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THREE NEW KILLIFISH SPECIES (CYPRINODONTIFORMES: NOTHOBRANCHIIDAE) FROM EQUATORIAL GUINEA

Francisco J. Malumbres[1], Rainer Sonnenberg[2] & Jouke R. Van der Zee[3,*]

[1] Gabriela Mistral 16 / 7 E, Madrid 2, Spain. Email: fran.malumbres@icloud.com – ORCID iD: https://orcid.org/0000-0002-4403-5956

[2] Zoologisches Forschungsmuseum Alexander Koenig, Department of Vertebrates, Adenauerallee 160, D–53113 Bonn, Germany. Email: r.sonnenberg.zfmk@uni-bonn.de – ORCID iD: https://orcid.org/0000-0003-3244-4880

[3] Royal Museum for Central Africa, Vertebrate Section, Ichthyology, Leuvensesteenweg 13, B-3080 Tervuren, Belgium. Email: joukevdz@hotmail.com – ORCID iD: https://orcid.org/0000-0002-7010-533X

[*] Corresponding author

ABSTRACT

Two new *Mesoaphyosemion* species and one new species of the '*Aphyosemion' herzogi* group are described from the inland of Equatorial Guinea. The results of mtDNA analyses of most of the known phenotypes of the genus *Mesoaphyosemion* and the '*Aphyosemion' herzogi* species group, respectively, are presented. Both new *Mesoaphyosemion* species have dark blotches on the posterior flanks and resemble *M. maculatum* from Gabon, yet they are not closely related to that species. Although the two new species occur in very close proximity, DNA results suggest no close relationship. The '*Aphyosemion' herzogi* species group has a similar distribution as the genus *Mesoaphyosemion*, but with its northern boundary in southern Cameroon. Based on mtDNA the new '*Aphyosemion'* from the Mitemele drainage in south west Equatorial Guinea is basal species to the remaining species group. It is distinguished from the two described congeners, '*A.' bochtleri* and '*A.' herzogi* by a diagnostic combination of colouration characters. Unpaired fins and flanks have a green background and caudal peduncle is often yellow to golden with dark red irregular dark red bars. The genetic data indicate that the species group contains several additional, genetically and by colour pattern well separated, potentially undescribed species.

Keywords: Mitemele drainage, Parque National de Monte Alén, National Park, Woleu-Ntem Plateau, Monts de Cristal, introgression, convergent evolution, '*Aphyosemion' mitemelense* sp. nov., '*Aphyosemion' herzogi* group, *Mesoaphyosemion losantosi* sp. nov., *Mesoaphyosemion montealenense* sp. nov.

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RESUMEN

Tres nuevas especies de killis (Cyprinodontiformes: Nothobranchiidae) de Guinea Ecuatorial

Se describen para la parte continental de Guinea Ecuatorial dos nuevas especies de *Mesoaphyosemion* y una nueva especie del grupo de especies *'Aphyosemion' herzogi*. También, se presentan los resultados de los análisis de DNAmt de casi todos los fenotipos conocidos del género *Mesoaphyosemion* y del grupo de especies '*Aphyosemion' herzogi*. Ambas nuevas especies de *Mesoaphyosemion* tienen manchas oscuras en los flancos posteriores y se parecen a *M. maculatum* de Gabón, pero no están estrechamente relacionadas con esa especie. Aunque las dos nuevas especies son próximas, los resultados del ADN sugieren que no existe entre ellas una relación cercana. El grupo de especies '*Aphyosemion' herzogi* tiene una distribución similar a la de *Mesoaphyosemion*, pero con su límite norte en el sur de Camerún. Basado en ADNmt, el nuevo '*Aphyosemion'* de la cuenca del río Mitemele, en el suroeste de la parte continental de Guinea Ecuatorial, es una especie basal al resto de las especies estudiadas. Se distingue de los dos congéneres descritos, '*A.' bochtleri* y '*A.' herzogi* por una combinación de caracteres diagnósticos de la coloración. Las aletas impares y los flancos tienen un fondo verde y el pedúnculo caudal suele ser de color amarillo a dorado con barras rojo oscuro irregulares. Los datos genéticos indican que el grupo de especies contiene varias especies más, que están genéticamente y por patrón de coloración bien delimitadas, y que no han sido formalmente descritas.

Palabras clave: Cuenca del Mitemele, Parque Nacional de Monte Alén, Parque Nacional, Meseta Woleu-Ntem, Montes de Cristal, introgresión, evolución convergente, 'Aphyosemion' mitemelense sp. nov., grupo *'Aphyosemion' herzogi*, Mesoaphyosemion losantosi sp. nov., Mesoaphyosemion montealenense sp. nov.

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Introduction

Equatorial Guinea is a small country surrounded in the north by Cameroon and east and south by Gabon. Its fish fauna belongs to the Lower Guinea ichthyoprovince (Stiassny *et al.*, 2007). Contrasting to the surrounding countries its fish fauna has been less studied. Pappenheim (1910) was the first to publish on a small collection made by Tessman at the end of the nineteenth century. The first ichthyological inventory of continental Equatorial Guinea was conducted by Roman (1971), followed by a comprehensive survey by Castelo (1994). Lasso *et al.* (1998) reported on the fish fauna of Monte Alén and listed 58 species in 36 genera and 16 families. In 2017 an expedition surveyed the freshwater fishes at 42 locations that resulted in the description of a new *Chiloglanis* species (Schmidt & Barrientos, 2019), two new *Distichodus* species (Schmidt *et al.*, 2021), and several new characid and cyprinid species will be described in the near future (R. Schmidt, pers. comm., Sep. 2019). These recent studies indicate that the diversity of freshwater fish is only partially known.

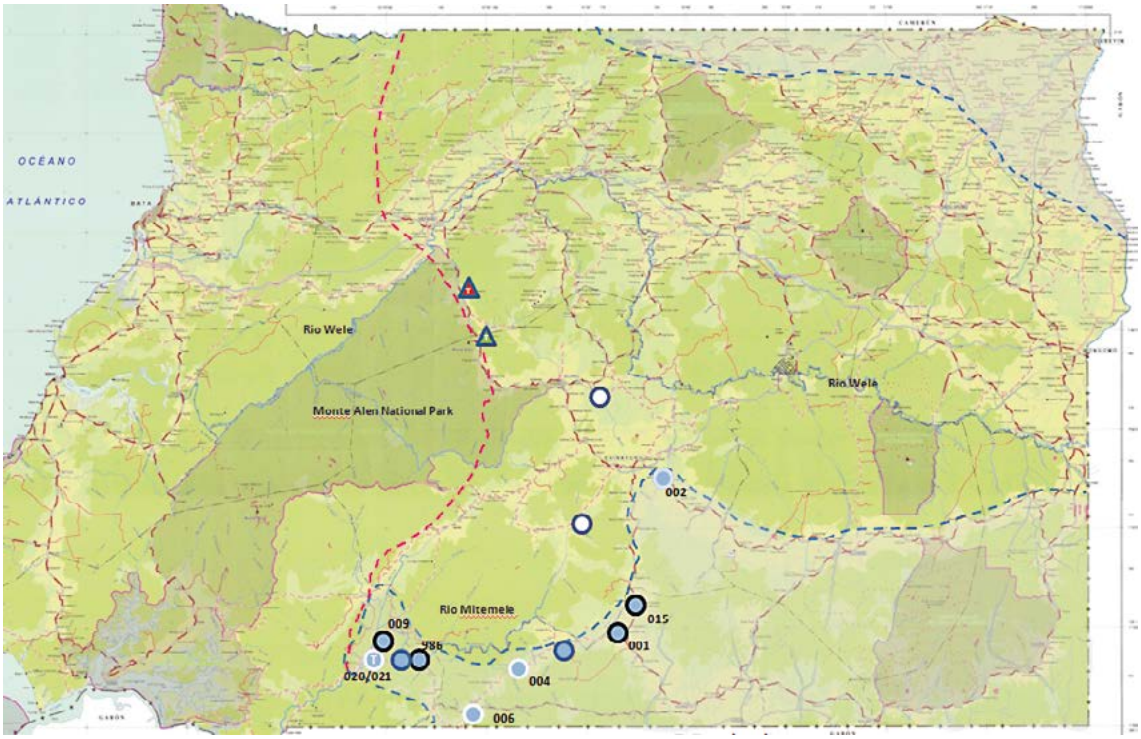

Fig. 1.– Distribution of the new species in Equatorial Guinea (based on a map issued by the government of Equatorial Guinea in 2017). Symbols indicate collection localities: red triangle: type locality of *Mesoaphyosemion losantosi* sp. nov., green triangle: type locality of *M. montealenense* sp. nov., blue circle: collection locality of '*A.*' *mitemelense* sp. nov., numbers correspond to DNA samples. T = type locality, white margin: southern clade, black margin: northern clade, light blue margin: population outside Mitemele basin, blue edge: not sequenced, white circle: unidentified species of the '*A*'. *herzogi* group collected by Castelo in 1989, but never collected again; red dotted line: western border of the *Mesoaphyosemion* distribution, blue dotted line: border of the distribution of the '*Aphyosemion*' *herzogi* species group. Recently a population of '*A.*' *mitemelense* sp. nov. was collected in northern Gabon 40 km south of the type locality (Schmidt, pers. comm.).

Fig. 1.– Distribución de las nuevas especies en Guinea Ecuatorial (basado en un mapa emitido por el gobierno de Guinea Ecuatorial en 2017). Los símbolos indican las localidades de recolección: triángulo rojo: localidad tipo de *Mesoaphyosemion losantosi* sp. nov., triángulo verde: localidad tipo de *M. montealenense* sp. nov., círculo azul: localidad de recolección de *'A.' mitemelense* sp. nov. Los números corresponden a muestras de ADN. T = localidad tipo, margen blanco: clado sur, margen negro: clado norte, margen azul claro: población esterna a la cuenca de Mitemele, borde azul: no secuenciado, círculo blanco: especie no identificada del grupo *'A'. herzogi* colectado por Castelo en 1989, pero no encontrada nunca más; línea punteada roja: frontera occidental de la distribución de *Mesoaphyosemion*, línea punteada azul: frontera de la distribución del grupo de especies *'Aphyosemion' herzogi*. Recientemente se recolectó una población de *'A.' mitemelense* sp. nov. en el norte de Gabón, 40 km al sur de la localidad tipo (Schmidt, com. pers.).

From 2000 to 2018 eighteen collecting trips were organized by Spanish aquarium keepers which focused especially on Nothobranchiidae. Many collection sites were sampled as well in the coastal plain as in the interior highlands. This resulted currently in the description of four new endemic nothobranchiid species (Legros & Zentz, 2007; Malumbres & Castelo, 2001; Sonnenberg, 2008) from the coastal plain. There is a sharp boundary between the Lower Guinean Cyprinodontiformes fauna of the low altitude coastal plain and the high altitude inland hills situated on the Woleu-Ntem plateau (McKenzie *et al.*, 2013). The collections of nothobranchiid fishes indicate the occurrence of different species groups in the inland, similar to the occurrence pattern in Cameroon and Gabon.

The Monte Alén National Park is situated in the south-west of the Woleu-Ntem inland plateau (Fig. 1). It has been a protected area since 1988 and contains a high biodiversity (De la Riva, 1994; Lasso, 1995; Lasso *et al.*, 2002) probably due to its assumed history as a Pleistocene rain forest refugium (Maley, 1987, 1991). On the Gabonese side it extends further south in the Monts de Cristal. These mountains, which are actually hills (under 1000 m), are famous for their botanical species diversity and a postulated Pleistocene refugium (Leal, 2005). This area also harbors several endemic nothobranchiid killifishes (Eberl, 2016; Huber, 2020; Stiassny *et al.*, 2007). Three of them were recently described from the Monts de Cristal (Huber, 1977; Sonnenberg & Blum, 2005; Sonnenberg *et al.*, 2006) of which two also occur in the adjacent part in Equatorial Guinea.

Several recent nothobranchiid collections made at the northern and southern fringes of the Monte Alén National park indicate that the inland area is inhabited by the same species groups or genera as the adjacent areas in Gabon and Cameroon. The majority of the collected species belong to the genus *Mesoaphyosemion* and to the '*Aphyosemion' herzogi* group. Only a few records of *Raddaella* sp. aff. *kunzi* and *Kathetys exiguum* (Boulenger, 1911) are known from the north-east and in the south-east *Diapteron cyanostictum* (Lambert & Géry, 1968), *Episemion callipteron* Radda & Pürzl, 1987 and *E. krystallinoron* Sonnenberg, Blum & Misof, 2006, respectively, were collected. At present the genus *Mesoaphyosemion* contains the following described species: *Mesoaphyosemion cameronense* (Boulenger, 1903), *M. obscurum* (Ahl, 1924), *M. amoenum* (Radda & Pürzl, 1976), *M. haasi* (Radda & Pürzl, 1976), *M. halleri* (Radda & Pürzl, 1976), *M. maculatum* (Radda & Pürzl, 1977), *M. mimbon* (Huber, 1977), and *M. etsamense* (Sonnenberg & Blum, 2005). Besides these species at least nine different undescribed phenotypes were recognized (Amiet, 1987; Dadaniak *et al.*, 1995; Sonnenberg *et al.*, submitted). The '*Aphyosemion' herzogi* group consists of two described species: '*A.*' *herzogi* Radda, 1975 and '*A.*' *bochtleri* Radda, 1975. Both species were synonimized by some authors (Seegers, 1997; Wildekamp *et al.*, 1986; Wildekamp, 1993). In addition many divergent phenotypes which might represent distinct species are known (Eberl, 2016). In the following we describe two endemic species of the genus *Mesoaphyosemion* and one endemic '*Aphyosemion' herzogi* group species from the Monte Alén area.

Material and methods

The description of the new species is based on specimens from the collections made by the first author and his co-travelers J.G. Poves and D. Gonzalez in 2018. The collections were licensed by Instituto Nacional de Desarrollo Forestal y Gestión del Sistema de Aéreas Protegidas (INDEFOR-AP), license number 281-018, issued 26 November 2018. Most specimens were preserved in 96% ethanol and some were preserved in 4% formalin first and transferred after one month into 70% ethanol.

DNA methods

A ca. 800 bp fragment of the mitochondrial cytochrome b was sequenced for 45 specimens of the '*Aphyosemion' herzogi* species group and 64 specimens of *Mesoaphyosemion*, as outgroup for both datasets we used a sample of '*A.' coeleste* Huber & Radda, 1977 which belongs to a different species group (Collier, 2006; Murphy & Collier, 1999). Part of the sequences were previously published in Sonnenberg & Blum (2005; GenBank acc. Nos AY748279–AY748296), and Van der Zee *et al.* (2018; MF155816), the new sequences are deposited in GenBank under the accession numbers ON166122–ON166212. DNA samples are listed in Table 1. DNA extraction, PCR, and sequencing methods follow Sonnenberg & Blum (2005) and Sonnenberg *et al.* (2006, 2007), sequences of the new species and additional samples from Equatorial Guinea were prepared by Advanced Identification Methods – AIM GmbH using the published primer combinations (Sonnenberg & Blum, 2005). Sequences were aligned and checked by eye in BioEdit 7.0.5.3 (Hall, 1999), translated into corresponding amino acids to confirm for functionality and tested for the anti-G bias of mitochondrial sequences (Zhang & Hewitt, 1996) for mitochondrial origin. Uncorrected p-distances with pairwise exclusion of missing data were calculated in MEGA 7 (Kumar *et al.*, 2016) and given in Supplementary files 1 and 2.

We performed Bayesian analyses (BA) for two parameter models (Nst = 2 and Nst = 6) for each species group with MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) and compare the results between the simpler model (Nst = 2) and the more complex

Table 1.– DNA samples used in this study with respectively DNA sample code, field code of collection, geographical coordinates, country, collectors and year of collection.

Tabla 1.– Muestras de ADN utilizadas en este estudio. Se indican, respectivamente, el código de muestra de ADN y el código de campo de recolección, las coordenadas geográficas, el país, los recolectores y el año de recolección.

Table 1.1 *Mesoaphyosemion*						
Mesoaphyosemion amoenum						
AMO-1011	C01/72	4°04'57.5''N	10°33'42.7''E	Cameroon	Sonnenberg *et al.*	2001
Mesoaphyosemion cf. *amoenum*						
AMO-242	CMM26	3°30'35.7''N	10°40'17.8''E	Cameroon	Sonnenberg *et al.*	2000
Mesoaphyosemion cameronense						
CAM-262	CMM40	2°48'56.1''N	10°40'35.0''E	Cameroon	Sonnenberg *et al.*	2000
CAM-560	G02-126	0°30'37.6''N	12°48'10.3''E	Gabon	Sonnenberg *et al.*	2002
CAM-575	BS02-1	0°37'12.0"N	12°58'15.6"E	Gabon	Blum & Sewer	2002
CAM-629	G02-132	0°33'59.8''N	12°38'31.6''E	Gabon	Sonnenberg *et al.*	2002
CAM-630	G02-133	0°31'23.2''N	12°28'44.0''E	Gabon	Sonnenberg *et al.*	2002
CAM-634	G02-138	0°46'32.6''N	11°32'28.4''E	Gabon	Sonnenberg *et al.*	2002
CAM-635	G02-139	0°49'47.1''N	11°29'25.9''E	Gabon	Sonnenberg *et al.*	2002
CAM-636	G02-141	0°57'05.9''N	11°16'06.0''E	Gabon	Sonnenberg *et al.*	2002
CAM-637	G02-143	0°59'21.3''N	11°21'04.1''E	Gabon	Sonnenberg *et al.*	2002
CAM-638	G02-155	1°00'22.8''N	10°54'13.0''E	Gabon	Sonnenberg *et al.*	2002
CAM-639	G02-153	0°57'38.7''N	11°08'25.3''E	Gabon	Sonnenberg *et al.*	2002
CAM-931	G02-154	0°58'18.8''N	11°05'13.2''E	Gabon	Sonnenberg *et al.*	2002
CAM-932	G02-150	0°59'11.2''N	11°14'15.3''E	Gabon	Sonnenberg *et al.*	2002
CAM-933	G02-152	0°57'06.1''N	11°11'13.5''E	Gabon	Sonnenberg *et al.*	2002
CAM-934	G02-151	0°58'34.3''N	11°13'13.6''E	Gabon	Sonnenberg *et al.*	2002
CAM-936	G02-148	1°24'58.4''N	11°26'41.3''E	Gabon	Sonnenberg *et al.*	2002
CAM-1284	GEMLBJ03-35	1°50'48.2"N	10°08'23.7"E	Equat.Guinea	Malumbres *et al.*	2003
CAM-1369	G02-140	0°54'11.4''N	11°19'34.5''E	Gabon	Sonnenberg *et al.*	2002
CAM 1371	G02-144	1°23'49.9''N	11°37'44.7''E	Gabon	Sonnenberg *et al.*	2002
CAM 1372	G02-145	1°27'12.0''N	11°35'45.8''E	Gabon	Sonnenberg *et al.*	2002
CAM-1439	GEMLBJ03-6	1°40'42.3"N	1°16'45.9"E	Equat.Guinea	Malumbres *et al.*	2003
CAM-1445	GEMLBJ03-13	2°03'56.2"N	11°19'11.5"E	Equat.Guinea	Malumbres *et al.*	2003
CAM-1647	BS02-3	0°34'01.2"N	12°38'36.5"E	Gabon	Blum & Sewer	2002
CAM-1717	G02-133	0°31'23.2''N	12°28'44.0''E	Gabon	Sonnenberg *et al.*	2002
CAM-1744	GEMLCG07-23	1°06'13.7"N	11°15'20.7"E	Equat.Guinea	Malumbres *et al.*	2007
CAM-1745	GEMLCG07-47	1°50'37.6"N	10°08'17"E	Equat.Guinea	Malumbres *et al.*	2007
Mesoaphyosemion etsamense						
ETS-545	G02-160	0°46'34.1''N	10°24'03.0''E	Gabon	Sonnenberg *et al.*	2002
ETS-577	BS02-12	0°46'34.1''N	10°24'02.0''E	Gabon	Blum & Sewer	2002
ETS-604	G02-160	0°46'34.1''N	10°24'03.0''E	Gabon	Sonnenberg *et al.*	2002
ETS-633	G02-160	0°46'34.1''N	10°24'03.0''E	Gabon	Sonnenberg *et al.*	2002
ETS-923	G02-160	0°46'34.1''N	10°24'03.0''E	Gabon	Sonnenberg *et al.*	2002
ETS-924	G02-160	0°46'34.1''N	10°24'03.0''E	Gabon	Sonnenberg *et al.*	2002
ETS-925	BS02-13	0°43'36.1''N	10°21'58.1"E	Gabon	Blum & Sewer	2002
ETS-1355	G02-161	0°42'58.1''N	10°21'37.0''E	Gabon	Sonnenberg *et al.*	2002
Mesoaphyosemion halleri						
HAL-1280	GEMLBJ03-12	2°09'24.7"N	11°17'12.0"E	Equat.Guinea	Malumbres *et al.*	2003
Mesoaphyosemion losantosi						
LOS-022	GEGVPO18-5	1°43'43.5''N.	10°17'29.4''E	Equat.Guinea	Ott *et al.*	2018
LOS-1175	GEMLBJ03-34	1°43'43.5''N.	10°17'29.4''E	Equat.Guinea	Malumbres *et al.*	2003
Mesoaphyosemion cf. *maculatum*						
MAC-564	G02-125	0°17'22.1''N	11°42'08.0''E	Gabon	Sonnenberg *et al.*	2002

Mesoaphyosemion maculatum						
MAC-632	G02-135	0°19‘53.9‘‘N	12°01‘53.4‘‘E	Gabon	Sonnenberg *et al.*	2002
MAC-1025	G02-135	0°19‘53.9‘‘N	12°01‘53.4‘‘E	Gabon	Sonnenberg *et al.*	2002
MAC-1026	G02-135	0°19‘53.9‘‘N	12°01‘53.4‘‘E	Gabon	Sonnenberg *et al.*	2002
Mesoaphyosemion mimbon						
MIM-003	GEGVPO18/8	1°24‘43.8“N	10°38‘04.2“E	Equat. Guinea	Ott *et al.*	2018
MIM-005	GEGVPO18/9	1°04‘31.7“N	10°20‘56.0“E	Equat. Guinea	Ott *et al.*	2018
MIM-008	GEGVPO18/12	1°03‘55.7“N	10°14‘47.3“E	Equat. Guinea	Ott *et al.*	2018
MIM-012	GEGVPO18/17	1°11‘32.4“N	10° 6‘00.6“E	Equat. Guinea	Ott *et al.*	2018
MIM-014	GEGVPO18/24	1°06‘05.9“N	10°45‘47.4“E	Equat. Guinea	Ott *et al.*	2018
MIM-570	G02-158	0°57‘29.7‘‘N	10°39‘01.2‘‘E	Gabon	Sonnenberg *et al.*	2002
MIM-571	G02-159	0°56‘46.0‘‘N	10°38‘14.2‘‘E	Gabon	Sonnenberg *et al.*	2002
MIM-640	G02-157	0°58‘06.3‘‘N	10°41‘33.5‘‘E	Gabon	Sonnenberg *et al.*	2002
MIM-929	G02/156	1°00‘06.0‘‘N	10°45‘50.9‘‘E	Gabon	Sonnenberg *et al.*	2002
MIM-930	G02-159	0°56‘46.0‘‘N	10°38‘14.2‘‘E	Gabon	Sonnenberg *et al.*	2002
MIM-974	GEMLBJ03/20	1°06‘46.8“N	10°46‘30.0“E	Equat. Guinea	Malumbres *et al.*	2003
Mesoaphyosemion cf. *montealenense*						
cMON-984	GEMLBJ03-31	1°28‘48.6“N	10°29‘29.6“E	Equat. Guinea	Malumbres *et al.*	2003
Mesoaphyosemion montealenense						
MON-018	GEGVPO18-6	1°39´05.5‘‘N	10°19´10.4‘‘E	Equat.Guinea	Ott *et al.*	2018
MON-019	GEGVPO18-7	1°34´23.7‘‘N	10°19´10.4‘‘E	Equat.Guinea	Ott *et al.*	2018
MON-985	GEMLBJ03-33	1°39´05.5‘‘N	10°19´10.4‘‘E	Equat.Guinea	Malumbres *et al.*	2003
Mesoaphyosemion sp. aff. *cameronense*, phenotype 1						
PT1-1238	CMM29	3°13‘30.5‘‘N	10°40‘37.2‘‘E	Cameroon	Sonnenberg *et al.*	2000
Mesoaphyosemion sp. aff. *cameronense*, phenotype 3						
PT3-1742	ABDK10-391	2°43‘30.5‘‘N	13°05‘37.2‘‘E	Cameroon	Agnese *et al.*	2010
Mesoaphyosemion sp. aff. *cameronense*, phenotype 5						
PT5-603	G02-136	0°12‘59.5‘‘ N	11°56‘03.9‘‘ E	Gabon	Sonnenberg *et al.*	2002
Mesoaphyosemion sp. aff. *cameronense*, phenotype 8						
PT8-635	G02-139	0°49‘47.1‘‘N	11°29‘25.9‘‘E	Gabon	Sonnenberg *et al.*	2002
Mesoaphyosemion sp. aff. *cameronense*, phenotype 9						
PT9-1743	KEK98-16	3°21‘51.6“N	10°45‘27.0“E	Cameroon	Kämpf *et al.*	1998
Mesoaphyosemion sp. aff. *cameronense*, phenotype 10						
PT10-377	BBS99-16	1°59‘39.8“N	11°59‘06.6“E	Gabon	Blum *et al.*	1999

Table 1.2 *“Aphyosemion”herzogi* group

‘Aphyosemion’ bochtleri						
BOC-631	G02-134	0°25‘08.9‘‘N	12°15‘58.8‘‘E	Gabon	Sonnenberg *et al.*	2002
BOC-1024	G02-134	0°25‘08.9‘‘N	12°15‘58.8‘‘E	Gabon	Sonnenberg *et al.*	2002
BOC-1243	G02-134	0°25‘08.9‘‘N	12°15‘58.8‘‘E	Gabon	Sonnenberg *et al.*	2002
BOC-1244	G02-134	0°25‘08.9‘‘N	12°15‘58.8‘‘E	Gabon	Sonnenberg *et al.*	2002
BOC-1245	G02-134	0°25‘08.9‘‘N	12°15‘58.8‘‘E	Gabon	Sonnenberg *et al.*	2002
‘Aphyosemion’ herzogi						
HER-1252	G02-137	0°35‘56.2‘‘N	11°27‘56.2‘‘E	Gabon	Sonnenberg *et al.*	2002
HER-1498	GBG 92-26	0°36“N	11°29‘E	Gabon	Bitter & Grell	1992
‘Aphyosemion’ sp. aff. *herzogi* (multiple species)						
cHER-007	GEGVPO18-12	1°03´55.6“N	10°14´47.5“E	Equat. Guinea	Ott *et al.*	2018
cHER-010	GEGVPO18-16	1°08´57.9“N	10°04´58.0“E	Equat. Guinea	Ott *et al.*	2018

cHER-011	GEGVPO18-17	1°11′32.8“N	10°06′00.9“E	Equat. Guinea	Ott *et al.*	2018
cHER-013	GEGVPO18-21	1°02’06.4”N	10°40’44.7“E	Equat. Guinea	Ott *et al.*	2018
cHER-016	GEGVPO18-28	2°08′43.6“N	10°38′59.4“E	Equat. Guinea	Ott *et al.*	2018
cHER-017	GEMLH16-12	1°07′00“N	11°15′35“E	Equat. Guinea	Malumbres *et al.*	2016
cHER-277	BBS99-21	0°56′90“N	11°09′44“E	Gabon	Blum *et al.*	1999
cHER-280	BBS98-9	no coordinates		Gabon	Blum *et al.*	1998
cHER-281	BBS99-14	1°35‘46.4“N	11°39‘48.8“E	Gabon	Blum *et al.*	1999
cHER-572	G02-159	0°56‘46.0“N	10°38‘14.2“E	Gabon	Sonnenberg *et al.*	2002
cHER-576	BS02-11	no coordinates		Gabon	Blum & Sewer	2002
cHER-926	G02-149	1°23‘48.7“N	11°24‘22.5“E	Gabon	Sonnenberg *et al.*	2002
cHER1223	G02-133	0°31‘23.2“N	12°28‘44.0“E	Gabon	Sonnenberg *et al.*	2002
cHER-1226	G02-136	0°12‘59.5“N	11°56‘03.9“E	Gabon	Sonnenberg *et al.*	2002
cHER-1229	G02-135	0°19‘53.9“N	12°01‘53.4“E	Gabon	Sonnenberg *et al.*	2002
cHER-1232	G02-141	0°57‘05.9“N	11°16‘06.0“E	Gabon	Sonnenberg *et al.*	2002
cHER-1234	G02-153	0°57‘38.7“N	11°08‘25.3“E	Gabon	Sonnenberg *et al.*	2002
cHER-1237	G02-157	0°58‘06.3“N	10°41‘33.5“E	Gabon	Sonnenberg *et al.*	2002
cHER-1246	G02-146	1°47‘42.3“N	11°44‘58.4“E	Gabon	Sonnenberg *et al.*	2002
cHER-1249	BBS99-11	0°19‘53.9“N	12°01‘53.4“E	Gabon	Blum *et al.*	1999
cHER-1250	G02-159	0°56‘46.0“N	10°38‘14.2“E	Gabon	Sonnenberg *et al.*	2002
cHER-1254	G02-141	0°57‘05.9“N	11°16‘06.0“E	Gabon	Sonnenberg *et al.*	2002
cHER-1258	BBS99-24	no coordinates		Gabon	Blum *et al.*	1999
cHER-1279	GEMLBJ03-30	1°6‘15.86“N	10°35‘37.4“E	Equat.Guinea	Malumbres *et al.*	2003
cHER-1281	GEMLBJ03-12	2°9‘24.72“N	11°17‘12.0“E	Equat.Guinea	Malumbres *et al.*	2003
cHER-1282	GEMLBJ03-13	2°3‘56.16“N	11°19‘11.5“E	Equat.Guinea	Malumbres *et al.*	2003
cHER-1440	BLLMC05-20	2°30‘53.29“N	11°08‘8.78“E	Cameroon	Bogaerts *et al.*	2005
cHER-1733	BS02-02	no coordinates		Gabon	Blum & Sewer	2002
‘Aphyosemion’ mitemelense						
MIT-001	GEGVP16-9	1°08′53,8“N	10°31′27.7“E	Equat.Guinea	Gonzaléz *et al.*	2016
MIT-002	GEGVPO18-8	1°24‘43.8“N	10°38‘04.21“E	Equat.Guinea	Ott *et al.*	2018
MIT-004	GEGVPO18-9	1°04‘31.7“N	10°20‘56.0“E	Equat.Guinea	Ott *et al.*	2018
MIT-006	GEGVPO18-10	1°00′23,3“N	10°17′42,2“E	Equat.Guinea	Ott *et al.*	2018
MIT-009	GEGVPO18-15	1°07′20,1“N	10°09′34,8“E	Equat.Guinea	Ott *et al.*	2018
MIT-015	GEGVPO18-25	1°12′08,2“N	10°35′20,1“E	Equat.Guinea	Ott *et al.*	2018
MIT-020	GEMPG18-14	1°06′00.6“N	10°08′25.9“E	Equat.Guinea	Malumbres *et al.*	2018
MIT-021	GEMPG18-14	1°06′00.6“N	10°08′25.9“E	Equat.Guinea	Malumbres *et al.*	2018
MIT-986	GEMLBJ03-27	1°05‘37.68“N	10°13‘18.3“E	Equat.Guinea	Malumbres *et al.*	2003

6 parameter model GTR (Nst = 6). If both analyses gave compatible results we can assume not to introduce error due to over- or under parameterized models. For all analyses the settings were rate=gamma and ngammacat=4 as the suggested default value for MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001), number of generations were set to 10 000 000 with sampling of every 1000 generation. The results of the first ten percent of the sampling were discarded as burn-in time after confirming that the runs had reached the stationary phase by plotting the values of the p-files in Excel. All trees were rooted with ‘*Aphyosemion’ coeleste* as outgroup species. Only nodes with a posterior probability (PP) of 0.95 or higher in at least one analysis are considered as statistically supported.

Morphometrics and meristics

Morphometric measurements were taken with a digital caliper using a dissecting microscope, and rounded to the nearest 0.1 mm. Measurements are presented as percentages of standard length (SL). Counts and methods follow Amiet (1987), except for standard length (SL) that was measured from the tip of the snout and not from the tip of the lower jaw, eye diameter and interorbital width and scales that were not registered by Amiet. Eye diameter and inter orbital distance were measured at the orbital rim at the centre of the eye. 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. Transversal scales were counted in an oblique line from the first scale anterior to the dorsal fin base to ventral edge of the body. Circum caudal peduncle scales were counted around the body. All visible fin rays were counted. The relative position of first dorsal fin ray to anal fin (D/A) was estimated as in Sonnenberg & Schunke (2010). Nomenclature for the cephalic lateral line system follows Clausen (1967) and Van Bergeijk & Alexander (1962), and

that for the supraorbital (frontal) squamation follows Hoedeman (1958). X-rays were taken with a Faxitron LX-60 Digital Specimen Radiography System at the Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK). Colour pattern elements of fins were measured on digital pictures of preserved specimens by means of MB-Ruler 5.3.3.7, an application to measure distances on a computer screen.

Taxonomic concept

In earlier papers the second and third authors (Sonnenberg, 2007; Van der Zee & Sonnenberg, 2010) suggested to use *Aphyosemion* only for the monophyletic species group containing the generic type of the genus, *A. castaneum* Myers, 1924, i.e. the *A. elegans* species group. For the remaining species groups we recommended using published generic level names or, in the case of unnamed species groups, within parentheses as *'Aphyosemion'*, indicating the provisional generic status.

Naming of genus level groups is regulated by the ICZN rules and the descriptions are based on the diagnoses and can be validated, falsified or extended, however, for the usage of the taxonomic rank (genus/subgenus) there are no kind of regulations or technical advice.

Our arguments are, that the generic level of species groups enhances taxonomic stability and carries more information about the named group than an all including genus *Aphyosemion* s.l. with 100+ species.

Currently published molecular phylogenies do not fully resolve the relationships between the different species groups with statistically significant support values (Agnèse *et al.*, 2006, 2009; Collier, 2006; Murphy & Collier, 1999), however, species groups are always recovered as monophyletic units with good support. Actually, morphology based studies of nothobranchiid phylogeny lack resolution due to absence of useful characters (Aarn & Shepherd, 2001; Costa, 2009, 2015; Huber, 2013). Independent of their phylogenetic relationships, species groups are currently the most stable units in nothobranchiid taxonomy. They carry more detailed information about the species group, e.g. like distribution, morphology, behavior, life history, and even in changing phylogenetic hypotheses, names at the species group level have no need to change. In addition, their diagnosis is usually better defined than for a taxon *Aphyosemion* s.l., with the latter most difficult to delineate to allow inclusion of the different species groups, which makes it in turn more complicated to separate it from related nothobranchiid genera like e.g. *Fundulopanchax*, *Fenerbahce*, *Foerschichthys* or *Fundulosoma* with a convincing argumentation.

The new nothobranchiid species of the *'A.' herzogi* species group is here described within *Aphyosemion* to follow ICZN Article 5.3, but will appear as *'Aphyosemion' mitelemense* sp. nov., in latter paragraphs indicating that it is not placed within *Aphyosemion* s.s.

We adopted the pragmatic approach by Moritz *et al.* (2000) as a species concept.

Comparative material examined

Mesoaphyosemion cameronense, Syntypes, BMNH 1902.11.12.148–149 (2), Cameroon, Kribi River, 1903.7.28.119–122 (4); 1903.7.28.123 (1) Kribi River, 1903.7.28.211 (1) Ja River. — MRAC 93-108-0472–491, Cameroon, Ntem River basin, small river at 4 km from Akoabass in the direction of Bitche, A. Kamdem Toham 29 June 1993; Cameroon, 42 km west of Ebolowa along N17, W. Herzog, H-J. Joachim & R. Roth, November 1992, field code HJRK92-11 (offspring, aquarium material, JZ collection).

Mesoaphyosemion etsamense, Holotype, ZFMK 39832, male, 32,4 mm SL; Gabon, western slopes of the Monts de Cristal, a small river at the village Etsam I, crossing the road N5 from Medouneu to Kougouleu, T. Blum, G. Fleck & R. Sonnenberg, 29 July 2002, field code G02/160. — Paratypes, MRAC A4-42-P-1-4, same data as holotype; Paratypes, ZFMK 39843, male, Gabon, north of the village Assok, T. Blum and P. Sewer, 11 September 2002, field code BS 02/13.

Mesoaphyosemion halleri, Equatorial Guinea, Ntem River basin, 5 km west of Ebebeyin, F. Malumbres, F. García Lora, J. Blanco Barles & M. Juhl 13 April 2003, field code GEMLBJ03-12 (offspring, aquarium material, RS collection). The types of *M. halleri* couldn't be studied since they are not present in NMW (pers. comm. A. Palandacic, NMW, Nov. 2019).

Mesoaphyosemion maculatum, Holotype, NMW 90757. Gabon, rainforest creek at the Koumameyong-Ovan National highway No.4, 33 kilometers east Koumameyong, 20 kilometers west Ovan, Radda & Pürzl, 1977; Gabon, Ivindo River basin, Mvoung River, Mva creek, near the village of Ebé, O. Legros, W. Eberl and A. Cerfontaine, 8 January 1993, field code LEC93-4 (offspring, aquarium material, JZ collection).

Mesoaphyosemion mimbon, Equatorial Guinea, small tributary of Rio Ncomo, between Bas and Oveng, north-east of Acurenam, F. Malumbres, F. García Lora, J. Blanco Barles & M. Juhl, 16 April 2003, field code GEMLBJ03-20 (offspring, aquarium material RS collection). — Gabon, small tributary of Komo River, 6 km west of Medouneu along the N5, T. Blum, G. Fleck and R. Sonnenberg, 29 July 2002, field code G02/156 (RS collection).

'Aphyosemion' bochtleri, Holotype, NMW 77332, Gabon, Mvoung River basin, Brook in rainforest about 300 meters on right side of a village near Mintoum, Herzog *et al.* 1972. — Gabon, Mvoung River basin, Mintoum, P. Wagner and R. Wendel, January 1986, field code GWW86-11 (JZ collection).

'Aphyosemion' herzogi, Holotype, NMW 77327, Gabon, fast running brook about 3 kilometers north

of Zoumoukou or 16 kilometers north of Lalara on the road to Mitzic and Oyem, Herzog *et al.* 1972. — Gabon, Anveme River basin, 26 km from Zomoko to Oveng, offspring of fish collected in 1993, G. Passaro and W. Eberl, field code PEG93-15 (JZ collection).

'Aphyosemion' sp. aff. *herzogi* "Cameroon", Cameroon, Ntem River basin, Mvila River, Nsessoum, P. Venstermans, J. Aerts and M. Bogaerts, 8 July 2016, field code VAB16-22 (JZ collection). — Cameroon, Ntem River basin, Kyé River, Mefoub, P. Venstermans, J. Aerts and M. Bogaerts, 16 July 2018, field code VAB18-52 (JZ collection).

'Aphyosemion' sp. aff. *herzogi* "S.E. Eq. Guinea", Equatorial Guinea, Komo River basin, Mbé River drainage, 3km south of Ngolensok, offspring of fish collected in 2000, F. Malumbres, J. Sanjuán and G-J. van Huijgevoort, field code GEMSH00-10 (DNA specimen, JZ collection). — Equatorial Guinea, Komo River basin, unnamed tributary, 1 km east of Acurenam, A. González, C. Vizcaíno, F. Portal and H. Ott, 21 August 2018, field code GEGVPO18-21 (DNA specimen).

'Aphyosemion' sp. aff. *herzogi* "Nviayong", Equatorial Guinea, Mitemele River basin, Rio Mven, near the village of Nviayong, A. González, C. Vizcaíno, F. Portal and H. Ott, 19 August 2018, field code GEGVPO18-12 (DNA specimen).

'Aphyosemion' sp. aff. *herzogi* "Mitemele right bank ", Equatorial Guinea, Mitemele River basin, unnamed right bank tributary, A. González, C. Vizcaíno, F. Portal and H. Ott, 20 August 2018, field code GEGVPO18-16 (DNA specimen). — Equatorial Guinea, Mitemele River basin, unnamed tributary of Rio Midjobo, A. González, C. Vizcaíno, F. Portal and H. Ott, 20 August 2018, field code GEGVPO18-17 (DNA specimen).

Results

DNA analysis. *Mesoaphyosemion*

The complete sequence alignment has a length of 778 bp for 64 *Mesoaphyosemion* specimens from 58 populations and '*Aphyosemion' coeleste* as outgroup. The alignment including the outgroup has 264 variable positions of which 215 are parsimony informative. Within *Mesoaphyosemion* 248 positions are variable, 208 of them parsimony informative. The observed uncorrected pairwise distances between specimens are listed in Supplementary file 1. The values between the outgroup species and *Mesoaphyosemion* range from 11.83%–14.40%, the maximum observed value within *Mesoaphyosemion* is 12.08%, the average uncorrected sequence divergence is 7.25%. The base composition shows with A = 27.8%, C = 24.8%, G = 13.3%, and T = 34.1% the typical A/T bias of mitochondrial DNA sequences (Zhang & Hewitt, 1996). The sequences translate into 258 amino acids of which 37 are variable and 14 parsimony informative. *Mesoaphyosemion losantosi* sp. nov., and *M. montealenense* sp. nov., are nested within an only partially resolved mtDNA phylogeny of the genus. Both are not closely related to each other or to samples identified as *M. maculatum, M.* cf. *maculatum,* or *M. mimbon*. *Mesoaphyosemion maculatum* and *M.* cf. *maculatum* occur in close proximity in central Gabon but are genetically not closely related. *Mesoaphyosemion montealenense* sp. nov., forms a well supported clade with several other samples identified as *M. cameronense* (Fig. 2). Observed pairwise sequence divergence within *M. losantosi* sp. nov., is 0.26%, within *M. montealenense* sp. nov., 0.13%–0.39%, between *M. losantosi* sp. nov., and *M. montealenense* sp. nov., is 6.68%–7.07%, between *M. losantosi* sp. nov. and *M. maculatum* 5.40%–6.30%, between *M. montealenense* sp. nov. and *M. maculatum* 8.10%–8.87%, and between the two new species and all other samples the divergence range between 4.37%–10.67%.

DNA Analysis. *'Aphyosemion' herzogi* group

The full sequence alignment of the '*Aphyosemion' herzogi* group dataset has a length of 778 bp for 45 specimens from 38 populations of the ingroup and '*A.' coeleste* as outgroup species. The alignment including the outgroup has 252 variable positions of which 188 are parsimony informative, excluding the outgroup there are 217 variable and 181 parsimony informative positions. The uncorrected pairwise distances between all included specimens are listed in Supplementary file 2. The values between the outgroup species and the '*A.' herzogi* group range from 16.71%–18.25%, the maximum observed value in the ingroup is 12.72%, the average uncorrected sequence divergence for the whole dataset is 7.39%. The base composition shows the typical A/T bias of mitochondrial sequences (Zhang & Hewitt, 1996) with A = 27.0%, C = 23.6%, G = 13.7%, and T = 35.7%. The sequences translate into 258 amino acids of which 32 are variable and 18 parsimony informative.

Within the resulting trees, not all nodes are resolved with significant statistical posterior probability value, however, '*Aphyosemion' mitemelense* sp. nov. turns out as sister species to all remaining samples/species of the '*A.' herzogi* group (Fig. 3). Pairwise distances range between 0.00%–5.01% within '*A.' mitemelense* sp. nov., and from 10.28%–12.72% between this species and all other samples/species of the group.

The DNA sample from a population just outside the Mitemele basin (MIT002) is placed basal to all remaining population samples located inside the basin, but the specimens are by morphology and colour pattern indistinguishable from the populations inside the basin and observed distance values are 4.37%–5.01% between MIT002 and the remaining samples and 0.00%–4.11% within the latter. Inside the basin two clades are present (Fig. 1): MIT004, MIT006,

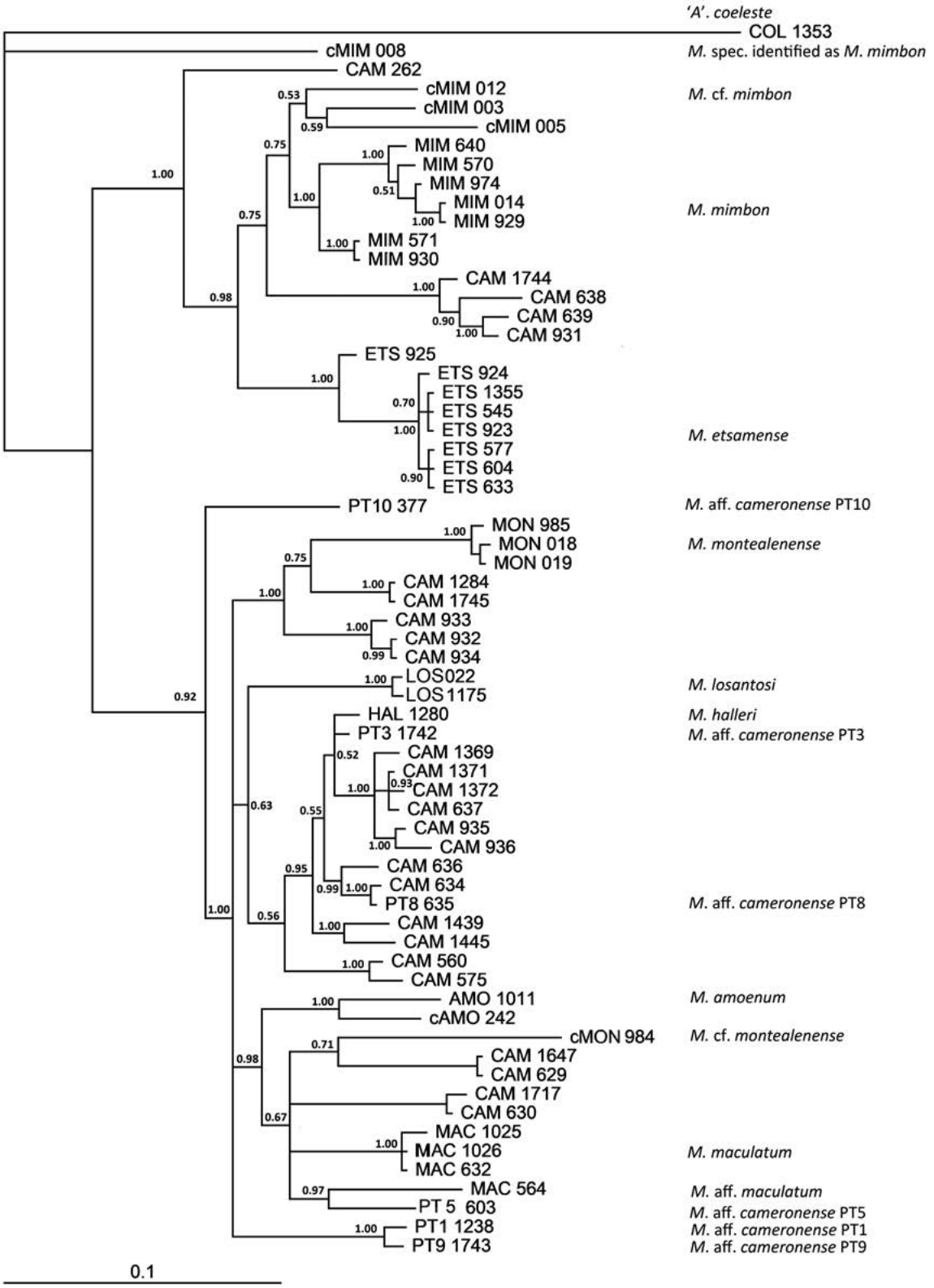

Fig. 2.– Results of the Bayesian analyses (BA) of 64 *Mesoaphyosemion* specimens. Shown are the results of the NST2 analysis, number at nodes representing the posterior probabilities (PP), the results of the NST6 analysis differ only slightly in PP values, but all statistically supported nodes are recovered in both analyses. The tree is rooted with '*Aphyosemion' coeleste* as outgroup species.

Fig. 2.– Resultados de los análisis bayesianos (BA) de 64 especímenes de *Mesoaphyosemion*. Se muestran los resultados del análisis NST2, número en los nodos que representan las probabilidades posteriores (PP), los resultados del análisis NST6 difieren solo ligeramente en los valores de PP, pero todos los nodos respaldados estadísticamente se recuperan en ambos análisis. El árbol se enraíza con *'Aphyosemion' coeleste* como grupo externo.

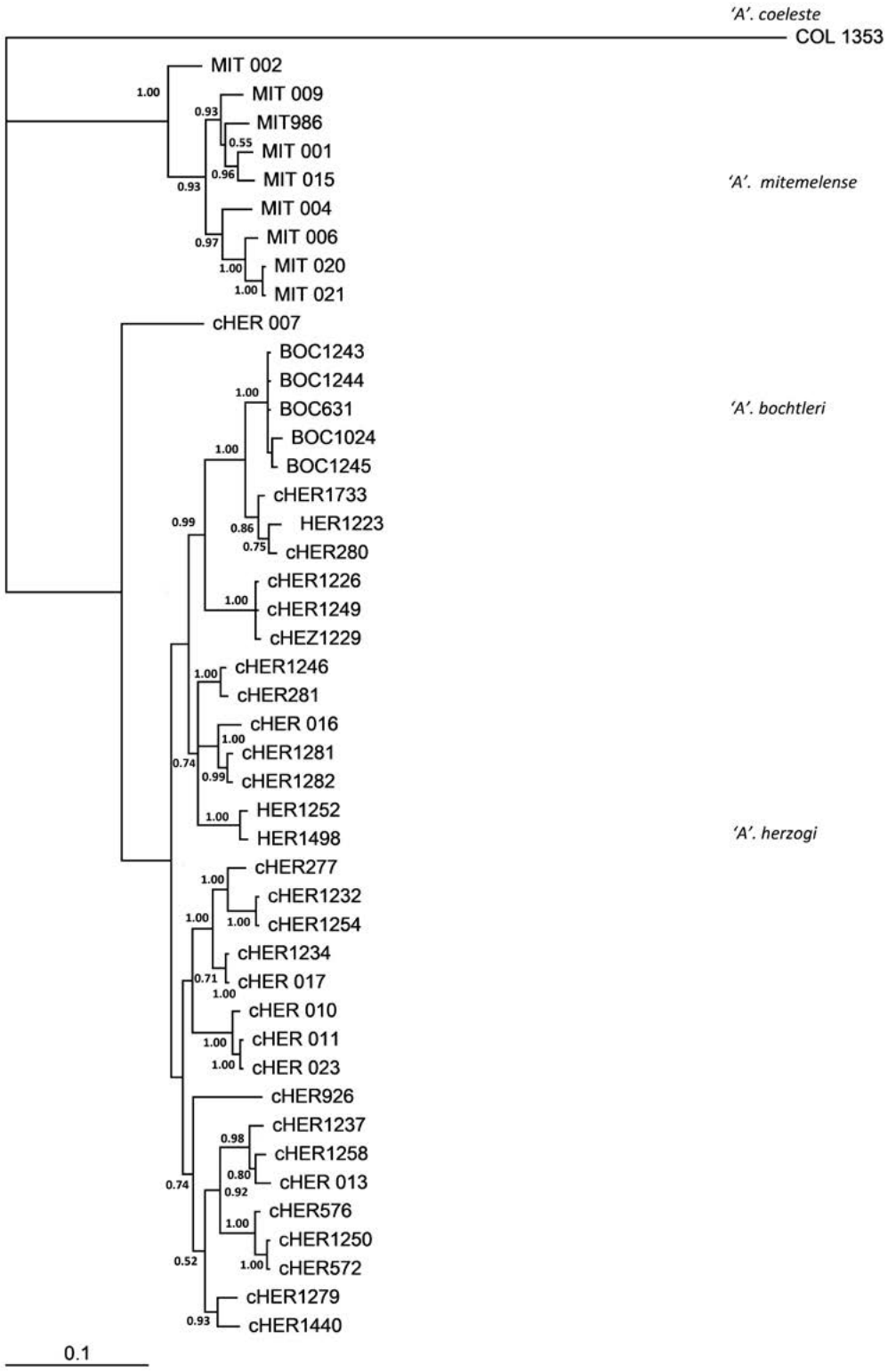

Fig. 3.– Results of the Bayesian analyses (BA) of 45 specimens of the '*Aphyosemion' herzogi* species group. Shown are the results of the NST2 analysis, number at nodes representing the posterior probabilities (PP) of the NST2 analysis, the results of the NST6 analysis differ only slightly in PP values. The tree is rooted with the outgroup species '*Aphyosemion' coeleste*. The samples of '*A.' bochtleri* are from the area of the type locality, the samples of '*A.' herzogi* are close to the type locality and show the same diagnostic colour pattern (sample HER 1498 is offspring of an aquarium strain from a collection made in 1992) (see Table 1).

Fig. 3.– Resultados de los análisis bayesianos (BA) de 45 especímenes del grupo de especies *'Aphyosemion' herzogi*. Se muestran los resultados del análisis NST2, número en los nodos que representan las probabilidades posteriores (PP) del análisis NST2, los resultados del análisis NST6 difieren solo ligeramente en los valores de PP. El árbol está enraizado con *'Aphyosemion' coeleste* como grupo externo. Las muestras de *'A.' bochtleri* provienen del área de la localidad tipo, las muestras de *'A.' herzogi* de las cercanías de la localidad tipo y presentan el mismo patrón de color diagnóstico (la muestra HER 1498 desciende de una cepa de acuario de una recolección realizada en 1992) (ver Tabla 1).

MIT021, and MIT022 (the latter two from the type locality) occur in the southern part of the distribution area and MIT001, MIT009, MIT015, and MIT986 in the northern part. Peculiar is that MIT 009 resembles MIT021 and MIT 022 (type locality) in colour pattern but is genetically not close. MIT001, MIT015 and MIT986 resemble MIT004 and MIT006, but are also not genetically close. Genetics reflects geographical origin (outside the basin, northern part or southern part within the basin) but not colour pattern differences.

Mesoaphyosemion losantosi sp. nov.

urn:lsid:zoobank.org:act:6D227B78-38D2-4208-99F0-9E2455D2287B

Figs 4–5, Tables 2–3

Aphyosemion maculatum – American Killifish Association, no date.
Aphyosemion maculatum – Castelo, 1994. — Huber, 2007.

Type material

Holotype. MRAC 2019.008.P.0001, male, 31.0 mm SL, Equatorial Guinea, Centro Sur province, affluent of Rio Nfogoyi, a left bank tributary of Rio Wele, 100 meter west of the village of Bisun, 1º43´43.5´´N, 10º17´29.4´´E. Collected on 5 December 2018 by F. Malumbres, J. G. Povez, and D. González, Field code GEMPG18-20.

Paratypes. MRAC 2019.008.P.0002–0005, 2 males and 2 females, 30.7–36.5 mm SL, collected with the holotype. — MNCN_ICTIO 296.758, 1 male, 22.2 mm SL, MNCN_ICTIO 296.759, 1 female, 21.8 mm SL, collected with the holotype. — ZSM 47573, 1 male and 2 females, 27.0–37.9 mm SL, collected with the holotype. — AMNH 275003, 1 male and 2 females, 29.6–34.3 mm SL, collected with the holotype.

Not measured type material. MRAC 2019.008.P.0006–00011, 6 juvenile specimens.

Diagnosis

Mesoaphyosemion losantosi (Figs 4–5) belongs, according to DNA results, morphology and colouration characters to the genus *Mesoaphyosemion*. It shares with other *Mesoaphyosemion* species the combination of the following characters: a mostly metallic light blue, blue or blue green colouration on side of males; an often variable pattern of red dots on sides, in most species dots are irregularly distributed, in others forming more or less regular stripes or larger blotches; unpaired fins without or only short fin extensions, caudal-fin in males nearly straight, slightly rounded or weakly trilobate. No conspicuous markings on ventral side of the head; all adult males have a yellow snout. None of these characters alone is diagnostic and they are also known from other nothobranchiid genera or species groups, but in combination they are diagnostic for the genus. In most *Mesoaphyosemion* species a broad latero-ventral red line on the caudal peduncle is present, but this line is absent in all species with large dark red blotches on the flanks: *M. mimbon, M.* cf. *maculatum*, *M. maculatum*, *M. losantosi*, and *M. montealenense* sp. nov. *M. losantosi* is distinguished from all other *Mesoaphyosemion* species by having 7 transversal scales (vs. 8). In *M. montealenense* sp. nov., the anal fin has a light blue background with a more or less checkerboard pattern of square and rectangular blotches. In other species the background colour is darker and the less distinct submarginal band often fuses with the background colour and/or the blotches in the anal fin, or the anal fin is complete dark red or yellow.

Description

See Figs 4 and 5 for general appearance and colour pattern, see Table 2 for morphometric and Table 3 for meristic data of types. Small to medium sized member of *Mesoaphyosemion* with strong sexual dimorphism, males being colourful and females light brownish. Dorsal profile nearly straight, slightly convex from nape to dorsal fin base. Ventral profile gently convex from head to end of anal fin base. Caudal peduncle slightly concave from end of both anal and dorsal fin base to base of caudal fin. Snout slightly pointed, mouth directed upwards; lower jaw longer than upper; posterior end of mouth at approximately same level as anterior edge of eye. On both jaws unicuspid, slightly posteriorly directed teeth, some larger teeth on outer row. Open frontal cephalic sensory system with two pits separated by a low ridge, each neuromast accompanied by 2 very small lobes. Preopercular system tubular with 6 pores, supra-orbital system open groove with 3 neuromasts on both sides, pre- and post-orbital system short tube with 2 large pores.

Dorsal fin in males rounded, anal fin pointed in older males. Fins in females smaller and anal fin rounded. Dorsal and anal fins located posterior to mid-body. Dorsal fin with 11–12 rays; anal fin with 14–16 rays, first ray of dorsal fin above anal fin ray 8–9. Caudal fin slightly spade shaped. Pectoral fin reaching origin of pelvic fin in males, not in females; pelvic fin reaching origin of anal fin in males, not in females. Frontal squamation of G-type, with small elongated G scale. Scales on mid-longitudinal series 30–32 + 3–4 on caudal fin base. Transverse rows of scales above pelvic fin 7; circumpeduncular scale row 12.

Live colouration

Males (Fig. 4a). Snout yellow, dorsum brownish with copper shining. Flank metallic light blue with green shining. Operculum with three dark red stripes, top stripe very short and sometimes hardly visible, middle stripe longest and most prominent. In some specimens a narrow suborbital dark red stripe. Anterior flank with two short rows of small dark red spots, followed posterior flank with very large irregular dark red to purple brown blotches, forming up to three vertical or oblique bars on caudal peduncle in most specimens. Pectoral fins transparent with a white margin, pelvic fins white to light blue with narrow white margin and wider dark red submarginal band.

Table 2.– Morphometrics of *Mesoaphyosemion losantosi* sp. nov., based on 13 specimens. M(6) mean = mean of 6 males, F(7) = mean of 7 females, SD = standard deviation. All measurements in percentages of standard length (SL), except standard length in mm. BD = body depth, HL = head length, ED = eye diameter, IO = interorbital width, PD = pre dorsal length, PA = pre anal length, PP = pre pelvic length, DB = dorsal base, AB = anal base, CL= caudal peduncle length, CD = caudal peduncle depth, CR = caudal peduncle ratio.

Tabla 2.– Variables morfométricas de *Mesoaphyosemion losantosi* sp. nov., en base a 13 especímenes. M(6) media = media de 6 machos, F(7) = media de 7 hembras, SD = desviación estándar. Todas las medidas en porcentajes de longitud estándar (SL), excepto la longitud estándar en mm. BD = altura del cuerpo, HL = longitud de la cabeza, ED = diámetro del ojo, IO = anchura interorbitaria, PD = longitud predorsal, PA = longitud preanal, PP = longitud prepélvica, DB = base dorsal, AB = base anal, CL = longitud del pedúnculo caudal, CD = altura del pedúnculo caudal, CR = proporción del pedúnculo caudal.

	Holotype	M(6) mean (SD)	median	range	F(7) mean (SD)	median	range
SL	31.0	30.7 (5.2)	31.8	30.7–32.1	31.2 (6.2)	32.0	21.8–37.9
BD	19.4	20.2 (0.9)	20.0	19.4–21.4	21.5 (1.5)	22.3	19.1–22.7
HL	23.2	22.4 (0.8)	22.6	22.5–23.2	22.4 (0.8)	21.4	20.0–25.7
ED	8.4	8.0 (0.6)	8.0	7.3–8.4	7.5 (0.7)	7.8	6.6–9.0
IO	11.0	11.7 (0.4)	11.7	11.0–12.1	12.5 (0.7)	12.5	11.7–13.7
PD	66.8	66.7 (1.9)	67.1	64.2–68.2	68.1 (1.9)	68.4	59.2–70.1
PA	56.1	55.6 (1.9)	55.4	52.8–58.6	59.0 (1.2)	59.2	55.4–60.5
PP	41,6	43.5 (1.2)	43.5	41.6–44.2	45.6 (1.4)	45.9	43.2–47.3
DB	13.9	13.7 (0.7)	13.8	12.6–14.0	13.4 (0.9)	13.4	12.4–14.8
AB	21,3	20.8 (1.1)	21.1	20.8–22.1	19.5 (1.2)	19.0	18.1–21.5
CL	21.9	22.3 (1.8)	22.3	20.1–23.8	23.0 (1.3)	22.5	21.4–25.3
CD	11.0	11.8 (0.7)	11.7	11.0–13.1	12.1 (0.4)	12.2	11.6–12.8
CR	2.0	2.0 (0.2)	2.0	1.6–2.1	1.9 (0.2)	1.9	1.7–2.1

Table 3.– Meristics of *Mesoaphyosemion losantosi* sp. nov., based on 13 specimens. SD = standard deviation, D = number of dorsal fin rays, A = number of anal fin rays, D/A = dorsal / anal fin position, C = number of caudal fin rays, LS = number of mid longitudinal line scales, TS = number transversal scales, CS = number of scales around caudal peduncle.

Tabla 3.– Variables merísticas de *Mesoaphyosemion losantosi* sp. nov., basadas en 13 especímenes. SD = desviación estándar, D = número de radios de la aleta dorsal, A = número de radios de la aleta longitudinal media, D/A = posición de la aleta dorsal / anal, C = número de radios de la aleta caudal, LS = número de escamas de la línea lateral, TS = número de escamas transversales, CS = número de escamas alrededor del pedúnculo caudal

	holotype	all types mean (SD)	median	all types range
D	10	11.1 (0.6)	11	10–12
A	13	14.5 (0.9)	14	13–16
D/A	7	7.9 (0.9)	8	7–9
LS	30	31.1 (0.8)	31	30–32
TS	7	7 (0.7)	7	7
CS	12	12 (0)	12	12

Dorsal and anal fins light blue or whitish with white narrow margins. Dorsal fin with a variable number of dark red interradial spots and with some larger dark red blotches at its base. Anal fin with dark red submarginal band and a variable number of elongated, sometimes round, dark red interradial blotches. Caudal fin grayish to light blue with a variable number of dark red spots and blotches with white dorsal and ventral margins, ventral margin wide with dark red submarginal band, dorsal margin narrow.

Females (Fig. 4b). Dorsum and flanks brownish, dorsally darker, ventrally light grey or brownish. Flanks with blueish shining. Scales on dorsum and sides with irregular narrow dark edges, forming a reticulate pattern. All fins hyaline; unpaired fins with many red spots, often arranged in rows. All fins, except caudal fin with narrow white edges.

Colour after 3 months in 96% ethanol

Males (Fig. 5a). Dorsally and laterally dark brown to black, ventrally lighter brown. Vague dark bars on caudal peduncle. Flank scales with dark margins, resulting in a reticulated pattern. All fins dark with some lighter dots. Head dark. Females (Fig. 5b). Dorsally and laterally brown, ventrally lighter brown to yellow. Flank scales with posterior dark edges with larger dark centre. Lower jaw brown, operculum brown. Unpaired and pelvic fins light grey, with dark rounded to oval spots, arranged in irregular rows, pectoral fin hyaline.

Fig. 4.– *Mesoaphyosemion losantosi* sp. nov. a: male from type locality, not preserved. Photo by H. Ott; b: female paratype. Photo by J. Hernández Corral.

Fig. 4.– *Mesoaphyosemion losantosi* sp. nov. a: macho de la localidad tipo, no preservado. Foto de H. Ott; b: paratipo hembra. Foto de J. Hernández Corral.

Colour after 1 month in formalin and then transferred to 70% ethanol

Males. Dorsum grey. Flanks light grey. Ventrum white. Flank and ventrum scales with narrow dark margin. Unpaired fins dark grey with vague dark red markings. Pelvic fins black. Pectoral fins light grey with wide dark grey margin. Irregular dark red bars on posterior flanks and caudal peduncle. Head dark grey, ventrally white. Lower lip black. Females. Dorsum and lateral flanks light grey, ventral flank light brownish. All scales with dark margins. Small dark red spots on posterior flanks and caudal peduncle. Unpaired fins grey with rounded to oval dark grey spots. Pelvic fins dark grey. Pectoral fins light grey. Head light grey, ventrally white. Lower lip dark grey.

Distribution and habitat

The new species is currently known from a stretch of 6 kilometers north and south Bisun along the road from Niefang to Bicurga in central Equatorial Guinea (Fig. 1). It is not unlikely, however, that it also occurs east and west of the present distribution area, since no collections were made there. North and south of the distribution area several collections were made, but *M. losantosi* was not found. The type locality is a small creek in humid forest, slowly running, slightly amber coloured clear water, close to source, water 15 cm deep and 1 m wide, loamy soil covered with leaves, pH 6.0, conductivity 0.5 Microsiemens, water temperature 23.9 ºC.

Etymology

The name *losantosi* is in honour of Félix Losantos (1967–2020), a very good friend and co-traveler of the first author on collection trips to Equatorial Guinea. Félix was a tireless, passionate and helpful person.

Mesoaphyosemion montealenense sp. nov.

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Figs 6–7, Tables 4–5

Aphyosemion maculatum – American Killifish Association, no date.
Aphyosemion maculatum – Huber, 2007.
Aphyosemion aff. *maculatum* – Ott, 2019: 41.

Type material

Holotype. MRAC 2019.008.P.0012, male, 37.3 mm SL, Equatorial Guinea, Centro Sur province, stream Biñogo affluent of Río Laña, a left bank tributary of Río Wele, just west of the village of Moka, 1º39´05.5´´N, 10º19´10.4´´E, coordinates were taken in the village of Moka, not on the type locality itself, collected on 5 December 2018 by F. Malumbres, J. G. Povez and D. González, Field code GEMPG18-19.

Fig. 5.– *Mesoaphyosemion losantosi* sp. nov., specimens preserved; a: holotype 31.0 mm SL, b: female paratype, 37.2 mm SL.

Fig. 5.– *Mesoaphyosemion losantosi* sp. nov., especímenes conservados; a: holotipo 31,0 mm SL, b: paratipo hembra, 37,2 mm SL.

Paratypes. MRAC 2019.008.P.0013–0016, 2 males and 2 females, 35.1–19.5 mm SL. — MNCN_ICTIO 296.760, male, 31.0 mm SL. — MNCN_ICTIO 296.761, female, 30.4 mm SL. All collected with the holotype.

Diagnosis

Mesoaphyosemion montealenense (Figs 6–7) belongs to the genus *Mesoaphyosemion* based on characters mentioned in the diagnosis of *M. losantosi*. It differs from all other species of *Mesoaphyosemion* (except for *M. losantosi*, *M. maculatum*, *M.* cf. *maculatum,* and *M. mimbon*), by having large dark blotches on flanks and lacking a broad latero-ventral red line on the caudal peduncle. It differs from all other species with large blotches on flanks by having a checkerboard pattern of elongated and square spots in unpaired fins on a light blue background. In some specimens spots merged, forming inter-radial stripes.

Description

See Figs 6 and 7 for general appearance and colour pattern, see Table 4 for morphometric and Table 5 for meristic data. Medium sized *Mesoaphyosemion* with strong sexual dimorphism, males being colourful and females dull. Dorsal profile nearly straight, slightly convex from nape to dorsal fin base. Ventral profile gently convex from head to end of anal fin base. Caudal peduncle slightly concave from end of both anal and dorsal fin base to base of caudal fin. Snout slightly pointed, mouth directed upwards; lower jaw longer than upper; posterior end of mouth at approximately same level as anterior edge of eye. On both jaws unicuspid, slightly posteriorly directed teeth, some larger teeth on outer row. Open frontal cephalic sensory system with two separate pits, each neuromast accompanied by 2 small lobes, preopercular system tubular with 6 large oval pores, supra-orbital system open groove with 3 neuromasts on both sides, 3 low distal lobes and 2 low proximal lobes, pre- and post-orbital system short tube with 2 large pores.

Dorsal fin and anal fin pointed in older males. Fins in females smaller and never pointed. Dorsal and anal fins located posterior to mid-body. Dorsal fin with 11–13 rays; anal fin with 14–16 rays, first ray of dorsal fin above anal fin ray 6–8. Caudal fin spade shaped in older males with 25–28 fin rays. Pectoral fin reaching origin of pelvic fin in males, not in females; pelvic fin reaching origin of anal fin in males, not in females. Frontal squamation of G-type; scales on mid-longitudinal series 30–33 + 3–4 on caudal fin base. Transverse rows of scales above pelvic fin 8; circumpeduncular scale row 12.

Live colouration

Males (Fig. 6a). Snout and lower jaw yellow. Dorsum brownish with copper shining. Flank metallic

Table 4.– Morphometrics of *Mesoaphyosemion montealenensis* sp. nov., based on 6 specimens. M(4) mean = mean of 4 males, F(2) = mean of 2 females, SD = standard deviation. All measurements in percentages of standard length (SL), except standard length in mm. BD = body depth, HL = head length, ED = eye diameter, PD = pre dorsal length, PA = pre anal length, PP = pre pelvic length, DB = dorsal base, AB = anal base, CL= caudal peduncle length, CD = caudal peduncle depth, CR = caudal peduncle ratio.

Tabla 4.– Variables morfométricas de *Mesoaphyosemion montealenensis* sp. nov., basadas en 6 especímenes. M(4) media = media de 4 machos, F(2) = media de 2 hembras, SD = desviación estándar. Todas las medidas en porcentajes de longitud estándar (SL), excepto la longitud estándar en mm. BD = altura del cuerpo, HL = longitud de la cabeza, ED = diámetro del ojo, PD = longitud predorsal, PA = longitud preanal, PP = longitud prepélvica, DB = base dorsal, AB = base anal, CL= longitud del pedúnculo caudal, CD = altura del pedúnculo caudal, CR = proporción del pedúnculo caudal.

	holotype	M(4) mean (SD)	median	range	F(2) mean* (SD)	range
SL	37.3	30.9 (4.5)	29.4	27.6–37.3	31.7 (1.8)	30.4–33.0
BD	20.6	21.2 (0.5)	21.2	20.6–22.4	22.5 (0.1)	22.4–22.6
HL	22.3	22.5 (0.7)	22,2	22.0–24.0	22.8 (1.8)	21.5–24.0
ED	7.8	8.2 (0.4)	8,2	7.8–8.7	8.0 (1.4)	7.0–8.9
IO	10.7	11.7 (0.7)	12,0	10.7–12.3	11.7 (0.6)	11.1–12.2
PD	66.0	64.3 (4.2)	66,0	58.1–67.1	67.7 (0.6)	67.3–68.1
PA	60.9	61.6 (3.1)	61,2	58.3–65.8	60.5 (2.4)	58.8–62.2
PP	44.5	45.0 (0.8)	44,7	44.4–46.1	44.5 (1.3)	43.6–45.4
DB	17.2	16.3 (0.8)	16,3	15.5–17.2	15.3 (2.1)	13.8–16.7
AB	20.9	22.3 (1.6)	21,9	20.9–24.5	21.1 (1.4)	18.1–21.7
CL	22.5	22.5 (0.8)	22,7	21.3–23.2	21.1 (1.0)	20.1–22.1
CD	12.8	12.4 (0.3)	12,3	12.0–12.8	12.2 (0.5)	11.8–12.5
CR	1.9	1.9 (0.1)	1,9	1.7–1.9	1.8 (0.1)	1.8–1.9

* only 2 females: mean = median

Table 5.– Meristics of *Mesoaphyosemion montealenensis* sp. nov., based on 6 specimens. SD = standard deviation, D = number of dorsal fin rays, A = number of anal fin rays, D/A = dorsal / anal fin position, C = number of caudal fin rays, LS = number of mid longitudinal line scales, TS = number transversal scales, CS = number of scales around caudal peduncle.

Tabla 5.– Variables merísticas de *Mesoaphyosemion montealenensis* sp. nov., con base en 6 especímenes. SD = desviación estándar, D = número de radios de la aleta dorsal, A = número de radios de la aleta anal, D/A = posición de la aleta dorsal / anal, C = número de radios de la aleta caudal, LS = número de escamas de la línea longitudinal media, TS = número de escamas transversales, CS = número de escamas alrededor del pedúnculo caudal.

	holotype	all types mean (SD)	median	all types range
D	12	11.8 (0.4)	12	11–12
A	16	15.8 (0.8)	16	15–17
D/A	6	7.2 (1.0)	7.5	6–8
LS	32	31.7 (0.8)	31.5	31–33
TS	8	8 (0)	8	8
CS	12	12 (0)	12	12

light blue with green shining. Operculum with three dark red stripes, top stripe very short and sometimes hardly visible, the other two stripes of equal length. In some specimens a narrow suborbital dark red stripe. Anterior flank with no or only a few small dark red spots, posterior flank with large irregular dark red to purple brown blotches forming three to nine vertical or oblique bars on caudal peduncle. In some specimens these blotches form a marbled pattern whereas in other specimens these blotches are reduced. Pectoral fins transparent with a narrow white margin, pelvic fins with narrow white margin and dark red submarginal band, provided with two or three dark red blotches. Dorsal and anal fins light blue or whitish with only a very narrow white margin in dorsal fin, no white margin in anal fin. Dorsal fin with a variable pattern of dark red spots, in some specimens up to 40 smaller rounded spots all over the fin, in others small spots at the base of the fin and interradial stripes distally. Anal fin also variable; in some specimens a checkerboard pattern of more or less square dark red spots at the base and stripes distally, in others only alternating stripes. Caudal fin with asymmetric colour pattern. No or very narrow white dorsal margin in most specimens, in some specimens this margin is much wider. Ventral white margin always wider than dorsal margin. Central fin with 15 to 40 small dark red spots or short stripes.

Fig. 6.– *Mesoaphyosemion montealenense* sp. nov. a: male from type locality, not preserved. Photo by H. Ott; b: female paratype. Photo by J. Hernández Corral.

Fig. 6.– *Mesoaphyosemion montealenense* sp. nov. a: macho de la localidad tipo, no preservado. Foto de H. Ott; b: paratipo hembra. Foto de J. Hernández Corral.

Females (Fig. 6b). Dorsum and flanks brownish, dorsally darker, ventrally light grey or brownish. Scales on dorsum and sides with irregular narrow dark margins, forming a reticulate pattern. All fins hyaline; unpaired and pelvic fins with many red spots, often arranged in rows. All fins, except posterior caudal fin with narrow white margins.

Colour after 3 months in 96% ethanol

Males (Fig. 7a). Dorsum completely black. Flank scales with many small melanophores and black edges. Black bars on caudal peduncle. Operculum scales also with many small black spots, but without black margins. Throat and ventrum light brown, scales with black edges. Unpaired fins as in live specimens, but light blue colour now dark grey and dark red colour black. Pelvic fins black, pectoral fins translucent dark grey. Females (Fig. 7b). Dorsum dark brown, flanks and ventrum light brown. All dorsal and flank scales with prominent black margins, resulting in a reticulated pattern. Ventral side not reticulated. Unpaired fins greyish with dark spots. Pectoral and pelvic fins dark grey hyaline.

Distribution and habitat

The new species is restricted to a small area just southeast of Monte Alén in central Equatorial Guinea (Fig. 1). All collections were made 2–3 kilometers southeast of this mountain along the road from Niefang to Bicurga. It is not unlikely, however, that it also occurs east and west of the present distribution area, since no collections were made there. North and south of the distribution area was frequently sampled and no further occurrence of *M. montealenense* was found. Habitat: slowly running shallow clear water; pH 6.5; conductivity 3 Microsiemens, water temperature 22.5ºC.

Etymology

The specific epithet is derived from Monte Alén, the highest mountain in Equatorial Guinea (1250 m). The new species occurs at the southeastern foot of this mountain at an altitude of ca. 690 meter.

Aphyosemion mitemelense sp. nov.

urn:lsid:zoobank.org:act:B1C83E77-5B71-4373-9C83-DA769AE6A3A4

Figs 8–10, Tables 6–7

Aphyosemion aff. *herzogi* – Ott, 2019: 43–45.

Type material

Holotype. MRAC 2019.008.P.0017, male 34.9 mm SL, Equatorial Guinea, Province Litoral, Mitimele River basin, Nki-Besua creek, 1º06´00.6´´N, 10º08´25.9´´E, collected on 4 December 2018 by F. Malumbres, J. G. Povez & D. González, Field code GEMPG18-14.

Fig. 7.– *Mesoaphyosemion montealenense* sp. nov., specimens preserved. a: holotype 37.3 mm SL, b: female paratype, 30.4 mm SL.

Fig. 7.– *Mesoaphyosemion montealenense* sp. nov., especímenes conservados. a: holotipo 37,3 mm SL, b: paratipo hembra, 30,4 mm SL.

Paratypes. MRAC 2019.008.P.018–0021, 3 males and 1 female, 23.8–30.2 mm SL. — MNCN _ICTIO 296.762, male, 35.8 mm SL. — MNCN_ICTIO 296.763, female, 24.4mm SL. — ZSM 47593, 1 male 24.9 mm SL and 1 female, 23.0 mm SL. — AMNH 275004, 1 male, 30.2 mm SL and 1 female, 28.4 mm SL. All collected with the holotype.

Additional material. *Aphyosemion mitemelense*, Equatorial Guinea, Komo River basin, Rio Mbé, Yama, F. Malumbres, J. Sanjuán & G-J. van Huijgevoort, 1 July 2000, field code GEMHS00-10 (JZ collection). — Equatorial Guinea, Wele River basin, Rio Chiguo drainage, A. González, C. Vizcaíno, F. Portal & H. Ott, 18 August 2018, field code GEGVPO18-8 (RS collection). — Equatorial Guinea, Mitemele River basin, unnamed tributary between Rio Nkiém and Rio Mba, Esakora (Acurnam), A. González, C. Vizcaíno, F. Portal & H. Ott, 19 August 2018, field code GEGVPO18-9 (JZ collection). — Equatorial Guinea, Mitemele River basin, Rio Nkién, Ayem II, A. González, C. Vizcaíno, F. Portal & H. Ott, 19 August 2018, field code GEGVPO18-10 (JZ collection). — Equatorial Guinea, Mitemele River basin, unnamed left bank tributary, A. González, C. Vizcaíno, F. Portal & H. Ott, 19 August 2018, field code GEGVPO18-15 (RS collection). — Equatorial Guinea, Mitemele River basin, Rio Be drainage, A. González, C. Vizcaíno, F. Portal & H. Ott, 22 August 2018, field code GEGVPO18-25 (DNA specimen).

Diagnosis

Aphyosemion mitemelense belongs to the '*Aphyosemion' herzogi* group, based on the oval shaped caudal fin, broad flat head, low number of anal fin rays and females that are less dull coloured and show often parts of male colour patterns than in other *Aphyosemion* s.l. It can be distinguished from all other members of the species group by a unique combination of colour pattern characters.

Aphyosemion mitemelense can be distinguished from both described species of the '*A.' herzogi* species group by the male colour pattern of body and unpaired fins. In *A. mitemelense* the caudal fin has several more or less horizontal relatively wide dark red bands on centre and lower part of fin vs. whole fin with many red interradial lines on blue centre and lower part of fin in '*A.' bochtleri* and two red lines edging a broad yellow band on fin centre in '*A.' herzogi*. Background of anal fin green to blue green in *A. mitemelense* vs. yellow to orange, at least basally, in the other two species.

Aphyosemion mitemelense can be distinguished from neighboring undescribed species from the '*A.' herzogi* group by having a greenish appearance and a green anal fin with irregular red blotches and/or stripes and a large dark red spot at the base of pectoral fin vs. a reddish appearance and an orange anal fin with regular interradial lines and lacking a red spot at the base of pectoral fin in populations on the right bank of the Mitemele River in southern Equatorial Guinea; by lacking a yellow horizontal band at the base of anal fin vs. having such a band in a population that occur sympatric near the village of Mveayong in the extreme southeast of Equatorial Guinea. *Aphyosemion mitemelense* differs from populations between Ngolensok and Acurenam in southeastern Equatorial Guinea by absence of more or

less regular red interradial lines in caudal fin centre, lower half of caudal greenish versus yellow and upper part of fin with many irregular red spots versus most part of upper half of caudal fin with red interradial lines; it differs also by the presence of broad red blotches, stripes or bands on greenish or blue-greenish anal fin versus more narrow, short red interradial lines on yellow or orange anal fin.

Description

See Figs 8 and 9 for general appearance and colour pattern, see Table 6 for morphometric and Table 7 for meristic data. Medium sized *Aphyosemion* s.l. with strong sexual dimorphism, males being colourful and females rather dull but showing faint traces of male colour pattern. Dorsal profile convex from nape to halfway dorsal fin base. Ventral profile straight convex from operculum to end of anal fin base. Caudal peduncle straight from end of both anal and dorsal fin base to base of caudal fin. Snout slightly pointed, mouth directed upwards; lower jaw longer than upper; posterior end of mouth at approximately same level as anterior edge of eye. On both jaws unicuspid, slightly posteriorly directed teeth, some larger teeth on outer row. Open frontal cephalic sensory system with two separate pits accompanies by 2 small lobes, large distance between pits (more than twice the length of a pit). Preopercular system tubular with 6 small pores, anterior most pore reduced and sometimes hardly visible. Supra-orbital system open groove with 3 neuromasts on both sides,

Table 6.– Morphometrics of *Aphyosemion mitemelensis* sp. nov., based on 11 specimens. M(7) mean = mean of 7 males, F(4) = mean of 4 females, SD = standard deviation. All measurements in percentages of standard length (SL), except standard length in mm. BD = body depth, HL = head length, ED = eye diameter, PD = pre dorsal length, PA = pre anal length, PP = pre pelvic length, DB = dorsal base, AB = anal base, CL= caudal peduncle length, CD = caudal peduncle depth, CR = caudal peduncle ratio.

Tabla 6.– Variables morfométricas de *Aphyosemion mitemelensis* sp. nov., basadas en 11 especímenes. M(7) media = media de 7 machos, F(4) = media de 4 hembras, SD = desviación estándar. Todas las medidas en porcentajes de longitud estándar (SL), excepto la longitud estándar en mm. BD = altura del cuerpo, HL = longitud de la cabeza, ED = diámetro del ojo, PD = longitud predorsal, PA = longitud preanal, PP = longitud prepélvica, DB = base dorsal, AB = base anal, CL= longitud del pedúnculo caudal, CD = altura del pedúnculo caudal, CR = proporción del pedúnculo caudal.

	holotype	M(7) mean (SD)	median	range	F(4) mean (SD)	median	range
SL	34.9	30,5 (4.9)	30,2	23.8–35.8	26,8 (3.2)	26,9	23.0–30.2
BD	23.5	21,1(2.2)	20,9	18.7–24.2	21,0 (2.7)	20,6	18.7–24.0
HL	24.4	24,5 (1.7)	25,1	21.8–25.8	24,8 (1.7)	25,3	22.5–26.1
ED	6.6	7,4 (0.8)	7,5	6.6–8.4	8,0 (1.0)	8,4	6.6–8.7
IO	14.9	13,6 (0.6)	13,7	10.7–14.2	13,1 (0.6	13,1	11.2–13.5
PD	68.2	67,5 (1.2)	67,5	63.5–68.2	66,4 (3.9)	68,0	60.6–69.0
PA	60.2	60,1 (2.9)	60,2	52.3–62.6	59,6 (3.5)	58,7	45.7–50.0
PP	50.7	47,5 (2.3)	47,3	45.0–50.7	47,7 (1.8)	47,5	43.2–55.7
DB	15.2	15,6 (1.1)	15,2	14.8–17.5	15,4 (1.0)	15,3	14.4–16.5
AB	21,8	20,5 (1.1)	20,5	19.2–21.8	19,1 (1.7)	19,1	17.3–20.9
CL	20.1	21,7 (1.4)	21,2	18.1–23.7	21,8 (2.1)	21,8	19.2–24.3
CD	15.5	13,9 (1.1)	14,1	12.5–15.5	12,9 (0.8)	12,7	12.3–13.9
CR	1.3	1,6 (0.1)	1,7	1.3–1.7	1,7 (0.1)	1,7	1.6–1.8

Table 7.– Meristics of *Aphyosemion mitemelensis* sp. nov., based on 11 specimens. SD = standard deviation, D = number of dorsal fin rays, A = number of anal fin rays, D/A = dorsal / anal fin position, C = number of caudal fin rays, LS = number of mid longitudinal line scales, TS = number transversal scales, CS = number of scales around caudal peduncle.

Tabla 7.– Variables merísticas de *Aphyosemion mitemelensis* sp. nov., basadas en 11 especímenes. SD = desviación estándar, D = número de radios de la aleta dorsal, A = número de radios de la aleta anal, D/A = posición de la aleta dorsal / anal, C = número de radios de la aleta caudal, LS = número de escamas de la línea longitudinal media, TS = número de escamas transversales, CS = número de escamas alrededor del pedúnculo caudal.

	holotype	all types mean (SD)	median	all types range
D	11	10.3 (0.8)	11	9–11
A	14	13.1 (0.7)	13	12–14
D/A	7	6.4 (0.5)	6	6–7
LS	29	30.4 (0.7)	30	29–31
TS	8	8 (0)	8	8
CS	12	11.7 (0.5)	12	11–12

Fig. 8.– *'Aphyosemion' mitemelense* sp. nov. a: male from type locality, not preserved. Photo by A. Garvía; b: female paratype. Photo by J. Hernandez Corral.

Fig. 8.– *'Aphyosemion' mitemelense* sp. nov. a: macho de la localidad tipo, no conservado. Foto de A. Garvía; b: paratipo hembra. Foto de J. Hernández Corral.

with 2 proximal lobes and 3 very small distal lobes, pre- and post-orbital system short tube with 2 large pores.

All fins rounded. Dorsal and anal fins located posterior to mid-body. Dorsal fin with 9–11 rays; anal fin with 12–14 rays, first ray of dorsal fin above anal fin ray 6–7. Pectoral fin reaching origin of pelvic fin in males, not in females; pelvic fin reaching origin of anal fin in males, not in females. Frontal squamation of G-type, with medium sized round to rather square G scale, not much smaller than E-scales. Scales on mid-longitudinal series 29–31. Transverse rows of scales above pelvic fin 8; circumpeduncular scale row 12.

Live colouration

Males (Fig. 8a). Top of head and dorsum brownish with dark green shining. Flank metallic green. Caudal peduncle with yellow to golden shine except western-most populations with blueish green caudal peduncle. Operculum with three dark red stripes, top stripe very short and sometimes hardly visible, lower stripe longest and most prominent and posteriorly often connected to the red spot in pectoral fin (Fig. 9a–c). This spot varies from small and rounded to large and triangle shaped. The lower opercular stripe anteriorly forming a suborbital stripe. Flank with 3 (very rarely 4) longitudinal lines with red spots. Top line reaching caudal fin base, other lines getting shorter from dorsum to ventrum. Caudal peduncle in western-most populations with broad longitudinal ventral red line, in other populations with irregular narrow red bars or blotches and often yellow to orange ventral edge. Pectoral fins transparent grayish to yellow with a dark red spot at the base, pelvic fins yellow to green with some larger red spots and/or streaks. Dorsal and anal fins green, sometimes with a narrow white margin. Dorsal fin with many dark red spots. Anal fin with dark red submarginal band and narrow blue or whitish edge and variable median band of elongated blotches, sometimes forming a band. Dorsal part of caudal fin with same colour pattern as dorsal fin, separated from rest of this fin by a straight narrow red line. Mid section of caudal fin light green to yellow-green with 2 or 3 red lines, mid section separated from ventral edge of fin by a broader red line. Ventral margin of caudal fin blue. Pelvic fin very variable, with or without red spots and/or stripes, generally green, but also populations with red and yellow are known. Pectoral fin hyaline, yellow or green with diagnostic red marking at base.

Females (Fig. 8b). Relatively colourful compared to other *Aphyosemion* s.l. females as all females of the '*Aphyosemion' herzogi* group. Dorsum and flanks brownish, dorsally darker, ventrally light grey or whitish. Sides with 3 or 4 relatively regular rows of small red spots. Unpaired fins light grey and yellowish with narrow light grey edge. Anal fin and caudal fin

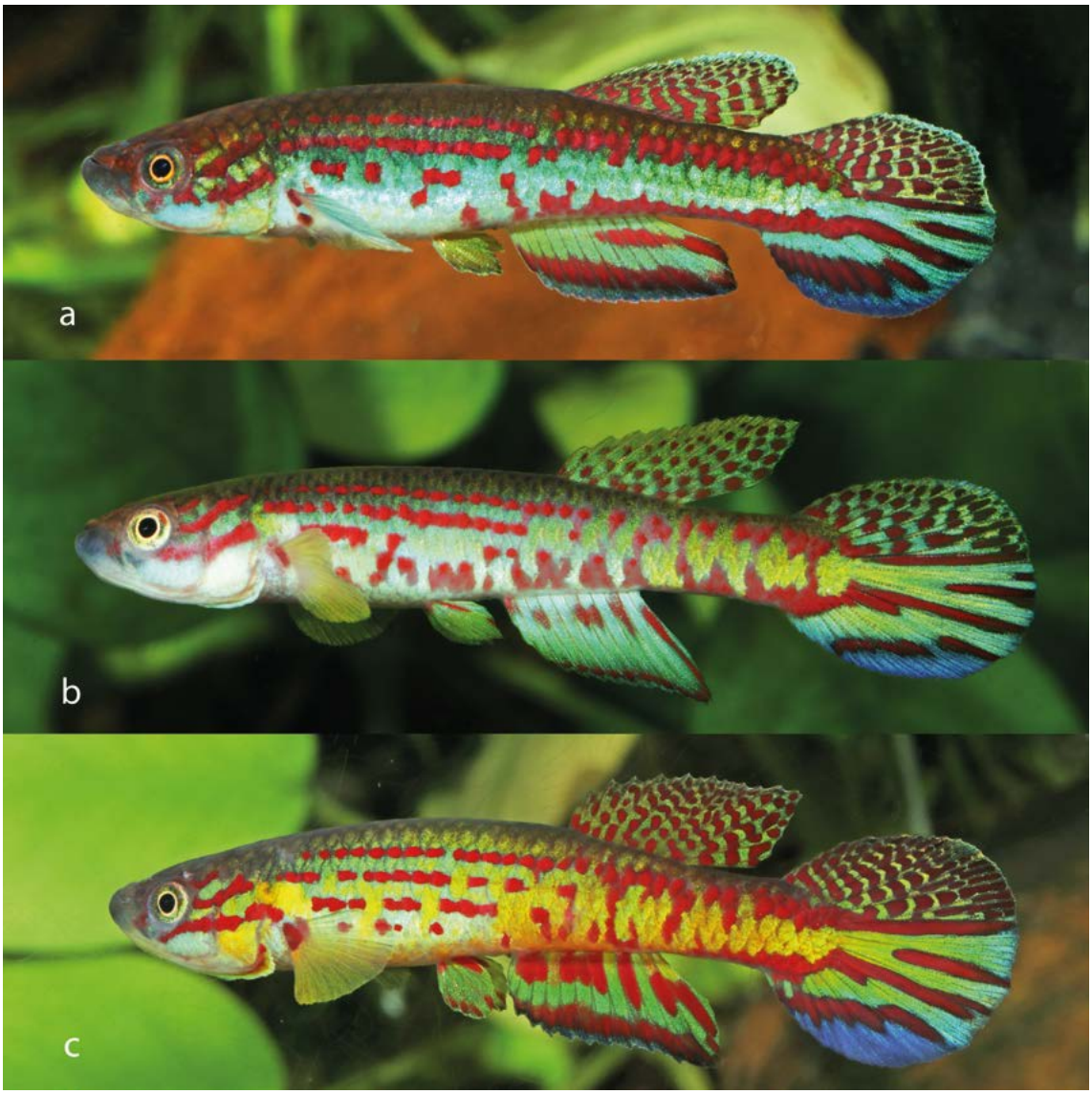

Fig. 9.– *'Aphyosemion' mitemelense* sp. nov., phenotypes of other locations than the type locality; a: 3 km northeast of type locality, field code GEGVPO18-15, DNA sample MIT009; b: 51 km east of type locality, field code GEGVPO18-25, DNA sample MIT015; c: 19 km southeast of type locality, Ayem II, field code GEGVPO18-10, DNA sample MIT006.Specimens from Figs 9 a and b are genetically closely related whilst that of Fig. 9 c is genetically closer to the specimens of the type locality. Photos by H. Ott.

Fig. 9.– *'Aphyosemion' mitemelense* sp. nov., fenotipos de localidades distintas a la localidad tipo; a: 3 km al noreste de la localidad tipo, código de campo GEGVPO18-15, muestra de ADN MIT009; b: 51 km al este de la localidad tipo, código de campo GEGVPO18-25, muestra de ADN MIT015; c: 19 km al sureste de la localidad tipo, Ayem II, código de campo GEGVPO18-10, muestra de ADN MIT006. Los especímenes de las Figs. 9a y b están genéticamente estrechamente relacionados, mientras que el de la Fig. 9c es genéticamente más próximo a los especímenes de la localidad tipo. Fotos de H. Ott.

with red inter-radial red stripes at the base (not well visible in Fig. 8). Dorsal fin and dorsal part of caudal fin with many red spots. Pectoral fin hyaline often with orange or yellow spot at the base.

Colour after 3 months in 96% ethanol

Males (Fig. 10a). Dorsum black, flanks dark grey, flanks and caudal peduncle ventrally light brown, ventrum light brown. Flanks scales with dark grey posterior margin. Third, fourth and fifth scale row with rounded red spots. Third row complete red, fourth row to onset dorsal fin, fifth row shorter and sometimes incomplete. Ventral scale row caudal peduncle with irregular dark red band. All fins black, red pigmentation hardly visible. Head dark grey, ventrally light brown. Space between pectoral fin and operculum marked light brown. Lower lip anteriorly black.

Females (Fig. 10b). Dorsum and dorsal part of flanks dark grey. Ventrum, ventral part of flanks and caudal peduncle light brown. Three to four irregular rows of red spots on anterior flank, some posterior flank scales with narrow crescent shaped red markings.

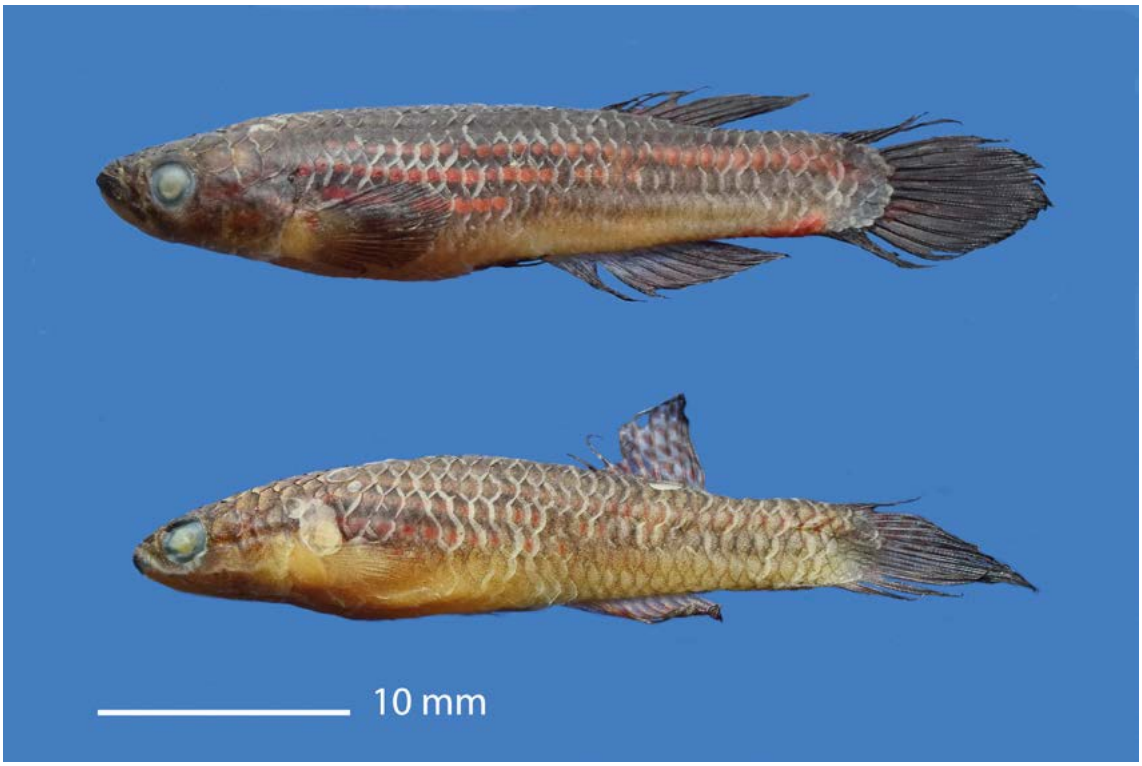

Fig. 10.– *'Aphyosemion' mitemelense* sp. nov., specimens preserved, a: holotype, male, 34.9 mm SL, b: paratype, female, 30.2 mm SL.

Fig. 10.– *'Aphyosemion' mitemelense* sp. nov., ejemplares conservados, a: holotipo, macho, 34,9 mm SL, b: paratipo, hembra, 30,2 mm SL.

Unpaired fins grey. Dorsal fin with many red spots, anal and caudal fin with red spots and/or interradial red stripes. Pelvic and pectoral fin hyaline. Space between pectoral fin and operculum marked light brown. Lower lip anteriorly black.

Colour after 1 month in formalin and then transferred to 70% ethanol

Males. Dorsum grey-brown, flanks and caudal peduncle ventrally light grey. Flank scales without dark posterior margin. Third, fourth, and fifth scale row with rounded red spots. Third row complete red, fourth row to onset dorsal fin, fifth row shorter and sometimes incomplete. Ventral scale row caudal peduncle with irregular red band. All fins dark grey to black. Anal with dark red median band. Caudal fin ventrally with red interradial stripes, dorsally with red spots. Dorsal fin with elongated red spots. Pectoral fin with dark red spot at base. Head grey. Space between pectoral fin and operculum white. Lower lip anteriorly black.

Females. As preserved directly in 96% ethanol.

Distribution and habitat

'*Aphyosemion' mitemelense* was thought to be restricted to small left bank tributaries of the southeastern Mitemele River basin in Equatorial Guinea (Fig. 1). One population, however, is known from just outside the basin 8 km southeast of Evinayong and recently the species was collected in north-west Gabon, where it was found in a small creek 13 km north of the village of Song in the Noya River basin (personal communication L. Chirio, August 2020). This species is restricted to a small area at the southwestern range of the species group. The currently described species are found at the southern ('*A.' herzogi* within the Okano River drainage) or southeastern ('*A.' bochtleri*, Mvoung River drainage) range of the species groups distribution area. Water conditions at the type locality (altitude 354 meter): pH 6.6; conductivity 11 µS; water temperature 24ºC. Sympatric occurring Cyprinodontiformes: *Mesoaphyosemion mimbon* and *Plataplochilus* sp. aff. *pulcher*.

Etymology

The specific epithet is derived from the Mitemele River (Equatorial Guinea), to which its distribution is restricted, except for one population occurring in adjacent northern Gabon.

Discussion

The freshwater fish fauna of Equatorial Guinea is still incompletely known as shown by the descriptions of killifish species in the last years (Legros & Zentz, 2007; Malumbres & Castelo, 2001; Sonnenberg,

2008; this paper) and the results of recent surveys which still find new reports or undescribed species (Roman, 1971; Castelo, 1994; Lasso *et al.*, 1998; Schmidt & Barrientos, 2019; R. Schmidt, pers. comm., Sep. 2019). The recently described killifish species are often endemic to Equatorial Guinea or shared with usually only one of the neighboring countries (Malumbres & Castelo, 2001; Legros & Zentz, 2007; Sonnenberg, 2008; this paper). All are usually known from a relative small distribution area.

Mesoaphyosemion is a large and complex group of species living in small rainforest streams in southern Cameroon, northern Gabon, eastern Equatorial Guinea, and northern Republic of Congo (Amiet, 1987; Wildekamp, 1993; Huber, 2007; Stiassny *et al.*, 2007). Currently eight species are accepted as valid, but actually nine more or less distinct male colour pattern phenotypes are recognized. In combination with the here published genetic data this indicates the occurrence of a large number undescribed species. One obstacle is the repeated recurrence of certain male colour pattern characters within the genus, like the marbled pattern of *M. maculatum*, *M. losantosi*, *M. montealenense*, and *M. mimbon*, or a yellow caudal peduncle in *M. amoenum*, *M. halleri*, and *M.* sp. aff. *cameronense* phenotypes 3, 4, 5, 9, and 10 (see also Sonnenberg *et al.*, submitted). However, the genetic data do not support a single, monophyletic origin of these characters, but a repeated independent occurrence in different genetic lineages (Fig. 2). Despite their similarity, *M. losantosi*, *M. montealenense*, *M. maculatum*, and *M. mimbon* do not represent a single species, nor a distinct lineage within *Mesoaphyosemion*. The genetic data also indicate a quite high genetic divergence between them of more than 5% observed pairwise distance, which is in the range found in other nothobranchiid species groups for cytochrome b or COI mtDNA datasets between different species. Similar colour pattern characters are not uncommon in unrelated nothobranchiid species, e.g. a pattern of narrow bars on the caudal peduncle in different genera (*Aphyosemion* s.s., '*A.*' *ogoense* species group, *Diapteron*, *Fundulopanchax, Kathetys, Scheelsemion*). Huber (1980) coined the term "frontier species" for similarities in colour pattern of neighboring *Aphyosemion* s.l. populations. Probably it will be possible in future to use Quantative Trait Locus (QTL) mapping and next generation sequencing to find out if these similar colour pattern characters in different species are based on the same genes and 'switched' on and off due to small mutations independently.

'Aphyosemion' herzogi and '*A.*' *bochtleri* were the first described species of the '*A.*' *herzogi* group. Although both species differ considerably in colour pattern, several authors considered '*A.*' *bochtleri* as a subspecies of '*A.*' *herzogi*, since populations showing sometimes mixed combinations of colour patterns as in the above species where found (Seegers, 1997; Wildekamp, 1993; Wildekamp *et al.*, 1986). These discussions were summarized by Eberl (2016). The phylogenetic tree in this paper (Fig. 3) shows that similar to the results of *Mesoaphyosemion* the '*A.*' *herzogi* group most probably consists of a potentially larger number of distinct species, which is in agreement with the different male colour phenotypes found (Eberl, 2016; Seegers, 1997; own observations).

'Aphyosemion' mitemelense is genetically quite distinct from the remaining samples of the group. Within this species, the DNA sample from the collection site GEGVPO18-8 (DNA sample MIT002), which is outside the Mitemele basin, is genetically quite divergent from the other samples of the species. It differs up to 4.76% from populations inside the basin which is considerably high. Since it cannot be distinguished from the nearest population inside the basin, based on external morphology and colour pattern, we consider it yet as conspecific with '*A.*' *mitemelense.* A similar case is e.g. *Chromaphyosemion riggenbachi* (Ahl, 1924) in Cameroon, which contains several distinct lineages with quite high genetic distances between them (haplotype groups I-V with uncorrected mean distances between 3.99%-7.24%) (Völker *et al.*, 2006), which are all very similar by male colour pattern, but have quite different chromosome numbers.

Inside the Mitemele basin two colour patterns occur in *'Aphyosemion' mitemelense* (Fig. 9). The majority of known populations have a colour pattern as seen in Fig.9b and 9c: a barred caudal peduncle with a more or less yellow to golden colour. Two populations at the extreme south-west of the distribution area lack the bars and have a blue-greenish caudal peduncle (Fig. 9a): the type locality (DNA samples MIT020 and 021) and a population just 3 km north-east of it (DNA sample MIT009). Yet they are not closely related with a genetic distance of almost 4%. In the other (golden and barred) populations there is also a genetic gap, although they all look very similar. The two southern-most populations (DNA samples 004 and 006) group with the type specimens (MIT020 and MIT021) and the more northern populations group with the MIT009 population (Fig.1). Maybe the species developed the two different phenotypes in allopatric populations, that came together in secondary contact and between the phenotypes some exchange of genetic material took place. Actually the mitochondrial genetic data and the male colour pattern of the two different phenotypes do not show a congruent pattern. Probably population genetic data sets based on highly variable nuclear marker might be able to determine if mitochondrial introgression has occurred across the two '*A.' mitemelense* phenotypes.

Small scale species distribution in nothobranchiids is quite common. This is usually also reflected in genetic data. Even within species quite some genetic divergence can occur between populations (Völker *et al.*, 2006; own unpublished data). Possible causes

for low genetic exchange between populations might be low dispersal capabilities, local adaptations (McKenzie *et al.*, 2013), and strong mate choice preference (see references in Sonnenberg, 2007).

Different views exist about the relationships of the *'A.' herzogi* species group. Amiet (1987) assumes a closer relationship to *Scheelsemion franzwerneri* (Scheel, 1971), based on: a) less sexual colour dimorphism as in other *Aphyosemion* s.l., b) a racket shaped caudal, c) dark post-cephalic mark, d) few dorsal and anal fin rays, dorsal inserted above 6-7 anal fin ray, e) dark crossbars as chevrons or Xs in stressed specimens. Both species share similar habitat preferences (wells, shallow water in rain forest), behavior (mainly bottom orientated, less free swimming) and very faint similarity in colour pattern (Amiet, 1987; Huber, 2013, own observations, pers. comm. T. Blum). However, these characters are not unique to these species and genetic data include *S. franzwerneri* into the *'A.' calliurum* species group (Agnèse *et al.*, 2009; Collier, 2006). Recently, Huber (2013: fig. 2) presented a morphological study and places the *'A.' herzogi* group in a clade with *'A.' tirbaki*, which in turn is the sister group to the *'A.' calliurum* group (in a basal position), *Chromaphyosemion, Diapteron,* and *Kathetys [(('A.' tirbaki, 'A.' herzogi) (Scheelsemion, (Chromaphyosemion, (Diapteron, Kathetys)))))]* This is not supported by the phylogenetic tree based on mitochondrial DNA sequences as published in Collier (2006: fig. 5), in which the nodes of *'A.' bochtleri, A.' herzogi,* and *'A.' tirbaki* are printed close to the samples of *Scheelsemion,* but a relationship of these species groups has no statistical significant support. Huber's (2013) inclusion of the *'A.' herzogi* species group and *'A.' tirbaki* into a subgenus *Scheelsemion* in his sense makes it a para- or probably polyphyletic taxon.

Collier (2006) coined the term 'orphan' taxa for those species and species groups that do not show any closer relationship to any other group. Interestingly even the phylogenetic tree published by Huber (2013: fig. 2), based on morphological characters does not allow the inclusion of *'A.' tirbaki* and the *'A.' herzogi* group into a monophyletic *Scheelsemion* (see also above). A preliminary analysis of Huber's (2013) published dataset indicates that of the 91 morphological characters only 50 are informative. A simple Maximum Parsimony analysis of the dataset results in 10 equally short trees, the 50% consensus tree of a bootstrap analyses with 1000 replicates did not show any significant support for a taxon *Scheelsemion* as proposed in Huber (2013). Of all presented characters only one is informative for a grouping of the *'A.' calliurum* group with *'A.' herzogi* and *'A.' tirbaki* (character 65, isolated yellow spot surrounded by red, postopercular, above pectoral fin insertion), two additional (characters 31 and 35, dorsal longitudinal fin shape and anal posterior fin shape in females) are also found either in *Chromaphyosemion* or *Diapteron* and one (character 3, Body-outline (longitudinal) in male: higher at about ventral fin level) supports a grouping of *'A.' tirbaki, 'A.' herzogi, Scheelsemion, Chromaphyosemion, Diapteron,* and *Kathetys* (Huber, 2013). At the moment it seems that character 65, the occurrence of a yellow spot is not diagnostic, because this is not found in several populations and species of the *'A.' calliurum* group and in *'A.' tirbaki*, but present in other species, as e.g. *'A.' coeleste* or *'A.' wachtersi*, the same might be the case for character 3, but needs further study, as characters 31 and 35.

Huber (2013) diagnoses *Scheelsemion* (including the *'A.' herzogi* group and *'A.' tirbaki*) with several characters, of which some are only diagnostic for the *'A.' calliurum* species group, which includes the type species of Scheelsemion, like a dark blotch under the chin (see Fig. 11) and long extensions of unpaired fins in males or only diagnostic for the *'A.' herzogi* group like red lower lip and the three-part colour pattern of the caudal fin. Some characters are not really useful as diagnostic characters (e.g. incomplete rows of red dots or blotches, fin extensions of unpaired fins in males, red lower lip) as they occur in several other species groups, which can't be distinguished by the given characters. The problem with the diagnosis of *Scheelsemion* is, that it has to include three different species groups ('*A.*' *calliurum*, '*A.*' *herzogi*, '*A.*' *tirbaki*) that do not share some unique diagnostic features and, by DNA studies, are most probably not closely related.

Currently, all published molecular genetic studies of the relationships within *Aphyosemion* s.l. that include members of both species groups do not support a closer relationship of the *'A.' herzogi* species group with or a placement within *Scheelsemion* (Agnèse *et al.*, 2009; Collier, 2006). If *Scheelsemion* is restricted to the '*A.*' *calliurum* group, this is supported by the available DNA studies (Agnèse *et al.*, 2009; Collier, 2006)

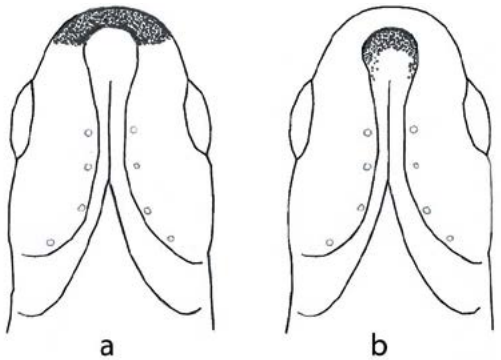

Fig. 11.– Sketch of the pigmented markings on the ventral head of a) the *'A.' herzogi* species group and b) *'A.' calliurum* species group.

Fig. 11.– Bosquejo de las marcas pigmentadas en la cabeza ventral de a) grupo de especies *'A.' herzogi* y b) grupo de especies *'A.' calliurum*.

and it is diagnosed by the dark blotch on chin and a unique pattern of the frontal neuromast system in all species. In turn, the *'A.' herzogi* complex seems to be a separate species groups that needs further studies.

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References

Aarn, S. & Shepherd, M. 2001. Descriptive anatomy of *Epiplatys sexfasciatus* (Cyprinodontiformes: Aplocheilidae) and a phylogenetic analysis of Epiplatina. *Cybium,* 25(3): 209–225

Agnèse, J.-F., Brummett, R., Camindae, P., Catalan, J. & Kornobis, E. 2009. Genetic characterization of the *Aphyosemion calliurum* species group and description of a new species from this assemblage: *A. campomaanense* (Cyprinodontiformes: Aplocheiloidei: Nothobranchiidae) from Southern Cameroon. *Zootaxa*, 2045(1): 43–59. https://doi.org/10.11646/zootaxa.2045.1.4

Agnèse, J.-F., Zentz, F., Legros, O. & Sellos, D. 2006 Phylogenetic relationships and phylogeography of the Killifish species of the subgenus *Chromaphyosemion* (Radda, 1971) in West Africa, inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 40(2): 332–346. https://doi.org/10.1016/j.ympev.2006.03.018

American Killifish Association. No date. *Aphyosemion maculatum*. Available from http://www.wak.aka.org/Ref_Library/Aphyosemion/A.maculatum.htm [accessed 29 Oct. 2019].

Amiet, J.L. 1987. *Le genre Aphyosemion Myers (Pisces, Teleostei, Cyprinodontiformes)*. Faune du Cameroun, Volume 2. Sciences Nat, Compiègne.

Castelo Álvarez, R. 1994. Fauna ictiológica epicontinental de las repúblicas de Guinea Ecuatorial y Santo Tomé-Príncipe. Doctoral dissertation, Universidade de Santiago de Compostela.

Clausen, H.S. 1967. *Tropical old world cyprinodonts, reflections on the taxonomy of tropical old world cyprinodonts, with remarks on their biology and distribution*. Akademisk Forlag. Kopenhagen. 64 pp.

Collier, G.E. 2006. The genus *Aphyosemion*. Taxonomic history and molecular phylogeny. *Journal of the American Killifish Association*, 39(5-6): 147–168.

Costa, W.J.E.M. 2009. Osteology of the African annual killifish genus *Callopanchax* (Teleostei: Cyprinodontiformes: Nothobranchiidae) and phylogenetic implications. *Vertebrate Zoology*, 59(1): 31–40.

Costa, W.J.E.M. 2015. Comparative morphology, phylogeny, and classification of West African callopanchacine killifishes (Teleostei: Cyprinodontiformes: Nothobranchiidae). *Zoological Journal of the Linnean Society*, 175(1): 134–149. https://doi.org/10.1111/zoj.12270

Dadaniak, N., Lütje, R. & Eberl, W. 1995. *Faszination Killifische: Die "Aphyosemion cameronense"-Gruppe*. Published by the authors. Germany. 480 pp.

De La Riva, I. 1994. *Informe sobre la diversidad faunística del Parque Nacional de Monte Alén, Guinea Ecuatorial*. Informe Técnico AGRECO–C.T.F.T. Asociación Amigos del Coto de Donaña. 51 pp.

Eberl, W. 2016. Die *Aphyosemion herzogi*-Artengruppe. *Deutsche Killifisch Gemeinschaft-Journal*, 48: 1–60.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41: 95–98.

Hoedeman, J. 1958. The frontal scalation pattern in some groups of toothcarps (Pisces: Cyprinodontiformes). *Bulletin of Aquatic Biology*, 1: 23–28.

Huber, J.-H. 1977. Une chaine de deux *Aphyosemion* sympatriques dans les Monts de Cristal, Gabon, avec description d'une espèce nouvelle: *A mimbon* n. sp. *Revue Française d'Aquariologie et Herpetologie*, 4: 3–10.

Huber, J.-H. 1980. Die *Aphyosemion* Arten des Ivindo Beckens im Kongo, mit der Beschreibung von *Aphyosemion* (*Diapteron*) *seegersi* nov. spec. *Deutsche Killifisch Gemeinschaft-Journal*, 12(1): 1–10.

Huber, J.H. 2007. *Killi Data 2007. Updated checklist of taxonomic names, collecting localities and bibliographic references of oviparous cyprinodont fishes (Cyprinodontiformes)*. Killi-Data Editions. Paris. 410 pp.

Huber, J.-H. 2013. Reappraisal of the phylogeny of the African genus *Aphyosemion* (Cyprinodontiformes) focused on external characters, in line with molecular

data, with new and redefined subgenera. *Killi-Data Series*, 2013: 4–20.

Huber, J.-H. 2020. Killi-Data (all information on Killies). Available from www.killi-data.org, [accessed 12 Apr. 2020].

Huelsenbeck, J.P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, 17(8): 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Kumar, S., Stecher, G. & Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33(7): 1870-1874. https://doi.org/10.1093/molbev/msw054

Lasso, C.A. 1995. *Biodiversidad Animal del Parque Nacional de Monte Alén. República de Guinea Ecuatorial, Proyecto ECOFAC – Componente Guinea Ecuatorial*. Informe Técnico AGRECO–C.T.F.T. Asociación Amigos del Coto de Donaña. 78 pp.

Lasso, C.A., Castelo, R. & Rial, A. 1998. Peces del Parque Nacional de Monte Alén, cuenca del río Uoro (Río Muni), Guinea Ecuatorial. *Publicaciones de la Asociación de Amigos de Donana*, 11: 1–33.

Lasso, C.A., Rial, A.I., Castroviejo, J. & De la Riva, I. 2002. Herpetofauna del Parque Nacional de Monte Alén (Río Muni, Guinea Ecuatorial). *Graellsia*, 58(2): 21–34. https://doi.org/10.3989/graellsia.2002.v58.i2.276

Leal, M.E. 2005. *The biodiversity of Mont Mbilan, preliminary results and observations*. Missouri Botanical Garden. 12 pp.

Legros, O. & Zentz, F. 2007. *Aphyosemion malumbresi* n. sp., un nouveau *Chromaphyosemion* (Teleostei: Aplocheilidae) originaire de Guinée Equatoriale continentale. *Association Killiphile Francophone de Belgique, Killi Contact*, 34: 1–27.

Maley, J. 1987. Fragmentation de la forêt dense humide africaine et extension des biotopes montagnards au Quaternaire récent: nouvelles données polliniques et chronologiques. Implications paléoclimatiques et biogéographiques. *Palaeocology of Africa*, 18: 307–334.

Maley, J. 1991. The African rain forest vegetation and palaeoenvironments during late Quaternary. *Climatic Change*, 19: 79–98. https://doi.org/10.1007/BF00142216

Malumbres, F. & Castelo, W. 2001. Descripción de una nueva especie del género *Fundulopanchax* Myers, 1924 (Cyprinodontiformes, Aplocheilidae), para la ictiofauna continental de Guinea Ecuatorial. *Graellsia*, 57(2): 175–180. https://doi.org/10.3989/graellsia.2001.v57.i2.285

McKenzie, D.J., Estivales, G., Svendsen, J.C., Steffensen, J.F. & Agnèse, J.-F. 2013. Local adaptation to altitude underlies divergent thermal physiology in tropical killifishes of the genus *Aphyosemion*. *PLoS ONE*, 8(1): e54345. https://doi.org/10.1371/journal.pone.0054345

Moritz, C., Patton, J.L., Schneider, C.J. & Smith, T.B. 2000. Diversification of rainforest faunas: an integrated molecular approach. *Annual Review of Ecology and Systematics*, 31: 533–563. https://doi.org/10.1146/annurev.ecolsys.31.1.533

Murphy, W.J. & Collier, G.E. 1999. Phylogenetic relationships of African killifishes in the genera *Aphyosemion* and *Fundulopanchax* inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 11(3): 351–360. https://doi.org/10.1006/mpev.1998.0566

Ott, H. 2019. Ein kleines afrikanisches Land mit großartigen Fischen. *Amazonas*, 83: 41–47.

Pappenheim, P. 1910. Zoologische Ergebnisse der Expedition des Herren G. Tessmann nach Süd-Kamerun und Spanisch-Guinea. *Mitteilungen aus dem Zoologischen Museums in Berlin*, 5: 507–528.

Román, B. 1971. *Peces de Río Muni, Guinea Ecuatorial (aguas dulces y salobres)*. Fundación la Salle de Ciencias Naturales. Madrid.

Schmidt, R.C. & Barrientos, C. 2019. A new species of suckermouth catfish (Mochokidae: *Chilogolanis*) from the Rio Mongo in Equatorial Guinea. *Zootaxa*, 4652(3): 507–519. https://doi.org/10.11646/zootaxa.4652.3.7

Schmidt, R.C., Knobloch, E.C. & Barrientos, C. 2021. Cast netting new species: Integrative taxonomy of *Distichodus notospilus* (Characiformes: Distichodontidae) discovers new species and overlooked areas of endemism in Central Africa. *Zootaxa*, 4952(2): 291–313. https://doi.org/10.11646/zootaxa.4952.2.5

Seegers, L. 1997. *Killifishes of the World. Old World Killis II*. Aqualog, Verlag A.C.S., Mörfelden-Walldorf.

Sonnenberg, R. 2007. Description of three new species of the genus *Chromaphyosemion* Radda, 1971 (Cyprinodontiformes: Nothobranchiidae) from the coastal plains of Cameroon with a preliminary review of the *Chromaphyosemion splendopleure* complex. *Zootaxa*, 1591: 1–38

Sonnenberg, R. 2008. Two new species of *Chromaphyosemion* (Cyprinodontiformes: Nothobranchiidae) from the coastal plain of Equatorial Guinea. *Ichthyological Exploration of Freshwaters*, 18(4): 359–373.

Sonnenberg, R. & Blum, T. 2005. *Aphyosemion* (*Mesoaphyosemion*) *etsamense* (Cyprinodontiformes: Aplocheiloidei: Nothobranchiidae), a New Species from the Monts de Cristal, Northwestern Gabon. *Bonner zoologische Beiträge*, 53 (1/2): 211–220.

Sonnenberg, R., Blum, T. & Misof, B. 2006. Description of a new *Episemion* species (Cyprinodontiformes: Nothobranchiidae) from northern Gabon and southeastern Equatorial Guinea. *Zootaxa*, 1361: 1–20. https://doi.org/10.11646/zootaxa.1361.1.1

Sonnenberg, R., Nolte, A.W. & Tautz, D. 2007. An evaluation of LSU rDNA D1–D2 sequences for their use in species identification. *Frontiers in Zoology*, 4: 6. https://doi.org/10.1186/1742-9994-4-6

Sonnenberg, R. & Schunke, A.C. 2010. On the taxonomic identity of *Fundulus beauforti* Ahl, 1924 (Cyprindontiformes: Aplocheiloidei). *Zoosystematics and Evolution*, 86(2): 337–341. https://doi.org/10.1002/zoos.201000015

Stiassnym M.L., Teugels, G.G., Hopkins, C.D. & Paugy, D, 2007. *The fresh and brackish water fishes of lower Guinea, west-central Africa*. Volume 1. IRD Editions. Paris.

Van der Zee, J.R. & Sonnenberg, R. 2010. *Aphyosemion teugelsi* (Cyprinodontiformes: Nothobranchiidae), a new species from a remote locality in the southern Democratic Republic of Congo. *Zootaxa*, 2724: 58–68. https://doi.org/10.11646/zootaxa.2724.1.4

Van der Zee, J.R. Walsh, G., Boukaka Mikembi, V., Jonker, M., Alexandre, M. & Sonnenberg, R. 2018. Three new endemic *Aphyosemion* species (Cyprinodontiformes: Nothobranchiidae) from the Massif du Chaillu in the upper Louessé River system, Republic of the Congo. *Zootaxa*, 4369(1): 63–92. https://doi.org/10.11646/zootaxa.4369.1.3

Van Bergeijk, W.A. & Alexander, S. 1962. Lateral line canal organs on the head of *Fundulus heteroclitus*. *Journal of Morphology*, 110: 333–346. https://doi.org/10.1002/jmor.1051100303

Völker, M., Sonnenberg, R. & Kullmann, H. 2006. Karyotype differentiation in *Chromaphyosemion* killifishes (Cyprinodontiformes, Nothobranchiidae). II: Cytogenetic and mitochondrial DNA analyses demonstrate karyotype differentiation and its evolutionary direction in *C. riggenbachi*. *Cytogenetic and Genome Research*, 115: 70–83. https://doi.org/10.1159/000094803

Wildekamp, R.H. 1993. *A world of killies. Atlas of the oviparous cyprinodontiform fishes of the World*. American Killifish Association, Elyria

Wildekamp, R.H., Romand, R. & Scheel, J.J. 1986. Cyprinodontidae. *In*: Daget, J., Gosse, J-P. & Thys van den Audenaerde, D.F. *Checklist of the freshwater fishes of Africa (CLOFFA)*. ORSTOM, Paris & MRAC, Tervuren.

Zhang, D.-X. & Hewitt, G.M. 1996. Nuclear integrations: challenges for mitochondrial DNA markers. *Trends in Ecology and Evolution*, 11(6): 247–251. https://doi.org/10.1016/0169-5347(96)10031-8

Supplementary files

Supp. file 1.— Uncorrected pairwise distance (in %) for the *Mesoaphyosemion* cytochrome b sequence samples. For sample abbreviations refer to Table 1.

Supp. file 2.— Uncorrected pairwise distance (in %) for the '*Aphyosemion' herzogi* species group cytochrome b sequence samples. For sample abbreviations refer to Table 1.