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Revisão taxonômica das espécies do gênero *Odontostilbe* Cope, 1870 (Characidae: Cheirodontinae) da Bacia do Rio da Prata

Dissertação apresentada ao Programa de Pós-graduação em Biologia Animal, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Mestre em Biologia Animal

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Sumário

Resumo

O gênero *Odontostilbe* distribuído na bacia do rio da Prata é revisado. *Odontostilbe microcephala* e *O. paraguayensis* são redescritos e cinco espécies novas são reconhecidas. Revisando-se o material da redescrição de *Odontostilbe microcephala* feito por Eigenmann, foram encontradas duas espécies novas: *Odontostilbe* sp. n. A e *Odontostilbe* sp. n. W que durante muitos anos foram, erroneamente, identificadas como *O. microcephala*. *Odontostilbe microcephala* ficou restrita para a vertente andina da bacia do rio Paraguai e parte baixa da bacia do rio Paraná. *Odontostilbe* sp. n. A e *Odontostilbe* sp. n. W apresentam distribuição restrita, ocorrendo somente na porção superior do rio Paraná. As duas espécies novas distinguem-se de seus congêneres pela presença de dentes no mesopterigoide, agrupados em uma fileira na parte central. A partir da redescrição de *O. paraguayensis* foi possível descrever uma nova espécie, *Odontostilbe* sp. n. B. *Odontostilbe paraguayensis* é amplamente distribuído na bacia do baixo Paraná e rio Paraguai e é facilmente reconhecida pela presença de um pequeno espinho ósseo projetado anteriormente (sob a pele), sustentado pelos supraneurais fusionados. *Odontostilbe* sp. n. B apresenta distribuição restrita à porção superior do rio Paraná, distinguindo-se de seus congêneres pela presença da linha lateral incompleta e pela presença de ganchos em quase todos os raios ramificados da nadadeira anal em machos maduros. Na bacia do rio Uruguai foram reconhecidas duas espécies novas: *Odontostilbe* sp. n. R, endêmica do alto rio Uruguai, e *Odontostilbe* sp. n. U distribuída na parte baixa. Estas espécies distinguem-se de suas congêneres pela boca subterminal e formato dos supraneurais. *Odontostilbe* sp. n. U distingue-se de *Odontostilbe* sp. n. R pela contagem das escamas perfuradas da linha lateral e pelo padrão de coloração da mácula do pedúnculo caudal.

Palavras chaves: Peixes, Dentes do mesopterigoides, Nova espécies, Redescrição, VARSEDIG.

Abstract

The genus *Odontostilbe* distributed in the River Plate basin is reviewed. *Odontostilbe microcephala* and *O. paraguayensis* are redescribed and five new species are recognized. Reviewing the material of the redescription of *Odontostilbe microcephala* by Eigenmann, two new species were found: *Odontostilbe* sp. n. A and *Odontostilbe* sp. n. W that for many years were erroneously identified as O. microcephala restricted to the Andean slope of the rio Paraguay basin and lower part of the rio Paraná basin. *Odontostilbe* sp. n. A and *Odontostilbe* sp. n. W present restricted distribution, occurring only in the upper portion of the rio Paraná. The two new species are distinguished from their congeners by the presence of teeth in the mesopterigoid, grouped in a row in the central part. From the redescription of *Odontostilbe paraguayensis* it was possible to describe a new species, *Odontostilbe* sp. n. B. *Odontostilbe paraguayensis* is widely distributed in the basin of the lower rio Paraná and rio Paraguay and is easily recognized by the presence of a small bony spine previously projected (under the skin), supported by the supraneural fused. *Odontostilbe* sp. n. B presents a restricted distribution to the upper portion of the rio Paraná, distinguishing itself from its congeners by the presence of the incomplete lateral line and the presence of hooks in almost all the branched rays of the anal fin in mature males. Two new species were recognized in the rio Uruguay basin: *Odontostilbe* sp. n. R, endemic to the upper rio Uruguay, and *Odontostilbe* sp. n. U distributed in the lower part. These species are distinguished from their congeners by the subterminal mouth and shape of the supraneural. *Odontostilbe* sp. n. U is distinguished from *Odontostilbe* sp. n. R by counting the perforated scales of the lateral line and by the staining pattern of the spot of the caudal peduncle.

Keyword : Fishes, Mesopterigoid teeth, New species, Redescription, VARSEDIG

Introdução

Cheirodontinae species are found in most river drainages of Central and South America, occurring from Costa Rica to central Chile and Argentina, in both Atlantic and Pacific drainages of the Andes (Malabarba 2003). The subfamily includes 18 genera: *Amazonspinther* Bührnheim, Carvalho, Malabarba & Weitzman, *Acinocheirodon* Malabarba & Weitzman, *Aphyocheirodon* Eigenmann, *Cheirodon* Girard, *Cheirodontops* Schultz, *Compsura* Eigenmann, *Ctenocheirodon* Malabarba & Jerep, *Heterocheirodon* Malabarba, *Kolpotocheirodon* Malabarba & Weitzman, *Macropsobrycon* Eigenmann, *Nanocheirodon* Malabarba, *Odontostilbe* Cope, *Prodontocharax* Eigenmann & Pearson, *Protocheirodon* Vari, *Pseudocheirodon* Meek & Hildebrand, *Saccoderma* Schultz, *Serrapinnus* Malabarba, *Spintherobolus* Eigenmann and one genus fossil *Megacheirodon* M. C. Malabarba. Currently are recognized 66 valid species, of these, three genus and nine species were described in the last ten years (Malabarba 1998, 2003; Bührnheim *et al*. 2008; Malabarba & Jerep 2012; Vari *et al*. 2016).

Cope (1970) describes the genus *Odontostilbe* by teeth in a single series on the premaxillary and dentary, anal fin elongate and lateral line complet *versus* interrupted in *Cheirodon* (describing as type species *Odontostilbe fugitiva* Cope). Currently there are 12 valid species of *Odontostilbe* (Bührnheim & Malabarba 2006, 2007): *O. dierythrura* Fowler; *O. ecuadorensis* Bührnheim & Malabarba; *O. euspilurus* (Fowler); *O. fugitiva*; *O. microcephala* Eigenmann; *O. nareuda* Bührnheim & Malabarba; *O. pao* Bührnheim & Malabarba; *O. paraguayensis* Eigenmann & Kennedy; *O. parecis* Bührnheim & Malabarba, *O. pequira* (Steindachner); *O. pulchra* (Gill); and *O. splendida* Bührnheim & Malabarba. Eigenmann (1915) considered the species "*Odontostilbe" dialeptura* (Fink & Weitzman) and "*Odontostilbe" mitoptera* (Fink & Weitzman) from Pacific rivers in Costa Rica and Panama are possibly not part of the genus *Odontostilbe*, observation latter corroborated by Malabarba (1998). The two species are currently hypothesized to be related to compsurin cheirodontines, but remain referred to *Odontostilbe* in the lack of a proper generic name (Bührnheim & Malabarba 2006). Describing in the last ten years five species, between Amazon basin (Bührnheim & Malabarba 2006) and Orinoco river drainage (Bührnheim & Malabarba 2007).

Historically the validity of *Odontostilbe* has been discussed; Lutken (1875) includes *Odontostilbe* as a subgenus of *Chirodon* [unjustified modification of *Cheirodon* for *Chirodon* by Gunther (1864)]. Subsequently, Steindachner (1882) described *Chirodon*

pequira. Eigenmann & Eigenmann (1891) considering *Odontostilbe* as a subgenus of *Cheirodon*. Eigenmann (1915) makes the first review of Cheirodontinae, based on morphology of teeth, scales and infra-orbital bone, recognizing *Odontostilbe* with eight species (*O. fugitiva*, *O. microcephala*, *O. pulchra*, *O. paraguayensis*, *O. drepanon* Fowler [synonym of *O. fugitiva*], *O. madeira* Fowler [synonym of *O. fugitiva*], *O. hastata* Eigenmann [valid as *Saccoderma hastata* (Eigenmann)] and *O. melandeta* Eigenmann [valid as *Aphyocharacidium melandetum* (Eigenmann)]. Eigenmann (1915) affirms the possibility that *O. hastata* and *O. melandeta* are not part of the genus *Odontostilbe*, observation corroborated by Malabarba (1998).

Fink & Weitzman (1974) review Cheirodontinae of Central America, considering group polyphyletic; the genera *Odontostilbe*, *Pseudocheirodon*, and *Compsura* are synonomized with *Cheirodon*, based on reevaluation of the characters. Géry (1977) affirms that to accept that indicated by Fink & Weitzman (1974) requires complete revision of the genus including the type species (*Cheirodon pisciculus* and *Odontostilbe fugitiva*) and considers genus *Odontostilbe* as valid. Weitzman & Fink (1983) discuss the classification of Eigenmann (1915, 1917) who classify genus of Characiformes following two criteria: one a group of closely related species, descended from a common ancestor and other a polyphyletic type, consist of species having a combination of characters in common. The latter classification criterion caused many problems for the recognition of genus in subsequent years, as was the case of Cheirodontinae, in addition, they mention that to recognize, as valid *Odontostilbe* and *Cheirodon* is necessary make a taxonomic revision of *Cheirodon* and study the phylogenetic relationships of all the species currently placed in *Cheirodon*.

Uj (1987) reviews Cheirodontinae of Paraguay, redefines *Odontostilbe*, based on characters of the type species, and regroups species within of *Odontostilbe* and *Cheirodon* by morphological similarity, transferring *Cheirodon piaba*, *C. kriegi*, *C. stenodon*, *C. microdon*, and *C. notomelas* to *Odontostilbe*.

Malabarba (1998) proposes four synapomorphies support the monophyly of the Cheirodontinae: presence of a large, nearly triangular hiatus of muscles covering the anterior chamber of the swim bladder between the first and second pleural ribs (pseudotympanum) (Character 1). Lack of humeral spot (Character 64). The teeth are pedunculated, largely expanded and compressed distally (Character 56). A single regular

tooth row with teeth perfectly aligned and similar in shape and cusp number in the premaxilla (Character 55), these character was utilized for include a species previously assigned to *Leptagoniates* (Aphyocharacinae) in a new genus *Protocheirodon* (Cheirodontinae) (Vari *et al.* 2016). Also determines two diagnostic characters for *Odontostilbe*: the second unbranched dorsal-fin ray is elongate (Character 11) and the unbranched pelvic-fin ray is elongate (Character 15); also placed *Holoshesthes* as a junior synonym of *Odontostilbe.* Bührnheim & Malabarba (2006, 2007) redescribed *O. fugitiva* and *O. pulchra*, and described five new species, affirming that a reevaluation of the diagnosis of *Odontostilbe* is necessary because of the discovery of several new species not previously used in a phylogenetic analysis of the genus by Malabarba (1998). Miquelarena *et al.* (2008) makes a review of all species of Cheirodontinae of Mesopotamian Region in Argentina, including the species of *Odontostilbe* basin La Plata River.

Herein we provide a redescription of *Odontostilbe microcephala* and *O. paraguayensis*, in order to present a better diagnosis for these species. We also describe five new species from the upper rio Paraná and rio Uruguay, for Brazil and Uruguay.

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Capítulo 1

Two new species of *Odontostilbe* Cope historically hidden under *O. microcephala* Eigenmann (Characiformes: Cheirodontinae)

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Remark: cited as Chuctaya *et al.* in press.A

Two new species of *Odontostilbe* historically hidden under *O. microcephala* (Characiformes: Cheirodontinae)

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Abstract

Specimens historically identified since the Cheirodontinae monograph of Eigenmann as *Odontostilbe microcephala* from the upper rio Paraná and Andean piedmont tributaries of the rio Paraguay are reviewed along with new samples, and split in three species. The statistical analyses were performed using the statistical package VARSEDIG, a free function of the R program to define the morphometric variables that differed significantly between species. The distribution of *O. microcephala* is restricted to the Andean slope of the rio Paraguay basin. The species is distinguished from congeners with subterminal mouth by the elongate body, usually 10-12 gill rakers on upper branch and smaller horizontal orbital diameter (24.6-32.8 % HL, mean 28.7%). Specimens from upper rio Paraná constitute two new species, diagnosed from other Cheirodontinae by the presence of mesopterygoid teeth, grouped on median portion and forming a continuous row. One new species is diagnosed by having premaxillary teeth usually with five cusps and the second species by bearing premaxillary teeth usually with nine cusps.

Keywords: Biodiversity, Characidae, La Plata River basin, Taxonomy, VARSEDIG.

Resumo

Espécimes do rio Paraná e tributários do rio Paraguay, historicamente identificados como *Odontostilbe microcephala* desde a monografia de Eigenman, foram revisados com amostras novas e separados em três espécies. Análises estatisticas foram realizadas usando o pacote estatístico VARSEDIG, uma função do programa R para definir as variáveis morfométricas que diferem significativamente entre as espécies. A distribuição de *O. microcephala* é restrita ao sopé andino da bacia do rio Paraguay. A espécie é distinta das congeners com boca subterminal pela forma alongada, geralmente 10-12 rastros branquiais no ramo superior e menor diâmetro horizontal da orbita (24,6-

32,8 % CC, média 28,7%). Espécimes do alto rio Paraná constituem duas espécies novas diagnosticadas de outros Cheirodontinae pela presença de dentes no mesopterigóide, agrupados em sua porção média e formando uma fileira continua. Uma das espécies novas é diagnosticada por ter dentes pré-maxilares geralmente com cinco cúspides e a segunda por apresentar dentes pré-maxilares geralmente com nove cúspides.

Palavras-chave: Biodiversidade, Characidae, Bacia do rio da Prata, Taxonomia, VARSEDIG.

Running Head: Two new species of *Odontostilbe*

Introduction

The species of Cheirodontinae are found in most river drainages of Central and South America, occurring from Costa Rica to central Chile and Argentina, in both Atlantic and Pacific drainages of the Andes (Malabarba, 2003). The subfamily includes 18 genera and 66 valid species, being almost half (eight) of these genera and almost one third (19) of its species described in the last twenty years (Malabarba, 1998, 2003; Malabarba, Bertaco, 1999; Malabarba, Weitzman, 1999, 2000; Weitzman, Malabarba, 1999; Malabarba *et al*. 2004; Bührnheim, Malabarba, 2006, 2007; Bührnheim *et al*. 2008; Malabarba, Jerep, 2012, 2014; Jerep, Malabarba, 2014; Zarske, 2012; Vari *et al*. 2016; Jerep *et al*. 2016).

Currently there are 14 valid species of *Odontostilbe* (Bührnheim, Malabarba, 2006, 2007): *O*. *dierythrura* Fowler; *O*. *ecuadorensis* Bührnheim & Malabarba; *O*. *euspilurus* (Fowler); *O*. *fugitiva* Cope; *O*. *microcephala* Eigenmann; *O*. *nareuda* Bührnheim & Malabarba; *O*. *pao* Bührnheim & Malabarba; *O*. *paraguayensis* Eigenmann & Kennedy; *O*. *parecis* Bührnheim & Malabarba, *O*. *pequira* (Steindachner); *O*. *pulchra* (Gill); and *O*. *splendida* Bührnheim & Malabarba. Eigenmann (1915) considered the species "*Odontostilbe" dialeptura* (Fink & Weitzman) and "*Odontostilbe" mitoptera* (Fink & Weitzman) from Pacific rivers in Costa Rica and Panama are possibly not part of the genus *Odontostilbe*, observation latter corroborated by Malabarba (1998). The two species are currently hypothesized to be related to compsurin cheirodontines, but remain

referred to *Odontostilbe* in the lack of a proper generic name (Bührnheim, Malabarba, 2006).

Odontostilbe microcephala was described by Eigenmann in Eigenmann, Ogle (1907) based on two specimens from rio Pilcomayo, Bolivia, a tributary of the rio Paraguay. Later, the species was redescribed by Eigenmann (1915) in his monograph on Cheirodontinae, with the examination of additional material from the rio Tietê, expanding the distribution of the species to the upper rio Paraná. After Eigenmann, several studies referred *O. microcephala* from both rio Paraguay and/or upper rio Paraná drainages (e.g. Uj, 1987; Miquelarena *et al*. 2008; Oyakawa, Menezes, 2011).

The examination of Eigenmann's (1907, 1915) specimens along with additional new material proved the specimens from upper rio Paraná to belong to two new species, and that *Odontostilbe microcephala* occurs exclusively in tributaries of rio Paraguay. Herein we provide a redescription of *Odontostilbe microcephala* and the description of the two new species of *Odontostilbe* following the definition of Malabarba (1998).

Material and Methods

Counts and measurements follow Fink, Weitzman (1974) primarily on the left side of the specimen. Length of the base of the anal fin is from the origin of the first ray of the anal fin to the insertion of last anal-fin ray. Measurements corresponding to parts of the head were performed in stereomicroscope. Horizontal measurements were taken projected on the long axis of the body. Head length is the distance between the tip of the snout and the posterior end of subopercle, which is slight posterior to the margin of the opercle (Bührnheim, Malabarba, 2006). The measurements presented in tables are expressed as percent of the standard length (SL) except for subunits of the head, which are recorded as percent of the head length (HL).

Precaudal, caudal and total vertebrae counts include the four vertebrae of the Weberian apparatus, and the terminal "half centrum" as outlined by Malabarba, Weitzman (1999). The gill raker at the junction of ceratobranchial and epibranchial is counted jointly with the gill rakers on the lower branch as in Bührnheim, Malabarba (2006). In the counting of the rays of anal fin, the last two rays are counted as a single element by being on the same pterygiophore. For the description of secondary sexual

characteristics (presence of hooks in the fins), the fin is divided into three sectors, proximal (from the base of the ray to the first bifurcation), median (from the first to second bifurcation) and distal (from second bifurcation to ray tip).

Specimens were cleared and stained (c&s) according to Taylor, Van Dyke (1985), and were used for counting vertebrae, teeth, gill rakers, denticulation of gill rakers, supraneurals and proximal radios. Radiographs of the types of *Odontostilbe microcephala* were made by The California Academy of Sciences-Ichthyology Section, and were used for counting vertebrae and fin rays of the types. Scanning Electron Microscope (SEM) images were obtained from teeth and denticulation of gill rakers. Pictures of supraneurals were taken from cleared and stained specimens with a Nikon AZ100M camera attached a stereomicroscope. Nomenclature follows Weitzman (1962) for bones. To standardize the description of fish color in live and alcohol preserved specimens, the scale color of package "DAAG" (Maindonald, Braun, 2011) was used as a reference, with the function: Show.color ("Single") for colors that do not have intensities and Show.color ("Shades") for colors if they have different intensities.

The following institutions provided material for the study: ANSP, Academy of Natural Sciences, Philadelphia, USA; CAS, California Academy of Sciences, San Francisco, USA; FMNH, Field Museum of Natural History, Chicago, USA; MHNG, Muséum d'histoire naturelle, Genève, Switzerland; INPA, Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil; INHS, Illinois Natural History Survey, Illinois, USA; MCP, Museu de Ciências e Tecnologia da PUCRS, Porto Alegre, Brazil; MZUSP, Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil; USNM, National Museum of Natural History, Washington D.C., USA. UMSS, Universidad Mayor de San Simon, Cochabamba, Bolivia; UFRGS, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

Statistical analyses. The standard length was not used as a variable and the remaining morphometric variables were standardized as the ratio against the standard length and coded as follows: M1 (snout to anal-fin origin), M2 (snout to dorsal-fin origin), M3 (snout to pelvic-fin origin), M4 (snout to pectoral-fin origin), M5 (dorsal-fin origin to caudal-fin origin), M6 (orbit to dorsal-fin origin), M7 (anal-fin base length), M8 (length of caudal peduncle), M9 (depth of caudal peduncle), M10 (body depth at dorsal fin), M11 (dorsal-fin length), M12 (pelvic-fin length), M13 (pectoral-fin length), M14 (head

length), M15 (snout length), M16 (upper jaw length), M17 (horizontal orbit diameter) and M18 (interorbital width).

The combined measurement data were examined with Principle Components Analysis (PCA) calculated in Past 3.x version 2016 (Hammer *et al*. 2001) to determine if distinct groups were identified. Defined groups were then analyzed with the algorithm of Variable selection to discriminate among taxonomic groups (VARSEDIG) (Guisande *et al*. 2016; Guisande, 2016). VARSEDIG was used to identify the morphometric characters that significantly discriminate separately two taxa and validate the morphological distinctness between them via a Monte-Carlo method, that has had an extraordinary success for conducting inference in phylogenetic, and indeed throughout the sciences (Leigh, Bryant, 2015). For analysis with the VARDESIG algorithm 18 morphometric variables were selected, grouped by species and sex. Since this method only allows discriminate between two groups, species 1 *vs*. 2, 2 *vs*. 3 and 1 *vs*. 3 were contrasted. Moreover, female and male individuals for each species were contrasted. Overlap methods were used to prioritize the variables according to their capacity for discrimination. It was obtained a density curve for each variable and the overlap of the area under the curve between the two groups was estimated for all variables. The variables with lower overlap should have better discrimination capacities and, hence, all variables ordered from lowest to highest overlap. To eliminate those variables that are not significant it is applied the logistic regression by steps. VARSEDIG default established other parameters. These are the estimated mean X and Y polar coordinates for both groups and the Euclidean distance of these means is calculated between both groups. Algorithm for variable selection were following Guisande *et al*. (2016) and Guisande (2016). Figures show plots obtained with VARSEDIG, and the variables that better discriminate between two compared species were selected; a density plot was depicted for the quantitative variable with lower overlap between both groups and, thus, the highest discrimination capacity. For two compared species, the scatterplot of the polar coordinates using variables selected with highest discrimination capacity was performed. Two figures were obtained with the results of a bivariate randomization test, one with the value of the individual of species 1 (red point), with the higher probability (p-value) of belonging to species 2 and the second with value of the individual of species 2 (red point) with the highest p-value of belonging to species 1, respectively. If when comparing group 1 with group 2 and group 2 with 1, the p-value is close to, or

lower than, 0.05 for the X or Y polar coordinates, it is concluded that the selected variables are significantly contributing to discriminating between both species.

Quantitative variables were represented by graphical boxplot, a version of this type of graphic is to add an element, called "notch" representing approximate confidence intervals 95% for median: two medians are different (the groups are different) if intervals or notches corresponding boxes do not overlap.

All statistical analyses were completed using R (R Core Team, 2016), with the exception of Principle Components Analysis (PCA) which were calculated in Past 3.x version 2016 (Hammer *et al*. 2001). The boxplot graph was performed using “sm” package. To use VARSEDIG is necessary to download the following packages: adehabitatHR, adehabitatHS, adehabitatLT and adehabitatMA packages (Calenge, 2006); ade4 package (Chessel *et al*. 2004; Dray *et al*. 2007; Dray *et al*. 2015); sp package (Pebesma, Bivand, 2005; Bivand *et al*. 2013); deldir package (Turner, 2016); CircStats package (Lund, Agostinelli, 2012); MASS package (Venables, Ripley, 2002); boot package (Davison, Hinkley, 1997; Canty, Ripley, 2016); kulife package (Ekstrom *et al*. 2013) and car package (Fox, Weisberg, 2011). Geographic distribution map was performed in the Quantum GIS version 2.8 software (Quantum GIS Development Team, 2016).

Results

Odontostilbe sp. n. W

urn:lsid:zoobank.org:pub:EE245067-1C76-47A5-94BE-836B4711CE5C

Figs. 1-4

Odontostilbe microcephala non Eigenmann, 1907. -Eigenmann, 1915 [in part]: 94-95 [three smaller specimens from CM 6854a-c (FMNH 131317, 35.9-38.8 mm SL)] and ten specimens from CM 6855 [CAS 60508 (3, 25.0-39.4 mm SL) and FMNH 57872 (7, 21.4-23.9 mm SL)] from rio Tietê at Salto Avanhandava above the falls, Penápolis, São Paulo, Upper Paraná basin]. -Uj, 1987: 132 (key), 138 (description), 154 (osteological description of skull, dorsal and anal fins, generic position), 160 (distribution). -Casciotta *et al*. 1992:10-13 (compared with *Odontostilbe yatai* based on Uj (1987) description), 14 (key).

Odontostilbe sp. - Silva *et al*. 2017: e20160196, 4, fig 3(t) [Inventory of Ichthyofauna]

Holotype: MZUSP 121648, 42.5 mm SL, Itirapina, Lapa stream near the mouth and along the seawall of rock and the bridge on the road, 22°22′ 35.5″ S 47°46′55.6″W, 31 Jan 2002, E. N. Fragoso.

Paratypes. All from Brazil, upper rio Paraná basin. **São Paulo State:** CAS 60508, 3, 25.0-39.4 mm SL, rio Tietê at Salto Avanhandava below the falls, Penápolis, São Paulo, 21°10′3.5″ S 50°7′3.22″W, 14 Sep 1908, J. D. Haseman. FMNH 131317, 3, 35.9-38.8 mm SL, rio Tietê at Salto Avanhandava below the falls 21°10′03″S 50°7′03″W, 15 Sep 1908, J. D. Haseman. FMNH 57872, 7, 21.4-23.9 mm SL, rio Tietê at Salto Avanhandava below the falls, Penápolis, São Paulo, 21°10′03″S 50°7′03″W, 15 Sep 1908, J. D. Haseman. MCP 32277, 2, 37.9-42.3 mm SL, rio Piracicaba, Piracicaba, tributary of rio Tietê, 22°43′19″S 47°39′21″W, 29 Jan 2001, C. Lucena, J. Silva, E. Pereira & A. Cardoso. MCP 12108, 2, 42.3-43.8 mm SL, Island Solteira, rio Paraná, 20°22′58″ S 51°25′56″ W, 19 Oct 1969, Expedition Department of Zoology USP leg. MZUSP 42657, 186, 33.3-43.4 mm SL, rio Mogi Guaçu, Emas, Pirassununga, 21°55′00″S 47°23′00″W, EEBP-Emas. MZUSP 16851, 13, 36.8-41.8 mm SL, rio Mogi Guaçu, Emas, Pirassununga, 21°55′00″S 47°23′00″W, 22 Oct 1963, H. A. Britski. MZUSP 87947, 10, 42.5-50.5 mm SL, Itirapina, stream of Lapa near the mouth and along the seawall of rock and the bridge on the road, 22°15′00″S 47°51′48″ W, 31 Jan 2002, E. N. Fragoso. **Mato Grosso do Sul State:** MCP 12105, 2, 34.2-37.2 mm SL, rio Paraná, in front of Jupiá, MT, 21°08′00″S 51°46′00″W, 11-13 Oct 1969, Expedition Department of Zoology USP leg. MZUSP 4011, 2199, 25.3-40.0 mm SL, rio Paraná, in front of Jupiá, MT, Jupiá, 21°08′00″S 51°46′00″W, 4 Dec 1960, P. E. Vanzolini & S. Saiar. **Goiás State:** MCP 27814, 9, 39.5-0.8 mm SL, rio São Marcos, road Catalão/Davinópolis, Catalão, 18°06′15″S 47°41′35″W, 23 Jan 2001, C. Lucena, J. Silva, E. Pereira & A. Cardoso. MCP 26001, 1, 40.3 mm SL, rio Corumbá, downstream UHE Corumbá, rio Paranaíba, Caldas Novas, 17°29′00″S 48°23′00″W, 15 Oct 1996, Nupelia staff. MCP 20337, 50, 36.8-54.2, rio Corumbá, affluent of rio Paranaíba. region of the municipalities of Caldas Novas, Corumbaína, Pires do Rio and Ipameri, 18°00′00″S 49°00′00″W, 07 Sep 1996, Nupelia staff.

Non types: All from Brazil, upper rio Paraná basin. **Goiás State:** MCP25999, 2, 56.1-56.3 mm SL, rio Corumbá, near the mouth of rio Pirapitinga, (tributary of rio Paranaíba), 17°47′00″S 48°32′60″ W, 18 Sep 1996, Nupelia. MCP26000, 2, 55.1-56. 8

mm SL, rio Corumbá, near the mouth of rio do Peixe (tributary of rio Paranaíba), 17°36′00″S 48°27′00″W, 21 Sep 1996, Nupelia staff.

Diagnosis. *Odontostilbe* sp. n. W differs from all species of the genus, except from *Odontostilbe* sp. n. A, by the presence of mesopterygoid teeth (Fig. 3), grouped on median portion of the bone, forming a continuous row (*vs*. absent). *Odontostilbe* sp. n. W is distinguished from *Odontostilbe* sp. n. A by premaxillary teeth with 5(13) or 7(3) cusps [usually 5], with central cusp wider and larger than lateral cusps (Fig. 2) (*vs*. premaxillary teeth bearing 7-11 [usually 9] cusps, all approximately with the same size). Additionally *Odontostilbe* sp. n. W is distinguished from *O. dierythrura*, *O. euspilurus* and *O. microcephala* by the terminal mouth (*vs*. subterminal mouth); from *O. pulchra*, and *O. ecuadorensis* by the presence of hooks on first to seventh, [mostly fifth to 6^{th}] anal-fin branched rays of males (*vs*. hooks on first to 22^{nd} anal-fin branched rays of males in *O. pulchra*, first to sixteenth in *O. ecuadorensis*); from *O. splendida* by the origin of adipose fin positioned at vertical through last anal-fin ray insertion (*vs*. origin of adipose fin, at vertical through last 2 or 3 anal-fin rays insertion); from *O. pao* and *O. parecis* by the presence of 5-7 [mostly 5] cusps in premaxillary teeth (Fig. 2) (*vs*. 8-10 cusps in *O. pao* and *O. parecis*); from *O. pequira* by anterior dentary teeth with 5 cusps, being central cusp larger and longer than laterals cusps (*vs*. dentary with 4 anterior teeth with 3 large and equally longer compressed cusps and 2-3 lateral small cusps); and from *O. paraguayensis* by absence of predorsal spine (*vs*. presence of predorsal spine).

FIGURE 1. . *Odontostilbe* sp. n. W: (a) holotype, MZUSP 121648, 1, 42.5 mm SL, Itirapina, São Paulo State. (b) paratype, MCP 32277, female, 42.2 mm SL, rio Piracicaba, São Paulo. (c) paratype, FMNH 131317, female, 38.7 mm SL, rio Tietê at Salto Avanhandava below the falls, Penápolis, São Paulo, Brazil, was listed under *O. microcephala* by Eigenmann (1915).

Description. Morphometric data on Tab. 1. Body elongate and slightly compressed (Fig. 1). Greatest body depth at vertical through dorsal-fin origin. Snout slightly elongated and round. Dorsal profile of head convex from snout to vertical through anterior bony orbital margin, slightly concave from there to distal tip of supraoccipital

bone. Predorsal profile slightly convex from posterior end of supraoccipital to dorsal-fin origin. Dorsal-fin origin located anteriorly at midlength of SL. Body profile in base of dorsal fin straight to slightly convex and slightly convex from there to origin of adipose fin. Body profile between adipose-fin base and first rays of caudal fin slightly concave. Ventral profile of head slightly convex from snout to origin of pelvic fins and slightly convex from there to origin of anal fin. Body profile along base of anal fin straight or slightly convex. Ventral profile of caudal peduncle straight to slightly concave. Caudal peduncle slightly longer than deep.

Head small relative to body length, 22.8-27.7% SL, mean 24.4%. Mouth terminal, opening below horizontal line through middle of eye pupil. Upper and lower jaws approximately same length. Maxilla short, posteroventrally angled, posterior tip surpassing vertical through anterior border of eye and surpassing horizontal through ventral border of eye. Teeth pedunculated distally expanded, all similar in shape. Premaxilla with five teeth aligned in one single row, with 5(13) or 7(3) cusps [mostly 5]. Maxilla with two teeth with 5(7) cusps. Dentary teeth nine gradually decreasing in size posteriorly; anterior six teeth, larger, bearing 5-7 cusps; followed posteriorly by smaller teeth with 1-3 cusps (Fig. 2). All jaw teeth with central cup larger and wider than lateral cusps. Ventral surface of mesopterygoid with 18-19 teeth with one, two and three cusps grouped on median portion forming a continuous row (Fig. 3).

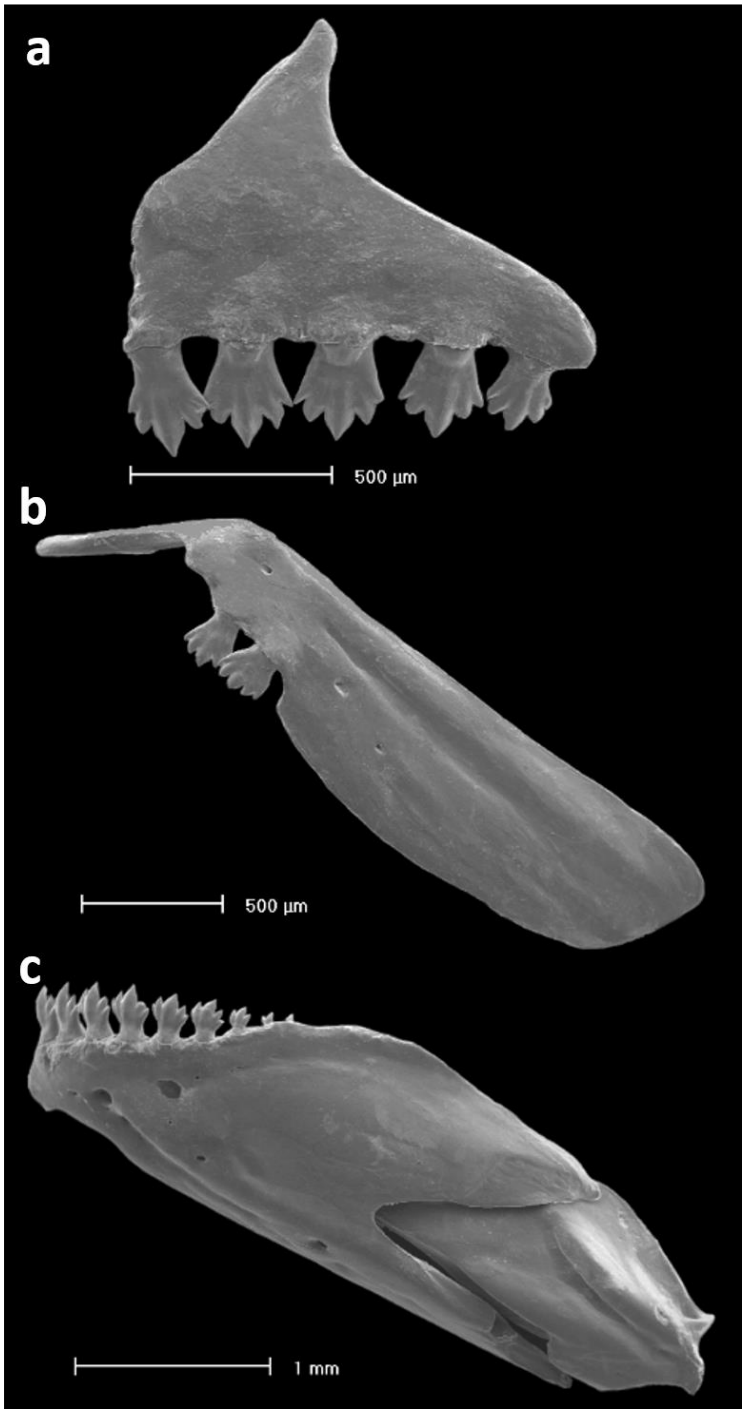

FIGURE 2. Dentition of **.** *Odontostilbe* sp. n. W, paratype MCP 20337 male: (a) left side premaxilla, (b) maxilla, and (c) dentary, lateral view. Scanning electron micrographs (SEM).

TABLE 1. Morphometric data for *Odontostilbe sp. n. W*. Number of individuals (N), mean, minimum (min), maximum (max) and standard deviation (SD) include values of the holotype (male) MZUSP121648.

	Holotype	Females					Males					Unsexed				
	MZUSP121648	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD
Standard length (mm)	42.5	37	29.94	50.81	39.28	-	17	29.8	43.86	38.04	-	9	21.41	25.15	23.45	-
Percents of Standard Length																
Snout to anal-fin origin	62.3	36	61.6	67.3	63.5	1.21	15	60.6	63.9	62.4	0.88	7	63.3	66.6	64.4	1.15
Snout to dorsal-fin origin	46.1	36	45.3	51.7	47.9	1.14	15	45.4	49.9	47.2	1.49	7	47.9	52.1	49.9	1.47
Snout to pelvic-fin origin	43.9	36	43.3	49.3	45.4	1.15	15	43.3	45.5	44.6	0.63	7	45.5	49.5	47.3	1.39
Snout to pectoral-fin origin	21.9	36	20.7	25.8	23.1	1.07	15	21.9	26.6	23.1	1.24	7	24.1	26.4	25.5	0.81
Dorsal to caudal-fin origin	51.9	36	47.7	54.2	50.9	1.72	15	47.3	53.8	51.6	1.80	7	48.1	51.8	49.9	1.12
Orbit to dorsal-fin origin	32.1	36	31.3	36.1	33.0	1.20	15	30.0	34.7	32.2	1.35	7	29.6	34.9	32.9	1.80
Anal fin base length	23.2	36	20.9	27.9	23.8	1.17	15	21.9	27.4	23.9	1.27	7	22.4	26.0	23.8	1.23
Peduncle length	13.9	36	10.8	14.5	12.5	1.00	15	11.3	14.1	12.9	0.84	7	12.5	14.6	13.6	0.89
Peduncle depth	10.5	36	9.3	11.1	10.3	0.43	15	9.5	11.0	10.2	0.53	7	9.6	11.7	10.4	0.67
Body depth at dorsal fin	28.8	36	23.1	32.9	27.9	1.98	15	23.6	30.9	26.3	1.89	7	27.3	31.0	28.2	1.28
Dorsal fin length	27.2	36	23.5	27.2	25.4	1.11	15	23.5	29.9	26.6	2.17	7	23.5	25.7	25.0	0.85
Pelvic fin length	17.1	36	14.0	18.5	16.1	0.95	15	15.0	21.9	17.5	1.98	7	13.7	17.2	15.3	1.30
Pectoral fin length	22.3	36	18.6	21.8	20.1	0.70	15	19.6	22.6	21.0	0.85	7	16.4	20.8	18.0	2.05
Head length	23.2	36	23.1	25.3	24.0	0.57	15	23.2	26.4	24.6	0.78	7	25.8	27.4	26.7	0.61
Percents of Head Length																
Snout length	23.8	36	21.1	29.2	24.2	1.70	15	20.9	27.5	24.7	1.72	7	22.8	28.2	25.0	2.15
Upper Jaw length	35.3	36	30.7	37.9	34.0	1.53	15	31.0	36.9	34.0	1.72	7	31.5	35.0	33.3	1.36
Horizontal orbit diameter	36.9	36	34.0	40.5	36.9	1.65	15	35.0	40.9	37.2	1.70	7	34.9	40.7	37.5	1.85
Interorbital width	32.6	36	29.0	35.1	32.5	1.54	15	29.4	34.1	31.4	1.30	7	29.1	34.6	31.1	1.73

Dorsal-fin rays ii,9(60). First unbranched dorsal-fin ray about half-length of second unbranched dorsal-fin ray; branched rays gradually decreasing in size posteriorly. Dorsal fin with 10(5) pterygiophores. First dorsal-fin pterygiophore supports one supernumerary spine. Proximal radial of first pterygiophore of dorsal fin posterior to neural spine of ninth precaudal vertebra. Medial radial absent from first to fourth pterygiophores and visible from fifth to tenth pterygiophores, ossified. Proximal radial of first to ninth pterygiophore with lateral projections, absent in 10th.

Adipose-fin origin posterior to vertical through base of last anal-fin ray. Unbranched anal-fin rays ii(1), iii(33), iv(21) or v(4); branched anal-fin rays 17(3), 18(20), 19(30) or 20(6) with one row of scales on base of first anal-fin ray. Profile of distal margin of anal fin distinctively concave forming an angle in tenth branched ray. Anal fin with 18(1), 20(3) or 21(1) pterygiophores. Medial radial absent in first to sixth pterygiophore and visible from there to last pterygiophore. Proximal radial of first pterygiophore in contact with hemal spine of first caudal vertebra.

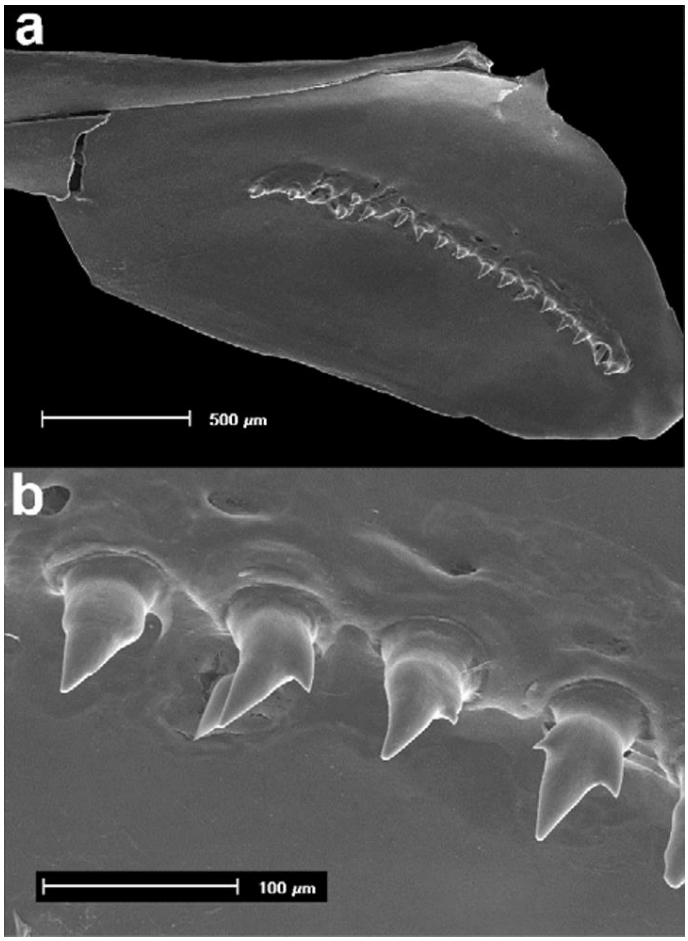

FIGURE 3. Mesopterygoid of **.** *Odontostilbe* sp. n. W paratype, male (MCP 20337) ventral view: (a) mesopterygoid with teeth forming a row, (b) detailed image of the teeth of mesopterygoid with one, two and three cusps.

Pectoral fin i(59), 11(22), 12(30) or 13(7). Tip of extended pectoral fin not reaching pelvic-fin origin. Pelvic-fin origin anterior to vertical through dorsal-fin origin. Pelvic-fin rays I,7(60). Principal caudal-fin rays 19(58). Dorsal procurrent caudal-fin rays 8(7), 9(2), 11(8) or 12(1) and ventral procurrent caudal-fin rays 7(2), 8(5), 9(15) or 10(4).

Scales cycloid; lateral line complete with 34(22), 35(18), 36(4) or 37(2) pored scales. Pre-dorsal scales in regular series with 10(2), 11(2), 12(30), 13(17) and 14(3) scales; scale rows between lateral line and dorsal line origin 5(51); scale rows between lateral line and pelvic-fin origin 3(9) or 4(42); circumpeduncular scale rows 14(14).

Counts based on c&s specimens (3): supraneurals 4(3) without lateral projections; 5 upper gill rakers and 10 lower gill rakers (2 on hypobranchial). Upper gill rakers with

2-5 denticles on anterolateral border, and 0-2 on posterolateral border, mainly on basal portion of upper gill rakers. Lower gill rakers with 3 or 6 denticles on anterolateral border, and none on posterolateral border. Gill raker at junction of ceratobranchial and epibranchial with 3-4 denticles on dorsolateral and 3 on ventrolateral borders (Fig. 4). Precaudal vertebrae 16(3); caudal vertebrae 18(3); total vertebrae 34(3).

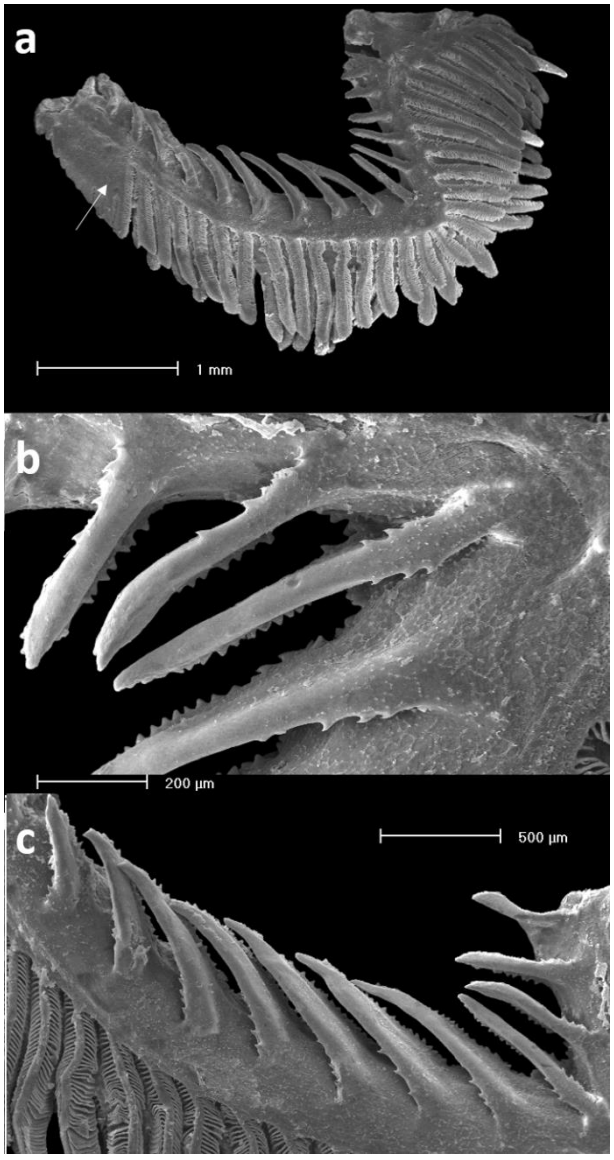

FIGURE 4. First gill arch of **.** *Odontostilbe* sp. n. W: (a) left side, lateral view, showing gill gland (arrow) on anteriormost portion of lower branch of MCP 20337 male. In detail (b) gill rakers near the junction of ceratobranchial and epibranchial, and (c) gill rakers on lower branch of gill arch. Scanning electron micrographs (SEM).

Color in alcohol. Overall body coloration dark-golden-rod (shade 3). Dorsal surface of head from snout to posterior limit of frontal golden-rod (shade 3) coloration with scattered light grey chromatophores. Dark-golden-rod (shade 4) color in region of parietal and supraoccipital. Opercular apparatus and infraorbital region of beige silver color extending to cleithrum. Ventral region between pectoral fin and pelvic fin with lighter area with golden-rod (shade 1) color. Humeral region with a darkened triangular area due muscular hiatus of pseudotympanum. Scales of dorsal portion of body from supraoccipital to caudal peduncle with small dots dim-gray concentrated in posterior margin. Region posterior of pseudotympanum to caudal peduncle with a silvery lateral thin band above lateral line, top edge of chromatophores dim-gray forming a dark band reaching caudal spot. A conspicuous oval horizontally black caudal spot continued on base of middle caudal-fin rays (Fig. 1).

Dorsal fin with scattered black chromatophores slightly darker and more numerous on mid-distal portion of dorsal fin, extending from 2nd unbranched to 8th branched dorsal-fin rays. Pectoral and pelvic fins with dark grey chromatophores scattered mainly in unbranched ray. Anal fin with dark grey dots slightly scattered in all rays, concentrated in basal region. Adipose fin unpigmented.

Sexual dimorphism. Mature males have second unbranched ray of dorsal fin and unbranched ray of pelvic fin slightly longer than remaining ones, differing from females, with those rays not elongated.

Mature males with small hooks on pelvic and anal fins. Pelvic-fin rays with small hooks in all branched rays, with one retrorse bony hook per segment on medioventral border of both lepidotrichia in median and distal portions of pelvic fin. Tip of bony hooks not reaching proximal border of segment of lepidotrichia where inserted. Anal-fin rays with one pair of small retrorse bony hooks per segment symmetrically arranged (exceptionally two pairs), inserted along of last unbranched ray to fourth or seventh branched rays, decreasing in number from first ray until disappearing. Hooks mostly distributed along middle third of anal ray length in posterolateral border. Distal tip of bony hooks not reaching proximal border of segment of lepidotrichia where inserted.

Males bear a gill gland (Fig. 4) similar in size and number of modified filaments (8) to those observed in externally fertilized species of Cheirodontinae (Oliveira *et al*. 2012).

Morphometric analysis of sexual dimorphism with VARSEDIG shows a high overlap between measurements that best discriminate sex of *Odontostilbe* sp. n. W: M2 (snout to dorsal-fin origin), M6 (orbit to dorsal-fin origin) and M10 (body depth at dorsal fin). Bivariate randomization test of male *vs.* female (p= 0.281) and female *vs.* male (p= 0.25) showed no significant difference between sexes (p> 0.05).

Geographic distribution: *Odontostilbe* sp. n. W is known to inhabit tributaries of the upper rio Paraná basin, in the states of Goiás, Minas Gerais, and São Paulo, Brazil and Paraguay (Fig. 5).

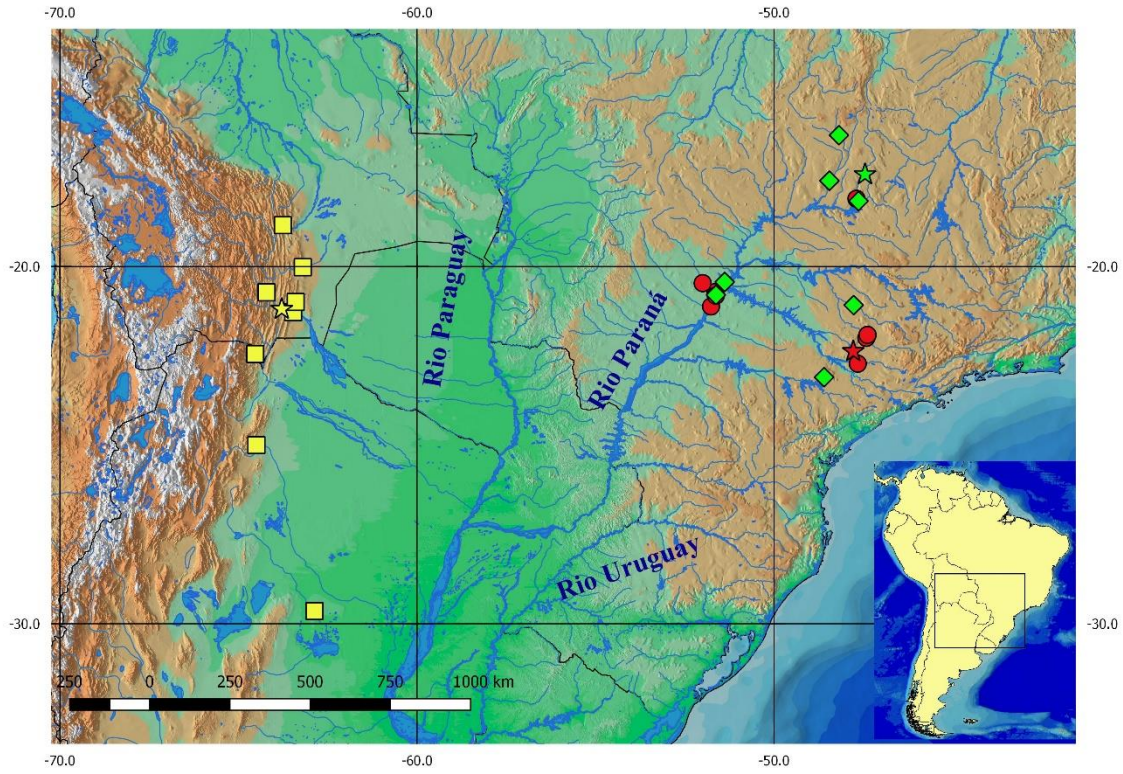

FIGURE 5. Southern South America showing the distribution of **.** *Odontostilbe* sp. n. A (diamond), *O. microcephala* (square) and **.** *Odontostilbe* sp. n. W (circle). Type locality (star).

Etymology: The specific epithet is named for Stanley H. Weitzman, in recognition of his work on the systematics of Neotropical characiforms, particularly of the characid subfamily Cheirodontinae.

Conservation status: *Odontostilbe* sp. n. W is abundant in the places where is found. *Odontostilbe* sp. n. W was categorized as Least Concern (LC), according to the IUCN criteria for evaluation on threatening status, version 3.1 (IUCN, 2001).

Remarks: Besides the type specimens from the upper rio Paraná basin, on which we based the above description, we have obtained additional lots of what appear to be *Odontostilbe* sp. n. W (female, 55.1-56.8 mm SL) from two localities along the rio Corumbá, Goiás (MCP 25999 and MCP26000). These specimens have the same number of cusps of premaxillary teeth, number of branched anal-fin rays and other morphometric characters as the type specimens, but can be differentiated in the length of caudal peduncle (9.9-11.8% SL, mean 10.6% SL *vs*. 10.8-14.5% SL, mean 12.5% SL in *Odontostilbe* sp. n. W type specimens) and body depth at dorsal-fin (35.0-37.1% SL, mean 36.0, *vs*. 23.1-32.9% SL, mean 27.9% SL in *Odontostilbe* sp. n. W type specimens). We suggest that this difference is related to the largest size of individuals from Goiás. We noted also that the stretches of the rivers where these specimens were collected have been changed from lotic to lentic environments by the construction of hydroelectric power dams, and this may possibly influence the changes in body size, body depth and caudal peduncle length of studied populations.

Several specimens of *Odontostilbe* sp. n. W collected in the upper rio Paraná basin are found among the material used by Eigenmann (1915) to redescribe *Odontostilbe microcephala* (Fig. 1c; CM 6854a-c, now FMNH 131317; CM 6855a-p, now CAS 60508 and FMNH 57872), and since then, specimens of *Odontostilbe* sp. n. W from the upper rio Paraná have been erroneously identified as *O. microcephala*.

***Odontostilbe* sp. n. A**

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Figs. 6, 7, 8

Odontostilbe microcephala non Eigenmann, 1907. -Eigenmann, 1915 [in part]: 94-95 (the largest specimen from CM 6854a-c (FMNH 57871) from rio Tietê at Salto Avanhandava above the falls, Penápolis, São Paulo, Brazil, Upper rio Paraná basin).

Holotype: LIRP 3239, 48.7 mm SL, female, ribeirão da Batalha, farm Batalha (Pedro Queresma), rio Paranaíba, Paracatu, Minas Gerais State, Brazil, 17°25′25″S 47°27′11″W, 27 Apr 2002, C. A. A. Figueiredo & E. S. S. Rego.

Paratypes: All from Brazil, upper rio Paraná basin. **São Paulo State**: FMNH 57871, 1, 62.1 mm SL, rio Tietê at Salto Avanhandava below the falls, Penápolis, 21°10′04″S 50°07′03″W, 14 Sep 1908, J. Haseman [Material reviewed by Eigenmann (1915)]. LIRP 00314, 2, 47.4-51.1 mm SL, rio Pardo, Ribeirão Preto, 21°04′49″S 47°46′13″W, 15 Oct 1991, W. E. Alvear. MCP 12103, 1, 56.1 mm SL, Ilha Solteira, rio Paraná, 20°26′03″S 51°22′60″W, Set 1965. MCP 26004, 2, 39.3-57.9 mm SL, rio Paranapanema, at Jurumirim dam reservoir, Itatinga, 23°06′00″S 48°36′00″W, UNESP de Botucatu. **Goiás State:** MCP 26003, 1, 43.8 mm SL, rio do Peixe, Linígrafo (tributary of rio Corumbá), rio Paranaíba, Caldas Novas, 17°36′00″S 48°27′00″W, 19 Oct 1996, Nupelia staff. MCP 26002, 1, 57.7 mm SL, rio do Peixe, Next to Foz (tributary of rio Corumbá), rio Paranaíba, Caldas Novas, 17°36′00″S 48°27′00″W, 21 Sep 1996, Nupelia staff. MNRJ 19718, 1, 54.2 mm SL, rio São Bento, a tributary of the left margin of the rio São Marcos, downstream of the future AHE Serra dam, Paranaíba basin, Divinópolis, 18°10′10″S 47°38′07″W, 27 Sep 1999, C. A. Figueiredo, F. A. Bockmann & A. P. R. Pires. MZUSP 80112, 2, 66.4-77.7 mm SL, Ensecadeira da Usina Hidroeletrica de Corumbá IV, rio Corumbá, Luziania, 16°19′31″S 48°10′59″W, H. L. R. Silva. **Mato Grosso do Sul State:** MCP 12101, 1, 30.2 mm SL, lakes along of the margin of rio Paraná, in front of Jupiá, 20°47′44″S 51°37′45″W, 11-23 Sep 1964.

Diagnosis. *Odontostilbe* sp. n. A differs from its congeners, except of *Odontostilbe* sp. n. W, by the presence of mesopterygoid teeth, grouped on median portion forming a continuous row, covering half of mesopterygoid bone (*vs*. absent). *Odontostilbe* sp. n. A is distinguished from *Odontostilbe* sp. n. W and all species of the genus, except *O. fugitiva*, *O. splendida*, *O. parecis*, *O. pao* by the presence of teeth with 9 or 11 cusps in the premaxilla (Fig. 7) [mostly 9, a few teeth with 7 cusps may be also present] (*vs*. 3-7 cusps; teeth with 9 or 11 cusps always absent). *Odontostilbe* sp. n. A is distinguished from *O. fugitiva*, *O. splendida*, *O. pao* by the subterminal mouth (*vs.* terminal mouth); additionally from *O. parecis* and *O. fugitiva* by the presence of 16-18 anal-fin branched rays [mostly 17] (*vs.* 20-21 in *O. parecis* and 19-24 [mostly 21-22] in *O, fugitiva*); from *O. splendida* by the anterior position of the adipose fin, positioned at vertical through last anal-fin ray insertion (*vs.* at vertical through last 2nd or 3rd anal-fin ray insertions); from *O. pao* by the number of scale rows between lateral line and dorsal-fin origin 5 (*vs.* 6-7 [mostly 7]).

FIGURE 6. *Odontostilbe* sp. n. A: (a) holotype LIRP 3239, female, 48.7 mm SL; rio Paranaíba, Paracatu, Minas Gerais, Brazil. (b) paratype, FMNH 57871, male 62.1 mm SL, rio Tietê at Salto Avanhandava below the falls, Penápolis, São Paulo, Brazil; specimen reviewed by Eigenmann (1915).

Description. Morphometric data on Tab. 2. Body elongate and slightly compressed. Greatest body depth at vertical through dorsal-fin origin. Snout elongated and round. Dorsal profile of head convex from snout to posterior margin of frontal, slightly concave from there to distal tip of supraoccipital bone. Predorsal profile slightly convex from posterior end of supraoccipital to dorsal fin origin. Dorsal-fin origin located at midlength of SL. Body profile in base of dorsal fin straight and slightly convex from there to origin of adipose fin. Body profile between adipose-fin base and dorsal procurrent caudal-fin rays slightly concave. Ventral profile of head slightly convex from snout to origin of pelvic fins and convex from there to origin of anal fin. Body profile along base of anal fin straight or slightly convex. Ventral profile of caudal peduncle slightly concave. Caudal peduncle slightly longer than deep.

Head small relative to body length, 21.3-24.0% SL, mean 22.5%. Mouth subterminal, opening oriented forward and somewhat ventrally, in which upper jaw and snout clearly extend beyond the lower jaw. Maxilla posteroventrally angled, posterior

end surpassing vertical through anterior border of eye and surpassing horizontal through ventral border of eye. Two or three maxillary teeth, with 7-9 [mostly 9] cusps nearly of equal size. Premaxilla with one single row; with 5-6 teeth slightly inclined to inside mouth (Fig. 7), bearing 7-11 [mostly 9] cusps nearly of equal size. Dentary with seven or eight teeth, anterior six teeth larger, bearing seven cusps nearly of equal size, followed posteriorly by one or two smaller teeth conical, rarely tricuspidate. Dentary teeth inclined anteriorly. Presence of 14 mesopterygoid teeth, grouped on median portion forming a continuous row, covering half-length of ventral surface of mesopterygoid bone.

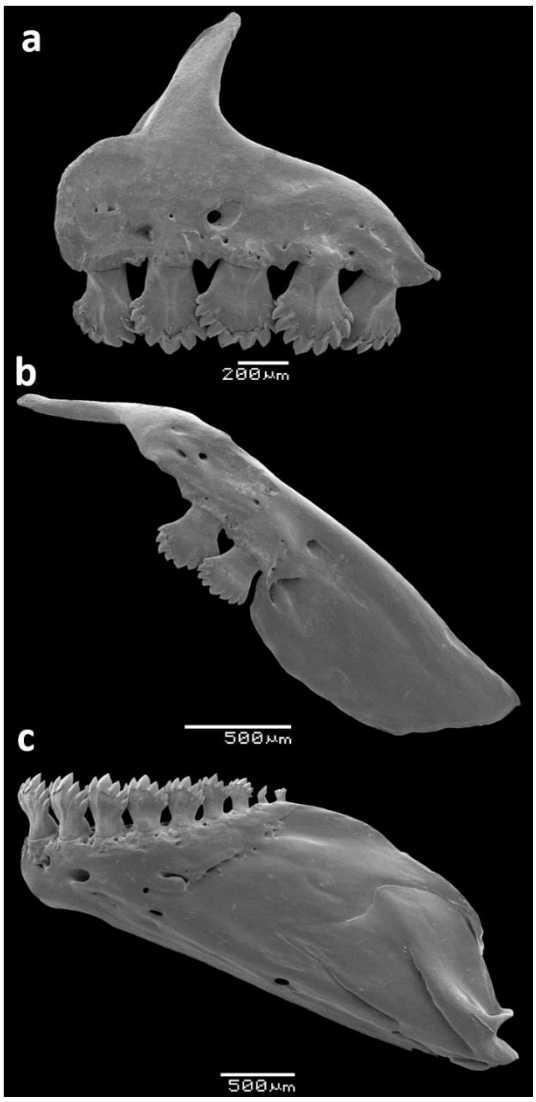

FIGURE 7. Dentition of *Odontostilbe* sp. n. A, MCP 26004, paratype: (a) premaxilla, (b) maxilla, and (c) dentary, left side, lateral view. Scanning electron micrographs (SEM).

TABLE 2. Morphometric data for *Odontostilbe* sp. n. A, number of individuals (N), mean, minimum (min), maximum (max) and standard deviation (SD) include values of holotype (female) LIRP3239

	Holotype	Paratypes				
	LIRP3239	N	Min	Max	Mean	SD
Standard length (mm)	48.65	14	30.17	70.73	52.98	-
Percents of Standard Length						
Snout to anal-fin origin	67.1	14	66.9	71.6	68.3	1.39
Snout to dorsal-fin origin	47.2	14	46.2	50.7	48.3	1.43
Snout to pelvic-fin origin	47.2	14	46.5	50.8	48.3	1.31
Snout to pectoral-fin origin	21.1	14	18.7	24.2	21.6	1.78
Dorsal to caudal-fin origin	49.2	14	48.9	53.5	51.3	1.59
Orbit to dorsal-fin origin	32.4	14	31.8	38.0	34.9	1.74
Anal-fin base length	18.2	14	17.4	22.4	20.0	1.47
Length of caudal peduncle	12.4	14	10.3	13.2	12.1	0.88
Depth of caudal peduncle	15.9	14	9.7	15.9	11.1	1.49
Body depth at dorsal-fin	25.4	14	25.4	32.9	29.2	2.26
Dorsal-fin length	24.2	14	21.6	26.5	24.2	1.31
Pelvic-fin length	16.5	14	14.4	17.7	16.1	0.86
Pectoral-fin length	19.5	14	17.9	20.9	19.1	0.97
Head length	22.2	14	21.3	24.0	22.5	0.81
Percents of Head Length						
Snout length	25.5	14	23.3	28.4	25.5	1.42
Upper Jaw length	31.0	14	30.8	36.3	32.8	1.65
Horizontal orbit diameter	38.3	14	31.0	39.7	35.5	2.42
Interorbital width	34.9	14	30.9	36.1	33.6	1.86

Dorsal-fin rays ii,9(14) in all examined specimens. Dorsal-fin origin in vertical at pelvic-fin insertion. Profile of distal margin of dorsal fin slightly concave. First unbranched dorsal-fin ray less that half-length of second, following branched rays gradually decreasing in size posteriorly. First unbranched ray of dorsal-fin inserted in first pterygiophore and last two branched rays inserted in tenth pterygiophore. Proximal radial of first pterygiophore in contact with neural spine of eleventh precaudal vertebrae. Dorsal fin with medial radial absent from first to fourth pterygiophore and visible from fifth to tenth pterygiophore. Proximal radial of first to ninth pterygiophores with lateral projections.

Adipose-fin origin posterior to vertical through base of the last anal-fin ray. Unbranched anal-fin rays ii(1), iii(12) or iv(1); branched anal-fin rays 16(4), 17(8), 18(2), with two rows of scales covering base of anterior six branched rays. Anal-fin origin posterior of vertical through base of last ray of dorsal fin. Profile of distal margin of anal fin concave. Anal fin with 18 pterygiophores, anterior three unbranched rays associated with first pterygiophore, last unbranched ray associated with second pterygiophore. Medial radial absent from first to fifth pterygiophores, visible from that point to last pterygiophore. Proximal radial of first pterygiophore in contact with hemal spine of first caudal vertebra.

Pectoral fin rays i(14), 11(2), 12(1), 13(5), 14(4) or 15(2). First pectoral-fin ray reaching anterior edge of pelvic bone. Pelvic fin i-7(14) rays; insertion located approximately at vertical of origin of dorsal fin. In mature males, first unbranched ray prolonged in filament, usually surpassing origin of anal fin. Principal caudal fin rays 19(14). Procurrent caudal fin rays: dorsal 7(3), 8(9), 9(1) or 11(1) rays and ventral with 6(2), 7(4), 8(3), 9(4) or 10(1) rays.

Scales cycloids; pored scales on lateral line complete with 36(1), 37(4), 38 (4) or 39(2). Pre-dorsal scales arranged in a regular series with 11(2) or 12(6) scales; scale rows between lateral line and dorsal line origin 5(14); scales rows between lateral line and pelvic fin origin 4(14); scales rows around caudal peduncle 14(11). Supraneurals 5(1).

Precaudal vertebrae 17; caudal vertebrae 19; total of vertebrae 36. Gill rakers upper 6, lower 12 (2 on hypobranchial). Upper gill rakers with 6-8 denticles on anterolateral border, and 0-2 denticles on posterolateral border. Lower gill rakers with 1 7-12 denticles on anterolateral border, and 0-1 denticles on posterolateral border. Gill raker inserted on junction of ceratobranchial and epibranchial with 10 denticles on ventrolateral and 3 on dorsolateral borders (Fig. 8).

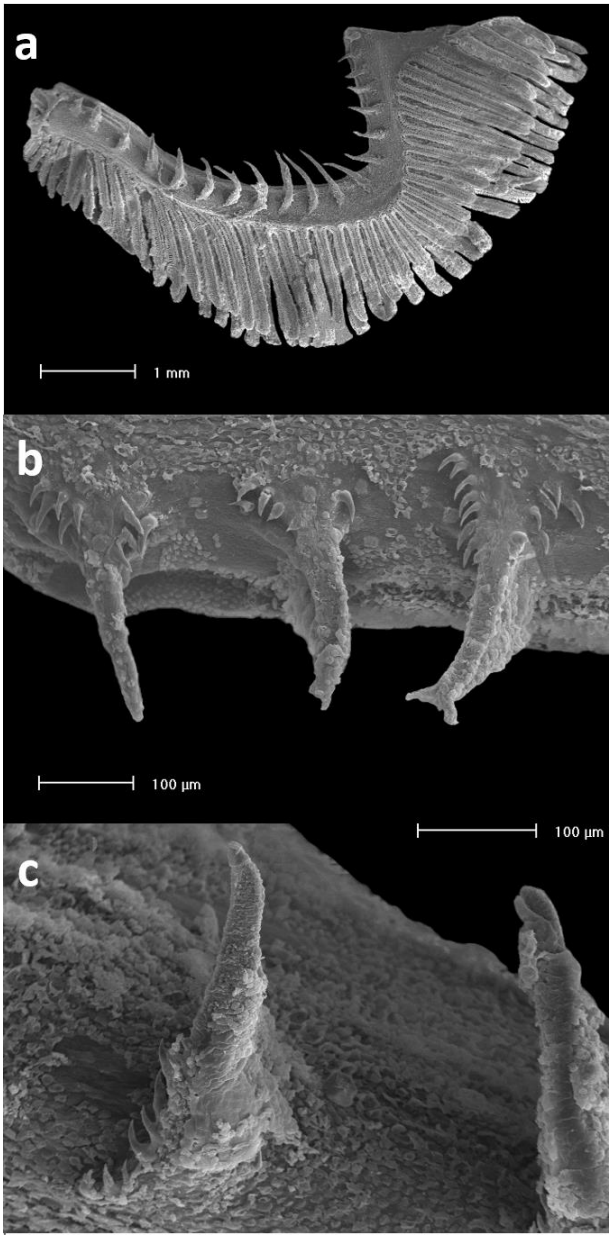

FIGURE 8. (a) First gill arch of *Odontostilbe* sp. n. A, MCP 26004, paratype, left side, lateral view. In detail (b) gill rakers on upper branchial branch, and (c) gill rakers on lower branchial branch. Scanning electron micrographs (SEM).

Color in alcohol. Overall body coloration gold (shade 1). Dorsal surface of head from snout to posterior limit of frontal with chromatophores scattered giving a goldenrod coloration. Chromatophores noticeably more concentrated dark red in region of parietal and supraoccipital. Opercular apparatus and infraorbital region silver beige. Branchiostegal rays silver beige. Ventral region between pectoral and pelvic fins with a lighter area with light-goldenrod color. Humeral region with a darkened triangular area

due to muscular hiatus of pseudotympanum. Scales of dorsal portion of body from supraoccipital to caudal peduncle with small dots goldenrod color concentrated in posterior margin. Region posterior of pseudotympanum to caudal peduncle with silvery lateral thin band, top edge and inferior of gray color, chromatophores forming a blank band reaching the caudal spot. A conspicuous oval horizontally black caudal spot continued on base of middle caudal fin rays.

Dorsal fin with scattered black chromatophores slightly darker and more numerous on mid-distal portion of dorsal fin, extending on the first unbranched dorsal fin ray and ninth branched fin ray. Pectoral and pelvic fins hyaline. Anal fin with dark dots scattered slightly in all rays, concentrated in the basal region. Adipose fin unpigmented.

Sexual dimorphism. Mature males with small hooks on the pelvic and anal fins. Pelvic-fin rays with small hooks in all branched rays, with one bony retrorse hook per segment, on mesoventral border of both lepidotrichia in median portion and distal portions of pelvic fin. Tip of bony hooks not reaching proximal border of segment of lepidotrichia where inserted. Anal fin rays with one pair small of retrorse bony hooks per segment symmetrically disposed, on last unbranched ray and first to fourth branched rays, decreasing in number posteriorly until disappearing. Bony hooks on median portion of posterior branch (never on anterior branch). Tip of bony hooks not reaching proximal border of segment of lepidotrichia where inserted.

Geographic distribution: *Odontostilbe* sp. n. A is known to inhabit tributaries of the upper rio Paraná basin, in the states of Mato Grosso, Goiás, Minas Gerais, and São Paulo, Brazil (Fig. 5).

Etymology: In the submitted article

Conservation status: *Odontostilbe* sp. n. A presents the same distribution area as *Odontostilbe* sp. n. W. It is corresponds to a species elongated, is characterized by being the species that reaches the largest size within *Odontostilbe*. Currently this species not is very abundant in scientific collections, but presents an ample distribution in upper rio Paraná. *Odontostilbe* sp. n. A is categorized as Least Concern (LC), according to the IUCN criteria for evaluation on threatening status, version 3.1 (IUCN, 2001).

Remarks. A specimen of *Odontostilbe* sp. n. A collected in the upper rio Paraná basin was found in the material used by Eigenmann (1915) to redescribe *O. microcephala* (FMNH 57871), and since then, specimens of *Odontostilbe* sp. n. A from the upper rio Paraná have been erroneously identified as *O. microcephala*.

Wasko *et al*. (2001) performs a cytogenetic study of one species based on specimens collected in the rio Mogi-Guaçu (municipality of Luiz Antônio, São Paulo State, Brazil). The material examined by Wasko and co-authors is possibly *Odontostilbe* sp. n. W or *Odontostilbe* sp. n. A, these authors, however, do not provide any descriptive information or photo of the fish he examined, not allowing confirmation of the identifications.

***Odontostilbe microcephala* Eigenmann, 1907**

Figs. 9-11

Odontostilbe microcephalus Eigenmann, in Eigenmann & Ogle, 1907: 10 (original description; type locality: rio Pilcomayo, Bolivia). -Fowler, 1940: 61-62 (color description in alcohol; collected rio Pilcomayo, tributary of the rio Paraguay, Villa Montes, Department of Tarija).

Odontostilbe microcephala. -Eigenmann, 1910:429 (cited, habitat: Pilcomayo).-Eigenmann, 1915 (in part): 94-95 (type and paratype from rio Pilcomayo, Bolivia); 94 (figure maxilla, premaxilla and mandible). -Ringuelet *et al*. 1967: 94 (key diagnosed), 95-96 (brief description).-Géry, 1972: 70-71 (comparative material). -Géry, 1977: 558 (key). -Malabarba, 2003:218 (distribution: rio Pilcomayo basin, Bolivia). -Bührnheim, Malabarba, 2006: 172 (comparative material). -Bührnheim, Malabarba, 2007: 5 (comparative material). -Miquelarena *et al*. 2008: 57-58 (Key), 82-83 (description, distribution). -Mirande, 2010 (comparative material).

Diagnosis. *Odontostilbe microcephala* is distinguished from all species of the genus by the subterminal mouth (*vs*. terminal mouth, except in *O dierythrura*, *O. euspilurus* and *Odontostilbe* sp. n. A); usually 10 gill rakers on upper branch and 14-15 on lower branch (*vs*. 11-12 on lower branch in *O. dierythrura*, 5-6 on upper branch and 9-10 on lower branch in *O. euspilurus*). *Odontostilbe microcephala* differs from *Odontostilbe* sp. n. A in horizontal orbit diameter (24.6-32.8 % HL, mean 28.7% *vs*. 31.0-39.7% HL, mean 35.5 %), number of scales between lateral line and dorsal-fin origin 6 (*vs*. 5 in

Odontostilbe sp. n. A). *Odontostilbe microcephala* further differs from *Odontostilbe* sp. n. W in horizontal orbit diameter (24.6-32.8 % HL, mean 28.7% *vs*. 34.0-40.9% HL, mean 37.0 %).

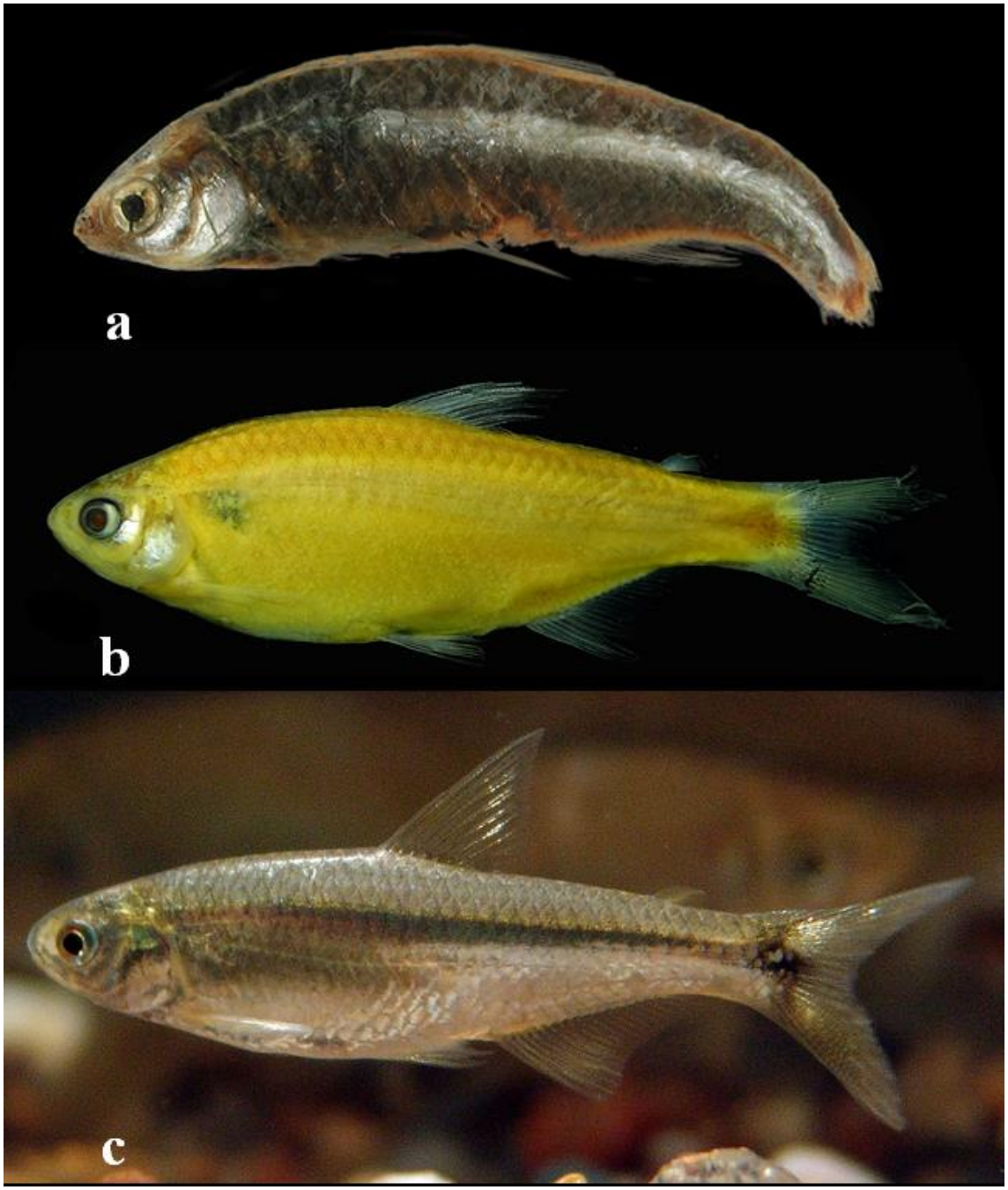

FIGURE 9. *Odontostilbe microcephala*: (a) holotype CAS 59790, male 32.7 mm SL, río Pilcomayo, Bolivia, tributary of río Paraguay (photo by The California Academy of Sciences - Ichthyology Section); (b) UMSS 473, female, 42.1 mm SL, rio Masicurí, Mamoré, rio Madera, Río Amazonas basin, Bolivia; (c) male , río Salí, in Tucumán, Argentina (not preserved, photo by Gaston Aguilera).

TABLE 3. Morphometric data for *Odontostilbe microcephala*. Males range, females range, unsexed range, number of individuals (N), mean, minimum (min), maximum (max) and standard deviation (SD) include values of the paratype CAS59791 and Standard length of holotype CAS59790 (male).

	Holotype	Paratype	Females					Males				
	CAS59790	CAS59791	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD
Standard length (mm)	32.7	33.7	39	29.66	51.97	38.75	-	22	30.37	43.59	36.44	-
Percents of Standard Length												
Snout to anal-fin origin		67.06	36	61.6	69.1	64.2	1.44	18	61.7	64.7	63.4	1.03
Snout to dorsal-fin origin		48.07	36	45.5	50.8	48.3	1.11	18	45.7	50.1	48.0	1.03
Snout to pelvic-fin origin		45.99	36	42.7	48.8	45.7	1.22	18	44.0	49.2	45.9	1.41
Snout to pectoral-fin origin		21.07	36	20.1	23.0	21.7	0.73	18	20.7	23.8	22.3	0.91
Dorsal to caudal-fin origin		50.45	36	48.0	56.8	52.1	1.90	18	48.7	55.0	52.2	1.67
Orbit to dorsal-fin origin		39.47	36	33.7	39.6	37.1	1.35	18	32.8	38.6	36.2	1.42
Anal fin base length		23.15	31	20.1	24.4	21.9	0.96	18	20.2	25.2	22.6	1.23
Peduncle length		14.84	32	11.5	16.9	14.4	1.23	18	11.2	16.2	14.0	1.43
Peduncle depth		10.39	32	9.8	12.5	10.8	0.67	18	8.6	11.7	10.5	0.88
Body depth at dorsal fin			34	24.3	34.0	28.2	2.39	18	24.7	30.7	27.5	1.97
Dorsal fin length			27	22.8	28.1	24.6	1.15	17	23.8	32.9	27.2	3.13
Pelvic fin length			29	13.9	17.4	16.0	0.89	18	16.3	21.4	18.3	1.72
Pectoral fin length			29	17.3	25.9	20.2	1.63	18	17.8	23.2	20.9	1.40
Head length		23.44	36	20.5	23.9	22.4	0.82	18	21.2	23.6	22.7	0.64
Percents of Head Length												
Snout length		22.78	36	18.8	24.8	22.6	1.43	18	20.1	25.1	23.2	1.37
Upper Jaw length		30.38	33	29.0	35.7	31.5	1.91	18	29.6	35.8	32.1	2.00
Horizontal orbit diameter		25.32	36	25.3	32.8	28.6	1.88	18	24.6	31.8	28.8	2.01
Interorbital width		30.38	36	28.4	35.6	32.4	1.96	18	29.4	35.5	32.0	1.72

Description. Morphometric data in Tab.3. Body relatively elongate and compressed. Greatest body depth at or immediately anterior to dorsal-fin origin. Dorsal profile of head relatively convex from snout to vertical through posterior border of nares, straight or slightly convex from that point to rear of supraoccipital spine. Predorsal profile of body slightly convex from posterior end of supraoccipital to dorsal-fin origin located at vertical of midlength of standard length, dorsal-fin base straight to slightly convex. Dorsal profile of body between last dorsal-fin ray and adipose-fin slightly convex, slightly concave from that point to caudal-fin origin. Caudal-peduncle slightly longer than deep. Ventral profile of head slightly convex from mouth to pectoral-fin origin; convex from that point to ventral-fin origin, with an obtuse angle in the prepelvic region. Ventral profile straight from pelvic-fin origin to anal-fin origin. Anal fin base straight to slightly concave. Caudal-peduncle ventral profile straight.

Head small (20.5-23.9 % SL, mean 22.5%). Mouth subterminal, opening oriented forward and somewhat ventrally, in which upper jaw and snout clearly extend beyond the lower jaw. Short maxilla, positioned obliquely at angle inferior of 45 degrees relative to body axis. Premaxillary dentition in single row with five teeth, slightly inclined to inside mouth, bearing seven cusps, exceptionally nine; central cusp slightly longer and wider than lateral cusps. Maxilla with two teeth bearing seven cusps, similar

in shape to premaxillary teeth. Dentary with eight or nine teeth, anterior seven teeth larger, bearing seven cusps, with central cusp slightly longer and wider than lateral cusps, followed posteriorly by one or two smaller and conical teeth. Dentary teeth inclined anteriorly (Fig. 10).

Dorsal-fin rays ii,9(23) in all examined specimens. Dorsal-fin origin posterior of vertical through pelvic-fin insertion. Profile of distal margin of dorsal fin slightly concave. First unbranched dorsal-fin ray about half-length of second, following branched rays gradually decreasing in size posteriorly. In mature males, second unbranched ray is prolonged in filament; its length three times longer than first unbranched ray, reaching origin of adipose fin in some specimens (Fig. 11b). First unbranched ray of dorsal fin inserted in first pterygiophore and last two branched rays inserted in tenth pterygiophore. Proximal radial of first pterygiophore in contact with neural spine of eleventh or twelfth precaudal vertebrae. Dorsal fin with medial radial absent from first to fifth pterygiophore and visible from sixth to ninth pterygiophore. All proximal radials with lateral projections. Origin of adipose fin anterior to vertical through base of the last rays of anal fin.

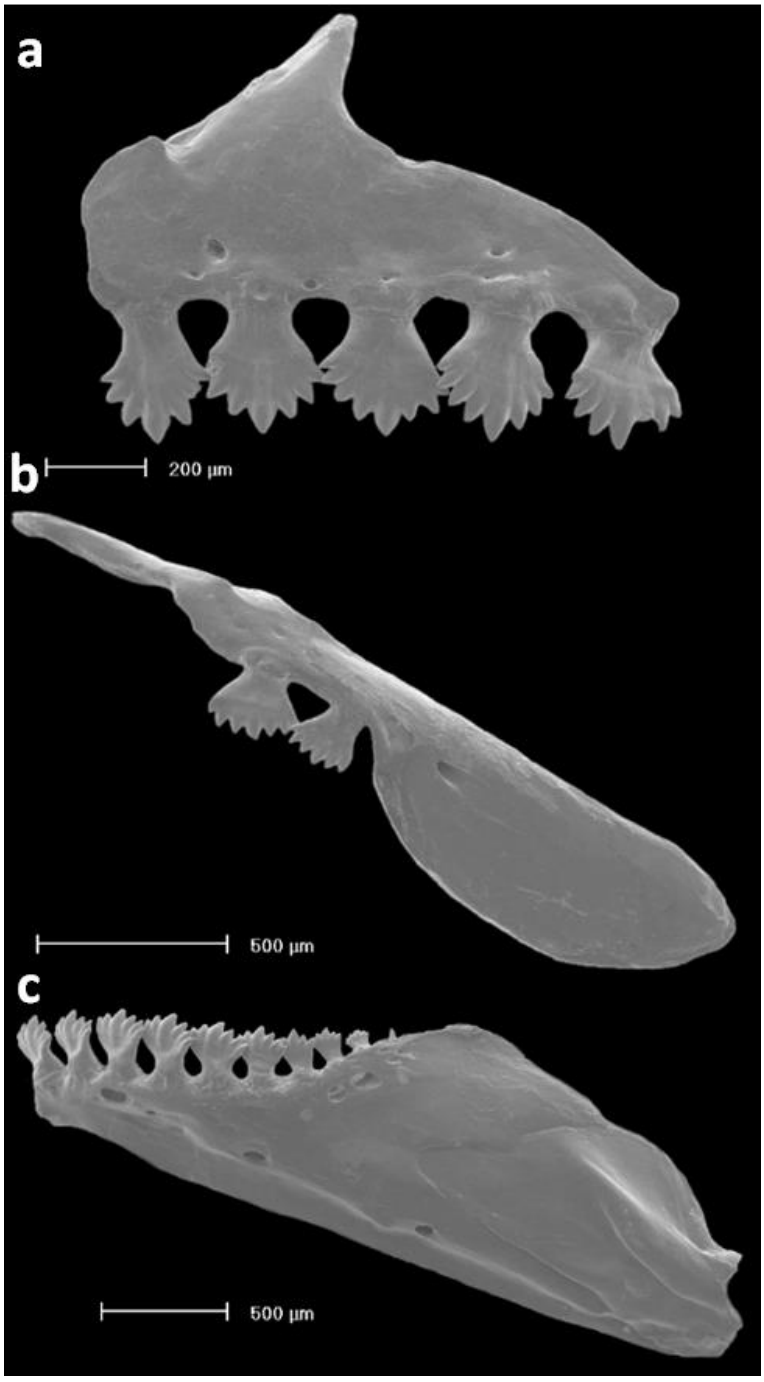

FIGURE 10. Dentition of *Odontostilbe microcephala*, paratype USNM 32473, left side, lateral view: (a) premaxilla, (b) maxilla, and (c) dentary. Scanning electron micrographs (SEM).

Unbranched anal-fin rays iii(7), iv(10) or v(4); branched anal-fin rays 16(1), 17(6), 18(8), 19(7), with two rows of scales covering base of anterior five branched rays. Anal-fin origin posterior to vertical through base of last rays of dorsal fin. Profile

of distal margin of anal fin concave. Anal fin with 20 pterygiophores, anterior three or four unbranched rays associated with first pterygiophore. First four to seven pterygiophores with medial radial absent, visible from that point to last pterygiophore. Proximal radial of first pterygiophore in contact with hemal spine of first caudal vertebra.

Pectoral-fin rays i(23), 10(1), 11(11), 12(9) or 13(2). Longest pectoral-fin ray reaching anterior edge of pelvic bone and reaching origin of pelvic fin in mature males. Pelvic fin i,7(23) rays; insertion slightly anterior to vertical through origin of dorsal fin. In mature males, first unbranched ray prolonged in filament, usually surpassing origin of anal fin. Long thin hook present on all branched pelvic-fin rays in mature males. Principal caudal-fin rays 19(23). Procurrent caudal-fin rays: dorsal 9(5), 10(9), 11(5) or 12(3) and ventral 6(1), 7(1), 8(7), 9(10) or 10(3).

Scales cycloids; lateral line complete with 35(3), 36(4) or 37(8) pored scales. Predorsal scales arranged in a regular series with 11(4), 12(5) or 13(1) scales; scale rows between lateral line and dorsal-fin origin 6(15); scales rows between lateral line and pelvic-fin origin 4(14) exceptionally 3(1); scales rows around caudal peduncle 14(23). Supraneurals 5(11) or 6 (3).

Precaudal vertebrae 17(4) or 18(5); caudal vertebrae 17(4), 18(4) or 19(1); total vertebrae 34(1), 35(6), 36(1) or 37(1). Upper gill rakers 10, lower 14-15 (2 on hypobranchial). Upper gill rakers with three or four denticles on anterolateral border, and two or three denticles on posterolateral border. Lower gill rakers with four denticles on anterolateral border, and one or two denticles on posterolateral border. Denticulation mainly on basal portion of gill rakers.

Color in alcohol. Overall body color pale yellow to light brown. Dorsal surface of head from snout to anterior limit of frontal pale yellow, black chromatophores noticeably more concentrated in region of parietal and supraoccipital. Region of third-fourth infraorbital and opercular apparatus silver color. Dorsum from posterior limit of supraoccipital to caudal peduncle with dark gray chromatophores more concentrate on scales border. Ventral region between pectoral and pelvic fins light yellow. Humeral region with slightly darkened triangular area due muscular hiatus of pseudotympanum. Body with silver lateral band between pseudotympanum and caudal peduncle. Caudal

peduncle with brown rhomboid spot, not reaching dorsal and ventral borders of caudal peduncle, covering basal portion of central rays of caudal fin.

Dorsal fin translucent, with few chromatophores scattered in anterior rays. Black chromatophores along first unbranched pectoral-fin ray. Pelvic fin hyaline. Anal fin hyaline with light gray chromatophores in basal portion of anterior rays. Adipose fin hyaline with light gray chromatophores basally. Caudal fin ray hyaline with basal portion covered by caudal peduncle spot.

The coloration of the paratype CAS 59791 is badly preserved, being remarkable only the presence of a long silver lateral band extending from the region immediately posterior to pseudotympanum to near the caudal fin.

Color in life. Caudal spot black, not reaching upper and lower margin of caudal peduncle and extending to base of caudal fin, followed by silvery pigments in each lobe of the caudal fin. Medial part of the dorsal and anal fin gold silvery. Lateral band dark green with gold line on upper margin, extending from pseudotympanum to caudal spot. Body silvery, with scattered dark chromatophores on scales and fins.

Sexual dimorphism. Sexually mature males with small hooks on anal and pelvic fins. Anal-fin rays (Fig. 11c,d) with one pair of retrorse bony hooks per ray segment symmetrically disposed, with a robust base and curved or straight tips, present on last unbranched ray and anterior six to eight branched rays, decreasing in number posteriorly until disappearing. Bony hooks present on posterior branch in median part, never on its anterior branch. Tip of bony hooks not reaching proximal border of segment of lepidotrichia where inserted. Pelvic-fin rays (Fig. 11e,f) with small hooks in all branched rays; hooks retrorse with robust base and curved tips inserted on median and distal parts of fin rays, some very small hooks on distal border of proximal portion. One bony hook, per segment on posterolateral border of anterior and posterior lepidotrichia of first segmentation in median portion and on posterolateral border of posterior lepidotrichia of second segmentation in distal portion. Tip of bony hooks not reaching proximal border of segment of lepidotrichia where inserted.

Morphometric analysis of differences with VARSEDIG shows a high overlap between the measurements that best discriminate for sex in *O. microcephala*, M12 (Dorsal-fin length) and M11 (Pelvic-fin length). These being not significant in the bivariate randomization test of male *vs.* female (p=0.269) and female *vs.* male (p=0.333)

showing no significant difference between sex. These measurements become significant when evaluating mature males that have the second unbranched ray of dorsal fin and the unbranched ray of pelvic fin elongate, prolonged in filament. The length of second unbranched ray of dorsal fin is three times length of first unbranched ray, reaching in some specimens, the origin of the adipose fin. The unbranched pelvic-fin ray elongate, reaching first or second branched anal-fin rays.

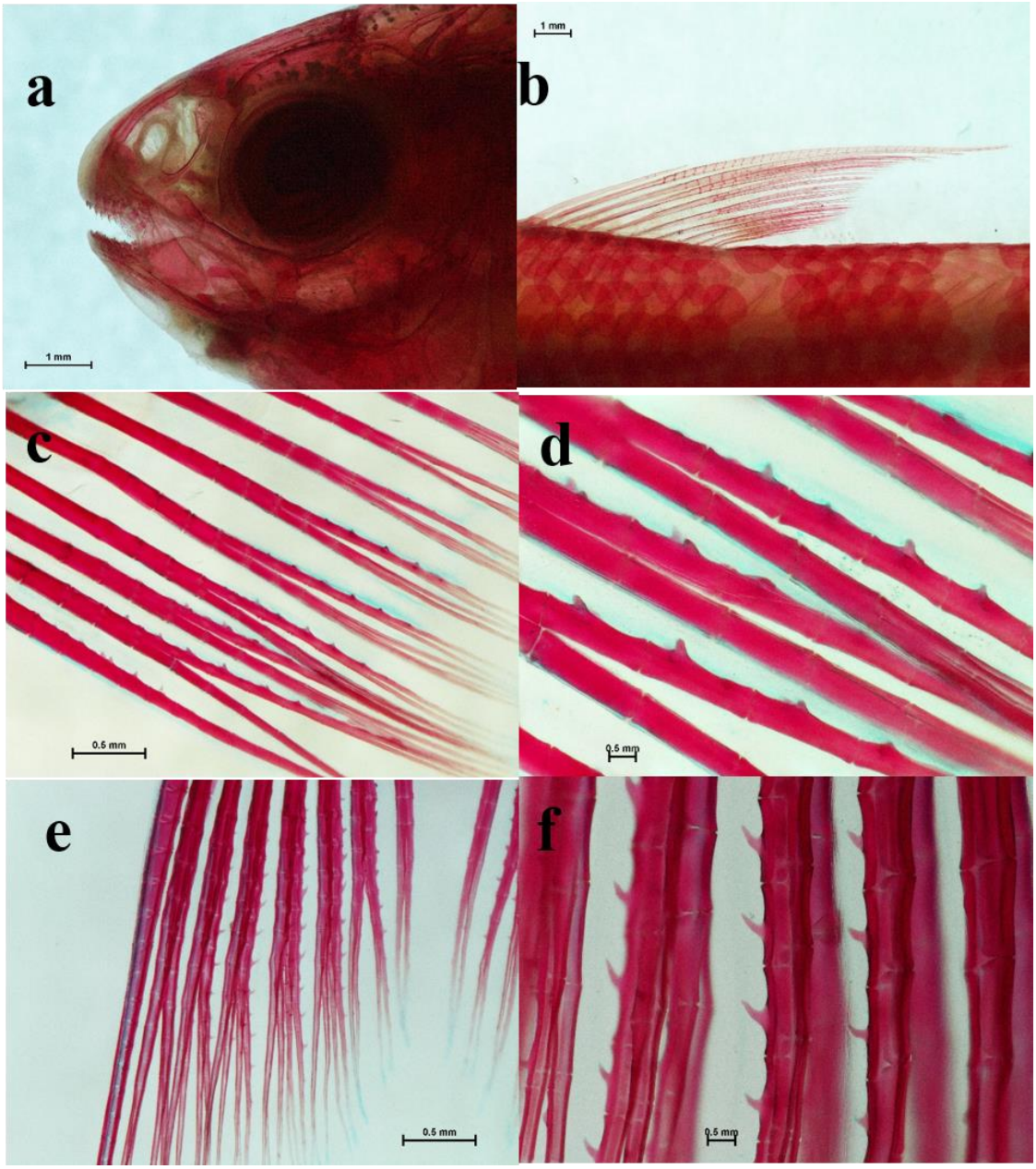

FIGURE 11. *Odontostilbe microcephala*. Male (MCP 38311): (a) Subterminal mouth (b) Second unbranched of dorsal fin elongate. Male (USNM 321173): (c) and (d) hooks in anal-fin rays; (e) and (f) hooks in pelvic-fin rays.

Geographic distribution. *Odontostilbe microcephala* is known from rio Pilcomayo, Paraguay basin, in Bolivia and Argentina and rio Masicuri, headwaters of rio Mamoré, Amazon basin, in Bolivia (Fig. 5). Carvalho, Albert, (2011) discuss the distribution of *Odontostilbe microcephala* between the Paraguay and Mamoré basins that is located mainly in the Chaco Plain and traversed by several major rivers (i.e., Parapetí, Grande, and Pilcomayo), where a mixture occurs of taxa typical of the La Plata and Upper Madeira basins, due to an important exchange of water during the rainy season.

Ecological notes. Examination of the intestinal contents of two specimens of *Odontostilbe microcephala* (USNM 305484) revealed the species feeds on insect larvae, composed mainly of nymphs of Ephemeroptera and pupa of Diptera in higher numbers, followed by larvae of Trichoptera and allochthone adults of Hymenoptera (Family Vespidae).

Rondineli *et al*. (2011) described the species as insectivorous based on specimens collected in the rio Corumbataí (São Paulo, Brazil). The material examined by Rondineli and co-authors is possibly *Odontostilbe* sp. n. A or *Odontostilbe* sp. n. W since *O*. *microcephala* does not occur in the upper rio Paraná. These authors, however, do not provide any descriptive information or photo of the fish he examined, not allowing confirmation of the identifications.

Conservation status. *Odontostilbe microcephala* is categorized as Least Concern (LC), according to the IUCN criteria for evaluation on threatening status, version 3.1 (Reis & Lima, 2009), due to its wide distribution and the lack of any known major threats across its range.

Remarks. *Odontostilbe microcephala* was briefly described by Eigenmann (in Eigenmann & Ogle, 1907) based on two specimens from the rio Pilcomayo in Bolivia. Eigenmann (1915) redescribed the species with more detail based on the type specimens plus some specimens collected in the rio Tietê, São Paulo, Brazil (CAS 60508, FMNH 57871, FMNH 57872, FMNH 131317), and extended its distribution to the upper rio Paraná. Specimens examined by Eigenmann (1915), however, were analyzed herein and belong to the two new species described in this paper, and no specimens identifiable as *O*. *microcephala* were found among all the material available from the upper rio Paraná. Due to the redescription of Eigenmann (1915), however, the name of *O*. *microcephala* has been cited for the upper rio Paraná in different studies and as a component of the

fish species from that drainage (Oyakawa, Menezes, 2011). This species occurs only in the Andean slope of the rio Paraguay basin (Bolivia).

Fowler (1940) described in detail the color pattern in 48 alcohol preserved specimens identified as *O. microcephala,* collected in the rio Pilcomayo, but did not present morphometric and meristic data of the specimens examined, not allowing a clear corroboration of this identification.

Uj (1987) redescribed *Odontostilbe microcephala* including osteological description of the skull, dorsal and anal fins with specimens collected in the upper rio Paraná in Puerto Bertoni. The specimens of Uj seems to belong to *Odontostilbe* sp. n. W, instead of *O. microcephala*.

Miquelarena *et al*. (2008) makes key diagnosed Cheirodontinae species of Argentina makes description using one specimen, and complementing information with description by Ringuelet *et al*. (1967).

Material examined. Holotype CAS 59790, 1, 46.0 mm SL, rio Pilcomayo, Bolivia, tributary of rio Paraguay, 21°11′32″S 63°47′32″W, 1900-1901. **Paratype** CAS 59791, 1, 45.0 mm SL, rio Pilcomayo, Bolivia, tributary of rio Paraguay, 20°43′30″S 64°12′55″W, 1900-1901. **Non-type specimens:** MCP 38311, 4, 38.9-43.6 mm SL, Endorheic of rio Uruena, drainage of Bajo Paraná, Rosario de la Frontera, Salta, Argentina, 25°00′00″S 64°30′00″W, 2 Mar 2001, G. Monasterio de Gonzo & M. Mosqueira. USNM 176033, 1, 48.9 mm SL, Rio Dulce, endorheic, afluent Mar Chiquita Lake Argentina, 29°38′47″S 62°52′19″W, 1 Aug 1933, T. Marini. UMSS 473, 6, 37.4-43.0 mm SL, rio Masicurí, farm Piraymiri, rio Grande, rio Mamoré, rio Madera, rio Amazonas, Bolivia, 18°50′36″S 63°45′32″W, 22 nov 2005, L. Cordova, M. Maldonado, M. Arraya, USNM 306349, 2, 41.7-47.4 mm SL, rio Bermejo, 4-5 km S, Pueblo Salado, 30 air KM NW Bermejo, Border of department of Tarija, rio Paraguay, Argentina-Bolivia, 22°27′00″S 64°32′00″W, 5 Oct 1988, W. Starnes, L. Starnes, J. Sarmiento & R. Vasquez. USNM 305484, 109, 33.54-51.97 mm SL, rio Pilcomayo, Department of Tarija, at Villamontes RR BRIDGE, rio Paraguay, Bolivia, 21°16′48″S 63°28′12″W, 1 Oct 1988 Cols W. Starnes, L. Starnes, J. Sarmiento & R. Vasquez. USNM 321173, (2 c&s, 30.6-44.0 mm SL) 49, 29.7-44.0 mm SL, rio Camatindi, 8 Km N Border Department of Tarija, 40 Km N Villamontes, Department Chuquisaca, rio Paraguay, Bolivia, 20°59′34″S 63°23′51″W, 2 Oct 1988 Cols W. Starnes, L. Starnes, J. Sarmiento

& R. Vasquez. USNM 319279, 200, 21.96-38.04 mm SL, rio Parapeti, At Rr Bridge At San Antonio, 40 Air km E Camiri, Department Santa Cruz, Amazon and Paraguay, Bolivia, 20°01′12″S 63°12′00″W, 30 Sep 1988, Cols W. Starnes, L. Starnes, J. Sarmiento & R. Vasquez.

Comparative material examined. ***Odontostilbe fugitiva***: **Perú** : ANSP 178908, 2 c&s, 29.5-32.5 mm SL. **Brazil:** INPA 18506, 3 c&s, 24.5-32.4 mm SL. INPA 18512, 1 c&s, 34.9 mm SL. **Ecuador:** MZUSP 77844, 2 c&s, 36.9-40.0 mm SL. ***Odontostilbe pulchra:*** **Trinidad and Tobago**: INHS 40101, 3 c&s, 32.5-34.4 mm SL. INHS 40081, 1 c&s, 30.5 mm SL. ***Odontostilbe naeruda*****: Brazil:** MZUSP 87759, 1 c&s, 27.5 mm SL. **Bolivia**: FMNH 106433, 1 c&s, 31.6 mm SL. ***Odontostilbe dierythrura***: **Bolivia:** MCP 38624, 7 (2 c&s, 33.5-38.4 mm SL), 32.7-34.5 mm SL. ***Odontostilbe euspilurus***: **Perú:** ANSP 143702, 2 c&s, 29.5-33.1 mm SL. **Ecuador:** MCP 38420, 13, 35.2-41.2 mm SL. ***Odontostilbe paraguayensis***: **Brazil:** MCP 35618, 54, 28.5-33.9 mm SL. **Paraguay**: MCP 12031, 3, 30.92-33.90 mm SL. ***Odontostilbe pequira***: **Brazil**: UFRGS 7022, 2 c& 36.7-38.7 mm SL, UFRGS 8641, 14, 32.0-46.6 mm SL. UFRGS 5589, 5, 31.5-34.0 mm SL. UFRGS 13365, 22, 37.6-33.9 mm SL. UFRGS 13006, 99, 26.6-34.7 mm SL. Brazil. MZUSP 21067, 1 c&s, 31.0 mm SL.

Morphometric comparisons.

Results of the PCA of morphometric data (Fig. 12) are congruent with the morphological analysis in identifying three species, *Odontostilbe* sp. n. A, *O. microcephala* and *Odontostilbe* sp. n. W. Although overlapping, boxplot graphic lateral line scale counts shows the differences between *O. microcephala* vs. *Odontostilbe* sp. n. W and *Odontostilbe* sp. n. A *vs*. *Odontostilbe* sp. n. W are significant, and that *Odontostilbe* sp. n. A and *O. microcephala* present slight overlap. Again, although overlapping, branched anal-fin rays counts are significant between *Odontostilbe* sp. n. A vs. *Odontostilbe* sp. n. W and slight different between *Odontostilbe* sp. n. A and *O. microcephala* (Fig. 13).

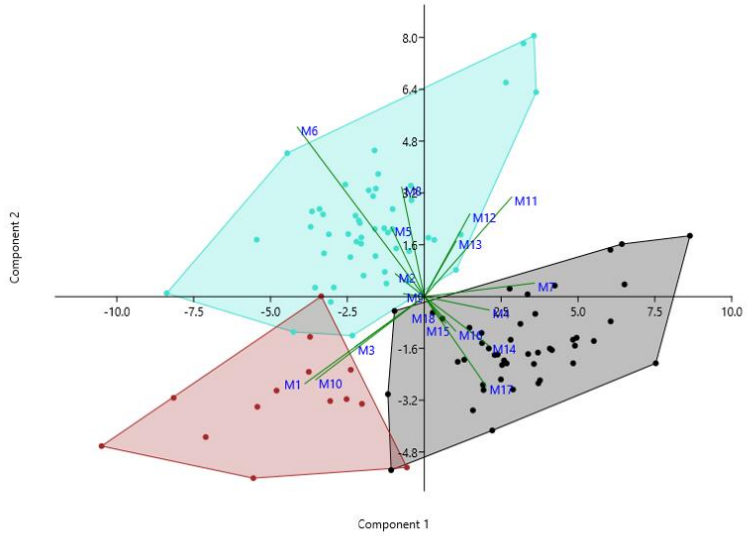

FIGURE 12. Principle Components Analysis (PCA) between the species *Odontostilbe* sp. n. A (red) *O. microcephala* (green) and **.** *Odontostilbe* sp. n. W (black).

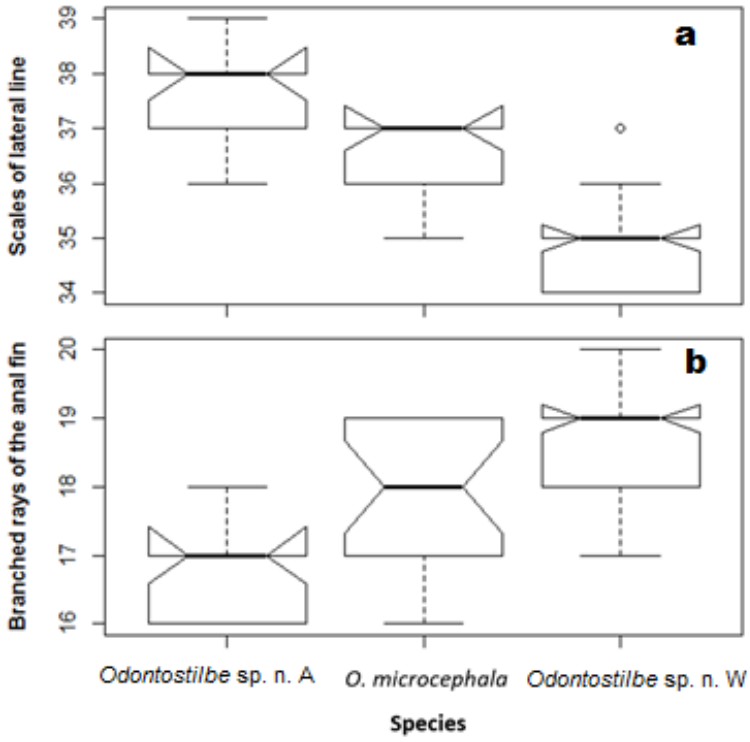

FIGURE 13. Box-plots between *Odontostilbe* sp. n. A, *O. microcephala* and **.** *Odontostilbe* sp. n. W: lateral line series of scales (top); branched rays of the anal fin (bottom).

Testing the morphometric differences between *O. microcephala* and *Odontostilbe* sp. n. W (Fig. 14), the variable that best discriminate the two species is M17 (horizontal orbit diameter), not presenting overlap between both species and showing a high discrimination capacity (Fig. 14a). Other variable with high discrimination capacity is M6 (orbit-dorsal fin origin). The scatterplot of polar coordinates obtained for both species using M17 and M6 (Fig. 14b) shows the differences between *O. microcephala* and *Odontostilbe* sp. n. W. The bivariate randomization test also separates the individuals of the two species. The individual of *O. microcephala* (Fig. 14c, red point) with the higher probability of belonging to *Odontostilbe* sp. n. W, with p= 0.019 for X-axis, is not included among the specimens belonging to *Odontostilbe* sp. n. W, rejecting the null hypothesis. Consequently, the X polar coordinates of all individuals of *O. microcephala* are significantly different from those of *Odontostilbe* sp. n. W and, therefore, none of the individuals identified as *O. microcephala* could be designated as belonging to *Odontostilbe* sp. n. W. The same is found for the individual of *Odontostilbe* sp. n. W (Fig. 14d, red point) with higher probability of belonging to *O. microcephala* (p = 0.025 for X-axis), rejecting the null hypothesis, consequently the X polar coordinates of all individuals of *Odontostilbe* sp. n. W are significantly different from those of *O. microcephala*.

The variable that best discriminate *Odontostilbe* sp. n. A and *O. microcephala* is M1 (Snout to anal- fin distance) (Fig. 15a). Other variable with high discrimination capacity is M17 (Horizontal orbit diameter). The scatterplot of polar coordinates using M1 and M17 (Fig. 15b) also discriminate the two species. The bivariate randomization test shows the individual of *Odontostilbe* sp. n. A (Fig. 15c, red point) with higher probability of belonging to *O. microcephala* (p=0.025 for Y-axis) not included among the individuals of this species, rejecting the null hypothesis. Consequently, the Y polar coordinates of all individuals of *Odontostilbe* sp. n. A are significantly different from those of *O. microcephala* and, therefore, none of the individuals identified as *Odontostilbe* sp. n. A may be designated as *O. microcephala*.

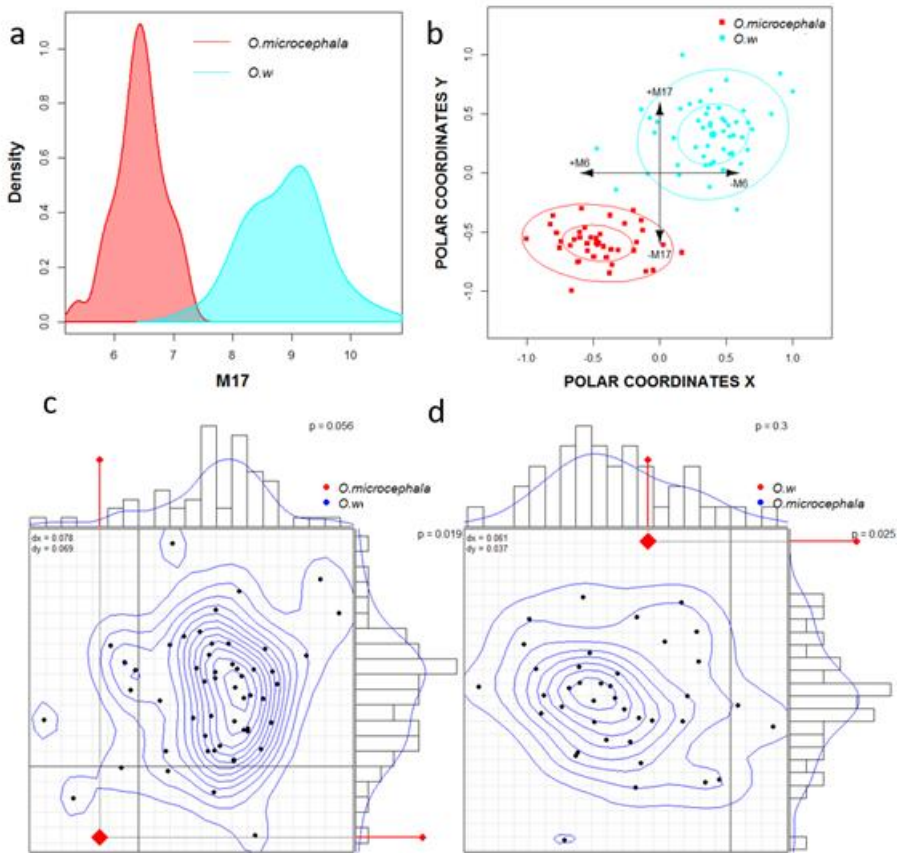

FIGURE 14. Morphometric comparison of *Odontostilbe microcephala* and **.** *Odontostilbe* sp. n. W. (a) Density plot with the overlap of the variable M17 (horizontal orbit diameter), that best discriminates the two species. (b) Scatterplot of the polar coordinates obtained for both species using variables M17 and M6 (orbit to dorsal-fin origin); the arrows show the vector of variables. (c) Bivariate randomization test, showing the individual (red point) with a higher probability of belonging to **.** *Odontostilbe* sp. n. W among all included individuals identified as *O. microcephala*. (d) Bivariate randomization test, showing the individual (red point) with a higher probability of belonging to *O. microcephala* among all individuals identified as **.** *Odontostilbe* sp. n. W.

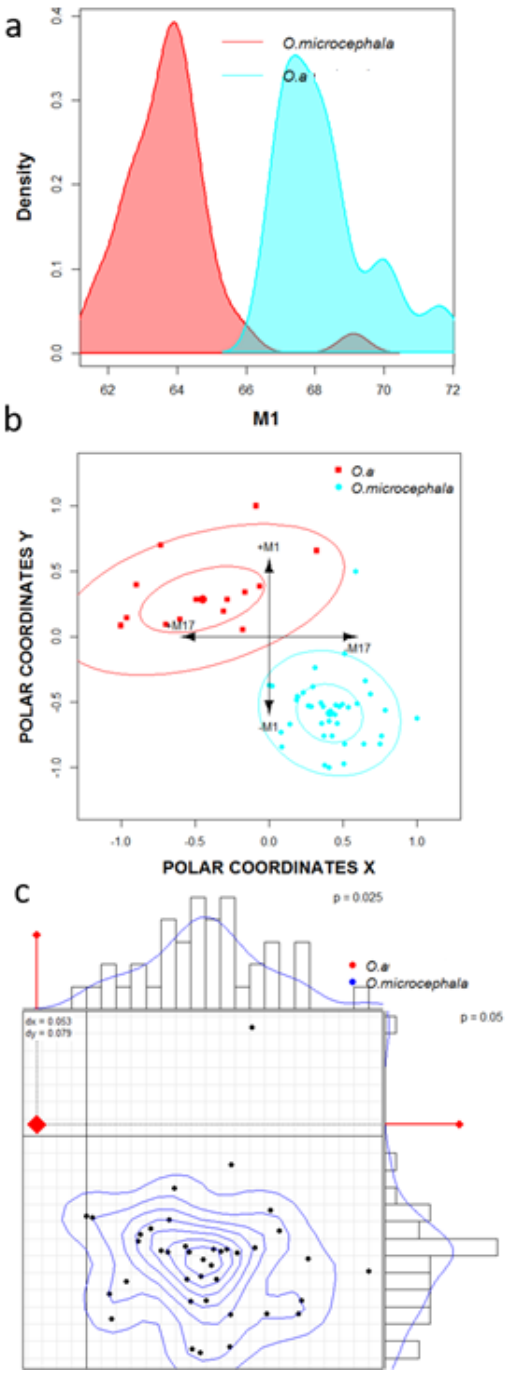

FIGURE 15. Morphometric comparison of *Odontostilbe* sp. n. A and *O. microcephala*. (a) Density plot with the overlap of the variable M1 (snout to anal-fin origin), that best discriminates the two species. (b) Scatterplot of the polar coordinates obtained for both species using variables M1 and M17 (horizontal orbit diameter); the arrows show the vector of variables. (c) Bivariate randomization test showing the individual (red point) with a higher probability of belonging to *O. microcephala* among all individuals identified as *Odontostilbe* sp. n. A.

The variable that best discriminate *Odontostilbe* sp. n. A and *Odontostilbe* sp. n. W is M1 (snout-anal fin distance) (Fig. 16a). Other variables with high discrimination capacity is M7 (anal-fin base length). The scatterplot of polar coordinates using M1 and M7 (Fig. 16b) also discriminate the two species. The bivariate randomization test shows the individual of *Odontostilbe* sp. n. A (Fig. 16c, red point) with higher probability of belonging to *Odontostilbe* sp. n. W (p=0.037 for Y-axis) not included among the individuals of this species, rejecting the null hypothesis. Consequently, the Y polar coordinates of all individuals of *Odontostilbe* sp. n. A are significantly different of those of *Odontostilbe* sp. n. W and, therefore, none of the individuals identified as *Odontostilbe* sp. n. A could be designated as *Odontostilbe* sp. n. W.

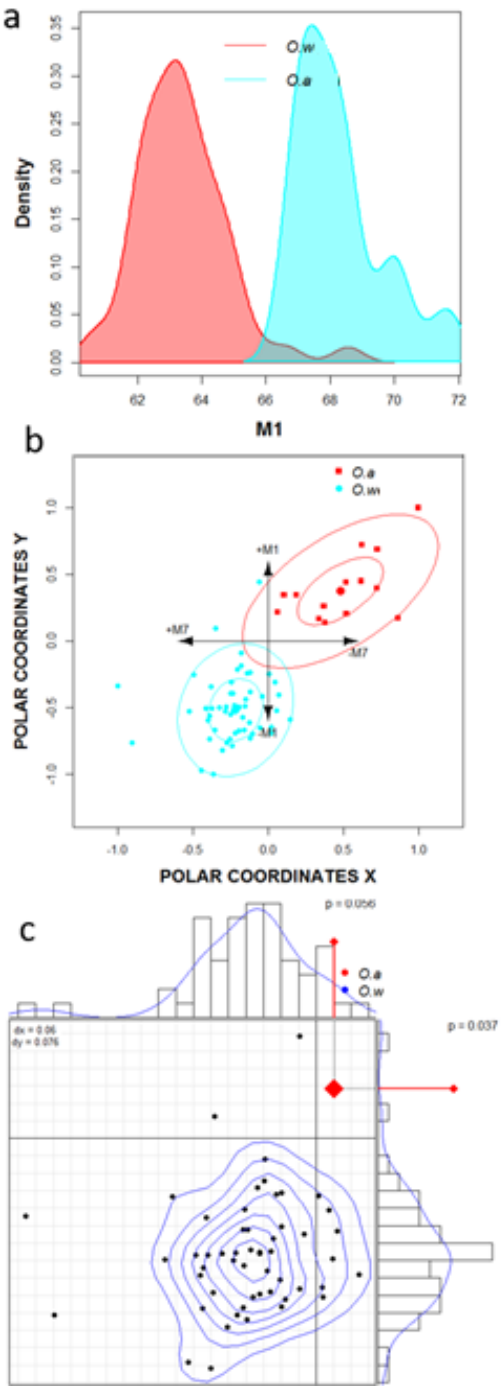

FIGURE 16. Morphometric comparison of *Odontostilbe* sp. n. A and **.** *Odontostilbe* sp. n. W. (a) Density plot with the overlap of the variable M1 (snout to anal-fin origin), that best discriminates the two species. (b) Scatterplot of the polar coordinates obtained for both species using variables M1 and M7 (anal-fin base length); the arrows show the vector of variables. (c) Bivariate randomization test showing the individual (red point) with a higher probability of belonging to **.** *Odontostilbe* sp. n. W among all individuals identified as *Odontostilbe* sp. n. A.

Discussion

Over the course of history, species of *Odontostilbe* have been wrongly identified, mainly due to the non-diagnostic original descriptions made at the beginning of the XX Century. It is the case *Odontostilbe microcephala* described originally from Bolivia (Eigenmann, 1907), but redescribed by Eigenmann (1915) including material from the upper rio Paraná. The most part of this material was reviewed, concluding that it belongs to *Odontostilbe* sp. n. A and *Odontostilbe* sp. n. W, two new species endemic to the upper rio Paraná. Uj (1987) in his review of the Cheirodontinae from Paraguay repeated Eigenmann´s mistake, redescribing *Odontostilbe microcephala* based on specimens of *Odontostilbe* sp. n. W, collected in the upper rio Paraná in Paraguay, noting that the morphometric results of the specimens analyzed by him matched with the redescription given by Eigenmann (1915).

Odontostilbe sp. n. A, *O. microcephala* and *Odontostilbe* sp. n. W can be clearly diagnosed among themselves based in the mouth position, tooth cusp number, number of gill rakers in the upper and lower branch, horizontal orbit diameter, head size, snout to anal-fin origin distance, number of branched rays of the anal-fin and perforated scales of lateral line and format of hooks in the pelvic fin.

The hooks on the fins are variable in Cheirodontinae and their number, size, shape, arrangement on fin-rays or presence in a specific fin have been described as diagnostic for some genera of the subfamily, like *Acinocheirodon* (Malabarba, Weitzman, 1999), *Kolpotocheirodon* (Malabarba *et al*. 2004), *Macropsobrycon* (Jerep, Malabarba, 2011), *Ctenocheirodon* (Malabarba, Jerep, 2012), and *Serrapinnus* (Malabarba, Jerep, 2014). The presence and morphology of these bony hooks represent an important data source for comparative phylogenetic, reproductive and behavioral studies (Bertaco, Malabarba, 2005; Viera *et al*. 2016). Even though they are usually less developed and less informative in the species of *Odontostilbe*, their number and size were useful to diagnose *Odontostilbe* sp. n. A, *O. microcephala* and *Odontostilbe* sp. n. W.

Difference in the mouth position were found among species of *Odontostilbe*, with terminal mouths observed in *O. ecuadorensis*, *O. fugitiva*, *O. nareuda*, *O. pao*, *O. paraguayensis*, *Odontostilbe* sp. n. W, *O. parecis*, *O. pequira*, *O. pulchra*, and *O. splendida*, and subterminal mouths in *Odontostilbe* sp. n. A, *O. dierythrura*, *O.*

euspilurus and *O. microcephala*. This variation in mouth position may reflect differences in feeding habits, such as mode of foraging, orientation, or diet composition. Species that feed in midwater often have terminals mouths, while benthic feeders exhibit subterminal mouths (Keast, Webb, 1966; Langerhans *et al*. 2003).

Odontostilbe sp. n. A, *O. microcephala* and *Odontostilbe* sp. n. W are characterized by having a fusiform and elongated body (more elongated in *Odontostilbe* sp. n. A). This characteristic is related to the ability to accelerate rapidly, prolonged swimming, direction change, among other features (Webb, 1984) and could be influenced by the type of habitat where they live.

The shape and number of the gill rakers in the lower and upper branch of the first branchial arch has been described recently for some species of *Odontostilbe*, being a character that varies slightly between species (Bührnheim, Malabarba, 2006, 2007). *Odontostilbe microcephala*, however, shows the highest number of gill rakers (upper 10, lower 14-15), comparatively to other species of the genus: *Odontostilbe* sp. n. A (upper 6, lower 12), *O. ecuadorensis* (upper 5-6, lower 10-11), *O. fugitiva* (upper 6-8, lower 11-14), *O. nareuda* (upper 5-6, lower 10-12), *O. pao* (upper 6, lower 11), *Odontostilbe* sp. n. W (upper 5, lower 10) , *O. parecis* (upper 5-6, lower 9-10), *O. pulchra* (upper 6-7, lower 11-12), *O. splendida* (upper 5-6, lower10-12). The number of gill rakers, may play an important role in food-particle retention, particularly with respect to zooplankton feeding (Mac Neil, Brandt, 1990; Amundsen *et al*. 2004), and may reflect differences in the feeding habits of *O. microcephala*.

Description of denticles on the gill rakers was provided for the first time for species of the Cheirodontinae by Bührnheim, Malabarba (2006); this character was considered in the phylogeny of Characidae by Mirande (2010: character 201). The denticles on gill rakers of the first branchial arch are found among all groups of Characiformes, especially in Characidae, being its absence observed in some Characidae as for example the Gymnocharacinae, *Axelrodia lindae*, and *Pseudochalceus kyburzi* (Mirande, 2010). When present, the denticles may be restricted to one margin or be distributed along of the entire surface of gill rakers as in *Astyanax puka* Mirande *et al*. (2007). The number of denticles on anterolateral border of gill raker presents large intraspecific variation: *O. ecuadorensis* (upper 1-6, lower 1-6), *Odontostilbe fugitiva* (upper 1-5, lower 1-4), *O. nareuda* (upper 1-3, lower 0-3), *O. pao*

(upper 1-2, lower 0-2), *O. paraguayensis* (upper 0-2, lower 4-6), *O. parecis* (upper 1-3, lower 0-5), *O. pulchra* (upper 1-4, lower 1-6), and *O. splendida* (upper 0-3, lower 1-4). *Odontostilbe microcephala* (upper 3-4, lower 4) presents the denticulation mainly on basal portion of gill rakers, differing from *Odontostilbe* sp. n. W (upper 4-5, lower 3-6) that presents the denticles situated also on the lateral surfaces of gill rakers.

Mesopterygoid teeth were reported in some Characiformes, in *Hoplerythrinus unitaeniatus* (Spix & Agassiz) (Weitzman, 1964), *Boulengerella* (Vari, 1995), *Crenuchus* and *Ammocryptocharax vintonae* (Eigenmann) (Buckup, 1993), *Acestrorhynchus* and *Cynodontinae* (Lucena, Menezes, 1998; Toledo-Piza, 2000), *Roeboides dispar* (Lucena, 2001). The mesopterygoid teeth can present interspecific and/or ontogenetic variation, being present in some individuals and absent in others within the same species (Toledo-Piza, 2000). In *Odontostilbe*, this character is known only in *Odontostilbe* sp. n. A and *Odontostilbe* sp. n. W in other undescribed species of the genus.

There are several characters useful in diagnosing cheirodontine species to be deeply explored or even discovered, as exemplified herein. This seems to be valid also for other characid species, whose diagnoses are usually limited to scales and fin-rays counts, and little tooth morphology information.

Generic assignment of the new species. Cope (1970) diagnosed *Odontostilbe* based on three characters that are currently shared with other species of Cheirodontinae: the teeth spatulate and crenate in a single series on the premaxilla and dentary, the anal fin elongate and the lateral line continued to the caudal fin. Malabarba (1998) proposed two synapomorphies to diagnose the genus *Odontostilbe* related to the length of the second branched ray of the dorsal fin and length of the first unbranched ray of the pelvic fin in males. More recently, Bührnheim & Malabarba (2006, 2007) used this criterion to describe five new species to this genus (*O. ecuadorensis*, *O. nareuda*, *O. pao*, *O. parecis*, and *O. splendida*), along with the redescription of its type species *Odontostilbe fugitiva* and of *O. pulchra*. *Odontostilbe* sp. n. W, and *O. microcephala* present the two synapomorphies proposed by Malabarba (1998).

The elongation of second branched ray of the dorsal fin and of the unbranched ray of the pelvic fin in males is not an exclusive character of *Odontostilbe*. It has been recorded in *Cheirodontops geayi* (Bührnheim, Malabarba, 2006), but this species differs

from *Odontostilbe* by the premaxillary teeth with 3 cusps only. It is also found in *Serrapinnus tocantinensis* Malabarba, Jerep (2014), but this species shares with other *Serrapinnus* the caudal peduncle conspicuously arched ventrally in mature males, and several other synapomorphies of the Cheirodontini, not shared with the species of *Odontostilbe* (Malabarba, Jerep 2014).

Recently, Mariguela *et al*. (2013) proposed *Odontostilbe* as polyphyletic in a phylogenetic analysis based on molecular data. According to Mariguela *et al*. (2013), *O. pequira* and an undescribed species (*O*. sp. 2) are grouped in their Clade (8), including species of *Serrapinnus*, *Acinocheirodon* and *Aphyocheirodon*: (*Aphyocheirodon* + *Acinocheirodon*) form a sister group of (*Serrapinnus microdon* (*O. pequira* + (*O*. sp 2 + *S. heterodon*))). Five species were recovered as belonging to their clade (11), including the type species of the genus: (*O. splendida* + *O. pulchra*) form a sister group of (*Ctenocheirodon pristis* (*S. kriegi* (*O. fugitiva* + (*O. ecuadorensis* + *O. paraguayensis*) + ((*S. piaba* + *Compsura heterura*) + *S. calliurus* (*S. calliurus* + *S. notomelas*))))). Finally, the two species provisionally housed in *Odontostilbe* were recovered as related to *Saccoderma* and an undescribed *Compsura* in their clade (4): (*Saccoderma melanostigma* ("*Odontostilbe*." *mitoptera* + *C. gorgonae*) + ("*O*." *dialeptura* + *Compsura* sp.)). Based on these results, Mariguela *et al*. (2013) proposed a new classification of the tribes in Cheirodontinae, but ignoring numerous evidences, including those associated to the primary and secondary sexual characters, that refute the monophyly of their clade 1 and clade 2, and supporting Cheirodontinae and Compsurini *sensu* Malabarba (1998). The clade (3) presents "*Odontostilbe" dialeptura* and "*Odontostilbe" mitoptera* as belonging of another clade, related to fishes of Central America, corroborating those obtained by Malabarba (1998). Bührnheim, Malabarba (2006) considered that these species need to be excluded from *Odontostilbe* and in the lack of a proper generic name they are provisionally kept in *"Odontostilbe"*. Characters diagnosing *Odontostilbe* and its relationships must be reviewed in an integrative analysis, given the recent addition of new species to the genus and the conflicting hypotheses presented in morphological and molecular analyses.

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Capítulo 2

A new species of *Odontostilbe* and redescription of *Odontostilbe paraguayensis* Eigenmann & Kennedy (Characidae: Cheirodontinae)

(Manuscrito a ser submetido para o periódico Zootaxa)

Remark: cited as Chuctaya *et al.* in press.B

A new species of *Odontostilbe* and redescription of *Odontostilbe paraguayensis* Eigenmann & Kennedy (Characidae: Cheirodontinae)

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Abstract

Species of *Odontostilbe* from Paraná and Paraguay basin are revised and one new species is described. For the description of the species were reviewed morphological, meristic and morphometric characters. The statistical analysis to define the morphometric variables differed significantly between species were performed using the statistical package VARSEDIG, a free function of the R program. *Odontostilbe* sp. n. B is distinguished from most of his congeners by the presence lateral line incomplete, presence of mesopterygoid teeth arranged in two groups, presence of hook in anal fin in mature males present on all unbranched ray. *Odontostilbe paraguayensis* is distinguished from all its congeners by the presence of partially fused supraneurals. The distribution of *O. paraguayensis* is limited for the rio Paraguay basin and *Odontostilbe* sp. n. B for the upper rio Paraná basin.

Introduction

Eigenmann (1915) makes the first review of Cheirodontinae, based on morphology of teeth, scales and infra-orbital bone, recognizing *Odontostilbe* with eight species (*O. fugitiva*, *O. microcephala*, *O. pulchra*, *O. paraguayensis*, *O. drepanon* Fowler [synonym of *O. fugitiva*], *O. madeira* Fowler [synonym of *O. fugitiva*], *O. hastata* Eigenmann [valid as *Saccoderma hastata* (Eigenmann)] and *O. melandeta* Eigenmann [valid as *Aphyocharacidium melandetum* (Eigenmann)]. Eigenmann (1915) says the possibility that *O. hastata* and *O. melandeta* are not part of the genus *Odontostilbe*, observation corroborated by Malabarba (1998).

Uj (1987) reviews Cheirodontinae of Paraguay, redefines *Odontostilbe*, based on characters of the type species, and regroups species within of *Odontostilbe* and *Cheirodon* by morphological similarity, transferring *Cheirodon piaba*, *C. kriegi*, *C. stenodon*, *C. microdon* and *C. notomelas* to *Odontostilbe*. Currentley, all these species belong to *Serrapinnus* genus (Malabarba ____).

Herein we provide a redescription of *Odontostilbe paraguayensis*, in order to present a better diagnosis for is species. We also describe one new species from major tributaries of the Paraná basin, in Brazil.

Material and Methods

Counts and measurements follow Fink & Weitzman (1974) and Chuctaya *et al*. (in press), unless otherwise specified. Precaudal, caudal and total vertebrae counts include the four vertebrae of the Weberian apparatus, and the terminal "half centrum" as outlined by Malabarba & Weitzman (1999). The gill raker at the junction of ceratobranchial and epibranchial is counted jointly with the gill rakers on the lower branch as in Bührnheim & Malabarba (2006). In the counting of the rays of anal fin, the last two rays are counted as a single element by being on the same pterygiophore. For the description of secondary sexual characteristics (presence of hooks in the fins) follow Chuctaya *et al*. (in press). Specimens were cleared and stained (c&s) according to Taylor & Van Dyke (1985), and were used for counting vertebrae, teeth, gill rakers, denticulation of gill rakers, supraneurals and proximal radios. Radiographs of the types of *Odontostilbe paraguayensis* were made by The California Academy of Sciences – Ichthyology Section, and were used for counting vertebrae and fin rays of the types. Scanning Electron Microscope (SEM) images were obtained from teeth and denticulation of gill rakers. Pictures of supraneurals were taken from cleared and stained specimens with a Nikon AZ100M camera attached a stereomicroscope. Nomenclature follows Weitzman (1962) for bones. To standardize the description of fish color in live and alcohol preserved specimens, the scale color of package "DAAG" (Maindonald & Braun 2015) was used as a reference, with the function: Show.color ("Single") for colors that do not have intensities and Show.color ("Shades") for colors if they have different intensities.

The following institutions provided material for the study: ANSP – Academy of Natural Sciences, Philadelphia, USA; CAS – California Academy of Sciences, San

Francisco, USA; FMNH – Field Museum of Natural History, Chicago, USA; INPA – Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil; INHS – Illinois Natural History survey, Illinois, USA; MCP – Museu de Ciências e Tecnologia, Porto Alegre, Brazil; MZUSP – Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil; UFRGS – Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. Measurements and counted specimens are indicated between parentheses in the list of paratypes or comparative material.

Statistical analyses. The standard length was not used as a variable and the remaining morphometric variables were standardized against standard length, as the ratio of every variable against the standard length and coded following Chuctaya *et al.* (in press).

The combined measurement data were examined with Principle Components Analysis (PCA) calculated in Past 3.x version 2016 (Hammer *et al*. 2001) to determine if distinct groups were identified. Defined groups were then analyzed with the algorithm of Variable selection to discriminate among taxonomic groups (VARSEDIG) (Guisande *et al*. 2016; Guisande 2016), used in the morphometric analysis of *Odontostilbe* species recently described (Chuctaya *et al*. in press). Algorithm for variable selection were following Guisande *et al.* (2016), Guisande (2016), Chuctaya *et al.* (in press). Figures are obtained with VARSEDIG, and the variables that better discriminate between two compared species were selected, using a density plot, a scatterplot of the polar coordinates and two figures results of a bivariate randomization test. If when comparing group 1 with group 2 and group 2 with 1, the p-value is close to, or lower than, 0.05 for the X or Y polar coordinates, it is concluded that the selected variables are significantly contributing to discriminating between both species.

Quantitative variables were represented by graphical boxplot, a version of this type of graphic is to add an element, called "notch" representing approximate confidence intervals 95% for median: two medians are different (the groups are different) if intervals or notches corresponding boxes do not overlap.

All statistical analyses were completed using R (R Core Team, 2016), with the exception of Principle Components Analysis (PCA) which were calculated in Past 3.x version 2016 (Hammer *et al*., 2001). The boxplot graph is performed using “sm” package. To use VARSEDIG is necessary to download the following packages: adehabitatHR, adehabitatHS, adehabitatLT and adehabitatMA packages (Calenge 2006,

Calenge 2015); ade4 package (Chessel *et al*., 2004; Dray *et al*., 2007); sp package (Pebesma & Bivand 2005; Bivand *et al*., 2013); deldir package (Turner, 2016); CircStats package (Lund & Agostinelli 2012); MASS package (Venables & Ripley 2002); boot package (Davison & Hinkley 1997; Canty & Ripley 2016); kulife package (Ekstrom *et al*., 2013) and car package (Fox & Stanford 2011, Fox *et al*., 2014). Geographic distribution map was performed in the Quantum GIS version 2.8 software (Quantum GIS Development Team, 2016).

Results

Odontostilbe sp. n. B, new species

Figs. 1–**4**

Holotype. MCP 12111, 24.9 mm SL, male, marginal pools of the rio Corumbataí, rio Tietê basin, São Paulo, Brazil, 22.241489° S, 47.608446 W, 23 Feb 1963, H. A. Britski.

Paratypes. All from Brazil, upper rio Paraná basin. **São Paulo State:** MCP 12109, (2, 23.4–24.7 mm SL), marginal pools of the rio Corumbataí, rio Tietê basin, 22.241489° S, 47.608446 W, 25 Jan 1963, H. A. Britski. & M. Vander. MCP 12110, marginal pools of the rio Corumbataí, rio Tietê basin, 22.241489° S, 47.608446 W, H. A. Britski. MCP 12111, (17.2–25.4 mm SL), marginal pools of the rio Corumbataí, rio Tietê basin, 22.241489° S, 47.608446 W, 23 Feb 1963, H. A. Britski. MZUSP 42803 (20, 23.0–26.4 mm SL), Lagoa da Ponta Seca, Corumbatai. 22.233334° S, 47.616665° W, 20 Apr 1964, H.A. Britski & N.A. Menezes, MZUSP 16751 (1, 23.9 mm SL) marginal pools of the rio Corumbataí, rio Tietê basin, 22.241489° S, 47.608446 W, 25 Jan 1963, H. A. Britski. & M. Vander. UFRGS 22870 (20, 22.6–26.6 mm SL), Lagoa da Ponta Seca, Corumbataí, 22.233334° S, 47.616665° W, 20 Apr 1964, H.A. Britski & N.A. Menezes.

Diagnosis. *Odontostilbe* sp. n. B differs from all species of the genus, by the presence of mesopterygoid teeth present in two groups on median portion of the bone (*vs.* mesopterygoid teeth present in one group in *Odontostilbe* sp. n. W and *Odontostilbe* sp. n. A, mesopterygoid teeth absent in all other congeners). This new species differs by presenting: Mouth terminal (*vs.* subterminal mouth in *O. dierythrura* and *O. euspilurus*, *O. microcephala* and *Odontostilbe* sp. n. A). Incomplete lateral line (*vs.* complete lateral line in all other congeners). Hook on 1st to 15th anal fin branched rays of males (*vs.* hook

on 1st to 22nd anal fin branched rays of males in *O. pulchra*, 1st to 7th in *Odontostilbe* sp. n. W, 1st to 9th in *O. nareuda* , 1st to 8th in *O. parecis* , 1st to 7th in *O. splendida*, 1st to 8th in *O. pao*, 1st to 7th in *O. paraguayensis* and 1st to 8th in *O. microcephala*). Premaxillary teeth with 7 cups (*vs.* 5-7 cups [mostly 5] in *Odontostilbe* sp. n. W, 7 -11 [mostly 9] cups in *Odontostilbe* sp. n. A, 8-11 cups in *O. splendida*, 8-10 cups in *O. pao*, 6-11 [mostly 8-10] cups in *O. fugitiva*, 5-9 [mostly 7-9]cups in *O. ecuadorensis*, 7-9 cups in *O. nareuda*, 8-10 cups in *O. parecsis*, 7-9 [mostly 7] cups in *O. microcephala* and 9-11 cups in *O. paraguayensis*). Five separate supraneurals (*vs*. 2nd – 4th fusioned supraneurals in *O. paraguayensis).* Five to six dentary teeth with five cusps, followed by one to two lower teeth tricuspid (*vs*. four large dentary teeth with three large and compressed central cusp and two, three lateral small cusps in *O. pequira*).

FIGURE 1. *Odontostilbe* sp. n. B: (a) holotype, MCP 12111, male, 24.9 mm SL, marginal pools of the rio Corumbataí, rio Tietê basin, São Paulo, Brazil; (b) paratype, MCP 12111, female, 25.2 mm SL, marginal pools of the rio Corumbataí, rio Tietê basin, São Paulo, Brazil.

Description. Morphometric data for *Odontostilbe* sp. n. B in Table 1. Body slightly short and compressed with maximum standard length 26.59 mm. Greatest body depth at or immediately anterior dorsal fin origin. Dorsal profile of head convex from snout to rear of supraoccipital spine. Predorsal profile of body slightly convex from posterior end of supraoccipital to dorsal-fin origin located at vertical of midlength of standard length, dorsal-fin base straight. Dorsal profile of body between last dorsal fin ray and adipose-fin slightly convex, slightly concave from that point to caudal-fin origin. Caudal peduncle longer than deep. Ventral profile of head convex from mouth to ventral-fin origin, with an obtuse angle in the region of pectoral-fin origin. Ventral profile straight of pelvic-fin origin to anal-fin origin. Anal fin base straight to slightly concave. Caudal-peduncle ventral profile slightly concave.

Head slightly small 25.7-30.0% SL, mean 27.7. Short snout y slightly rounded. Mouth terminal opening at the anterior tip of head. Short maxilla, positioned obliquely

at angle of approximately 45 degrees relative to axial axis of body, posterior tip reaching vertical through anterior border of eye and surpassing ventrally horizontal through ventral border of eye. Premaxilla with five teeth, aligned in one single row, bearing 5 cusp; each with a greater central cusp wider than lateral cusps. Maxilla with 2 broad teeth bearing 5 cusp, similar to cusps of premaxillary teeth. Dentary with 7 teeth gradually decreasing in size posteriorly; anterior 5-6 teeth larger, bearing 5 cusps; followed posteriorly by 1-2 teeth, bearing 3-5 cusp (Fig. 2). Mesopterygoid with teeth with one cusps, forming two groups in median portion (Fig. 3).

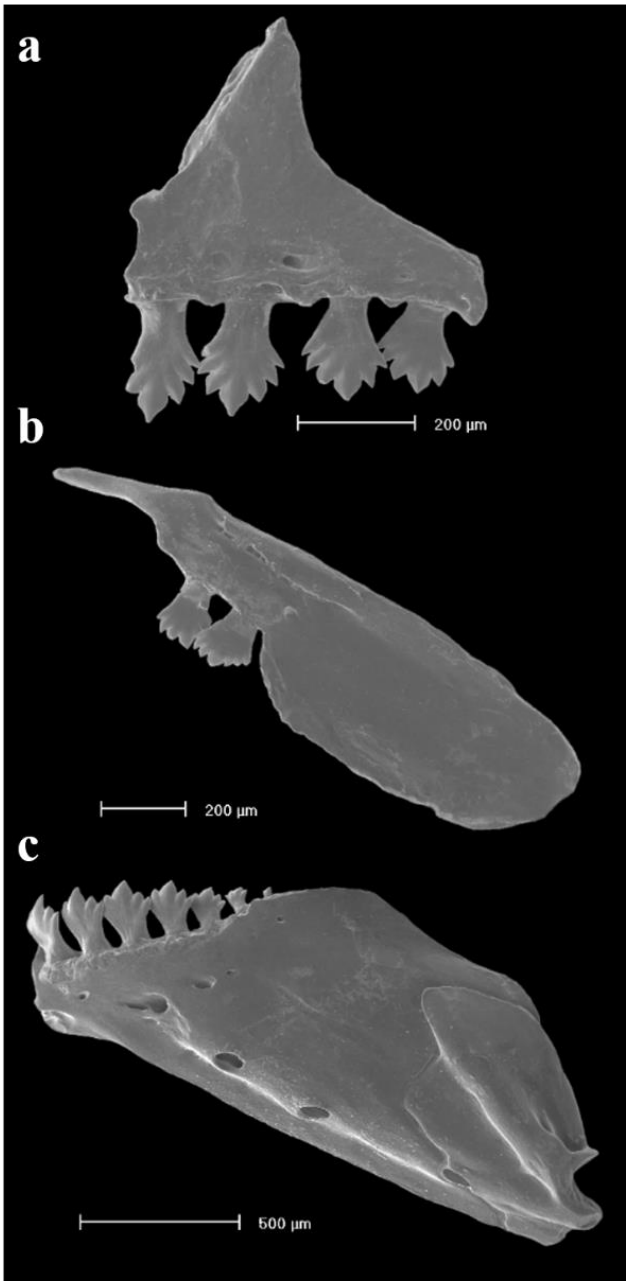

FIGURE 2. Dentition of *Odontostilbe* sp. n. B, paratype MCP 12110: (a) left side premaxilla, (b) maxilla, and (c) dentary, lateral view. Scanning electron micrographs (SEM).

Dorsal fin rays ii + 9 (35). First unbranched dorsal fin ray about half-length of second, following branched rays gradually decreasing in size posteriorly. Origin of dorsal fin anterior of middle of standard length. In mature males, second unbranched ray is prolonged filament reaching, in some specimens, origin of adipose fin. First ray unbranched of dorsal fin inserted in first pterygiophore and last two branched rays

inserted in tenth pterygiophore. Proximal radial of first pterygiophore in contact with neural spine of ninth precaudal vertebrae. Dorsal fin with medial radial absent from first to fourth pterygiophore and visible from fifth to tenth pterygiophore. All proximal radial pterygiophore with lateral projections. Origin of adipose fin vertical through base of last rays of anal fin.

Unbranched anal-fin rays ii (1), iii (24) or iv (10); branched anal fin rays 15 (11), 16(19) or 17 (5). Profile anal fin concave. Anal-fin origin posterior of vertical through base of last rays of dorsal fin. Rays of anal fin in mature males with fine and long hooks, present in last unbranched ray to of 12^{th} or 16^{th} rays branched, Two pairs per segment of lepidotrichia, tip of extended hook not reach limit anterior of segment. Anal fin 17 pterygiophores. First three unbranched radios associated with first pterygiophore. Medial radial absent from first to fifth pterygiophore and visible from sixth to last pterygiophore. Proximal radial of first pterygiophore in contact with hemal spine of first caudal vertebra.

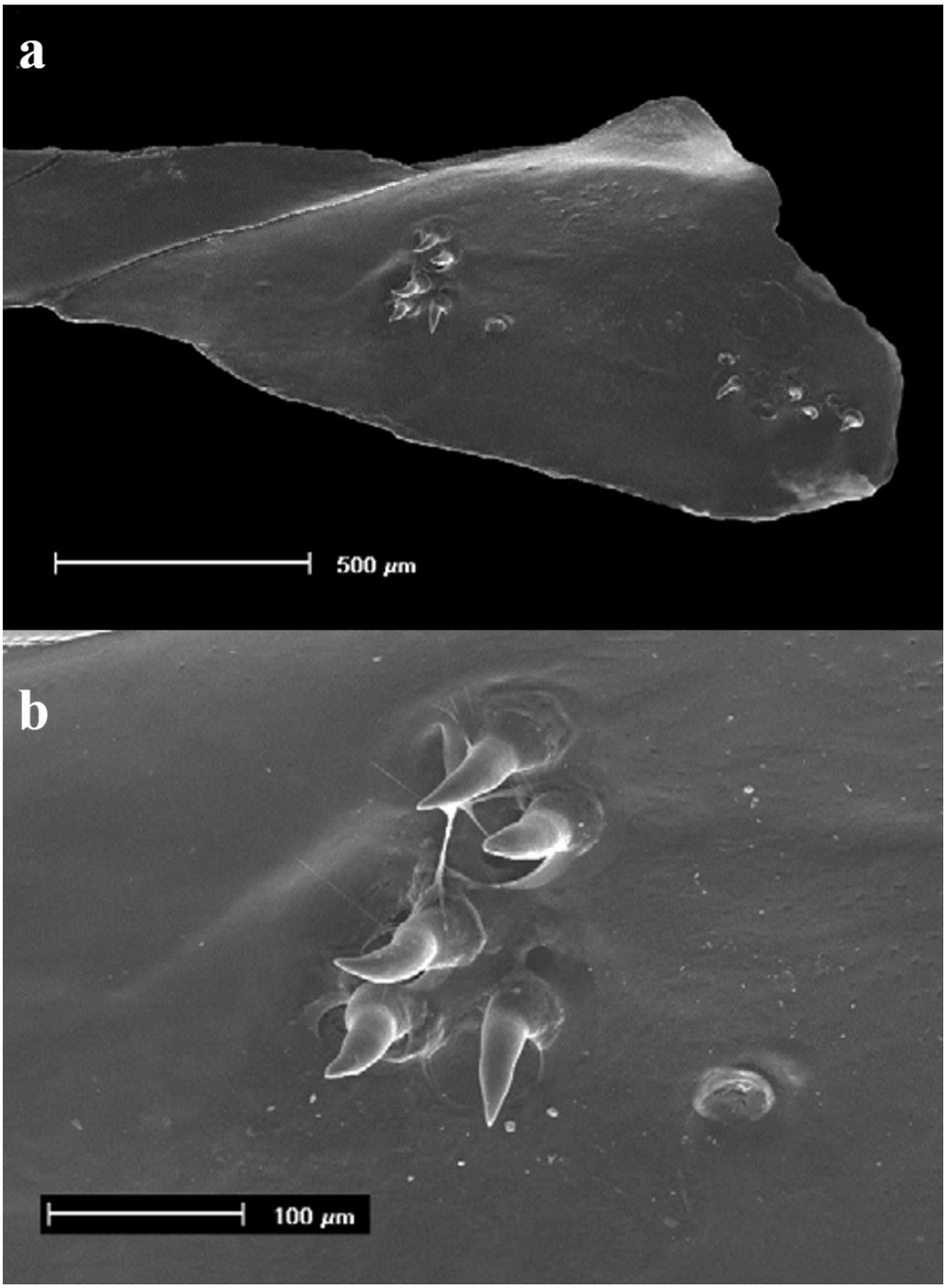

FIGURE 3. Mesopterygoid of *Odontostilbe* sp. n. B, MCP 12110, paratype, ventral view: (a) mesopterygoid with teeth forming two group, (b) detailed image of the teeth of mesopterygoid with one cusps.

Pectoral fin rays i (35), 11(19), 12(10) or 13(2), branched pectoral fin ray reaching pelvic fin origin. Pelvic fin i,7 (35) rays; its origin located approximately below vertical passing through origin of dorsal fin. In sexual mature males, unbranched pelvic fin ray

prolonged filament surpassing the origin of anal fin. Pelvic fin rays in males with long thin hook, present on all branched rays of pelvic fin, one per segment of lepidotrichia, tip of extended hook reach limit anterior of segment. Principal caudal fin rays 19(35). Procurrent caudal fin rays: dorsal with 9 (2), 10(13), 11(19) or 13(1) and ventral with 8(2), 9(3), 10(13), 11(14) or 12 (3).

Scales cycloids; pored scales on lateral line incomplete with 6 (1), 7(5), 8(11), 9(8), 10(4), 11(1) or 12(1). Scales of lateral series with 30 (1), 31 (5), 32 (10), 33 (12) or 34(1); scales pre-dorsal 10 (21), 11(1) or 12(1); scale rows between lateral line and dorsal line origin 5(35); scales rows between lateral line and anal fin origin 4(35); scales rows around caudal peduncle 14(35). One row scales at base of 1st unbranched to 5th branched anal fin rays.

Precaudal vertebrae 15(4), caudal vertebrae 17 (4); total of vertebrae 33(1), 34(3) or 35 (1). Supraneurals 4 (3) or 5(1) without lateral projections. Gill rakers upper 6, lower 9 (2 hypobranchial). Upper gill rakers with three or four denticles on anterolateral border, and three denticles on posterolateral border. Lower gill rakers with four denticles on anterolateral border, and one or two denticles on posterolateral border. Denticulation mainly on basal portion of gill rakers.

Color in alcohol. Overall body coloration pale yellow. Dorsal surface of head from snout to anterior limit of frontal of coloration yellow, chromatophore slightly more concentrated dark in region of parietal and supraoccipital. Region of Opercular apparatus of silver color. Dorsal part from the posterior limit of supraoccipital to caudal peduncle darker with chromatophores slightly dispersed on scales. Ventral region between pectoral fin and pelvic fin with a clearer area. Humeral region with a slightly darkened triangular area due muscular hiatus of pseudotympanum. Lateral region with a silver lateral band between pseudotympanum to caudal peduncle. A conspicuous rounded black caudal spot continued on the base of the middle caudal rays, usually reaching upper and lower border of caudal peduncle. Dorsal fin hyaline, with spot dark scattered in region mean of dorsal fin between second unbranched and last branched ray, reducing in last branched ray. Pectoral and pelvic fins hyaline- Anal fin hyaline with chromatophores slightly scattered in median part of anal rays. Adipose and caudal fins hyaline (Fig. 1).

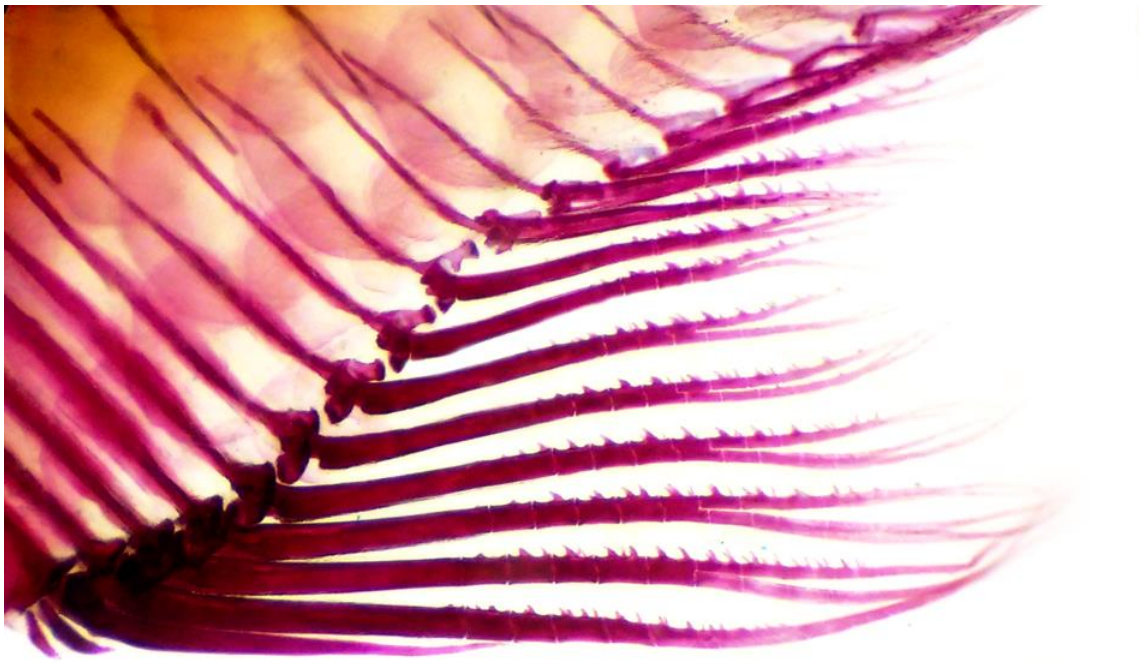

FIGURE 4. Anal fin rays with two pair of retrorse bony hooks per segment, symmetric disposed, with a robust base and curved or straight tips inserted along of last unbranched ray and on 12th or 15th branched rays in *Odontostilbe* sp. n. B, male UFRGS 22870,paratype.

Sexual dimorphism. Is easily recognizable by the difference in length of second unbranched, where in males are longer than females. In sexually mature males, presenting hooks small on anal and pelvic fins. Pelvic fin ray with hook along of all branched rays inserted on median and distal parts, some vestigial hook on distal border de la proximal portion. Hook one per segment of lepidotrichia on posterolateral border, retrorse hook with a robust base and curved tip reach proximal border of segment where inserted. Anal fin rays (Fig. 4) with two pair of retrorse bony hooks per segment, symmetric disposed, with a robust base and curved or straight tips inserted along of last unbranched ray and on 12th or 15th branched rays, decreasing in number until disappearing. Bony hooks present between posterior part of proximal region to distal part, principally on posterior lepidotrichia of median part, exceptionally on its anterior lepidotrichia. Distal tips of bony hook does not reach proximal border of segment of lepidotrichia where inserted.

Distribution: *Odontostilbe* sp. n. B known upper rio Paraná basin, Brazil (Fig. 5).

Etymology. Included in the article

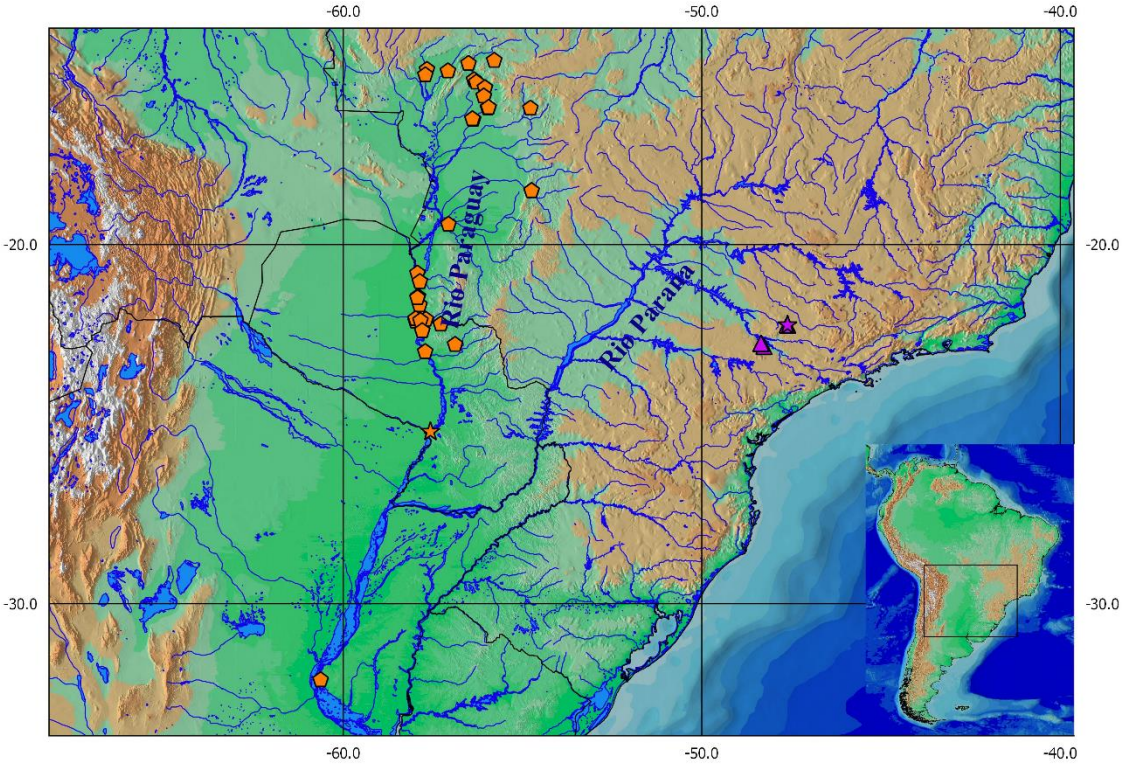

FIGURE 5. Southern South America showing the distribution of *Odontostilbe* sp. n. B (triangle) and *O. paraguayensis* (diamond). Type locality (star).

Odontostilbe paraguayensis Eigenmann & Kennedy, 1903

Figs. 6-11

Odontostilbe paraguayensis Eigenmann & Kennedy, 1903: 512-513 [original description; type material; type locality: Asuncion, Paraguay]. – Eigenmann, Mcatee and Ward, 1907:125 [listed, Corumbá]. –Eigenmann, 1910:429 [listed, geographic distribution Paraguay]. –Bertoni, 1914:11 [listed geographic distribution Paraguay]. –Eigenmann, 1915: 12 [distribution], 90 [key diagnosis], 96 [description], Plate XVI [figure specimen]. –Pearson, 1924:34 [listed, headwaters of the Beni river, Bolivia]. –Pearson, 1937:108 [listed, geographic distribution Bolivia]. –Fowler, 1940:99 [listed, geographic distribution Bolivia]. –Fowler, 1941: 183[comparative material, description *Odontostilbe iheringi*, valid as *Serrapinnus heterodon* (Eigenmann)] –Fowler, 1948:198-199 [listed, including

figured of Eigenmann (1915)]. –Ringuelet *et al*. 1967: 94-95 [key diagnosed], 95-97 [description with specimens collected by Ringuelet & Aramburu (1962)], 97 [listed, notes on distribution, Bermejo Basin in Salta and Formosa, Argentina]. Ringuelet, 1975:65[listed, upper Parana and Paraguay river], 75 [listed, Bermejo basin and Parana basin], 78 [listed, Paraguay river part inferior of Bahia Negra], –Géry, 1977: 554 [figure of Eigenmann (1915)], 558 [key diagnostic]. –Arratia *et al*. 1983:80 [listed, Bermejo and Juramento rivers, Argentina]. – Uj, 1987: 132[key diagnosed], 136-138 [description, erroneously referred to as type locality stream Tramantina, Paraguay], 153-154 [osteological description of the skull, dorsal and anal fins, generic position], 160 [distribution]. –Ibarra & Stewart, 1987: 61 [listed, paratype:Arroyo Trementina].- Casciotta *et al*. 1992: 10-13 [comparative material, description *Odontostilbe yatai*, valid as *Heterocheirodon yatai* (Casciotta, Miquelarena & Protogino 1992)], 14[Key diagnosed]. –Britski *et al*. 1999:47 [diagnosis key, brief description].–Toledo-Piza *et al.* 2001: 73-79 [collected, distribution, ecology for Upper and Lower rio Paraguay Basin and the rio Apa sub-basin Paraguay]. –Malabarba, 2003:218 [listed, notes on distribution: Paraguay and lower Paraná River basins]. –López *et al*. 2003: 31 [listed for Parana River basin, province of Corrientes, Argentina]. - Liotta, 2005: 222[listed: notes on geographic distribution in Argentina]. –López *et al.* 2005:226 [listed, Parana river], 236 [listed, affluent of Parana river]. –Bührnheim & Malabarba, 2006:172 [Comparative material, redescription *Odontostilbe fugitiva*], 188 [ecological note: makes a review of the collection of Aquarap Expedition, correct the identification, described *Odontostilbe nareuda*]. –Buhrnheim & Malabarba, 2007: 5 [comparative material, redescription *Odontostilbe pulchra*]. –Miquelarena *et al*. 2008: 57-58[key diagnosed Cheirodontinae species of Argentina], 83-85 [description, distribution]. –Mirande, 2010 [Comparative material, phylogeny of the family Characidae]. –Mariguela *et al.* [Phylogeny molecular of Cheirodontinae]. –Almiron *et al.* 2015: 125 [brief description, distribution Delta del Paraná].

Diagnosis. *Odontostilbe paraguayensis* (Fig. 6) is distinguished from all the other species of the genus by the presence of supraneurals partially fused (*vs*. four to six separate supraneurals in all the other species of the genus). Additionally for the following characters, mouth terminal, mouth slit at horizontal below middle of eye (*vs.*

subterminal, mouth in *Odontostilbe* sp. n. A, *O. euspilurus*, *O. microcephala* and *O dierythrura*). Pectoral fin extending beyond pelvic-fin origin (*vs.* never extending beyond in males of *O. fugitiva*). First to seventh anal fin branched rays of males with hooks (*vs.* 1st to 20th anal fin branched rays of males with hooks in *O. pulchra*;1st to 15th in *Odontostilbe* sp. n. B). Maxilla with two teeth (*vs.* maxillar with four teeth in *O. pao*). Branched anal-fin rays 19 -21 [mean 20] (*vs.* 19-24 [mostly 21-22] in *O. fugitiva* and 23-26 in *O. nareuda*). Absent of mesopterygoid teeth (*vs* presence and grouped on median portion forming a continuous row in *Odontostilbe* sp. n. W and *Odontostilbe* sp. n. A, forming two group in *Odontostilbe* sp. n. B). Only two other *Odontostilbe* species, *O. microcephala* and *O. pequira*, are knowns to occur sympatrically with *O. paraguayensis*. Neither of those species has 2nd – 4th fusioned supraneurals, differs from *O. microcephala* to present terminal mouth, of *O. pequira* for the absence of spot dark in the dorsal fin..

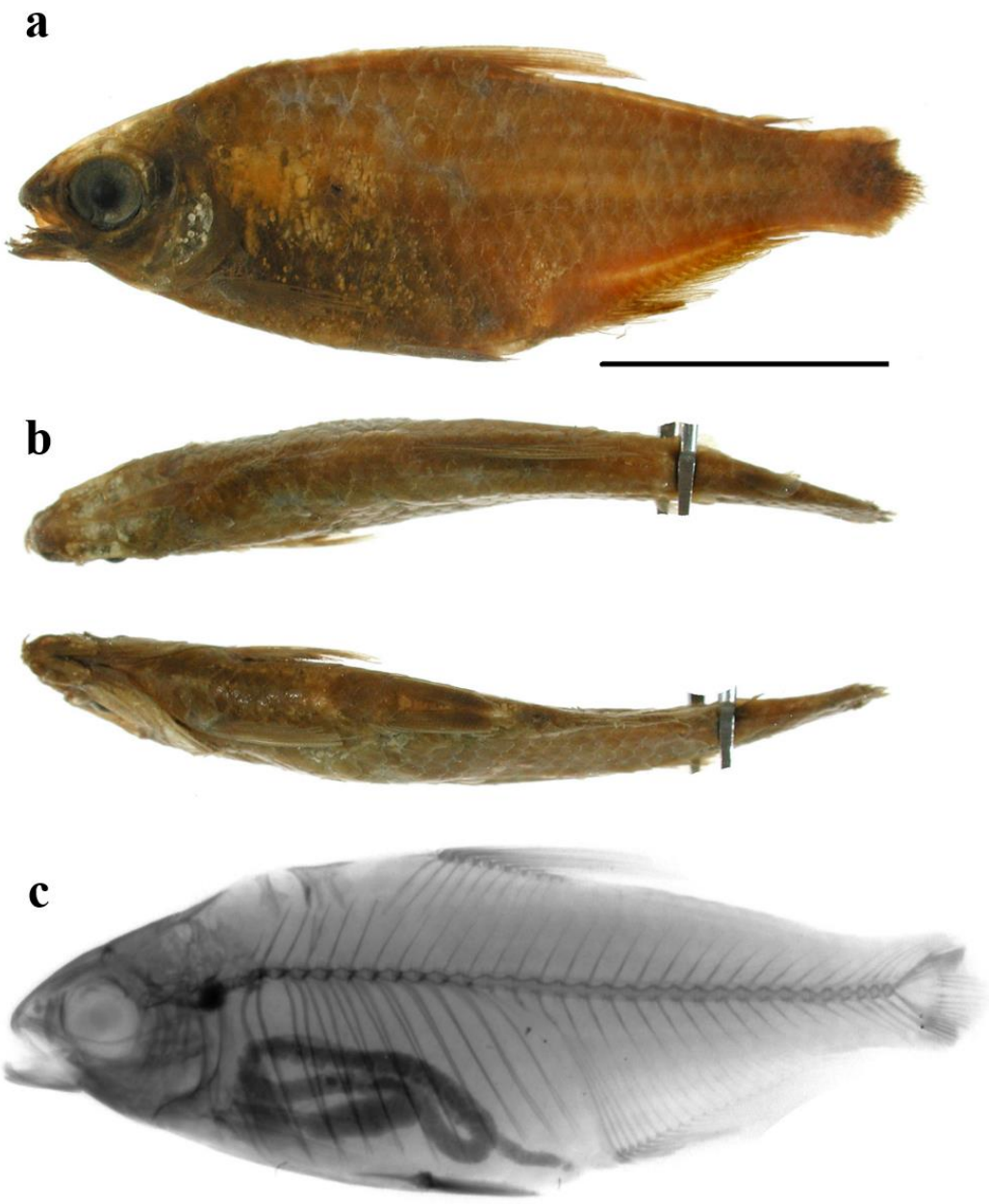

FIGURE 6. *Odontostilbe paraguayensis*, holotype, CAS 59785, female, 30.5 mm SL; Asuncion, Paraguay: (a) Left side view, (b) dorsal and ventral view, (c) radiographs of the type left side view. Photographed by the California Academy of Sciences-Ichthyology Section. Scale bar ??

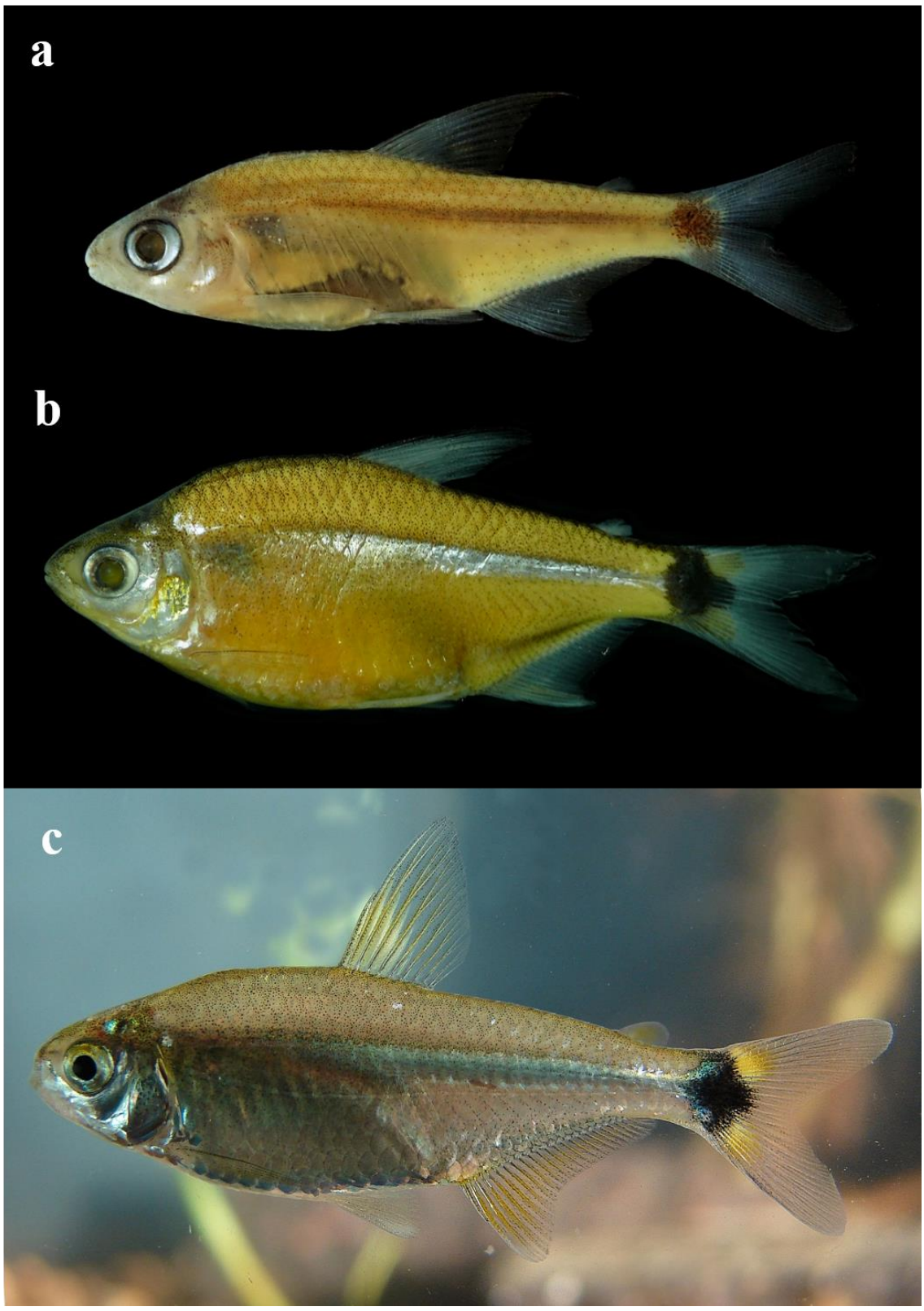

FIGURE 7. *Odontostilbe paraguayensis*: (a) MZUSP 90666, male, 30.8 mm SL, rio Sepotuba, Paraguay basin, Cáceres, Mato Grosso, (b) UFRGS 13019, female, 32.3 mm SL, Córrego Pinheiros, Paraguay River, (c) not preserved, Pre-Delta National Park, Argentina (photo by Liliana Ciotek and Pablo Giorgis).

Description. Morphometric data for *Odontostilbe paraguayensis* in Table 2. Body slightly short and compressed. Predorsal profile of body irregular, with protuberance on dorsal-medium line between second and fourth predorsals scale, with small spine directed forward (under skin) and sustained by fused of supraneurals second to fourth. Dorsal profile straight to head, accentuated concave from supra-occipital process to spines of supraneurals and straight, generally parallel to axis of body, from there to origin of dorsal fin. Greatest variable body depth between projection of supraneurals spine and origin of dorsal fin. Body profile between base of last ray of dorsal fin and adipose-fin straight or slightly concave, and slightly concave from there to base of first rays of caudal fin. Pre-ventral area flat, with well-marked lateral angles. Convex ventral profile of snout to origin of pelvic fins, more pronounced in females and slightly convex from there to origin of anal fin, body profile along the straight base anal fin and ventral profile of caudal peduncle slightly concave (Fig. 7).

Small head 21.1–28.1% SL, mean 24.6. Short snout y slightly rounded. Mouth terminal opening at the anterior tip of head, upper and lower jaws are approximately the same length. Short maxilla, positioned obliquely at angle of approximately 45 degrees relative to axial axis of body, maxillary reaching vertical of anterior margin of eye. Premaxilla with five teeth exceptionally 6, aligned in one single row, bearing 9–11 cusp; each with a prominent central cusp wider than lateral cusps. Maxilla with 2 broad teeth bearing 7 cusps, similar to cusps of premaxillary teeth. Dentary with 7–10 teeth gradually decreasing in size posteriorly; anterior 5-6 teeth larger, bearing 7–9 cusps; followed posteriorly by 1–2 teeth, bearing 3–5 cusp and 2 smaller teeth conical. Teeth generally asymmetric, with central cusp slightly longer and wider than the other, slightly inclined to inside mouth. Dentary teeth inclined to front and out of mouth (Fig. 8).

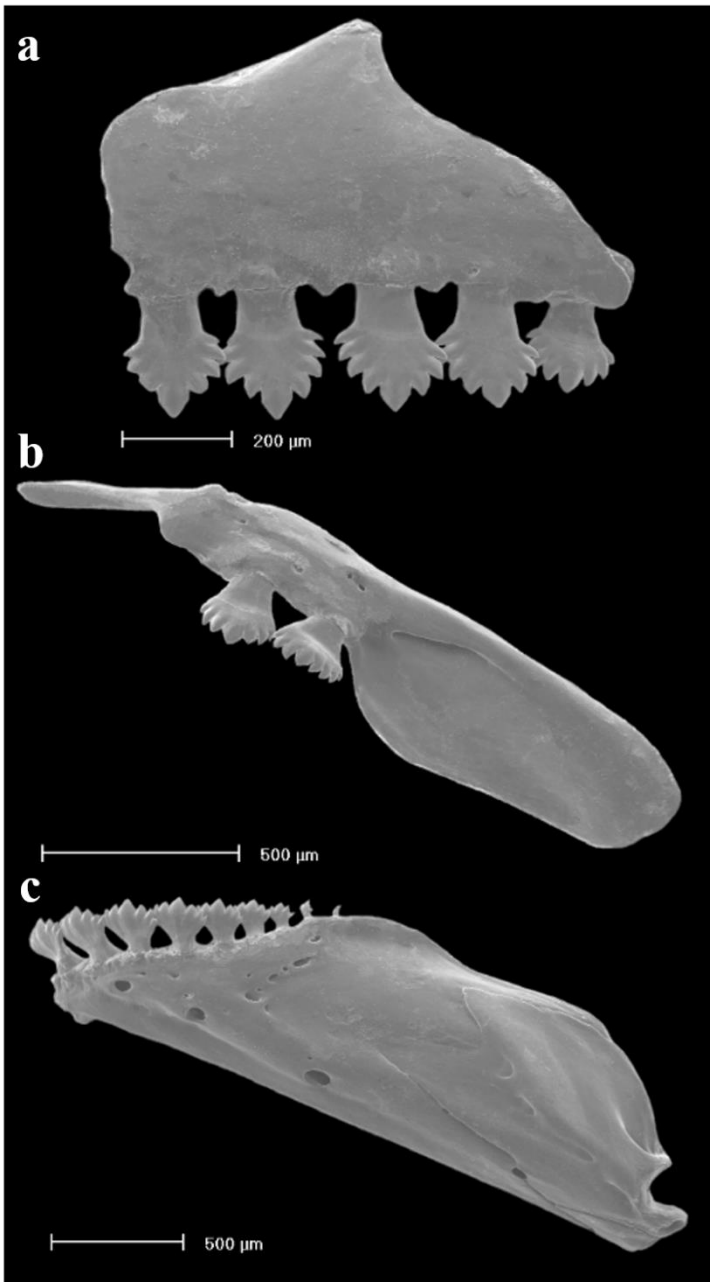

FIGURE 8. Dentition of *Odontostilbe paraguayensis*, MCP 12032, (a) premaxilla, (b) maxilla, and (c) dentary, left side, lateral view. Scanning electron micrographs (SEM).

Dorsal fin rays ii + 9 (32). First unbranched dorsal fin ray about half-length of second, following branched rays gradually decreasing in size posteriorly. Origin of dorsal fin anterior of middle of standard length. In mature males, second unbranched ray is prolonged filament reaching, in some specimens, origin of adipose fin. First ray

unbranched of dorsal fin inserted in first pterygiophore and last two branched rays inserted in tenth pterygiophore. Proximal radial of first pterygiophore in contact with neural spine of ninth precaudal vertebrae. Dorsal fin with medial radial absent from first to fifth pterygiophore and visible from sixth to tenth pterygiophore. Proximal radial of 1st to 8th pterygiophore with lateral projections, absent from ninth to tenth. Origin of adipose fin vertical through base of last rays of anal fin.

Unbranched anal-fin rays iii (3), iv (22) or v (5); branched anal fin rays 18 (1), 19(9), 20 (17) or 21(3), with a few scales at base of first anal rays. Profile anal fin concave. Anal-fin origin posterior of vertical through base of last rays of dorsal fin. Rays of anal fin in mature males with fine and long hooks, present in last unbranched ray to of 5th or 7th rays branched. Anal fin 21-23 pterygiophores. First three or four unbranched radios associated with first pterygiophore. Medial radial absent from first to fifth pterygiophore and visible from sixth to last pterygiophore. Proximal radial of first pterygiophore in contact with hemal spine of first caudal vertebra.

Pectoral fin rays i (22), 11(3), 12(17) or 13(2), branched pectoral fin ray reaching anterior edge of pelvic bone, extending beyond that point in males. Pelvic fin i,7 (33) rays; its origin located approximately below vertical passing through origin of dorsal fin. In sexual mature males, unbranched pelvic fin ray prolonged filament reaching the origin of anal fin. Pelvic fin rays in males with long thin hook, arranged in medial ventral surface of rays and curved to proximal region thereof; present in all radios branched. Principal caudal fin rays 19(27) exceptionally 20 (2). Procurrent caudal fin rays: dorsal with 8 (3), 9(1), 10(5), 11(4) or 12(5) and ventral with 8(5), 9(10), 10(3) or 11(1).

Scales cycloids; pored scales on lateral line complete with 34 (7), 35 (9) or 36 (3); pre-dorsal scales usually not arranged in regular series; scale rows between lateral line and dorsal line origin 6 (27); scales rows between lateral line and anal fin origin 4(27); scales rows around caudal peduncle 14(27). Scales on anal fin base 3–5, covering base of unbranched rays and of 1st to 6th branched rays.

Precaudal vertebrae 15(1), 16 (3) or 17(1); caudal vertebrae 18 (5); total of vertebrae 33(1), 34(3) or 35 (1). Supraneurals 4, most anterior of form lamellar and three subsequent, lamellar and fused, carrying a spinous process anterior directed to

front and with protrusion profile dorsal usually between third and fourth predorsal scale (Fig. 9).

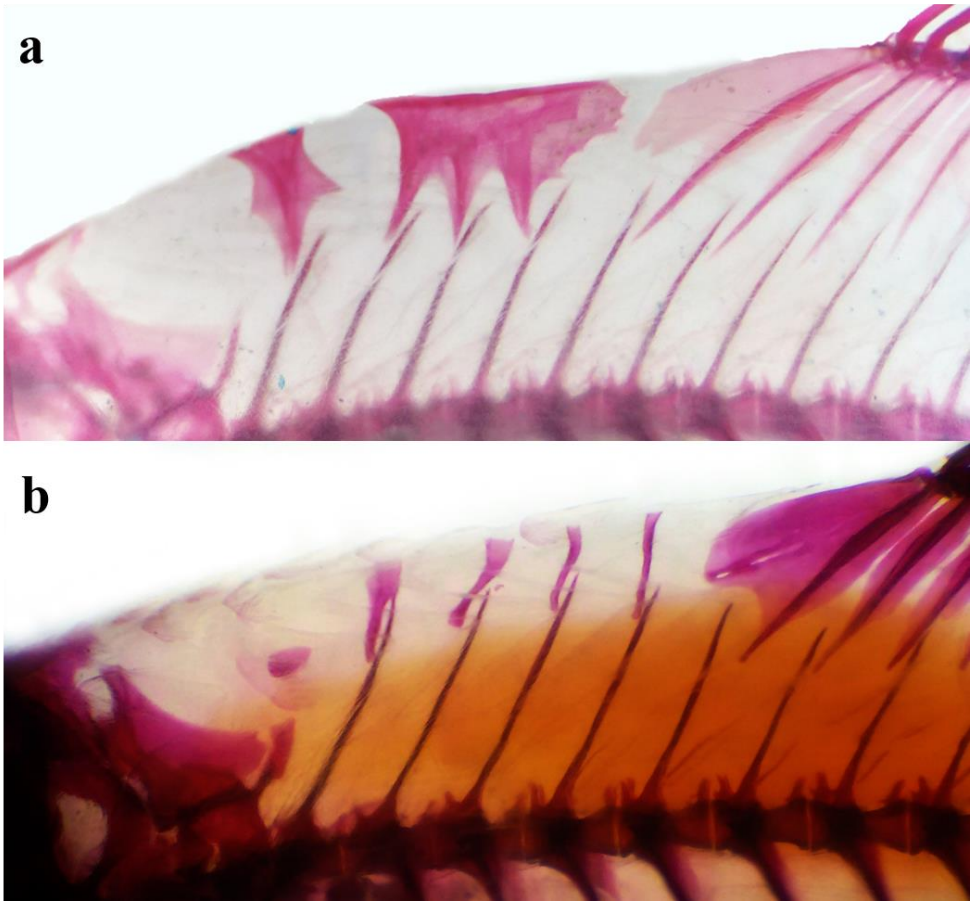

FIGURE 9. (a) Supraneurals fused of *Odontostilbe paraguayensis*, MCP 35618, left side, lateral view; (b) supraneurals of *Odontostilbe* sp. n. B, UFRGS 22870, paratype, left side, lateral view.

Gill rakers, upper 6, lower 10-11, Upper gill rakers with 0-2 denticles on anterolateral border, and 2 or 3 denticles on posterolateral border. Lower gill rakers with 4-6 denticles on anterolateral border, and 1 denticle on posterolateral border highly developed. Denticulation mainly on basal portion of gill rakers (Fig. 10).

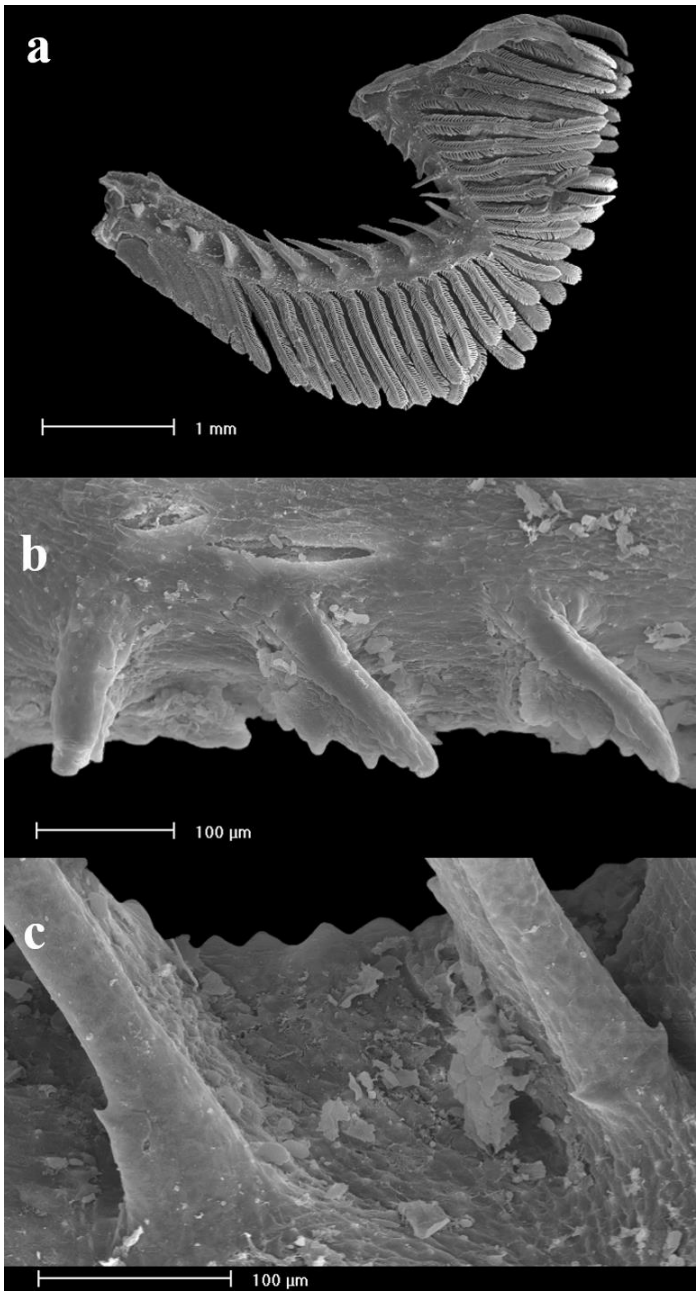

FIGURE 10. (a) First gill arch of *Odontostilbe paraguayensis*, MCP 12032, left side, lateral view. In detail (b) gill rakers on upper branchial branch, and (c) gill rakers on lower branchial branch. Scanning electron micrographs (SEM).

Color in alcohol. Overall body coloration golden-rod (shade 3). Dorsal surface of head from snout to posterior limit of frontal with chromatophores sparsely scattered giving a golden-rod coloration. Dark-golden color in region of parietal and supraoccipital. Opercular apparatus, infraorbital region and branchiostegal rays of white silver color. Opercular apparatus with patches of golden color chromatophores. Ventral

region between pectoral fin and pelvic fin with a clearer area. Humeral region with a slightly darkened triangular area due muscular hiatus of pseudotympanum. Lateral region with a white silvery lateral band, top edge of dark chromatophores forming a black line between pseudotympanum to caudal peduncle, scales top and bottom of the lateral band with scattered dark chromatophores. Dorsal region of supraoccipital to caudal peduncle with small dark chromatophores. A conspicuous rounded black caudal spot continued on the base of the middle caudal rays, usually reaching upper border of caudal peduncle.

Dorsal fin with dark-gray chromatophores along first and second unbranched ray and scattered in branched rays. Dark-gray chromatophore along first unbranched pectoral fin ray. Pelvic and anal fins with dark chromatophores scattered in all rays. Adipose fin hyaline with small dark spots widely scattered. Caudal fin covered scattered dark-gray chromatophores on part posterior and along the edges of fin rays, basal part of the lobes the caudal fin with clear chromatophores ,just behind the spot of the caudal peduncle covering the basal portion of the central rays caudal fin.

Color in life. It presents black caudal spot, which reaches the upper margin of the caudal peduncle and extends to the base of the caudal fin, followed by yellow pigments in each lobe of the caudal fin. Basal and medial part of the dorsal and anal fin yellow. Presence of a lateral band silvery covered with blue chromatophores, reached the anterior margin of the caudal spot. Yellow body, with scattered dark chromatophores on the scales and fins, region lateral and ventral of head silver colored (Fig. 7c).

Sexual dimorphism. Is easily recognizable in sexually mature males, by the presence of a series of secondary sexual characteristics, presenting hooks at the pelvic and anal fins (Fig. 11). Pelvic fin ray with small, elongate and retrorse hook with a robust base and curved tip, inserted along of all branched rays from the middle of proximal portion to distal portion, with one bony hook per segment on posterolateral border, which continues on anterior and posterior lepidotrichia of first and second ramification. Distal tips of bony hook of medial and distal portion reaches or exceeds proximal border of segment of lepidotrichia where inserted. Anal fin rays with retrorse hooks with a robust base and curved or straight tips inserted along of last unbranched ray and on first five-six branched rays decreasing in number until disappearing. Hooks present only on median portion, on posterior ramification of rays, never on its anterior

ramification. One pair of bony hooks per ray segment symmetric, disposed two pairs or asymmetrical arrangement occur on some segments of last unbranched and first posterior ramification of branched rays. Distal tips of bony hook does not reaches proximal border of segment of lepidotrichia where inserted. In addition, the mature males have these fins with ray-prolonged filament, the second unbranched ray in dorsal fin and the unbranched ray in pelvic fin. Morphometric analysis of differences with VARSEDIG show the variable that best discriminate the sex of *O. paraguayensis* is M12 (Pelvic fin length), not presenting overlap and high discrimination capacity. Additionally were determinate other variables with low overlap and high discrimination capacity M11 (Dorsal fin length), M13 (Pectoral fin length), M16 (Upper Jaw length) and M4 (Snout-pectoral fin origin).

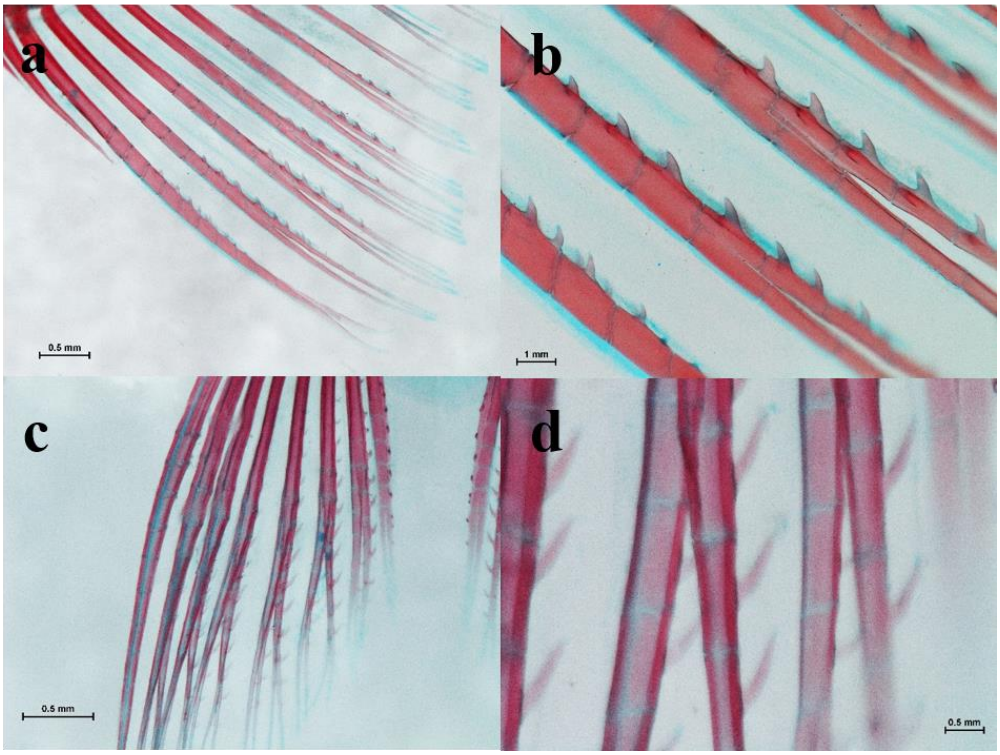

FIGURE 11. *Odontostilbe paraguayensis*, male (MCP 12031); (a) anal fin with hook, (b) hook on leptotrichia of anal fin, (c) ventral fin with hook, (d) hook on ventral fin reaching the limit anterior of leptotrichia.

Distribution. *Odontostilbe paraguayensis* known from Paraguay River system (Toledo-Piza *et al*. 2001) and various localities along of river Paraná, in Argentina, Brazil and Paraguay. In Argentina, distributed in the provinces of Salta, Formosa,

Chaco, Corrientes, Santa Fé, Entre Rios, Buenos Aires (Miquelarena *et al*. 2008) (Fig. 5).

Ecological notes. *Odontostilbe paraguayensis* collected in aquatic environments with clear water, weak stream speed and moderate marginal vegetation, substrate with rocks, gravel and sand. Toledo-Piza *et al.* (2001) collected this specie in shallow water habitats such as beaches, backwaters, and flooded forests. Examination of the intestinal contents of two specimen of *O. paraguayensis* (UFRGS 13019) reveals that the species feeds mainly on detritus, followed by filamentous algae, and lesser amount found plant remains, rotifers, cladocerans, tecameba and pupa of diptera. Miquelarena *et al.* (2008) considering this species as Iliophagus-phytophagous, algae ingested in significant quantities and sand. Corrales de Jacobo & Canon Veron (1995) consider this species as detritivorous in specimens collected in the basin of the Oriental Chaco. Wantzen *et al*. (2002) with samples of the Pantanal, using isotope analyse, classified *O. paraguayensis* as omnivorous.

Material examined. Holotype: CAS 59785 (1, 40 mm SL), Asunción, Paraguay River Paraguay, 25.21050° S 57.57166° W, 1900-1901, Col J.D. Anisits. (Photo). **Paratype:** CAS 59786 (originally IUM 10111) , female (1, 31.5 mm SL), stream Trementina, a tributary of rio Aquido, Canigi and Lagunitas along the stream, Paraguay River, Paraguay, 22.78359° S - 56.88308° W, 1900-1901, J.D. Anisits.

Non-types. Brazil, Mato Grosso State: MCP 35618, (54, 28.5–33.9 mm SL), River Jauquara in Jauquara (afluente of river Dos Pássaros, rio Paraguay), Barra do Bugres, rio Paraguay , 15.16667° S 57.08333° W, 10 Oct 1991, R.E. Reis, L.R. Malabarba & N.A. Menezes. MCP 37799, (31, 26.2–29.6 mm SL), Stream of the Bagres, on highway MT-120 between BR-174 and Jauru, *ca*. from 3 km to N of BR-174, Jauru. Paraguay, 15.63944° S 58.73500° W, 11 Jul 2004, R.E. Reis, P.A. Buckup, F. Lanjeani & E.H.L. Pereira. MCP 38155, (82, 27.2 - 32.2 mm SL), Stream of the Bagres na BR-174, *ca*. from 3km to NW of the road to Jauru (MT-388), Figueiropolis de Oeste, Paraguay River, 15.64667°S 58.74722° W, 11-Jul-04, R.E. Reis, P.A Buckup. F. Lanjeani & E.H.L. Pereira. MCP 39366, (1, 28.8 mm SL), rio Paraguai, in front of the camping Dois de Ouro. Caceres, Paraguay River, 16.14417° S 57.72972° W, 10-Jun-05, P. Lehman, V. Bertaco, F. Lima, C. Galina *et al*. MZUSP 79012, (1, 25.9 mm SL), rio Cachoeirinha, en la carretera Cáceres / Porto Estrela, Cáceres, 15.741111° S

57.33139° W, 27 Feb 2002, H. A. Britski, F.A. Machado & O. Froehlich. MZUSP 18298, (1, 20.9 mm SL), Rio Cuiabá, Barão de Melgaço, 16.183332° S 55.950001° W, CEPIPAM. MZUSP 18580, (26, 25.4–29.7 mm SL), Rio Coxipó da Ponte, Coxipó da Ponte, 15.633333° S 56.049999° W, 27–28 Oct 1975, K. de Silimon. MZUSP38073 (14, 29.9–31.4 mm SL), Ilha da Goiaba, rio Taquari, Coxim, 18.5° S 54.75° W, 1 Oct 1979, A. Carvalho Filho. MZUSP 4391, (9, 25.3–28.7 mm SL), Rio Cuiabá, Santo Antônio do Leverger, 15.866667° S 56.083332°W, G. Olson. MZUSP 74372, (1, 22.0 mm SL), Rio Cuiabá, boca do Croará, 20 km de Barão de Melgaço, Barão de Melgaço, 16.183332° S 55.950001° W, 3 May 1977, CEPIPAM & H.A. Britski. MZUSP 74392, (39, 25.2–32.1 mm SL), Rio Cuiabá (Viveiro de pássaros), Poconé, 16.5° S 56.400002° W, 15-16 Oct 1977, CEPIPAM. MZUSP 79015, (8, 31.5–35.6 mm SL), rio Jaurú, Bridge on the road, Araputangá/Jaurú, Indiavaí, 29 Fev 2002, F.A. Machado, F.C.T. Lima, G. Borges & J. Nakaga. MZUSP 79030, (19, 19.6–25.5 mm SL), Rio Cuiabá, boca do Croará, 20 km de Barão de Melgaço, Barão de Melgaço, 16.183332°S 55.950001° W, 3 May 1977, CEPIPAM & H.A. Britski. MZUSP 79116, (1, 29.1 mm SL), Baía do Chacoraré, Barão de Melgaço, 18 Oct 1977, CEPIPAM. MZUSP 79126, (3, 27.4–29.6 mm SL), Baía do Chacoraré, Barão de Melgaço, 18 Oct 1977, CEPIPAM. MZUSP 79134, (135, 25.4–30.5 mm SL), Boca do Crocura, aprox. 20km de Barão de Melgaço, Barão de Melgaço, 16.1944° S 55.9675° W, 31 Jan 1977, CEPIPAM. MZUSP 79136, (131, 24.0–28.6 mm SL), Rio Cuiabá, cerca de 3 km de Santo Antônio do Leverger, Santo Antonio Leverger, 15.866667° S 56.083332° W, 28 Aug 1976, CEPIPAM. MZUSP 79183, (1, 29.0 mm SL) Barão de Melgaço, 16.183332° S 55.950001° W, CEPIPAM. MZUSP 90717, (2, 31.1–32.3 mm SL), Rio Sepotuba (trecho medio), drenagem Paraguay, Barra dos Bugres, 15.125000° 57.668056° W, 3 Jan 2002, F. Marquez. MZUSP 90666, (23, 29.3–33.0 mm SL), Rio Sepotuba (trecho medio), drenagem Paraguay. Caceres, 15.276389° S 57.713889° W, 5 Mar 2002, H. A. Britski, O. Froehlich, A.C. Catella & F. Marquez. UFRGS 12934, (9, 18.2–27.2 mm SL), River Tugoré, BR-163, Rondonópolis Paraguay, 16.20361° S - 54.78139° W, 9 Jun 2010, V.R. Lampert, & S. Scherer. UFRGS 12995, (50, 22.2–34.3 mm SL), Ribeirão Esmerial, Br-163, Paraguay River, 15.48028° S - 56.29083° W, 10 Jun 2010, V.R.Lampert & S. Scherer. UFRGS 13019, (56, 29.8–34.2 mm SL), Córrego Pinheiros, Paraguay River, 15.41250° S 56.35556° W, 11 Jun 2010, V.R Lampert & S. Scherer. UFRGS 13040, (34, 25.8–31.6 mm SL), Ribeirão Chiqueirinho, Paraguay River, 14.95778° S 56.50972° W, 11 Jun 2010, V.R. Lampert, & S. Scherer. UFRGS 13360,

(33, 28.8–33.8 mm SL), River Manso, downstream of the bridge of UHE do Manso, Cuiabá, Paraguay River, 14.87197° S 55.79139° W, 23 Oct 2010, C.M. Baicere-Silva, F.C. Jerep, V.A. Bertaco, R. Baicere-Silva, R. & F.R. Carvalho. UFRGS 13469, (2, 26.1–31.1 mm SL), River Cuiabá, SESC Pantanal, upstream and down on beaches, Poconé, Paraguay River, 16.51530° S 56.37772° W, 24 Oct 2010, C.M. Baicere-Silva, F.C. Jerep, V.A. Bertaco, & J. Silva. **Mato Grosso do Sul State:** MZUSP 27742, (5, 29.1–31.6 mm SL), Rio Taquari, Coxim, 24 Oct 1980. A. Carvalho Filho. MZUSP 28553, (10, 30.2–33.3 mm SL), Rio Taquari, junto à cidade de Coxim, Coxim, 18.5° S 54.75° W, Oct 1983, A. Carvalho Filho. MZUSP 38055, (79, 29.4–32.4 mm SL), Ilha do Pisca, rio Taquari, Coxim, 2 Oct 1979, A. Carvalho Filho. UFRGS 11163, (10, 20.5–29.8 mm SL), Porto Murtinho, Paraguay River, 21.68306° S 57.87583° W, 31 Aug 2009, Y. Suarez. **Paraguay, Alto Paraguay Departament:** MCP 12031, (3, 30.9–33.9 mm SL), River Apa, Estância Estrellas, Paraguay River, 22.08333° S - 57.70000° W, 15 oct 1979, Miss. Zool. do Museu de Geneve. MCP 12032, (7, 30.4–33.3 mm SL), Arroio 15 km Oeste de San Luis, San Luis, Paraguay River, 15 Oct 1979, cols Miss. Zool. do Museu de Geneve. MZUSP 54372, (1, 21.91 mm SL), Rio Paraguay, em frente a Fuerte Olympo, 21.033333° S 57.866665° W, 8 Sep 1997, J. Sarmiento, M.T. Piza, C. Magalhães & A. Narváez. MZUSP 54373, (14, 20.8–26.1 mm SL), Riacho Lechuza, second tributary stream of the Paraguay River, below Boquerón, 20.799999° S 57.933334° W, 07 Sep 1997, D. Mandelburguer, M. Medina, M.T. Piza & student. MZUSP 54374, (56, 17.9–29.7 mm SL), Riacho que desemboca no Rio Paraguay, na frente de Valle Mi, 22.133333° S 57.983334° W, 12 Sep 1997, J. Sarmiento, M. T. Piza, C. Magalhães & A. Narváez. MZUSP 54375, (20, 20.1–27.7), Rio Paraguay, em frente a Fuerte Olympo, na praia, 21.033333°–57.866665°, 09 Sep 1997, J. Sarmiento, M.T. Piza, C. Magalhães & A. Narváez. MZUSP 54377, (25, 17.9–32.9 mm SL), Rio Paraguay, margem direita, ca 1500 m acima da Estância Cerrito, 21.450001° S 57.916668° W, 11 Sep 1997, J. Sarmiento, M.T. Piza, C. Magalhães & A. Narváez. MZUSP 54380 (6, 23.8–30.9 mm SL), Charco inundado no Riacho Puerto Esperanza, 22.066668° S 57.983334° W, 14 Sep 1997, D. Mandelburguer, M. Medina & S. Villanueva. MZUSP 54383, (18, 22.7–31.8 mm SL), Rio Paraguay, *ca.* 1 km below the Estancia Cerrito, Puerto Coeyú, 21.483334° S 57.933334° W, D. Mandelburguer, M. Medina & S. Villanueva. **Concepcion Departament**: MZUSP 54376 (20, 31.8–34.5 mm SL), rio Apa, Left margin, just above of San Carlos, 22.216667° S, 57.283333° W, J, Sarmiento, M. T. Piza, C. Magalhães & A. Narváez. MZUSP 54378, (8, 16.9–25.2

mm SL), Riacho Napegue, margem esquerda, 22.983334° S 57.716667° W, 17 Sep 1997, J. Sarmiento, M.T. Piza, C. Magalhães & A. Narváez. MZUSP 54379, (1, 27.9 mm SL), Arroyo La Paz, margem direita, 22.4° S 57.799999° W, 16 Sep 1997, D. Mandelburguer, M. Medina & S. Villanueva. MZUSP 54381, (84, 25.2–27.2 mm SL), Rio Apa, in pond located ca. 500 m from the river, 22.116667° S 57.849998° W, 15 Sep 1997, D. Mandelburguer, M. Medina & S. Villanueva. MZUSP 54382, (21, 25.8–34.5 mm SL), The Paz stream, on an arm of the river that enters the right bank, ca 80 m from the bank, 22.4° S 57.799999° W, 16 Sep 1997, D. Mandelburguer, M. Medina & S. Villanueva.

Morphometric comparisons.

Results of the PCA of morphometric data (Figs. 12a,b) are congruent with the morphological analysis in identifying four species, three that are distributed in the upper Parana river, *Odontostilbe* sp. n. A, *Odontostilbe* sp. n. W and *Odontostilbe* sp. n. B, and one species that is distributed in the Rio Paraguay, *O. paraguayensis*. Although overlapping, boxplot graphic lateral line scale counts and branched rays of the anal fin shows the differences between *O. paraguayensis* vs. *Odontostilbe* sp. n. B (Figs. 13a, b).

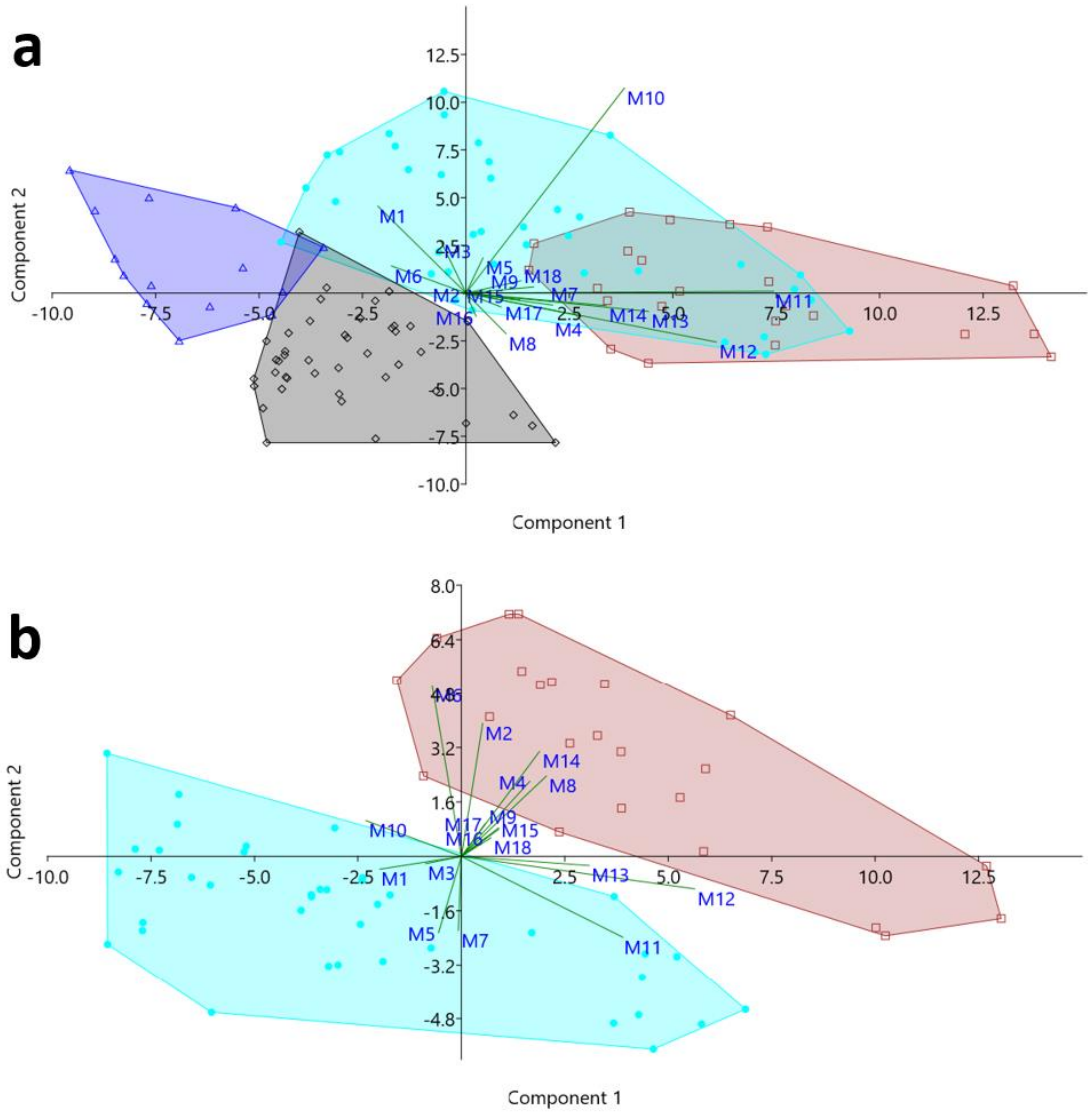

FIGURE 12. Principle Components Analysis: a) PCA between the species *Odontostilbe* sp. n. B (dots) and O. *paraguayensis* (square), *Odontostilbe* sp. n. W, (diamond) and *Odontostilbe* sp. n. A (triangle). b) PCA between *Odontostilbe* sp. n. B (dots) and *O. paraguayensis* (square).

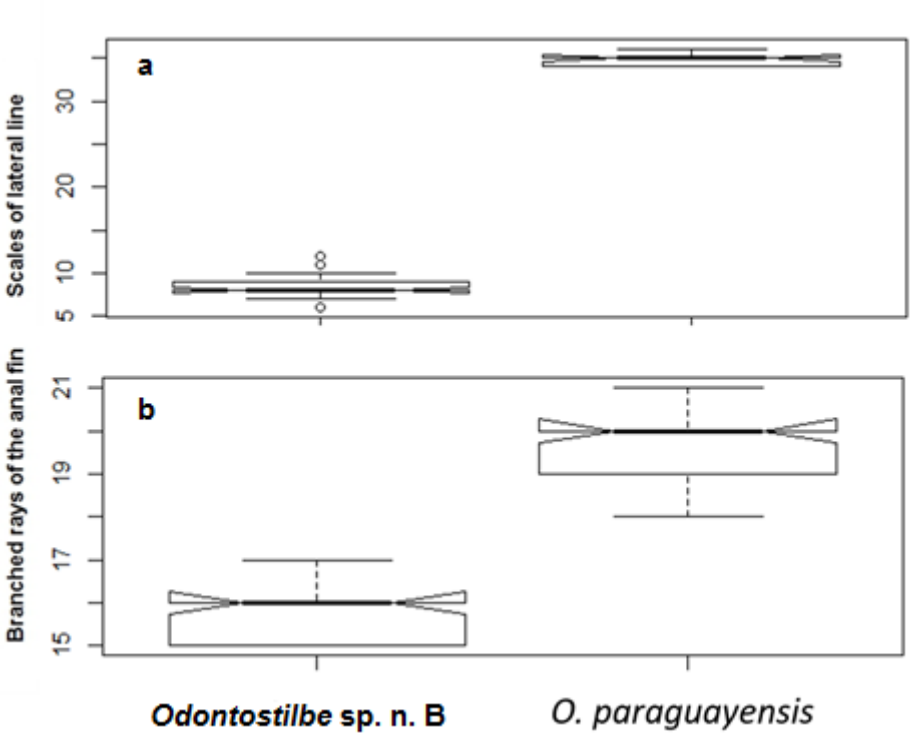

FIGURE 13. Box-plots between *Odontostilbe paraguayensis* and *Odontostilbe* sp. n. B: a) scales of lateral line, b) branched rays of the anal fin.

Testing the morphometric differences between *O. paraguayensi* and *Odontostilbe* sp. n. B (Fig. 14), the variable that best discriminate the two species is M12 (Pelvic-fin length) and M15 (Snout length), presenting slight overlap between both species and showing a high discrimination capacity (Fig. 14a). Other variable with high discrimination capacity is M8 (Length of caudal peduncle). The scatterplot of polar coordinates obtained for both species using M15 and M8 (Fig. 14b) shows the differences between *Odontostilbe* sp. n. B and *O. paraguayensis*. The bivariate randomization test also separates the individuals of the two species. The individual of *Odontostilbe* sp. n. B (Fig. 14c, red point) with the higher probability of belonging to *O. paraguayensis*, with p= 0.045 for Y-axis, is not included among the specimens belonging to *O. paraguayensis*, rejecting the null hypothesis. Consequently, the Y polar coordinates of all individuals of *Odontostilbe* sp. n. B are significantly different from those of *O. paraguayensis* and, therefore, none of the individuals identified as *Odontostilbe* sp. n. B may be designated as belonging to *O. paraguayensis*. The same is found for the individual of *O. paraguayensis* (Fig. 14d, red point) with higher probability of belonging to *Odontostilbe* sp. n. B (p = 0.042 for X-axis), rejecting the

null hypothesis, consequently the X polar coordinates of all individuals of *O. paraguayensis* are significantly different from those of *Odontostilbe* sp. n. B.

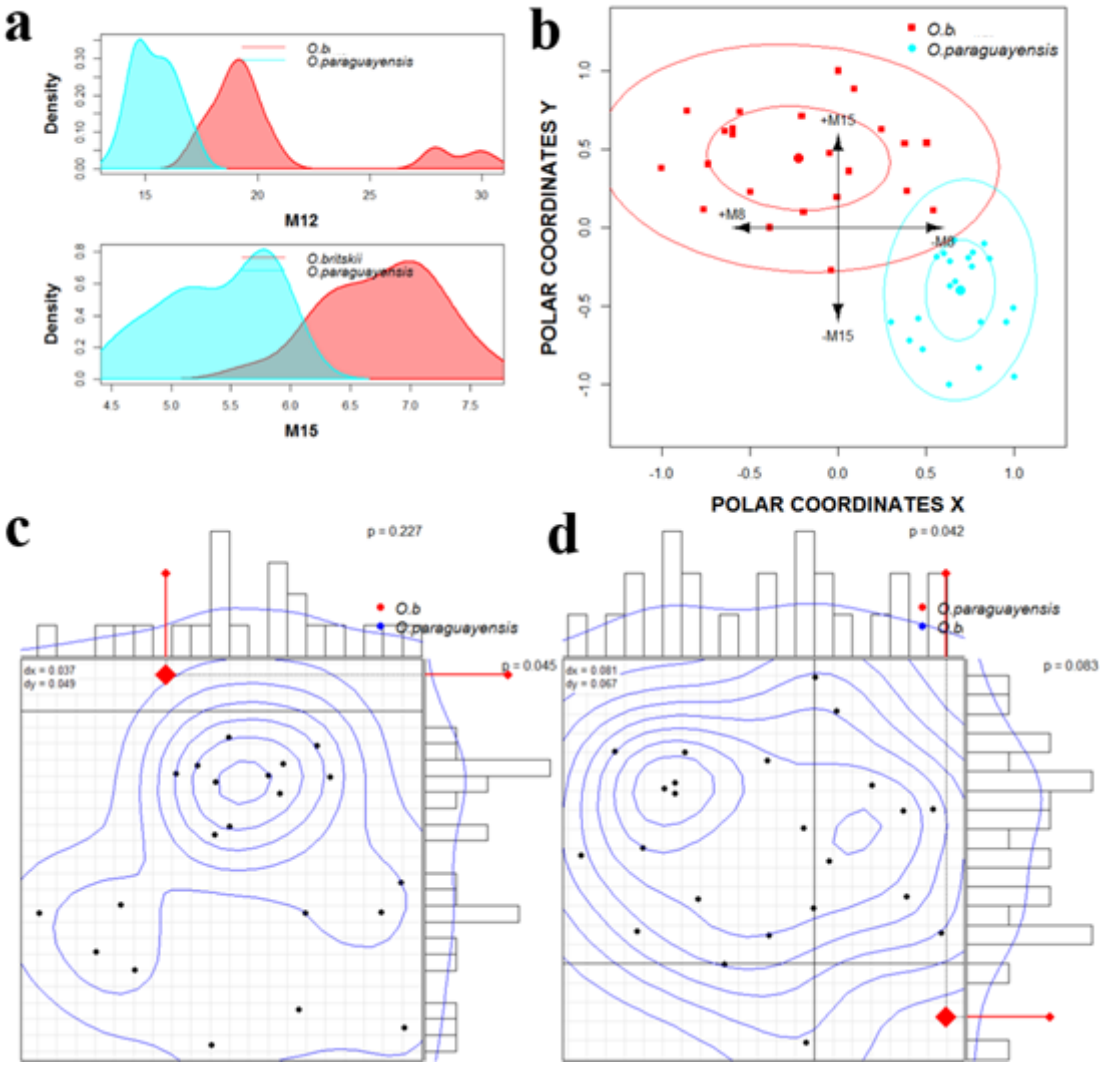

FIGURE 14. Morphometric comparison of *Odontostilbe* sp. n. B and *Odontostilbe paraguayensis*. (a) Density plot with the overlap of the variable M12 (Pelvic-fin length) and M15 (Snout length) that best discriminates the two species. (b) Scatterplot of the polar coordinates obtained for both species using variables M15 and M8 (Length of caudal peduncle); the arrows show the vector of variables. (c) Bivariate randomization test, showing the individual (red point) with a higher probability of belonging to *O. paraguayensis* among all included individuals identified as *Odontostilbe* sp. n. B. (d) Bivariate randomization test, showing the individual (red point) with a higher probability of belonging to *Odontostilbe* sp. n. B among all individuals identified as *O. paraguayensis*.

Discussion

In recent years, knowledge of the taxonomy, diversity and ecology of *Odontostilbe* species has increased (Burns *et al*. 1997; Malabarba 1998; Bührnheim and Malabarba 2006, 2007; Oliveira *et al.* 2012; Tondado *et al.* 2014), but many species continue to present information gaps, knowing only their original descriptions whose characteristics overlap with the description of other species as *O. euspilurus*, *O. dierythrura*, *O. roloffi* and *O. paraguayensis*; currently many of these species continue to be misidentified. With the recent work in *Odontostilbe* (Burns *et al*. 1997; Malabarba 1998; Bührnheim & Malabarba 2006, 2007, Chuctaya *et al.* in press), has demonstrated the need to include other features that help to correctly identify these species, such as those related to sexual dimorphism, secondary sexual characters, teeth shape, gill arch, configuration of the dorsal and ventral fins, pattern pigmentation in the fins, supraneural shape, and morphometric features of the head and body that vary between species and sometimes between sexes.

Odontostilbe paraguayensis is clearly diagnosed in part, by format supraneurals, endochondral bone that is median and located in the dorsal skeletogenous septum between the cranium and the dorsal fin (Dahdul *et al.* 2010). Initially there were two hypotheses on the origin of the supraneurals: 1) supraneurals are serial homologues of median neural spines; and 2) teleostean supraneurals are serial homologues of pterygiophores, these hypotheses were rejected by Mabee (1988), concluding that supraneurals and predorsal bones in fish are phylogenetic homologues. Supraneurals develop from anterior to posterior, from small cluster of chondrocytes dorsal to the anterior precaudal neural spines and exhibit perichondral ossification (Bird & Mabee 2003). The first and fourth supraneural related to the Weberian Apparatus. Supraneurals 2 bone in otophysans is part of the neural complex and supraneurals 3 is part of the neural complex and fuses in development with neural arches 3 and 4 (Dahdul *et al.* 2010). Shape, presence and absence has studied to determine interrelationships of Ostariophysan fishes (Fink & Fink 1981), in *Odontostilbe* this structure presents variations in shape and number, the fused form is a diagnostic character of *Odontostilbe paraguayensis*, while in *Odontostilbe* sp. n. B it is separated and varies from the other species in the number.

Other character is the format and number of hooks in the pelvic fin and anal fin, and the combination of characters morphometric. The presence of hooks on the fins were previously described in other species of Cheirodontinae, being an important source of data for phylogenetic, reproductive and behavioral comparative studies (Chuctaya *et al.* in press), demonstrating a high variation of shape of this structure between the genus. In *Odontostilbe* the presence of hooks in branched anal-fin rays presents an interspecific variation. *Odontostilbe pulchra* (1^{st} to 22^{nd}), *O. ecuadorensis* (1^{st} to 16^{th}) and *Odontostilbe* sp. n. B (1^{st} -16^{th}) are distinguished from *O. splendida* (1^{st} to 6^{th}); *O. pao* (1^{st} to 8^{th}); *O. fugitiva* (1^{st} to 9^{th}); *O. nareuda* (1^{st} to 9^{th}), *O. parecis* (1^{st} to 8^{th}), *O. paraguayensis* (1^{st} to 6^{th}); *O. microcephala* (1^{st} to 8^{th}); *Odontostilbe* sp. n. W (1^{st} to 7^{th}) and *Odontostilbe* sp. n. A (1^{st} to 4^{th}).

Mesopterygoid teeth were reported in some Characiformes (Weitzman 1964, Vari 1995, Buckup 1993, Lucena & Menezes 1998 and Toledo-Piza 2000) and were recently described for two species of *Odontostilbe* distributed in the upper Paraná River (Chuctaya *et al.* in press). *Odontostilbe* sp. n. W and *Odontostilbe* sp. n. A present mesopterygoid teeth grouped in a row (Chuctaya *et al.* in press), differing from *O. britskii* that presents mesopterygoid teeth forming two groups, on median portion of the bone.

Generic assignment of the new species. *Odontostilbe* sp. n. B presents two synapomorphies proposed by Malabarba (1998) to diagnose the genus *Odontostilbe*, related to the length of the second branched ray of the dorsal fin and the length of the ray unbranched fin pelvic in males. This criterion were used to redescription of *O. fugitiva* and *O. pulchra*, and description of new species in *Odontostilbe*: *O. nareuda*, *O. ecuadorensis*, *O. pao*, *O. splendida* and *O. parecsis* (Bührnheim & Malabarba 2006, 2007).

Odontostilbe sp. n. B differs from all species of the genus because it presents incomplete lateral line. In Cheirodontinae the genus that present species with incomplete lateral line are *Cheirodon*, *Nanocheirodon*, *Spintherobulus*, *Serrapinnus*, *Heterocheirodon*, *Compsura*, *Macropsobrycon*, *Acinocheirodon*, *Aphyocheirodon* and *Odontostilbe mitoptera* (Malabarba 1998). *Odontostilbe* sp. n. B differs from these genus by the following characteristics: number of ventral procurrent caudal-fin ranges from 8-12 (*vs.* 16-30 in *Cheirodon* and *Nanocheirodon* (Malabarba 1998)); premaxilla

teeth with 5 cusps (*vs.* Premaxillar teeth elongate and conical or tricusp in *Spintherobolus* (Malabarba 1998; Weitzman & Malabarba 1999) and *Macropsobrycon* (Malabarba 1998; Jerep & Malabarba 2011)); caudal peduncle in straight mature males (*vs.* deeply arched ventrally, the last vertebrae reaching a 45 degrees angle relative to the first vertebrae in *Serrapinnus* (Malabarba 1998; Malabarba & Jerep 2014; Jerep & Malabarba 2014); rays of the caudal fin without hooks (*vs.* small and flexible hooks present along the proximal portion of the lower lobe principal caudal-fin rays in *Macropsobrycon* (Malabarba 1998; Jerep & Malabarba 2011) and *Acinocheirodon* (Malabarba 1998; Malabarba & Weitzman 1999)); absence of modified in last scales of lateral line (*vs*. last scale of the longitudinal series of scales just below lateral line modified in *Compsura* (Malabarba 1998)). Lepidotrichia form a "V" shaped bone in frontal view with two halves fused only distally (*vs.* anteriormost 2, 3 or 4 ventral procurrent caudal-fin rays are laminar in *Heterocheirodon* (Malabarba 1998; Malabarba & Bertaco 1999)); dentary teeth with 5 cups (*vs*. dentary teeth with three central cups larger, compressed and in a row forming a sharp cutting edge in *Aphyocheirodon*, this character is also observed in *O. pequira*, but is considered non-homologous on the basis of overall parsimony (Malabarba 1998)).

The elongation of second branched ray of the dorsal fin and of the ray unbranched fin pelvic in males in *Odontostilbe*, was also previously recorded in other genus (Chuctaya *et al.* in press) but these species present other characteristics that clearly distinguish of *Odontostilbe.*

The taxonomic revision of *Odontostilbe* is still necessary for several basins, it is important to update the diagnosis of species that were described more than 100 years ago with very general characteristics, which in the last years has generated confusion, mainly observed in ecology, biogeographic, cytogenetic and phylogenetic. The redescription of Cheirodontinae species is the basis of phylogenetic studies, correctly identifying these species will allow us to obtain better hypotheses of phylogenetic relationships within this subfamily. In addition to correctly determining the conservation status of these species.

Comparative material examined. *Odontostilbe microcephala*: MCP 38311, 4 ex, 38.86–43.59 mm SL, Endorheic of Uruena River, drenaje of Bajo Paraná, Rosario de la Frontera, Salta, Bajo Paraná, Argentina, 25.00000 S 64.50000 W, 2 Mar 2001, Cols

Gladys Monasterio de Gonzo and Mario Mosqueira. USNM 321173, 5, (2 c&s) of 49 ex, 29.66–44.02 mm SL, Camatindi River, 8 Km N Border Dept. Tarija, 40 Km N Villamontes, Dept Chuquisaca. Rio Paraguay, Bolivia, 20.99271 S 63.39922 W, 2 Oct 1988 Cols W. Starnes, L. Starnes, J. Sarmiento & R. Vasquez. ***Odontostilbe* sp. n. A:** LIRP 3239, 48.7 mm SL, ribeirão da Batalha, Fazenda Batalha (Pedro Queresma), rio Paranaíba, Paracatu, Minas Gerais State, Brazil, 17.423611° S, 47.453056° W, 27 Apr 2002, C. A. A. Figueiredo & E. S. S. Rego. FMNH 57871 (1, 62.1 mm SL), rio Tietê at Salto Avanhandava below the falls, Penápolis, São Paulo, 21.167648° S, 50.117562° W, 14 Sep 1908, J. Haseman. ***Odontostilbe* sp. n. W**: MZUSP 16851, 38.9 mm SL, male, rio Mogi Guaçu, Emas, Pirassununga, São Paulo, Brazil, 21.916666° S 47.383335° W, 22 Oct 1963, H. A. Britski. MZUSP 87947, (11, 42.5–50.5 mm SL), Itirapina, córrego da Lapa near the mouth and along the seawall of rock and the bridge on the road, 22.249918° S, 47.863376° W, 31 Jan 2002, E. N. Fragoso. ***Odontostilbe fugitiva*:** ANSP 178908, 2 c&s (1 female, 1 male) of 12, Perú, Loreto, Maynas, [lower rio Itaya] at bridge on Iquitos-Nauta highway, approximately 25 miles SSW of Iquitos. INPA 18465, 4 c&s (2 males, 2 females) of 73, Brazil, Amazonas, Ilha da Marchantaria. INPA 18506, 3 c&s (1 male, 1 female, 1 unsexed) of 50. Brazil, Amazonas, Paraná do Xiborena. INPA 18512, 1 male c&s, Brazil, Amazonas, lago Pirapora, Catalão. MZUSP 77844, 2 c&s, 36.9-40.0 mm SL, rio Pastaza, Pastaza, Ecuador. ***Odontostilbe pulchra**:* INHS 40101, 2 c&s (1 male, 1 female) of 20 m. Trinidad, Cumuto River, 5 km S Brazil on the road to Talparo. INHS 40081, 4m (2 females 32.6-33.3 mm SL, 2 unsexed 26.3-29.9 mm SL), Quare River, 1km E Valencia on road to Arima. ***Odontostilbe naeruda*:** MZUSP 87759, (1 female 35.3 mm SL), Calama, M. Goulding, 2 Feb 1981. FMNH 106433, 1 male c&s of 30, Bolivia, Pando, rio Madeira basin, creek at right margin of rio Nareuda, ca. 3-4 km upstream of the mouth of rio Tahuamanu. ***Odontostilbe dierythrura***: MCP 38624, 2 c&s (1 male, 1 female) of 7, Bolívia, Cochabamba, rio Madeira, rio Ichilo basin, rio Samusabety, system Isiboro-Mamoré-Madeira. ***Odontostilbe euspilurus***: ANSP 143702, 2 c&s (1 male, 1 female) of 8, Peru, Cuzco/Madre de Dios, mouth of rio Carbon, below Atalaya on N/S road, above and below ford. MCP 38420, 13 ex, Distributary of Rio Payamino, few Km upstream from San Jose de Payamino Napo, Equador. ***Odontostilbe pequira***: UFRGS 8641, 14 ex, Rio Uruguai, junto a um clube, Uruguaiana, Rio Grande do Sul, Brazil. UFRGS 5589, 5 ex, arroio do Salso, Uruguaiana, Rio Grande do Sul, Brazil UFRGS 13365, 22 ex, Afluente da margem esquerda do rio Soberbo, rodovia para o lago do Manso, Cuiabá, Mato

Grosso, Brazil. UFRGS 13006, 99 ex, ribeirão Esmerial, Br-163. Mato Grosso, Brazil. MZUSP 21067, 1 male, 1 unsexed c&s of 53, Brazil, Paraná, rio Paraná below Sete Quedas, CETESB.

Acknowledgments

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Table 1. Morphometric data for *Odontostilbe* sp. n. B. Males range, females range, unsexed range, number of individuals (N), mean, min, max, and standard deviation (SD) include values of the holotype (male) MCP 12111.

	Holotype	Females					Males				
	MCP 12111	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD
Standard length (mm)	31.5	19	17.63	26.59	24.37	-	12	22.6	25.38	23.98	-
Percents of Standard Length											
Snout to anal-fin origin	62.4	19	62.6	65.7	64.2	1.14	12	60.8	64.2	62.5	1.11
Snout to dorsal-fin origin	47.8	19	47.6	51.5	49.8	1.08	12	46.0	50.4	48.1	1.41
Snout to pelvic-fin origin	44.9	19	43.8	47.5	45.9	1.09	12	42.9	47.1	45.2	1.16
Snout to pectoral-fin origin	25.7	19	24.1	28.8	26.1	1.32	12	23.4	26.8	25.2	1.19
Dorsal to caudal-fin origin	51.8	19	47.6	52.9	50.3	1.56	12	48.8	54.8	51.8	1.52
Orbit to dorsal-fin origin	31.8	19	32.5	37.0	35.2	1.33	12	31.2	35.3	32.2	1.23
Anal fin base length	24.8	19	20.6	24.7	23.0	1.17	12	19.4	25.9	23.3	1.95
Peduncle length	14.7	19	11.6	15.3	13.3	0.97	12	11.5	16.8	15.1	1.43
Peduncle depth	13.6	19	10.9	12.6	11.9	0.51	12	12.1	14.4	13.0	0.60
Body depth at dorsal fin	32.2	19	31.7	37.4	34.1	1.60	12	30.5	34.4	32.3	1.15
Dorsal fin length	34.6	19	25.4	31.3	29.3	1.33	12	28.3	36.9	31.9	2.66
Pelvic fin length	30.3	19	17.2	20.4	18.6	0.94	12	18.4	30.3	22.6	4.77
Pectoral fin length	26.2	19	19.6	25.8	21.7	1.59	12	23.2	26.2	24.7	1.10
Head length	25.8	19	25.8	30.0	28.0	1.15	12	25.7	28.8	27.2	0.92
Percents of Head Length											
Snout length	23.6	19	21.4	26.8	23.8	1.47	12	23.0	27.0	24.7	1.31
Upper Jaw length	28.4	19	26.1	33.9	29.8	2.30	12	28.2	34.1	30.3	1.89
Horizontal orbit diameter	36.0	19	33.1	40.6	36.7	2.25	12	35.5	39.2	36.8	1.07
Interorbital width	35.4	19	29.6	36.6	33.1	2.20	12	31.6	36.8	34.9	1.46

Table 2. Morphometric data for *Odontostilbe paraguayensis.* Males range, females range, unsexed range, number of individuals (N), mean, min, max, and standard deviation (SD) include values of the paratype CAS 59786 and SL of holotype CAS 59785.

	Holotype CAS 59785	Paratype CAS 59786	Females					Males				
			N	Min	Max	Mean	SD	N	Min	Max	Mean	SD
Standard length (mm)	30.5	31.5	33	25.8	37.56	30.85	-	12	29.3	33.87	31.67	-
Percents of Standard Length												
Snout to anal-fin origin		66.29	29	63.2	68.7	65.7	1.40	11	61.7	66.2	64.1	1.36
Snout to dorsal-fin origin		46.70	29	44.1	49.4	46.8	1.28	11	44.8	49.4	46.8	1.63
Snout to pelvic-fin origin		47.30	29	44.8	48.9	46.9	0.93	11	44.8	48.0	46.3	1.01
Snout to pectoral-fin origin		22.89	29	19.8	26.1	23.1	1.40	11	23.4	25.9	24.5	0.97
Dorsal to caudal-fin origin		54.89	29	50.1	55.0	52.4	1.51	11	49.5	55.2	52.1	1.64
Orbit to dorsal-fin origin		32.10	29	29.5	35.5	32.1	1.63	11	28.5	33.2	29.9	1.44
Anal fin base length		24.41	29	23.1	26.5	24.4	0.93	11	23.8	26.7	25.7	0.87
Peduncle length		14.29	29	9.7	14.3	11.0	0.95	11	10.1	12.4	11.5	0.70
Peduncle depth		10.79	29	9.9	12.3	11.0	0.57	11	10.3	11.6	10.8	0.43
Body depth at dorsal fin		34.00	29	30.1	39.1	34.8	2.60	11	29.1	35.1	31.7	1.81
Dorsal fin length			29	23.6	30.4	27.7	1.68	11	29.6	35.0	32.9	1.64
Pelvic fin length		15.56	29	12.7	18.1	15.5	1.14	11	16.5	23.6	20.7	1.94
Pectoral fin length			29	17.1	23.3	20.5	1.28	11	19.2	25.5	23.4	1.74
Head length		25.08	29	21.1	28.1	24.4	1.48	11	23.6	26.9	25.3	0.96
Percents of Head Length												
Snout length			29	19.1	26.4	22.3	1.58	11	23.1	29.0	25.5	1.81
Upper Jaw length			29	25.3	36.8	30.1	2.68	11	27.9	33.9	30.2	1.60
Horizontal orbit diameter		32.91	29	32.2	41.5	36.9	2.47	11	34.7	39.2	36.4	1.36
Interorbital width		34.18	29	31.2	39.5	34.8	1.95	11	34.1	39.5	36.4	1.52

Capitulo 3

Two new species of *Odontostilbe* Cope (Characiformes: Characidae: Cheirodontinae) from the rio Uruguay basin, a probable allopathic speciation

(Manuscrito a ser submetido para o periódico Zootaxa)

Two new species of *Odontostilbe* Cope (Characiformes: Characidae: Cheirodontinae) from the rio Uruguay basin, a probable allopathic speciation

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Abstract

Species of the genus *Odontostilbe* from the rio Uruguay basin is revised and two new specie described. The subterminal mouth and shape of the supraneurals, diagnoses this two new species. These two species are distributed in two different regions in the rio Uruguay basin, limited by the Salto do Yucumã. The species of the lower part of the rio Uruguay differs from the upper species because it has an incomplete lateral line (*vs*. complete lateral lineal) and a caudal spot that extends covering the middle rays of the caudal fin (*vs*. spot flow that concentrates in the caudal peduncle, reaching the base of the central rays of the caudal fin). The importance of the Salto do Yucumã as a geographic barrier, which influences the presence of endemic species in the upper part of the rio Uruguay and a probable cause of allopathic speciation, is highlighted.

Introduction

Accurate data on the geographical distribution of the fish in a region are necessary and prerequisite for understanding ecological interrelations between species. This information contributes to know the historical component of the region, raising hypotheses of centers of origin and establishment of patterns of diversity and endemism, allowing us to make inferences about the mechanisms of speciation in different fish species in continental aquatic environments. Bertaco *et al.* (2016) presents an updated inventory of the freshwater fish species of the rio Uruguay (partial) in Brazil, laguna dos Patos system (complete) and rio Tramandaí basin (complete), showing that of the 275 species known for the rio Uruguaybasin, 78 (28%) are endemic. A geographic barrier, the Salto do Yucumã, segments the rio Uruguay basin in two part, upper and lower; many authors have emphasized the importance of the upper rio Uruguay basin because

it has a high number of endemic species, one of the possible causes being the Salto do Yucumã (Carvalho & Reis 2009; Bertaco *et al*. 2016).

Miquelarena *et al.* (2008) makes a review of all species of Cheirodontinae of Mesopotamian Region in Argentina, including the species of *Odontostilbe* from the Rio da Plata, Paraná, Uruguay and Paraguay river basins. Currently the only species of *Odontostilbe* previously recorded from the lower rio Uruguay basin is *Odontostilbe pequira* (Steindachner) and *Odontostilbe* sp. n. (Bertaco *et al*. 2016). Herein we present the description of two new species of *Odontostilbe*, which are distributed to the upper and lower parts of the rio Uruguay basin.

Material and Methods

Counts and measurements follow Fink & Weitzman (1974), Chuctaya *et al.* (in press), unless otherwise specified. Precaudal, caudal and total vertebrae counts include the four vertebrae of the Weberian apparatus, and the terminal "half centrum" as outlined by Malabarba & Weitzman (1999). The gill raker at the junction of ceratobranchial and epibranchial is counted jointly with the gill rakers on the lower branch as in Bührnheim & Malabarba (2006). In the counting of the rays of anal fin, the last two rays are counted as a single element by being on the same pterygiophore. For the description of secondary sexual characteristics (presence of hooks in the fins) follow Chuctaya *et al*. (in press). Specimens were cleared and stained (c&s) according to Taylor & Van Dyke (1985), and were used for counting vertebrae, teeth, gill rakers, denticulation of gill rakers, supraneurals and proximal radios. Scanning Electron Microscope (SEM) images were obtained from teeth premaxilla of *Odontostilbe* sp. n. R. Pictures of supraneurals were taken from cleared and stained specimens with a Nikon AZ100M camera attached a stereomicroscope. Nomenclature follows Weitzman (1962) for bones. To standardize the description of fish color in live and alcohol preserved specimens follow Chuctaya *et al*. (in press).

The following institutions provided material for the study, ANSP – Academy of Natural Sciences, Philadelphia, USA; CAS – California Academy of Sciences, San Francisco, USA; FMNH – Field Museum of Natural History, Chicago, USA; MHNG – Muséum d'histoire naturelle, Genève,Geneva, Switzerland; INPA – Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil; INHS – Illinois Natural History survey, Illinois, USA; MCP – Museu de Ciências e Tecnologia, Porto Alegre, Brazil; MZUSP –

Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil; USNM – National Museum of Natural History, Washington D.C., USA.UMSS – Universidad Mayor de San Simon, Cochabamba, Bolivia; UFRGS – Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. ZVC-P – Facultad de Ciencias de Uruguay; MNHN– Museo Nacional de Historia Natural de Montevideo, Uruguay.

Statistical analyses. The standard length was not used as a variable and the remaining morphometric variables were standardized against standard length, as the ratio of every variable against the standard length and coded following Chuctaya *et al.* (in press).

The combined measurement data were examined with Principle Components Analysis (PCA) calculated in Past 3.x version 2016 (Hammer *et al*. 2001) to determine if distinct groups were identified. Defined groups were then analyzed with the algorithm of Variable selection to discriminate among taxonomic groups (VARSEDIG) (Guisande *et al*. 2016; Guisande 2016), used in the morphometric analysis of *Odontostilbe* species recently described by Chuctaya *et al.* (in press A, B). Algorithm for variable selection were following Guisande *et al*. (2016), Guisande (2016), Chuctaya *et al.* (in press A, B). Figures are obtained with VARSEDIG, and the variables that better discriminate between two compared species were selected, using a density plot, a scatterplot of the polar coordinates and two figures results of a bivariate randomization test. If when comparing group 1 with group 2 and group 2 with 1, the p-value is close to, or lower than, 0.05 for the X or Y polar coordinates, it is concluded that the selected variables are significantly contributing to discriminating between both species.

Quantitative variables were represented by graphical boxplot, a version of this type of graphic is to add an element, called "notch" representing approximate confidence intervals 95% for median: two medians are different (the groups are different) if intervals or notches corresponding boxes do not overlap.

All statistical analyses were completed using R (R Core Team 2016), with the exception of Principle Components Analysis (PCA) which were calculated in Past 3.x version 2016 (Hammer *et al*. 2001). The boxplot graph is performed using “sm” package. To use VARSEDIG is necessary to download the following packages: adehabitatHR, adehabitatHS, adehabitatLT and adehabitatMA packages (Calenge 2006); ade4 package (Chessel *et al*. 2004; Dray *et al*. 2007; Dray *et al.* 2015); sp package (Pebesma & Bivand 2005; Bivand *et al*. 2013); deldir package (Turner 2016);

CircStats package (Lund & Agostinelli 2012); MASS package (Venables & Ripley 2002); boot package (Davison & Hinkley 1997; Canty & Ripley 2016); kulife package (Ekstrom *et al*. 2013) and car package (Fox & Weisberg 2011). Geographic distribution map was performed in the Quantum GIS version 2.8 software (Quantum GIS Development Team 2016).

Results

Odontostilbe sp. n. R, new species

Figs. 1–**4**

Odontostilbe sp. n. –Bertaco *et al*. 2016: 414 [Inventory of the freshwater fishes from Rio Grande do Sul, Brazil, MCP 13312]

Holotype. MCP 12893, 46.28 mm SL, male, rio Canoas at Passo do Canoas, rio Uruguay basin, road (SC–458) between Tupitinga and Celso Ramos, Campos Novos, Santa Catarina, Brazil, 27.586390° S 51.402500° W, 21 Jan 1989, C. A. S. Lucena, L. A. Bergmann, P. Azevedo & E. H. L. Pereira.

Paratypes. Rio Grande do Sul State: UFRGS 9728, (1, 34.5 mm SL), rio Marmeleiro, afluent rio Uruguay, PCH-Ouro, Barracão, 27.636111° S 51.494722° W, 25 Feb 2008, G, Neves. MCP 33481, (3, 43.1–47.3 mm SL), rio Uruguay in Espigão Alto, Barracão, 27.606112° S 51.461113° W, 1 Jan 1988, E. Lerner, E. Pereira, L. Bergmann & P. Azevedo. MCP 13334, (5, 40.9 –54.0 mm SL), rio Uruguay in the confluence of the rivers Canoas and Pelotas, Barracão, 27.606112° S 51.461113° W, 18 Feb 1989, R. Reis, E. Pereira, L. Bergmann & P. Azevedo. MCP 41332, (14, 37.2–42.7 mm SL), rio Forquilha, road Maximiliano de Almeida –Machadinho, Camping Casa de Pedra, Machadinho, 27.613056° S 51.753333° W, 27 Mar 2007, C. A. Lucena, J. P. Silva, V. Bertaco &T. P. Carvalho. MCP 13312, (4 c&s, 44.9-50.4 mm SL), rio Ligeiro in road Marcelino Ramos / Maximiliano de Almeida (affluent of rio Uruguay), Marcelino Ramos, 27.633333° S 51.866665° W, 17 Feb 1989, R. Reis, E. Pereira, L. Bergmann & P. Azevedo. **Santa Catarina State:** MCP 12925, (5, 34.8–37.7 mm SL), rio do Peixe in Volta Grande (afluent of rio Uruguay), Concórdia, 27.466667° S 51.883335° W, 9 Dec 1988, R. Reis, E. Pereira, L. Bergmann, P. Azevedo. MCP 33483, (2, 47.8 –49.5 mm SL), rio Canoas, Passo do Canoas, Road Tupitinga/Celso Ramos, Tupitinga, 27.586390°S 51.402500° W, 25 May 1988, E. Pereira, L. Bergmann,

P. Azevedo & R. Reis. MCP 12975, (4, 36.2–37.6 mm SL), rio do Peixe in Volta Grande (affluent of rio Uruguay), Concórdia, 27.466667° S 51.883335° W, 9 Dec 1988, R. Reis, E. Pereira, L. Bergmann & P. Azevedo. MCP 13307, (23, 33.4–44.5 mm SL), rio do Peixe em Volta Grande (affluent of rio Uruguay), Concórdia, 27.466667° S 51.883335° W, 16 Feb 1989, R. Reis, E. Pereira, L. Bergmann & P. Azevedo. MCP 33482, (31.3–33.1 mm SL), rio do Peixe em Volta Grande (affluent of rio Uruguay, Piritiba, 27.466667° S 51.883335° W, 1 Jan 1988, R. Reis, E. Pereira, L. Bergmann & P. Azevedo.

Diagnosis. The following character distinguish *Odontostilbe* sp. n. R from all the other species of the genus: subterminal mouth (*vs*. mouth terminal in all congeners, except in *Odontostilbe euspilurus*, *O. dierythrura*, *O. microcephala*, *Odontostilbe* sp. n. A., and *Odontostilbe* sp. n. U); gill rakers usually 6 on upper branch and 12 on lower branch (*vs*. 5 – 6 on upper branch and 9–10 on lower branch in *O. euspilurus*, usually 10–12 on upper branch and 14 – 16 on lower branch in *O microcephala*); horizontal orbit diameter 34.8 – 44.4% HL, average 40.1 % (*vs*. horizontal orbit diameter 24.6 – 32.8 % HL, average 28.6% in *O. microcephala*); 5 scales of lateral line-dorsal fin origin (*vs*. 6 in *O. dierythrura*); absence of mesopterygoid teeth (*vs*. presence of mesopterygoid teeth in *Odontostilbe* sp. n. A); additionaly from *Odontostilbe* sp. n. A by the presence of 18–23 anal-fin branched rays [mostly 20] (*vs.* 16–18 [mostly 17]) and from *Odontostilbe* sp. n. U by lateral line complete (*vs.* lateral line incomplete).

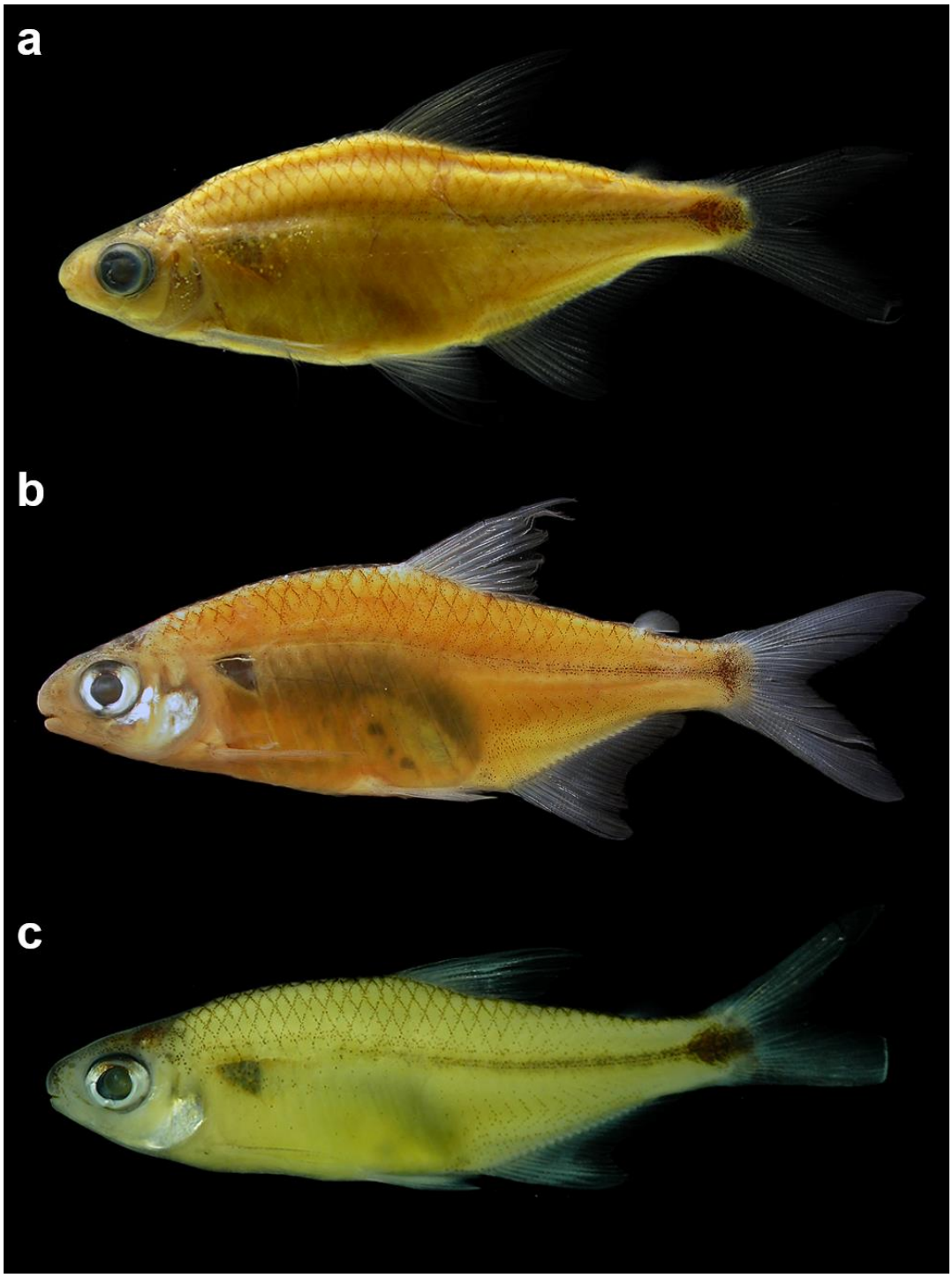

FIGURE 1. *Odontostilbe* sp. n. R: (a) MCP 12893, male, 46.28 mm SL, rio Canoas at Passo do Canoas, road (SC-458) between Tupitinga and Celso Ramos, Campos Novos, Uruguay River, Santa Catarina, Brazil; (b) paratype, MCP 13334, female, 48.3 mm SL, Uruguay River in the confluence of the rivers Canoas and Pelotas, Barracão. (c) Paratype, UFRGS 9728, male, 34.5 mm SL, Marmeleiro River, tributary river Uruguay, PCH-Ouro, Barracão.

Description. Morphometric data on Table 1. Body elongate and slightly compressed. Greatest body depth at the origin of the dorsal fin. Pre-dorsal convex profile, with a slight concavity in the base of the process supraoccipital. Body profile in the base of dorsal fin straight or slightly convex, and slightly convex from there to the origin of adipose fin. Body profile between the base adipose fin and the first rays of the caudal fin slightly concave. Slightly convex ventral profile of the snout to origin of pelvic fins with well-marked lateral angles, and straight or concave from there to the origin of the anal fin. Profile body along the base of the anal fin straight or slightly convex. Caudal peduncle ventral profile straight to slightly concave, slightly longer than deep (Fig. 1).

Small head relative to the body. Snout slightly elongated with premaxilla more elongated than the jaw. Subterminal or inferior mouth opening under his snout, mouth slit at horizontal near lower eye edge. Short maxilla, positioned obliquely at an angle of approximately 45 degrees relative to the axial axis of the body; posterior tip reaching the vertical line passing between the anterior margin of the eye and the pupil. Teeth pedunculated distally expanded, with similar shape and cusp number. Premaxilla with five teeth aligned in one single row, bearing nine or 11 small and regular cusp. Extremity of the medial teeth sharply arched, with the central cusp wider than the most lateral cusps. Ends of the lateral teeth markedly expanded to two or more times the base width of the tooth. Maxilla with three or four teeth bearing seven cusp, similar to the cusps of the premaxillary teeth. Dentary teeth nine gradually decreasing in size posteriorly; anterior six teeth, larger, bearing seven to nine cusps; followed posteriorly by teeth smaller with five to seven cusp. Central cusp large than lateral cusp (Fig. 2).

Dorsal fin rays ii,9–10 (exceptionally 10). First unbranched dorsal-fin ray about half-length of second unbranched dorsal-fin ray, following branched rays gradually decreasing in size posteriorly. In sexually mature males, the second unbranched dorsal fin ray is slightly elongate. Dorsal-fin origin on midlength of standard length. Dorsal fin with ten pterygiophore. The first dorsal fin pterygiophore support one supernumerary spine. Proximal radial of first pterygiophore of dorsal fin in contact with the neural spine of the 12^{th} or 13^{th} precaudal vertebrae. Medial radial absent from first to fourth pterygiophore and visible from the fifth to tenth pterygiophore. Proximal radial of first to eighth pterygiophore with lateral projections, absent from ninth to tenth.

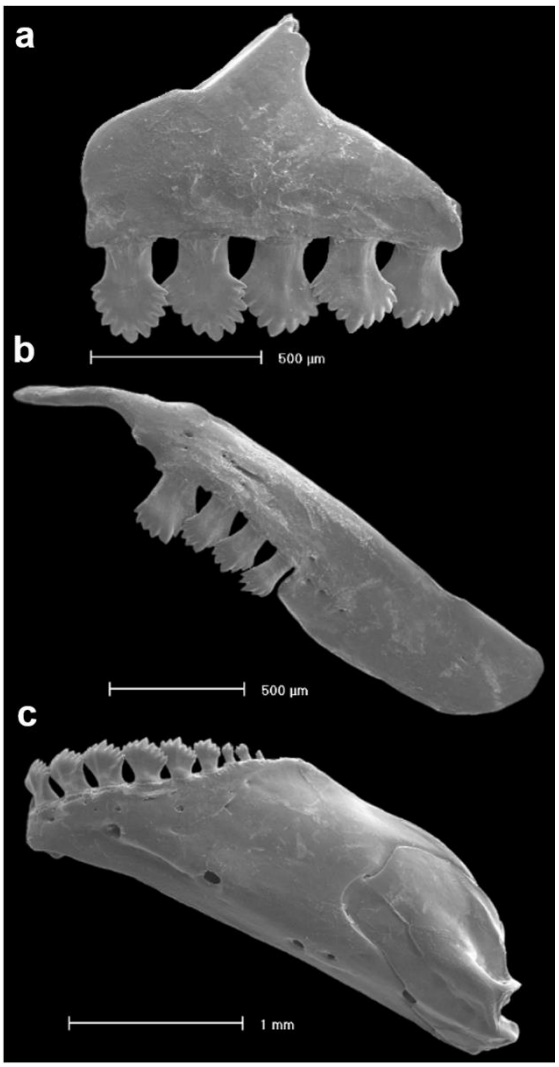

FIGURE 2. Dentition of *Odontostilbe* sp. n. R, paratype, MCP 13307: (a) left side premaxilla, (b) maxilla, and (c) dentary, lateral view. Scanning electron micrographs (SEM).

Adipose-fin origin posterior to vertical through base of last anal fin. Anal–fin ray ii–v, 18–23 with one row of scales on the base of the first ray anals Profile of distal margin of anal fin distinctively concave. Hooks typically present in the last unbranched ray and of six first rays branched in mature males. Anal fin 20–21 pterygiophores. Medial radial absent in the first to third or fourth pterygiophore and visible from there to the last pterygiophore. Proximal radial of first pterygiophore in contact with hemal spine of the first caudal vertebra.

Pectoral fin rays i, 9–12. Tip of extended pectoral fin does not reach the pelvic-fin origin. Pelvic-fin rays i,6,i. In sexual mature males, the unbranched pelvic fin ray elongate in form of filament, going beyond the insertion of the anal fin, with hook

present on all branched rays of pelvic fin. Principal caudal fin rays 19. Procurrent caudal fin rays: dorsal 8–12 rays and ventral with 7–11 rays.

Scales cycloids; scales in the lateral line 34(3), 35(16), 36(44), 37(16) and 38 (1). Pre-dorsal scales arranged in a regular series with 10–14 scales; scale rows between lateral line and dorsal line origin 5 (61) or 6 (5); scales rows between lateral line and pelvic fin origin 4; scales rows around caudal peduncle 14. Supraneurals 5–8 (mode 6) (Fig. 3).

Precaudal vertebrae 17–19 (mode 17); caudal vertebrae 17–18 (mode 18); total of vertebrae 34–37 (mode 35). Gill rakers upper 6, lower 12 (2 hypobranchial), with 7-9 denticles on basal portions of gill raker.

FIGURE 3. Supraneurals of *Odontostilbe* sp. n. R, c&s paratype, MCP 33482, presence of six supraneurals separated with lateral projections, in the dorsal part, lateral view.

Color in alcohol. Overall body coloration dark yellow. Dorsal surface of head from snout to posterior limit of frontal with chromatophores scattered giving a light brown coloration, concentrated chromatophores extends laterally to margin of orbit, with conspicuous black band covering the upper region of the iris. Dark coloration in region of parietal and supraoccipital. Opercular apparatus and infraorbital region of silver color extending to the cleithrum. Opercular apparatus with patches of golden color chromatophores. Region anterior ventral of pectoral fin with a clearer area. Ventral region between pectoral fin and pelvic fin with a clearer area. Humeral region with a darkened triangular area due muscular hiatus of pseudotympanum. Dark

pigmentation from supraoccipital to caudal peduncle, scales of dorsal portion of body with small dots dark brown to black concentrated in posterior margin. Scales posterior region of the lateral band with slightly scattered dots. Region pseudotympanum to caudal peduncle with a silvery lateral thin band, top edge of dark chromatophores forming a black band that becomes wider from the vertical projection of the last dorsal radius to the caudal peduncle. Scales of lateral portion of body under the lateral band between vertical projection of the last dorsal ray to the caudal peduncle with small dots dark brown to black concentrated in posterior borded. Scales basal region of anal fin with slightly scattered dots. A conspicuous rhomboid black caudal reaching the base of the middle caudal rays, usually reaching upper border of caudal peduncle.

Dorsal fin with dark dots scattered in all rays more concentrated at the first ray unbranched. Pectoral and pelvic fins with dark chromatophores scattered principally in the ray unbranched. Anal fin with dark dots scattered in all rays more concentrated in the basal region. Adipose fin hyaline. Caudal fin covered scattered dark chromatophores on part posterior of the central radios and along the edges of unbranched rays, spot of the caudal peduncle covering the basal portion of the central caudal fin rays.

Sexual dimorphism. Mature males with hooks small on the pelvic and anal fins. Pelvic fin ray with small hook along of all branched rays, with one pair bony retrorse hook per segment, on medioventral border of both lepidotrichia in median and distal portion of pelvic fin. Distal tips of bony hook not reaches proximal border of segment of lepidotrichia where inserted (Fig. 4).

Anal fin rays with one pair small of retrorse bony hooks per segment, inserted along of last unbranched ray to 6th branched rays, decreasing in number until disappearing. Bony hooks present principally on posterior branch in median part, never on its anterior branch, and on the first two segments of posterior branch in distal part. Distal tips of bony hook does not reach proximal border of segment of lepidotrichia where inserted. The mature males have the second unbranched ray of dorsal fin and the unbranched ray of pelvic fin slightly elongate.

Distribution. *Odontostilbe* sp. n. R is known to upper rio Uruguay basin, in the states of Rio Grande do Sul and Santa Catarina, South Brazil.

Etymology. Included in the article.

Ecological notes. Examination of the intestinal contents of two specimen of *Odontostilbe* sp. n. R (MCP 33482) reveals high detritus content.

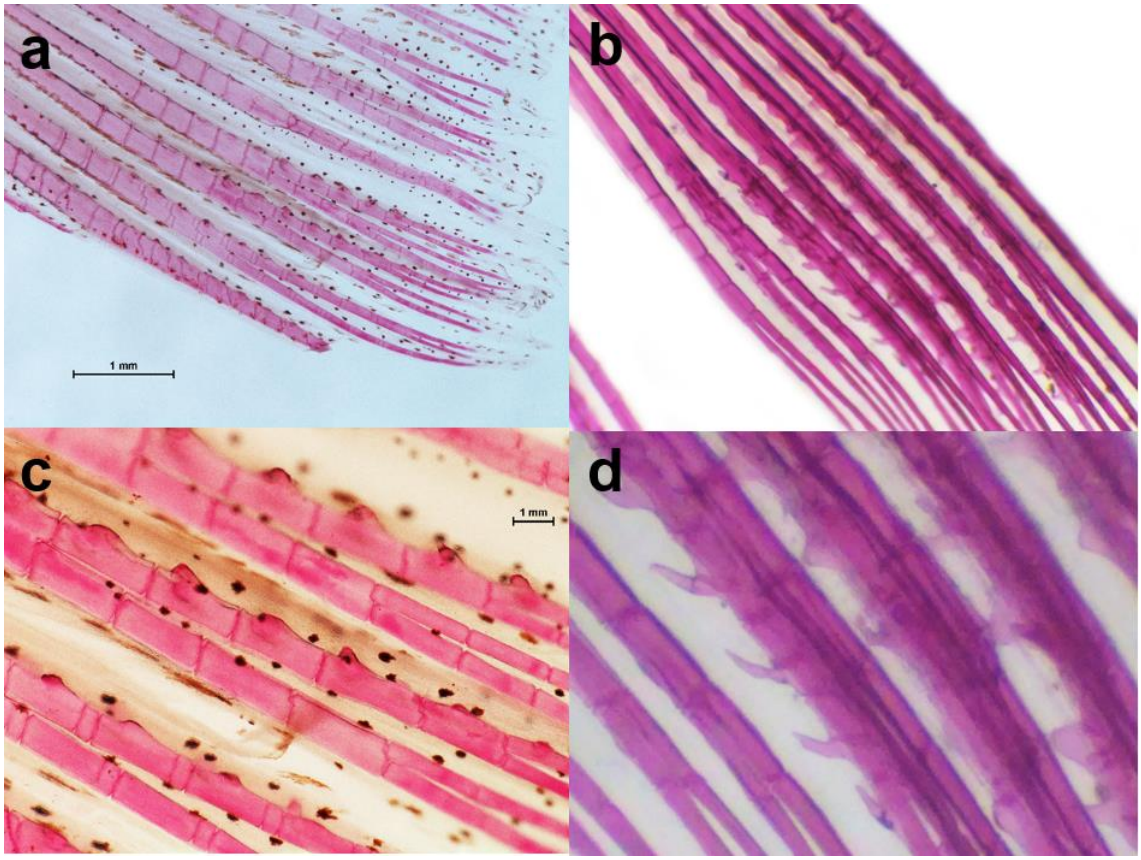

FIGURE 4. *Odontostilbe* sp. n. R, male (MCP 13334): (a) anal fin, (b) pelvic fin, (c) hooks in anal-fin rays, (d) hooks in pelvic-fin rays.

***Odontostilbe* sp. n. U, new species**

Figs. 5–8

Holotype. ZVC-P 13291, female, 46.5 mm SL, Rio Queguay, Paso del Sauce, 32.28207°S 56.75479°W, Departament Paysandu, Uruguay, 19 Dec 2014, A. Duarte, W. S, Serra, M. Loureiro.

Paratype. Brazil, Rio Grande do Sul State: MZUSP 37840, (1, 40.4 mm SL), rioPiratini, Coimbra Distric, Santo Ângelo, Rio Uruguay, 28.700001° S 54.416668° W, J.R. Stehmann. MCP 11136, (1, 37.0 mm SL), rio Piratini in fazenda dos Hinz, Coimbra Distric (affluent of rio Uruguay), Santo Ângelo, 28.700001° S 54.416668° W, 19 Dec 1985, L. R. Malabarba, R. E. Reis & S. B. Mallmann. MCP 11137, (2, 37.6–50.5 mm

SL), Rio Piratini in fazenda dos Hinz, Coimbra Distric (affluent of Rio Uruguay), Santo Ângelo, 28.700001° S 54.416668° W, 19 Dec 1985, L. R. Malabarba, R. E. Reis & S. B. Mallmann. UFRGS 21476, (2, 47.4–48.6 mm SL), Rio Piratini in fazenda dos Hinz, Coimbra Distric (affluent of Rio Uruguay), Santo Ângelo, 28.700001° S 54.416668° W, 19 Dec 1985, L. R. Malabarba, R. E. Reis & S. B. Mallmann. MZUSP 37841, (2, 46.7–50.0mm SL), Rio Piratini in fazenda dos Hinz, Coimbra Distric (affluent of Rio Uruguay), Santo Ângelo, 28.700001° S 54.416668° W, 19 Dec 1985, L. R. Malabarba, R. E. Reis & S.B. Mallmann. **Uruguay: Departament of Artigas:** ZVC-P 10688, (1, 39 mm SL), Rio Cuareim NW of Paso Ramos, Cuareim basin, 30.10592° S 56.81950° W, Feb 2006, M. Loureiro; F. Teixerira de Melo, I. Gonzales & F. Quintans. MHNM 3883, (2, 47.6–49.2 mmSL) Rio Cuareim al NW de Paso Ramos, Cuareim basin , 30.10592° S 56.81950° W, Feb 2006, M. Loureiro; F. Teixerira de Melo, I. Gonzales & F. Quintans. **Departament Paysandu**: ZVC-P 13291, (5, 45.8–48.8 mm SL) Rio Queguay, Paso del Sauce, Queguay, 32.28207° S 56.7547919° W, Dec 2014, A. Duarte, W.S, Serra, M. Loureiro. MHNM 3884, (4, 36.7–38.7 mmSL) Rio Queguay, Paso del Sauce, Queguay, 32.28207° S 56.75479° W, 19 Dec 2014, A. Duarte, W.S, Serra, M. Loureiro.

Diagnosis. *Odontostilbe* sp. n. U differs from its congeners, except of *Odontostilbe* sp. n. B by presenting lateral line incomplete (*vs.* lateral complete). *Odontostilbe* sp. n. U is distinguished from *Odontostilbe* sp. n. B by the absence of mesopterygoid teeth (vs. present); by the presence of hook on 1st to 6th anal fin branched rays of males (*vs.* hook on 1st to 15nd anal fin branched rays of males). *Odontostilbe* sp. n. U is distinguished from all species of the genus, except *O. microcephala*, *Odontostilbe* sp. n. A, *O. dierythrura*, *O. euspilurus* and *Odontostilbe* sp. n. R by subterminal mouth (vs. terminal mouth). Additionally *Odontostilbe* sp. n. U differs of *O. microcephala* by horizontal orbit diameter 32.2–41.0 % HL [mean 35.7) (*vs.* 24.6–32.8 % HL [mean 28.7]); from *Odontostilbe* sp. n. A by the absence of mesopterygoid teeth (*vs.* present), by snout–dorsal fin distance 61.2–66.7 % SL [mean 63.7] (*vs.* 66.9–71.6 % SL [mean 68.3]); from *O. dierythrura* by the number of scales of lateral line–dorsal fin origin 5 (*vs.* 6); from *O. euspilurus* by gill rakers upper 8, lower 14 (*vs.* 5–6 on upper branch and 9–10 on lower branch); from *Odontostilbe* sp. n. R by presence of spot in the caudal peduncle that extends to the central rays of the caudal fin (*vs.* spot concentrated in the caudal peduncle).

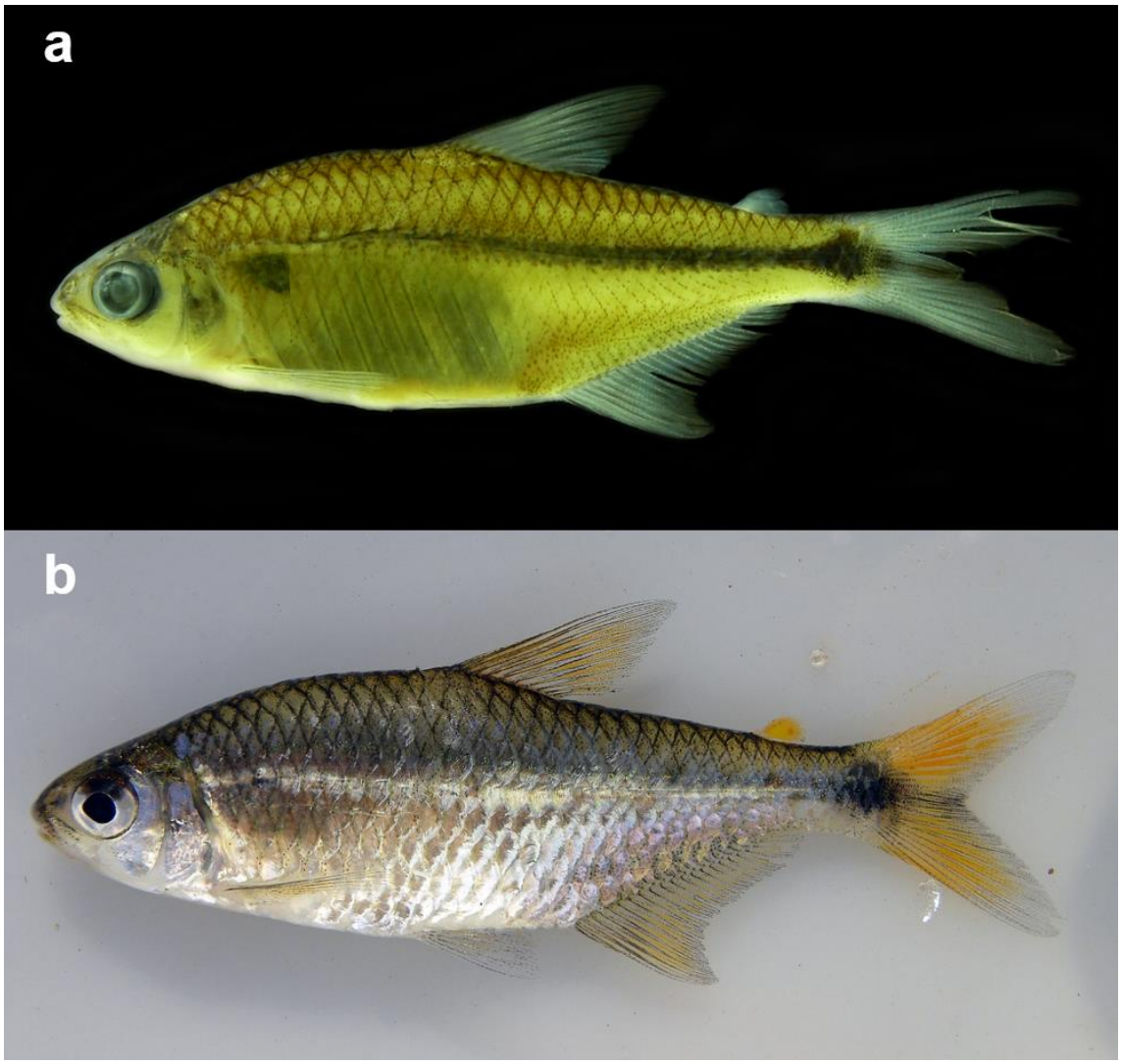

FIGURE 5. *Odontostilbe* sp. n. U: (a) holotype, ZVC-P 13291, female, 46.5 mm SL; and (b) paratype, ZVC-P 13291, female, rio Queguay , Paso del Sauce, Departament Paysandu, Uruguay.

Description. Morphometric data on Table 2. Body elongate and slightly compressed. Greatest body depth at vertical through dorsal-fin origin. Snout elongated and round. Dorsal profile of head convex from snout to posterior margin of frontal, slightly concave from there to distal tip of supraoccipital bone. Predorsal profile slightly convex from posterior end of supraoccipital to dorsal fin origin. Dorsal-fin origin located at midlength of SL. Body profile in base of dorsal fin straight and slightly convex from there to origin of adipose fin. Body profile between adipose-fin base and dorsal

procurrent caudal-fin rays slightly concave. Ventral profile of head slightly convex from snout to origin of pelvic fins and convex from there to origin of anal fin. Body profile along base of anal fin straight or slightly convex. Ventral profile of caudal peduncle slightly concave. Caudal peduncle slightly longer than deep (Fig. 5).

Head small relative to body length, 23.4–26.7% SL, mean 24.7%. Mouth subterminal, opening oriented forward and somewhat ventrally, in which upper jaw and snout clearly extend beyond the lower jaw. Maxilla posteroventrally angled, posterior end surpassing vertical through anterior border of eye and surpassing horizontal through ventral border of eye. Three to four maxillary teeth, with 7–9 [mostly 9] cusps nearly of equal size. Premaxilla with one single row; with 5–6 teeth slightly inclined to inside mouth, bearing 9–11 [mostly 11] cusps nearly of equal size. Dentary with nine teeth, anterior seven teeth larger, bearing seven cusps nearly of equal size, followed posteriorly by one or two smaller teeth with five and three cups. Dentary teeth inclined anteriorly (Fig. 6). Absence of mesopterygoid teeth.

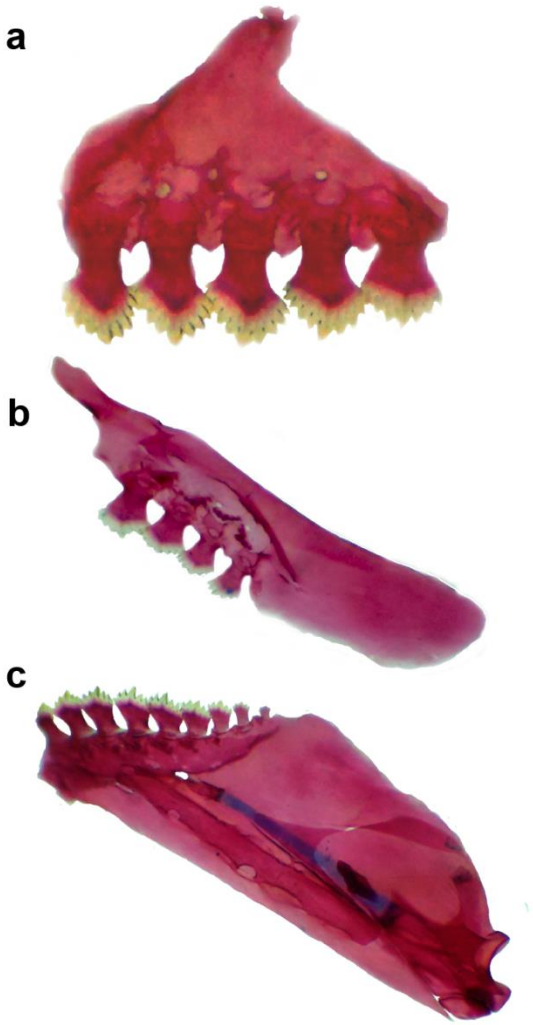

FIGURE 6. Dentition of *Odontostilbe* sp. n. U, ZVC-P 10688, male, 37.6 mm SL, paratype: (a) premaxilla, (b) maxilla, and (c) dentary, left side, lateral view. Specimens cleared and stained.

Dorsal-fin rays ii, 9(31) in all examined specimens. Dorsal-fin origin in vertical at pelvic-fin insertion. Profile of distal margin of dorsal fin slightly concave. First unbranched dorsal-fin ray less that half-length of second, following branched rays gradually decreasing in size posteriorly. First unbranched ray of dorsal-fin inserted in first pterygiophore and last two branched rays inserted in tenth pterygiophore. Proximal radial of first pterygiophore in contact with neural spine of 11^{th} or 12^{th} precaudal vertebrae. Dorsal fin with medial radial absent from first to sixth pterygiophore and visible from seventh to tenth pterygiophore. Proximal radial of first to ninth pterygiophores with lateral projections.

Adipose-fin origin posterior to vertical through base of the last anal-fin ray. Unbranched anal-fin rays ii (1), iii (9), iv(20) or v(1); branched anal-fin rays 18(1), 19(11), 20(7), 21(6) or 22(2) with one rows of scales covering base of anterior five branched rays. Anal-fin origin posterior of vertical through base of last ray of dorsal fin. Profile of distal margin of anal fin concave. Anal fin with 20 or 21 pterygiophores, anterior three unbranched rays associated with first pterygiophore, last unbranched ray associated with second pterygiophore. Medial radial absent from first to fifth or sixth pterygiophores, visible from that point to last pterygiophore. Proximal radial of first pterygiophore in contact with hemal spine of first caudal vertebra.

Pectoral fin rays i(31), 10 (1), 11(1), 12(14) or 13(13). First pectoral-fin ray reaching the insertion of pelvic fin. Pelvic fin i,7(31) rays; insertion located anterior of vertical of origin of dorsal fin. In mature males, first unbranched ray prolonged in filament, usually surpassing origin of anal fin. Principal caudal fin rays 19(31). Procurrent caudal fin rays: dorsal 8(7), 9(8), 10(6), 11(9) or 12(1) rays and ventral with 8(6), 9(10), 10(11) or 11(4) rays.

Scales cycloids; pored scales on lateral line incomplete with 8 (1), 9 (4), 10(16), 11(7), 12(3), 13 (11), 14 (3), 15(3), 16(2), 17(2), 22(1), 23(2), 24(2), 27(1) or 29(1). Pre-dorsal scales arranged in a regular series with 11(16), 12 (34), 13(2) or 14 (1) scales; scale rows between lateral line and dorsal line origin 5(59); scales rows between lateral line and pelvic fin origin 4(59); scales rows around caudal peduncle 14(59). Supraneurals 5(2) (Fig. 8b).

Precaudal vertebrae 16 or 17; caudal vertebrae 18 or19; total of vertebrae 35. Gill rakers upper 8, lower 14 (3 on hypobranchial). Upper gill rakers with 3–4 denticles on anterolateral border, and 0–1 denticles on posterolateral border. Lower gill rakers with 3–5 denticles on anterolateral border, and 0–1 denticles on posterolateral border. Gill raker inserted on junction of ceratobranchial and epibranchial with 3 denticles on ventrolateral and 2 on dorsolateral borders.

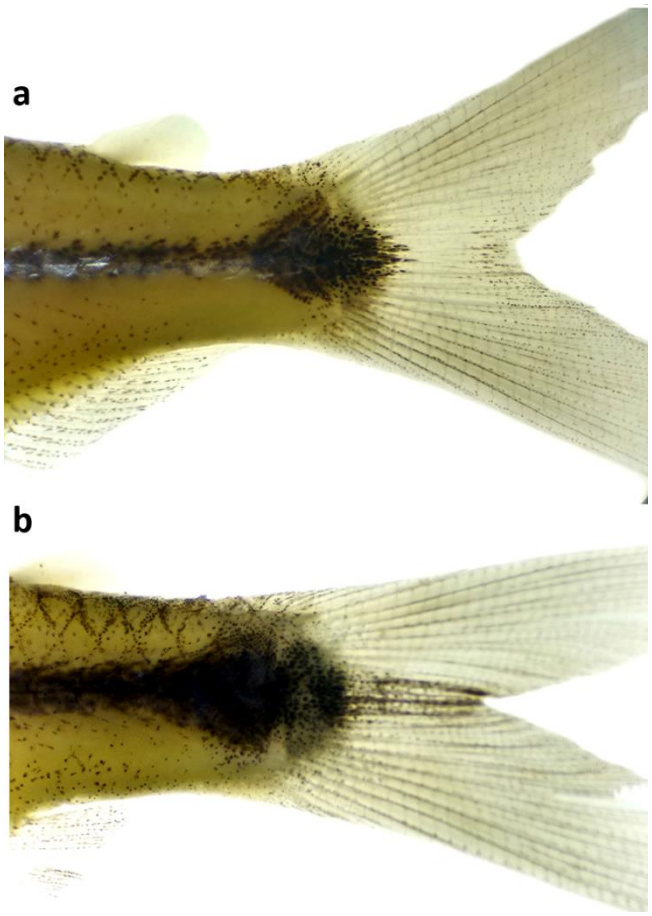

FIGURE 7. (a) *Odontostilbe* sp. n. R, MCP 41332, paratype, rhomboid spot concentrated in the caudal peduncle. (b) *Odontostilbe* sp. n. U, ZVC-P 13291, rhomboidal stain of the caudal peduncle extending to the central rays of the caudal fin.

Color in alcohol. Overall body coloration goldenrod (shade 3). Dorsal surface of head from snout to supraoccipital with dim gray coloration. Opercular apparatus, infraorbital region and branchiostegal of light yellow coloration. Ventral region between pectoral and pelvic fins with dim gray color. Humeral region with a darkened triangular area due to muscular hiatus of pseudotympanum. Scales of dorsal portion of body from supraoccipital to caudal peduncle with small dots dim gray color concentrated in posterior margin. Region posterior of pseudotympanum to caudal peduncle with black lateral band reaching the caudal spot. A conspicuous oval horizontally black caudal spot that reaching middle caudal fin rays (Fig. 7b). Dorsal, pectoral and pelvic fins with scattered black chromatophores slightly darker and more numerous, concentrated in tissue that joins rays. Adipose fin unpigmented.

Color in life. Caudal spot black, not reaching upper and lower margin of caudal peduncle and extending for the central rays of the caudal fin. Lobes of the caudal fin orange and hyalina the tips of the lobules. Medial part of the dorsal and anal fins of orange colors with chromatophores dispersed black color. Lateral band silvery, extending from pseudotympanum to caudal spot. Silver body, with golden chromophores dispersed in the scales of the dorsal region and dark chromatophores concentrated in the scales edges of the dorsal region. Presence of dark chromatophores in the part dorsal of head, extending laterally to margin of orbit, with conspicuous black band covering the upper region of the iris (Fig. 1b).

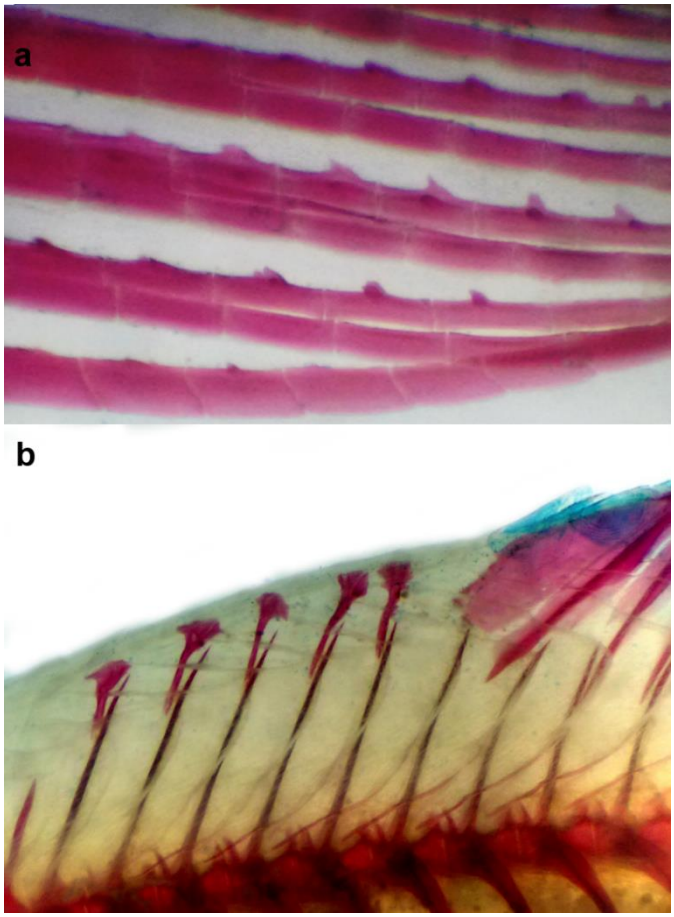

FIGURE 8. *Odontostilbe* sp. n. U, ZVC-P 10688, male, 37.6 mm SL, c&s paratype: (a) hooks in pelvic-fin rays, (b) presence of five supraneurals separated, with lateral projections in the dorsal part, lateral view.

Sexual dimorphism. Mature males with small hooks on pelvic and anal fins (Fig. 8a). Pelvic-fin rays with small hooks in all branched rays, with one retrorse bony hook per segment on medioventral border of both lepidotrichia in median and distal portions of pelvic fin. Tip of bony hooks not reaching proximal border of segment of lepidotrichia where inserted. Anal-fin rays with one pair of small retrorse bony hooks per segment symmetrically arranged (Fig. 8), inserted along of last unbranched ray to sixth branched rays, decreasing in number from first ray until disappearing. Hooks mostly distributed along middle third of anal ray length in posterolateral border. Distal tip of bony hooks not reaching proximal border of segment of lepidotrichia where inserted.

Distribution. *Odontostilbe* sp. n. U is known to inhabit tributaries of the lower rio Uruguay basin, between Brazil and Uruguay (Fig. 9).

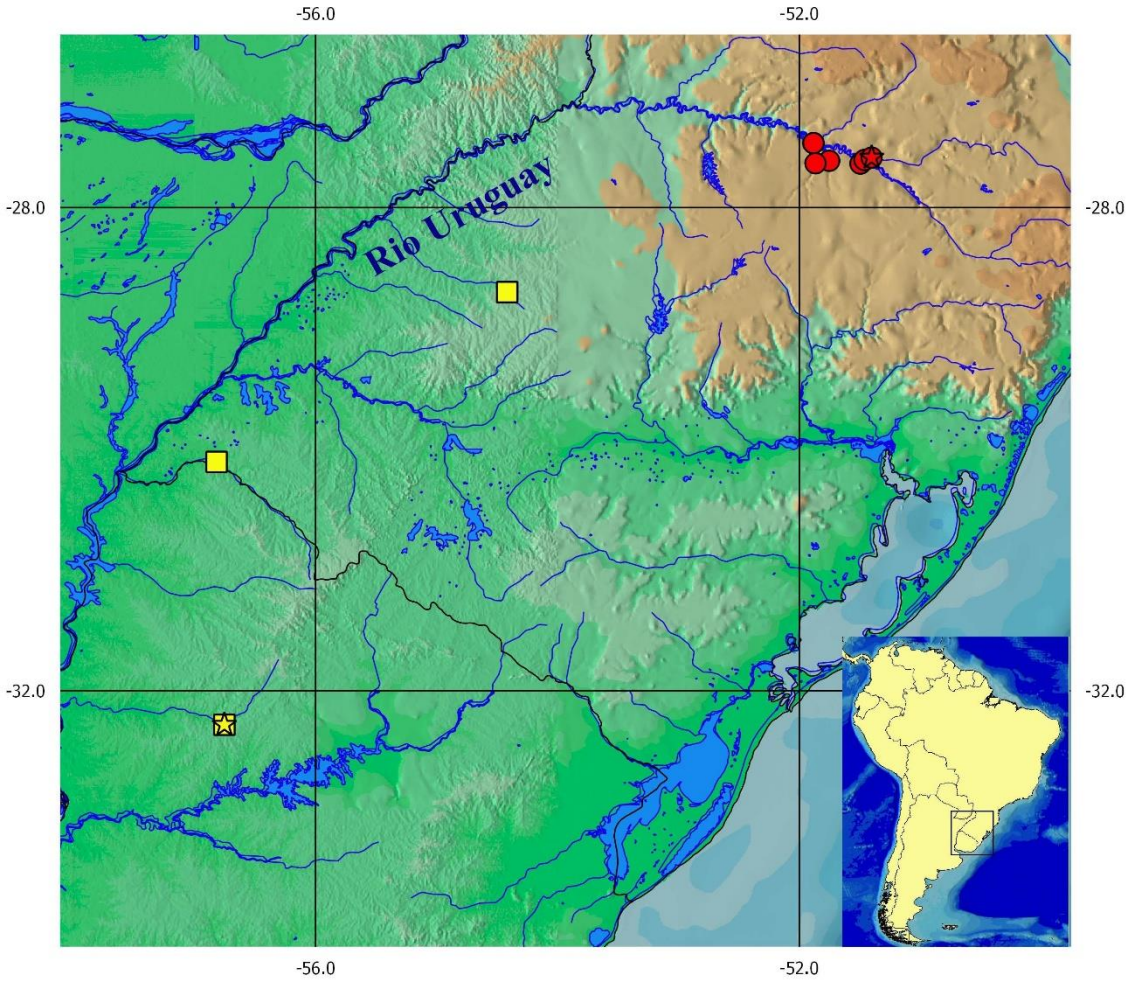

FIGURE 9. Southern South America showing the distribution of *Odontostilbe* sp. n. R (circles) and *Odontostilbe* sp. n. U (squares). Star: type locality.

Etymology. Included in the article.

Morphometric comparisons.

Results of the PCA of morphometric data (Fig. 10) are congruent with the morphological analysis in identifying two species, *Odontostilbe* sp. n. R and *Odontostilbe* sp. n. U. Although overlapping, boxplot graphic lateral line scale counts shows the differences this species (Fig. 11).

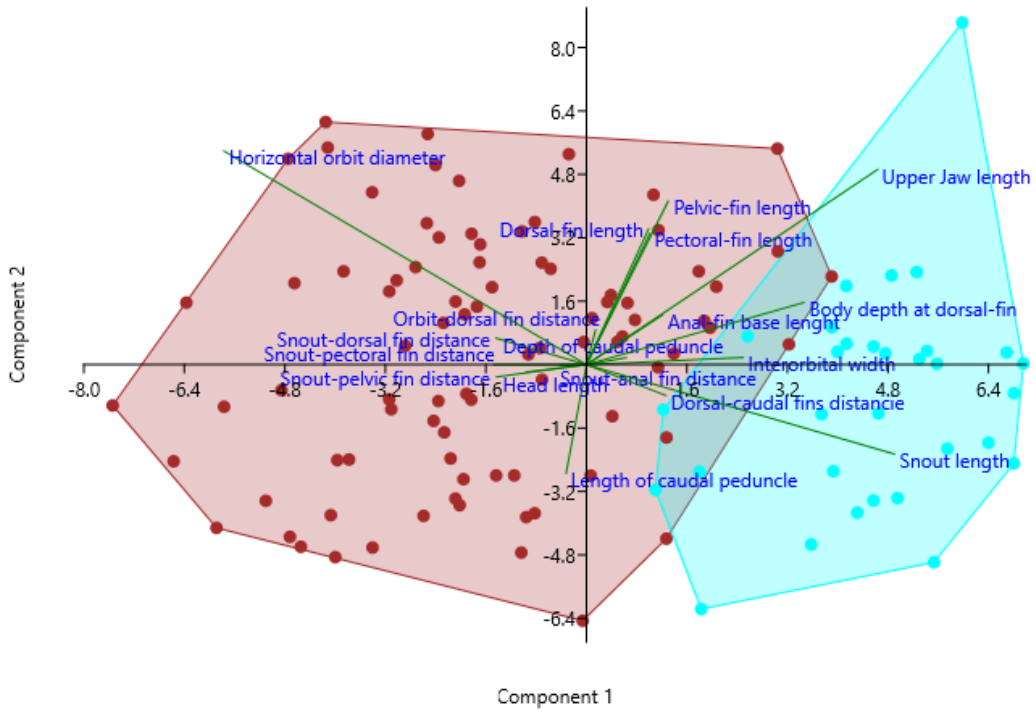

FIGURE 10. Principle Components Analysis (PCA) between the species *Odontostilbe* sp. n. R (red) and *Odontostilbe* sp. n. U (blue).

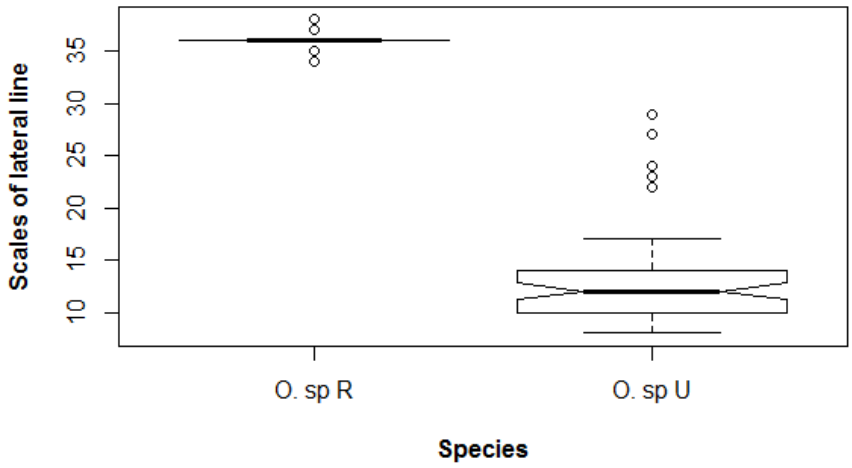

FIGURE 11. Box-plots between *Odontostilbe* sp. n. R and *Odontostilbe* sp. n. U of lateral line series of scales.

Testing the morphometric differences between *Odontostilbe* sp. n. R and *Odontostilbe* sp. n. U (Fig. 12), the variable that best discriminate the two species is M17 (horizontal orbit diameter), presenting slightly overlap between both species and showing a high discrimination capacity (Fig. 12a). Other variable with high discrimination capacity is M15 (snout length). The scatterplot of polar coordinates obtained for both species using M17 and M15 (Fig. 12b) shows the differences between *Odontostilbe* sp. n. R and *Odontostilbe* sp. n. U. The bivariate randomization test not separates the individuals of the two species. The individual of *Odontostilbe* sp. n. U (Fig. 12c, red point) with the higher probability of belonging to *Odontostilbe* sp. n. R, and the individual of *Odontostilbe* sp. n. R (Fig. 12d, red point) with higher probability of belonging to *Odontostilbe* sp. n. U, presented $p > 0.05$. Consequently, the polar coordinates X and Y of some individuals for both species present similar body morphometry.

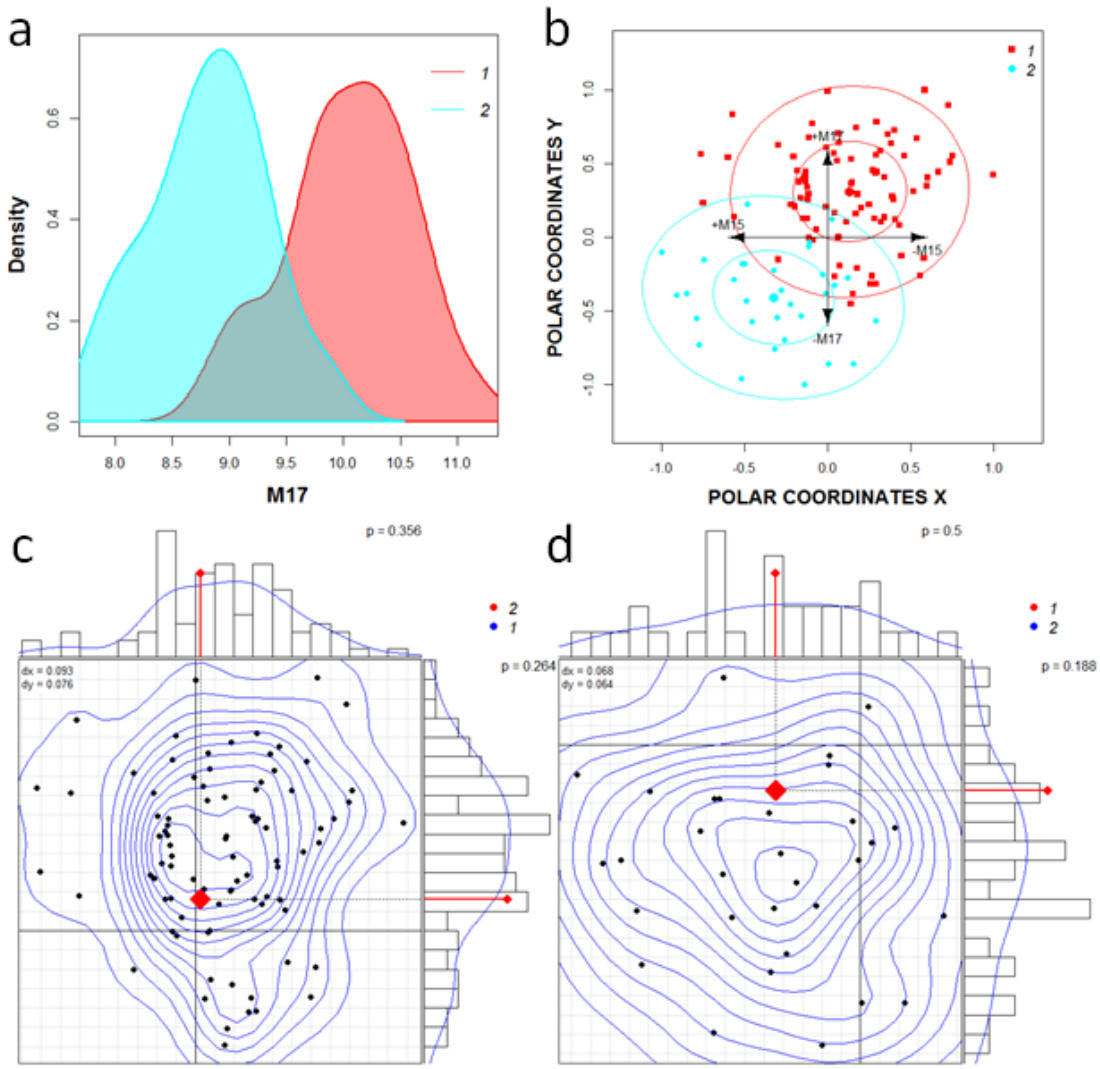

FIGURE 12. Morphometric comparison of *Odontostilbe* sp. n. R (1) and *Odontostilbe* sp. n. U (2). (a) Density plot with the overlap of the variable M17 (horizontal orbit diameter), that best discriminates the two species. (b) Scatterplot of the polar coordinates obtained for both species using variables M17 and M15 (snout length), the arrows show the vector of variables. (c) Bivariate randomization test, showing the individual (red point) with a higher probability of belonging to *Odontostilbe* sp. n. R among all included individuals identified as *Odontostilbe* sp. n. U (d) Bivariate randomization test, showing the individual (red point) with a higher probability of belonging to *Odontostilbe* sp. n. U among all individuals identified as *Odontostilbe* sp. n. R

Discussion

The recent work published on the genus *Odontostilbe* (Bührnheim & Malabarba 2006, 2007, Chuctaya *et al.* in press A, Chuctaya *et al.* in press B) shows us that much remains to be known within this genus. Cope (1978) defined *Odontostilbe* for the following characteristics: the teeth spatulate and crenate in a single series on the premaxilla and dentary, the anal fin elongate and the lateral line continued to the caudal fin. Malabarba (1998) defines two synapomorphies to diagnose the genus *Odontostilbe*. Length of the second branched ray of the dorsal fin and length of the first unbranched ray of the pelvic fin in males, which were corroborated in the rewriting of *Odontostilbe fugitiva* (Bührnheim & Malabarba 2006), *O. pulchra* (Bührnheim & Malabarba 2007), *O. microcephala* (Chuctaya *et al.* in press A) and *O. paraguayensis* (Chuctaya *et al.* in press B). In addition, they were the diagnostic characteristics for the description of new species, *O. ecuadorensis*; *O. nareuda*; *O. pao*; *O. parecis*, *O. splendida*, *Odontostilbe* sp. n. W, *Odontostilbe* sp. n. A. and *Odontostilbe* sp. n. B (Bührnheim & Malabarba 2006, 2007, Chuctaya *et al.* in press A, B). Although *Odontostilbe* sp. n. U, has lateral line incompletely pored, characteristic previously described for a species that is distributed in the upper Parana River, *Odontostilbe* sp. n. B. (Chuctaya *et al*, in press B). The inclusion of a species with incomplete lateral line within the genus *Odontostilbe* was previously discussed in Chuctaya *et al.* (in press B), but the incomplete lateral line also needs to be evaluated in a phylogenetic framework.

Odontostilbe sp. n. U differs from *Odontostilbe* sp. n. B by the following characteristics, subterminal mouth (*vs.* terminal mouth), absence of teeth in the mesopterygoid (*vs.* presence), hooks in the anal fin, present from the first to the sixth unbranched ray (*vs.* present on almost all unbranched ray). Within *Odontostilbe*, the species of *O. microcephala*, *O. euspilura*, *O. derytrhura* and *Odontostilbe* sp. n. A, share with the two new species one distinctive features, the presence of subterminal mouth, these character, however, are not restricted to these genus, subterminal mouth also occurs for example in *Heterocheirodon* (Malabarba & Bertaco 1999) and *Prodontocharax* (Eigenmann & Pearson 1924).

The two species new are distributed in the rio Uruguay basin, but they are not sympatric, and are easily distinguishable. *Odontostilbe* sp. n. R differs from *Odontostilbe* sp. n. U, due to the following characteristics, incomplete lateral line (*vs.*

complete lateral line), and a conspicuous rhomboid black caudal spot continued on the base of the middle caudal rays, (*vs.*, caudal spot extending to the central rays of the caudal fin). *Odontostilbe* sp. n. R was recorded for the upper rio Uruguay basin (upstream of Salto do Yucumã), while *Odontostilbe* sp. n. U is recorded for the lower part. This limitation of rio Uruguay was discussed by Bertaco *et al.* (2016), highlighting the importance of the upper rio Uruguay for harboring a representative number of endemic species. The difference in diversity of ichthyofauna of upper Uruguay River in relation to lower Uruguay River and basin Paraná / Paraguay Rivers would be related to the characteristic geomorphologic of the high part, type of substrate and speed of the water. Rapids and waterfalls (as Yucumã Falls) are dispersion barriers for the different species and probably cause of allopatric speciation during the drainage evolution process (Carvalho & Reis 2009). For different Orders, species have been registered that are only distributed in the upper Uruguay River, for example, in Cichliformes we have representatives of the genus: *Crenicichla* (Lucena & Kullander 1992), *Australoheros* (Rican & Kullander 2008) and *Gymnogeophagus* (Malabarba *et al.* 2015). In Siluriformes, the genus: *Hypostomus* (Reis *et al.* 1990), *Tatia* (koch & Reis 1996), *Microglanis* (Malabarba & Mahler 1998), *Pimelodus* (Vildal & Lucena, 1999); *Hemiancistrus* (Cardoso & Malabarba 1999); *Pogonopoma* (Quevedo & Reis 2002), *Pareiorhaphis* (Pereira & Reis 2002), *Rineloricaria* (Ghazzi 2008) and *Hisonotus* (Carvalho & Reis 2009). In Characiformes, representatives of *Oligosacus* (Menezes 1987); *Bryconamericus* (Malabarba & Malabarba 1994), *Characidium* (Buckup & Hahn, 2000), *Diapoma* (Menezes & Weitzman 2011) and currently *Odontostilbe.* This high diversity of endemic species, allows emphasizing to upper Uruguay River like priority zone of conservation.

Odontostilbe sp. n. R was recorded for the upper part of the Uruguay River basin (upstream of Salto do Yucumã), while *Odontostilbe* sp. n. U is recorded for the lower part. Bertaco *et al.* (2016) discusses this limitation of Uruguay River, highlighting the importance of the upper Uruguay River for harboring a representative number of endemic species.

Finally, for the rio Uruguay basin knows three species, *Odontostilbe pequira*, *Odontostilbe* sp. n. U and *Odontostilbe* sp. n. R. As no specimens found of *Odontostilbe* sp. n. R in the lower rio Uruguay, we limited the distribution of this species to upper rio Uruguay, being endemic for this region. The limits of *Odontostilbe* sp. n. U, are still

unknown, a greater number of collection is necessary to determine its limit in the rio de la Plata basin, and if they reach the same distribution currently known for *O. pequira*. It is necessary to emphasize that the diversity of *Odontostilbe* is currently underestimated, since it is a genus with a wide distribution, *Odontostilbe* may well prove to be one of the richest genera within the subfamily Cheirodontinae.

Comparative material examined. *Odontostilbe paraguayensis*: MCP 35618 (54, 28.5–33.9 mm SL), River Jauquara in Jauquara (afluente of river Dos Pássaros, rio Paraguay), Barra do Bugres, Paraguay River, 15.16667° S 57.08333° W, 10 Oct 1991, R.E. Reis, L.R. Malabarba & N.A. Menezes. MCP 37799, (31, 26.2–29.6 mm SL), Córrego dos Bagres, na estrada MT-120 entre a BR-174 e Jauru, ca. de 3 Km ao N da BR-174, Jauru. Paraguay, 15.63944° S 58.73500° W, 11 Jul 2004, R.E. Reis, P.A. Buckup, F. Lanjeani & E.H.L. Pereira. ***Odontostilbe* sp. n. B:** MCP 12111, 24.9 mm SL, male, marginal pools of the rio Corumbataí, rio Tietê basin, São Paulo, Brazil, 22.241489° S, 47.608446 W, 23 Fev 1963, H. A. Britski. MZUSP 42803 (20, 23.0–26.4 mm SL), Lagoa da Ponta Seca, Corumbatai. 22.233334° S, 47.616665° W, 20 Apr 1964, H.A. Britski & N.A. Menezes. ***Odontostilbe microcephala*:** MCP 38311, 4ex, 38.86–43.59 mm SL, Endorheic of Uruena River, drenaje of Bajo Paraná, Rosario de la Frontera, Salta, Bajo Paraná, Argentina, 25.00000 S 64.50000 W, 2 Mar 2001, Gladys Monasterio de Gonzo and Mario Mosqueira. USNM 321173, 5, (2 c&s) of 49ex, 29.66–44.02 mm SL, Camatindi River, 8 Km N Border Dept. Tarija, 40 Km N Villamontes, Dept Chuquisaca. Rio Paraguay, Bolivia, 20.99271 S 63.39922 W, 2 Oct 1988 W. Starnes, L. Starnes, J. Sarmiento & R. Vasquez. ***Odontostilbe* sp. n. A:** LIRP 3239, 48.7 mm SL, ribeirão da Batalha, Fazenda Batalha (Pedro Queresma), rio Paranaíba, Paracatu, Minas Gerais State, Brazil, 17.423611° S, 47.453056° W, 27 Apr 2002, C. A. A. Figueiredo & E. S. S. Rego. FMNH 57871 (1, 62.1 mm SL), rio Tietê at Salto Avanhandava below the falls, Penápolis, São Paulo, 21.167648° S, 50.117562° W, 14 Sep 1908, J. Haseman. ***Odontostilbe* sp. n. W**: MZUSP 16851, 38.9 mm SL, male, rio Mogi Guaçu, Emas, Pirassununga, São Paulo, Brazil, 21.916666° S 47.383335° W, 22 Oct 1963, H. A. Britski. MZUSP 87947, (11, 42.5–50.5 mm SL), Itirapina, córrego da Lapa near the mouth and along the seawall of rock and the bridge on the road, 22.249918° S, 47.863376° W, 31 Jan 2002, E. N. Fragoso. ***Odontostilbe fugitiva*:** ANSP 178908, 2 c&s (1 female, 1 male) of 12, Perú, Loreto, Maynas, [lower rio Itaya] at bridge on Iquitos-Nauta highway, approximately 25 miles SSW of Iquitos. INPA

18465, 4 c&s (2 males, 2 females) of 73, Brazil, Amazonas, Ilha da Marchantaria. INPA 18506, 3 c&s (1 male, 1 female, 1 unsexed) of 50. Brazil, Amazonas, Paraná do Xiborena. INPA 18512, 1 male c&s, Brazil, Amazonas, lago Pirapora, Catalão. MZUSP 77844, 2 c&s, 36.9–40.0 mm SL, rio Pastaza, Pastaza, Ecuador. ***Odontostilbe pulchra****:* INHS 40101, 2 c&s (1 male, 1 female) of 20m. Trinidad, Cumuto River, 5km S Brazil on the road to Talparo. INHS 40081, 4m (2 females 32.6–33.3 mm SL, 2 unsexed 26.3–29.9mm SL), Quare River, 1km E Valencia on road to Arima. ***Odontostilbe naeruda*****:** MZUSP 87759, (1 female 35.3 mm SL), Calama, M. Goulding, 2 Feb 1981. FMNH 106433, 1 male c&s of 30, Bolivia, Pando, rio Madeira basin, creek at right margin of rio Nareuda, *ca*. 34 km upstream of the mouth of rio Tahuamanu. ***Odontostilbe dierythrura***: MCP 38624, 2 c&s (1 male, 1 female) of 7, Bolívia, Cochabamba, rio Madeira, rio Ichilo basin, rio Samusabety, system Isiboro-Mamoré-Madeira. ***Odontostilbe euspilurus***: ANSP 143702, 2 c&s (1 male, 1 female) of 8, Peru, Cuzco/Madre de Dios, mouth of rio Carbon, below Atalaya on N/S road, above and below ford. MCP 38420, 13ex, Distributary of Rio Payamino, few Km upstream from San Jose de Payamino Napo, Equador. ***Odontostilbe pequira***: UFRGS 8641, 14 ex, Rio Uruguai, junto a um clube, Uruguaiana, Rio Grande do Sul, Brazil. UFRGS 5589, 5 ex, arroio do Salso, Uruguaiana, Rio Grande do Sul, Brazil UFRGS 13365, 22 ex, Afluente da margem esquerda do rio Soberbo, rodovia para o lago do Manso, Cuiabá, Mato Grosso, Brazil. UFRGS 13006, 99 ex, ribeirão Esmerial, Br-163. Mato Grosso, Brazil. MZUSP 21067, 1 male, 1 unsexed c&s of 53, Brazil, Paraná, rio Paraná below Sete Quedas, CETESB.

Acknowledgments

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Table 1. Morphometric data for *Odontostilbe* sp. n. R, number of individuals (N), mean, min, max, and standard deviation (SD) include values of holotype MCP 12893

	Holotype	Paratypes				
	MCP 12893	N	Min	Max	Mean	SD
Standard length (mm)	46.28	87	25.32	53.96	35.49	-
Percents of Standard Length						
Snout to anal-fin origin	29.01	87	60.2	68.3	64.0	1.57
Snout to dorsal-fin origin	23.67	87	48.0	54.5	51.3	1.47
Snout to pelvic-fin origin	20.83	87	43.9	50.1	47.3	1.37
Snout to pectoral-fin origin	10.54	87	22.5	28.4	24.9	1.35
Dorsal to caudal fins origin	22.59	87	44.9	52.0	48.3	1.67
Orbit to dorsal-fin origin	16.25	87	32.1	39.2	35.6	1.69
Anal-fin base lenght	12.43	87	21.3	29.0	25.6	1.38
Length of caudal peduncle	5.2	87	8.9	14.3	11.4	1.46
Depth of caudal peduncle	5.02	87	8.6	12.0	10.2	0.64
Body depth at dorsal-fin	15.48	87	27.5	35.7	31.3	1.64
Dorsal-fin length	14.18	87	25.0	31.4	27.9	1.39
Pelvic-fin length	9.4	87	14.2	21.0	17.5	1.74
Pectoral-fin length	10.73	87	18.1	24.8	21.4	1.53
Head length	11.07	87	23.3	27.1	25.1	0.95
Percents of Head Length						
Snout length	2.56	87	17.9	27.5	22.3	1.82
Upper Jaw length	4.04	87	27.7	38.1	32.7	2.26
Horizontal orbit diameter	4.53	87	34.8	44.4	40.1	1.98
Interorbital width	3.37	86	24.4	34.0	28.3	1.83

Table 2. Morphometric data for *Odontostilbe* sp. n. U, number of individuals (N), mean, min, max, and standard deviation (SD) include values of holotype ZVC-P 13291

	Holotype	Paratypes				
	ZVC-P 13291	N	Min	Max	Mean	SD
Standard length (mm)	46.53	59	33.67	50.72	40.47	-
Percents of Standard Length						
Snout to anal-fin origin	28.98	31	61.2	66.7	63.7	1.30
Snout to dorsal-fin origin	23.21	31	46.4	52.9	50.7	1.33
Snout to pelvic-fin origin	22.03	31	43.9	48.5	46.2	1.06
Snout to pectoral-fin origin	11.84	31	22.1	26.7	24.2	1.06
Dorsal to caudal fins origin	23.29	31	46.6	51.7	49.1	1.34
Orbit to dorsal-fin origin	16.56	31	34.0	38.0	36.2	0.90
Anal-fin base lenght	12.47	31	23.6	28.7	25.9	1.39
Length of caudal peduncle	4.71	31	10.1	13.5	11.8	1.03
Depth of caudal peduncle	4.81	31	9.6	11.2	10.5	0.46
Body depth at dorsal-fin	15.24	31	30.6	34.9	32.9	1.42
Dorsal-fin length	14	31	25.0	34.1	28.3	1.95
Pelvic-fin length	8.54	31	16.0	21.3	18.1	1.24
Pectoral-fin length	11.42	31	19.3	26.7	22.2	1.56
Head length	11.51	31	23.4	26.7	24.7	0.84
Percents of Head Length						
Snout length	2.9	31	21.5	27.9	25.3	1.81
Upper Jaw length	3.84	31	31.0	43.8	34.6	2.79
Horizontal orbit diameter	4.25	31	32.2	41.0	35.7	2.00
Interorbital width	3.46	31	27.1	31.3	29.5	1.15

Considerações finais

A revisão taxonômica do gênero *Odontostilbe* da bacia do rio da Prata, revelou a presença de cinco espécies novas: *Odontostilbe* sp. n. A, *Odontostilbe* sp. n. B e *Odontostilbe* sp. n. U, apresentam uma distribuição restrita ocorrendo somente na porção mais superior do rio Paraná; *Odontostilbe* sp. n. R e *Odontostilbe* sp. n. U apresentam uma distribuição para o rio Uruguai, sendo *Odontostilbe* sp. n. R. endêmica para a porção superior do rio Uruguai. Foram realizadas as redescrições de *Odontostilbe microcephala* e *O. paraguayensis*. *Odontostilbe microcephala* foi limitada para a vertente dos andes do rio Paraguai e parte inferior do rio Paraná; *Odontostilbe paraguayensis* apresenta uma ampla distribuição nos cursos inferiores do rio Paraná e na bacia do rio Paraguai. Se destaca a ocorrência de novos caracteres diagnósticos para as espécies de *Odontostilbe* como a presença de dentes no mesopterigoide em *Odontostilbe* sp. n. A, *Odontostilbe* sp. n. B e *Odontostilbe* sp. n. W; também foram descritas as primeiras espécies de *Odontostilbe* com linha lateral incompleta, *Odontostilbe* sp. n. B e *Odontostilbe* sp. n. U. Uma nova ferramenta que contribuiu favoravelmente para o analises do datos morfométricos é utilizada permitindo escolher quales são as medidas que melhor separam duas especies, VARSEDIG, um pacote do programa R. *Odontostilbe* é um gênero que apresenta uma ampla distribuição, com alto potencial de ser o mais?? diverso de Cheirodontinae. Este estudo apresentou informação base que ajudará em novos estudos taxonômicos do gênero em outras bacias e, posteriormente, em estudos filogenéticos.

ANEXO

Anexo 1

Normas para publicacao no periódico Zootaxa

Preparation of manuscripts

1) *General.* All papers must be in English. Authors whose native language is not English are encouraged to have their manuscripts read by a native English-speaking colleague before submission. Nomenclature must be in agreement with the *International Code of Zoological Nomenclature* (4th edition 1999), which came into force on 1 January 2000. Author(s) of species name must be provided when the scientific name of any animal species is first mentioned (the year of publication needs not be given; if you give it, then provide a full reference of this in the reference list). Authors of plant species names need not be given. Metric systems should be used. If possible, use the common font New Times Roman and use as little formatting as possible (use only **bold** and *italics* where necessary and indentions of paragraphs except the first). Special symbols (e.g. male or female sign) should be avoided because they are likely to be altered when files are read on different machines (Mac versus PC with different language systems). You can code them as m# and f#, which can be replaced during page setting. The style of each author is generally respected but they must follow the following general guidelines.

2) The **title** should be concise and informative. The higher taxa containing the taxa dealt with in the paper should be indicated in parentheses: e.g. A taxonomic revision of the genus *Aus* (Order: family).

3) The **name(s) of all authors** of the paper must be given and should be typed in the upper case (e.g. ADAM SMITH, BRIAN SMITH & CAROL SMITH). The address of each author should be given in *italics* each starting a separate line. E-mail address(es) should be provided if available.

4) The **abstract** should be concise and informative. Any new names or new combinations proposed in the paper should be mentioned. Abstracts in other languages may also be included in addition to English abstract. The abstract should be followed by a list of **key words** that are not present in the title. Abstract and key words are not needed in short correspondence.

5) The arrangement of the **main text** varies with different types of papers (a taxonomic revision, an analysis of characters and phylogeny, a catalogue etc.), but should usually start with an **introduction** and end with a list of **references**. References should be cited in the text as Smith (1999), Smith & Smith (2000) or Smith *et al.* (2001) (3 or more authors), or alternatively in a parenthesis (Smith 1999; Smith & Smith 2000; Smith *et al*. 2001). All literature cited in the text must be listed in the references in the following format (see a sample page here in PDF).

A) **Journal paper**:
Smith, A. (1999) Title of the paper. *Title of the journal in full*, volume number, page range.

B) **Book chapter**:
Smith, A. & Smith, B. (2000) Title of the Chapter. *In*: Smith, A, Smith, B. & Smith, C. (Eds), *Title of Book*. Publisher name and location, pp. x–y.

C) **Book**:
Smith, A., Smith, B. & Smith, C. (2001) *Title of Book*. Publisher name and location, xyz pp.

D) **Internet resources**
Author (2002) Title of website, database or other resources, Publisher name and location (if indicated), number of pages (if known). Available from: http://xxx.xxx.xxx/ (Date of access).

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