UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE BIOLOGÍA

SISTEMÁTICA

PATRONES BIOGEOGRÁFICOS Y DE FORMACIÓN DE ESPECIES EN EL GÉNERO *CHIROPTEROTRITON* (CAUDATA: PLETHODONTIDAE)

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

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MÉXICO, Cd. Mx. **ENERO, 2019**

UNAM POSGRADO
Ciencias Biológicas

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OFICIO CPCB/1162/2018

Asunto: Oficio de Jurado para Examen de Grado.

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Me permito informar a usted que en la reunión del Subcomité por Campo de Conocimiento de Ecología y Manejo Integral de Ecosistemas del Posgrado en Ciencias Biológicas, celebrada el día 15 de octubre de 2018, se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la alumna **GARCÍA CASTILLO MIRNA GRISEL** con número de cuenta **300173675** con la tesis titulada: **"PATRONES BIOGEOGRÁFICOS Y DE FORMACIÓN DE ESPECIES EN EL GÉNERO *CHIROPTEROTRITON* (CAUDATA: PLETHODONTIDAE)"**, realizada bajo la dirección de la **DRA. GABRIELA PARRA OLEA:**

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Sin otro particular, me es grato enviarle un cordial saludo.

ATENTAMENTE
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, Cd. Mx., a 30 de noviembre de 2018.

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AGRADECIMIENTOS

Agradezco al Posgrado en Ciencias Biológicas, de la Universidad Nacional Autónoma de México, por la oportunidad cursar el programa de Doctorado en Ciencias Biológicas, así como el apoyo otorgado para asistir a una estancia de investigación en Occidental College, Los Ángeles, California, EUA (octubre de 2015), la participación en el XI Congreso Latinoamericano de Herpetología, Quito, Ecuador (agosto 2017) y el apoyo otorgado como parte del fomento a la graduación.

De la misma manera, agradezco al Consejo Nacional de Ciencia y Tecnología CONACyT (CVU/Becario: 413761/262662) por la beca brindada durante los cuatro años correspondientes al programa de Doctorado (2014-2018) y el apoyo de beca mixta otorgado para la estancia de investigación en octubre de 2015 en Occidental College, Los Ángeles, California, EUA. También, al apoyo del proyecto de investigación PAPIIT UNAM IN203617 de la Dra. Gabriela Parra Olea, por el financiamiento del trabajo de laboratorio y campo desarrollado durante mi proyecto.

Al comité Tutoral conformado por: Dra. Gabriela Parra Olea, Dr. Daniel Piñero Dalmau y Dr. Oscar Flores Villela

AGRADECIMIENTOS PERSONALES

Gracias a los miembros del jurado por sus valiosos comentarios y disposición en el proceso de graduación. A mi tutora Gabriela Parra por la oportunidad de pertenecer a su laboratorio y contribuir con desarrollo como estudiante e investigadora. Muchas gracias a Sean Rovito por sus consejos, sus aportes y su disposición ante las dudas que surgieron a lo largo del doctorado. También agradezco a Laura Márquez por si invaluable ayuda por cada vez que la necesité. Mil gracias a Rocío González Acosta por su amabilidad y su apoyo durante maestría y sobre todo por su ayuda en el proceso de graduación en doctorado.

Gracias a mis compañeros de laboratorio por las risas, por su apoyo como equipo y también enriquecer mi conocimiento con charlas, debates y colaboraciones. Especial agradecimiento a mis compañeros de doctorado: Aldo López Velázquez y Erick Arias Piedra por vivir esta experiencia de principio a fin con su apoyo académico y amistoso, que hicieron más llevadero los momentos difíciles de mi trabajo.

Le agradezco a mi mamá por si sus cuidados, por confiar en mí en todo momento y guiarme en los momentos difíciles. Gracias Martín García Varela por tu apoyo y tus consejos.

Agradezco a todos los que estuvieron conmigo cuando tuve problemas de salud, sin duda fue un altibajo que terminó por impulsarme en más de un sentido. Gracias a los que estuvieron al pendiente de mí, los que ayudaron, los que me visitaron y los que me apoyaron en la recuperación.

DEDICATORIA

Son tus paredes, tus aulas, los verdes pastos,
Son tu grandeza, tu belleza y tu espíritu.
Mi segunda casa, mi refugio, mi camino,
Es mi amada Universidad Nacional Autónoma de México,
En ti confió y en ti mi alivio, gracias por iluminar mi destino,
A ti debo mi más grande orgullo, pertenecer a ti y crecer contigo,
Gracias por la grandeza, la inspiración y cada aprendizaje.
Por ti fui, por ti soy y por ti también seré.

Índice

RESUMEN

Las salamandras neotropicales o bolitoglosinos son un grupo de caudados muy diverso (42% de todas las especies de salamandras vivientes), el cual se encuentra distribuido desde Mesoamérica hasta América del Sur; su notable diversidad se asocia con una radiación adaptativa que ocurrió en Mesoamérica. El territorio mexicano alberga 12 de los 14 géneros que conforman el grupo de los bolitoglossinos, por lo que es una región con alta diversidad de salamandras a nivel mundial. Uno de estos géneros es *Chiropterotriton,* endémico de México, representado por 18 especies distribuidas en los bosques de la Sierra Madre Oriental, Faja Volcánica Transmexicana y montañas del norte de Oaxaca.

Las primeras reconstrucciones filogenéticas de *Chiropterotriton* mostraban dos agrupamientos principales de especies (norte-centro y centro-sur de México), y sugerían además la existencia de al menos ocho especies nuevas. Es por ello que este grupo ha sido objeto de trabajos taxonómicos recientes, donde se han combinado evidencias morfológicas y moleculares para la descripción de cuatro especies: *C. miquihuanus* (2014), *C. cieloensis* y *C. infernalis* (2015), y *C. chico* (2017). Sin embargo, aún existen revisiones taxonómicas pendientes para conocer la diversidad de especies total para este género. Por otra parte, las reconstrucciones filogenéticas moleculares propuestas incluyen marcadores nucleares o mitocondriales, pero no representan hipótesis robustas para resolver las relaciones filogenéticas del género, las cuales son débilmente apoyadas sobre todo en nodos profundos. Por lo tanto, sin una filogenia robusta las inferencias sobre el origen, historia biogeográfica y los procesos involucrados en la especiación del género son inciertas.

En este trabajo realizamos una revisión taxonómica del grupo, que se presenta en el Capítulo I, el cual incluye la descripción de ocho nuevas especies, las cuales son producto de especies previamente propuestas e incluso de taxones que no habían sido reportados anteriormente. Además, realizamos tres re-descripciones de especies que requerían de una revisión taxonómica por conflictos resultantes de descripciones poco detalladas y sinonimias anteriores. Dichas descripciones constituyen tres artículos (dos de ellos publicados), que incluyen datos moleculares, morfológicos y morfométricos.

En el Capítulo II se presenta una filogenia del género *Chiropterotriton* basada en datos nucleares y mitocondriales, generados mediante secuenciación masiva. Los resultados

mostraron filogenias mayormente concordantes entre sí, exceptuando las relaciones filogenéticas de uno de los clados de la Faja Volcánica Transmexicana, lo que puede explicarse por un sorteo incompleto de linaje o introgresión en el DNA mitocondrial. Asi mismo, estimamos que el género *Chiropterotriton* se originó hace aproximadamente 24 millones de años, con dos series de eventos de especiación principales; el primero durante el Mioceno temprano, cuando se originaron las especies más antiguas en la Sierra Madre Oriental, y el segundo fue durante el Mioceno tardío y Plioceno, cuando se originaron las especies más recientes en esta misma sierra, así como las especies en la Faja Volcánica Transmexicana y montañas del norte de Oaxaca.

ABSTRACT

Neotropical salamanders or bolitoglossines are the most diverse group of Caudata (42% of all species of living salamanders), which have a current distribution from Mesoamerica to South America and its remarkable diversity is associated with an adaptive radiation that occurred in Mesoamerica. Mexican territory holds 12 of the 14 bolitoglossine genera, making it a globally important region with a high diversity of salamanders. One of these genera is *Chiropterotriton*, endemic to Mexico, represented by 18 species distributed in forests from the Sierra Madre Oriental, the Trans-Mexican Volcanic Belt and into the highlands of northern Oaxaca.

The first phylogenetic reconstructions of *Chiropterotriton* showed two main species groupings (north-central and south-central Mexico), and suggested the existence of at least eight new species. Thus, this group has been the subject of recent taxonomic investigations, where morphological and molecular evidence have been used for the description of four species: *C. miquihuanus* (2014), *C. cieloensis* and *C. infernalis* (2015), and *C. chico* (2017). However, there is still pending taxonomic working order to accurately establish the total species diversity for this genus. Existing molecular phylogenetic reconstructions based on nuclear or mitochondrial markers are weakly supported at deeper nodes and therefore do not represent robust phylogenetic hypotheses of the genus. Inferences about biogeographic history as well as the processes involved in diversification of the genus are uncertain without a robust phylogeny.

In this work, we attended the outstanding taxonomic tasks of the group, which are contained in Chapter I, and include the description of eight new species. These species descriptions are the product of previously proposed species and even taxa that had not been suggested in previous works. In addition, three re-descriptions of species that required a taxonomic revision were made due to conflicts originating from unspecified descriptions and previous synonymizations. These contributions were included in three articles, which include molecular, morphological and morphometric data.

In Chapter II, we present a robust phylogeny for *Chiropterotriton,* based on nuclear and mitochondrial data generated via high-throughput DNA sequencing (massive parallel sequencing). The results showed phylogenies mostly concordant with each other, except in

relationships between species one clade of the Trans-Mexican Volcanic Belt, which could be explained by incomplete lineage sorting or introgression in the mitochondrial DNA. We estimated that *Chiropterotriton* originated approximately 24 million years ago, with two series of major speciation events; the first during the Early Miocene when the oldest species originated in the Sierra Madre Oriental, and the second during the Late Miocene and Pliocene, when the most recent species of the Sierra Madre Oriental originated, as well as the species in the Trans-Mexican Volcanic Belt and northern Oaxaca.

INTRODUCCIÓN GENERAL

El Orden Caudata es el segundo grupo más diverso de los anfibios y actualmente cuenta con 717 especies que se encuentran contenidas en 10 familias (AmphibiaWeb, 2019). La familia Plethodontidae es la más numerosa con 473 especies; 309 de ellas pertenecen a la tribu Bolitoglossini que representa el 43% de todos los caudados siendo el grupo más diverso de salamandras (Wake, 2012). Ello ha promovido el interés de los científicos para evaluar las razones de dicha diversidad, la cual se explica en parte como resultado de una radiación adaptativa de gran dimensión que ocurrió en Mesoamérica (Desde México hasta Panamá) (Rovito et al., 2015; Rovito & Parra-Olea, 2016; Kozak, 2017). En México habitan 148 especies de caudados; 85.8% corresponden a bolitoglossinos, representando 12 de los 14 géneros descritos para este grupo (Wake, 2012; Rovito et al., 2015), es decir que el territorio alberga una porción significativa de la biodiversidad de bolitoglossinos (Parra-Olea, Flores-Villela, & Mendoza-Almeralla, 2014).

El estudio de esta notable diversidad de bolitoglossinos inició en México a partir de los estudios de carácter científico post-Linneano, cuando hubo un auge importante de múltiples recolectas y descripciones de herpetofauna en el país (Flores Villela, 1993). Autores del siglo XIX como Edward D. Cope y John E. Gray realizaron las primeras descripciones de bolitoglossinos (*p. ej.,* Gray, 1831; Cope, 1865), seguidas en el siglo XX por autores como Emmett R. Dunn, Edward H. Taylor, Hobart M. Smith y George B. Rabb (*p. ej.,* Dunn, 1924; Taylor, 1944; Bumzahem & Smith, 1955; Rabb, 1960). Por otro lado, en tiempos más recientes el conocimiento de la riqueza de especies ha cambiado gracias al uso de herramientas moleculares (Vences & Wake, 2007; Rovito et al., 2015; Rovito & Parra-Olea, 2016), las cuales han revelado un gran número de especies crípticas y han abierto un panorama más claro sobre los procesos de diversificación en los bolitoglosinos (*p. ej.,* Rovito et al., 2013). Aunado a ello, la llamada "Era Genómica" ha revolucionado los estudios más recientes sobre genética, filogeografía y evolución (Glenn, 2011). En particular, la secuenciación de nueva generación ha permitido obtener datos de ADN de forma rápida y masiva, así como el desarrollo de estudios sobre límites entre especies, filogeografía y biogeografía de salamandras (*p. ej.,* Newman & Austin, 2016; Bryson et al., 2018).

Chiropterotriton es uno de los 14 géneros que representan a la tribu bolitoglossini y es endémico de México. Tiene una distribución geográfica a lo largo de la Sierra Madre Oriental (SMO; en los estados de Nuevo León, Tamaulipas, San Luis Potosí, Hidalgo, Querétaro y Puebla), la Faja Volcánica Transmexicana (FVTM; en los estados de Querétaro, Estado de México, Ciudad de México, Hidalgo, Tlaxcala, Puebla y Veracruz) alcanzando su distribución más sureña en las montañas del norte de Oaxaca (Darda, 1994; Parra-Olea, 2003). Actualmente este género contiene 18 especies descritas: *C. arboreus* (Taylor), *C. aureus* García-Castillo et al., *C. cieloensis* Rovito & Parra-Olea, *C. chico* García-Castillo et al., *C. chiropterus* (Cope), *C. chondrostega* (Taylor), *C. cracens* Rabb, *C. dimidiatus* (Taylor), *C, infernalis* Rovito & Parra-Olea, *C. lavae* (Taylor), *C. magnipes* Rabb, *C. miquihuanus* Campbell et al*.,* *C. mosaueri* (Woodall), *C. multidentatus* (Taylor), *C. nubilus* García-Castillo et al., *C. orculus* (Cope), *C. priscus* Rabb y *C. terrestris* (Taylor). Sin embargo, estudios basados en análisis moleculares han sugerido al menos 10 especies más que requieren de una descripción formal (Darda, 1994; Parra-Olea, 2003; García-Castillo, 2014).

El género *Chiropterotriton* está representado por salamandras de cuerpo esbelto con una notable agilidad en sus movimientos, su tamaño corporal va desde los 2–6 cm de hocico-cloaca y además posee una diversidad morfológica que incluye especies terrestres, cavernícolas y arborícolas, las cuales muestran adaptaciones en forma y proporciones corporales asociadas a su microhábitat (Darda & Wake, 2015). La diferenciación morfológica intraespecífica es muy notable en las especies que se encuentran al norte y centro de su distribución en la SMO, mientras que las especies de la FVTM y de las montañas del norte de Oaxaca conservan una forma similar (Darda & Wake, 2015). Recientemente se describieron seis especies para este género (Campbell et al., 2014; Rovito & Parra-Olea, 2015; García-Castillo et al., 2017; García-Castillo et al., 2018); cuatro de ellas se distribuyen en la SMO y corresponden a formas terrestres y cavernícolas, mientras que dos son de la FVTM y son de hábitos arborícolas. Particularmente, la similitud de formas de las especies de la FVTM y las montañas del norte de Oaxaca han complicado la tarea taxonómica, la cual no ha avanzado desde que se sugirieron especies genéticamente válidas (Darda, 1994; Parra-Olea, 2003). Asimismo, además las diagnosis pueden dificultarse dado que no existen caracterizaciones detalladas de algunas de las especies descritas.

Las relaciones filogenéticas de este grupo han sido propuestas con base en aloenzimas (Darda, 1994) y genes mitocondriales (Parra-Olea, 2003; Rovito & Parra-Olea, 2015), las cuales mostraron una estructura geográfica de las especies y han servido para validar especies nuevas. Sin embargo, las reconstrucciones filogenéticas propuestas no presentan apoyos óptimos de ramas, principalmente en los nodos profundos (Parra-Olea, 2003; García-Castillo, 2014). Por esta razón, obtener una hipótesis filogenética resuelta podría ser la evidencia necesaria para entender los procesos evolutivos que han promovido la diversificación en este grupo.

Por todo lo anterior, el presente trabajo plantea resolver una pregunta central sobre este grupo: ¿Cómo y cuándo se originaron las especies del género *Chiropterotriton*? Sin embargo, para poder resolverla es necesario conocer a todas las especies que conforman el grupo y proponer una filogenia robusta que sirva como base para reconstruir su historia biogeográfica. En el Capítulo I de esta tesis se realizó una revisión taxonómica sobre el grupo, incluyendo la descripción de ocho especies y re-descripción de tres. En el Capítulo II se proponen hipótesis filogenéticas basadas en ADN mitocondrial y nuclear, las cuales se obtuvieron a partir de un método de secuenciación masiva. La hipótesis filogenética nuclear fue la base para reconstruir la historia biogeográfica del género y con ello aportar información sobre el origen y riqueza de los bolitoglossinos en México.

OBJETIVO GENERAL

Describir los patrones biogeográficos y filogenéticos del género *Chiropterotriton* (Caudata: Plethodontidae).

OBJETIVOS PARTICULARES

1. Describir especies del género *Chiropterotriton* mediante la integración de los datos morfológicos, morfométricos y moleculares.
2. Proponer una filogenia molecular del género *Chiropterotriton* con base en secuencias de genes mitocondriales y nucleares obtenidos por secuenciación masiva.
3. Estimar los tiempos de divergencia filogenética de las especies que conforman el género *Chiropterotriton.*
4. Estimar el área ancestral de distribución del género *Chiropterotriton*.

CAPÍTULO I

Revisión taxonómica y descripción de especies del género *Chiropterotriton* con base en caracteres morfológicos, morfométricos y moleculares

1) A new terrestrial species of *Chiropterotriton* (Caudata: Plethodontidae) from central Mexico. Publicado en la revista *Zootaxa*

2) Two new species of *Chiropterotriton* (Caudata: Plethodontidae) from central Veracruz, Mexico. Publicado en la revista *Amphibian & Reptile Conservation*

3) Revision of the genus *Chiropterotriton* with description of 5 new species (Caudata: Plethodontidae).

Zootaxa 4363 (4): 489–505
http://www.mapress.com/j/zt/

Article

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

https://doi.org/10.11646/zootaxa.4363.4.2
http://zoobank.org/urn:lsid:zoobank.org:pub:520C0F4D-E187-4162-84C8-5A72396EB748

A new terrestrial species of *Chiropterotriton* (Caudata: Plethodontidae) from central Mexico

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Abstract

Chiropterotriton is a relatively small genus that comprises 15 species with great morphological and ecological diversity. In previous studies, molecular data provided evidence for a considerable number of species that remain undescribed. In this study, we describe one new species, *Chiropterotriton chico* **sp. nov.** based on molecular and morphological characters. We present mtDNA phylogenetic analyses using Bayesian inference and maximum likelihood that include all described and several undescribed species. Morphometric data from eight recognized species provide evidence for the distinctiveness of the new taxon. Description of this new species adds to the already high salamander diversity of the state of Hidalgo, which is an important area for the diversification of the genus.

Key words: salamanders, bolitogossines, morphology, taxonomy, Hidalgo

Introduction

The tribe Bolitoglossini (Wake 2012) is composed of 305 species (AmphibiaWeb 2017), which represents more than 65% of the known Plethodontid species. It includes 14 genera that show extensive morphological diversification in the Neotropics (Rovito *et al.* 2015). Although this highly diverse group has been studied for many years, molecular tools have shown that the diversity of the group has been underestimated and many species of bolitoglossine salamanders have been recently described based on morphological and molecular data (Townsend *et al.* 2010; Boza-Oviedo *et al.* 2012; Acevedo *et al.* 2013; Rovito & Parra-Olea 2015; Parra-Olea *et al.* 2016).

The genus *Chiropterotriton* currently contains 15 described species, which occur exclusively in Mexico from the northern states of Coahuila, Nuevo León, and Tamaulipas to the south in Oaxaca (AmphibiaWeb 2017). This group shows substantial morphological diversity, including extensive variation in degree of foot webbing and body size. Ecological diversity is also evident in the arboreal, terrestrial, and cave-dwelling habitats of these salamanders. This diversity is largely a product of size change and heterochrony, which is common in plethodontid evolution (Darda 1994; Darda & Wake 2015). The first molecular phylogeny of *Chiropterotriton,* based on allozyme data, was published by Darda (1994) who analyzed samples from 25 populations that included all but two (*C. mosaueri* (Woodall) and *C. multidentatus* (Taylor)) of the then described species. His analysis grouped all species in two geographic assemblages (northern and southern) and suggested the existence of 13 undescribed species. Parra-Olea (2003) presented a mitochondrial phylogeny, which also lacked sequences of *C. mosaueri* and *C. multidentatus,* and included 27 populations. Parra-Olea's phylogeny was in general in agreement with most of the allozyme results and supported seven of Darda's undescribed species. More recently, Campbell *et al.* (2014) described one new species from northern Mexico. Rovito & Parra-Olea (2015) presented a phylogeny based on two mitochondrial genes that included 13 species of *Chiropterotriton,* as well as many undescribed species, and described two more species from northern Mexico. Of these three newly described species, one corresponds to one

Accepted by M. Vences: 21 Aug. 2017; published: 13 Dec. 2017

of the 13 undescribed species reported for Darda (1994) while two of them were not included in the phylogenetic analyses of Darda (1994) or Parra-Olea (2003). In general, all published phylogenies agree on the existence of two clades, concordant with the geographic distribution of the species, and support the existence of multiple undescribed species.

In the present paper, we describe a species from El Chico National Park in the state of Hidalgo, which long have been assigned to *Chiropterotriton multidentatus* (Rabb 1958; Wake & Lynch 1976). Only recently has tissue been available from topotypic *C. multidentatus* (Rovito & Parra-Olea 2015), and it has proven crucial in the present study. The description is based on both mtDNA phylogenetic analyses and morphological characters.

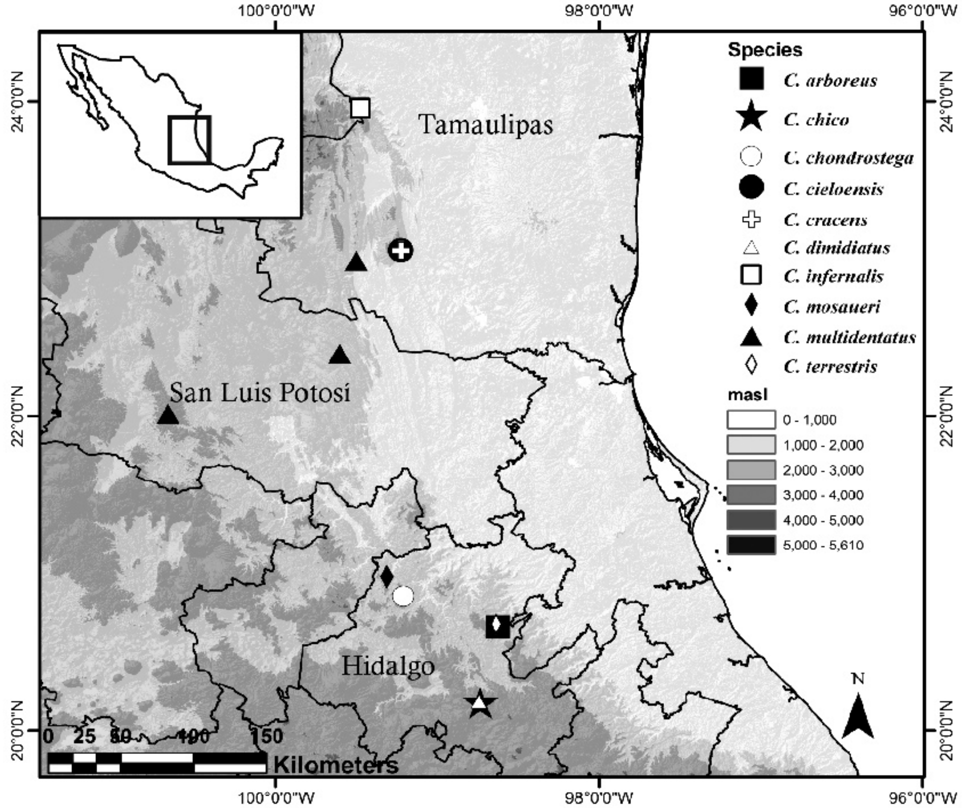

FIGURE 1. Map of *Chiropterotriton* localities in central and northern Mexico used for phylogenetic and morphological comparisons. Distributional ranges for all species of *Chiropterotriton* used in this study are a small area surrounding the locality shown here.

Methods

We analyzed a total of 30 previously published sequences of the large subunit ribosomal RNA (16S) mitochondrial gene (Parra-Olea 2003; Rovito & Parra-Olea 2015). Voucher information and Genbank numbers are given in Table 1. We aligned the sequences with Muscle v.3.8 (Edgar 2004) and used jModelTest2 (Darriba *et al.* 2012) to select the best-fitting nucleotide substitution model substitution model (GTR+I+G). We performed a Bayesian phylogenetic analysis using the program MrBayes 3.2 (Ronquist *et al.* 2012). Two separate runs were done for 20,000,000 generations, with three hot and one cold chains, sampled every 1000 generations, and the first 5000 samples were discarded as burn-in. A maximum likelihood analysis was run in RAxML v.8.0 (Stamatakis 2006). Nodal support was assessed through 1000 bootstrap replicates. *Isthmura boneti* (Alvarez & Martín) and *Thorius* *magnipes* Hanken & Wake were used as outgroups in both analyses. Both phylogenetic analyses were run on the CIPRES Science Gateway (Miller *et al.* 2010). Finally, we obtained corrected genetic distances (Kimura two-parameter, K2P) using Mesquite v3.04 (Maddison & Maddison 2015), which are shown in Table 2.

TABLE 1. Voucher information and GenBank numbers for specimens included in phylogenetic analyses from the Museum of Vertebrate Zoology, UC Berkeley (MVZ) and Instituto de Biología, UNAM (IBH). Locality abbreviations: DF: Distrito Federal, Hgo: Hidalgo, NL: Nuevo León, Oax: Oaxaca, Pue: Puebla, SLP: San Luis Potosí, Tamps: Tamaulipas, Ver: Veracruz.

Species	Locality	Voucher number	Genbank Accessions
C. arboreus	Hgo: 4.3 km E of Hwy 105 junction on road to Tianguistengo	MVZ200622	AY522474
C. chico	Hgo: 3.8 km S Mineral del Chico	MVZ200679	AY522471
C. chiropterus	Ver: Huatusco	MVZ163724	AY522495
C. chondrostega	Hgo: La Venta, 3.6 km NE of jct to hwy Mx 85 on road to La Encarnación	IBH22266	AY523996
C. chondrostega	Hgo: 1.0 km S (by rd) of La Encarnación	IBH28195	KT820673
C. cieloensis	Tamps: 5.6 km (air) NW Gómez Farías	MVZ158570	AY522473
C. cieloensis	Tamps: 5.6 km (air) W Gómez Farías	MVZ173380	AY522459
C. cracens	Tamps: 1.3 km NE (by air) of San José, Reserva de la Biósfera El Cielo	IBH28192	KT820674
C. dimidiatus	Hgo: 4.9 km S Mineral del Chico	IBH22344	AY522463
C. dimidiatus	Hgo: 4.1 km S (by rd) of Mineral del Chico	IBH28196	KT820675
C. infernalis	Tamps: Cueva del Brinco, Conrado Castillo	MVZ269665	KT820694
C. lavae	Ver: La Joya	IBH22342	AY522466
C. lavae	Ver: La Joya	IBH28180	KT820676
C. magnipes	SLP: 1.1 km W Ahuacatlán	MVZ200678	AY522469
C. miquihuanus	NL: 1.8 km S (by rd) of La Encantada on road from La Bolsa to Zaragoza	MVZ269643	KT820697
C. mosaueri	Hgo: 900 m SSE Durango	IBH28179	KT820677
C. multidentatus	SLP: 900 m NE Valle de los Fantasmas	IBH28177	KT820678
C. multidentatus	SLP: 26.2 km E (by rd) of center of Ciudad del Maíz	IBH28194	KT820680
C. multidentatus	Tamps: 22.0 km E (by rd) Tula on road to Ocampo	MVZ269654	KT820681
C. orculus	DF: 8.8 km SW La Venta, Parque Nacional Desierto de los Leones	MVZ138677	AY522444
C. priscus	NL: Cerro Potosí	gp011	AY522476
C. priscus	NL: Cerro Potosí, 30.2 km NW (by rd) of center of Galeana	MVZ269655	KT820682
C. terrestris	Hgo: 4 km NNE Zacualtipán	MVZ172149	AY522455
C. sp. C	Ver: 3.2 km S Puerto del Aire	MVZ163635	AY522453
C. sp. F	Pue: Xicotepec de Juárez	MVZ178707	AY522479
C. sp. G	Pue: 4 km S Chignahuapan	MVZ178703	AY522481
C. sp. H	Ver: 15.9 km S Las Vigas on road to RMO Las Lajas	IBH23066	AY522482
C. sp. I	Pue: Santa Cruz de Texmalaquilla	MVZ201387	AY522488
C. sp. J	Oax: La Esperanza	gp086	AY522489
C. sp. K	Oax: Cerro San Felipe	MVZ173232	AY522491
Isthmura boneti	Oax: Cerro Zempoaltepetl	MVZ163873	AY864714
Thorius magnipes	Pue: Lagunas de San Bernardino	IBH22918	KC884063

TABLE 2. Sequence divergence with Kimura two-parameter distances (upper half) and uncorrected (*p*) distances (lower half).

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	*C. arboreus*		0.069	0.119	0.115	0.062	0.073	0.122	0.066	0.101	0.124	0.083	0.113
2	*C. chico*	0.065		0.128	0.105	0.075	0.075	0.121	0.062	0.104	0.107	0.088	0.095
3	*C. chiropterus*	0.110	0.117		0.122	0.143	0.119	0.113	0.117	0.083	0.139	0.113	0.127
4	*C. chondrostega*	0.106	0.097	0.112		0.127	0.087	0.118	0.099	0.115	0.110	0.092	0.099
5	*C. cieloensis*	0.059	0.071	0.129	0.116		0.066	0.129	0.069	0.119	0.122	0.092	0.103
6	*C. cracens*	0.068	0.071	0.109	0.082	0.063		0.108	0.068	0.110	0.110	0.074	0.098
7	*C. dimidiatus*	0.112	0.111	0.104	0.108	0.118	0.100		0.123	0.085	0.133	0.102	0.123
8	*C. infernalis*	0.063	0.060	0.108	0.093	0.065	0.065	0.112		0.103	0.120	0.092	0.103
9	*C. lavae*	0.094	0.096	0.078	0.105	0.109	0.101	0.080	0.096		0.127	0.103	0.108
10	*C. magnipes*	0.113	0.098	0.125	0.102	0.111	0.101	0.119	0.110	0.114		0.104	0.109
11	*C. miquihuanus*	0.078	0.083	0.104	0.087	0.086	0.070	0.094	0.086	0.096	0.096		0.094
12	*C. mosaueri*	0.104	0.089	0.116	0.092	0.096	0.092	0.112	0.096	0.099	0.099	0.088	
13	*C. multidentatus*	0.039	0.065	0.116	0.098	0.057	0.065	0.114	0.065	0.104	0.110	0.077	0.114
14	*C. multidentatus*	0.037	0.061	0.111	0.102	0.067	0.066	0.110	0.063	0.092	0.105	0.078	0.112
15	*C. multidentatus*	0.033	0.053	0.105	0.100	0.057	0.059	0.102	0.059	0.086	0.109	0.074	0.110
16	*C. orculus*	0.080	0.087	0.080	0.109	0.096	0.088	0.080	0.090	0.068	0.115	0.086	0.097
17	*C. priscus*	0.094	0.085	0.129	0.102	0.102	0.105	0.108	0.096	0.099	0.109	0.063	0.080
18	*C. terrestris*	0.062	0.028	0.113	0.091	0.067	0.069	0.103	0.056	0.093	0.101	0.080	0.087
19	*C.* sp. C	0.094	0.100	0.070	0.107	0.113	0.105	0.078	0.096	0.014	0.114	0.096	0.101
20	*C.* sp. F	0.119	0.111	0.072	0.114	0.137	0.110	0.104	0.116	0.088	0.125	0.116	0.124
21	*C.* sp. G	0.084	0.095	0.080	0.107	0.105	0.088	0.082	0.092	0.066	0.122	0.082	0.103
22	*C.* sp. H	0.096	0.098	0.082	0.109	0.111	0.107	0.084	0.098	0.012	0.112	0.098	0.099
23	*C.* sp. I	0.090	0.103	0.074	0.110	0.111	0.103	0.080	0.102	0.016	0.115	0.094	0.102
24	*C.* sp. J	0.102	0.112	0.021	0.111	0.121	0.105	0.098	0.104	0.076	0.120	0.098	0.103
25	*C.* sp. K	0.092	0.099	0.072	0.109	0.108	0.088	0.092	0.094	0.062	0.097	0.094	0.100

……*continued on the next page*

TABLE 2. (Continiued)

		13	14	15	16	17	18	19	20	21	22	23	24	25
1	*C. arboreus*	0.040	0.038	0.034	0.085	0.101	0.065	0.101	0.131	0.089	0.103	0.096	0.110	0.099
2	*C. chico*	0.069	0.064	0.056	0.093	0.090	0.028	0.109	0.121	0.102	0.106	0.111	0.123	0.107
3	*C. chiropterus*	0.127	0.121	0.115	0.085	0.143	0.123	0.074	0.077	0.085	0.087	0.079	0.022	0.077
4	*C. chondrostega*	0.106	0.110	0.108	0.119	0.110	0.098	0.117	0.124	0.117	0.119	0.120	0.121	0.120
5	*C. cieloensis*	0.060	0.071	0.060	0.103	0.110	0.071	0.124	0.153	0.114	0.121	0.122	0.133	0.117
6	*C. cracens*	0.068	0.070	0.062	0.094	0.115	0.073	0.114	0.119	0.094	0.116	0.112	0.114	0.094
7	*C. dimidiatus*	0.125	0.120	0.110	0.085	0.118	0.112	0.083	0.113	0.087	0.090	0.085	0.106	0.099
8	*C. infernalis*	0.069	0.066	0.062	0.096	0.104	0.058	0.103	0.127	0.099	0.105	0.110	0.112	0.101
9	*C. lavae*	0.112	0.098	0.091	0.072	0.108	0.100	0.014	0.094	0.070	0.012	0.016	0.081	0.066
10	*C. magnipes*	0.120	0.115	0.120	0.127	0.120	0.109	0.127	0.139	0.137	0.124	0.127	0.134	0.106
11	*C. miquihuanus*	0.081	0.083	0.079	0.092	0.066	0.084	0.103	0.127	0.087	0.105	0.101	0.106	0.102
12	*C. mosaueri*	0.125	0.122	0.120	0.106	0.085	0.093	0.110	0.137	0.113	0.108	0.111	0.112	0.108
13	*C. multidentatus*		0.028	0.028	0.099	0.108	0.067	0.117	0.129	0.096	0.115	0.115	0.121	0.110
14	*C. multidentatus*	0.027		0.020	0.089	0.115	0.065	0.107	0.129	0.091	0.105	0.103	0.116	0.096
15	*C. multidentatus*	0.027	0.019		0.083	0.108	0.056	0.101	0.122	0.085	0.098	0.098	0.109	0.094
16	*C. orculus*	0.092	0.084	0.078		0.122	0.097	0.070	0.084	0.022	0.076	0.072	0.076	0.047
17	*C. priscus*	0.100	0.105	0.099	0.111		0.102	0.112	0.146	0.114	0.110	0.110	0.135	0.134
18	*C. terrestris*	0.064	0.062	0.054	0.091	0.095		0.104	0.114	0.107	0.102	0.107	0.123	0.105
19	*C.* sp. C	0.108	0.099	0.094	0.066	0.103	0.097		0.087	0.068	0.018	0.006	0.074	0.063
20	*C.* sp. F	0.118	0.117	0.112	0.078	0.131	0.105	0.082		0.083	0.096	0.095	0.072	0.072
21	*C.* sp. G	0.090	0.086	0.080	0.021	0.105	0.099	0.064	0.078		0.070	0.068	0.072	0.044
22	*C.* sp. H	0.106	0.097	0.092	0.072	0.101	0.095	0.017	0.090	0.066		0.020	0.085	0.066
23	*C.* sp. I	0.106	0.096	0.092	0.068	0.101	0.099	0.006	0.088	0.064	0.019		0.074	0.066
24	*C.* sp. J	0.112	0.107	0.101	0.072	0.123	0.113	0.070	0.068	0.068	0.080	0.070		0.068
25	*C.* sp. K	0.102	0.090	0.088	0.045	0.121	0.097	0.060	0.068	0.043	0.062	0.062	0.064	

TABLE 3. Mean ± standard deviation and range of morphological measurements (in mm) and tooth counts for males. Sample size given when different from total number of specimens measured for each species. * Data taken from Rovito & Parra-Olea 2015.

MALES	*C. arboreus* *N* = 9	*C. chondrostega* *N* = 10	*C. cieloensis** *N* = 8	*C. chico* *N* = 15	*C. cracens** *N* = 4	*C. dimidiatus* *N* = 15	*C. infernalis** *N* = 8	*C. multidentatus** *N* = 7	*C. terrestris* *N* = 15
SVL	33.4±3.62 (27.8–39.5)	23.1±1.07 (21.0–24.6)	32.6±1.96 (30.2–36.1)	38.4±1.99 (36.2–42.6)	25.7±1.98 (23.7–28.0)	24.6±0.97 (23.3–26.7)	36.4±3.84 (31.0–41.6)	33.6±2.92 (29.3–36.7)	24.2±1.33 (21.6–26.9)
TL	41.5±5.91 (32.0–50.4) *N* = 8	25.6±3.91 (19.4–32.4)	38.3±5.25 (32.9–48.1) *N* = 7	45.4±2.81 (40.4–49.5)	30.6±3.65 (26.6–35.4)	22.0±1.71 (18.4–24.1)	44.6±4.72 (38.1–51.9) *N* = 7	37.8±7.52 (30.3–48.9) *N* = 6	25.3±2.26 (19.8–29.2)
AX	16.5±1.74 (13.7–19.7)	11.62±0.47 (11.1–12.4)	16.7±1.27 (15.2–18.9)	19.8±1.19 (17.5–21.7)	13.3±0.99 (12.3–14.7)	13.1±0.74 (11.6–13.9)	18.6±2.25 (15.0–21.1)	17.4±1.72 (15.2–20.0)	12.1±0.76 (10.8–13.3)
FLL	8.2±0.73 (7.2–9.2)	4.9±0.31 (4.5–5.5)	8.6±0.68 (7.7–9.8)	8.4±0.54 (7.2–9.4)	6.1±1.06 (5.1–7.6)	4.5±0.33 (3.7–4.9)	9.8±1.37 (8.0–11.8)	8.8±1.63 (6.8–11.5)	4.7±0.47 (3.9–5.5)
HLL	9.5±0.73 (8.4–10.3)	5.4±0.43 (5.1–6.5)	9.8±0.68 (9.0–10.9)	10.1±0.73 (8.0–10.8)	7.0±1.07 (6.0–8.5)	5.2±0.33 (4.8–5.9)	11.2±1.28 (9.1–12.9)	10.0±2.05 (7.7–13.2)	5.2±0.53 (4.3–6.0)
HL	7.9±0.80 (6.5–9.2)	5.6±0.30 (5.1–6.1)	7.1±0.33 (6.7–7.8)	8.8±0.61 (7.2–9.8)	5.5±0.78 (4.6–6.4)	5.2±0.31 (4.8–5.8)	8.2± 1.07 (6.9–10.1)	7.4±0.87 (6.4–8.9)	5.7±0.33 (5.0–6.1)
HW	5.0±0.49 (4.4–5.7)	3.4±0.19 (3.2–3.8)	4.8±0.30 (4.3–5.4)	5.6±0.24 (5.0–5.9)	3.8±0.52 (3.2–4.4)	3.4±0.20 (2.9–3.7)	5.7± 0.62 (4.6–6.4)	4.9±0.56 (4.1–5.7)	3.5±0.27 (3.2–4.0)
HD	2.5±0.28 (1.9–2.8)	1.8±0.09 (1.68–1.97)	—	2.6±0.20 (2.2–2.9)	—	1.8±0.08 (1.7–1.9)	—	—	1.8±0.21 (1.5–2.3)
SW	4.2±0.52 (3.5–4.9)	3.04±0.19 (2.7–3.4)	—	4.8±0.35 (4.0–5.5)	—	2.9±0.29 (2.3–3.5)	—	—	2.7±0.32 (2.1–3.3)
IN	1.8±0.24 (1.3–2.0)	1.3±0.08 (1.2–1.4)	1.7±0.08 (1.6–1.9)	2.1±0.18 (1.8–2.5)	1.3±0.16 (1.1–1.5)	1.1±0.08 (1.0–1.2)	1.9±0.19 (1.5–2.1)	1.7±0.25 (1.3–2.1)	1.3±0.13 (1.1–1.5)
FW	3.4±0.30 (2.8–3.9)	1.8±0.21 (1.5–2.2)	3.2±0.40 (2.7–3.8)	4.1±0.35 (3.4–4.6)	2.2±0.34 (1.9–2.7)	1.7±0.19 (1.4–2.0)	4.2±0.70 (3.2–5.3)	3.6±0.39 (3.1–4.1)	1.9±0.29 (1.3–2.4)
NL	0.16±0.019 (0.13–0.18)	0.13±0.009 (0.11–0.14)	0.19±0.020 (0.16–0.21) *N* = 4	0.16±0.012 (0.13–0.18)	0.15±0.010 (0.14–0.16)	0.25±0.010 (0.24–0.28)	0.24±0.030 (0.19–0.28) *N* = 4	0.22±0.020 (0.19–0.23) *N* = 4	0.12±0.008 (0.10–0.13)
NW	0.08±0.009 (0.07–0.08)	0.06±0.005 (0.06–0.08)	0.12±0.000 (0.12–0.12) *N* = 4	0.08±0.006 (0.06–0.08)	0.11±0.020 (0.09–0.12)	0.25±0.012 (0.23–0.28)	0.17±0.030 (0.12–0.21) *N* = 4	0.16±0.010 (0.14–0.16) *N* = 4	0.07±0.006 (0.06–0.08)
ND	2.1±0.24 (1.8–2.5)	1.9±0.19 (1.6–2.2)	1.6±0.19 (1.4–1.8) *N* = 4	1.9±0.21 (1.6–2.5)	1.5±0.23 (1.2–1.8)	1.0±0.06 (0.8–1.1)	1.5±0.18 (1.1–1.7) *N* = 4	1.4±0.22 (1.1–1.6) *N* = 4	1.7±0.17 (1.4–2.0)
LI	0.2±0.36 (0–1)	2.0±0.00 (2–2)	-0.2±0.46 (-1–0.5)	0.6±0.30 (0–1)	1.8±0.65 (1–2.5)	3.8±0.35 (3–4)	–0.7± 0.98 (–2–1)	0.1±1.60 (–3–1.5)	1.9±0.32 (1.5–2.5)
PMT+MT	26.5±3.39 (20–30)	22.5±3.71 (18–29)	31.4±3.66 (26–35)	42.3±5.08 (32–54)	36.8±0.96 (36–38)	9.4±2.58 (5–14)	49.3±7.11 (36–56)	40.0±11.06 (26–56)	21.2±3.82 (15–30)
VT	11.2±2.59 (9–17)	7.9±1.52 (6–11)	11.5±2.00 (9–15)	13.6±2.58 (10–19)	12.5±2.08 (10–15)	5.6±1.34 (4–8)	17.6±3.16 (13–22)	13.3±1.70 (11–16)	8.8±1.82 (6–14)

TABLE 4. Mean ± standard deviation and range of morphological measurements (in mm) and tooth counts for females. Sample size given when different from total number of specimens measured for each species. * Data taken from Rovito & Parra-Olea 2015.

FEMALES	*C. arboreus* *N = 5*	*C. chondrostega* *N = 11*	*C. cieloensis** *N = 4*	*C. chico* *N = 15*	*C. cracens** *N = 15*	*C. dimidiatus* *N = 15*	*C. infernalis** *N = 1*	*C. multidentatus** *N = 9*	*C. terrestris* *N = 15*
SVL	32.2±3.47 (29.3–38.0)	25.4±1.73 (23.5–28.2)	31.1±2.73 (28.3–34.4)	39.3±2.59 (35.9–44.3)	27.4±3.30 (23.2–34.0)	25.8±1.55 (23.0–29.0)	29.7	34.0±6.27 (24.5–41.9)	23.0±1.92 (21.6–27.6)
TL	37.4±5.82 (33.7–47.6)	24.2±3.70 (17.6–31.0)	31.9±5.32 (24.5–36.0)	44.1±3.65 (37.0–49.0)	28.8±6.17 (17.2–38.4)	22.4±1.84 (19.8–25.1)	34.5	34.9±5.81 (26.5–43.6)	22.4±1.89 (21.0–28.5)
AX	16.7±1.55 (15.3–19.2)	13.4±0.89 (12.5–14.9)	16.8±1.98 (14.1–18.9)	21.8±1.64 (19.0–23.9)	14.5±1.99 (11.5–18.5)	14.7±1.23 (12.5–17.2)	14.2	17.5±3.59 (11.5–22.5)	12.0±1.22 (11.2–15.3)
FLL	7.4±0.93 (6.8–9.0)	5.0±0.30 (4.3–5.3)	8.1±0.98 (7.1–9.4)	8.2±0.59 (7.2–9.0)	6.4±1.53 (4.4–8.8)	4.3±0.43 (3.7–5.1)	7.2	8.6±2.12 (5.9–11.7)	4.3±0.36 (3.9–5.4)
HLL	8.9±0.61 (8.3–9.6)	5.5±0.42 (4.9–6.1)	9.4±0.95 (8.8–10.8)	9.6±0.84 (8.4–11.1)	7.3±1.46 (5.5–9.7)	4.9±0.47 (4.3–6.1)	8.9	9.8±2.29 (6.7–13.5)	4.8±0.44 (4.3–5.8)
HL	7.6±0.87 (6.9–9.0)	5.7±0.33 (5.4–6.5)	6.9±0.65 (6.3–7.7)	8.7±0.62 (7.9–9.9)	5.8±0.84 (4.8–7.1)	5.0±0.33 (4.4–5.6)	6.2	7.4±1.37 (5.2–9.2)	5.2±0.35 (5.1–6.1)
HW	4.9±0.51 (4.5–5.7)	3.7±0.19 (3.4–4.0)	4.9±0.59 (4.5–5.8)	5.7±0.41 (5.1–6.7)	4.1±0.62 (3.2–5.1)	3.5±0.24 (3.1–3.9)	4.3	5.1±0.89 (3.7–6.2)	3.3±0.48 (2.2–4.2)
HD	2.1±0.24 (1.8–2.4)	1.9±0.09 (1.7–2.0)	—	2.8±0.28 (2.4–3.4)	—	2.0±0.20 (1.7–2.2)	—	—	1.8±0.20 (1.6–2.3)
SW	4.0±0.42 (3.6–4.7)	3.13±0.26 (2.8–3.8)	—	5.1±0.36 (4.6–5.8)	—	3.1±0.26 (2.7–3.5)	—	—	2.8±0.29 (2.6–3.6)
IN	1.8±0.06 (1.7–1.8)	1.2±0.11 (1.1–1.4)	1.6±0.13 (1.5–1.8)	2.1±0.13 (1.9–2.3)	1.3±0.19 (1.0–1.6)	1.2±0.14 (1.0–1.6)	1.4	1.6±0.23 (1.3–2.0)	1.2±0.14 (1.1–1.5)
FW	3.5±0.62 (2.9–4.4)	1.8±0.18 (1.5–2.2)	3.1±0.55 (2.3–3.6)	4.2±0.58 (3.4–5.5)	2.5±0.67 (1.8–3.5)	1.7±0.25 (1.2–2.2)	2.8	3.5±0.80 (2.3–4.6)	1.7±0.25 (1.4–2.2)
NL	0.16±0.009 (0.15–0.17)	0.12±0.010 (0.11–0.14)	0.21±0.020 (0.19– 0.23) N = 3	0.15±0.008 (0.14–0.16)	0.19±0.030 (0.14–0.23) N = 14	0.21±0.026 (0.19–0.26)	0.20	0.21±0.020 (0.19–0.23) N = 5	0.11±0.009 (0.10–0.13)
NW	0.08±0.007 (0.07–0.08)	0.06±0.007 (0.04–0.08)	0.12±0 (0.12–0.12) N = 3	0.08±0.015 (0.06–0.11)	0.12±0.030 (0.09–0.19) N = 14	0.24±0.020 (0.19–0.28)	0.19	0.14±0.020 (0.12–0.16) N = 5	0.07±0.009 (0.07–0.08)
ND	2.00±0.19 (1.8–2.2)	1.97±0.21 (1.6–2.3)	1.80±0.20 (1.6–2.0) N = 3	1.95±0.27 (1.4–2.2)	1.60±0.27 (1.2–2.0) N = 14	0.89±0.10 (0.8–1.0)	1.13	1.5±0.18 (1.3–1.8) N = 5	1.5±0.22 (1.2–2.0)
LI	1.0±0 (1–1)	3.0± 0.0 (3–3)	0.1±0.85 (-1–1)	2.1±0.39 (1.5–3.0)	2.1±0.89 (0.5–3.5)	4.9±0.25 (4.0–5.0)	–0.5	1.0±0.97 (0.5–2)	2.6±0.28 (2–3)
PMT +MT	33.4±3.43 (28–36)	40.9±5.33 (33–48)	46.8±6.40 (42–56)	56.5±4.79 (48–67)	49.5±11.07 (32–74)	34.4±4.11 (27–41)	53	47.8±11.24 (34–66)	38.1±7.04 (27–53)
VT	12.4±2.41 (9–15)	9.54±2.29 (6–14)	12.8±1.71 (11–15)	15.6±1.79 (13–19)	12.0±2.48 (7–16)	8.33±1.34 (6–11)	15	13.1±3.02 (10–19)	9.9±1.92 (8–14)

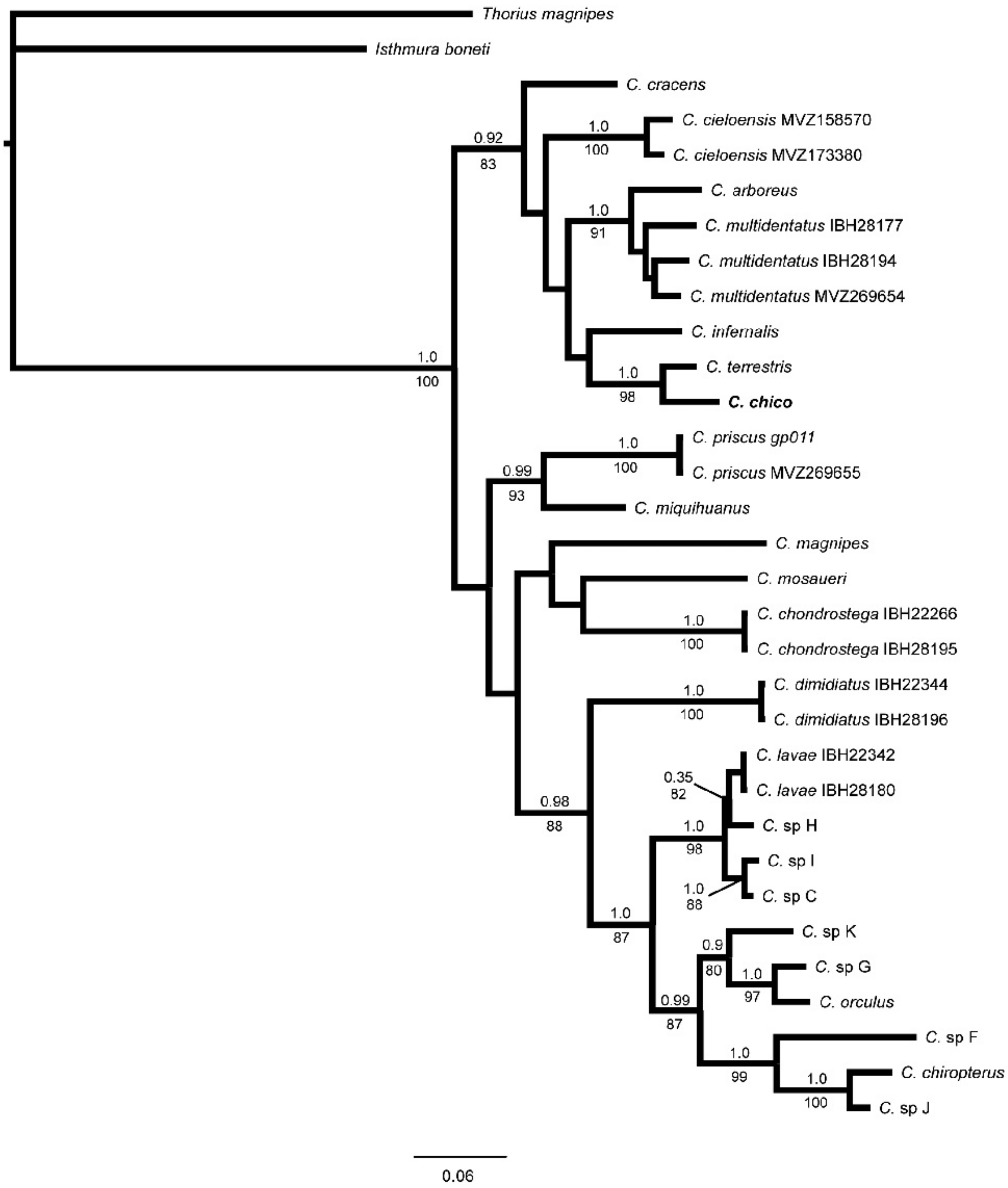

FIGURE 2. Mitochondrial DNA gene tree of *Chiropterotriton* from Bayesian analysis of 16S sequences. Numbers above branches represent posterior probabilities and numbers below branches are boostrap values from maximum likelihood phylogenetic analysis.

Species descriptions follow the format used by Lynch and Wake (1989) for species of Neotropical plethodontids and include the same basic characters and measurements. The comparisons for the new species were made with the other five members of its clade from our phylogenetic analyses (see Results) as well as with other geographically proximate species. We examined specimens of other species that occur in Hidalgo, *Chiropterotriton* *arboreus* (Taylor), *C. chondrostega* (Taylor), *C. dimidiatus* (Taylor) and *C. terrestris* (Taylor) (Appendix 1), and we used the published measurements of *C. mosaueri* (Woodall 1941; Rabb 1958; Rovito & Parra-Olea 2015), *C. cracens* Rabb, *C. cieloensis* Rovito & Parra-Olea, *C. infernalis* Rovito & Parra-Olea, and *C. multidentatus* (Rovito & Parra-Olea 2015) that occur in Hidalgo, Tamaulipas and San Luis Potosí.

We compared measurements of 181 specimens (91 males and 90 females) from nine species of *Chiropterotriton* from central and northern Mexico (Fig. 1, Tables 3 and 4, Appendix 1). We took measurements of 11 characters to the nearest 0.1 mm with a Vernier calipers: 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 angle of jaw (HW), head depth (HD), shoulder width (SW), internarial distance (IN), and right foot width (FW). We also measured the widest and narrowest nostril dimensions (nostril length, NL, and nostril width, NW) using an ocular micrometer and calculated a ratio of nostril dimensions (ND = NL/NW) as an index of nostril shape. We counted maxillary (MT), premaxillary (PMT), and vomerine teeth (VT) and we present counts for PMT and MT together because of the difficulty in distinguishing them in some specimens. We also measured limb interval (LI) as the number of costal folds between adpressed limbs, with positive values meaning grooves between limbs and negative values meaning overlap between limbs. We separated males and females in order to account for sexual dimorphism in the statistics. Twelve additional measurements were obtained for the holotype: 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 vent, snout to forelimb, tail depth at base, and tail width at base.

Results

Our phylogenetic hypothesis based on mtDNA (Fig. 2) shows that the populations from Hidalgo are not close relatives of topotypic *C. multidentatus*, but instead are the sister species of the much smaller *C. terrestris*, from farther north in Hidalgo. Such a relationship was suggested by studies of allozymes and mtDNA in previous studies (Darda 1994; Parra-Olea 2003; Rovito & Parra-Olea 2015), but topotypic samples of *C. multidentatus* were lacking in the former two studies. In our analysis *C. chico* is well supported as the sister species of *C. terrestris* (Posterior probability = 1.0, bootstrap = 97) and both are placed in a clade that contains five species (*C. arboreus*, *C. cieloensis*, *C. cracens*, *C. infernalis,* and *C. multidentatus*), which range across the states of Hidalgo, San Luis Potosí, and Tamaulipas. Genetic divergence between *C. chico* and its sister species *C. terrestris* is 2.8% for both K2P distance and uncorrected (*p*) distance (Table 2).

Taxonomy

Amphibia

Caudata

Plethodontidae

Chiropterotriton chico sp. nov.

El Chico Salamander
Salamandra de El Chico
(Figures 3A and 4A)

Chiropterotriton multidentata (part): Taylor, 1944.
Chiropterotriton cf multidentatus (part): Rabb, 1958; Darda, 1994.
Chiropterotriton sp. 15: Parra-Olea, 2003; Rovito & Parra-Olea, 2015

Holotype. MVZ 118888, an adult male from El Chico National Park, Hidalgo, Mexico, 2950–3050 m, 20.184154° N, 98.734437° W (maximum error distance 5477 m). Collected 31 July 1972 by James Lynch and Lynne D. Houck.

Paratypes. Twenty-nine specimens, all from Hidalgo, Mexico. Fifteen males: MVZ 118752, 118804, 118811, 118821, 118827, 118842, 118869, 118900, 119053, 119057, 119078, 119166, 119212, 119216, El Chico National Park; MVZ 138852, road to El Chico National Park, 1.0 km N (by road) junction Hwy. 105. Fourteen females: MVZ 114436, El Chico National Park, 4 km S (by road) Mineral del Chico; MVZ 118740, 118791, 118793, 118798, 118800, 118816, 118901, 118905, 119016, 119034, 119062, 119179, 119193, El Chico National Park.

Referred specimens. CAS 13112–13113; ENCB 139-142; IBH 23105; LACM 68955–69037, 69066–69071, 124057–124087, 168383–168388; MCZ A-25605–25606, A-93296 –93297; MVZ 46909, 97959, 98956–99022, 103938–103960, 106596–106655, 114292–114297, 114420–114435, 114437–114489, 118047–118739, 118741–118751, 118753–118790, 118792, 118794–118797, 118799, 118801–118803, 118805–118810, 118812–118815, 118817–118820, 118822–118826, 118828–118841, 118843–118868, 118870–118887, 118889–118899, 118902–118904, 118906–119015, 119017–119033, 119035–119052, 119054–119056, 119058–119061, 119063–119077, 119079–119165, 119167–119178, 119180–119192, 119194–119211, 119213–119215, 119217–119220, 128972–128977, 138853–138866, 143864–143893, 163620–163629, 172142–172147, 183645–183654, 195833–195835, 199175–199190, 200679, 219569–219571; UCM 41242–41252; UMMZ 151186–151192; USNM 116324–116338, 134284–134286, 201055-201057, 204910–204913, 249001-249004; UTEP 657–658.

Diagnosis. A plethodontid salamander assigned to *Chiropterotriton* because of small size, presence of sublingual fold, and distinctive shape of the digits of the hand and foot (with a relatively long outer digit; Wake & Elias 1983), as well as on the basis of analyses of mtDNA sequence data. Phylogenetically most closely related to *C. terrestris*, also from the state of Hidalgo. Morphological differences between *C. chico* and its sister taxon *C. terrestris* are much larger size (mean SVL 38.4 in males and 39.3 in females of *C. chico* vs. 24.1 in males, 23.0 in females of *C. terrestris*), relatively longer tail (mean TL/SVL 1.18 in males and 1.12 in females of *C. chico* vs. 1.05 in males and 0.97 in females of *C. terrestris*), longer limbs (mean LI 0.6 costal folds in males and 2.1 in females of *C. chico* vs. 1.9 in males, 2.6 in females of *C. terrestris*), longer head (mean HL 8.8 in males and 8.7 in females of *C. chico* vs. 5.7 in males and 5.2 in females of *C. terrestris*), broader head (mean HW 5.6 in males and 5.7 in females of *C. chico* vs. 3.5 in males and 3.3 in females of *C. terrestris*), broader feet (mean FW 4.1 in males and 4.2 in females of *C. chico* vs. 1.9 in males and 1.6 in females of *C. terrestris*), more maxillary and premaxillary teeth (mean MT+PMT 42.3 for males and 56.5 for females of *C. chico* vs. 21.2 for males and 38.2 for females of *C. terrestris)*, and more vomerine teeth (mean VT 13.6 in males and 15.6 in females of *C. chico* vs. 8.8 in males and 9.9 in females of *C. terrestris)*.

Chiropterotriton chico differs from *C. arboreus* in the following characteristics: larger size (mean SVL 38.4 in males, 39.3 in females of *C. chico* vs. 33.4 in males, 32.2 in females of *C. arboreus*), relatively longer tail (mean TL/SVL 1.18 in males, and 1.12 in females of *C. chico* vs. 0.83 in males and 0.87 in females of *C. arboreus*), shorter limbs (mean LI 0.6 in males, 2.1 in females of *C. chico* vs. 0.2 in males, 1.0 in females of *C. arboreus*), longer head (mean HL 8.8 in males and 8.7 in females of *C. chico* vs. 7.9 in males and 7.5 in females of *C. arboreus*), broader head (mean HW 5.6 in males and 5.7 in females of *C. chico* vs. 5.0 in males and 4.8 in females of *C. arboreus*), broader feet (mean FW 4.1 in males and 4.2 in females of *C. chico* vs. 3.4 in males and 3.5 in females of *C. arboreus*), more maxillary and premaxillary teeth (mean MT+PMT 42.3 for males and 56.5 for females of *C. chico* vs. 26.5 for males and 33.4 for females of *C. arboreus*) and more vomerine teeth (mean VT 13.6 in males and 15.6 in females of *C. chico* vs. 11.2 in males, and 12.4 in females of *C. arboreus*).

Chiropterotriton chico differs from *C. chondrostega* in the following characteristics: larger size (mean SVL 38.4 in males, 39.3 in females of *C. chico* vs. 23.0 in males, 25.4 in females of *C. chondrostega*), longer tail (mean TL/SVL 1.18 in males and 1.12 in females of *C. chico* vs. 0.92 in males and 1.07 in females of *C. chondrostega*), longer limbs (mean LI 0.6 in males and 2.1 in females of *C. chico* vs. 2.0 in males, 3.0 in females of *C. chondrostega*), longer head (mean HL 8.8 in males and 8.7 in females *C. chico* vs. 5.5 in males and 5.7 in females of *C. chondrostega*), broader head (mean HW 5.6 in males and 5.7 in females of *C. chico* vs. 3.4 in males and 3.7 in females of *C. chondrostega*), broader feet (mean FW 4.1 in males and 4.2 in females of *C. chico* vs. 1.8 in males and females of *C. chondrostega*), more maxillary teeth (mean MT+PMT 42.3 for males and 56.5 for females of *C. chico* vs. 22.5 for males and 40.9 for females of *C. chondrostega*), and more vomerine teeth (mean VT 13.6 in males and 15.6 in females of *C. chico* vs. 8 in males and 10 in females of *C. chondrostega*).

Chiropterotriton chico differs from *C. mosaueri* by its relatively shorter tail (mean TL/SVL 1.18 in males and 1.12 in females of *C. chico* vs. 1.31 in males and 1.39 in a female of *C. mosaueri*), narrower head (mean HW 5.6 in males and 5.7 in females of *C. chico* vs. 6.8 in males and 7.0 in a female of *C. mosaueri*), fewer maxillary and premaxillary teeth (mean MT+PMT 42.3 for males and 56.5 for females of *C. chico* vs. mean MT 69.0 for males and 70 for a female of *C. mosaueri*), and fewer vomerine teeth (mean VT 13.6 in males and 15.6 in females of *C. chico* vs. 28 in males and 20 in a female of *C. mosaueri*) (Woodall 1941; Rabb 1958; Rovito & Parra-Olea 2015).

Chiropterotriton chico differs from *C. multidentatus,* by its larger size (mean SVL 38.4 in males, 39.3 in females of *C. chico* vs. 33.6 in males, 34.0 in females of *C. multidentatus*), slightly longer tail (mean TL/SVL 1.18 in males, and 1.12 in females of *C. chico* vs. 1.13 in males and 1.03 in females of *C. multidentatus*), shorter limbs (mean LI 0.6 in males, 2.1 in females of *C. chico* vs. 0.1 in males, 1.0 in females of *C. multidentatus)*, longer head (mean HL 8.8 in males and 8.7 in females *C. chico* vs. 7.4 in both males and females of *C. multidentatus*), broader head (mean HW 5.6 in males and 5.7 in females of *C. chico* vs. 4.9 in males and 5.1 in females of *C. multidentatus*), broader feet (mean FW 4.1 in males and 4.2 in females of *C. chico* vs. 3.6 in males and 3.5 in females of *C. multidentatus*), and fewer maxillary and premaxillary teeth (mean MT+PMT 26.0 for males and 36.0 for females of *C. chico* vs. 40.0 for males and 49.0 for females of *C. multidentatus*).

Chiropterotriton chico differs from *C. cieloensis* by its larger size (mean SVL 38.4 in males, 39.3 in females of *C. chico* vs. 32.6 in males, 31.1 in females of *C. cieloensis*), shorter limbs (mean LI 0.6 in males, 2.1 in females of *C. chico* vs. -0.2 in males, 0.1 in females of *C. cieloensis)*, longer head (mean HL 8.8 in males and 8.7 in females of *C. chico* vs. 7.1 in males and 6.9 in females of *C. cieloensis*), broader head (mean HW 5.6 in males and 5.7 in females of *C. chico* vs. 4.8 in males and 4.9 in females of *C. cieloensis*), broader feet (mean FW 4.1 in males and 4.2 in females of *C. chico* vs. 3.2 in males and 3.1 in females of *C. cieloensis*); more maxillary and premaxillary teeth (mean MT+PMT 42.3 for males and 56.5 for females of *C. chico* vs. 31.0 for males and 47.0 for females of *C. cieloensis*), and more vomerine teeth (mean VT 13.6 in males and 15.6 in females of *C. chico* vs. 11.5 in males, and 12.8 in females of *C. cieloensis*).

Chiropterotriton chico differs from *C. infernalis* by its larger size (mean SVL 38.4 in males, 39.3 in females of *C. chico* vs. 36.4 in males, 29.7 in a female of *C. infernalis*), shorter limbs (mean LI 0.6 in males, 2.1 in females of *C. chico* vs. -0.7 in males, -0.5 in a female of *C. infernalis)*, less extensive feet webbing (moderate webbing that extends just onto penultimate phalanx in *C. chico* vs. extensive foot webbing onto penultimate phalange in *C. infernalis*; Fig. 3A, I), and slightly longer head (mean HL 8.8 in males and 8.7 in females *C. chico* vs. 8.2 in males and 6.2 in a female of *C. infernalis*).

Chiropterotriton chico differs from *C. cracens* in body size (mean SVL 38.4 in males, 39.3 in females of *C. chico* vs. 25.7 in males, 27.4 in females of *C. cracens*) and by having shorter limbs in males (mean LI 0.6 of *C. chico* vs. 1.8 of *C. cracens*), a longer head (mean HL 8.8 in males and 8.7 in females *C. chico* vs. 5.5 in males and 5.8 in females of *C. cracens*), broader head (mean HW 5.6 in males and 5.7 in females of *C. chico* vs. 3.8 in males and 4.1 in females of *C. cracens*), broader feet (mean FW 4.1 in males and 4.2 in females of *C. chico* vs. 2.2 in males and 2.5 in females of *C. cracens*), and more maxillary and premaxillary teeth (mean MT+PMT 42.3 for males and 56.5 for females of *C. chico* vs. 36.8 for males and 49.5 for females of *C. cracens*).

Chiropterotriton chico differs from its sympatric species *C. dimidiatus* by its larger size (mean SVL 38.4 in males, 39.3 in females of *C. chico* vs. 24.6 in males, 25.8 in females of *C. dimidiatus*), relatively longer tail (mean TL/SVL 1.18 in males, and 1.12 in females vs. 0.89 in males, and 0.86 in females of *C. dimidiatus*), longer limbs (mean LI 0.6 in males, 2.1 in females of *C. chico* vs. 3.8 in males, 4.9 in females of *C. dimidiatus*), longer head (mean HL 8.8 in males and 8.7 in females of *C. chico* vs. 5.2 in males and 5.0 in females of *C. dimidiatus*), broader head (mean HW 5.6 in males and 5.7 in females of *C. chico* vs. 3.4 in males and 3.5 in females of *C. dimidiatus*), broader feet (mean FW 4.1 in males and 4.2 in females of *C. chico* vs. 1.7 in both males and females of *C. dimidiatus*), more numerous maxillary and premaxillary teeth (mean MT+PMT 42.3 for males and 56.5 for females of *C. chico* vs. 9.4 for males and 34.4 for females of *C. dimidiatus*), and vomerine teeth (mean VT 13.6 in males and 15.6 in females of *C. chico* vs. 5.6 in males, and 8.3 in females of *C. dimidiatus*). Moreover, *C. chico* is easily distinguished for relative narrow nostril size (mean NW/HW 0.01 in both males and females of *C. chico* vs. 0.07 in both males and females of *C. dimidiatus*), this character is evident even in very small individuals.

Chiropterotriton chico differs from most of the remaining species of the genus, in having relatively large size (mean SVL 38.4 males, 39.3 females). Exceptions are the much stouter and somewhat larger *C. priscus* Rabb (mean SVL 38.5 males, 41.8 females; Rovito & Parra-Olea 2015) and *C. magnipes* Rabb (range SVL 40–53 males, 51–60 females; Campbell *et al.* 2014). *C. chico* has more maxillary teeth than most other species of this genus (mean MT+PMT 42.3 for males and 56.5 for females), except for *C. multidentatus* (mean MT+PMT 40.0 for males and 47.8 for females; Rovito & Parra-Olea 2015) and *C. infernalis* (mean MT+PMT 42.3 for males and 56.5 for females; Rovito & Parra-Olea 2015) which have similar numbers of teeth, and *C. magnipes* (males average 79; Campbell *et al.* 2014) and *C. mosaueri* (range in males 56–69; Campbell *et al.* 2014), which have more maxillary teeth.

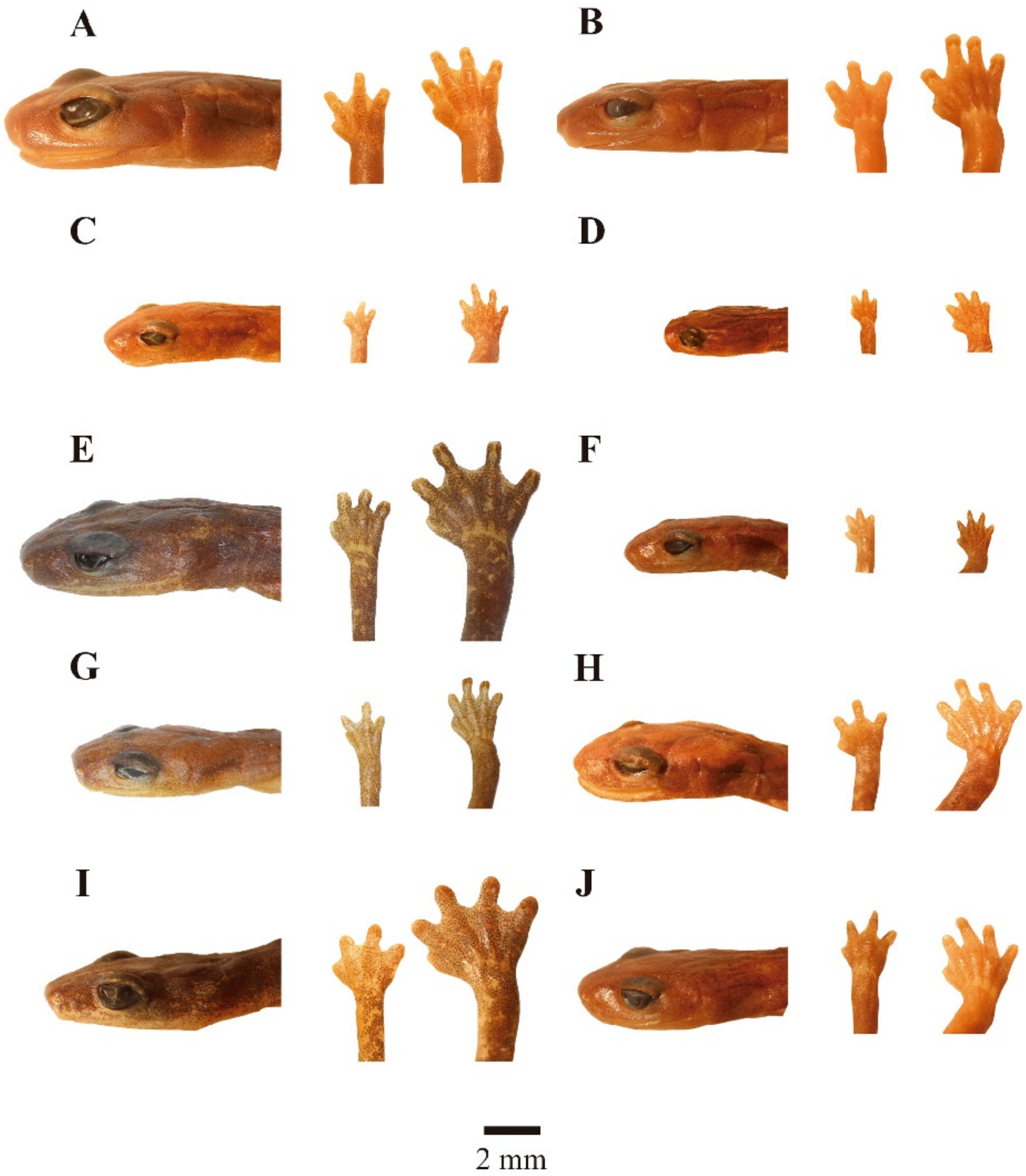

FIGURE 3. Head, hand and foot morphology of *Chiropterotriton* specimens from Hidalgo **A)** *C. chico,* holotype MVZ 118888, **B)** *C. arboreus,* MVZ 172159, **C)** *C. chondrostega* MVZ 106660, **D)** *C. dimidiatus* MVZ 178671, **E)** *C. mosaueri* IBH 28179, **F)** *C. terrestris,* MVZ 106700, and Tamaulipas and San Luis Potosí **G)** *C. cieloensis,* holotype IBH 29561, **H)** *C. cracens* MVZ 129005, **I)** *C. infernalis,* holotype MVZ 269665, **J)** *C. multidentatus* MVZ 163908. Scale bar corresponds to 2 mm.

FIGURE 4. Photos in life of *Chiropterotriton.* **A)** *Chiropterotriton chico,* DBW (Jan 1974) **B)** *C. arboreus,* DBW, **C)** *C. chondrostega,* SMR (IBH 28195), **D)** *C. dimidiatus,* DBW 1342-45, **E)** *C. mosaueri* SMR (IBH 28179), **F)** *C. terrestris,* DBW, **G)** *C. cieloensis,* SMR holotype (IBH 29561), **H)** *C. cracens,* SMR (IBH 28192), **I)** *C. infernalis,* SMR holotype (MVZ 269665), **J)** *C. multidentatus* SMR (IBH 29571).

Description. This is a medium sized species of *Chiropterotriton*; mean SVL 38.4 in fifteen adult males (range 36.2–42.6) and 39.3 in fifteen adult females (range 35.9–44.3). The head is relatively narrow and moderately long (Fig. 3), HW averages 14% of SVL in both males and females (range 11–16). In males, the snout is broad and truncated. Jaw muscles are pronounced and visible as a bulging mass immediately behind the eyes. Eyes are moderately protuberant and extend laterally beyond the jaw margin in ventral view. There are a moderate number of maxillary and premaxillary teeth in males (mean MT+PMT 42.3, range 32–52) and more in females (mean MT+PMT 56.5, range 48–67). There are few vomerine teeth in males (mean 13.6, range 10–19) and females (mean 15.6, range 13–19). The tail is long; mean TL equals 1.18 of SVL in males (range 0.96–1.27) and 1.12 of SVL in females (range 0.98–1.24). Limbs are moderate and slender; (FLL+ HLL)/SVL average 0.48 in males (range 0.82–1.11) and 0.98 in females (range 0.87–1.13). Adpressed limbs approach closely on males (mean LI 0.6, range 0–1) but they are separated by as many as two costal folds in females (mean 2.1, range 1.5–3.0). Digits are slender and expanded distally, with distinct subterminal pads and moderate webbing at the base. All digits are discrete, however the first barely extends beyond the margins of the webbing and it extends just onto penultimate phalanx of the longest toe (Fig. 3). The outermost toes are particularly well developed. The smallest male with a mental gland is 36.2 SVL. Parotoid glands not evident. Prominent oval-shaped mental gland is present in all adult males. Vomerine teeth arranged in a well-defined line extending to outer margin of choanae. Digits in order of increasing length: hand I-IV-II-III, foot I-V-II-IV-III. Phalangeal formulae: hand 1-2-3-2, foot 1-2-3-3-2.

Coloration of the holotype in alcohol. Holotype uniform dark tannish brown dorsally becoming paler laterally and very pale cream ventrally. There is a central reddish brown stripe flanked by yellow lines. The stripe extends from to the postocular region, where it has yellow spots, to the base of the tail where it also leaves two yellow spots. The entire tail is a slightly darker brown than the body. Limbs are lighter in color than dorsum but still dark brown. Snout is mottled with dark and light brown. Dorsal surface of hands and feet only slightly lighter than body.

Measurements of the holotype (in mm), tooth counts and limb interval.— SVL 42.2, TL 46.1, AX 22.4, SW 5.2, HL 9.8, HW 6.3, HD 3.1, projection of snout beyond mandible 1.4, anterior rim of orbit to snout 2.7, interorbital distance 2.6, eyelid length 2.2, eyelid width 1.4, horizontal orbit diameter 2.7, NL 0.14, NW 0.06, FLL 8.5, HLL 10.7, snout to forelimb 12.8, snout to anterior angle of vent 42.1, tail width at base 3.1, tail depth at base 3.2, FW 4.0, length of fifth toe 0.7, length of third (longest) toe 1.2. MT+PMT 60, VT 6-7 (right-left sides). Adpressed limbs separated by two costal folds.

Habitat and range. This species is only known from Parque Nacional El Chico in Hidalgo, Mexico at an elevation between 2400 and 3050 m, in pine-oak forest. It is unlikely to occur more widely, because surrounding areas have been extensively surveyed.

Etymology. The species name *chico* is in reference to the national park where the species occurs. Parque Nacional El Chico in Hidalgo, Mexico has been a protected area since 1922. The species name is used as an invariable noun in apposition to the generic name.

Remarks. This species was previously considered as conspecific with *C. multidentatus* and occurs in sympatry with *C. dimidiatus* and *Aquiloeurycea cephalica*. Likewise, *Isthmura bellii* has been collected very near sites where *C. chico* was once common (MVZ 118953, 118954, 128978), but it is unknown if the two species occur in syntopy.

Discussion

Molecular tools have been of great help in uncovering a large diversity of species that were missed using only traditional (morphological) approaches because of the outward similarity of species. For Mexican salamanders, there has been a steady pace of species description leading to an increase of about 55% in the known diversity in the last 24 years (Flores-Villela & Canseco-Marquez 2004; AmphibiaWeb 2017). This study contributes with the description of one species, increasing the number of *Chiropterotriton* species from 15 to 16. Historically, this genus has been considered to represent a relatively small number of species despite the multiple undescribed taxa proposed in previous studies (Rabb 1958; Wake & Lynch 1976; Darda 1994; Parra-Olea 2003). Recently, three new species have been described with morphological comparisons (Campbell *et al.* 2014) and also molecular phylogenetic analyses (Rovito & Parra-Olea 2015), but at least seven undescribed taxa remain: *C.* sp. C, *C.* sp. H, and *C.* sp. I from Veracruz; *C.* sp. F and *C.* sp. G from Puebla; and *C.* sp. J and *C.* sp. K from Oaxaca.

Chiropterotriton chico was first referenced by Taylor (1938) who listed specimens from El Chico, Hidalgo as paratypes of *Oedipus multidentata.* Later, they were assigned to *C. multidentatus* by Taylor (1944) as its southernmost population. Rabb (1958) noted morphological differences between this population and those from Tamaulipas. Allozyme evidence (Darda 1994) identified the El Chico population as a new species (*C.* sp. 15, population 15) and Parra-Olea (2003) agreed, based on her mtDNA phylogeny (*C.* sp. 15, population 12). Although *C. chico* is the sister species of *C. terrestris,* they are easily differentiated by morphological characters. The body size of *C. chico* makes it one of the largest species of this genus; only *C. magnipes, C. mosaueri* and *C. priscus* are larger.

From the 1950s into the 1970s, *Chiropterotriton chico* was incredibly abundant locally. D. Wake visited the park in August 1971 (field notes, Museum of Vertebrate Zoology), and recorded a very high density of salamanders. At high elevation (3000 m) salamanders were found under roadside rocks on barren soil. A salamander was found under every 10th to 12th rock and more than 75 *C. chico* were seen (as well as 10 *C. dimidiatus*). At lower elevation (estimated from topographic maps as 2400 m), in oak forest, Wake wrote: "I have never seen anything like the density of salamanders encountered here! I'm sure we could have collected thousands if we had wished". Any shelter had salamanders, and it was a rare log than had none. Only five bromeliads were opened but one contained a *C. chico*. Many cover objects housed multiple specimens: seven under a log 1.2 m (four feet) long, twelve under a log 3.0 m (ten feet) long, ten under a flat piece of wood (0.3x0.6 m in dimensions), etc. Yet, at another site less than 4 km away, no salamanders were found.

High numbers were encountered on other trips, but by the mid-1970s salamanders became uncommon and then rare. Apparently, the decline of *C. chico* is not related to habitat loss or disturbance. Parra-Olea *et al.* (1999) did not report important changes in habitat quality or availability in El Chico National Park, however, they found a lower density of salamanders than observed in the 1970s. Parra-Olea found one individual of *C. chico* and two of *C. dimidiatus* in 2002 while S. Rovito found one of *C. chico* and several for C. *dimidiatus* in 2016. While these declines are not well understood, one suggested factor is the arrival of the pathogenic chytrid fungus *Batrachochytrium dendrobatidis*. The fungus was absent in a sample of 30 individuals sampled in 1972, but present in a sample of 40 from 1974 (Cheng *et al*. 2011).

Given the standard criteria used by the International Union for the Conservation of Nature for its assessment of species to be added to the Red List of Threatened Species (IUCN 2017), we think that a listing of Critically Endangered is warranted for *C. chico* according to criterion B1 ab(v), where B1 corresponds to the geographic range that is estimated to be less than 100 km^2, at only a single location (a) and continuing decline (b) for number of mature individuals (v). The species has declined severely in abundance, with declines exceeding 80% based on the number of individuals seen in field surveys, in the limited area from which it is known. Extensive exploration has taken place in the immediate vicinity of the park and throughout much of the state of Hidalgo, but no additional populations have been found.

Hidalgo includes an unusual region where two of the main mountain complexes of Mexico meet: the Trans-Mexican Volcanic Belt (TMVB) and Sierra Madre Oriental (SMO). These ranges are known to have a high degree of topographic, geologic and climatic variability than has promoted a high biodiversity (Luna *et al.* 2004; Luna *et al.* 2007). This is especially true for the herpetofauna from Hidalgo (Lemos-Espinal & Smith 2015), which is the state with the highest number of species of *Chiropterotriton* (6), representing 37.5% of the described species of this genus.

Acknowledgments

This study will be submitted by Mirna G. Garcia-Castillo in partial fulfillment of the requirements to obtain the degree of *Doctor en Ciencias* in the *Posgrado en Ciencias Biológicas*, *Universidad Nacional Autónoma de México*. We thank Carol Spencer for help provided in the Museum of Vertebrate Zoology for specimen measurements. Rocío Alanís-Anaya helped with map design. This research was supported by grants from the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT-UNAM) IN209914 and UC MEXUS-CONACyT to GPO. MGC thanks the support of the "Programa de Posgrado en Ciencias Biológicas, UNAM" and CONACyT (CVU/Becario: 413761/262662) for granting a scholarship to complete her PhD program. This paper was written during the sabbatical leave of GPO to the University of Otago, New Zealand. Thanks are due to Dr. Phil Bishop for facilities provided in his laboratory, and to DGAPA-UNAM and CONACyT for their support through their sabbatical programs.

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APPENDIX 1. Specimens examined for morphological analysis.

Chiropterotriton arboreus: Mexico, Hidalgo: MVZ 106381, 11 km S Tianguistengo (1 km N Zacualtipán); MVZ 114333–114335, 2.4 km SSW (by road) Tianguistengo along road to Zacualtipán; MVZ 163917, 163919, 172159, 172160, 172162–172163, 3.4 km S Tianguistengo; MVZ 200621, 200623–200625, 4.3 km E of junction of Mexico Hwy. 105 and Old Hwy. to Tianguistengo.

Chiropterotriton chico: Mexico, Hidalgo: MVZ 114436, El Chico Parque Nacional, 4 km S (by road) Mineral del Chico; MVZ 118740, 118752, 118791, 118793, 118798, 118800, 118804, 118811, 118816, 118821, 118827, 118842, 118869, 118888, 118900–118901, 118905, 119016, 119034, 119053, 119057, 119062, 119078, 119166, 119179, 119193, 119212, 119216, El Chico Parque Nacional; MVZ 138852, road to El Chico National Park, 1.0 km N (by road) junction Hwy. 105. *Chiropterotriton chondrostega*: Mexico, Hidalgo: MVZ 106656–106657, 106659–106666, 11 km S Tianguistengo, ca. 1 km N Zacualtipán; MVZ 163987–163988, 172148–172154, 172156–172157, 4 km NNE Zacaultipan.

Chiropterotriton dimidiatus: Mexico, Hidalgo: MVZ 98939, 98942, 98947, El Chico Parque Nacional, 9.7 mi (by winding road) from Hwy. 105 and 5.5 mi (by winding road) from Park Custodian house; MVZ 103961–103963, 103968, El Chico Parque Nacional, N slope along Rd. between summit and town of El Chico; MVZ 106460, 106465, 106468, 106471, 106474, 106493, 106495, 106514–106515, El Chico Parque Nacional, 4 km S Mineral del Chico; MVZ 114248, 114251, 114261, 114265, 114268, 114274, 114291–114292, El Chico Parque Nacional, between Park Headquarters and Mineral del Chico; MVZ 118727, 118729, 118732, 118735, 185962, 195832, El Chico Parque Nacional.

Chiropterotriton terrestris: Mexico, Hidalgo: MVZ 106668–106669, 106672, 106677–106678, 106681, 106683, 106685, 106690–106692, 106694–106695, 106700, 106705–106706, 106712–106713, 106718–106720, 106722, 106724, 11 km S Tianguistengo, ca. 1 km N Zacualtipán; MVZ 114302, 114304, 114316, 114339, 114343, 114345, 12.8 km SSW (by road) Tianguistengo (along road to Zacaultipan); MVZ 200636, 4.3 km E of junction of Mexico Hwy. 105 and Old Hwy. to Tianguistengo.

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Amphibian & Reptile Conservation
12(2) [Special Section]: 37–54 (e167).

urn:lsid:zoobank.org:pub:440CB3D6-450A-463B-B3D3-1CCBCBD8670E

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

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***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

Citation: García-Castillo MG, Soto-Pozos ÁF, Aguilar-López JL, Pineda E, Parra-Olea G. 2018. Two new species of *Chiropterotriton* (Caudata: Plethodontidae) from central Veracruz, Mexico. *Amphibian & Reptile Conservation* 12(2) [Special Section]: 37–54 (e167).

Received: 30 October 2018; **Accepted:** 01 December 2018; **Published:** 31 December 2018

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

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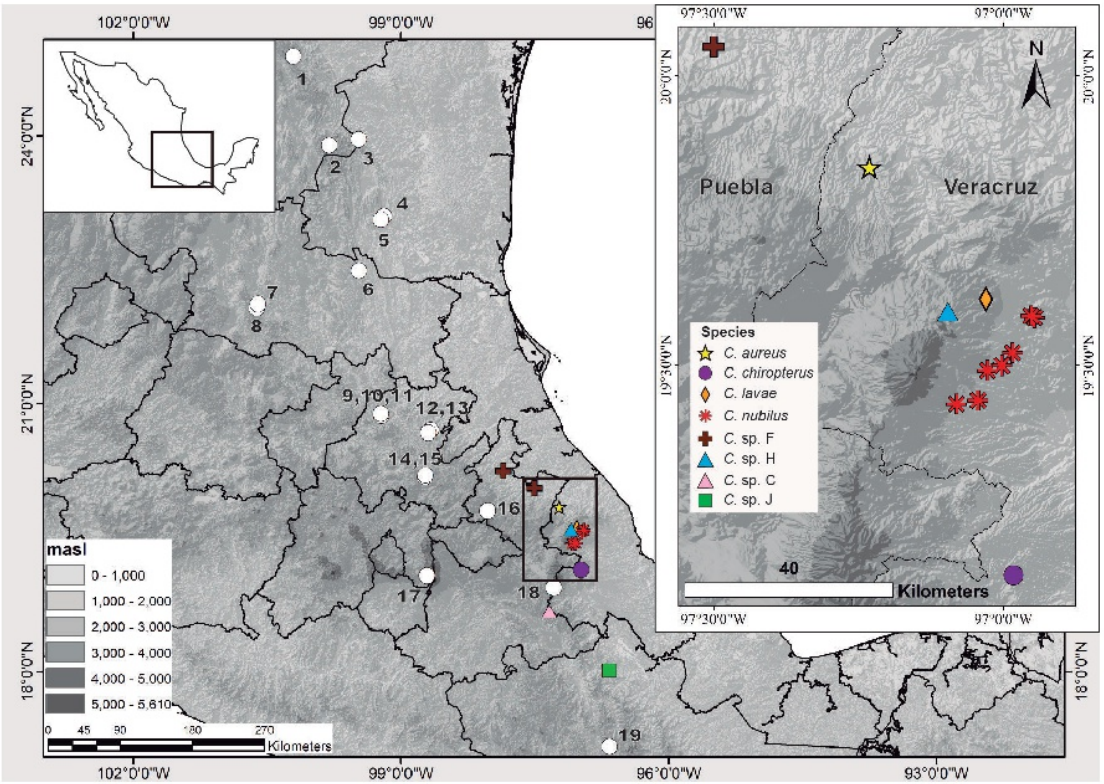

Fig. 1. Map of sampled localities for phylogenetic analyses of the genus *Chiropterotriton*. White circles correspond to: **1)** *C. priscus*, **2)** *C. miquihuanus*, **3)** *C. infernalis*, **4)** *C. cieloensis*, **5)** *C. cracens*, **6)** *C. multidentatus* (Cd. Maiz), **7)** *C. multidentatus* (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 °C (120 s), 55 °C (60 s), and 72 °C (300 s), and COI, 35 cycles of 94 °C (30 s), 50 °C (30 s), and 72 °C (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 Potosí	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 2nd 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.

urn:lsid:zoobank.org:act:A288BF9A-589E-42D5-8675-2AA9E6E55865

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).

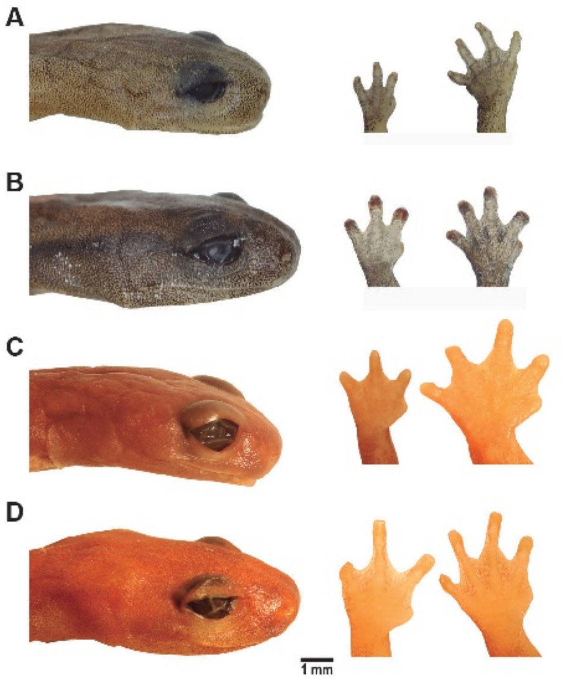

Fig. 3. Head, hand, and foot morphology of preserved specimens of *Chiropterotriton* species from central Veracruz. Ventral view from right hand and foot. **A)** *C. aureus* holotype IBH 31042, **B)** *C. nubilus* holotype IBH 31048, **C)** *C. lavae* MVZ 106436, and **D)** *C. chiropterus* MVZ 85590.

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.

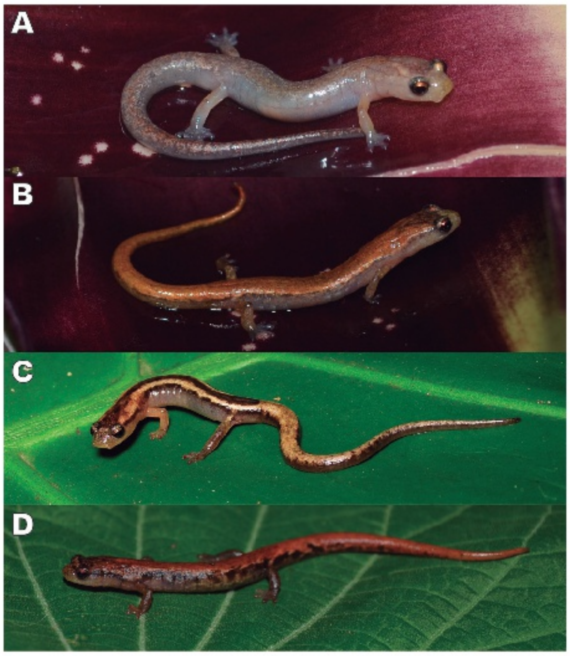

Fig. 4. Photos in life of two new species from central Veracruz. **A)** *C. aureus* (male) holotype IBH 31042, **B)** *C. aureus* (female) paratype IBH 31044, **C)** *C. nubilus* (male) paratype CARIE 0739, and **D)** *C. nubilus* (female) holotype IBH 31048. *Photo credit: Maria Delia Basanta (A, B,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

Chiropterotriton nubilus sp. nov.

urn:lsid:zoobank.org:act:F785D7FD-301F-4D53-BD7F-AC680A0BB875

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

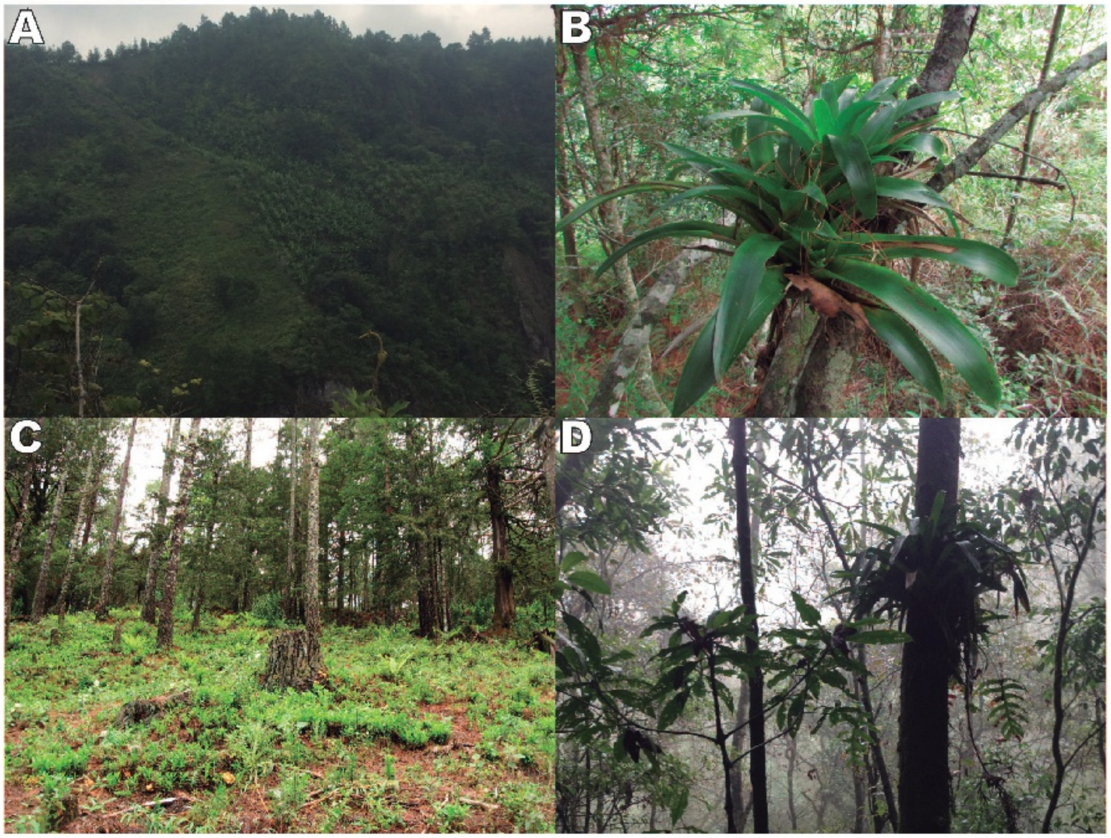

Fig. 5. Microhabitat and landscape photographs for new species from central Veracruz. **A)** Landscape from type locality of *C. aureus* (Atzalan, Veracruz), **B)** bromeliad from type locality of *C. aureus*, **C)** view of type locality of *C. nubilus* (Coxmatla, Veracruz), and **D)** bromeliad from 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.

Acknowledgements.—MGGC thanks the Posgrado en Ciencias Biológicas program, Universidad Nacional Autónoma de México, and CONACyT for a scholarship grant (CVU/Becario: 413761/262662). A supporting grant for our research was kindly provided by Programa de Apoyo a Proyectos de Investigación e Innnovación Tecnológica (PAPIIT-UNAM) IN203617. We also would like to thank Laura Márquez-Valdemar and Andrea Jimenez for assisting us with laboratory work. Special thanks to Aldo López-Velázquez for the excellent photography instruction and the use of his equipment. We thank Jean H. Raffaëlli, David Darda, and an anonymous reviewer who all made valuable comments to improve our manuscript. Finally, we thank Omar Becerra-Soria, Fabiola A. Herrera-Balcázar, Adriana Sandoval-Comte, and Flor Vázquez for field assistance and the Soto-Pozos family for providing lodging in Teocelo, Veracruz. Collection permits were provided by the Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT): SGPA/DGVS/00947/16, SGPA/DGVS/03038/17 and FAUT-0303.

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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.

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Revision of the genus *Chiropterotriton* with description of 5 new species (Caudata: Plethodontidae)

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Abstract**:** *Chiropterotriton* is a salamander genus endemic to Mexico with a geographical distribution along the Sierra Madre Oriental, Trans Mexican Volcanic Belt and northern Oaxaca. The recent use of molecular tools has shown that Mexico's amphibian diversity is highly underestimated. *Chiropterotriton* has 16 described species including terrestrial, arboreal and cave dweller species. However, according to previous studies there are several undescribed species. We present a phylogenetic hypothesis based on two mitochondrial markers (L2 and COI), which includes all described species and four new species. We implemented Maximum Likelihood and Bayesian analysis on 71 sequences of mtDNA and we measured a total of 124 individuals for statistical morphometrics. Based on morphological and molecular data, we describe five new species and re-describe three species of *Chiropterotriton.* By increasing the species diversity of the genus from 16 to 21, this study represents a considerable contribution to Mexican plethodontid richness.

Key words: plethodontids, phylogeny, taxonomy, Mexico, bolitoglossines

INTRODUCTION

Salamanders of the genus *Chiropterotriton* are one of the main components of the large endemic plethodontid fauna of México. This genus occurs from the highlands of Nuevo León and Tamaulipas, southward along the Sierra Madre Oriental, across the Trans

Mexican Volcanic Belt, and into the cloud forests of southwestern Veracruz and northern Oaxaca (Casas-Andreu et al., 1996; Darda, 1994; Wake, 1987). The taxonomy of the group has been stable for many years, with only four species recently described (Campbell et al., 2014, Rovito and Parra-Olea, 2015, García-Castillo et al 2017), even though the existence of several more undescribed taxa has been known for decades (Wake and Lynch, 1976, Darda 1994).

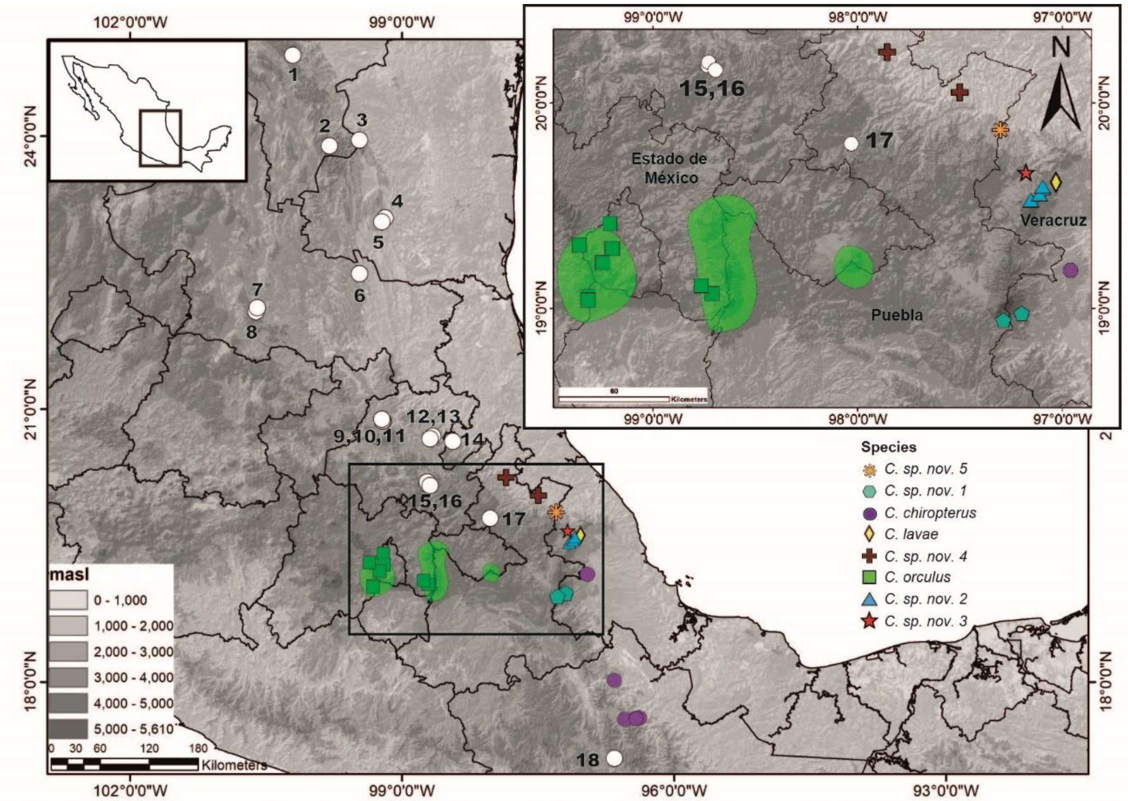
Results from molecular analyses based on protein electrophoresis (Darda, 1994) and mitochondrial DNA (mtDNA) sequences (Parra-Olea 2003, Rovito and Parra-Olea 2015, Garcia-Castillo et al 2017) find support for two geographically discrete clades, a northern and a southern clade. The northern clade is formed by 12 species that occur from central Mexico in the state of Hidalgo to the northern states of Nuevo Leon and Tamaulipas while the southern clade is formed by four species, plus seven previously recognized undescribed taxa that occur also from the central state of Hidalgo southwards to the state of Oaxaca (Figure 1). The southern clade contains two species complexes: *C. chiropterus* and *C. orculus*.

Chiropterotriton chiropterus complex has suffered from taxonomic re-arrangements, mostly due to imprecise type localities and the lack of adequate samples from those localities. Based on external morphology, Wake and Lynch (1976) defined the *chiropterus* group as comprising *C. chiropterus, C. chondrostega, C. dimidiatus* and *C. lavae*. Later, on the basis of immunological data, Maxson and Wake (1981) recognized the *chiropterus* group as consisting only of *C. chiropterus* and *C. lavae*. Based on allozyme data, Darda (1994) recognized a group of populations found along the Trans Mexican Volcanic Belt, which he called the *chiropterus* complex. This group was formed by *C. chiropterus* from La Joya Veracruz, *C. orculus* from Zacualtipan, Hidalgo, and nine additional undescribed species. However, Parra-Olea (2003) concluded that *C. chiropterus* applies exclusively to the low-elevation populations located in or near the Huatusco region.

Chiropterotriton orculus complex is represented by a relatively widespread species of the genus. Based on morphological characters, *C. orculus* was described by Cope (1865) as *Spelerpes orculus* from Mexican Table Land, but four years later was placed in synonymy with *C. chiropterus* (Cope, 1989). The allozyme data gave species status to *C. orculus,* which was contained in two populations (Darda 1994). Parra-Olea (2003) added one more population to *C. orculus,* and emphasized the differentiation levels discordance with mtDNA between populations. In this way, *C. orculus* is characterized by several morphological uniform populations around Mexico City.

In this paper, we present a phylogenetic hypothesis based on mtDNA, which includes all 16 described species plus 7 undescribed taxa, including all the populations identified in previous studies as new species within complexes. Based on the molecular and morphological data, we describe 5 new species of the genus *Chiropterotriton.* These increase the number of described species from 16 to 21 and still recognize two candidate species yet undescribed. In order to make morphological comparison between species of the complex we re-describe three species.

Figure 1.



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 (Zacualtipán), 14.- C. arboreus (Zilacatipán), 15.- C. dimidiatus, 16.- C. chico, 17.- C. sp. G, 18.- C. sp K.

MATERIAL AND METHODS

Amplification and sequencing

Whole genomic DNA was extracted from liver, intestine or tail tissue using DNeasy tissue Kit (Qiagen). Although a full molecular analysis of the genus *Chiropterotriton* is beyond the scope of the present work, several mitochondrial sequences were generated in order to compare the new species (when available) to other members of the genus. PCR amplification was done using primers LX12SN1 and LX16S1R for mitochondrial fragment L2; it includes partial sequences from the 12S ribosomal subunit, the tRNA and the large subunit16S (Zhang *et al.* 2008). PCR reaction were as follow: 35 cycles of 96ºC (2 min), 55ºC (1 min) and 72ºC (5 min). We also amplificated another mitochondrial fragment using primers dgLCO y dgHCO for COI (Meyer 2003). PCR conditions were as follow: 35 cycles of 94ºC (30 s), 50ºC (30 s) and 72ºC (45 s). We cleaned PCR products with ExoSap-IT (USB Corporation, Cleveland, OH) and sequencing reaction with BigDye Terminator v3.1 cycle kit (Applied Biosystems, Foster City, CA). The products were purified using Sephadex G-50 (GE Heathcare) and run on an ABI3730 capillary sequencer in Instituto de Biología, UNAM.

Sequence alignment and phylogenetical analyses

Editing and assembly of sequences were performed in Sequencher 5.0.1 (Gene Codes Corporation). We used Muscle 3.8 (Edgar, 2004) to align L2 and COI sequences. The alignment from ribosomal fragment (L2) included 34 *Chiropterotriton* individuals sequenced in this study, 35 sequences available from GenBank and two additional sequences from *Aquiloeurycea cephalica* and *Thorius* as outgroups. The alignment for COI included seven sequences from this study and 17 from Genbank (Table 1). We used Mesquite v3.40 (Maddison & Maddison 2018) to concatenate and review the data matrix; missing base pairs were coded as missing data (?). We used PartitionFinder v1.0 (Lanfear *et al.* 2012) to determine best-fit substitution models and a partitioning scheme using the Bayesian Information Criterion (BIC). We ran Maximum Likelihood and Bayesian inference phylogenetical analyses throught CIPRES data portal (Miller *et al.* 2010). We run RAxML v8.2 (Stamatakis 2014) to infer a Maximum Likelihood tree, with 1000 bootstrap replicates for assessing nodal support. We run MrBayes v3.2 (Ronquist *et al.* 2012) for

Bayesian inference analysis, with 20 million of generations, sampling every 1000 generations, with four chains to obtain a majority consensus tree. Tracer v.1.7 (Rambaut *et al.* 2018) was used to assess the convergence and stability of the chains.

Table 1. Voucher information and GenBank numbers for the specimens of *Chiropterotriton* used in this study. *** Not available for phylogenetic analysis**

Species	**Voucher Number**	**Locality**	**L2/16S Genbank**	**COI Genbank**
C. arboreus	IBH28191	Hidalgo: 6.8 km SW (by rd) of Zacualtipan on road to Tianguistengo	XXXXX	XXXXX
C. arboreus	IBH22847	Veracruz: 3.2 km S Zilacatipan		_
**C. sp. nov. 5*	MVZ92874	Veracruz: 13 mi SW Tlapacoyan	_	_
C. sp. nov. 1	IBH30987	Veracruz: 1.1 km N Xometla		
C. sp. nov. 1	IBH30988	Veracruz: 1.1 km N Xometla		
C. sp. nov. 1	MVZ201387	Puebla: Santa Cruz de Texmalaquilla	AY522488	_
C. sp. nov. 1	MVZ201389	Puebla: Santa Cruz de Texmalaquilla	AY522487	_
C. chico	MVZ200679	Hidalgo: 3.8 km S Mineral del Chico	AY522471	_
C. chiropterus	CARIE0777	Veracruz: Huatusco	XXXXX	XXXXX
C. chiropterus	CARIE0719	Veracruz: Huatusco	XXXXX	_
C. chiropterus	IBH22736	Oaxaca: San Bernardo, ca. 5km SW (by rd) of La Esperanza on MX 175		_
C. chiropterus	IBH30088	Oaxaca: ca. 400 m from MX175 on road to San Isidro Yolox		_
C. chiropterus	IBH22550	Oaxaca: La Galera, 11.0km SW (by rd) of La Esperanza on MX175		_
C. chiropterus	IBH30099	Oaxaca: San Bernardo, 4.8 km SW (by rd) of La Esperanza on MX177	XXXXX	XXXXX
C. chiropterus	GP088	Oaxaca: 67 Km N Guelatao, trail to San Isidro, La Esperanza	AY522490	_
C. chondrostega	IBH28195	Hidalgo: 1.0 km S (by rd) of La Encarnacion on road to MX85, Parque Nacional los Marmoles		_
C. chondrostega	IBH30098	Hidalgo: 1.0 km S (by rd) of La Encarnacion on road to MX85, Parque Nacional los Marmoles	XXXXX	XXXXX
C. cieloensis	IBH28190	Tamaulipas: 0.2 km E (by air) of Rancho El Cielo, 6.9 km NNW (by air) of center of Gomez Farías, Reserva de la Biosfera El Cielo		_

C. cieloensis	IBH28181	Tamaulipas: 0.2 km E (by air) of Rancho El Cielo, 6.9 km NNW (by air) of center of Gomez Farías, Reserva de la Biosfera El Cielo	XXXXX	XXXXX
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	XXXXX	XXXXX
C. dimidiatus	IBH28196	Hidalgo: 4.1km S (by rd) of Mineral del Chico on road to Pachuca, Parque Nacional El Chico	XXXXX	XXXXX
C. dimidiatus	IBH22344	Hidalgo: 4.3 km N hwy 105 at Mineral del Monte.		_
C. infernalis	MVZ269665	Tamaulipas: Cueva del Brinco, Conrado Castillo, ca. 43.5 km SW (by rd) of Ejido Guayabas	XXXXX	XXXXX
C. infernalis	IBH29575	Tamaulipas: Conrado Castillo, ca. 43.5 km SW (by rd) of Ejido Guayabas		
C. lavae	IBH22360	Veracruz: 200 m N hwy 140 at La Joya		_
C. lavae	IBH22351	Veracruz: 200 m N hwy 140 at La Joya		_
C. lavae	IBH22369	Veracruz: 200 m N hwy 140 at La Joya	XXXXX	XXXXX
C. lavae	IBH22349	Veracruz: 200 m N hwy 140 at La Joya		_
C. magnipes	IBH30093	Hidalgo: "El Coní", 900m SSE of center of Durango, Municipio Zimapan, Parque Nacional los Marmoles.		_
C. magnipes	IBH28176	Hidalgo: "El Coní", 900m SSE of center of Durango, Municipio Zimapan, Parque Nacional los Marmoles.	XXXXX	XXXXX
C. sp. nov. 4	IBH30112	Puebla: 7.1 km N (by rd) of center of Cuetzalan on road to Yohualichán	XXXXX	XXXXX
C. sp. nov. 4	MVZ178706	Puebla: 3.9km S Xicotepec de Juárez	AY522477	_
C. sp. nov. 4	MVZ200723	Puebla: Xicotepec de Juárez	AY522478	_
C. miquihuanus	IBH30329	Nuevo León: 1.8km S (by rd) of La Encantada on road from La Bolsa to Zaragoza	XXXXX	XXXXX
C. miquihuanus	IBH30330	Nuevo León: 22.6 km N (by rd) of La Bolsa on road to Zaragoza		_
C. mosaueri	IBH28179	Hidalgo: "El Coní", 900m SSE of center of Durango, Municipio Zimapan, Parque Nacional los Marmoles	XXXXX	XXXXX
C. multidentatus	IBH28177	San Luis Potosí: Cueva el Madroño, 900m NW (by air) of entrance to Valle de los Fantasmas on MX70, Sierra de Alvarez	XXXXX	_
C. multidentatus	IBH30102	San Luis Potosí: Cueva el Madroño, 900m NW (by air) of entrance to Valle de los Fantasmas on MX70, Sierra de Alvarez	XXXXX	_

C. multidentatus	IBH28193	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	XXXXX	_
C. multidentatus	IBH30104	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	XXXXX	_
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	XXXXX	_
C. multidentatus	IBH23111	San Luis Potosí: Rancho Borbortón	XXXXX	_
C. orculus	IBH30765	Estado de México: Amecameca, road to Popocatepetl volcano	XXXXX	XXXXX
C. orculus	IBH30943	Estado de México: Amecameca, road to Popocatepetl volcano	XXXXX	XXXXX
C. orculus	IBH30746	Estado de México: Amecameca, road to Popocatepetl volcano		_
C. orculus	IBH22866	Estado de México: Amecameca, road to Popocatepetl volcano		_
C. orculus	IBH22210	Ciudad de Mexico: Colonia Prolongación Miguel Hidalgo		_
C. orculus	AMH300	Ciudad de Mexico: Desierto de los Leones		_
C. orculus		Ciudad de Mexico: Bosque de Tlalpan		_
C. orculus	IBH29851	Morelos: Parque Nacional Lagunas de Zempoala		_
C. orculus	GP2039	Morelos: Parque Nacional Lagunas de Zempoala		_
C. orculus	IBH26478	Ciudad de Mexico: El Ajusco, km 29.4 from Picacho-Ajusco road.		_
C. orculus	MVZ138672	Ciudad de Mexico: Desierto de Los Leones Nat'l Park, 8.8 km [Rd.] SW La Venta by Mexico Hwy. 15	AY522442	_
C. sp. nov. 2	IBH23066	Veracruz: 15.9 km on microondas road, Las Vigas, Ver		_
C. sp. nov. 2	IBH22395	Veracruz: 15.9 km on microondas road, Las Vigas, Ver		_
C. sp. nov. 2	IBH31032	Veracruz: Conejo, road to the peak of Cofre de Perote		_
C. sp. nov. 2	IBH31033	Veracruz: Conejo, road to the peak of Cofre de Perote		_
C. sp. nov. 2	IBH31034	Veracruz: Conejo, road to the peak of Cofre de Perote		_

C. sp. nov. 2	IBH31035	Veracruz: Conejo, road to the peak of Cofre de Perote		
C. sp. nov. 2	IBH31036	Veracruz: Conejo, road to the peak of Cofre de Perote		_
C. sp. nov. 2	IBH31037	Veracruz: 2km (by air) al NE de LLanillo redondo camino a Valle Alegre.		_
C. sp. nov. 2	IBH31038	Veracruz: 2km (by air) al NE de LLanillo redondo camino a Valle Alegre.		_
C. sp. nov. 2	IBH31039	Veracruz: 2km (by air) al NE de LLanillo redondo camino a Valle Alegre.		
C. sp. nov. 2	IBH22568	Veracruz: Microondas las Lajas	KP886893	_
C. priscus	IBH22367	Nuevo León: 9.7 Km W 18 de Marzo, Cerro Potosi,	XXXXX	XXXXX
C. terrestris	GP215	Hidalgo: 5.3 km N hwy 105 at Zacualtipan, Hgo.	XXXXX	XXXXX
C. sp. nov. 3	IBH31030	Veracruz: El Polvorín, 5km SW of Villa Aldama		
C. sp. nov. 3	IBH31031	Veracruz: El Polvorín, 5km SW of Villa Aldama		
C. sp. G	MVZ178700	Puebla: along Rd. to Tetela, 10.3 km E Mexico Hwy. 119 at Point 4 km S Chingnahuapan	AY522480	_
C. sp. K	MVZ173231	Oaxaca: Cerro San Felipe	AY522493	_
Aquiloeurycea cephalica	IBH30253	Hidalgo: 1.0 km S (by rd) of La Encarnación on road to MX85, Parque Nacional los Mármoles	XXXXX	XXXXX
Thorius sp	IBH30942	Oaxaca: Clemencia, Cuauhtemoc, Chilchotla, Sierra Mazateca		_

Morphological analyses and species descriptions

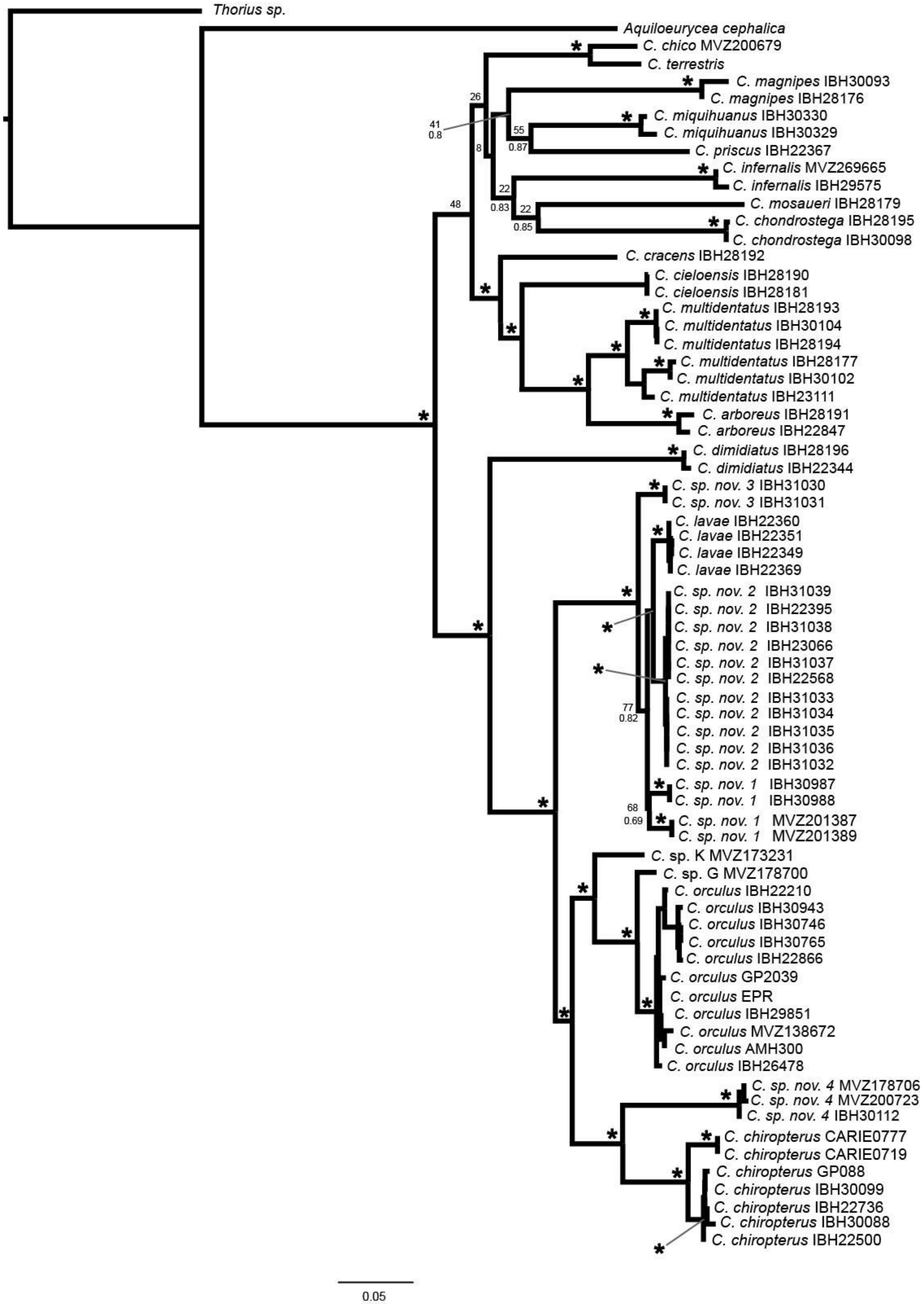
Species descriptions follow the format used by Lynch & Wake (1989) for species of Neotropical plethodontids and include the same basic characters and measurements, including coloration and external measurements. We used an electronic vernier caliper to measure 11 characters: snout-vent length (SVL), 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 angle of jaw (HW), head depth (HD), shoulder width (SW), internarial distance (IN), and right foot width (FW). In order to obtain an index for nostril shape, we used an ocular micrometer to measure the longest and shortest nostril dimensions (nostril length, NL, and nostril width, NW) and we calculated a ratio of nostril dimensions (ND = NL/NW). We also counted maxillary (MT), premaxillary (PMT), and vomerine teeth (VT) and we present counts for PMT and MT together because of the difficulty in distinguishing them in some specimens. We also measured limb interval (LI) as the number of costal folds between adpressed limbs, with positive values meaning grooves between limbs and negative values meaning overlap between limbs. We separated males and females to consider the sexual dimorphism. Finally, twelve additional measurements were obtained for the 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 vent, snout to forelimb, tail depth at base, and tail width at base. We examined 124 individuals from eight species and we used published measurements for comparisons to remaining species of *Chiropterotriton*. All material is deposited at the United States National Museum (USNM), Colección Nacional de Anfibios y Reptiles, Instituto de Biología (IBH) and Museum of Vertebrate Zoology, UC Berkeley (MVZ) collections (Appendix 1).

RESULTS

Molecular Analyses

A total of 71 individuals were included on phylogeny, for the taxon distributed in Tlapacoyan, Veracruz there was not DNA data available (Table 1). Bayesian analyses support the existence of two main clades in the genus *Chiropterotriton* (Fig 2). This grouping had already been proposed previously by Darda (1994) and Parra-Olea (2003), based on allozymes and mtDNA data and corresponds to the two main discrete units in the geographic distribution of the species, that is a northern and southern clade. Clade I, the northern clade, includes *C. priscus, C. magnipes, C. chondrostega, C. terrestris, C. chico, C. cracens, C, cieloensis, C. multidentatus, C. arboreus, C. miquihuanus* and *C. infernalis*. This clade is weakly supported (bs=48), with only one larger subclade has good support (Fig 1). Clade II, , the southern clade, has strong support, with *C. dimidiatus* as the sister species to the remaining members of the clade. This clade is formed by two subclades, including three groups which we call the *lavae*, the *orculus* and the *chiropterus* clades respectively. The *lavae* clade has strong support and is formed by 5 species: *C. sp. nov. 3, C. lavae*, *C. sp. nov. 2* and *C. sp. nov. 1* . The *orculus* clade with strong support is formed by four species, *C. orculus, C.* sp. G, *C.* sp. K . The *chiropterus* clade with strong suport is formed by two species: *C. chiropterus* and *C. sp. nov. 4*.

Figure 2. Phylogeny of *Chiropterotriton* from bayesian analysis of mtDNA.



Systematics

Chiropterotriton ***sp. nov. 1***

Texmalaquilla salamander

Salamandra de Texmalaquilla

Chiropterotriton chiropterus (part): Gadow, 1905

Chiropterotriton sp. I: Darda, 1994 (pop 22); Parra-Olea, 2003; Rovito and Parra-Olea 2015, Garcia-Castillo *et al*. 2017.

Holotype. USNM 224212, an adult male from Santa Cruz Texmalaquilla (4.7 mi by road NE of Atzitzintla), ca. 1 km NE of, on south slope of Pico de Orizaba, Puebla, Mexico, 3110 masl, 18.9484º N, 97.2802º W. Collected September 3rd, 1975, by R.W. McDiarmid.

Paratypes. Twenty specimens, all from Puebla, Mexico. Ten males: MVZ 201393 Santa Cruz Texmalaquilla, S side of Mt. Orizaba; USNM 224202, 224207–08, 224211, 224218–20, 224230, 224236, Santa Cruz Texmalaquilla (4.7 mi by road NE of Atzitzintla), ca. 1 km NE of, on south slope of Pico de Orizaba. Ten females: 224240–41, 224247, 224250, 224252–53, 224257, 224259, 224275–76, Santa Cruz Texmalaquilla (4.7 mi by road NE of Atzitzintla), ca. 1 km NE of, on south slope of Pico de Orizaba.

Referred specimens. Santa Cruz Texmalaquilla, Puebla: MVZ 201387–92. USNM 224193–201, 224203–06, 224209–10, 224213–17, 224221–29, 224231–35, 224237–39, 224242–46, 224248–49, 224251, 224254–56, 224258, 224260–74. Xometla, Veracruz: CAS 98934–36, 98939, 98953, 98957. KU 106641–65. IBH 30987–88. LACM 117161–230. MVZ 114378–82, 138759, 138761–63, 143910–17, 163583–97, 163601–06, 163612, 184830, 195827–30, 198914–17, 198919, 198921, 231345–47, 233032–34. USNM 492145–47.

Diagnosis. A plethodontid salamander assigned to the genus *Chiropterotriton* due to its slender body, shape of hand and feet digits, presence of sublingual fold, and based on mtDNA sequence data. Phylogenetically related to *C. sp. nov. 2, C. sp. nov. 3* and *C. lavae*. *Chiropterotriton sp. nov. 1* differs from *C. lavae* in being slightly larger (mean SVL 33.9 in males, 34.9 in females of *C. sp. nov. 1* vs. 32.4 in males, 31.6 in females of *C. lavae*), a shorter tail (mean TL/SVL 1.0 in males, 0.97 in females of *C. sp. nov. 1* vs. 1.19 in males, 1.02 in females of *C. lavae*), shorter limbs (mean LI 0.0 in males, 1.5 in females of *C. sp. nov. 1* vs. -0.6 in males, 0.6 in females of *C. lavae*), more maxillary teeth (mean MT 11.0 in males, 47.7 in females of *C. sp. nov. 1* vs. 7.0 in males, 20.8 in females of *C. lavae*) and more vomerine teeth (mean VT 13.0 in males, 15.9 in females of *C. sp. nov. 1* vs. 8.9 in males, 11.4 in females of *C. lavae*).

Chiropterotriton sp. nov. 1 differs from *C. sp. nov. 2* by its larger size (mean SVL 33.9 in males, 34.9 in females of C. *sp. nov. 1* vs. 29.7 in males, 31.7 in females of *C. sp. nov. 2)*, longer limbs (mean LI 0.0 in males, 1.5 in females of *C. sp. nov. 1* vs. 2.5 in males, 3.3 in females of *C. sp. nov. 2)*, larger head (mean HL 7.5 in males, 7.1 in females *C. sp. nov. 1* vs. 6.6 in males, 6.7 in females of *C. sp. nov. 2*), broader head (mean HW 5.1 in both males and females of *C. sp. nov. 1* vs. 4.2 in males, 4.4 in females of *C. sp. nov. 2*), broader feet (mean FW 3.8 in males, 3.5 in females of *C. sp. nov. 1* vs. 2.6 in both males and females of *C. sp. nov. 2*), more maxillary teeth (mean MT 11.0 for males, 47.7 for females of *C. sp. nov. 1* vs. 7.2 for males, 27.9 for females of *C. sp. nov. 2)*, and more vomerine teeth (mean VT 13.0 in males, 15.9 in females of *C. sp. nov. 1* vs. 9.0 in males, 11.1 in females of *C. sp. nov. 2)*.

Chiropterotriton sp. nov. 1 differs from *C. sp. nov. 3* in being slightly smaller (mean SVL 33.9 in males, 34.9 in females of *C. sp. nov. 1* vs. 35.7 in males, 35.5 in females of *C. sp. nov. 3*) and by having a shorter tail (mean TL/SVL 1.0 in males, 0.97 in females of *C. sp. nov. 1* vs. 1.16 in males, 1.20 in females of *C. sp. nov. 3*), shorter limbs (mean LI 0.0 in males, 1.5 in females of *C. sp. nov. 1* vs. -0.6 in males, 0.0 in females of *C. sp. nov. 3*), and fewer maxillary teeth (mean MT 11.0 for males, 47.7 for females of *C. sp. nov. 1* vs. 32.9 for males, 52.6 for females of *C. sp. nov. 3*).

Chiropterotriton sp. nov. 1 differs from *C. chiropterus* in males being smaller (mean SVL 33.9 in males of *C. sp. nov. 1* vs. 37.5 in males of *C. chiropterus*), by having a shorter tail (mean TL/SVL 1.0 in males, 0.97 in females of *C. sp. nov. 1* vs. 1.25 in males, 1.19 in females of *C. chiropterus*), longer limbs (mean LI 0.0 in males, 1.5 in females of *C. sp. nov. 1* vs. 0.3 in males, 2.0 in females of *C. chiropterus*), and fewer maxillary teeth (mean

MT 11.0 for males, 47.7 for females of *C. sp. nov. 1* vs. 12.6 for males, 48.0 for females of *C. chiropterus*).

Chiropterotriton sp. nov. 1 differs from *C. sp. nov. 4* by its larger size (mean SVL 33.9 in males, 34.9 in females of C. *sp. nov. 1* vs. 29.2 in males, 28.5 in females of *C. sp. nov. 4)*, longer limbs in males (mean LI 0.0 in males of *C. sp. nov. 1* vs. 2.3 in males of *C. sp. nov. 4)*, larger head (mean HL 7.5 in males, 7.1 in females *C. sp. nov. 1* vs. 6.3 in males, 6.4 in females of *C. sp. nov. 4*), broader head (mean HW 5.1 in both males and females of *C. sp. nov. 1* vs. 4.3 in males, 4.2 in females of *C. sp. nov. 4*), broader feet (mean FW 3.8 in males, 3.5 in females of *C. sp. nov. 1* vs. 2.4 in males, 2.6 in females of *C. sp. nov. 4*), more maxillary teeth (mean MT 11.0 for males, 47.7 for females of *C. sp. nov. 1* vs. 9.5 for males, 31.0 for females of *C. sp. nov. 4)*, and more vomerine teeth (mean VT 13.0 in males, 15.9 in females of *C. sp. nov. 1* vs. 11.0 in males, 13.0 in females of *C. sp. nov. 4*).

Chiropterotriton sp. nov. 1 differs from *C. sp. nov. 5* being smaller (mean SVL 33.9 in males, 34.9 in females of *C. sp. nov. 1* vs. 37.8 in males, 40.9 in one female of *C. sp. nov. 5*) and by having smaller head (mean HL 7.5 in males, 7.1 in females of *C. sp. nov. 1* vs. 8.3 in males, 8.6 in one female of *C. sp. nov. 5*), narrower head (mean HW 5.1 in both males and females of *C. sp. nov. 1* vs. 5.8 in males, 5.9 in one female of *C. sp. nov. 5*), longer limbs in males (mean LI 0.0 in males of *C. sp. nov. 1* vs. 0.8 in males of *C. sp. nov. 5*), more maxillary teeth (mean MT 11.0 for males, 47.7 for females of *C. sp. nov. 1* vs. 9 for males, 30 for one female of *C. sp. nov. 5*) and more vomerine teeth (mean VT 13.0 for males, 15.9 for females of *C. sp. nov. 1* vs. 9 for males, 13 for one female of *C. sp. nov. 5*).

Chiropterotriton sp. nov. 1 differs from *C. orculus* in being smaller (mean SVL 33.9 in males, 34.9 in females of *C. sp. nov. 1* vs. 35.9 in males, 39.0 in females of *C. orculus*), longer limbs (mean LI 0.0 in males, 1.5 in females of *C. sp. nov. 1* vs. 1.9 in males, 2.9 in females of *C. orculus)*, more maxillary teeth (mean MT 11.0 for males, 47.7 for females of *C. sp. nov. 1* vs. 8.2 for males, 28.8 for females of *C. orculus)*, and more vomerine teeth (mean VT 13.0 in males, 15.9 in females of *C. sp. nov. 1* vs. 8.6 in males, 12.0 in females of *C. orculus*).

Description. This is a medium-sized species of *Chiropterotriton*, mean SVL 33.9 in ten adult males (range 30.6–36.2) and 34.9 in ten adult females mean (range 33.3–38.3). The head is of moderate width HW averages 15% of SVL in both males and females (range 14–16). In males the snout is broad and truncated. Jaw muscles are pronounced and visible as a bulging mass immediately behind the eyes. Eyes are moderately protuberant and extend laterally beyond the jaw margin in ventral view. There are few maxillary teeth in males (mean MT 11.0, range 7–18) but are relatively numerous in females (mean MT 47.7, range 36–56). There are few vomerine teeth in males (mean VT 13.0, range 11–17) and females (mean VT 15.9, range 13–22) and arranged in a curved line that does not extend past outer margin of choanae. The tail is moderate in length, mean TL equals 1.0 of SVL in males (range 0.89–1.12) and 0.97 of SVL in females (range 0.85–1.07). Limbs are moderate length (FLL+HLL)/SVL average 54% of SVL in males (range 48–57) and 50% in females (range 45–54). Adpressed limbs approach closely or overlap slightly in males (mean LI 0.0, range -0.5–1) but they are separated by as many as two costal folds in females (mean LI 1.5, range 1–2). Digits are slender and expanded distally, with distinct subterminal pads and moderate webbing at the base. All digits are discrete, including the first, which extends beyond the margins of the webbing. The outermost toes are particularly well developed. The smallest male with a mental gland is 30.6 SVL. The mental gland is prominent and oval (nearly round) to round. Parotoid glands are not evident.

Variation. The population of C. *sp. nov. 1 from* Xometla differs from type locality in being relatively smaller and having a relatively longer tail (mean SVL 33.9 in males, 34.9 in females of *C. sp. nov. 1* from Texmalaquilla vs. 31.0 in males, 32.0 in females of *C. sp. nov. 1* from Xometla, mean TL/SVL 1.0 in males, 0.97 in females of *C. sp. nov. 1* from Texmalaquilla vs. 1.17 in males, 1.08 in females of *C. sp. nov. 1 from* Xometla).

Color in alcohol. Holotype uniform dark tannish brown dorsally becoming paler laterally and very pale cream color ventrally. Dark brown extends to tip of tail. Limbs yellowish. Mental gland beige. Nine paratypes are uniform dorsally ranging from golden tan to very dark grey, in some cases tail is slightly paler than dorsum. All of them have lateral surfaces paler than dorsal and ventral surfaces are much lighter than lateral surfaces. All remaining have a stripe of some sort; the stripe is always paler than immediate lateral parts but can be very obscure and seen mainly in the tail or can extend all the way from the back of the head to the tip of the tail. In some individuals this stripe is bright yellow but typically it is darker; in some instance there is a suffusion of black in the middle of the stripe. All individuals are paler ventrally but in some of the very dark animals the venter is dark gray and only the gular area is pale. Mental gland is usually pale.

Measurements of the holotype (in mm), tooth counts and limb interval. SVL 36.2, TL 34.3, AX 17.9, SW 3.4, HL 8.1, HW 5.3, HD 2.6, projection of snout beyond mandible 0.8, anterior rim of orbit to snout 2.0, interorbital distance 2.6, eyelid length 1.8, eyelid width 1.3, horizontal orbit diameter 1.6, nostril diameter 0.3, FLL 10.0, HLL 10.3, snout to forelimb 11.5, snout to anterior angle of vent 35.2, tail width at base 2.4, tail depth at base 2.6, FW 4.6, length of fifth toe 0.7, length of third (longest) toe 1.3, mental gland length 2.0, mental gland width 1.7. Premaxillary teeth 3, maxillary 5-4 (right-left sides) and vomerine 5-6 (right-left sides). Adpressed limbs are separated by 2 costal folds.

Habitat and range. Southern slopes of Pico de Orizaba, in the states of Puebla and Veracruz from 2600 m to approximately 3100 masl. Specimens have been found in arboreal bromeliads as well as under terrestrial cover objects.

Etymology. Named after the Ceron family who have assisted generations of biologists collecting salamanders in the general region of Orizaba.

Remarks. This species is found in sympatry with *Pseudoeurycea gadovi*, *P. leprosa*, *Thorius spilogaster* and *T. lunaris*. Much of the habitat has been destroyed making the finding of this species difficult in recent years.

Chiropterotriton ***sp. nov. 2***

Valle Alegre salamander
Salamandra de Valle Alegre

C. chiropterus (part): Wake *et al*. 1992.
Chiropterotriton sp. D: Darda, 1994.

Chiropterotriton sp. H: Darda, 1994; Parra-Olea, 2003; Parra-Olea & Wake, 2001; Rovito & Parra-Olea, 2015; García-Castillo *et al.* 2017.
Chiropterotriton sp.: Rovito *et al.* 2015.

Holotype. MVZ 200693 an adult female from 14.4 km S (by Rock Rd.) Las Vigas de Ramírez at Microwave Station, Valle Alegre, Veracruz, Mexico, 3020 masl, 19.56917ºN, 97.09528ºW (EPE = max. error distance 1.142 km). Collected 26 Aug 1982 by D. M. Darda and S. Sessions.

Paratypes. Nineteen specimens, all from Veracruz, Mexico. Twelve males: MVZ 114356, 114359, road from Las Vigas de Ramírez to Microwave Station on N Flank Cofre de Perote, 11.6 km S (by road), Las Vigas; MVZ 173428–29, Las Vigas de Ramírez, Microondas Rd.; MVZ 178661, 178663–65, 8–15.5 km S (via Microondas Rd.), Las Vigas de Ramírez; MVZ 200681–83, 200698, 14.4 km S (by Rock Rd.), Las Vigas de Ramírez at Microwave Station. Seven females: MVZ 173438–39, Las Vigas de Ramírez, Microondas Rd.; MVZ 186711, road to Microwave Station, 15 km S (by road) Las Vigas de Ramírez; MVZ 200691, 200694–95, 200702. 14.4 km S (by Rock Rd.) Las Vigas de Ramírez at Microwave Station.

Referred specimens. IBH 16778–82, 22384, 22391, 22395, 23062, 23066, 23072, 29853, 29857, 29863–64, 29866, 29872, 30840–41, 30844, 30847, 31032–39, 31055–62. KU 100747–54. MVZ 114351, 114355, 114357, 114358, 173440–41, 178659–60, 178662, 178666–68, 200684–86, 200688–90, 200692, 200695–97, 200699–701, 200703.

Diagnosis. A plethodontid salamander assigned to the genus *Chiropterotriton* due to its slender body, shape of hand and feet digits, presence of sublingual fold, and based on mtDNA sequence data. Phylogenetically related to *C. lavae*, *C. sp. nov. 1* and *C. sp. nov. 3* which differs morphologically from them as follows: *C. sp. nov. 2* differs from *C. lavae* in having smaller size in males (mean SVL 29.7 in males of *C. sp. nov. 2* vs. 32.4 in males of *C. lavae*), by having shorter limbs (mean LI 2.5 in males, 3.3 in females of *C. sp. nov. 2* vs. -0.6 in males, 0.6 in females of *C. lavae*), slightly narrower head (mean HW 4.2 in males, 4.4 in females of *C. sp. nov. 2* vs. 4.9 in males, 4.7 females of *C. lavae*), shorter head (mean HL 6.6 in males, 6.7 in females of *C. sp. nov. 2* vs. 7.5 in males, 7.0 in females of *C. lavae*), narrower feet (FW 2.6 in both males and females of *C. sp. nov. 2* vs. 3.7 in males, 3.3 in females of *C. lavae*), and more maxillary teeth in females (mean MT 27.9 for females of *C. sp. nov. 2* vs. 20.8 for females of *C. lavae*).

Chiropterotriton sp. nov. 2 differs from *C. sp. nov. 1* in being smaller (mean SVL 29.7 in males, 31.7 in females of *C. sp. nov. 2* vs. 33.9 in males, 34.9 in females of *C. sp. nov. 1*), and by having shorter limbs (mean LI 2.5 in males, 3.3 in females of *C. sp. nov. 2* vs. 0.0 in males, 1.5 in females of *C. sp. nov. 1*), smaller head (mean HL 6.6 in males, 6.7 in females of *C. sp. nov. 2* vs. 7.5 in males, 7.1 in females of C. *sp. nov. 1*), narrower head (mean HW 4.2 in males, 4.4 in females of *C. sp. nov. 2* vs. 5.1 in both males and females of *C. sp. nov. 1*), smaller feet (mean FW 2.6 in both males and females of *C. sp. nov. 2* vs. 3.8 in males, 3.5 in females of *C. sp. nov. 1*), fewer maxillary teeth (mean MT 7.2 for males, 27.8 for females vs. 11.0 for males and 47.7 for females of *C. sp. nov. 1*), and fewer vomerine teeth (VT 9.0 in males, 11.1 in females, of *C. sp. nov. 2* vs. 13.0 in males, 15.9 in females of *C. sp. nov. 1*).

Chiropterotriton sp. nov. 2 differs from *C. sp. nov. 3* in being smaller (mean SVL 29.7 in males, 31.7 in females of *C. sp. nov. 2* vs. 35.7 in males, 35.5 in females of *C. sp. nov. 3*) and by having a shorter tail (mean TL/SVL 1.0 in both males and in females of *C. sp. nov. 2* vs. 1.16 in males, 1.20 in females of *C. sp. nov. 3*), shorter limbs (mean LI 2.5 in males, 3.3 in females of *C. sp. nov. 2* vs. -0.60 in males, 0.0 in females of *C. sp. nov. 3*), shorter head (mean HL 6.6 in males, 6.7 in females of *C. sp. nov. 2* vs. 8.5 in males, 7.6 in females of *C. sp. nov. 3*), narrower head (mean HW 4.2 in males, 4.4 in females of *C. sp. nov. 2* vs. 5.2 in both males and females of *C. sp. nov. 3*), smaller feet (mean FW 2.6 in both males and females of *C. sp. nov. 2* vs. 4.2 in males, 4.0 in females of *C. sp. nov. 3*), fewer maxillary teeth (mean MT 7.2 for males, 27.9 for females of *C. sp. nov. 2* vs. 32.9 for males, 52.6 for females of *C. sp. nov. 3*), and fewer vomerine teeth (mean VT 9.0 in males, 11.1 in females of *C. sp. nov. 2* vs. 11.6 in males, 13.7 in females of *C. sp. nov. 3*).

Chiropterotriton sp. nov. 2 differs from *C. orculus* in being smaller (mean SVL 29.7 in males, 31.7 in females of *C. sp. nov. 2* vs. 35.9 in males, 39.0 in females of *C. orculus*) and by having slightly shorter limbs (mean LI 2.5 in males, 3.3 in females of *C. sp. nov. 2* vs. 1.9 in males, 2.9 in females of *C. orculus*), shorter head (mean HL 6.6 in males, 6.7 in females of *C. sp. nov. 2* vs. 7.4 in males, 8.0 in females of *C. orculus*), narrower head (mean HW 4.2 in males, 4.4 in females of *C. sp. nov. 2* vs. 5.0 in males, 5.2 in females of *C. orculus*), smaller feet (mean FW 2.6 in both males and females of *C. sp. nov. 2* vs. 3.2 in males, 3.4 in females of *C. orculus*), and fewer maxillary teeth (mean MT 7.2 for males, 27.9 for females of *C. sp. nov. 2* vs. 8.2 for males, 28.8 for females of *C. orculus*).

Chiropterotriton sp. nov. 2 is very similar in morphological proportions to *C. sp. nov. 4* differing from it by females having shorter limbs (mean LI 3.3 in females of *C. sp. nov. 2* vs. 1.8 in females of *C. sp. nov. 4*), less maxillary teeth (mean MT 7.2 for males, 27.9 for females of *C. sp. nov. 2* vs. 9.5 for males, 31.0 for females of *C. sp. nov. 4*), and fewer vomerine teeth (mean VT 9.0 in males, 11.1 in females of *C. sp. nov. 2* vs. 11.0 in males, 13.0 in females of *C. sp. nov. 4*).

Chiropterotriton sp. nov. 2 differs from *C. chiropterus* in being smaller (mean SVL 29.7 in males, 31.7 in females of *C. sp. nov. 2* vs. 37.5 in males, 33.5 in females of *C. chiropterus*) and by having a shorter tail (mean TL/SVL 1.0 in both males and in females of *C. sp. nov. 2* vs. 1.25 in males, 1.19 in females of *C. chiropterus*), shorter limbs (mean LI 2.5 in males, 3.3 in females of *C. sp. nov. 2* vs. 0.30 in males, 2.0 in females of *C. chiropterus*), shorter head (mean HL 6.6 in males, 6.7 in females of *C. sp. nov. 2* vs. 8.1 in males, 7.3 in females of *C. chiropterus*), narrower head (mean HW 4.2 in males, 4.4 in females of *C. sp. nov. 2* vs. 5.6 in males and 4.8 in females of *C. chiropterus*), smaller feet (mean FW 2.6 in both males and females of *C. sp. nov. 2* vs. 3.7 in males, 3.1 in females of *C. chiropterus*), fewer maxillary teeth (mean MT 7.2 for males, 27.9 for females of *C. sp. nov. 2* vs. 12.6 for males, 48.0 for females of *C. chiropterus*), and fewer vomerine teeth (mean VT 9.0 in males, 11.1 in females of *C. sp. nov. 2* vs. 10.6 in males, 12.5 in females of *C. chiropterus*).

Chiropterotriton sp. nov. 2 differs from *C. sp. nov. 5* in being smaller (mean SVL 29.7 in males, 31.7 in females of *C. sp. nov. 2* vs. 37.8 in males, 40.9 in one female of *C. sp. nov. 5*) and by having shorter limbs (mean LI 2.5 in males, 3.3 in females of *C. sp. nov. 2* vs. 0.80 in males, 1.0 in one female of *C. sp. nov. 5*), shorter head (mean HL 6.6 in males, 6.7 in females of *C. sp. nov. 2* vs. 8.3 in males, 8.6 in one female of *C. chiropterus*), narrower head (mean HW 4.2 in males, 4.4 in females of *C. sp. nov. 2* vs. 5.8 in males and 5.9 in one female of *C. sp. nov. 5*), smaller feet (mean FW 2.6 in both males and females of *C. sp. nov. 2* vs. 3.7 in both males and one female of *C. sp. nov. 5*), fewer maxillary teeth (mean MT 7.2 for males, 27.9 for females of *C. sp. nov. 2* vs. 9.0 for males, 30.0 in one female of *C. sp. nov. 5*), and fewer vomerine teeth in females (11.1 in females of *C. sp. nov. 2* vs.13.0 in one female of *C. sp. nov. 5*).

Description. This is a small but stout species of *Chiropterotriton*, mean SVL 29.7 in 12 adult males (range 26.5–32.8) and 31.7 in eight adult females (range 27.4–34.3). The head is of moderate width, HW averages 14% of SVL in both males and females (range 13–15). The snout is short. Eyes are small and typically do not protrude laterally beyond the jaw margin in ventral view; they are less prominent than in most other species of *Chiropterotriton*. Jaw muscles behind eyes are variably developed but generally pronounced. There are few maxillary teeth in males (mean MT 7.2, range 2–17) and moderate numbers in females (mean MT 27.9, range 19–36). There are few vomerine teeth in both males (mean VT 9.0, range 7–12) and females (mean MT 11.1, range 10–13) arranged in a curved line, not extending past outer margin of choanae. The tail is moderate-sized, mean TL equals 1.03 of SVL in males (range 0.92–1.16) and 1.0 of SVL in females (range 0.79–1.11). Limbs are short; (FLL+HLL)/SVL average 47% of SVL in males (range 44–50) and 43% of SVL in females (range 41–46). Adpressed limbs never overlap and are widely separated in both males (mean LI 2.5, range 1–3), and in females (mean LI 3.3, range 2–4). Manus and pes are relatively small for the genus. Webbing ranges from absent to slight and, when present, is limited to the metatarsal region. The first digit is small and usually included within the webbing, although a small portion of it may be free at the tip. The outermost digit is less prominent than in other species, digit 5 (pes) is distinctly shorter than digits 2–4. Subterminal pads are present but not prominent. The smallest male with a mental gland is 29.3 SVL. Oval-shaped mental gland present in males, not particularly prominent. Paratoid glands are present in many individuals, and sometimes prominent.

Color in alcohol. Holotype uniform dark brown dorsally and laterally becoming blackish brown on the tail. Venter much paler, than dorsum becoming dark brown under the tail. Limbs dark brown. No other distinguishing color. Two of the paratypes have a hint of a dorsal stripe slightly paler than surrounding areas. Manus and pes are paler but in general are brown to blackish brown.

Color in life. All dark, dark reddish-brown stripe, other have obscure brown to grey brown stripe (six specimens: IBH 29853, 29857, 29863, 29864, 29866, 29872, 15 km S of Las Vigas on road to Valle Alegre, these specimens are not in type series) lots of small guanophores over the mainly very dark pigment dorsally. Iris golden brown to dark brown, reddish stripe is brightest laterally with darker pigment medially. Venter dark to very dark. In one adult there is a complete melanophore network; in another, dense punctuations. Some white guanophores prominent in the darker individual.
IBH 22384, 22395, 23062, 23066, 23072, 15.9 km on microondas road, Las Vigas (these specimens are not in type series): Adults very dark almost black dorsally with fine speckling of obscure white overlying ground color. Fine background mottling of dark brown on black. Limbs black with some paler highlights becoming brown distally. Dark brownish black iris. Venter dark, dense mainly punctate melanophores, with very fine superficial sprinkling of white ventral-laterally. Gular area a bit paler. Juveniles have indistinct brown stripe, which apparently become obscure as animals grow.

Measurements of the holotype (in mm), tooth counts and limb interval. SVL 31.1, TL 30.7, AX 16.4, SW 3.1, HL 6.8, HW 4.2, HD 2.0, projection of snout beyond mandible 0.4, anterior rim of orbit to snout 1.7, interorbital distance 1.8, eyelid length 2.2, eyelid width 0.8, horizontal orbit diameter 1.4, FLL 6.5, HLL 6.7, snout to forelimb 8.8, snout to anterior angle of vent 29.5, tail width at base 2.1, tail depth at base 2.6, FW 2.5, length of fifth toe 0.5, length of third (longest) toe 1.2, Premaxillary teeth 6, maxillary 15-16 (right-left sides) and vomerine 7-6 (right-left sides). Adpressed limbs are separated by 4 costal folds.

Habitat and range. This species is found in pine and fir forest as well as above the tree line on Cofre de Perote. It ranges from 2950 to 4015 m, reaching the summit of Cofre de Perote. Specimens have been found on road banks at night, under terrestrial objects, and active on boulders at night. Occurs in sympatry with *Aquiloeurycea cephalica*, *Isthmura naucampatepetl*, *Pseudoeurycea leprosa* and *P. melanomolga*.

Etymology. — The species name is a noun in the genitive case and refers to the Cofre de Perote volcano, on the slopes of which this species is found.

Chiropterotriton ***sp. nov. 3***

Cruz Blanca salamander
Salamandra de Cruz Blanca

Chiropterotriton sp. E: Darda, 1994.
Chiropterotriton chiropterus (part): Wake *et al.*, 1992.
Chiropterotriton chiropterus: Parra-Olea *et al.*, 2001.

Holotype. MVZ 163945 an adult female from 6 km W Las Vigas de Ramírez, Veracruz, Mexico, 2420 masl, 19.635º N, 97.159166º W (EPE = max. error distance 5.71 km). Collected 25 Jul 1979 by D. B. Wake

Paratypes. Nineteen specimens, all from Veracruz, Mexico. Ten males: MVZ 163947–49, 163989–90, 163993, 171903, 171905, 171907, 171909, 6 km W Las Vigas de Ramírez. Nine females: MVZ 136981–82, 136986, pine forest along Mexico Hwy. 140, 4 km W Las Vigas de Ramírez; MVZ 138703–04, 138716, 138765, Mexico Hwy. 140, 4.5 km W (by road) Las Vigas de Ramírez; MVZ 163943, 171910, 6 km W Las Vigas de Ramírez.

Referred specimens: IBH 00122, 31030–31031, MVZ 136983–85, 137029, 138702, 138705–15, 138717–19, 163942, 163944, 163946, 163991–92, 163994, 171904, 171906, 171908, 171911–31.

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. lavae*, *C. sp. nov. 2* and *C. sp. nov. 1* . which differs morphologically from them as follows: *C. sp. nov. 3* differs from *C. lavae* in being larger (mean SVL 35.7 in males, 35.5 in females of *C. sp. nov. 3* vs. 32.4 in males, 31.6 in females of *C. lavae*), and in having relatively longer tails in females (mean TL/SVL 1.20 in females of *C. sp. nov. 3* vs. 1.02 in females of *C.*

lavae), longer limbs in females (mean LI 0.0 in females of *C. sp. nov. 3* vs. 0.6 in females of *C. lavae*), a longer head (mean HL 8.5 in males and 7.6 in females of C. *sp. nov. 3* vs.7.5 in males, 7.0 in females of *C. lavae*), a slightly broader head (mean HW 5.2 in both males and females of *C. sp. nov. 3* vs. 4.9 in males, 4.7 in females of *C. lavae*), larger feet (mean FW 4.2 in males, 4.0 in females of *C. sp. nov. 3* vs. 3.7 in males, 3.3 in females of *C. lavae*), more maxillary teeth (mean MT 32.9 for males, 52.6 for females of *C. sp. nov. 3* vs. 7.0 for males, 20.8 for females of *C. lavae*), and more vomerine teeth (mean VT 11.6 in males, 13.7 in females of *C. sp. nov. 3* vs. 8.9 in males, 11.4 in females of *C. lavae*).

Chiropterotriton sp. nov. 3 differs from *C. sp. nov. 2* in having larger size (mean SVL 35.7 in males, 35.5 in females of *C. sp. nov. 3* vs. 29.7 in males, 31.7 in females of *C. sp. nov. 2*), a longer tail (mean TL/SVL 1.16 in males, 1.20 in females of *C. sp. nov. 3* vs. 1.0 in both males and in females of *C. sp. nov. 2*), longer limbs (mean LI -0.60 in males, 0.0 in females of *C. sp. nov. 3* vs. 2.5 in males, 3.3 in females of *C. sp. nov. 2*), a longer head (mean HL 8.5 in males, 7.6 in females of C. *sp. nov. 3* vs. 6.6 in males, 6.7 in females of *C. sp. nov. 2*), a broader head (mean HW 5.2 in both males and females of *C. sp. nov. 3* vs. 4.2 in males, 4.4 in females of *C. sp. nov. 2*), a larger feet (mean FW 4.2 in males, 4.0 in females of *C. sp. nov. 3* vs. 2.6 in both males and females of *C. sp. nov. 2*), more maxillary teeth (mean MT 32.9 for males, 52.6 for females of *C. sp. nov. 3* vs. 7.2 for males, 27.9 for females of *C. sp. nov. 2*), and more vomerine teeth (mean VT 11.6 in males, 13.7 in females of *C. sp. nov. 3* vs. 9.0 in males, 11.1 in females of *C. sp. nov. 2*).

Chiropterotriton sp. nov. 3 differs from *C. sp. nov. 1* having larger size (mean SVL 35.7 in males, 35.5 in females of *C. sp. nov. 3* vs. 33.9 in males, 34.9 in females of *C. sp. nov. 1)*, a longer tail (mean TL/SVL 1.16 in males, 1.20 in females of *C. sp. nov. 3* vs. 1.0 in males, 0.97 in females of *C. sp. nov. 1)*, longer limbs (mean LI -0.6 in males, 0.0 in females of *C. sp. nov. 3* vs. 0.0 in males, 1.5 in females of *C. sp. nov. 1),* a longer head (mean HL 8.5 in males, 7.6 in females of C. *sp. nov. 3* vs. 7.5 in males and 7.1 in females of *C. sp. nov. 1)*, slightly larger feet (mean FW 4.2 in males, 4.0 in females of *C. sp. nov. 3* vs. 3.8 in males, 3.5 in females of *C. sp. nov. 1),* more maxillary teeth (mean MT 32.9 for males, 52.6 for females of *C. sp. nov. 3* vs. 11.0 for males, 47.7 for females of *C. sp. nov. 1)*, and fewer vomerine teeth (mean VT 11.6 in males, 13.7 in females of *C. sp. nov. 3* vs. 13.0 in males, 15.9 in females of *C. sp. nov. 1)*.

C. sp. nov. 3 differs from *C. orculus* in females being smaller (mean SVL 35.5 in females of *C. sp. nov. 3* vs. 39.0 in females of *C. orculus*), having a longer tail (mean TL/SVL 1.16 in males, 1.20 in females of *C. sp. nov. 3* vs. 1.0 in both males and in females of *C. orculus*), longer limbs (mean LI -0.60 in males, 0.0 in females of *C. sp. nov. 3* vs. 1.9 in males, 2.9 in females of *C. orculus*)*,* a longer head in males (mean HL 8.5 in males of C. *sp. nov. 3* vs. 7.4 in males, of *C. orculus*), larger feet (mean FW 4.2 in males, 4.0 in females of *C. sp. nov. 3* vs. 3.2 in males, 3.4 and females of *C. orculus*)*,* more maxillary teeth (mean MT 32.9 for males, 52.6 for females of *C. sp. nov. 3* vs. 8.2 for males, 28.8 for females of *C. orculus*), and more vomerine teeth (mean VT 11.6 in males, 13.7 in females of *C. sp. nov. 3* vs. 8.6 in males, 12.0 in females of *C. orculus*).

Chiropterotriton sp. nov. 3 differs from *C. sp. nov. 4* in having larger size (mean SVL 35.7 in males, 35.5 in females of *C. sp. nov. 3* vs. 29.2 in males, 28.5 in females of *C. sp. nov. 4*), having a longer tail in females (mean TL/SVL 1.20 in females of *C. sp. nov. 3* vs. 1.11 in females of *C. sp. nov. 4*), longer limbs (mean LI -0.60 in males, 0.0 in females of *C. sp. nov. 3* vs. 2.3 in males, 1.8 in females of *C. sp. nov. 4*)*,* a longer head (mean HL 8.5 in males, 7.6 in females of C. *sp. nov. 3* vs. 6.3 in males, 6.4 in females of *C. sp. nov. 4*), a broader head (mean HW 5.2 in both males and females of *C. sp. nov. 3* vs. 4.3 in males, 4.2 in females of *C. sp. nov. 4*), larger feet (mean FW 4.2 in males, 4.0 in females of *C. sp. nov. 3* vs. 2.4 in males, 2.6 in females of *C. sp. nov. 4*)*,* and more maxillary teeth (mean MT 32.9 for males, 52.6 for females of *C. sp. nov. 3* vs. 9.5 for males, 31.0 for females of *C. sp. nov. 4*).

C. sp. nov. 3 differs from *C. chiropterus* in males being smaller (mean SVL 35.7 in males of *C. sp. nov. 3* vs. 37.5 in males, of *C. chiropterus*), having a shorter tail (mean TL/SVL 1.16 in males, 1.20 in females of *C. sp. nov. 3* vs. 1.25 in males, 1.19 in females of *C. chiropterus*), longer limbs (mean LI -0.60 in males, 0.0 in females of *C. sp. nov. 3* vs. 0.3 in males, 2.0 in females of *C. chiropterus*)*,* longer head (mean HL 8.5 in males, 7.6 in females of C. *sp. nov. 3* vs. 8.1 in males, 7.3 in females of *C. chiropterus*), larger feet in males (mean FW 4.2 in males of *C. sp. nov. 3* vs. 3.7 in males of *C. chiropterus*)*,* more maxillary teeth (mean MT 32.9 for males, 52.6 for females of *C. sp. nov. 3* vs. 12.6 for males, 48.0 for females of *C. chiropterus*), and more vomerine teeth (mean VT 11.6 in males, 13.7 in females of *C. sp. nov. 3* vs. 10.6 in males, 12.5 in females of *C. chiropterus*).

Chiropterotriton sp. nov. 3 differs from *C. sp. nov. 5* in being smaller mean SVL 35.7 in males, 35.5 in females of *C. sp. nov. 3* vs. 37.8 in males, 40.9 in one female of *C. sp. nov. 5*) and by having longer limbs (mean LI -0.6 in males, 0.0 in females of *C. sp. nov. 3* vs. 0.80 in males, 1.0 in one female of *C. sp. nov. 5*), narrower head (mean HW 5.2 in both males and females of *C. sp. nov. 3* vs. 5.8 in males and 5.9 in one female of *C. sp. nov. 5*), larger feet (mean FW 4.2 in males, 4.0 in females of *C. sp. nov. 3* vs. 3.7 in both males and one female of *C. sp. nov. 5*), and fewer maxillary teeth (mean MT 32.9 for males, 52.6 for females of *C. sp. nov. 3* vs. 9.0 for males, 30.0 in one female of *C. sp. nov. 5*).

Description. This is a medium-sized species of *Chiropterotriton*, mean SVL 35.7 in ten adult males (range 32.0–38.6) and 35.5 in ten adult females (range 31.8–38.3). The head is of moderate width, HW averages 15% of SVL in both males and females (range 14–16). Jaw muscles are prominent in both males and females. Adult males have a broad, blunt snout with pronounced nasolabial protuberances that extend below the lip. Eyes are large and prominent and extend laterally beyond the jaw margin in ventral view. There are numerous numbers of maxillary teeth in males (mean MT 32.9, range 18–48) and many teeth in females (mean MT 52.6, range 45–60). There are few vomerine teeth in both males (mean VT 11.6, range 10–15) and females (mean MT 13.7, range 9–17) arranged in a curved line, not extending past outer margin of choanae. The tail is long and slender and typically exceeds SVL, mean TL equals 1.16 of SL in males (range 0.92–1.24) and 1.20 in females (range 1.06–1.38). Limbs are of moderate length; (FLL+HLL)/SVL average 59% of SVL in males (range 55–64) and 57% in females (range 53–62). Adpressed limbs closely approach or overlap in males (mean LI -0.6, range -1–1) and females (mean LI 0.0, -1–1). Manus and pes are relatively wide with digital tips somewhat expanded, and there are distinct subterminal pads. Webbing extends to base of terminal phalanx. The first (innermost) digit, while distinct, is included in the web except at its tip. The smallest male with a mental gland is 32.0 SVL. Mental gland is large, oval-shaped and relatively prominent. Parotoid glands are well marked in some individuals but less evident in others.

Color in alcohol. Holotype medium brown with an obscure dorsal stripe, darker brown along the margin and more reddish brown on the stipe with a narrow darker median line. Head medium brown with a light bar extending between the eyes and snout mottled with dark cream and brown. Limbs mottled with light brown upper limbs especially near the body, darker lower limbs with light tan digits. Venter is mainly pale with some mottled darker brown. Gular region mottled with dark cream and brown. Undersides of tail paler than lateral surfaces of tail. One individual (MVZ 193943) has a distinct yellowish stripe bordered laterally by a very dark band of pigment, with the stripe extending to the tip of the tail. Most others are uniformly pale brown to tan dorsally with some darker brown. One individual (MVZ 1639547) is generally paler gray brown.

Measurements of the holotype (in mm), tooth counts and limb interval. SVL 35.8, TL 49.2, AX 18.3, SW 3.7, HL 7.7, HW 5.3, HD 2.4, projection of snout beyond mandible 0.7, anterior rim of orbit to snout 2.2, interorbital distance 2.0, eyelid length 2.2, eyelid width 1.2, nostril diameter 0.2, FLL 9.9, HLL 11.5, snout to forelimb 12.4, snout to anterior angle of vent 33.5, tail width at base 3.0, tail depth at base 2.7, FW 4.6, length of fifth toe 0.8, length of third (longest) toe 1.8. Premaxillary teeth 6, maxillary 27-23 (right-left sides) and vomerine 7-7 (right-left sides). Tips of adpressed limbs touch.

Habitat and range. This species ranges from 2200 to 2450 on the ridge between Cruz Blanca and Las Vigas. It also occurs in La Joya at 2000 masl.

Etymology. The specific epithet refers to the native Totonac culture of the central region of Veracruz.

Remarks. Recently, we found two specimens on a secondary pine forest near the type locality at Cruz Blanca. It is important to note that this secondary forest is the only place where they are currently known to occur given that nearly all forest from the type locality has been logged. The few trees remaining are in the “Bosque Estatal San Juan del Monte” This species is found in sympatry with *Aquiloeurycea cephalica, Pseudoeurycea leprosa,* and *Thorius munificus.*

Chiropterotriton ***sp. nov. 4*** sp. nov.

Xicotepec salamander

Salamandra de Xicotepec

Chiropterotriton sp. F: Darda, 1994; Parra-Olea, 2003; Rovito & Parra-Olea, 2015; Garcia-Castillo *et al.*, 2017.

Holotype. MVZ 200726, an adult male from Xicotepec de Juárez, 3.3 km S of Hotel Mi Ranchito on Mexico Hwy. 130, 2.1 km E on road to La Unión Veracruz, México, 1080 masl, 20.227755º N, 97.953269º W (EPE = max. error distance 1.0 km). Collected 8 December 1983 by D. M. Darda and P. A. Garvey.

Paratypes. Seven specimens, all from Puebla. Four males: MVZ 178706, 178708, 3.9 km S of Xicotepec de Juárez on Hwy. 130; MVZ 200723–24, Xicotepec de Juárez, Hwy. 130, 21 km E on road to La Unión Veracruz. Three females: MVZ 178707, 3.9 km S of Xicotepec de Juarez on Hwy. 130; MVZ 185972, 2.2 km on road to Patla from junction with Hwy. 120 SW out of Xicotepec de Juárez; MVZ 200725, Xicotepec de Juárez, Hwy. 130, 21 km E on road to La Unión Veracruz.

Referred specimens. Cuetzalan, Puebla: IBH 30112, MVZ 133019.

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. chiropterus*. It differs from *C. chiropterus* in being smaller (mean SVL 29.2 in males, 28.5 in females of *C. sp. nov. 4* vs. 37.5 in males, 33.5 in females of C. *chiropterus*) and having a shorter tail in males (mean TL/SVL 1.16 in males of *C. sp. nov. 4* vs. 1.25 in males of C. *chiropterus*), a shorter head (mean HL 6.3 in males, 6.4 in females of *C. sp. nov. 4* vs. 8.1 in males, 7.3 in females of *C. chiropterus*), a narrower head (mean HW 4.3 in males, 4.2 in females of *C. sp. nov. 4* vs. 5.6 in males, 4.8 in females of *C. chiropterus*), smaller limbs in males (mean LI 2.3 in males of *C. sp. nov. 4* vs. 0.3 in males of *C. chiropterus*), a narrower feet (mean FW 2.4 in males, 2.6 in females of *C. sp. nov. 4* vs. 3.7 in males, 3.1 in females of *C. chiropterus*), and fewer maxillary teeth (mean MT 9.5 in males and 31.0 in females of *C. sp. nov. 4* vs. 12.6 in males and 48.0 in females of *C. chiropterus*).

It differs from *C. sp. nov. 1* in being smaller (mean SVL 29.2 in males, 28.5 in females of *C. sp. nov. 4* vs. 33.9 in males, 34.9 in females of *C. sp. nov. 1*) and having a shorter tail (mean TL/SVL 1.16 in males, 1.11 in females of *C. sp. nov. 4* vs. 1.0 in males, 0.97 in females of *C. sp. nov. 1*), a shorter head (mean HL 6.3 in males, 6.4 in females of *C. sp. nov. 4* vs. 7.5 in males, 7.1 in females of *C. sp. nov. 1*), a narrower head (mean HW 4.3 in males, 4.2 mm in females of *C. sp. nov. 4* vs. 5.1 in both males, and females of *C. sp. nov. 1*), smaller limbs in males (mean LI 2.3 in males of *C. sp. nov. 4* vs. 0.0 in males of *C. sp. nov. 1*), a narrower feet (mean FW 2.4 in males, 2.6 in females of *C. sp. nov. 4* vs. 3.8 in males, 3.5 in females of *C. sp. nov. 1*), fewer maxillary teeth (mean MT 9.5 in males and 31.0 in females of *C. sp. nov. 4* vs. 11.0 in males and 47.7 in females of *C. sp. nov. 1*), and fewer vomerine teeth (mean VT 11.0 in males and 13.0 in females of *C. sp. nov. 4* vs. 13.0 in males and 15.9 in females of *C. sp. nov. 1*).

It differs from *C. sp. nov. 2* in being slightly smaller (mean SVL 29.2 in males, 28.5 in females of *C. sp. nov. 4* vs. 29.7 in males, 31.7 in females of *C. sp. nov. 2*) and having a shorter tail (mean TL/SVL 1.16 in males, 1.11 in females of *C. sp. nov. 4* vs. 1.0 in both males and females of *C. sp. nov. 2*), a shorter head (mean HL 6.3 in males, 6.4 in females of *C. sp. nov. 4* vs. 6.6 in males, 6.7 in females of *C. sp. nov. 2*), more maxillary teeth (mean MT 9.5 in males and 31.0 in females of *C. sp. nov. 4* vs. 7.2 in males and 27.9 in females of *C. sp. nov. 2*), and fewer vomerine teeth (mean VT 11.0 in males and 13.0 in females of *C. sp. nov. 4* vs. 9.0 in males and 11.1 in females of *C. sp. nov. 2*).

It differs from *C. lavae* in being smaller (mean SVL 29.2 in males, 28.5 in females of *C. sp. nov. 4* vs. 32.4 in males, 31.6 in females of *C. lavae*) and having a shorter head (mean HL 6.3 in males, 6.4 in females of *C. sp. nov. 4* vs. 7.5 in males, 7.0 in females of *C. lavae*), a narrower head (mean HW 4.3 in males, 4.2 in females of *C. sp. nov. 4* vs. 4.9 in males, 4.7 in females of *C. lavae*), smaller limbs (mean LI 2.3 in males, 1.8 in females of *C. sp. nov. 4* vs. -0.6 in males, 0.6 in females of *C. lavae*), a narrower feet (mean FW 2.4 in males, 2.6 in females of *C. sp. nov. 4* vs. 3.7 in males, 3.3 in females of *C. lavae*), more maxillary teeth (mean MT 9.5 in males and 31.0 in females of *C. sp. nov. 4* vs. 7.0 in males and 20.8 in females of *C. lavae*), and more vomerine teeth (mean VT 11.0 in males and 13.0 in females of *C. sp. nov. 4* vs. 8.9 in males and 11.4 in females of *C. lavae*).

It differs from *C. sp. nov. 3* in being smaller (mean SVL 29.2 in males, 28.5 in females of *C. sp. nov. 4* vs. 35.7 in males, 35.5 in females of *C. sp. nov. 3*) and having a shorter head (mean HL 6.3 in males, 6.4 in females of *C. sp. nov. 4* vs. 8.5 in males, 7.6 in females of *C. sp. nov. 3*), a narrower head (mean HW 4.3 in males, 4.2 in females of *C. sp. nov. 4* vs. 5.2 in both males, and females of *C. sp. nov. 3*), smaller limbs (mean LI 2.3 in males, 1.8 in females of *C. sp. nov. 4* vs. -0.6 in males, 0.0 in females of *C. sp. nov. 3*), a narrower feet (mean FW 2.4 in males, 2.6 in females of *C. sp. nov. 4* vs. 4.2 in males, 4.0 in females of *C. sp. nov. 3*), and more maxillary teeth (mean MT 9.5 in males and 31.0 in females of *C. sp. nov. 4* vs. 32.9 in males and 52.6 in females of *C. sp. nov. 3*).

It differs from *C. orculus* in being smaller (mean SVL 29.2 in males, 28.5 in females of *C. sp. nov. 4* vs. 35.9 in males, 39.0 in females of *C. orculus*) and having a shorter tail (mean TL/SVL 1.16 in males, 1.11 in females of *C. sp. nov. 4* vs. 1.02 in both males and females of *C. orculus*), a shorter head (mean HL 6.3 in males, 6.4 in females of *C. sp. nov. 4* vs. 7.4 in males, 8.0 in females of *C. orculus*), a narrower head (mean HW 4.3 in males, 4.2 mm in females of *C. sp. nov. 4* vs. 5.0 in males, 5.2 in females of *C. orculus*), smaller limbs in males (mean LI 2.3 in males of *C. sp. nov. 4* vs. 1.9 in males of *C. orculus*), a narrower feet (mean FW 2.4 in males, 2.6 in females of *C. sp. nov. 4* vs. 3.2 in males, 3.4 in females of *C. orculus*), more maxillary teeth (mean MT 9.5 in males and 31.0 in females of *C. sp. nov. 4* vs. 8.2 in males and 28.8 in females of *C. orculus*), and more vomerine teeth (mean VT 11.0 in males and 13.0 in females of *C. sp. nov. 4* vs. 8.6 in males and 12.0 in females of *C. orculus*).

It differs from *C. sp. nov. 5* in being smaller (mean SVL 29.2 in males, 28.5 in females of *C. sp. nov. 4* vs. 37.8 in males, 40.9 in one female of *C. sp. nov. 5*) and having a shorter tail in males (mean TL/SVL 1.16 in males of *C. sp. nov. 4* vs. 1.0 in males of *C. sp. nov. 5*), a shorter head (mean HL 6.3 in males, 6.4 in females of *C. sp. nov. 4* vs. 8.3 in males, 8.6 in one female of *C. sp. nov. 5*), a narrower head (mean HW 4.3 in males, 4.2 mm in females of *C. sp. nov. 4* vs. 5.8 in males, 5.9 in one female of *C. sp. nov. 5*), smaller limbs (mean LI 2.3 in males, 1.8 in females of *C. sp. nov. 4* vs. 0.8 in males, 1.0 in one female of *C. sp. nov. 5*), and narrower feet (mean FW 2.4 in males, 2.6 in females of *C. sp. nov. 4* vs. 3.7 in both and in one female of *C. sp. nov. 5*).

Description. This is a small species of *Chiropterotriton*, mean SVL 29.2 in four adult males (range 26.4–31.4) and 28.5 in three adult females (range 27.1–29.8). The head is of moderate width, HW averages 15% of SVL in both males and females (range 14–15). Adults have a broad, bluntly rounded snout with broad and adult males have moderately developed nasolabial protuberances. Eyes are large and prominent and extend laterally beyond the jaw margin in ventral view. There are few maxillary teeth in males (mean MT 9.5, range 7–12) and moderate numbers in females (mean MT 31.0, range 25–34). There are few vomerine teeth in both males (mean VT 11.0, range 8–15) and females (mean VT 13.0, range 9–19) arranged in a line not extending past outer margin of choanae. The tail is long and slender and exceeds SVL in all adults with complete tails, mean TL equals 1.16 of SVL in males (range 1.10–1.22) and 1.11 in females (1.03–1.18). Limbs are short; (FLL+HLL)/SVL averages 46% of SVL in males (range 39–50) and 49% in females (range 46–52). Adpressed limbs are widely separated and never overlap in males (mean LI 2.3, range 2–2.5) or females (mean LI 1.8, range 1.0–2.5). Manus and pes are relatively small, digits are slender and their tips only slightly expanded. Webbing ranges from slight to absent and is limited to the metatarsal region. The first digit is distinct but largely included in the webbing. Subterminal pads are small but well developed. The smallest mature male (pigmented testes) is 26.4 SVL, and the smallest male with a mental gland is 28.5 SVL. Relatively small, rounded to oval-shaped mental gland present in adult males. Parotoid glands are not evident.

Color in alcohol. Holotype is faded but is generally bright yellow to yellowish tan. Bright yellow dorsal stripe extending from eyes all the way to the tip of tail, bordered laterally by a pale brown stripe that becomes paler ventrolaterally. Venter very pale, almost pigmentless. Tail has some light brown pigment along lateral margins. The snout is pale yellow with a little scattered brown pigment. Dark stripe bordering the broad yellow dorsal stripe arises at eye and extends posteriorly onto tail. Paratypes all faded but yellowish tan with a pale yellowish tan dorsal stripe evident in all individuals to some degree. Dorsal stripe always bordered by a thin dorsal lateral light brown stripe. Venter very pale. Manus and pes are pale.

Measurements of the holotype (in mm), tooth counts and limb interval. SVL 28.5, TL 31.4, AX 15.5, SW 3.3, HL 6.3, HW 4.1, HD 2.1, projection of snout beyond mandible 0.7, anterior rim of orbit to snout 1.5, interorbital distance 1.4, distance between corners of eyes 2.2, interorbital width 1.3, eyelid length 1.7, eyelid width 0.9, nostril diameter 0.2, FLL 5.1, HLL 6.1, snout to forelimb 8.4, snout to anterior angle of vent 24.4, snout to gular fold distance 6.3, tail depth at base 2.7, FW 2.2. Premaxillary teeth 3, maxillary 4-4 (right-left sides) and vomerine 7-8 (right-left sides). Adpressed limbs are separated by 2.5 costal folds.

Habitat and range. This species occurs in Cuetzalan, Xocoyolo and Xicotepec de Juarez from 690 to 1420 m. It occurs in banana plants and bromeliads. It has been found in sympatry with *Aquiloeurycea quetzalanensis.* This species of *Chiropterotriton* reaches the lowest elevation of any other known species of the genus.

Etymology. The word Xicotepec is derived from in Nahuatl meaning "place of the jicotes" Jicotes are bumble bees of the genus. The name used for this species is a noun in apposition referring to the genus.

Chiropterotriton ***sp. nov. 5*** sp. nov.
Tlapacoyan salamander
Salamandra de tlapacoyan

Holotype. MVZ 92874 an adult male from 13 mi SW Tlapacoyan Veracruz, Mexico, 19.868483º N, 97.301500º W (EPE = max. error distance 2 km). Collected 26 december 1969 by R. Altig.

Paratypes. Six specimens, all from Tlapacoyan, Veracruz. Four males: MVZ 92875, 92877–79, 13 mi SW Tlapacoyan Veracruz. One female: MVZ 92876.

Diagnosis. A plethodontid salamander assigned to the genus *Chiropterotriton* due to its slender body, shape of hand and feet digits, presence of sublingual fold, and based on mtDNA sequence data. It´s phylogenetic position is unknown due to the lack of tissue for phylogenetic analyses. Geographically associated species include *C. chiropterus, C. sp.*

nov. 4, C. sp. nov. 2, *C. sp. nov. 3*, *C. sp. nov. 1* and *C. lavae*. It differs from *C. chiropterus* in females being larger (mean SVL 40.9 in one female of *C. sp. nov. 5* vs. 33.5 in females of *C. chiropterus*) and having a shorter tail (mean TL/SVL 1.04 in males of *C. sp. nov. 5* vs. 1.25 in males of *C. chiropterus*), a larger head (mean HL 8.3 in males, 8.6 in one female of *C. sp. nov. 5* vs. 8.1 in males, 7.3 in females of *C. chiropterus*), a narrower head (mean HW 5.8 in males, 5.9 in one female of *C. sp. nov. 5* vs. 5.6 in males, 4.8 in females of *C. chiropterus*), smaller limbs in males (mean LI 0.8 in males of *C. sp. nov. 5* vs. 0.3 in males of *C. chiropterus*), and fewer maxillary teeth (mean MT 9.0 in males and 30.0 in one female of *C. sp. nov. 5* vs. 12.6 in males and 48.0 in females of *C. chiropterus*).

It differs from *C. lavae* in being larger (mean SVL 37.8 in males, 40.9 in one female of *C. sp. nov. 5* vs. 32.4 in males 31.6 in females of *C. lavae*) and males having a shorter tail (mean TL/SVL 1.04 in males, of *C. sp. nov. 5* vs. 1.19 in males, of *C. lavae*), a larger head (mean HL 8.3 in males, 8.6 in one female of *C. sp. nov. 5* vs. 7.5 in males, 7.0 in females of *C. lavae*), a broader head (mean HW 5.8 in males, 5.9 mm in one female of *C. sp. nov. 5* vs. 4.9 in males, 4.7 in females of *C. lavae*), smaller limbs in (mean LI 0.8 in males, 2.0 in one female of *C. sp. nov. 5* vs. -0.6 in males, 0.6 in females of *C. lavae*), and more maxillary teeth in females (mean MT 30.0 in one female of *C. sp. nov. 5* vs. 20.8 in females of *C. lavae*).

It differs from *C. sp. nov. 4* in being larger (mean SVL 37.8 in males, 40.9 in one female of *C. sp. nov. 5* vs. 29.2 in males, 28.5 in females of *C. sp. nov. 4*) and having a shorter tail (mean TL/SVL 1.04 in males of *C. sp. nov. 5* vs. 1.16 in males of *C. sp. nov. 4*), a larger head (mean HL 8.3 in males, 8.6 in one female of *C. sp. nov. 5* vs. 6.3 in males, 6.4 in females of *C. sp. nov. 4*), a broader head (mean HW 5.8 in males, 5.9 in one female of *C. sp. nov. 5* vs. 4.3 in males, 4.2 in females of *C. sp. nov. 4*), longer limbs (mean LI 0.8 in males, 1.0 in one female of *C. sp. nov. 5* vs. 2.3 in males, 1.8 in females of *C. sp. nov. 4*), and broader feet (mean FW 3.7 in both males and in one female of *C. sp. nov. 5* vs. 2.4 in males, 2.6 in females of *C. sp. nov. 4*).

It differs from *C. sp. nov. 3* in being larger (mean SVL 37.8 in males, 40.9 in one female of *C. sp. nov. 5* vs. 35.7 in in males, 35.5 in females of *C. sp. nov. 3*) and having a shorter tail (mean TL/SVL 1.0 in males, of *C. sp. nov. 5* vs. 1.16 in males, of *C. sp. nov. 3*), a larger head in females (mean HL 8.6 in one female of *C. sp. nov. 5* vs. 7.6 in females of *C. sp. nov. 3*), a broader head in females (mean HW 5.9 mm in one female of *C. sp. nov. 5* vs. 5.2 in females of *C. sp. nov. 3*), shorter limbs (mean LI 0.8 in males, 1.0 in one female of *C. sp. nov. 5* vs. -0.6 in males, 0.0 in females of *C. sp. nov. 3*), and smaller feet (mean FW 3.7 in both males and one female of *C. sp. nov. 5* vs. 4.2 in males, 4.0 in females of *C. sp. nov. 3*) and fewer maxillary teeth (mean MT 9.0 in males and 30.0 in one female of *C. sp. nov. 5* vs. 32.9 in males and 52.6 in females of *C. sp. nov. 3*).

It differs from *C. sp. nov. 2* in being larger (mean SVL 37.8 in males, 40.9 in one female of *C. sp. nov. 5* vs. 29.7 in in males, 31.7 in females of *C. sp. nov. 2*) and having a larger head in females (mean HL 8.3 in males, 8.6 in one female of *C. sp. nov. 5* vs. 6.6 in males 6.7 in females of *C. sp. nov. 2*), a broader head (mean HW 5.8 in males, 5.9 in one female of *C. sp. nov. 5* vs. 4.2 in males, 4.4 in females of *C. sp. nov. 2*), longer limbs (mean LI 0.8 in males, 1.0 in one female of *C. sp. nov. 5* vs. 2.5 in males, 3.3 in females of *C. sp. nov. 2*), and larger feet (mean FW 3.7 in both males and one female of *C. sp. nov. 5* vs. 2.6 in both males and females of *C. sp. nov. 2*).

It differs from *C. sp. nov. 1* in being larger (mean SVL 37.8 in males, 40.9 in one female of *C. sp. nov. 5* vs. 33.9 in males, 34.9 in females of *C. sp. nov. 1*) and having a larger head (mean HL 8.3 in males, 8.6 in one female of *C. sp. nov. 5* vs. 7.5 in males, 7.1 females of *C. sp. nov. 1*), a broader head (mean HW 5.8 in males, 5.9 in one female of *C. sp. nov. 5* vs. 5.1 in both males and females of *C. sp. nov. 1*), and shorter limbs in males (mean LI 0.8 in males of *C. sp. nov. 5* vs. 0.0 in males of *C. sp. nov. 1*).

It differs from *C. orculus* in being larger (mean SVL 37.8 in males, 40.9 in one female of *C. sp. nov. 5* vs. 35.9 in males, 39.0 in females of *C. orculus*), and having larger head (mean HL 8.3 in males, 8.6 in one female of *C. sp. nov. 5* vs. 7.4 in males, 8.0 in females of *C. orculus*), a broader head (mean HW 5.8 in males, 5.9 in one female of *C. sp. nov. 5* vs. 5.0 in males, 5.2 in females of *C. orculus*), and longer limbs in males (mean LI 0.8 in males, 1.0 in one female of *C. sp. nov. 5* vs. 1.9 in males, 2.9 in females of *C. orculus*).

Description.

This is a relatively large species of *Chiropterotriton*, mean SVL 37.8 in four adult males (range 34.5–42.0). Only one female has been collected, with a SVL of 40.9. The head is of moderate width, HW averages 16% of SVL in males (range 13–17) and 14% in the female. In males the snout is broad and truncated. Jaw muscles are pronounced and visible as a bulging mass immediately behind the eyes. Eyes are moderately protuberant and extend laterally beyond the jaw margin in ventral view. There are few maxillary teeth in males (mean MT 9.0, range 6–13) but are relatively numerous in the female (MT 30.0). There are few vomerine teeth in males (mean VT 9.0, range 8–11) and female (VT 13.0) arranged in a line extending to, or just past, inner margin of choanae. The tail is moderate in length, mean TL equals 1.0 of SVL in males (range 0.90–1.15). Limbs are short and slender; (FLL+HLL)/SVL average 57% of SVL in males (range 55–60) and 55% in one female. Adpressed limbs approach closely (mean LI 0.8, range 0.0–1) and they are separated by one costal fold in one female. Digits are long and slender with blunt tips, distinct subterminal pads, and moderate webbing extending onto penultimate phalanx of third toe. Digits II–V are discrete, while digit I is very short and does not extend beyond the margin of webbing. The outermost toes are particularly well developed. The smallest male with a mental gland is 37.2 SVL. The mental gland is oval-shaped. Parotoid glands are not evident.

Measurements of the holotype (in mm), tooth counts and limb interval.

SVL 41.95, TL 37.55, AX 20.4, SW 3.8, HL 8.8, HW 5.6, HD 2.8, projection of snout beyond mandible 0.2, anterior rim of orbit to snout 1.7, interorbital distance 2.4, eyelid length 2.1, eyelid width 1.1, horizontal orbit diameter 2.1, nostril diameter 0.4, FLL 10.7, HLL 12.6, snout to forelimb 11.4, snout to anterior angle of vent 36.6, tail width at base 3.3, tail depth at base 3.9, FW 4.0 length of fifth toe 0.8, length of longest (third) toe 1.2, Mental gland length 1.3, mental gland width 1.3. Numbers of teeth: premaxillary 4, maxillary 4-4, vomerine 4-4. Adpressed limbs are separated by 0 costal folds.

Color in alcohol. Faded brown, dorsally and laterally. No sign of dorsal stripe. Limbs mottled. Head is uniform pale brown with some mottling on the snout. The paratypes present some variation. The entire body of MVZ 92876 is mottled with faded pale and dark brown. There is a pale band extending between the anterior part of the eyes, with a very mottled snout. Posterior part of the body is strongly mottled and the anterior part of the tail with an irregularly bordered light dorsal stripe. MVZ 92875 is less boldly mottled but has some mottling. All of them have a paler venter than dorsum. MVZ 92877 also has a pale bar extending between the eyes.

Habitat and range. Known only from the type locality. It has not been found since the original collection in 1969, and nearly all of the primary forest at the type locality has been cut down.

Etymology. Named after Gustavo Casas Andreu, a Mexican herpetologist who has dedicated his career to describe the biodiversity of amphibians and reptiles in Mexico.

REDESCRIPTIONS

Redescription of *Chiropterotriton chiropterus*

Cope 1863 Proc. Acad. Nat. Sci. Philadelphia 15: 54

Neotype. MVZ 85590, an adult male from 1.4 mi southwest by road southwest edge of Huatusco de Chicuellar, Veracruz, Mexico, 19.141388º N, 96.98083º W (EPE = max. error distance 1.202 mi). Collected 16 Jan 1969 by R. W. McDiarmid and R. D. Worthington.

Specimens examined. Eleven specimens, all from 1.4 mi southwest by road southwest edge of Huatusco de Chicuellar, Veracruz, Mexico. Seven males: MVZ 85588–89, 85591–92, 85594, 85599, 85613. Four females: MVZ 85597–98, 85605, 85632.

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. sp. nov. 4*. It differs from *C. sp. nov. 4* in having a longer body (mean SVL 37.5 in males, 33.5 in females of *C. chiropterus* vs. 29.2 in males, 28.5 in females of *C. sp. nov. 4*), a longer tail (mean TL/SVL 1.25 in males, 1.19 in females of *C. chiropterus* vs. 1.16 in males, 1.11 in females of *C. sp. nov. 4*), a longer head (mean HL 8.1 in males, 7.3 in females of *C. chiropterus* vs. 6.3 in males, 6.4 in females of *C. sp. nov. 4*), a wider head (mean HW 5.6 in males, 4.8 in females of *C. chiropterus* vs. 4.3 in males, 4.2 in females of *C. sp. nov. 4*), longer limbs in males (mean LI 0.3 in males of *C. chiropterus* vs. 2.3 in males of *C. sp. nov. 4*), wider feet (mean FW 3.7 in males, 3.1 in females of *C. chiropterus* vs. 2.4 in males, 2.6 in females of *C. sp. nov. 4*), and more maxillary teeth (mean MT 12.6 in males, 48.0 in females of *C. chiropterus* vs. 9.5 in males, 31.0 in females of *C. sp. nov. 4*).

Chiropterotriton chiropterus differs from *C. orculus* in having a longer tail (mean TL/SVL 1.25 in males, 1.19 in females of *C. chiropterus* vs. 1.02 in both males and females of *C. orculus*), a longer head in males (mean HL 8.1 in males of *C. chiropterus* vs. 7.4 in males of *C. orculus*), a wider head in males (mean HW 5.6 in males of *C. chiropterus* vs. 5.0 in males of *C. orculus*), longer limbs (mean LI 0.3 in males, 2.0 in females of *C. chiropterus* vs. 1.9 in males, 2.9 in females of *C. orculus*), wider feet in males (mean FW 3.7 in males of *C. chiropterus* vs. 3.2 in males of *C. orculus*), and more maxillary teeth (mean MT 12.6 in males, 48.0 in females of *C. chiropterus* vs. 8.2 in males, 28.8 in females of *C. orculus*).

Chiropterotriton chiropterus differs from *C. sp. nov. 2* in being larger (mean SVL 37.5 in males, 33.5 in females of *C. chiropterus* vs. 29.7 in males, 31.7 in females of *C. sp. nov. 2*) and in having a longer tail (mean TL/SVL 1.25 in males, 1.19 in females of *C. chiropterus* vs. 1.0 in both males and in females of *C. sp. nov. 2*), longer limbs (mean LI 0.3 in males, 2.0 in females of *C. chiropterus* vs. 2.5 in males, 3.3 in females of *C. sp. nov. 2*), a larger head (mean HL 8.1 in males, 7.3 in females *C. chiropterus* vs. 6.6 in males, 6.7 in females of *C. sp. nov. 2*), a broader head (mean HW 5.6 in males, 4.8 in females of *C. chiropterus* vs. 4.2 in males, 4.4 in females of *C. sp. nov. 2*), broader feet (mean FW 3.7 in males, 3.1 in females of *C. chiropterus* vs. 2.6 in both males and females of *C. sp. nov. 2*), fewer maxillary teeth (mean MT 12.6 for males and 48.0 for females of *C. chiropterus* vs. 7.2 for males, 27.9 for females of *C. sp. nov. 2*) and more vomerine teeth (mean VT 10.6 in males, 12.5 in females of *C. chiropterus* vs. 9.0 in males, and 11.1 in females of *C. sp. nov. 2*).

Chiropterotriton chiropterus differs from *C. sp. nov. 1* in males being larger (mean SVL 37.5 in males of *C. chiropterus* vs. 33.9 in males of *C. sp. nov. 1*) and by a longer tail (mean TL/SVL 1.25 in males, 1.19 in females of *C. chiropterus* vs. 1.0 in males, 0.97 in females of *C. sp. nov. 1*), shorter limbs (mean LI 0.3 in males, 2.0 in females of *C. chiropterus* vs. 0.0 in males, 1.5 in females of *C. sp. nov. 1*), a larger head (mean HL 8.1 in males, 7.3 in females *C. chiropterus* vs. 7.5 in males, 7.1 in females of *C. sp. nov. 1*), a broader head in males (mean HW 5.6 in males *C. chiropterus* vs. 5.1 in males of *C. sp. nov. 1*), and fewer vomerine teeth (mean VT 10.6 in males, 12.5 in females of *C. chiropterus* vs. 13.0 in males, 15.9 in females of *C. sp. nov. 1*).

Chiropterotriton chiropterus differs from *C. lavae* in being larger (mean SVL 37.5 in males, 33.5 in females of *C. chiropterus* vs. 32.4 in males, 31.6 in females of *C. lavae*), a longer tail (mean TL/SVL 1.25 in males, 1.19 in females of *C. chiropterus* vs. 1.19 in males, 1.02 in females of *C. lavae*), shorter limbs (mean LI 0.3 in males, 2.0 in females of *C. chiropterus* vs. -0.6 in males, 0.6 in females of *C. lavae*), a larger head (mean HL 8.1 in males, 7.3 in females *C. chiropterus* vs. 7.5 in males, 7.0 in females of *C. lavae*), a broader head (mean HW 5.6 in males, 4.8 in females *C. chiropterus* vs. 4.9 in males, 4.7 in females of *C. lavae*), more maxillary teeth (mean MT 12.6 for males, 48.0 for females of *C. chiropterus* vs. 7.0 for males, 20.8 for females of *C. lavae*) and more vomerine teeth (mean VT 10.6 in males, 12.5 in females of *C. chiropterus* vs. 8.9 in males, 11.4 in females of *C. lavae*).

Chiropterotriton chiropterus differs from *C. sp. nov. 3* in males being larger (mean SVL 37.5 in males, of *C. chiropterus* vs. 35.7 in males of *C. sp. nov. 3*), in having longer tails (mean TL/SVL 1.25 in males, 1.19 in females of *C. chiropterus vs* 1.16 in males, 1.20 in females of *C. sp. nov. 3)*, shorter limbs (mean LI 0.3 in males, 2.0 in females of *C. chiropterus vs* -0.60 in males, 0.0 in females of *C. sp. nov. 3*), shorter head (mean HL 8.1 in males, 7.3 in females of *C. chiropterus vs* 8.5 in males, 7.6 in females of C. *sp. nov. 3*), smaller feet in males (mean FW 3.7 in males of *C. chiropterus vs* 4.2 in males of *C. sp. nov. 3*), less maxillary teeth (mean MT 12.6 for males, 48.0 for females of *C. chiropterus vs* 32.9 for males, 52.6 for females of *C. sp. nov. 3*), and less vomerine teeth (mean VT 10.6 in males, 12.5 in females of *C. chiropterus vs* 11.6 in males, 13.7 in females of *C. sp. nov. 3*).

Chiropterotriton chiropterus differs from *C. sp. nov. 5* in females being larger (mean SVL 33.5 in females of *C. chiropterus vs* 40.9 in one female of *C. sp. nov. 5*) and having a longer tail (mean TL/SVL 1.25 in males, of *C. chiropterus vs* 1.04 in males, of *C. sp. nov. 5*), a shorter head (mean HL 8.1 in males, 7.3 in females of *C. chiropterus* vs 8.3 in males, 8.6 in one female of *C. sp. nov. 5*), a broader head (mean HW 5.6 in males, 4.8 in females of *C. chiropterus* vs. 5.8 in males, 5.9 in one female of *C. sp. nov. 5*), longer limbs in males (mean LI 0.3 in males of *C. chiropterus* vs 0.8 in males of *C. sp. nov. 5*), and more maxillary teeth (mean MT 12.6 in males and 48.0 in females of *C. chiropterus vs* 9.0 in males and 30.0 in one female of *C. sp. nov. 5*).

Description. This is a medium-sized species of *Chiropterotriton*, mean SVL 37.5 in eight adult males (range 36.1–38.8) and 33.5 in four adult females (range 30.7–36.7). The head is of moderate width, HW averages 15% of SVL in both males and females (range 14–16). In males the snout is broad and bluntly rounded. Jaw muscles are relatively pronounced. Eyes are moderately protuberant and extend laterally beyond the jaw margin in ventral view. There are few maxillary teeth in males (mean MT 12.6, range 9–17) and numerous numbers in females (mean MT 48.0, range 42–57). There are few vomerine teeth in both males (mean VT 10.6, range 9–12) and females (mean VT 12.5, 10–15) arranged in a line that does not reach or barely reach inner margin of choanae. The tail is long and slender and exceeds SVL by a considerable amount in nearly all specimens; mean TL equals 1.25 of SVL in males (range 1.13–1.38) and 1.19 in females (1.01–1.26). Limbs are short to moderate; (FLL+HLL)/SVL average 52% of SVL in males (range 48–54) and 50% in females (range 47–53). Adpressed limbs closely approach or overlap slightly in males (mean LI 0.3, range -0.5–1) but they are more widely separated in females (mean LI 2.0, range 1.5–2.5). Manus and pes are relatively small, digits are slender. Subterminal pads are small but well developed. Webbing ranges from slight to absent and is limited to the metatarsal region. The first digit is distinct but largely included in the webbing. Digital tips are only slightly expanded. The smallest mature male (pigmented testes) is 36.1 SVL, the smallest male with a mental gland is 33.3 SVL. The mental gland is oval-shaped and not especially prominent. Parotoid glands are not evident.

Color in alcohol. Dorsum relatively pale brown, either uniform or with an indistinct, broad brown dorsal stripe bordered by thin, darker brown dorsolateral lines extending from behind eye to base of tail. Dorsal surface of tail relatively bale brown with some darker mottling; head sometimes with a small amount of darker mottling. Venter and gular region uniform pale tan, ventral side of tail uniform, slightly darker brown.

Redescription of *Chiropterotriton orculus*

Neotype. MVZ 138783, an adult male from the ridge between Popocatepetl and Iztaccihuatl, along Mex Hwy 196, 16.2 km by road east jct MX Hwy 115., Mexico, Mexico, 3300 masl, 19.0973ºN, 98.6829º W. Collected 26 July 1976 by J. F. Lynch, D. B. Wake and M. E. Feder.

Specimens examined. Nineteen specimens, all from ridge between Popocatepetl and Iztaccihuatl, México, Mexico. Nine males: MVZ 76161, 138694, 138696–97, 138700, 138778, 138784, 138804, 200630. Ten females: MVZ 138686, 138688, 138776–77, 138779, 138781, 138793, 138796–97, 200629.

Diagnosis. A plethodontid salamander assigned to the genus *Chiropterotriton* due to its slender body, shape of hand and feet digits, presence of sublingual fold, and based on mtDNA sequence data. *Chiropterotriton orculus* differs from *C. chiropterus* in having a shorter tail (mean TL/SVL 1.02 of SVL in both males and females of *C. orculus* vs. 1.25 in males, 1.19 in females of *C. chiropterus*), a shorter head in males (mean HL 7.4 in males of *C. orculus* vs. 8.1 in males of *C. chiropterus*), a narrower head in males (mean HW 5.0 in males of *C. orculus* vs. 5.6 in males of *C. chiropterus*), shorter limbs (mean LI 1.9 in males, 2.9 in females of *C. orculus* vs. 0.3 in males, 2.0 in females of *C. chiropterus*), narrower feet in males (mean FW 3.2 in males of *C. orculus* vs. 3.7 in males of *C. chiropterus*), and fewer maxillary teeth (mean MT 8.2 in males, 28.8 in females of *C. orculus* vs. 12.6 in males, 48.0 in females of *C. chiropterus*).

Chiropterotriton orculus differs from *C. sp. nov. 4* in having a larger body (mean SVL 35.9 in males, 39.0 in females of *C. orculus* vs. 29.2 in males, 28.5 in females of *C. sp. nov. 4*), a shorter tail (mean TL/SVL 1.02 in both males and females of *C. orculus* vs. 1.16 in males 1.11 in females of *C. sp. nov. 4*), a larger head (mean HL 7.4 in males, 8.0 in females of *C. orculus* vs. 6.3 in males, 6.4 in females of *C. sp. nov. 4*), a broader head (mean HW 5.0 in males, 5.2 in females of *C. orculus* vs. 4.3 in males, 4.2 in females of *C. sp. nov. 4*), and broader feet (mean FW 3.2 in males, 3.4 in females of *C. orculus* vs. 2.4 in males, 2.6 in females of *C. sp. nov. 4*).

Chiropterotriton orculus differs from *C. sp. nov. 1* in being larger (mean SVL 35.9 in males, 39.0 in females of *C. orculus vs* 33.9 in males, 34.9 in females of *C. sp. nov. 1*), shorter limbs (mean LI 1.9 in males, 2.9 in females of *C. orculus vs* 0.0 in males, 1.5 in females of *C. sp. nov. 1)*, less maxillary teeth (mean MT 8.2 for males, 28.8 for females of *C. orculus vs* 11.0 for males, 47.7 for females of *C. sp. nov. 1)*, and less vomerine teeth (mean VT 8.6 in males, 12.0 in females of *C. orculus vs* 13.0 in males, 15.9 in females of *C. sp. nov. 1*).

Chiropterotriton orculus differs from *C. sp. nov. 2* in being larger (mean SVL 35.9 in males, 39.0 in females of *C. orculus* vs 29.7 in males, 31.7 in females of *C. sp. nov. 2*) and by having slightly longer limbs (mean LI 1.9 in males, 2.9 in females of *C. orculus vs* 2.5 in males, 3.3 in females of *C. sp. nov. 2*) longer head (mean HL 7.4 in males, 8.0 in females of *C. orculus vs* 6.6 in males, 6.7 in females of *C. sp. nov. 2*), broader head (mean HW 5.0 in males, 5.2 in females of *C. orculus vs* 4.2 in males, 4.4 in females of *C. sp. nov. 2*), larger feet (mean FW 3.2 in males, 3.4 in females of *C. orculus vs* 2.6 in both males and females of *C. sp. nov. 2*), and more maxillary teeth (mean MT 8.2 for males, 28.8 for females of *C. orculus vs* 7.2 for males, 27.9 for females of *C. sp. nov. 2*).

Chiropterotriton orculus differs from *C. sp. nov. 3* in females being larger (mean SVL 39.0 in females, of *C. orculus vs* 35.5 in females of *C. sp. nov. 3*), having a shorter tail (mean TL/SVL 1.02 in both males and in females of *C. orculus vs* 1.16 in males, 1.20 in females of *C. sp. nov. 3*), shorter limbs (mean LI1.9 in males, 2.9 in females of *C. orculus* vs -0.60 in males, 0.0 in females of *C. sp. nov. 3*)*,* a smaller head in males (mean HL 7.4 in males, of *C. orculus vs* 8.5 in males of C. *sp. nov. 3*), smaller feet (mean FW 3.2 in males, 3.4 and females of *C. orculus vs* 4.2 in males, 4.0 in females of *C. sp. nov. 3*)*,* less maxillary teeth (mean MT 8.2 for males, 28.8 for females of *C. orculus vs* 32.9 for males, 52.6 for females of *C. sp. nov. 3*), and less vomerine teeth (mean VT 8.6 in males, 12.0 in females of *C. orculus vs* 11.6 in males, 13.7 in females of *C. sp. nov. 3*).

Chiropterotriton orculus differs from *C. lavae* in being larger (mean SVL 35.9 in males, 39.0 in females, of *C. orculus vs* 32.4 in males, 31.6 in females of *C. lavae*), having a shorter tail (mean TL/SVL 1.02 in both males and in females of *C. orculus vs* 1.19 in males, 1.02 in females of *C. lavae*), shorter limbs (mean LI 1.9 in males, 2.9 in females of *C. orculus* vs -0.60 in males, 0.6 in females of *C. lavae*), and more maxillary teeth (mean MT 8.2 for males, 28.8 for females of *C. orculus vs* 7.0 for males, 20.8 for females of *C. lavae*).

Chiropterotriton orculus differs from *C. sp. nov. 5* in being smaller (mean SVL 35.9 in males, 39.0 in females of *C. orculus vs* 37.8 in males, 40.9 in one female of *C. sp. nov. 5*), having smaller head (mean HL7.4 in males, 8.0 in females of *C. orculus* vs 8.3 in males, 8.6 in one female of *C. sp. nov. 5*), a narrower head (mean HW 5.0 in males, 5.2 in females of *C. orculus vs* 5.8 in males, 5.9 in one female of *C. sp. nov. 5*), and shorter limbs in males (mean LI 1.9 in males, 2.9 in females of *C. orculus vs* 0.8 in males, 1.0 in one female of *C. sp. nov. 5*).

Chiropterotriton orculus differs from *C. dimidiatus* in being larger (mean SVL SVL 35.9 in males, 39.0 in females of *C. orculus* vs. 24.7 in males, 25.8 in females of *C. dimidiatus*) and by having a longer tail (mean TL/SVL 1.02 in both males and females of *C. orculus* vs. 0.89 in males, 0.87 in females of *C. dimidiatus*), longer limbs (mean LI 1.90 in males, 2.90 in females of *C. orculus* vs. 3.9 in males, 4.9 in females of *C. dimidiatus*), a longer head (mean HL 7.4 in males, 8.0 in females of *C. orculus* vs. 5.3 in males, 5.1 in females of *C. dimidiatus*), a broader head (mean HW 5.0 in males, 5.2 in females of *C. orculus* vs. 3.5 in both males and females of *C. dimidiatus*), broader feet (mean FW 3.2 in males, 3.4 in females of *C. orculus* vs. 1.7 in males, 1.8 in females of *C. dimidiatus*), more maxillary teeth (mean MT 8.2 in males, 28.8 in females of *C. orculus* vs. 3.8 in males, 17.0 in females of *C. dimidiatus*) and more vomerine teeth (mean VT 8.6 in males, 12.0 in females of *C. orculus* vs. 5.6 in males, 8.3 in females of *C. dimidiatus*).

Chiropterotriton orculus differs from *C. chico* in males being smaller (mean SVL 35.9 in males of *C. orculus* vs. 38.4 in males of *C. chico*) and by having a shorter tail (mean TL/SVL 1.02 in both males and females of *C. orculus* vs. 1.18 in males, 1.12 in females of *C. chico*), shorter limbs (mean LI 1.90 in males, 2.90 in females of *C. orculus* vs. 0.7 in males, 2.1 in females of *C. chico*), a shorter head (mean HL 7.4 in males, 8.0 in females *C. orculus* vs. 8.8 in both males and females of *C. chico*), a narrower head (mean HW 5.0 in males, 5.2 in females *C. orculus* vs. 5.6 in males, 5.7 in females of *C. chico*), narrower feet (mean FW 3.2 in males, 3.4 in females of *C. orculus* vs. 4.1 in males, 4.3 in females of *C. chico*), and fewer vomerine teeth (mean VT 8.6 in males, 12.0 in females of *C. orculus* vs. 13.7 in males, 15.7 in females of *C. chico*).

Chiropterotriton orculus differs from *C. arboreus* in being larger (mean SVL 35.9 in males, 39.0 in females of *C. orculus* vs. 33.4 in males, 32.2 in females of *C. arboreus*) and by having a longer tail (mean TL/SVL 1.02 in both males and females of *C. orculus* vs. 0.83 in males, 0.87 in females of *C. arboreus*) and shorter limbs (mean LI 1.90 in males, 2.90 in females of *C. orculus* vs. 0.20 in males, 1.0 in females of *C. arboreus*).

Chiropterotriton orculus differs from *C. terrestris* in being larger (mean SVL 35.9 in males, 39.0 in females of *C. orculus* vs. 24.2 in males, 23.0 in females of *C. terrestris*) and by having a longer head (mean HL 7.4 in males, 8.0 in females *C. orculus* vs. 5.7 in males, 5.2 in females of *C. terrestris*), a broader head (mean HW 5.0 in males, 5.2 in females *C. orculus* vs. 3.5 in males, 3.4 in females of *C. terrestris*) and broader feet (mean FW 3.2 in males, 3.4 in females of *C. orculus* vs. 1.9 in males, 1.7 in females of *C. terrestris*).

Description. This is a medium sized species of *Chiropterotriton*, mean SVL 35.9 in 10 adult males (range 33.6–38.9) and 39.0 in 10 adult females (range 34.9–43.0). The head is of moderate width, HW averages 14% of SVL in males (range 13–15) and 13% in females (range 12–14). Jaw muscles are prominent in both males and females. Adult males have a broad, bluntly rounded snout with broad and moderately developed nasolabial protuberances. Eyes are large and relatively prominent and extend slightly beyond the jaw margin in ventral view. There are few maxillary teeth in males (mean MT 8.2, range 5-11) and moderate numbers in females (mean MT 28.8, range 23–35). There are few vomerine teeth in both males (mean VT 8.6, 5–11) and females (mean VT 12.0, range 9–15) arranged in a curved line that does not extend past outer margin of choanae. The tail is of moderate length and exceeds SVL by a slightly amount in most of the specimens, mean TL equals 1.02 of SVL in both males (range 0.86–1.15) and females (range 0.87–1.12). Limbs are short to medium length in both females and males; (FLL+HLL)/SVL averages 51% of SVL in males (range 43–56) and 47% in females (range 44–50). Adpressed limbs are close in males (mean LI 1.9, range 0.0–3.0) and widely separated in females (mean LI 2.9, range 2.0–3.0). Manus and pes are relatively small, digits are broad. Subterminal pads are well developed. Webbing ranges from slight to moderate, extending to base of penultimate phalanx on third toe. The first digit is distinct but barely emerges from the webbing. Digital tips are only slightly expanded. The smallest mature male is 33.6 SVL. Mental gland is prominent, relatively large, and oval shaped (nearly round).

Color in alcohol. Dorsum, head, and tail uniform medium brown. Upper side of limbs and feet paler brown. Venter, gular region, and underside of fore limbs tan to pale brown; underside of hind limbs and tail slightly darker brown.

Redescription of *C. lavae*

Neotype. MVZ 163812, an adult male from La Joya, Veracruz, Mexico, 2125 masl, 19.19618º N, 97.02722º W (EPE = max. error distance 1.292 km). Collected on 25 July 1979 by David B. Wake.

Specimens examined. Eighteen specimens, all from La Joya, Veracruz, Mexico. Nine males: MVZ 163913, 163915, 171873–74, 173394–95, 173398, 178685, 192789. Nine females: MVZ 106537, 106548, 171876, 171881, 171885, 171901, 192788, 197788, 200638.

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. sp. nov. 3*, *C. sp. nov. 2* and *C. sp. nov. 1*. *Chiropterotriton lavae* differs from *C. sp. nov. 3* in being smaller (mean SVL 32.4 in males, 31.6 in females of *C. lavae* vs. 35.7 in males, 35.5 in females of *C. sp. nov. 3*) and by having a shorter tail in females (mean TL/SVL 1.02 in females of *C. lavae* vs. 1.20 in females of *C. sp. nov. 3*), shorter limbs in females (mean LI 0.6 in females of *C. lavae* vs. 0.0 in females of *C. sp. nov. 3*), a shorter head (mean HL 7.5 in males, 7.0 in females of *C. lavae* vs. 8.5 in males, 7.6 in females of C. *sp. nov. 3*), a slightly narrower head (mean HW 4.9 in males, 4.7 in females of *C. lavae* vs. 5.2 in both males and females of *C. sp. nov. 3*), smaller feet (mean FW 3.7 in males, 3.3 in females of *C. lavae* vs. 4.2 in males, 4.0 in females of *C. sp. nov. 3*)*,* fewer maxillary teeth (mean MT 7.0 for males, 20.8 for females of *C. lavae* vs. 32.9 for males and 52.6 for females of *C. sp. nov. 3*), and less vomerine teeth (mean VT 8.9 in males, 11.4 in females of *C. lavae* vs. 11.6 in males, 13.7 in females of *C. sp. nov. 3*).

Chiropterotriton lavae differs from *C. sp. nov. 2* in males of *C. lavae* being larger (mean SVL 32.4 in males of *C. lavae* vs. 29.7 in males of *C. sp. nov. 2*) and by having longer limbs (mean LI -0.6 in males, 0.6 in females of *C. lavae* vs. 2.5 in males, 3.3 in females of *C. sp. nov. 2*), a slightly wider head (mean HW 4.9 in males, 4.7 females of *C. lavae* vs. 4.2 in males, 4.4 in females of *C. sp. nov. 2*), a longer head (mean HL 7.5 in males, 7.0 in females of *C. lavae* vs. 6.6 in males, 6.7 in females of *C. sp. nov. 2*), wider feet (FW 3.7 in males, 3.3 in females of *C. lavae* vs. 2.6 in both males and females of *C. sp. nov. 2*), and slightly fewer maxillary teeth in females (mean MT 20.8 for females of *C. lavae* vs. 27.9 for females of *C. sp. nov. 2*).

Chiropterotriton lavae differs from C. *sp. nov. 1* from in being slightly smaller (mean SVL 32.4 in males, 31.6 in females of *C. lavae* vs. 33.9 in males, 34.9 in females of *C. sp. nov. 1*) and by having a longer tail (mean TL/SVL 1.19 in males, 1.02 in females of *C. lavae* vs. 1.0 in males, 0.97 in females of *C. sp. nov. 1*), longer limbs (mean LI -0.6 in males, 0.6 in females of *C. lavae* vs. 0.0 in males, 1.5 in females of *C. sp. nov. 1*), fewer maxillary teeth (mean MT 7.0 for males, 20.8 for females of *C. lavae* vs. 11.0 for males and 47.7 for females of *C. sp. nov. 1*) and fewer vomerine teeth (mean VT 8.9 in males, 11.4 in females of *C. lavae* vs. 13.0 in males and 15.9 in females of *C. sp. nov. 1*).

Chiropterotriton lavae differs from *C. chiropterus* in being smaller (mean SVL 32.4 in males, 31.6 in females of *C. lavae* vs. 37.5 in males, 33.5 in females of *C. chiropterus*) and by having a shorter tail (mean TL/SVL 1.19 in males, 1.02 in females of *C. lavae* vs. 1.25 in males, 1.19 in females of *C. chiropterus)*, longer limbs (mean LI -0.6 in males, 0.6 in females of *C. lavae* vs. 0.3 in males, 2.0 in females of *C. chiropterus*), a smaller head (mean HL 7.5 in males, 7.0 in females of *C. lavae* vs. 8.1 in males, 7.3 in females *C. chiropterus*), a narrower head (mean HW 4.9 in males, 4.7 in females of *C. lavae* vs. 5.6 in males, 4.8 in females *C. chiropterus*), fewer maxillary teeth (mean MT 7.0 in males, 20.8 in females of *C. lavae* vs. 12.6 in males, 48.0 for females of *C. chiropterus*) and fewer vomerine teeth (mean VT 8.9 in males, 11.4 in females of *C. lavae* vs. 10.6 in males, 12.5 in females of *C. chiropterus*).

Chiropterotriton lavae differs from *C. orculus* in being smaller (mean SVL 32.4 in males, 31.6 in females of *C. lavae* vs. 35.9 in males, 39.0 in females of *C. orculus*) and by having a longer tail in males (mean TL/SVL 1.19 in males of *C. lavae* vs. 1.02 in males of *C. orculus*), and longer limbs (mean LI -0.6 in males, 0.6 in females of *C. lavae* vs. 1.9 in males, 2.9 in females of *C. orculus*).

Chiropterotriton lavae differs from *C. sp. nov. 4* in being larger (mean SVL 32.4 in males, 31.6 in females of *C. lavae* vs 29.2 in males, 28.5 in females of *C. sp. nov. 4*), having a longer head (mean HL 7.5 in males, 7.0 in females of *C. lavae vs* 6.3 in males, 6.4 in females of *C. sp. nov. 4*), a broader head (mean HW 4.9 in males, 4.7 in females of *C. lavae vs* 4.3 in males, 4.2 in females of *C. sp. nov. 4*), longer limbs (mean LI -0.6 in males, 0.6 in females of *C. lavae vs* 2.3 in males, 1.8 in females of *C. sp. nov. 4*), a broader feet (mean FW 3.7 in males, 3.3 in females of *C. lavae vs* 2.4 in males, 2.6 in females of *C. sp. nov. 4*), less maxillary teeth (mean MT 7.0 in males and 20.8 in females of *C. lavae vs* 9.5 in males and 31.0 in females of *C. sp. nov. 4*), and less vomerine teeth (mean VT 8.9 in males and 11.4 in females of *C. lavae vs* 11.0 in males and 13.0 in females of *C. sp. nov. 4*).

Chiropterotriton lavae differs from *C. sp. nov. 5* in being smaller (mean SVL 32.4 in males 31.6 in females of *C. lavae* vs 37.8 in males, 40.9 in one female of *C. sp. nov. 5*) and males having a longer tail (mean TL/SVL 1.19 in males, of *C. lavae vs* 1.04 in males of *C. sp. nov. 5*), a smaller head (mean HL 7.5 in males, 7.0 in females of *C. lavae vs* 8.3 in males, 8.6 in one female of *C. sp. nov. 5*), a narrower head (mean HW 4.9 in males, 4.7 in females of *C. lavae vs* 5.8 in males, 5.9 in one female of *C. sp. nov. 5*), longer limbs (mean LI -0.6 in males, 0.6 in females of *C. lavae vs* 0.8 in males, 1.0 in one female of *C. sp. nov. 5*), and less maxillary teeth in females (mean MT 20.8 in females of *C. lavae vs* 30.0 in females of *C. sp. nov. 5*).

Description. This is a medium sized species of *Chiropterotriton*, mean SVL 32.4 in 10 adult males (range 31.06–33.8) and 31.6 in 10 adult females (range 27.9–34.9). The head is of moderate width, HW averages 15% of SVL in males (range 14–17) and 15% in females (range 14–16). Jaw muscles are prominent in both males and females. Adult males and females have a bluntly rounded snout with moderately developed nasolabial protuberances. Eyes are large and prominent and extend laterally well beyond the jaw margin in ventral view. There are few maxillary teeth in males (mean MT 7.0, range 1-10) and moderate numbers in females (mean MT 20.8, range 13–36). There are few vomerine teeth in both males (mean VT 8.9, 7–10) and females (mean VT 11.4, range 8–15) arranged in a short line not reaching or barely reaching inner margin of choanae. The tail is of moderate length and exceeds SVL by a slightly amount in most of the specimens, mean TL equals 1.19 of SVL in males (range 1.11–1.27) and 1.02 in females (range 0.85–1.15). Limbs are of moderate length to large both females and males; (FLL+HLL)/SVL averages 59% of SVL in males (range 53–65) and 54% in females (range 50–59). Adpressed limbs are close or overlap in males (mean LI -0.60, range -1.0–0.0) and more separated in females (mean LI 0.6, range 0.0–2.0). Manus and pes are moderate in size. Subterminal pads are well developed. Webbing limited, reaching only to base of penultimate phalanx on third toe. The first digit is included entirely in the webbing. Digital tips are slightly expanded. The smallest male with a mental gland is 31.2 SVL. The mental gland is oval-shaped (nearly round), somewhat prominent, and moderately sized.

Color in alcohol. Dorsum, tail, and head relatively pale to dark brown, often with a paler, broad dorsal band bordered by darker brown coloration. Paler background color often faintly mottled with darker brown. Venter uniform tan to pale brown, ventral side of tail and limbs slightly darker brown. Gular region tan to pale brown, sometimes with a small amount of mottling.

Habitat and range: This species is known only from between Toxtlacoya and La Joya, along the road from Perote to Xalapa, Veracruz. It occurs in bromeliads in the cloud forest and has been found in somewhat disturbed habitat in and around the town of La Joya.

Figure 3. Heads and foot morphology.

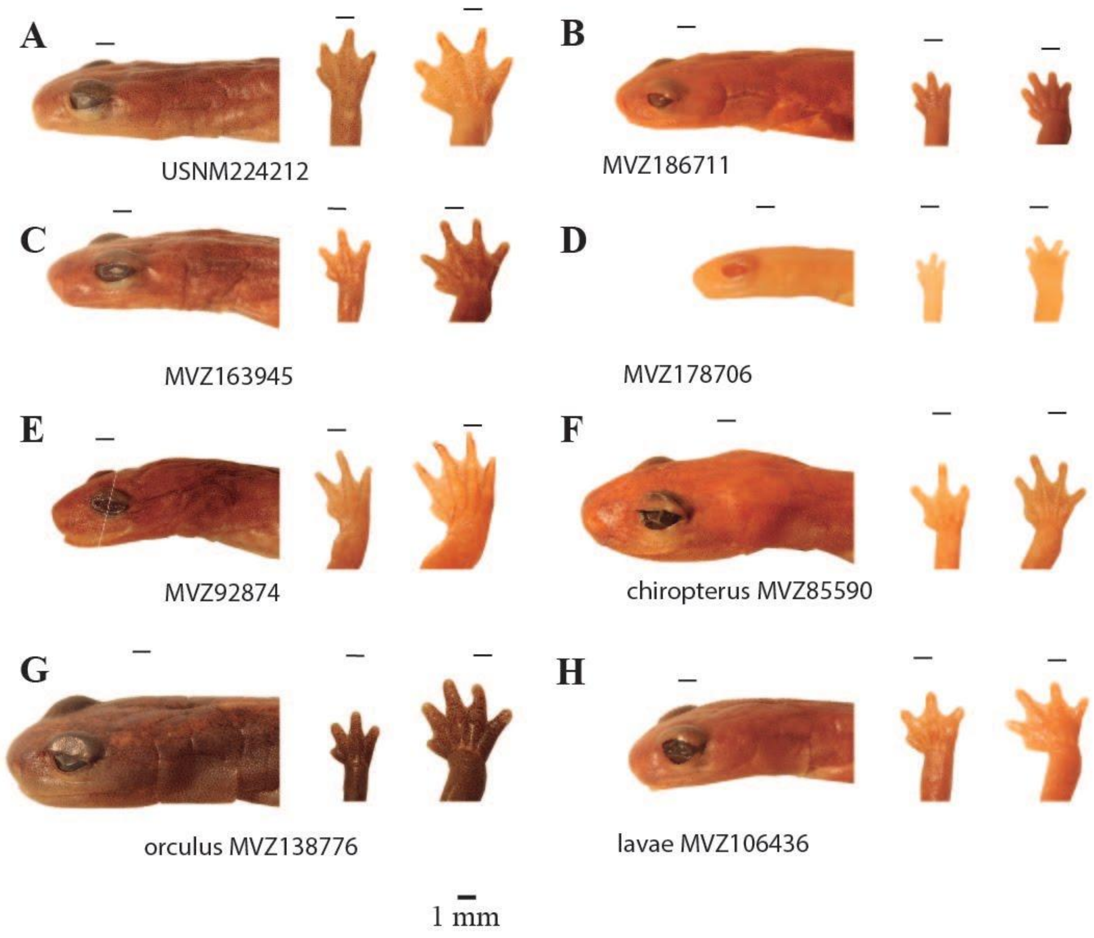


Figure 4. Photos in life

DISCUSSION

Our study represents the most extended sampling of the *Chiropterotriton* genus, and evidences the linages than we are presenting as new species with an remarkable geographical structure. The resulting phylogenetic tree is similar to the topology presented by Parra-Olea (2003), but shows a weak support in some nodes. In order to resolve the phylogenetic relationships between these species, especially at deeper nodes, we recommend a phylogenetic study with more data, including nuclear markers.

Chiropterotriton is comprised of terrestrial, arboreal and cave-dwelling species. Terrestrial species are commonly found in high elevation pine and pine-oak forests under logs or rocks. Arboreal species are often found in cloud forests between 1200 and 2500 masl, where they occupy bromeliads as microhabitats. These species differ from the terrestrial species in having longer limbs, longer tails and broader hands and feet. Cave-dwelling species are the most morphologically specialized species of the genus, having extremely broad webbed feet, large legs and tail.

Currently, there is a continued discovery of new species of amphibians worldwide (e. g. AmphibiaWeb 2019; Canseco-Márquez and Parra-Olea, 2003; Garcia-Paris et al., 2002; Campbell *et al.,* 2014; Rovito and Parra-Olea, 2015). Adding these five new species to the genus *Chiropterotriton* increases its diversity by at least 23 species, thus represents a significant contribution to the number of species now recorded for Mexican plethodontids (from 127 to 132 species). In addition, the complexes described in previous studies have been resolved with the combination of morphological and molecular characters. The molecular tools have certainly been precursors of a broader view of the biodiversity of species on the planet (Wake, 2009).

Chiropterotriton genus is endemic to Mexico and according to the Red List IUCN and NOM 059, most of its described species are threatened, mainly due to anthropogenic activity, climate change, and the incidence of the pathogen fungus *Batrachochytrium dendrobatidis* (Rovito et al., 2009). The situation for the species described herein is unknown, so it is important to continue studies of these populations to increase our knowledge about them, since these organisms have a high degree of ecological vulnerability (García -Vázquez et al., 2006). Biogeographical studies of this kind can contribute valuable information for decision-making on conservation issues, mainly by the occurrence of areas of endemism and the associated recognition of "hotspots" (Morrone and Crisci, 1992).

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CAPITULO II.

Filogenia multilocus y reconstrucción biogeográfica del género *Chiropterotriton*.

1) Molecular systematics and phylogenomic insights into the biogeography of the Mexican genus *Chiropterotriton* (Caudata: Plethodontidae). Será sometido a la revista *Journal of Biogeography*

Molecular systematics and phylogenomic insights into the biogeography of the Mexican genus *Chiropterotriton* (Caudata: Plethodontidae).

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1. INTRODUCTION

The Neotropical salamanders of the Tribe Bolitoglossini conform one of the nine main clades of the family Plethodontidae (Wake, 2012). This clade is the most diverse group in the family with 42.9% of all salamander species worldwide (AmphibiaWeb, 2018). Bolitoglossines are related to the Californian genus *Batrachoseps* (tribe Batrachosepini; Wake, 2012) and according to recent phylogenetic studies, these two related groups diverged in Mesoamerica (defined by Johnson et al., 2000 as the area from Mexico to Panamá), where bolitoglossines had a major evolutionary radiation resulting in a large number of species distributed from Mexico to middle South America (Rovito et. al, 2015; Rovito & Parra-Olea, 2016). Bolitoglossines include 14 genera that are characterized by having high levels of endemism with a great number of species restricted just to a single site or mountain (Rovito et. al, 2015; Rovito & Parra-Olea, 2016).

The restricted pattern of salamander distribution is explained by intrinsic factors such as low vagility, high philopatry and limited osmotic tolerance (Vences & Wake, 2007). In this way, environmental conditions play an important role given the dependence of these organisms to the ideal conditions of humidity. Thus, montane zones offer a gradient of conditions that have promoted geographic isolation and consequently speciation (Kozak & Wiens, 2007). In particular, bolitoglossines have the most species richness in mid-elevational habitats (1000-2000 m asl), which could be explained by early colonizations in this elevational zonation and the niche conservatism shown by this group (Wiens et. al, 2007). One of the main challenges presented by this group is the lack fossil record, that limits the knowledge about historical biogeographic processes that drove their current geographic distribution. However, bolitoglossines are beginning to be understood by means of phylogenetic analysis based on molecular data (*p. eg.* Rovito et. al., 2012; Rovito et. al., 2015; Bryson et. al., 2018).

The first bolitoglossine phylogenetic hypothesis tried to reconstruct species relationships based on few mitochondrial DNA genes (mtDNA) (*p. eg.* García-París & Wake, 2000; Parra-Olea et al., 2002). Subsequently, mitochondrial markers have been extensively used for species descriptions and delimitations (e. g. McCranie & Rovito, 2014; García-Castillo et al., 2017; Arias & Kubicki, 2018). More recently, nuclear data (nDNA) has been incorporated into systematic studies (Rovito et al., 2013; Rovito et al., 2015), proving to be informative for deep phylogenetic relationships and to estimate divergence times for biogeographical hypothesis (Shen et. al. 2016). The understanding of bolitoglossine diversification patterns and evolution requires a strong phylogenetic hypothesis, preferably obtained with large amounts of molecular data, which could help in hypothesis testing for particular geological events on a finer timescale (Rovito & Parra-Olea, 2016). At present, new sequencing technologies offer the possibility to get massive nDNA datasets. The sequence capture of ultraconserved elements (UCEs) allows getting a great quantity of nDNA data and it has been successfully used in salamanders (Newman & Austin, 2016; Bryson et al., 2018). Furthermore, UCE capture also offers the possibility of getting mitochondrial genomes from the subproducts of the massive data (do Amaral et al., 2015).

Chiropterotriton, with only 23 species, is a small genus of bolitoglossine salamanders compared to *Bolitoglossa,* which consists of 132 species (AmphibiaWeb, 2018). *Chiropterotriton* is restricted to Mexico and occurs along the Sierra Madre Oriental (SMO), from central to eastern Trans-Mexican Volcanic Belt (TMVB), and in the south in the highlands of northern Oaxaca. Despite the extreme morphological homoplasy present in the genus, which is associated to their ecological specializations (Darda & Wake, 2015), this group proved to be monophyletic since the very first molecular study (Darda, 1994; Parra-Olea, 2003). The species richness in *Chiropterotriton* increased by 50% in the last four years (Campbell et. al., 2014; Rovito & Parra-Olea, 2015; García-Castillo et. al., 2017; García-Castillo et. al., 2018; Parra-Olea et. al. unpublish). The phylogenies presented in recent studies are based on mtDNA and they provided strong evidence in support for the current taxonomy. However, there is not a robust phylogeny that fully resolves the relationships in this group and consequently, the biogeographical and speciation patterns cannot be accurately explained.

In this study, we used a protocol for sequence capture of ultraconserved elements (UCEs) to reconstruct a nDNA time-calibrated phylogeny of *Chiropterotriton.*Additionally, we obtained a large part of the mitochondrial genome from UCE residues. With the combined data, we obtained a robust phylogeny that includes all described species plus most known candidate species and shed light on the biogeographical processes that yielded the contemporary distribution patterns of this group.

2. MATERIALS AND METHODS

2.1 Laboratory protocols

Liver, intestine and tail tissues were obtained from the 35 specimens of the genus *Chiropterotriton*, representing 19 of currently recognized species (*C. aureus, C. chico, C. sp. nov. 4* and *C. sp. nov. 5* are not included because tissue was not available at the time of the study), plus six undescribed taxa; *C.* sp. C, *C.* sp. G, *C.* sp. 1, *C.* sp. 2, *C.* sp. 3 and *C.* sp. 4 (except *C.* sp. K, which tissue was not available) (Table S1). *Aquiloeurycea cephalica* was used as outgroup. Qiagen DNeasy Blood & Tissue kit (Qiagen, Valencia, CA, USA) was used for genomic DNA extraction. Library preparation and enrichment for UCEs was carried out at the Moore Laboratory of Zoology at Occidental College, Los Angeles, California, USA. First, genomic DNA was fragmented with a sonicator (Qsonica 125) with amplitude of 35% in a time of 1:45–4:00 minutes. An agarose gel was run to verify that the fragments did not exceed 800 base pairs (bp) and a concentration measurement was taken per microliter through a flourometer (Qubit). A dilution of 20ng/µl was carried out and the library preparation was done with the Kapa Hiper Prep Kit (KK8505), following the supplier's protocol. Amplification of the libraries was carried out with 6–16 cycles depending on the concentration of the ligation product, and unique adapters were added to each sample. Six pools of 500 ng were prepared and dried in a vacuum centrifuge to be resuspended with 3.4 µl of water. The enrichment with UCEs was done through the My Baits Kit (Target Enrichment of Illumina Libraries), with 5,060 RNA probes (Mycroarray, Inc) directed to 2386 ultraconserved elements and their contiguous sequences, following the protocol of Faircloth (2012). The library size range was verified with an Agilent 2100 Bioanalyzer (Palo Alto, CA). The qPCR was used to to quantify the enriched pools and they were combined in one megapool. Finally, a single pool was sent to the University of California Santa Cruz Genome Technology Center to be sequenced on an Illumina HiSeq 2000 lane.

2. 2 Data assembly and nuclear (UCEs) data extraction

Illumina raw reads (demultiplexed) were verified for quality control with FASTQC v0.11.4. The adapter contamination and low-quality bases were removed with TRIMMOMATIC 0.32 (Bolguer et. al., 2014). Five raw sequences data were added from a published UCEs study in order to use more outgroups: *Aquiloeurycea cephalica, Isthmura bellii, I. boneti, I. gigantea* and *Pseudoeurycea unguidentis* from Bryson et al. (2018), and *Plethodon cinereus* and *P. serratus* from Newman & Austin (2016). PHYLUCE 1.5.0 pipeline (Faircloth, 2016) was used to assembly the clean reads using Trinity method (Grabherr et al., 2013). Phylogenetically useful sequences were obtained by aligning assembled the contigs to probes and trimming ultra conserved sites to recover the informative flanks. Finally, MAFFT 7.130 (Katoh et. al., 2005) was used to align the multiple sequences for loci and data sets were compiled in one concatenated matrix.

2. 3 Mitochondrial DNA data extraction

While the enrichment with UCEs technique allows us to get a massive nuclear data, this method is able to obtain nearly complete mitochondrial genomes (Smith et. al., 2013; Zarza et. al., 2017). A resume list from assembly results was used for identify the long contigs that could belong to mitochondrial genome. Each long contig was tested using Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi), and the positive results were submitted to MITOchondrial genome annotation Server (MITOS; Bernt et. al., 2013). In order to get each taxon covered, we used all MITOS result files to create an addressed reference in BLAST executable pipeline (ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/), and an independent BLAST was made using all mitochondrial genes in each taxon. In order to inquire the phylogenetical position from the excluded species (*C. aureus, C. chico, C. sp. nov 4, C. sp. nov 5* and *C.* sp. K), available mitochondrial sequences were added from GenBank (except *C. sp. nov 5,* which has no molecular data available).

2. 4 Phylogenetic analyses and divergence time estimation

Maximum-likelihood (ML) and Bayesian inference (BI) were used to infer phylogenetic trees from the mtDNA and nDNA matrices. Both analyses were run in the CIPRES data portal (Miller et. al., 2010). We used RAxML v.7.4.2 (Stamatakis, 2006) for ML analyses with 1000 bootstrap replicates. PartitionFinder v1.0 (Lanfear et al., 2012) was used to substitution model selection for each mitochondrial locus and PhyML 3.0 (Guindon et al., 2010) for the entire nuclear matrix; both analysis were done using Bayesian Information Criterion (BIC). BEAST 1.8.2 (Drummond et. al., 2012) was used for BI analyses with 20 million generations, sampled every 1000 generations with four chains. A consensus tree was estimated with the first 5000 samples discarded as burn-in. BEAST 1.8.2 was used to obtain a time-calibrated tree with nDNA data, the mtDNA was not used to avoid time overestimation in mitochondrial estimates (Shen et al., 2016). A secondary calibration point was used to date the early divergence from the Most Recent Common Ancestor (MRCA) of *Chiropterotriton* genus. Based on Rovito et al. (2015), normal prior distribution with a mean of 24 Ma and a standard deviation of 4 Ma was assigned due to the 95% of the prior distribution on 20–28 Ma. Uncorrelated lognormal relaxed clock, Yule process tree prior and a GTR+G+I model of sequence evolution were used as priors. One hundred generations were ran, they were sampled every 10,000 steps and the first 25% of samples were discarded as burn-in. Tracer v.1.6.0 (Rambaut & Drummond, 2007) was used to assess convergence of the runs, appropriate burn-in and that effective sample size values were sufficiently high (>200). Finally, TreeAnnotator 1.8.2 (Drummond et. al., 2012) was used to construct maximum clade credibility tree with median heights.

2. 5 Ancestral range estimations

Dispersal-extinction-cladogenesis was used with jump dispersal (DEC+j) model to estimate the ancestral range for *Chiropterotriton*, based on stochastic likelihood models of geographic range. BioGeoBEARS 0.2.1 package was ran on R (Matzke, 2013). Ultrametric tree from BEAST analysis was used as input tree with ingroup only (35 taxa) and biogeographical regions were assigned for each terminal. World Wildlife Fund - US ecoregions were used in order to assign a representative regionalization, which based on biodiversity analyses and expert reviews (Olson et. al., 2004). Every terminal branch was assigned to one of the six ecoregions: Oaxacan montane forests (OMF), Sierra Madre de Oaxaca pine-oak forests (SMOax), Sierra Madre Oriental pine-oak forests (SMO), Trans-Mexican Volcanic Belt pine-oak forests (TMVB), Veracruz moist forests (VMF) and Veracruz montane forest (Vmon). Given the low dispersal capabilities of these salamanders, all range sizes were constrained to be composed of at most of two regions.

3. RESULTS

3.1 Genetic data

We captured nuclear data with an average of 1119.4 UCE loci per sample (range 384–1617), and 249,452.8 bp (range 97,487–311,605; Table S1). The final nDNA data set contained 1209 loci and was 347,481 bp in length (gaps included). Additionally, twelve coding and one ribosomal genes were captured from the mitochondrial genome (Table S2). We recovered an average of 10.9 loci (range 1–13), with an average of 9,520.2 bp (range 415–12,681; Table S1). The final mitochondrial matrix (gaps included) was 12,794 bp in length. The partition scheme with substitution model results are shown in Table S2.

3.2 Phylogenetic inferences

The phylogenetic reconstruction with mtDNA is similar to the nDNA results (Figure 1). Both contain an initial split that corresponds to groups that are currently found in the northern and southern portions of the distribution range of *Chiropterotriton*. Results from both datasets also show support for five groups with the same taxa composition. Group I (Figures 1 and 2) is formed by 13 species all occurring in the northern part of the distribution of *Chiropterotriton*. mtDNA Bayesian and ML analysis group these 13 species into two subclades, but the relationships between species are different and only Bayesian analysis has strong support (Figure 1 and Figure S1). Both ML and Bayesian nDNA reconstructions are almost identical and fully resolved (only one node is weak in ML tree, BS=64; Figure 1). According to nDNA analyses Group I is formed by: *C. arboreus* represented by three populations (Xilitla, Zacualtipán and Tlalchinol) and is the sister species of *C. multidentatus* also represented by three populations (Cd. Maiz, Borbotón and El Cielo). They are subsequently related *C.* sp. 1, *C. cieloensis, C. cracens* and *C. chondrostega.* These are the sister clade of *C. miquihuanus* + *C. priscus* and both are the sister clade of *C. infernalis.* Subsequent positions are taken by *C. magnipes, C. mosaueri* and *C. terrestris.* Finally, *C. chico* is the sister species of *C. terrestis* (based only on mt reconstruction). Group II (Figures 1 and 2) is formed by one single species *C. dimidatus,* which is always sister to groups III, IV and V in all analyses. Phylogenetic relationships between groups III, IV and V are not full concordant in all analyses. In ML mtDNA analysis (Figure S1) Group IV is sister clade of Group III + Group V. However, mtDNA Bayesian analysis and both nDNA analyses support two clades: Group III + Group IV as sister clades of Group V (Figure 1). Group III is formed by five species supported by concordant phylogenetic relationships in both data analysis: *C.* sp. 2 is the sister species of the rest of the group, *C.* sp. K is the sister species of *C.* sp. G + *C.* sp. 3 and *C. orculus* that is represented by five localities (Popocatépetl, Desierto de los Leones, Llano Grande, Bosque de Tlalpan and Lagunas de Zempoala; Figure 2, Table 1). The grouping of the populations of *C. orculus* is slightly different between mtDNA and nDNA analysis (Figure 1), however, the rest of the group is fully concordant.

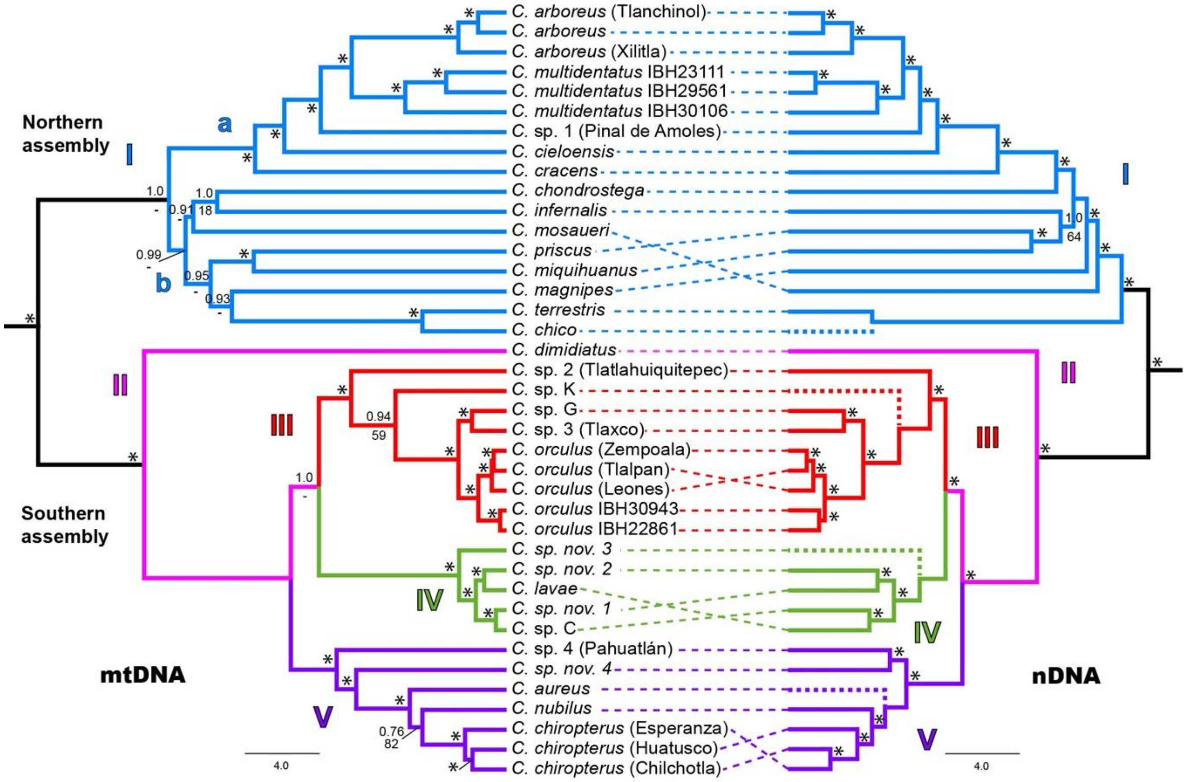

Figure 1. Mitochondrial (left) and nuclear (right) phylogenetic reconstruction based on ML and BI analyses. Colors means groups as follows: I blue, II pink, III red, IV green, V purple. Node support is given in posterior probabilities (above) and bootstrap (below). Asterisks mean strong node support (PP > 0.95, BS > 75). Discontinuous lines indicate taxon belong branch. Dotted line is the hypothetical taxa position in nDNA based on mtDNA data.

Group IV (Figures 1 and 2) has five species and they are represented by well-supported nodes on mtDNA and nDNA analysis, however, the topology is not concordant between both reconstructions and branch lengths are notably different. *Chiropterotriton sp. nov.* 3 is the sister species of the rest of this group (based only on mt data), *C. sp. nov.* 2 is the sister species of *C. lavae*, and *C.* sp. C is the sister species of *C. sp. nov.* 1*,* but there are different associations in nuclear results (*C. sp. nov. 2* + *C. sp. nov. 1, C.* sp. C + *C. lavae*; Figure 1). Finally, group V (Figures 1 and 2) contains five species, but mtDNA and nDNA data are contrasting in the relationship between *C.* sp 4 and *C. sp. nov. 4*, which are sister taxa in the nDNA results but not in the mtDNA results (Figure 1). *Chiropterotriton aureus* is the sister species of *C. nubilus* + *C. chiropterus,* which is represented by three populations (La Esperanza, Huatusco and Chilchotla) and the population structure between them vary on the position of Huatusco.

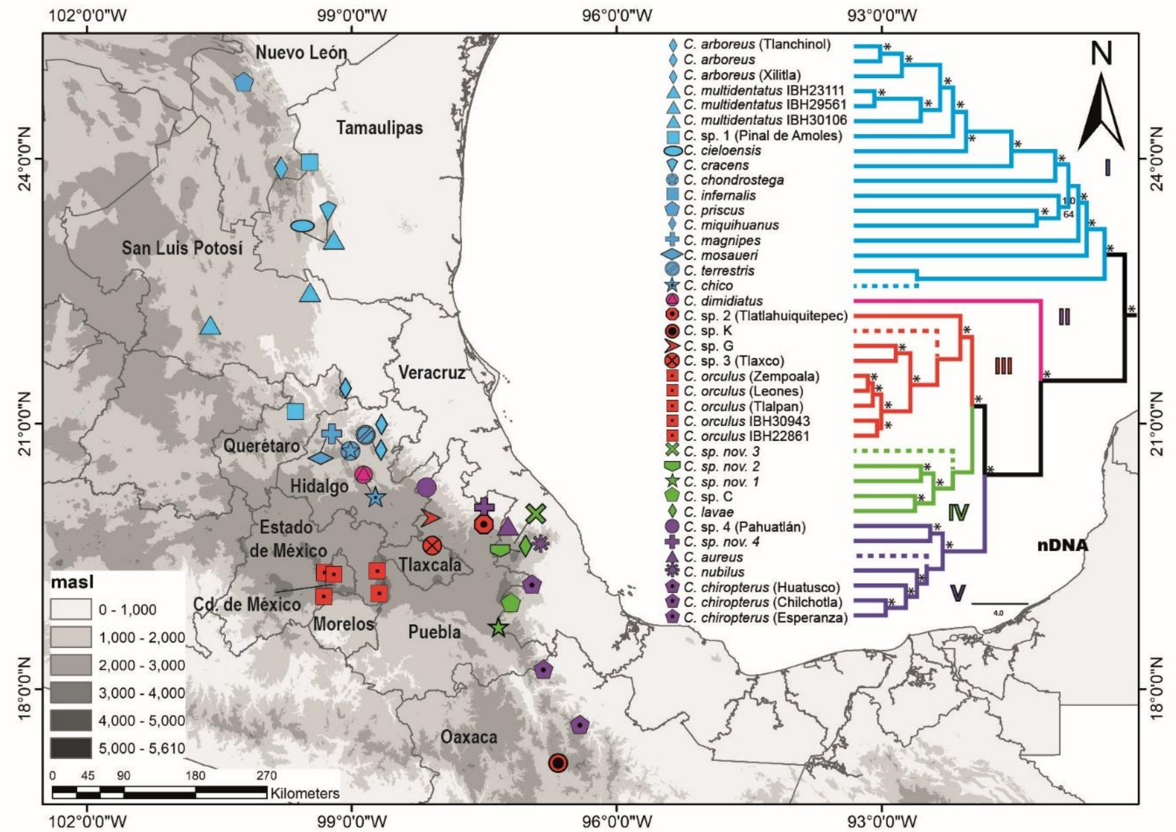

Figure 2. Spatial representation from all species of *Chiropterotriton*. Tree represents the Bayesian topology from nuclear reconstruction with five groups.

3.3 Divergence times

The nDNA Bayesian analysis produced a phylogenetic reconstruction for *Chiropterotriton* with high resolution and strong node support (100% of nodes with PP > 0.95; Figure 1). Our analyses, based on a secondary calibration point, suggest that the origin of this group dates back of the Late Oligocene, approximately 23.7 Ma (95% Highest Posterior Density, HPD: 19.8–27.7 Ma, Figure 3), but most of the diversification of the group occurred during the Miocene (23.03–5.33 Ma). Group I has the oldest extant species of the genus, where seven subsequent splits occurred during the Early Miocene (23.03–15.97 Ma) giving rise to *C. terrestris, C. mosaueri, C. magnipes, C. infernalis, C. priscus, C. miquihuanus* and *C. chondrostega* (Figure 3). Later, there were four more splits during Middle and Late Miocene where *C. cracens, C. cieloensis, C.* sp. 1 and *C. multidentatus* evolved and another split event during the Pliocene (15.97–2.58 Ma) that gave rise to *C.*

arboreus. Finally, during the Pleistocene, most of the population divergences occurred in *C. arboreus* and *C. multidentatus.*

The first split in Group II began in the Early Miocene with *C. dimidiatus* approximately 16.8 Ma (13.5–20.2). Group III had an initial split during Late Miocene arraund 9.7 Ma (7.7–11.8), and gave rise to *C.* sp. 2 and two more splits that occurred during Pliocene gave rise to *C.* sp. 3, C. sp. G and *C. orculus* (5.2–2.5 Ma). The isolation of *C. orculus* populations occurred during the Pleistocene (2.1–1.3 Ma). Group IV had an initial split approximately 7.2 Ma (9.6–14.4) and two more around 6.1 Ma (4.0–7.2) and 5.5 Ma (4.6–7.8), originating *C.* sp. C + *C. lavae* and *C. sp. nov.* 1 + *C. sp. nov.* 2 respectively during the Late Miocene. Finally, three species of group V arose in the Late Miocene approximately 8.0 Ma (6.3–9.9) with the following species; *C. sp. nov. 4. C.* sp. 4, *C. nubilus* and *C. chiropterus.*

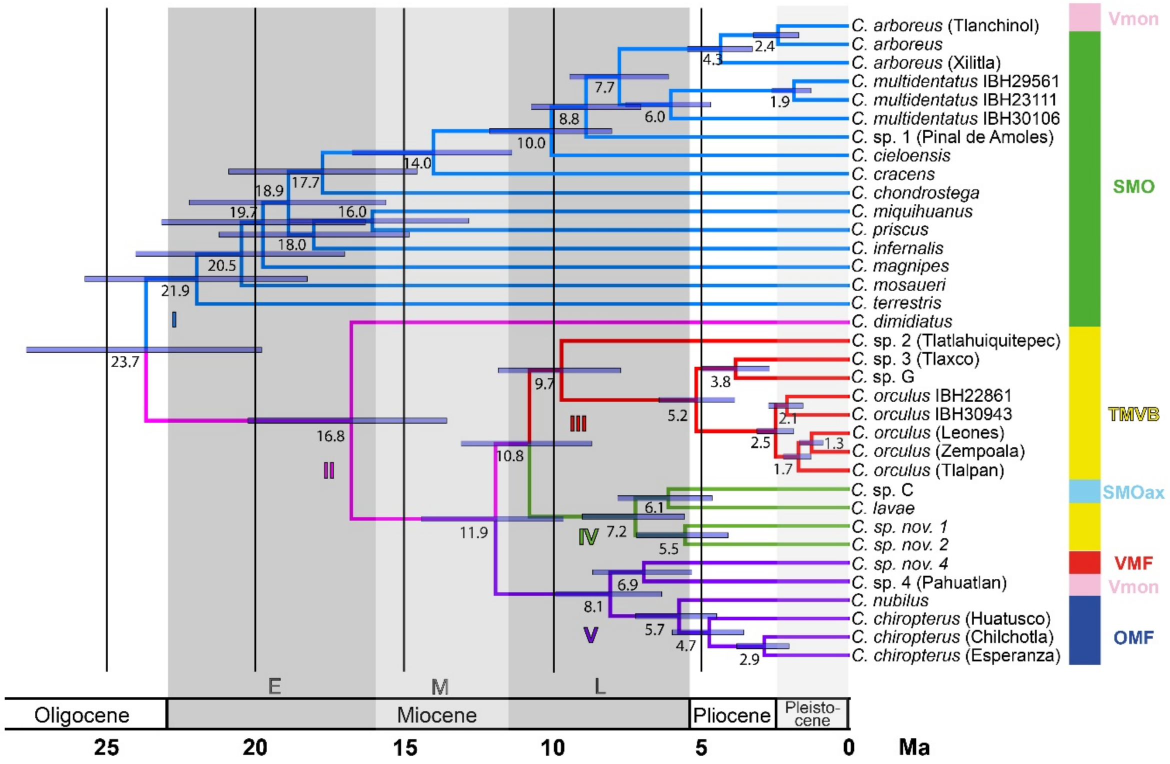

Figure 3. Divergence time estimates for *Chiropterotriton* estimated from the nuclear dataset using BEAST. Bars indicate 95% highest posterior densities of divergence dates, with mean estimates in millions of years ago (Ma) given at nodes. Arabic numbers indicate the median of age estimated for each node. Roman numbers mean the species groups. Color bar in right side indicate the current ecoregion occupied by each taxon; green (SMO), pink (Vmon), yellow (TMVB), blue (SMOax), red (VMF), dark blue (OMF).

3.4 Ancestral range estimation

Ancestral range estimations are given for statistically supported nodes in tree of Figure 4; corners represent the ranges instantaneously after cladogenesis and estimations at the nodes correspond to ranges instantaneously before cladogenesis for the ancestral biogeographic range analyses of nuclear dataset. The most probable ancestral area for *Chiropterotriton* was the SMO (prob=0.43) at 23.7 Ma (Figure 4). For Group I the most probably ancestral area is SMO (prob= 1.0), likewise Group II but with lower probability (prob=0.49). The TMVB is the most probable ancestral area of Group III and IV (prob=0.97) and group V could have occurred on OMF (prob=0.5).

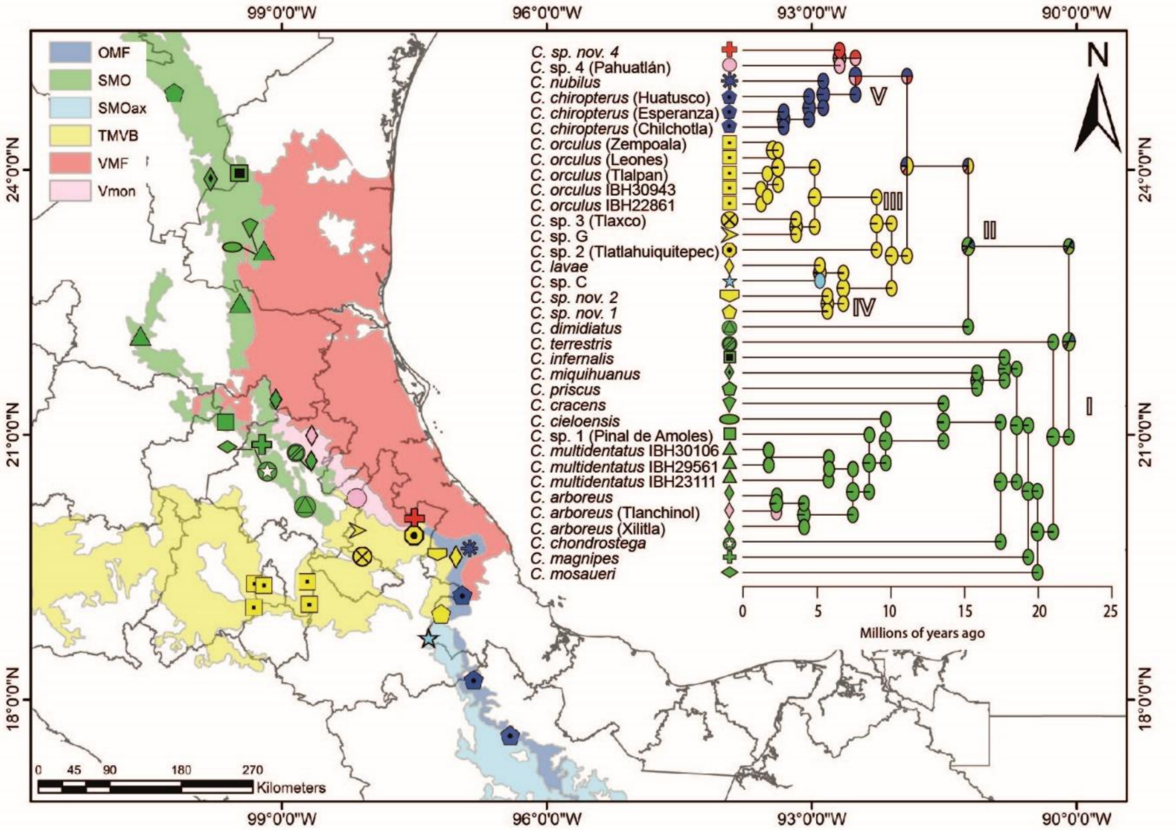

Figure 4. Map represent the geographical distribution for *Chiropterotriton* on the different ecoregions: Oaxacan montane forests (OMF), Sierra Madre Oriental pine-oak forests (SMO), Sierra Madre de Oaxaca pine-oak forests (SMOax), Trans-Mexican Volcanic Belt pine-oak forests (TMVB), Veracruz moist forests (VMF) and Veracruz montane forest (Vmon). Tree shows ancestral area reconstructions analysis from BioGeoBEARS with time scale.

4. DISCUSSION

4.1 Molecular systematics

Our phylogenetic reconstructions based on molecular data are the most highly supported hypothesis for *Chiropterotriton* published to date (Darda 1994; Parra-Olea, 2003; Parra-Olea & Rovito, 2015; Garcia-Castillo et al. 2017; García-Castillo et al. 2018). This study also represents the first study of any bolitoglossine genus that includes both a large portion of the mitochondrial genome and genome-wide nuclear DNA data. Previous phylogenies agree with the existence of two main clades that correspond with the geographic distribution of the species of *Chiropterotriton*, that is, a northern and a southern clade. These reconstructions were based on one or two mtDNA fragments, except the allozyme study by Darda (1994), and their results were strong enough to propose the existence of at least 10 undescribed species (Darda 1994, Parra-Olea & Rovito, 2015; Garcia-Castillo et al. 2017). However, our main goal here was to obtain resolution for deeper nodes, which had not been obtained before and represented a problem for any hypothesis about origin and historical biogeography for this group.

There is a general agreement between mtDNA and nDNA phylogenies, but mtDNA-based hypotheses are not fully resolved, and exhibit some differences between the ML and BI analyses (Figure 1). The main discrepancy between mtDNA and nDNA phylogenies is the relationships within groups I and IV, but species composition in all groups is fully concordant. In one hand, Group I show deeper nodes with low support in mtDNA, which could be explained by the inherent low resolution of mtDNA data for deep divergences (Shen et al., 2016). Our results based on nDNA data suggest a general agreement with nuclear large-scale benefits, given than in nDNA phylogeny most of the clades have strong support. On the other hand, Group IV showed different topologies with well-supported nodes in both mtDNA and nDNA phylogenies. This could be explained by incomplete lineage sorting or introgression in mtDNA. Both processes could show similar phylogenetic patterns (paraphyletic taxa) and is very difficult to determine which of them are responsible for the discrepancies (Funk & Omland, 2003). Incomplete lineage sorting refers to the retention of an ancestral polymorphism due to stochastic sorting which could affect any taxa and loci (Funk & Omland, 2003). This phenomenon could explain paraphyletic results when species divergence is very recent or if the alleles are geographically random distributed (McGuire et al., 2007; Peters et al., 2007). mtDNA introgression could also explain discrepant results in Group IV. Given that the species that form this group occur in neighboring areas, thye could have has a secondary contact and introgression could have occurred. This has been been reported for salamanders previously (Canestrelli et. al., 2014; Johnson et. al., 2015; Bisconti et. al., 2018). However, genetic population framework with coalescent genealogy analyses have been used to discriminate between incompleate lineage sorting and introgression (Peters et al., 2007; Choleva et al., 2014). Because we only had one sample in most of species sampling, there is not possible to test it, but maybe these approaches could explain the most plausible reason of mtDNA and nDNA discrepancies in Group IV.

The remaining differences between mtDNA and nDNA analyses are subtler and they involve mainly population-level structure. *Chiropterotriton orculus* and its sister species, *C.* sp. G and *C.* sp. 3, have slightly shorter branches in mtDNA trees, compared to the nDNA. The differences between *C. orculus* population structure could be associated with contact zones and continued gene flow between populations.

4.2 Historical biogeography

The lack fossil record in plethodontids is one of the main challenges in historical biogeography studies about this group (Wake, 2013; Rovito et al., 2015; Rovito & Parra-Olea 2016). In the absence of accurate calibration points, postulated hypothesis have carried uncertainties derived from distant taxa-divergence times (Rovito et al., 2015). This also entails cautious historical hypothesis, which includes wide-time intervals that limit the inferences about explicit cladogenesis causes. Thus, our hypothesis about historical biogeography of *Chiropterotriton,* which is based on a single calibration point, represents an approximation that is mainly supported by large-scale DNA data analyses. Moreover, the well-studied region where this group occurs and/or other taxa historical reconstructions have allowed to support the hypothesis suggested herein.

The SMO has been subject of biodiversity studies due to its high species richness and notably high endemism, explained by the complex topography, geological and climatic mosaic that offers a great habitat variability (Luna et al., 2004; Sánchez-González et al., 2008; Del Conde Juárez, et al., 2009). In terms of herpetofauna, the SMO has more than

50% of the families that occur in Mexico (Canseco-Márquez et al., 2004). According with our analysis, the SMO is the most likely ancestral area for *Chiropterotriton* (Figure 4). This hypothesis is also supported by the occurrence of 14 species (47% of the extant species), and includes the oldest cladogenesis events in the group (Figure 3). Based on our phylogeny, older lineages correspond to terrestrial species. According to morphological studies, terrestrial forms were considered the primitive for *Chiropterotriton* (Rabb, 1957; Wake & Lynch, 1976), in a time when the most northern-distributed species (*C. priscus*) was considered the most primitive form. Since *C. priscus* and other terrestrial species have the oldest divergence times, we also agree with the hypothesis about terrestrial species as a primitive form in this group.

Our results suggest that the MRCA of *Chiropterotriton* diverged in two lineages and had different dispersal routes: one from central Mexico to the north through the SMO and the second from central Mexico to the south through TMVB and continuing into Oaxaca. The first hypothesis about bolitoglossines origin assumed a North America ancestry, and involved a northern colonization from northern Mexico through SMO to Central America, however, Rovito et al. (2015) do not find any evidence from ancestral range analysis to support this. Instead of that, they suggested a Pacific route of bolitoglossine ancestor that could successfully diversify in TMVB and Nuclear Central America. Even though our results suggest the key role of SMO in *Chiropterotriton* diversification, we also support that the most plausible route was from Central Mexico to Northern and Southern portions.

The SMO ended its formation in the Early Oligocene (32 Ma; Ferrusquia-Villafranca, 1993), before *Chiropterotriton* had the first main split (approximately 23.7 Ma) in the SMO. The global temperature conditions during the Late Oligocene were warmer (≈6°C) than Early and Middle Oligocene (≈2°C), and conditions remained stable through the Early Miocene (Zachos et al., 2001). During the Early Miocene, there were forests of *Abies, Pinus* and *Quercus* forest already in the SMO (Graham, 1999). Our phylogenetic evidences suggest that the oldest cladogenesis events in the group gave rise to eight species of *Chiropterotriton* (Group I + *C. dimidiatus*; Figure 1), most of them are terrestrial (*C. terrestris, C. chondrostega, C. dimidiatus, C. priscus,* and *C. miquiahuanus*) and some cave-dwelling (*C. magnipes, C. mosaueri, C. infernalis*). Even if our estimations are not highly accurate given that they are based on a secondary calibration, environmental conditions support the feasible existence of these species during Miocene and they could have successfully diversified in those suitable conditions. Later on, during Middle to Late Miocene, global climate underwent a cooling trend (Mastretta-Yanes et al., 2015) that could have driven continuous dispersal and isolation that explain the progressive cladogenesis events in SMO, giving place to terrestrial species; *C. cracens, C. cieloensis, C.* sp 1, and more recently (7.7 Ma) *C. multidentatus* and *C. arboreus* (the only arboreal species on SMO).

The TMVB has a notable biodiversity for different groups (Luna et al., 2007). Due to its geographical heterogeneity, geological and biotic history (Mastretta-Yanes et al., 2015), this area is characterized by a wide range of climates and have the most types of vegetation described in Mexico (Rzedowski, 1981). In terms of herpetofauna, it hosts approximately 25% of Mexican species, of which 80% are endemic (Flores-Villela and Canseco-Márquez, 2007). Two divergence events of *Chiropterotriton* (Groups III and IV) occurred in the TMBV during the Late Miocene. On one hand, Group III shows a pattern of biogeographic history similar to that reported for *Pseudoeurycea leprosa*, a sympatric species (Parra-Olea et al., 2012; Velo-Antón et al., 2013). The structure between *P. leprosa* populations show ancestral lineages that occur in northeast TMBV in Tlatlahuiquitepec and Tlaxco, the same localities for *C.* sp. 2 and *C.* sp. 3. Furthermore, the genetic structure in recent populations corresponds to the structure showed in *C. orculus* populations. In this way, the mountain ranges in the TMBV provided habitat refugia during Late Miocene for *C.* sp. 2 and Pleistocene for *C.* sp. 3, *C.* sp. G and the structured populations of *C. orculus* more recently. Group IV diverged on the eastern part of TMVB in the Late Miocene (7.2 Ma) with two arboreal species (*C.* sp. C and *C. lavae*) and two terrestrial (*C. sp. nov. 1* and *C. sp. nov. 2*). The primary (7.2 Ma) and secondary (6.1 and 5.5 Ma) divergences events of this group are older than the stratovolcanoes formation where they now occur (Late Pliocene-Holocene; Ferrari et al., 2012). Apparently, arboreal and terrestrial species could have colonized different microhabitats, and sister species were separated by geographical distance. Later, volcanic structures could arise an altitudinal stratification where terrestrial species established in high elevations while arboreal established in cloud forest at mid elevations.

Group V includes arboreal species that arose in the Late Miocene (8.1 Ma), with species from mid elevations in Puebla and Oaxaca. This group is associated with cloud forest that already occurred during the Miocene (Ornelas et al., 2010), where bromeliads represent a suitable microhabitat for amphibians (Vences & Wake, 2007). There are two main cladogenesis events in this group, one of them in the northern cloud forest and the other likely a colonization pattern from Veracruz to Oaxaca, with most recent divergences in *C. chiropterus* (Figure 3).

4.3 Taxonomic implications

Nuclear DNA was used only by Darda´s study (1994). Subsequent phylogenetic studies were based on few mtDNA loci and they gave rise to multiple species descriptions (Rovito & Parra-Olea, 2015; García-Castillo et. al., 2017; García-Castillo et. al., 2018; Parra-Olea et. al. unpublish). Our analyses with large-scale sequence data support all described species for this group and two of the candidate species previously proposed: *C.* sp C and *C.* sp G (Darda, 1994; Parra-Olea, 2003) but we consider Darda's candidate species *C.* sp J (La Esperanza, Oaxaca), as part of *C. chiropterus* due to the very short genetic divergence and lack of morphological differentiation. The phylogenetic hypotheses of this study are remarkable geographically structured (Figure 2, Figure 3 and Figure 4), except for *C.* sp. K that according with the geographical patterns it would have more affinity for Group V instead of Group III (Figure 2). This current locality information of *C.* sp. K corresponds to the unique collect did in 1980, and has never been found again despite many visits to the locality (Cerro San Felipe, Oaxaca). The uncertainty for this species about its taxonomy and biogeography will remain until a new specimen collection.

Chiropterotriton has notably increased the species number since last five years, following tendencies about amphibian diversity expansion (AmphibiaWeb, 2019). During 2014 to 2019, have been described 11 new species of *Chiropterotriton* (Campbell et al., 2014; Rovito & Parra-Olea, 2015; García-Castillo et al., 2017; García-Castillo et al., 2018; Parra-Olea, et al), most of them based on molecular data as primary criterion. In this istudy, we included four more populations which could be considered as candidate species based on mtDNA and nDNA sequence data; *C.* sp. 1, *C.* sp. 2, *C.* sp. 3 and *C.* sp. 4. Due to our hypothesis is based only on genetic evidence, we recommend a pertinent morphological examination to diagnose these species. Currently, *Chiropterotriton* has 23 described species and seven more candidate species (*C.* sp C, *C.* sp G, *C.* sp K, *C.* sp. 1, *C.* sp. 2, *C.* sp. 3 and *C.* sp. 4), corresponding to 30 species for this group.

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Supplementary material

Table 1S. Voucher information, locality, results from genetic capture and ecoregion assigned to *Chiropterotriton* 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. Asterisks means the data was added from available sequences (GenBank).

Species	**Voucher Number**	**Locality**	**mt genes**	**mtDNA (bp)**	**UCE loci**	**nucDNA (bp)**	**Ecoregion**
C. arboreus	IBH30090	Hidalgo: 6.8 km N (by rd) of Zacualtipán on road to Tianguistengo	13	12250	1381	295050	Vmon
C. arboreus		San Luis Potosí: Xilitla	13	12605	1396	300133	SMO
C. arboreus		Higalgo: Tlanchinol.	13	12662	1403	261680	Vmon

C. aureus	IBH310140	Veracruz: 6.5km (by air) N from Atzalan, ejido de desarrollo urbano Quetzalcoatl	2*	2147*	–	–	OMF
C. sp. nov. 1	IBH30987	Veracruz: 1.1 km N Xometla	13	12473	1449	280400	TMVB
C. chico	MVZ200679	Hidalgo: 3.8 km S Mineral del Chico	1*	529*	–	–	SMO
C. chiropterus	CARIE0719	Veracruz: Huatusco	6	3071	927	240577	OMF
C. chiropterus	GP088	Oaxaca: 67 Km N Guelatao, trail to San Isidro, La Esperanza	6	3947	1132	279333	OMF
C. chiropterus		Oaxaca: Santa María Chilchotla, Sierra Mazateca.	11	7334	1423	298125	OMF
C. chondrostega	IBH30081	Hidalgo: 1.0 km S (by rd) of La Encarnacion on road to MX85, Parque Nacional los Marmoles	13	12472	749	198309	SMO
C. cieloensis	IBH28181	Tamaulipas: 0.2 km E (by air) of Rancho El Cielo, 6.9 km NNW (by air) of center of Gomez Farías, Reserva de la Biosfera El Cielo	13	12120	878	231681	SMO

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	13	12469	1360	284519	SMO
C. dimidiatus	IBH22344	Hidalgo: 4.3 km N hwy 105 at Mineral del Monte.	5	3005	957	243024	SMO
C. infernalis	MVZ269665	Tamps: Cueva del Brinco, Conrado Castillo, ca. 43.5 km SW (by rd) of Ejido Guayabas	13	12560	384	97487	SMO
C. lavae	IBH22342	Veracruz: 200 m N hwy 140 at La Joya	11	7976	1390	297803	TMVB
C. magnipes	IBH30097	Hidalgo: "El Coní", 900m SSE of center of Durango, Municipio Zimapan, Parque Nacional los Marmoles.	13	11831	1516	310526	SMO
C. sp. nov. 4	IBH30112	Puebla: 7.1 km N (by rd) of center of Cuetzalan on road to Yohualichán	13	12551	1515	290366	VMF
C. miquihuanus	IBH30330	Nuevo León: 22.6 km N (by rd) of La Bolsa on road to Zaragoza	13	12224	1002	252087	SMO

C. mosaueri	IBH30108	Hidalgo: "El Coní", 900m SSE of center of Durango, Municipio Zimapan, Parque Nacional los Marmoles	13	12432	1617	309221	SMO
C. multidentatus	IBH23111	San Luis Potosí: Rancho Borbortón	11	10015	1421		SMO
C. multidentatus	IBH30106	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	13	12673	1243	284846	SMO
C. multidentatus	IBH29561	Tamaulipas: 0.2 km E (by air) of Rancho El Cielo, 6.9 km NNW (by air) of center of Gomez Farias, Reserva de la Biosfera El Cielo	13	12673	1385	297457	SMO
C. nubilus	CARIE0739	Veracruz: Bosque Banderilla, Banderilla.	1	1523	874	229389	OMF
C. orculus	IBH22861	Mexico: road to Popocatepetl volcano.	13	10375	1280	296927	TMVB
C. orculus		Ciudad de Mexico: Desierto de los leones	8	6402	1212	291339	TMVB
C. orculus	IBH30943	Mexico: Llano Grande	13	12634	1335	302120	TMVB

C. orculus		Ciudad de Mexico: Bosque de Tlalpan	13	12458	1394	309120	TMVB
C. orculus	IBH31023	Morelos: Lagunas de Zempoala	12	11096	1177	283899	TMVB
C. sp. nov. 2	IBH22395	Veracruz: 15.9 km on microondas road, Las Vigas.	8	3864	1337	280831	TMVB
C. priscus	IBH22253	Nuevo León: 20.7 Km W 18 de Marzo, Cerro Potosi.	13	12504	839	221121	SMO
C. terrestris	GP215	Hidalgo: 5.3 km N hwy 105 at Zacualtipan.	13	9339	983	243962	SMO
C. sp. nov. 3	IBH31030	Veracruz: El Polvorín, 5km W of Villa Aldama	2*	2180*	–	–	SMO
C. sp. C	IBH 14317	Veacruz: Puerto del Aire, El Sumidero.	8	4331	683	182352	SMOax
C. sp. G	IBH31028	Puebla: 20 km S Chignahuapan	13	12572	1125	277809	TMVB
C. sp. K	MVZ173232	Oaxaca: Cerro San Felipe	1*	529*	–	–	SMOax
C. sp. 1		Querétaro: W Pinal de Amoles.	13	12680	1178	281954	SMO
C. sp. 2		Puebla: Tlatlahuiquitepec.	13	12491	1529	311605	TMVB
C. sp. 3	IBH18185	Tlaxcala: Tlaxco.	13	12539	1380	298859	TMVB

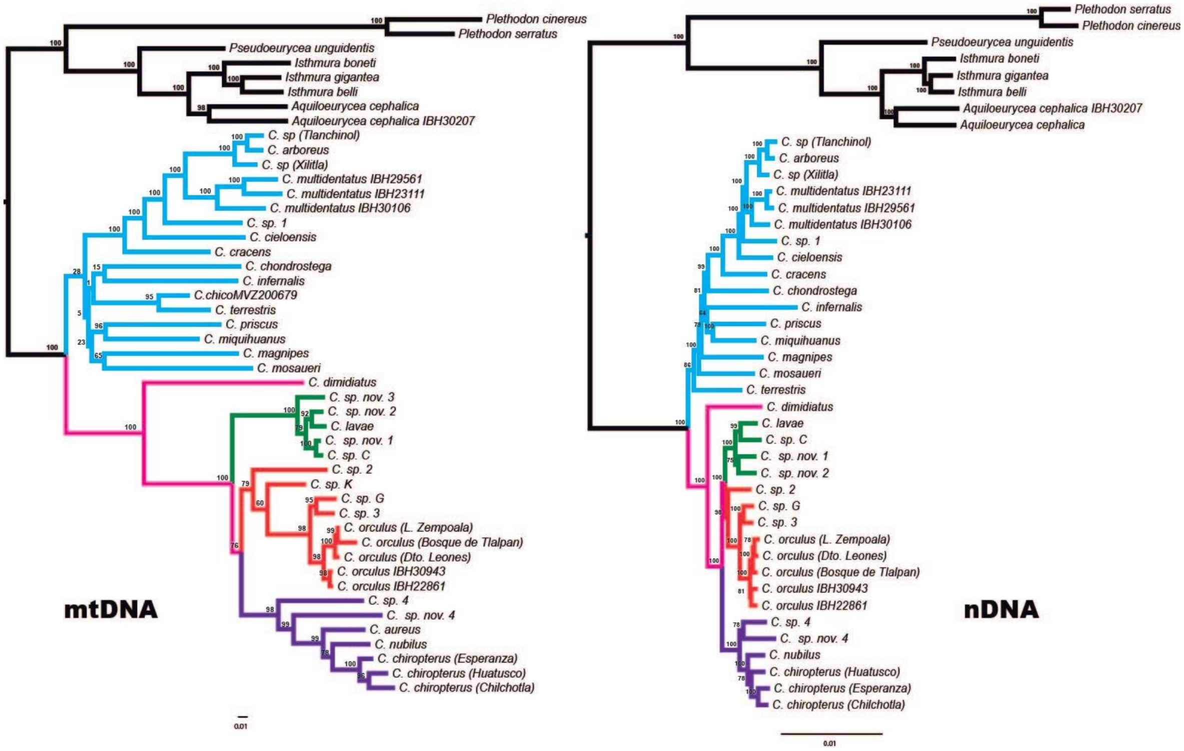
C. sp. 4		Puebla: Pahuatlán del Valle.	13	12641	634	169121	Vmon
Aquiloeurycea cephalica	IBH30207	Hidalgo: 4.1 km S (by rd) of Mineral del Chico on road to Pachuca, Parque Nacional El Chico	13	12681	974	236229	–
Aquiloeurycea cephalica			13	12551	873	222143	–
Isthmura belli			13	11721	959	232136	–
Isthmura boneti			8	5809	616	164200	–
Isthmura gigantea			11	8079	748	195381	–
Plethodon cinereus			1	415	990	135783	–
Plethodon serratus			10	7782	1209	164298	–
Pseudoeurycea unguidentis			6	2495	537	139411	–

Table 2S. Results from substitution model selection with partition scheme, averages of nucleotides recovered by genes (above) and ranges (below).

MT GENES	CHARSET	SUSTITUTION MODEL	NUCLEOTIDS (BP)
ATP	atp6_1	GTR+I+G	630.2 (287–618)
	atp6_2	GTR+I+G	
	atp6_3	GTR+I+G	
COB	cob_1	GTR+I+G	1059.4 (404–1125)
	cob_2	GTR+I+G	
	cob_3	HKY+I+G	

COX 1	cox1_1	GTR+I+G	1277.6 (310–1525)
	cox1_2	HKY+I+G	
	cox1_3	GTR+G	
COX 2	cox2_1	GTR+I+G	607.5 (126–678)
	cox2_2	GTR+I+G	
	cox2_3	GTR+G	
COX 3	cox3_1	GTR+I+G	752.2 (471–783)
	cox3_2	HKY+I+G	
	cox3_3	GTR+I+G	
NAD 1	nad1_1	GTR+I+G	856.8 (244–954)
	nad1_2	HKY+I+G	
	nad1_3	GTR+I+G	
NAD 2	nad2_1	GTR+I+G	947.4 (432–1035)
	nad2_2	GTR+I+G	
	nad2_3	GTR+I+G	
NAD 3	nad3_1	GTR+I+G	327.9 (198–345)
	nad3_2	GTR+I+G	
	nad3_3	GTR+G	
NAD 4	nad4_1	GTR+I+G	1216.9 (379–1365)
	nad4_2	HKY+I+G	
	nad4_3	GTR+I+G	
NAD 4L	nad4l_1	GTR+I+G	284.6 (129–294)
	nad4l_2	GTR+I+G	
	nad4l_3	GTR+I+G	
NAD 5	nad5_1	GTR+I+G	1523.3 (305–1800)
	nad5_2	GTR+I+G	
	nad5_3	GTR+I+G	
NAD 6	nad6_1	GTR+G	498.9 (195–521)
	nad6_2	GTR+G	
	nad6_3	HKY+I+G	
RRNL	rrnL	GTR+I+G	1492.6 (749–1583)

Figure S1. Maximum Likelihood analysis from mtDNA and nDNA data. Node numbers mean bootstrap values.



DISCUSIÓN GENERAL Y CONCLUSIONES

El número de especies de salamandras pletodontidas en México continúa incrementándose de manera constante debido a nuevos descubrimientos y revisiones taxonómicas de complejos de especies (*p. ej.,* Rovito et al., 2015; Parra-Olea et al., 2016; Sandoval-Comte et al., 2017). Gracias a ello, el género *Chiropterotriton* ha incrementado notablemente el número de especies descritas en los últimos cinco años, de 12 a 23 especies (considerando las últimas cinco especies que se encuentran en proceso de publicación). Sin embargo, aún existen siete especies candidatas que no han sido descritas formalmente. Tres de ellas propuestas por Darda (1994) hace más de una década: *C.* sp. C (Veracruz), *C.* sp. G (Puebla) y *C.* sp. K (Oaxaca). Y cuatro que son el parte de los resultados del presente trabajo: *C.* sp. 1 (Querétaro), *C.* sp. 2 (Puebla), *C.* sp. 3 (Tlaxcala) y *C.* sp. 4 (Puebla).

A pesar de que existen muchas especies candidatas en salamandras a partir de herramientas moleculares, una de las principales causas en el rezago en las descripciones de es la falta de material suficiente para las diagnosis. Esto ocurre dado a que algunas especies son raras (*p. ej., Bolitoglossa jacksonii*), incluso existen registros de especies que después de su descripción no han sido encontradas nuevamente (*p. ej., Pseudoeurycea aquatica*). Idealmente las diagnosis deben consistir en una serie de individuos representativos de las especies, sin embargo, algunas de las especies descritas han sido diagnosticadas con muy pocos ejemplares (de 1 a 3 individuos) tales como *Bolitoglosa aurae, Isthmura corrugata, Cryptotriton necopinus, Nototriton costaricense,* y *Nototriton tomamorum*. Aunque las descripciones basadas en pocos individuos no son ideales, éstas suelen incluir evidencia sólida tanto morfológica como molecular, la cual diagnostica adecuadamente a la especie a pesar de que la variación de sus caracteres se mantenga desconocida. Asi mismo, algunas veces las revisiones taxónomicas son necesarias para resolver complejos de especies. Tal es el caso de *Chiropterotriton,* que al igual que otros géneros de salamandras como *Batrachoseps* (*p. ej.,* Jockush et al., 2001), *Eurycea* (*p. ej.,* Wray et al., 2017), *Plethodon* (*p. ej.* Highton & Peabody, 2000), requirieron de revisiones taxonómicas para poder describir una serie de especies.

Los resultados taxonómicos desarrollados en este trabajo involucran tablas morfométricas, imágenes detalladas de caracteres morfológicos e hipótesis filogenéticas basadas en caracteres moleculares, los cuales apoyan la validez de los taxones descritos y detalla los caracteres de especies anteriormente descritas. Además, los datos han quedado disponibles para la identificación de especímenes en campo e incluso para la descripción de nuevas especies, lo que representa un acervo significativo de información para el conocimiento y desarrollo de la taxonomía en salamandras mexicanas.

Chiropterotriton está formado por dos grupos principales con afinidad filogenética y geográfica. Por un lado, están las especies distribuidas en la SMO que fueron originadas tras los eventos de cladogénesis más antiguos del grupo y presentan una diferenciación morfológica notable, de las cuales se describieron cuatro recientemente; *C. miquihuanus, C. cieloensis, C. infernalis* y *C. chico* (Campbell et al., 2014; Rovito & Parra-Olea, 2015; García-Castillo et al., 2017). Por otro lado, las especies de la FVTM y de las montañas del norte de Oaxaca son más recientes y morfológicamente conservadas, y particularmente fueron la principal fuente del estudio taxonómico de este trabajo. Se re-describieron tres especies (*C. chiropterus, C. lavae* y *C. orculus*) y se describieron siete especies nuevas (*C. aureus, C. nubilus, C. sp. nov. 1, C. sp. nov. 2, C. sp. nov. 3, C. sp. nov. 4, C. sp. nov. 5*).

La secuenciación masiva de ADN representa una herramienta rápida y eficaz para obtener una gran cantidad de caracteres que ayudan a descifrar preguntas de carácter evolutivo (Liu et al., 2012). Particularmente, el enriquecimiento con los elementos genómicos ultraconservados (UCEs por sus siglas en inglés) está dirigido a obtener ADN nuclear en grandes cantidades, y con atributos de variabilidad a nivel inter e intraespecífico (Faircloth et al., 2012). Este método ha sido utilizado para el estudio de aves (McCormack, Tsai, & Faircloth, 2016), peces (Faircloth et al., 2013; Gilbert et al., 2015), tortugas (Crawford et al., 2015), artrópodos (Faircloth et al., 2015) y mamíferos (McCormack et al. 2012). Aunado a ello, este método ofrece la posibilidad de obtener el genoma mitocondrial que permite tener un panorama más amplio sobre los procesos evolutivos que ocurren en los distintos taxones (Zarza et al., 2017). De esta manera, el enriquecimiento con UCEs fue eficiente para poder obtener una filogenia robusta que resolviera las preguntas de este trabajo. Cabe mencionar que dadas las características del tamaño genómico de las salamandras (~10Gb a ~120Gb; Gregory, 2019), no son aptas para la secuenciación masiva con algunos de los métodos actuales. La obtención de una gran parte del genoma mitocondrial fue la clave para poder detectar el contacto secundario que ocurrió en el género *Chiropterotriton* y además permitió incluir taxones que no pudieron ser integrados a la preparación de librerías para UCEs.

Gracias a la obtención de la gran cantidad de datos genómicos, fue posible la reconstrucción biogeográfica del género *Chiropterotriton*. Los análisis de áreas ancestrales revelaron que lo más probable es que el ancestro de este grupo se haya distribuido en la SMO, y posteriormente fue colonizando la FVTM de Centro-Oeste, para continuar por la porción norte de las montañas de Oaxaca. Esto apoya la hipótesis sobre la diversificación de algunos bolitoglossinos desde el centro de México (Rovito et al., 2015). Uno de los estudios más comparables con el presente trabajo es el que se realizó con el género *Isthmura* (Bryson et. al., 2018), el cual también sugiere una dispersión desde el centro México, pero con dirección Este-Oeste y hasta el Noroeste del país. Otros géneros como *Bolitoglossa, Pseudoeurycea* y *Thorius*, tienen una distribución interesante y además están conformados por numerosas especies, por lo que son sin duda eslabones clave para reconstruir la historia biogeográfica de los bolitoglossinos en México.

Por otra parte, las estimaciones de tiempos de divergencia en este trabajo también son aportaciones valiosas al conocimiento de la historia evolutiva de las salamandras en México. Sin embargo, estas inferencias están basadas en un solo punto de calibración secundaria, lo que representa un amplio intervalo de incertidumbre que limita la precisión en los tiempos de divergencia estimados (Rovito & Parra-Olea, 2016). A pesar de esto, generamos una hipótesis en donde sugerimos que este género tuvo dos series de eventos de diversificación; el primero ocurrió en el Mioceno temprano donde los linajes de la SMO se dispersaron hacia el norte, y la segunda ocurrió durante el Mioceno tardío y el Plioceno, dando origen a los linajes más recientes de la SMO, casi simultáneamente también se originaron los linajes en la FVTM y montañas del norte de Oaxaca. Durante esta época, procesos geológicos y climáticos pudieron dirigir la diversificación de *Chiropterotriton* tal como ocurrió en otros grupos biológicos (Mastretta-Yanes et al., 2015). Particularmente, las salamandras pletodontidas tienen una afinidad por los hábitats húmedos y templados, con una fuerte asociación a los bosques de pino, pino-encino y bosques nublados. Y ciertamente, estos ambientes estaban establecidos durante los primeros eventos de cladogénesis (Mioceno Temprano) estimados en este trabajo, por lo cual pudieron haber representado un corredor para su dispersión, tal como se ha propuesto en otras hipótesis de salamandras (Rovito & Parra-Olea, 2016; Rovito, 2017).

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