovery (ABGD) algorithm (Puillandre *et al.* 2011), has been recently recommended as a reliable barcode cluster identification algorithm (Paz & Crawford 2012). Therefore, we also evaluated our sequences applying this method, using the Web interface at http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html. The following settings were chosen: steps=20, distance= Kimura 2-parameter 2.0, and the setting for the minimum relative gap width (X) was moved to different values between 0 and 1.5.

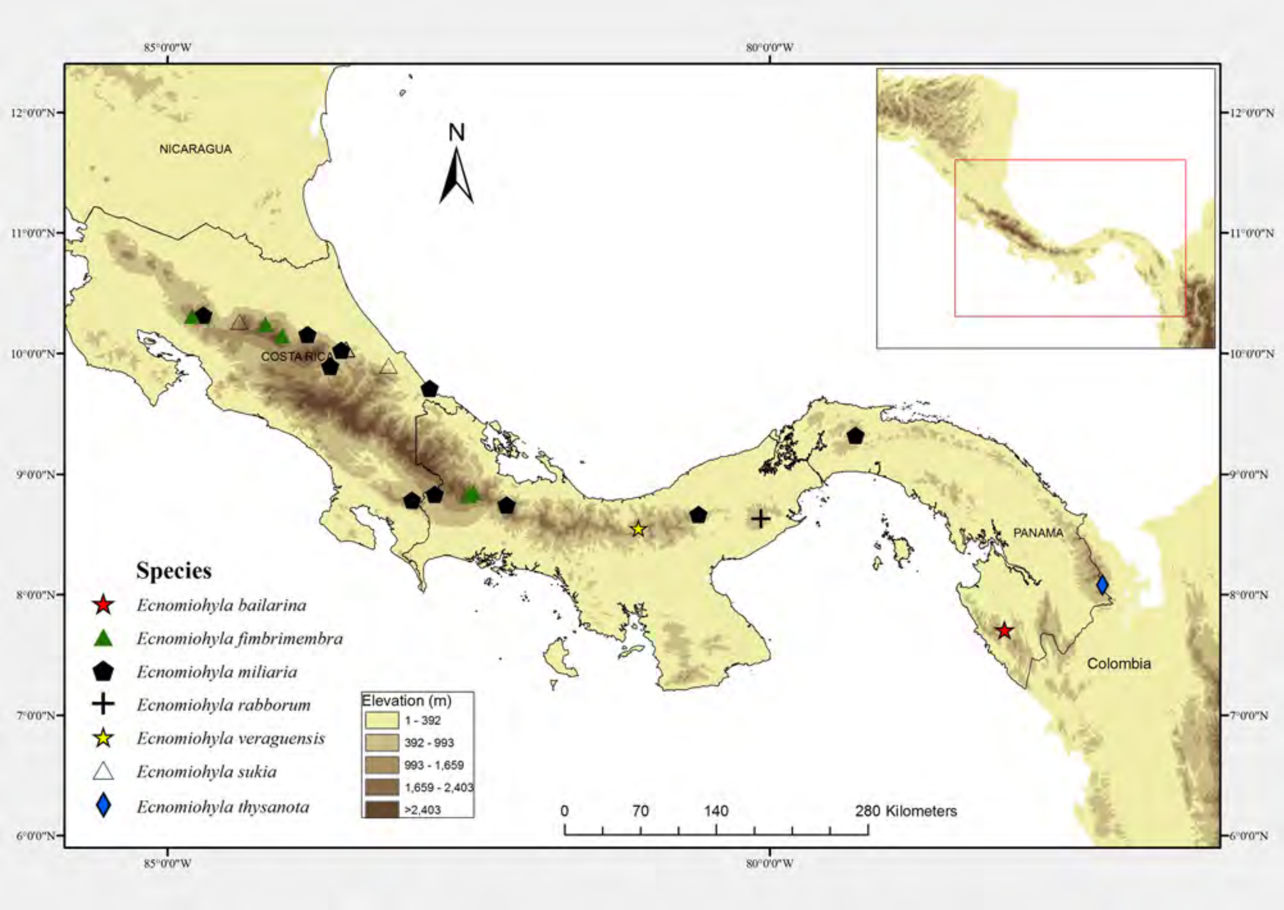

FIGURE 1. Distribution of the *Ecnomiohyla* spp. in lower Central America (main map). See Table 1 for detailed information on the localities.

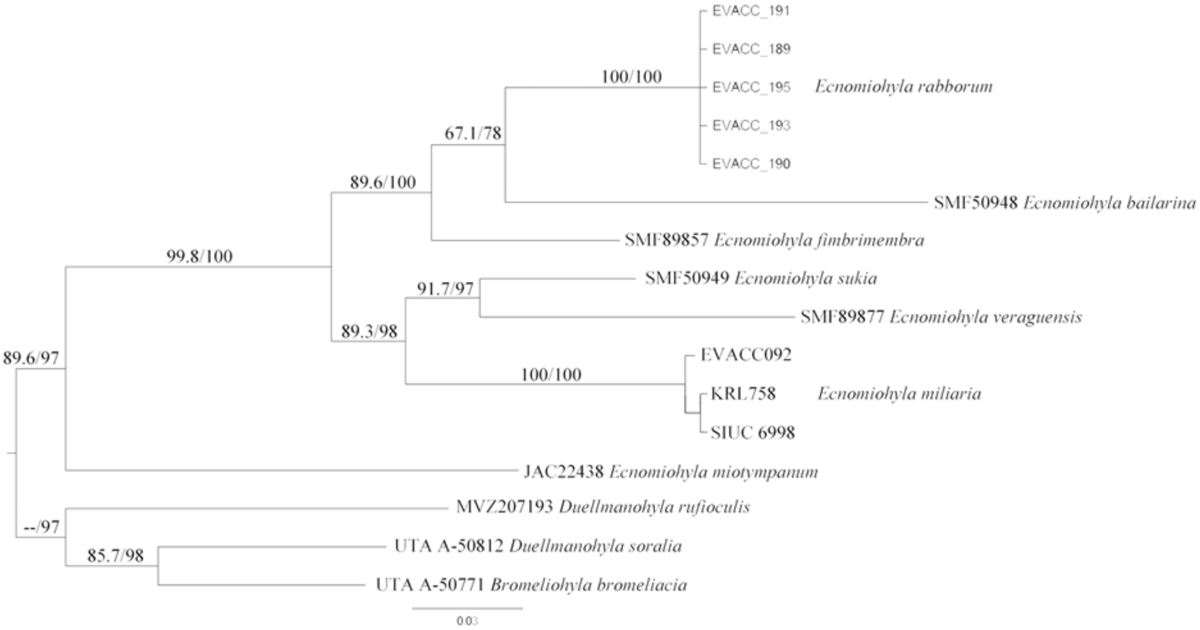

FIGURE 2. Maximum likelihood consensus tree of the 16S mtDNA gene, for the *Ecnomiohyla* spp. from lower Central America. Specimen labels refer to collection or museum number. Scale bar refers to number of substitutions per site. Maximum likelihood bootstrap values are shown in front of slash mark, Bayesian posterior probabilities (multiplied by 100) behind slash mark.

TABLE 1. Genbank accessions and detailed information on the localities of specimens mentioned in the text.

Museum number	Accession 16S	Species	Locality	Country	Coordinates N	Coordinates W	Elev.
UTA A-50771	AY843612.1	*Bromeliohyla bromeliacia*	Huehuetenango, Sierra de los Cuchumatanes, Finca Chiblac (now Aldea Buenos Aires)	Guatemala			
MVZ 207193	AY843583.1	*Duellmanohyla rufioculis*	Guanacaste, Volcan Cacao				
UTA A-50812	AY843584.1	*Duellmanohyla soralia*	Izabal, Morales, Sierra de Caral, Finca Quebradas-Cerro Pozo de Agua	Guatemala			
SMF97398	KF924240	*Ecnomiohyla bailarina*	North slope of the Jingurudó mountain range, about ca.14.6 Km S from Pavarandó village, Sambú, Comarca Emberá-Wounaam N°2	Panama	7.70903	-78.04882	750
SMF 89857	KF924242	*Ecnomiohyla fimbrimembra*	Boquete/Bajo Mono Sendero La Cascada	Panama	8.82629	-82.49907	1820
LACM 149980		*Ecnomiohyla fimbrimembra*	Pantanosa Trail, Monte Verde	Costa Rica	10.310388	-84.798056	1600
LACM 149979		*Ecnomiohyla fimbrimembra*	Northern slope of Volcan Barba, Heredia	Costa Rica	10.144033	-84.052224	1800
FMNH 191784		*Ecnomiohyla fimbrimembra*	Isla Bonita, Alajuela, Costa Rica	Costa Rica	10.241438	-84.190483	1300
CHP1036		*Ecnomiohyla fimbrimembra*	Cerro Horqueta, Boquete Panama	Panama	8.850493	-82.46196	1600
SIUC 6998	AY843777.1	*Ecnomiohyla miliaria*	EL Copé, Parque Nacional Omar Torrijos, Loop, Stream 2, Coclé.	Panama	8.667	-80.592	800
KRL758	DQ055824	*Ecnomiohyla miliaria*	EL Copé, Parque Nacional Omar Torrijos, Loop, Stream 2, Coclé.	Panama	8.667	-80.592	800
EVACC092	KC014814.1	*Ecnomiohyla miliaria*	Chagres National Park, Cerro Brewster Stream, Panamá	Panama	9.31985	-79.2889	818
KU 98451		*Ecnomiohyla miliaria*	Santa Clara, Renacimiento, Chiriquí	Panama	8.834816	-82.783559	1100
KU30404		*Ecnomiohyla miliaria*	Cartago: 2.5 km east of Turrialba	Costa Rica	9.8936	-83.6521	602
USNM 331414		*Ecnomiohyla miliaria*	Siquirres	Costa Rica	10.0269	-83.5602	62

TABLE 1. (Continued)

Museum number	Accession 16S	Species	Locality	Country	Coordinates N	Coordinates W	Elev.
UCR 4979		*Ecnomiohyla miliaria*	Estacion Biologia Las Cruces, Puntarenas	Costa Rica	8.787262	-82.972649	1200
LACM 150152		*Ecnomiohyla miliaria*	Río Peñas Blancas, Alajuela	Costa Rica	10.319491	-84.704758	800
UCR 12678		*Ecnomiohyla miliaria*	Río Blanco area, Provincia Limón.	Costa Rica	10.157077	-83.840814	450
UMMZ 149201		*Ecnomiohyla miliaria*	Comadre de Cahuita, Limón	Costa Rica	9.710069	-82.82482	20
AMNH 94887		*Ecnomiohyla miliaria*	RF fortuna Río Chiriquí	Panama	8.747156	-82.186534	1100
JAC22438	AY843645.1	*Ecnomiohyla miotympanum*	Puebla, Sierra Norte, Cuetzalan, Hotel Villas Cuetzalan	Mexico			1250
SMF94908		*Ecnomiohyla miotympanum*	Cuetzalan, Apuleo, hacienda km 7, sierra norte, Puebla	Mexico			
EVACC191	KC014813.1	*Ecnomiohyla rabborum*	El Valle, Rio Maria, Panama	Panama	8.63312	-80.0767	990
EVACC189	KC014811.1	*Ecnomiohyla rabborum*	El Valle, Rio Maria, Panama	Panama	8.63312	-80.0767	990
EVACC195	KC014809.1	*Ecnomiohyla rabborum*	El Valle, Rio Maria, Panama	Panama	8.63312	-80.0767	990
EVACC193	KC014807.1	*Ecnomiohyla rabborum*	El Valle, Rio Maria, Panama	Panama	8.63312	-80.0767	990
EVACC190	KC014812.1	*Ecnomiohyla rabborum*	El Valle, Rio Maria, Panama	Panama	8.63312	-80.0767	990
SMF94578	KF924239	*Ecnomiohyla sukia*	San Carlos, Cerro Chato, Alajuela	Costa Rica	10.2632	-84.4052	922
UCR 12787		*Ecnomiohyla sukia*	Guayacán: Alto Colorado,	Costa Rica	10.037139	-83.522889	710
UCR 10966		*Ecnomiohyla sukia*	5km from Moravia de Siquirres toward Turrialba	Costa Rica	10.033333	-83.516667	710
UCR 17024		*Ecnomiohyla sukia*	S Río Blanco (town): Fila Asunción	Costa Rica	9.9	-83.166667	400
USNM 151080		*Ecnomiohyla thysanota*	Cerro Malí, 1265 m (holotype of E. thysanota), Darién.	Panama	8.080757	-77.235448	1265
SMF82418		*Ecnomiohyla tuberculosa*					
SMF 89877	KF924241	*Ecnomiohyla veraguensis*	Cerro Negro/PN Santa Fe	Panama	8.5533	-81.09261	540

TABLE 2. Estimates of evolutionary divergence among 16S mtDNA gene sequences of the *Ecnomiohyla* spp. used in the phylogenetic analysis. Numbers below diagonal are for uncorrected p-distances and numbers above are standard error estimates.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1	*E. bailarina* (SMF97398)		0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.01	0.02
2	*E. veraguensis* (SMF89877)	0.14		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
3	*E sukia* (SMF94578)	0.15	0.07		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
4	*E. rabborum* (EVACC191)	0.11	0.10	0.10		0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.02
5	*E. rabborum* (EVACC189)	0.11	0.10	0.10	0.00		0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.02
6	*E. rabborum* (EVACC195)	0.11	0.10	0.10	0.00	0.00		0.00	0.00	0.01	0.01	0.01	0.01	0.02
7	*E. rabborum* (EVACC193)	0.11	0.10	0.10	0.00	0.00	0.00		0.00	0.01	0.01	0.01	0.01	0.02
8	*E. miliaria* (EVACC190)	0.11	0.10	0.10	0.00	0.00	0.00	0.00		0.01	0.01	0.01	0.01	0.02
9	*E. miliaria* (SIUC6998)	0.14	0.09	0.10	0.12	0.12	0.12	0.12	0.12		0.00	0.00	0.01	0.02
10	*E. miliaria* (KRL 0758)	0.14	0.09	0.10	0.12	0.12	0.12	0.12	0.12	0.00		0.00	0.01	0.02
11	*E. miliaria* (EVACCC092)	0.14	0.09	0.10	0.11	0.11	0.11	0.11	0.11	0.00	0.00		0.01	0.02
12	*E. fimbrimembra* (SMF89857)	0.12	0.10	0.10	0.08	0.08	0.08	0.08	0.08	0.11	0.11	0.11		0.02
13	*E. miotympanum* (JAC22438)	0.19	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.15	0.15	0.16	0.15	

TABLE 3. Principal differential traits of the *Ecnomiohyla* species from lower Central America and Colombia.

Trait	*E. rabborum*	*E. bailarina* sp. n.	*E. fimbrimembra*	*E. miliaria*	*E. sukia*	*E. veraguensis*	*E. phantasmagoria*	*E. thysanota*
SVL males	62.8-97.3	68	NA	86.0-110.0	56.7-63.2	57.8	95	NA
SVL females	61.3-79.9	NA	71.0 – 91.0	86.2	58.1-68.1	NA	NA	95
dorsum	granular	tuberculate	granular	tuberculate	tuberculate	finely tuberculated	tuberculate	granular
Cephalic skin co-ossified with skull	-	-	+	-	-	-	-	-
Cranial osteoderms	-	+	-	+	+	+	+	-
Dorsal osteoderms	-	+	-	+	+	+	+	-
Humeral projection in males	+	-	-	-	-	-	-	-
Prepollex (males)	blunt	rounded	blunt	recurved	obtuse	recurved	recurved	NA
Prepollical bony projection (males)	rounded	bluntly pointed. directed medially	rounded	spine	spadelike, directed laterally	spadelike, directed laterally	spine	NA
Keratinized black spines on prepollex and thumbs (males)	+	+	+	-	-	+	-	NA
Finger webbing	S	EX	C	EX	EX	EX	C	EX
Toe webbing	EX	EX	C	EX	EX	EX	EX	EX
Heel	smooth	scalloped fringe	pointed tubercles	pointed tubercles	scalloped fringe	scalloped fringe	pointed tubercles	smooth
Color in life		green with brownish flecks	brown with darker markings	brown to mottled brown and green				uniformly green

See methods for finger and toe webbing abbreviations; “-“= absent; “+”= present; NA= no specimen available for comparison.

Results

According to the barcode analysis with ABGD, we found that the comparison of all included *Ecnomiohyla* samples resulted in seven distinct species with a prior intraspecific divergence of 4.9% (Fig. 2). The overall genetic p-distance between the samples was 10.0%. Our two newly sequenced *Ecnomiohyla* specimens, one from western and one from eastern Panama, are genetically distinct and have no morphological characters that would assign them to any previously described species in the genus (Savage & Kubicki 2010; Köhler 2011). The specimen from western Panama (SMF89877) forms a sister clade to *E. sukia* from Costa Rica with an estimated evolutionary divergence of 7% (Table 2). The specimen from eastern Panama (SMF97398) is most closely related to *E. rabborum* and *E. fimbrimembra,* but is genetically distinct by 11 and 12% p-distance in the 16S gene (Table 2), respectively. SMF97398 shows the highest p-distance of 15% to *E. sukia*. *Enomiohyla miotympanum* is revealed as sister taxon to all *Ecnomiohyla* from lower Central America (Fig. 2, Table 2), separated by a p-distance of 14–19%. According to our findings of significant genetic and morphological differences in two of our newly obtained specimens (Table 3), we proceed to describe them as two species new to science.

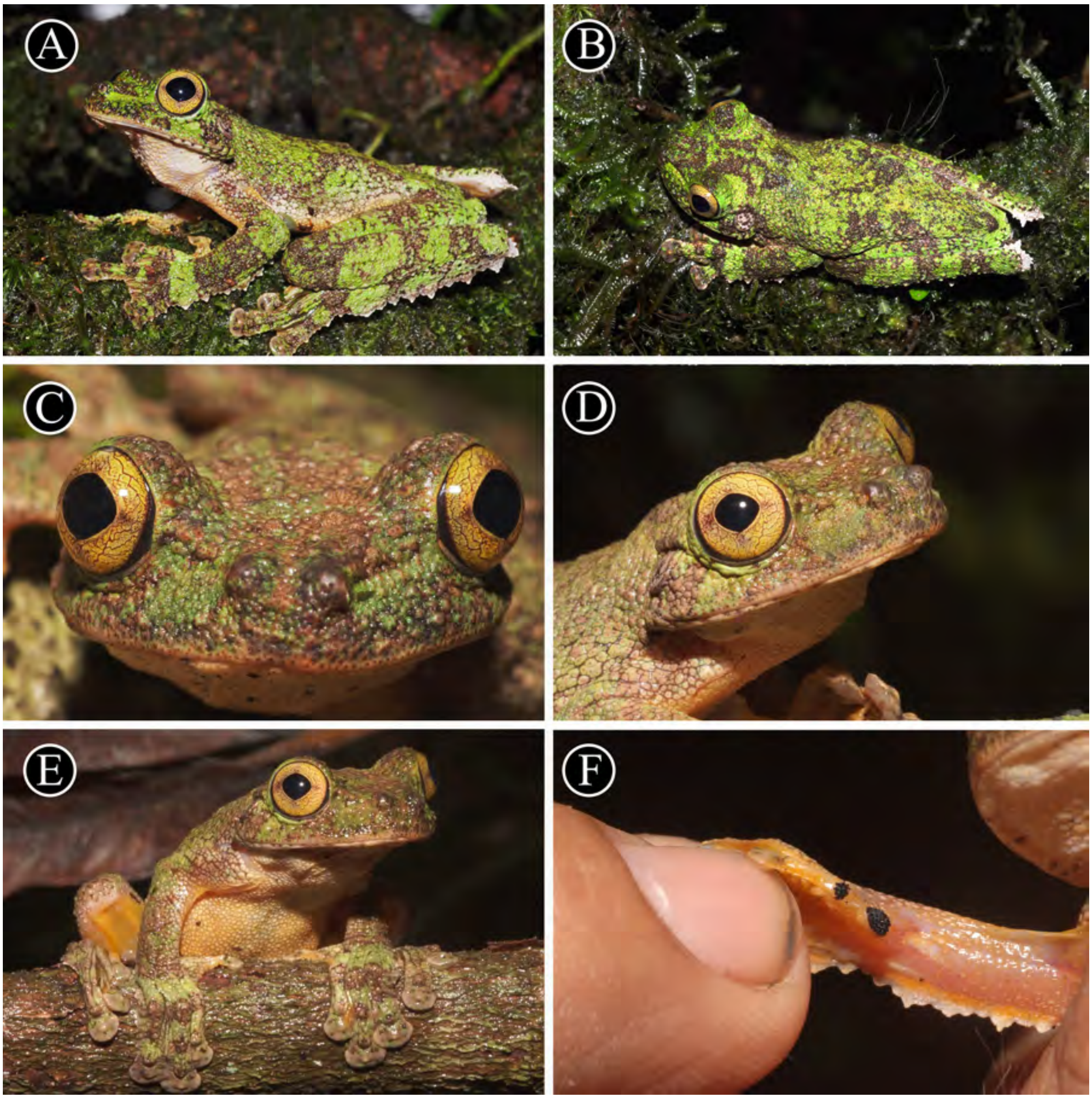

FIGURE 3. Photographs of the holotype of *Ecnomiohyla bailarina* in life. A) lateral view; B) dorsal view; C) frontal view; D) profile; E) ventral coloration; F) prepollical spines on right hand.

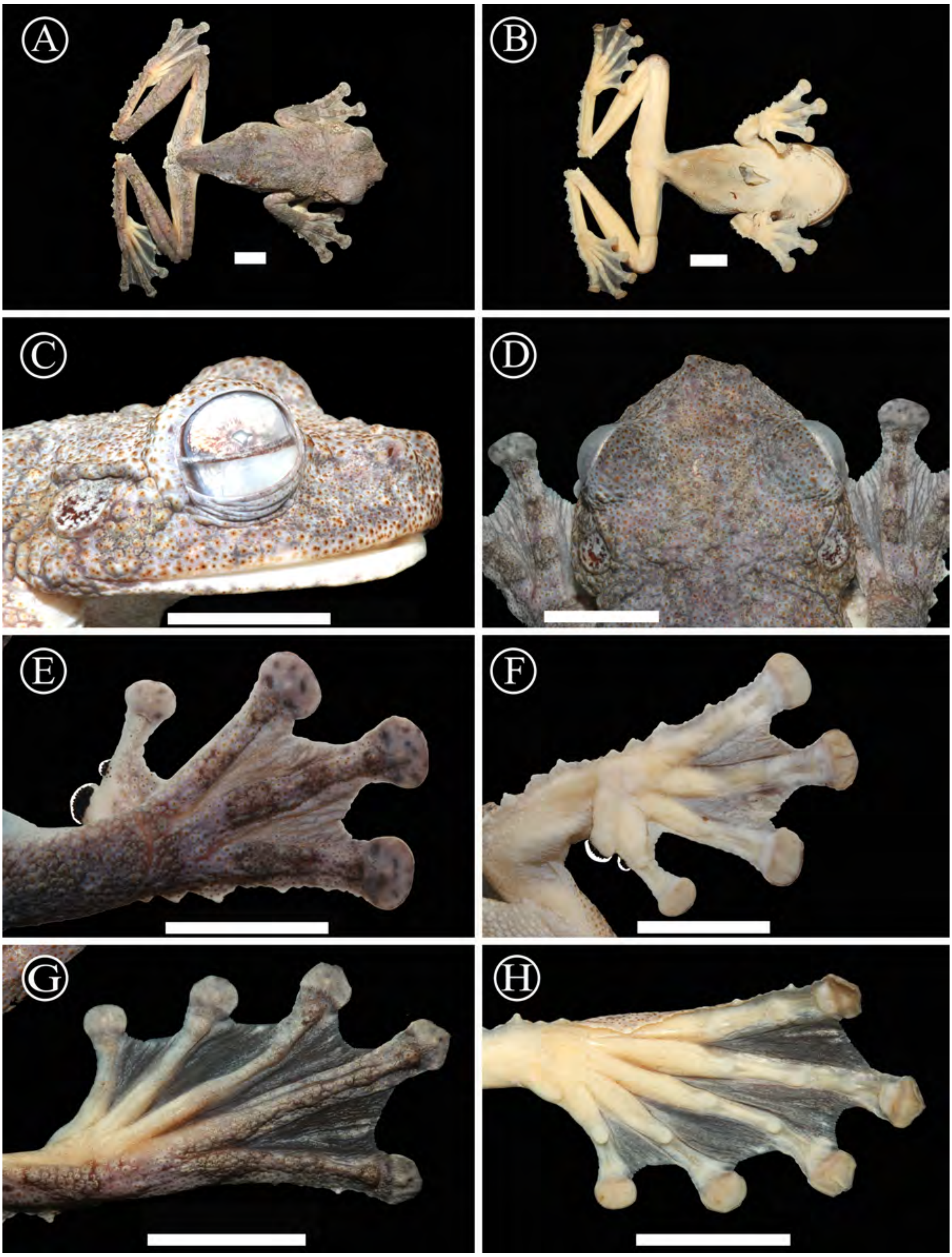

FIGURE 4. Photographs of preserved holotype of Ecnomiohyla bailarina. A) dorsal view; B) ventral view; C) head in profile; D) head dorsally; E) right hand dorsally; F) right hand ventrally; G) right foot dorsally; H) right foot ventrally. Scale bars = 10 mm.

Ecnomiohyla bailarina sp. nov.

Holotype. SMF97398 (original field number AB297; Fig. 3–4), an adult male from the north slope of the Jingurudó mountain range (Fig. 5), about 14.6 km S from Pavarandó village (7.70903°N, -78.04882°W, 750 m a.s.l.), Sambú, Comarca Emberá-Wounaan N°2, Darién, Panama, collected by Abel Batista and Milan Vesely on 25 September 2011 at 21:27 hrs.

Diagnosis. A medium-sized *Ecnomiohyla* (single known specimen is an adult male 68.1 mm in SVL; Figs. 3–4), differing from other known species in the genus by the following combination of characters: 1) finger webbing extensive, web reaching the finger disk on at least one side on two fingers (Fig. 4); 2) toes extensively webbed as well, web reaching the toe disk at least on one side of four toes (Fig. 4); 3) skin on dorsum strongly tuberculate; 4) cranial and dorsal osteoderms present; 5) skin on upper surface of head not co-ossified with underlying cranial elements; 6) humerus without enlarged *crista lateralis*; 7) prepollex distinct, obtuse, with bony prepollical projection rounded distally, bluntly pointed at side adjacent to thumb; 8) two clusters of nuptial spines at the distal end of prepollical tubercle and at the end of the first phalanx of the thumb; 9) a distinct scalloped fringe with pointed tubercles on a ventral surface of heel flaps, continuing almost to the disc of the 5th toe; 10) dorsal coloration in life green with scattered brownish or black flecks.

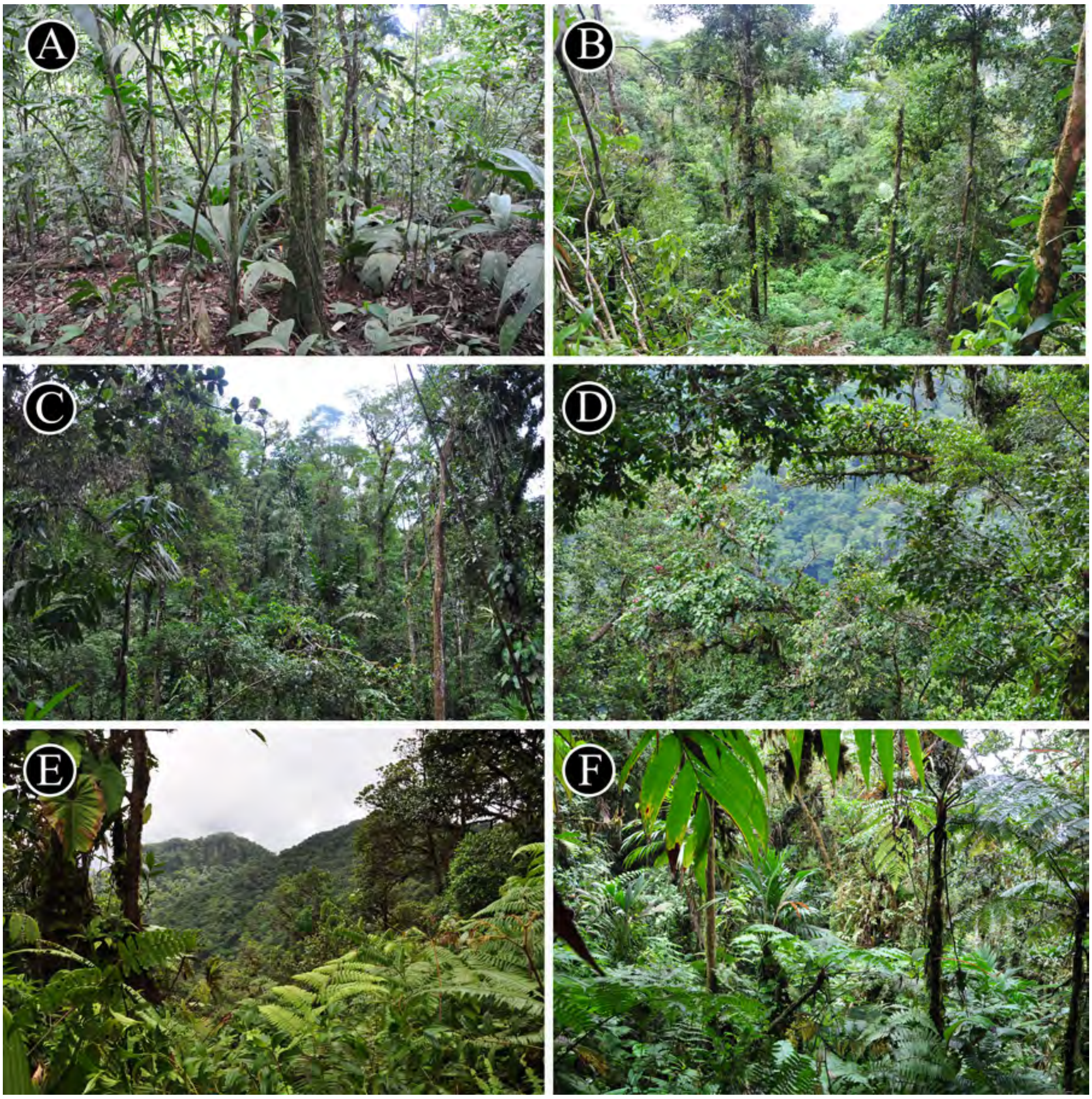

FIGURE 5. Habitat of *Ecnomiohyla bailarina* A) understory area where holotype was caught; B-C) forest structure from an open area; D) canopy forest; E) Cerro Bailarín, view from a ridge to 900 m a.s.l.; F) understory at Cerro Bailarín.

Comparison with other species of *Ecnomiohyla*. *Ecnomiohyla bailarina* can be distinguished from other species of *Ecnomiohyla* by the following characters (with contrasting features for *E. bailarina* in parentheses, see Table 3 for more details): *Ecnomiohyla minera, E. thysanota* (see Fig. 6) and *E. rabborum* are easily distinguished from the new species by having smooth heels without a scalloped fringe (triangular serrate fringe with pointed tubercles on a ventral surface of heel flaps); *E. rabborum* and *E. minera* are further distinct in having a humeral projection in males (no humeral projection); *E. rabborum* has a substantial webbing on one finger only, reaching base of disk on one finger (webbing extensive reaching base of disk on two fingers); *E. echinata, E. fimbrimembra, E. minera, E. salvaje* and *E. valancifer* lack of cranial or dorsal osteoderms (well developed cranial and dorsal osteoderms); the type locality of the only known specimen of *E. thysanota,* a female collected at Cerro Malí, Darién (Duellman 1966), is only 100 km northeast of the type locality of *E. bailarina*, but *E. thysanota* lacks cranial and dorsal osteoderms (well developed cranial and dorsal osteoderms, see Figs. 3−4, and 6), skin on dorsum is granular (strongly tuberculate), coloration in life is reported to be uniformly green (green with scattered brown or blackish flecks); in addition, these potentially sympatric species would probably differ also in size, as the *E. thysanota* specimen is a female that is much larger (95 mm vs 68.1 mm SVL, see Table 3) than our male *E. bailarina*; males in *Ecnomiohyla* spp. tend to be bigger or at least the same size as females (Table 3, Savage & Kubicki 2010), and hence, an adult male *E. thysanota* is presumed to be considerably larger than the adult male holotype of *E. bailarina; E. fimbrimembra* (see Fig. 9), *E. miliaria*, and *E. phantasmagoria* also lack a fringe on heels (present), but have pointed heel tubercles; in addition, males of *E. miliaria* and *E. phantasmagoria* have a sharp prepollical spine directed laterally (prepollical spine vestigial, bluntly pointed and directed to the thumb); *E. fimbrimembra* and *E. salvaje* have the skin on the head co-ossified with the cranium, (Fig. 9 E–H) (skin not co-ossified with cranium); males of *E. miliaria, E, phantasmagoria, E. sukia, E. tuberculosa* and *E. valancifer* have no nuptial black spines on prepollex (numerous small black keratinized spines present on prepollex); *E. miotympanum* lacks of scalloped dermal fringes on the outer margin of the forearm and foot, large digital disks, and enlarged prepollices (present in *E. bailarina*); *E. tuberculosa* does not have a prepollical projection in adult males (prepollical projection present); in *E. sukia*, the prepollical spine has a similar size and direction, but is rather spade-like, not forming a sharp spine as in *E. bailarina*; *E. veraguensis* (sp. nov., see below) can be distinguished from *E. bailarina* by having only a few large, widely spaced nuptial black keratinized spines, dorsolaterally on the base of the pollex and none on the prepollex in adult males (thickly clustered smaller spines on prepollex and pollex; Fig. 10); further, it has a finely tuberculated dorsum (strongly tuberculated dorsum), and keratinized tubercles on the ventral side of the scalloped fringe on the heels are absent (present in *E. bailarina*).

TABLE 4. Measurements and morphological proportions for the holotypes of the new *Ecnomiohyla* species described herein.

Trait	Measurements (mm)		Trait	Proportions (%)	
	E. veraguensis	*E. bailarina*		*E. veraguensis*	*E. bailarina*
SVL	57.8	68.1	IND/SVL	9.7	8.2
HL	20.2	22.0	HL/SVL	34.9	32.3
HW	23.6	24.1	HW/SVL	40.8	35.4
IOD	14.7	14.1	HL/HW	85.6	91.3
ED	6.0	6.8	IOD/SVL	25.4	20.7
TD	3.6	4.2	ED/SVL	10.4	10.0
HAL	20.8	21.2	TD/SVL	6.2	6.2
FAL	11.8	16.7	HAL/SVL	36.0	31.1
IND	5.6	5.6	FAL/SVL	58.4	75.9
TL	32.8	35.8	TL/SVL	56.7	52.6
FL	28.0	27.6	FL/SVL	48.4	40.5
3FW	2.2	3.1	3FW/SVL	3.8	4.6
3FD	3.4	4.4	3FD/SVL	5.9	6.5
4TW	2.0	3.4	4TW/SVL	3.5	5.0
4TD	2.7	3.4	4TD/SVL	4.7	5.0
3TW	2.1	2.9	3TW/SVL	3.6	4.3
3TD	2.7	3.1	3TD/SVL	4.7	4.6
BW	30.5	19.7	BW/SVL	52.8	28.9

Description of the holotype. An adult male, as indicated by the presence of keratinized nuptial spines. Measurements of the holotype are shown in Table 4. Head rounded in dorsal view, slightly wider than long (HL/HW = 91.3%); snout truncate in dorsal and lateral views; nearly terminal nostrils directed laterally; top of head flat; canthus rostralis concave; loreal region concave; skin on dorsal surface of head and body tuberculate, tubercles formed by osteoderms; tubercles on upper lip, loreal and supraorbital area tipped with tiny blunt keratinous spines; lower eyelid with transparent upper part; a well-developed supratympanic fold running from midpoint of posterior margin of eye above the upper margin of tympanum, slightly curved around its upper posterior edge, tympanum prominent, opaque, smooth, 51.5% of ED, separated from eye by 3.20 mm; upper surfaces of body and limbs tuberculate, intermixed with scattered larger tubercles, cluster of tubercles above the insertion of arms; a triangular serrate-like fringe extends from the elbow along the ventrolateral margin of the forearm and continues along the outer edge of Finger IV to the base of the disk; serrate fringe largest on forearm, less evident serration along fingers; hands moderate in length (HAL/SVL = 31.1%); Finger lengths I<II<IV<III, terminal disk on Finger I 79% of diameter of disks on Fingers II–IV; which are almost the same size as tympanum (3FD/TD 1.04 times); distal subarticular tubercles on Fingers I–III large, rounded; bifid at Finger IV, larger than proximal subarticular tubercles on Fingers III–IV; indistinct supernumerary tubercles; prepollex enlarged and rounded; bony prepollical projection rounded distally, bluntly pointed at side adjacent to thumb; two clusters of nuptial spines at the distal end of prepollical tubercle and at the end of the first phalanx of the thumb; fingers extensively webbed, web extending to base of disk on at least two fingers; webbing formula: **I** $1^{3/4}$–2 **II** $^{3/4}$–$1^{1/2}$ **III** $1^{1/2}$–$1^{1/4}$ **IV**; legs relatively long and slender (TL/SVL = 52.6%), heels of adpressed limbs overlapping about 1/4 length of tibia, thigh 30.00 mm long; distinct fleshy, triangular serrate like fringe begins on heel by a striking flap and extends along ventrolateral margin of tarsus and outer margin of Toe V to base of disk; scallops deeply incised and pointed, largest on tarsus, smaller along toe; small tubercles with keratinized tips present on dorsal and ventral surface of fringe on heel; tarsal fold and outer metatarsal tubercle absent, inner metatarsal tubercle moderately large (same size as 3TD), ovoid, flat, and spadelike distally; toe lengths I<II<III=V<IV; disks on toes 75% of diameter of those on fingers, equal on Toes III–V, decreasing in size on toes II–I; subarticular tubercles rounded; supernumerary tubercles indistinct; toes extensively webbed, webs extending to base of disks on at least four toes; webbing formula: **I** $^{3/4}$–$1^{1/4}$ **II** $^{3/4}$–$1^{1/4}$ **III** $^{3/4}$–1 **IV** $1^{1/4}$–$^{3/4}$ **V**; gular area and venter strongly granulate, fine granulation on undersides of arms and proximal thighs, smooth skin on anterior surfaces of thighs and ventral parts of legs; cloacal opening directed posteriorly at mid-level of thighs, two distinct granular dermal folds under the vent; tongue slightly cordiform; vomerine ridges transverse, narrowly separated medially, placed between the posterior margins of the moderately large ovoid choanae; vomerine teeth 12–13; vocal slits not present.

Coloration of holotype in life (Fig. 3). Dorsal ground colour Light Grass Green (color 109 of Köhler 2012) with irregular Vandyke Brown (281) flecks scattered all over the head and body giving the animal a "moss cryptic" appearance; Raw Umber (22) bands present on dorsal surfaces of arms and legs, edges of scalloped fringes on arms and fleshy flaps on heels Cream Color (12); toe webbing Tawny Olive (17); tops of some dorsal granules and tubercles Orange-Rufous (56). After metachrosis (day and night coloration), ground coloration faded to Pale Emerald Green (141), brown areas to Dark Salmon color (59), pattern did not change; throat, chest, venter and ventral surfaces of arms and legs Cream Color (12) grading into Salmon (83) ventrolaterally and Orange Yellow (8) on anterior surface of thigh; a few small dark blotches on the edge of lower lip; iris Light Yellow Ocher (13), finely reticulated with Dark Brownish Olive (127); tympanum Pale Mauve (204) with scattered irregular Vinaceous Pink (245) blotches.

Coloration in preservative (Fig. 4). Dorsal surfaces Glaucous (272) with Sepia (279) mottling on upper surfaces of hind limbs; tympanum Pratt's Payne's Gray (293) with scattered irregular Maroon (39) blotches, cloacal region Pratt's Payne's Gray (293) dorsally and Cream (12) ventrally; posterior surfaces of thighs Light Yellow Ocher (13); ventral surfaces of body and limbs Cream (12); toe webbing Amber (51).

Distribution and natural history. *Ecnomiohyla bailarina* is known only from the type locality, in the eastern Panamanian montane forest (Fund & Hogan 2012; Fig. 5 A-D). The potential area of distribution of *E. bailarina* comprises the vicinities of Jingurudó and Sapo mountain ranges, between 400 to 1400 m a.s.l. (Fig. 1). Although the type locality is in a primary forest, there are some open areas with successional secondary forest. The area is on a ridge, so the trees could be affected by strong winds. In the surroundings we saw four fallen large trees probably overthrown by the wind that left clearings in the otherwise pristine forest. The largest trees in this area reached more than 20 m in height having branches in the canopy covered by bromeliads and other epiphytes (e. g., orchids and Lorantaceae), Tree trunks were almost bare or with just a little epiphytic growth. In the understory, palms and vines were predominant. The holotype was found on a ridge in a water conserving posture (see Fig 1B in Pough *et al.* 1983) on the bark of a small tree (Fig. 5A), approximately 1.5 m above the ground. The day before the night of the capture was dry except for a drizzle that had fallen in the afternoon between 14:00–15:00 hrs. During the encounter, a slight breeze was blowing. Other amphibian species observed in the area that day were: *Colostethus* aff. *pratti* (Boulenger, 1899), *Craugastor opimus* (Savage and Myers, 2002), *Pristimantis cruentus* (Peters, 1873), *P. taeniatus* (Boulenger, 1912), *Rhinella alata* (Thominot, 1884), and *Sachatamia ilex* (Savage, 1967).

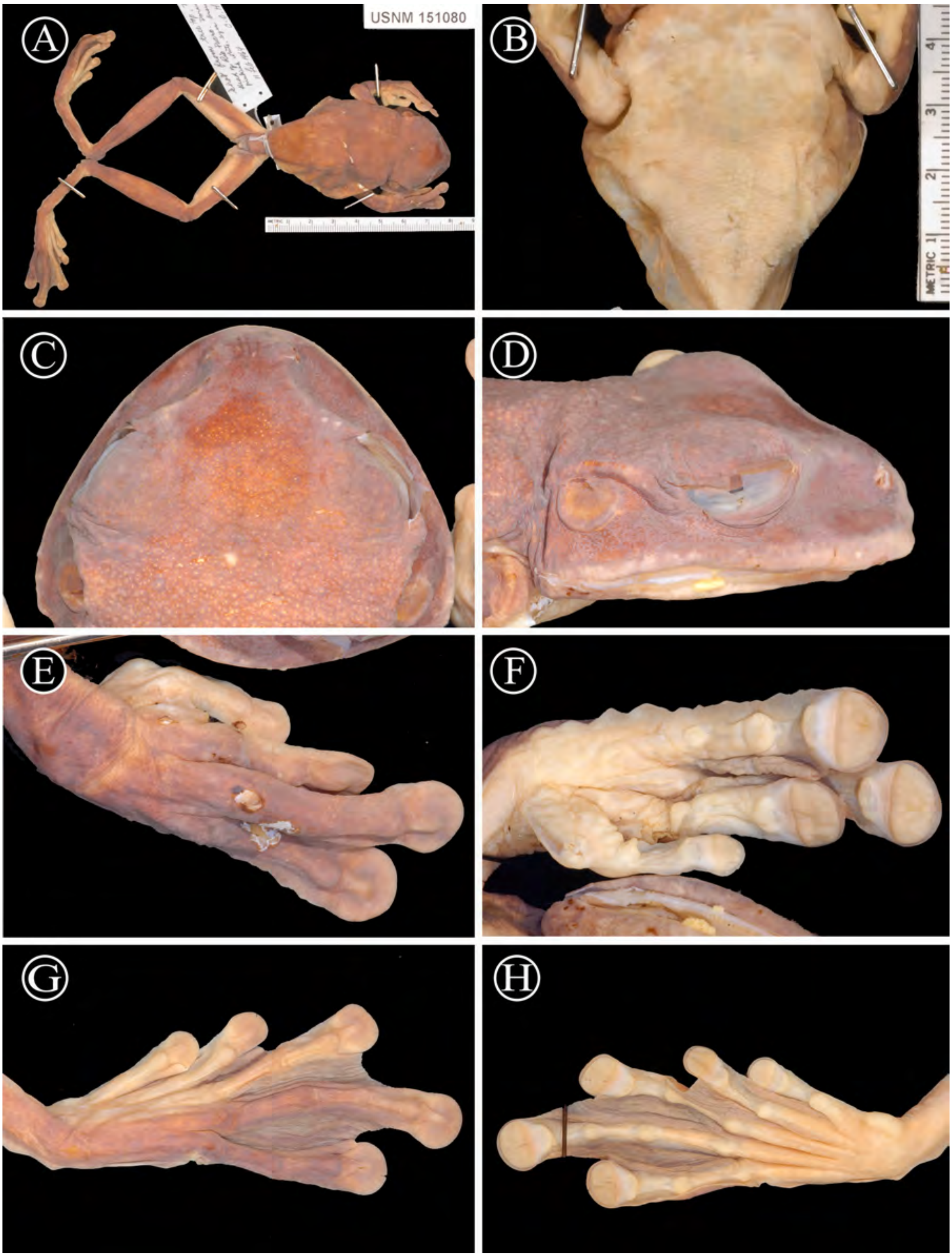

FIGURE 6. *Ecnomiohyla thysanota*, Holotype (USNM151080), preserved specimen. A) dorsal view; B) ventral view; C) head dorsally; D) head in profile; E) right hand dorsally; F) right hand ventrally; G) right foot dorsally; H) right foot ventrally.

Etymology. The name *bailarina* is a noun in apposition in reference to the hill where the specimen was found. The indigenous people of the Embera call it "Cerro Bailarín", in addition, the English translation of "bailarina" is ballerina, so the name also refers to the resemblance of the fringes on arms and feet of the frog to the tutu skirt that a ballerina wears.

Conservation status. The secretive habits of *Ecnomiohyla bailarina* make the assessment of the population size difficult, as in other *Ecnomiohyla* species. Considering that the status of the *E. bailarina* population is unknown, the data deficient (DD) criterion, according the IUCN (IUCN 2013), seems appropriate for this species, until data on its population trend become available. Moreover, due to fact that *E. bailarina* and *E. thysanota* occur in a region affected by social problems and political conflicts along the border between Panama and Colombia, it is unlikely that there will be sufficient opportunity to visit the region to assess population sizes in the near future.

Ecnomiohyla veraguensis sp. nov.

Ecnomiohyla rabborum–Köhler 2011: p. 224 Fig. 537; p. 226 Fig. 541 b.

Holotype. SMF89877 (original field number AH210) an adult male (Figs. 7–8) collected near Cerro Negro (8.5533 °N, -81.09261 °W, 540 m a.s.l.), Santa Fé National Park, Veraguas, Panama, on 31 March 2009 at 12:00 hrs, collected by Smelin Abrego, Arcadio Carrizo, Andreas Hertz, and Sebastian Lotzkat.

Diagnosis. A medium-sized species of *Ecnomiohyla*. The single known specimen is an adult male, 57.8 mm in SVL) differing from other known species of the genus by following combination of characters: 1) finger webbing extensive, web touching the finger disk on at least one side on Fingers II–IV; 2) toes extensively webbed; web reaching the toe disk at least on one side on four toes; 3) skin on dorsum finely tuberculate with scattered minute keratin tipped tubercles posteriorly; 4) cranial and dorsal osteoderms present; 5) skin on upper surface of head not co-ossified with underlying cranial elements; 6) humerus without enlarged *crista lateralis*; 7) prepollex distinct, recurved, with distinct bony prepollical projection, spadelike and directed laterally; 8) 6–8 widely spaced, keratinized black spines present bordering the outer side of the thumb; 9) a distinct scalloped fringe without pointed tubercles on its ventral surface, arising at the heel and continuing on the outer side of Toe V and reaching almost to the disk of Toe V (Fig. 8); 10) dorsal coloration in life smoke gray, with upper surface of forearms bearing a suggestion of lime green (Fig. 7).

Comparison with other species of *Ecnomiohyla*. *Ecnomiohyla veraguensis* can be distinguished from other species of *Ecnomiohyla* by the following characters (with contrasting features for *E. veraguensis* in parentheses; see Table 3 for more details): *E. echinata, E. minera*, *E. rabborum, E. salvaje, E. thysanota,* and *E. valancifer* can be distinguished from the new species by the lack of cranial and dorsal osteoderms (both present); *E. rabborum* and *E. minera* are further distinct in having a humeral projection in males (no humeral projection); *E. rabborum* has substantial finger webbing, web reaching base of disk on one finger (extensive webbing, web touching the finger disk on at least one side on Fingers II–IV); *E. fimbrimembra* (Fig. 7 E-F), *E. miliaria* and *E. phantasmagoria* lack scalloped fleshy fringes on heels and have pointed heel tubercles instead (scalloped fleshy fringes present, no heel tubercles); *E. fimbrimembra* and *E. salvaje* have the skin on the head co-ossified with the cranium, (skin not co-ossified with cranium); males of *E. miliaria* and *E. phantasmagoria* have a sharp prepollical spine protruding from the prepollex (prepollex recurved, no protruding spine); *E. bailarina* has a strongly tuberculate dorsum (finely tuberculate) and two clusters of numerous, small nuptial spines at the distal end of the prepollex and the base of the pollex (only 6–8 larger, widely spaced nuptial spines along the outer side of the pollex; Fig. 10); *E. tuberculosa* lacks an enlarged prepollical bony projection or keratinized black spines on the prepollex in adult males (enlarged prepollical bony projection and 6–8 widely spaced, keratinized black spines presents); *E. miotympanum* lacks of scalloped dermal fringes on the outer margin of the forearm and foot, large digital disks, and enlarged prepollices (present in *E. veraguensis*); in terms of general appearance, *E. sukia* is most similar to the new species, but differs by a genetic distance in the 16S gene of 7% and the lack of nuptial spines in adult males (6–8 widely spaced nuptial spines on the outer side of the pollex; see Fig. 9 (B, D) and 10); further, *E. sukia* lacks keratin tipped tubercles on the dorsum (presence of keratin tipped tubercles on the dorsum).

FIGURE 7. Photographs of the holotype of *Ecnomiohyla veraguensis* in life. A) lateral view (night time); B) dorsal view (day time); C) frontal view; D) at the moment of encounter; E-F) voucher specimen of *E. fimbrimembra* (SMF89857).

Description of the holotype. An adult male, as determinated by the presence of nuptial spines, and vocal slits. Measurements of the holotype are indicated in Table 4. Head rounded in dorsal view, wider than long (HL/HW= 85.6%); snout truncate in dorsal and lateral views; nostrils directed laterally; top of head flat; canthus rostralis concave; loreal region concave; a well-developed supratympanic fold running from above the upper margin of tympanum, slightly curved around its upper posterior edge; tympanum prominent, smooth, same color as dorsum, and 60.0% of ED; separated from eye by 3.04 mm; upper surface of body finely tuberculated, scattered tubercles present on dorsal surfaces of the limbs; arms robust, hypertrophied; a fleshy scalloped fringe extends from the elbow along the ventrolateral margin of forearm and continues along the outer edge of Finger IV to base of disk; scallops of fringe largest on forearm, weak scallops along finger; hands moderate in length (HAL/SVL= 36.0%); finger lengths I<II<IV<III, terminal disk on Finger I 70% of diameter of disks on Fingers II–IV, which are slightly smaller in size than tympanum (3FD/TD= 94%); distal subarticular tubercles on fingers rounded and elevated; a row of supernumerary tubercles present under the first phalanges on Fingers II–III; palmar tubercles rounded and low; prepollex enlarged and recurved, bony prepollical projection spadelike, directed laterally at side adjacent to thumb; 6–7 nuptial spines along the outer side of the pollex; fingers extensively webbed, web extending to base of disks on at least two fingers; webbing formula: **I** $1^{3/4}$–2 **II** $^{3/4}$–$1^{1/4}$ **III** $1^{1/4}$–$^{3/4}$ **IV**; legs relatively long and slender (FL/SVL= 48.4%), heels of adpressed limbs overlapping about 1/3 length of tibia, thigh 26.70 mm long; distinct fleshy,

scalloped fringe begins on heel and extends along ventrolateral margin of tarsus and outer margin of Toe V to base of disk; scallops sinuously serrated, widest on tarsus, smaller along toe; tarsal fold slightly evident; outer metatarsal tubercle barely distinct, inner metatarsal tubercle large (1.12 times 3TD), ovoid, slightly elevated, and spadelike distally; toe lengths I<II<III>V<IV; disks on toes 80–91% of diameter of those on fingers, disk on Toe IV same size as disks on Toes III and V, decreasing in size on Toes II–I; subarticular tubercles rounded and slightly elevated; a row of 5–10 supernumerary tubercles barely distinct under the proximal phalanges on toes; extensive toe webbing , webs extending to base of disks on at least four toes; webbing formula: $\mathbf{I}\ {}^{3/4}\text{–}1^{1/2}\ \mathbf{II}\ {}^{3/4}\text{–}1^{1/4}\ \mathbf{III}\ {}^{3/4}\text{–}{}^{3/4}\ \mathbf{IV}\ {}^{1/4}\text{–}{}^{3/4}\ \mathbf{V}$; gular area and venter granulate, fine granulation on undersides of arms and proximal thighs, smooth skin on anterior surfaces of thighs and ventral parts of legs; cloacal opening directed posteriorly at mid-level of thighs, a distinct granular dermal fold under the vent. Tongue slightly cordiform, broader at the base; vomerine ridges large and transverse, well separated medially, placed between the posterior margins of choanae; vomerine teeth 10–14; paired vocal slits extending posteriorly from posterior lateral base of tongue toward angle of jaws.

Coloration of holotype in life (Fig. 7). Coloration in life was recorded at daytime: Dorsal ground color Smoke Gray (44); snout, canthus rostralis, and supraorbital regions Brownish Olive (29) suffused with Olive Green (Auxiliary 47); upper surfaces of forearms with a suggestion of Lime Green (59); dorsal surfaces of finger webbing like dorsal coloration on body, but toe webbing Vandyke Brown (121); ventral surfaces of chin and body Cream Color (54), spotted with Raw Sienna (136); ventral surfaces of hindlimbs True Cinnamon (139); ventral coloration of toe and finger webbings Vandyke Brown (121).

Coloration in preservative (Fig. 8). Dorsal surfaces Grayish Horn Color (268); snout, canthus rostralis, and supraorbital regions Medium Plumbeus (294); darker bars on upper surfaces of limbs Medium Plumbeus (294); cloacal region Pale Buff (1), suffused with Medium Plumbeus (294); groin and posterior surfaces of thighs mottled with Maroon (39) on a Pale Buff (1) ground; ventral regions Pale Buff (1); chin suffused with Maroon (39); toe and finger webbing Burnt Umber (48).

Distribution and natural history. *Ecnomiohyla veraguensis* is known only from the type locality in the Isthmian-Pacific moist forests (Fund & Hogan 2012). The holotype was found at noon on a sunny day at the end of the dry season. Relative air humidity at the moment of encounter was 68% at a temperature of 21.8 °C and it was slightly windy. The frog was sitting in a water conserving posture (Fig. 7D) on a fern leaf approximately 0.5 m above the ground, next to a water tube that is used by local people to obtain drinking water. Other amphibian species that were observed at Cerro Negro on this expedition conducted between March 31 and April 03 2009 include *Atelopus varius* (Lichtenstein & Martens, 1856), *Bolitoglossa colonnea* (Dunn, 1924), *Craugastor gollmeri* (Peters, 1863), *C. megacephalus* (Cope, 1875“1876“), *Diasporus citrinobapheus* Hertz, Hauenschild, Lotzkat & Köhler, 2012, *Lithobates warszewitschii* (Schmidt, 1857), *Pristimantis caryophyllaceus* (Barbour, 1928), *P. cerasinus* (Cope, 1875 “1876“), *P. cruentus*, *P. museosus* (Ibáñez, Jaramillo & Arosemena, 1994), *P. pardalis* (Barbour, 1928), *Rhaebo haematiticus* Cope, 1862, and *Sachatamia albomaculata* (Taylor, 1949). Since *E. veraguensis* is only known from a single specimen from a single locality, the distribution is unknown. It is expected to occur along mid-elevations of the Serranía de Tabasará.

Etymology. The species name is derived from the province name Veraguas where the holotype was found, with the Latin suffix *-ensis* donating a place or locality. The species name has been chosen to accentuate the particular role the province of Veraguas plays in terms of amphibian conservation. It is the only Panamanian province with Atlantic and Pacific coasts, thus encompassing a great variety of habitats for many amphibian species.

Conservation status. As other *Ecnomiohyla* species, *E. veraguensis* could be considered as a rare species, due to the habitats it uses, this fact makes it difficult to assess its populations. Like *E. bailarina*, the data deficient (DD) criterion, according the IUCN (IUCN 2013), seems appropriate for *E. veraguensis* too, until data on its population trend become available.

Discussion

We describe *Ecnomiohyla bailarina* based on both molecular and morphological data. This spectacular species appears to be very distinct from all other known members of the genus. The type locality of *E. bailarina* is relatively close to that of *E. thysanota* (Fig. 6), and our first assumption in the field was, that they could be conspecific. However, after comparing pictures of the *E. thysanota* holotype with our specimen we easily detected several substantive differences in morphology, mainly demonstrated by the presence of cranial and dorsal osteoderms in *E. bailarina* and not in *E. thysanota*, the different fringe shape on the heel as well as a different skin texture, which argue for two distinct species despite of the lack of molecular genetic data from *E. thysanota*.

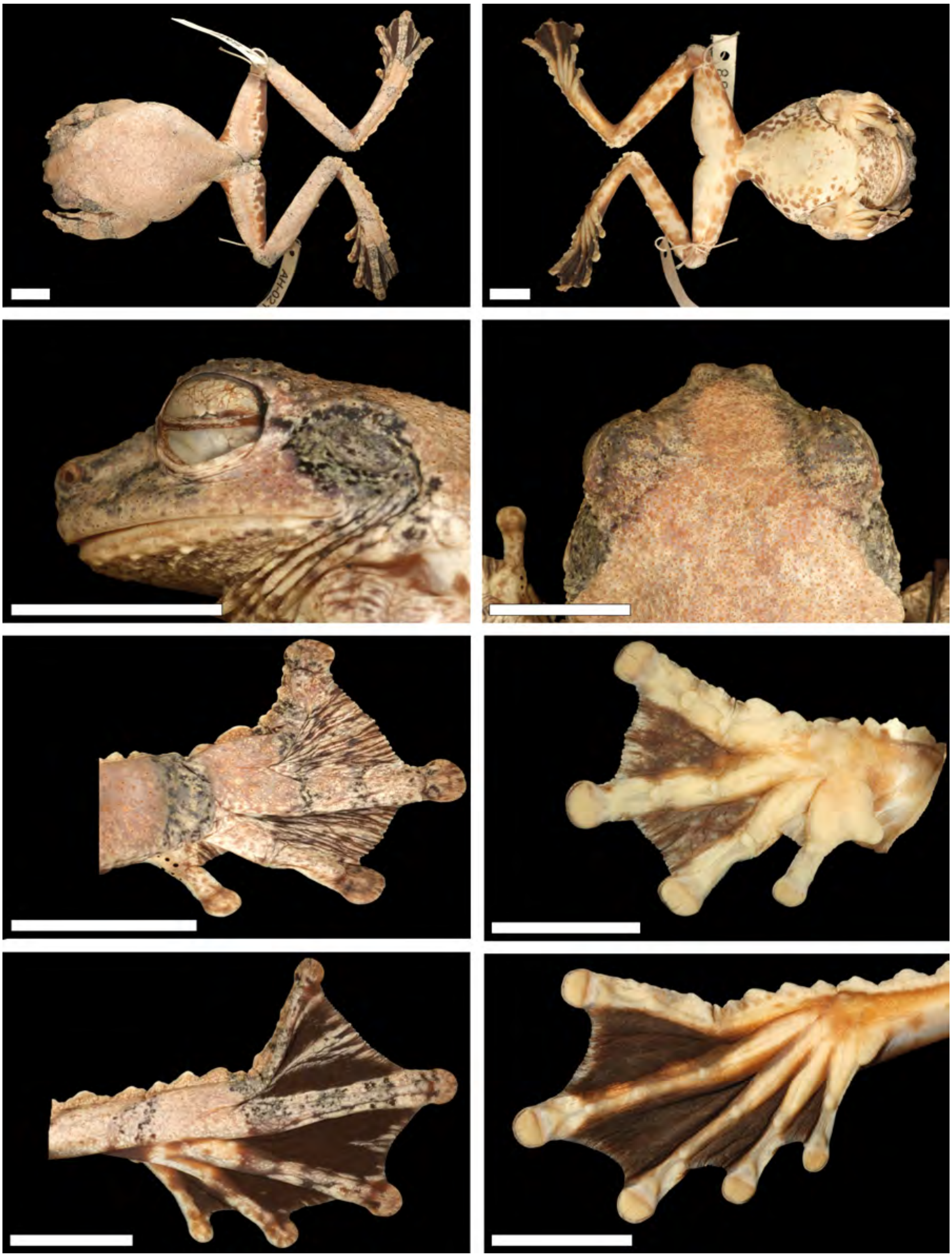

FIGURE 8. Holotype of *Ecnomiohyla veraguensis* in preservation. A) dorsal view; B) ventral view; C) head in profile; D) head dorsally; E) right hand dorsally; F) right hand ventrally; G) right foot dorsally; H) right foot ventrally. Scale bars= 10 mm.

Ecnomiohyla veraguensis is similar to *E. sukia* in overall appearance and both species clusters as sister clades in the 16S tree. However, the genetic distance between them is 7% and thus far above the threshold of 3%, that is commonly used to identify potential candidate species with 16S mtDNA barcoding in the tropics (Vences *et al.*

2005; Fouquet *et al*. 2007; Jansen *et al.* 2011; Crawford *et al*. 2010, 2013). Additionally, *E. sukia* lacks nuptial spines in all examined males (Fig. 10D; Brian Kubicki pers. comm. 2011). Although the appearance of spines and tubercles could be influenced by seasonality in some other species (Mendelson *et al.* 2008), this seems not to be the case in *E. sukia*, for which two adult males have been examined, and one was kept in captivity for four years without evidence of developing any nuptial spines (Savage & Kubicki 2010). Moreover, the holotype of *E. sukia*, an adult male, was collected on 25 March 1999, thus in the same season (see ETESA 2009, and IMN 2009) as the holotype of *E. veraguensis*, but showed no nuptial spines.

So far, this is the most comprehensive phylogenetic study of the genus *Ecnomiohyla* based on molecular data. DNA sequences of few species of fringed frogs were already used in several older large-scale phylogenies (Faivovich at al. 2005, Wiens et al. 2010; Pyron & Wiens 2011) to uncover the phylogenetic relationships inside the Hylidae, but without comments to on relations inside the genus. Herein we used sequence data for six of the seven *Ecnomiohyla* species known from lower Central America and for *E. miotympanum*. Since our motivation is to identify species delimitations through genetic barcoding, we used only the widely accepted mitochondrial 16S marker (Monaghan *et al.* 2009; Vieites *et al.* 2009). Deeper analyses, including nuclear markers to support the phylogenetic relationships between the species are certainly needed.

Anyway, we found incongruence between our molecular phylogenetic analysis and the three morphological groups suggested by Savage & Kubicki (2010). In their concept *Ecnomiohyla bailarina* would clearly meet the Group 2 criteria, whereas *E. veraguensis* meets the criteria of Group 3, assuming that nuptial spines are not necessarily absent, but only largely reduced. Our phylogenetic analyses revealed two major clades in the genus. One clade contains only *E. miotympanum* and stands opposed to the rest of the species in the genus. The latter is divided into two subclades, separated by a well-supported node in both trees (bootstrap value, bs: 99.8; posterior probability, pp: 100): Sub-clade 1 contains *E. rabborum*, *E. bailarina*, and *E. fimbrimembra*; and subclade 2 contains *E. sukia*, *E. veraguensis*, and *E. miliaria*. Thus, *E. bailarina* appears to be closer related to *E. rabborum* (Group 1), than to *E. fimbrimembra* (Group 2). Our subclade 2 also contains all available species assigned to Group 3 by Savage & Kubicki (2010) (Fig. 2). As a consequence of these results, we modify the groups proposed by Savage & Kubicki (2010) into two consequent groups, characterized as follows (for the remaining species, see below): In members of the *E. fimbrimembra* species group (Group 1), male frogs may have a bony humeral projection or not, but always have conspicuous cluster of black keratinized nuptial spines on thumb and prepollex, as demonstrated in species *E. bailarina, E. echinata, E. fimbrimembra, E. minera*, *E. rabborum*, and *E. salvaje.* While in the *E. miliaria* species group (Group 2), male frogs have neither humeral projection nor black nuptial spines on the prepollex. A few nuptial spines may be present on the thumb, but if this is the case these are fewer than ten, usually light brown (not black) and widely spaced (not building a cluster); this group contains *E. miliaria*, *E. phantasmagoria*, *E. sukia*, *E. valancifer*, and *E. veraguensis* (Fig. 10).

In our phylogeny *E. miotympanum* is the only member of an own species group within *Ecnomiohyla* and appears as a sister clade to other *Ecnomiohyla* what is strongly supported (bs: 89.6; pp: 97). Thus the molecular monophyly of all members of the genus we have data for, is confirmed here. Other recent studies suggested *Ecnomiohyla* may not be monophyletic with regard to *E. miotympanum*, even though this was not strongly supported (Wiens *et al.* 2010; Pyron & Wiens 2011). While our taxon sampling of *Ecnomiohyla* species is large, the molecular dataset is restricted to a single marker, so a deeper analysis is still needed. However, as a consequence from our results we continue to treat *E. miotympanum* as a member of the genus *Ecnomiohyla* for the moment. Savage & Kubicki (2010) pointed out that *E. tuberculosa* should not be included in the genus as it lacks the enlarged prepollex and prepollical bony projection, which is present in all other species in the genus. Pro tem, we are including *E. tuberculosa* within the genus, until further data becomes available. Further, we could not assign *E. thysanota* to one of the species groups, since the male of this species is not known yet and molecular data is lacking.

In Table 4, we have summarized the morphological characteristics of the two species described here compared to the other *Ecnomiohyla* species that are present in Lower Central America (Table 3). The genus is distributed as follows: In Lower Central America, Colombia and Ecuador, the fringe-limbed frog representatives are *E. bailarina, E. fimbrimembra, E. miliaria, E. phantasmagoria, E. rabborum*, *E. sukia, E. thysanota, E. tuberculosa,* and *E. veraguensis* (Ortega-Andrade *et al.* 2010; Savage & Kubicki 2010; Köhler 2011; Ron 2012; this paper); in Nuclear Central America the fringe-limbed frogs are *E. echinata, E. miliaria, E. minera*, *E. salvaje*, and *E. valancifer*; The only known species reaching North America in eastern and central Mexico is *E. miotympanum.* The most widespread species seems to be *E. miliaria*, which is found in Nuclear and Lower Central America, distributed from south-eastern Honduras to central Panama (Köhler 2011). However, it seems likely that *E. miliaria* represents more than one species (Solís *et al.* 2010). One evidence for this assumption is that there are two specimens from Panama assigned to *E. miliaria*, one from the Reserva Forestal Fortuna dam site (Myers & Duellman 1982) and one from El Copé (Savage & Kubicki 2010), which indeed are different to *E. miliaria* from Nuclear Central America (revised by Savage & Kubicki 2010), *E. sukia* or *E. veraguensis* (different in skin texture and fringe shape, see Fig. 13 in: Myers & Duellman 1982).

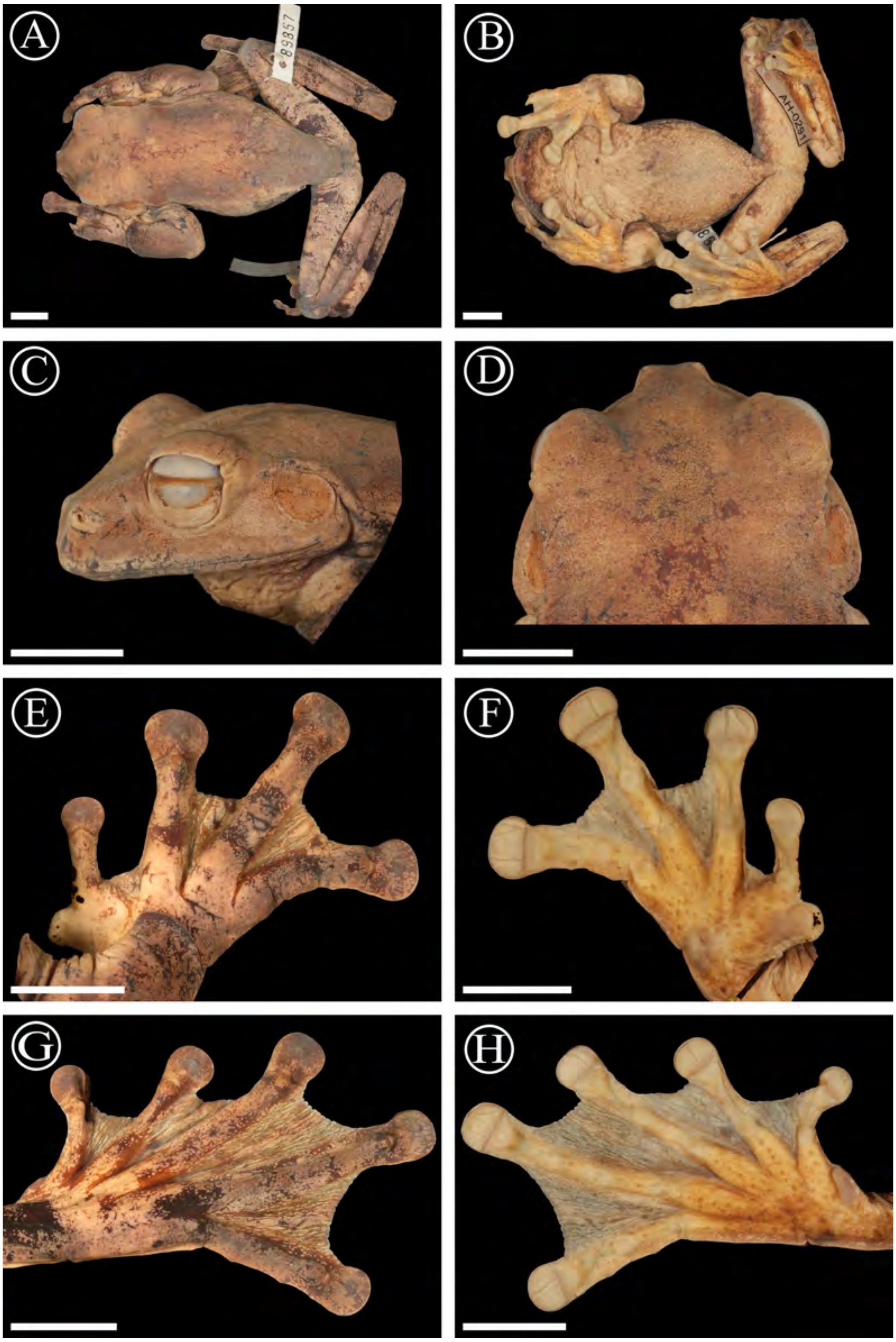

FIGURE 9. *Ecnomiohyla fimbrimembra* (SMF89857), preserved male specimen from Panama. A) dorsal view; B) ventral view; C) head in profile; D) head dorsally; E) right hand dorsally; F) right hand ventrally; G) right foot dorsally; H) right foot ventrally. Scale bars= 10 mm.

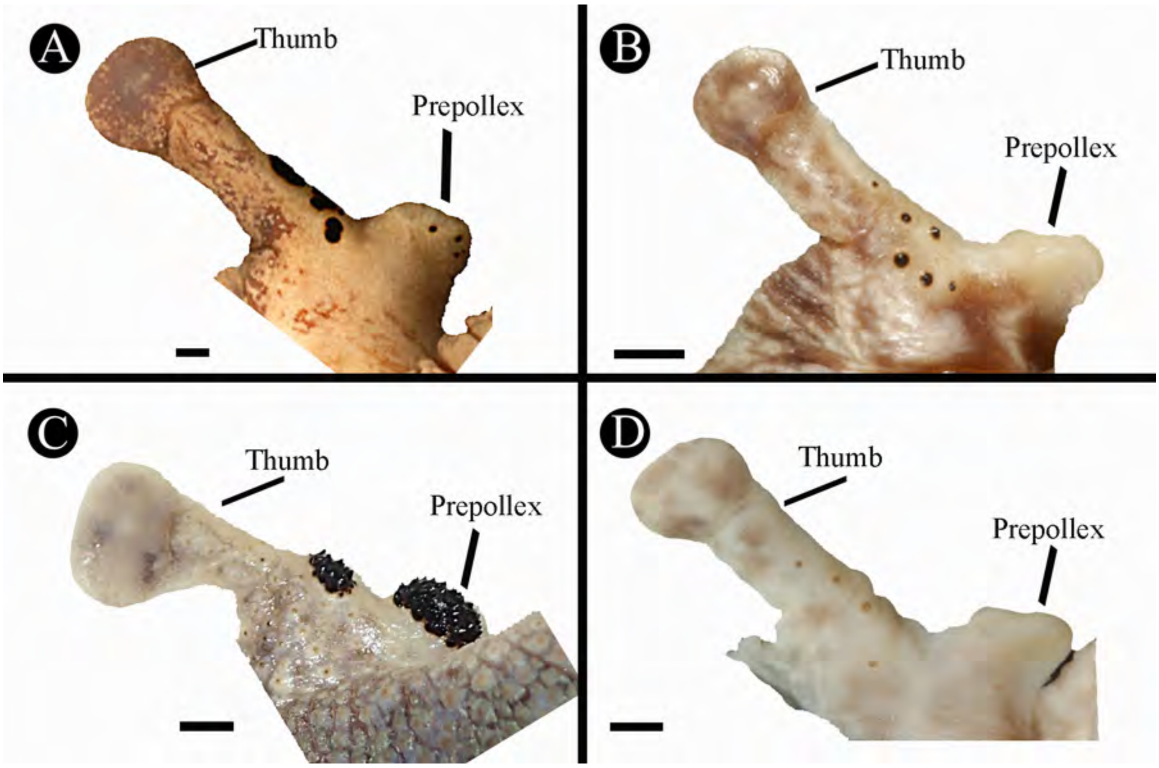

FIGURE 10. Details of the thumb and the keratinized black spines on prepollex, A) *Ecnomiohyla fimbrimembra* (SMF89857); B) *E. veraguensis* (SMF89877); C) *E. bailarina* (SMF97398); D) *E. sukia* (SMF94578). Scale bars= 1 mm

Key to the species of the genus *Ecnomiohyla*.

1a. No scalloped dermal fringes on the outer margin of the forearm and foot . . . . *E. miotympanum*

b. Scalloped dermal fringes on the outer margin of the forearm and foot present 2

2a. Males without an enlarged prepollex and prepollical bony projection . . . .*E. tuberculosa*

b. Males with an enlarged prepollex and prepollical bony projection 3

3a. Webbing between Finger II–IV not extending beyond penultimate subarticular tubercle on Finger III (Fig. 9F); supratympanic fold continuing posteriorly behind tympanum to terminate above axilla (Fig. 9C); dermal fringe along lateral edge of forearm and tarsus narrow and not or only weakly scalloped; dorsal skin smooth to minutely granular; skin of dorsal surface of head co-ossified with skull . . . . *Enomiohyla frimbrimembra*

b. Webbing between Finger II–IV extending well beyond penultimate subarticular tubercle on Finger III; supratympanic fold not continuing posteriorly behind tympanum to terminate above axilla; dermal fringe along lateral edge of forearm and tarsus usually prominent and scalloped; dorsal of variable texture; skin of dorsal surface of head co-ossified with skull or not 4

4a. Dorsum uniform green in life; skin of dorsal surface of head not co-ossified with skull, and granular; heel without tubercles but with a well-defined, scalloped dermal fold (Fig. 6A) . . . .*Ecnomiohyla thysanota*

b. Dorsum brown, reddish brown, or brown with green or darker brown markings or mottling; dorsum granular or tuberculate; skin of dorsal surface of head in adults co-ossified with skull or not; condition of heel variable 5

5a. Dorsum tuberculate; osteoderms usually present 6

b. Dorsum granular or smooth; without osteoderms 12

6a. Humeral projection present; heel without tubercles; prepollex in adult males with scattered small black spines . . . . *Ecnomiohyla minera*

b. No humeral projection; heel with one or several tubercles or, if without tubercles then with a scalloped fringe; prepollex in adult males with or without small black spines. 7

7a. Dorsum tuberculate without cranial or dorsal osteoderms; heel with one large tubercle; males with spade-like prepollex and flattened prepollical bony projection. . . . . *Ecnomiohyla valancifer*

b. Dorsum tuberculate with cranial or dorsal osteoderms; heel with one or several pointed tubercles or, if without tubercles then with a scalloped fringe; males with variable prepollex and prepollical bony projection 8

8a. Webbing on fingers not reaching the base of disk on any digit . . . . *Ecnomiohyla phantasmagoria*

b. Webbing on fingers reaching to base of disk on two to four but not all digits 9

9a. Heel with one or several pointed tubercles; black keratin tipped tubercles over most of flanks and venter; prepollical bony projection in males terminating in a sharp spine in adults . . . . *Ecnomiohyla miliaria*

b. Heel without tubercles but with a scalloped fringe; without black keratin tipped tubercles over most of flanks and venter; prepollical bony projection in males, variable 10

10a. Males without keratinized black spines on prepollex; without black keratin tipped tubercles on dorsum (Fig. 10D). . . . . *Ecnomiohyla sukia*

b. Males with keratinized black spines on prepollex; black keratin tipped tubercles over most of dorsum 11

11a. Dorsum strongly tuberculated; two clusters of nuptial spines at the distal end of prepollical tubercle and the base of prepollex in males (Fig. 10C) . . . . *Ecnomiohyla bailarina*

b. Dorsum slightly tuberculated; without nuptial spines arranged in clusters, instead 6-7 nuptial spines scattered along the pollex (Fig. 10B) . . . . *Ecnomiohyla veraguensis*

12a. Cephalic skin co-ossifed with skull; webbing on fingers extensive, reaching to the base of disk on two to four but not all digits; toe webbing full, reaching to the base of disks on all digits . . . . *Ecnomiohyla salvaje*

b. Cephalic skin not co-ossifed with skull; finger webbing usually not reaching to the base of disk and if, then only on one digit; toe webbing never reaching to the base of disk on all digits. 13

13a. Dorsum smooth; humeral projection absent in males; heel with few small tubercles; SVL of adult females 60.2 mm, SVL of adult males 57 mm . . . . *Ecnomiohyla echinata*

b. Dorsum granular; humeral projection present in males; heel smooth; SVL of adult females 61.3–79.9 mm, SVL of adult males 62.8–97.3 mm . . . . *Ecnomiohyla rabborum*

Acknowledgements

Scientific permits: 2009 (SC/A-8-09, SC/A-28-09), 2011 (SC/A-37-11), 2012 (SC/A-33-12), and exportation permits 2012 (SC/A-33-12), 2013 (SEX/A-7-13) were provided by ANAM, Panama, and T. Quintana (Cacique General del área de Sambú) from the “despacho del cacique Regional” Comarca Emberá-Wounaan, Panama. Special thanks go to the indigenous people of Embera from Puerto Indio and Pavarandó, especially to D. Berrugate (Secretary of the Emberá-Wounaan congress, Sambú); to L. Caibera (Noko of Pavarando village) and his family who allowed us to enter their autonomous territory and kindly supported our work logistically. We are very grateful to Don Faustino, Hermelinda, and family, who gave us shelter on their nice sustainable farm at la Moneda's village during our travels to Darien. For transportation to Santa Fé National Park, we thank Rafael González; to Arcadio Carrizo and Smelin Abrego we are grateful for field assistance at that site. For assistance in the field at Bajo Mono that led to the discovery of the *Ecnomiohyla fimbrimembra* specimen, we thank Andreas Uselis, Caroline Judith, Falk Ortlieb, Frank Hauenschild, and Joe-Felix Bienentreu. We thank Yorlis Cáceres, Daniel Cáceres, Isaac Pizarro, Gustavo Dogirama, Mario Cuñapa, Anselmo Caicedo, Hugo Martínez, Elacio Méndez, and Gilberto Torres for field assistance. We thank Connie Cochrane, Jack Seigel, Glenn Lee, Victor Orrico and two anonymous reviewer for insightful comments on an early draft of the manuscript. B. Kubicki and J. Savage made helpful comments on some morphological characters of *E. sukia*. J. Poindexter II (AMNH) kindly took photos of the *E. thysanota* holotype. José Edilson Espitia Barrera kindly (MLS-BOG) took photos of the *E. phantasmagoria* holotype. This work was supported financially by the Secretaría de Ciencia y Tecnología (SENACYT), Instituto para la Formación y Aprovechamiento de los Recursos Humanos (IFARHU), Panama, and MWH, Panama. Andreas Hertz was supported financially by the FAZIT-Stiftung, Sebastian Lotzkat by the Studienstiftung des deutschen Volkes and the Vereinigung der Freunde und Förderer der Goethe-Universität, and Milan Vesely by institutional support of Palacky University.

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Příloha 9.

Batista, A., Köhler, G., Mebert, K., Hertz, A., **Veselý, M.,** 2016: An integrative approach to reveal speciation and species richness in the genus *Diasporus* (Amphibia: Anura: Eleutherodactylidae) in eastern Panama. *Zoological Journal of the Linnean Society* 178(10): 267–311.

The contribution in words:

AB, GK and MV conceived and designed the study. AB, MV and KM carried out the fieldwork, AB and MV analysed morphological data and performed the molecular analyses. AB and AH performed the analysis of bioacoustics. AB and MV wrote the manuscript. All authors contributed to manuscript revision. All authors approved the final version of the manuscript. MV supervised the work.

Ranking: IF_{2016} – 2.711; **D1** (Zoology)
Citace na WoS (Core collection; bez autocitací všech spoluautorů): 3
Citace celkem (Google Scholar): 6

The LINNEAN SOCIETY of London ZOOLOGICAL Journal of the Linnean Society

Zoological Journal of the Linnean Society, 2016, **178**, 267–311. With 17 figures

An integrative approach to reveal speciation and species richness in the genus *Diasporus* (Amphibia: Anura: Eleutherodactylidae) in eastern Panama

ABEL BATISTA[1,2]*, GUNTHER KÖHLER[1], KONRAD MEBERT[3], ANDREAS HERTZ[1,2] and MILAN VESELÝ[4]

[1]*Senckenberg Forschungsinstitut und Naturmuseum Frankfurt, Senckenberganlage 25, 60325 Frankfurt am Main, Germany*
[2]*Institute for Ecology, Evolution & Diversity, Biologicum, Goethe-University, Building C, Max-von-Laue-Straße 13, 60438 Frankfurt am Main, Germany*
[3]*Department of Environmental Sciences, Section of Conservation Biology, University of Basel, St. Johanns-Vorstadt 10, CH-4056 Basel, Switzerland*
[4]*Department of Zoology, Faculty of Natural Sciences, Palacký University, 17. Listopadu 50, 77146 Olomouc, Czech Republic*

Received 4 August 2015; revised 27 January 2016; accepted for publication 7 February 2016

We have applied an integrative taxonomic approach, including bioacoustics, ecology, morphology, and molecular genetics (barcoding and phylogeography), to explore species richness in the genus *Diasporus* in eastern Panama, from where only *Diasporus quidditus* (Lynch, 2001) was previously known. During fieldwork in eastern Panama in 2011 and 2012 we found six additional species, four of which we are describing here as new to science, plus two species that are new for this region. We have evaluated the presence of *Diasporus diastema* (Cope, 1875) in eastern Panama by comparing morphological, genetic, and bioacoustic characters of specimens from near the type locality in central Panama with specimens from eastern Panama. We further describe and compare male advertisement calls of most *Diasporus* species. The phylogeographic analysis suggests the allopatric speciation of *Diasporus* species in eastern Panama following the completion of the Panamanian isthmus in the middle Miocene. Subsequent geological events concur with the vicariant evolution of different lineages *in situ*, suggesting eastern Panama to be a centre of endemism for this group of frogs. We present an integrative analysis of the species from eastern Panama and include an identification key for all species of the genus.

doi: 10.1111/zoj.12411

ADDITIONAL KEYWORDS: barcoding – bioacoustics – *Diasporus* – *Diasporus darienensis* sp. nov. – *Diasporus majeensis* sp. nov. – *Diasporus pequeno* sp. nov. – *Diasporus sapo* sp. nov. – ecology – integrative taxonomy – Panama – phylogeography.

INTRODUCTION

Cryptic species are defined as 'two or more distinct species that are erroneously classified under one species name' (Bickford *et al.*, 2007). Frogs of the genus *Diasporus* are a good example of cryptic diversity, as most species resemble each other externally, and are difficult to distinguish by morphological methods alone; however, integrative analysis (the combination of several methods and lines of evidence) reveals the true diversity in this species complex (Hertz *et al.*, 2012). In recent years, three new species of the genus *Diasporus* (formerly *Eleutherodactylus diastema* group; *sensu* Hedges, Duellman & Heinicke, 2008) have been described from Costa Rica and western Panama (Chaves *et al.*, 2009; Batista, Ponce &

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*Corresponding author. E-mail: abelbatista@hotmail.com

Hertz, 2012; Hertz *et al.*, 2012), and several more species presumably await discovery and description (Lynch & Duellman, 1997; Ibáñez, Rand & Jaramillo, 1999; Savage, 2002; Hertz *et al.*, 2012). There is evidence of divergence between populations in traits other than morphology, for example on geographic isolation, ecology, bioacoustics, and molecular genetics, but it is difficult to distinguish them based on morphology alone. The application of an integrative approach offers a more balanced solution (Dayrat, 2005; Vieites *et al.*, 2009; Padial *et al.*, 2010; Hertz *et al.*, 2012) that can solve the taxonomic problems within this genus.

The genus *Diasporus* comprises small frogs with short limbs and expanded disc pads, with or without lanceolate or papillate tips, that inhabit rainforests from eastern Honduras to north-western South America as far as north-western Ecuador (Hedges *et al.*, 2008). Adult males possess well-developed vocal slits and a single external subgular vocal sac. Their characteristic 'dink' call (subjective general acoustic description), which can be more like a whistle in some species, is commonly heard in wet forests throughout the distribution range of the genus. Males usually call from vegetation growing at ground level up to several metres above the ground (Savage, 2002).

To date, the genus *Diasporus* contains 11 described species (Frost, 2014). Seven species are currently known to be endemic to Central America: *Diasporus citrinobapheus* Hertz *et al.*, 2012; Serranía de Tabasará, Panama; *Diasporus diastema* (Cope, 1875), widespread between central Panama and Honduras; *Diasporus hylaeformis* (Cope, 1875), cordilleras (mountain ranges) of Costa Rica and western Panama; *Diasporus igneus* Batista *et al.*, 2012; Serranía de Tabasará, Panama; *Diasporus tigrillo* (Savage, 1997), Atlantic slopes of the Cordillera de Talamanca, Costa Rica; *Diasporus ventrimaculatus* Chaves *et al.*, 2009; Cordillera de Talamanca, Costa Rica; and *Diasporus vocator* (Taylor, 1955), western Panama and southern Costa Rica. *Diasporus quidditus* (Lynch, 2001) occurs in eastern Panama and north-western Colombia (Lynch, 2001; Köhler, 2011). The remaining three species are distributed along the Pacific side of northern South America, from Colombia to north-western Ecuador: *Diasporus anthrax* (Lynch, 2001), along the eastern foothills of the Cordillera central and the western slope of the Cordillera Oriental, Colombia; *Diasporus gularis* (Boulenger, 1898), lowlands of western Colombia and north-western Ecuador; and *Diasporus tinker* (Lynch, 2001), Pacific slopes of Colombia (IUCN, 2013; Frost, 2014).

Eastern Panama (EP) is known as an important biodiversity hot spot with high endemism, as a result of the great variety of habitats. Still largely unknown forests, that are suffering from rapid habitat loss (Parker, Carrión & Samudio, 2004). The high endemism in EP is likely to be the result of the complex geohistory of the Isthmus of Panama. EP represents the northernmost part of the Chocó biogeographical region (Duque-Caro, 1990), and can be subdivided into three main geographical units: the massifs of the Dabeiba Arc in the north-east (San Blas and Darién mountain ranges) and the Baudó Arc in the south-west (Jingurudó, Majé, Pirre, and Sapo mountain ranges). Between these mountains lies the Chucunaque Basin, a sedimentary basin that forms the central part of the Choco Block, with the drainage of Chucunaque River and Tuira Basin in the lowlands (Duque-Caro, 1990; Coates & Obando, 1996). The uplift of the Choco Block is the result of the collision of the Panama Arc with South America since the middle Miocene (as early as 11 Mya; Farris *et al.*, 2011). The continuous uplift of the Choco Block shallowed the water depth in the Atrato and Chucunaque basins, as they were steadily filled with sediments (Duque-Caro, 1990; Coates *et al.*, 2004). These geohistorical dynamics periodically separated land masses (e.g. the isolation of the mountains in the Baudo and Dabeiba arcs in EP during the Middle Miocene) that promoted speciation events and an increased species diversity in this region (Batista *et al.*, 2014b).

During the last 4 years we have conducted several expeditions and collected numerous specimens and associated materials of amphibians and reptiles across large parts of EP. Besides two recent discoveries and publications on regional anuran fauna (Batista *et al.*, 2014a,b), preliminary barcoding analysis of *Diasporus* frogs from different localities in EP revealed several distinct lineages. Herein, we apply the first integrative approach on *Diasporus* taxa to evaluate the status of these lineages. We use information from bioacoustics, ecology, morphology, and biogeography to evaluate the divergence among genetic lineages and draw taxonomic conclusions. Furthermore, we discuss the distribution pattern of *Diasporus* taxa from EP in a biogeographical context by comparing analyses of regional geological events and molecular clock calibrations.

MATERIAL AND METHODS

Fieldwork was carried out in the Chucunaque and Tuira basins of the eastern Panamanian lowlands (EPLL), and in all major eastern Panamanian mountain ranges. Eastern Panama (EP) defines the eastern half of the country, corresponding to the area east of the Panama Canal. It comprises two important ecoregions of the western hemisphere, the eastern Panamanian montane forests (EPMF) in the highlands and the Chocó-Darién moist forests (CDMF) in the lowlands (Fund, 2014). The EPMF is further split into several mountain ranges: San Blas mountain range (SBM), Darién mountain range (DM), Jingurudó-Sapo mountain range (JSM), Majé mountain range (MM), and Pirre mountain range (PM) (Figs 1 and 2). The lowlands (EPLL) are dissected by a few large rivers, the Balsas, Chucunaque, Sambú, and Tuira rivers, within the CDMF. There are a few additional, smaller (<500 m a.s.l.) mountain ranges, such as the Filo del Tallo-Canglón in middle of Darién, and the Bagre in the south-east of Darién along the Sambú River. All geographical coordinates were recorded in the WGS 1984 datum given in decimal degrees. The maps were created with ArcGIS 10 (ESRI, 2009). The voucher specimens collected were killed with agent T61 and subsequently fixed with a preservative mixture of 5 mL of formalin (40%) in 1 L of ethanol (94%), and then stored in ethanol (70%). All figures have been digitally modified for improved visibility and combined using Adobe CS3. For candidate species and their delimitation we follow the integrative concept for amphibians of Vieites *et al.* (2009).

MORPHOMETRICS

Morphological nomenclature, measurements, and diagnoses follow Duellman & Lehr (2009). All measurements were made using digital calipers and were rounded to the nearest 0.01 mm. Measurements are given as mean ± SD and range in parentheses (Table 1). Specimens were deposited in the Museo Herpetológico de Chiriquí at the Universidad Autónoma de Chiriquí, David, Panama, and at the Senckenberg Research Institute and Nature Museum, Frankfurt, Germany. The abbreviations for museum collections follow Sabaj Pérez (2013), with field numbers AB from the abbreviated name Abel Batista. Morphological data of similar *Diasporus*

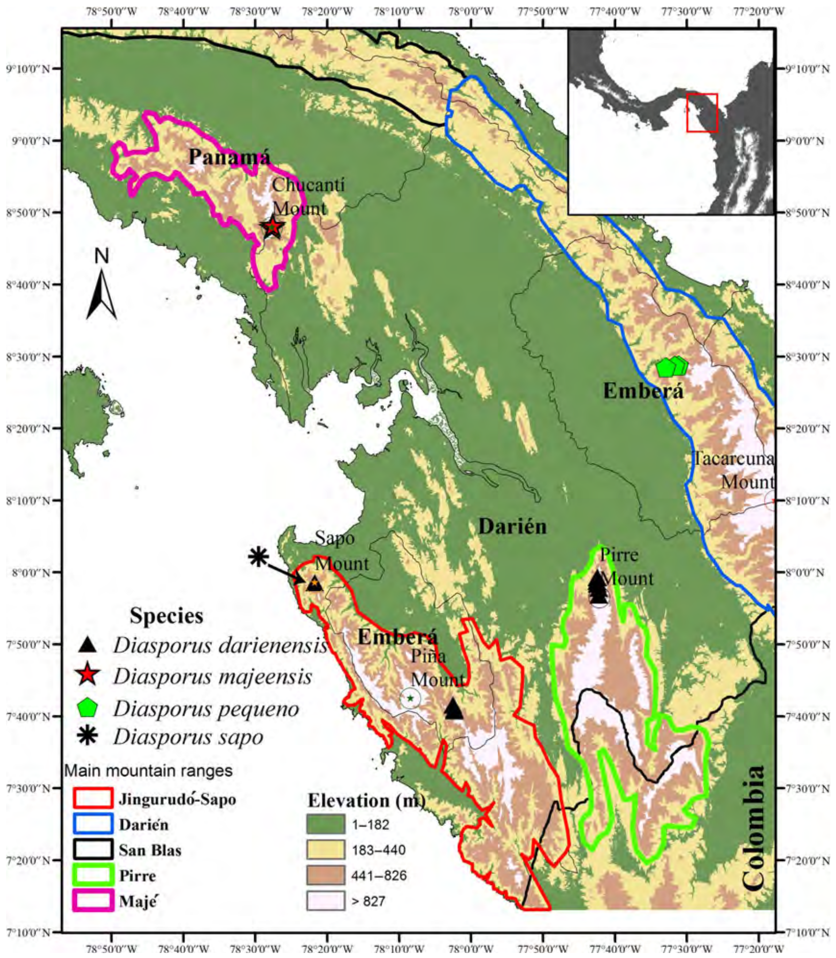

Figure 1. Map of the Darién region, eastern Panama, showing the distribution of the species of *Diasporus* described herein.

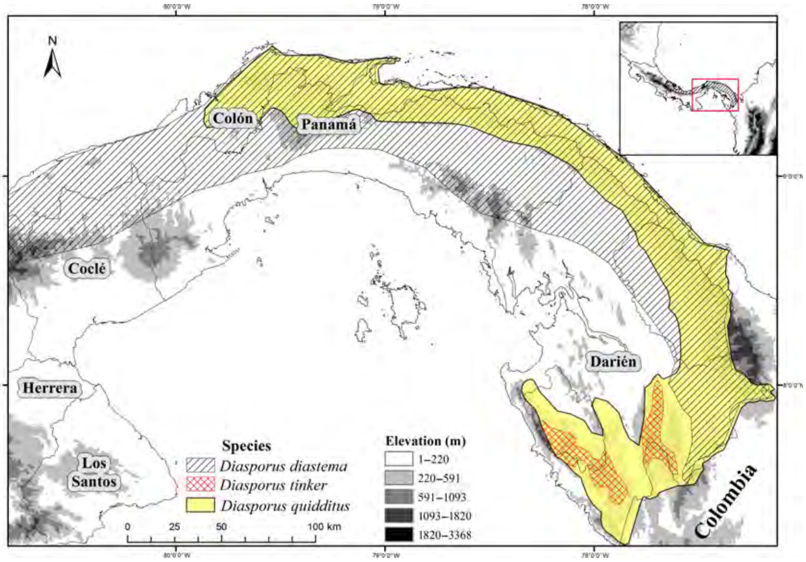

Figure 2. Map of eastern Panama (EP), showing the distribution of EP species that inhabit variable elevations, including lowlands: *Diasporus diastema* complex, *Diasporus* aff. *quidditus*, and *Diasporus tinker*.

Table 1. Genetic p–distances (in percentages) in the *16S* mtDNA between the *Diasporus* spp. used in the phylogenetic analysis

		p–distance (in %)											
	Species	1	2	3	4	5	6	7	8	9	10	11	12
1	*D. majeensis* sp. nov.												
2	*D. darienensis* sp. nov.	5.0											
3	*D. pequeno* sp. nov.	10.3	8.5										
4	*D. sapo* sp. nov.	6.5	2.6	9.5									
5	*D. diastema* CWP	10.0	9.1	9.6	8.3								
6	*D.* aff. *diastema* EPL	11.0	9.2	9.7	9.2	4.6							
7	*D.* aff. *diastema* MM	10.8	8.0	7.1	8.0	5.0	4.2						
8	*D. citrinobapheus*	11.0	9.0	9.7	9.2	3.8	5.1	4.6					
9	*D. hylaeformis*	8.4	5.5	10.0	6.5	10.7	10.6	10.5	10.1				
10	*D.* aff. *quidditus*	10.2	6.9	9.2	7.0	7.0	6.3	5.2	7.0	8.9			
11	*D. tinker*	9.9	7.2	8.5	8.0	7.8	7.4	7.1	7.5	9.6	7.4		
12	*D. vocator*	10.5	8.9	11.1	8.3	10.7	10.8	9.8	10.1	8.2	9.2	10.6	

species for comparison were taken from holotypes (*D. anthrax*, *D. citrinobapheus*, *D. igneus*, *D. quidditus*, and *D. tinker*), as well as from original descriptions and published literature (Savage, 1997; Lynch, 2001; Chaves *et al.*, 2009; Batista *et al.*, 2012; Hertz *et al.*, 2012; Jiménez *et al.*, 2013). A list of specimens examined is presented in Appendix 1.

The following morphometric measurements were taken (with abbreviations indicated, see Duellman & Lehr, 2009): snout-vent length (SVL); head length (HL), measured diagonally from angle of jaw to tip of snout; head width (HW), measured between angles of jaws; interorbital distance (IOD); eye length (EL), measured from anterior to posterior edge of externally accessible eye; hand length (HAL), measured from proximal edge of palmar tubercle to tip of third finger; tibia length (TL), the distance from knee to distal end of the tibia; foot length (FL), measured from proximal edge of outer metatarsal tubercle to tip of fourth toe; width of third finger (3FW), at penultimate phalanx just anterior to disc; width of disc of third finger (3FD), at greatest width; width of third toe (3TW), at penultimate phalanx just anterior to disc; width of disc of third toe (3TD), at greatest width; width of fourth toe (4TW), at penultimate phalanx just anterior to the disc; width of disc of fourth toe (4TD), at greatest width; tympanum diameter (TD), measured horizontally, based on an estimated circular tympanum; and body width (BW), at greatest width of body. We determined the sex of adults by the presence of vocal slits in males and the presence of eggs in females. Specimens without vocal slits or eggs, and with the correspondingly smaller SVL (relative to the standard size of confirmed adult females or males for each species), were classified as juveniles. For the description of the ungual flap we followed Lynch (2001) and Savage (2002). The ungual flap is known as the 'more or less free anterior portion of the disk cover' (see Savage, 1987), it represents the anteriormost margin of the finger/toe tip, which can be hanging in a loose flap (sheet of skin). Generalized coloration summaries were derived directly from live specimens or indirectly from photos of live specimens. For the standardized color descriptions of selected individuals, the capitalized color and color codes (with the latter in parentheses) are taken from Köhler (2012).

BIOACOUSTICS

Male advertisement calls were recorded using a Marantz Professional (PMD 620) and/or a Panasonic RR-XS410 digital recorder, with a Sennheiser ME 66 shotgun microphone capsule and a Sennheiser K6 powering module. The microphone was positioned between 0.5 and 1.5 m from the calling frog. Recordings were made at a sampling rate of 44 kHz with 16–bit resolution in uncompressed pulse-code modulation (PCM) format and saved as .wav files. The spectral and temporal parameters were analysed and the power spectra were calculated in RAVEN PRO 1.4 (Blackman DFT window; 2048 samples; 3–dB filter bandwidth of 158 Hz; grid spacing of 21.5 Hz; overlap 70.1%; Charif, Clark & Fristrup, 2004). The lowest and highest frequencies were measured at 20 dB below the peak frequency, thereby avoiding overlapping with background noise (Simões & Lima, 2011). Because our original recordings of two male *Diasporus* sp. nov. from Bajo Pequeno were lost, we extracted the calls from the background of another recording that was targeted at a *Pristimantis* sp. nov. (A. Batista, unpubl. data), using Adobe Audition 5.0. We amplified the sound (using the amplitude function) to extract details that were difficult to see in the original file. The terminology used in the description of advertisement calls follows Duellman & Trueb (1994), and follows Ursprung, Ringler & Hödl (2009) for the description of calling in bouts. The call rate was calculated as (total number of calls − 1)/(time from the beginning of the first call to the beginning of the last call) (Cocroft & Ryan, 1995). Because the dominant frequency (DF) is correlated with SVL in many frog species (Gerhardt & Huber, 2002; Bradbury & Vehrencamp, 2011), we used the non-standardized residuals between these variables for the statistical analyses. The environmental temperature and humidity were measured using an Oakton digital thermo-hygrometer. Because temporal parameters are temperature-dependent in many frog species, statistical adjustments are required (Gerhardt & Huber, 2002). In cases where we found no correlation between temperature and temporal parameters among species, we used raw data (e.g. call rate, note duration, note interval). We ran a discriminant function analysis to classify the advertisement calls of different species. The species for which SVL and/or temperature were not available were excluded from this analysis; however, all specimens were included in scatter plots of raw data that correlate DF against call rate and DF against note duration (Gerhardt & Huber, 2002; Padial *et al.*, 2008). The statistical analyses were performed using SPSS 21.0. Acoustic data for *D. anthrax* and *D. ventrimaculatus* were taken from Chaves *et al.* (2009) and Jiménez *et al.* (2013), respectively. As the terms DF, peak frequency, and high frequency were obviously confused by Jiménez *et al.* (2013), we re-estimated the real DF value from the spectrogram shown in that publication. The spectrogram figure was produced with the SEEWAVE package in R.

MOLECULAR LABORATORY WORK AND PHYLOGENETIC INFERENCE

DNA was extracted from fresh muscle or liver tissue in the Grunelius-Möllgaard Laboratory for Molecular Evolution, Senckenberg, Germany, using the protocol of Ivanova, Dewaard & Hebert (2006). The samples were amplified using a Mastercycler pro S (Eppendorf, Hamburg, Germany), performing an initial denaturation for 1 min at 94 °C, followed by 35 steps with denaturation for 9 s at 94 °C, annealing for 27 s at 45 °C, and with elongation for 1.5 min at 72 °C. Final elongation proceeded for 7 min at 94 °C. For the nuclear *recombination activating gene 1* (*RAG1*), we used: one cycle of 2 min at 96 °C; 45 cycles of 20 s at 95 °C, 25 s at 52 °C, and 2 min at 72 °C; and one cycle of 7 min at 72 °C. The reaction mixture contained 1 μL of mitochondrial DNA (mtDNA) template, 2.5 μL of reaction buffer ×10 (PeqGold), 4 μL of 2.5 mM dNTPs, 0.4 μL (containing 2.5 units) of Taq Polymerase (PeqLab), 14.1 μL of H_2O, 1 μL of 25 mM $MgCl_2$, and 1 μL per primer for *16S* (10 pmol; forward primer, L2510, 5′-CGCCTGTTTATCAAAAACAT-3′; reverse primer, H3056, 5′-CCGGTCTGAACTCAGATCACGT-3′; eurofins MWG Operon), and 3 μL per primer for *RAG1* (forward, R182, 5′-GCCATAACTGCTGGAGCATYAT-3′; reverse, R270, 5′-AGYAGATGTTGCCTGGGTCTTC-3′; eurofins MWG Operon (Heinicke, Duellman & Hedges, 2007). Sequencing of the *16S* rRNA and *RAG1* was performed in the molecular laboratory of the Senckenberg Biodiversität und Klima Forschungszentrum (BIK–F), Germany. The mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene was sequenced in the Southern China DNA Barcoding Center. In total we extracted DNA from 48 *Diasporus* frogs from EP and received 41 sequences for *16S*, 30 sequences for *COI*, and 45 sequences for *RAG1* (Appendix 2). We compared the mtDNA data of our specimens with *Diasporus* sequences published in GenBank. The sequences were aligned with CLUSTAL W (Larkin *et al.*, 2007) and were edited by eye using GENEIOUS 4.8.5 (Drummond *et al.*, 2010). A list of specimens included in the genetic analysis with corresponding GenBank accession numbers is presented in Appendix 2. GenBank sequences of *Colostethus panamansis* (Dunn, 1933), *Craugastor opimus* (Savage and Myers, 2002), *Craugastor fitzingeri* (Schmidt, 1857), *Eleutherodactylus planirostris* (Cope, 1862), *Eleutherodactylus thorectes* Hedges, 1988, and *Pristimantis caryophyllaceus* (Barbour, 1928) were used as out-groups. The final alignment (including GenBank sequences) of the *16S* mtDNA comprised 66 sequences (excluding out-groups) and consisted of 449 positions, of which 251 sites were variable and 158 sites were parsimony-informative. The final alignment of *COI* comprised 38 sequences consisting of 552 positions, of which 250 sites were variable and 224 sites were parsimony-informative. The final alignment of *RAG1* comprised 48 sequences consisting of 571 positions, of which 172 sites were variable and 76 sites were parsimony-informative. A total of 73 samples, combining the mitochondrial genes and the nuclear *RAG1*, were obtained (excluding out-groups), consisting of 1537 positions, of which 630 sites were variable and 447 were parsimony-informative. Using MEGA 6 (Tamura *et al.*, 2011) we calculated uncorrected pairwise genetic p–distances for *COI* and *16S* both separately and combined. For each gene and for the combined gene data set (*COI*, *16S*, and *RAG1*). We selected the substitution model for the Bayesian analysis using JModeltest 0.1.1 (Posada, 2008) under the corrected Akaike information criterion (AICc; Akaike, 1974). The three-parameter model with rate heterogeneity, TPM1uf+I+G (Kimura, 1981), was implemented for the combined gene data set. We ran a Bayesian phylogenetic analysis in MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) for 10 000 000 generations with four default chains, sampling every 1000 generations. In the final consensus tree 25% were discarded as burn-in. To test species delimitation among the *Diasporus* species, we applied the automatic barcode gap discovery (ABGD) algorithm (Puillandre *et al.*, 2011) with the following settings: 20 steps; distance calculated using the Kimura two-parameter model with a transversion/transition ratio of 2.0; and the setting for the minimum relative gap width (*X*) was set to different values between 0 and 1.5.

PHYLOGEOGRAPHY AND DIVERGENCE TIME ANALYSIS

We have used defined biogeographic areas of EP (see first paragraph in the Material and methods section) in the phylogeographic analysis. The phylogenetic relationships and divergence times were estimated for the mtDNAs *16S* and *COI* and nuclear DNA *RAG1* combined (1537 bp, 20 *Diasporus* samples included) in BEAST 1.5.4 (Drummond & Rambaut, 2007), with a relaxed clock, allowing substitution rates to vary according to an uncorrelated log-normal distribution, assuming a Yule tree prior (Drummond *et al.*, 2006). The prior distribution on substitution parameters was set to the default. To calibrate the root and one node age respectively, we used the age of approximately 57 Mya with a standard deviation of 15 Myr for the most common recent ancestor between *Pristimantis* and *Eleutherodactylus-Diasporus* (Heinicke *et al.*, 2009; Pinto-Sánchez *et al.*, 2012); and with the estimated crown ages of 32 Mya for the *Eleutherodactylus-Diasporus* (Heinicke *et al.*, 2009), and 17 Mya for *D. vocator* and *D. hylaeformis* (Pinto-Sánchez *et al.*, 2012). Parameters were estimated using 100 million generations with a burn-in of 50 million generations and trees were sampled every 10 000 generations. Results were visualized and compared using TRACER 1.5 (Rambaut & Drummond, 2009), and summary trees were generated using TreeAnnotator 1.5.4.

RESULTS

The phylogenetic analysis of the two mitochondrial genes revealed substantial molecular differences between groups with p–distances above 4.0% in *16S* mtDNA, 11% in *COI* mtDNA (Tables 1–3), and 7.0% when both genes were combined (Table 3). The divergence of these groups are further supported by substantial differences in male advertisement call and morphometric characters. The ABGD analysis generated 12 distinct phylogenetic groups for *16S* with a divergence threshold of 0.033 and a relative barcoding gap of 0.05 (*X*–value). For *COI*, it produced 12 groups assuming an a priori intraspecific divergence threshold of 0.021 and a relative gap width of 0.05 (*X*–value). The two analyses (ABGD for *16S* and *COI*) lumped all samples in one group, with a prior intraspecific divergence of 0.050 and 0.010, respectively. For both mitochondrial genes, the groups resulting from the ABGD analysis were consistent with the species units named in this study, except for the lumping of *Diasporus* sp. nov. from Cerro Sapo and *Diasporus* sp. nov. from DM and JSM, which is also present at Cerro Sapo.

The Bayesian consensus tree of all genetic markers combined (*16S*, *COI*, and *RAG1*) as well the divergence time analyses showed two well-differentiated clades of *Diasporus* in EP, with one containing the highland species (MM, PM, and JSM) that are monophyletic and well supported (Bayesian probability >0.95). The second clade includes species primarily distributed in the lowlands, including also *D. citrinobapheus* (from western Panama, WP), various lineages of *D.* aff. *diastema* (from central Panama, CP, the eastern Panamanian lowlands, EPL, and Majé MM, below 1000 m a.s.l.) and *D.* aff. *vocator* (from WP and south-eastern Costa Rica, CR). The divergence time analyses indicated that the EP lineages split from the WP lineages around 15 Mya (95% confidence interval, 95% CI, 6–29 Myr).

Most *Diasporus* species can be differentiated morphologically by a combination of SVL, disc shape, and color pattern (Table 4). In bioacoustics, a discriminant function analysis correctly classified all species included ($P < 0.01$; $N = 26$; Table 5). These results proved that seven species of the genus *Diasporus* occur in EP and can be diagnosed by some selected traits (e.g. SVL, ungual flap, color pattern, dominant frequency, and distribution; for more details, see Figs 3–7 and Table 6), with a new record for the species *D. tinker* in Panama. A detailed comparison of the type series of *D. tinker* from Colombia left no doubt that the specimens collected in EP are conspecific. We took advantage of these new records and include molecular, bioacoustic, and morphological data of *D. tinker* to increase the knowledge of morphological variation in this species in Panama. Furthermore, we used this data to distinguish *D. tinker* from other *Diasporus* species in EP. Addi-

Table 2. Genetic p–distances (in percentages) in the *COI* mtDNA between the *Diasporus* spp. used in the phylogenetic analysis

		p–distance (in %)										
	Species	1	2	3	4	5	6	7	8	9	10	11
1	*D. majeensis* sp. nov.											
2	*D. darienensis* sp. nov.	12.3										
3	*D. pequeno* sp. nov.	15.2	16.9									
4	*D. sapo* sp. nov.	13.0	10.0	14.7								
5	*D. diastema* CP	16.1	17.6	13.9	16.5							
6	*D.* aff. *diastema* EPL	16.7	16.5	14.4	17.0	10.4						
7	*D.* aff. *diastema* MM	15.2	16.0	12.3	16.2	9.6	10.0					
8	*D. hylaeformis*	18.3	19.7	18.3	18.7	18.9	19.1	17.0				
9	*D.* aff. *quidditus*	16.7	16.9	14.5	17.5	12.6	14.3	13.1	19.0			
10	*D. tinker*	16.4	16.9	14.6	17.1	14.7	14.1	14.0	20.6	16.2		
11	*D. vocator*	17.8	18.7	17.7	17.6	18.5	20.1	16.5	19.1	21.1	18.6	

Table 3. Genetic p–distances (in percentages) in the *16S* and *COI* mtDNA combined between the *Diasporus* spp. used in the phylogenetic analysis

		p–distance (in %)						
	Species	1	2	3	4	5	6	7
1	*D.* aff. *diastema* EPL							
2	*D. majeensis* sp. nov.	14						
3	*D.* aff. *quidditus*	10.4	14.3					
4	*D. darienensis* sp. nov.	13.5	9.9	12.6				
5	*D. tinker*	11.5	14.1	12.2	13.2			
6	*D. sapo* sp. nov.	13.4	10.3	12.7	7	13.6		
7	*D. pequeno* sp. nov.	12.1	14.1	12.4	14.3	12.5	12.9	

tionally, four species that clearly belong to the genus *Diasporus* on the basis of molecular (Figs 8, 9), bioacoustic, and morphological evidence cannot be assigned to any described species. Thus, we describe them as new species hereafter.

SPECIES ACCOUNTS

DIASPORUS DARIENENSIS SP. NOV.

FIGS 10, 14A, B

ELEUTHERODACTYLUS DIASTEMA – MYERS 1969: FIG. 19B

Holotype

SMF 97304 (original field number AB 1144), an adult female (Fig. 10), collected by Abel Batista & Konrad Mebert on the ridge of Pirre mountain range, ~3 km north from the peak of Cerro Pirre, Parque Nacional Darién (PND), Distrito de Pinogana, Darién, Panama, on 8 December 2012 at 20:35 h (7.97312 N, 77.70785 W; 1143 m a.s.l.).

Paratypes

MHCH 2840–41, 2844–47, 2862, SMF 97303, 97314, 97306–08, 97661–62, with same collecting data as holotype; MHCH 2850–52, SMF 97309–10, 97312, collected by Abel Batista & Milan Veselý in the Jingurudo mountain range, on a ridge between Aldo Creek and Sambú River, between the Comarca Emberá-Wounaan and the PND, Distrito de Sambú, Darién, Panama, 26–30 September 2011 (7.69271 N, 78.04200 W; 869 m a.s.l.); SMF 97313, collected by Abel Batista & Gustavo Dojirama at the top of Cerro Sapo, PND, Distrito de Garachiné, Darién, Panama, on 4 December 2011, at 22:06 h (7.97618 N, 78.36263 W; 1169 m a.s.l.).

Diagnosis

Diasporus darienensis sp. nov. is characterized by the following combination of traits (see Table 1): (1) dorsal skin texture smooth and/or with rounded or pointed scattered tubercles; (2) tympanic annulus concealed by skin, tympanic membrane absent; (3) snout acuminated in dorsal view and rounded in profile; (4) usually with a slightly enlarged and conical supraocular tubercle, cranial crests absent; (5) dentigerous processes of vomers triangular, diagonal to the eyes, near to the middle of the mouth and posteriorly separated about three-quarters of their total length from each other; (6) vocal sac moderately developed, with longitudinal gular folds evident, vocal slits present on the posterior part of the jaw, halfway under the tongue and ending at the midlevel of the eyes, no nuptial pads; (7) finger II longer than finger I, ungual flap mostly expanded, rounded, and more evident on fingers II–IV; (8) no fringes or webbing on fingers; (9) palmar tubercle ovoid, flattened, and slightly larger than thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular; no supernumerary tubercles, palmar accessory tubercles small, rounded, and almost indistinguishable; (10) heel smooth; (11) no fringes or webbing on toes, ungual flap slightly expanded to rounded, more evident on toes II–V; (12) plantar tubercle indistinguishable, between one and three non-protuberant subarticular tubercles present (one on toes I and II, two on toes III and V, and three on toe IV); inner metatarsal tubercle elongated; outer metatarsal tubercles conical and smaller than inner; tarsal ridge absent; (13) dorsal ground color in life brown to reddish, some specimens with pale reticulations on a dark background color; usually with a pair of red or pale dorsolateral lines, venter translucent or suffused with dark color, vocal sac yellow; (14) SVL 18.1 ± 21.3 (14.9–22.9, $N = 21$), males 17.1 ± 1.11 (14.9–18.5, $N = 15$), females 20.7 ± 1.86 (18.5–22.9, $N = 6$); (15) advertisement call composed of a single, amplitude-modulated short note (49.1–51.7 ms) with harmonic structure. The dominant frequency is also the fundamental frequency, with most energy emitted at 3.34–3.81 kHz.

Description of the holotype

An adult female (SVL 17.40, Fig. 10) with slender body; dorsal skin smooth with small scattered tubercles, ventral skin smooth, discoidal fold not evident, low anal warts present; one small conical supraocular tubercle; eye twice as long as snout; tympanum of moderate size, ratio TD/EL 34%; tympanum indistinguishable, annulus tympanicus concealed by skin, tympanic membrane absent, positioned above the junction of jaws and behind the orbit; head as long as wide (HL/HW 1.03), greatest head width between angles of jaw 40% of SVL; snout subacuminate from above and rounded in profile; nares situated near tip of snout and slightly dorsolaterally directed, clearly visible in frontal view, also visible dorsally but not ventrally; canthus rostralis rounded; loreal region feebly concave; dentigerous processes of vomer clearly visible, orbit in an oblique outline in frontal of eyes, each with five teeth; vocal slits absent; tongue long (25% of SVL) and knobbed at the end, first third attached to floor of mouth; hands moderate in size, 20% of SVL; relative lengths of adpressed fingers I < II < IV < III; finger II subequal in size to finger VI, finger II reaching the disc on finger IV when adpressed; finger III disc 1.6 times wider than distal end of adjacent phalanx; palmar tubercle low and rounded, larger than thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular; no supernumerary tubercles;

Table 4. Morphological proportions for *Diasporus* species

Species (*N*)	SVL	HW	HL
D. anthrax (2)*	16.5–18.7	–	–
D. tigrillo (2)*	16.8–17.5		
D. citrinobapheus (7)	19 ± 1.41 (17.3–21.8)	7 ± 0.47 (6.5–7.8)	7.6 ± 0.54 (7–8.7)
D. darienensis sp. nov. (21)	18.1 ± 2.13 (14.9–22.9)	6.5 ± 0.82 (5.2–8.4)	6.5 ± 0.64 (5.6–7.9)
D. aff. *diastema*, CP (49)	19.2 ± 2.78 (14.6–27.7)	7 ± 1.2 (5.4–10.9)	7.6 ± 0.99 (6–10.6)
D. aff. *diastema*, MM (5)	19.8 ± 3.11 (16–24.5)	7.3 ± 0.93 (6.1–8.7)	7.3 ± 1.04 (6–8.9)
D. aff. *diastema*, EPL (20)	21.3 ± 1.82 (18.3–25.2)	7.9 ± 0.83 (6.1–9.6)	7.4 ± 0.63 (6.7–8.4)
D. gularis (3)	22.1 ± 1.75 (20.4–23.9)	–	–
D. hylaeformis (28)	20.3 ± 1.51 (16.9–23.1)	7.4 ± 0.74 (5.9–8.8)	8 ± 0.71 (6.8–9.5)
D. igneus (4)	26.1 ± 0.5 (25.5–26.6)	9.9 ± 0.17 (9.7–10.1)	8.5 ± 0.31 (8.2–8.8)
D. majeensis sp. nov. (15)	21.5 ± 2.64 (15.3–25.5)	8.1 ± 0.92 (6.2–9.7)	7.8 ± 0.84 (6–9.4)
D. pequeno sp. nov. (13)	19.3 ± 2.38 (16.9–24.8)	7.2 ± 0.96 (6.2–8.9)	7.2 ± 1.04 (5.9–9.1)
D. aff. *quidditus* (51)	14.5 ± 1.44 (11.5–17.9)	5.4 ± 0.48 (4–6.5)	5.6 ± 0.48 (4.5–6.3)
D. sapo sp. nov. (11)	22.6 ± 2.86 (18.8–29.1)	8 ± 0.82 (7.1–9.7)	8.2 ± 1.05 (6.6–10.2)
D. tinker (39)	17.3 ± 1.55 (14.6–20.4)	6.4 ± 0.42 (5.6–7.3)	6.2 ± 0.58 (5.3–7.2)
D. ventrimaculatus (4)	22.8 ± 1.89 (20.2–24.7)	–	–
D. vocator (12)	14.4 ± 1.46 (12–17.2)	4.9 ± 0.41 (4–5.4)	5.4 ± 0.66 (4.4–6.3)
Species	**TL**	**HAL**	**HW/SVL**
D. anthrax	–	–	–
D. tigrillo		–	0.36 (0.34–0.37)
D. citrinobapheus	7.9 ± 0.68 (7–9.2)	–	–
D. darienensis sp. nov.	7.7 ± 0.73 (6.5–9.5)	6.8 ± 0.89 (5.4–9)	4.4 ± 0.39 (3.7–5.1)
D. aff. *diastema*, CP	8 ± 1.32 (5.5–12)	4.5 ± 0.93 (3.2–6)	0.4 ± 0.02 (0.3–0.4)
D. aff. *diastema*, MM	8.4 ± 0.96 (7.3–9.9)	4 ± 0.58 (3.5–5)	0.4 ± 0.01 (0.4–0.4)
D. aff. *diastema*, EPL	8.3 ± 0.77 (7.3–9.4)	3.9 ± 0.5 (3.4–4.8)	0.4 ± 0.02 (0.3–0.4)
D. gularis	–	–	–
D. hylaeformis	8 ± 0.75 (6.6–10)	–	–
D. igneus	11.7 ± 0.5 (11.3–12.4)	11.5 ± 0.27 (11.1–11.7)	11.9 ± 0.36 (11.5–12.2)
D. majeensis sp. nov.	9.1 ± 1.14 (6.8–10.9)	8.3 ± 1.01 (5.9–9.6)	5.1 ± 0.63 (3.5–6.1)
D. pequeño	8.4 ± 0.71 (7.5–10)	7.1 ± 0.69 (6.1–8.5)	4.4 ± 0.51 (3.7–5.5)
D. aff. *quidditus*	6.9 ± 0.56 (5.9–8)	5.5 ± 0.54 (4.3–6.3)	3.3 ± 0.32 (2.7–4)
D. sapo sp. nov.	10.4 ± 1.02 (8.3–12.1)	9.5 ± 1.07 (7.3–11.5)	5.5 ± 0.6 (4.2–6.4)
D. tinker	7.3 ± 0.66 (5.3–8.8)	6.2 ± 0.42 (5.3–7.4)	3.8 ± 0.31 (3.2–4.6)
D. ventrimaculatus	–	–	–
D. vocator	5.8 ± 0.43 (5.2–6.6)	4.9 ± 0.88 (3.9–5.5)	2.9 ± 0.38 (2.5–3.2)
Species	**HW/HL**	**HL/SVL**	**TL/SVL**
D. anthrax	–	–	–
D. aff. *tigrillo*	0.92 (0.85–0.99)	0.39 (0.38–0.40)	0.48 (0.46–0.50)
D. citrinobapheus	0.9 ± 0.04 (0.9–1)	0.4 ± 0.02 (0.4–0.4)	0.4 ± 0.01 (0.4–0.4)
D. darienensis sp. nov.	1 ± 0.06 (0.9–1.1)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.02 (0.4–0.5)
D. aff. *diastema*, CP	0.9 ± 0.07 (0.8–1)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.04 (0.4–0.6)
D. aff. *diastema*, MM	1 ± 0.02 (1–1)	0.4 ± 0.01 (0.4–0.4)	0.4 ± 0.03 (0.4–0.5)
D. aff. *diastema*, EPL	1 ± 0.05 (0.9–1.1)	0.4 ± 0.01 (0.3–0.4)	0.4 ± 0.02 (0.4–0.4)
D. gularis	–	–	–
D. hylaeformis	0.9 ± 0.05 (0.8–1)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.03 (0.3–0.5)
D. igneus	1.2 ± 0.03 (1.1–1.2)	0.3 ± 0.01 (0.3–0.3)	–
D. majeensis sp. nov.	1 ± 0.03 (1–1.1)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.02 (0.4–0.5)
D. pequeño	1 ± 0.05 (1–1.1)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.02 (0.4–0.5)
D. aff. *quidditus*	0.9 ± 0.07 (0.8–1.2)	0.2 ± 0.19 (0–0.4)	0.5 ± 0.03 (0.4–0.5)
D. sapo sp. nov.	1 ± 0.05 (0.9–1.1)	0.4 ± 0.02 (0.3–0.4)	0.5 ± 0.02 (0.4–0.5)
D. tinker	1 ± 0.06 (0.9–1.2)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.03 (0.3–0.5)
D. ventrimaculatus	–	–	–
D. vocator	0.9 ± 0.07 (0.8–1.1)	0.4 ± 0.03 (0.3–0.4)	0.4 ± 0.04 (0.4–0.5)

Mean ± SD (range); see Material and methods for abbreviations. Numbers in parenthesis next to the species names represents the number of specimens analysed.

*Measurements taken from original descriptions and literature.

Table 5. Variations in advertisement call parameters in 11 species of *Diasporus*

	Traits				
Species	DF (kHz)	Low freq. (Hz)	High freq. (Hz)	Delta freq. (Hz)	Note duration (s)
*D. anthrax**	3.81	3.19 ± 0.35 (2.94–3.44)	4.45 ± 0.29 (4.25–4.65)	1.31–1.22	0.06
D. citrinobapheus (2)	2.86–3.04	2.77–2.95	2.95–3.42	0.2–0.5	0.11–0.17
D. darienensis sp. nov. (2)	3.57 ± 0.33 (3.34–3.81)	3 ± 0.29 (2.79–3.21)	4.07 ± 0.33 (3.83–4.3)	1.07 ± 0.04 (1.04–1.09)	0.05 ± 0 (0.05–0.05)
D. aff. *diastema*, EPL (7)	3.3 ± 0.12 (3.2–3.5)	2.9 ± 0.07 (2.8–3)	3.8 ± 0.11 (3.6–3.9)	0.9 ± 0.05 (0.8–0.9)	0.1 ± 0.01 (0.1–0.1)
D. aff. *diastema*, MM (4)	3.1 ± 0.2 (3–3.4)	2.7 ± 0.1 (2.7–2.9)	3.5 ± 0.15 (3.4–3.7)	0.8 ± 0.06 (0.7–0.9)	0.1 ± 0 (0.1–0.1)
D. aff. *diastema*, CP (7)	3.3 ± 0.16 (3.2–3.5)	2.97	3.82	0.86	0.09
*D. igneus** (1)	2.4	2	2.7	0.7	0.05–0.10
D. majeensis sp. nov. (1)	2.47–2.71	2.38–3.03	2.85–3.14	0.50–0.93	0.01–0.02
D. pequeño (1)	3.44–3.48	3.20–3.23	3.67–3.63	0.39–0.46	0.09–0.15
D. aff. *quidditus* (22)	4.81 ± 0.14 (4.55–5.08)	4.56 ± 0.18 (4.35–4.84)	4.97 ± 0.19 (4.77–5.29)	0.41 ± 0.02 (0.39–0.45)	0.34 ± 0.04 (0.25–0.38)
D. tinker (9)	3.5 ± 0.19 (3.14–3.71)	3.16 ± 0.16 (2.84–3.32)	3.8 ± 0.2 (3.42–4.07)	0.64 ± 0.06 (0.56–0.75)	0.17 ± 0.02 (0.14–0.19)
*D. ventrimaculatus**	2.50–2.61	2.14	2.9	0.76	0.07
D. vocator (5)	4.6 ± 0.3 (4.35–5.1)	3.83 ± 0.17 (3.71–3.94)	4.94 ± 0.25 (4.77–5.12)	1.12 ± 0.08 (1.06–1.18)	0.02 ± 0 (0.01–0.02)
Species	**Note interval (s)**	**Call rate (calls/min)**	**Notes/bouts**	**Bout duration**	**Interbout duration**
D. anthrax	0.55–5.77	23.4–44.2			
D. citrinobapheus	16.58 ± 0.47 (16.25–16.91)	3.61 ± 0.1 (3.54–3.68)	19–30	19.30–55.50	38.47–156.43
D. darienensis sp. nov.	3.08 ± 0.9 (1.65–4.84)	20.29 ± 6.17 (12.04–34.41)	–	–	–
D. aff. *diastema*, EPL	3.1 ± 0.68 (2.2–3.9)	19.7 ± 4.43 (14.8–25.8)	8.5	28.4	40.26
D. aff. *diastema*, MM	3.4 ± 1.08 (2.5–4.8)	17.9 ± 5.02 (12–22.8)			
D. aff. *diastema*, CP	1.65	34.41	10.2	17.33	30.25
D. igneus	6.40–9.67	8.18	–	–	–
D. majeensis sp. nov.	2.67–6.02	12.32	–	–	–
D. pequeño	3.51–6.85	11.61	–	–	–
D. aff. *quidditus*	4.71 ± 0.78 (3.27–5.44)	12.19 ± 2.24 (10.35–16.71)	–	–	–
D. tinker	2.96 ± 2.05 (1.35–7.55)	25.38 ± 11.84 (7.76–39.16)	11–13	15.97–27.85	19.71–30.58
*D. ventrimaculatus**	5.15	11.45			
D. vocator	1.91 ± 0.57 (1.4–2.52)	32.98 ± 9.35 (23.7–42.39)	13.23 ± 10.51 (5.8–20.67)	19.25 ± 13.31 (9.84–28.66)	311.02 ± 304.7 (95.56–526.47)

Mean ± SD (range). Number in parentheses next to the species names represents the number of individuals analysed. DF, dominant frequency.
*Information obtained from literature.

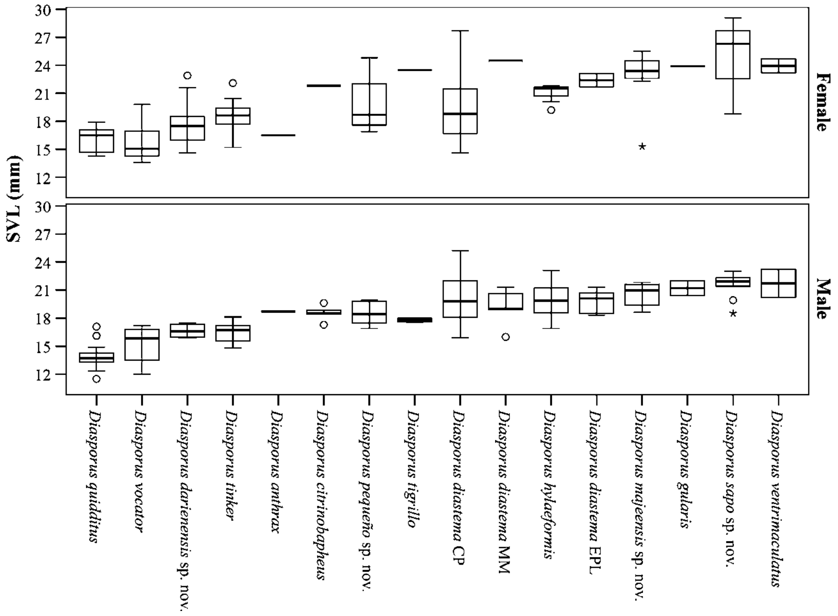

Figure 3. Differences in snout-vent length (SVL) of *Diasporus* species, separated by sex. The bottom and top of the box are the first and third percentile, and the band inside the box is the median, whiskers are the extreme values; open circles above or below the boxes represent outliers.

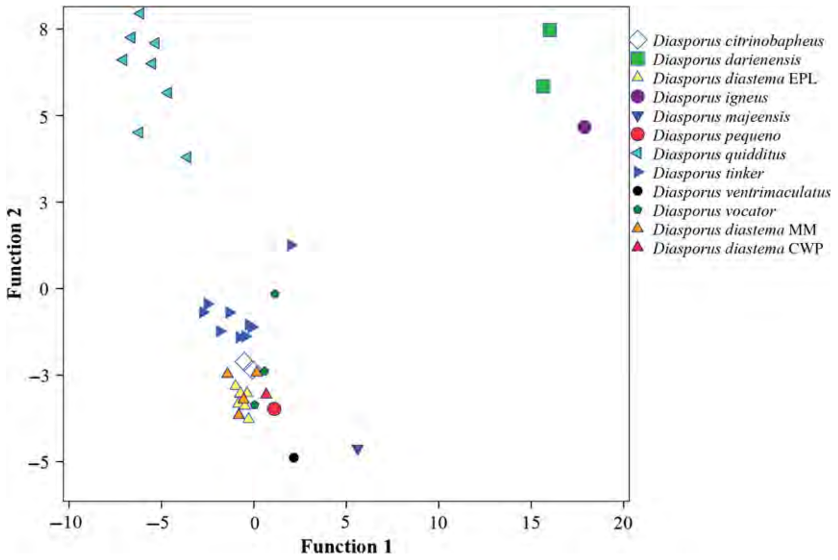

Figure 4. Discriminant function analyses of the acoustic characters of *Diasporus* species. Variables included in the analysis: note duration, note interval, dominant frequency (DF, corrected by snout-vent length), low frequency, high frequency, and call rate (temporal characters are corrected for temperature).

palmar and plantar accessory tubercles indistinguishable; no nuptial pads; no fringes on fingers; hindlimbs of moderate length, TL 43% of SVL; relative lengths of adpressed toes I < II < III < V < IV; when adpressed, tip of toe I reaches to tubercle of toe II; disc of toe IV slightly expanded, 1.3 times wider than distal end of adjacent phalanx; no fringes on toes; between one and three nonprotuberant subarticular tubercles present (one each on toes I and II, two on toes III and V, and three on toe IV); inner metatarsal tubercle ovoid; outer metatarsal tubercles slightly pointed and smaller than inner; tarsal ridge absent; hands and feet without webbing; finger and toe discs even, broadened; ungual flap expanded, almost

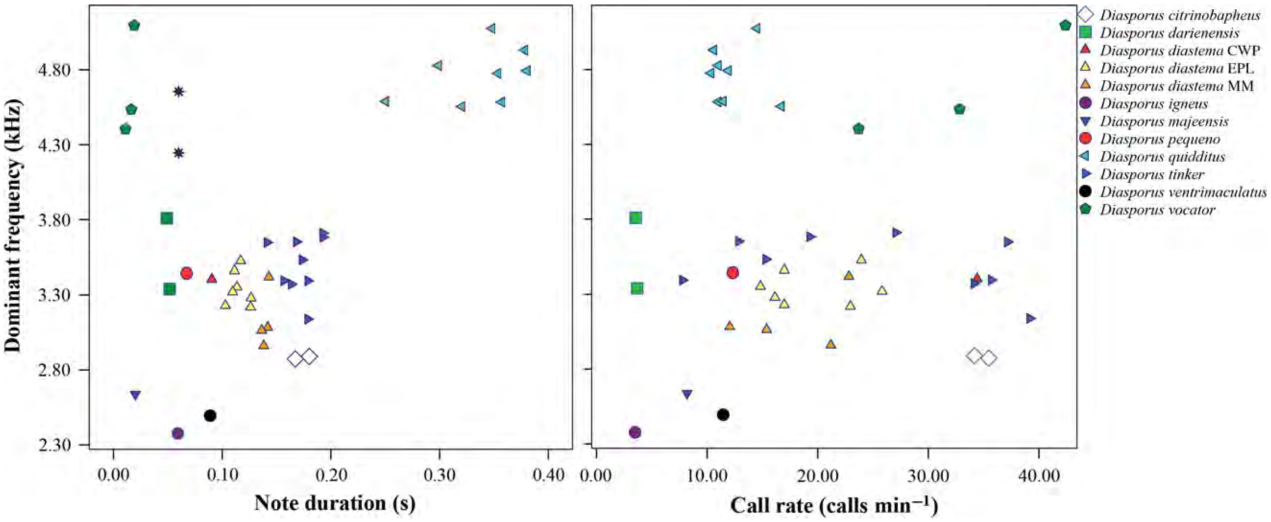

Figure 5. Scatter plot for dominant frequency/note duration (left) and dominant frequency/call rate (right) in 11 species of *Diasporus*.

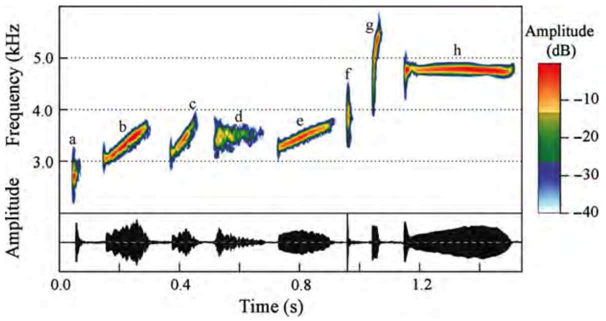

Figure 6. Spectrograms (only the harmonic containing the dominant frequency is shown) and oscillograms (below) of the advertisement calls of *Diasporus* species from eastern Panama (EP): a, *Diasporus majeensis* sp. nov. (SMF 97658); b, *Diasporus* aff. *diastema* MM (MHCH 2809); c, *Diasporus diastema* from Colón, Panama (SMF 97287, 9.26020 °N, 79.93540 °W, 36 m a.s.l.; ~9 km south-west from type locality); d, *Diasporus pequeno* sp. nov. (Bajo Pequeño, Cerro Pechito Parado, not collected); e, *Diasporus tinker* (SMF 97315); f, *Diasporus darienensis* sp. nov. (SMF 97313); g, *Diasporus vocator* (not collected; from Celmira, Bugaba, Panama, 8.55348 °N, 82.81525 °W, 242 m a.s.l.; ~60 km east from type locality); h, *Diasporus* aff. *quidditus* (SMF 97292).

rounded; pads broadened and globular in profile (Figs 7A, 10).

Coloration of holotype in life

Holotype (SMF 97304; Figs 10, 14A, B) recorded as follows: iris light Pratt's rufous (71) with a couple of lateral and irregular lines tawny (60), lumbar region tawny (60), bordered by two lines light buff (2); flanks brick red (36); groin, axilla, and ventral areas mottled with brick red (36).

Coloration in preservative

Dorsal ground color raw amber (23), with a couple of dorsolateral lines light buff (2); groin and ventral areas buff (5), with small points sepia (279); ungual flaps dark drab (45).

Measurements of holotype (mm)

SVL 17.40; HL 6.70; HW 6.30; IOD 3.24; EL 2.72; TD 0.92; FL 6.42; TL 7.50; HAL 3.47; 3FW 0.40; 3FD 0.64; 3TW 0.36; 3TD 0.49; 4TW 0.31; 4TD 0.67; BW 5.22 (for variation of the species, see Table 1).

Vocalization

The calls produced by two specimens from Cerro Sapo (Fig. 6; Table 2), one paratype (SMF 97313, environmental temperature 21.5 °C; humidity 84%; 22:06 h) and an uncollected specimen (environmental temperature 21.7 °C; humidity 80%; 21:00 h) were analysed. The calls consist of single, short, monophasic notes that are reminiscent of a 'whistle' (Fig. 6). Note duration is 0.04–0.05 s, with an interval between calls of 16.91–16.25 s, and with a call rate of four calls per minute. The peak frequency band ranges from 2.79 to 4.30 kHz; the first harmonic contains the dominant frequency at 3.34–3.81 kHz.

Natural history

This species is found in the eastern Panamanian montane forest (Fund & Hogan, 2012) along the PM and JSM (Fig. 1). The vegetation consists predominantly of trees covered with moss, bromeliads (*Werauhia* spp.

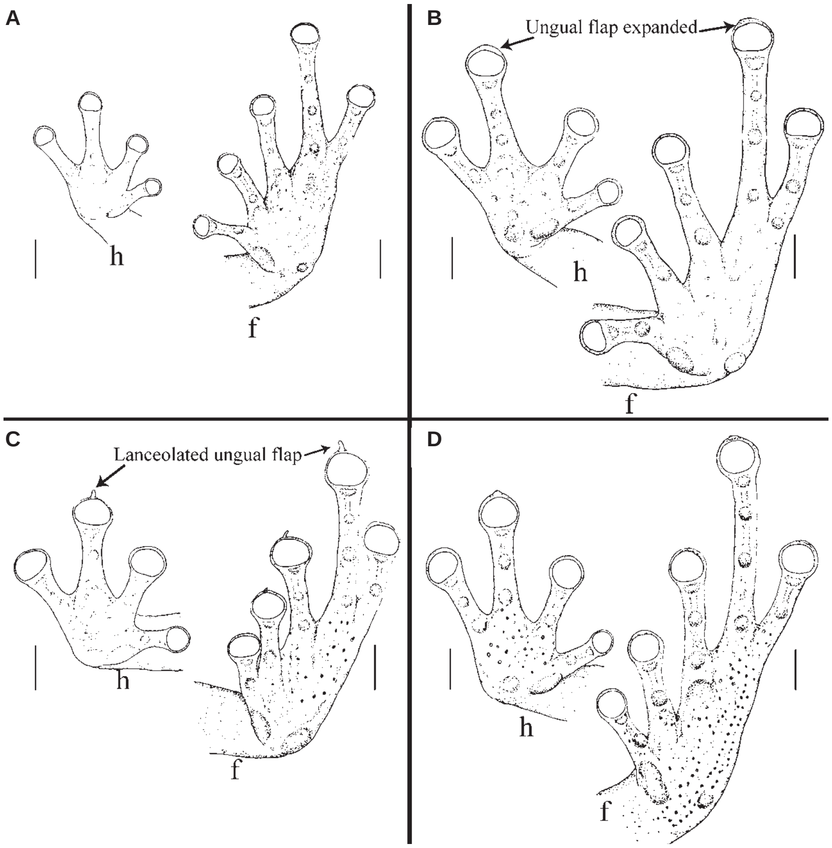

Figure 7. Drawings of ventral view of right hand and left foot of the new *Diasporus* species described here; h, hand; f, foot. Arrows indicate two examples of ungual flap shape. A, *Diasporus darienensis* sp. nov. (MHCH 2852). B, *Diasporus majeensis* sp. nov. (MHCH 2835). C, *Diasporus pequeno* sp. nov. (MHCH 2826). D, *Diasporus sapo* sp. nov. (SMF 97331). Scale bars: 1 mm.

and *Guzmania* spp.), giant ferns (*Cyathea* spp.), and orchids. *Diasporus darienensis* sp. nov. is an inhabitant of the cloud forest (869–1169 m a.s.l.), usually found 1–5 m above ground. During the day, specimens seek retreats between bromeliad leaves. At night they actively move across tree bark and bromeliads. Only two males were encountered calling, both during the end of the rainy season (December) at the top of Cerro Sapo. One male (SMF 97313) was observed calling from the underside of a leaf in a tree about 5 m above ground, the other was calling from a branch on a ridge 3 m above the ground on the same day. Diet is not known, but as with other *Diasporus* it may eat small arthropods (Batista, 2009).

Etymology

The species name is derived from the province name Darién where the holotype was found, with the Latin suffix *-ensis* donating a place or locality. The species is known to occur only in this province, and it is present in the main mountain ranges of the region.

DIASPORUS MAJEENSIS SP. NOV.

FIGS 11, 14C, D

Holotype

SMF 97293 (original field number AB 1030), an adult male (Fig. 11) collected by Abel Batista & Konrad Mebert on the top of Cerro Chucantí, at Majé mountain range, Río Congo Arriba, Distrito de Chepigana, Darién, Panama, on 2 December 2012 at 20:35 h (8.79936 N, 78.46156 W; 1380 m a.s.l.).

Paratypes

MHCH 2832–39, SMF 97655–60, with same collection data as the holotype.

Diagnosis: Diasporus majeensis sp. nov. is characterized by the following combination of characters (Figs 11, 14C, D; Table 1): (1) dorsal skin smooth with small dispersed warts, ventral skin smooth; (2) only lower part of the tympanic annulus barely visible, tympanic membrane absent; (3) snout

Table 6. Main diagnostic characters and character states to differentiate members of the *Diasporus* genus in Central and South America

Species	SVL	Ungual flap	Dorsal color pattern	Ventral color pattern	DF (kHz)	Distribution
*D. anthrax**	16.5–18.7	Palmate to rounded, or expanded	Dark or blackish	Black with white flecks	3.81	North-western Ecuador and Colombia
*D. tigrillo**	16.8–17.5	Spadate	Yellow to orange	White, granules on venter with base greyish	No data	Alto Lari, SE Costa Rica, 300–400 m a.s.l.
D. citrinobapheus	19 ± 1.41 (17.3–21.8)	Palmate to rounded, or spadate	Yellowish	Almost transparent	2.86–3.04	Western Panama, 680–790 m a.s.l.
D. darienensis sp. nov.	18.1 ± 2.13 (14.9–22.9)	Palmate to rounded, or spadate	Reddish, with or without reticulations	Reddish or small black speckles	3.57 ± 0.33 (3.34–3.81)	Serranía de Pirre and Jingurudó-Sapo, Darién, Panama, 869–1169 m a.s.l.
D. aff. *diastema*, CWP	19.2 ± 2.78 (14.6–27.7)	Palmate to rounded, or spadate	Yellowish	White with dark spots	3.3 ± 0.16 (3.2–3.5)	Lowlands of central and western Panama
D. aff. *diastema*, MM	19.8 ± 3.11 (16–24.5)	Palmate to rounded	Yellowish	White with dark spots	3.1 ± 0.2 (3–3.4)	Serranía de Maje, Panama
D. aff. *diastema*, EPL	21.3 ± 1.82 (18.3–25.2)	Palmate to rounded, or spadate	Yellowish	White with dark spots	3.3 ± 0.12 (3.2–3.5)	Lowlands of eastern Panama
D. gularis	22.1 ± 1.75 (20.4–23.9)	Palmate to rounded, or spadate	Pale brown with vague markings	Cream with brown stippling on throat	No data	Lowlands of western Colombia and north-western Ecuador
D. hylaeformis	20.3 ± 1.51 (16.9–23.1)	Palmate to rounded	Suffused with pink or red	Translucent, suffused with yellow or red	No data	Serranía de Talamanca Costa Rica and Panama, 1500–2500 m a.s.l.
D. igneus	26.1 ± 0.5 (25.5–26.6)	Palmate to rounded	Brownish with yellow to orange reticulations	Yellow	2.4	Western and eastern slopes of Cerro Santiago, Panama, above 1500 m a.s.l.
D. majeensis sp. nov.	21.5 ± 2.64 (15.3–25.5)	Palmate to rounded, or spadate	Reddish, with or without reticulations	Unpigmented venter	2.47–2.71	Top of Cerro Chucantí, Panama, 1400 m a.s.l.
D. pequeno sp. nov.	19.3 ± 2.38 (16.9–24.8)	Lanceolate to papillate	Brown, cream, with dark reticulations	Venter translucent, with a dark speckle and sky blue spots	3.44–3.48	Serranía de Darién, Panama, above 472 m a.s.l.
D. aff. *quidditus*	14.5 ± 1.44 (11.5–17.9)	Lanceolate to papillate	Brown	Brown	4.81 ± 0.14 (4.55–5.08)	Eastern Panama and north-western Colombia, above 100 m a.s.l.

Table 6. *Continued*

Species	SVL	Ungual flap	Dorsal color pattern	Ventral color pattern	DF (kHz)	Distribution
D. sapo sp. nov.	22.6 ± 2.86 (18.8–29.1)	Palmate to rounded, or spadate	Uniform red	Translucent, suffused with red	No data	Cerro Sapo, Darién, Panama, 1169 m a.s.l.
D. tinker	17.3 ± 1.55 (14.6–20.4)	Lanceolate to papillate	Grey	Brown to orange	3.5 ± 0.19 (3.14–3.71)	Eastern Panama to north-western Colombia, in Panama 800–1350 m a.s.l., in Colombia, up to 1880 m a.s.l.
D. ventrimaculatus	22.8 ± 1.89 (20.2–24.7)	Spadate	Red to pink	White with red spots in males and white with dark spots in females	2.50–2.61	Valle del Silencio, Costa Rica, 2550 m a.s.l.
D. vocator	14.4 ± 1.46 (12–17.2)	Lanceolate to papillate	Pigmented with dark mottling and light areas	Brown	4.6 ± 0.3 (4.35–5.1)	South-western Costa Rica to central Panama, 2–1220 m a.s.l.

*Information obtained from literature. DF, dominant frequency.

rounded in dorsal and profile view; (4) conical supraocular tubercle or cranial crests absent; (5) dentigerous processes of vomers with between one and four teeth each, straight in outline, in frontal to the orbit; (6) vocal sac small, but with visible longitudinal gular folds, vocal slits present, situated beside the tongue, from the middle side of the tongue to near the junctions of jaws, no nuptial pads; (7) finger II longer than finger I, ungual flap mostly expanded, rounded, more evident on fingers II–IV; (8) no fringes or webbing on fingers; (9) palmar tubercle ovoid, flattened, and slightly larger than thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular, first tubercle more evident; one or two supernumerary tubercles, palmar accessory tubercles small and rounded; (10) heel smooth; (11) no fringes or webbing on toes, ungual flap slightly expanded to rounded, more evident on toes II–V; (12) plantar tubercle indistinguishable, subarticular tubercles present (one on toes I and II, two on toes III and V, and three on toe IV), first tubercle more evident; small and rounded supernumerary tubercles; inner metatarsal tubercle elongated; outer metatarsal tubercles conical and smaller than inner; tarsal ridge absent; (13) dorsal ground color in life brown to reddish, some specimens with dark reticulations on a reddish background color, venter translucent, vocal sac same color as venter (Fig. 14C–D); (14) SVL 21.5 ± 2.64 (15.3–25.5, $N = 15$), males 19.9 ± 2.1 (15.3–21.8, $N = 9$), females 23.9 ± 1.22 (22.3–25.5, $N = 6$); (15) advertisement call composed of a single, amplitude-modulated short note with duration of 0.01–0.02 s, and with the DF ranging between 2.47 and 2.71 kHz (Fig. 6; Table 2).

Description of the holotype

An adult female (SVL 20.90), with slender body; dorsal skin smooth with small dispersed warts, ventral skin smooth, discoidal fold not evident; eye 1.30 times longer than snout; tympanum small, ratio TD/EL 21%; only lower part of the tympanic annulus barely visible, tympanic membrane absent, positioned 2 mm behind orbit; head slightly wider than long (HL/HW 0.85), greatest head width between angles of jaw 38% of SVL; snout rounded from above and in profile; nares situated near tip of snout and slightly dorsolaterally directed, visible in frontal view, and also visible dorsally but not ventrally; canthus rostralis rounded; loreal region feebly concave; dentigerous processes barely visible, in frontal of the orbit of eyes in a straight outline, each with four teeth; vocal slits absent; tongue long (20% of SVL) and broadening to the end, first third attached to floor of mouth; hands moderate in size, 23% of SVL; relative lengths of adpressed fingers I < II < IV < III; finger II smaller

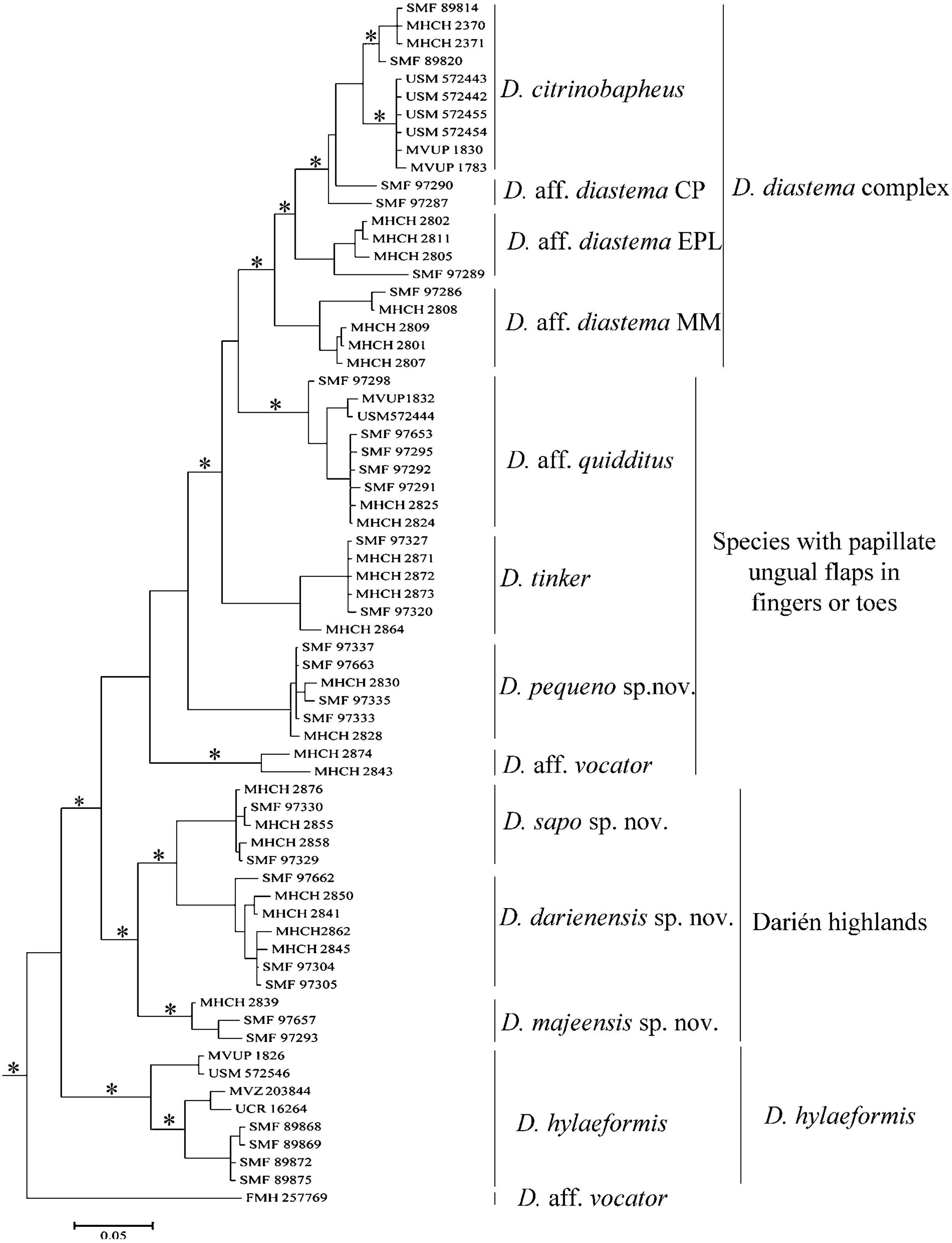

Figure 8. Bayesian consensus tree of the genus *Diasporus* based on *16S*, *COI*, and *RAG1* genes. Out-groups are not shown (*Pristimantis caryophyllaceus*, *Craugastor gollmeri*, *Craugastor fitzingeri*, *Colostethus pratti*, *Eleutherodactylus planirostris*, and *Eleutherodactylus thorectes*). Asterisks on nodes indicate estimated posterior probabilities: $P \geq 0.90$.

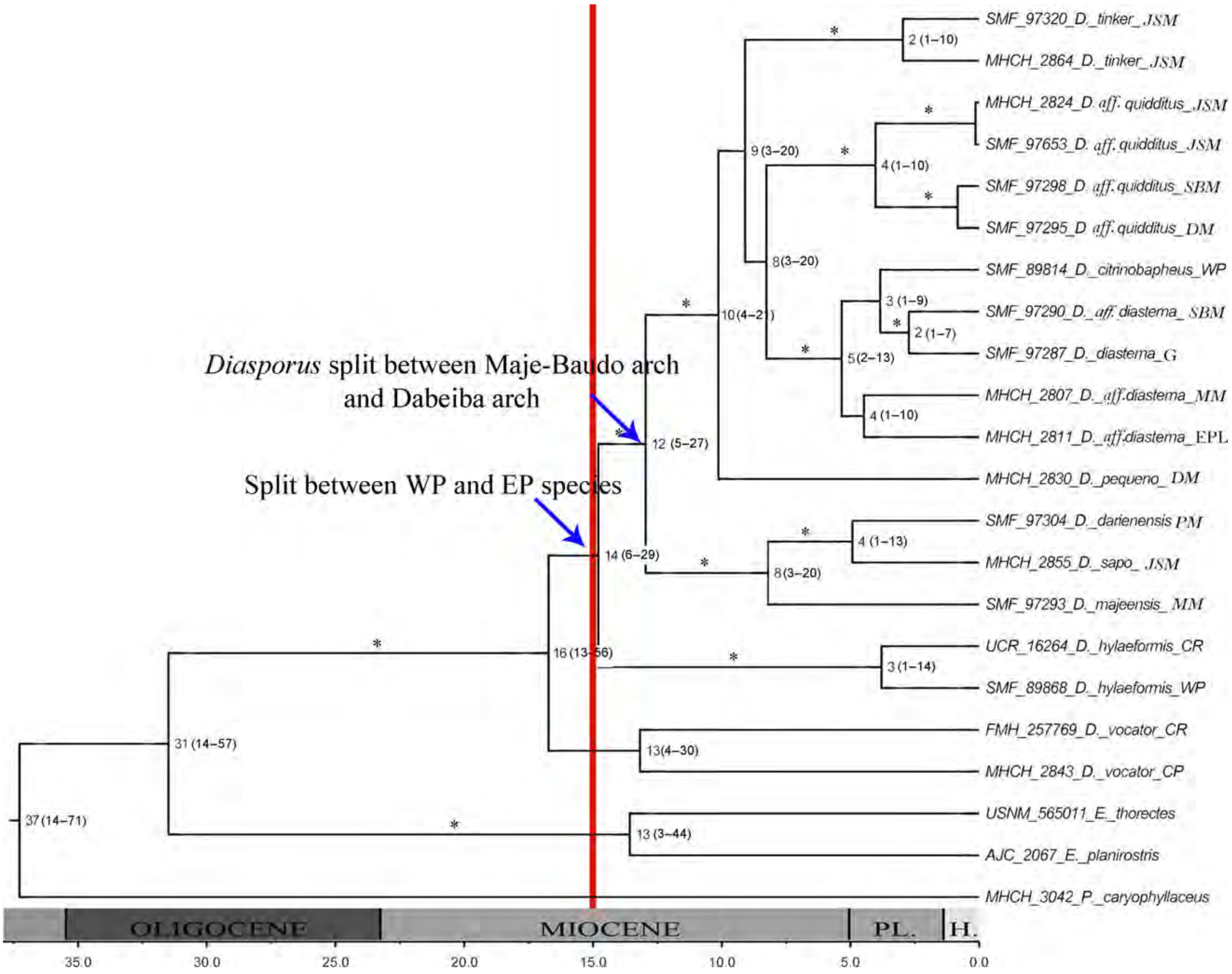

Figure 9. A chronogram of *Diasporus* species based on *16S*, *COI*, and *RAG1*, derived from a relaxed-clock Bayesian analysis, using BEAST software. The scale indicates time in Mya. The red line indicates the hypothesized completion, 15 Mya, of the Isthmus of Panama. Asterisks on nodes indicate estimated posterior probabilities: $P \geq 0.95$. Numbers at nodes represent estimated ages of diversification (SD in parenthesis). Letters at the end of species names represent biogeographic areas (for an explanation, see Material and methods); CR, Costa Rica; CP, central Panama; DM, Darién mountain range; G, Gatún lake at CP; JSM, Jingurudó-Sapo mountain range; MM, Majé mountain range; PM, Pirre mountain range; SBM, San Blas mountain range; WP, western Panama.

than finger VI, finger II reaching the base of disc on finger IV when adpressed; finger III disc 1.6 times wider than distal end of adjacent phalanx; palmar tubercle low and rounded, larger than thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular; no supernumerary tubercles; palmar and plantar accessory tubercles small and rounded; no nuptial pads; no fringes on fingers; hindlimbs of moderate lengths, TL 46% of SVL; relative lengths of adpressed toes I < II < III < V < IV; when adpressed, tip of toe I reaches the last third of distal phalanx of toe II; disc of toe IV slightly expanded, 1.3 times wider than distal end of adjacent phalanx; no fringes on toes; subarticular tubercles present (one each on toes I and II, two on toes III and V, and three on toe IV), first subarticular tubercles more visible than the rest; inner metatarsal tubercle ovoid; outer metatarsal tubercles rounded, slightly pointed, and smaller than inner; tarsal ridge absent; hands and feet without webbing; finger and toe discs slightly triangular; ungual flap expanded, even, rounded; pads globular in profile (Fig. 7B).

Coloration of holotype in life

Holotype (SMF 97293, Fig. 11) recorded as follows: iris light orange yellow (7) with middle area light Pratt's rufous (71); dorsal ground color chestnut (30) with peach red (70) areas in the occipital, flanks, and

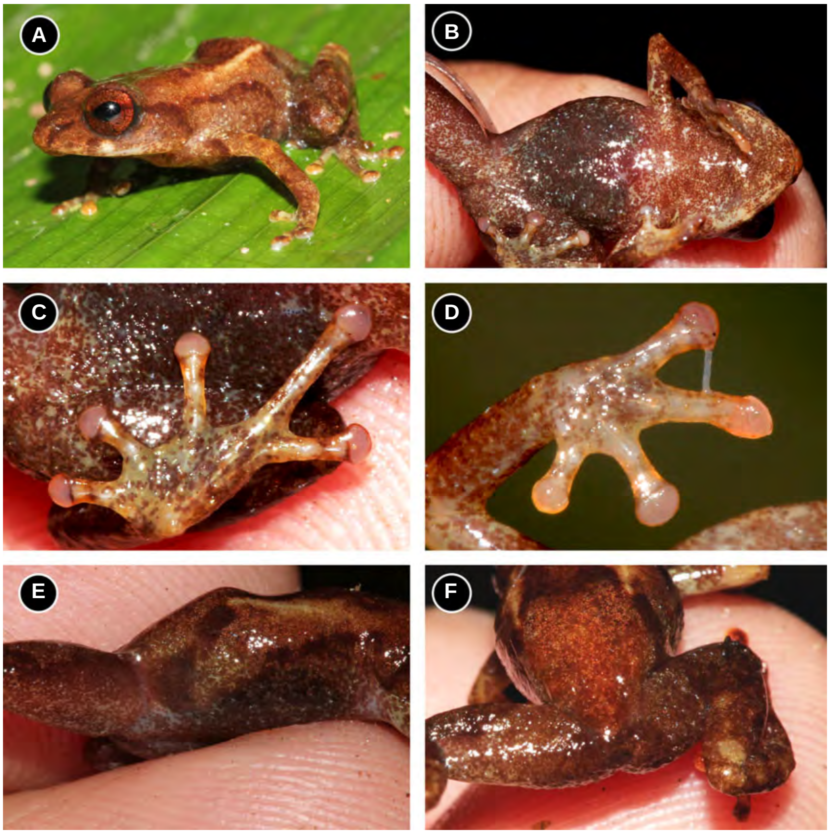

Figure 10. Holotype of *Diasporus darienensis*> sp. nov.: A, frontal view; B, ventral view; C, left foot ventrally; D, right hand ventrally; E, flanks; F, posterior side of thighs and rear.

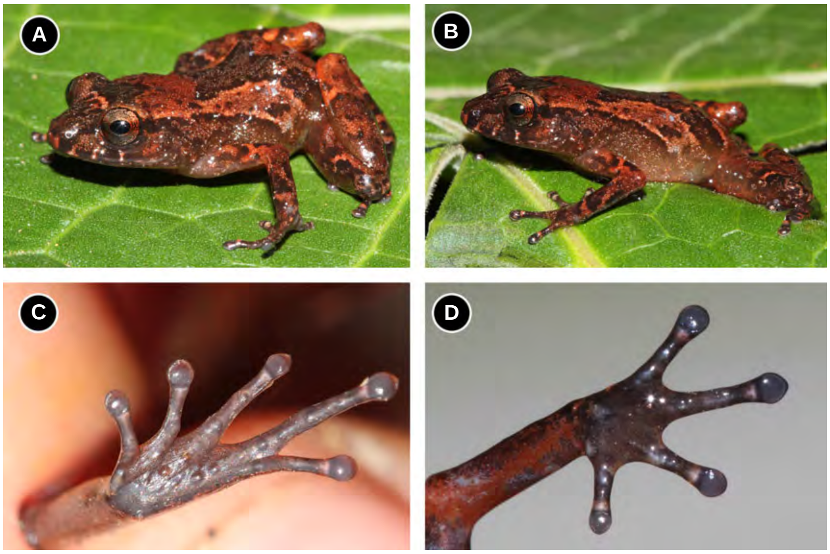

Figure 11. Holotype of *Diasporus majeensis* sp. nov.: A, B, frontal and lateral view, respectively; C, left foot ventrally; D, right hand ventrally.

lumbar region; a spectrum red (67) interorbital band, bordered posteriorly by a sepia (286) band; axilla and groin slightly pigmented with chestnut (30); limbs same as dorsum; ventral areas translucent slightly pigmented with sepia (286); ventral part of fingers and toes dark carmine (61).

Coloration in preservative
Dorsal ground color burnt sienna (38) with flesh ocher (57) areas in the occipital, flanks, and lumbar region; interorbital band flesh ocher (57), groin and venter light buff (2), ventral surfaces of limbs light orange yellow (7).

Measurements of holotype (mm)
SVL 20.90; HL 6.53; HW 7.68; IOD 2.21; EL 2.97; TD 0.62; FL 8.41; TL 9.51; HAL 4.79; 3FW 0.47; 3FD 0.75; 3TW 0.47; 3TD 0.65; 4TW 0.42; 4TD 0.53; BW 6.77 (for variation of the species, see Table 1).

Vocalization
The calls produced by one specimen (SMF 97658, environmental temperature 18.5 °C; 3 December 2012, 18:19 h) were analysed. The call consisted of single, short, monophasic notes that are reminiscent of a 'whistle' (Fig. 6). Note duration is 0.01–0.02 s, with an interval between calls of 2.67–6.02 s and a call rate of 12.32 calls/min; the low frequency was 2.38–3.03 kHz, the high frequency was 2.85–3.14 kHz, and the first harmonic contains the dominant frequency at 2.47–2.71 kHz.

Natural history
This species is found in the eastern Panamanian montane forest (Fund & Hogan, 2012) of the Majé mountain ranges (Fig. 1). Cloud forest in this area has vegetation consisting predominantly of trees covered with moss and a large variety of understory bromeliads (*Werauhia* spp. and *Guzmania* spp.). At night, *D. majeensis* sp. nov. was found 0.5–2.0 m above ground on tree bark in bromeliad foliage. During the daytime, individuals were found hiding between bromeliad leaves. At the top of Cerro Chucantí, males were calling during the end of the rainy season (December). The recorded male was observed calling between dry bromeliad leaves 1.5 m above ground. The diet is not known, but as with other *Diasporus* it is likely to eat small crickets, cockroaches, ants, and isopods (Batista, 2009).

Etymology
The species name is derived from the name of the mountain range, Majé, where the holotype was found, with the Latin suffix *-ensis* donating a place or locality.

DIASPORUS PEQUENO SP. NOV.
FIGS 12, 14 E, F

Holotype
SMF 97663 (original field number AB 857), an adult female (Fig. 12) collected by Abel Batista, Marcial Sabugara, and Amadiel Chaquí at Cerro Pechito Parado, at the Darién mountain range, Río Tuquesa, Bajo Pequeño, Cémaco, Comarca Embera Wounaan, Darién, Panama, on 5 November 2012 at 22:35 h (8.47553 N, 77.54883 W; 472 m a.s.l.).

Paratypes
SMF 97333–34, same locality as holotype; MHCH 2828–31, SMF 97635–38, collected at Cerro Pechito Parado on 7 November 2012 at 19:00–

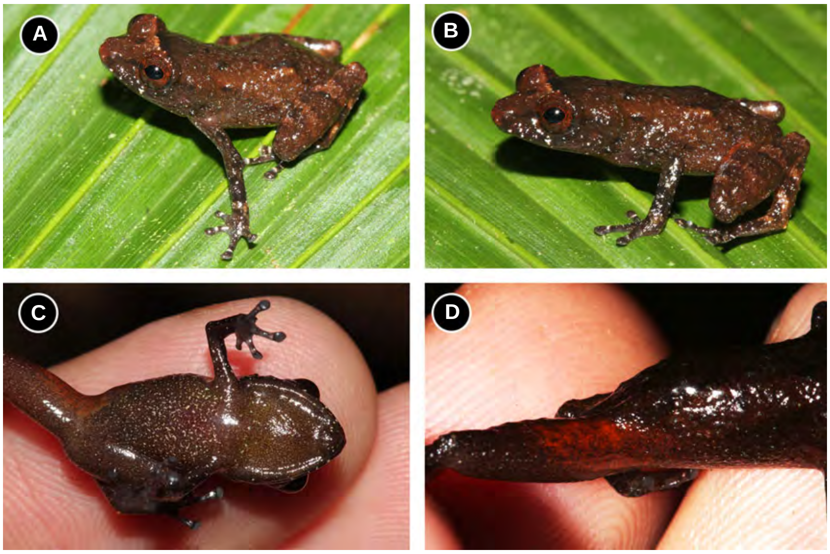

Figure 12. Holotype of *Diasporus pequeno* sp. nov.: A, B, frontal and lateral view, respectively; C, ventral view; D, flanks.

00:30 h (8.47911 N, 77.52799 W; 718 m a.s.l.), with same collectors as for holotype; MHCH 2826–27 collected at Cerro Pechito Parado, on 6 November 2012 at 19:00–01:30 h (8.47996 N, 77.51941 W; 858 m a.s.l.), with same collectors as for holotype.

Diagnosis

Diasporus pequeno sp. nov. is characterized by the following combination of characters (see Tables 4–6): (1) dorsal and ventral skin texture smooth, with small scattered tubercles, anal warts present; (2) tympanic annulus present, but with only the lower part clearly visible, tympanic membrane absent; (3) snout rounded in dorsal view and in profile; (4) rounded supraocular tubercle present, cranial crests absent; (5) dentigerous processes of vomers with between four and six teeth each, straight in outline, near to the frontal border of the orbit; (6) vocal sac well developed (Fig. 14E, F), vocal slits present, situated under the centre of the orbit level, no nuptial pads; (7) finger II longer than finger I; disc pads even broadened; ungual flap expanded, lanceolate, more evident on finger III (Fig. 7C); (8) no fringes or webbing on fingers; (9) palmar tubercle rounded, flattened, and larger than thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular, first tubercle more evident; supernumerary tubercles barely evident, palmar accessory tubercles small and rounded; (10) heel smooth; (11) no fringes or webbing on toes, ungual flap expanded, lanceolate, more evident on toes II–V; (12) plantar tubercle indistinguishable, subarticular tubercles present (one on toes I and II, two on toes III and V, and three on toe IV), first tubercle more evident; small and rounded supernumerary tubercles; inner metatarsal tubercle elongated, outer metatarsal tubercles rounded and smaller than inner; tarsal ridge absent; (13) dorsal ground color in life brown, cream, with dark reticulations, venter translucent (Wolffian duct visible), with a dark speckle and sky-blue spots, vocal sac bright yellow (Fig. 14E, F); (14) SVL 19.3 ± 2.38 (16.9–24.8, *N* = 13), males 18.2 ± 1.09 (16.9–19.9, *N* = 10), females 22.9 ± 1.69 (21.5–24.8, *N* = 3); (15) advertisement call composed of a single, amplitude-modulated short note (0.09–0.15 s) with harmonic structure, and with most energy emitted with the first harmonic call (3.44–3.48 kHz).

Description of the holotype

An adult female (SVL 22.68) with a slender body; dorsal skin smooth with scattered tubercles, with a small supraciliary tubercle, ventral skin smooth, discoidal fold not evident; eye 1.70 times longer than snout; tympanum of moderate size, ratio TD/EL 36%, tympanic annulus present, but only the lower part clearly visible, tympanic membrane absent; head as wide as long (HL/HW 0.95), greatest head width between angles of jaw 39% of SVL; snout rounded from above and in profile; nares situated near tip of snout and slightly dorsolaterally directed, visible in frontal view, also visible dorsally but not ventrally; canthus rostralis rounded; loreal region feebly concave; dentigerous processes in front of the orbit of eyes, perpendicular in direction to the centre of roof of mouth, in a straight outline, each with seven teeth; vocal slits absent; tongue long (18% of SVL) and broadening to the tip, first third attached to floor of mouth; hands moderate in size, 18% of SVL; relative lengths of adpressed fingers I < II < IV < III; finger II smaller than finger VI, finger II reaching the disc on finger IV when adpressed; finger III disc 2.16 times wider than distal end of adjacent phalanx; palmar tubercle rounded to ovoid, larger than thenar tubercle; thenar tubercle elongate; subarticular tubercles rounded and globular, first tubercle more evident; supernumerary tubercles rounded and small; palmar and plantar accessory tubercles small and rounded; no nuptial pads; no fringes on fingers; hindlimbs of moderate length, TL 43% of SVL; relative lengths of adpressed toes I < II < III < V < IV; when adpressed, tip of toe I reaches the last third of distal phalanx of toe II; disc of toe IV expanded, 1.73 times wider than distal end of adjacent phalanx; no fringes on toes; subarticular tubercles present (one each on toes I and II, two on toes III and V, and three on toe IV), first subarticular tubercles more evident than the rest; inner metatarsal tubercle ovoid; outer metatarsal tubercles rounded, slightly pointed, and smaller than inner; tarsal ridge absent; hands and feet without webbing; finger and toe discs even broadened; ungual flap expanded, fingers and toes III and IV lanceolated; pads globular in profile (Fig. 7C).

Coloration of holotype in life

Holotype (SMF 97663; Fig. 12) recorded as follows: iris geranium (66) with fine sepia (286) reticulations; dorsal ground color walnut brown (27), with sepia (286) blotches, and small sky-blue (192) dots; a flesh ocher (57) interorbital band, bordered posteriorly by a sepia (286) band; groin Pratt's ruby (68); axilla and venter walnut brown (27) mottled with pale pinkish buff (3), throat suffused with buff (5); fingers and toes with a pale buff (1) band just before the disc cover.

Coloration in preservative

Dorsal ground color drab (19), with a pair of dorsolateral lines light orange yellow (7); groin and ventral areas light buff (2), with small points sepia (279); ungual flaps cinnamon–drab (50).

Measurements of holotype (mm)

SVL 22.68; HL 8.43; HW 8.89; IOD 2.52; EL 3.33; TD 1.20; FL 8.12; TL 9.75; HAL 4.49; 3FW 0.50; 3FD 1.08; 3TW 0.51; 3TD 0.91; 4TW 0.52; 4TD 0.90; BW 7.81 (see Table 4).

Vocalization

Through call amplification of a recording containing several species (4–dB amplified; for an explanation, see Material and methods), we were able to extract the calls of three species: *Pristimantis* sp. (DF 2.76 kHz), *D. diastema* (DF 2.99 kHz), and *D. pequeno* sp. nov. (environmental temperature 24 °C; 8 October 2012, 18:17 h). Eleven calls were analysed (because the similarity of the call intervals indicates a single individual), consisting of single, short, monophasic notes that are reminiscent of a 'tink' (Fig. 6). Note duration is 0.09–0.15 s, with an interval between calls of 3.51–6.85 s and a call rate of 11.61 call/min; the low frequency was 3.20–3.23 kHz, the high frequency was 3.63–3.67 kHz, and the fundamental frequency is also the dominant frequency at 3.44–3.48 kHz.

Natural history

This species is found in the eastern Panamanian montane forest (Fund & Hogan, 2012) of the Darién mountain range (Fig. 1). Most specimens were found at 0.2–1.0 m above ground, over green leaves, between branches with dry leaves or in bromeliads. At the first location (472 m a.s.l.) the understory was open. The predominant vegetation were palms, vines, and small trees; at the second location above 700 m a.s.l. bromeliads were predominant; *D.* aff. *pequeno* sp. nov. was found to be sympatric with *D. diastema* and *D. quidditus*, and all species were actively calling.

Etymology

The species name *pequeno* is derived from the name Bajo Pequeño (or Bajo Chiquito), the last village at Río Tuquesa, where this species was found.

DIASPORUS SAPO SP. NOV.

FIGS 13, 14G, H

ELEUTHERODACTYLUS SP. – MYERS 1969: FIG. 19C.

Holotype

SMF 97329 (original field number AB 429), an adult female (Fig. 13) collected by Abel Batista & Gustavo Dojirama at the top of Cerro Sapo, PND, Distrito de Garachiné, Darién, Panama, on 4 December 2011, at 20:00 h (7.97618 N, 78.36263 W; 1169 m a.s.l.).

Paratypes

MHCH 2853–58, SMF 97328, SMF 97330–32; same collecting data as for holotype.

Diagnosis

Diasporus sapo sp. nov. is characterized by the following combination of characters (see Tables 4–6): (1) dorsal skin texture slightly tuberculate, venter smooth; (2) tympanum indistinguishable, annulus tympanicus and tympanic membrane absent; (3) snout rounded in dorsal view and in profile; (4) conical supraocular tubercle and cranial crests absent; (5) dentigerous processes of vomers with between seven and 11 teeth each, straight in outline, from the centre of the orbit to the centre of the roof of mouth; (6) vocal sac and vocal slits not differentiated, only a slightly differentiated fold beside the tongue, no nuptial pads; (7) finger II longer than finger I, ungual flap expanded, spadate, more evident on fingers II–IV; (8) no fringes or webbing on fingers; (9) palmar tubercle ovoid or rounded, flattened and almost the same size as thenar tubercle; thenar tubercle elongate; subarticular tubercles rounded and globular; two or three supernumerary tubercles; (10) heel smooth; (11) no fringes or webbing on toes, ungual flap on toes expanded, spadate, more evident on toes IV and V; (12) plantar tubercle indistinguishable, subarticular tubercles rounded and globular (one on toes I and II, two on toes III and V, and three on toe IV); foot without supernumerary tubercles; inner metatarsal tubercle elongated, outer metatarsal tubercles rounded and

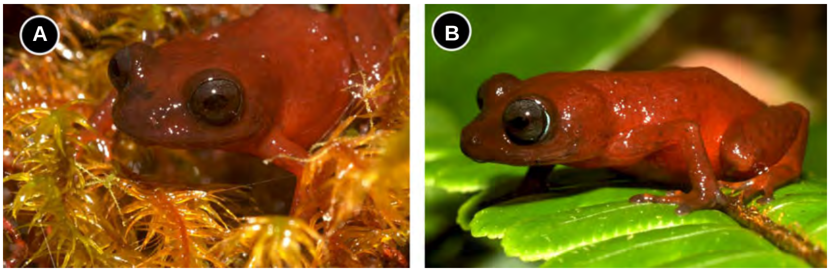

Figure 13. Holotype of *Diasporus sapo* sp. nov.: A, B, frontal and lateral views, respectively.

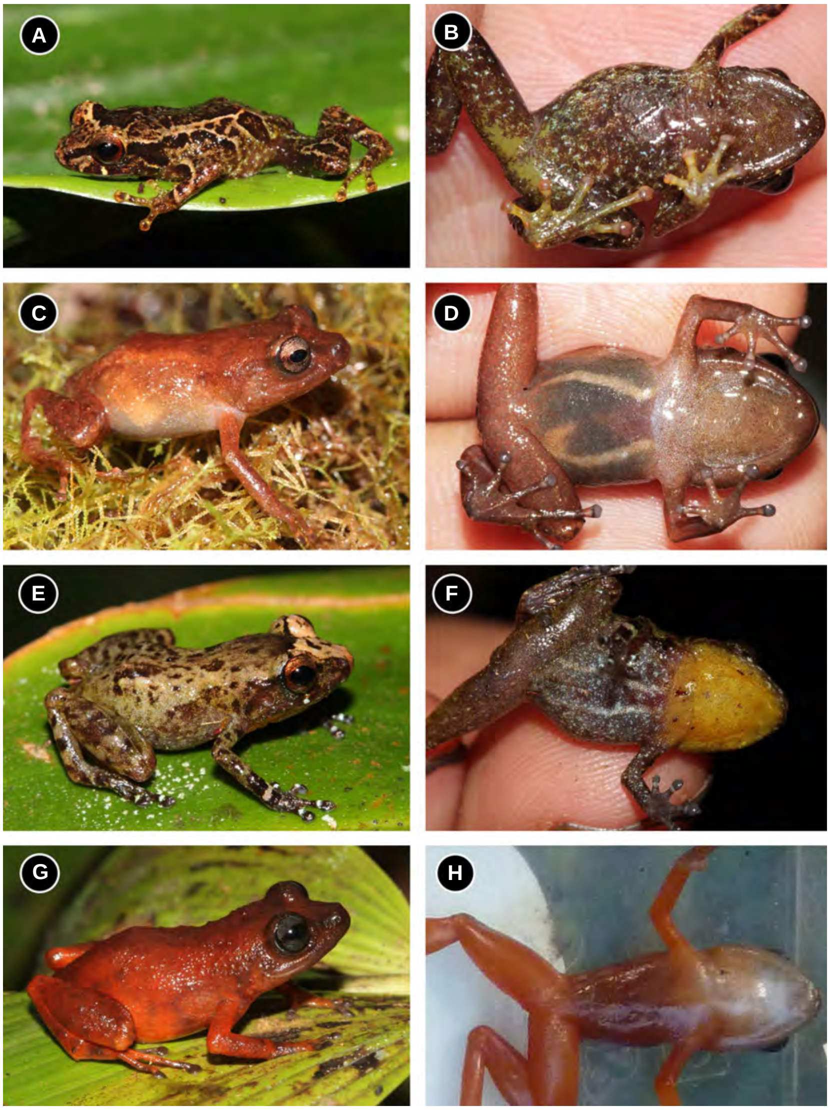

Figure 14. Color variation of the new *Diasporus* species: A, B, *Diasporus darienensis* sp. nov. (SMF 97305); C, D, *Diasporus majeensis* sp. nov. (SMF 97658); E, F, *Diasporus pequeno* sp. nov. (MHCH 2830); G, H, *Diasporus sapo* sp. nov. (G, not collected; H, MHCH 2854).

globular, smaller than inner; tarsal ridge absent; (13) dorsal ground color in life reddish and patternless, venter translucent, vocal sac not visible (Fig. 14H); (14) SVL 22.6 ± 2.86 (18.8–29.1, $N = 11$), males 22.6 ± 2.59 (19.9–29.1, $N = 9$), females 22.6 ± 5.3 (18.8–26.3, $N = 2$); (15) advertisement call unknown.

Description of the holotype

An adult female (SVL 28.91), with slender body; dorsal skin texture slightly tuberculate, venter skin smooth, discoidal fold not evident; protuberant eyes 1.78 times longer than snout; tympanum small, ratio TD/EL 23%; tympanum indistinguishable, annulus tympanicus and tympanic membrane absent, positioned 2.6 mm behind the orbit; head as wide as long (HL/HW 0.95), greatest head width between angles of jaw 35% of SVL; snout rounded from above and in profile; nares situated near tip of snout and slightly dorsolaterally directed, visible in frontal view, also visible dorsally but not ventrally; canthus rostralis rounded; loreal region concave; dentigerous processes of vomers with ten (right) and eight (left) teeth each side, straight in outline, from the centre of the orbit to the centre of the roof of mouth, and separated by a space of half of its total length; vocal slits absent; tongue long (26% of SVL) and broadening to the end, first third attached to floor of mouth; hands moderate in size, 22% of SVL; relative lengths of adpressed fingers

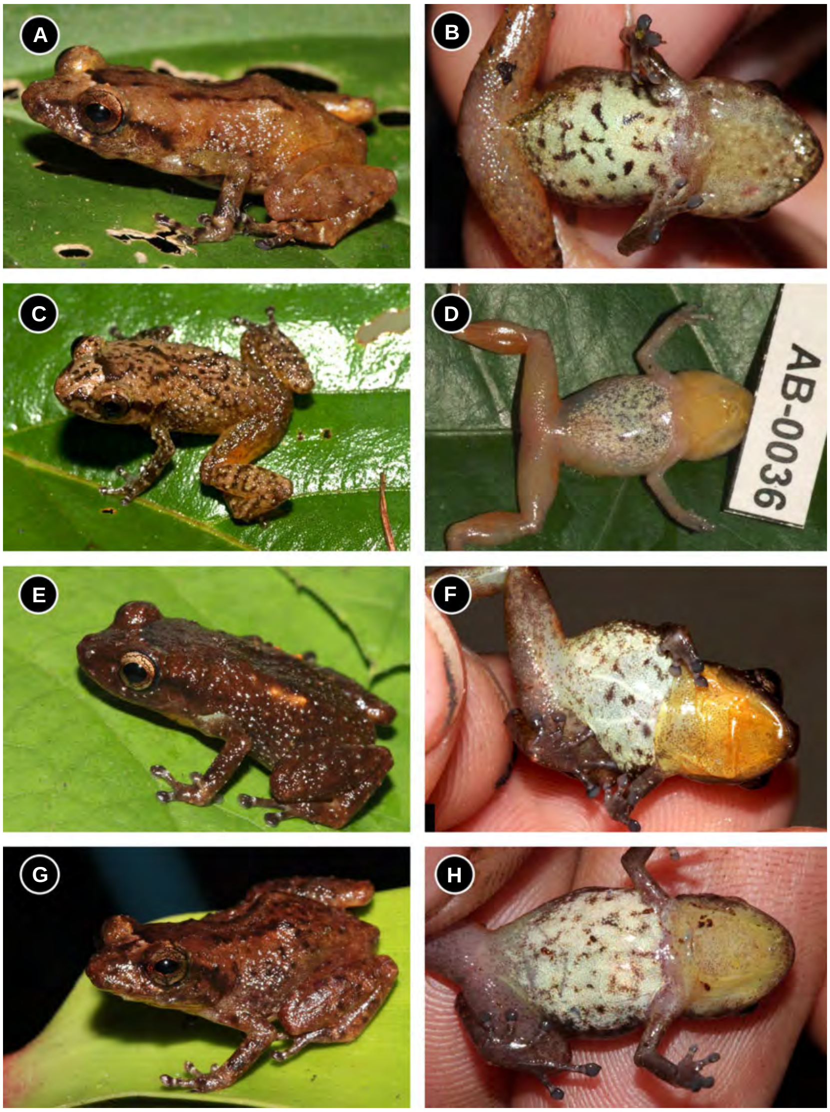

Figure 15. *Diasporus* aff. *diastema*, dorsal and ventral views: A, B, Majé mountain range (MM), near Ambroya (MHCH 2801); C, D, Eastern Panamanian lowlands (EPL), Río Mono, near Bayano (MHCH 2806); E, F, Gatún, Colón, near type locality (SMF 97287); G, H, Darién mountain range (DM), Bajo Pequeño, Río Tuquesa (SMF 97289).

I < II < IV < III; finger II smaller than finger VI, finger II reaching the middle of disc on finger IV when adpressed; finger III disc 2.07 times wider than distal end of adjacent phalanx; palmar tubercle ovoid to rounded, flattened, and almost the same size as thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular; no supernumerary tubercles; two palmar accessory tubercles small and rounded; no nuptial pads; no fringes on fingers; hindlimbs of moderate lengths, TL 43% of SVL; relative lengths of adpressed toes I < II < III < V < IV; when adpressed, tip of toe I reaches the disc base of toe II; disc of toe IV expanded, 2.11 times wider than distal end of adjacent phalanx; no fringes on toes; subarticular tubercles rounded and globular (one each on toes I and II, two on toes III and V, and three on toe IV); inner metatarsal tubercle elongated; outer metatarsal tubercles rounded, globular, and smaller than inner; tarsal ridge absent; hands and feet without webbing; finger and toe discs even broadened and slightly globular in profile (Fig. 7D); ungual flap on toes expanded, spadate, more evident on toes IV and V.

Coloration of holotype in life

Coloration recorded as follows (Fig. 13): iris medium neutral gray (298) with reticulations sepia (286), iris periphery jet black (300), eye periphery sky blue (192); dorsal ground color uniform Pratt's ruby (68), becoming darker to the front as dark carmine (61); venter and limbs chrome orange (74), throat pale buff (1).

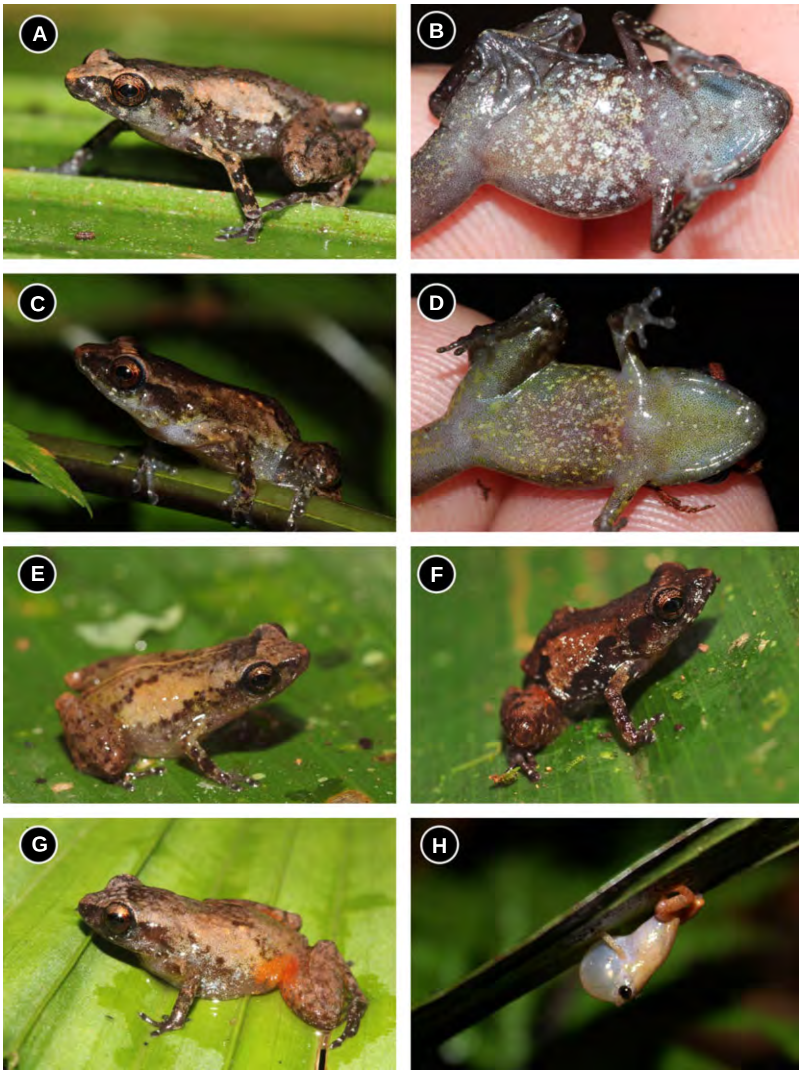

Figure 16. *Diasporus* aff. *quidditus*: A, B, Pirre mountain range (PM), Perresenico stream (MHCH 2824); C, D, Jingurudó-Sapo mountain range (JSM), near Pavarandó (SMF 97653); E, PM, Cana Field Station (MHCH 2813); F, PM, Pirre ridge (SMF 97292); G, San Blas mountain range (SBM), Taintidu river (SMF 97298); H, calling male under leaf, 20 cm from ground, SBM, Burbayar Private Reserve.

Coloration in preservative

Dorsal ground color cinnamon–drab (50), becoming darker to warm sepia (40) to the tip of snout; limbs and venter cream color (12), throat buff (5), hand and foot drab (19).

Measurements of holotype (mm)

SVL 28.91; HL 9.63; HW 10.19; IOD 2.93; EL 4.29; TD 0.98; FL 11.58; TL 12.33; HAL 6.37; 3FW 0.52; 3FD 1.8; 3TW 0.61; 3TD 1.12; 4TW 0.57; 4TD 1.20; BW 9.99 (for variation in the species, see Tables 4–6).

Natural history

This species is known only from the top of Cerro Sapo, which is covered by elfin forest. The vegetation predominantly consists of small trees (roughly 10 m in height) fully covered with moss and bromeliads. *Diasporus sapo* sp. nov. was most often found at 1–2 m above ground during the night; individuals were seen walking over tree branches and tree bark.

Etymology

The species name is derived from the name of Cerro Sapo, where the species was found.

DISCUSSION

The application of an integrative approach has resulted in the description of four new species within the genus. Additionally, the historical reconstruction

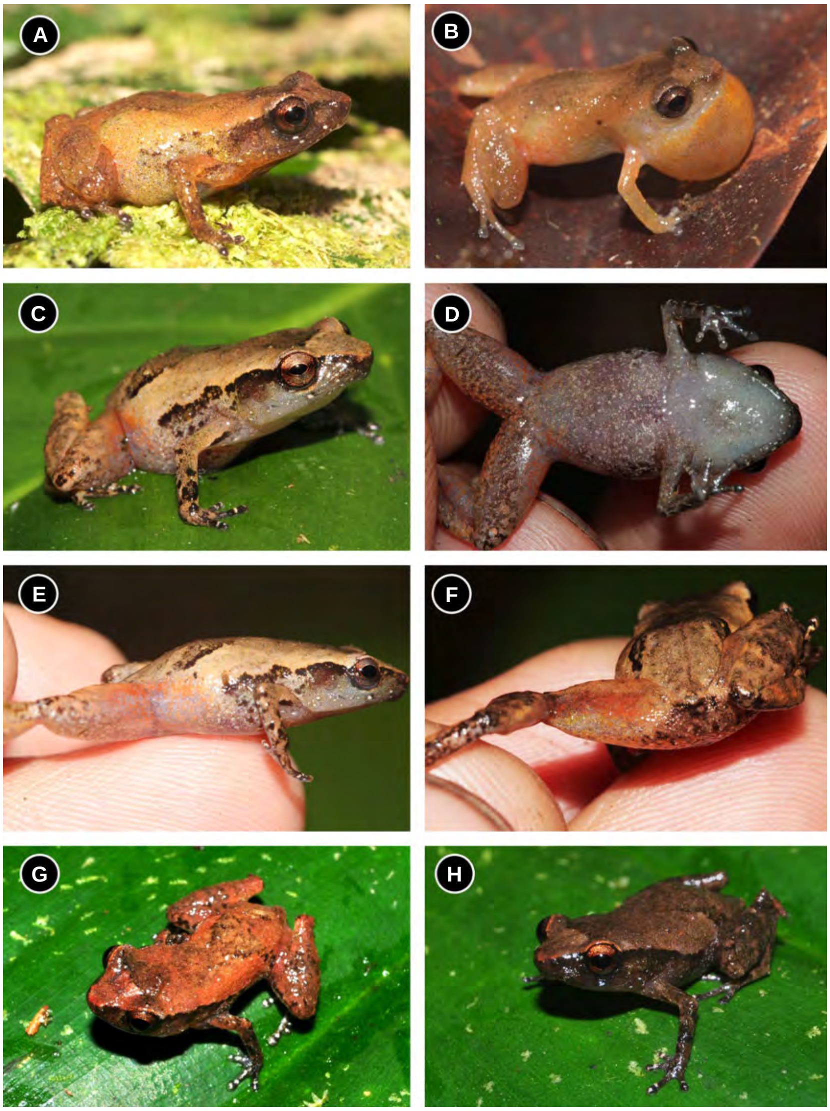

Figure 17. *Diasporus tinker*: A, B, Pirre mountain range (PM), Pirre ridge (MHCH 2864); C, F, PM, Pirre ridge (SMF 97324); G, H, Jingurudó-Sapo mountain range (JSM), showing metachrosis (same specimen); G, night coloration; H, day coloration.

of the biogeography for the *Diasporus* species confirms a Middle American origin of the genus, as revealed by previous studies (e.g. Pinto-Sánchez, Crawford & Wiens, 2014), as the older clade is represented by species distributed in western Panama (Fig. 2). Moreover, the 11 amphibian species endemic to EP (Ibáñez & Crawford, 2004; Crawford, Ryan & Jaramillo, 2010a; Crawford, Lips & Bermingham, 2010b; Batista *et al.*, 2014a; this paper) support the hypothesis of EP being a centre of endemism rather than just a pathway between two continents during the Great American Biotic Interchange (GABI; Crawford *et al.*, 2010a,b).

MORPHOLOGY AND ECOLOGY

Most diagnostic characters used to differentiate species within the genus *Diasporus* are morphological traits: usually SVL (body size), ungual flap (disc cover), and color pattern (Savage, 1997; Lynch, 2001; Chaves *et al.*, 2009; Batista *et al.*, 2012; Hertz *et al.*, 2012). Although SVL appears to be helpful in identifying species because of remarkable interspecific variation (Fig. 3), this character is valid only to differentiate between species with no overlap in SVL, or supported along with other morphological characters. In EP there are two groups of *Diasporus*, separated by the ungual flap shape (see results, Fig. 3). The lanceolate or papillate ungual flap present in *D. pequeno* sp. nov., *D. quidditus*, *D.* aff. *vocator*, and *D. tinker* seems to have evolved from one common ancestor. Although those species build a monophyletic clade with members of the *D. diastema* complex, the latter do not possess lanceolate or papillate ungual flaps. This possibly means that a lanceolate/papillate ungual flap was subsequently lost in *D. diastema* and *D. citrinobapheus*. The function of the ungual flap in these frogs is not known, but interestingly all species with lanceolate or papillate ungual flaps are inhabitants of the lower understory up to 1 m above ground (including the western Panamanian *D. vocator*), and only populations of *D. quidditus* from Colombia are usually found above this height. There are other rain frogs or Terrarana (Hedges *et al.*, 2008; former genus *Eleutherodactylus*) with lanceolate or papillate ungual flaps [*Craugastor gollmeri* group, *Pristimantis chalceus* (Peters, 1873), *Pristimantis scolodiscus* (Lynch & Burrowes, 1990), *Noblella* spp.] that are also inhabitants of the forest floor or the low understory (Savage, 1987; Lynch & Duellman, 1997; Duellman & Lehr, 2009). In contrast, lanceolate or papillate ungual flaps are usually not present in other Terrarana members with more arboreal habits (e.g. *Pristimantis* spp.; Savage, 2002; Duellman & Lehr, 2009). So we suggest that the expression of this character is related to the understory habit of these frogs.

Diasporus diastema has more palmate than spadate disc covers, as stated by Savage (1997, 2002), Lynch (2001), and Köhler (2011); however, we found specimens at the mouth of Chagres River, Colón, Panama (near the type locality) and in EP with spadate rather than palmate finger disc covers. We presume that the authors mentioned above did not examine *D. diastema* specimens from central Panama, but only examined specimens from Costa Rica that are likely to represent at least one different species (Savage, 1997, 2002: plate 127; Lynch, 2001: fig. 2D KU 35149; Köhler, 2011: figs 437, 39). Moreover, the disc cover shape can be highly variable as a result of different preservation techniques (Lynch, 2001). Such high variation in disc shape has been described within and between populations of *Pristimantis caryophyllaceus* (Barbour, 1928) (Batista *et al.*, 2014b), among other species of Terrarana, leaving this trait as a controversial tool to identify species in preserved specimens.

Most species of *Diasporus* are inhabitants of the understory of tropical forests. Nevertheless, there are differences in the spatial distribution of species: whereas *D. pequeno* sp. nov., *D. quidditus* (Fig. 16), and *D. tinker* (Fig. 17) occupy the zone from near ground level up to 1 m above the forest floor, *D. diastema*, *D. majeensis* sp. nov., and *D. sapo* sp. nov. are usually found higher, 1.0–2.5 m above the ground, and *D. darienensis* sp. nov. has been found even higher, from 1 m up to 5 m above ground. There is also controversy about the smallest species, *D. quidditus*, which was originally described from Colombia, with specimens from Panama referred to this species (Lynch, 2001). Although the populations from Panama and Colombia are almost similar in appearance (size and color), they vary substantially in ecology. Specimens from Colombia are reported to inhabit the mid-level of the forest, with males calling from the undersides of leaves at 1.5–4.0 m above ground (Lynch, 2001). After more than 3 years of field experience with this species in EP and observing more than a hundred calling males, we never encountered *D. quidditus* (Fig. 16) calling from a position higher than 0.5 m above the ground. Unfortunately, we have neither acoustic nor molecular data of topotypic specimens of *D. quidditus* to compare with our specimens. Therefore, the taxonomic relevance of these geographical behavioural variations remains unclear. Thus, we consider the populations from EP as *D.* aff. *quidditus* until more detailed molecular and bioacoustics comparison between the populations from EP and Colombia become available.

BIOACOUSTIC

To the human ear, the calls of *Diasporus* species are very similar to each other. A typical advertisement call was described as a simple 'tink tink' or 'dink dink' (Savage, 1997, 2002; Lynch, 2001). With such a simple subjective call description it was difficult to distinguish among the calls of different species; however, in recent years detailed descriptions of advertisement calls for 12 of the 15 described species have been published (Chaves *et al.*, 2009; Batista *et al.*, 2012; Hertz *et al.*, 2012; Jiménez *et al.*, 2013; this paper). It has been shown that comparison of acoustic parameters is a powerful tool for species identification in the genus *Diasporus*. To date, only call descriptions of *D. gularis*, *D. sapo* sp. nov., and *D. tigrillo* are pending.

Spectral and temporal parameters of the calls have been used successfully to evaluate the taxonomic status in Terrarana frogs. Even with a limited sample size these parameters show differences between *Diasporus* spp. (Padial *et al.*, 2008; Figs 4 and 5). The discriminant function analysis properly sorted species when we compared dominant frequency (DF) against note duration, but not if we used DF against call rate (Figs 4 and 5). Some species, such as *D. citrinobapheus*, *D. diastema* (at least for Panamanian populations of *D.* aff. *diastema*), *D. tinker*, and

D. vocator have calls that are organized in bouts. In species with calls organized in bouts, the note interval decreases from the beginning towards the end of the call group. Thus, the high variation of DF versus call rate in those species is a result of this modulation of the note interval. We recommend that more call recordings are made in future studies (with at least ten individuals and ten calls/individual per population) to evaluate the effect of the note interval modulation on the call rate. Four species of *Diasporus* are known to call in bouts, but we did not evaluate whether this is a phylogenetic character of related species. Usually, temporal parameters can be related to evolutionary constraints or to environmental selection (Ryan, Cocroft & Wilczynski, 1990; Bosch & De la Riva, 2004); however, calling in bouts is often used to facilitate note alternation between conspecific neighbours (Schwartz, 1991), as well as to restore energetic deficits during a calling session (Leary *et al.*, 2004). It is known that orthopterans that call in bouts can affect female preferences (Hendrick, 1986), but in *Diasporus* spp. the reason for this calling pattern remains unknown.

The call of *D. diastema* from central Panama was described by Fouquette (1960) and Wilczynski & Brenowitz (1988), but their temporal and spectral measurements are highly variable, which might be an artifact of involving several species in the recording (Hertz *et al.*, 2012). We have analysed a sample of *D. diastema* from a site near the type locality (Figs 6C and 16E, F), and its DF ranged between 3.34 and 3.47 kHz, which agrees well with data from Wilczynski & Brenowitz (1988). Nevertheless, some incongruence has been detected in the note duration (Hertz *et al.*, 2012). According to our experiences, measuring temporal parameters can sometimes lead to erroneous results. This is especially true when trying to determine the end point of the call on the oscillogram, or if the recording includes considerable background noise. The *D. diastema* specimen (SMF 97287; Fig. 15E, F) that we found at the mouth of the Chagres River was the only *Diasporus* specimen found within a radius of 2 km from this site, which is approximately 9 km from the type locality near Margarita, Colón, Panama (Dunn, 1942; Taylor, 1955; Savage, 1973; Hertz *et al.*, 2012; Fig. 16E, F). Therefore, we assume that this specimen belongs to *D. diastema* as originally described by Cope (1876). Nevertheless, it is difficult to test for conspecificity by comparing the morphology of our specimen with that of the holotype of *D. diastema*, as it is in poor condition (Cochran & Goin, 1970; A. Hertz, pers. observ.); however, it should be noted that the specimen from the mouth of Chagres River and the holotype of *D. diastema* are at least of almost similar size (*D. diastema* holotype, SVL 20.0 mm; *Diasporus* sp. from Chagres River, SVL 19.0 mm). In this area the only other congeneric frog is *D.* aff. *vocator* (see also Ibáñez *et al.*, 1999), which is significantly smaller than *D. diastema*, (Fig. 3; Table 4), and calls at a higher DF.

BARCODING AND PHYLOGENETIC INFERENCE

DNA barcoding is a useful tool for species identification (Hebert *et al.*, 2004; Crawford *et al.*, 2010b; Jörger *et al.*, 2012; Paz & Crawford, 2012); however, the straightforward application of this approach could yield misleading interpretations of biodiversity (see Trewick, 2008; Huang *et al.*, 2013; Shen, Chen & Murphy, 2013). DNA sequence information in the absence of other lines of evidence should never be used for species delimitations (DeSalle, 2006). Here we are using molecular barcoding along with other methods to reveal unnamed species within the genus *Diasporus* from EP. We found high genetic divergence between lineages above the suggested threshold to identify candidate species in Neotropical amphibians (>3.0% in *16S* and >10% in *COI*; Vences *et al.*, 2005; Fouquet *et al.*, 2007; Crawford *et al.*, 2013), and most of them were supported by the barcoding analysis (ABGD; Puillandre *et al.*, 2011). Differences in the barcoding genes were additionally supported by bioacoustics, ecology, morphology, and phylogeography. According to the integrative analysis, most lineages identified as species showed considerable genetic distances and are monophyletic in the reconstructed tree; however, the polyphyly in members of the *D. diastema* complex is problematic (Fig. 8). Whereas *D. citrinobapheus* is monophyletic, although consisting of two subclades, and is well differentiated from *D. diastema*, *D. diastema* itself is paraphyletic. We included two specimens from central Panama, of which SMF 97287 is most probably a 'true' *D. distema*, whereas SMF 97290 could represents another genetic lineage. We were not able to clearly distinguish between separate lineages of *D.* aff. *diastema* from MM or EPL, nor raise any of them to species level (Tables 4–6). Despite the fact that they showed a genetic distance above the threshold used to recognize candidate species within this group (e.g. >4.0% genetic p–distance in mtDNA *16S*; Table 1), we prefer treating these populations as unconfirmed candidate species (Vieites *et al.*, 2009), and label all specimens other than SMF 97287 as *Diasporus* aff. *diastema* until more comparative data from more widespread populations become available.

PHYLOGEOGRAPHY

The results of our chronological tree indicate that species from EP are younger than those from WP

(Fig. 9). Thus, the ancestors of the genus *Diasporus* have originated somewhere in Lower Central America (see also Pinto-Sánchez *et al.*, 2014). Recent hypotheses on the formation of the land bridge between South America and North America suggest that the Isthmus of Panama was connected with the north-western landmass of South America via an island arc during the mid-Miocene around 15 Mya (Montes *et al.*, 2012a,b). *Diasporus* species from WP and EP split around this time (15 Mya), promoting the subsequent evolution into numerous *Diasporus* spp. within EP. Later on, another vicariant event took place *in situ*, probably induced by eustatic fluctuations during the middle and late Miocene (as early as 11 Mya), such as the flooding of the Atrato and Chucunaque basins (Duque-Caro, 1990; Coates *et al.*, 2004). This consequently separated populations, including the predecessors of the three closely related species *D. darienensis* sp. nov., *D. majeensis* sp. nov., and *D. sapo* sp. nov. that became isolated on separate land masses of EP during this period (5–8 Mya), and evolved allopatrically into distinct species. Around the same time (5–6 Mya), *D.* aff. *diastema* from MM split from the rest of the *D.* aff. *diastema* complex and remained isolated within the foothills of MM, evolving only minor morphological changes.

A hypothetical route of colonization and speciation for *Diasporus* frogs in EP is as follows: *Diasporus* ingresses into EP through the San Blas peninsula when it started to uplift (around 20 Ma, Montes *et al.*, 2012a). The Chucunaque and Atrato basins isolated the islands of Maje-Baudo in the south and Dabeiba (e.g. the San Blas mountain range) in the north. *Diasporus* populations colonized those islands either by over sea dispersal (debris rafting) and/or via a temporary land connection. The clade of *D. dariensis*, *D. sapo* sp. nov., and *D. majeensis* sp. nov. evolved on the southern island, whereas the remainder species (*D.* aff. *diastema*, *D. pequeno* sp. nov., *D.* aff. *quidditus*, and *D. tinker*) evolved on the northern island. *Diasporus pequeno* sp. nov. is the oldest lineage of the latter clade, whereas the other species from the southern island dispersed more recently when continuing tectonic events and sedimentation allowed occasional migrations over more shallow and narrow water bodies. Ancestors of *D. tinker* expanded east and crossed the Atrato corridor. The ancestors of *D.* aff. *quidditus* evolved during an extended period on the northern island, and began dispersing into South America when the Isthmus of Panama was nearly completed. The *D.* aff. *diastema* clade including *D. citronobapheus* expanded in both western and eastern directions.

CONCLUSION

Many species within the genus *Diasporus* in EP have been difficult to differentiate: as they were based solely on external appearance, a new approach became necessary. Based on a comprehensive analysis of *Diasporus* samples from EP, we bring new insights into bioacoustics, ecology, molecular diversification, and morphology, and reconstruct the phylogeography of the genus in this region. All new species described herein were well supported by the integration of these approaches. Although this study substantially raises the number of known species of the genus, the diversity of *Diasporus* spp. still promises to grow in the future. During the last 4 years seven species have been described (including those described here); therefore, we can expect that integrative taxonomical approaches on the genus in western Panama, Costa Rica, and Colombia may further raise the number of species.

KEY TO THE SPECIES OF THE GENUS *DIASPORUS*

1a. Ungual flap lanceolate or papillate at least on the third finger or third and fourth toe. 2
1b. Fingers and toes with rounded or spadate ungual flap. 6
2a. Very small frogs; SVL usually <17 mm. 3
2b. Small frogs; SVL usually >17 mm 4
3a. Dorsum shagreen; fingers without thick lateral fringes; toe V not partially fused with toe IV; SVL of adult males 14.0–16.0 mm, adult females 16.5–18.0 mm; calls with DF of 4.35–5.10 kHz . . . . *Diasporus vocator*
3b. Dorsum with scattered low warts; fingers with thick lateral fringes; toe V partially fused with toe IV; SVL of adult males 10.9–14.8 mm, adult females 13.2–16.9 mm; calls with DF of 4.55–5.08 kHz . . . *Diasporus* aff. *quidditus* (populations from EP)
4a. Disk expanded with cuspidate pads, skin smooth aside from low flattened warts, no perianal warts; vocal sac pale brown or orange in males; calls with DF of 3.14–3.71 kHz . . . . *Diasporus tinker*
4b. Disk expanded with rounded pads, skin texture smooth, with small scattered tubercles, perianal warts may or may not be present, vocal sac bright yellow in males 5
5a. Finger III with a small papillate ungual flap; dorsal pattern brownish with dark blotches; ventral areas translucent with dark speckles and small sky-blue blotches; males with bright

yellow vocal sac; calls with DF of 3.44–3.48 kHz . . . . *Diasporus pequeno* sp. nov.

5b. Fingers without papillate ungual flap; dorsal color yellowish tan, with brown markings; ventral surfaces white . . . . *Diasporus gularis*

6a. Reddish color pattern on dorsum, venter translucent or with distinct black and white blotches 7

6b. Dorsal color pattern variable, pale, dark brown, or yellowish, venter white or cream in color, with dark blotches or suffused (or speckled) with dark color 10

7a. Venter translucent. 8

7b. Venter usually with distinct black and white blotches, males have white venters with red spots and females have white venters with black spots; calls with DF of 2.50–2.61 kHz. . . . . *Diasporus ventrimaculatus*

8a. Outer edge of the tibia and forearm smooth, without a series of tubercles. 9

8b. Outer edge of the tibia and forearm covered with a series of tubercles; calls with DF of 2.4 kHz . . . . *Diasporus igneus*

9a. Dorsal color uniform red; eye periphery sky blue; species restricted to the Sapo-Jingurudó mountain range . . . . *Diasporus sapo* sp. nov.

9b. Dorsal color reddish with brown or pale reticulations; eye periphery black; species restricted to the Majé mountain range; calls with DF of 2.47–2.71 kHz . . . . *Diasporus majeensis* sp. nov.

10a. Dorsal pattern yellowish, usually suffused with pink or red; venter translucent without blotches, or with speckled pattern 11

10b. Dorsal pattern dark or pale brown, venter cream with dark spots or dark with white flecks 13

11a. Dorsum uniformly bright yellow to orange, color of posterior surface of thigh same color as dorsum; adults with vomerine teeth 12

11b. Posterior surface of thigh often suffused with pink or red in life; adults without vomerine teeth; calls with DF of 2.35–3.05 kHz. . . . *Diasporus hylaeformis*

12a. Dorsum smooth, uniformly bright yellow to orange, sometimes with irregularly distributed dark blotches; distal subarticular tubercle on finger I and toe I flat and rounded; SVL of adult males 17.3–19.7 mm; calls with DF of 2.86–3.04 kHz . . . . . *Diasporus citrinobapheus*

12b. Dorsum with scattered low pustules, dorsum yellow to orange with dark-brown spots confined to pustules; distal subarticular tubercle on finger I and toe I weakly bifid; SVL of adult males 16.0–17.5 mm. . . . . . . *Diasporus tigrillo*

13a. Dorsal pattern pale brown or reddish; venter cream with dark spots or suffused with reddish color; axilla and groin cream in color or same color as dorsum 14

13b. Dorsal pattern black with short red lines; axilla and groin scarlet; ventral surfaces black with white flecks; calls with DF of 3.81 kHz . . . .*Diasporus anthrax*

14a. Dorsal pattern pale brown with dark spots; venter cream with dark spots; axilla and groin cream in color; calls with DF of 2.96–3.55 kHz.. . . . .*Diasporus* aff. *diastema* (populations from CP and EP)

14b. Dorsal pattern reddish with pale lines or blotches; venter suffused with reddish color; axilla and groin unpigmented or same color as dorsum; calls with DF of 3.34–3.81 kHz . . . . . *Diasporus darienensis* sp. nov.

ACKNOWLEDGEMENTS

Collection permits in 2009 (SC/A-8-09, SC/A-28-09) and 2011 (SC/A-37-11), 2012 (SC/A-33-12), and exportation permits in 2012 (SC/A-33-12) and 2013 (SEX/A-7-13), were provided by ANAM, Panama, and T. Quintana (Cacique General del área de Sambú) from the ‘despacho del cacique Regional’ Comarca Emberá-Wounaan, Panama. Special thanks go to the indigenous people of Embera from Puerto Indio and Pavarandó, especially to D. Berrugate (Secretary of the Emberá-Wounaan congress, Sambú); and to L. Caibera (Noko of Pavarando village) and his family, who allowed us to enter their autonomous territory and kindly supported our work logistically. We are very grateful to Don Faustino, Hermelinda, and family, who gave us shelter on their nice sustainable farm at la Moneda’s village during our travels to Darien. We thank Yorlis Cáceres, Daniel Cáceres, Isaac Pizarro, Gustavo Dogirama, Mario Cuñapa, Anselmo Caicedo, Hugo Martínez, Elacio Méndez, and Gilberto Torres for assistance in the field. We thank Adrían García, Javier Sunyer, Don Filipiak, and one anonymous reviewer for insightful comments on an earlier draft of the article. This work was supported financially by the Secretaría de Ciencia y Tecnología (SENACYT), Instituto para la Formación y Aprovechamiento de los Recursos Humanos (IFARHU), Panama, and MWH, Panama, and Milan Veselý by institutional support of Palacky University.

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APPENDICES

Appendix 1. Details of the museum voucher numbers (when available) and collecting locality for all *Diasporus* samples used in this study.

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
SMF 89819	*D.* aff. *citrinobapheus*	Alto de Piedra, Santa fé National Park	Panama	8.51449	81.1171	878
MHCH 2373	*D.* aff. *citrinobapheus*	Alto de Piedra, Santa fé National Park	Panama	8.51449	81.1171	878
LSt 018	*D.* aff. *citrinobapheus*	Cerro Mariposa, Santa fé National Park	Panama	8.51545	81.119	930
LSt 085	*D.* aff. *diastema*	Cerro Mariposa, Santa fé National Park	Panama	8.50128	81.11868	1215
LSt 120	*D.* aff. *diastema*	Cerro Mariposa, Santa fé National Park	Panama	8.52556	81.13168	652
ICN 41696 (holotype)	*D. anthrax*	Campamento la Miel II, near junction of quebrada Tasajos with Río la Miel, km 23 carretera la Victoria-Samaná; Caldas, Colombia	Colombia			700
ICN 41697 (paratype)	*D. anthrax*	Bosque de San Rafaél, Municipio San Rafael, Antioquia, Colombia	Colombia			1200
MHCH 2840	*D. darienensis* sp. nov.	Pirre Mountain top (1400 m a.s.l.) to camp 2; Rancho Frío Field station, Pirre mountain range	Panama	7.94739	77.7042	1317
MHCH 2841	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98792	77.70774	1127
MHCH 2844	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98792	77.70774	1127
MHCH 2845	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98771	77.70783	1149
MHCH 2846	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.97976	77.70843	1133
MHCH 2847	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98	77.70839	1139
MHCH 2850	*D. darienensis* sp. nov.	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68289	78.03846	959
MHCH 2851	*D. darienensis* sp. nov.	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.67942	78.03868	946
MHCH 2852	*D. darienensis* sp. nov.	Bailarín mount, Jingurudó mountain range	Panama	7.69312	78.04226	865
MHCH 2862	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98724	77.70785	1140
SMF 97303	*D. darienensis* sp. nov.	Pirre Mountain top (1400 m a.s.l.) to camp 2; Rancho Frío Field station, Pirre mountain range	Panama	7.94739	77.7042	1317
SMF 97304	*D. darienensis* sp. nov.	Camp 2 (ridge 1300 m a.s.l.); Rancho Frío Field station, Pirre mountain range	Panama	7.9632	77.70432	1267
SMF 97305	*D. darienensis* sp. nov.	From mirador 2 to Perresenico Stream to Camp 2 (ridge 1300 m a.s.l.); Rancho Frío Field Station	Panama	7.97312	77.70785	1143
SMF 97306	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98887	77.70739	1100
SMF 97307	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.97864	77.70851	1112

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
SMF 97308	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.97935	77.70872	1085
SMF 97309	*D. darienensis* sp. nov.	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
SMF 97310	*D. darienensis* sp. nov.	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
SMF 97312	*D. darienensis* sp. nov.	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68357	78.03848	948
SMF 97313	*D. darienensis* sp. nov.	Sapo Mountain	Panama	7.97632	78.36269	1152
SMF 97314	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98741	77.70787	1137
SMF 97661	*D. darienensis* sp. nov.	Camp 2 (ridge 1300 m a.s.l.); Rancho Frío Field station, Pirre mountain range	Panama	7.9632	77.70432	1267
SMF 97662	*D. darienensis* sp. nov.	Pirre Mountain top (1400 m a.s.l.) to camp 2; Rancho Frío Field Station, Pirre mountain range	Panama	7.94719	77.7042	1326
MHCH 2801	*D. diastema*	Amborlla, La Javillosa ridge	Panama	8.91587	78.62897	906
MHCH 2802	*D. diastema*	Taintidu River, Chucunaque River	Panama	9.03547	78.02637	289
MHCH 2803	*D. diastema*	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
MHCH 2804	*D. diastema*	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
MHCH 2805	*D. diastema*	Río Mono, 5.7 km SE from Bayano Bridge	Panama	9.1772	78.74551	107
MHCH 2806	*D. diastema*	Río Mono, 5.7 km SE from Bayano Bridge	Panama	9.17816	78.74582	100
MHCH 2807	*D. diastema*	Maje mountain range, Amborlla	Panama	8.89224	78.56029	943
MHCH 2808	*D. diastema*	Maje mountain range, Amborlla	Panama	8.89182	78.56268	788
MHCH 2809	*D. diastema*	Maje mountain range, Amborlla	Panama	8.89427	78.56509	622
MHCH 2810	*D. diastema*	Maje mountain range, Amborlla	Panama	8.8972	78.56762	610
MHCH 2811	*D. diastema*	La Moneda, Meteti, Darién	Panama	8.5974	78.04934	69
SMF 97286	*D. diastema*	Maje mountain range, Amborlla	Panama	8.91681	78.61779	485
SMF 97287	*D. diastema*	400 m W from the Gatún Locks, Colón	Panama	9.26021	79.9354	36
SMF 97288	*D. diastema*	Maje mountain range, Amborlla	Panama	8.92267	78.6253	852
SMF 97289	*D. diastema*	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
SMF 97290	*D. diastema*	Río Terable, Chepo	Panama	9.28399	78.98383	322
MHCH 1440	*D. diastema*	Donoso, Colón	Panama			
MHCH 1469	*D. diastema*	Donoso, Colón	Panama			
SMF 80781	*D. diastema*		Panama			
SMF 81961	*D. diastema*		Panama			
SMF 79796	*D. diastema*		Panama			
SMF 79797	*D. diastema*		Panama			
SMF 83391	*D. diastema*		Panama			
SMF 85135	*D. diastema*		Panama			
SMF 78965	*D. diastema*		Panama			
SMF 82033	*D. diastema*		Panama			

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
SMF 82032	*D. diastema*		Panama			
SMF 82035	*D. diastema*		Panama			
SMF 81812	*D. diastema*		Panama			
SMF 78187	*D. diastema*		Panama			
SMF 78188	*D. diastema*		Panama			
SMF 78189	*D. diastema*		Panama			
SMF 78190	*D. diastema*		Panama			
SMF 78186	*D. diastema*		Panama			
SMF 78191	*D. diastema*		Panama			
LSt 018	*D. diastema*		Panama			
LSt 085	*D. diastema*		Panama			
MHCH 1360	*D. diastema*	Bocas del Toro Island, Bocas del Toro	Panama			
MHCH 1379	*D. diastema*	Donoso, Colón	Panama			
MHCH 1427	*D. diastema*	Donoso, Colón	Panama			
SMF 85938	*D. diastema*		Panama			
SMF 79794	*D. diastema*		Panama			
SMF 79800	*D. diastema*		Panama			
SMF 79799	*D. diastema*		Panama			
SMF 83390	*D. diastema*		Panama			
SMF 83389	*D. diastema*		Panama			
SMF 85068	*D. diastema*		Panama			
SMF 84997	*D. diastema*		Panama			
SMF 80977	*D. diastema*		Panama			
SMF 80978	*D. diastema*		Panama			
SMF 80979	*D. diastema*		Panama			
SMF 82034	*D. diastema*		Panama			
SMF 82031	*D. diastema*		Panama			
SMF 29859	*D. diastema*		Panama			
SMF 29874	*D. diastema*		Panama			
SMF 81811	*D. diastema*		Panama			
SMF 78561	*D. diastema*		Panama			
SMF 78185	*D. diastema*		Panama			
SMF 78184	*D. diastema*		Panama			
SMF 77231	*D. diastema*		Panama			
LSt 123	*D. diastema*		Panama			
SMF 85939	*D. diastema*		Panama			
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
ICN 19306	*D. gularis*	Quebrada La Miquera, Vereda Venados, Parque Natural Nacional Las Orquideas, Antioquia	Colombia	6.53802	76.30345	1060
ICN 53771	*D. gularis*		Colombia			
ICN 45169	*D. gularis*	Centro Forestal Bajo Calima, Valle del Cauca	Colombia	3.98333	76.95	50
AH 242	*D. hylaeformis*	Bajo Mono, Boquete	Panama	8.82515	82.50204	1820
AH 244	*D. hylaeformis*	Bajo Mono, Boquete	Panama	8.82515	82.50204	1872
AH 245	*D. hylaeformis*	Bajo Mono, Boquete	Panama	8.82515	82.50204	1780
HAU 012	*D. hylaeformis*	Bajo Mono, Boquete	Panama	8.82511	82.49813	1778
HAU 013	*D. hylaeformis*	Bajo Mono, Boquete	Panama	8.82595	82.49904	1800
HAU 018	*D. hylaeformis*	Cerro Guayaba, Comarca Ngöbe buglé	Panama	8.75797	82.2572	1358
AH 486	*D. hylaeformis*	Cerro Sagui, Comarca Ngöbe buglé	Panama	8.5639	81.8221	2033
AH 175	*D. hylaeformis*	Reserva Forestal Fortuna	Panama	8.67685	82.19606	1750
AH 178	*D. hylaeformis*	Reserva Forestal Fortuna	Panama	8.67685	82.19606	1750
AH 176	*D. hylaeformis*	Reserva Forestal Fortuna	Panama	8.67685	82.19606	1750
AH 177	*D. hylaeformis*	Reserva Forestal Fortuna	Panama	8.67685	82.19606	1750
AH 115	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.6775	82.198	1760
AH 116	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.6775	82.198	1760
AH 117	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.6775	82.198	1760

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
AH 118	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.6775	82.198	1760
AH 380	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.67857	82.19329	1793
HAU 007	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.67885	82.20037	1810
HAU 011	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.67885	82.20037	1750
AH 381	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.67857	82.19329	1793
SMF 89872	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.91138	82.71288	2068
SMF 89867	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.91844	82.72325	2332
	D. hylaeformis	Jurutungo, Río Sereno, Renacimientos	Panama	8.91844	82.72325	2332
	D. hylaeformis	Jurutungo, Río Sereno, Renacimientos	Panama	8.91844	82.72325	2332
	D. hylaeformis	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
SMF 89874	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
SMF 89873	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
MHCH	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
SMF 89868	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
SMF 89869	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
SMF 89875	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.91152	82.71253	2070
SMF 89876	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.91152	82.71253	2070
AH 042	*D. hylaeformis*	La Nevera, Comarca Ngöbe buglé	Panama	8.49966	81.77238	1700
AH 343	*D. hylaeformis*	La Nevera/Cerro Santiago Westhang	Panama	8.49546	81.76718	1815
SMF 89871	*D. hylaeformis*	Las Nubes, Cerro Punta, Chiriquí	Panama	8.89418	82.6149	2117
SMF 89870	*D. hylaeformis*	Las Nubes, Cerro Punta, Chiriquí	Panama	8.89418	82.6149	2117
NH 0034	*D. hylaeformis*	Lost and Found, Reserva Forestal Fortuna	Panama	8.67445	82.2193	1283
AH 236	*D. hylaeformis*	Volcán Barú/Sendero Quezales	Panama	8.84944	82.51538	2134
MHCH 1327 (holotype)	*D. igneus*		Panama			
MHCH 1388 (paratype)	*D. igneus*		Panama			
MHCH 2072 (paratype)	*D. igneus*		Panama			
SMF 89821 (paratype)	*D. igneus*		Panama			
SMF 89821 (paratype)	*D. igneus*	La Nevera, Comarca Ngöbe buglé	Panama	8.49546	81.76718	1815
MHCH 2832	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
MHCH 2833	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
MHCH 2834	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
MHCH 2835	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
MHCH 2836	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
MHCH 2837	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
MHCH 2838	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
MHCH 2839	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
SMF 97293	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
SMF 97655	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
SMF 97656	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
SMF 97657	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
SMF 97658	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
SMF 97659	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
SMF 97660	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
MHCH 2826	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47997	77.51941	859
MHCH 2827	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47997	77.51941	859
MHCH 2828	*D. pequeno* sp. nov.	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
MHCH 2829	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
MHCH 2830	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
MHCH 2831	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
SMF 97333	*D. pequeno* sp. nov.	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
SMF 97334	*D. pequeno* sp. nov.	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
SMF 97335	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
SMF 97336	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
SMF 97337	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
SMF 97338	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
SMF 97663	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
MHCH 2813	*D. quidditus*	Rio Cana, Cana field station, Chimenea trail	Panama	7.75602	77.68565	525
MHCH 2814	*D. quidditus*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68338	78.03844	943
MHCH 2815	*D. quidditus*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.67978	78.03856	947
MHCH 2816	*D. quidditus*	Sapo Mountain	Panama	7.98013	78.35546	796
MHCH 2817	*D. quidditus*	Púcuro River, Darién	Panama	8.0575	77.37022	1043
MHCH 2818	*D. quidditus*	Púcuro River, Darién	Panama	8.0575	77.37022	1043
MHCH 2819	*D. quidditus*	Púcuro River, Darién	Panama	8.0575	77.37022	1043
MHCH 2820	*D. quidditus*	Taintidu River, Chucunaque River	Panama	9.03434	78.022	228
MHCH 2821	*D. quidditus*	Taintidu River, Chucunaque River	Panama	9.03547	78.02637	289
MHCH 2822	*D. quidditus*	From Taintidu River to the ridge, San Blas mountain range	Panama	9.04897	77.99753	433
MHCH 2823	*D. quidditus*	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
MHCH 2824	*D. quidditus*	Near Perresenico creek Rancho Frío Field station	Panama	7.99706	77.71084	558
SMF 97291	*D. quidditus*	Near Perresenico creek Rancho Frío Field station	Panama	7.99241	77.70941	871

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
SMF 97292	*D. quidditus*	Pirre mountain range	Panama	7.98728	77.70785	1135
SMF 97294	*D. quidditus*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.67962	78.03859	956
SMF 97295	*D. quidditus*	Camp 2 Pícuro River	Panama	8.04887	77.37004	787
SMF 97296	*D. quidditus*	Camp 4 Pícuro River	Panama	8.0575	77.37022	1043
SMF 97297	*D. quidditus*	Camp 4 Pícuro River	Panama	8.0575	77.37022	1043
SMF 97298	*D. quidditus*	Taintidu River, Chucunaque River	Panama	9.03547	78.02637	289
SMF 97299	*D. quidditus*	San Blas mountain range	Panama	9.05929	77.98421	553
SMF 97300	*D. quidditus*	Bajo pequeño, camp 2 Pechito parao Mount	Panama	8.47553	77.54884	472
SMF 97301	*D. quidditus*	Bajo pequeño, camp 2 Pechito parao Mount	Panama	8.47997	77.51941	859
SMF 97302	*D. quidditus*	Camp 2 (ridge 1300 m a.s.l.); Rancho Frío Field station	Panama	7.9632	77.70432	1267
SMF 97653	*D. quidditus*	Pavarandó, from camp 1 to stream; Garra Garra Mountain	Panama	7.75898	78.09228	643
SMF 97654	*D. quidditus*	Pavarandó, from Camp 1 to stream; Garra Garra Mountain	Panama	7.75898	78.09228	643
MHCH 2825	*D. quidditus*	Pirre mountain range	Panama	7.99207	77.70947	871
ICN 38150 (paratype)	*D. quidditus*	3 km NE de la cabecera municipal, via Cerro Macana, Bahía Solano, Chocó	Colombia			200
ICN 38151 (paratype)	*D. quidditus*	4 km. NE de la cabecera municipal, via Cerro Macana, Bahía Solano, Chocó	Colombia			200
ICN 38152 (paratype)	*D. quidditus*	5 km NE de la cabecera municipal, via Cerro Macana, Bahía Solano, Chocó	Colombia			200
ICN 45173 (holotype)	*D. quidditus*	Centro Forestal Bajo Calima, Buenaventura, Valle del Cauca	Colombia	3.98333	76.94999	50
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
MHCH 2853	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97616	78.36097	1063
MHCH 2854	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97614	78.36285	1148
MHCH 2855	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
MHCH 2856	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
MHCH 2857	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
MHCH 2858	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
SMF 97328	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
SMF 97329	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97606	78.36289	1158
SMF 97330	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97589	78.36254	1160

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
SMF 97331	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97594	78.36265	1158
SMF 97332	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
MHCH 2812	*D. tinker*	Pirre mountain range	Panama	7.98923	77.7074	1149
MHCH 2848	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68412	78.03866	962
MHCH 2849	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68412	78.03866	962
MHCH 2863	*D. tinker*	Pirre mountain range	Panama	7.96258	77.70401	1253
MHCH 2864	*D. tinker*	Pirre mountain range	Panama	7.96256	77.70393	1243
MHCH 2865	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69463	78.0426	818
MHCH 2866	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
MHCH 2867	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68423	78.03867	971
MHCH 2868	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68405	78.03865	969
MHCH 2869	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68393	78.0386	970
MHCH 2870	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68393	78.0386	970
MHCH 2871	*D. tinker*	Bailarin mount, Jingurudó mountain range	Panama	7.66911	78.03802	1133
MHCH 2872	*D. tinker*	Bailarin mount, Jingurudó mountain range	Panama	7.66911	78.03802	1133
MHCH 2873	*D. tinker*	Bailarin mount, Jingurudó mountain range	Panama	7.66911	78.03802	1133
SMF 97311	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68412	78.03866	962
SMF 97315	*D. tinker*	Pirre mountain range	Panama	7.96256	77.70393	1243
SMF 97316	*D. tinker*	Pirre mountain range	Panama	7.96256	77.70393	1243
SMF 97317	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69463	78.0426	818
SMF 97318	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
SMF 97319	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
SMF 97320	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
SMF 97321	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68405	78.03865	969

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
SMF 97322	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.67947	78.03861	955
SMF 97323	*D. tinker*	Bailarin mount, Jingurudó mountain range	Panama	7.66911	78.03802	1133
SMF 97324	*D. tinker*	Camp 2 (ridge 1300 m a.s.l.); Rancho Frío Field station, Pirre mountain range	Panama	7.9615	77.70426	1303
SMF 97325	*D. tinker*	Camp 2 (ridge 1300 m a.s.l.) to stream; Rancho Frío Field station, Pirre mountain range	Panama	7.95947	77.70365	1230
SMF 97326	*D. tinker*	Pirre top (1400) to camp 2; Rancho Frío Field station, Pirre mountain range	Panama	7.94719	77.7042	1326
SMF 97327	*D. tinker*	Bailarin mount, Jingurudó mountain range	Panama	7.66911	78.03802	1133
ICN 45174 (holotype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45175 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45176 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45177 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45178 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45179 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45181 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
UCR 20491	*D. ventrimaculatus*	Valle del Silencio at the east edge of the Cordillera de Talamanca 20 km west from the Costa Rica–Panama border	Costa Rica	9.1116	82.96172	2550
UCR 20492	*D. ventrimaculatus*	Valle del Silencio at the east edge of the Cordillera de Talamanca 20 km west from the Costa Rica–Panama border	Costa Rica	9.1116	82.96172	2550
UCR 20493	*D. ventrimaculatus*	Valle del Silencio at the east edge of the Cordillera de Talamanca 20 km west from the Costa Rica–Panama border	Costa Rica	9.1116	82.96172	2550
UCR 20504	*D. ventrimaculatus*	Valle del Silencio at the east edge of the Cordillera de Talamanca 20 km west from the Costa Rica–Panama border	Costa Rica	9.1116	82.96172	2550
MHCH 2874	*D. vocator*	Narices Mountain, Santa fé National Park	Panama	8.56315	81.05242	841
SMF 97339	*D. vocator*	San Lucas, Donoso, colón	Panama	8.98843	80.58243	149
SMF 89949	*D. vocator* cf.	Cerro Negro, Santa fé National Park	Panama	8.5691	81.09875	730

Appendix 1. *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
SMF 89950	*D. vocator* cf.	Cerro Negro, Santa fé National Park	Panama	8.5691	81.09875	730
AH 364	*D. vocator* cf.	San San Pond Sak, Bocas del Toro	Panama	9.50555	82.52417	5
SMF 89865	*D. vocator* cf.	San San Pond Sak, Bocas del Toro	Panama	9.50814	82.52843	5
SMF 89820	*D. citrinobapheus*	Cerro Negro, Santa fé National Park	Panama	8.5691	81.09875	730
SMF 89814 (holotype)	*D. citrinobapheus*	Paredón	Panama	8.48507	81.17273	788
MHCH 2370 (paratype)	*D. citrinobapheus*	Paredón	Panama	8.48507	81.17273	788
SMF 89816 (paratype)	*D. citrinobapheus*	Paredón	Panama	8.48507	81.17273	788
MHCH 2371 (paratype)	*D. citrinobapheus*	Paredón	Panama	8.48507	81.17273	788
MHCH 2372	*D. citrinobapheus*	Willi Mazu, Palo seco national park	Panama	8.79028	82.19893	681
SMF 89817	*D. citrinobapheus*	Willi Mazu, Palo seco national park	Panama	8.79028	82.19893	681

n.a. = voucher number not available.

Appendix 2. Voucher numbers and GenBank accession numbers for all *Diasporus* samples included in the phylogenetic analyses.

			GenBank accession no.				Coordinates	
Species	Museum no.	Field number	*16S*	*COI*	*RAG1*	Country	Latitude	Longitude
D. aff. *diastema*	MHCH 2801	AB 637	KT186624	KT186572	KT119471	Panama	8.91587	78.62896
D. aff. *diastema*	MHCH 2802	AB 675	KT186617	KT186563	KT119472	Panama	9.03547	78.02637
D. aff. *diastema*	MHCH 2805	AB 035		KT186555	KT119461	Panama	9.17720	78.74551
D. aff. *diastema*	MHCH 2807	AB 073		KT186580	KT119438	Panama	8.89224	78.56029
D. aff. *diastema*	MHCH 2808	AB 084	KT186627	KT186578	KT119439	Panama	8.89182	78.56268
D. aff. *diastema*	MHCH 2809	AB 086	KT186633	KT186588		Panama	8.89427	78.56509
D. aff. *diastema*	MHCH 2811	AB 218		KT186571	KT119459	Panama	8.59740	78.04934
D. aff. *quidditus*	MHCH 2824	AB 1130	KT186621	KT186569	KT119443	Panama	7.99706	77.71084
D. aff. *quidditus*	MHCH 2825	B 131		KT186560	KT119454	Panama	7.99207	77.70947
D. pequeno sp. nov.	MHCH 2828	AB 822		KT186556	KT119475	Panama	8.47553	77.54884
D. pequeno sp. nov.	MHCH 2830	AB 860		KT186559	KT119478	Panama	8.47911	77.52799
D. majeensis sp. nov.	MHCH 2839	AB 1065	KT186629		KT119442	Panama	8.80462	78.45951
D. darienensis sp. nov.	MHCH 2841	AB 1268	KT186618		KT119449	Panama	7.98771	77.70783
D. vocator	MHCH 2843	AB 1240			KT119448	Panama	8.86528	80.64383
D. darienensis sp. nov.	MHCH 2845	AB 151		KT186561	KT119456	Panama	7.98771	77.70783
D. darienensis sp. nov.	MHCH 2850	AB 329	KT186626	KT186576	KT119460	Panama	7.68289	78.03846
D. sapo sp. nov.	MHCH 2855	AB 430	KT186619	KT186568	KT119464	Panama	7.97618	78.36263
D. sapo sp. nov.	MHCH 2856	AB 431	KT186616		KT119465	Panama	7.97618	78.36263
D. sapo sp. nov.	MHCH 2858	AB 439	KT186628		KT119467	Panama	7.97618	78.36263
D. sp.	MHCH 2859	AB 032	KT186614	KT186558		Panama	8.71893	82.23161
D. darienensis sp. nov.	MHCH 2862	AB 159		KT186579	KT119458	Panama	7.98724	77.70785
D. tinker	MHCH 2871	AB 1270	KT186620		KT119451	Panama	7.66911	78.03802
D. tinker	MHCH 2872	AB 1271	KT186623		KT119452	Panama	7.66911	78.03802
D. tinker	MHCH 2873	AB 1272	KT186615		KT119453	Panama	7.66911	78.03802
D. vocator	MHCH 2874	AB 564	KT186622		KT119469	Panama	8.56315	81.05242
D. diastema	SMF 97287	AB 602		KT186566	KT119470	Panama	9.26021	79.93540
D. diastema	SMF 97289	AB 818		KT186586	KT119474	Panama	8.47553	77.54884
D. diastema	SMF 97290	AB 979		KT186577	KT119481	Panama	9.28399	78.98383
D. aff. *quidditus*	SMF 97291	AB 138	KT186613		KT119455	Panama	7.99241	77.70941
D. aff. *quidditus*	SMF 97292	AB 158	KT186634	KT186589	KT119457	Panama	7.98728	77.70785
D. majeensis sp. nov.	SMF 97293	AB 1030		KT186565	KT119440	Panama	8.79936	78.46156
D. aff. *quidditus*	SMF 97295	AB 499			KT119468	Panama	8.04887	77.37004
D. aff. *quidditus*	SMF 97298	AB 689	KT186625		KT119473	Panama	9.03547	78.02637
D. darienensis sp. nov.	SMF 97304	AB 1144		KT186581	KT119445	Panama	7.96320	77.70432
D. darienensis sp. nov.	SMF 97305	AB 1134		KT186582	KT119444	Panama	7.97312	77.70785
D. darienensis sp. nov.	SMF 97312	AB 323	KT186631	KT186585		Panama	7.68357	78.03848
D. darienensis sp. nov.	SMF 97313	AB 425			KT119462	Panama	7.97632	78.36269
D. tinker	SMF 97320	AB 308	KT186632	KT186587		Panama	7.69271	78.04200

Appendix 2. *Continued*

Species	Museum no.	Field number	GenBank accession no.			Country	Coordinates	
			16S	*COI*	*RAG1*		Latitude	Longitude
D. tinker	SMF 97326	AB 1184			KT119446	Panama	7.94718	77.70420
D. tinker	SMF 97327	AB 1269	KT186635		KT119450	Panama	7.66911	78.03802
D. sapo sp. nov.	SMF 97329	AB 429		KT186557	KT119463	Panama	7.97606	78.36289
D. sapo sp. nov.	SMF 97330	AB 435	KT186630	KT186584	KT119466	Panama	7.97589	78.36254
D. pequeno sp. nov.	SMF 97335	AB 856		KT186583	KT119476	Panama	8.47911	77.52799
D. pequeno sp. nov.	SMF 97337	AB 861		KT186570	KT119479	Panama	8.47911	77.52799
D. vocator	SMF 97339	AB 028		KT186573		Panama	8.98843	80.58243
D. aff. *quidditus*	SMF 97652	AB 931		KT186562		Panama	7.75898	78.09228
D. majeensis sp. nov.	SMF 97653	AB 1031		KT186567	KT119480	Panama	8.79936	78.46156
D. majeensis sp. nov.	SMF 97657	AB 1033			KT119441	Panama	8.79936	78.46156
D. darienensis sp. nov.	SMF 97662	AB 1185		KT186564	KT119447	Panama	7.94719	77.70420
D. pequeno sp. nov.	SMF 97663	AB 857		KT186575	KT119477	Panama	8.47911	77.52799
D. citrinobapheus	SMF 89814	AH 449	JQ927333			Panama	8.48500	81.17300
D. citrinobapheus	SMF 89820	AH 211	JQ927334			Panama	8.56900	81.09900
D. citrinobapheus	MHCH 2370	AH 450	JQ927335			Panama	8.48500	81.17300
D. citrinobapheus	MHCH 2371	AH 452	JQ927336			Panama	8.48500	81.17300
D. hylaeformis	SMF 89868	AH 267	JQ927337			Panama	8.93100	82.71400
D. hylaeformis	SMF 89869	AH 268	JQ927338			Panama	8.93100	82.71400
D. hylaeformis	SMF 89872	AH 124	JQ927339			Panama	8.91100	82.71300
D. hylaeformis	SMF 89875	AH 282	JQ927340			Panama	8.91200	82.71300
D. citrinobapheus	USNM 572442	KRL 0902	FJ784425			Panama	8.66700	80.59200
D. citrinobapheus	USNM 572443	KRL 1181	FJ784484			Panama	8.66700	80.59200
D. citrinobapheus	USNM 572454	KRL 0900	FJ784423			Panama	8.66700	80.59200
D. citrinobapheus	USNM 572455	KRL 0901	FJ784424			Panama	8.66700	80.59200
D. citrinobapheus	MVUP 1783	KRL 0694	FJ784338			Panama	8.66700	80.59200
D. citrinobapheus	MVUP 1830	KRL 0840	FJ784395			Panama	8.66700	80.59200
D. quidditus	USNM 572444	KRL 0647	FJ784326			Panama	8.66700	80.59200
D. quidditus	MVUP 1832	KRL 0856	FJ784405			Panama	8.66700	80.59200
D. vocator	FMNH 257769	AJC 0127	JN991419	JN991348		Costa Rica	8.79000	82.96000
D. hylaeformis	USNM 572546	KRL 0782	FJ784369	FJ766810		Panama	8.66700	80.59200
D. hylaeformis	MVUP 1826	KRL 0831	FJ784390	FJ766809		Panama	8.66700	80.59200
D. hylaeformis	MVZ 203844	1999	EU186682			Costa Rica	9.75000	83.80400
D. hylaeformis	UCR 16264	AJC 0468	JN991418	JN991347		Costa Rica	10.22000	84.54000

Příloha 10.

Veselý, M & Batista, A., 2021: A New Species of *Atelopus* (Amphibia: Bufonidae) from eastern Panama. *Zoological Research* 42(3): 272-279.

The contribution in words:

MV and AB designed the study. Both authors equally participated in all aspects of the study, including fieldwork, morphometry, and molecular genetics, and wrote the manuscript. MV finalised molecular genetic outputs (trees and haplotype networks) and submitted sequences to GenBank. MV and AB revised the manuscript. Both authors read and approved the final version of the manuscript.

Ranking: IF_{2019} – 2.638; **D1** (Zoology 2019)

Veselý & Batista. *Zool. Res.* 2021, 42(3): 272−279
https://doi.org/10.24272/j.issn.2095-8137.2020.319

ZR Zoological Research

Letters to the editor **Open Access**

A new species of *Atelopus* (Amphibia: Bufonidae) from eastern Panama

A new species of the genus *Atelopus*, *Atelopus fronterizo* **sp. nov.**, from eastern Panama is described herein based on molecular, morphological, and bioacoustic evidence. The new species can be distinguished from its congeners occurring in the region by a combination of the following characters: (1) phalangeal reduction in thumb; (2) SVL (females only) (35.1–50.1; *n*=13), HW/SVL (0.23–0.34; *n*=59), EYND/HW (0.27–0.39; *n*=60), TIBL/SVL (0.41–0.56; *n*=58), and HAL/SVL (0.22–0.28; *n*=49); (3) dorsal color pattern with green or yellow background and extensive dark olive blotches forming transversal bands or mottling; (4) advertisement call duration 176–235 ms with 19–34 pulses, average pulse rate 131.69 pulses/s, and dominant frequency 2 422.50–2 606.50 Hz. The new species is nested within the Central American clade of *Atelopus*. The minimum Kimura-2-parameter (K2P) genetic divergence between *Atelopus fronterizo* **sp. nov.** and its most phylogenetically similar congeners (*A. certus* and *A. glyphus*) is >2.6% for 16S and >4.9% for *COI* (Table 1). The phylogenetic relationship is strongly supported by ultrafast bootstrap values for the maximum-likelihood trees of both genetic markers (16S, 96; *COI*, 100, Figure 1A). Bayesian analysis of the concatenated sequences resulted in a tree with similar topology and high posterior probability support (0.99; Supplementary Figure S1). In addition, haplotype networks inferred from *COI* and 16S (Supplementary Figure S2) showed a well-separated clade containing the new species (two for *COI*, four for 16S). The number of mutational steps between haplotypes for the new species samples is very low (1–4 in 16S; one in *COI*), and the minimum number of mutational steps from the nearest species is nine for 16S (distance to *A. certus*) and 28 for *COI* (distance to *A. glyphus*).

Harlequin frogs occur from Costa Rica to Bolivia (Lötters, 1996; Lötters et al., 2011; Savage, 2002). Nearly a hundred species (AmphibiaWeb, 2020; Frost, 2020) are currently recognized, six of which occur in Panama: i.e., *Atelopus certus* Barbour, 1923; *Atelopus chiriquiensis* Shreve, 1936; *Atelopus glyphus* Dunn, 1931; *Atelopus limosus* Ibáñez R., Jaramillo, and Solís, 1995; *Atelopus varius* (Lichtenstein and von Martens, 1856); and *Atelopus zeteki* Dunn, 1933. The geographical distribution of the new species and other Panamanian *Atelopus* species is shown in Figure 1B.

During recent field surveys in the mountain ranges of eastern Panama, we collected five *Atelopus* specimens, along with recordings of calling males. Subsequent molecular analysis revealed that our samples were clustered together, comprising a clade well separated from all known species. Comparison of our specimens with material in museum collections demonstrated that the species has a long scientific history. It was first collected in 1911 by naturalist Henri Pittier near Puerto Obaldia on the Caribbean coast of Darién (Heckadon-Moreno, 1996). This oldest known specimen, stored in the United States National Museum (catalog No. USNM 48 594), was presented by Dunn (1931) as a paratype of *Atelopus glyphus*. Since then, the species has been repeatedly collected and examined (Breder, 1946; Cocroft et al., 1990; Savage, 1972) without any taxonomic consequences. Recently, several authors have identified this taxon as *Atelopus* cf. *limosus* (Lewis et al., 2019) or *Atelopus* sp. "Puerto Obaldía-Capurganá" (Ramírez et al., 2020).

Field work was carried out from 2011 to 2017 in the Chucunaque and Tuira Basins of the eastern Panamanian lowlands, and in all principal eastern Panamanian mountain ranges. The collected specimens were euthanized with T61 and fixed with a preservative mixture of 5 mL formalin (40%) and 1 L ethanol 96 (94%), then stored in ethanol (70%). All geographical coordinates were recorded based on the WGS 1984 datum. A map was created using QGIS 2.18 (Las Palmas) with the OpenStreetMap layer (OSM Contributors, 2015). Abbreviations for museum collections followed Sabaj (2016).

Morphometric data were taken following Bravo-Valencia & Rivera-Correa (2011). Abbreviations of measurements are as

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Received: 01 November 2020; Accepted: 24 March 2021; Online: 26 March 2021

Foundation items: This work was supported by the Sistema Nacional de Investigación (SNI) of SENACYT, Panama, and M.V. received institutional support from Palacky University

Table 1 K2P distances for mitochondrial fragments of 16S and *COI* genes of *Atelopus* species from Panamá and Colombian samples of *A spurrelli.*

	Species	1 *n*=2	2 *n*=2	3 *n*=3	4 *n*=1	5	6 *n*=7	7 *n*=19
1	*A. fronterizo* **sp. nov.**	**0.24**	**5.18–5.69**	**4.93–5.70**	**8.88–9.16**		**8.64–10.02**	**9.74–11.49**
	n=4	0.50–2.04						
2	*A. certus*		**0.71**	**2.41–3.14**	**9.48–9.74**		**9.98 - 10.02**	**9.98–10.57**
	n=2	2.55–3.08	1.01					
3	*A. glyphus*			**0.00–0.47**	**8.88–9.14**		**9.18–9.74**	**8.64–9.72**
	n=3	2.55–3.60	1.52–2.55	0.00–0.50				
4	*A. spurrelli*				**0.00**		**8.04–8.85**	**8.85–11.71**
	n=2	4.67–5.22	5.75	5.74–6.29	0.00			
5	*A. varius*							
	n=5	3.61–4.67	4.67–5.75	4.15–5.22	4.12–4.65	0.00–0.50		
6	*A. zeteki*						**0.00–3.90**	**0.00–6.50**
	n=7	3.60–5.77	4.66–6.87	4.13–6.33	4.11–5.74	0.00–2.03	0.00–2.55	
7	*A. limosus*							**0.00–6.23**
	n=23	4.15–5.22	4.67–6.31	4.15–5.77	4.12–5.19	0.50–2.03	0.50–3.08	0.00–1.52
8	*A. chiriquiensis*							
	n=1	4.15–4.69	4.13–4.67	3.61–4.15	4.12	1.52–2.03	1.52–3.08	1.52–2.04

Ranges represent minimum and maximum observed distances. Intraspecific genetic distances are shown along diagonal of matrix. Values correspond to interspecific genetic distances of 16S and *COI* genes (*COI* in bold); *COI* sequences of *A. varius* and *A. chiriquiensis* were not available.

follows: SVL (snout-vent length), TIBL (shank length), FTL (foot length), HL (head length from point behind angle of jaw to tip of snout), HW (head width at widest point), EYDM (eye diameter), EYND (eye to nostril distance), IOID (interorbital internal distance), IND (internarial distance), FAL (length of flexed forearm), HAL (hand length from proximal edge of outer metacarpal tubercle to tip of finger III), and THBL (thumb length from outer metacarpal tubercle to tip of finger I). Measurements of frogs were taken to the nearest 0.1 mm with digital calipers. Webbing formulae followed Myers & Duellman (1982) and Savage & Heyer (1997). Museum and field numbers of voucher specimens are listed in Supplementary Table S1.

The DNA extraction protocols followed Batista et al. (2016). Details on final alignment, maximum-likelihood (ML) analysis, Bayesian analysis, and haplotype network construction are provided in the Supplementary Notes. GenBank accession Nos. of the sequences used (including those submitted by us) are listed in Supplementary Tables S2, S3.

For confirmed candidate species (CCS) and their delimitation, we followed the integrative concept for amphibians of Vieites et al. (2009). For species-level analysis, genetic divergences were calculated using the Kimura 2-parameter (K2P) model (Kimura, 1980) for 16S and *COI* separately in MEGA 6. Default values were retained for all parameters. Statistical analyses of morphometric characters were performed using SPSS 21.0.

Details on call recordings and bioacoustic methodology are described in the Supplementary Notes.

Molecular analysis (mitochondrial DNA (mtDNA) 16S and *COI*) of *Atelopus* specimens from the San Blas Mountains (two specimens from Nurra) and Cerro Tacarcuna slopes (one specimen from Pechito Parao near Río Tuquesa and one specimen from Río Púcuro) confirmed that these samples formed a monophyletic lineage and sister clade to the clade containing *A. certus* and *A. glyphus* from eastern Panama (Figure 1A). Discriminant function analysis (DFA) of morphometric characters of *Atelopus* from eastern Panama (Supplementary Figure S3) correctly classified the new species, with 80.4% of specimens separated from related species according to *a priori* groupings (correct classification for 85.3% of *Atelopus fronterizo* **sp. nov.**; 70% of *A. glyphus*; 50% of *A. certus*). The principal morphological variables contributing to the grouping in order of relevance were: (1) HW/SVL, (2) EYND/HW, (3) TIBL/SVL, (4) HAL/SVL; first function: DS=0.23xHW/SVL±0.18xEYND/HW–0.65xTIBL/SVL+4.24xHAL/SVL; second function: DS=–0.22xHW/SVL±0.45xEYND/HW+1.72xTIBL/SVL+5.07xHAL/SVL. When including SVL alone, DFA correctly classified 87% of the specimens. Moreover, the new species can be distinguished from all other species of *Atelopus* occurring in eastern Panama by additional morphological and bioacoustic characters. According to our findings, we describe the species as new to science.

Taxonomic account

Atelopus fronterizo **sp. nov.** (for synonymy see Supplementary Notes)

Holotype: MHCH 3110 (AB 543; Figure 1C–H), from Río Púcuro, Pinogana, Provincia Darién, Panama (N8°1'44.40", W77°22'10.20"; 132 m a.s.l.), collected by Abel Batista on 10 June 2012.

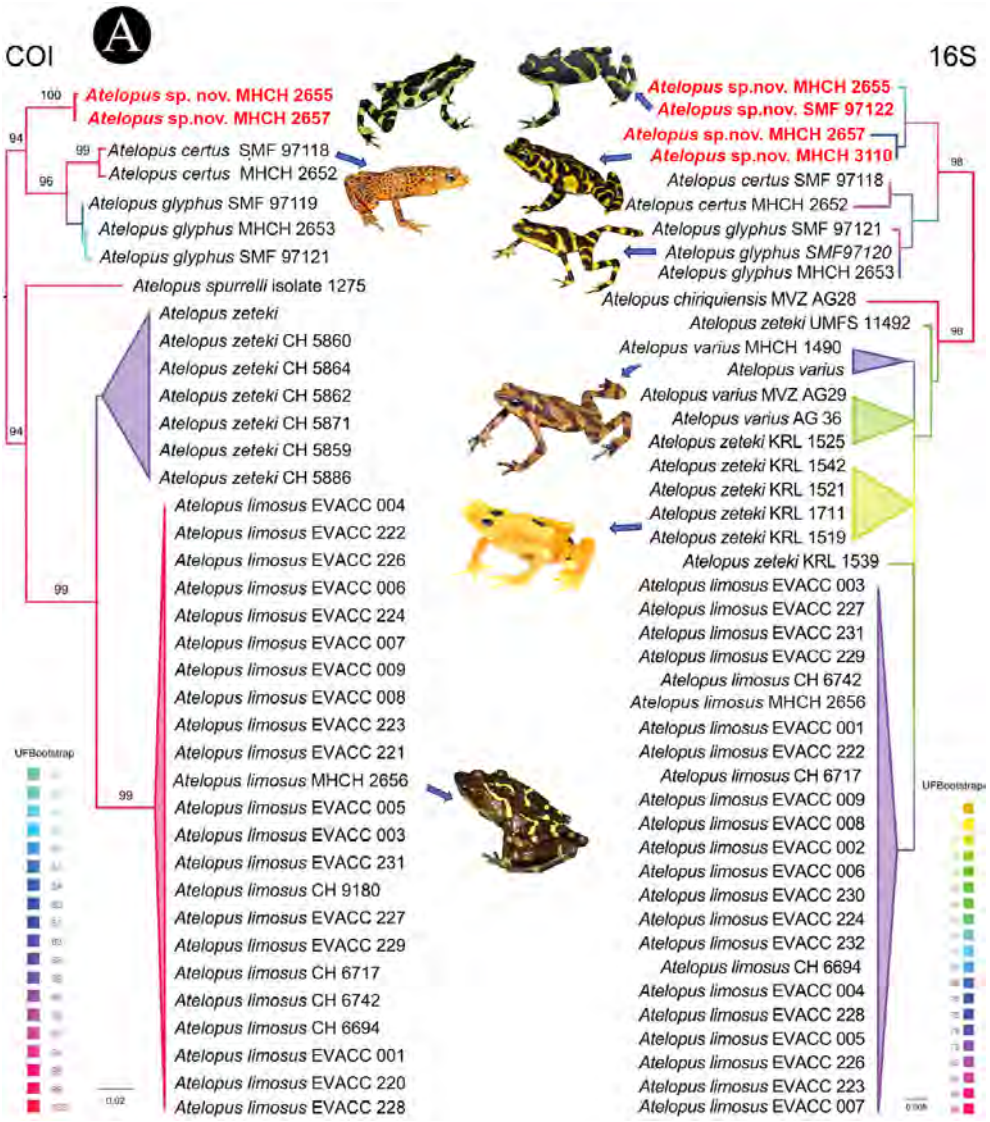
Paratypes: Two adult males, SMF 97122 (AB720) and MHCH 2655 (AB733), both from Nurra, Comarca Wargandi, collected by Milan Veselý and Abel Batista on 2 October 2012 (N9°3'36.00", W77°58'44.4"; 339 m a.s.l.); adult male MHCH

2657 (AB830) from Cerro Pechito Parao, Bajo Pequeño, Lajas Blancas, Cémaco, Comarca Embera-Wounaan (N8°28'31.80", W77°31'9.84"; 472 m a.s.l.), collected by Abel Batista and Milan Veselý on 5 November 2012 (Supplementary Figure S4).

Etymology: The species epithet "*fronterizo*" refers to the area in which the species is distributed, i.e., the border between Panama and Colombia. Panamanian people use "*fronterizo*" to refer to someone living at the Panamanian border police SENAFRONT (Servicio Nacional de Fronteras) guarding this region, including the habitat of the camouflaged harlequin frog. SENAFRONT has helped increase knowledge of the area's wildlife by reporting sightings, recording sounds, and photographing observations.

Diagnosis: Medium-sized *Atelopus* [average SVL (mm): all 33.7±6.8 (24.2-50.1; *n*=50); females 43.8±4.1 (35.1-50.1; *n*=13); males 30.4±2.6 (24.2-34.8; *n*=37)] characterized by the following combination of characters: (1) body slender, snout protruding, with tip rounded; (2) neural spines weakly visible externally; (3) hindlimbs long, tibiotarsal articulation reaching anterior corner of eye when leg is outstretched forward along body (average in males TIBL/SVL 0.47; *n*=37); (4) foot shorter than shank (average in males FTL/TIBL 0.81; *n*=31); (5) tympanic annulus and tympanic membrane absent; (6) dorsal parts of body and limbs smooth to slightly shagreen (visible under stereomicroscope); (7) foot webbing formula I0–0II0–2^{-}III1–3^{+}IV3^{+}–1^{-}V; (8) thumb short (average THBL/HAND 0.32; *n*=20); (9) plantar and palmar surfaces mostly smooth with subarticular tubercles poorly defined.

Description of holotype: Body slender; neural spines weakly visible externally; head longer than wide (HW/HL=0.94); head length 27% of SVL; snout protruding, with tip rounded, dorsally subelliptical; upper jaw extending beyond lower jaw; nostrils lateral, slightly visible from above; tongue twice as long as wide, anterior two-thirds attached to floor of mouth; canthus rostralis slightly concave from nostril to tip of snout, concave from eye to nostril; nostril closer to tip of snout than to eye; eye diameter slightly larger than distance from eye to nostril; tympanic annulus and tympanum not visible; supratympanic crests slightly visible; parotid glands small and barely visible;



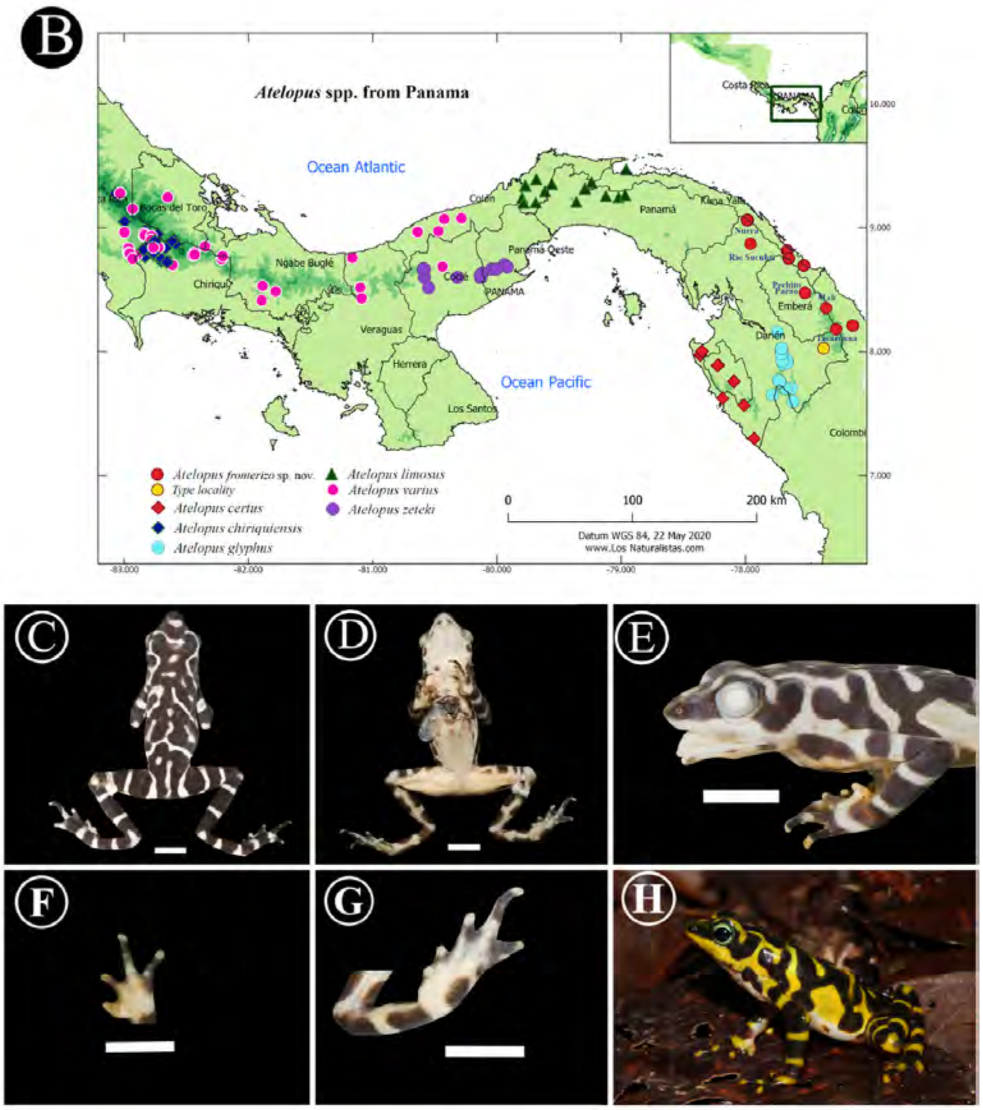


Figure 1 Molecular comparison and geographic distribution of Panamanian species of *Atelopus*, and main features of holotype of *Atelopus fronterizo* sp. nov.

A: Maximum-likelihood trees based on *COI* and 16S sequences of *Atelopus* species from Panamá. Colors of branches indicate bootstrap support, values >90 are marked on branches. B: Map of Panama showing distribution of *Atelopus* species occurring in the country; C: *Atelopus fronterizo* **sp. nov.** holotype (MHCH 3110), dorsal view of preserved specimen; D: Ventral view; E: Head laterally; F: Left hand; G: Left foot; H: Live holotype, from Púcuro River (MHCH 3110).

vomers without teeth or odontoids; choanae rounded and broadly separated in between. Shank moderately long (TIBL/SVL=0.41); foot shorter than shank (FL/TIBL=0.85); relative length of toes I<II<III<V<IV; foot webbing formula I0–0II0–2^{-}III1–3^{+}IV3^{+}–1^{-}V; outer metatarsal tubercle round, weakly prominent; inner metatarsal tubercle poorly defined, oval, flattened, larger than outer; subarticular tubercles low and rounded. Forelimb long, slender; relative length of fingers I<II<IV<III; hand webbing formula I2–3^{-}II3–4III4–3IV; outer palmar tubercle distinct, rounded, larger than oval inner; subarticular tubercles moderately marked and rounded; thumb moderately short, distance from tip to outer edge of inner palmar tubercle less than length of tubercle (THBL/HAND=0.27). Skin of dorsal surfaces of body and limbs smooth, glandular and weakly rugose in texture (observed under stereomicroscope); ventral surfaces smooth.

Color in life: Dorsum pale yellow with irregular, well-defined, dark brown to almost black blotches, bars, and vermiculation (Figure 1H); upper surfaces of limbs yellow with broad dark olive bands; upper lip yellow, suffused slightly with dark pigmentation; lower lips with small dark olive blotches; throat, chest, and venter creamy white; lower venter and lower surfaces of thighs bright orange; remaining ventral parts of legs yellow with interrupted dark olive transverse bands, extending from dorsal parts; palmar and plantar surfaces orange suffused with dark olive blotches; ventral color of arms white with dark olive transverse bands extending from dorsal surfaces; iris black, with narrow, pale green ring surrounding pupil, reticulated with dark color in external borders.

Color in preservative: Dorsal surfaces dirty white to pale gray, with irregular, well-defined, dark to almost black blotches, bars, and vermiculation; dark bars on exposed areas of limbs and flanks; throat, venter, ventral surfaces of thighs, and palmar and plantar surfaces cream; two tiny, round, dark brown blotches on chest, another on venter (around midlevel) on right side; edges of lower lip with small dark brown blotches; throat with two barely visible diffused dark brown blotches (Figure 1C–G).

Measurements (in mm): Holotype followed by range of males (holotype plus three male paratypes): SVL 29.5 (27.5–34.1); HL 8.5 (8.2–9.3); HW 8.0 (7.7–9.2); IND 2.6 (2.5–3.5); EYND 2.5 (2.3–3.0); EYDM 3.0 (2.5–3.4); TIBL 12.1 (12.1–15.7); FTL 10.3 (9.4–13.8); HAL 6.5 (6.5–8.5); THBL 1.8 (1.8–2.8).

Variation: Measurements and intraspecific variation of known populations are shown in Supplementary Table S4 (males) and Supplementary Table S5 (females). We identified two color morphs, i.e., Tacarcuna and Sucubti morphs. The Tacarcuna morph (Figure 1H) has a dark vermiculation pattern, which agrees well with the holotype description; this population is distributed around Cerro Tacarcuna southward to Colombia (Figure 1B). The Sucubti morph populations occur northwards of Cerro Tacarcuna, with most specimens having broader dark transverse bands on the dorsum compared with the Tacarcuna morph, often with a narrow (sometimes incomplete) light inverted chevron just anterior to the interorbital area, a light chevron across the mid-dorsum, and a light band across the postsacral region, with a definite elongated light area in the suprascapular region; these light blotches share a dorsal ground color, which can be yellow, pale green, or green (Supplementary Figure S5).

Call description: We recorded an *Atelopus fronterizo* **sp. nov.** male near Nurra on 3 October 2012, 0949 h, 25.5 °C (locality of paratype MHCH 2655). The male was calling towards another male sitting about 1 m away. The vocalization produced by the Sucubti morph consists of pulsed calls (buzz-like sounds) emitted at 2 505.94±66.05 Hz (2 422.5–2 605.5; *n*=8), with a call duration of 209.75±20.25 ms (176–235; *n*=8), pulses of 28.0±5.48 (19–34, *n*=7), and pulse rate of 131.69±16.39 pulses/s (97.44–144.68; *n*=7; Supplementary Table S6). In the recording, we identified three call bouts, with four, five, and eight calls, respectively. A male recorded by C. Myers in 1967 emitted partially pulsed short calls with a downward modulated frequency (Cocroft et al., 1990: Figure 4K–L). That call demonstrated a dominant frequency in the same range as the call recorded by our male but was substantially shorter (~55% of the duration of normal advertisement pulse call recorded in our study). An oscillogram and spectrogram of the *Atelopus fronterizo* **sp. nov** advertisement call are presented in Supplementary Figure S6.

Distribution and natural history: *Atelopus fronterizo* **sp. nov.** occurs in the Darien Mountain range in northeastern Panama and northwestern Colombia (Figure 1B), as well as in the eastern Panamanian montane forests (World Wildlife Fund, 2014) and Chocó-Darién moist forests (Hogan & World Wildlife Fund, 2014). Most specimens were active during the daytime along small streams, although the holotype was found along the moderately sized Púcuro River (Supplementary Figure S7). We encountered three specimens at night at Cerro Pechito Parao (ca. 1–2 km from the Tuquesa River, Bajo Pequeño), sleeping approximately 20–30 cm above the ground in low bushes and far from any stream or river. On the Caribbean side of Nurra, we found 22 individuals during a nighttime search along a 200 m transect following a stream. The next day, we also recorded one calling male and observed one amplectant pair. Several males were observed to use forelimb waving signals during visual interactions with other individuals.

Conservation status: Due to the current declines in *Atelopus* populations caused by the chytrid fungus *Batrachochytrium dendrobatidis* (Flechas et al., 2017b; La Marca et al., 2005; Lewis et al., 2019), we argue that *Atelopus fronterizo* **sp. nov.** should be included in the Critically Endangered category (A3ce), as also suggested by Lewis et al. (2019). *Atelopus fronterizo* **sp. nov.** faces the same risks of decimation and potential extinction as other *Atelopus* species that occur in eastern Panama.

Similar species: *Atelopus fronterizo* **sp. nov.** can be differentiated from other species of *Atelopus* occurring in the Panamanian region and adjacent areas of Colombia by a combination of the following characters: dorsal coloration showing irregular, dark brown bands and blotches on green or yellow background (Figure 1H; Supplementary Figure S5); phalangeal reduction in thumb; larger sized females (SVL); and characteristic advertisement calls. The advertisement call oscillogram of *Atelopus fronterizo* **sp. nov.** is easily distinguishable from those of Panamanian congeners by the envelope shape of the call, where the fall time ends abruptly compared to other species in the region (Supplementary Figure S6). From its geographically closest congeners (*A. certus*, *A. glyphus*), the new species differs in molecular genetics, with K2P genetic divergences of >2.6% for 16S and >4.9% for *COI* (Table 1). Additionally, *Atelopus fronterizo* **sp. nov.** can be distinguished by the following characters (contrasting features for the new species in parentheses (see Supplementary Tables S4–S6 and Supplementary Figure S8)). *Atelopus certus*: coloration in life brick red with black spots (vs. pale green or yellow with dark olive irregular vermiculations or transversal bars), head almost as broad as long HL/HW 1.03±0.09 (vs. slightly broader HL/HW 0.94±0.06), shorter shank length TIBL=13.1±0.71 mm (vs. 17.1±3.7 mm), longer thumb in proportion to SVL 0.10±0.01 (vs. 0.08±0.01); longer call duration 0.30±0.046 ms (vs. 0.21±0.02 ms), with higher number of pulses per call 38.5±6.75 (vs. 27.5±5.82), and broader bandwidth 710.4±195.9 Hz (vs. 318.6±254.4 Hz). *Atelopus glyphus*: smaller in size, females 38.7±3.7 mm, *n*=10; males 26.5±3.5 mm, *n*=124 (vs. females 43.8±4.1 mm; males 30.4±2.6 mm); head as broad as long HL/HW 1.01±0.05 (vs. slightly broader HL/HW 0.94±0.06), shorter shank length TIBL 14.9±2.56 mm (vs. 17.07±3.7 mm); longer call duration 0.25±0.013 ms (vs. 0.21±0.02 ms), higher number of pulses per call 35.1±2.77 (vs. 27.5±5.82), and broader bandwidth 623.2±50.1 Hz (vs. 318.6±254.4 Hz). *Atelopus limosus*: dorsal ground color dark brown, black, or solid olive with variable bright green markings (vs. pale green or yellow with dark olive irregular reticulations or bars), smaller size, females 36.2±0.8 mm, *n*=5; males 26.3±2.5 mm, *n*=22 (vs. females 43.8±4.1 mm, males 30.4±2.6 mm), higher call pulse rate (pulses/s) 151.6±3.1 (vs. 131.69±16.39), and maximum frequency 2 689±93 Hz (vs. 2 505.94±66.05 Hz). *Atelopus spurrelli*: smaller size, females 30–34 mm, males 26 mm (Rivera-Correa, 2005) (vs. females 43.8±4.1 mm, males 30.4±2.6 mm). *Atelopus varius*: dorsal color highly variable, mostly black or chocolate brown with yellow or red bands and blotches (vs. pale yellow or green yellow with dark olive irregular blotches or bands), longer call duration 413–431 ms (vs. 176–235 ms), and more pulses per call 48–50 (vs. 19–34). *Atelopus zeteki*: in life dorsal color uniformly golden yellow, usually with black bands or blotches (vs. pale yellow or green yellow with dark olive irregular reticulations or bands).

Remarks: Many species in the genus *Atelopus* are convergent in color, shading, and pattern. When coupled with high (but poorly understood) intraspecific variation and cryptic species diversity, this renders species delimitation through morphological characters alone particularly challenging (Coloma et al., 2000; De la Riva et al., 2011; Guayasamin et al., 2010). This likely prevented earlier recognition and description of *Atelopus fronterizo* **sp. nov.** by other herpetologists. As outlined above, the identity of this species has raised taxonomic doubts for more than a century, and has been repeatedly confused with *A. limosus*, *A. glyphus* and *A. spurrelli* (Flechas et al., 2017a; Rivera-Correa, 2005; https://www.inaturalist.org/, accessed on 19 January 2021).

Our molecular analysis relied on comparisons of two genetic markers, 16S and *COI*, which are consistently used across systematic studies of amphibians (e.g., Acosta-Galvis et al., 2020; Batista et al., 2014a, 2014b, 2014c; Chambers & Hebert, 2016; Jorge et al., 2020; Lötters et al., 2011; Nagy et al., 2012; Vieites et al., 2009). The K2P genetic distance of *Atelopus fronterizo* **sp. nov.** to its genetically closest neighbors, *A. certus* and *A glyphus*, is slightly smaller than the commonly accepted threshold for these markers in amphibians (i.e., 2.6%/2.6% for 16S and 4.9%/5.2% for *COI*). Nevertheless, the morphologically distinct and widely accepted species *A. glyphus* and *A. certus* show even lower distances between each other (minimum K2P distance 1.5% in 16S and 2.4% in *COI*) and low distances have also been reported between other *Atelopus* species in Panama (Lewis et al., 2019; Table 1). Other morphologically similar species, which extend, at least partly, to the Darién/Chocó border area between Panama and Colombia (*A. limosus*, *A. spurrelli*), are genetically much more distinct (Table 1).

Bioacoustic signals are still poorly studied in *Atelopus*. Four call types (pulsed calls, pure tone calls, pulsed short calls, and pure tone short calls) are currently recognized within the genus (Lötters et al., 2019). The advertisement call pattern of *Atelopus fronterizo* **sp. nov.** resembles that of other *Atelopus* species from Panama in having a pulsed call and pulsed short call (Cocroft et al., 1990; Ibáñez et al., 1995; Jaslow, 1979; Lewis et al., 2019, this study). Pure tone calls and pure tone short calls have not yet been recorded for *Atelopus fronterizo* **sp. nov.**, but the former is present in *A. chiriquiensis* (Jaslow, 1979) and the latter is present in *A. zeteki* (Cocroft et al., 1990, Lotters et al., 2019). Calls by *Atelopus fronterizo* **sp. nov.** remain well differentiated from those of other *Atelopus* species in eastern Panama after temperature correction (Supplementary Table S6; Supplementary Figure S6).

The observed *Atelopus fronterizo* **sp. nov.** populations were not affected by the chytrid fungus. Likewise, Flechas et al. (2017a) did not find *B. dendrobatidis* in swab samples from wild populations in Capurganá, Colombia. Although the current situation may be different due to the rapid spread of the pathogen in Panama over the last two decades (Crawford et al., 2010; Lewis et al., 2019; Lips et al., 2006), there is evidence that some species can, at least partially, resist *B. dendrobatidis* with the help of anti-*B. dendrobatidis* activity from symbiotic skin bacteria. Such effects have been shown for skin bacteria in *Atelopus fronterizo* **sp. nov.** (Flechas et al., 2017a).

With the immediate need for protection of the vulnerable genus *Atelopus* (La Marca, 2005; Ramírez et al., 2020; Zippel et al., 2006), we appeal for an urgent conservation plan for *Atelopus fronterizo* **sp. nov.**, including captive breeding programs and subsequent establishment of a founder population (Lewis et al., 2019). Conservation efforts for *Atelopus fronterizo* **sp. nov.** may serve to protect biodiversity in the borderland jungle and help to secure the future of this and other yet to be recognized species in the Darién Gap.

Nomenclatural acts registration

The electronic version of this article in portable document format represents a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone (see Articles 8.5–8.6 of the Code). This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information can be viewed through any standard web browser by appending the LSID to the prefixhttp://zoobank.org/.

Publication LSID: urn:lsid: zoobank.org:pub:06959E87-EE29-44BD-AA0A-43BE0CCE0183 *Atelopus fronterizo* LSID:
urn:lsid: zoobank.org:act:0DCD680B-2D5B-4EFF-8831-F6F07A0B7F21

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Collection permits 2011 (SC/A-37-11) and 2012 (SC/A-93633-12) and exportation permits 2012 (SC/A-33-12) and 2013 (SEX/A-7-13) were provided by UNARGEN-Ministerio de Ambiente, Panama.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

M.V. and A.B. designed the study. Both authors equally participated in all aspects of the study, including fieldwork, morphometry, and molecular genetics, and wrote the manuscript. A.B. performed bioacoustic analysis. M.V. finalized molecular genetic outputs (trees and haplotype networks) and submitted sequences to GenBank. M.V. and A.B. revised the manuscript. Both authors read and approved the final version of the manuscript.

ACKNOWLEDGEMENTS

We thank Yorlis Cáceres, Daniel Cáceres, Isaac Pizarro, Gustavo Dogirama, Mario Cuñapa, Anselmo Caicedo, Hugo Martínez, Elacio Méndez, Gilberto Torres, and the people from Bajo Pequeño for field assistance. Michal Motyka and Dominik Kusý kindly helped with molecular data processing; Madian Miranda helped with photography and creating the appendix figures of preserved specimens from the American Museum of Natural History (AMNH). David Kizirian kindly allowed revision of the specimens at the AMNH. We also thank Dennis Voeten, Taylor Cooper, and Joseph Mendelson III for language assistance.

Milan Veselý[1], Abel Batista[2,3,4,*]

[1] *Department of Zoology*, *Faculty of Natural Sciences*, *Palacky University*, *Olomouc* 77146, *Czech Republic*
[2] *Universidad Autónoma de Chiriquí*, *Ciudad Universitaria El Cabrero David*, *Chiriquí* 427, *Panamá*
[3] *Asociación ADOPTA el Bosque Panamá*, *Gamboa* 119*x*, *Panamá*
[4] *Fundación Los Naturalistas*, *David*, *Chiriquí* 426-01459, *Panamá*
*Corresponding author, abel.batista@unachi.ac.pa

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Supplementary Materials

Supplementary Notes

A) Molecular laboratory work, phylogenetic inference and haplotype network construction

The final 16S alignment comprised 11 sequences obtained in this study and 34 sequences from GenBank consisting of 540 bp, of which 428 sites were variable, 387 parsimony-informative, and 41 singletons; the final alignment for the *COI* gene comprised 8 sequences from our material and 30 ones from GenBank, consisting of 567 bp, of which 293 sites were variable, 282 parsimony-informative, and 11 singletons. A maximum likelihood (MA) analysis was conducted for both genetic markers using IQ-TREE (Nguyen *et al.*, 2015; Trifinopoulos *et al.*, 2016). The substitution model was not defined a priori; instead, the implemented ModelFinder (Kalyaanamoorthy *et al.*, 2017) was applied to find the best models during analysis under the Bayesian information criterion. For estimates of support, 5000 replicates of ultrafast bootstrapping (Minh *et al.*, 2013) were performed.

To infer a Bayesian (BA) phylogenetic tree from a concatenated 2-gene dataset, we conducted the analysis by applying the site-heterogeneous mixture model CAT + GTR + Γ4 implemented in the Phylobayes MPI software (Lartillot *et al.*, 2013). Two independent Monte Carlo Markov chains (MCMC) were run. We checked for the convergence in the tree space with bpcomp program and generated output of the largest (maxdiff = 0.062493) and mean (meandiff = 0.00214141) discrepancy observed across all bipartitions and generated majority-rule consensus tree using a burn-in of 10 000 trees and sub-sampling every 10 trees. Additionally, we used program Tracecomp to check for convergence of the continuous parameters of the model.

We constructed a haplotype network using the 95% limit of parsimony as implemented in TCS 1.21 (Clement *et al.*, 2002) for the available eastern Panama samples and the geographically closest GenBank sample of *Atelopus spurreli* (DQ502895.1) from Colombia, for 16S and COI separately. The raw output of TCS was visualized in PopART version 1.7 (Leigh & Bryant, 2015). The descriptive statistics for nucleotide diversity was produced also in PopART version 1.7.

B) Bioacoustics

Male advertisement calls of *Atelopus fronterizo* and *A. certus* were recorded from frogs sitting on rocks, using Panasonic RRXS410 digital recorder, and Sennheiser ME66 microphone with

K6 powering module. The microphone was held at a distance 1–1.5 m from the calling male. *Atelopus glyphus* was recorded using an Android phone, with the RecForge II App. Recordings were made at the sampling rate of 44 kHz and 16 bits resolution in uncompressed PCM format and saved as wav files. Call recordings of *A. varius* from Panama highlands were provided by E. Griffith, recorded in captivity at El Valle Amphibian Conservation Center (EVACC), the call of *A. zeteki* was recorded by A. Batista using a cellphone with the RecForge II App in October 11th 2019 at EVACC, the call of *A. limosus* was provided by R. Ibañez,. For each call, we analyzed call duration, pulse duration, pulses per second, call interval, and minimum, maximum, and dominant frequencies. The spectral and temporal parameters analysis follow Batista *et al.* (2016). Temporal measurements of calls such as call duration (msec), call interval (msec), pulse rate (pulses/sec), number of pulses and calls/bouts were taken on the waveforms view of Raven. Terminology used in the advertisement call description follows Zimmermann (1990) and Erdtmann & Amézquita (2009). The call repetition rate was calculated as the number of calls per call bout duration. The environmental temperature and humidity were measured using a digital thermo-hygrometer Garden HighPro Medium.

C) Synonomy

***Atelopus fronterizo* sp. nov.**

Atelopus glyphus – Puerto Obaldia: Guna Yala: Panama (USNM 48594-5 adult) Dunn (1931)
Atelopus varius glyphus Rio Sucubti, Chalichiman's Cr. – Breder (1946)
Atelopus sp. – Rio Sucubti, Chalichiman's Cr., Tacarcuna Village: Darien; Puerto Obaldia: Armila: Guna Yala – Savage (1972)
Atelopus sp. – Sasardi camp, Guna Yala – Cocroft *et al.* (1990)
Atelopus sp. – Lötters (1996)
Atelopus spurrelli – Rivera_Correa (2005)
Atelopus aff. *limosus* – Capurganá: Colombia – Flechas *et al.* (2017a)
Atelopus aff. *limosus* – Cerro Tarcacuna, Darien National Park: Darién – Lewis *et al.* (2019)
Atelopus sp. Puerto Obaldía – Capurganá – Ramírez et al. (2020)

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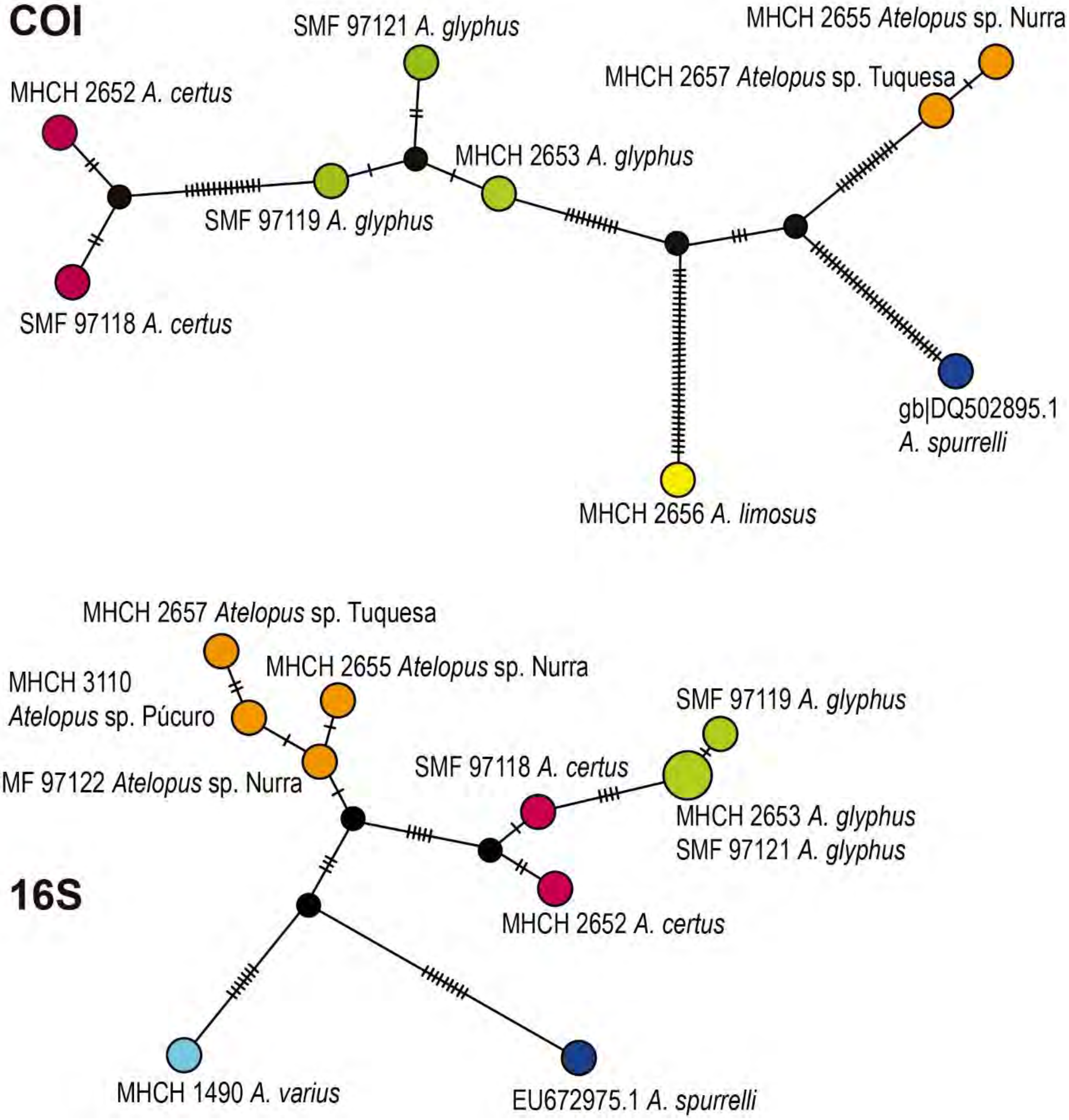
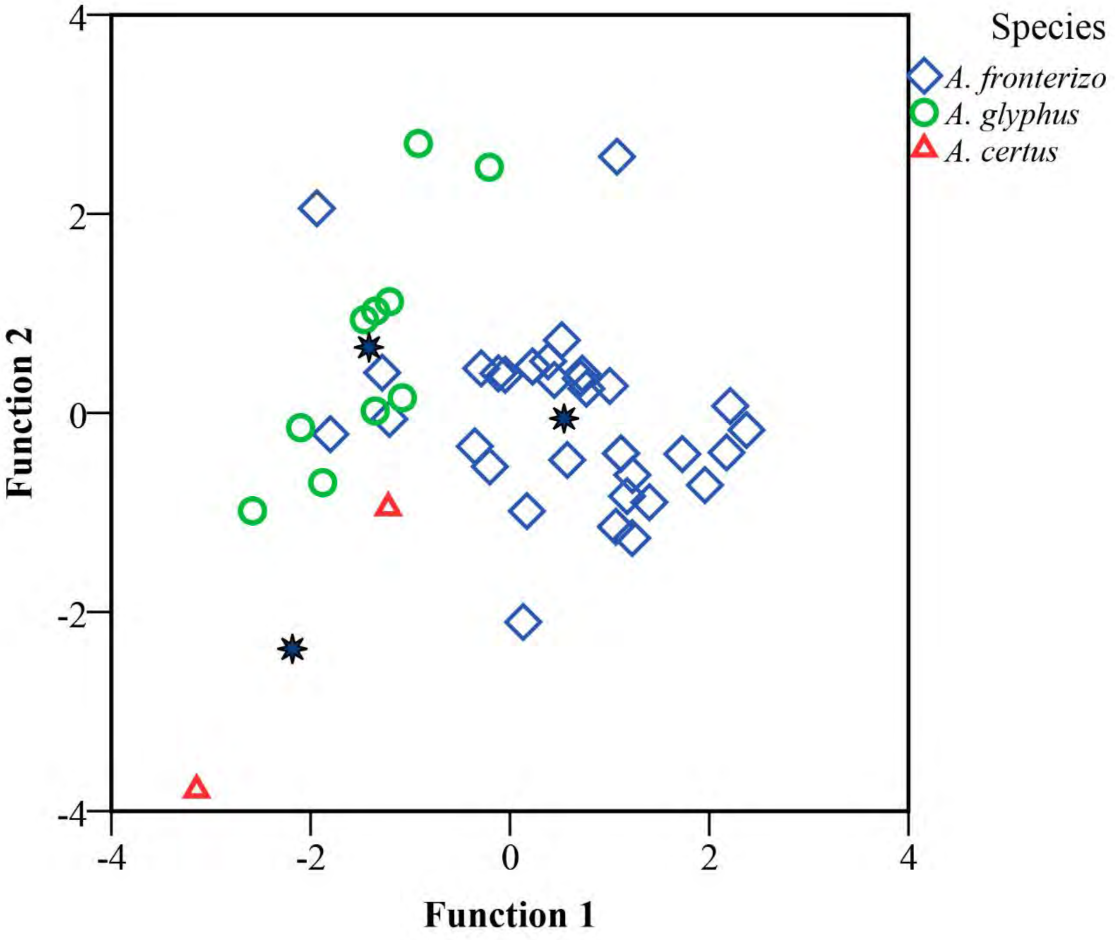
Supplementary Figures

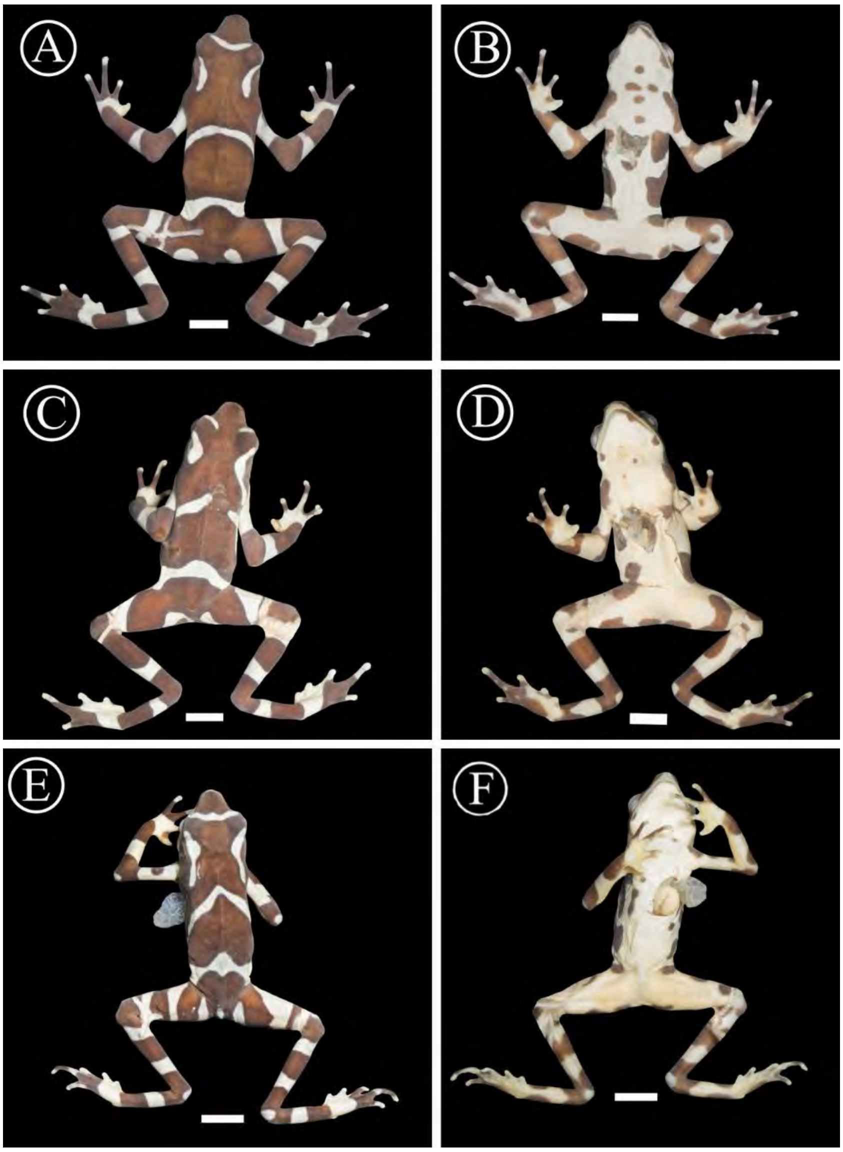
Supplementary Figure S1. Bayesian tree based on concatenated sequences of 16S and COI of *Atelopus* from Panamá and adjacent Colombia (*A. spurrelli*). Posterior probabilities >0.80 are shown above branches. Specimens are indicated by their museum voucher numbers. Scale represents estimated substitutions per site.



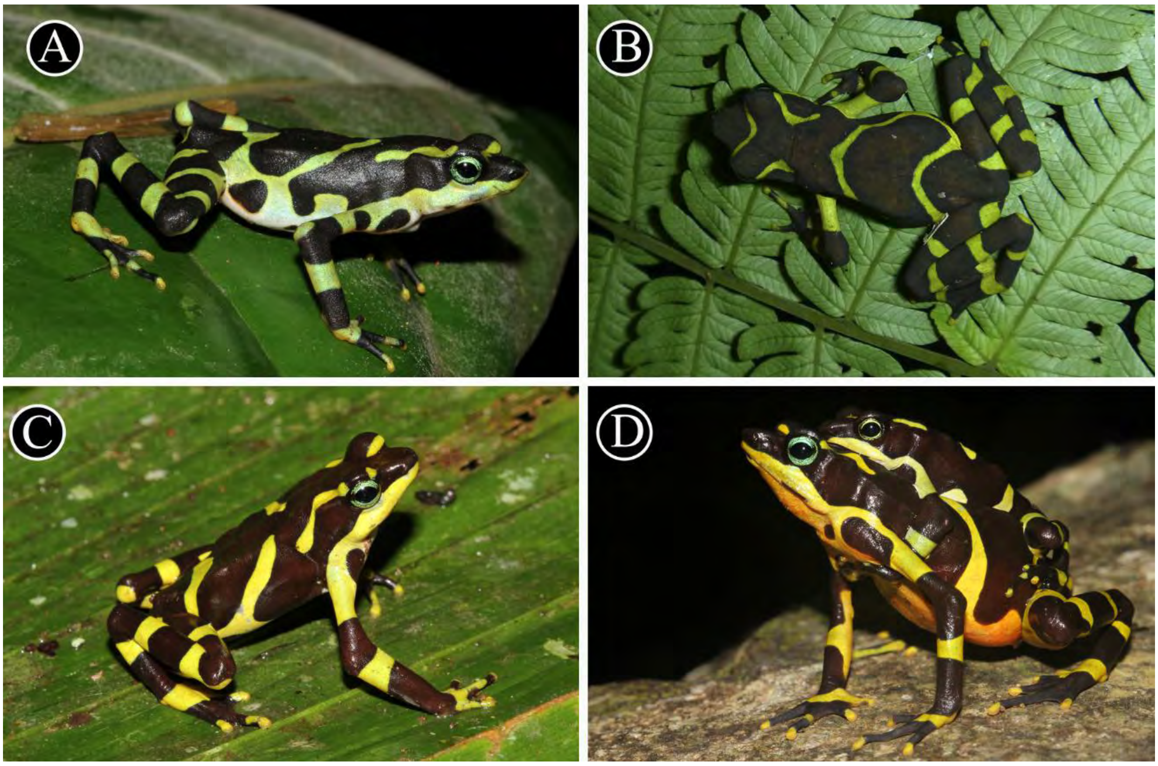
Supplementary Figure S2. Haplotype networks of mitochondrial COI and16S genes. Species are presented by colors. Sizes of circles are proportional to sample size. Each hatch mark on lines connecting haplotypes represents a single nucleotide substitution. Small black circle represents inferred intermediate haplotype.



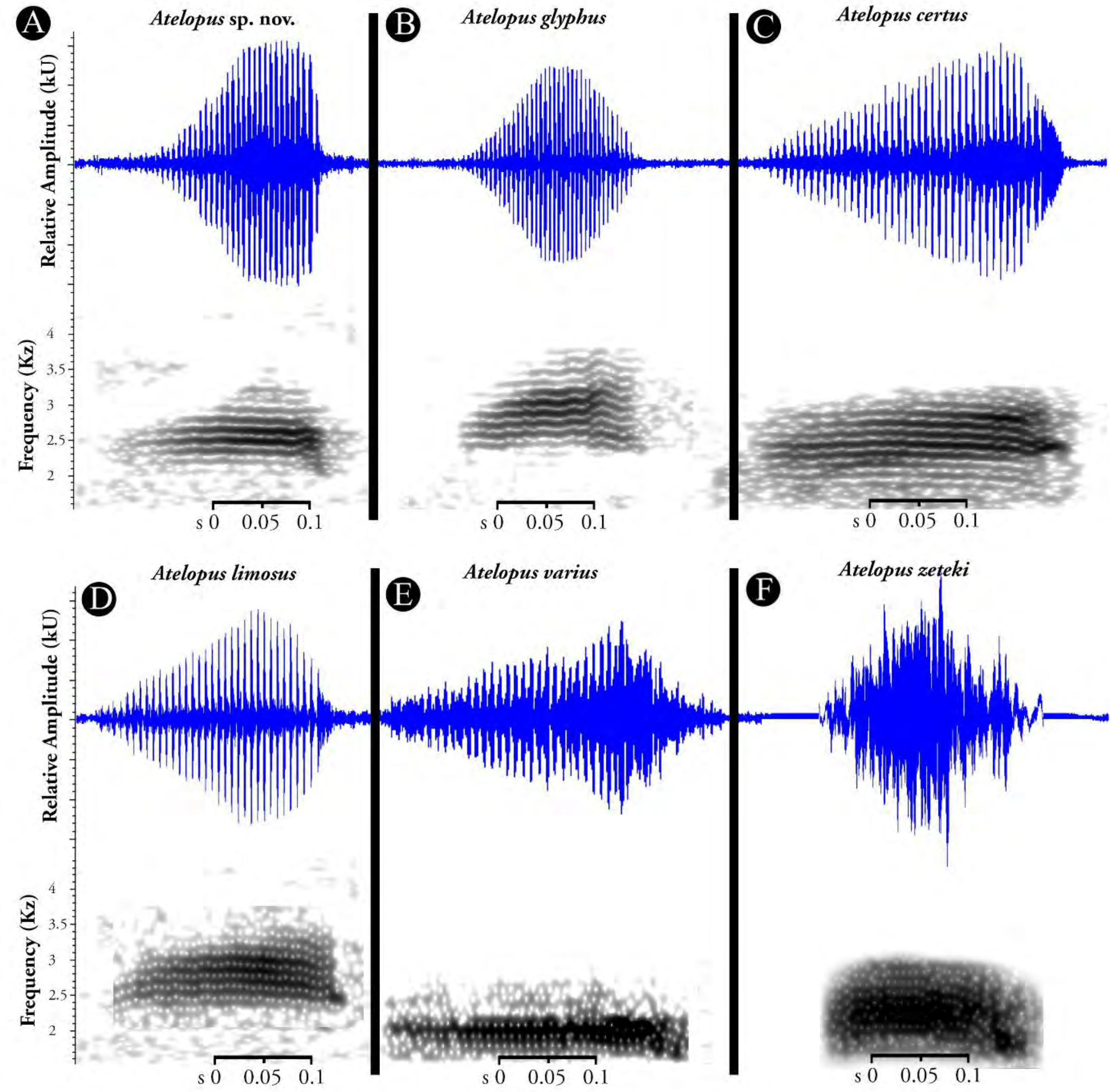
Supplementary Figure S3. Discriminant function analysis of morphometric characters of *Atelopus* species from eastern Panama. Variables included in analysis: HW/SVL, EYND/HW, TIBL/SVL, HAL/SVL, THBL/SVL, IND/IOD, HL/HW, FTL/SVL, FTL/ TIBL, THBL/HAL.



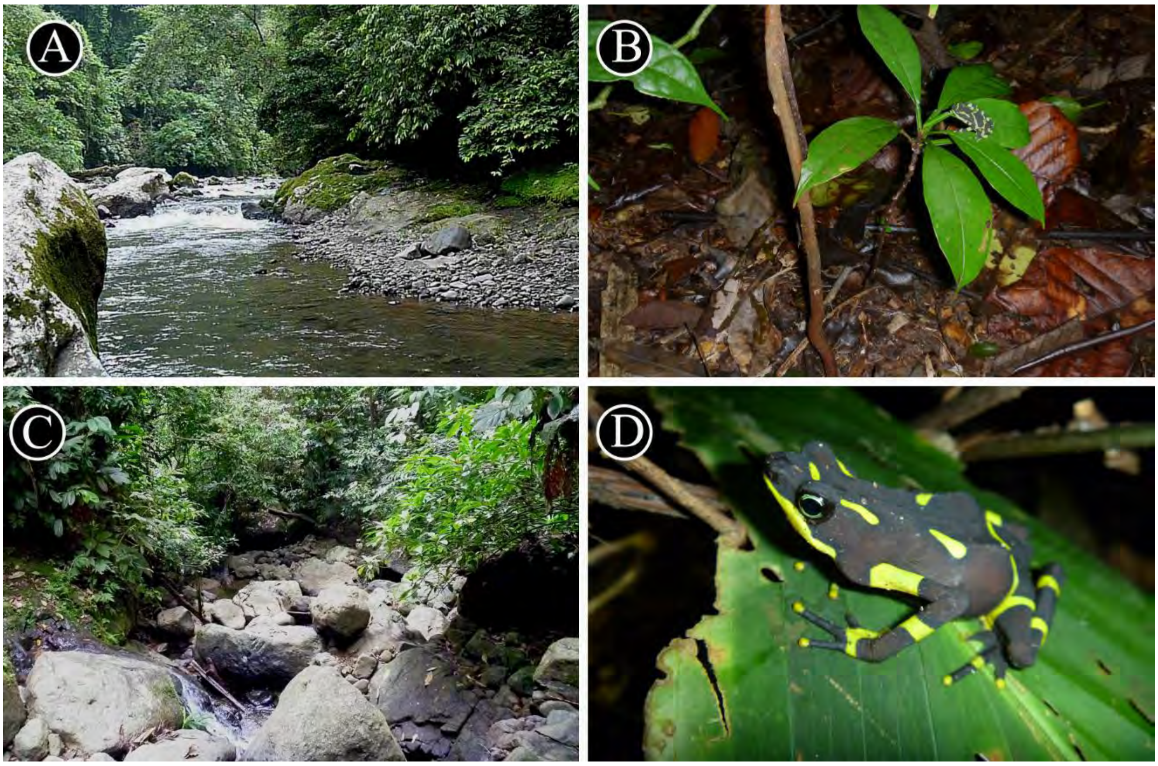
Supplementary Figure S4. Paratype specimens of *Atelopus fronterizo* **sp. nov.** in preservative **A-B)** SMF 97122; **C-D)** MHCH 2655; **E-F)** MHCH 2657.



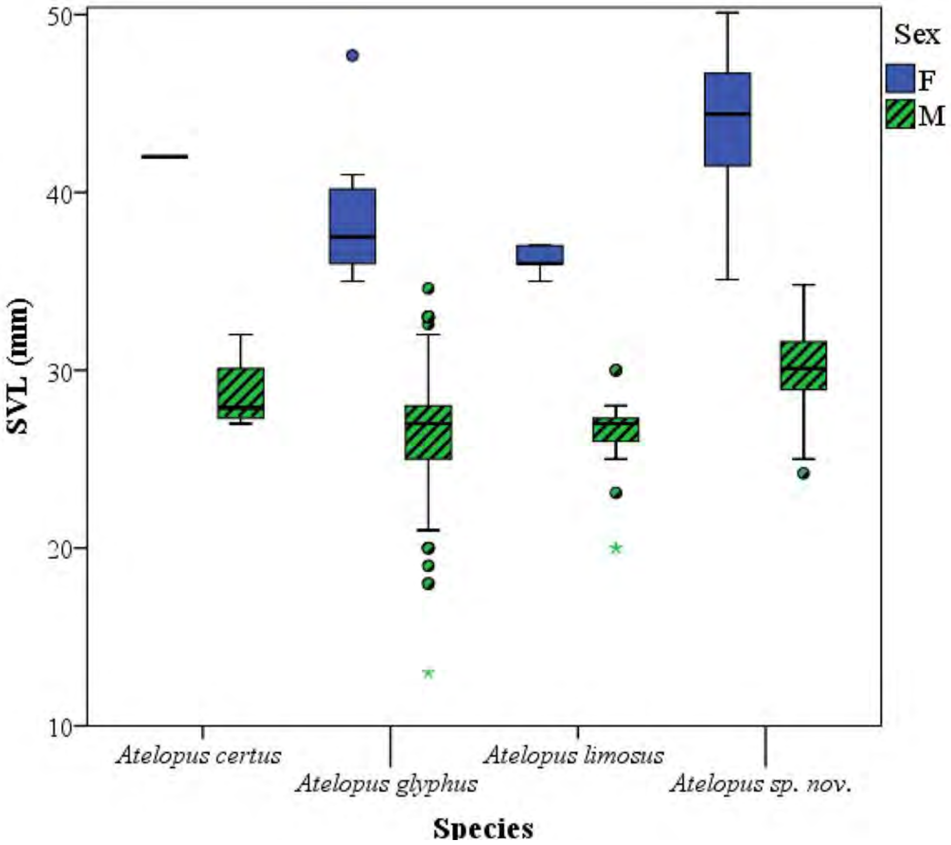
Supplementary Figure S5. Color variation of *Atelopus fronterizo* **sp. nov.** in life: A) Paratype, Pechito Parao near Tuquesa river (MHCH 2657); B) Male, from Nurra Caribe (not collected); C) Paratype, Nurra Caribe (MHCH 2655); D) Amplexus from Nurra Caribe (not collected).



Supplementary Figure S6. Oscillograms (above) and spectrograms (below) of advertisement calls of six species of *Atelopus* from Panama. A) *Atelopus fronterizo* **sp. nov.** (from San Blas Mountain range, 3 October 2021, 09:49 h, 339 m asl, 25.5 °C; B) *A. glyphus* (from Darien National Park, 250 m asl, 12 December 2019,↰ ↱ 11:13 h); C) *A. certus* (from Garachiné, Chepigana, Darién, Panamá, 200 m asl, 10 April 2014, 18:00 h, 28.1 °C; D) *A. limosus*, (from Panama Central, provided by R. Ibañez); E) *A. varius* (from Panama highlands, provided by E. Griffith); F) *A. zeteki* (in captivity at El Valle Amphibian Conservation Center, EVACC). See Supplementary Notes 1 for more details.



Supplementary Figure S7. Habitat of *Atelopus fronterizo* **sp. nov.** A) Type locality at Pucuro River; B) Pechito Parado Mount, at Rio Tuquesa, male found at night in understory, at least 1 km from nearest stream; C) Nurra Caribe, Taintidu stream; D) Female from Nurra Caribe, photographed at night near Taintidu stream.



Supplementary Figure S8. Differences in snout-vent length (SVL) of *Atelopus* species from eastern Panama, separated by sex. Bottom and top of box are first and third percentiles respectively, and band inside box represents median, whiskers show extreme values; circles and asterisk above or below boxes represent outliers. *Atelopus fronterizo* **sp. nov.** *n*: females=13, males=37*; A. glyphus, n*: females=10, males=124; *A. certus*, *n*: females=1, males=4; *A. limosus*, *n*: females=5, males=22.

Supplementary Tables

Supplementary Table S1. Numbers of voucher specimens included in morphological analysis or comparison. Specimens not collected were captured, measured, and released under a monitoring project. Those with field numbers were documented photographically.

Museum number	Field number	Species	Province	Locality
Not collected		*Atelopus certus*	Darién	Qda. Perresenico
SMF97118	AB401	*Atelopus certus*	Darién	Rio San Antonio, Cerro Sapo.
Not collected		*Atelopus certus*	Darién	Cerro Sapo
Not collected		*Atelopus certus*	Darién	Cerro Sapo
MHCH 2652		*Atelopus certus*	Darién	Pavarando
MHCH 3110	AB 543	*Atelopus fronterizo*	Darién	Rio Púcuro
SMF97122	AB 720	*Atelopus fronterizo*	Darién	Nurra
MHCH2655	AB 733	*Atelopus fronterizo*	Darién	Nurra
MHCH2657	AB 830	*Atelopus fronterizo*	Darién	Río Tuquesa
SMF 97125	AB 911	*Atelopus fronterizo*	Darién	Río Tuquesa
AMNH 104422		*Atelopus fronterizo*	Darién	Malí
AMNH 104427		*Atelopus fronterizo*	Darién	Malí
AMNH 104428		*Atelopus fronterizo*	Darién	Malí
AMNH 104429		*Atelopus fronterizo*	Darién	Malí
AMNH 104430		*Atelopus fronterizo*	Darién	Malí
AMNH 104431		*Atelopus fronterizo*	Darién	Malí
AMNH 104432		*Atelopus fronterizo*	Darién	Malí
AMNH 104433		*Atelopus fronterizo*	Darién	Malí
AMNH 104434		*Atelopus fronterizo*	Darién	Malí
AMNH 104421		*Atelopus fronterizo*	Darién	Malí
AMNH 104423		*Atelopus fronterizo*	Darién	Malí
AMNH 104424		*Atelopus fronterizo*	Darién	Malí
AMNH 104425		*Atelopus fronterizo*	Darién	Malí
AMNH 104426		*Atelopus fronterizo*	Darién	Malí
AMNH 104435		*Atelopus fronterizo*	Darién	Río Púcuro
AMNH 104436		*Atelopus fronterizo*	Darién	Río Púcuro
AMNH 104437		*Atelopus fronterizo*	Darién	Río Púcuro
AMNH 104438		*Atelopus fronterizo*	Darién	Río Púcuro
AMNH 104438		*Atelopus fronterizo*	Darién	Río Púcuro
AMNH 39764		*Atelopus fronterizo*	Darién	Sucubtí
AMNH 40901		*Atelopus fronterizo*	Darién	Sucubtí
AMNH 39762		*Atelopus fronterizo*	Darién	Sucubtí
AMNH 39763		*Atelopus fronterizo*	Darién	Sucubtí
AMNH 40506		*Atelopus fronterizo*	Darién	Sucubtí
AMNH 40520		*Atelopus fronterizo*	Darién	Sucubtí
AMNH 40521		*Atelopus fronterizo*	Darién	Sucubtí
AMNH 40535		*Atelopus fronterizo*	Darién	Sucubtí
AMNH 40889		*Atelopus fronterizo*	Darién	Sucubtí

AMNH 40890		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40891		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40892		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40893		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40894		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40898		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40899		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40900		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40902		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40903		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40904		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40905		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40906		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40907		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40908		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40909		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40910		*Atelopus fronterizo*	Darién	Sucubtí	
AMNH 40912		*Atelopus fronterizo*	Darién	Sucubtí	
Not collected	1	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	2	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	3	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	4	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	5	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	6	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	7	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	8	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	9	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	10	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	11	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	13	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	14	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	15	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	16	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	17	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	18	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	19	*Atelopus glyphus*	Darién	Qda Uruseca	PND
Not collected	20	*Atelopus glyphus*	Darién	Qda Uruseca	PND
SMF 97119	AB 0126	*Atelopus glyphus*	Darién	Qda. Perresenico	
SMF 97120	AB 0153	*Atelopus glyphus*	Darién	Qda. Perresenico	
SMF 97121	AB 1174	*Atelopus glyphus*	Darién	Qda. Perresenico	
	AB 1186	*Atelopus glyphus*	Darién	Qda. Perresenico	
	AB0146	*Atelopus glyphus*	Darién	Qda. Uruseca	
	AB0180	*Atelopus glyphus*	Darién	Qda. Uruseca	
	AB0337	*Atelopus glyphus*	Darién	Qda. Uruseca	
	AB0346	*Atelopus glyphus*	Darién	Qda. Uruseca	
	AB1173	*Atelopus glyphus*	Darién	Cerro Pirre	

SMF97121	AB1174	*Atelopus glyphus*	Darién	Cerro Pirre
	AB1174	*Atelopus glyphus*	Darién	Cerro Pirre
	AB1185	*Atelopus glyphus*	Darién	Cerro Pirre
MHCH2654	AB1186	*Atelopus glyphus*	Darién	Cerro Pirre
	AB1186	*Atelopus glyphus*	Darién	Cerro Pirre
SMF97119	AB126	*Atelopus glyphus*	Darién	Qda Perresenico
MHCH2653	AB127	*Atelopus glyphus*	Darién	Qda Perresenico
SMF97120	AB153	*Atelopus glyphus*	Darién	Qda Perresenico
	AB776	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	M1	*Atelopus glyphus*	Darién	Qda. Almendro
Not collected	M11	*Atelopus glyphus*	Darién	Qda. Almendro
Not collected	M32	*Atelopus glyphus*	Darién	Qda. Almendro
Not collected	PA04	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA07	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA08	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA09	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA10	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA13	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA14	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA16	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA16	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA17	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA19	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA20	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA21	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA22	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA23	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA32	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA34	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA35	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA36	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA38	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA38	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA40	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA42	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA43	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA44	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA45	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA46	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA47	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA48	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA50	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA51	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA53	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA54	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA55	*Atelopus glyphus*	Darién	Qda. Almendro

Not collected	PA55	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PA57	*Atelopus glyphus*	Darién	Qda. Almendro
Not collected	PA58	*Atelopus glyphus*	Darién	Qda. Almendro
Not collected	PA59	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA60	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA61	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA62	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA63	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA64	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA65	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA65	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA66	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA67	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA68	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA69	*Atelopus glyphus*	Darién	Qda. Perresenico
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Not collected	PA73	*Atelopus glyphus*	Darién	Qda. Perresenico
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Not collected	PA76	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PA77	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP102	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PP103	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PP105	*Atelopus glyphus*	Darién	Qda. Uruseca
Not collected	PP111	*Atelopus glyphus*	Darién	Qda. Almendro
Not collected	PP112	*Atelopus glyphus*	Darién	Qda. Almendro
Not collected	PP113	*Atelopus glyphus*	Darién	Qda. Almendro
Not collected	PP114	*Atelopus glyphus*	Darién	Qda. Almendro
Not collected	PP115	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP117	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP118	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP119	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP120	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP121	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP122	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP126	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP127	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP129	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP131	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP132	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP57	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP72	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP73	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP79	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP80	*Atelopus glyphus*	Darién	Qda. Perresenico

Not collected	PP82	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP83	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP84	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP86	*Atelopus glyphus*	Darién	Qda. Perresenico
Not collected	PP94	*Atelopus glyphus*	Darién	Qda Uruseca PND
Not collected	PP95	*Atelopus glyphus*	Darién	Qda Uruseca PND
Not collected	PP98	*Atelopus glyphus*	Darién	Qda Uruseca PND
Not collected	PP99	*Atelopus glyphus*	Darién	Qda Uruseca PND
	AB1375	*Atelopus glyphus*	Darién	Qda. Uruseca
	AB1376	*Atelopus glyphus*	Darién	Qda. Uruseca
	AB1376	*Atelopus glyphus*	Darién	Qda. Uruseca
	AB1377	*Atelopus glyphus*	Darién	Qda. Uruseca
	AB1377	*Atelopus glyphus*	Darién	Qda. Uruseca
	AB1378	*Atelopus glyphus*	Darién	Qda. Uruseca
MHCH2656	AB 984	*Atelopus limosus*	Panama	Río Terable
Not collected	ALI-038	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	ALI-069	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	ALI-101	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	ALI-114	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	Atelopus 01	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	Atelopus 02	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	Atelopus 03	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	Atelopus 04	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	Atelopus 05	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	Atelopus 06	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	CREA 125	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	CREA 138	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	CREA 184	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	CREA 217	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	CREA 242	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	CREA 243	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	CREA 247	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	MP 110	*Atelopus limosus*	Panama	Cocobolo Nature Private

				Reserve
Not collected	MP 111	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	MP 160	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	MP 161	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	MP 165	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	MP 176	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	MP 178	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	MP 201	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve
Not collected	MP 205	*Atelopus limosus*	Panama	Cocobolo Nature Private Reserve

Supplementary Table S2. Tissue samples and sequences (16S, COI) of Panamanian *Atelopus* species used in this study.

Field No.	**Mus. No.**	**Locality**	**species**	**Coordinates**		**Source**	**GenBank accession number**	
				N	**E**		**16S**	***COI***
AB126	SMF97119	Qda Perresenico	Atelopus glyphus	7,99764	-77,71086	This study		MT540972
AB127	MHCH2653	Qda Perresenico	Atelopus glyphus	7,99764	-77,71086	This study	MT560720	MT540973
AB153	SMF97120	Serrania de Pirre.	Atelopus glyphus	7,98746	-77,70778	This study	MT560719	
AB401	SMF97118	Rio San Antonio, Cerro Sapo.	Atelopus certus	8,00447	-78,34854	This study	MT560713	
AB543	MHCH 3110	Río Púcuro	Atelopus fronterizo sp. nov.	8,02908	-77,41318	This study	MT560718	MT540974
AB720	SMF97122	Serrania San Blas	Atelopus fronterizo sp. nov.	9,06105	-77,97971	This study	MT560717	
AB733	MHCH2655	Serrania San Blas	Atelopus fronterizo sp. nov.	9,06105	-77,97971	This study	MT560715	
AB830	MHCH2657	Río Tuquesa, Bajo pequeño	Atelopus fronterizo sp. nov.	8,47553	-77,54883	This study	MT560716	MT540975
AB939	MHCH2652	Pavarandó, Garra garra	Atelopus certus	7,76310	-78,10156	This study	MT560721	MT540976
AB1174	SMF97121	Pirre top (1400); Rancho Frío	Atelopus glyphus	7,94194	-77,70252	This study	MT560712	MT540977
	MHCH 1490	Donoso, Colón, Panama	Atelopus varius			This study	MT560714	MT540979
AB984	MHCH2656	Río Terable, Burbayar	Atelopus limosus	9,28298	-78,98370	This study		MT540978

Supplementary Table S3. List of GenBank sequences (with GenBank accession numbers and their localities) used for phylogenetic analyses.

GenBank Accession Number		**species**	**Locality**	**Departamento**	**Authors**	**Reference**
COI	**16S**					
KC129184.1		*Atelopus_limosus_voucher_CH_9180*	Chagres PN,Cerro Brewster	Chepo	Crawford et al.	Mol Ecol Resour 13 (6), 1005-1018 (2013)
KC129183.1	KC129291.1	*Atelopus_limosus_voucher_CH_6742*				
KC129182.1	KC129290.1	*Atelopus_limosus_voucher_CH_6717*				
KC129181.1	KC129289.1	*Atelopus_limosus_voucher_CH_6694*				
KC014635.1	KC014780.1	*Atelopus_limosus_voucher_EVACC_223*	Chagres PN,Sierra Lorona	Chepo	Crawford et al.	Mol Ecol Resour 13 (6), 1005-1018 (2013)
KC014634.1	KC014779.1	*Atelopus_limosus_voucher_EVACC_222*				
KC014633.1		*Atelopus_limosus_voucher_EVACC_221*				
KC014632.1		*Atelopus_limosus_voucher_EVACC_220*				
KC014631.1	KC014778.1	*Atelopus_limosus_voucher_EVACC_009*				
KC014630.1	KC014777.1	*Atelopus_limosus_voucher_EVACC_008*				
KC014629.1	KC014776.1	*Atelopus_limosus_voucher_EVACC_007*				
KC014628.1	KC014775.1	*Atelopus_limosus_voucher_EVACC_006*				
KC014627.1	KC014774.1	*Atelopus_limosus_voucher_EVACC_005*	Mamoni, Madroño	Chepo		
KC014626.1	KC014773.1	*Atelopus_limosus_voucher_EVACC_004*	Chagres PN,Cerro Brewster	Chepo	Crawford et al.	Mol Ecol Resour 13 (6), 1005-1018 (2013)
KC014625.1	KC014772.1	*Atelopus_limosus_voucher_EVACC_003*				
	KC014771.1	*Atelopus_limosus_voucher_EVACC_002*				
KC014624.1	KC014770.1	*Atelopus_limosus_voucher_EVACC_001*				
KC014623.1	KC014768.1	*Atelopus_limosus_voucher_EVACC_231*				
KC014622.1	KC014767.1	*Atelopus_limosus_voucher_EVACC_229*				
KC014621.1	KC014766.1	*Atelopus_limosus_voucher_EVACC_228*				
KC014620.1	KC014765.1	*Atelopus_limosus_voucher_EVACC_227*				
KC014619.1	KC014764.1	*Atelopus_limosus_voucher_EVACC_226*				
KC014618.1	KC014763.1	*Atelopus_limosus_voucher_EVACC_224*				
	KC014762.1	*Atelopus_limosus_voucher_EVACC_230*				
	KC014769.1	*Atelopus_limosus_voucher_EVACC_232*				
FJ766577.1		*Atelopus_zeteki_voucher_CH_5859*	Omar Torrijos PN	Coclé	Crawford et al.	Proc. Natl. Acad. Sci. U.S.A. 107 (31), 13777-13782 (2010)
FJ766576.1		*Atelopus_zeteki_voucher_CH_5860*				
FJ766575.1		*Atelopus_zeteki_voucher_CH_5862*				
FJ766574.1		*Atelopus_zeteki_voucher_CH_5886*				
FJ766573.1		*Atelopus_zeteki_voucher_CH_5864*				

FJ766572.1		*Atelopus_zeteki_voucher_CH_5871*				
DQ502895.1		*Atelopus_spurrelli_isolate_1275*	Bahía Solano	Chocó, Colombia	Grant et al.	Bull. Am. Mus. Nat. Hist. 299, 1-261 (2006)
DQ502857.1		*Atelopus_zeteki*	Las Filipinas Near Sora	Coclé	Frost et al.	Bull. Am. Mus. Nat. Hist. 297, 1-370 (2006)
	U52780.1	*Atelopus_chiriquiensis_MVZ_AG28*		Chiriquí	Graybeal, A.	Mol Ecol Resour 13 (6), 1005-1018 (2013)

Supplementary Table S4. Male intraspecific variation in basic morphometric characters and ratios among three populations of *Atelopus fronterizo* **sp. nov.** from eastern Panama. Data follow the format Minimum-Maximum (Average±Standard Deviation (SD)). All measurements are in mm, all ratios are in %. For abbreviations, please see Methods section.

Population	**Púcuro (n=3)**	**Malí (n=5)**	**Sucubtí/ Nurra/ Tuquesa (n=29)**
Measurements			
SVL	24.2 – 31.6 (28.4 ± 3.8)	31.9 – 34.8 (33.8 ± 1.2)	25.6 – 34.1 (30.0 ± 2.1)
HW	7.2 – 8.8 (8.0 ± 0.8)	9.3 – 10.0 (9.7 ± 0.3)	7.5 – 9.4 (8.7 ± 0.4)
HL	7.2 – 8.5 (7.8 ± 0.7)	8.4 – 9.5 (8.9 ± 0.4)	6.2 – 9.3 (7.8 ± 0.7)
IND	2.1 – 2.8 (2.5 ± 0.3)	2.8 – 3.6 (3.1 ± 0.3)	2.4 – 3.5 (2.9 ± 0.2)
IOID	2.3 – 2.9 (2.6 ± 0.3)	2.7 – 3.5 (3.3 ± 0.3)	2.3 – 3.4 (2.8 ± 0.2)
EYDM	2.3 – 3.0 (2.7 ± 0.3)	2.5 – 3.8 (3.2 ± 0.4)	2.1 – 3.6 (2.7 ± 0.3)
EYND	2.5 – 3.0 (2.7 ± 0.2)	2.6 – 3.8 (3.1 ± 0.3)	2.3 – 3.2 (2.7 ± 0.2)
TIBL	12.1 – 17.0 (14.0 ± 2.7)	14.5 – 17.0 (16.3 ± 0.9)	12.3 – 15.7 (13.8 ± 0.7)
FTL	10.3 – 14.0 (12.0 ± 1.9)	12.3 – 15.4 (14.0 ± 1.1)	9.4 – 13.8 (11.0 ± 0.9)
FAL	7.4 – 10.4 (8.5 ± 1.7)	9.3 – 10.6 (10.3 ± 0.5)	7.8 – 9.5 (8.5 ± 0.4)
THBL	1.8 – 2.8 (2.3 ± 0.5)	2.7 – 3.2 (3.0 ± 0.2)	1.9 – 2.8 (2.3 ± 0.3)
HAL	6.4 – 8.8 (7.3 ± 1.1)	8.2 – 9.4 (9.0 ± 0.4)	6.6 – 8.5 (7.2 ± 0.4)
Ratios			
HW/SVL	27.1 – 29.7 (28.2 ± 1.3)	27.9 – 29.4 (28.7 ± 0.6)	27.2 – 34.0 (29.1 ± 1.5)
EYND/HW	31.3 – 35.9 (33.8 ± 2.4)	28.2 – 31.0 (29.9 ± 1.1)	26.5 – 36.0 (30.9 ± 2.6)
TIBL/SVL	41.0 – 54.0 (49.4 ± 7.3)	45.3 – 49.6 (47.7 ± 1.6)	41.5 – 55.6 (46.4 ± 3.3)
HAL/SVL	22.0 – 27.7 (25.3 ± 2.9)	25.8 – 26.8 (26.4 ± 0.5)	21.9 – 27.9 (23.9 ± 1.6)
THBL/SVL	6.1 – 9.0 (8.0 ± 1.7)	8.4 – 9.5 (8.9 ± 0.4)	6.3 – 10.7 (8.0 ± 1.1)
IND/IOID	89.7 – 103.8 (95.5 ± 7.4)	82.4 – 108.6 (96.8 ± 10.8)	85.5 – 121.6 (103.0 ± 9.4)
HL/HW	95.2 – 106.3 (100.5 ± 5.6)	85.5 – 95.7(92.4 ± 4.0)	70.7 – 106.5 (90.2 ± 9.4)
FTL/SVL	34.9 – 42.7 (39.9 ± 4.4)	37.5 – 45.0(41.2 ± 3.1)	32.1 – 44.2 (36.9 ± 3.1)
HAL/SVL	25.8 – 32.9 (29.8 ± 3.7)	29.3 – 31.3 (30.4 ± 0.8)	25.0 – 35.6 (29.0 ± 2.3)

Supplementary Table S5. Female intraspecific variation in basic morphometric characters and ratios among three populations of *Atelopus fronterizo* **sp. nov.** (no female was available for populations from Nurra and Río Tuquesa). Data follow the format Minimum-Maximum (Average±SD). All measurements are in mm, all ratios are in %. For abbreviations, please see Methods section.

Population	**Púcuro (n=2)**	**Malí (n=9)**	**Sucubtí (n=2)**
Measurements			
SVL	43.2– 44.4 (43.8 ± 0.9)	41.4 – 50.1 (45.4 ± 2.9)	35.1 – 38.3 (36.7 ± 2.2)
HW	10.5– 11.2 (10.8 ± 0.5)	11.0 – 12.0 (11.5 ± 0.4)	10.1 – 11.1 (10.6 ± 0.7)
HL	8.7 – 10.8 (9.8 ± 1.5)	10.0 – 11.4 (10.6 ± 0.5)	10.0 – 10.7 (10.4 ± 0.5)
IND	3.3 – 3.3 (3.3 ± 0.0)	3.2 – 4.0 (3.6 ± 0.3)	3.4 – 3.6 (3.5 ± 0.1)
IOID	4.4 – 4.7 (4.6 ± 0.2)	3.6 – 4.5 (4.2 ± 0.3)	3.0 – 3.6 (3.3 ± 0.5)
EYDM	2.8 – 3.5 (3.2 ± 0.5)	3.1 – 4.0 (3.6 ± 0.3)	2.7 – 3.4 (3.1 ± 0.5)
EYND	3.0 – 3.9 (3.5 ± 0.7)	3.4 – 4.5 (3.9 ± 0.3)	3.0 – 3.6 (3.3 ± 0.4)
TIBL	19.0 – 23.6 (21.3 ± 3.3)	18.9 – 23.0 (20.6 ± 1.4)	19.4 – 19.7 (19.5 ± 0.2)
FTL	16.4 – 20.4 (18.4 ± 2.8)	16.9 – 21.2 (18.8 ± 1.5)	16.1 – 19.8 (18.0 ± 2.6)
FAL	13.8 – 14.1 (14.0 ± 0.2)	12.3 – 14.8 (13.4 ± 0.8)	11.9 – 11.9 (11.9 ± 0.0)
THBL	3.5 – 4.6 (4.0 ± 0.8)	3.5 – 5.0 (4.0 ± 0.5)	3.7
HAL	10.2 – 12.5 (11.4 ± 1.6)	10.2 – 12.6 (11.7 ± 0.8)	not available
Ratios			
HW/SVL	24.3 – 25.2 (24.7 ± 0.6)	23.4 – 26.8 (25.4 ± 1.1)	28.8 – 29.0 (28.9 ± 0.1)
EYND/HW	28.6 – 35.2 (31.9 ± 4.7)	28.5 – 38.8 (33.5 ± 2.8)	29.9 – 32.0 (31.0 ± 1.5)
TIBL/SVL	44.0 – 53.2 (48.6 ± 6.5)	40.5 – 49.2 (45.4 ± 2.8)	50.7 – 56.0 (53.4 ± 3.7)
HAL/SVL	23.6 – 28.2 (25.9 ± 3.2)	22.7 – 27.2 (25.7 ± 1.5)	not available
THBL/SVL	8.0 – 10.4 (9.2 ± 1.6)	8.1 – 9.9 (8.8 ± 0.6)	9.6
IND/IOID	83.0 – 96.5 (89.8 ± 9.6)	73.3 – 104.0 (85.4 ± 8.8)	99.5 – 115.3 (107.4 ± 11.2)
HL/HW	83.0 – 96.5 (89.8 ± 9.6)	86.1 – 97.1 (92.0 ± 3.2)	96.5 – 98.9 (97.7 ± 1.7)
FTL/SVL	46.0 – 46.0 (46.0 ± 0.0)	37.7 – 44.5 (41.4 ± 2.2)	42.1 – 56.4 (49.2 ± 10.1)
HAL/SVL	31.1 – 32.7 (31.9 ± 1.2)	27.3 – 31.6 (29.4 ± 1.6)	31.2 – 33.8 (32.5 ± 1.9)

Supplementary Table S6. Spectral and temporal parameters of advertisement call of *Atelopus* spp. from eastern Panama. No specimen was measured or collected. Measurements are: Mean±SD (range; n). *data from Ibáñez et al. (1995). ** data from a single call (see Methods for details on specimens).

Trait	***A. fronterizo* sp. nov. (25.5 °C)**	***A certus* (28.1 °C)**
Call duration (msec)	209.8 ± 20.3 (176–235; 8)	296.7 ± 46.1 (163–351; 14)
Call interval (msec)	3036.3 ± 521.3 (2504–3878; 7)	2602.2 ± 734.5 (1519–4052; 13)
Pulse rate (pulses/sec)	131.7 ± 16.4 (97.4–144.7; 7)	132.3 ± 3.3 (122.7–136.8; 14)
Pulses #	28 ± 5.5 (19–34; 7)	39.6 ± 6.7 (20–48; 14)
Low Freq (Hz)	2367.1 ± 134.5 (2114.2–2547.9; 8)	2153.2 ± 44.6 (2076.2–2222; 15)
High Freq (Hz)	2685.7 ± 137.6 (2541.5–3006.2; 8)	2863.6 ± 196.3 (2498.3–3010.4; 15)
Dom Freq (Hz)	2505.9 ± 66.1 (2422.5–2605.5; 8)	2506.5 ± 195.3 (2314.8–2820.8; 15)
Bandwidth (Hz)	318.6 ± 254.4 (116.3-892; 8)	710.4 ± 195.9 (354.5-915.2; 15)
Calls/bouts	5–8 n: 3	2–14; n: 3

Trait	***A. glyphus* (25.7 °C)**	***A. limosus* (26.0 °C) ***
Call duration (msec)	250.1 ± 13.2 (225–272; 14)	260 ± 30 (205–295; 13)
Call interval (msec)	2714.8 ± 663.3 (2140–4606; 13)	
Pulse rate (pulses/sec)	139.4 ± 4.4 (128.9–143.4; 13)	151.6 +– 3.1 (146.4–156.3; 13)
Pulses #	34.9 ± 2.7 (29–38; 13)	40 ± 5 (31–45; 13)
Low Freq (Hz)	2548.9 ± 46.3 (2463.7–2630.7; 14)	2160 ± 49 (2080–2200; 13)
High Freq (Hz)	3172.1 ± 34.7 (3074.5–3200.2; 14)	3409 ± 174 (3080–3760; 13)
Max Freq (Hz)	2988.1 ± 41.8 (2874.7–3025.4; 14)	2689 ± 93 (2600–2800; 13)
Bandwidth (Hz)	623.2 ± 50.1 (528.4-670; 14)	729.1**
Calls/bouts	7–13; n: 2	