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Anolis marsupialis Taylor 1956, a valid species from southern Pacific Costa Rica (Reptilia, Squamata, Dactyloidae)

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Abstract

The examination of the holotype of *Anolis marsupialis* Taylor 1956 along with recently collected specimens reveals that *A. marsupialis* is a valid species. It differs from its closest congeners *A. humilis* Peters 1863 and *A. quaggulus* Cope 1885, in male dewlap coloration, scalation, body size, and hemipenial morphology. These findings are supported by preliminary molecular genetic analysis.

Key words: *Anolis humilis, A. marsupialis*, *A. quaggulus*, synonymy, taxonomy

Introduction

In 1956, Edward H. Taylor described *Anolis humilis marsupialis* based on a male holotype (KU 40893) and seven paratypes (KU 40889–92, 40894–96) from "about 15 km. WSW of San Isidro del General along the Dominical Road.", a collecting site situated in the Fila Costeña. Savage (1974) provided the following comments on the type locality of *A. marsupialis*: "1.5 km NW Alfombra, Cantón de Pérez Zeledón, Provincia de San José: 970 m. E.H. Taylor collected in this vicinity on several occasions. The swamp and meadow that he mentions at 15 km (by road) WSW of San Isidro de El General drains into the Río Pacurito and lies east of the road to Dominical. Premontane Pluvial bioclimate."

This taxon was treated as a subspecies of *A. humilis* Peters 1863 by Peters & Donoso-Barros (1970) but was placed in synonymy of the latter species by Savage & Villa (1986), Savage (2002), and Köhler *et al.* (2003, 2006). Wilson & Johnson (2010) briefly discussed this issue, and, just as Sasa *et al.* (2010), retained *A. marsupialis* in the synonymy of *A. humilis*. Bolaños & Savage (2009) and Bolaños et al. (2011) treated this form as a full species, pointing to Taylor (1956) for a diagnosis.

Recent field work in the area of the type locality of *Anolis humilis marsupialis* produced additional specimens that allow for reconsideration of this taxonomic issue.

Material and methods

Before preservation, the dewlap of male individuals was extended with small forceps, and the extended dewlap was photographed in life. Sampled specimens were euthanized by injection of T61 (Intervet Deutschland GmbH, Unterschleißheim, Germany). Tissue samples were taken from the forearm, tail tip, or liver and stored in Eppendorf tubes filled with 96% ethanol. Samples were stored at -20 °C after return from the field. In males, hemipenes were everted by manually applying gentle pressure to the base of the tail. This, however, results only in partial eversion of the organs. In order to reach full eversion, 70% ethanol was injected into the hemipenis pockets just posterior to

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the cloacal opening with a fine injection needle. To avoid partial retraction of the lobes, the specimen was submerged in 70% ethanol for about 30 seconds before removing the injection needle. Preparation and preservation of the specimens follow Köhler (2001).

A list of the comparative specimens examined is provided in Appendix I. Abbreviations for museum collections follow Sabaj Pérez (2010). Nomenclature of scale characters follows Köhler (2008). 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. Dorsal and ventral scales were counted along the midline of the body. 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 4^{th} toe.

For analyses of the mitochondrial 16S gene, DNA extractions were carried out by vacuum extraction using a Pall vacuum extraction Kit (Pall GmbH, Dreieich, Germany) and following the instructions of the manufacturer. DNA concentration was checked electrophoretically. A 532 bp fragment of the mitochondrial 16S gene was amplified by PCR, using primers as published by Vences *et al.* (2005). PCR reactions were run with 1 µL DNA template, 2.5 µL Reaction Buffer (PeqGold), 2.5 µL 2.5 mM dNTPs, 0.5 µL Taq Polymerase (PeqLab), 16.5 µL H_2O, and 10 pmol of each primer. Reaction mixes were processed by the following protocol: Initial denaturation, 180 s at 94 °C; denaturation, 15 s at 94 °C; hybridization, 60 s at 51 °C; elongation, 60 s at 72 °C; repeated for 39 cycles; final elongation, 120 s at 72 °C. PCR products were sent for sequencing to the Grunelius-Möllgaard-Laboratory for Molecular Evolution at the Senckenberg Research Institute, Frankfurt am Main, Germany.

Sequences were automatically aligned applying the ClustalW algorithm (Larkin *et al.,* 2007) as implanted in MEGA 5 software (Tamura *et al.* 2011), and alignments were checked manually. A matrix of uncorrected, pairwise distances was computed. Gaps were deleted pairwise. The alignment consisted of two sequences of specimens from the vicinity of the type locality of *Anolis marsupialis* (SMF 76030, 76031) and specimens of *A. humilis* (SMF 96125) and *A quaggulus* (SMF 96101) from other localities that were obtained in another study. The Genbank accession numbers of these sequences are provided in Appendix I.

We also performed a phylogenetic analysis of data from the mitochondrial COI gene including specimens from the type locality of *Anolis marsupialis*, specimens of *Anolis humilis* from three localities, eight additional Central American *Norops* clade *Anolis*, and *Anolis sagrei*. Tissues for this analysis were preserved in 95% ethanol and sent to the Barcode of Life Data Systems (BOLD; Ratnasingham & Hebert 2007) for COI sequencing and alignment at the Canadian Center for DNA Barcoding (CCDB). The CCDB alignment was checked using the codon models of Muscle in MEGA. Sequences were analyzed using maximum likelihood in RaxML (GUI; Silvestro & Michalak 2012). The GTRGAMMA model was used with separate models for each codon position. An "ML + thorough bootstrap" analysis was run with 100 repetitions. The resulting trees were rooted on *A. sagrei*.

Results

Our recently collected specimens of *Anolis humilis*-like anoles from the Pacific versant of southern Costa Rica (hereafter referred to as *A. marsupialis*) differ in several morphological characters from *A. humilis* and *A. quaggulus* as follows: Adult males of *A. marsupialis* have a red dewlap with several dark brownish red semicircular streaks (versus dewlap orange-red with a yellowish orange or greenish yellow margin in *A. humilis* and *A. quaggulus*; Fig. 1). *Anolis marsupialis* has about 10 greatly enlarged middorsal scale rows, the median two rows larger than or subequal to the adjacent rows (versus moderately enlarged middorsal scale rows, the median rows with scattered scales that are smaller than paravertebral scales; Fig. 2). The completely everted hemipenis of *A. marsupialis* is a medium-sized stout bilobed organ with well-developed, bulbous lobes (versus a large bilobed organ with well-developed, elongate lobes in *A. humilis*; and a small, slightly bilobed organ with rudimentary lobes in *A. quaggulus*; Fig. 3). The axillary pocket is partly covered with scales and pigmented (versus without scales and unpigmented in *A. humilis* and *A. quaggulus*; Fig. 4). Finally, males of *A. marsupialis* show a distinct a nuchal crest and a dorsal ridge (see Fig. 5) when handled (versus no such fold in *A. humilis* and *A. quaggulus*).

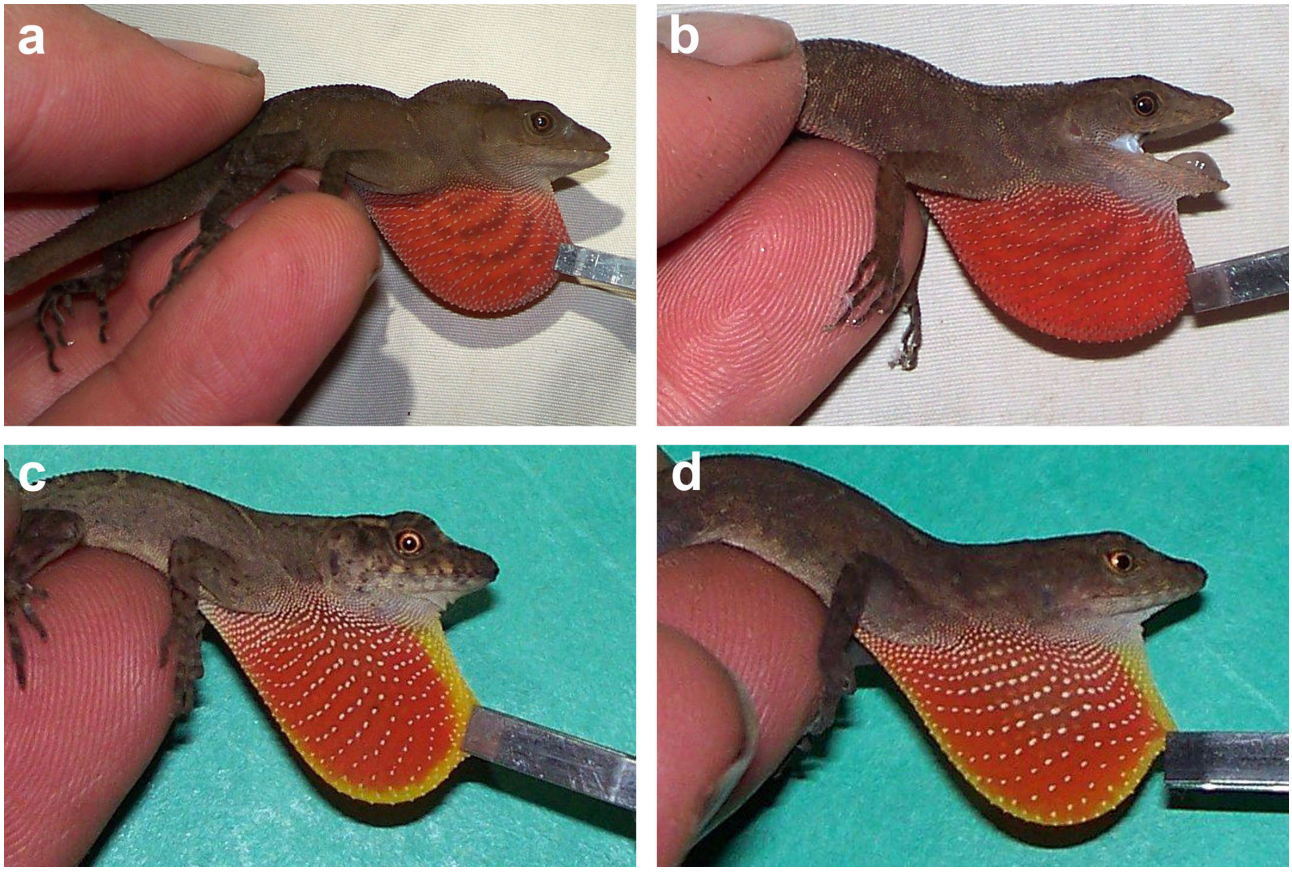

FIGURE 1. Adult males with extended dewlaps in life (a) *Anolis marsupialis* SMF 76030; (b) *A. marsupialis* SMF 76031; (c) *A. humilis* SMF 96188; (d) *A. quaggulus* SMF 96157.

The comparison of the 532 bp fragment of the mitochondrial 16S gene revealed a high degree of genetic divergence between *Anolis marsupialis* and its assumed closest relatives *A. humilis* and *A. quaggulus.* The uncorrected p-distance between sequences of *A. marsupialis* and *A. humilis* was 0.129. Distances between *A. marsupialis* and *A. quaggulus* were 0.108 and 0.112, respectively. The p-distance values between the samples of *A. humilis* and *A. quaggulus* were notably lower (0.027).

Phylogenetic analysis of COI resulted in reciprocal monophyly of *A. marsupialis* and *A. humilis* (Fig. 6). This sample of *A. humilis* includes individuals from north (Monteverde, Costa Rica) and south (Fortuna pass, Panama; El Copé, Panama) of the type locality of *A. marsupialis*. Average COI p-distance between samples of *A. marsupialis* and *A. humilis* was 0.175.

Based on the morphological and genetic evidence presented above, we recognize *A. marsupialis* as specifically distinct from *A. humilis* and *A. quaggulus*. In morphometrics and scalation, our specimens of *A. marsupialis* agree well with the description provided by Taylor (1956). However, Taylor described the male dewlap coloration in life of *A. marsupialis* as "dark reddish with a yellow border" (Taylor 1956:99), which is typical for *A. humilis* and *A. quaggulus*, but unlike the dewlap of our specimens of *A. marsupialis* (Fig. 1; see above). Either the dewlap in this taxon shows considerable variation, including a coloration typically observed in *A. humilis* and *A. quaggulus*, or this statement of Taylor is simply an error. In the three adult males of *A. marsupialis* for which the dewlap coloration in life was documented, variation was negligible (see also Fig. 1a,b). The type locality of *A. marsupialis* is just a few kilometers away from the place where we collected our series. Thus, despite the discrepancy in dewlap coloration, we feel confident that our specimens have to be referred to as *A. marsupialis*. We support the assessment of Bolaños *et al.* (2011) that *A. marsupialis* represents a distinct species, for which we provide the following description.

Anolis marsupialis is a small anole (maximum recorded SVL 48.4 mm in males, 45.4 mm in females); dorsal head scales in internasal region keeled, in prefrontal, parietal, and frontal areas mostly keeled, some rugose (Fig. 7c); a moderately deep frontal depression and a shallow parietal depression present; 6–8 postrostrals; anterior nasal

divided, the lower scale in contact with rostral and first supralabial (Fig. 7b); 5–7 internasals; canthal ridge sharply defined; scales comprising supraorbital semicircles moderately keeled, largest scale in semicircles as large as largest supraocular scale; supraorbital semicircles weakly defined; 2–3 scales separating supraorbital semicircles at narrowest point; 2–4 scales separating supraorbital semicircles and interparietal at narrowest point; interparietal well defined, greatly enlarged relative to adjacent scales, surrounded by scales of moderate size, longer than wide, usually smaller than ear opening; enlarged supraoculars separated from supraorbital semicircles by a complete row of small scales; 2–3 elongate superciliaries, anterior one longest; 2–3 enlarged canthals; 9–10 scales between second canthals; 11–13 scales present between posterior canthals; loreal region slightly concave, 30–33 mostly keeled (some tuberculate) loreal scales in 4–7 horizontal rows; 6–7 supralabials to level below center of eye; suboculars keeled, separated from supralabials by a complete row of keeled scales; ear opening vertically oval; scales anterior to ear opening keeled granulars, slightly larger than those posterior to ear opening; 4–8 postmentals, outer pair about twice as large as median postmental scales; sublabials not differentiated; keeled, elongate granular scales present on chin and throat; male dewlap large, extending onto chest; 13–14 horizontal gorgetal-sternal rows with 19–24 scales per row; female dewlap very small; an erectable nuchal crest and a dorsal ridge present in males when aroused; about 8–12 middorsal scale rows enlarged, distinctly keeled, scales of median two rows larger than or of same size as scales of adjacent rows (Figs. 2a,b); flank scales homogeneous; 25–30 dorsal scales along vertebral midline between levels of axilla and groin; 18–21 dorsal scales along vertebral midline contained in one head length; scales on midventer keeled, subimbricate, smaller than dorsal scales; 34–38 ventral scales along midventral line between levels of axilla and groin; 26–28 ventral scales contained in one head length; 72–80 scales around midbody; tube-like axillary pocket well developed, partly covered with scales and pigmented; precloacal scales tuberculate; no enlarged postcloacal scales present; tail laterally compressed in cross section. Basal subcaudal scales keeled; lateral caudal scales keeled; dorsal medial caudal scale row keeled, not enlarged, not forming a crest; scales on lateral surface of antebrachium keeled, subimbricate; 17–20 subdigital lamellae on Phalanges ii–iv of 4th toe of hind limbs. Longest toe of adpressed hind leg reaches nostril (in SMF 76030), anterior border of eye (SMF 76028 and SMF 76031), or center of eye (SMF 76029). For variation in selected scalation and morphometric characters see Table 1.

TABLE 1. Selected measurements, proportions and scale characters *Anolis humilis, A. quaggulus,* and *A. marsupialis.* Range is followed by mean and standard deviation in parentheses. For abbreviations see text.

		A. humilis ♂ 31 ♀ 16	*A. quaggulus* ♂ 26 ♀ 19	*A. marsupialis* ♂ 24 ♀ 9
maximum SVL	males	42	40.4	48.4
	females	48	43.7	45.4
tail length / SVL	males	1.26–1.79 (1.59±0.14)	1.35–1.73 (1.53±0.10)	1.14–1.62 (1.43±0.19)
	females	1.31–1.55 (1.43±0.10)	1.30–1.49 (1.40±0.07)	1.17–1.43 (1.33±0.11)
HL / SVL	males	0.24–0.33 (0.28±0.02)	0.23–0.33 (0.27±0.02)	0.24–0.33 (0.28±0.02)
	females	0.23–0.30 (0.27±0.02)	0.22–0.30 (0.26±0.02)	0.24–0.33 (0.28±0.02)
HL / HW	males	1.39–1.65 (1.54±0.06)	1.39–1.66 (1.54±0.06)	1.42–1.68 (1.58±0.06)
	females	1.38–1.72 (1.53±0.09)	1.37–1.63 (1.51±0.07)	1.48–1.61 (1.54±0.04)
ear height / length of interparietal		0.82–3.66 (1.67±0.46)	1.00–2.79 (1.62±0.39)	1.19–2.74 (1.74±0.41)
length / width of interparietal		0.87–2.21 (1.53±0.27)	1.25–3.67 (1.83±0.44)	0.65–4.33 (1.67±0.62)
rows of enlarged dorsal scales		7–12 (9.23±1.00)	7–11 (8.60±1.03)	8–12 (10.09±1.18)
postrostral scales		5–9 (6.96±0.78)	7–10 (7.93±0.81)	6–8 (6.69±0.71)
postmental scales		3–7 (5.21±1.17)	4–8 (6.18±0.81)	4–8 (6.45±1.18)
loreal rows		5–8 (6.09±0.82)	5–9 (6.78±0.88)	4–7 (5.90±0.89)
loreal scales		20–46 (33.43±6.27)	26–52 (38.09±6.60)	16–45 (30.38±6.84)

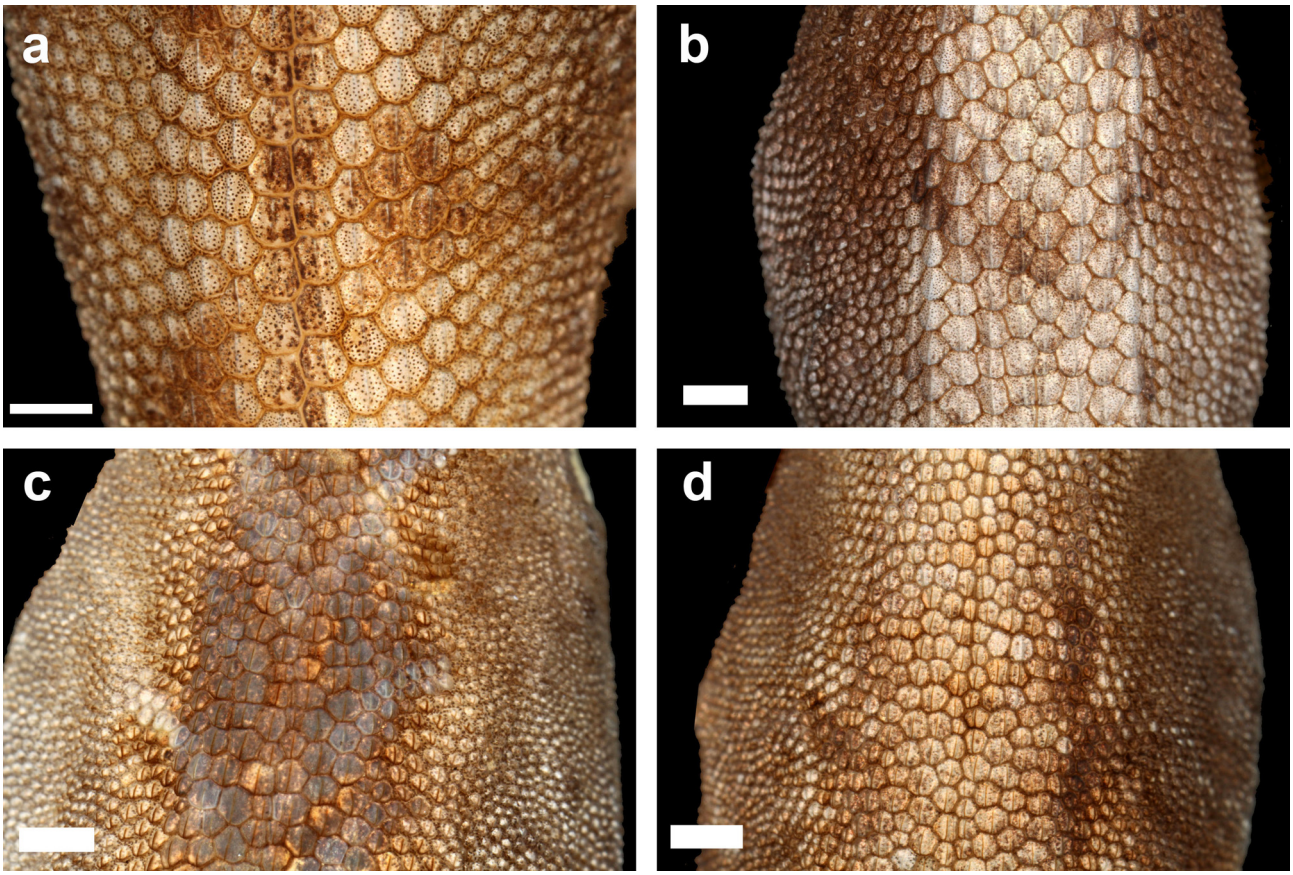

FIGURE 2. Middorsal scalation in (a) *Anolis marsupialis* SMF 76029; (b) *A. marsupialis* SMF 76031; (c) *A. humilis* SMF 96118; (d) *A. quaggulus* SMF 96181. Scale bars equal 1 mm.

The completely everted hemipenis (SMF 76030) is a moderately sized organ with well-developed bulbous lobes; sulcus spermaticus bordered by well-developed lips; on the sulcal side of the apex it opens directly into two sulcal fields that are void of ornamentation; asulcal side of the lobes covered with transverse folds that gradually merge into fine calyces toward the tips; no pronounced asulcal processus present; asulcal side of the truncus with transverse folds.

Distribution. As currently understood, the geographic distribution of *Anolis marsupialis* is restricted to the Pacific versant of southern Costa Rica (Fig 8).

Natural History Notes. In the vicinity of the type locality, *Anolis marsupialis* inhabits semideciduous, premontane rain, and premontane wet forests (Fig. 9). Occasionally, individuals can be found near forest seeps and along small streams, but are more common in the forest interior. The following observations are unpublished field notes from MJR taken at Tinamastes, Costa Rica. *Anolis marsupialis* is a diurnal species found in the leaf litter and on low perches up to 50 cm above the ground. Males tend to be observed on elevated perches more often than females. When perched, males usually have their heads oriented downward towards the leaf litter. Adults are most commonly observed in deep shade near tree buttresses, large rotting logs, or dense stands of palms with deep litter. Rarely are they found more than 1.5 meters from such habitat features. Hatchling lizards have been observed in every month of the wet season (April – November) and juveniles (>20 mm SVL) can be found in every month of the year. Known predators include wolf spiders (family Ctenidae) and the snakes *Bothrops asper, Leptodeira septentrionalis, Nothopsis rugosus, Oxyrhopus petolarius,* and *Rhadinaea decorata.* This species occurs sympatrically with *A. aquaticus*, *A. capito, A. lemurinus,* and *A. polylepis*.

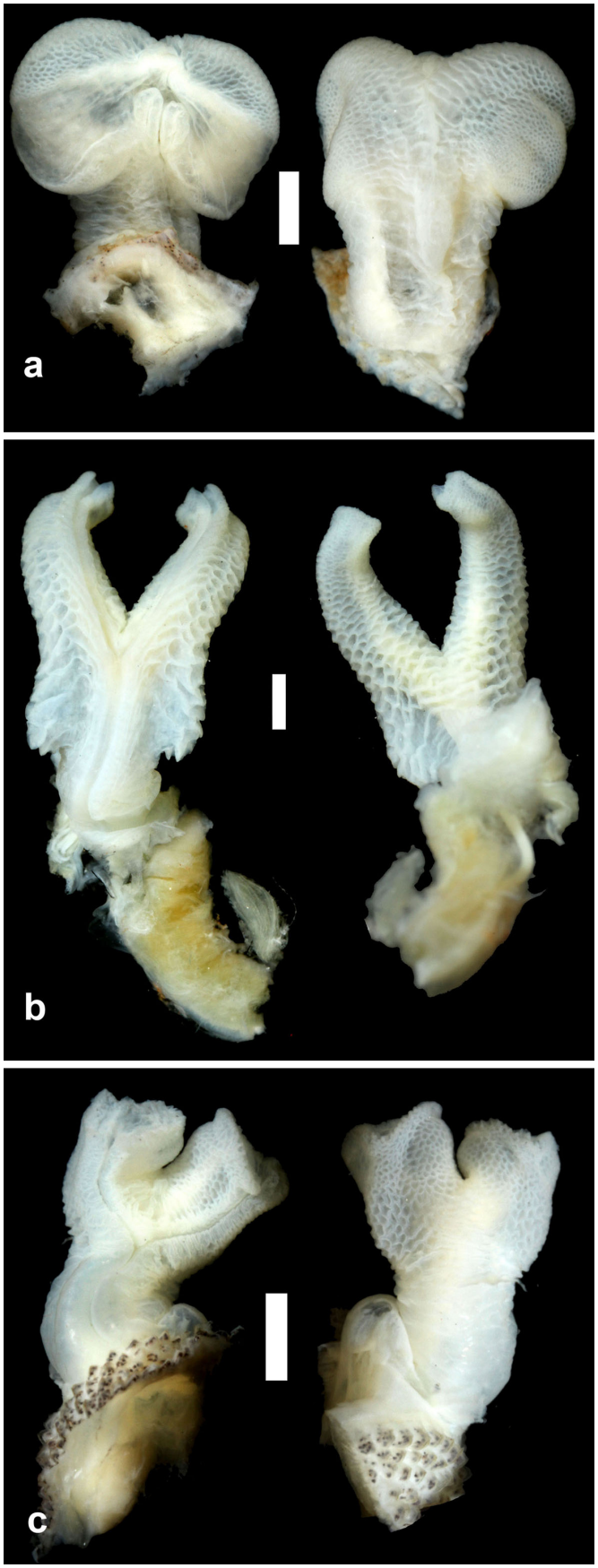

FIGURE 3. Hemipenis in (a) *Anolis marsupialis* SMF 76030; (b) *A. humilis* SMF 80845; (c) *A. quaggulus* SMF 96172. Scale bars equal 1 mm.

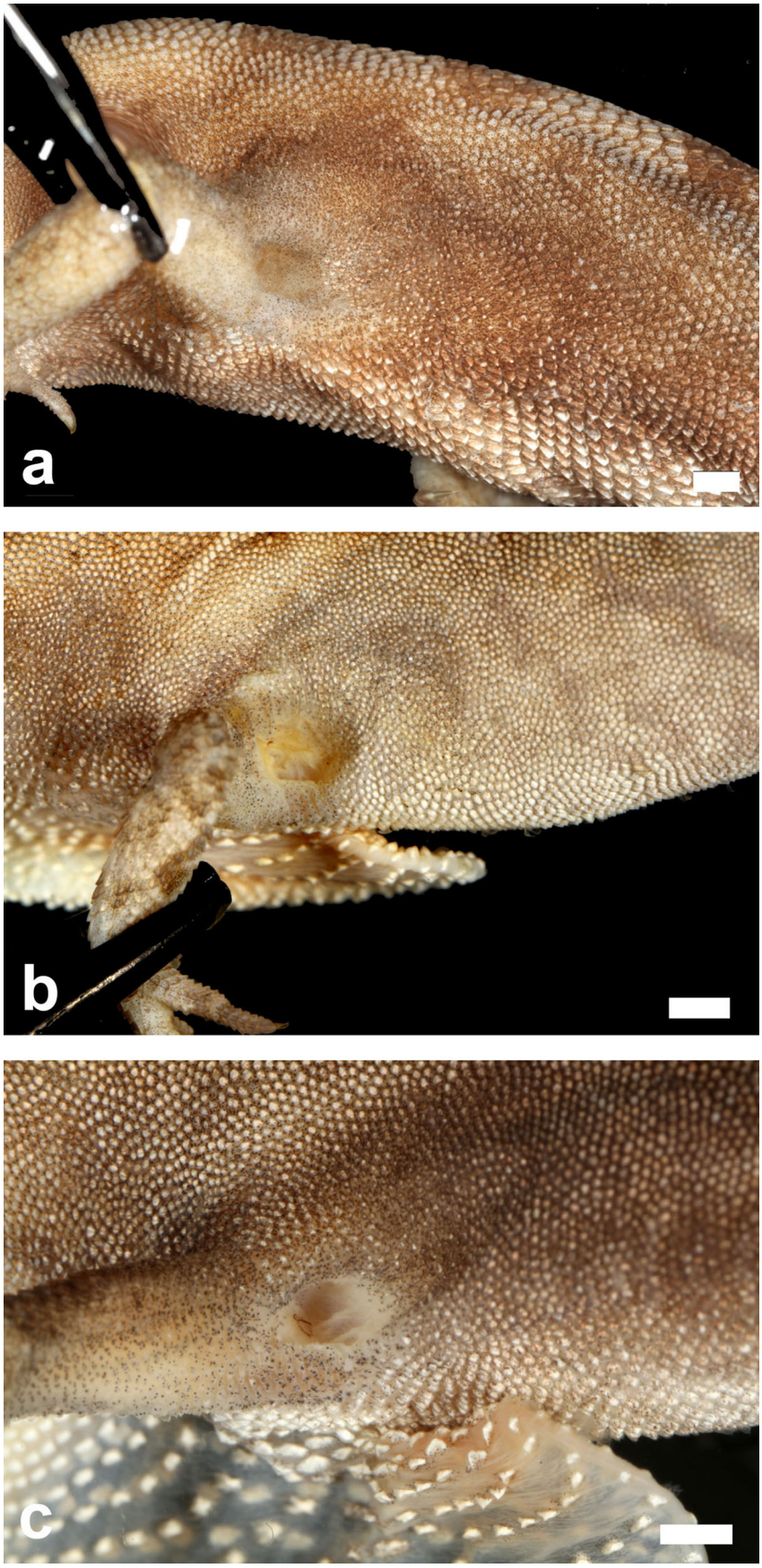

FIGURE 4. Axillary pocket in (a) *Anolis marsupialis* SMF 76031; (b) *A. humilis* SMF 96188; (c) *A. quaggulus* SMF 96181. Scale bars equal 1 mm.

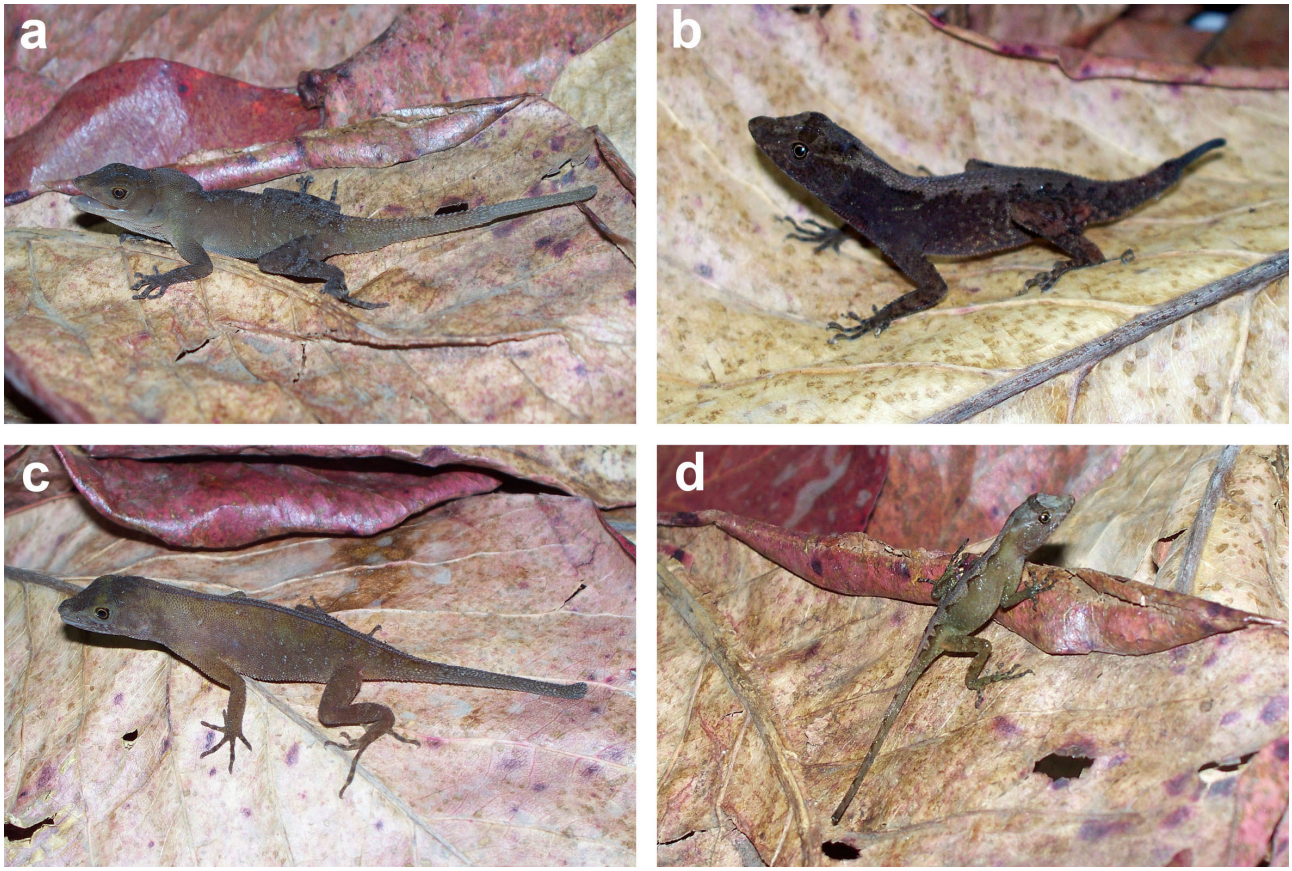

FIGURE 5. *Anolis marsupialis* in life (a) adult male SMF 76030; (b) adult male SMF 76031; (c) adult female SMF 76029; (d) juvenile female SMF 76032.

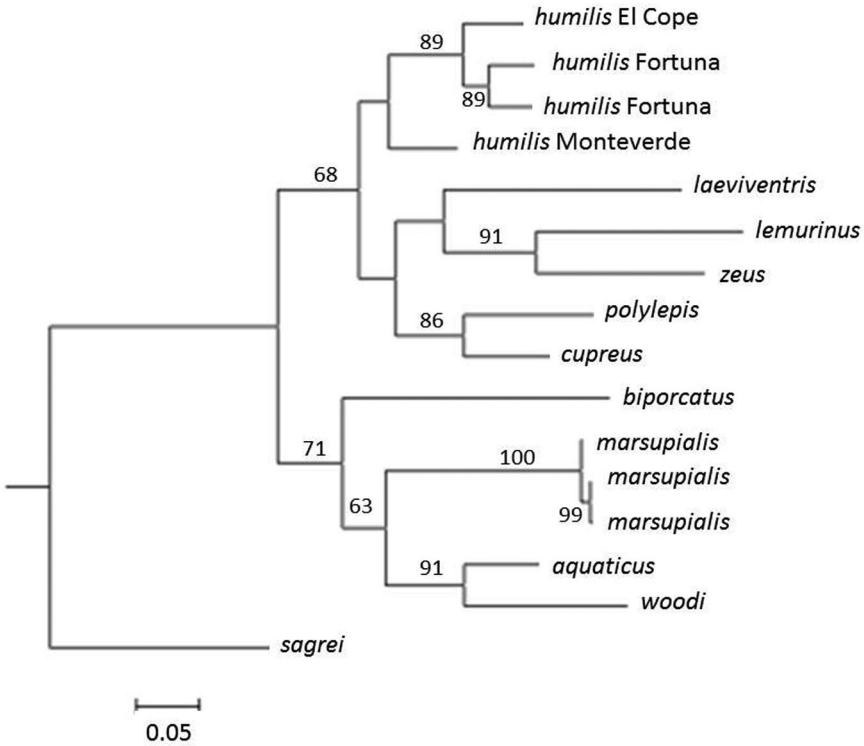

FIGURE 6. Phylogenetic estimate of *Anolis humilis* and other Central American *Anolis* based on maximum likelihood. Numbers on clades are bootstrap values.

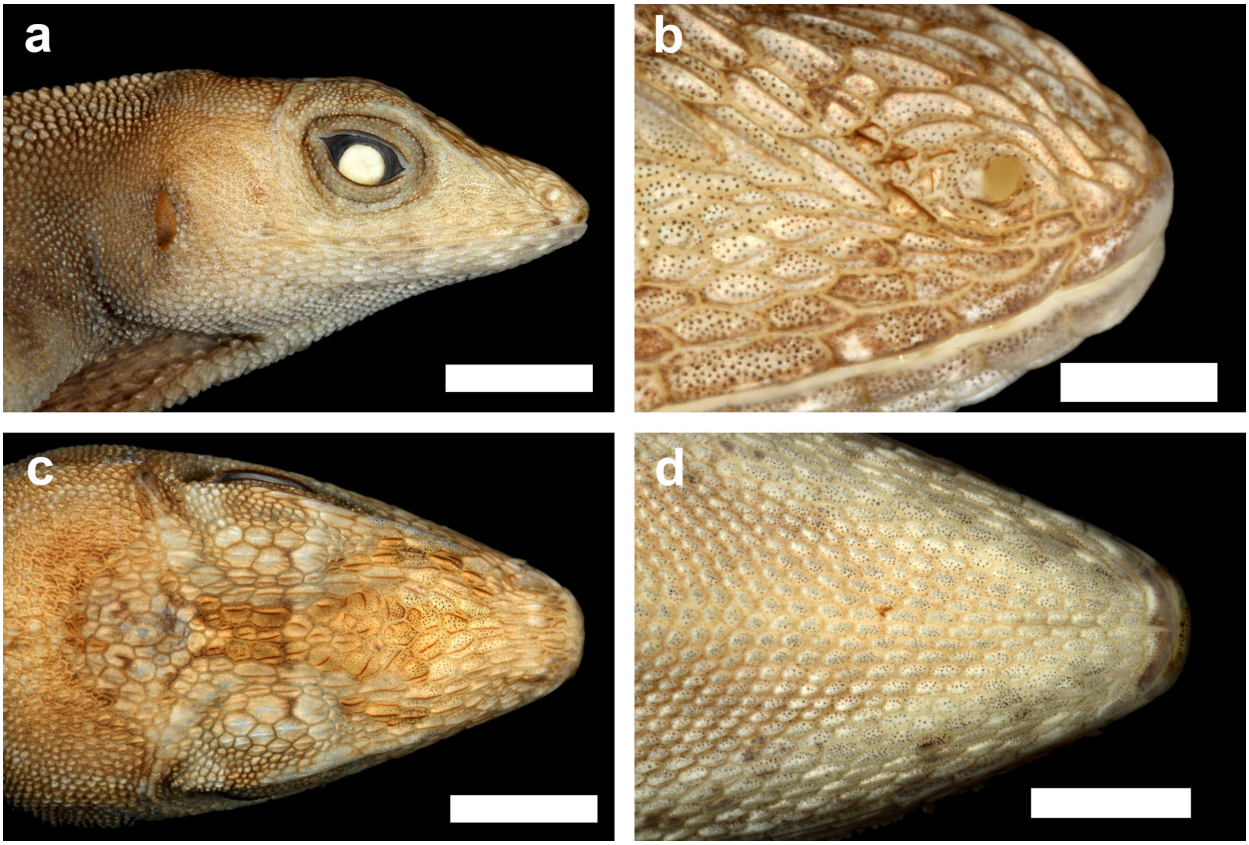

FIGURE 7. Head scalation in *Anolis marsupialis* SMF 76031 (a) lateral view; (b) nasal region; (c) dorsal view; (d) ventral view. Scale bars equal 5 mm in a,c,d, and 1 mm in b.

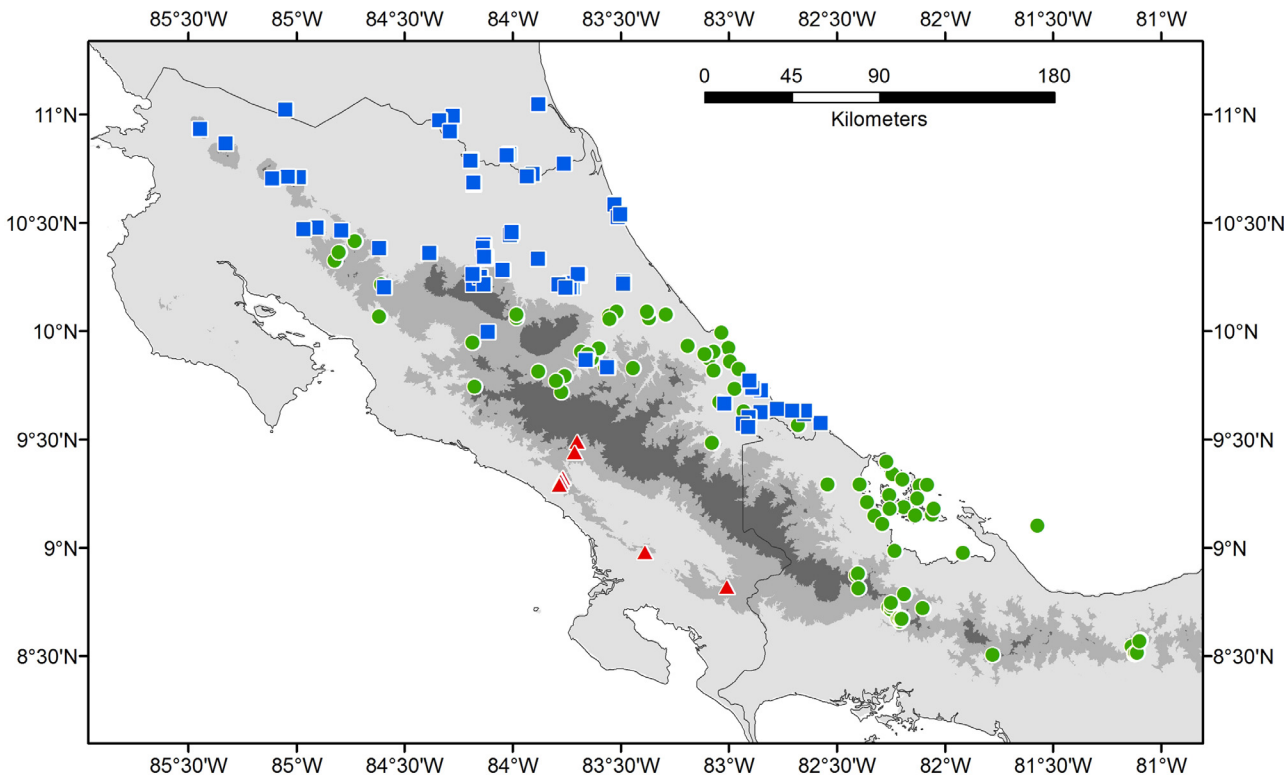

FIGURE 8. Map indicating collecting localities of species related to *Anolis humilis* in southern Nicaragua, Costa Rica, and western Panama. Each symbol can represent one or more adjacent localities. Green circles: *Anolis humilis*; blue squares: *A. quaggulus*; red triangles: *A. marsupialis*. Areas >1000 masl shaded pale gray, those >2000 masl shaded dark gray.

FIGURE 9. Habitat of *Anolis marsupialis* near Platanillo, 710 masl, Province San José, Costa Rica.

Discussion

The most conspicuous morphological differences between *Anolis marsupialis* and its supposedly closest congeners *A. humilis* and *A. quaggulus* are dewlap coloration and hemipenial morphology, characters likely to be sexually selected. However, hemipenial morphology is not accessible in preserved specimens without everted hemipenes. More field work is needed in order to better map the geographic distribution of *A. marsupialis* relative to these forms. According to Savage (1974), the type locality of *A. marsupialis* is about 1.5 km NW of the village Alfombra. We roughly estimated the GPS coordinates of this area to be 9.3167°N, 83.7833°W, which would be about 3 km N of the locality where our SMF specimens were collected. Taylor already pointed out some of the diagnostic characters that we used to differentiate *A. marsupialis* from *A. humilis* and *A. quaggulus*, such as the different dorsal scalation, the condition of the axillary pocket, and the presence versus absence of a dorsal ridge in males. However, we were unable to discern the relative size differences between dorsal and ventral scales indicated by Taylor (1956:91). In all our specimens of *A. marsupialis*, *A. humilis*, and *A. quaggulus*, the largest dorsal scales are larger than the ventral scales.

Our phylogenetic results clearly show *A. marsupialis* and *A. humilis* to be distinct mitochondrial lineages. Surprisingly, these species are not members of a single clade in our estimate. The great morphological similarity of these forms suggests either a case of extreme convergence or that our tree is in error. The COI gene used in our analyses is known to evolve quickly, and might be more suitable for the species-level problem addressed in this paper than for resolving deeper levels of evolutionary history. We expect that future phylogenetic work, including morphological and nuclear DNA analyses, will find *A. marsupialis* and *A. humilis* to be close relatives.

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APPENDIX. Comparative material examined.

Specimens used in COI analysis (GenBank accession numbers in parentheses):

Anolis marsupialis: **Costa Rica**: San Jose: Alfombra: POE 2239 (KP100429), 2567 (KP100427), 2240 (KP100428); *Anolis humilis*: **Costa Rica**: Guanacaste: Monteverde: Sunset Lodge: POE 3344 (KP100432); **Panama**: Chiriqui: Fortuna Pass: POE 1461 (KP100433), 1836 (KP100431); Colon: Parque Omar Torrijos: POE 1965 (KP100430); *Anolis laeviventris*: **Costa Rica**: Guanacaste: Monteverde: Sunset Lodge: POE 3350 (KP100442); *Anolis lemurinus*: **Honduras**: Yoro: near Locomapa: POE 3617 (KP100434); *Anolis zeus*: **Honduras**: Atlantida: Lancetilla Botanicl Garden: POE 3468 (KP100435); *Anolis polylepis*: **Costa Rica**: Puntarenas: Esquinas Rainforest Lodge: POE 2276 (KP100436); *Anolis cupreus*: **Costa Rica**: Puntarenas: Guapil: POE 2264 (KP100437); *Anolis biporcatus*: **Belize**: Cayo: Caracol: POE 1194 (KP100438); *Anolis aquaticus*: **Costa Rica**: Puntarenas: near San Vito: POE 2282 (KP100439); *Anolis woodi*: **Costa Rica**: Monteverde: Sunset Lodge: POE 3357 (KP100440); *Anolis sagrei*: **USA**: Florida: Crandon Park: POE 3739 (KP100441).

Specimens used in 16S analysis (GenBank accession numbers in parentheses):

Anolis humilis: **Costa Rica**: Puntarenas: Santa Elena: SMF 996125 (KC329690); *Anolis quaggulus*: **Costa Rica**: Alajuela: Rincon de la Vieja: SMF 96101 (KC329673); *Anolis marsupialis*: **Costa Rica**: San Jose: 2.5 km N Platanillo: 9.29°N, 83.78°W: SMF 76030 (KJ028217), 76031 (KJ028218).

Specimens used in morphological comparisons.

Specimens with everted hemipenes are indicated by an H following the collection number.

Anolis humilis—**Costa Rica**: Cartago: Navarro: KU 40793; Morravia de Chirripo: KU 40795–96; Instituto Interamericano de Ciencias Agrigolas: KU 40800; Parque Nacional Tapanti: 9.72°N, 83.77°W: MD 072^{H}; Tapanti: Río Grande: Orosi Puente: 9.79°N, 83.76°W: UCR 12396, 2809^{H}; Morravia de Chirripo: KU 40794; Limon: Batan: KU 40802–05, 40807–08, 40810; RB Hitoy Cerere: 9.67°N, 83.04°W: MD 089^{H}; N slope Cerro Nimaso: Distrito Bratsi: 9.49°N, 83.08°W: UCR 8475^{H} ; San Andres: 9.83°N, 82.96°W: SMF 96188^{H}; Vicinity of Aguas Zarcas: 9.88°N, 83.10°W: SMF 96118^{H}; Puntarenas: Monteverde: Ecolodge: 10.36°N, 84.80°W: UCR 17040^{H}; **Panama:** Bocas del Toro: Bocas del Toro: POE 1836; Cerro Brujo: 9.19°N, 82.19°W: SMF 85112^{H}; Río Uyama: 9.15°N, 82.32°W: SMF 85116; Chiriqui: Fortuna: POE 1461; Reserva Forestal Fortuna: 8.73°N, 82.26°W: SMF 85101^{H}, 85103–04$^{both\ H}$, 85107–09, 85113^{H}–14; Cocle: El Cope: POE 1612, 1625, 1836, 1891, 1895, 1965; El Valle: KU 75848–50, 75852–54; El Valle de Antón: Cerro Gaitál: 8.62°N, 80.13°W: SMF 80782–83$^{both\ H}$; San Blas: Nusagandi: Vicinity of field station: 9.34°N, 78.99°W: SMF 80787^{H}, 80845–46$^{both\ H}$, 80847–49, 80850^{H}.

Anolis marsupialis— **Costa Rica**: San Jose: Alfombra: AUM 34953–56, 34958–62, 34964, 34966; LACM 155626–27; MCZ 186166; MCZ 186140–41; POE 2238; 1.5 km NW Alfombra: 9.32°N, 83.78°N, (estimated type locality): KU 40889, 40890, KU 40892–93, 40895; San Isidro del General: MCZ 56244–45; Tinamaste: AUM 34957; Tinamaste: Distrito Baru: 9.30°N, 83.78°W: UCR 14512^{H}, 14514^{H}, 14523^{H}, 14805^{H}, 15933^{H}; 2.5 km N Platanillo: 9.29°N, 83.78°W: SMF 76028^{H}–29, 76030–31^{bothH}, 76032; Puntarenas: Fila Cedro Distrito San Vito: 8.82°W, 83.01°N: UCR 12784^{H}.

Anolis quaggulus— **Costa Rica:** Alajuela: Cinchona: 10.23°N, 84.17°W: SMF 85530^{H}; Heredia: Reserva Biológica La Tirimbina: Sarapiquí: 10.40°N, 84.13°W: MD 083^{H}; Puerto Viejo: 10.46°N, 84.00°W: UCR 4662^{H}; Limón: Playa Gandoca,: Distrito Sixaola; 9.58°N, 82.57°W: UCR 12959^{H}; Manzanillo: 9.64°N, 82.65°W: SMF 96157^{H}; La Pera: 9.60°N, 82.93°W: SMF 96181^{H}; Selva de Guacimo: 10.20°N, 83.72°W: SMF 96172^{H}; **Honduras**: Olancho: Quebrada El Guásimo: 14.58°N, 85.30°W: SMF 80819^{H}; **Nicaragua**: Atlántico Norte: Rancho Alegre,Junto a rio Rancho Grande: 13.66°N, 85.03°W: SMF 84745^{H}; Jinotega: Kilambe: 13.58°W, 85.72°N: JS 144^{H}; 13.59°N, 85.70°W: JS 283^{H}; 13.61°N, 85.74°W: SMF 84746^{H}; 13.58°N, 85.72°W: SMF 84747–58$^{boh\ H}$; 13.60°N, 85.71°W: SMF 84749^{H}; 13.59°N, 85.70°W: SMF 84754^{H}, 84755–56; Datanli–El Diablo: 13.14°N, 85.86°W: JS 206^{H}; 13.16°N, 85.87°W: JS 230; 13.17°N, 85.86°W: SMF 84750–51, 84753; Matagalpa: Selva Negra: 13.00°N, 85.91°W: SMF 77457, SMF 77480–81$^{both\ H}$, 77483^{H}, 77485, 78279^{H}, 78516^{H}, 79821, 79823^{H}; Río San Juan: Bartola: 10.97°N, 84.34°W: SMF 80944, 80946; Río San Juan, at junction with Río Sarapiqui: 10.71°N, 83.93°W: SMF 83151, 83172; at junction with Río Chimurria: 10.73°N, 83.91°W: SMF 83153–54^{H}; Río El Chancho, 5–6 km above junction with Río San Juan: 10.82°N, 84.02°W: SMF 83156, 83158^{H}, 83162^{H}.