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Aphyosemion musafirii (Cyprinodontiformes: Nothobranchiidae), a new species from the Tshopo Province in the Democratic Republic of Congo, with some notes on the *Aphyosemion* of the Congo Basin

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Abstract. *Aphyosemion musafirii*, new species, is described from specimens collected near Ubundu (Ruiki River, Congo Basin, Tshopo Province, Democratic Republic of the Congo). Another population of *A. musafirii* is known from the Romée River, 50 km West of Kisangani. The Ruiki and Romée Rivers are small tributaries on the left bank of the Congo River. *Aphyosemion musafirii* can be distinguished from its closest relative *A. castaneum* by the male colour pattern. A preliminary DNA analysis demonstrates that *Aphyosemion* s.s. consists of two major clades. *Aphyosemion musafirii* is in a clade with *A. castaneum*, *A. polli*, *A. lamberti*, *A. rectogoense,* and *A. congicum*. The distribution of all species of *Aphyosemion* s.s. is discussed.

Resumé. *Aphyosemion musafirii*, nouvelle espèce, est décrite à partir de spécimens récoltés près de Ubundu (rivière Ruiki, bassin du Congo, Province Tshopo, République Démocratique du Congo). Une autre population de *A. musafirii* est connue de la rivière Romée, 50 km à l'Ouest de Kisangani. Les rivières Ruiki et Romée sont de petits affluents rive gauche du fleuve Congo. *Aphyosemion musafirii* peut être distingué de son plus proche parent *A. castaneum* par le patron de coloration mâle. Une analyse ADN préliminaire démontre que *Aphyosemion s.s*. consiste en 2 clades majeurs. *Aphyosemion musafirii* est dans un clade avec *A. castaneum*, *A. polli*, *A. lamberti*, *A. rectogoense* et *A. congicum*. La distribution de toutes les espèces de *Aphyosemion s.s.* est discutée.

Key words. Killifish, eastern Congo basin, Ubundu, systematics, taxonomy, biogeography.

INTRODUCTION

The genus *Aphyosemion* was erected by Myers in 1924 with the type species *A. castaneum*, described in the same publication, from Kisangani (Democratic Republic of Congo). At present the taxonomy of the genus is still not settled, here we use *Aphyosemion* as proposed in two recent publications of the authors (Sonnenberg, 2007; Van der Zee & Sonnenberg, 2010). This is identical with the subgenus *Aphyosemion* of other authors (e.g. Collier, 2007; Huber, 2007; Murphy & Collier, 1999; Wildekamp, 1993) and consists of 16 species currently accepted as valid, which are, with the exception of two species from Gabon, endemic to the Congo drainage.

Only three species are currently known to occur in the eastern part of the Congo Basin: *A. christyi* (Boulenger, 1915), *A. schoutedeni* (Boulenger, 1920), and *A. castaneum* Myers, 1924 (Fig. 1). A fourth species, *A. margaretae* Fowler, 1936, is currently considered as a synonym to *A. christyi* (Van der Zee & Huber, 2006). The majority of museum collections of these species originate from the right bank of the Congo River. *Aphyosemion schoutedeni* was assumed to be restricted to the type locality "Medje at the Naya River", a tributary to the Aruwimi Basin, about 300 km northeast of Kisangani. Although the types are in good condition, all colour has disappeared. Since nothobranchiid species, at least within species groups or genera, differ little in morphological characters (Scheel, 1968, 1990), colour pattern of the male is crucial for species identification. Topotypes collected by Lang and Chapin in 1910, however, still have their colour pattern preserved (Van der Zee & Huber, 2006) and it is close to that of *A. castaneum* with the exception of the anal fin colour pattern. This colour pattern is also present in several *Aphyosemion* collections in the Royal Museum for Central Africa (MRAC) (Tervuren, Belgium) originating from the Aruwimi Basin, east of the Kisangani-Buta road

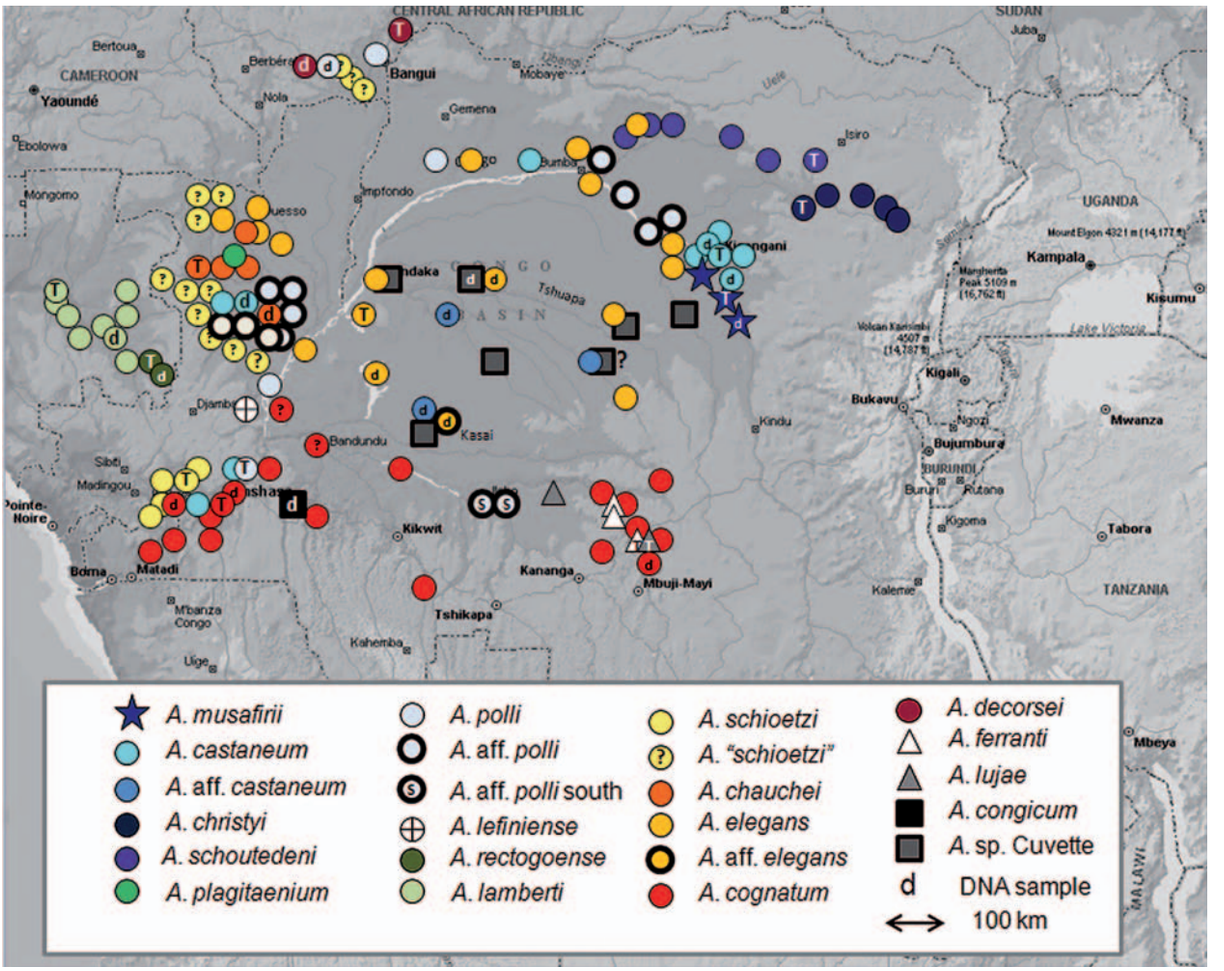

Fig. 1. Map showing the distribution of the genus *Aphyosemion*. Type localities are indicated by a T in the species symbol. The type locality for *A. congicum* is unknown and for *A. lefiniense* and *A. plagitaenium* only collections from the type locality or in proximity are currently known.

and may well represent *A. schoutedeni*. *Aphyosemion christyi* is restricted to the Epulu and Ituri drainages (about 350 km east of Kisangani) and is the only species that can be identified by morphological characters (i.e., higher number of dorsal fin rays than all other species) (Boulenger, 1915; Van der Zee & Sonnenberg, unpubl. data).

Aphyosemion castaneum has long been misidentified as *A. christyi* in literature and only recently Van der Zee & Huber (2006) demonstrated that *A. christyi* is restricted to elevations above 500 m over a distance of 180 km northeast of the type locality, Bafwasende. *Aphyosemion castaneum* is widespread around Kisangani, but additional records of this species are known from Salonga Park by Scheel (1990), who didn't specify the exact location within the park, Lompole, and Yaka in the central Congo Basin (Huber, 2005b). According to Huber (2005b) it is likely that these populations represent a different, undescribed species. Consequently, *A. castaneum* might be restricted to the right bank of the Congo River (Huber, 2005b), with the exception of some populations that were found close to the left bank, and the specimens collected on the left bank with a wide distribution in the central basin were distinguished here as *A.* sp. aff. *castaneum* (Fig. 1).

From the Romée and Ruiki Rivers on the left bank of the Congo River in the eastern part of the central Congo drainage, three collections of *Aphyosemion* are known from the MRAC, originally identified as *A. christyi*. The colour pattern of the anal and caudal fins in preserved specimens differs from *A. castaneum,* which is widespread around Kisangani. In 2007, A. Van Deun (Leuven, Belgium) collected a species of *Aphyosemion* with the same colour pattern as the three previous collections from the Romée and Ruiki Rivers, at two localities just north of

Table 1. List of specimens used for the DNA analyses with locality information and GenBank accession numbers. Abbreviations: DRC = Democratic Republic of Congo; RCA = Republic of Central Africa; US = sample provided by Uli Schliewen, ZSM, Munich; AS = aquarium bred strain; CI = commercial import; WC = wild caught sample.

Species	sample no.	Country	Collection locality	GenBank acc. no.
Aphyosemion castaneum AS	RS1408	DRC	Kisangani	JF307802
A. castaneum AS	RS1499	Republic Congo	Oyo	JF307797
A. castaneum WC	RS1790	DRC	AVD 3	JF307803
A. cf. *chauchei* AS	RS1527	Republic Congo	Olombo	JF307796
A. cf. *decorsei* AS	RS1521	RepublicCongo	Lobaye	JF307795
A. cognatum WC	RS1515	Republic Congo	Mbonza II (US 107)	JF307791
A. cognatum AS	RS1520	DRC	Lake Fwa	JF307793
A. cognatum AS	RS1529	DRC	Kinsuka	JF307794
A. congicum AS	RS1617	DRC	Z 82/17	JF307798
A. elegans WC	RS1747	DRC	Boende CI 2006	JF307792
A. elegans WC	RS1513	DRC	Inongo (US 24)	JF307790
A. elegans WC	RS1514	DRC	Inongo (US 66)	JF307789
A. lamberti AS	RS1256	Gabon	BSWG 97/9	JF307781
A. musafirii WC	RS1787	DRC	AVD 1	JF307804
A. polli AS	RS1584	DRC	CI	JF307800
A. polli AS	RS1479	RCA	RCA 91/1, Kapou 1	JF307801
A. rectogoense AS	RS1419	Gabon	PEG 95/16	JF307799
A. sp. aff. *castaneum* WC	RS1506	DRC	Lompolé (US 74)	JF307782
A. sp. aff. *castaneum* WC	RS1507	DRC	Lompolé (US 79)	JF307783
A. sp. aff. *castaneum* WC	RS1510	DRC	Yaka (US 33)	JF307786
A. sp. aff. *castaneum* WC	RS1511	DRC	Yaka (US 45)	JF307787
A. sp. aff. *castaneum* WC	RS1512	DRC	Yaka (US 61)	JF307788
A. sp. aff. *elegans* WC	RS1508	DRC	Lui Kotalé (US 75)	JF307784
A. sp. aff. *elegans* WC	RS1509	DRC	Lui Kotalé (US 77)	JF307785
A. sp. Cuvette AS	RS1019	DRC	Boende 2002	JF307780
Mesoaphyosemion cameronense	RS262	Cameroon	CMM 40	AY748282

Ubundu on the left bank of the Congo River. Based on live male colour pattern and a preliminary mitochondrial DNA analysis this species is described here as A*phyosemion musafirii,* new species.

MATERIAL AND METHODS

Morphometric measurements were taken with a digital calliper, partly under a dissecting microscope, and rounded to the nearest 0.1 mm. Counts and methods follow Amiet (1987). Measurements, including subunits of head, are presented as percentages of standard length (SL). The number of all visible rays of dorsal, anal, caudal, pelvic, and pectoral fins were counted, the abbreviation D/A means the relative position of the first dorsal fin ray with regard to the opposite anal fin ray. Count of scales on the mid-longitudinal series is the number of scales between the upper attachment of the opercular membrane and the caudal fin base. Excluded are the scales posterior to the hypural junction, which were counted separately. Nomenclature for the neuromast system on the head follows Scheel (1968) and Van Bergeijk & Alexander (1962), and that for the supraorbital (frontal) squamation follows Hoedeman (1958).

Total DNA was extracted from fin clips or muscle tissue from the caudal peduncle of ethanol preserved specimens, following a modified DNA extraction protocol after Gustinicich et al. (1991). Specimens used for DNA analyses are listed in Table 1 with GenBank accession numbers. A fragment of the mitochondrial cytochrome b gene was sequenced for 25 specimens of *Aphyosemion* and *Mesoaphyosemion cameronense* (GenBank accession number

Table 2. Uncorrected p-distances for the mitochondrial cytochrome b sequences with pairwise exclusion of missing data. Abbreviations for the DNA samples: CAM = *M. cameronense*, CAS = *A. castaneum*, cCHA = *A.* cf. *chauchei*, COG = *A. cognatum*, CON = *A. congicum*, cDEC = *A.* cf. *decorsei*, ELE = *A. elegans*, LAM = *A. lamberti*, MUS = *A. musafirii*, POL = *A. polli*, REC = *A. rectogoense*, sCAS = *A.* sp. aff. *castaneum*, sELE = *A.* sp. aff. *elegans*, spCU = *A.* sp. Cuvette.

species	CAM 262	spCU 1019	CON 1617	CAS 1499	CAS 1408	CAS 1790	MUS 1787	POL 1479	POL 1584	LAM 1256	REC 1419	sCAS 1506	sCAS 1507	sELE 1513	sELE 1514	sCAS 1510	sCAS 1511	sCAS 1512	ELE 1747	sELE 1508	sELE 1509	cDEC 1521	cCHA 1527	COG 1520	COG 1529
spCU1019	12.6																								
CON1617	14.9	5.9																							
CAS1499	13.0	7.9	6.9																						
CAS1408	13.4	7.0	6.5	2.0																					
CAS1790	13.4	7.1	6.3	2.1	1.7																				
MUS1787	13.7	6.6	5.8	3.4	3.3	3.2																			
POL1479	12.9	7.9	7.2	6.3	5.7	5.5	5.0																		
POL1584	13.0	7.8	7.6	6.1	5.4	5.5	5.0	1.1																	
LAM1256	13.0	7.5	6.7	5.9	5.8	5.7	4.6	5.1	5.1																
REC1419	13.7	8.2	7.1	6.4	6.3	5.9	5.4	5.1	5.1	3.0															
sCAS1506	13.2	8.2	7.8	7.1	7.2	6.8	6.6	7.6	7.6	6.3	6.2														
sCAS1507	13.2	8.6	7.9	7.0	7.2	6.8	6.6	7.6	7.6	6.6	6.2	0.7													
sELE1513	13.2	8.3	7.6	7.1	7.2	6.8	6.6	7.4	7.4	6.3	5.9	0.4	0.3												
sELE1514	13.4	8.4	7.8	7.0	7.1	6.7	6.4	7.5	7.5	6.4	6.1	0.7	0.3	0.3											
sCAS1510	13.3	9.1	8.4	7.4	7.5	7.1	7.1	8.2	8.2	7.1	6.7	1.2	0.8	0.8	0.8										
sCAS1511	13.6	9.1	8.4	7.4	7.5	7.1	7.1	8.2	8.2	7.1	6.7	1.2	0.8	0.8	0.8	0.3									
sCAS1512	13.3	9.1	8.4	7.4	7.5	7.1	7.1	8.2	8.2	7.1	6.7	1.2	0.8	0.8	0.8	0.0	0.3								
ELE1747	13.3	8.8	8.0	7.1	7.2	7.1	6.8	7.5	7.5	6.6	6.4	0.8	0.7	0.4	0.7	0.9	0.9	0.9							
sELE1508	13.6	8.7	8.3	7.0	7.1	6.7	7.0	7.5	7.8	7.0	6.6	1.6	1.4	1.2	1.4	1.7	1.7	1.7	1.3						
sELE1509	13.3	8.7	8.3	7.2	7.4	7.0	7.0	7.5	7.8	7.0	6.6	1.6	1.4	1.2	1.4	1.7	1.7	1.7	1.3	0.3					
cDEC1521	13.5	8.3	8.0	7.9	8.0	7.6	7.4	8.0	8.0	7.1	6.7	2.0	1.9	1.6	1.9	1.9	1.9	1.9	1.7	2.3	2.3				
cCHA1527	13.3	8.9	8.4	7.6	7.8	7.4	7.1	7.6	7.6	6.8	6.2	1.8	1.7	1.4	1.7	2.0	2.0	2.0	1.6	2.4	2.1	1.5			
COG1520	13.4	8.4	8.2	7.5	7.6	7.5	6.8	7.9	7.9	6.6	6.4	3.8	3.7	3.4	3.6	3.9	3.9	3.9	3.3	4.1	4.1	3.9	3.6		
COG1529	13.4	8.4	8.2	7.5	7.6	7.5	6.8	7.9	7.9	6.6	6.4	3.8	3.7	3.4	3.6	3.9	3.9	3.9	3.3	4.1	4.1	3.9	3.6	0.0	
COG1515	12.8	7.9	7.2	6.7	6.8	6.4	6.2	7.0	7.0	6.3	5.8	2.9	2.8	2.5	2.6	3.0	3.0	3.0	2.7	3.2	3.2	2.7	2.5	3.0	3.0

Fig. 2. *Aphyosemion musafirii*, male, collected with the types by A. Van Deun, 22.10. 2007, not preserved. Type locality, 67 km on the road from Kisangani to Ubundu, Democratic Republic of Congo. Photo: H. Ott.

AY748282, published in Sonnenberg & Blum [2005]) was used as outgroup, for lab protocols see Sonnenberg et al. (2006).

Resulting sequences were aligned with ClustalX 1.8 (Thompson et al., 1997) and checked by eye in BioEdit 7.0.5.3 (Hall, 1999). All sequences were translated into the corresponding amino acids and tested for the anti-G bias of mitochondrial sequences (Zhang & Hewitt, 1996) to confirm for functionality and mitochondrial origin. Uncorrected p-distances with pairwise exclusion of missing data were calculated in MEGA 4.1 beta 3 (Tamura et al., 2007) and are given in Table 2.

Fig. 3. *Aphyosemion musafirii,* male*,* 7 km north of Ubundu, Democratic Republic of Congo, collected by A. Van Deun, 22.10.2007, not preserved. Photo: H. Ott.

Analyses of sequence data were performed with PAUP 4.0b10 (Swofford, 1998) by maximum parsimony and with MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) by a Bayesian approach, all analyses with the identical respective settings given in Sonnenberg & Busch (2010). In addition, we performed a maximum parsimony analysis with the same parameters as the previous, but with exclusion of the third protein coding position. Only nodes, which are supported with bootstrap values above 75% or posterior probabilities above 95% were considered as supported by the data.

We used the software SplitsTree (Huson & Bryant, 2006) to calculate a split decomposition network representation of the dataset.

As species concept we adopted the approach by Moritz et al. (2000), which is similar to the Evolutionary or Phylogenetic species concepts (as discussed in Kottelat, 1997).

RESULTS

Aphyosemion musafirii, new species

(Figs 2–6, Tables 3–5)

Holotype. MRAC 2011-007-P-1, male, 36.7 mm SL, Democratic Republic of Congo, Tshopo Province, 67 km on the road from Kisangani to Ubundu (1°30' N, 25°21' E), 450 m altitude, north-eastern Congo basin, A. Van Deun, 22 October 2007.

Fig. 4. *Aphyosemion musafirii*, male, F1 from specimens collected 7 km north of Ubundu, Democratic Republic of Congo, showing a different anal fin pattern than most of the wild collected males from that population. Photo: W. Grell.

Paratypes. MRAC 2011-007-P-2-5, 4 females, 30.1–33.5 mm SL, collected with the holotype; MRAC 2011-007-P-6-9, 4 males, 32.9–34.6 mm SL, collected with the holotype.

Additional non-type material. MRAC 90-30-P-1471, labelled as *A. christyi* (Boulenger, 1915), Democratic Republic of Congo, Ubundu, L. De Vos, February 1990.

MRAC 90-47-P-846-853, labelled as *A. christyi* (Boulenger, 1915), Democratic Republic of Congo, Riv. Romée, km 30 route Kisangani-Opala, L. De Vos & C. Danadu, 07.02.1990.

Fig. 5. *Aphyosemion musafirii*, female, 7 km north of Ubundu, Democratic Republic of Congo, collected by A. Van Deun, 22.10.2007, not preserved. Photo: H. Ott.

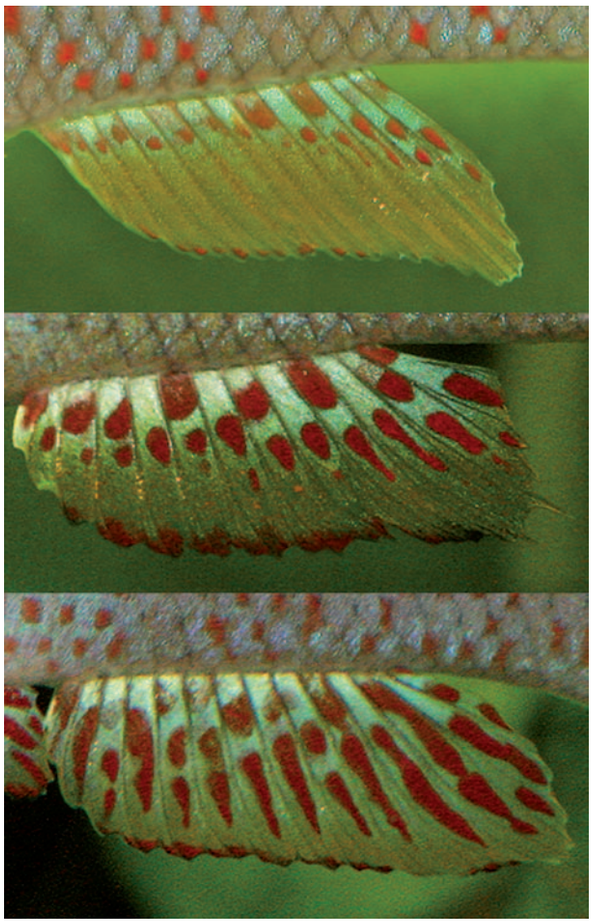

Fig. 6. Variability of the anal fin pattern in *Aphyosemion musafirii* (all examples from wild caught specimens).

MRAC 90-47-P-854-863, labelled as *A. christyi* (Boulenger, 1915), Democratic Republic of Congo, Riv. Romée km 3, rive gauche, route Kisangani-Opala, L. De Vos & C. Danadu, 10.–17.04.1990.

Diagnosis. *Aphyosemion musafirii* (Fig. 2–6) is placed within the genus *Aphyosemion* by the combination of the following characters: preopercular neuromast system with 6 pores, slender body, posterior origin of dorsal fin with less than 10 fin rays, females with a strong reticulation due to dark scale borders, and the extended edges of the caudal fin in males (Huber, 2005a).

Males of *A. musafirii* differ from all other representatives of *Aphyosemion* in the north-eastern Congo Basin by the in average broader red margin of the dorsal fin. This red border is narrow (up to 10%) in all other species in this area and is often absent at the distal end of the fin.

Males of *A. musafirii* differ from *A. castaneum* by the absence of a red band in the approximate centre of the anal fin, absence of a red infra-buccal band, absence of red edges around the light ventral zone in the caudal fin, absence of, or only very narrow, yellow margin of pelvic fins and a higher average number of red dots on side (*A. musafirii*: min. 51, max. 117, average = 79, sd = 23, number of specimens = 11; *A. castaneum*: min: 13, max. 57, average = 30, sd = 15, number of specimens = 20). These dots are arranged in more or less regular, interrupted rows in *A. musafirii*, whereas these dots are irregularly distributed in *A. castaneum*. The higher number of red dots on the side also distinguishes males of *A. congicum* (25–40), *A. schoutedeni* (17–28) and *A. polli* (11–49) from *A. musafirii*. It can also be distinguished by the more or less regular interrupted rows of red dots from *A.* sp. Cuvette, *A. elegans* (Boulenger 1899), and *A. plagitaenium*, which have either a pattern of vertical streaks or oblique bars on the side in males. Males of *A. musafirii* differ from *A. schoutedeni* by the same characters as *A. castaneum*, with the exception that *A. schoutedeni* lacks a red band in the anal fin. Males of *A. musafirii* have less spots in the caudal fin than *A. christyi* (35–55 in *A. musafirii*; over 70 in *A. christyi*). The background colour of the flanks is blue-green in *A. musafirii* and purple-blue in *A. christyi*. In addition to colouration characters, males of *A. musafirii* can be distinguished from *A. christyi* by the lower number of dorsal fin rays (7–9 in *A. musafirii* versus 10–11 in *A. christyi).*

Description. See Figures 2–6 for general appearance and Tables 3–5 for morphometric and meristic data of the type series. *Aphyosemion musafirii* shows strong sexual dimorphism, males more colourful, unpaired fins larger, dorsal and anal fins with posterior fin rays extended. A medium sized, slightly laterally compressed species; dorsal profile slightly convex, greatest body depth approximately at pelvic fins. Ventral profile slightly convex from head to end of anal fin, concave on caudal peduncle. Snout slightly rounded, mouth directed upwards, lower jaw longer than upper jaw. Dentary bears an outer row of large and inner irregular rows of smaller unicuspid, curved teeth; the premaxilla bears some larger and several smaller unicuspid and curved teeth.

Frontal (after Scheel, 1968) or nasal (after van Bergeijk & Alexander, 1962) neuromasts in separate grooves, the preopercular canal with six pores.

Scales cycloid, entirely scaled except ventral surface of head; frontal squamation of G-type; scales on mid-longitudinal series 29–30, with 1–2 scales posterior to the hypural plate; 7 transversal scales, 12 scales around the caudal peduncle.

Table 3. Morphometrics of *Aphyosemion musafirii*, new species, (H = holotype, P = paratypes: 4 males and 4 females). All measurements in percentages of standard length, standard length in mm.

	H ♂	P ♂	P ♂	P ♂	P ♂	P ♀	P♀	P ♀	P ♀
Standard length	36.7	34.6	34.2	33.3	32.9	33.5	32.6	31.3	30.1
Body depth	21.0	18.4	19.6	19.5	18.8	18.8	20.8	20.1	21.4
Head length	21.5	20.5	21.6	21.0	20.4	19.1	20.5	19.5	19.0
Eye diameter	6.3	6.3	6.4	6.3	6.1	5.7	6.4	6.4	6.9
Interorbital width	12.0	12.7	11.1	11.4	12.5	10.7	11.3	11.2	13.0
Pre-dorsal length	63.2	61.0	66.0	66.4	62.1	60.2	66.2	65.8	63.5
Pre-anal length	55.0	54.9	53.5	54.3	50.8	55.2	56.1	54.6	57.3
Dorsal fin base	11.2	12.1	10.2	11.1	11.2	11.0	10.1	10.9	11.0
Anal fin base	19.1	18.8	17.5	19.2	18.2	17.3	16.1	17.3	17.2
Caudal peduncle depth	12.5	11.5	11.1	11.7	11.6	10.7	11.7	11.5	11.9

Small dorsal fin with 7–9 fin rays, first dorsal fin ray inserts above the 7–10th anal fin ray; anal fin with 13–15 rays; posterior dorsal and anal fin rays slightly elongated in males; caudal fin with 21–24 rays, with extensions on upper and lower fin rays. Pectoral fin with 12–14, pelvic fin with 5 rays.

Live colouration Males. (Figs 2–4 & 6) Flanks greyish brown with blue-green iridescence. Edges of scales on the flanks with dark pigmentation resulting in a reticulated pattern. Dorsally the scales have broader pigmented edges than ventrally. Flanks with approximately 50 to 120 red spots. These spots are mainly situated at the anterior edge of the scales and are mostly irregularly distributed in lines, usually forming up to five parallel lines. Three red streaks on opercle in an approximate 45° angle. Infra-buccal band absent or only present at the sides of the jaw.

Table 4. Meristics of *Aphyosemion musafirii*, new species. Numbers indicate observed values; numbers in parentheses frequency of occurrence; values found for the holotype are indicated by an asterisk.

meristic count	**values (frequency)**
dorsal fin rays	9 (3*), 8 (4) 7 (2)
anal fin rays	13 (5*), 14 (3), 15(1)
D/A	+7 (1*), +8 (1), +9 (3), +10 (4 fem.)
caudal fin rays	21 (3*), 22 (2), 23 (3), 24 (1)
pelvic fin rays	5 (9*)
pectoral fin rays	12 (2*), 13 (6), 14 (1)
lateral line scales	29 (2), 30 (6*), 31 (1)
transversal row scales	7 (9*)
circumpenduncul. scales	12 (9*)

Pectoral fin yellow, unspotted or just with some tiny spots in the centre. Pelvic fin yellow with several prominent red spots. Dorsal and anal fin blue iridescent at the base and yellow distally, provided with red spots; spots more numerous and larger at the base than distally. Dorsal fin edged with a broad dark red band (up to 30% of fin length at mid section). Anal fin edged with a narrow red band. Spots on anal fin rounded to elongated, sometimes missing at the distal part of the fin, leaving a broad yellow subdistal band. Caudal fin light blue with yellowish distal edges provided with rounded and/or 35–55 elongated red spots. Dorsal and ventral edge of the caudal fin provided with a broad dark red band.

Females. (Fig. 5) Flanks grey with darker reticulation. Red streaks on opercle reduced and infra-buccal band absent. All fins transparent. Edge of anal fin and distal part of ventral fins light blue. Faint spots on anal fin, more prominent spots on base of dorsal and dorsal part of caudal fin.

After one year of preservation in ethanol. Males. Flanks light brown with transition to light ventral side. Reticulation, buccal band, and red streaks on opercle as in live specimens. Spots on flanks as in live specimens, but smaller and more vague. All fins transparent greyish, provided with spots as in live specimens, but vague and pink.

Females. As in live specimens, but spots on anal fin hardly visible.

Distribution. (Fig. 1) *Aphyosemion musafirii* is restricted to brooks in the Ruiki and Romée River systems on the left bank of the Congo River between Kisangani and Ubundu, Tshopo Province, Democratic Republic of Congo.

Table 5. Comparison of the morphometric values for *Aphyosemion musafirii*, new species, and members of the subgenus *Aphyosemion*. Abbreviations used in the table: CAS = *A. castaneum*, CHR = *A. christyi*, COG = *A. cognatum*, CON = *A. congicum*, LEF = *A. lefiniense*, MEL = *A. melanopteron*, MUS = *A. musafirii*, POL = *A. polli*, TEU = *A. teugelsi*, E = eye diameter, I = interorbital width, BD = body depth, HL = head length, HW = head width, pD = predorsal fin distance, pA = preanal fin distance, DB = dorsal fin base, AB = anal fin base, CD = caudal peduncle depth, sd = standard deviation.

species	location	sex	SL	E	I	BD	HL	pD	pA	DB	AB	CD
COG	Z 91/3,	♂	33.5	8.0	11.0	22.9	18.9	67.1	62.2	11.5	21.9	14.3
CHR	HZ 85/14,	♂	29.1	7.9	9.3	18.4	21.9	65.7	59.0	11.6	19.6	11.2
	Epulu	♀	31.2	7.8	9.3	17.1	22.1	64.5	58.7	11.3	19.9	10.9
POL	RCA 91/1,	♂	36.0	7.5	10.6	22.5	23.3	68.3	59.4	12.1	21.7	13.1
	Kapou	♀	29.1	6.9	8.2	23.6	22.7	67.0	61.9	11.7	19.9	12.6
CON	type of MEL	♂		7.8	9.5	21.6	27.8	67.3	61.5	12.1	20.9	13.4
CAS	HZ 85/13	♂	20.8	7.7	13.4	20.4	20.2	70.2	53.8	12.0	22.1	11.1
		♀	19.4	7.7	11.9	20.8	19.8	68.3	52.1	11.5	20.0	12.3
LEF	Luna River	♂	23.1	8.7	12.1	19.0	24.1	66.6	54.9	7.0	13.0	9.6
		♀	19.2	8.3	11.4	21.0	23.6	69.9	57.0	8.6	16.2	11.8
mean				7.8	10.6	20.8	22.2	67.7	58.1	11.0	19.6	12.2
sd				0.5	1.4	2.0	2.6	1.9	3.4	1.6	2.7	1.4
range				6.9–8.7	8.2–13.4	17.1–23.6	18.9–27.8	64.5–70.2	52.1–62.2	8.6–12.1	13.0–22.1	9.6–14.3
MUS												
mean				6.3	12.1	19.2	30.9	68.3	61.1	14.6	19.0	12.4
sd				0.3	0.5	2.2	1.0	1.6	1.5	0.7	1.2	0.2
range				5.4–16.9	11.3–12.8	16.6–21.2	29.3–32.0	66.0–70.5	59.2–62.8	13.8–15.3	17.4–20.7	12.1–12.7

Etymology. *Aphyosemion musafirii* is named after Dr. Jean Musafiri (Ubundu, Democratic Republic of Congo), coordinator for the national tuberculosis and leprosy control programme in the "Province Orientale Occidentale", the huge forest area around Kisangani. The name Musafiri means "traveller" in Swahili. Indeed, he travels around the area under very difficult circumstances, covering enormous distances by jeep, motorised canoe or small motorcycle. Dr. Musafiri was born in Ubundu and has always stayed in the province to help his people, in spite of the very difficult living conditions and the atrocities of the war in the eastern Democratic Republic of Congo. He made it possible that the type material of *A. musafirii* and a new species of *Fenerbahce* (Sonnenberg, Woeltjes & Van der Zee, submitted) could be collected by A. Van Deun (Institute of Tropical Medicine, Antwerp) at the occasion of an external evaluation visit of the tuberculosis/leprosy programme.

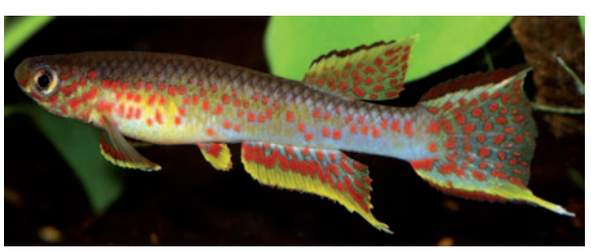

Fig. 7. *Aphyosemion castaneum*, 11 km west of Kisangani, Democratic Republic of Congo, not preserved. Photo: H. Ott.

Fig. 8. *Aphyosemion castaneum*, Wani Rukula, 65 km southeast of Kisangani, Democratic Republic of Congo. Photo: H. Ott.

DNA analyses. The resulting sequence alignment has a final length of 760 bp, the base composition shows the, for mitochondrial sequences typical, A/T bias (Zhang & Hewitt, 1996). In two sequences (RS1747 and RS1521), up to 13 N were introduced at the start of the alignment for equal sequence length. We found 192 variable and 130 phylogenetic informative positions. The DNA fragment translates into 253 amino acids, of which 27 are variable and 15 phylogenetic informative, and contains no unexpected stop codon. Uncorrected pairwise distances be-

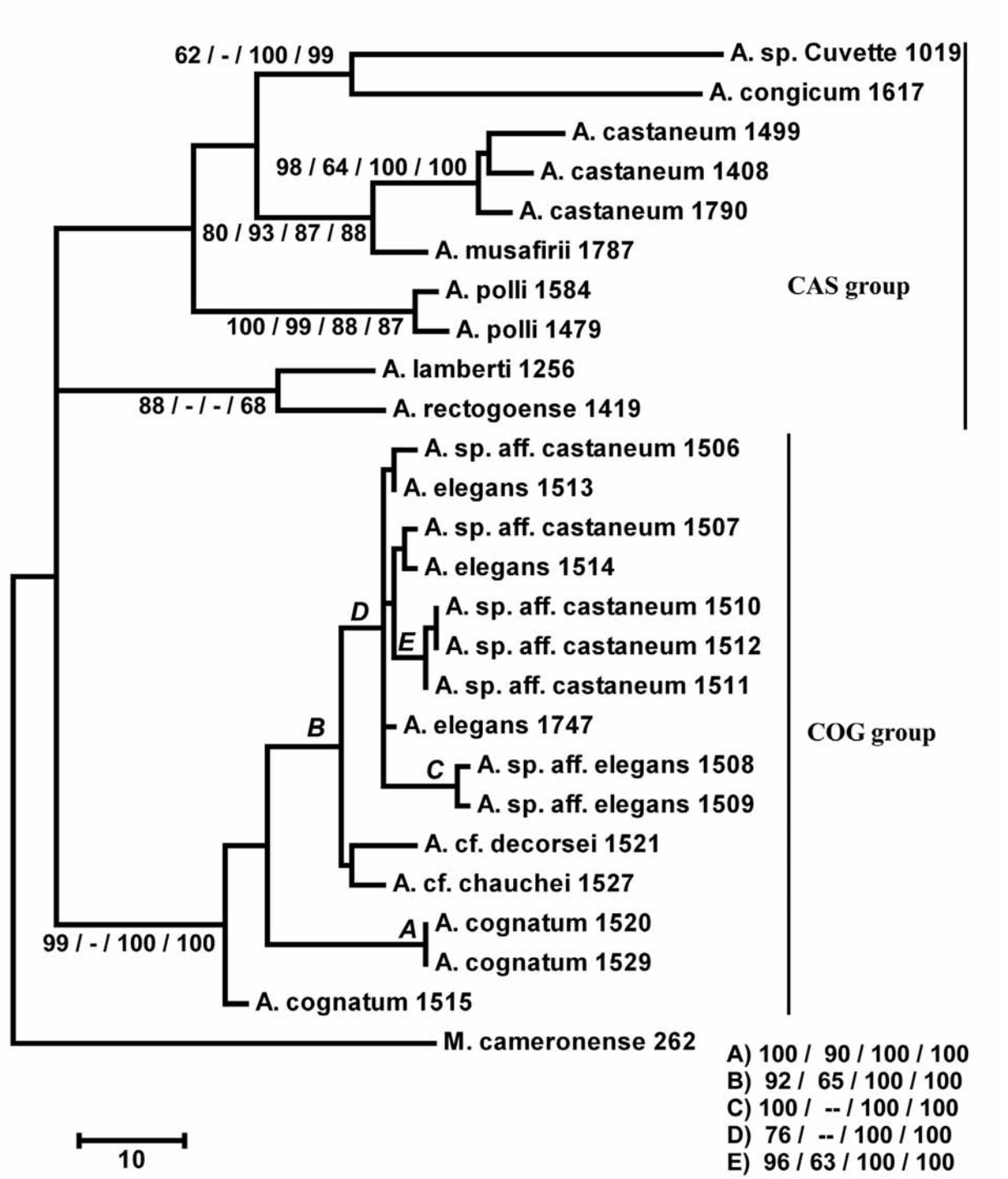

Fig. 9. Phylogenetic tree by maximum parsimony, tree length = 283, consistency index = 0.5194, homoplasy index = 0.4806, retention index = 0.7467, and rescaled consistency index = 0.3879. Shown is one of 36 equally short trees, on the left side of nodes support values are shown for maximum parsimony bootstrap analyses and Bayesian posterior probabilities in the following order: maximum parsimony / maximum parsimony with exclusion of third position / Bayes with Nst = 2 / Bayes with Nst = 6. Only support values for nodes are shown, for which at least in one analysis a bootstrap value of 75% or posterior probabilities of 95% were reached. No value given means that the support value in all analyses is below the previous given values or the node is not recovered in the analysis. Some values are given below the tree and are indicated on the respective nodes by a capital letter for better readability.

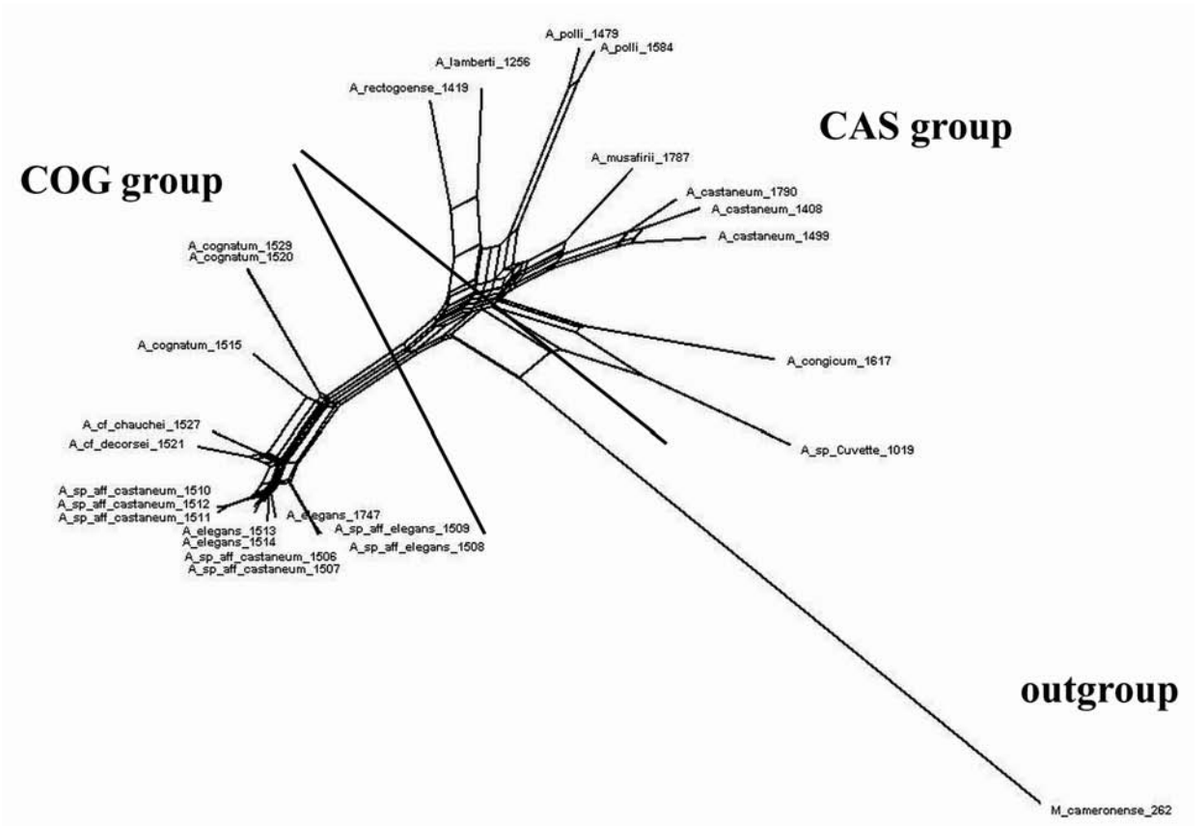

Fig. 10. SplitsTree network representation of the *Aphyosemion* dataset. Number of taxa = 26, 760 base pairs, fit = 98.01, uncorrected p-distances, neighborNet.

tween sequences are presented in Table 2, the observed values found between *Aphyosemion* and *Mesoaphyosemion* as the outgroup range from 12.6–14.9%, the maximum observed value within *Aphyosemion* is 9.1%.

The samples of *A. cognatum* from aquarium strains originating from Kinsuka and Lake Fwa are, despite nearly 900 km distance, identical, and also with the shorter sequence of an *A. cognatum* from Lake Fwa published by Murphy & Collier (1999, GenBank acc. no. AF002324), therefore it might be possible that the strains were erroneously mixed or mislabelled since their introduction in the killifish hobby. A test with a second specimen from the Kinsuka strain resulted in the same sequence (data not shown).

The *A. cognatum* 1515 sample is only represented by a female, so the species identification is tentative based on the knowledge of other *Aphyosemion* collections around this area and the resulting sequence is identical with a shorter sequence of Murphy & Collier (1999, GenBank acc. no. AF002327), which they have published as *A. elegans* from Naoimda, and slightly different from their sequence of what they call *A. elegans* from Madimba (GenBank acc. no. AF002328). It is not clear if Naoimda is a misspelling of Madimba. However, the specimens from Madimba belong to *A. cognatum* (e.g. see Seegers, 1997, p. 74), the sequences differ in only two bases.

The phylogenetic analyses (Fig. 9) give an only partially resolved tree of the analysed specimens. In addition, there are differences in support for nodes; excluding the third protein coding positions gives no bootstrap support above 50% for several nodes, which were recovered by the other analyses (Fig. 9). *Aphyosemion musafirii* seems to be closer to *A. castaneum,* however, this node is only supported by the maximum parsimony analyses. Comparing the phylogenetic hypotheses summarized in the tree (Fig. 9) with the split-decomposition network representation (Fig. 10), the network indicates a clear separation of the analysed specimens into two main groups, which is not that prominent in the dichotomous phylogenetic hypotheses (Fig. 9). One group, in the following called the *A. castaneum* group (CAS) is characterised by longer branches between the species (range 1.1–8.2% p-distance), whereas the second group, the *A. cognatum* group (COG) has

shorter internal branch length (0.0–4.1% p-distance), the divergence between both groups ranges between 5.8–9.1% p-distance. The CAS group contains, according to the network (Fig. 10), five species, which were found from the western to the eastern Congo Basin, and two species from Gabon. However, this group is not recovered by the phylogenetic analyses with any significant support (Fig. 9). The second group in the network (Fig. 10) is recovered with high support (Fig. 9) at least by three analyses and contains samples from the western and central Congo Basin.

DISCUSSION

Colour pattern

The main criterion for distinguishing species in nothobranchiids and especially *Aphyosemion* is the male colour pattern. All Nothobranchiidae show a polygamous mating system and a high degree of sexual dimorphism. Amiet (1987) therefore assumed that male colouration might be important in mate choice of females in nothobranchiid fishes. Van der Zee et al. (2007) assumed that especially the colour pattern of the caudal peduncle and unpaired fins plays an important role in female mating preferences. Kullmann & Klemme (2007) were able to demonstrate for *Chromaphyosemion* that females prefer to mate with their own males, for *Diapteron* this was studied by Brosset & Lachaise (1995). Thus female mate choice on divergent male colour characters may enable speciation by sexual selection. After secondary contact of previously allopatric populations, species cohesion is then easily maintained in parapatry or sympatry.

The current study shows that the genus *Aphyosemion* remains taxonomically problematic, which is in part due to old type material with no traces of the most important characters, the male colouration pattern, and descriptions, which do not give detailed information about live or preserved colouration. On the other hand, many species show a certain degree of variation in colour pattern within and between populations, which makes identification in some cases difficult. Differing species identification between authors further complicates killifish literature, as for example in the case of the *A. elegans* samples in the study of Murphy & Collier (1999) mentioned above. A third sample listed as *A. elegans* in their study, an aquarium strain from Epoma (Murphy & Collier, 1999) was later described as *A. plagitaenium* by Huber (2004).

Phylogeny

Our preliminary molecular phylogeny of this group gives a very complex pattern. The CAS (*A. castaneum*) group might be not a monophyletic unit, but consists of several species with deep divergences, which is reflected by the large p-distances found in this group (Table 2). The root of the group might be placed close to this species complex (Fig. 10). The COG (*A. cognatum*) group consists of species with lower within-group p-distances (Table 2), appears to be monophyletic and might contain species of more recent origin. Both groups cover large ranges in the Congo and adjacent river basins in Gabon (*A. lamberti* and *A. rectogoense*), have a kind of mixed distribution and occur in some cases in sympatry (e.g. *A. elegans* and *A*. sp. Cuvette) or even syntopically (*A*. sp. Cuvette and *A*. sp. aff. *castaneum; A. chauchei* and *A. elegans*; *A. "schioetzi*" and *A. polli*).

Interestingly some phenotypically different species (*A.* sp. aff. *castaneum* and *A. elegans* / *A*. sp. aff. *elegans*) in the Cuvette centrale turn out to be closely related and not genetically separated by the mitochondrial DNA data (Fig. 9). Compared with some additional nuclear 28S rDNA (LSU) sequences (unpublished data), it turns out that the studied samples can be separated into two groups: one including *A. castaneum* and *A*. sp. aff. *castaneum* and on the other hand the samples of *A. elegans* / *A*. sp. aff. *elegans.* This indicates that most probably mitochondrial introgression between species of the CAS and COG group has occurred in an area were both groups are at least parapatric. However, despite the potential of hybridization as is indicated by the mitochondrial introgression, both species or species groups live at least in sympatry and have stable distinguishing characters.

Distribution of the genus *Aphyosemion*

With the addition of *A. musafirii* the number of species in the eastern Congo Basin is increased to four (see Introduction). The study of the *Aphyosemion* specimens in the MRAC by the first author also added some collections of other species from this area (Fig. 1), in part due to misidentified samples. However, the maximum number of eight species is found in the westernmost part of the Congo Basin. An artificial factor, which might explain the concentration of species here, is the higher number of samples from this area. A natural cause of high species diversity might be the proposed existence of a forest refuge during dryer periods in the past (Leal, 2004; Maley, 1996; Plana, 2004). Two of these eight species are widely distributed in the northern part of the basin: *A. castaneum* Myers, 1924 and *A. polli* Radda & Pürzl, 1987. These two species were probably transported downstream by the Congo and Ubangui rivers, and both can also be found at Pool Malebo. *Aphyosemion elegans* is here present in the northern tributaries of the Likouala River, but is widespread in the central Congo Basin (Cuvette centrale) and north of the middle Congo section up to Buta.

Three species have a more restricted distribution in the Central Republic of Congo: *A. chauchei* Huber & Scheel, 1981 is found in the Likouala and Alima drainages, *A. lefiniense* Woeltjes, 1984 is restricted to the southern tributaries of the Lefini River, and *A. plagitaenium* Huber, 2004 is only known from the type locality at Epoma in the northern Central Republic of Congo.

Aphyosemion schioetzi Huber & Scheel, 1981 has a disjunctive distribution: a rather restricted area in the south around the type locality and a huge area in the north, separated by a gap of almost 280 km where only *A. lefiniense* is found (Huber & Scheel, 1981). The present authors suggest that the northern populations (labelled here in the following "*schioetzi*", Fig. 1) do not belong to *A. schioetzi sensu strictu*, since all representatives are lacking the dark red edge of the anal fin and seem to be built more slender than *A. schioetzi*. The northern populations might represent a new species or be conspecific with *A. decorsei* (Pellegrin, 1904). The status of *A. decorsei* has been subject to discussion for a long time (Huber, 1994, 2004, 2005a; Scheel, 1968, 1990; Wildekamp, 1993). Poll (1951) even placed it in *Epiplatys* and in the description of *Haplochilus decorsei* Pellegrin assumed it to be close to *Aplocheilichthys spilauchen*. Myers (1924) placed it in *Aphyosemion* with some hesitation. Scheel (1968, 1990), Huber (1994, 2004, 2005a), and Wildekamp (1993) all have seen the types and they confirmed Myers's statement. The types from the Central African Republic are in poor condition with the colour pattern lost. Huber (2004, 2005a) stated that *A. decorsei* has only few red spots on the flanks and might be conspecific with *A. polli*, the latter being a junior synonym. Wildekamp (1993), however, is convinced that *A. decorsei* has many spots on the flanks, based on the light spots on the scales of the syntypes. Red pigmentation pattern, after preservation in formalin and transfer in ethanol, leaves corresponding patterns of lighter areas than the body colouration (Van der Zee & Sonnenberg, 2010). *Aphyosemion polli* has not only few spots on the flanks, but also has very few spots (or no spots) on the anal fin, arranged near the base of this fin. In the original description of *A. decorsei* Pellegrin writes: "la dorsale, l'anale et des ventrales avec des petits points carmins plus ou moins nombreux" (dorsal, anal, and ventral fins with small more or less numerous carmine spots). The present authors agree with Wildekamp's argumentation: *A. decorsei* is a species with many spots at least on the anal fin.

Aphyosemion cognatum Meinken, 1951 has a huge distribution area in the south from the right bank of the Congo River to Lodja in the upper Lukenie basin. Sympatric with *A. cognatum* occur *A. ferranti* (Boulenger, 1910), *A. congicum* (Ahl, 1924), and *A. lujae* (Boulenger, 1911).

In the large Cuvette centrale only three species occur: a blue species that resembles *A. castaneum* (as *A.* sp. aff. *castaneum* in Fig. 1), *A. elegans,* and another undescribed species (*A*. sp. Cuvette) that can occur sympatrically with *A. elegans*. This species, with a characteristic dark dorsal fin, has long been taken for *A. elegans* in many publications (Radda & Pürzl, 1987; Huber, 2004, 2005a,b; Wildekamp, 1993). However, the description and original drawing do not mention this dark dorsal fin that is very prominent even in preserved specimens (Boulenger, 1899). Our DNA analysis shows that *A. elegans* and *A.* sp. Cuvette are not closely related (Fig. 9). Additionally, the specimens from Lui Kotale (Fig. 1) differ by male colour pattern and are tentatively named *A*. sp. aff. *elegans* to indicate the differences and their potential status as separate species.

Historical influences on current pattern

The distribution pattern within the group is very complex. Several species are found over large areas (Fig. 1), often mixed with congeners. This complexity is probably related to the history of the Congo Basin.

From our data it cannot be determined when *Aphyosemion* reached the Congo Basin. The ancestors of the extant species must have come from the west where their closest relatives live. With the current data it is not possible to decide if they were already dispersed around the endorheic lake or if they entered the basin after a river capture to the west. Both scenarios will result in different distribution and dispersal patterns.

In later stages forest refuges probably played an important role in the establishment of the mosaic distribution pattern of *Aphyosemion* during glacial dry periods. Almost all species of *Aphyosemion* s.l. are strictly bound to forest cover (Brosset, 1982; Kamdem Toham & Teugels, 1997, 1998, 1999). During glacial periods, the majority of the forest in the Congo Basin was replaced by savannah (see Leal, 2004; Maley, 1996). Only in the western part of the basin a relatively large refuge was present from where dispersal might have originated during more humid periods. Leal (2004) proposed that besides larger well known refuges also micro refuges must have existed, e.g. in Gabon. A combination of one or more larger and several micro refuges from where repeatedly dispersal could have occurred might explain the very complex distribution pattern of *Aphyosemion* in the Congo Basin.

Prospect

Further DNA studies can contribute to alpha taxonomy and towards a better understanding of the phylogeny and bio-

geography of this group and add to our understanding of its evolution. However, much more samples from the huge distribution area of *Aphyosemion* will be needed and the occurrence of mitochondrial introgression makes it necessary to include several nuclear markers into such a study to get reliable and well supported results.

COMPARATIVE MATERIAL

Part of the comparative material is listed in Van der Zee & Sonnenberg (2010); additional material is listed here and in the Online Appendix:

Aphyosemion sp. Cuvette: MRAC 79-09-P-720-722, labelled as *A. christyi* (Boulenger, 1915), Democratic Republic of Congo, Iteli River, terr. Opala, J. Lambert, 12.05.1958.

Aphyosemion sp.: MRAC 90-30-P-1471, labelled as *A. christyi* (Boulenger, 1915), Democratic Republic of Congo, Yangambi, J. Lambert, 08.05.1957; MRAC 119855-856, labelled as *A. christyi* (Boulenger, 1915), Democratic Republic of Congo, Yangambi, J. Lambert, 09.05.1957.

Aphyosemion castaneum Myers, 1924: MRAC 22555-22561, Democratic Republic of Congo, Stanleyville, Dr. Richard, 1930; MRAC 89-043-P-547-612, labelled as *A. christyi* (Boulenger, 1915), Democratic Republic of Congo, Libuku River near Kisangani, L. De Vos & M. Katembo, April 1988.

Aphyosemion schoutedeni (Boulenger, 1920): MRAC15664-15665, identified by David (1936) as *Epiplatys boulengeri,* Democratic Republic of Congo, Medje, Lang & Chapin, 1910; MRAC 25529, Democratic Republic of Congo, Medje, H. Schouteden, no year.

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The Online Appendix is available at http://www.zfmk.de/web/Forschung/Buecher/Beitraege/index.en.html

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