

Description of a New Species of Killifish of the Genus *Profundulus* (Atherinomorpha: Profundulidae) from the Mexican State of Oaxaca

Wilfredo A. Matamoros¹, Sara E. Domínguez-Cisneros¹, Ernesto Velázquez-Velázquez¹, and Caleb D. McMahan²

The Middle American killifish genus *Profundulus* occurs in most Pacific and Atlantic drainages from the Mexican state of Guerrero to the Chamelecón River in Honduras, with highest species diversity in southern Mexico. In this study, we describe a new member of the genus, *Profundulus parentiae*, new species, from the Mexican state of Oaxaca. It is distinguished from *P. guatemalensis* and *P. kreiseri* by having rows of dots on the sides of the body. *Profundulus parentiae*, new species, can be distinguished from *P. oaxacae* by having 33 lateral scales, versus 29–31 in *P. oaxacae*. *Profundulus parentiae*, new species, can be distinguished from *P. balsanus*, *P. oaxacae*, and *P. mixtlanensis* by the presence of long epiotic processes that extend beyond the epipleural ribs of the first vertebra, versus short epiotic processes not reaching the epipleural ribs of the first vertebra. *Profundulus parentiae*, new species, can be differentiated from *P. punctatus* by the presence of a dorso-ventrally compressed Meckel's cartilage with a relatively straight medial process and a narrow and strongly concave sesamoid articular, versus a dorso-ventrally expanded Meckel's cartilage with concave medial process and a wide and moderately concave sesamoid articular. Based on the phylogenetic analysis of molecular sequence data, the new species is recovered as the sister taxon to *P. balsanus*. The discovery and description of this new species in southeastern Mexico, raises the number of species from this region to five and suggests that this area has been an important center for diversification within this lineage of killifishes.

El género *Profundulus* son escamudos de América-Media que ocurren en la mayoría de cuencas del Pacífico y Atlántico, desde el estado de Guerrero hasta la zona central de Honduras, presentando los niveles más altos de diversidad en el sureste de México. En este estudio describimos un nuevo miembro de este género, *Profundulus parentiae* sp. nov., del estado de Oaxaca, México. Se distingue de *P. guatemalensis* y *P. kreiseri* por tener líneas de puntos a los lados del cuerpo. *Profundulus parentiae* se puede distinguir de *P. oaxacae* por tener 33 escamas laterales, versus 29–31 en *P. oaxacae*. *Profundulus parentiae* sp. nov. se puede distinguir de *P. balsanus*, *P. oaxacae* y *P. mixtlanensis* por la presencia de un proceso epiotico alargado que se extiende por debajo de la costilla epipleural de la primera vertebra, versus un proceso epiotico corto que no llega a la costilla epipleural de la primera vertebra. *Profundulus parentiae* sp. nov. se puede diferenciar de *P. punctatus* por la presencia de un cartílago de Meckel comprimido dorso-ventralmente con un proceso medial relativamente recto y un angosto y fuertemente cóncavo sesamoideo articular, versus un cartílago de Meckel expandido dorso-ventralmente con un proceso medial cóncavo y un sesamoideo articular ancho y moderadamente cóncavo. Basados en los análisis filogenéticos con datos de secuenciaciones moleculares, la nueva especie se recobró como taxón hermano de *P. balsanus*. El descubrimiento y descripción de esta nueva especie en el sureste de México, aumenta el número de especies en esta región a cinco, y sugiere que esta área ha sido un importante centro para la diversificación para este linaje de escamudos.

UNTIL recently, Profundulidae was comprised of a single genus, *Profundulus*, with two subgenera, *Profundulus* and *Tlaloc* (Miller, 1955). Based on phylogenetic relationships recovered using molecular data, these two subgenera were elevated to the generic level by Morcillo et al. (2016). Morcillo et al. (2016) provided a combination of morphological characters to define the two genera such as presence or absence of a humeral spot, a lachrymal bone with or without scales, and patterns of scale cover on the caudal fin. Furthermore, the two genera can be largely separated by their geographic distributions. Members of *Profundulus* are mainly found in rivers and streams that drain into the Central American Pacific slope, from the Río Papagayo in the Costa Chica in the Mexican state of Guerrero to the Río Lempa in Honduras and El Salvador (McMahan et al., 2013; Jamangapé et al., 2016); however, in some parts of its distribution, species of this genus are also found in the Central American Atlantic slope. This is the case with *Profundulus kreiseri*, widely distributed in Pacific rivers of Honduras and El Salvador (McMahan et al., 2013; Jamangapé et al., 2016) but also present in rivers draining to the Atlantic

slope (e.g., Chamelecón, Ulúa, and Motagua in Honduras; Matamoros et al., 2012; Jamangapé et al., 2016) and the Río Polochic in Guatemala (Jamangapé et al., 2016; Morcillo et al., 2016). Conversely, members of the genus *Tlaloc* are mainly restricted to the Central American Atlantic slope from the Río Grijalva-Usumacinta basin in Mexico and Guatemala (Gómez-González et al., 2015; Velázquez-Velázquez [sic] et al., 2016) to the Río Ulúa in Honduras (Matamoros and Schaefer, 2010; Matamoros et al., 2012; Jamangapé et al., 2016), with the exception of *T. portillorum* which is also found in the Río Nacaome and Río Choluteca in the Pacific slope of Honduras (Matamoros and Schaefer, 2010).

Little work has been published focused on understanding the evolutionary history of the Profundulidae. Miller (1955) published a revision of the entire family in which all Mexican members of *Profundulus* were placed into the synonymy of a single species, *P. punctatus*. However, recent molecular studies have demonstrated that Mexican populations of *P. punctatus* (*sensu* Miller, 1955) include at least seven distinct lineages (Doadrio et al., 1999; Morcillo et al., 2016). Morcillo et al. (2016) recovered these populations in three clades (A, B, and

¹ Colección de Ictiología, Instituto de Ciencias Biológicas, Universidad de Ciencias y Artes de Chiapas, México, CP, 29039, Tuxtla Gutiérrez, Chiapas, México; Email: (WAM) wilmatamoros@yahoo.com; (SED-C) sara.dominguez@unicach.mx; and (EV-V) ernesto.velazquez@unicach.mx. Send reprint requests to WAM.

² The Field Museum, 1400 S. Lake Shore Drive, Chicago, Illinois 60605; Email: cmcmahan@fieldmuseum.org.

Submitted: 21 August 2017. Accepted: 5 March 2018. Associate Editor: J. F. Schaefer.

© 2018 by the American Society of Ichthyologists and Herpetologists DOI: 10.1643/CI-17-677 Published online: 3 May 2018

Table 1. Morphometric and meristic data from the holotype and paratypes of *P. parentiae*. SD = standard deviation, Min = minimum, Max = Maximum. Measurements for holotype in millimeters.

Morphometric characters	Holotype (<i>n</i> = 1)	Paratypes (<i>n</i> = 36)			
		% of standard length			
		Mean	SD	Min	Max
Standard length (SL)	65.9	—	—	—	—
Head length (HL)	17.7	29.4	1.7	26.9	35.3
Pre-dorsal length (PDL)	43.6	67.1	1.1	64.3	69.3
Pre-pelvic length (PPL)	32.8	59.1	8.6	49.2	70.5
Anal origin to caudal base (AOCD)	22.2	32.2	3.8	16.9	36.2
Body-greatest depth (BGD)	20.4	29.8	1.9	25.4	33.7
Body-greatest width (BGW)	12.2	17.9	1.9	13.3	21.4
Caudal peduncle length (CPL)	10.6	17.9	1.2	14.3	20.0
Caudal peduncle depth (PLD)	9.6	15.2	0.8	14.1	16.9
Dorsal-fin base length (DFBL)	9.9	14.4	1.3	11.6	16.8
Anal-fin base length (AFBL)	11.3	15.7	1.1	13.2	17.5
Head depth (HD)	12.8	20.1	1.2	17.7	23.4
Head width (HW)	12.9	20.0	1.0	18.6	22.7
Interorbital bony width (IOLBW)	9.4	14.8	1.2	12.3	17.4
Orbital length (OL)	4.58	7.6	0.5	6.6	8.5
Snout length (SNL)	5.6	8.3	0.6	7.2	9.7
Upper jaw length (UJL)	3.2	4.8	0.8	3.1	6.4
Meristic characters		Mode	SD	Min	Max
Dorsal-fin rays (DFR)	12	12	0.7	10	13
Anal-fin rays (AFR)	14	15	0.7	13	15
Caudal-fin rays (CFR)	20	20	0.6	18	20
Pectoral-fin rays (PCR)	16	16	0.9	11	17
Pelvic-fin rays (PLR)	6	6	0.0	6	6
Lateral line scales (LLS)	33	33	0.0	33	33
Pre-dorsal scales (PDS)	22	22	0.7	18	22
Scales around the body (ARB)	29	29	0.8	25	29
Scales around the caudal peduncle (ARC)	19	19	0.9	17	21

D). Clade A included four species, two of which are already described (*P. punctatus* and *P. oaxacae*) and two putatively new species. Clade B included *P. balsanus*, recently re-described and resurrected from synonymy by Jamangapé et al. (2016), and an undescribed lineage labeled *P. aff. punctatus*. *Profundulus aff. punctatus* and *P. balsanus* possess the northernmost distributions for the genus. Clade D, *P. mixtlanensis*, was recently described by Ornelas-García et al. (2015).

In 2015, we made collections of *Profundulus* from the Mexican states of Guerrero and Oaxaca, which already harbor four known species (Jamangapé et al., 2016; Morcillo et al., 2016): *P. balsanus*, *P. mixtlanensis*, *P. oaxacae*, and *P. punctatus*. Effort was made to collect specimens belonging to the sub-clade “*P. aff. punctatus*” of Morcillo et al. (2016). We gathered morphometric, meristic, osteological, and molecular data with the aim of assessing distinctiveness and taxonomic status of this population of *P. aff. punctatus*.

MATERIALS AND METHODS

Sampling was performed from 21 April to 1 May 2015 at 23 localities in drainages of the Pacific slope of the Mexican states of Chiapas, Oaxaca, and Guerrero. Fishes were captured using a combination of collection gear that included seines, cast-nets, and electrofishing. After capture, fishes were euthanized with an overdose of MS222. In the field, a fin clip was excised from specimens and preserved in 95% ethanol. Fishes were then fixed in a solution of 10% formalin

for at least 72 hours, then rinsed for 24 hours in tap water and immediately transferred to a solution of 70% ethanol for final preservation. All samples were deposited at the Universidad de Ciencias y Artes de Chiapas (UNICACH-MZ-P), Universidad Nacional Autónoma de México (CNPE-IBUNAM), and the Field Museum of Natural History (FMNH). Institutional abbreviations follow Sabaj (2016), except for UNICACH-MZ-P.

Morphological analyses.—A total of 106 individuals including the putative new species (*n* = 36) and three geographically close congeners, *P. balsanus* (*n* = 22), *P. oaxacae* (*n* = 34), and *P. punctatus* (*n* = 14), were examined for morphological analysis. See Material Examined section for collection details. Measurements and counts followed Miller (1948). A set of 17 standardized morphometric measurements and nine meristic counts were collected for all specimens (Table 1). Measurements were taken with digital calipers to the nearest 0.1 mm and are presented as percentage of standard length (SL). As indicated by Miller (1948), the last two fin rays of the anal and dorsal fins bifurcate internally; thus, counts of anal- and dorsal-fin rays were performed accordingly by counting all rays in the fin and subtracting one ray. A summary of the names of each morphometric measurement and count with their acronyms is provided in Table 1.

A principal component analysis (PCA) was applied to explore body shape variability and summarize meristic data. To avoid potentially confounding effects of allometry, the

Table 2. GenBank accession numbers. Species identifiers match those in phylogeny (Fig. 3). Newly produced sequences in bold font. Locality details provided in Supplemental Material 1 (see Data Accessibility).

Species	Cyt- <i>b</i>	COI	ND2
<i>P. parentiae</i> CT196	MH144355	MH114094	MH114085
<i>P. parentiae</i> CT210	MH144353	MH114092	MH114084
<i>P. parentiae</i> CT229	MH144354	MH114093	N/A
<i>P. parentiae</i> CT251	MH144351	MH114091	MH114083
<i>P. parentiae</i> CT497	MH144356	MH114095	MH114086
<i>P. balsanus</i> 1	KX611586	KX611579	N/A
<i>P. balsanus</i> 2	KX611587	KX611580	N/A
<i>P. balsanus</i> 3	KX611588	KX611581	N/A
<i>P. balsanus</i> 4	KX611589	KX611582	N/A
<i>P. balsanus</i> 5	KX611590	KX611583	N/A
<i>P. kreiseri</i> HN	JQ254934	N/A	N/A
<i>P. kreiseri</i> 173	MH144359	N/A	MH114089
<i>P. kreiseri</i> 168	MH144352	N/A	MH114090
<i>P. kreiseri</i> 166	MH144358	MH114097	MH114088
<i>P. guatemalensis</i>	AY155568	N/A	KJ878766
<i>P. punctatus</i>	KX611591	KX611585	KJ878760
<i>P. mixtlanensis</i>	N/A	N/A	KJ878776
<i>P. mixtlanensis</i> CT470	MH144357	MH114096	MH114087
<i>P. oaxacae</i>	JQ254933	KX611584	KJ878754
<i>Tlaloc portillorum</i>	JQ254930	N/A	KJ878788
<i>Tlaloc labialis</i>	AY155567	JN028283	KJ878784
<i>Tlaloc candalarius</i>	JQ254931	KX632152	KJ878780
<i>Tlaloc hildebrandi</i>	JQ254932	KX632151	KJ878779

raw morphometric data, including head subunits, were standardized by converting them into ratios by dividing all linear measurements by SL (Atchley et al., 1976; Winemiller, 1991). Analyses were implemented in R v.3.0.2 (R Core Team, 2013) using the package VEGAN 2.2-1 (Oksanen et al., 2015).

Molecular analyses.—We obtained mitochondrial sequence data from 23 individuals representing 11 species (Table 2); seven in the genus *Profundulus* (*P. balsanus*, *P. guatemalensis*, *P. kreiseri*, *P. mixtlanensis*, *P. oaxacae*, *P. punctatus*, and *Profundulus parentiae*, new species), and four more from the genus *Tlaloc* (*T. candalarius*, *T. hildebrandi*, *T. labialis*, and *T. portillorum*) that were used as outgroups. Choice of outgroup taxa was based on previous molecular analyses of *Profundulidae* (e.g., Matamoros et al., 2012; Jamangapé et al., 2016; Morcillo et al., 2016). DNA sequences included three mitochondrial genes (Cytochrome *b* = Cyt-*b*, Cytochrome oxidase subunit I = COI, and NADH dehydrogenase subunit 2 = ND2). Twenty-four new sequences were produced and deposited in GenBank (Table 2). Amplification conditions, primers, and sequencing protocols are described by Jamangapé et al. (2016) and Morcillo et al. (2016). Molecular data were concatenated in Mesquite 3.2 (Maddison and Maddison, 2016). We partitioned the concatenated dataset by locus and assigned the best fitting models of molecular evolution selected by jModelTest (Posada, 2008) using the Akaike Information Criterion. Phylogenetic relationships were estimated using Bayesian inference in the program MrBayes 3.2 (Ronquist et al., 2012). Two analyses of one million generations each were performed with four chains (one cold, three heated) sampling every 1000 generations. We used Tracer 1.5 (Rambaut and Drummond, 2009) to check the trace files and ensure the chains had reached convergence, and discarded the first 25% of trees as burn-in. A 50% majority rule consensus tree was generated from post-burn-in trees.

RESULTS

The putative new species was found in only four of the 23 sampled localities, all in the state of Oaxaca (Fig. 1). The PCA based on meristic characters (Fig. 2A) explained 57.3% of the variance, with 29.5% explained by PC1 and 27.8% explained by PC2. *Profundulus balsanus* and *Profundulus parentiae*, new species, occupied almost the same multivariate space and strongly loaded to the negative of PC1, which is consistent with higher ARB counts (scales around body). Conversely, *P. oaxacae* and *P. punctatus* loaded strongly toward the positive end of PC1; these are the species with the lowest ARB counts. In the PCA based on morphometric characters (Fig. 2B), PC1 explained 82.3% of the variance and PC2 explained 7.2% for a total of 89.6% of variance explained by this analysis. Characters that loaded heavily along the positive of PC2 were related to head morphology such as upper jaw length, snout length and head depth. In this PCA plot, *P. balsanus*, *P. punctatus*, and *Profundulus parentiae*, new species, overlapped in multivariate space, strongly loading toward the negative of PC2, suggesting that these species are very similar at least from a morphometric approach. The negative PC2 was related to HD and UJL size; the three aforementioned species represent the smaller UJL and HD. *Profundulus oaxacae* was found almost exclusively within the positive of PC2.

A total of three mitochondrial genes were included in the analyses (Cyt-*b* = 1055 bp; COI = 638 bp; ND2 = 1047 bp) that totaled 2740 bp in length. The Bayesian topology (Fig. 3) had high posterior probabilities throughout the tree. *Profundulus parentiae*, new species, was recovered sister to *P. balsanus*. The ranges of sequence divergence between *Profundulus parentiae*, new species, and its congeners varied across the three mitochondrial loci, with the smaller ranges of divergence in COI (2.9–7.1%) and Cyt-*b* genes (3.7–5.4%) and the largest with ND2 (4.9–5.8%). The average sequence divergence between *P. balsanus* and *Profundulus parentiae*, new species,

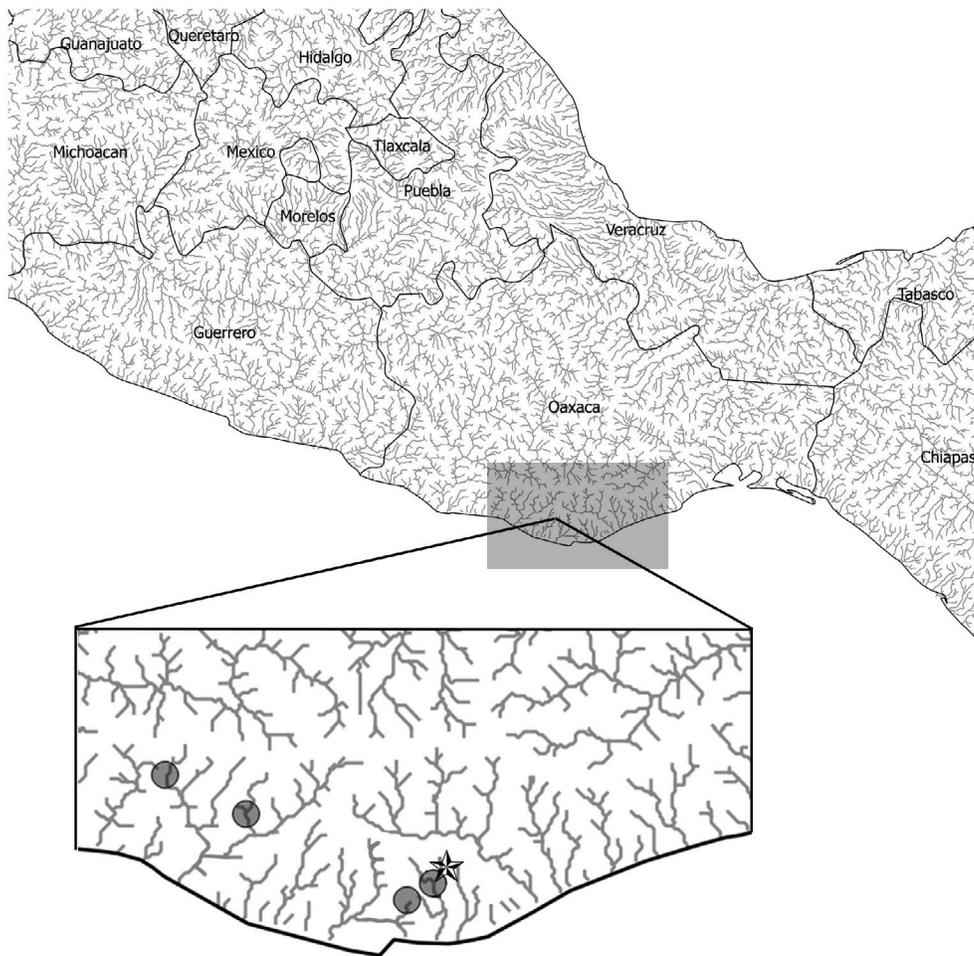


Fig. 1. Map showing collection localities for *Profundulus parentiae*. Type locality depicted by a star.

across loci was 2.8–4.6% (COI 2.8% \pm 0.7, Cyt-*b* 3.6% \pm 0.05, ND2 4.6% \pm 0.4).

These two lineages form a clade with *P. guatemalensis* and *P. kreiseri*. *Profundulus punctatus* and *P. oaxacae* form a clade sister to *Profundulus parentiae*, new species, *P. balsanus*, *P. guatemalensis*, and *P. kreiseri*. Finally, *P. mixtlanensis* was recovered as sister to all other species of *Profundulus* (Fig. 3).

***Profundulus parentiae*, new species**

urn:lsid:zoobank.org:act:552BB934-D1FE-4D8C-BA2A-4E78A8DB2527

Parenti's Killifish

Escamudo de Parenti

Figures 4, 5

Profundulus aff. punctatus (Morcillo et al., 2016)

Holotype.—CNPE-IBUNAM 22804, female, 65.9 mm SL, México, State of Oaxaca, Santa María Huatulco, Municipality of Santa María Huatulco, Toma de agua Río Huatulco, on the way to the Benito Juárez community, small creek of about 20 m width with an average depth of ~30 cm, 15. 862172N, 96.312999W, W. A. Matamoros, M. de J. Anzueto, J. A. Jamangapé, and I. Aguilar, 25 April 2015.

Paratypes.—CNPE-IBUNAM 22805, 3, FMNH 131755, 2, UNICACH-MZ-P 7236, 1, same locality and collecting event as holotype; CNPE-IBUNAM 22901, 2, UNICACH-MZ-P 6576, 3, UNICACH-MZ-P 7238, 4, Mexico, State of Oaxaca, Municipality of Pochutla, arroyo de la calle central, colonia San

Miguel Figueroa, Pochutla, 15.790005N, 96.401313W, W. A. Matamoros, M. de J. Anzueto, J. A. Jamangapé, and I. Aguilar, 21 April 2015; CNPE-IBUNAM 22903, 3, Mexico, State of Oaxaca, Municipality of Santa María Huatulco, La Loma River, on the road to Comitlan, 16.017916N, 96.844287W, W. A. Matamoros, M. de J. Anzueto, J. A. Jamangapé, and I. Aguilar, 22 April 2015; UNICACH-MZ-P 6574, 4, UNICACH-MZ-P 7237, 5, Mexico, State of Oaxaca, Santa María Huatulco, Municipality of Santa María Huatulco, Hondura de toro at 100 meters from bridge, Huatulco, 15.834580N, 96.329944W, W. A. Matamoros, M. de J. Anzueto, J. A. Jamangapé, and I. Aguilar, 21 April 2015; UNICACH-MZ-P 6713, 5, Mexico, State of Oaxaca, Santa María Colotepec, Camino a Comitlan, Rio la Loma, close to Santa María Colotepec, 16.017916N, 96.844287W, W. A. Matamoros, M. de J. Anzueto, J. A. Jamangapé, and I. Aguilar, 22 April 2015.

Non-type material.—UNICACH-MZ-P 6574, 4, Mexico, State of Oaxaca, Santa María Huatulco, Municipality of Santa María Huatulco, Huatulco, Hondura de toro at 100 meters of bridge, 15.834580N, 96.329944W, W. A. Matamoros, M. de J. Anzueto, J. A. Jamangapé, and I. Aguilar, 21 April 2015; UNICACH-MZ-P 7239, 28, Mexico, State of Oaxaca, Municipality of Santa María Huatulco, La Loma River, on the road to Comitlan, 16.017916N, 96.844287W, W. A. Matamoros, M. de J. Anzueto, J. A. Jamangapé, and I. Aguilar, 22 April 2015. Cleared and stained material: UNICACH-MZ-P 6575, 5, 43.2–68.4 mm SL; UNICACH-MZ-P 6713, 3, 36.6–37.6 mm SL.

Diagnosis.—*Profundulus parentiae* can be distinguished from congeners *P. balsanus*, *P. oaxacae*, and *P. mixtlanensis* by the

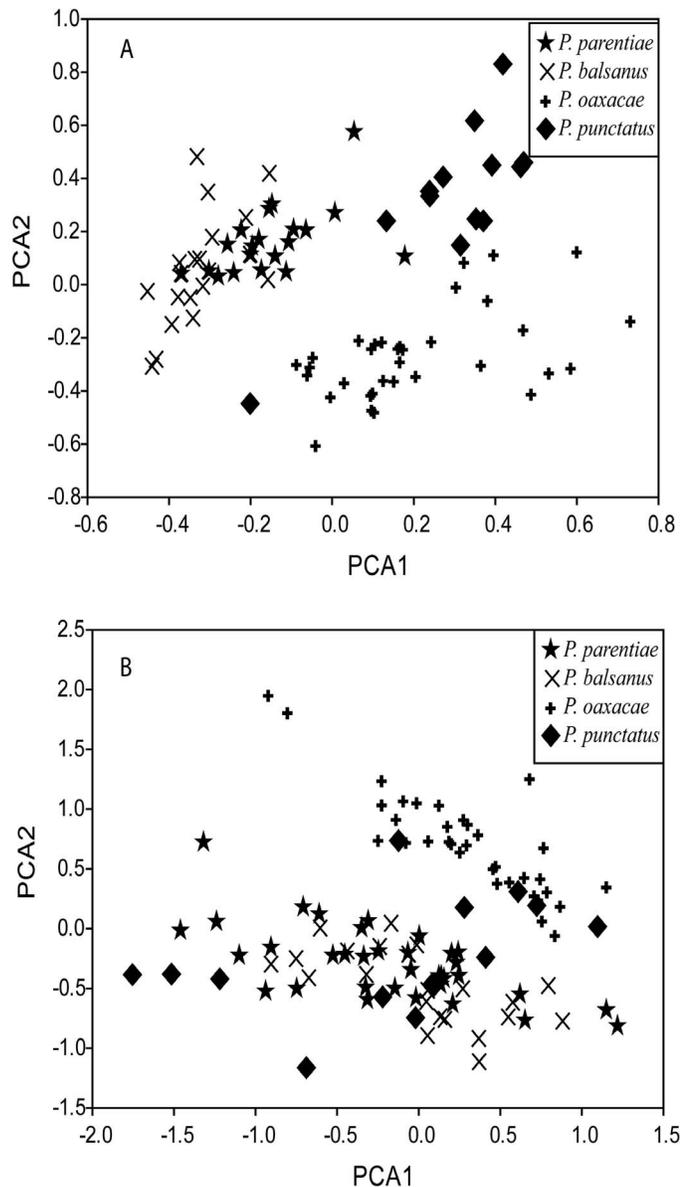


Fig. 2. Results of principal component analysis (PCA) based on meristic (A) and morphometric (B) data for four species of *Profundulus*: *P. balsanus*, *P. oaxacae*, *P. punctatus*, and *P. parentiae*, new species.

presence of long epiotic processes that extend beyond the epipleural ribs of the first vertebra (Fig. 6A), versus short epiotic process not reaching the epipleural ribs of the first vertebra (Fig. 6B). *Profundulus parentiae* can be differentiated from *P. guatemalensis* and *P. kreiseri* by the presence of rows of dark dots along the sides of the body versus the absence of rows of dark dots in *P. guatemalensis* and *P. kreiseri*. *Profundulus parentiae* can be differentiated from *P. punctatus* by the presence of a dorso-ventrally compressed Meckel's cartilage with a relatively straight ventral edge and a narrow and strongly concave sesamoid articular (Fig. 7A), versus a dorso-ventrally expanded Meckel's cartilage with convex ventral edge and a wide and moderately concave sesamoid articular in *P. punctatus* (Fig. 7B). *Profundulus parentiae* can be further distinguished from *P. oaxacae* by the number of scales in the lateral series; unequivocally 33 in *P. parentiae* and 29–31 in *P. oaxacae*.

Description.—Morphometric and meristic data are summarized in Table 1. Mouth is terminal with lower jaw protruding

forward and including the upper jaw. Snout rounded, 8.3% of SL. Lower jaw terminal, posterior tip of maxilla below inferior edge of the orbit. Head length 29.4% of SL, head width (HW) 20.0% of SL, and head depth (HD) 20.0% of SL. Orbital length 7.6% of SL. Greatest body depth approximately 29.8% of SL. Head and pre-dorsal profiles straight from end of the head to the anterior insertion of the dorsal fin. Dorsal fin positioned posteriorly on the body with anterior and posterior insertions slightly anterior of those of the anal fin. Caudal fin rounded. Pectoral fins moderately elongate and rounded at tips, inserted below midline of body; pelvic fins short and inserted mid-way between the insertions of the anal and pectoral fins. Maximum reported SL 68.3 mm (UNICACH-MZ-P 6575).

Preserved coloration.—Preserved specimens brownish laterally and yellowish ventrally. Five rows of dots present along the side of the body from the operculum to the caudal-fin base; this line is less conspicuous in life and ends in a semicircle formed by dots. The pelvic and pectoral fins transparent. Dorsal fin transparent with small dark spots. Anal fin yellowish with a dark submarginal line. Posterior half of the caudal fin clear and yellowish with some dots at the anterior half (Fig. 4).

Live coloration.—Live coloration description is largely based on a single live female specimen (Fig. 5). This species is brownish to yellowish above the midline of the body, whereas below is pale. A stripe formed by rows of dots is present on the side of the body and extends from the base of the caudal peduncle to near the posterior tip of the pectoral fin. This stripe is formed by 3–5 rows of dark dots; these dots are more conspicuous in preserved specimens (Fig. 4). A golden yellow blotch covers the operculum and reaches the base of the pectoral fin. Pelvic and pectoral fins transparent. Dorsal and anal fin yellowish. Anal fin yellowish with a dark submarginal line more conspicuous in preserved coloration. Caudal fin yellowish in the base and transparent posteriorly (Fig. 5).

Distribution and habitat.—The type locality (Fig. 1) is near the town of Santa María de Huatulco, along the lower reaches of the Río Huatulco, a tributary of the Río Coyula, at an altitude of 260 meters. Environmental conditions at the type locality during sampling were as follows: water temperature 27°C, pH 6.7, conductivity 10.9 mS, dissolved oxygen 12.3 mg/L, river width 20 m, and capture depth 0.5 m. Current was slow, and there was no canopy cover. *Poeciliopsis pleurospilus*, *Poecilia sphenops*, and *Sicydium salvini* were collected along with *P. parentiae* at the type locality and collecting event.

The known distribution of *P. parentiae* extends from the lower reaches of the Huatulco River in the Mexican state of Oaxaca close to the city of Santa María de Huatulco to tributaries of the Grande River in the vicinity of the town of Comitlan, in Oaxaca (Fig. 1). The species was captured in a range of altitudes from 188 to 420 meters.

Phylogenetic relationships.—*Profundulus parentiae* is the sister species to *P. balsanus* (Fig. 3). Average mitochondrial sequence divergence between *P. parentiae* and *P. balsanus* is 2.5–4.7%.

Etymology.—This species is named in honor of Dr. Lynne Parenti, who has made many important contributions to our knowledge of the systematics, biogeography, biology, and morphology of cyprinodontiforms as well as numerous other groups of fishes.

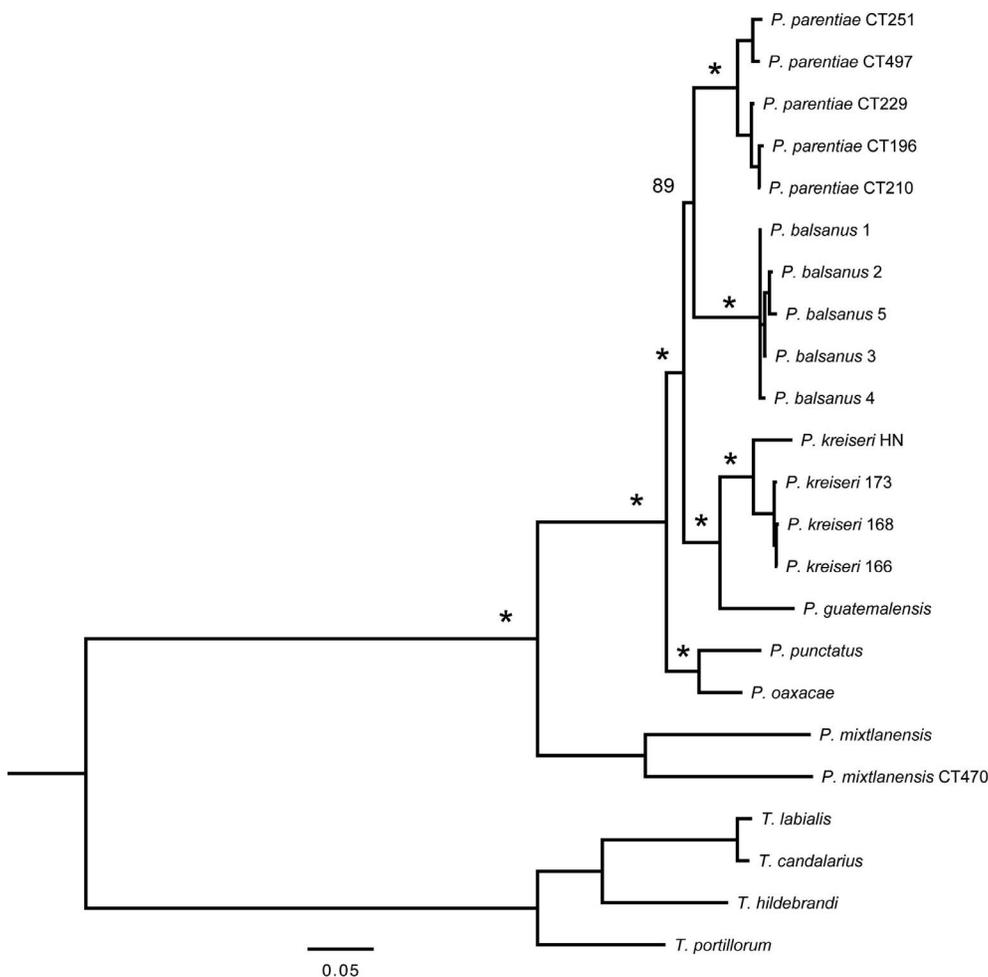


Fig. 3. Bayesian phylogeny showing relationships of *Profundulus parentiae*, new species, with other species of *Profundulus* and *Tilaloc* in Central America and southern Mexico. Numbers above nodes represent Bayesian posterior probabilities; * indicates posterior probability ≥ 95 .

DISCUSSION

Much progress has been made in understanding the systematics and taxonomy of Profundulidae since Miller's (1955) revision of the group. Molecular data have not only allowed for the detection of undescribed species but also offered new evidence to resurrect others that were previously placed in synonymy, leading to more accurate delimitation of distributional ranges for species. A notable feature of the freshwater ichthyofauna of southern Pacific drainages in Mexico is the relatively low intra-generic diversity, which is normally limited to only one or two species. The genus *Profundulus* now contains five species in this region. Additional data and

exploration with no doubt will offer new avenues to explore evolutionary and biogeographic patterns in this region.

Our results suggest that *P. parentiae* is sister to *P. balsanus*. This relationship was expected since *P. parentiae* was split from the latter species. Although this relationship is consistent with the study of Morcillo et al. (2016), other phylogenetic relationships recovered in these two studies were not. For instance, in Morcillo et al. (2016), the clade formed by *P. punctatus* and *P. oaxaca* is sister to *P. balsanus* and *P. parentiae*. However, in our tree, *P. punctatus* and *P. oaxaca* are sister to a clade comprising (*P. balsanus* and *P. parentiae*) + (*P. guatemalensis* and *P. kreiseri*). Both studies recovered *P. mixtlanensis* as the sister taxon to all other



Fig. 4. *Profundulus parentiae*, holotype, CNPE-IBUNAM 22804, 65.9 mm SL.



Fig. 5. Live specimen of *Profundulus parentiae*, UNICACH-MZ-P 6576.

Profundulus. Differences found in both phylogenies may be due to artifacts created by differences in the number or choice of molecular markers used in both studies.

Although the genus *Profundulus* occurs mainly in the Pacific slope of Central America, populations of some species are shared with the Atlantic slope. For example, populations of *P. punctatus* are found in the Coatzacoalcos River in southern Mexico and populations of *P. kreiseri* are found in the Ulúa, Chamelecón, Motagua, and Polochic rivers in Honduras and Guatemala. The prevalence of species of *Profundulus* in the Pacific slope of Central America could be the result of niche conservatism, and the occurrence of subsets of populations in the Atlantic slope can be explained by recent dispersal events, although dispersal mechanisms and routes remain unclear. River-capture in upper portions of rivers and anastomosis in lowlands are possible causes of inter-slope dispersal that should be considered in future studies.

MATERIAL EXAMINED

Profundulus balsanus: UNICACH-MZ-P 6712, 10, 48.67–64.68 mm SL, Guerrero, Malinaltepec, Río Malinaltepec, 17. 2307N, 98.66698W.

Profundulus mixtlanensis: UNICACH-MZ-P 6719, 2, Mexico, State of Oaxaca, Municipality of Santa María Zacatepequec,

puente el Platanar, 16.749385N, 97.992423W, W. A. Matamoros, M. de J. Anzueto, J. A. Jamangapé, and I. Aguilar, 29 April 2015.

Profundulus oaxacae: UNICACH-MZ-P 6714, 46, 26.46–63.19 mm SL, Oaxaca, Mitla, Río Salado-Subcuenca del Atoyac, 16.926607N, 96.3343525W.

Profundulus punctatus: UNICACH-MZ-P 6355, 18, 29.73–78.33 mm SL, Chiapas, Cacahoatán, Río Zapata-Puente Col. Emiliano Zapata, 14.986563N, 92.170663W.

Cleared and stained material

Profundulus balsanus: UNICACH-MZ-P 6712, 5, 31.6–58.3 mm SL.

Profundulus kreiseri: UNICACH-MZ-P 7231, 2, 48.1–59.6 mm SL.

Profundulus punctatus: UNICACH-MZ-P 6326, 2, 68.1–70.7 mm SL; UNICACH-MZ-P 6355, 1, 67.3 mm SL.

DATA ACCESSIBILITY

Supplemental material is available at <http://www.copeiajournal.org/ci-17-677>.

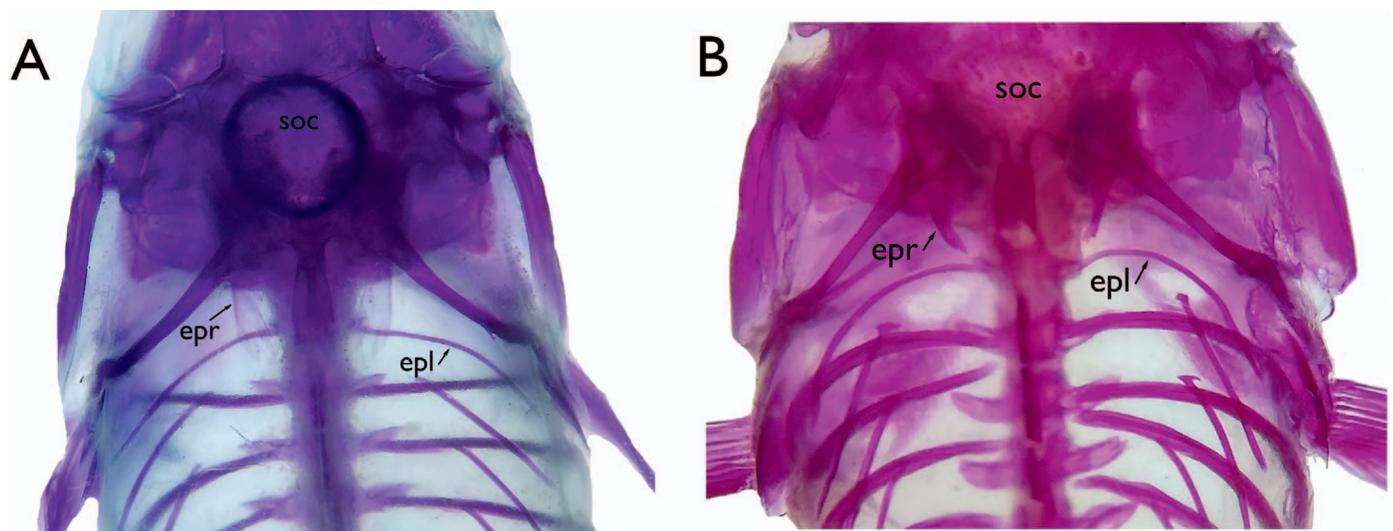


Fig. 6. Cleared and stained specimens showing long epiotic processes that extend beyond the epipleural ribs of the first vertebra in *P. parentiae* (4A; UNICACH-MZ-P 6575), versus short epiotic processes not reaching the epipleural ribs of the first vertebra in *P. balsanus* (4B; UNICACH-MZ-P 6712). epl = epipleural rib, epr = epiotic process, soc = supraoccipital.

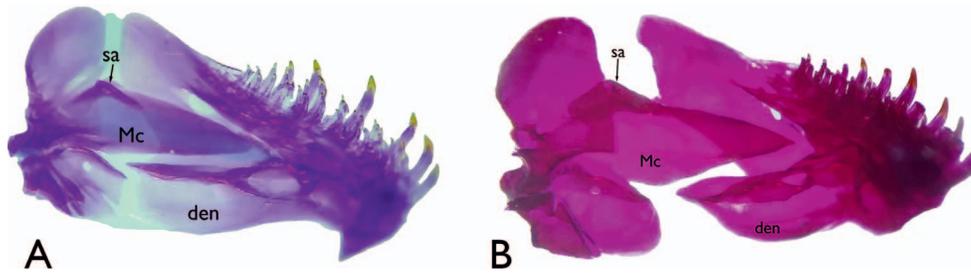


Fig. 7. Cleared and stained specimens showing dorso-ventrally compressed Meckel's cartilage with relatively straight ventral edge and narrow and strongly concave sesamoid articular in *P. parentiae* (5A; UNICACH-MZ-P 6575), versus dorso-ventrally expanded Meckel's cartilage with convex ventral edge and wide, moderately concave sesamoid articular in *P. punctatus* (5B; UNICACH-MZ-P 6355). den = dentary, Mc = Meckel's cartilage, sa = sesamoid articular.

ACKNOWLEDGMENTS

We are thankful to M. Anzueto, A. Jamangapé, and I. Aguilar for assistance with fieldwork. We thank James Egan for assistance with map preparation and C. L. Miceli and M. Peralta from the Direction of the Instituto de Biología at UNICACH for continued support to WAM. We thank SAGARPA personnel for issuing collecting permits in the states of Guerrero, Oaxaca, and Chiapas. Portions of this work were carried out in the Pritzker Laboratory for Molecular Systematics and Evolution at the Field Museum of Natural History, with support from the Pritzker Foundation. This study was partially financed by funds to WAM from PRODEP and the Visiting Scholarships Program of the Field Museum of Natural History.

LITERATURE CITED

- Atchley, W. R., C. T. Gaskins, and D. Anderson. 1976. Statistical properties of ratios. I. Empirical results. *Systematic Zoology* 25:137–148.
- Doadrio, I., J. A. Carmona, E. Martínez, and A. de Sostoa. 1999. Genetic variation and taxonomic analysis of the subgenus *Profundulus*. *Journal of Fish Biology* 55:751–756.
- Gómez-González, A. E., E. Velázquez-Velázquez, M. Anzueto-Calvo, and M. F. Maza-Cruz. 2015. Fishes of the Grijalva River basin of Mexico and Guatemala. *Check List* 11:1726.
- Jamangapé, J. A., E. Velásquez-Velázquez, E. Martínez-Ramírez, M. Anzueto-Calvo, E. Gómez, S. E. Dominguez-Cisneros, C. D. McMahan, and W. A. Matamoros. 2016. Validity and redescription of *Profundulus balsanus* Ahl, 1935 (Cyprinodontiformes: Profundulidae). *Zootaxa* 4173: 55–65.
- Maddison, W. P., and D. R. Maddison. 2016. Mesquite: a modular system for evolutionary analysis: 3.11. Retrieved from <http://mesquiteproject.org>
- Matamoros, W. A., and J. F. Schaefer. 2010. A new species of *Profundulus* (Cyprinodontiformes: Profundulidae) from the Honduran central highlands. *Journal of Fish Biology* 76: 1498–1507.
- Matamoros, W., J. Schaefer, C. Hernández, and P. Chakrabarty. 2012. *Profundulus kreiseri*, a new species of Profundulidae (Teleostei, Cyprinodontiformes) from northwestern Honduras. *ZooKeys* 227:49–62.
- McMahan, C. D., W. A. Matamoros, F. S. A. Calderón, W. Y. Henríquez, H. M. Recinos, P. Chakrabarty, and N. Herrera. 2013. Checklist of the inland fishes of El Salvador. *Zootaxa* 3608:440–456.
- Miller, R. R. 1948. The cyprinodont fishes of the Death Valley system of eastern California and southwestern Nevada. *Miscellaneous Publications of the Museum of Zoology of the University of Michigan* 68:1–155.
- Miller, R. R. 1955. A systematic review of the Middle American fishes of the genus *Profundulus*. *Miscellaneous Publications of the Museum of Zoology of the University of Michigan* 92:1–64.
- Morcillo, F., C. P. Ornelas-García, L. Alcaraz, W. M. Matamoros, and I. Doadrio. 2016. Phylogenetic relationships and evolutionary history of the Mesoamerican endemic freshwater fish family Profundulidae (Cyprinodontiformes: Actinopterygii). *Molecular Phylogenetics and Evolution* 94:242–251.
- Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. Henry, H. Stevens, and H. Wagner. 2015. *vegan*: community ecology package. R package version 2.2-1. <http://CRAN.R-project.org/package=vegan>
- Ornelas-García, C. P., E. Martínez-Ramírez, and I. Doadrio. 2015. A new species of killifish of the family Profundulidae from the highlands of the Mixteca region, Mexico. *Revista Mexicana de Biodiversidad* 86:926–933.
- Posada, D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25:1253–1256.
- R Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- Rambaut, A., and J. Drummond. 2009. Tracer 1.5. MCMC Trace File Analyser. <http://tree.bio.ed.ac.uk/software/tracer/>
- Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61:539–542.
- Sabaj, M. H. (Ed.). 2016. Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an Online Reference. Version 6.5 (16 August 2016). Electronically accessible at <http://www.asih.org/>, American Society of Ichthyologists and Herpetologists, Washington, D.C.
- Velázquez-Velázquez [sic], E., J. H. López-Vila, A. E. Gómez-González, E. I. Romero-Berny, J. L. Lievano-Trujillo, and W. A. Matamoros. 2016. Checklist of the continental fishes of the state of Chiapas, Mexico, and their distribution. *ZooKeys* 632:99–120.
- Winemiller, K. O. 1991. Ecomorphological diversification in lowland freshwater fish assemblages from five biotic regions. *Ecological Monographs* 61:343–365.