



Amphibians and Reptiles from Cloud Forest at Cumbre de Tonalixco in the Central Veracruz Highlands of Mexico

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Veracruz has many geographical and geological elements that produce wide environmental variation and promote high biodiversity (Soto-Esparza and Geissert-Kientz 2011). This wide biodiversity is reflected in several biological groups such as seed plants (Rzedowski 1991, 1993), birds (Navarro-Sigüenza et al. 2014), and herpetofauna (Flores-Villela and García-Vázquez 2014; Parra-Olea et al. 2014). The amphibian richness of Veracruz ranks third among Mexican states with a total of 96 species, 55 of them endemic (Parra-Olea et al. 2014). The reptilian diversity of Veracruz also ranks third with 200 species (Flores-Villela and García-Vázquez 2014).

Floral biodiversity in Veracruz is also notable, with 18 types of primary vegetation (Castillo-Campos et al. 2011). The cloud forest is one of the most complex and diverse vegetative assemblies in the state (Williams-Linera 2007; Williams-Linera et al. 2007; Goyenechea and Gual-Díaz 2014) with its humid climate covering elevations of 900–2,300 m asl across 135–271 ha (Castillo-Campos et al. 2011). Unfortunately, the cloud forest is also one of the most threatened ecosystems in Mexico due to exhaustive deforestation (CONABIO 2010), and its area has been continually reduced over the years in central Veracruz. Nevertheless some preserved areas still support unexplored biodiversity.

The municipality of Rafael Delgado in central Veracruz is located near some historically important localities, such as Cuautlapan in the municipality of Ixtaczoquitlán (Smith 1939; Taylor 1940; Cerón de la Luz et al. 2016) and Petlalcala in the municipality of San Andrés Tenejapan (de la Torre-Loranca 1999, Kelly-Hernández 2017) (Fig. 1). Recently, several species have been reported for the first time in localities near Rafael Delgado: Townsend’s Salamander, *Parvimolge townsendi* (Dunn 1922); Firschein’s False Brook Salamander, *Pseudoeurycea firscheini* Shannon and Werler 1955; Veracruz

Pigmy Salamander, *Thorius pennatulus* Cope 1869; and the Coffee Grove Salamander, *Aquiloerycea cafetalera* (Parra-Olea, Rovito, Márquez-Valdelmar, Cruz, Murrieta-Galindo, and Wake 2010) (Contreras-Calvario et al. 2019). Another species, the Greater Bromeliad Treefrog, *Bromeliohyla dendroscarta* (Taylor 1940), was rediscovered at its type locality in Cuautlapan after 47 years (García-Bañuelos et al. 2017), and the Granite-colored Salamander, *Pseudoeurycea granitum* García-Bañuelos, Aguilar-López, Kelly-Hernandez, Vásquez-Cruz, Pineda-Arredondo, and Rovito 2020, newly described. Finally, Rafael Delgado also claims the first records of the

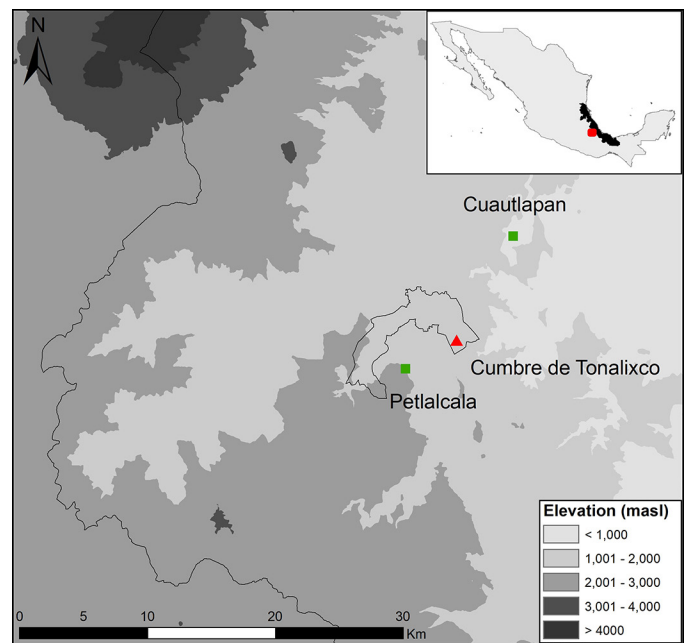


Fig. 1. Map showing the location of the study site at Cumbre de Tonalixco (red triangle), Municipality of Rafael Delgado (thin outline), Veracruz, and sites of previous herpetofaunal studies (green squares).

Veracruz Pigmy Salamander, *Thorius pennatulus*, Gloomy Mountain Stream Frog, *Ptychohyala zophodes* Campbell and Duellman 2000, and Scheide’s Anole, *Anolis schiedii* (Wiegmann 1834), which were recently published (Peralta-Hernández and Perea-Pérez 2019; Peralta-Hernández et al. 2019; Taval-Velázquez et al. 2020). The lack of sampling in areas with probable high biodiversity, such as Rafael Delgado, highlights the importance of new field surveys and revisions of herpetofaunal occurrence records.

In this study, we sampled the locality of Cumbre de Tonalixco (18°47'47.00"N, 97°3'45.00"W; WGS 84; elev. 1,406 m asl), located in eastern Rafael Delgado, Veracruz (Fig.

1). The locality has several streams, cloud forest vegetation dominated by oaks (*Quercus*) and sweetgums (*Liquidambar*), and a number of coffee plantations. We conducted five field trips from September 2019 to January 2020 in an area of approximately 5.4 km² at elevations from 1,350–1,635 m asl. All surveys were daytime only, with visual searching under rocks, logs, leaf litter, and on epiphytic vegetation.

Individuals were identified and photographed; all identities were confirmed using Flores-Villela et al. (1995). Due to uncertainty when identifying two salamanders, we collected a tissue sample (tail fragment) from each specimen for molecular analysis. In the laboratory, we extracted DNA using the

Table 1. Amphibians and reptiles from the locality Cumbre de Tonalixco, Municipality of Rafael Delgado, Veracruz, Mexico. Risk categories of the IUCN Red List (CR = Critically Endangered, EN = Endangered, VU = Vulnerable, NT = Near Threatened, LC = Least Concern, DD = Data Deficient, NE = not evaluated) and under Mexican law (NOM-059-SEMARNAT-2010) (P = extinction risk, A = threatened, Pr = special protection, NI = not included). For new records for the Municipality of Rafael Delgado, distances to the nearest previously documented record (source) and range extensions according to IUCN Red List polygons (source).

Taxon	IUCN Red List	Mexican Law	Distance (km) (source)	Range Extension (source)
AMPHIBIA				
Caudata: Plethodontidae				
<i>Parvimolge townsendi</i>	CR	P	4.3 NE (Kelly-Hernández 2017)	Parra-Olea et al. (2008)
<i>Pseudoeurycea granitum</i>	NE	NI	4.3 NE (García-Bañuelos et al. 2020)	
<i>Pseudoeurycea werleri</i>	EN	Pr	143 NW (Gual et al. 2013)	IUCN SSC Amphibian Specialist Group (2016)
Anura: Hylidae				
<i>Bromeliohyla dendroscarta</i>	EN	Pr	4.8 SE (Flores-Villela 1998)	IUCN SSC Amphibian Specialist Group (2020)
<i>Ptychohyala zophodes</i>	VU	NI		
<i>Rheohyla miotympanum</i>	NT	NI	4.3 NE (Kelly-Hernández 2017)	
Anura: Craugastoridae				
<i>Craugastor spatulatus</i>	EN	Pr	8.4 SW (Feeney 2019)	Santos-Barrera and Parra-Olea (2004)
<i>Craugastor rhodopis</i>	VU	NI		
REPTILIA				
Squamata: Dactyloidae				
<i>Anolis laevis</i>	NE	NI	4.3 NE (Kelly-Hernández 2017)	
<i>Anolis schiedii</i>	DD	Pr		
Squamata: Phrynosomatidae				
<i>Sceloporus variabilis</i>	LC	NI		
Squamata: Colubridae				
<i>Stenorrhina degenhardtii</i>	LC	NI		
Squamata: Dipsadidae				
<i>Coniophanes fissidens</i>	LC	NI	3.4 NE (Feeney 2019)	Flores-Villela et al. (2019)
Squamata: Viperidae				
<i>Metlapilcoatlus nummifer</i>	LC	A	4.3 NE (Kelly-Hernández 2017)	

modified protocol of phenol-chloroform (Sambrook and Russell 2006) and amplified the mitochondrial gene cytochrome b (cyt *b*). We used primers MVZ15 and MVZ16 (Moritz et al. 1992) with reactions at 94 °C for 5 min, 38 cycles of 94 °C for 30 sec, 48 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 8 min. Sequences were assembled and exported to fasta files in Sequencher 5.0.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and we used the BLAST analysis tool (National Center for Biotechnology Information, Bethesda, Maryland, USA) to compare our sequences with GenBank data. The BLAST matched Townsend's Salamander (*Parvimolge townsendi*) and Werler's Salamander (*Pseudoeurycea werleri* Darling and Smith 1954) sequences, with 94.9 and 94.2% identities, respectively (0% gaps and an e-value of 0.0 in both analy-

ses). Sequences were deposited in the GenBank repository (MW206671–2).

We recorded 14 herpetofaunal species at Cumbre de Tonalixco, nine for the first time in the municipality of Rafael Delgado. The newly reported taxa include six amphibian species in three families: Craugastoridae (1), Hylidae (2), and Plethodontidae (3), and three reptilian species in three families: Dactyloidae (1), Dipsadidae (1), and Viperidae (1) (Table 1). We found only two species previously reported from Cumbre de Tonalixco (Peralta-Hernández and Perea-Perez 2019; Peralta-Hernández et al. 2019), although we did not find any record for this locality in GBIF (2020). However, 13 additional records were found for Rafael Delgado (GBIF 2020; Serna-Lagunes 2005; Taval-Velázquez et al. 2020), resulting in a total of 24 species recorded in the municipality.

Nine species are registered for the first time in the municipality of Rafael Delgado (Figs. 2–4), of which *Parvimolge townsendi* (Fig. 2A) was the most abundant with seven registered individuals, followed by *Pseudoeurycea werleri* (Fig. 2C) with four individuals, then the Small-eared Treefrog (*Rheohyla myotimpanum*) (Fig. 3F) and Yellow-bellied Snake (*Coniophanes fissidens*) (Fig. 4I) with two each. Finally, we found only single individuals of the Greater Bromeliad Treefrog (Fig. 2A), Spatulate Robber Frog (*Craugastor spatulatus*) (Fig. 3D), Granite-colored Salamander (Fig. 2B), White Anole (*Anolis laevis*) (Fig. 4G), and Jumping Pitviper (*Metlapilcoatlus nummifer*) (Fig. 4H).

Our work increases the available knowledge of herpetofaunal diversity in a cloud forest in central Veracruz and provides an opportunity to promote the conservation of biodiversity in the region. Unfortunately, most of the amphibian species recorded for the first time in Rafael Delgado are listed in IUCN Red List risk categories (IUCN 2020) and are, along with many species of amphibians, at risk of disappearing (Wake and Vredenburg 2008). However, Cumbre de Tonalixco represents an important area for species conservation, especially for two species of salamanders (*Pseudoeurycea granitum* and *P. werleri*. due to its relatively well-preserved



Fig. 2. Species recorded for the first time in the Municipality of Rafael Delgado, Veracruz, Mexico (salamanders): (A) Townsend's Salamander (*Parvimolge townsendi*), (B) Granite-colored Salamander (*Pseudoeurycea granitum*), and (C) Werler's Salamander (*P. werleri*). Photographs by Aldo López-Velázquez (A, C) and Rafael Peralta-Hernández (B).

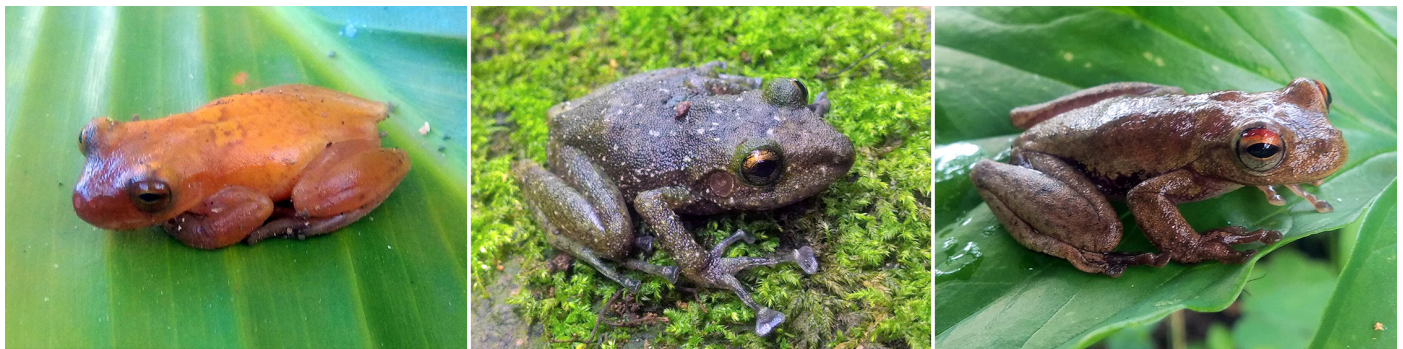


Fig. 3. Species recorded for the first time in the Municipality of Rafael Delgado, Veracruz, Mexico (anurans): (D) Greater Bromeliad Treefrog (*Bromeliophyla dendrocarta*), (E) Spatulate Robber Frog (*Craugastor spatulatus*), and (F) Small-eared Treefrog (*Rheohyla myotimpanum*). Photographs by Rafael Peralta-Hernández.



Fig. 4. Species recorded for the first time in the Municipality of Rafael Delgado, Veracruz, Mexico (reptiles): (G) White Anole (*Anolis laeiventris*), (H) Jumping Pitviper (*Metlapilcoatlus nummifer*), and (I) Yellow-bellied Snake (*Coniophanes fissidens*). Photographs by Rafael Peralta-Hernández.

forest. For *P. granitum*, we add a new locality to the three recorded in the recent description of the species (García-Bañuelos et al. 2020). For *P. werleri*, our new record represents a considerable extension of the previously known distribution and the high genetic divergence found is suggestive of a structured population. Future studies and fieldwork are needed to find more of these salamanders in areas between sites that could provide valuable information regarding its conservation status.

Despite the perilous state of cloud forest in the highlands of central Veracruz, it is a continuous source of new biological information. Even our records from a single locality increased the known number of herpetofaunal species in the municipal-

ity by nearly 40%, highlighting the importance of exploring remaining forested areas.

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