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REVIEW ARTICLE

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# Phylogeny and species diversity of the genus *Herichthys* (Teleostei: Cichlidae)

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## Abstract

We provide a review of the systematics of *Herichthys* by evaluating the usefulness of several mitochondrial and nuclear genetic markers together with morphological data. The nDNA next-generation sequencing ddRAD analysis together with the mtDNA *cytochrome b* gene provided well-resolved and well-supported phylogenies of *Herichthys*. On the other hand, the nDNA S7 introns have limited resolution and support and the *COI* barcoding analysis completely failed to recover all but one species of *Herichthys* as monophyletic. The *COI* barcoding as currently implemented is thus insufficient to distinguish clearly distinct species in the genus *Herichthys* that are supported by other molecular markers and by morphological characters. Based on our results, *Herichthys* is composed of 11 species and includes two main clades (the *H. labridens* and *H. cyanoguttatus* species groups). *Herichthys bartoni* is in many respects the most plesiomorphic species in the genus and has a conflicting phylogenetic position between mtDNA and nDNA markers, where the robust nDNA ddRAD data place it as a rather distant basal member of the *H. labridens* species group. The mtDNA of *H. bartoni* is on the other hand only slightly divergent from the sympatric and syntopic *H. labridens*, and the species thus probably have hybridized in the relatively recent past. The sympatric and syntopic *Herichthys steindachneri* and *H. pame* are supported as sister species. The *Herichthys cyanoguttatus* species group shows two well-separated basal species (the northernmost *H. minckleyi* and the southernmost *H. deppii*) followed by the closely related and centrally distributed species *H. cyanoguttatus*, *H. tepehua*, *H. carpintis*, and *H. tamasopoensis* whose relationships differ between analyses and show likely hybridizations between themselves and the two basal species as suggested by conflicts between DNA analyses. Several instances of introgressions/hybridizations have also been found between the two main clades of *Herichthys*.

**KEYWORDS**
barcoding, ddRAD, geometric morphometrics, morphology, neotropical, next-generation sequencing

## 1 | INTRODUCTION

Fishes of the family Cichlidae have experienced repeated and spectacular adaptive radiations and show high levels of phenotypic plasticity and diversity that make them excellent models in ecology and evolution (Barlow, 2000; Fryer & Iles, 1972; Near et al., 2013). In African cichlids, the high diversity is associated with and often explained by the great amount of ecological niches present in lakes (Fryer & Iles, 1972; Joyce et al., 2005; Koblmüller, Sefc, & Sturmbauer, 2008). While rivers offer a lesser amount of niches than lakes

  

riverine cichlid diversifications are also known and one of the very good examples among the neotropical cichlids are the Middle American cichlids (Říčan, Piálek, Dragová, & Novák, 2016). There are four areas in Middle America (the Usumacinta, the San Juan, the Tuira, and the Pánuco river basins) where cichlid diversifications appear to have happened in sympatry (in individual river basins as opposed to the better known lake diversifications) and where there are sympatric and even syntopic sister species. Sympatry of congeneric and especially of sister species is otherwise rare among riverine cichlids because of the predominance of allopatric speciation (e.g., Kullander, 1986; Kullander & Ferreira, 2007; Kullander & Nijssen, 1989; Musilová, Říčan, Janko, & Novák, 2008; Musilová et al., 2015; Říčan, Piálek, Zardoya, Doadrio, & Zrzavý, 2013; Říčan et al., 2016; Willis, Nunes, Montana, Farias, & Lovejoy, 2007). The sympatric species in all these areas of Middle America differ substantially in their morphological and ecological adaptations and probably evolved in sympatry through ecomorphological divergence (Říčan et al., 2013, 2016).

The northernmost postulated cases of sympatric ecomorphological divergence in Middle American cichlids are in the genus *Herichthys* (Kornfield, Smith, Gagnon, & Taylor, 1982; Kornfield & Taylor, 1983; Taylor & Miller, 1983), which is distributed on the Atlantic slope of Mexico from north-central Veracruz to southern Texas (Miller, 1976). The species of *Herichthys* that has received most attention in this regard is the polymorphic *H. minckleyi* (Hulsey & García de León, 2013; Hulsey et al., 2006; Kornfield & Taylor, 1983; Kornfield et al., 1982; Magalhaes, Ornelas-García, Leal-Cardin, Ramírez, & Barluenga, 2015) with at least three distinct sympatric and partially syntopic trophic morphotypes, or ecomorphs (Kornfield & Taylor, 1983). *Herichthys*, however, also includes similarly morphologically and ecologically distinct syntopic forms, which are, however, classified as separate species (contrary to the situation in the supposedly conspecific morphs of *H. minckleyi*) living in two sub-basins of the Rio Pánuco river basin (Taylor & Miller, 1983). These sympatric and syntopic species are believed to form two species pairs, one including the piscivorous *H. steindachneri* (Jordan & Snyder, 1899) and the molluscivorous *H. pame* De La Maza-Benignos & Lozano-Vilano, 2013; and the other the molluscivorous *H. labridens* (Pellegrin, 1903) and the possibly polymorphic (Říčan et al., 2016) *H. bartoni* (Bean, 1892). In contrast to *H. minckleyi*, these species remain virtually unstudied and even their phylogenetic relationships and supposed species-pair relationships remain to be fully ascertained.

The species diversity overall is rather poorly known in *Herichthys*, especially for the allopatric species in regard to their geographical and morphological boundaries. This limitation derives from the still poor understanding of the phylogenetic relationships within *Herichthys* (Artigas Azas, 1993; Kornfield & Taylor, 1983; Mejía, Pérez-Miranda, León-Romero, Soto-Galera, & Luna, 2015; Miller, Minckley, & Norris, 2005; Říčan et al., 2016). The monophyly of the genus *Herichthys* is on the other hand clearly supported by several different molecular markers (Concheiro-Pérez et al., 2006; Hulsey, García de León, Johnson, Hendrickson, & Near, 2004; Hulsey, Hollingsworth, & Fordyce, 2010; López-Fernández, Winemiller, & Honeycutt, 2010; Říčan, Zardoya, & Doadrio, 2008; Říčan et al., 2013, 2016), by analysis of morphological characters (Říčan et al., 2008, 2016), as well as by total-evidence combined analyses (Říčan et al., 2008, 2016). *Herichthys* is based on these results divided into two monophyletic groups, one including *H. bartoni*, *H. labridens*, *H. steindachneri*, *H. pame*, and *H. pantostictus* (Taylor & Miller, 1983) (*H. labridens* group) and the other including *H. cyanoguttatus* Baird & Girard, 1854; *H. carpintis* (Jordan & Snyder, 1899); *H. minckleyi*, *H. tamasopoensis* Artigas Azas, 1993; *H. tepehua* De la Maza-Benignos, Ornelas-Garcia, Lozano-Vilano, Garcia-Ramirez, & Doadrio, 2015; and *H. deppii* (Heckel, 1840) (*H. cyanoguttatus* group).

Until recently (2013), the genus species-level systematics was stable and comprised nine species. In the last few years, the genus has experienced a significant taxonomic upheaval and inflation that included the formal descriptions of several previously postulated species, one putatively new species, and the segregation of some species into a new genus. In a first instance, De La Maza-Benignos and Lozano-Vilano (2013) and De la Maza-Benignos et al. (2015) described three species previously informally known as "white labridens" (*H. pame*), "green labridens" (*H. pratinus* De La Maza-Benignos & Lozano-Vilano, 2013), and "turquoise *Herichthys*" (*H. tepehua*) plus one putatively new species *H. molango* De La Maza-Benignos & Lozano-Vilano, 2013 and elevated *H. teporatus* (Fowler 1903) to a valid species. There is a problem with all these species descriptions because they lack diagnostic characters and additionally were not supported by the presented (mtDNA) phylogenies. Secondly, De la Maza-Benignos et al. (2015) segregated the species formerly included in the *H. labridens* species group into a new genus named *Nosferatu*, again in spite of lack of monophyly in their presented mtDNA phylogeny and with a questionable morphological diagnosis that additionally contradicted the only available morphological phylogeny of the genus (Říčan et al., 2008). The genus *Herichthys* has thus been recently through several taxonomical changes that are not supported by presented data and are in many cases refuted by additional data (e.g., Mejía, Pérez-Miranda, León-Romero, Soto-Galera, & De Luna, 2015; Říčan et al., 2016).

This study has thus been formulated as a necessary first step toward reviewing the species diversity and phylogenetic relationships within the genus *Herichthys* (i.e., including *Nosferatu* sensu De la Maza-Benignos et al., 2015). To achieve this goal, we have reviewed all previously used molecular markers (two mitochondrial markers and two nuclear datasets, the latter including a reduced-genome representation analysis) and morphological characters together with a novel morphological and morphometric analysis (of the *H. cyanoguttatus* group while Mejía et al., 2015 have provided the same analyses for the *H. labridens* species group) including coloration patterns to provide a firm systematic background for future studies on the genus. The focus of this study was thus to find well-resolving robust markers that in future studies with a much denser specimen sampling will stabilize the systematics of the genus *Herichthys* and reveal its diversification patterns.

## 2 | MATERIAL AND METHODS

### 2.1 | Molecular datasets

#### 2.1.1 | Mitochondrial DNA analyses

***Cytochrome c oxidase subunit 1 (COI)***

A subset of 178 individuals from 31 localities of the *H. cyanoguttatus* species group were used for the molecular analysis; additionally, 88 previously generated sequences (Mejía et al., 2015) of the *H. labridens* species group were included together with 11 COI sequences of the genera *Paraneetroplus*, *Theraps*, and *Thorichthys* that served as out-groups. Sequences were deposited in GenBank and are available from BOLD projects "Freshwater fishes of the Pánuco-Tamesí system" and "DNA barcode and geometric morphometrics of the genus *Herichthys*". A small amount of muscle from ethanol-preserved individuals were removed to generate DNA barcode sequences following the protocol previously described by Ivanova, Zemlak, Hanner, and Hebert (2007) in Ecosur, Chetumal. Only fragments longer than 500 bp were incorporated in the final analysis. The taxon sampling includes all previously recognized species except *H. molango*.

***Cytochrome b (cytb)***

The mtDNA *cytb* dataset includes 85 specimens (109 terminals with the inclusion of out-group taxa; average is seven specimens per *Herichthys* species) representing all species except *H. molango* (sample not available). The mtDNA *cytb* dataset has 1,137 bp of which 340 are parsimony-informative. The sequences were generated and previously used in the review study of all Middle American cichlids (Říčan et al., 2016) but are here for the first time fully explored and compared with other DNA markers in a review of the genus *Herichthys*.

#### 2.1.2 | Nuclear DNA analyses

The nDNA datasets include a reduced number of the same specimens as used in the *cytb* analysis: 22 specimens for the data set of nucelar introns (*S7i*) and 28 specimens in the ddRAD analyses, with a minimum of two specimens per the majority of species (See Table 1 for the *cytb*, *S7i* and ddRAD sampling).

**S7 introns**

Introns 1 and 2 of the ribosomal protein S7 gene (*S7i*) were newly sequenced for this study following the protocol of Říčan et al. (2008). GenBank accession numbers are MF625511–MF625554 (see Table 1). The *S7i* dataset includes 22 specimens representing most species except *H. molango* (sample not available) and *H. minckleyi* (no success in amplifying the samples). Both introns were sequenced in their complete length, and the *S7* introns 1 and 2 dataset includes 2671 characters of which 180 (including indels) are parsimony-informative in *Herichthys*.

**Reduced-genome representation**

The double-digest restriction-site associated DNA sequencing (ddRADseq; Peterson, Weber, Kay, Fisher, & Hoekstra, 2012) method was used to acquire a sufficient amount of nuclear markers. We used the ddRAD library prepared and sequenced for the Middle American cichlid study of Říčan et al. (2016) which we reduced here to a *Herichthys*-specific dataset. The dataset included 32 terminals, 28 of them were *Herichthys* specimens that represented all species except *H. molango* and four were closely related out-group taxa. Narrowing the dataset enabled us to call for genus-specific SNPs and thus to obtain data matrices with higher resolution at the intrageneric level than in the previous Říčan et al. (2016) study. The number of newly extracted SNPs (compared to the previous study) was further significantly increased by aligning the obtained RAD tags to the genome of the much more closely related Middle American cichlid species *Amphilophus citrinellus* (GenBank GCA_000751415.1; overall alignment rate 97.7 % compared to 52.1 % in the distantly related African cichlid *Oreochromis niloticus* genome used in Říčan et al., 2016). The obtained RAD tags were processed in Stacks v1.35 (Catchen, Amores, Hohenlohe, Cresko, & Postlethwait, 2011) following the procedure with reference-genome assembly described in Říčan et al. (2016).

### 2.2 | Phylogenetic analyses

All phylogenetic analyses were rooted using specimens from several related genera. The mtDNA *COI*, *cytb*, and the nDNA *S7i* 1 and 2 data matrices were analyzed using Bayesian phylogenetic inference with MRBAYES 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Sequences were aligned in Clustal X 2.0 (Larkin et al., 2007) using default parameters and five iterations. An optimal model of evolution for each data matrix according to Akaike's information criterion was selected using jModelTest (Posada, 2008).

The *cytb* was analyzed with divided codon positions (1st + 2nd vs. 3rd). Bayesian analyses of the data were performed with the HKY + gamma (*COI* data), GTR + I + G (*cytb* data), and GTR + I + G (plus indels with standard model; *S7i* 1, 2 data) substitution models and the following parameters: two independent analyses, each comprising two runs with eight chains, 2 million generations with trees sampled and saved every 1,000 generations for the *COI* data and 10 million generations with trees sampled and saved every 1,000 generations for the *cytb* and *S7i* data. Convergence of the runs was estimated with the use of: (i) diagnostic criteria produced by the "sump" command in MRBAYES; (ii) graphical visualization and diagnostics in TRACER 1.5.0 (Rambaut & Drummond, 2007). The first 25% of trees from each run were discarded as burn-in; the remaining trees were used for reconstruction of the 50% majority-rule consensus trees with posterior probability (PP) values of the branches. The *COI* data were additionally due to the large number of specimens (266) analyzed with terminals collapsed into haplotypes. The collapsing to haplotypes was done in DAMBE (Xia, 2013) and resulted in 124 different haplotypes (see Appendix S1).

The ddRAD data were phylogenetically analyzed using maximum likelihood (ML) in RaxML v8.2.4 (Stamatakis, 2014). The analyzed datasets were generated from loci presented in min. 90 % of individuals and stack depth 10, and the matrix with all variable SNPs included 42,979 characters of which 16,509 were parsimony-informative. We

**TABLE 1** Data table with specimens and localities used for mtDNA *cytb* and nDNA *S7i* and ddRAD analyses

| Lab. No. | Specimen ID | Species (*Herichthys*) | Field species ID | Locality | River basin (Figure 1) | Number of Specimens in lot | Number of analyzed specimens | | | Specimen ID (FSFC) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Cyt *b* | S7 intron 1 and 2 | ddRAD | |
| H1 | 91-1-1 | *bartoni* | *bartoni* | M91 | Verde-Laguna Media Luna area | 19 | 1 | MF625511, MF625533 | | M91-1-9515 |
| H53 | 91-1-2 | *bartoni* | *bartoni* | M91 | Verde-Laguna Media Luna area | 1 | 1 | | 1 | M91-1-9516 |
| H2 | 93-1-1 | *bartoni* | *bartoni* | M93 | Verde-Laguna Media Luna area | 7 | 1 | MF625512, MF625534 | | M93-1-9609 |
| H54 | 93-1-2 | *bartoni* | *bartoni* | M93 | Verde-Laguna Media Luna area | 1 | 1 | | 1 | M93-1-9610 |
| H3 | 74-1-1 | *cyanoguttatus* × *carpintis* | *carpintis* | M74 | Tempoal | 1 | 1 | MF625513, MF625535 | | M74-1-8658 |
| H55 | 74-1-2 | *carpintis* | *carpintis* | M74 | Tempoal | 1 | 1 | | 1 | M74-1-8659 |
| H4 | 75-1-1 | *carpintis* | *carpintis* | M75 | Tempoal | 2 | 1 | | | M75-1-8682 |
| H56 | 75-1-2 | *carpintis* | *carpintis* | M75 | Tempoal | 1 | 1 | | | M75-1-8683 |
| H5 | 76-4 | *carpintis* | *carpintis* | M76 | Tempoal | 1 | 1 | | | M76-4-8793 |
| H6 | 76-5 | *carpintis* | *carpintis* | M76 | Tempoal | 1 | 1 | | | M76-5-8812 |
| H7 | 77-2-1 | *carpintis* | *carpintis* | M77 | Moctezuma | 2 | 1 | | 1 | M77-2-8844 |
| H8 | 77-3-1 | *carpintis* | *carpintis* | M77 | Moctezuma | 2 | 1 | | | M77-3-8844 |
| H9 | 78-1 | *carpintis* × *tamasopoensis* | *carpintis* | M78 | Panuco | 1 | 1 | MF625514, MF625536 | | M78-1-8898 |
| H10 | 79-1-1 | *carpintis* | *carpintis* | M79 | Tamesi | 1 | 1 | | 1 | M79-1-8910 |
| H57 | 79-1-2 | *carpintis* | *carpintis* | M79 | Tamesi | 1 | 1 | MF625531, MF625553 | | M79-1-8911 |
| H11 | 80-1 | *carpintis* | *carpintis* | M80 | Tamesi | 1 | 1 | | 1 | M80-1-8956 |
| H12 | 81-1-1 | *carpintis* | *carpintis* | M81 | Panuco | 1 | 1 | MF625515, MF625537 | | M81-1-8968 |
| H58 | 81-1-2 | *carpintis* × *tamasopoensis* | *carpintis* | M81 | Panuco | 1 | 1 | | | M81-1-8969 |
| H13 | 89-1-1 | *carpintis* | *carpintis* | M89 | Verde-Laguna Media Luna area (introduced) | 3 | 1 | | | M89-1-9477 |
| H14 | 89-2-1 | *carpintis* | *carpintis* | M89 | Verde-Laguna Media Luna area (introduced) | 2 | 1 | | | M89-2-9478 |
| H15 | 90-1-1 | *carpintis* | *carpintis* | M90 | Verde-Laguna Media Luna area (introduced) | 4 | 1 | | | M90-1-9491 |
| H59 | 90-1-2 | *carpintis* | *carpintis* | M90 | Verde-Laguna Media Luna area (introduced) | 1 | 1 | | | M90-1-9492 |
| H16 | 92-1 | *carpintis* | *carpintis* | M92 | Verde-Laguna Media Luna area (introduced) | 1 | 1 | MF625516, MF625538 | | M92-1-9576 |
| H17 | 83-1 | *carpintis* | *cf. carpintis* | M83 | Panuco-El Salto | 1 | 1 | | | M83-1-9085 |
| H75 | HCRA | *cyanoguttatus* | *cyanoguttatus* | M98 | Bravo del Norte-San Juan | 1 | 1 | | | M98-865 |
| H73 | HCLE | *carpintis* × *cyanoguttatus* | *cyanoguttatus* | M97 | San fernando-Conchos | 1 | 1 | | 1 | M97-859 |
| H76 | HCSI | *cyanoguttatus* | *cyanoguttatus* | M97A | San fernando-Conchos | 1 | 1 | | 1 | M97A-867 |
| H27 | 68-1 | *deppii* | *deppii* | M68 | Nautla | 1 | 1 | MF625519, MF625541 | 1 | M68-1-8525 |

(Continues)

**TABLE 1** (Continued)

| Lab. No. | Specimen ID | Species (*Herichthys*) | Field species ID | Locality | River basin (Figure 1) | Number of Specimens in lot | Number of analyzed specimens | | | Specimen ID (FSFC) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Cyt *b* | S7 intron 1 and 2 | ddRAD | |
| H28 | 69-1 | *deppii* | *deppii* | M69 | Nautla | 1 | 1 | MF625520, MF625542 | 1 | M69-1-8544 |
| H29 | 69-2-1 | *deppii* | *deppii* | M69 | Nautla | 2 | 1 | | | M69-2-8551 |
| | 69-3-1 | *deppii* | *deppii* | M69 | Nautla | 3 | 1 | | | M69-3-8552 |
| H30 | 89-3-1 | *labridens* | *labridens* | M89 | Verde-Laguna Media Luna area | 4 | 1 | | | M89-3-9449 |
| H31 | 89-4-1 | *labridens* | *labridens* | M89 | Verde-Laguna Media Luna area | 4 | 1 | MF625521, MF625543 | | M89-4-9450 |
| H32 | 89-5-1 | *labridens* | *labridens* | M89 | Verde-Laguna Media Luna area | 5 | 1 | MF625522, MF625544 | | M89-5-9451 |
| H33 | 91-2-1 | *labridens* | *labridens* | M91 | Verde-Laguna Media Luna area | 1 | 1 | | | M91-2-9541 |
| H62 | 91-2-2 | *labridens* | *labridens* | M91 | Verde-Laguna Media Luna area | 1 | 1 | | 1 | M91-2-9542 |
| H34 | 93-2-1 | *labridens* | *labridens* | M93 | Verde-Laguna Media Luna area | 2 | 1 | MF625523, MF625545 | 1 | M93-2-9393 |
| H63 | 93-2-2 | *labridens* | *labridens* | M93 | Verde-Laguna Media Luna area | 1 | 1 | | | M93-2-9394 |
| H71 | HMIN | *minckleyi* | *minckleyi* | M99 | Bravo del Norte-Cuatrocienegas | 1 | 1 | | 1 | M99-854 |
| H18 | 84-1-1 | *pame* | *sp. Tamul* | M84 | Gallinas | 2 | 1 | MF625517, MF625539 | 1 | M84-1-9128 |
| H19 | 84-3-1 | *pame* | *sp. Tamul* | M84 | Gallinas | 5 | 1 | | | M84-3-9180 |
| H20 | 85-1-1 | *pame* | *sp. Tamul* | M85 | Gallinas | 4 | 1 | | 1 | M85-1-9267 |
| H21 | 85-2-1 | *pame* | *sp. Tamul* | M85 | Gallinas | 4 | 1 | | | M85-2-9268 |
| | 85-3-1 | *pame* | *sp. Tamul* | M85 | Gallinas | 4 | 1 | | | M85-3-9269 |
| H22 | 86-1 | *pame* | *sp. Tamul* | M86 | Gallinas | 1 | 1 | | | M86-1-9373 |
| H23 | 87-1-1 | *pame* | *sp. Tamul* | M87 | Gallinas | 4 | 1 | | | M87-1-9398 |
| H60 | 87-1-2 | *pame* | *sp. Tamul* | M87 | Gallinas | 1 | 1 | | | M87-1-9399 |
| H61 | 88-1-2 | *pame* | *sp. Tamul* | M88 | Gallinas | 1 | 1 | | | M88-1-9417 |
| H35 | 76-1 | *pantostictus* | *labridens "blue"* | M76 | Tempoal | 1 | 1 | MF625524, MF625546 | | M76-1-8706 |
| H36 | 76-2-1 | *pantostictus* | *labridens "blue"* | M76 | Tempoal | 1 | 1 | | | M76-2-8701 |
| H64 | 76-2-2 | *pantostictus* | *labridens "blue"* | M76 | Tempoal | 1 | 1 | | | M76-2-8702 |
| | 76-3-1 | *pantostictus* | *labridens "blue"* | M76 | Tempoal | 3 | 1 | | | M76-3-8721 |
| H37 | 77-1 | *pantostictus* | *labridens "blue"* | M77 | Moctezuma | 1 | 1 | | | M77-1-8820 |
| H38 | 77-4-1 | *pantostictus* | *labridens "blue"* | M77 | Moctezuma | 1 | 1 | | 1 | M77-4-8862 |
| H65 | 77-4-2 | *pantostictus* | *labridens "blue"* | M77 | Moctezuma | 1 | 1 | | | M77-4-8863 |
| | 77-5-1 | *pantostictus* | *labridens "blue"* | M77 | Moctezuma | 2 | 1 | | | M77-5-8862 |
| | 77-6-1 | *pantostictus* | *labridens "blue"* | M77 | Moctezuma | 3 | 1 | | | M77-5-8863 |
| H39 | 78-2 | *pantostictus* | *labridens "blue"* | M78 | Panuco | 1 | 1 | | | M78-2-8905 |
| H25 | 81-2-1 | *pantostictus* | *cf. labridens/pantostictus* | M81 | Panuco | 2 | 1 | | | M81-2-8977 |
| H26 | 81-3-1 | *pantostictus* | *cf. labridens/pantostictus* | M81 | Panuco | 2 | 1 | | | M81-3-8978 |

(Continues)

**TABLE 1** (Continued)

| Lab. No. | Specimen ID | Species (*Herichthys*) | Field species ID | Locality | River basin (Figure 1) | Number of Specimens in lot | Number of analyzed specimens | | | Specimen ID (FSFC) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Cyt *b* | S7 intron 1 and 2 | ddRAD | |
| | 81-4-1 | *pantostictus* | *cf. labridens/pantostictus* | M81 | Panuco | 2 | 1 | | | M81-4-8979 |
| H40 | 82-1 | *pantostictus (pratinus)* | *labridens "green"* | M82 | Panuco-El Salto | 1 | 1 | | 1 | M82-1-9030 |
| H41 | 82-2-1 | *pantostictus (pratinus)* | *labridens "green"* | M82 | Panuco-El Salto | 2 | 1 | MF625525, MF625547 | | M82-2-9043 |
| H66 | 82-2-2 | *pantostictus (pratinus)* | *labridens "green"* | M82 | Panuco-El Salto | 1 | 1 | | | M82-2-9044 |
| H42 | 83-2 | *pantostictus (pratinus)* | *labridens "green"* | M83 | Panuco-El Salto | 1 | 1 | | 1 | M83-2-9091 |
| H43 | 80-2-1 | *pantostictus* | *pantostictus* | M80 | Tamesi | 3 | 1 | MF625526, MF625548 | 1 | M80-2-8927 |
| H44 | 80-3-1 | *pantostictus* | *pantostictus* | M80 | Tamesi | 2 | 1 | | | M80-3-8946 |
| H74 | HPGU | *pantostictus* | *pantostictus* | M96 | Tamesi | 1 | 1 | | 1 | M96-862 |
| H47 | 84-2-1 | *steindachneri* | *steindachneri* | M84 | Gallinas | 1 | 1 | | 1 | M84-2-9164 |
| H48 | 84-2-2 | *steindachneri* | *steindachneri* | M84 | Gallinas | 1 | 1 | MF625529, MF625551 | 1 | M84-2-9165 |
| H24 | 88-1-1 | *steindachneri* | *steindachneri* | M88 | Gallinas | 2 | 1 | MF625518, MF625540 | | M88-1-9437 |
| H49 | 84-4 | *tamasopoensis* | *tamasopoensis* | M84 | Gallinas | 1 | 1 | | | M84-4-9221 |
| H50 | 85-4-1 | *tamasopoensis* | *tamasopoensis* | M85 | Gallinas | 3 | 1 | MF625530, MF625552 | 1 | M85-4-9291 |
| H67 | 85-4-2 | *tamasopoensis* | *tamasopoensis* | M85 | Gallinas | 1 | 1 | MF625532, MF625554 | 1 | M85-4-9292 |
| H72 | HTAM | *tamasopoensis* | *tamasopoensis* | M85A | Gallinas | 1 | 1 | | | M85A-1857 |
| H51 | 87-2-1 | *tamasopoensis* | *tamasopoensis* | M87 | Gallinas | 5 | 1 | | | M87-2-9429 |
| H68 | 87-2-2 | *tamasopoensis* | *tamasopoensis* | M87 | Gallinas | 1 | 1 | | | M87-2-9430 |
| H52 | 88-2-1 | *tamasopoensis* | *tamasopoensis* | M88 | Gallinas | 1 | 1 | | | M88-2-9444 |
| H69 | 88-2-2 | *tamasopoensis* | *tamasopoensis* | M88 | Gallinas | 1 | 1 | | | M88-2-9445 |
| H46 | 73-1 | *tepehua* | *sp. Tuxpan/Pantepec* | M73 | Tuxpan | 1 | 1 | MF625528, MF625550 | 1 | M73-1-8626 |
| | 73-2 | *tepehua* | *sp. Tuxpan/Pantepec* | M73 | Tuxpan | 1 | 1 | | | M73-2-8627 |
| | 73-3 | *tepehua* | *sp. Tuxpan/Pantepec* | M73 | Tuxpan | 1 | 1 | | | M73-3-8628 |
| H45 | 64-1 | *tepehua* | *sp. Cazones* | M64 | Cazones | 1 | 1 | MF625527, MF625549 | 1 | M64-1-8441 |
| H70 | HSPC | *tepehua* | *sp. Cazones or Turquoise* | M94 | Cazones | 1 | 1 | | 1 | M94-850 |
| H77 | HSPP | *tepehua* | *sp. Pantepec* | M95 | Tuxpan | 1 | 1 | | 1 | M95-869 |
| Collection (FSFC) University of South Bohemia, České Budějovice, Czech Republic | | | | | | | 85 | 22 | 28 | |
| For cytb GenBank accession numbers see Říčan et al. (2016) | | | | | | | | | | |

also prepared a reduced dataset of solely fixed (homozygous) SNPs (masking thus within-individual polymorphism) which included 23,955 characters of which 12,665 were parsimony-informative. Analyses were run with optimization of equilibrium frequencies and using the GTR substitution model, and we used 100 bootstrap replicates to evaluate statistical branch supports of ML trees. TreeMix (Pickrell & Pritchard, 2012) was also used to study historical relationships among populations in likely hybridization events.

All Bayesian and ML analyses were run at Metacentrum computational resources (http://www.metacentrum.cz).

## 2.3 | Morphological methods

A total of 528 individuals from the *Herichthys cyanoguttatus* species group from 94 localities representing all seven previously recognized valid species (*H. minckleyi*, *H. cyanoguttatus*, *H. teporatus*, *H. carpintis*, *H. tamasopoensis*, *H. tepehua*, *H. deppii*) were analysed using both traditional morphological and geometric morphometrics approaches (Figure 1); 509 of the examined specimens are deposited in the Colección Nacional de Peces Dulceacuícolas Mexicanos de la Escuela Nacional de Ciencias Biológicas (ENCB-P) and 19 in the Colección Nacional de Peces del Instituto de Biología de la Universidad Nacional Autónoma de México (IBUNAM; see examined material section below the references). All specimens were identified using the diagnostic characters proposed in original species descriptions, in Miller et al. (2005), and in De la Maza-Benignos et al. (2015). The same analysis of both traditional morphological and geometric morphometrics analysis of species in the *Herichthys labridens* group was previously performed by Mejía et al. (2015).

### 2.3.1 | Traditional morphometrics

A total of 25 morphometric characters were measured with a precision of 0.01 mm with a digital calliper: total length of the anal fin (LAF), total length of the dorsal fin (LDF), length of the dorsal fin spines (DFE), length of the dorsal fin rays (DFR), length of the anal fin spines (AFE), length of the anal fin rays (AFR), length of the pectoral fin (LPF), length of the pelvic fin (LVF), predorsal length (PDL), preanal length (PAL), postorbital length (POL), length of the upper jaw (UML), length of the lower jaw (LLM), length of the caudal

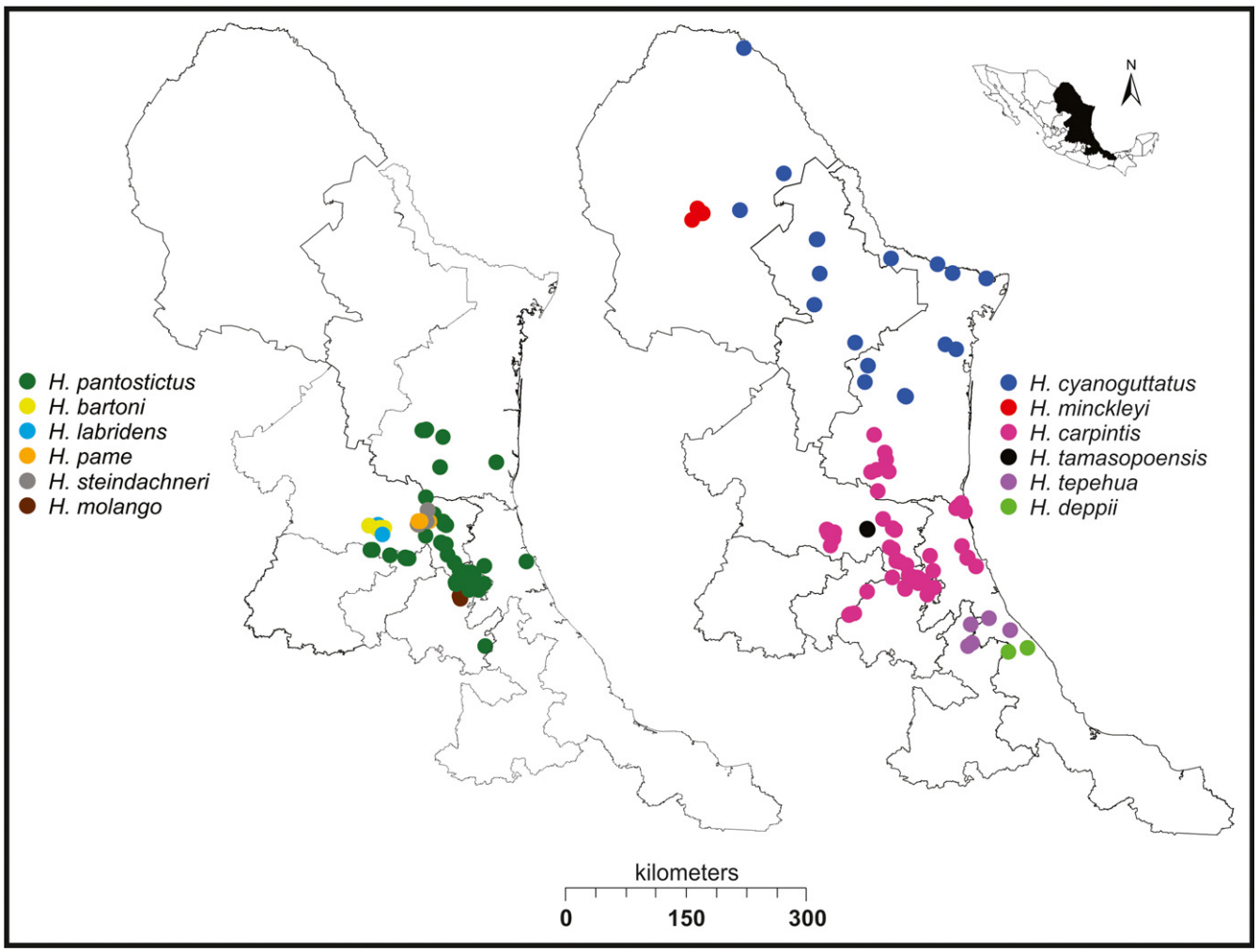


**FIGURE 1** Geographic distribution and sampling localities of *Herichthys* species. The outlines show Mexican states, and the black area in the inset figure shows the location of the *Herichthys* distribution area within Mexico

peduncle (LCP), length of the dorsal fin base (LDB), length of the anal fin base (LAB), head length (HLE), snout length (SNL), length of the ascending premaxillary process (LPP), length of the post ascending premaxillary process (PPP), distance between the anal fin and the base of the pelvic fins (DBF), body height (BHE), height of the caudal peduncle (HCP), eye diameter (EYD), and interocular distance (IOD). Morphometric characters were standardized in two ways to remove the size effect: as proportion of standard length (22 characters) or as a proportion of head length (eight characters) and through the Mosimann method (Butler & Losos, 2002). Additionally, a total of 12 meristic characters were recorded in each specimen: number of dorsal fin spines, number of dorsal fin rays, number of anal fin spines, number of anal fin rays, number of pectoral fin rays, number of pelvic fin rays, number of gill rakers on the first arm, number of scales in a longitudinal series, number of circumpeduncular scales, number of scales in the upper portion of the lateral line, number of scales in the lower portion of the lateral line, and total number of scales in the lateral line. Morphometric characters were compared through a one-way ANOVA with a Tukey's multiple comparison test; meanwhile, meristic data were evaluated with a Kruskal–Wallis test. Analyses were performed in STATISTICA ver. 10 (Statsoft inc.).

#### 2.3.2 | Geometric morphometrics

For each specimen, landmarks were taken from photographs of the left side; 25 landmarks were digitized for the body and 15 landmarks for the head in TPSDIG (Rohlf, 2010). To eliminate the effect of curvature caused by preservation, a regression with the "unbend specimens" option was performed in the TPSUTIL software (Rohlf, 2012) using landmarks as in Mejía et al. (2015). The generated Bookstein coordinates were converted to Procrustes distances using MORPHO J 1.03C (Klingenberg, 2011). To eliminate the allometric effect associated with growth, we performed a multivariate regression analysis using the Procrustes distances as the dependent variable and the size of the centroid as the independent variable. The adjusted Procrustes distances were used as descriptors of the level of differences among body and head shapes between the species; the significance of the differences was evaluated using a permutation test with 10,000 iterations (Elmer, Kusche, Lehtonen, & Meyer, 2010) in MorphoJ 1.03C (Klingenberg, 2011). Finally, the residuals of the regression analysis were used in a CVA and PCA analysis to compare the seven putative species included in the *Herichthys cyanoguttatus* species group. Similar to the Procrustes distances, the significance of the differences was evaluated using a permutation test with 10,000 iterations in MorphoJ 1.03C (Klingenberg, 2011). A similar procedure was used to compare between *Herichthys cyanoguttatus* and *H. labridens* species groups.

#### 2.3.3 | Coloration patterns

Coloration patterns were assessed using photographs of live specimens collected during and outside the breeding season to capture the full range of variation of the coloration patterns.

#### 2.3.4 | Feeding specializations

Feeding specializations of the individual species have been studied by combining our field observations with cranial and tooth morphology (Říčan et al., 2016) and with literature describing observation of feeding (Artigas Azas, 1992, 1994, 1996, 1998a,b, 2005, 2006, 2008, 2012; J. M. Artigas Azas personal communication; Swanson, Gibb, Marks, & Hendrikson, 2003) and analyzing ecomorphology and stomach contents (Buchanan, 1971; Díaz-Pardo & Guerra-Magaña, 1994; Hulsey et al., 2006; Kornfield & Taylor, 1983; Kornfield et al., 1982; Magalhaes et al., 2015; Sage & Selander, 1975; Taylor & Miller, 1983). These two aspects (direct observations and analyses) have been combined in the case of *Herichthys* in only the one well-studied species (*H. minckleyi*; e.g., Swanson, Gibb, Marks, & Hendrikson, 2008) and we attempt here a synthesis for all species, with much more work needed to be done. In the polymorphic *H. minckleyi*, there are three distinct feeding morphs based on synthesis of feeding behavior (Artigas Azas, 1998a,b; J. M. Artigas Azas personal communication) and ecomorphology including stomach contents. The specialized and rare piscivorous morph (Artigas Azas, 1998a,b; Kornfield & Taylor, 1983) appears to have escaped the attention of all above studies focusing solely on the analytical part without field observation.

## 3 | RESULTS

### 3.1 | Phylogenies based on individual datasets

#### 3.1.1 | Mitochondrial DNA markers

**Cytochrome oxidase I**

The analysis of the *COI* data collapsed into distinct haplotypes (see Methods and Appendix S1) resulted in a poorly resolved and supported topology at the species level with only one of the presently recognized species (*H. deppii*) found monophyletic and exclusive of other species (Figure 2). At deeper nodes, the resolution and support is good and *Herichthys* is recovered as a monophyletic well-supported group (BPP = 1.0). Two well-supported clades were recovered within *Herichthys* (BPP = 1.0), one including most of the haplotypes of the *H. cyanoguttatus* species group, the other most haplotypes of the *H. labridens* species group. Several haplotypes of *H. labridens* (or *H. pantostictus*; determination of this specimen from the Santa María river basin is equivocal; see Appendix S2), *H. pantostictus*, and *H. pame* were, however, found in the first group and the second includes one haplotype of *H. tamasopoensis* (Figure 2) and the two species groups are thus not monophyletic in this molecular marker. Most of the conflicts appear between sympatric or parapatric species. In the *H. labridens* species group, three well-supported clades were recovered. The first includes haplotypes of the sympatric *H. bartoni* and *H. labridens* (BPP = 1.0), the second includes haplotypes of the sympatric *H. steindachneri* and *H. pame* (BPP = 1.0), and the last one includes haplotypes of the allopatric *H. pantostictus*, *H. pratinus*, *H. labridens*, and the one haplotype of *H. tamasopoensis* (BPP = 0.92). In the *Herichthys cyanoguttatus* species group, only the haplotypes of *H. deppii* were recovered as

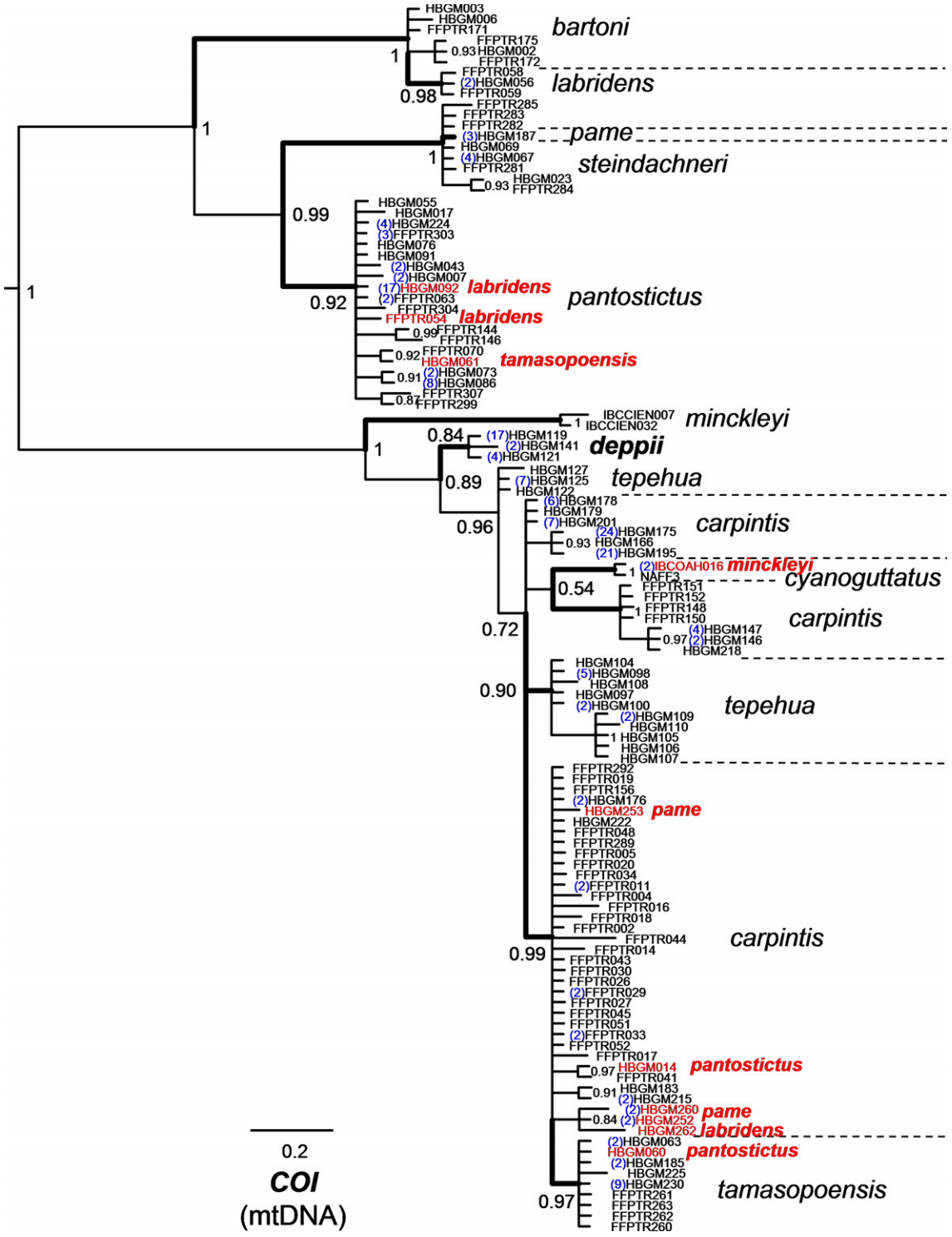


**FIGURE 2** Phylogenetic tree of the *Herichthys* species based on the mtDNA partial *COI* marker with specimens collapsed into haplotypes (numbers of collapsed specimens for each haplotype are shown with blue color; see Methods and Appendix S1). Clades with bold branches correspond to species-level taxa. Only one species (*H. deppii*; in bold) is unequivocally supported by the COI data. All other species are either found non-monophyletic, nested within other species, or include specimens from other species (shown in red). Bayesian posterior probabilities (BPP) are shown for all clades

monophyletic, but the clade lacks support (BPP = 0.84). The haplotypes of *H. minckleyi* were found in two clades, one basal and monophyletic and one in a clade with *H. cyanoguttatus*. The haplotypes of *H. cyanoguttatus*, *H. carpintis* (including *H. teporatus*), and *H. tepehua* were recovered unresolved at the species level. Haplotypes of *H tamasopoensis* form a single clade (BPP = 0.97) within *H. carpintis*, but this clade also includes one haplotype of *H. pantostictus*.

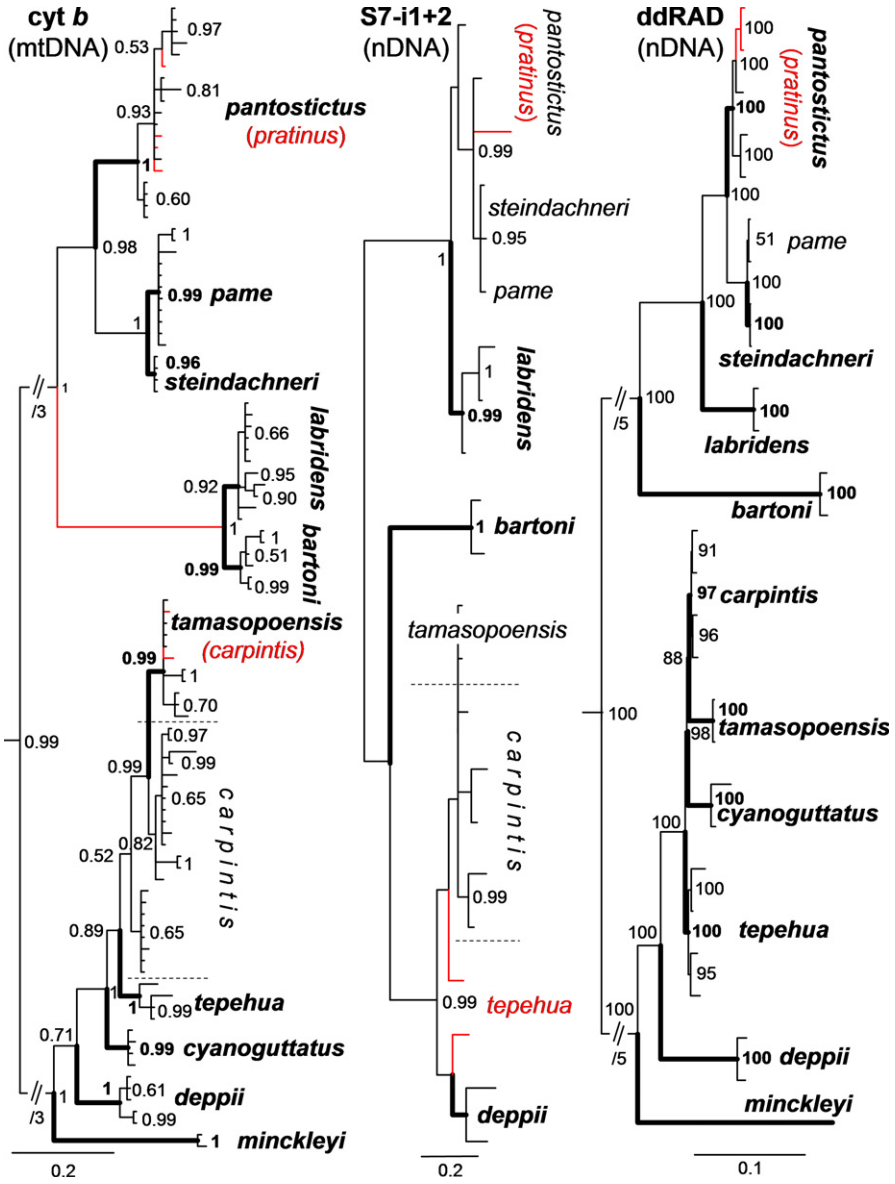


**FIGURE 3** Phylogenetic analyses based on the mtDNA *cytb* marker, the nDNA *S7* introns 1 and 2, and the genomic ddRAD dataset. The ddRAD results show analysis based on fixed SNPs, analysis including also variable SNPs is shown in Figure S2. See Table 1 for cross-linking of specimens used in the *cytb*, *S7i*, and ddRAD analyses. Taxa shown in bold are supported as monophyletic by the particular analysis. Taxa shown with red color are not supported as separate species by the particular analysis. *Herichthys pratinus* is in all cases nested within *H. pantostictus* (see Text and Appendix S2 and Figure S5). The red branch connecting *H. bartoni* and *H. labridens* in the mtDNA analysis shows conflict with nDNA analyses, which do not find these two species as sister taxa

### Cytochrome b

Compared to the poor species resolution of the *COI* marker, this other studied mtDNA marker provides a well-resolved and well-supported phylogeny of *Herichthys* (Figure 3). The topology of the *cytb* marker supports the reciprocal monophyly of the two main species groups (the *H. cyanoguttatus* group and the *H. labridens* group) with strong support (BPP = 0.99). Within the *H. cyanoguttatus* species group, *H. minckleyi* is supported as the basal-most species (BPP = 1.0), followed by the monophyletic *H. deppii* (BPP = 0.99), *H. cyanoguttatus* (BPP = 0.99), and *H. tepehua* (BPP = 0.99). Reciprocal monophyly was not found in the terminal clade (BPP = 1.0) where *H. tamasopoensis* (BPP = 0.99) is nested within the paraphyletic *H. carpintis*. Samples from the nominal *Herichthys teporatus* were not included in the analysis. Within the *H. labridens* species group, *Herichthys bartoni* (BPP = 0.99) is found as the sister species of *H. labridens* (BPP = 0.90) in a basal clade (BPP = 1.0), followed by the sister-species (BPP = 1.0) *H. pame* (BPP=0.99) and *H. steindachneri* (BPP=0.96) as the sister group of the last supported species *H. pantostictus* (BPP = 1.0). Samples of *H. molango* were not available at time of the analysis and were thus not included. *Herichthys pratinus* is found polyphyletic within samples of *H. pantostictus*.

## 3.1.2 | Nuclear DNA markers

### S7 introns

The *S7* introns 1 and 2 provided a resolved but poorly supported phylogeny of *Herichthys* (Figure 3; Figure S1). The analysis does not divide *Herichthys* strictly into the two main species groups because *H. bartoni* is unlike in the mtDNA phylogeny placed equidistantly between the two main groups with marginal support as the sister group of the *H. cyanoguttatus* group (Figure 3). Both the *H. labridens* (BPP = 1.0) and the *H. cyanoguttatus* species groups (BPP = 0.99) are well supported. Within the *H. cyanoguttatus* group, *H. deppii* is found monophyletic, *H. tepehua* poly/paraphyletic on a basal node, and

*H. tamasopoensis* is found within *H. carpintis* (as in mtDNA *cytb*). Within the *H. labridens* group, *H. labridens* is found in a basal position to a paraphyletic *H. pantostictus* that also includes *H. pratinus* (as in mtDNA *cytb*) and also in a clade *H. pame* and *H. steindachneri* without reciprocal monophyly.

### Reduced-genome representation ddRAD analysis

The genomic ddRAD analysis based on fixed SNPs provided well-resolved and very well-supported results (Figure 3). The most notable difference to the two mtDNA markers is the position of *H. bartoni* which is not the sister species of *H. labridens* (as in mtDNA) but is the basal species of the whole *H. labridens* species group (BS = 100; contra to the *H. cyanoguttatus* group in the nDNA *S7* introns analysis). The internal structure of the *H. labridens* species group is as in the nDNA *S7* introns analysis and (except *H. bartoni*) as in the mtDNA analyses with *H. labridens* (BS = 100) basal to a clade (BS = 100) including *H. pantostictus* (BS = 100) as the sister-clade (BS = 100) of *H. pame* plus *H. steindachneri*, which are recovered as reciprocally monophyletic (as in mtDNA *cytb* but unlike in nDNA *S7* introns). *Herichthys pratinus* is as in all other analyses nested within *H. pantostictus* and is thus not supported by any analysis as a phylogenetically separate species. Samples of *H. molango* were not available at time of the analysis and were thus not included. *Herichthys minckleyi* is the basal species of the *H. cyanoguttatus* group (BS = 100), followed by *H. deppii* (BS = 100). The internodes between the remaining four species of the *H. cyanoguttatus* are very short, but the relationships are statistically robust and all species are supported as monophyletic with BS = 100 except in *H. carpintis* (BS = 97). *Herichthys carpintis* is the sister species of *H. tamasopoensis* (BS = 88), this clade is sister to *H. cyanoguttatus* (BS = 98), and the basal of these four species is *H. tepehua* (BS = 100).

We have further analyzed the ddRAD data with the inclusion of all variable SNPs (see Figure S2) to look for more recent gene flow. While results are very similar, the analysis including variable SNPs differs in two points. (i) *Herichthys tepehua* is not supported as monophyletic but is found paraphyletic between *H. deppii* and *H. cyanoguttatus* and (ii) the analysis has increased resolution of branch lengths at the species level compared to the fixed SNPs analysis. TreeMix analyses (results not shown) suggest that the non-monophyly of *H. tepehua* is due to SNP sharing with *H. deppii* and *H. carpintis*. The analyses thus show that the gene pool of *H. tepehua* has been secondarily compromised by the two neighboring species.

## 3.2 | Morphometric and meristic delimitation of *Herichthys* species

The analysis of both traditional morphological and geometric morphometrics analysis of species in the *Herichthys labridens* group was previously performed by Mejía et al. (2015), and we report here the results of the analyses for the *H. cyanoguttatus* species group. In the meristic dataset, the number of pectoral rays, number of rays in the dorsal fin, and number of rays in the anal fin allowed separation of

**TABLE 2** Descriptive statistics for the 12 meristic data used in this study. The mode, minimum and maximum for each character are presented. The numbers shaded represent the characters that showed significant differences between the species

| Character | *H. carpintis* | | | *H. cyanoguttatus* | | | *H. deppii* | | | *H. minckleyi* | | | *H. tamasopoensis* | | | *H. tepehua* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Min | m | Max | Min | m | Max | Min | m | Max | Min | m | Max | Min | m | Max | Min | m | Max |
| Number of spines in the dorsal fin | 15 | 16 | 17 | 15 | 16 | 17 | 15 | 16 | 17 | 15 | 16 | 17 | 15 | 16 | 17 | 15 | 16 | 17 |
| Number of rays in the dorsal fin | 7 | 10 | 12 | 8 | 10 | 11 | 10 | 11 | 13 | 9 | 11 | 12 | 9 | 10 | 10 | 10 | 11 | 12 |
| Number of spines in the anal fin | 4 | 5 | 6 | 4 | 5 | 6 | 5 | 6 | 7 | 4 | 5 | 6 | 5 | 5 | 5 | 5 | 6 | 8 |
| Number of rays in the anal fin | 4 | 8 | 10 | 7 | 8 | 10 | 7 | 9 | 11 | 7 | 8 | 11 | 7 | 8 | 8 | 8 | 9 | 10 |
| Number of rays in the pectoral fins | 12 | 14 | 15 | 10 | 14 | 15 | 12 | 14 | 15 | 12 | 14 | 15 | 13 | 13 | 14 | 13 | 14 | 14 |
| Number of rays in the pelvic fins | 4 | 5 | 5 | 2 | 5 | 5 | 5 | 5 | 5 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Number of gill rakers in the first arm | 6 | 7 | 9 | 6 | 8 | 10 | 5 | 7 | 8 | 6 | 7 | 9 | 7 | 8 | 10 | 5 | 7 | 9 |
| Number of scales in a longitudinal series | 21 | 26 | 30 | 22 | 27 | 30 | 26 | 27 | 29 | 26 | 28 | 31 | 24 | 25 | 28 | 26 | 27 | 28 |
| Number of circumpeduncular scales | 14 | 16 | 19 | 16 | 16 | 18 | 16 | 18 | 18 | 16 | 18 | 19 | 16 | 16 | 18 | 16 | 18 | 18 |
| Number of scales in the first portion of the lateral line | 12 | 18 | 22 | 13 | 19 | 21 | 17 | 18 | 21 | 16 | 20 | 22 | 14 | 18 | 20 | 16 | 18 | 21 |
| Number of scales in the second portion of the lateral line | 2 | 10 | 13 | 2 | 11 | 13 | 8 | 13 | 14 | 10 | 13 | 16 | 8 | 10 | 12 | 8 | 10 | 14 |
| Total number of scales in the lateral line | 14 | 28 | 32 | 17 | 30 | 33 | 26 | 31 | 35 | 27 | 30 & 33 | 35 | 23 | 27 | 32 | 25 | 30 | 34 |

**TABLE 3** Descriptive statistics for the 18 morphometric characters adjusted as proportions of the standard length (SL) and the seven morphometric characters adjusted as proportions of the head length (HL) used in this study. The mean, minimum and maximum for each species are expressed as percentages. The numbers shaded represent the characters that showed significant differences between the species

| | *H. carpintis* | | | *H. cyanoguttatus* | | | *H. deppii* | | | *H. minckleyi* | | | *H. tamasopoensis* | | | *H. tepehua* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Min | X | Max | Min | X | Max | Min | X | Max | Min | X | Max | Min | X | Max | Min | X | Max |
| **SL** | | | | | | | | | | | | | | | | | | |
| Total length of the anal fin (LAF) | 32 | 40 | 54 | 34 | 43 | 52 | 27 | 40 | 48 | 28 | 35 | 48 | 27 | 34 | 40 | 34 | 41 | 51 |
| Total length of the dorsal fin (LDF) | 52 | 72 | 88 | 65 | 75 | 95 | 63 | 72 | 80 | 59 | 64 | 72 | 58 | 69 | 85 | 66 | 72 | 77 |
| Length of the dorsal fin of spines (DFE) | 41 | 55 | 63 | 49 | 58 | 69 | 49 | 54 | 59 | 47 | 51 | 70 | 53 | 55 | 58 | 50 | 54 | 59 |
| Length of the dorsal fin of rays (DFR) | 8 | 17 | 30 | 8 | 17 | 26 | 10 | 17 | 29 | 4 | 12 | 17 | 5 | 14 | 30 | 12 | 17 | 22 |
| Length of the anal fin of spines (AFE) | 18 | 25 | 31 | 19 | 26 | 33 | 18 | 26 | 29 | 19 | 24 | 28 | 19 | 23 | 28 | 20 | 25 | 28 |
| Length of the anal fin of rays (AFR) | 7 | 15 | 29 | 9 | 16 | 25 | 8 | 14 | 21 | 6 | 11 | 25 | 4 | 10 | 13 | 6 | 15 | 24 |
| Length of the pectoral fin (LPF) | 18 | 26 | 31 | 20 | 25 | 29 | 23 | 27 | 30 | 20 | 24 | 29 | 24 | 27 | 30 | 23 | 27 | 31 |
| Length of the pelvic fin (LVF) | 19 | 28 | 40 | 19 | 28 | 34 | 23 | 28 | 36 | 18 | 24 | 27 | 21 | 24 | 27 | 23 | 27 | 39 |
| Predorsal length (PDL) | 14 | 33 | 47 | 24 | 32 | 37 | 27 | 32 | 38 | 31 | 36 | 41 | 29 | 33 | 38 | 28 | 32 | 36 |
| Preanal length (PAL) | 50 | 67 | 73 | 59 | 66 | 72 | 61 | 64 | 70 | 59 | 66 | 76 | 64 | 68 | 72 | 59 | 64 | 67 |
| Length of the caudal peduncle (LCP) | 8 | 11 | 16 | 8 | 11 | 13 | 10 | 12 | 13 | 10 | 12 | 15 | 11 | 13 | 15 | 9 | 12 | 14 |
| Length of the dorsal fin at its base (LDB) | 35 | 54 | 65 | 50 | 56 | 65 | 50 | 55 | 62 | 18 | 49 | 54 | 46 | 54 | 58 | 50 | 55 | 75 |
| Length of the anal fin at its base (LAB) | 16 | 20 | 25 | 16 | 22 | 29 | 19 | 22 | 27 | 12 | 18 | 45 | 15 | 18 | 23 | 18 | 23 | 38 |
| Head length (HLE) | 29 | 34 | 39 | 30 | 34 | 39 | 30 | 34 | 36 | 31 | 36 | 41 | 29 | 34 | 37 | 30 | 33 | 36 |
| Length of the post ascending premaxillary process (PPP) | 21 | 27 | 33 | 24 | 28 | 32 | 24 | 27 | 30 | 24 | 27 | 31 | 26 | 28 | 31 | 22 | 26 | 29 |
| Distance between the anal fin and the base of the pelvic fins (DBF) | 23 | 29 | 37 | 23 | 29 | 34 | 23 | 26 | 29 | 23 | 28 | 33 | 26 | 30 | 35 | 24 | 26 | 30 |
| Body height (BHE) | 39 | 46 | 58 | 40 | 47 | 61 | 38 | 42 | 46 | 34 | 42 | 49 | 42 | 44 | 47 | 40 | 43 | 47 |
| Height of the caudal peduncle (HCP) | 13 | 16 | 22 | 15 | 17 | 19 | 15 | 16 | 17 | 13 | 14 | 17 | 14 | 15 | 16 | 15 | 16 | 17 |
| **HL** | | | | | | | | | | | | | | | | | | |
| Postorbital length (POL) | 32 | 44 | 54 | 36 | 44 | 53 | 39 | 45 | 52 | 38 | 43 | 52 | 39 | 45 | 57 | 38 | 46 | 57 |
| Length of the upper maxilla (UML) | 12 | 24 | 33 | 19 | 26 | 31 | 17 | 24 | 30 | 18 | 25 | 32 | 18 | 24 | 27 | 17 | 22 | 28 |
| Length of the lower maxilla (LLM) | 12 | 24 | 33 | 15 | 26 | 31 | 17 | 24 | 30 | 18 | 25 | 34 | 18 | 24 | 27 | 17 | 22 | 28 |
| Snout length (SNL) | 21 | 32 | 43 | 26 | 35 | 45 | 18 | 30 | 38 | 22 | 37 | 52 | 21 | 30 | 36 | 24 | 31 | 39 |
| Length of the ascending premaxillary process (LPP) | 33 | 43 | 54 | 36 | 43 | 56 | 41 | 46 | 53 | 41 | 46 | 57 | 36 | 43 | 51 | 40 | 47 | 50 |
| Eye diameter (EYD) | 22 | 30 | 40 | 20 | 27 | 34 | 22 | 32 | 43 | 19 | 27 | 34 | 26 | 32 | 36 | 21 | 30 | 42 |
| interocular distance (IOD) | 27 | 35 | 48 | 30 | 38 | 50 | 26 | 34 | 40 | 26 | 33 | 46 | 28 | 33 | 41 | 26 | 35 | 42 |

*H. tamasopoensis* from the rest of the species, while the number of scales in the lower lateral line allowed separation of *H. minckleyi* (Table 2). Among the morphometric data differences in eight characters allowed discrimination between the species, seven are diagnostic for *H. minckleyi* (length of the dorsal fin, length of the dorsal fin of spines, total length of the pectoral fin, predorsal length, head length, length of the dorsal fin base, and height of the caudal peduncle) and one (height of the caudal peduncle) is diagnostic for *H. cyanoguttatus* (Table 3). Five nominal species could not be separated in the morphometric analyses from each other (*H. cyanoguttatus*, *H. carpintis*, *H. teporatus*, *H. tepehua*, *H. deppii*; the *H. cyanoguttatus* morphogroup).

ANOVA found statistical differences in two characters associated with the head length and in ten characters associated with the standard length. *Herichthys cyanoguttatus* showed the highest interocular distance, while *H. minckleyi* showed the biggest snout (Table 3). *Herichthys minckleyi* differs in length of the dorsal fin, length of the dorsal fin spines, length of the pectoral fin, predorsal length, length of the dorsal fin at its base, head length, and height of the caudal peduncle, while *H. cyanoguttatus* differs from the rest of the species in the height of the caudal peduncle and in body height (Table 3).

The analysis of Procrustes distances revealed significant differences in body shape in six taxa (Table 4). In the canonical variable

**TABLE 4** Procrustes distances among taxa of the *Herichthys cyanoguttatus* species group. Above the diagonal the results for the 15 landmarks of the head, below the diagonal the results for the 25 landmarks of the body

| | *H. carpintis* | *H. cyanoguttatus* | *H. deppii* | *H. minckleyi* | *H. tamasopoensis* | *H. tepehua* |
|---|---|---|---|---|---|---|
| *H. carpintis* | | 0.0664* | 0.0376* | 0.0634* | 0.0622* | 0.0376* |
| *H. cyanoguttatus* | 0.0184* | | 0.0909* | 0.1094* | 0.1093* | 0.0847* |
| *H. deppii* | 0.0254* | 0.0309* | | 0.0530* | 0.0479* | 0.0383 |
| *H. minckleyi* | 0.0366* | 0.0459* | 0.0414* | | 0.0872* | 0.0450* |
| *H. tamasopoensis* | 0.0220* | 0.0318* | 0.0276* | 0.0425* | | 0.0703* |
| *H. tepehua* | 0.0280* | 0.0344* | 0.0133* | 0.0414* | 0.0377* | |

*Significant $p$ values ($p < .05$) after 10,000 iterations

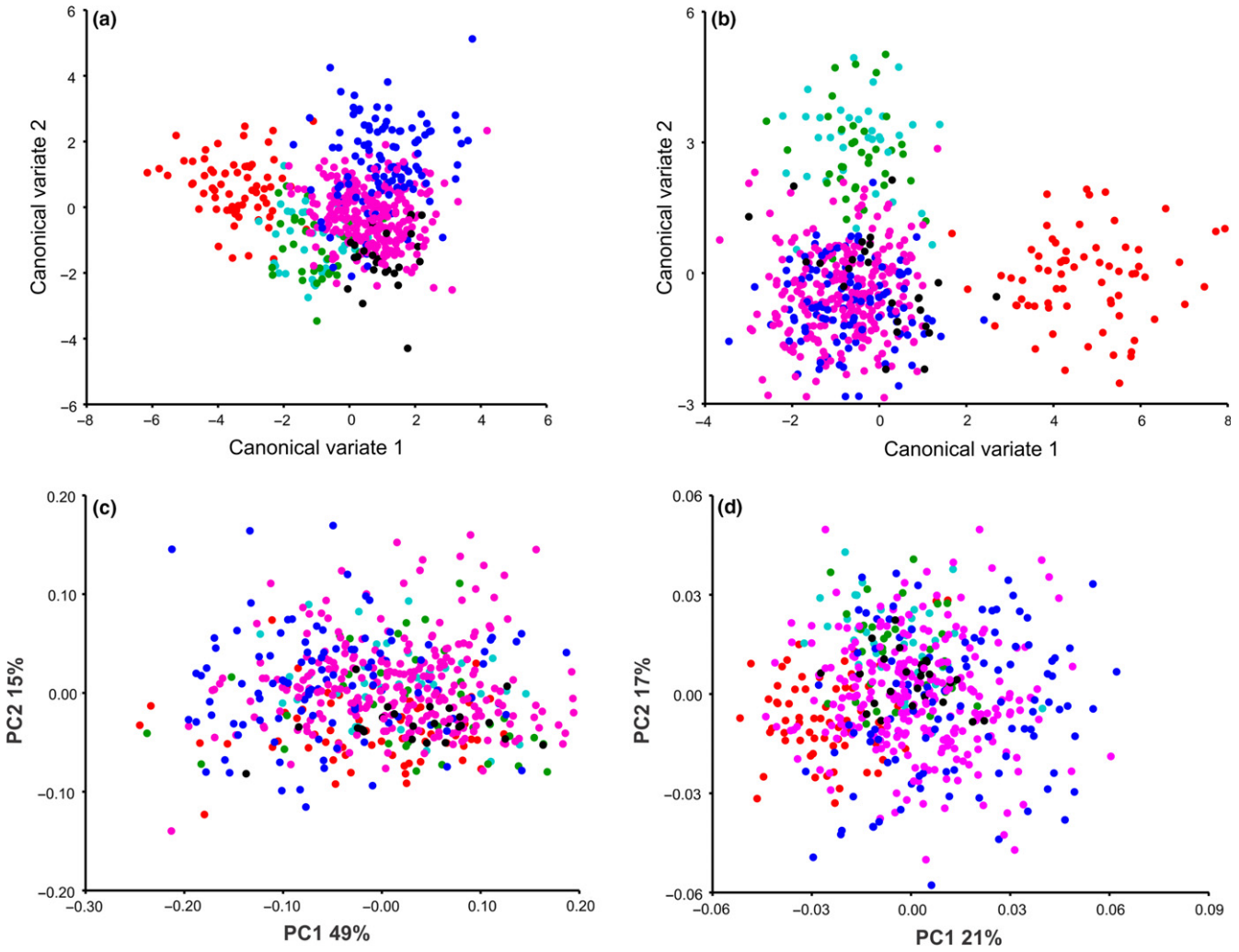


**FIGURE 4** Canonical variable and principal component analysis of species in the *H. cyanoguttatus* species group according to the results of geometric morphometrics. (a) Canonical variables for the head; (b) canonical variables for the body; (c) principal components for the head; (d) principal components for the body. Symbology: *H. deppii* green, *H. carpintis* (including *H. teporatus*) pink, *H. cyanoguttatus* blue, *H. minckleyi* red, *H. tamasopoensis* black, *H. tepehua* aqua

analysis, *H. minckleyi* showed a different shape for the head and the body, *H. deppi* and *H. tepehua* showed similar shapes, *H. tamasopoensis* showed a slightly different head shape, while *H. cyanoguttatus* and *H. carpintis* (including *H. teporatus*) showed no differences between them (Figure 4). In contrast, the principal component analysis from the Procrustes distances showed no significant differences in head and body shape for either species with the exception of body shape in *H. minckleyi* (Figure 4).

Comparisons of meristic characters between the *H. labridens* species group (=*Nosferatu*) and the *H. cyanoguttatus* species group (=*Herichthys*) with the Mann–Whitney U-test showed differences in eight of the twelve analyzed characters (Table 5), while for the

**TABLE 5** Mann–Whitney comparison test between the two species groups included in the genus *Herichthys* for the twelve meristic characters analyzed. The numbers shaded represent the characters that showed significant differences

| Character | Rank Sum *Nosferatu* | Rank Sum *Herichthys* | *U* | *Z* | *p* |
|---|---|---|---|---|---|
| Number of spines in the dorsal fin | 299322.0 | 276879.0 | 138278.0 | 1.3552 | .1753 |
| Number of rays in the dorsal fin | 344503.5 | 231697.5 | 93096.5 | 11.1775 | .0000 |
| Number of spines in the anal fin | 295083.5 | 281117.5 | 142516.5 | 0.3250 | .7452 |
| Number of rays in the anal fin | 375285.0 | 200916.0 | 62315.0 | 17.6673 | .0000 |
| Number of rays in the pectoral fins | 380303.5 | 195897.5 | 57296.5 | 19.0947 | .0000 |
| Number of rays in the pelvic fins | 292217.0 | 283984.0 | 142339.0 | −1.4283 | .1532 |
| Number of gill rakers in the first arm | 268320.0 | 307881.0 | 118442.0 | −5.3511 | .0000 |
| Number of scales in a longitudinal series | 359435.0 | 216766.0 | 78165.0 | 13.4452 | .0000 |
| Number of circumpeduncular scales | 264206.0 | 311995.0 | 114328.0 | −6.6347 | .0000 |
| Number of scales in the first portion of the lateral line | 306520.5 | 269680.5 | 131079.5 | 2.6032 | .0092 |
| Number of scales in the second portion of the lateral line | 270612.5 | 305588.5 | 120734.5 | −4.6524 | .0000 |
| Total number of scales in the lateral line | 284754.0 | 291447.0 | 134876.0 | −1.7927 | .0730 |

morphometric characters the t-test showed differences in twenty of the twenty-five characters (Table 6). However, the ranges of all these characters showed high levels of overlap that preclude their use as diagnostic characters for the two groups. The results of the principal component analysis of meristic data, morphometric data adjusted by Mosimann method, and shapes of the head and the body derived from the geometric morphometrics analysis failed to recover both species groups as discrete entities (Figure 5).

## 3.3 | Delimitation of *Herichthys* species using coloration patterns

Among morphological characters, the best discrimination of species in *Herichthys* is achieved through coloration patterns and especially breeding coloration patterns which are virtually species specific (Figure 6; for details see next section). In general, *Herichthys* species develop during courtship and breeding strikingly different coloration patterns compared to the normal coloration that they have outside of the breeding season. In *Herichthys*, the female is predominantly responsible for the care and protection of the offspring and the female thus develops the species-specific breeding dress much better than the male, whose major responsibility is the protection of the breeding territory (see Říčan et al., 2016 for a summary).

The breeding coloration in *Herichthys* is composed of a darkening (blackening) of the ventral portion of the head, ventral portion of anterior body and whole posterior part of body, while the rest of the head and body turns white to snow-white (or yellow in one species; *H. labridens*). The species-specific breeding coloration patterns are observed in the details of the extent of the blackening on the head (from completely missing in *H. minckleyi* and *H. cyanoguttatus* to maximum extent in the *H. labridens* group and *H. bartoni*) and the patterns of blackening on the body (either as vertical bars or black zones, the first typical in the *H. cyanoguttatus* group, the second in the *H. labridens* group plus *H. bartoni* and to major extent also in *H. carpintis*) and the ventral fins (Figure 6; see next section).

## 3.4 | Revised diagnoses of *Herichthys* species

Based on our results, we review the diagnostic characters of all here supported *Herichthys* species.

### 3.4.1 | The *Herichthys labridens* species group

All species are found in the Pánuco river basin.

*Herichthys bartoni* is distinguished from all other *Herichthys* species by a breeding coloration composed of the whole suborbital part of head and two-thirds to three quarters of body flanks uniformly black, while the rest of the dorsal part of body is snow-white (Figure 6). *Herichthys bartoni* is distinguished from the remaining species in the *H. labridens* species group by having opalescent markings (otherwise present only in the *H. cyanoguttatus* group species) on posterior part of body, caudal peduncle and unpaired fins (especially in breeding individuals), and by lacking the naked (scaleless) red-violet colored area in the axil of the pectoral fin diagnostic for all other species of the *H. labridens* group. *Herichthys bartoni* does have a group of rusty-colored spots on the posterior area of head and anterior portion of body including the axil of the pectoral fin, but the spot in the axil is on a scaled area and is thus not homologous to the naked red-colored area present in the *H. labridens* species group. The same rusty-colored dots found along the border between the head and body and not associated with a naked area in the axil of the pectoral fins are except for *H. bartoni* also found in *H. deppii* and in some *H. tepehua*. *Herichthys bartoni* is clearly distinguished from the sympatric *H. labridens* by black-white vs. black-yellow breeding coloration (unique for *H. labridens*; Figures 6 and S3) where the black area in *H. bartoni* includes all of ventral head and all of ventral body, by lacking the stout, molariform lower pharyngeal jaw, by having a larger head, larger mouth with a prognathous lower jaw (vs. isognathous or hypognathous with upper lip reaching over the lower lip), by having brown to black small and widely separated dots on head, cheek, and opercular series instead of much more tightly spaced dots

**TABLE 6** Comparison test between the two species groups included in the genus *Herichthys* for the 18 morphometric characters adjusted as proportions of the standard length (SL) and the seven morphometric characters adjusted as proportions of the head length (HL) used in this study. The numbers shaded represent the characters that showed significant differences within the species

| | Mean *Nosferatu* | Mean *Herichthys* | *t*-value | *p* |
|---|---|---|---|---|
| Characters adjusted as a percentage of the standard length | | | | |
| Total length of the anal fin (LAF) | 0.3595 | 0.3974 | −15.5328 | .0000 |
| Total length of the dorsal fin (LDF) | 0.6724 | 0.7149 | −13.7433 | .0000 |
| Length of the dorsal fin of spines (DFE) | 0.4934 | 0.5482 | −26.0060 | .0000 |
| Length of the dorsal fin of rays (DFR) | 0.1740 | 0.1618 | 5.4542 | .0000 |
| Length of the anal fin of spines (AFE) | 0.1969 | 0.2483 | −31.2082 | .0000 |
| Length of the anal fin of rays (AFR) | 0.1578 | 0.1449 | 5.8968 | .0000 |
| Length of the pectoral fin (LPF) | 0.2368 | 0.2587 | −18.2524 | .0000 |
| Length of the pelvic fin (LVF) | 0.2388 | 0.2713 | −19.8043 | .0000 |
| Predorsal length (PDL) | 0.3537 | 0.3326 | 12.0466 | .0000 |
| Preanal length (PAL) | 0.6561 | 0.6666 | −6.2068 | .0000 |
| Length of the caudal peduncle (LCP) | 0.1155 | 0.1131 | 3.1484 | .0017 |
| Length of the dorsal fin at its base (LDB) | 0.5079 | 0.5380 | −14.6905 | .0000 |
| Length of the anal fin at its base (LAB) | 0.1906 | 0.2009 | −6.6058 | .0000 |
| Head length (HLE) | 0.3418 | 0.3431 | −1.0646 | .2873 |
| Length of the post ascending premaxillary process (PPP) | 0.2577 | 0.2739 | −12.3366 | .0000 |
| Distance between the anal fin and the base of the pelvic fins (DBF) | 0.2875 | 0.2862 | 0.7002 | .4840 |
| Body height (BHE) | 0.4082 | 0.4507 | −24.7118 | .0000 |
| Height of the caudal peduncle (HCP) | 0.1592 | 0.1593 | −0.1954 | .8451 |
| Characters adjusted as a percentage of the head length | | | | |
| Postorbital length (POL) | 0.4411 | 0.4399 | 0.5933 | .5531 |
| Length of the upper maxilla (UML) | 0.2214 | 0.2413 | −10.7384 | .0000 |
| Length of the lower maxilla (LLM) | 0.2228 | 0.2417 | −10.0869 | .0000 |
| Snout length (SNL) | 0.3382 | 0.3263 | 4.6999 | .0000 |
| Length of the ascending premaxillary process (LPP) | 0.4393 | 0.4403 | −0.3542 | .7233 |
| Eye diameter (EYD) | 0.2494 | 0.2898 | −18.8707 | .0000 |
| interocular distance (IOD) | 0.3357 | 0.3529 | −7.2442 | .0000 |

and often vermiculated black lines (or no dots at all) on a blue-green background with blue lips, and by always lacking blue coloration in unpaired fins (the fins are orange to rusty). *Herichthys bartoni* is also distinguished from all other *Herichthys* species by being the only species with a modal count of only four anal fin spines. *Herichthys bartoni* is together with the sympatric and syntopic *H. labridens* endemic to the lagoons of the Laguna de la Media Luna area (San Luis Potosí) at elevations between 1,000 and 1,100 m a.s.l. and also (though much rarer) in parts of the Upper Rio Verde.

*Herichthys labridens* is unique among all *Herichthys* species in having instead of a white and black breeding coloration a yellow and black combination. This combination is also unique among all heroine cichlids. The distribution of the yellow and black fields of coloration on body is typical of the spectrum found in the *H. labridens* species group, namely a black ventral portion of head below the eye continuing to mid body, where replaced with white/yellow and a second area of black extending forward onto the body from the caudal peduncle. Live coloration outside of the breeding season is also unique among *Herichthys* with dark blue-green head and cheeks and blue lips, with blue markings also in unpaired fins (as opposed to all other species in the *H. labridens* group). *Herichthys labridens* is clearly distinguished from the sympatric *H. bartoni* (see above). *Herichthys labridens* is together with the sympatric and syntopic *H. bartoni* endemic to the lagoons of the Laguna de la Media Luna area (San Luis Potosí) at elevations between 1,000 and 1,100 m a.s.l. and also (though much rarer) in parts of the Upper Rio Verde.

*Herichthys steindachneri* is a slender-bodied piscivorous species (Figure S3) unique among all *Herichthys* species by its very large, long and pointed head, which is usually greater (always above 90%) than body depth over the base of pelvic fin. The lower jaw is prognathous. The head is larger than even in the piscivorous morphs of *H. minckleyi* and *H. bartoni*. The body of *H. steindachneri* is also much longer than in the piscivorous morphs of *H. minckleyi* and *H. bartoni*. Breeding coloration of the species is very faint but diagnostic from all species of *Herichthys* except the sympatric and syntopic *H. pame* which shares an almost identical configuration of the black and

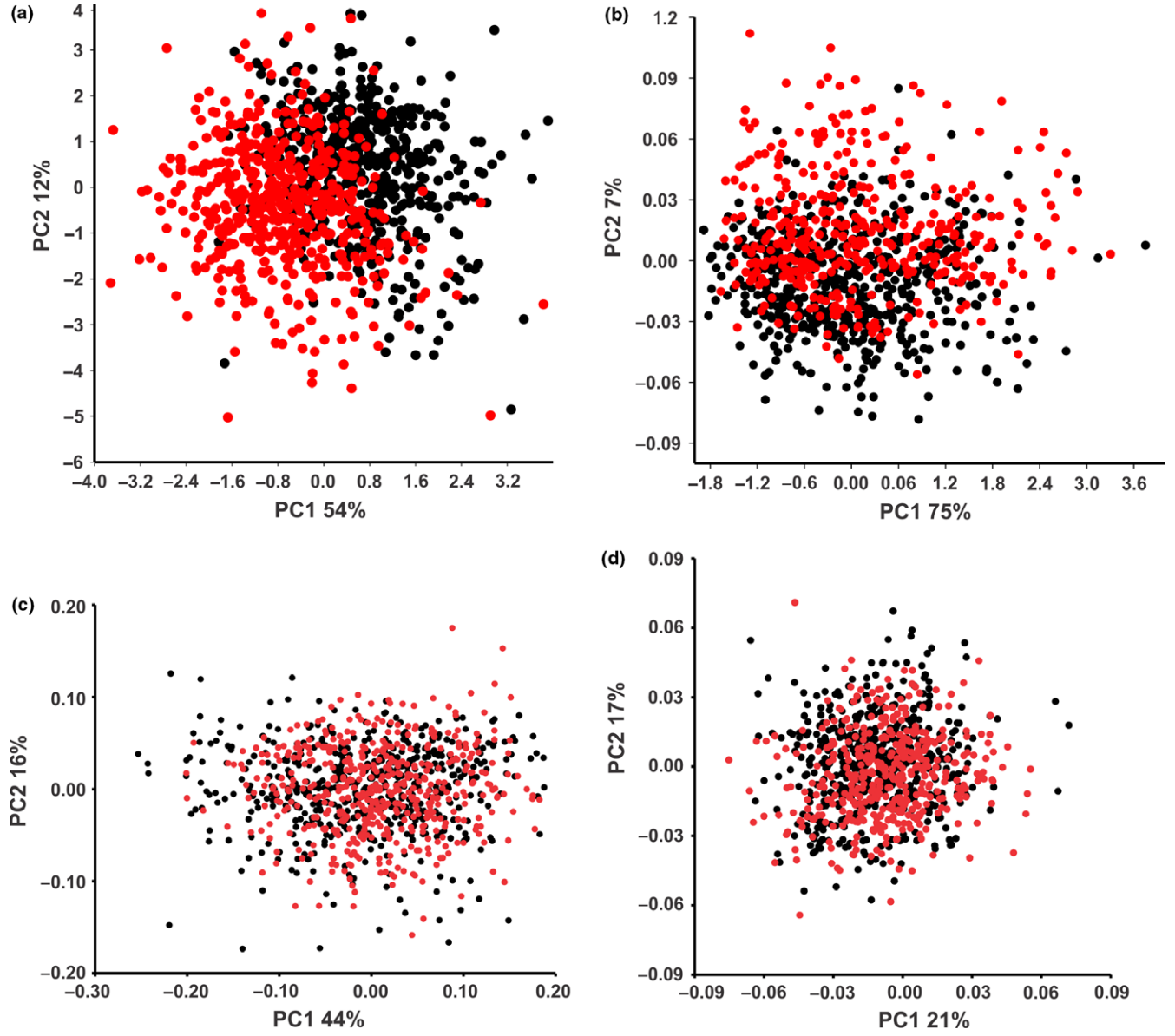


**FIGURE 5** Principal component analysis of the *Herichthys* species groups. (a) Principal component of the meristic data; (b) principal component of the morphometric data adjusted by the method of Mosimann; (c) principal component of the geometric morphometrics data of the head; (d) principal component of the geometric morphometrics data of the body. Symbology Red = *H. cyanoguttatus* group and Black = *H. labridens* group

white fields making up the breeding coloration. The breeding coloration is, however, very lightly developed, probably because the much larger adult size of *H. steindachneri*. The unique feature in the breeding coloration of both *H. steindachneri* and *H. pame* is the interruption of the mid-dorsal white field by a black extension from both the cranial and the caudal peduncle black fields (riverine populations of *H. pantostictus* also show this feature, but all other species and populations of *Herichthys* have either white dorsum or remnants of vertical bars on dorsum). *Herichthys steindachneri* is together with its sympatric and syntopic sister species *H. pame* endemic to the Rio Gallinas and its tributaries (except the Rio Tamasopo above the Tamasopo falls where only *H. pame* is found) above the 105 m high Tamul waterfall.

*Herichthys pame* shares a similar breeding coloration with other species of the *H. labridens* group and shares with *H. steindachneri* (and riverine populations of *H. pantostictus*) an almost identical configuration of the black and white fields. *Herichthys pame* shares with all species in the *H. labridens* group the red/magenta spot in the naked axil of the pectoral fin. It is distinguished from *H. labridens* by breeding coloration (white-black in *H. pame* vs. yellow-black in *H. labridens*), by having pale yellow instead of blue cheeks, and by a more elongated snout and no blue-green markings in the dorsal fin. *Herichthys pame* is distinguished from its predatory sister-species *H. steindachneri* by lacking the elongated enlarged head and by having shorter and subequal oral jaws (vs. lower jaw prognathous) and by having a stout lower pharyngeal jaw with molariform teeth.

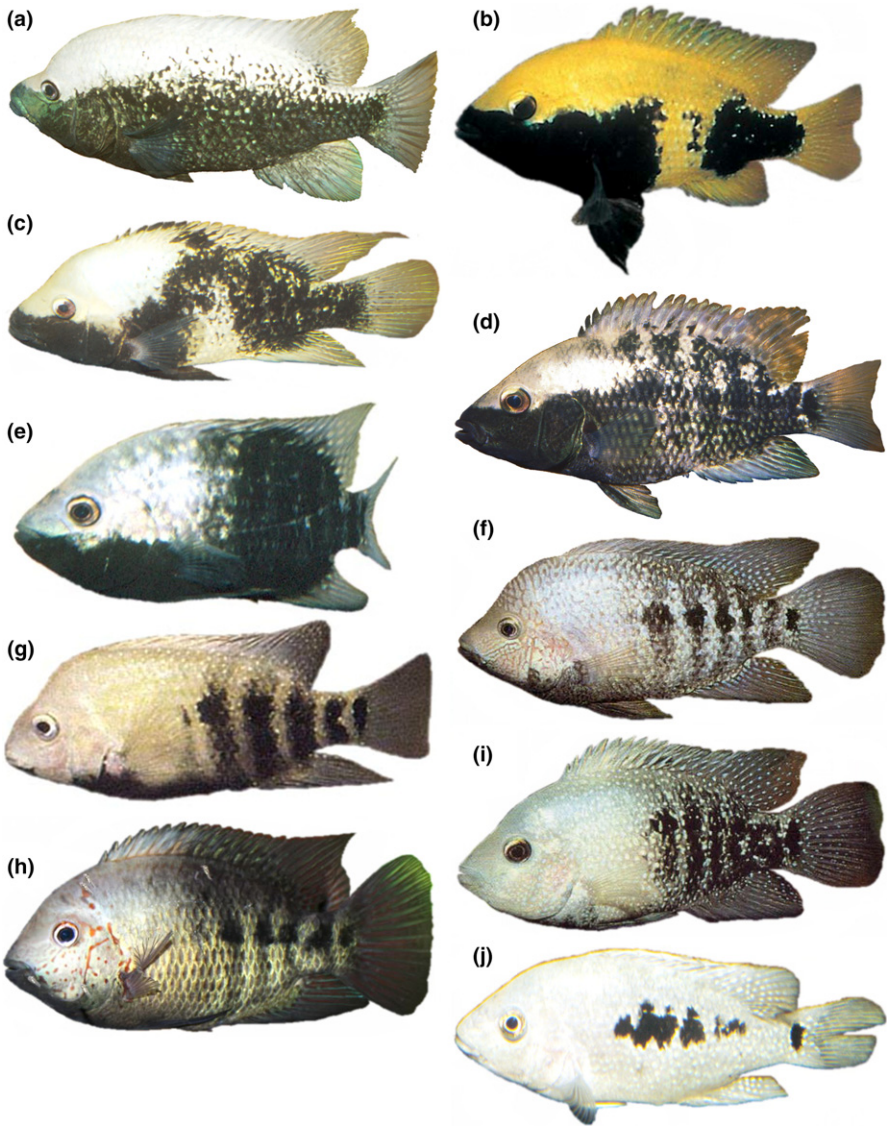


**FIGURE 6** Diversity of breeding coloration patterns in *Herichthys* species. (a) *Herichthys bartoni*, (b) *H. labridens*, (c) *H. pame*, (d) *H. pantostictus* (lacustrine population), (e) *H. carpintis*, (f) *H. tepehua*, (g) *H. tamasopoensis*, (h) *H. deppii*, (i) *H. cyanoguttatus*, (j) *H. minckleyi* (female; male is all black). See species diagnoses for additional information. Photographs are from Říčan et al. (2016)

*Herichthys pame* is distinguished from the most similar riverine populations of *H. pantostictus* (Figures S4 and S5) in having no red markings in fins (except the red outline of the dorsal and anal fins that can be present in both species) and no red dots on body, in having a purple/magenta (vs. red) colored spot in the axil of the pectoral fin, in breeding coloration by having a pure white instead of a yellowish-white breeding coloration background, and by the uniformly well-developed robust lower pharyngeal jaw and molariform teeth (vs. intermediate or present in only some populations). *Herichthys pame* is together with its sympatric and syntopic sister species *H. steindachneri* endemic to the Rio Gallinas and its tributaries (including the Rio Tamasopo above the Tamasopo falls where only *H. pame* is found) above the 105 m high Tamul waterfall.

*Herichthys pantostictus* (junior synonym: *Herichthys pratinus*) is a dimorphic species with neither morph being monophyletic and thus clearly representing ecological adaptation to two different environments in this widespread species. The lowland lacustrine form is distinguished from all *Herichthys* species in having the entire body in normal coloration (except abdomen and ventral surface of head) uniformly pale and covered with small dark dots. This lowland form is also distinguished from all species in the *H. labridens* species group plus *H. bartoni* by the presence of vertical bars in the breeding coloration. The presence of vertical bars in the breeding coloration is, however, a plesiomorphy, as it is also present in the majority of species of the *H. cyanoguttatus* species group and is the norm in breeding colorations of Middle American cichlids and cichlids in general. Except for the presence of vertical bars, the breeding coloration of the lowland lacustrine form of *Herichthys pantostictus* is identical to *H. bartoni* in being composed of the whole suborbital part of head and two-thirds of body flanks uniformly black, dorsal rest of body white. The riverine form of *H. pantostictus* is most similar to *H. pame* but is distinguished in coloration details and in the degree of specialization in cranial morphology and in diet. *Herichthys pantostictus* is the only widespread species in the *H. labridens* species group being endemic to virtually the whole Tamesí-Pánuco river basin including Rio El Salto (the nominal *H. pratinus* considered here conspecific

with *H. pantostictus*) but except the high-elevation areas of Rio Verde and Media Luna (where replaced by *H. bartoni* and *H. labridens*), Rio Gallinas (where replaced by *H. pame* and *H. steindachneri*) and the headwaters of the Rio Moctezuma.

### 3.4.2 | The *Herichthys cyanoguttatus* species group

*Herichthys minckleyi* is unique among all *Herichthys* species by having a completely snow-white whole head and body in breeding females without any traces of blackened areas. This breeding coloration is a continuation of the condition found in *H. cyanoguttatus*, which has lost black coloration on the bottom of head. *Herichthys minckleyi* has additionally and uniquely lost also the black coloration on belly and posterior flanks. The males in breeding coloration are conversely and uniquely completely black. Outside of the breeding season easily diagnosed by the combination of pointed oral jaw teeth (otherwise only found in *H. bartoni* and the *H. labridens* group) and the blue iridescent spots on body (otherwise only found in the *H. cyanoguttatus* group and in *H. bartoni*). Distinct from other species in the *H. cyanoguttatus* group in having a plesiomorphic tooth morphology of pointed teeth that are labiolingually flattened only at their tips (vs. truncated labiolingually flattened teeth). *Herichthys minckleyi* is based on our results the only species in the *H. cyanoguttatus* group with a distinct head and body shape found in both of its two morphs differing in pharyngeal teeth. *Herichthys minckleyi* has a longer head (36% versus 33%–34%), longer snout (37% versus 30%–35%), longer predorsal length (36% versus 32%–33%), shorter dorsal fin (64% versus 69%–75%), shorter dorsal fin base (49% versus 54%–56%), shorter dorsal fin spines (51% versus 54–58%), shorter pectoral fin (24% versus 25%–27%), and more scales in the lateral line (33 versus 27–31) than species in the *H. cyanoguttatus* group. Biogeographically originally unique by being restricted to the endorheic basin of Cuatro Ciénegas, Coahuila, a disjunct part of the Rio Bravo basin.

*Herichthys cyanoguttatus* (junior synonyms: *Heros pavonaceus* Garman, 1881; *Parapetenia cyanostigma* Hernández-Rolón, 1990) is unique among *Herichthys* in its breeding coloration that combines a completely white head and ventral fins (both shared only with *H. minckleyi*) with a blackened belly and black posterior vertical bars (shared with other species of the *H. cyanoguttatus* group). Apomorphic labiolingually compressed truncated anterior jaw teeth shared with other species of the *H. cyanoguttatus* group. Distinguished from the southern parapatric *H. carpintis* by small (vs. large) opalescent spots on body and head and by breeding coloration. Our results further show that *H. cyanoguttatus* have a larger interocular distance than the other species (38% versus 33%–35%) and generally have a higher body (47% versus 42%–46%), although variation in these characters is very large. *Herichthys cyanoguttatus* is distributed between the ranges of *H. minckleyi* in the NW and *H. carpintis* to the S from the Rio Bravo and Rio Nueces in Texas to the South including the San Fernando and Soto la Marina basins.

*Herichthys carpintis* (junior synonyms: *Heros teporatus* Fowler, 1903; *Cichlosoma laurae* Regan, 1908) is unique among species of the *H. cyanoguttatus* group in having a breeding coloration where the blackened area reaches on the head much higher up to the suborbital series and includes the upper lip (i.e., almost below the eye vs. only the ventral part of the head to the level of the lower or rarely upper lip). Distinguished from the species of the *H. labridens group* and *H. bartoni* in which the blackened area on head in breeding coloration reaches even higher to the eye and includes also the nasal area of the head. *Herichthys carpintis* is distributed between the ranges of *H. cyanoguttatus* to the N and *H. tepehua* to the S and its distribution includes the whole Pánuco basin except upper reaches below 1,000 m a.s.l. (probably much lower before artificial introductions).

*Herichthys tamasopoensis* is distinguished from the most similar, most closely related and parapatric *H. carpintis* by breeding coloration with blackened areas on head limited to the ventral part of mouth excluding the lips, preoperculum all white in lateral aspect interrupting the blackened anterior and posterior area, which includes the ventral part of the suboperculum and the ventral part of the whole body combined with black posterior body bars. The breeding coloration on head is also diagnostic against *H. deppii* in having white lips and otherwise being intermediate between *H. carpintis* and *H. cyanoguttatus* plus *H. minckleyi*, which both lack the blackened ventral part of the mouth. Distinguished from its closest relative *H. carpintis* apart from the breeding coloration by having the opalescent spots on body and head much smaller and more whitish, in size more similar to the other species of the *H. cyanoguttatus* group. Based on our results, *Herichthys tamasopoensis* can also be distinguished from the rest of the species in the *H. cyanoguttatus* species group by having 13 versus 14 pectoral fin rays. Biogeographically unique being isolated from closely related species in the Gallinas river basin including the Rio Tamasopo above the Tamasopo falls by the 105 m tall Cascada Tamul, sharing its distribution with the species pair *H. pame*/*H. steindachneri* from the *H. labridens* species group.

*Herichthys tepehua* is unique among all *Herichthys* in having an aquamarine-turquoise body coloration caused by an over-expression and fusion of the *H. cyanoguttatus* group-typical opalescent dots and spots. This coloration is, however, present only in a small number of specimens referred to *H. tepehua* (see Appendix S2). Breeding coloration is intermediate between the northern and southern neighboring species *H. carpintis* and *H. deppii* in its extent of black coloration on the cheek. Some populations of *Herichthys tepehua* (not those with the diagnostic aquamarine-turquoise body coloration) share with *H. deppii* the large orange/rust-brown dots, but they are unlike in *H. deppii* also found on the posterior of body and in some populations on the head the dots form two conspicuous parallel uninterrupted lines from the lip fold to the orbit. *Herichthys tepehua* is found in between the distribution areas of *H. carpintis* to the N and *H. deppii* to the S and is found in the Tuxpan/Pantepec, Cazones, Tecolutla, Tenixtepec, and the Solteros river systems.

*Herichthys deppii* (junior synonyms: *Heros montezuma* Heckel, 1840; *Herichthys geddesi* Regan, 1905) is unique among *Herichthys* (except some populations of *H. tepehua*) in having conspicuous rather large orange/rust-brown dots on the face, cheek, and anterior

sides of body as opposed to the blue markings found in other species of the *Herichthys cyanoguttatus* species group. The iridescent blue, turquoise, or silvery spots on head and body are absent (vs. present in *H. cyanoguttatus*, *H. carpintis*, *H. minckleyi*, *H. tamasopoensis*, and *H. tepehua*). Breeding coloration with blackened areas on head limited to the ventral part of mouth including the lips (diagnostic from *H. minckleyi*, *H. cyanoguttatus*, and *H. tamasopoensis*), preoperculum all white in lateral aspect interrupting the blackened anterior and posterior area, which includes the ventral part of the subopercu-lum and the ventral part of the whole body combined with black posterior body bars. The breeding coloration on head is diagnostic against *H. tamasopoensis* in having black lips and otherwise intermediate between *H. carpintis* and *H. cyanoguttatus* plus *H. minckleyi*, which both lack the blackened ventral part of the mouth. *Herichthys deppii* is further distinguished from all other species except *H. tepehua* by having more anal fin spines (usually six, sometimes seven, rarely five). *Herichthys deppii* is the southernmost *Herichthys* species distributed in the Nautla and Misantla rivers in the state of Veracruz (see also De la Maza-Benignos et al., 2015 and Paepke, Morgenstern, & Schindler, 2014).

## 4 | DISCUSSION

### 4.1 | Performance and utility of studied molecular and morphological datasets

Among the studied molecular markers, the mtDNA complete *cytb* sequences and nDNA ddRAD markers provide well-resolved and well-supported phylogenetic trees that enable to distinguish species and their phylogenetic relationships within the genus *Herichthys*. On the other hand, the partial sequences of the mtDNA *COI* marker and the complete nDNA *S7* introns 1 and 2 markers provide much poorer discrimination of species and their phylogenetic relationships. The results of the nDNA *S7* introns are still compatible with the nDNA ddRAD analyses, while the mtDNA *COI* analysis clearly suffers from being based only on partial sequences and is among our DNA datasets the only one without the power to distinguish species in the genus *Herichthys*. The *COI* barcoding approach had proven to be useful to discriminate near to 90% of the fish species essayed worldwide (Hubert et al., 2008; Kim, Eo, Koo, Choi, & Kim, 2010; Valdez-Moreno, Ivanova, Elías-Gutiérrez, Contreras-Balderas, & Hebert, 2009); however, our study together with several previous studies (Breman, Loix, Jordaens, Snoeks, & Van Steenberge, 2016; De la Maza-Benignos et al., 2015; León-Romero, Mejía, & Soto-Galera, 2012; Mejía, León-Romero, & Soto-Galera., 2012) clearly demonstrates its limited use in *Herichthys*. Future studies of *Herichthys* will thus need to attempt barcoding using the whole *COI* marker or using the whole *cytb* gene (as successfully demonstrated in the present study) and will need to compare results with a nuclear marker with sufficient resolution and robustness, which currently is based on our results only the genomic ddRAD marker set. No so far tested single-locus nDNA marker (*S7* introns, RAG 1 and 2 exons) or their concatenation (Říčan et al., 2008, 2013, 2016) has enough resolution and robustness to study species diversity and relationships of species within *Herichthys*. The *COI*, *cytb*, and *S7* molecular markers used here are single loci and beyond the question of their resolution at the species level outlined above they additionally cannot be expected to fully represent a species tree. The mtDNA *COI* and *cytb* are additionally clonally and uniparentally (maternally) inherited. The ddRAD dataset is thus the most inclusive with respect to the amount of separate loci and the phylogenetic tree from this dataset therefore best approximates the species tree of the genus *Herichthys*. The combination of well-resolving mtDNA and nDNA markers will in future studies also be needed to fully understand the here and in the literature revealed introgressions/hybridizations between many of the *Herichthys* species.

Our results in agreement with previously published morphological analyses of *Herichthys* (De La Maza-Benignos & Lozano-Vilano, 2013; De la Maza-Benignos et al., 2015; Mejía et al., 2015) show a limited potential of morphological characters for the study of allopatric species diversity in *Herichthys*, contrary to the well-diagnosable morphologically distinct sympatric species (Taylor & Miller, 1983). Separation of allopatric species in *Herichthys* based on geometric morphometrics is rare, the exceptions being partial separation of *H. minckleyi* and *H. cyanoguttatus* from the *H. cyanoguttatus* species group (see Results and Figure 4). Separation based on meristic characters is also rare; the exceptions are again *H. minckleyi* in scale numbers, *H. tamasopoensis* in pectoral fin rays, and *H. deppii* and *H. bartoni* as the opposite extremes in anal fin spine numbers. Separation of the two main molecular clades within *Herichthys* (and hence separation into two genera sensu De la Maza-Benignos et al., 2015; see below) is also not possible using meristics, principal component analysis, geometric morphometric analyses (Figure 5), and distribution of morphological character states (see below and the morphological phylogeny in Říčan et al., 2008, 2016). Among morphological characters, the best discrimination of species in *Herichthys* is achieved through coloration patterns and especially breeding coloration patterns, which are virtually species specific (Figure 6). The other morphological characters, together with the geometric morphometric analysis, add discriminatory information, when combined with color and genetic data (see Revised Diagnoses of species in Results).

### 4.2 | Phylogenetic relationships and species diversity in *Herichthys*

*Herichthys* is made up of two lineages of morphologically and phylogenetically closely related species groups (the *H. cyanoguttatus* and *H. labridens* groups) plus two phylogenetically more basal and morphologically more ancestral species (*H. minckleyi*, *H. bartoni*) that appear to be the basal members of the respective species groups. *Herichthys* is based on our results composed of 11 species (*H. minckleyi*, *H. deppii*, *H. cyanoguttatus*, *H. tepehua*, *H. carpintis*, *H. tamasopoensis*, *H. bartoni*, *H. labridens*, *H. pame*, *H. steindachneri*, and *H. pantostictus*). Samples of *H. molango* were not available for molecular analyses, and further, preferably genomic work is needed to test

its status. Appendix S2 provides discussion of the here supported and also unsupported species including *H. molango*.

All here studied mtDNA and nDNA markers show conflicts with previously advocated *Herichthys* taxonomy. All DNA markers agree that *H. pratinus* is not a separate species from *H. pantostictus* in agreement with its lack of morphological diagnostic characters. Nuclear DNA markers suggest that *H. tepehua* has hybridized with *H. carpintis* and *H. deppii*, which is also evident in its coloration pattern variability. Much denser specimen sampling in both mtDNA and nDNA markers is needed to ascertain the status of *H. tepehua* but our so far specimen-limited ddRAD analyses suggest that it ancestrally was a distinct monophyletic species only later compromised by hybridizations.

MtDNA markers show several introgressions between sympatric or parapatric species (*H. tamasopoensis* into *H. pantostictus*, and *H. pantostictus* and *H. pame* into *H. carpintis* and *H. tamasopoensis* in *COI* and *H. tamasopoensis* into *H. carpintis* in *cytb*) that render the former species polyphyletic in these markers. Most of these introgressions are actually between (and not within) the two species groups. Whether these conflicts represent solely limited introgressions or significant hybridizations remains to be studied using a much denser specimen sampling in analyses of the well-resolving mtDNA (*cytb*) and nDNA (ddRAD) markers. The *COI* marker has a much better specimen sampling and suggests that future analyses of other markers with a more extensive specimen sampling will encounter many more incongruences than did our limited specimen sampling in most markers in the present study, which was focused on the search for the most suitable markers.

The biogeography of the *H. cyanoguttatus* group appears to be entirely vicariant from an ancestrally wide area, while in the *H. labridens* group, it is more complicated with one widespread species (*H. pantostictus*) and two localized sympatric species pairs (*H. labridens* plus *H. bartoni* and *H. steindachneri* plus *H. pame*; Figures 6 and S3), of which however only the latter is formed by sister species (Figure 3). The sympatry of *H. bartoni* and *H. labridens* is probably a relict from a wider ancestral area of the whole *H. labridens* group that was later fragmented by vicariance. This is because the two species are not sister species based on nDNA markers and their sister-group relationship in the mtDNA *cytb* marker is interpreted here as mtDNA introgression from *H. labridens* into *H. bartoni* (see also Říčan et al., 2016). The degree and possibly the context of this ancient hybridization (4 Mya based on Concheiro Pérez et al., 2007 and Říčan et al., 2013) remains to be studied by genomic markers (e.g., ddRAD) using a larger specimen sampling of the two species from localities without the presence of the introduced *H. carpintis* (see Appendix S2) whose genomic signatures would further complicate such study. The potential of hybridizations (both with *H. labridens* and *H. carpintis*) in generating the observed variability and possible polymorphism in *H. bartoni* also remains to be studied.

The sympatry of *H. steindachneri* and *H. pame* could be based on their sister-species relationship in all DNA markers interpreted as the result of sympatric speciation. The situation, however, requires further investigation with a much denser specimen sampling and in comparison with *H. minckleyi* (and *H. bartoni*) where similarly diverse trophic morphs are considered conspecific (Hulsey & García de León, 2013; Hulsey et al., 2006; Kornfield & Taylor, 1983; Kornfield et al., 1982; Magalhaes et al., 2015), while in *H. steindachneri* and *H. pame*, they are treated as separate species. This dichotomy of classification might be entirely artificial, but despite this the situation has not attracted any research nor has it even been mentioned in the literature to our knowledge.

There are two main phenotypic types within *Herichthys*: (i) the nominotypical type with spatulate teeth, semi-herbivorous diet, and blue opalescent body markings represented by all species of the *H. cyanoguttatus* group except the basal *H. minckleyi* (which only shares the blue opalescent body markings with these species and deviates in tooth morphology and diet), and (ii) the second type represented by species which also have labiolingually flattened teeth, but only on their tips which are pointed (not truncated), these species are predominantly detritivorous or molluscivorous, one species (*H. steindachneri*) is a piscivore, and these species (the monophyletic *H. labridens* group) lack the opalescent markings of the first group but have a red-magenta colored scaleless area in the axil of the pectoral fin (the last character is, however, absent from the most basal species *H. bartoni* which additionally has some opalescent markings; see Results). The basal species *H. minckleyi* and *H. bartoni* are thus intermediate between both phenotypic groups in having the ancestral tooth type, the coloration of opalescent blue markings, and in lacking the red-magenta colored scaleless area in the axil of the pectoral fin.

As the two basal species (*H. minckleyi*, *H. bartoni*) are intermediate between the two species groups, they preclude separation of *Herichthys* into two genera and they do not enable diagnosis of two genera using apomorphic characters. The suggested dichotomy of generic diagnosis (De la Maza-Benignos et al., 2015) of the *H. cyanoguttatus* group plus *H. minckleyi* by the opalescent markings and of the *H. labridens* group plus *H. bartoni* by the red-magenta colored scaleless area in the axil of the pectoral fin thus does not exist nor does a diagnosis of the latter species group as a genus based on breeding dresses without vertical bars as these are present in lacustrine populations of *H. pantostictus*. The opalescent markings are thus most likely ancestral for the whole genus *Herichthys* (and not apomorphic for the *H. cyanoguttatus* group plus *H. minckleyi*; but see another possibility below) and are also common in other Middle American cichlid genera. The only distinguishing character between the two monophyletic groups and possible genera (the *H. cyanoguttatus* group including *H. minckleyi* vs. the *H. labridens* group including *H. bartoni*) is the extent of black coloration on head. In the *H. labridens group* including *H. bartoni*, the blackened area on head in breeding coloration reaches highest, up to the eye and includes also the nasal area of the head. This character is, however, part of a gradient from the completely white head of *H. minckleyi* through partial and increasing blackening of lower head in a series of species in the *H. cyanoguttatus* group (from *H. cyanoguttatus* to *H. carpintis*) where *H. carpintis* is clearly intermediate between the situation in the *H. cyanoguttatus* group and the *H. labridens* group (see Figure 6). Additionally, none of the coloration patterns including this last one is unique for *Herichthys* among

Middle American cichlids (Říčan et al., 2016). Based on our re-examination, all original diagnostic characters of the proposed genus *Nosferatu* are thus invalid and the evidence for splitting *Herichthys* into two genera is thus very weak to nonexistent, especially when compared to the diversity in Middle American cichlids in general and the consistent criteria applied in their classification (Říčan et al., 2016). Additionally, the name *Nosferatu* is inappropriate because it is given after a plesiomorphic tooth morphology ancestral to the genus *Herichthys* and its sister-group the *Theraps-Paraneetroplus* clade (Říčan et al., 2016). Lamentably, the separation of *Herichthys* into two genera was also done despite lack of support from the only studied molecular marker (partial *COI* analysis; De la Maza-Benignos et al., 2015). Based on the above considerations, we thus reject classification of *Herichthys* into two genera and treat *Nosferatu* De la Maza-Benignos et al., 2015 as a junior synonym of *Herichthys* Baird and Girard, 1854.

## 4.3 | Key to the species of *Herichthys* (most characters only apply for adult specimens)

**1.** Anal fin with only four spines; breeding coloration of females a continuous black on lower part of head and body and continuous white on upper part of head and body; brown to black small and widely separated dots on head, cheek, and opercular series; head and mouth large, lower jaw projecting in front of upper . . . . . *H. bartoni* (Figure 6a; endemic to the lagoons of the Laguna de la Media Luna area (San Luis Potosí) at elevations between 1000 and 1100 m a.s.l.)

**1'.** Anal fin with six to seven spines; conspicuous rather large orange/rust-brown dots on the face, cheek and anterior sides of body; the blue markings found in other species of the *Herichthys cyanoguttatus* species group are absent . . . . . *H. deppii* (Figures 6h and S3a,c; the southernmost species found endemic to the Nautla and Misantla rivers in the state of Veracruz)

**1".** Anal fin with modally five spines . . . . . 2

**2.** Head large, long and pointed, length usually greater (always above 90%) than body depth over pelvic base; mouth very large, lower jaw projecting in front of upper; body long and narrow . . . . . *H. steindachneri* (Figure S3f; endemic to the Rio Gallinas, a waterfall-isolated tributary to the Rio Pánuco)

**2'.** Head shorter, length usually much less than body depth over pelvic base; jaws equal or upper projecting slightly; mouth small . . . . . 3

**3.** Non-breeding specimens with blue cheeks and lips but body without blue markings; breeding coloration yellow-black instead of white-black; pharyngeal teeth along midline of lower pharyngeal jaw (LPJ) strongly molariform in all specimens . . . . . *H. labridens* (Figures 6b and S3b,d; endemic to the lagoons of the Laguna de la Media Luna area (San Luis Potosí) at elevations between 1000 and 1100 m a.s.l.)

**3'.** Non-breeding specimens with blue opalescent spots on body; breeding specimens with black coloration on head limited to below a line from mouth angle . . . . . 4

**3".** Non-breeding specimens without blue opalescent spots on body; breeding specimens with black coloration on head reaching high-up and touching lower margin of eye . . . . . 7

**4.** Oral jaw teeth pointed; breeding females entirely white, breeding males entirely black; relatively long head (36% in SL versus 33%–34%), snout (37% versus 30%–35%), predorsal distance (36% versus 32%–33%); relatively short dorsal fin (64% versus 69%–75%), dorsal fin base (49% versus 54%–56%), dorsal fin spines (51% versus 54–58%), shorter pectoral fin (24% versus 25%–27%); more scales in the lateral line (33 versus 27–31); polymorphic in head shape, LPJ robustness, and dentition. . . . . *H. minckleyi* (Figure 6j; endemic to the endorheic desert basin of Cuatro Ciénegas, Coahuila, a disjunct part of the Rio Bravo basin, together with *H. cyanoguttatus* the northernmost species)

**4'.** Oral jaw teeth truncated, incisor-like; breeding females with varying amount of black on lower half of head and body; relatively short head (33%–34% in SL versus 36%), snout (30%–35% versus 37%), predorsal distance (32%–33% versus 36%); relatively long dorsal fin (69%–75% versus 64%), dorsal fin base (54%–56% versus 49%), dorsal fin spines (54–58% versus 51%), longer pectoral fin (25%–27% versus 24%); less scales in the lateral line (27–31 versus 33) . . . . . 5

**5.** Pectoral fin rays 13; opalescent spots on body and head very small and whitish; black breeding coloration on head very limited, preoperculum all white . . . . . *H. tamasopoensis* (Figure 6g; endemic to the Rio Gallinas, a waterfall-isolated tributary to the Rio Pánuco)

**5'.** Pectoral fin rays 14 . . . . . 6

**6.** Opalescent spots on body and head small; black breeding coloration on head very limited, preoperculum, and whole area bellow mouth white . . . . . *H. cyanoguttatus* (Figure 6i; the northernmost reaching species, distributed between the ranges of H. *minckleyi* in the NW and *H. carpintis* to the S from the Rio Bravo and Rio Nueces in Texas to the South including the San Fernando and Soto la Marina basins)

**6'.** Opalescent spots on body and head large; black breeding coloration on head most developed among the *H. cyanoguttatus* group reaching up to the mouth angle with the whole lower part of head black . . . . . *H. carpintis* (Figure 6e; distributed between *H. cyanoguttatus* to the N and *H. tepehua* to the S in the whole Pánuco basin except its upper reaches (where artificially introduced)

**6".** Opalescent spots so enlarged that they fuse into a unique aquamarine-turquoise body coloration; some specimens with orange/rust-brown dots on the face and cheek (otherwise only present in H. deppii, where much better developed and larger); amount of black coloration on head intermediate between *H. carpintis* and *H. cyanoguttatus*. . . . . *H. tepehua* (Figure 6f; distributed between the *H. carpintis* to the N and *H. deppii* to the S in the Tuxpan/Pantepec, Cazones, Tecolutla, Tenixtepec, and the Solteros river systems)

**7.** Red markings in unpaired fins and red dots on body; red (versus purple/magenta) spot in the axil of the pectoral fin; breeding

coloration with a yellowish-white background; variable development of molariform LPJ teeth; dimorphic species with distinct lacustrine and riverine forms in terms of coloration and body shape . . . . . *H. pantostictus* (Figure 6d, lacustrine form; the only widespread species in the *H. labridens* species group being endemic to virtually the whole Tamesí-Pánuco river basin including Rio El Salto but except the high-elevation areas of Rio Verde, Rio Gallinas, and the headwaters of the Rio Moctezuma)

**7'.** No red markings in unpaired fins and on body; a purple/magenta (versus red) spot in the axil of the pectoral fin; breeding coloration with a pure white background; invariably well developed molariform LPJ teeth . . . . . *H. pame* (Figure 6c; endemic to the Rio Gallinas, a waterfall-isolated tributary to the Rio Pánuco)

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# APPENDIX

## EXAMINED MATERIAL IN THIS STUDY (FOR THE *H. LABRIDENS* GROUP MATERIAL SEE MEJÍA ET AL., 2015)

### *Herichthys minckleyi* (Kornfield & Taylor, 1983)

MÉXICO: ENCB-P P1760 (*n* = 3) [Entre el Mojarral y Cuatro Ciénegas, Coah.]; P2109 (*n* = 18) [Poza de las Becerras, Coah.]; P2607 (*n* = 10) [Poza de las Becerras, Coah.]; P2798 (*n* = 18) [Cuatro Ciénegas, Coah.]; P3693 (*n* = 6) [Escobedo a 1 km de Cuatro Ciénegas, Coah.]; P3694 (*n* = 10) [Poza de las Becerras, Coah.]; P4192 (*n* = 3) [Laguna el Mojarras, Coah.].

### *Herichthys deppii* (Heckel, 1840)

MÉXICO: ENCB-P P6369 (*n* = 27) [Rancho el Jobo, Ver.]; P6370 (*n* = 9) [Santa Clara, Misantla, Ver.].

### *Herichthys tamasopoensis* Artigas-Azas, 1993

MÉXICO: ENCB-P P6010 (*n* = 3) [Balñeario Cascadas de Tamasopo, SLP]; P6114 (*n* = 13) [Puente Tamasopo, SLP]; P6443 (*n* = 9) [Cascadas de Tamasopo, SLP].

### *Herichthys cyanoguttatus* Baird and Girard, 1854

MÉXICO: ENCB-P P1573 (*n* = 3) [Río Purificación, Tamps.]; P1824 (*n* = 4) [Río Ramos, NL]; P1826 (*n* = 3) [Río Santa Engracia, Tamps.]; P2300 (*n* = 12) [Presa Marte, Tamps.]; P2365 (*n* = 4) [Rancho la Reforma, Tamps.]; P2547 (*n* = 10) [Río Ramos, NL]; P2606 (*n* = 6) [Arroyo los Mimbre, Coah.]; P2610 (*n* = 2) [Arroyo Dr. Chapa, Coah.]; P2611 (*n* = 1) [Presa Don Martín, Coah.]; P2804 (*n* = 5) [Canal de Anzalduas, Tamps.]; P2817 (*n* = 2) [Laguna de la Puerta, Tamps.]; P2930 (*n* = 1) [Río Bravo, Tamps.]; P3225 (*n* = 2) [Arroyo Las Sabinas, NL]; P3433 (*n* = 4) [Sabinas Hidalgo, NL]; P3453 (*n* = 3) [Ciénaga de Flores, NL]. CNPE-IBUNAM P4749 (*n* = 1) [Río Conchos, NL]; 12578 (*n* = 11) [Río San Fernando, Tamps.]; 12604 (*n* = 2) [Río San Fernando, Tamps.]; P2811 (*n* = 4) [Río Purificación, Tamps.]; UANL 6677 (*n* = 2) [San Fernando, Tamps.].

### *Herichthys carpintis* (Jordan & Snyder, 1899)

MÉXICO: ENCB-P P870 (*n* = 15) [Lago Tequesquitengo, Mor.]; P1305 (*n* = 1) [Laguna del Chairel, Tamps.]; P1833 (*n* = 4) [Laguna del Chairel, Tamps.]; P1980 (*n* = 2) [Villa González, Tamps.]; P2040 (*n* = 3) [Laguna del Chairel, Tamps.]; P2173 (*n* = 5) [Presa los Patos, Tamps.]; P2541 (*n* = 7) [Río Guayalejo, Tamps.]; P4778 (*n* = 1) [Río San Pedro, Hgo.]; P4785 (*n* = 1) [Afluente Río Atlapexco, Hgo.]; P4879 (*n* = 12) [Puente Plazuela, SLP]; P4951 (*n* = 4) [Río Atlapexco, Hgo.]; P4958 (*n* = 8) [Río San Pedro, Hgo.]; P4971 (*n* = 3) [Río San Pedro, Hgo.]; P4979 (*n* = 3) [Platón Sánchez, Ver.]; P4980 (*n* = 2) [Huejutla de Reyes, Hgo.]; P4982 (*n* = 11) [Arroyo en Vinazco, Hgo.]; P4989 (*n* = 2) [Río Coy en Aquismón, SLP]; P5050 (*n* = 1) [Embalse Zimapan, Qro.]; P5053 (*n* = 9) [Embalse Zimapan, Hgo.]; P5067 (*n* = 6) [Río San Juan, Hgo.]; P5153 (*n* = 12) [Río San Juan, Hgo.]; P5904 (*n* = 3) [Río Moctezuma, Hgo.]; P5906 (*n* = 1) [Río Moctezuma, Hgo.]; P5918 (*n* = 1) [Río Claro, Hgo.]; P5919 (*n* = 4) [Río Claro, SLP]; P5920 (*n* = 3) [Río Claro, Hgo.]; P5922 (*n* = 12) [Río San Pedro, Hgo.]; P5955 (*n* = 3) [Puente Xilitla, SLP]: P5956 (*n* = 1) [Aquismón, SLP]; P5960 (*n* = 2) [Río Valles, SLP]; P5966 (*n* = 2) [La Ceiba, SLP]; P5986 (*n* = 9) [Ocampo, Tamps.]; P5988 (*n* = 7) [El Mante, Tamps.]; P5997 (*n* = 5) [Río Sabinas, Tamps.];

P5999 (*n* = 7) [Llera de Canales, Tamps.]; P6003 (*n* = 4) [Entre Jaumave y San Vicente, Tamps.]; P6005 (*n* = 2) [Nuevo Morelos, Tamps.]; P6011 (*n* = 3) [Canal perpendicular a la Media Luna, SLP]; P6012 (*n* = 9) [Canal perpendicular a la Media Luna, SLP]; P6013 (*n* = 1) [Los Anteojitos, SLP]; P6015 (*n* = 3) [Río Verde, SLP]; P6099 (*n* = 1) [Camino a la Estación, Ver.]; P6104 (*n* = 5) [Río Tempoal, Ver.]; P6106 (*n* = 1) [Estación Mascareña, Ver.]; P6108 (*n* = 1) [Los Anteojitos, SLP]; P6109 (*n* = 12) [Orizatlán, Hgo.]; P6116 (*n* = 3) [Puente Xilitla, SLP]; P6117 (*n* = 2) [Río Axila, SLP]; P6120 (*n* = 3) [Río Valles, SLP]; P6121 (*n* = 1) [Puente Covadonga, SLP]; P6364 (*n* = 4) [Pedernales, Ver.]; P6365 (*n* = 3) [Puente Terrero, Ver.]; P6366 (*n* = 1) [Camino al Colorado, Ver.]; P6367 (*n* = 11) [Puente Tanconchin, Ver.]; P6373 (*n* = 1) [Canal perpendicular a la Media Luna, SLP]; P6438 (*n* = 20) [Naranjos de Amatlán, Ver.]; P6440 (*n* = 14) [Ozuluama, Ver.]; P6442 (*n* = 1) [Río Micos, SLP]. CNPE-IBUNAM 13147 (*n* = 3) [Río Guayalejo, Tamps.].

### *Herichthys tepehua* De la Maza-Benignos, Ornelas-García, Lozano-Vilano, García-Ramírez and Doadrio, 2015

MÉXICO: ENCB-P P2020 (*n* = 5) [Río Cazones, Ver.]; P6363 (*n* = 13) [Piedras Negras, Pue.]; P6368 (*n* = 8) [Poblado Arroyo de Cañas, Ver.]; P6378 (*n* = 9) [Puente Buenos Aires, Pue.].

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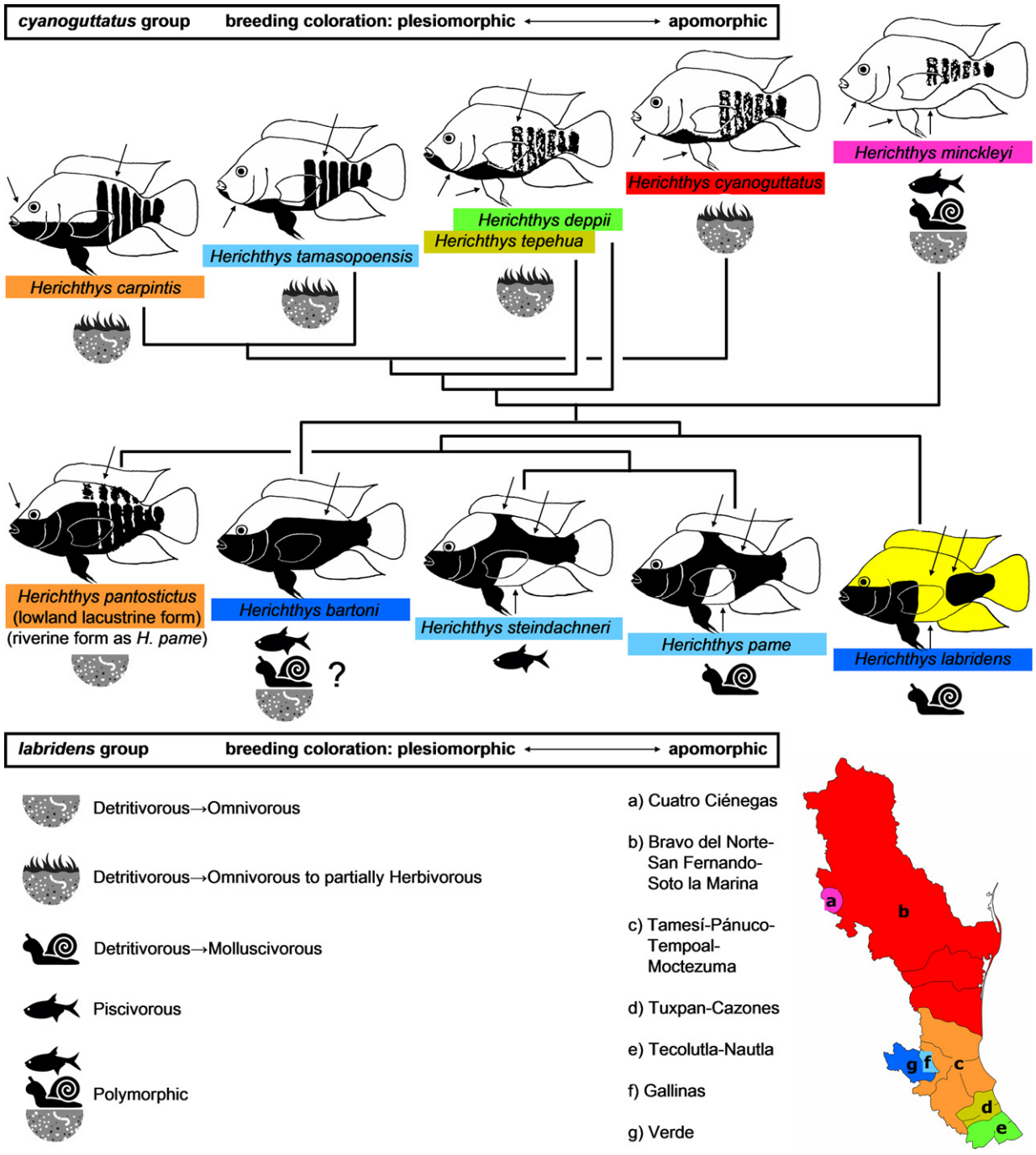


A review of *Herichthys* species diversity is provided based on phylogenetic analyses of several mitochondrial and nuclear genetic markers together with analyses of morphological data. *Herichthys* is composed of 11 species and includes two main clades. *Herichthys* species are re-diagnosed based on well-diagnostic characters that include traditional morphological characters together with breeding coloration patterns, trophic ecomorphology, and biogeography.