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Leeches from Chiapas, Mexico, with a new species of *Erpobdella* (Hirudinida: Erpobdellidae)

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ABSTRACT

Freshwater leeches were collected from Chiapas, Mexico. Among these collections and prior records, a total of nine species were found, comprising six genera and four families. One species, *Diestecostoma octannulata*, represents a new record for Mexico and two species, *Helobdella elongata* and *H. octatestisaca*, represent new records for Chiapas. Additionally, a new species of *Erpobdella* was discovered from a single stream in the park El Arcotete near San Cristobal de las Casas. Here it is described as *Erpobdella adani* and it is morphologically distinguished from Mexican *Erpobdella* species by the combination of having the whole body strongly dorsoventrally flattened, three annuli between gonopores, an enlarged male gonopore, and no preatrial loops for the male reproductive system. Based on sequence data (mitochondrial cytochrome *c* oxidase subunit I and 12S; nuclear 18S), this new species appears most closely related to a clade formed by *E. coastalis* and *E. ochoterenai*. Both mitochondrial cytochrome *c* oxidase subunit I molecular barcodes and morphological descriptions of the collected species are presented.

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INTRODUCTION

Mexico is well known as one of the world's biodiversity hotspots (Ramamoorthy, 1993). Although collecting efforts for leeches have grown slowly, it is beginning to become clear that leeches are also diverse in Mexico. In the past decade a massive effort to collect leeches throughout the central states of Mexico has taken place (Oceguera-Figueroa and León-Règagnon, 2014). These explorations have been fruitful and clearly establish Mexico as ripe for leech discovery, with many endemics uncovered. Furthermore, these leeches are phylogenetically diverse, with representatives from over 70% of leech families (Oceguera-Figueroa and León-Règagnon, 2014).

No other area in North America has had so many recent leech species discoveries, and there is no sign of this rapid rate of discovery slowing (Oceguera-Figueroa and León-Règagnon, 2014). Concerted efforts to further collect and systematize Mexican leech diversity have filled in many of the gaps in records, collections, and undescribed diversity, especially from Central Mexico. Chiapas, the southernmost state of Mexico, has had few surveying efforts. Still, this work has resulted in interesting discoveries of endemic species, such as the mammalophilic leech *Pintobdella chiapasensis* Caballero, 1957, known only from Custepeques and Lagunas de Montebello (Caballero, 1957; Phillips et al., 2010), and a turtle-feeding species, *Placobdella ringueleti* López-Jiménez and Oceguera-Figueroa, 2009, from Laguna Bélgica, Bonampak, Rancho Alejandría, Malpaso Dam, Tuxtla Gutierrez, and Reforma. Furthermore, *Haementeria acuecueyetzin* Oceguera-Figueroa, 2008, was found parasitizing a manatee in Palenque, Chiapas, which is the first and only record of a leech feeding on a manatee (Pérez-Flores et al., 2016). In addition to these records, two species of leeches are known from the Colección Nacional de Helmintos (CNHE), Instituto de Biología, UNAM: *Erpobdella triannulata* Moore, 1908, from Lagunas de Montebello and *Helobdella socimulcensis* Caballero, 1931, from Presa Belisario Domínguez.

Overall, Chiapas has largely remained unexplored (Oceguera-Figueroa and León-Règagnon, 2014). In the present study, we provide a brief description of the morphology and genetic barcodes—mitochondrial cytochrome *c* oxidase subunit I fragment (cox1)—for each species newly collected in Chiapas. We present new localities for three species, a new country record, as well as the description and phylogenetic placement of a new species of macrophagous leech of the genus *Erpobdella*.

MATERIALS AND METHODS

Leeches were collected in 2014 and 2015 from localities across Chiapas (fig. 1) under the permit SEMARNAT 12099 to A.O.-F. Collection techniques primarily followed two standard survey strategies: (1) bare-legged wading in water to attract blood-feeding leeches; and (2) a more active search for both blood-feeding and non-blood-feeding leeches by flipping over rocks, sticks, and vegetation both in and at the edge of the water. Both lentic and lotic sites were checked, but a greater focus was made on lentic sites due to their generally higher leech diversity. Additionally, terrestrial leeches in the Xerobdellidae were sought in moist forests by flipping over rocks and logs. Specimens were gradually relaxed in water with increasing concentrations of ethanol, and eventually fixed in 70%–90% ethanol. Collected leeches were identi-

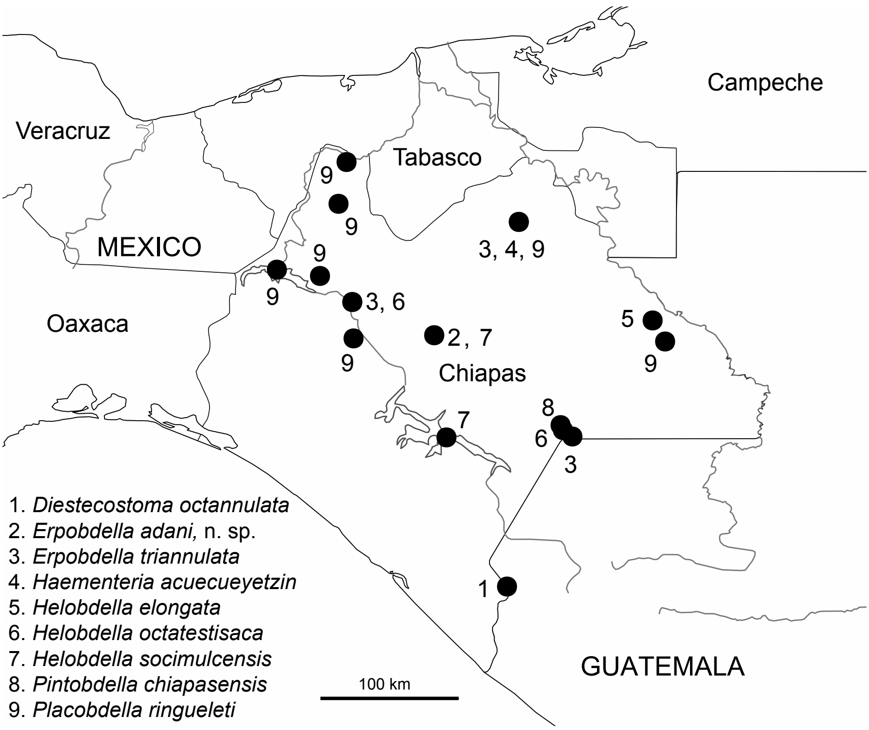


FIGURE 1. Map of the State of Chiapas, Mexico, with the collecting localities of leeches.

fied and photographed under a stereomicroscope. In addition, in this study we include samples collected by other biologists as well as previously published records.

At least one specimen from each species collected was genetically barcoded with cox1. DNA extractions, amplifications, and sequencing protocols followed prior studies (Apakupakul et al., 1999; Borda and Siddall, 2004). For the new species of *Erpobdella* we amplified a longer fragment of cox1 using HHCO1 (developed at the AMNH) 5′-GCTGCAAAAATRGCAAATACTGC-3′ instead of HCO2198, but otherwise kept identical protocols (Apakupakul et al., 1999). For the new species, we also amplified the mitochondrial 12S following Simon et al. (1994) using 12Sa and 12Sb primers and nuclear 18S (amplified and sequenced using primers "A," "B," and "Y") with the protocols found in Borda and Siddall (2004) and Apakupakul et al. (1999), respectively. Sequences were reconciled in Geneious version 6.1.8 (Biomatters Limited).

To determine the phylogenetic position of the new species within *Erpobdella*, a matrix was constructed for those *Erpobdella* species with sequence data available for cox1, 12S, and/or 18S (table 1). Sequences were aligned using MUSCLE (Edgar, 2004a, 2004b) through the "muscle" package in R (www.r-project.org/). Both parsimony via TNT (Goloboff et al., 2008) and maximum likelihood (ML) via Garli (Zwickl, 2006) were then used to infer the phylogeny. For ML reconstruction, loci were partitioned using models found with jModelTest 2.1.4 (Darriba et al., 2012). For both inferences, support was assessed using 1000 bootstrap pseudoreplicates, summarized on the ML tree using SumTrees (Sukumaran and Holder, 2010). Trees were visualized in FigTree v1.4.2 (Rambaut, 2014). Additionally, cox1 sequences were used to calculate genetic distances with the Kimura two-parameter using PAUP* 4.0b10 (Swofford, 2002).

TABLE 1. Molecular data used to determine the phylogenetic placement of *Erpobdella adani* within the genus *Erpobdella*. GenBank accession numbers are given for each sequence.

Family	Species	Cox1	12S	18S	Country
Erpobdellidae	*Erpobdella adani* n. sp. 1	MG745144	MG745141	MG745138	Mexico
	Erpobdella adani n. sp. 2	MG745145	MG745142	MG745139	Mexico
	Erpobdella adani n. sp. 3	MG745146	MG745143	MG745140	Mexico
	Erpobdella annulata	HQ336345	—	HQ336379	USA
	Erpobdella borisi	KP749904	—	—	Iran
	Erpobdella bucera	AF116024	AF462026	AF115998	USA
	Erpobdella bychowskii	DQ009667	AF169372	—	Slovenia
	Erpobdella coastalis 1	AY425460	AY425442	AY425478	USA
	Erpobdella coastalis 2	—	HQ336354	HQ336381	USA
	Erpobdella dubia	AF116023	AF462022	AF115997	USA
	Erpobdella haskonis	DQ009668	—	—	Germany
	Erpobdella [Trocheta] intermedia	DQ009669	—	—	Germany
	Erpobdella japonica 1	AB679654	AB679655	AB663648	Japan
	Erpobdella japonica 2	AF116026	AF462023	AF116000	Korea
	Erpobdella johanssoni	HM246605	—	—	Spain
	Erpobdella krilata	HM246629	—	—	Albania
	Erpobdella latestriata	HM246610	—	—	Greece
	Erpobdella [Dina] lepinja	HM246597	—	—	Macedonia
	Erpobdella lineata 1	—	AF099952	AF099950	Denmark
	Erpobdella lineata 2	HM246611	—	—	Macedonia
	Erpobdella [Dina] lyhnida	HM246595	—	—	Macedonia
	Erpobdella melanostoma	AF116025	AF462027	AF115999	USA
	Erpobdella mexicana 1	DQ235595	DQ235585	DQ235605	Mexico
	Erpobdella mexicana 2	DQ235601	DQ235591	DQ235611	Mexico
	Erpobdella mexicana 3	DQ235597	DQ235587	DQ235607	Mexico
	Erpobdella monostriata	DQ009665	—	—	Germany
	Erpobdella montezuma	GQ368760	GQ368820	GQ368802	USA
	Erpobdella nigricolis	DQ009664	—	—	Germany
	Erpobdella obscura	AF003273	AF462028	AF116004	Canada
	Erpobdella ochoterenai 1	DQ235603	DQ235593	DQ235613	Mexico
	Erpobdella ochoterenai 2	DQ235599	DQ235589	DQ235609	Mexico
	Erpobdella ochoterenai 3	DQ235600	DQ235590	DQ235610	Mexico
	Erpobdella octoculata 1	AF003274	AF099954	AF116001	France
	Erpobdella octoculata 2	HQ336344	—	HQ336378	Uzbekistán
	Erpobdella [Dina] ohridana	HM246633	—	—	Macedonia
	Erpobdella [Dina] cf. profunda	HM246581	—	—	Macedonia
	Erpobdella [Trocheta] pseudodina	EF125041	—	—	Germany
	Erpobdella punctata 1	HQ336346	HQ336352	HQ336380	Canada

Family	Species	Cox1	12S	18S	Country
	Erpobdella punctata 2	AF003275	AF462024	AF116002	Canada
	Erpobdella subviridis	—	AF169374	—	Croatia
	Erpobdella svilesta	HM246598	—	—	Macedonia
	Erpobdella testacea	AF116027	AF462025	AF116003	France
	Erpobdella triannulata 1	DQ235602	DQ235592	DQ235612	Mexico
	Erpobdella triannulata 2	DQ235604	DQ235594	DQ235614	Mexico
	Erpobdella triannulata 3	HQ336347	HQ336353	—	Mexico
	Erpobdella vilnensis	DQ009663	—	—	Germany
Gastrostomobdellidae	*Gastrostomobdella monticola*	AB679656	AB679657	AB663649	Malaysia
	Orobdella dolichopharynx	AB679680	AB679681	AB663665	Japan
Salifidae	*Barbronia weberi*	DQ235598	DQ235588	DQ235608	Mexico
	Linta be	AY786460	—	AY786466	Madagascar
	Mimobdella japonica	AB679658	AB679659	AB663650	Japan
	Odontobdella blanchardi	AB938004	AB937995	AB663651	Japan
	Salifa motokawai	LC029431	LC029432	LC029434	Vietnam

SYSTEMATICS

Order Arhynchobdellida Blanchard, 1894

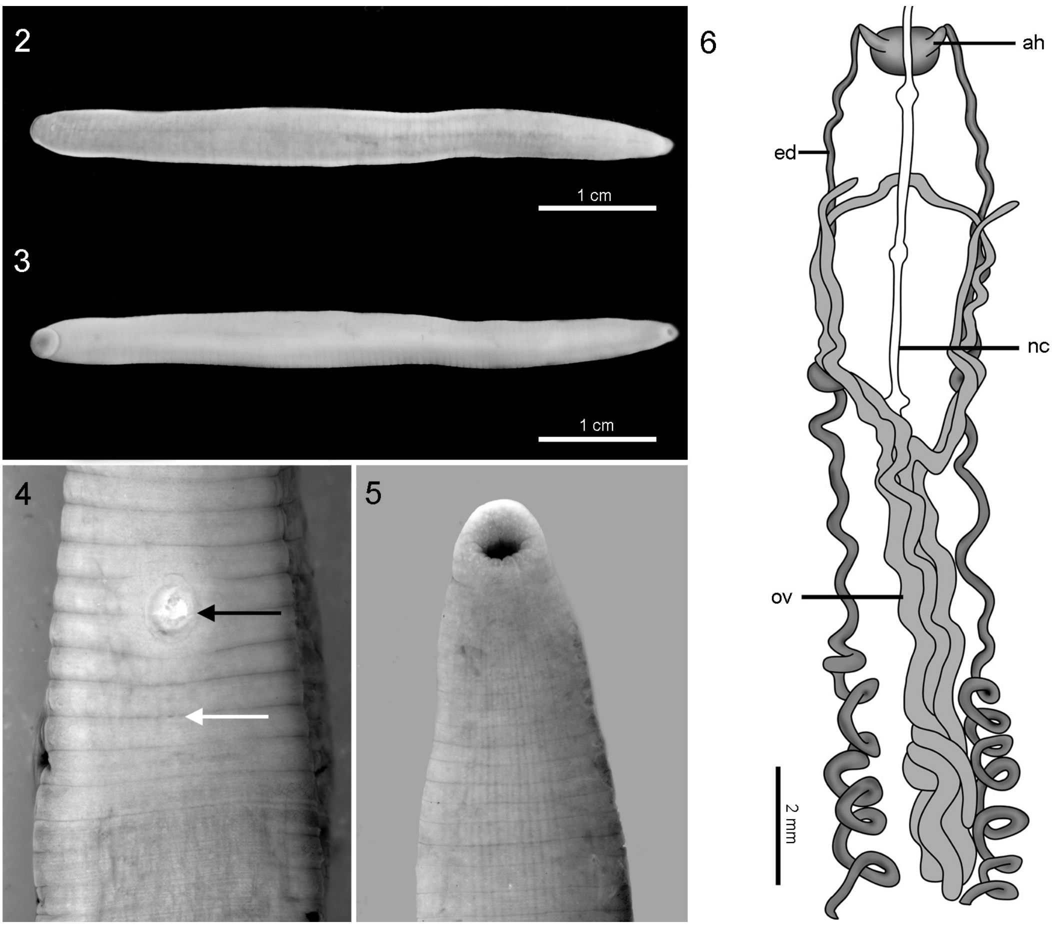
Family Erpobdellidae Blanchard, 1894

Erpobdella adani, n. sp.

Figures 2–6

Material Examined: Fifteen specimens collected in Río Fogótico, in Arcotete, Chiapas, Mexico, on January 23, 2016, by Michael Tessler, Samantha Contreras, Jairo Arroyave, and Alejandro Oceguera-Figueroa. Specimens were found beneath submerged rocks in moderately flowing water. Non-blood-feeding (macrophagous).

Description: External morphology based on 15 specimens. Body strongly dorsoventrally flattened, vermiform, slender. Dorsal surface dark gray, ventral surface lighter; some specimens with three longitudinal lines of black pigment, others lacking lines. Dorsal surface smooth, without conspicuous papillae or other sensory structures; ventral surface of some specimens covered with minute papillae (figs. 2, 3). Average body length 51 mm (35–64 mm), average body width 4 mm (2–5 mm). Complete somite five annulate. Clitellum conspicuous, thick both dorsally and ventrally, comprising 15 annuli, from X b5 to XIII a2. Male gonopore at XII b2/a2, female gonopore XII/XIII; three annuli between gonopores (fig. 4). Male gonopore large, conspicuous, and surrounded by an integumental disc; female gonopore small. Three pairs of eyespots in the classic "erpobdellid" arrangement with the anterior labial pair on III and two buccal pairs at IV. Mouth wide, occupying entire anterior sucker; anterior lip blunt (fig. 5). Anus large, on XXVI/XXVII. Posterior sucker weakly developed,



FIGURES 2–6. *Erpobdella adani*. **2.** Holotype, dorsal view. **3.** Holotype, ventral view. **4.** Paratype, male gonopore (black arrow), female gonopore (white arrow). **5.** Paratype, ventral anterior end, anterior sucker. **6.** Illustration of the male (dark gray) and female (light gray) reproductive systems, atrial horns (ah), ejaculatory duct (ed), ventral nerve cord (nc), ovary (ov); testisacs not shown.

ventrally directed, narrower than the posterior part of the body. Somites I–III unianulate, IV–V biannulate, VI three annulate, VII four annulate, VIII–XXIV five annulate, XXV three annulate, XXVI biannulate, XXVII unianulate.

Internal morphology based on the dissection of three specimens. Agnathous, pharynx strepsilaematous, muscular, extending to XIII/XIV. Crop tubular, acaecate, extending from XIV to XIX/XX; intestine acaecate extending to XXVI/XXVII. Male medial reproductive system with paired atrial horns directed anterolaterally, without preatrial loops (fig. 6). Ejaculatory ducts tubular, extending posteriorly to XVIII/XIX, continuing with multiple testisacs (forming grapelike clusters) reaching XXIV. Female medial reproductive system formed by tubular ovaries with a lateral first portion in XIII and then joining the median line of the body mass extending to XVI/XVII where the ovaries redirect anteriorly to XIII and then they follow the distribution of the lateral first portion of the ovaries ending laterally at XII/XIII (fig. 6).

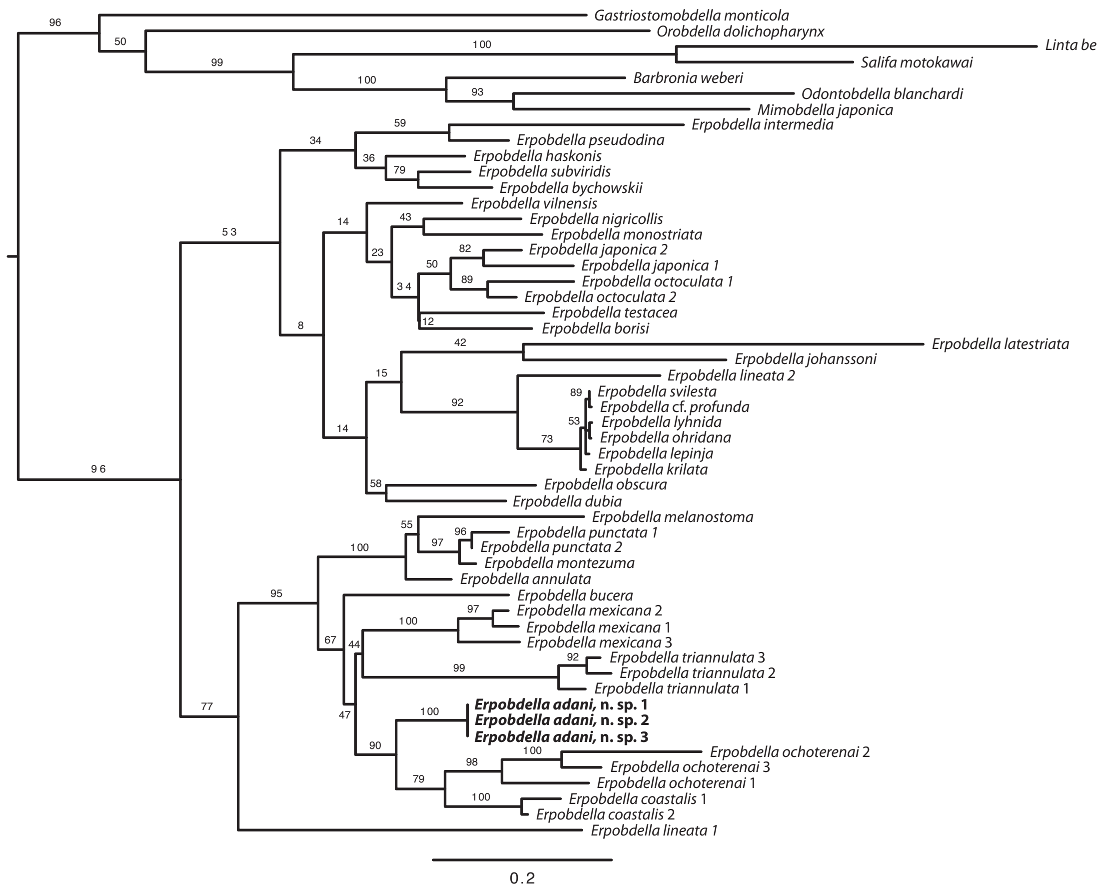


FIGURE 7. Maximum likelihood phylogenetic reconstruction of the family Erpobdellidae focused on establishing the closest relatives to *Erpobdella adani*. Support values are based on 1000 bootstrap pseudoreplicates.

Etymology: This species is named to remember Adán Enrique Gómez González (1980–2018). Adán was an enthusiastic biologist with a deep passion and knowledge of biodiversity from Mexico, in particular from Chiapas, where he was born and where the new species of *Erpobdella* was collected.

Holotype: Colección Nacional de Invertebrados, Instituto de Biología, UNAM (catalog number 21).

Paratypes: Eleven specimens, Colección Nacional de Invertebrados, Instituto de Biología, UNAM (catalog number 22); three specimens, American Museum of Natural History (AMNH_IZC 250222; GenBank accession numbers presented in table 1).

Type Locality: Río Fogótico, Arcotete, Chiapas, Mexico (16°44′46.5″N, 92°34′17.24″W; 2298 m).

Phylogenetic Analyses: The three loci reviewed did not differ for the three *Erpobdella adani* specimens sequenced. *Erpobdella adani* was found nested in an exclusively North American clade. It was found to be sister to an *E. coastalis*/*E. ochoterenai* Caballero, 1932, clade with moderate support (fig. 7). This combined clade was sister to *E. mexicana* (Dugès, 1876) in parsimony and sister to an *E. mexicana*/*E. triannulata* clade in ML analyses; however, these relationships had weak support in both analyses.

More generally, the phylogenetic inference resulted in two principal clades within the genus *Erpobdella*; these results are similar to previous analyses (Siddall, 2002; Oceguera-Figueroa et al., 2005, 2011). For both analyses, the two specimens of *E. lineata* (O.F. Müller, 1774) are not sister; *E. lineata* 2 (cox1 data only; specimen from Macedonia) grouped with the principally European clade, while *E. lineata* 1 (12S and 18S data only; specimen from Denmark) varied in its positioning.

Remarks: Only three other species of *Erpobdella* are known from Mexico: *E. mexicana*, *E. ochoterenai*, and *E. triannulata*, with only *E. triannulata* known from Chiapas. The new species, *E. adani*, is distinguished from *E. mexicana* and *E. triannulata* based on the male reproductive structures. While *E. mexicana* and *E. triannulata* have ejaculatory ducts forming a preatrial loop extending to XI before entering the atrial horns, in *E. adani* the preatrial loop is absent (fig. 6). In addition, *E. mexicana* has two annuli separating the gonopores while *E. adani* has three. In Central America, including Costa Rica, Guatemala, and Honduras (Oceguera-Figueroa and Pacheco-Chaves, 2012; Cornejo et al., 2015), only *E. triannulata* has been reported from this region, the southern limit of the distribution of the species of the genus.

Erpobdella ochoterenai, from Central Mexico, and *E. coastalis* and *E. microstoma* (Moore, 1901), both from the southeastern United States, are morphologically the most similar species to *Erpobdella adani*. These species have three annuli between gonopores and lack preatrial loops of the ejaculatory ducts. However, in *E. adani* the body is strongly dorsoventrally flattened, from the preclitellar region to the posterior sucker. This characteristic is constant in the specimens studied and apparently not related to the process of narcotization-fixation. This contrasts with *E. ochoterenai*, *E. coastalis*, and *E. microstoma*, which are terete for the anterior two thirds of the body and dorsoventrally flattened only for the last third of the body (Moore, 1945; A.O.-F., personal obs.). Additionally, *E. adani* has the male gonopore unusually enlarged, in some cases occupying almost all of b2 and half of a2 of somite XII, a character not seen in other members of the genus.

The average cox1 genetic distances for samples of the new species is 13% divergent from *Erpobdella ochoterenai* and 18% from *E. coastalis*, values that independent of morphology indicate that both groups likely represent separate species.

Other Taxa

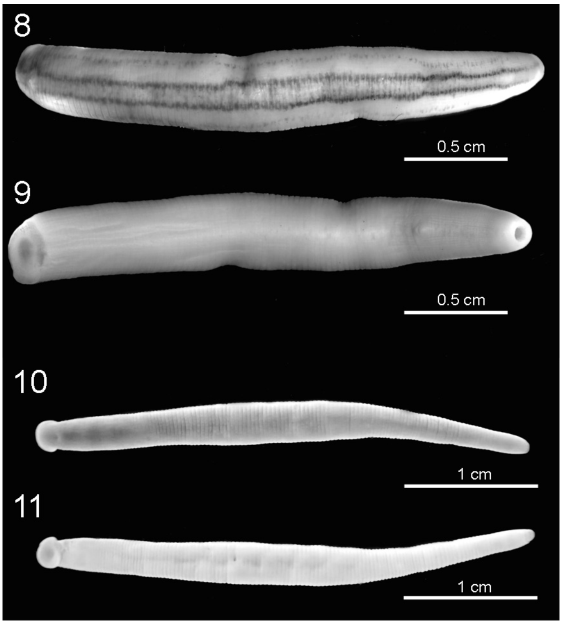
Order Arhynchobdellida Blanchard, 1894

Family Erpobdellidae Blanchard, 1894

Erpobdella triannulata Moore, 1908

Figures 8–9

The presence of three pairs of eyespots, two pairs of longitudinal lines on the dorsal surface, three annuli between gonopores, ejaculatory ducts forming preatrial loops, and medium body



FIGURES 8–11. Arhynchobdellida from Chiapas, Mexico. *Erpobdella triannulata*: **8.** dorsal view, **9.** ventral view. *Diestecostoma octannulata*: **10.** dorsal view, **11.** ventral view.

size correspond to the description of *Erpobdella triannulata* (Oceguera-Figueroa et al., 2005). Found on the underside of submerged rocks and roots of aquatic plants. Non-blood-feeding (macrophagous). New locality records: Palenque (17°29′10″N, 92°01′10″W; 77 m), four specimens collected March, 2015, by J. Pérez-Flores, (CNI 23); Osumacinta Dam (16°57′47.5″N, 93°06′21.82″W; 207 m), 22 specimens collected January 24, 2016, by M. Tessler, J. Arroyave, S. Contreras, and A. Oceguera-Figueroa, (CNHE 24). GenBank cox1 barcode sequence accessions: MG821606 and MG821607.

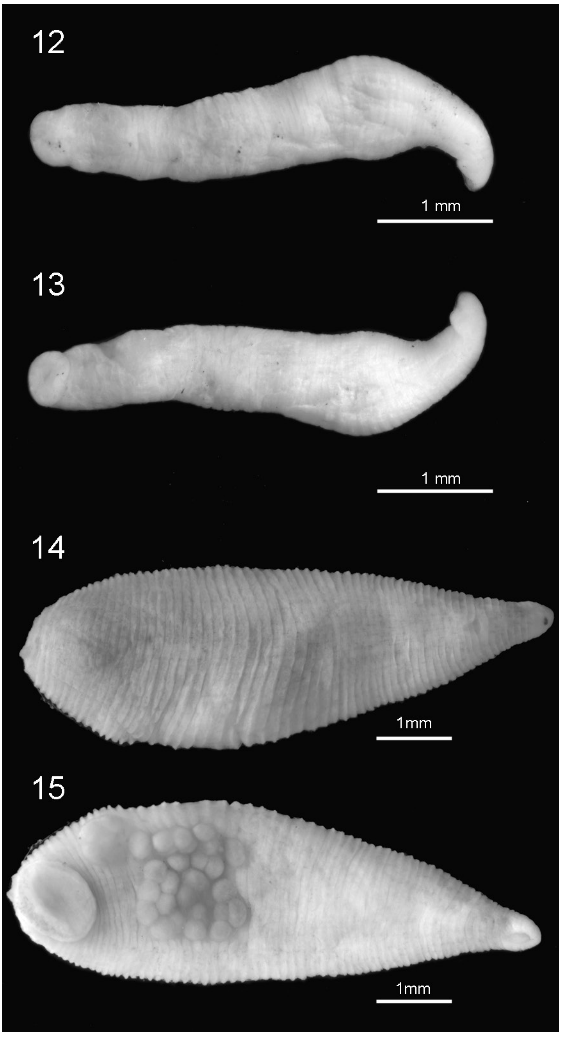
Family Xerobdellidae Moore, 1946

Borda, Oceguera-Figueroa, and Siddall, 2008

Diestecostoma octannulata Moore, 1946

Figures 10–11

The presence of five eyespot pairs arranged in a parabolic arc (first four pairs on contiguous annuli and two annuli separating fourth and fifth pair), complete somite eight annulate (16 + 1/2 annuli between gonopores) is consistent with the description of *Diestecostoma octannulata* Moore, 1946. Found in a rotten tree under the bark. Terrestrial leech; presumably non-blood-feeding (macrophagous). New country record; new state record; new locality record: Mexico, Volcán Tacaná, la Caracola (15°06′19.39″N, 92°05′47.27″W; 2452 m), one specimen collected July, 25, 2015, by Alejandro Oceguera-Figueroa (CNI 15). GenBank cox1 barcode sequence accession: MG821605.



FIGURES 12–15. Rhynchobdellida from Chiapas, Mexico. *Helobdella elongata*: **12.** dorsal view, **13**. ventral view. *Helobdella octatestisaca*: **14.** dorsal view, **15.** ventral view.

Order Rhynchobdellida Blanchard, 1894

Family Glossiphoniidae Vaillant, 1890

Helobdella elongata Castle, 1900

Figures 12–13

The absence of a chitinous nuchal scute; presence of an unpigmented, nonpapillated, and subcylindrical body in cross section; and weak anterior and posterior suckers all correspond to the description of *Helobdella elongata*. Specimens lacked eyespots, a characteristic noticed before for some individuals of this species from other localities (Klemm, 1982). Found on the underside of submerged rocks and accidentally attached to skin. Non-blood-feeding (liquidosomatophagous). Cox1 genetic distances of *H. elongata* from Chiapas are around 10% divergent from other samples of the same species from other localities. This suggests that samples from Chiapas may represent an undescribed species. New state record; new locality records: Tres Lagunas, Chiapas (16°50′33.9″N, 91°08′46.69″W; 370 m) 4 specimens collected January 27, 2016 by M. Tessler, J. Arroyave, S. Contreras and A. Oceguera-Figueroa (CNI 25); Bosque Azul, Chiapas (16°07′37.83″N, 91°43′53.68″W; 1462 m) 3 specimens collected January 25, 2016 by M. Tessler, J. Arroyave, S. Contreras and A. Oceguera-Figueroa (CNI 26). GenBank cox1 barcode sequence accessions: MG821608, MG821609, and MG821610.

Helobdella octatestisaca Lai and Chang, 2009

Figures 14–15

The presence of a chitinous nuchal scute; dorsal and ventral surfaces with inconspicuous papillae; no metameric pigmentation patterns; eyespots in one pair, each shaped somewhat triangularly, almost touching each other is consistent with the description of *Helobdella octatestisaca* (see Salas-Montiel et al., 2014). Found on the underside of submerged rocks and plants. Non-blood-feeding (liquidosomatophagous). New state record; new locality records: Bosque Azul, Lagunas de Montebello (16°07′37.83″N, 91°43′53.68″W; 1462 m) 30 specimens collected January 25, 2016, by M. Tessler, J. Arroyave, S. Contreras, and A. Oceguera-Figueroa (CNI 27); Osumacinta Dam (16°57′47.5″N, 93°06′21.82″W; 207 m) 16 specimens collected January 24, 2016, by M. Tessler, J. Arroyave, S. Contreras, and A. Oceguera-Figueroa (CNI 28). GenBank cox1 barcode sequence accessions: MG821611, MG821612, MG821613, and MG821614.

Helobdella socimulcensis Caballero, 1931

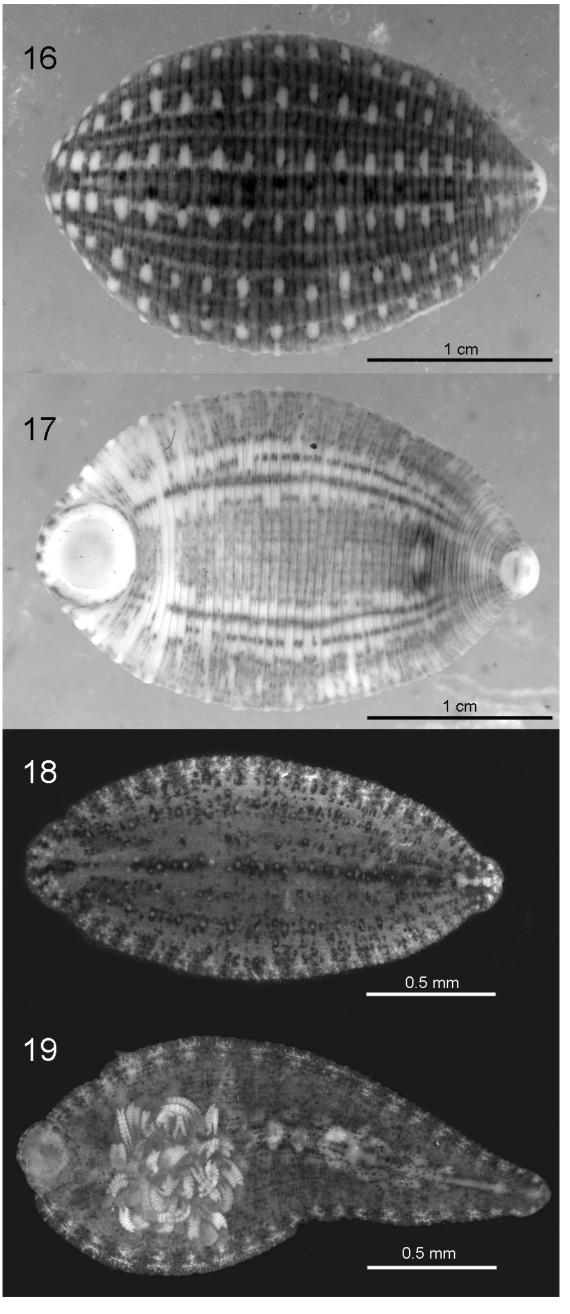
Figures 16–17

The presence of dorsal surface with complex pattern of longitudinal stripes and metameric papillae; nuchal scute absent; eyespots in one pair, punctiform, well separated from each other is consistent with the description of *Helobdella socimulcensis* (see Salas-Montiel et al., 2014). Found on the underside of submerged rocks and plants. Non-blood-feeding (liquidosomatophagous). New locality records: Río Fogótico, Arcotete, Chiapas, Mexico (16°44′46.5″N, 92°34′17.24″W; 2298 m), collected January 23, 2016, by M. Tessler, J. Arroyave, S. Contreras, and A. Oceguera-Figueroa (CNI 29). GenBank cox1 barcode sequence accessions: MG821615 and MG821616.

Placobdella ringueleti López-Jiménez and Oceguera-Figueroa, 2009

Figures 18–19

The presence of two pairs of eyespots in close contact, a single dorsal longitudinal stripe, and a papillated dorsal surface is consistent with the description of *Placobdella ringueleti*. New locality record: Palenque (17°29′10″N, 92°01′10″W; 68 m), collected March, 2015, by J. Pérez-Flores (CNHE 10455). GenBank cox1 barcode sequence accession: MG821617.



FIGURES 16–19. Rhynchobdellida from Chiapas, Mexico. *Helobdella socimulcensis*: **16.** dorsal view, **17.** ventral view. *Placobdella ringueleti*: **18.** dorsal view, **19.** ventral view.

CONCLUSIONS

With the description of *Erpobdella adani* there are now 33 known leech species from Mexico (Oceguera-Figueroa and León-Règagnon, 2014; Salas-Montiel et al., 2014). Accordingly, the nine species found in Chiapas (six non-blood-feeding and three blood-feeding) represent 27% of the leeches found in Mexico. Four of the 10 leech families known from Mexico (40%) were found as well. The diversity found thus far in Chiapas is similar to that known from the relatively well-surveyed central states of Mexico. However, the diversity of the state is surely higher than presently known. For example, the marine species of the Piscicolidae and Ozobranchidae have yet to be surveyed in the area, as far as we are aware.

All but two leech species found are aquatic. The terrestrial leech *Diestecostoma octannulata* is previously known only from Volcán Tajumulco, Guatemala (Moore, 1946), and thus the record reported here expands the known distribution of this species to Mexico. Another terrestrial leech is potentially known for the state: *D. magna* Moore, 1945 (Oceguera-Figueroa and León-Règagnon, 2014); however, the type locality of this species is ambiguous, being between the states of Veracruz and Chiapas (Moore, 1945). Two *Helobdella* species were found in addition to the previous record of *H. socimulcensis*—these three species are relatively widespread in Mexico and correspond to half of the known Mexican *Helobdella* species (Salas-Montiel et al., 2014). However, *H. elongata* from Chiapas might be a new taxon given the elevated genetic distances in comparison with samples from other localities in Mexico. The three blood-feeding species, *Pintobdella chiapasensis, Placobdella ringueleti*, and *Haementeria acuecueyetzin* were found in prior works (Caballero, 1957; López-Jiménez and Oceguera-Figueroa, 2009; Phillips et al., 2010; Pérez-Flores et al., 2016).

The addition of the new species, *Erpobdella adani*, along with current and prior records of *E. triannulata* represent half of the four *Erpobdella* species now known from Mexico (Oceguera-Figueroa and León-Règagnon, 2014). *Erpobdella adani* is phylogenetically distinct from those *Erpobdella* species included in the analysis. It is generally closely related to the other three species known from Mexico, as well as most of the North American *Erpobdella*. Of the three other Mexican species, *E. adani* is most closely related to *E. ochoterenai*. The relationships recovered for Mexican *Erpobdella* in our analyses are largely consistent with prior work (Oceguera-Figueroa et al., 2005, 2011), insofar as the positions of *E. mexicana* and *E. ochoterenai* are consistent while *E. triannulata* varies in its position depending on the analysis.

Unlike the other Mexican species that are broadly distributed (Oceguera-Figueroa and León-Règagnon, 2014), *Erpobdella adani* was found only in a single stream. Future collecting efforts for this species should concentrate on more streams in Chiapas and possibly in nearby Guatemala, which, like much of Central America, remains largely unsurveyed for leech diversity. Until then, it will be hard to determine how restricted the range is of this putative endemic. It is also worth noting that leech-collecting efforts generally focus on lacustrine habitats, while *E. adani* was found in lotic conditions. Furthermore, these conditions made it generally hard to spot and collect.

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