

Reappraisal of *Rhamdia branneri* Haseman, 1911 and *R. voulezi* Haseman, 1911 (Siluriformes: Heptapteridae) from the rio Iguaçu with notes on their morphometry and karyotype

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The species *Rhamdia branneri* Haseman, 1911 and the subspecies *Rhamdia branneri voulezi* Haseman, 1911 from rio Iguaçu are currently recognized as synonyms of *Rhamdia quelen* (Quoy & Gaimard, 1824). However, recent karyotype and ecomorphology studies distinguish *R. branneri* and *R. voulezi* as different species. Examination of *Rhamdia* populations from rio Iguaçu, including type specimens, together with other congeners from rio Tibagi, allowed to properly reexamine the situation of these Haseman's taxa and references given to *Rhamdia* in the Iguaçu. The species *R. branneri* and *R. voulezi* have strong serrae with large basis on both margins of the pectoral-fin spine, uncommon in the remaining species of *Rhamdia* and different from the fine serrate margins of the pectoral spine of *R. quelen*; a regular dorsal profile, slightly curved between supraoccipital and dorsal fin; with dorsal dark-brown or light-gray coloration along body, abdomen pale, without profuse small black spots, common in populations of the upper Paraná species and the type-material of *R. quelen*. Also the following morphometric characters discriminate these species: *Rhamdia voulezi*, adipose fin elongate; smaller distance between dorsal and adipose fin; smaller length between adipose fin to base of caudal fin and smaller distance from posterior margin of eye to opercular border. *Rhamdia branneri*, larger distance from dorsal and adipose fins; deeper caudal peduncle; higher trunk depth in the vertical distance through adipose, between pelvic and anal fins; larger scapular bridge; shorter maxillary barbel; shorter external mental barbel; shorter interorbital distance; shorter length of dorsal fin basis and shorter adipose-fin base length. A PCA between the populations of *Rhamdia* from Iguaçu and a population from rio Tibagi, upper Paraná basin, discriminates the population from Tibagi on basis of dorsal to adipose fin distance, dorsal-fin spine length, maxillary barbel length, eye diameter, and pectoral-fin spine length. This morphometric study allied to the karyotype known differences suggest *R. branneri* and *R. voulezi* as valid species. The complex state of *R. quelen* with the neotype recently designated from rio Samiria recommend new studies on basis of molecular genetics and provision of the names *R. branneri* and *R. voulezi* in the Iguaçu basin.

A espécie *Rhamdia branneri* Haseman, 1911 e sua subespécie *Rhamdia branneri voulezi* Haseman, 1911 são atualmente consideradas sinônimos de *Rhamdia quelen* (Quoy & Gaimard, 1824). Recentemente, estudos de cariótipos e de ecomorfologia distinguiram *R. branneri* e *R. voulezi* em espécies distintas. Exame de populações de *Rhamdia* do rio Iguaçu, incluindo espécimes-tipo, em conjunto com outras congêneres do rio Tibagi, permitiu reexaminar apropriadamente a situação destas espécies de Haseman e das referências dadas ao gênero *Rhamdia* para a bacia do rio Iguaçu. *Rhamdia branneri* e *R. voulezi* diferem de outras espécies de *Rhamdia* do rio Iguaçu por possuírem ambas as bordas do raio indiviso peitoral fortemente serrada com unidades rombudas, diferentemente da delicada serra do espinho peitoral de *R. quelen*; combinam o perfil dorsal em suave curva regular, levemente convexo entre o supraoccipital e a nadadeira dorsal e o padrão de colorido marrom escuro ou cinza claro dorsal com abdome pálido, sem qualquer vestígio de colorido densamente pintalgado de negro, comum em outras espécies de *Rhamdia* da bacia do alto Paraná e ao material tipo de *R. quelen*. Adicionalmente, as seguintes características discriminam estas espécies: *Rhamdia voulezi*, nadadeira adiposa alongada; menor distância entre as nadadeiras dorsal e adiposa; menor comprimento entre a nadadeira adiposa e a base da nadadeira caudal e menor distância entre a margem posterior da órbita e a margem do opérculo. *Rhamdia branneri*, maior distância da nadadeira dorsal à adiposa; maior altura do pedúnculo caudal; maior altura do tronco na vertical que passa pela nadadeira adiposa e entre as nadadeiras ventral e anal; maior largura da cintura escapular; menor comprimento do barbilhão maxilar; menor comprimento do barbilhão mentoniano externo; menor distância interorbital; menor comprimento da base da nadadeira dorsal e menor comprimento da base da adiposa. Uma análise de componentes principais realizada entre as populações de *Rhamdia* do rio Iguaçu e uma população do rio Tibagi, bacia do alto rio Paraná, distinguiu a população do Tibagi com base na distância entre a nadadeira dorsal e a adiposa, comprimento do raio indiviso dorsal, comprimento do barbilhão maxilar, diâmetro orbital e comprimento do raio indiviso peitoral. O estudo morfométrico aliado às diferenças de cariótipo conhecidas sugerem *R. branneri* e *R. voulezi* como espécies válidas. A complexa situação de *R. quelen*, que possui ampla distribuição e com o neótipo recém designado para o rio Samiria, recomenda a realização de novos estudos com base em genética molecular e a manutenção dos nomes *R. branneri* e *R. voulezi* para o rio Iguaçu.

Keywords: Endemism, Ostariophysi, Revision, Taxonomy.

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Introduction

Rhamdia Bleeker, 1858 is recognized by the combination of the following characters proposed by Silfvergrip (1996): three pair of barbels; vomer bone edentulous; double lip fold; posterior process of the fourth parapophysis expanded distally with one large and several smaller indentations; supraoccipital process free from supraneural plate; orbital rim free; adipose fin with free posterior margin, posterior fontanel closed and posterior process of cleithrum (humeral process) well developed. One additional feature that diagnoses *Rhamdia* is the forked caudal fin with rounded lobes. None of these characters, however, are exclusive to *Rhamdia* and there is no attempt to test the monophyly of *Rhamdia* or to determine the synapomorphies that support the genus.

On the other hand, during the last century the genus *Rhamdia* appeared with 66 described species, as listed by Gosline (1945) and accepted by Mees (1974). Silfvergrip (1996) revised the genus and recognized only 11 valid species, distributed from Mexico to Argentina and confined to the Cis-Andean region of South America as accepted by Bockmann & Guazelli (2003) and Ferraris (2007). With respect to the Iguaçú *Rhamdia*, Shibatta & Garavello (1995) studied populations of this genus in accord that this area perhaps retain exclusive species of *Rhamdia*. Also Garavello & Shibatta (2007), Garavello *et al.* (2012) and Baumgartner *et al.* (2012), inform the Iguaçú basin as an area of medium endemism with native species inclusive those of *Rhamdia*.

From this area, Haseman (1911) described *Rhamdia branneri*, *Rhamdia branneri voulezi* and identified other sympatric species as *Rhamdia quelen* (Quoy & Gaimard, 1824), combining the following characters: supraoccipital longer in *R. branneri voulezi* compared to *R. branneri*; head proportionally larger than trunk; and adipose fin and barbels relatively shorter than in *R. quelen*. As originally identified by Haseman, the subspecies *R. branneri voulezi* was distinguished from *R. branneri* on basis in the very elongate post-mentonian barbel that surpass the pectoral-fin insertion, and the long maxillary barbel reaching to adipose-fin origin. Silfvergrip (1996) refute the characters utilized by Haseman arguing they are labile and put Haseman's species in the synonymy of *R. quelen*.

Further, Abucarma & Martins-Santos (2001) studying on *Rhamdia* karyotypes from Iguaçú, and Garcia *et al.* (2010) on karyotype evolution trends, origin and differentiation of supernumerary chromosomes in *R. quelen*, favored to reappraise the species of *Rhamdia* from the rio Iguaçú. Recently, Mise *et al.* (2013), also in a collection from the Iguaçú, revealed that morphological characters split the species *R. branneri* and *R. voulezi*.

In the present study, collections of *Rhamdia* undertaken by LISDEBE in the 1980s and NUP in 1990s at rio Iguaçú, allow us to reexamine the populations of *Rhamdia* from this area on basis of morphometry and karyotype available data. The result herein provided is the reappraisal and redescription of *R. branneri* and *R. voulezi* and the recommendation for the maintenance of these names for the *Rhamdia* species from Iguaçú basin.

Material and Methods

The morphometric data were obtained with help of a vernier caliper with 0.05 mm of precision. The following set of measurements were taken point to point (Fig. 1): standard length (SL); pre-dorsal length (DPD); dorsal-fin base length (CBD); dorsal-fin height (AD); dorsal-fin to adipose-fin distance (DDAd); adipose-fin base length (CBAAd); adipose-fin to caudal-fin base distance (DAdBC); pre-pelvic length (DPV); pelvic-fin to anal-fin distance (DVA); anal-fin base length (CBA); anal-fin to caudal-fin base distance (DAC); caudal-peduncle depth (APC); trunk depth at vertical through adipose fin between pelvic and anal fins (ATAA); first dorsal-fin ray to first pelvic-fin ray distance (DIRD1RV); first pelvic-fin ray to last dorsal-fin ray distance (DIRVUD); last dorsal-fin ray to first anal-fin ray distance (DURD1A); pectoral-girdle width (LCE); pectoral-fin spine length (CEP); dorsal-fin spine length (CED); maxillary-barbel length (CBM); external mental-barbel length (CBME); head length (CC); snout length (CF); eye diameter (DO); eye-operculum distance (DOOp); interorbital distance (DIO); and mouth width (LB). Counts taken included: 1) dorsal-fin rays; 2) pectoral-fin rays; 3) pelvic-fin rays; 4) anal-fin rays; 5) caudal-fin rays; 6) gill-rakers on first left branchial arch.

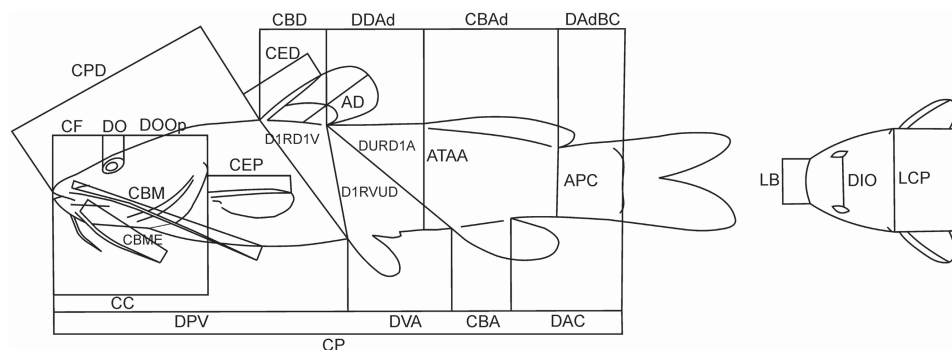


Fig. 1. Diagram of measurements taken from *Rhamdia* specimens for the principal component analysis. See Materials and Methods for explanation of abbreviations.

Morphometric analysis. Body proportions were obtained for each character in relation to standard length (SL) except subunits of head that were taken in relation to head length (HL). The principal component analysis (PCA), that included the holotype measurements of *R. branneri* and *R. voulezi*, was applied to discriminate the body forms of these two *Rhamdia* species from rio Iguaçu. Also these two species were compared with a population of *Rhamdia* from rio Tibagi of the upper Paraná basin adjacent to the Iguaçu. Patterns of size and shape variation among the morphometric variables listed above were used from the studied samples that were evaluated through PCA, a multivariate statistical procedure designed to summarize major patterns of variation among metric characters. The values for all characters were transformed to logarithms to equalize variances of allometric relationships (Bookstein *et al.*, 1985). The principal component scores for the first axis were plotted to assess patterns of size and shape variation within and among *R. branneri* and *R. voulezi* populations. We investigate the species delimitation of these two species based on distinctive morphology, morphometrics, meristics and coloration to redescribe them. In all these cases, reliable morphometric differentiation requires attention to allometry. Statistical analysis were performed using the statistical program PAST (Hammer *et al.*, 2001).

Institutional abbreviations: LISDEBE, Laboratório de Ictiologia Sistemática do Departamento de Ecologia e Biologia Evolutiva da Universidade Federal de São Carlos, São Carlos; MZUEL, Museu de Zoologia da Universidade Estadual de Londrina, Londrina; NUP, Núcleo de

Pesquisas em Limnologia, Ictiologia e Aquicultura-Coleção Ictiológica, Maringá; CAS-IC, California Academy of Sciences-Ichthyological Collection, San Francisco; FMNH, Field Museum of Natural History, Chicago; NRM, Swedish Museum of Natural History, Stockholm. A large collection of *Rhamdia* from rio Iguaçu housed in these institutions and listed below was studied and 46 specimens were chosen for morphometric analysis, diagnosis, descriptions and photographs. Main karyotype data were taken from Abucarma & Martins-Santos (2001), Garcia *et al.* (2010) and Martinez *et al.* (2011). The examined type material of *R. branneri* and *R. branneri voulezi* come from FMNH and that of *Rhamdia quelen* from NRM.

Results

Rhamdia branneri Haseman, 1911

(Figs. 2-4)

Rhamdia branneri Haseman, 1911: 377 pl. LXXV; Creek of rio Iguassú (current Iguaçu), near Serrinha, Paraná State, Brazil; -Gosline, 1945: 36 (literature compilation); -Fowler, 1954: 558 (literature compilation); -Ibarra & Stewart, 1987: 74 (literature compilation); -Severi & Cordeiro, 1994: 67 fig. (literature compilation); -Garavello *et al.*, 1997: 76 (citation); -Abucarma & Martins-Santos, 2001: 300 (cytogenetic description); -Baumgartner *et al.*, 2012:134 (citation).

Rhamdia sebae (non Cuvier, 1829): Severi & Cordeiro, 1994: 69 (literature compilation).



Fig. 2. Lateral view of *Rhamdia branneri*, LISDEBE 5534, 228.5 mm SL, Brazil, Paraná, rio Timbó, Colônia São Pedro.



Fig. 3. Lateral view of paratype of *Rhamdia branneri*, FMNH 54237, 100.0 mm SL, Brazil, Paraná, Serrinha, rio Iguaçu.

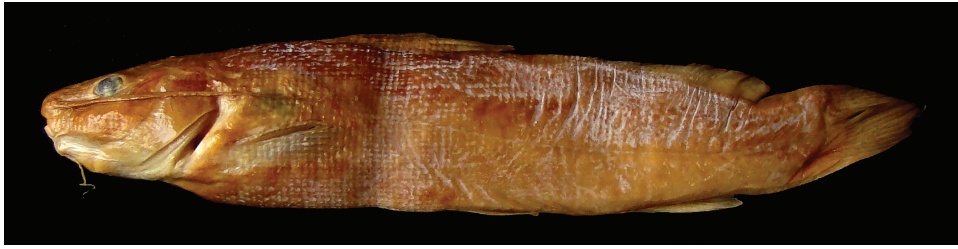


Fig. 4. Lateral view of holotype of *Rhamdia branneri*, FMNH 54235, 100.3 mm SL, Brazil, Paraná, Serrinha, rio Iguaçú.

Diagnosis. *Rhamdia branneri* differs from the remaining *Rhamdia* species, with exception of *R. voulezi*, and *Rhamdia* cf. *quelen* from rio Tibagi by having both sides anterior and posterior of the pectoral-fin spine serrate in large dents (vs. slightly serrate in fine dents in *Rhamdia* cf. *quelen*). *Rhamdia branneri* is also distinct by its dorsal-fin length not reaching the origin of the adipose fin when adpressed (vs. adpressed dorsal fin reaching adipose-fin origin in *R. voulezi* and *R. cf. quelen*); maxillary barbel short not reaching the origin of adipose fin (vs. maxillary barbel long, surpassing adipose fin origin in *R. voulezi* and longer than the SL in *R. cf. quelen*); *R. branneri* dorsal profile straight between the snout and supraoccipital process and convex from this point to the dorsal fin insertion (vs. dorsal profile almost straight from tip of snout to dorsal-fin insertion in *R. voulezi* and *R. cf. quelen*). Ground color of preserved *R. branneri* specimens dark or light gray with few large spots and without small black dots over trunk (vs. ground color grayish on dorsum to light orange on abdomen in *R. voulezi* and dark brown spotted in *R. cf. quelen*); dorsal-fin without a hyaline horizontal stripe immediately dorsal to base (vs. a hyaline horizontal stripe higher to base of dorsal fin in *R. voulezi* and *R. cf. quelen*). Differs in corporal proportions from *R. voulezi* in the higher distance between dorsal and adipose fins; higher distance between adipose ending and caudal fin base; higher distance between orbit and opercular bone and larger scapular bridge width; *R. branneri* also differs in chromosome number and morphology: $2n=58-62$ chromosomes, plus one submetacentric B chromosome and multiple NOR system (vs. $2n=58-60$ plus one metacentric B chromosome and a simple nucleolus organizer region (NOR) in *R. voulezi*). *Rhamdia branneri* differs from *R. voulezi*, *R. foina* (Müller & Troschel, 1849) and *R. itacaiunas* Silfvergrip, 1996 by having 9-14 gill rakers (mode 10) on the first gill arch (vs. 9-14, 12 in mode in *R. voulezi*; 15-20 in *R. foina* and 5-7 in *R. itacaiunas*); differs from *R. laukidi* Bleeker, 1858 by having absence of a diagonal light bar from superior corner of operculum to base of pelvic fin; *R. branneri* also differs from *R. xetequepeque*, *R. jequitinhonha* Silfvergrip, 1996, *R. muelleri* (Günther, 1864) and *R. poeyi* Eigenmann & Eigenmann, 1888 by having shorter orbital diameter: 12.9-17.6% of HL (vs. 12.1-20.2% in *R. voulezi*, 30.5-36.9% in *R. xetequepeque*, 20.4% in *R. jequitinhonha*, 12.1-27.2% in *R. muelleri* and 20.1-26.2% in *R. poeyi*). *Rhamdia branneri* is further distinguished from *R. jequitinhonha* by their longer caudal peduncle (18.4-21.8% SL vs. 16.3%) and longer interorbital distance: *R. branneri*

30.1-41.8% HL (vs. 29.8-39.9% in *R. voulezi*, 24.2% in *R. muelleri*) and by its shorter maxillary barbel length: 129.9-213% in *R. branneri* (vs. 178.5-374.2% in *R. voulezi*, and 73.9-129.0% in *R. muelleri*). It differs from *R. poeyi* by strong and well developed serrae on both sides of pectoral spine (vs. serrae just on posterior margin of pectoral spine); *R. branneri* also differs by a rounded margin of lower lobe (vs. longer and pointed lower caudal fin lobe in *R. poeyi*).

Description. Morphometric characters of *R. branneri* are summarized in Table 1 and the meristic characters in Table 2. Dorsal profile almost straight from tip of snout to supraoccipital and slight convex from this point to dorsal-fin insertion; body depth at anterior vertical through adipose fin and between pelvic and anal fin shorter than the distance between the ending of dorsal fin insertion and pelvic fin. Distance from dorsal to adipose fin generally shorter than the length of dorsal-fin base. Caudal peduncle depth small nearly 7.8-12.0, mean 10.7% SL. Head almost conic in dorsal view, covered by thick skin, except for eyes with free margin, occipital process covered by thick skin (not visible). Orbit relatively large nearly 12.9-17.6, mean 15.3% SL in head length. Upper maxillae elongate longer than dentary; premaxillary dental plate distally semicircular; dental plate of dentary narrowing posteriorly with a gap over the mandibular symphysis; small teeth nearly of same size in premaxillary and dentary. Maxillary barbel short and depressed reaching dorsal-fin insertion, but not reaching adipose-fin insertion; mental external barbel reaching origin of pectoral-fin base; internal mental barbel not surpassing the gill opening. Posterior cleithral process short. Dorsal-fin spine smooth on both anterior and posterior faces. Pectoral-fin elongate, both sides anterior and posterior of the serrate pectoral-fin spine; posterior border of pectoral fin reaching vertical through the first branched dorsal-fin ray; pelvic-fin insertion generally beginning at vertical through the last dorsal-fin ray; pelvic-fin rounded; caudal fin furcated; distal lobes rounded, ventral lobe larger than dorsal.

Color pattern of preserved specimens. Ground color of trunk and abdomen dark gray or light gray with few and sparse large brown spots and without small black dots over trunk and abdomen; ground color light gray. Dorsally light brown; interradiation membranes hyaline; dorsal fin without a hyaline horizontal stripe immediately dorsal to base. Remaining fins with nearly the same ground color of body.

Table 1. Body proportions of *Rhamdia branneri* and *Rhamdia voulezi* from the rio Iguaçú. SD = Standard deviation.

	<i>Rhamdia branneri</i> (n=16)		<i>Rhamdia voulezi</i> (n=30)	
	Min.-max.	Mean±SD	Min.-max.	Mean±SD
Standard length (mm)	150.4-327.8	216.9±50.98	101.7-350.2	207.6±61.14
Percents of standard length				
Head length	20.5-28.1	25.6±1.60	24.8-29.0	26.2±0.93
Predorsal distance	33.8-39.9	36.5±1.44	34.1-39.5	35.9±1.42
Dorsal-fin base length	13.5-18.9	15.7±1.38	14.3-19.0	16.6±1.28
Dorsal fin to adipose fin distance	7.1-14.5	10.2±2.45	3.1-8.4	5.9±1.34
Adipose-fin base length	23.8-34.9	30.1±2.94	18.7-38.8	34.5±3.53
Adipose fin to caudal fin distance	9.5-13.0	11.2±0.93	8.3-12.0	10.3±1.01
Pre-pelvic distance	47.9-54.8	51.3±2.09	38.9-82.1	51.2±6.40
Pelvic fin to anal fin distance	18.0-22.3	20.0±1.20	17.9-22.9	20.5±1.09
Anal-fin base length	10.8-15.3	12.5±1.10	9.8-16.1	12.2±1.14
Anal fin to caudal fin distance	17.2-20.4	19.4±0.79	17.1-22.2	19.9±1.32
Caudal peduncle depth	7.8-12.0	10.7±1.14	9.5-13.2	11.0±0.94
Pectoral girdle width	18.0-22.4	20.1±0.99	17.4-21.8	20.0±1.04
Dorsal-fin spine length	6.5-9.2	7.6±0.77	6.9-12.1	8.8±1.24
Pectoral-fin spine length	9.7-15.7	12.0±1.39	10.7-15.4	13.2±1.11
Body depth	14.5-21.5	17.4±2.06	15.5-23.5	18.6±1.89
Percents of head length				
Snout length	35.6-47.9	39.9±2.90	37.0-42.7	40.1±1.47
Orbital diameter	12.9-17.6	15.3±1.57	12.1-20.2	16.4±1.95
Interorbital distance	30.1-41.8	33.7±3.59	29.8-39.9	35.2±2.79
Maxillary barbel length	128.9-213.3	167.8±24.17	178.5-374.2	261.3±53.33
Mouth width	40.5-57.1	45.4±4.15	42.1-57.0	47.1±3.19

Table 2. Meristic characters of *Rhamdia branneri* and *R. voulezi*.

	<i>R. branneri</i>		<i>R. voulezi</i>	
	Counts	Mode	Counts	Mode
1. Dorsal fin	1,6-8	1,6	1,6-7	1,6
2. Pectoral fin	1,7-9	1,8	1,7-9	1,9
3. Pelvic fin	1,4-5	1,5	1,5	1,5
4. Anal fin	i-iii,6-9	ii,7 and ii,8	ii-v,7-9	i,8
5. Caudal fin	1,6-9/8-10,I	1,7/9,I	1,6-9/8-10,I	1,8
6. Gill-rakers on outermost arch	9-14	10	9-14	12

Distribution. Known from the rio Iguaçú, rio Paraná basin in Brazil (Fig. 5).

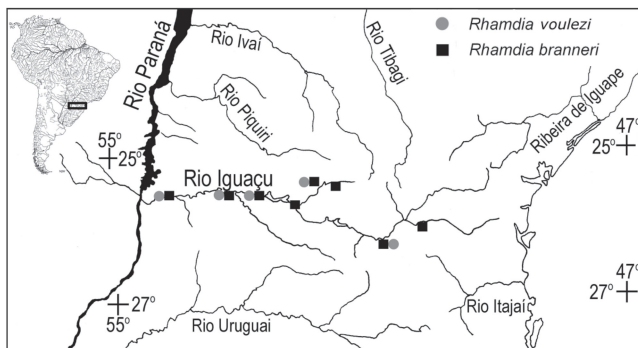


Fig. 5. Geographical distribution of *Rhamdia branneri* and *R. voulezi* in rio Iguaçú. Each symbol may represent more than one sample.

Material examined. Paraná: FMNH 54235, holotype of *Rhamdia branneri*, Serrinha, Iguassu (current Iguaçú), J. D. Haseman, 24 Dec 1909; FMNH 54237, 1, paratype of *Rhamdia branneri*, Iguassu (current Iguaçú), J. D. Haseman, 30 Dec 1909; LISDEBE 5530, 1, rio Negro, under the bridge of BR 116-Rio Negro, collectors of Ichthyology DCB- UFSCar, 2 Apr 1985; LISDEBE 2294, 2, rio Iguaçú, Porto Lupion, collectors of Ichthyology Surehma, Dec 1979-Jan 1980; NUP5715,1, reservoir of Segredo, municipality of Pinhã, border of Mangueirinha, collectors of Nupélia, 19 May 1993; NUP5713,1, Segredo reservoir, municipality of Pinhã, border of Mangueirinha, collectors of Nupélia, 18 Jun 1993; NUP 5719, 2, rio Iguaçú, down river from hydroelectric Segredo, municipality of Pinhã, border of Mangueirinha, collectors of Nupélia, 7 Sept 1993; NUP 5676, 1, rio Butiã tributary of rio Iguaçú, municipality of Palmas, border of Mangueirinha, collectors of Nupélia, 7 Aug 1993; NUP5720, 2, rio São Pedro, municipality of Pinhã, collectors of Nupélia, 12 Sept 1993; NUP 5718, 2, rio Floresta, municipality

of Pinhão, collectors of Nupélia, 12-14 Aug 1993; LISDEBE 5531, 3, Porto Lupion, rio Iguçu, J.C. Garavello & collectors of Surehna, May 1979; LISDEBE 5532, 1, rio Jordão, tributary of rio Iguçu at Estância Santa Clara, Candói, J. C. Garavello, 20 Jan 1997; LISDEBE 5533, 1, rio Cavernoso, tributary of rio Iguçu near Candói, J. C. Garavello & A. S. Soares, 19-23 Jun 1998. **Santa Catarina:** Porto União: CAS-SU 62523, 3, rio Iguçu at Porto União, George S. Myers & Antenor L. Carvalho, 1944; CAS 62686, 1, rio Iguçu at Porto União, George S. Myers & Antenor L. Carvalho, 1944; LISDEBE 5534, 4, rio Timbó, tributary of rio Iguçu, Colônia São Pedro, J. C. Garavello & UFSCar collectors, 20-21 Nov 1986; LISDEBE 5535, 1, rio Timbó, tributary of rio Iguçu, Colônia São Pedro, J. C. Garavello & UFSCar collectors, 18-19 Nov 1986.

Rhamdia voulezi Haseman, 1911

(Figs. 6-7)

Rhamdia branneri voulezi Haseman, 1911: 378, pl. LXXXVI; (Porto União da Vitória (current União da Vitória), rio Iguassu (current Iguçu), Paraná State, Brazil); -Gosline, 1945: 37 (literature compilation); -Fowler, 1954: 558 (literature compilation); -Ibarra & Stewart, 1987: 74 (literature compilation).

Rhamdia voulezi. -Garavello *et al.*, 1997: 77 (citation); -Abucarma & Martins-Santos, 2001: 300 (cytogenetic description); -Baumgartner *et al.*, 2012:135 (citation).



Fig. 6. Lateral view of *Rhamdia voulezi*, LISDEBE 5529, 214.0 mm SL, Brazil, Paraná, São José dos Pinhais, rio Iguçu, marginal pound and lagoons at Torres Avenue, sides of Reserva do Cambuí.



Fig. 7. Lateral view of holotype of *Rhamdia branneri voulezi*, FMNH 54238, 122.0 mm SL, Brazil, Paraná, Porto União da Vitória, rio Iguçu.

Diagnosis. *Rhamdia voulezi* is distinguished from the remaining *Rhamdia* species, with exception of *R. branneri* and *Rhamdia* cf. *quelen* from rio Tibagi, by having both sides anterior and posterior of the pectoral-fin strong serrate in large dents (*vs.* slightly serrate in fine dents in *Rhamdia* cf. *quelen*). Differ from *R. branneri* by the dorsal profile almost straight from tip of snout, through supraoccipital and to dorsal-fin insertion (*vs.* dorsal profile straight between the snout and supraoccipital process and convex from this point to the dorsal fin insertion in *R. branneri*). *Rhamdia voulezi* is also distinct from *R. branneri* by its dorsal-fin

length reaching the origin of the adipose fin when adressed (*vs.* adressed dorsal fin not reaching adipose-fin origin in *R. branneri*); maxillary barbel long reaching the origin of adipose fin (*vs.* maxillary barbel short, not surpassing adipose-fin origin in *R. branneri*; longer than the SL in *R. cf. quelen*). Ground color of preserved *R. voulezi* specimens grayish on dorsum to light orange on abdomen (*vs.* dark or light gray with few large spots and without small black dots over trunk in *R. branneri* and dark brown spotted in *R. cf. quelen*). *Rhamdia voulezi* differs in corporal proportions from *R. branneri* by the longer maxillary barbel (178.5 to

374.2% of HL, mean=261.3%; vs. 128.9 to 213.3% of HL, mean= 167.8% in *R. branneri*); larger interorbital distance (29.8 to 39.9% of HL, mean 35.2% vs. 30.1 to 41.8%, mean 33.7% in *R. branneri*); longer dorsal-fin base (14.3 to 19.0% of SL, mean=16.6% vs. 13.5 to 18.9%, mean=15.7% in *R. branneri*); and longer adipose fin length (18.7 to 38.8% of SL, mean 34.5% vs. 23.8 to 34.9%, mean 30.1% in *R. branneri*). *Rhamdia voulezi* also differ in chromosome number and morphology: 2n=58-60 plus one metacentric B chromosome and a simple nucleolus organizer region (NOR) (vs. 2n=58-62 chromosomes, plus one submetacentric B chromosome and multiple NOR system in *R. branneri*). *Rhamdia voulezi* also differ from *R. branneri*, *R. foina* and *R. itacaiunas* by having 9-14 gill rakers (12 in mode) on the first gill arch (vs. 9-14, 10 in mode in *R. branneri*, 15-20 in *R. foina* and 5-7 in *R. itacaiunas*); differ from *R. laukidi* by having absence of a diagonal light bar from superior corner of operculum to base of pelvic fin; differ from *R. branneri*, *R. xetequepeque*, *R. jequitinhonha*, *R. muelleri* and *R. poeyi* by having shorter orbital diameter: *R. voulezi* 12.1-20.2% of HL (vs. 12.9-17.6% in *R. branneri*, 30.5-36.9% in *R. xetequepeque*, 20.4% in *R. jequitinhonha*, 12.1-27.2% in *R. muelleri* and 20.1-26.2% in *R. poeyi*). *Rhamdia voulezi* is further distinguished from *R. jequitinhonha* by their longer caudal peduncle (18.4-21.8% vs. 16.3% SL) and from *R. muelleri* by the longer interorbital distance 29.8-39.9% HL in *R. voulezi* (vs. 24.2% in *R. muelleri*) and shorter maxillary barbel length: 178.5-374.2% HL in *R. voulezi* (vs. 73.9-129.0% in *R. muelleri*); *R. poeyi* with small serrae of spines on posterior margin of pectoral spine differ from *R. voulezi* with strong and well developed serrae on both sides; *R. voulezi* also differ from *R. poeyi* in caudal fin upper lobe shorter than lower and margin of lower lobe rounded (vs. longer and pointed lower caudal fin lobe in *R. poeyi*).

Description. Morphometric and meristic characters are summarized in Tables 1-2. Dorsal profile almost straight from tip of snout through supraoccipital and to dorsal-fin insertion. Body height between ventral and anal-fin smaller than distance between ending of dorsal fin and anterior insertion of anal fin; caudal peduncle depth small, height near 9.5-13.2, mean 11.0% SL. Distance from ending dorsal and anterior insertion of adipose fin always shorter than dorsal fin basis; head almost conic, occipital process covered by thick skin. Orbits relatively large, near 12.1-20.2, mean 16.4% HL. Upper maxillae slightly longer than dentary; distal portion of premaxillary dental plate slightly circular; dental plate of mandible slightly decreasing distally and with gap over the mandibular symphysis; small teeth, almost with same size on premaxillae and dentary. Maxillary barbel flattened, longer and surpassing beginning of adipose-fin insertion. In young specimens, this barbel surpasses anal-fin base; external mental barbel surpassing beginning of pectoral-fin; inner mental barbel surpassing gill opening in juveniles, but not in adults. Posterior cleithral process short. Dorsal-fin spine smooth

on both sides. Posterior border of pectoral-fin reaching vertical through the first dorsal-fin branched ray. Pelvic-fin beginning in vertical through anterior or over last ray of dorsal-fin. Anal-fin with rounded distal border. Caudal fin forked; ventral lobe larger than dorsal.

Color pattern of preserved specimens. Ground color generally grayish and with small dispersed black dots; abdomen generally yellowish-orange. Dorsal-fin with a light horizontal line upper to its base; over to this light line the interradial membranes with the same ground color and the branched rays may appear blackish highlighted. Remaining fins with nearly same ground color of body and trunk; interradial membranes hyaline.

Distribution. Known from rio Iguaçu, rio Paraná basin in Brazil (Fig. 5).

Material examined. FMNH 54238, holotype of *Rhamdia branneri voulezi*, Porto União da Victória, rio Iguassu (current Iguaçu), J. D. Haseman, 24 Dec 1909. **Paraná:** LISDEBE 2292, 3, rio Iguaçu, Porto Lupion, collectors of Ichthyology Surehma, Dec 1979 – Jan 1980; LISDEBE 2296, 4, rio Iguaçu, Porto Lupion, Surehma collectors, Dec 1979 – Jan 1980; LISDEBE 2293, 1, rio Iguaçu, Porto Lupion, J. C. Garavello & Surehma collectors, May 1979; LISDEBE 5529, 2, São José dos Pinhais, rio Iguaçu, marginal lagoons at Torres Avenue, beside of Cambuí reserve, UFSCar collectors, 29 Dec 1984; LISDEBE 5527, 1, rio Iguaçu, downriver from the Salto Osorio hydroelectric power dam, municipality of Quedas do Iguaçu, J. C. Garavello, A. S. Soares & J. C. Soares, 14-17 Jan 1990; LISDEBE 2297, 4, rio Iguaçu, downriver from Salto Santiago hydroelectric power dam, between municipalities of Laranjeiras do Sul and Chopinzinho, J. C. Garavello, A. S. Soares, R. J. Rodrigues, M. J. Veber & M. M. Paschoa, 5-10 Nov 1990; LISDEBE 2295, 3, rio Cavernoso, tributary of rio Iguaçu, near Cândói city, J. C. Garavello & A. S. Soares, 19-23 Jun 1998; NUP-MU 3893, 1, rio Ocoi, Vila Ipiranga, São Miguel do Iguaçu, Nupélia collectors (25°13'S 54°12'W), 10 Nov 1988; NUP-MU 2664, 1, riacho na Fazenda Picui, São Miguel do Iguaçu, Nupélia collectors (25°15'S 54°15'W) 22 Mar 1989; NUP 5675, 3, Pinhão, Segredo reservoir at Mangueirinha border, Nupélia collectors, 10 May 1993; NUP 5674, 7, Pinhão, Segredo reservoir at Mangueirinha municipality border, Nupélia collectors, 17-18 Jun 1993; NUP 5673, 6, Pinhão, Segredo reservoir at Mangueirinha border, Nupélia collectors, 24 Mar 1993; NUP 5678, 3, Palmas, rio Iratim, border with Pinhão and Mangueirinha municipalities, Nupélia collectors, 21 May 1993; NUP 5674, 7, Pinhão, rio São Pedro, Nupélia collectors, 22 Jun 1993; NUP 2673, 1, Pinhão, Foz do Areia reservoir, border with municipality of Bituruna, Nupélia collectors, 1 Jul 2001; NUP 5716, 1, Pinhão, Foz do Areia reservoir, border with municipality of Bituruna, Nupélia collectors, 28 Aug 1993; NUP 5677, 3, Pinhão, Foz do Areia reservoir, border with municipality of Bituruna, Nupélia collectors 12-13 Aug 1993; NUP 5679, 2, Pinhão, Foz do Areia reservoir, border with municipality of Bituruna, Nupélia collectors 12-13 Sep 1993; NUP 5305, 1 of

29, rio Iguaçu, downriver of Segredo reservoir, municipality of Pinhão at Mangueirinha border, Nupélia collectors, 12 Jul 1993. **Santa Catarina:** Porto União: LISDEBE 2347, 2, rio Timbó, tributary of rio Iguaçu at colônia São Pedro, J. C. Garavello & UFSCar collectors, 18-19 Nov 1986; LISDEBE 5528, 1, rio Timbó, tributary of rio Iguaçu, colônia São Pedro, J. C. Garavello & UFSCar collectors, 19-20 Nov 1986.

Discussion

The Silfvergrip (1996) decision to include the species *Rhamdia branneri* and *R. branneri voulezi* under the synonymy of *Rhamdia quelen* (Quoy & Gaimard, 1854) contrast with the present study. The examination of specimens from the type series of the neotype of *Rhamdia quelen* from rio Samiria (Fig. 8) gives opportunity to get in touch with differences between this species and *R. branneri* and *R. voulezi*. They differ concerning to morphology of dorsal and adipose fins, structure and length of mental and maxillary barbels, pigmentation and morphometric traits from the Iguaçu species and the population of *Rhamdia* cf. *quelen* from rio Tibagi basin. Also, the examination of the Iguaçu specimens studied by Silfvergrip (1996) revealed that the specimens from São Miguel do Iguaçu (NUP-MU 2664 and NUP-MU 3893) in fact belong to *R. voulezi*. In addition, the examination of the specimens from rio Iguaçu at Porto União, CAS-SU 62523 and CAS-SU 62686 revealed to be *R. branneri*. The pectoral spine double serrate, the karyotype differences and ecomorphological data, as soon as color pattern and morphometric trends herein studied, suggest that *R. branneri* and *R. voulezi* are significantly distinct justifying the present adjustment.

As first consideration, it is important to recognize the analysis of metaphase cells of these species by Abucarma & Martins-Santos (2001) revealing 58 - 62 (2n) chromosomes for *R. branneri* and 58 - 60 (2n) chromosomes for *R. voulezi*. These authors make clear the variation on karyotypes of *Rhamdia* from rio Iguaçu within 36 metacentric chromosomes, 14 sub-metacentric chromosomes, 4 sub-telocentric chromosomes and 4 acrocentric chromosomes.

They also recognize the presence of two types of B chromosomes: a medium sized B metacentric chromosome and a sub-metacentric chromosome, more frequent in *R. branneri*; 0 to 2 medium sized metacentric and a B sub-metacentric chromosome in *R. voulezi*. These differences in karyotype of these Iguaçu *Rhamdia*, including one submetacentric B chromosome in *R. branneri*, vs. one metacentric B chromosome in *R. voulezi*, plus a simple nucleolus organizer region (NOR) in *R. voulezi*, vs. multiple NOR system in *R. branneri* may suggest that differentiation occurred on the karyotype structure of these species. These described chromosomes are considered by the authors as safeguard for inter-specific variation between *R. branneri* and *R. voulezi*.

It is also important to recognize the karyotype description of a *Rhamdia quelen* population by Moraes *et al.* (2007) from Bodoquena Plateau, Mato Grosso, Brazil, composed by a diploid number of chromosomes $2n=58$: 36 metacentric; 16 sub-metacentric and 6 sub-telocentric chromosomes. One to three B chromosomes of medium size and the nucleolus organizer region (NOR) situated in terminal region of the shorter arm of the sub-metacentric chromosome pair 20. Also, Martinez *et al.* (2011) offered a study on three populations of a species defined as *Rhamdia quelen* from two large Brazilian rivers: Paraná and Araguaia, showing that the diploid number found for the three analyzed populations is 58 stick chromosomes. Following Garcia *et al.* (2010), "*Rhamdia quelen* invariably presents 58 (2n) chromosomes in all populations that have been analyzed so far. Since *R. quelen* is the most widely species cytogenetically studied among the Heptapteridae, the stability of this diploid number can be regard as characteristic of this species". They observed various karyotype formulae in populations identified as *R. quelen* from the upper and lower Paraná, and from Paraíba do Sul rivers. In both cases the karyotype formula: 36m, 10sm and 12st (see Garcia *et al.*, 2010) was the most frequent in identifying *R. quelen* populations from Paraná, while 40m, 10sm and 8st was the commonest formula in the Paraíba do Sul populations.



Fig. 8. Lateral view of *Rhamdia quelen*, neotype series, NRM 33452, 108.3 mm SL, Peru, Loreto, right bank quebradita tributary to rio Samiria between caño Pastos and Hamburgo.

Perdices *et al.* (2002) based on sequencing data of mitochondrial genes show that the genus *Rhamdia* has diverged from other heptapterids very recently, suggesting that *Rhamdia* is undergoing an initial process of karyotype differentiation, reinforcing their reproductive isolation leading to speciation. For those authors *R. quelen*, *sensu* Silfvergrip, is polyphyletic under mitochondrial DNA perspective.

As second consideration, concerning to morphometry, Mise *et al.* (2013) advanced a PCA on populations of *R. branneri* and *R. voulezi* that indicates an obvious segregation between these two species along the first axis of their figure 2. Those authors also revealed that most of the morphologic characters that separate the *Rhamdia* species from Iguaçu are related to body shape. They clearly discussed that *R. voulezi* can be characterized by having more compressed and less depressed bodies, longer caudal peduncle, wider mouth and larger aspect ratio of the anal fin. On the other hand, *R. branneri* can be characterized by having a higher mouth opening and higher anal fin.

Our morphometric analysis corroborate Mise *et al.* (2013), concluding that there are two distinct species of *Rhamdia* in the rio Iguaçu basin. The sheared principal component analysis, herein applied to populations of *Rhamdia* from Iguaçu, mainly suggest that *R. branneri* and *R. voulezi* may be considered independent specific taxa (Tables 3-4 and Fig. 9). Additionally, a comparison through a principal component analysis between populations of *R. branneri*, *R. voulezi* from Iguaçu *versus* a population of *Rhamdia cf. quelen* from rio Tibagi highlighted their differences as showed in Table 5 and Figs. 9-10.

Table 3. Principal component analysis of *Rhamdia branneri* (n=16) and *R. voulezi* (n=30). Sheared Principal Component Analysis and Eigenvalues (Prior to Shearing).

	Eigenvalues	Total variance (%)	Total variance (cumulative %)
1	0.298	83.993	83.993
2	0.036	10.204	94.197
3	0.004	1.240	95.437
4	0.003	0.815	96.252
5	0.003	0.727	96.978
6	0.002	0.642	97.621
7	0.002	0.505	98.126
8	0.002	0.424	98.549
9	0.001	0.301	98.851
10	0.001	0.292	99.143
11	0.001	0.223	99.366
12	0.001	0.152	99.518
13	0.000	0.109	99.626
14	0.000	0.099	99.725
15	0.000	0.077	99.802
16	0.000	0.069	99.871
17	0.000	0.049	99.920
18	0.000	0.041	99.961
19	0.000	0.024	99.985
20	0.000	0.015	100.000

Table 4. Sheared principal component loadings of the principal component analysis of *Rhamdia branneri* (n=16) and *R. voulezi* (n=30).

	PC1	Sheared Principal Component 2
Head length	0.225387	-0.010014
Predorsal distance	0.232133	-0.049210
Dorsal-fin base length	0.218160	0.032738 (> <i>R. voulezi</i>)
Dorsal fin to adipose fin distance	0.170089	-0.807027 (> <i>R. branneri</i>)
Adipose-fin base length	0.223615	0.164383 (> <i>R. voulezi</i>)
Adipose fin to caudal fin distance	0.208639	-0.128385
Pre-pelvic distance	0.248891	-0.070166
Pelvic fin to anal fin distance	0.231776	-0.007022
Anal-fin base length	0.222427	-0.066667
Anal fin to caudal fin distance	0.199487	0.000423
Caudal peduncle depth	0.269678	0.002440
Pectoral girdle width	0.245498	-0.040196
Dorsal-fin spine length	0.181536	0.104746
Pectoral-fin spine length	0.197812	0.074551
Body depth	0.275806	0.048665
Snout length	0.243877	0.007242 (> <i>R. branneri</i>)
Orbital diameter	0.184111	0.071448
Interorbital distance	0.267512	0.076605 (> <i>R. voulezi</i>)
Maxillary barbel length	0.131916	0.508441 (> <i>R. voulezi</i>)
Mouth width	0.238400	0.043075 (> <i>R. branneri</i>)

Table 5. Principal Component loadings between *R. branneri*, *R. voulezi* and *Rhamdia cf. quelen*.

	Axis 1	Axis 2	Axis 3
Predorsal distance	0.2293	-0.04746	0.03899
Dorsal-fin base length	0.222	0.01773	-0.01941
Dorsal fin to adipose fin distance	0.1867	-0.774	0.1647
Adipose-fin base length	0.2207	0.1554	0.02703
Adipose fin to caudal fin distance	0.2119	-0.1108	0.08364
Pre-pelvic distance	0.2438	-0.05166	0.1038
Ventral fin to anal fin distance	0.2256	0.002937	0.08632
Anal-fin base length	0.2252	-0.04737	-0.01762
Anal fin to caudal fin distance	0.2008	0.006726	-0.01687
Caudal peduncle depth	0.2672	0.01019	0.04614
Snout length	0.2428	0.008378	-0.008869
Eye diameter	0.1818	0.07199	0.3203
Interorbital distance	0.2592	0.07494	0.02412
Pectoral girdle width	0.2445	-0.02995	0.04604
Maxillary barbel length	0.1381	0.5729	0.3686
Mouth width	0.2383	0.03858	-0.06656
Dorsal-fin spine length	0.1909	0.09137	-0.7721
Pectoral-fin spine length	0.2049	0.06548	-0.3136
Body depth at adipose fin	0.2709	0.05207	-0.04057
Head length	0.2241	-0.006128	0.02304

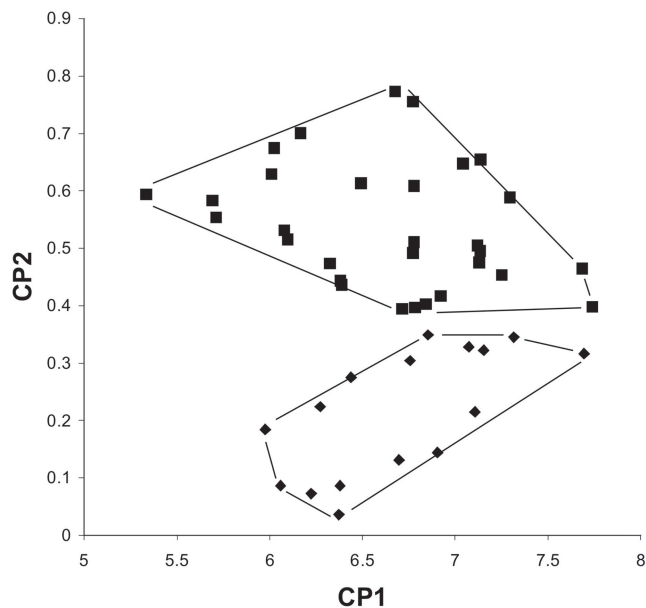


Fig. 9. Scatter plot of combined samples of *Rhamdia branneri* (n=16, diamonds) and *Rhamdia voulezi* (n=30, squares) from rio Iguaçu on the first and second principal component axis.

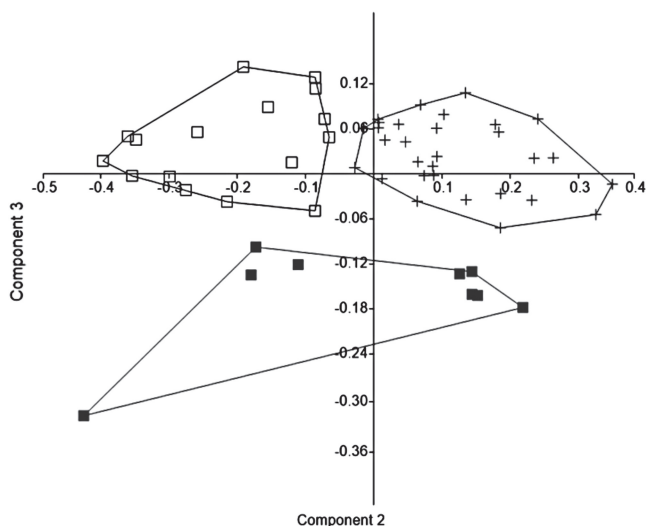


Fig. 10. Scatter plot of combined samples of *Rhamdia branneri* (n=16, open squares), *R. voulezi* (n=30, crosses) and *R. cf. quelen*. (n=9, filled squares) from rio Iguaçu and rio Tibagi (Upper Paraná basin) on the second and third principal component axis.

On basis of the accurate examination of the holotype specimens of *R. branneri* and *R. branneri voulezi*, the Iguaçu material studied by Silfvergrip (1996) from CAS and NUP plus a large number of fresh specimens, we conclude that the serrae on both sides of the pectoral spine, the length of the internal and external mental barbels and the length of adipose fin are the main characters to distinguish these species and are in agreement with the pattern established by Haseman for *R. branneri* and *R. voulezi*.

Also *R. branneri* have the ground color of trunk and abdomen pale brown with rare large dark dots dispersed through body and dorsal-fin without a hyaline horizontal strip immediately dorsal to base. *Rhamdia voulezi* has the ground color generally gray and without large dispersed black dots; abdomen generally orangish-yellow and the dorsal fin with a light horizontal line dorsal to its base, distinct from *R. branneri* and other species. As a result, the main differential morphometric characters between *R. branneri* and *R. voulezi* that could be seen in Table 4 are: *R. branneri* have smaller interorbital distance, smaller dorsal-fin base length, smaller adipose-fin base length, larger mouth and larger snout length; while *R. voulezi* have: larger interorbital distance; larger dorsal-fin base length; larger adipose -fin base length, smaller distance between adressed dorsal fin and adipose-fin base.

Studies of Shibatta & Garavello (1995) on morphology, Abucarma & Martins-Santos (2001), Moraes *et al.* (2007), Garcia *et al.* (2003, 2010) and Martinez *et al.* (2011) on karyotypes, and Mise *et al.* (2013) on ecomorphology, strength this study on the *Rhamdia* species from Iguaçu. Given that Perdices *et al.* (2002) suggest that *Rhamdia* is undergoing an initial process of karyotype differentiation, it is also important to attempt the molecular data to fulfill gaps of morphology in allowing revisions of the species now identified as *Rhamdia quelen sensu* Silfvergrip (1996).

Furthermore, the overwhelming endemism verified in the Iguaçu basin, corroborates the distinctiveness of its *Rhamdia* species. Recent studies on *Trichomycterus* by Wosiacki & Garavello (2004), *Steindachneridion* by Garavello (2005), *Pimelodus* by Garavello & Shibatta (2007), *Astyanax* by Garavello & Sampaio (2010) and *Hypostomus* by Garavello *et al.* (2012) provides strong evidence that the Iguaçu retains exclusive species found nowhere else. Essential to observe that the reference by Severi & Cordeiro (1994) of *Rhamdia sebae*, described from Cayenne, Suriname, is inapplicable for any *Rhamdia* species from Iguaçu.

Finally, the taxonomical simplification proposed by Silfvergrip (1996) when strongly reduced the number of species of *Rhamdia* recognizing many species as synonym of *R. quelen* (Quoy & Gaimard, 1824) may be re-evaluated. Perdices *et al.* (2002) already advanced in this way considering the identification by Silfvergrip of *R. quelen* from Central America a mistake, because there are morphological and molecular evidence to consider the Central America species different from *Rhamdia* species of South America. So, we agree with Perdices *et al.* (2002) that genus *Rhamdia* needs revision under molecular genetic analysis, which may lead to the evaluation of the species actually in synonymy of *Rhamdia quelen*.

Comparative material examined. *Rhamdia quelen*: Peru, Loreto: paratypes: NRM 33542, 3, 101.5-122.5 mm SL, right bank quebradita tributary to rio Samiria between caño Pastos and Hamburgo; NRM 16085, 2, 52.3-58.0 mm SL, Amazon

basin, rio Samiria drainage, left bank quebradita halfway between Hamburgo and Santa Helena. *Rhamdia jequitinhonha*: **Brazil, Minas Gerais**: holotype: MZUSP 38.630, 160.9 mm SL, Santa Rita, rio Araçuaí; MZUEL 3617, 2, São Gonçalo do Rio Pardo, rio Preto, rio Jequitinhonha basin, Parque Estadual Rio Preto. *Rhamdia foina*: **Brazil, Roraima**: INPA 7988, 2, 113.2-122.5 mm SL, rio Branco drainage, rio Uraricoera, Ilha de Maracá, furo de Maracá. **Brazil, Pará**: INPA 7967, 2, 91.8-97.4 mm SL, Oriximiná, rio Trombetas, montante CPT; INPA 7973, 1, 84.3 mm SL, rio Mapuera, Cachoeira Porteira, last rapid before rio Trombetas. *Rhamdia cf. quelen*: **Brazil, Paraná**: Upper Paraná: rio Tibagi: MZUEL 1587, 1, rio Iapó, tributary of Tibagi; MZUEL 1522, 1, Londrina; MZUEL 1034, 2, Londrina; MZUEL 3686, 1, Ponta Grossa; MZUEL 2, 4, Ipiranga; MZUEL 4, 3, Ipiranga; MZUEL 1670, 1, Assai; MZUEL 8, 1, Jataizinho; MZUEL 5, 2, Uvaia; MZUEL 6, 3, Telêmaco Borba; MZUEL 7, 1, Telêmaco Borba.

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