

Opening the Trojan horse: phylogeny of *Astyanax*, two new genera and resurrection of *Psalidodon* (Teleostei: Characidae)

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The freshwater fish genus *Astyanax* is one of the most diverse among the Characidae. The genus is defined by a combination of character states that are widely distributed in Characidae. In addition, the genus has the broadest geographical distribution in the family, being found in a great variety of environments of the Neotropical region. Although phylogenetic relationships were treated only partially, many authors agree that the genus is not monophyletic. In this contribution, we study the phylogenetic relationships of *Astyanax* in the context of the family Characidae, by combining morphological and molecular data. A total of 520 morphological characters, nine molecular markers and 608 taxa are analysed, of which 98 belong to *Astyanax*. According to our results, *Astyanax* is not monophyletic. We recovered species attributed to *Astyanax* in different subfamilies: Gymnocharacinae (including the type species), Stevardiinae and Tetragonopterinae. Among the species recovered in Gymnocharacinae, most (including the type species, the resurrected *Psalidodon*, and the new genus ***Andromakhe* gen. nov.**) were recovered in Gymnocharacini, while the remaining ones were recovered in Probolodini (transferred to *Deuterodon* or the new genus ***Makunaima* gen. nov.**).

ADDITIONAL KEYWORDS: fishes – Gymnocharacinae – Gymnocharacini – implied weighting – parsimony – Probolodini – Stevardiinae – systematics – taxonomy – Tetragonopterinae.

INTRODUCTION

Freshwater fishes of the genus *Astyanax* Baird & Girard, 1854 comprise more than 170 valid species (Fricke *et al.*, 2019). As currently defined, it is the most species-rich genus of the highly diverse family Characidae (Mirande, 2010; Oliveira *et al.*, 2011). This is also the most widely distributed genus in the family, with species occurring from Texas to the cis-Andean of northern Patagonia (i.e. *A. argentatus* Baird & Girard, 1854 and *A. pampa* Casciotta *et al.*, 2005, respectively). Indeed, the occurrence of species of *Astyanax* covers almost the entire distribution of Characidae, including

also Pacific drainages from Mexico to Ecuador. Species of the genus can be found from sea level to high mountain streams (e.g. *Astyanax tumbayaensis* Miquelarena & Menni, 2005 was recorded at 2094 m a.s.l.). Species of *Astyanax* are morphologically conservative and have medium to large sizes (about 40 to 200 mm standard length), when compared with the remaining members of the family. The Characidae had an explosive radiation in the Neotropics, attributable to the absence of carps of the family Cyprinidae in this region, which are dominant in all other continents except Australia (Géry, 1977; Mirande 2019). In the Neotropical region, species of *Astyanax* are especially abundant in peripheral areas relative to the more diverse basins of the Amazon and Orinoco rivers. The distribution area comprises large river systems such as the Rio de la Plata, São Francisco and Magdalena, small basins including most coastal basins of Central

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and South America and also the headwaters and tributaries of the Amazon and Orinoco systems.

According to [Eigenmann \(1917, 1921\)](#), *Astyanax* is diagnosed by the presence of two series of teeth in the premaxilla (the first series with a variable number of teeth and the second series with usually five), crowns of premaxillary and mandibular teeth usually ridged and denticulated, maxilla with 0–9 teeth, a complete lateral line, absence of a predorsal spine and a naked caudal fin. Most of these characters are broadly distributed among characids, suggesting that a phylogenetic definition of the genus is necessary ([Mirande et al., 2004, 2006, 2007; Marinho & Lima, 2009](#)). [Eigenmann \(1914\)](#) attempted to define characid genera as sets of species derived from common ancestors and sharing uniquely derived character states, but he also recognized that some of his genera were polyphyletic, due to the shared possession of character states that may have appeared in parallel during evolution of the group. [Eigenmann \(1917\)](#) explicitly said that he could not represent the evolution of Characidae as a branching tree and illustrated it, instead, as a radial scheme with *Astyanax* and *Moenkhausia* [Eigenmann, 1903](#) in the centre and independent radiations to the remaining genera. Such a situation was decades later defined as paraphyly ([Hennig, 1966; Farris, 1983](#)).

The revision by [Eigenmann \(1917\)](#) is still the most complete and comprehensive treatment of the genus, but it is outdated in terms of synonymy, generic reassignments and advances of phylogenetic systematics. Also, the number of taxa included in *Astyanax* has grown significantly since that revision, with approximately 20% of the species described in the past decade ([Rossini et al., 2016; Fricke et al., 2019](#)). [Eigenmann \(1917\)](#) defined three subgenera in *Astyanax* (*Astyanax* [Baird & Girard, Poecilurichthys](#) [Gill and Zyogaster](#) [Eigenmann](#)), but those subgenera were, in practice, difficult to distinguish from each other. [Géry \(1977\)](#) followed the classification proposed by [Eigenmann](#), but highlighted this difficulty: ‘we get thus a paradoxical situation: in most cases, while identifying a fish of this group, we got to deduce its subgeneric name from its species name’. Indeed, there are species that are currently considered as synonyms, which were assigned to different subgenera by [Eigenmann \(1917\)](#). Many species of *Astyanax* are difficult to distinguish from each other and are frequently identified in ichthyological collections only at the generic level or into species complexes, such as the *Astyanax bimaculatus* group, *A. fasciatus* gr., *A. orthodus* gr., *A. paucidens* gr. or *A. scabripinnis* gr. ([Géry, 1977; Moreira-Filho & Bertollo, 1991; Melo & Buckup, 2006; Garutti & Langeani, 2009; Ruiz-C et al., 2018](#)).

PHYLOGENETIC BACKGROUND

As implicitly proposed by [Eigenmann \(1917\)](#), the current phylogenetic hypotheses show *Astyanax* as a non-monophyletic unit, including several species of other genera of Characidae, such as *Hyphessobrycon anisitsi* ([Eigenmann, 1907](#)) ([Mirande, 2010](#)) and excluding some species, such as *Astyanax festae* ([Boulenger, 1898](#)) and *A. vermillion* [Zanata & Camelier, 2009](#) ([Mirande 2019: 12; Supporting Information, Appendix S7](#)).

Indeed, many authors agreed that *Astyanax* does not represent a monophyletic unit ([Rosen, 1972; Calcagnotto et al., 2005; Ornelas-García et al., 2008; Mirande, 2009, 2010, 2019; Javonillo et al., 2010; Oliveira et al., 2011; Schmitter-Soto, 2016](#)). Recently, [Rossini et al. \(2016\)](#) published a comprehensive approach based on DNA sequences of cytochrome c oxidase I (*COI*), proposing the existence of five *Astyanax* lineages. However, [Rossini et al. \(2016\)](#) focused on species delimitation through *COI* sequences rather than proposing a phylogenetic hypothesis for *Astyanax*. Therefore, they did not include outgroups, and neither the monophyly of the genus nor the relationships of each of those clades with the remaining members of Characidae were tested.

In this study we provide a hypothesis of the phylogenetic relationships of species currently assigned to *Astyanax* in the context of the family Characidae. This constitutes the most comprehensive analysis to date for the genus, based on a dataset combining morphological and molecular data. Some nomenclatural changes are presented for the cases in which we recovered stable and diagnosable clades that could represent a genus.

MATERIAL AND METHODS

TAXON SAMPLING

We analysed an expanded version of the dataset by [Mirande \(2019\)](#) with a total of 520 morphological characters and data for nine molecular markers (12S, 16S, *ATP6*, *COI*, *CYTB*, *MYH6*, *PTCHD1*, *RAG1* and *RAG2*). The list of characters is provided as [appendix S1](#) of [Mirande \(2019\)](#) and their detailed descriptions and illustrations were published either in [Mirande \(2010\)](#) or [Mirande \(2019\)](#). The dataset includes morphological data for 344 terminal taxa, of which only the fossil taxa †*Bryconetes enigmaticus* [Weiss et al., 2014](#), †*Paleotetra aiuruoca* [Weiss et al., 2012](#) and †*P. entrecorregos* [Weiss et al., 2012](#) were coded from literature and the remaining ones were coded by the authors. We include data for 98 species currently assigned to *Astyanax*, of which 24 were analysed previously by [Mirande \(2019\)](#). Among them, we coded the morphological

data of 80 species, while the remaining ones have only information of DNA. Relative to [Mirande \(2019\)](#), this dataset expands the total number of species from 473 to 608 and the number of species coded for morphology from 263 to 344. Species of *Astyanax* were selected to sample the distribution range of the genus, representatives of the three subgenera of Eigenmann, informal species groups and the clades proposed by [Rossini et al. \(2016\)](#). Osteological and myological preparations were made following [Taylor & Van Dyke \(1985\)](#) with modifications by [Datovo & Castro \(2012\)](#). The list of examined material is provided as [Supporting Information, Appendix S1](#). Osteological nomenclature follows [Weitzman \(1962\)](#) with modifications by [Zanata & Vari \(2005\)](#), which were mostly based on [Nelson \(1969\)](#), [Patterson \(1975\)](#) and [Fink & Fink \(1981, 1996\)](#). Myological nomenclature follows [Datovo & Castro \(2012\)](#). Authorities of family-level groups have been corroborated with [Van der Laan et al. \(2014\)](#).

Abbreviations mentioned in the list of examined material are as follows: ANSP (Academy of Natural Sciences of Philadelphia, USA), CI-FML (Colección Ictiológica de la Fundación Miguel Lillo, Argentina), CPUFMT (Coleção de Peixes da Universidade Federal de Mato Grosso, Brazil), CZUT-IC (Colección de Zoología de la Universidad del Tolima, Colombia), LBP (Laboratório de Biologia de Peixes da Universidade Estadual Paulista 'Júlio de Mesquita Filho', Brazil), MCP (Museu de Ciências e Tecnologia da Pontificia Universidade Católica do Rio Grande do Sul, Brazil), IUQ (Laboratorio de Ictiología de la Universidad del Quindío, Colombia), LACM (Natural History Museum of Los Angeles County, USA), MCNi (Museo de Ciencias Naturales de Salta, Argentina), MNHN (Muséum National d'Histoire naturelle, Paris, France), MHNG (Musée d'histoire naturelle, Genève, Switzerland), MZUSP (Museu de Zoologia da Universidade de São Paulo, Brazil), UFRGS (Universidade Federal do Rio Grande do Sul, Brazil), UMMZ (Museum of Zoology, Ann Arbor, Michigan, USA), USNM (Smithsonian National Museum of Natural History, Washington, DC, USA) and ZUEC (Museu de Zoologia da Universidade Estadual de Campinas, Brazil).

DNA SEQUENCES

Sequences have mainly been obtained from GenBank. Additional *COI* sequences for 23 species were produced especially for this article. The molecular partition was built with the aid of GB-to-TNT software ([Goloboff & Catalano, 2012](#)) and each marker aligned with MUSCLE ([Edgar, 2004](#)). Sequence alignments produced none or a few gaps, which were considered as missing entries. The taxonomic identity of each species, obtained from GenBank, was updated following [Fricke et al. \(2019\)](#) and, when possible, corroborated with the distribution

of the taxon. Possibly contaminated sequences have been detected through separate phylogenetic analyses of each marker and the use of BLAST ([Altschul et al., 1990](#)). New sequences (*COI*) were generated for species of *Astyanax* without published sequences (listed in [Supporting Information, Appendix S2](#)). We chose to sequence *COI*, which is the marker with data for more species in the genus. The complete dataset is available as [Supporting Information, Appendix S3](#).

Total genomic DNA was extracted from ethanol-preserved muscle tissue, using the Qiagen DNeasy kit. PCR amplifications were carried out in 30- μ L reactions using 0.2 μ L Taq (Genbiotech). A 650 bp DNA sequence from the 5' region of mitochondrial gen *COI* were amplified using the cocktail primers: VF2_t1; FishF2_t1; FishR2_t1; Fr1d_t1 ([Ivanova et al., 2007](#)). The PCR protocol consisted of an initial denaturation step at 95 °C (2 min), 30 cycles consisting of 94 °C (30 s) for denaturation, 54 °C (30 s) for annealing and 72 °C (1 min) for extension, followed by a final extension step at 72 °C (10 min). PCR-amplified products were cleaned using AccuPrep PCR Purification Kit. Products were sequenced with an automated sequencer (Macrogen, Korea) and all samples were sequenced in both directions to check for potential errors. Chromatograms obtained from the automated sequencer were processed and edited using GENEIOUS PRO 4.8.5 (Biomatters Ltd). Consensus sequences were deposited in GenBank under the accession numbers listed in [Supporting Information, Appendix S2](#).

Phylogenetic analysis

Phylogenetic analyses were made under parsimony, using extended implied weighting ([Goloboff, 1993, 2014](#)) in a broad set of conditions with TNT software ([Goloboff et al., 2008](#)). Five weighting schemes [in the sense of [Mirande \(2019\)](#)] for the coding (non-ribosomal) molecular sequences were explored and defined as follows: SEP – each character weighted according to its own homoplasy; COD – sequences divided in sets of three contiguous sites (codons) and each character weighted by the average homoplasy of its set; GRO – sequences divided in sets of 30 contiguous sites (ten codons) and each character weighted by the average homoplasy of its set; BLK – each character weighted by the average homoplasy of entire data partition (markers); and POS – sets formed by codon positions for each partition and each character weighted according to its position. Justification of the use of these weighting schemes is available in the literature ([Goloboff, 2014; Mirande, 2017, 2019](#)). Each of the five schemes was combined with nine weighting strengths (K-values; [Goloboff, 1993](#)), totalling 45 analytical conditions. The selected K-values (20, 21, 23, 26, 30,

35, 41, 48 and 56) were intended to sample regularly decreasing weighting strengths (see: [Mirande, 2009](#)). From this point, the analytical condition is denoted with the abbreviation for the weighting scheme followed by the weighting strength (value of K). For instance, in GRO41, 30 contiguous sites are grouped to be collectively weighted with a K = 41.

Searches in each analytical condition were done from Wagner trees and TBR with the help of parsimony ratchet ([Nixon, 1999](#)), sectorial searches, tree drifting and tree fusing ([Goloboff, 1999](#)). Each search concluded when the same (provisionally optimal) fit was hit three times for each condition. Successive refinements were subsequently done using the most parsimonious trees from all searches as sources for rounds of tree fusing for each of the analytical conditions, with the addition of sectorial searches when trees were improved by the fusing. These rounds of searches were carried out until the most parsimonious trees under all the conditions remained stable.

The final hypothesis was obtained in a two-steps process consisting of (1) parsimony searches on different analytical conditions and (2) a strict consensus from selected most parsimonious trees (MPTs) among those obtained in the first step. In previous analyses of the Characidae (e.g. [Mirande, 2009, 2010, 2019](#)) different criteria were used for each of those steps: parsimony for the first one (the main criterion) and topological stability for the second (a metacriterion). The same happens in the analyses that are followed by a sensitivity analysis to choose the final hypothesis (e.g. [Wheeler et al., 1995](#); [Whiting et al., 1997](#); [Prendini, 2000](#); [Giribet, 2003](#)). Instead, in this article we used the same criterion (parsimony) in both steps of the analysis. The general idea is to select, among the MPTs obtained in the first step, the trees that are overall the most parsimonious in the whole explored set of analytical conditions. This is the same as evaluating how strongly contradicted each MPT is under the conditions in which it is not optimal. By this procedure, a MPT for a given condition that is close to being optimal (weakly contradicted) in all the remaining ones will be preferred over a MPT that is comparatively far from being optimal (strongly contradicted) for the conditions in which it is suboptimal. This idea is similar to that of the decay index ([Bremer, 1994](#)), but is applied to entire trees instead of each clade independently.

Relative optimality of the MPTs from different searches cannot be calculated simply by summing up the fit of each MPT for each of the conditions and selecting the lowest one, because the values of fit are progressively lower at higher K-values and, arguably, the differences in fit between the MPTs and the closest suboptimals. Therefore, for every condition, each of the

MPTs (one for each analytical condition) was scaled between 0 and 100 according to its degree of optimality. Among that set of MPTs, the tree(s) with best fit for the evaluated condition was assigned a value of 100, while the worst (i.e. less parsimonious) was assigned a value of 0. Thus, each of the 45 MPTs was assigned one value between 0 and 100 for each of the 45 analytical conditions. Those values were averaged for each MPT and the tree with the highest value was selected as the overall most parsimonious tree. It is expected that some correlation of this criterion with the stability, regularly used in sensitivity analyses, but results of both criteria may differ. Only one MPT for each analytical condition was compared, to prevent overweight of the conditions in which a higher number of MPTs were found. All these calculations were done with a TNT script (by JMM), available in [Supporting Information, Appendix S3](#). Analyses were performed in a single Manjaro Linux platform with eight processors and 8 Gb of RAM. Clade support was calculated through 300 replications of symmetric resampling, searching with sectorial searches and tree fusing ([Goloboff, 1999](#)) in each of the resampled matrices. Supports are expressed as differences of frequencies 'Group present/Contradicted' (GC-values) ([Goloboff et al., 2003](#)).

The morphological partition of the dataset, the combined matrix, the script used to calculate the overall MPTs, the complete final hypothesis, the consensus of the most parsimonious trees obtained both under implied and equal weighting and the complete list of synapomorphies are available online at MorphoBank P-3458 ([O'Leary & Kaufman, 2011](#)).

RESULTS

A total of 45 phylogenetic analyses were performed, as combinations of the five weighting schemes and nine weighting strengths. The resulting MPTs ranged from 68 697 to 69 176 steps (COD56 and POS20, respectively). Most parsimonious trees under equal weights had 68 618 steps (more than 10 000 MPTs). The overall most parsimonious tree (see Material and Methods section) was obtained under BLK41 (72.07 points), followed by GRO30 (71.55) and GRO26 (71.33). Among the MPTs, the overall less parsimonious ones were under SEP20 (22.60), SEP21 (24.96) and POS20 (29.25). The final hypothesis is the strict consensus of 63 trees of 68 730 steps (Fit = 937.19053; CI = 11.29; RI = 64.07), obtained under BLK41 ([Figs 1, 2](#)). The complete final hypothesis with support values is provided graphically (in.svg format) in [Supporting Information, Appendix S4](#). MPTs obtained in each of the analytical conditions of extended implied weighting and the consensus tree of the MPTs obtained

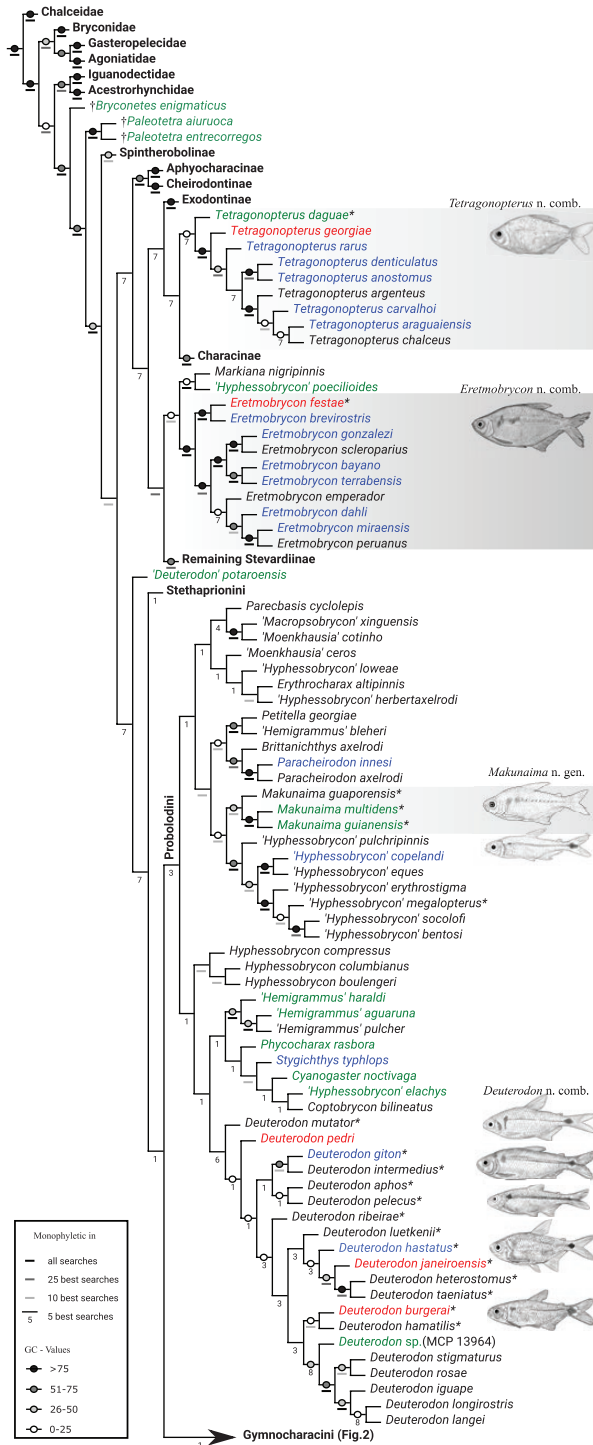


Figure 1. Phylogenetic relationships of species attributed to *Astyanax* included in the Tetragonopterinae, Stevardiinae, and Probolodini, as obtained from the combined phylogenetic analysis under parsimony and extended implied weighting. Consensus tree of 63 MPTs obtained under extended implied weighting (BLK; K = 41; Fit = 931.19053; Length = 68 730 steps). Taxa with their

under equal weighting are included in [Supporting Information, Appendix S3](#).

The Characidae and its subfamilies as classified by [Mirande \(2019\)](#) were obtained as monophyletic. The fossil genera †*Bryconetes* and †*Paleotetra* were recovered as successive sister-groups of the clade of extant analysed species. Most species of *Astyanax*, including its type species, were obtained in the Stethaprioniinae: Gymnocharacini. However, the genus, as in previous hypotheses ([Mirande, 2009, 2010, 2019](#)), was not monophyletic and several members were found to be only distantly related to the clade, including the type species of *Astyanax*. Only the clades including species currently assigned to *Astyanax* are herein discussed, but complete results and the list of synapomorphies are listed in [Supporting Information, Appendix S5](#).

‘*Astyanax*’ *daguae* [Eigenmann, 1913](#) and ‘*Astyanax*’ *festae*, formerly in the Stethaprioniinae, were reallocated to Tetragonopterinae and Stevardiinae, respectively.

TAXONOMY

TETRAGONOPTERINAE

TETRAGONOPTERUS [CUVIER, 1816](#)

Type species: *Tetragonopterus argenteus* [Cuvier, 1816](#).

New combinations: *Tetragonopterus daguae* ([Eigenmann, 1913](#)), **comb. nov.**

Diagnosis: *Tetragonopterus* is diagnosed by the following combination of characters: a deep and compressed body, two rows of premaxillary teeth with the inner row generally consisting of five or more teeth, a flattened prepelvic area that is bounded laterally by well-marked angles and a complete lateral line with an anterior portion that is strongly bent downwards ([Eigenmann, 1917](#)). Species of this genus are also recognizable, as mentioned by [Silva et al. \(2017\)](#), by a deep body, complete lateral line and the presence of pentacuspitate teeth in the inner premaxillary row.

Tetragonopterus daguae does not share with the remaining species in the genus the branched

name in black were analysed from both morphological and molecular data; taxa in blue from several DNA markers plus some morphological characters coded from literature; taxa in red from just one DNA marker (*COI*) plus some morphological characters coded from literature; and taxa in green analysed only from morphological data. The expression ‘best searches’ refers to the calculations of overall parsimony explained in the text.

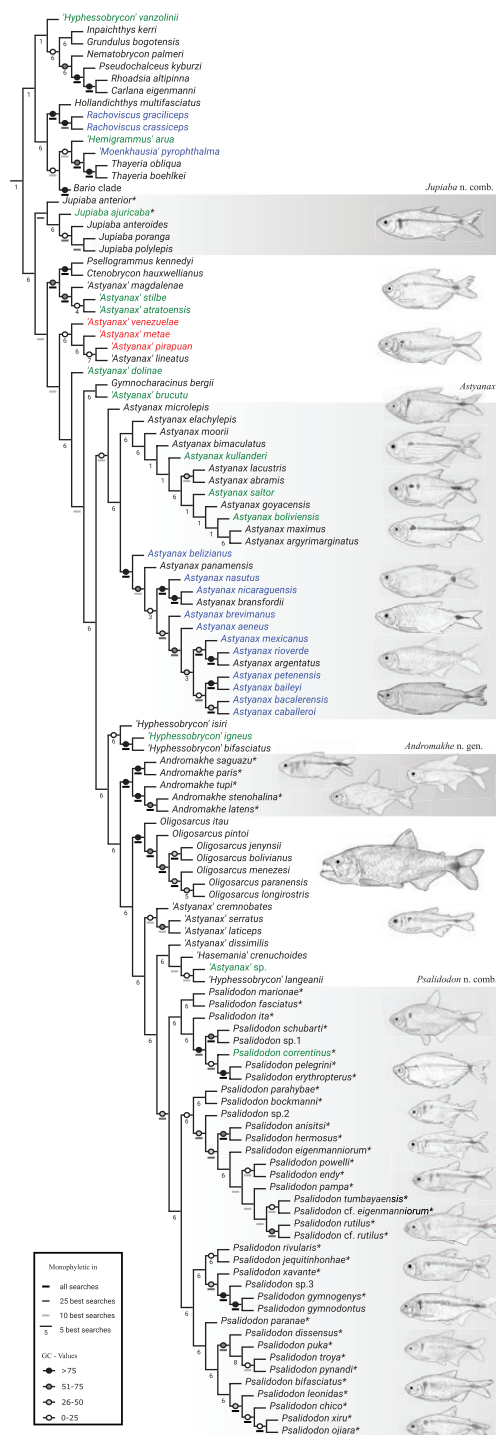


Figure 2. Phylogenetic relationships of species attributed to *Astyanax* included in the Gymnocharacini, as obtained from the combined phylogenetic analysis under parsimony and extended implied weighting. Consensus tree of 63 MPts obtained under extended implied weighting (BLK; K = 41; Fit = 931.19053; Length = 68 730 steps). Taxa with their name in black were analysed from both morphological

and molecular data; taxa in blue from several DNA markers plus some morphological characters coded from literature; taxa in red from just one DNA marker (*COI*) plus some morphological characters coded from literature; and taxa in green analysed only from morphological data. The expression 'best searches' refers to the calculations of overall parsimony explained in the text.

Synapomorphies: With the addition of *Tetragonopterus daguae*, the genus is supported by: ten ethmoid cartilage distant from lateral ethmoids (40:1), sphenotic spine long and reaching hyomandibular dorsal margin (47:1), supraoccipital spine extended dorsal to entire neural complex of Weberian apparatus (61:0), laterosensory pore anterior to dilatator fossa oriented dorsomedially (115:0), five teeth in the inner premaxillary series (182:0), dentary teeth abruptly decreasing in size (207:1), metapterygoid foramen for passage of the pseudobranch artery as an oblique canal through the metapterygoid (228:1), axis of supraneurals dorsally bifurcated (396:1), bony lamellae developed on supraneurals (397:1) and the presence of iii dorsal-fin rays with the anterior one visible only in c&s specimens (404:1).

STEVARDIINAE: ERETMOBRYCONINI

ERETMOBRYCON FINK, 1976

Type species: *Eretmobyron bayano* Fink, 1976.

New combination: *Eretmobyron festae* (Boulenger, 1898) **comb. nov.**

Diagnosis: *Eretmobyron* is recognizable by having four teeth in the inner premaxillary row, iii+9 dorsal-fin rays supported by ten pterygiophores, only one row of scales covering up to half the anal-fin base* and scales covering only the base of the caudal fin*. *Eretmobyron* was supported in the phylogenetic analysis only by molecular data (44 synapomorphies) given that the characters (marked above with an asterisk) distinguishing it from *Markiana* Eigenmann, 1903, its sister-group, were optimized as plesiomorphies for Stevardiinae.

Synapomorphies: The Eretmobyronini are supported by 25 molecular and seven morphological

and molecular data; taxa in blue from several DNA markers plus some morphological characters coded from literature; taxa in red from just one DNA marker (*COI*) plus some morphological characters coded from literature; and taxa in green analysed only from morphological data. The expression 'best searches' refers to the calculations of overall parsimony explained in the text.

synapomorphies: absence of a bony rhinosphenoid (34:0), a long sphenotic spine that reaches the dorsal margin of hyomandibula (47:1), the sixth infraorbital almost completely covering the dilatator fossa (94:0), tubule for passage of blood vessels on lamellar maxillary portion with an anterior branch (142:1), ventral margin of posttemporal situated more posterior than lateral margin of epioccipital (336:1), three unbranched dorsal-fin rays articulating with first dorsal-fin pterygiophore (404:1) and two pairs of uroneurals (427:1).

'*Astyanax festae*' was consistently recovered within the *Eretmobrycon* clade, even though its relationships were herein analysed only using *COI* data. This species has four teeth in the inner premaxillary row (Eigenmann, 1917), which is typical in Stevardiinae. *Eretmobrycon festae* was recovered as the sister-group of *E. brevisrostris* (Günther, 1860). Eigenmann (1917) already noticed a close resemblance between these species (see Discussion). Herein we transfer *A. festae* to *Eretmobrycon*, which has been implicitly resurrected by Thomaz *et al.* (2015), but without providing a diagnosis of the genus.

STETHAPRIONINAE: PROBOLODINI

MAKUNAIMA gen. nov.

LSID: urn:lsid:zoobank.

org:act:F38D65AB-344D-4752-84B3-801D7F9E613B

Type species: *Astyanax guaporensis* Eigenmann, 1911.

New combinations: ***Makunaima guaporensis* (Eigenmann, 1911) comb. nov., *Makunaima guianensis* (Eigenmann, 1909) comb. nov., *Makunaima multidentis* (Eigenmann, 1908) comb. nov.**

Diagnosis: *Makunaima* is diagnosed from the remaining Stethaprioninae by the following combination of characters: presence of two rows of teeth in the premaxilla, with five or six in the inner row, a naked caudal fin, *circuli* absent on posterior field of scales, more than five maxillary teeth, dorsal expansion of the rhinosphenoid present, base of the teeth from inner premaxillary row as broad as distal portion, a single tubule for passage of blood vessels on lamellar portion of the maxilla, complete lateral line with 31 to 35 perforated scales and the caudal fin hyaline or with a blotch but always lacking a black longitudinal stripe.

Synapomorphies: *Makunaima* is recovered as monophyletic, supported by four morphological synapomorphies: posterior laterosensory pore of

pterotic aligned with ventral pore (119:1), dentary teeth abruptly decreasing in size (207:1), posterior margin of quadrate reaching a vertical line through tip of symplectic (234:0) and the opercle with pronouncedly concave posterior margin (252:1).

Etymology: Makunaima (also spelled as Macunaima or Makonaima) is a god of creation in the mythology of several Amazonian tribes. According to legend, Makunaima created animals and a great tree from which all food plants grew. Gender: masculine.

DEUTERODON EIGENMANN, 1907

Type species: *Deuterodon iguape* Eigenmann, 1907

Junior synonyms: *Myxiops Zanata & Akama, 2004* (type species *Myxiops aphos* Zanata & Akama, 2004). *Probolodus Eigenmann, 1911* (type species *Probolodus heterostomus* Eigenmann, 1911).

New combinations: ***Deuterodon aphos* (Zanata & Akama, 2004) comb. nov., *Deuterodon burgerai* (Zanata & Camelier, 2009) comb. nov., *Deuterodon giton* (Eigenmann, 1908) comb. nov., *Deuterodon hamatilis* (Camiel & Zanata, 2014) comb. nov., *Deuterodon hastatus* (Myers, 1928) comb. nov., *Deuterodon heterostomus* (Eigenmann, 1911) comb. nov., *Deuterodon intermedius* (Eigenmann, 1908) comb. nov., *Deuterodon janeiroensis* (Eigenmann, 1908) comb. nov., *Deuterodon luetkenii* (Boulenger, 1887) comb. nov., *Deuterodon mutator* (Eigenmann, 1909) comb. nov., *Deuterodon oyakawai* (Santos & Castro, 2014) comb. nov., *Deuterodon pelecus* (Bertaco & Lucena, 2006) comb. nov., *Deuterodon ribeirae* (Eigenmann, 1911) comb. nov., *Deuterodon sazimai* (Santos & Castro, 2014) comb. nov., *Deuterodon taeniatus* (Jenyns, 1842) comb. nov.**

Diagnosis: With the proposed composition, an expanded genus *Deuterodon* is distinguished from the remaining Stethaprioninae by the following combination of characters: a naked caudal fin, *circuli* absent on posterior field of scales, laterosensory pore anterior to dilatator fossa oriented dorsomedially (except in *D. mutator*), base of the teeth from the inner premaxillary row smaller than their distal portion (except in *D. heterostomus*, *D. oyakawai* and *D. sazimai*), rhinosphenoid expanded dorsally between olfactory nerves and the presence of a single tubule for passage of blood vessels on the lamellar portion of the maxilla.

Synapomorphies: *Deuterodon* is herein obtained as monophyletic, supported by the three molecular and four morphological synapomorphies, mostly related

with dentition: presence of five or more cusps on teeth from outer premaxillary row (177:1), seven or more cusps on first maxillary tooth (194:1), six or more cusps on dentary teeth (202:1) and presence of two pairs of uroneurals (427:1).

Several species currently assigned to *Astyanax*, mostly from coastal basins of southern Brazil were found to be closely related to *Deuterodon* as defined by Lucena & Lucena (1992). These species are herein transferred to *Deuterodon*, expanding the taxonomic definition of the genus. Also, the genera *Myxiops* Zanata & Akama and *Probolodus* Eigenmann are synonymized with *Deuterodon*. '*Hyphessobrycon*' *luetkenii* was also consistently recovered in this clade and transferred to *Deuterodon*. '*Deuterodon*' *potaroensis* Eigenmann, 1909 was not found in this clade and remains as *incertae sedis* in the Stethaprioninae.

JUPIABA ZANATA, 1997

Type species: *Jupiaba poranga* Zanata, 1997.

New combinations: ***Jupiaba ajuricaba* (Marinho & Lima, 2009) comb. nov., *Jupiaba anterior* (Eigenmann, 1908) comb. nov.**

Diagnosis: The new composition herein proposed for a monophyletic genus *Jupiaba* is diagnosed from the remaining Stethaprioninae by the following combination of characters: rhinosphenoid present and expanded dorsally between the olfactory nerves, *circuli* present on posterior field of scales, the base of the inner premaxillary teeth approximately as wide as their distal portion, absence of bony hooks in mature males, and by the presence of two series of teeth in the premaxilla, a complete lateral line and a naked caudal fin.

Synapomorphies: The clade of *Jupiaba*, including its type species, is supported by 12 molecular and two morphological synapomorphies: abrupt decrease of dentary teeth (207:1) and presence of 25 or more gill filaments attached to leading edge of first ceratobranchial (304:1).

Jupiaba was herein obtained in two separate clades, with three species, including the type species, *Jupiaba poranga*, in this clade, and three species in the Stethaprionini [*Jupiaba*' *acanthogaster* (Eigenmann, 1911), '*Jupiaba*' *mucronata* (Eigenmann, 1909) and '*Jupiaba*' *scologaster* (Weitzman & Vari, 1986)]. Because several species of *Jupiaba* were not analysed, the complete composition of the genus is still unknown.

STETHAPRIONINAE: GYMNOCHARACINI

CTENOBRYCON CLADE

Composition: *Ctenobrycon* Eigenmann, *Psellogrammus* Eigenmann and probably *Zygogaster* Eigenmann.

This clade is highly supported and stable in all analyses. It is supported by 33 molecular and three morphological synapomorphies: long supraoccipital spine that extends dorsal to entire neural complex of the Weberian apparatus (61:0), anterior tip of pelvic bone reaching a position anterior to second pleural rib (376:0) and four or fewer supraneurals (392:0).

CF. ZYGOGASTER EIGENMANN 1913

Type species: *Zygogaster filiferus* Eigenmann, 1913 (not analysed herein).

Because the type species of *Zygogaster* was not analysed, the putative generic assignment of the included species may vary in the future. The species herein analysed share the presence of an oval humeral spot (which is absent in *Astyanax filiferus*) and the following morphological synapomorphies: presence of a posteriorly oriented epioccipital spine (66:0), five or more cusps on outer premaxillary teeth (193:1), longitudinal ridge on medial surface of opercle reaching 60% or more of its length (254:0), presence of denticles on entire surface of the gill rakers (301:1) and presence of 25 or more gill filaments attached to leading edge of first ceratobranchial (304:1).

Zygogaster was proposed as a genus by Eigenmann (1913) and shortly after considered a subgenus of *Astyanax* (Eigenmann, 1917). This subgenus included five species [*Astyanax atratoensis* (Eigenmann, 1907), *A. caucanus* (Steindachner, 1879), *A. filiferus*, *A. magdalenae* Eigenmann & Henn, 1916 and *A. stilbe* (Cope, 1870)]. We find three of these species to form a clade that does not include the type species of *Astyanax*. *Zygogaster* is, thus, a candidate genus to be resurrected. However, we do not have enough evidence to do so, because we could not analyse the type species of the genus (*Zygogaster filiferus*).

'ASTYANAX' LINEATUS CLADE

Composition: '*Astyanax*' *lineatus* (Perugia, 1891), '*Astyanax*' *metae* Eigenmann, 1914, '*Astyanax*' *pirapuan* Tagliacollo et al., 2011 and '*Astyanax*' *venezuelae* Schultz, 1944.

The monophyly and, eventually, the composition of this clade should be further investigated. In this analysis it is supported by ten molecular and

one morphological synapomorphy: sheath of scales covering three-quarters or more of the anal-fin base (469:0).

Among the species in this clade, only ‘*Astyanax*’ *lineatus* has both morphological and molecular data available. The remaining species were analysed only from sequences of *COI*. The obtained relationships of this clade suggest the need of creating a new generic name for it. However, given the low amount of available data for most of the analysed species, we prefer to keep these species provisionally as *incertae sedis* in Gymnocharacini.

‘ASTYANAX’ DOLINAE GRAÇA ET AL., 2017

This species was obtained as the sister-group of a large clade composed of most members of the Gymnocharacini. ‘*Astyanax*’ *dolinae* has a combination of pentacuspitate teeth on the anterior premaxillary row and *circuli* on the posterior field of scales, which is uncommon among the species classified in *Astyanax* prior to this study (Graça et al., 2017). This species was analysed only from morphology and its relationships should be further tested from additional data.

GYMNOCHARACINUS CLADE

Composition: This clade includes *Gymnocharacinus bergii* Steindachner, 1903 and ‘*Astyanax*’ *brucutu* Zanata et al., 2017. It is most probably an artefact resulting from the highly divergent morphology of both species and/or the lack of molecular data for *A. brucutu*. The latter species oscillated in the different analyses between this clade and the *Psalidodon* clade (see below), being the last option more plausible from a biogeographical point of view. No analysis recovered *Astyanax brucutu* as part of the containing the type of *Astyanax*, and we leave it as *incertae sedis* in Gymnocharacini. This clade is supported by three morphological synapomorphies: the presence of four teeth in the inner premaxillary row (181:1), the possession of eight or fewer dentary teeth (198:0) and cartilage from the first epibranchial attached to the middle region of the second pharyngobranchial (278:0).

ASTYANAX BAIRD & GIRARD, 1854

Type species: *Astyanax argentatus* Baird & Girard, 1854.

Junior synonyms: *Anoptichthys* Hubbs & Innes, 1936 (type species *Anoptichthys jordani* Hubbs & Innes, 1936). *Astyanacinus* Eigenmann, 1907 (type species

Tetragonopterus moorii Boulenger, 1892). *Bertoniolus* Fowler, 1918 (type species *Bertoniolus paraguayensis* Fowler, 1918). *Bramocharax* Gill & Bransford, 1877 (type species *Bramocharax bransfordii* Gill, 1877). *Catemaco* (subgenus of *Bramocharax*) Contreras-Balderas & Rivera-Teillery, 1985 (type species *Bramocharax caballeroi* Contreras-Balderas & Rivera-Teillery, 1985). *Poecilurichthys* Gill, 1858 (type species *Poecilurichthys brevoortii* Gill, 1858).

Diagnosis: A monophyletic *Astyanax* can be diagnosed from the remaining Stethaprioninae by the following combination of characters: laterosensorial pore anterior to dilatator fossa oriented lateroventrally, rhinosphenoid (when present) lacking a dorsal expansion between olfactory nerves, presence of *circuli* on posterior field of scales, presence of an anterior branch of the tubule for passage of blood vessels on lamellar portion of maxilla, naked caudal fin and a complete lateral line.

Synapomorphies: According to the present analysis, the synapomorphies of this clade are 14 from molecular data and two from morphology: cusps of second premaxillary row of teeth forming an anteriorly concave semicircle from ventral view (179:0) and reduced lateral urohyal bony expansions (305:1).

Astyanax was recovered as monophyletic with this composition in most analyses, but it included ‘*Astyanax*’ *dolinae* in some of them. The obtained support for the monophyly of *Astyanax* is poor, but the clade is stable with the exception of the occasional inclusion of *A. dolinae*. In the final hypothesis herein obtained, *Astyanax* is composed of *A. microlepis* Eigenmann, 1913 as the sister-group of two large clades, one of them (the *Astyanax argentatus* clade) containing the type species of the genus and including the Central- and North-American species. The other clade was composed of the *Astyanax bimaculatus* (Linnaeus, 1758) group and related species.

The *Astyanax argentatus* clade is recovered as monophyletic under all explored conditions, as supported by 34 molecular synapomorphies. This clade includes the type species of *Astyanax* and most of the trans-Andean species.

The *Astyanax bimaculatus* clade, supported by 14 molecular synapomorphies, includes all analysed species assigned to the *Astyanax bimaculatus* group. Also, it contains two members of the *Astyanax orthodus* group and *Astyanax maximus* (Steindachner, 1876). The species with a horizontally ovate humeral spot are, in the final hypothesis, paraphyletic in terms of *A. maximus*, which is obtained as the sister-species of *A. argyrimarginatus* Garutti, 1999. However, the position of *A. maximus* is variable among the different

analytical conditions, resulting in the monophyly of the *A. bimaculatus* group in some analyses.

'HYPHESSOBRYCON' BIFASCIATUS CLADE

Composition: This clade is composed of *Hyphessobrycon* *bifasciatus* Ellis, 1911, *Hyphessobrycon* *isiri* Almirón *et al.*, 2006 and *Hyphessobrycon* *igneus* Miquelarena *et al.*, 1980, among the analysed species. However, the taxon sampling is not oriented to investigate the relationships of these species and this clade could include other taxa that were not analysed here. The diagnosis herein provided is based only on these three species and may have to be adjusted with the inclusion of more related taxa. Even though this clade is stable and well-supported, we prefer not to erect a new genus for it given the low taxon sampling in *Hyphessobrycon* and that, under certain conditions, the morphologically divergent species *Gymnocharacinus bergii* was obtained as sister-group of *H. isiri* (see Discussion).

The species included herein share the presence of a rhinosphenoid lacking a dorsal projection, a relatively long supraoccipital process projected dorsal to middle length of Weberian apparatus neural complex, presence of an anterior branch in the maxillary tubules for blood vessels and an incomplete lateral line. This clade is supported by two molecular and ten morphological synapomorphies: anterior extension of nasal extending only slightly anterior to mesethmoid wing (11:0), fourth infraorbital longer dorsoventrally than longitudinally (92:1), sixth infraorbital leaving a conspicuous naked area in anterior region of dilatator fossa (94:1), laterosensory pore anterior to dilatator fossa oriented dorsomedially (115:0), interrupted lateral line (123:1), canal of lateral line on caudal-fin membrane absent (125:0), coronomeckelian bone mainly lateral to Meckelian cartilage (159:0), eight or fewer dentary teeth (198:0), posterior tip of quadrate not reaching posterior margin of symplectic (234:1) and presence of 17 or fewer gill filaments attached to leading edge of first ceratobranchial (303:0).

ANDROMAKHE gen. nov.

LSID:lsid:zoobank.org:act:74BC5B28-B3E7-46D2-A5FA-532728B096E2AQ17

Type species: *Astyanax latens* Mirande *et al.*, 2004.

New combinations: *Andromakhe latens* (Mirande *et al.*, 2004) **comb. nov.**, *Andromakhe paris* (Azpelicueta *et al.*, 2002b) **comb. nov.**, *Andromakhe saguazu* (Casciotta *et al.*, 2003a) **comb. nov.**, *Andromakhe stenohalina* (Messner, 1962) **comb. nov.**, *Andromakhe tupi* (Azpelicueta *et al.*, 2003)

comb. nov.

Diagnosis: *Andromakhe* is distinguished from the remaining Stethaprioninae by the following combination of characters: *circuli* absent in the posterior field of scales, rhinosphenoid lacking a dorsal projection between olfactory nerves, anal-fin origin anterior to the vertical line through the last dorsal-fin ray, presence of an anterior branch on maxillary tubule for passage of blood vessels, presence of two to four maxillary teeth, a complete lateral line, a naked caudal fin and the presence of two series of teeth in the premaxilla.

Synapomorphies: This genus is supported by 17 molecular synapomorphies.

Etymology: The genus is named for *Ἀνδρομάχη*, *Andromakhe* ('battle of men'), a character in Greek mythology where she is the wife of Hector, Prince of Troy, and, in Homer's epic poem *Iliad*, the mother of *Ἄστυάναξ*, *Astyanax* ('city protector'). Gender: feminine.

The monophyly of *Andromakhe* is stable and has high support in all analyses.

PSALIDODON EIGENMANN, 1911

Type species: *Psalidodon gymnodontus* Eigenmann, 1911.

New combinations: *Psalidodon anisitsi* (Eigenmann, 1907) **comb. nov.**, *Psalidodon bifasciatus* (Garavello & Sampaio, 2010) **comb. nov.**, *Psalidodon bockmanni* (Vari & Castro, 2007) **comb. nov.**, *Psalidodon chico* (Casciotta & Almirón, 2004) **comb. nov.**, *Psalidodon correntinus* (Holmberg, 1891) **comb. nov.**, *Psalidodon dissensus* (Lucena & Thofehrn, 2013) **comb. nov.**, *Psalidodon eigenmanniorum* (Cope, 1894) **comb. nov.**, *Psalidodon endy* (Mirande *et al.*, 2006) **comb. nov.**, *Psalidodon erythropterus* (Holmberg, 1891) **comb. nov.**, *Psalidodon fasciatus* (Cuvier, 1819) **comb. nov.**, *Psalidodon gymnogenys* (Eigenmann, 1911) **comb. nov.**, *Psalidodon hermosus* (Miquelarena, Protogino & López, 2005) **comb. nov.**, *Psalidodon ita* (Almirón, Azpelicueta & Casciotta, 2002) **comb. nov.**, *Psalidodon jequitinhonhae* (Steindachner, 1877) **comb. nov.**, *Psalidodon leonidas* (Azpelicueta *et al.*, 2002b) **comb. nov.**, *Psalidodon marionae* (Eigenmann, 1911) **comb. nov.**, *Psalidodon ojiara* (Azpelicueta & Garcia, 2000) **comb. nov.**, *Psalidodon pampa* (Casciotta *et al.*, 2005) **comb. nov.**, *Psalidodon parahybae* (Eigenmann, 1908) **comb.**

nov., *Psalidodon paranae* (Eigenmann, 1914) **comb. nov.**, *Psalidodon pelegri* (Eigenmann, 1907) **comb. nov.**, *Psalidodon powelli* (Terán *et al.*, 2017) **comb. nov.**, *Psalidodon puka* (Mirande *et al.*, 2007) **comb. nov.**, *Psalidodon pynandi* (Casciotta *et al.*, 2003b) **comb. nov.**, *Psalidodon rivularis* (Lütken, 1875) **comb. nov.**, *Psalidodon rutilus* (Jenyns, 1842) **comb. nov.**, *Psalidodon schubarti* (Britski, 1964) **comb. nov.**, *Psalidodon troya* (Azpelicueta *et al.*, 2002b) **comb. nov.**, *Psalidodon tumbayaensis* (Miquelarena & Menni, 2005) **comb. nov.**, *Psalidodon xavante* (Garutti & Venere, 2009) **comb. nov.**, *Psalidodon xiru* (Lucena *et al.*, 2013) **comb. nov.**

Diagnosis: The resurrected and expanded genus *Psalidodon* is diagnosed from the remaining Stethaprioninae by the following combination of characters: laterosensory pore anterior to dilatator fossa oriented lateroventrally, rhinosphenoid lacking a dorsal projection between olfactory nerves, *circuli* absent on posterior field of scales (excepting *P. erythropterus* and *P. pelegri*), presence of an anterior branch in the maxillary tubules for blood vessels, possession of 0–3 maxillary teeth (usually 1), anal-fin origin posterior to a vertical line through last dorsal-fin ray and a naked caudal fin.

Synapomorphies: *Psalidodon* is obtained as monophyletic with moderate support. The monophyly of the genus is obtained from four molecular and two morphological synapomorphies: the presence of five or more cusps on teeth both from the outer premaxillary row (177:1) and the maxilla (193:1).

Psalidodon has been erected by Eigenmann (1911) as a monotypic genus for its type species *P. gymnodontus*. That combination was used by many subsequent authors, as Géry (1977), Malabarba (1998), Pavanelli (2003) and Mirande (2009), until Pavanelli & Oliveira (2009) presented a redescription of the type species and synonymized *Psalidodon* with *Astyanax*. We hereby resurrect it from this synonymy and consider *Psalidodon* as a valid genus. This expanded definition of *Psalidodon* includes many taxa previously classified in *Astyanax*, including most species of the *Astyanax eigenmanniorum*, *A. fasciatus* and *A. scabripinnis* groups.

All nomenclatural changes in this paper are summarized in Supporting Information, Appendix 6.

DISCUSSION

A phylogenetic definition of *Astyanax* is one of the main challenges in the systematics of Neotropical fishes. This highly diverse genus includes many species that

are hardly distinguishable from each other, which are highly conservative even in their finest details. The known molecular data are similarly conservative. We observed that the *COI* marker, vastly used for ‘molecular taxonomy’, does not discriminate between some evidently different species or has intraspecific variations that preclude its use for taxonomy [in agreement with Rossini *et al.* (2016)]. For instance, among the DNA data produced for this article, we found exactly the same *COI* sequence in some specimens of *Psalidodon powelli* and *P. endy*, which are easily distinguished from each other by morphology. The fact of being in the limit of resolution of both morphology and *COI* sequences produced many clades in the *Astyanax* clade (*s.l.*) to have moderate to low support. Therefore, some of the results herein presented should be further corroborated through the analysis of more information (species and/or characters). We consider the available data are enough to restrict *Astyanax* and to propose the genera *Andromakhe* and *Makunaima*, but not to erect further new genera for the ‘*Astyanax*’ *lineatus* or the ‘*Hyphessobrycon*’ *bifasciatus* clades.

Two species classified in *Astyanax* were obtained in different subfamilies than the true *Astyanax*. ‘*Astyanax*’ *festae* was recovered in the *Eretmobrycon* clade (Stevardiinae: Eretmobryconini) of Mirande (2019) and is transferred to this genus. This species was found in the clade 5 of Rossini *et al.* (2016), together with specimens of taxa distantly related both to *E. festae* and between them. Actually, according to the present results, the most accurate previous evaluation of the relationships of *Eretmobrycon festae* (as treated herein) was by Eigenmann (1917: 392), who also noted that the species had four teeth in the posterior premaxillary row and stated for *Eretmobrycon brevirostris* (the sister-group of *E. festae* according to our results) that: ‘This species connects the genus *Astyanax* through *A. festae* with the genus *Bryconamericus*’. Note that the genus *Eretmobrycon* had not been erected yet and the species now included in this genus were classified in *Bryconamericus* Eigenmann at that time. ‘*Astyanax*’ *daguae* was herein recovered in the Tetragonopterinae and transferred to *Tetragonopterus*. However, this species is analysed only from morphological data and some DNA information may be useful to test the current hypothesis and classification.

Several species attributed to *Astyanax* are placed in Probolodini. Three of them are herein included in the new genus *Makunaima*. These species were recently redescribed and considered to be closely related to each other in a series of papers led by Marinho (Marinho & Birindelli, 2013; Marinho & Ohara, 2013; Marinho *et al.*, 2015). Marinho *et al.* (2015) mentioned several species of *Astyanax* sharing the high number of maxillary teeth with *Makunaima guianensis*,

in addition to *M. guaporensis* and *M. multidens*: *Astyanax angustifrons* (Regan, 1908), *A. aurocaudatus* Eigenmann, 1913, *A. gisleni* Dahl, 1943, *A. goyanensis* (Miranda-Ribeiro, 1944), *A. henseli* Melo & Buckup, 2006, *A. leopoldi* Géry, Planquette & Le Bail, 1988, *A. nasutus* Meek, 1907, *A. nicaraguensis* Eigenmann & Ogle, 1907, *A. superbis* Myers, 1942 and *A. totae* Ferreira Haluch & Abilhoa, 2005. Among those species, *A. nasutus* and *A. nicaraguensis* were obtained in the clade of *Astyanax argentatus*, while ‘*Astyanax aurocaudatus*’ is actually a Stevardiinae, transferred again to *Carlastyanax* by Mirande et al. (2013). Some of the remaining species listed by Marinho et al. (2015) may be related to *Makunaima*, but this issue should be addressed in specific studies. The remaining probolodin species attributed in the literature to *Astyanax* are here found to be related to *Deuterodon* and transferred to that genus. This agrees with the results of Silva et al. (2017), who found that several coastal species of *Astyanax* plus *Hyphessobrycon luetkenii* were closely related to *Deuterodon*, *Myxiops* and *Probolodus*. All species of that clade are, in this contribution, transferred to *Deuterodon*, which has temporal priority over the remaining genera. These nomenclatural changes include the synonymy of *Myxiops* and *Probolodus* with *Deuterodon*.

The remaining analysed taxa currently classified in *Astyanax*, including its type species, are placed in Gymnocharacini sensu Mirande (2019). Within this tribe, we obtained nine genus-level clades plus ‘*Astyanax dolinae*’, which was recovered in a pectinate position. ‘*Astyanax ajuricaba*’ and ‘*Astyanax anterior*’ were obtained in a clade with three of the six analysed species of *Jupiaba*, including *J. poranga*, the type species of that genus, and these species are this transferred to it. The non-monophyly of *Jupiaba* was already obtained (Oliveira et al., 2011; Mirande, 2019), but its type species was herein analysed for the first time. The following clade is composed of *Ctenobrycon*, *Psellogrammus* and the three analysed species of the subgenus *Zygogaster*, which was not herein resurrected because we did not include its type species (*A. filiferus*). The clade composed of ‘*Astyanax lineatus*’, ‘*Astyanax metae*’, ‘*Astyanax pirapuan*’ and ‘*Astyanax venezuelae*’ is not comparable to any clade proposed in the literature, given that only *A. lineatus* was previously analysed (e.g. Mirande, 2010, 2019) and that ‘*Astyanax metae*’, ‘*Astyanax pirapuan*’ and ‘*Astyanax venezuelae*’ were obtained in clade 4 of Rossini et al. (2016).

The sister-group relationship between ‘*Astyanax brucutu*’ and *Gymnocharacinus bergii* obtained in the present analyses is most probably an artefact, produced by morphological convergences. However, it is interesting that this analysis supports a close relationship of both species with *Astyanax s.l.*, as it was proposed in the literature (Zanata et al., 2017;

Mirande, 2019). As noted by Zanata et al. (2017), ‘*Astyanax brucutu*’ shares with the Stevardiinae the possession of only four teeth in the inner premaxillary row and, especially with *Creagrutus* Günther, several features presumably related to durophagy. However, it was obtained in the Gymnocharacini, supporting the conclusions of Zanata et al. (2017:503). In tests removing each of those species from the analyses, *G. bergii* was obtained as the sister-group of ‘*Hyphessobrycon isiri*’, in the clade including also *H. bifasciatus* and *H. igneus*. These relationships of *G. bergii* would also be odd from a morphological point of view, but biogeographically more plausible than its sister-group relationship with *A. brucutu*. In a test removing *G. bergii* from the analysis, *A. brucutu* was obtained as the sister-group of ‘*Hasemanianus crenuchoides*’ and ‘*Hyphessobrycon langeanii*’, forming with *A. cremnobates*, *A. dissimilis*, *A. laticeps*, *A. serratus* and an undescribed species, the sister-group of *Psolidodon*. Thus, the independent removal of both *A. brucutu* and *G. bergii* from the analyses resulted in topologies that in some sense seem more congruent with the morphology and/or distribution of those species. This issue would probably be resolved with the addition of DNA data for *A. brucutu*. The relationships of the monotypic *G. bergii* are especially important from a taxonomic point of view, given that *Gymnocharacinus* is the nominotypical genus of Gymnocharacini.

According to our results, *Astyanax* is restricted to the Central- and North-American clade (including the type species of the genus), the *Astyanax bimaculatus* group and related species, plus *Astyanax microlepis* as the sister-group of both clades. The Central- and North-American species of *Astyanax* were recently reviewed by Schmitter-Soto (2017). However, that author did not consider some important characters in the diagnoses or in the description, like the number of maxillary teeth or the number of teeth cusps. Little importance was also given to the number of anal-fin rays and the presence or absence of *circuli* on posterior field of scales was not even mentioned. Instead, he put much importance on some osteological characters extracted from Schmitter-Soto (2016), many of which are intraspecifically variable in other characids and maybe also in *Astyanax*. This renders the identification of Central- and North-American species of *Astyanax* rather difficult unless by their geographical locality. Thus, the validity of some species should be further evaluated.

The new genus *Andromakhe* includes several species with an unusually high number of maxillary teeth among those classified in *Astyanax*. Most of the species in this genus were relatively recently described (Azpelicueta et al., 2002, 2003; Casciotta et al., 2003a; Mirande et al., 2004), as also ‘*Astyanax taurorum*’ (Lucena et al., 2017) that differs from *Andromakhe paris* mainly by the presence of secondary sexual

hooks in mature males (vs. absence in *A. paris*) and probably should also be transferred to *Andromakhe*. ‘*Astyanax*’ *bagual* Bertaco & Vigo, 2015 and ‘*Astyanax*’ *douradilho* Bertaco, 2014 may also belong to this genus, but the phylogenetic relationships of these species are pending further investigation.

The resurrection of *Psalidodon* is herein proposed even if moderately supported in its current definition, given that its monophyly was rather stable, with only the inclusion or exclusion of one or a few taxa in some of the analyses performed. *Psalidodon*, with this definition, becomes the richest genus in the Gymnocharacini, with 37 examined species, but probably including more taxa whose phylogenetic relationships still have to be evaluated. The sister-group of *Oligosarcus* Günther in our analysis is a weakly supported clade composed of *Psalidodon* and two smaller clades with, respectively, part of the *Astyanax scabripinnis* (Jenyns, 1842) group and a set of species including ‘*Astyanax*’ *dissimilis* Garavello & Sampaio, 2010, *Hasemanian crenuchoides* Zarske & Géry, 1999, ‘*Hyphessobrycon*’ *langeanii* Lima & Moreira, 2003 and an undescribed species attributable to *Astyanax*, according to the traditional definition of the genus. The first of those clades, composed of ‘*Astyanax*’ *cremnobates* Bertaco & Malabarba, 2001, ‘*Astyanax*’ *laticeps* (Cope, 1894) and ‘*Astyanax*’ *serratus* Garavello & Sampaio, 2010, had a relatively good support, while the second one was weakly supported and unstable. We decided to restrict *Psalidodon* to a less inclusive clade in order to gain stability and support for the genus, but apparently there is no other available generic name for the species included in the successive sister-groups of this genus (we did not analyse the type species of *Hasemanian*). Therefore, if the relationships herein obtained are further corroborated, the entire sister-group of *Oligosarcus* could be transferred to *Psalidodon*.

The available phylogenetic hypotheses of *Astyanax* previous to this study were restricted to general analyses of the Characidae, including a few taxa of this genus (Mirande, 2009, 2010, 2019; Mirande *et al.*, 2011; Oliveira *et al.*, 2011), a molecular study based on *COI* gene that was oriented mainly to the utility of that marker in the delimitation of species (Rossini *et al.*, 2016) and studies focused on Central- and North-American species of the genus (Ornelas-García *et al.*, 2008; Schmitter-Soto, 2016). Results of articles in the first and second groups are hardly comparable with the present ones, given the low information content regarding *Astyanax* in the first case and the lack of outgroups in Rossini *et al.* (2016). The phylogenetic hypothesis by Ornelas-García *et al.* (2008) is also difficult to compare, because many DNA samples they used were obtained from species or populations that were reclassified by Schmitter-Soto (2017). In this contribution we tentatively reassigned those sequences

to the species of Schmitter-Soto (2017) according to their locality, but their identification should be further corroborated, as the validity of the taxonomy proposed by that author. The phylogenetic analysis of Schmitter-Soto (2016) was based on 52 morphological characters and 35 populations of *Astyanax* and *Bramocharax* considered as different terminal taxa, plus a limited outgroup composed of three species. We surveyed those characters to evaluate if they were informative for the present analyses and found that, among those not already considered by Mirande (2019), most characters were intraspecifically variable, not definable in unambiguous discrete states, or even flawed, as his character 2, whose state 1 described a presumably broken lower fifth ceratobranchial tooth plate (Schmitter-Soto, 2016: fig. 2b). However, even considering those shortcomings, the synonymy of *Bramocharax* with *Astyanax* is an important point of congruence between the present analysis and the conclusions of both Ornelas-García *et al.* (2008) and Schmitter-Soto (2016).

CONCLUSION

In this paper we did not intend to propose a definitive phylogeny of *Astyanax*, because we are aware that the amount of evidence per species is still low and that several species currently attributed to the genus were not included in our analyses. However, results from the different analyses herein performed are highly stable and congruent with the final hypothesis proposed. The complete replacement of the classic definition of *Astyanax* with a phylogeny-based taxonomy will require further expansion of this dataset, both in the number of species and characters (morphology and molecular). This will eventually lead to an evaluation of the generic assignment of all remaining species currently attributed to the genus.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Appendix S1. List of examined specimens.

Appendix S2. List of new DNA sequences generated for this study.

Appendix S3. TNT files, including the analysed dataset (astyanax.tnt), the script to select the overall most parsimonious trees (ompt.run), the list with the points for each MPT according to that script (overallMPT.txt) and samples of ten MPTs obtained on each of the explored analytical condition (alltrees.zip). This includes the final hypothesis (BLK41.tre) and the consensus of the MPT under equal weights (equal.tre). All trees are in parenthetical TNT format.

Appendix S4. Graphical trees in.svg format, including the final hypothesis showing number of nodes (final_nodes.svg) and GC-values (supports.svg) and the consensus of the MPTs under equal weights (equal_nodes.svg).

Appendix 5. List of synapomorphies. Node numbers correspond to the consensus tree of the Appendix S4 (final_nodes.svg).

Appendix 6. List of nomenclatural changes herein proposed.