The Sierra Madre del Sur in Oaxaca and Guerrero, Mexico, supports a diverse herpetofauna that includes numerous anuran species. Despite the presence of the frog pathogen *Batrachochytrium dendrobatidis* for at least the past 15 years, many of the native anuran species still are present and obviously are reproducing successfully. In the following contribution the authors present their results of recent fieldwork on adult frogs and tadpoles along the south-facing side of the Sierra Madre del Sur in Oaxaca. Pictured here is an adult individual of *Lithobates sierramadrensis* trying to hide underwater in a rocky stream near San Gabriel Mixtepec.

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A survey of tadpoles and adult anurans in the Sierra Madre del Sur of Oaxaca, Mexico (Amphibia: Anura)

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Abstract: We conducted a survey of tadpoles and adult anurans along several streams in the Sierra Madre del Sur, Oaxaca, Mexico. We collected tadpoles of one species of bufonid (Incilius occidentalis, \( n = 14 \) tadpoles), four species of hylids (Exerodonta juanitae, \( n = 15 \); Megastomatohyla pellita, \( n = 5 \); Ptychohyla leonhardschultzei, \( n = 56 \); Sarcohyla pentheter, \( n = 24 \)), and two species of ranids (Lithobates forreri, \( n = 9 \); L. sierramadrensis, \( n = 13 \)). Furthermore, we collected adults of Craugastor rugulosus, Exerodonta juanitae, E. sumichrasti, Lithobates sierramadrensis, Megastomatohyla pellita, Ptychohyla leonhardschultzei, and Sarcohyla pentheter. We determined the taxonomic identities of the tadpoles by matching the tadpole DNA barcodes (16S) with the respective barcodes of adults. We provide descriptions and illustrations of the larval morphology of these anurans, including their coloration in life. The tadpoles we collected were screened for symptoms of *Batrachochytrium dendrobatidis* (*Bd*) infection, by visually inspecting the keratinous mouth parts under a dissecting microscope. Except for *Incilius occidentalis* and *Megastomatohyla pellita*, we found evidence of *Bd* infection in the tadpoles of all the species collected. The symptoms ranged from minor loss of keratodont rows to complete loss of these rows and a varying degree of bleaching on the beak. Generally, we found that larger tadpoles (considering both staging and size) tend to show a higher frequency and severity of the chytrid symptoms. In March of 2013, *Ptychohyla leonhardschultzei* and *Sarcohyla pentheter* were the most abundant species at our study sites, both in the number of tadpoles and adults.

Key Words: Anuran larvae, *Batrachochytrium dendrobatidis*, Craugastor rugulosus, Exerodonta juanitae, Exerodonta sumichrasti, Incilius occidentalis, Lithobates forreri, Lithobates sierramadrensis, Megastomatohyla pellita, Ptychohyla leonhardschultzei, Sarcohyla pentheter

Resumen: Realizamos una búsqueda de renacuajos y adultos de anuros a lo largo de varios arroyos en la Sierra Madre del Sur en Oaxaca. Colectamos renacuajos de una especie de bufónido (*Incilius occidentalis*, \( n = 14 \) renacuajos), cuatro especies de hylidos (*Exerodonta juanitae*, \( n = 15 \); *Megastomatohyla pellita*, \( n = 5 \); *Ptychohyla leonhardschultzei*, \( n = 56 \); *Sarcohyla pentheter*, \( n = 24 \)); y dos especies de ránidos (*Lithobates forreri*, \( n = 9 \); *L. sierramadrensis*, \( n = 13 \)). Además, colectamos adultos de *Craugastor rugulosus*, *Exerodonta juanitae*, *E. sumichrasti*, *Lithobates sierramadrensis*, *Megastomatohyla pellita*, *Exerodonta juanitae*, *E. sumichrasti*, *Lithobates sierramadrensis*, *Megastomatohyla pellita*, *Ptychohyla leonhardschultzei*, and *Sarcohyla pentheter*. 

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**Ptychohyla leonhardschultzei** y **Sarcohyla pentheter.** La identidad taxonómica de los renacuajos se determinó comparando los códigos de barras de DNA (16S) con el de los adultos. Se muestran descripciones e ilustraciones de la morfología de las larvas de los anuros, incluyendo la coloración en vida. Los renacuajos colectados fueron revisados para buscar síntomas de infección de *Batrachochytrium dendrobatidis* (*Bd*) mediante inspección visual de la parte queratinizada de la boca utilizando un microscopio de disección. Con excepción de Incilius occidentalis y Megastomatohyla pellita encontramos evidencias de infecciones de (*Bd*) en los renacuajos de todas las especies colectadas. Los síntomas variaban desde una pérdida menor de las hileras queratodontas hasta la pérdida total de éstas y varios grados de blanqueamiento del pico. Generalmente encontramos la tendencia de que los renacuajos más grandes (considerando tanto en talla como en estadio larval) mostraban una mayor frecuencia y severidad de los síntomas del quitridio. En marzo de 2013 *Ptychohyla leonhardschultzei* y *Sarcohyla pentheter* fueron las especies más abundantes en nuestros sitios de estudio, tanto de cantidad de larvas como de adultos.

**Palabras Claves:** *Batrachochytrium dendrobatidis*, *Craugastor rugulosus*, *Exerodonta juanitae*, *Exerodonta sumichrasti*, *Incilius occidentalis*, *Lithobates forrerii*, *Lithobates sierramadrensis*, *Mega-stomatohyla pellita*, *Ptychohyla leonhardschultzei*, renacuajos, *Sarcohyla pentheter*

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**INTRODUCTION**

The Sierra Madre del Sur is a mountain chain than extends about 600 km parallel to the coastline of Mexico in the states of Guerrero and Oaxaca, and covers an area of ca. 9,000 km². The lowlands of the Balsas-Tepalcatepec Valley and the Isthmus of Tehuantepec, respectively, define the western and eastern ends of the sierra. The highest peak in the Sierra Madre del Sur is 3,703 m, and is located in central Guerrero. In general, the western portion of this mountain chain is drier than the more mesic eastern portion, with the annual precipitation ranging from 800 to 1,600 mm, depending on the location.

The primary vegetation associations in this highland area are oak forest, pine-oak forest and some dispersed pine forest, as well as cloud forest in the more mesic regions. In Oaxaca, the Sierra Madre del Sur supports a rich anuran fauna with a total of 42 currently recognized anuran species, of which many are endemic to this region (Mata-Silva et al. 2015). The presence of the pathogen *Batrachochytrium dendrobatidis* (*Bd*) in this area was documented about 15 years ago, along with evidence for the decline and extirpation of populations of several species of frogs in the Sierra Madre del Sur of Oaxaca (Lips et al. 2004). Recent field surveys, however, have shown that most frog species remain present in this region (Delia et al., 2013; Caviedes-Solis et al., 2014; Köhler et al., 2015). During field studies along the Pacific slopes of the Sierra Madre del Sur in Oaxaca, we collected adults and tadpoles of anurans along several streams, and screened the tadpoles for symptoms of *Bd* infection. Furthermore, we provide descriptions of the collected tadpoles, both in life and in preservative.

**METHODS**

In March of 2013, GK and RGTP conducted fieldwork in Oaxaca (see Appendix I and Fig. 1 for visited localities). We searched for frogs and tadpoles along streams at night and during the day, and used nets for collecting
the tadpoles. Before preservation, we photographed the tadpoles and their mouthparts using a small glass tank and a digital SLR camera with a Canon ME 65 mm macro lens. We took photographs of the lateral view (left side of a specimen to show the spiracle) of untreated or anaesthetized specimens, and those of the mouth parts using freshly killed specimens after their mouth parts had been fixed in an open position with a drop of full-strength formalin. The tadpoles we collected were anaesthetized and euthanized using an aqueous solution of tricaine methane sulphonate (MS-222), buffered with sodium bicarbonate. We fixed and preserved all of the tadpoles in 4% formalin, collected tissue samples (tip of the tail or one hind leg in advanced tadpole stages) and preserved these in 98% non-denatured ethanol for DNA extraction, prior to treatment with formalin. We preserved the adult frogs by injecting a solution of 5 mL of absolute (i.e., 36%) formalin in 1 L of 96% ethanol into the body cavity and thighs of the animal, which was stored immediately after fixation in 70% ethanol. We deposited the specimens collected in the Instituto de Biología (IBH), Universidad Nacional Autónoma de México (UNAM), México D.F., Mexico, and in the herpetological collection of the Senckenberg Research Institute Frankfurt (SMF), Germany. See Table 1 and Appendix 1 for the samples sizes of the specimens collected. We verified the species identification of the collected tadpoles by matching 16S barcodes with the respective barcodes of adult frogs via a Maximum-Likelihood analysis. We generated the barcodes of the adult frogs from specimens we secured at the same sites where the tadpoles were collected. For species where we failed to collect adult conspecifics, we matched the tadpole barcodes with sequences of conspecifics we collected elsewhere (i.e., for *Lithobates forreri*, IBH 26979–80) or with 16S sequences deposited at GenBank (i.e., for *Incilius occidentalis*, UTA 13543). We identified the adult frogs encountered in this study based on the keys and descriptions in Webb (1978), Duellman (1970; 2001), and Hillis and de Sá (1988). The capitalized colors

![Fig. 1. Study sites in the Sierra Madre del Sur (Oaxaca, Mexico). Site numbers correspond to those in Table 1.](image-url)
Table 1. Collecting sites visited during the present study. The site numbers correspond to those in Fig. 1.

<table>
<thead>
<tr>
<th>Site No.</th>
<th>Locality</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation</th>
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<tr>
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</tr>
<tr>
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<td>-96.5321</td>
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<td>Near Buena Vista</td>
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</tr>
<tr>
<td>9</td>
<td>Near La Soledad</td>
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<tr>
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<tr>
<td>11</td>
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<td>1,290 m</td>
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<td>Tierra Blanca</td>
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<td>-96.57749</td>
<td>1,369 m</td>
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The morphological data of tadpoles of species not studied by us but used for constructing the identification key to the tadpoles of the Pacific versant of the Sierra Madre del Sur of Oaxaca, Mexico, were taken from Duellman (1970; 2001), Caldwell (1974), Kaplan et al. (2006), and Köhler (2011). In the color descriptions, the terminology of markings follows Köhler (2012). The terminology of tadpole morphology follows Altig (1970) and McDiarmid and Altig (1999), except for using the term keratodonts instead of teeth, following Dubois (1995). We used Gosner’s (1960) classification to identify the developmental stages. We also used the abbreviations LKRF (labial keratodont row formula) and Bd (Batrachochytrium dendrobatidis). We present all of the coordinates in decimal degrees and WGS 1984 datum, and all the elevations in meters above sea level. We screened all of the tadpoles collected for symptoms of infection with Bd by visual inspecting the keratinous mouth parts under a dissecting microscope. Based on the degree of loss of keratinous mouth structures, we classified the severity of the symptoms in four categories (i.e., no symptoms, mild symptoms, moderate symptoms, and severe symptoms; Fig. 2).

We extracted DNA following the protocol of Ivanova et al. (2006). To eliminate potential PCR-inhibiting contaminants, we incubated the tissue samples for 14 h at 4 °C in 200 µL low PBS buffer (20 µL PBS in 180 µL of water) before overnight digestion with the vertebrate lysis buffer at 56 °C. After extraction, DNA was eluted in 50 µL TE buffer. A fragment of the mitochondrial 16S rRNA gene was amplified in an Eppendorf Mastercycler®pro using the following protocol: initial denaturation for 2 min at 94 °C; followed by 40 cycles with denaturation for 35 s at 94 °C, hybridization for 35 s at 48.5 °C, and elongation for 60 s at 72 °C; and final elongation for 10 min at 72 °C. The reaction mix for each sample contained 1 µL DNA template, 14 µL water, 2.5 µL PCR-buffer, 1 µL 25 mM MgCl₂, 4 µL 2.5 mM dNTPs (Invitrogen), 0.5 µL (containing 2.5 units) Taq Polymerase (PeqLab), and 1 µL of each primer (forward: L2510, 5’–CGCCTGTTTATCAAAAACAT–3’; and reverse: H3056, 5’–CCGGTCTGAACTCAGATCACGT–3’; eurofins MWG Operon). We analyzed a total of 43 16S sequences of eight species of stream-associated anurans (see Appendix I for specimens examined and Appendix II for GenBank accession numbers). We aligned the sequences with MUSCLE (Edgar, 2004) using the default settings in Geneious (Drummond et al., 2010). Using MEGA 6.06 (Tamura et al., 2013), we conducted a Maximum-Likelihood analysis for matching the 16S sequences of the tadpoles and adult anurans. We performed the best-fit model selection in MEGA 6.06, resulting in the use of the evolutionary model T92 + G. We provided branch support in the trees by standard bootstrap analysis (10,000 replicates).
RESULTS

During our surveys, we collected representatives of eight anuran species assigned to the families Bufonidae (1 species), Craugastoridae (1), Hylidae (5), and Ranidae (2): *Incilius occidentalis* (Camerano, 1879), *Craugastor rugulosus* (Cope, 1870), *Exerodonta juanitae* (Snyder, 1972), *E. sumichrasti* (Brocchi, 1879), *Megastomatoxyyla pellita* (Duellman, 1968), *Ptychohyla leonhardschulzei* (Ahl, 1934), *Sarcohyla pentheter* (Adler, 1965), *Lithobates forreri* (Boulenger, 1883), and *L. sierramadrensis* (Taylor, 1939). See Fig. 3 for images of the adult frogs collected in this study. Figure 4 demonstrates the relationship of tadpole size to Gosner stage in the two species with the largest sample sizes.

Except for *Incilius occidentalis* and *Megastomatoxyyla pellita*, we found evidence of tadpoles infected with *Bd* in all of the collected species (Fig. 5). The symptoms ranged from a minor loss of keratodont rows to a complete loss of these rows and a varying degree of bleaching on the beak. Generally, we found that larger tadpoles (considering both staging and size) tend to show a higher frequency and severity of chytrid symptoms (Fig. 6). In March of 2013, *Ptychohyla leonhardschulzei* and *Sarcohyla pentheter* were the most abundant species at our study sites, both of the number of tadpoles and adults.
Figure 7 shows the results of a Maximum-Likelihood analysis we used to match the 16S barcode sequences obtained from the tadpoles and adult frogs. The tadpole sequences match nicely with the adult frog sequences in all the species, with the in-group distances in most species ranging from 0.0% to 0.2%. Exceptions are *Lithobates forreri* and *Exerodonta juanitae* with a genetic distance of 1.5% and 1.9%, respectively, between the geographically distant populations. In the following discussion we provide descriptions of the external morphology and coloration in life of the tadpoles we studied.

**Incilius occidentalis**

Fig. 8a

The following measurements are based on five tadpoles (SMF 99714) at Gosner stages 33 (2 specimens), 34 and 35 (2), respectively: the total length = 34.20–38.50 mm (36.20 ± 1.62); the body length = 13.00–13.50 mm (13.38 ± 0.22); the tail length = 20.80–25.00 (22.82 ± 1.65); the maximum body height = 7.22–8.81 mm; the maximum
body width = 8.17–9.63 mm; the internarial distance = 1.90–2.29 mm; the interorbital distance = 4.20–4.72 mm; the eye diameter = 1.86–2.66 mm; the oral disc width = 3.42–4.21 mm; the snout–nostril distance = 1.79–2.39 mm; the snout–spiracle distance = 7.51–8.40 mm; the maximum tail height = 5.81–8.44 mm; the maximum dorsal fin height = 2.26–3.23 mm; the maximum ventral fin height = 1.76–2.34 mm; the maximum tail muscle height = 4.08–4.66 mm; the maximum tail muscle width = 3.04–3.87 mm; the body length relative to the total length = 35.06%–39.18%; and the eye diameter relative to the total length = 5.18%–6.91%.

**External morphology:** The body is ovoid in dorsal view. The snout is round in dorsal view, and blunt in lateral view. The oral disc is emarginated, situated, and directed ventrally. The oral disc contains a large gap of papillae anteriorly and posteriorly, but one lateral row of triangular papillae and some isolated conical submarginals are present. The jaw sheaths are serrated, with the upper one M-shaped and the lower one widely V-shaped. The LKRF is 2/3. The anterior keratodont rows are slightly longer than the posterior rows, and A-2 contains a median gap. The posterior keratodont rows are nearly the same length. The eyes are small and situated in a dorsal position, and directed dorsolaterally. The nostrils are positioned dorsally and directed dorsolaterally, are ovoid to reniform.

![Diagram showing the relationship of tadpole size and Gosner stage.](image)

**Fig. 4.** Diagram showing the relationship of tadpole size and Gosner stage. (A) *Ptychohyla leonhardschultzei*; and (B) *Sarcohyla pentether.*
in shape, and are elevated on the inner fringe, forming a triangular operculum. The spiracle is centric sinistral and directed posterodorsally, with the opening directed posterolaterally, and the tip of the tube is adnate and slightly less wide than the base of the spiracle. The cloacal tube is situated medially, and is longer than wide and attached to the ventral fin. The attached side of tube is somewhat longer than the free side, and contains a dextral orientated opening. The dorsal and ventral fins rise at the base of the tail, reach their maximum height at the midlength of the tail, and decrease again toward the tip of the tail. The tip of tail is rounded.

The following color description is based on one tadpole (SMF 96483) at Gosner stage 35: the dorsal body is Dark Neutral Gray (299); the lateral body is Brownish Olive (292) with suffusions of Prout’s Brown (47), Medium Neutral Gray (298), and with scattered Geranium (66) colored markings in the gill region; the whole body is covered with Light Smoke Gray (263) stipples that grade into white, and also increase in density in the ventrolateral region; the nostrils are Smoke Gray (266); the iris is Jet Black (300), bordered by Pratt’s Payne’s Gray (293); the pupil is edged with Cream White (52); the tail muscle is Smoky White (261), with a blurred Prout’s Brown (47) and Sepia (279) reticulum and with Smoke Gray (267) dots ventrally; the fins are transparent; the dorsal fin contains Dark Drab (45) mottling and capillary lines; and only a few Dark Drab (45) stipples are present on the ventral fin, in the posterior region.

![Diagram showing the frequency distribution of Bd symptoms among tadpoles of the anuran species examined in this study. Color codes: green = without symptoms; yellow = mild symptoms; orange = moderate symptoms; and red = severe symptoms.](image)

**Lithobates forreri**

Figs. 8b, 9a

The following measurements are based on four tadpoles (SMF 99712) at Gosner stages 25, 30, 31 and 36, respectively: the total length = 35.30–51.10 mm (45.90 ± 7.33); the body length = 14.50–19.30 mm (17.40 ± 2.30); the tail length = 20.80–31.80 (28.50 ± 5.18); the maximum body height = 7.21–10.43 mm; the maximum body width = 7.95–11.89 mm; the internarial distance = 2.73–3.55 mm; the interorbital distance = 4.66–6.59 mm; the eye diameter = 2.08–3.18 mm; the oral disc width = 2.77–3.41 mm; the snout–nostril distance 2.45–3.21 mm; the snout–spiracle
distance = 9.66–12.81 mm; the maximum tail height = 6.63–10.61 mm; the maximum dorsal fin height = 2.63–4.45 mm; the maximum ventral fin height = 1.57–2.20 mm; the maximum tail muscle height = 3.56–6.85 mm; the maximum tail muscle width = 2.65–5.88 mm; the body length relative to the total length = 35.55%–41.08%; and the eye diameter relative to the total length = 5.48%–6.36%.

**External morphology:** The body is ovoid in dorsal view. The snout is oval in dorsal view, and blunt in lateral view. The oral disc is relatively small, situated and directed anterodorsally, and not emarginated. The oral disc contains a large anterior gap of papillae anteriorly and one row posteriorly. The papillae are triangular. Very few submarginal papillae are present, and grouped on the labium near the lateral ends. The jaw sheaths are serrated, with the upper one arc- to slightly M-shaped and the lower one widely V-shaped. The LKRF is 2/3. The anterior keratodont rows are the same size as the posterior rows, and a clear median gap is present in A-2 and P-1. P-3 is shorter than the other posterior rows. The eyes are small, and situated in a dorsolateral position and direction. The nostrils are positioned anterodorsally and directed anterolaterally, circular, with an elevated torus on the inner fringe, and form a small operculum dorsally. The spiracle is centric sinistral, with the opening directed slightly posterolaterally, and the tube is adnate except for a free distal end. The cloacal tube is situated medially, and is longer than wide and attached to the ventral fin. The attached side of the tube is longer than the free side, with a dextral orientated opening.

Fig. 6. Diagram showing the relationship of the severity of *Bd* symptoms and the Gosner stage in *Ptychohyla leonhardschultzei*. Color codes: green = without symptoms; yellow = mild symptoms; orange = moderate symptoms; and red = severe symptoms.
Fig. 7. Maximum-Likelihood analysis of the 16S barcode sequences obtained from tadpoles (marked here with an asterisk) and adult frogs.
The dorsal fin rises at the juncture between the body and tail and has a straight margin, and is slightly higher than the body in the posterior region. The distance of the dorsal and ventral fins from the tail muscle to the fin border increases in a posterior direction, but decreases to the tip of the tail. The ventral fin is low throughout its length. The tip of the tail is tapered.

The following color description is based on one tadpole (SMF 99712) at Gosner stage 30: The dorsal body is Raw Umber (22) with suffusions of Natal Brown (49), and the lateral body is Pearl Gray (262) interspersed with Dusky Brown (285) mottling; the upper snout region is Tawny (60), and the ventral body is Cream White (52); the entire body an tail are covered with Smoky Gray (261) clumped stipples, which are denser ventrally; the iris is Jet Black (300) with Straw Yellow (153) stipples, and with Cream White (52) edging on the pupil; the tail muscle is Light Sky Blue (191) with a reticulate structure in Prout’s Brown (47) and Burnt Umber (48), and with visible muscle segments bordered by Plumbeous (295), which are more dense dorsally; the upper fin is transparent and contains Dark Brownish Olive (127) stipples on a reticulate pattern in Buff (5), of which the latter is more dense; the lower fin is transparent and contains a cream white reticulate pattern and sporadic Dark Brownish Olive (127) stipples.

**Lithobates sierramadrensis**

Figs. 8c, 9b

The following measurements are based on five tadpoles (SMF 96499 [1], SMF 99711 [3], and SMF 99694 [1]) at Gosner stages 30, 31, 33, 34 and 36, respectively: the total length = 51.10–63.70 mm (57.44 ± 5.42); the body length = 20.50–23.30 mm (21.76 ± 1.18); the tail length = 30.60–41.00 mm (35.68 ± 4.42); the maximum body height = 9.59–11.71 mm; the maximum body width = 11.71–14.04 mm; the internarial distance = 4.12–4.65 mm; the interorbital distance = 6.91–8.20 mm; the eye diameter = 3.21–4.15 mm; the oral disc width = 6.02–6.91 mm; the snout–nostril distance = 4.29–5.05 mm; the snout–spiracle distance = 13.88–16.48 mm; the maximum tail height = 9.98–13.21 mm; the maximum dorsal fin height = 3.95–4.42 mm; the maximum ventral fin height = 1.85–2.96 mm; the maximum tail muscle height = 6.62–8.49 mm; the maximum tail muscle width = 6.39–7.71 mm; the body length relative to the total length = 35.64%–40.12%; and the eye diameter relative to the total length = 15.29%–18.28%.

**External morphology:** The body is ovoid in dorsal and lateral views. The snout is oval in dorsal view. The oral disc is prominent, situated and directed anteroventrally, and not emarginated. The oral disc contains a large anterior gap of papillae, and one row posteriorly. The papillae are triangular in shape, with the inner papillae in the lateral region covered with keratodont teeth. The jaw sheaths are serrated, with the upper one arc-shaped and the lower one V-shaped. The LKRF is 7/3. A-2 to A-7 and P-1 contain a median gap, with A-3 to A-7 separated by the beak. The anterior rows are longer than the posterior rows, but keratodont rows are present within the anterior and posterior labium, respectively, and are nearly the same length. The eyes are small, and situated in a dorsolateral position and direction. The nostrils are positioned anterodorsally and directed anterolaterally, circular, and form a small torus but lack an operculum. The spiracle is centric sinistral, with the opening directed slightly posterolaterally, with the tube adnate except at the tip, and the opening is slightly less wide than the base of the spiracle. The cloacal tube is situated medially, and is slightly longer than wide and attached to the ventral fin. The attached side of tube is longer than the free side, which contains a dextral orientated opening.

The dorsal fin rises at the juncture between the body and tail, and has a straight margin and is slightly higher than the body. The distance of the dorsal and ventral fins from the tail muscle to the fin border increases in a posterior direction. The ventral fin is lower than dorsal fin, but is similar in shape. The tip of the tail is tapered.

The following color description is based on one tadpole (SMF 96499) at Gosner stage 30: The anterior body/head is Pearl Gray (262), with suffusions of Dark Neutral Gray (299); the posterior body is Dark Brownish Olive (127), with Light Yellow Ocher (13) suffusions; the gill region is Dark Carmine (61); the body is covered with lichen-like groups of stipples of Pearl Gray (262); the iris is Cream White (52) with Jet Black (300) corners; the tail muscle is Olive Yellow (117), with visible muscle segments bordered in Hair Brown (277) and lichen-like groups of Light Sky Blue (191) stipples, which are more compacted than on the body, and sporadic blotches of Warm Sepia (40); the fins are transparent, with capillary lines of Smoke Gray (266) starting from the tail muscle; and the dorsal fin contains sporadic Olive (126) mottling.
**Exerodonta juanitae**  
Figs. 8e, 9c

The following measurements are based on five tadpoles (SMF 100340) at Gosner stages 26, 28 (3 specimens) and 30: The total length = 23.80–35.50 mm (30.94 ± 4.44); the body length = 9.40–12.10 mm (11.05±1.04); the tail length = 14.40–23.40 mm (19.88 ± 3.44); the maximum body height = 4.43–6.95 mm; the maximum body width = 5.56–7.39 mm; the internarial distance = 2.59–3.96 mm; the interorbital distance = 4.25–5.28 mm; the eye diameter = 1.41–1.54 mm; the oral disc width = 3.11–4.04 mm; the snout–nostril distance = 1.72–3.02 mm; the snout–spiracle distance = 6.55–9.00 mm; the maximum tail height = 4.53–6.59 mm; the maximum dorsal fin height = 1.67–2.02 mm; the maximum ventral fin height = 0.95–1.73 mm; the maximum tail muscle height = 4.44–4.80 mm; the maximum tail muscle width = 4.44–4.80 mm; the body length relative to the total length = 34.06%–39.50%; and the eye diameter relative to the total length = 4.17%–5.92%.

**External morphology:** The body is ovoid in dorsal view. The snout is oval in dorsal view, and tapers in lateral view to a blunt end. The oral disc is prominent, situated and directed anteroventrally, and not emarginated. The oral disc is bordered by at least two rows of marginal papillae. The papillae in lateral view are long, thin, and cylindrical. Submarginal papillae are present, and grouped on the labium near the lateral ends. The jaw sheaths are serrated, with the upper one arc-shaped and the lower one widely V-shaped. The LKRF is 2/5. A-2 and P-1 contain a median gap. Keratodont rows within the anterior and posterior labium, respectively, are nearly the same length. The eyes are small and situated in a dorsolateral position, and directed dorsolaterally. The nostrils are positioned dorso-laterally and directed anterolaterally, circular, and form a small torus but lack an operculum. The spiracle is centric sinistral, the tube is directed posterodorsally, with the opening directed laterally, and the tip of the tube is adnate and slightly less wide than the base of the spiracle. The cloacal tube is situated medially, and is longer than wide and attached to the ventral fin. The attached side of tube is longer than the free side, with a dextral orientated opening.

The dorsal fin rises slightly before the juncture between the body and tail, and has a straight margin. The distance of the ventral fin from the tail muscle to the fin border is equal throughout its length. The dorsal and ventral fins are similar in shape. The tip of the tail tapers to a blunt end.

The following color description is based on one tadpole (IBH 27011) at Gosner stage 28: the body is Plumbeous (295) with Smoky White (261) stippled laterally, which turn into Smoke Gray (266) stippled dorsally and with fewer stipple in gill region; the region under the eye contains Carmine (64) colored suffusions; the snout region is of Olive Horn Color (16), and ventrally turns into Light Sky Blue (191) on the anterior part; the iris is Jet Black (300) with Orange Yellow (8) and Cyan White (156) stipple; the tail muscle is lighter than the body, which is Smoky White (261) with the visible muscle segments bordered with Olive-Brown (278), and the base of the tail muscle lacks Grayish Horn Color (268) stipple but contains an increasing number in a posterodorsal direction; the dorsal fin is transparent with scattered Smoke Gray (267) stipple, and the ventral fin is uniformly transparent.

**Ptychohyla leonhardschultzei**  
Figs. 8d, 9f

The following measurements are based on five tadpoles (SMF 99701–02, 100344 [3], SMF 100343 [2]) at Gosner stages 34, 35 (2 specimens), 36, and 37, respectively: the total length = 39.10–43.00 mm (41.74 ± 1.52); the body length = 12.20–13.80 mm (12.80 ± 0.65); the tail length = 26.00–30.60 mm (28.94 ± 1.81); the maximum body height = 5.82–6.38 mm; the maximum body width = 6.38–6.70 mm; the internarial distance = 1.88–2.61 mm; the interorbital distance = 4.90–5.78 mm; the eye diameter = 1.49–1.91 mm; the oral disc width = 3.17–4.18 mm; the snout–nostril distance = 1.10–2.07 mm; the snout–spiracle distance = 6.99–8.11 mm; the maximum tail height = 6.26–6.91 mm; the maximum dorsal fin height = 1.47–2.03 mm; the maximum ventral fin height = 1.15–1.90 mm; the maximum tail muscle height = 4.44–4.80 mm; the maximum tail muscle width = 3.53–4.38 mm; the body length relative to the total length = 28.84%–33.50%; and the eye diameter relative to the total length = 3.52%–4.58%.

**External morphology:** The body is ovoid in dorsal view, and slightly compressed in lateral view. The snout is oval in dorsal view, and blunt in lateral view. The oral disc is directed almost ventrally, and is not emarginated. The oral disc is bordered by two staggered rows of marginal papillae. The papillae, in lateral view, are cylindrical and sometimes contain a tapered top. Submarginal papillae are present, and grouped on the labium near the lateral
ends. The jaw sheaths are serrated, with the upper one arc-shaped and lower one widely V-shaped. The LKRF is 4/6, but younger individuals contain one less row anteriorly and/or posteriorly. All the keratodont rows are about the same length, and A-4 and P-1 contain a median gap. The eyes are small and situated dorsolaterally, and directed laterally. The nostrils are positioned anterolaterally and directed anteriorly, are circular in shape, and form a small torus but lack an operculum. The spiracle is centric sinistral, with the opening directed posterolaterally, and the tip of the tube is adnate and slightly less wide than the base of the spiracle, with the inner wall of the spiracle present as a slight ridge. The cloacal tube is situated medially, and is longer than wide and attached to the ventral fin. The attached side of the tube is longer than the free side, and contains a dextral orientated opening.

Fig. 8. Oral discs of preserved tadpoles. (A) *Incilius occidentalis* (SMF 99705); (B) *Lithobates forreri* (SMF 99712); (C) *Lithobates sierramadrensis* (SMF 96499); (D) *Pychohyla leonhardschultzei* (SMF 99695); (E) *Exerodonta juanitae* (SMF 100340); and (F) *Megastomatohyla pellita* (SMF 99433).
The dorsal fin rises slightly after the juncture between the body and tail, is almost invisible on the first one-third of the tail, and has a straight margin. The ventral fin is low throughout its length, and only is slightly higher than the body. The tip of the tail is rounded.

The following color description is based on one tadpole (SMF 99695) at Gosner stage 31: The dorsal body is Brownish Olive (276) with suffusions of Clay Color (18) and Smoke Gray (267), with lateral stipplings turning into Smoke Gray (266) stipplings dorsally and with a large Lilac (222) blotch present in the gill region; the iris is Light Orange Yellow (7) with Jet Black (300) corners; the tail muscle is Smoky White (261) with Burnt Umber (48) blotches and speckles, and contains groups of white stipplings; the dorsal and ventral fins are transparent with Medium Neutral Gray (298) speckles.

*Sarcohyla pentheterr*
Figs. 2, 9e

The following measurements are based on five tadpoles at Gosner stages 30 (2 specimens) 31, 34 and 35, respectively (SMF 99700 [2], SMF 100332–33 [2], SMF 100343 [1]): the total length = 51.85–63.60 mm (59.80 ± 4.93); the body length = 17.25–19.50 mm (18.38 ± 0.88); the tail length = 34.60–45.40 mm (41.42 ± 4.50); the maximum body height = 9.68–11.46 mm; the maximum body width = 9.72–12.36 mm; the internarial distance = 2.93–3.56 mm; the interorbital distance = 6.48–7.72 mm; the eye diameter = 2.15–2.59 mm; the oral disc width = 5.10–6.15 mm; the snout–nostril distance = 2.47–4.95 mm; the snout–spiracle distance = 10.76–12.03 mm; the maximum tail height = 11.62–12.91 mm; the maximum dorsal fin height = 3.52–4.06 mm; the maximum ventral fin height = 2.49–3.52 mm; the maximum tail muscle height = 7.15–8.55 mm; the maximum tail muscle width = 5.97–6.78 mm; the body length relative to the total length = 29.25%–33.33%; and the eye diameter relative to the total length = 3.43%–4.40%.

**External morphology:** The body is ovoid in dorsal view. The snout is oval in dorsal view, and blunt in lateral view. The oral disc is prominent, situated and directed almost ventrally, and not emarginated. The oral disc is bordered by two rows of marginal papillae, and also by an inner row of larger papillae. The inner papillae are larger than the marginal papillae and conical in shape. Submarginal papillae are present, and grouped on the labium near the lateral ends. The jaw sheaths are serrated, with the upper one arc-shaped and lower one widely V-shaped. The LKRF is 2/3. The anterior keratodont rows are distinctly longer than the posterior rows, and A-2 contains a clear median gap. The keratodont rows within the anterior and posterior labium, respectively, are nearly the same length. The eyes are small and situated in a dorsal position, and directed laterally. The nostrils are positioned dorsolaterally and directed anterodorsally, ovoid, and form a small torus but lack an operculum. The spiracle is centric sinistral, with the opening directed posterolaterally, and the tip of the tube is adnate and slightly less wide than the base of the spiracle. The cloacal tube is situated medially, and is longer than wide and attached to the ventral fin. The attached side of tube is longer than the free side, and contains a dextral orientated opening.

The low dorsal fin rises slightly before the juncture between the body and tail, has a straight margin, and is slightly higher than the body. The ventral fin is low throughout its length, and only slightly higher than the body. The dorsal and ventral fins are similar in shape. The tip of the tail is rounded.

The following color description is based on a tadpole (SMF 100332) at Gosner stage 30: The dorsal body is Olive (126), with the upper snout region Light Drab (269) and the lower snout region Pearl Gray (269); the posterolateral body is Dark Blue Gray (194) with Light Smoke Gray (263) suffusions, and contains a large Dark Bluish Purple (230) blotch in the gill region; the posterior body is covered with Salmon (83) and Color Pale Greenish White (97) speckles; the iris is Light Straw Yellow (95) with Vandyke Brown (282) motting, and contains interrupted circles and a Sky Blue (192) and Medium Blue Gray (193) marginal area; the tail muscle is Cream White (52), with visible muscle segments bordered in Spinel Pink (235); the tail muscle and fins are covered with Warm Sepia (40) blotches, except on the anterior tail muscle and dorsal fin, and the anterior half of the ventral fin; and the fins are transparent, except for the anterior upper fin.
Fig. 9. Tadpoles in life (lateral view). (A) *Lithobates forreri* (SMF 99712); (B) *Lithobates sierramadrensis* (SMF 96499); (C) *Exerodonta juanitae* (IBH 27011); (D) *Megastomatohyla pellita* (SMF 99433); (E) *Sarcohyla penther* (IBH 26995); and (F) *Ptychohyla leonhardschultzei* (SMF 99697).
Key to the stream-breeding anuran tadpoles on the Pacific slopes of the Sierra Madre del Sur in Oaxaca, Mexico

Note: The tadpole of *Sarcohyla miahuatlanensis* is unknown, and therefore is not included in this key.

1a Tooth row formula 2/3. ........................................................................................................ 2
1b Tooth row formula with more rows of keratodont teeth than 2/3 ........................................ 10

2a Posterior gap in marginal papillae present; oral disc laterally emarginate ............................. 3
2b No posterior gap in marginal papillae; oral disc not laterally emarginate ................................. 4

3a Caudal musculature with a brown reticulum, no distinct pale saddles or blotches; caudal fin with dark stipples

.................................................................................................................................................. *Incilius occidentalis*

3b Caudal musculature with distinct pale saddles or blotches; caudal fin with dark reticulum  

.................................................................................................................................................. *Incilius marmoreus*

4a Vent tube medial; spiracle sinistral but located more posteriorly, at 72–81% of body length toward vent; body greatly depressed; overall coloration reddish in life .................................................. *Hyalinobatrachium fleischmanni*

4b Vent tube dextral; spiracle sinistral but located near midlength of body; body not greatly depressed; overall coloration not reddish in life ................................................................................. 5

5a Anterior gap in marginal papillae present ............................................................................. 6
5b No anterior gap in marginal papillae ....................................................................................... 7

6a Gap in A2 row broad, its width greater than either segment length; fins with dark stipples on a reticulate pattern ......................................................................................................................... *Lithobates forreri*

6b Gap in A2 row narrow, its width about $\frac{1}{5}$ of either segment length; fins with dark blotches  

.................................................................................................................................................. *Sarcohyla hazelae*

7a Enlarged papillae medially adjacent to posterior marginal papillae present. ........................... 8
7b No conspicuously enlarged papillae medially adjacent to posterior marginal papillae ................. 9

8a Enlarged papillae medially adjacent to posterior marginal papillae evenly spaced and in a regular row; posterior tooth rows about the same length as or only slightly shorter than anterior rows; dorsal tail fin with dense pattern of dark splotches .................................................................................................................. *Sarcohyla pentheter*

8b Enlarged papillae medially adjacent to posterior marginal papillae irregularly arranged; posterior tooth rows much shorter than anterior rows; dorsal tail fin with only a few scattered dark splotches ............................................................................. *Sarcohyla thorectes*

9a Lateral fold densely covered with submarginal papillae; dorsal tail fin with fine dark speckling ..........................................................  

.................................................................................................................................................. *Charadrahyla altipotens*

9b Only a few submarginal papillae present in lateral fold; tail fin with few small brown (pale yellow and copper in life) flecks along margin.  

.................................................................................................................................................. *Sarcohyla cembra*

10a Tooth row formula 2/5. ........................................................................................................... 11
10b Tooth row formula with more rows of keratodont teeth than 2/5 .............................................. 13
DISCUSSION

The descriptions and illustrations of the tadpoles of the species reported in this study, along with the provided identification key, should facilitate tadpole identification during surveys along the Pacific slopes of the southern Sierra Madre del Sur in Mexico. Tadpole surveys are efficient tools for rapid inventories and for the long-term monitoring of species. The tadpoles that develop in mid-elevation streams require several months to complete development, and therefore can be detected more easily than their adult conspecifics. Also, tadpoles can be sampled during the day, whereas the adult frogs of many species are nocturnal and surveys must be conducted at night. Furthermore, the relatively stable conditions of a given stream indicate that tadpoles can be found reliably over a long period of time, whereas the activity of adult frogs is affected by short-term climatic conditions resulting in low activity (e.g., during cold, dry, windy periods). In this study, we detected two species (i.e., the bufonid *Incilius occidentalis* and the ranid *Lithobates forreri*) only in their larval stage, and no adult frogs of these species were found despite intensive nocturnal surveys. Conversely, we collected only adults but no tadpoles of the hylid frog *Exerodonta sumichrasti*. In direct-developing species such as *Craugastor rugulosus*, nighttime searches for adults were the only option for their detection. Tadpole surveys, therefore, should be seen as complementary to nocturnal opportunistic searches, and are valuable tools for monitoring stream-breeding montane frogs, and particularly suited for uncommon or rarely found species. These surveys have become even more important as *Bd* already is established in this region (Lips et al., 2004; this study), and tadpoles showing anomalies in their keratinized mouthparts are reliable indicators of this infection.

In this study we found evidence of the presence of *Bd* in most of the anuran species we collected in the various streams in the southern Sierra Madre del Sur in Mexico. Despite the presence of this pathogen for at least the past 15 years (Lips et al., 2004), however, these anurans still were present and obviously have reproduced successfully. At least some of the species (in particular, *Pychohyla leonhardschultzei* and *Sarcohyla pentheter*) were abundant at our study sites, with hundreds of tadpoles and dozens of adults seen during a single night survey. These observations appear to indicate that the pathogen and the anuran host have established a means of coexistence. The dynamics and the underlying evolutionary mechanisms of this coexistence mostly are unknown. Promising areas of research to shed light on these issues are the study of variation and evolution of skin peptides and the microbial fauna in the mucus of adult frogs. In general, more fieldwork is necessary on frog populations that have survived and are recovering from *Bd* infection. Many questions, however, remain to be answered. For example, it is unknown whether individuals that were infected by *Bd* in the larval stage have a different survival perspective after metamorphosis, as compared to non-infected ones. Whereas in tadpoles the fungus apparently is restricted to the keratinous mouthparts, it can spread across the whole integument when it is keratinized after metamorphosis.
The morphological data reported in this study mostly agree well with the published tadpole descriptions for the hylid and ranid species we encountered (Webb, 1978; Duellman, 1970; 2001; Snyder, 1972; Köhler et al., 2015). The tadpole of Incilius occidentalis was described and illustrated in Canseco-Márquez and Gutiérrez-Mayén (2010). In the material we studied, however, the A2-gap is much narrower than depicted for this species in the cited publication. Whether this discrepancy reflects geographic variation present in this species is not clear at this point.

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**Appendix 1. Specimens examined.**

**A. Adult frogs**

*Craugastor rugulosus*—**MEXICO**: OAXACA: Río Sal, 3 km N San Gabriel Mixtepec: 690 m: IBH 27007, SMF 96475–76.

*Exerodonta juanitae*—**MEXICO**: OAXACA: 25 km N San Gabriel Mixtepec, 1,490 m: SMF 96515

Buena Vista, 1,490 m: SMF 96516.

*Exerodonta sumichrasti*—**MEXICO**: OAXACA: 25 km N San Gabriel Mixtepec, 1,490 m: SMF 96481; Buena Vista, 1,490 m: SMF 96482; Tierra Blanca, 1,369 m: SMF 96764–65; road to El Vidrio, 1,481 m: SMF 96766–68.

*Lithobates sierramadrensis*—**MEXICO**: OAXACA: Río Sal, 3 km N San Gabriel Mixtepec, 690 m: IBH 27010, 27025, SMF 96500; near La Soledad, 1,350 m: SMF 96501; 25 km N San Gabriel Mixtepec, 1,490 m: SMF 96503.

*Megastomatohyla pellita*—**MEXICO**: OAXACA: 25 km N San Gabriel Mixtepec, 1,490 m: IBH 27027, SMF 96479.

*Ptychohyla leonhardschulzei*—**MEXICO**: OAXACA: near Pluma Hildago, 1,348 m: IBH 27005; 30 km N San Gabriel Mixtepec, 1,600 m: IBH 26502, 27026, SMF 96520; 35 km N San Gabriel Mixtepec, 1,830 m: SMF 96518; 37 km N San Gabriel Mixtepec, 1,900 m: SMF 96519; Santiago la Galera, 1,160 m: SMF 96283; near Pluma Hildago, 1,348 m: SMF 96517; near La Soledad, 1,350 m: IBH 27042, SMF 96521–22, 96524; Buena Vista, 1,299 m: SMF 96793–94; Buena Vista, 1,490 m: SMF 96526–27; road to El Vidrio, 1,481 m: SMF 96795–97.

*Sarcohyla pentheter*—**MEXICO**: OAXACA: Buena Vista, 1,299 m: SMF 96787–88.

**B. Tadpoles**

*Exerodonta juanitae*—**MEXICO**: OAXACA: 25 km N San Gabriel Mixtepec, 1,490 m: IBH 27011, SMF 100339; near Jalatengo, 1,355 m: SMF 100340; 30 km N San Gabriel Mixtepec, 1,600 m: SMF 100346.

*Incilius occidentalis*—**MEXICO**: OAXACA: near Jalatengo, 1,355 m: IBH 27045, SMF 99705.

*Lithobates forreri*—**MEXICO**: OAXACA: near Jalatengo, 1,355 m: SMF 100341; Río Sal, 3 km N San Gabriel Mixtepec, 690 m: SMF 99712.

*Lithobates sierramadrensis*—**MEXICO**: OAXACA: near Pluma Hidalgo, 1,295 m: IBH 26996, SMF 96498, 99708; Río Sal, 3 km N San Gabriel Mixtepec, 690 m: IBH 27006, SMF 99694, 96499, 99711.

*Megastomatohyla pellita*—**MEXICO**: OAXACA: 25 km N San Gabriel Mixtepec, 1,490 m: SMF 99433.

*Ptychohyla leonhardschulzei*—**MEXICO**: OAXACA: 25 km N San Gabriel Mixtepec, 1,490 m: SMF 96560; between La Soledad and Buena Vista, 1,505 m: IBH 27040, SMF 99700; near Pluma Hidalgo, 1,295 m: SMF 96480; near Pluma Hildago, 1,348 m: SMF 99710, 100347; near Tierra Blanca, 1,290 m: IBH 27044, SMF 99703; 25 km N San Gabriel Mixtepec, 1,490 m: IBH 27012, SMF 99695–96; between La Soledad and Buena Vista, 1,485 m: SMF IBH 27041, 99701–02, 100344; near La Soledad, 1,350 m: SMF 99697: IBH 27033–34, 27036; 30 km N San Gabriel Mixtepec, 1,600 m: SMF 99704.

*Sarcohyla pentheter*—**MEXICO**: OAXACA: 25 km N San Gabriel Mixtepec, 1,490 m: IBH 27009, SMF 100335–37; between La Soledad and Buena Vista, 1,565 m: SMF 100338; between La Soledad and Buena Vista, 1,485 m: SMF 100342; La Soledad: SMF 96523; near La Soledad, 1,350 m: IBH 27035, SMF 100332–34; near Pluma Hidalgo, 1,295 m: IBH 26995; near Tierra Blanca, 1,290 m: SMF 100343; near Tierra Blanca, 1,287 m: SMF 100345.
## Appendix 2. GenBank accession numbers for the 16S sequences used in this study.

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</table>
Gunther Köhler received a degree in Veterinary Medicine (Staatsexamen) at the University Gießen, Germany, in 1993, and a Doctoral degree at Goethe University Frankfurt am Main, Germany, in 1995; since that time, he has been the Curator of Herpetology at the Senckenberg Research Institute, Frankfurt am Main, Germany. His research focuses on the Neotropical herpetofauna, primarily that of Central America and Mexico. To date, Gunther has authored 27 books and 205 research papers on amphibians and reptiles.

Raúl Gómez Trejo-Pérez studied biology at the Universidad Nacional Autónoma de México. His primary interests are in learning about the amphibians and reptiles of Mexico and their conservation, and during the course of his studies has conducted research stays at the Smithsonian Institute, in Panama, and the Senckenberg Research Institute, in Germany. Raúl has co-authored four papers in which seven new species of lizards were described. Currently, he is pursuing a Master’s degree at the Instituto de Biología, Universidad Nacional Autónoma de México; his thesis focuses on the thermal biology of montane anoles.

Victoria Reuber graduated with a B.Sc. in Biology and recently started her M.Sc. in Ecology and Evolution at the Goethe University Frankfurt, Germany. She is particularly interested in biodiversity, evolution, and systematics. During an internship at Gunther Köhler’s lab at Senckenberg Museum, her fascination for the field of herpetofauna amplified. As a conservation volunteer, Victoria visited Honduras and took part in a project to preserve the endemic spiny-tailed iguana on the island of Utila. During her Bachelor’s studies she was a laboratory assistant in plant physiology and a member of the biology student council.

Gerrit Wehrenberg graduated with a B.Sc. in Biology at the Johannes-Gutenberg University of Mainz in Germany, and recently started his M.Sc. studies in ecology and evolution at the Goethe University Frankfurt, Germany. He is interested in systematics, biogeography, conservation, and zoo biology. As a conservation volunteer, he visited Costa Rica and worked with sea turtles. In 2016 he participated in a Senckenberg Research Institute herpetological expedition to Panama. Gerrit has experience in aquaristics and herpetoculture, and also worked in the scientific department at the Zoological Garden in Frankfurt, Germany. To date, he has authored two popular science articles.

Fausto R. Méndez-de la Cruz is a full professor at the Universidad Nacional Autónoma de México, in Mexico City. He received his Ph.D. in 1989 at the Universidad Nacional Autónoma de México, and conducted postdoctoral research at the University of Florida, and a sabbatical at Virginia Polytechnic Institute and State University, Virginia, both in the United States. Fausto’s research focuses on the evolution of reproductive systems in reptiles, mainly in the evolution of viviparity and parthenogenesis, and more recently in the vulnerability of amphibians and reptiles to climate change in Mexico and South America.