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Two new species of *Australoheros* (Teleostei: Cichlidae), with notes on diversity of the genus and biogeography of the Río de la Plata basin

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Abstract

Two new species of *Australoheros* Říčan and Kullander are described. *Australoheros ykeregua* **sp. nov.** is described from the tributaries of the río Uruguay in Misiones province, Argentina. *Australoheros angiru* **sp. nov.** is described from the tributaries of the upper rio Uruguai and middle rio Iguaçu in Brazil. The two new species are not closely related, *A. ykeregua* is the sister species of *A. forquilha* Říčan and Kullander, while *A. angiru* is the sister species of *A. minuano* Říčan and Kullander. The diversity of the genus *Australoheros* is reviewed using morphological and molecular phylogenetic analyses. These analyses suggest that the described species diversity of the genus in the coastal drainages of SE Brazil is overestimated and that many described species are best undestood as representing cases of intraspecific variation. The distribution patterns of *Australoheros* species in the Uruguay and Iguazú river drainages point to historical connections between today isolated river drainages (the lower río Iguazú with the arroyo Urugua–í, and the middle rio Iguaçu with the upper rio Uruguai). Molecular clocks are used to date these and other biogeographic patterns.

Key words: *Australoheros*, new species, Cichlidae, phylogeny, South America, biogeography, Brazilian shield

Resumen

Dos nuevas especies de *Australoheros* Říčan y Kullander son descriptas. *Australoheros ykeregua* **sp. nov.** es descripta de tributarios del río Uruguay en la provincia de Misiones, Argentina. *Australoheros angiru* **sp. nov.** es descripta de tributarios del rio Uruguai superior y rio Iguaçu medio en Brasil. Las dos especies nuevas no se encuentran estrechamente relacionadas, *A. ykeregua* is la especie hermana de *A. forquilha* Říčan y Kullander, mientras que *A. angiru* es la especie hermana de *A. minuano* Říčan y Kullander. La diversidad del género *Australoheros* es revisada usando análisis filogenéticos morfológicos y moleculares. Estos análisis sugieren que la diversidad específica del género en las cuencas costeras del sudeste del Brasil se encuentra sobreestimada. Los patrones de distribución de las especies de *Australoheros* en las cuencas de los ríos Uruguay e Iguazú señalan una conexión histórica de cuencas que no se mantiene en la actualidad (río Iguazú inferior con el arroyo Urugua-í y rio Iguaçu medio con el rio Uruguai superior). Relojes moleculares son usados para datar estos y otros patrones biogeográficos.

Introduction

The genus *Australoheros* Říčan & Kullander with at present 20 valid species is rapidly becoming one of the most speciose genera of heroine cichlids. Twelve new species from the Atlantic coastal drainages of Brazil (Ottoni & Costa 2008; Ottoni *et al.* 2008; Ottoni & Cheffe 2009; Ottoni 2010), and seven new species from the Río de la Plata basin (Uruguay, Iguazú and Paraná river drainages) (Casciotta *et al.* 1995; Casciotta *et al.* 2006; Říčan & Kullander 2003, 2008) were described recently.

Říčan and Kullander (2006, 2008) have reviewed the species diversity of the genus *Australoheros* in the Río de la Plata basin. The authors reported a considerable diversity of this cichlid fish genus in this river drainage. Based

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on personal observation and also according to Ottoni and Costa (2008), Ottoni *et al.* (2008), Ottoni and Cheffe (2009) and Ottoni (2010), the *Australoheros* species from the rivers of the Atlantic coast of Brazil are rather similar to each other, with exception of *A. taura* Ottoni and Cheffe. The species from the Río de la Plata river drainages, on the other hand, show a wider spectrum of morphological and color pattern variation.

The highest diversity of *Australoheros* in the Río de la Plata basin is so far known from the río Uruguay drainage, which has four endemic species; *A. scitulus* (Říčan and Kullander), *A. charrua* Říčan and Kullander, *A. forquilha* Říčan and Kullander, *A. minuano* Říčan and Kullander. The río Paraná drainage has two endemic species (*A. guarani* Říčan and Kullander, *A. tembe* [Casciotta *et al.*]). Only two species are (in the Río de la Plata basin) presently known to occur in two separate river drainages (*A. facetus* [Jenyns], *A. kaaygua* Casciotta *et al.*).

New data have recently become available and demonstrate that the diversity described above is still underestimated, since *A. kaaygua* and *A. forquilha* as presently understood hide considerable variation, which better corresponds to four rather than two species. The aim of this paper is to describe this variation and to demonstrate that the species of *Australoheros* from the Río de la Plata basin reveal some interesting biogeographic patterns.

Material and methods

River names terminology. Rivers flowing through both Spanish and Portuguese speaking countries (*e.g.* Argentina *vs.* Brazil) usually vary in their names. Typical examples in our case are the río Iguazú (in Argentina), but rio Iguaçu (in Brazil), or the río Uruguay (in Argentina and Uruguay), but rio Uruguai in Brazil. We keep this difference in names throughout the text because it helps in pointing out which part of the river in which country we mean without the necessity to repeat the name of the country. If the river drainage is meant in general, the Spanish version is used. The rio Uruguai (Brazil) is not to be confused with the arroyo Urugua–í, which is a tributary of the río Paraná in Misiones, Argentina.

Morphological methods. In this work, we use character-based and tree-based approaches to analyze morphological characters as two tests of species delimitation.

Character-based delimitation. Character-based species delimitation involves finding diagnostic character states that represent seemingly fixed differences between the putative species, or differences that are at least non overlapping (*e.g.* Říčan & Kullander 2006). This approach is useful but lacks the clear relationship to estimated patterns of gene flow that the phylogenetic component of the tree-based approach offers.

Tree-based delimitation. Tree-based delimitation with morphology, although advocated by some authors (*e.g.* Baum & Donoghue 1995), has rarely been used by empirical systematists (*e.g.* Hollingsworth 1998; Wiens & Penkrot 2002; Říčan & Kullander 2006, 2008). The tree-based approach provides the parsimonious solution of character distribution, a homology hypothesis, and presents monophyletic groups, which are compared with results of the character-based approach. This two-step system, combining character- and tree-based approaches, has multiple advantages over a single step system (see Říčan and Kullander, 2006, 2008).
We complement our tree-based morphological delimitation with molecular data.

Characters. Measurements and counts were taken as described by Kullander (1986). Measurements were taken with digital calipers to 0.1 mm and are made point to point except for head length and snout length, which are projections from the anterior tip of the premaxilla to the orbital margin and the posterior margin of the gill cover, respectively. Scale rows are numbered as described by Kullander (1990), *i.e.* the horizontal row including the lower lateral line is designated as row E0, and the rows are counted as E1, E2 *etc.* dorsally, and H1, H2 *etc.* ventrally. Dorsal and anal fin rays, pterygiophores and vertebrae were counted on X-radiographs. Vertebral counts include the last halfcentrum. Color marking terminology follows Kullander (1983, 1986) and Říčan *et al.* (2005). Bars are counted and numbered in postero-anterior succession (Kullander 1983; Kullander & Silfvergrip 1991; Říčan *et al.* 2005). In the Description sections the number of specimens is indicated in parentheses, values of the holotype are indicated by an asterisk. Body length is expressed as standard length (SL).

Institutional abbreviations are as listed in Leviton *et al.* (1985) and Leviton and Gibbs (1988), except for AI (Asociación Ictiológica, La Plata, Argentina) and MACN-ict (Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Argentina).

Characters used in the present study include the following (plus color pattern) characters (HL: head length; SL: standard length): HL/SL, snout L/HL, body depth/SL, orbital diameter/HL, head width/HL, interorbital dist./HL,

preorbital dist./HL, caudal peduncle L/caudal peduncle depth, pectoral fin L/SL, ventral fin L/SL, last dorsal fin spine L/SL, and the following counts: scale counts (E0, L1, L2, scales between anterior insertion of the dorsal fin and the upper lateral line, scales between the posterior end of the upper lateral line and the dorsal fin, cheek scale rows), first ceratobranchial gill-rakers, caudal vertebrae, caudal peduncle vertebrae, anal pterygiophores anteriorly from the first haemal spine, anal-fin spines, anal-fin rays, anal-fin total, dorsal-fin spines, dorsal-fin rays, dorsal-fin total, pectoral-fin rays.

Molecular characters include the mitochondrial cytochrome *b* gene.

Molecular methods. Sequences of the mitochondrial cytochrome *b* gene from 38 specimens representing eight *Australoheros* species (and three outgroup taxa) make up our molecular data set (Table 1). New sequences have been deposited in GenBank under the following accession numbers: HQ197686–HQ197712.

DNA was extracted from small pieces of muscle or gill (10 to 25 mg) using the DNeasy™ Tissue Kit (Qiagen). The entire cytochrome *b* gene (1.3 kb) was PCR amplified with primers GLuDGL-TGA CTT GAA RAA CCA YCG TTG (Palumbi *et al.* 1991) and H15915-AAC TGC AGT CAT CTC CGG GTT ACA AGA C (Irwin *et al.* 1991). PCR reactions were carried out with initial denaturation at 94°C for 5 min, followed by 30 cycles with denaturation at 94°C for 1 min, primer annealing at 45 to 50°C for 40 s and primer extension at 72°C for 1 min. PCR was finished by final extension at 72°C for 5 min. PCR products were purified by ethanol precipitation or using Microcon PCR Filter Units (Millipore) and directly sequenced on an automated DNA sequencer using BigDye™ Terminator Cycle Sequencing Kit v.3.1 (PE Applied Biosystems). Sequencing reaction products were cleaned by ethanol precipitation or with DyeEx 2.0 Spin Kit (QIAGEN) and then resolved on ABI Prism 310 Genetic Analyser (Perkin Elmer). Except the amplification primers, the following additional primers were used for sequencing: modified L14952 of Lydeard *et al.* (1995; TCA TCC GTC GCC CAC AT), modified L15162 of Taberlet *et al.* (1992; CCA TGA GGA CAA ATA TC), and L15299 (Lydeard & Roe 1997). Chromatograms were assembled and checked by eye for potential mistakes using SEQMAN II of the DNASTAR software package (http://www.dnastar.com). Edited sequences were aligned using the default settings in ClustalX software (Thompson *et al.* 1997). The alignment was manually revised in BIOEDIT (Biological sequence alignment editor v5.0.9, http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The alignment includes no gaps.

Phylogenetic analyses. The morphological data set is coded with populations as terminal units (PTU) to enable tree-based species delimitation. The morphological matrix inludes 39 characters, of which 26 are multistate and 20 are ordered. See Appendices 1 and 2 for details. Morphological data for the Atlantic coast species of Brazil are taken from the respective species descriptions.

Qualitative characters were coded using the majority approach. Some characters, such as the number of abdominal bars have been coded using the scaled coding (Campbell & Frost 1993). The states are ordered under the assumption that traits pass through a polymorphic stage between absence and fixed presence. The scaled method is advantageous in that it allows polymorphisms to act as synapomorphies.

Quantitative characters have been coded using the gap weighting method (GW) of Thiele (1993). Thiele's implementation of gap weighting involves finding (for a given character) the mean value of the trait in each species in the analysis, the range of mean species values among taxa (*i.e.* the species with the greatest mean value and the species with the lowest), and then dividing this range into smaller ranges or segments equal to the maximum number of character states allowed by the phylogenetic software program (*e.g.* 32 for PAUP*; Swofford 2001). We have used a less fine grained spacing, thus having in most cases less than 32 states. Species are then assigned states based on these ranges, and the character is ordered. Evolving from low to high mean trait values (or vice versa) therefore requires passing through many intermediate states and requires many steps, whereas smaller changes in trait values involve fewer state changes and fewer steps. An important advantage of the gap-weighting method is that it incorporates information on the distance between states, weighting the changes according to the difference between mean species values.

We have used the between-state scaling (Wiens 2001) to weight quantitative characters against qualitative characters. This weighting scheme assigns transformations between species with fixed, adjacent values of meristic variables (*e.g.* 13 to 14 vertebrae) the same weight as changes in binary variables (0 to 1), and species with intermediate mean values (*e.g.* 13.5) receive proportionally intermediate weights. The consistency index is reported with uninformative characters excluded.

The phylogenetic analyses were performed using PAUP* 4b.10 (Swofford 2001) with maximum parsimony (MP). Analyses included 500 random sequence additions, 10 trees kept per addition, and a hs (heuristic) search on

TABLE 1. Locality data for DNA samples. Accession numbers highlighted in bold indicate new sequences generated for this study.

Species	DNA label	Locality	Drainage	GPS	GenBank
A. angiru		Brazil, Santa Catarina	rio Iguaçu		AY998658
A. facetus	A24	Paraguay, Itapua, P09-03	río Paraná	27°05'26.16"S, 55°53'13.02"W	**HQ197709**
A. facetus	A25	Paraguay, Itapua, P09-04	río Paraná	27°05'26.16"S, 55°53'13.02"W	**HQ197710**
A. facetus	A26	Argentina, Corrientes, Laguna Iberá	río Paraná	28°32'47.28"S, 57°11'44.70"W	**HQ197711**
A. facetus	A27	Argentina, Corrientes, Laguna Iberá	río Paraná	28°32'47.28"S, 57°11'44.70"W	**HQ197712**
A. facetus	H18	Argentina, Catamarca	río Paraná	28°28'08.37"S, 65°46'44.30"W	**HQ197703**
A. facetus	H19	Argentina, Catamarca	río Paraná	28°28'08.37"S, 65°46'44.30"W	**HQ197704**
A. facetus		Argentina, Entre Ríos	río Uruguay		AY843387
A. facetus		Argentina, Entre Ríos	río Uruguay		AY998665
A. facetus		Argentina, Entre Ríos	río Uruguay		AY998667
A. facetus		Uruguay, Maldonado	Río de la Plata		AY998666
A. forquilha	A22	Brazil, Rio Grande do Sul, B902, río forquilha	rio uruguai	27°37'26.34"S, 51°45'00.12"W	**HQ197707**
A. forquilha	A23	Brazil, Rio Grande do Sul, B902, río forquilha	rio uruguai	27°37'26.34"S, 51°45'00.12"W	**HQ197708**
A. kaaygua	H1	A07-02, arroyo Lobo	río Iguazú	25°42'34.79"S, 54°05'39.42"W	**HQ197686**
A. minuano		Uruguay, Salto	río Uruguay		AY998659
A. scitulus	A20	Argentina, Misiones, arroyo Itacaruare, A09-01	río Uruguay	27°52'33.80"S, 55°16'35.07"W	**HQ197705**
A. scitulus	A21	Argentina, Misiones, arroyo Itacaruare, A09-02	río Uruguay	27°52'33.80"S, 55°16'35.07"W	**HQ197706**
A. scitulus	H16	Argentina, Corrientes, A07-23	río Uruguay	29°36'49.60"S, 58°07'6.12"W	**HQ197701**
A. scitulus	H17	Argentina, Corrientes, A07-24	río Uruguay	29°36'49.60"S, 58°07'6.12"W	**HQ197702**
A. scitulus		Uruguay, Colonia	Río de la Plata	34°19'07" S, 59°20'13" W	AY998662

continued next page

TABLE 1. (continued)

Species	DNA label	Locality	Drainage	GPS	GenBank
A. scitulus		Uruguay, Colonia	Río de la Plata	34°19'07" S, 59°20'13" W	AY998661
A. scitulus		Argentina, Entre Ríos	Río Uruguay	31°53'55" S, 58°19'55" W	AY998663
A. tembe	H2	Argentina, Misiones, A07-04, arroyo Falso Urugua–í	arroyo Urugua–í, río Paraná	25°58'26.20"S, 54°15'28.78"W	**HQ197687**
A. tembe	H3	Argentina, Misiones, A07-04, arroyo Falso Urugua–í	arroyo Urugua–í, río Paraná	25°58'26.20"S, 54°15'28.78"W	**HQ197688**
A. tembe		Argentina, Misiones, arroyo Tirica	arroyo Urugua–í, río Paraná		AY998660
A. tembe		Argentina, Misiones	arroyo Urugua–í, río Paraná		AY843373
A. ykeregua	H4	Argentina, Misiones, A07-08, arroyo Fortaleza	río Uruguay	26°45'56.63"S, 54°10'57.43"W	**HQ197689**
A. ykeregua	H5	Argentina, Misiones, A07-08, arroyo Fortaleza	río Uruguay	26°45'56.63"S, 54°10'57.43"W	**HQ197690**
A. ykeregua	H6	Argentina, Misiones, A07-10A, arroyo Paraiso	río Uruguay	27°14'15.07"S, 54°02'38.49"W	**HQ197691**
A. ykeregua	H7	Argentina, Misiones, A07-10A, arroyo Paraiso	río Uruguay	27°14'15.07"S, 54°02'38.49"W	**HQ197692**
A. ykeregua	H8	Argentina, Misiones, A07-10B, arroyo Paraiso	río Uruguay	27°14'15.07"S, 54°02'38.49"W	**HQ197693**
A. ykeregua	H9	Argentina, Misiones, A07-10B, arroyo Paraiso	río Uruguay	27°14'15.07"S, 54°02'38.49"W	**HQ197694**
A. ykeregua	H10	Argentina, Misiones, A07-11, arroyo Shangai	río Uruguay	27°28'13.83"S, 54°41'24.52"W	**HQ197695**
A. ykeregua	H11	Argentina, Misiones, A07-11, arroyo Shangai	río Uruguay	27°28'13.83"S, 54°41'24.52"W	**HQ197696**
A. ykeregua	H12	Argentina, Misiones, A07-12, arroyo Guerrero	río Uruguay	27°45'57.45"S, 55°09'33.75"W	**HQ197697**
A. ykeregua	H13	Argentina, Misiones, A07-12, arroyo Guerrero	río Uruguay	27°45'57.45"S, 55°09'33.75"W	**HQ197698**
A. ykeregua	H14	Argentina, Misiones, A07-13, arroyo Tamandua	río Uruguay	27°05'56.53"S, 54°45'48.89"W	**HQ197699**
A. ykeregua	H15	Argentina, Misiones, A07-13, arroyo Tamandua	río Uruguay	27°05'56.53"S, 54°45'48.89"W	**HQ197700**

the saved trees to find all the shortest trees. Bootstrap analyses were done using the same approach, with 5 random sequence additions per one bootstrap. Bootstrap analyses were run with 1000 replications.

Characters have been mapped onto phylogeny using the software package Mesquite (Maddison and Maddison 2004).

Since the sister group of *Australoheros* is not established (Říčan *et al.* 2008), we have used a composite outgroup based on a reconstructed ancestor of the CAM heroine cichlids (Říčan *et al.* 2008).

Molecular data set. The molecular cytochrome *b* matrix was analyzed using MP in PAUP* 4b.10 with the same settings as the morphological data set and with Bayesian inference (BI) using MrBayes version 3.01 (Huelsenbeck & Ronquist 2001). The evolutionary model that best fits the analyzed sequence data set was selected using Modeltest and the Akaike information criterium (Posada & Crandall 1998). The Bayesian tree was inferred using the selected GTR+I+G model with partitioning by codon, with two MCMC chains for 5 million generations, sampling each hundredth tree, and discarding first 25% trees as burn-in. Statistical support for recovered clades was assessed using posterior probabilities (BI) and bootstrap (MP).

All molecular divergences mentioned in this text are uncorrected pairwise divergences reported by PAUP* with the use of the command 'showdist'.

Results

Tree-based delimitation. The phylogenetic analysis of the morphological matrix of 39 characters (Appendices 1 and 2) resulted into two MP trees (L=693; CI=0.51; RI=0.66) (Fig. 1). The two trees differ only in the internal topology of *A. angiru*. *Australoheros ykeregua* is found as the sister group of *A. forquilha*. *Australoheros kaaygua* and *A. angiru* are not conspecific, and not even sister groups. The validity of all species, including *A. ykeregua* and *A. angiru*, are supported by this morphological tree-based delimitation.

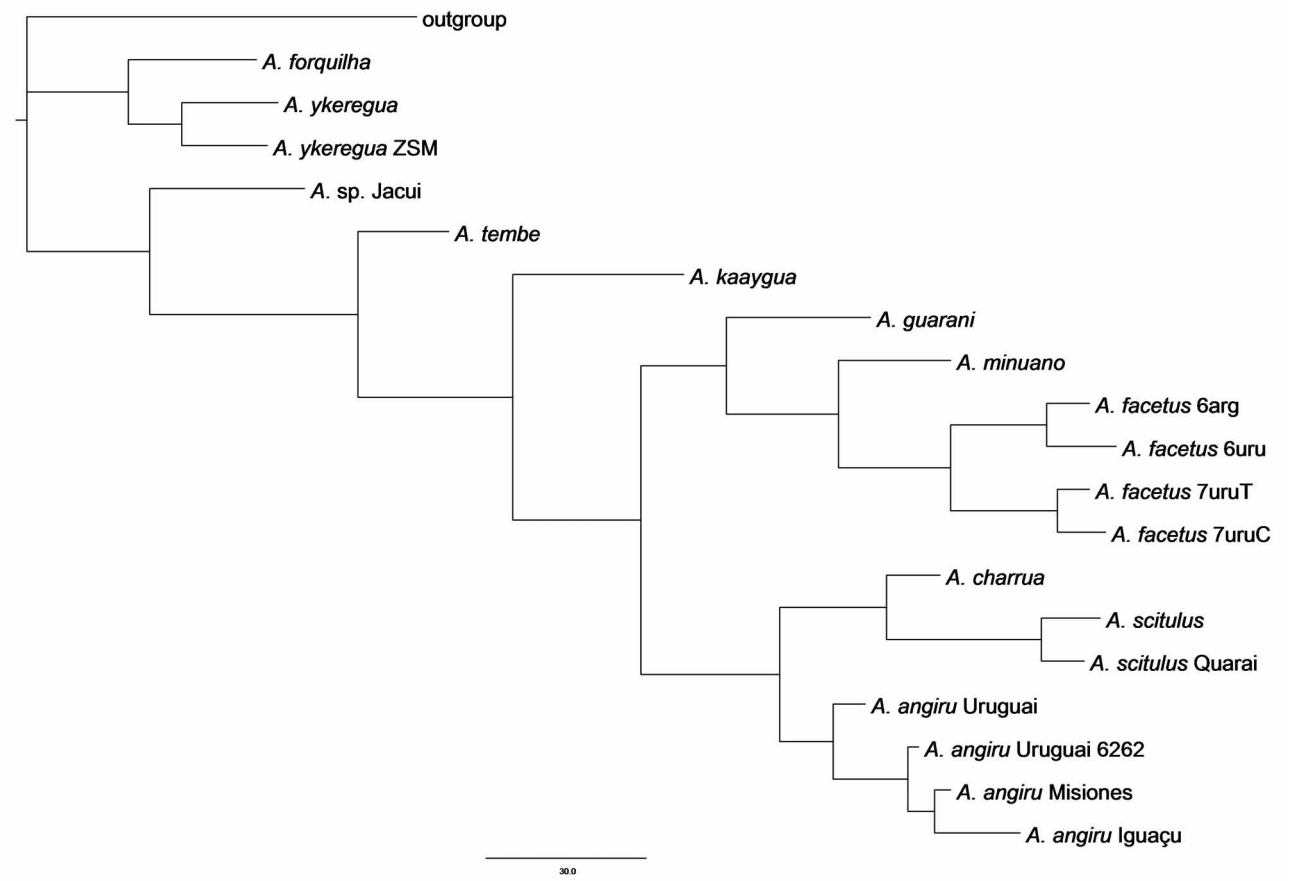

FIGURE 1. Tree-based delimitation using MP phylogenetic analysis of morphological data. The tree shown is one of two MP trees (L= 693; N=2; CI=0.51; RI=0.66), which differ only in the internal topology of *A. angiru*. Branch lengths represent morphological divergences.

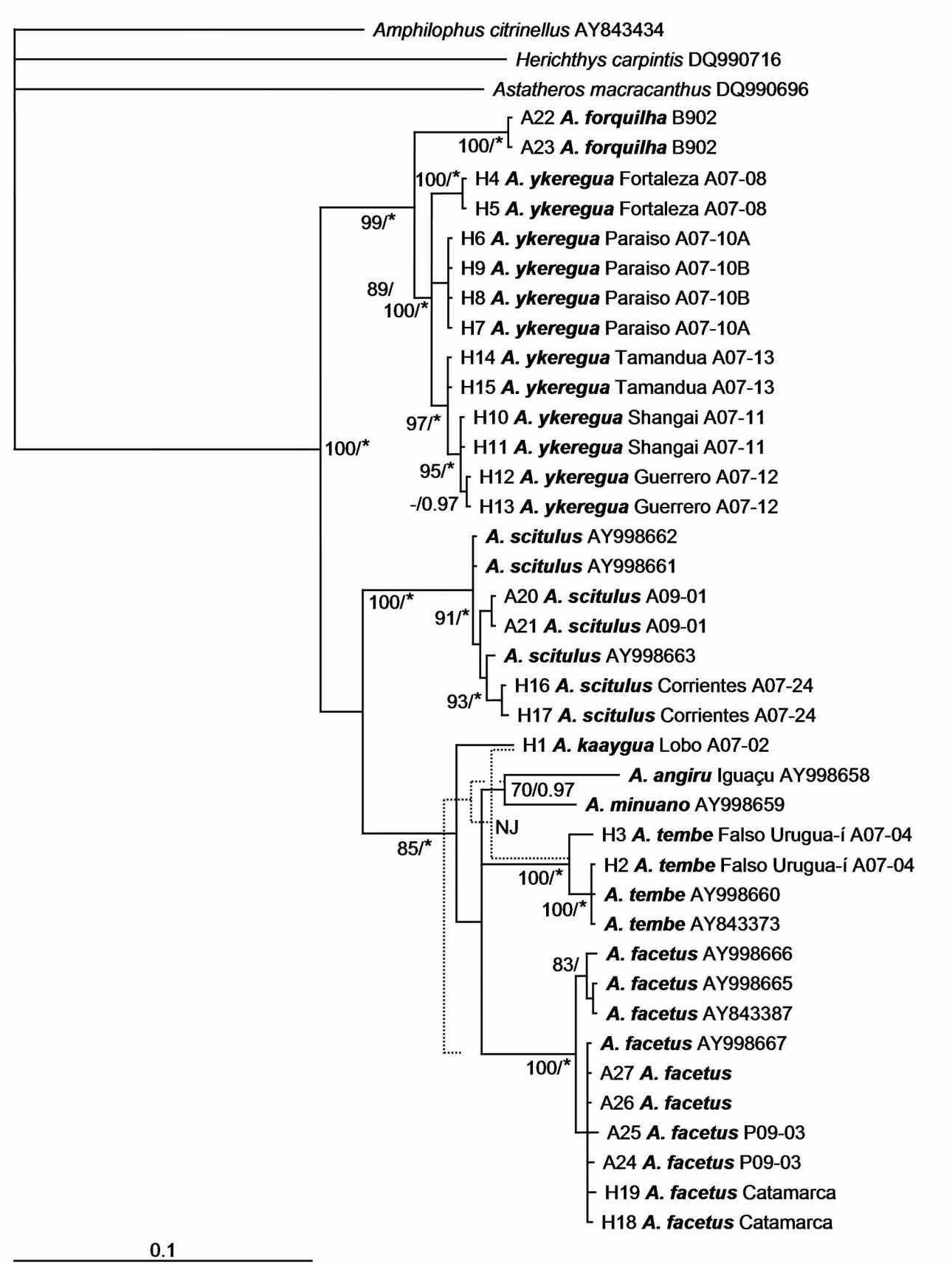

FIGURE 2 . Molecular phylogeny of the Río de la Plata basin *Australoheros* species using BI. Node support values shown for MP/BI analyses. The alternative dotted topology represents neighbor-joining (NJ) analysis. Asterisk denotes posterior probability of 1.00.

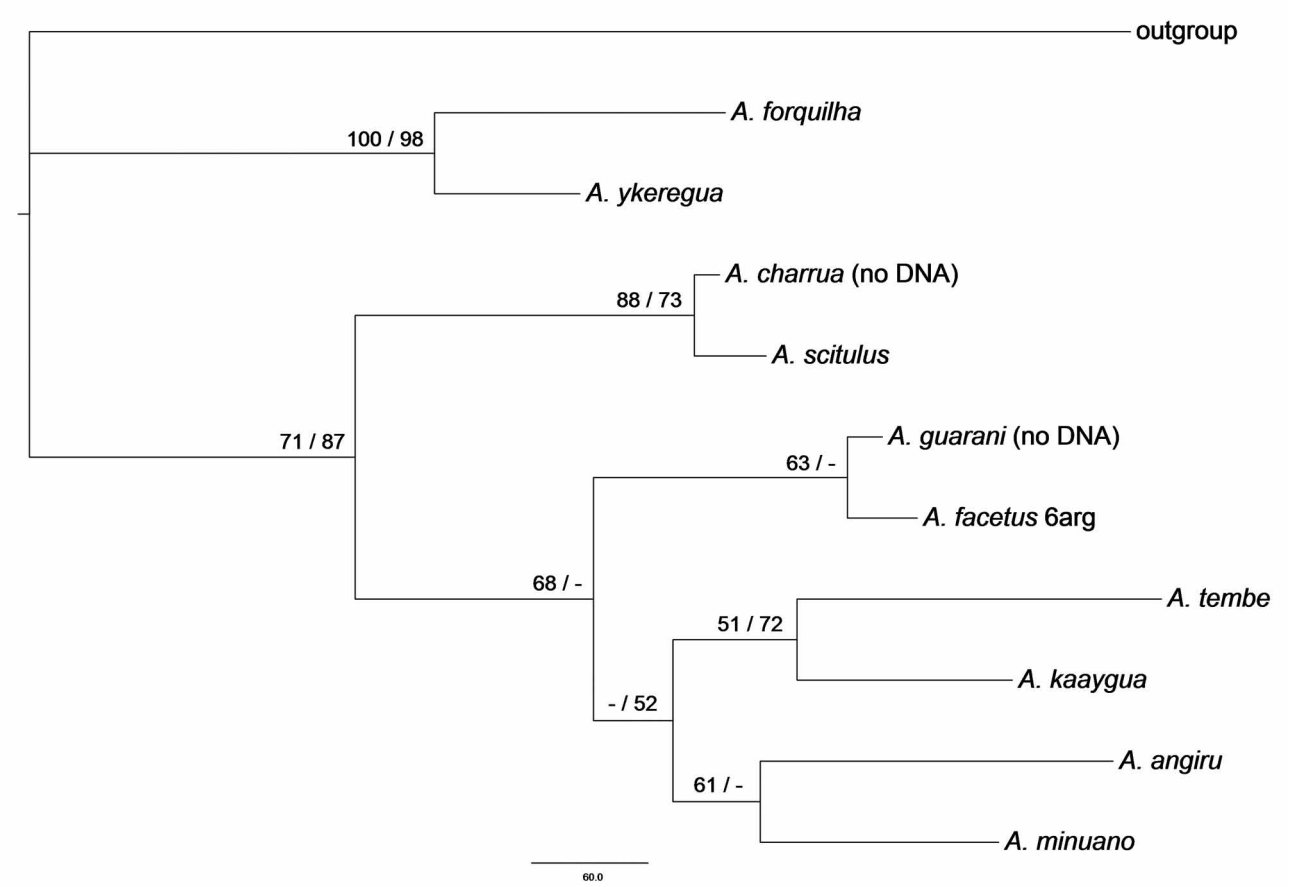

FIGURE 3. Combined MP morphological-molecular phylogeny with between-state scaling internal weighting between morphological and molecular data (L=2457; N=1; CI=0.58; RI=0.49). Node support values show MP bootstrap for two types of analyses (left: between-state scaling internal weighting structure / right: all characters weighted equally).

The phylogenetic analysis of the molecular cyt*b* matrix is shown in Fig. 2. The results are similar to those from the morphological analysis, with *A. ykeregua* and *A. forquilha* as sister groups, and *A. kaaygua* and *A. angiru* as not conspecific and not immediately related. The validity of all species is again supported.

The combined morphological-molecular phylogenetic analysis (Fig. 3) supports the results of the independent analyses (Figs 1 and 2). *Australoheros ykeregua* and *A. forquilha* are sister groups, with a mean divergence of 2.3% in the cyt*b* gene. *Australoheros kaaygua* and *A. angiru* are not conspecific, separated by a divergence of 4.8% in the cyt*b* gene. *Australoheros angiru* is the sister species of *A. minuano* (cyt*b* divergence of 4.2%). *Australoheros kaaygua* is the sister group of *A. tembe* (mean cyt*b* divergence of 3.8%). *Australoheros guarani* and *A. facetus*, and *A. scitulus* and *A. charrua* are additional sister groups (DNA data not available for *A. guarani* and *A. charrua*).

Our tree-based delimitation analyses thus support the distinctivness of *A. ykeregua* from *A. forquilha*, and of *A. angiru* from *A. kaaygua*.

Character-based delimitation. Character based delimitation, in agreement with tree-based delimitation, supports the distinctivness of *A. ykeregua* from *A. forquilha*, and of *A. angiru* from *A. kaaygua* (Tables 2 and 3). For separating characters see the taxonomy section below.

TABLE 2. Meristics of the two new species (*A. ykeregua*, *A. angiru*) and the two species with which they have been previously associated (*A. forquilha*, *A. kaaygua*).

Dorsal fin count frequences		XIV 12	XV 8	XV 9	XV 10	XV 11	XVI 7	XVI 8	XVI 9	XVI 10	XVI 11	XVII 7	XVII 8	XVII 9	XVII 10
A. ykeregua					3	3			3	16	22			1	2
A. forquilha						1				5	5				
A. angiru	upper rio Uruguai								16	13			2		
	rio Iguaçu								3	1					
A. kaaygua			1	2			1	8	1					1	

Anal fin count frequences		V 7	V 8	V 9	VI 6	VI 7	VI 8	VI 9	VII 7	VII 8	VII 9	VIII 6	VIII 7	VIII 8
A. ykeregua			3	1		14	29	3						
A. forquilha						1	7	1		2				
A. angiru	upper rio Uruguai					2	3		17	8		1		
	rio Iguaçu								1	3				
A. kaaygua		2			7	5								

		vertebrae 13 12	13 13	13 14	13 15	14 12	14 13	14 14	pectoral fin rays 12	13	14	15	C1 gill rakers 5	6	7	8	9
A. ykeregua			3	19			3			14	18	15			3	13	4
A. forquilha				5	6					6	3				4	5	
A. angiru	upper rio Uruguai		29	2					11	5			4	11	1		
	rio Iguaçu	1	3							4			1	2	1		
A. kaaygua			1						9	5				1			

		caudal peduncle vertebrae -2	-1.5	-1	-0.5	0	0.5	1	1.5	2	2.5	3	3.5
A. ykeregua								1	5	11	6	1	
A. forquilha										3	3	5	
A. angiru	upper rio Uruguai			3	1	4	3	10	1				
	rio Iguaçu		2	2									

		anal pterygiophores 11 1	11 2	12 1	12 2	13 1	13 2	13 3	14 1	14 2	14 3	15 2	dorsal pterygiophores 9	10	11	12
A. ykeregua		10	5	3	4									6	16	
A. forquilha			1	6	2	2									5	6
A. angiru	upper rio Uruguai	1	1	7	8	3	3						1	13	9	
	rio Iguaçu			1		1	2							3	1	
A. kaaygua				1												

		E0 scale counts 23	24	25	26	L1 scale counts 13	14	15	16	17	18	19	L2 scale counts 6	7	8	9	10	11
A. ykeregua			1	32	13		1	1	5	15	19	5		1	4	26	10	5
A. forquilha					9					2	5	2			4	3	2	
A. angiru	upper rio Uruguai	3	16	4					1	6	8			4	7	4		
	rio Iguaçu																	

Taxonomy

Australoheros ykeregua sp. nov.

(Figs. 4, 5, 6, 7).

"Cichlasoma" cf. *tembe* (arroyo Fortaleza)—Casciotta et *al.* 2003: 68, 70
"Cichlasoma" cf. *tembe*—Stawikowski and Werner 2004: 455
Australoheros sp. Forquilha—Říčan and Kullander 2006: 6
Australoheros forquilha (non-type material from ZSM)—Říčan and Kullander 2008: 16

TABLE 3. Proportional measurements in percents of standard length (SL) of the two new species (*A. ykeregua, A. angiru*) and the two species with which they have been previously associated (*A. forquilha, A. kaaygua*). SD=standard deviation.

		A. forquilha			*A. ykeregua*	
	N	Min-Max	Mean ± SD	N	Min-Max	Mean ± SD
Head length	10	31.5 – 34.6	33.2 ± 1.2	49	33.2 – 39.1	36.2 ± 1.2
Snout length	10	7.6 – 12.6	10.5 ± 1.6	49	8.8. – 18.4	14.9 ± 2.3
Body depth	10	40.9 – 46.6	43.9 ± 1.9	49	41.7 – 47.8	44.9 ± 1.5
Orbital diameter	10	9.3 – 12.6	11.3 ± 0.8	49	8.1 – 13.8	10.5 ± 1.4
Head width	10	15.6 – 18.0	16.5 ± 0.7	49	16.0 – 19.1	17.6 ± 0.6
Interorbital width	10	8.7 – 11.5	10.1 ± 0.9	49	8.7 – 14.3	10.9 ± 1.6
Preorbital distance	10	6.4 – 10.8	9.1 ± 1.4	49	6.4 – 12.3	9.3 ± 1.3
Caudal peduncle depth	10	16.6 – 18.3	17.4 ± 0.5	49	15.6 – 18.8	17.2 ± 0.7
Caudal peduncle length	10	8.9 – 11.1	10.2 ± 0.7	49	8.4 – 13.9	10.9 ± 1.3
Pectoral fin length	10	25.6 – 29.5	26.9 ± 1.2	49	25.9 – 32.5	29.4 ± 1.6
Ventral fin length	10	22.1 – 29.6	26.1 ± 2.2	49	23.3 – 34.7	29.6 ± 2.0

continued.

		A. angiru			*A. kaaygua*	
	N	Min-Max	Mean ± SD	N	Min-Max	Mean ± SD
Head length	16	31.7 – 36.2	33.3 ± 1.5	13	35.2 – 38.4	37.0 ± 1.02
Snout length	16	7.8 – 11.4	9.5 ± 0.9	13	8.9 – 13.0	10.9 ± 1.16
Body depth	16	46.2 – 51.5	49.6 ± 1.2	13	40.7 – 46.7	43.8 ± 1.71
Orbital diameter	16	10.8 – 13.5	11.8 ± 0.8	13	9.8 – 12.9	11.2 ± 1.19
Head width	16	16.4 – 20.5	17.7 ± 1.2	13	17.9 – 23.4	19.6 ± 1.4
Interorbital width	16	10.3 – 12.7	11.8 ± 0.6	13	10.1 – 15.1	11.7 ± 1.42
Preorbital distance	16	6.3 – 8.3	7.3 ± 0.6	13	7.3 – 11.0	8.9 ± 1.25
Caudal peduncle depth	16	17.8 – 19.4	18.5 ± 0.5	13	13.9 – 17.6	16.2 ± 1.0
Caudal peduncle length	16	5.5 – 9.2	7.4 ± 0.9	13	8.9 – 11.0	10.4 ± 0.79
Pectoral fin length	16	28.1 – 32.4	30.3 ± 1.4	13	27.3 – 31.7	29.0 ± 1.38
Ventral fin length	16	28.3 – 37.6	32.4 ± 3.1	13	26.4 – 35.3	28.8 ± 2.81

Holotype. MACN-ict 9467, 102.0 mm SL, Argentina, río Uruguay basin, arroyo Paraiso (or Canal Muerto), 27°14'15.1" S, 54°02'38.5" W, col: Říčan *et al.*, December 2007.

Paratypes. 30 specimens, 39.5–136.8 mm SL, all from Argentina, Misiones province, río Uruguay basin. MACN-ict 9468, 4 ex., 39.5–108.7 mm SL, same data as holotype. MACN-ict 9469, 3 ex., 101.1–136.8 mm SL, arroyo Fortaleza, 26°45'56.6" S, 54°10'57.4" W, col: Říčan *et al.*, December 2007. AI 270, 3 ex. (C&S), 57.0–64.0 mm SL, arroyo Fortaleza, 26°45'56.6" S, 54°10'57.4" W, col: Casciotta *et al.*, April 2000. MACN-ict 9470, 3 ex., 90.5–112.0 mm SL, arroyo Guerrero, 27°45'57.4" S, 55°09'33.7" W, col: Říčan *et al.*, December 2007. MACN-ict 9471, 4 ex., 86.5–102.1 mm SL, arroyo Shangai or arroyo Pindaiti, 27°28'13.8" S, 54°41'24.5" W, col: Říčan *et al.*, December 2007. MACN-ict 9472, 13 ex., 47.0–86.3 mm SL, arroyo Tamandua, 27°05'56.5" S, 54°45'48.9" W, col: Říčan *et al.*, December 2007.

Additional non-type material. ZSM 23060b, 6 ex., río Soberbio, El Soberbio, col: J. Foerster, 1966. ZSM 23482b, 13 ex., río Soberbio, El Soberbio, col: J. Foerster, 1966. ZSM 23482c, 2 ex. (C&S), río Soberbio, El Soberbio, col: J. Foerster, 1966.

Diagnosis. *Australoheros ykeregua* is distinguished from all *Australoheros* species except *A. forquilha* (with which it was previously associated) in having a series of opalescent pale blue dots along the postero-lateral border

of the suborbital series (dark markings in preserved specimens), in having checkerboard-spotted dorsal, anal and caudal fins (red spots in live animals and dark grey in preserved specimens), a red to orange branchiostegal membrane, mouth and lower head area and base of pectoral fin, by having comparativelly thick lips (shared also with *A. tembe*), the lower jaw shorter than the upper, by having 25–26 E0 scales (vs. less than 25), by having the longest dorsal fin scale cover (shared also with *A. tembe*), and by the narrowest head (head width less than 50% *vs.* more than 50% of HL), shortest interorbital (10.9% of SL) and longest preorbital (9.3% of SL) distances.

Australoheros ykeregua is distinguished from *A. forquilha* by not having opalescent pale blue dots on each body scale, by not having them widely distributed on the head, but limited to a single line below the suborbital series, and in having a red coloration limited to the head region and the base of the pectoral fin (*vs.* red coloration on the whole belly to the end of the anal fin). Further distinguished by lower counts of caudal vertebrae (13–14 *vs*. 14–15), less caudal peduncle vertebrae (modally 2 *vs.* modally 3), lower total dorsal fin counts (25–26 *vs.* 26–27) and 25 *vs.* 26 E0 scales.

Australoheros ykeregua is distinguished from the only other similar species, *Australoheros tembe*, by the above listed unique characters and by coloration (shared only with *A. forquilha*) and additionally by a shorter caudal peduncle (including 2 *vs.* 3 vertebrae) and more dorsal fin rays (10–11 *vs.* 9).

For distinguishing characters from all other *Australoheros* species see the Notes section.

FIGURE 4. *Australoheros ykeregua*, MACN-ict 9467, 102.0 mm SL, holotype, right side (reversed). This specimen does not show vertical bars after preservation, but see Fig. 7 of the same specimens photographed alive.

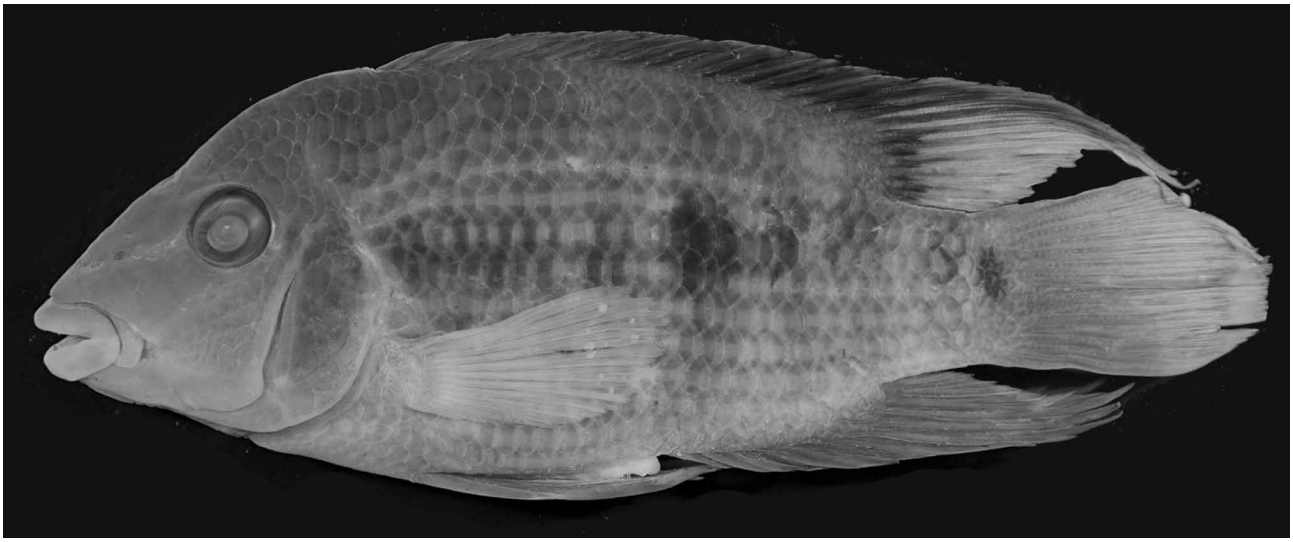

FIGURE 5. *Australoheros ykeregua*, MACN-ict 9470, 90.5 mm SL. This specimens shows the dark color of the dorsal fin and the midlateral blotch and vertical bars.

FIGURE 6. *Australoheros ykeregua*, MACN-ict 9472, 66.2 mm SL. This specimen shows a continuous lateral band extending beyond the midlateral spot and the checker-board spot pattern of unpaired fins (also evident in the holotype and the majority of specimens).

Description. Based on specimens over 60 mm SL. Meristic data are summarized in Table 2, morphometric data are summarized in Table 3.

Body rather slender (44.9% SL), head with a rounded profile, mouth subterminal with comparativelly thick lips, short interorbital (10.9% SL) and long preorbital (9.3% SL) distances. Lacrimal bone deeper than wide. A rather long caudal peduncle containing modally two vertebrae, 14 caudal vertebrae. Caudal peduncle considerably deeper than long (mean length 63% of depth).

Scales on chest smaller than half the size of the biggest scales in the E0 row above the pectoral fin. About 8 scale rows between the opercular flap and the anterior insertion of the pelvic fin in the holotype. Scales in E0 row 24(1), 25(32*), 26(13). Upper lateral line scales 14(1), 15(1), 16(5), 17(15), 18(19*), 19(5). Lower lateral line scales 7(1), 8(4), 9(26*), 10(10), 11(5). Scales between upper lateral line and dorsal-fin scale cover 3 posteriorly, 4 plus two small parallel scales anteriorly, forming a sheath of smaller scales arranged in pairs per scale row, along the insertion of the dorsal fin. Cheek scale rows 4(2), 5(16*), 6(1). Dorsal fin with interradial scales appearing from 13^{th}(1), 14^{th}(6), 15^{th}(8), 16^{th}(4*) spine membrane, in single rows. One (5), two (10*) or three (4) last interradial membranes without scales. Anal fin with one basal scale row; interradial scales in single rows, from the 4^{th}(1), 5^{th}(8), 6^{th}(10*) spine membrane lacking on one (17*) or two (2) last interradial membranes. Caudal fin densely scaled, scales ctenoid; interradial scales in one or two rows; posterior margin of scaly area concave, extending to between one-third and middle of caudal fin.

Soft dorsal fin pointed, extending to the middle or almost to the end of the caudal fin. D. XV,10(3), XV,11(3), XVI,9(2), XVI,10(16), XVI,11(22*), XVII,9(1), XVII,10(2). Soft anal fin pointed, of about the same length as dorsal fin. A. V,8(3), V,9(1), VI,7(14), VI,8(29*), VI,9(3). Anal fin pterygiophores 11(15), 12(7). Pelvic fin base slightly posterior of pectoral fin base; first branched ray longest. Pelvic fin not reaching (2), reaching (10) or surpassing (7*) anal fin origin. Pectoral fin shorter than pelvic fin, with a rounded tip. P. 13(14), 14(18), 15(15*). Caudal fin with rounded corners.

Oral jaw teeth caniniform, slightly curved. Outer row teeth increasing in size symphysiad, upper-jaw anterior teeth more robust, lower-jaw anterior teeth subequal.

Lower pharyngeal tooth plate in a dissected specimen about one quarter wider than long (length 59–62% of width). Dentigerous area wider than long. 7–9 teeth along midline, 22–26 teeth along posterior margin. Posterior teeth tend to be progressively more compressed, except for medial teeth. Larger teeth medially and posteriorly, gradually smaller anteriad and laterad. Posterior teeth with forwards curved posterior cusp and subapical anterior shelf. Large laterally compressed teeth with a second cusp projecting anteriorly from shelf.

Gill rakers externally on first gill arch: 1-2 epibranchial, 1 in angle, 7(3), 8(13), 9(4) ceratobranchial.

Vertebrae 13+13=26(3), 13+14=27(19), 14+13=27(3). Caudal peduncle contains 1(1), 1.5(5), 2(11), 2.5(6), 3(1) vertebrae.

Color pattern in alcohol. Six or seven vertical flank bars, a midlateral blotch in the fourth flank bar (sensu Říčan *et al.* 2005), a caudal fin spot, and the caudal peduncle bar make up the principal markings. Base of caudal spot at level of the lower lateral line. Lateral band 1, 1/2 or 2 scales deep posteriorly from the posterodorsal edge of opercular to the midlateral blotch (not clearly visible in the holotype). Lateral band extending behind the midlateral blotch, widening towards the end of dorsal-fin base level in five adult specimens and in eight juveniles (arroyo Tamandua, MACN-ict 9472).

Vertical bars are relatively wide, indistinct in their ventral parts. The fourth bar, bearing the midlateral blotch is centered above the anteriormost portion of the anal fin. Many thin parallel stripes on flanks, more evident on lower half of body.

Dorsal fin with a dark pigmentation from interradial membranes from 8th or 9th spine to 3rd to 4th branched ray. This pigmentation extended to the tip of the dorsal filament. Same dark pigmentation on basal third of the remaining branched rays. Soft dorsal and anal fins, and caudal fin with dark spots in a checker-board pattern on interradial membranes (missing in some specimens).

One (MACN-ict 9468, MACN-ict 9472), two (in the holotype MACN-ict 9467 and in MACN-ict 9468, MACN-ict 9472) or three (MACN-ict 9472) small and inconspicuous dark blotches below the orbit along the postero-lateral border of the suborbital series.

Life coloration. The most distinct color markings include the diagnostic 1) red/orange branchiostegal membrane, base of pectoral fin, mouth and lower head area, 2) the single interrupted line of blue dots along the suborbital series (dark blotches in preserved specimens), and 3) the checkerboard pattern of red dots on unpaired fins (Fig. 7). This character combination is unique among *Australoheros*. The most similar species, *A. forquilha*, is easily distinguished in that the blue dots are not limited to a single line below the orbit. Instead, they cover the whole head and are present in all body scales and are also present on all fins except the pectorals (see Fig. 7 and also *"Cichlasoma"* cf. *tembe* in Stawikowski & Werner 2004, p. 455).

Distribution. *Australoheros ykeregua* is so far known only from Argentinean territory in the tributaries of the río Uruguay below the Salto Moconá, province of Misiones.

Etymology. The Guaraní word *ykeregua* means neighbor (vecino in Spanish). The etymology is based on the fact that *A. ykeregua* and *A. forquilha* have been preliminarily treated as conspecific (Říčan & Kullander 2008). New data have however demonstrated that they are two sister group species living in the same river drainage (río Uruguay), though not sympatrically.

Notes. Říčan and Kullander (2006, 2008) treated part of the ZSM non-type material from Argentina as conspecific with *A. forquilha*. New fresh material collected in 2007 has revealed that the Argentinean and Brazilian material do not represent the same species. The ZSM lots 23060 and 23482 have been divided since they contained two different species and lots ZSM 23060b, 23482b and 23482c hold *A. ykeregua*.

We hypothesize that the barrier between the two species, *A. forquilha* and *A. ykeregua*, is formed by the Salto Moconá on the río Uruguay just below the confluence with the río Pepirí Guazú (which forms the international border between Argentina and Brazil). The two species are closely related, but important differences in morphology and DNA demonstrate that there is no gene flow between them and they are thus evolutionarily independent units.

Additional diagnostic characters that separate *Australoheros ykeregua* from all other species except *A. forquilha* and *A. tembe* are as follows. From *A. facetus*, by having more caudal vertebrae (14 *vs*. 13), more caudal peduncle vertebrae (2 *vs*. 0–1), more E0 scales (25–26 *vs*. 24), and by a longer snouth (14.9 *vs*. 9.4 % SL) and a longer preorbital distance (9.3 *vs*. 5.7 % SL).

Australoheros ykeregua is additionally distinguished from *A. kaaygua* by having more caudal vertebrae (14 *vs*. 13), more C1 gill rakers (8 *vs*. 6), more caudal peduncle vertebrae (2 *vs*. 0–1), more E1 scales (18 *vs*. 16) and by a slightly longer snouth (14.9 *vs*. 10.9 % SL). It is additionally distinguished from *A. minuano* by lacking a pinkish body coloration of live specimens, by having more caudal vertebrae (14 *vs*. 13), more pectoral fin rays (14 *vs*. 12), more C1 gill rakers (8 *vs*. 6), more caudal peduncle vertebrae (2 *vs*. 0–1), more E0 scales (25–26 *vs*. 24), and by a longer snouth (14.9 *vs*. 10.6 % SL) and a longer preorbital distance (9.3 *vs*. 6.0 % SL).

Australoheros ykeregua is distinguished from *A. guarani* by also having more caudal vertebrae (14 *vs*. 13), more pectoral fin rays (14 *vs*. 13), more C1 gill rakers (8 *vs*. 7), more E0 scales (25–26 *vs*. 24), more caudal peduncle vertebrae (2 *vs*. 0–1), and by a shorter head (36.2 *vs*. 32.4 % SL), longer snouth (14.9 *vs*. 8.5 % SL), and less deep body (44.9 *vs*. 48.1 % SL). It is additionally distinguished from *A. charrua* by lacking a pinkish body coloration of live specimens, by less anal fin spines (5–6 *vs*. 7–8), more C1 gill rakers (8 *vs*. 6), more caudal peduncle

vertebrae (2 *vs*. 0–1), by a slightly longer head (36.2 *vs*. 32.4 % SL), slightly longer preorbital distance (9.3 *vs*. 7.3 % SL) and by a longer snouth (14.9 *vs*. 8.5 % SL).

FIGURE 7. Color plate. Horizontaly from upper left to lower right. *Australoheros forquilha*, rio Forquilha, rio Uruguai drainage, Rio Grande do Sul, Brazil (not preserved). *Australoheros ykeregua* (MACN-ict 9467, holotype), río Uruguay drainage, arroyo Paraiso (or Canal Muerto), Misiones province, Argentina. *Australoheros kaaygua* (MACN-ict 9473), río Iguazú drainage, small stream 7 km SW from Andresito, Misiones province, Argentina. *Australoheros angiru*, male in neutral colors, rio Chopim, rio Iguaçu drainage, Paraná, Brazil (not preserved). *A. angiru*, male and female in breeding colors guarding fry, same locality (not preserved). All *A. angiru* photographs courtesy of Wolfgang Staeck.

Australoheros ykeregua is additionally distinguished from *A. scitulus* in lacking the dark spot-markings on the head and anterior part of body, less dorsal fin spines (16 *vs*. 17), more dorsal fin rays (10–11 *vs*. 9–10), less anal fin spines (5–6 *vs*. 8–9), more pectoral fin rays (14 *vs*. 13), by more C1 gill rakers (8 *vs*. 6), more caudal peduncle vertebrae (2 *vs*. 0) and less deep body (44.9 *vs*. 47.7 % SL). It is also distinguished from *A. angiru* by lacking the yellow background coloration, yellow iris and red dorsal and ventral margins and corners of the caudal fin in live specimens, by having more dorsal fin rays (10–11 *vs*. 9–10), less anal fin spines (6 *vs*. 7), more caudal vertebrae (14 *vs*. 13), more pectoral fin rays (14 *vs*. 12), more C1 gill rakers (8 *vs*. 6), more E0 scales (25–26 *vs*. 24), more caudal peduncle vertebrae (2 *vs*. 0–1), a longer head (36.2 *vs*. 33.3 % SL), a longer snouth (14.9 *vs*. 9.5 % SL), a less deep body (44.9 *vs*. 49.6 % SL) and a longer preorbital distance (9.3 *vs*. 7.3 % SL).

Australoheros ykeregua is distinguished from *A. acaroides* by also having more caudal vertebrae (14 *vs*. 13), more caudal peduncle vertebrae (2 *vs*. 0–1), less anal fin spines (6 *vs*. 7), more E0 scales (25 *vs*. 23–24), more C1 gill rakers (8 *vs*. 6), and a smaller interorbital distance (33 *vs*. 43 % HL). It is additionally distinguished from *A. taura* by lacking a pink to red body coloration of live specimens, more caudal vertebrae (14 *vs*. 13), more C1 gill rakers (8 *vs*. 7), and a deeper body (44.9 *vs*. 41.4 % SL) and a smaller interorbital distance (33 *vs*. 41% HL).

Australoheros ykeregua is additionally distinguished from all the Atlantic coast species north of *A. acaroides* and *A. taura* (*A. autrani*, *A. barbosae*, *A. capixaba*, *A. ipatinguensis*, *A. macacuensis*, *A. macaensis*, *A. muriae*, *A. paraibae*, *A. ribeirae*, *A. robustus*, *A. saquarema*) by having more caudal vertebrae (14 *vs*. 12 or 13), more caudal peduncle vertebrae (2 *vs*. 0), less anal fin spines (6 *vs*. 7), a smaller interorbital distance (33 *vs*. 41% HL), and a shorter pelvic fin (<30 *vs*. >30 % SL).

Australoheros angiru **sp. nov.**

(Figs 7, 8, 9).

"Cichlasoma" facetum—Staeck 1998a: 62–63; 1998b: 81–85
"Cichlasoma" sp. Iguaçu—Staeck 2003: 64–65
"Cichlasoma" sp. Iguaçu—Stawikowski and Werner 2004: 455
Australoheros sp. jacutinga—Říčan and Kullander 2006: 6
Australoheros kaaygua—Říčan and Kullander 2008: 28 (in part)

Holotype. MCP 13937, 73.2 mm SL, Brazil, Santa Catarina State, rio Uruguai drainage, rio Jacutinga, road BR 283 from Ceará to Concordia, col: Bergmann *et al.*, October 1988.

Paratypes. 13 specimens, 24.6–77.0 mm SL, all from Brazil. Santa Catarina State, rio Uruguai drainage: MCP 13383, 6 ex., 24.6–77.0 mm SL, rio Jacutinga, road BR 283 from Ceará to Concordia, col: Reis *et al.*, February 1989. MCP 12509, 1 ex., 75.0 mm SL, same data as holotype. MCP 13011, 6 ex., 44.2–61.4 mm SL, rio Jacutinga, road BR 283 from Ceará to Concordia, col: Reis *et al.*, December 1988.

Additional non-type material. Paraná State, rio Iguaçu drainage: NUP 3913, 2 ex., rio São Pedro, tributary to rio Iguaçu, Pinhão county, 26º05´S, 51º45'W, col: Nupélia staff, March 1993. NUP 3914, 1 ex, rio Iratim (Linígrafo), tributary to rio Iguaçu, Palmas county, boundary with Pìnhão-PR, 26º05´S, 51º45´W, col: Nupélia staff, April 1993. NUP 3915, 1 ex, rio São Pedro, tributary to rio Iguaçu, Pinhão county, 26º05´S, 51º45'W, col: Nupélia staff, March 1993. Rio Grande do Sul State, rio Uruguai drainage: MCP 46328, 13 ex., Sanga das Aguas Frias, Irai, col: Malabarba *et al.*, 1985. Argentina, Misiones province, río Uruguay drainage: ZSM 23482a, 1 ex., P, río Soberbio, El Soberbio, col: J. Foerster, 1966. ZSM 23060a, 4 ex., río Soberbio, El Soberbio, col: J. Foerster, 1966. ZSM 23060c, 2 ex. (C&S), río Soberbio, El Soberbio, col: J. Foerster, 1966.

Diagnosis. *Australoheros angiru* is one of the most deep-bodied species of *Australoheros* (body depth in SL >49%; shared with *A. guarani* and *A. facetus*). It has been previously associated with *A. kaaygua*, but it is the sister species of *A. minuano* based on DNA characters.

Australoheros angiru is distinguished from *A. kaaygua* by having less scale rows between anterior end of dorsal fin and upper lateral line (ch4 states 1–2 *vs.* 0), by a very narrow or missing caudal base spot, by a pure yellow ground color (*vs.* yellowish-green), by yellow eyes (*vs.* dark green), by more scales between anterior end of dorsal fin and upper lateral line (5 *vs.* 4), more anal fin spines (7 *vs.* 6), more anal fin rays (> 7 *vs.* < 7), more dorsal fin rays (9 *vs*. 8), less E0 scales (24 *vs.* > 25), more L1 scales (> 17–18 *vs.* 16), less L2 scales (8 *vs.* > 9), and by a being more deep-bodied (49.6% *vs.* 43.8% SL), and having a shorter caudal peduncle (7.4% *vs.* 10.4% SL).

Australoheros angiru is distinguished from *A. minuano* by a large and dominant midlateral blotch, very narrow or missing caudal base spot, by lacking a pinkish body coloration, by a small terminal or subterminal mouth (*vs*. large supraterminal), by more scales between the anterior end of the dorsal fin and the upper lateral line (5 *vs.* 4), less anal fin rays (7 *vs.* 8), less dorsal fin rays (9 *vs.* 10), and by slight differences in body depth (49.6% *vs.* 46.9% SL) and in preorbital distance (7.3% *vs.* 6.0% SL).

For distinguishing characters to all other *Australoheros* species see the Notes section.

FIGURE 8. *Australoheros angiru*. Holotype, MCP 13937, 73.2 mm SL, rio Jacutinga, rio Uruguai drainage, Brazil.

FIGURE 9. *Australoheros angiru*. Paratype, MCP 13011, 48.1 mm SL, rio Jacutinga, rio Uruguai drainage, Brazil.

Description. Based on specimens over 60 mm SL, with notes on smaller specimens. Meristic data are summarized in Table 2, morphometric data are summarized in Table 3.

Comparatively deep bodied (mean body depth 49.6% SL). Snout short, straight in lateral view. Jaws isognathous. Mouth small.

Scales on head and chest not distinctly smaller than on flanks. Scales in E0 row 23(3), 24(16*), 25(4). Upper lateral line scales 16(1), 17(6*), 18(8). Lower lateral line scales 7(4), 8(7*), 9(4). Scales between upper lateral line and dorsal fin 4 anteriorly, 1 large plus 1 small posteriorly. Cheek scale rows 3(14*), 4(2). About 8 scale rows between the opercular flap and the anterior insertion of the pelvic fin. Dorsal fin with one basal scale row, starting from the 7th or 8th spine and running posteriad; interradial scales appear from 14th or 15th spine membrane, in single rows. Anal fin with one basal scale row; interradial scales in single rows, from penultimate spine. Caudal fin densely scaled, scales ctenoid; interradial scales in single rows; hind margin of scaly area concave, extending to between one-third and middle of caudal fin.

Soft dorsal fin pointed, extending beyond middle of caudal fin. D. XVI,9(16*), XVI,10(13), XVII,8(2). Soft anal fin pointed, of about the same length as dorsal fin. A. VI,7(2), VI,8(3), VII,7(17*), VII,8(8), VIII,6(1). Anal fin pterygiophores 11(2), 12(22*), 13(7). First pelvic fin ray longest, extending up to the second anal fin spine. Pectoral fin with a rounded tip, third and fourth rays longest, extending just to the midlateral blotch. P. 12(11*), 13(5). Caudal fin rounded to subtruncate.

All teeth caniniform, slightly curved. Outer row teeth increasing in size symphysiad, upper jaw anterior teeth longest, lower jaw anterior teeth subequal. Number of lower jaw teeth up to 16 in one outer hemiseries, upper jaw tooth row much shorter, with about 7 or 8 teeth in one outer hemiseries. Lower pharyngeal tooth plate not studied. Gill rakers externally on first gill arch, 2 epibranchial, 1 in angle, 5(4), 6(11*), 7(1) ceratobranchial.
Vertebrae 13+13=26(29*), 13+14=27(2). Caudal peduncle with no vertebrae (10) or containing 0.5(4), 1(14*), 1.5(1) vertebrae.

Color pattern in alcohol. Six to seven vertical flank bars, a caudal peduncle bar confluent with the caudal-base bar, and a midlateral stripe bearing the midlateral blotch in the fourth flank bar (sensu Říčan *et al.* 2005) make up the principal markings. All fins and body are without conspicuous spots or blotches. The midlateral stripe is more distinct anteriorly from the midlateral blotch than posteriorly, and the midlateral blotch itself is a dominant coloration pattern element. Vertical bars are relatively wide, faint, indistinct in their ventral parts. The midlateral stripe posteriorly from the midlateral blotch does not align with the lower lateral line and aligns with the E1 scale row and does not continue in the E0 scale row. Posteriorly from the midlateral blotch, the stripe is slightly decomposed into two blotches in the respective vertical flank bars. The blotch posterior from the midlateral blotch is centered in the same scale row as the midlateral blotch (*i.e.* E1 scale row), whereas the second blotch is more elongate along the vertical axis and centered in the E2 scale row, making the impression that the midlateral stripe makes a dorsally directed turn at its posterior end. The arrangement of the bars on the body in essentially the same as described for *A. scitulus* (Říčan & Kullander 2003). Very small spots present on the bases of some body scales in adult specimens. In juveniles the spotted pattern of the body is much more pronounced, with virtually every scale on the body having a spot at its base, including those in the anterior part of the E4 scale row (*i.e.* as in adult *A. scitulus*).

Life coloration. Coloration of life specimens from the rio Uruguai drainage is unknown to us. Staeck (1998a, 1998b, 2003: p. 64) photographed specimens from the rio Iguaçu drainage (Fig. 7). These specimens have a yellow ground coloration with dark vertical bars and a dark horizontal stripe. Several other species of *Australoheros* have a yellowish ground color, but it is best developed in *A. angiru*. The iris is also yellow. The caudal fin has red dorsal and ventral margins and corners. This character is not unique for *A. angiru*, and can also be seen in *A. kaaygua* and in populations of *A. facetus* from the state of Uruguay. Breeding animals have the typical *Australoheros* breeding coloration with the horizontal interruption of the black vertical bars in their dorsal portion between the opercle and the midlateral blotch (Říčan & Kullander 2003; Staeck 1998a: p. 82, 1998b: p. 62, 2003: p. 65). Females in breeding coloration develop a black blotch in the dorsal fin. Staeck (1998b, 2003) describes behavior and spawning under aquarium conditions.

Distribution. *Australoheros angiru* has a disjunct distribution in the rio Iguaçu and in the upper rio Uruguai. One locality is so far known from the middle río Uruguay in Misiones province, Argentina (Fig. 10).

Etymology. The Guaraní word *angirû* means friend, partner (amigo or compañero in Spanish). The etymology is based on the fact that *A. angiru* and *A. kaaygua* have been confused as one species (Říčan & Kullander 2008). New data have however demonstrate that they are two non-sister group species living in the same river drainage (río Iguazú), though not sympatrically.

Notes. Part of *Australoheros angiru* material (MCP 6262) has been previously considered conspecific with *A. kaaygua* (Říčan & Kullander 2008). The authors were aware of the morphological variation within *A. kaaygua* (sensu Říčan & Kullander 2008), but lack of DNA data and of first hand examination of the type series of *A. kaaygua* made them sceptical about describing a new species with an additionally unusual distribution (occuring in the same river basin, río Iguazú as *A. kaaygua*, but not in sympatry, and at the same time also in the río Uruguay). DNA data from the rio Iguaçu populations in Brazil (*A. angiru*) however show no relationship to *A. kaaygua* in the río Iguazú in Argentina (Fig. 2). DNA data from the río Uruguay are so far lacking. A more detailed morphological analysis (Fig. 1) also supports the notion of two unrelated species, with populations of *A. angiru* from both the rio Iguaçu and from the río Uruguay forming a homogenous clade with short intraspecific branch lengths. The sister species of *A. angiru* is *A. minuano*, while that of *A. kaaygua* is *A. tembe* (Fig. 3).

The MCP 6262 lot additionally included two species (Říčan and Kullander, 2008). Nine specimens from this lot are paratypes of *Australoheros forquilha* Říčan and Kullander, 2008. Thirteen specimens from this lot represent *A. angiru* (previously erroneously treated as *A. kaaygua* in Říčan and Kullander, 2008) and were separated into a new lot MCP 46328.

Additional diagnostic characters of *Australoheros angiru* that separate it from all other species except *A. kaaygua* and *A. minuano* are as follows. It is distinguished (in decreasing order of overall similarity; except for species from coastal drainages treated as last) from *A. charrua* and *A. scitulus* by having less scale rows between posterior end of upper lateral line and dorsal fin (ch3 state 2 *vs.* 0 *vs.* 1), less caudal vertebrae (13 *vs*. 14), in being more deep-bodied (50 *vs*. 45% SL), and in having less E0 scales (24 *vs*. >25). Additionally distinguished from *A. charrua* by details in the shape of the midlatral stripe (see description) and by lacking a pinkish body coloration. Additionally distinguished from *A. scitulus* by lacking black blotches on the opercular series, having less anal fin spines (7 *vs*. 8), less dorsal fin spines (16 *vs*. 17), less caudal vertebrae (13 *vs*. 14), in being more deep-bodied (50 *vs*. 45% SL), and in having less pectoral fin rays (12–13 *vs*. 13–14).

Australoheros angiru is distinguished from *A. tembe* by having less scale rows between anterior end of dorsal fin and upper lateral line (ch4 states 1–2 *vs.* 0), by a very narrow or missing caudal base spot, a shorter dorsal fin scale cover (ch1 state 1 *vs.* 0), less scale rows between the posterior end of the upper lateral line and dorsal fin (ch3 state 2 *vs.* 0), by lacking thick lips, by having more anal fin spines (7 *vs*. 6), less caudal vertebrae (13 *vs*. 14), and less caudal peduncle vertebrae (0 *vs.* 3). It is distinguished from *A. guarani*, *A. facetus*, *A. acaroides* and *A. taura* by a large and dominant midlateral blotch (except *A. facetus*), very narrow or missing caudal base spot, and details in the shape of the midlatral stripe (see description).

Australoheros angiru is additionally distinguished from *A. guarani* by a small terminal or subterminal mouth (*vs*. large supraterminal), more anal fin spines (7 *vs*. 6), shorter preorbital distance (21 *vs.* 25% HL), and less C1 gill rakers (6 *vs*. 7). Additionally distinguished from *A. facetus* by a longer dorsal fin scale cover (ch1 state 1 *vs.* 2), more anal fin spines (7 *vs*. 6), less anal fin rays (7 *vs*. 8), less pectoral fin rays (12–13 *vs*. 13–14), and less C1 gill rakers (6 *vs*. 7–8). It is additionally distinguished from *A. acaroides* by a longer dorsal fin scale cover (ch1 state 1 *vs.* 2), shorter caudal peduncle (40% CPD *vs*. 50–60% CPD), by being more deep-bodied (50 *vs*. 45% SL), and having a narrower interorbital distance (35 *vs*. 40–45% HL). It is distinguished from *A. taura* by also lacking a pinkish body coloration, by a small terminal or subterminal mouth (*vs*. large supraterminal), shorter caudal peduncle (40% CPD *vs*. 50% CPD), by being more deep-bodied (50 *vs*. 40% SL), by a narrower interorbital distance (35 *vs*. 40% HL), less pectoral fin rays (12–13 *vs.* 13–14), and less E0 scales (24 *vs*. >25).

Australoheros angiru is distinguished from *A. ykeregua* and *A. forquilha* by a shorter dorsal fin scale cover (ch1 state 1 *vs.* 0), a different scale pattern along anterior border of dorsal fin (ch2 state 0 *vs.* 1), less scale rows between posterior end of upper lateral line and dorsal fin (ch3 state 2 *vs.* 0), very narrow or missing caudal base spot, absence of opalescent spots below orbit, unpaired fins without checker-board spotted pattern, absence of red colored lower head area and opercular membrane, by a small terminal or subterminal mouth (*vs*. large supraterminal), less dorsal fin rays (9 *vs*. 10), less caudal peduncle vertebrae (0 *vs*. 2 *vs*. 2.5), shorter caudal peduncle (40% CPD *vs*. 60% CPD), by being more deep-bodied (50 *vs*. 45 *vs*. 40% SL), with a wider head (55 *vs*. <50% HL), and in having less pectoral fin rays (12–13 *vs*. 13–14). Additionally distinguished from *A. ykeregua* by a large and dominant midlateral blotch, and more anal fin spines (7 *vs*. 6). Additionally distinguished from *A. forquilha* by less scale rows between anterior end of dorsal fin and upper lateral line (ch4 state 1 *vs.* 0), absence of opalescent scale rows on body, and less pectoral fin rays (12–13 *vs*. 13–14).

Australoheros angiru is distinguished from all the Atlantic coast species north of *A. acaroides* and *A. taura* (*A. autrani*, *A. barbosae*, *A. capixaba*, *A. ipatinguensis*, *A. macacuensis*, *A. macaensis*, *A. muriae*, *A. paraibae*, *A. ribeirae*, *A. robustus*, *A. saquarema*) by a longer dorsal fin scale cover (ch1 state 1 *vs.* 2), a large and dominant midlateral blotch, details in the shape of the midlatral stripe (see description), shorter caudal peduncle (40% CPD *vs*. >50% CPD), in being more deep-bodied (50 *vs*. 45% SL), with a narrower interorbital distance (35 *vs*. 40% HL), less pectoral fin rays (12–13 *vs*. 13–14), and less E0 scales (24 *vs.* >25).

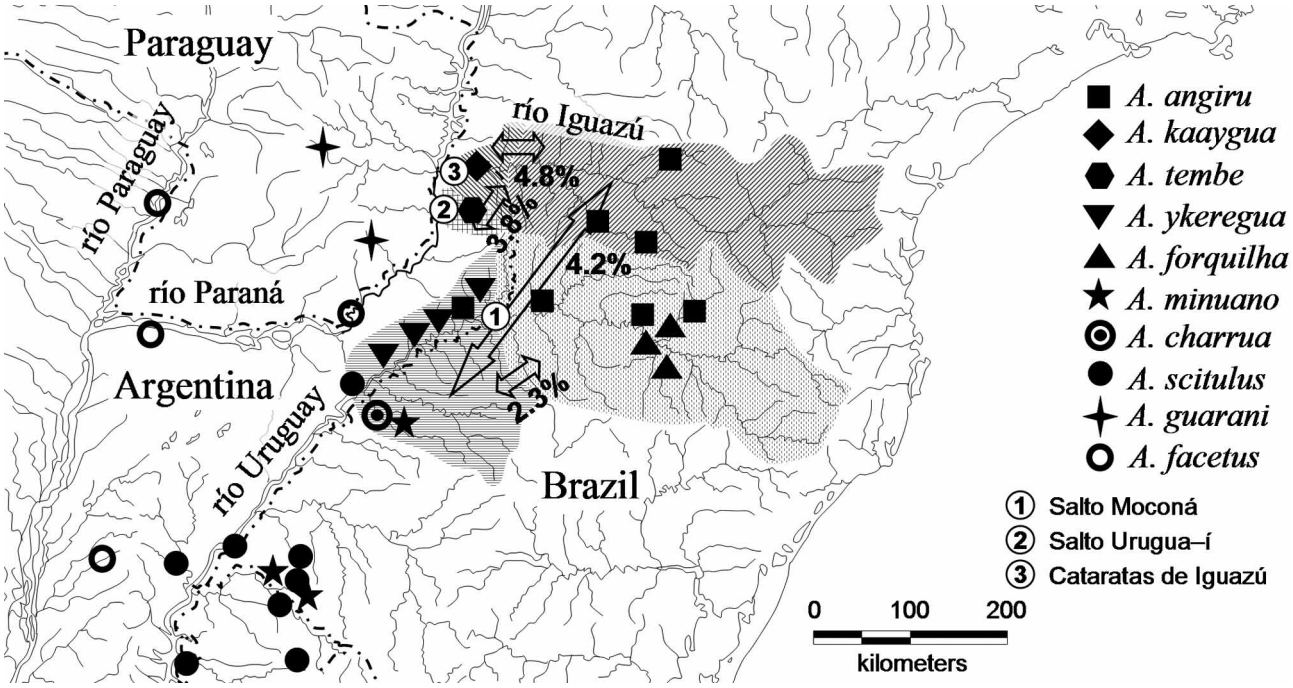

FIGURE 10. Map of the middle Río de la Plata basin. Distributions of the two new species (*A. angiru* and *A. ykeregua*) and their relatives, as well as five areas of endemism are shown. Percent values and corresponding arrows demonstrate sequence divergences in the cyt*b* gene (see Fig. 2) between the species and areas of endemism in the río Iguazú and río Uruguay river drainages (plus the arroyo Urugua–í). Divergence of *A. ykeregua* from its sister species *A. forquilha* is 2.3%. This divergence probably represents the minimum age of the Salto Moconá. Divergence of *A. kaaygua* from its sister species *A. tembe* is 3.8%, and of *A. angiru* from *A. tembe* is similarly 3.6–3.7%. This probably represents the age of the division of the arroyo Urugua–í from the río Iguazú. Divergence of *A. angiru* from its sister species *A. minuano* is 4.2%. This is likely a divergence of the rio Iguaçu and río Uruguay drainages. Divergence of *A. angiru* from *A. kaaygua* (4.8%), two unrelated species endemic to the río Iguazú river drainage, demonstrates an old divergence within the Iguazú drainage basin itself. See Discussion for more detailed description of the biogeography.

Discussion

Biogeography. The cyt*b* data reveal some interesting intraspecific geographical structure within *A. ykeregua*, amounting up to 1.5% divergences. The cytb data sugest that upstream populations (Fig. 2: 13) are potentially ancestral to downstream populations (Fig. 2: 11, 12). This pattern is in good agreement with theoretical prediction since the upstream population does not have a unique haplotype, compared to the downstream populations 11 plus 12. Upstream populations are divided from downstream populations (in this case by river rapids and waterfalls) and the only possible dispersal is downstream. *Australoheros ykeregua* is the only *Australoheros* species common in the tributaries of the río Uruguay in Misiones. This observation has two biogeographical and evolutionary implications (given the presence of waterfalls and a number of rapids on these tributaries and the presence of other *Australoheros* species in the mainstream of the río Uruguay in Misiones and in tributaries further south). First, *A. ykeregua* is the oldest *Australoheros* species in the río Uruguay drainage of Misiones, older than the respective barriers, which are impenetrable for the later immigrating species into the area (*A. angiru*, *A. minuano*, *A. scitulus*, and *A. charrua*). Second, its divergence from its sister species (*A. forquilha*) corresponds to a barrier between them, probably the Salto Moconá, which is not the case for *A. angiru*. *Australoheros angiru* (as presently understood) is partly sympatric with both *A. forquilha* and *A. ykeregua*, but its occupation of the río Uruguay in Misiones is much younger, and we predict that its molecular divergences (presently unknown) of populations below and above Salto Moconá will be much lower than in the case of *A. ykeregua* and *A. forquilha*. The biogeography of *A. angiru* suggests (in the absence of molecular data) that its original distribution area was the rio Iguaçu, and that its presence in the río Uruguay is secondary.

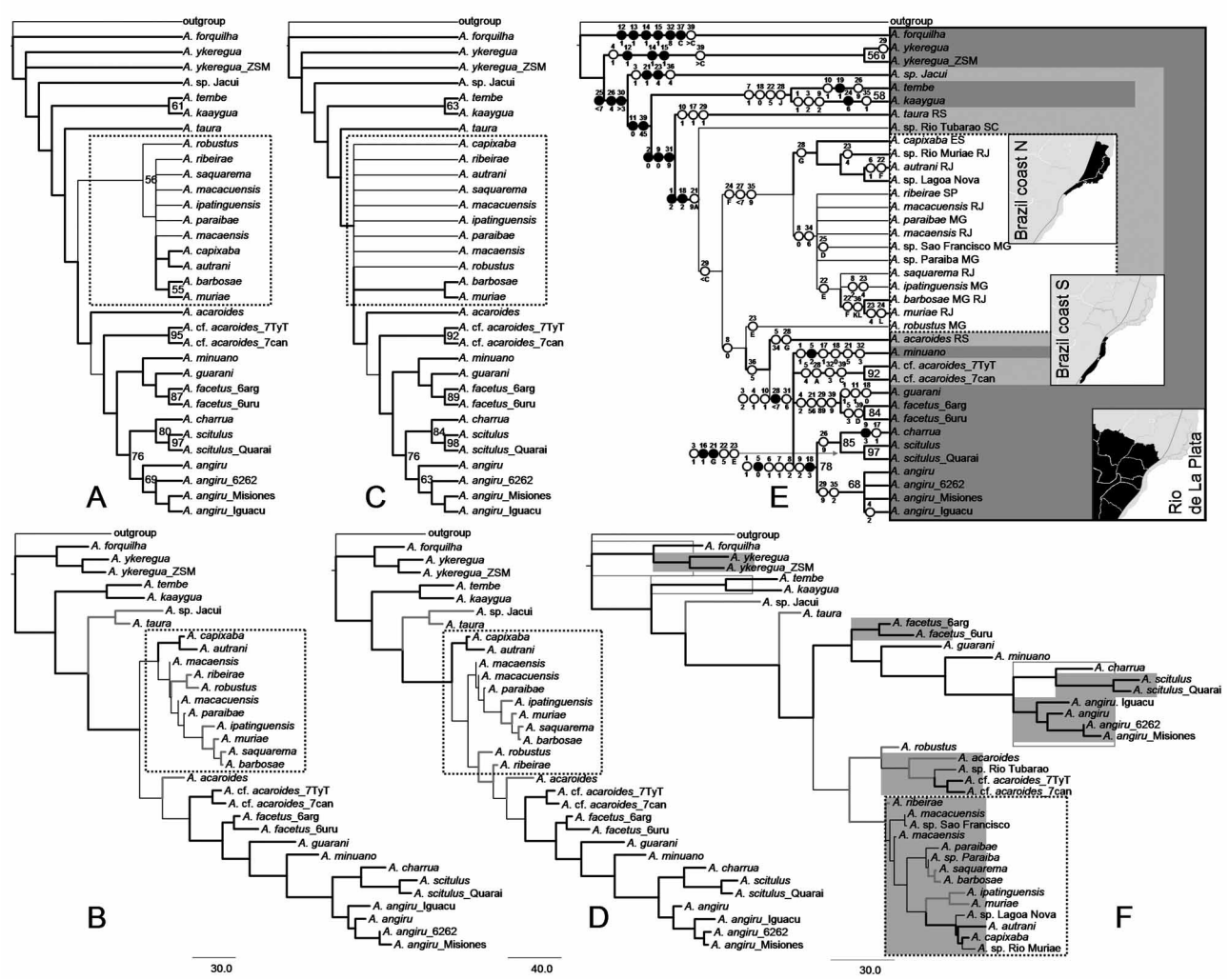

FIGURE 11. Phylogeny of all valid and one putative species of *Australoheros* based on 38 morphological characters. Ottoni and Costa (2008), Ottoni *et al.* (2008), Ottoni and Cheffe (2009) and Ottoni (2010) have diagnosed the Brazilian coastal species by a unique combination of 14 + 12 vertebrae. Our examination of material from some of the drainages (see Figs E and F) instead shows a combination of 13 + 13 vertebrae, which is not unique among *Australoheros*. Our phylogenetic analyses have thus been performed with both combinations (14 + 12 in Figs A and B; 13 + 13 in Figs C – F). The three upper Figs (A, C, E) show maximum parsimony (MP) topologies, the lower three show neighbour joining (NJ) topologies (with branch lengths showing amount of morphological divergence; B, D, F). Numbers at nodes show bootstrap support. Bold black nodes and branches show agreement between all analyses (MP and NJ separately), bold grey nodes and branches agreement between two of three analyses. The interrupted-line boxes show the relationships and branch lengths among the northern Brazilian coastal species. Notable is the collaps of their relationships under the 13 + 13 scenario (Figs C – F) and the markedly short branches separating these species (Figs B, D, F). The short branches separating these species are much more similar to intraspecific variability among other species of *Australoheros* (grey boxes in Fig. F) than to interspecific branch lengths (grey-line boxes in Fig. F). This low differenciation of the northern Brazilian coastal species is also evident from Fig. E, where the morphological matrix (Appendices 1 and 2) is mapped onto the phylogeny (geographical distribution of the species is also shown). Most species, with the exception of the northern Brazilian coastal species, are diagnosed by unique characters or unique combinations of characters. The average number of changes among interspecific pairs described by Říčan and Kullander (2003, 2008, this study) is 98.5, while among intraspecific comparisions it is 20.7. The average for comparisons among the species described by Ottoni and Costa (2008), Ottoni *et al.* (2008), Ottoni and Cheffe (2009) and Ottoni (2010) is 20.5, i.e. corresponding to variation within species of Říčan and Kullander (op. cit.). Based on these considerations we believe that the number of described species from the northern Brazilian coastal drainages is a case of excessive splitting and that the species diversity is actually much lower.

As proposed above, the barrier responsible for the divergence of *A. ykeregua* and *A. forquilha* is probably the Salto Moconá on the río Uruguay, just below the mouth of the río Pepirí Guazú, which marks the international boundary between Argentina and Brazil. The divergence between *A. ykeregua* and *A. forquilha* amounts to 2.3% uncorrected distance in the cyt*b* gene. Translated into time units this corresponds roughly to 2.3–3.3 Mya (based on calibration of the cyt*b* gene by Concheiro Pérez *et al.* 2007).

The divergence patterns found in the río Iguazú drainage are even more complex than those in the río Uruguay drainage. The two *Australoheros* species from this drainage are not sister species (*A. kaaygua* and *A. angiru*), and correspondingly their divergence amounts to a higher distance (than in the case of *A. ykeregua* and *A. forquilha*) of 4.8% (*i.e.* 4.8–6.8 Mya). The presence of two separate and non-overlapping fish faunas in the Iguazú again suggests a barrier within the river basin (as the Salto Moconá in the Uruguay river basin). This time, however, each fauna has a different sister group in a separate, but at the same time adjacent river drainage. The sister group of *A. kaaygua* is *A. tembe*, found in the adjacent arroyo Urugua–í river drainage (see Fig. 10) south from the lower río Iguazú where *A. kaaygua* is found. The divergence between the two species is 3.8% (*i.e.* 3.8–5.4 Mya). The sister group of *A. angiru* from the middle Iguaçu river drainage in Brazil is *A. minuano*, found in the middle río Uruguay river drainage, south from the middle rio Iguaçu. The divergence between the two species is 4.2% (*i.e.* 4.2–6.0 Mya). Not only are the relationships of the two non-related species from the río Iguazú drainage (*A. kaaygua* and *A. angiru*) with species in adjacent river drainages to the south (*A. tembe*, *A. minuano*), but also the estimated times of divergence closely match one another (3.8% *vs.* 4.2% divergence). This scenario is complicated by the fact that *A. angiru* occurs not only in the rio Iguaçu basin but also in the upper rio Uruguai basin. Absence of molecular data from the latter populations at the moment prohibits our understanding of additional details responsible for this distribution pattern.

The above described biogeographic and time-frame patterns are likely more than just coincidence. We believe that the fishes are starting to reveal some ancient history of the river drainages themselves. That waterfalls form barriers to dispersal, and that increasing height (and also age?) of the waterfalls increases isolation is evident from our data. Waterfalls in the case of *Australoheros* mostly divide unrelated species from each other. The two highest waterfalls (Cataratas de Iguazú, Salto Urugua–í) divide endemic species (*A. kaaygua* and *A. tembe*) from an unrelated species (*A. guaraní*) (Fig. 3). The same is true vice-versa, since *A. guaraní* is divided from these two species by the equally high Salto Monday in Paraguay (Fig. 10). None of the three species is known from the río Paraná itself below these three waterfalls (where *A. facetus* occurs because there is no barrier for its upstream migration through the río Paraná (see *A. facetus* A24, A25 in Fig. 2; cf. also Table 1). A rather low waterfall (Salto Moconá on the río Uruguay) on the other hand divides two sister species (*A. forquilha* and *A. ykeregua*). Unfortunatelly, we have so far no clue as to the localization of the barrier within the today heavilly dammed rio Iguaçu.

Prominent waterfalls thus in *Australoheros* generally divide unrelated species, while at the same time related species are in most cases separated by drainage divides. This suggests that waterfalls delimit the boundaries of a given fauna, while river captures and drainage translocations are responsible for the evolution of the diversity per se. Our data would thus suggest that the lower río Iguazú and the arroyo Urugua–í were once connected (*A. kaaygua vs. A. tembe*), as was the middle rio Iguaçu with the río Uruguay (*A. angiru vs. A. minuano*). The postulated connection between the lower río Iguazú and the arroyo Urugua–í is additionally supported by several other fish species or species pairs (*Astyanax leonidas*, *Glanidium riberoi*, *Hypostomus myersi*, *H. derbyi*, *Corydoras carlae*, *Crenicichla yaha vs. C.* cf. *yaha* [Casciotta *et al.* 2006b, Piálek *et al.* 2010] *Bryconamericus ikaa vs. B.* cf. *ikaa*) distributed only in the two river drainages. The connection between the middle rio Iguaçu and rio Uruguai is more enigmatic, to our knowledge so far supported only by the distribution of *A. angiru*, and lack of DNA data prohibits our knowledge of additional details of this distribution.

Diversity. Ten species of *Australoheros* are presently known from the Río de la Plata basin (Figs 1, 2, 3, 10) and 13 species from the Atlantic coast drainages of Brazil (Ottoni & Costa 2008; Ottoni *et al.* 2008; Ottoni & Cheffe 2009; Ottoni 2010). Neither the Río de la Plata basin nor the Atlantic coast drainage species of *Australoheros* seem to be a monophyletic group (Fig. 11). The little known *A.* sp. Jacui does not seem to be conspecific with *A. taura* (Ottoni & Cheffe 2009) from the same river drainage, and these two species are probably not related to the remaining species of the Atlantic coast drainages of Brazil (Fig. 11). *Australoheros facetus* seems to have phylogenetic affinities with the remaining species described from the Atlantic coast drainages of Brazil (Ottoni & Costa 2008; Ottoni *et al.* 2008; Ottoni 2010). The interspecific branch lengths between the Atlantic coast species (Ottoni & Costa 2008; Ottoni *et al.* 2008; Ottoni 2010) are much shorter than interspecific branch lengths between the

remaining species, and equal approximately intraspecific branch lengths within *e.g. A. ykeregua*, *A. angiru* or *A. scitulus* (Fig. 11). The Atlantic coast species also lack clear unique diagnostic characters (Ottoni & Costa 2008; Ottoni *et al.* 2008; Ottoni 2010; pers. obs.), which rises questions about the validity and the number of species involved. Under the two-step system of species delimitation employed in the present study (character- and tree-based delimitation), only one species instead of 11 species would be recognized. What is presently understood as *A. facetus* from Argentina and Uruguay shows a much higher diversity (judging from the branch lengths in Fig. 11) than the 11 species from the Atlantic coast of Brazil. Clearly, the *A. facetus* lineage of *Australoheros* (which probably includes the Atlantic coast species of Brazil), requires further study.

The identity of four nominal species, treated variously as synonyms of *A. facetus*, has variously been adressed in studies focusing on species from the Atlantic coast drainages. One of these names, *Heros jenynsii* Steindachner from Montevideo has been synonymized with *A. facetus* (Schindler et al., 2010). Another available name is *Heros acaroides* Hensel from Porto Alegre, Brasil. This nominal species was redescribed by Schindler et al. (2010). Our phylogenetic results (Fig. 11) support its separate status from *A. facetus*. The other two nominal species either have no precise locality (*Heros autochthon* Günther from "Brazil") or the locality is doubtfull (*Chromys oblonga* Castelnau from the rio Tocantins in Goiás, Brazil) and their status remains uncertain.

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APPENDIX 1: Morphological character list. Character 28 is used only in the 32 taxon phylogenetic analysis (Fig. 11).

1. Length of dorsal fin scale cover. states: long, reaching anterior insertion of dorsal fin [0]; intermediate, covering the bases of the middle portion of the hard part of the dorsal fin [1]; short, only covering the bases of the two last spines [2]; outgroup [0] –unordered.

Scale pattern along anterior dorsal fin border. states: scale row terminating with 1 small scale [0]; scale row terminating with 2 small scales arranged horizontally [1]. outgroup [?].

Scale rows between posterior end of upper lateral line and dorsal fin. states. 2 large 1 small or more [0]; 1 large and 1 of almost the same size, 1 additional small from 13–14th dorsal spine [1]; 1 large 1 small, 1 additional small from 13–14th dorsal spine [2]; 1 large 1 small, 1 additional small from 9th spine [3]. outgroup [0] –unordered.

Scale rows between anterior end of dorsal fin and upper lateral line. states. 5 [0]; 4 [1]; 3 [2]. outgroup [0]. –unordered.

Abdominal bars. states. 3 in all developmental steps and also in adults [0]; 4 in about 50% of juveniles, 3 in all adults [1]; 4 in about 50% of juveniles, 4 about 50% of adults [2]; 4 in all juveniles, 4 in more than 80% of adults, but only in less than 20% completely separated [3]; 4 in all juveniles, 4 in more than 80% of adults, completely separated in more than 80% of adults [4]. outgroup [0] -unordered

Distinct and dominant midlateral stripe between operculum and midlateral spot continuous, not fragmented into spots. states. no [0]; yes [1]. outgroup [?].

Large, dominant and well circumscribed midlateral blotch in juveniles and adults: no [1]; yes [0]. outgroup [0].

Caudal base spot. states: distinct, rounded spot [0]; weakly developed [1]; very narrow or completely missing [2]. outgroup [?]-unordered.

Midlateral stripe posterior from the midlateral blotch. states: running in scale rows 0 and E1 as anterior of the blotch [0]; The midlateral stripe runs in scale rows E0, E1 and E2 posterior to the midlateral blotch—*i.e.* the midlateral stripe gets wider posterior of the midlateral blotch [1]; midlateral stripe bend upwards posterior from the midlateral blotch—the blotch posterior to the midlateral stripe is centered in the same scale row as the midlateral bar (*i.e.* E1 scale row), and the last blotch is high on the body [2]; midlateral stripe bend upwards posterior from the midlateral blotch—the midlateral blotch is centered in the E1 scale row, while the next posterior blotch is centered in the E2 scale row and the blotch in the last body bar is centered in the E3 scale row. The midlateral stripe does not run in the 0 scale row posterior from the midlateral blotch [3]; outgroup [1] –unordered.

Midlateral stripe. states: without distinct borders [0]; clearly bordered [1]; outgroup [?]

Spots in scales arranged into stripes (at least one) also ventral from the 0 scale row. states: no [1]; yes, at least in the posterior part of the body [0]; outgroup [?]

Opalescent line below the circumorbital series. states: absent [0]; present [1]. outgroup [0].

Opalescent scales on body and head. states: absent [0]; present [1]. outgroup [0].

Checkerboard spotted unpaired fins (*i.e.* soft part of dorsal, caudal and soft part of anal fins). states: absent [0]; present [1]. outgroup [0].

Red ventral part of head, preoperculum and opercular membrane. states: absent [0]; present [1]. outgroup [0].

Opercular spots. states: absent [0]; present [1].

Pink body coloration. states: absent [0]; present [1].

Mouth position and size. states: mouth proportionally large, terminal [0]; mouth proportionally large, pointing down, lower jaw proportionally shorter [1]; mouth proportionally large, pointing up, lower jaw projecting in front of upper [2]; mouth very small, terminal or slightly pointing down [3]. –unordered.

Species develops thick lips. no [0]; yes [1].

Anal pterygiophores. Range 11–15. Frequency bins spaced at 0.2. states: 11.0–11.2 [0]; 11.2–11.4 [1]; ... [2,3,4,5,6,7,8,9,A,B,C,D,E,F,G,H,J,K]. –ordered.

Anal spines. Range 5–9. Frequency bins spaced at 0.2. states: 5.0–5.2 [0]; 5.2–5.4 [1]; ...[2,3,4,5,6,7,8,9,A,B,C,D,E,F,G,H,J,K]. –ordered.

Anal rays. Range 6–9. Frequency bins spaced at 0.2. states: 6.0–6.2 [0]; 6.2–6.4 [1]; ...[2,3,4,5,6,7,8,9,A,B,C,D,E,F,G,H,J,K]. –ordered.

Dorsal spines. Range 14–18. Frequency bins spaced at 0.2. states: 14.0–14.2 [0]; 14.2–14.4 [1]; ...[2,3,4,5,6,7,8,9,A,B,C,D,E,F,G,H,J,K]. –ordered.

Dorsal rays. Range 7–12. Frequency bins spaced at 0.2. states: 7.0–7.2 [0]; 7.2–7.4 [1]; ...[2,3,4,5,6,7,8,9,A,B,C,D,E,F,G,H,J,K,L,M,N,P,Q]. –ordered.

Dorsal total. Range 24–27. Frequency bins spaced at 0.2. states: 24.0–24.2 [0]; 24.2–24.4 [1]; ...[2,3,4,5,6,7,8,9,A,B,C,D,E]. –ordered.

Caudal vertebrae. Range 12–15. Frequency bins spaced at 0.2. states: 12.0–12.2 [0]; 12.2–12.4 [1]; ...[2,3,4,5,6,7,8,9,A,B,C,D,E]. –ordered.

Caudal peduncle vertebrae. Range -2-(+3.5). Frequency bins spaced at 0.2. states: -2-(-1.8) [0]; -1.8-(-1.6) [1]; ...[2,3,4,5,6,7,8,9,A,B,C,D,E,F,G,H,J,K,L,M,N,P,Q]. –ordered.

Caudal peduncle length / caudal peduncle depth. Range 0.28–0.74. Frequency bins spaced at 0.2 states. 0.28–0.30 [0]; 0.30–0.32 [1]; … ...[2,3,4,5,6,7,8,9,A,B,C,D,E,F,G,H,J,K,L,M,N].]. –ordered.

Body depth / SL. Range 0.40–0.53. Frequency bins spaced at 0.1. states: 0.40–0.41 [0]; 0.41–0.42 [1]; ...[2,3,4,5,6,7,8,9,A,B,C]. –ordered.

Head width / HL. Range 0.44–0.64. Frequency bins spaced at 0.2. states: 0.44–0.46 [0]; 0.46–0.48 [1]; ...[2,3,4,5,6,7,8,9]. –ordered.

Interorbital distance / HL. Range 0.22–0.46. Frequency bins spaced at 0.2. states: 0.22–0.24 [0]; 0.24–0.26 [1]; ...[2,3,4,5,6,7,8,9,A,B]. –ordered.

Preorbital distance / HL. Range 0.10–0.36. Frequency bins spaced at 0.2. states: 0.10–0.12 [0]; 0.12–0.14 [1]; ...[2,3,4,5,6,7,8,9,A,B,C]. –ordered.

Pectoral fin length / SL. Range 0.24–0.36. Frequency bins spaced at 0.2. states: 0.24–0.26 [0]; 0.26–0.28 [1]; ...[2,3,4,5]. –ordered.

Ventral fin length / SL. Range 0.22–0.48. Frequency bins spaced at 0.2. states: 0.22–0.24 [0]; 0.24–0.26 [1]; ...[2,3,4,5,6,7,8,9,A,B,C]. –ordered.

Pectoral fin rays. Range 12–14. Frequency bins spaced at 0.2. states: 12.0–12.2 [0]; 12.2–12.4 [1]; ...[2,3,4,5,6,7,8,9]. –ordered.

E0 scales. Range 23–26. Frequency bins spaced at 0.2. states: 23.0–23.2 [0]; 23.2–23.4 [1]; ...[2,3,4,5,6,7,8,9,A,B,C,D,E]. –ordered.

L1 scales. Range 13–19. Frequency bins spaced at 0.4. states: 13.0–13.4 [0]; 13.4–13.8 [1]; ...[2,3,4,5,6,7,8,9,A,B,C,D,E]. –ordered.

L2 scales. Range 6–11. Frequency bins spaced at 0.2. states: 6.0–6.2 [0]; 6.2–6.4 [1]; ...[2,3,4,5,6,7,8,9,A,B,C,D,E,F,G,H,J,K,L,M,N,P,Q]. –ordered.

C1 gill rakers. Range 5–9. Frequency bins spaced at 0.2. states: 5.0–5.2 [0]; 5.2–5.4 [1]; ...[2,3,4,5,6,7,8,9,A,B,C,D,E,F,G,H,J,K]. –ordered.

APPENDIX 2. Morphological character matrix.

outgroup	0?000?1?1? ?000000?0K ??CQEER?90 63???EEQA
'A forquilha '	0100101110 1111100106 699HCCPF32 48226ECDC
'A ykeregua '	0101100110 110110010? 4AAHD{789}{HJKL}?01 5610BCAG{EF}
'A ykeregua ZSM '	0101100110 1101100101 478H89KE52 263369BFF
'A sp Jacui '	0110?00110 100000?101 1A4F54KC53 552364A98
'A tembe'	0000101111 0000000011 559949RK43 4622?9???
'A charrua'	1001011231 0000001307 A8BDA9A665 66434BAC5
'A kaaygua'	1?20{123}01120 0000000{02}0? 429604?J34 47331C7H4
'A angiru'	1021011221 0000000306 979C75D593 653425BA4
'A angiru 6262'	1021011221 0000000305 96AB64C?95 654602A82
'A angiru Misiones'	1021011221 0000000305 96A954779{345} 65{34}{456}{012}{2345}{AB}{89A}{234}
'A angiru Iguacu'	1022011221 0000000308 989B63339{345} 65{34}{456}{012}{2345}{AB}{89A}4
'A scitulus'	1011011221 000001030C H5EA9AB764 434359AA5
'A scitulus Quarai'	1011011221 000001030B G5E9999674 664459AF4
'A minuano'	1021200011 0000001008 6CAD949664 5333058D4
'A guarani'	1022100101 1000000005 599C74D795 873444979
'A facetus 6arg'	2022300001 0000000206 6A9E94C595 6534859FD
'A facetus 6uru'	2022300001 0000000205 5C9FA6A584 543487ACE
'A facetus 7TyT'	2021400001 0000000208 A8BB76FA65 53335677C
'A facetus 7can'	2021400001 0000000209 A8AB65FA45 4233476BC
'A capixaba'	????{12}0010? 000000020? 997G84?G53 9?359B9H?
'A taura'	????{12}00101000000110?{56789}9{ABCDE}{ABCDEFGHJ}?4?C13 9?33{56789}{ABCDEFGHJKLMNPQ}{789}{ABCDEFGHJK}?
'A ribeirae'	2???{12}000?0000000020?{56789}99{ABCDE}?4?9{789A}{234} {9AB}??{567}9{56789ABCDE}{789ABC}{56789ABCDE}?
'A autrani'	2???{12}10100000000020?{ABCDE}{FGHJK}{56789}{FGHJKLMNPQ}?4?J{56789A}{45} {9ABC}?{01234}{456}9{ABCDEFGHJKLMPQ}{56789ABC}{0123456789ABCDEFGHJK}?
'A saquarema'	2???{12}???????????0?0?9E9{FGHJK}?4?{6789ABC}{45678}{45} {AB}?{123456}{789}9{ABCDEFGHJK}{ABC}{56789ABCDE}?

'A macacuensis'	2???{12}00000000000020?{ABCDE}{ABCDE}9{FGHJK}?4?{3456789A}{6789}{456}{789}?{123456}{67}9{ABCDEFGHJKLMNPQ}{56789}{56789ABCDE}?
'A ipatinguensis'	2???{12}00200000000020?9E4{FGHJK}?4?{78}{789A}{123}{78}?{345}{3456789ABC}9{ABCDE}{789}{56789ABCDE}?
'A barbosae'	2???{12}00100000000020?{ABCDE}{FGHJK}9{FGHJK}?4?{3456789AB}{456789}{345}{9ABC}?{2345}{234567}{ABCDE}{KLMNPQ}{56789}{56789ABCDE}?
'A paraibae'	2???{12}00000000000020?{ABCDE}{ABCDE}{56789}E?4?{678}{23456}{234}{9A}?{123}{23456}{56789}{FGHJKLMNPQ}{789}{56789ABCDE}?
'A macaensis'	2???{12}00000000000020?{ABCDE}{ABCDE}9{FGHJK}?4?{ABCDEF}{45678}{3456}{9ABC}?{2345}{2345678}9{ABCDEFGHJKLMNPQ}{789}{ABCDEFGHJK}?
'A robustus'	2???{12}00000000000020?{ABCDE}{56789}E{56789}?4?{ABC}{345}{3456}{9ABC}?{34}{2567}{56789}{FGHJKLMNPQ}{789}{56789}?
'A muriae'	2???{12}00{01}00000000020?{56789ABCDE}{FGHJK}4{LMNPQ}?4?9{3456789A}{345678}{89ABC}?{2345}?{ABCDE}{LMNPQ}{789}{FGHJKLMNPQ}?
'A acaroides'	2???{34}00{01}?0 000000020? 99BB74?G54 A?22628E4
'A sp Sao Francisco'	2???{12}00?00 000000020B AAAHD44??? ?????????
'A sp Lagoa Nova'	2???{12}00?00 000000020B 9B7E627??? ?????????
'A sp Paraiba'	2???{12}00?00 000000020C 9C9E945??? ?????????
'A sp Rio Muriae'	2???{12}00?00 000000020A 994F566??? ?????????
'A sp Rio Tubarao'	2???{12}00?00 0000000207 A79C74G??? ?????????