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# Morphology, molecules and the phylogeny of Characidae (Teleostei, Characiformes)

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**Abstract**

This is the most comprehensive phylogenetic analysis of the Characidae to date and the first large-scale hypothesis of the family, combining myriad morphological data with molecular information. A total of 520 morphological characters were analysed herein, of which 98 are newly defined. Among the analysed taxa, 259 species were coded by examining specimens, three fossil species were coded from the literature, one species was coded almost completely from published figures, 122 were partially coded from the literature, and 88 were analysed exclusively from molecular data. The total number of species in the analysed dataset is 473. Analyses were made by parsimony under equal and extended implied weighting with a broad range of parameters. The final hypothesis was selected using a stability criterion that chooses among the most parsimonious trees of all searches. It was found by weighting molecular characters with the average homoplasy of entire partitions (markers). The resulting hypothesis is congruent with previous molecular-based phylogenies of the family. The Characidae are monophyletic, with four main clades: the Spintherobolinae new subfamily; an expanded Stethaprioninae including the Grundulini, Gymnocharacini, Rhoadsiini and Stethaprionini; the Stevardiinae; and a clade composed of the Aphyocharacinae, Characinae, Cheirodontinae, Exodontinae and Tetragonopterinae. Also, a stem Characidae was found, as formed by the Eocene–Oligocene genera †*Bryconetes* and †*Paleotetra* as successive sister groups of extant members of the family. A subfamilial classification is proposed, but deep changes in the systematics that are beyond the scope of this study are still needed to classify the Characidae into monophyletic genera.



## Introduction

The Characidae are the most diverse family of Neotropical fishes, with more than 1150 known species, of which 231 were described in the last 10 years (Eschmeyer and Fong, 2017), suggesting that many species are still to be discovered and described. Most members of the Characidae are small-sized fishes, <8 cm in standard length (SL), reaching as much as 20 cm in some predatory genera. There also are several miniature species reaching 26 mm SL or less (Weitzman and Vari, 1988). Many fishes of the family are kept as ornamental fishes and known in the aquarium market under the popular name of "tetras".

Characids are primarily distributed in virtually all freshwater basins from southern USA to northern Patagonia, in Argentina, but they are especially diverse in tropical South America. The Characidae are ranked fourth among actinopterygian fishes in terms of number of species, after Cyprinidae, Gobiidae and Cichlidae, and are geographically the most restricted of these. In the Actinopterygii, only the African cichlids (Pseudocrenilabrinae) show a comparable radiation: 1104 species restricted to the African continent (e.g. Muschick et al., 2012; Santos and Salzburger, 2012; Wagner et al., 2012). Considering other vertebrates, the radiation of the Characidae in South America is comparable to that of the marsupials in Australia, both exploiting habitats that in most of the remaining continents were inhabited by other dominant groups—respectively, the Cypriniformes (Briggs, 1979, 2005) and placental mammals (Clemens, 1968; Nilsson et al.,

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2010). The Alestidae (Characiformes), often considered the counterpart of the Characidae in Africa (e.g. Géry, 1977; Zanata and Vari, 2005), evolved under the competitive pressure of the barbs and relatives (Cyprinidae), and their extant richness is less than one tenth the number of characid species (Briggs, 2005; Eschmeyer and Fong, 2017).

The morphology of the Characidae is highly conservative, with most of their variation related to some extent with either miniaturization events or ecological habits, including breeding and feeding (Mirande, 2010). Miniaturization events in the Characidae (with species smaller than 26 mm SL; Mattox et al., 2013) are most common in the tropical environments of South America. Most miniature characids show reductive characters (i.e. loss of laterosensory canals or even entire bones) (e.g. Weitzman and Fink, 1983; Rüber et al., 2007; Mirande, 2010; Mattox et al., 2013), whose correlation with the phylogeny may only be evaluated in comprehensive analyses. Many characids exhibit features that are either autapomorphic or support clades composed of a few species, such as the loss of scales in the Patagonian naked characin *Gymnocharacinus* or the conspicuously red snout and head of the rummy-nose tetras *Hemigrammus bleheri*, *Hemigrammus rhodostomus* and *Petitella georgiae*. Given the conservative morphology of the Characidae and the numerous features arguably correlated with ecological traits (i.e. predation), convergences derived from a single evolutionary process (e.g. miniaturization), or specialized and/or autapomorphic features (e.g. loss of scales), the discovery of additional morphological characters relevant to the phylogeny is increasingly difficult. Deep nodes in the Characidae had low support in previous hypotheses (e.g. Mirande, 2010; Ohara et al., 2017), which renders this search for new data necessary to gain resolution, nodal supports and diagnoses based on synapomorphies.

Several lines of research have been focused on different potential sources of phylogenetic characters in the Characidae: musculature (Datovo and Castro, 2012), sperm (e.g. Baicere-Silva et al., 2011a,b; Ferreira et al., 2011; Santana et al., 2013), gill-derived glands (Oliveira et al., 2012; Terán et al., 2014) and the alimentary system (Alonso et al., 2015). However, most of the morphological data used in the literature for phylogenetic analyses of the Characidae refer to the skeleton and musculature (e.g. Mirande, 2010; Vanegas-Rios, 2018).

The temporal diversification of the Characidae is unclear. Fossil characids are scarce, with only four described species, all of them from freshwater deposits: †*Bryconetes enigmaticus* and †*Paleotetra* spp. from the Eocene–Oligocene (Entre-Córregos Formation, Minas Gerais, Brazil) and †*Megacheirodon unicus* from the Oligocene–Miocene (Tremembé Formation, São Paulo, Brazil). †*M. unicus* was hypothesized to be related to *Spintherobolus* (Malabarba, 1998). †*Brycon avus* and †*Lignobrycon ligniticus* are also from the Tremembé Formation, and after the systematic proposal of Oliveira et al. (2011) are classified in the Bryconidae and Triportheidae, respectively. †*Bryconetes* and †*Paleotetra* are morphologically generalized taxa, like most members of the Characidae. However, †*Bryconetes* has a supraorbital bone, as in most non-characid Characiformes, which is absent in †*Paleotetra*. This supported the hypothesis of †*Bryconetes* as a stem characid, proposed by Weiss et al. (2014). Relationships of †*Paleotetra* were not explicitly stated (Weiss et al., 2012).

Phylogenetic relationships within the Characidae were poorly known until recently, when different approaches using both molecular (Ortí and Meyer, 1997; Calcagnotto et al., 2005; Javonillo et al., 2010; Oliveira et al., 2011; Arcila et al., 2017) and morphological (Mirande, 2009, 2010; Mirande et al., 2011, 2013; Ohara et al., 2017) data were published. Two alternative groups of hypotheses and classifications for the Characidae have been proposed, corresponding to the morphological (Mirande, 2010; Mirande et al., 2011) and molecular approaches (Oliveira et al., 2011). Those classifications were surprisingly congruent, given the difference in data sources and taxon sampling upon which the phylogenetic analyses were based. However, some incompatibilities in those results and the different nomenclatural decisions that they produced resulted in relatively deep discrepancies in the current classifications of the family (compare Mirande, 2010 and Oliveira et al., 2011). At the moment, no global phylogeny of the Characidae including most of the available molecular and morphological data has been published.

Mirande (2010) treated the Characidae as including most of the genera historically classified in the family, such as *Agoniates*, *Brycon* and *Salminus*. Oliveira et al. (2011), conversely, restricted the Characidae to a clade diagnosed by the lack of a supraorbital bone, which also was recovered in the morphology-based hypotheses (e.g. Mirande, 2010), transferring many species traditionally included in the Characidae to the families Bryconidae, Chalceidae, Iguanodectidae and Triportheidae. Oliveira et al. (2011) also resurrected and expanded the Acestrorhynchidae to include the Heterocharacinae and Roestinae. These subfamilies were previously included in the Characidae and Cynodontidae, respectively (Lucena and Menezes, 1998; Mirande, 2010).

Although the restriction of the Characidae to the clade of species lacking a supraorbital bone was compatible with both the molecular and morphological approaches and could be considered a solution to reach stability in the definition of the family, the new families defined by Oliveira et al. (2011) were not supported by analyses based on morphological data.

According to Mirande (2009, 2010) and Mirande et al. (2011), the Acestrorhynchidae, Bryconidae and Triportheidae, as treated by Oliveira et al. (2011), were not monophyletic, whereas *Chalceus* was the sister group of the African Alestidae (as originally proposed by Zanata and Vari, 2005).

Mirande (2010) also discussed a possible classification of the Characidae in terms of subfamilies or subfamily-level clades and the possible phylogenetic relationships of the *incertae sedis* genera of Characidae sensu Lima et al. (2003). More recently, there have been several approaches to assessing the phylogenetic relationships of different clades of Characidae, based on either molecular or morphological data. Mattox and Toledo-Piza (2012) published a comprehensive phylogeny of the Characinae based on morphological characters. Tagliacollo et al. (2012) proposed a mostly molecular phylogeny of the Aphyocharacinae, but also provided some new morphological characters for the subfamily. Santana et al. (2013) published a phylogenetic analysis of *Moenkhausia* based on sperm morphology, providing data from reproductive cells and habits. Mariguela et al. (2013) treated the phylogeny of the Cheirodontinae based on molecular data. Thomaz et al. (2015) published a molecular phylogenetic hypothesis of the Stevardiinae, proposing many nomenclatural changes with various levels of support. In the latter contribution, a tribal classification of the Stevardiinae was proposed, leaving ten genera not analysed as *incertae sedis* within the subfamily. Among the molecular phylogenetic studies of the Characidae, Tagliacollo et al.'s (2012) was the only one that combined that information with some morphological characters.

The aim of the present article is to produce an updated phylogenetic hypothesis of the Characidae in the light of additional morphological information and to combine it with published molecular data. The morphological partition of data includes the characters from Mirande (2009, 2010), Mirande et al. (2011, 2013) and Ohara et al. (2017), plus 133 morphological characters coded for 53 more species than the most taxon-dense previous morphological analysis (Ohara et al., 2017). This total evidence study is the most comprehensive phylogenetic analysis of the family to date; in conjunction with the above-mentioned morphology-based articles, this comparative anatomy of the Characidae involves the largest ever number of characters and species.

## Material and methods

### *Morphological characters*

A total of 520 morphological characters are analysed herein. Among them, 387 were examined by the author in previous contributions (Mirande, 2009, 2010; Mirande et al., 2011, 2013; Ohara et al., 2017), 34 were taken from other published phylogenetic studies of the Characidae (Vari and Harold, 2001; Menezes and Weitzman, 2009; Mattox and Toledo-Piza, 2012), and 98 are newly defined characters. Fourteen characters were modified from Mirande (2010). The list of characters with figures and explanations is provided as Appendix S1. Eight characters of Mirande (2010) and 13 of Mirande et al. (2013), were removed from the present analysis for various reasons (Appendix S1). Most characters are osteological (474), with the remaining ones derived from myology (18), coloration (13), external features (6), cytogenetics (5) or histology (4). Virtually all osteological characters have the potential to be coded in fossils. Indeed, †*B. enigmaticus* was coded for 155 characters, †*Paleotetra aiuruoca* for 134 and †*Paleotetra entrecorregos* for 160. Fossil taxa were coded for the characters of Mirande (2010) in their descriptions (Weiss et al., 2012, 2014), but the specimens were not available to be coded for the new characters defined herein. Therefore, an examination of the specimens of †*Bryconetes* and †*Paleotetra* may eventually increase the available data for these taxa.

The morphological dataset includes 263 terminal taxa, of which 259 were coded for this study through the examination of museum specimens, three fossil species were coded by Weiss et al. (2012, 2014) for the characters of Mirande (2010), and one species was herein coded from figures published in Mattox et al. (2013). The dataset also includes 122 species coded partially from the literature in order to expand overlap between morphological and molecular data. Relative to previous phylogenetic analyses, *Puntigrus tetrazona* and *Brycon meeki* were excluded from this analysis. The former species was used to root all previous morphology-based analyses, but given the uncertain relationships between the Cypriniformes and the Characiformes, and the proposed nonmonophyly of the latter order (e.g. Chakrabarty et al., 2017; Mirande, 2017), it was herein preferred to root this analysis with *Distichodus maculatus*. After the analyses, trees were re-rooted with *Citharinus congicus*, to obtain a monophyletic Distichodontidae. *Brycon meeki* was coded by Mirande (2009) exclusively from the literature (Weitzman, 1962), and it had many missing entries in the morphological partition. As there are no available DNA sequences for that species and it forms part of the outgroup, after the restriction of the Characidae by Oliveira et al. (2011), it was preferred to exclude *B. meeki* from this analysis. With those two exclusions, the total number of additional species was raised to 51, instead of the 53 coded for this study.

All of the extant species whose morphological data were analysed have been examined by the author, excepting *Cyanogaster noctivaga*, which was coded from the figures provided by Mattox et al. (2013).

Codings of *Ectrepopterus uruguayensis* and *Hyphessobrycon compressus* were partially based on Malabarba and Jerep (2012). *Erythrocharax altipinnis* was completely re-coded given incongruences found in the coding with the text and figures provided by Netto-Ferreira et al. (2013). Many species had available molecular data, but their morphology could not be coded herein. Among those taxa, 122 species of Characidae with at least four available DNA markers were partially coded for various morphological characters from data in the literature and included in the analyses. *Moenkhausia australe* has been considered in the literature either as a synonym of *Moenkhausia sanctaefilomenae* (Mirande and Koerber, 2015) or as a valid species (Benine et al., 2009; Azevedo-Santos and Benine, 2016) and was herein treated as a separate terminal taxon.

Osteological and myological preparations followed Taylor and van Dyke (1985) with modifications by Datovo and Castro (2012). The analysed histological and cytogenetic characters were coded from the literature. The complete list of examined material and museum acronyms is provided as Appendix S2. The list of species coded from literature and the articles taken as references are listed in the Appendix S3.

Osteological nomenclature follows Weitzman (1962) with modifications by Zanata and Vari (2005), which were based mostly on Nelson (1969), Patterson (1975), and Fink and Fink (1981, 1996). Myological nomenclature follows Datovo and Castro (2012). Authorities of family-level groups were corroborated in Van der Laan et al. (2014). The use of "stem" refers to fossil *incertae sedis* clade(s) or species that are successive sister groups of a "crown" group composed of the extant species (Budd and Jensen, 2000). A valid alternative to the use of stem/crown groups is to define new suprageneric taxa for such fossil taxa. However, given that the fossils were not examined for this study and their codings were taken from literature (Weiss et al., 2012, 2014), it was preferred to use this more informal nomenclature, pending corroboration of the results proposed herein.

### *Molecular data*

Analysed markers include four mitochondrial (cox1, cytb, and ribosomal 12S and 16S) and four nuclear (myh6, ptchd1, rag1 and rag2). Those markers were chosen because they are best represented among the analysed species. DNA sequences were extracted from GenBank using Gb-to-TNT (Goloboff and Catalano, 2012) and aligned with Muscle (Edgar, 2004) using default settings. Alignment of molecular data was trivial for coding sequences (cox1, cytb, myh6, ptchd1, rag1 and rag2), which almost lacked gaps. Ribosomal sequences had some stable and some gap regions, but the overall alignments were clean enough to allow the inclusion of all the available information, without the need to crop part of the sequences. No manual editing of the alignments was done. Gaps were considered as missing data. Several tests to detect wrongly attributed sequences were performed, such as comparing the results of phylogenetic analyses of each individual marker and the use of Blast (Altschul et al., 1990). The list of sequences removed from the dataset due to possible contamination or problems in the species identification is provided as Appendix S4. When two or more reliable sequences of the same gene and species were available, they were merged using IUPAC codes for polymorphisms with Asado software (Nixon, 2004). The purpose of analysing consensus sequences was to sample molecular polymorphisms in the same manner that is regularly done with morphological characters, diminishing as much as possible spurious resolution provided by intraspecifically variable sites. Datasets corresponding to each individual marker, including all of the GenBank Accession numbers from which the consensus sequences for each species were obtained are provided as Appendix S5.

### *Dataset and analysis*

With the inclusion of all species having at least one available sequence of the analysed molecular markers, the complete dataset contained 859 terminal taxa and 6653 characters, of which 520 were morphological. The dataset was subsequently reduced for the analyses in order to obtain as many overlapping data as possible. Thus, the analysed dataset contains all of the species coded for morphological characters and ingroup species having information for at least four markers, even when not coded for morphology. Outgroup species were selected according to their systematic diversity, including representatives of the highest possible number of families and genera having at least four DNA markers in the matrix. The complete dataset and the TNT script to select which species were included in the final dataset are provided as Appendix S5. From these, 259 species were coded for morphology by examining specimens, whereas the coding of three fossil species were extracted from literature (Weiss et al., 2012, 2014), one species was coded from published figures (Mattox et al., 2013), 122 were coded only partially, from diverse publications (Appendix S4), and 88 were analysed exclusively from molecular data. The analysed dataset contains 473 species. The morphological partition of data and the remaining supplementary appendices also are available online at MorphoBank P-2722 (O'Leary and Kaufman, 2011, 2012). The origin of the data herein analysed and comparisons with previous phylogenetic analyses of the Characidae are shown in Fig. 1. The missing entries are 45% in the

analysed dataset and 56% in the complete dataset. In the analysed dataset, the morphological and molecular partitions have 44% and 45% of missing entries, respectively (Fig. 1). The list of species in the complete dataset, showing which taxa have data for each block, is provided as Appendix S6.

Parsimony-informative and total numbers of characters by partitions of data (morphology and each molecular marker) are provided in Table 1. All of the analyses were done under parsimony using TNT (Goloboff et al., 2008). Searches included rounds of tree fusing, sectorial searches, tree drifting (Goloboff, 1999) and tree ratchet (Nixon, 1999), stopping each search after the optimal fit had been hit three times. Results under different searches were checked *a posteriori* with tree fusing, using as source trees the most parsimonious trees (MPTs) obtained under all of the remaining analyses. Searches were performed under extended implied weighting (Goloboff, 1993, 2014). The original implied weighting method (Goloboff, 1993) had (at least potentially) a bias to give artificially higher weights to characters with many missing entries (Goloboff, 2014). When combining morphological and molecular data, this bias is usually higher and uneven given that entire blocks of data are often missing for many species. Thus, with the same proportion of homoplasy, the less the known data for a marker, the higher weights the characters (columns) of that marker will receive. The extended implied weighting method corrects the weighting strength during the calculations by assigning homoplastic steps to the missing characters, which are proportional to the homoplasy of the observed ones (Goloboff, 2014). This reduces the effect of missing data in the final calculations of character weights.

Molecular phylogenetic analyses (either under parsimony or probabilistic approaches) treat each aligned column as a separate character. Thus, weights are applied to each column independently from its neighbours. However, molecular characters are the product of alignments and the data in a given position for different taxa are often hardly comparable (e.g. Wheeler

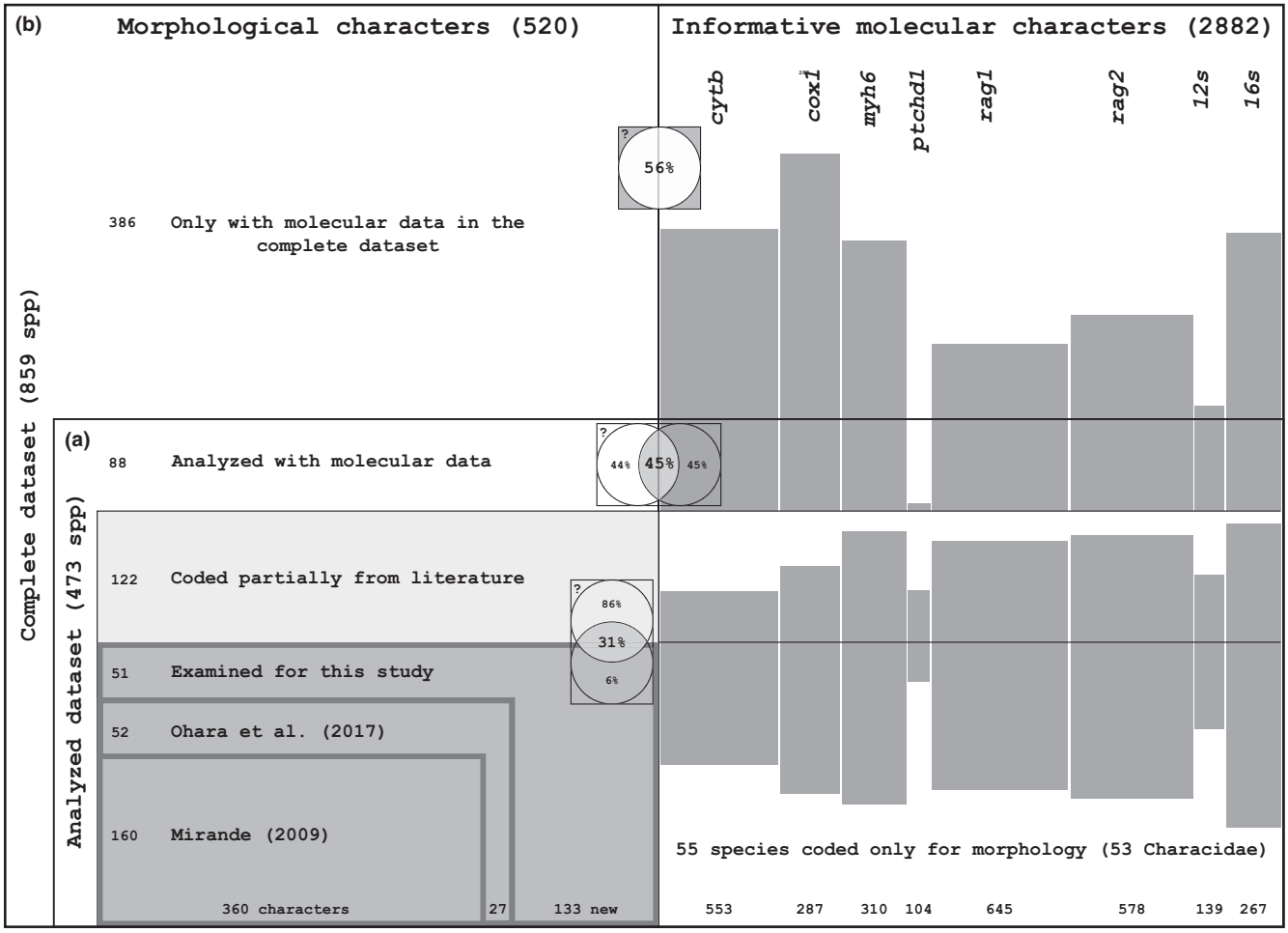


Fig. 1. Origin of the analyzed data in comparison with previous contributions. A: analyzed dataset; B: complete dataset. Percentage of missing entries of different sets of data are shown in circles, highlighting in larger fonts those of the examined dataset and those of the complete dataset.

Table 1
Partitions of data analysed, indicating the number of informative characters and maximum and minimum numbers of steps for each one and for the complete dataset.

| Block | Sites-characters | Informative | Min. steps | Max. steps |
|---|---|---|---|---|
| Morphology | 520 | 500 | 530 | 12 724 |
| cox1 | 651 | 287 | 620 | 22 956 |
| cytb | 990 | 553 | 1270 | 34 224 |
| 12s | 361 | 139 | 296 | 3420 |
| 16s | 547 | 267 | 598 | 14 075 |
| myh6 | 750 | 310 | 597 | 12 329 |
| ptchd1 | 537 | 104 | 184 | 1567 |
| rag1 | 1265 | 645 | 1482 | 22 313 |
| rag2 | 1032 | 578 | 1222 | 18 223 |
| Total | 6653 | 3383 | 6799 | 141 831 |

et al., 1995; Wheeler, 2001). This is partially solved using conservative sequences, amino acids instead of nucleotides, or consensus of sequences by species in order to consider polymorphic sites. The extended implied weighting (Goloboff, 2014) not only corrects the weighting strength according to the missing entries, but also considers the average homoplasy of either gene regions, codon positions or whole markers during the calculations of weights. Implied weighting calculations based either on the homoplasy of single molecular characters or on different groupings of characters are herein named "weighting schemes". Variations on the value of $k$ (concavity constant; Goloboff, 1993) are referred to as "weighting strengths".

Different weighting strengths within each scheme were sampled by using five reference values of $k$ in which an average character had, respectively, 60, 65, 70, 75 and 80% of the weight of a completely hierarchical one (i.e. without homoplasy). Those values of $k$ (19, 24, 30, 38 and 51, respectively) were combined with four weighting schemes for molecular characters: (1) SEP: each column weighted separately according to its own homoplasy; (2) COD: groups of three columns (codons, in the case of coding sequences) weighted according to their average homoplasy; (3) BLK: all characters of each molecular block weighted according to the average homoplasy of the whole marker; and (4) POS: sites of each codon position of the coding (nonribosomal) sequences weighted according to the average homoplasy of all characters of the same position in each block. In the last scheme, ribosomal characters were weighted according to the average homoplasy of their partititions. Morphological characters were weighted according to their own homoplasy under all of the weighting schemes. Combining the five weighting strengths and the four described weighting schemes, 20 analytical conditions were explored under extended implied weighting, along with a search under equal weighting. MPTs from the 20 searches under extended implied weighting were submitted to a kind of sensitivity analysis (Wheeler et al., 1995; Whiting et al., 1997; Prendini, 2000; Giribet, 2003) to assess stability of clades by comparing tree topologies. Such comparisons were made through SPR distances (Goloboff, 2008) and the distortion coefficient (Farris, 1989). As in Mirande (2009), the criterion to select a final hypothesis was to evaluate which of the most parsimonious topologies obtained in the different analyses, as an average, was more similar to the remaining ones. Similarity was used as a measure of the global stability of each of the most parsimonious hypotheses. The aim was not to find a particular set of conditions to derive the final hypothesis (as in a typical sensitivity analysis), but to obtain a series of values of $k$ producing relatively stable results and to test how the clades were affected by variations in the weighting parameters (Mirande, 2009). After selecting one of the 20 initial most parsimonious hypotheses as the globally most stable, an additional exploration of contiguous $k$-values of 24–46 under the weighting scheme selected in the first round of searches was done. The purpose of this procedure was to obtain the broadest possible range of $k$-values whose MPTs were similar enough to produce a reasonably well-resolved strict consensus.

This sensitivity analysis differs from either selecting some particular set of parameters (under equal or implied weighting) or condensing the results obtained under all of the explored conditions. Compared with selecting some particular conditions, this procedure risks losing some resolution (informativeness) to gain corroboration (robustness) (Mirande, 2009). Support was calculated through 300 replicates of symmetric resampling (probability of change 0.33) with searches of each resampled matrix using sectorial searches and tree fusing (Goloboff, 1999). Results are expressed as differences of frequencies "Group present/Contradicted" (GC-values) (Goloboff et al., 2003).

## Results

The 20 analytical conditions of the first round of searches under extended implied weighting resulted in MPTs ranging from 58 148 to 58 397 steps. MPTs

from the different searches were rather divergent, but shared 234 out of 472 possible nodes. Grouping columns by contiguous sectors to collectively weight characters (BLK and COD) produced more stable results than grouping by positions or weighting each column separately (POS and SEP). This agrees with the analyses by Mirande (2017), in which the globally most stable results were obtained by grouping every three contiguous positions (COD). Minimum SPR movements to convert the MPTs obtained under some particular conditions to those obtained in all the remaining ones was used as a measure of stability (see Materials and Methods section and Mirande, 2009 for details). Grouping entire blocks (BLK) needed an average of 67 movements, with a minimum of 60, when analysing with $k = 38$. Grouping each three positions (COD) needed an average of 69 movements, whereas not grouping columns (SEP) and grouping by codon positions (POS) needed 71 and 72 SPR movements, respectively. Those results agree with the comparisons made with the distortion coefficients and allowed one to choose BLK as the preferred weighting scheme for this analysis.

In the second round of searches, exploring contiguous values of $k$ between 24 and 46 under BLK obtained three similar topologies under $k = 34$–45, ranging from 58 161 to 58 158 steps. Among them, the same set of MPTs of 58 159 steps were obtained with $k = 35$–42 (fit of 863.96817 under $k = 39$). The final hypothesis is the strict consensus of the set of trees obtained between $k = 34$ and $k = 45$, but some results also are discussed in light of a wider range of $k$-values. As $k = 39$ is the mean value from which the final hypothesis was condensed, this value was used to estimate clade supports through resampling. MPTs from both rounds of searches are provided in Appendix S5. The final hypothesis is herein illustrated in Figs 2–5 and provided both in parenthetical (TNT and Newick) and .svg formats in the Appendices S5 and S7, respectively.

Under equal weights (EQW) more than 1000 trees of 58 097 steps were obtained. Their strict consensus is provided in parenthetical TNT and Newick (Appendix S5), and .svg formats (Appendix S7). The complete dataset, with 859 species analysed under $k = 39$ and BLK, produced more than 1000 MPTs with fit = 1008.28569 and 78 672 steps. The strict consensus tree (Appendix S5 in TNT and Newick; Appendix S7 in .svg format) and an agreement subtree containing 812 terminal taxa (Appendix S7, in .svg format) are provided. Major relationships between families and characid subfamilies are summarized in Fig. 2. The complete final hypothesis with support values is provided in Appendix S7.

Most of the characiform families (sensu Oliveira et al., 2011) were obtained as monophyletic. Only the Alestidae was not found as a clade, with *Arnoldichthys* as the sister group of *Hepsetus* and the remaining African alestids. The Neotropical genus *Chalceus*, included in the Alestidae by Zanata and Vari (2005), was obtained in a different clade than the African Alestidae and classified in the Chalceidae (sensu Oliveira et al., 2011). In the complete dataset, *Lepidarchus* (not analysed in the reduced dataset) was obtained as the sister group of *Arnoldichthys* and both form a clade with *Hepsetus*. The clade composed of the Alestidae and Hepsetidae includes all of the African representatives of the Characoidei. This African monophyletic group was obtained as the sister group of a large Neotropical clade, composed of the Cynodontidae, Erythrinidae, Hemiodontidae, Parodontidae, Serrasalmidae and the Anostomoidea.

The Characidae were obtained as monophyletic. The (Agoniatidae + Gasteropelecidae), the Bryconidae, the (Acestrorhynchidae + Iguanodectidae) and the Chalceidae are successive sister groups of the Characidae. The clade including all of those families is well-supported (GC = 95) and may be defined as the Characoidea. The Iguanodectidae include *Bryconops*, in addition to *Iguanodectes* and *Piabucus*. The Acestrorhynchidae, along with the nominotypical genus of the family, includes *Gilbertolus*, *Roestes* and the Heterocharacinae of Mirande (2010). The Bryconidae are composed of *Brycon*, *Chilobrycon*, *Henochilus* and *Salminus*, with the Central American and trans-Andean members of *Brycon* plus *Chilobrycon* as the sister group of *Salminus* plus a clade including *Brycon falcatus*, the type species of the genus. As in Mirande (2010), the Gasteropelecidae include *Engraulisoma* as the sister group of the remaining members of the family. The Agoniatidae are composed of *Lignobrycon* as the sister group of two clades, formed by (*Agoniates* + *Clupeacharax*) and *Triportheus*, respectively.

A stem Characidae (sensu Budd and Jensen, 2000) was obtained composed of the Brazilian Eocene–Oligocene genera †*Bryconetes* and †*Paleotetra* as successive sister groups of the eight subfamilies of the crown Characidae. Two large clades were obtained within the crown Characidae, one composed of the very diverse Stethaprioninae and the other composed of the remaining seven subfamilies. Under some of the explored parameters and in the complete dataset, however, the Spintherobolinae (see below) were obtained as the sister group of all of the remaining subfamilies.

The clade herein attributed to the Stethaprioninae (Fig. 4) includes most of the tetras traditionally classified in the Tetragonopterinae, such as the diverse *Astyanax*, *Hemigrammus*, *Hyphessobrycon* and *Moenkhausia*, along with many other less diverse genera (see Table 2). This subfamily, however, had relatively low support in this analysis. In the current definition, the Stethaprioninae are the most diverse

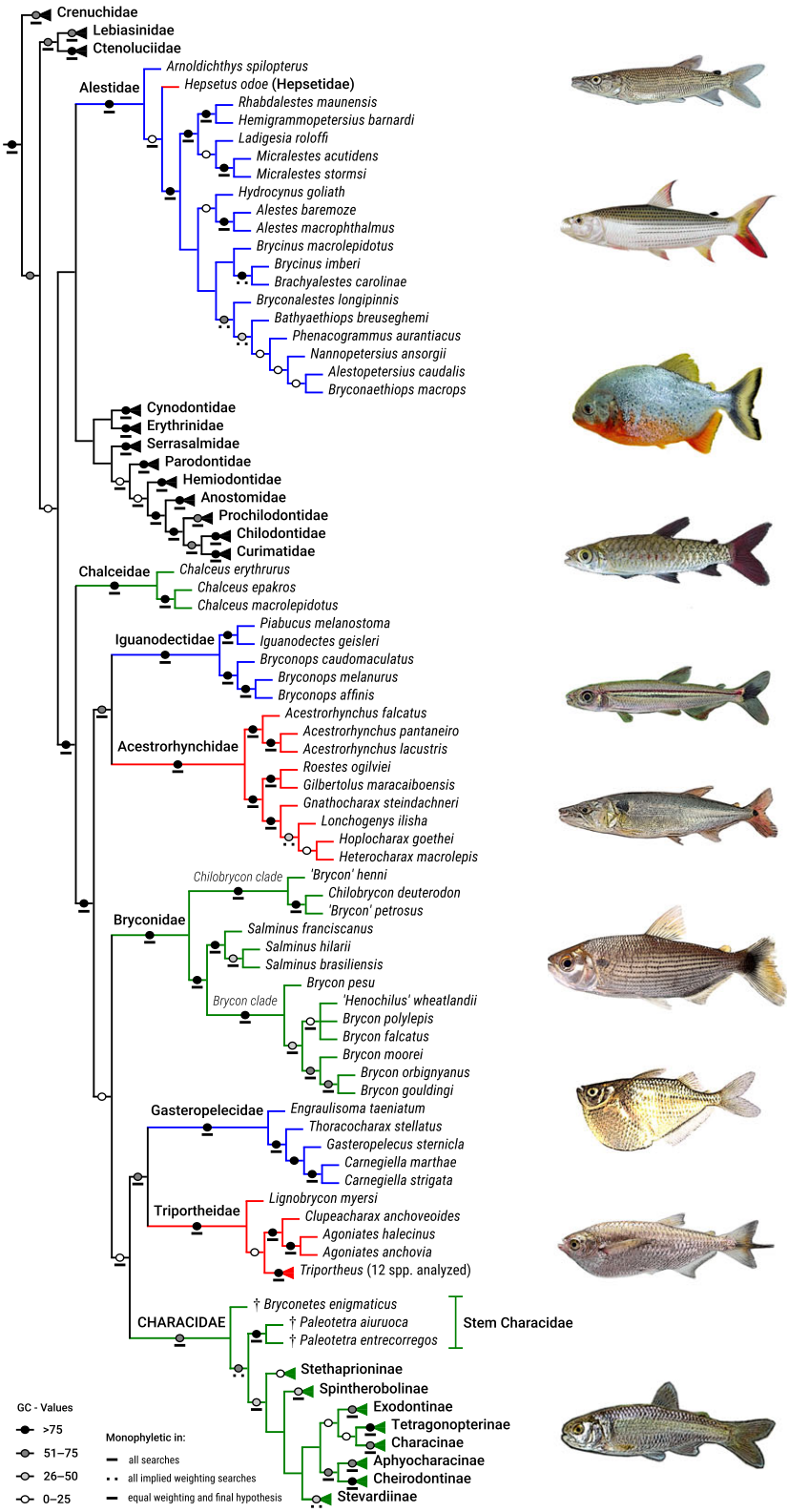


Fig. 2. General topology of the final hypothesis as obtained from the combined phylogenetic analysis under parsimony and extended implied weighting (BLK; $k$ = 39), showing relationships between characiform families, characid subfamilies, and details of the Alestidae, Acestrorhynchidae, Bryconidae, Gasteropelecidae, Agoniatidae, stem Characidae and Spintherobolinae. (Fit = 863.96817; Length = 58 159 steps). Figures 3–5 show details of the subfamilies of Characidae. [Colour figure can be viewed at wileyonlinelibrary.com]

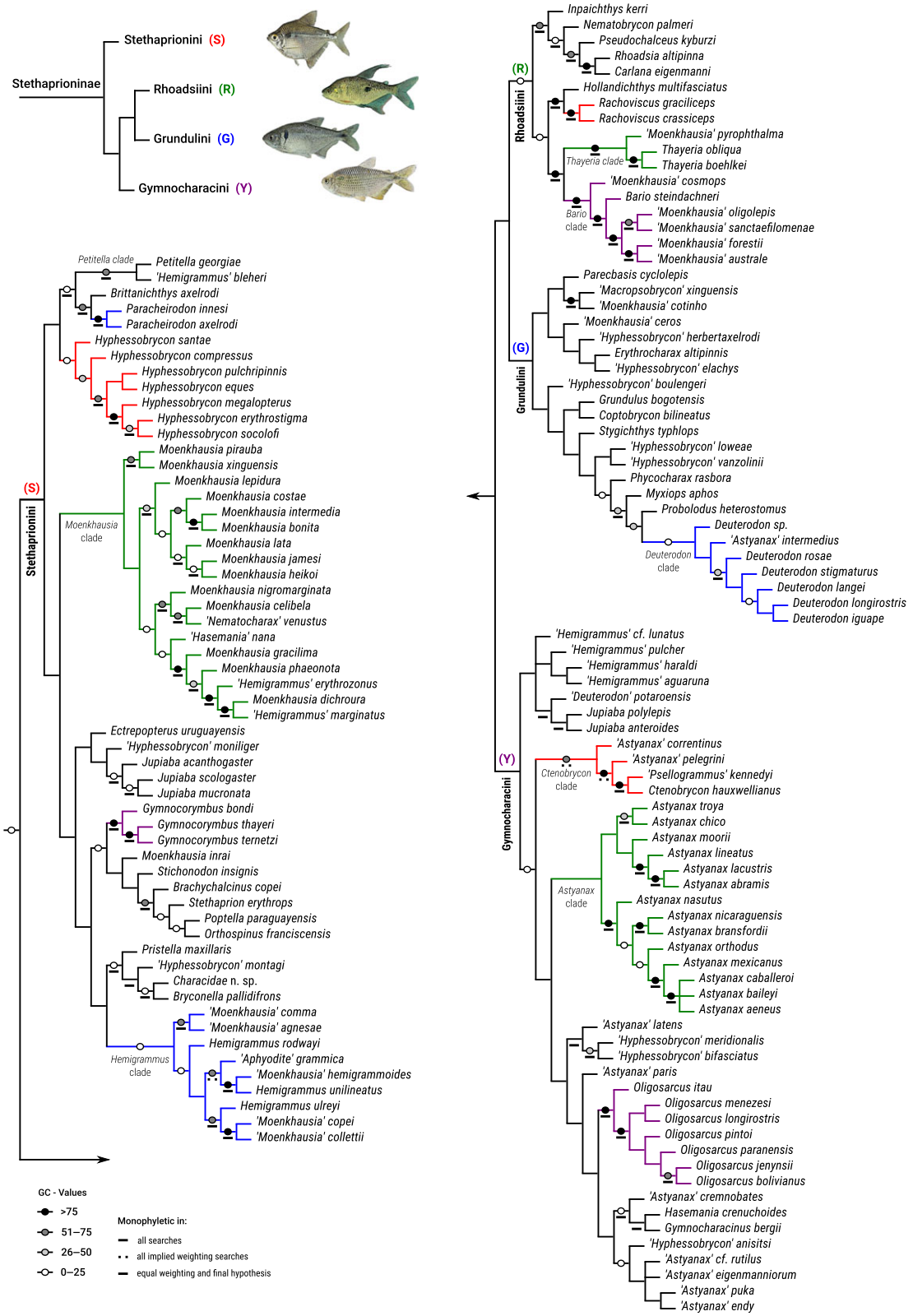


Fig. 3. Phylogenetic relationships among the Stethaprioninae as obtained from the combined phylogenetic analysis under parsimony and extended implied weighting. (BLK; $k$ = 39; Fit = 863.96817; Length = 58 159 steps). Excerpt of the phylogenetic tree shown in Fig. 2. Informal names are given to some generic clades to help in the discussion. [Colour figure can be viewed at wileyonlinelibrary.com]

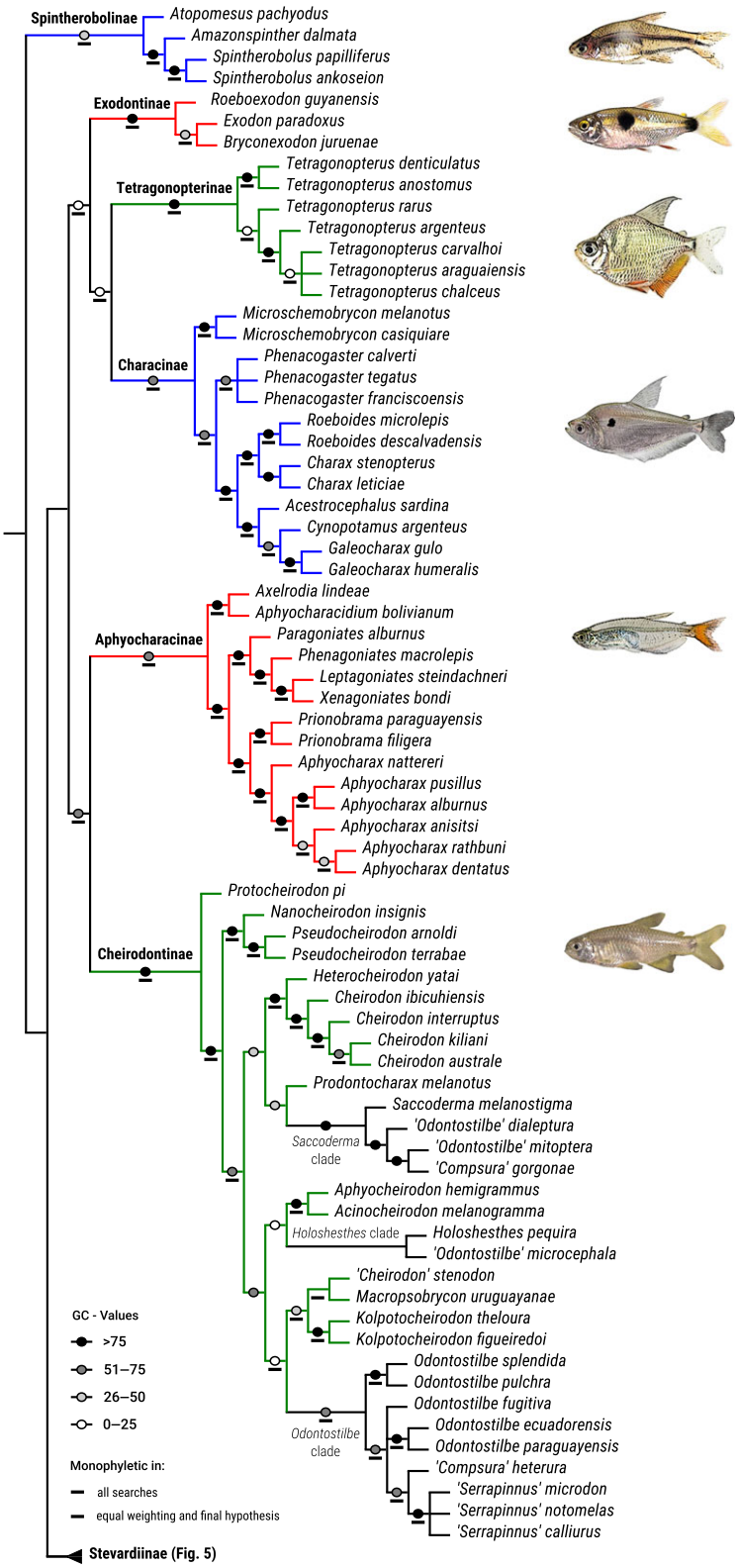


Fig. 4. Phylogenetic relationships among the Spintherobolinae, Aphyocharacinae, Cheirodontinae, Exodontinae, Tetragonopterinae, Characinae and Stevardiinae, with details of these six subfamilies. Obtained from the combined phylogenetic analysis under parsimony and extended implied weighting (BLK; $k$ = 39; Fit = 863.96817; Length = 58 159 steps). Excerpt of the phylogenetic tree shown in Fig. 2. Informal names are given to some genus-level clades to help in the discussion. [Colour figure can be viewed at wileyonlinelibrary.com]

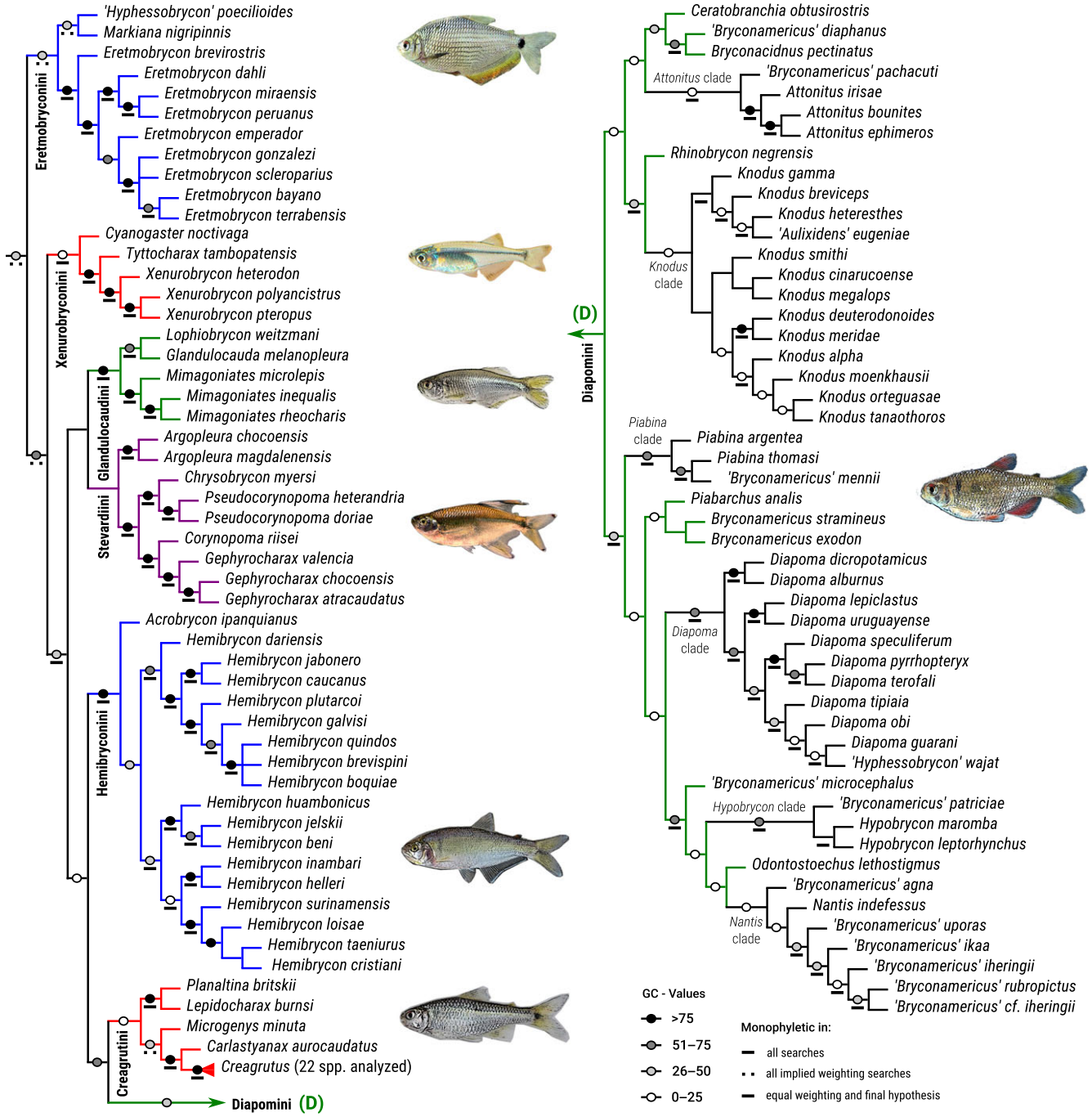


Fig. 5. Phylogenetic relationships of the Stevardiinae as obtained from the combined phylogenetic analysis under parsimony and extended implied weighting. (BLK; $k$ = 39; Fit = 863.96817; Length = 58 159 steps). Excerpt of the phylogenetic tree shown in Fig. 2. Informal names are given to some generic clades to help in the discussion. [Colour figure can be viewed at wileyonlinelibrary.com]

characid subfamily and, in order to define it in smaller units, four tribes are herein recognized: Stethaprionini Eigenmann, 1907 (Fig. 3), Grundulini Fowler, 1958, Gymnocharacini Eigenmann, 1909, and Rhoadsiini Fowler, 1911 (Fig. 3). The Stethaprioninae, but not their tribes, also were obtained as monophyletic in the complete dataset. Neither the Stethaprioninae nor their tribes were recovered under equal weights. The Stethaprionini (Fig. 3) are the most diverse tribe of Stethaprioninae, including the taxa classified in the subfamily by Reis (1989), diagnosed mainly by the presence of an anterior projection of the first unbranched dorsal-fin ray, plus the highly diverse *Hemigrammus*, *Hyphessobrycon* and *Moenkhausia*, and

several minor genera. Deep nodes of this tribe have low support and there are only a few moderate to highly supported clades, as the one composed of *Brittanichthys*, *Paracheirodon* and *Petitella* (including *H. bleheri*). A "*Moenkhausia* clade" was recovered as including most analysed species of the genus, along with *Hasemania nana*, *Hemigrammus erythrozonus*, *Hemigrammus marginatus* and *Nematocharax venustus*. This clade is weakly supported but rather stable among different searches. In the complete dataset, the "*Moenkhausia* clade" also includes *Astyanax vermilion* and *Hyphessobrycon parvellus*. A "*Hemigrammus* clade" is recovered as moderately well-supported and contains, in addition to some species of *Hemigrammus* (including its type species), *Aphyodite grammica* and five species currently classified in *Moenkhausia*. In the complete dataset this clade includes also *Moenkhausia grandisquamis* and *Moenkhausia melogramma*. The Stethaprionini contains the "true" *Hyphessobrycon* as a rather well-supported clade. However, there are many species currently attributed to *Hemigrammus*, *Hyphessobrycon* and *Moenkhausia* distributed among different clades.

The Rhoadsiini is the tribe with best support among the Stethaprioninae and includes three clades composed of *Rhoadsia* and relatives, *Hollandichthys* and *Rachoviscus*, and a group including the *Bario* and *Thayeria* clades. The "*Bario* clade" is composed of *Bario steindachneri* and five species of the *Moenkhausia oligolepis* group. The "*Thayeria* clade" includes *Moenkhausia pyrophthalma*, together with the species of *Thayeria*. Despite the high support of the Rhoadsiini in the reduced dataset, this tribe was not obtained as monophyletic in the complete dataset, due to the exclusion of the clade containing *Rhoadsia*. The Grundulini are composed of *Deuterodon*, some species currently classified in *Astyanax*, *Hyphessobrycon* and *Moenkhausia*, and other less diverse genera (Fig. 3; Table 1). In the complete dataset this tribe was not recovered, but noticeably, several species currently classified in *Astyanax* (mostly having only one *cox1* sequence) were related to *Deuterodon*, *Myxiops*, *Probolodus* and *Stygichthys*, all of them included in the Grundulini. The Gymnocharacini include mostly *Astyanax* and related genera, plus a weakly supported clade composed of some species classified in *Hyphessobrycon* plus *Deuterodon potaroensis* and two species of *Jupiaba*. The analysis of a cox1 sequence resulted in important changes in the relationships of the naked characin *Gymnocharacinus bergii* from previous hypotheses (e.g. Mirande, 2010), involving its inclusion in the clade of *Astyanax*, related to *Astyanax cremnobates* and *Hasemania crenuchoides*. Species of the predatory genus *Oligosarcus* were recovered as a well-supported monophyletic unit, in a clade that also includes several species of tetras with rather generalized morphology, such as *Astyanax*, *Hasemania* and *Hyphessobrycon*, but not the type species of *Astyanax* and *Hyphessobrycon* (that of *Hasemania* was not analysed). The clade including *Astyanax argentatus*, the type species of the genus, and other taxa from North and Central America, including species formerly in *Bramocharax*, is well-supported and stable across the analyses.

The Stethaprioninae were obtained as the sister group of a large clade composed of the seven remaining subfamilies. In that clade, the Spintherobolinae were found as the sister group of a large clade composed of the (Exodontinae (Characinae + Tetragonopterinae)), the (Aphyocharacinae + Cheirodontinae),

Table 2
Subfamilial status of the 141 genera of Characidae recognized in this study. Taxa with an asterisk were not examined herein, but some of them are attributed to a subfamily from information in the literature.

| Subfamilies of Characidae |
|---|
| **Aphyocharacinae:** *Aphyocharacidium*, *Aphyocharax*, *Leptagoniates*, *Paragoniates*, *Phenagoniates*, *Xenagoniates*; **Characinae:** *Acanthocharax**, *Acestrocephalus*, *Charax*, *Cynopotamus*, *Galeocharax*, *Microschemobrycon*, *Phenacogaster*, *Priocharax**, *Roeboides*. **Cheirodontinae:** *Acinocheirodon*, *Aphyocheirodon*, *Cheirodon*, *Cheirodontops**, *Compsura*, *Ctenocheirodon**, *Heterocheirodon*, *Kolpotocheirodon*, *Macropsobrycon*, *Nanocheirodon*, *Odontostilbe*, *Prodontocharax*, *Protocheirodon*, *Pseudocheirodon*, *Saccoderma*, *Serrapinnus*; **Exodontinae:** *Bryconexodon*, *Exodon*, *Roeboexodon*; **Spintherobolinae:** *Amazonspinther*, *Atopomesus*, *Spintherobolus*; **Stethaprioninae:** *Astyanax*, *Bario*, *Brachychalcinus*, *Brittanichthys*, *Bryconella*, *Carlana*, *Coptobrycon*, *Ctenobrycon*, *Deuterodon*, *Ectrepopterus*, *Erythrocharax*, *Grundulus*, *Gymnocharacinus*, *Gymnocorymbus*, *Hemigrammus*, *Hollandichthys*, *Holoshesthes*, *Hyphessobrycon*, *Inpaichthys*, *Moenkhausia*, *Myxiops*, *Nematobrycon*, *Oligosarcus*, *Orthospinus*, *Paracheirodon*, *Parastremma**, *Parecbasis*, *Petitella*, *Phycocharax*, *Poptella*, *Pristella*, *Probolodus*, *Psellogrammus*, *Rachoviscus*, *Rhoadsia*, *Stethaprion*, *Stichonodon*, *Stygichthys*, *Thayeria*; **Stevardiinae:** *Acrobrycon*, *Argopleura*, *Attonitus*, *Boehlkea**, *Bryconacidnus*, *Bryconamericus*, *Caiapobrycon**, *Carlastyanax*, *Ceratobranchia*, *Chrysobrycon*, *Corynopoma*, *Creagrutus*, *Cyanogaster*, *Diapoma*, *Eretmobrycon*, *Gephyrocharax*, *Glandulocauda*, *Hemibrycon*, *Histeronotus**, *Hypobrycon*, *Iotabrycon**, *Knodus*, *Landonia**, *Lepidocharax*, *Lophiobrycon*, *Markiana*, *Microgenys*, *Mimagoniates*, *Monotocheirodon**, *Nantis*, *Odontostoechus*, *Othonocheirodus**, *Piabarchus*, *Piabina*, *Planaltina*, *Phallobrycon**, *Phenacobrycon**, *Pseudocorynopoma*, *Pterobrycon**, *Ptychocharax**, *Rhinobrycon*, *Rhinopetitia**, *Scopaeocharax**, *Tyttocharax*, *Xenurobrycon*; **Tetragonopterinae:** *Tetragonopterus*; ***incertae sedis*:** *Axelrodia*, †*Bryconetes*, *Dectobrycon**, *Genycharax**, *Hasemania*, *Leptobrycon**, †*Megacheirodon**, *Mixobrycon**, *Oligobrycon**, *Oxybrycon**, †*Paleotetra*, *Parapristella**, *Schultzites**, *Scissor**, *Serrabrycon**, *Thrissobrycon**, *Trochilocharax**, *Tyttobrycon**, *Tucanoichthys** |

and the Stevardiinae (Fig. 4). In the Spintherobolinae, *Atopomesus*, whose phylogenetic relationships were herein evaluated for first time, was obtained as the sister group of *Amazonspinther* and *Spintherobolus*. The Exodontinae are recognizable for their teeth orientated to outside the mouth, associated with lepidophagous habits, and include only *Bryconexodon*, *Exodon* and *Roeboexodon*. The Tetragonopterinae are restricted to the species currently recognized in its nominotypical genus. The composition of the mostly predatory Characinae is congruent with that proposed by Mattox and Toledo-Piza (2012), after the inclusion of *Microschemobrycon* (Fig. 4). The Aphyocharacinae include *Aphyocharacidium*, as proposed by Tagliacollo et al. (2012), but also *Axelrodia lindeae*, in addition to *Aphyocharax*, *Leptagoniates*, *Paragoniates*, *Prionobrama* and *Xenagoniates*. Most nodes of the Aphyocharacinae are well-supported and stable. The Cheirodontinae are congruent with their current definition, with *Protocheirodon* as the sister group of the remaining members. Nodes in the Cheirodontinae are highly supported except in a distal clade composed mostly of *Odontostilbe* and *Serrapinnus*. In the present analysis, *Odontostilbe* is not monophyletic, including *Compsura heterura* and *Serrapinnus* and excluding *Odontostilbe microcephala*. The latter species was found related to *Holoshesthes pequira*.

The composition of the Stevardiinae (Fig. 5) is congruent with current classifications, but including also *Hyphessobrycon poecilioides* as the sister species of *Markiana nigripinnis*. With some exceptions, species in this subfamily have ii,8 (two unbranched plus eight branched) dorsal-fin rays and four teeth in the second premaxillary row. Tribes Eretmobryconini, Xenurobryconini, Glandulocaudini, Stevardiini, Hemibryconini, Creagrutini and Diapomini were recovered almost with the same compositions as in Thomaz et al. (2015). The reduced dataset weakly supported the inclusion of *Cyanogaster* (analysed only for morphology) in the Xenurobryconini, whereas in the complete matrix, this genus was excluded from the mentioned tribe but still included in the Stevardiinae. The Glandulocaudini and Stevardiini, sharing the presence of a glandular caudal-fin organ, were obtained as monophyletic. The Stevardiini, however, was weakly supported in the final hypothesis and paraphyletic in the complete dataset due to the exclusion of *Argopleura* spp. In the Diapomini, the morphologically odd genus *Aulixidens* was included in *Knodus*. The clade composed of the species of *Diapoma* included also *Hyphessobrycon wajat*, whereas an '*Attonitus* clade' was composed also of *Bryconamericus pachacuti*. The '*Piabina* clade' included also *Bryconamericus mennii*. *Bryconamericus exodon*, the type species of its genus, was obtained in a clade with *Bryconamericus stramineus* and *Piabarchus analis* but excluding all of the remaining analysed species of *Bryconamericus*. The "*Diapoma* clade" was related to a monophyletic group composed of species currently classified in *Bryconamericus* or recently proposed to be transferred to this genus (Thomaz et al., 2015). Those transfers are unsupported by this analysis. This group includes *Odontostoechus lethostigmus*, the "*Hypobrycon* clade" composed also of *Bryconamericus patriciae*, and the "*Nantis* clade", composed of *Nantis indefessus* and species classified in *Bryconamericus*, such as *Bryconamericus iheringii* and *Bryconamericus rubropictus*. In the complete dataset, *Astyanax festae* was obtained within *Eretmobrycon*, *Knodus tiquiensis* was the sister group of *Rhinobrycon*, *Landonia* was included in *Knodus*, and *Bryconamericus turiuba* was included in the clade of the "true" *Bryconamericus*. Relationships of *Hypobrycon*, *Nantis* and *Odontostoechus* as separate from *Bryconamericus* and, hence, valid genera, were corroborated by the complete dataset.

Five morphological synapomorphies were found for the Characidae: character 53(1), the pterotic spine restricted to attachment site of hyomandibular ligament; 62(0), supraoccipital reaching at least to middle length of neural complex of the Weberian apparatus; 85(0), the second infraorbital not overlapping maxilla; 120(1), the absence of posterior branch of post-temporal laterosensory canal; and 395(0), the possession of seven or fewer supraneurals. Crown characids, including all of the analysed extant members of the family, are diagnosed by three synapomorphies: character 263(1), the presence of notches in anterior ceratohyal for articulation of branchiostegal rays; 310(1), the neural pedicle of third vertebra reduced and not synchondrally articulated with neural complex; and 392 (0), the presence of four or fewer supraneurals. The absence of supraorbital (75(0)), which was considered as a synapomorphy of the Characidae by Oliveira et al. (2011), was found as a synapomorphy of †*Paleotetra* plus the crown characids. The presence of a foramen in the anterior ceratohyal for exit of the hyoid artery (262(1)), considered also a synapomorphy of the "distal" characids of Mirande (2010), which corresponds to the Characidae of Oliveira et al. (2011), is unknown for †*Bryconetes* and †*Paleotetra* and optimized as an ambiguous synapomorphy for both the Characidae and the clade of crown members of the family. Diagnoses of the main clades and the complete list of synapomorphies, are provided as Appendix S8.

The strict consensus obtained under equal weights was much less resolved than under implied weighting. The Characidae also were obtained as monophyletic, with a basal trichotomy composed of †*Bryconetes*, †*Paleotetra* and the crown members of the family. The Aphyocharacinae, Characinae, Cheirodontinae, Exodontinae, Spintherobolinae and Tetragonopterinae were obtained as monophyletic. The Stevardiinae

excluded the Eretmobryconini and Xenurobryconini, whereas the Stethaprioninae and its tribes were not monophyletic, due to large polytomies in the internal nodes of the family. The agreement subtree of the MPTs obtained under equal weighting contained 451 species (out of 473 in the analysed dataset), meaning that the polytomies found in the strict consensus are produced by just a few floating taxa. The agreement subtree under equal weighting showed some odd results, such as the inclusion of *Astyanax moorii* in a clade with *Deuterodon*, but most of the relationships are congruent or have small differences with the final hypothesis proposed herein.

## Systematics

The new subfamily Spintherobolinae is proposed herein. To comply with the ICZN code, its diagnosis and comparisons are provided below. Diagnoses of the Characidae and all of its subfamilies, as herein recognized, are provided in the Appendix S8.

**Spintherobolinae** subfam.n.

Type genus: *Spintherobolus* Eigenmann, 1911.

Genera included: *Amazonspinther* Bührnheim, Carvalho, Malabarba and Weitzman, 2008; *Atopomesus* Myers, 1927; and *Spintherobolus* Eigenmann, 1911.

Diagnosis: (1) absence of a bony rhinosphenoid; (2) short ascending process of the premaxilla, reaching only the anterior end of the nasal; (3) short maxilla, not reaching the posterior margin of Meckelian cartilage; (4) one row of premaxillary teeth; (5) maxillary teeth conical, with a single cusp; (6) lack of contact between ectopterygoid and quadrate; (7) absence of transitional vertebrae with haemal canal but lacking a haemal spine; (8) absence of haemal prezygapophyses on anterior caudal vertebra; (9) three or fewer unbranched anal-fin rays; (10) mandibular accessory tendon attached below middle length of Meckelian cartilage or anterior to it.

Members of the Spintherobolinae differ from representatives of all remaining subfamilies excepting the Aphyocharacinae and Cheirodontinae by the possession of only one aligned premaxillary row of teeth and the presence of a pseudotympanum between ribs of the fifth and sixth vertebrae. The lack of a bony rhinosphenoid (vs. presence) and the presence of only three unbranched anal-fin rays (vs. 4–6) distinguishes the Spintherobolinae from members of the Aphyocharacinae and Cheirodontinae.

## Discussion

A total evidence study of the Characidae is herein presented for the first time, being the most comprehensive phylogenetic analysis of this family to date. This analysis includes molecular data for eight markers and a morphological partition of 520 characters, of which approximately one fifth were newly defined for this study. Analyses under extended implied weighting (Goloboff, 2014) explored a broad sample of conditions, including four different schemes and several *k*-values. Also, an analysis under equal weights was performed. The exploration made among different weighting schemes produced results congruent with those of Mirande (2017) in the preference of methods grouping contiguous nucleotides to collectively weight them. This kind of weighting has two important effects. First, it gives third positions the same weight as other positions, discarding the influence of their high homoplasy in the calculation of their weights. Secondly, uninformative sites, although not considered as characters during searches, are used to calculate the weight of the informative positions of the set. Therefore, they are not completely ignored during searches (Goloboff, 2014). The higher stability of the results produced by schemes grouping contiguous sites to assign weights should be confirmed by subsequent analyses, but it may give some clue about how to consider third positions and character weighting in molecular analyses in general.

Mirande (2010) recognized 15 subfamilies of Characidae plus six clades of subfamilial level that were not defined as subfamilies given their low support and/or stability. Among those subfamilies, five are recognized as such in this study: Aphyocharacinae, Characinae, Cheirodontinae, Stevardiinae and Tetragonopterinae. The proposed classification differs from Mirande (2010), among other things, in the recognition of the Stethaprioninae, which was included in the Tetragonopterinae in that proposal. Also differing from Mirande (2010), the Aphyoditeinae are completely disaggregated in the present hypothesis, with all of their genera related to different species or clades and *Aphyodite*, the nominotypical genus of that subfamily, included in the Stethaprioninae and proposed to be closely related to the type species of *Hemigrammus*. The Stethaprioninae, in this hypothesis, is the most diverse subfamily of Characidae and it includes most of the taxa formerly assigned to Tetragonopterinae, Gymnocharacinae and Rhoadsiinae, the "*Astyanax* clade", the "*Bramocharax* clade", the "*Pseudochalceus* clade", the "*Hyphessobrycon luetkenii* clade" and the "*Astyanax paris* clade" of Mirande (2010). This subfamily is now congruent with clade 54 of Oliveira et al. (2011: fig. 10), although its internal relationships are somewhat different. Four tribes are proposed in the Stethaprioninae—the Grundulini, Gymnocharacini, Rhoadsiini and Stethaprionini—which are not congruent with Oliveira et al. (2011) and need further corroboration. Also, the Grundulini

lack morphological synapomorphies, whereas the Stethaprionini have only one, which support the need for additional studies.

The phylogenetic position of the naked characin *G. bergii* is controversial. This deeply morphologically divergent species is an emblematic Patagonian fish and the southernmost distributed species of Characidae. It was obtained by Mirande (2010) as related to other odd taxa from distant localities (*Coptobrycon*, from Eastern Brazil, and *Grundulus*, from Magdalena river basin in Colombia) and arguably grouped in the same clade based on resemblances in some of the many morphological features from which they diverge from remaining characids. Many studies have been performed on different aspects of *G. bergii* (e.g. Lozada et al., 2000; Cussac and Ortubay, 2002; Miquelarena et al., 2005), but none of them assessed their phylogenetic relationships until Mirande (2009, 2010). Even after the present analysis, the phylogenetic relationships of *G. bergii* appear to be far from conclusive, but its close relationship with species currently classified in *Astyanax* is, at least biogeographically, more plausible than previous hypotheses. The Stethaprioninae include some of the most diverse and problematic genera of the family (*Astyanax*, *Hemigrammus*, *Hyphessobrycon* and *Moenkhausia*) that, according to the present hypothesis, will need deep nomenclatural changes and the definition of several new genera to become monophyletic.

The comparatively better studied subfamilies Aphyocharacinae, Characinae, Cheirodontinae and Stevardiinae (Lucena and Menezes, 1998; Malabarba, 1998; Mattox and Toledo-Piza, 2012; Tagliacollo et al., 2012; Mariguela et al., 2013; Thomaz et al., 2015) were recovered almost exactly with the same compositions as proposed in the literature. The Exodontinae Fowler, 1958 are resurrected and the Spintherobolinae are proposed as a new subfamily. The Exodontinae groups the lepidophagous genera *Bryconexodon*, *Exodon* and *Roeboexodon*, which also were supported as monophyletic by Mirande (2009, 2010) and Mattox and Toledo-Piza (2012). The Spintherobolinae are congruent with the hypothesis proposed by Bührnheim et al. (2008) of a close relationship between *Amazonspinther* and *Spintherobolus* and to that of Oliveira et al. (2011) regarding the exclusion of the latter genus from the Cheirodontinae. The lack of a mesocoracoid bone was found as a synapomorphy relating *Amazonspinther* and *Spintherobolus*, in addition to those proposed by Bührnheim et al. (2008). *Atopomesus*, which was not previously included in a phylogenetic analysis, was herein found to be a member of the Spintherobolinae as supported by ten morphological synapomorphies. However, *Atopomesus* was analysed only for morphological characters and molecular data could modify those results, even if stable and well supported in this study.

The Eocene–Oligocene genera †*Paleotetra* and †*Bryconetes* were obtained as successive sister groups of the crown Characidae. The phylogenetic position of †*Bryconetes* as stem Characidae was already proposed by Weiss et al. (2014), but in a more restricted analysis. Relationships of †*Paleotetra* were instead not conclusive in its original description (Weiss et al., 2012) and this analysis gives the first insight about its phylogenetic relationships. The other known fossil characid, †*M. unicus*, was related to *Spintherobolus* by Malabarba (1998), who included both genera in the Cheirodontinae. With the phylogenetic hypothesis herein proposed plus the hypothesis by Malabarba (1998), the inclusion of †*Megacheirodon* in the Cheirodontinae is challenged and a close relationship with the Spintherobolinae should not be discarded. The age of the main radiation of the Characidae that led to their great extant diversity is difficult to estimate given the scarce fossil record, but may be as old as the Eocene or Oligocene, especially if †*Megacheirodon* is part of the crown Characidae, as currently argued.

In the molecular hypothesis by Oliveira et al. (2011), four clades were obtained within the Characidae. One of them is composed solely of *Spintherobolus* (they did not analyse *Amazonspinther* and *Atopomesus*) and three diverse clades that had been found previously by Javonillo et al. (2010) and named in that article as clades "A", "B", and "C". The same four clades were obtained in this analysis, but differed in their relationships.

The "clade A" of Javonillo et al. (2010) and Oliveira et al. (2011) was composed of the Stevardiinae, their "clade B" included the Stethaprioninae (as recognized herein), and their "clade C" was composed of the Aphyocharacinae, Characinae, Cheirodontinae, Exodontinae and Tetragonopterinae (as recognized herein). Results of this analysis are congruent with those obtained from different DNA sequences and taxon sampling by Arcila et al. (2017), which did not analyse members of the Spintherobolinae. Thus, although many clades are still weakly supported and there are some differences in the deep nodes, some stability is reached in the large-scale phylogeny of the Characidae, considering the convergence of results from analyses based on different sets of data.

The generic assignment of many species of Characidae still follows the traditional classification by Eigenmann (1912, 1915, 1917, 1918, 1921, 1927) and Eigenmann and Myers (1929). The replacement of that classification with a new one based on monophyletic groups depends on the availability of comprehensive phylogenetic hypotheses producing not only stable and well-supported results, but also morphologically diagnosable subfamilies and genera. Among the Characidae, genera of Aphyocharacinae (Tagliacollo et al., 2012), Characinae (Mattox and Toledo-Piza, 2012),

Cheirodontinae (Malabarba, 1998) and most tribes of Stevardiinae (Thomaz et al., 2015) have been defined from phylogenetic analyses. Those definitions stem from the examination of representatives of all genera of each subfamily and subsequent morphological diagnoses. However, the bulk of the nomenclatural problems in the Characidae, involving species grouped into the Stethaprioninae and the Diapomini (Stevardiinae) in this analysis, remain relatively far from resolution.

The need to consider several sources of information and to employ the best possible taxon sampling before doing any generic rearrangement became evident after the contribution by Thomaz et al. (2015). In that paper, they proposed the synonymy of *Hypobrycon*, *Nantis* and *Odontostoechus* with *Bryconamericus* after the weakly supported inclusion of *Bryconamericus exodon* (type species of its genus) in the clade of the three former genera. Those relationships were odd from a morphological point of view, given that *Bryconamericus exodon* is hardly distinguishable from *Bryconamericus stramineus*, which was obtained by Thomaz et al. (2015) in a relatively distant clade and, indeed, the latter species was proposed to be transferred to *Piabarchus*. In the present analysis, *B. exodon* was recovered as the sister group of *B. stramineus* (which is consequently restored to *Bryconamericus*), and *Hypobrycon*, *Nantis* and *Odontostoechus* are resurrected, at least provisionally, until the Diapomini can be analysed more exhaustively. Several genera of Stevardiinae, as happens in the Stethaprioninae and the *incertae sedis* Characidae, have never been the subjects of a phylogenetic analysis. As some of those genera may have temporal precedence over the members of the Stevardiinae analysed by Thomaz et al. (2015) or herein, any new generic transfers in this subfamily and, especially, in the Diapomini, would be difficult to justify at present.

*Holoshesthes* (with *H. pequira* as type species) had been synonymized with *Odontostilbe* by Malabarba (1998), but both the present study and the molecular phylogeny by Mariguela et al. (2013) contradict that synonymy. Therefore, *Holoshesthes* is herein resurrected. *Odontostilbe microcephala* was obtained as the sister species of *H. pequira* but, as in other similar cases, no generic transfer is proposed until specific studies are completed.

This analysis is the most comprehensive to date for the Characidae, with about one third of the living species of the family and three of the four known fossil species, and combines for the first time myriad of morphological data with molecular information. Although results are rather congruent with the literature, there are still many species and several genera that are virtually unknown beyond their descriptions and whose phylogenetic affinities are not predictable even at the subfamilial level.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** List of morphological characters, with figures, discussions of the new ones, and explanations on the removed characters relative to previous analyses.

**Appendix S2.** List of examined material.

**Appendix S3.** List of species coded from literature and papers from which the information was extracted.

**Appendix S4.** List of sequences removed from the analysis given to possible contamination or misidentifications.

**Appendix S5.** TNT files, including the complete dataset, the script to select the analysed species, the gen-by-gen datasets including GenBank accessions, and all trees from both rounds of searches. Also, it includes the final hypothesis (final), the consensus of the MPT from the complete dataset (BLK, $k = 39$; complete), and the consensus of the MPT under equal weights (equal) are presented in parenthetical TNT and Newick formats.

**Appendix S6.** List of species in the dataset showing which one has information for each partition of data. Species in the final dataset are highlighted in yellow.

**Appendix S7.** Graphical trees in .svg format, including the final hypothesis showing number of nodes (final_nodes.svg) and GC-values (supports.svg), a strict consensus of the MPT with the complete dataset (complete.svg), the agreement subtree of the complete dataset (completed_pruned.svg), and the consensus of the MPT under equal weights (equal_nodes.svg).

**Appendix S8.** Diagnoses of the Characidae, its subfamilies and tribes. Also a list of synapomorphies of the final hypothesis is provided. Node numbers correspond to the consensus tree of the Appendix S7 (final_nodes.svg).