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## Three new endemic *Aphyosemion* species (Cyprinodontiformes: Nothobranchiidae) from the Massif du Chaillu in the upper Louessé River system, Republic of the Congo

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### Abstract

Three new '*Aphyosemion*' species are described from the upper Louessé River in the Massif du Chaillu, Republic of the Congo, based on a combination of DNA, habitat preference, male colour pattern, and morphological data. '*Aphyosemion*' *cyanoflavum*, new species, is a member of the '*A. ogoense*' group. It differs from its congeners by a unique colour pattern and cephalic sensory system which contains a wide supra-orbital groove with large, densely pigmented anterior neuromasts and dark frontal neuromasts housed in one pit with one central anterior lobe. '*Aphyosemion*' *mandoroense*, new species, and '*A. cryptum*', new species, are members of the '*A. coeleste*' group. '*Aphyosemion*' *cryptum*, new species, is in appearance very similar to '*A. coeleste*', but lacks the typical post opercular metallic green blotch and is generally larger in body size. Initial DNA analyses demonstrate that '*A. cryptum*', new species, is more closely related to '*A. mandoroense*', new species, than to '*A. coeleste*', despite similarity in appearance. '*Aphyosemion*' *cryptum*, new species, and '*A. coeleste*' occur syntopic in several locations in a sub-catchment of the upper Louessé system, however differ in microhabitat preference. '*Aphyosemion*' *mandoroense*, new species, differs by male body and fin colour pattern from all species of the '*A. coeleste*' group except '*A. citrineipinnis*'. From the latter, it can be distinguished by the absence of red pigmentation and a dark grey to black margin in the unpaired fins.

### Résumé

Trois nouvelles espèces de '*Aphyosemion*' sont décrites de la Louessé Supérieure dans le Massif du Chaillu en République du Congo, sur base d'une combinaison d'analyse d'ADN, préférence dans les habitats, couleur des mâles et données morphologiques. '*Aphyosemion*' *cyanoflavum*, une espèce nouvelle, est un membre du groupe '*A. ogoense*'. Il diffère de ses congénères par une palette de couleur unique et un ensemble sensoriel céphalique qui exhibe une large rainure supra-orbitale, des neuromasts antérieurs densément pigmentés et des neuromasts frontaux sombre logés dans un puit avec un lobe antérieur central. '*Aphyosemion*' *mandoroense*, nouvelle espèce, et '*A. cryptum*', nouvelle espèce, sont des membres du groupe '*A. coeleste*'. '*Aphyosemion*' *cryptum*, nouvelle espèce, est en apparence très similaire à '*A. coeleste*', mais manque cependant la typique tache vert métallique post operculaire et il présente généralement une taille corporelle supérieure. Les analyses d'ADN initiales démontrent que '*A. cryptum*', nouvelle espèce, est plus proche de '*A. mandoroense*', nouvelle espèce, que de '*A. coeleste*', malgré des similarités en apparence. '*Aphyosemion*' *cryptum*, nouvelle espèce, et '*A. coeleste*' cohabitent de manière syntopique sur plusieurs localisations du sous bassin de la Louessé supérieure mais diffèrent dans les préférences de micro habitats. '*Aphyosemion*' *mandoroense*, nouvelle espèce, diffère de par la taille corporelle des mâles et par la palette et motif de coloration des nageoires des autres espèces du groupe '*A. coeleste*' excepté pour '*A. citri-*

*neipinnis*. Il est aussi possible de le distinguer de ce dernier par l'absence de pigmentation rouge et une marge gris foncée à noire sur les nageoires non paires.

**Key words:** ‘*Aphyosemion*’ *cyanoflavum*, new species, ‘*A. mandoroense*, new species, ‘*A. cryptum*, new species, ‘*A. ogoense* group, ‘*A. coeleste* group, Louessé, Mandoro, Niari, mtDNA, colour pattern, morphology, cephalic sensory system, neuromasts, ecology, Pleistocene rainforest refuge, distribution, syntopic occurrence

## Introduction

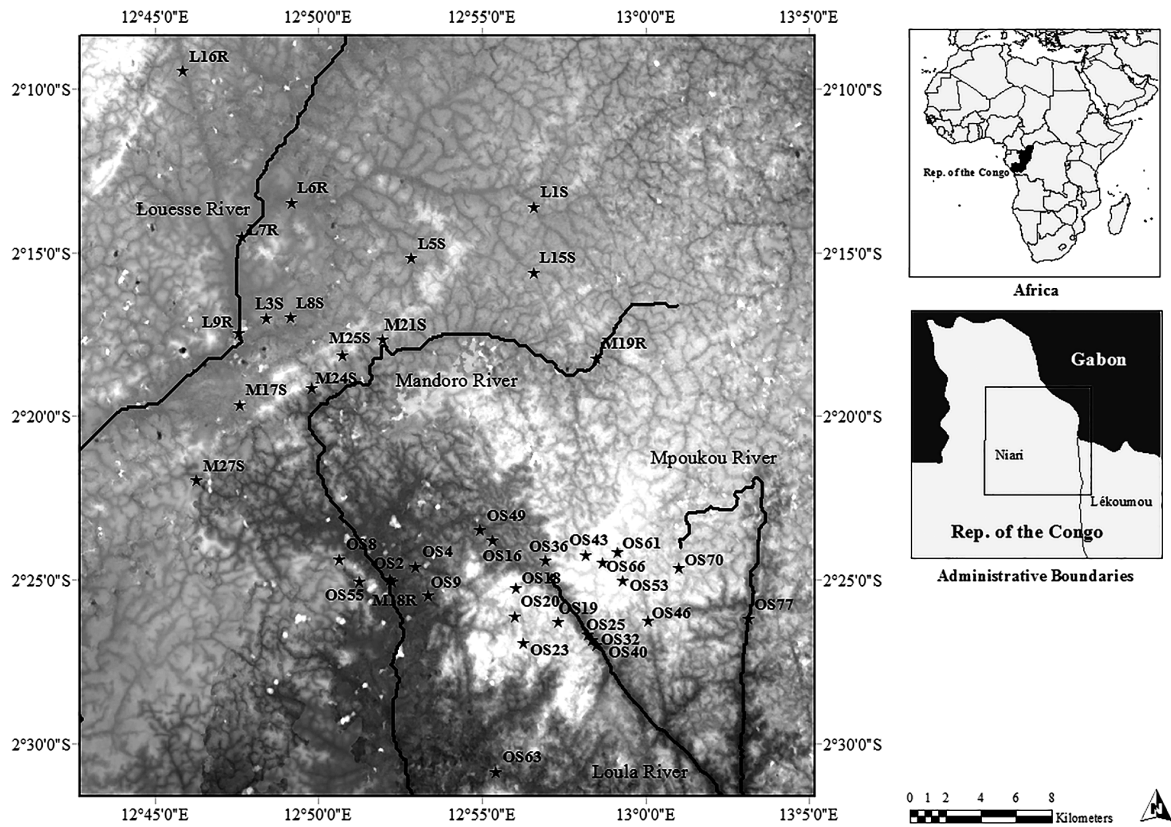
The genus *Aphyosemion* Myers, 1924 s.l. (see Sonnenberg 2007; Van der Zee & Sonnenberg 2010 for taxonomic concept) currently consists of 117 recognized species or subspecies of which 31 occur in the Republic of the Congo (Costa 2008; Huber 2013a,b; Valdesalici & Eberl 2015; Van der Zee *et al.* 2007; Wildekamp 1993). In the following, the genus name *Aphyosemion* is only used for the *Aphyosemion elegans* (Boulenger, 1899) species group. For the remaining species groups either the published generic name is used or, for unnamed groups, *Aphyosemion* within single quotes is used as suggested by Sonnenberg (2007) and Van der Zee & Sonnenberg (2010).

With respect to the killifish ichthyofauna, the Republic of the Congo can be subdivided into three main areas: the coastal plain, the Congo Cuvette, and the inland plateau (Huber 1982). The latter can be further subdivided into three regions, namely the Upper Ivindo River drainage in the north, the hills of central Congo and the Massif du Chaillu in the southwest, which have little overlap of nothobranchiid species or species groups (Van der Zee *et al.* 2007; Wildekamp 1993). The central hills are part of the Congo ichthyofaunal province, whilst the Upper Ivindo and the Massif du Chaillu are part of the Lower Guinea province (Stiassny *et al.* 2007). Fifty-two percent of *Aphyosemion* s.l. species reported from the Republic of the Congo, including the species described in this paper, are endemic to the Massif du Chaillu, a mountain range covered with dense forest reaching altitudes of 900 meters in southern Gabon and north-western Congo. The southern Massif du Chaillu is covered by a savannah and forest mosaic and is considered a highly diverse Pleistocene refuge area with a high number of endemic plants and animals (Born *et al.* 2011; Maley 1996; Plana *et al.* 2004). The area has a dry season from June to September with less than 50 mm rainfall, and a wet season from October till May with 1800-1900 mm average rainfall (Petit 1975). There is an all year permanent cloud cover that shelters from solar radiation, resulting in high humidity and relatively low temperatures (Maley 1996).

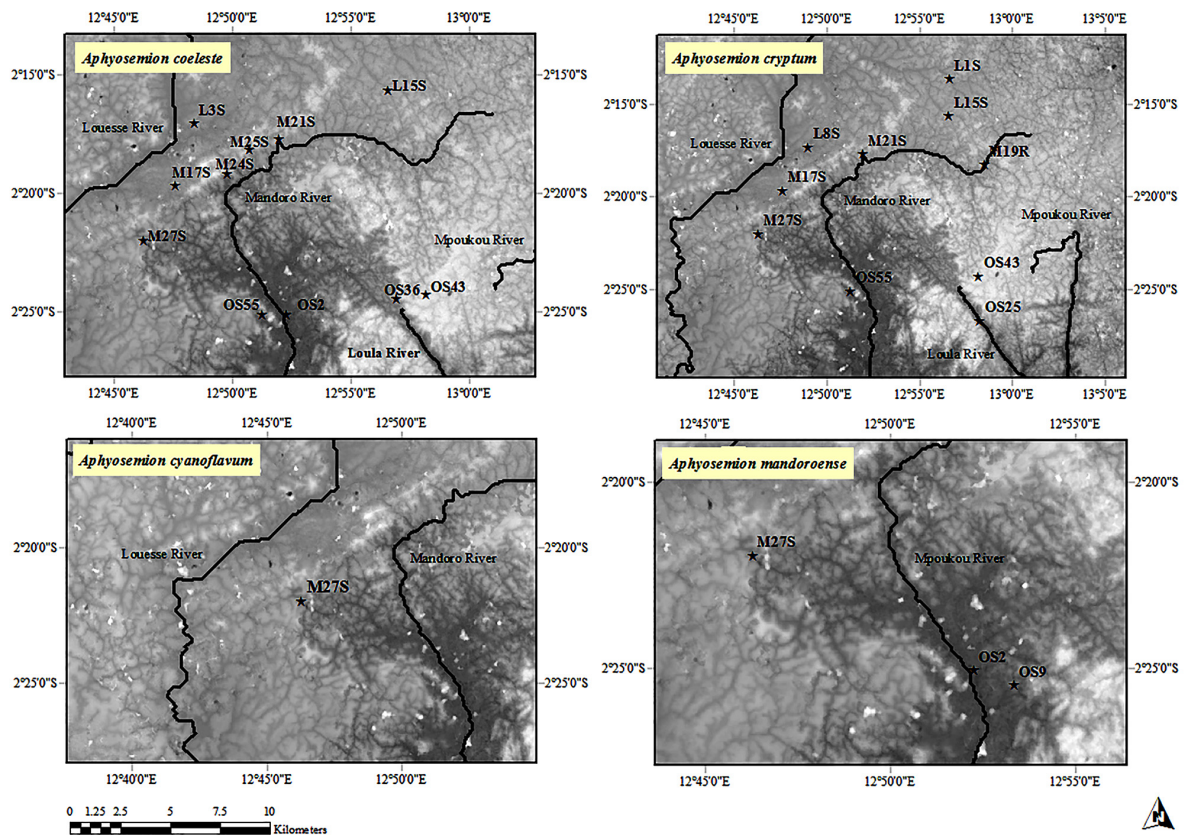
Until 1978, when the Massif du Chaillu was explored for killifishes independently by Buytaert and Wachtters (Buytaert 1980) and Huber (1982) only two nothobranchiid species were known from the Congolese region, namely, ‘*A. louessense* (Pellegrin, 1931) and ‘*A. ogoense* (Pellegrin, 1930). The expeditions resulted in the description of several new species (Huber & Radda 1979; Radda & Huber 1978; Van der Zee *et al.* 2007).

The nothobranchiid fauna of the southern part of the Massif du Chaillu is represented by three different species groups (‘*A. coeleste* group, ‘*A. ogoense* group, and ‘*A. wachtersi* group). ‘*Aphyosemion*’ *coeleste* Huber & Radda, 1977 is a wide spread species in the area between Moanda in Gabon, to Titi in the Republic of the Congo (Huber 2016; Van der Zee *et al.* 2007; Wildekamp 1993) and the most common nothobranchiid species in the research area. Although there is some regional variation, ‘*A. coeleste* has a rather uniform colour pattern all over its distribution range (Seegers 1997; Wildekamp 1993). ‘*Aphyosemion*’ *ocellatum* Huber & Radda, 1979 is only known from a small area at the border of Gabon and is currently the only additional species of this group occurring in the Congolese part of the Massif du Chaillu.

Recently, several papers on the ichthyofauna of the Massif du Chaillu were published, including the descriptions of four new nothobranchiid species and a report of collections from the upper Louessé basin (Mamonekene & Stiassny 2012; Valdesalici & Eberl 2013, 2014, 2015, 2016). In 2012 and 2013 four of the authors (GW, MJ, MA, and VBM), together with V. Mamonekene, surveyed the freshwater fish fauna of the upper Louessé River at the highest part of the Massif du Chaillu in the Republic of the Congo, including the Louessé, Mandoro, and Mpoukou rivers, which ultimately drain into the Niari River (Figs 1 & 2). From this collection, three unknown *Aphyosemion* s.l. species were noted in small tributaries in the study area, and in instances were recorded in syntopy with ‘*A. coeleste*. In the following paper, we describe these three ‘*Aphyosemion*’ species based on the combination of DNA, ecological partition, male colour pattern, and morphological data.



**FIGURE 1.** Map of the study area in the upper Louessé River basin in the Republic of the Congo and the 38 collections sites of the freshwater fauna survey.



**FIGURE 2.** Collection sites of the four collected ‘*Aphyosemion*’ species in the studied area.

## Material and methods

The description of the new species is based on the collections made by GW, MA, MJ VBM, and V. Mamonekene in the Louessé drainage in the Republic of the Congo. Preserved specimens are deposited in the collection of the American Museum of Natural History, New York (AMNH). The DNA or tissue samples (Table 1) are deposited in the collection of AMNH and Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK). Additional comparative material, mostly from the Royal Museum for Central Africa (RMCA), private collections of Rainer Sonnenberg (RSC) and Jouke van der Zee (JZC) are listed below.

**Fish collection.** Thirty-eight sites within the Louessé, Mandoro, and Mpoukou catchments of the upper Niari system were sampled (Figs 1 & 2). Sites included various habitat types such as rivers in lowland settings, and forest streams and swamp forests in mid and headwater settings. The study area was assessed in September and October 2012 and in October and November 2013 immediately after the onset of the wet season.

Fish sampling effort was standardised and dependant on the habitat type and various physical and chemical parameters. For forest streams and swamp forest systems, relatively shallow depths allowed electro-fishing, dip-netting, and ichthyotoxin sampling methods to be employed. For larger river systems scoop nets and electro-fishing were utilised off of the banks. The length of stream sampled at each site was typically around 150 m and covered all habitat units in the reach.

Fishes were collected according to the guidelines for the use of fishes in research (AFS/AIFRB/ASIH 2003) and were lodged at the AMNH in New York, USA. Data are accessible online at the AMNH vertebrate zoology database at <http://sci-web-001.amnh.org/db/emuwebamnh/>.

**Morphometrics and meristics.** Morphometric measurements were taken with digital callipers using a dissecting microscope, and rounded to the nearest 0.1 mm. Counts and methods follow Amiet (1987) except for Standard Length (SL), which was measured from the anterior tip of the snout and not from the tip of the lower jaw. Measurements are presented as percentages of SL. Count of scales on the mid-longitudinal series refer to 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. All visible fin rays were counted, relative position of the first dorsal fin ray to anal fin (D/A) is 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 supra-orbital (frontal) squamation follows Hoedeman (1958).

The supra-orbital cephalic lateral line system houses neuromasts in two longitudinal grooves. The grooves are partially surrounded by enlarged scales that were alphabetically coded by Hoedeman (1958) from A to H. In *Aphyosemion* s.l. the G-scale is on top of all other enlarged scales. Width of the supra-orbital groove and shape of the G-scale were measured for two to six specimens of 40 *Aphyosemion* s.l. species. Distances were measured on digital photographs of the dorsal part of the head by using MB Ruler 5.3 software to measure objects on the computer screen (Bader 2016).

**Environmental data.** Data for a total of twenty-two environmental attributes were collected at each site. Physico-chemical analyses undertaken included pH, temperature (°C), electrical conductivity (µS/cm), dissolved oxygen (%), turbidity (NTU), Nitrate (mg/l) and Nitrate as N (mg/l). Channel width, average velocity and discharge were measured in the field to one decimal place with an electronic flow meter. The presence of organic material (detritus, leaf litter or allochthonous material) and the percentage of canopy cover were also recorded as percentage estimates. The fish habitat assessment (Kleynhans 2007) was used to assess fish habitat diversity of various velocity-depth classes (slow-deep, slow-shallow, fast-deep, fast-shallow), and the occurrence of cover types (water column, over-hanging vegetation, substrate, undercut banks, root-wads, aquatic macrophytes) at each velocity-depth class in the representative stream reach.

**Statistical analyses of environmental data.** For the purpose of visually illustrating the relationship between the collected '*Aphyosemion*' species and environmental data for habitat associations, respective data were subjected to Detrended Canonical Correspondence Analysis (DCCA) to determine whether data was linear or unimodal. A linear Redundancy Analysis (RDA) of species-environment data was undertaken using Canoco version 4.5 based on gradient length of the DCCA (Ter Braak & Smilauer 2002). Redundancy analysis was carried out on log transformed presence/absence species and environmental data. Forward selection with partial Monte Carlo permutation testing was used to assess the significance of each environmental predictor variable for extending the subset of explanatory variables used in the ordination model. The significance of axes was tested using unrestricted Monte Carlo permutation testing (499 permutations,  $p \leq 0.05$ ).

**TABLE 1.** List of specimens used for DNA sequencing with collection localities, voucher sample number, and GenBank accession numbers. AS = aquarium specimen of commercial import, the exact origin in nature is not known (not all are from a commercial import, but received without collection code), M = voucher specimens are deposited in the AMNH, New York, USA, RS = voucher specimens and/or DNA samples are deposited in the ZFMK, Bonn, Germany..

Species	Sample no. or field label	Collection locality	Country	GenBank acc.no.
<i>'A. citrineipinnis</i>	RS 1449	BBW 00/5	Gabon	MF155815
<i>'A. coeleste</i>	M 276	M 27S	Republic of the Congo	MF155818
	M 301	OS 02A	Republic of the Congo	MF155819
	M 303	L 15S	Republic of the Congo	MF155820
	M 307	OS 43	Republic of the Congo	MF155821
	M 310	OS 55	Republic of the Congo	MF155822
	M 319	OS 36	Republic of the Congo	MF155823
	RS 1353	BB 04/6	Gabon	MF155816
	RS 1721	BB 04/7	Gabon	MF155817
<i>'A. cryptum</i>	M 304	M 21S	Republic of the Congo	MF155809
	M 306	OS 43	Republic of the Congo	MF155810
<i>'A. cf. cryptum</i>	M 260	L 8S	Republic of the Congo	MF155808
<i>'A. hanneloreae</i>	RS 1688	GEB 94/19	Gabon	MF155824
<i>'A. mandoroense</i>	M 268	M 27S	Republic of the Congo	MF155811
	M 274	M 27S	Republic of the Congo	MF155812
	M 298	OS 02	Republic of the Congo	MF155813
	M 311	OS 09	Republic of the Congo	MF155814
<i>'A. ocellatum</i>	RS 283	BBW 00/11	Gabon	MF155826
	RS 1727	BBW 00/17	Gabon	MF155825
<i>'A. passeroi</i>	RS 287	BBW 00/16	Gabon	MF155828
	RS 289	BBW 00/16	Gabon	MF155829
	RS 1722	BBW 00/14	Gabon	MF155827
<i>'A. wuendschi</i>	RS 1689	BB 04/16	Gabon	MF155830
<i>M. cameronense</i>	RS 262	CMM 40	Cameroon	AY748282
<i>'A. cyanoflavum</i>	M 275	M 27S	Republic of the Congo	MF155839
<i>'A. louessense</i>	RS 1152	AS	Republic of the Congo	MF155834
<i>'A. ogoense</i>	RS 1255	BSW 99/4	Gabon	MF155836
	RS 1699	BB 04/4	Gabon	MF155837
	RS 1700	BB 04/4	Gabon	MF155838
<i>'A. ottogartneri</i>	RS 1157	AS	Republic of the Congo	MF155833
<i>'A. pyrophore</i>	RS 1160	AS	Republic of the Congo	MF155835
<i>'A. thysi</i>	RS 221	AS	Republic of the Congo	MF155832
<i>I. striatum</i>	RS 1714	G 02/84	Gabon	MF155831

**DNA methods.** Table 1 lists the specimens for the DNA study with GenBank accession and DNA sample or voucher numbers. Total DNA was extracted from the ethanol preserved specimens using fin clips or muscle tissue from the caudal peduncle. Voucher numbers starting with RS were extracted following a modified CTAB/chloroform-isoamylalcohol extraction after Gustinicich *et al.* (1991). The partial mitochondrial cytochrome *b* (*cytb*) gene was amplified and sequenced for 23 specimens of the *'A. coeleste* species group and *Mesoaphyosemion cameronense* (Boulenger, 1903) as an outgroup species, and eight specimens of the *'A. ogoense* species group and *Iconisemion striatum* (Boulenger, 1911) as outgroup. We have chosen the cytochrome *b* fragment because of an existing dataset of one of the authors and the observed amount of variation within species

groups. Additional data from GenBank were not included as the majority of relevant species have only shorter cytochrome *b* sequences in the database and the resolution between species groups is not sufficient for a resolved phylogeny (see. e.g. Collier 2007, Murphy & Collier 1999). Details for methods and primers were given in Sonnenberg & Blum (2005) and Sonnenberg *et al.* (2007). All specimens recently collected in the Republic of the Congo were prepared and sequenced by Inqaba Biotechnical Industries, Hatfield, Pretoria, South Africa with the same PCR and sequencing primers.

Resulting sequences were aligned and checked in BioEdit 7.0.5.3 (Hall 1999). The protein coding sequences were translated into the corresponding amino acids to check for the presence of pseudogenes. The nucleotide composition of the mitochondrial DNA sequences was tested for an anti-G bias characteristic for this organelle DNA (Zhang & Hewitt 1996). Uncorrected p-distances with pairwise deletion of missing data were calculated in MEGA 5.05 (Tamura *et al.* 2011) and presented in Table 2 for the '*A.* *ogoense*' group and in Table 3 for the '*A.* *coeleste*' group.

**TABLE 2.** Uncorrected p-distances for the '*A.* *ogoense*' group. CYF = '*A.* *cyanoflavum*'; LOU = '*A.* *louessense*'; OGO = '*A.* *ogoense*'; OTT = '*A.* *ottogartneri*'; PYR = '*A.* *pyrophore*'; STR = '*I.* *striatum*'.

	STR_1714	OGO_1255	OGO_1699	OGO_1700	CYF_M275	PYR_1160	LOU_1152	OTT_1157
OGO_1255	18.00							
OGO_1699	17.73	6.85						
OGO_1700	17.73	6.85	0.00					
CYF_M275	18.67	9.49	10.28	10.28				
PYR_1160	17.87	12.25	13.04	13.04	11.07			
LOU_1152	17.20	12.25	13.57	13.57	12.91	11.59		
OTT_1157	18.53	12.78	13.18	13.18	14.23	12.52	7.64	
THY_221	17.20	12.52	12.52	12.52	12.38	10.67	6.72	8.96

Data were analyzed for each species group separately with a Bayesian approach. Bayesian analyses (BA) were performed with Mr Bayes 3.1.2 (Huelsenbeck & Ronquist 2001) with one run for each parameter models Nst2 and Nst6. For both we used the settings rate=gamma and ngammacat=4, number of generations was set to 10,000,000 with sampling of every 1,000 generations. The results of the first 1,000,000 generations were discarded as burn-in time after checking that the runs had reached the stationary phase at this point.

Only nodes with  $\geq 0.95$  posterior probabilities in the BA analyses were considered as supported by the data.

**Taxonomic concept.** According to the proposal published by two of the authors on the usage of generic names in nothobranchiids related to the genus *Aphyosemion* (Sonnenberg *et al.* 2007; Van der Zee & Sonnenberg 2010), we suggest using *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 recommend using either published generic level names or, in the case of unnamed species groups, '*Aphyosemion*' within single quotes. The new nothobranchiid species are here described within *Aphyosemion* to follow ICZN Article 5.3, but will further appear as '*Aphyosemion*' in this paper, because the phylogenetic placement of the respective species groups within this complex is currently not clear and the taxonomy not fully revised. Recently Huber (2013c) described the two new subgenera *Scheelsemion* Huber, 2013c and *Iconisemion* Huber, 2013c and reorganized *Mesoaphyosemion* Radda, 1977 for a clade consisting of *Mesoaphyosemion* s.s., the '*A.* *coeleste*', and '*A.* *wildekampi*' Berkenkamp, 1973 species groups, which are related to *Raddaella* Huber, 1977 and *Aphyosemion* sensu stricto (Collier 2007; Murphy & Collier 1999). The '*A.* *ogoense*' group was placed within *Iconisemion* (Huber 2013c). We do not follow these steps as no clear diagnostic characters uniting these different species groups into the proposed subgenera were presented, and the currently available genetic (Collier 2007; Murphy & Collier 1999) and morphological analyses (Huber 2013c) are not congruent with, or at least do not support, a monophyletic *Mesoaphyosemion*, *Iconisemion*, nor *Scheelsemion* sensu Huber (2013c).

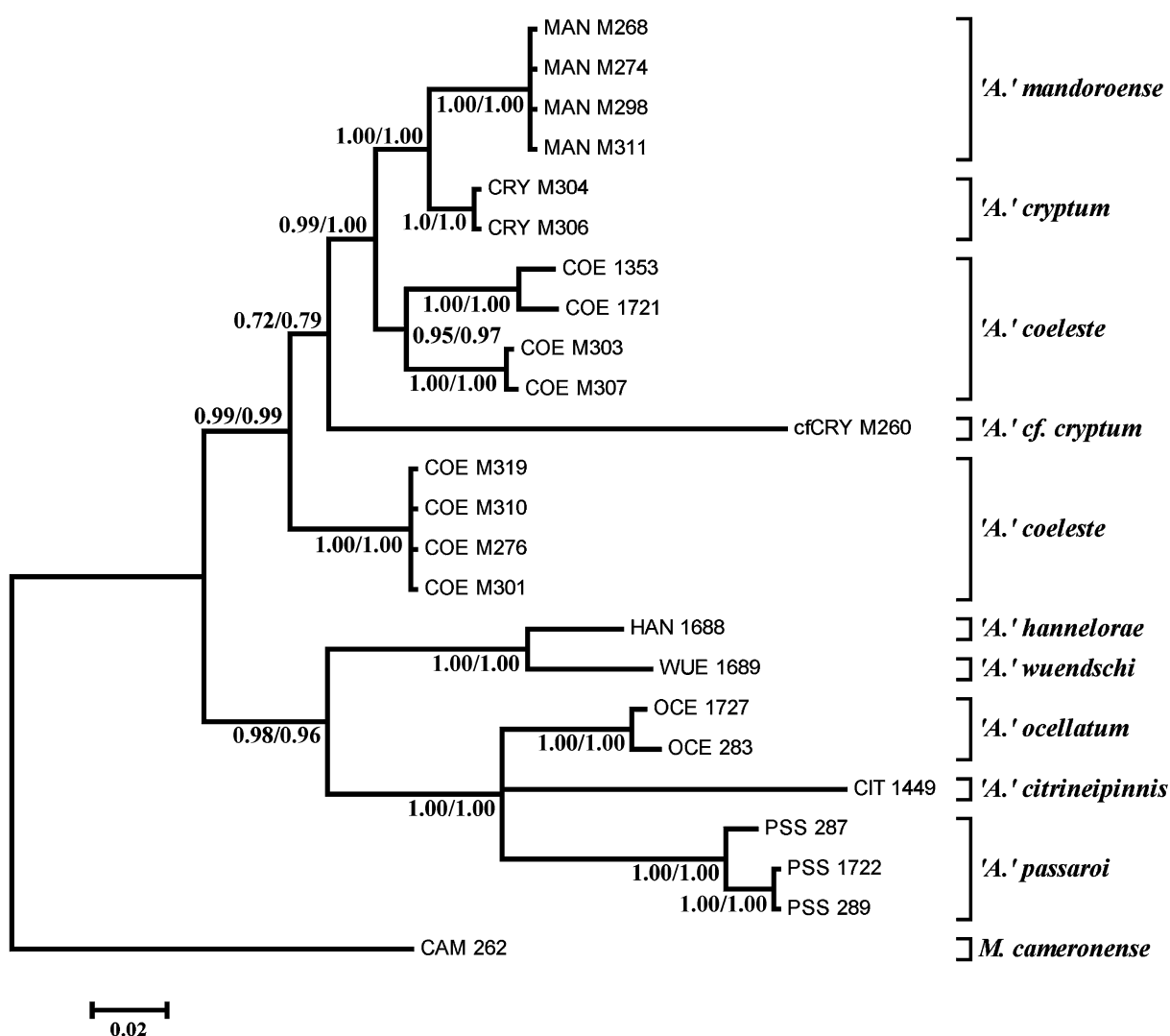
Therefore, we restrict the recently described subgenera to the species group including their type species and treat them as genera. We adopted the pragmatic approach by Moritz *et al.* (2000) as a species concept.







including as one monophyletic clade '*A. hanneloreae*' and '*A. wuendschi*' Radda & Pürzl, 1985 and as second clade '*A. ocellatum*', '*A. citrineipinnis*', and '*A. passaroi*'.

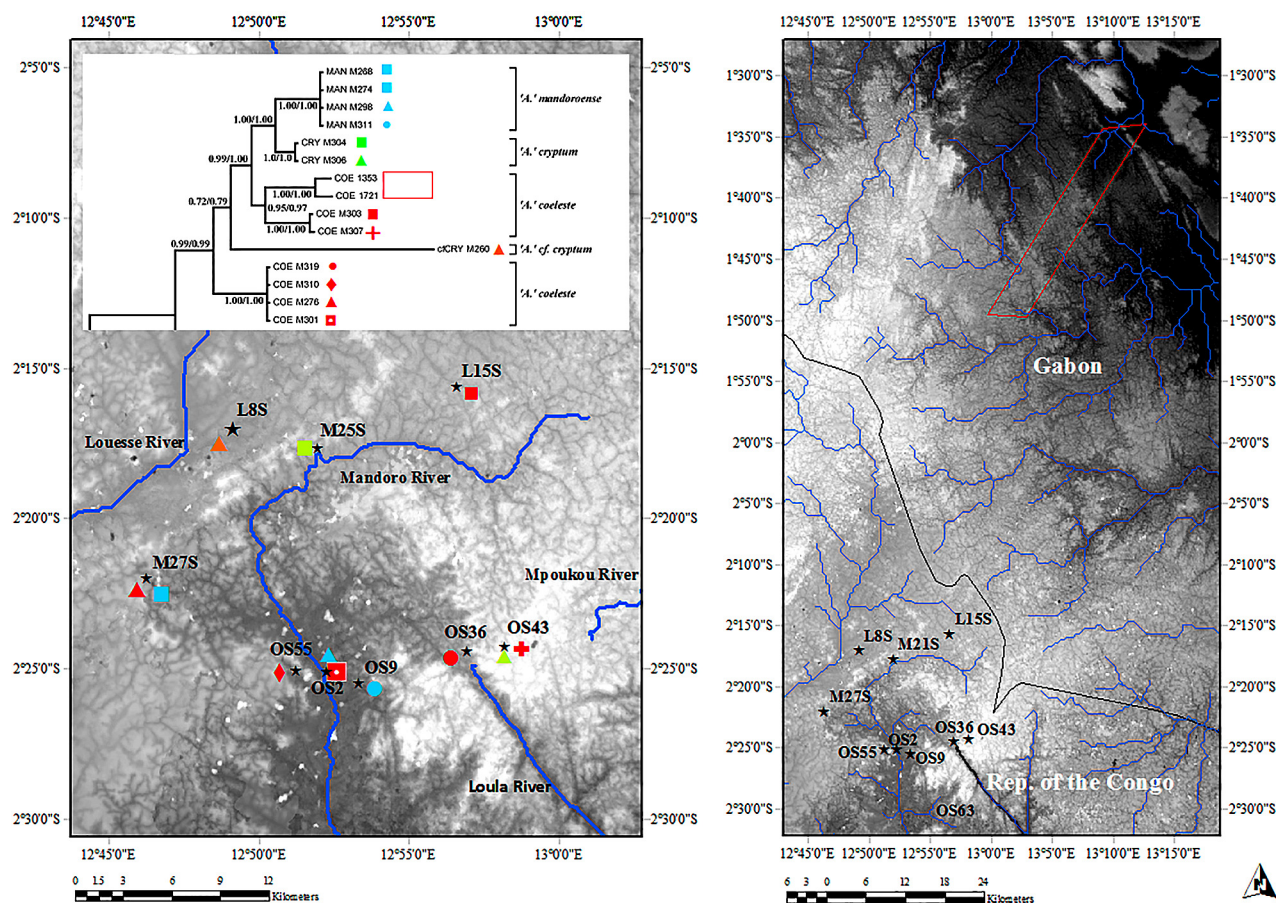


**FIGURE 4.** Phylogenetic results of the cytochrome *b* data for the '*A. coeleste*' group. Bayesian analyses (BA): numbers at nodes represent posterior probability values for both analyses (NST2/NST6). All trees are rooted with *M. cameronense* (CAM 262). Sample abbreviations are given in Table 1.

All analyses place the '*A. hanneloreae*' clade as sister clade to the '*A. ocellatum*' clade. The '*A. ocellatum*' clade contains in this dataset '*A. citrineipinnis*', '*A. ocellatum*', and '*A. passaroi*'. In a recent publication (Collier, 2007) the only species missing in this analysis of the '*A. coeleste*' group, '*A. aureum*' Radda, 1980a, is placed as sister species to '*A. ocellatum*'. The relationship between '*A. ocellatum*', '*A. citrineipinnis*', and '*A. passaroi*' is here not well resolved (Fig. 4). The topology of our results is similar to the published phylogeny of Collier (2007), however, in that paper, only the relationships between '*A. aureum*', '*A. citrineipinnis*', and '*A. ocellatum*' receive a significant bootstrap support, the relationships of the other two species of the '*A. coeleste*' group included in this analysis, '*A. coeleste*' and '*A. hanneloreae*', are uncertain due to low bootstrap results.

Within the '*A. coeleste*' clade, there are several distinct genetic lineages: one is a clade of four '*A. coeleste*' samples from the localities M27S, OS02A, OS5, and OS36 (Figs 4 & 5) that show no differences in their cytochrome *b* sequences. This is probably the sister clade to all remaining within the '*A. coeleste*' clade (Fig. 4). A distinct lineage is represented by the sequence of a specimen '*A. cf. cryptum*' (sample M260), which is by genetic distance values well separated from all other samples (Table 3), its detailed relationship to the other lineages, however, is statistically not well supported. The two new species are well supported sister taxa, together they form

a monophyletic group with the '*A. coeleste*' specimens (RS 1353 and RS 1721) from south-eastern Gabon from the Ogooué River basin and two specimens from the study area in the Republic of the Congo. Interestingly for all major genetic lineages within the '*A. coeleste*' clade, the specimens from the study area show no or very low genetic differences within a clade, but the genetic distances between the lineages are relatively large (Table 3).



**FIGURE 5.** The collection localities of the '*A. coeleste*', '*A. cryptum*', and '*A. mandoroense*' DNA samples are indicated with coloured symbols on maps of the upper Louessé (left) and upper Louessé and upper Ogooué Rivers (right), and within the part of the resulting tree of the BA including these samples. For the two Gabon samples (COE 1353 and COE 1721) no exact locality data are known, the red surrounded area indicates their collection area.

### *Aphyosemion cyanoflavum*, new species

(Figs 6–9; Table 4)

**Holotype.** AMNH 263406, male, 27.8 mm SL, Republic of the Congo, 1.5 km south of the village of Lisoukou (10 km south of Mayoko) on the road R1 to Mossendjo, Leyou River, tributary of the Mandoro River, tributary of the Louessé River, Niari River drainage (2°21'57" S, 12°46'16" E), field code M27, field label M275, collected by G. Walsh, M. Alexandre & V. Boukaka Mikembi, 22 October 2013.

**Paratypes.** AMNH 262589, 2 males, 25.5–28.0 mm, 1 juvenile male, 20.7 mm SL, not measured, Republic of the Congo, collected with the holotype.

**Diagnosis.** '*Aphyosemion*' *cyanoflavum* (Figs 6–9, Table 4) is a member of the '*A. ogoense*' group based on DNA analysis. It can be distinguished from all other *Aphyosemion* s.l. species by its cephalic lateral line system, having unique large, dark coloured anterior supra-orbital neuromasts in very wide supra-orbital grooves (18.3–19.4 % of inter-orbital width versus 7.3–16.9 % in other species, Fig. 8, Table 5), very large pre- and postorbital canals with large pores (Fig. 7) and frontal neuromasts housed in one pit with one anterior lobe; by its wide upper jaw (due to enlarged lacrimal bones), wider than inter-orbital distance versus narrower in all other species, making the dorsal and lateral view of the head much more reminiscent of an *Epiplatys* Gill, 1862 than an *Aphyosemion* s.l. (Figs 7–8).

Males are distinguished from all other *Aphyosemion* s.l. species by their unique lateral colour pattern: anterior part of body on side yellow and posterior side of body purple-grey. The transition between yellow and purple-grey is very sharp and situated just behind the pelvic fin (Fig. 9).

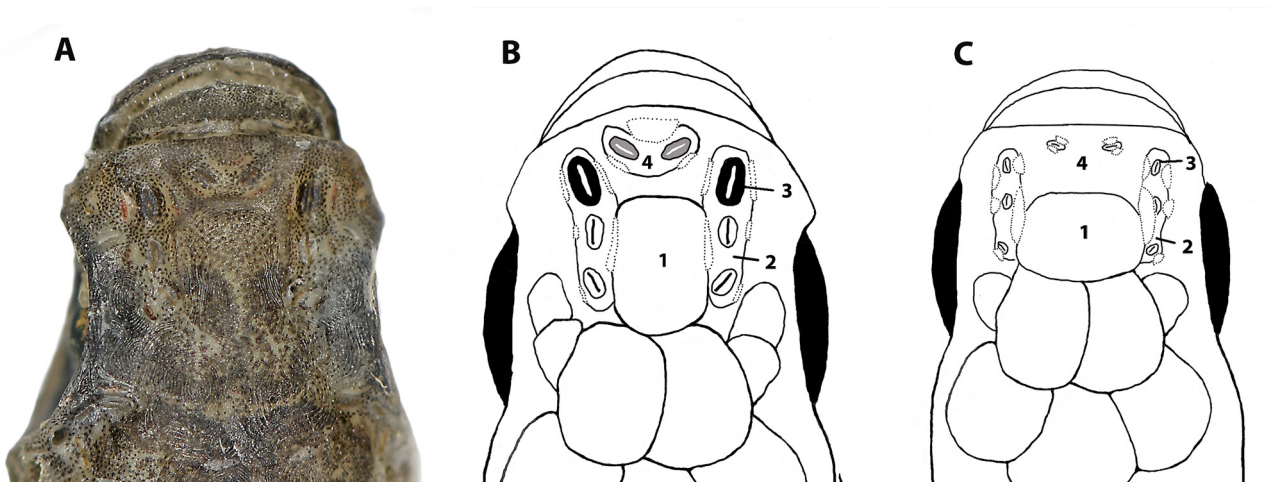
Males are distinguished from all other members of the '*A. ogoense* group (except for '*A. caudofasciatum* Huber & Radda, 1979, '*A. pyrophore*, and '*A. thysi*) by the presence of narrow dark red bars in the posterior part of the flanks and on the caudal peduncle. Males are distinguished from the latter three species by the above mentioned wide supra-orbital grooves and the unique colour pattern of anterior yellow and posterior purple grey on the flanks.



**FIGURE 6.** '*Aphyosemion*' *cyanoflavum*, male, 27.8 mm SL, holotype, AMNH 263406.



**FIGURE 7.** Head of '*Aphyosemion*' *cyanoflavum*, male, 25.5 mm SL, paratype, AMNH 262589, lateral view.



**FIGURE 8.** Dorsal view of frontal and supra-orbital cephalic lateral line system. '*Aphyosemion*' *cyanoflavum*, male, 28.0 mm SL, paratype, AMNH 262589 (A & B), and generalized '*Aphyosemion*' (C). Dotted lines: dermal tissue lobes; 1: G-scale narrow versus wide; 2: wide supra-orbital groove with large neuromasts versus narrow groove with small neuromasts; 3: large black anterior neuromast versus small light neuromast; 4: two large and darker frontal neuromasts in one pit, anteriorly bordered with one dermal tissue lobe versus two small light neuromasts, each in a separate pit with two dermal tissue lobes,



**FIGURE 9.** '*Aphyosemion*' *cyanoflavum*, male, 39.5 mm SL, holotype, AMNH 263407.

**Description.** See Figs 6 & 9 for general appearance and colour pattern, Table 4 for morphometric data of types. Medium sized *Aphyosemion* s.l. species, largest observed specimen in type series 35.5 mm total length.

Dorsal and ventral profile slightly convex, greatest body depth approximately at base of pelvic fin, ventral profile slightly convex, caudal peduncle dorsally and ventrally concave. Mouth directed upwards, lower jaw longer than upper jaw, upper jaw wider than inter-orbital distance.

Frontal neuromasts in one groove with one central anterior lobe, pre-opercular, pre- and postorbital cephalic lateral line system with prominent tubular canals with large pores (Fig.7). Two supra-orbital grooves with three neuromasts each. Grooves wider and deeper than in other *Aphyosemion* s.l. species (Table 5). Anterior part of supra-orbital groove strongly widened with many melanophores (Figs 7 & 8). Anterior-most supra-orbital neuromasts large and dark grey to black due to dense concentration of melanophores (Fig. 7). Dermal lobes bordering the grooves very small, shallow and narrow.

Teeth on jaws unicuspid, outer row large and inward curved, inner teeth smaller and more irregularly placed. Scales cycloid, body completely scaled except ventral head surface. Frontal squamation variable, one specimen of

G-type, one specimen of E-type (third specimen lost its G- and E-scales). G-scale narrow and elongated due to wide supra-orbital grooves. Two F-scales present (Fig. 8). No scales on dorsal and anal fin base. Caudal fin base with two to three scale rows. Scales in mid longitudinal series 29–30, transverse series of scales before dorsal fin nine, circumpeduncular scale row 14. All unpaired fins pointed in males. Number of dorsal fin rays 11–12, anal fin rays 14–15; first dorsal fin ray above anal fin ray seven to eight. Pectoral fin not reaching pelvic fin, pelvic fin just reaching anal fin.

**Live colouration.** Males (Fig. 9). Snout dark grey to black. Eye iris light with some metallic green areas on outer margin. Three red streaks on opercle in approximate 45° angle, top red streak behind eye can be reduced to red spot, lower red streak short or reduced to red spot at the posterior tip of the operculum. Anterior part of body yellow with green iridescence, posterior part purple grey, metallic ice blue iridescence, anteriorly in patches, otherwise concentrated at scale border. Most scales on upper half of anterior side of body with small red rounded spot forming up to four irregular horizontal lines, lower being very short. Lower half of anterior body without or with sparse red spots. Majority of scales on posterior part of body with variable larger dark red spots, forming irregular bars. Body dorsally brownish, ventrally from chin to halfway between pelvic and anal fin bright yellow.

Unpaired fins light blue with metallic sheen and dark black or red margin (narrow in anal fin and broad in caudal and dorsal fin), dark red dots and/or short streaks between fin rays. On caudal fin two larger dark red dots at centre of fin base and one large red dot at the ventral caudal fin base. Pelvic fin light metallic blue, sometimes with few red spots. Pectoral fin hyaline with blue metallic margin.

**TABLE 4.** Morphometric and meristic data of '*A. cyanoflavum*, new species. Abbreviations: TL = total length, SL = standard length, HL = head length, BD = body depth, E = eye diameter, IO = inter-orbital distance, pD = pre-dorsal length, pA = pre-anal length, CL = caudal peduncle length, CD = caudal peduncle depth, CL/D = caudal peduncle length/depth ratio, DB = dorsal fin base, AB = anal fin base, D = number of dorsal fin rays, A = number of anal fin rays, D/A = position of first dorsal fin ray relative to the opposite anal fin ray, C = number of caudal fin rays, P = number of pectoral-fin rays, LLS = number of scales on the lateral line, TS = number of transversal scales on one flank, CS = number of scales around the caudal peduncle. SL is given in mm, other measurements as percentages of SL, sd = standard deviation.

	Holotype Male	Paratype range (2 males)	Mean all types (sd)
TL	127.7	126.2 – 127.9	127.3 (0.9)
SL	27.8	25.5 – 28.0	27.1 (1.4)
HL	28.4	27.5 – 27.9	27.9 (0.5)
BD	20.9	19.6 – 20.4	20.3 (0.7)
E	9.4	8.6 – 9.0	9.0 (0.4)
IO	12.2	11.4 – 11.8	11/8 (0.4)
pD	68.0	64.7 – 67.5	66.7 (1.8)
pA	62.2	61.4 – 62.4	62.0 (0.5)
CL	26.3	24.7 – 26.1	25.7 (0.9)
CD	12.2	11.4 – 12.9	12.2 (0.8)
CL/D	2.2	2.0 – 2.2	2.1 (0.1)
DB	15.8	14.5 – 16.4	15.6 (1.0)
AB	18.7	18.4 – 18.9	18.7 (0.3)
D	12	11	11.3 (0.6)
A	15	14 – 15	14.7 (0.6)
D/A	8	7 – 8	27.7 (0.6)
C	26	26	26 (0)
P	14	14 – 15	14.7 (0.6)
LLS	29	29 – 30	29.7 (0.6)
TS	9	9	9 (0)
CS	14	14	14 (0)

**After 3 months in 70 % ethanol.** (Fig. 6). Lower lip black to dark grey. Operculum and head below eyes light grey, red streaks on operculum as in live specimens. Flanks grey with sharp transition to unpigmented whitish ventral side anterior of pelvic fin. Large dark grey to black blotch on anterior flank. Melanophores of posterior flank scales concentrated on posterior margin of scale. Dorsal third of body with irregular lines of red spots. Dorsal, anal, and caudal fins light grey, with irregular red dots and streaks between fin rays, and with narrow dark margins as in live specimens. Pelvic and pectoral fins light grey at base and dark grey at margin.

**Distribution and habitat.** ‘*Aphyosemion*’ *cyanoflavum* is currently only known from the type locality in the southern Massif du Chaillu. The Leyou River is a small stream flowing into the upper Mandoro River, a tributary of the Louessé River in the south-western part of the Republic of the Congo. It is found syntopic with ‘*A. coeleste*’ and ‘*A. mandoroense*’, new species, where they occur in more or less similar microhabitats. ‘*Aphyosemion*’ *cyanoflavum* seems to be rare in the study area. Although the area was thoroughly sampled by four of the authors (GW, MA, MJ & VBM) during two field seasons and many *Aphyosemion* s.l. specimens were collected; it was only present at the type locality in the upper Mandoro River system. It cannot be excluded that it occurs in rivers east of the Mandoro, since no fish collections were made there.

**Etymology.** The name *cyanoflavum* refers to the bicoloured flanks in live males, the anterior part of the body being yellow (Latin: *flavum*, meaning yellow an adjective) and the posterior part predominantly blue (*ciano* for the Greek *kýanos*, meaning blue, an adjective).

**TABLE 5.** Relative width of supra-orbital groove of cephalic sensory system (width groove in percentage of inter-orbital distance) and relative width of G-scale (width of G-scale in percentage of length of G-scale).

Taxon	Relative width groove	Relative width G scale
‘ <i>A. cyanoflavum</i> ’	18.3–19.4	59.5–68.4
‘ <i>A. ogoense</i> ’ group*	11.3–14.1	87.2–104.8
‘ <i>A. mandoroense</i> ’	13.9–15.0	67.6–75.5
‘ <i>A. ocellatum</i> ’	15.9–16.9	57.5–76.2
‘ <i>A. coeleste</i> ’ group**	10.1–13.1	85.8–118.3
‘ <i>A. punctatum</i> ’ group	9.9–11.4	130.3–135.3
‘ <i>A. herzogi</i> ’ group	10.5–11.6	109.1–123.4
<i>Aphyosemion</i>	10.3–10.6	111.7–117.0
<i>Chromaphyosemion</i>	8.5–12.1	116.5–139.9
<i>Diapteron</i>	7.3–9.4	114.5–123.7
<i>Episemion</i>	14.9–15.5	51.5–54.9
<i>Iconisemion</i>	9.9–11.1	106.1–116.9
<i>Kathetys</i>	9.7–13.4	95.1–110.1
<i>Mesoaphyosemion</i>	10.3–11.0	91.6–93.3
<i>Raddaella</i>	10.0–12.2	89.7–113.5
<i>Scheelsemion</i>	10.1–10.8	114.3–134.4
‘ <i>A. hera</i> ’	9.2–9.3	118.0–148.6
‘ <i>A. hofmanni</i> ’	14.2–16.9	104.9–117.2
‘ <i>A. tirbaki</i> ’	12.6	106.9
‘ <i>A. wachtersi</i> ’	11.8–11.9	77.6–95.2

\* except ‘*A. cyanoflavum*’

\*\* except ‘*A. mandoroense*’ and ‘*A. ocellatum*’

### ***Aphyosemion mandoroense*, new species**

(Figs 10–11; Table 6)

**Holotype.** AMNH 263405, male, 34.0 mm SL, Republic of the Congo, Mandoro River, swamp forest flood

benches, tributary of the Louessé River, Niari River drainage (2°25'27" S, 12°53'19" E), collection locality OS9, field label M311, collected by G. Walsh, M. Alexandre & V. Boukaka Mikembi, 11 November 2013.

**Paratypes.** AMNH 262592, 1 male, 37.1 mm SL, collected with the holotype; AMNH 262591, 2 males, 24.3–26.8 mm SL, Republic of the Congo, Mandoro River, recently flooded swamp forest flood bench, field code OS2, field label M298, G. Walsh, M. Alexandre & V. Boukaka Mikembi, 26 November 2013; AMNH 262590, 3 males 24.3–26.4 mm SL and 1 female, 37.5 mm SL, Republic of the Congo, about 1.5 km south of the village of Lisoukou (10 km south of Mayoko) on the road R1 to Mossendjo, Leyou River, tributary of the Mandoro River, tributary of the Louessé River, Niari River drainage, field code M27, field labels M268 & M274, G. Walsh, M. Alexandre & V. Boukaka Mikembi, 22 October 2013; AMNH 262593, 2 males, not measured, Republic of the Congo, tributary of Mandoro River, field code M21S, G. Walsh, M. Alexandre & V. Boukaka Mikembi, 18 October 2013.

**Diagnosis.** (Figs 10–11, Table 6) Males of '*A. mandoroense*' are distinguished from all other *Aphyosemion* s.l. species by the lack of red pigmentation on its fins, except for the posterior margin of the dorsal fin in some specimens. DNA data (Fig. 4) and male colour pattern place it into the '*A. coeleste*' group.



**FIGURE 10.** '*Aphyosemion*' *mandoroense*, male, 34.0 mm SL, holotype, AMNH 263405.

*Aphyosemion* *mandoroense* can be distinguished by its diagnostic male colouration pattern from all other species of the '*A. coeleste*' group by the nearly complete absence of red in unpaired fins, versus red margins or sub-marginal stripes in all other species except '*A. ocellatum*', which shows faint red areas in the unpaired fin centre of some individuals. The new species is distinguished from all other species except '*A. ocellatum*' and '*A. passaroi*' by its dark grey to black margins of unpaired fins. It is distinguished from '*A. passaroi*' by the nearly complete absence of red in fins, blue side and opercle versus basal broad red stripe in anal fin and orange to brownish or pinkish side and opercle.

*Aphyosemion* *mandoroense* is distinguished from the '*A. ocellatum*' populations with blue sides by the three or four rows of red dots on anterior side, the light blue opercle versus a dark red blotch posterior to operculum, and yellow or orange operculum, and from those with a more brownish to orange body colour by the blue side colour, absence of dark red blotch posterior to operculum and blue operculum. '*Aphyosemion*' *ocellatum* in addition has darker and broader fin margins on pelvic, anal, dorsal, and upper and lower caudal fin.

The superficially most similar '*A. citrineipinnis*' and '*A. ocellatum*' also have a reduced red pigmentation on fins, but usually at least traces of a red stripe at the base of the anal fin is present. Base of anal fin in '*A. citrineipinnis*' blue, central to distal part yellow versus completely yellow in '*A. mandoroense*'. Unpaired fin margins in '*A. mandoroense*' dark grey to black versus no or very narrow dark fin margin in '*A. citrineipinnis*'. Many '*A. citrineipinnis*' populations usually show a prominent "wound mark", a group of dark red scales just



behind the operculum, that is always absent in '*A. mandoroense*', which shows three or four rows of red dots on the anterior side above the pelvic fin. The operculum is light blue in '*A. mandoroense*' versus yellow to orange in '*A. citrineipinnis*'. In '*A. mandoroense*', the yellow colour of the caudal fin is extended in the dorsal and ventral edge of the caudal peduncle, versus no yellow on caudal peduncle in '*A. citrineipinnis*'. In '*A. mandoroense*', the dorsal fin inserts more posteriorly than in '*A. citrineipinnis*' (D/A = 1/10–11 versus 1/7–8).

**Description.** See Figures 10–11 for general appearance and colour pattern and Table 6 for morphometric data of type series. Largest observed specimen in type series 37.1 mm standard length. Strong sexual dimorphism, adult males more colourful than females and have larger unpaired fins.



**FIGURE 11.** '*Aphyosemion*' *mandoroense*, female, 37.5 mm SL, paratype, AMNH 262590.

Dorsal profile straight or slightly convex, greatest body depth at base of pelvic fin, ventral profile slightly convex. Caudal peduncle dorsally and ventrally slightly concave. Snout rounded, mouth directed upwards, lower jaw longer than upper jaw, posterior end of rictus at level of dorsal third of eye.

Frontal neuromasts in two separate grooves, pre-opercular, pre- and post-orbital system with tubular canals. Two rather wide supra-orbital grooves (13.9–15.0 % of inter-orbital width, Table 6) with three neuromasts each.

Teeth on jaws unicuspid, outer row large and inward curved, inner teeth smaller and more irregularly placed. Scales cycloid, body completely scaled except ventral head surface. Frontal squamation of G-type. No scales on dorsal and anal fin base, two to three scale rows on caudal fin base, two scale rows beneath the eye; scales on mid longitudinal series 31–32; transverse series of scales before dorsal fin nine; circumpeduncular scale row 14. All unpaired fins rounded in males, no elongated rays at the caudal fin edges; caudal fin posterior margin rounded. Number of dorsal fin rays 11–12, anal fin rays 16–17; first dorsal fin ray above anal fin ray 10–11. Pectoral fin just reaching pelvic fin, pelvic fin not reaching anal fin.

**Live colouration.** Males (Fig. 10). Side of body and operculum light blue. Lips and throat bright lemon to yellow. Dorsal side yellow brown, ventrally yellow. Majority of scales on anterior, approximately dorsal half of side with small red spot forming up to four rather regular horizontal lines, posterior scales with faint reddish margin forming weak reticulated pattern. Three red streaks on operculum typical for *Aphyosemion* s.l. (Van der Zee 2002) reduced or absent. All fins bright yellow. Dorsal fin with narrow grey margin, becoming red on tip and posterior four fin rays. Caudal fin with broad grey posterior margin, anal fin with narrow dark grey margin. Iris greyish with some metallic blue areas, central part surrounding black pupil has narrow yellowish to whitish ring.

Females (Fig. 11). Body light brown to grey-brownish, dorsally darker and ventrally light brown, with golden hue dorsally. Pre-operculum light, sometimes almost whitish, operculum with many melanophores. Lips and sometimes chin yellow. Scales on sides, especially along the anterior lateral line, with a dark border, forming a reticulated pattern. Most scales on dorsal half of sides with a very small red dot at the transition of two scales.

Dorsal fin hyaline with numerous red dots, in some female's dorsal fin with narrow dark edge, last four fin rays with red edge. Anal fin hyaline, unspotted, sometimes with red hue. Caudal hyaline, unspotted, membrane between the first three to four dorsal and ventral fin rays red. Pelvic and pectoral fins hyaline, unspotted.

**TABLE 6.** Morphometric and meristic data of '*A. mandoroense*', new species. SL is given in mm, other measurements as percentages of SL. Abbreviations see Table 4.

	Holotype (male)	Paratypes Range (8 males)	Mean (sd) All males	Paratype (1 female)
TL	119.7	118.3 – 132.9	127.1 (4.8)	124.0
SL	34.0	24.3 – 37.1	28.2 (4.4)	37.5
HL	26.5	21.0 – 28.0	26.0 (2.2)	20.8
BD	18.5	19.1 – 27.2	20.9 (2.4)	23.2
E	7.9	7.3 – 9.5	8.8 (0.7)	7.2
IO	11.5	11.2 – 13.6	12.2 (0.7)	12.5
pD	70.3	63.4 – 72.0	67.7 (2.6)	68.3
pA	56.2	57.1 – 62.5	58.4 (2.1)	61.3
CL	20.3	19.1 – 25.8	22.4 (2.6)	23.5
CD	12.1	11.1 – 12.3	11.7 (0.4)	12.0
CL/D	1.7	1.7 – 2.1	1.9 (0.2)	2.0
DB	12.9	12.1 – 18.1	14.4 (1.8)	12.3
AB	20.3	20.2 – 23.4	21.9 (1.4)	19.7
D	11	11 – 12	11.2 (0.4)	12
A	16	16 – 17	16.8 (0.4)	17
D/A	11	10 – 11	10.6 (0.5)	11
C	28	28 – 29	28.2 (0.4)	28
P	18	17 – 18	17.3 (0.5)	18
LLS	32	31 – 32	31.3 (0.3)	31
TS	9	9	9 (0)	9
CS	14	14	14 (0)	14

**After 3 months in 70 % ethanol.** Males. Side and dorsal grey with sharp transition to unpigmented light ventral anterior to the anal fin. Head dorsally dark grey with a sharp transition to whitish ventrally on head and throat. Lips grey. All fins except caudal fin hyaline, unspotted. Caudal fin hyaline with broad dark grey posterior edge and dark blotch at base; inter-radial membrane of centre four to five fin rays with dark spots.

Females. Side grey with gradual transition to light grey ventrally. No sharp transition of dark dorsal and light ventral of head as in males. All scales with a dark margin, most pronounced along lateral line. Pectoral fins hyaline, all other fins with many very small dark spots on inter-radial membrane. Dorsal fin with red spots, anal and caudal fin unspotted. Caudal fin with red markings at dorsal and ventral insertion.

**Distribution and habitat.** '*Aphyosemion*' *mandoroense* is currently only known from the tributaries of the upper Mandoro River (Louessé basin) in south-western Congo, where it occurs sympatric and sometimes syntopic with '*A. coeleste*' or '*A. cryptum*', new species. In the Leyou River it occurs syntopic with '*A. coeleste*' and '*A. cyanoflavum*'.

**Etymology.** The species is named after the Mandoro River, a tributary of the Louessé River in the southern Massif du Chaillu in the Republic of the Congo.

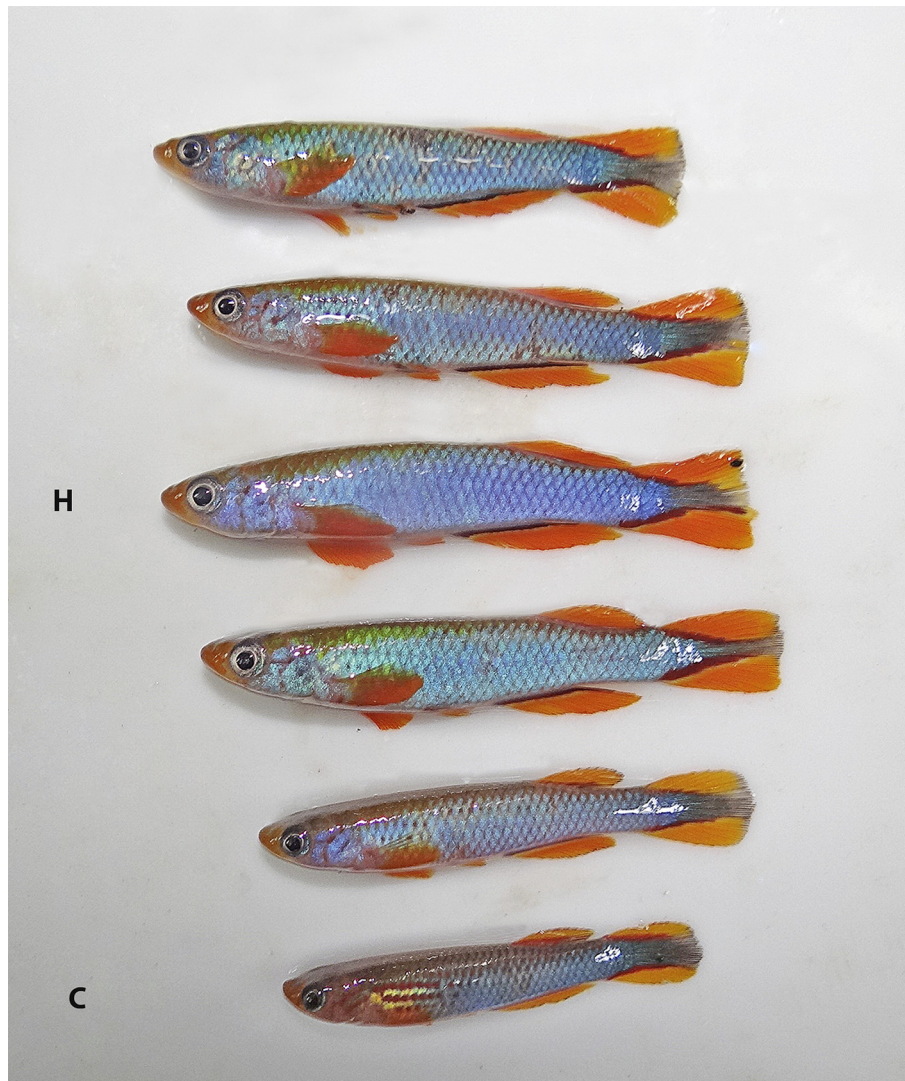
***Aphyosemion cryptum*, new species**

(Figs 12–13; Table 7)

*Aphyosemion coeleste* non Huber & Radda, 1977: Wildekamp, 1993: p. 129, fig. of specimen from Mbinda, western Congo.

**Holotype.** AMNH 263407, male, 39.5 mm SL, Republic of the Congo, tributary of the Mandoro River, tributary of the Louessé River, Niari River drainage (2°21'57" S, 12°46'16" E), field code OS43, field label M331, collected by G. Walsh, M. Alexandre & V. Boukaka Mikembi, November 2013.

**Paratypes.** AMNH 262594, 4 males (field label 306, 329, 330, 332), 34.0–41.2 mm, 1 female (field label 308) 39.4 mm SL, collected with the holotype; AMNH 262593, 1 male (field label 304), 34.6 mm SL, 1 female (field label 305), 31.1 mm SL, Republic of the Congo, tributary of Mandoro River; field code M21S, G. Walsh, V. Mamonekene, M. Jonker & V. Boukaka Mikembi, January 2012; AMNH 258825, 7 specimens, Republic of the Congo, Lipia River, tributary of Louessé River, field code L8S, G. Walsh, V. Mamonekene, M. Jonker & V. Boukaka Mikembi, January 2012; AMNH 258849, 10 specimens, Republic of the Congo, Tributary of Leala River, tributary of Louessé River, field code L15S, G. Walsh, V. Mamonekene, M. Jonker & V. Boukaka Mikembi, January 2012; AMNH 258498, 10 specimens, Republic of the Congo, tributary of Leala River, tributary of Louessé River, field code L5S, G. Walsh, V. Mamonekene, M. Jonker & V. Boukaka Mikembi, 10 January 2012; AMNH 258888, 10 specimens, Republic of the Congo, Tributary of Mandoro River, field code M21S, G. Walsh, V. Mamonekene, M. Jonker & V. Boukaka Mikembi, January 2012.



**FIGURE 12.** Males of '*Aphyosemion*' *cryptum*, holotype (AMNH 263407) indicated by the letter H, syntopic paratypes (AMNH 262594), and syntopic '*A. coeleste*' (indicated by the letter C, AMNH 262596).



**FIGURE 13.** '*Aphyosemion*' *cryptum*, male, 34.6 mm SL, paratype, AMNH 262593.

**Diagnosis.** Males of '*Aphyosemion*' *cryptum* (Figs 12–13, Table 7) are distinguished from all other *Aphyosemion* s.l. except for '*A. coeleste*' by its unique combination of colouration characters in males: a light blue body with a few red markings on flanks and caudal peduncle; yellow to orange on distal part of unpaired fins; base of dorsal and anal fins blue, distally followed by a red band, two parallel or converging red lines along the blue centre of caudal fin. At least in anal and ventral part of caudal fin between red band and yellow to orange distal parts a narrow white line. By mitochondrial DNA results and male colour pattern it clearly belongs to the '*A. coeleste*' group. It differs from '*A. coeleste*' by the lack of a green or yellow iridescent blotch posterior to the operculum. In '*A. coeleste*' three to four less regular rows of red spots on anterior part of side within the green or yellow blotch versus rows of red spots absent or reduced to a few irregular spots in '*A. cryptum*'.

**Description.** See Figures 12–13 for general appearance and colour pattern and Table 7 for morphometric data of type series. Largest observed specimen in type series 39.5 mm standard length. Strong sexual dimorphism, adult males more colourful than females and have larger unpaired fins.

Dorsal profile slightly convex, greatest body depth at base of pelvic fin, ventral profile slightly convex, caudal peduncle dorsally and ventrally concave. Snout rounded, mouth directed upwards, lower jaw longer than upper jaw, posterior end of rictus at level of dorsal third of eye.

Frontal neuromasts in two separate grooves, pre-opercular, pre- and post-orbital system with tubular canals. Two supra-orbital grooves (12.1–13.1 % of inter-orbital width, Table 5) with three neuromasts each. Teeth on jaws unicuspid, outer row large and inward curved, inner teeth smaller and more irregularly placed. Scales cycloid, body completely scaled except ventral head surface. Frontal squamation of G-type. No scales on dorsal and anal fin base; two to three scale rows on caudal fin base; scales on mid longitudinal series 30–32; transverse series of scales before dorsal fin nine; circumpeduncular scale row 14.

All unpaired fins rounded in males, adult males have no elongated rays at caudal fin edges. Number of dorsal-fin rays 9–12, anal fin rays 14–16; first dorsal fin ray above anal fin ray seven to eight. Pectoral fin not reaching pelvic fin, pelvic fin not reaching anal fin.

**Colouration.** Live specimens. Males (Figs 12–13). Body on side blue, only anteriorly some irregular red spots, in some populations these spots are completely absent. Ventrally light blue to greyish, dorsally brown. Snout yellow. Up to three red streaks on operculum in an approximate 45° angle, however, in most populations reduced or completely absent. Anal, dorsal, and pelvic fin distally yellow to orange, with red band above narrow blue base, sometimes with light blue narrow edge. Caudal fin dorsally and ventrally yellow to orange, separated from the blue fin centre by two parallel or converging red bands. Red band in fins distally often followed by narrow white line. Pectoral fin yellow to orange.

Females. Body light brown to grey-brownish, dorsally darker and ventrally light brown. Scales on sides, especially on dorsal part and on caudal peduncle, with dark border, forming reticulated pattern. Most scales on dorsal half of sides with small red dot at transition of two scales. Dorsal fin hyaline with inter-radial red streaks, sometimes spotted at base, anal fin hyaline with inter-radial reddish-grey streaks, caudal fin hyaline, unspotted, pelvic and pectoral fins hyaline, unspotted.

**TABLE 7.** Morphometric and meristic data of '*A. cryptum*', new species. SL is given in mm, other measurements as percentages of SL. Abbreviations see Table 4.

	Holotype (male)	Paratypes Range (5 males)	Mean (sd) All males	Paratypes Range (2 females)	Mean (sd) All females
TL	118.2	117.3–121.6	118.7 (1.7)	120.8–123.2	122.0 (1.7)
SL	32.8	34.0–39.5	37.4 (3.0)	31.1–39.4	37.4 (5.8)
HL	23.5	25.1–26.4	25.2 (1.2)	26.1–26.3	26.2 (0.1)
BD	20.6	20.0–21.2	20.7 (0.5)	21.1–23.9	22.5 (2.0)
E	8.3	7.4–8.7	8.3 (0.5)	8.0–8.1	8.0 (0.1)
IO	11.8	10.7–11.9	11.4 (1.2)	10.9–11.5	11.2 (0.4)
pD	66.6	66.7–68.0	67.4 (0.6)	67.6–71.2	69.4 (2.6)
pA	59.8	59.7–63.1	60.3 (1.2)	61.6–62.0	61.8 (0.3)
CL	23.4	19.0–23.8	22.4 (2.0)	22.1–22.4	22.3 (0.2)
CD	12.5	11.6–12.9	12.3 (0.5)	11.4–12.4	11.9 (0.7)
CL/D	1.8	1.6–2.0	1.8 (0.2)	1.8–1.9	1.8 (0.1)
DB	13.9	14.6–17.5	14.3 (1.5)	13.5–14.7	14.1 (0.8)
AB	19.7	18.2–19.8	18.9 (1.0)	16.8–18.3	17.6 (1.1)
D	11	9–12	10.2 (1.3)	11–12	11.5 (0.7)
A	15	14–16	14.8 (0.8)	14–16	15 (1.4)
D/A	8	7–8	7.3 (0.5)	7	7 (0)
C	25	24–26	24.8 (0.8)	25	25 (0)
P	17	17–18	17.5 (0.5)	17–19	18 (1.4)
LLS	32	30–32	31.2 (1.0)	30–32	31 (1.4)
TS	9	9	9 (0)	9	9 (0)
CS	14	14	14 (0)	14	14 (0)

**After 3 months in 70 % ethanol.** Males. Body dark grey, ventrally lighter from pelvic fins to ventral part of head and throat. Chin and lips light grey. Dorsal, anal, and pelvic fin whitish with red band at the base. Caudal fin whitish with dark grey central part, edged with red bands. Pectoral fin whitish.

Females. Dorsal part of flanks grey-brown with gradual transition to lighter ventral side. All scales with dark border, especially on the caudal peduncle. Pelvic and pectoral fin grey. Dorsal fin with inter-radial red streaks, anal and caudal fin grey with inter-radial dark grey pigmentation.

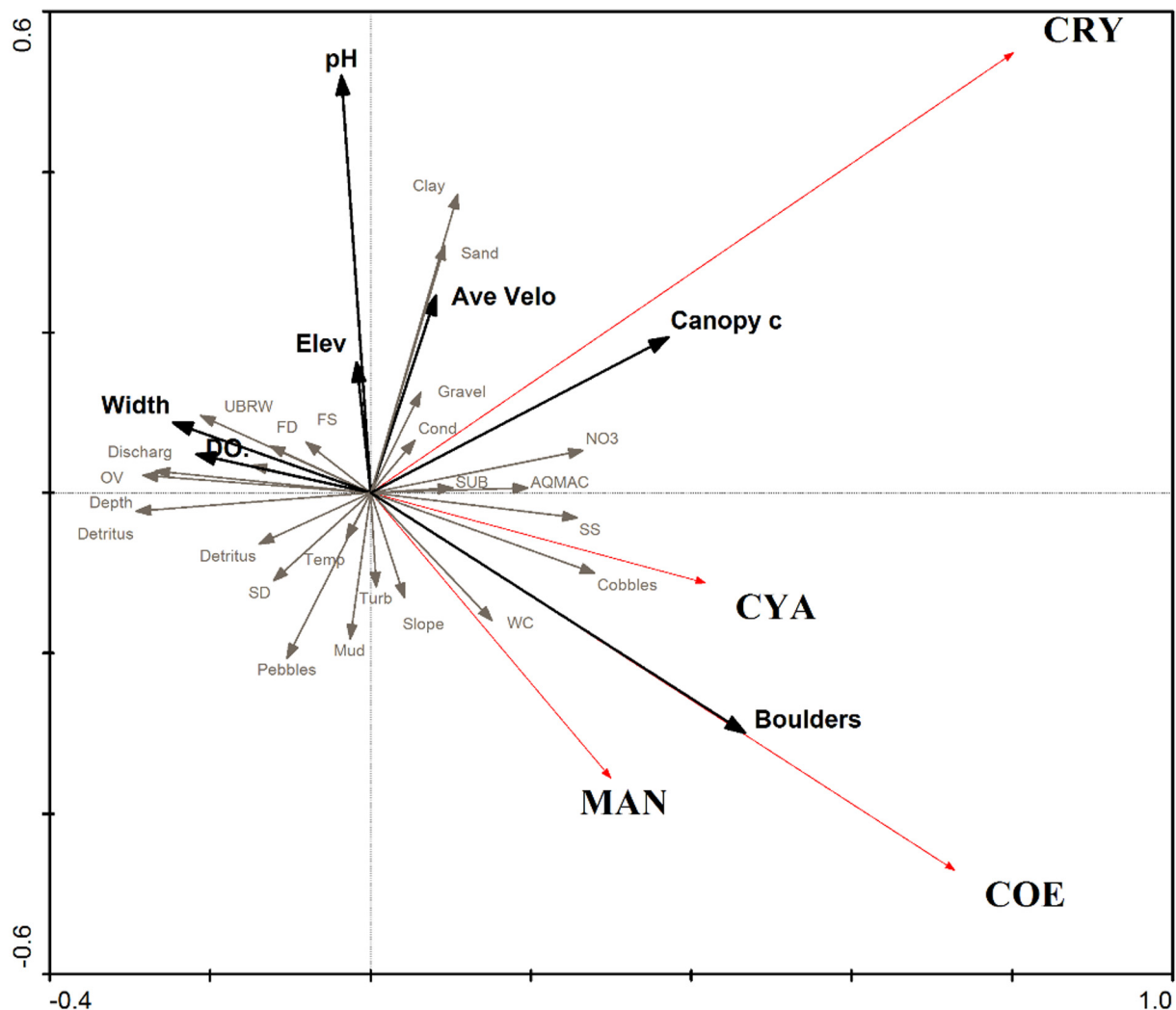
**Distribution and habitat.** '*Aphyosemion*' *cryptum* is known from shallow parts of small tributaries of the upper Louessé and Mandoro Rivers in the southern Massif du Chaillu within the Republic of the Congo. It can sometimes be found syntopic with '*A. coeleste*' or '*A. mandoroense*'. However, sites characterised by sand and clay substrate associated with higher average velocity, higher elevation and relatively higher pH values were more likely to yield '*A. cryptum*' than '*A. coeleste*' or '*A. mandoroense*'.

**Etymology.** The name *cryptum* (an adjective from the Greek *krypton*, meaning "hidden") refers to the fact that the species was first thought to represent just a colour variation of '*A. coeleste*' and not a species of its own.

## Environmental data

Of the 38 sites sampled (Fig. 1), a total of 16 of the sites had '*Aphyosemion*' species (42 %) with 24 occurrences of the various species recorded (Fig. 2). The RDA showed a cumulative percentage variance of species data of 51.8 % explained on the first two axes. A cumulative species-environment relation showed that 81.3% was explained on the first two axes. Environmental variables significantly ( $p < 0.05$ ) contributing towards the measured variance in the species data include the presence of boulders, increased canopy cover, average velocity, elevation, channel width and dissolved oxygen.

All new '*Aphyosemion*' species grouped to the right on the Y-axis (Fig. 14). The presence of these species was predicted by relatively smaller watercourses (forest stream and swamp forests) with low dissolved oxygen and slow flowing water associated with cobbles and aquatic macrophytes. The occurrence and abundance of '*A. cryptum*' was strongly predicted by canopy cover, where the presence of detritus and deep, slow-flowing water showed a strong negative relationship with the occurrence of this species. Sites characterised by sand and clay substrate associated with higher average velocity, higher elevation, and relatively higher pH values were more likely to yield '*A. cryptum*', than other sites assessed. The ordination of sites which yielded '*A. cryptum*', from other *Aphyosemion* s.l. yielding sites was predominantly influenced by more alkaline condition and higher elevations.



**FIGURE 14.** Redundancy Analysis (RDA) of the occurrence of '*Aphyosemion*' species in relation to various environmental attributes, where attributes highlighted in black are significant ( $p \leq 0.05$ ). CYA = '*A. cyanoflavum*'; MAN = '*A. mandoroense*'; CRY = '*A. cryptum*'; COE = '*A. coeleste*'; Elev = Elevation; Ave Velo = Average Velocity; Canopy c = Canopy Cover; DO = Dissolved Oxygen.

'*Aphyosemion*' *cyanoflavum*, '*A. coeleste*, and '*A. mandoroense* ordinated in the bottom right quadrant and reflected similar habitat requirements. The presence of boulders strongly predicted the presence of '*A. coeleste*, while also reflecting a moderately strong covariance with the presence of '*A. cyanoflavum* and '*A. mandoroense*.

Some marginal variation within environmental variables associated with '*A. cyanoflavum*, '*A. coeleste*, and '*A. mandoroense* was measured. '*Aphyosemion*' *cyanoflavum* reflected a weak positive correlation with the presence of clay and sand, while '*A. mandoroense* sites reflected a weak negative correlation with the same

environmental variables. Similarly, '*A. mandoroense*' were more likely to have detritus and slow flowing deep water associated with their presence. Due to the small sample size of '*A. cyanoflavum*' this result needs further corroboration by larger samples.

## Discussion

**Endemic species diversity.** The Massif du Chaillu has a high diversity of endemic killifish species and species groups. All currently known *Aphyosemion* s.l. species of the Massif du Chaillu are endemic to these mountains. From the *Aphyosemion* s.l. species groups or ungrouped species ('*A. coeleste* group, '*A. ogoense* group, '*A. wachtersi* group, '*A. grelli* group, '*A. hofmanni*, '*A. joergenscheeli*, and '*A. tirbaki*) occurring in the area, only two are not restricted to the Massif du Chaillu: '*A. joergenscheeli* is probably related to a group of species of the coastal plain (Collier 2007), and the '*A. ogoense* group also occurs south and east of the Massif.

Based on DNA, nine species are grouped into the endemic '*A. coeleste* group (Collier 2007; this study): '*A. aureum*, '*A. citrineipinnis*, '*A. coeleste*, '*A. cryptum*, '*A. hanneloreae*, '*A. mandoroense*, '*A. ocellatum*, '*A. passaroi*, and '*A. wuendschi*. The '*A. ogoense* group also contains nine species of which seven are endemic to the Massif (Collier, 2007; Valdesalici & Eberl, 2015; this study): '*A. caudofasciatum*, '*A. cyanoflavum*, '*A. jeanhuberi* Valdesalici & Eberl, 2015, '*A. louessense*, '*A. ogoense*, '*A. ottogartneri*, '*A. pyrophore*, '*A. thysi*, and '*A. zygaima* ('*A. ottogartneri* and '*A. zygaima* occur just south of the Massif).

Three recently described species belong to the '*A. grelli* group based on assumption of their authors on colour pattern characters: '*A. bitteri* Valdesalici & Eberl, 2016, '*A. grelli* Valdesalici & Eberl, 2013, and '*A. mengilai* Valdesalici & Eberl, 2014. '*Aphyosemion wachtersi* Radda & Huber, 1978, and '*A. buytaerti* are close relatives, but their relationship to other groups remains unclear (Collier 2007). Further studies are necessary on the taxonomic status of the subspecies '*A. wachtersi mikeae* Radda, 1980b, which might represent a distinct species. DNA data indicate, that '*A. schluppi* Radda & Huber, 1978 is also a member of this group (unpublished data), contradicting the hypothesis that it is related to '*A. thysi* (e.g. Huber 2007). The relationship of '*A. hofmanni* and '*A. tirbaki* within *Aphyosemion* s.l. is currently not established (Collier 2007).

**Cephalic lateral line system.** Although DNA analyses demonstrate that '*A. cyanoflavum* is part of the '*A. ogoense* group, this species differs from all other *Aphyosemion* s.l. species with respect to the morphology of the dorsal anterior part of the head, frontal squamation and the cephalic lateral line system. The lateral line system is a close-range detection system found exclusively in fishes and larval and purely aquatic amphibians. It supports behaviour involved in courtship, orientation to water currents, and prey detection (Coombs & Brown 2003). In *Aphyosemion* s.l. the dorsal part of the cephalic lateral line system consists of a frontal system with two pits, each containing one neuromast, and a supra-orbital system with two shallow grooves with each three neuromasts (Fig. 8).

*Aphyosemion* s.l. species hardly differ in morphology (Scheel 1968; Van der Zee et al. 2007). The cephalic lateral line system in *Aphyosemion* s.l. is also rather uniform compared to Poeciliidae (Sonnenberg et al. 2014; Van der Zee et al. 2015), however, the present study shows that at least the width of the supra-orbital grooves differs in some genera and species groups.

In '*A. cyanoflavum* the anterior most neuromast of the supra-orbital system is enlarged, a character that is shared by all *Diapteron* Huber & Seegers, 1977 species, but in contrast to '*A. cyanoflavum*, the latter genus has the narrowest grooves of all *Aphyosemion* s.l. species (Table 5). According to Brosset (1982) and Brosset & Lachaise (1995), *Diapteron* differs in food ecology from other syntopic nothobranchiids by feeding at dawn and mainly or exclusively on aquatic invertebrates versus diurnal feeding mainly on terrestrial arthropods in the other species. Currently only four '*A. cyanoflavum* specimens are known, therefore research of stomach contents was not conducted. The juvenile male (20.7 mm SL) however had the exoskeleton of a relatively large terrestrial beetle protruding from its cloaca, indicating that '*A. cyanoflavum* feeds on terrestrial arthropods as do most other studied *Aphyosemion* s.l. (Brosset 1982).

The dorsal and lateral view of the head of '*A. cyanoflavum* looks more like an *Epiplatys* than an *Aphyosemion* s.l. Furthermore, '*A. cyanoflavum* is the only *Aphyosemion* s.l. species that has its frontal neuromasts housed in one pit protected by one central anterior lobe. All other species have separated pits with two small lobes for each frontal neuromast. In rare cases the two anterior lobes are fused, but still can be recognized as two lobes. Except for

the *Epiplatys sexfasciatus* Gill, 1862 species group that occurs in the coastal plain from the Dahomey gap to the Ogowe estuary, all *Epiplatys* species have frontal neuromasts housed in one pit with one central anterior lobe. *Epiplatys* species are widespread in the Lower Guinea ichthyofaunal province (Van der Zee *et al.* 2007) and in the Congo ichthyofaunal province (Van der Zee *et al.* 2013), but are completely lacking from the Massif du Chaillu, except for some isolated populations of *E. sp. aff. multifasciatus* in upper Niari tributaries. *Episemion callipteron* Radda & Pürzl, 1987 and *E. krystallinoron* Sonnenberg, Blum & Misof, 2006 also look more similar to *Epiplatys* than to *Aphyosemion* s.l. Although *Episemion* Radda & Pürzl, 1987 is nested within *Aphyosemion* s.l. (Van der Zee 2002; Sonnenberg *et al.* 2006) it was initially described as a subgenus within *Epiplatys* (Radda & Pürzl 1987; Neumann 2000). Both species are restricted to the Monts de Cristal in northern Gabon and south-eastern Equatorial Guinea, where *Epiplatys* is mostly lacking at altitudes above ca. 220 m, too. Additional research of feeding behaviour in '*A. cyanoflavum*' and *Episemion* may shed light on this peculiar coincidence.

'*Aphyosemion cyanoflavum*' has the widest supra-orbital grooves of all *Aphyosemion* s.l. species. However, many *Aphyosemion* s.l. species of the Massif du Chaillu show more or less widened supra-orbital grooves compared to species outside the Massif. Outside this mountain chain only *Episemion* of the Monts de Cristal shows similar widened grooves (Table 5). Widened supra-orbital grooves are often related to an elongated shape of the G scale (Table 5). Whether these characters are related to a different feeding behaviour in the absence of *Epiplatys* or if it is related to higher water velocity in mountainous brooks should be subject for further research.

'*Aphyosemion coeleste*' and relatives. The '*A. coeleste*' species group has its largest species diversity in southern Gabon. All species living in Gabon, except '*A. coeleste*', represent a monophyletic group (Fig. 4), which is the sister group to the two new species currently only known from the Republic of the Congo and '*A. coeleste*'.

The syntopic occurrence of two or three different species of one species group is not uncommon in the '*A. coeleste*' group (e.g. '*A. coeleste*', '*A. hanneloreae*', and '*A. ocellatum*' at Malinga, Gabon), but these belong to phenotypically and genetically more distinct taxa. Whereas '*A. mandoroense*' is easily recognized as a distinct species based on male colouration characters, the syntopic existence of two genetically distinct species of the same species group like '*A. cryptum*' and '*A. coeleste*' with a remarkably similar colour pattern is unique in *Aphyosemion* s.l.

'*Aphyosemion cryptum*' was probably first found but not recognized as a distinct species by Huber (1982) who collected it in 1978 at Mbinda, 20 km north of the locality where the AMNH 262593 paratypes were collected. Wildekamp (1993) recognized the differences in colour pattern of the Mbinda population and regarded it as a colour morph of '*A. coeleste*'. Initially all '*A. cryptum*' collections were tentatively identified as '*A. coeleste*' by us based on field pictures made by GW. However, the small differences in colour pattern of the two syntopic occurring species and the genetic data supported the hypothesis of an undescribed cryptic species.

The first genetic data indicate a complex phylogeographic pattern within the monophyletic group including '*A. coeleste*', '*A. cryptum*', and '*A. mandoroense*' (Figs 4 & 5). Beside the new species, two genetically quite distinct clades of '*A. coeleste*' are found in the same area (Fig. 5). A possible explanation might be the independent dispersal of two distinct genetic lineages of '*A. coeleste*' into the distribution area of the two new species. In addition, it might be possible that the two distinct '*A. coeleste*' lineages represent two cryptic species in a secondary contact zone. However, further collections and genetic data from the distribution area of the '*A. coeleste*' clade are necessary to study these hypotheses.

The slightly divergent preference of different habitat parameters by '*A. cryptum*' (Fig. 14) might be interpreted as competition avoidance with the quite distinct coloured '*A. mandoroense*' and the very similar '*A. coeleste*' in the case of syntopic occurrence. Unfortunately, currently nothing is known about the habitat preferences and potential ecological differences of species in the other cases of syntopic occurrences in the '*A. coeleste*' species group.

## Comparative material examined

'*Aphyosemion bochleri*' Radda, 1975: RSC, Gabon, Ogooué-Ivindo, Mintoum, T. Blum, G. Fleck & R. Sonnenberg, 23 July 2002.

'*Aphyosemion buytaerti*' Radda & Huber, 1978: MRAC 1978-22-P-448, holotype, Republic of the Congo, on the road Zanaga-Voula II, village Ogouée, brook Ekouma (op de weg Zanaga-Voula II, dorp Ogouée, beek Ekouma), W. Wachters & J. Buytaert, 23 July 1978.



- Aphyosemion castaneum* Myers, 1924: MRAC 22555–22561, Democratic Republic of the Congo, Stanleyville, Dr. Richard, 1930; MRAC 1989-043-P-547–612, labelled as *A. christyi* (Boulenger, 1915), Democratic Republic of the Congo, Kisangani, Libuku River, L. De Vos & M. Katembo, April 1988.
- ‘*Aphyosemion*’ *coeleste* Huber & Radda, 1979: MRAC 1978-22-P-1390–1566, Republic of the Congo, on the road Mbinda-Mossendjo, village Mayala, brook Niolo (op de weg Mbinda-Mossendjo, dorp Mayala, beek Niolo), W. Wachters, 18 July 1978; MRAC 1978-22-P-1567–1578, Republic of the Congo, on the road Mbinda-Mossendjo, village Mambengue, brook Kobe (op de weg Mbinda-Mossendjo, dorp Mambengue, beek Kobe), W. Wachters, 18 July 1978; MRAC 1978-22-P-1579–1592, Republic of the Congo, on the road Mossendjo-Gnimi in the direction of Komono, village Ngamaka, brook Liwélé (op de weg Mossendjo-Gnimi, richting Komono, dorp Ngamaka, beek Liwélé, W. Wachters), 19 July 1978; MRAC 1978-22-P-1290–1307, Republic of the Congo, Titi, W. Wachters, 13 July 1978; AMNH 258483, Republic of the Congo, Bakoulou River, tributary of Louessé River, field code L3S, G. Walsh, V. Mamonekene, M. Jonker & V. Boukaka Mikembi, 10 January 2012; AMNH 258487, Republic of the Congo, Mipoundi River, tributary of Louessé River, field code L4S, G. Walsh, V. Mamonekene, M. Jonker & V. Boukaka Mikembi, 10 January 2012; AMNH 258864, Republic of the Congo, Lekoumoumayoko River, tributary of Mandoro River, field code M17S, G. Walsh, V. Mamonekene, M. Jonker & V. Boukaka Mikembi, 10 January 2012; AMNH 258902, Republic of the Congo, tributary of Mandoro River, field code M24S, G. Walsh, M. Jonker, M. Alexandre & V. Boukaka Mikembi, 10 January 2012; AMNH 258904, Republic of the Congo, tributary of Mandoro River, field code M25S, G. Walsh, V. Mamonekene, M. Jonker & V. Boukaka Mikembi, 10 January 2012; AMNH 258916, Republic of the Congo, tributary of Mandoro River, field code M26S, G. Walsh, V. Mamonekene, M. Jonker & V. Boukaka Mikembi, 10 January 2012; AMNH 262595, Republic of the Congo, tributary of Mandoro River, field code OS36, G. Walsh, M. Alexandre & V. Boukaka Mikembi, October 2013; AMNH 262600, Republic of the Congo, swamp forest tributary of Mandoro River, field code OS55, G. Walsh, M. Alexandre & V. Boukaka Mikembi, November 2013; AMNH 262596, Republic of the Congo, about 1.5 km south of the village of Lisoukou (10 km south of Mayoko) on the road R1 to Mossendjo, Leyou River, tributary of the Mandoro River, Niari River drainage, field code M27, collected by G. Walsh, M. Alexandre & V. Boukaka Mikembi, 22 October 2013.
- ‘*Aphyosemion*’ cf. *cryptum*: AMNH 258825, Republic of the Congo, Lipia River, tributary of Louessé River, field code L8S, G. Walsh, M. Jonker, V. Boukaka Mikembi & V. Mamonekene, 10 January 2012.
- ‘*Aphyosemion*’ *citrineipinnis* Huber & Radda, 1977: RSC, Gabon, north of Yeno, field code BBW 00/5, F. Bitter, H. Weder & T. Blum, September 2000.
- Aphyosemion cognatum* Meinken, 1951: MRAC P-100505–100510, Democratic Republic of the Congo, Stanleyville, P. Brichard, 1 January 1955; MRAC 2007-009-P-0182, Democratic Republic of the Congo, village Kinsende, Inkisi basin, Bimpuampolo River, S. Wamuini, 9 October 2005.
- Aphyosemion elegans* (Boulenger, 1899): MRAC P-990–992, syntypes, Democratic Republic of the Congo, Equateur Province, Tshuapa Basin, Bikoro, coll. P. Delhez, no year.
- ‘*Aphyosemion*’ *escherichi* (Ahl, 1924a): RSC, Gabon, Ngounié, small road to the right at the road N1 from Fougamou to Lambarene, field code G 02/109, T. Blum, G. Fleck & R. Sonnenberg, 12 July 2002; RSC, Gabon, Estuaire, at the L 107 before Nzog Binzog, field code G 02/119, T. Blum, G. Fleck & R. Sonnenberg, 14 July 2002.
- Aphyosemion ferranti* (Boulenger, 1910): MRAC 1976-1-P-639–641, Democratic Republic of the Congo, Kasai occidental, Bena Tshadi, Lukibu (Mukangala), J. S. Robertson, 10 May 1975
- ‘*Aphyosemion*’ *hanneloreae* Radda & Pürzl, 1985: JZC, Gabon, Malinga, offspring of specimens collected at locality with field code GJS 00/14, R. Guggenbühl, P. Sewer & M. Juhl, 4 August 2000.
- ‘*Aphyosemion*’ *hera* Huber, 1998a: RSC, Gabon, Moyen-Ogooué, small river at the road N1 north of Lambarene, near Bengui 3, T. Blum, G. Fleck & R. Sonnenberg, 13 July 2002.
- ‘*Aphyosemion*’ *hofmanni* Radda, 1980a: RSC, Gabon, Ngounié, Mimongo-Mbigou road, 21 km after Bitsago’o, field code BBW 00/20, F. Bitter, T. Blum & H. Weder, September 2000.
- ‘*Aphyosemion*’ *joergenscheeli* Huber & Radda, 1977: MRAC 1983-005-P-032–033, Republic of the Congo, 8 km NNW Mbigou, on the road to Mimongo (aan de weg naar Mimongo), Di Banga River, J. Pap, 24 July 1980.
- ‘*Aphyosemion*’ *labarrei* Poll, 1951: MRAC P-77212–77215, paratypes, Democratic Republic of the Congo, Kingemba, Ngufu basin, River Mausso, A. Dubois, 1951.

- Aphyosemion lefiniense* Woeltjes, 1984: MRAC 1980-20-P-183–185, Congo, affl. marécageux rive droite Louna, affl. Léfini, au camp PPG Abio 1, E. Vreven & I. Zamba, September 2008.
- ‘*Aphyosemion*’ *louessense* (Pellegrin, 1931): MRAC 1978-20-P-450–465, Republic of the Congo, on the road Komono-Sibiti, 600 m beyond location 24, a small concrete bridge (op de weg Komono-Sibiti, 600m voorbij punt 24, bruggetje in beton), W. Wachters & J. Buytaert, 21 July 1978; MRAC 1978-22-P-1732, Republic of the Congo, on the road Komono-Sibiti, 1 km from the village Lekoli Magogo, brook Lecesse (op de weg Komono-Sibiti, op 1 km van Lekoli Magogo, beek Lecesse), W. Wachters, 20 July 1978.
- Aphyosemion lujae* (Boulenger, 1911): MRAC P-3419–3430, Democratic Republic of the Congo, Kondue, Sankuru basin, E. Luja, no year.
- Aphyosemion musafirii* Van der Zee & Sonnenberg, 2011: MRAC 2011-007-P-1, holotype, Democratic Republic of the Congo, Tshopo Province, 67 km on the road from Kisangani to Ubundu, A. Van Deun, 22 October 2007.
- ‘*Aphyosemion*’ *ocellatum* Huber & Radda, 1979: MRAC 1985-048-P-543–595, Gabon, Epamboua, 6 km after Epamboua coming from Lebamba, Pürzl, 1 June 1985; JZC, Gabon, 10 km south Egombi Ferry, offspring of material collected by G. Passaro & W. Eberl, July 1993; RSC, Gabon, field code BBW00/8, F. Bitter, T. Blum & H. Weder, September 2000.
- ‘*Aphyosemion*’ *ogoense* (Pellegrin, 1930): MRAC 1980-54-P-1321–1367, Gabon, Boumango, Pürzl & Hofmann, 1 June 1980; MRAC 1980-54-P-1260–1310, Gabon, 61 km beyond Franceville in the direction of Boumango (nach Franceville Richtung Boumango), Pürzl & Hofmann, 1 June 1980; RSC, Gabon, Haut-Ogooué, field code BB 04/4, F. Bitter & T. Blum, 2004.
- ‘*Aphyosemion*’ *ottogartneri* Radda, 1980b: MRAC P-125311, Republic of the Congo, entre Mindouli et Dolisie sur le Congo Océan, bassin Niari-Kouilou, P. Brichard, 1 September 1958; MRAC 1976-060-P-094–115, Republic of the Congo, Lutete, bassin Niari, collector and date unknown.
- ‘*Aphyosemion*’ *passaroi* Huber, 1994: RSC, Gabon, field code BBW 00/16, F. Bitter, T. Blum & H. Weder, September 2000.
- Aphyosemion pseudoelegans* Sonnenberg & Van der Zee, 2012: MRAC P-37840–378843, Democratic Republic of the Congo, Équateur Province, Flandria riv., Momboyo, G. Hulstaert, 15 July 1931.
- ‘*Aphyosemion*’ *punctatum* Radda & Pürzl, 1977: RSC, Gabon, Ogooué-Ivindo, small tributary of the Ivindo at the CENAREST Research Center at Makokou, field code G 02/126, T. Blum, G. Fleck & R. Sonnenberg, 18 July 2002. RSC, Gabon, Ogooué-Ivindo, Kolé River near Malassa, field code G 02/128, T. Blum, G. Fleck & R. Sonnenberg, 20 July 2002.
- ‘*Aphyosemion*’ *pyrophore* Huber & Radda, 1979: MRAC 1978-22-P-466–472, Republic of the Congo; on the road Gnimi-Komono, 15 km beyond Gnimi in the direction of Moetché (op de weg Gnimi-Komono, 1,5 km voorbij Gnimi, richting Moetché), W. Wachters & J. Buytaert, 20 July 1978; MRAC 1978-22-P-136–144, Republic of the Congo, dorp Gnimi, quartier Mbaya, beek Moupoutoulou, prov. Lékoumbu, W. Wachters & J. Buytaert, 22 July 1978.
- ‘*Aphyosemion*’ *teugelsi* Van der Zee & Sonnenberg, 2010: MRAC P-62053, holotype, Democratic Republic of the Congo, Bandundu Province, 10 km north of Panzi, Lukula Creek, M. Bequaert, 22 February 1939.
- ‘*Aphyosemion*’ *thysi* Radda & Huber, 1978: MRAC 78-22-P-304, holotype, Republic of the Congo, op de weg Titi-Mossendjo, op 500 m van Ngala en 17 km van Titi, W. Wachters & J. Buytaert, 14 July 1978; MRAC 1978-22-P-114–119, paratypes, Republic of the Congo, 13 km beyond Komono in the direction of Mbila (13 km voorbij Komono, richting Mbila), W. Wachters & J. Buytaert, 20 July 1978; MRAC 1978-22-P-304, holotype, Republic of the Congo, op de weg Titi-Mossendjo, op 500 m van Ngala en 17 km van Titi, W. Wachters & J. Buytaert, 14 July 1978.
- ‘*Aphyosemion*’ *tirbaki* Huber, 1999: MRAC 1997-44-P-007–010, paratypes, Gabon, a village on the road from Lastourville to Moanda, 82.7 km from Lastourville, or about 38 km from Moanda, Joumini River, a part of Youmidié, then of Lékoudi basin, Tirbak, Eberl & Blum, 3 March 1996.
- ‘*Aphyosemion*’ *wachtersi* Radda & Huber, 1978: MRAC 1978-22-P-318–403, Republic of the Congo, on the road Zanaga-Ogouée at 9 km from Zanaga, village Voula I, brook Lesala (op de weg Zanaga-Ogouée, op 9 km van Zanaga, richting Ogouée, dorp Voula I, beek Lesala), W. Wachters & J. Buytaert, 23 July 1978.
- ‘*Aphyosemion*’ *wildekampi* Berkenkamp, 1973: JZC, Central African Republic, West of Nola, near border with Cameroon, field code GKCAR 90/7, W. Grell & K. H. Kohler, 1990.
- ‘*Aphyosemion*’ *zygaima* Huber, 1981: MRAC 1973-39-P-1635, Republic of the Congo, Mindouli, Upper Niari, P.

- Brichard, 1 February 1965; MRAC 73-39-P-1636–1645, Republic of the Congo, Mindouli, Upper Niari, P. Brichard, 1 February 1965.
- Chromaphyosemion alpha* (Huber, 1998b): ZFMK 40366–40374, Gabon, Estuaire, road L 101 between Libreville and Cap Esterias, T. Blum, G. Fleck & R. Sonnenberg, 07 July 2002.
- Chromaphyosemion poliaki* (Amiet, 1991): ZFMK 40174–40186, Cameroon, small river close to Boanda, U. Kämpf, R. Sonnenberg & A. Tränkner, 21 January 2000
- Chromaphyosemion riggenbachii* (Ahl, 1924b): ZFMK 40312–40332, Cameroon, Province Littoral, Ndokama, H. Kullmann, B. Misof & R. Sonnenberg, 09. December 2001.
- Diapteron abacinum* (Huber, 1976): DNA vouchers RS 680, 682, 1215, Gabon, Ogooué-Ivindo, Zoula, Calobais River, T. Blum, G. Fleck & R. Sonnenberg, 19 July 2002.
- Diapteron cyanostictum* (Lambert & Géry, 1968): DNA vouchers RS 685, 808, Gabon, Ogooué-Ivindo, small creek at CENAREST research center near Makokou, T. Blum, G. Fleck & R. Sonnenberg, 18 July 2002; MRAC 1998-029-P-0472–490, Gabon, Ivindo basin, Nyame Pende creek behind IRET Station M. Stiassny, J. Sullivan & E. Vreven, 31 January 1998
- Diapteron fulgens* (Radda, 1975): Gabon, DNA vouchers RS 624, 625, 629, Gabon, Ogooué-Ivindo, road N 4 between Ovan and Makokou, T. Blum, G. Fleck & R. Sonnenberg, 22 July 2002; DNA vouchers RS 693, 694, 696, Gabon, Ogooué-Ivindo, road N 4 between Ovan and Makokou, T. Blum, G. Fleck & R. Sonnenberg, 22 July 2002.
- Diapteron georgiae* (Lambert & Géry, 1968): MRAC P-153831–836 paratypes, Gabon, Nyabarelé, PK 15 route de Libreville après Makokou, bassin de l'Ivindo, J. Géry, 14 September 1964; DNA vouchers RS 563, 678, 679, Gabon, Ogooué-Ivindo, road N 4 between Mitzi-Makokou, Matora, Bomalonga River, T. Blum, G. Fleck & R. Sonnenberg, 17 July 2002.
- Episemion krystallinoron* Sonnenberg, Blum & Misof, 2006: ZFMK 39942, holotype, Gabon, northern part of the Monts de Cristal, a river near village of Nkinèn, crossing the road N5 from Médoneu to Kougouleu, T. Blum, G. Fleck & R. Sonnenberg, 29 July 2002.
- Iconisemion striatum* (Boulenger, 1911): RSC, Gabon, Estuaire, Goma, Biyemam River, G. Fleck & R. Sonnenberg, 03 July 2002; JZC, Gabon, aquarium strain.
- Kathethys dargei* (Amiet, 1987): JZC, Cameroon, Central Province, Mbam River ferry, aquarium strain.
- Kathethys elberti* (Ahl, 1924a): JZC, Cameroon, Tignere, upper Benoue Basin, field code JVC 2013-25, J. Vlaming, January 2014; JZC, Cameroon, Centre Province, Ntui, aquarium strain.
- Mesopahyosemion cameronense* (Boulenger, 1903): RSC, Gabon, Ogooué-Ivindo, Mintoum, T. Blum, G. Fleck & R. Sonnenberg, 23 July 2002.
- Mesopahyosemion maculatum* (Radda & Pürzl, 1977): RSC, Gabon, Ogooué-Ivindo, Nzenkelle, T. Blum, G. Fleck & R. Sonnenberg, 23 July 2002.
- Raddaella batesii* (Boulenger, 1911): RSC, Gabon, Ogooué-Ivindo, Malassa, Kolé River, T. Blum, G. Fleck & R. Sonnenberg, 20 July 2002.
- Scheelsemion calliurum* (Boulenger, 1911): RSC, Cameroon, on the road from Miundemba to Ekondo-Titi, south of Mundemba, U. Kämpf, R. Sonnenberg & A. Tränkner, 26 January 2000.
- Scheelsemion franzwernerii* (Scheel, 1971): RSC, Cameroon, River Ngo Njock near Ndog Nyang, U. Kämpf, R. Sonnenberg & A. Tränkner, 05 January 2000.

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## References

- Ahl, E. (1924a) Über neue afrikanische Zahnkarpfen der Gattung *Panchax*. *Zoologischer Anzeiger*, 60, 303–312.
- Ahl, E. (1924b) Neue afrikanische Zahnkarpfen aus dem Zoologischen Museum Berlin. *Zoologischer Anzeiger*, 61, 135–145.
- Amiet, J.-L. (1987) *Le Genre Aphyosemion Myers (Pisces, Teleostei, Cyprinodontiformes)*. *Faune du Cameroun*. Sciences Naturelles, Compiegne, 262 pp.
- Amiet, J.-L. (1991) Diagnoses de deux espèces nouvelles d'*Aphyosemion* du Cameroun (Teleostei: Aplocheilidae). *Ichthyological Exploration of Freshwaters*, 2, 83–95.
- AFS/AIFRB/ASIH. (2003) Guidelines for the use of fishes in research. The American Fisheries Society American Institute of Fisheries Research Biologists American Society of Ichthyologists and Herpetologists, Bethesda, Maryland. Available from: <http://www.asih.org/sites/default/files/documents/resources/guidelinesfishresearch2003-draft.pdf> (accessed 14 November 2016)
- Bader, M. (2016) MB-Ruler - the triangular screen ruler 5.3. Available from: <http://www.markus-bader.de/MB-Ruler/index.php> (accessed 14 November 2016)
- Berkenkamp, H.-O. (1973) Beschreibung eines neuen Prachtkärpflings aus Ost-Kamerun, *Aphyosemion wildekampi* spec. nov. *Aquarienfreund*, 2, 107–120.
- Born, C., Alvarez, N., McKey, D., Ossari, S., Wickings, E.J., Hossaert-McKey, M. & Chevallier, M.H. (2011) Insights into the biogeographical history of the lower Guinea forest domain: evidence for the role of refugia in the intraspecific differentiation of *Aucoumea klaineana*. *Molecular Ecology*, 20, 131–142. <https://doi.org/10.1111/j.1365-294x.2010.04919.x>
- Boulenger, G.A. (1899) Matériaux pour la faune du Congo. Poissons nouveaux du Congo. Cinquième Partie. Cyprins, Silures, Cyprinodontes, Acanthoptérygiens. *Annales du Musée du Congo*, Series Zoology, 1, 97–128, pls. 40–47.
- Boulenger, G.A. (1903) Descriptions of new freshwater fishes from southern Cameroon. *Annals and Magazine of Natural History*, Series 7, 12, 435–441. <https://doi.org/10.1080/00222930308678878>
- Boulenger, G.A. (1910) Poisson Cyprinodonte nouveau du Bas-Congo. *Bulletins Mensuels / Société des naturalistes Luxembourgeois*, 4, 285.
- Boulenger, G.A. (1911) Descriptions of new African cyprinodont fishes. *Annals and Magazine of Natural History*, Series 8, 8, 260–268. <https://doi.org/10.1080/00222931108693023>
- Boulenger, G.A. (1915) *Catalogue of the fresh-water fishes of Africa in the British Museum (Natural History)*. Vol. 3. Printed by order of the Trustees [by Taylor and Francis], London, XII + 526 pp.
- Brosset, A. (1982) Le peuplement de Cyprinodontes du bassin de l'Ivindo, Gabon. *Terre et La Vie - Revue d'Écologie Appliquée*, 36, 233–292.
- Brosset, A. & Lachaise, D. (1995) Evolution as a lottery conflicting with evolution via sexual selection in African rainforest dwelling killifishes (Cyprinodontidae, Rivulinae, Diapteron). *Evolutionary Biology*, 28, 217–264. [https://doi.org/10.1007/978-1-4615-1847-1\\_6](https://doi.org/10.1007/978-1-4615-1847-1_6)
- Buytaert, J. (1980) Auf Suche nach neuen Fischen in Kongo-Brazzaville. *Deutsche Killifisch Gemeinschaft Journal*, 12, 11–14.
- 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. (2007) The genus *Aphyosemion*: taxonomic history and molecular phylogeny. *Journal of the American Killifish Association*, 39, 147–168.
- Coombs, S. & Brown, C.P. (2003) Information processing by the lateral line system. In: Collin, S.P. & Marshall, N.J. (Eds.), *Sensory processing in aquatic environments*. Springer Verlag, New York, pp. 122–138. [https://doi.org/10.1007/978-0-387-22628-6\\_7](https://doi.org/10.1007/978-0-387-22628-6_7)
- Costa, W.J.E.M. (2008) *Catalog of aplocheiloid killifishes of the world*. Rio de Janeiro Reproarte, Departamento de Zoologia, Rio de Janeiro, 127 pp.
- Gill, T.N. (1862) On the West African genus *Hemichromis* and descriptions of new species in the museums of the Academy and Smithsonian Institution. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 14, 134–139.
- Gustinich, S., Manfioletti, G., Del Dal, G., Schneider, C. & Caninci, P. (1991) A fast method for high quality genomic DNA extraction from whole human blood. *BioTechniques*, 11, 298–302.
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.

- Hoedeman, J. (1958) The frontal scalation pattern in some groups of toothcarps (Pisces: Cyprinodontiformes). *Bulletin of Aquatic Biology*, 1, 23–28.
- Huber, J.H. (1976) Un nouveau killi du Gabon nord-oriental *Aphyosemion abacinum* nov. spec. (athériniforme, cyprinodontidé, rivuliné). *Revue française d'Aquariologie Herpétologie*, 3, 79–82.
- Huber, J.H. (1977) Liste nominale annotée de *Aphyosemion* Myers, avec description de *Raddaella* et *Kathetys* deux nouveaux sous-genres nouveaux à la biologie originale. *Killi Revue*, 4 (Supplément), 16 pp.
- Huber, J.H. (1981) A new but well-known *Aphyosemion* species from the southern Congolese Plateau, *Aphyosemion zygaima* n. sp. *Tropical Fish Hobbyist*, 29 (8), 23–30.
- Huber, J.H. (1982) Rapport de synthèse sur l'expédition au Congo (1978) Cyprinodontidés récoltés et *Micropanchax silvestris* synonyme de *stictopleuron*. *Revue Française d'Aquariologie*, 9, 1–12.
- Huber, J.H. (1994) *Aphyosemion passerai*, espèce inédite du Gabon sud-oriental, au patron de coloration unique et description complémentaire de *Aphyosemion decorsei* (Pellegrin). Cyprinodontiformes, Aplocheilidae. *Revue française d'Aquariologie Herpétologie*, 20, 77–79.
- Huber, J.H. (1998a) A new cyprinodont species with a uniquely-colored female, *Aphyosemion hera* n. sp. (Cyprinodontiformes, Pisces), from northwestern Gabon. *Revue Suisse de Zoologie*, 105, 331–338.
- Huber, J.H. (1998b) *Aphyosemion alpha* n. sp. eine neue Art der Untergattung *Chromaphyosemion* mit einem ausgeprägten Färbungsmuster und einer besonders südlichen Verbreitung. *Das Aquarium: Monatsmagazin für Vivaristik*, 32, 15–23.
- Huber, J.H. (1999) A new species of cyprinodont fish, *Aphyosemion tirbaki* n. sp. (Cyprinodontiformes: Aplocheilidae) from Gabon, with further evidence of the frontier species concept. *Freshwater and Marine Aquarium*, 22, 104, 106, 108, 110–113, 116, 118.
- Huber, J.H. (2013a) List of all valid species per country. Killi-Data International. Available from: <http://www.killi-data.org/mem12bers/aa-header-list-countries.php> (accessed 3 November 2013)
- Huber, J.H. (2013b) List of valid species names by genus with page links. Killi-Data International. Available from: <http://www.killi-data.org/> (accessed 3 November 2013)
- Huber, J.H. (2013c) 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, 31–35.
- Huber, J.H. (2016) *Aphyosemion coeleste*. *Killi-Data International*. Available from: <http://www.killi-data.org/mem12bers/coelesteAphy.php> (accessed 27 September 2016)
- Huber, J.H. & Radda, A.C. (1977) Cyprinodontiden-Studien in Gabun. IV. Das Du Chaillu-Massiv. *Aquaria: Vivaristische Fachzeitschrift für die Schweiz und Österreich*, 24, 99–110.
- Huber, J.H. & Radda, A.C. (1979) Die Rivulinae des südlichen Kongo (Brazzaville). 2. Der *Aphyosemion lujae*-Komplex. *Aquaria: Vivaristische Fachzeitschrift für die Schweiz und Österreich*, 26, 175–185.
- Huber, J.H. & Seegers, L. (1977) Vorläufige Beschreibung von *Diapteron*, nov. subgen. *Deutsche Killifisch Gemeinschaft Journal*, 9, 146–148.
- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, 17, 754–755. <https://doi.org/10.1093/bioinformatics/17.8.754>
- Kleynhans, C.J. (2007) Module D: Fish Response Assessment Index in River EcoClassification: Manual for EcoStatus Determination (version 2). Water Research Commission and Department of Water Affairs and Forestry, Pretoria, South Africa. Available from: [https://www.dwa.gov.za/iwqsr/rhp/eco/EcoStatus/ModuleD\\_FRAI/Module%20D\\_FRAI.pdf](https://www.dwa.gov.za/iwqsr/rhp/eco/EcoStatus/ModuleD_FRAI/Module%20D_FRAI.pdf) (accessed 14 November 2016)
- Lambert, J.G. & Géry, J. (1968) Poissons du bassin de l'Ivindo: III -- le genre *Aphyosemion*. *Biologia Gabonica*, 3, 291–318, pls. 1–3.
- Maley, J. (1996) The African rain forest – main characteristics of changes in vegetation and climate from the Upper Cretaceous to the Quaternary. *Proceedings of the Royal Society of Edinburgh*, 104 (B), 31–73. <https://doi.org/10.1017/S0269727000006114>
- Mamonekene, V. & Stiassny, M.L.J. (2012) Fishes of the Du Chaillu Massif, Niari depression, and Mayombe Massif (Republic of Congo, west-central Africa): a list of species collected in the tributaries of the upper Ogowe and middle and upper Kouilou-Niari River basins. *Check List*, 8, 1172–1183. <https://doi.org/10.15560/8.6.1172>
- Meinken, H. (1951) Mitteilungen der Fischbestimmungsstelle des VDA. VI. *Aphyosemion cognatum*, eine neue Zahnkarpfenart aus dem Kongo-Gebiet. *Die Aquarien- und Terrarienzeitschrift (DATZ)*, 4, 255–257.
- 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, 351–360. <https://doi.org/10.1006/mpev.1998.0566>
- Myers, G.S. (1924) A new poeciliid fish from the Congo, with remarks on funduline genera. *American Museum Novitates*, 116, 1–9.
- Neumann, W. (2000) Die Hechtlinge. *Deutsche Killifisch Gemeinschaft Journal*, Supplementheft Nr. 6, 1–114.

- Pellegrin, J. (1930) Poissons de l'Ogôoué, du Kouilou, de l'Alima et de la Sangha recueillis par M. A. Bandon. Description de cinq espèces et cinq variétés nouvelles. *Bulletin de la Société Zoologique de France*, 55, 196–210.
- Pellegrin, J. (1931) Poissons de la Louessé (Kouilou) recueillis par M. A. Bandon. Description d'une variété nouvelle. *Bulletin de la Société Zoologique de France*, 56, 219–221.
- Petit, M. (1975) Le massif granitique du Chaillu et ses enveloppes gréseuses. Etude de géographie physique. *Travaux et Documents de Géographie Tropicale*, 22, 3–108.
- Plana, V., Gascoigne, A., Forrest, L., Harris, D. & Pennington, T. (2004) Pleistocene and pre-Pleistocene *Begonia* speciation in Africa. *Molecular Phylogenetics and Evolution*, 31, 449–461.  
<https://doi.org/10.1016/j.ympev.2003.08.023>
- Poll, M. (1951) Notes sur les Cyprinodontidae du Musée du Congo belge. Première partie: les Rivulini. *Revue de Zoologie et de Botanique Africaines*, 45, 157–171.
- Radda, A.C. (1975) Contribution to the knowledge of the Cyprinodonts of Gabon, with the description of four new species and one new subspecies of the genus *Aphyosemion* Myers. *British Killifish Association, Killi News*, Separate (June 1975), 1–20.
- Radda, A.C. (1977) Vorläufige Beschreibung von vier neuen Subgenera der Gattung *Aphyosemion*. *Aquaria*, 24, 209–216.
- Radda, A.C. (1980a) Beschreibung zweier neuer *Aphyosemion*-Arten aus Süd-Gabun. *Aquaria: Vivaristische Fachzeitschrift für die Schweiz und Österreich*, 27, 201–205.
- Radda, A.C. (1980b) Die Rivulinae des südlichen Kongo (Brazzaville). 3. Revision des *Aphyosemion-ogoense*-Komplexes. *Aquaria: Vivaristische Fachzeitschrift für die Schweiz und Österreich*, 27, 193–200.
- Radda, A.C. & Huber, J.H. (1977) Cyprinodontiden-Studien in Gabun. V. Das Tiefland West-Gabuns und die Mayumbe-Berge. *Aquaria: Vivaristische Fachzeitschrift für die Schweiz und Österreich*, 24, 137–150.
- Radda, A.C. & Huber, J.H. (1978) Die Rivulinae des südlichen Kongo (Brazzaville) 1, Beschreibung von vier neuen Arten der Gattung *Aphyosemion* Myers. *Aquaria: Vivaristische Fachzeitschrift für die Schweiz und Österreich*, 25, 173–187.
- Radda A.C. & Pürzl, E. (1977) Cyprinodontiden-Studien in Gabun. II. Nordgabun. *Aquaria: Vivaristische Fachzeitschrift für die Schweiz und Österreich*, 24, 21–31.
- Radda, A.C. & Pürzl, E. (1985) Zwei neue Formen der Gattung *Aphyosemion* aus Süd-Gabun. *Aquaria: Vivaristische Fachzeitschrift für die Schweiz und Österreich*, 32, 157–160.
- Radda, A.C. & Pürzl, E. (1987) *Episemion callipteron*, ein neuer Killifisch aus Nordgabun. *Deutsche Killifisch Gemeinschaft Journal*, 19, 17–22.
- Scheel, J.J. (1968) *Rivulins of the Old World*. TFH Publications, New Jersey, 480 pp.
- Scheel, J.J. (1971) *Aphyosemion franzwerneri* and *Aphyosemion celiae*, two new rivulins from Cameroon. *Tropical Fish Hobbyist*, 19, 48–49, 52–57, 60–66.
- Seegers, L. (1997) *Killifishes of the World. Old World Killifishes I*. Aqualog A.C.S GmbH, Mörfelden-Walldorf, 73 pp.
- 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. & Blum, T. (2005) *Aphyosemion (Mesoaphyosemion) etsamense* (Cyprinodontiformes: Aplocheiloidei: Nothobranchiidae), a new species from the Monts de Cristal, north-western Gabon. *Bonner zoologische Beiträge*, 53, 211–220.
- Sonnenberg, R., Blum, T. & Misof, B. (2006) Description of a new *Episemion* species (Cyprinodontiformes: Nothobranchiidae) from northern Gabon and south-eastern Equatorial Guinea. *Zootaxa*, 1361, 1–20.
- 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, 1–12.  
<https://doi.org/10.1186/1742-9994-4-6>
- Sonnenberg, R. & Schunke, A.C. (2010) On the taxonomic identity of *Fundulus beauforti* Ahl, 1924 (Cyprinodontiformes, Aplocheiloidei). *Zoosystematics and Evolution*, 86, 337–341.  
<https://doi.org/10.1002/zoos.201000015>
- Sonnenberg, R., Friel, J.P. & Van der Zee, J.R. (2014) Description of *Hylopanchax paucisquamatus* (Cyprinodontiformes: Poeciliidae), a new lampeye species from the Odzala-Kokoua National Park, Republic of Congo. *Zootaxa*, 3846 (4), 577–583.  
<https://doi.org/10.11646/zootaxa.3846.4.6>
- Sonnenberg, R. & Van der Zee, J.R. (2012) *Aphyosemion pseudoelegans* (Cyprinodontiformes: Nothobranchiidae), a new killifish species from the Cuvette centrale in the Congo basin (Democratic Republic of Congo). *Bonner zoologische Beiträge*, 61, 3–13.
- Stiassny, M.L.J., Teugels, G.G. & Hopkins, C.D. (Eds.) (2007) *The Fresh and Brackish Water Fishes of Lower Guinea, West-Central Africa. Vol. I*. IRD Éditions, Paris, 800 pp.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28, 2731–2739.  
<https://doi.org/10.1093/molbev/msr121>
- Ter Braak, C.J.F. & Smilauer, P. (2002) *CANOCO reference manual and Conodraw for Windows user's guide: Software for*

canonical community ordination. Version 4.5. Microcomputer Power, New York, 500 pp.

- Valdesalici, S. & Eberl, W. (2013) *Aphyosemion grelli* (Cyprinodontiformes: Nothobranchiidae), a new species from the Massif du Chaillu, southern Gabon. *Vertebrate Zoology*, 63, 155–160.
- Valdesalici, S. & Eberl, W. (2014) *Aphyosemion mengilai*, a new killifish species from the northern Massif du Chaillu, central Gabon (Cyprinodontiformes: Nothobranchiidae). *Aqua International Journal of Ichthyology*, 20, 111–116.
- Valdesalici, S. & Eberl, W. (2015) *Aphyosemion jeanhuberi*, a new killifish species of the *Aphyosemion ogoense* species group (Cyprinodontiformes: Nothobranchiidae), with remarks on the identity of *Aphyosemion louessense* (Pellegrin, 1931). *Aqua International Journal of Ichthyology*, 21, 110–119.
- Valdesalici, S. & Eberl, W. (2016) *Aphyosemion bitteri* (Cyprinodontiformes: Nothobranchiidae), a new killifish species from the northern Massif du Chaillu, Gabon. *Aqua International Journal of Ichthyology*, 22, 61–68.
- 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>
- Van der Zee, J. (2002) Neue Einsichten in die Verwandtschaft der Gattung *Episemion*. *Deutsche Killifisch Geimeinschaft Journal*, 34, 135–142.
- Van der Zee, J.R., Mbimbi, J.J. & Sonnenberg, R. (2013) *Epiplatys atratus* (Cyprinodontiformes: Nothobranchiidae), a new species of the *E. multifasciatus* species group from the Lulua Basin (Kasaï drainage), Democratic Republic of Congo. *Zootaxa*, 3700 (3), 411–427.  
<https://doi.org/10.11646/zootaxa.3700.3.5>
- 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.
- Van der Zee, J.R. & Sonnenberg, R. (2011) *Aphyosemion musafirii* (Cyprinodontiformes: Nothobranchiidae), a new species from the Tshopo Province in the Democratic Republic of Congo, with some notes on the *Aphyosemion* of the Congo Basin. *Bonn zoological Bulletin*, 60, 73–87.
- Van der Zee, J.R., Sonnenberg, R. & Mbimbi, J.J. (2015) *Hypsopanchax stiassnyae*, a new poeciliid fish from the Lulua River (Democratic Republic of Congo) (Teleostei: Cyprinodontiformes). *Ichthyological Exploration of Freshwaters*, 26, 87–96.
- Van der Zee, J.R., Woeltjes, T. & Wildekamp, R.H. (2007) Aplocheilidae. In: Stiassny, M. L.J., Teugels, G.G. & Hopkins, C.D. (Eds.), *The Fresh and Brackish Water Fishes of Lower Guinea, West-Central Africa. Vol. II*. IRD Éditions, Paris, pp. 80–240.
- Wildekamp, R.H. (1993) *A World of Killies: Atlas of the Oviparous Cyprinodontiform Fishes of the World. Vol. 1*. The American Killifish Association, Mishawaka, Indiana, 311 pp.
- Woeltjes, T. (1984) *Aphyosemion lefiniense* spec. nov. (Pisces, Atheriniformes, Rivulinae) aus dem Lefini-Subsystem des Kongo-Flusses in der Volksrepublik Kongo. *Deutsche Killifisch Gemeinschaft Journal*, 16, 3–14.
- Zhang, D.X. & Hewitt, G.M. (1996) Nuclear integrations: challenges for mitochondrial DNA markers. *Trends in Ecology and Evolution*, 11, 247–251.  
[https://doi.org/10.1016/0169-5347\(96\)10031-8](https://doi.org/10.1016/0169-5347(96)10031-8)