

## Systematics and Taxonomy of *Chapalichthys* (Cyprinodontiformes: Goodeidae), a Small Genus of Live-Bearers from Central Mexico

Kyle R. Piller<sup>1</sup>, Devin D. Bloom<sup>2</sup>, John Lyons<sup>3</sup>, and Norman Mercado-Silva<sup>4</sup>

**The genus *Chapalichthys* (Cyprinodontiformes: Goodeidae) consists of three allopatrically distributed species that occur on the Mesa Central, Mexico. *Chapalichthys encaustus* primarily occurs in the Río Lerma-Santiago basin, whereas both *C. peraticus* and *C. pardalis* have restricted distributions in the adjacent Río Balsas basin. Taxonomic issues in the genus center around the validity of *C. peraticus*. A formal systematic and taxonomic assessment of the genus inclusive of all three species of *Chapalichthys* has never been conducted. Therefore, the objectives of this study were two-fold: 1) to assess the phylogenetic relationships among multiple populations and all three species of *Chapalichthys* using 1,047 bp of mtDNA (ND2) sequence data, and 2) in light of the phylogenetic results, to re-examine the taxonomic status of *C. peraticus* using meristic and pigmentation characters. The phylogeny indicates two clades, each consisting of a valid species. One clade includes multiple populations of *C. encaustus*, and a second clade consists of multiple individuals of *C. pardalis* and *C. peraticus*. *Chapalichthys pardalis* and *C. peraticus* possess nearly identical mitochondrial sequences for ND2. Morphologically, meristic counts of all characters examined showed overlap for all three species and provide no species-specific diagnostic information. *Chapalichthys encaustus* can be differentiated from *C. pardalis* and *C. peraticus* based on the presence of vertical bars along the lateral flank versus a spotted pattern in the other two species. *Chapalichthys pardalis* and *C. peraticus* cannot be differentiated from one another based on pigmentation or meristics. The results from this study support the recognition of only two species of *Chapalichthys*: *C. encaustus* and *C. pardalis*.**

THE Mexican Mesa Central, an elevated plateau located between the Sierra Occidental and the Sierra Oriental, is an ichthyologically distinct area that harbors many endemic taxa. One of the predominant groups of fishes is the family Goodeidae (subfamily Goodeinae) that consists of 19 genera and approximately 40 species (Lyons et al., 2019). Recent studies investigating the phylogenetic relationships among goodeid fishes have generally been congruent and provide robust hypotheses regarding the relationships among the goodeid genera. However, higher-level relationships based on mitochondrial (Doadrio and Domínguez, 2004; Webb et al., 2004) and nuclear DNA (Parker et al., 2019) are not in complete agreement. Greater sampling within particular goodeid genera is needed, not only to better understand the alpha-level diversity within the Goodeinae (Domínguez-Domínguez et al., 2008; Corona-Santiago et al., 2015; Piller et al., 2015), but also to better understand the distribution of genetic and morphological variation within various species groups and genera.

The genus *Chapalichthys* (subfamily Goodeinae) consists of three allopatrically distributed species that occur on the Mesa Central (Jordan and Snyder, 1899; Álvarez del Villar, 1963; Domínguez-Domínguez et al., 2005; Fig. 1). *Chapalichthys encaustus* was described by Jordan and Snyder (1899) from Lake Chapala, near Ocotlán, Jalisco, Mexico. *Chapalichthys encaustus* occurs in the lower portion of the Río Lerma and the upper portion of the Río Santiago (upstream from the falls at Juanacatlán), but, historically, has been most abundant in Lake Chapala. In addition, *C. encaustus* also occurs in several isolated but formerly connected lakes in the region. *Chapalichthys encaustus* is sympatric with many

species of *Chirostoma* (Atherinopsidae), one of the most economically important groups of fishes in Mexico (Vital-Rodríguez et al., 2017). Species of *Chirostoma* from Lake Chapala have been unintentionally introduced across Mexico and, as a result, several populations of *C. encaustus* likely were introduced into other lakes in the region. Hieronimus (1995) reported the occurrence of *C. encaustus* from the Río San Juan del Río in Queretáro, but Miller et al. (2005) suggested that these records were likely the result of human introductions. Lyons et al. (2019) also noted the occurrence of an introduced population of *C. encaustus* in Presa la Vega (Río Ameca basin).

The remaining two species in the genus, *C. peraticus* and *C. pardalis*, both were described by Álvarez del Villar (1963) in the same publication. Both species have restricted distributions in the Balsas River drainage. *Chapalichthys peraticus* is endemic to Presa San Juanico, near Cotija, Michoacán, Mexico, and *C. pardalis* only occurs at the spring (Balenario Ojo de Agua) in Tocumbo, Michoacán, Mexico. These localities are geographically proximate, less than 15 km in distance from one another.

From a taxonomic and systematic perspective, goodeids have been a challenging group to study due the lack of morphological and molecular divergence among closely related species (Hubbs and Turner, 1939; Doadrio and Domínguez, 2004; Foster and Piller, 2018; Parker et al., 2019). This is likely due to their recent (Lower-Middle Miocene) diversification in central Mexico (Doadrio and Domínguez, 2004). *Chapalichthys peraticus* and *C. pardalis* are primarily differentiated from *C. encaustus* by changes in pigmentation patterns. *Chapalichthys pardalis* and *C. peraticus*

<sup>1</sup> Southeastern Louisiana University, Department of Biological Sciences, Hammond, Louisiana 70402; Email: (KRP) kyle.piller@selu.edu. Send reprint requests to KRP.

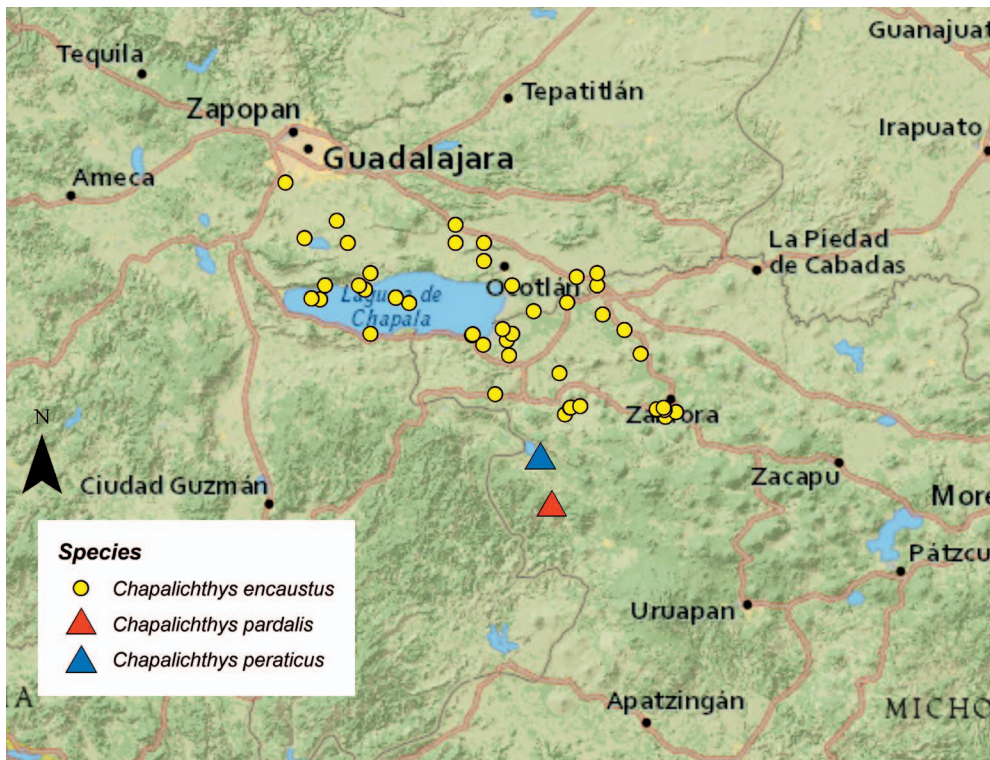
<sup>2</sup> Department of Biological Sciences, Western Michigan University, Kalamazoo, Michigan 49008.

<sup>3</sup> University of Wisconsin Zoological Museum, Madison, Wisconsin 53706.

<sup>4</sup> Centro de Investigación en Biodiversidad y Conservación, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico, C.P. 62209.

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**Fig. 1.** Distribution of the three species of *Chapalichthys* based on vouchered museum records ([www.fishnet2.org](http://www.fishnet2.org), March 2020).

both possess similar meristic counts (Álvarez del Villar, 1963). As a result, there has been some question regarding the taxonomic validity of these forms (Table 1). Webb (1998), in an unpublished dissertation chapter, subsumed *C. peraticus* with *C. encaustus*, but presented no data; whereas Miller et al. (2005) recognized *C. peraticus* as a synonym of *C. pardalis*, but again presented no data. Most other recent studies recognize the validity of *C. encaustus* and *C. pardalis* (Miller et al., 2005; Lyons et al., 2019; Parker et al., 2019), but not *C. peraticus*. The morphological and molecular differences within and among the three taxa of *Chapalichthys* have never been adequately quantified.

The objectives of this study are two-fold. First, we investigated the phylogenetic relationships among species of *Chapalichthys* by including multiple individuals and/or populations of all three currently recognized species. Although previous phylogenetic studies of goodeids have included *Chapalichthys*, no studies have included all three species of *Chapalichthys* (Doadrio and Domínguez, 2004; Webb et al., 2004; Parker et al., 2019). Second, as a result of the taxonomic issues with *C. peraticus*, we used the genetic data along with meristic and pigmentation characters to reassess the taxonomic status of *C. peraticus*. There has long been doubt regarding the validity of *C. peraticus* (Uyeno et al.,

1983; Miller et al., 2005), but there has been no formal treatment of its taxonomic status since its original description.

**MATERIALS AND METHODS**

**DNA sequencing.**—Specimens for genetic analysis were sampled from all three species with standard seines in 2005. All specimens were preserved in 95% ethanol. Specimens of *C. encaustus* were obtained from multiple localities, whereas specimens of *C. pardalis* and *C. peraticus* are only known from single localities in the Río Balsas basin (Table 2). Total genomic DNA was extracted from ethanol preserved tissues using the DNeasy Tissue Kit (Qiagen). The complete NADH dehydrogenase subunit 2 gene (ND2) were isolated using the polymerase chain reaction and primers identified in Kocher et al. (1995) using the following temperature profile: 94°C for 2 min, followed by 30–35 cycles of 94°C (45 sec), 51°C (45 sec), and 72°C (1 min). PCR products were purified using the QIAquick PCR purification kit (Qiagen) or ExoSAP (USB, Corp.) and used in cycle sequencing reactions (Applied Biosystems, Foster City, CA) according to the manufacturers’ recommendations. Excess dye terminators, primers, and nucleotides were removed by gel filtration (Edge Biosystems) prior to sequencing. Chromatographs were initially checked for ambiguities by eye and then both strands aligned using Sequencher (v.4.2; GeneCodes).

A partitioned mixed-model Bayesian analysis (pMM) was conducted to assess relationships among the populations and species of *Chapalichthys*. PartitionFinder2.0 (Lanfear et al., 2016) was used to compare multiple models of DNA substitution to infer the best model of DNA sequence evolution by partition (codon position) using AICc. Each data partition was assigned a distinct model of evolution in Mr.Bayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Posterior probabilities were estimated using the Metropolis-coupled

**Table 1.** Species of *Chapalichthys* recognized by different authorities.

Reference	<i>C. encaustus</i>	<i>C. pardalis</i>	<i>C. peraticus</i>
Uyeno et al., 1983	X	X	
Doadrio and Domínguez, 2004	X		X
Webb et al., 2004	X	X	
Miller et al., 2005	X	X	
Lyons et al., 2019	X	X	
Parker et al., 2019	X	X	

**Table 2.** Locality information for selected populations of goodeids and GenBank accession numbers.

	Species	Locality	GenBank-ND2
1	<i>Chapalichthys encaustus</i>	Laguna de Chapala at Ajijic, Jalisco, MX	MT811849
2	<i>Chapalichthys encaustus</i>	Laguna de Chapala at San Cristobal Zapotitlán, Jalisco, MX	MT811850
3	<i>Chapalichthys encaustus</i>	Río Duero at El Capulín, Michoacán, MX	MT811851
4	<i>Chapalichthys encaustus</i>	Lago de Los Negritos 10km, E. Sayhauo, Michoacán, MX	MT811842
5	<i>Chapalichthys encaustus</i>	Lago Cajititlán, S, Guadalajara, Jalisco, MX	MT811844
6	<i>Chapalichthys encaustus</i>	Lago Cajititlán, S, Guadalajara, Jalisco, MX	MT811845
7	<i>Chapalichthys encaustus</i>	Presa San Antonio, Huaracha, Michoacán, MX	MT811843
8	<i>Chapalichthys encaustus</i>	Río Duero at La Luz, Michoacán, MX	MT811847
9	<i>Chapalichthys encaustus</i>	Río Duero at La Luz, Michoacán, MX	MT811846
10	<i>Chapalichthys encaustus</i>	Presa Arcina, La Arcina, Jalisco, MX	MT811848
11	<i>Chapalichthys peraticus</i>	Laguna de San Juanico, NE corner, Cotija de la Paz, Michoacán, MX	MT811854
12	<i>Chapalichthys peraticus</i>	Laguna de San Juanico, NE corner, Cotija de la Paz, Michoacán, MX	MT811852
13	<i>Chapalichthys peraticus</i>	Laguna de San Juanico, NE corner, Cotija de la Paz, Michoacán, MX	MT811853
14	<i>Chapalichthys pardalis</i>	Parque Ojo de Agua at Tocumbo, Michoacán, MX	MT811855
15	<i>Ameca splendens</i>	El Rincon at Teuchitlán, Jalisco, MX	MT811856
16	<i>Xenotoca eiseni</i>	Río Compostela at Compostela, Nayarit, MX	MT811857
17	<i>Ilyodon furcidens</i>	Puente Arroyo Estanzuela, Río de la Pola, Jalisco, MX	MT811858

Markov chain Monte Carlo (Huelsenbeck et al., 2001). Bayesian analyses were run for 5 million generations and trees were sampled every 100 generations resulting in 50,000 saved trees per analysis, and 25% of trees were removed as burn-in. After confirming that all analyses reached stationarity at similar likelihood values, the remaining (non-discarded) trees were used to calculate branch support as posterior probabilities (Holder and Lewis, 2003). Results from four separate analyses were compared to provide additional confirmation of convergence among likelihood values, tree topologies, and posterior distributions. Three other goodeid species were included, *Ameca splendens*, *Ilyodon furcidens*, and *Xenotoca eiseni*. *Ilyodon furcidens* was designated as the outgroup.

**Morphology.**—Material examined in this study was obtained from the Royal D. Suttkus Fish Collection at the Tulane University Biodiversity Research Institute (TU) and the Field Museum of Natural History (FMNH). Institutional abbreviations follow Sabaj (2020). The following material was examined: *C. encaustus* (TU 30789 and FMNH3561), *C. peraticus* (TU 31943), and *C. pardalis* (UMMZ 202427). Counts and measurements follow Hubbs and Lagler (1958). Meristic data were gathered for the following characters: lateral line scales, scales around the caudal peduncle, and dorsal-fin, anal-fin, and pectoral-fin elements. Color and pigmentation patterns were described from live, freshly preserved, and museum specimens.

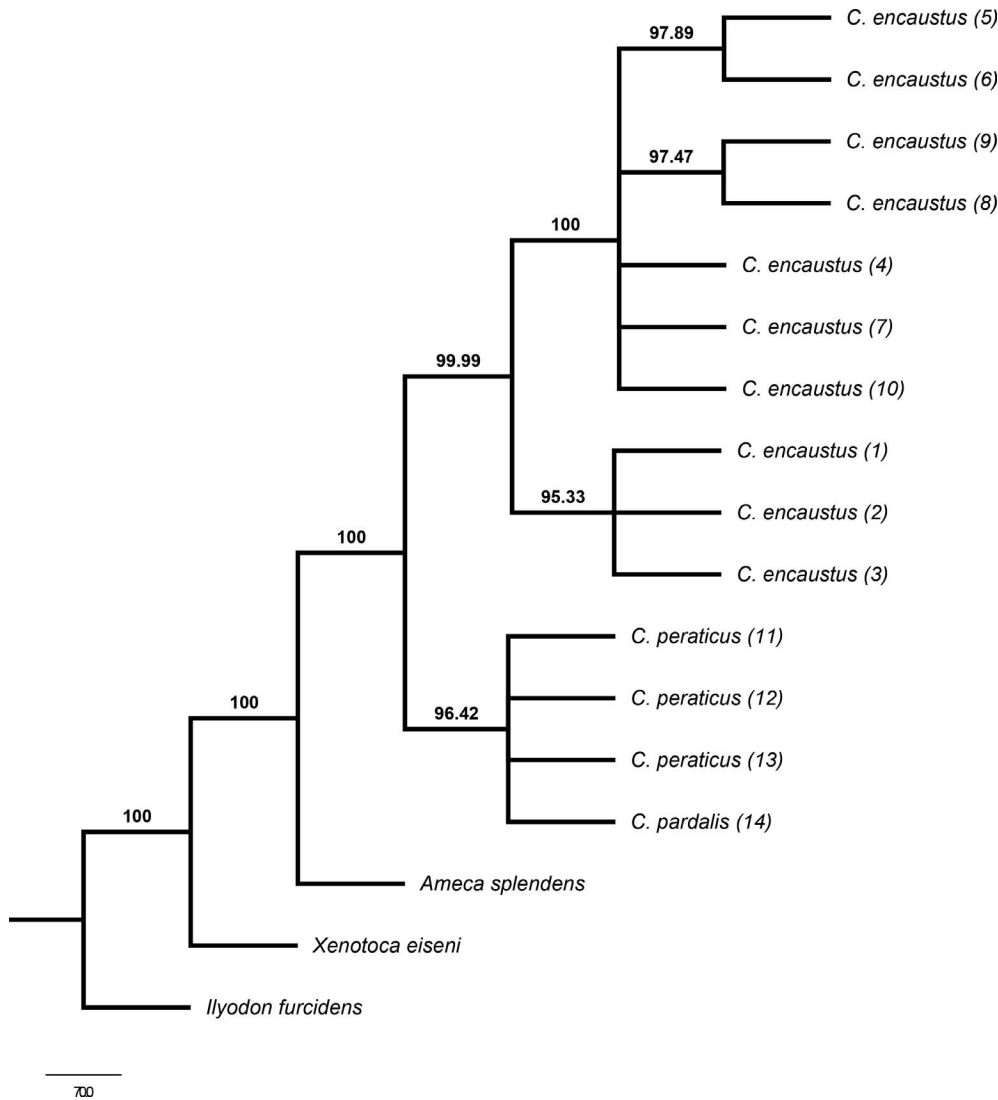
## RESULTS

**Molecular data.**—The complete data set consisted of 1,047 bp recovered from 14 specimens of *Chapalichthys* from eight localities (Table 2). All sequences are available on GenBank AF (MT811842–MT811858). Uncorrected pairwise distances among ingroup taxa was low, 0.00% to 1.10% for ND2 (uncorrected p-distance,  $\bar{x}$  = 0.60%). The largest degree of pairwise genetic divergence (uncorrected p-distance = 2.0–2.4%) was between *C. encaustus* and all individuals of *C. pardalis* and *C. peraticus*. ND2 sequences of *C. pardalis* and *C. peraticus* were nearly identical, with these two species differing from one another by a single, third codon position nucleotide change (uncorrected p-distance = 0.00–0.01%).

The Bayesian analysis of ND2 supported two clades. The Río Lerma-Santiago basin-Inland lake clade included all individuals of *C. encaustus* and was supported by high posterior probabilities (BP > 0.95; Fig. 2). There is a low degree of resolution within the *C. encaustus* clade due to lack of sequence divergence among the sequences of *C. encaustus* (uncorrected p-distance = 0.00–0.02%). The Río Balsas basin clade was also well supported (BP > 0.95), and included all individuals of *C. peraticus/pardalis*, but had no resolution among individuals in this clade.

**Meristic variation.**—There was little variation in meristic counts across the three species of *Chapalichthys*, as meristic characters overlapped for all enumerated characters (Tables 3A–E). Lateral-line scale counts were higher for *C. encaustus* ( $\bar{x}$  = 36.08, range = 34–37, mode = 37) in comparison to *C. pardalis* ( $\bar{x}$  = 34.33, range = 33–36, mode = 35) and *C. peraticus* ( $\bar{x}$  = 34.80, range = 34–37, mode = 34), which were similar to one another. *Chapalichthys encaustus* also possesses higher caudal-peduncle scale counts ( $\bar{x}$  = 19.32, range = 18–21, mode = 19) in comparison to *C. pardalis* ( $\bar{x}$  = 18.65, range = 18–20, mode = 18) and *C. peraticus* ( $\bar{x}$  = 17.91, range = 17–19, mode = 18). Dorsal-fin elements varied slightly with *C. pardalis* possessing the highest average number of elements ( $\bar{x}$  = 15.67, range = 15–17, mode = 15,16), followed closely by *C. encaustus* ( $\bar{x}$  = 15.62, range = 15–17, mode = 16) and *C. peraticus* ( $\bar{x}$  = 14.89, range = 14–16, mode = 15). Mean anal-fin element counts also were similar across species: *C. encaustus* ( $\bar{x}$  = 15.60, range = 14–17, mode = 16), *C. peraticus* ( $\bar{x}$  = 14.93, range = 14–16, mode = 15), and *C. pardalis* ( $\bar{x}$  = 14.93, range = 14–16, mode = 15). Finally, mean pectoral-fin element counts were nearly identical for all *C. encaustus* ( $\bar{x}$  = 13.16, range = 12–14, mode = 13), *C. peraticus* ( $\bar{x}$  = 13.37, range = 13–14, mode = 13), and *C. pardalis* ( $\bar{x}$  = 13.67, range = 13–14, mode = 13).

**Pigmentation characters.**—There are substantial differences in pigmentation patterns between *C. encaustus* and *C. pardalis/peraticus* (Fig. 3). Adult *Chapalichthys encaustus* have at least eight short, dark, vertically aligned bars along the mid-lateral region of the body, with the last two pigmentation markings before the caudal fin often being irregular to circular in



**Fig. 2.** Partitioned mixed-model Bayesian tree of *Chapalichthys*. Values above the nodes refer to posterior probabilities. Numbers in parentheses correspond to populations identified in Table 2.

shape. The belly lacks pigment and appears white-cream colored in live specimens. The dorsal, anal, and caudal fins have melanophores along the fin rays, which typically extend from the base of the fin distally to approximately one-half to three-fourths of the length of the fin. The posterior edge of the caudal, anal, and dorsal fins is typically yellow-orange in color, while the remaining portion of the fins are generally unpigmented. The operculum is generally immaculate with an occasional scattering of melanophores on the dorsal region.

*Chapalichthys pardalis* and *C. peraticus* are identical in pigmentation patterns with both species possessing a peppered pattern of melanophores along the flank from the operculum to the origin of the caudal fin. The peppered pattern primarily covers the lower one-half to two-thirds of the body. Melanophores on the dorsal, anal, and caudal fins mimic what is seen in *C. encaustus*. Like *C. encaustus*, the edge of the caudal, dorsal, and anal fins of both *C. pardalis* and *C. peraticus* are bordered by a yellow-orange band.

## DISCUSSION

**Phylogenetic relationships.**—The genus *Chapalichthys* was recovered as monophyletic in the first study to include all

three species in the genus. Previous molecular studies have provided incongruent results regarding the monophyly of *Chapalichthys*. Webb et al. (2004), using cytochrome oxidase I sequences (mtDNA), recovered a monophyletic *Chapalichthys*, and noted a close relationship between *Chapalichthys* and *Allophorus*. Doadrio and Domínguez (2004) used cytochrome *b* sequences (mtDNA) and recovered a sister group relationship between several populations of *C. encaustus* and *A. splendens*. Most recently, Parker et al. (2019) recovered *Chapalichthys* as monophyletic based on eight nuclear loci. Our results provide further support for a monophyletic *Chapalichthys*.

There are two clades of *Chapalichthys*: one clade includes samples of *C. encaustus* from Lake Chapala and other nearby and currently or formerly connected inland lakes in central Mexico; the other clade includes all individuals of *C. pardalis* and *C. peraticus* from the Río Balsas basin. *Chapalichthys pardalis* and *C. peraticus*, both found in the Río Balsas basin and residing only 15 km apart, possess nearly identical haplotypes for ND2. There was also little genetic divergence among the ten individuals of *C. encaustus*. Two explanations likely account for this. First, recent isolation of populations of *C. encaustus* in the inland lakes on the Mexican Mesa Central may have played a role in the lack of genetic

**Table 3.** Meristic data for species of *Chapalichthys*.

(A)	Lateral line scales	33	34	35	36	37	<i>n</i>	SD	Mean	Mode
	<i>C. encaustus</i>		1	11	10	16	38	0.91	36.08	37
	<i>C. pardalis</i>	6	5	12	1		24	0.91	34.33	35
	<i>C. peraticus</i>		13	5	6	1	25	0.96	34.80	34
(B)	Caudal peduncle scales	17	18	19	20	21	<i>n</i>	SD	Mean	Mode
	<i>C. encaustus</i>		10	15	9	7	41	1.04	19.32	19
	<i>C. pardalis</i>		12	7	4		23	0.78	18.65	18
	<i>C. peraticus</i>	6	12	4			22	0.68	17.91	18
(C)	Dorsal-fin elements	14	15	16	17		<i>n</i>	SD	Mean	Mode
	<i>C. encaustus</i>	3	15	23	4		45	0.75	15.62	16
	<i>C. pardalis</i>		12	12	3		27	0.67	15.67	15, 16
	<i>C. peraticus</i>	4	22	1			27	0.42	14.89	15
(D)	Anal-fin elements	14	15	16	17		<i>n</i>	SD	Mean	Mode
	<i>C. encaustus</i>	1	18	20	3		42	0.66	15.60	16
	<i>C. pardalis</i>	8	13	6			27	0.73	14.93	15
	<i>C. peraticus</i>	6	16	5			27	0.65	14.96	15
(E)	Pectoral-fin elements	12	13	14			<i>n</i>	SD	Mean	Mode
	<i>C. encaustus</i>	3	32	10			45	0.52	13.16	13
	<i>C. pardalis</i>		9	18			27	0.48	13.67	14
	<i>C. peraticus</i>		17	10			27	0.49	13.37	13

divergence. Goodeinae is a relatively recent clade, with molecular age estimates suggesting diversification primarily occurred during the Late Pliocene-Pleistocene (Doadrio and Domínguez, 2004). Recent crown ages for Goodeinae are supported by the fossil record, with the oldest fossils of *C. encaustus* known from late-Pliocene-early Pleistocene deposits from Lake Chapala (Smith et al., 1975). Furthermore, Barbour (1973) noted that several of the inland lakes around Lake Chapala are of recent origin due to geologic uplift during the Pleistocene. Other fish clades from the Mexican Mesa Central, such as silversides (Bloom et al., 2013; Campanella et al., 2015; Corona-Santiago et al., 2015), also show relatively recent diversification. Second, since *C. encaustus* co-occurs with species of *Chiostoma* (Atherinopsidae) in Lake Chapala, it is plausible that *C. encaustus* has been incidentally introduced throughout central Mexico along with species of *Chiostoma* that have been stocked in the region. Fertilized eggs of *Chiostoma* from Lake Chapala were previously introduced into two Río Grande reservoirs by Mexican officials (Contreras and Escalante, 1984), so the hypothesis of artificial transfer is feasible. During our sampling, we examined the catches of commercial fisherman from Lake Chapala. Many of these catches contained by-catch specimens of *C. encaustus* and other species. Higher resolution molecular markers and a larger sample size would be needed to test this hypothesis.

Based on similarities of the ichthyofauna, the valley between Tocumbo and Cotija, Michoacán, Mexico was formerly occupied by a northward flowing tributary of Lake Chapala (Álvarez del Villar, 1963; Barbour, 1973). This area is now occupied by Presa de San Juanico (Balsas River basin) and contains several unique species, including two endemic silversides (*C. consocium reseratum* and *C. melanococcus*) and one

species of *Chapalichthys*. The cessation of gene flow between the Balsas and Lerma-Santiago basins likely was responsible for the diversification of a Balsas form of *Chapalichthys* (*C. pardalis/peraticus*) and a Lerma-Santiago form (*C. encaustus*).

Meristic characters also provide little support for the diagnosis of any of the species of *Chapalichthys*, as there is overlap in all characters analyzed. This is also the case for many other species of goodeids, which show little variation in meristic characters (Miller et al., 2005). Pigmentation characters, however, are informative and separate *C. encaustus* from *C. pardalis* and *C. peraticus*.

The lack of morphological and molecular variation between *C. pardalis* and *C. peraticus* suggests that *C. pardalis* and *C. peraticus* are conspecific. Although *C. pardalis* and *C. peraticus* were originally described in the same publication, the description of *C. pardalis* (p. 119) appears prior to the description of *C. peraticus* (p. 123) in Álvarez del Villar (1963). Therefore, *C. peraticus* is recognized as a junior synonym of *C. pardalis*. A re-diagnosis of the two valid species of *Chapalichthys* is provided below.

#### ***Chapalichthys encaustus* (Jordan and Snyder, 1899)**

Barred Splitfin, Pintito de Ocotlán

*Characodon encaustus* (original description, Jordan and Snyder, 1899).

**Type.**—*Characodon encaustus* was described by Jordan and Snyder (1899), Laguna Chapala, near Ocotlan, Jalisco, Mexico.

**Synonyms.**—*Characodon encaustus* Jordan and Snyder, 1899.



**Fig. 3.** Photographs of live specimens of (A) *Chapalichthys encaustus* (Lake Chapala, Chapala, Jalisco, MX), (B) *C. pardalis* (Balenario Ojo de Agua, Tocumbo, Michoacán, MX), and (C) *C. peraticus* (Presa San Juanico, Cotija, Michoacán, Mexico).

**Diagnosis.**—*Chapalichthys encaustus* can be differentiated from *C. pardalis* primarily by differences in body pigmentation. *Chapalichthys encaustus* possess eight or more vertical bars along the lateral portion of the body, whereas the body of *C. pardalis* contains dark spots and speckles and is “leopard-like” in appearance.

**Distribution.**—Río Lerma-Santiago basin, several inland lakes and rivers and streams in Jalisco and Michoacán including but not limited to the Upper Santiago and lower Lerma and Duero rivers, and lakes La Luz, Orandino, Chapala, Cajititlán, and Los Negritos.

**Conservation status.**—Populations of *C. encaustus* are vulnerable (Domínguez-Domínguez et al., 2005; Lyons et al., 2019), and declining. Widespread pollution and environmental degradation, along within the introduction of exotic species (e.g., *Tilapia* and *Oreochromis* spp.) are the major threats to the long-term persistence of this species.

### ***Chapalichthys pardalis* Álvarez del Villar, 1963**

Polka-Dot Splitfin, Pintito de Tocumbo

**Type.**—*Chapalichthys pardalis* was described by Álvarez del Villar (1963) from the spring at Tocumbo, Michoacán, Mexico.

**Synonyms.**—*Chapalichthys peraticus* Álvarez del Villar, 1963 (published in the same manuscript as *C. pardalis*, but later in the document) is recognized as a junior synonym of *C. pardalis*.

**Diagnosis.**—*Chapalichthys pardalis* differs from its congener *C. encaustus* on the basis of its peppered pigmentation pattern along the lateral region of the body and its restricted distribution in the Río Balsas basin of central Mexico.

**Distribution.**—*Chapalichthys pardalis* is restricted to the Balsas River drainage in central Mexico. *Chapalichthys pardalis* is restricted to the spring (Ojo de Agua) at Tocumbo, Michoa-

cán, Mexico and Presa San Juanico near Cotija, Michoacán, Mexico.

**Conservation status.**—Both populations of *C. pardalis* should be recognized as critically endangered (*sensu* Lyons et al., 2019) due to their restricted distribution and the ongoing environmental changes in the region (Domínguez-Domínguez et al., 2005).

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