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Two new species of *Chiropterotriton* (Caudata: Plethodontidae) from central Veracruz, Mexico

[1]Mirna G. García-Castillo, [2]Ángel F. Soto-Pozos, [3]J. Luis Aguilar-López, [4]Eduardo Pineda, and [5]Gabriela Parra-Olea

[1,2,5]*Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, AP 70-153, Tercer Circuito Exterior s/n, Ciudad Universitaria, México, Distrito Federal, MÉXICO* [3,4]*Red de Biología y Conservación de Vertebrados, Instituto de Ecología, A.C., Carretera Antigua a Coatepec No. 351, El Haya, CP. 91070, Xalapa, Veracruz, MÉXICO*

***Abstract.*—The lungless salamanders of the tribe Bolitoglossini show notable diversification in the Neotropics, and through the use of molecular tools and/or new discoveries, the total number of species continues to increase. Mexico is home to a great number of bolitoglossines primarily distributed along the eastern, central, and southern mountain ranges where the genus *Chiropterotriton* occurs. This group is relatively small, with 16 described species, but there remains a considerable number of undescribed species, suggested by the use of molecular tools in the lab more than a decade ago. Most of these undescribed species are found in the state of Veracruz, an area characterized by diverse topography and high salamander richness. Described herein are two new species of *Chiropterotriton* based on molecular and morphological data. Both new species were found in bromeliads in cloud forests of central Veracruz and do not correspond to any previously proposed species. Phylogenetic reconstructions included two mitochondrial fragments (L2 and COI) and identified are two primary assemblages corresponding to northern and southern species distributions, concordant with previous studies. The two new species are closely related but morphologically and molecularly differentiated from other species of the *C. chiropterus* group.**

Keywords. Salamanders, bolitoglossines, bromeliads, phylogenetics, cryopreservation, living tissue, biobanking

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Introduction

Due to their unique topography and geological history, the Mexican highlands have played an important role in the evolution of plethodontid salamanders (Wake and Lynch 1976; Darda 1994). Particularly, the tribe Bolitoglossini (Wake 2012) underwent an adaptive radiation and diversification in the mountainous regions of Mexico (Wake and Lynch 1976; Wake 1987), resulting in 40% of the representative biodiversity of the group (AmphibiaWeb 2018). With the aid of molecular tools and recent expedition activity, the number of described species has increased in recent years (Parra-Olea et al. 2016; García-Castillo et al. 2017; Sandoval-Comte et al. 2017).

In Mexico, plethodontid richness is concentrated in regions with rugged topography and a corresponding great diversity of habitats and microhabitats (Wake et al. 1992; Rovito et al. 2009). These characteristics are found in the central region of Veracruz, where two important mountain systems converge: the Trans Mexican Volcanic Belt (TMVB) and the Sierra Madre Oriental (SMO). The state of Veracruz has the second highest salamander diversity in Mexico with 36 species, after Oaxaca with 42 species (Parra-Olea et al. 2014).

The genus *Chiropterotriton* includes 16 described species with seven populations suggested as candidate species in previous phylogenetic analyses: *C.* sp. C, *C.* sp. F, *C.* sp. G, *C.* sp. H, *C.* sp. I, *C.* sp. J, and *C.* sp. K (Darda 1994; Parra-Olea 2003). Two of the described species, *C. lavae* (Taylor) and *C. chiropterus* (Cope), and two candidate species (*C.* sp. C and *C.* sp. H) occur in Veracruz (Fig. 1). Describe herein are two new species

Correspondence. [1]*biol.mirnagarcia@gmail.com* [2]*angelfsotop90@gmail.com* [3]*jlal.herp@gmail.com* [4]*eduardo.pineda@inecol.mx* [5]*gparra@ib.unam.mx* (Corresponding author)

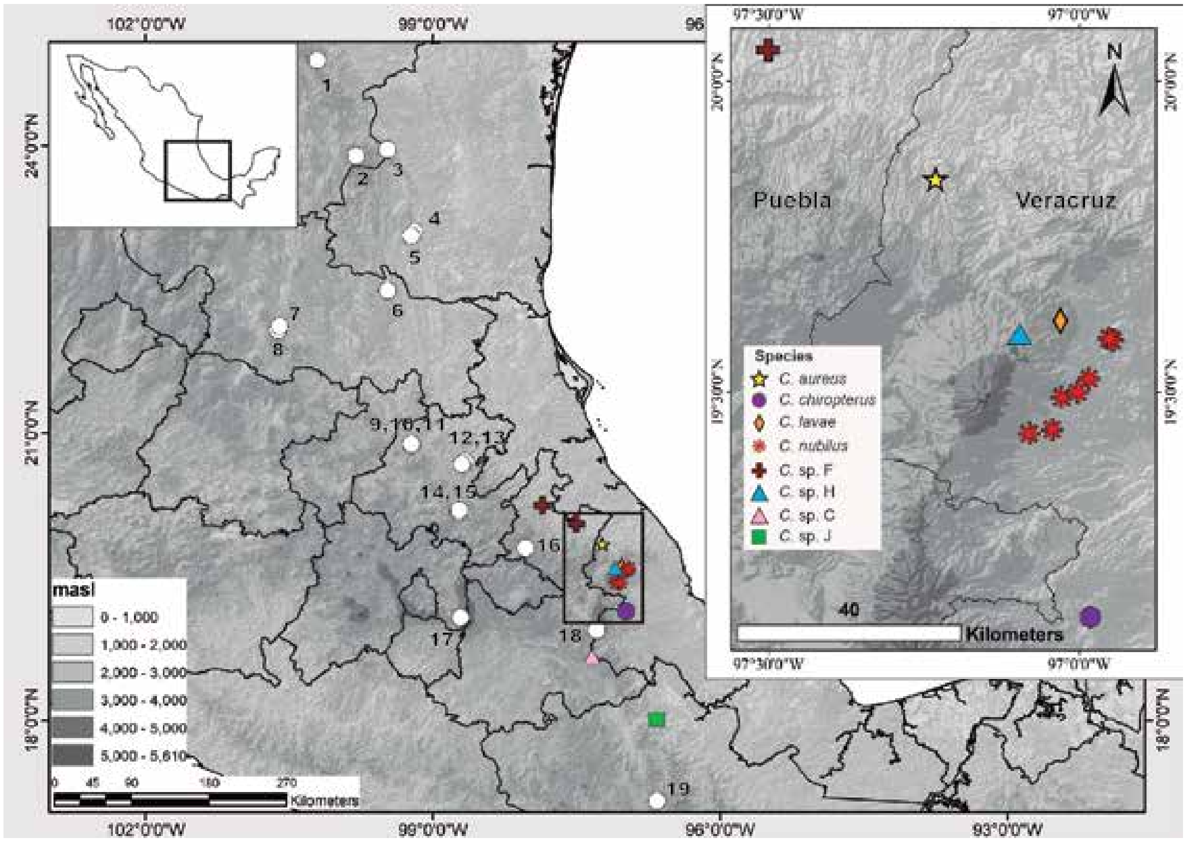

Fig. 1. Map of sampled localities for phylogenetic analyses of the genus *Chiropterotriton*. White circles correspond to: **1)** *C. priscus*, **2)** *C. miquihuanus*, **3)** *C. infernalis*, **4)** *C. cieloensis*, **5)** *C. cracens*, **6)** *C. multidentatus* (Cd. Maiz), **7)** *C. multidentatus* (Rancho Borbotón), **8)** *C. multidentatus* (Sierra de Álvarez), **9)** *C. magnipes*, **10)** *C. mosaueri*, **11)** *C. chondrostega*, **12)** *C. terrestris*, **13)** *C. arboreus*, **14)** *C. dimidiatus*, **15)** *C. chico*, **16)** *C.* sp. G, **17)** *C. orculus*, **18)** *C.* sp. I, and **19)** *C.* sp. K.

of *Chiropterotriton* based on analysis of two mitochondrial fragments (L2 and COI) and differing morphological characteristics. Specimens were discovered during recent expeditions in the mountainous regions of central Veracruz but could not be assigned to any current species due to their unique morphological and genetic differentiation. Furthermore, these proposed new salamanders do not belong to any candidate species postulated by Darda (1994) and Parra-Olea (2003).

Methods

Molecular Analyses

Genomic DNA was extracted from liver, intestine, and tail tissue samples from 38 *Chiropterotriton* individuals and *Aquiloeurycea cephalica* and *Parvimolge townsendi* using a DNeasy tissue kit (Qiagen, Valencia, California, USA). Amplified two mitochondrial fragments using primers LX12SN1 and LX16S1R for L2 (partial 12S ribosomal subunit, the tRNA, and large subunit16S; Zhang et al. 2008) and dgLCO and dgHCO for COI (Meyer 2003). PCR conditions were as follows: L2, 35 cycles of 96 ºC (120 s), 55 ºC (60 s), and 72 ºC (300 s), and COI, 35 cycles of 94 ºC (30 s), 50 ºC (30 s), and 72 ºC (45 s). PCR products were cleaned with ExoSap-IT (USB Corporation, Cleveland, Ohio, USA) and sequenced with a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, California, USA). Products were purified using Sephadex G-50 (GE Healthcare) and an ABI3730 capillary sequencer to run sequences. Additionally, 13 *Chiropterotriton* sequences were obtained from previous studies (Parra-Olea 2003; Rovito et al. 2015) to complete the study. Voucher information for all sequences are shown in Table 1.

Sequencher 5.0.1 (Gene Codes Corporation) was used to edit and assemble sequences and Muscle 3.8 (Edgar 2004) to align fasta files. Mesquite v3.40 (Maddison and Maddison 2018) was applied to review and concatenate data matrices and calculate Kimura 2-parameter (K2P) corrected genetic distances (Table 2). DNA substitution models were calculated using PartitionFinder v1.0 (Lanfear et al. 2012) under the Bayesian information criterion (BIC), and estimated a maximum likelihood (ML) tree from RAxML v8.2 (Stamatakis 2014) with 1,000 bootstrap replicates and a GTR+G substitution model. Additionally, MrBayes v3.2 (Huelsenbeck and Ronquist 2001) was applied for Bayesian analysis with 20 million generations, sampling every 1,000 generations, and four chains used to construct a majority consensus tree. Tracer v.1.7 (Rambaut et al. 2018) was administered to check stationarity and convergence of chains. Lastly, both phy-

Table 1. Voucher information, localities, GenBank accessions, coordinates and elevation data from specimens used for phylogenetic analyses. Collection abbreviations: CARIE, Colección de Referencia de Anfibios y Reptiles del Instituto de Ecología, A.C.; IBH, Colección Nacional de Anfibios y Reptiles, Instituto de Biología, UNAM; MVZ, Museum of Vertebrate Zoology, University of California, Berkeley, California, USA. NOTE: Asterisks indicate data inferred indirectly from the available information.

Species	Voucher Number	Locality	16S GenBank	COI GenBank	Latitude	Longitude	Elevation m asl
C. arboreus	IBH28191	Hidalgo: 6.8 km SW (by rd) of Zacualtipan on road to Tianguistengo	MK335386	MK335232	20.702	-98.667	2029
C. aureus	IBH31040	Veracruz: 6.5 km N from Atzalan, ejido de desarrollo urbano Quetzalcoatl	MK335395	MK335241	19.843	-97.231	1249
C. aureus	IBH31041	Veracruz: 6.5 km N from Atzalan, ejido de desarrollo urbano Quetzalcoatl	MK335398	MK335244	19.843	-97.231	1249
C. aureus	IBH31042	Veracruz: 6.5 km N from Atzalan, ejido de desarrollo urbano Quetzalcoatl	MK335396	MK335242	19.843	-97.231	1249
C. aureus	IBH31043	Veracruz: 6.5 km N from Atzalan, ejido de desarrollo urbano Quetzalcoatl	MK335394	MK335240	19.843	-97.231	1249
C. aureus	IBH31044	Veracruz: 6.5 km N from Atzalan, ejido de desarrollo urbano Quetzalcoatl	MK335397	MK335243	19.843	-97.231	1249
C. chico	MVZ200679	Hidalgo: 3.8 km S Mineral del Chico	AY522471	–	20.180	-98.731	2630
C. chiropterus	CARIE0777	Veracruz: Huatusco	MK335407	MK335253	19.185	-96.959	1280
C. chiropterus	CARIE0719	Veracruz: Huatusco	MK335408	–	19.185	-96.959	1280
C. chondrostega	IBH30098	Hidalgo: 1.0 km S (by rd) of La Encarnacion on road to MX85, Parque Nacional los Marmoles	MK335383	MK335229	20.866	-99.219	2471
C. cieloensis	IBH28181	Tamaulipas: 0.2 km E (by air) of Rancho El Cielo, 6.9 km NNW (by air) of the center of Gomez Farías, Reserva de la Biosfera El Cielo	MK335385	MK335231	23.100	-99.190	1174
C. cracens	IBH28192	Tamaulipas: Road from Alta Cima to San Jose, 1.3 km NE (by air) of San Jose, Reserva de la Biosfera El Cielo	MK335384	MK335230	23.059	-99.226	1320
C. dimidiatus	IBH28196	Hidalgo: 4.1 km S (by rd) of Mineral del Chico on the road to Pachuca, Parque Nacional El Chico	MK335390	MK335236	20.198	-98.727	2768
C. infernalis	MVZ269665	Tamps: Cueva del Brinco, Conrado Castillo, ca. 43.5 km SW (by rd) of Ejido Guayabas	MK335382	MK335228	23.959	-99.474	1920
C. lavae	IBH22369	Veracruz: 200 m N hwy 140 at La Joya	MK335393	MK335239	19.614	-97.030	2060
C. magnipes	IBH28176	Hidalgo: "El Coní," 900 m SSE of the center of Durango, Municipio Zimapan, Parque Nacional los Marmoles	MK335387	MK335233	20.888	-99.226	2234
C. miquihuanus	IBH30329	Nuevo León: 1.8 km S (by rd) of La Encantada on road from La Bolsa to Zaragoza	MK335381	MK335227	23.893	-99.803	2803

Table 1 (continued). Voucher information, localities, GenBank accessions, coordinates and elevation data from specimens used for phylogenetic analyses. Collection abbreviations: CARIE, Colección de Referencia de Anfibios y Reptiles del Instituto de Ecología, A.C.; IBH, Colección Nacional de Anfibios y Reptiles, Instituto de Biología, UNAM; MVZ, Museum of Vertebrate Zoology, University of California, Berkeley, California, USA. NOTE: Asterisks indicate data inferred indirectly from the available information.

Species	Voucher Number	Locality	16S GenBank	COI GenBank	Latitude	Longitude	Elevation m asl
C. mosaueri	IBH28179	Hidalgo: "El Coní," 900 m SSE of center of Durango, Municipio Zimapan, Parque Nacional los Marmoles	MK335388	MK335234	20.888	-99.226	2234
C. multidentatus	IBH28177	San Luis Potosí: Cueva el Madroño, 900 m NW (by air) of the entrance to Valle de los Fantasmas on MX70, Sierra de Alvarez	MK335416	–	22.071	-100.614	2297
C. multidentatus	IBH30102	San Luis Potosí: Cueva el Madroño, 900 m NW (by air) of entrance to Valle de los Fantasmas on MX70, Sierra de Alvarez	MK335417	–	22.071	-100.614	2297
C. multidentatus	IBH28193	San Luis Potosí: 26.2 km E (by rd) of the center of Ciudad del Maíz on MX80, at turnoff to RMO Las Antenas San Luis Potosí	MK335412	–	22.487	-99.473	1223
C. multidentatus	IBH30104	San Luis Potosí: 26.2 km E (by rd) of the center of Ciudad del Maíz on MX80, at turnoff to RMO Las Antenas San Luis Potosí	MK335414	–	22.487	-99.473	1223
C. multidentatus	IBH28194	San Luis Potosí: 26.2 km E (by rd) of center of Ciudad del Maíz on MX80, at turnoff to RMO Las Antenas San Luis Potosí	MK335413	–	22.487	-99.473	1223
C. multidentatus	IBH23111	San Luis Potosí: Rancho Borbortón	MK335415	–	22.116	-100.601	2098
C. nubilus	IBH31045	Veracruz: 8.2 km W of Xico, Coxmatla	MK335405	MK335251	19.433	-97.080	2023
C. nubilus	IBH31046	Veracruz: 8.2 km W of Xico, Coxmatla	MK335399	MK335245	19.433	-97.080	2023
C. nubilus	IBH31048	Veracruz: 8.2 km W of Xico, Coxmatla	MK335402	MK335248	19.433	-97.080	2023
C. nubilus	IBH31049	Veracruz: 8.2 km W of Xico, Coxmatla	MK335403	MK335249	19.433	-97.080	2023
C. nubilus	IBH31050	Veracruz: 8.2 km W of Xico, Coxmatla	MK335400	MK335246	19.433	-97.080	2023
C. nubilus	IBH31052	Veracruz: 8.2 km W of Xico, Coxmatla	MK335401	MK335247	19.433	-97.080	2023
C. nubilus	IBH31053	Veracruz: 4 km W of Xico, road to Xico Viejo	MK335404	MK335250	19.439	-97.043	1583
C. nubilus	CARIE0739	Veracruz: Bosque Banderilla, Banderilla	MK335411	–	19.586	-96.946	1580
C. nubilus	CARIE0740	Veracruz: Bosque Rancho Viejo, Tlalnehuayocan	MK335406	MK335252	19.521	-96.984	1520
C. nubilus	CARIE1162	Veracruz: Rancho la Mesa, Banderilla	KP886894	–	19.582	-96.945	1577
C. orculus	IBH30765	Estado de México: Amecameca, road to Popocatepetl volcano	MK335391	MK335237	19.072	-98.711	2800*
C. orculus	IBH30746	Estado de México: Amecameca, road to Popocatepetl volcano	MK335392	MK335238	19.072	-98.711	2800*

Table 1 (continued). Voucher information, localities, GenBank accessions, coordinates and elevation data from specimens used for phylogenetic analyses. Collection abbreviations: CARIE, Colección de Referencia de Anfibios y Reptiles del Instituto de Ecología, A.C.; IBH, Colección Nacional de Anfibios y Reptiles, Instituto de Biología, UNAM; MVZ, Museum of Vertebrate Zoology, University of California, Berkeley, California, USA. NOTE: Asterisks indicate data inferred indirectly from the available information.

Species	Voucher Number	Locality	16S GenBank	COI GenBank	Latitude	Longitude	Elevation m asl
C. priscus	IBH22367	Nuevo León: 19.4 Km W 18 de Marzo, Cerro Potosi	MK335380	MK335226	24.891	-100.208	2600
C. terrestris	GP215	Hidalgo: 5.3 km N hwy 105 at Zacualtipan.	MK335389	MK335235	20.674	-98.696	1860
C. sp. C	MVZ163635	Veracruz: 3.2 km S Puerto del Aire	AY522453	–	18.670	-97.338	2406*
C. sp. C	IBH 14317	Veracruz: 3.0 km S Puerto del Aire	AY522454	–	18.670	-97.338	2400
C. sp. F	IBH30112	Puebla: 7.1 km N (by rd) of the center of Cuetzalan on road to Yohualichán	MK335410	MK335255	20.050	-97.500	965
C. sp. F	MVZ178706	Puebla: 3.9 km S Xicotepec de Juárez	AY522477	–	20.246	-97.854	1135
C. sp. F	MVZ200723	Puebla: Xicotepec de Juárez, 3.3 km S of Hotel M. Ranchito on Mexico Hwy. 130, 21 km E on road to La Union	AY522478	–	20.246	-97.854	1152
C. sp. F	MVZ178707	Puebla: 3.9 km S Xicotepec de Juárez	AY522479	–	20.246	-97.854	1135
C. sp. G	MVZ178700	Puebla: 4 km S Chignahuapan	AY522480	–	19.801	-98.030	2750
C. sp. G	MVZ178703	Puebla: 4 km S Chignahuapan	AY522481	–	19.801	-98.030	2750
C. sp. H	IBH22568	Veracruz: Microondas las Lajas	KP886893	–	19.593	-97.095	3127
C. sp. I	MVZ201387	Puebla: Santa Cruz de Texmalaquilla	AY522488	–	18.942	-97.287	3100*
C. sp. I	MVZ201389	Puebla: Santa Cruz de Texmalaquilla	AY522487	–	18.942	-97.287	3100*
C. sp. J	IBH30099	Oaxaca: San Bernardo, 4.8 km SW (by rd) of La Esperanza on MX177	MK335409	MK335254	18.015	-96.660	1672
C. sp. K	MVZ173231	Oaxaca: Cerro San Felipe	AY522493	–	17.160	-96.661	3010*
Aquiloeurycea cephalica	IBH30253	Hidalgo: 1.0 km S (by rd) of La Encarnación on road to MX85, Parque Nacional los Mármoles	MK335378	–	20.866	-99.219	2407
Parvimolge townsendi	IBH31063	Veracruz: 4 km W Xico, road to Xico Viejo	MK335379	MK335225	19.439	-97.043	1583

Table 2. Sequence divergence with Kimura two-parameter distances for 16S (left) and COI (right).

	C. aureus	***C. chiropterus***	***C. lavae***	***C. nubilus***	***C. sp. C***	***C. sp. F***	***C. sp. G***	***C. sp. H***	***C. sp. I***	***C. sp. J***	***C. sp. K***
C. aureus	–	5%/10%	10%/13%	4%/7%	9%/–	6%/11%	6%/–	9%/–	9%/–	5%/8%	5%/–
C. chiropterus	5%/10%	–	7%/16%	3%/10%	7%/–	6%/13%	7%/–	7%/–	7%/–	1%/5%	6%/–
C. lavae	10%/13%	7%/16%	–	8%/15%	1%/–	9%/13%	7%/–	1%/–	1%/–	8%/15%	7%/–
C. nubilus	4%/7%	3%10%	8%/15%	–	7%/–	5%/12%	7%/–	8%/–	7%/–	3%/8%	6%/–
C. sp. C	9%/–	7%/–	1%/–	7%/–	–	9%/–	7%/–	2%/–	1%/–	7%/–	7%/–
C. sp. F	6%/11%	6%/13%	9%/13%	5%/12%	9%/–	–	8%/–	9%/–	8%/–	7%/12%	7%/–
C. sp. G	6%/–	7%/–	7%/–	7%/–	7%/–	8%/–	–	7%/–	7%/–	7%/–	4%/–
C. sp. H	9%/–	7%/–	1%/–	8%/–	2%/–	9%/–	7%/–	–	1%/–	8%/–	7%/–
C. sp. I	9%/–	7%/–	1%/–	7%/–	1%/–	8%/–	7%/–	1%/–	–	7%/–	6%/–
C. sp. J	5%/8%	1%/5%	8%/15%	3%/8%	7%/–	7%/12%	7%/–	8%/–	7%/–	–	7%/–
C. sp. K	5%/–	6%/–	7%/–	6%/–	7%/–	7%/–	4%/–	7%/–	6%/–	7%/–	–

logenetic methods were ran through the CIPRES data portal (Miller et al. 2010).

Morphological Analyses

Analysis compared new taxa morphology with phylogenetically and geographically related species (see Results). Further comparisons included measurements taken from seven adult specimens of the two new species, twelve *C. chiropterus*, nineteen *C. lavae*, twenty *C. orculus* (Cope), and published measurements of *C. dimidiatus* (Taylor) from García-Castillo et al. (2017) [Table 3; Appendix 1]. Male and female comparisons were completed separately due to sexual dimorphic differences.

Basic characters and measurements follow the format used by Lynch and Wake (1989): snout-vent length (SVL), tail length (TL), axilla-groin distance (AX), forelimb length (FLL), hind limb length (HLL), snout to gular fold distance (head length, HL), head width at the angle of the jaw (HW), head depth (HD), shoulder width (SW), internarial distance (IN), and right foot width (FW). In addition, twelve measurements were taken from holotypes: anterior rim of orbit to snout, eyelid length, eyelid width, horizontal orbital diameter, interorbital distance, length of third (longest) toe, length of fifth toe, projection of snout beyond mandible, snout to anterior angle of the vent, snout to forelimb, tail depth at the base, and tail width at the base (All measurements are given in mm, except tooth counts and adpressed limbs). Maxillary plus premaxillary (MT+PMT) and vomerine teeth (VT) were recorded for all specimens. Finally, measurements were documented for the limb interval (LI) as the number of costal folds between adpressed limbs (positive values as grooves and negative values as the overlap between limbs). Descriptions are based on the color catalogue from Köhler (2012).

Results

Mitochondrial DNA (mtDNA) dataset included the 16 described species of *Chiropterotriton* plus seven previously proposed candidate species (Darda 1994; Parra-Olea 2003). Obtained were a 1,477-bp matrix for ribosomal 12S, tRNA, and 16S genes (including gaps) and 658 bp for COI gene. The estimated substitution models were as follows: GTR+G for 12S, tRNA, 16S, the 3rd codon position of COI, K80+G for the 1st codon position of COI, and HKY+1 for the 2^{nd} codon position of COI. Our concatenated phylogeny has a similar topology as shown in previous studies (Darda 1994; Parra-Olea 2003; Rovito and Parra-Olea 2015; García-Castillo et al. 2017), which show two main groups, a northern and southern species groups. (Fig. 2). The northern assemblages have a distribution from central Mexico in Hidalgo to Nuevo Leon, the most northern limit for the genus, and include the following species: *C. terrestris* (Taylor), *C. chico* García-Castillo et al., *C. infernalis* Rovito and Parra-Olea, *C. chondrostega* (Taylor), *C. mosaueri* (Woodall), *C. priscus* Rabb, *C. miquihuanus* Campbell et al., *C. magnipes* Rabb, *C. cracens* Rabb, *C. cieloensis* Rovito and Parra-Olea, *C. arboreus* (Taylor), and *C. multidentatus* (Taylor). Whereas, the southern assemblages (PP = 1.0, BS = 100) occur from central Mexico in Hidalgo to the south in Oaxaca and only have four described species: *C. dimidiatus*, *C. orculus*, *C. lavae*, and *C. chiropterus*. However, this clade includes seven previously proposed candidate species: *C*. sp. G, *C*. sp. K, *C*. sp. H, *C*. sp. I, *C*. sp. C, *C*. sp. F, and *C*. sp. J (Fig. 2). Results support the distinctiveness of two additional taxa genetically divergent from all others and correspond to specimens collected in central Veracruz. One occurs in only one locality (Atzalan) on the western side of Sierra de Chiconquiaco, but the second was found in six localities (Coxmatla, Xico, Banderilla, Cinco Palos, La Cortadura, and Tlalnehuayocan) on the eastern slope of Cofre de Perote (Fig. 2). There is no molecular data for the Cinco Palos and La Cortadura populations, but these specimens were assigned to the new taxa according to morphological characters and concordant geographical distributions (Fig. 1).

The new taxa are phylogenetically related to *C. chiropterus*, *C*. sp. J, and *C*. sp. F. The genetic distance (K2P) between specimens from Atzalan and their closely related taxa are as follows: *C. chiropterus* 5% (16S) and 10% (COI), *C*. sp. J 5% (16S) and 8% (COI), *C*. sp. F 6% (16S) and 11% (COI), and the Cofre de Perote specimens (average for all 4 populations) 4% (16S) and 7% (COI). The genetic distance between specimens from Cofre de Perote (all 4 populations) and their closely related taxa are as follows: *C. chiropterus* 3% (16S) and 10% (COI), *C*. sp. J 3% (16S) and 8% (COI), *C*. sp. F 5% (16S) and 12% (COI), and Atzalan specimens 4% (16S) and 7% (COI) [Table 2]. According to the phylogenetic analysis, *C. chiropterus* and *C*. sp. J are sister taxa (PP = 1.0, BS = 100) with 1% (16S) and 5% (COI) genetic divergence between them, and a sister clade to specimens from Cofre de Perote, although with little support (PP = 0.61, BS = 40). These three taxa are the sister group to specimens from Atzalan (PP = 1.0, BS = 100). *Chiropterotriton* sp. F is the sister taxon of all the aforementioned taxa (PP = 1.0, BS = 100) [Fig. 2]. Given the molecular evidence and morphological comparisons, proposed herein are the Atzalan and Cofre de Perote populations as new species.

Systematics

Chiropterotriton aureus sp. nov.

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Atzalan Golden Salamander
Salamandra Dorada de Atzalan
(Figs. 3A, 4A, and 4B)

Holotype. IBH 31042, an adult male from Atzalan, Veracruz, 6.5 km N from Atzalan, ejido de desarrollo urbano

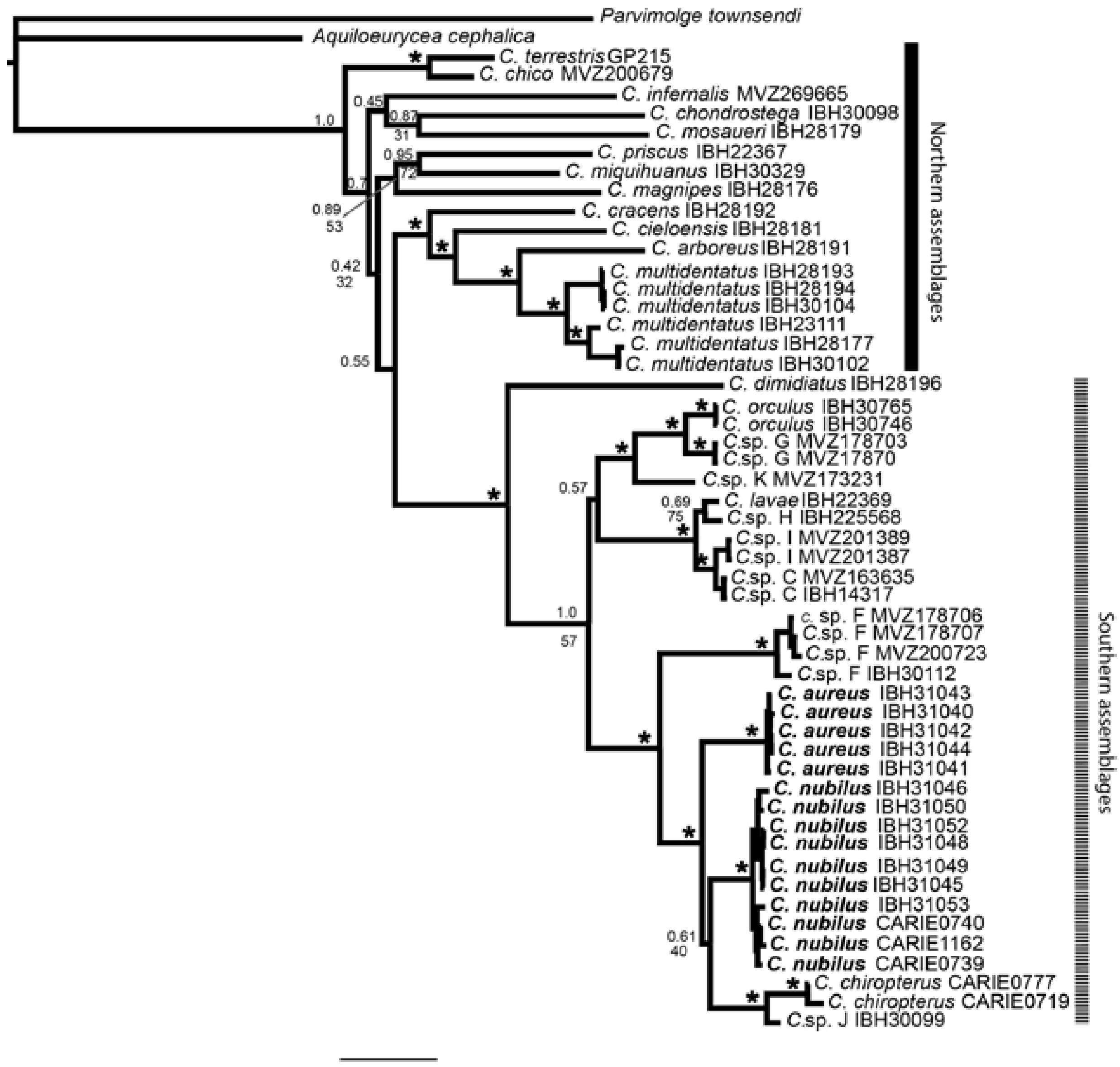

Fig. 2. Bayesian analysis tree for mitochondrial loci. Numbers above branches correspond to posterior probability, and numbers below branches are bootstrap values from maximum likelihood analysis. Asterisks indicate significant support (posterior probability, PP > 0.95 and bootstrap, BS > 70) in both analyses. The topology is grouped into northern and southern assemblages according to species distributions.

Quetzalcóatl, Mexico, 1,249 meters (m) above sea level (asl), 19.843138N, 97.231194W. Collected on 11 July 2016 by Ángel F. Soto-Pozos, M. Delia Basanta, Omar Becerra-Soria, and Mirna G. García-Castillo.

Paratypes. Three specimens from Atzalan, Veracruz, Mexico. All females: IBH 31041, 31043–44, 6.5 km N from Atzalan, ejido de desarrollo urbano Quetzalcóatl, Atzalan, Veracruz, Mexico.

Referred specimens. IBH 31040, 6.5 km N from Atzalan, ejido de desarrollo urbano Quetzalcóatl, Atzalan, Veracruz, Mexico.

Diagnosis. A plethodontid salamander assigned to the genus *Chiropterotriton* due to its small size, slender body, shape of hand and feet digits (relatively long outer digit), relatively long tail, presence of sublingual fold, and based on mtDNA sequence data. Phylogenetically related to *C. nubilus*, *C. chiropterus*, *C.* sp. F, and *C.* sp. J (Fig. 2). *Chiropterotriton aureus* differs from *C. nubilus* in being shorter (SVL 28.5 in one male, mean 26.8 in females of *C. aureus* vs. 29.4 in one male, 30.5 in females of *C. nubilus*) with a shorter head (HL 6.4 in one male, mean 6.0 in females of *C. aureus* vs. 6.6 in one male, 7.4 in females of *C. nubilus*), narrower head in females (mean HW 3.6 in females of *C. aureus* vs. 4.4 in females of *C. nubilus*), relatively shorter limbs in females (mean LI 2.3 in females of *C. aureus* vs. 1.5 in females of *C. nubilus*), and smaller feet (FW 2.4 in one male, mean 1.8 in females of *C. aureus* vs. 2.6 in one male, 2.3 in females of *C. nubilus*). Digits are narrower at the tip and with

Table 3. Mean ± standard deviation (above) and range (below) of morphometric variables from males and females of *C. aureus*, *C. chiropterus*, *C. dimidiatus*, *C. lavae*, *C. nubilus*, and *C. orculus*. Measurements are given in millimeters (mm), except TL/SLV (proportional value), LI (limb interval), and tooth counts. NOTE: Data taken from García-Castillo et al. 2017.

Males	***C. aureus*** ***N*=1**	***C. chiropterus*** ***N*=8**	***C. dimidiatus**** ***N*=15**	***C. lavae*** ***N*=10**	***C. nubilus*** ***N*=1**	***C. orculus*** ***N*=10**
SVL	28.5	37.5±0.98 (36.1–38.8)	24.7±0.97 (23.3–26.7)	32.4±0.92 (31.0–33.8)	29.4	35.9±1.36 (33.6–38.9)
TL	36.5	47.3±3.24 (42.6–52.3) *N*=7	22.0±1.72 (18.5–24.1)	38.5±2.11 (36.2–42.3)	40.2	36.6±2.87 (33.3–41.0) *N*=9
TL/SLV	1.28	1.25±0.08 (1.13–1.38) *N*=7	0.89±0.08 (0.7–1.0)	1.2±0.06 (1.11–1.27)	1.37	1.02±0.08 (0.86–1.15) *N*=9
AX	15.5	19.6±0.59 (18.7–20.8)	13.1±0.75 (11.7–14.0)	16.2±0.87 (14.7–17.4)	15.9	18.6±1.04 (17.1–20.5)
FLL	5.9	9.1±0.44 (8.2–9.5)	4.5±0.34 (3.8–5.0)	9.3±0.59 (8.4–10.2)	6.4	8.9±0.65 (7.4–9.6)
HLL	7.5	10.3±0.47 (9.5–10.8)	5.2±0.34 (4.9–5.9)	9.9±0.72 (8.5–11.0)	7.1	9.3±0.64 (8.2–10.4)
HL	6.4	8.1±0.41 (7.7–8.9)	5.3±0.32 (4.8–5.8)	7.5±0.33 (7.2–8.1)	6.6	7.4±0.47 (6.7–8.1)
HW	4.0	5.6±0.22 (5.4–6.0)	3.5±0.21 (3.0–3.7)	4.9±0.31 (4.5–5.6)	4.0	5.0±0.35 (4.5–5.5)
HD	1.8	2.7±0.07 (2.6–2.8)	1.8±0.09 (1.7–2.0)	2.5±0.19 (2.3–2.9)	2.0	2.4±0.13 (2.2–2.7)
SW	3.4	4.0±0.35 (3.2–4.4)	2.9±0.29 (2.3–3.6)	3.1±0.30 (2.6–3.5)	3.4	3.4±0.30 (3.1–4.0)
IN	1.0	1.9±0.13 (1.7–2.1)	1.2±0.08 (1.0–1.3)	2.3±0.20 (1.9–2.5)	1.2	2.2±0.19 (1.9–2.5)
FW	2.4	3.7±0.33 (3.3–4.4)	1.7±0.20 (1.4–2.1)	3.7±0.39 (3.1–4.2)	2.6	3.2±0.22 (2.8–3.5)
LI	2.0	0.3±0.53 (-0.5–1.0)	3.9±0.35 (3.0–4.0)	-0.6±0.52 (-1.0–0.0)	2.0	1.9±0.88 (0.0–3.0)
PMT+MT	14.0	16.3±3.69 (11.0–21.0)	9.4±2.59 (5.0–14.0)	10.3±3.62 (3.0–15.0)	20.0	10.9±2.47 (7.0–14.0)
VT	15.0	10.6±1.06 (9.0–12.0)	5.7±1.35 (4.0–8.0)	8.9±1.10 (7.0–10.0)	10.0	8.6±1.90 (5.0–11.0)

Females	***C. aureus*** ***N*=3**	***C. chiropterus*** ***N*=4**	***C. dimidiatus**** ***N*=15**	***C. lavae*** ***N*=9**	***C. nubilus*** ***N*=2**	***C. orculus*** ***N*=10**
SVL	26.8±0.86 (26.0–27.7)	33.5±2.55 (30.7–36.7)	25.8±1.56 (23.1–29.1)	31.6±2.46 (27.9–34.9)	30.5±3.89 (27.7–33.2)	39.0±2.70 (34.9–43.0)
TL	31.1±1.41 (30.1–32.1)	39.5±2.35 (37.0–42.6)	22.4±1.85 (19.9–25.2)	32.5±4.89 (25.7–40.1)	34.3±5.16 (30.6–37.9)	39.2±3.64 (34.7–44.7) *N*=9
TL/SLV	1.16±0.00 (1.16–1.16)	1.19±0.12 (1.01–1.26)	0.87±0.06 (0.7–1.0)	1.0±0.10 (0.85–1.15)	1.12±0.03 (1.10–1.14)	1.02±0.08 (0.87–1.12) *N*=9
AX	15.0±0.49 (14.7–15.6)	18.5±2.27 (15.4–20.7)	14.8±1.24 (12.6–17.3)	16.3±1.68 (13.9–18.5)	16.4±2.69 (14.5–18.3)	21.2±1.58 (18.6–23.2)
FLL	5.3±0.42 (4.8–5.6)	7.8±0.48 (7.1–8.2)	4.3±0.43 (3.8–5.1)	8.2±0.72 (7.1–9.5)	6.5±0.28 (6.3–6.7)	8.9±0.63 (7.6–10.0)
HLL	6.7±0.35 (6.4–7.1)	8.9±0.31 (8.4–9.1)	5.0±0.47 (4.4–6.1)	8.8±0.73 (7.5–9.8)	7.2±0.14 (7.1–7.3)	9.5±0.57 (8.6–10.4)

Table 3 (continued). Mean ± standard deviation (above) and range (below) of morphometric variables from males and females of *C. aureus*, *C. chiropterus*, *C. dimidiatus*, *C. lavae*, *C. nubilus*, and *C. orculus*. Measurements are given in millimeters (mm), except TL/SLV (proportional value), LI (limb interval), and tooth counts. NOTE: Data taken from García-Castillo et al. 2017.

Females	*C. aureus* *N*=3	*C. chiropterus* *N*=4	*C. dimidiatus** *N*=15	*C. lavae* *N*=9	*C. nubilus* *N*=2	*C. orculus* *N*=10
HL	6.0±0.31 (5.7–6.3)	7.3±0.56 (6.5–7.8)	5.1±0.34 (4.5–5.6)	7.0±0.42 (6.3–7.6)	7.4±0.99 (6.7–8.1)	8.0±0.52 (7.4–8.9)
HW	3.6±0.10 (3.5–3.7)	4.8±0.21 (4.5–5.0)	3.5±0.25 (3.2–4.0)	4.7±0.30 (4.1–5.0)	4.4±0.14 (4.3–4.5)	5.2±0.29 (4.7–5.6)
HD	1.8±0.02 (1.8–1.8)	2.5±0.14 (2.3–2.6)	2.0±0.20 (1.7–2.2)	2.3±0.18 (2.1–2.7)	2.0±0.07 (1.9–2.0)	2.6±0.32 (2.3–3.4)
SW	3.1±0.17 (3.0–3.3)	3.6±0.38 (3.3–4.1)	3.1±0.26 (2.8–3.5)	3.3±0.33 (2.8–3.8)	3.3±0.28 (3.1–3.5)	3.9±0.46 (3.4–4.8)
IN	1.1±0.06 (1.0–1.1)	1.7±0.38 (1.4–2.1)	1.3±0.15 (1.1–1.7)	1.8±0.13 (1.6–2.0)	1.2±0.02 (1.2–1.2)	2.1±0.25 (1.7–2.5)
FW	1.8±0.21 (1.6–2.0)	3.1±0.37 (2.6–3.5)	1.8±0.26 (1.3–2.2)	3.3±0.27 (3.0–3.7)	2.3±0.57 (1.9–2.7)	3.4±0.37 (2.6–3.9)
LI	2.3±0.58 (2.0–3.0)	2.0±0.41 (1.5–2.5)	4.9±0.26 (4.0–5.0)	0.6±0.73 (0.0–2.0)	1.5±0.71 (1.0–2.0)	2.9±0.32 (2.0–3.0)
PMT+MT	44.7±2.08 (43.0–47.0)	54.3±8.08 (47.0–63.0)	34.4±4.12 (27.0–41.0)	28.0±8.19 (17.0–45.0)	48.0±2.83 (46.0–50.0)	35.9±4.46 (29.0–43.0)
VT	12.3±1.53 (11.0–14.0)	12.5±2.38 (10.0–15.0)	8.3±1.35 (6.0–11.0)	11.4±2.30 (8.0–15.0)	13.5±0.71 (13.0–14.0)	12.0±1.94 (9.0–15.0)

less webbing (just onto the penultimate phalanx) than *C. nubilus* (Fig. 3).

Chiropterotriton aureus differs from *C. chiropterus* in being shorter (SVL 28.5 in one male, mean 26.8 in females of *C. aureus* vs. 37.5 in males, 33.5 in females of *C. chiropterus*), relatively shorter limbs in males (LI 2.0 in one male of *C. aureus* vs. 0.3 in males of *C. chiropterus*), shorter head (HL 6.4 in one male, mean 6.0 in females of *C. aureus* vs. 8.1 in males, 7.3 in females of *C. chiropterus*), narrower head (HW 4.0 in one male, 3.6 in females of *C. aureus* vs. 5.6 in males, 4.8 in females of *C. chiropterus*), and smaller feet (FW 2.4 in one male, mean 1.8 in females of *C. aureus* vs. 3.7 in males, 3.1 in females of *C. chiropterus*). *Chiropterotriton aureus* has narrower digits at the tip and smaller feet and hands than *C. chiropterus* (Fig. 3).

Chiropterotriton aureus differs from its geographically close species *C. lavae* by being shorter (SVL 28.5 in one male, mean 26.8 in females of *C. aureus* vs. 32.4 in males, 31.6 in females of *C. lavae*), shorter head (HL 6.4 in one male, mean 6.0 in females of *C. aureus* vs. 7.5 in males, 7.0 in females of *C. lavae*), narrower head (HW 4.0 in one male, 3.6 in females of *C. aureus* vs. 4.9 in males, 4.7 in females of *C. lavae*), shorter limbs (LI 2.0 in one male, mean 2.3 in females of *C. aureus* vs. -0.6 in males, 0.6 in females of *C. lavae*), and smaller feet (FW 2.4 in one male, mean 1.8 in females of *C. aureus* vs. 3.7 in males, 3.3 in females of *C. lavae*) with less webbing in *C. aureus* than in *C. lavae* (Fig. 3).

Chiropterotriton aureus differs from *C. orculus* by being shorter (SVL 28.5 in one male, mean 26.8 in females of *C. aureus* vs. 35.9 in males, 39.0 in females of *C. orculus*), longer tail (TL/SVL 1.28 in one male, mean 1.16 in females of *C. aureus* vs. 1.02 in both males and females of *C. orculus*), relatively larger limbs in females (mean LI 2.3 in females of *C. aureus* vs. 2.9 in females of *C. orculus*), shorter head (HL 6.4 in one male, mean 6.0 in females of *C. aureus* vs. 7.4 in males, 8.0 in females of *C. orculus*), narrower head (HW 4.0 in one male, 3.6 in females of *C. aureus* vs. 5.0 in males, 5.2 in females of *C. orculus*), and smaller feet (FW 2.4 in one male, mean 1.8 in females of *C. aureus* vs. 3.2 in males, 3.4 in females of *C. orculus*).

Chiropterotriton aureus differs from *C. dimidiatus* in being longer (SVL 28.5 in one male, mean 26.8 in females of *C. aureus* vs. 24.7 in males, 25.8 in females of *C. dimidiatus*), longer tail (TL/SVL 1.28 in one male, mean 1.16 in females of *C. aureus* vs. 0.89 in males, 0.87 in females of *C. dimidiatus*), longer head (HL 6.4 in one male, mean 6.0 in females of *C. aureus* vs. 5.3 in males, 5.1 in females of *C. dimidiatus*), longer limbs (LI 2.0 in one male, mean 2.3 in females of *C. aureus* vs. 3.9 in males, 4.9 in females of *C. dimidiatus*), and more maxillary teeth (PMT+MT 14.0 in one male, mean 44.7 in females of *C. aureus* vs. 9.4 in males, 34.4 in females of *C. dimidiatus*).

Chiropterotriton aureus is phylogenetically related to members of the southern assemblages (Fig. 2), which includes seven undescribed taxa previously suggested by allozyme data (Darda 1994) and mtDNA (Parra-Olea 2003). *Chiropterotriton aureus* differs genetically from its close relatives as follows: 6% (16S) and 11% (COI) to *C.* sp. F; 5% (16S) and 8% (COI) to *C.* sp. J; 9% (16S) to *C.* sp. H, *C.* sp. I, and *C.* sp. C; 6% (16S) to *C.* sp. G; and 5% (16S) to *C.* sp. K (Table 2).

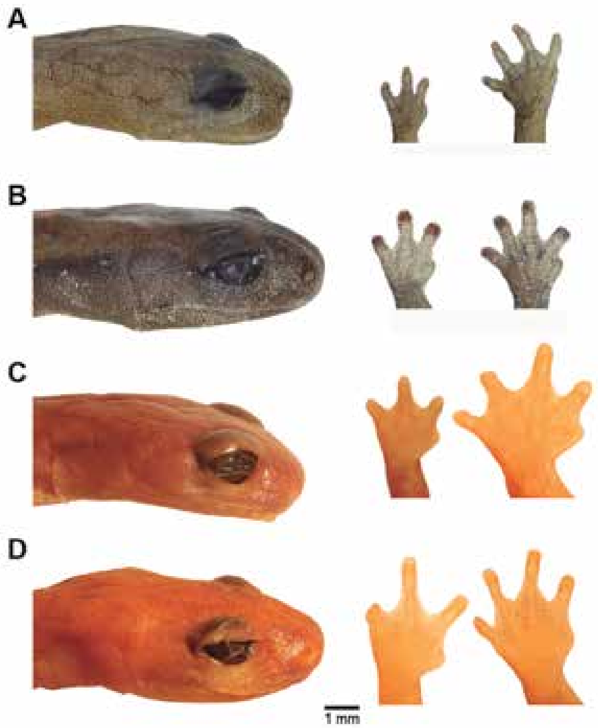

Fig. 3. Head, hand, and foot morphology of preserved specimens of *Chiropterotriton* species from central Veracruz. Ventral view from right hand and foot. **A)** *C. aureus* holotype IBH 31042, **B)** *C. nubilus* holotype IBH 31048, **C)** *C. lavae* MVZ 106436, and **D)** *C. chiropterus* MVZ 85590.

Chiropterotriton aureus differs from other members of *Chiropterotriton* by its smaller body size (SVL 28.5 in one male, mean 26.8 in females), while *C. arboreus* (mean SVL 33.4 in males, 32.2 in females; García-Castillo et al. 2017), *C. cieloensis* (mean SVL 32.6 in males, 31.1 in females; Rovito and Parra-Olea 2015), *C. chico* (mean SVL 38.4 in males, 39.3 in females; García-Castillo et al. 2017), *C. infernalis* (mean SVL 36.4 in males, 29.7 in one female; Rovito and Parra-Olea 2015), *C. magnipes* (mean SVL 46.8 in males, 57.5 in females; Rabb 1965), *C. miquihuanus* (mean SVL 33.3 in males, 36.5 in females; Rovito and Parra-Olea 2015), *C. mosaueri* (mean SVL 42.8 in males; Woodall 1941), *C. multidentatus* (mean SVL 33.6 in males, 34.0 in females; Rovito and Parra-Olea 2015), and *C. priscus* (mean SVL 38.5 in males, 41.8 in females; Rovito and Parra-Olea 2015). However, this species is longer than *C. chondrostega* (mean SVL 23.1 in males, 25.4 in females; García-Castillo et al. 2017), *C. cracens* (mean SVL 25.7 in males, 27.4 in females; Rovito and Parra-Olea 2015), and *C. terrestris* (mean SVL 24.2 in males, 23.0 in females; García-Castillo et al. 2017). *Chiropterotriton aureus* has smaller feet (FW 2.4 in one male, mean 1.8 in females) than *C. arboreus* (mean FW 3.4 in males, 3.5 in females; García-Castillo et al. 2017), *C. cieloensis* (mean FW 3.2 in males, 3.1 in females; Rovito and Parra-Olea 2015), *C. chico* (mean FW 4.1 in males, 4.2 in females; García-Castillo et al. 2017), *C. infernalis* (mean FW 4.2 in males, 2.8 in one female; Rovito and Parra-Olea 2015), *C. multidentatus* (mean FW 3.6 in males, 3.5 in females; Rovito and Parra-Olea 2015), and *C. priscus* (mean FW 3.2 in males, 3.5 in females; Rovito and Parra-Olea 2015). *Chiropterotriton aureus* has shorter limbs (LI 2.0 in one male, mean 2.3 in females) than *C. arboreus* (mean LI 0.2 in males, 1.0 in females; García-Castillo et al. 2017), *C. cieloensis* (mean LI -0.2 in males, 0.1 in females; Rovito and Parra-Olea 2015), *C. infernalis* (mean LI -0.7 in males, -0.5 in one female; Rovito and Parra-Olea 2015), *C. multidentatus* (mean LI 0.1 in males, 1.0 in females; Rovito and Parra-Olea 2015), but it has longer limbs than *C. dimidiatus* (mean LI 3.8 in males, 4.9 in females; García-Castillo et al. 2017), *C. miquihuanus* (mean LI 4.2 in males, 4.3 in females; Rovito and Parra-Olea 2015), and *C. priscus* (mean LI 3.2 in males, 3.7 in females; Rovito and Parra-Olea 2015).

Description. A small species of *Chiropterotriton*, mean SVL 28.5 in one adult male (with mental gland) and 26.8 in three adult females (range 26.0–27.7). Head narrow and moderately long (HW 4.0 in one male, mean 3.6 in females; HL 6.4 in one male, mean 6.0 in females), HW/SVL=14% in one male a mean of 13% in females (range 13–14), and is wider than the shoulders (SW 3.4 in one male, mean 3.1 in females). Nostril oval shaped. Mental gland in one male small and almost circular shaped. Snout narrow and squared shaped. Eyes slightly protuberant. Jaw muscles are visible as grooves in the "V" behind the eyes. Few maxillary teeth in one male (mean MT 10.0) but a moderately large number in females (mean MT 38.3, range 37–40). Premaxillary teeth in one male are not enlarged and not piercing the lip. Few vomerine teeth in one male (VT 15.0) and females (mean VT 12.3, range 11–14), and arranged in a well-defined line nearly to outer margin of the choanae. Tail is longer than SVL, TL/SVL 1.28 in one male and 1.16 in females. Limbs are short and slender, FLL+HLL 47% of SVL in one male and 45% in females (range 43–46). Adpressed limbs separated by 2.0 costal folds in one male (LI 2.0) and 2.3 in females (mean LI 2.3, range 2.0–3.0). Digits slender and narrower at the tip with moderate webbing just onto the penultimate phalanx. Subterminal pads present. Phalangeal formulae: hand 1-2-3-2, foot 1-2-3-3-2. Digits in order of increasing length: hand I-IV~II-III, foot I-V-II-IV-III.

Coloration in life (from photos). Upper side of head Buff (5) or Yellow Ocher (14) on Dark Carmine (61) surface, Cream Yellow (82) on the tip of head and part of the eyelids, and lateral and gular region Pale Buff (1). Dorsum Buff (5), Yellow Ocher (14) or Olive Horn (16) on Pale Buff (1) surface, venter and costal sides Pale Buff (1). Upper side of tail with progressively darker Dark Carmine (61) with Buff (5) and Light Pratt's Rufous (71) speckles, or uniform Yellow Ocher (14), or Olive Horn (16) with Peach Red (70) speckles. Underside of tail Pale Buff (1). Forelimbs Chamois (84), and hands nearly translucent. Hindlimbs Buff (5), feet nearly translucent. Underside of limbs Pale Buff (1). Iris Orange-Rufous (56).

Coloration in alcohol. Upper side of head and dorsum Drab (19) and underside of head Pale Horn Color (11). Venter Pale Pinkish Buff (3) and costal region Cream Color (12) or Cinnamon-Drab (50). Upper side of tail Dark Drab (45), Cinnamon (225) or Hair Brown (277), and underside of tail Buff (5) or Drab (19). Upper side of limbs Drab (19) and underside of limbs Cream Color (12).

Measurements of the holotype, tooth counts, and limb intervals. SVL 28.5, TL 36.5, AX 15.5, SW 3.4, HL 6.4, HW 4.0, HD 1.8, projection of snout beyond mandible 0.7, anterior rim of orbit to snout 1.8, interorbital distance 1.9, eyelid length 1.7, eyelid width 1.3, horizontal orbit diameter 0.8, distance between corners of eyes 3.6, FLL 5.9, HLL 7.5, snout to forelimb 9.2, snout to anterior angle of vent 26.7, tail width at base 2.0, tail depth at base 1.9, FW 2.4, length of fifth toe 0.5, length of third (longest) toe 0.9, mental gland length 1.2, and mental gland width 1.0. Premaxillary teeth four, maxillary 4-6 (right-left sides) and vomerine 8-7 (right-left sides). Adpressed limbs separated by two costal folds.

Habitat and distribution. Western side of Sierra de Chiconquiaco, part of the Sierra Madre Oriental in central Veracruz. Specimens found in a cloud forest with extensive deforestation (near crops and paddocks), exclusively in arboreal bromeliads over oaks at 1,249 m asl (Figs. 5A and 5B).

Natural History. *Chiropterotriton aureus* was found exclusively in bromeliads in cloud forest around 1,200 m asl. Examined were approximately 40 bromeliads and found only five specimens, including four adults (one male and three females). Sampled bromeliads were at 1.5–3.0 m from the ground and small (approximately 20–40 cm in diameter). Sampling site was disturbed and deforested, but adjacent zones with similar environmental conditions could be explored to delimit the distributional range of this species. Species possibly sympatric with *C. aureus* may be *Aquiloeurycea cafetalera*, *Bolitoglossa platydactyla*, *Isthmura gigantea*, *Pseudoeurycea lynchi*, and *Thorius minydemus.*

Etymology. Latin epithet aureus (feminine *aurea*, neuter *aureus*) is derived from "*aurum*" gold + derivational suffix "-*eus*," meaning made of gold or gold in color, which is the featured characteristic color of the species.

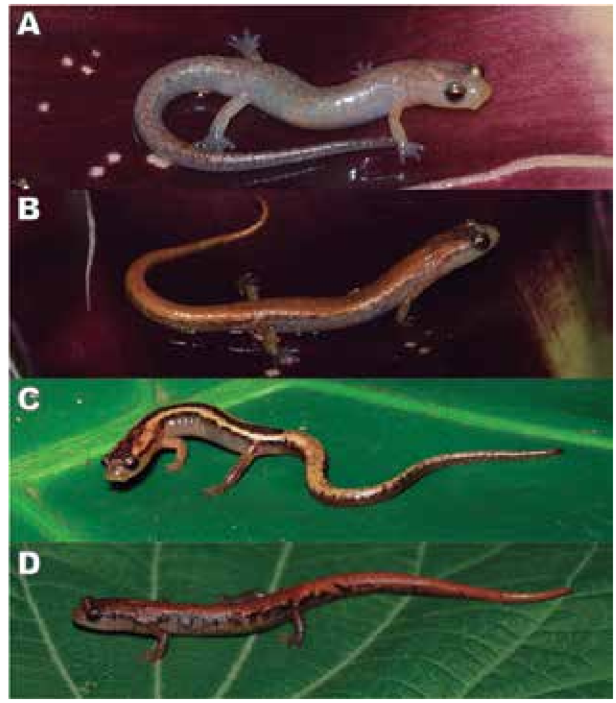

Fig. 4. Photos in life of two new species from central Veracruz. **A)** *C. aureus* (male) holotype IBH 31042, **B)** *C. aureus* (female) paratype IBH 31044, **C)** *C. nubilus* (male) paratype CARIE 0739, and **D)** *C. nubilus* (female) holotype IBH 31048. *Photo credit: Maria Delia Basanta (A, B, D) and J. Luis Aguilar-López (C).*

Chiropterotriton nubilus sp. nov.

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Cloud Forest Salamander from Cofre de Perote
Salamandra del Bosque de Niebla del Cofre de Perote
(Figs. 3B, 4C, and 4D)

Chiropterotriton sp.: Rovito et al. 2015

Holotype. IBH 31048, an adult female from Coxmatla, Veracruz, 8.2 km W of Xico, Veracruz, Mexico, 2,023 m asl, 19.433264N, 97.080639W. Collected 25 June 2017 by Ángel F. Soto-Pozos, Fabiola A. Herrera-Balcázar, M. Delia Basanta, Omar Becerra-Soria, and Mirna G. García-Castillo.

Paratypes. One male: CARIE 0739, Banderilla, 19.586667N, 96.946111W. One Female: IBH 31049, Coxmatla, 8.2 km W of Xico.

Referred specimens. IBH 31045–46, IBH 31050–52, Coxmatla, 8.2 km W of Xico; IBH 31047, IBH 31053 4 km W of Xico, road to Xico Viejo; CARIE 0718, La Cortadura, Coatepec, 19.491389N, 97.027778W; CARIE 0740, CARIE 1269, Bosque Rancho Viejo, Tlalnehuayocan; CARIE 1162, Rancho La Mesa, Banderilla; CARIE

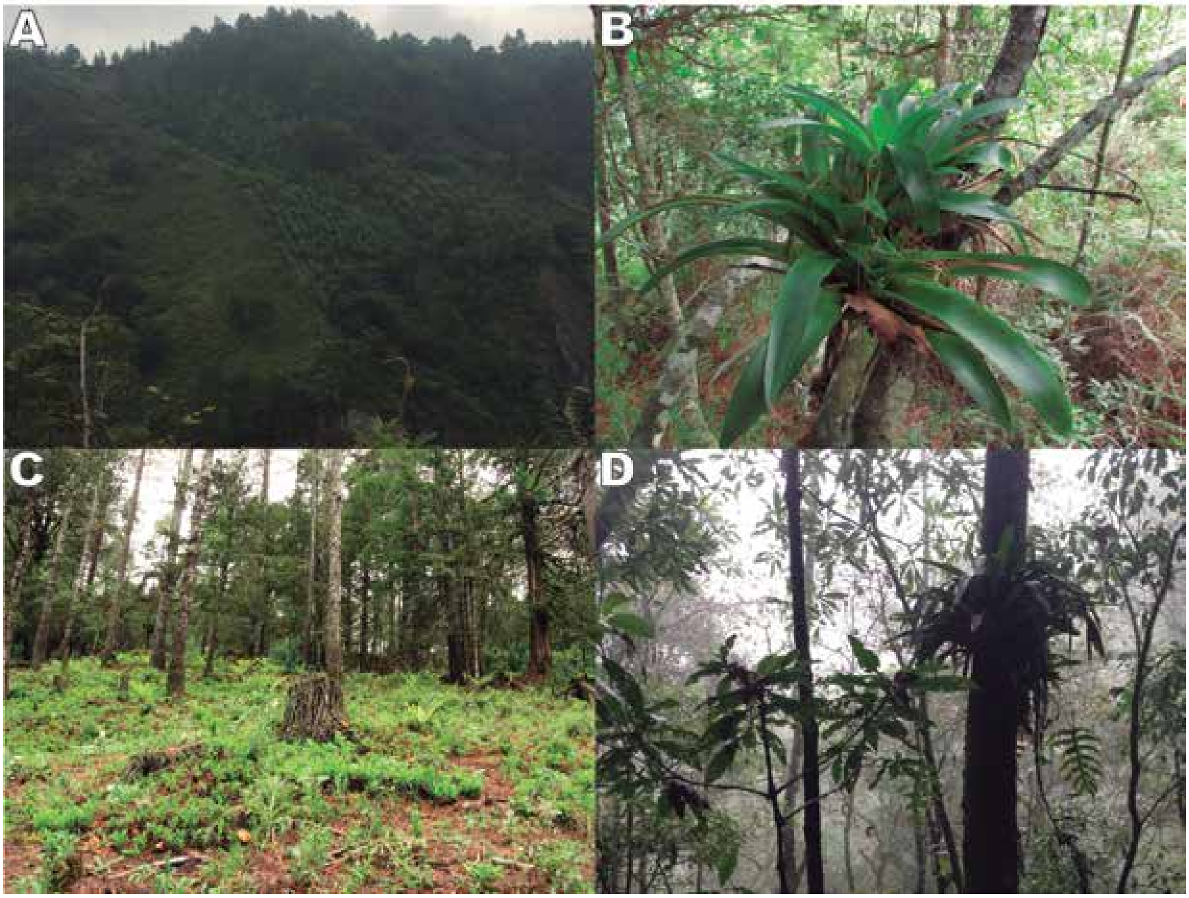

Fig. 5. Microhabitat and landscape photographs for new species from central Veracruz. **A)** Landscape from type locality of *C. aureus* (Atzalan, Veracruz), **B)** bromeliad from type locality of *C. aureus*, **C)** view of type locality of *C. nubilus* (Coxmatla, Veracruz), and **D)** bromeliad from locality of *C. nubilus* (Xico, Veracruz). *Photo credit: Mirna G. García-Castillo and Ángel F. Soto-Pozos.*

1267, Banderilla; CARIE 1272, Cinco Palos, Coatepec, 19.5N, 97.002778W.

Diagnosis. A plethodontid salamander assigned to the genus *Chiropterotriton* due to its slender body with a relatively long tail, shape of hand and feet digits, presence of sublingual fold, and based on mtDNA sequence data. Phylogenetically related to *C. aureus*, *C. chiropterus*, *C.* sp. F, and *C.* sp. J (Fig. 2). *Chiropterotriton nubilus* differs from *C. aureus* in females being longer (mean SVL 30.5 in females of *C. nubilus* vs. 26.8 in females of *C. aureus*), longer tail in males (TL/SVL 1.37 in one male of *C. nubilus* vs. 1.28 in one male of *C. aureus*), relatively longer limbs in females (mean LI 1.5 in females of *C. nubilus* vs. 2.3 in females of *C. aureus*), a longer head (mean HL 7.4 in females of *C. nubilus* vs. 6.0 in females of *C. aureus*), and broader head (mean HW 4.4 in females of *C. nubilus* vs. 3.6 in females of *C. aureus*). *Chiropterotriton nubilus* has longer feet (mean FW 2.3 in females of *C. nubilus* vs. 1.8 in females of *C. aureus*) with more rounded digits and slightly more webbing (just above penultimate phalanx) than *C. aureus* (Fig. 3).

Chiropterotriton nubilus differs from *C. chiropterus* by being shorter (SVL 29.4 in one male, mean 30.5 in females of *C. nubilus* vs. 37.5 in males, 33.5 in females of *C. chiropterus*), with relatively shorter limbs in males (LI 2.0 in one male of *C. nubilus* vs. mean 0.3 in males of *C. chiropterus*), shorter head in males (HL 6.6 in one male of *C. nubilus* vs. mean 8.1 in males of *C. chiropterus*), narrower head (HW 4.0 in one male, mean 4.4 in females of *C. nubilus* vs. 5.6 in males, 4.8 in females of *C. chiropterus*), jaw muscles less pronounced and eyes less protuberant than *C. chiropterus* (Fig. 3). *Chiropterotriton nubilus* has smaller feet (FW 2.6 in one male, mean 2.3 in females of *C. nubilus* vs. 3.7 in males, 3.1 in females of *C. chiropterus*), with rounded digits, and fourth finger of hand and fifth toe of foot longer than *C. chiropterus*. Likewise, *C. nubilus* has more webbing that covers just above the penultimate phalanx while *C. chiropterus* has webbing under the penultimate phalanx (Fig. 3).

Chiropterotriton nubilus differs from geographically proximate species *C. lavae* in males being shorter (SVL 29.4 in one male of *C. nubilus* vs. mean 32.4 in males of *C. lavae*), a longer tail (TL/SVL 1.37 in one male, mean 1.12 in females of *C. nubilus* vs. 1.2 in males, 1.0 in females of *C. lavae*), narrower head (HW 4.0 in one male, mean 4.4 in females of *C. nubilus* vs. 4.9 in males, 4.7 in females of *C. lavae*), relatively shorter limbs (LI 2.0 in one male, mean 1.5 in females of *C. nubilus* vs. -0.6 in males, 0.6 in females of lavae), and more maxillary teeth

(PMT+MT 20.0 in one male, mean 48.0 in females of *C. nubilus* vs. 10.3 in males, 28.0 in females of *C. lavae*). In general, *C. nubilus* is morphologically similar to *C. lavae* in body size and proportions (Table 3), but *C. nubilus* has smaller feet (FW 2.6 in one male, mean 2.3 in females of *C. nubilus* vs. 3.7 in males, 3.3 females of *C. lavae*) and less webbing (Fig. 3).

Chiropterotriton nubilus differs from *C. orculus* in being shorter (SVL 29.4 in one male, mean 30.5 in females of *C. nubilus* vs. 35.9 in males, 39.0 in females of *C. orculus*), longer tail (TL/SVL 1.37 in one male, mean 1.12 in females of *C. nubilus* vs. 1.02 in both males and females of *C. orculus*), relatively longer limbs in females (mean LI 1.5 in females of *C. nubilus* vs. 2.9 in females of *C. orculus*), shorter head (HL 6.6 in one male, mean 7.4 in females of *C. nubilus* vs. 7.4 in males, 8.0 in females of *C. orculus*), narrower head (HW 4.0 in one male, mean 4.4 in females of *C. nubilus* vs. 5.0 in males, 5.2 in females of *C. orculus*), more maxillary teeth (PMT+MT 20.0 in one male, mean 48.0 in females of *C. nubilus* vs. 10.9 in males, 35.9 in females of *C. orculus*), and smaller feet (FW 2.6 in one male, mean 2.3 in females of *C. nubilus* vs. 3.2 in males, 3.4 in females of *C. orculus*).

Chiropterotriton nubilus differs from *C. dimidiatus* in being shorter (SVL 29.4 in one male, mean 30.5 in females of *C. nubilus* vs. 24.7 in males, 25.8 in females of *C. dimidiatus*), longer tail (TL/SVL 1.37 in one male, mean 1.12 in females of *C. nubilus* vs. 0.89 in males, 0.87 in females of *C. dimidiatus*), longer head (HL 6.6 in one male, mean 7.4 in females of *C. nubilus* vs. 5.3 in males, 5.1 in females of *C. dimidiatus*), broader head (HW 4.0 in one male, mean 4.4 in females of *C. nubilus* vs. 3.5 in both males and females of *C. dimidiatus*), relatively longer limbs (LI 2.0 in one male, mean 1.5 in females of *C. nubilus* vs. 3.9 in males, 4.9 in females of *C. dimidiatus*), more maxillary teeth (PMT+MT 20.0 in one male, mean 48.0 in females of *C. nubilus* vs. 9.4 in males, 34.4 in females of *C. dimidiatus*), more vomerine teeth (VT 10.0 in one male, mean 13.5 in females of *C. nubilus* vs. 5.7 in males, 8.3 in females of *C. dimidiatus*), and longer feet (FW 2.6 in one male, mean 2.3 in females of *C. nubilus* vs. 1.7 in males, 1.8 in females of *C. dimidiatus*).

Chiropterotriton nubilus is related to an undescribed taxon of the southern assemblages with genetic divergences as follows: 5% (16S) and 12% (COI) to *C.* sp. F; 3% (16S) and 8% (COI) to *C.* sp. J; 8% (16S) to *C.* sp. H; 7% (16S) to *C.* sp. I, *C.* sp. C, and *C.* sp. G; and 6% (16S) to *C.* sp. K (Table 2).

Chiropterotriton nubilus differs from other species of *Chiropterotriton* by being shorter (SVL 29.4 in one male, mean 30.5 in females) other than *C. arboreus* (mean SVL 33.4 in males, 32.2 in females; García-Castillo et al. 2017), *C. chico* (mean SVL 38.4 in males, 39.3 in females; García-Castillo et al. 2017), *C. magnipes* (mean SVL 46.8 in males, 57.5 in females; Rabb 1965), *C. miquihuanus* (mean SVL 33.3 in males, 36.5 in females; Rovito and Parra-Olea 2015), *C. mosaueri* (mean SVL 42.8 in males; Woodall 1941), *C. multidentatus* (mean SVL 33.6 in males, 34.0 in females; Rovito and Parra-Olea 2015), and *C. priscus* (mean SVL 38.5 in males, 41.8 in females; Rovito and Parra-Olea 2015). *Chiropterotriton nubilus* has a longer body size than *C. chondrostega* (mean SVL 23.1 in males, 25.4 in females; García-Castillo et al. 2017), *C. cracens* (mean SVL 25.7 in males, 27.4 in females; Rovito and Parra-Olea 2015), *C. dimidiatus* (mean SVL 24.6 in males, 25.8 in females; García-Castillo et al. 2017), and *C. terrestris* (mean SVL 24.2 in males, 23.0 in females; García-Castillo et al. 2017). *Chiropterotriton nubilus* has smaller feet (FW 2.6 in one male, mean 2.3 in females) other than *C. arboreus* (mean FW 3.4 in males, 3.5 in females; García-Castillo et al. 2017), *C. cieloensis* (mean FW 3.2 in males, 3.1 in females; Rovito and Parra-Olea, 2015), *C. chico* (mean FW 4.1 in males, 4.2 in females; García-Castillo et al. 2017), *C. infernalis* (4.2 in males, 2.8 in one female; Rovito and Parra-Olea, 2015), and *C. priscus* (mean FW 3.2 in males, 3.5 in females; Rovito and Parra-Olea 2015). *Chiropterotriton nubilus* has relatively shorter limbs (LI 2.0 in one male, mean 1.5 in females) other than *C. arboreus* (mean LI 0.2 in males, 1.0 in females; García-Castillo et al. 2017), *C. cieloensis* (mean LI -0.2 in males, 0.1 in females; Rovito and Parra-Olea 2015), *C. infernalis* (mean LI -0.7 in males, -0.5 in one female; Rovito and Parra-Olea 2015), *C. multidentatus* (mean LI 0.1 in males, 1.0 in females; Rovito and Parra-Olea 2015), but relatively longer limbs than *C. dimidiatus* (mean LI 3.8 in males, 4.9 in females; García-Castillo et al. 2017), *C. miquihuanus* (mean LI 4.2 in males, 4.3 in females; Rovito and Parra-Olea 2015), and *C. priscus* (mean LI 3.2 in males, 3.7 in females; Rovito and Parra-Olea 2015).

Description. Moderate-sized species of *Chiropterotriton*, SVL 29.4 in one adult male and mean 30.5 in two adult females (range 27.7–33.2). Head relatively narrow and moderately long (HW 4.0 in one male, mean 4.4 in females; HL 6.6 in one male, mean 7.4 in females), 14% of HW/SVL in one male and 15% in females (range 14–16), and wider shoulders (SW 3.4 in one male, mean 3.3 in females). Nostrils moderately sized and oval shaped. Snout narrow and truncated. Eyes slightly protuberant. Jaw muscles appear as a bulging mass behind the eyes and beyond the margin of the jaw, when viewed from above. Premaxillary teeth in one male not enlarged and not piercing lip. Few maxillary teeth in males (MT 13.0) but many in females (mean MT 41.5, range 40–43). Few vomerine teeth in males (VT 10.0) and females (mean VT 13.5, range 13–14), arranged in a well-defined line nearly to outer margin of the choanae. Tail large, mean TL/SVL 1.37, in one male and moderate, 1.12, in females (range 1.10–1.14). Limbs short and slender, FLL+HLL 46% of SVL in one male and 45% in females (range 42–48). Adpressed limbs separated by 2.0 costal folds in one male (LI 2.0) and 1.5 in females (mean LI 1.5, range 1.0–2.0). Digits slender with distinct terminal pads and moderate webbing just above the penultimate phalanx. Phalangeal formulae: hand 1-2-3-2, foot 1-2-3-3-2. Digits in order of increasing length: hand I-IV-II-III, foot I-V-II-IV-III.

Coloration in life (from photos). Predominating colors on the upper side of the head and dorsum are Flesh Ocher (57) or Salmon (58) on Sepia (286) background. Lateral side of the head is Cream White (52), and underside of head and venter are Cream White (52) background with Glaucous (291) marks. Dorsum flanks Glaucous (291) on Cream White (52) surface with Smoky White (261) stipples. Tail Flesh Ocher (57) with Sepia (286) marks on flanks and underside Perl Gray (262) with Glaucous (291) marks. Upper side of limbs Maroon (39) with toe tips Magenta (236) and underside of limbs Cream White (52) surface with Glaucous (291) marks. Iris Gem Roby (65) [Fig. 4D].

Variation of coloration in life (from photos). CARIE 0739 adult male. Upper side of head Pale Horn Color (11) on Dark Brownish Olive (127) surface, lateral head Cream White (52) and underside of head Pale Buff (1). Dorsum with two stripes Pale Horn Color (11) on Sepia (286) surface, lateral dorsum Light Lavender (201) and underside of dorsum Pinkish White (216) with Medium Bluish Purple (212) small dots. Upper side of tail Pale Horn Color (11) on Sepia (286) surface and underside of tail Pinkish White (216) with Medium Bluish Purple (212) small dots and some Pale Horn Color (11) speckles. Forelimbs Cream Color (12) and hindlimbs Fawn Color (258) with toe tips Magenta (236). Iris Light Yellow Ocher (13) [Fig. 4C].

Coloration in alcohol. Upper side of head Drab (19), lateral Dusky Brown (285) line and underside Smoke Gray (266) with Smoky White (261) marks. Upper side of dorsum and tail Dark Yellow Buff (54) on Dusky Brown (285) surface, dorsum flanks Olive-Gray (265) and underside of dorsum Smoke Gray (266). Underside of tail Grayish Horn Color (268). Upper side of limbs Olive-Brown (278) and upper side of limbs Smoke Gray (266).

Variation in alcohol preserved coloration. Three specimens: one adult male (CARIE 0739) and two juvenile (CARIE 0740, CARIE 1267). Upper side of head Cream White (52) on Raw Umber (23) surface and underside of head Smoky White (261). Dorsum with two stripes Cream White (52) on Raw Umber (23) surface, flanks and underside of dorsum Smoky White (261). Upper side of tail Cream White (52) on Raw Umber (23) surface and underside Smoky White (261). Upper side of forelimbs Olive Horn Color (16), hindlimbs Fawn Color (258) and underside of limbs Smoky White (261).

Measurements of holotype, tooth counts, and limb intervals. SVL 33.2, TL 37.9, AX 18.3, SW 3.5, HL 8.1, HW 4.5, HD 2.0, projection of snout beyond mandible 0.8, anterior rim of orbit to snout 2.0, interorbital distance 3.9, eyelid length 1.9, eyelid width 1.5, horizontal orbit diameter 0.7, distance between corners of eyes 2.5, FLL 6.7, HLL 7.3, snout to forelimb 10.0, snout to anterior angle of vent 31.4, tail width at base 2.2, tail depth at base 2.3, FW 2.7, length of fifth toe 0.5, and length of third (longest) toe 0.8. Premaxillary teeth 23, maxillary 7–20 (right-left sides) and vomerine 7–6 (right-left sides). Adpressed limbs are separated by two costal folds.

Habitat and distribution. Eastern slopes of Cofre de Perote in central Veracruz among cloud forest between 1,520 and 2,023 m asl. Specimens found in arboreal bromeliads of cloud forest fragments with low or moderate disturbance of habitat. The majority of the specimens found were juveniles so the possibility of finding them in terrestrial environments (under cover objects) is not rejected (Figs. 5C and 5D). Two localities where *C. nubilus* occurs are within protected areas: municipal (La Cortadura) and the other under private ownership (Rancho Viejo).

Natural History. *Chiropterotriton nubilus* was exclusively found in bromeliads and six localities on the eastern slope of Cofre de Perote. Distribution could include a fragmented band along cloud forests from Coxmatla to Banderilla at 1,500–2,000 m asl. Samples included three collections in three study locations (Banderilla, La Cortadura, and Rancho Viejo) for a total of nine sampling events. Each sampling event applied 16 person-hours for a total sampling effort of 144 person-hours. In four of the nine sampling events collected were *C. nubilus*, varying between one to three specimens per sampling event. Bromeliads where *C. nubilus* were found measured 1.5–5.0 m from the ground and were medium in size (approximately 40–60 cm in diameter). Species found in sympatry with *C. nubilus* were *Aquiloeurycea cafetalera*, *Parvimolge townsendi*, *Pseudoeurycea lynchi*, and *Thorius pennatulus*. It is conceivable that *C. nubilus* could be found in sympatry with *C. lavae* because distributions converge at the W slope of Cofre de Perote at 2,000 m asl. However, *C. lavae* (La Joya) is found eight km away from the nearest location (Banderilla) *C. nubilus* occurs.

Etymology. Latin epithet *nubilus* (adjective: feminine *nubile*, neuter *nubilum*) means cloudy or rain clouds, referring to the cloud forest of Cofre de Perote where it occurs.

Discussion

Due to recent systematic reviews, expeditions to poorly explored areas, and recurrent field samplings in relatively well-studied regions, the number of described species of bolitoglossine salamanders has increased at a slow but steady pace in recent years (e.g., Rovito et al. 2015; Kubicki and Arias 2016; Parra-Olea et al. 2016; García-Castillo et al. 2017; Arias and Kubicki 2018). The Cofre de Perote area has been well studied and is notable for its salamander richness, which now includes 20 species representing 16% of Mexican bolitoglossines (Wake et al. 1992; Parra-Olea et al. 2001). The description of these two new species increases the salamander diversity in the state of Veracruz from 37 to 39 (Parra-Olea et al. 2014), including the recently described *Isthmura corrugata* (Sandoval-Comte et al. 2017).

The number of species in the genus *Chiropterotriton* has increased by approximately 50% in the last four years (Campbell et al. 2014; Rovito and Parra-Olea 2015; García-Castillo et al. 2017), but phylogenetic relationships are not fully resolved. Although the phylogeny exhibited in this study includes a greater number of well-supported clades (PP > 0.95, BS > 70), some relationships still lack strong support. However, previous studies (Parra-Olea 2003; Rovito and Parra-Olea 2015; García-Castillo et al. 2017) and results here show a well-supported clade with species from central and southern Mexico, in which *C. dimidiatus* is sister to the group. This group also includes three more described species (*C. chiropterus*, *C. lavae*, and *C. orculus*) plus seven previously proposed candidate species (Figs. 1 and 2). Within the southern assemblages, *Chiropterotriton* species form two subclades with three sister taxa groups. The first group includes two terrestrial forms, sister taxa *C. orculus* + *C*. sp. G and *C*. sp. K, for which only juveniles are known. The second group includes *C. lavae* + *C*. sp. H and *C*. sp. I + *C*. sp. C. The first sister pair occur in geographical proximity to Cofre de Perote but in different elevation ranges and different environmental conditions (one terrestrial and one arboreal): *Chiropterotriton lavae* at 2,000 m asl in cloud forest and *C*. sp. H at 3,000 m asl in pine forest. The second sister pair (*C*. sp. C + *C*. sp. I) occur near Pico de Orizaba, with *C*. sp. C at 2,400 m asl in cloud forest and *C*. sp. I at 3,000 m asl in pine forest, again one species being arboreal and the other terrestrial. In contrast to the previous two groups, the third group is formed by five arboreal species (*C*. sp. F, *C. aureus*, *C. nubilus*, *C. chiropterus*, and *C*. sp. J), all distributed in similar elevation ranges (1,200 to 2,000 m asl), and similar environmental conditions along the cloud forest from Sierra Madre Oriental to Sierra de Juárez, Oaxaca. This continuous cloud forest belt may have promoted a progressive colonization process enabling species formation through time and isolation and could very well explain the phylogenetic link between the species of Veracruz and Oaxaca, a pattern also seen in other bolitoglossine groups like *Thorius* (Rovito et al. 2013) and *Isthmura* (Sandoval-Comte et al. 2017).

The two new species of *Chiropterotriton* have not been previously reported, although a sequence of *C. nubilus* (GenBank number KP886894) was used as a representative of *Chiropterotriton* in a bolitoglossine study (Rovito et al. 2015). The discovery of these specimens in a relatively well-studied area is reason to continue explorations, especially if localities are progressively being deforested (Williams-Linera 2007). Likewise, salamander diversity numbers are likely underestimated for central Veracruz (*C*. sp. C and *C*. sp. H), Puebla (*C*. sp. F, *C*. sp. G and *C*. sp. I.) and Oaxaca (*C*. sp. J and *C*. sp. K) and investigations should therefore continue as species knowledge is more completely appreciated.

Tropical salamanders are at high risk of extinction (Rovito et al. 2009), including the genus *Chiropterotriton*. It is imperative, now more than ever, to make the best use of available bioresources by biobanking genetic material and living tissue for current and future uses (Hassapakis and Clark 2017; Zimkus et al. 2018). Cryobanked genetic material has been essential for systematic and evolutionary studies of tropical salamanders, and allowed the description of taxa thought to be extinct (i.e., *Isthmura naucampatepetl*), make taxonomic rearrangements (Wake et al. 2012), discover cryptic taxa (Parra-Olea et al. 2016), and propose large genus level phylogenies (Parra-Olea et al 2004; Rovito et al. 2013) but may now benefit and contribute to best practices in species conservation (Zimkus et al. 2018). Biobanking for amphibian conservation may enable us to mitigate or prevent the complete loss of species already at high risk (e.g., *Bolitoglossa jacksoni*, *Cryptotriton alvarezdeltoroi*) and archive these bioresources (e.g., cryopreserved sperm, cell cultures, somatic tissue) and make them available for present and future conservation technologies (i.e., Assisted Reproductive Technologies [ART]; Kouba et al. 2012; Kouba and Vance 2013) and methodologies. Those with access to specimens (e.g., field biologists, zoo and aquarium personnel, et al.) should consider in their research activities and grant proposals to allow resources and time for biobanking and preserving amphibian genetic resources and living tissues to enhance species conservation efforts (Zimkus et al. 2018). Finally, the existence of the Genome Resource Banks (GRBs) can alleviate other issues not related to the biology of species but rather to pressing political (difficulties of obtaining field sampling due to safety) issues and economic troubles faced by many countries worldwide.

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Appendix 1. Specimens examined for morphological comparisons.

Chiropterotriton aureus: Mexico, Veracruz: IBH 31041–44, 6.5 km N of Atzalan, ejido de desarrollo urbano Quetzalcóatl.

Chiropterotriton chiropterus: Mexico, Veracruz: MVZ 85588–92, 85594, 85597–99, 85605, 85613, 85632, 1.4 miles (mi) SW (by road), SW edge of Huatusco de Chicuellar.

Chiropterotriton lavae: Mexico, Veracruz: MVZ 106537, 106548, W edge of La Joya along Highway (Hwy). 140; MVZ 163912–13, 163915, 171873–74, 171876, 171881, 171885, 171901, 173394–95, 173398, 192788–89, 197788, La Joya; 178685, La Joya, Mexico Hwy. 140; MVZ 200638, forest W of La Joya.

Chiropterotriton nubilus: Mexico, Veracruz: IBH 31048–49, Coxmatla, 8.2 km W of Xico. CARIE 0739, Banderilla.

Chiropterotriton orculus: Mexico, Estado de México: MVZ 76161, 138686, 138688, 138694, 138696–97, 138700, 138776–79, 138781, 138783–84, 138793, 138796–97, 138804, 200629–30, Ridge between Volcanoes Popocatepetl and Iztaccihuatl along Mexico Hwy. 196, 16.2 km E (by road) of hwy. 115.

Mirna Grisel García-Castillo is a Mexican Ph.D. student from Universidad Nacional Autónoma de México in Mexico City. She began her study of salamanders in Dr. Parra´s Lab in 2011 where she received a Master's degree focused on molecular phylogeny of the genus *Chiropterotriton*. Since that time, she's worked on molecular systematics, taxonomy, morphology, biogeography, and evolution of this group as part of her doctoral thesis she is currently finishing.

Angel Fernando Soto-Pozos received a Master's degree in Biological Sciences in 2018 from the Instituto de Biología, Universidad Nacional Autónoma de México. His research interests include edge effect in communities of amphibians and reptiles, diversity, conservation of endangered species of salamanders in fragmented forest from Veracruz State, and the historic role of diseases in salamander declines.

José Luis Aguilar López received his Master´s degree in 2010 from the Instituto de Ecología A.C. and is currently a doctoral student at the same institution. His research interests include diversity patterns, taxonomy, and conservation of amphibians and reptiles in tropical forests.

Eduardo Pineda is a titular researcher at the Instituto de Ecología, A.C. in Xalapa, Mexico. His research is focused on understanding the relationship between the transformation of tropical forest and biodiversity at different spatial scales, recognizing the importance of conserved areas and modified habitats to maintain amphibian diversity, and assess the current situation, through fieldwork, of amphibian species in imminent danger of extinction. Currently he has several graduate students addressing topics in ecology and/or conservation of amphibians in Mexico and Latin America.

Gabriela Parra Olea is a titular researcher at the Instituto de Biología, UNAM, Mexico. Her research is focused on molecular systematics and conservation of Mexican amphibians. Her laboratory is formed by students and postdocs from different countries as Mexico, Guatemala, Costa Rica, Colombia, and Argentina, all working on research projects in systematics and taxonomy, conservation genetics, and the impact of infectious diseases, specifically chytridiomycosis, on the conservation of amphibians.