FEMALE MORPHOMETRIC AND GENETIC ANALYSIS OF POTENTIAL NEW BRYCINUS SPECIES (TELEOSTEI: CHARACIFORMES: ALESTIIDAE) FROM CUANZA (QUANZA, KWANZA) RIVER CATCHMENT, BIÉ, ANGOLA

PAVLINA KURIKOVA, JIRI PATOKA, LUKAS KALOUS
Department of Zoology and Fisheries
Czech University of Life Sciences
Kamycka 129, 165 21 Praha
CZECH REPUBLIC

kurikovap@af.czu.cz

Abstract: African characiform fish species from genus Brycinus Valenciennes (family Alestiidae) greatly vary in morphological characteristics. Moreover, recent genetic analyses suggest that validity and systematic position of certain species are uncertain and confused, and further revision of entire genus is required. Therefore, we considered to publish our finding from Cuanza River catchment in Angola. In 2007, we captured four adult females of Brycinus sp. in Cuquema River, one of the main tributaries of Cuanza River. These fishes were morphologically and genetically analysed and B. imberi (Peters) and B. lateralis (Boulenger) were identified as the most similar species. Both mentioned species can be easily distinguished from found Brycinus sp. by certain morphological characteristics and by genetic sequences. Since adult males absent in our data set, we recommend future collecting trip focused especially on mentioned males to confirm validity of suggested potentially new species.

Key Words: morphology, DNA, African characids, Cuquema River, taxonomy, new species

INTRODUCTION

The order Characiformes, one of the major lineages of ostariophysan fishes, is widely distributed in freshwaters throughout the most of South and Central America, and continental Africa. New World characiform fishes occur from the southern boundary regions of the United States to the central parts of Chile and Argentina (Lundberg et al. 1998, López et al. 2008, Froese and Pauly 2016). Characiform fishes inhabit freshwater ecosystems within broad regions of sub-Saharan Africa with the exception of the southern portions of the continent and the Horn of Africa (Orti 1997, Calcagnotto et al. 2005). Several species occur through the Sahara Desert along the length of the Nile River basin (Stewart 2009). Phylogenetic studies in recent decades determined three subunits among African characiform fishes: (i) the clade consisting of the families Distichodontidae and Citharinidae (Vari 1979); (ii) the clade formed by the putatively monotypic family Hepsetidae; and (iii) the assemblage recognized alternatively as 'African Characidae', Alestiinae, Alestiidae or Alestidae (hereafter referred to as Alestiidae) (Vari 1979). These studies revealed that mentioned three subunits of Characiformes do not jointly constitute a monophyletic unit, being instead dispersed across the entire order phylogeny. This conclusion is interesting both phylogenetically and in terms of the historical biogeographical relationships of the South American and African freshwater ichthyofauna (Zanata and Vari 2005). The latter mentioned subunit Alestiidae had been previously classified as subfamily of family Characidae (e.g., Greenwood et al. 1966, Géry 1977, Orti 1997, Weitzman and Malabarba 1998). Nevertheless, Buckup (1998) considered Alestiidae to be a valid family belonging to superfamily Alestioidea. Thus, this group of characiform fishes formed one of the few freshwater fish families occurring in both South America and Africa (Murray and Stewart 2002). This family currently comprises dwarf members generally grouped in the "Petersiini", and the genera *Alestes* Müller and Troschel, *Brycinus* Valenciennes, *Bryconaethiops* Günther and *Hydrocynus* Cuvier (Murray and Stewart, 2002). In total, 18 genera of the family (Froese and Pauly 2016) are actually divided into three tribes: the Hydrocinini; the Alestini and the miniaturized Petersiini (Hubert et al. 2005).

Circa 118 valid species from family Alestiidae greatly vary in body and fin sizes, shapes and occupied ecological niches (Froese and Pauly 2016). Alestiidae is the most speciose of all African characiform families (Paugy and Schaefer 2007, Arroyave and Stiassny 2011). Many morphological (Vari 1979, Buckup 1998, Zanata and Vari 2005) and molecular (Orti and Meyer 1998, Murray and Stewart 2002, Hubert et al. 2005, Calcagnotto et al. 2005) studies considered this family to be monophyletic. Contrary, certain recent studies focused on the Alestiidae phylogenetics this monophyly rejected (Arroyave and Stiassny 2011, Decru et al. 2016).

The genus *Brycinus* belongs to the tribe Alestiini and is characterized by presence of a rudimentary adipose eyelids, and by having two rows of pluricuspid teeth on the upper jaw (Paugy 2003). This genus (together with *Alestes*) is divided into three groups: (i) *B. longipinnis* (Günther) – small sized (maximum 140 mm standard length, SL), with fronto-parietal fontanelle always present; (ii) *B. nurse* (Rüppell) – medium sized (maximum 270 mm SL), with fronto-parietal fontanelle present in juveniles but closed in adults; and (iii) *B. macrolepidotus* Valenciennes – large sized (maximum 530 mm SL), with fronto-parietal fontanelle always absent (Paugy 1986, Decru et al. 2016).

In total, seven *Brycinus* species are native to Angola: *B. grandisquamis* (Boulenger), *B. humilis* (Boulenger), *B. imberi* (Peters), *B. kingsleyae* (Günther), *B. longipinnis*, *B. macrolepidotus*, and *B. lateralis* (Boulenger). The last species, *B. imberi*, is very widespread (Paugy 1986) with native range in coastal watershed of the Gulf of Guinea and in the Congo basin in West and South Africa (Paugy, 2003); in tributaries of the Great Lakes region in East Africa; and in the middle and lower Zambezi River (Balon 1971, Bell-Cross 1976) and in Phongolo River catchment (Skelton 2001) in southern Africa. Aforementioned *B. imberi* is perceived as a species complex (Paugy 1986).

Since numerous original descriptions and taxonomic statutes of African characids are to a certain degree confused in light of recent genetic analyses, the revision is strongly recommended by many authors (Murray and Stewart 2002, Hubert et al. 2005, Stiassny and Mamonekene 2007, Arroyave and Stiassny 2011, Decru et al. 2016). In order to contribute to the knowledge of fish from genus *Brycinus* we considered to publish our findings from Cuanza River catchment in Angola with suggestion of one potentially new species.

MATERIAL AND METHODS

Study locality and data collecting

During ichthyological survey in 2007 we sampled Cuanza River catchment on Bié Plateau in Angola (Figure 1). The fishes were collected mainly by seine, rod and hand netting. Fish were euthanized with overdose of the anaesthetic Phenoxy-2-ethanol diluted in water. All individuals were fin-clipped for later DNA analyses, and specimens were fixed in formaldehyde and deposited in the Aquatic Organism Collection at the Czech University of Life Sciences Prague, Czech Republic.

Figure 1 African characid (Brycinus sp.) from Cuanza River, province Bié, Angola

Morphological analysis

All specimen morphometric measurements were made with a digital calliper (accuracy of 0.01 mm). According to Boulenger (1900), Poll (1967) and Skelton (2001) following characteristics were measured: number of scales in lateral line (l. l.), number of perforated scales in canal on lateral line between the head and the base of the caudal fin; number of scale rows above lateral line (Squ. sup.), number of rows from dorsal fin base diagonally forward to the lateral line; number of rows below lateral line (Squ. inf.), number of rows from pelvic fin base diagonally forward to lateral line; number of unbranched dorsal fin rays (D-Du); number of branched dorsal fin rays (D-Db); number of unbranched

anal fin rays (A-Au); number of branched anal fin rays (A-Ab); standard body length (SL), distance between end of snout and end of scaled caudal fin base; total body length (TL), distance from front of snout to end of longest caudal fin ray; head length (lc), lateral distance from top of snout being closed to mouth to posterior margin of operculum without gill membrane; maximum body height (H), distance from highest point of ridge vertically down; predorsal distance (pD), distance from top of snout in straight line to beginning of dorsal fin base; preanal distance (pA), distance from top of snout in straight line to beginning of anal fin base; preventral distance (pV), distance from top of snout in straight line to origin of pelvic fin base; length of caudal peduncle (lpc), distance from to posterior end of scaled caudal part of body; height of caudal peduncle (hpc), distance vertically between end of anal fin base and upper margin of body; length of dorsal fin (lD); length of anal fin (lA); length of pectoral fin ray (lP), distance between beginning and end of longest base of pectoral fin ray; length of pelvic fin ray (lV), distance between beginning and end of longest base of pelvic fin ray; head width (lac), transverse distance between margins at widest area of the head; horizontal diameter of eye (Oh), corneal diameter.

Genetic analysis

DNA was extracted in two individuals from small pieces of fish pectoral or ventral fins using DNeasyTM Tissue Kit (Qiagen, Valencia, CA, USA). We amplified one mitochondrial gene - cytochrome oxidase subunit I. Following primers were used for COI: forward primers 5′-TCA ACC AAC CAC AAA GAC ATT GGC AC-3′ and reverse 5′-TAG ACT TCT GGG TGG CCA AAG AAT CA-3′. PCR was performed in a volume of 25 µl. An initial denaturation step for COI gene an initial denaturation step was of 94 °C for 2 min, followed by 36 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min and elongation at 72 °C for 1 min. The terminal extension was at 72 °C for 10 minutes. PCR products were sent the Macrogen service in South Korea (www.macrogen.com).

The chromatograms obtained were assembled by hand and eye check for potential errors. The comparison specimens from Cuanza were obtained 61 sequences from the GenBank database. This dataset was aligned using Clustal W is done in the software package BioEdit (Hall 1999). Both sequences were submitted to GenBank (KX853170, KX853171). Family relationships were estimated using Bayesian methods of analysis (BI) using MrBayes ver. 3.0 (Huelsenbeck and Ronquist 2001).

RESULTS AND DISCUSSION

In total, we captured four adult females of *Brycinus* sp. in Cuquema River, one of the main tributaries of Cuanza River (Figure 2).

Detailed morphological measurements of analysed individuals are given in Table 1. Head twice longer than wide; head width 9.7 to 10.5 times in TL; snout shorter than eye; eye width 2.9 to 3.1 in head; depth of the body 4.6 to 5.0 times in TL. Head and body depressed dorsoventrally, eye pigmented. Jaw not reaching front edge of the eye. Two rows of teeth on the upper and lower jaws: 16 teeth (8/8) upper, 8 (6/2) lower. Number of dorsal-fin rays II-III 7; dorsal fin originating at the base of pelvic fins; predorsal distance 1.9 to 2.0 times and preventral distance 2.1 times to SL. Number of anal-fin rays II 15. Pectoral fin 1.1 to 1.2 times of the head length, reaching ventral fin base. Caudal fin deeply forked. Caudal peduncle 1.3 to 1.5 times longer than deep. Scales cycloid. Lateral line scales without arborescent or anastomosing canals, 20 to 22 in total, 2 scales between lateral line and ventral fin base and 5 scales between lateral line and dorsal fin base. Body colouration of live individuals silvery-greenish with prominent dark stripe on lateral line reaching posteriorly behind caudal fin base, large black blotch laterally on caudal peduncle not reaching the end of caudal fin. Individuals deposited at the Czech University of Life Sciences (No. AOCAO1, AOCAO2, AOCAO3 and AOCAO4).

Based on genetic and morphological matching, *B. imberi* and *B. lateralis* (occurring also in Cuanza River catchment) were identified as the most similar species to described *Brycinus* sp. Both mentioned species can be distinguished from found *Brycinus* sp. by: (i) different depth of the body which is 2.7 to 3.5 times in TL in *B. imberi* and 3.8 to 4.5 times in *B. lateralis*; (i) numbers of teeth in lower jaws which is 10 (8/2) in both *B. imberi* and *B. lateralis*; (iii) number of dorsal-fin rays which is II 8 in *B. imberi* and II-III 8 in *B. lateralis*; (iv) number of anal-fin rays which is II-III 14-16 in *B. imberi* and III-IV 15-16 in *B. lateralis*; (v) number of lateral line scales which is 30-33 in *B. lateralis*; and (vi) colouration: *B. imberi* has a dark spot laterally behind the head above lateral line and large black blotch laterally on caudal peduncle, and *B. lateralis* has a black lateral stripe extending to median rays of caudal

peduncle and large black blotch laterally on caudal peduncle reaching the end of caudal fin (Boulenger 1909).

The final result of genetic analyses of the COI sequences consisted of 578 characters. Phylogenetic reconstruction method recovered tree of genera *Brycinus* and *Alestes*. Both individuals from Cuanza River identified as *Brycinus* sp. belong in the same group of *B. imberi*. Detailed results of molecular analysis are shown in Figure 3.

Table 1 Detailed morphological measurements of analysed individuals

Characters	AOCAO1	AOCAO2	AOCAO3	AOCAO4
l.l.	22	22	22	20
Squ. sup.	5	5	5	5
Squ. inf.	2	2	2	2
D-Du	3	2	3	2
D-Db	7	7	7	7
A-Au	2	2	2	2
A-Ab	15	15	15	15
SL	66.43	79.4	77.14	69.77
TL	81.44	96.82	93.65	86.09
lc	16.94	19.24	18.8	18.6
H	18.92	21.17	19.92	18.48
pD	35.52	39.33	39.56	37.37
pA	49.82	56.58	54.92	49.77
pV	31,0	38.12	37.86	35.16
lpc	10.85	13.13	12.9	10.94
hpc	8.25	8.78	8.45	7.61
lD	5.88	8.65	8.57	6.82
lA	8.49	10.66	11.29	10.61
lP	15.58	17.78	17.43	15.85
lac	8.19	9.95	9.23	8.23
Oh	5.83	6.41	6.25	5.97

Figure 2 Map of Angola with locality where individuals of Brycinus sp. were captured (indicated by black triangle)

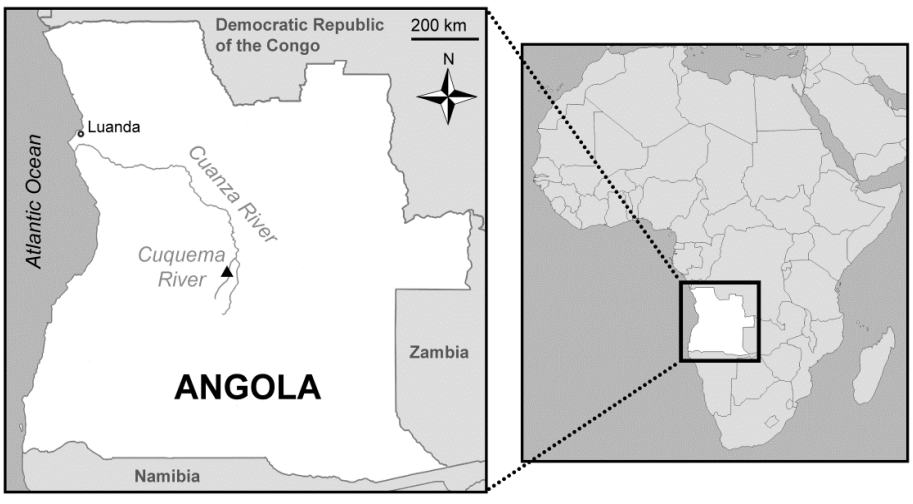
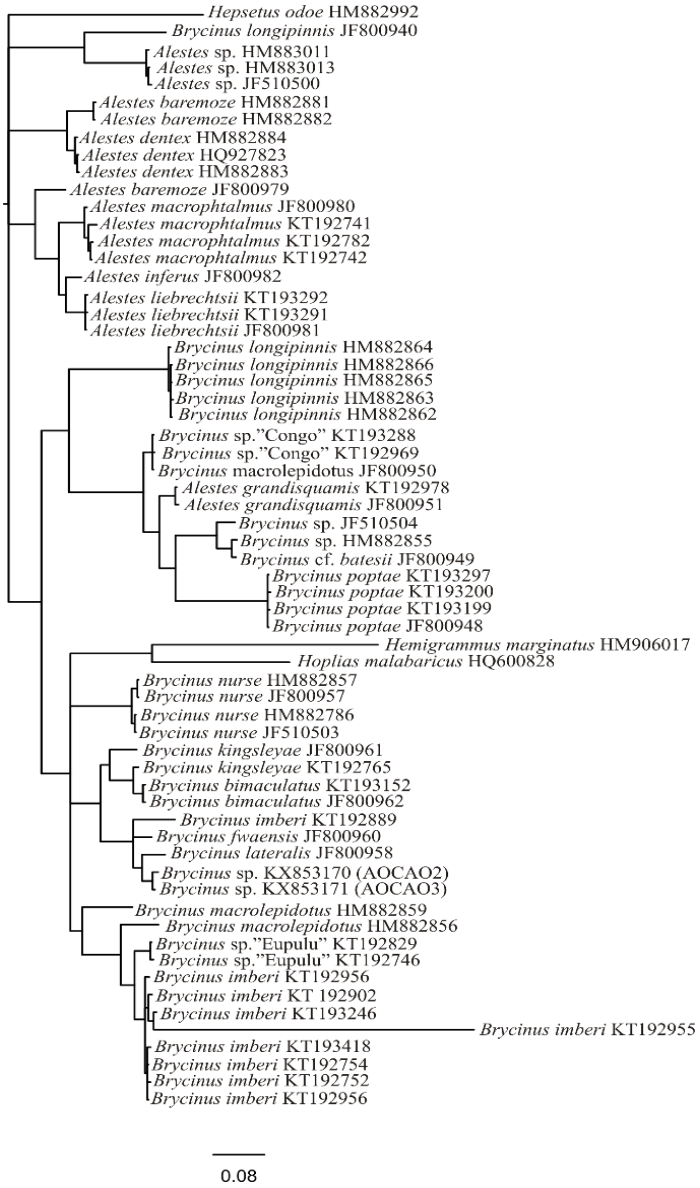


Figure 3 Detailed results of molecular analysis of the program MrBayes. Hepsetus odoe (Bloch) was used as the outgroup



CONCLUSION

Since genetic and morphological analyses suggested that found fishes belong to a new species of *Brycinus*, we cannot validate the new taxon due to the absence of adult males in our data set. Therefore, we recommend further collecting trip focused especially on mentioned males within the Cuanza River catchment.

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