

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

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Electronic Supporting information

Appendix S1.

List of specimens collapsed into unique haplotypes in analysis of the *COI* data (Fig. 2).

Each haplotype is shown by blue color, followed by all specimens with the same haplotype. Specimens shown in red color share haplotype with a different species.

IBOH016Herichthys_minckleyi_IBIEN018Herichthys_minckleyi

HBM063Herichthys_tamasopoensis_HBM064Herichthys_tamasopoensis

HBM187Herichthys_pame_HBM188Herichthys_pame_HBM189Herichthys_pame

HBM224Herichthys_pantostictus_HBM263Herichthys_pantostictus_HBM223Herichthys_pantostictus_HBM264Herichthys_pantostictus

FFPR303Herichthys_pantostictus_FFPR078Herichthys_pantostictus_FFPR300Herichthys_pantostictus

HBM073Herichthys_pantostictus_HBM090Herichthys_pantostictus

HBM086Herichthys_pantostictus_HBM087Herichthys_pantostictus_HBM083Herichthys_pantostictus_HBM071Herichthys_pantostictus_HBM072Herichthys_pantostictus_HBM081Herichthys_pantostictus_HBM082Herichthys_pantostictus_HBM085Herichthys_pantostictus

HBM043Herichthys_pantostictus_HBM016Herichthys_pantostictus

HBM007Herichthys_pantostictus_FFPR298Herichthys_pantostictus

HBM092Herichthys_pantostictus_FFPR296Herichthys_pantostictus_FFPR055Herichthys_labridens_FFPR056Herichthys_labridens_HBM078Herichthys_pantostictus_HBM077Herichthys_pantostictus_HBM059Herichthys_pantostictus_HBM013Herichthys_pantostictus_FFPR057Herichthys_labridens_FFPR302Herichthys_pantostictus_HBM048Herichthys_pantostictus_HBM046Herichthys_pantostictus_HBM047Herichthys_pantostictus_HBM022Herichthys_pantostictus_FFPR061Herichthys_pantostictus_HBM015Herichthys_pantostictus_FFPR062Herichthys_pantostictus

HBM067Herichthys_steindachneri_HBM024Herichthys_steindachneri_HBM068Herichthys_steindachneri_HBM027Herichthys_steindachneri

FFPR063Herichthys_pantostictus_FFPR297Herichthys_pantostictus

HBM056Herichthys_labridens_HBM058Herichthys_labridens

HBM175Herichthys_carpintis_HBM150Herichthys_carpintis_HBM156Herichthys_carpintis_HBM144Herichthys_carpintis_HBM173Herichthys_carpintis_HBM174Herichthys

s_carpintis_HBM162Herichthys_carpintis_HBM149Herichthys_carpintis_HBM168Herichthys_carpintis_HBM164Herichthys_carpintis_HBM145Herichthys_carpintis_HBM163Herichthys_carpintis_HBM177Herichthys_carpintis_HBM161Herichthys_carpintis_HBM180Herichthys_carpintis_HBM160Herichthys_carpintis_HBM159Herichthys_carpintis_HBM151Herichthys_carpintis_HBM157Herichthys_carpintis_HBM165Herichthys_carpintis_HBM155Herichthys_carpintis_HBM153Herichthys_carpintis_HBM154Herichthys_carpintis_HBM171Herichthys_carpintis

HBM178Herichthys_carpintis_HBM172Herichthys_carpintis_HBM182Herichthys_carpintis_HBM181Herichthys_carpintis_HBM170Herichthys_carpintis_HBM169Herichthys_carpintis

HBM119Herichthys_deppii_HBM112Herichthys_deppii_HBM118Herichthys_deppii_HBM115Herichthys_deppii_HBM116Herichthys_deppii_HBM138Herichthys_deppii_HBM117Herichthys_deppii_HBM131Herichthys_deppii_HBM142Herichthys_deppii_HBM135Herichthys_deppii_HBM139Herichthys_deppii_HBM137Herichthys_deppii_HBM132Herichthys_deppii_HBM120Herichthys_deppii_HBM133Herichthys_deppii_HBM134Herichthys_deppii_HBM114Herichthys_deppii

HBM141Herichthys_deppii_HBM143Herichthys_deppii

HBM125Herichthys_tepehua_HBM126Herichthys_tepehua_HBM123Herichthys_tepehua_HBM128Herichthys_tepehua_HBM130Herichthys_tepehua_HBM129Herichthys_tepehua_HBM124Herichthys_tepehua

HBM109Herichthys_tepehua_HBM111Herichthys_tepehua

HBM098Herichthys_tepehua_HBM096Herichthys_tepehua_HBM103Herichthys_tepehua_HBM102Herichthys_tepehua_HBM099Herichthys_tepehua

HBM185Herichthys_tamasopoensis_HBM186Herichthys_tamasopoensis

HBM176Herichthys_carpintis_HBM184Herichthys_carpintis

HBM147Herichthys_carpintis_HBM158Herichthys_carpintis_HBM167Herichthys_carpintis_HBM148Herichthys_carpintis

HBM146Herichthys_carpintis_HBM152Herichthys_carpintis

HBM121Herichthys_deppii_HBM113Herichthys_deppii_HBM140Herichthys_deppii_HBM136Herichthys_deppii

HBM195Herichthys_carpintis_HBM202Herichthys_carpintis_HBM197Herichthys_carpintis_HBM221Herichthys_carpintis_HBM219Herichthys_carpintis_HBM217Herichthys_carpintis_HBM216Herichthys_carpintis_HBM214Herichthys_carpintis_HBM213Herichthys_carpintis_HBM212Herichthys_carpintis_HBM191Herichthys_carpintis_HBM19

2Herichthys_carpintis_HBM203Herichthys_carpintis_HBM204Herichthys_carpintis_HBM205Herichthys_carpintis_HBM206Herichthys_carpintis_HBM207Herichthys_carpintis_HBM208Herichthys_carpintis_HBM209Herichthys_carpintis_HBM211Herichthys_carpintis_HBM210Herichthys_carpintis

HBM201Herichthys_carpintis_HBM196Herichthys_carpintis_HBM198Herichthys_carpintis_HBM199Herichthys_carpintis_HBM194Herichthys_carpintis_HBM193Herichthys_carpintis_HBM190Herichthys_carpintis

HBM215Herichthys_carpintis_HBM200Herichthys_carpintis

HBM230Herichthys_tamasopoensis_HBM228Herichthys_tamasopoensis_HBM231Herichthys_tamasopoensis_HBM229Herichthys_tamasopoensis_HBM232Herichthys_tamasopoensis_HBM233Herichthys_tamasopoensis_HBM227Herichthys_tamasopoensis_HBM234Herichthys_tamasopoensis_HBM226Herichthys_tamasopoensis

HBM260Herichthys_pame_HBM257Herichthys_pame

HBM252Herichthys_pame_HBM256Herichthys_pame_HBM258Herichthys_pame_HBM254Herichthys_pame_HBM259Herichthys_pame_HBM255Herichthys_pame_HBM261Herichthys_pame

HBM100Herichthys_tepesua_HBM101Herichthys_tepesua

FFPR011Herichthys_carpintis_FFPR028Herichthys_carpintis

FFPR029Herichthys_carpintis_FFPR003Herichthys_carpintis

FFPR033Herichthys_carpintis_FFPR039Herichthys_carpintis

Appendix S2.

Discussion of species-level taxonomy of *Herichthys*.

The *Herichthys cyanoguttatus* group

Within the *H. cyanoguttatus* group there has historically been some confusion in the literature regarding *Herichthys carpintis* and *H. cyanoguttatus*. In the original description of *H. carpintis* by Jordan and Snyder (1899) is a mention that some specimens not included in the type series were collected near Rioverde, San Luis Potosí, far away from the type locality of “Laguna del carpintero” in Tamaulipas, meanwhile, other specimens collected in the lagoons near to Tampico were identified as *H. cyanoguttatus* (Jordan and Snyder 1899). There is also a historical controversy regarding the validity of *H. cyanoguttatus* and *H. carpintis* as distinct species. For example, for LaBounty (1974) *H. carpintis* replace *H. cyanoguttatus* in the coastal lagoons of the Atlantic basin. For Miller et al. (2005) the main difference between both species relies on body height and body shape, although as has been reported earlier in the results of this study both species showed similar shapes. On the other hand, for Regan (1905) and Miller (1976) *H. carpintis* is a synonym of *H. cyanoguttatus*. Finally, in the recent redescription of the species included in the *H. cyanoguttatus* species group, De La Maza-Benignos et al. (2015) distinguish *H. carpintis*, *H. cyanoguttatus* and even *H. teporatus* as separate taxa. Their study however does not provide any evidence that they are distinct taxa. They claim that male *H. carpintis* are distinct in developing a nuchal hump (gibe) that makes them different from the males of *H. cyanoguttatus* but not from the males of *H. teporatus*. In our examination all *Herichthys* can develop a nuchal hump and this character is not diagnostic among Middle American cichlids (see Řičan et al. 2016). The morphometric character differences reported for these two (three) species by De La Maza-Benignos et al. (2015) have not been corroborated in the present study and the main distinguishing characters between *H. carpintis* and *H. cyanoguttatus* are coloration patterns (see Results and Fig. 6) as has been the case throughout most of the history of classification of these two species. Genetically (both in mtDNA and in nDNA) and morphologically (mainly in coloration patterns) *H. carpintis* and *H. cyanoguttatus* are distinct species as shown in the present study except in the too short fragment of the mtDNA *COI* marker (which lacks resolution at the species level in all species of *Herichthys*). *Herichthys carpintis* and *H. cyanoguttatus* show signs of hybridization in the studied DNA markers. *Herichthys cyanoguttatus* has also recently been found in secondary contact within with the originally parapatric *H. minckleyi* due to possible introduction into its originally endemic area and the two species are now known to have hybridized (Hulse and García de León 2013). *Herichthys carpintis* has on the other hand been introduced into the high-elevation areas of Rio Verde including the Laguna Media Luna area (Taylor and Miller 1983) where it hybridizes with the native endemic *H. labridens* (Artigas Azas 1996). These hybrid specimens are very obvious and easily recognized and are very common in the Laguna Media Luna area (pers. obs., Artigas Azas 1996; Artigas Azas pers. com.) and now only isolated small lagoons (e.g. the here studied El Aguaje M91, Charco Azul M93; Table 1) hold non-hybridized *H. labridens* populations. Hybridization with *H. bartoni* has so far not been mentioned in the literature but if present the hybrids are less obvious than in *H. labridens*. The so far unstudied polymorphism observed in *H. bartoni* (pers. obs.; Artigas Azas pers. com.) plus the presence of opalescent spots in its coloration however

call for future studies of possible hybridization with *H. carpintis* as hybridization has been one of the possibilities of explaining the polymorphisms of the much better studied *H. minckleyi* (Hulsey and García de León 2013; Magalhaes et al. 2015).

Herichthys teporatus (Fowler 1903) is here treated as a junior synonym of *Herichthys carpintis* (Jordan and Snyder 1899) due to complete lack of any diagnostic characters from this species. Fowler (1903) regarded *H. teporatus* as closely related with *H. cyanoguttatus* with the only difference in coloration pattern (shared with *H. carpintis*). Burgess (2000) considered *H. teporatus* as a synonym of *H. carpintis* and Kullander (2003; without any analysis or justification) as a synonym of *H. cyanoguttatus*. De la Maza-Benignos et al. (2015) treated *H. teporatus* as a valid species but all diagnostic characters (development of nuchal humps, size of iridescent spots, proportional measurements) are within the variation of either *H. cyanoguttatus* or *H. carpintis*. Molecular support for distinctiveness is lacking in both De la Maza-Benignos et al. (2015) and our *COI* phylogeny. *Herichthys teporatus* haplotypes are non-monophyletic and interspersed between haplotypes of *H. carpintis*, *H. tepehua* and *H. tamasopoensis*. Miller et al. (2005) mentioned that *H. cyanoguttatus* and *H. carpintis* possibly hybridize within sections of the Soto la Marina River Basin (the range of *H. teporatus*) and we have indeed found some possibly hybrid specimens between the two species in our molecular analyses (Figure 3) and in the examined material from the geographic range of the nominal *H. teporatus*. NGS ddRAD studies in preparation should demonstrate the evolutionary status of the Soto La Marina *Herichthys*.

Herichthys tepehua also appears to be a complicated species. The multivariate morphometric analyses of De la Maza-Benignos et al. (2015) and in the present study do not support *H. tepehua* as a distinct species from *H. carpintis* and *H. deppii* nor does their and our here presented mtDNA *COI* analysis based on partial sequences. *Herichthys tepehua* does have unique coloration pattern characters but these are present in only some populations (see Results). The problem with *H. tepehua* is that De la Maza-Benignos et al. (2015) did not acknowledge that they did not discover the species and that they do not cite the first publications discovering it (i.e. Heijns 1991a, b). Heijns already in the first publications recognized the presence of two forms in the intervening distribution area between *H. carpintis* and *H. deppii*. The two forms were called '*Cichlasoma*' sp. "Pantepec" and '*Cichlasoma*' sp. "Poza Rica" (Heijns 1991a, b). The two forms differ in coloration patterns, the form from the Cazonas, Solteros, and Tecolutla basins differs from the Pantepec and Tenixtepec in having a unique aquamarine ground colour (vs. an undiagnostic brownish in the others). This difference in coloration could however be to some extent due to chemical water composition and transparency as in *H. pame* and *H. pantostictus* (see below). Our nDNA ddRAD analyses based on fixed SNPs support the monophyly of *H. tepehua* as does the mtDNA *cytb* gene, but other nDNA markers and analyses (*S7* introns, ddRAD analyses based on variable SNPs) find *H. tepehua* paraphyletic as two clades placed between *H. deppii* and *H. carpintis*. The ancestrally genetically distinct species *H. tepehua* thus appears to have been compromised by hybridizations with *H. carpintis* and *H. deppii* which possibly also eroded its morphological distinctiveness. Some of the specimens do indeed show strong similarity to *H. carpintis*, others rather to *H. deppii* (and both may be hybrids with these species as suggested by the DNA data) and only the aquamarine-turquoise specimens are truly unique. Since De la Maza-Benignos et al. (2015) do not acknowledge any previous

knowledge about what they refer to as *H. tepehua* connecting their information with what was published before is not possible. Their holotype is figured only in preserved state and live colours are thus not visible but the holotype is from the Rio Pantepec and is thus likely not of the diagnostic aquamarine-turquoise form. While the taxon currently known as *Herichthys tepehua* is represented by a unique phylogenetic lineage (compromised secondarily by hybridization) and represents a good species diagnosable by live coloration patterns much further study is needed since the holotype and part of the type series may actually be hybrids with *H. carpintis* and/or *H. deppii* and the description may even be invalidated in future studies because only the distinct aquamarine-turquoise specimens should be treated as a separate species, which has however become extremely rare and is seldom captured in the Czones, Tecolutla, and Solteros Rivers.

The *Herichthys labridens* species group

Both *H. bartoni* and *H. labridens* are virtually only known from lacustrine habitats in the upper Rio Verde Basin but Artigas Azas (1996) mentions a riverine population of *H. labridens* from the Rio Santa María up to 1000 meters above sea level. Mejía et al. (2015) also lists *H. labridens* from the upper Rio Santa Maria (río Jalpan and río Conca, Querétaro), and additionally from the río Calabazas (San Luis Potosí) and río Manzanares (Guanajuato). Our reexamination of some of these specimens reveals a complex situation since they cannot clearly be attributed neither to *H. labridens* nor *H. pantostictus* and they require further study. Some of the specimens have the *H. pantostictus* diagnostic black or brown dots on the head and anterior body, others have a yellow ground coloration and bluish head and cheeks with vermiculated patterns as in *H. labridens*, which is again a situation suggestive of hybridizations.

De la Maza-Beningos and Lozano-Vilano (2013) have designated a lectotype for *H. bartoni* but this designation is in our re-examination invalid because the original description and drawing of one of the type specimens (Bean 1892) is clearly sufficient for the recognition and diagnosis of the species which have been further extended in the present study. The only specimen of *H. bartoni* shown in De la Maza-Beningos and Lozano-Vilano (2013) is not a specimen agreeing with the original diagnosis and with our rediagnosis of *H. bartoni* but is intermediate between *H. bartoni* and *H. labridens*.

Herichthys pantostictus features a type of allopatric dimorphism in coloration and especially body shape (see Results) between lowland lacustrine and highland riverine populations (the latter considered to be *H. labridens* by Taylor and Miller 1983) and the same type of dimorphism is in a less distinct form mirrored in the sympatric *H. carpintis* (see Artigas Azas 2013). This type of dimorphism clearly has to do with the different habitats found in the lowlands (type areas of both *H. pantostictus* and *H. carpintis*) and in the highland and mountain streams. These two species have the largest elevational gradients between their populations within the genus.

Herichthys pratinus De la Maza-Beningos and Lozano-Vilano 2013 was described as a separate species but there are no diagnostic characters distinguishing it from riverine populations of *H. pantostictus*. The multivariate morphometric analysis of De la Maza-Beningos and Lozano-Vilano (2013) do not distinguish *H. pratinus* as a distinct species from *H. pantostictus* nor does the mtDNA *COI* analysis of De la Maza-Beningos et al. (2015) and nor do any of our molecular analyses. The studies of De la Maza-Beningos and Lozano-Vilano (2013) and De la Maza-Beningos et al. (2015) thus did not provide

any basis on which to describe *H. pratinus* as a new species. *Herichthys pratinus* was first recognized as potentially a different species from the then described species *H. labridens* and *H. pantostictus* not by De la Maza-Benignos and Lozano-Vilano (2013) but by Artigas Azas and Wessel in 2000 (Wessel 2001) and was called *Herichthys* sp. “green labridens” (because of its greenish and pale background coloration). Later these authors however considered *Herichthys* sp. “green labridens” as conspecific with other riverine populations of *H. pantostictus* (referred to as “blue labridens”) which were previously considered to be *H. labridens* by Taylor and Miller (1983). Artigas Azas (O.Ř. pers. comm., November 2008) considers both the “green labridens” (= *H. pratinus*) and “blue labridens” (= riverine *H. pantostictus*) as riverine populations of *H. pantostictus*, a view confirmed by all DNA markers in the present study. *Herichthys* sp. “green labridens” (= *H. pratinus*) was believed to be isolated from other *Herichthys* species in the labridens group by the 90 m high Cascadas de Micos, but our DNA results demonstrate that these waterfalls must be young (or were recently circumvented by drainage basin evolution) since there is no monophyly of mtDNA haplotypes and no species isolation above the falls and *H. pratinus* is thus conspecific with *H. pantostictus* and its junior synonym. The pale greenish base coloration of *H. pratinus* is the result of the very milky and highly alkaline karstic waters of the Rio El Salto above the Cascade de Micos (see photos in Supporting information Figure S4 and S5).

Herichthys molango De la Maza-Benignos and Lozano-Vilano, 2013 from Laguna Atezca in the headwaters of the Rio Moctezuma Basin, Hidalgo (1280 m a.s.l.) has not been available to us for study of DNA markers but its morphological traits and especially coloration patterns which while distinctive seem to be a combination of traits found in both main clades of *Herichthys*. Breeding coloration of *H. molango* is the same as in riverine populations of *H. pantostictus*. The live coloration of *H. molango* is distinctive with a olive background and scales on flanks fringed by diamond-shaped dark-purple outlines (scale-pockets), thicker over postero-ventral half, giving the flanks a reticulate appearance. All specimens have a red marking at the naked axil behind pectoral fin as is typical for the *H. labridens* species group. Dorsal, anal and caudal fins are olive green with green and purple intermingled blotches. This combination of traits from both main clades of *Herichthys* strangely coincides with the findings of De la Maza-Benignos et al. (2015) where *H. molango* had mitochondrial DNA affinity to the *Herichthys cyanoguttatus* species group. These sequences of *H. molango* have however not been deposited in any available database nor have been any other sequences from De la Maza-Benignos et al. (2015) which precludes us from reanalysing their data. The indications are however pointing in the direction that *H. molango* may in future studies well prove to be a hybrid population between the two *Herichthys* species groups, most likely between the parapatric *H. pantostictus* and *H. carpintis* (or *H. tepehua*). De la Maza-Benignos et al. (2015, Supplementary Material 3) actually show a possible hybrid specimen between *Herichthys pantostictus* and *Herichthys carpintis* and this specimen is indistinguishable in coloration and coloration patterns from *H. molango*. The indications are very strong that *H. molango* is not a species but a hybrid population(s). We have studied for the mtDNA *COI* marker and for morphological characters (Mejía et al. 2015) populations from the immediate vicinity of the laguna Atezca and these are in all our results riverine populations of *H. pantostictus* (the specimens were in Mejía et al. 2015 confusingly referred to as *H. molango*, but since they are not directly from the type lagoon and since

they agree in diagnostic characters with *H. pantostictus* we refer to them here as the latter species).

Additional references

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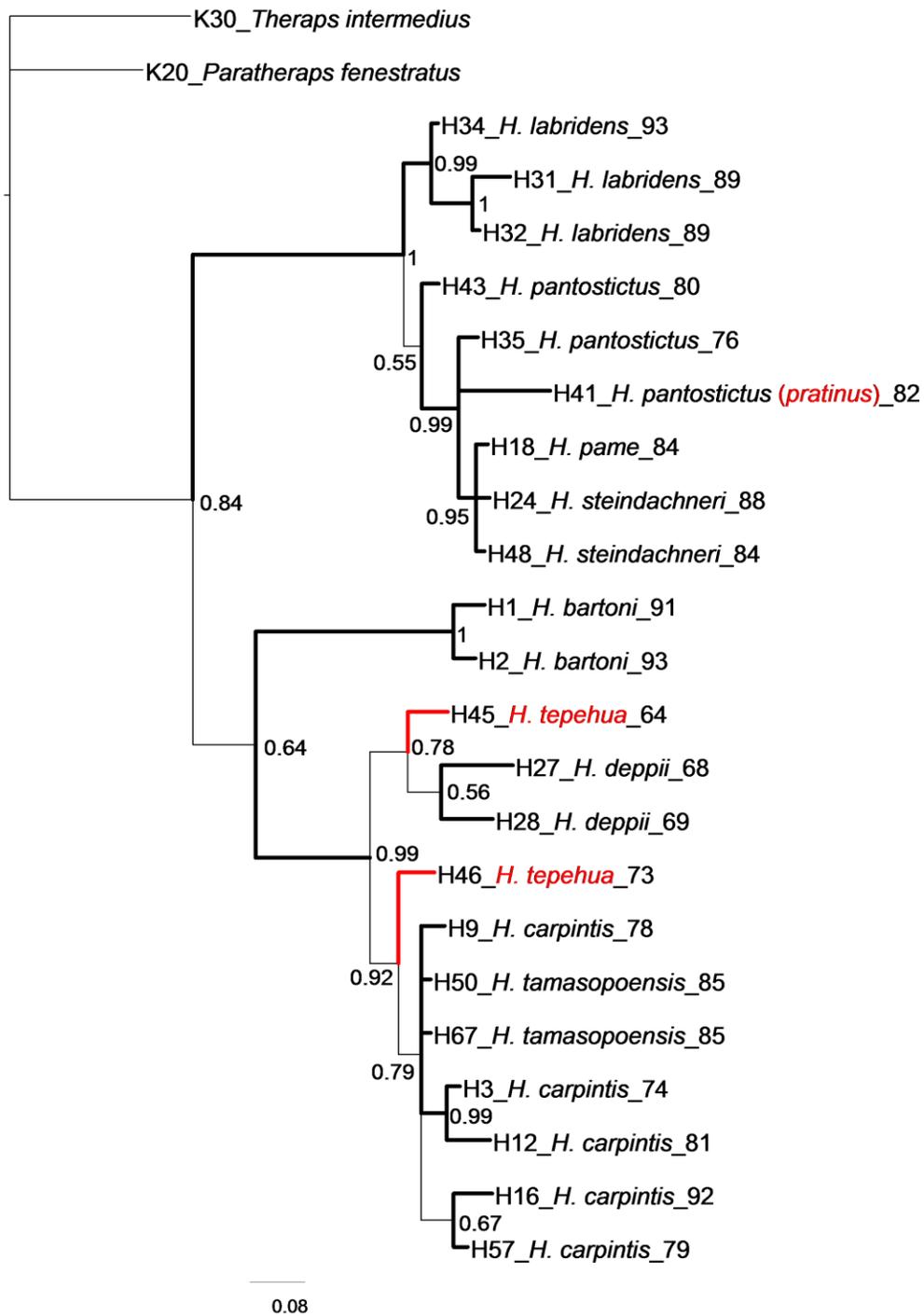


Figure S1. S7 intron 1 and 2 phylogeny. *Herichthys pratinus* is not recovered as a separate species from *H. pantostictus*, nor is *H. tepehua* supported as a species.

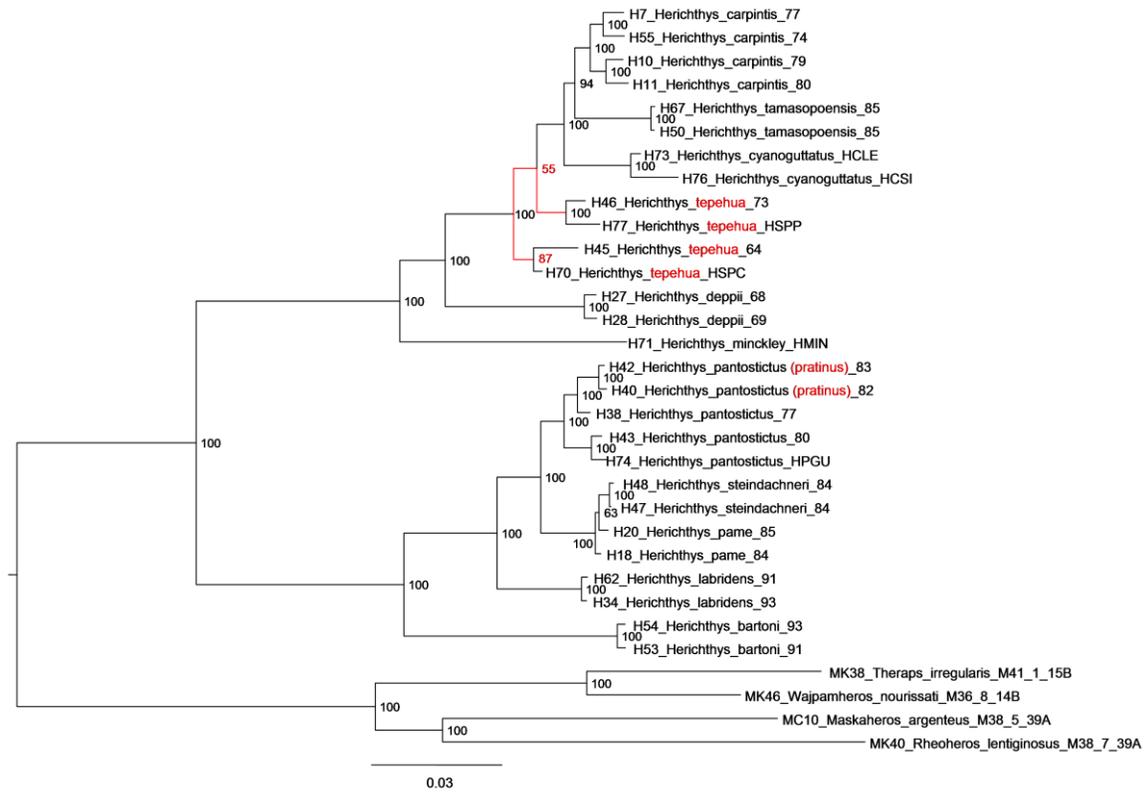


Figure S2. nDNA ddRAD tree analysis based on inclusion of variable SNPs. The tree is also a maximum-likelihood analysis with the same parameters as analysis with only fixed SNPs in Figure 3. Note that the analysis including variable SNPs does not support monophyly of *H. tepehua* (which in TreeMix analysis of the same data (not shown) shows hybrid signals of one clade with *H. carpintis* and the other clade with *H. deppii*; hence the here reconstructed paraphyly of *H. tepehua*), and also of *H. pame*. Note also that the analysis including variable SNPs has increased resolution of branch-lengths at the species level compared to the fixed SNPs analysis in Figure 3. *Herichthys pratinus* is as in fixed SNPs analysis recovered nested within *H. pantostictus*.

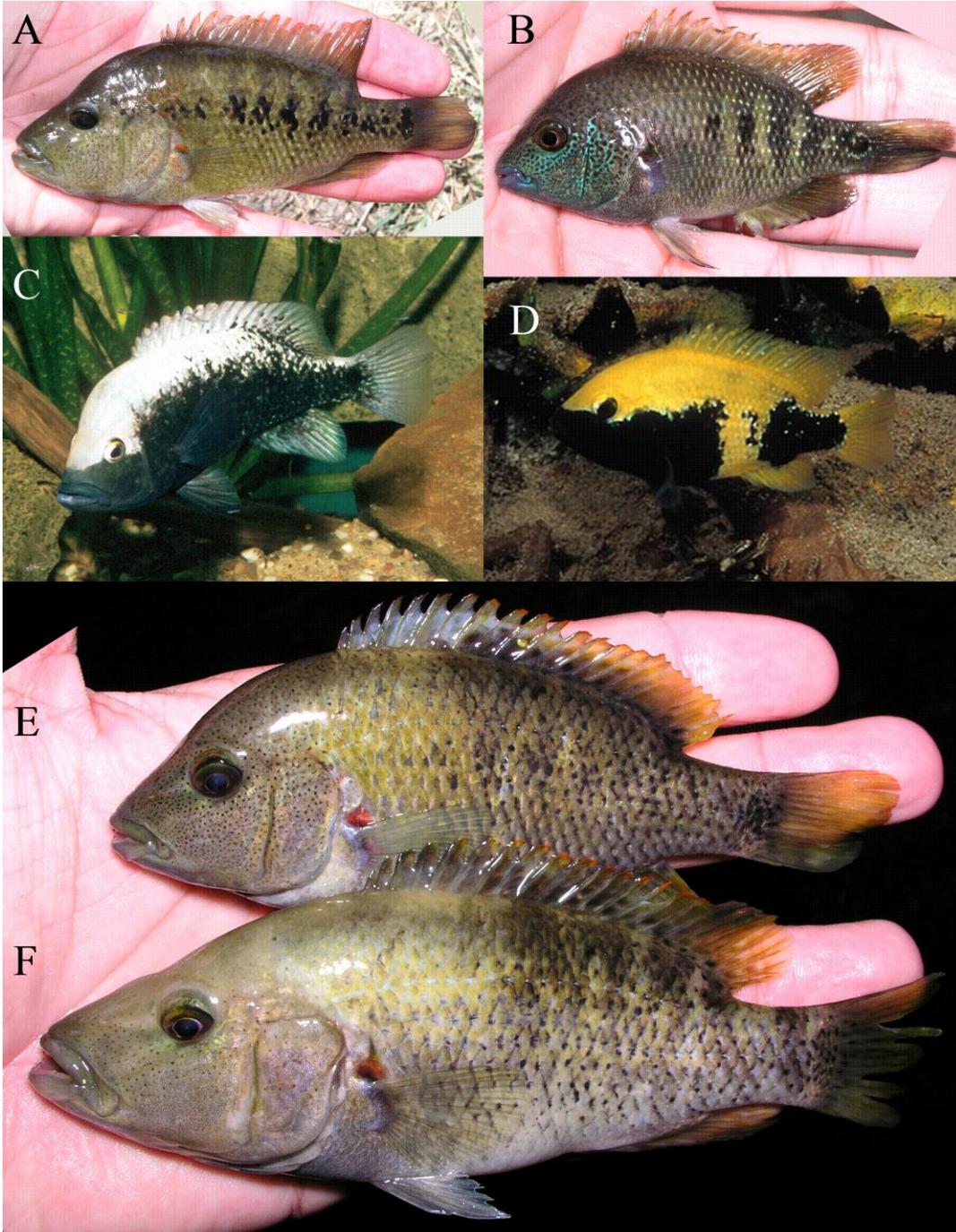


Figure S3. Sympatric species-pairs. A, B) *Herichthys bartoni* and *H. labridens* in normal coloration, freshly caught wild specimens. C, D) *Herichthys bartoni* and *H. labridens* in breeding coloration, C aquarium-kept female, D female photographed in natural habitat. C and D courtesy of Uwe Werner and Juan Miguel Artigas Azas, respectively. E, F) *Herichthys steindachneri* (foreground) and *H. pame* in normal coloration, freshly caught wild specimens. Note differences in head shape and size between the predatory *H. steindachneri* and *H. bartoni* (A, F) and the molluscivorous *H. labridens* and *H. pame* (B, E).



Figure S4. Variability in coloration of *H. pame* based on locality and habitat (mainly water tint and transparency). Specimens from highly alkaline waters (e.g. locality 86) are very pale with indistinct coloration (the same pale coloration is present in specimens of *H. pantostictus* – described as *H. pratinus* – from the same type of water; see Figure S5)



Figure S5. Variability in coloration of riverine populations of *H. pantostictus* based on locality and habitat (mainly water tint and transparency). Note pale specimens from a highly alkaline locality (82) described as a separate species *H. pratinus* (here considered as synonymous with *H. pantostictus*).