

Description of *Mesoaphyosemion izyarae* (Cyprinodontiformes: Nothobranchiidae), a new killifish species from southern Equatorial Guinea

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Abstract

Mesoaphyosemion izyarae **sp. nov.**, is described from a small left-bank tributary of the Mitemele River in southern Equatorial Guinea. The new species is distinguished from all other described *Mesoaphyosemion* species by a unique combination of male colouration characters: dorsal fin yellow with red streaks between fin rays; anal and pelvic fins yellow with black or dark red margin, basally light blue, caudal fin in centre black with red streaks between fin rays, broad yellow area on dorsal and ventral margin, body metallic light blue with more or regular rows of red dots on side, posteriorly denser and forming a reticulated pattern. The species status is supported by mtDNA analyses, including nine of the eleven described valid *Mesoaphyosemion* species.

Key words: phylogeny, colour pattern, taxonomy, mtDNA, Rio Mitemele, Pleistocene rainforest refuge

Resumen

Se describe la nueva especie *Mesoaphyosemion izyarae*, procedente de un pequeño afluente de la margen izquierda del río Mitemele, en el sur de Guinea Ecuatorial. La nueva especie se distingue de todas las demás especies descritas de *Mesoaphyosemion* por una combinación única en el patrón de coloración de los machos: aleta dorsal amarilla con vetas rojas entre los radios de las aletas; aletas anal y pélvica amarillas con margen negro o rojo oscuro, basalmente azul claro y aleta caudal, negra en el centro, con vetas rojas entre los radios de las aletas, amplia zona amarilla en el margen dorsal y ventral, cuerpo azul claro metálico con hileras más o menos regulares de puntos rojos a los lados, posteriormente más densos formando un patrón reticulado. El estatus de especie está respaldado por análisis de ADNmt, que incluyen nueve de las once especies de *Mesoaphyosemion* válidas descritas.

Résumé

Mesoaphyosemion izyarae, nouvelle espèce, est décrite à partir de spécimens provenant d'un petit affluent de la rive gauche de la rivière Mitemele, dans le sud de la Guinée équatoriale. La nouvelle espèce se distingue de toutes les autres espèces de *Mesoaphyosemion* décrites par une combinaison unique de caractères de coloration mâles: nageoire dorsale jaune avec des stries rouges entre les rayons des nageoires; nageoires anale et pelvienne jaunes avec une marge noire ou

rouge foncé, à la base bleu clair, nageoire caudale au centre noire avec des stries rouges entre les rayons des nageoires, large zone jaune sur les marges dorsale et ventrale, corps bleu clair métallique avec des rangées plus ou régulières de points rouges sur le côté, en arrière plus dense et formant un motif réticulé. Le statut de l'espèce est étayé par des analyses d'ADNmt, incluant neuf des onze espèces valides décrites de *Mesoaphyosemion*.

Introduction

The freshwater fish fauna of Equatorial Guinea belongs to the Lower Guinea ichthyofaunal province (Stiassny *et al.* 2007). The country's continental part is bounded by Cameroon to the north and Gabon to the east and south. The first comprehensive ichthyofaunal inventories were published by Roman (1971) and later by Castelo (1994) and, restricted to the Monte Alén region, by Lasso *et al.* (1998). Scheel (1972, 1974) published some information based on his collections, including karyotypes and crossing experiments. Therefore, until about two decades ago, relatively little data had been published on the nothobranchiid fauna of continental Equatorial Guinea (see summary in Malumbres *et al.* 2022). This situation changed at the onset of the present century due to several collection trips conducted by interested aquarium keepers, mostly focused on killifishes (see González *et al.* 2019; Legros & Zentz 2007; Malumbres & Castelo 2001; Malumbres *et al.* 2022; Ott 2020; Sonnenberg 2008; Valdesalici & Malumbres 2022, 2023). A more general fish inventory occurred in 2017 (Schmidt & Barrientos 2019).

Until recently, it was assumed that the killifish fauna of continental Equatorial Guinea did not include endemic species (Castelo 1994; Roman 1971; Scheel 1972, 1974, 1990). However, currently, 24 nothobranchiid species, 7 of them endemics, have been reported (Huber 2024; Legros & Zentz 2007; Malumbres & Castelo 2001; Malumbres *et al.* 2022; Sonnenberg 2008; Stiassny *et al.* 2007; Valdesalici & Malumbres 2022, 2023). Species shared with Cameroon and/or Gabon are '*Aphyosemion*' *escherichi* (Ahl 1924), '*A.*' *herzogi* Radda 1975, *Epiplatys* cf. *sangmelinensis* (Ahl 1928), *E. grahami* (Boulenger 1911), *E. infra fasciatus* (Günther 1866), *E. sexfasciatus* Gill 1862, *Episemion callipteron* Radda & Pürzl 1987, *Ep. krystallinoron* Sonnenberg, Blum & Misof 2006, *Iconisemion striatum* (Boulenger 1911), *Kathetys exiguum* (Boulenger 1911), *Mesoaphyosemion cameronense* (Boulenger 1903), *M. halleri* (Radda & Pürzl 1976), *M. mimbon* (Huber 1977), *Raddaella kunzi* (Radda 1975), and *Scheelsemion ahli* (Myers 1933). The greatest diversity of nothobranchiid species is found in the inland region with 13 species, compared to eight in the coastal lowland (Malumbres *et al.* 2022; Valdesalici & Malumbres 2022, 2023).

Of all genera or species groups, *Mesoaphyosemion* Radda 1977, is represented by six reported species, of which three are endemic, and is the most diverse genus/species group in Equatorial Guinea (Huber 2024; Malumbres *et al.* 2022; Valdesalici & Malumbres 2022, 2023). *Mesoaphyosemion* is widespread in southern Cameroon, northern Gabon, eastern Equatorial Guinea and north-western Republic of the Congo (Amiet 1987; Dadaniak *et al.* 1995; Huber 2007; Malumbres *et al.* 2022; Scheel 1990; Van der Zee *et al.* 2007; Wildekamp 1993) and consists of thirteen described species of which eleven are regarded as valid (Costa 2008; Huber 2007; Malumbres *et al.* 2022; Seegers 1986, 1988; Sonnenberg & Blum 2005; Sonnenberg *et al.* submitted; Sonnenberg & Schunke 2010; Wildekamp 1993). Current genetic data (Malumbres *et al.* 2022) and phenotypic differences in male colour pattern indicate a high number of potentially undescribed species (e.g. Amiet 1987; Dadaniak *et al.* 1995; Malumbres *et al.* 2022; Sonnenberg & Blum 2005).

Based on an assemblage of specimens collected in part by the authors, as well as the results of a recent DNA study (Malumbres *et al.* 2022), we consider the specimens of a *Mesoaphyosemion* population from the Rio Mveñ drainage (Mitemele basin) with a distinct phenotype, as representing a new species which we describe herein as *M. izyarae*, Ott, Sonnenberg & Van der Zee.

Materials and Methods

The description of the new species is based on specimens collected in 2018 in Equatorial Guinea, deposited at the Royal Museum for Central Africa, Tervuren, Belgium (RMCA), and the Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN). Collection permits were issued by Instituto Nacional de Desarrollo Forestal Y Gestión del Sistema de Aéreas Protegidas (INDEFOR.AP, 308, issued 18 August 2018). Additional *Mesoaphyosemion* samples for DNA studies from aquarium-bred and wild-caught specimens from Cameroon, Equatorial Guinea, and Gabon are listed in Malumbres *et al.* (2022) and Sonnenberg & Blum (2005).

The comparative material studied is listed in Malumbres *et al.* (2022) and Sonnenberg & Blum (2005).

Meristics and Morphometrics. Counts and measurements follow Bragança *et al.* (2020) and were partly made with a dissecting microscope. Nomenclature for supraorbital (frontal) squamation follows Hoedeman (1958) and, for the neuromast system on the head, Scheel (1968) and Van Bergeijk & Alexander (1962). The abbreviation D/A refers to the position of the first dorsal fin ray relative to the opposing first anal fin ray (see Sonnenberg & Schunke 2010).

DNA methods. Details for specimens used in the DNA study, methods, analyses and GenBank accession numbers are published in Malumbres *et al.* (2022), Sonnenberg & Blum (2005), and Sonnenberg *et al.* (2007). A fragment of the mitochondrial cytochrome b gene was sequenced for 64 *Mesoephyosemion* specimens from 58 localities, including nine of the eleven described and valid species, and samples of six undescribed phenotypes (PT 1, 3, 5, 8, 9, and 10) mentioned in Amiet (1987), Dadaniak *et al.* (1995), and Malumbres *et al.* (2022). In the phylogeny published in Malumbres *et al.* (2022), the new species was labelled cMIM008.

Taxonomic concepts. The taxonomic concept employed at the genus level in Nothobranchiidae is published in Malumbres *et al.* (2022) and Sonnenberg (2007). As species concept, we adopted the pragmatic approach by Moritz *et al.* (2000).

Results

It should be noted that the authors of this new taxon are different from the authors of this paper (reference to Article 50.1 and Recommendation 50A of the International Code of Zoological Nomenclature; ICZN 1999).

***Mesoephyosemion izyarae* sp. nov., Ott, Sonnenberg & Van der Zee**
urn:lsid:zoobank.org:act:9F1611AB-7789-43A1-AD85-2AABA0A4DA04
(Figs 1–2, Table 1)

Mesoephyosemion cf. *mimbon* (non-Huber 1977) in part: Malumbres *et al.* (2022)

Holotype. MRAC 2022.028.P.0001, male, 38.6 mm SL, Equatorial Guinea, Centro Sur province, Mitemele drainage, small creek in the Rio Mveñ basin, near the village of Efulan, 1°03'55.6" N, 10°14'47.5"S, coll. A. González, C. Vizcaino, F. Portal & H. Ott, 19.08.2018.

Paratypes. MRAC 2022.028.P.0002–0004, 2 males, 36.7–37.4 mm SL, and 1 female, 41.9 mm SL, collected with the holotype; MNCN-ICTIO 297.332–297.333, 2 males, 34.5–37.9 mm SL, collected with the holotype.

Diagnosis. *Mesoephyosemion izyarae* (Figs 1–2) shares with other species of the genus a combination of the following characteristics in the male: a mostly metallic pale blue, blue or blue-green colouration on the sides; an often variable pattern of red dots on sides, in most species irregularly distributed, in others forming more or less regular stripes or blotches; a latero-ventral red line on the caudal peduncle; unpaired fins without, or with only short, fin extensions, caudal fin nearly straight, slightly rounded or weakly trilobate; no conspicuous markings on ventral side of the head; adults have a yellow snout. Unfortunately, none of these characteristics is diagnostic on its own as they are also known from other nothobranchiid genera or species groups but, in combination, they are significant.

Mesoephyosemion izyarae is distinguished from all other known *Mesoephyosemion* species by a unique combination of colour pattern features in the male: dorsal fin yellow with red streaks between fin rays; anal and pelvic fins yellow with black or dark red margin, basally light blue, caudal fin black in centre with red streaks between fin rays, broad yellow area on dorsal and ventral margins; body metallic light blue with more or less regular rows of red dots on side, posteriorly denser and forming a reticulated pattern; body ventrally red.

Within the genus *Mesoephyosemion*, yellow fins are only known from a few populations of *M. mimbon*. Still, these have a broad red band in the pectoral and anal fins and red spots or stripes on the flanks, which are absent in *M. izyarae*. Also a population of *M. aff. cameronense* from the Ivindo Basin has yellow fins (Sonnenberg & Blum, 2005 Fig. 16) but it has a broad red band at the base of the anal fin. It lacks the dark red to black margins of the pectoral and anal fins and black in the centre of the caudal fin of *M. izyarae*. In addition, *Mesoephyosemion izyarae* is distinguished from *M. amoenum* (Radda & Pürzl 1976), *M. halleri*, and the undescribed phenotypes 3, 5, 9, and 10 by the absence of a yellow caudal peduncle (see also Sonnenberg *et al.* submitted). From *M. lorai* (Valdesalici & Malumbres 2022, 2023), *M. losantosi* Malumbres, Sonnenberg & Van der Zee 2022, *M. maculatum* (Radda & Pürzl

1977), *M. sp. aff. maculatum*, *M. mimbon*, and *M. montealenense* Malumbres, Sonnenberg & Van der Zee 2022, *M. izyarae* is distinguished by the absence of red vertical stripes or blotches on the caudal peduncle.

Description. See Figures 1 and 2 for overall appearance and Table 1 for morphometric data of the type series. Like most cyprinodontiform fishes, *M. izyarae* shows strong sexual dimorphism. Adult males are more colourful and have larger and more pointed fins than females. A slender, laterally slightly compressed and elongated nothobranchiid species. Dorsal profile nearly straight on head, slightly convex from nape to dorsal fin. Ventral profile convex from head to anal fin. Caudal peduncle slightly concave from anal and dorsal fin to base of caudal fin. Snout slightly pointed, mouth directed upwards; tip of the lower jaw protrudes past that of the upper jaw; posterior end of rictus at approximately same level as centre of eye. On both jaws irregular rows of unicuspid, slightly posteriorly curved teeth, with some larger teeth on outer row. Open frontal neuromast system with two separate grooves, preopercular neuromast system with 6 pores.

TABLE 1. Morphometric and meristic data of *Mesoaphyosemion izyarae*. All measurements are in standard length (SL) percentages, except standard length in mm. TL = total length, PD = pre-dorsal fin distance, HL = length of head, pPD = pre-pelvic fin distance, pAD = pre-anal fin distance, DB = greatest body depth, HC = height of caudal peduncle, CL = length of caudal peduncle, CR = caudal peduncle ratio, BD = base of dorsal fin, BA = base of anal fin, E = eye diameter, I = interorbital width, D* = dorsal fin rays, A* = anal fin rays, D/A* = dorsal / anal fin position, C = caudal fin rays, PC = pectoral fin rays, PL = pelvic fin rays, LLS = lateral line scales + number of scales on caudal fin base, TS = transverse row of scales, CS = scales around caudal peduncle, PS = predorsal scales, SD = standard deviation.

	holotype	mean (5 males)	SD	range (5 males)	1 female
SL	39.0	37.3	1.7	34.3–39.0	43.2
TL	121.9	123.2	1.1	122.0–124.5	121.8
PD	70.7	67.9	2.0	64.8–70.7	64.8
HL	25.3	23.4	1.5	21.0–25.3	24.8
pPD	46.6	46.4	1.5	42.0–46.4	46.3
pAD	56.2	56.6	1.5	56.1–59.2	61.4
DB	19.2	19.8	0.7	19.4–21.4	19.2
HC	10.4	11.0	1.1	9.3–12.5	12.1
CL	20.8	21.8	1.2	20.5–23.7	23.4
CR	2.0	2.0	0.2	1.6–2.3	1.9
BD	12.4	12.9	0.7	12.0–13.9	11.8
BA	21.6	18.6	1.2	16.9–19.9	14.9
E	8.0	7.8	0.5	7.0–8.4	6.7
I	11.1	10.4	0.8	9.1–11.4	10.0
Meristics males and females					
	holotype	mean	SD	range	
C	24	21.2	2.0	18–24	
PC	13	12.8	0.4	12–13	
PL	6	5.8	0.4	5–6	
LLS	31	30.5	0.8	29–31	
TS	8	8	0	8	
CS	12	12	0	12	
PS	19	19.2	0.4	19–20	
D*		10.4	0.7	9–11	
A*		14.6	0.8	13–16	
D/A*		8.6	0.5	1/8–1/9	

* D, A and D/A were not counted on preserved samples because the fins of all specimens were folded. Therefore, these were determined from photographs of 10 live specimens.



FIGURE 1. *Mesoaphyosemion izyarae*. Male (1) and female (2) from the type locality, Rio Mveñ basin, near the village of Efulan, Equatorial Guinea (not preserved).

Dorsal and anal fin in males slightly pointed; caudal fin slightly rounded or trilobate, no filamentous extensions. Fins in females are generally more rounded. Dorsal and anal fin located posterior to mid-body. Dorsal fin with 10–12 rays; anal fin with 15–16 rays, first ray of dorsal fin above anal fin ray 5–7, caudal fin with 18–24 fin rays. Pectoral fin origin posterior to opercle, below mid-lateral, extending posteriorly and may reach origin of pelvic fin level; pelvic fin origin ventral, extending posteriorly and may reach origin of anal fin.

Scales cycloid; body and head entirely scaled, except on ventral surface of head. Frontal squamation of G-type; scales on mid-longitudinal series 29–31. Transverse row of scales above pelvic fin 8; circum-peduncular scale row 12.

Live colouration. Male (Fig. 1.1). Back grey to brown, in adult males often with a yellow hue between head and dorsal fin. Side metallic light blue to light green-blue; on side posterior of the opercle up to five more or less complete rows of red dots. Belly ventrally light grey, separated from the blue side by a continuous red stripe from base of pectoral to base of caudal fin. Red pigmentation on caudal peduncle denser, forming a reticulated pattern or a large red blotch at posterior end. Snout yellow to greyish. Eye iris light metallic blue. On opercle posterior to the eye, up to three more or less continuous oblique red stripes, a red line under the eye and red dots or short narrow stripes at snout in some specimens. Pectoral fin transparent, light grey with a white or light blue margin, pelvic fin

yellow to orange on centre, fin margin dark red to almost black, small metallic blue spot on fin base. Dorsal fin yellow to orange, some small red spots or short streaks between fin rays, very narrow light blue margin in some specimens; anal fin yellow to orange, metallic blue fin base, fin margin red to almost black, some red dots or short streaks between fin rays mostly on posterior half of fin. Caudal fin centre dark grey to nearly black, some red pigmentation at fin base between fin rays, broad yellow to orange dorsal and ventral margin.

Female (Fig. 1.2). Back and side brownish, dorsally darker, ventrally light-grey or brown. Scales on back and sides with irregular red or dark markings, forming a reticulate pattern; some scales on side with red dots. Faint red oblique lines on the opercle. Distal part of the peduncle with a dark brown to black dot. All fins hyaline, dorsal and anal fins distally sometimes with yellowish hue, sometimes faint blue at the base. Dorsal and anal fin with red lines or spots between the fin rays.



FIGURE 2. *Mesoaphyosemion izyarae*. Top: holotype, 39.0mm SL. Bottom: female paratype, 43.2 mm SL.

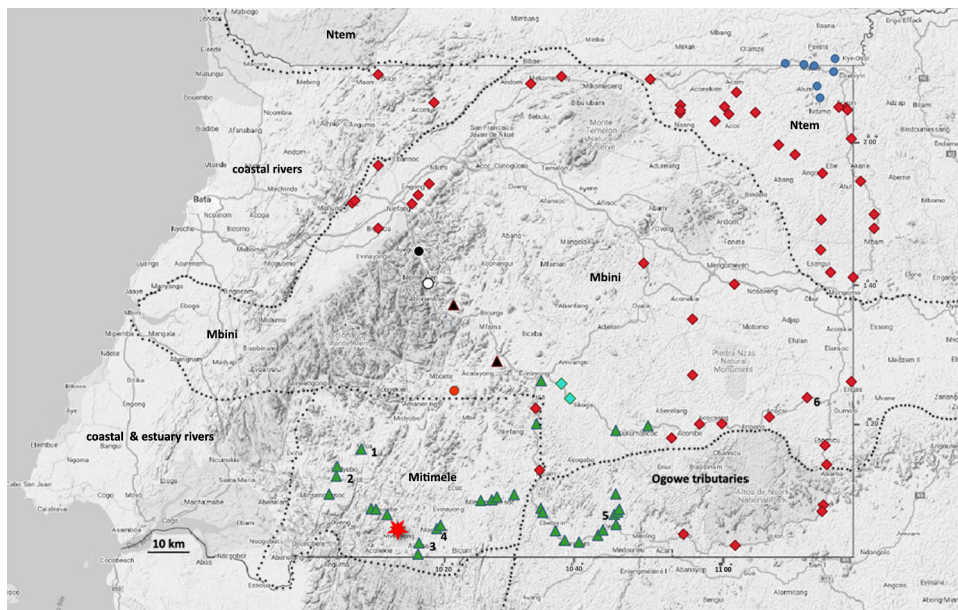


FIGURE 3. Distribution of *Mesoaphyosemion* species in Equatorial Guinea based on museum records and aquarist collections. Symbols: red star *M. izyarae* type locality; red circle *M. sp. aff. izyarae* collected by R. Schmidt, 2017; green triangle *M. mimbon* and *M. aff. mimbon*; red diamond *M. sp. aff. camerounense*; blue circle *M. halleri*; light blue diamond *M. lorai*; black circle *M. losantosi*; white circle *M. montealeenense*; black triangle, *M. cf. montealeenense*; numbers associated with certain symbols refer to those *Mesoaphyosemion* species illustrated in FIGURE 5: *M. mimbon*, field code GEGVPO18-20 [1]; *Mesoaphyosemion sp. aff. mimbon*, field code GEGVPO18-17 [2]; *M. sp. aff. mimbon*, field code GEGVPO18-10 [3]; *M. sp. aff. mimbon*, field code GEGVPO18-9 [4]; 5. *M. mimbon*, field code GEGVPO18/24 [5]; *M. sp. aff. camerounense*, field code GEGVPO18-22 [6]. A black dotted line indicates the borders of the inland river basins (names in black).

Colour in ethanol. Male (Fig. 2). Dorsally and laterally brown, belly light brown or beige. Scales on back and sides with a darker margin, forming a reticulated pattern. Anterior-laterally some light-brown dots, forming irregular rows on the sides. Unpaired and pelvic fins with brown pigmentation on fin rays; distally darker brown, especially on anal, pectoral and pelvic fins. Dark brown basal margin followed by a broader light band on anal fin. Caudal fin centre dark grey, ventral and dorsal margin lighter.

Female (Fig. 2). Dorsally and laterally brown, belly light brown. Scales with a dark-brown, narrow margin, forming a reticulated pattern. Unpaired and pelvic fins with brown pigmentation on fin rays; dorsal fin and dorsal part of caudal fin with some small, dark brown dots. Pectoral fin hyaline.

Etymology. *Mesoaphyosemion izyarae* is named after Izyar, the daughter of Adolfo González, who conducted several killifish collection trips in Equatorial Guinea.

Distribution and habitat. *Mesoaphyosemion izyarae* is at present known only from a single locality in primary rainforest in southern Equatorial Guinea near the village Efulan, 536 m above sea level (Fig. 3). The habitat is a slow-flowing creek, 1–2 m wide and up to 50 cm deep at the time of collection in August 2018. The stream bed consisted of bare sand and clay, with no water plants. Water conditions were: pH 6, total hardness 3 (Ott, 2020). Syntopic with the new species is a fish of the ‘*Aphyosemion*’ *herzogi* species group (Fig. 4). It should be noted that a *Mesoaphyosemion*, very similar to *M. izyarae*, was caught in 2017 by R. Schmidt in the Nfua River (Laña drainage, Mbini Basin) near the village of Nfeme (1°23′45.96″N, 10°22′52.54″E). However, further studies are needed to determine if it represents the same species.

DNA analyses. Details and results of the DNA analyses were published in Malumbres *et al.* (2022). The sample cMIM008 in Figure 2 of this paper represents *Mesoaphyosemion izyarae*. The phylogeny comprises two major clades. One contains Monts de Cristal and Monte Alén species (*M. sp. aff. cameronense* samples CAM 638, 639, 931, and 1744, *M. etsamense* (Sonnenberg & Blum 2005), *M. lorai*, *M. mimbon*), and *M. cameronense* sample from southern Cameroon (CAM 262). The other clade contains most samples, from the largest part of the genus distribution area. The sample of *M. izyarae* is in an isolated position. The genetic divergence between this species and the remaining *Mesoaphyosemion* samples ranges from 9.25 % (samples *M. etsamense* (ETS925), *M. sp. aff. cameronense* PT3 and 8 (PT3 1742, PT8 635) and 11.41% (sample *M. cf. mimbon* (cMIM005)). Because of the distinct position of the cytochrome b sequence of *M. izyarae* within *Mesoaphyosemion*, we checked with BLAST (Altschul *et al.* 1990) searches and translation of the reading frame of the sequence into amino acids that it is a functional sequence and not a nuclear pseudogene or contamination. The closest BLAST results are *Mesoaphyosemion* samples, and the protein-coding part of the sequence does not show any unexpected stop codon or reading frame.



FIGURE 4. ‘*Aphyosemion*’ aff. *herzogi*, male, Rio Mveñ basin, near the village of Efulan, Equatorial Guinea, occurring syntopically with *Mesoaphyosemion izyarae*.



FIGURE 5. *Mesoaphyosemion* phenotypes near the *M. izyarae* type locality (numbers in brackets in the foregoing correspond to those associated with certain symbols on the map of FIGURE 3, thereby indicating their locations): 1. *M. mimbon*, Mitemele basin, southwest of the village of Oveng [1], field code GEGVPO18-20 (1°15'5," N, 10°09'34,2" E); 2. *Mesoaphyosemion* sp. aff. *mimbon*, Mitemele basin, southwest of the village of Midyobo [2], field code GEGVPO18-17 (1°11'32,8" N, 10°06'00,9" E); 3. *M. sp. aff. mimbon*, Mitemele basin, village of Ayem II [3], field code GEGVPO18-10 (1°00'23,3" N, 10°17'42,2" E); 4. *M. sp. aff. mimbon*, Mitemele basin, village of Esakora [4], field code GEGVPO18-9 (1°04'31,4" N, 10°20'55,6" E); 5. *M. mimbon*, north of village Oveng [2], Komo basin, Rio Mbé [5], field code GEGVPO18/24 (1°06'05,2" N, 10°45'47,8" E); 6. *M. sp. aff. cameronense*, Rio Wele basin, village of Mie/Nvom [6], field code GEGVPO18-22 (1°22'39,52" N, 11°13'12,8" E).

Discussion

Based on genetic data and differences in male colour patterns (Amiet 1987; Dadaniak *et al.* 1995; Malumbres *et al.*, 2020) we regard *Mesoaphyosemion* as a rather species-rich genus, for which most species are still undescribed. In the case of *M. izyarae*, we have a distinct male phenotype and large genetic distances of 9.25–11.41%, which is far over a theoretical species delimitation of 2% (Avise & Aquadro, 1982, Johns & Avise, 1998), especially compared to nearby species (see Fig. 2 in Malumbres *et al.*, 2022). The colour pattern of the caudal fin is similar to that of some *M. mimbon* populations, but the body and fin colouration is unique and resembles that of a *Mesoaphyosemion* from the Ivindo basin in Gabon (Sonnenberg & Blum, 2005, Fig. 16). In addition, the syntopic species of the '*A. herzogi*' species group (Fig. 4) also shows a distinct phylogenetic placement and a distinct male phenotype compared with the geographically close populations of the same species (Malumbres *et al.*, 2022). This area requires further study of its fish fauna and its population genetics, drainage patterns and geological history. It may be that the area represents a local, small-scale Pleistocene refuge for two distinct lineages of killifish genera/species groups. This would be consistent with the hypothesis that the Monte Alén/Monts de Cristal region might have been a Pleistocene rainforest refuge (Maley 1987, 1991), as indicated by botanical species diversity (Leal 2005). In addition, this area currently hosts seven endemic nothobranchiid species, and available/published data indicate the occurrence of further, as yet undescribed, endemics.

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