INSTITUTO DE BIOCÊNCIAS
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CAROLINA SANTOS VIEIRA

DIVERSIFICAÇÃO DOS CARACTERES SEXUAIS SECUNDÁRIOS E SUAS IMPLICAÇÕES FILOGENÉTICAS EM APHYOCHARACINAE (CHARACIDAE: CHARACIFORMES)

PORTO ALEGRE
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Tese apresentada ao Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Doutor em Biologia Animal.

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Orientador: Prof. Dr. Luiz Roberto Malabarba
Coorientadora: Dra. Priscilla Caroline Silva

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RESUMO

Os peixes exibem amplos padrões de diversificação. Entre os Ostariophysi, uma das maiores radiações em vertebrados, Characiformes representa um de seus grupos mais diversos, onde as espécies de água doce estão amplamente distribuídas na região Neotropical. Várias formas de estruturas sexualmente dimórficas são conhecidas como caracteres sexuais secundários, que estão intimamente relacionadas aos ciclos reprodutivos dos peixes. Características sexualmente dimórficas foram relatadas em muitas espécies de Characidae e podem estar associadas a uma variedade de estruturas modificadas de machos adultos e, às vezes, fêmeas. Os atributos do sistema sexual e as variações morfológicas desses caracteres foram aqui implementados como fontes de novos dados. Modificações do sistema sexual secundário foram o foco deste estudo, que correlacionou a evolução dos caracteres sexuais secundários à diversificação filogenética na subfamília Aphyocharacinae. Através da análise da reconstrução de estados de caráter ancestral com base em uma hipótese filogenética das relações evolutivas dos afiocaracíneos, propusemos novos conjuntos de caracteres envolvendo: a modificação de estruturas corporais em espécimes adultos, e a presença/ausência na ocorrência de ganchos ósseos nos raios das nadadeiras pélvicas e anal em machos sexualmente dimórficos. As informações disponíveis sobre os estados de caráter dos caracteres sexuais secundários revelaram possível reconstruir os eventos históricos da evolução dos táxons de Aphyocharacinae ao longo do tempo. As histórias evolutivas dos caracteres recuperadas aqui corroboraram hipóteses de relações filogenéticas dentre os Aphyocharacinae. A presença ancestral de ganchos ósseos nos raios das nadadeiras pélvicas e anal foi recuperada na maioria das espécies da subfamília, onde a ausência dessas estruturas foi considerada uma novidade evolutiva que suporta a monofilia de um clado composto por *Leptagoniates*, *Paragoniates*, *Phenagoniates* e *Xenagoniates*; características sexualmente dimórficas evoluíram uma vez no ancestral comum do *Prionobrama*, e sustentam a monofilia e o diagnose do gênero; a distribuição dos ganchos ósseos ao longo dos raios da nadadeira pélvica recupera as relações filogenéticas entre as espécies de *Aphyocharax*. Nossas principais conclusões suportam o grande potencial das informações associadas aos caracteres do sistema sexual, onde investigações adicionais, incluindo novos conjuntos de caracteres morfológicos, devem ser consideradas em futuras análises filogenéticas.

Palavras-chave: Peixes de água doce; Dimorfismo sexual; Métodos comparativos filogenéticos; Evolução de carcateres; Biologia evolutiva.

ABSTRACT

Fishes exhibit broad patterns of diversification. Among the Ostariophysi, one of the greater radiations in vertebrates, Characiformes represents one of its most diverse groups where the freshwater species are extensively distributed across Neotropical region. Vast arrays of sexually dimorphic structures are known as secondary sexual characteristics that are closely related to the reproductive cycles of fishes. Sexually dimorphic characteristics have been reported in many species of Characidae, and can be associated to a variety of modified structures of adult males, and sometimes females. Attributes of the sexual system and morphological variations of these characters were implemented herein as sources of new data. Modifications of the secondary sexual system were the focus of this study, which correlated the evolution of secondary sexual characters to the phylogenetic diversification within the subfamily Aphyocharacinae. Through the analyses of reconstruction of ancestral character states based on a phylogenetic hypothesis of evolutionary relationships of the aphyocharacins, we proposed new suites of characters involving: the modification of body structures in adult specimens, and the presence/absence on the occurrence of bony hooks on pelvic and anal-fin rays in sexually dimorphic males. Available information on the character states of secondary sexual traits revealed possible to reconstruct the historical events of the evolution of Aphyocharacinae taxa over time. Evolutionary character histories recovered here corroborated hypotheses of phylogenetic relationships within the Aphyocharacinae. The ancestral presence of bony hooks on pelvic and anal-fin rays was recovered in most species of the subfamily, where the absence of these structures were considered an evolutionary novelty supporting the monophyly of a clade composed by *Leptagoniates*, *Paragoniates*, *Phenagoniates* and *Xenagoniates*; sexually dimorphic features have evolved once in the common ancestor of *Prionobrama*, and further supports the monophyly and the diagnosis of the genus; the distribution of bony hooks along pelvic-fin rays recover the phylogenetic relationships among species of *Aphyocharax*. Our main conclusions supports the great potential of information associated with characters the sexual system, where further investigations by including new sets of morphological characters should be considered in future phylogenetic analyses.

Key-words: Freshwater fishes; Sexual dimorphism; Phylogenetic comparative methods; Character evolution; Evolutionary biology.

EVOLUTION OF SECONDARY SEXUAL CHARACTERS: DESCRIPTION OF SEXUALLY DIMORPHIC STRUCTURES AND PHYLOGENETIC IMPLICATIONS IN THE SUBFAMILY APHYOCHARACINAE (CHARACIFORMES: CHARACIDAE)

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INTRODUCTION

Fishes account for more than one-half of the world's living vertebrates (~60,000 species) with more than 32,000 species. They also exhibit the most diverse patterns of morphology, habitat use, behavior and overall biology (Nelson *et al.*, 2016) in vertebrates. It is estimated that about a third of all freshwater fishes worldwide are distributed in approximately 12% of the total continental surface area, i.e. South America, of the planet (Reis *et al.*, 2016). Within Actinopterygii, teleosts are the most species-rich and diverse group, and among them the superorder Ostariophysi accounts for about 68% of the known freshwater species (Nelson *et al.*, 2016; Betancur-R *et al.*, 2017); and is one of the exceptional radiations in vertebrates (Alfaro *et al.*, 2009). Easily recognized by endemism, the Neotropical fishes are mostly comprised by ostariophysans; representing around 70% of its diversity (Albert *et al.*, 2011).

The order Characiformes represents one of the most diverse groups of Ostariophysi and occurrs in Africa, southern North America, and is most diverse in Central and South America. Characiformes are currently comprised of more than 2200 valid species (Nelson *et al.*, 2016; Fricke *et al.*, 2020). The family Characidae comprises approximately 50% of species belonging to neotropical characiforms (Fricke *et al.*, 2020, Table 1), with a wide spectrum of morphological and biological variations, including the prevalence of small sized fishes (Azevedo, 2010).

This amazing diversity can also be observed in the strategies and reproductive tactics utilized by characids (Wootton, 1984; Winemiller, 1989; Taphorn, 1992). Among species of this lineage, the most common mode of reproduction are the externally fertilizing species with minimal evidence of external sexual dimorphism (Weitzman & Malabarba, 1998). Others, however, exhibit a vast array of sexually dimorphic structures that make genders distinguishable, and display elaborate anatomical modifications possibly associated with complex reproductive courtship behaviors (*e.g*. Burns *et al.*, 1995, 1997; Bushmann *et al.*, 2002; Malabarba, 1998; Malabarba & Weitzman, 1999, 2000, 2003; Malabarba *et al.*, 2004; Oliveira *et al.*, 2012; Weitzman *et al.*, 2005; Abrahão *et al.*, 2019). Secondary sexual characteristics may be directly or indirectly related to the reproductive act (Vazzoler, 1996) and are predominantly found in adult males (Wiley & Collette, 1970; Burns & Weitzman, 1996; Malabarba & Weitzman, 2003; Oliveira *et al*., 2012). Dimorphic sexual characteristics have been reported in many species of Characidae, and include variation in structures such as fin shape, size, coloration, bony hooks on fin rays, and hypertrophied tissues (*e.g.* Burns & Weitzman, 1996; Burns *et al.*, 1997, 1998; Malabarba, 1998; Malabarba & Weitzman, 1999, 2003; Bushmann *et al.*, 2002; Malabarba *et al.*, 2004; Lima & Sousa, 2009; Menezes & Weitzman, 2009; Lucena & Malabarba, 2010; Miquelarena & López, 2010; Zanata & Camelier, 2010; Jerep & Malabarba, 2011; Oliveira *et al.*, 2012; Dala-Corte & Fialho, 2014; Camelier & Zanata, 2014; Vieira *et al.*, 2016; Longoni *et al.*, 2018). Modifications of the secondary sexual system are a data rich source of characters for phylogenetic studies for those characids that bear such modifications; an observation that is equally true and important whether they utilize insemination or external fertilization strategies (Weitzman & Malabarba, 1998; Malabarba, 1998; Menezes & Weitzman, 2009).

The analysis of ancestral character states based on explicitly measured characteristics allows the estimation of the history of examined characters when based on a phylogenetic hypothesis of evolutionary relationships (Joy *et al.*, 2016). Available information on the character states of contemporary species combined with a phylogeny makes it possible to reconstruct the historical events of the evolution of taxa over time, thereby providing a way to test hypotheses about evolution and adaptation (Pagel, 1999). These analyses depend on the application of evolutionary models that are sufficiently realistic to recover ancestral states, and are generally applied based on a phylogenetic hypothesis inferred from the same data (Joy *et al.*, 2016).

Due to the incredibly large number of species and the variety of forms, the phylogeny of Characidae continues to be investigated (*e.g.* Mirande, 2009, 2010; Oliveira *et al.*, 2011). Over the past decade, the characids have had the highest number of described species of all characiforms (Oliveira *et al.*, 2011; Fricke *et al.*, 2020). Recent morphological (Malabarba & Weitzman, 2003; Mirande, 2009, 2010) and molecular studies (Calcagnotto *et al.*, 2005; Javonillo *et al.*, 2010; Oliveira *et al.*, 2011), including a total evidence approach (Mirande, 2019), have been conducted to clarify the phylogenetic relationships among species of Characidae. Results from these studies on the species composition and phylogenetic relationships among the subfamilies and genera of Characidae, and its relationship with other families of the order, unfortunately remains unresolved (Oliveira *et al.*, 2011). To date, only a few genera and subfamilies of characids have been recovered as monophyletic. The limitations for a better understanding of phylogenetic relationships of characids is likely having an adequately focused sampling of internal (characids) and external (characiforms) taxa. Further, using attributes of the sexual system (Weitzman & Malabarba, 1998) and morphological modifications of this system (Malabarba, 1998; Lima & Sousa, 2009) as sources of character data may provide new information and perspectives that will likely aid our understanding for the phylogeny of complex or little-known groups (Weitzman & Malabarba, 1998; Lima & Sousa, 2009).

Javonillo *et al.* (2010) proposed “Clade B” characids as the sister lineage to Stevardiinae (*sensu* Thomaz *et al.*, 2015). Clade B includes species of *Exodon*, Cheirodontinae, Aphyocharacinae, Tetragonopterinae and Characinae (excluding *Gnathocharax*). This clade has been recovered in studies asking different phylogenetic questions using morphological (Mirande, 2009, 2010) and molecular data (Oliveira *et al.*, 2011), as well as a total evidence analysis (Mirande, 2019), and presently “Clade B” includes the subfamilies Exodontinae, Tetragonopterinae, Characinae, Aphyocharacinae and Cheirodontinae. The Exodontinae was first proposed by Fowler (1958), and was recently resurrected as a subfamily of Characidae comprised as monophyletic by *Roeboexodon* Géry, 1959; *Exodon* Müller & Troschel, 1844 and *Bryconexodon* Géry, 1980 (Mirande, 2019; Fricke *et al.*, 2020). Tetragonopterinae has been recognized for a long time as a large assemblage of fishes and is currently recognized as a monophyletic group comprised only by the genus *Tetragonopterus* Cuvier, 1816 (Melo *et al.*, 2016; Fricke *et al.*, 2020). Characinae holds the type species of the family and order, currently recognized as a monophyletic group of nine genera (*Acanthocharax* Eigenmann, 1912; *Acestrocephalus* Eigenmann, 1910; *Charax* Scopoli, 1777; *Cynopotamus* Valenciennes, 1849; *Galeocharax* Fowler, 1910; *Microschemobrycon* Eigenmann, 1915; *Phenacogaster* Eigenmann, 1907; *Priocharax* Weitzman & Vari, 1987; *Roeboides* Günther, 1864) (Mattox & Toledo-Piza, 2012; Mirande, 2019; Fricke *et al.*, 2020). Aphyocharacinae has been recently revised with *Axelrodia lindeae* plus seven genera currently assigned to the subfamily (*Aphyocharacidium* Géry, 1960; *Aphyocharax* Günther, 1868; *Leptagoniates* Boulenger, 1887; *Paragoniates* Steindachner, 1876; *Phenagoniates* Eigenmann & Wilson, 1914; *Prionobrama* Fowler, 1913; *Xenagoniates* Myers, 1942) (Tagliacollo *et al.*, 2012; Mirande, 2019). Cheirodontinae is the most diverse group of "Clade B" and to date is one of the most extensively studied subfamilies of characids, with 16 genera currently assigned (*Acinocheirodon* Malabarba & Weitzman, 1999; *Aphyocheirodon* Eigenmann, 1915; *Cheirodon* Girard, 1855; *Cheirodontops* Schultz, 1944; *Compsura* Eigenmann, 1915; *Ctenocheirodon* Malabarba & Jerep, 2011; *Heterocheirodon* Malabarba, 1998; *Kolpotocheirodon* Malabarba & Weitzman, 2000; *Macropsobrycon* Eigenmann, 1915; *Nanocheirodon* Malabarba, 1998; *Odontostilbe* Cope, 1870; *Prodontocharax* Pearson, 1924; *Protocheirodon* Vari, Melo & Oliveira, 2016; *Pseudocheirodon* Meek & Hildebrand, 1916; *Saccoderma* Schultz, 1944; *Serrapinnus* Malabarba, 1998) (Mirande, 2019; Fricke *et al.*, 2020), but there is still controversy concerning hypotheses of internal phylogenetic relationships (Malabarba, 1998; Bührnheim *et al.*, 2008; Mirande, 2009, 2010, 2019; Oliveira *et al.*, 2011; Mariguela *et al.*, 2013).

Table 1 – Characidae's diversity across lineages (Fricke *et al.*, 2020).

Subfamily	**Number of valid species**	**Last 10-year descriptions***
Stethaprioninae	610	122
Characids *incertae sedis*	37	-
Aphyoditeinae	10	3
Spintherobolinae	6	-
Exodontinae	4	-
Tetragonopterinae	13	10

Characinae	96	9
Aphyocharacinae	23	1
Cheirodontinae	65	10
Stevardiinae	347	51

*2011–2020

First proposed by Eigenmann (1909), the subfamily Aphiocharacinae (*sic*), included fishes with a single series of well-developed teeth on the premaxilla, mandible and maxilla, large parietal and frontal fontanels, gill-membranes free from the isthmus and each other, and presence of an adipose fin. Eigenmann (1910) assigned the genera *Coelurichthys* Miranda Ribeiro, 1908 (= *Mimagoniates* Regan, 1907), *Odontostilbe*, *Holoshesthes* Eigenmann, 1903 (= *Odontostilbe*), *Cheirodon*, *Aphyocharax* and *Holoprion* Eigenmann, 1903 (= *Aphyocharax*), later including *Aphyodite* (Eigenmann, 1912) and *Prionobrama* (Fowler, 1913) to the subfamily. Eigenmann (1915) later classified Aphyocharacinae as members of the subfamily Cheirodontinae, and Weitzman (1962), based on the lack of strong evidence on differences in cranial anatomy, included them as part of Characinae. More recently Aphyocharacinae was revalidated as a subfamily of Characidae (Géry, 1972, 1977) composed only by *Aphyocharax* and subsequent studies at taxonomic level (Souza-Lima, 2004) and about relationships were carried out by cladistic methods (Mirande, 2009, 2010). Although not phylogenetically tested, a close relationship between genera of Aphyocharacinae and Paragoniatinae was mentioned by Géry (1977), and further investigated by Quevedo (2006) with members of Paragoniatinae and a few representatives of Characidae (including three species of *Aphyocharax*). The latter reserved definitive conclusions of interrelationships between the subfamilies in lieu of more information. Based on morphological data, Mirande (2009, 2010) recovered a monophyletic Aphyocharacinae, composed of *Aphyocharax* and members of Paragoniatinae (*Leptagoniates*, *Paragoniates*, *Phenagoniates*, *Prionobrama*, *Rachoviscus* Myers, 1926 and *Xenagoniates*) plus *Inpaichthys* Géry & Junk, 1977. In a total evidence analysis, Tagliacollo *et al.* (2012) proposed a new hypothesis of relationships for the Aphyocharacinae, removing *Inpaichthys* and *Rachoviscus*, and proposed adding *Aphyocharacidium*. Following the total-evidence approach by combining the available information of morphology and molecular-based studies, Mirande (2019) presented new relationships of species of Characidae, including the addition of *Axelrodia lindeae* in Aphyocharacinae.

Regarding secondary sexual characters and patterns of reproduction, numerous questions remain unanswered for many species of Characidae. Yet, there is unprecedented potential for understanding the inter- and intrafamilial relationships from characteristics of the sexual systems among groups of Characidae (Weitzman & Malabarba, 1998). Their utility has been demonstrated as essential for corroboration of hypotheses of phylogenetic relationships in monophyletic groups of Characidae, *e.g.* Xenurobryconini (Weitzman & Fink, 1985), Cheirodontinae (Malabarba, 1998), and Glandulocaudinae (Menezes & Weitzman, 2009). Within the groups of "Clade B", the Cheirodontinae is the most well-documented in the literature, with several studies concerning their reproductive biology and dimorphic structures (*e.g.* Fink & Weitzman, 1974; Burns *et al.*, 1997; Malabarba, 1998; Malabarba *et al.*, 2004; Gonçalves *et al.*, 2005; Azevedo *et al.*, 2010; Oliveira *et al.*, 2010, 2012; Malabarba & Jerep, 2014). Discussions focused on the secondary sexual characteristics for members of Aphyoditeinae, Tetragonopterinae, Characinae and Aphyocharacinae are scarce, and more commonly found in species descriptions or generic revisions.

This study is designed to analyze morphological and molecular characters for members of Aphyocharacinae. One primary goal is characterizing the occurrence of secondary sexual characters and diagnosing the patterns of evolution of these features among this highly diverse group of fishes.

MATERIAL AND METHODS

Datasets include morphological features and DNA sequences from five genes (two mitochondrial and three nuclear) of characid species were generated for this study. Phylogenetic analyses utilizing Bayesian Inference (*Beast2.6; *RevBayes1.0) were conducted to investigate the subfamily Aphyocharacinae and to recover and propose hypotheses for the internal relationships of constituent taxa. Based on the interrelationships of aphyocharacins, the morphological features and the hypothesis framework were used to conduct ancestral reconstruction analyses to explore the patterns of evolution of secondary sexual characters within Aphyocharacinae through time.

Ingroup and outgroup Sampling

The ingroup used to test the relationships of members from Aphyocharacinae includes eight species of *Aphyocharax* (*Ap. alburnus*, *Ap. anisitsi*, *Ap. avary*, *Ap. colifax*, *Ap. dentatus*, *Ap. erythrurus*, *Ap. gracilis*, *Ap. nattereri*, *Ap. pusillus*, *Ap. rathbuni*, *Ap. yekwanae*), *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, and species of the genera *Leptagoniates*, *Paragoniates*, *Phenagoniates*, *Prionobrama*, *Xenagoniates*. Those taxa follow the most recent phylogenetic hypotheses for the subfamily, based on morphological and molecular evidence (Oliveira *et al.*, 2011; Tagliacollo *et al.*, 2012; Mirande, 2019).

Outgroup taxa were selected based on recent phylogenies proposed by Oliveira *et al.* (2011) and Tagliacollo *et al.* (2012). According to their hypotheses, Characidae is a strongly supported clade comprised by four monophyletic units. Species from the subfamilies Exodontinae (*Exodon paradoxus* Müller & Troschel, 1844; *Roeboexodon guyanensis* (Puyo, 1948)), Tetragonopterinae (*Tetragonopterus argenteus* Cuvier, 1816; *Tetragonopterus chalceus* Spix & Agassiz, 1829), Characinae (*Charax leticiae* Lucena, 1987; *Cynopotamus kincaidi* (Schultz, 1950); *Microschemobrycon casiquiare* Böhlke, 1953) and Cheirodontinae (*Cheirodon ibicuhiensis* Eigenmann, 1915; *Compsura heterura* Eigenmann, 1915; *Heterocheirodon yatai* (Casciotta, Miquelarena & Protogino, 1992); *Kolpotocheirodon theloura* Malabarba & Weitzman, 2000; *Macropsobrycon uruguayanae* Eigenmann, 1915; *Odontostilbe fugitiva* Cope, 1870; *Serrapinnus calliurus* (Boulenger, 1900); *Serrapinnus piaba* (Lütken, 1875)) were selected as outgroups and, in addition, members from Spintherobolinae (*Spintherobolus ankoseion* Weitzman & Malabarba, 1999; *Spintherobolus broccae* Myers, 1925; *Spintherobolus leptoura* Weitzman & Malabarba, 1999) were included as additional outgroups. The molecular sequence data are based on a novel set of 58 sequences from five genes of 34 Characidae taxa (Supporting Information 1).

Morphological data

Counts and measurements followed Fink & Weitzman (1974), with the addition and modifications of the following: anal-fin base length measured from the anal-fin origin to the last anal-fin ray; horizontal scale rows below lateral line counted to pelvic-fin insertion. Morphometric data were acquired point-to-point with a digital caliper (0.01 mm). Measurements and counts were taken on the left side of specimens, except when measurements and/or counts would be skewed by an abnormal or damaged specimen. In a case like this, the data were obtained from the right side of the specimens. Morphometric data are included in Support Information 5.

Osteological nomenclature follows Weitzman (1962), following modifications from Zanata & Vari (2005). Counts of supraneurals, teeth, fin hooks, vertebrae, procurrent caudal-fin rays were taken from x-ray images on Faxitron X-ray LX60, and cleared and stained (c&s) specimens were prepared according to the protocol of Taylor & Van Dyke (1985). Vertebral counts include the Weberian apparatus as four elements, and the fused PU1+U1 of the caudal region as a single element (Weitzman, 1954). Precaudal vertebrae and caudal vertebrae definitions follow Weitzman (1962).

Definitions concerning the explicit localization of primary and secondary branching of pelvic-fin and anal-fin rays in sexually dimporphic taxa are proposed herein for the first time in characids (Figure 1). Primary branching is considered here as the first split of a fin ray, which consequently differentiates each ramification: medial and lateral primary ramifications on pelvic-fin rays; anterior and posterior primary ramifications on anal-fin rays. Secondary branching corresponds to all subsequent splits and derived ramifications of pelvic and anal-fin rays.

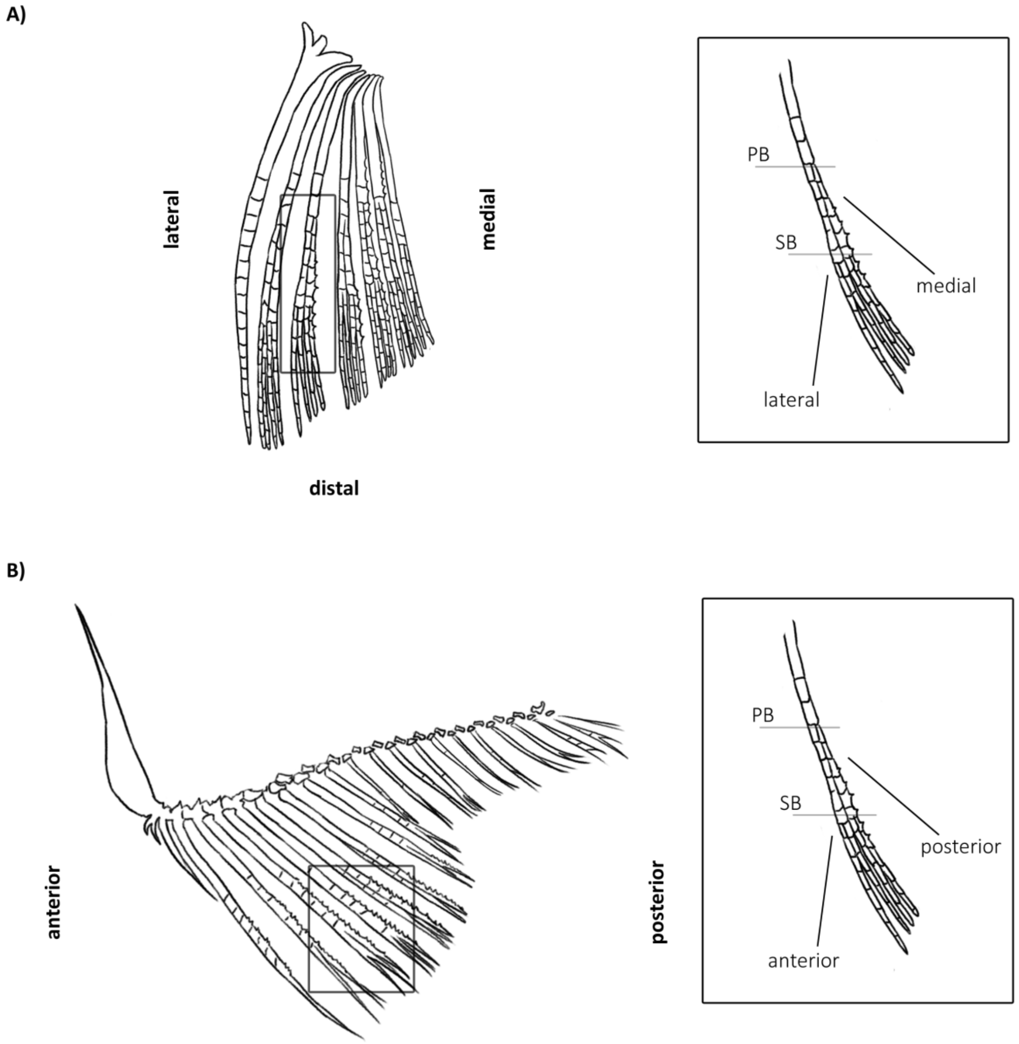

Figure 1 – Definitions on primary and secondary branching and on the use of lateral and medial or anterior and posterior branches of pelvic-fin and anal-fin rays. PB= Primary branching; SB= Secondary branching.

Cleared and stained (c&s) and whole specimens were imaged on a Nikon Multizoom AZ100.

Examined material (Supporting Information 2) came from the following Institutions: Academy of Natural Sciences of Philadelphia, Philadelphia (ANSP); Auburn University Museum of Natural History, Auburn (AUM); California Academy of Sciences, San Francisco (CAS); Coleção Ictiológica do Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (NUPELIA), Universidade Estadual de Maringá, Maringá (NUP); Cornell University Museum of Vertebrates, Ithaca (CUMV); Field Museum of Natural History, Chicago

(FMNH); Illinois Natural History Survey, University of Illinois at Urbana-Champaign, Champaign (INHS); Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Botucatu (LBP); Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (MCP); Museum of Comparative Zoology, Harvard University, Cambridge (MCZ); Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima (MUSM); Museu de Zoologia, Universidade de São Paulo, São Paulo (MZUSP); Naturhistoriska riksmuseet, Departments of Vertebrate Zoology and Palaeozoology, Stockholm (NRM); Royal Ontario Museum, Department of Natural History, Toronto (ROM); Universidade Federal do Rio Grande do Sul, Porto Alegre (UFRGS). Institutional acronyms follow Sabaj (2019).

Secondary sexual characters

Observations for the analyses of secondary sexual charactheristics consisted of viewing specimens under a stereomicroscope to detect and describe the occurrence of external morphological features related to sexual dimorphism, primarily focused on the characterization of bony hooks on fin rays. Sexes of adult specimens were checked from previously granted authorization for dissection from Examined material.

Morphological descriptions of the bony hooks on the fin rays provide detailed descriptions across species about the number, form (tip and base shape), distribution (concentration of bony hooks on fin ray regions), development and orientation (insertion along the fin rays) of these characters. The terminology used to describe the bony hooks follows Weitzman & Fink (1985), Reis (1989), Malabarba (1998), Malabarba & Weitzman (2000), and Vieira *et al.* (2016).

Molecular data

Sequences of the mitochondrial genes 16S rRNA (16S), cytochrome b (cyt *b*), and nuclear genes myosin heavy chain 6 gene (Myh6), recombination activating gene 1 (RAG 1) and recombination activating gene 2 (RAG 2) from the studies of Oliveira *et al.* (2011) and Tagliacollo *et al.* (2012) are available on GenBank database (Benson *et al.*, 2012), and were retrieved for this study. Additional sequences of the mitochondrial gene 16S were added for 16 specimens of *Aphyocharax alburnus* (n=2), *Aphyocharax avary* (n=2), *Aphyocharax erythrurus* (n=5), *Aphyocharax pusillus* (n=1), *Leptagoniates steindachneri* (n=2) and *Paragoniates alburnus* (n=4), with the remaining genes assigned as missing data. DNA sequences were deposited in GenBank database (Accession Nº. XXXX). Tissue samples and sequence information are listed in Supporting Information 1.

DNA extraction was completed with the QIAGEN DNeasy Blood & Tissue Kit by Qiagen© per the manufacturer's instructions, and all work was carried out at the Nancy and Larry Fuller Lab of Evolutionary Biology (Cornell University Museum of Vertebrates – Lab of Ornithology, Ithaca – EUA).

Sequences of the mitochondrial gene 16S rRNA (16S) were amplified by polymerase chain reaction (PCR) (Table 2). PCR amplifications were performed in 25μl reactions using Phusion® High-Fidelity PCR Master Mix with HF Buffer by New England BioLabs® Inc., consisting of: 12.5μl reaction buffer (with correction of 1.25μl $MgCl_2$ in some samples), 1.25μl of each primer at 10μM, 1–2μl DNA, and 6.75–9μl of double-distilled water. Amplification of 16S sequences were retrieved using one complete round of PCR with cycles of amplification as follows: (1) 30 seconds at 98°C (initial denaturation), (2) 10 s at 98°C, (3) 30 s at 54°C, (4) 30 s at 72°C, and 5 min at 72°C (final elongation), with steps (2) through (4) repeated 35 times. PCR products were checked by electrophoresis in an agarose gel and sequenced in both directions at Biotechnology Resource Center – BRC facility (Institute of Biotechnology, Cornell University, Ithaca – EUA).

Table 2 – Sequences of primers.

Gene	Primer name	Primer sequence (5'–3')	Source
16S rRNA	16Sar	ACGCCTGTTTATCAAAAACAT	[1–2]
	16Sbr	CCGGTCTGAACTCAGATCACGT	[1–2]

[1]Palumbi, 1996; **[2]**Palumbi *et al.*, 2002.

Phylogenetic analysis

Molecular based data

Each gene sequence was independently aligned using the MUSCLE algorithm (Edgar, 2004) with default parameters in MEGA7 software (Kumar *et al.*, 2016), subsequently, alignments were inspected by eye for any possible misalignment.

The species tree was estimated using BEAST v2.6.0 (Bouckaert *et al.*, 2014) and utilizing the StarBeast template on XSEDE v2.6.1 (Drummond & Rambaut, 2007; Suchard &

Rambaut, 2009) through CIPRES Science Gateway (Miller *et al.*, 2010). DNA alignments from each gene were considered as partitions, and models of molecular evolution and gene trees were unlinked. Substitution models for DNA alignments were estimated with the Bayesian Information Criterion (BIC) method (Schwartz, 1978) implemented by PartitionFinder v1.1.1 (Lanfear *et al.*, 2012) under default parameters, generating the information necessary on Site Model settings for priors on substitution models for the partitions. The multi-species Coalescent was set for Population function as "constant". Specifications for priors in the Species Tree was set to Birth-Death Model, and Gamma distributions were determined for partitions Clock rates. The species tree was estimated in two runs, and each run was performed with 400000000 MCMC generations with trees stored every 20000 iteration and pre burn-in at 0 (zero) with 20,000 trees retained. Evolution of log likelihood scores and checks for the stationary of all model parameters of each run via ESS values were visualized and completed using Tracer v1.7.1 (Rambaut *et al.*, 2018), with 10% of the initial states discarded as burn-in. The tool TreeAnnotator on XSEDE (Drummond & Rambaut, 2007) was used on CIPRES to summarize the trees post 10% burn-in.

The concatenated aligned DNA sequence data matrix of 58 specimens (34 species) based on a multilocus dataset (16S, CytB, Myh6, RAG1, RAG2) (Table 3) resulted in a matrix with 4,303 aligned base pairs (bp) with taxa from the five subfamilies that are currently known as "Clade B" of Characidae (Exodontinae, Tetragonopterinae, Characinae, Cheirodontinae and Aphyocharacinae) plus Spintherobolinae.

Table 3 – Descriptive information of genes and substitution models.

	Gene				
	16S	**cyt *b***	**Myh6**	**RAG1**	**RAG2**
Number of sequences	58	42	42	41	42
bp after alignment	553	791	731	1236	992
Substitution model	SYM+I+G	SYM+I+G**[1]** HKY+I+G**[2]** TrN+G**[3]**	HKY+I+G**[1,2]** K80+G**[3]**	GTR+G**[1]** HKY+I+G**[2]** K80+G**[3]**	GTR+G**[1]** HKY+I+G**[2]** K80+G**[3]**

[1]1st codon; **[2]**2nd codon; **[3]**3rd codon.

Mean completeness of character are ~70% for most taxa, The influence of missing data on phylogentic analyses have been thouroughly considered, e.g. Wiens & Morrill (2011), wherein no evidence has been found for inaccuracy of Bayesian estimates and phylogenetic placement when >90% of missing data are present.

Morphology based data

A morphological data matrix of the subfamily Aphyocharacinae and other subfamilies within Characidae was assembled using Mesquite version 3.31 (Maddison & Maddison, 2017). Bayesian Inference of the character matrix was conducted in RevBayes v1.0.13 software (Höhna *et al.*, 2016). The Mk model proposed by Lewis (2001) was implemented for estimating phylogenetic trees from discrete morphological data. This model is a generalization of the Jukes-Cantor substitution model (Jukes & Cantor, 1969) assuming a Markov process for character change, allowing for multiple character-state symmetrical changes along a single branch (Wright & Hillis, 2014). "Relaxed-clock models" were implemented including a constant-rate birth-death branching process by applying a Birth-Death model as a prior on the distribution of tree topologies and node ages (Höhna *et al.*, 2015), where diversification, turnover, speciation and extinction rates were estimated. Rho was included to model incomplete taxon sampling, considered as the probability of sampled taxa (this study) and known described species of Characiformes (numbers from Fricke *et al.*, 2020).

Considering the computational efforts needed for such large-scale data analyses, the estimation of BI was performed using clusters at the Centro Nacional de Supercomputação (CESUP) of Universidade Federal do Rio Grande do Sul (UFRGS).

The morphology-based analysis was performed using an extended matrix of Mirande (2019). Herein, the addition of nine taxa currently assigned to the subfamily Aphyocharacinae (*Aphyocharacidium* n. sp., *Aphyocharax alburnus, Ap. avary, Ap. colifax, Ap. erythrurus, Ap. gracilis, Ap. pusillus, Ap. rathbuni, Ap. yekwanae*) and twenty three new characters were added to the matrix previously published by Mirande (2019). This resulted in a morphological character matrix wtih 543 characters for 54 taxa (Supporting Information 3). The new characters were coded in all of the species noted above and left as missing data for the remaining characidae that were selected from the original matrix. The codification of characters 34, 45, 90, 91, 92, 123, 124, 125, 143, 189, 190, 203, 324, 325, 326, 384, 385, 391, 392, 393, 394, 395, 408, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 432, 433, 434, 441, 442, 446, 447, 448, 449, 450, 451, 452, 453, 512 and 513 were checked for members of Aphyocharacinae available from Mirande (2019), and the differences observed are highlighted in the Results. Characters 92 and 450 in Mirande (2019) were necessarily modified and are also addressed in the Results. The above listed characters were also coded for the newly added taxa.

Though the addition of new characters are assigned as missing data for the remaining taxa of Mirande's (2019) original matrix, increasing data set size can improve phylogenetic estimation methods (Wright & Hillis, 2014). The influence of missing data on morphological super matrix was tested by Dillman *et al.* (2016), and results show that matrices composed by more than 60% of missing data are still viable to reconstruct strongly supported and highly resolved hypotheses of relationships under Parsimony analysis.

The tree was estimated using two independent runs, each run performed with 400000 MCMC generations with trees stored every 100 iteration and pre burnin at 10% with 8000 trees retained. The distribution of log likelihood scores was examined to determine stationarity for each run and achieve convergence through ESS values using Tracer v1.7.1 (Rambaut *et al.*, 2018), with 10% of the initial states discarded as burn-in. The Bayesian inference hypothesis based on morphological data was obtained as the Maximum Clade Credibility tree (MCC).

Ancestral character state reconstruction (ACSR)

From the Bayesian phylogenetic hypotheses generated with the data collected during this study, an analyses of ancestral character reconstruction was performed through the application of scripts using the R packages: ape (Paradis *et al.*, 2004), phytools (Revell, 2012), parallel (R Core Team, 2017), geiger (Harmon *et al.*, 2008). The ACSR analyses were conducted under the following models of discrete character evolution: "ER" – equal rates for all permitted transitions; "ARD" – all-rates-different for permitted transitions; "SYM" – symmetric backward and forward rates for all permitted transitions (Harmon *et al.*, 2020). When comparing models fitted by maximum likelihood, the best model was assessed under the Akaike Information Criterion method (AIC) with a correction to small sample size (AICc; Burnham & Anderson, 2002). AICc values were calculated for each applied fitted model. Akaike weights were retrieved from AICc values, and were used for selection of best fitted model (see Results).

RESULTS

4. Secondary sexual characters in Aphyocharacinae

The occurrence of secondary sexual characters in members of the subfamily Aphyocharacinae is demonstrated for adult males of the genera *Aphyocharacidium*, *Aphyocharax* and *Prionobrama* genera, as well as in *Axelrodia lindeae*. Examined material from the genera *Leptagoniates*, *Paragoniates*, *Phenagoniates* and *Xenagoniates* showed no evidence of bony hooks. Through careful examination of their external morphology, no conspicuous features or consistent patterns of sexual dimorphism were observed in these genera. Character information on *Aphyocharax gracilis* were gathered only from the holotype (ANSP68864), where no evidence of secondary sexual characters was observed. With no support or indication of the absence/presence of sexually dimorphic structures in this species, all characters related to those features were coded as unknown.

Gradients of development were observed throughout the examined material, providing robust information on variants of the morphology and distribution of the sexually dimorphic characters. The shape of bony hooks were carefully observed among species, and could be broadly sorted into a variation of sizes on the following three categories: Weakly developed ("nodule-like") – bony hooks seen as a conspicuous protuberance resembling a nodule (small rounded lump) on fin ray surface; Intermediate – bony hooks not quite prominent, but conspicuously visible with clear distinction from proximal base and distal tip; Well-developed – prominent structure with base and distal tip fully evident. Bony hooks were absent on dorsal, pectoral and caudal-fin rays of adult males, and on all fins of females. These categories (Figure 2), as defined, are not intended to relate to gonadal development, i.e. direct external observations of secondary sexual characters were not intended to approximate gonadal developmental and sexual maturity.

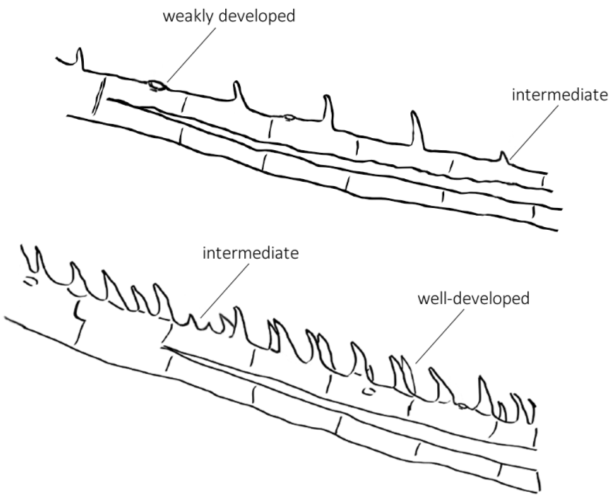

Figure 2 – Three categories based on variation of sizes of bony hooks on fin rays.

Observations on the findings of the presence of bony hooks on fin rays of adult males and comments on other dimorphic features are described below.

Aphyocharacidium bolivianum Géry, 1973

Pelvic fin

Pelvic fin sexually dimorphic, with no elongation of pelvic-fin rays in adult males. Pelvic-fin bony hooks were observed on the unbranched ray and from the first to the sixth branched ray. Bony hooks on fin rays occurring on a symmetric, paired and regularly arranged fashion, similar in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks distributed on proximal-middle length of rays, including the long basal segment of lepidotrichia. Bony hooks usually attached to ventral borders of medial and lateral branches on the secondary branches of the rays. Hook-bearing segments usually exhibiting 1 to 2 bony hooks per segment of lepidotrichia, which seems to be more numerous in first rays becoming scarcer in number on last rays.

Pelvic-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a straight tip. On all rays more developed hooks project from ray segments with tips directed to the medial plane.

Well-developed and intermediate hooks are found accumulated in more proximal portions of the rays, while weakly developed hooks become more predominant distally. When occurring on the long basal segment, bony hooks are found in similar number when compared to segmented portions of the ray. Bony hooks occurring on secondary branches of the first to fifth branched rays are as numerous as on medial primary branches, usually bearing 1 to 2 hooks per segment of lepidotrichia.

Anal fin

Anal fin sexually dimorphic, with no elongation of anal-fin rays in adult males. Anal-fin bony hooks were observed on the last unbranched ray and from the first to the tenth branched ray. Sexually dimorphic males exhibit anterior branched anal-fin rays bearing hooks more expanded in the sagittal plane than comparable to other anal-fin rays. Bony hooks on fin rays occurring on an asymmetric, unpaired and irregularly arranged fashion, varying in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks distributed on middle-distal length of rays, beginning after primary branching (usually first appearing on the 5th or 6th most proximal segment of lepidotrichia). Bony hooks usually attached to lateral borders of anterior and posterior branches on the secondary branches of the rays. Hook-bearing segments usually with 1 to 3 bony hooks per segment of lepidotrichia, and conspicuously more numerous in first rays becoming abruptly reduced in number on most posterior ray.

Anal-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays, more developed hooks project from ray segments with tips directed to the anal-fin base.

Well-developed and intermediate hooks are found concentrated in the middle portion of the rays, while intermediate and weakly developed hooks were observed more distally. Bony hooks occurring on secondary branches from first to sixth branched rays are as numerous as on primary branches, displaying 1 to 2 hooks (sometimes 3) per segment of lepidotrichia.

Aphyocharacidium n. sp. (new species)

Pelvic fin

Pelvic fin sexually dimorphic, with no elongation of pelvic-fin rays in adult males (Figure 3). Pelvic-fin bony hooks occurring on the unbranched ray and from first to the sixth branched ray. Bony hooks on fin rays are asymmetrically arranged, occurring only in the medial hemitrichia of rays. Bony hooks distributed along the entire length of the rays, beginning from the long basal segment to the most distal segment of lepidotrichia. Bony hooks usually attached to ventral borders of medial and lateral branches on the secondary branches of the rays. Hook-bearing segments usually with 1 to 3 bony hooks per segment of lepidotrichia, which seems to be higher in number in middle rays (usually from 2nd to 4th branched rays) reducing in number on last rays.

Pelvic-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays more developed hooks project from ray segments with tips directed to the medial plane.

Well-developed and intermediate hooks are found through the entire length of most rays, while weakly developed hooks become more predominant on last rays (usually on the 6th branched ray). Bony hooks on the long basal segment are numerous, similar to segmented portions of the rays. Bony hooks occurring on secondary branches of first to fifth branched rays are as numerous as on medial primary branches, regularly exhibiting 1 to 2 hooks (sometimes 3) per segment of lepidotrichia.

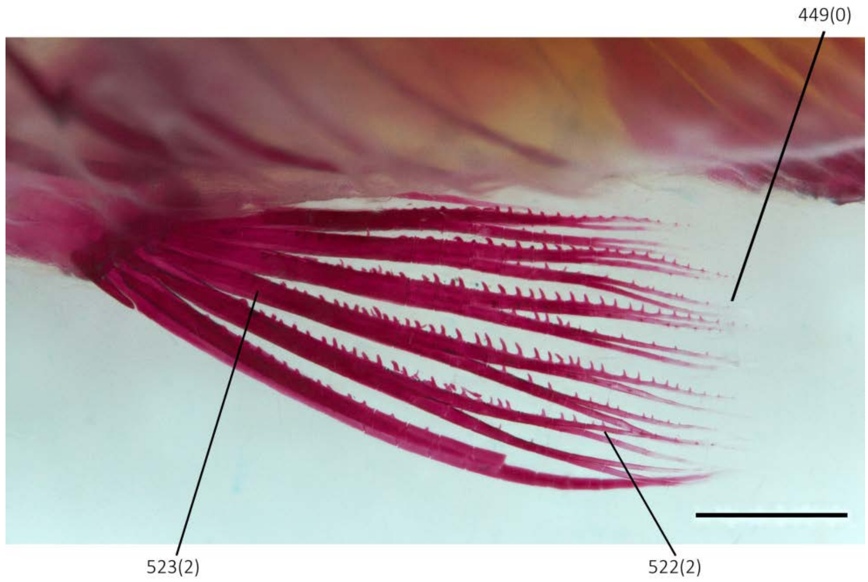

Figure 3 – Pelvic fin of sexually dimorphic male of *Aphyocharacidium* n. sp. (MUSM68942), 27.8 mm SL. Scale bar= 1mm.

Anal fin

Anal fin sexually dimorphic, with no elongation of anal-fin rays in adult males (Figure 4). Anal-fin bony hooks were observed on the last unbranched ray and from the first to the seventh branched ray. Sexually dimorphic males exhibit anterior branched anal-fin rays bearing hooks more expanded in the sagittal plane than comparable to other anal-fin rays. Bony hooks on fin rays occuring on an asymmetric, unpaired and irregular arrangement, varying in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks distributed on middle-distal length of rays, beginning before primary branching (first appearing from 2nd to 5th most proximal segment of lepidotrichia). Bony hooks attached to posterolateral border of the rays. Hook-bearing segments usually with 1 to 4 bony hooks per segment of lepidotrichia, and conspicuously more numerous in first rays becoming abruptly reduced in number in most posterior ray.

Anal-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays, more developed hooks project from ray segments with tips directed to the anal-fin base.

Well-developed hooks are found near middle portions and intermediate hooks usually observed at distal portions of most rays, while weakly developed hooks become more predominant on last rays. Bony hooks absent on secondary branches of the rays.

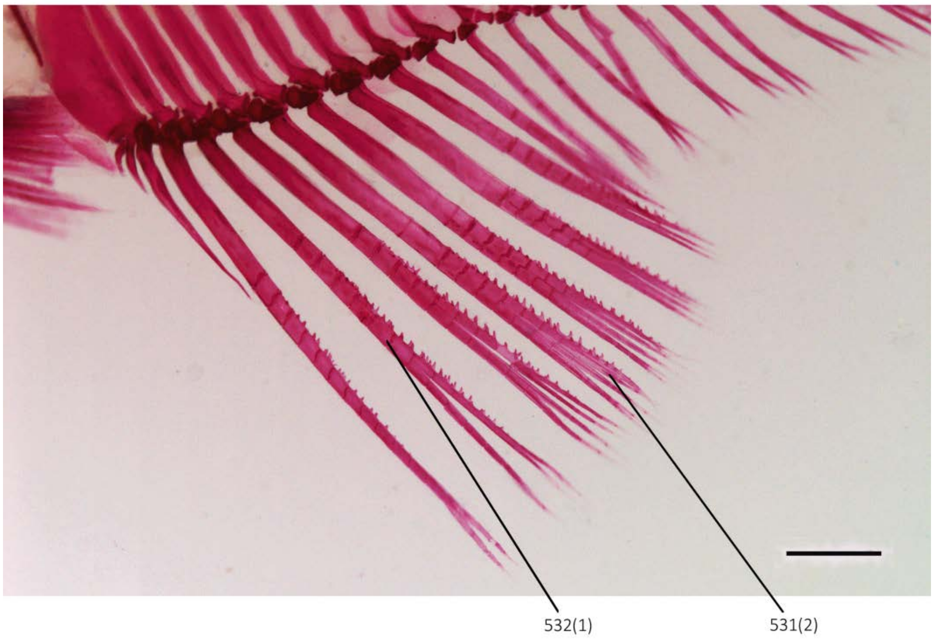

Figure 4 – Anal fin of sexually dimorphic male of *Aphyocharacidium* n. sp. (MUSM68942), 27.8 mm SL. Scale bar= 1mm.

Caudal peduncle

Procurrent caudal-fin rays are fully observed in males and females of this species, projecting between muscles and skin on ventral margin of caudal-peduncle. The anterior elements (1 to 5) of the ventral procurrent caudal-fin rays series of adult males are hypertrophied and more expanded in the sagittal plane when related to remaining rays. All procurrent caudal-fin rays are slender on females.

Axelrodia lindeae Géry, 1973

Pelvic fin

Pelvic fin sexually dimorphic, with no elongation of pelvic-fin rays observed in adult males (Figure 5). Pelvic-fin bony hooks occurring on the first to the sixth branched ray. Bony hooks on fin rays on a symmetric, paired and regularly arranged manner, similar in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks distributed on proximal-middle length of rays, beginning from the long basal segment of lepidotrichia. Bony hooks usually attached to ventral borders of medial and lateral branches on the secondary branches of the rays. Hook-bearing segments usually with 1 to 2 bony hooks per segment of lepidotrichia, which are numerous in most rays with reduced number on last ray.

Pelvic-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays more developed hooks project from ray segments with tips directed to pelvic fin insertion.

Well-developed hooks are found near middle portions of most rays, while intermediate and weakly developed hooks are usually observed more distally. Weakly developed hooks become more predominant on last rays (usually on the 6th branched ray). Bony hooks on the long basal segment are numerous, similar to segmented portions of the ray. Bony hooks occurring on secondary branches of first to fourth branched rays are as numerous as on medial primary branches, regularly exhibiting 1 to 2 hooks (usually 1) per segment of lepidotrichia.

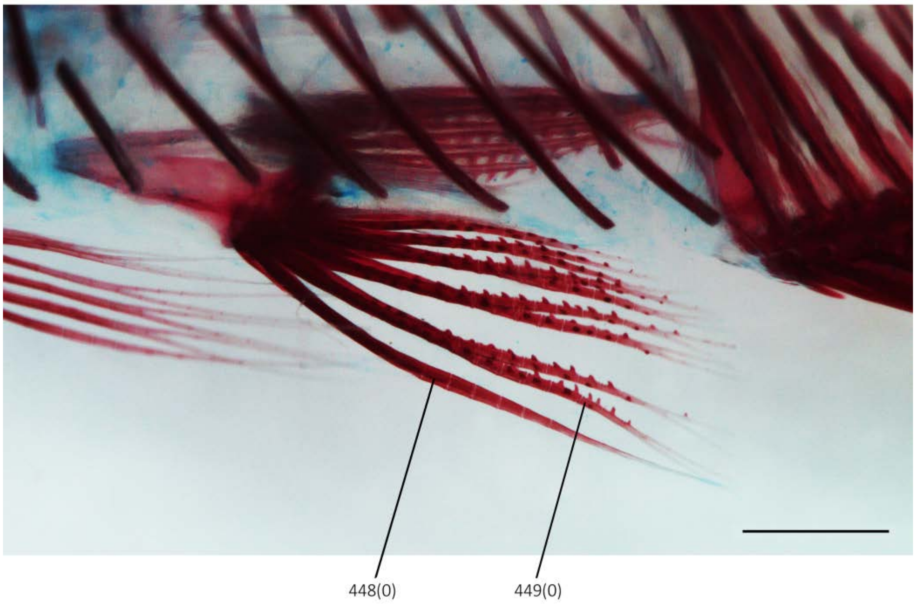

Figure 5 – Pelvic fin of sexually dimorphic male of *Axelrodia lindeae* (MCP37314), 22.5 mm SL. Scale bar= 1mm.

Anal fin

Anal fin sexually dimorphic, with no elongation of anal-fin rays in adult males (Figure 6). Anal-fin bony hooks were observed on the last unbranched ray and from the first to the fifth branched ray. Sexually dimorphic males exhibit anterior branched anal-fin rays bearing hooks more expanded in the sagittal plane than comparable to other anal-fin rays. Bony hooks on fin rays occurring on a symmetric, paired and regular arrangement, similar in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks occurrence restricted to the middle length of rays, beginning before primary branching (usually first appearing on the 2nd or 3rd most proximal segment of lepidotrichia). Bony hooks attached to posterolateral border of the rays. Hook-bearing segments usually with 1 to 4 bony hooks per segment of lepidotrichia, conspicuously more numerous in anterior rays reduced in number on most posterior ray.

Anal-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays, more developed hooks project from ray segments with tips directed to the anal-fin base.

Well-developed hooks are found near middle portions of first rays, while intermediate and weakly developed hooks usually observed in most posterior rays. Bony hooks absent on secondary branches of the rays.

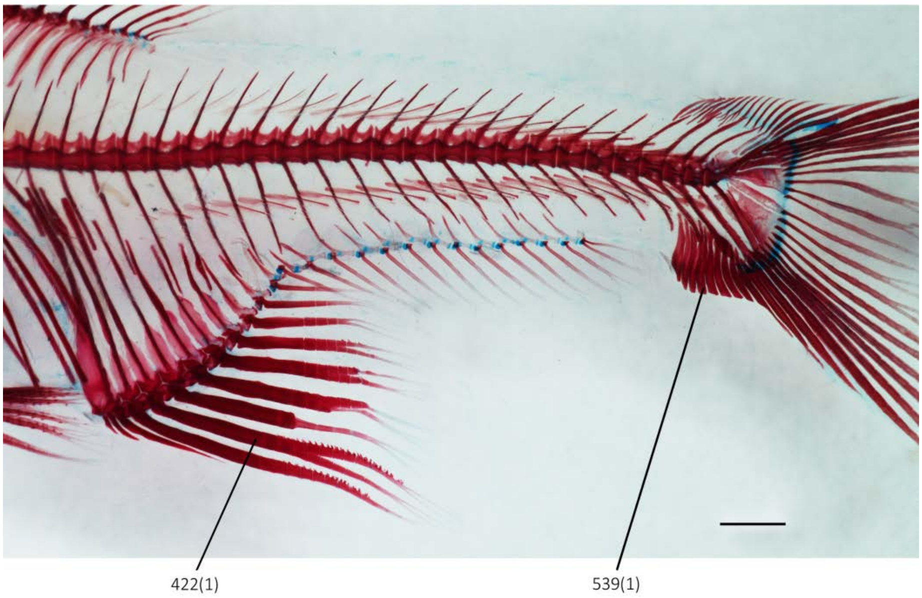

Figure 6 – Anal-fin rays and ventral procurrent caudal-fin rays of sexually dimorphic male of *Axelrodia lindeae* (MCP37314), 22.5 mm SL. Scale bar= 1mm.

Caudal peduncle

Procurrent caudal-fin rays are fully observed in males and females of this species, projecting between muscles and skin on ventral margin of caudal-peduncle. The anterior elements (1 to 9) of the ventral procurrent caudal-fin rays series of adult males are hypertrophied and more expanded in the sagittal plane when related to remaining rays (Fig. 6). All procurrent caudal-fin rays are slender on females.

Aphyocharax alburnus Günther, 1869

Pelvic fin

Pelvic fin sexually dimorphic, with no elongation of pelvic-fin rays in adult males. Pelvic-fin bony hooks occurring on the first to the fourth branched ray. Bony hooks on fin rays on a symmetric, paired and regular arrangement, similar in position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed restricted to the middle length of rays, beginning before primary branching (first appearing from 2nd to 6th most proximal segment of lepidotrichia). Bony hooks usually attached to medioventral border of the rays.

Hook-bearing segments usually with 1 to 2 bony hooks per segment of lepidotrichia, which show little variation in number progressively reduced from first to last rays.

From examined material, pelvic-fin bony hooks were consistently observed as a conspicuous protuberance resembling a nodule (small rounded lump) on hook-bearing rays' surface. Though all adult males of this species displayed “nodule-like” hooks, the description of pelvic-fin bony hooks shape was carefully considered. Characters concerning “shape” of bony hooks (ch. 525 and ch. 526 – see details below) were coded as unknown.

Bony hooks were observed throughout middle portions of hook-bearing rays. Bony hooks absent on secondary branches of the rays.

Anal fin

Anal fin sexually dimorphic, with no elongation of anal-fin rays in adult males. Anal-fin bony hooks were observed on the last unbranched ray and from the first to the sixth branched ray. Bony hooks on fin rays occuring on an asymmetric, unpaired and irregular arrangement, varying in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks presence are restricted to the middle length of rays, beginning after primary branching (first appearing from 5th to 9th most proximal segment of lepidotrichia). Bony hooks attached to posterolateral border of the rays. Hook-bearing segments with 1 bony hook per segment of lepidotrichia, with branched rays showing little variation in number on anterior rays, with reduced number on most posterior ray. Last unbranched ray bearing fewer hooks when compared to branched rays.

Anal-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays, more developed hooks project from ray segments with tips directed to the anal-fin base.

Well-developed hooks are found near middle portions of most rays, while intermediate and weakly developed hooks are usually observed more distally. Weakly developed hooks become more predominant on most posterior rays. Bony hooks absent on secondary branches of the rays.

Aphyocharax anisitsi Eigenmann & Kennedy, 1903

Pelvic fin

Pelvic fin sexually dimorphic, with no elongation of pelvic-fin rays observed in adult males (Figure 7). Pelvic-fin bony hooks occurring on the unbranched ray and from first to the sixth branched ray. Bony hooks on fin rays on a symmetric, paired and regularly arrangement, similar in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed on proximal-middle length of rays, beginning from the long basal segment of lepidotrichia. Bony hooks usually attached to medioventral border of the rays. Hook-bearing segments usually with 1 to 2 bony hooks per segment of lepidotrichia, which are numerous in most rays decreasing in number on last rays.

Pelvic-fin bony hooks showed a base with same width as middle length of hook sharpening into a retrorse tip. On all rays more developed hooks project from ray segments with tips directed to pelvic fin insertion.

Well-developed hooks are found from long basl segment to middle portions of most rays, while intermediate are usually observed more distally. Weakly developed hooks become more predominant on last rays (usually on the 5th or 6th branched ray). Bony hooks on the long basal segment are numerous, similar to segmented portions of the ray. Bony hooks absent on secondary branches of the rays.

Examined paratype (CAS059718) is an adult male, showing distribution of pelvic-fin bony hooks on unbranched ray and from first to fifth branched ray. Remaining descriptions are consistent with non-type examined material.

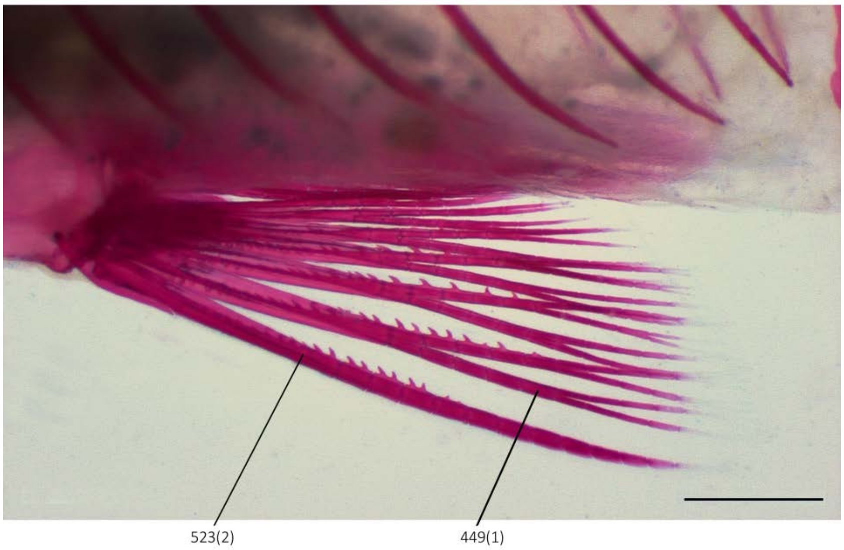

Figure 7 - Pelvic fin of sexually dimorphic male of *Aphyocharax anisitsi* (FMNH107797). Scale bar= 1mm.

Anal fin

Anal fin sexually dimorphic, with no elongation of anal-fin rays in adult males (Figure 8). Anal-fin bony hooks were observed on the last unbranched ray and from the first to the nineteenth branched ray. Bony hooks on fin rays on an asymmetric, unpaired and irregular arrangement, varying in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks occurence are restricted to the middle length of rays, beginning before primary branching (first appearing from 2nd to 4th most proximal segment of lepidotrichia). Bony hooks attached to posterolateral border of the rays. Hook-bearing segments with 1 bony hook per segment of lepidotrichia, showing little variation in number on most rays, with reduced number on most posterior ray.

Anal-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays, more developed hooks project from ray segments with tips directed to the anal-fin base.

Well-developed hooks are found on middle portions of most rays, while intermediate and weakly developed hooks are usually observed more distally. Bony hooks absent on secondary branches of the rays.

A thickened layer of what may be epithelial tissue (histology evidence are needed to confirm) are usually observed in association with hook-bearing portions of most anal-fin rays.

Anal fin from examined paratype (CAS059718) is in poor state condition with many of the anal-fin rays broken from middle portion on. Anal-fin bony hooks were observed on last unbranched ray and from first to seventeenth branched ray. Remaining descriptions are consistent with non-type examined material.

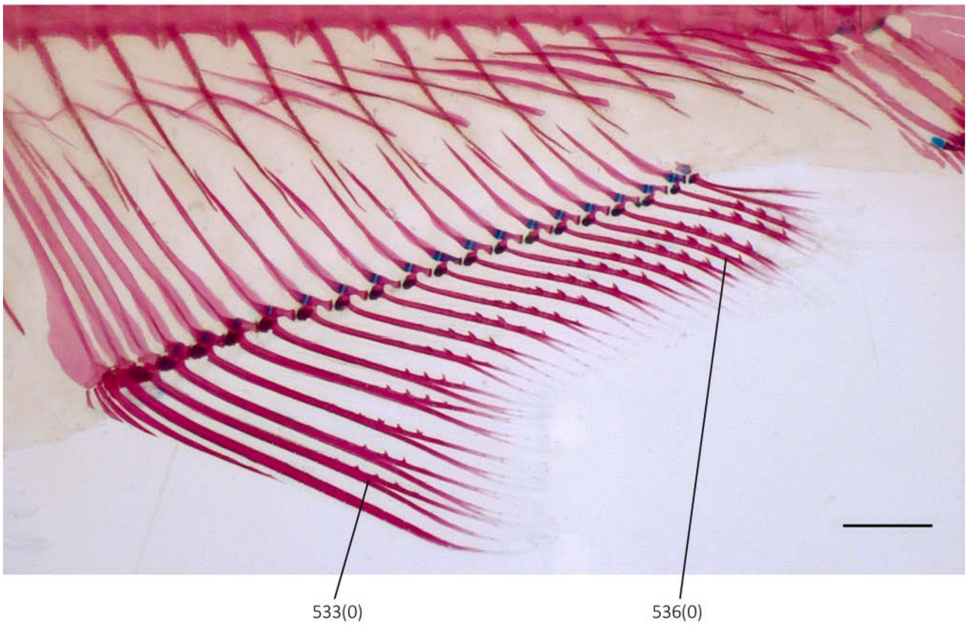

Figure 8 – Anal fin of sexually dimorphic male of Aphyocharax anisitsi (FMNH107797). Scale bar= 1mm.

Aphyocharax avary Fowler, 1913

Pelvic fin

Pelvic fin sexually dimorphic, with no elongation of pelvic-fin rays in adult males. Pelvic-fin bony hooks occurring on the first to the fifth branched ray. Bony hooks on fin rays on an asymmetric, unpaired and irregular arrangement, varying in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed restricted to the middle length of rays, beginning after primary branching (first appearing from 2nd to 7th most proximal segment of lepidotrichia). Bony hooks usually attached to medioventral border of the rays. Hook-bearing segments usually with 1 to 2 bony hooks per segment of lepidotrichia, which are numerous in most rays progressively decreasing in number from first to last rays.

Pelvic-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays more developed hooks project from ray segments with tips directed to the medial plane.

Well-developed hooks are found near middle portions of most rays, while intermediate and weakly developed hooks are usually observed more distally. Weakly developed hooks become more predominant on last rays. Bony hooks absent on secondary branches of the rays.

Examined holotype (ANSP39217) is an adult male, showing distribution of pelvic-fin bony hooks on first to fifth branched ray. Remaining descriptions are consistent with non-type examined material.

Anal fin

Anal fin sexually dimorphic, with no elongation of anal-fin rays in adult males. Anal-fin bony hooks were observed on the last unbranched ray and from the first to the thirteenth branched ray. Bony hooks on fin rays on an asymmetric, unpaired and irregular arrangement, varying in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed restricted to the middle length of rays, beginning before primary branching (first appearing from 2nd to 5th most proximal segment of lepidotrichia). Bony hooks attached to posterolateral border of the rays. Hook-bearing segments usually with 1 to 2 bony hooks per segment of lepidotrichia, showing little variation in number on most rays, progressivelly reducing in number on most posterior rays.

Anal-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays, more developed hooks project from ray segments with tips directed to the anal-fin base.

Well-developed hooks are found on middle portions of most rays, while intermediate and weakly developed hooks are usually observed more distally. Weakly developed hooks become more predominant on most posterior rays. Bony hooks absent on secondary branches of the rays.

Anal fin from examined holotype (ANSP39217) is in good state condition but with some of the anal-fin rays broken or damaged from middle portion on. Anal-fin bony hooks could not be observed on last unbranched ray (broken), occuring from first to fifth branched ray. Remaining descriptions are consistent with non-type examined material.

Aphyocharax colifax Taphorn & Thomerson, 1991

Pelvic fin

Pelvic fin sexually dimorphic, with no elongation of pelvic-fin rays in adult males. Pelvic-fin bony hooks occurring on the first to the fifth branched ray. Bony hooks on fin rays occurring on a symmetric, paired and regular arrangement, similar in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed restricted to the middle length of rays, beginning before primary branching (first appearing from 2nd to 4th most proximal segment of lepidotrichia). Bony hooks usually attached to medioventral border of the rays. Hook-bearing segments usually with 1 to 2 bony hooks per segment of lepidotrichia, which are numerous in first rays progressively decreasing in number from first to last rays.

Pelvic-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays more developed hooks project from ray segments with tips directed to pelvic fin insertion.

Well-developed hooks were observed middle portions of most rays, while intermediate and weakly developed hooks are usually observed more distally. Weakly developed hooks become more predominant on last rays. Bony hooks absent on secondary branches of the rays.

Anal fin

Anal fin sexually dimorphic, with no elongation of anal-fin rays in adult males. Anal-fin bony hooks were observed on the last unbranched ray and from the first to the seventh branched ray. Bony hooks on fin rays on a symmetric, paired and regularly arrangement, similar in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed restricted to the middle length of rays, beginning before primary branching (first appearing from 2nd to 5th most proximal segment of lepidotrichia). Bony hooks usually attached to lateral borders of posterior and anterior branches on the secondary branches of the rays. Hook-bearing segments with 1 bony hook per segment of lepidotrichia, displaying little variation in number on most rays gradually decreasing in number on most posterior rays.

Anal-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays, more developed hooks project from ray segments with tips directed to the anal-fin base.

Well-developed hooks are found on middle portions of most rays, while intermediate hooks are usually observed more distally. Weakly developed hooks become more predominant on most posterior rays. Bony hooks occurring on secondary branches of rays are reduced in number compared to primary branches, regularly exhibiting 1 hook per segment of lepidotrichia.

Aphyocharax dentatus Eigenmann & Kennedy, 1903

Pelvic fin

Pelvic fin sexually dimorphic, with no elongation of pelvic-fin rays in adult males. Pelvic-fin bony hooks occurring on the unbranched ray and from the first to the sixth branched ray. Bony hooks on fin rays occurring on a symmetric, paired and regular arrangement, similar in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed on proximal-middle length of rays, beginning from the long basal segment of lepidotrichia. Bony hooks usually attached to ventral borders of medial and lateral branches on the secondary branches of the rays. Hook-bearing segments usually with 1 to 2 bony hooks per segment of lepidotrichia, more numerous in middle rays (usually 2nd to 4th branched rays), reduced in number on last ray.

Pelvic-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays more developed hooks project from ray segments with tips directed to the medial plane.

Well-developed hooks were observed middle portions of most rays, while intermediate and weakly developed hooks are usually observed more distally. Intermediate and weakly developed hooks become more predominant on last rays. Bony hooks occurring on secondary branches of second to fifth branched rays are as numerous as on medial primary branches, regularly exhibiting 1 to 2 hooks (usually 1) per segment of lepidotrichia.

Examined paratypes (CAS076471 ; CAS059724 ; CAS059726 ; CAS059725 ; CAS059723) are adult specimens, with the presence of bony hooks on pelvic-fin rays detected in five specimens (CAS059726 – 1 spp. ; CAS059725 – 1 spp. ; CAS059723 – 2 spp.). Pelvic-fin bony hooks could be observed from the first to the sixth branched ray, absent on the unbranched ray. Remaining descriptions are consistent with non-type examined material.

Anal fin

Anal fin sexually dimorphic, with no elongation of anal-fin rays in adult males. Anal-fin bony hooks were observed on the last unbranched ray and from the first to the ninth branched ray. Bony hooks on fin rays on an asymmetric, unpaired and irregular arrangement, varying in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed restricted to the middle length of rays, beginning after primary branching (usually first appearing from 5th to 7th most proximal segment of lepidotrichia). Bony hooks usually attached to lateral borders of posterior and anterior branches on the secondary branches of the rays. Hook-bearing segments usually with 1 to 2 bony hooks per segment of lepidotrichia, displaying little variation in number on most rays and progressively reducing in number on most posterior rays.

Anal-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays, more developed hooks project from ray segments with tips directed to the anal-fin base.

Well-developed hooks are found on middle portions of most rays, while intermediate hooks are usually observed more distally. Intermediate and weakly developed hooks become more predominant on most posterior rays. Bony hooks occurring on secondary branches of rays are reduced in number compared to primary branches, usually exhibiting 1 to 2 hooks per segment of lepidotrichia.

Anal fin from examined paratypes (CAS076471 ; CAS059724 ; CAS059726 ; CAS059725 ; CAS059723) vary on state condition, with some of the specimens with anal-fin rays broken or damaged from middle portion on. Anal-fin bony hooks were detected on last unbranched ray, occuring from first to ninth branched ray. Remaining descriptions are consistent with non-type examined material.

Aphyocharax erythrurus Eigenmann, 1912

Pelvic fin

Pelvic fin sexually dimorphic, with no elongation of pelvic-fin rays in adult males. Pelvic-fin bony hooks occurring on the first to the sixth branched ray. Bony hooks on fin rays occuring on a symmetric, paired and regular arranged manner, similar in position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed restricted to the middle length of rays, beginning after primary branching (first appearing from 7th to 9th most proximal segment of lepidotrichia). Bony hooks usually attached to medioventral border of the rays. Hook-bearing segments with 1 bony hook per segment of lepidotrichia, showing variation in number progressively reduced from first to last rays.

From examined material, pelvic-fin bony hooks were consistently observed as a conspicuous protuberance resembling a nodule (small rounded lump) on hook-bearing rays' surface. Though all adult males of this species displayed "nodule-like" hooks, the description of pelvic-fin bony hooks shape was carefully considered. Characters concerning "shape" of bony hooks (ch. 525 and ch. 526 – see details below) were coded as unknown.

Bony hooks were observed throughout middle portions of hook-bearing rays. Bony hooks absent on secondary branches of the rays.

Anal fin

Anal fin sexually dimorphic, with no elongation of anal-fin rays in adult males. Anal-fin bony hooks were observed on the last unbranched ray and from the first to the eleventh branched ray. Bony hooks on fin rays on an asymmetric, unpaired and irregular arrangement, varying in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks presence are restricted to the middle length of rays, beginning after primary branching (first appearing from 5th to 7th most proximal segment of lepidotrichia). Bony hooks usually attached to lateral borders of posterior and anterior branches on the secondary branches of the rays. Hook-bearing segments with 1 bony hook per segment of lepidotrichia, with unbranched ray showing reduced number of hooks and branched rays showing little variation in number on anterior rays, with reduced number on most posterior rays.

Anal-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays, more developed hooks project from ray segments with tips directed to the anal-fin base.

Well-developed hooks are found near middle portions of most rays, while intermediate and weakly developed hooks are usually observed more distally. Weakly developed hooks become more predominant on most posterior rays. Bony hooks occurring on secondary branches of rays are reduced in number compared to primary branches, usually exhibiting 1 to 2 hooks (usually 1) per segment of lepidotrichia.

Comments

Examined paratypes (CAS-SU021912) were included in this analysis, and no bony hooks were detected on pelvic-fin and anal-fin rays.

Aphyocharax nattereri (Steindachner, 1882)

Pelvic fin

Pelvic fin sexually dimorphic, with no elongation of pelvic-fin rays in adult males. Pelvic-fin bony hooks were observed on the unbranched ray and from the first to the fifth branched ray. Bony hooks on fin rays on a symmetric, paired and regular arrangment, similar in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks distributed on proximal-middle length of rays, beginning from the long basal segment of lepidotrichia. Bony hooks usually attached to ventral borders of medial and lateral branches on the secondary branches of the rays. Hook-bearing segments usually with 1 to 2 bony hooks per segment of lepidotrichia, which are more concentrated in number on most branched rays becoming abruptly reduced in number on last ray.

Pelvic-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays more developed hooks project from ray segments with tips directed to pelvic fin insertion.

Well-developed and intermediate hooks are found accumulated in more proximal portions of the rays, while weakly developed hooks become more predominant at distal portions of the rays. When occurring on the long basal segment, bony hooks are found in reduced number when compared to segmented portions of the ray. Bony hooks occurring on secondary branches of the first to fifth branched rays are as numerous as on medial primary branches, Bony hooks occurring on secondary branches from first to second branched rays are reduced in number compared to medial branches, displaying 1 hook per segment of lepidotrichia.

Anal fin

Anal fin sexually dimorphic, with no elongation of anal-fin rays in adult males. Anal-fin bony hooks were observed on the last unbranched ray and from the first to the seventh branched ray. Bony hooks on fin rays occurring on an asymmetric, unpaired and irregularly arranged fashion, varying in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed restricted to the middle length of rays, beginning before primary branching (first appearing from 2nd to 5th most proximal segment of lepidotrichia). Bony hooks attached to posterolateral border of the rays. Hook-bearing segments usually with 1 to 2 bony hooks per segment of lepidotrichia, showing little variation in number on anterior rays, reduced in number on most posterior rays.

Anal-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays, more developed hooks project from ray segments with tips directed to the anal-fin base.

Well-developed and intermediate hooks are found concentrated in the middle portion of the rays, while intermediate and weakly developed hooks were observed more distally.

Weakly developed hooks become more predominant on most posterior rays. Bony hooks absent on secondary branches of the rays.

Aphyocharax pusillus Günther, 1868

Pelvic fin

Pelvic fin sexually dimorphic, with no elongation of pelvic-fin rays in adult males. Pelvic-fin bony hooks occurring from the first to the sixth branched ray. Bony hooks on fin rays occurring on a symmetric, paired and regular arrangement, similar in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed restricted to the middle length of rays, beginning after primary branching (first appearing from 2nd to 5th most proximal segment of lepidotrichia). Bony hooks usually attached to ventral borders of medial and lateral branches on the secondary branches of the rays. Hook-bearing segments usually with 1 to 3 bony hooks (usually 2) per segment of lepidotrichia, more numerous in first rays gradually decreasing in number on last rays.

Pelvic-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a straight tip. On all rays more developed hooks project from ray segments with tips directed to pelvic fin insertion.

Well-developed and intermediate hooks were observed near middle portions of most rays, while weakly developed hooks are usually observed more distally. Intermediate and weakly developed hooks become more predominant on last rays. Bony hooks occurring on secondary branches of second to fourth branched rays are reduced in number when compared to medial primary branches, regularly exhibiting 1 hook per segment of lepidotrichia.

Anal fin

Anal fin sexually dimorphic, with no elongation of anal-fin rays in adult males. Anal-fin bony hooks were observed on the last unbranched ray and from the first to the eleventh branched ray. Bony hooks on fin rays on an asymmetric, unpaired and irregular arrangement, varying in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed restricted to the middle length of rays, beginning before primary branching (usually first appearing from 3rd to 5th most proximal segment of lepidotrichia). Bony hooks attached to posterolateral border of the rays. Hook-bearing segments usually with

1 to 2 bony hooks per segment of lepidotrichia, displaying little variation in number on most anterior rays progressively reducing in number on most posterior rays.

Anal-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays, more developed hooks project from ray segments with tips directed to the anal-fin base.

Well-developed and intermediate hooks are found concentrated in the middle portion of the rays, while intermediate and weakly developed hooks were observed more distally. Intermediate and weakly developed hooks become more predominant on most posterior rays. Bony hooks absent on secondary branches of the rays.

Aphyocharax rathbuni Eigenmann, 1907

Pelvic fin

Pelvic fin sexually dimorphic, with no elongation of pelvic-fin rays observed in adult males. Pelvic-fin bony hooks occurring on the unbranched ray and from first to the sixth branched ray. Bony hooks on fin rays occuring on a symmetric, paired and regularly arrangement, similar in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed on proximal-middle length of rays, beginning from the long basal segment of lepidotrichia. Bony hooks usually attached to medioventral border of the rays. Hook-bearing segments usually with 1 to 2 bony hooks per segment of lepidotrichia, with numerous hooks on first branched rays (usually from 2nd to 4th branched rays) and decreasing in number on last rays. Unbranched ray bearing fewer hooks when compared to branched rays.

Pelvic-fin bony hooks showed a base with same width as middle length of hook sharpening into a retrorse tip. On all rays more developed hooks project from ray segments with tips directed to pelvic fin insertion.

Well-developed hooks are found from long basl segment to middle portions of most rays, while intermediate are usually observed more distally. Weakly developed hooks become more predominant on last rays (usually on the 5th or 6th branched ray). Bony hooks on the long basal segment are numerous, similar to segmented portions of the ray. Bony hooks absent on secondary branches of the rays.

Anal fin

Anal fin sexually dimorphic, with no elongation of anal-fin rays in adult males. Anal-fin bony hooks were observed on the last unbranched ray and from the first to the nineteenth branched ray. Bony hooks on fin rays on an asymmetric, unpaired and irregular arrangement, varying in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks occuring on proximal-middle length of rays, beginning from the long basal segment of lepidotrichia. Bony hooks attached to posterolateral border of the rays. Hook-bearing segments usually with 1 to 2 bony hooks per segment of lepidotrichia, showing little variation in number on most rays, with reduced number on most posterior ray.

Anal-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays, more developed hooks project from ray segments with tips directed to the anal-fin base.

Well-developed hooks are found on middle portions of most rays, while intermediate hooks are usually observed near proximal portions of hook-bearing rays. Intermediate and weakly developed hooks become more predominant on most posterior rays. Bony hooks absent on secondary branches of the rays.

Aphyocharax yekwanae Willink, Chernoff & Machado-Allison, 2003

Pelvic fin

Pelvic fin sexually dimorphic, with no elongation of pelvic-fin rays in adult males. Pelvic-fin bony hooks occurring on the unbranched rays and from the first to the sixth branched ray. Bony hooks on fin rays occurring on a symmetric, paired and regular arrangement, similar in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed restricted to the middle length of rays, beginning after primary branching (usually first appearing from 6th or 7th most proximal segment of lepidotrichia). Bony hooks usually attached to ventral borders of medial and lateral branches on the secondary branches of the rays. Hook-bearing segments usually with 1 to 2 bony hooks per segment of lepidotrichia, more numerous in first rays gradually decreasing in number on last rays.

Pelvic-fin bony hooks showed a base with same width as middle length of hook sharpening into a straight tip. On examined material, pelvic-fin rays of adult males exhibited quite small bony hooks, but it was possible to observe tips projecting towards the medial plane.

Bony hooks were observed throughout middle portions of most rays, almost reaching dital portions of rays. Bony hooks usually occurring on secondary branches of first to fourth branched rays, reduced in number when compared to medial primary branches, regularly exhibiting 1 hook per segment of lepidotrichia.

Anal fin

Anal fin sexually dimorphic, with no elongation of anal-fin rays in adult males. Anal-fin bony hooks were observed on the last unbranched ray and from the first to the fifteenth branched ray. Bony hooks on fin rays were observed on a symmetric, paired and regular arrangement, similar in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed restricted to the middle length of rays, beginning before primary branching (usually first appearing from 4th to 6th most proximal segment of lepidotrichia). Bony hooks attached to posterolateral border of the rays. Hook-bearing segments with 1 bony hook per segment of lepidotrichia, exhibiting little variation in number on most anterior rays progressively reducing in number on most posterior rays.

Anal-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays, more developed hooks project from ray segments with tips directed to the posterior region.

Well-developed hooks are found near middle portion of the rays, while intermediate hooks were observed more distally. Intermediate and weakly developed hooks become more predominant on most posterior rays. Bony hooks absent on secondary branches of the rays.

Comments

Data from this species were gathered from examined paratypes (FMNH109275 ; FMNH109277). Paratypes are adult specimens, in good state condition, with the presence of bony hooks observed on pelvic-fin and anal-fin rays detected on one specimen from FMNH109275.

Prionobrama filigera (Cope, 1870)

Pelvic fin

Pelvic fin with elongation of unbranched ray and first branched ray becoming filamentous in adult males and females. Pelvic fin sexually dimorphic in adult males. Pelvic-fin bony hooks occurring from the first to the sixth branched ray. Bony hooks on fin rays on an asymmetric, unpaired and irregular arrangement, varying in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks were detected restricted to the middle length of rays, beginning after primary branching (first appearing from 2nd to 5th most proximal segment of lepidotrichia). Bony hooks usually attached to ventral borders of medial and lateral branches on the secondary branches of the rays. Hook-bearing segments usually with 1 to 3 bony hooks (usually 2) per segment of lepidotrichia, displaying little variation in number from first to last rays.

Pelvic-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays more developed hooks project from ray segments with tips directed to the medial plane.

Well-developed and intermediate hooks were observed near middle portions of most rays, while weakly developed hooks are usually observed more distally. Intermediate and weakly developed hooks are more predominant on last rays. Bony hooks occurring on secondary branches of third to fifth branched rays are reduced in number when compared to medial primary branches, exhibiting 1 to 2 hooks (usually 1) per segment of lepidotrichia.

Anal fin

Anal-fin rays of adult specimens exhibiting elongation of last unbranched ray, first and second branched rays becoming filamentous (Figure 9). The extension in length of elongated unbranched ray usually meeting most posterior rays (19th to 29th branched ray), but can sometimes surpasses the anal fin reaching the caudal fin. Elongated first and second branched rays can reach usualy extending to middle (12th to 23rd branched ray) and most anterior rays (8th to 12th branched ray), respectively.

Anal fin sexually dimorphic in adult males. Anal-fin bony hooks were observed on the last unbranched ray and from the first to the eighth branched ray. Bony hooks on fin rays occurring on an asymmetric, unpaired and irregular arrangement, varying in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed restricted to the middle length of rays, beginning before primary branching (first appearing from 2nd to 7th most proximal segment of lepidotrichia). Bony hooks usually attached to lateral borders of posterior and anterior branches on the secondary branches of the rays. Hook-bearing segments with 1 to 3 bony hooks (usually 2) per segment of lepidotrichia, displaying little variation in number on most rays reduced in number on most posterior ray.

Anal-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays, more developed hooks project from ray segments with tips directed to the anal-fin base.

Well-developed hooks are found on middle portions of most rays, while intermediate hooks are usually observed more distally. Weakly developed hooks become more predominant on most posterior rays. Bony hooks occurring on secondary branches of rays are reduced in number compared to primary branches with 1 to 3 hooks (usually 1) per segment of lepidotrichia.

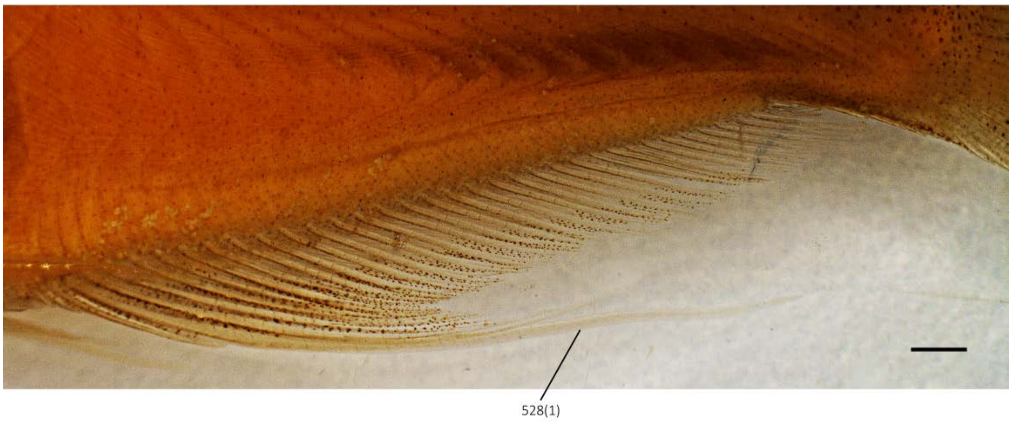

Figure 9 – Most anterior anal-fin ray elongation on adult specimen of *Prionobrama filigera* (FMNH106490), 40.2 mm SL. Scale bar= 1mm.

Comments

Examined type material (ANSP8059 – holotype ; ANSP8073 – paratype). Paratypes are adult specimens, in very poor state condition, with most fin rays broken or damaged. The presence of bony hooks on pelvic-fin and anal-fin rays on those specimens were not observed.

Prionobrama paraguayensis (Eigenmann, 1914)

Pelvic fin

Occurrence of elongation of pelvic-fin rays could not be consistently observed between adult males and females of this species. Pelvic fin sexually dimorphic in adult males. Pelvic-fin bony hooks occurring on the unbranched ray and from the first to the sixth branched ray. Bony hooks on fin rays observed on a symmetric, paired and regular arrangement, similar in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks distributed along the entire length of the rays, beginning from the long basal segment to the most distal segment of lepidotrichia. Bony hooks usually attached to ventral borders of medial and lateral branches on the secondary branches of the rays. Hook-bearing segments usually with 1 to 3 bony hooks (usually 2) per segment of lepidotrichia, exhibiting little variation in number from first to last rays.

Pelvic-fin bony hooks have a wider base than compared to the width at middle length of hook, gradually sharpening into a retrorse tip. On all rays more developed hooks project from ray segments with tips directed to the medial plane.

Well-developed and intermediate hooks are found through the entire length of most rays, while intermediate and weakly developed hooks are usually observed on most distal portions of the rays. Weakly developed hooks become more predominant on most posterior rays (usually on the 5th to 6th branched ray). Bony hooks on the long basal segment were detected in reduced number when compared to segmented portions of the rays. Bony hooks occurring on secondary branches of second to sixth branched rays, with numerous hooks similar to medial primary branches and usually 1 to 2 hooks (sometimes 3) per segment of lepidotrichia.

Examined paratypes (CAS059710 ; FMNH56682) are adult specimens, with the presence of bony hooks on pelvic-fin rays detected in one specimen (FMNH56682 – 1 spp.). Pelvic-fin bony hooks could be observed fom the first to the sixth branched ray, absent on the unbranched ray. Remaining descriptions are consistent with non-type examined material.

Anal fin

Anal-fin rays of adult specimens exhibiting elongation of last unbranched ray, first and second branched rays becoming filamentous. The extension in length of elongated unbranched ray and first branched ray usually meeting middle rays (11th to 16th branched ray). Elongated second branched ray extending from most anterior to middle rays (6th to 11th branched rays).

Anal fin sexually dimorphic in adult males. Anal-fin bony hooks were observed on the last unbranched ray and from the first to the nineteenth branched ray. Bony hooks on fin rays occuring on an asymmetric, unpaired and irregular arrangement, varying in shape, size and position on each hemitrichia and segment of lepidotrichia. Bony hooks were observed restricted to the middle length of rays, beginning before primary branching (first appearing from 2nd to 8th most proximal segment of lepidotrichia). Bony hooks usually attached to lateral borders of posterior and anterior branches on the secondary branches of the rays. Hook-bearing segments with 1 to 3 bony hooks (usually 2) per segment of lepidotrichia, more numerous on most anterior rays gradually decreasing in number on most posterior rays.

Anal-fin bony hooks have a base with same width as middle length of hook sharpening into a retrorse tip. On all rays, more developed hooks project from ray segments with tips directed to the anal-fin base.

Well-developed and intermediate hooks are found near middle portions of most rays, while intermediate and weakly developed hooks are usually observed more distally. Intermediate and weakly developed hooks become more predominant on most posterior rays. Bony hooks occurring on secondary branches of rays are reduced in number when compared to primary branches with 1 to 2 hooks (usually 1) per segment of lepidotrichia.

Anal fin from examined paratypes (CAS059710 ; FMNH56682) are in overall good state condition, with some of the specimens with anal-fin rays broken from middle portion on. Anal-fin bony hooks were detected from second to eighteenth branched ray. Bony hooks could not be observed on last unbranched ray, and first branched ray (both rays were broken). Remaining descriptions are consistent with non-type examined material.

2. Characters

The examination of 11 species of *Aphyocharax* plus two species of *Aphyocharacidium*, *Axelrodia lindeae*, and species of the genera *Leptagoniates, Paragoniates, Phenagoniates, Prionobrama, Xenagoniates* allowed the description of 23 new characters, mostly related to secondary sexual characters.

Some characters are reinterpretations of Malabarba (1998), and others are firstly proposed herein. The new characters follow the description of secondary sexual characters in Aphyocharacinae based on evidence collected from this study (see above). New characters start at number 521 to allow for a fuller integration into the character literature on this hyperdiverse lineage of Neotropical fishes. Characters observed in other studies, and incorporated herein, retain their original character number from these studies. Characters modified from their original designation bear the designation “md” for modified. Unknown characters were coded as “?” and inapplicable characters were coded as “-”.

PELVIC FIN

521 – Distribution of bony hooks on branched pelvic-fin rays of adult males: (0) From 1st to the 4th branched ray; (1) From 1st to the 5th branched ray; (2) From 1st to the 6th branched ray.

(0) From 1st to the 4th branched ray: *Aphyocharax alburnus*

(1) From 1st to the 5th branched ray: *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax nattereri*

(2) From 1st to the 6th branched ray: *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax anisitsi*, *Aphyocharax dentatus*, *Aphyocharax erythrurus*, *Aphyocharax pusillus*, *Aphyocharax rathbuni*, *Aphyocharax yekwanae*, *Prionobrama filigera*, *Prionobrama paraguayensis*

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

522 – Distribution of bony hooks along branched and unbranched pelvic-fin rays of adult males: (0) Restricted to the middle length of rays; (1) Distributed on proximal-middle length of rays; (2) Distributed along the entire length of the ray.

(0) Restricted to the middle length of rays: *Aphyocharax alburnus*, *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax erythrurus*, *Aphyocharax pusillus*, *Aphyocharax yekwanae*

(1) Distributed on proximal-middle length of rays: *Aphyocharacidium bolivianum*, *Axelrodia lindeae*, *Aphyocharax anisitsi*, *Aphyocharax dentatus*, *Aphyocharax nattereri*, *Aphyocharax rathbuni*, *Prionobrama filigera*

(2) Distributed along the entire length of the ray: *Aphyocharacidium* n. sp., *Prionobrama paraguayensis*

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

523 – Proximal pelvic-fin ray segment bearing bony hooks on adult males: (0) On segment after primary branching; (1) On segment before primary branching; (2) On the long basal segment.

(0) On segment after primary branching: *Aphyocharax avary*, *Aphyocharax erythrurus*, *Aphyocharax pusillus*, *Aphyocharax yekwanae*, *Prionobrama filigera*

(1) On segment before primary branching: *Aphyocharax alburnus*, *Aphyocharax colifax*

(2) On the long basal segment: *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax anisitsi*, *Aphyocharax dentatus*, *Aphyocharax nattereri*, *Aphyocharax rathbuni*, *Prionobrama paraguayensis*

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

524 – Position of insertion of bony hooks on pelvic-fin rays of adult: (0) Only medioventral; (1) Both medio- and lateroventral.

(0) Only medioventral: *Aphyocharax alburnus*, *Aphyocharax anisitsi*, *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax erythrurus*, *Aphyocharax rathbuni*, *Prionobrama filigera*

(1) Both medioventral and lateroventral: *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax dentatus*, *Aphyocharax nattereri*, *Aphyocharax pusillus*, *Aphyocharax yekwanae*, *Prionobrama paraguayensis*

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

525 – Width of bony hook at base on pelvic-fin rays of adult males: (0) Same width as middle length of hook; (1) Wider than width at middle length of hook.

(0) Same width as middle length of hook: *Aphyocharax anisitsi*, *Aphyocharax rathbuni*, *Aphyocharax yekwanae*

(1) Wider than width at middle length of hook: *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax avary*, *Aphyocharax colifax*,

Aphyocharax dentatus, *Aphyocharax nattereri*, *Aphyocharax pusillus*, *Prionobrama filigera*, *Prionobrama paraguayensis*

This character was coded as unknown for *Aphyocharax gracilis*, *Aphyocharax alburnus*, *Aphyocharax erythrurus*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

526 – Distal tip of bony hooks on pelvic-fin rays of adult males: (0) Retrorse; (1) Straight.

(0) Retrorse: *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax anisitsi*, *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax dentatus*, *Aphyocharax nattereri*, *Aphyocharax rathbuni*, *Prionobrama filigera*, *Prionobrama paraguayensis*

(1) Straight: *Aphyocharacidium bolivianum*, *Aphyocharax pusillus*, *Aphyocharax yekwanae*

This character was coded as unknown for *Aphyocharax gracilis*, *Aphyocharax alburnus*, *Aphyocharax erythrurus*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

527 – Number of bony hooks per pelvic-fin ray segment of adult males: (0) Maximum of one hook per segment; (1) Maximum of two hooks per segment; (2) Maximum of three hooks per segment.

(0) Maximum of one hook per segment: *Aphyocharax erythrurus*

(1) Maximum of two hooks per segment: *Aphyocharacidium bolivianum*, *Axelrodia lindeae*, *Aphyocharax alburnus*, *Aphyocharax anisitsi*, *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax dentatus*, *Aphyocharax nattereri*, *Aphyocharax rathbuni*, *Aphyocharax yekwanae*

(2) Maximum of three hooks per segment: *Aphyocharacidium* n. sp., *Aphyocharax pusillus*, *Prionobrama filigera*, *Prionobrama paraguayensis*

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

ANAL FIN

528 – Anal fin shape profile in adult specimens (char. 20 md.: Malabarba, 1998): (0) Lacking elongation of the last unbranched ray, first and second branched rays; (1) Last unbranched ray, first and second branched rays elongated and filamentous.

(0) Lacking elongation of the last unbranched ray, first and second branched rays: *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax alburnus*, *Aphyocharax anisitsi*, *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax dentatus*, *Aphyocharax erythrurus*, *Aphyocharax gracilis*, *Aphyocharax nattereri*, *Aphyocharax pusillus*, *Aphyocharax rathbuni*, *Aphyocharax yekwanae*, *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*

(1) Last unbranched ray, first and second branched rays elongated and filamentous: *Prionobrama filigera*, *Prionobrama paraguayensis*

529 – Bony hooks on unbranched anal-fin rays of adult males: (0) Absent; (1) Present.

(1) Present: *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax alburnus*, *Aphyocharax anisitsi*, *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax dentatus*, *Aphyocharax erythrurus*, *Aphyocharax nattereri*, *Aphyocharax pusillus*, *Aphyocharax rathbuni*, *Aphyocharax yekwanae*, *Prionobrama filigera*, *Prionobrama paraguayensis*

In Aphyocharacinae, anal-fin bony hooks were absent in *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi* and were coded as non applicable. Still, the investigation of further data regarding the variation of this character within Characidae is proposed herein.

This character was coded as unknown for *Aphyocharax gracilis*.

530 – Distribution of bony hooks on branched anal-fin rays of adult males (ch. 29–30 md.: Malabarba, 1998): (0) From 1st to the 10th branched ray or fewer; (1) From 1st to the 11th branched ray or more.

(0) From 1st to the 10th branched ray or fewer: *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax alburnus*, *Aphyocharax colifax*, *Aphyocharax dentatus*, *Aphyocharax nattereri*, *Prionobrama filigera*

(1) From 1st to the 11th branched ray or more: *Aphyocharax anisitsi*, *Aphyocharax avary, Aphyocharax erythrurus*, *Aphyocharax pusillus*, *Aphyocharax rathbuni*, *Aphyocharax yekwanae*, *Prionobrama paraguayensis*

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

531 – Distribution of bony hooks along anal-fin rays of adult males (ch. 27 md.: Malabarba, 1998): (0) Restricted to middle length of rays; (1) Distributed on proximal-middle length of rays; (2) Distributed on middle-distal length of rays.

(0) Restricted to middle length of rays: *Axelrodia lindeae*, *Aphyocharax alburnus Aphyocharax anisitsi*, *Aphyocharax avary, Aphyocharax colifax*, *Aphyocharax dentatus*, *Aphyocharax erythrurus*, *Aphyocharax nattereri*, *Aphyocharax pusillus*, *Aphyocharax yekwanae*, *Prionobrama filigera*, *Prionobrama paraguayensis*

(1) Distributed on proximal-middle length of rays: *Aphyocharax rathbuni*

(2) Distributed on middle-distal length of rays: *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp.

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

532 – Proximal anal-fin ray segment bearing bony hooks on adult males: (0) On segment after primary branching; (1) On segment before primary branching; (2) On the long basal segment.

(0) On segment after primary branching: *Aphyocharacidium bolivianum*, *Aphyocharax alburnus*, *Aphyocharax dentatus*, *Aphyocharax erythrurus*

(1) On segment before primary branching: *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax anisitsi*, *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax nattereri*, *Aphyocharax pusillus*, *Aphyocharax yekwanae*, *Prionobrama filigera*, *Prionobrama paraguayensis*

(2) On the long basal segment: *Aphyocharax rathbuni*

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

533 – Position of insertion of bony hooks on anal-fin rays of adult males (ch. 26 md.: Malabarba, 1998): (0) Only posterolateral; (1) Both posterolateral and anterolateral.

(0) Only posterolateral: *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax alburnus*, *Aphyocharax anisitsi*, *Aphyocharax avary*, *Aphyocharax dentatus*, *Aphyocharax nattereri*, *Aphyocharax pusillus*, *Aphyocharax rathbuni*, *Aphyocharax yekwanae*

(1) Both posterolateral and anterolateral: *Aphyocharacidium bolivianum*, *Aphyocharax colifax*, *Aphyocharax erythrurus*, *Prionobrama filigera*, *Prionobrama paraguayensis*

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

534 – Width of bony hook at base on anal-fin rays of adult males: (0) Same width as middle length of hook; (1) Wider than width at middle length of hook.

(0) Same width as middle length of hook: *Prionobrama paraguayensis*

(1) Wider than width at middle length of hook: *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax alburnus*, *Aphyocharax anisitsi*, *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax dentatus*, *Aphyocharax erythrurus*, *Aphyocharax nattereri*, *Aphyocharax pusillus*, *Aphyocharax rathbuni*, *Aphyocharax yekwanae*, *Prionobrama filigera*

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

535 – Distal tip of bony hooks on anal-fin rays of adult males: (0) Retrorse; (1) Straight.

(0) Retrorse: *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax alburnus*, *Aphyocharax anisitsi*, *Aphyocharax avary*, *Aphyocharax*

colifax, *Aphyocharax erythrurus*, *Aphyocharax nattereri*, *Aphyocharax pusillus*, *Aphyocharax rathbuni*, *Aphyocharax yekwanae*, *Prionobrama paraguayensis*

(1) Straight: *Aphyocharax dentatus*, *Prionobrama filigera*

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

536 – Number of bony hooks per anal-fin ray segment on adult males (ch. 22 md.: Malabarba, 1998): (0) Maximum of one hook per segment; (1) Maximum of two hooks per segment; (2) Maximum of three hooks per segment; (3) Maximum of four hooks per segment.

(0) Maximum of one hook per segment: *Aphyocharax alburnus*, *Aphyocharax anisitsi*, *Aphyocharax colifax*, *Aphyocharax yekwanae*

(1) Maximum of two hooks per segment: *Aphyocharax avary*, *Aphyocharax dentatus*, *Aphyocharax erythrurus*, *Aphyocharax nattereri*, *Aphyocharax pusillus*, *Aphyocharax rathbuni*

(2) Maximum of three hooks per segment: *Aphyocharacidium bolivianum*, *Prionobrama filigera*, *Prionobrama paraguayensis*

(3) Maximum of four hooks per segment: *Aphyocharacidium* n. sp., *Axelrodia lindeae*

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

537 – Bony hooks on unbranched anal-fin ray: (0) Reduced in number; (1) As numerous as in branched rays.

(0) Reduced in number: *Aphyocharax alburnus*, *Aphyocharax erythrurus*, *Prionobrama filigera*, *Prionobrama paraguayensis*

(1) As numerous as in branched rays: *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax anisitsi*, *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax dentatus*, *Aphyocharax nattereri*, *Aphyocharax pusillus*, *Aphyocharax rathbuni*, *Aphyocharax yekwanae*

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

538 – Bony hooks along branched anal-fin rays (ch. 28 md.: Malabarba, 1998): (0) Progressively reduced in number from anterior to posterior rays; (1) Reduced in number in most posterior rays.

(0) Progressively reduced in number from anterior to posterior rays: *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax dentatus*, *Aphyocharax pusillus*, *Aphyocharax yekwanae*, *Prionobrama paraguayensis*

(1) Reduced in number in most posterior rays: *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax alburnus*, *Aphyocharax anisitsi*, *Aphyocharax erythrurus*, *Aphyocharax nattereri*, *Aphyocharax rathbuni*, *Prionobrama filigera*

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

PROCURRENT CAUDAL-FIN RAYS

539 – Shape of anterior ventral procurrent caudal-fin rays of adult males (ch. 46 md.: Malabarba, 1998): (0) Slender; (1) Hypertrophied, expanded in sagittal plane.

(0) Slender: *Aphyocharacidium bolivianum*, *Aphyocharax alburnus*, *Aphyocharax anisitsi*, *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax dentatus*, *Aphyocharax erythrurus*, *Aphyocharax nattereri*, *Aphyocharax pusillus*, *Aphyocharax rathbuni*, *Aphyocharax yekwanae*, *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Prionobrama filigera*, *Prionobrama paraguayensis*, *Xenagoniates bondi*

(1) Hypertrophied, expanded in sagittal plane: *Aphyocharacidium* n. sp., *Axelrodia lindeae*

This character was coded as unknown for *Aphyocharax gracilis*.

CAUDAL FIN

By examining Aphyocharacinae species we noticed different characteristics on the arrangement and display of superficial caudal-fin muscles. Hypertrophied muscle tissue described here could represent variable configurations and modifications of caudal-fin muscles, similar to what Weitzman & Fink (1985) mentioned in their study on Xenurobryconini. As observed in Weitzman & Fink (1985; figs. 44–49), the arrangements and distribution of the *interradialis* muscles on caudal fin vary among aphyocharacins throughout the principal caudal-fin rays. Patterns of sexual dimorphism in these characters were not observed in Aphyocharacinae members.

540 – Hypertrophied muscle tissue on caudal-fin rays base: (0) Absent, base of principal rays and procurrent rays visible; (1) “Half moon” shaped, covering base of 5th to 16th principal rays; (2) Bifurcated (v-shaped), covering base of 5th to 17th principal rays; (3) Throughout base, covering base of all principal rays and procurrent rays.

(0) Absent, base of principal rays and procurrent rays visible: *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax anisitsi*, *Aphyocharax nattereri*, *Aphyocharax rathbuni*, *Aphyocharax yekwanae*, *Phenagoniates macrolepis*, *Prionobrama paraguayensis*, *Xenagoniates bondi*

(1) “Half moon” shaped, covering base of 5th to 16th principal rays: *Aphyocharax alburnus*, *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax gracilis*, *Aphyocharax pusillus*, *Leptagoniates steindachneri*

(2) Bifurcated (v-shaped), covering base of 5th to 17th principal rays: *Aphyocharax dentatus*, *Aphyocharax erythrurus*, *Prionobrama filigera*

(3) Throughout base, covering base of all principal rays and procurrent rays: *Paragoniates alburnus*

541 – Distribution of *interradialis* muscles in upper caudal fin lobe: (0) From 3rd to the 10th principal ray; (1) From 4th to the 10th principal ray; (2) From 5th to the 10th principal ray.

(0) From 3rd to the 10th principal ray: *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*

(1) From 4th to the 10th principal ray: *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax anisitsi*, *Aphyocharax nattereri*, *Aphyocharax rathbuni*, *Prionobrama paraguayensis*

(2) From 5th to the 10th principal ray: *Aphyocharax alburnus*, *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax dentatus*, *Aphyocharax erythrurus*, *Aphyocharax gracilis*, *Aphyocharax pusillus*, *Aphyocharax yekwanae*, *Prionobrama filigera*

542 – Distribution of *interradialis* muscles in lower caudal fin lobe: (0) From 11th to the 16th principal rays; (1) From 11th to the 17th principal rays; (2) From 11th and going to the 18th principal rays; (3) From 11th and going to the 19th principal rays.

(0) From 11th to the 16th principal rays: *Aphyocharacidium* n. sp., *Axelrodia lindeae*

(1) From 11th to the 17th principal rays: *Aphyocharacidium bolivianum, Aphyocharax alburnus*, *Aphyocharax anisitsi*, *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax dentatus*, *Aphyocharax erythrurus*, *Aphyocharax gracilis*, *Aphyocharax pusillus*, *Aphyocharax rathbuni*, *Aphyocharax yekwanae*, *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Prionobrama filigera*

(2) From 11th and going to the 18th principal rays: *Prionobrama paraguayensis*, *Xenagoniates bondi*

(3) From 11th and going to the 19th principal rays: *Aphyocharax nattereri*, *Phenagoniates macrolepis*

543 – Symmetry of caudal-fin rays: (0) Symmetrical, rays of upper and lower lobes proportional; (1) Asymmetrical, upper lobe rays slightly longer than lower lobe rays; (2) Asymmetrical, lower lobe rays slightly longer than upper lobe rays.

(0) Symmetrical, rays of upper and lower lobes proportional: *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax alburnus*, *Aphyocharax anisitsi*, *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax dentatus*, *Aphyocharax erythrurus*, *Aphyocharax gracilis*, *Aphyocharax nattereri*, *Aphyocharax pusillus*, *Aphyocharax rathbuni*, *Aphyocharax yekwanae*, *Phenagoniates macrolepis*, *Prionobrama filigera*, *Prionobrama paraguayensis*, *Xenagoniates bondi*

(1) Asymmetrical, upper lobe rays slightly longer than lower lobe rays: *Paragoniates alburnus*

(2) Asymmetrical, lower lobe rays slightly longer than upper lobe rays: *Leptagoniates steindachneri*

Characters modified from Mirande (2019):

The following character headings and states were retrieved as presented in Mirande (2019). Statements included here were based on observations from our examined material over Mirande's (2019) morphological characters.

92 – Form of fourth infraorbital: (0) Approximately square or more developed longitudinally than dorsoventrally; (1) Longer dorsoventrally than longitudinally.

Added state: (2) Absent or triangular with posterior margin meeting third and fifth infraorbitals.

Mirande (2010, char. 67) first proposed this character with two states: (0) Approximately square or more developed longitudinally than dorsoventrally; (1) Longer dorsoventrally than longitudinally. In Aphyocharacinae, the presence of a well developed fourth infraorbital bone (IO4) is observed in *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, and *Xenagoniates bondi*. When present, most *Aphyocharax* species and *Prionobrama filigera* exhibit a much reduced IO4, bordered posteriorly by the third and fifth infraorbital bones. With the addition of the state two to this previously described character, information on the variation of the form of IO4 observed in Aphyocharacinae members is now included.

450 – Distribution of bony hooks on pelvic-fin segments of adult males: (0) Bilateral, at least in some segments; (1) Unpaired, oriented medially.

Added state: (2) Present only in the medial hemitrichia.

The distribution of bony hooks along pelvic-fin rays of adult males in Characidae shows great variability. We were able to observe the occurrence of the two described states in members of Aphyocharacinae, as well as a different distribution that didn't fit with the previous description provided by Mirande (2019). Some species display paired and symmetrically distributed bony hooks on both pelvic-fin rays' hemitrichia, and others were observed exhibiting unpaired and asymmetrically distributed bony hooks on both sides of the lepidotrichia. In *Aphyocharacidium bolivianum* and *Aphyocharacidium* n. sp., however, bony hooks were distributed asymmetrically, occurring on just the medial portion of the hemitrichia of the pelvic-fin ray.

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

Comments on other characters from Mirande (2019):

34 – Rhinosphenoid: (0) Absent; (1) Present.

Amongst newly added Aphyocharacinae, we observed the presence of this bone for *Aphyocharacidium* n. sp.. The rhinosphenoid is absent in *Leptagoniates steindachneri*, and unknown in *Aphyocharax gracilis*.

45 – Large foramen on pterosphenoid: (0) Absent; (1) Present, well developed.

This feature has been observed in species of *Aphyocharacidium* and *Axelrodia lindeae*. Mirande (2010) observed the presence of a “large foramen situated in the middle of the pterosphenoid” in specimens of *Aphyocharacidium bolivianum* and *Axelrodia lindeae*.

The presence of a large foramen on the pterosphenoid bone is herein coded as absent for *Leptagoniates steindachneri*, and as missing for *Aphyocharax gracilis*.

90 – Fourth infraorbital: (0) Present; (1) Absent.

Species of *Aphyocharax* are more variable among the examined members of Aphyocharacinae concerning this character. The presence of this bone is herein noted as intraspecifically variable in *Aphyocharax pusillus* and *Prionobrama paraguayensis*, and, as such, the character is coded as polymorphic.

91 – Form of fourth infraorbital: (0) Reaching posterior margin of third and fifth infraorbitals; (1) Excluded from posterior margin of infraorbital series.

In Mirande’s (2013) study this character had two states descriptions: (0) quadrangular, reaching posterior margin of third and fifth infraorbitals; (1) triangular, excluded from posterior margin of infraorbital series. In this previous description, the second state brought more information on the variation of shape of the fourth infraorbital bone within Characidae. This information was further atomized and is now the foucs of character 92 (Mirande, 2019 – see comments on this character above).

123 – Lateral line: (0) Complete; (1) Interrupted.

Variations of this character are observed among Aphyocharacinae species, which shows different conditions within this group. Mirande (2010) pointed out that even though this character is probably hilghly homoplastic amongst Characidae, there is some evidence of its informative potentitial in phylogenetic studies.

124 – Isolated pored scale in caudal peduncle: (0) Absent; (1) Present.

Proposed by Tagliacollo *et al.* (2012) for species with an incomplete lateral line where the posteriormost scale of the lateral series is pored. This was noted by the author as a synapomorphy of *Aphyocharax* and *Prionobrama*. This character is usually related to the presence of a canal of the lateral line on caudal-fin interradial membrane (Mirande, 2019 – ch. 125: see comment below). This character is coded as inapplicable to species of Aphyocharacinae with a complete lateral line.

125 – Canal of lateral line on caudal-fin membrane: (0) Absent; (1) Present.

When present in species of Aphyocharacinae, the canal of the lateral line on the caudal fin is located on the interradial membrane between the 10th and 11th principal caudal-fin rays. This character is herein coded as present for *Aphyocharax avary*, *Aphyocharax colifax*, *Aphyocharax erythrurus* and *Aphyocharax gracilis*. It is absent in *Aphyocharacidium* n. sp., *Aphyocharax yekwanae*, *Leptagoniates steindachneri* and *Xenagoniates bondi*. The presence of this structure is variable among the examined specimens of *Aphyocharax rathbuni*, and was teated as polymorphic.

143 – Posterior extent of maxilla: (0) Not reaching second infraorbital; (1) Reaching second infraorbital.

There are some degrees of the posterior extent of maxillary bone on Aphyocharacinae members. *Leptagoniates steindachneri*, *Phenagoniates macrolepis*, *Xenagoniates bondi* exhibits the posterior end of the maxillary reaching the anterior margin of the second infraorbital (IO2). Interestingly, *Paragoniates alburnus* display the posterior portion of the maxillary exceeding the IO2 posterior margin, reaching the anterior margin of the third infraorbital (IO3).

189 – Number of maxillary teeth: (0) Only one, or absent; (1) Two or more.

190 – Number of maxillary teeth: (0) Up to three; (1) Four or more.

Six or more teeth on the maxillary are observed in most of the species in Aphyocharacinae. From examined material, *Aphyocharax anistsi* counts to two to four teeth, where in *Aphyocharax rathbuni* 1 or no teeth were observed.

Teeth of *Aphyocharax erythrurus* and *Aphyocharax yekwanae* were not examined, and were coded as unknown.

203 – Inner row of minute dentary teeth: (0) Absent or restricted to lateral region of dentary; (1) Present from anterior region of dentary.

The presence of an inner row of numerous minute teeth on the dentary bone is herein observed for *Aphyocharacidium bolivianum* and *Aphyocharacidium* n. sp. When proposing this genus, Géry (1960) described the presence of an inner row of dentary teeth inserted in a crest visibly separated from the outer one; and without proper examination this inner row of minute teeth could be sometimes perceived as “replacement teeth”.

324 – Relative number of precaudal vertebrae: (0) exceeding caudal vertebrae in two or more elements; (1) equal or less numerous than caudal vertebrae.

In most *Aphyocharax* species numbers of pre-caudal vertebrae elements were slighty lower than caudal vertebrae units. Counts from *Axelrodia lindeae*, and species of *Aphyochracidium*, *Leptagoniates*, *Paragoniates*, *Phenagoniates*, *Prionobrama* and *Xenagoniates* genera showed a much higher number of caudal vertebrae elements compared to pre-caudal ones. Examined speciemens of *Aphyocharax pusillus* had similar counts from pre-caudal and caudal vertebrae elements.

Data on *Aphyocharax gracilis* vertebrae counts could not be retrieved, and was therefore coded as unknown (also for ch. 325 and ch. 326).

325 – Total number of vertebrae: (0) 33 or fewer; (1) 34 or more.
326 – Total number of vertebrae: (0) 40 or fewer; (1) 41 or more.

Vertebrae elements were observed in higher number among *Leptagoniates steindachneri* (46–48), *Paragoniates alburnus* (38–42), *Phenagoniates macrolepis* (41–43) and *Xenagoniates bondi* (49–51). Similar counts were found on *Aphyocharacidium bolivinaum* (34), *Aphyocharacidium* n. sp. (34), *Aphyocharax alburnus* (33–38), *Ap. anistsi* (33–34), *Ap. avary* (33–38), *Ap. colifax* (35–37), *Ap. dentatus* (34–38), *Ap. erythrurus* (35–37), *Ap. nattereri* (33–34), *Ap. pusillus* (36–38), *Ap. rathbuni* (33–36), *Ap. yekwanae* (37–39), *Axelrodia lindeae* (27), *Prionobrama filigera* (35–37) and *Prionobrama paraguayensis* (35–38).

384 – Number of branched pelvic-fin rays: (0) Six or less; (1) Seven or more.

385 – Number of branched pelvic-fin rays: (0) Seven or less; (1) Eight or more.

Branched pelvic-fin ray counts are lower in *Phenagoniates macrolepis* (5) and *Xenagoniates bondi* (5). Coding for remaining Aphyocharacinae followed examined material counts from *Aphyocharacidium bolivianum* (6–7), *Aphyocharacidium* n.sp. (6–7), *Axelrodia lindeae* (6), *Aphyocharax alburnus* (6–7), *Ap. anisitsi* (5–7), *Ap. avary* (6–7), *Ap. colifax* (6–7), *Ap. dentatus* (7), *Ap. erythrurus* (7), *Ap. gracilis* (7), *Ap. nattereri* (6), *Ap. pusillus* (6–8), *Ap. rathbuni* (6–7), *Ap. yekwanae* (7), *Leptagoniates steindachneri* (5–6), *Paragoniates alburnus* (6–7), *Prionobrama filigera* (6–7) and *Pr. paraguayensis* (7).

391 – Number of supraneurals: (0) Three; (1) Four or more.

392 – Number of supraneurals: (0) Four or fewer; (1) Five or more.

393 – Number of supraneurals: (0) Five or fewer; (1) Six or more.

394 – Number of supraneurals: (0) Six or more; (1) Seven or fewer.

395 – Number of supraneurals: (0) Seven or fewer; (1) Eight or more.

Mirande (2019) adopted an expansion of characters related to the number of supraneurals (see Mirande, 2009; 2010) to convey closer ranges of variation, considering the greater diversity of this trait within Characidae.

Counts on supraneurals were higher on *Leptagoniates steindachneri* (9–11), *Paragoniates alburnus* (8–10), *Phenagoniates microlepis* (10–12) and *Xenagoniates bondi* (10–12), and lower on *Aphyocharacidium bolivianum* (4), *Aphyocharacidium* n. sp. (4–5), *Axelrodia lindeae* (4). Similar numbers were retievied from *Aphyocharax alburnus* (6–7), *Ap. anisitsi* (6), *Ap. avary* (5–7), *Ap. colifax* (6–7), *Ap. dentatus* (5–7), *Ap. erythrurus* (6–7), *Ap. nattereri* (6–7), *Ap. pusillus* (6), *Ap. rathbuni* (5–7), *Ap. yekwanae* (6–7), *Prionobrama filigera* (7) and *Pr. Paraguayensis* (8).

Data on *Aphyocharax gracilis* supraneural counts could not be retrieved, and was coded as unknown.

408 – Number of branched rays on dorsal fin: (0) Seven or fewer; (1) Eight or more.

409 – Number of branched rays on dorsal fin: (0) Eight or fewer; (1) Nine or more.

Similar counts of branched dorsal-fin rays were registered in *Aphyocharax alburnus* (8–9), *Ap. anisitsi* (9–10), *Ap. avary* (8–10), *Ap. colifax* (9–10), *Ap. dentatus* (9), *Ap. erythrurus* (9), *Ap. gracilis* (9), *Ap. pusillus* (8–9), *Ap. rathbuni* (9–10), *Ap. yekwanae* (9), *Aphyocharacidium bolivianum* (8–9), *Aphyocharacidium* n.sp. (9), *Axelrodia lindeae* (9), *Leptagoniates steindachneri* (8), *Paragoniates alburnus* (8–9), *Prionobrama filigera* (9) and *Pr. paraguayensis* (8–9). Examined material from *Aphyocharax nattereri*, *Phenagoniates macrolepis* and *Xenagoniates bondi* showed a variation of 7 to 8 branched rays on dorsal fin.

415 – Anal-fin position: (0) Posterior or almost posterior to vertical through last dorsal-fin ray; (1) Extended anteriorly ventral to dorsal fin.

416 – Anal-fin position: (0) Posterior to or at vertical through base of last dorsal-fin ray; (1) Anterior to vertical through base of last dorsal-fin ray.

On *Axelrodia lindeae*, *Prionobrama filigera* and species of *Aphyocharacidium* and *Aphyocharax* the anal fin origin is vertically aligned or posterior to first dorsal-fin ray insertion. Among *Prionobrama paraguayensis*, and species of *Leptagoniates*, *Paragoniates*, *Phenagoniates* and *Xenagoniates* genera the anal fin is positioned anterior to the insertion of the first dorsal-fin ray.

417 – Number of unbranched anal-fin rays: (0) Three or fewer; (1) Four or more.

The number of unbranched anal-fin rays showed little variation within *Aphyocharax alburnus* (2–3), *Ap. avary* (3–4), *Ap. dentatus* (3–4), *Ap. erythrurus* (3–4), *Ap. pusillus* (3–4) and *Ap. rathbuni* (3–4). Similar counts were registered between *Aphyocharacidium bolivianum* (3), *Aphyocharacidium* n. sp. (3), *Aphyocharax colifax* (3), *Ap. gracilis* (4) and *Ap. yekwanae* (3). From examined material, more variation was observed in *Axelrodia lindeae* (2–4), *Aphyocharax anisitsi* (2–4), *Ap. nattereri* (2–4), *Leptagoniates steindachneri* (2–4), *Paragoniates alburnus* (2–4), *Phenagoniates macrolepis* (2–4), *Prionobrama filigera* (4–6), *Pr. paraguayensis* (3–6) and *Xenagoniates bondi* (2–4).

418 – Number of branched anal-fin rays: (0) Ten or fewer; (1) 11 or more.

419 – Number of branched anal-fin rays: (0) 17 or fewer; (1) 18 or more.

420 – Number of branched anal-fin rays: (0) 24 or fewer; (1) 25 or more.

421 – Number of branched anal-fin rays: (0) 34 or fewer; (1) 35 or more.

The longer anal fin is a conspicuous feature among the aphyocharacins *Leptagoniates steindachneri* (57–69), *Paragoniates alburnus* (40–50), *Phenagoniates macrolepis* (45–55) and *Xenagoniates bondi* (57–66). Similar variations on counts were found on *Aphyocharax alburnus* (15–20), *Ap. anisitsi* (16–19), *Ap. avary* (14–19), *Aphyocharax colifax* (15–19), *Ap. dentatus* (15–19), *Ap. erythrurus* (15–19), *Ap. pusillus* (15–19), *Aphyocharacidium bolivianum* (17–20), *Ap. nattereri* (17–20), *Ap. rathbuni* (17–20), *Ap. gracilis* (18), *Ap. yekwanae* (18) and *Aphyocharacidium* n. sp. (19–22). Slightly higher number of anal-fin rays are observed on *Axelrodia lindeae* (22–26), *Prionobrama filigera* (27–33) and *P. paraguayensis* (27–32).

422 – Form and length of anterior anal-fin rays: (0) Similar to posterior rays; (1) Longer and more compressed laterally than posterior rays.

Malabarba (1998) described this elaborate anatomical modification of the anal fin rays on sexually dimorphic males within Cheirodontini's Clade B members (ch. 17). Similar to those cheirodontines, the hook-bearing anal-fin rays become expanded in the sagittal plane when compared to the other rays of examined adult males in *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp. and *Axelrodia lindeae*. On remaining Aphyocharacinae, no evidence of sexual dimorphism was observed on anal-fin ray shape, where rays are usually circular in cross section and progressively reduced in diameter from anteriormost to posteriormost element on all analyzed specimens.

423 – Number of rays on last anal pterygiophore: (0) Two; (1) One.

All aphyocharacins analyzed here have two rays on last anal pterygiophore.

424 – Form of caudal peduncle in mature males: (0) not decurved ventrally; (1) decurved ventrally in mature males.

Malabarba (1998) noted that the curvature of the caudal peduncle could be associated to the development of the *hypaxialis* muscles over anal-fin pterygiophores and the ventral portion of the caudal peduncle, and of the *infracranialis posterior* muscle that unites the last anal-fin pterygiophores to the ventral procurrent caudal-fin rays. It was also speculated that this modification is related to courtship behaviors of mature males.

Within Aphyocharacinae, most species did not show modifications to the external morphology of the caudal peduncle. Mature males of *Axelrodia lindeae* were observed exhibiting degrees of curvature of the caudal peduncle, where it becomes ventrally arched in relation to the main body axis.

432 – Ventral procurrent caudal-fin rays of adult males: (0) Slender; (1) Laminar.

Sexually dimorphic males of *Aphyocharacidium* n. sp. and *Axelrodia lindeae* showed a hypertrophy of the most anterior elements of the ventral procurrent caudal-fin rays in relation to remaining ones (referred as “laminar” in Mirande, 2010; 2019). In most members of Aphyocharacinae, slender ventral procurrent caudal-fin rays were observed in all examined specimens.

This character was coded as unknown for *Aphyocharax gracilis*.

433 – Number of ventral procurrent caudal-fin rays: (0) 11 or fewer; (1) 12 or more.

The number of ventral procurrent caudal-fin rays showed more variation among *Aphyocharax alburnus* (6–9), *Ap. avary* (6–10), *Ap. colifax* (6–9), *Ap. dentatus* (6–10), *Ap. erythrurus* (7–10), *Ap. pusillus* (6–10), *Ap. rathbuni* (6–9), *Paragoniates alburnus* (3–7) and *Prionobrama filigera* (6–9). Similar counts were observed in *Aphyocharax anisitsi* (5–8), *Ap. nattereri* (6–8), *Ap. yekwanae* (8–9), *Leptagoniates steindachneri* (5–8), *Phenagoniates macrolepis* (5–8), *Pr. paraguayensis* (5–8) and *Xenagoniates bondi* (6–8). Higher numbers of ventral procurrent caudal-fin ray elements were found on *Aphyocharacidium bolivianum* (9–11), *Aphyocharacidium* n. sp. (12–15), *Axelrodia lindeae* (10–13) and *Aphyocharax gracilis* (9).

434 – Ventral procurrent caudal-fin rays of adult males: (0) Not projecting through musculature and skin of peduncle; (1) Projecting ventrally through peduncle musculature and skin.

Based on our observations, the visibility of ventral procurrent caudal-fin rays through muscles and skin was not considered a dimorphic feature among Aphyocharacinae members. When the series is fully visible, bones project between the muscles and skin of the caudal peduncle on males and females of *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax rathbuni* and *Ap. yekwanae*. On remaining aphyocharacins, most of those elements are buried on the musculature and skin of the caudal peduncle and are not visible.

441 – Anal-fin bony hooks in adult males of species bearing hooks on fins: (0) Absent; (1) Present.

Bony hooks on anal-fin rays were absent on *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis* and *Xenagoniates bondi*. Sexually dimorphic males were observed bearing bony hooks of pelvic-fin rays of *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax alburnus*, *Ap. anisitsi*, *Ap. avary*, *Ap. colifax*, *Ap. dentatus*, *Ap. erythrurus*, *Ap. nattereri*, *Ap. pusillus*, *Ap. rathbuni*, *Ap. yekwanae*, *Prionobrama filigera* and *Pr. paraguayensis*.

This character was coded as unknown for *Aphyocharax gracilis*.

442 – Pelvic-fin bony hooks in adult males of species bearing hooks on fins: (0) Absent; (1) Present.

Bony hooks on pelvic-fin rays were absent on *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis* and *Xenagoniates bondi*. Sexually dimorphic males were observed bearing bony hooks of pelvic-fin rays of *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax alburnus*, *Ap. anisitsi*, *Ap. avary*, *Ap. colifax*, *Ap. dentatus*, *Ap. erythrurus*, *Ap. nattereri*, *Ap. pusillus*, *Ap. rathbuni*, *Ap. yekwanae*, *Prionobrama filigera* and *Pr. paraguayensis*.

This character was coded as unknown for *Aphyocharax gracilis*.

446 – Bony hooks on base of pelvic-fin rays of adult males: (0) Absent, or in small number compared to on segmented portion of rays; (1) As numerous as on segmented portion of rays.

The presence of a similar or higher number of bony hooks on the long basal segment in relation to other portions of the ray were found on adult males of *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Aphyocharax anisitsi*, *Ap. dentatus* and *Ap. rathbuni*. Bony hooks on base of pelvic-fin rays were observed in fewer numbers compared to segmented portion of rays on dimorphic males of *Axelrodia lindeae*, *Aphyocharax nattereri* and *Prionobrama paraguayensis*.

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Ap. alburnus*, *Ap. avary*, *Ap. colifax*, *Ap. erythrurus*, *Ap. pusillus*, *Ap. yekwanae*, *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Prionobrama filigera*, *Xenagoniates bondi*.

447 – Bony hooks on last pelvic-fin ray of adult males: (0) Absent or reduced in number; (1) As numerous as in other rays.

On sexually dimorphic males of *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Aphyocharax anisitsi*, *Ap. dentatus*, *Ap. erythrurus*, *Ap.pusillus*, *Ap. rathbuni* and *Ap. yekwanae* the number of bony hooks on last pelvic-fin ray are reduced when compared to other rays. From examined material, adult males of *Axelrodia lindeae*, *Prionobrama filigera* and *Pr. paraguayensis* showed a similar number of bony hooks on all pelvic fin hook-bearing rays. Bony hooks on last pelvic-fin ray were absent on adult males of *Aphyocharax alburnus*, *Ap. avary*, *Ap. colifax* and *Ap. nattereri*.

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

448 – Bony hooks on first pelvic-fin ray of adult males: (0) Absent; (1) Present.

The presence of bony hooks on the first pelvic-fin ray (unbranched ray) was registered on adult males of *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Aphyocharax anisitsi*, *Ap. dentatus*, *Ap. nattereri*, *Ap. rathbuni*, *Ap. yekwanae* and *Prionobrama paraguayensis*. From examined material, we observed no evidence of this trait on sexually dimorphic males of *Axelrodia lindeae*, *Aphyocharax alburnus*, *Ap. avary*, *Ap. colifax*, *Ap. erythrurus*, *Ap. pusillus* and *Prionobrama filigera*.

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

449 – Distribution of bony hooks on pelvic-fin branched rays of males: (0) On all branches; (1) Restricted to medial branches.

Bony hooks were observed on secondary branches of pelvic-fin rays of adult males on *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax dentatus*, *Ap. nattereri*, *Ap. pusillus*, *Ap. yekwanae*, *Prionobrama filigera* and *Pr. paraguayensis*. On examined adult males of *Aphyocharax alburnus*, *Ap. anisitsi*, *Ap. avary*, *Ap. colifax*, *Ap. erythrurus* and *Ap. rathbuni* bony hooks were retricted to the medial primary branching of pelvic-fin rays, attached to the medioventral border.

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

451 – Position of anal-fin bony hooks of adult males: (0) Paired and ordered laterally or posterolaterally; (1) Medially positioned and oriented posteriorly; (2) Asymmetrically disposed and irregularly arranged.

According to Malabarba (1998: ch. 25), anal-fin bony hooks of similar shape displayed on a symmetrical and paired arrangement on each segment of hemitrichia of rays was observed as the common condition among characid and most Cheirodontinae members. Assymetrical and irregularly arranged hooks on each anal-fin ray hemitrichia were detected as a particular variation of anal-fin hooks on Cheirodontini's Clade B (Malabarba, 1998), and previously proposed as a synapomorphy of the Stethaprioninae by Reis (1989: ch. 2).

When proposing this character, Mirande (2010) included the condition "medially positioned and oriented posteriorly" (ch. 316: state 1) based on Malabarba's (1998) conclusions, and were only observed on the cheirodontines *Heterocheirodon yatai* (Casciotta, Miquelarena & Protogino, 1992), *Serrapinnus calliurus* (Boulenger, 1900), *Serrapinnus notomelas* (Eigenmann, 1915) and *Serrapinnus microdon* (Eigenmann, 1915) (see Mirande, 2019).

Among Aphyocharacinae species, sexually simorphic males of *Axelrodia lindeae*, *Aphyocharax alburnus*, *Ap. colifax* and *Ap. dentatus* exhibited bony hooks on a symmetrical and paired arrangement on anal-fin rays. Examined adult males of *Aphyocharacidium bolivianum*, *Aphyocharacidium* n. sp., *Aphyocharax anisitsi*, *Ap. avary*, *Ap. erythrurus*, *Ap. nattereri*, *Ap. pusillus*, *Ap. rathbuni*, *Ap. yekwanae*, *Prionobrama filigera* and *Pr. paraguayensis* showed the presence of an asymmetrical and unpaired disposition on anal-fin rays bearing hooks.

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

452 – Number of anal-fin hooks on each ray bearing hooks: (0) Three or more on each ray with hooks; (1) Only one or two hooks on each ray.

This character was proposed by Weitzman & Menezes (2009: ch. 10) to illustrate the variation of the number of anal-fin hooks on rays on species from “Clade A”, where the most common condition is having “two or more” hooks on each anal-fin ray. In his most recent hypothesis of phylogenetic relationships among Characidae, Mirande (2019) included and modified this character accounting for the found diversity within this family.

As most characins (see Mirande, 2019), the presence of “three or more” bony hooks on each anal-fin ray bearing hooks was observed as a general pattern on all sexually dimorphic Aphyocharacinae members.

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

453 – Distribution of bony hooks on anal-fin branches of adult males: (0) on all branches; (1) restricted, or almost restricted, to posterior primary branch.

Bony hooks were observed on secondary branches of anal-fin rays of adult males on *Aphyocharacidium bolivianum*, *Aphyocharax colifax*, *Ap. erythrurus*, *Prionobrama filigera* and *Pr. Paraguayensis*. On remaining aphyocharacins, examined adult males of *Aphyocharacidium* n. sp., *Axelrodia lindeae*, *Aphyocharax alburnus*, *Ap. anisitsi*, *Ap. avary*, *Ap. dentatus*, *Ap. nattereri*, *Ap. pusillus*, *Ap. rathbuni* and *Ap. yekwanae* exhibited bony hooks retricted to the posterior primary branching of anal-fin rays, attached to the posterolateral border.

This character was coded as unknown for *Aphyocharax gracilis*, and non applicable to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Xenagoniates bondi*.

512 – Adipose fin: (0) Present; (1) Absent.

The absence of an adipose fin is registered for *Phenagoniates macrolepis*. Remaing Aphyocharacinae members have a conspicuous adipose fin.

513 – Enlarged pouch scale: (0) Absent or similar to a regular lateral-line scale; (1) Present and enlarged, with numerous radii.

There was no evidence of an enlarged pouch scale among all examined aphyocharacins. Mirande (2019) relates this condition to the majority of Characiformes, where “the last scale of the lateral line is similar to the remaining ones”. Scales on caudal fin of Aphyocharacinae are similar in shape and slightly smaller in size compared to body scales.

3. Phylogenetic relationships of Aphyocharacinae and “Clade B”

Molecular phylogenetic analysis

StarBeast Bayesian inference recovered Aphyocharacinae as a monophyletic clade composed by *Aphyocharacidium bolivianum*, *Aphyocharax*, *Prionobrama*, *Leptagoniates*, *Paragoniates*, *Phenagoniates* and *Xenagoniates* genera (Figure 10). Exodontinae, Tetragonopterinae and Characinae were closely related in a monophyletic unit (posterior probability 1) and recovered as sister clade to Aphyocharacinae with strong support (posterior probability 0.92). The hypothesis of relationships amongst Cheirodontinae and remaining “Clade B” member were weakly supported and will not be the focus of further comments.

The genus *Aphyocharax* was not recovered as monophyletic, with *Aphyocharax nattereri* more closely related to *Prionobrama* species (posterior probability 0.73). The remaining *Aphyocharax* species were recovered with a strong support (posterior probability 1) composed by two monophyletic units. The newly added *Aphyocharax erythrurus* appears closely related to *Aphyocharax pusillus* (posterior probability 0.99) and was recovered as part of a monophyletic group (posterior probability 0.77) with *Aphyocharax alburnus* and *Aphyocharax avary*. Their sister group was recovered with a low posterior probability in a clade with *Aphyocharax anistsi*, *Aphyocharax rathbuni* and *Aphyocharax dentatus*. A strong support can be observed among the remaining *Aphyocharax* species (posterior probability 1) and the monophyletic group comprised of *Prionobrama*+*Aphyocharax nattereri*.

The monophyletic group composed of *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Leptagoniates steindachneri* and *Xenagoniates bondi* were recovered with strong support (posterior probability 1) recovered as a separate clade of *Aphyocharax* and *Prionobrama*. The hypothesis of relationship among Aphyocharacinae clades were weakly supported and are will be refrained of further discussions.

Aphyocharacidium bolivianum is recovered as a single lineage sister group to all recognized Aphyocharacinae members with strong support (posterior probability 1).

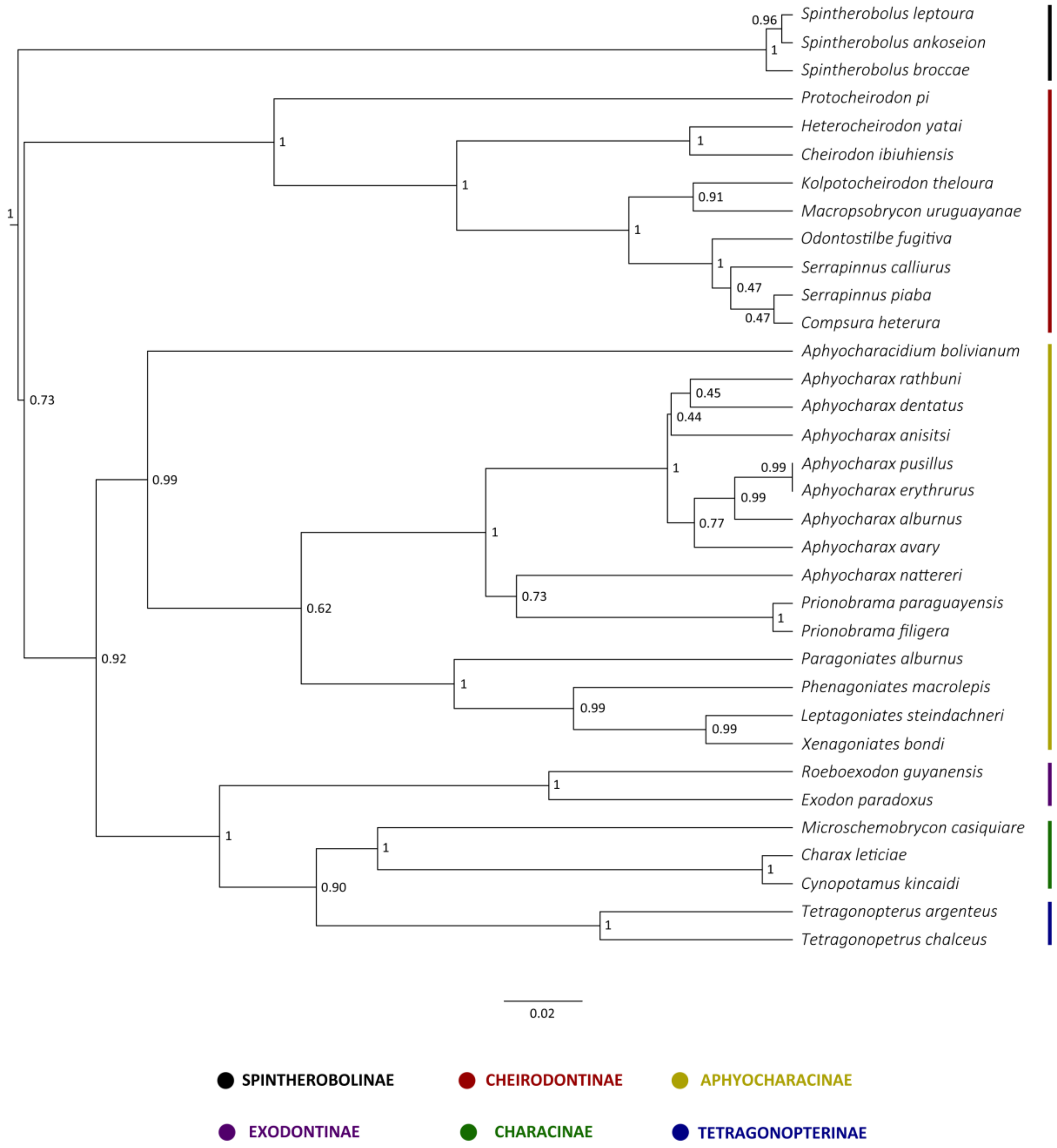

Figure 10 – Species tree based on Bayesian Inference (BI) from five genes (16S, CytB, Myh6, RAG1, RAG2). Numbers at nodes are branch support based on posterior probability. Hypothesis of relationships of Characidae's "Clade B" plus Spintherobolinae based on molecular evidence, with yellow band representing Aphyocharacinae members.

Morphological phylogenetic analysis

The morphological analysis under Bayesian Inference recovered a large monophyletic clade, similar to the one obtained from the analysis recovered from molecular data (Figure 11). From a morphological standpoint, the monophyly of the genus *Aphyocharax* was strongly supported (posterior probability 0.98), similarto the findings from molecular evidence, the genus is composed of two monophyletic units plus *Ap. natterreri*. We were able to observe a slight change in topologies with the inclusion of *Aphyocharax gracilis*, *Aphyocharax colifax* and *Aphyocharax yekwanae*, which were not available in the molecular analysis. *Aphyocharax yekwanae* was recovered as part of a monophyletic group (posterior probability 0.94) with *Ap. pusillus*, *Aphyocharax gracilis*, *Ap. erythrurus*, *Ap. avary*, *Aphyocharax colifax* and *Ap. alburnus*. *Aphyocharax colifax* appears closely related to *Ap. alburnus* with strong support (posterior probability 0.94). Still, weak support values within this clade should be further investigated to corroborate those relationships. The other monophyletic group accounts for a strongly supported relationship between *Ap. dentatus* (posterior probability 0.84) sister group was to *Ap.anistsi* and *Ap. rathbuni* (posterior probability 1). A similar relationship was recovered with the molecular dataset, but with much stronger support values when applied to morphological data.

Pionobrama species were closely related in a monophyletic unit, recovered as the sister group of *Aphyocharax*, as observed from the hypothesis obtained from molecular data but with lower support. *Aphyocharax nattereri* was found to be more closely related to all *Aphyocharax* species, now as the single lineage sister group to its congeners.

The monophyly of the group composed of *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Leptagoniates steindachneri* and *Xenagoniates bondi* were once again recovered with strong support (posterior probability 1). The morphology hypothesis of relationships between the latter and the clade comprised of *Aphyocharax* and *Prionobrama* genera were strongly supported (posterior probability 1).

Based on this morphological evidence, *Protocheirodon pi* is recovered as the single lineage sister group to all recognized Aphyocharacinae members with good support (posterior probability 0.88).

The relationships among Exodontinae, Tetragonopterinae and Characinae with sister clade Aphyocharacinae were recovered with good support (posterior probability 0.81). The hypothesis of relationships concerning other “Clade B” members as sister clade to Cheirodontinae were weakly supported, but as similarly recovered from molecular data.

A strongly supported monophyletic clade (posterior probability 1) comprised of *Aphyocharacidium bolivianum* plus *Aphyocharacidium* n. sp., *Axelrodia lindeae* and *Microschemobrycon casiquiare* were found as sister group to all known Cheirodontinae members. With the exception of *A. bolivianum*, the aforementioned taxa weren’t available in the molecular dataset. This evidence represents the striking difference found between topologies from molecular and morphological data set analysis.

The lower support and major differences concerning the relationships within Aphyocharacinae and “Clade B” based on morphology and molecular evidences should be carefully considered in further studies.

The reasons for not adopting the morphology hypothesis for the ancestral character state reconstruction are addressed in the discussion.

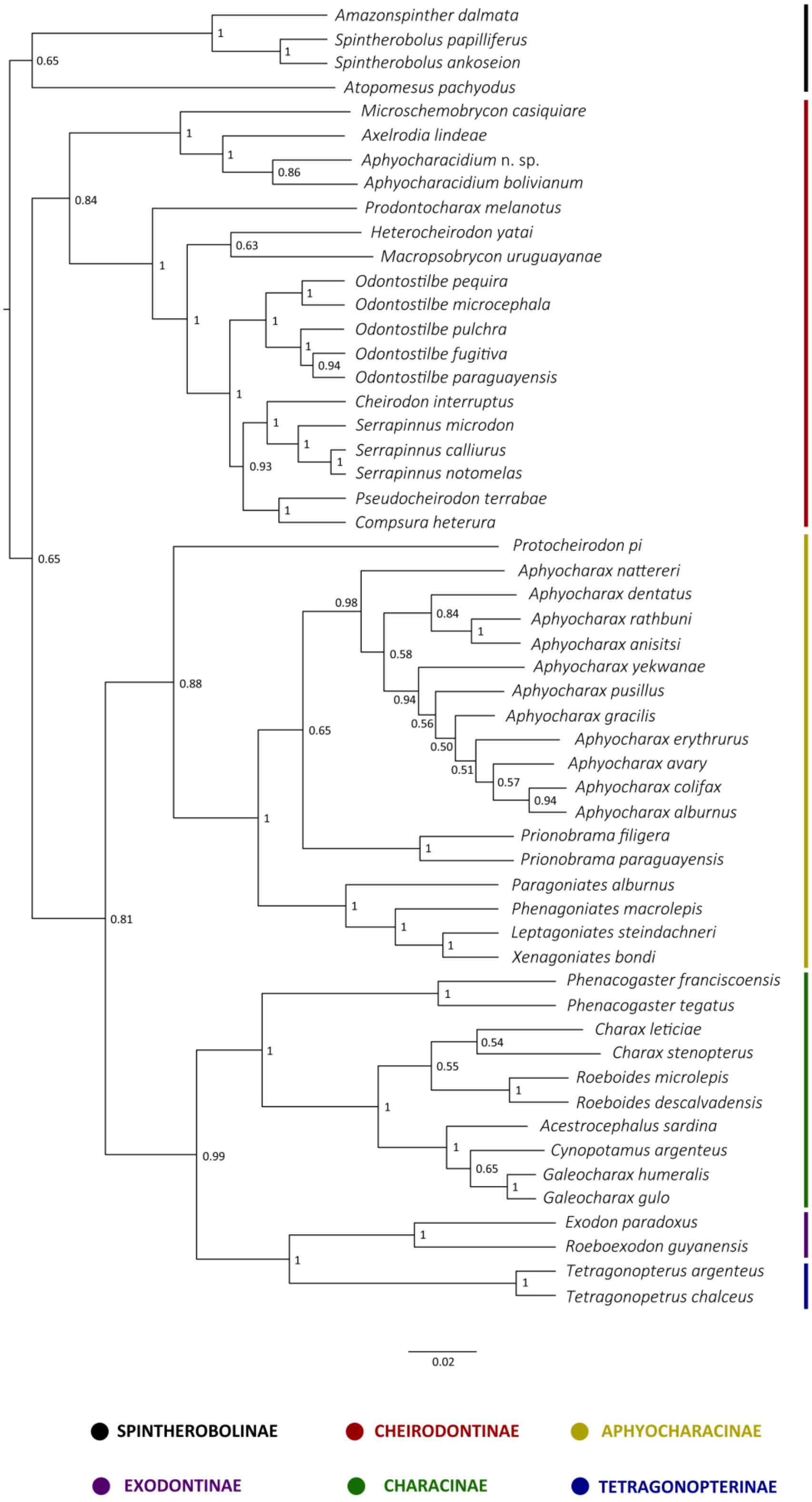

Figure 11 – Maximum Clade Credibility tree generated under Mk model with Bayesian Inference (BI). Numbers refer to branch support based on posterior probability. Hypothesis of relationships of Characidae's "Clade B" plus Spintherobolinae based on morphological evidence, with yellow band representing Aphyocharacinae members.

4. Ancestral character state reconstruction of secondary sexual characters

4.1. Phylogenetic framework

For all comparative analyses, we used the species tree phylogeny for "Clade B". The 'drop.tip' function (ape – Paradis *et al.*, 2004) was used to trim the phylogeny to retain only the species from Aphyocharacinae subfamily (Figure 12) associated with our morphological dataset.

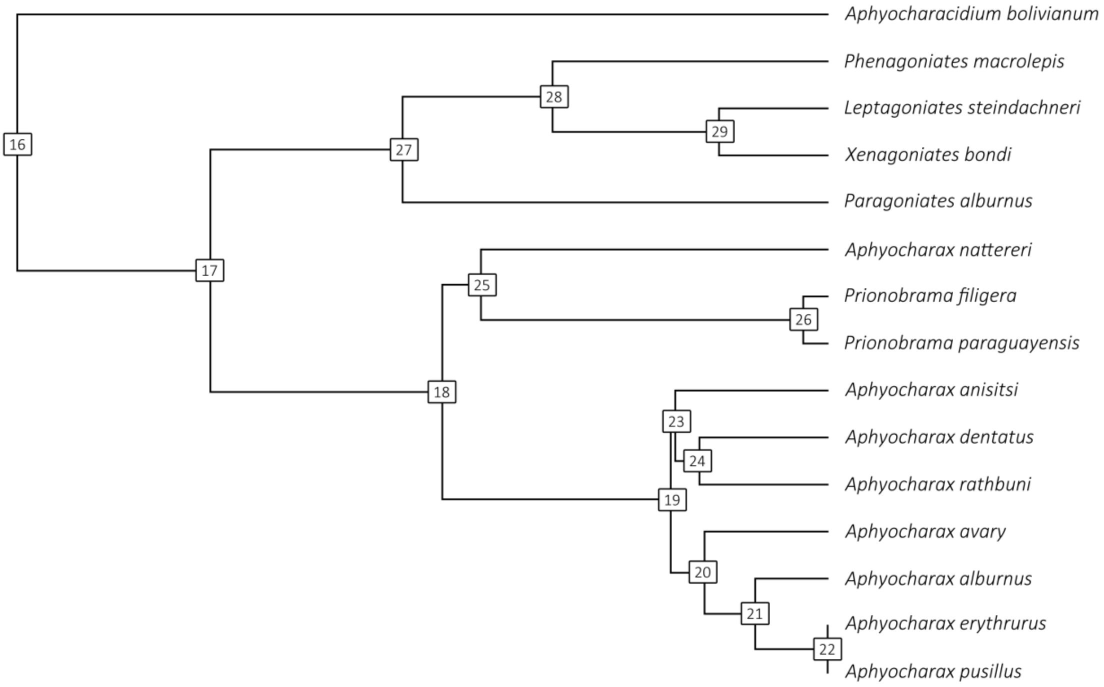

Figure 12 – Phylogenetic relationships of Aphyocharacinae subfamily. "Clade B"'s trimmed phylogenetic tree from Bayesian Inference based on molecular data used in ancestral reconstruction analyses. Tree nodes are informed.

Characters were here considered independent, where analyses of evolutionary history for each trait were conducted separately. For this approach, *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis* and *Xenagoniates bondi* were coded as absent (ch. 449 – 453 ; ch. 521 – 527 ; ch. 531 – 538).

Discrete character models of evolution were estimated using 'fitMk' function (phytools – Revell, 2012), and models with the lowest Akaike weight (AICc weight) were selected as the best model (Table 4).

Table 4 – Best model fits for secondary sexual characters in Aphyocharacinae.

Character	Best model	AICc	AICc weight
440 – 442	ER / SYM	11.11050	0.4377491
446	ER / SYM	18.79073	0.4024427
447	ER / SYM	10.80906	0.3975337
448	ER / SYM	21.72958	0.4346668
449*	SYM	32.02561	0.99734040
450	ER	33.80732	0.99917836
451	ER	31.08573	0.99898031
452	ER	13.69157	0.88598545
453*	SYM	32.02558	0.99723256
521	ER	34.35999	0.99977763
522	ER	35.63109	0.99964546
523	ER	41.14358	0.997113
524*	SYM	32.02561	0.83412742
525	ER	25.82722	0.77362367
526	ER	21.59041	0.92987388
527	ER	49.29108	0.6146242
528	ER / SYM	10.80906	0.3975337
529	ER / SYM	11.11050	0.4377491
530 – 531	ER	24.54086	0.99991829
532*	ER	43.89654	0.99223609
533	ER	40.42185	0.77283551
534	ER	23.83540	0.94595987
535	ER	29.35911	0.65291578

536	ARD	-76.22604	1
537*	SYM	32.02558	0.99683502
538*	SYM	32.02559	0.83383423

The evolutionary history of 23 secondary sexual characters (see below) were estimated for 1,000 trees by stochastic character mapping (Bollback, 2006; Huelsenbeck *et al.*, 2003) with the 'make.simmap' function (phytools – Revell, 2012). Estimates on the evolutionary history of secondary sexual characters were reconstructed from 1,000 random trees based on the posterior distribution from our tree to account for phylogenetic uncertainty.

Best fitted models from characters 449, 453, 524, 532, 537 and 538 were retrieved, however the ACSR could not be completed due to computationally demanding analyses.

4.2. Evolutionary history of sexually dimorphic structures in Aphyocharacinae

Bony hooks on fin rays (ch. 440)

The transition rate between the states "absent" and "present" was estimated on 1.732149. Changes between states within sampled trees slightly varied from best fitted models, as observed on average changes between states from "absent" to "present" (ER: 0.67 ; SYM: 0.659) and from "present" to "absent" (ER: 0.947 ; SYM: 0.999).

Stochastic mapping of ancestral states were similarly estimated under both models of evolution (Supporting Information 4). Under those scenarios, bony hooks on fin rays have undergone transitions from "present" to "absent" in Aphyocharacinae one time, with no reversals (Figures 13–14).

The presence of bony fin hooks on rays was recovered with a high probability as the ancestral condition in Aphyocharacinae (node 16, Figs. 13–14). Most nodes with strong probabilities (PP= >98%) for the "present" state as the ancestral condition. Reversal on ancestral nodes of *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Leptagoniates steindachneri* and *Xenagoniates bondi* (nodes 27–29) were based on high probabilities (PP= >90%).

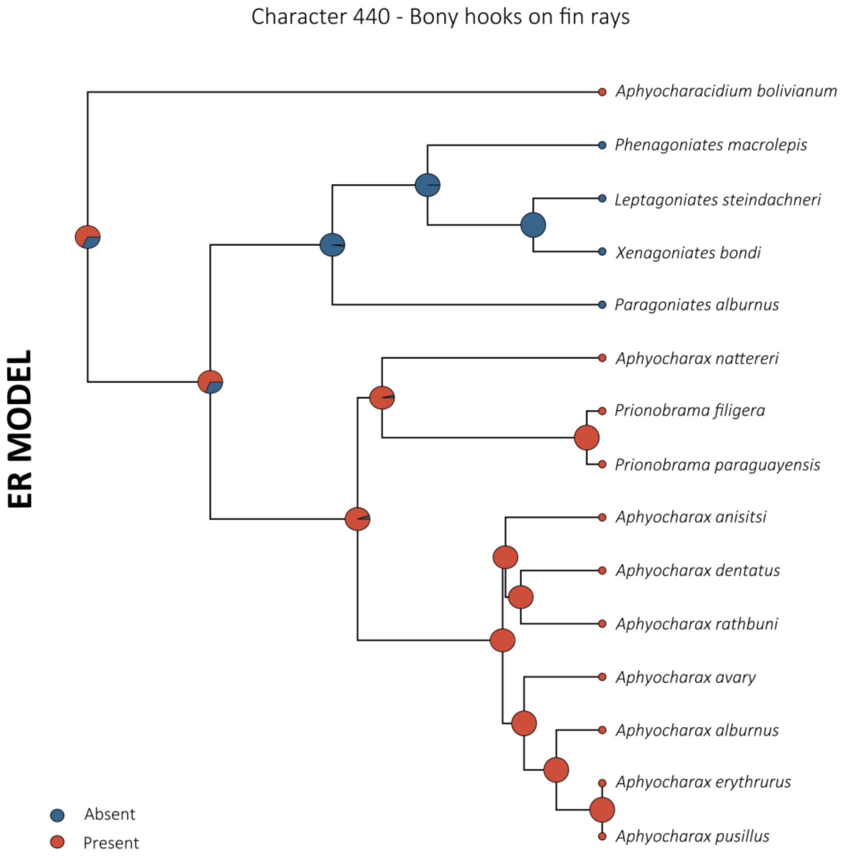

Figure 13 – Ancestral state reconstruction of bony hooks on fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under "ER" model.

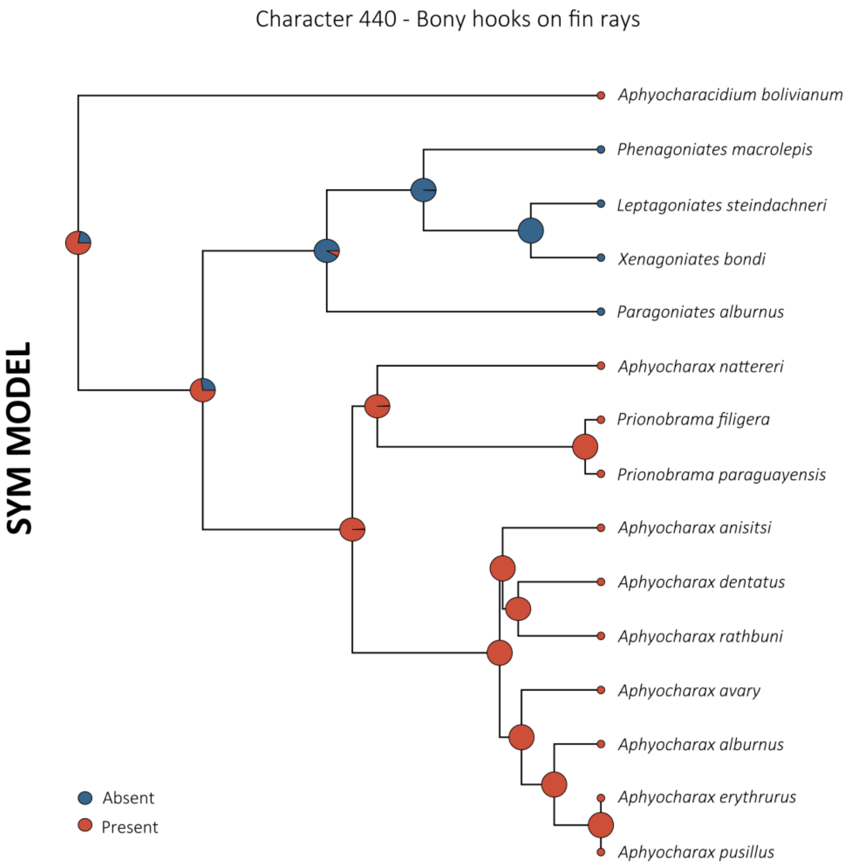

Figure 14 – Ancestral state reconstruction of bony hooks on fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under "SYM" model.

Anal-fin bony hooks in adult males (ch. 441)

The estimated evolutionary history of this character follows similar trends than the ones observed for the overall presence of bony hooks on fin rays (ch. 440) of Aphyocharacinae subfamily.

Transition rate between the states "absent" and "present" was estimated on 1.732149. Changes between states within sampled trees varied from best fitted models, as observed on average changes between states from "absent" to "present" (ER: 0.628 ; SYM: 0.576) and from "present" to "absent" (ER: 0.949 ; SYM: 0.976).

Under both models of evolution (Supporting Information 4), stochastic mapping of ancestral states were similarly estimated, where anal-fin bony hooks in adult males transitioned once from "present" to "absent" through Aphyocharacinae's evolution (Figures 15–16).

The presence of anal-fin bony hooks in adult males was recovered with a high probability as the ancestral condition at the root (node 16, Figs. 15–16). Within Aphyocharacinae the “present” state was recovered in most of the ancestral nodes with strong probabilities (PP= >99%). The occurrence of this trait at common ancestors of *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Leptagoniates steindachneri* and *Xenagoniates bondi* (nodes 27–29) were estimated as “absent” (PP= >90%).

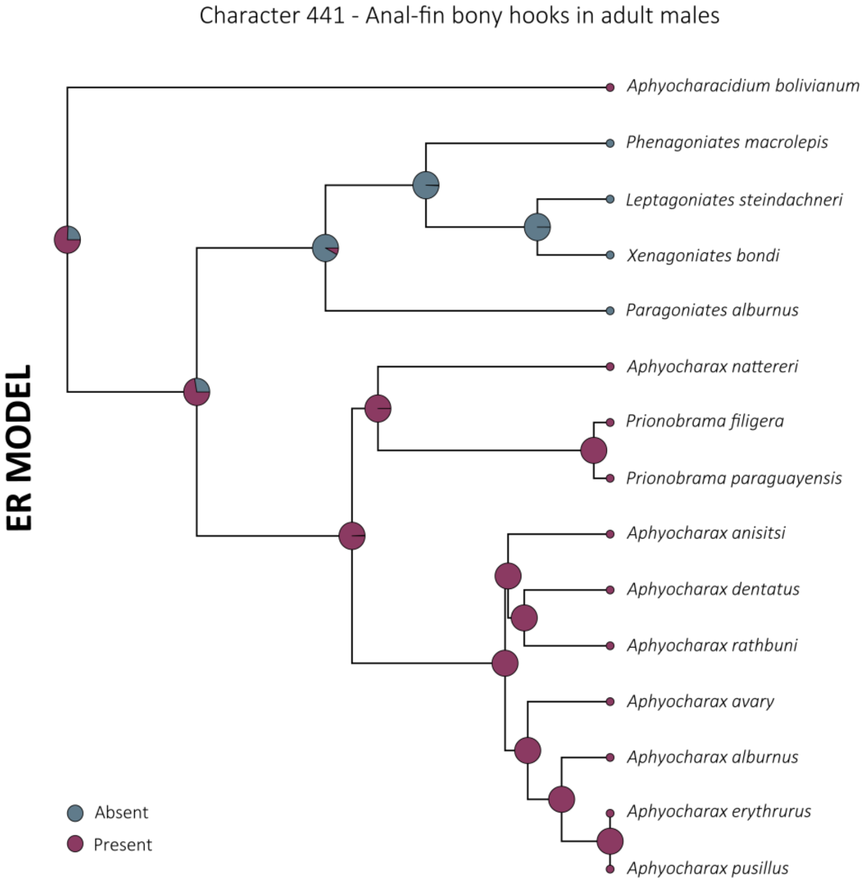

Figure 15 - Ancestral state reconstruction of anal-fin bony hooks on fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under “ER” model.

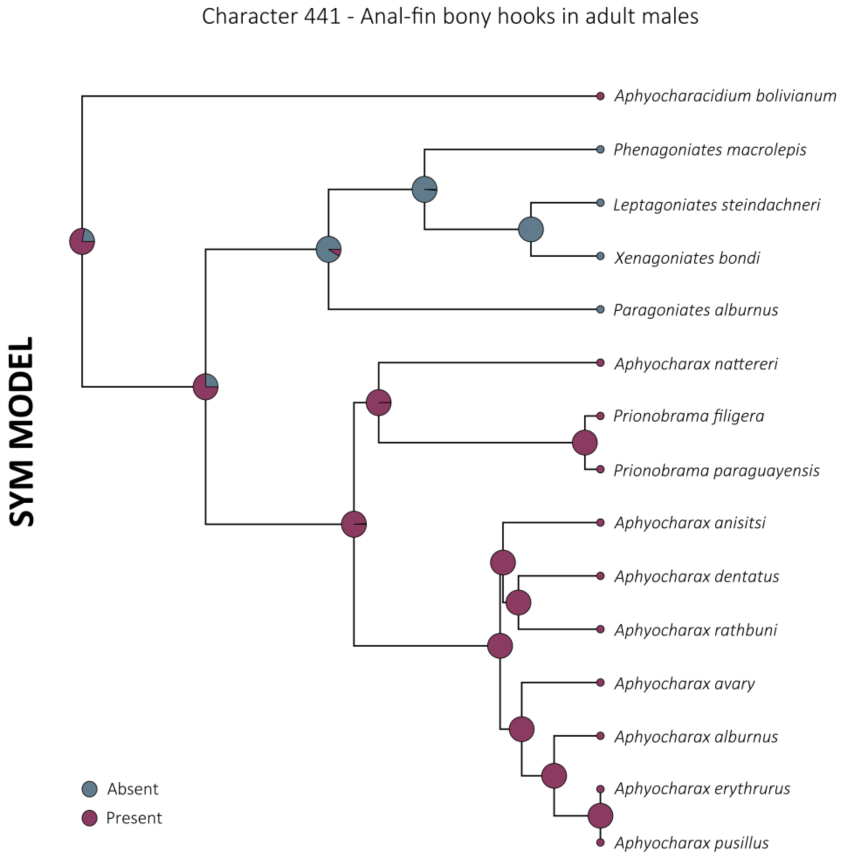

Figure 16 – Ancestral state reconstruction of anal-fin bony hooks on fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under "ER" model.

Pelvic-fin bony hooks in adult males (ch. 442)

The occurrence and evolutionary history of this trait showed similar patterns to the ones recovered for bony hooks on fin rays (ch. 440) and anal-fin bony hooks (ch. 441) within Aphyocharacinae subfamily.

Transition rate between the states "absent" and "present" was estimated on 1.732149. Changes between states within sampled trees varied from best fitted models, as observed on average changes between states from "absent" to "present" (ER: 0.597 ; SYM: 0.662) and from "present" to "absent" (ER: 0.965 ; SYM: 0.971).

Through "ER" and "SYM" stochastic mapping of ancestral states estimates (Supporting Information 4), pelvic-fin bony hooks in adult males transitioned from "present" to "absent" one time in Aphyocharacinae (Figures 17–18).

The presence of pelvic-fin bony hooks in adult males was estimated as the ancestral condition at all Aphyocharacinae's common ancestor with a high probability (node 16, Figs. 17–18). Most nodes were recovered with strong probabilities (>99%) for the "present" state as the ancestral condition. The condition "absent" as the most probable condition on the ancestral nodes of *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Leptagoniates steindachneri* and *Xenagoniates bondi* (nodes 27–29) based on high probabilities (PP= >90%).

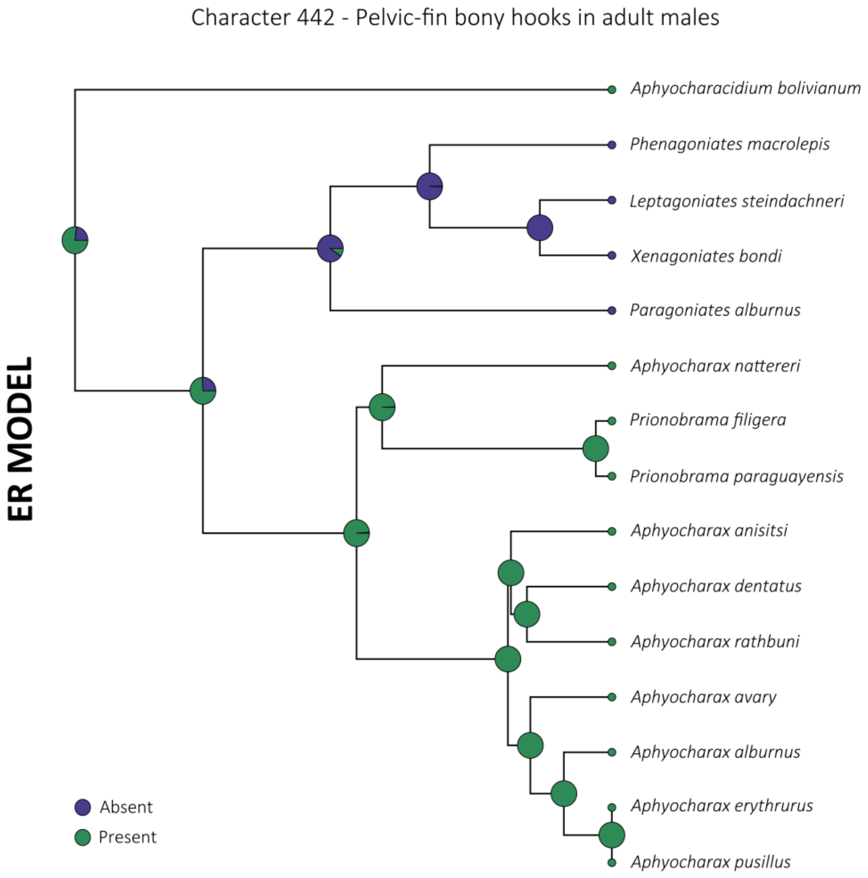

Figure 17 – Ancestral state reconstruction of pelvic-fin bony hooks on fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under "ER" model.

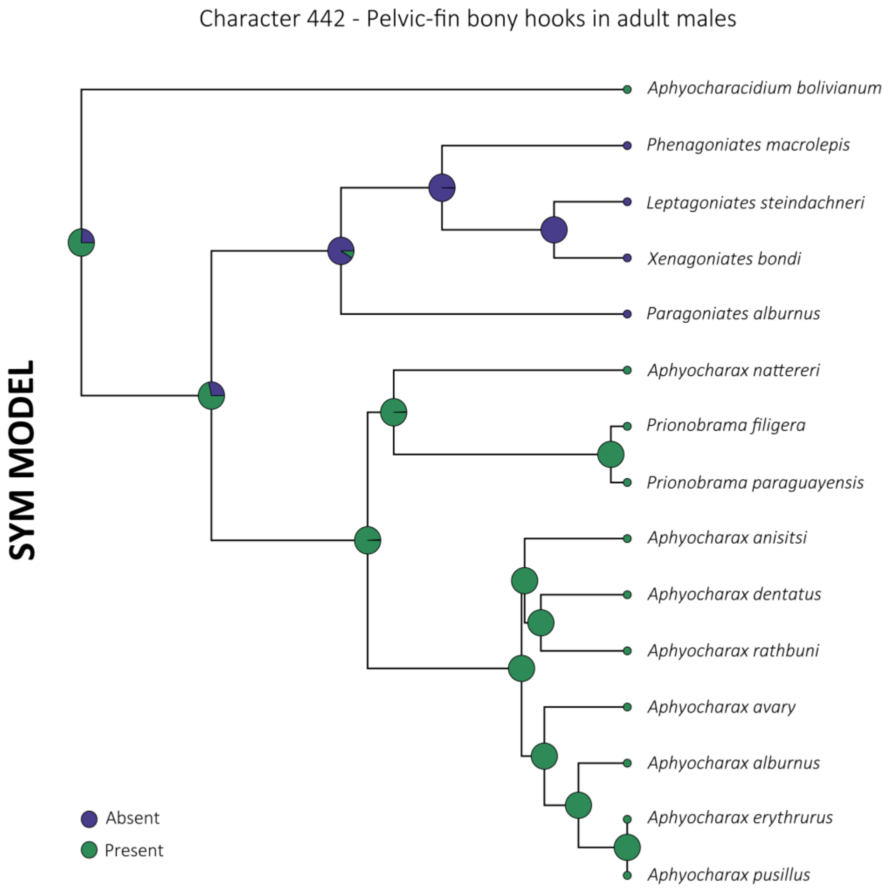

Figure 18 – Ancestral state reconstruction of pelvic-fin bony hooks on fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under “SYM” model.

Bony hooks on base of pelvic-fin rays of adult males (ch. 446)

Transition rate between states was estimated on 5.028054. Within sampled trees, changes between the two conditions were similarly estimated on best fitted models, with average changes between states from “absent or fewer” to “similar”[1] (ER: 2.559 ; SYM: 2.556) and from “similar” to “absent or fewer” (ER: 2.041 ; SYM: 1.977).

Stochastic mapping estimates (Supporting Information 4) inferred some interesting patterns on the condition states on ancestor nodes within Aphyocharacinae. On node 16 we observe a moderate probability of the ancestor condition being “absent or fewer” (PP= ER: 0.663 ; SYM: 0.679), increasing in the descending internal nodes 17 (PP= ER: 0.835 ; SYM:0.856) and 18 (PP = ER: 0.846 ; SYM: 0.869).

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[1] See Supporting Information 4 for all character states’ caption reference.

The transitions between "absent or fewer" to "similar" and vice-versa were similarly estimated under both models of evolution (Supporting Information 4) returning ambiguous probabilities on the condition of *Aphyocharax* species' most common ancestor (node 19, Figures 19–20). A reversal to the "similar" state was estimated at a high probability at the common ancestor of *Ap. ansitsi*, *Ap. dentatus* and *Ap. rathbuni* (node 23, Figs. 19–20).

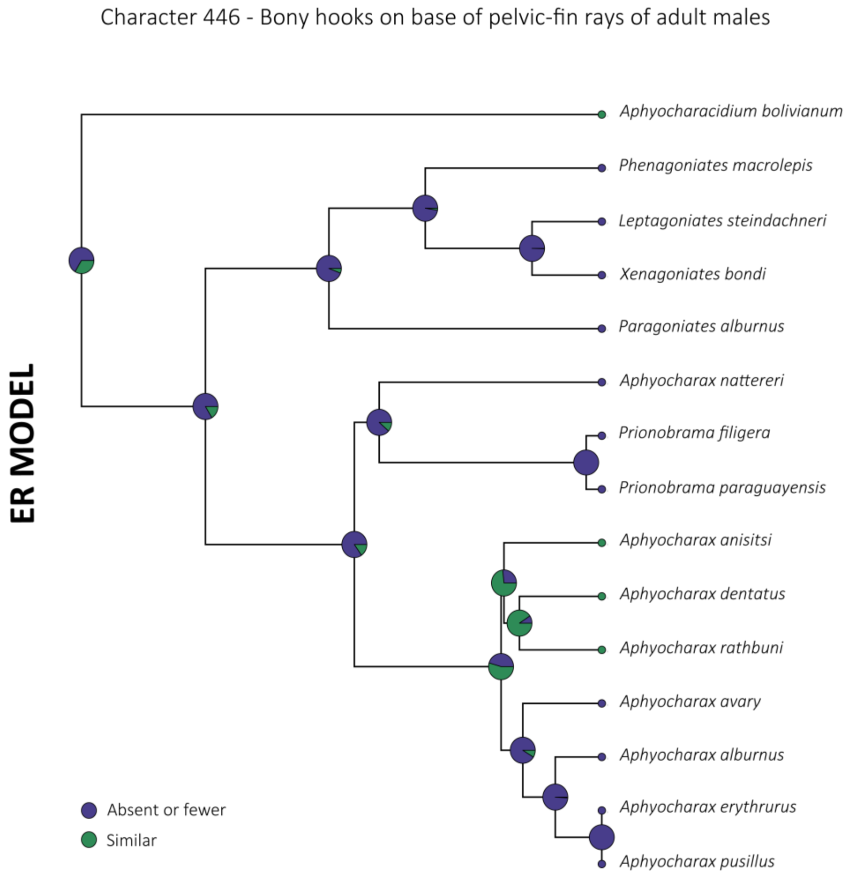

Figure 19 – Ancestral state reconstruction of bony hooks on base of pelvic-fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under "ER" model.

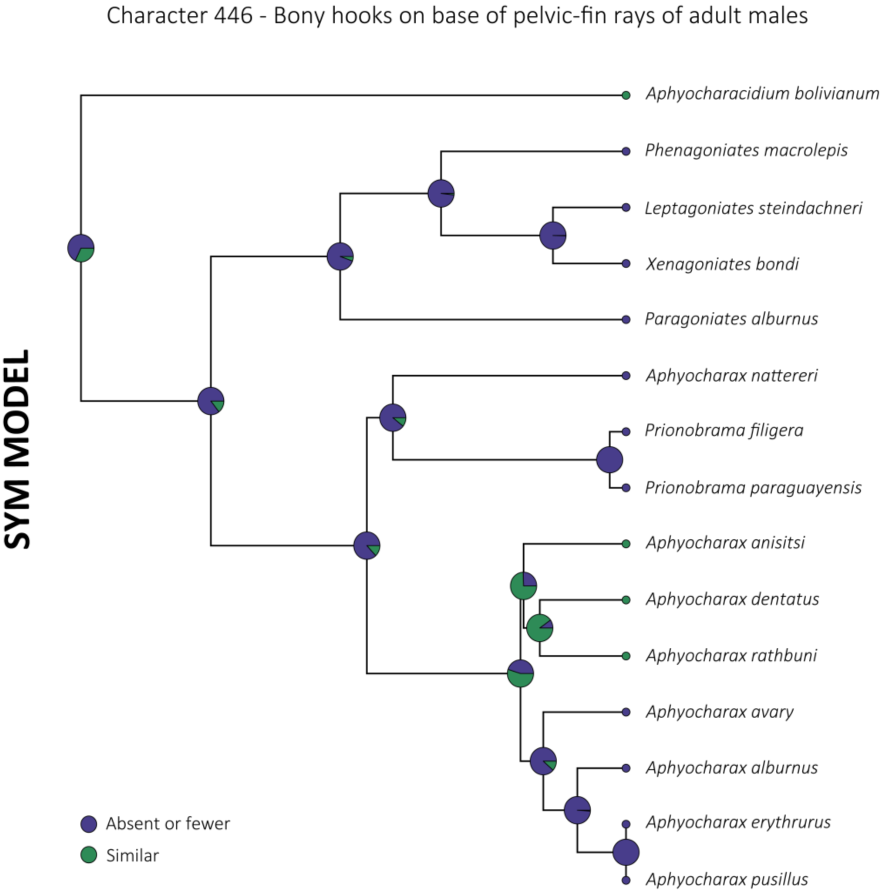

Figure 20 – Ancestral state reconstruction of bony hooks on base of pelvic-fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under "SYM" model.

Bony hooks on last pelvic-fin ray of adult males (ch.447)

Transition rate between sates was estimated on 1.252819. Evolutionary history of bony hooks on last pelvic-fin ray within Aphyocharacinae was correspondingly estimated on best fitted models. Similar patterns of average swaps were observed between states from "absent or reduced" to "similar" (ER: 1.047 ; SYM: 1.041) and from "similar" to "absent or reduced" (ER: 0.089 ; SYM: 0.079).

Having similar number of bony hooks on last pelvic-fin ray in adult males was recovered with strong probabilities as the ancestral condition at most of the Aphyocharacinae's ancestral nodes (PP= >99%, Supporting Information 4). A reversal on node 25 (PP = ER: 0.990 ; SYM: 0.988, Figures 21–22), where in *Prionobrama* species bony hooks were observed in reduced number compared to remaining hook-bearing pelvic-fin rays.

The absence of this trait is attested here in *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Leptagoniates steindachneri* and *Xenagoniates bondi*.

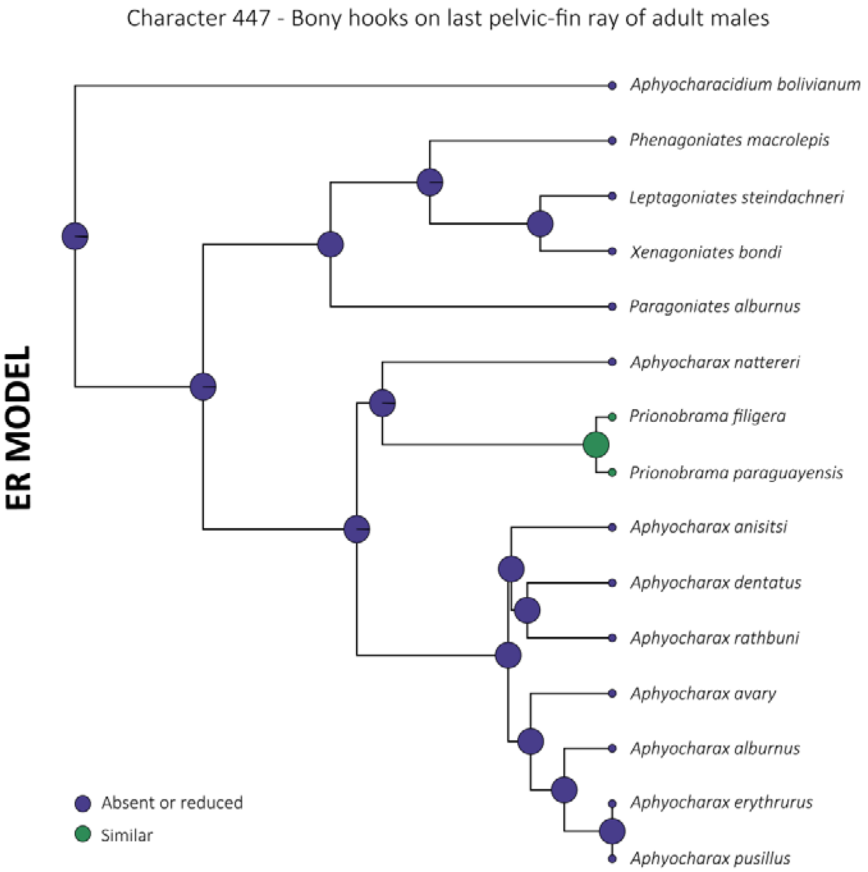

Figure 21 – Ancestral state reconstruction of bony hooks on last pelvic-fin ray in Aphyocharacinae from 1,000 simulations with stochastic mapping under "ER" model.

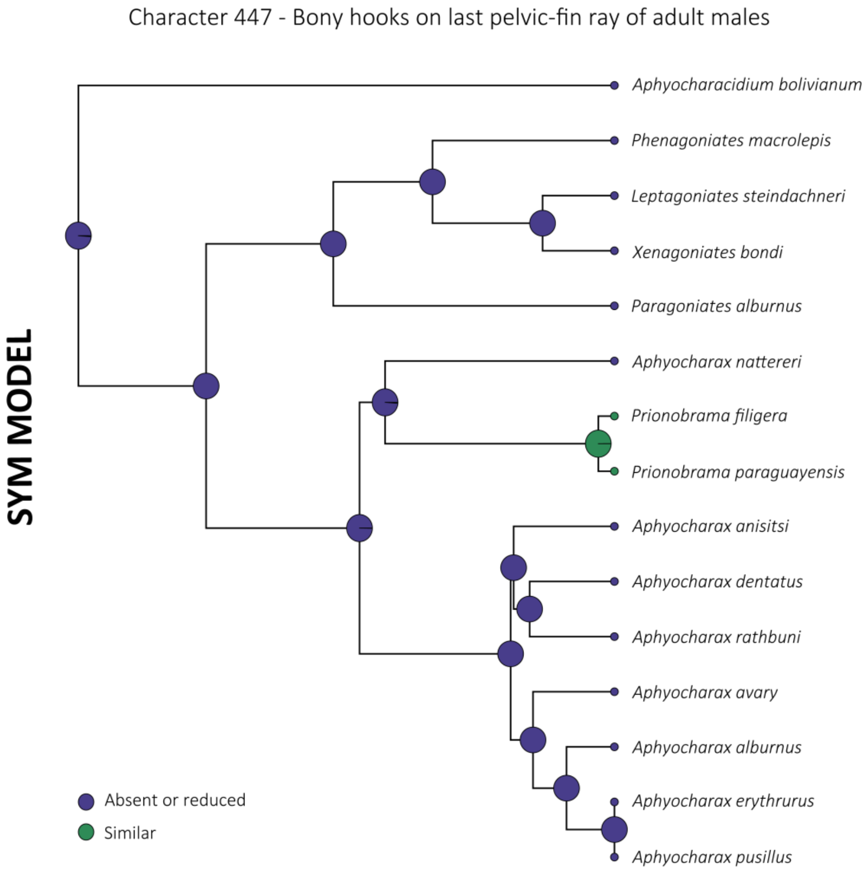

Figure 22 – Ancestral state reconstruction of bony hooks on last pelvic-fin ray in Aphyocharacinae from 1,000 simulations with stochastic mapping under "SYM" model.

Bony hooks on first pelvic-fin ray of adult males (ch.448)

Transition rate between the states "absent" and "present" was estimated on 354.6687. From best fitted models, average changes between states within sampled trees were congruent with observed transitions from "absent" to "present" (ER: 160.908 ; SYM: 160.902) and from "present" to "absent" (ER: 161.924 ; SYM: 161.909).

From sampled trees, stochastic mapping estimate analysis returned ambiguous probabilities (Supporting Information 4) on the ancestral condition of this character at the root and most of the Aphyocharacinae's ancestral nodes. A transition from "present" to "absent" was recovered with strong probability on node 22 (PP= 100%, Figures 23–24).

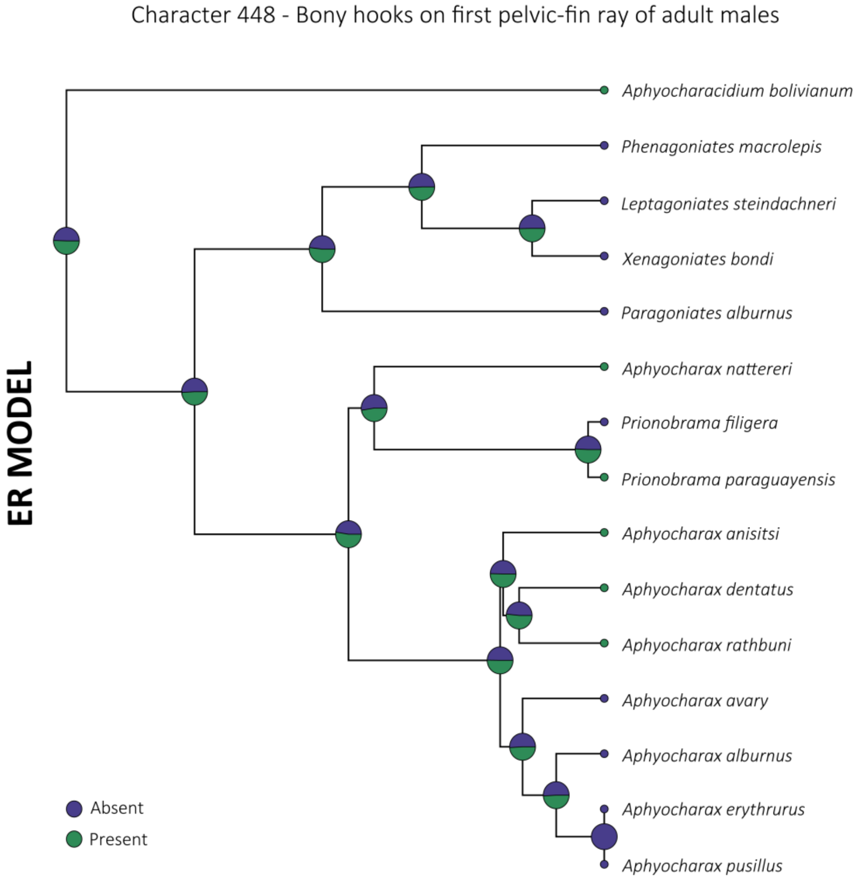

Figure 23 – Ancestral state reconstruction of bony hooks on first pelvic-fin ray in Aphyocharacinae from 1,000 simulations with stochastic mapping under "ER" model.

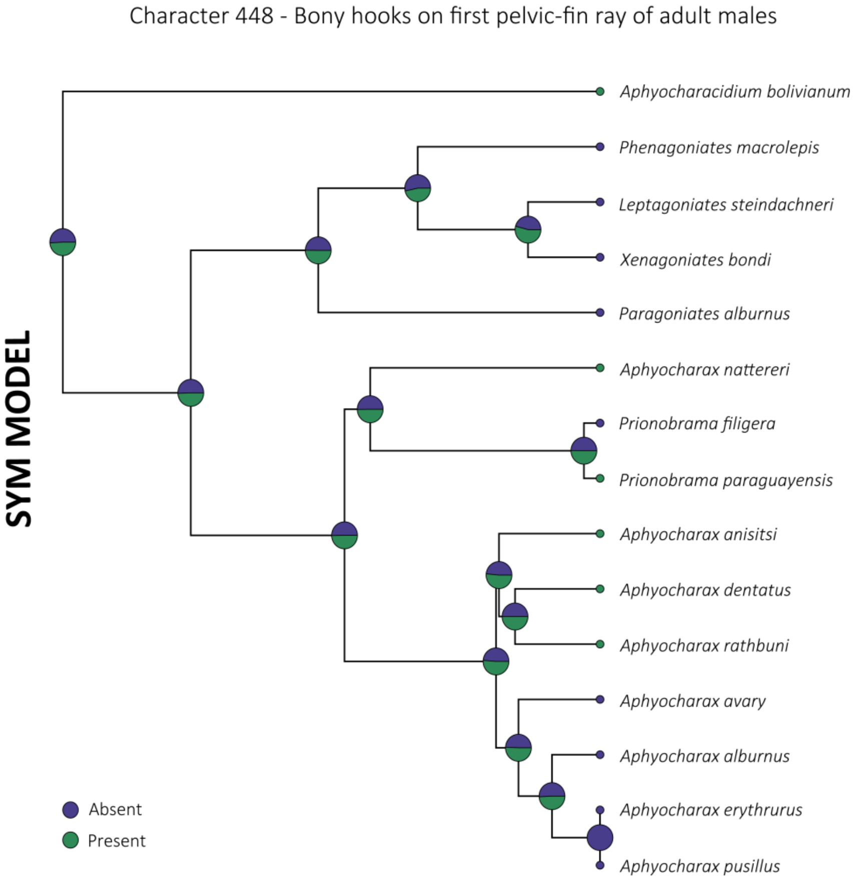

Figure 24 – Ancestral state reconstruction of bony hooks on first pelvic-fin ray in Aphyocharacinae from 1,000 simulations with stochastic mapping under "SYM" model.

Distribution of bony hooks on pelvic-fin segments of adult males (ch.450)

Transition rate between states was estimated on 2.157510. Accumulated average changes between states were observed over trees at different proportions. According to this evolutionary hypothesis, occurring from "bilateral" to "unpaired" state were higher (2.045) compared to all others, whereas going from the condition "unpaired" to "bilateral" rendered fewer changes (0.34) over sampled trees. Remaining changes between states were observed as "medial hemitrichia" to "unpaired" (0.102), "absent" to "unpaired" (0.188), "unpaired" to "medial hemitrichia" (0.202), "unpaired" to "absent" (0.214), "medial hemtrichia" to "absent" (0.247), "medial hemitrichia" to bilateral" (0.261), "absent" to "medial hemitrichia" (0.42), "bilateral" to "medial hemitrichia" (0.441), "absent" to "bilateral" (0.59) and "bilateral" to "absent" (0.687).

Having bony hooks on pelvic-fin segments distributed on both hemitrichia of rays ("bilateral" state) was recovered with high probabilities (PP= >95%, Supporting Information 4) at the common ancestors of *Aphyocharax* and *Prionobrama* species. Absence of this trait was estimated as the most probable ancestral condition at node 27 and all its descendant nodes and lineages (Figure 25).

Ambiguous probabilities on the conditions "absent", "bilateral" and "medial hemtrichia" were recovered at nodes 16 and 17, where the possible arrangement of pelvic-fin bony hooks on those remain uncertain. The patterns of distribution of bony hooks on pelvic-fin segments of adult males on ancestor nodes have also showed lower probabilities of occurring on an unpaired arrangement among Aphyocharacinae's most common ancestors.

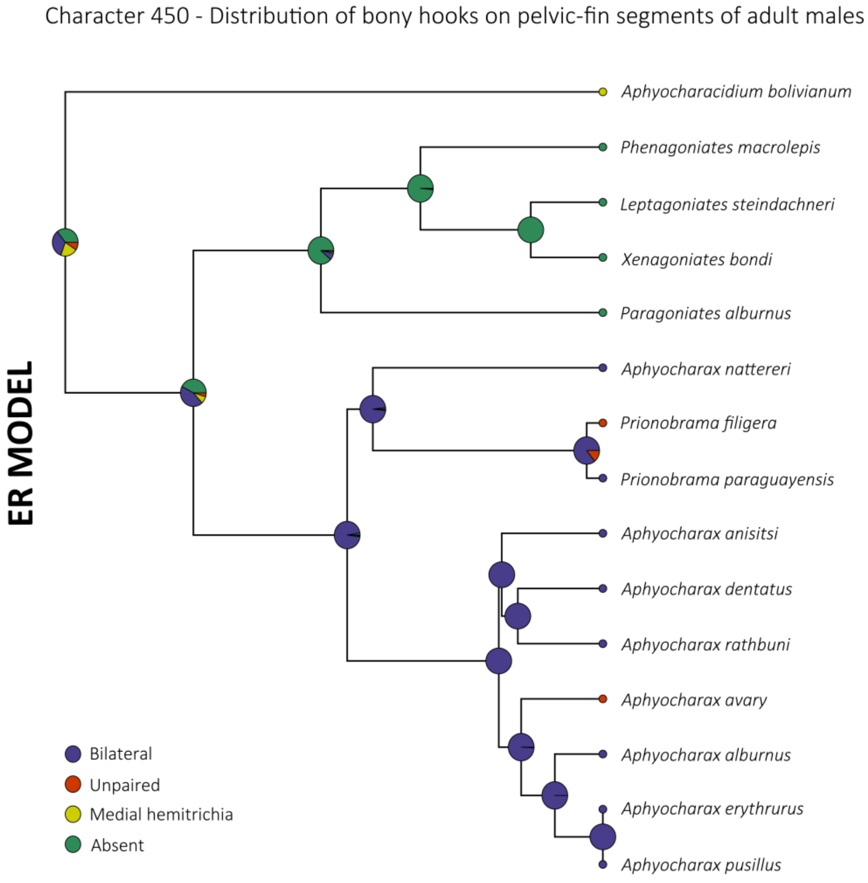

Figure 25 – Ancestral state reconstruction of the distribution of bony hooks on pelvic-fin segments in Aphyocharacinae from 1,000 simulations with stochastic mapping under "ER" model.

Position of anal-fin bony hooks of adult males (ch.451)

Transition rate between states was estimated on 1.622828. Changes occurring from “asymmetric” to “paired” were higher (2.016) than from “paired” to “asymmetric” (0.211) based on average changes between states over sampled trees. Other state changes were related from “asymmetric” to “absent” (0.856), “absent” to “asymmetric” (0.6), “medial” to “asymmetric” (0.165), “asymmetric” to “medial” (0.119), “absent” to “paired” (0.118), “medial” to “absent” (0.1), “paired” to “absent” (0.09), “absent” to “medial” (0.05), “medial” to “paired” (0.04) and “paired” to “medial” (0.02).

Moderate probabilities point to an “asymmetric” state as the probable condition at the root (PP= ~65%) and node 17, where estimations still considered a smaller but fair possibility at the “absent” state. However, the presence of an asymmetrical disposition of bony hooks on pelvic-fin rays returned as the most likely ancestral condition at the common ancestors of *Aphyocharax* and *Prionobrama* species, showing a consistent pattern of evolution through time along Aphyocharacinae’s diversification (Supporting Information 4). Reversals to the “paired” state were observed in *Ap. dentatus* and *Ap. alburnus*.

The “medial” state returned at lower probabilities at a few nodes within this subfamily, which could be an inherited condition from outgroup (see considerations for ch. 451). Node 27 was recovered at an “absent” condition (Figure 26), where this transition was estimated to have occurred one time through Aphyocharacinae’s evolutionary process, with no reversals.

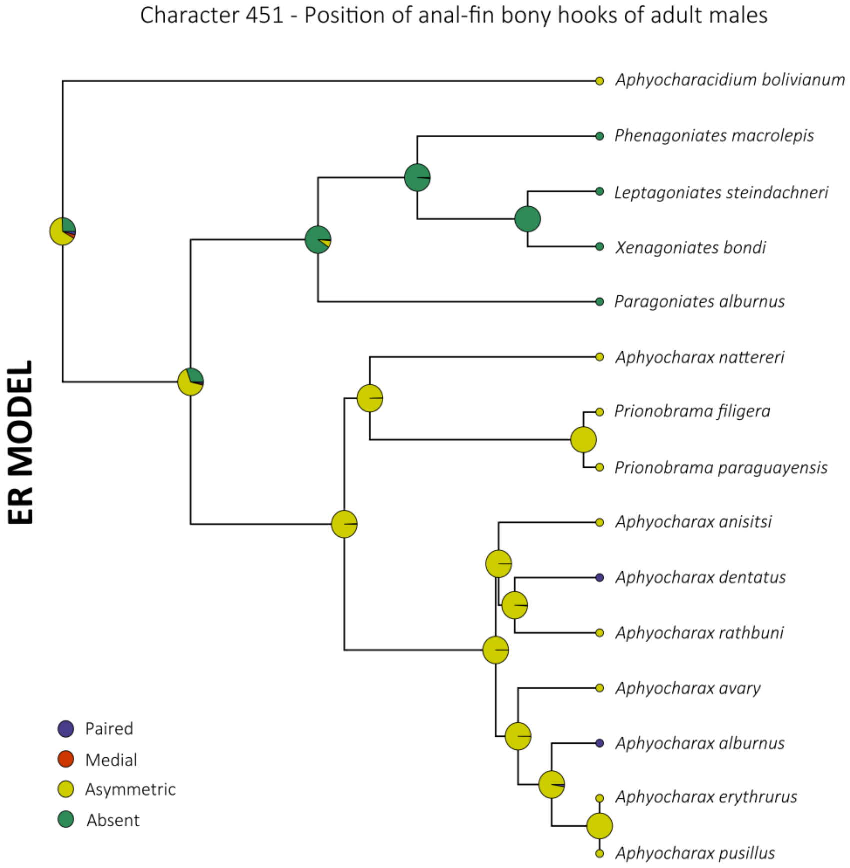

Figure 26 – Ancestral state reconstruction on the position of anal-fin bony hooks in Aphyocharacinae from 1,000 simulations with stochastic mapping under "ER" model.

Number of anal-fin hooks of adult males (ch.452)

Transition rate between states was estimated on 0.7259296. Among sampled trees, changes from the state "three or more" to "absent" were higher (0.915). Remaining state changes occurred between "absent" to "three or more" (0.28), "one or two" to "three or more" (0.035), "three or more" to "one or two" (0.034), "one or two" to "absent" (0.027) and "absent" to "one or two" (0.01).

Throughout Aphyocharacinae's evolutionary process, the state "three or more" was estimated at most nodes (Figure 27), where this ancestral condition was recovered at the common ancestors of *Aphyocharax* and *Prionobrama* species with strong support (Supporting Information 4). The number of bony hooks on anal-fin rays at the state "one or two" was not observed among aphyocharacins, and returned at lower probabilities at a few nodes as a probable inherited condition from “Clade A” within Characidae (see considerations for ch. 452).

The absence of this trait was estimated as the most likely state at the ancestors of *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Leptagoniates steindachneri* and *Xenagoniates bondi* (nodes 27–29, Fig. 27).

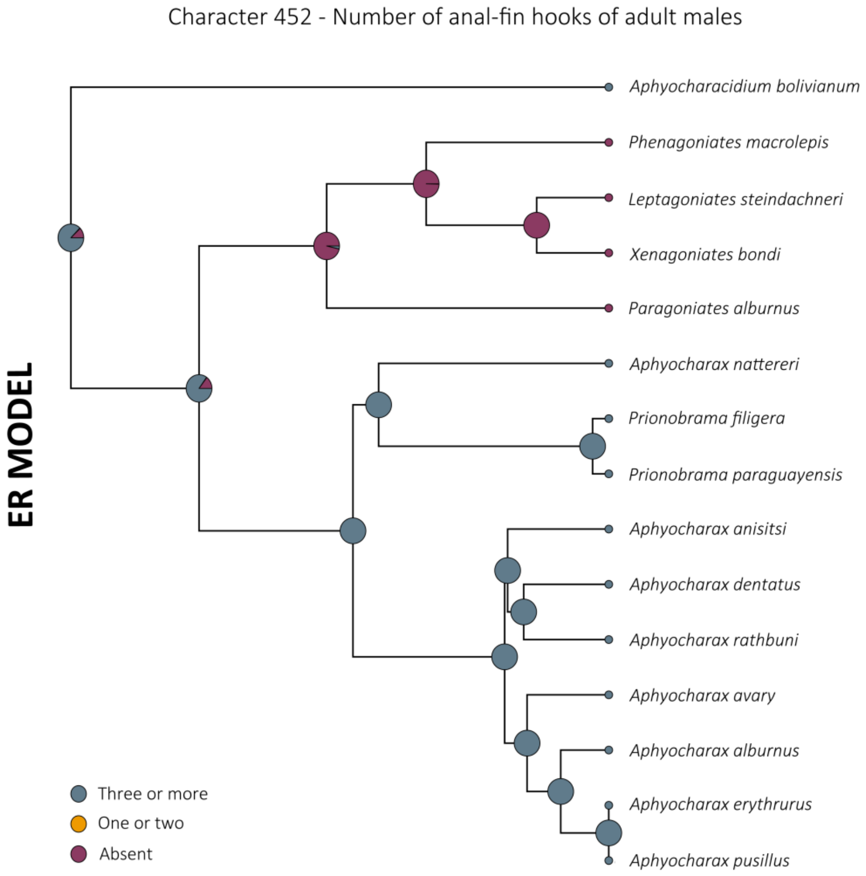

Figure 27 – Ancestral state reconstruction of the number of anal-fin bony hooks in Aphyocharacinae from 1,000 simulations with stochastic mapping under “ER” model.

Distribution of bony hooks on branched pelvic-fin rays of adult males (ch.521)

Transition rate between the states was estimated on 2.784045. Changes between states were measured at different proportions, where changes from “first to sixth” to “first to fifth” (1.838) and the state “first to sixth” to “first to fourth” were higher (1.217). State transformations occurring between “first to sixth” to “absent” (0.853), “absent” to “first to sixth” (0.809) and “first to fifth” to “first to sixth” (0.623) recovered similar trends. Other changes observed from “first to fourth” to “first to sixth” (0.457), “first to fifth” to “absent” (0.383), “absent” to “first to fifth” (0.362), “first to fourth” to “absent” (0.291), “first to fourth” to “first to fifth” (0.258), “absent” to “first to fourth” (0.223) and “first to fifth” to “first to fourth” (0.147) accumulated fewer transitions over sampled trees.

Moderate probabilities point to the presence of bony hooks on the first to sixth branched rays (“fifth to sixth” state) as the likely condition at the root (node 16, PP= ~48%). The distribution of bony hooks on branched pelvic-fin rays in adult males was estimated to have transitioned to the “absent” condition one time during Aphyocharacinae’s diversification with no reversals (node 27, Figure 28). However, the absence of this trait was also recovered with moderate probabilities of occurrence at the common ancestor of most aphyocharacins (node 17, Supporting Information 4).

Trends pointing to the occurrence of bony hooks on fewer branched pelvic-fin rays rendered lower probabilities among most ancestor nodes (Supporting Information 4). A single transformation to the “first to fourth” state was observed on *Aphyocharax alburnus*, and the presence of bony hooks from the first to fifth branched pelvic-fin rays (“first to fifth” state) have appeared two times in *Ap. nattereri* and *Ap. avary*.

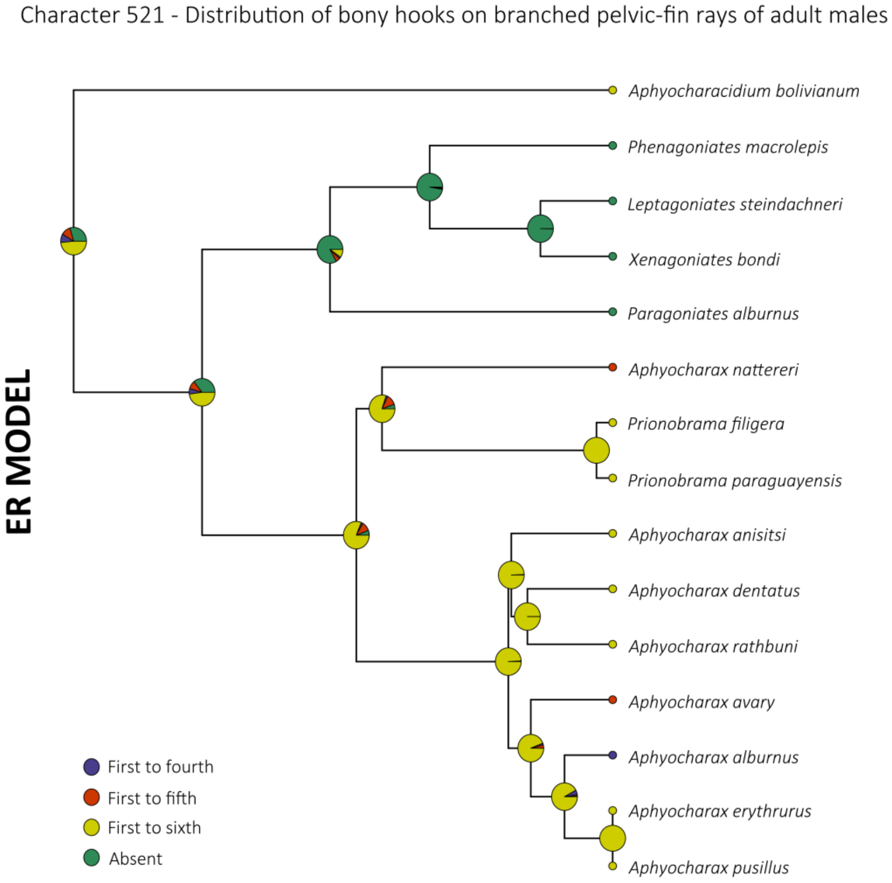

Figure 28 – Ancestral state reconstruction on the distribution of bony hooks on branched pelvic-fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under “ER” model.

Distribution of bony hooks along pelvic-fin rays of adult males (ch.522)

Transition rate between states was estimated on 1.774861. Average changes between states from trees showed more variations from “proximal to middle” to “restricted to middle” (1.169) and “proximal to middle” to “entire length” (1.09), where from “restricted to middle” to “entire length” (0.037) and “entire length” to “restricted to middle” (0.04) were less observed in relation to other transformations. Accumulated changes from “proximal to middle” to “absent” (0.797), “absent” to “proximal to middle” (0.636), “entire length” to “proximal to middle” (0.355), “restricted to middle” to “proximal to middle” (0.301), “restricted to middle” to “absent” (0.141), “entire length” to “absent” (0.139), “absent” to “entire length” (0.103) and “absent” to “restricted to middle” (0.092) were recovered following similar patterns of transition.

The distribution of bony hooks along pelvic-fin rays of adult males revealed a rather structured diversification across evolutionary history. The “proximal to middle” state was indicated as the likely ancestral condition with moderate probabilities at common ancestors of the Aphyocharacinae (nodes 16 and 17, Supporting Information 4), and were recovered with strong support at most descending nodes (PP= >92%, Figure 29).

Patterns of character diversification within this subfamily have returned lower probabilities on the incidence of the states “absent”, “entire length” and “restricted to middle” at most common ancestors (nodes 16 and 17, Fig. 29). The absence of this trait was assessed as the most probable condition at ancestor nodes of *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Leptagoniates steindachneri* and *Xenagoniates bondi* (nodes 27–29, Fig. 29). A single transformation to the “entire length” state was observed in *Prionobrama filigera*, where the transition to “restricted to middle” occurred one time at node 20 and descendant lineages with no reversals.

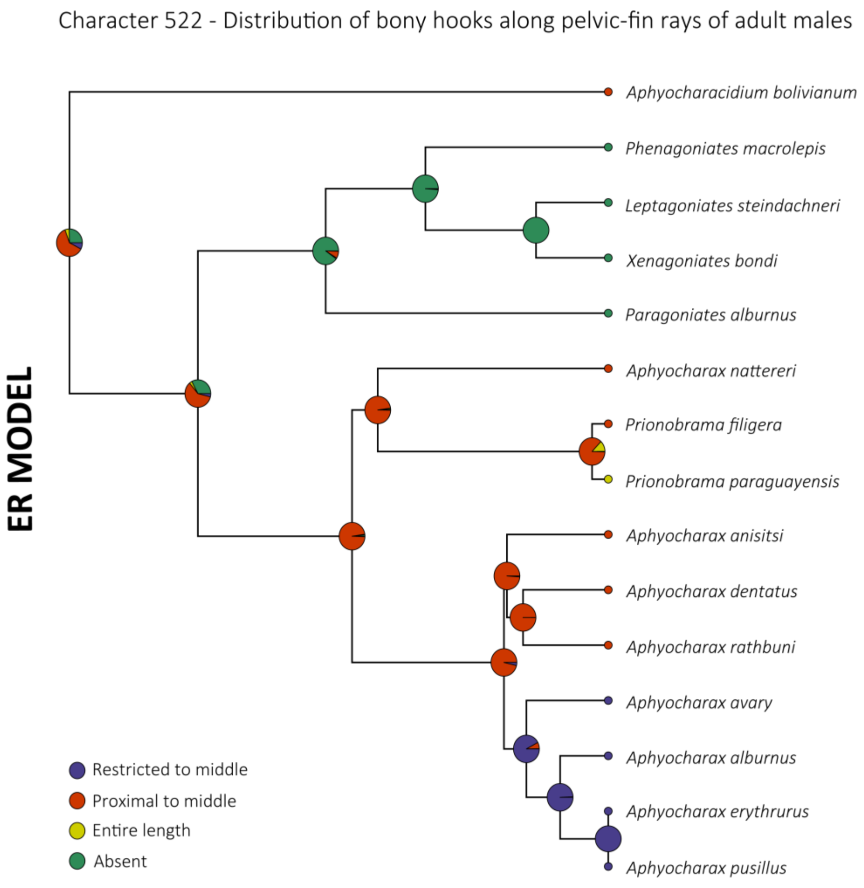

Figure 29 – Ancestral state reconstruction on the distribution of bony hooks along pelvic-fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under “ER” model.

Proximal pelvic-fin ray segment bearing bony hooks on adult males (ch.523)

Transition rate between states was estimated on 167.0951. Changes occurring from “before” to “basal” (38.471), “absent” to “basal” (38.395), “after” to “absent” (38.371), “after” to “basal” (38.368), “before” to “absent” (38.077), “before” to “after” (37.938), “basal” to “after” (37.796), “absent” to “before” (37.658), “basal” to “absent” (37.574), “absent” to “after” (37.423), “basal” to “before” (37.26) and “after” to “before” (37.041) returned similar transitions over sampled trees

Ambiguous probabilities were recovered on most of Aphyocharacinae’s ancestral nodes (Supporting Information 4), where the diversification of this trait within this subfamily needs further investigation. According to this evolutionary hypothesis, a transition to the “after” state was retrieved with strong support on node 22 (PP= 100%, Figure 30).

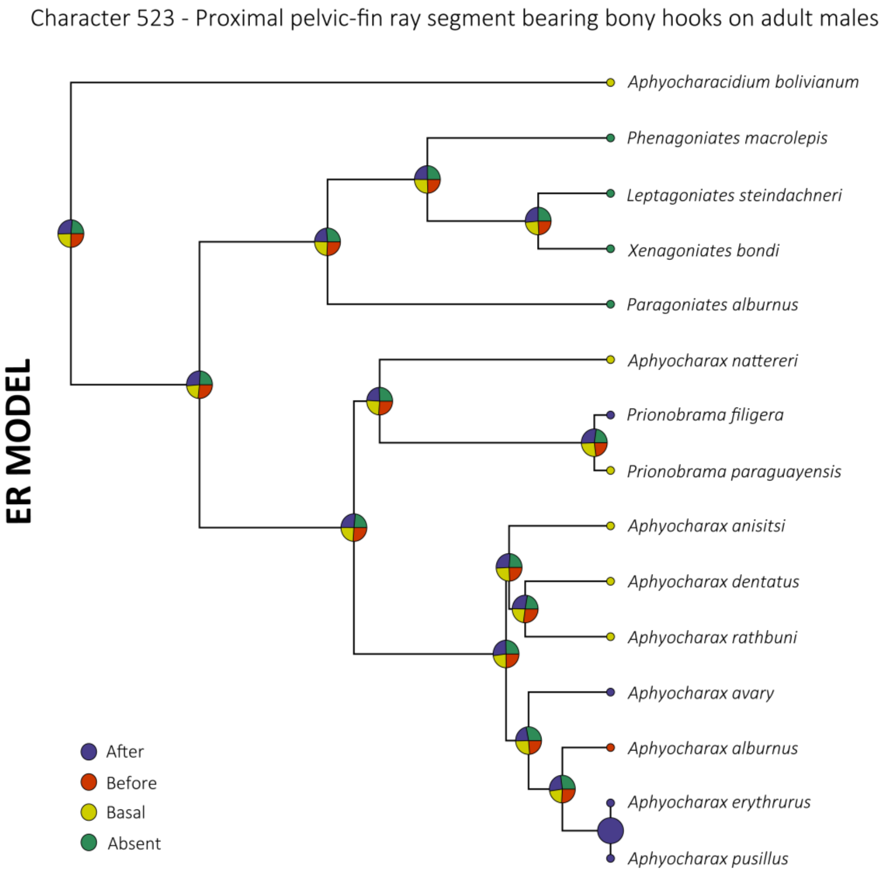

Figure 30 – Ancestral state reconstruction of the most basal pelvic-fin ray segment bearing bony hooks in Aphyocharacinae from 1,000 simulations with stochastic mapping under “ER” model.

Width of bony hook at base on pelvic-fin rays of adult males (ch.525)

Transition rate between the states was estimated on 2.916658. Average changes between states were observed at different proportions, when occurring from “wider” to “same width” state were higher (2.116) compared to all others. Transformations from “wider” to “absent” (0.999) and “absent” to “wider” (0.971) were observed at similar patterns, whereas fewer changes were observed from “same width” to “wider” (0.577), “same width” to “absent” (0.313) and “absent” to “same width” (0.253).

The “wider” state was estimated as the most likely condition at the common ancestor of *Aphyocharax* and *Prionobrama* species and descending lineages with strong support (PP= >90%). The presence of pelvic-fin bony hooks wider at its base was recovered at the root (node 16) and node 17 with moderate probabilities (Supporting Information 4).

During Aphyocharacinae's diversification, the absence of this trait was likely to have transitioned one time with no reversals (node 27, Figure 31). Still, moderate probabilities of occurrence at the "absent" state were recovered at most common ancestors of this subfamily (node 16 and 17, Supporting Information 4).

Trends pointing to the occurrence of pelvic-fin bony hooks with a "same width" base returned at lower probabilities on most ancestor nodes (Supporting Information 4). According to estimations, higher probabilities suggests that *Aphyocharax alburnus* (PP= 0.943) and *Ap. erythrurus* (PP= 1) should exhibit pelvic-fin bony hooks at the "wider" condition. Two transformations to the "same width" state were observed on *Ap. anisitsi* and *Ap. rathbuni*.

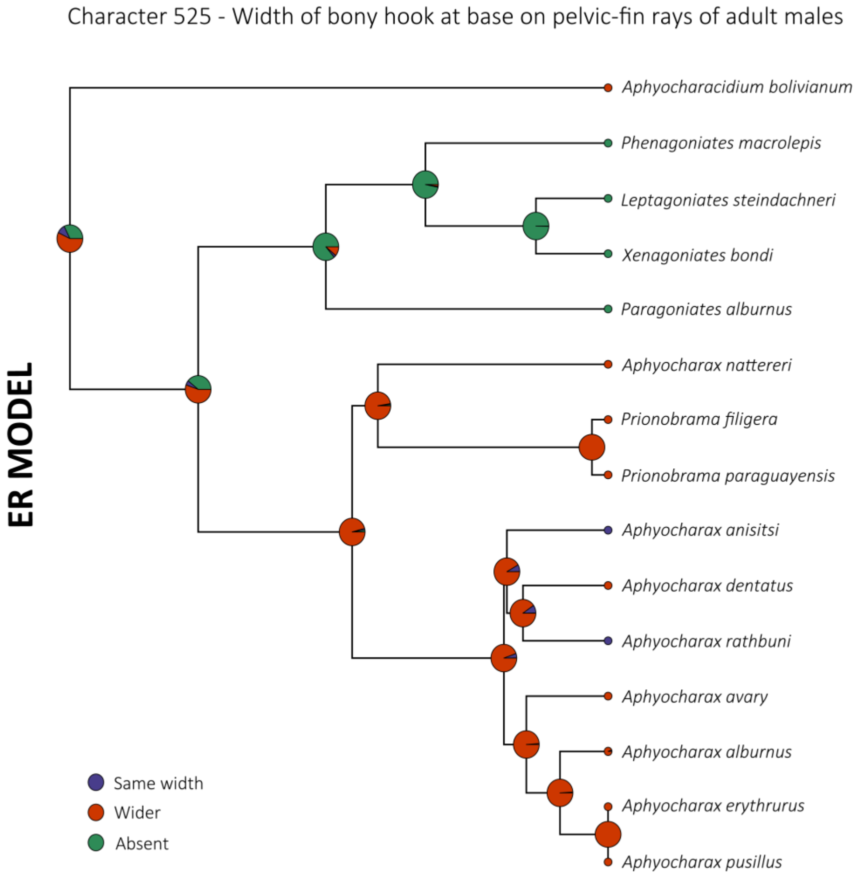

Figure 31 – Ancestral state reconstruction on the width of bony hook at base on pelvic-fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under "ER" model.

Distal tip of bony hooks on pelvic-fin rays of adult males (ch.526)

Transition rate between the states was estimated on 2.204538. Accumulated changes over trees were detected at distinct patterns, where transitions between states “retrorse” to “straight” (1.456) were more observed. Transformations from “retrorse” to “absent” (0.734) and “absent” to “retrorse” (0.694) were similarly estimated, while fewer changes were monitored from “absent” to “straight” (0.534), “straight” to “absent” (0.304) and “straight” to “retrorse” (0.3).

Ambiguous probabilities on all states were recovered at nodes 16 and 17, where the shape of the distal tip of pelvic-fin bony hooks on most common ancestors of Aphyocharacinae members remains uncertain. The absence of this trait was estimated as the most probable condition at the ancestors of *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Leptagoniates steindachneri* and *Xenagoniates bondi* (nodes 27 – 29, Figure 32).

The “retrorse” state was observed on most Aphyocharacinae members, and was recovered with strong support as the likely ancestral condition at node 18 and most of its descendant lineages (Supporting Information 4). The presence of pelvic-fin bony hooks with a straight tip rendered lower probabilities on most ancestor nodes of *Aphyocharax* and *Prionobrama* genera (Supporting Information 4). However, this state was recovered with a moderate probability at node 21 (PP= ~42%), followed by a strong support of full transition at node 22 (Fig. 32). According to this hypothesis, higher probabilities suggests that *Ap. erythrurus* (PP= 0.999) displays pelvic-fin hook tips at the “straight” state, whereas ambiguous ratios were recovered for *Aphyocharax alburnus* and its condition could not be inferred.

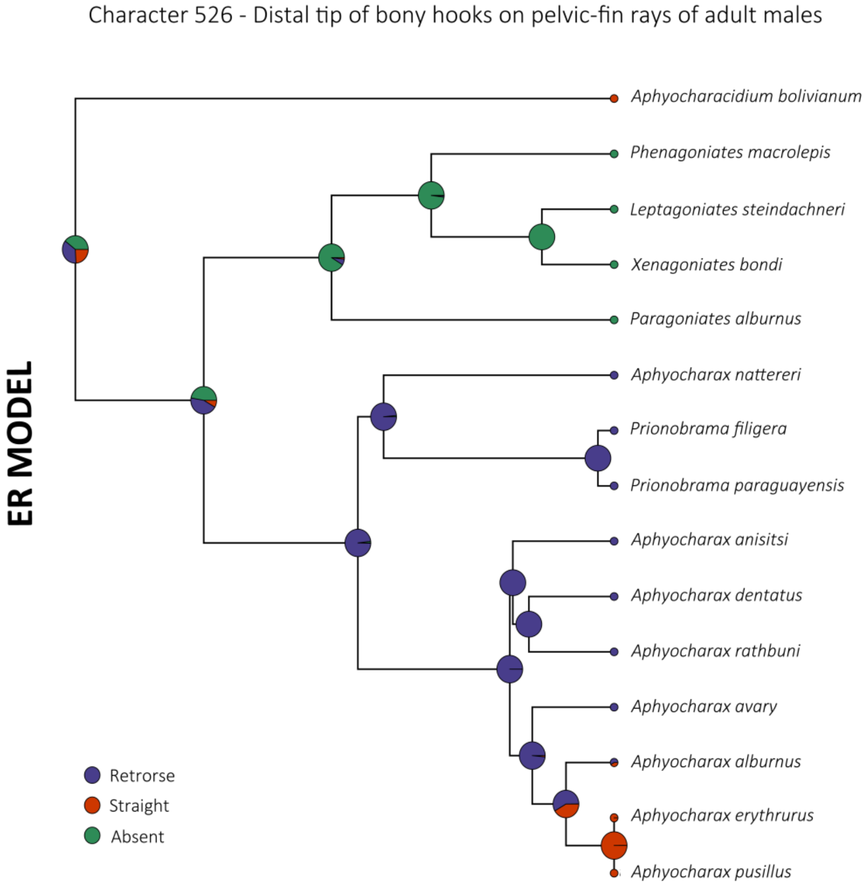

Figure 32 – Ancestral state reconstruction of the distal tip of bony hooks on pelvic-fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under “ER” model.

Number of bony hooks per pelvic-fin ray segment of adult males (ch.527)

Transition rate between states was estimated on 2.691844. Average changes over sampled trees returned at different proportions, where conversions from the state “two” to “three” were higher (1.368) when compared to changes between “three” to “two” (0.554). Transformations from “two” to “absent” (0.865), “absent” to “two” (0.819), “two” to “one” (0.74), “one” to “three” (0.713) and “three” to “one” (0.614) were recovered following similar patterns of transition. Changes occurring from “one” to “two” (0.467), “absent” to “three” (0.335), “three” to “absent” (0.329), “one” to “absent” (0.291) and “absent” to “one” (0.224) were less observed in relation to other transformations.

Across Aphyocharacinae’s evolutionary process, the state “two” was estimated at most nodes (Supporting Information 4) with strong support. This condition was recovered as the most probable occurrence at the common ancestors of *Aphyocharax* and *Prionobrama* genera

(Figure 33). Within this clade, a transition to having up to three bony hooks per pelvic-fin ray segment was retrieved at the ancestor node of *Prionobrama* species. Ambiguous probabilities were recovered at node 22, where the number of pelvic-fin bony hooks was closely estimated between states “one” and “three”.

Moderate probabilities point to the presence of up to two bony hooks attached to pelvic-fin ray segments as the likely condition at the root (node 16, PP= ~46%), with estimates pointing to slightly lower probabilities at remaining states. Ambiguous ratios on the absence or the occurrence of the state “two” were recovered at the common ancestor of most aphyocharacins (node 17, Supporting Information 4).

The number of bony hooks on pelvic-fin rays in adult males was estimated to have transitioned to the “absent” condition one time during Aphyocharacinae’s diversification with no reversals (node 27, Fig. 33). A single transition to having “one” hook per pelvic-fin ray segment was observed in *Aphyocharax erythurus*.

Character 527 - Number of bony hooks per pelvic-fin ray segment of adult males

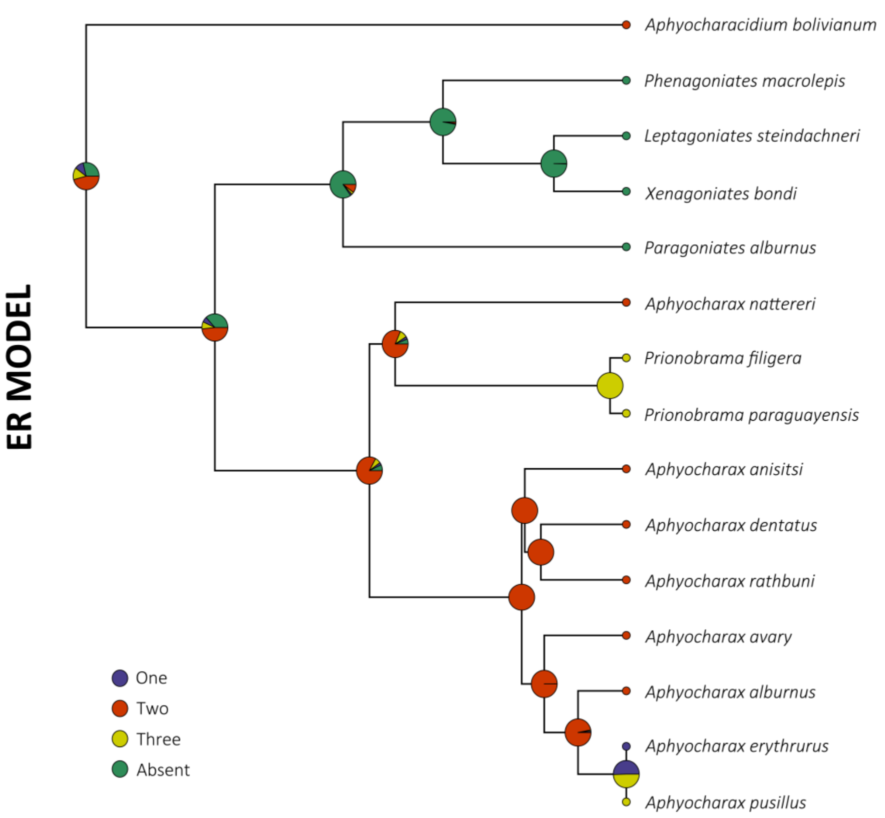

Figure 33 – Ancestral state reconstruction on the number of bony hooks per pelvic-fin ray segment in Aphyocharacinae from 1,000 simulations with stochastic mapping under "ER" model.

Anal fin shape profile in adult specimens (ch.528)

Transition rate between the states "no elongation" and "elongated rays" was estimated on 1.252819. Accumulated changes between states within sampled trees varied from best fitted models, where conversions from "no elongation" to "elongated rays" (ER: 1.056 ; SYM: 1.049) where higher when compared to transformation from "elongated rays" to "no elongation" (ER: 0.089 ; SYM: 0.113).

Under both models of evolution (Supporting Information 4), stochastic mapping of ancestral states were similarly estimated. Among the Aphyocharacinae, the "no elongation" state was retrieved in most of the ancestral nodes with strong probabilities (PP= >99%).
According to these hypotheses, the elongation of the most anterior anal-fin rays in adult specimens has transitioned once through Aphyocharacinae's diversification with no reversals

(Figures 34–35). The anal fin shape exhibiting elongated rays was recovered with strong support as the ancestral condition at the common ancestor of *Prionobrama* species.

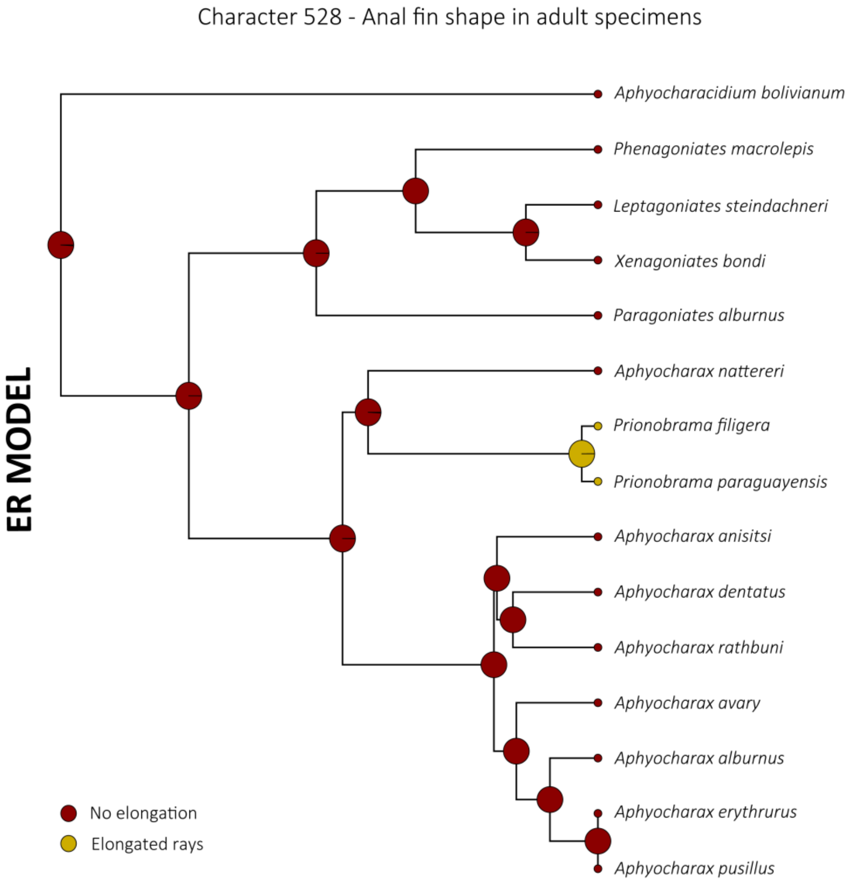

Figure 34 – Ancestral state reconstruction of the anal fin shape in Aphyocharacinae from 1,000 simulations with stochastic mapping under “ER” model.

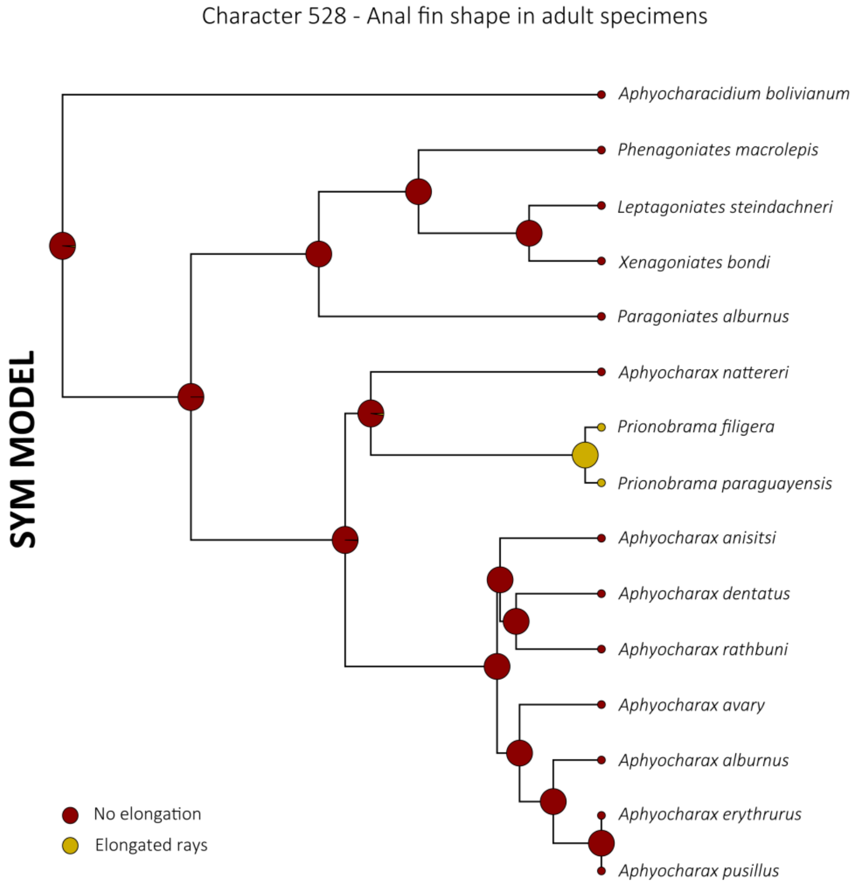

Figure 35 – Ancestral state reconstruction of the anal fin shape in Aphyocharacinae from 1,000 simulations with stochastic mapping under "SYM" model.

Bony hooks on unbranched anal-fin rays of adult males (ch.529)

Character evolutionary history followed similar patterns to the ones recovered for bony hooks on fin rays (ch. 440), anal-fin bony hooks (ch. 441) and pelvic-fin bony hooks (ch. 442) within Aphyocharacinae subfamily.

Transition rate between the states "absent" and "present" was estimated on 1.732149. Changes between states within sampled trees were similarly recovered from best fitted models, with fewer transformations from "absent" to "present" (ER: 0.597 ; SYM: 0.583) in relation to changes from "present" to "absent" (ER: 0.936 ; SYM: 0.989).

Bony hooks on unbranched anal-fin rays had undergone single a transition from "present" to "absent" throughout Aphyocharacinae's diversification (Figures 36–37). The absence of this trait was recovered with strong probabilities at node 27 and descending lineages with no reversals (PP= >99%).

The presence of pelvic-fin bony hooks in adult males was estimated with high probabilities as the ancestral condition at most common ancestors of the aphyocharacins (Supporting Information 4). This condition was recovered with strong support as the most likely state at the common ancestors of all *Aphyocharax* and *Prionobrama* species (Figs. 36–37). Still, moderate probabilities of occurrence at the "absent" state were recovered at most common ancestors of this subfamily (node 16 and 17, Supporting Information 4).

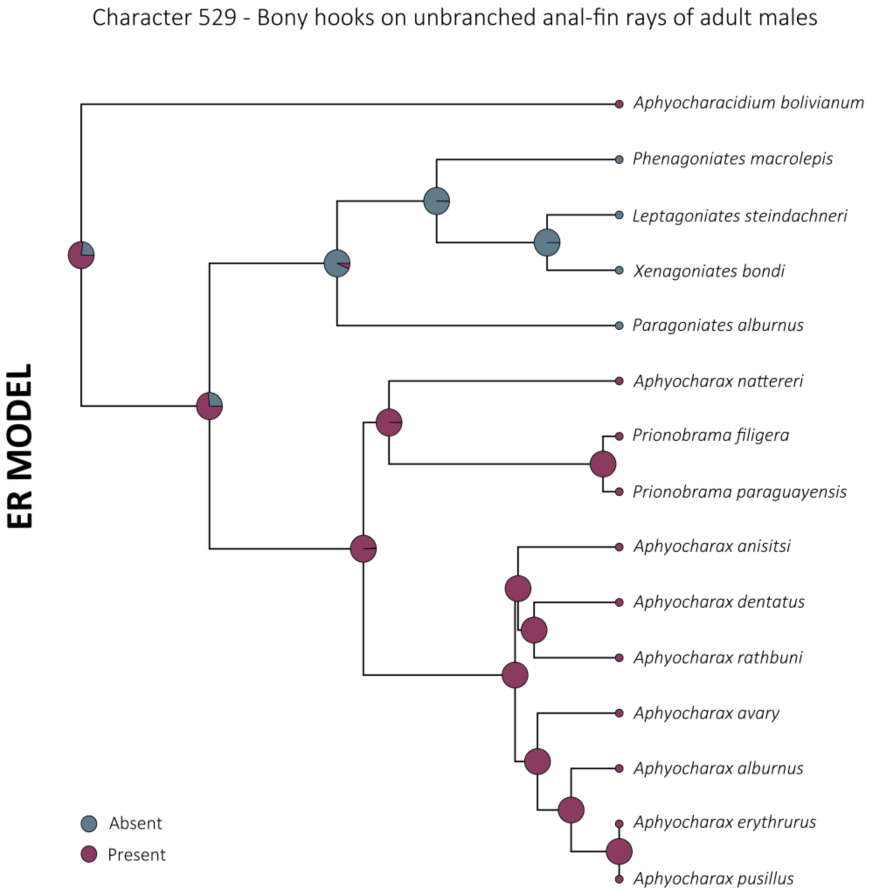
Character 529 - Bony hooks on unbranched anal-fin rays of adult males

Figure 36 – Ancestral state reconstruction of bony hooks on unbranched anal-fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under "ER" model.

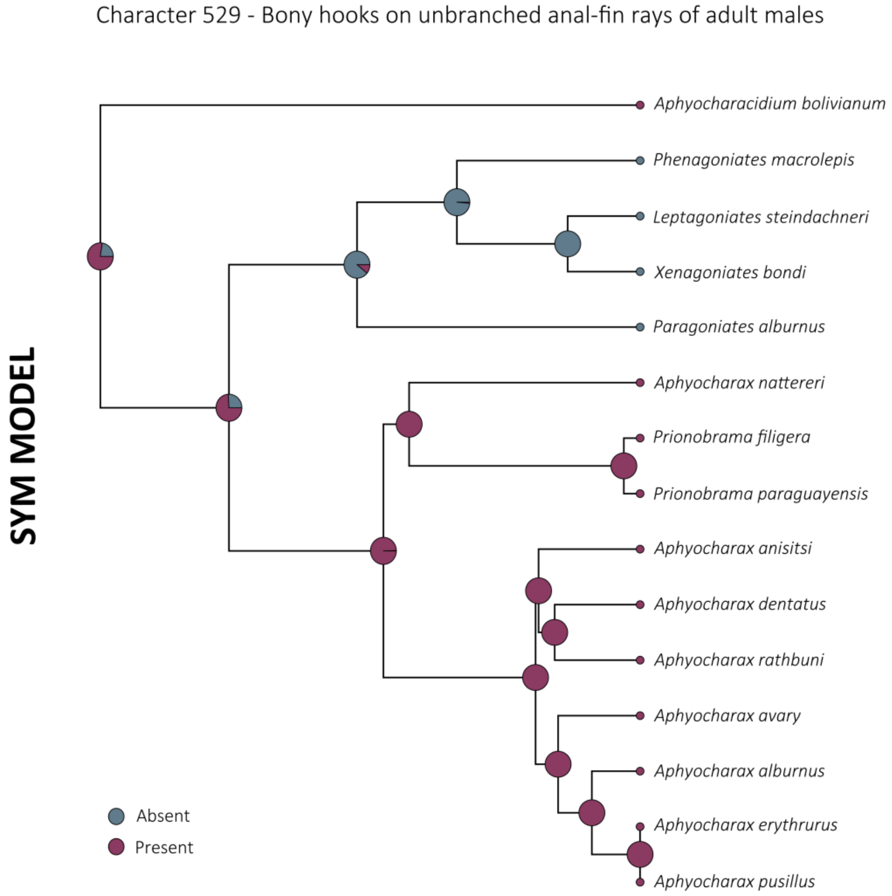

Figure 37 – Ancestral state reconstruction of bony hooks on unbranched anal-fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under "SYM" model.

Distribution of bony hooks on branched anal-fin rays of adult males (ch.530)

Transition rate between the states was estimated on 226.7617. Changes over trees between states from "first to tenth" to "first to eleventh" (68.807), "absent" to "first to tenth" (68.793), "first to eleventh" to "first to tenth" (68.599), "absent" to "first to eleventh" (68.545), "first to eleventh" to "absent" (68.343) and "first to tenth" to "absent" (68.244) were observed at similar patterns

Ambiguous probabilities were recovered on most of Aphyocharacinae's ancestral nodes (Supporting Information 4), where the diversification of this trait within this subfamily could not be inferred and needs further investigation. However, a transition to the presence of bony hooks from the first to the eleventh branched anal-fin rays ("first to eleventh" state) was retrieved at node 22 with a strong support (Figure 38).

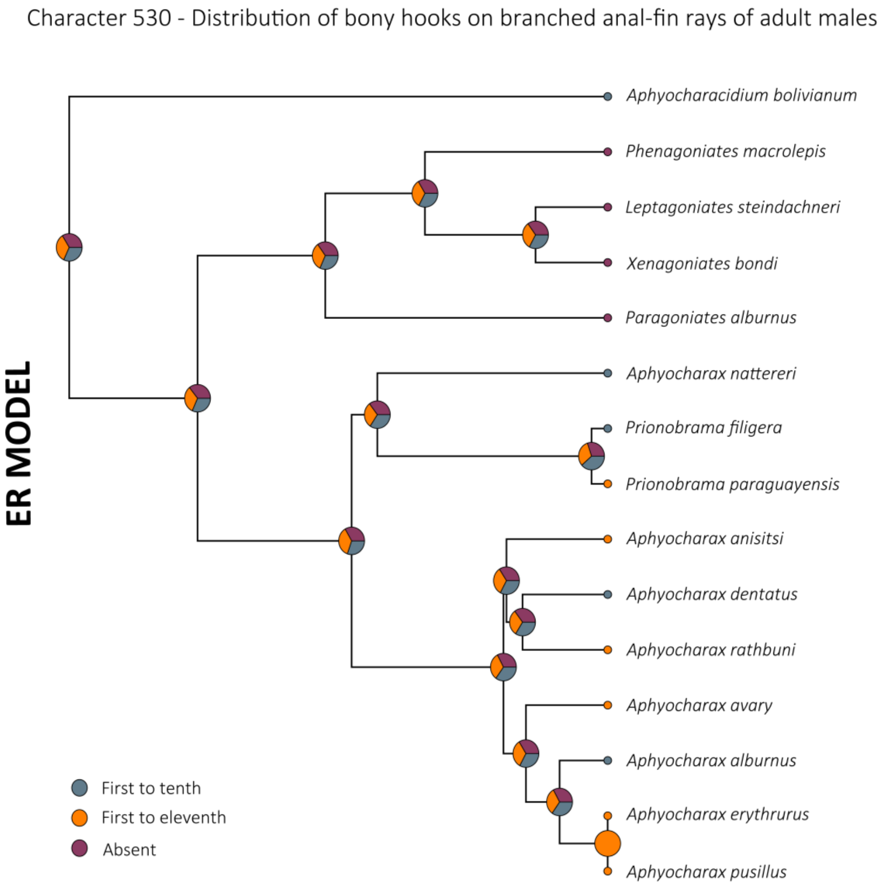

Figure 38 – Ancestral state reconstruction on the distribution of bony hooks on branched anal-fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under "ER" model.

Distribution of bony hooks along anal-fin rays of adult males (ch.531)

Transition rate between states was estimated on 1.416272. Average changes between states from trees revealed more variations from "restricted to middle" to "proximal to middle" (1.041) when compared to transformations from "proximal to middle" to "restricted to middle" (0.129). Accumulated changes from "restricted to middle" to "absent" (0.557), "absent" to "restricted to middle" (0.514), "restricted to middle" to "middle to distal" (0.369) and "absent" to "middle to distal" (0.365) were recovered following similar patterns of transition. Changes occurring from "middle to distal" to "absent" (0.231), "middle to distal" to "restricted to middle" (0.22), "proximal to middle" to "middle to distal" (0.167), "proximal to middle" to "absent" (0.123), "absent" to "proximal to middle" (0.113) and "middle to distal” to “proximal to middle” (0.056) were less observed in relation to other transformations.

The distribution of bony hooks along anal-fin rays of adult males revealed a structured diversification across evolutionary history. The “restricted to middle” state was indicated as the likely ancestral condition with moderate probabilities at common ancestors of the Aphyocharacinae (nodes 16 and 17, Supporting Information 4), and were recovered with strong support at most descending nodes (PP= >94%, Figure 39). The absence of this trait was retrieved as the most probable condition at ancestor nodes of *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Leptagoniates steindachneri* and *Xenagoniates bondi* with no reversals (nodes 27–29, Figure 39).

Trends of character diversification within this subfamily have rendered lower probabilities on the occurrence of the states “proximal to middle” and “middle to distal” at most ancestor nodes. A single transformation to the “proximal to middle” state was observed in *Aphyocharax rathbuni*, and to the “middle to distal” condition in *Aphyocharacidium bolivianum* (Fig. 39).

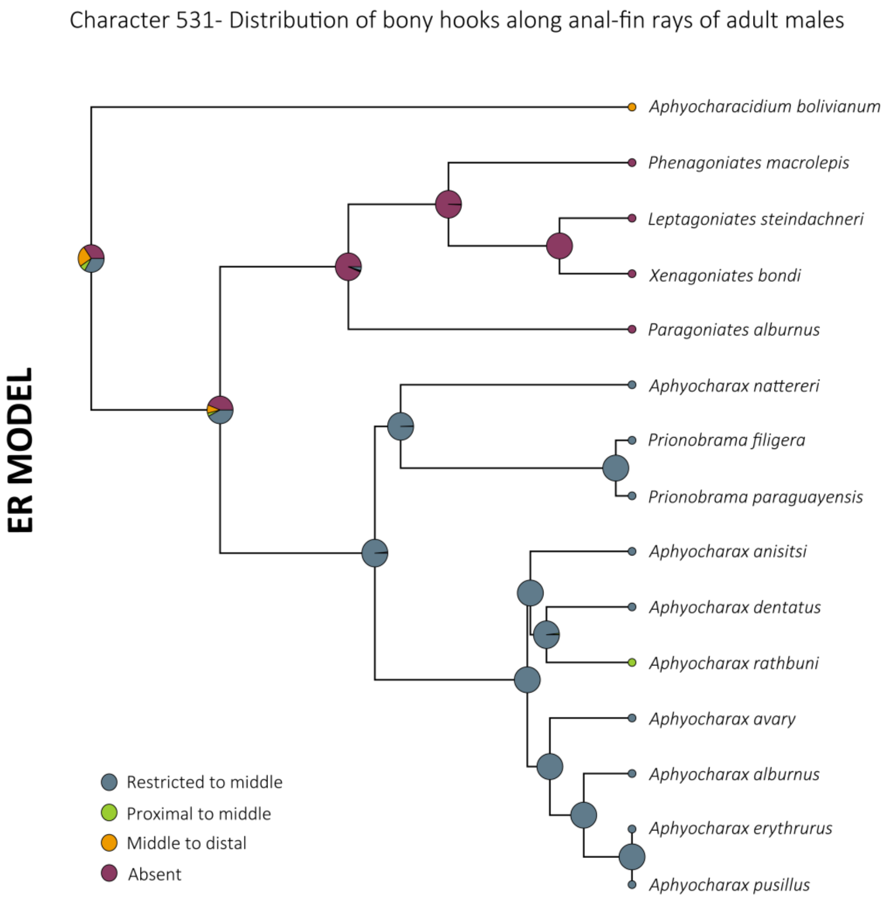

Figure 39 – Ancestral state reconstruction on the distribution of bony hooks along anal-fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under "ER" model.

Position of insertion of bony hooks on anal-fin rays of adult males (ch.533)

Transition rate between states was estimated on 3.413500. Changes occurring from "posterolateral" to "both" were higher (2.293) than from "both" to "posterolateral" (0.709) based on average changes between states over sampled trees. Other state changes were related from "posterolateral" to "absent" (0.843), "absent" to "posterolateral" (0.833), "absent" to "both" (0.81) and "both" to "absent" (0.62).

Ambiguous probabilities on all states were recovered at nodes 16 and 17, where the position of insertion of bony hooks on anal-fin rays on most common ancestors of Aphyocharacinae members remains uncertain. However, the presence of an attachment of bony hooks to the posterolateral margin of anal-fin rays returned as the most likely ancestral condition at the common ancestors of *Aphyocharax* and *Prionobrama* species, showing a consistent pattern of evolution across Aphyocharacinae's diversification (Supporting

Information 4). A reversal to the attachment of bony hooks on both anal-fin ray borders (anterolateral and posterolateral) was estimated at node 26 and its descendant lineages (Figure 40).

Node 27 was recovered at an “absent” condition (Fig. 40), where this transition was estimated to have occurred one time within Aphyocharacinae, with no reversals.

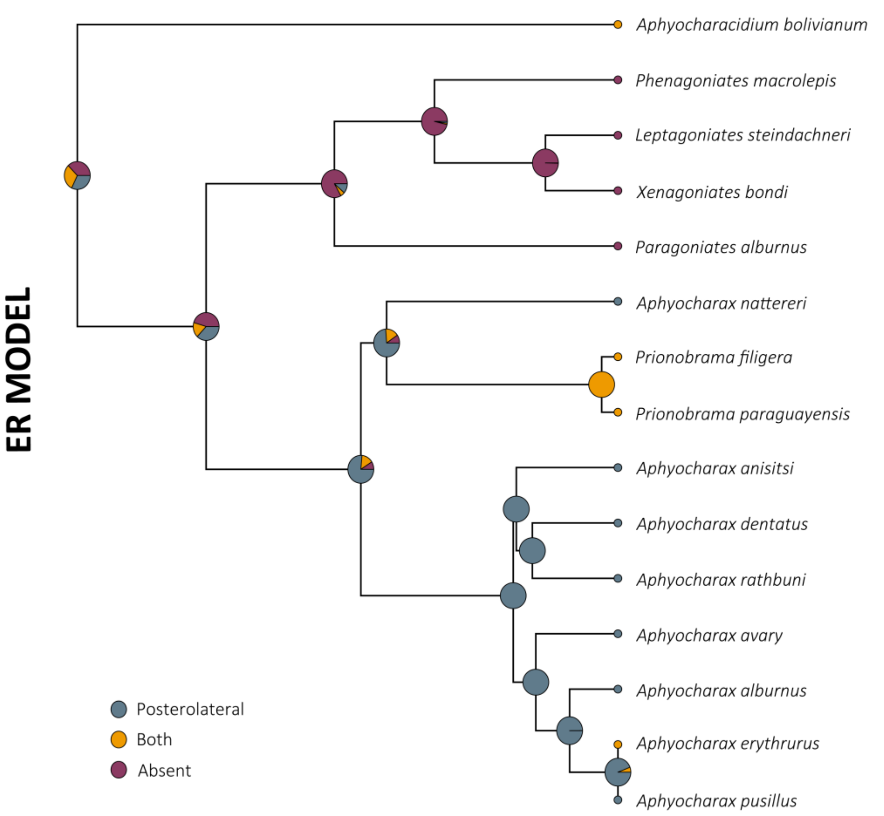
Character 533 - Distribution of bony hooks on branched anal-fin rays of adult males

Figure 40 – Ancestral state reconstruction on the distribution of bony hooks on branched anal-fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under “ER” model.

Width of bony hook at base on anal-fin rays of adult males (ch.534)

Transition rate between the states was estimated on 2.916658. Average changes between states were observed occurring from “wider” to “same width” (1.068) and “wider” to “absent” (0.871) at higher proportions, whereas fewer changes were detected from “absent” to "wider" (0.596), "same width" to "wider" (0.306), "same width" to "absent" (0.118) and "absent" to "same width" (0.102).

The "wider" state was estimated as the most probable condition at the common ancestor of *Aphyocharax* and *Prionobrama* species and descending lineages with strong support (PP= >90%). The presence of anal-fin bony hooks displaying a wider base was recovered at the root (node 16) and node 17 with moderate probabilities (Supporting Information 4). A single transition to the "same width" condition was observed in *Prionobrama paraguayensis*, and the occurrence of anal-fin bony hooks at this state had returned lower probabilities on most ancestor nodes (Supporting Information 4).

Within Aphyocharacinae, the absence of this character was likely to have transitioned one time with no reversals (node 27, Figure 41). Still, moderate probabilities at the "absent" state were recovered at most common ancestors of this subfamily (node 16 and 17, Supporting Information 4).

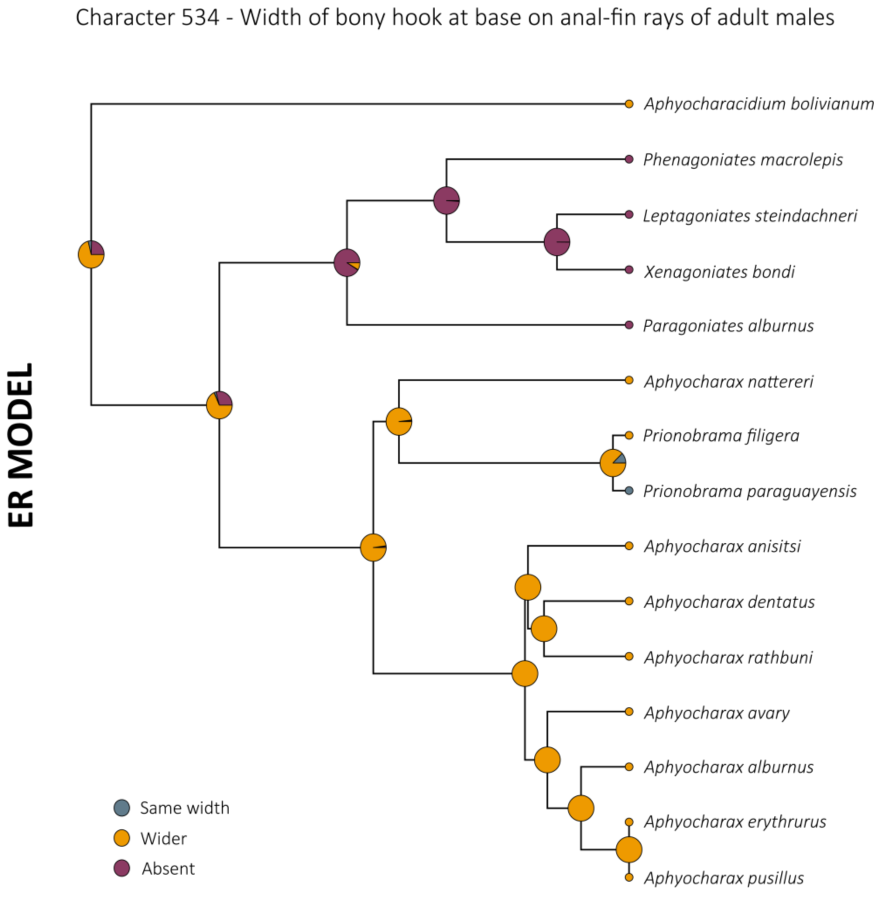

Figure 41 – Ancestral state reconstruction of the width of bony hook at base on anal-fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under "ER" model.

Distal tip of bony hooks on anal-fin rays of adult males (ch.535)

Transition rate between the states was estimated on 3.071134. Accumulated changes over trees were detected at distinct patterns, where transitions between states “retrorse” to “straight” (2.198) were more observed. Transformations from “retrorse” to “absent” (1.076), “absent” to “retrorse” (0.973) and “straight” to “retrorse” (0.767) were similarly estimated, while fewer changes were monitored from “straight” to “absent” (0.355) and “absent” to “straight” (0.303).

Moderate probabilities on “retrorse” state were recovered at nodes 16 and 17, although slightly lower estimations at the “absent” state were still considered as the ancestral condition (Supporting Information 4). The absence of this trait was estimated as the most likely state at the ancestors of *Paragoniates alburnus*, *Phenagoniates macrolepis*, *Leptagoniates steindachneri* and *Xenagoniates bondi* (nodes 27–29, Figure 42) with no reversals.

The “retrorse” state was observed on most Aphyocharacinae members, and was retrieved with strong support as the probable ancestral condition at node 18 and most of its descending lineages. The presence of anal-fin bony hooks with a straight tip rendered lower probabilities on most ancestor nodes among aphyocharacins (Supporting Information 4). Across Aphyocharacinae’s diversification the “straight” condition was recovered to have occurred two times, once in *Prionobrama filigera* and another in *Aphyocharax dentatus* (Fig. 42).

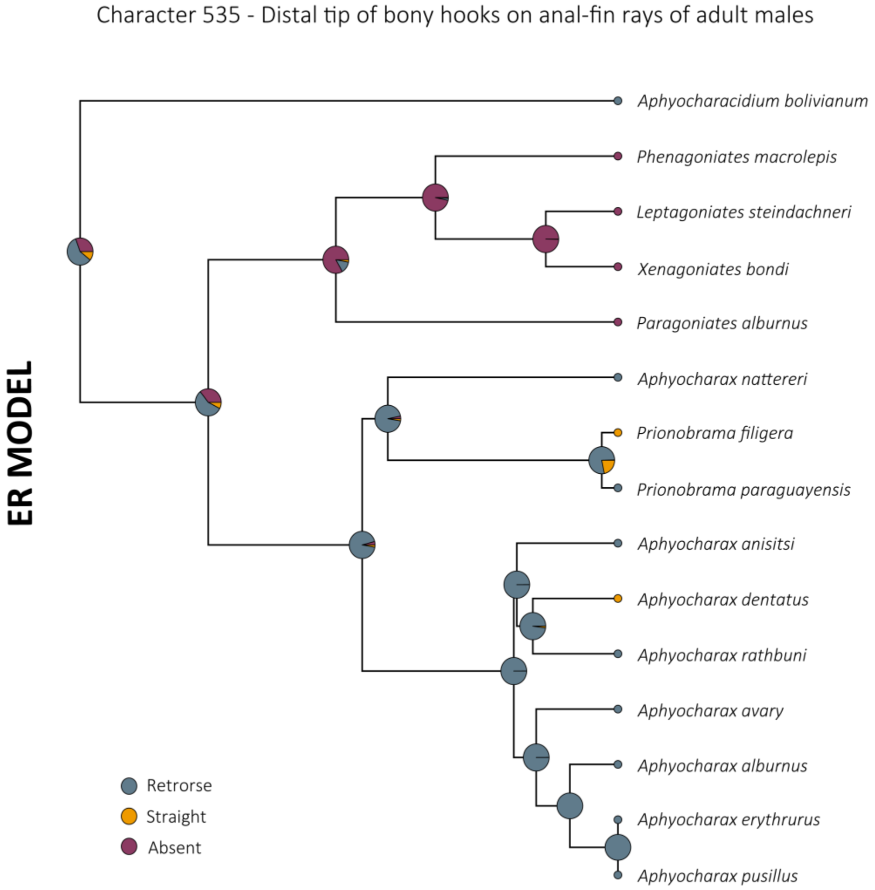

Figure 42 – Ancestral state reconstruction of the distal tip of bony hooks on anal-fin rays in Aphyocharacinae from 1,000 simulations with stochastic mapping under "ER" model.

Number of bony hooks per anal-fin ray segment on adult males (ch.536)

Transition rate between states was estimated on 2.180444. Average changes over sampled trees returned at different proportions, where conversions from the state "two" to "one" (12.421) and "one" to "two" (11.473) were higher when compared to other changes. Transformations from "one" to "three" (1.906) and "three" to "two" (1.108) were recovered following similar patterns of transition. Changes occurring from "four" to "one" (0.226), "four" to "two" (0.385) and "four" to "three" (0.097) were less observed in relation to other transformations. According to best fitted model of evolution, remaining changes were not permitted.

Across Aphyocharacinae's evolutionary process, the state "two" was recovered with good probabilities as the likely ancestral condition at most nodes (Supporting Information 4). Ambiguous ratios on the occurrence of the states "two" and "four" were recovered at the common ancestor of most aphyocharacins (node 16, Supporting Information 4). Within this subfamily, the presence of "four" bony hooks on anal-fin ray segments were only observed in *Axelrodia lindeae* and *Aphyocharacidum* n. sp. (not included in ACSR analyses).

Moderate probabilities point to the presence of "one" bony hook attached to anal-fin ray segments among the Aphyocharacinae, with lower probabilities at node 27 and descendant lineages. Reversals to "one" hook per anal-fin ray segment were observed in *Aphyocharax alburnus* and *Ap. anisitsi*. A transition to having up to three bony hooks on each anal-fin ray segment was recovered at the common ancestral of *Prionobrama* species, and in *Aphyocharacidium bolivianum* (Figure 43).

The number of bony hooks on anal-fin rays in adult males was estimated to have transitioned to the "absent" condition one time during Aphyocharacinae's diversification with no reversals (node 27, Fig. 43).

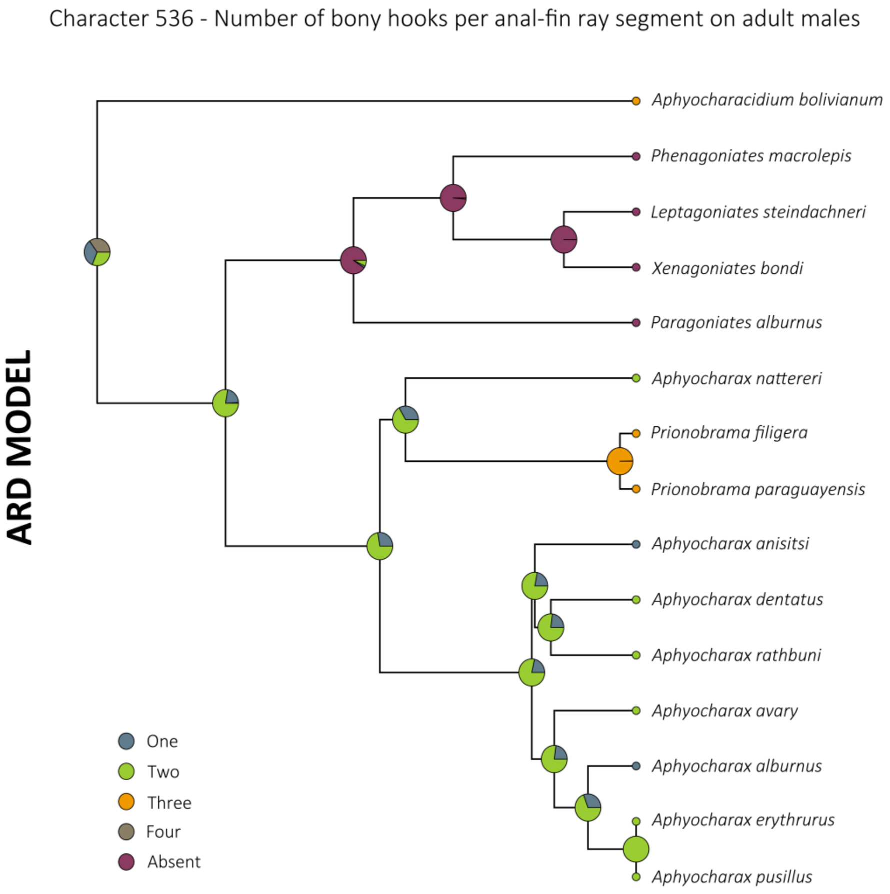

Figure 43 – Ancestral state reconstruction on the number of bony hooks per anal-fin ray segment in Aphyocharacinae from 1,000 simulations with stochastic mapping under "ARD" model.

DISCUSSION

The evolutionary histories of characters on distinct traits of the sexual system, mostly concerning bony hooks on pelvic and anal-fin rays first proposed herein, is the demonstrade with the most complete sampling of Aphyocharacinae to date. Previously considered by Weitzman & Fink (1985), we have analyzed the evolution of secondary sexual characters at the great variation within these features through independant characters and modern comparative methods across the subfamily Aphyocharacinae.

As main results we find that the evolution of these characters are mostly consistent with the phylogenetic history of these taxa, i.e., sexually dimorphic features may recover hypothetical lineages based on other data (morphological or molecular). These main findings, listed below, will be discussed herein:

1 – Most Aphyocharacinae members have retained ancestral presence of bony hooks on pelvic and anal-fin rays in males. The lack of bony hooks on pelvic and anal-fin rays in males in *Leptagoniates*, *Paragoniates*, *Phenagoniates* and *Xenagoniates* represents an evolutionary novelty that arose once in the common ancestor of those genera and further supports the monophyly of this group (previously proposed as a separate subfamily, the Paragoniatinae).

2 – Besides the anal fin shape that is usually employed as a diagnostic character for the genus, several sexually dimorphic characters have evolved once in the common ancestor of the species of *Prionobrama*, and further support the monophyly and the diagnosis of the genus. These include characters that have been neglected in species descriptions, phylogenetic analysis or in taxonomic reviews: the presence of a similar number of bony hooks on hook-bearing pelvic-fin rays, the number of bony hooks per pelvic-fin ray segment, the distribution of bony hooks on branched anal-fin rays, and the number of bony hooks per anal-fin ray segment.

3 – Characters as simple as the distribution of bony hooks along pelvic-fin rays are congruent and recover relationships among species of *Aphyocharax*, and should be considered in phylogenetic analyses.

The occurrence of sexually dimorphic structures in Characidae is increasingly being explored by researchers when proposing new species, and these interesting features are being addressed in the context of taxonomic reviews and phylogenetic studies. Malabarba & Weitzman (2003) suggested the presence of bony hooks on fin rays as a derived condition synapomorphic to the Gasteropelecidae and most Characidae, including members of “Clade B”. Wiley & Colette (1970) referred to the presence of “bony fin-ray contact organs, or hooks” on males of many species, suggesting that it would be a way to keep close contact between male and female during the spawning act. As seen here, most members of Aphyocharacinae have retained ancestral the presence of bony hooks on pelvic and anal-fin rays in males, and the absence of these structures in *Leptagoniates*, *Phenagoniates*, *Paragoniates* and *Xenagoniates* represents an evolutionary novelty that arose once in the common ancestor of those genera.

Géry (1977) mentions a “so-called regressive evolution” to explain the loss of a trait from descendant lineages. The author remarked that this regression pattern is usually polyphyletic, probably occurring several times in distinct lineages of Characidae. Interestingly, the supposed absence of bony hooks on fin rays is not an exclusive attribute of this lineage in Aphyocharacinae. Within Characidae, the lack of bony hooks on fin rays was addressed for *incertae sedis* members, as well as in the subfamilies Stethaprioninae, Stevardiinae, Aphyoditeinae, Tetragonopterinae, Spintherobolinae, Exodontinae, and Characinae (Malabarba & Weitzman, 2003; Mirande, 2009, 2010, 2019).

Remarks on sexualyl dimorphic features are consistently absent in reference to *Leptagoniates steindachneri*, *Paragoniates alburnus*, *Phenagoniates macrolepis* and *Xenagoniates bondi* (Géry, 1977; Quevedo, 2006). We are in agreement that the external morphology provides no conspicuous characters that allow differentiation between adult males of females of these species. The absence of pelvic and anal-fin bony hooks was once recovered as a synapomorphy of a clade composed by *L. steindachneri*, *Phenagoniates wilsoni* (= *Ph. macrolepis*), *P. alburnus* and *X. bondi* (Quevedo, 2006). Even though the reconstruction of ancestral characters of bony hooks on pelvic and anal fins may show repetitive patterns for *Leptagoniates*, *Paragoniates*, *Phenagoniates* and *Xenagoniates* genera (node 27), the absence of those sexual traits in this clade highlights an interesting shift in the evolution of Aphyocharacinae. In remaining aphyocharacins, the observed diversity in bony hook shape, position, and distribution on pelvic and anal-fin frays can be strongly related to a successful radiation when considering the ecological trade-offs linked to particular reproductive tactics adopted by those species. Bony hooks on pelvic and anal-fin rays were consistently observed in adult males from members of *Aphyocharacidium*, *Aphyocharax*, *Prionobrama* plus *Axelrodia lindeae*, all of which are currently assigned to the subfamily Aphyocharacinae (*sensu* Mirande, 2019). Variation in the presence and distribution of these sexually dimorphic features are considered and discussed here in more detail.

Among the Aphyocharacinae, the presence of bony hooks on pelvic and anal-fin rays were feature mostly known in the sexually dimorphic males of species of *Aphyocharax*. Malabarba & Weitzman (2003) described the presence of these sexually dimorphic structures as unknown in the following genera of Aphyocharacinae: *Aphyocharacidium*, *Leptagoniates*, *Paragoniates*, *Phenagoniates* and *Xenagoniates,* including *Axelrodia*. The presence of bony hooks on pelvic and anal-fin rays in *Aphyocharacidium bolivianum* and *Axelrodia lindeae* was included by Mirande (2009) without comment. These structures are considered herein not only to attest to the presence of these structures, but to describe in greater detail the occurrence of secondary sexual characters in species of *Aphyocharacidium*, and *Ax. lindeae*.

Within the members of Aphyocharacinae examined by Quevedo (2006), bony hooks were observed on both anal and pelvic-fin rays in males *Prionobrama* species (char. 70:1). These findings are not entirely corroborated by our data. With the exception of members of “clade 27”, the presence of bony hooks on pelvic-fin rays (char. 442:1) were found in all examined members of Aphyocharacinae herein. The distribution of bony hooks occurring from the last unbranched ray, varying in distribution along branched pelvic-fin (char. 75:0) and anal-fin rays (char. 72:0) was reported in *Ap. anisitsi*, *Ap. nattereri*, *Ap. pusillus* and *Prionobrama* species (Quevedo, 2006). Our data support that observation, reinforcing that even when variable, characterization on the range of distribution of bony hooks on fin rays (char. 521; char. 530) are an incredibly resource of information for the identification of species of Aphyocharacinae.

Quevedo (2006) reported 1–2 bony hooks per segment for pelvic-fin rays (char. 74:0), and 2–5 hooks per segment for anal-fin rays (char. 71:1) in species of *Prionobrama*. The counts in Quevedo (2006) do not exactly match our observations. Amongst the Aphyocharacinae, adult males were observed exhibiting a maximum of 3 bony hooks per segment of pelvic-fin ray (char. 527), and a maximum of 3 or 4 bony hooks per segment of anal-fin ray (char. 536). The higher number of bony hooks per ray segment noted by Quevedo (2006) were observed on pelvic-fin rays of *Ap. pusillus* and *Prionobrama* species (char. 527:2), and on anal-fin rays in *A. bolivianum*, *Pr. filigera*, *Pr. paraguayensis* (char. 536:2), *Aphyocharacidium* n. sp. and *Ax. lindeae* (char. 536:3), where the most anterior anal-fin rays showed higher counts of hooks gradually reducing in number on posterior rays. The occurrence of 1–4 bony hooks per segment attached to the posterolateral border of anal-fin rays detected on adult males of *Aphyocharacidium* n. sp., *A. bolivianum*, *Ax. lindeae*, *Pr. filigera* and *Pr. paraguayensis* were similarly reported by Malabarba (1998) on Clade B Cheirodontini's (ch. 22). As previously mentioned, reproductive timing is intrinsically related to secondary sexual character development, and we believe that maturation phase of males could be a factor explaining the variation in count range of bony hooks on fins rays.

Given the absence of *Aphyocharacidium* n. sp. and *Ax. lindeae* on the herein proposed molecular hypothesis, ancestral character states reconstructions regarding sexually dimorphic structures observed among these species could not be analyzed. The most anterior hook-bearing branched rays of the anal-fin more expanded in the sagittal plane in raelation to posterior rays were only observed on adult males of *Aphyocharacidium* species plus *Ax. lindeae*. Moreover, dimorphic males displayed a ventral curvature of the caudal peduncle in *Ax. lindeae*, and a conspicuous hypertrofiation of the most anterior elements of the ventral procurrent caudal fin ray series in *Aphyocracidium* n. sp. and *Ax. lindeae*. These features were observed as exclusive to the aforementioned taxa when compared to remaining Aphyocharacinae, where similar patterns on the morphology and development of these structures were reported by Malabarba (1998) when examining the Cheirodontinae.

Unpaired and asymmetrical distributions of the anal-fin bony hooks was the observed condition found in mature males of *Ap. anisitsi*, *Ap. nattereri*, *Ap. pusillus* and *Prionobrama* species (char. 73:1; Quevedo, 2006). We detected a similar pattern, where dimorphic males exhibited an unpaired asymmetrical arrangement of bony hooks between contralateral segments of lepidotrichia on both pelvic fin and anal fin of *Pr. filigera*, and on anal fin of *Pr. paraguayensis* (char. 450:1 ; char. 451:2). However, pelvic-fin hooks in *Pr. paraguayensis* were observed on a paired symmetrical arrangement between hemitrichia of rays (char. 450:0). Among aphyocharacins, a paired and symmetric arragenment of bony hooks on pelvic-fin rays was also detected on adult males in *A. bolivianum*, *Ax. lindeae*, *Ap. anisitsi*, *Ap. colifax*, *Ap. dentatus*, *Ap. erythrurus*, *Ap. nattereri*, *Ap. pusillus*, *Ap. rathbuni* and *Ap. yekwanae*.

Descriptions and subsequent studies on the relationships of *Prionobrama* within Characidae, refer to the conspicuous elongation of the most anterior rays of the anal fin as characterizing species in this genus is the (Cope, 1870; Fowler, 1913; Eigenmann, 1915). The presence of these "filamentous rays" were ascribed as a feature of males (Géry, 1977), where later Quevedo (2006) reported slightly different results from *Prionobrama* species. Quevedo (ibid.) observed the elongation of the most anterior anal-fin rays in *Aphyocharax anisitsi* and *Prionobrama* species. Within Characidae, the presence of an elongation of the last unbranched ray, and first to second branched rays on anal fin was also observed on males of *Nanocheirodon* by Malabarba (char. 20: 1998). Although usually considered as a sexually dimorphic structures, Quevedo (2006) concluded that elongated anal-fin rays were present in both adult male and female specimens, and as such that terminology could not be correctly applied to *Prionobrama* (char. 63:1). Our findings support the same conclusion, and are considered here as secondary sexual characters related to sexual maturation in both sexes. Further analyses should be carried out regarding this particular character, correlating the development of these structures to the reproductive period and gonadosomatic index (GSI). No further comments were made regarding the occurrence of elongated anal-fin rays in *Ap. anisitsi* (it is present in both males and females?). These structures were not observed within our examined material and should be investigated further.

Our analyses showed that traits concerning the presence of a similar number of bony hooks on last pelvic fin rays of adult males (char. 447) and elongated anal-fin rays in adult specimens (char. 528) appear to have evolved independently in the ancestor of *Prionobrama* within Aphyocharacinae (Figs. 21–22, 34–35).

Aphyocharax colifax (Taphorn & Thomerson, 1986) was described as having bony hooks on the first pelvic-fin rays, and on almost all branched rays of anal fin. Within our examined material, we were able to observe a broader distribution of bony hooks on pelvic-fin rays, and relatively shorter range on anal-fin rays of adult males. In agreement with Géry’s (1977) hypotheses on possible relationships among *Aphyocharax* species, Taphorn & Thomerson (1986) included remarks on the informative potential of bony hooks, where based on their distinctive distributions of anal-fin hooks and color pattern *Ap. colifax* could be easily differentiated from *Ap. alburnus*. Variation in the distribution of bony hooks on rays within a single species are commonly observed, and should be carefully assessed for taxonomic identities. When dealing with very similar species, as useful as the distribution of bony hooks on anal-fin rays are for identification, the characters proposed herein are also a good source of information. In this particular case, by analyzing the anal fin we could identify dimorphic males of *Ap. colifax* by the presence of first bony hooks attached to the segment before the primary branching of rays (char. 532), occurrence of hooks on secondary branches of rays (char. 453) and the attachment of hooks on posterolateral and anterolateral borders of rays (char. 533) when compared to adult males of *Ap. alburnus*. In spite of the absence of *Ap.*

colifax in our molecular hypothesis, a close relationship between these species was herein recovered through morphological evidence for the first time (see Results), suggesting *Ap. alburnus* and *Ap. colifax* as sister taxa. Testing this hypothesis with DNA sequence data will be important in future studies.

Multiple nodes of reproduction exist across the most diverse radiation of fishes (Breder & Rosen, 1966) and sexual dimorphism comes in many forms such as size, color pattern, modified structures, nest building, among others. Winemiller (1989) proposed a characterization of reproductive patterns based on life history attributes. In that proposal most species of Characidae were associated with seasonal reproduction. The “seasonal strategy” seems to ensure the exploitation of temporal and spatial use of habitats for enhanced survival and development of juveniles, characterized by distinctly periodic reproduction events during the year (sometimes once), long generation times coinciding with reproductive cycles, large clutches and very little or no parental care (Winemiller, 1989; Taphorn, 1992). The mechanisms of seasonal and opportunistic strategies in fishes, largely associated to Characiformes members, are closely related to Pianka’s (1970) theory on “r-selected” organisms (Winemiller, 1989). Among the Aphyocharacinae, studies on the reproductive patterns of *Ap. alburnus*, *Ap. anistsi*, *Ap. dentatus* and *Paragoniates alburnus* showed these species as seasonal strategists (see Winemiller, 1989; Taphorn, 1992; Vazzoler & Menezes, 1992; Vazzoler, 1996; Gonçlaves *et al.*, 2005; Azevedo, 2010).

Cues on the development of bony hooks on fin rays, although not fully understood, have been related to the onset of sexual maturity in many species of Characidae. The absence or a reduced distribution of hooks on fin rays was correlated with young males captured on specific months of the year (Gonçalves *et al.*, 2005; Vieira *et al.*, 2016; Longoni *et al.*, 2018) and translates into strong evidence for the influence of the tempo of reproduction concerning the presence, variation in distrituion on fin rays and development of secondary sexual characters.

During maturation, the development of secondary sexual characters must be closely associated with metabolic demands of energy (see Makiguchi *et al.*, 2017) and, such allocation of investment would likely imply a cost in terms of natural selection (Meyer *et al.*, 1994). Given the selective pressures, the evidence for evolutionary novelty by convergence between members across different taxa is bound to chance (Tinkle *et al.*, 1970; Stearns, 1983; Lynch & Wagner, 2010). That noted, there is still a long way towards understanding which traits incur adaptative advantages as key variables during the evolutionary process (Winemiller, 1989).

Seasonal variation of bony hooks in taxa remains part of an ongoing discussion. The presence of bony hooks on fin rays are usually assigned to adult males of Characidae, and their development often related to the reproductive season (von Ihering & Azevedo, 1936; Collette, 1977; Garutti, 1990; Dala-Corte, 2014). At the same time, and with increased observation, these structures are consisistenly found in adult specimens (Andrade *et al*., 1984; Silva *et al.*, 1996; Azevedo *et al.*, 2000; Lampert, 2004, 2007; Gonçalves *et al.*, 2005; Longoni *et al.*, 2018). Many of these studies attributed the development of bony hooks as a permanent trait, and that once developed by the male the hooks are retained on the anal-fin rays. From dimorphic males within the Aphyocharacinae analyzed herein, no pattern on seasonal variation of the secondary sexual characters could be found.

The development of secondary sexual characters, such as fin hooks, are related to steroid hormones that are synthesized and released into the bloodstream triggering endocrine effects (Pankhurst, 2008; Planas & Swanson, 2008). Another common sexually dimorphic feature in Characidae is the modification of the anterior-most gill filaments, where developing a gill-derived gland on the first gill arch found in mature males. These sexually modified structures are hypothesized to be linked in chemical signaling during courtship and/or aggressive territorial behavior (Bushmann *et al*., 2002). Gill-derived glands are reported in many genera within Characidae (*e.g.* Oliveira *et al*., 2012; Bushmann *et al*., 2002; Weitzman *et al*., 2005; Téran *et al.*, 2015). In members of Aphyocharacinae, the gill-derived gland has been reported for mature males of *Ap. anisitsi*, althought the occurrence of the gland was not consistent (Gonçalves *et al.*, 2005). Based on the examination of their external morphology, gill-derived glands were not observed within the Aphyocharacinae and should be further explored. However, it is our opinion that evidence on gill-derived glands should be carefully examined through histological and ultrastructure techniques in order to properly characterize the presence and development of these fine structures in Characidae species (see Oliveira *et al.*, 2012; Téran *et al.*, 2015).

Taphorn (1992) sees the specialized life history strategies adopted by characiforms as ecological trade-offs, pulling species in different “directions”. The intrinsic relationship of life history traits and the absence/presence of secondary sexual characters with evolutionary process in Aphyocharacinae could be such that these adaptations are a product of selective pressures resulting in phylogenetic diversification.

While the origin, development, and function of bony hooks on fin rays remains under investigation, proposing new suites of characters based on studies of behavioral, reproductive, physiological (and other) life-history aspects would contribute to our understanding of this group and would help answer many of these questions, thereby paving the way for future studies and new testable hypotheses of relationships, and character states within species of Characidae.

Variation on the morphology of the IO4 in Aphyocharacinae was already observed in recent taxonomic reviews (Souza-Lima, 2004; Quevedo, 2006) and phylogenetic studies (Mirande, 2009, 2010, 2019; Tagliacollo *et al.*, 2012). The range of phenotypic variation for this character within this subfamily was detected as absent or in three different shapes when present. Species in *Leptagoniates, Paragoniates, Phenagoniates* and *Xenagoniates* exhibited a well-developed approximately square IO4 (char. 92:0), wheareas in *A. bolivianum*, *Aphyocoharacidium* n. sp. and *Axelrodia lindeae* the form of the IO4 is longer dorsoventrally (char. 92:1). In the aforementioned groups, the morphologically distinct forms of the IO4 bone were observed meeting the margins of IO3 and IO5 (char. 91:0). Species of *Aphyocharax* and *Prionobrama* showed a trigular shaped IO4 or the absence of the bone (char. 92:2), that when present the reduced form of the bone is limted anteriorly and does not meet the posterior margins of IO3 and IO5 (char. 91:1). The similatrity over the infraorbital series (and overall morphology) was earliear suggested by Eigenmann (1915) as evidence of a close relationship between these genera. Ontogenetic approaches to phenotypic variation and the polymorphic condition concerning the absence/presence of the IO4, observed here in *Ap. pusillus* and *Pr. paraguayensis*, could yield novel information on their development and variation within Aphyocharacinae.

By recovering a close relationship between Aphyocharacinae and most ‘Paragotiniatinae’ members, Quevedo (2006) considered that the taxa might be invalid, discarding Géry’s (1977) hypothesis. A clade comprised by *Ap. nattereri*, *Ap. anisitsi*, *Ap. pusillus*, *P. alburnus*, *Phenagoniates wilsoni* Eigenmann, 1914 (= *Ph. macrolepis*), *L. steindachner*i, *Pr. filigera*, *Pr. paraguayensis* and *X. bondi*. The monophyly of *L. steindachneri*, *P. alburnus*, *Ph. wilsoni* (= *Ph. macrolepis*), *Pr. filigera*, *Pr. paraguayensis* and *X. bondi* recognized by Quevedo (2006) within Aphyocharacinae was not corroborated in our analyses. Instead, molecular and morphological evidence were congruent by recovering two monophyletic clades, where one clade is composed by *Prionobrama* species close related to *Aphyocharax* genus, and the other comprising the genera *Leptagoniates*, *Paragoniates*, *Phenagoniates* and *Xenagoniates*.

Knowledge of the phylogenetic placement and data concerning members of Aphyocharacinae has been gradually increasing as new phylogenies are proposed in members of Characidae and as more taxa are examined (Mirande, 2009, 2010, 2019; Javonillo *et al.*, 2010; Oliveira *et al.*, 2011; Tagliacollo *et al.*, 2012). Most recent hypotheses concerning subfamily relationships of Aphyocharacinae based on a total evidence approach (Tagliacollo *et al.*, 2012; Mirande, 2019) are mostly in agreement with our molecular hypothesis. Diverging results envolve the non-monophyly of *Aphyocharax*, where *Ap. nattereri* was herein recovered as sister taxa to *Prionobrama* species.

The internal relationships of Aphyocharacinae based on our molecular and morphological evidence have, however, revealed considerable incongruences between hypotheses of relationships with Cheirodontinae (see Tagliacollo *et al.*, 2012; Mirande, 2019). The phylogenetic relationships among currently assigned Aphyocharacinae members are complex and should remain under investigation. Further efforts on phylogenetic studies should absolutely include morphological information; especially given the phenotypic variation within species of Aphyocharacinae and the broad Neotropical distribution of this subfamily. Inclusion of more diversity of taxa in Aphyocharacinae together with the herein proposed characters and new sources of information, additional phylogenetic studies are encouraged in order to elucidate at finer scales the complex and intriguing relationships within this subfamily.

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LISTA DE ANEXOS

ANEXO 1: Supporting Information 1 – Collection vouchers and associated information of material used for molecular dataset of 58 sequences from five genes of 34 Characidae taxa.

ANEXO 2: Supporting Information 2 – List of examined material of the subfamily Aphyocharacinae (Characiformes: Characidae).

ANEXO 3: Supporting Information 3 – Morphological data based on an extended matrix of Mirande (2019). Matrix based on 543 characters for 54 species of the "Clade B" plus Spintherobolinae of the family Characidae. Coding as follows: Polymorphic characters "(01) or (012)" ; Unknown characters "?" ; Non-applicable "-".

ANEXO 4: Supporting Information 4 – Posterior probabilities of marginal ancestral state estimation from 1,000 simulations under stochastic mapping with "make.simmap" function (phytools – Revell, 2012). Discrete character models of evolution: "ER"– equal rates; "ARD" – all-rates-different; "SYM" – symmetric.

ANEXO 5: Supporting Information 5 – Morphometric data from examined material of the subfamily Aphyocharacinae (Characiformes: Characidae). Body measurements are percentages of Standard length; Subunits of the head are percentages of Head length.

ANEXO 6: Morphology of Aphyocharacinae: comparative table of overall counts.

ANEXO 7: Secondary sexual characters in Aphyocharacinae: comparative table of overall morphology and ditribution.

ANEXO 1: Supporting Information 1 – Collection vouchers and associated information of material used for molecular dataset of 58 sequences from five genes of 34 Characidae taxa.

Table 5 – List of species and locality information. Collection vouchers of members of the "Clade B" plus Spintherobolinae of the family Characidae.

Subfamily	**Species**	**Voucher**	**Locality**	**Coordinates**
Aphyocharacinae	*Aphyocharacidium bolivianum*	LBP 9055	Arara. Roraima, Brasil.	S 09°36'39.5" W 64°55'38.9"
	Aphyocharax alburnus	LBP 2293	Rio Orinoco. Caicara del Orinoco, Bolivar, Venezuela.	N 07°39'06" W 66°10'34"
	Aphyocharax alburnus	CUMV 82626	Rio Las Marias. Portuguesa, Venezuela.	N 9°11'60" W 69°42'0"
	Aphyocharax alburnus	CUMV 82627	Rio Portuguesa. Portuguesa, Venezuela.	N 8°55'59.88" W 69°5'60"
	Aphyocharax anisitsi	LBP 3764	Rio Negro. Aquidauana Mato Grosso do Sul, Brasil.	S 19°34'33.7" W 56°14'49.5"
	Aphyocharax anisitsi	LBP 4750	Rio Guaíba. Barra do Ribeiro, Rio Grande do Sul, Brasil.	S 30°17'07.0" W 51°18'01.1"
	Aphyocharax avary	ROM 107212	Rio Orteguaza. Colombia.	N 1°31'9.552" W 75°32'19.104"
	Aphyocharax dentatus	LBP 1587	Rio das Garças. Barra do Garça, Mato Grosso, Brasil.	S 15°54'18.1" W 2°19'24.2"
	Aphyocharax dentatus	LBP 5112	Lagoa Bairro caiçara. Rio Paraguai. Cáceres, Mato Grosso, Brasil.	S 16°06'66" W 57°44'33"
	Aphyocharax dentatus	LBP 20	Rio Miranda. Rio Paraguai. Corumbá, Mato Grosso, Brasil.	S 19°34' W 57°01'
	Aphyocharax dentatus	LBP 2480	Rio Araguaia. Aragarças, Goiás, Brasil.	S 15°53'35" W 52°15'00"

Aphyocharacinae	*Aphyocharax erythrurus*	ROM 88558	East Berbice. Guyana.	N 5°0'28.476" W 58°14'46.932"
	Aphyocharax erythrurus	ROM 96222	Rapids at shelf of rock, large wide river. Takutu River, Upper Takutu-Upper Essequibo. Guyana.	N 2°50'9.480" W 59°59'25.560"
	Aphyocharax erythrurus	ROM 95892	Mouth of Marudi Creek at Kuyuwini River, downstream from Parabara, Marudi Creek. Upper Takutu-Upper Essequibo. Guyana.	N 2°9'33.480" W 59°17'33.600"
	Aphyocharax erythrurus	ROM 97120	Near Mango Landing. Essequibo River. Potaro-Siparuni, Guyana.	N 5°18'53.532" W 58°54'21.420"
	Aphyocharax nattereri	LBP 3734	Lagoa Marginal Rio Negro. Drenagem do Rio Paraguai. Aquidauana, Mato Grosso do Sul, Brasil.	S 19°34'54.6' W 56°15'16.5"
	Aphyocharax nattereri	LBP 3786	Lagoa marginal do Rio Negro. Rio Paraguai. Aquidauna, Mato Grosso do Sul, Brasil.	S 19°34'17" W 56°14'44"
	Aphyocharax pusillus	LBP 4046	Rio Moa. Cruzeiro do Sul, Acre, Brasil.	S 7°37'20" W 2°47'42.2"
	Aphyocharax pusillus	LBP 4097	Rio Japim. Rio Juruá. Mâncio Lima, Acre, Brasil.	S 07°34'28" W 72°55'24"
	Aphyocharax pusillus	ROM 92238	Sand beach along small island and exposed rocky shoal across channel from Puerto Venado (near Samariapo), 56.7 km south-southwest of Puerto Ayacucho. Rio Orinoco. Amazonas, Venezuela.	N 5°12'34.272" W 67°48'34.344"
	Aphyocharax rathbuni	LBP 7608	Lagoa marginal do rio Cuiabá. La Plata basin. Barão de melgaço, Mato Grosso, Brasil.	S 16°11'39" W 55°48'25"
	Aphyocharax rathbuni	LBP 8457	Lagoa Marginal rio Paraguai. Rio Paraguai, La Plata Basin. Cáceres, Mato Grosso, Brasil.	S 16°03'13.6' W 57°48'31.8"
	Leptagoniates steindachneri	LBP 4137	Rio Moa. Mâncio Lima, Acre, Brasil.	S 07°26'35.5" W 73°03'33.5"
	Leptagoniates steindachneri	ANSP 180645	Rio Acre, at town of Inapari on border with Brazil. Purus drainage. Madre de Dios, Peru.	S 10°56'39" W 69°34'39"
	Paragoniates alburnus	AUM 71167	Rio Utcubamba, 11 km ESSE of Bagua Grande. Rio Marañón. Rio Amazonas drainage. Amazonas, Peru.	S 5°47'16.476" W 78°20'49.596"
	Paragoniates alburnus	CUMV 82705	Rio Portuguesa. Portuguesa, Venezuela.	N 8°55'59.88" W 69°5'60"

Aphyocharacinae	*Paragoniates alburnus*	LBP 9208	Rio Manapire. Cabruta, Guárico, Venezuela.	N 7°52'04.1" W 66°12'40.1"
	Paragoniates alburnus	ROM 94132	15 km south-southwest of Guanare in Nuestra Señora de Coromoto National Sanctuary, Río Tucupido. Guanare, Portuguesa, Venezuela.	N 8°54'50.796" W 69°45'39.960"
	Phenagoniates macrolepis	LBP 6105	Rio Apon Medio. Machiques de Perijá, Zulia, Venezuela.	N 10°09'42" W 72°25'58"
	Prionobrama filigera	LBP 4139	Rio Japim. Rio Juruá. Mâncio Lima, Acre, Brasil.	S 07°26'35" W 73°03'33"
	Prionobrama paraguayensis	LBP 3230	Lagoa marginal. Nobres, Mato Grosso, Brasil.	S 14°40'32.8" W 56°13'14"
	Xenagoniates bondi	LBP 3074	Rio Orinoco. Caicara del Orinoco. Bolivar, Venezuela.	N 07°38'11.6" W 66°19'04.2"
Characinae	*Charax leticiae*	LBP 1480	Rio Taquari - Pesqueiro Recanto Alegre. Coxim, Mato Grosso do Sul, Brasil.	S 18°25'42.5" W 54°50'02.8"
	Cynopotamus kincaidi	LBP 3225	Lagoa marginal. Nobres, Mato Grosso, Brasil.	S 14°40'32.8" W 56°13'14"
	Microschemobrycon casiquiare	LBP 8161	Rio Tapajós. Pimental, Pará, Brasil.	S 04°32'25" W 56°15'15"
Cheirodontinae	*Cheirodon ibicuhiensis*	LBP 4777	Rio Guaíba. Barra do Ribeiro, Rio Grande do Sul, Brasil.	S 30°18'03.9" W 51°20'40.8"
	Compsura heterura	LBP 4733	Rio Ceará-Mirim. Natal, Rio Grande do Norte, Brasil.	S 05°37'47" W 35°37'09"
	Heterocheirodon yatai	LBP 4872	Rio Yi. Durazno, Durazno, Uruguai.	S 33°23'49" W 56°24'10"
	Kolpotocheirodon theloura	LBP 5033	Ribeirão Bananal. Distrito Federal, Brasil.	S 15°43'42.7" W 47°54'39.4"
	Macropsobrycon uruguayanae	LBP 6039	Rio Piquiri. Cachoeira do Sul, Rio Grande do Sul, Brasil.	S 30°14'46" W 52°45'53"
	Odontostilbe fugitiva	LBP 4052	Rio Moa. Cruzeiro do Sul, Acre, Brasil.	S 7°37'20" W 72°47'42.2"

Cheirodontinae	*Protocheirodon pi*	LBP 10565	Rio Acre/Amazonas. Rio Branco, Acre, Brasil.	S 10°03'28" W 67°51'25"
	Serrapinnus calliurus	LBP 3731	Lagoa Marginal Rio Negro. Aquidauana, Mato Grosso do Sul, Brasil.	S 19°34'54.6" W 56°15'16.5"
	Serrapinnus piaba	LBP 8972	Córrego da Mata. Pedro Leopoldo, Minas Gerais, Brasil.	S 19°37'59.7" W 44°06'25.5"
Exodontinae	*Exodon paradoxos*	LBP 4006	Lago Morto. São Félix do Araguaia, Mato Grosso, Brasil.	S 11°40'9" W 50°51'0.30"
	Roeboexodon guyanensis	LBP 5315	Igarapé Uiratapuru. Laranjal do Jari, Amapá, Brasil.	S 00°34'03" W 52°34'41"
Tetragonopterinae	*Tetragonopterus argenteus*	LBP 3758	Rio Negro. Aquidauana, Mato Grosso do Sul, Brasil.	S 19°34'33.7" W 56°14'49.5"
	Tetragonopterus chalceus	LBP 8268	Rio Verde Grande. Jaíba, Minas Gerais, Brasil.	S 15°19'24.2" W 43°39'52.5"
Spintherobolinae	*Spintherobolus ankoseion*	LBP 4725	Arroio que desaguá no lago Acaraí. São Francisco do Sul, Santa Catarina, Brasil.	S 26°17'35" W 48°35'21"
	Spintherobolus broccae	LBP 3916	Riacho sem nome afluente do rio Vermelho. Bertioga, São Paulo, Brasil.	S 23°46'16.2" W 46°00'37.2"
	Spintherobolus leptoura	LBP 7544	Afluente rio Mumuna. Iguape, São Paulo, Brasil.	S 24°42'57.8" W 47°41'28.3"

Table 6 – List of species and accession number on GenBank. Collection vouchers of members of the “Clade B” plus Spintherobolinae of the family Characidae.

Subfamily	**Species**	**Voucher**	**Specimen**	**GenBank acession number**				
				16S	***cyt b***	**Myh6**	**RAG1**	**RAG2**
Aphyocharacinae	*Aphyocharacidium bolivianum*	LBP 9055	42219	HQ171424	HQ289710	HQ289132	HQ289325	HQ289517
	Aphyocharax alburnus	LBP 2293	15819	JQ820097	JQ820076	JQ820064	JQ820054	JQ820034
	Aphyocharax alburnus	LBP 2293	15820	JQ820098	JQ820077	JQ820065	JQ820055	JQ820035
	Aphyocharax alburnus	CUMV 82626	1		–	–	–	–
	Aphyocharax alburnus	CUMV 82627	1		–	–	–	–
	Aphyocharax anisitsi	LBP 3764	22190	HQ171292	HQ289581	HQ289002	HQ289196	HQ289389
	Aphyocharax anisitsi	LBP 4750	25524	JQ820095	JQ820081	JQ820067	JQ820049	JQ820040
	Aphyocharax avary	ROM 107212	T24762		–	–	–	–
	Aphyocharax avary	ROM 107212	T24763		–	–	–	–
	Aphyocharax dentatus	LBP 1587	11774	HQ171242	HQ289533	HQ288952	HQ289149	HQ289340
	Aphyocharax dentatus	LBP 5112	26163	JQ820091	JQ820082	JQ820068	JQ820047	JQ820036
	Aphyocharax dentatus	LBP 20	3604	JQ820092	JQ820083	JQ820069	JQ820048	JQ820037
	Aphyocharax dentatus	LBP 1587	11774	HQ171242	HQ289533	HQ288952	HQ289149	JQ820039
	Aphyocharax dentatus	LBP 2480	16349	JQ820090	JQ820084	JQ820061	JQ820050	JQ820038
	Aphyocharax erythrurus	ROM 88558	T08146		–	–	–	–

Aphyocharacinae	*Aphyocharax erythrurus*	ROM 96222	T14958		–	–	–	–
	Aphyocharax erythrurus	ROM 95892	T16229		–	–	–	–
	Aphyocharax erythrurus	ROM 97120	T18119		–	–	–	–
	Aphyocharax erythrurus	ROM 97120	T18124		–	–	–	–
	Aphyocharax nattereri	LBP 3734	22132	JQ820089	JQ820071	JQ820058	JQ820043	JQ820030
	Aphyocharax nattereri	LBP 3786	22345	JQ820088	JQ820070	JQ820057	JQ820042	JQ820029
	Aphyocharax pusillus	LBP 4046	22920	HQ171301	HQ289590	HQ289011	HQ289203	HQ289397
	Aphyocharax pusillus	LBP 4097	23546	JQ820096	JQ820078	JQ820066	JQ820053	JQ820033
	Aphyocharax pusillus	ROM 92238	T09118		–	–	–	–
	Aphyocharax rathbuni	LBP 7608	36496	JQ820093	JQ820079	JQ820062	JQ820051	JQ820031
	Aphyocharax rathbuni	LBP 8457	40434	JQ820094	JQ820080	JQ820063	JQ820052	JQ820032
	Leptagoniates steindachneri	LBP 4137	23661	HQ171311	HQ289600	HQ289021	HQ289213	HQ289407
	Leptagoniates steindachneri	ANSP 180645	1202		–	–	–	–
	Leptagoniates steindachneri	ANSP 180645	1203		–	–	–	–
	Paragoniates alburnus	AUM 71167	AUFT10595		–	–	–	–
	Paragoniates alburnus	AUM 71167	AUFT10596		–	–	–	–
	Paragoniates alburnus	CUMV 82705	1		–	–	–	–
	Paragoniates alburnus	LBP 9208	43156	HQ171426	HQ289712	HQ289134	HQ289326	HQ289519
	Paragoniates alburnus	ROM 94132	T08996		–	–	–	–

Aphyocharacinae	*Phenagoniates macrolepis*	LBP 6105	35623	HQ171391	HQ289678	HQ289099	HQ289292	HQ289484
	Prionobrama filigera	LBP 4139	23663	JQ820086	JQ820074	JQ820059	JQ820044	JQ820027
	Prionobrama filigera	LBP 4139	23664	JQ820087	JQ820075	JQ820060	JQ820045	JQ820028
	Prionobrama paraguayensis	LBP 3230	19465	HQ171272	JQ820073	HQ288982	JQ820046	HQ289369
	Prionobrama paraguayensis	LBP 3230	19468	HQ171273	JQ820072	HQ288983	HQ289179	HQ289370
	Xenagoniates bondi	LBP 3074	19694	HQ171274	HQ289563	HQ288984	–	–
Characinae	*Charax leticiae*	LBP 1480	12700	HQ171244	HQ289535	HQ288954	HQ289151	HQ289342
	Cynopotamus kincaidi	LBP 3225	19449	HQ171271	HQ289560	HQ288981	HQ289178	HQ289368
	Microschemobrycon casiquiare	LBP 8161	38058	HQ171409	HQ289696	HQ289117	HQ289310	HQ289502
Cheirodontinae	*Cheirodon ibicuhiensis*	LBP 4777	25598	HQ171334	HQ289623	HQ289043	HQ289236	HQ289430
	Compsura heterura	LBP 4733	24984	HQ171332	HQ289621	HQ289041	HQ289234	HQ289428
	Kolpotocheirodon theloura	LBP 5033	25982	HQ171336	HQ289625	HQ289045	HQ289238	HQ289432
	Macropsobrycon uruguayanae	LBP 6039	29061	HQ171355	HQ289644	HQ289064	HQ289257	HQ289450
	Odontostilbe fugitiva	LBP 4052	22932	HQ171302	HQ289591	HQ289012	HQ289204	HQ289398
	Protocheirodon pi	LBP 10565	49267	JQ820099	JQ820085	JQ820056	JQ820041	JQ820026
	Serrapinnus calliurus	LBP 3731	22121	HQ171291	HQ289580	HQ289001	HQ289195	HQ289388
	Serrapinnus piaba	LBP 8972	41813	HQ171416	HQ289703	HQ289124	HQ289317	HQ289509
Exodontinae	*Exodon paradoxus*	LBP 4006	23040	HQ171306	HQ289595	HQ289016	HQ289208	HQ289402
	Roeboexodon guyanensis	LBP 5315	26921	HQ171345	HQ289634	HQ289054	HQ289247	HQ289440

Tetragonopterinae	*Tetragonopterus argenteus*	LBP 3758	22029	HQ171289	HQ289578	HQ288999	HQ289193	HQ289386
	Tetragonopterus chalceus	LBP 8268	37556	HQ171405	HQ289692	HQ289113	HQ289306	HQ289498
Spintherobolinae	*Spintherobolus ankoseion*	LBP 4725	24957	HQ171331	HQ289620	HQ289040	HQ289233	HQ289427
	Spintherobolus broccae	LBP 3916	22558	HQ171294	HQ289583	HQ289004	HQ289198	HQ289391
	Spintherobolus leptoura	LBP 7544	36098	HQ171393	HQ289680	HQ289101	HQ289294	HQ289486

ANEXO 2: Supporting Information 2 – List of examined material of the subfamily Aphyocharacinae (Characiformes: Characidae).

BOLIVIA – *Aphyocharax avary*: ANSP 84380, 30, 25.9–52.9 mm SL (1 male c&s, 33.1 mm SL; 2 females c&s, 30.7–36.2 mm SL), Cochabamba, Todos Santos, Rio Chapare; altitude ca. 1000 ft. *Aphyocharax pusillus*: ANSP 84379, 20, 26.5–49.0 mm SL (1 male c&s, 44.2 mm SL; 2 females c&s, 45.7–49.0 mm SL),Cochabamba, Boca Chapare, mouth of the Rio Chapare. CAS 068332, 1, 51.0 mm SL, El Beni, R. Iniqui, Tributary to Rio Beni ca. midway between Rurrenabaque and Huachi (=San Miguel de Huachi?), 15°27’35.986”S, 67°15’38.606”W. *Paragoniates alburnus*: FMNH 106460, 1, 58.8 mm SL, Pando, R. Manuripi; +/- 12 km rio arriba de Puerto Rico, 11°09’06.1”S, 67°33’41”W. *Prionobrama filigera*: FMNH 106490, 10, 35.7–41.0 mm SL, Pando, Aserradero Rutina, 77 km SO de Cobija, 11°25’54.8”S, 69°00’09”W.

BRAZIL – *Aphyocharacidium bolivianum*: LBP 12010, 8, 23.1–30.2 mm SL, Amazonas, Lábrea, Afluente rio Purus, Madeira drainage, 7°56.110'S, 63°27.353'W. LBP 18008, 10, 19.3–24.8 mm SL, Amazonas, Itacoatiara, Igarapé Grande, Rodovia AM–010, Rio Amazonas drainage, 3°7’7’’S, 58°27’14.7”W. MCP 39961, 15, 21.2–28.1 mm SL (1 female c&s, 24.1 mm SL), Amazonas, Humaitá, igarapé do Vinte e Dois, Recanto do Sanari, 20 km west of Humaitá, 7°35’36”S, 63°10’27”W. MCZ 56010, 10, 16.6–25.4 mm SL, Amazonas, small dark water creek flowing into the Paraná de Janauacá, 3°25’S, 61°21’W. *Aphyocharax alburnus*: UFRGS 24116, 4, 36.5–45.1 mm SL, Rondônia, Porto Velho, Foz do rio Jatuarana com rio Madeira, próximo a cachoeira do Teotônio, Madeira drainage, 8°49’54”S, 64°2’45”W. UFRGS 24198, 10, 37.8–44.7 mm SL (1 male c&s, 43.7 mm SL; 1 female c&s, 44.7 mm SL), Rondônia, Porto Velho, Foz do rio Jatuarana com rio Madeira, próximo a cachoeira do Teotônio. Madeira drainage, 8°49’54”S, 64°2’45”W. *Aphyocharax anisitsi*: LBP 6568, 9, 21.0–30.8 mm SL, Paraná, Marilena, Lagoa marginal Rio Paraná. Rio Paraná/Bacia do Prata, 22°37’57.3”S, 53°03’09.4”W. LBP 4750, 4, 25.2–28.2 mm SL, Rio Grande do Sul, Barra do Ribeiro, Rio Guaíba, 30°17’07.0”S, 51°18’01.1”W. UFRGS 9315, 1 (1 female c&s, 29.3 mm SL), Rio Grande do Sul, São Sepé, açude em propriedade destinada à silvicultura, 29°57’14”S, 53°45’24”W. UFRGS 6485, 2 (2 females c&s, 25.1–27.1 mm SL), Rio Grande do Sul, Rosário do Sul, arroio do Salso, 30°22’26”S, 55°2’5”W. UFRGS 10440, 6, 26.2–29.3 mm SL, Rio Grande do Sul, Rosário do Sul, Rio Ibicuí da Faxina, entre Santana do Livramento e Rosário do Sul, Uruguay basin, 30°47’22”S, 55°12’41”W. UFRGS 23453, 6, 24.4–27.6 mm SL, Rio Grande do Sul, Uruguaiana, Arroio Itapitocaí na BR 472 entre Uruguaiana e Barra do Quaraí. Lower Uruguay basin. Brazil. 29°53’2.7”S, 57°6’56.7”W. *Aphyocharax avary*: ANSP 39217 (Holotype), 1, 42.9 mm SL, Madeira River about 200 miles east of Long, 62°20’W. UFRGS 25829, 7, 32.8–43.0 mm SL (1 male c&s, 32.8 mm SL; 1 female c&s, 39.5 mm SL), Rondônia, Porto Velho, Foz do rio Jaci-Paraná com o rio Madeira, Madeira drainage, 9°9’41”S, 64°23’55”W. *Aphyocharax dentatus*: LBP 20, 3, 45.6–61.8 mm SL, Mato Grosso do Sul, Corumbá, Rio Miranda. Rio Paraguay drainage, 19°34.630’S, 57°01.123’W. LBP 5112, 2, 50.7–51.0 mm SL, Mato Grosso, Cáceres, Lagoa Bairro Caiçara, Rio Paraguai drainage, 16°06’66”S, 57°44’33”W. LBP1587, 1, 34.3 mm SL, Mato Grosso, Barra do Garça, Rio das Garças, Rio Araguaia drainage, 15°54’18.1”S, 52°19’24.2”W. NUP 3459, 8, 34.0–38.5 mm SL (1 male c&s, 36.0 mm SL; 1 female c&s, 37.8 mm SL), Mato Grosso, Rosário Oeste, Rio Quilombo (Qu1), 15°6’50”S, 55°40’38”W. *Aphyocharax nattereri*: MCP 34718, 5, 22.6–24.2 mm SL, Pará, Almeirim, Comunidade de São Raimundo, Pesqueiro, quadra 32, 2°32’S, 52°34’W. NUP 3385, 3, 24.5–25.2 mm SL, Mato Grosso, Barão De Melgaço, Baia Sinhá Mariana, 16°19’48”S, 55°54’0”W. FMNH 108377, 5, 20.0–23.0mm SL, Mato Grosso do Sul, Corumbá, lower Rio Negro swamp, 19°20’17.16”S, 56°57’42.12”W. UFRGS 17455, 2 (1 female c&s, 22.6 mm SL; 1 male c&s, 23.6 mm SL), Mato Grosso do Sul, Corumbá, Açude próximo a torre da BEP (base experimental do pantanal), 19°34’4.42”S, 57°1’20.49”W. *Aphyocharax pusillus*: LBP 4046, 16, 27.8–46.8 mm SL, Acre, Cruzeiro do Sul, Rio Moa, Rio Juruá, Amazonas drainage, 7°37’20.0”S, 72°47’42.2”W. MCP 32769, 6, 41.2–50.8 mm SL, Amazonas, Alvarães, Rio Solimões, na ilha do Içé. Confluência Solimões-Japurá, 3°16'36"S, 64°41’1”W. *Aphyocharax rathbuni*: MCP 15681, 3, 20.0–24.6 mm SL, Mato Grosso, Cáceres, Rio Paraguai em Cáceres e arredores, 16°3’S, 57°42’W. FMNH108378, 25, 14.7–21.8 mm SL, Mato Grosso do Sul, Rio Verde de Mato Grosso, Rio Negro, Corrego Anhuma at road; hole, minor connection with main channel, 19°9’39.96”S, 55°17’52.08”W. LBP8457, 2, 19.5–19.9 mm SL, Mato Grosso, Cáceres, Lagoa Marginal rio Paraguai. Rio Paraguai/La Plata Basin, 16°03’13.6”S, 57°48’31.8”W. LBP7608, 2, 19.1–24.2 mm SL, Mato Grosso, Barão de Melgaço, Lagoa Margina rio Cuiabá. La Plata Basin, 16°11’39.5”S, 55°48’25.1”W. UFRGS 13635, 1, 26.1 mm SL, Mato Grosso, Poconé, Fazenda na rodovia que liga Cuiabá à Poconé, várzea do rio Bento Gomes, Paraguay basin, 15°59’56”S, 56°28’50”W. *Axelrodia lindeae*: ANSP 139717 (Partype), 2, 15.0–18.0 mm SL, Rio Curucamba, about 9 km N of Obidos (3 km from the Obidos airport), lower Amazon basin, 1°49’55.499”S, 55°29’30.678”W. MPEG 27576, 8, 20.0–25.0 mm SL, Juruti, Pará, igarapé Rio Branco, Arapiuns basin, 2°20’57.4”S 56°1’28”W. MCP 37314, 10, 17.7–22.6 mm SL (1 male c&s, 22.5 mm SL; 3 females c&s, 23.8–18.7 mm SL), Acre, Bujari, igarapé Marizinho, BR–364 highway, rio Antimari drainage, 9º36’41”S, 68º14’40”W. MCP 37317, 7, 19.1–24.3 mm SL, Rondônia, Candeias de Jamari, riacho na estrada 364 entre Ariquemes e Candeias do Jamari, afluente do rio Candeias, 8º47’14”S, 63º36’23”W. *Paragoniates alburnus*: MCP 17081, 1 (1 c&s, 62.1 mm SL), Amazonas, Manacabi, rio Jupurá, cerca de 50 km da foz, 2°45’S, 64°52’W. MCP 25500, 1, 69.2 mm SL, Guárico, Rio Tiznados, Portuguesa Drainage., 9°04’N, 67°22’60”W. MCP 28797, 1, 66.7 mm SL, Acre, Bujari, Igarapé Marizinho, na estrada BR 364, 86 km a SE de Sena Madureira (alf. rio Atimari), 9°36’41”S, 68°14’44”W. MCP 29613, 1, 59.8 mm SL, Amazonas, Tefé, Rio Solimões, margem sul da ilha Panamim, Solimões drainage, 3°19’19”S, 64°40’25”W. MCP 29850, 1, 65.5 mm SL, Amazonas, Alavarães, Rio Japurá, na comunidade Nova Colômbia; Solimões drainage, 2°54’46”S, 64°54'26”W. *Prionobrama filigera*: MCP 17091, 1 (1 c&s, 41.8 mm SL), Rondônia, Porto Velho, rio Madeira, cachoeira Santo Antônio, 8°49’59”S, 64°W. MCP 29567, 1 (1 c&s, 41.3 mm SL), Amazonas, Iranduba, Alvarães, canal do lago Mamirauá Estação A na comunidade Boca do lago Mamirauá, sistema do lago Mamirauá, 3°6’37”S, 64°47’49”W. LBP 4139, 3, 31.4–37.9 mm SL, Acre, Mâncio Lima, Rio Moá, Rio Juruá drainage, 7°26’35.5”S, 73°3’33.5”W. *Prionobrama paraguayensis*: MCP 15580, 6, 29.5–32.4 mm SL (2 c&s, 31.5–32.4 mm SL), Mato Grosso, Cáceres, rio Paraguai em Cáceres e arredores, 16°3’S, 57°42’W. MZUSP 42748, 1 (1 c&s, 31.4 mm SL), Mato Grosso, Barão de Melgaço, rio Cuiabá, 16°11’S, 55°57’W. LBP 3230, 5, 26.4–34.1 mm SL, Mato Grosso, Nobres, Rio Cuiabazinho, Lagoa marginal, 14°40’32.8”S, 56°13’14”W.

COLOMBIA – *Aphyocharax avary*: ROM 107212, 1, 36.8 mm SL, Rio Orteguaza, 1°31’9.552”N, 75°32’19.104”W. *Paragoniates alburnus*: NRM 26051, 1, 52.0 mm SL, Meta, Orinoco basin, Río Meta drainage: Río Negro, where crossed by road Villavicencio - Puerto López at village La Balsa. *Phenagoniates macrolepis*: CAS 70950, 1, 24.7 mm SL, Choco, Rio Truando, a western tributary emptying into the Atrato near Rio Sucio, Atrato basin, 7°23’20.9”N, 77°07’54.1”W. NRM 23751, 2, 28.6–29.7 mm SL, Norte de Santander, Cucuta, La Silla, small brook. *Prionobrama filigera*: ANSP 135939, 4, 39.8–44.8 mm SL, Leticia, backwater (The Lakes) area cut off from Amazon River. *Xenagoniates bondi*: MCP 15136, 4, 35.5–41.7 mm SL, Meta, Puerto Lopéz, Quebrada Venturosa, 1/4 creek mi above road between La Balsa and Puerto Lopéz (drains into rio Meta), 4°05’N, 72°58’W. ANSP 128849, 1 (1 female c&s, 40.6 mm SL), Meta, Quebrada Venturosa, 1/4 creek mi above road between La Balsa and Puerto Lopez; drains into Rio Meta, 4°3’50.350”N, 72°58’52.975”W.

ECUADOR – *Leptagoniates steindachneri*: FMNH 97120, 4, 32.1–54.0 mm SL (1 c&s, 54.0 mm SL), Napo, rio Jivino, lower 4 km (mostly) to ca. 6 km upstream from mouth (most between the two “ports” for Limoncocha), 0°24’36”S, 76°39’W. ANSP 130577, 1, 66.2 mm SL, Napo, Rio Aguarico at Santa Cecilia, 0°06’N, 76°51’W. FMNH 97115, 4, 43.1–56.2 mm SL, Napo,Qbda Apoalla, tributary to lower Rio Shushufindi, 0°16’59.9”S, 76°27’W. FMNH 97116, 3, 53.1–56.7 mm SL, Napo, Rio Aguarico, few km upstream from mouth of Rio Eno, 0°10’59.9”S, 76°30’W. *Paragoniates alburnus*: FMNH 100346, 1 (1 c&s, 70.0 mm SL), Napo, Quebrada Apoalla, tributary to lower rio Shushufindi, 0°16’59”S, 76°27’W. *Prionobrama filigera*: FMNH 100347, 9, 46.2–56.2 mm SL, Napo, Rio San Miguel at Tipishca [km. 54.5], 0°12’29.9”N, 76°13’00.1”W.

GUYANA – *Aphyocharax erythrurus*: CAS-SU 021912 (Paratype), 2, 26.5–35.0 mm SL, Rockstone sand bank. Río Essequibo, 5°58’50.466”N, 58°33’14.504”W. ANSP 190645, 10, 25.0–45.7 mm SL, Rupununi, Rupununi River (Essequibo Dr.), sand beach and inlet at Karanambo Ranch, 3°45’0”N, 59°18’30”W. ANSP 190620, 7, 26.6–32.0 mm SL (3 c&s), Rupununi, Pirara River, trib. Ireng River (Takutu-Negro Dr.), beach at Pirara Ranch on road to Lethem, 3°37’17”N, 59°40’29”W. ROM 88558, 2, 38.6–44.6 mm SL, East Berbice, 5°0’28.476”N, 58°14’46.932”W. ROM 96222, 4, 28.6–31.4 mm SL, Rapids at shelf of rock, large wide river, Takutu River, Upper Takutu-Upper Essequibo, 2°50’9.480”N, 59°59’25.560”W. ROM 95892, 4, 29.7–35.6 mm SL, Mouth of Marudi Creek at Kuyuwini River, downstream from Parabara, Marudi Creek, Upper Takutu-Upper Essequibo, 2°9’33.480”N, 59°17’33.600”W.

PANAMA – *Roeboides macrolepis* (Paratypes): FMNH12851, 1, 42.3 mm SL, Darien, Rio Cupe, Boca de Cupe. FMNH12852, 1, 43.7 mm SL, Darien, Rio Cupe, Boca de Cupe. FMNH12853, 1, 39.0 mm SL, Darien, Rio Cupe, Boca de Cupe.

PARAGUAY – *Bleptonema paraguayensis* (Paratypes): CAS 059710, 2, 38.5–38.6 mm SL, Mato Grosso do Sul, R. Paraguay, Corumba, 18°59’43.458”S, 57°38’5.928”W. FMNH56682, 3, 34.8–40.3 mm SL, Mato Grosso do Sul, R. Paraguay, Corumba, 18°59’43.458”S, 57°38’5.928”W. *Aphyocharax anisitsi*: CAS 059718 (Paratype), 1, 28.8 mm SL, Asuncion, Río Paraguay, 25°18’20.358”S, 57°40’16.075”W. FMNH 107797, 23, 23.7–31.2 mm SL (1 male, 1 female c&s), Alto Paraguay, Rio Paraguay at left margin, above Estancia Cerrito, 21°25’59.880”S, 57°55’0.120”W. *Aphyocharax dentatus*: CAS 076471 (Paratype), 2, 54.4–

59.3 mm SL, Laguna Pasito, At Asuncion, 25°18’20.358”S, 57°40’16.075”W. CAS 059723 (Paratype), 3, 25.8–45.7 mm SL, Laguna Pasito, At Asuncion, 25°18’20.358”S, 57°40’16.075”W. CAS 029724(Paratype), 1, 62.5 mm SL, Asuncion, Laguna Pasito, At Asuncion, 25°18’20.358”S, 57°40’16.075”W. CAS 059725(Paratype), 2, 39.9–47.6 mm SL, Asuncion, Laguna Pasito, At Asuncion, 25°18’20.358”S, 57°40’16.075”W. CAS 059726 (Paratype), 3, 32.9–61.7 mm SL, Aguada, near Arroyo Trementina, 22°47’0.913”S, 56°52’59.088”W. ANSP 174946, 6, 47.9–51.9 mm SL, Paraguari Dept., Rio Paraná, Mud beach on Parador Las Mercedes side of inlet (Río Tebicuary), 26°22’34”S, 62°47’38.71”W. *Aphyocharax nattereri*: FMNH 107808, 3, 20.1–23.0 mm SL, Concepcion, Rio Paraguay, Rio Apa at small embayment near its mouth, 22°7’0.12”S, 57°55’0.12”W. *Prionobrama paraguayensis*: FMNH 57903, 7, 34.0–38.1 mm SL, Aregua, 25°18’33.1”S, 57°23’10.3”W. FMNH 107946, 3, 30.1–35.4 mm SL (1 male c&s, 30.1 mm SL; 1 female c&s, 32.1 mm SL), Concepción, Rio Apa at small embayment. 22°’0.120”S, 57°49’0.120”W.

PERU – *Aphyocharacidium* n. sp.: MUSM 68942, 6, 22.0–29.1 mm SL (1 male c&s, 27.8 mm SL), Loreto, Andoas, Cuenca del Marañón, río Plantayacu, 3°08’8.40”S, 75°46’43.53”W. MUSM 68515, 1, 25.1 mm SL, Loreto, Morona, Datem del Marañón, río Morona, Quebrada Situche, 3°03’16.51”S, 77°26’57.31”W. MUSM 41944, 2, 25.7–24.3 mm SL, Loreto, Morona, Datem del Marañón, río Morona, Quebrada Situche, 3°03’16.51”S, 77°26’57.31”W. MUSM 69043, 5, 21.5–25.7 mm SL (1 female c&s, 23.8 mm SL), Loreto, Morona, Datem del Marañón, río Morona, Quebrada Katirnaetnsa, 3°04’1.58”S, 77°20’42.73”W. UFRGS 28200, 2, 23.6–26.7 mm SL, Loreto, Morona, Datem del Marañón, río Morona, Quebrada Katirnaetnsa, 3°04’1.58”S, 77°20’42.73”W. MUSM42077, 4, 22.0–23.4 mm SL, Loreto, Morona, Datem del Marañón, río Morona, Quebrada Katirnaetnsa, 3°06’10.30”S, 77°22’28”W. *Aphyocharax alburnus*: ANSP 185206, 10, 26.5–57.5 mm SL, (2 c&s, 43.8–52.0 mm SL), Madre de Dios, Rio Manuripe (Orton-Madre de Dios Dr.), road crossing town of Mavila, 11°55’44”S, 69°7’15”W. *Aphyocharax filigerus*: ANSP8059 (Holotype), 1, 45.9 mm SL, Pebas, 3°19'21.439"S, 71°51’48.092”W. ANSP8073 (Paratype), 1, 41.1 mm SL, Pebas, 3°19’21.439”S, 71°51’48.092”W. *Aphyocharax pusillus*: CAS076367, 12, 44.9–53.7 mm SL, Loreto, R. Huallaga, Yurimaguas, 5°53’3.138”S, 76°6’29.606”W. *Leptagoniates steindachneri*: CUMV 94607, 1, 46.5 mm SL, Amazonas, Condorcanqui, Río Nieva, 7.4 km SSW Juan Velasco (Sta Maria de Nieva); Río Marañón, 4°39’38.52”S, 77°53’2.04”W. AUM 46648 , 4, 56.4–65.1 mm SL, Amazonas, Condorcanqui, Río Marañón, 4°39’38.448”S, 77°53’2.004”W. ANSP 180645, 13, 42.1–69.2 mm SL (2 females c&s, 47.6–53.6 mm SL), Madre de Dios, Rio Acre, at town of Inapari on border with Brazil, Purus drainage, 10°56’39”S, 69°34’39”W. NRM 25858, 1, 34.2 mm SL, Loreto, San Jacinto, quebrada at km 53, Amazon basin, rio Tigre drainage, 2°32’S, 75°43’60”W. *Paragoniates alburnus*: ANSP 178149, 2, 35.6–44.9 mm SL, Maynas, Loreto, Rio Napo (R. Amazonas Dr.), beach & backwater along right bank just upstream from mouth R. Mazan, near town of Mazan, 3°29’10”S, 73°06’24”W. AUM51390, 2, 41.5–51.3 mm SL, Madre de Dios, Rio Acre - Rio Madeira - Madre Dios, Quebrada Primavera, en el puente de la carretera interoceánica 3.6km ESE de Iñapari, 11°0’38.16”S, 69°33’20.484”W. *Prionobrama filigera*: ANSP 136835, 3, 33.4–45.1 mm SL, Loreto, Vicinity Iquitos, left bank Rio Amazonas (Maranon) due S of Isla Iquitos, Sta. 5. NRM 30441, 2, 28.4–30.4 mm SL, Ucayali, Yarinacocha, canal to Paca Cocha, Río Ucayali drainage, Amazon basin, 8°19’S, 74°34’W.

VENEZUELA – *Aphyocharax alburnus*: AUM 40124, 1, 31.5 mm SL, Amazonas, Río Orinoco, Rio Ventuari, beach below Raudales Tencua, 56 km ESE of San Juan de Manapiare, 5°2’58.848”N, 65°37’37.992”W. CAS 068353, 25, 27.7–42.6 mm SL, Anzoategui State, R. Guanipa, 25 km north of El Tigre, 9°6’36.346”N, 64°15’3.823”W. CUMV 76227 , 11, 30.2–38.5 mm SL (1 male c&s, 35.1 mm SL), Apure, Rio Caicara site #2, 15.5 km W of Bruzual-Elorza Hwy. on road parallelin Rio Caicara, 7°30’N, 69°30’W. CUMV 82627, 14, 27.7–37.1 mm SL (1 male c&s, 29.1; 1 female c&s, 35.2 mm SL), Portuguesa, Rio Portuguesa, 8°55’59.88”N, 69°5’60”W. CUMV 82626, 1, 36.2 mm SL, Portuguesa, Rio Las Marias, 9°11’60”N, 69°42’'0”W. CUMV 82628, 1, 38.9 mm SL, Portuguesa, Rio Las Marias, 9°11’60”N, 69°42’0”W. *Aphyocharax avary*: ANSP 159441, 30, 25.8–38.5 mm SL (3 females c&s, 30.3–38.5 mm SL), Bolivar, Cano Caiman, at crossing of Caicara - Puerto Ayacucho hwy, 19.2 km W of Ciudad Bolivar-Caicara hwy. FMNH 85496, 1, 31.8 mm SL, Apure, Rio Orinoco, Rio Aruaca, 32.5 km S Biruaca. *Aphyoharax colifax*: ANSP 139596, 19, 28.0–43.7 mm SL (1 female c&s, 36.1 mm), Bolivar, Sand bar along Rio Mato, 6°47’53.927”N, 65°14’44.722”W. ANSP 161038, 2026.5–39.3 mm SL, Bolivar, Rio Cariapo, just above and below bridge on Caicara - Ciudad Bolivar hwy., 7°53’0”N, 63°49’42”W. INHS 31513, 3, 26.2–32 mm SL, Monagas, Rio Morichal Largo, 6 km SE El Silencio at bridge, 9°8’58.456”N, 62°47’38.710”W. INHS 31307, 5, 26.9–32.3 mm SL, Anzoategui, Rio Aisme, 35 km NE El Tigre, 9°4’31.372”N, 64°3’36.263”W. *Aphyocharax yekwanae*: FMNH 109275 (Paratype), 2, 23.3–41.0 mm SL (1 c&s), Bolívar, Caño Suadijitu, 05°29’35”N, 64°35’10”W. FMNH109277 (Paratype), 128.2 mm SL, Bolivar,Rio Caura at beach in Raudal Cejiato, 5°33’28.08”N, 64°18’48.96”W. *Paragoniates alburnus*: ANSP 149991, 2, 55.9–60.8

mm SL, Rio Apure at San Fernando, 7°54’02.7”N, 67°28’16.1”W. CAS 64412, 5, 54.0–82.3 mm SL, Portuguesa, Caño Maraca en el puente 60 km via Guanare-Guaranito Rd. R. Orinoco basin, 8°49’40.3”N, 69°20’38.9”W. CUMV71737, 4, 34.0–81.2 mm SL, Monagas, Gulf of Paria at Guyata R., under bridge where Hwy. 1 (Maturin to Curipe) crosses it. CUMV82705, 5, 54.6–71.7 mm SL (1 c&s), Portuguesa, Rio Portuguesa, 8°55’59.88”N, 69°5’60”W. FMNH 103533, 7, 43.6–60.5 mm SL, Barinas, Rio Anaro ca. 10 minutes from mouth in Rio Suripa; small island and collected small arms of river, 7°53’15.4”N, 70°20’22.2”W. *Phenagoniates macrolepis*: INHS55464, 2, 32.5–34.6 mm SL, Zulia, Caño El Padre, on road from Hwy. 2 to town of KM 35, 8°45’41.796”N, 71°45’47.304”W. ANSP 150124, 5, 31.4–39.1 mm SL (2 c&s, 35.2–26.8 mm SL), Motatan, 30 km N Trujillo. INHS 28803, 9, 30.7–41.9 mm SL (1 female c&s, 35.3 mm SL), Trib. Trujillo, Rio Monay (Rio Monatan - Lake Maracaibo Dr.) 8 km N Monay at bridge, 09°36’19”N, 70°27’06”W. INHS 34701, 1, 28.3 mm SL, Zulia, Caño (Lago Maracaibo Dr.) just S of Machiques, 09°45’N, 72°30’42”W. INHS 35463, 1, 27.9 mm SL, Zulia, Rio Santa Rosa (Rio Santa Ana - Lago Maracaibo Dr.) hwy. 6 bridge, 09°39’06”N, 72°35’W. ANSP 150122, 1 (1 c&s, 28.0 mm SL), Zulia, Motilones, 30 km NW Machiquies; tributary Rio Apon. LBP 6105, 1, 25.0 mm SL, Zulia, Rio Apon Medio, Lago Maracaibo, Machiques de Perijá, 10°01’42.0”N, 72°25’58.0”W. *Xenagoniates bondi*: CAS-SU036487 (Paratype), 2, 50.7–50.9 mm SL, Monagas State, R. Amana (Amanda?), 6 km east of Santa Barbara, 35 km west of Maturin, 9°38’42.220”N, 63°28’59.239”W. ANSP 150421, 1 (1 c&s, 53.8 mm SL), Guárico, Orituco River at the bridge crossing Calabozo-Carorla highway. ANSP 165260, 1 (1 c&s, 36.4 mm SL), Apure, rio Matiyure, at Achaguas, 7°45’N, 68°11’W. FMNH 85494, 1 (1 c&s, 45.0 mm SL), Apure, rio Aruaca, 32.5 km S Biruaca, 7°32’18”N, 67°31’29”W. FMNH 103538, 1 (1 c&s, 45.1 mm SL), Barinas, Caño La Indiacita near mouth in rio Suripa ca. 35 minutes up river from boat launch in Hato Mercedes, 7°47’50”N, 69°54’33”W. ANSP 165151, 3, 50.8–57.8 mm SL, Guárico, Rio Orituco: cas. 15 km SE of Cabozo (depth to 5 ft.), 8°50’N, 67°22’W. ANSP 160008, 3, 36.7–42.3 mm SL, Bolívar, Caño Caiman, at crossing of Caicara - Puerto Ayacucho highway, 19.2 km W of Ciudad Bolivar-Caicara highway. INHS 54562, 3, 40.0–46.5 mm SL (1 male c&s, 46.5 mm SL; 1 female c&s, 40.0 mm), Portuguesa, Caño Maraca, on rd. from Guanare to Guanarito at 60 km marker. LBP9937, 2, 37.5–37.9 mm SL, Guárico, Cabruta, Rio Manapire, Orinoco drainage, 7°52’04.1”N, 66°12’40.1”W.

ANEXO 3: Supporting Information 3 – Morphological data based on an extended matrix of Mirande (2019). Matrix based on 543 characters for 54 species of the "Clade B" plus Spintherobolinae of the family Characidae. Coding as follows: Polymorphic characters "(01) or (012)" ; Unknown characters "?" ; Non-applicable "-".

Acestrocephalus sardina

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Aphyocharacidium bolivianum

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Aphyocharax anisitsi

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Aphyocharax dentatus

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Aphyocharax nattereri

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Atopomesus pachyodus

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Axelrodia lindeae

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Charax leticiae

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Charax stenopterus

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Cheirodon interruptus

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Cynopotamus argenteus

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Exodon paradoxus

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Galeocharax humeralis

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Galeocharax gulo

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Heterocheirodon yatai

1010101-00000000010100-0-010-010010010000?00000000101000000011(01)1010-0110001-00100--
00100011--101000---1-??01010000?01-0110??0-101001000?0000101010000100000000 0(01)0-1?1100000--?--
-111000000011001110-
00001100000?000000000000000000110000001001000010000 0101??0100010001100100000101000????0001?1

0001110000000001??0000010010010010000110000110001010001000?0000000000000000000100010001001110
0000010000001111(01)0000100110001110000011110000010001010(01)001100111000110??10?00101100000
00(01)0?0001000101???0010?0000000000000000??0?1101000????????????????????????????

Microschemobrycon casiquiare

101000100000000000101000000010-0100100100000000(01)000001010000000011(01)1010-0110001-
00000000010000000101000-00000000011--000011110100-
1010010000000000010100001000000000000101000000------1--111100111101000-
01100000000110000000000100000110000001001000010(01)0001001(01)010001000110010000010100010100
0011100001111000000010000000101100100100001(01)0000110001010001000000000000000000000001000100
010011000000010000001000000001001000011100000(01)11(01)00000100010100001100??????????????0010
11010010000?(01)0010001011100010000000000000000000??00??0?00??00000??????????????????????

Odontostilbe microcephala

1010001000000100000110100010-01001001000000000000000101000000001111010-0110001-
00100000001000000100010011000000000010000100101100-
101010010000000001010100001000000000100101100000------111110000011001110-
0(01)0011000000010000100000010000011001000100100001000001011001000100011101000001010001100000
11100?111100000000100000001001001001000011000011000101000100000000000000000000000010001000100
111000001100000011000000010011001111000000111000001000101000011001110000100?00?00101100000
0000000010001011100010-0000000000000000??00010100??11100?????????????????????????

Odontostilbe paraguayensis

101000100000000000000100100010-0100100100000(01)0000000101000000001011010-0110001-
00101000010000010001000-00000001010000100101100-
101010010000000001010100001000000000100101100000------111100000011001110-
0(01)00110000000100000000001000001100(01)00010010000100000010110010001000111010000010100011000
0011100?0011100000001000000010010010010000100000110001010001000000000000000001-
0001000100010011000000011000000010000110010011001111000000011100000010001010000110011100001001
0010010110000?00(01)0000010001010100010-
00000000000000000?00(01)101000011100???????????????????????

Odontostilbe pequira

1010001-0000010(01)000110100010-01001001000000000000000101000000001111010-0110001-
001000000010000010001000-00000000010000100101100-
101010010000000001010000001000000000100101100000------111100000011001110-
0(01)001000000?10000000000(01)00000110000001001000010000010(01)10010001000110010000010100011
000001110000001100000000100000001001001001000011000011000101000100000000000000000(01)010001000
100010011(01)0000011000000110000000100110011110000000111000001000101000011001110000100000100
10110000000000000010001011100010-000000000000000000?000101000011100?????????????????????

Paragoniates alburnus

100?00??10?0000?0?01?01?1010-010000-0010000?0?00001?1?????001001010-011?001-
0????1?001?000000?01000-11000(01)0?010000?000?1101110101?010??000000-
100000?1000000000000101000000?0?--?1--
100000?11111000000100000000??000???00?000?0011000000010010000(01)?00001001001000100?11001000
001010?0001000011100000??100000001???000010010?10?100?011(01)0011?010??0000100001000000000000
1-
0(01)(01)???0?0001101(01)000?000(01)000000111110000100100001(01)1001011(01)11110001000101000011

000-------------001?????00?0001?0001000????100???-00000000000000000?00??0?0000?????-------0----------
03011

Phenacogaster franciscoensis

1000001000000000000010000000000100100100000000000000111000000001001010-0110101-
000000000?0100000101000-000000000100001001011100-1010(01)001000000000-
100000010001001000001010000010001-
10111111(01)000111100000010000000000100000000000(01)000001100000000010000000000100100100010000
11001010001010000001000011(01)0000011100010110000010100100110100001100001100011100000000000000
0000000010100010001000110000000000100000001000000000100100001110000111111000010001010000110000
11100010000000100101100000000000000100010111000110000000000000000??00100100????????????????????
???????????

Phenacogaster tegatus

1000001000000000000010000000001010010010000000000000011100000001001010-0110101-
0000000002010001010100 0-00000000010000100-0110100-101001000000000-
100000010001000000000101000010001-
1011011110001111000000100000000001000000000000(01)00000110000001001000000000001101001000100011
00101000101000101000011100000111000101100100101001001101000011000011010101000000000000000000
00001000001000100010(01)000000001000000110001100100100001110000111111000100010100001100110
0100-----
00?0010110000?0000?0001000101110001100000000000000000??00??0100?????????????????????????????

Phenagoniates macrolepis

10000010-00000000-?1001010(01)00010000-001000000?00011(01)1100000001011010-0110?01-
00000100010000000101000-010000000100?001100-10111010010100000110-
100000?1001000000000101000000------
11111(01)000?11111000001000000000?10000000000000000011000000110100001000001001001000010001100
1000001010001110000111000000111000000010000000100100100100001110001?010100000010000000000000000
0001-000???0100010010000?000000000011111000000010000(01)01001011(01)11110001001101000011000--
-----------00000---00(01)000??0001100??11?00000-00000000000000000?00110?100??????-------0----------
00030

Prionobrama filigera

100?00??100000000001?0101010-010010100(01)00000000000011111000?001111010-0110001-
00001(01)0001000112-101000-1100010(01)0100001110?11011101011010000000000-
100000010000000000000101000000------1--
11100001111100000100000000000100000000000010000011000000100100001000001001001000100011001000 0
010100010000001?1000001100000011??000001001001001000011000011000100000100000000000000000111-
000??0010001?0?????0000(01)0000001111000000100100001110000111111000010001010000110011100000100
1200001?110000000?0000010001011??0????0000000000000000??00??0100??11100210010211001111201022
10

Prionobrama paraguayensis

100000--1000000000001001010 10-0100101001000000000000111110000001111010-0110001-
0000110001000(01)12-101000-1100000000010000(01)110011011101011010000000000-
100000010000000000000101000000------1--
1111001111100000010000000000010000(01)000001000001100000010010000100000100100100010001100100
0001010001000000011100000110000100100(01)000010010010010000110000110001000000100000000000000000
101-

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000?0?01000100?????000000000000111110000000100001(01)1000011(01)111000000000101000011001110000
1101200001?110000?000000001000??10100000-
00000000000000000?001?010000111002221102111011002000120
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Prodontocharax melanotus

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10100011000001000001100(01)00010-010010010000(01)(01)00?0000101000000001111010-0110001-
00?0000001000001010100-0000000(01)010000100101100-10101001000000010-
1010000100000000(01)100101000000------1111000000011101(01)00-
110000000001100000000001000001100000001001000010000001011001000101011001000000101100110000011
1000111(01)000000010000000100100100100001100001100010100010000000000000000000000010001000100
110000001100000011000110010011000111000000110000001000101000011001110000(01)00??00?001011000
00000000001000??10100010?0000000000000000??000101000?????????????????????????????
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Roeboexodon guyanensis

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11000?10000100000001010000001010011010000000000010010100000001(01)01010-0110001-0000101?0-
000000000100(01)-00?00000010000100001100-??10--01000100000-1000000100000-00(01)0001110100??-----
--------0001111000000?0-
0000000?0000000000?000000110100001001100(01)100000100100100010001100100000100100000000001110
000011100000001100000001011001001000001(01)0000100001010001000000000000000000000001000100010011
0000000100000001100000001001000011100000011100000010001010000110011?????????????001?110000?0000
?0001??0??11100010?0000000000000000???0??0?00??????????????????????????????
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Roeboides descalvadensis

```
100-0010001000100001-100000010101100100000000001010111000(01)0000001000-0100001-
000010000201011--0011011000101000100(01)0100101100-11100001000000000-
1000000100000001000001000100??--------------00111100000010000000000000000000001000000011101100-
0011000100000110100100010001000101010100100000000011100000011(01)0001011101011010010011010000
0110000110001110(01)1000000000000000000(01)1000100010001100000000000100000010000110010001(01)0
001110110111111100010(01)01010000110011----???--??-001011000-
01000000010011111100011000000000000000000??000?0000??11100???????????????????????
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Roeboides microlepis

```
100000100010001000010000000001010100-1010000000101011100010000001000-0100001-001010000201011-
-001101100010(01)00010000100101100-11100001000000000-1000000100000001000001000100??-------------
00111100000010000000000000000000001000000011111110(01)-
00110011010001101000000100010001010101001001000000111000000110000010111100110100100110100001
10000110001110110000000000000000000000000010001000110000000000010000000100001100100110001110110
111111100010101010(01)001100110000-----00-
0000110000000011000100111111000110000001000000000000??000?0000??11100?????????????????????
```

Serrapinnus calliurus

```
101?00100000000-010110100010-0100100100000(01)000000001010000000001111010-0110001-
001000000100000010001000-00000000101000010010110100-
1010010000000001010100001000000000100101100000------111100000011001110-
000011000000010000000000010000011001000100100001000000101100100010101110100000101000110000011
10000011100000001000000010010010010000100000100001010001000000000000000000000100010001000100(01
)00000000100000001000001001001100011100000011100101100010111101100111000110001010010110000?0
0(01)000001000101010001000000000000000000?00(01)?01000011100???????????????????????
```

Serrapinnus notomelas

1010001000000000010110100010-0100100100000100(01)000101000000001111010-0110001-
0010000000100000101010000-0000000101000010(01)10110100-
101001000000001010100001000000000100101100000------11110000000011001110-
000011000001100000000000100000110010(01)10010000(01)000001011001000100011(01)01000001010001 1
00000111000000111000000010000000100100100100001000001000010100010000000000000000000?000100010
0010010000000001000000100001100100110001110000000111001011000101111011001110001100010100101 1
0000000(01)0000010001010100010000000000000000000??000?01000011100??????????????????????

Spintherobolus ankoseion

1010100-0000000(01)000---000010-010000-100000000000001011000?0011(01)0-10-0110001-00--0---0--1-11-
-10-------001---011--0?11--110100-101001000000000010100000-0010000000000101000000------
111111(01)00111(01)00000-011000000000001000?000000(01)0000-0--0001010010100100-
0010011010001100(01)10010000010100011001001 1000??111?????0?1000000001011001001 00001(01)000011
01011-000100-100?-01-01---00(01)001--
001000100(01)000000000000000010000000001001(01)00011100100101000100 1(01)00101111011001010001 01
??---001011000??-0(01)---0-110-101???0010000000000000000-000??0?????100??????????????????????????????

Spintherobolus papilliferus

1010100-0000000000000100000010-010000-1000000000000001011000000011(01)1(01)10-0110001-00--0---0--1-
11--10-------001---011--0?11--110100-1010010000000000(01)0100000-001000010001010000000------
111111(01)00111(01)00000-01000000000001000000000000010000-0--0001010010100100-
0010010010001100(01)1001000000101000?????001?000????1???????1??00000100100100100001100001101 01
1-000----100?-01-01---001101--
0010001001(01)0000000000000000111000000010010000111000000100000?001000101?1?0110010100010111---
?????????????00???0?110?101???0010000000000000000?000??0?00??100????????????????????????????

Tetragonopterus argenteus

10000010000000000(01)01000000000101001(01)01001000000100010101100000001010-0110001-
0000000000001000101010011000000000100000000011100-
111000010000000000110000001000000000000001010000010000-10110100000011(01)01000-
0(01)00100000110000000000000000000011100000000100001000000100100100010001100100000010100000000000
1110010011000000011101000010010010010000 1(01)000010000101000000000000000000000000000000000000001
10(01)000000001000000100001100100110001110010011111(01)00010101010(01)001100111000000001100?01
10110100000(01)11100100010111000000-00000010000000000000001000000?11100??????????????????????

Tetragonopterus chalceus

1000001000(01)000000000100000000010100110100100000001000101011000(01)0001010-0111001-
00000000000010001000100110000000000100000000011100-
111000010000000000110000001000000000000001010000010000-10110100000011(01)01000-
0100100000110000000000000000000001110000000010000100000010011010001000110010000010 1000(01)000000
111001001100000001110000000100100100100001000001000010100000000000000000000000000000100010001(01
)01000000000100000000000011001001100011100000001111000010101010000110011 1000????????0110110100
000???1001000101???0????000000100000000000???0??0?00??11100??????????????????????

Xenagoniates bondi

100000--10000000000110010101 0-010001-001000000000000111110000000001001010-0110001-
00???100010?00000?0?000-1100000?010000(01)0000-100-1?10??01000?00110-
100000?100100000?011101000000--?---
1111??0000111110 0000(01)000000000?10000???10000000001100000001101000010000010010010 00100011001
00000100?10101000011100000011100000000100?0000100100100100001110001101 0??00001100000000000000000

0000(01)1--001000110??????0000000000111110010000100000(01)01001011(01)11110001000101000011000---
----------001?110000?0000?000111???11??0????0000000000000000?00??0?0000?????-------0----------00020

Macropsobrycon uruguayanae

101000110000000000?0100000010-01001001001000?0000001(01)10????001101010-?110?01-
001(01)00000100000101010000---00000001000010(01)--1101?0-1?1001000000---
01010000100000000010010(01)000000------1111(01)000000000---0-
00000000000110000000000000000011001000100100001000001011001000100011001000001010000????0001?1
0000110000000001101000010000010010000110000110(01)0101000100000000000000000101000010010001001
100000001000000111100000100110001110000011110000010001010000110011100101001000000101100000?0
0?0?0?010001010100??????00000000000000??0001010010????????????????????????????

Protocheirodon pi

1010001(01)(01)0000000010100(01)00010-01001000000000000----1010000000000101??0110?01-00??0-
?0010?01---?01000---000(01)01010000000--1100-
??101(01)01000000000001010000100000000001001010000000------111111100011001000-
0(01)000000000?00001000000000000010-
0000(01)1001000010000010010010001000110010000010110O1(01)1000011100000111000(01)??1??00000100
100100100001100001101010000010000000000000000011-
00001?0100010110000000000000000010000110010010000111000?01111111000100010100001100?????????????
?????????????0????00100?101??????????0000000000000??00????00??????????????????????????????

Odontostilbe fugitiva

1010001000000000100011010001 0-0100100100000000000000101000000001111010-0110001-
001110000100000100010000-000000010100001001011000-
??1010010000000001010100001000000000100101100000------111110000011001110-
01001100000(01)10000000000(01)000001100100000010000100000101100100010001110100000101000110000
00111000001110000001000000010010010010000110000110001010001000000000000000000010001000100 01
0011?0000001000000100001100100110011110000001110000010001010000110011100 00??????????????????
0????001000101??????????0000000000000??00????0000????????????????????????????

Odontostilbe pulchra

1010001000000(01)00010100100010-01001001000000000000001010000000011(01)1010-0110001-
0010(01)000010000010001000-0000000101000010010110 0-
101010010000000001010100001000000000100101100000------111100000011001110-
0(01)001100000?100000000001000001(012)0010001001000010000010110010001 00011(01)0100000101000 1
100000111000000111000000 1(01)00000010(01)100100100001(01)000011000101000100000000000000000010
00100010001001(01)000000(01)1000000100000100100110001110000001110000010001010000110011100001
0000000010110000?00????001000101??????????0000000000000??00????0000????????????????????????????

Pseudocheirodon terrabae

1000001000000(01)00000100100010-0100100100000(01)0000000101000000001011010-0110001-
0000(01)0000100000101010 00-
0000000001000010010110??10101001000(01)000110101000010000000001001011000 00------
111100000011 1(01)1110-
(01)1001100000?00000000000(01)000001100000010010000100000101100100010101110100000101000110 00
001110000011000000011000000100100100100001(01)0000110001010001000000000000000001-
00010001000100 1(01)0000000100000010000 0(01)00100110001110000001110000010001010000110011100 01
1000000001011000 0-00----001000101??????????0000000000000??0001??000??????????????????????????????

Compsura heterura

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10100010000000000010100100010-0100100100000000000000101000000001111010-0110001-00100000010001--
-101000-000000010100001001 0110--0-10100100000000101010000100000000100101100000------
111100000011(01)01110-
00001100000?100000000000100000110010001001000010000010110010001010 11(01)0100000101000110000 0
11100000111000000010000000100100100100001000001100010100010000000000000000001-
00010001000100100000000100000001000000001001100011100000011100000100010100001100111000111 00
0000010110000-00----011000101??????????00000000000000??00????001??????????????????????????????
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Amazonspinther dalmata

```
10?000??0000000000001000000 10-0?0000-100?00000000000 10100??00011(01)0-10-0110001-00--0---0--1-11--
10-------1-1---011--0111--110100-1010010000000000?0100000-0010000?000101000000---------
110000111100000-0(01)000000000010000000000100000 0--0000010010100100-
0010010010001000(01)000101100100100110010 01?0000?111?00001-
1??10000100100100100001(01)00001101010-000100-1000001-01---00-101--
0010001001000000 0?0000000100000000100110001110 0?001000000000100010100001100???????????????????
???????0????001?????????001000000000000000?000??0???0?000??????????????????????????????
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Serrapinnus microdon

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101000100000000000001(01)0100010-0100100100000000000000101000000001111010-0110001-
0010000001000001 0(01)01000-00000000010000100101 10100-
101001000000001010100001000000000100101100000------1111(01)000001(01)001100-
00001000000(01)10000000000(01)000001100(01)00010010000100000101100100010001 1(01)0100000101000
1100000111000000110000000100000001001001001000010000 01(01)0001010001(01)00000000000000000001000
?00010001001(01)0000000100000011(01)0000001001100011100000011100101100010111101100111000 1110
1101001011000000 0?00000100010101000100000000000000000??000101000011100??????????????????????
?
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Leptagoniates steindachneri

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??????????????????????????????????0??????????0?????????????????????????????????????????????000?????????
?????????????????????0-1????????????????-1?????????????????????1000000-----?-
??1111???1111??????1????000???????????????????????????????????????????????????????????????????????????
??????????????????????????????????????????????111?????????????????????????????????????1????????????????
????00?????11111???????1????101????11(01)1111000???????000?????0-------------
0????????????0???00??????????????????00??0?????????????????0???????-------0----------01012
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Aphyocharacidium n. sp.

```
??????????????????????????????????1??????????1?????????????????????????????????????????????001?????????
?????????????????????0-
0?????????????????1?????????????????????????????00????????????????111????????????1??????????????????????
????????????????????????????????????????????????????????????????????????????????????????????????????110
????????????????????????????????????????????????????????????(01)0?????1(01)000????????????10?????0101(01
)00100???????111?????11100010102201 0???????????????????????????????????????????????????????????00????
???22211020102101031110100
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Aphyocharax alburnus

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??????????????????????????????????1??????????0?????????????????????????????????????????????012?????????
?????????????????????111?????????????????1???????????????????????????00????????????????111????????????
```

0???
???????????????????1(01)0???(01)0?????111(01)0??
??????????1(01)?????0001(01)00000???????000?????111000000100010????????????????????????????????????
?????????????????????00???????0010(01)(01)10100001000101210

Aphyocharax avary

?????????????????????????????????1??????????0??012?????????
?????????????????????111?????????????????1??????????????????????????00????????????????111????????????
0???
???????????????????1(01)0???(01)0?????11(01)(01)
0????????????1(01)????00(01)1(01)00000???????000?????111000000112010???????????????????????????????
???????????????????????????00???????10001010110101011001210

Aphyocharax colifax

?????????????????????????????????1??????????0??012?????????
?????????????????????111?????????????????1??????????????????????????00????????????????111????????????
0???
???????????????????110???(01)0?????111(01)0????
????????11?????0001(01)00000???????000?????111000000100000??
?????????????????00???????10101010100111001001210

Aphyocharax erythrurus

?????????????????????????????????1??????????0??012?????????
?????????????????????111?????????????????1??????????????????????????00????????????????1??????????????
0???
???????????????????110???10?????111(01)0???????
?????11?????00(01)1(01)00000???????000?????111000000102000???
????????????????00???????2000(01)(01)00110011010102210

Aphyocharax gracilis

??0??012?????????
?????????????????????111?????????????????1??????????????????????????00????????????????111????????????
0???
???10????????????????????
??11?????001110000??????????00??????????????????0???
??????00??????????????0???????????1210

Aphyocharax pusillus

?????????????????????????????????1??????????0??(01)12??????
????????????????????????111?????????????????1??????????????????????????00????????????????111?????????
???0??
??????????????????????110???(01)(01)?????11100?
???????????1(01)?????00(01)1(01)00000???????000?????111000000002010????????????????????????????????
?????????????????????????00???????20011120110101011001210

Aphyocharax rathbuni

?????????????????????????????????0??????????0??012?????????
?????????????????????1(01)(01)?????????????????1??????????????????????????00????????????????10-

????????????0??
???????????????????????????????1(01)0???(01)0???
??11(01)(01)0????????????11?????01(01)1(01)00000???????001?????111000101102010????????????????????
????????????????????????????????????00???????21200010111201011100110

Aphyocharax yekwanae

?????????????????????????????????0??????????0??012?????????
?????????????????????100?????????????????1????????????????????????00????????????????1??????????????
0??
???????????????????110???10?????11100?????????
???11?????0001100000???????001?????111000001002010???
?????????00???????20010110110101001000210

ANEXO 4: Supporting Information 4 – Posterior probabilities of marginal ancestral state estimation from 1,000 simulations under stochastic mapping with “make.simmap” function (phytools – Revell, 2012). Discrete character models of evolution: “ER”– equal rates; “ARD” – all-rates-different; “SYM” – symmetric.

Ch. 440 – Bony hooks on fin rays: (0) absent ; (1) present.

Tree nodes	**ER**		**SYM**	
	“absent”	“present”	“absent”	“present”
16	0.251	0.749	0.241	0.759
17	0.292	0.708	0.286	0.714
18	0.012	0.988	0.007	0.993
19	0.000	1.000	0.000	1.000
20	0.000	1.000	0.000	1.000
21	0.000	1.000	0.000	1.000
22	0.000	1.000	0.000	1.000
23	0.000	1.000	0.000	1.000
24	0.000	1.000	0.000	1.000
25	0.007	0.993	0.003	0.997
26	0.000	1.000	0.000	1.000
27	0.920	0.080	0.897	0.103
28	0.991	0.009	0.985	0.015
29	0.999	0.001	1.000	0.000

Ch. 441 – Anal-fin bony hooks in adult males of species bearing hooks on fins: (0) absent ; (1) present.

Tree nodes	**ER**		**SYM**	
	“absent”	“present”	“absent”	“present”
16	0.241	0.759	0.220	0.780
17	0.276	0.724	0.250	0.750
18	0.007	0.993	0.007	0.993
19	0.000	1.000	0.000	1.000
20	0.000	1.000	0.000	1.000
21	0.000	1.000	0.000	1.000
22	0.000	1.000	0.000	1.000
23	0.000	1.000	0.000	1.000
24	0.000	1.000	0.000	1.000
25	0.001	0.999	0.003	0.997
26	0.000	1.000	0.000	1.000
27	0.920	0.080	0.913	0.087
28	0.994	0.006	0.987	0.013
29	0.999	0.001	1.000	0.000

Ch. 442 – Pelvic-fin bony hooks in adult males of species bearing hooks on fins: (0) absent ; (1) present.

Tree nodes	ER		SYM	
	“absent”	“present”	“absent”	“present”
16	0.231	0.769	0.243	0.757
17	0.259	0.741	0.283	0.717
18	0.010	0.990	0.009	0.991
19	0.000	1.000	0.000	1.000
20	0.000	1.000	0.000	1.000
21	0.000	1.000	0.000	1.000
22	0.000	1.000	0.000	1.000
23	0.000	1.000	0.000	1.000
24	0.000	1.000	0.000	1.000
25	0.005	0.995	0.006	0.994
26	0.000	1.000	0.000	1.000
27	0.902	0.098	0.912	0.088
28	0.994	0.006	0.995	0.005
29	1.000	0.000	1.000	0.000

Ch. 446 – Bony hooks on base of pelvic-fin rays of adult males: (0) absent, or in small number compared to on segmented portion* ; (1) as numerous as on segmented portion of rays**.

Tree nodes	ER		SYM	
	“absent or fewer”	“similar”	“absent or fewer”	“similar”
16	0.663	0.337	0.679	0.321
17	0.835	0.165	0.856	0.144
18	0.846	0.154	0.869	0.131
19	0.454	0.546	0.451	0.549
20	0.912	0.088	0.885	0.115
21	0.990	0.010	0.989	0.011
22	1.000	0.000	1.000	0.000
23	0.266	0.734	0.256	0.744
24	0.093	0.907	0.098	0.902
25	0.885	0.115	0.894	0.106
26	1.000	0.000	1.000	0.000
27	10.941	0.059	0.946	0.054
28	0.975	0.025	0.982	0.018
29	0.995	0.005	0.995	0.005

Referred as: *“absent or fewer”; **“similar”.

Ch. 447 – Bony hooks on last pelvic-fin ray of adult males: (0) absent or reduced in number* ; (1) as numerous as in other rays**.

Tree nodes	**ER**		**SYM**	
	“absent or reduced”	“similar”	“absent or reduced”	“similar”
16	0.988	0.012	0.988	0.012
17	0.997	0.003	1.000	0.000
18	0.996	0.004	0.997	0.003
19	1.000	0.000	1.000	0.000
20	1.000	0.000	1.000	0.000
21	1.000	0.000	1.000	0.000
22	1.000	0.000	1.000	0.000
23	1.000	0.000	1.000	0.000
24	1.000	0.000	1.000	0.000
25	0.990	0.010	0.988	0.012
26	0.000	1.000	0.001	0.999
27	1.000	0.000	1.000	0.000
28	0.999	0.001	1.000	0.000
29	1.000	0.000	1.000	0.000

Referred as: *“absent or reduced”; **“similar”.

Ch. 448 – Bony hooks on first pelvic-fin ray of adult males: (0) absent ; (1) present.

Tree nodes	**ER**		**SYM**	
	“absent”	“present”	“absent”	“present”
16	0.495	0.505	0.509	0.491
17	0.510	0.490	0.506	0.494
18	0.475	0.525	0.503	0.497
19	0.497	0.503	0.487	0.513
20	0.510	0.490	0.494	0.506
21	0.509	0.491	0.508	0.492
22	1.000	0.000	1.000	0.000
23	0.496	0.504	0.485	0.515
24	0.484	0.516	0.500	0.500
25	0.526	0.474	0.502	0.498
26	0.493	0.507	0.498	0.502
27	0.482	0.518	0.499	0.501
28	0.509	0.491	0.534	0.466
29	0.503	0.497	0.459	0.541

Ch. 450 – Distribution of bony hooks on pelvic-fin segments of adult males: (0) bilateral, at least in some segments* ; (1) unpaired, oriented medially** ; (2) present only in the medial hemitrichia** ; (3) absent.

Tree nodes	**ER**			
	"bilateral"	"unpaired"	"medial hemitrichia"	"absent"
16	0.340	0.093	0.212	0.355
17	0.445	0.047	0.084	0.424
18	0.957	0.009	0.010	0.024
19	1.000	0.000	0.000	0.000
20	0.992	0.007	0.001	0.000
21	0.999	0.001	0.000	0.000
22	1.000	0.000	0.000	0.000
23	1.000	0.000	0.000	0.000
24	1.000	0.000	0.000	0.000
25	0.965	0.008	0.008	0.019
26	0.855	0.143	0.000	0.002
27	0.083	0.015	0.017	0.885
28	0.006	0.005	0.003	0.986
29	0.000	0.000	0.000	1.000

*Referred as: *"bilateral"; **"unpaired"; ***"medial hemitrichia"

Ch. 451 – Position of anal-fin bony hooks of adult males: (0) paired and ordered laterally or posterolaterally* ; (1) medially positioned and oriented posteriorly** ; (2) asymmetrically disposed and irregularly arranged*** ; (3) absent.

Tree nodes	**ER**			
	"paired"	"medial"	"asymmetric"	"absent"
16	0.039	0.044	0.661	0.256
17	0.022	0.017	0.658	0.303
18	0.001	0.002	0.990	0.007
19	0.001	0.000	0.999	0.000
20	0.002	0.000	0.998	0.000
21	0.026	0.001	0.972	0.001
22	0.000	0.000	1.000	0.000
23	0.001	0.000	0.999	0.000
24	0.009	0.001	0.990	0.000
25	0.000	0.000	0.995	0.005
26	0.000	0.000	1.000	0.000
27	0.007	0.006	0.081	0.906
28	0.000	0.001	0.011	0.988
29	0.000	0.000	0.000	1.000

Referred as: *" paired"; **" medial"; ***"asymmetric".

Ch. 452 – Number of anal-fin hooks of adult males: (0) three or more on each ray with hooks* ; (1) only one or two hooks on each ray** ; (2) absent.

Tree nodes	**ER**		
	"three or more"	"one or two"	"absent"
16	0.873	0.006	0.121
17	0.849	0.004	0.147
18	1.000	0.000	0.000
19	1.000	0.000	0.000
20	1.000	0.000	0.000
21	1.000	0.000	0.000
22	1.000	0.000	0.000
23	1.000	0.000	0.000
24	1.000	0.000	0.000
25	1.000	0.000	0.000
26	1.000	0.000	0.000
27	0.035	0.002	0.963
28	0.004	0.000	0.996
29	0.000	0.000	1.000

Referred as: *"three or more"; **"one or two".

Ch. 453 – Distribution of bony hooks on anal-fin branches of adult males: (0) on all branches* ; (1) restricted, or almost restricted, to posterior primary branch** ; (2) absent.

Tree nodes	**ER**		
	"all branches"	"restricted"	"absent"
16	0.324	0.309	0.367
17	0.172	0.356	0.472
18	0.154	0.744	0.102
19	0.000	1.000	0.000
20	0.001	0.999	0.000
21	0.003	0.997	0.000
22	0.049	0.951	0.000
23	0.000	1.000	0.000
24	0.000	1.000	0.000
25	0.179	0.738	0.083
26	0.999	0.001	0.000
27	0.064	0.086	0.850
28	0.020	0.024	0.956
29	0.001	0.002	0.997

Referred as: *"all branches"; **"restricted".

Ch. 521 – Distribution of bony hooks on branched pelvic-fin rays of adult males: (0) from 1st to the 4th branched ray* ; (1) from 1st to the 5th branched ray** ; (2) from 1st to the 6th branched ray*** ; (3) absent.

Tree nodes	**ER**			
	"first to fourth"	"first to fifth"	"first to sixth"	"absent"
16	0.101	0.118	0.485	0.296
17	0.064	0.098	0.479	0.359
18	0.022	0.110	0.812	0.056
19	0.001	0.007	0.992	0.000
20	0.021	0.037	0.941	0.001
21	0.057	0.017	0.921	0.005
22	0.000	0.000	1.000	0.000
23	0.001	0.005	0.994	0.000
24	0.000	0.001	0.999	0.000
25	0.021	0.126	0.801	0.052
26	0.000	0.000	1.000	0.000
27	0.021	0.051	0.096	0.832
28	0.008	0.007	0.012	0.973
29	0.001	0.000	0.002	0.997

Referred as: *"first to fourth"; **"first to fifth"; ***"first to sixth".

Ch. 522 – Distribution of bony hooks along pelvic-fin rays of adult males: (0) restricted to the middle length of rays* ; (1) distributed on proximal-middle length of rays** ; (2) distributed along the entire length of the ray*** ; (3) absent.

Tree nodes	**ER**			
	"restricted to middle"	"proximal to middle"	"entire length"	"absent"
16	0.076	0.617	0.053	0.254
17	0.039	0.599	0.034	0.328
18	0.006	0.971	0.006	0.017
19	0.037	0.963	0.000	0.000
20	0.922	0.075	0.003	0.000
21	0.994	0.006	0.000	0.000
22	1.000	0.000	0.000	0.000
23	0.011	0.989	0.000	0.000
24	0.001	0.999	0.000	0.000
25	0.003	0.974	0.013	0.010
26	0.005	0.866	0.128	0.001
27	0.005	0.082	0.011	0.902
28	0.003	0.006	0.003	0.988
29	0.000	0.000	0.000	1.000

Referred as: *" restricted to middle"; **" proximal to middle"; ***" entire length".

Ch. 523 – Proximal pelvic-fin ray segment bearing bony hooks on adult males: (0) on segment after primary branching* ; (1) on segment before primary branching** ; (2) on the long basal segment*** ; (3) absent.

Tree nodes	**ER**			
	“after”	“before”	“basal”	“absent”
16	0.271	0.252	0.244	0.233
17	0.272	0.267	0.220	0.241
18	0.266	0.260	0.241	0.233
19	0.266	0.249	0.232	0.253
20	0.224	0.237	0.259	0.280
21	0.252	0.259	0.218	0.271
22	1.000	0.000	0.000	0.000
23	0.270	0.243	0.249	0.238
24	0.273	0.272	0.232	0.223
25	0.235	0.262	0.247	0.256
26	0.279	0.229	0.265	0.227
27	0.232	0.258	0.246	0.264
28	0.248	0.245	0.256	0.251
29	0.264	0.242	0.243	0.251

Referred as: *“after”; **“before”; ***“basal”.

Ch. 525 – Width of bony hook at base on pelvic-fin rays of adult males: (0) same width as middle length of hook* ; (1) wider than width at middle length of hook** ; (2) absent.

Tree nodes	**ER**		
	“same width”	“wider”	“absent”
16	0.105	0.571	0.324
17	0.054	0.553	0.393
18	0.018	0.959	0.023
19	0.052	0.946	0.002
20	0.011	0.989	0.000
21	0.009	0.990	0.001
22	0.000	1.000	0.000
23	0.080	0.918	0.002
24	0.093	0.905	0.002
25	0.011	0.974	0.015
26	0.000	1.000	0.000
27	0.028	0.110	0.862
28	0.011	0.021	0.968
29	0.001	0.003	0.996

Referred as: *“same width”; **“wider”.

Ch. 526 – Distal tip of bony hooks on pelvic-fin rays of adult males: (0) retrorse ; (1) straight ; (2) absent.

Tree nodes	**ER**		
	“retrorse”	“straight”	“absent”
16	0.363	0.242	0.395
17	0.444	0.083	0.473
18	0.977	0.004	0.019
19	0.999	0.001	0.000
20	0.984	0.016	0.000
21	0.582	0.416	0.002
22	0.001	0.999	0.000
23	1.000	0.000	0.000
24	1.000	0.000	0.000
25	0.985	0.001	0.014
26	1.000	0.000	0.000
27	0.068	0.019	0.913
28	0.015	0.006	0.979
29	0.000	0.000	1.000

Ch. 527 – Number of bony hooks per pelvic-fin ray segment of adult males: (0) maximum of one hook per segment* ; (1) maximum of two hooks per segment** ; (2) maximum of three hooks per segment*** ; (3) absent.

Tree nodes	**ER**			
	“one”	“two”	“three”	“absent”
16	0.111	0.458	0.143	0.288
17	0.064	0.483	0.085	0.368
18	0.029	0.829	0.072	0.070
19	0.000	1.000	0.000	0.000
20	0.000	0.999	0.000	0.001
21	0.016	0.969	0.013	0.002
22	0.505	0.000	0.495	0.000
23	0.000	1.000	0.000	0.000
24	0.000	1.000	0.000	0.000
25	0.031	0.816	0.094	0.059
26	0.000	0.000	1.000	0.000
27	0.021	0.100	0.029	0.850
28	0.004	0.018	0.006	0.972
29	0.001	0.003	0.001	0.995

Referred as: *“one”; **“two”; ***“three”.

Ch. 528 – Anal fin shape profile in adult specimens: (0) lacking elongation of the last unbranched ray, first and second branched rays* ; (1) last unbranched ray, first and second branched rays elongated and filamentous**.

Tree nodes	**ER**		**SYM**	
	“no elongation”	“elongated rays”	“no elongation”	“elongated rays”
16	0.990	0.010	0.983	0.017
17	0.999	0.001	0.999	0.001
18	0.998	0.002	0.993	0.007
19	1.000	0.000	1.000	0.000
20	1.000	0.000	1.000	0.000
21	1.000	0.000	1.000	0.000
22	1.000	0.000	1.000	0.000
23	1.000	0.000	1.000	0.000
24	1.000	0.000	1.000	0.000
25	0.991	0.009	0.978	0.022
26	0.001	0.999	0.000	1.000
27	0.999	0.001	1.000	0.000
28	1.000	0.000	1.000	0.000
29	0.999	0.001	1.000	0.000

Referred as: *“no elongation”; **“elongated rays”.

Ch. 529 – Bony hooks on unbranched anal-fin rays of adult males: (0) absent ; (1) present.

Tree nodes	**ER**		**SYM**	
	“absent”	“present”	“absent”	“present”
16	0.228	0.772	0.226	0.774
17	0.265	0.735	0.258	0.742
18	0.009	0.991	0.004	0.996
19	0.000	1.000	0.000	1.000
20	0.000	1.000	0.000	1.000
21	0.000	1.000	0.000	1.000
22	0.000	1.000	0.000	1.000
23	0.000	1.000	0.000	1.000
24	0.000	1.000	0.000	1.000
25	0.003	0.997	0.000	1.000
26	0.000	1.000	0.000	1.000
27	0.933	0.067	0.892	0.108
28	0.996	0.004	0.988	0.012
29	0.999	0.001	1.000	0.000

Ch. 530 – Distribution of bony hooks on branched anal-fin rays of adult males: (0) from 1st to the 10th branched ray or fewer* ; (1) from 1st to the 11th branched ray or more** ; (2) absent.

Tree nodes	**ER**		
	"first to tenth"	"first to eleventh"	"absent"
16	0.320	0.341	0.339
17	0.317	0.325	0.358
18	0.304	0.362	0.334
19	0.349	0.324	0.327
20	0.329	0.333	0.338
21	0.348	0.322	0.330
22	0.000	1.000	0.000
23	0.329	0.329	0.342
24	0.339	0.308	0.353
25	0.343	0.303	0.354
26	0.386	0.313	0.301
27	0.318	0.332	0.350
28	0.328	0.336	0.336
29	0.329	0.321	0.350

Referred as: *"first to tenth"; **"first to eleventh".

Ch. 531 – Distribution of bony hooks along anal-fin rays of adult males: (0) restricted to middle length of rays* ; (1) distributed on proximal-middle length of rays** ; (2) distributed on middle-distal length of rays*** ; (3) absent.

Tree nodes	**ER**			
	"restricted to middle"	"proximal to middle"	"middle to distal"	"absent"
16	0.332	0.074	0.248	0.346
17	0.424	0.037	0.098	0.441
18	0.987	0.001	0.000	0.012
19	1.000	0.000	0.000	0.000
20	1.000	0.000	0.000	0.000
21	1.000	0.000	0.000	0.000
22	1.000	0.000	0.000	0.000
23	1.000	0.000	0.000	0.000
24	0.984	0.015	0.001	0.000
25	0.994	0.000	0.000	0.006
26	1.000	0.000	0.000	0.000
27	0.045	0.008	0.010	0.937
28	0.006	0.000	0.001	0.993
29	0.000	0.000	0.000	1.000

Referred as: *"restricted to middle"; **"proximal to middle"; ***"middle to distal".

Ch. 533 – Position of insertion of bony hooks on anal-fin rays of adult males: (0) only posterolateral* ; (1) both posterolateral and anterolateral** ; (2) absent.

Tree nodes	**ER**		
	"posterolateral"	"both"	"absent"
16	0.322	0.304	0.374
17	0.371	0.175	0.454
18	0.764	0.146	0.090
19	1.000	0.000	0.000
20	1.000	0.000	0.000
21	0.999	0.001	0.000
22	0.942	0.058	0.000
23	1.000	0.000	0.000
24	1.000	0.000	0.000
25	0.738	0.167	0.095
26	0.000	1.000	0.000
27	0.113	0.053	0.834
28	0.021	0.016	0.963
29	0.002	0.002	0.996

Referred as: *"posterolateral"; **"both".

Ch. 534 – Width of bony hook at base on anal-fin rays of adult males: (0) same width as middle length of hook* ; (1) wider than width at middle length of hook** ; (2) absent.

Tree nodes	**ER**		
	"same width"	"wider"	"absent"
16	0.043	0.712	0.245
17	0.026	0.681	0.293
18	0.006	0.979	0.015
19	0.000	1.000	0.000
20	0.000	1.000	0.000
21	0.000	1.000	0.000
22	0.000	1.000	0.000
23	0.000	1.000	0.000
24	0.000	1.000	0.000
25	0.007	0.982	0.011
26	0.121	0.877	0.002
27	0.005	0.085	0.910
28	0.002	0.008	0.990
29	0.001	0.001	0.998

Referred as: *"same width"; **"wider".

Ch. 535 – Distal tip of bony hooks on anal-fin rays of adult males: (0) retrorse ; (1) straight ; (2) absent.

Tree nodes	**ER**		
	“retrorse”	“straight”	“absent”
16	0.587	0.107	0.306
17	0.568	0.075	0.357
18	0.931	0.028	0.041
19	0.999	0.000	0.001
20	0.999	0.000	0.001
21	1.000	0.000	0.000
22	1.000	0.000	0.000
23	0.999	0.000	0.001
24	0.977	0.022	0.001
25	0.943	0.025	0.032
26	0.777	0.219	0.004
27	0.138	0.029	0.833
28	0.026	0.008	0.966
29	0.003	0.001	0.996

Ch. 536 – Number of bony hooks per anal-fin ray segment on adult males: (0) maximum of one hook per segment* ; (1) maximum of two hooks per segment** ; (2) maximum of three hooks per segment*** ; (3) maximum of four hooks per segment**** ; (4) absent.

Tree nodes	**ARD**				
	“one”	“two”	“three”	“four”	“absent”
16	0.339	0.311	0.000	0.350	0.000
17	0.215	0.777	0.000	0.008	0.000
18	0.275	0.725	0.000	0.000	0.000
19	0.216	0.784	0.000	0.000	0.000
20	0.230	0.770	0.000	0.000	0.000
21	0.306	0.694	0.000	0.000	0.000
22	0.000	1.000	0.000	0.000	0.000
23	0.220	0.780	0.000	0.000	0.000
24	0.230	0.770	0.000	0.000	0.000
25	0.333	0.667	0.000	0.000	0.000
26	0.003	0.000	0.997	0.000	0.000
27	0.025	0.069	0.000	0.000	0.906
28	0.001	0.012	0.000	0.000	0.987
29	0.000	0.001	0.000	0.000	0.999

Referred as: *“one”; **“two”; ***“three”; ****“four”.

ANEXO 5: Supporting Information 5 – Morphometric data from examined material of the subfamily Aphyocharacinae (Characiformes: Characidae). Body measurements are percentages of Standard length; Subunits of the head are percentages of Head length.

Table 7 – Morphometric data from examined material of *Aphyocharacidium bolivianum* (n=43). SD = Standard deviation.

	Min	**Max**	**Mean**	**SD**
Standard length (mm)	16.6	30.2	-	-
Percents of Standard Length (SL)				
Snout-anal fin distance	9.7	17.5	13.1	1.4
Snout-dorsal fin distance	8.3	14.5	10.9	1.2
Snout-pelvic fin distance	7.2	12.9	9.9	1.0
Snout-pectoral fin distance	4.6	7.9	6.1	0.6
Dorsal-caudal fin distance	8.9	16.9	12.7	1.3
Orbit-dorsal fin distance	5.6	10.0	7.7	0.8
Anal-fin base length	5.5	9.9	7.4	0.7
Length of caudal peduncle	2.0	4.0	3.0	0.4
Depth of caudal peduncle	1.6	2.8	2.1	0.2
Body depth at dorsal fin	4.3	7.7	6.0	0.7
Dorsal-fin length	4.6	7.7	6.4	0.6
Pelvic-fin length	2.9	4.3	3.6	0.3
Pectoral-fin length	3.3	5.3	4.3	0.4
Head length	4.9	8.0	6.2	0.5
Percents of Head Length (HL)				
Snout length	1.4	2.4	1.8	0.2
Upper Jaw length	1.9	2.9	2.3	0.2
Horizontal orbit diameter	1.9	3.3	2.5	0.2
Interorbital width	1.6	2.3	1.9	0.1

Table 8 – Morphometric data from examined material of *Aphyocharacidium* n. sp. (n=20). SD = Standard deviation.

	Min	**Max**	**Mean**	**SD**
Standard length (mm)	21.5	29.1	-	-
Percents of Standard Length (SL)				
Snout-anal fin distance	12.7	17.5	14.5	1.5
Snout-dorsal fin distance	11.1	14.8	12.6	1.2
Snout-pelvic fin distance	9.7	12.8	11.0	1.0
Snout-pectoral fin distance	6.0	8.0	6.7	0.6
Dorsal-caudal fin distance	11.3	15.7	13.2	1.4
Orbit-dorsal fin distance	7.8	10.5	8.9	1.0
Anal-fin base length	6.2	8.5	7.2	0.7
Length of caudal peduncle	2.5	3.7	3.1	0.3
Depth of caudal peduncle	2.1	3.0	2.5	0.3
Body depth at dorsal fin	6.0	9.0	7.2	0.9
Dorsal-fin length	6.5	8.5	7.3	0.7
Pelvic-fin length	3.7	5.0	4.2	0.4
Pectoral-fin length	4.2	5.7	4.9	0.5
Head length	12.7	17.5	14.5	1.5
Percents of Head Length (HL)				
Snout length	6.0	7.9	6.8	0.6
Upper Jaw length	1.7	2.2	2.0	0.2
Horizontal orbit diameter	2.3	3.0	2.5	0.2
Interorbital width	2.5	3.2	2.8	0.2

Table 9 – Morphometric data from examined material of *Aphyocharax alburnus* (n=75). SD = Standard deviation.

	Min	**Max**	**Mean**	**SD**
Standard length (mm)	26.5	57.5	-	-
Percents of Standard Length (SL)				
Snout-anal fin distance	17.0	39.6	23.6	4.3
Snout-dorsal fin distance	14.1	31.5	19.5	3.2
Snout-pelvic fin distance	12.7	28.7	17.1	3.1
Snout-pectoral fin distance	7.1	14.3	9.3	1.3
Dorsal-caudal fin distance	12.7	27.8	17.7	3.0
Orbit-dorsal fin distance	10.7	24.9	15.4	2.6
Anal-fin base length	6.0	12.4	8.1	1.2
Length of caudal peduncle	3.6	7.4	5.3	1.0
Depth of caudal peduncle	2.4	6.0	3.7	0.6
Body depth at dorsal fin	5.7	14.2	8.9	1.5
Dorsal-fin length	6.3	13.6	8.7	1.3
Pelvic-fin length	3.9	8.8	5.8	0.9
Pectoral-fin length	5.3	12.2	7.6	1.3
Head length	6.7	15.5	9.3	1.5
Percents of Head Length (HL)				
Snout length	1.6	4.1	2.5	0.4
Upper Jaw length	2.4	6.0	3.3	0.6
Horizontal orbit diameter	2.3	4.3	3.0	0.4
Interorbital width	2.1	4.3	2.8	0.4

Table 10 – Morphometric data from paratype (CAS059718) and non-type examined material of *Aphyocharax anisitsi* (n=48). SD = Standard deviation. All measurements from paratype are in mm.

	Paratype	**Min**	**Max**	**Mean**	**SD**
Standard length (mm)	28.8	21.0	31.2	-	-
		Percents of Standard Length (SL)			
Snout-anal fin distance	17.9	13.2	19.9	17.7	1.4
Snout-dorsal fin distance	17.1	11.7	17.1	15.5	1.2
Snout-pelvic fin distance	12.5	9.7	14.7	12.9	1.0
Snout-pectoral fin distance	7.4	5.5	8.6	7.4	0.6
Dorsal-caudal fin distance	12.6	10.1	15.4	13.6	1.1
Orbit-dorsal fin distance	13.5	9.1	13.7	12.3	1.0
Anal-fin base length	6.5	5.0	7.7	6.9	0.6
Length of caudal peduncle	4.6	3.1	4.5	3.9	0.3
Depth of caudal peduncle	3.1	2.0	3.3	2.9	0.3
Body depth at dorsal fin	8.1	5.5	8.9	7.7	0.7
Dorsal-fin length	7.3	5.4	8.0	7.1	0.6
Pelvic-fin length	5.3	3.5	5.3	4.8	0.4
Pectoral-fin length	6.0	4.4	6.8	6.1	0.5
Head length	7.4	5.6	8.6	7.5	0.6
		Percents of Head Length (HL)			
Snout length	1.7	1.4	2.2	1.9	0.2
Upper Jaw length	2.2	1.8	2.6	2.3	0.2
Horizontal orbit diameter	2.7	2.0	2.8	2.5	0.2
Interorbital width	2.3	1.8	2.7	2.4	0.2

Table 11 – Morphometric data from holotype (ANSP39217) and non-type examined material of *Aphyocharax avary* (n=68). SD = Standard deviation. All measurements from holotype are in mm.

	Holotype	**Min**	**Max**	**Mean**	**SD**
Standard length (mm)	42.9	25.8	43.0	-	-
		Percents of Standard Length (SL)			
Snout-anal fin distance	28.2	16.5	29.1	21.3	3.0
Snout-dorsal fin distance	21.8	13.7	22.7	17.4	2.2
Snout-pelvic fin distance	20.2	11.9	20.8	15.3	2.1
Snout-pectoral fin distance	10.3	7.0	10.2	8.4	0.9
Dorsal-caudal fin distance	21.7	12.4	21.5	16.0	2.2
Orbit-dorsal fin distance	17.9	10.6	18.5	13.9	1.8
Anal-fin base length	8.2	5.7	9.3	7.5	0.9
Length of caudal peduncle	7.8	3.7	6.2	4.7	0.6
Depth of caudal peduncle	4.5	2.5	4.8	3.3	0.5
Body depth at dorsal fin	9.9	5.8	10.4	8.2	1.1
Dorsal-fin length	9.9	6.3	10.5	8.0	1.0
Pelvic-fin length	6.6	3.9	6.9	5.2	0.6
Pectoral-fin length	8.5	5.1	9.4	6.8	0.9
Head length	10.5	6.5	10.4	8.4	1.0
		Percents of Head Length (HL)			
Snout length	3.1	1.7	2.8	2.2	0.3
Upper Jaw length	3.8	2.1	3.7	2.9	0.4
Horizontal orbit diameter	3.5	2.3	3.4	2.8	0.3
Interorbital width	3.1	2.0	3.3	2.6	0.3

Table 12 – Morphometric data from examined material of *Aphyocharax colifax* (n=47). SD = Standard deviation.

	Min	**Max**	**Mean**	**SD**
Standard length (mm)	26.2	43.7	-	-
Percents of Standard Length (SL)				
Snout-anal fin distance	16.7	28.7	22.2	3.3
Snout-dorsal fin distance	14.1	23.4	18.3	2.6
Snout-pelvic fin distance	12.2	20.5	16.1	2.4
Snout-pectoral fin distance	6.8	10.9	8.9	1.2
Dorsal-caudal fin distance	12.8	21.8	16.9	2.5
Orbit-dorsal fin distance	11.0	18.5	14.3	2.1
Anal-fin base length	6.2	10.4	8.0	1.0
Length of caudal peduncle	3.7	6.8	5.1	0.8
Depth of caudal peduncle	2.5	4.4	3.5	0.6
Body depth at dorsal fin	6.2	11.1	8.7	1.3
Dorsal-fin length	5.9	10.0	8.4	1.1
Pelvic-fin length	3.7	6.6	5.4	0.8
Pectoral-fin length	5.0	9.5	7.1	1.1
Head length	6.9	11.1	9.0	1.1
Percents of Head Length (HL)				
Snout length	1.9	3.1	2.4	0.3
Upper Jaw length	2.3	4.0	3.2	0.4
Horizontal orbit diameter	2.3	3.5	2.9	0.3
Interorbital width	2.2	3.5	2.7	0.4

Table 13 – Morphometric data from analyzed paratypes (CAS076471 ; CAS059724 ; CAS059726 ; CAS059725 ; CAS059723) of *Aphyocharax dentatus* (n=11). SD = Standard deviation.

	Min	**Max**	**Mean**	**SD**
Standard length (mm)	25.8	62.5	-	-
Percents of Standard Length (SL)				
Snout-anal fin distance	16.3	42.5	30.6	9.6
Snout-dorsal fin distance	14.5	36.2	25.5	7.6
Snout-pelvic fin distance	11.8	31.0	22.1	6.9
Snout-pectoral fin distance	6.9	15.8	12.0	3.1
Dorsal-caudal fin distance	12.1	30.5	22.2	6.3
Orbit-dorsal fin distance	11.8	27.6	20.3	5.8
Anal-fin base length	5.1	13.6	9.6	2.8
Length of caudal peduncle	4.5	8.4	6.7	1.3
Depth of caudal peduncle	2.6	6.5	4.7	1.4
Body depth at dorsal fin	6.0	16.7	11.9	3.8
Dorsal-fin length	6.5	15.1	10.7	2.8
Pelvic-fin length	4.0	10.1	7.1	2.0
Pectoral-fin length	5.3	12.3	9.4	2.5
Head length	6.7	16.3	12.0	3.5
Percents of Head Length (HL)				
Snout length	2.0	4.5	3.4	0.9
Upper Jaw length	2.3	7.5	5.3	1.8
Horizontal orbit diameter	2.5	4.5	3.7	0.7
Interorbital width	2.0	5.0	3.7	1.1

Table 14 – Morphometric data from non-type examined material of *Aphyocharax dentatus* (n=20). SD = Standard deviation.

	Min	**Max**	**Mean**	**SD**
Standard length (mm)	34.0	61.8	-	-
Percents of Standard Length (SL)				
Snout-anal fin distance	21.8	42.3	29.0	6.0
Snout-dorsal fin distance	18.2	33.2	23.7	4.4
Snout-pelvic fin distance	15.9	30.9	21.0	4.5
Snout-pectoral fin distance	8.1	16.0	11.2	2.1
Dorsal-caudal fin distance	16.6	30.3	21.7	4.2
Orbit-dorsal fin distance	14.4	26.4	18.7	3.5
Anal-fin base length	6.4	12.4	9.7	1.6
Length of caudal peduncle	5.1	9.3	6.6	1.1
Depth of caudal peduncle	3.4	6.2	4.4	0.8
Body depth at dorsal fin	8.1	14.0	10.6	1.6
Dorsal-fin length	8.0	13.8	10.2	1.9
Pelvic-fin length	5.2	9.6	6.5	1.4
Pectoral-fin length	6.8	13.0	8.9	2.0
Head length	8.4	16.3	11.4	2.2
Percents of Head Length (HL)				
Snout length	2.3	4.5	3.0	0.6
Upper Jaw length	2.8	7.4	4.5	1.5
Horizontal orbit diameter	2.8	4.8	3.6	0.6
Interorbital width	2.7	5.2	3.6	0.7

Table 15 – Morphometric data from paratypes (CAS-SU021912) (n=2) and non-type examined material of *Aphyocharax erythrurus* (n=27). SD = Standard deviation. All measurements from paratypes are in mm.

	Paratypes	**Min**	**Max**	**Mean**	**SD**
Standard length (mm)	26.5–35.0	25.0	45.7	-	-
		Percents of Standard Length (SL)			
Snout-anal fin distance	17.0–22.0	15.7	29.9	21.0	3.1
Snout-dorsal fin distance	13.9–18.7	13.4	24.7	17.3	2.5
Snout-pelvic fin distance	12.6–16.6	11.7	22.2	15.2	2.3
Snout-pectoral fin distance	7.2–9.3	6.5	11.4	8.2	1.2
Dorsal-caudal fin distance	13.0–17.2	12.4	22.1	16.0	2.2
Orbit-dorsal fin distance	10.8–14.5	10.2	19.6	13.5	2.0
Anal-fin base length	6.3–8.2	5.4	10.1	7.1	1.1
Length of caudal peduncle	3.7–5.1	4.2	7.2	5.0	0.7
Depth of caudal peduncle	2.6–3.4	2.4	4.4	3.1	0.5
Body depth at dorsal fin	6.1–8.3	5.5	10.8	7.7	1.2
Dorsal-fin length	6.4–8.3	6.0	10.8	7.8	1.1
Pelvic-fin length	4.0–5.3	4.0	7.1	5.0	0.7
Pectoral-fin length	5.1–6.7	4.7	8.9	6.6	0.9
Head length	7.4–9.7	6.2	12.0	8.4	1.2
		Percents of Head Length (HL)			
Snout length	1.9–2.5	1.7	3.2	2.3	0.3
Upper Jaw length	2.5–3.2	2.3	4.4	3.0	0.4
Horizontal orbit diameter	2.4–2.9	2.3	3.9	2.7	0.3
Interorbital width	2.1–2.7	1.9	3.5	2.5	0.3

Table 16 – Morphometric data from holotype (ANSP68864) of *Aphyocharax gracilis*. All measurements are in mm.

	Holotype
Standard length	42.5
Snout-anal fin distance	27.3
Snout-dorsal fin distance	22.4
Snout-pelvic fin distance	19.5
Snout-pectoral fin distance	10.1
Dorsal-caudal fin distance	20.9
Orbit-dorsal fin distance	18.5
Anal-fin base length	9.4
Length of caudal peduncle	7.1
Depth of caudal peduncle	4.3
Body depth at dorsal fin	8.3
Dorsal-fin length	9.3
Pelvic-fin length	5.7
Pectoral-fin length	8.8
Head length	9.7
Snout length	2.7
Upper Jaw length	3.8
Horizontal orbit diameter	2.7
Interorbital width	2.8

Table 17 – Morphometric data from examined material of *Aphyocharax nattereri* (n=20). SD = Standard deviation.

	Min	**Max**	**Mean**	**SD**
Standard length (mm)	16.2	25.2	-	-
Percents of Standard Length (SL)				
Snout-anal fin distance	9.8	15.8	13.7	1.5
Snout-dorsal fin distance	9.1	14.7	12.7	1.4
Snout-pelvic fin distance	7.9	12.0	10.6	1.0
Snout-pectoral fin distance	4.8	7.9	6.5	0.7
Dorsal-caudal fin distance	7.6	12.3	10.3	1.1
Orbit-dorsal fin distance	7.1	11.4	10.0	1.1
Anal-fin base length	4.7	7.6	6.6	0.8
Length of caudal peduncle	2.5	3.7	3.1	0.3
Depth of caudal peduncle	1.6	2.7	2.1	0.3
Body depth at dorsal fin	4.3	6.8	6.0	0.6
Dorsal-fin length	4.2	6.6	5.6	0.5
Pelvic-fin length	2.6	3.9	3.4	0.3
Pectoral-fin length	3.9	6.2	5.2	0.5
Head length	4.7	7.3	6.5	0.7
Percents of Head Length (HL)				
Snout length	1.2	2.0	1.6	0.2
Upper Jaw length	1.9	3.0	2.6	0.3
Horizontal orbit diameter	1.9	2.3	2.1	0.1
Interorbital width	1.3	2.2	1.8	0.2

Table 18 – Morphometric data from examined material of *Aphyocharax pusillus* (n=57). SD = Standard deviation.

	Min	**Max**	**Mean**	**SD**
Standard length (mm)	26.5	54.8	-	-
Percents of Standard Length (SL)				
Snout-anal fin distance	17.5	36.7	28.6	4.7
Snout-dorsal fin distance	14.2	29.3	22.9	3.5
Snout-pelvic fin distance	12.4	26.8	20.6	3.2
Snout-pectoral fin distance	6.3	13.2	10.7	1.4
Dorsal-caudal fin distance	12.9	27.1	21.1	3.4
Orbit-dorsal fin distance	11.0	23.8	18.3	2.9
Anal-fin base length	5.9	12.0	9.1	1.4
Length of caudal peduncle	3.4	8.0	6.0	1.0
Depth of caudal peduncle	2.6	5.8	4.5	0.8
Body depth at dorsal fin	6.3	14.1	10.4	1.8
Dorsal-fin length	6.1	12.3	9.9	1.3
Pelvic-fin length	4.4	8.3	6.6	1.0
Pectoral-fin length	5.6	11.1	8.7	1.2
Head length	6.9	14.0	10.7	1.4
Percents of Head Length (HL)				
Snout length	1.7	3.8	2.7	0.4
Upper Jaw length	2.1	5.1	3.6	0.6
Horizontal orbit diameter	2.4	4.0	3.2	0.3
Interorbital width	2.2	4.1	3.3	0.4

Table 19 – Morphometric data from examined material of *Aphyocharax rathbuni* (n=31). SD = Standard deviation.

	Min	**Max**	**Mean**	**SD**
Standard length (mm)	14.7	26.1	-	-
Percents of Standard Length (SL)				
Snout-anal fin distance	8.7	16.6	11.8	1.6
Snout-dorsal fin distance	7.8	14.4	10.3	1.3
Snout-pelvic fin distance	6.6	12.8	8.7	1.3
Snout-pectoral fin distance	4.1	8.4	5.4	0.8
Dorsal-caudal fin distance	7.2	13.6	9.8	1.2
Orbit-dorsal fin distance	5.9	11.7	7.9	1.2
Anal-fin base length	3.9	6.9	5.1	0.6
Length of caudal peduncle	2.1	3.8	2.8	0.4
Depth of caudal peduncle	1.1	2.7	1.6	0.4
Body depth at dorsal fin	3.4	8.5	5.0	0.9
Dorsal-fin length	3.5	7.2	5.1	0.7
Pelvic-fin length	2.4	4.8	3.4	0.4
Pectoral-fin length	2.3	5.4	3.5	0.6
Head length	4.0	7.6	5.2	0.7
Percents of Head Length (HL)				
Snout length	1.0	1.9	1.3	0.2
Upper Jaw length	1.0	2.2	1.4	0.3
Horizontal orbit diameter	1.8	2.5	2.1	0.2
Interorbital width	1.2	2.2	1.6	0.2

Table 20 – Morphometric data from paratypes of *Aphyocharax yekwanae* (n=2). All measurements are in mm.

	FMNH109275	**FMNH109277**
Standard length	23.3	28.2
Snout-anal fin distance	14.5	18.0
Snout-dorsal fin distance	12.3	15.0
Snout-pelvic fin distance	10.8	13.2
Snout-pectoral fin distance	6.2	7.1
Dorsal-caudal fin distance	11.0	13.5
Orbit-dorsal fin distance	9.3	11.4
Anal-fin base length	5.0	6.0
Length of caudal peduncle	3.1	4.1
Depth of caudal peduncle	2.1	2.5
Body depth at dorsal fin	5.3	6.5
Dorsal-fin length	5.6	6.7
Pelvic-fin length	3.7	4.4
Pectoral-fin length	4.4	5.6
Head length	6.2	7.6
Snout length	1.5	1.8
Upper Jaw length	2.1	2.5
Horizontal orbit diameter	2.2	2.6
Interorbital width	1.9	2.3

Table 21 – Morphometric data from paratypes (ANSP139717) (n=2) and non-type examined material of *Axelrodia lindeae* (n=21). SD = Standard deviation. All measurements from paratypes are in mm.

	Paratypes	**Min**	**Max**	**Mean**	**SD**
Standard length (mm)	15.1–18.0	17.7	25.0	-	-
		Percents of Standard Length (SL)			
Snout-anal fin distance	8.5–10.3	9.8	14.2	11.8	1.4
Snout-dorsal fin distance	8.0–9.7	8.8	12.5	10.4	1.1
Snout-pelvic fin distance	6.7–8.3	7.3	10.8	8.8	1.0
Snout-pectoral fin distance	4.8–5.5	5.1	7.1	6.0	0.6
Dorsal-caudal fin distance	7.9–9.3	9.6	13.9	11.6	1.3
Orbit-dorsal fin distance	5.2–6.1	5.8	8.7	7.1	0.8
Anal-fin base length	5.8–6.7	6.3	8.5	7.3	0.7
Length of caudal peduncle	1.4–1.6	1.6	3.1	2.4	0.4
Depth of caudal peduncle	1.3–1.5	1.5	2.5	1.9	0.3
Body depth at dorsal fin	4.3–5.0	4.7	7.9	6.1	1.0
Dorsal-fin length	4.4–5.4	4.5	7.0	5.7	0.8
Pelvic-fin length	2.2–2.6	2.6	3.9	3.2	0.4
Pectoral-fin length	2.9–3.5	2.8	4.7	3.7	0.6
Head length	4.6–5.5	5.2	7.3	6.1	0.6
		Percents of Head Length (HL)			
Snout length	1.3–1.5	1.3	2.2	1.7	0.2
Upper Jaw length	1.8–2.1	1.8	2.7	2.2	0.3
Horizontal orbit diameter	2. 1–2.6	2.2	3.2	2.6	0.3
Interorbital width	1.1–1.4	1.4	2.1	1.7	0.2

Table 22 – Morphometric data from examined material of *Leptagoniates steindachneri* (n=29). SD = Standard deviation.

	Min	**Max**	**Mean**	**SD**
Standard length (mm)	32.1	69.2	-	-
Percents of Standard Length (SL)				
Snout-anal fin distance	11.1	24.5	17.3	3.1
Snout-dorsal fin distance	17.4	38.5	27.9	4.7
Snout-pelvic fin distance	9.4	19.5	14.2	2.5
Snout-pectoral fin distance	6.5	12.4	9.3	1.5
Dorsal-caudal fin distance	15.5	32.4	24.8	4.5
Orbit-dorsal fin distance	13.8	32.2	23.2	4.1
Anal-fin base length	19.9	42.2	31.8	5.6
Length of caudal peduncle	1.9	4.5	3.4	0.6
Depth of caudal peduncle	2.2	5.5	3.9	0.8
Body depth at dorsal fin	6.5	16.0	11.1	2.4
Dorsal-fin length	6.3	14.2	9.9	1.8
Pelvic-fin length	2.4	5.8	3.8	0.8
Pectoral-fin length	5.4	11.2	8.6	1.5
Head length	6.4	12.1	9.3	1.5
Percents of Head Length (HL)				
Snout length	1.7	3.3	2.5	0.4
Upper Jaw length	1.9	3.7	2.8	0.4
Horizontal orbit diameter	2.9	4.7	3.7	0.5
Interorbital width	1.8	3.3	2.5	0.4

Table 23 – Morphometric data from examined material of *Paragoniates alburnus* (n=43). SD = Standard deviation.

	Min	**Max**	**Mean**	**SD**
Standard length (mm)	34.0	91.8	-	-
Percents of Standard Length (SL)				
Snout-anal fin distance	18.7	52.7	33.5	7.5
Snout-dorsal fin distance	20.5	56.0	36.7	8.3
Snout-pelvic fin distance	13.9	40.6	25.8	5.7
Snout-pectoral fin distance	9.2	23.5	15.7	3.1
Dorsal-caudal fin distance	14.7	38.7	26.1	5.5
Orbit-dorsal fin distance	15.8	45.2	29.3	7.0
Anal-fin base length	15.7	42.5	27.7	6.0
Length of caudal peduncle	2.3	6.3	4.2	1.0
Depth of caudal peduncle	3.2	10.6	6.5	1.6
Body depth at dorsal fin	10.7	33.1	20.1	5.3
Dorsal-fin length	9.6	28.2	17.7	4.1
Pelvic-fin length	6.4	17.7	12.2	2.5
Pectoral-fin length	8.4	23.9	15.9	3.5
Head length	8.8	22.8	15.2	3.1
Percents of Head Length (HL)				
Snout length	2.3	6.7	4.4	0.9
Upper Jaw length	4.7	12.6	8.1	1.7
Horizontal orbit diameter	3.0	7.4	5.2	0.9
Interorbital width	2.7	7.5	4.7	1.0

Table 24 – Morphometric data from paratypes of *Phenagoniates macrolepis* (n=3). All measurements are in mm.

	FMNH12853	**FMNH12851**	**FMNH12852**
Standard length	39.0	42.3	43.7
Snout-anal fin distance	16.2	17.1	17.8
Snout-dorsal fin distance	23.6	24.5	25.6
Snout-pelvic fin distance	13.2	13.7	14.4
Snout-pectoral fin distance	8.6	8.8	9.3
Dorsal-caudal fin distance	17.1	20.2	20.3
Orbit-dorsal fin distance	18.8	19.5	20.7
Anal-fin base length	22.2	24.1	25.1
Length of caudal peduncle	1.7	2.3	2.4
Depth of caudal peduncle	3.5	3.1	3.8
Body depth at dorsal fin	10.0	11.6	12.9
Dorsal-fin length	9.0	10.0	10.4
Pelvic-fin length	3.6	3.8	4.0
Pectoral-fin length	7.2	7.9	8.3
Head length	8.3	8.7	9.6
Snout length	2.2	2.4	2.7
Upper Jaw length	2.5	2.8	2.9
Horizontal orbit diameter	3.2	3.5	3.8
Interorbital width	2.4	2.4	2.7

Table 25 – Morphometric data from non-type examined material of *Phenagoniates macrolepis* (n=22). SD = Standard deviation.

	Min	**Max**	**Mean**	**SD**
Standard length (mm)	24.7	41.9	-	-
Percents of Standard Length (SL)				
Snout-anal fin distance	10.3	17.4	13.4	1.8
Snout-dorsal fin distance	13.7	23.1	17.9	2.5
Snout-pelvic fin distance	8.7	14.2	11.0	1.3
Snout-pectoral fin distance	5.7	8.6	7.1	0.7
Dorsal-caudal fin distance	12.0	20.1	15.9	2.2
Orbit-dorsal fin distance	10.6	19.0	14.6	2.0
Anal-fin base length	12.4	24.0	18.8	2.9
Length of caudal peduncle	1.3	2.8	1.9	0.4
Depth of caudal peduncle	1.9	3.7	2.8	0.5
Body depth at dorsal fin	5.9	12.3	9.4	1.6
Dorsal-fin length	5.6	8.8	7.4	0.9
Pelvic-fin length	2.4	3.8	3.1	0.4
Pectoral-fin length	3.8	7.0	5.9	0.8
Head length	5.8	9.0	7.2	0.8
Percents of Head Length (HL)				
Snout length	1.3	2.5	2.0	0.3
Upper Jaw length	1.6	2.7	2.2	0.3
Horizontal orbit diameter	2.4	3.5	2.9	0.3
Interorbital width	1.6	2.5	2.1	0.2

Table 26 – Morphometric data from holotype (ANSP8059) and paratype (ANSP8073) of *Prionobrama filigera*. All measurements are in mm.

	Holotype	**Paratype**
Standard length	45.9	41.1
Snout-anal fin distance	26.0	24.5
Snout-dorsal fin distance	25.3	23.5
Snout-pelvic fin distance	19.2	18.1
Snout-pectoral fin distance	10.9	10.4
Dorsal-caudal fin distance	22.0	19.2
Orbit-dorsal fin distance	20.3	19.4
Anal-fin base length	15.8	14.1
Length of caudal peduncle	6.2	4.4
Depth of caudal peduncle	4.0	3.3
Body depth at dorsal fin	11.3	10.8
Dorsal-fin length	-	-
Pelvic-fin length	-	-
Pectoral-fin length	-	-
Head length	9.7	-
Snout length	2.6	-
Upper Jaw length	4.2	4.2
Horizontal orbit diameter	3.6	-
Interorbital width	2.9	-

Table 27 – Morphometric data from non-type examined material of *Prionobrama filigera* (n=29). SD = Standard deviation.

	Min	**Max**	**Mean**	**SD**
Standard length (mm)	28.4	56.2	-	-
Percents of Standard Length (SL)				
Snout-anal fin distance	16.5	32.1	23.2	3.9
Snout-dorsal fin distance	15.4	30.0	22.7	3.6
Snout-pelvic fin distance	12.8	23.7	17.8	2.8
Snout-pectoral fin distance	7.7	12.9	10.1	1.4
Dorsal-caudal fin distance	13.2	29.1	20.3	4.0
Orbit-dorsal fin distance	12.4	25.1	18.5	3.0
Anal-fin base length	10.0	21.0	15.6	2.8
Length of caudal peduncle	3.4	7.3	4.8	1.0
Depth of caudal peduncle	2.7	6.0	4.3	0.8
Body depth at dorsal fin	7.5	17.6	11.8	2.5
Dorsal-fin length	8.0	16.6	11.8	2.3
Pelvic-fin length	5.2	17.3	9.9	3.1
Pectoral-fin length	7.1	13.5	10.2	1.7
Head length	7.1	12.3	9.4	1.3
Percents of Head Length (HL)				
Snout length	1.8	3.4	2.6	0.4
Upper Jaw length	2.8	5.3	3.9	0.6
Horizontal orbit diameter	2.8	4.7	3.5	0.5
Interorbital width	2.0	3.7	2.8	0.5

Table 28 – Morphometric data from paratypes (CAS059710 ; FMNH56682) of *Prionobrama paraguayensis* (n=5). SD = Standard deviation.

	Min	**Max**	**Mean**	**SD**
Standard length (mm)	34.8	40.3	-	-
Percents of Standard Length (SL)				
Snout-anal fin distance	20.0	23.6	22.1	1.3
Snout-dorsal fin distance	20.0	23.5	22.4	1.3
Snout-pelvic fin distance	15.3	17.7	16.8	0.9
Snout-pectoral fin distance	8.9	10.2	9.7	0.5
Dorsal-caudal fin distance	15.3	17.3	16.8	0.8
Orbit-dorsal fin distance	16.5	19.3	18.3	1.0
Anal-fin base length	13.6	15.1	14.7	0.6
Length of caudal peduncle	3.2	4.0	3.6	0.3
Depth of caudal peduncle	3.1	3.7	3.5	0.2
Body depth at dorsal fin	10.0	11.9	11.1	0.8
Dorsal-fin length	9.6	11.6	10.4	0.7
Pelvic-fin length	6.8	8.8	7.9	0.8
Pectoral-fin length	9.8	11.2	10.7	0.6
Head length	7.8	9.1	8.6	0.5
Percents of Head Length (HL)				
Snout length	1.9	2.2	2.1	0.1
Upper Jaw length	3.1	3.6	3.4	0.2
Horizontal orbit diameter	2.9	3.5	3.3	0.2
Interorbital width	2.3	2.8	2.6	0.2

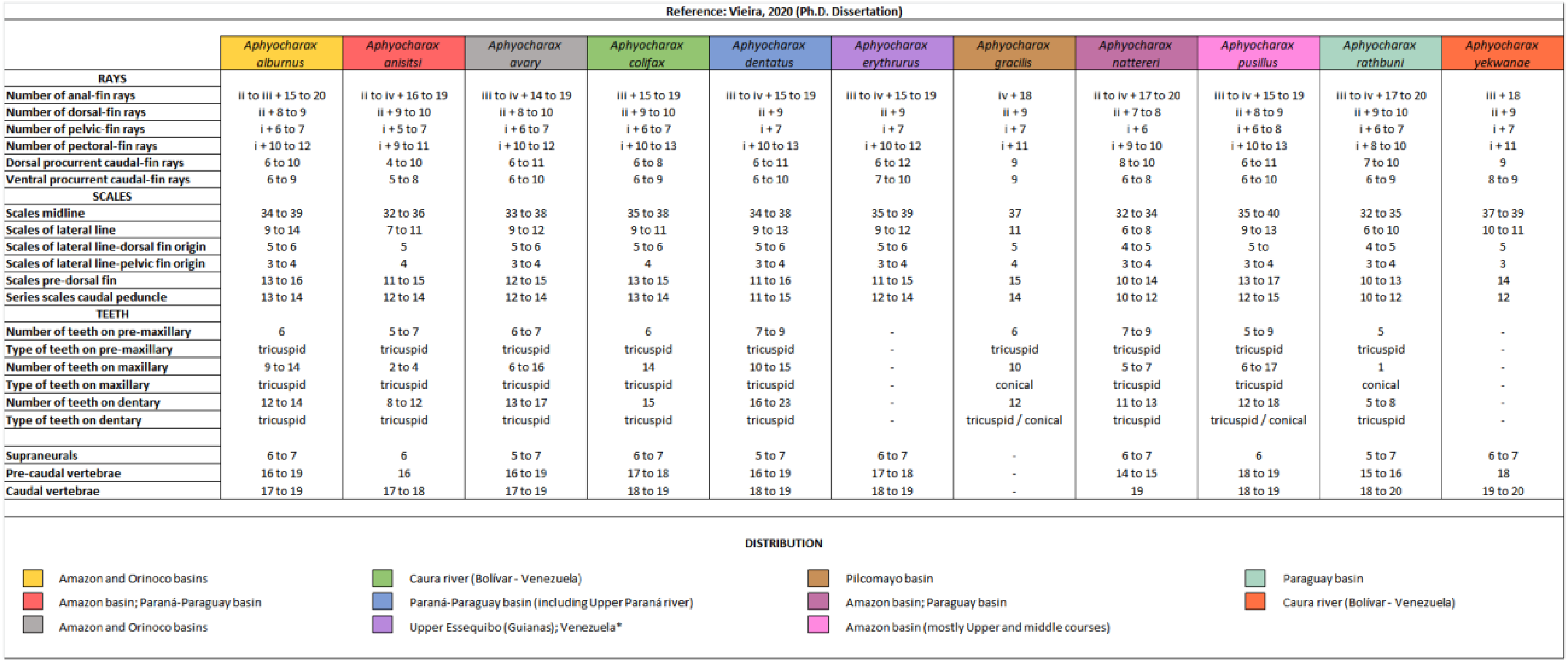
Table 29 – Morphometric data from non-type examined material of *Prionobrama paraguayensis* (n=21). SD = Standard deviation.

	Min	**Max**	**Mean**	**SD**
Standard length (mm)	26.4	38.1	-	-
Percents of Standard Length (SL)				
Snout-anal fin distance	14.8	21.5	18.5	1.7
Snout-dorsal fin distance	15.2	21.7	18.5	1.7
Snout-pelvic fin distance	11.6	16.3	14.2	1.3
Snout-pectoral fin distance	6.7	9.7	8.4	0.7
Dorsal-caudal fin distance	12.4	17.8	14.9	1.5
Orbit-dorsal fin distance	12.6	18.0	15.2	1.5
Anal-fin base length	9.7	14.6	12.2	1.4
Length of caudal peduncle	3.0	4.2	3.5	0.4
Depth of caudal peduncle	2.2	3.7	2.9	0.4
Body depth at dorsal fin	7.3	11.3	9.2	1.1
Dorsal-fin length	7.4	10.8	8.8	1.0
Pelvic-fin length	5.2	8.2	6.5	0.8
Pectoral-fin length	7.4	11.4	9.3	1.0
Head length	6.5	8.6	7.7	0.6
Percents of Head Length (HL)				
Snout length	1.6	2.3	2.0	0.2
Upper Jaw length	2.6	3.5	3.1	0.3
Horizontal orbit diameter	2.2	3.3	2.9	0.2
Interorbital width	1.9	2.6	2.3	0.2

Table 30 – Morphometric data from paratypes (CAS-SU036487) (n=2) and non-type examined material of *Xenagoniates bondi* (n=16). SD = Standard deviation. All measurements from paratypes are in mm.

	Paratypes	**Min**	**Max**	**Mean**	**SD**
Standard length (mm)	50.7–50.9	35.5	57.8	-	-
		Percents of Standard Length (SL)			
Snout-anal fin distance	17.4–19.0	12.8	20.8	15.4	2.3
Snout-dorsal fin distance	32.5–33.6	22.7	35.7	26.8	4.3
Snout-pelvic fin distance	15.0–15.7	10.8	17.2	12.8	1.9
Snout-pectoral fin distance	9.6–10.0	7.0	10.9	8.4	1.1
Dorsal-caudal fin distance	19.7–20.1	13.9	23.3	17.1	2.7
Orbit-dorsal fin distance	27.8–28.3	18.9	30.5	22.6	3.8
Anal-fin base length	31.3–29.6	21.9	35.3	26.6	4.4
Length of caudal peduncle	2.9–2.9	1.7	3.2	2.2	0.4
Depth of caudal peduncle	3.4–3.3	2.2	4.4	2.9	0.6
Body depth at dorsal fin	11.8–11.4	7.8	13.5	9.9	1.8
Dorsal-fin length	10.7–12.9	6.5	12.9	8.5	1.9
Pelvic-fin length	3.9–3.9	2.4	4.3	3.0	0.6
Pectoral-fin length	9.4–9.7	6.2	10.1	7.5	1.2
Head length	9.4–9.8	7.2	10.1	8.3	1.0
		Percents of Head Length (HL)			
Snout length	2.5–2.4	1.6	2.8	2.2	0.3
Upper Jaw length	2.7–2.6	2.0	2.6	2.2	0.2
Horizontal orbit diameter	3.6–3.5	2.7	3.7	3.2	0.3
Interorbital width	2.4–2.4	1.8	2.8	2.2	0.3

ANEXO 6: Morphology of Aphyocharacinae: comparative table of overall counts.



Reference: Vieira, 2020 (Ph.D. Dissertation)

	Aphyocharax alburnus	*Aphyocharax anisitsi*	*Aphyocharax avary*	*Aphyocharax colifax*	*Aphyocharax dentatus*	*Aphyocharax erythrurus*	*Aphyocharax gracilis*	*Aphyocharax nattereri*	*Aphyocharax pusillus*	*Aphyocharax rathbuni*	*Aphyocharax yekwanae*
RAYS											
Number of anal-fin rays	ii to iii + 15 to 20	ii to iv + 16 to 19	iii to iv + 14 to 19	iii + 15 to 19	iii to iv + 15 to 19	iii to iv + 15 to 19	iv + 18	ii to iv + 17 to 20	iii to iv + 15 to 19	iii to iv + 17 to 20	iii + 18
Number of dorsal-fin rays	ii + 8 to 9	ii + 9 to 10	ii + 8 to 10	ii + 9 to 10	ii + 9	ii + 9	ii + 9	ii + 7 to 8	ii + 8 to 9	ii + 9 to 10	ii + 9
Number of pelvic-fin rays	i + 6 to 7	i + 5 to 7	i + 6 to 7	i + 6 to 7	i + 7	i + 7	i + 7	i + 6	i + 6 to 8	i + 6 to 7	i + 7
Number of pectoral-fin rays	i + 10 to 12	i + 9 to 11	i + 10 to 12	i + 10 to 13	i + 10 to 13	i + 10 to 12	i + 11	i + 9 to 10	i + 10 to 13	i + 8 to 10	i + 11
Dorsal procurrent caudal-fin rays	6 to 10	4 to 10	6 to 11	6 to 8	6 to 11	6 to 12	9	8 to 10	6 to 11	7 to 10	9
Ventral procurrent caudal-fin rays	6 to 9	5 to 8	6 to 10	6 to 9	6 to 10	7 to 10	9	6 to 8	6 to 10	6 to 9	8 to 9
SCALES											
Scales midline	34 to 39	32 to 36	33 to 38	35 to 38	34 to 38	35 to 39	37	32 to 34	35 to 40	32 to 35	37 to 39
Scales of lateral line	9 to 14	7 to 11	9 to 12	9 to 11	9 to 13	9 to 12	11	6 to 8	9 to 13	6 to 10	10 to 11
Scales of lateral line-dorsal fin origin	5 to 6	5	5 to 6	5 to 6	5 to 6	5 to 6	5	4 to 5	5 to	4 to 5	5
Scales of lateral line-pelvic fin origin	3 to 4	4	3 to 4	4	3 to 4	3 to 4	4	3 to 4	3 to 4	3 to 4	3
Scales pre-dorsal fin	13 to 16	11 to 15	12 to 15	13 to 15	11 to 16	11 to 15	15	10 to 14	13 to 17	10 to 13	14
Series scales caudal peduncle	13 to 14	12 to 14	12 to 14	13 to 14	11 to 15	12 to 14	14	10 to 12	12 to 15	10 to 12	12
TEETH											
Number of teeth on pre-maxillary	6	5 to 7	6 to 7	6	7 to 9	-	6	7 to 9	5 to 9	5	-
Type of teeth on pre-maxillary	tricuspid	tricuspid	tricuspid	tricuspid	tricuspid	-	tricuspid	tricuspid	tricuspid	tricuspid	-
Number of teeth on maxillary	9 to 14	2 to 4	6 to 16	14	10 to 15	-	10	5 to 7	6 to 17	1	-
Type of teeth on maxillary	tricuspid	tricuspid	tricuspid	tricuspid	tricuspid	-	conical	tricuspid	tricuspid	conical	-
Number of teeth on dentary	12 to 14	8 to 12	13 to 17	15	16 to 23	-	12	11 to 13	12 to 18	5 to 8	-
Type of teeth on dentary	tricuspid	tricuspid	tricuspid	tricuspid	tricuspid	-	tricuspid / conical	tricuspid	tricuspid / conical	tricuspid	-
Supraneurals	6 to 7	6	5 to 7	6 to 7	5 to 7	6 to 7	-	6 to 7	6	5 to 7	6 to 7
Pre-caudal vertebrae	16 to 19	16	16 to 19	17 to 18	16 to 19	17 to 18	-	14 to 15	18 to 19	15 to 16	18
Caudal vertebrae	17 to 19	17 to 18	17 to 19	18 to 19	18 to 19	18 to 19	-	19	18 to 19	18 to 20	19 to 20

DISTRIBUTION

■ Amazon and Orinoco basins
■ Amazon basin; Paraná-Paraguay basin
■ Amazon and Orinoco basins
■ Caura river (Bolívar - Venezuela)
■ Paraná-Paraguay basin (including Upper Paraná river)
■ Upper Essequibo (Guianas); Venezuela*
■ Pilcomayo basin
■ Amazon basin; Paraguay basin
■ Amazon basin (mostly Upper and middle courses)
■ Paraguay basin
■ Caura river (Bolívar - Venezuela)

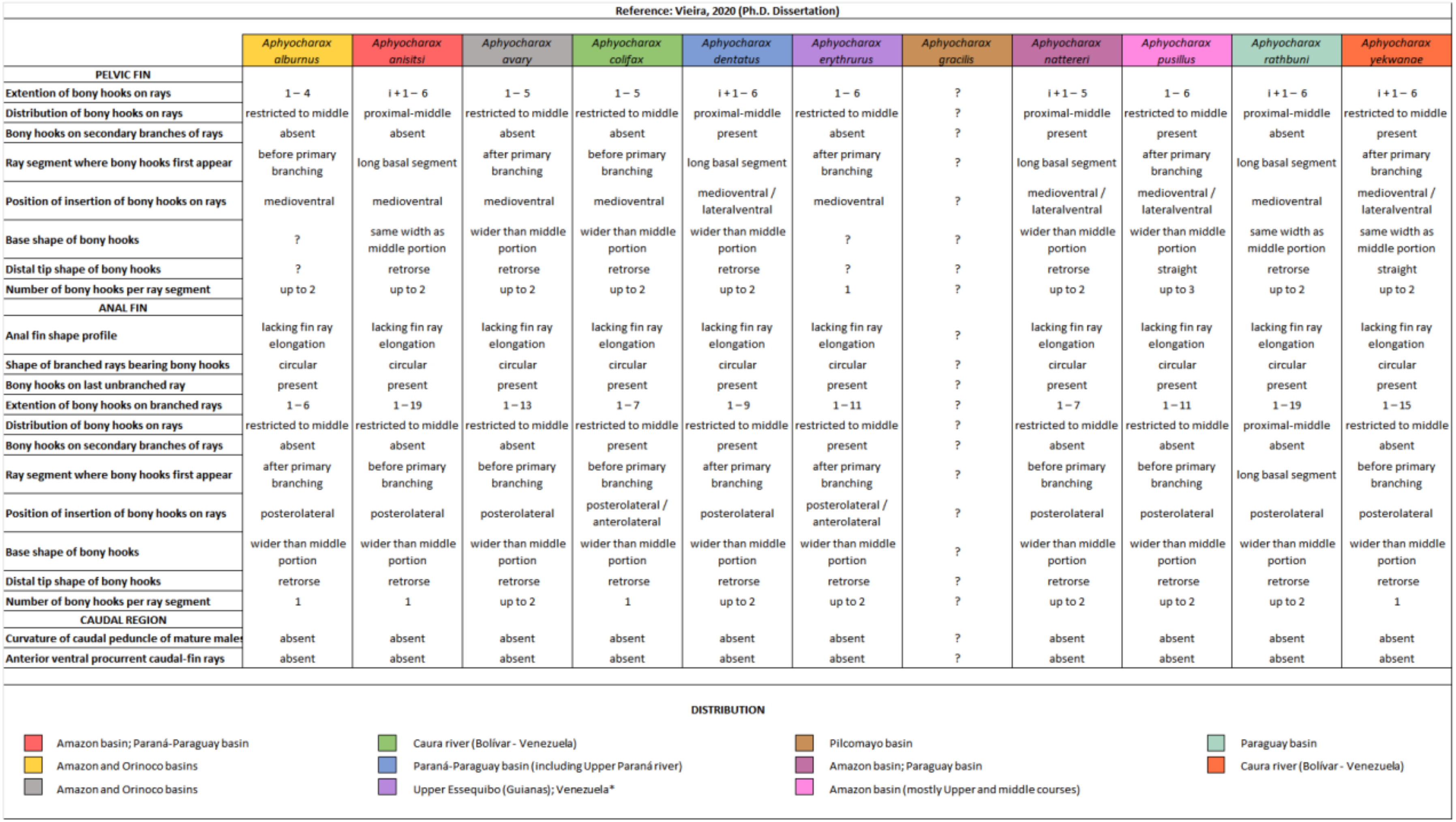
Reference: Vieira, 2020 (Ph.D. Dissertation)

	Aphyocharacidium bolivianum	*Aphyoharacidium* n. sp.*	*Axelrodia lindeae*	*Leptagoniates steindachneri*	*Paragoniates alburnus*	*Phenagoniates macrolepis*	*Prionobrama filigera*	*Prionobrama paraguayensis*	*Xenagoniates bondi*
RAYS									
Number of anal-fin rays	iii + 19 to 22	iii + 17 to 20	ii to iv + 22 to 26	ii to iv + 57 to 69	ii to iv + 40 to 50	ii to iv + 45 to 55	iv to vi + 27 to 33	iii to vi + 27 to 32	ii to iv + 57 to 66
Number of dorsal-fin rays	ii + 8 to 9	ii + 9	ii + 9	ii + 8	ii + 8 to 9	ii+ 7 to 8	ii + 9	ii + 8 to 9	ii + 7 to 8
Number of pelvic-fin rays	i + 6 to 7	i + 6 to 7	i + 6	i + 5 to 6	i + 6 to 7	i + 5	i + 6 to 7	i + 7	i + 5
Number of pectoral-fin rays	i + 9 to 11	i + 10 to 11	i + 7 to 10	i + 9 to 11	i + 10 to 13	i + 9 to 11	i + 10 to 12	i + 10 to 12	i + 10 to 11
Dorsal procurrent caudal-fin rays	8 to 14	12 to 15	11 to 14	6 to 10	2 to 9	7 to 10	6 to 11	6 to 9	7 to 9
Ventral procurrent caudal-fin rays	9 to 11	12 to 15	10 to 13	5 to 8	3 to 7	5 to 8	6 to 9	5 to 8	6 to 8
SCALES									
Scales midline	-	-	32 to 39	-	40 to 45	41 to 47	36 to 41	35 to 41	-
Scales of lateral line	32 to 35	31 to 32	8 to 12	46 to 52	11 to 17	7 to 15	9 to 14	7 to 10	50 to 56
Scales of lateral line-dorsal fin origin	3 to 5	5	5 to 6	6 to 7	6 to 8	6 to 7	5 to 6	4 to 6	7 to 8
Scales of lateral line-pelvic fin origin	3 to 4	4	4 to 5	6 to 7	5 to 6	6 to 7	4	4	6 to 7
Scales pre-dorsal fin	8 to 10	9 to 10	9 to 11	19 to 26	19 to 25	16 to 23	14 to 19	14 to 17	19 to 29
Series scales caudal peduncle	10 to 12	12 to 13	10 to 12	10 to 13	12 to 18	11 to 14	12 to 15	11 to 13	10 to 13
TEETH									
Number of teeth on pre-maxillary	10 to 11	12 to 13	9	7	5 to 8	6 to 7	6 to 9	6 to 9	7
Type of teeth on pre-maxillary	tricuspid	tricuspid	conical	tricuspid	tricuspid	tricuspid	tricuspid	tricuspid	tricuspid
Number of teeth on maxillary	6 to 7	6 to 7	6 to 7	11	17 to 26	9 to 10	15 to 21	14 to 22	8 to 11
Type of teeth on maxillary	tricuspid	tricuspid	conical	tricuspid / conical	tricuspid	tricuspi / conical	tricuspid	tricuspid	tricuspid / conical
Number of teeth on dentary	outter row: 17 to 19 / inner row: 19 to 20	outter row: 18 to 20 / inner row: 22 to 23	16 to 19	12	18 to 25	9 to 11	13 to 19	11 to 15	6 to 8
Type of teeth on dentary	tricuspid / conical	tricuspid / conical	conical	tricuspid / conical	tricuspid	tricuspid / conical	tricuspid	tricuspid	tricuspid
Supraneurals	4	4 to 5	4	9 to 11	8 to 10	10 to 12	7	8	10 to 12
Pre-caudal vertebrae	14	14	13	11	16	13	16 to 17	15 to 16	11 to 12
Caudal vertebrae	20	20	24	35 to 37	22 to 26	28 to 30	20 to 21	20 to 22	38 to 39

DISTRIBUTION

🟩 Amazon basin (Upper course)
🟧 Lower Marañón river
🟫 Madeira river
🟪 Amazon basin (Upper course)
🟥 Amazon basin; Orinoco basin
🟨 Lake Maracaibo basin; Atrato river; Panamá*
⬜ Amazon basin
🟦 Paraná-Paraguay basin
🟫 Orinoco basin; Coastal drainages of Venezuela

ANEXO 7: Secondary sexual characters in Aphyocharacinae: comparative table of overall morphology and distribution.

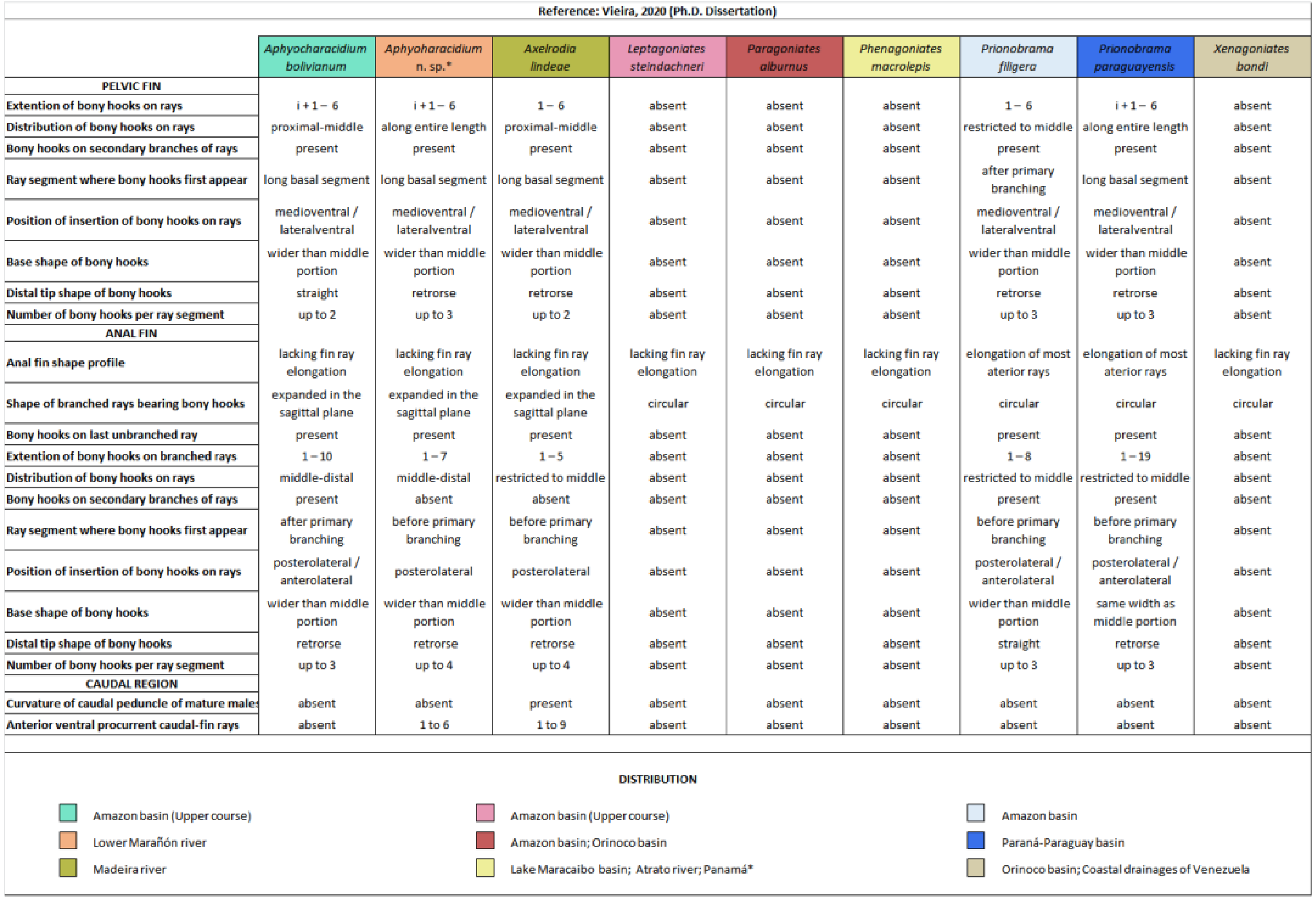


Reference: Vieira, 2020 (Ph.D. Dissertation)

	Aphyocharax alburnus	*Aphyocharax anisitsi*	*Aphyocharax avary*	*Aphyocharax colifax*	*Aphyocharax dentatus*	*Aphyocharax erythrurus*	*Aphyocharax gracilis*	*Aphyocharax nattereri*	*Aphyocharax pusillus*	*Aphyocharax rathbuni*	*Aphyocharax yekwanae*
PELVIC FIN											
Extention of bony hooks on rays	1 – 4	i + 1 – 6	1 – 5	1 – 5	i + 1 – 6	1 – 6	?	i + 1 – 5	1 – 6	i + 1 – 6	i + 1 – 6
Distribution of bony hooks on rays	restricted to middle	proximal-middle	restricted to middle	restricted to middle	proximal-middle	restricted to middle	?	proximal-middle	restricted to middle	proximal-middle	restricted to middle
Bony hooks on secondary branches of rays	absent	absent	absent	absent	present	absent	?	present	present	absent	present
Ray segment where bony hooks first appear	before primary branching	long basal segment	after primary branching	before primary branching	long basal segment	after primary branching	?	long basal segment	after primary branching	long basal segment	after primary branching
Position of insertion of bony hooks on rays	medioventral	medioventral	medioventral	medioventral	medioventral / lateralventral	medioventral	?	medioventral / lateralventral	medioventral / lateralventral	medioventral	medioventral / lateralventral
Base shape of bony hooks	?	same width as middle portion	wider than middle portion	wider than middle portion	wider than middle portion	?	?	wider than middle portion	wider than middle portion	same width as middle portion	same width as middle portion
Distal tip shape of bony hooks	?	retrorse	retrorse	retrorse	retrorse	?	?	retrorse	straight	retrorse	straight
Number of bony hooks per ray segment	up to 2	up to 2	up to 2	up to 2	up to 2	1	?	up to 2	up to 3	up to 2	up to 2
ANAL FIN											
Anal fin shape profile	lacking fin ray elongation	lacking fin ray elongation	lacking fin ray elongation	lacking fin ray elongation	lacking fin ray elongation	lacking fin ray elongation	?	lacking fin ray elongation	lacking fin ray elongation	lacking fin ray elongation	lacking fin ray elongation
Shape of branched rays bearing bony hooks	circular	circular	circular	circular	circular	circular	?	circular	circular	circular	circular
Bony hooks on last unbranched ray	present	present	present	present	present	present	?	present	present	present	present
Extention of bony hooks on branched rays	1 – 6	1 – 19	1 – 13	1 – 7	1 – 9	1 – 11	?	1 – 7	1 – 11	1 – 19	1 – 15
Distribution of bony hooks on rays	restricted to middle	restricted to middle	restricted to middle	restricted to middle	restricted to middle	restricted to middle	?	restricted to middle	restricted to middle	proximal-middle	restricted to middle
Bony hooks on secondary branches of rays	absent	absent	absent	present	present	present	?	absent	absent	absent	absent
Ray segment where bony hooks first appear	after primary branching	before primary branching	before primary branching	before primary branching	after primary branching	after primary branching	?	before primary branching	before primary branching	long basal segment	before primary branching
Position of insertion of bony hooks on rays	posterolateral	posterolateral	posterolateral	posterolateral / anterolateral	posterolateral	posterolateral / anterolateral	?	posterolateral	posterolateral	posterolateral	posterolateral
Base shape of bony hooks	wider than middle portion	wider than middle portion	wider than middle portion	wider than middle portion	wider than middle portion	wider than middle portion	?	wider than middle portion	wider than middle portion	wider than middle portion	wider than middle portion
Distal tip shape of bony hooks	retrorse	retrorse	retrorse	retrorse	retrorse	retrorse	?	retrorse	retrorse	retrorse	retrorse
Number of bony hooks per ray segment	1	1	up to 2	1	up to 2	up to 2	?	up to 2	up to 2	up to 2	1
CAUDAL REGION											
Curvature of caudal peduncle of mature males	absent	absent	absent	absent	absent	absent	?	absent	absent	absent	absent
Anterior ventral procurrent caudal-fin rays	absent	absent	absent	absent	absent	absent	?	absent	absent	absent	absent

DISTRIBUTION

- 🟥 Amazon basin; Paraná-Paraguay basin
- 🟨 Amazon and Orinoco basins
- ▨ Amazon and Orinoco basins
- 🟩 Caura river (Bolívar - Venezuela)
- 🟦 Paraná-Paraguay basin (including Upper Paraná river)
- 🟪 Upper Essequibo (Guianas); Venezuela*
- 🟫 Pilcomayo basin
- 🟪 Amazon basin; Paraguay basin
- 🟪 Amazon basin (mostly Upper and middle courses)
- 🟩 Paraguay basin
- 🟧 Caura river (Bolívar - Venezuela)



Reference: Vieira, 2020 (Ph.D. Dissertation)

	Aphyocharacidium bolivianum	*Aphyoharacidium* n. sp.*	*Axelrodia lindeae*	*Leptagoniates steindachneri*	*Paragoniates alburnus*	*Phenagoniates macrolepis*	*Prionobrama filigera*	*Prionobrama paraguayensis*	*Xenagoniates bondi*
PELVIC FIN									
Extention of bony hooks on rays	i + 1 – 6	i + 1 – 6	1 – 6	absent	absent	absent	1 – 6	i + 1 – 6	absent
Distribution of bony hooks on rays	proximal-middle	along entire length	proximal-middle	absent	absent	absent	restricted to middle	along entire length	absent
Bony hooks on secondary branches of rays	present	present	present	absent	absent	absent	present	present	absent
Ray segment where bony hooks first appear	long basal segment	long basal segment	long basal segment	absent	absent	absent	after primary branching	long basal segment	absent
Position of insertion of bony hooks on rays	medioventral / lateralventral	medioventral / lateralventral	medioventral / lateralventral	absent	absent	absent	medioventral / lateralventral	medioventral / lateralventral	absent
Base shape of bony hooks	wider than middle portion	wider than middle portion	wider than middle portion	absent	absent	absent	wider than middle portion	wider than middle portion	absent
Distal tip shape of bony hooks	straight	retrorse	retrorse	absent	absent	absent	retrorse	retrorse	absent
Number of bony hooks per ray segment	up to 2	up to 3	up to 2	absent	absent	absent	up to 3	up to 3	absent
ANAL FIN									
Anal fin shape profile	lacking fin ray elongation	lacking fin ray elongation	lacking fin ray elongation	lacking fin ray elongation	lacking fin ray elongation	lacking fin ray elongation	elongation of most aterior rays	elongation of most aterior rays	lacking fin ray elongation
Shape of branched rays bearing bony hooks	expanded in the sagittal plane	expanded in the sagittal plane	expanded in the sagittal plane	circular	circular	circular	circular	circular	circular
Bony hooks on last unbranched ray	present	present	present	absent	absent	absent	present	present	absent
Extention of bony hooks on branched rays	1 – 10	1 – 7	1 – 5	absent	absent	absent	1 – 8	1 – 19	absent
Distribution of bony hooks on rays	middle-distal	middle-distal	restricted to middle	absent	absent	absent	restricted to middle	restricted to middle	absent
Bony hooks on secondary branches of rays	present	absent	absent	absent	absent	absent	present	present	absent
Ray segment where bony hooks first appear	after primary branching	before primary branching	before primary branching	absent	absent	absent	before primary branching	before primary branching	absent
Position of insertion of bony hooks on rays	posterolateral / anterolateral	posterolateral	posterolateral	absent	absent	absent	posterolateral / anterolateral	posterolateral / anterolateral	absent
Base shape of bony hooks	wider than middle portion	wider than middle portion	wider than middle portion	absent	absent	absent	wider than middle portion	same width as middle portion	absent
Distal tip shape of bony hooks	retrorse	retrorse	retrorse	absent	absent	absent	straight	retrorse	absent
Number of bony hooks per ray segment	up to 3	up to 4	up to 4	absent	absent	absent	up to 3	up to 3	absent
CAUDAL REGION									
Curvature of caudal peduncle of mature males	absent	absent	present	absent	absent	absent	absent	absent	absent
Anterior ventral procurrent caudal-fin rays	absent	1 to 6	1 to 9	absent	absent	absent	absent	absent	absent

DISTRIBUTION

■ Amazon basin (Upper course)
■ Lower Marañón river
■ Madeira river
■ Amazon basin (Upper course)
■ Amazon basin; Orinoco basin
■ Lake Maracaibo basin; Atrato river; Panamá*
■ Amazon basin
■ Paraná-Paraguay basin
■ Orinoco basin; Coastal drainages of Venezuela

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APÊNDICE 1:

A new sexually dimorphic species of *Aphyocharacidium* Géry, 1960 (Ostariophysi: Characidae: Aphyocharacinae) from the Marañón river, Peru

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Abstract

Aphyocharacidium n. sp. is described from tributaries of the Marañón River, Amazon River basin, Peru. The new species is distinguished from *Aphyocharacidium melandetum* (Eigenmann, 1912) and *Aphyocharacidium bolivianum* Géry, 1973 by the shape of ventral procurrent caudal-fin rays of sexually dimorphic males, expanded on sagittal plane, projecting between muscles and skin, being visible along the ventral margin of caudal peduncle. Additionally, the new species can be diagnosed from its congeners by the following combination of characters: the presence of a long and pointed ascending process on the premaxilla; 12–13 teeth on the premaxilla; 31–32 perforated scales on the lateral line; 17–20 branched anal-fin rays; well-developed bony hooks on the anal and pelvic-fin rays of mature males. A discussion about secondary sexual characters on *Aphyocharacidium* is presented.

Key words: Secondary sexual characters; Neotropical region; Amazon basin; taxonomy.

Resumo

Aphyocharacidium n. sp. é descrita para afluentes do rio Marañón, bacia do rio Amazonas, Peru. A nova espécie distingue-se de *Aphyocharacidium melandetum* (Eigenmann, 1912) e *Aphyocharacidium bolivianum* Géry, 1973 pela forma dos raios procorrentes ventrais da nadadeira caudal de machos sexualmente dimórficos, com elementos hipertrofiados expandidos em plano sagital, projetando-se entre os músculos e pele, sendo visíveis ao longo da margem ventral do pedúnculo caudal. Além disso, a nova espécie pode ser diagnosticada de seus congêneres pela seguinte combinação de caracteres: presença de um processo ascendente longo e pontiagudo na pré-maxila; 12–13 dentes na pré-maxila; 31–32 escamas perfuradas na linha lateral; 17–20 raios ramificados na nadadeira anal; ganchos ósseos bem desenvolvidos nos raios da nadadeira anal e pélvica de machos sexualmente maduros. Uma discussão sobre a presença de caracteres sexuais secundários em *Aphyocharacidium* é apresentada.

Palavras chave: Caracteres sexuais secundários; Região Neotropical; Bacia Amazônica; taxonomia.

Resumen

Aphyocharacidium n. sp. es descrita de los afluentes del río Marañón, cuenca del rio Amazonas, Perú. La nueva especie se distingue de *Aphyocharacidium melandetum* (Eigenmann, 1912) y *Aphyocharacidium bolivianum* Géry, 1973 por la forma de los radios procurrentes ventrales de la aleta caudal em machos sexualmente dimorfos, con elementos hipertrofiados expandidos en plano sagital, proyectándose entre los músculos y piel, siendo visible a lo largo de la margen ventral del pedúnculo caudal. Adicionalmente, la nueva especie puede ser diagnosticada de sus otros congéneres por la siguiente combinación de caracteres: presencia de un proceso ascendente largo e puntiagudo en la premaxila; 12–13 dientes en la premaxila; 31– 32 escamas perforadas en la línea lateral; 17–20 radios ramificados en la aleta anal; ganchos óseos bien desarrollados en los radios de la aleta anal y pélvica de machos sexualmente maduros. Una discusión sobre la presencia de caracteres sexuales secundarios en *Aphyocharacidium* es presentada.

Palabras clave: Caracteres sexuales secundarios, Región Neotropical, Cuenca Amazónica, taxonomía.

Introduction

The genus *Aphyocharacidium* was proposed by Géry (1960) to allocate *Odontostilbe melandetus* Eigenmann, 1912, the type species by original designation and monotypy. The new genus was diagnosed based on the description of a long combination of several characters, not exclusive and commonly found in other characids, related to the completeness of the lateral line and circumorbital series; presence of adipose fin, pseudotympanum, large fontanels, gill-rakers normal and “interhaemals” (ventral procurrent caudal-fin rays); lack of scales on caudal fin; shape of anal fin (short and falciform); premaxillary with an ascending apophysis; teeth numerous, narrow, tricuspid; and maxillary moderate, toothed. Géry (1960) further describes the presence of a second series of functional minute conical teeth in the dentary, besides the anterior series of tricuspid teeth, and considers this character unique among the known forms of Cheirodontines “and allied”, which, according to Géry, indicates it is a “border genus leading to the Characidiinae”.

Later, Géry (1965, 1972) assigned *Aphyocharacidium* to a new subtribe of Cheirodontidi [sic], the Aphyoditeini, including *Aphyodite* Eigenmann, 1912, *Axelrodia* Géry, 1965, *Brittanichthys* Géry, 1965, *Leptobrycon* Eigenmann, 1915, *Macropsobrycon*

Eigenmann, 1915, *Microschemobrycon* Eigenmann, 1915, *Oxybrycon* Géry, 1964, *Parecbasis* Eigenmann, 1914, *Prodontocharax* Pearson, 1924, and *Thrissobrycon* Böhlke, 1953. On a new contribution on the Aphyoditeina, Géry (1973) describes the second species of the genus: *Aphyocharacidium bolivianum* Géry, 1973, from Central Bolivia, occurring on tributaries of the Mamoré River.

Malabarba (1998) refuted the relationships of *Aphyocharacidium* to the Cheirodontinae and considered the genus *incertae sedis* among characids. Mirande (2009, 2010) found *Aphyocharacidium* as closely related to *Axelrodia*, sister to *Aphyodite*, *Microschemobrycon*, and *Parecbasis*, and possibly related to *Leptobrycon*, *Oxybrycon* and *Tyttobrycon* Géry, 1973, classifying all these genera in the redefined subfamily Aphyoditeinae. Oliveira *et al.* (2011) and Tagliacollo *et al.* (2012), instead, refute a close relationship between *Aphyocharacidium* and *Aphyodite*, including *Aphyocharacidium* in the subfamily Aphyocharacinae, along with *Aphyocharax* Günther, 1868, *Leptagoniates* Boulenger, 1887, *Paragoniates* Steindachner, 1876, *Phenagoniates* Eigenmann & Wilson, 1914, *Prionobrama* Fowler, 1913 and *Xenagoniates* Myers, 1942. Such a classification was followed later by Mirande (2019), corroborating the close relationship of *A. bolivianum* and *Axelrodia lindeae*, adding the latter species to Aphyocharacinae.

During the examination of small characid fishes collected in the Marañón River, Peru, we found a new characid fish species with two teeth rows on the dentary, indicating a close relationship to *Aphyocharacidium*. The new species, however, possesses highly developed secondary sexual characters, differing from the two valid species of *Aphyocharacidium* that lack such dimorphic features (Géry 1960, 1973). The new species is described herein, compared to *Aphyocharacidium* and putatively related species in order to uncover its relationships.

Material and Methods

Counts and measurements followed Fink & Weitzman (1974), with the addition and modifications of the following: anal-fin base length measured from the anal-fin origin to the base of the last anal-fin ray; horizontal scale rows below lateral line counted to pelvic-fin insertion. Measurements were taken point to point with a digital caliper (precision 0.01 mm), and measurements of the subunits of the head were performed under a stereomicroscope. The standard length (SL) is expressed in mm, all other measurements are expressed as percentages of SL, except subunits of the head recorded as percentages of head length (HL). Counts of supraneurals, gill-rakers on first gill arch, teeth, vertebrae, and teeth morphology were observed in cleared and stained (c&s) specimens prepared according to Taylor & van Dyke (1985). Counts of unbranched anal-fin rays, procurrent caudal-fin rays, and fin hooks were registered for all available specimens. Precaudal vertebrae and caudal vertebrae definitions follows Weitzman (1962). Gut content observed under stereomicroscope. On the description, counts are followed by their frequencies in parentheses; asterisks indicate the counts of the holotype. The terminology used to describe the secondary sexual characters of dimorphic males followed Weitzman & Fink (1985) and Malabarba (1998). Pictures of jaws, teeth, fin rays and ventral procurrent caudal-fin rays were taken from cleared and stained specimens on a Nikon Multizoom AZ100. Images of jaws and teeth were taken on a Scanning Electron Microscope (SEM) JEOL JSM-6060. Extent of Occurrence (EOO) calculation was performed using GeoCAT (Bachman *et al.* 2011; http://geocat.kew.org/editor). Institutional acronyms follows Sabaj (2019).

The data included for *Aphyocharacidium melandetum* was retrieved from the available information in Eigenmann (1912, 1915) and Géry (1960).

Results

***Aphyocharacidium* n. sp., new species**

(Figs. 1–6)

Holotype. MUSM 68515, male, 25.1 mm SL, Situche stream, Morona river, Morona, Datem del Marañón, Loreto, Peru, 03°03’16”S, 77°26’57”W, A. Cortijo, 10 Dec 2011.

Paratypes. MUSM 41944, 2, 24.3–25.7 mm SL, collected with holotype; MUSM 68942, 6, 22.0–29.1 mm SL (1 male c&s, 27.8 mm SL), Platanoyacu River tributary of the Corrientes River, Tigre River sub-basin Marañón basin, Andoas, Loreto, Peru, 03°08’08”S, 75°46’43”W, R. Quispe, 18 Sept 2008; MUSM 69043, 5, 21.5–25.7 mm SL (1 female c&s, 23.8 mm SL), Katirnaentsa stream, Morona River, Morona, Datem del Marañón, Loreto, Peru, 03°04’01”S, 77°20’42”W, A. Cortijo, 29 Nov 2011; MUSM 42077, 4, 22.0–23.4 mm SL, Katirnaentsa stream, Morona River, Morona, Datem del Marañón, Loreto, Peru, 03°06’10.3”S, 77°22’28”W, A. Cortijo, 2 Dec 2011. UFRGS 28200, 2, 23.6–26.7 mm SL, Katirnaentsa stream, Morona River, Morona, Datem del Marañón, Loreto, Peru, 03°04’01”S, 77°20’42”W, A. Cortijo, 29 Nov 2011.

Diagnosis. *Aphyocharacidium* n. sp. is distinguished from the two congeners by the presence of hypertrophy of the anterior elements of the procurrent caudal-fin rays series (*vs.* all ventral procurrent caudal-fin rays slender). Furthermore, *Aphyocharacidium* n. sp. can be diagnosed by the fewer number of perforated scales in lateral line (31–32 *vs.* 33–35 in *A. bolivianum,* 33 in *A. melandetum*); the ascending process on the premaxilla long and pointed (*vs.* short and slightly pointed in *A. bolivianum*, short and rounded in *A. melandetum*); spaced chromatophores, if present, after pseudotympanum (*vs.* conspicuous dark humeral spot in *A. bolivianum*, absent in *A. melandetum*).

Description. Morphometric data presented on Table 1. Body laterally compressed, elongated; maximum size 29.1 mm SL. Greatest body depth at vertical through dorsal-fin origin. Dorsal profile of head convex from tip of snout to vertical through posterior border of nare, slightly convex from that point to posterior tip of supraoccipital bone. Predorsal profile slightly elevated and convex from posterior tip of supraoccipital bone to dorsal-fin insertion. Dorsal profile straight and abruptly posteroventrally slanted along dorsal-fin base; slightly convex from dorsal-fin terminus to adipose-fin origin, and slightly concave along caudal peduncle. Caudal peduncle longer than deep. Ventral profile of head slightly convex from lower jaw to pelvic-fin insertion. Ventral region straight to slightly concave from pelvic-fin insertion to anal-fin origin. Anal-fin base straight to slightly concave in females, slightly convex anteriorly and straight posteriorly in sexually dimorphic males. Ventral margin of caudal peduncle slightly concave.

Head pointed anteriorly in lateral view. Snout rounded. Mouth terminal, slit situated below horizontal through middle of pupil. Nostril dorsolateral, anterior opening roundish, posterior opening crescent-shaped. Medial projection of quadrate articulated with anguloarticular. Coronomeckelian situated on Meckelian cartilage. Infraorbital series complete with IO1 to IO6. Third infraorbital ventrally extended, covering the cheek and reaching the horizontal arm of the preopercle. Fourth infraorbital somewhat developed, longer dorsoventrally than longitudinally with uneven margins. Pterosphenoid with large median foramen. Posterior margin of opercle sinusoidal with upper portion concave and lower portion convex.

Tooth narrow, tricuspid, with similar shape and cusp, except for dentary conical teeth irregularly arranged on posteriormost region. Central median cusp in all teeth slightly longer than lateral cusps. Premaxilla elongated, ascending process developed and pointed, 12(1) or 13(1) teeth aligned in a single row, 5(2) replacement teeth. Maxilla elongated, slightly angulated, with anterior end of ascending process pointed, lamellar portion extending to dorsal portion of Meckelian cartilage, reaching or almost reaching the dorsal margin of the coronomeckelian, 6–7(2) teeth at angulated portion. Dentary slightly projecting forward when the mouth is closed, teeth in two regular series. Outer row more developed, 14(1) or 16(1) teeth decreasing in size laterally; inner series backwards oriented, inserted in a crest distinctively separated from the outer series, with 18(2) tricuspid teeth decreasing in size laterally, and 8(1) or 9(1) minute conical teeth on posteriormost region (Fig. 2–3).

Dorsal-fin origin on midlength of standard length. Dorsal-fin rays ii*, 9*(20). First unbranched dorsal-fin ray approximately half-length of second unbranched dorsal-fin ray, following branched rays gradually decreasing in size posteriorly. Adipose-fin origin anterior to vertical through base of last anal-fin ray, well developed, almost reaching the dorsal procurrent caudal-fin rays. Anal-fin origin posterior to vertical through base of last dorsal-fin ray. Anal-fin rays iii*, 17(2), 18*(9), 19(6), or 20(3). Anal fin distal profile rounded on anterior lobe, concave posteriorly on females, and deeply concave posteriorly on males. Last unbranched and anterior three branched anal-fin rays longer than remaining rays, gradually decreasing in size posteriorly. Mature males with retrorse hooks on posterolateral border on last unbranched to 5th, 6th or 7th branched anal-fin rays. No hypertrophied soft tissue associated to hook bearing anal-fin rays. Pectoral-fin rays i*, 10*(13) or 11(7). Unbranched pectoral-fin ray reaching pelvic-fin origin. Pelvic-fin origin slightly anterior to vertical through dorsal-fin origin. Pelvic-fin rays i*, 6(2), 7*(18). Distal tip of pelvic fin reaching anal-fin origin. Pelvic-fin rays of mature males with retrorse hooks on ventral border of medial branches, and sometimes on ventral border of medial and lateral secondary branches. Bony hooks on last unbranched to 5th or 6th branched pelvic-fin rays. No hypertrophied soft tissue associated to hook bearing pelvic-fin rays. Caudal-fin lobes pointed, principal caudal-fin rays 19*(20). Dorsal procurrent caudal-fin rays 12(1), 13*(10), 14(8) or 15(1); ventral procurrent caudal-fin rays 12(1), 13*(15), 14(3) or 15(1). Mature males with anterior ventral procurrent caudal-fin rays hypertrophied, projecting between muscles and skin on ventral margin of caudal-peduncle.

Scales cycloid. Lateral line complete, 31(7) or 32*(13); predorsal scales 9(1) or 10*(19); scale rows from lateral line to dorsal-fin origin 5*(20); scale rows from lateral line to pelvic-fin origin 4*(20); scale rows around caudal peduncle 12(5) or 13*(15). One row of scales along anal-fin base, covering from first to 4th or 5th, rarely 6th branched anal-fin rays. Two scales covering caudal-fin base.

Supraneurals 4(1) or 5(1); precaudal vertebrae 10(2); caudal vertebrae 20(2). Gill rakers on first arch, 10 on lower limb, 7 on upper limb; on 2nd arch, 9 on lower limb, 4 on upper limb; on 3rd arch, 8 on lower limb, 4 on upper limb.

Color in alcohol. Overall body coloration yellowish to light brown. Head darker over dorsal region of neurocranium. Dark brown chromatophores heavily concentrated on parietals and supraoccipital region. Snout and maxilla with concentration of black chromatophores. Dark brown chromatophores scattered on upper portion of opercle. Ventral region of head and abdomen yellowish with no chromatophores. Humeral region with triangular dark area due to conspicuous pseudotympanum muscular gap. Occasionally, scattered dark chromatophores resembling faded humeral spot posterodorsal to pseudotympanum. Dorsum darker than lateral of body; scales with black chromatophores on distal margins, forming reticulated pattern. Subcutaneous black dots aligned on longitudinal line of body below adipose fin to vertical through first dorsal and ventral procurrent caudal-fin rays. Conspicuous dotted black line along base of anal fin. Black mark bordering caudal peduncle terminus, along base of procurrent and principal caudal-fin rays. Dorsal, anal, pectoral, and caudal fins mostly hyaline, with dark chromatophores scattered on interadial membrane. Unbranched pectoral-fin ray with higher concentration of black chromatophores. Pelvic and adipose fins hyaline.

Sexual dimorphism. Anterior ventral procurrent caudal-fin rays (4 to 6) of sexually dimorphic males more expanded in the sagittal plane than comparable to remaining rays, projecting between muscles and skin, visible along ventral margin of caudal peduncle (Fig. 4). All procurrent caudal-fin rays slender on females.

Anal-fin bony hooks from the last unbranched ray and from first up to the 7th branched rays of adult males (Fig. 5). The hooks are present on two thirds of anal-fin rays' length, beginning on the 2nd to 3rd proximal segment of lepidotrichia before primary branching of branched rays, inserted along the posterolateral border of the rays on posteriormost branch only, progressively fewer hooks posteriorly. One to four hooks (usually two) found per segment of lepidotrichia, with a wider base than the median portion of the hook, gradually sharpening to a retrorse tip. The anal-fin hooks are bilaterally asymmetric, irregularly arranged and unpaired, differing in number, size and/or position on each hemitrichia and segment of lepidotrichia of rays. In three specimens the hooks are not fully developed, found on first to 2nd(1), first to 3rd(1), and first to 4th(1) branched rays only.

Anterior branched anal-fin rays more expanded in the sagittal plane than comparable to other anal-fin rays in mature males bearing hooks.

Pelvic-fin bony hooks present on the unbranched ray and from first up to the sixth branched rays of adult males (Fig. 6). The hooks are distributed all along the length of the rays, beginning from the long basal segment to the most distal segment of lepidotrichia, attached on the ventral border of medial branch of the fin ray, progressively fewer hooks medially. Bony hooks usually attached to ventral and lateral borders of medial and lateral branches on the secondary branches of the rays. One to three hooks (usually two) per segment of lepidotrichia, with a wider base than the median portion of the hook, gradually sharpening to a retrorse tip. Pelvic-fin hooks bilaterally asymmetric, occurring on one hemitrichia of rays. In three specimens the hooks are not fully developed, found on first to 4th(2) or 5th(1) branched rays only.

Bony hooks absent on dorsal, pectoral and caudal-fin rays of mature males, and on all fins of females.

Distribution. *Aphyocharacidium* n. sp. is known to inhabit Morona River (Morona district) and Corrientes River tributary of the Tigre River (Andoas district) in Datem del Marañón, Loreto, Peru (Fig. 7). Both are tributaries of the lower Marañón River, upper Amazon River basin, located on Amazonas Lowlands ecoregion (Abell *et al.* 2008, ecoregion 316; WWF & TNC 2015).

Ecological notes. Gut contents of six specimens revealed a diet based mainly on aquatic insect larvae (mostly Chironomidae and Ephemeroptera).

Conservation status. *Aphyocharacidium* n. sp. is known from only four localities corresponding to an Extent of Occurrence (EOO) of approximately 52710 km^2, included in the calculations are the confluences of Morona River and Tigre River with the Marañón River. Although the species appears to be rare and known only from habitats with preserved riparian vegetation, the habitats in this region are currently preserved. Therefore, according to the IUCN criteria (IUCN, 2019), *Aphyocharacidium* n. sp. can be classified as a Least Concern (LC) species.

Discussion

The presence of markedly secondary sexual characters in *Aphyocharacidium* is recorded for the first time. We attest here the presence of bony hooks on anal and pelvic-fin rays on sexually dimorphic males of *Aphyocharacidium* n. sp.. Fin hooks are defined as bony spinous processes developed on the surface of individual segments of lepidotrichia and surrounded by the epidermis (Wiley & Collette 1970; Malabarba & Weitzman 2003). In Characidae, these projections compose a dimorphic feature of mature males with few records of its presence on females, and are most commonly present in the anal and pelvic fins, more rarely in the dorsal, pectoral, and caudal fins. Function is still unknown, although it is believed that it allows the male to recognize with precision its position in relation to the female (Foster 1967) or work as contact organs for maintaining male and female in direct contact during prespawning behavior or spawning act (Wiley & Collette 1970; Weitzman & Fink 1985).

There is great potential for understanding the inter- and intrafamilial relationships arising from evidence of the sexual systems among groups of Characidae (Wiley & Collette 1970; Weitzman & Malabarba 1998; Vieira *et al.* 2016). The presence of hooks on fins has been proposed as a synapomorphy of a group of Characiformes, including Characidae (see Malabarba & Weitzman 2003). Studies exploring the size, shape, position, and arrangement of bony hooks represents a great source of informative data on these features for many Characidae groups (*e.g.* Fink & Weitzman 1974; Weitzman & Fink 1985; Malabarba 1998; Menezes & Weitzman 2009). The variation of this structure has great potential in helping to understand the systematics of complex and poorly known characin groups (Lima & Sousa

2009). Their utility has been demonstrated on the corroboration of hypotheses of phylogenetic relationships in monophyletic groups of Characidae, *e.g.* Xenurobryconini (Weitzman & Fink 1985), Cheirodontinae (Malabarba 1998), and Glandulocaudinae (Menezes & Weitzman 2009). However, some characid taxa are assumed to lack fin hooks, such is the case reported for *Aphyocharacidium* and related genera (*Axelrodia*, *Leptagoniates*, *Phenagoniates*, *Paragoniates* and *Xenagoniates*) (Malabarba & Weitzman 2003). Géry (1960, 1973) originally described *Aphyocharacidium melandetum* and *A. bolivianum* as lacking bony hooks on all fins. Here we attest the presence of bony hooks on anal and pelvic-fin rays on mature males of *Aphyocharacidium* n. sp., as well in *Aphyocharacidium bolivianum* and *Axelrodia lindeae* (also described as not bearing fin hooks). Concerns of presence or seasonal variation on the occurrence of fin hooks in Characidae has been debated in many studies (*e.g.* Wiley & Collette 1970; Fink & Weitzman 1974; Weitzman & Fink 1985; Ferreira & Lima 2006; Lima & Sousa 2009). This issue remains unclear to a considerable number of genera within Characidae, mainly because many taxa are represented by few lots in collections and the analysis should encompass seasonality in samples and developmental stage of specimens in relation to their reproductive period (Ferreira & Lima 2006; Vieira *et al.* 2016). Thus, the availability of vouchers including mature males was essential for the positive identification on the presence of the sexually dimorphic features in these species.

Mature males of *Aphyocharacidium* n. sp. have the anterior branched anal-fin rays (those bearing bony hooks) more expanded in the sagittal plane than comparable to other anal-fin rays. This condition is also found in *Aphyocharacidium bolivianum* and *Axelrodia lindeae,* specimens examined herein, and similar to Clade B members of the Cheirodontinae (see Malabarba 1998: 211, ch. 17). One to four bony hooks (usually two) were observed on the posterolateral border of each anal-fin ray segment bearing hooks as the common condition in mature males of *Aphyocharacidium* n. sp., *A. bolivianum* and *Axelrodia lindeae*. They are more numerous on the anterior branched rays and progressively decreasing in number in the posterior anal-fin rays, as well as in Clade B cheirodontines (Malabarba 1998: 211, ch. 22). The three species also display anal-fin hooks bilaterally asymmetric, irregularly arranged and unpaired, differing in number, size and position between contralateral segments of lepidotrichia (left and right) as described for Clade B Cheirodontini species (Malabarba 1998: 212–213, ch. 25).

In mature males of *Aphyocharacidium* n. sp. and *Axelrodia lindeae*, the anterior ventral procurrent caudal-fin rays are hypertrophied, more expanded in the sagittal plane than comparable to remaining rays (4 to 5 in the former, and 1 to 9 in the latter). In the examined material of *A. bolivianum*, the ventral procurrent caudal-fin rays were all slender, showing no sexual dimorphism. In the examined specimens, the dorsal and ventral procurrent caudal-fin rays of *Aphyocharacidium* n. sp., *A. bolivianum* and *Axelrodia lindeae* are always projected between the muscle and skin, and visible along ventral margin of caudal peduncle.

Aphyocharacidium bolivianum has been consistently hypothesized as belonging to "Clade B" characids and to the Aphyocharacinae. Its position in a clade that is sister group to the remaining genera of this subfamily has been strongly supported both with molecular (Tagliacollo *et al.*, 2012) and morphological data (Mirande, 2019), these hypotheses differing only in the addition of *Axelrodia lindeae* as sister group to *A. bolivianum* in the morphological analysis (this species is missing in the molecular analysis).

The presence of an inner series of teeth on the dentary was addressed as a unique character state among characids. A second series of dentary teeth can also be found in *Oxybrycon parvulus* Géry, 1964 (see also Géry 1977), leading Mirande (2010) to interpret this as a potential synapomorphy of a clade containing *Aphyocharacidium* and *Oxybrycon*. The available information on *Oxybrycon parvulus* shows that despite the similarities on the arrangement into two rows of teeth on the dentary, the teeth seems very small and fragile on both premaxilla and dentary, and of scattered insertion on the lamellar portion of maxilla (Géry 1964: fig. 12). Also, a reduction of the infraorbital series with the fourth infraorbital absent or reduced to an extremely thin bone lamella, the lateral line practically absent (0–2 perforated scales) (Géry 1964), and other possible reductive characters that strongly distinguishes this small-bodied species. Though a careful analysis is needed, by considering the distinctiveness of these features it seems unlikely that a close relationship of these two genera would be solely based on the presence of two rows of dentary teeth.

Axelrodia lindeae Géry, 1973 was described as "at the limit of the genus" (Géry 1973: 114), given the striking differences between its congeners *Axelrodia stigmatias* (Fowler, 1913) (type species of the genus) and *Axelrodia riesei* Géry, 1966. Although not strictly similar, *Axelrodia lindeae* shares some interesting features in resemblance with *Aphyocharacidium bolivianum* and *Aphyocharacidium* n. sp.. The anterior portions of the branchiostegal rays slender near their articulation with ceratohyals (ch. 214), presence of a large foramen on pterosphenoid (ch. 43) and shape of fourth infraorbital longer dorsoventrally than longitudinally (ch. 67) (Mirande 2010), raises questions on the hidden relationships amongst these species. The arrangement of the dentary teeth could be one of them. Despite the fact that *Axelrodia lindeae* presents only one row of conical teeth, the series is placed on the inner region of the dentary and its base is bordered anteriorly by a continuous ridge (Mirande 2010). This disposition of the dentary teeth could be a similar condition to what Géry (1960) mentioned as "being inserted in a crest clearly separated from the outer one". Moreover, the presence of large a foramen on the pterosphenoid was only observed among *Aphyocharacidium* species and *Axelrodia lindeae*. Pending further investigations, perhaps a new combination would be in order, allocating *'Axelrodia' lindeae* in *Aphyocharacidium*.

Comparative material. Brazil – *Aphyocharacidium bolivianum*: MCP 39961, 15, 21.2–28.1 mm SL (1 female c&s, 24.1 mm SL), Amazonas, Humaitá, igarapé do Vinte e Dois, Recanto do Sanari, 20 km west of Humaitá, 7°35’36”S, 63°10’27”W. MCZ 56010, 10, 16.6–25.4 mm SL, Amazonas, small dark water creek flowing into the Paraná de Janauacá, 3°25’S, 61°21’W. LBP 12010, 8, 23.1–30.2 mm SL, Amazonas, Lábrea, afluente rio Purus, rio Madeira drainage, 7°56’S 63°27’W. LBP 18008, 10, 19.3–24.8 mm SL, Amazonas, Itacoatiara, igarapé Grande, AM–010 highway, rio Amazonas drainage, 3°7’7”S 58°27’14.7”W. *Aphyocharacidium* sp.: MCP 37959, 9, 22.5–26.0 mm SL (1 female c&s, 23.2 mm SL), Acre, igarapé Marizinho, BR–364 highway, rio Antimari drainage, 9°36’41”S, 68°14’40”W. MCP 37960, 13, 20.6–27.4 mm SL (1 female c&s, 24.8 mm SL), Acre, Sena Madureira, igarapé Taquari, entre o rio Antimari e Sena Madureira, BR–364 highway, 9°27’48”S, 68°22’42”W. *Aphyocharax alburnus*: UFRGS 24198, 2 (1 male c&s, 43.7 mm SL; 1 female c&s; 44.7 mm SL), Rondônia, Porto Velho, foz do rio Jatuarana com rio Madeira, próximo a cachoeira do Teotônio, 8°49’54”S, 64°2’45”W. UFRGS 25829, 2 (2 females c&s, 32.8–39.5 mm SL), Rondônia, Porto Velho, foz do rio Jaci-Paraná com o rio Madeira, 9°9’41”S, 64°23’55”W. *Aphyocharax anisitsi*: UFRGS 9315, 1 (1 female c&s, 29.3 mm SL), Rio Grande do Sul, São Sepé, açude em propriedade destinada à silvicultura, 29°57’14”S, 53°45’24”W. UFRGS 6485, 2 (2 females c&s, 25.1–27.1 mm SL), Rio Grande do Sul, Rosário do Sul, arroio do Salso, 30°22’26”S, 55°2’5”W. *Axelrodia lindeae*: MPEG 27576, 8, 20.0–25.0 mm SL, Juruti, Pará, igarapé Rio Branco, Arapiuns basin, 2°20’57.4”S 56°1’28”W. MCP 37314, 10, 17.7–22.6 mm SL (1 male c&s, 22.5 mm SL; 3 females c&s, 23.8–18.7 mm SL), Acre, Bujari, igarapé Marizinho, BR–364 highway, rio Antimari drainage, 9º36’41”S, 68º14’40”W. MCP 37317, 7, 19.1–24.3 mm SL, Rondônia, Candeias de Jamari, riacho na estrada 364 entre Ariquemes e Candeias do Jamari, afluente do rio Candeias, 8º47’14”S, 63º36’23”W. *Paragoniates alburnus*: MCP 17081, 1 (1 c&s, 62.1 mm

SL), Amazonas, Manacabi, rio Jupurá, cerca de 50 km da foz, 2°45’S, 64°52’W. *Prionobrama filigera*: MCP 17091, 1 (1 c&s, 41.8 mm SL), Rondônia, Porto Velho, rio Madeira, cachoeira Santo Antônio, 8°49’59”S, 64°W. MCP 29567, 1 (1 c&s, 41.3 mm SL), Amazonas, Iranduba, Alvarães, canal do lago Mamirauá Estação A na comunidade Boca do lago Mamirauá, sistema do lago Mamirauá, 3°6’37”S, 64°47’49”W. *Prionobrama paraguayensis*: MCP 15580, 2 (2 c&s, 31.5–32.4 mm SL), Mato Grosso, Cáceres, rio Paraguai em Cáceres e arredores, 16°3’S, 57°42’W. MZUSP 42748, 1 (1 c&s, 31.4 mm SL), Mato Grosso, Barão de Melgaço, rio Cuiabá, 16°11’S, 55°57’W. Ecuador – *Leptagoniates steindachneri*: FMNH 97120, 1 (1 c&s, 54.0 mm SL), Napo, rio Jivino, lower 4 km (mostly) to ca. 6 km upstream from mouth (most between the two “ports” for Limoncocha), 0°24’36”S, 76°39’W. *Paragoniates alburnus*: FMNH 100346, 1 (1 c&s, 70.0 mm SL), Napo, Quebrada Apoalla, tributary to lower rio Shushufindi, 0°16’59”S, 76°27’W. Venezuela – *Phenagoniates macrolepis*: ANSP 150124, 2 (2 c&s, 35.2–26.8 mm SL), Motatan, 30 km N Trujillo. *Xenagoniates bondi*: ANSP 150421, 1 (1 c&s, 53.8 mm SL), Guárico, Orituco River at the bridge crossing Calabozo-Carorla highway. ANSP 165260, (1 c&s, 36.4 mm SL), Apure, rio Matiyure, at Achaguas, 7°45’N, 68°11’W. FMNH 85494, 1 (1 c&s, 45.0 mm SL), Apure, rio Aruaca, 32.5 km S Biruaca, 7°32’18”N, 67°31’29”W. FMNH 103538, 1 (1 c&s, 45.1 mm SL), Barinas, Caño La Indiacita near mouth in rio Suripa ca. 35 minutes up river from boat launch in Hato Mercedes, 7°47’50”N, 69°54’33”W.

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Legends of illustrations

Figure 1. *Aphyocharacidium* n. sp., new species: A) holotype, MUSM 68515, 25.1 mm SL; B) paratype, MUSM 41944, 25.7 mm SL; both from Situche stream, Morona river, Morona, Datem del Marañón, Loreto, Peru.

Figure 2. Images of jaws of *Aphyocharacidium* n. sp., paratype, MUSM 69043, 23.8 mm SL: A) premaxilla; B) maxilla; C) dentary, lateral view; D) dentary, teeth inner series. Scale bars = 1 mm.

Figure 3. Scanning Electron Microscopy (SEM) images of jaws and dentition detail of *Aphyocharacidium* n. sp., paratype, female, MUSM 69043, 23.8 mm SL: A) maxilla; B) premaxilla; C) dentary; Lateral view, anterior to the left, Scale bar = 500 ɥm; D) maxillary teeth; E) premaxillary teeth; F) dentary teeth; Overview, Scale bar = 50 ɥm.

Figure 4. Ventral procurrent caudal-fin rays of *Aphyocharacidium* n. sp.: A) paratype, male, MUSM 68942, 27.8 mm SL; B) paratype, female, MUSM 69043, 23.8 mm SL. Lateral view, anterior to the left. Scale bars = 1 mm.

Figure 5. Anal fin of mature male of *Aphyocharacidium* n. sp.: paratype, male, MUSM 68942, 27.8 mm SL; hypertrophied anal-fin rays and bony hooks distribution. Lateral view, anterior to the left. Scale bar = 1 mm.

Figure 6. Pelvic fin of *Aphyocharacidium* n. sp.: A) paratype, male, MUSM 68942, 27.8 mm SL, pelvic-fin rays bearing bony hooks; B) paratype, female, MUSM 69043, 23.8 mm SL. Lateral view, anterior to the left. Scale bar = 1 mm.

Figure 7. Map of the upper Amazon basin, showing type locality (star) of *Aphyocharacidium* n. sp.. Distribution localities (dot) can indicate more than one collection event.

Table 1. Morphometric data for type material of *Aphyocharacidium* n. sp. (new species). N = number of specimens; SD = Standard deviation. Male ranges includes the holotype.

		Males					Females				
	Holotype	**N**	**Min**	**Max**	**Mean**	**SD**	**N**	**Min**	**Max**	**Mean**	**SD**
Standard length (mm)	25.1	8	22.0	29.1	25.2	–	12	21.5	28.9	24.3	–
Percents of Standard Length (SL)											
Snout-anal fin distance	58.8	8	57.6	59.1	58.4	0.5	12	57.6	60.7	59.4	0.8
Snout-dorsal fin distance	49.5	8	49.5	52.0	50.9	0.8	12	50.0	52.2	51.3	0.7
Snout-pelvic fin distance	44.6	8	43.4	45.6	44.4	0.7	12	44.3	46.3	45.1	0.7
Snout-pectoral fin distance	27.1	8	26.8	28.1	27.4	0.5	12	26.7	28.3	27.4	0.5
Dorsal-caudal fin distance	54.0	8	52.7	54.4	53.6	0.5	12	52.5	53.9	53.2	0.5
Orbit-dorsal fin distance	35.1	8	35.1	37.2	35.9	0.7	12	35.4	37.8	36.5	0.7
Anal-fin base length	30.0	8	29.1	30.2	29.4	0.4	12	27.7	29.7	28.9	0.5
Length of caudal peduncle	12.8	8	12.3	12.9	12.6	0.3	12	11.6	12.9	12.3	0.4
Depth of caudal peduncle	10.4	8	9.8	10.9	10.3	0.4	12	9.3	10.2	9.8	0.3
Body depth at dorsal fin	28.2	8	27.0	30.3	28.4	0.9	12	27.1	31.2	29.5	1.4
Dorsal-fin length	28.7	8	28.5	30.6	29.6	0.8	12	27.8	30.7	29.4	0.8
Pelvic-fin length	17.3	8	16.7	17.3	17.1	0.3	12	16.8	17.8	17.3	0.3
Pectoral-fin length	20.3	8	19.5	20.6	20.0	0.4	12	18.6	20.7	19.5	0.6
Head length	27.4	8	27.0	28.2	27.5	0.5	12	26.5	28.0	27.5	0.5
Percents of Head Length (HL)											
Snout length	30.5	8	27.3	30.5	29.1	1.1	12	28.2	30.8	29.3	0.8
Upper Jaw length	36.2	8	36.2	38.1	37.2	0.7	12	36.4	38.7	37.5	0.8
Horizontal orbit diameter	40.1	8	40.1	41.4	40.8	0.5	12	40.2	42.8	41.5	0.9
Interorbital width	29.9	8	29.6	31.9	30.4	0.8	12	29.3	31.1	30.1	0.7

Illustrations

Figure 1. *Aphyocharacidium* n. sp., new species: A) holotype, MUSM 68515, 25.1 mm SL; B) paratype, MUSM 41944, 25.7 mm SL; both from Situche stream, Morona river, Morona, Datem del Marañón, Loreto, Peru.

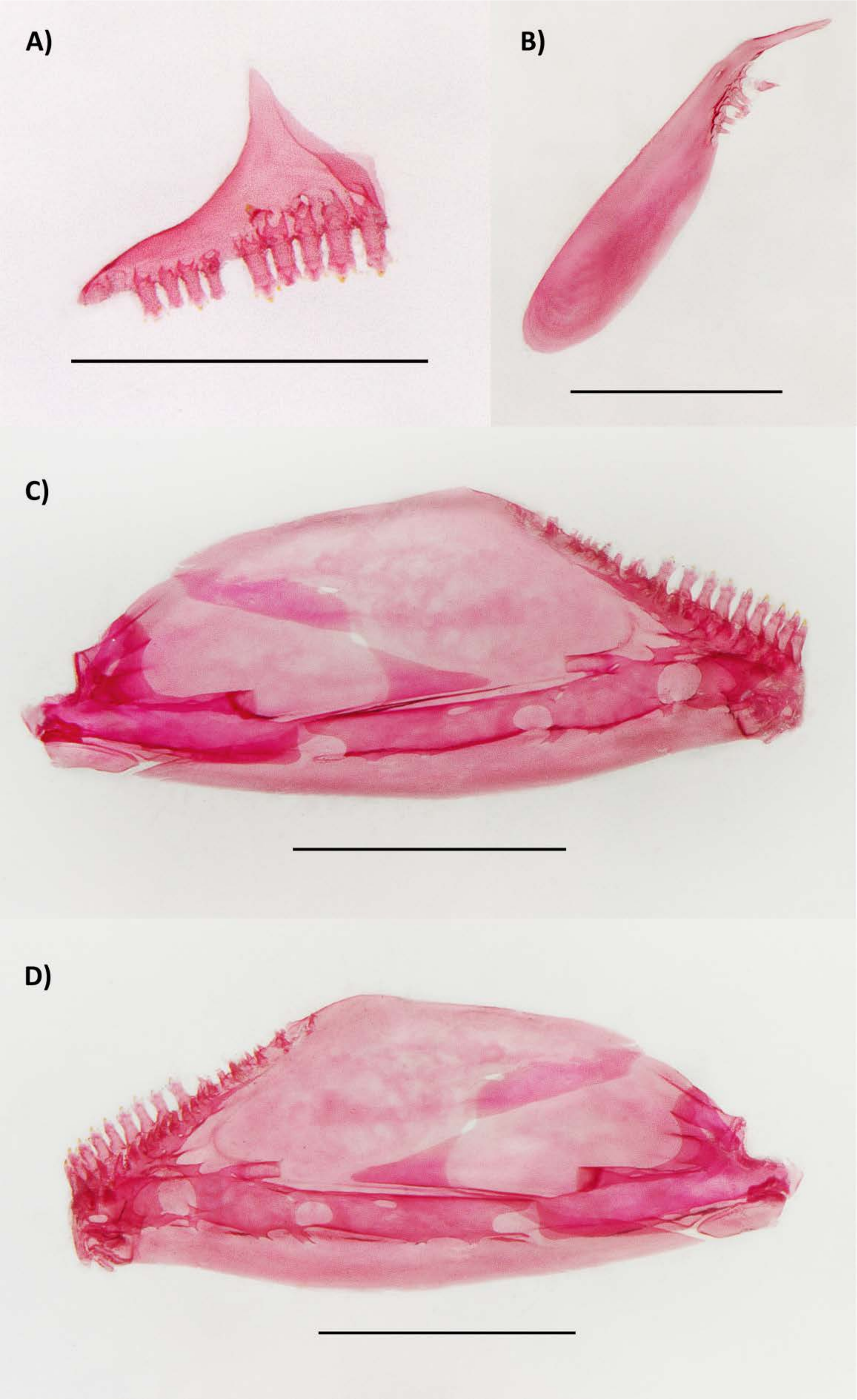

Figure 2. Images of jaws of *Aphyocharacidium* n. sp., paratype, MUSM 69043, 23.8 mm SL: A) premaxilla; B) maxilla; C) dentary, lateral view; D) dentary, teeth inner series. Scale bars = 1 mm.

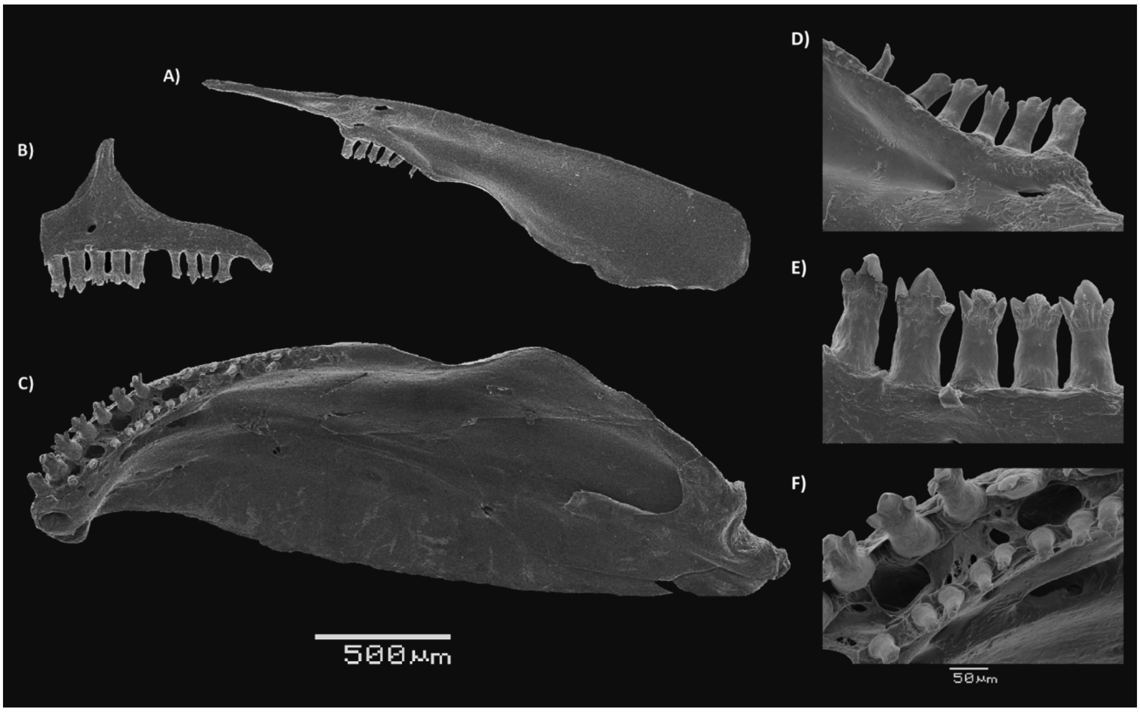

Figure 3. Scanning Electron Microscopy (SEM) images of jaws and dentition detail of *Aphyocharacidium* n. sp., paratype, female, MUSM 69043, 23.8 mm SL: A) maxilla; B) premaxilla; C) dentary; Lateral view, anterior to the left, Scale bar = 500 ɥm; D) maxillary teeth; E) premaxillary teeth; F) dentary teeth; Overview, Scale bar = 50 ɥm.

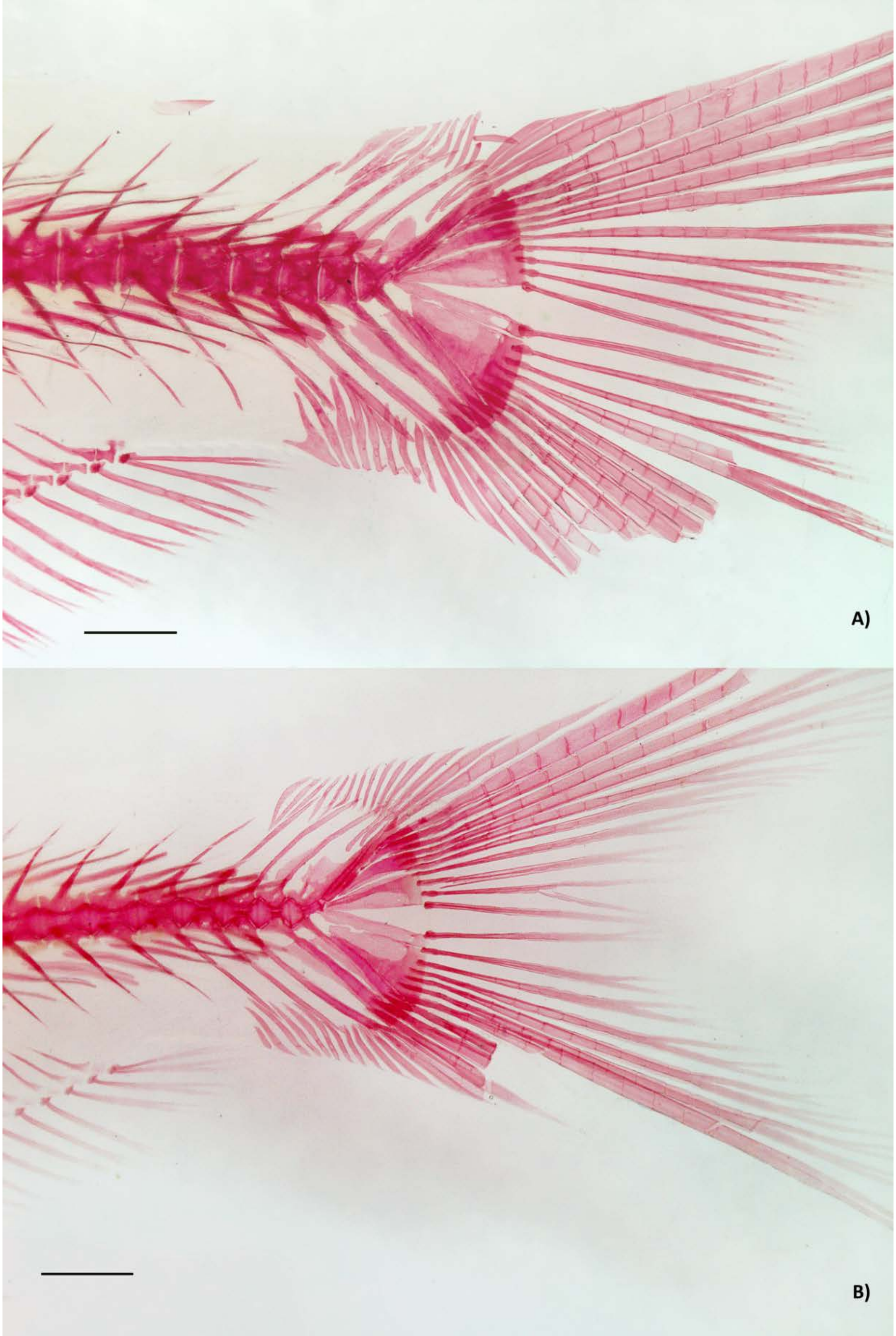

Figure 4. Ventral procurrent caudal-fin rays of *Aphyocharacidium* n. sp.: A) paratype, male, MUSM 68942, 27.8 mm SL; B) paratype, female, MUSM 69043, 23.8 mm SL. Lateral view, anterior to the left. Scale bars = 1 mm.

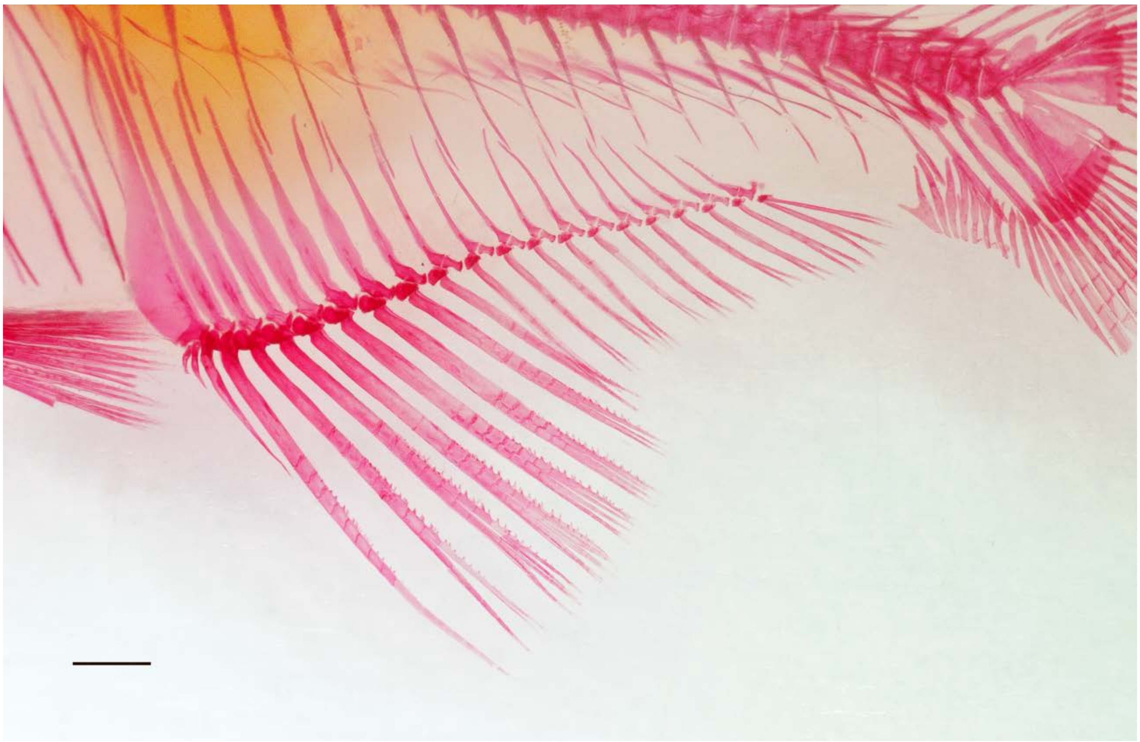

Figure 5. Anal fin of mature male of *Aphyocharacidium* n. sp.: paratype, male, MUSM 68942, 27.8 mm SL; hypertrophied anal-fin rays and bony hooks distribution. Lateral view, anterior to the left. Scale bar = 1 mm.

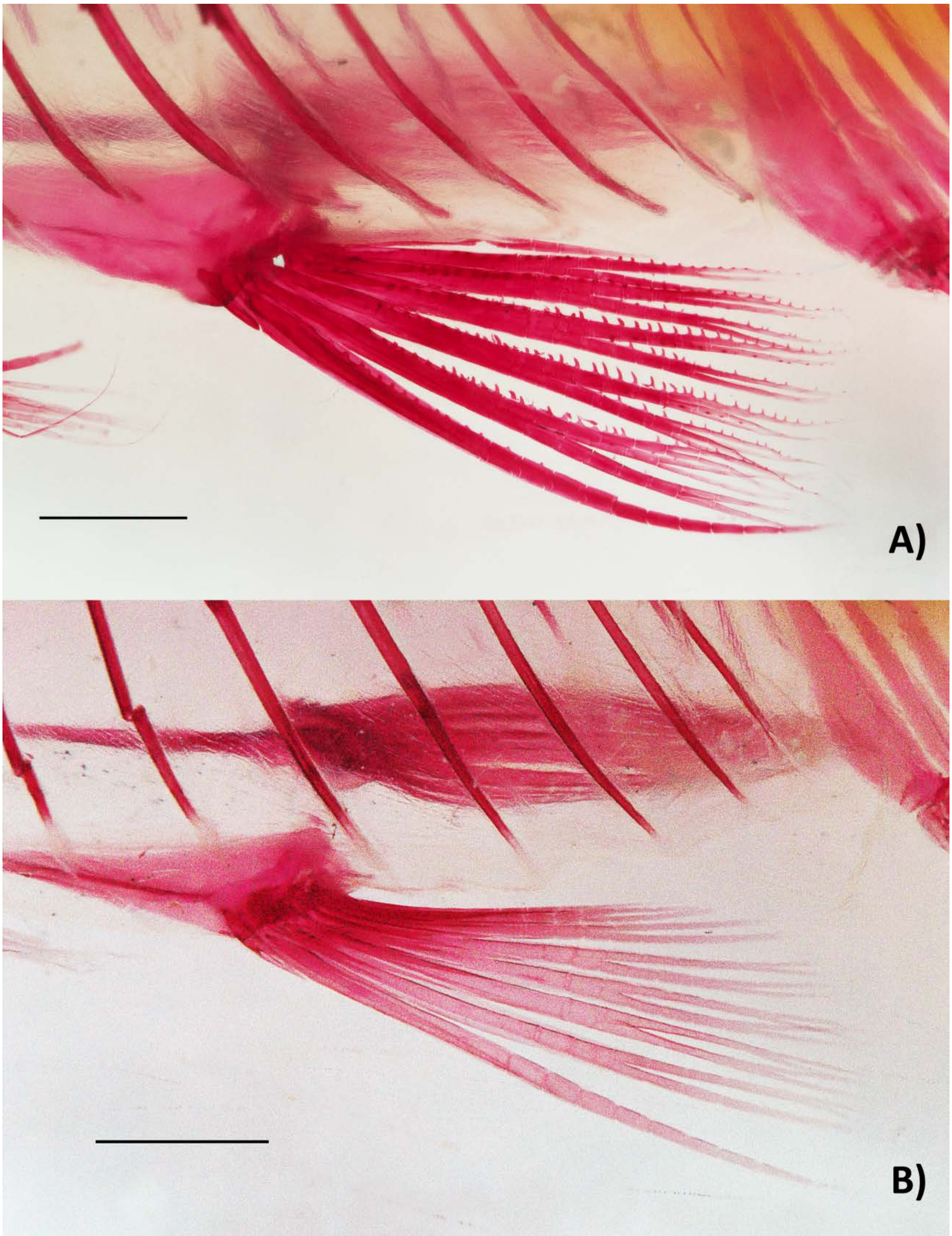

Figure 6. Pelvic fin of *Aphyocharacidium* n. sp.: A) paratype, male, MUSM 68942, 27.8 mm SL, pelvic-fin rays bearing bony hooks; B) paratype, female, MUSM 69043, 23.8 mm SL. Lateral view, anterior to the left. Scale bar = 1 mm.

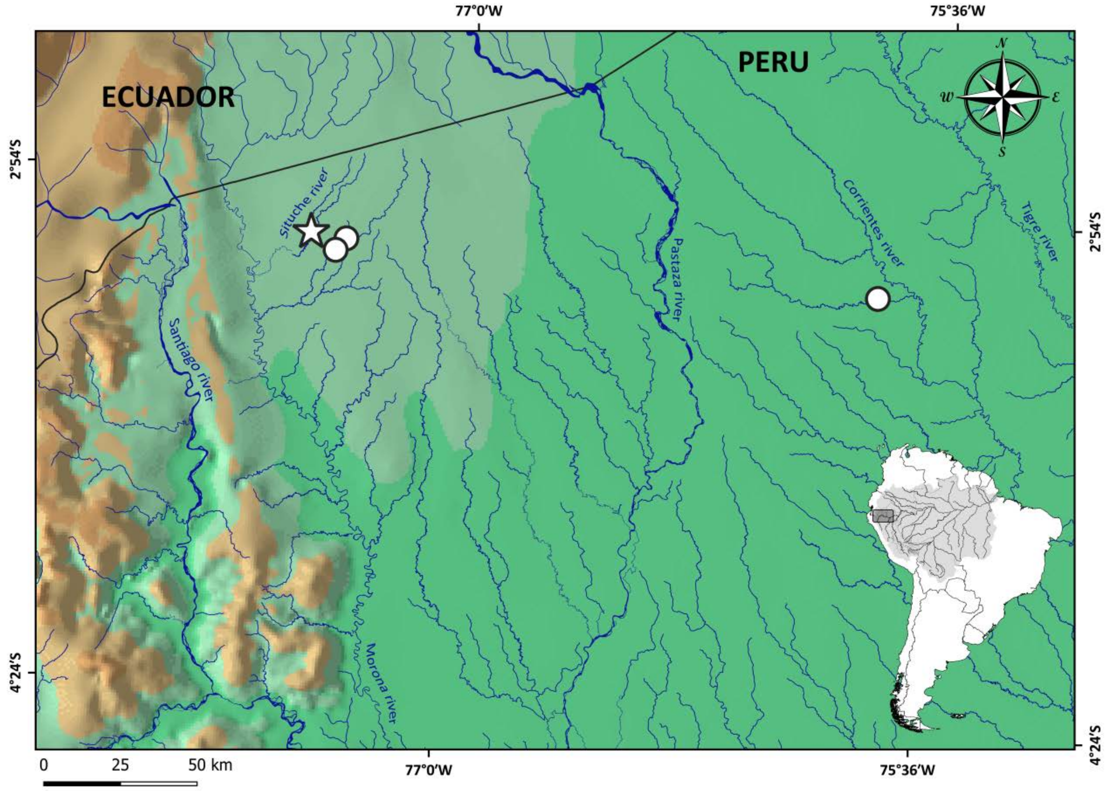

Figure 7. Map of the upper Amazon basin, showing type locality (star) of *Aphyocharacidium* n. sp.. Distribution localities (dot) can indicate more than one collection event.