Special *Nothobranchius* Issue

- Caprivi *Nothobranchius* Described
- Distribution and Migration of the Caprivi Killifish
- *Nothobranchius* Habitat Classification
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Contents

The Cover: *Nothobranchius capriviensis*, new species; male of the Salambala NA 07-1 population from the Salambala Conservancy, Zambezi Region, Namibia. Photograph by Brian Watters (© 2015).

Description and biogeography of *Nothobranchius capriviensis*, a new species of annual killifish from the Zambezi Region of Namibia (Cyprinodontiformes: Nothobranchiidae)

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Abstract

Nothobranchius capriviensis, new species, is described. This annual killifish inhabits seasonal pools in the area between the Zambezi and Chobe rivers and is restricted to the eastern part of the Zambezi Region (formerly the Caprivi Strip) of Namibia, in southern Africa. It is closely related to *N. kafuensis*, which occurs in neighboring Zambia, and can be distinguished from it by differences in male and female coloration, morphometric and meristic characters, and mtDNA. For comparison purposes, new morphometric and meristic data, and updated color pattern descriptions are provided for *N. kafuensis*. Consideration of biogeographical relationships suggests that these two species were derived from a common ancestor associated with a major ancient drainage system, the Paleo-Chambeshi, the middle and lower reaches of which comprised the Proto-Kafue River. The primary event that led to isolation of the Caprivi populations, and consequent divergent development, was a rearrangement of major ancient drainage, in particular that of the evolving Zambezi River, that occurred during Late Pliocene to Early Pleistocene times. In view of the very restricted distribution of *N. capriviensis*, and because it is here shown to be distinct from the more widely-distributed *N. kafuensis* in Zambia, it is suggested that its conservation status in the IUCN Red List of Threatened Species be upgraded.

Introduction

During August, 1973, while conducting an investigation of the freshwater fishes of the eastern Caprivi Strip (now part of the Zambezi Region) of Namibia, Van der Waal discovered three locations of a *Nothobranchius* species (Van der Waal, 1976; Van der Waal and Skelton, 1984). The work was carried out in collaboration with the Caprivi Region government during 1973-1977. Subsequent field work has revealed the presence of this *Nothobranchius* species at numerous other locations in the eastern

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Zambezi Region (D. Tweddle and B.C.W. van der Waal, personal communication, 2015).

The late Dr. Rex Jubb, then of the Albany Museum in Grahamstown, South Africa, initially regarded this fish as a variant of *Nothobranchius brieni* Poll, 1939 (Van der Waal, 1976; Jubb, 1976). This identification was based primarily on a published photograph of a *Nothobranchius* species identified as *N. brieni* that had been collected near Likasi, Democratic Republic of Congo (Van den Nieuwenhuizen, 1960). The latter is now regarded as a population of *Nothobranchius polli* Wildekamp, 1978. The Caprivi *Nothobranchius* was regarded as an undescribed species by Wildekamp (1978) and this interpretation was accepted by Van der Waal and Skelton (1984), Skelton (1993), and Watters and Wood (1996).

Jubb had intended to describe the Caprivi fish as a new species but wanted to delay the description until the identification of the so-called "Kayuni State Farm" *Nothobranchius* had been studied and described (personal communications with R.H. Wildekamp, 1980-1983). The Kayuni population had been collected by John Rosenstock in December, 1979 (Rosenstock, 1981). However, it was not until 1989, two years after Rex Jubb had passed away, that this and other populations originating from the Kafue River basin were described as *Nothobranchius kafuensis* by Wildekamp and Rosenstock (1989). Prior to the present work, no attempts had been made to study the Caprivi *Nothobranchius* in depth. This was, in part, due to the fact that it was regarded by some as synonymous with *N. kafuensis* (Tweddle, 2007), a species that occurs, as three

different phenotypes, to the northeast of the eastern Caprivi primarily in the system of the Kafue River in Zambia. Figure 1 shows the geographic distribution of the two species as discussed herein.

In the present study, the Caprivi *Nothobranchius* is compared with four populations of *N. kafuensis* representing all known phenotypes. One of these, originating from Mambova in southwestern Zambia, is only about 54 km from the nearest collection site of the Caprivi fish. The latter was found to differ in male and female coloration, morphometric and meristic characters, and mtDNA. Based on the results of this comparative study the Caprivi *Nothobranchius* is described herein as *Nothobranchius capriviensis*, new species.

Materials and Methods

Descriptions are based on specimens deposited at the South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown, South Africa and the Musée Royal de l'Afrique Centrale (Royal Museum of Central Africa), Tervuren, Belgium (MRAC). Live specimens were maintained in aquaria for observation of maximum attainable size, male and female breeding coloration and breeding biology. Data for additional material for comparative studies, comprising four *N. kafuensis* populations, are presented as additional material below.

Measurements were made with digital callipers (to the nearest 0.1 mm), in part under a dissecting microscope. Methods for counts and measurements were carried out as in Wildekamp *et al.* (2009) and Nagy (2014a). Counts of rays of the dorsal and anal fins include all visible rays. The count of scales in the mid-longitudinal se-
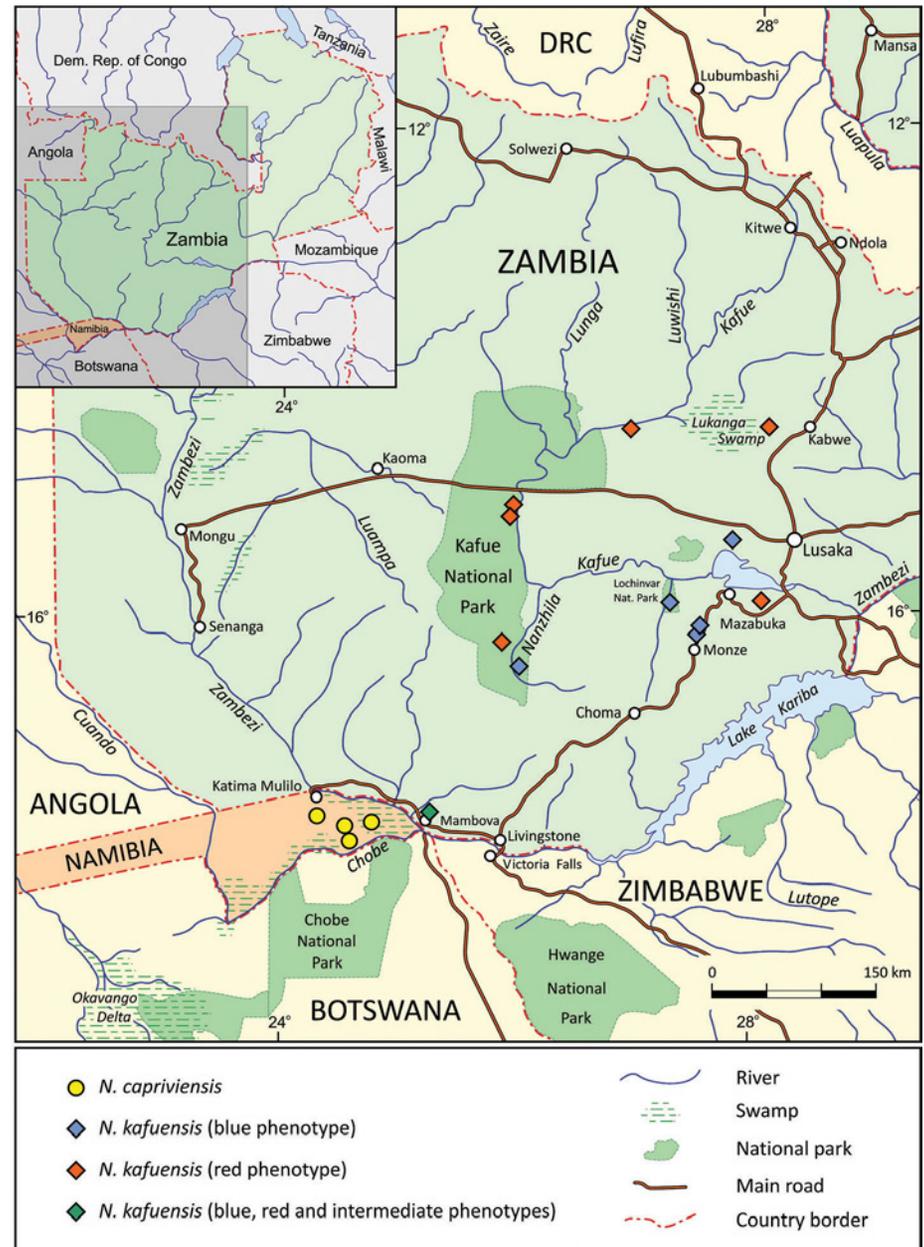
Figure 1: Distribution of *Nothobranchius capriviensis* and *Nothobranchius kafuensis* in Namibia (Zambezi Region) and Zambia, respectively. Individual symbols may represent multiple sites where occurrences are in close proximity to one another.

ries is the number of scales between the upper attachment of the opercular membrane and the base of the caudal fin. The small scales posterior to the hypural junction were counted separately. Terminology for the cephalic neuromast series follows Stenholt Clausen (1967), and that for the frontal squamation follows Hoedeman (1958).

Genomic DNA was extracted from muscle tissue of the caudal peduncle from both fresh and ethanol-preserved samples. DNA isolation was performed by the standard phenol-chloroform method. Partial sequences of two mitochondrial genes were examined: the large ribosomal sub-unit (16S) rDNA (655–663 b.p.) and the protein-coding ND2 gene (743 b.p.) translating to 247 amino acids; the combined dataset of both mitochondrial sequences has a total length of 1,414 b.p.

Polymerase chain reaction (PCR) was carried out with a set of specifically designed primers in different combinations. PCR conditions were: 94°C, 2 minutes initial denaturation followed by 35 cycles of 94°C, 20 seconds, denaturation; 48–55°C, 20 seconds, annealing; 72°C, 90 seconds extension; and a final extension step of 4 minutes at 72°C. PCR products were checked on a 1.0 % agarose gel (100-120V, 400 mA, 35–45 minutes). Purified PCR products were sequenced on an Applied Biosystems Avant 3130 in both directions according to the recommendations of the manufacturer.

Both mitochondrial genes were sequenced for one population of the Caprivi *Nothobranchius* and six populations of *N. kafuensis*. Also included in the analyses were populations of *N. polli*, *N. malaissei*, *N. hassoni*, *N. rosenstocki* and *N. symoensi*,

species from northeastern Zambia and the Katanga Province of the Democratic Republic of Congo, which occur in drainage systems that may once have had links to those presently hosting the Caprivi *Nothobranchius* and *N. kafuensis*. For comparison purposes, *N. kirki* was also analysed, representing a species that occurs in a more remote drainage system associated with the rift valley system in neighbouring Malawi. As an outgroup, *Pronothobranchius kiyawensis* was chosen as a representative of a genus closely related to *Nothobranchius*. Both genera are distinguished from all other cyprinodontiforms by the interarcual cartilage attaching directly to the cartilage of the second pharyngobranchial, and oval rather than round eggs; and from other aplocheiloids by an uncovered preopercular canal (e.g. Parenti, 1981). A list of all specimens analysed, with location data and GenBank accession numbers, is presented in Table 1.

Sequence alignment was performed with CLUSTAL W (Thompson *et al.*, 1994) as implemented in the "DNAStar" software package. Neighbour-joining (NJ) phylogenetic trees were calculated for each set of sequences with MEGA 4.0.1. The maximum parsimony (MP) analysis was performed with PAUP 4.0b10 (Swofford, 2003). Heuristic tree searches were performed with random addition of sequences for 500 replicates and gaps coded as fifth character state; a bootstrap test was performed with 10,000 replicates and identical tree search settings. The maximum likelihood (ML) analysis was carried out with 500 bootstrap replicates.

The results for the three datasets (16S, ND2, and combined) gave similar tree topologies with the three methods used (NJ,

Species	Population code	General location	Location coordinates	GenBank No. 16s/ND2
N. capriviensis	NA 01-1	Sakamanduna Pan, Namibia	17°38.654'S 24°17.664'E	KP842672/KP842693
N. kafuensis	ZAM 97-9	Kayuni, Zambia	16°07.529'S 27°33.085'E	KP842669/KP842682
N. kafuensis	ZAM 03-3	Nega Nega, Zambia	15°55.933'S 28°05.478'E	KP842668/KP842688
N. kafuensis	ZAM 03-1	Mambova, Zambia	17°38.367'S 25°14.410'E	KP842666/KP842686
N. kafuensis	ZMTW '94	Nanzhila River, Zambia	16°23.8'S 26°00.0'E	KP842667/KP842687
N. kafuensis		Chunga, Zambia	15°03'S 26°00'E	KP842664/KP842680
N. kafuensis		Lukanga Swamp, Zambia	14°28.333'S 28°17.850'E	KP842665/KP842684
N. malaissei	DRCH 2008-6	Sange, DRC	10°24.169'S 28°06.938'E	KP842671/KP842685
N. hassoni	DRCH 2008-10	Bunkeya, DRC	10°24.593'S 26°57.934'E	KP842663/KP842681
N. polli	DRCH 2008-2	Kyembe, DRC	11°01.790'S 27°17.965'E	KP842673/KP842689
N. rosenstocki	ZAM 07-8	Mansa, Zambia	11°15.845'S 29°02.571'E	KP842676/KP842695
N. rosenstocki	ZAM 07-7	Luapula River area, Zambia	12°02.413'S 29°28.074'E	KP842675/KP842690
N. rosenstocki	ZAM 07-10	Kasanka Nat. Park, Zambia	12°33.133'S 30°12.576'E	KP842674/KP842694
N. symoensi	ZAM 07-4	Luapula River area, Zambia	12°18.895'S 29°24.480'E	KP842677/KP842696
N. kirki	MW 91-15	Lake Chiuta area, Malawi	14°44'S 35°49'E	KP842670/KP842683
P. kiyawensis	GH 06-5	Ada, Ghana	05°46'N 00°37'E	EU401665/EU401645

Table 1: Specimens used for mitochondrial DNA analyses, with location data.

ML and MP). As the topologies of neighbour-joining, maximum parsimony (MP) and maximum likelihood (ML) trees were identical, only the NJ tree based on the combined dataset is presented in Figure 25.

Additional material used for comparison purposes

- *Nothobranchius kafuensis*: SAIAB 200553, six males 23.7–42.9 mm SL, three females 20.2–21.6 mm SL; Zambia: large seasonal pool with fringing reeds and grasses in a seasonal drainage system, adjacent to,

but not within, the present-day floodplain of the Zambezi River; 14.4 km west of the junction to the Kazungula ferry terminal, on the Livingstone–Sesheke road; near the village of Mambova; 17°38.367'S 25°14.410'E; population/collection code "Mambova ZAM 03-1"; B.R. Watters, J. Ippel and J. Bornman, 23 February 2003.
- *Nothobranchius kafuensis*: MRAC B5-01-P-1-4, three males 21.8–27.1 mm SL, one female 21.3 mm SL; collection data as for SAIAB 200553 above.

- *Nothobranchius kafuensis*: SAIAB 200554, four males 36.0–50.9 mm SL, one female 42.5 mm SL; Zambia: moderately large seasonal pool (dembo) with fringing grasses and reeds on west side of Monze–Mazabuka road, approximately 21 km north of Monze; 16°07.529'S 27°33.085'E; population/collection code "Kayuni ZAM 97-9"; B.R. Watters, T. Wood, P. Kearney, O. Schmidt, J. Ippel and N. Nathan, 2 April 1997.
- *Nothobranchius kafuensis*: SAIAB 200555, four males 31.8–39.4 mm SL, three females 29.4–31.5 mm SL; Zambia: small seasonal pool (dembo) with fringing reeds on Farm #19, close to the Monze–Mazabuka road, approximately 11 km north of Monze; 16°10.745'S 27°31.025'E; population/collection code "Kayuni ZAM 07-1"; B.R. Watters, B.J. Cooper, O. Schmidt and W. Bishopp, 18 March 2007.
- *Nothobranchius kafuensis*: MRAC B5-01-P-5-6, one male 39.2 mm SL, one female 33.9 mm SL; collection data as for SAIAB 200555 above.
- *Nothobranchius kafuensis*: SAIAB 200556, two males 42.9–47.0 mm SL, one female 35.1 mm SL; Zambia: large pool at a ford across the seasonal Nega Nega River (non-flowing at time of collection), on the south side of a minor road to the village of Nega Nega, 4.5 km northeast of the junction with the Mazabuka to Kafue River bridge road (junction 18 km south of Kafue River bridge); 15°55.933'S 28°05.478'E; population/collection code "Nega Nega ZAM 03-3"; B.R. Watters, J. Ippel, and J. Bornman, 23 February 2003.

Nothobranchius capriviensis, new species
(Figures 2–8)

Nothobranchius brieni non Poll, 1938: Van der Waal, 1976
Nothobranchius species Chobe River: Wildekamp, 1978
Nothobranchius species: Van der Waal and Skelton, 1984; Skelton, 1993
Nothobranchius species Caprivi: Watters and Wood, 1996
Nothobranchius kafuensis non Wildekamp and Rosenstock, 1989: Tweddle, 2007

Holotype. SAIAB 200550, male 39.3 mm SL; Namibia: Sakamanduna Pan, Gunkwe district, eastern Zambezi Region; two shallow seasonal pools on opposite sides of road, about 16 km directly south of Katima Mulilo (23 km by road) on the road to Linyanti via Gunkwe; pools with thick vegetation, black mud substrate, fringing reeds and grasses, and some relatively open areas with *Nymphea*; specimen was collected from the northeastern pool; 17°38.654'S 24°17.664'E; population/collection code "Caprivi NA 01-1"; J. Ippel and J. Bornman, 18 December 2001.

Paratypes. SAIAB 200551, two males, 37.2–37.9 mm SL, two females, 40.6–43.5 mm SL; same data as for holotype.

Paratypes. SAIAB 200552, five males, 32.1–49.7 mm SL, three females , 18.3–20.4 mm SL; Namibia: extensive seasonal pool in the Salambala Conservancy, about 50 km directly southeast of Katima Mulilo, eastern Zambezi Region; pool with dense vegetation, black mud substrate, fringing reeds and grasses, and a few relatively open areas with *Nymphea*;

17°50.008'S 24°35.998'E; population/collection code "Salambala NA 07-1"; B.R. Watters, B.J. Cooper, O. Schmidt, W. Bishopp and B.C.W. van der Waal, 15 March 2007.

Paratypes. MRAC B5-01-P-7-9, three males, 22.5–23.4 mm SL; MRAC B5-01-P-10 one female, 19.2 mm SL; same data as for paratypes SAIAB 200552 above.

Description

See Table 2 for morphometric and meristic data. This is a *Nothobranchius* species of moderate size with a pointed snout and subterminal mouth directed slightly upward. The head profile is slightly convex (to concave in older males) with no distinct transition to the dorsal profile. The dorsal profile is convex from the nape to the dorsal fin base. The caudal peduncle profile is slightly concave at the upper and lower sides.

The supra-orbital squamation is variable, but most specimens have G-type, partly covered with epidermal tissue anteriorly. The central supra-orbital neuromast systems are in two distinct shallow grooves, both sides of each lined with three shallow lobes; the posterior cephalic neuromast systems are in two curved pits. The dorsal fin rays number 15–18, the anal fin rays 16–18, and the position of the base of the first anal fin ray is under the base of dorsal fin ray 1 or 2. Scales in the median longitudinal line number 28–31, plus 1–3 on the caudal fin base, most with a shallow pit in the center and one neuromast. The transverse rows of scales above the pelvic fins number 12–14, and the scale count around the caudal peduncle is 14–16. The pelvic fins are short, not reaching the first anal fin

ray; the pectoral fins reach the first pelvic fin ray.

Male: Maximum size of specimens studied is 49.7 mm SL (61.0 mm TL). Maximum size observed in some wild-caught specimens and adult captive-bred specimens is up to about 65 mm TL. The body is laterally compressed and, compared to other *Nothobranchius* species, relatively deep. All unpaired fins are rounded, and the dorsal and anal fins are covered with a thin layer of epidermal tissue. Ctenii are present on all dorsal and anal fin rays, and project from the epidermis. The tips of the dorsal and anal fin rays project slightly from the fin membrane, more so in the Salambala population than in Sakamanduna population. The opercular membrane projects from the opercle.

Female: Smaller than male, with the maximum size of specimens studied being 35.1 mm SL (43.5 mm TL). Maximum size observed in wild-caught and adult captive-bred specimens is up to about 55 mm TL. The body is less compressed (mean 15.3 vs. 14.5% SL) and less deep than in the male (mean 30.0 vs. 35.4% SL). The dorsal and caudal fins are rounded, the anal fin is triangular with a rounded tip, rays 3–7 longer and more rigid. The anal fin is positioned more posteriorly than in the male (pre-anal length 63.9 versus 66.8% SL). No epidermal tissue or ctenii are present on the dorsal and anal fins. The opercular membrane does not project from opercle.

Coloration

Live male, Sakamanduna population (Figures 2–5): The body is light blue-gray with a brilliant blue-green iridescence, the abdomen white and the back gray-brown with a distinct yellow to golden

	N. capriviensis Sakamanduna NA 01-1					N. capriviensis Salambala NA 07-1			
	♂ H	♂ n = 3 (incl. H)		♀ n = 2		♂ n = 8		♀ n = 4	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
Standard length	39.3	37.2–39.3	38.1	34.1–35.1	34.6	22.5–49.7	29.8	18.3–20.4	19.3
Total length	118.6	118.6–124.5	122.3	119.1–123.9	121.5	122.7–129.9	125.1	116.1–124.0	119.3
Body depth	40.7	34.8–40.7	37.9	30.5–31.6	31.1	30.3–35.8	32.8	27.6–30.1	28.8
Body width	15.5	13.5–15.5	14.6	14.2–16.4	15.3	13.2–15.9	14.4	14.1–16.9	15.3
Head length	31.9	31.5–32.2	31.9	30.5–33.3	31.9	29.2–36.3	33.0	30.9–34.4	32.6
Eye diameter	8.9	8.1–8.9	8.6	9.1–9.4	9.3	8.0–10.9	9.3	9.3–9.9	9.5
Inter-orbital width	14.0	14.0–14.5	14.2	14.0–15.0	14.5	13.1–16.0	14.6	12.0–13.2	12.5
Snout to eye end	17.0	16.1–17.2	16.8	15.1–16.4	15.8	16.0–18.2	16.8	15.1–17.6	16.7
Snout length	10.4	8.2–10.4	9.3	6.0–7.3	6.7	6.2–8.7	7.0	6.9–8.2	7.7
Pre-dorsal length	63.1	62.1–63.1	62.8	60.4–64.4	62.4	56.3–60.2	58.2	55.7–64.4	60.0
Dorsal fin base	23.2	23.2–26.1	24.9	26.2–27.6	26.9	26.2–28.3	27.3	22.4–25.0	23.5
Pre-anal length	64.4	61.8–64.4	63.1	64.8–65.5	65.2	58.4–64.5	61.4	59.4–64.9	62.6
Anal fin base	21.6	21.6–25.0	22.9	16.4–16.5	16.5	20.0–23.7	21.9	15.2–17.5	16.5
Pre-ventral length	52.9	51.1–52.9	52.0	50.1–50.4	50.3	50.0–53.3	51.4	43.1–50.0	47.7
Caudal peduncle length	22.6	18.5–22.6	20.5	17.4–19.1	18.3	19.1–21.8	20.5	21.6–24.2	22.9
Caudal peduncle depth	15.5	14.0–15.9	15.1	12.0–13.4	12.7	13.2–14.8	14.1	12.0–13.2	12.7
Dorsal fin rays	16	16		17–18		15–17		16–17	
Anal fin rays	16	16–17		17–18		16–18		16–18	
Scales mid series	30(+1)	29–31(+1–2)		28–30(+1–2)		30–31(+2–3)		29–31(+1–2)	
Scales transverse	12	12		12		12–14		13–14	
Scales around caudal peduncle	14	14–16		16		15–16		15–16	

Table 2 (above): Morphometric and meristic data for males and females of the Sakamanduna NA 01-1 and Salambala NA 07-1 populations of *Nothobranchius capriviensis.* For morphometric data, Standard Length (SL) is in mm, all other measurements are expressed as a percentage of SL. The component of the mid-series scale count in parentheses represents the number of small scales on the hypural plate. H = holotype.

Table 3 (facing page): Morphometric and meristic data for males and females of various populations of *Nothobranchius kafuensis* used for comparison purposes. For morphometric data, Standard Length (SL) is in mm, all other measurements are expressed as a percentage of SL. The component of the mid-series scale count in parentheses represents the number of small scales on the hypural plate.

	N. kafuensis Nega Nega ZAM 03-3			N. kafuensis Kayuni ZAM 07-1				N. kafuensis Kayuni ZAM 97-9			N. kafuensis Mambova ZAM 03-1			
	♀ n = 1	♂ n = 2		♀ n = 4		♂ n = 5		♀ n = 1	♂ n = 4		♀ n = 4		♂ n = 9	
		Range	Mean	Range	Mean	Range	Mean		Range	Mean	Range	Mean	Range	Mean
Standard length	35.1	42.9–47.0	45.0	29.4–33.9	31.4	31.8–39.4	36.4	42.5	36.0–50.9	39.9	20.2–21.6	21.1	21.8–42.9	28.4
Total length	118.5	116.0–121.2	118.6	116.2–128.2	123.5	119.8–129.5	124.7	121.6	124.7–130.0	126.2	121.3–127.2	123.9	120.9–127.1	123.8
Body depth	28.8	30.3–33.3	31.8	26.8–27.6	27.3	31.7–35.5	34.1	30.4	32.4–34.2	33.4	26.9–29.7	28.5	25.1–38.9	31.9
Body width	14.2	14.7–17.9	16.3	12.9–15.0	13.8	14.0–18.6	15.3	15.8	11.0–14.2	13.0	14.9–17.3	16.2	11.0–16.1	13.3
Head length	28.8	27.4–29.8	28.6	29.9–32.7	30.4	30.7–36.0	33.4	30.4	30.8–32.9	31.9	31.6–36.2	33.7	29.5–33.5	31.6
Eye diameter	8.0	6.6–7.5	7.1	8.1–9.2	8.6	8.4–9.5	8.8	7.5	7.7–8.6	8.1	8.3–8.9	8.6	7.4–9.3	8.3
Inter-orbital width	10.5	12.1–12.4	12.3	9.8–12.9	11.3	12.8–14.1	13.5	11.5	13.0–15.5	14.6	13.9–15.0	14.2	11.9–14.8	13.6
Snout to eye end	19.1	15.6–16.2	15.9	14.1–16.7	15.3	15.5–17.3	16.6	16.2	14.5–16.1	15.2	16.4–17.6	17.0	14.7–16.8	15.8
Snout length	8.5	6.8–7.7	7.3	6.7–7.2	7.0	7.4–8.7	7.9	6.4	5.8–6.6	6.3	7.4–8.4	7.7	6.3–8.3	7.6
Pre-dorsal length	62.7	60.4–63.4	61.9	59.0–67.3	63.3	55.1–60.1	57.4	62.6	55.5–59.3	57.4	59.1–64.8	61.6	55.4–59.9	57.8
Dorsal fin base	25.1	23.8–24.9	24.4	22.4–24.1	23.1	29.6–31.4	30.6	23.5	28.5–32.2	30.9	20.2–23.3	22.0	22.9–29.0	24.9
Pre-anal length	67.0	59.9–61.5	60.7	63.8–68.0	65.6	60.4–62.8	61.3	68.9	60.5–62.5	61.9	61.1–67.6	64.6	57.9–63.3	60.3
Anal fin base	16.0	22.8–23.8	23.3	16.8–18.6	17.6	24.1–26.8	25.5	18.8	21.9–26.0	24.6	16.4–17.3	16.9	20.6–25.4	22.4
Pre-ventral length	50.4	49.7–50.9	50.3	53.4–54.6	53.9	51.3–57.8	53.2	52.7	50.3–54.4	51.6	50.5–55.8	52.8	47.4–52.5	50.6
Caudal peduncle length	16.2	18.3–19.1	18.7	18.0–20.1	19.2	17.6–20.5	18.9	18.1	14.7–18.1	16.6	21.6–23.8	22.6	15.8–24.8	18.4
Caudal peduncle depth	12.5	13.5–14.0	13.8	11.9–12.7	12.5	12.7–15.5	14.3	13.9	13.6–15.5	14.6	12.6–13.4	13.0	12.5–14.9	13.8
Dorsal fin rays	18	16–17		15–17		16–17		16	15–17		15–17		16–18	
Anal fin rays	16	15–16		16–17		17		16	16–17		16–18		16–18	
Scales mid series	30(+2)	30–31(+2–3)		29–31(+2–3)		30–31(+2–3)		28(+2)	29–32(+2–3)		29–31(+1–2)		30–31(+2–3)	
Scales transverse	13	13		12–13		12–13		13	13		13–14		13–14	
Scales around caudal peduncle	13	13		13		13		13	13		13–14		13–14	

hue. The posterior margins of most scales are pale gray to golden-yellow, the latter being a more prominent feature on the scales of the upper sides. The golden-yellow scale margin color becomes more extensively developed on the back resulting in an overall yellow-golden hue. The sides show a variable number of rearward-pointing, commonly irregular, chevron-shaped cross-bars of variable height, width and spacing. In general, these cross-bars are formed by dark red-brown to maroon scale margins. In some specimens the cross-bars may, in part, be arranged in pairs, producing a generally coarser pattern. The throat is pale blue-gray with no markings present. Two to three oblique bars, of the same color as the bars on the body, are present on the operculum. The projecting part of opercular membrane is light gray with a white margin.

The basal half of the caudal fin is light blue-gray to blue-green with a golden hue and irregularly dispersed dark red-brown to maroon spots. This component is followed distally by a dark red-brown or maroon band, a wider light blue submarginal band and a narrow black margin. The blue submarginal band may include some pale yellow flashes. The dorsal fin is light blue-gray to yellow-gray with irregular, dark brown to maroon stripes and spots which become denser and smaller distally. The base of the dorsal fin usually shows five to seven distinct, relatively large and angular, dark brown to maroon markings. Most specimens show a concentration of a few small black markings along the anterior marginal part of the dorsal fin. The proximal part of the anal fin is light blue-gray to yellow-gray with some dark red-brown to maroon spots. This zone is followed by an irregular, and commonly interrupted, dark red-brown to maroon band. The broad outer part of the fin, comprising approximately one-third, is usually light blue with yellow overtones. In some specimens the yellow color is strongly developed and may be the dominant color in this part of the fin. A narrow black margin may be present. The pelvic fins have a color pattern similar to that of the anal fin although the dark red-brown to maroon spots may be absent. The pectoral fins are hyaline with pale blue or blue and yellow posterior margins. The iris is golden-brown with a golden ring around the pupil and a vertical black bar.

Live male, Salambala population (Figures 6–7): In general, the color pattern is similar to that of males from the Sakamanduna population. The development of golden-yellow scale margins on the body is somewhat more prominent than in the Sakamanduna population, in some specimens extending from the back to the lower flanks. There is also a greater tendency for cross-bars on the body to be arranged in pairs, producing a generally coarser pattern. A yellow color in the outer part of the anal fin is more common in males of this population.

Live female (Figure 8): The body is pale gray-brown, darker on the back, grading to white on the abdomen. Scales on the back and upper sides have reflective light blue centers and narrow gray-brown margins, producing a faint reticulated pattern. Faint, narrow cross-bars may occur on the rear part of body, produced by a local widening of scale margins. Both the paired and unpaired fins are colorless. The iris is pale golden with an indistinct, relatively dark vertical bar.

Figure 2: *Nothobranchius capriviensis*, wild-caught male. Location Sakamanduna northeastern pan, eastern Zambezi Region, Namibia. Population code NA 01-1. Photograph by B.R. Watters.

Figure 2: *Nothobranchius capriviensis*, SAIAB 200550, holotype, male, 39.3 mm SL. Location Sakamanduna northeastern pan, eastern Zambezi Region, Namibia. Population code NA 01-1. Photograph by R.H. Wildekamp.

Figure 4: *Nothobranchius capriviensis*, wild-caught male. Location Sakamanduna northeastern pan, eastern Zambezi Region, Namibia. Population code NA 95-1. Photograph by B.R. Watters.

Figure 6: *Nothobranchius capriviensis*, wild-caught male. Location Salambala Conservancy, eastern Zambezi Region, Namibia. Population code NA 07-1. Photograph by B.R. Watters.

Figure 5: *Nothobranchius capriviensis*, wild-caught male. Location Sakamanduna northeastern pan, eastern Zambezi Region, Namibia. Population code NA 95-1. Photograph by B.R. Watters.

Figure 7: *Nothobranchius capriviensis*, wild-caught male. Location Salambala Conservancy, eastern Zambezi Region, Namibia. Population code NA 07-1. Photograph by B.R. Watters.

Figure 8: *Nothobranchius* capriviensis, wild-caught female. Location Sakamanduna northeastern pan, eastern Zambezi Region, Namibia. Population NA 95-1. Photograph by B.R. Watters.

Figure 9: *Nothobranchius kafuensis*, wild-caught male, Phenotype 2. Location Kayuni, Kafue Flats, southwestern Zambia. Population code ZAM 07-1. Photograph by B.R. Watters.

Diagnosis and Comparison

Nothobranchius capriviensis is known only as a blue form, in contrast to *N. kafuensis*, its closest relative, that occurs as red, blue and intermediate forms (Phenotypes 1, 2 and 3, respectively; see Appendix and Figures 9–17). While there are general similarities in male coloration between *N. capriviensis* and the blue form (Phenotype 2) of *N. kafuensis*, there are also consistent differences. The male *N. capriviensis* can be distinguished by the paler shade of blue than the blue phenotype of *N. kafuensis*, the lack of orange-red scale margins and the orange-red color on the head of the latter species, and a more prominent and consistent barring on the body of the former. Differences in female coloration (Figures 8 and 18) are not as obvious – scale centers of *N. capriviensis* are iridescent light blue, whereas those of female *N. kafuensis* are less prominently blue and have a silver hue.

Morphologically and meristically, the male *N. capriviensis* differs from that of *N. kafuensis* (Table 3) by a deeper body (mean 35.4 versus 31.4% SL), longer head (mean 32.5 versus 30.7% SL), a more posterior position of the anal fin (pre-anal length 62.3 versus 60.5% SL) and a higher number of scales around the caudal peduncle (14-16 versus 13-14). Female *N. capriviensis* have a more posterior position of the anal fin (pre-anal length 63.9 versus 66.8% SL) when compared with *N. kafuensis*.

Nothobranchius capriviensis can also be distinguished from *N. kafuensis* on the basis of mtDNA analyses (see Discussion section). The genetic data clearly show the close ancestral relationship between the two species.

Figure 10: *Nothobranchius kafuensis*, wild-caught male, Phenotype 1. Location Nega Nega, Kafue Flats, southwestern Zambia. Population code ZAM 92-1. Photograph by B.R. Watters.

Figure 11: *Nothobranchius kafuensis*, wild-caught male, Phenotype 2. Location Nanzhila River floodplain, southwestern Zambia. Population code ZMTW '94. Photograph by B.R. Watters.

Figure 13: *Nothobranchius kafuensis*, wild-caught male, Phenotype 3. Location Mambova, southwestern Zambia. Population code ZAM 03-1. Photograph by B.R. Watters.

Figure 12: *Nothobranchius kafuensis*, wild-caught male, Phenotype 2. Location Mambova, southwestern Zambia. Population code ZAM 03-1. Photograph by B.R. Watters.

Figure 14: *Nothobranchius kafuensis*, wild-caught male, Phenotype 1. Location Mambova, southwestern Zambia. Population code ZAM 03-1. Photograph by B.R. Watters.

Figure 15: *Nothobranchius kafuensis*, male, Phenotype 1. Location Lukanga Swamps, Kafue River system, southwestern Zambia. Photograph by A. Persson.

Figure 17: *Nothobranchius kafuensis*, male, Phenotype 1. Location Chunga, Kafue River floodplain, southwestern Zambia. Photograph by A. Persson.

Figure 16: *Nothobranchius kafuensis*, male, Phenotype 1. Location Lukanga Swamps, Kafue River system, southwestern Zambia. Photograph by A. Persson.

Figure 18: *Nothobranchius kafuensis*, female. Location Kayuni, Kafue Flats, southwestern Zambia. Population code ZAM 07-1. Photograph by B.R. Watters.

Etymology

The species name *capriviensis* represents a toponym after the Caprivi Strip, the historical name for the narrow extension of northeastern Namibia, where this species occurs. This part of Namibia was originally named after the German statesman Count Leo von Caprivi. It is suggested that the common name for this new species should be: Caprivi *Nothobranchius*.

Distribution

Nothobranchius capriviensis appears to have a very restricted range of distribution, confined to the eastern part of the Zambezi Region (formerly the Caprivi Strip) of Namibia, and situated between the Zambezi River to the north and the Chobe River to the south, in the western marginal zone of, and immediately west of, the present-day floodplain of those two rivers (Figure 1). The majority of habitats are underlain by alluvial deposits of an older floodplain. All known localities are within an approximate 26 km radius of one another, with Bukalo village approximately at the center of distribution. Principal occurrences reported prior to Tweddle *et al* (2014, this issue) are:

- Sakamanduna Pans in the Gunkwe District, about 16 km south of Katima Mulilo (Van der Waal and Skelton, 1984) (Figures 19 and 20), southwestern pan location 17°38.787'S 24°17.490'E, northeastern pan location 17°38.654'S 24°17.664'E.
- About 5 km southeast of Bukalo, or 36 km southeast of Katima Mulilo (Figure 21), location 17°45.067'S 24°33.617'E.
- Salambala Conservancy immediately north of the Chobe Swamp region, generally northwest of Ngoma Bridge (Figure 22), location 17°50.008'S 24°35.998'E.
- A small isolated pool in a seasonal stream associated with the permanent Mutwalwizi Channel, in the Lusese District (Van der Waal and Skelton, 1984), about 25 km east-northeast of Bukalo, location approximately 17°40.916'S 24°44.030'E (Tweddle *et al*., 2014, this issue).

Recent field work has revealed the presence of *Nothobranchius capriviensis* at additional locations in the eastern Zambezi Region, generally within the area covered by the above-mentioned sites (Tweddle *et al*., 2014, this issue).

Habitats

The Sakamanduna and Salambala sites, where the type specimens were collected, comprise large, relatively shallow ephemeral pools (Figures 19, 20 and 22). The former comprises two pools on either side of a road, but in the same extensive depression or "pan", and having a northeast-southwest alignment to one another. The type specimens were collected from the northeastern pool. As with all *Nothobranchius* habitats these pools dry up completely on a seasonal basis. *Nothobranchius capriviensis* is a typical annual cyprinodontiform fish. Eggs deposited in the mud substrate by the adult fish survive therein through the dry season, experiencing a number of phases of development with intervening diapauses. The eggs then hatch at the onset of the rainy season; growth of the fry is rapid and sexual maturity may be attained within four to six weeks. The substrate at both localities,

Figure 19: Type locality for *Nothobranchius capriviensis*, location Sakamanduna northeast pan, Gunkwe district, eastern Zambezi Region, Namibia; April 11, 1995. Photograph by B.R. Watters.

Figure 20: Habitat for *Nothobranchius capriviensis*, location Sakamanduna southwest pan, Gunkwe district, eastern Zambezi Region, Namibia; March 15, 2007. Photograph by B.R. Watters.

Figure 21: Habitat for *Nothobranchius capriviensis*, location Bukalo, eastern Zambezi Region, Namibia; March 15, 2007. Photograph by B.R. Watters.

Figure 22: Habitat for *Nothobranchius capriviensis*, location Salambala Conservancy, eastern Zambezi Region, Namibia; March 15, 2007. Photograph by B.R. Watters.

Figure 23: Typical black mud substrate of a *Nothobranchius capriviensis* habitat, location Sakamanduna southwestern pan, Gunkwe district, eastern Zambezi Region, Namibia; April 11, 1995. Photograph by B.R. Watters.

and also at the third major known locality near Bukalo, consists of fine, soft black mud rich in swelling clays and characteristic of *Nothobranchius* habitats (Watters, 2009) (Figure 23).

Habitat conditions at the Sakamanduna locality (Figure 19) were not measured at the time the type specimens were collected; however, the first author collected specimens from the same place in 1995 (Watters and Wood, 1996) at the end of the rainy season, and determined the following parameters:

- Northeastern pool (Figure 19): at about midday, April 11, 1995, approximately 50 x 30 m in areal extent, maximum water depth about 1 m, highly turbid, pH = 7.6, conductivity = 140 µS, TDS = 70 ppm, temperature = 28°C (82°F). Vegetation within the area of flooding comprised marginal grasses and sedges that in places extended into the central parts of the pool. Areas of open water included some *Nymphea*. It is estimated that when fully flooded, the area might be as much as 100 x 50 m.

- Southwestern pool (Figure 20): at 08:00 hours on April 11, 1995, residual pool about 5 m in diameter, water depth 20–30 cm, highly turbid, pH = 7.2, conductivity = 320 µS, Total Dissolved Solids (TDS) = 160 ppm, temperature = 19°C (66°F). It is estimated that when fully flooded, the area under water would approximate 130 x 40 m. The pool was densely vegetated with mainly short grasses and sedges, leaving only small areas of open water, some with *Nymphea*.

Habitat conditions at the Salambala locality (Figure 22), situated within the Salambala Conservancy, were measured at the time the type specimens were collected early in the rainy season (12:00 hours on March 15, 2007), as follows: water moderately turbid, pH = 6.6, conductivity = 153 μS, TDS = 78 ppm, temperature = 31°C (88°F) in marginal shallows where fish were most abundant. Areal extent of the pool was estimated at about 400 x 450 m and, because of the large size, maximum water depth could not be accurately determined; however, according to conservancy officials, the depth can be as much as 2 m. The pool was densely vegetated with a variety of aquatic plants and a thick growth of marginal grasses and sedges. Some relatively open areas of water had abundant *Nymphea*.

Biogeographical Relationships

The evolution of topography and drainage systems over time has been a major factor influencing the distribution and evolution of *Nothobranchius* fishes (Watters, 2009) and such factors have been important in determining the relationships between *N. capriviensis* and the closely related *N. kafuensis*, and also the present distribution of both species.

As suggested by Watters (2006, 2009), the two principal mechanisms involved in the dispersal of *Nothobranchius* fishes and, therefore, important factors controlling their distribution, are: flooding events on floodplains, and the evolution of landscape with consequent changes in drainage patterns. Topography and drainage are closely linked in that the former will determine the patterns of the latter and both will also be strongly controlled by geological setting and processes such as faulting and flexure leading to uplift or subsidence. Changing landscape and drainage can promote the dispersal of *Nothobranchius* fishes, and can also lead to the development of physical and ecological barriers that can separate and isolate populations of species, which may then evolve and diverge, leading to local variation or new species (Watters, 2009).

Most commonly, *Nothobranchius* habitats are situated in relatively low-lying flat areas, especially floodplains, where water can stand and form seasonal pools, and where a suitable habitat substrate is most likely to occur. Dispersal within any particular floodplain or system of linked floodplains will involve the fishes being transported from existing *Nothobranchius* habitats to other parts of the floodplain by flowing water and migration through shallow standing water. This will be a slow process but, over the long term (perhaps millions of years), all suitable sites will become inhabited. Features such as waterfalls, cataracts, rapids and broad strongly flowing permanent rivers can form barriers preventing the spread of a poor-swimming non-riverine fish such as *Nothobranchius* and may, therefore, constitute marked boundaries to the ranges of species.

One important component of drainage system evolution is the headward erosion of rivers into drainage divides, to the point where they connect with, or "capture", the water flowing in an adjacent drainage system. In that way, *Nothobranchius* species from the captured system can disperse into the other system and vice versa. Another result of river capture can be the isolation of fish populations in the cut-off sections of the original drainage system.

Geological and geomorphological processes such as these will be ongoing over long periods of time, millions of years in many cases. Therefore, in order to understand the broader, as well as more localized, aspects of the distribution of *Nothobranchius*, it is necessary to examine not only the present topography and drainage, but also the past configuration and evolution of these features through relatively recent geological time.

With only one exception, known populations of *N. kafuensis* in Zambia occur on the floodplain of the Kafue River and associated tributaries and swamps. The northeasternmost known localities are in the fringes of the Lukanga Swamp and the associated part of the upper Kafue River system. Following the river system downstream, the next group of localities occurs in the middle reaches in the northern and central parts of the Kafue National Park; for example, the Chunga locality (Figure 1). Further south, the Kafue River makes a sharp bend and flows eastwards, eventually joining the Zambezi River. In the lower part of the river system, downstream of the sharp bend, and specifically on the so-called Kafue Flats, numerous other populations occur (Figure 1); for example, Lochinvar National Park, Kayuni, Nega Nega and Shibuyunje. At the southern end of the Kafue National Park there are two populations presently indirectly associated with the Kafue system; one of these occurs on the floodplain of the Nanzhila River (Figure 1), a tributary to the Kafue.

Only one occurrence of this species is known from outside the present-day Kafue River system and that is the Mambova population, occurring to the south-southwest of the Kafue National Park region and only about 13 km distant from the Zambezi River (Figure 1). The Mambova locality occurs within a seasonal drainage system outside the present-day Zambezi floodplain, and at a somewhat higher elevation, but which flows in a southwestward direction terminating against the northeastern margin of it. The presence of the Victoria Falls and various rapids between the Mambova site and the confluence between the Kafue and Zambezi rivers rules out the possibility of this apparently disjunct population being the result of dispersal from lower reaches of the present-day Kafue River.

Populations of *N. capriviensis* are restricted to a small area in the eastern Caprivi between the Chobe and Zambezi rivers. The principal locations of this species lie between about 50 and 100 km west to west-southwest of the Mambova population of *N. kafuensis* but separated from it by the Zambezi River, a very large strongly-flowing river that constitutes a formidable barrier to the migration of a poor-swimming, non-riverine fish such as a *Nothobranchius* species.

Interpretation of the nature and distribution of ancient fluvial sediments, and information about the position and timing of crustal tectonic events that affect topography, make it possible to reconstruct the patterns of ancient drainage systems in south-central Africa (Moore and Larkin, 2001; Moore *et al.*, 2008; Moore *et al.*, 2012). Based on the geomorphological reconstructions of these authors it is possible to develop a plausible hypothesis for the distribution of, and relationships and evolutionary links between, *N. capriviensis* in the Zambezi Region of Namibia and populations of *N. kafuensis* in Zambia.

According to Moore and Larkin (2001) and Moore *et al*. (2012), during **Miocene times (23–5 Ma)** the drainage systems in south-central Africa were as shown in Figure 24A. Notably, the Proto-Kafue River constituted the lower part of the more extensive Paleo-Chambeshi River system, which drained southwestwards through the present-day Caprivi region, linking with the Paleo-Upper Zambezi, to drain into an endoreic or "closed" drainage basin, the so-called Kalahari Basin, in what is now northern Botswana. The Paleo-Middle Zambezi River was not, at that stage, linked to the Paleo-Upper Zambezi, but was undergoing headward erosion towards it. Significantly, the eastward-trending lower part of the present-day Kafue River did not exist at that time. Note that during pre-Miocene, late Paleogene, times the Paleo-Chambeshi and Upper Zambezi system drained into the Indian Ocean via the Proto-Limpopo River (Moore and Larkin, 2001). However, this link was disrupted by uplift along the Ovambo-Kalahari-Zimbabwe (OKZ) Axis (Figure 24A), together with subsidence of the Kalahari Basin, leading to the situation that existed during the early Miocene.

The superimposition of some representative present-day populations of *Nothobranchius* fishes on Figure 24A suggests that populations of a common ancestral form would have inhabited the floodplain of the Proto-Kafue River, ultimately giving rise to various populations of *N. kafuensis* (Lukanga Swamp, Kafue National Park, Nanzhila, Mambova) and also to *N. capriviensis*. Notably, at this stage no barriers existed between populations of the ancestral form situated at the southwestern end of the Proto-Kafue system and which

were eventually to evolve into two distinct species, *N. capriviensis* and *N. kafuensis*.

Headward erosion of the Paleo-Middle Zambezi resulted in the capture of the Paleo-Upper Zambezi and the Paleo-Chambeshi (including the Proto-Kafue component), diverting the flow of these major systems from the endoreic Kalahari Basin (Figure 24B). The timing of this capture event has been ascribed a **Late-Pliocene to Early-Pleistocene age (about 2.6 Ma)** (Moore and Larkin, 2001; Moore and Cotterill, 2010).

It is likely that this capture and rearrangement of drainage systems constituted the primary event that initiated the isolation of those populations of the ancestral *Nothobranchius* form occurring in what is now the Caprivi region (and which ultimately gave rise to *N. capriviensis*) from those to the northeast that gave rise to *N. kafuensis*. At this stage, the former would have been separated from the latter by the strongly-flowing Zambezi River, which would have constituted a significant barrier to a poor-swimming non-riverine fish such as *Nothobranchius*.

During **Early-Pleistocene times (about 1.8 Ma)** (Figure 24C) the link between the Paleo-Upper Zambezi and the Paleo-Middle Zambezi was severed as a result of uplift associated with the northeasterly-trending Linyanti and Chobe faults (along the line of the present-day Linyanti and Chobe river systems), transverse to the flow of that section of the Paleo-Zambezi. This, in turn, caused the Paleo-Upper Zambezi, and probably also the Proto-Kafue, to drain into a vast ancient lake system, the Makgadikgadi Paleo-Lake system, collectively referred to as Paleo-Lake Deception by Moore *et al*. (2012). This major

inland lake was also fed by other major rivers such as the Paleo-Cuando and the Paleo-Cubango/Okavango. During this time Paleo-Lake Deception would have flooded to the northeast across part of what is now the Caprivi region, into the Machili basin of what is now southwestern Zambia. The configuration of this flooding, and the uplift due to faulting, presumably took place in a manner that maintained the isolation of ancestral populations of *N. capriviensis* from those of *N. kafuensis*. It can be speculated that the former were confined to the shore regions of Paleo-Lake Deception lying immediately northwest of the uplifted zone associated with these faults, while the nearest populations of the *N. kafuensis* occurred in what is now the Mambova area then situated to the immediate east of the uplift associated with the Chobe fault (Figure 24C). Such a scenario could also account for the fact that no populations of *N. capriviensis* are known from the area in northern Botswana, immediately southeast of the Chobe River as that would have been on the uplifted zone or to the southeast of it.

Also during the Early-Pleistocene, the link between the Upper Chambeshi and the Proto-Kafue was severed due to uplift of the Congo-Zambezi watershed, the former initially establishing an endoreic lake (Paleo-Lake Bangweulu) before becoming a Congo River tributary draining into the Luapula River system (Moore *et al*., 2012).

During **Mid-Pleistocene times (about 0.8 Ma)**, as a result of headward erosion of the Paleo-Middle Zambezi into the fault-bounded Chobe horst, and sediment build-up in the northeastern part of the Paleo-Lake Deception basin, the link between the upper and middle Paleo-Zam-

bezi was restored (Figure 24D), a situation that exists to the present-day. Some other major rivers flowing into the ancient lake were also diverted (mainly to flow into the Zambezi River) causing Paleo-Lake Deception to shrink and form Paleo-Lake Makgadikgadi, and eventually, the Okavango Swamps and Makgadigadi Pans of present-day Botswana.

From Figure 24C it can be seen that the Mambova population of *N. kafuensis* is situated near the southwestern end of the "Paleo-Kafue" and, therefore, associated with the same ancient drainage system as the Nanzhila, Kafue National Park, and Lukanga Swamp populations of the species. The disjunct nature of this population, relative to other known populations of this species associated with the present-day Kafue River, is the result of a change in course of the Kafue that dates back to Mid-Pleistocene times. Notably, prior to the Mid-Pleistocene (Figures 24A, 24B and 24C) the eastward-trending lower part of the present-day Kafue River did not exist and, almost certainly, nor did the *N. kafuensis* populations that we now find associated with the lower reaches of that river (e.g. Lochinvar, Kayuni, Nega Nega, Shibuyunje).

Following the break between the Upper Paleo-Chambeshi and the Proto-Kafue, the latter continued to drain into the Paleo-Zambezi through the Machili Flats situated at the lowermost reaches of that system. However, that link was severed by tectonism causing uplift during the Mid-Pleistocene (Moore *et al*., 2012). The Paleo-Kafue then drained into, and maintained, a major endoreic lake, referred to as Paleo-Lake Patrick (Simms, 2000), situated over what represents the present-day
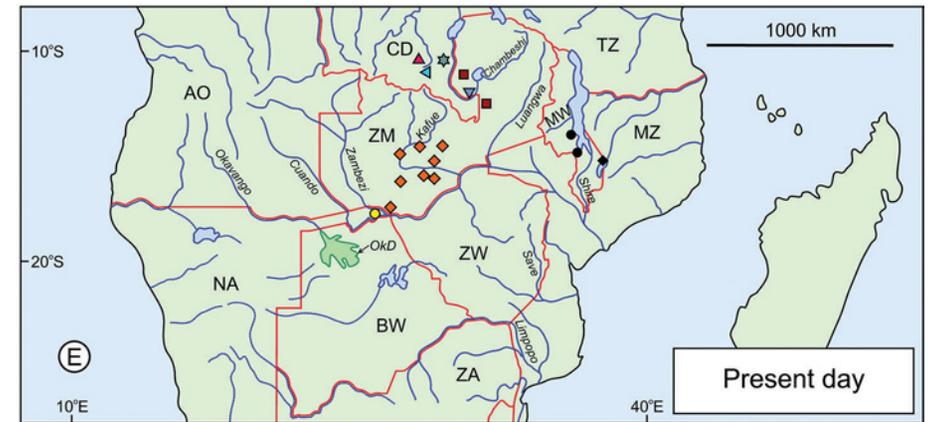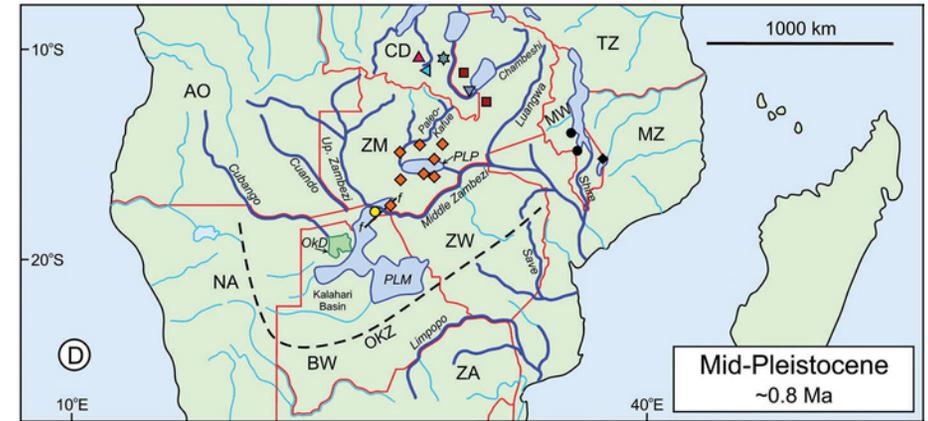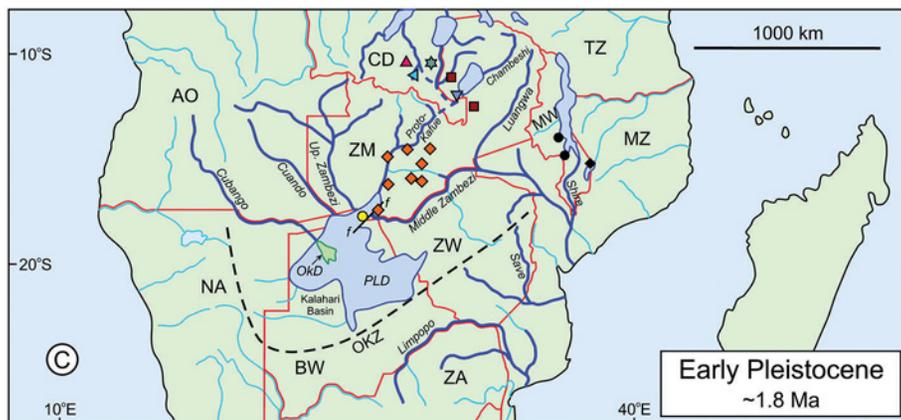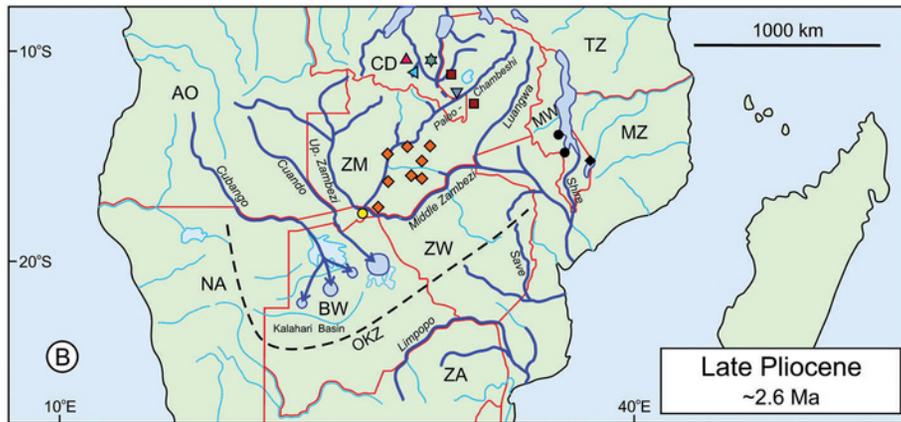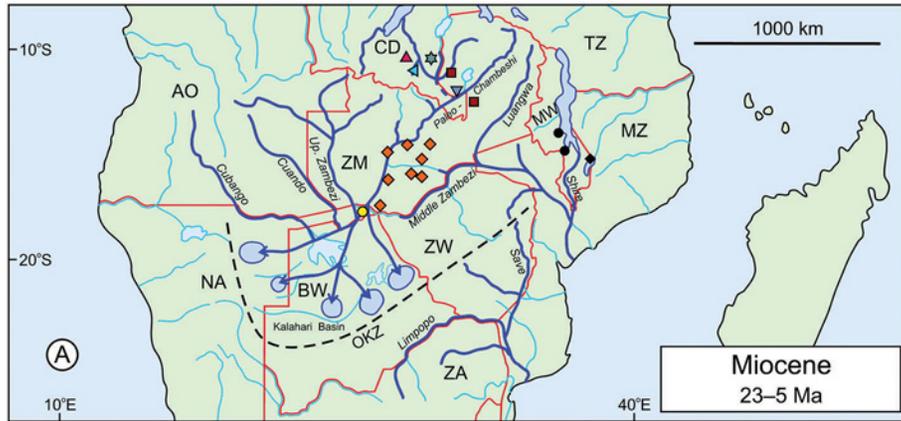
Figure 24 (facing page and above): Maps of central southern Africa showing the evolution of drainage systems from the Miocene (A), through Late Pliocene (B), Early Pleistocene (C) and Mid-Pleistocene (D), to the present-day (E). The present-day distribution of *N. capriviensis* and *N. kafuensis* populations is superimposed on the maps. Individual locality symbols may represent multiple populations that are in close proximity to one another. Relevant ancient drainage patterns, represented by heavy blue lines, have been superimposed on the present-day drainage (thin blue lines and pale blue-gray lakes). The blue areas edged in dark blue represent ancient lakes or drainage basins. Also shown are the locations of some other *Nothobranchius* species that were included in the genetic component of the present study. Ancient drainage systems not relevant to the present discussion have been omitted. Present-day country boundaries (red lines) are included for reference purposes. Configuration of ancient drainage systems adapted after Moore and Larkin (2001), Moore *et al*. (2008) and Moore *et al*. (2012).

Key to locality symbols and acronyms: yellow-filled circle = *N. capriviensis*; orange-filled diamond = *N. kafuensis*; rust-filled square = *N. rosenstocki*; blue-filled, downward-pointing triangle = *N. symoensi*; blue-green-filled, left-pointing triangle = *N. polli*; blue-gray-filled star = *N. malaissei*; red-filled triangle = N. hassoni; solid black circle = N. wattersi (Chia and Golomoti populations); solid black diamond = *N. kirki* (Chiuta population); OKZ = Ovambo-Kalahari-Zimbabwe Axis; OkD = Okavango Delta; PLD = Paleo-Lake Deception; PLM = Paleo-Lake Makgadigadi; PLP = Paleo-Lake Patrick; ZM = Zambia; ZW = Zimbabwe; ZA = South Africa; NA = Namibia; MZ = Mozambique; MW = Malawi; TZ = Tanzania; CD = Democratic Republic of Congo; AO = Angola; f—f = Chobe fault.

Kafue Flats (Figure 24D). This event effectively isolated the Mambova population of *N. kafuensis* from those situated in the middle and upper reaches of the Paleo-Kafue (i.e. Lukanga Swamp, Kafue National Park and Nanzhila populations) and may also have initiated the establishment of populations of *N. kafuensis* in areas peripheral to Paleo-Lake Patrick.

At that time there was also a tributary to the Paleo-Middle Zambezi which, by Mid-Pleistocene times, had eroded headward in a westerly direction, eventually to cause the complete draining of Paleo-Lake Patrick and the "capture" of the Paleo-Kafue system. This process resulted in the latter being diverted sharply eastward into the **present-day** drainage pattern (Figure 24E). This further allowed representatives of the populations of *N. kafuensis* in the middle and upper parts of the system to migrate downstream and populate the floodplain on the Kafue Flats associated with the new, eastward-trending lower component of the Kafue River system (i.e. Lochinvar National Park, Kayuni, Nega Nega, Shibuyunje). It is likely, therefore, that these populations of *N. kafuensis* represent the most recently established sites of the species, whereas the others, including the Mambova population, represent older populations.

Conservation Status

Nothobranchius capriviensis was first listed as "endangered" in the South African Red Data Book (Skelton, 1987). In the 1994 IUCN Red List of Endangered Species, *N. capriviensis* (listed as *Nothobranchius* sp. Caprivi) was included with "Endangered" status (Groombridge, 1994). However, in the 2013 list it was included under the name *Nothobranchius kafuensis*

in the category "Least Concern", based on the interpretation that it was a form of that species, which is relatively widespread in nearby Zambia (Tweddle, 2007).

Watters and Wood (1996) stated: "We consider this population of *Nothobranchius* [Caprivi] to be extremely rare in the wild and, therefore, susceptible to habitat destruction by human activities". Further proposals for the conservation of *N. capriviensis* were made by Tweddle (2009), advising that special attention be paid to the limited number of populations and their habitats, as any development project, such as road improvement, may threaten this species. Further, "The protection of *Nothobranchius kafuensis* 'Caprivi' [now *N. capriviensis*] population in the Salambala Pan and neighbouring pans in an effectively functioning conservancy, and the recognition by the conservancy committee of the importance of its presence in the conservancy area and the need to conserve it as a valuable contribution to biodiversity conservation" (Tweddle, 2009). Presently, most of the pans where *N. capriviensis* occurs are situated in the Salambala Conservancy and the Conservancy management committee is well aware of the presence of the fish and its conservation value. The present conservation status of *N. capriviensis* has been discussed in detail by Tweddle *et al.* (2014, this issue) who recommend that it be placed in the "Endangered" category.

Discussion

The phylogenetic tree shown in Figure 25 indicates a close, but divergent, relationship between *N. capriviensis* and *N. kafuensis*, supporting the contention that they represent two distinct species and that distinction is, as discussed earlier, also ex-

pressed by differences in morphometric and meristic character and color pattern (see section "Diagnosis and Comparison").

The genetic data as represented in Figure 25 supports a monophyletic origin for the *N. kafuensis* populations. Further, there is an indication of three clusters for the populations presently regarded as *N. kafuensis*. These genetic relationships can be explained on the basis of the biogeographic evolution as discussed earlier. One cluster includes those populations found in the upper and middle Kafue River basins, including the Lukanga Swamp and Chunga populations. These represent early populations derived from forms that inhabited the middle reaches of the Proto-Kafue system (Figure 24C). A second cluster comprises the Mambova and Nanzhila River populations, which were derived from forms associated with the lower reaches of the ancient Proto-Kafue system. When the lower section of the Proto-Kafue became disconnected from the middle section by uplift during Mid-Pleistocene times (Figure 24D), these populations became relict, especially in the case of the Mambova occurrence. The Nanzhila population was able to retain a connection with the middle Kafue system by virtue of being to the north of the zone of uplift where new tributary drainage, such as the Nanzhila River, then flowed northwards into the Kafue basin. The third cluster includes the Kayuni and Nega Nega populations, which represent a group of relatively recent sites established from the time when the middle Proto-Kafue was diverted to form Paleo-Lake Patrick and was eventually captured by a headward-eroding tributary of the Zambezi River (Figure 24D). Morphologically, all populations are similar (Table 3)

and, with the exception of the Mambova population, all include either Phenotype 1 (red/orange) forms or Phenotype 2 (blue) forms (see Appendix for a description of the phenotypes). The Mambova population is different in that it comprises all three known phenotypes, including Phenotype 3 (intermediate) which is unique to this occurrence. It would appear that isolation of the Mambova population led to a degree of separate development, as evidenced by the co-existing color forms. Populations that were associated with the middle reaches of the Proto-Kafue system include only Phenotype 1, whereas those associated with the more recently established eastward-flowing section of the present-day river, on the Kafue Flats, may comprise either Phenotype 1 or Phenotype 2 forms, but not both. The Nanzhila population includes Phenotype 2 forms only but these are somewhat different from the blue forms on the Kafue Flats in the lack of a barred pattern on the body. Males of this phenotype from the Mambova population also show this characteristic, which is consistent with the suggestion that the two populations represent relict occurrences once associated with the lower reaches of the ancient Proto-Kafue system.

The phylogenetic relationships shown by Figure 25 suggest that *N. capriviensis* and *N. kafuensis* form a clade with *N. malaissei*, *N. hassoni*, *N. polli*, *N. rosenstocki* and *N. symoensi*, with the latter two species showing a less close relationship than the former three. However, further elucidation of these relationships, and the biogeographical factors involved, are beyond the scope of this paper. *Nothobranchius kirki*, from Malawi, would not appear to constitute part of this clade.
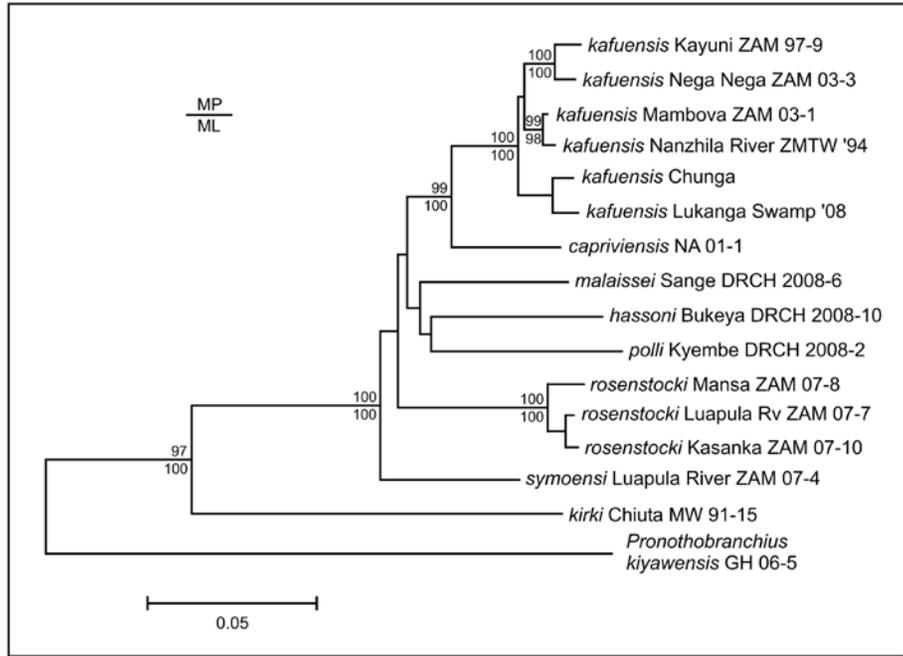
Figure 25: Neighbour-joining analysis of combined sequence dataset. Numbers at nodes are bootstrap values for the MP (above) and ML (below) analyses.

Nothobranchius capriviensis can be placed into the *N. brieni* species group, as based on the following set of defining characters suggested by Nagy (2014b): "caudal and anal fins proximal portion spotted, distal part with light margin or light sub-marginal band and dark margin; an absence of a dark distal margin on the dorsal fin; an absence of spots on head; and an absence of black posterior margin of scales." Based on the criteria of Radda (1969), *N. capriviensis* should be placed in the subgenus *Zononothobranchius*.

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Appendix:
Coloration of male
Nothobranchius kafuensis

Since the original description of *N. kafuensis* by Wildekamp and Rosenstock (1989), a number of new populations of this species have been discovered that expand our knowledge of the color variability of male specimens. Female specimens do not show any concomitant variability in color pattern. The discovery of a population near the village of Mambova in southwestern Zambia by one of the authors (BRW) and colleagues, has shown that the species includes three color morphs or phenotypes, rather than only the two previously known. For that reason, and to provide a reference for comparison with *N. capriviensis*, an updated definition of color characteristics for male *N. kafuensis* is provided below.

Phenotype 1, live male (Figures 10, 14–17): Specimens comprising this color form represent the so-called "red phenotype" described by Wildekamp and Rosenstock (1989). Scales on the body and head have light blue centers with orange-red margins forming a reticulated pattern. In some populations this reticulation may be slightly irregular and appear as irregular cross-bars that are most prominent on the rear part of the body. The width of the orange-red scale margins can be variable at both intra- and inter-population levels, resulting in some populations having a strong overall orange-red body color. Scale centers on the back may have an orange-red hue while those on the abdomen may be of a relatively lighter shade of blue or even white. The head, throat and jaws are deep orange-red. The part of the branchiostegal membrane projecting beyond the gill plate is orange-red with a narrow white margin. The iris is light golden-brown or golden-green with a golden ring around the pupil and a vertical black bar.

The dorsal fin is light blue-green with a heavy marbling resulting from an irregular pattern of red-brown spots and stripes that ranges from coarse near the fin base to fine distally.

The basal part of the anal fin is light blue to blue-green with a heavy, coarse marbling of red-brown markings. This basal component grades into the outer two-thirds to half of the fin that is commonly a clear orange-red with a black margin. Some populations may show a light blue submarginal band and males of this phenotype from the Mambova population may also have yellow flashes in the orange-red zone (Figure 14). The light blue submarginal band is most commonly narrow (Figures 14 and 15) but in some specimens from the Lukanga population it is broad, making up as much as one-third of the fin (Figure 16).

The coloration of the caudal fin is similar to that of the anal fin; the proximal one-third to two-thirds is orange-brown, followed distally by an orange component of variable width, and a black marginal band. In most populations the change from the inner orange-brown component to the outer orange zone is gradational (Figure 17), but in some populations it can be a sharp transition (e.g. the Mambova population, Figure 14). This fin can also have a light blue to, less commonly, white submarginal band, the development of which can vary from narrow and barely distinguishable (e.g. Nega Nega population, Figure 10) to broad and prominent, as in the Mambova population (Figure 14), and some males of the Lukanga Swamp populations. The width of the submarginal band in the latter populations is especially variable, ranging from light blue and narrow (Figures 15 and 16) to white and very broad (Østergaard, personal communication, 2015). Pelvic fins are similar to the anal fin and commonly have a light blue proximal part followed distally by an orange-red band; a very narrow light blue margin may or may not be present. The pectoral fins are hyaline with pale blue to, less commonly, white, posterior margins.

Phenotype 2, live male (Figures 9, 11 and 12): Specimens comprising this color form represent the so-called "blue phenotype" as described by Wildekamp and Rosenstock (1989). Scales on the body and head have light blue centers with orange-red margins forming a reticulated pattern. In some populations, such as the Kayuni and Lochinvar populations, this reticulation may be slightly irregular and appear as irregular cross-bars that are most prominent on the rear part of the body. Some males of the Shibuyunje population may show a more regular arrangement of narrow, chevron-shaped cross-bars while others have a regular reticulation on the body (Østergaard, personal communication, 2015). Males of the Nanzhila population (Figure 11), and those of this phenotype

from the Mambova population (Figure 12), consistently display a regular reticulation on the body without any cross-bars. Scale centers on the back may have a green-golden hue while those on the abdomen may be a lighter shade of blue or even white. The head, throat and jaws are red-brown to orange-red. The projecting part of the branchiostegal membrane is orange-red or red-brown with a narrow white margin. The iris is light golden-brown with a golden ring around the pupil and a vertical black bar.

The dorsal fin is light blue-green, with a golden hue in some cases, and a heavy marbling resulting from an irregular pattern of red-brown spots and stripes that ranges from coarse near the fin base to fine distally. A few small black markings are concentrated along the anterior margin of the dorsal fin.

The basal part of the anal fin is light blue, with a golden hue in some cases, and a heavy, coarse marbling of red-brown markings. The outer one-third to half of the fin is a clear light blue to, less commonly, white with a black margin and, in some specimens, a faint yellow hue or sparse yellow flashes (Figure 9). The marginal black band in this fin is usually fairly prominent but may be very poorly developed in some specimens or populations (e.g. Nanzhila; Figure 12). The transition from the inner to the outer components of the anal fin is variable, but is usually fairly sharp.

The coloration of the caudal fin is similar to that of the anal fin, the proximal part with red-brown to gray markings on a light blue to blue-green background. The markings in this part of the fin are more subdued than those in the anal fin and

tend to be elongated parallel to the fin rays. This zone is followed distally by a broad light blue to, less commonly, white band and a prominent black marginal band. The transition from the inner to the outer components of the caudal fin is variable, but usually fairly sharp. Pelvic fins are similar to the anal fin and most commonly have a light blue proximal part with some red-brown spots followed distally by a light blue component. The pectoral fins are hyaline with pale blue posterior margins.

Phenotype 3, live male (Figure 13): Specimens comprising this color form represent an intermediate phenotype that has only recently been recognized in a wild population. It is referred to here as an intermediate form because it displays color pattern characteristics of both the red and blue forms described above. It would appear, however, to be a discrete color form and not a transitional form between the red and blue phenotypes. To date, it is known only from the Mambova location where it is the dominant form existing together with lesser proportions of the blue and red forms. Although no statistical analysis was done, it was estimated that the distribution of the three color forms in the wild population was approximately: 90% Phenotype 3, 7% Phenotype 2 and 3% Phenotype 1. The Mambova population is the only presently-known polymorphic population of this species.

Scales on the body and head have light blue centers with orange-red margins forming a reticulated pattern. In some specimens this reticulation may be slightly irregular and appear as vague, irregular cross-bars which are most prominent on the rear part of the body. Scale centers on the back may have a green-golden hue

while those on the abdomen may be light blue or even white. The head, throat and jaws are red-brown to orange-red. The projecting part of the branchiostegal membrane is orange-red with a narrow white margin. The iris is light golden-brown with a golden ring around the pupil and a vertical black bar.

The dorsal fin is light blue-green with a golden hue and a heavy marbling resulting from an irregular pattern of red-brown spots and stripes that ranges from coarse near the fin base to fine distally. There is a concentration of a few small black markings along the anterior marginal part of the dorsal fin. Fin ray extensions may be black-tipped.

The anal fin is similar to that of Phenotype 2 males. The basal part of the fin is light blue with a coarse marbling of red-brown markings. This grades outward into a central zone having a light blue background color with yellow-green overtones and a dense pattern of red-brown markings, finer than those in the basal zone. The

outer approximately one-third of the fin is a clear light blue with the color becoming somewhat more intense toward the margin. A narrow black margin may be present.

The coloration of the caudal fin is generally similar to that of Phenotype 1 males. The proximal half to two-thirds is red-brown, followed distally by an orange-red component of variable width, a prominent light blue submarginal band and a black marginal band. Some blue-green markings may be present in the basal parts of the red-brown proximal component. Pelvic fins are similar to the anal fin and most commonly have a light blue proximal part with some red-brown spots followed distally by a light blue component. The pectoral fins are hyaline with pale blue posterior margins.