

Live coloration, habitat, biogeography and phylogenetic position of *Australoheros guarani* Řičan & Kullander, 2008 (Teleostei: Cichlidae).

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Abstract

The coloration of live specimens of *Australoheros guarani* is pictured and described for the first time. The species was originally described based on few preserved specimens from two separate tributaries of the Middle Paraná river in Paraguay. Apart from the photos and description of the live coloration of this uncommon species we also elucidate its possible habitat preferences and also its phylogenetic position and biogeography using a molecular phylogeny based on the mitochondrial cytochrome b marker.

Introduction

The genus *Australoheros* is one of three diverse cichlid genera (together with *Crenicichla* and *Gymnogeophagus*) found in the La Plata basin (the Paraná, Paraguay, Uruguay river system) and the coastal drainages of southern Brasil and Uruguay (Řičan & Kullander, 2006, 2008; Řičan et al., 2011; Piálek et al., 2012, 2015; Malabarba et al., 2015; Loureiro et al., 2016). *Australoheros* is the Southernmost genus of heroine cichlids (Řičan et al., 2016) and the only genus of this group found entirely south of the Amazon river basin. *Australoheros* are generalized heroine cichlids with a mostly allopatric distributional pattern between the species. The largest known diversity is found in the smallest La Plata tributary, the Uruguay river basin (six species, five endemic) followed by the Paraná basin (five species, three endemic).

The three endemic species of *Australoheros* in the Paraná river basin are all found in the short Middle Paraná section between the former Guairá or Setequeadas falls (Saltos del Guairá, Salto das Sete Quedas do Guairá; today replaced by the Itaipu hydroelectrical dam) in the North and the Apipé falls (Saltos de Yacyretá-Apipé; today replaced by the Yacyretá hydroelectrical dam) in the South. One of these endemic species is *A. tembe* found only in the arroyo Urugua-í, a left-hand tributary of the Paraná in Misiones, Argentina. The second species is *A. kaaygua* endemic to the lower Iguazú river, also a left-hand tributary of the Paraná in Misiones, Argentina and Paraná, Brazil. The third and least known species is *A. guarani* which is endemic to a wider area in two right-hand Paraná tributaries in Paraguay. *Australoheros guarani* is known from only a handful of preserved specimens and nothing is known about its biology or evolution.

The aim of this paper is to show and describe its live coloration, habitat, and phylogenetic position and biogeography based on seven specimens collected in September 2016, two of which have been included in a molecular phylogeny of the genus.

Examined Material

Australoheros guarani (figs. 1-5). 7 ex., not preserved, 3 juveniles-subadults, 4 adults, fin clips of 1 juvenile and 2 adults preserved for DNA study, side-lagoon on a tributary of the upper Tembey river, 4. 9. 2016, 17:20, S 26 20 52.22, W 55 13 47.69, 255 m above sea level, 9km north on RN6 from the Tembey river.



fig. 1. *Australoheros guarani*, subadult, live specimen immediately after capture (not preserved)

Methods

The phylogenetic position of *A. guarani* has been ascertained using a molecular phylogeny derived from the mitochondrial (mtDNA) cytochrome b (cytb) marker. DNA was extracted from two tissue samples (pelvic fin clips) of two *A. guarani* specimens. See Říčan et al. (2011) for methods of DNA isolation, cytb amplification and sequencing and cytb neighbour joining phylogenetic reconstruction.

Distribution

Australoheros guarani is so far only known from two separate tributaries of the middle Paraná in Paraguay (the Monday and Tembey rivers; Říčan & Kullander, 2008), each separated from the Middle Paraná by a waterfall (see Remarks). The here reported specimens were collected at a single locality, in a side-lagoon of a tributary of the upper Tembey river (04.09.2016, 17:20, 26°20'52.22"S - 55°13'47.69"W, 255 m above sea level) located 9km North on RN6 from the Tembey river.

Habitat

No detailed locality and habitat data are known for the type series of *A. guarani*. The newly collected specimens reported here were all collected in a side-lagoon (fig. 8) connected to the main stream (fig. 7) by a very small and shallow channel (fig. 9). This locality is the first recent record of the species and based on our field experience and studied localities in Paraguay (fig. 11) we believe that the lagoon-like habitat might actually be typical for the species. *Australoheros* are in general much more common in stagnant and slow-flowing water than in clear and running water (exceptions are e.g. *A. tembe* or *A. ykeregua* and *A. forquilha* where virtually all available habitats are clear-water streams with rocky bottoms). *Australoheros kaaygua* appears to have similar habitat preferences as *A. guarani* since all our localities of this species are slow-flowing or stagnant lagoons or river sections almost always with a soft bottom. We have never collected *A. kaaygua* in the much more common clear-water localities and rocky bottoms which are typical for the Iguazú river basin. In the same way we have never caught *A. guarani* in the also much more common clear-water localities and rocky bottoms which are typical for the streams in its distribution area in Paraguay. The high-bodied morphology of *A. guarani* with a short caudal peduncle and low meristics including very large scales (Říčan & Kullander, 2008) would support this argument since this type of morphology is among heroine cichlids associated with stagnant (lentic) habitats (Říčan et al., 2016). *Australoheros guarani* is in fact the most deep-bodied *Australoheros* species with one of the lowest meristics and the largest scales (Říčan & Kullander, 2006, 2008).



fig. 2. *Australoheros guarani*, adult, live specimen immediately after capture (not preserved)



fig. 3. *Australoheros guarani*, adult (same specimen as fig. 2).



fig. 4. *Australoheros guarani*, holotype, adult, MHNG 2237.58, Río Guyraugua, Río Paraná drainage, Paraguay.



fig. 5. *Australoheros guarani*, paratype, adult, NRM 33498, Río Tembey 4 km below the falls, Río Paraná drainage, Paraguay.

The lagoon where the here reported specimens of *A. guarani* were collected (4. 9. 2016; Fig. 8) was about 40m long and 6-10m wide (verified also from Google Earth satellite image dated closest to the date of collection on 19.05.2016) with a depth where the *A. guarani* specimens were collected of about 1m. Seven specimens were collected with a cast-net very easily within five minutes during not very favorable conditions just fifteen minutes before sunset after several days with some rain. *Australoheros guarani* was thus common in this lagoon at the time of collection which supports it as a favorable and possibly representative habitat for the species.

Collecting in the pool of the near-by (15m) Tembey river tributary with a mixture of rocky and muddy bottom on two separate occasions in two separate years (14.11.2009 and 04.09.2016) did not yield any specimens of *A. guarani* (only *Crenicichla mandelburgeri*) despite collecting for a much longer time (about one hour) with both a cast-net and three 20m long specialized gillnets nor did any other localities with a similar sampling effort throughout eastern Paraguay (white dots in fig. 11).

Live coloration

Live *A. guarani* feature an autapomorphic diagnostic coloration unique among *Australoheros* species that was previously unknown. Each scale-base insertion on body features an orange to dark-yellowish vertical bar-like blotch about one half the height of each scale (figs. 1-3). In areas where vertical bars underlie is the coloration of the scale-base blotches darker due to the presence of the melanin pigmentation of the vertical bars. The basal body coloration is whitish with an orange and violet (mostly on head) tinge in juveniles and yellowish in adults. The pectoral fins, lower portion of head, and bases of caudal, and soft parts of anal and dorsal fins are also yellowish to orange. The pelvic fins and the hard parts of dorsal and anal fins (and the iris and fine pigmentation on the head in juveniles) have a violet tinge. The posterior part of the caudal fin and the distal part of the soft dorsal fin are red. The iris is red to violet in adults with a yellow outline. Breeding coloration is unknown but assumed to consist of darkening of melanin coloration and whitening of rest of body as in virtually all heroine cichlids.



fig. 6. Río Tembey tributary at RN6, Río Paraná drainage, Paraguay, 14.11.2009, 14:55, 26°20'53.28"S - 55°13'46.30"W, 255 m. *Australoheros guarani* was not caught at this time neither with cast net nor with three 20m gillnets. The locality was collected in suboptimal conditions after several days of heavy rain (see high water level and turbid water).



fig. 7. Río Tembey tributary at RN6, Río Paraná drainage, Paraguay, 04.09.2016, 17:20, 26°20'53.28"S - 55°13'46.30"W, 255 m. *Australoheros guarani* was again as in 2009 (fig. 6) not caught in the main stream shown in the photo (neither with cast net nor with three 20m gillnets) but in a side lagoon (fig. 8) connected by a very small channel (fig. 9) following the path at the left hand side of this photo.



fig. 8. Lagoon by Río Tembey tributary at RN6, Río Paraná drainage, Paraguay, 4. 9. 2016, 17:20, 26°20'52.22"S - 55°13'47.69"W, 255 m. *Australoheros guarani* (7 specimens) was caught in this side lagoon and it was very easily collected by a cast net.



fig. 9. Small overflow stream connecting Río Tembey at RN6 (fig. 7) with the lagoon (fig. 8) where *A. guarani* was collected, Río Paraná drainage, Paraguay, 04.09.2016, 17:20, 26°20'52.55"S - 55°13'46.38"W, 255 m asl.

Remarks

Identification. The specimens of *A. guarani* were identified following Říčan & Kullander (2008). The species is distinctive by its high-bodied morphology (the most deep-bodied *Australoheros* species; figs. 1-3), one of the lowest meristics, a short caudal peduncle and the largest scales among *Australoheros* (figs. 1-3).

Phylogenetic position of *A. guarani*. The molecular mtDNA-based cytb phylogeny (fig. 10) shows *A. guarani* as a closely related species to the two other Middle Paraná tributaries endemics *A. tembe* and *A. kaaygua* (fig. 11). While nDNA-based phylogeny was not investigated in the present study (and is deferred to a separate study) the phylogenetic position of *A. guarani* as closely related to *A. tembe* and *A. kaaygua* is likely to be confirmed since the mtDNA cytb phylogeny (Říčan et al., 2008) and the nDNA ddRAD phylogeny (Říčan et al., 2016, Supplementary material 4) show good agreement with *A. kaaygua* and *A. tembe* as sister species in these phylogenies.

Biogeography of *A. guarani*. All three *Australoheros* endemics of the Middle Paraná tributaries (*A. guarani*, *A. tembe* and *A. kaaygua*; fig. 11) are closely related and form a distinct clade within *Australoheros* (fig. 10). The Middle Paraná basin thus in *Australoheros* forms a clear area of endemism. The three species are separated from each other by waterfalls that separate the Paraná river channel from the tributaries where these endemic species are found. *Australoheros kaaygua* is only known above the 75m high Iguazú falls (Cataratas de Iguazú; located 25 km upstream the Iguazú mouth into Paraná river) and *A. tembe* is only known above the former 28m high Urugua-í falls (Salto Urugua-í; located eight km from the confluence with the Paraná river; in 1989 replaced by a hydroelectric dam). *Australoheros guarani* is contrary to its two closely related species not found in a

single tributary of the Middle Paraná but based on present knowledge in two separate tributaries, each separated from the Middle Paraná by a waterfall. The type locality of *A. guarani* is in the Monday river basin which is isolated by the 45m high Monday falls (Saltos del Monday; fig. 11) located seven km from the mouth of the Monday into the río Paraná just opposite the mouth of the Iguazú. Some paratypes and the here reported specimens are from the Tembey river, which is not a directly neighbouring river basin to the Monday, being separated by the intervening Nacunday river and three other smaller river basins (fig. 11). The Tembey river also has a waterfall close to its mouth to the Paraná river, but these Tembey falls are probably not a barrier to fish dispersal due to their low height of only a few meters. During flooding in 2009 we have seen them to almost completely disappear. The 45m high Monday falls are however a formidable barrier to upstream fish dispersal. So far there are no records of *A. guarani* in the intervening river basins between the Monday and the Tembey.

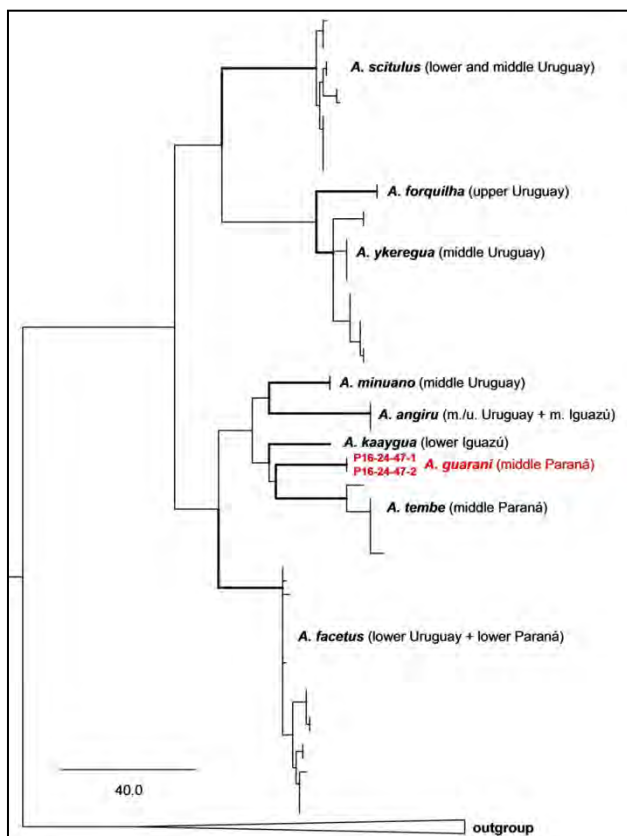


fig. 10. Phylogenetic position of *Australoheros guarani* as a closely related species of the two other known species found in tributaries of the Middle Paraná River (see map in fig. 11; *A. tembe* endemic to the arroyo Uruguá-i, Misiones, Argentina, and *A. kaaygua*, endemic to the Lower Iguazú river above Cataratas de Iguazú in Misiones, Argentina and Paraná, Brazil). See text for additional comments on the phylogenetic position. The tree is a neighbour joining topology derived from the mtDNA cytochrome b marker (see Říčan et al., 2011 and text for additional information).

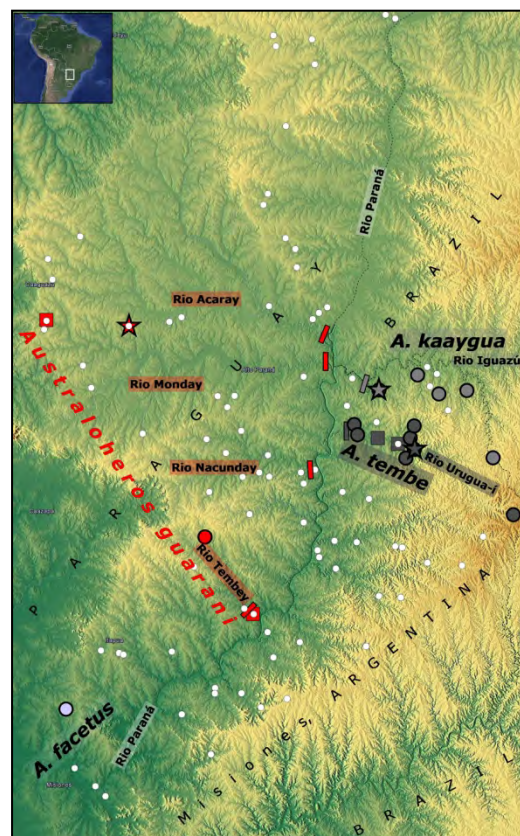


fig. 11. Map of the Middle Paraná river basin (between the former Quairá or Setequeadas falls in the north and the former Salto Apipé in the south) showing the geographical distribution of *A. guarani* (in red), of the two closely related Middle Paraná tributaries endemics (*A. tembe* and *A. kaaygua*) and of the more distantly related *A. facetus* which just reaches the southern border of the Middle Paraná basin. Stars show type localities, large colored circles localities with specimens examined for this study and collected by us, squares show records from literature, and small white dots show localities where we have not been successful in collecting *Australoheros* specimens. Bars show location of waterfalls (see text) on the Middle Paraná river tributaries.

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