



A new species of *Pseudoeurycea* from the cloud forest in Veracruz, México

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Abstract

We describe a new species of *Pseudoeurycea* from the cloud forests of Huatusco in the state of Veracruz, México. This species belongs to the *P. cephalica* complex. *Pseudoeurycea cafetalera* **sp. nov.** is sister to the clade formed by *P. cephalica* and *P. quetzalanensis*. The new species is diagnosed by a stout body, long stout legs, short digits, somewhat webbed hands and feet, a distinctive coloration and by divergent mitochondrial DNA sequences.

Key words: Caudata, México, new species, Plethodontidae, taxonomy

Introduction

The genus *Pseudoeurycea* is the most diverse clade of plethodontid salamanders in México. Most species have a generalized morphology and occur in terrestrial microhabitats in pine or pine-oak forests, above 2000 meters elevation. Of the 48 recognized species of *Pseudoeurycea*, only a few (*P. lineola*, *P. nigromaculata*, *P. praecellens*, *P. scandens* and *P. quetzalanensis*) occur in cloud forests between 1000 and 2000 meters elevation; only 4 more are known to occur lower than 1000 meters in tropical lowland or deciduous forest (*P. maxima*, *P. orchileucos*, *P. orchimelas*, and *P. werleri*). *Pseudoeurycea orchileucos* is the only species of *Pseudoeurycea* that is found near sea level in tropical forest; all others occur above 700 m.

The *Pseudoeurycea cephalica* group is formed by 4 described species: the widespread *P. cephalica*, which occurs in high elevation forests of the Trans-Mexican Volcanic Belt and Sierra Madre Oriental (Fig. 1); the terrestrial *P. galeanae* from northern México in the state of Nuevo León; the cave-dwelling *P. scandens*, which occurs in the northern state of Tamaulipas, and the terrestrial or semiarboreal *P. quetzalanensis* from the cloud forest in the Sierra Madre Oriental. *Pseudoeurycea cephalica sensu stricto* occurs in the highlands of central México (Uribe-Peña *et al.* 1999), and two subspecies have been described (*P. c. rubrimembris*, and *P. c. manni*) from the state of Hidalgo. Current field work and unpublished data have shown that there might be up to 5 new species in the *P. cephalica* complex, but insufficient material from type localities of the subspecies has kept us from doing a detailed taxonomic study of the entire group.

In order to evaluate salamander population declines, we began a multiyear period resurvey of the salamander fauna in the state of Veracruz, México. While trying to find the possibly extinct *P. praecellens* in the cloud forests of Veracruz, we came across a series of medium sized, stout *Pseudoeurycea* that cannot be referred to any known species. In this paper we describe the new species from Veracruz and discuss its phylogenetic relationships by reanalyzing the molecular data published by Parra-Olea (2002) and Wiens *et al.* (2007) with the addition of previously unpublished sequences for 6 species (*P. cephalica*, *P. aurantia*, *P. quetzalanensis*, *P. ruficauda*, *P. cafetalera* **sp. nov.**, and *Thorius magnipes*).

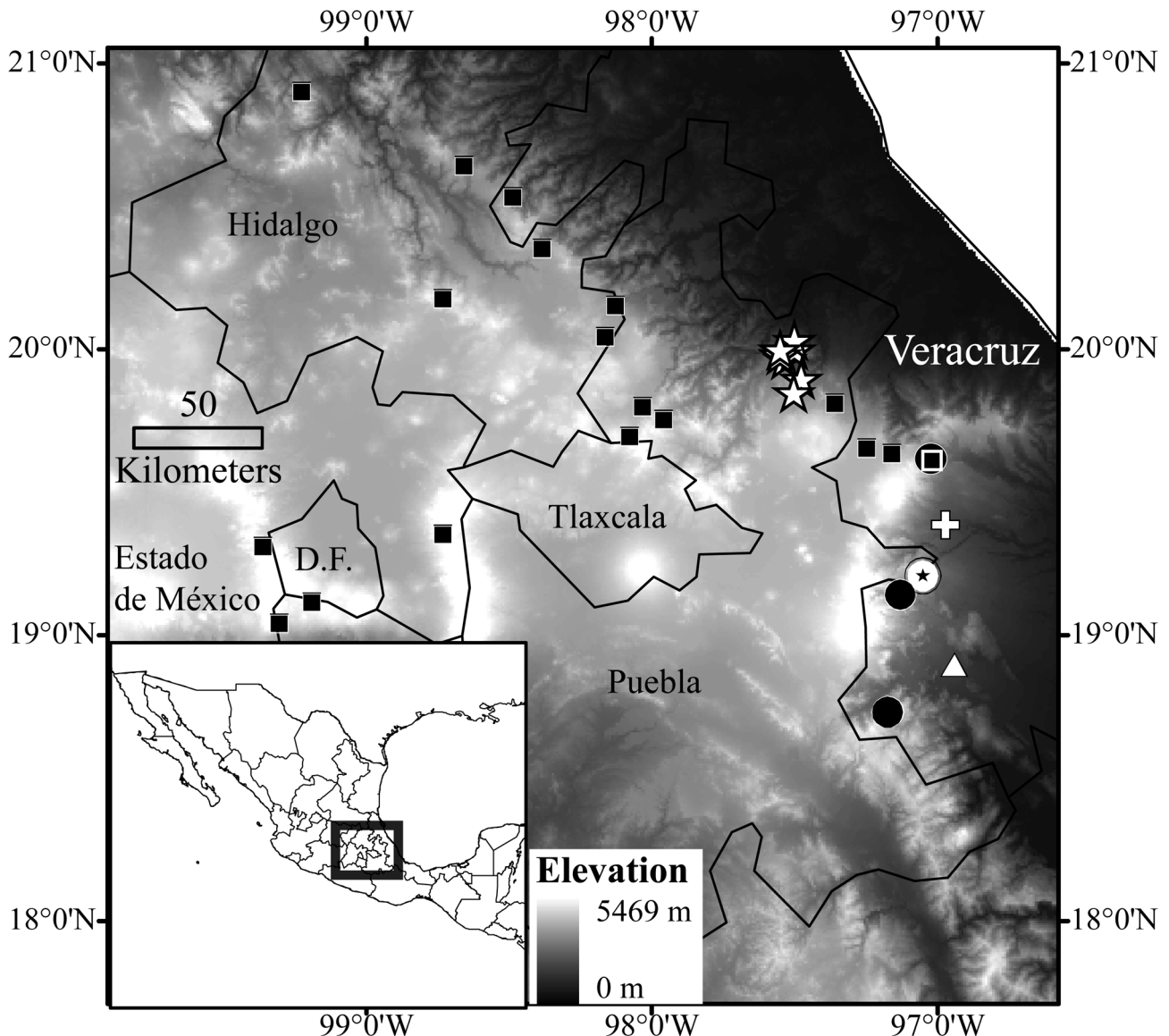


FIGURE 1. Map of species of the *Pseudoeurycea cephalica* complex in central México. Symbols: Black squares—*P. cephalica*; White stars—*P. quetzalanensis*; White circle with black star—*P. cafetalera* type locality; Black circles—other *P. cafetalera* localities; white cross—*Pseudoeurycea* cf *cafetalera* from Teocelo; white triangle—approximate type locality of *P. praezellens*. Overlapping black circle and black square indicate sympatry between *P. Cafetalera* and *P. Cephalica* at La Joya, Veracruz.

Material and methods

Morphology. The description follows the format used by Lynch & Wake (1989) for other species in the genus *Pseudoeurycea*, and includes the same basic characters and measurements. Larger measurements were taken using dial calipers (to the nearest 0.1 mm); measurements of feet, toes and some head dimensions (e.g. additional measurements of the holotype), as well as tooth counts (ankylosed teeth only), were taken under a stereoscopic microscope. All measurements are in mm and abbreviations are as follows: Standard length (SL), Tail length (TL), Snout-gular fold distance (SG), Head Width (HW), Axilla-groin distance (AG), Forelimb length (FLL), Hind limb length (HLL), Limb interval (LI), Shoulder Width (Sh-W), Foot Width (FW), Head Depth (HD), Nostril diameter (ND), Internarial Width (In-W), Premaxillary Teeth (PT), Maxillary Teeth (MT) and Vomerine Teeth (VT) (Table 1). Color notes are based on field notes taken from living specimens. Based

on body size and shape, and the presence of a sublingual fold, this species could be assigned only to either *Pseudoeurycea* or *Chiropterotriton* of the Mexican genera of salamanders. This species, like all other species of *Pseudoeurycea*, has pointed toes, rather than squared toes as in *Chiropterotriton*. Molecular data (presented herein) also support the inclusion of the new species in the genus *Pseudoeurycea*.

TABLE 1. Mean and standard error (above) and range (below) of all variables used in morphometric analyses and tooth counts for *P. cafetalera*, *P. cephalica*, and *P. quetzalanensis*, and *P. cf. cafetalera* (from Teocelo, Veracruz). All measurements are given in millimeters, except limb interval. Number of specimens used to calculate mean and variation noted if different from numbers in first row.

	<i>P. cafetalera</i>		<i>P. cephalica</i>		<i>P. quetzalanensis</i>		<i>P. cf. cafetalera</i>	
	females <i>N</i> = 6	males <i>N</i> = 2	females <i>N</i> = 15	males <i>N</i> = 8	females <i>N</i> = 4	males <i>N</i> = 2	females <i>N</i> = 2	males <i>N</i> = 2
SL	49.9 ± 6.4 (43.1–59.8)	45.5 ± 3.8 (42.8–48.1)	47.3 ± 4.5 (41.2–57.8)	45.3 ± 3.9 (40.1–51.4)	32.8 ± 4.3 (31.8–41)	35.7 ± 5.1 (32.1–39.3)	36.6 ± 0.5 (36.2–36.9)	43.4 ± 0.07 (43.3–43.4)
TL	39.5 ± 7.03 (31.8–45.6) <i>N</i> = 3	36.3 <i>N</i> = 1	36.9 ± 3.7 (33.3–42.6) <i>N</i> = 11	34.4 ± 2.6 (32.0–37.9) <i>N</i> = 6	27.7 ± 3.9 (22.4–31.1)	30.6 <i>N</i> = 1	24.0 ± 0.3 (23.8–24.2)	37.5 <i>N</i> = 1
SG	11.5 ± 1.2 (9.7–13.1)	11.0 ± 0.3 (10.7–11.2)	11.0 ± 0.9 (9.8–13.2)	11.0 ± 1.0 (9.6–13.1)	9.25 ± 1.4 (7.1–10.1)	9.45 ± 0.8 (8.9–10.0)	9.0 (9.0)	10.5 ± 0.7 (10.0–11.0)
HW	8.6 ± 0.9 (7.0–9.7)	7.7 ± 0.07 (7.7–7.8)	7.2 ± 0.6 (6.4–8.5)	7.01 ± 0.6 (6.2–7.9)	6.7 ± 0.6 (6.0–7.5)	6.6 ± 0.3 (6.4–6.8)	6.2 ± 0.1 (6.1–6.3)	7.3 ± 0.07 (7.3–7.4)
AG	25.2 ± 3.8 (22.1–31.6)	24.2 ± 0.8 (23.6–24.7)	24.5 ± 2.9 (19.7–30.3)	22.3 ± 2.4 (18.2–25.3)	20.1 ± 2.2 (17.1–22.2)	18.2 ± 2.8 (16.2–20.2)	18.3 ± 0.07 (18.3–18.4)	22.3 ± 0.07 (22.2–22.3)
FLL	13.2 ± 1.41 (11.3–14.8)	12.3 ± 0.14 (12.2–12.4)	11.3 ± 1.01 (9.5–12.9)	12.0 ± 1.54 (9.4–13.8)	9.7 ± 1.76 (7.1–11.0)	10.2 ± 1.32 (9.2–11.1)	9.25 ± 0.35 (9.0–9.5)	12.5 (12.5)
HLL	14.4 ± 1.6 (12.3–16.4)	14.1 ± 1.1 (13.3–14.9)	12.4 ± 1.0 (10.6–14.1)	13.1 ± 1.2 (11.4–14.6)	9.5 ± 1.5 (7.2–10.5)	9.7 ± 0.5 (9.3–10.0)	10.2 ± 0.2 (10.0–10.3)	13.3 ± 0.07 (13.2–13.3)
Sh-W	7.8 ± 0.6 (6.6–8.3)	7.0 ± 0.7 (6.5–7.5)	6.1 ± 0.8 (5.1–8.2)	5.4 ± 0.7 (4.7–6.6)	5.3 ± 0.5 (4.6–5.8)	5.1 ± 0.6 (4.6–5.5)	5.1 ± 0.2 (4.9–5.2)	5.9 ± 0.7 (5.4–6.4)
FW	4.8 ± 0.5 (4.2–5.5)	4.6 ± 0.6 (4.2–5.0)	4.4 ± 0.4 (3.6–5.3)	4.7 ± 0.5 (4.0–5.6)	3.0 ± 0.5 (2.3–3.4)	3.3 ± 0.3 (3.0–3.5)	3.3 ± 0.2 (3.1–3.4)	4.5 ± 0.2 (4.3–4.6)
HD	3.7 ± 0.7 (2.6–4.8)	3.3 ± 0.2 (3.1–3.4)	3.0 ± 0.3 (2.6–4.0)	2.8 ± 0.2 (2.6–3.4)	3.4 ± 0.42 (2.8–3.8)	2.9 ± 0.07 (2.8–2.9)	2.5 ± 0.2 (2.3–2.6)	3.3 ± 0.3 (3.0–3.5)
ND	0.3 ± 0.06 (0.2–0.4)	0.3 ± 0.07 (0.3–0.4)	0.4 ± 0.06 (0.2–0.4)	0.36 ± 0.1 (0.2–0.5)	0.1 ± 0.05 (0.1–0.2)	0.2 ± 0.07 (0.2–0.3)	0.2 (0.2)	0.3 ± 0.07 (0.3–0.4)
In-W	2.4 ± 0.2 (2.1–2.6)	2.3 ± 0.2 (2.1–2.4)	3.8 ± 0.2 (1.8–2.5) <i>N</i> = 14	2.6 ± 0.5 (2.0–3.3)	1.3 ± 0.1 (1.2–1.5)	1.5 ± 0.1 (1.4–1.6)	1.7 ± 0.3 (1.5–1.9)	2.2 ± 0.1 (2.1–2.3)
LI	0.91 ± 0.97 (-1–1.5)	0.3 ± 0.3 (0.0–0.5)	1.8 ± 0.7 (0.0–2.5)	0.5 ± 1.28 (-1–2.5)	1.4 ± 0.7 (1.0–2.5)	0.3 ± 0.3 (0.0–0.5)	1.0 (1.0)	0.0 (0.0)
PT	9.3 ± 2.0 (6–12)	3.5 ± 0.7 (3–4)	6.9 ± 2.2 (2–11)	3.9 ± 1.2 (2–6)	12.5 ± 1.29 (11–14)	3.5 ± 2.1 (2–5)	8.0 ± 1.41 (7–9)	3.0 (3)
MT	62.5 ± 7.0 (52–73)	49.0 ± 5.6 (45–53)	55.6 ± 6.3 (43–67) <i>N</i> = 14	56.1 ± 6.6 (44–64)	60.8 ± 9.0 (48–68)	60.0 ± 1.4 (59–61)	39.0 ± 4.2 (36–42)	52.5 ± 13.4 (43–62)
VT	31.8 ± 6.0 (25–43)	24 (24)	26.8 ± 4.3 (21–38)	26.0 ± 4.8 (20–36)	23.8 ± 4.0 (19–28)	27.0 ± 1.4 (26–28)	18.5 ± 2.1 (17–20)	23.0 ± 5.6 (19–27)

Phylogenetic analyses. We obtained partial sequences of the 16S rRNA mitochondrial gene for 10 specimens (Table 2). Whole genomic DNA was extracted from small amounts of ethanol preserved tissues using the Qiagen kit DNeasy extraction kit (Qiagen, Valencia, CA). We sequenced 534 base pairs of the large 16S subunit ribosomal mtDNA gene corresponding to positions 2510–3059 in the human mitochondrial genome (Anderson *et al.* 1981). Amplification was done via the polymerase chain reaction (PCR) (Saiki *et al.*

1988), using the primers “16Sar” and “16Sbr” (Palumbi, *et al.* 1991). PCR reactions consisted of 38 cycles with a denaturing temperature of 92°C (1 min), annealing at 48–50°C (1 min), and extension at 72°C (1 min) in a Techne PHC-1 thermocycler. PCR reactions were run in a total volume of 25 µl, using 0.5 pmol of each primer. Double strand templates were cleaned using QIAquick PCR purification kit (Qiagen, Valencia, CA). We used 1 µl of PCR product as the template for cycle sequencing reactions in a 10 µl total volume with the Perkin-Elmer Ready Reaction Kit to incorporate dye-labeled dideoxy terminators. Thermal cycling was performed using standard conditions. Cycle sequencing products were purified using ethanol precipitation and run on an ABI 310 capillary sequencer (Applied Biosystems, Foster City, CA). Genbank numbers for new sequences are given in Table 2.

TABLE 2. Catalogue numbers and Genbank accession numbers for 16S sequence data used for phylogenetic analyses. Species names are ordered alphabetically and * indicates previously published sequences. Voucher letters: IBH: Instituto de Biología, UNAM, Colección Nacional de Anfibios y Reptiles; MVZ: Museum of Vertebrate Zoology, University of California, Berkeley; EBUAP: Escuela de Biología, Universidad Autónoma de Puebla, GP and E=Unaccessioned specimen from Instituto de Biología, UNAM.

Species	Catalogue number	Locality	Genbank accession number
<i>Pseudoeurycea altamontana</i>	GP309	8.8 km E of Santa Marta, Edo. México, México	AF451245*
<i>P. anitae</i>	MVZ137939	San Vicente Lachixio, Oaxaca, México	AF451227*
<i>P. aurantia</i>	EBUAP2051	Peña Verde, Oaxaca, México	HM365056
<i>P. bellii</i>	GP048	Parque Nacional Morelos, Michoacán, México	AF451214*
<i>P. boneti</i>	E43.20	Sierra Monteflor, Oaxaca, Mexico	AY864709*
<i>P. brunnata</i>	MVZ137944	Laguna Chicabal, Quetzaltenango, Guatemala	AF451231*
<i>P. cafetalera</i>	IBH14341	Rancho dos Puentes, Veracruz, México	HM365064
<i>P. cafetalera</i>	IBH14343	Rancho dos Puentes, Veracruz, México	AF451216*
<i>P. cafetalera</i>	IBH14339	Zoncolco, Veracruz, Mexico	HM365063
<i>P. cafetalera</i>	MVZ196052	La Joya, Veracruz, México	HM365062
<i>P. cephalica</i>	GP049	La Marquesa, Edo. México, México	HM365060
<i>P. cephalica</i>	IBH22604	Tlaxlacoya, Veracruz, México	AF451218*
<i>P. cephalica</i>	MVZ143825	Lagunas de Zempoala, Morelos, México	HM365059
<i>P. cephalica</i>	MVZ178825	Mineral del Chico, Hidalgo, México	HM365061
<i>P. cochranæ</i>	GP231	San Vicente Lachixio, Oaxaca, México	AF451222*
<i>P. conanti</i>	MVZ158748	19 km S of Sola de Vega, Oaxaca, México	AF451240*
<i>P. exspectata</i>	MVZ160918	Mataquesuintla, Jalapa, Guatemala	AF451233*
<i>P. firscheini</i>	GP504	Atlahuilco, Veracruz, México	DQ642045*
<i>P. gadovii</i>	MVZ172741	Xometla, Veracruz, México	AF451229*
<i>P. galeanae</i>	GP047	Puerto de Cieneguillas, Nuevo León, México	AF451220*
<i>P. gigantea</i>	GP177	La Joya, Veracruz, México	AF451219*
<i>P. goebeli</i>	MVZ194276	Cerro Mozotal, Chiapas, México	AF451235*
<i>P. juarezi</i>	MVZ163771	Cerro Pelón, Oaxaca, México	AF380809*
<i>P. leprosa</i>	GP399	Parque Nacional La Malinche, Puebla, México	AF380820*
<i>P. lineola</i>	GP241	Barranca de San Miguel. Veracruz, México	AF380801*
<i>P. longicauda</i>	GP293	Lengua de Vaca, Edo. México, México	AF451246*
<i>P. lynchi</i>	GP160	Chiconquiaco, Veracruz, México	AF451225*
<i>P. maxima</i>	MVZ194327	6 km S of Putla de Guerrero, Oaxaca, México	AY864693*

continued next page

TABLE 2. (continued)

Species	Catalogue number	Locality	Genbank accession number
<i>P. melanomolga</i>	GP147	Las Vigas, Veracruz, México	AF451221*
<i>P. mixteca</i>	GP289	Tlaxiaco, Oaxaca, México	AF380829*
<i>P. mystax</i>	GP372	Tamazulapa, Oaxaca, México	AF380795*
<i>P. nigromaculata</i>	MVZ185977	Cerro Chicahuaxtla, Veracruz, México	AF451238*
<i>P. obesa</i>	IBH14196	Plan de Guadalupe, Oaxaca, Mexico	AY762354*
<i>P. orchimelas</i>	GP272	Volcán San Martin, Los Tuxtlas, Veracruz, México	AF380798*
<i>P. ruficauda</i>	IBH22610	Peña Verde, Oaxaca, México	HM365057
<i>P. papenfussi</i>	IBH14200	Cerro Pelon, Oaxaca, Mexico	AY762352*
<i>P. quetzalanensis</i>	IBH14208	Xocoyolo, Puebla, México	HM365058
<i>P. rex</i>	MVZ149701	El Retiro, Huehuetenango, Guatemala	AF451237*
<i>P. robertsi</i>	GP331	Raices, Edo. México, México	AF380831*
<i>P. ruficauda</i>	MVZ236762	Puerto Soledad, Oaxaca, México	AY354473*
<i>P. saltator</i>	GP084	La Esperanza, Oaxaca, México	AF380812*
<i>P. scandens</i>	MVZ173302	Rancho del Cielo, Tamaulipas, México	AF451243*
<i>P. smithi</i>	GP351	Llano de las Flores, Oaxaca, México	AF451224*
<i>P. tenchalli</i>	GP391	Corral de Bravo, Guerrero, México	AF451223*
<i>P. unguidentis</i>	GP352	Comaltepec, Oaxaca, México	AF380813*
<i>P. werleri</i>	GP282	Volcán San Martin, Veracruz, México	AF451226*
<i>Ixalotriton niger</i>	MVZ158823	12 km N of Berriozabal, Chiapas, México	AF451242*
<i>I. parvus</i>	MVZ194330	Cerro Baul, Chiapas, México	AF451249*
<i>Parvimolge townsendi</i>	MVZ190947	Barranca de San Miguel, Veracruz, México	AF451247*
<i>Thorius magnipes</i>	IBH22617	San Bernardino, Veracruz, México	HM367088

Fifty sequences of 534 bp of the 16S rRNA gene were used for phylogenetic analysis (Table 2). Sequences were aligned using Muscle 3.6 (Edgar 2004). Both Maximum Likelihood (ML) and Bayesian phylogenetic analyses were performed to determine the placement of the new species within the genus *Pseudoeurycea*. The GTR + I + G substitution model was selected using the Akaike Information Criterion (AIC) in the program MrModeltest2.2 (Nylander 2004). *Thorius magnipes* was used as the outgroup for all phylogenetic analyses. Maximum likelihood phylogenetic analysis was done using the program RAxML (Stamatakis 2006). One hundred bootstrap replicates were performed to assess node support. Bayesian phylogenetic analysis was done with the program MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The MCMC analysis was run for 20,000,000 generations with two separate runs, sampled every 1000 generations, and the first 5,000,000 generations were discarded as burn-in. The cumulative, sliding window, and compare plots from the program AWTY (Wilgenbusch *et al.* 2004) were examined to assess convergence. GTR + I + G distances were calculated using PAUP v4.0 (Swofford 2002).

Description of the new species

Pseudoeurycea cafetalera sp. nov.

Coffee Grove Salamander, Salamandra de cafetal

Figure 2A, 2C–2H.

Pseudoeurycea cephalica: Parra-Olea (2002). Table 1 (voucher: IBH14343)

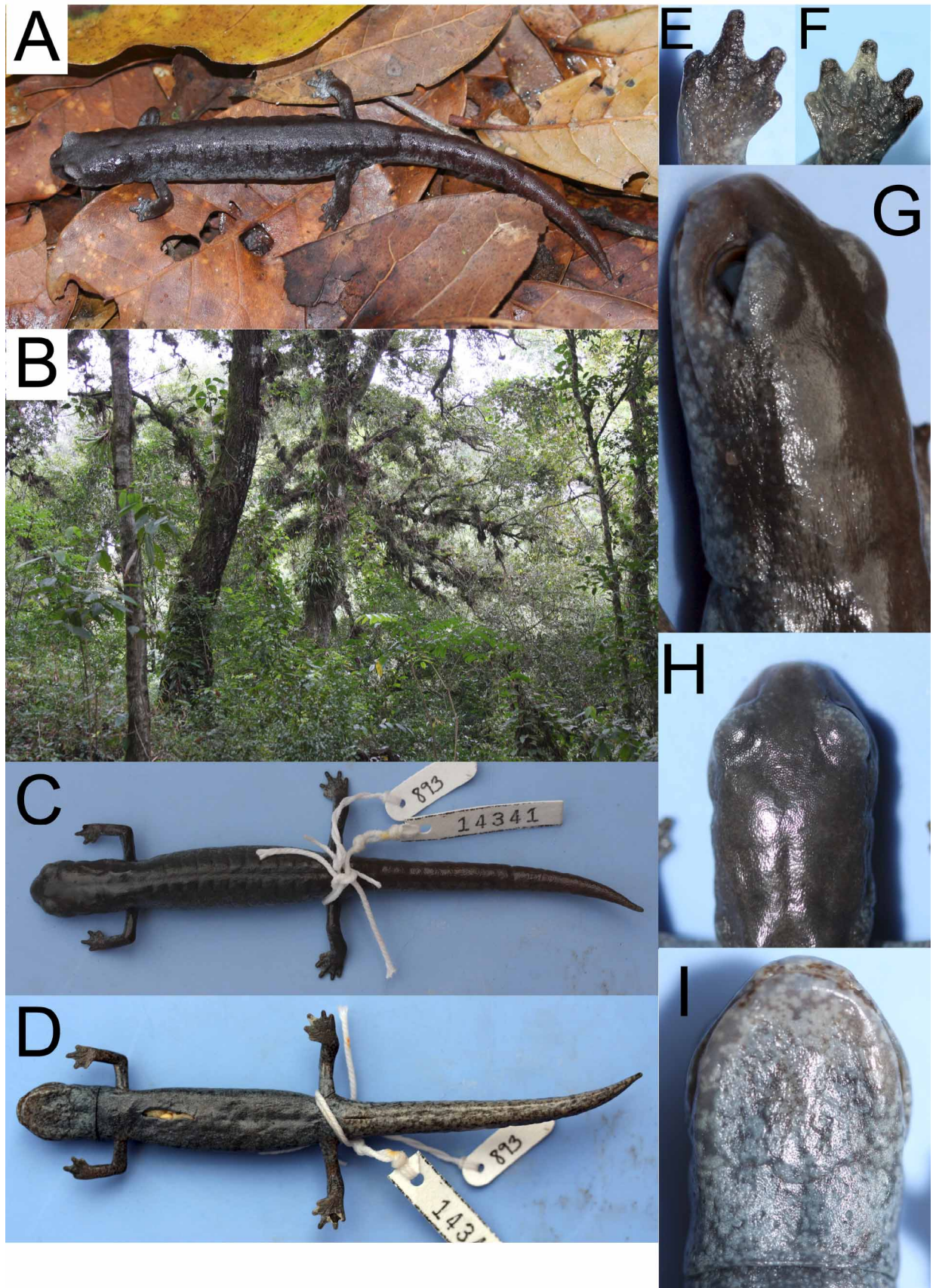


FIGURE 2. A) Holotype of *Pseudoeurycea cafetalera* (IBH14341). B) Forest habitat on edge of cafetal at type locality. C) Ventral and D) dorsal views of preserved holotype. E) Right hand and F) left foot of preserved holotype. G) Lateral, H) dorsal, and I) ventral views of head of preserved holotype.

Holotype. Colección Nacional de Anfibios y Reptiles IBH14341, an adult female from Rancho dos Puentes, 6.0 km (by road) north of entrance to Huatusco, Veracruz, México (19° 11.13' N, 96° 57.72' W, 1322 m elevation) (Fig. 1), collected by Sean M. Rovito on November 17, 2009.

Paratypes. Seven specimens. Two males: IBH14344, same data as holotype; IBH14349, from Las Cañadas, 5.4 km NW of center of Huatusco, Veracruz, México (19° 11.42' N, 96° 59.30' W, 1367 m elevation), collected by J. C. Winfield-Pérez. Five females: IBH14338-14340, Zoncolco, Veracruz, México (18° 46.4' N, 97° 07.61' W, 1600 m elevation); IBH14343, same locality as holotype, collected by GPO, DBW, M. García-París and J. Hanken; IBH14346, 3.5 km SW La Raya, road to Trincheras, Veracruz, México.

Referred specimens. Three juveniles: IBH14337, same data as holotype; IBH14345, Zoncolco, Veracruz, México (18° 46.4' N, 97° 07.61' W, 1600 m elevation); MVZ196052, La Joya, Veracruz, México (19° 36.65' N, 97° 1.63' W, 2125 m elevation).

Diagnosis. A medium sized species of *Pseudoeurycea*; distinguished from all other members of the genus *Pseudoeurycea* (except *P. cephalica* group) by the combination of: stout body, long stout legs, shorter digits, more webbed hands and feet with first and fourth fingers barely emerging from membrane. Distinguished from members of *P. bellii* group by lack of reddish spots on dorsum. Distinguished from its two sister taxa *P. quetzalanensis* and *P. cephalica* in the following characters: from *P. quetzalanensis* by its larger size (mean SL = 45 mm for males and 50 mm for females of *P. cafetalera* vs. 36 mm for males and 38 mm for females of *P. quetzalanensis*), longer limbs (adpressed limbs separated by -1–1.5 costal interspaces in females and they meet or are separated by 0.5 costal interspace in males of *P. cafetalera*; for *P. quetzalanensis*, separated by 1–2.5 for females and 0.5–1 for males), and wider feet (mean FW = 4.8 mm for females and 4.6 mm for males of *P. cafetalera* vs. 3.0 mm and 3.2 mm for females and males, respectively, of *P. quetzalanensis*). It differs from the other closely related species *P. cephalica* by its broader head (mean HW = 8.5 mm and 7.7 mm for females and males, respectively, of *P. cafetalera* and 7.1 mm and 6.9 mm for females and males, respectively, of *P. cephalica*), and more maxillary and vomerine teeth in females (mean MT = 62 in *P. cafetalera* vs. 56 in *P. cephalica*; mean VT = 32 in *P. cafetalera* vs. 27 in *P. cephalica*). It differs from other members of the *P. cephalica* group (*P. galeanae* and *P. scandens*) in being smaller; *P. galeanae* can reach 70 mm SL and *P. scandens* can reach 71 mm SL (Taylor, 1941; Walker, 1955). It also differs from these species in coloration; *P. galeanae* has large whitish spots on the tail, especially near the base, which are not present in *P. cafetalera*, and *P. scandens* has a uniform dark belly while *P. cafetalera* can have light grey flecks on the belly. Finally, *P. scandens* has longer legs with longer truncate digits (Walker 1955), and *P. galeanae* has more maxillary teeth (Taylor, 1941).

Description. A medium sized species; SL in 2 adult males 42.8–48.1 mm (mean = 45.5 mm), in 6 females 43.1–59.8 mm (mean = 49.9 mm), with robust habitus; head relatively broad, 0.16–0.18 SL in both males and females, snout broadly rounded in females and squared in males; neck region ill-defined, only slightly narrower than head. Parotoid glands not evident. Costal grooves 13, counting one each in axilla and groin. Limbs relatively long; digits relatively short and stout for *Pseudoeurycea*, bearing small subterminal pads; fifth toe much shorter than fourth. Digits in order of decreasing length: fingers 3-2-4-1; toes 3-4-2-5-1; basal webbing present. Tail missing in many specimens; when present, 0.69–0.76 (mean = 0.79) SL in females and 0.75 in males; tail stout, tapering rather abruptly toward tip. Maxillary teeth small, 45–53 (mean 49) in males, 58–73 (mean 62.5) in females; premaxillary teeth 3–4 (mean 3.5) and enlarged in adult males, 6–12 (mean 9.3) and smaller in females; vomerine teeth in long rows, 24 in males, 25–43 (mean 31.8) in females.

Measurements (in mm), limb interval and tooth counts of the holotype. HW 9.7; HD 4.8; eyelid length 4.1; eyelid width 2.2; anterior rim of orbit to snout 3.2; interorbital distance 3.2; distance between outer corners of eyes 8.3; snout to forelimb 16.8; ND 0.3; distance between external nares 2.6; projection of snout beyond mandible 0.5; SG 13.1; Sh-W 8.3; SL 59.8; snout to anterior angle of vent 54.4; AG 31.6; TL 45.6; tail depth at base 5.8; tail width at base 5.5; FLL 14.8; width of hand 4.5; HLL 16.4; FW 5.5; length of longest (third) toe 2.0; length of fifth toe 0.8. LI 1.5. Numbers of teeth: premaxillary 12; maxillary 65; vomerine 43.

Coloration (in life) of the holotype (Fig. 2A). Dorsum and dorsal surface of head solid dark chocolate brown. Grey specks on snout, mostly pale grey above eyes. Iris dark brown. Sides of head mostly brown with some grey speckling, with proportion of grey increasing toward back of head and mouth. Dorsal surface of tail

same color as dorsum at insertion of hindlimbs, transitioning gradually to medium reddish brown at tip. Sides of body dark brown above midline, with some pale grey flecks, and pale grey mottled with dark brown below midline. Dorsal surface of limbs lighter reddish brown (similar to dorsal surface of tail); dorsal surface of feet brown with grey specks as on lateral surface of body. Gular region, lateral and ventral surfaces of tail, and underside of limbs light grey with brown speckles throughout. Venter dark brown with very small grey flecks. Underside of feet solid brown.

Coloration (in alcohol) of the holotype. Dorsum and dorsal surface of head and limbs dark brown. Dorsal surface of tail solid medium red-brown. Small grey flecks above eyes, with larger grey flecks along labial surfaces, on rostrum and on sides of head below jaw. Sides of body mostly dark brown with few grey specks above midline and mostly grey with some dark brown specks below midline. Lateral and ventral surfaces of tail grey mottled with lighter brown compared to dorsum. Gular region and underside of limbs mostly pale grey mottled with some dark grey-brown. Venter slate grey with small specks of light grey throughout. Underside of feet slate grey.

Color variation. Paratype IBH14344 has a mostly grey venter, composed of tiny specks on a dark brown background. Dark brown blotches present on sides and at base of tail, at boundary between lateral and ventral coloration. Several lichen-like pale orange-brown blotches on dorsum. Lighter brown dorsal coloration compared to holotype. Almost no grey color above eyes. Other specimens lack grey mottling on ventrolateral surface and gular region, with underside of feet and ventral surface of tail light brown and light brownish color on rostrum and labial surfaces.

Distribution. This species is known from the vicinity of the type locality near Huatusco, Veracruz, north to La Joya where it occurs in sympatry with *P. cephalica*, and from the northern Sierra de Zongolica near the locality of Zoncolco, 49 km south-southwest of the type locality (Fig. 1). The species is presumed to occur in cloud forest to the south of Huatusco between Huatusco and Zoncolco, but appears not to occur on Cerro Chicahuaxtla and other well-collected sites near the city of Cordoba.

Natural history. *Pseudoeurycea cafetalera* appears to be a terrestrial species, like most members of its genus. It has been found under logs and in leaf litter in cafetal habitat with adjoining forest (Fig. 2B). Co-occurring species of salamanders at the type locality include *Bolitoglossa platydactyla*, *Chiropterotriton chiropterus*, *Parvimolge townsendi*, and *Thorius pennatulus*.

Etymology. This species is named for the coffee grove (cafetal) habitat where it is found. It is one of only three species of *Pseudoeurycea* (along with *P. quetzalanensis* and *P. lineola*) that has been found in this habitat so far.

Phylogenetic results. The 16S phylogeny (Fig. 3) provides strong support for the monophyly of *Pseudoeurycea cafetalera* (BS = 99, PP = 100). The *P. cephalica* group, which contains *P. cafetalera*, is also well supported (BS = 84, PP = 98) and is the sister clade to the *P. bellii* group (BS = 99, PP = 100). The relationship of *P. cafetalera* as the sister taxon to *P. cephalica* + *P. quetzalanensis* has little support (BS = 62, PP = 70). The divergence between *P. cafetalera* and *P. cephalica* ranged from 4.5–6.0% (mean = 5.4%), and the divergence between the two populations (Huatusco and Zongolica) of *P. cafetalera* is 2.6%. The divergence between *P. cafetalera* and *P. quetzalanensis* ranged from 5.0–5.8% (mean = 5.6%). The divergence between *P. cafetalera* and *P. cephalica* + *P. quetzalanensis* (4.4–6.0%, mean = 5.4%) is comparable to that between other species of *Pseudoeurycea* (Fig. 3). Sequence divergence within the *P. cephalica* group was as high as 10.1% (between *P. galeanae* and *P. cafetalera*).

Discussion

The genus *Pseudoeurycea* is a morphologically diverse group of 48 species distributed in México and Guatemala. Parra-Olea & Wake (2001) showed the genus *Lineatriton* to be polyphyletic and nested within *Pseudoeurycea* based on a mitochondrial DNA phylogeny, and the three species of *Lineatriton* are now considered to be part of *Pseudoeurycea* (Frost *et al.* 2006). The taxonomy of *Pseudoeurycea* is still not fully resolved, with 6 species recently described (in addition to *P. cafetalera*) and several more species awaiting

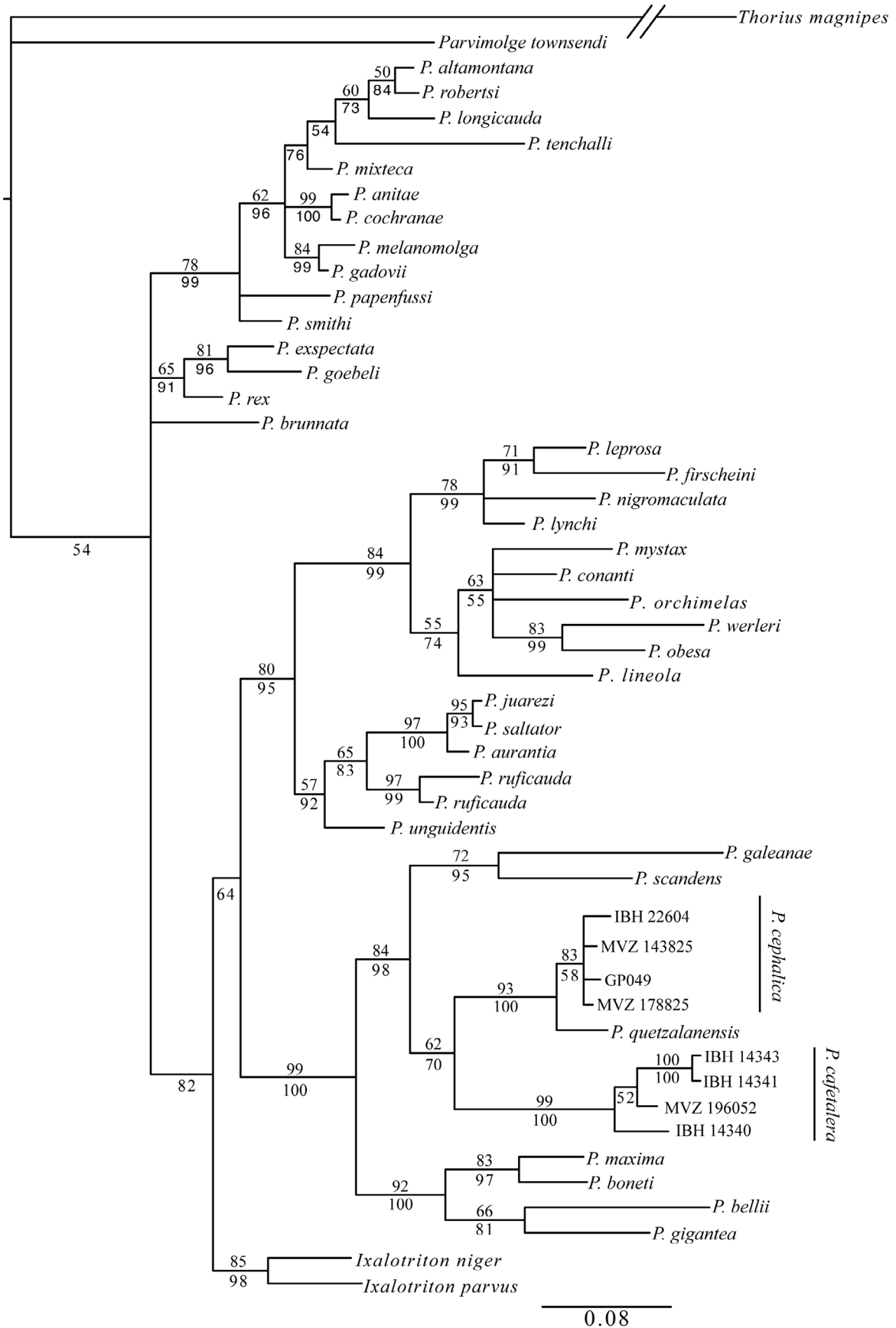


FIGURE 3. Phylogeny from Bayesian analysis of 16S rRNA gene. Maximum likelihood bootstrap values shown above branches, posterior probabilities (multiplied by 100) from Bayesian analysis shown below branches.

description. More importantly, the relationships between *Pseudoeurycea*, *Ixalotriton*, and *Parvimolge* are not yet resolved. The analyses of Parra-Olea (2002) and Wiens *et al.* (2007) found phylogenetic support for the recognition of three species groups within *Pseudoeurycea* (*P. bellii*, *P. gadovii* and *P. leprosa* species groups), with lower support for a clade composed of members of the *P. cephalica* group. In the present study, species in the *P. cephalica* group form a well-supported clade that is sister to the *P. bellii* species group in phylogenetic analyses. We include sequences of the recently described *P. quetzalanensis* (Parra-Olea *et al.* 2004) and *P. cafetalera*, both of which are in the same clade as other members of the *P. cephalica* group. In addition to *P. cafetalera* and *P. quetzalanensis*, high genetic divergences between populations of *P. cephalica* from the states of Hidalgo and Queretaro (Parra-Olea, unpublished) suggest that additional undescribed species are present within *P. cephalica*. Morphological and molecular study of populations of *P. cephalica* along its distributional range, including topotypic material for *P. c. rubrimembris* Taylor and Smith 1945 and *P. c. manni* Taylor 1939, is needed in order to resolve the taxonomic issues still pending for this group.

Mexican montane cloud forests are situated between 600 and 2000 m elevation and show a remarkable high biotic diversity (Rzedowsky 1978, 1996; Luna-Vega *et al.* 1999, 2000). The distribution of such forests has been highly fragmented historically, which caused each forest patch to have a different flora and fauna (Luna-Vega *et al.* 1999). Unfortunately, most of the cloud forest habitat in México has been transformed to cattle pasture or converted to cane, banana or coffee agriculture (Pimentel *et al.* 1992; Williams-Linera *et al.* 2002; Pineda & Halffter 2004; Williams-Linera 2007). The state of Veracruz ranks fifth among the Mexican states in the extent of remaining cloud forest habitats (Ortega & Castillo 1996); however, accelerated rates of deforestation and agricultural exploitation have dramatically reduced the size of each forest patch. The cloud forest in the state of Veracruz has been converted to coffee plantations for decades (Williams-Linera *et al.* 2002; Williams-Linera 2007). In some areas the forest has been completely converted for this purpose but in other parts, such as the type locality of *P. cafetalera*, the larger trees have been kept in order to provide shade for the coffee plants. Studies in the state of Veracruz have shown that about 50% of the expected amphibian cloud forest species have been found in coffee plantations (Macip-Ríos & Casas-Andreu 2008; Murrieta-Galindo 2007).

Salamander surveys in México and Guatemala have shown a dramatic decline in the last several decades (Rovito *et al.* 2009). Several species of salamanders once abundant in cloud forests in the state of Veracruz either can no longer be found or have become very rare. The area of Huatusco, the type locality of *P. cafetalera*, is exceptional in terms of salamander biodiversity because it contains representatives of five genera: *Bolitoglossa*, *Pseudoeurycea*, *Thorius*, *Parvimolge*, and *Chiropterotriton*. When *Lineatriton* is treated as a synonym of *Pseudoeurycea*, no other Neotropical site has as many sympatric salamander genera. The presence of four salamander genera (*Bolitoglossa*, *Parvimolge*, *Pseudoeurycea*, and *Thorius*) has been reported from the nearby site of Cuautlapan, near the city of Córdoba, Veracruz. This is one of the sites where a dramatic decline in salamander abundance has been observed (Rovito *et al.* 2009), and several of these genera (*Thorius*, *Pseudoeurycea*, and *Parvimolge*) have become extremely rare in the area. During our resurvey in the Huatusco area (8 visits to the site) we found only one *Parvimolge* and 5 specimens of *P. cafetalera*, and did not find any *Bolitoglossa*, *Chiropterotriton* or *Thorius*. The enigmatic *P. praecellens*, known only from a site near the city of Córdoba, has not been found since its original discovery (Rabb 1955).

North of Huatusco, in the area of Teocelo, six specimens of an undescribed species of *Pseudoeurycea* were collected between 1972 and 1979. Parra-Olea *et al.* (2004) compared these specimens to *P. quetzalanensis* and to the only specimen available of *P. praecellens*. They decided that the Teocelo specimens represented an undescribed species of *Pseudoeurycea* related to *P. cephalica* as Wake *et al.* (1992) suggested. We believe the Teocelo specimens (2 adult males, 2 subadult females, 2 juveniles) may belong to *P. cafetalera*. While the Teocelo specimens are smaller (SL = 43 mm for males, 36–37 mm for subadult females) and appear less robust than specimens of *P. cafetalera*, they are similar in overall morphology to *P. cafetalera* (Table 2) and share a similar coloration pattern to some of the paratypes from Zongolica. Additional adult specimens and molecular data will be necessary to resolve the taxonomic status of the Teocelo specimens.

Material examined

Pseudoeurycea cafetalera ($N = 10$), IBH14337, 14341, 14344, 14343, Rancho dos Puentes, 6.0 km (by road) north of entrance to Huatusco, Veracruz, México ($19^{\circ} 11.13' N$, $96^{\circ} 57.72' W$, 1322 m elevation); IBH14346, 3.5 km SW La Raya, road to Trincheras, Veracruz, México; IBH14338-14339, 14345, Zoncolco, Veracruz, México ($18^{\circ} 46.4' N$ $97^{\circ} 07.61' W$ elevation 1600 m); IBH14349, Las Cañadas, 5.4 km NW of center of Huatusco, Veracruz, México ($19^{\circ} 11.42' N$, $96^{\circ} 59.30' W$, 1367 m elevation).

P. cephalica ($N = 24$), MVZ114298, 147247, 173434, El Chico National Park, Hidalgo, México; MVZ138038, ca. 1.5 km N (by road) Mineral del Chico, Hidalgo, México; MVZ138039, road into El Chico National Park, 6.4 km N (by road) junction México Hwy 105, Hidalgo, México; MVZ172737, 1 km N (by road) Durango on México Hwy. 85, Hidalgo, México; MVZ178836, 4.9 km S Mineral del Chico, Hidalgo, México; IBH22604, Tlaxlacoya, 3.7 km W La Joya, Veracruz, México; IBH22607, 6 km N Hwy 105 at Zacualtipan, Hidalgo, México; IBH14335, 14342, 2 km W Tejocotal, Hidalgo, México; IBH22609, 22599, 22465, 2 km S Ahuazotepec, Puebla, México; IBH22606, 20 km S Chignahuapan, Puebla, México; IBH22605, 2 km N El Mirador, Teziutlan, Puebla, México; IBH22601, 17 km S Huayacocotla, Veracruz, México; IBH22600, 14348, 22608, Desierto de Los Leones, Los Ocotales, D.F., México; IBH14336, 14347, 5.4 km S Mineral del Chico, Hidalgo, México; IBH22602, 22603, km 35 on Apizaco-Chignahuapan Highway, Puebla, México.

Pseudoeurycea cf. cafetalera ($N = 4$), MVZ171336, 6.0 mi S (by road) Coatepec on road to Teocelo, Veracruz, México; MVZ172138, 172140, 172141, Barranca de Teocelo, N Teocelo, Veracruz, México.

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References

- Anderson, S., Bunkier, A.T., Barrell, B.G., Debruijn, M.H.L., Coulson, A.R., Drouin, J., Eperon, I.C., Nierlich, D.P., Roe, B.A., Sanger, F., Schreier, P.H., Smith, A.J.H., Staden, R. & Young I.G. (1981) Sequence and organization of the human mitochondrial genome. *Nature*, 290, 457–465.
- Edgar, R.C. (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, 5, 1–19.
- Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., de Sá, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, R., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M. & Wheeler, W.C. (2006) The amphibian tree of life. *Bulletin of the American Museum of Natural History*, 297, 1–370.
- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–755.
- Luna-Vega, I., Alcántara-Ayala, O., Espinosa-Organista, D., & Morrone, J.J. (1999) Historical relationships of the Mexican cloud forests: a preliminary vicariance model applying parsimony analysis of endemism to vascular plant taxa. *Journal of Biogeography*, 26, 1299–1305.
- Luna-Vega, I., Alcántara-Ayala, O., Morrone, J. J. & Espinosa-Organista, D. (2000) Track analysis and conservation priorities in the cloud forest of Hidalgo, México. *Diversity and Distributions*, 6, 137–143.
- Lynch, J.F. & Wake, D.B. (1989) Two new species of *Pseudoeurycea* (Amphibia: Caudata) from Oaxaca, México. *Contributions in Science, Natural History Museum of Los Angeles County*, 411, 11–22
- Macip-Ríos, R., & Casas-Andreu, G. (2008) Los cafetales de México y su importancia para la conservación de anfibios y reptiles. *Acta Zoológica Mexicana* (n.s.), 24, 143–159.
- Murrieta-Galindo, R. (2007) Diversidad de anfibios en cafetales en la zona centro del Estado de Veracruz, México. Ms Thesis. Manejo de Fauna Silvestre, Instituto de Ecología, A. C., México. 76 pp.
- Nylander, J.A.A. (2004) MrModeltest. Program distributed by the author. Evolutionary Biology Center, Uppsala

University, Uppsala, Sweden.

- Ortega, E.F. & Castillo, G. (1996) El bosque mesófilo de montaña y su importancia forestal. *Ciencias*, 43,32–39.
- Palumbi, S.R., Martin, A.P., Romano, S., McMillan, W.O., Stice, L. & Grabowski G. (1991) *The Simple Fool's Guide to PCR*. Special Publication, Department of Zoology, University of Hawaii, Honolulu, Hawaii, U.S.A.
- Parra-Olea, G. & Wake, D. B. (2001) Extreme morphological and ecological homoplasy in tropical salamanders. *Proceedings of the National Academy of Sciences of the USA*, 98, 7888–7891.
- Parra-Olea, G. (2002) Molecular Phylogenetic relationships of Neotropical salamanders of the genus *Pseudoeurycea*. *Molecular Phylogenetics and Evolution*, 22, 234–246.
- Parra-Olea, G., Canseco-Márquez, L. & García-París M. (2004) A morphologically distinct new species of *Pseudoeurycea* (Caudata: Plethodontidae) from the Sierra Madre Oriental of Puebla, México. *Herpetologica*, 64, 78–84
- Pimentel, D., Stachow, U., Takacs, D., Brubaker, H., Dumas, A., Meany, J., O'Neil, J., Onsi, D. & Corzilius, D. (1992) Conserving biological biodiversity in agricultural/forestry systems. *Bioscience*, 42, 354–362.
- Pineda, E. & Halffter, A.G. (2004) Species diversity and habitat fragmentation: frogs in a tropical montane landscape in México. *Biological Conservation*, 117, 499–508.
- Rabb, G.B. (1955) A new salamander of the genus *Parvimolge* from Mexico. *Breviora, Museum of Comparative Zoology*, 42, 1–9.
- Rovito, S.M., Parra-Olea, G., Vasquez-Almazan, C.R., Papenfuss T.J. & Wake, D.B. (2009) Dramatic declines in neotropical salamander populations are an important part of the global amphibian crisis. *Proceedings of the National Academy of Sciences*, 106, 3231–3236.
- Rzedowski, J. (1978) *Vegetación de México*. Limusa, México.
- Rzedowski, J. (1996) Análisis preliminar de la flora vascular de los bosques mesófilos de montaña de México. *Acta Botánica Mexicana*, 35, 25–44.
- Saiki, R.K., Delfand, D.H., Stooffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. & Erlich, H.A. (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, 239, 487–491.
- Stamatakis, A. (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22, 2688–2690.
- Swofford, D.L. (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.
- Taylor, E.H. (1939) "1938". Concerning Mexican salamanders. *University of Kansas Science Bulletin*, 25, 259–313.
- Taylor, E.H. (1941) Two new species of Mexican plethodontid salamanders. *Proceedings of the Biological Society of Washington*, 54, 81–86.
- Uribe-Peña, Z., Ramírez-Bautista, A. & Casas-Andreu, G. (1999) *Anfibios y reptiles de las serranías del Distrito Federal, México*. Cuadernos del Instituto de Biología, Universidad Nacional Autónoma de México, No. 32, 119 pp.
- Wake, D.B., Papenfuss, T.J. & Lynch, J.F. (1992) Distributions of salamanders along elevational transects in Mexico and Guatemala. *Tulane Studies in Zoology and Botany, Supplementary Publication*, 1, 303–319.
- Walker, C.F. 1955. A new salamander of the genus *Pseudoeurycea* from Tamaulipas. *Occasional Papers of the Museum of Zoology, University of Michigan*, 567: 1–8.
- Wiens, J.J., Parra-Olea, G., García-París, M. & Wake, D.B. (2007) Phylogenetic history explains elevational biodiversity patterns in tropical salamanders. *Proceedings of the Royal Society of London B*, 274, 919–928
- Wilgenbusch J.C., Warren D.L., & Swofford D.L. (2004) AWTY: A system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. <http://ceb.csit.fsu.edu/awty>.
- Williams-Linera, G., Manson R.H. & Isunza-Vera E. (2002). La fragmentación del bosque mesófilo de montaña y patrones de uso del suelo en la región oeste de Xalapa, Veracruz, México. *Madera y Bosque*, 8, 73–89
- Williams-Linera, G. (2007) *El bosque de niebla en el centro de Veracruz: ecología, historia y destino en tiempos de fragmentación y cambio climático*. CONABIO-Instituto de Ecología A. C., Xalapa, Veracruz, México. 208 pp.