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## *Nothobranchius sainthousei*, a new species of annual killifish from the Luapula River drainage in northern Zambia (Teleostei: Cyprinodontiformes)

Béla Nagy\*, Fenton P. D. Cotterill\*\* and Dirk U. Bellstedt\*\*\*

*Nothobranchius sainthousei*, new species, is described from a seasonal swamp on the floodplain of the small, seasonal Chimbembe River, near the Luongo-Luapula confluence in northern Zambia. It belongs to the *N. brieni* species group. Males of *Nothobranchius sainthousei* are distinguished from congeners by the following unique combination of characters: scales with broad orange posterior margin on the body; dorsal and anal fins with markings of orange-brown spots; anal fin with orange-brown margin; and head proportions. Genetic divergence in partial sequences of the protein-coding ND2 mitochondrial gene supports the distinction of the new species from its closest known relative, *N. chochamandai*, and confirms its position in the *N. brieni* species group.

### Introduction

The genus *Nothobranchius* Peters, 1868 currently includes about 71 valid species. Species occur mainly in river drainages of eastern and south-eastern Africa (Seegers, 1997; Watters, 2009). It is the most species rich and geographically widespread genus of seasonal nothobranchiid killifishes (Wildekamp, 2004). All known species have an annual or semi-annual life cycle (Peters, 1963) and reproduce in the seasonally arid savannah biome. They exploit temporary pools and swamps during the rainy season (Skelton, 2001); all species are critically dependent on montmoril-

lonite clays in these swampy depressions, where the eggs undergo development and diapause in the vertisol substratum through the dry season (Watters, 2009). *Nothobranchius* species are sexually highly dimorphic and dichromatic; the typically robust and colourful males contrast against the smaller and dull coloured females (Jubb, 1981; Wildekamp, 2004).

The south-eastern upper drainage of the Congo River basin has already been identified as a region with particularly complex phylogeographic patterns in aquatic organisms (Lévêque, 1997), reflecting active speciation and a high level of endemism (Balon & Stewart, 1983; Jackson,

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1986; Malaisse, 1997; Cotterill, 2005; Snoeks et al., 2011). This catchment of the Congo River drains the Katanga province, previously called Shaba, of Democratic Republic of the Congo and north-western Zambia. Several ichthyological studies have focused on the cyprinodontiform fishes of the region (Poll, 1963, 1976; Tait, 1965; Wildekamp, 1978; Valdesalici & Wildekamp, 2004, 2005; Valdesalici & Amato, 2011; Nagy, 2014a–c). The landscapes of the upper Congo basin are characterized by extensive gently sloping pediments, whose steep escarpments bound wide valleys with impeded drainage, characterized by seasonally inundated shallow wetlands, locally called as dambos, and floodplains (Flügel et al., 2015; Guillocheau et al., 2015). The Luapula River forms part of the Bangweulu-Mweru ecoregion (Thieme et al., 2005; Abell et al., 2008). The Bangweulu swamps are fed by a series of rivers, the largest of which is the Chambeshi, and the Bangweulu overflows to the Luapula, which in turn flows into Lake Mweru. The Mumbatuta and Mambilima falls are prominent knickpoints, which divide the 480 km channel of the Luapula into three main sections (Flügel et al., 2015). Both falls are effective barriers to fish dispersal (Worthington, 1933; Lavoué, 2012; Van Steenberge et al., 2014).

Four *Nothobranchius* species have been identified within the catchment of the Luapula: *N. chochamandai* Nagy, 2014, which is known from the Lufutishi system in the middle Luapula drainage; *N. malaissei* Wildekamp, 1978, found on the lower Luapula plain and in associated temporary rivers, downstream of Mambilima Falls; *N. symoensi* Wildekamp, 1978, known only from a relatively restricted part of the upper Luapula drainage, above the Mumbatuta Falls; and *N. rosenstocki* Valdesalici & Wildekamp, 2005, which inhabits several smaller river systems within the upper Luapula drainage, including its type locality, above the Mumbatuta Falls, as well as the headwaters of the Mansa River, a tributary of the Luapula between the Mambilima and Mumbatuta Falls (Rosenstock, 1991; Schmidt, 1999, 2008; Wood, 2001; Valdesalici & Wildekamp, 2005; Nagy, 2014b).

The four above-mentioned species, together with all other *Nothobranchius* species from other parts of the upper Congo and Zambezi drainages in Katanga and Zambia, were assigned by Valdesalici (2010) to a *N. brienii* species group, whereas five new species belonging to this species group have been described since. All currently known species of this species group have allopatric

distributions (Nagy, 2014a–c). In addition to the above-mentioned four species, the nine currently known other species of the *N. brienii* species group are: *N. boklundi* Valdesalici, 2010, from the Luangwa Valley in eastern Zambia; *N. brienii* Poll, 1938, from the middle Lualaba drainage in Katanga; *N. capriviensis* Watters, Wildekamp & Shidlovskiy, 2015 (Watters et al., 2014), from the upper Zambezi drainage in the Zambezi region (formerly Caprivi Strip) of Namibia; *N. flagrans* Nagy, 2014, from the lower Lufira drainage in Katanga; *N. hassoni* Valdesalici & Wildekamp, 2004, from the lower Lufira drainage in Katanga; *N. kafuensis* Wildekamp & Rosenstock, 1989, from the Kafue and upper Zambezi drainages in southern Zambia; *N. milvertzi* Nagy, 2014, from the Lake Mweru basin in northern Zambia; *N. oestergaardi* Valdesalici & Amato, 2011, from the Lake Mweru Wantipa basin in northern Zambia and *N. polli* Wildekamp, 1978, from the upper Lufira drainage in Katanga. These members of the *N. brienii* species group represent the southwest limit of the range of the genus in southern Africa (Skelton, 1994; Watters et al., 2014).

In April 2012, survey by the first author and F. Milvertz targeted oviparous cyprinodontiform fishes of northern Zambia; collecting in a seasonal swamp on the floodplain of the small seasonal Chimbembe River, about 5 km southwest from the influx of the Luongo into the Luapula, near Mweshi village, in northern Zambia, and found specimens of a new species of *Nothobranchius* population, which is herein described.

## Material and methods

The collected specimens used for morphometric and meristic determinations were initially fixed in the field in approximately 7 % formalin and transferred in 70 % ethanol for long-term conservation. Two males and one female of the type series were maintained in aquarium to observe colouration, behaviour, breeding biology and potential maximum size.

**Morphology.** Conventional measurements of bilaterally symmetrical features were taken on the left side of specimens with a digital calliper, partly under dissecting microscope, to a precision of 0.1 mm. Twenty-three measurements and five counts were taken as described in Nagy (2014a–b). The suborbital depth is the greatest vertical dis-



tance between the inferior margin of the orbit and the ventral surface of the head. Standard length (SL) is presented in mm; all other measurements are presented as percentages of standard length, except sub-units of head, which are expressed as proportions of head length (HL). Fin-ray counts include all visible elements. Scale counts do not include small scales on caudal-fin base. Terminology for cephalic sensitive system follows Huber (2000). The nomenclature for frontal squamation follows Hoedeman (1958). Osteological observations are based on a cleared and stained (c&s) specimen, according to Taylor & Van Dyke (1985), stained for bones only.

Uni- and multivariate biostatistical analyses of the morphometric and meristic variables were performed using Minitab 16, from Minitab, Inc. Proportions of measurements were calculated in order to remove size effects from variation in body shape. Scatter plot graphs with the proportions plotted against standard length or head length were accomplished to individually examine allometric growth effects. Morphometric data were log-transformed with base-10 log whereas meristic data were square-root transformed before the biostatistical tests were carried out in order to meet standards for statistical and hypothesis testing (Sokal & Rohlf, 1995, 2009; Zuur et al., 2007; McDonald, 2008; Zar, 2010). Because of pronounced sexual dimorphism, data of males and females were analysed separately.

Non-parametric Mann-Whitney U tests were used for univariate comparisons between species, whereas non-parametric Kruskal-Wallis tests were used to identify morphological variations among populations. Hypotheses of statistical significance were two-tailed, accomplished to compare differences of mean ranks among morphometric variables (Sokal & Rohlf, 1995, 2009; Zar, 2010; Dytham, 2011). The significance level was set a priori at  $P < 0.05$ , and  $P < 0.01$  was considered as highly significant result. Sequential Bonferroni test was applied to correct for multiple pairwise comparisons (Rice, 1989).

Principal component analysis (PCA) has been employed on the correlation matrix in order to visualize differences of morphometric characters between species. PCA is used as a distribution-free ordination method to graphically display uncorrelated linear combinations of the original variables in a multivariate dataset, explaining a maximal amount of variance of the variables (James & McCulloch, 1990; Zuur et al., 2007).

Shape variation among specimens was evaluated through the geometric morphometric technique of relative warp analysis (RWA). For the analysis, 16 homologous landmarks were predefined (Nagy, 2014c), based on positions of comparable morphological features in species (Bookstein et al., 1985; Strauss, 2010). Landmarks were digitized onto each specimen into pairs of Cartesian coordinates with tpsDig version 2.19 (Rohlf, 1999), whereas tpsRelw version 1.53 was used to perform standardization of Procrustes superimposition, by optimally translating, rotating and uniformly scaling specimens, as well as to generate relative warp analysis (Rohlf, 1993; Zelditch et al., 2012).

**Molecular analysis.** Fin clips of representative voucher specimens were stored in 99 % ethanol. Genomic DNA was extracted using the Qiagen DNeasy kit, following the standard protocol of the manufacturer. Partial sequences of the protein-coding ND2 mitochondrial gene were examined (700 base pairs). Polymerase chain reactions (PCR) were performed in a 30  $\mu$ l volume using standard reagents for 30 cycles. PCR products were visualized on an agarose gel and the purified products were cycle sequenced in both forward and reverse directions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) according to the manufacturer's instructions. Sections of the mtDNA ND2 gene were amplified using specific primers for *Nothobranchius* fishes (Wildekamp et al., 2009; Shidlovskiy et al., 2010). Sequence alignment was completed in Bioedit 7.2.5 (Hall, 1999). Phylogenetic analyses were performed by Bayesian inference using MrBayes V3.1 (Ronquist & Huelsenbeck, 2003). Using jModelTest (Posada, 2008) the GTR+I+G model was selected to account for patterns of molecular evolution. Chains were run for five million generations, and sampled every 1000 generations. This analysis was repeated in three independent runs. The first 20 % of sampled generations were discarded as 'burn-in'. The sampling performance and convergence of the Bayesian priors was assessed in Tracer V1.6 (Rambaut et al., 2014). Maximum clade credibility trees were viewed in Figtree V1.2.3. Maximum likelihood (ML) analyses were performed using RAxML on CIPRES (Stamatakis, 2006). Parsimony analyses were performed using PAUP Version 4.0a147 (Swofford, 2002). All phylogenetic analyses were rooted with species





from the northern distribution range of the genus in Africa as outgroups, namely *N. bojiensis* and *N. virgatus*. This is based on the assumption of Nilo-Sudanian origin of the genus (Dorn et al., 2014), supported by sister genera most closely distributed in western Africa. *N. rubroreticulatus* was included as a member of the inland clade sensu Dorn et al. (2014) to which the ingroup species are affiliated. Specimens included in the molecular analyses with sampling localities in drainage systems and GenBank accession numbers are summarized in Table 1.

Type series and comparative specimens, all of them wild collected specimens, are deposited at the MRAC, Royal Museum for Central Africa, Tervuren.

## Results

*Nothobranchius sainthousei* belongs to the *Nothobranchius brieni* species group, which includes another 13 species. Within this species group, *N. chochamandai*, *N. malaissei*, *N. rosenstocki* and *N. symoensi* are regarded most similar to *N. sainthousei*, based on close geographical distribution, representing the only other valid species known from the same ecoregion and their colour pattern. The uniqueness of *N. sainthousei* is confirmed by morphometric and molecular evidence, which we elaborate on below.

**Morphology.** Morphometric and meristic characters of *Nothobranchius sainthousei* are listed in Table 2, whereas measurements for *N. chochamandai*, *N. malaissei*, *N. rosenstocki* and *N. symoensi* are listed in Table 3. Divergences of morphological characters between males of *N. sainthousei* and

**Table 1.** *Nothobranchius* specimens used for molecular analysis, with collection locality, known distribution in drainage systems and GenBank accession numbers.

species	country	locality	population	drainage system	GenBank
<i>N. bojiensis</i>	Kenya	Ewaso Ng'iro	KEN 10-1	Ewaso Ng'iro drainage	KT716810
<i>N. bojiensis</i>	Kenya	Habaswein	KEN 10-2	Ewaso Ng'iro drainage	KT716811
<i>N. brieni</i>	DRC	Bukama	CD 13-4	middle Luapula drainage	KT716784
<i>N. brieni</i>	DRC	Bukama	CD 13-4	middle Luapula drainage	KT716785
<i>N. chochamandai</i>	DRC	Kasomeno	CD 13-11	middle Luapula drainage	KT716792
<i>N. chochamandai</i>	DRC	Kasomeno	CD 13-11	middle Luapula drainage	KT716793
<i>N. flagrans</i>	DRC	Kabungu River	CD 13-7	lower Lufira drainage	KT716788
<i>N. flagrans</i>	DRC	Kabungu River	CD 13-7	lower Lufira drainage	KT716789
<i>N. flagrans</i>	DRC	Kabungu River	CD 13-7	lower Lufira drainage	KT716790
<i>N. flagrans</i>	DRC	Kabungu River	CD 13-7	lower Lufira drainage	KT716791
<i>N. hassoni</i>	DRC	Bunkeya	CD 13-8	lower Lufira drainage	KT716786
<i>N. hassoni</i>	DRC	Bunkeya	CD 13-8	lower Lufira drainage	KT716787
<i>N. malaissei</i>	DRC	Sange	DRCH 2006-06	lower Luapula drainage	KT716797
<i>N. malaissei</i>	DRC	Sange	DRCH 2006-06	lower Luapula drainage	KT716798
<i>N. milvoertzi</i>	Zambia	Chienge	ZM 12-20	Lake Mweru basin	KT716794
<i>N. milvoertzi</i>	Zambia	Chienge	ZM 12-20	Lake Mweru basin	KT716795
<i>N. milvoertzi</i>	Zambia	Chienge	ZM 12-20	Lake Mweru basin	KT716796
<i>N. oestergaardi</i>	Zambia	Mweru Wantipa	ZAM 10-4	Lake Mweru Wantipa basin	KT716802
<i>N. polli</i>	DRC	Kyembe	DRCH 2006-02	upper Lufira drainage	KT716803
<i>N. polli</i>	DRC	Kyembe	CD 13-9	upper Lufira drainage	KT716804
<i>N. rosenstocki</i>	Zambia	Mansa	ZAM-07-8	upper Luapula drainage	KT716805
<i>N. rosenstocki</i>	Zambia	Mansa	ZAM-07-8	upper Luapula drainage	KT716806
<i>N. rosenstocki</i>	Zambia	Luapula River	ZAM-07-3	upper Luapula drainage	KT716807
<i>N. rubroreticulatus</i>	Chad	Zakouma N. P.	TD 05-1	Chari drainage	GU138047
<i>N. sainthousei</i>	Zambia	Mweshi	ZM 12-19	middle Luapula drainage	KT716799
<i>N. sainthousei</i>	Zambia	Mweshi	ZM 12-19	middle Luapula drainage	KT716800
<i>N. sainthousei</i>	Zambia	Mweshi	ZM 12-19	middle Luapula drainage	KT716801
<i>N. symoensi</i>	Zambia	Luapula River	ZAM-07-4	upper Luapula drainage	KT716808
<i>N. symoensi</i>	Zambia	Luapula River	ZAM-07-4	upper Luapula drainage	KT716809
<i>N. virgatus</i>	Sudan	Fula Azarga	SD 10-3	Wadi Al Ghallah system	KT716812



those of *N. chochamandai*, *N. malaissei*, *N. rosenstocki* and *N. symoensi* were found based on the Mann-Whitney U test with sequential Bonferroni correction (Table 3). Highly significant differences ( $P < 0.01$ ) were observed in five characters out of the 27 examined characters compared to *N. chochamandai*, in 10 characters compared to *N. malaissei*, in 12 characters compared to *N. rosenstocki*, and in nine characters compared to *N. symoensi*, whereas significant results ( $P < 0.05$ ) were observed in zero, one, two and two characters, respectively. The comparison of the morphological characters of *N. sainthousei* has shown highly significant differences to all species in head length and head width. Furthermore, highly significant population-level

divergence of morphological characters between males of *N. rosenstocki* was found based on the non-parametric Kruskal-Wallis test ( $P < 0.01$ ) with sequential Bonferroni correction between the upper Luapula populations and the Mansa River population in pre-pelvic length, caudal peduncle length and depth, and head depth, whereas among three upper Luapula populations all morphological traits were found to be similar (data not shown). Therefore, the upper Luapula and Mansa populations of *N. rosenstocki* were treated separately in statistical treatment and comparisons.

In order to visualize the examined biometric differences, PCA has been employed on the cor-

**Table 2.** Morphometric and meristic data of holotype and paratypes of *Nothobranchius sainthousei*. Holotype values included in range, mean and SD. H, holotype; SD, standard deviation.

	males (n=8)				females (n=3)		
	H	range	mean	SD	range	mean	SD
Standard length	32.9	24.1-40.8			25.4-29.9		
<b>Percent of standard length</b>							
Total length	123.7	117.6-123.7	121.2	2.6	119.3-121.7	120.7	1.3
Body depth at pelvic fin origin	32.2	26.1-32.2	29.2	2.5	26.9-31.8	28.6	2.7
Head length	27.4	25.1-29.3	27.1	1.2	28.7-30.1	29.5	0.7
Pre-anal length	59.9	58.0-61.5	60.2	1.2	65.1-69.9	67.7	2.4
Pre-dorsal length	58.4	51.9-58.5	56.1	2.2	57.1-61.9	59.9	2.5
Pre-pelvic length	48.9	48.9-54.4	50.5	1.9	51.6-52.0	51.8	0.2
Pre-pectoral length	29.2	25.1-29.4	27.9	1.5	26.8-30.5	28.4	1.9
Caudal peduncle length	18.2	18.0-20.1	18.6	0.6	16.2-19.3	17.4	1.6
Caudal peduncle depth	12.8	12.5-14.0	13.0	0.5	10.7-13.1	11.6	1.3
Dorsal-fin base length	28.9	26.5-29.2	28.0	1.0	24.7-26.9	25.7	1.1
Anal-fin base length	23.1	19.5-23.1	22.0	1.4	14.9-17.7	16.1	1.4
Caudal-fin length	23.7	17.9-24.1	21.4	2.7	19.6-21.7	20.9	1.1
<b>Percent of head length</b>							
Head width	72	71-79	74.6	2.4	65-69	67.1	1.8
Head depth	89	88-94	90.4	2.3	77-82	79.3	2.6
Interorbital width	41	40-48	44.7	3.0	35-39	37.2	2.2
Postorbital length	49	49-55	50.8	2.1	53-57	55.7	2.2
Suborbital depth	26	19-26	22.5	2.4	14-19	16.9	2.2
Eye diameter	29	26-32	29.2	2.1	29-32	30.4	1.4
Snout to eye end length	50	45-52	49.1	2.0	43-47	44.3	2.2
Snout length	20	18-21	19.5	1.0	14-16	15.2	0.8
<b>Ratios</b>							
Head width in % of its depth	81	79-86	82.5	2.7	84-86	84.6	0.9
Caudal peduncle length in % of its depth	143	140-147	143.3	2.5	147-152	150.3	2.7
<b>Meristics</b>		range	mode		range	mode	
Dorsal-fin rays	18	15-19	16		15	15	
Anal-fin rays	19	15-19	16		15-16	16	
Scales mid-longitudinal series	31	29-31	29		28-29	28	
Scales transverse	11	10-11	11		10-11	10	
Scales circumpeduncular	12	10-12	12		12	12	





relation matrix of seven distinctive morphometric characters and, including all examined male specimens in the analysis, this represents an 8 : 1 subject to item ratio. The first two principal components were retained, supported by eigenvalue-one criterion and proportion of the components in total variance. Proportion of variance explained by the retained principal components and factor loadings of each character are shown in Table 4. The first principal component (PC1) explains 42.2 % of the variation among specimens in the multivariate dataset, whereas PC2 represents 25.3 %. The PCA

reveals that *N. sainthousei* groups separately on the score plot of PC1 vs. PC2 (Fig. 1a). The first principal component explains much of the variation among specimens in head length, pre-pectoral length, head depth and snout length, whereas the second principal component is associated mainly with body depth, caudal peduncle depth and postorbital length. *Nothobranchius sainthousei* is entirely situated on the positive part of the first PC axis and without overlap to *N. malaissei* and the Mansa population of *N. rosenstocki*, which are situated entirely on the negative part of the

**Table 3.** Morphometric and meristic data of males of the comparative material of *Nothobranchius chochamandai*, *N. malaissei*, *N. rosenstocki* and *N. symoensi*. Results of Mann-Whitney U tests two-tailed statistical significance after Bonferroni correction are marked with \* for significant result (P < 0.05) and \*\* for highly significant result (P < 0.01). SD, standard deviation.

	<i>N. chochamandai</i> males (n=7)				<i>N. malaissei</i> males (n=8)			
	range	mean	SD	Mann-Whitney (P)	range	mean	SD	Mann-Whitney (P)
Standard length	23.7–35.2				33.8–43.2			
<b>Percent of standard length</b>								
Total length	118.9–123.9	121.7	1.7	0.8622	117.9–124.6	122.4	2.3	0.3720
Body depth at pelvic fin origin	30.8–35.1	32.5	1.7	0.0323	28.9–34.9	31.5	2.0	0.1563
Head length	29.6–32.5	31.2	1.4	0.0015**	32.7–35.4	33.4	1.1	0.0009**
Pre-anal length	56.4–62.3	59.4	2.1	0.4519	57.2–63.0	60.1	1.6	0.6365
Pre-dorsal length	53.6–57.7	56.4	1.4	0.9539	52.8–59.2	56.0	2.2	0.9581
Pre-pelvic length	45.8–51.2	48.8	2.2	0.2243	47.0–51.7	49.9	1.4	0.9581
Pre-pectoral length	27.5–34.0	30.9	2.1	0.0128	30.1–34.4	32.1	1.2	0.0009**
Caudal peduncle length	17.8–20.4	18.9	0.9	0.5244	17.3–19.6	18.6	0.9	0.8748
Caudal peduncle depth	12.9–14.4	13.6	0.5	0.0323	13.2–16.0	14.7	0.9	0.0039**
Dorsal-fin base length	25.9–31.2	29.1	1.9	0.1480	26.2–31.3	28.2	1.7	0.9581
Anal-fin base length	20.6–24.5	22.7	1.4	0.4519	19.9–23.8	22.1	1.2	0.9581
Caudal-fin length	18.9–23.9	21.7	1.7	0.8622	17.9–24.6	22.4	2.3	0.4309
<b>Percent of head length</b>								
Head width	58–62	60.4	1.9	0.0015**	56–62	59.3	2.3	0.0009**
Head depth	87–93	89.6	2.2	0.9538	78–87	82.2	2.7	0.0009**
Interorbital width	37–43	40.1	2.6	0.0176	33–36	34.1	1.2	0.0009**
Postorbital length	56–60	56.7	1.5	0.0015**	48–54	51.3	2.3	0.6365
Suborbital depth	21–27	23.0	2.3	0.9539	22–29	24.6	2.4	0.1563
Eye diameter	27–30	27.8	1.3	0.2243	24–26	24.8	0.7	0.0009**
Snout to eye end length	40–45	44.0	1.8	0.0015**	45–52	48.6	2.4	0.7132
Snout length	16–19	17.4	1.4	0.0323	22–28	24.9	2.3	0.0009**
<b>Ratios</b>								
Head width in % of its depth	65–69	67.4	1.5	0.0015**	68–75	72.2	2.5	0.0009**
Caudal peduncle length in % of its depth	135–142	138.6	2.5	0.0128	122–131	126.8	3.1	0.0009**
<b>Meristics</b>	range	mode			range	mode		
Dorsal-fin rays	15–17	16		0.2306	16–18	16		0.9558
Anal-fin rays	15–18	17		0.9539	16–18	16		0.8616
Scales mid-longitudinal series	26–30	28		0.0224	28–30	29		0.0678
Scales transverse	10–11	11		0.7773	11–12	12		0.0117*
Scales circumpeduncular	12	12		–	12	12		–

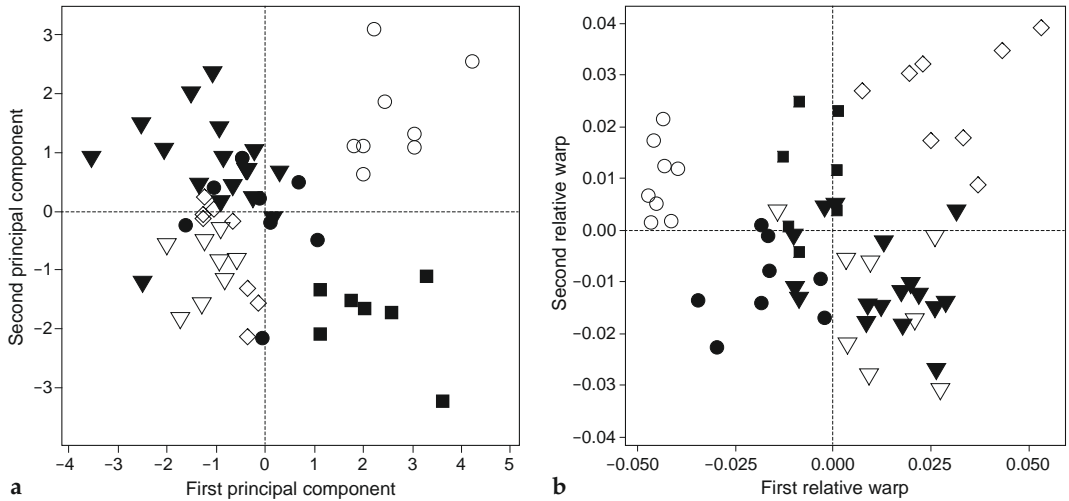


first axis, and without overlap to *N. symoensi* and the upper Luapula populations of *N. rosenstocki*, which are situated on the negative and slightly in the positive part of the first axis. *Nothobranchius sainthousei* is entirely situated on the positive part of the second axis and without overlap to *N. chochamandai*, which is situated entirely on the negative part of the second axis, and without overlap to *N. malaissei* and the Mansa population of *N. rosenstocki*, which are situated on the negative and slightly on the positive part of the second axis.

Relative warp analysis has been employed to evaluate the major trends of shape variation in the set of specimens. The value of the alpha parameter was set to zero ( $\alpha=0$ ), giving equal weight to the variation along the principal warps.

The first two relative warps were retained to document the variations among the examined specimens. Singular values and proportion of variance explained by the retained relative warps are shown in Table 5. The RWA reveals that *N. sainthousei* diverges in body shape and groups separately on the ordination plot of RW1 vs. RW2 (Fig. 1b). The first relative warp explains 35.0 % of shape variation among the examined specimens. *Nothobranchius sainthousei* is entirely situated on the negative part of the first relative warp and without overlap to the other species included in the study. RW1 is strongly associated with the landmarks at the posterior margin of operculum horizontal to centre of eye, the posterior margin of operculum on ventral lateral profile, and the

<i>N. rosenstocki</i> upper Luapula males (n=17)				<i>N. rosenstocki</i> Mansa River males (n=8)				<i>N. symoensi</i> males (n=8)			
range	mean	SD	Mann-Whitney (P)	range	mean	SD	Mann-Whitney (P)	range	mean	SD	Mann-Whitney (P)
21.5-34.9				20.9-25.8				25.6-39.3			
120.1-124.6	122.1	1.5	0.4316	121.7-123.6	122.6	0.7	0.7929	119.4-123.4	121.0	1.3	0.9581
26.0-32.6	29.3	1.8	0.8384	27.9-31.4	30.3	1.1	0.5635	29.6-34.6	31.5	1.6	0.1278
29.9-38.7	33.1	2.6	0.0001**	32.5-36.2	33.8	1.3	0.0009**	30.3-33.0	31.3	1.1	0.0009**
55.7-62.1	59.2	1.7	0.1535	58.9-62.9	60.5	1.3	0.9581	59.2-61.3	60.4	0.7	0.7929
52.0-63.4	55.4	2.7	0.2324	55.0-59.0	57.5	1.4	0.2271	55.1-60.1	57.9	1.5	0.0661
45.1-49.0	47.9	1.1	0.0001**	48.8-51.9	50.1	1.1	0.7929	48.2-51.4	49.7	1.0	0.7132
29.8-36.3	32.5	2.0	0.0001**	32.5-36.2	33.5	1.2	0.0009**	29.3-34.8	31.4	1.9	0.0019**
19.0-22.0	20.2	1.0	0.0010**	17.1-18.9	18.1	0.6	0.1893	18.7-21.2	20.1	0.7	0.0039**
11.9-13.4	12.6	0.5	0.0665	13.8-15.4	14.5	0.5	0.0014**	11.8-13.4	12.9	0.5	0.9581
20.1-32.2	28.7	3.0	0.1710	26.0-30.1	27.5	1.3	0.3184	25.8-29.6	28.0	1.5	0.6365
17.5-24.8	21.7	1.7	0.6622	20.5-25.8	21.9	1.7	0.5635	18.6-22.0	20.3	1.1	0.0313*
20.1-27.1	22.2	1.8	0.6622	21.7-23.6	22.6	0.7	0.9581	18.4-23.4	20.6	1.5	0.6365
53-60	56.3	2.2	0.0001**	53-59	55.0	1.8	0.0009**	55-60	57.4	1.9	0.0009**
78-84	79.6	2.3	0.0001**	75-77	75.8	0.8	0.0009**	74-78	76.5	1.6	0.0009**
31-35	32.9	1.2	0.0001**	33-37	34.2	1.6	0.0009**	32-40	37.5	2.6	0.0009**
49-54	51.2	1.5	0.4665	50-55	52.5	1.9	0.0831	49-55	52.5	2.3	0.1563
19-24	20.9	1.5	0.1535	19-24	20.5	1.5	0.1278	21-26	22.4	1.4	0.9581
23-29	25.9	1.7	0.0018**	21-25	23.7	1.7	0.0009**	23-26	24.6	1.2	0.0014**
46-51	48.7	1.6	0.6205	45-50	47.5	1.9	0.1036	44-51	47.1	2.6	0.1563
24-28	24.8	1.4	0.0001**	21-25	22.2	1.4	0.0009**	20-25	21.8	1.8	0.0209*
68-78	70.7	2.3	0.0001**	70-77	72.5	2.0	0.0009**	73-79	75.0	2.6	0.0009**
158-164	160.8	2.2	0.0001**	120-129	125.1	3.3	0.0009**	152-159	156.1	2.7	0.0009**
range	mode			range	mode			range	mode		
14-17	15		0.0231*	14-16	15		0.0209*	16-18	17		0.3184
14-16	15		0.0231*	14-15	14		0.0028**	17-18	17		0.0520
25-30	28		0.0004**	25-28	26		0.0009**	27-31	29		0.2936
10-11	11		0.9072	10-11	11		0.7132	10-12	10		0.5635
10-12	12		0.9768	12	12		-	12	12		-



**Fig. 1.** Comparative morphometry in males of *Nothobranchius sainthouei* (○), *N. chochamandai* (■), *N. malaissei* (◇), *N. rosenstocki* upper Luapula populations (▼), *N. rosenstocki* Mansa population (▽), *N. symoensi* (●). **a**, Score plot of principal component analysis on morphometric characters, first component vs. second component; **b**, Ordination plot of relative warp analysis on morphometric landmarks, first and second relative warp axes.

dorsal origin of pectoral fin, these landmarks located in more posterior position in specimens with higher score. The second relative warp accounts for 15.3 % of shape variation. Shape change on RW2 is mainly associated with the landmarks at the bases of the last dorsal and anal fins-rays, and the dorsal and ventral origins of caudal fin, specimens with higher score having a relatively higher caudal peduncle.

**Table 4.** Factor loadings for the first two principal component axes of seven distinctive morphometric characters, and proportions of variance explained by the selected principal components, in males of *Nothobranchius sainthouei*, *N. chochamandai*, *N. malaissei*, *N. rosenstocki* and *N. symoensi*.

Morphometric characters	PC1	PC2
Body depth at pelvic fin origin	0.087	-0.570
Head length	-0.491	-0.318
Pre-pectoral length	-0.484	-0.276
Caudal peduncle depth	0.003	-0.397
Head depth	0.469	0.035
Postorbital length	0.240	-0.518
Snout length	-0.489	0.267
<b>Eigenanalysis of the correlation matrix</b>		
Eigenvalue	2.9506	1.7691
Explained variance (% of total variance)	42.2	25.3
Cumulative variance (%)	42.2	67.4

The discrete position of *N. sainthouei* recovered in the PCA and RWA supports the hypothesis that it can be separated from the most similar species also based on morphological characters and body shape.

**Molecular analysis.** In order to assess if genetic separation would support the morphological and colour pattern differences, molecular analyses have been carried out. Partial sequences of the protein-coding ND2 mitochondrial gene were examined for 26 specimens, representing 11 species in the target study region of northern Zambia and Katanga province of DRC, as well as four specimens of three species used as outgroup. The examined specimens include all species currently known from the entire Luapula drainage, as well as all known species from the nearby Lake

**Table 5.** Singular values and proportion of variance explained by the first two relative warps of RWA, carried out on 16 homologous landmarks in males of *Nothobranchius sainthouei*, *N. chochamandai*, *N. malaissei*, *N. rosenstocki* and *N. symoensi*.

Relative warp analysis	RW1	RW2
Singular value	0.18960	0.12512
Explained variance (% of total variance)	35.0	15.3
Cumulative variance (%)	35.0	50.3



Mweru and Lake Mweru Wantipa basins and the neighbouring drainage of the Lufira. Specimens included in the molecular analyses are listed in Table 1. The phylogenetic tree resulting from of the Bayesian analysis is presented in Figure 2, and the recovered topology is congruent with the trees recovered using maximum likelihood and maximum parsimony (not presented).

The phylogenetic analysis confirms the evolutionary distinctiveness of *N. sainthousei* from all known species of the genus occurring in the study region. *Nothobranchius sainthousei* is most closely related to *N. chochamandai*, in the ND2 phylogeny. Significantly, *N. sainthousei* is phylogenetically distinct from its closest geographical neighbours, namely: *N. rosenstocki* and *N. symoensi* (upper Luapula drainage), *N. malaissei* (lower Luapula drainage), *N. milvertzi* (northern part of Lake Mweru basin), *N. oestergaardi* (Lake Mweru Wantipa basin), *N. hassoni*, *N. polli* and *N. flagrans* (Lufira drainage) and *N. brieni* (middle Lualaba drainage).

#### *Nothobranchius sainthousei*, new species

(Figs. 3–5)

**Holotype.** MRAC B5-027-P-0001, male, 32.9 mm SL; Zambia: Luapula Province: Luapula drainage: seasonal pools formed in small riverbed on floodplain of small seasonal Chimbembe River, about 5 km southwest from influx of Luongo into Luapula, near Mweshi village (10°43.51'S 28°38.22'E); B. Nagy & F. Milvertz, 6 Apr 2012, preserved in the field.

**Paratypes.** MRAC B5-027-P-0005-11, 5 males, 24.1–36.1 mm SL and 2 females, 25.4–27.5 mm SL; collected with holotype, preserved in the field. – MRAC B5-027-P-0002-4, 2 males, 40.0–40.8 mm SL and 1 female, 29.9 mm SL; collected with holotype and preserved after 3 months in captivity.

**Material examined** (non-type). MRAC B5-027-P-0012, male, 28.6 mm SL, c&s; collected with holotype, preserved in the field.

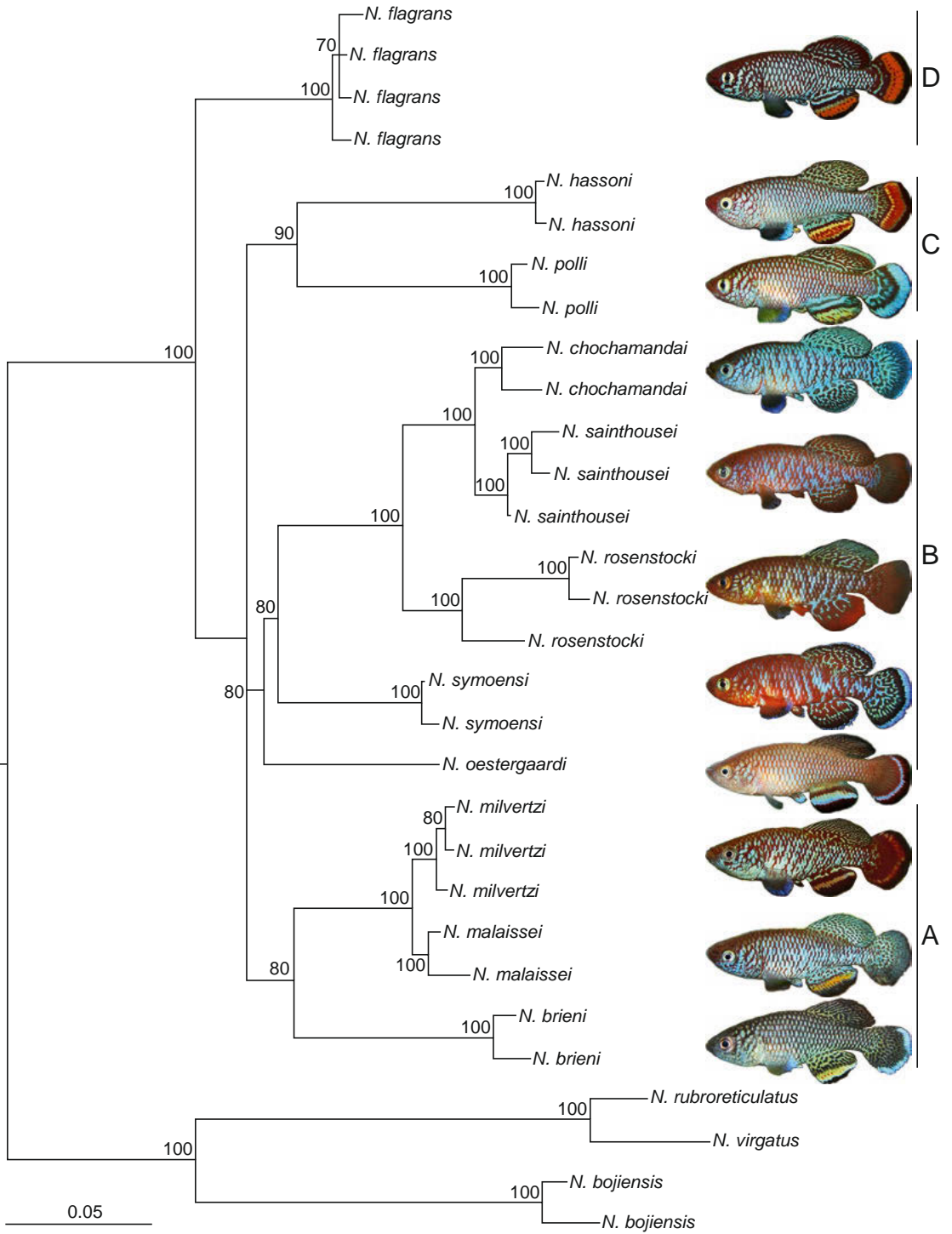
**Diagnosis.** *Nothobranchius sainthousei* is distinguished from the other *Nothobranchius* species from the drainages of the Upper Congo in northern Zambia and Katanga province of DRC (except *N. rosenstocki*) by having broad orange-brown posterior scale margins on the trunk (vs.

red) and an anal fin with orange-brown margin (vs. light blue or yellow or red-brown or black). It is distinguished from *N. rosenstocki* by having dorsal and anal fins with orange-brown spots (vs. irregular orange-brown bars). Furthermore, *N. sainthousei* is distinguished from all members of the *N. brieni* group by head length (25.1–29.3 % SL vs. 29.6–38.7) and head width (71–79 % HL vs. 52–62).

**Description.** General body features are illustrated in Figures 3–5. Morphometric and meristic characters are summarized in Table 2. General body shape robust, laterally compressed and deep. Maximum observed length in males 40.8 mm SL. Greatest body depth at vertical in front of pelvic-fin origin. Greatest body width at pectoral-fin base with body progressively narrowing towards caudal-fin base. Dorsal profile convex from tip of snout to base of last dorsal-fin ray, straight to slightly concave on caudal peduncle. Ventral profile convex from lower jaw to base of last anal-fin ray, straight to slightly concave on caudal peduncle. Caudal peduncle shallow, depth 1.4–1.5 times in its length.

Head short, laterally compressed, deeper than wide. Snout slightly pointed, smaller than eye diameter. Mouth supraterminal, slightly oblique in profile. Jaws subequal, lower jaw longer than upper, posterior end of rictus at same level or slightly ventral to centre of eye. Premaxilla and dentary with many irregularly distributed conical, slightly curved teeth at outer row of lower and upper jaws. Orbit large, in anterior half of head, pupil round. Branchiostegal membrane projecting posteriorly from opercle.

Dorsal-fin origin anterior to anal-fin origin, both fins originating posterior to mid-length of body. Extremity of dorsal and anal fins rounded, with small contact organs in form of papillae on fin rays and distal margin with short filamentous rays. Posterior extremity of dorsal fin reaching caudal-fin base in some specimens. Dorsal fin 15–19 rays; anal fin 15–17 rays. Pectoral fin subtriangular, insertion slightly posterior to margin of opercular opening, base slightly oblique, upper fin rays placed slightly anteriorly to lower fin rays, tip reaching or slightly overlapping base of pelvic fin. Pelvic fin subabdominal, origin at about mid-length of body, short, bases medially separated, tip reaching urogenital papilla. Caudal fin rounded.



**Fig. 2.** Phylogenetic tree based on sequences of the protein-coding ND2 mitochondrial gene and Bayesian analysis. Support values are posterior probabilities.





**Fig. 3.** *Nothobranchius sainthousei*, MRAC B5-027-P-0001, holotype, male, 32.9 mm SL; Zambia: Luapula Province: middle Luapula drainage: Chimbembe River floodplain.

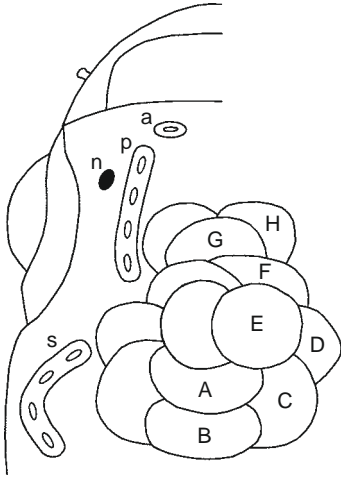


**Fig. 4.** *Nothobranchius sainthousei*, MRAC B5-027-P-0003, paratype, male, 40.8 mm SL; Zambia: Luapula Province: middle Luapula drainage: Chimbembe River floodplain. After 1 month in captivity.



**Fig. 5.** *Nothobranchius sainthousei*, MRAC B5-027-P-0002, paratype, female, 29.9 mm SL; Zambia: Luapula Province: middle Luapula drainage: Chimbembe River floodplain. After 1 month in captivity.





**Fig. 6.** Diagrammatic representation of the frontal squamation pattern and cephalic sensitive system in *Nothobranchius sainthousei*; MRAC B5-027-P-0001, holotype, male, 32.9 mm SL; dorsal view of head. **a**, anterior neuromasts; **n**, nostril; **p**, preorbital level; **s**, supraorbital level; **A–H**, frontal scales.

Scales cycloid, body and head entirely scaled, except for ventral surface of head. Scales in mid-longitudinal series 28–31 plus 2 or 3 small scales on caudal-fin base. Transverse rows of scales in front of dorsal-fin origin 10–11; scale rows around caudal peduncle 10–12.

Cephalic squamation pattern variable, holotype with E-type, and with E-scales and F-scales overlapping each other at median lateral margin. Nostril in front of eye, with single oblique aperture. Anterior neuromasts separate in two grooves. Cephalic sensory system at preorbital level in a continuous shallow groove, with four exposed neuromasts, whereas at supraorbital level in a curved groove, with four exposed neuromasts (Fig. 6). One neuromast on each scale along trunk mid-longitudinal series. Total number of vertebrae 29.

Female smaller than male, maximum observed length 29.9 mm SL. Body and head less laterally compressed and slightly more slender than in males (head width 65–69 % HL vs. 71–79). Dorsal and caudal fins rounded. Anal fin subtriangular, tip rounded, central rays longer and more rigid. Dorsal and anal fins positioned more posteriorly (pre-dorsal length 57.1–61.9 % SL vs. 51.9–58.5, pre-anal length 65.1–69.9 % SL vs. 58.0–61.5) than in male. Pelvic fin short, tip reaching anus. Caudal peduncle length smaller than in males. Branchio-

stegal membrane not projecting posteriorly from opercle. No papillae or epidermal tissue present on dorsal and anal fins.

Eggs slightly oval, measuring 1.40 mm long and 1.30 mm wide on average ( $n=10$ ), with short filaments on chorion.

**Colouration.** Live male: Scales on trunk and head light blue with broad orange-brown posterior margin, forming irregular reticulated pattern on body. Scales on abdomen light blue to silver, with narrow orange-brown margin. Snout, frontal and dorsal portions of head orange, throat blue. Exposed part of branchiostegal membrane orange. Iris golden to green, with dark grey vertical bar through the centre of eye. Dorsal fin light blue with irregular orange-brown spots, more distinct at base of fin and merging into striped pattern towards distal edge, parallel to fin rays, with black markings between the first and second and second and third fin rays at anterior margin. Anal fin light blue with irregular orange-brown spots, forming short stripes at base of fin, denser at distal part and merged into plain orange-brown margin. Caudal fin orange-brown with faint orange-brown spots proximally and a faint light blue, irregular narrow distal margin. Pelvic fin orange-brown with faint blue spots. Pectoral fin pale hyaline, faint orange at base, with light blue posterior margin.

Live female: Scales on trunk and head pale grey-brown, darker on dorsum and lighter to silver on venter. Dark grey reticulation on dorsal and posteroventral portions of flank. Blue iridescence on trunk and opercular region. Iris golden. All fins hyaline.

**Distribution.** *Nothobranchius sainthousei* is currently known only from within dambos (ephemeral pools) formed in the riverbed of the small Chimbembe River (Fig. 7). According to local people, the species is commonly found in other ephemeral habitats in the floodplain between the small seasonal Chimbembe and Kusake rivers. The floodplain with the type locality is situated about 5 km southwest of the confluence of the Luapula with the Luongo, the largest right-bank tributary, which joins the Luapula between two large natural barriers; upstream, the Mumbatua Falls isolates the river section from the upper Luapula and Bangweulu depression, while downstream the Mambilima Falls contains the lower part of the Luapula drainage and Lake Mweru (Fig. 8).



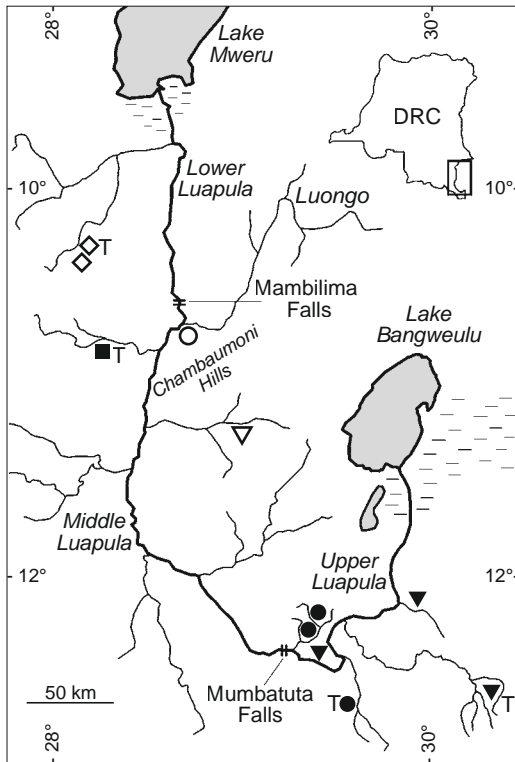
Fig. 7. Type locality of *Nothobranchius sainthousei*; Zambia: Luapula Province: middle Luapula drainage: Chimbembe River floodplain; 6 April 2012.

**Ecology.** The area of the middle Luapula drainage experiences a mean annual rainfall of 1020–1120 mm concentrated between December and May (Symoens, 1987; Hughes & Hughes, 1992), which maintains high waters peaking from March to May. Wetlands are at their lowest levels between September and January, when seasonal streams and most dambos are dry. At the type locality, *N. sainthousei* was the only *Nothobranchius* species observed. The accompanying fauna consisted of non-annual species, including representatives of Cyprinidae, Cichlidae and Mormyridae. The type locality consisted of a series of ephemeral pools formed in a small river bed, as standing fragments of a seasonal river draining a grassy dambo (Fig. 7). The pools were about 5–10 m wide, and less than 1 m deep at the deepest point. Several of these ephemeral pools were sampled, separated at the time of collecting but linked during the hot, wet season. The aquatic habitat was heavily overgrown with grass. The water of pH

6.98 contained 15 ppm of total dissolved solids. It was brown and turbid, so the pool substratum was only visible at shallower depths. The water temperature measured at midday was 23.0 °C.

**Conservation status.** Based on limited knowledge of its geographic distribution, the conservation status of *N. sainthousei* is uncertain. Present knowledge indicates, however, that the extent of occurrence (EOO) < 20000 km<sup>2</sup>; and the area of occupancy (AOO) are in continuing decline, as a result of the expansion of agriculture. Using IUCN (2014) criteria, the species appears to qualify as Vulnerable (B1bii). Additional collecting efforts targeting suitable habitats should be conducted in the region in order to better understand the geographic distribution of this species.

**Biology.** Aquarium maintenance of selected individuals was undertaken for observation of breeding behaviour and biology. *Nothobranchius*



**Fig. 8.** Luapula drainage showing the distribution of *Nothobranchius* populations used for morphologic comparisons in present study and presently known entire ranges of the respective species. Symbols may represent multiple adjacent locations. *Nothobranchius sainthousei* (○), *N. chochamandai* (■), *N. malaissei* (◇), *N. rosenstocki* upper Luapula populations (▼), *N. rosenstocki* Mansa population (▽), *N. symoensi* (●). T, type locality of respective species.

*sainthousei* has the annual mode of reproduction, typical of the genus. Under captive conditions, peat moss was used successfully as an artificial spawning substrate. An embryonic development period of three to four months was observed at about 22–24 °C. Stomach contents of three adult wild-caught specimens show that *N. sainthousei* is a micropredator, feeding on small aquatic crustaceans, worms, insect larvae and other zooplankton.

**Etymology.** The specific name honours Ian Sainthouse, renowned breeder and collector of killifish, for his special longstanding dedication to researches on the genus *Nothobranchius*. A noun in genitive.

## Discussion

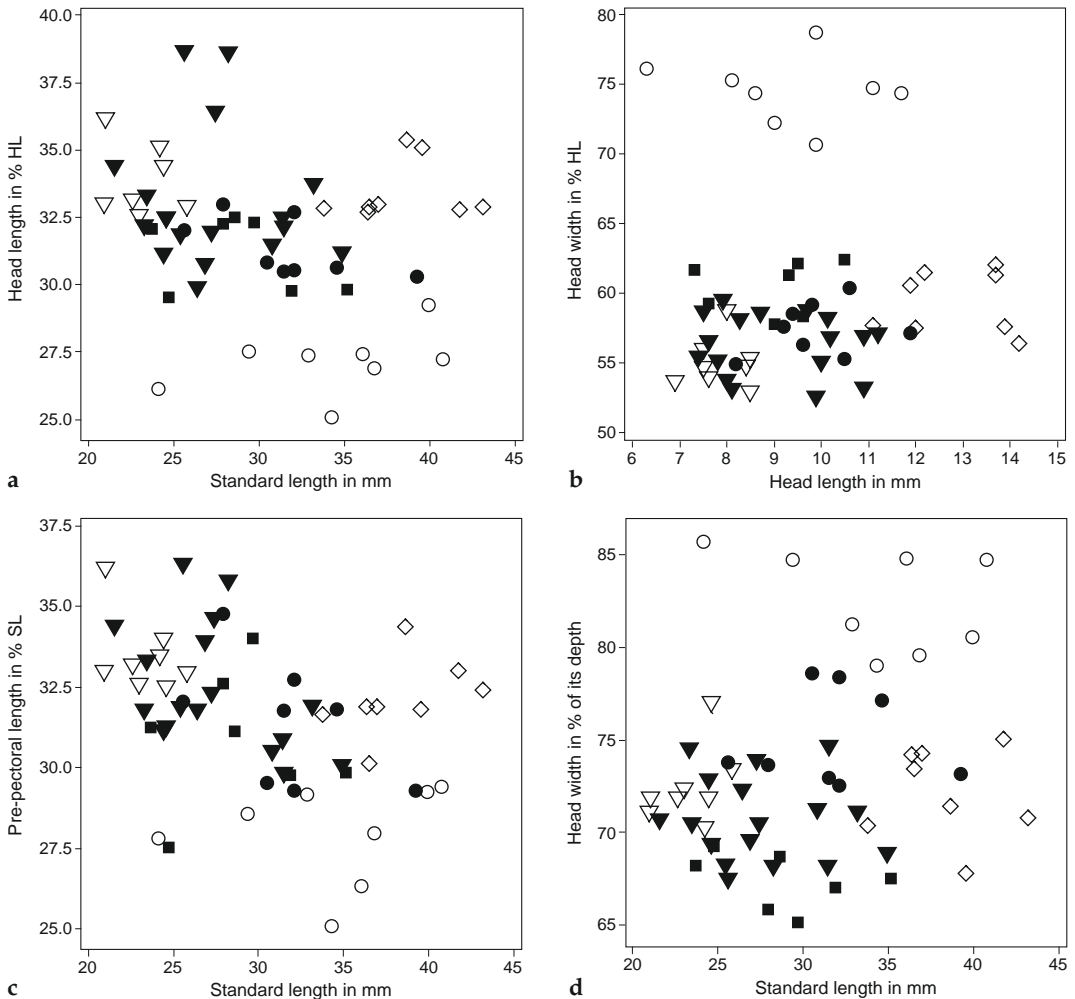
For a long time, information on the *Nothobranchius* fauna of the south-eastern upper drainage of the Congo River basin has been fragmentary (Sainthouse, 1985). Three species known from the Luapula drainage, *N. malaissei*, *N. symoensi* and *N. rosenstocki*, were first assigned by Valdesalici & Wildekamp (2005) to the *N. malaissei* species group, based on male colour pattern. They were then placed in the *N. brieni* species group (Valdesalici, 2010; Nagy, 2014b–c) together with all other *Nothobranchius* species from other parts of the upper Congo and Zambezi drainages in Katanga and Zambia (Nagy, 2014b).

*Nothobranchius sainthousei* also belongs to the *N. brieni* species group. Different diagnostic features are shared by diverse species within this species group. Based on the colour pattern, *N. sainthousei* is similar to *N. chochamandai*, *N. malaissei*, *N. rosenstocki* and *N. symoensi*, but this is not supported by the molecular analysis. As these species are closely related this may represent a pattern of lineage sorting that is occurring in the course of speciation in this group. Recent and repeated rifting in the drainages of the Upper Congo has generated waterfalls which separate the habitats in which these fishes occur and thereby drives allopatric speciation.

### Comparative morphometrics and colour pattern.

*Nothobranchius sainthousei* is distinguished from the four most closely related species as follows: Males of *Nothobranchius sainthousei* differ from those of *N. chochamandai* by having the dorsal fin with orange-brown spots (vs. red-brown stripes and spots); anal fin with orange-brown distal margin (vs. light blue distal margin); caudal fin orange-brown with faint orange-brown spots proximally (vs. light blue with irregular red-brown spots); pectoral fin hyaline with orange at base (vs. yellow hyaline); pelvic fin orange-brown with faint blue spots (vs. light blue with red-brown spots); relatively smaller head length (25.1–29.3 % SL vs. 29.6–32.5); and greater head width (71–79 % HL vs. 58–62).

Males of *N. sainthousei* differ from those of *N. malaissei* by having the dorsal fin with orange-brown spots (vs. red-brown spots); anal fin without submargin and with orange-brown distal margin (vs. orange submargin and red-brown distal margin); caudal fin orange-brown with faint orange-brown spots proximally (vs.



**Fig. 9.** Comparisons of morphometric characters in males for *Nothobranchius sainthousei* (○), *N. chochamandai* (■), *N. malaissei* (◇), *N. rosenstocki* upper Luapula populations (▼), *N. rosenstocki* Mansa population (▽), *N. symoensi* (●): **a**, head length in % SL plotted against standard length in mm; **b**, head width in % HL plotted against head length in mm; **c**, pre-pectoral length in % SL plotted against standard length in mm; **d**, head width in in % of its depth plotted against standard length in mm.

light blue completely covered with red-brown spots); pectoral fin hyaline with orange at base (vs. hyaline); pelvic fin orange-brown with faint blue spots (vs. light blue with red-brown spots); relatively smaller head length (25.1–29.3 % SL vs. 32.7–35.4); smaller pre-pectoral length (25.1–29.4 vs. 30.1–34.4); and greater head width (71–79 % HL vs. 56–62).

Males of *N. sainthousei* differ from those of *N. rosenstocki* by having the dorsal fin with irregular orange-brown spots (vs. irregular orange-brown bars); pectoral fin hyaline with orange at

base (vs. hyaline); relatively smaller head length (25.1–29.3 % SL vs. 29.9–38.7 in upper Luapula populations and 32.5–36.2 in Mansa population); smaller pre-pectoral length (25.1–29.4 vs. 29.8–36.3 in upper Luapula populations and 32.5–36.2 in Mansa population); and greater head width (71–79 % HL vs. 53–60 in upper Luapula populations and 53–59 in Mansa population).

Males of *N. sainthousei* differ from those of *N. symoensi* by having the dorsal fin without distinct margin (vs. light blue distinct margin); anal fin with orange-brown margin (vs. light blue





distinct margin); caudal fin orange-brown with faint orange-brown spots proximally and with irregular faint light blue narrow margin (vs. with red-brown irregular pattern and with light blue distinct margin); relatively smaller head length (25.1–29.3 % SL vs. 30.3–33.0); and greater head width (71–79 % HL vs. 55–60).

Morphometric comparisons with the four most closely related species reveal that males of *N. sainthousei* exhibit a shorter and wider head (Figs. 9a–b,d) and a smaller pre-pectoral length (Fig. 9c).

Furthermore, *N. sainthousei* differs from the other members of *N. brieni* species group by the following characters: anal fin without submarginal band and with orange-brown distal margin (vs. anal fin medial and distal portions yellow in *N. boklundi* and *N. polli*; anal fin with yellow submarginal band and red-brown distal margin in *N. brieni*; anal fin with orange-red submarginal band and dark grey distal margin in *N. flagrans*; anal fin with orange submarginal band and red-brown distal margin in *N. hassoni* and *N. milvertzi*; anal fin with cream to light blue submarginal band and black distal margin in *N. capriviensis* and *N. kafuensis*; anal fin with cream to light blue submarginal band and black distal margin in *N. oestergaardi*); caudal fin orange-brown and with faint orange-brown spots proximally and without submarginal band and with irregular faint light blue narrow margin (vs. light blue or orange with red-brown spots and narrow black distal margin in *N. boklundi*; light blue to yellowish with red-brown spots and without submarginal band and with distinct light blue distal margin in *N. brieni*; red-brown with light blue spots and orange-red submarginal band and distinct dark grey distal margin in *N. flagrans*; light blue with red-brown markings and with orange submarginal band and distinct dark red-brown distal margin in *N. hassoni*; light blue or plain orange without distinct markings and with cream to light blue submarginal band and distinct black distal margin in *N. capriviensis* and *N. kafuensis*; red with orange submarginal band and distinct dark red-brown distal margin in *N. milvertzi*; pale red or pale blue with cream to light blue submarginal band and red-brown distal margin in *N. oestergaardi*; light blue with red-brown irregular markings and with light blue submarginal band and narrow black distal margin in *N. polli*); head length 25–29 % SL (vs. 31–35 in *N. boklundi*; 29–32 in

*N. brieni*, *N. flagrans*, *N. kafuensis* and *N. milvertzi*; 32 in *N. capriviensis*; 29–34 in *N. hassoni*; 30–35 in *N. oestergaardi*; and 30–33 in *N. polli*).

**Molecular analysis.** To date, only a few phylogenetic reconstructions have been published that included some members of the *N. brieni* species group from Katanga province of DRC and Zambia. Shidlovskiy et al. (2010) analysed partial sequences of two mitochondrial genes that included two species of the *N. brieni* species group: *N. kafuensis* and *N. polli*. The relationship of the two species was resolved in a well-supported monophyletic clade and the analysis did not support any closer relationship to members of other species group.

Dorn et al. (2014) sequenced one mitochondrial locus and five nuclear loci of 46 valid species and four putative species of *Nothobranchius*. The analysis divided the genus in four geographically separated clades whose boundaries largely corresponded to the East African Rift system. The species from Zambia and DRC that have been included in the analysis, *N. boklundi*, *N. hassoni*, *N. kafuensis*, *N. malaissei*, *N. oestergaardi* and the putative species *N. spec. Lubumbashi*, formed a monophyletic group that was resolved as part of the geographically-structured Inland clade.

Watters et al. (2014) analysed partial sequences of the ND2 and 16S rDNA mitochondrial genes. The members of the *N. brieni* species group that have been included in the analysis (*N. capriviensis*, *N. kafuensis*, *N. hassoni*, *N. malaissei*, *N. polli*, *N. rosenstocki* and *N. symoensi*) are resolved in a well-supported monophyletic clade. The evolutionary relationships between those species evaluated in both Watters et al. (2014) and the taxa evaluated in the present study show similar topology.

Our results are presented here as preliminary to reporting a larger study (Cotterill, Bellstedt, Watters & Nagy, unpublished data) sufficient to evaluate *Nothobranchius sainthousei*. So it is premature to further evaluate the affinities of all these species, which is a subject beyond the terms of reference of the present paper.

**Geographical distribution.** The type locality of *N. sainthousei* on the floodplain of the Chimbembe is close to the Luongo-Luapula confluence. The river section inclusive of the Luongo-Luapula confluence is contained between the Mumbatuta Falls and the downstream Mambilima Falls,



which respectively isolate the aquatic habitats of the upper Luapula and Bangweulu and the lower Luapula and Lake Mweru wetlands, as argued by Jackson (1962, 1986), Skelton (1994) and Graf et al. (2014). The Luongo has a rich ichthyofauna and its diversity is attributed to the admixture of founder species from different regional drainage systems, where recent geologic processes (Cotterill 2004, 2005, 2006; Moore et al. 2012) have modified physiographic habitats along the river continuum. This evolutionary legacy of landscape evolution has been invoked to explain speciation of endemics in the Luongo (Balon & Stewart, 1983; Cotterill, 2003, 2006; Graf et al., 2014). Congruent biogeographical signals of aquatic biodiversity in the Luongo and neighbouring drainage basins point to widespread controls of drainage evolution of speciation and dispersals of the aquatic biota across the south-central African plateau. The modern regional drainage network preserves striking evidence of several major reorganizations (Moore & Larkin, 2001; Goudie, 2005; Moore et al., 2007, 2012), exemplified in the changes to precursors of the modern Luongo and Luapula rivers (Cotterill, 2006; Cotterill & de Wit 2011).

As presently known, it appears that the Mumbatuta Falls isolates the populations of *N. sainthousei* and *N. chochamandai* from populations of topotypical *N. rosenstocki* and *N. symoensi* confined within the upper Luapula drainage, while the Mambilima Falls isolates *N. malaissei* in the lower Luapula drainage (Fig. 8). *Nothobranchius sainthousei* is separated from the Mansa populations of *N. rosenstocki* by the watershed of the Chambaumoni Hills. The diverse terrain of Katanga supports uniquely diverse assemblages of biodiversity, as indicated by the relatively high proportion of endemic vertebrates and angiosperms (Malaisse, 1997; Broadley & Cotterill, 2003; Cotterill, 2005; Thieme et al., 2005). High diversity and endemism is exemplified in the ichthyofauna of the Luapula-Bangweulu and neighbouring freshwater ecosystems (Thieme et al., 2005; Lévêque et al., 2008). Political instability and weak infrastructure, however, rendered large parts of the region inaccessible (Nagy, 2014a-c), and biodiversity of the region remains incompletely surveyed. Present knowledge indicates that floodplains of different river systems in the region host diverse species of allopatric *Nothobranchius*, characterized by numerous local endemics. The

few sampled locations of the known population of *N. rosenstocki* from the Mansa River drainage exemplify large gaps in survey coverage, and there were no documented records of *Nothobranchius* along the Luapula drainage between the Mumbatuta Falls and the downstream Mambilima Falls, until the discovery and description of *N. chochamandai* (Nagy, 2014b). *Nothobranchius sainthousei* is now the second described species from this relatively unexplored area.

**Notes.** On its initial discovery, *N. sainthousei* was tentatively identified in the field as close to *N. rosenstocki*, based on some similarities in general appearance and male colour pattern. Aquarium maintenance of selected specimens was undertaken to observe breeding behaviour and biology. This included two males and one female of the type series and a few additional, non-preserved specimens. The offspring of these specimens were widely distributed among killifish hobbyists under the name of *N. cf. rosenstocki* or *N. species Mweshi*. The captive stock is uniquely recognized by the specific assigned field collection code: Mweshi ZM 12-19.

In Bemba language, spoken at the type locality of the species, species of *Nothobranchius* are commonly called sangemalale.

#### **Key to the species of *Nothobranchius brieni* species group**

Species of the genus *Nothobranchius* exhibit pronounced sexual dichromatism and dimorphism. Colour pattern elements and morphometric characters are based on mature males.

- 1 – Anal and caudal fins without distinct dark distal margin. .... 2
- Anal or caudal fins, or both, with distinct dark distal margin. .... 6
- 2 – Anal and caudal fins with distinct light blue distal margin. .... 3
- Anal fin with orange or yellow distal margin; caudal fin with distinct light blue margin. .... 4





- 3 – Dorsal fin without light blue distal margin; caudal fin without submarginal band; head depth 87–93 % HL; caudal peduncle length 1.4 times its depth (middle Luapula drainage).  
..... *N. chochamandai*
- Dorsal fin with distinct light blue distal margin; caudal fin with red-brown submarginal band; head depth 74–78 % HL; caudal peduncle length 1.5–1.6 times its depth (upper Luapula drainage).  
..... *N. symoensi*
- 4 – Anal fin with broad yellow distal margin; dorsal fin completely spotted with red-brown dots; caudal fin orange or spotted with red-brown dots and with a light blue distal margin (Luangwa drainage).  
..... *N. boklundi*
- Anal fin with irregular orange-brown markings and orange-brown distal margin; dorsal fin with orange-brown markings, with black markings between the first and second and second and third fin rays at anterior margin; caudal fin orange-brown (Luapula drainage).  
..... 5
- 5 – Dorsal and anal fins with irregular orange-brown spots; pectoral fin hyaline with orange at base; head length 25–29 % SL; head width 71–79 % HL (middle Luapula drainage).  
..... *N. sainthousei*
- Dorsal and anal fins with irregular orange-brown bars; pectoral fin entirely hyaline; head length 30–39 % SL; head width 53–60 % HL (upper Luapula drainage).  
..... *N. rosenstocki*
- 6 – Anal and caudal fins with cream to light blue submarginal band.  
..... 7
- Anal and caudal fins with yellow to orange submarginal band or caudal fin without submarginal band.  
..... 9
- 7 – Anal and caudal fins with red-brown distal margin (Lake Mweru Wantipa basin).  
..... *N. oestergaardi*
- Anal and caudal fins with black distal margin.  
..... 8
- 8 – Trunk scales with orange-red margin resulting in an evenly distributed bar pattern on trunk; head with orange-red colour (Kafue and upper Zambezi drainages).  
..... *N. kafuensis*
- Trunk scales without orange-red scale margin, and irregular bars on trunk; head without orange-red colour (upper Zambezi drainage).  
..... *N. capriviensis*
- 9 – Caudal fin with light blue distal margin.  
..... 10
- Caudal fin with dark red-brown, dark grey or black distal margin.  
..... 11
- 10 – Anal fin with orange submarginal band; head width 56–62 % HL (lower Luapula drainage).  
..... *N. malaissei*
- Anal fin with yellow submarginal band; head width 68–76 % HL (upper Lualaba drainage).  
..... *N. brieni*
- 11 – Dorsal fin with distinct light blue distal margin.  
..... 12
- Dorsal fin without distinct light blue distal margin.  
..... 13
- 12 – Anal fin with median and distal portions yellow; pre-pelvic length 46–49 % SL; caudal peduncle length 1.3–1.4 times its depth (upper Lufira drainage).  
..... *N. polli*
- Anal fin with orange-red submarginal band and distinct dark grey distal margin; pre-pelvic length 50–53 % SL; caudal peduncle length 1.5–1.6 times its depth (lower Lufira drainage).  
..... *N. flagrans*
- 13 – Caudal fin intense red with orange semicircular submarginal band; head width 63–72 % HL; snout length 18–20 % HL (Lake Mweru basin: Lushiba Marsh).  
..... *N. milvertzi*
- Caudal fin blue with irregular red-brown spots proximally and irregular yellow-orange submarginal stripe; head width 50–



59 % HL; snout length 20–26 % HL (lower Lufira drainage).

..... *N. hassoni*

**Comparative material.** *Nothobranchius chochamandai*: MRAC B3-022-P-0194, holotype, male, 29.7 mm SL; MRAC B3-022-P-0195-200, paratypes, 1 male, 27.9 mm SL, 6 females, 20.9–27.1 mm SL; MRAC B4-008-P-0003-11, paratypes, 4 males, 23.7–31.9 mm SL, 5 females, 22.0–27.8 mm SL; MRAC B4-008-P-0001-2, paratypes, 1 male, 35.2 mm SL, 1 female, 25.1 mm SL; DR Congo: Katanga Province: Luapula drainage, 10°46.03' S 28°17.00' E.

*N. malaissei*: MRAC 73-24-P-952, holotype, male, 33 mm SL; MRAC 73-24-P-947–951, 5, 22–30 mm SL; MRAC 73-24-P-914–919, 6, 28–39 mm SL; DR Congo: Katanga province: Luapula drainage, 10°17' S 28°09' E. – MRAC B4-008-0014, 1 male, 33.8 mm SL; DR Congo: Katanga province: Luapula drainage, 10°24.17' S 28°06.94' E.

*N. rosenstocki*: MRAC B3-028-P-0028–34, 7 males, 20.9–24.6 mm SL; MRAC B3-028-P-0035, male, 25.8 mm SL; Zambia: Mansa River drainage, 11°15.85' S 29°02.57' E. – MRAC B3-028-P-0013–7, 5 females, 18.1–20.5 mm SL; MRAC B3-028-P-0018–22, 5 males, 21.5–26.4 mm SL; Zambia: upper Luapula drainage, 12°09.93' S 29°55.87' E. – MRAC B3-028-P-0040–1, 2 males, 26.8–31.5 mm SL; Zambia: upper Luapula drainage, 12°09.73' S 29°55.64' E. – MRAC B3-028-P-0042–53, 5 males, 25.6–34.9 mm SL; 7 females, 21.7–24.8 mm SL; Zambia: upper Luapula drainage, 12°23.06' S 29°23.18' E. – MRAC B3-028-P-0036–9, 4 females, 24.2–27.7 mm SL; Zambia: upper Luapula drainage, 12°33.13' S 30°12.58' E. – MRAC B3-028-P-0054–9, 5 males, 23.3–33.2 mm SL; female, 25.9 mm SL, Zambia: upper Luapula drainage, 12°36.29' S 30°15.88' E.

*N. symoensi*: MRAC B3-028-P-0011-2, male, 31.5 mm SL; female, 28.8 mm SL; MRAC B4-008-P-0013, male, 39.3 mm SL; Zambia: upper Luapula drainage, 12°14.45' S 29°25.78' E. – MRAC B3-028-P-005, male, 32.1 mm SL; Zambia: upper Luapula drainage, 12°18.90' S 29°24.98' E. – MRAC B3-028-P-0006-10, 3 males, 25.6–32.1 mm SL; 2 females, 23.9–25.7 mm SL; MRAC B4-008-P-0012, male, 34.6 mm SL; Zambia: upper Luapula drainage, 12°18.89' S 29°24.48' E. – MRAC 73-25-P-1108-9; holotype and paratype, male and female, 21.5–30.5 mm SL; DR Congo: Katanga province: Malinde River drainage, 12°56' S 29°22' E.

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### Literature cited

- Abell, R., M. L. Thieme, C. Revenga, M. Bryer, M. Kottelat, N. Bogutskaya, B. Coad, N. Mandrak, S. C. Balderas, W. Bussing, et al. 2008. Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. *BioScience*, 58: 403–414.
- Balon, E. K. & D. J. Stewart. 1983. Fish assemblages in a river with unusual gradient (Luongo, Africa-Zaire system), reflections on river zonation, and description of another new species. *Environmental Biology of Fishes*, 9: 225–252.
- Bookstein, F. L., B. L. Chernoff, R. L. Elder, J. M. Humphries, G. R. Smith & R. E. Strauss. 1985. *Morphometrics in evolutionary biology: the geometry of size and shape change*. Special Publication, Academy of Natural Sciences of Philadelphia, 15: 1–277.
- Broadley, D. G., F. P. D. Cotterill. 2004. The reptiles of southeast Katanga, an overlooked 'hot spot'. *African Journal of Herpetology*, 53: 35–61.
- Cotterill, F. P. D. 2003. Geomorphological influences on vicariant evolution in some African mammals in the Zambezi basin: some lessons for conservation. Pp. 11–58 in: A. Plowman (ed.), *Ecology and conservation of small antelope*. Proceedings of an International Symposium on Duiker and Dwarf Antelope in Africa. Filander, Fürth.
- 2004. Drainage evolution in south-central Africa and vicariant speciation in swamp-dwelling weaver birds and swamp flycatchers. *The Honeyguide*, 25: 7–25.
- 2005. The Upemba lechwe, *Kobus anselli*: an antelope new to science emphasizes the conservation importance of Katanga, Democratic Republic of Congo. *Journal of Zoology*, London, 265: 113–132.
- 2006. The evolutionary history and taxonomy of the *Kobus leche* species complex of south-central Africa in the context of palaeo-drainage dynamics. PhD Thesis, University of Stellenbosch, 222 pp.
- Cotterill, F. P. D. & M. J. de Wit. 2011. Geocodynamics and the Kalahari epeirogeny: linking its genomic record, tree of life and palimpsest into a unified narrative of landscape evolution. *South African Journal of Geology*, 114: 493–518.
- Dorn, A., Z. Musilová, M. Platzer, K. Reichwald & A. Cellerino. 2014. The strange case of East African annual fishes: aridification correlates with diversification for a savannah aquatic group? *BMC Evolutionary Biology*, 14: 210.
- Dytham, C. 2011. *Choosing and using statistics: a biologist's guide*. 3rd Edition. Wiley-Blackwell, Chichester, 320 pp.



- Flügel, T., F. D. Eckardt & F. P. D. Cotterill. 2015. The present day drainage patterns of the Congo river system and their Neogene evolution. Pp. 315–337 in: M. J. de Wit, F. Guillocheau & M. C. J. de Wit (eds.), *Geology and resource potential of the Congo basin*. Springer, Berlin.
- Goudie, A. S. 2005. The drainage of Africa since the cretaceous. *Geomorphology*, 67: 437–456.
- Graf, D. L., A. J. Geneva, J. M. Pfeiffer & A. D. Chilala. 2014. Phylogenetic analysis of *Prisodontopsis* Tomlin, 1928 and *Mveruella* Haas, 1936 (Bivalvia: Unionidae) from Lake Mweru (Congo basin) supports a Quaternary radiation in the Zambian Congo. *Journal of Molluscan Studies*, 80: 303–314.
- Guillocheau, F., R. Chelalou, B. Linol, O. Dauteuil, C. Robin, F. Mvondo, Y. Callec & J-P. Colin. 2015. Cenozoic landscape evolution in and around the Congo basin: constraints from sediments and planation surfaces. Pp. 271–313 in: M. J. de Wit, F. Guillocheau & M. C. J. de Wit (eds.), *Geology and resource potential of the Congo basin*. Springer, Berlin.
- Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41: 95–98.
- Hoedeman, J. J. 1958. The frontal scalation pattern in some groups of tooth carps (Pisces, Cyprinodontiformes). *Bulletin of Aquatic Biology*, 1: 23–28.
- Huber, J. H. 2000. Killi-Data 2000. Updated checklist of taxonomic names, collecting localities and bibliographic references of oviparous Cyprinodont fishes (Cyprinodontiformes). *Société Française d'Ichtyologie*, Paris, 538 pp.
- Hughes, R. H. & J. S. Hughes. 1992. *A directory of African wetlands*. IUCN, Gland and Cambridge & UNEP, Nairobi, 820 pp.
- IUCN. 2012. *IUCN Red List Categories and Criteria: Version 3.1*. Second edition. IUCN, Gland and Cambridge, 32 pp.
- Jackson, P. B. N. 1962. Ecological factors affecting the distribution of freshwater fishes in Africa. *Annals of the Cape Provincial Museum*, 2: 223–228.
- 1986. Fish of the Zambezi system. Pp. 269–288 in: B. R. Davies & K. F. Walker (eds.), *The ecology of river systems*. Monographie Biologicae. Junk, Dordrecht.
- James, F. C. & C. E. McCulloch. 1990. Multivariate analysis in ecology and systematics. *Annual Review of Ecology and Systematics*, 21: 129–166.
- Jubb, R. A. 1967. *Freshwater fishes of southern Africa*. Balkema, Cape Town, 248 pp.
- 1981. *Nothobranchius*. T. F. H. Publication, Neptune, 61 pp.
- Lavoué, S. 2012. *Petrocephalus* Marcusen 1854 (Osteoglossomorpha, Mormyridae) of the Bangweulu-Mweru ecoregion (Luapula River System, Congo basin), with the description of a new species. *Journal of Natural History*, 46: 2159–2178.
- Lévêque, C. 1997. *Biodiversity and conservation: the freshwater fish of tropical Africa*. Cambridge University Press, Cambridge, 432 pp.
- Lévêque, C., T. Oberdorff, D. Paugy, M. L. J. Stiassny & P. A. Tedesco. 2008. Global diversity of fish (Pisces) in freshwater. *Hydrobiologia*, 595: 545–567.
- Malaisse, F. 1997. *Se nourrir en forêt claire africaine: approche écologique et nutritionnelle*. Presses Agronomiques de Gembloux, Gembloux, 384 pp.
- McDonald, J. H. 2008. *Handbook of biological statistics*. Sparky House Publishing, Baltimore, 319 pp.
- Moore, A. E. & P. A. Larkin. 2001. Drainage evolution in south-central Africa since the break-up of Gondwana. *South African Journal of Geology*, 104: 47–68.
- Moore, A. E., F. P. D. Cotterill, M. Main & H. Williams. 2007. The Zambezi River. Pp. 311–332 in: A. Gupta (ed.), *Large rivers: geomorphology and management*. Wiley, New York.
- Moore, A. E., F. P. D. Cotterill & F. D. Eckardt. 2012. The evolution and ages of Makgadikgadi palaeo-lakes: consistent evidence from Kalahari drainage evolution. *South African Journal of Geology*, 115: 385–413.
- Nagy, B. 2014a. *Nothobranchius milvertzi*, a new species of killifish from the Lushiba Marsh in the Lake Mweru drainage, Zambia (Teleostei: Cyprinodontiformes: Nothobranchiidae). *Ichthyological Exploration of Freshwaters*, 24: 347–360.
- 2014b. *Nothobranchius chochamandai*, a new species of annual killifish from the Luapula drainage, Democratic Republic of Congo (Cyprinodontiformes: Nothobranchiidae). *Ichthyological Exploration of Freshwaters*, 25: 167–183.
- 2014c. *Nothobranchius flagrans*, a new species of annual killifish from the Lufira drainage, Democratic Republic of Congo (Cyprinodontiformes: Nothobranchiidae). *Ichthyological Exploration of Freshwaters*, 25: 259–276.
- Parenti, L. R. 1981. A phylogenetic and biogeographic analysis of cyprinodontiform fishes (Teleostei, Atherinomorpha). *Bulletin of the American Museum of Natural History*, 168: 335–557.
- Peters, N. 1963. Zur Embryonalentwicklung bodenleuchtender Zahnkarpfen. *Die Aquarien und Terrarien Zeitschrift*, 16: 201–204.
- Peters, W. C. H. 1868. Über eine neue Untergattung (*Petronomus*) der Flederthiere und über neue Gattungen und Arten von Fischen. *Monatsberichte der Königlich-Preussische Akademie der Wissenschaften zu Berlin*, 1868: 145–148.
- Poll, M. 1938. Poissons du Katanga (bassin du Congo), récoltés par le professeur Paul Brien. *Revue de Zoologie et de Botanique Africaines*, 30: 389–423.
- 1963. Zoogéographie ichthyologique du cours supérieur du Lualaba. Colloque sur les problèmes biogéographiques du Parc National de l'Upemba. Publications de l'Université d'Elisabethville, 6: 95–104.
- 1976. Exploration du Parc National de l'Upemba. Mission G. F. de Witte & R. Verheyen (1946–1949). Poissons. *Fondation pour Favoriser les Recherches Scientifiques en Afrique*, Brussels, 73: 1–127.



- Posada, D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, 25: 1253–1256.
- Rambaut, A., M. Suchard, D. Xie & A. Drummond. 2014. Tracer v1.6. Available from <http://beast.bio.ed.ac.uk/tracer>.
- Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution*, 43: 223–225.
- Rohlf, F. J. 1993. Relative warp analysis and an example of its application to mosquito wings. Pp. 131–159 in: L. F. Marcus, E. Bello, & A. Garcia-Valdecasas (eds.), *Contributions to morphometrics. Monografías del Museo Nacional de Ciencias Naturales*, Madrid, 8: 1–264.
- 1999. Shape statistics: Procrustes superimpositions and tangent spaces. *Journal of classification*, 16: 197–223.
- Ronquist, F. & J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19: 1572–1574.
- Rosenstock, J. 1991. Zambia 1989. *Journal of the American Killifish Association*, 24: 213–220.
- Sainthouse, I. 1985. Notes on the *Nothobranchius* species from Zambia and the adjacent area of Zaire and Namibia. *British Killifish Association, Killi-News*, 247: 57–76.
- Schmidt, O. 1999. Auf Killi-Jagd nach Sambia. *Deutsche Killifisch Gemeinschaft Journal*, 31: 79–91.
- 2008. Zambia revisited, 2007. *Journal of the American Killifish Association*, 41: 3–18.
- Seegers, L. 1997. Killifishes of the world. Old world killis II. Aqualog, A.C.S., Mörfelden-Walldorf, 112 pp.
- Shidlovskiy, K. M., B. R. Watters & R. H. Wildekamp. 2010. Notes on the annual killifish species *Nothobranchius rachovii* (Cyprinodontiformes; Nothobranchiidae) with the description of two new species. *Zootaxa*, 2724: 37–57.
- Skelton, P. H. 1994. Diversity and distribution of freshwater fishes in east and southern Africa. *Annals of the Royal Central Africa Museum, Zoology*, 275: 95–131.
- 2001. A complete guide to the freshwater fishes of southern Africa. Struik Publishers, Cape Town, 395 pp.
- Snoeks, J., I. J. Harrison & M. L. J. Stiassny, 2011. The status and distribution of freshwater fishes. Pp. 43–91 in: W. R. T. Darwall, K. G. Smith, D. J. Allen, R. A. Holland, I. J. Harrison & E. G. E. Brooks (eds.), *The diversity of life in African freshwaters: under water, under threat. An analysis of the status and distribution of freshwater species throughout mainland Africa*. IUCN, Cambridge and Gland.
- Sokal, R. R. & F. J. Rohlf. 1995. *Biometry: The principles and practice of statistics in biological research*. 3rd edition. Freeman, New York, 880 pp.
- Sokal, R. R. & F. J. Rohlf. 2009. *Introduction to biostatistics*. 2nd edition. Dover Publications, Mineola, 382 pp.
- Stamatakis, A. 2006. RaxML-VI-HPC: maximum likelihood-based phylogenetic analysis with thousands of taxa and mixed models. *Bioinformatics*, 22: 2688–2690.
- Strauss, R. E. 2010. *Discriminating groups of organisms. Lecture Notes in Earth Sciences*, 124: 73–91.
- Swofford, D. L. 2003. *Phylogenetic analysis using parsimony (and other methods)*. Version 4. Sinauer Associates, Sunderland.
- Symoens J. J. 1987. Region 7: Zaïre basin. Pp. 401–456 in: M. J. Burgis, J. J. Symoens & P. Compère (eds.), *African wetlands and shallow water bodies*. Office de la Recherche Scientifique et Technique Outre-Mer, Paris.
- Tait, C. C. 1965. Notes on the species *Nothobranchius brieni* Poll (Cyprinodontidae). The Puku, Occasional Papers of the Department of Game and Fisheries, Zambia, 3: 125–131.
- Taylor, W. R. & G. C. Van Dyke. 1985. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybiurn*, 9: 107–119.
- Thieme, M. L., R. Abell, M. L. J. Stiassny, P. Skelton, N. Burgess, B. Lehner, E. Dinerstein, D. Olson, G. G. Teugels & A. Kamdem-Toham. 2005. *Freshwater ecoregions of Africa and Madagascar: a conservation assessment*. Island Press, Washington, 483 pp.
- Valdesalici, S. 2010. *Nothobranchius boklundi* (Cyprinodontiformes: Nothobranchiidae): a new annual killifish with two male colour morphs from the Luangwa River basin, Zambia. *Aqua, International Journal of Ichthyology*, 16: 51–60.
- Valdesalici, S. & R. H. Wildekamp. 2004. A new species of the genus *Nothobranchius* Peters, 1868 from the Lufwa river basin, Katanga province, Democratic Republic of Congo (Pisces, Cyprinodontiformes, Aplocheilidae). *Annali del Museo Civico di Storia Naturale Giacomo Doria*, 96: 241–251.
- Valdesalici, S. & R. H. Wildekamp. 2005. A new species of the genus *Nothobranchius* (Cyprinodontiformes: Nothobranchiidae) from Luapula River basin, Zambia. *Aqua, International Journal of Ichthyology*, 9: 89–96.
- Valdesalici, S. & G. Amato. 2011. *Nothobranchius oestergaardi* (Cyprinodontiformes: Nothobranchiidae), a new annual killifish from Mweru Wantipa Lake drainage basin, northern Zambia. *Aqua, International Journal of Ichthyology*, 17: 111–119.
- Van Steenberge, M., E. Vreven & J. Snoeks. 2014. The fishes of the Upper Luapula area (Congo basin): a fauna of mixed origin. *Ichthyological Exploration of Freshwaters*, 24: 329–345.
- Watters, B. R. 2009. The ecology and distribution of *Nothobranchius* fishes. *Journal of the American Killifish Association*, 42: 37–76.
- Watters, B. R., R. H. Wildekamp & K. M. Shidlovskiy. 2014. Description and biogeography of *Nothobranchius capriviviensis*, a new species of annual killifish from the Zambezi Region of Namibia



- (Cyprinodontiformes: Nothobranchiidae). Journal of the American Killifish Association, 47: 97–133.
- Wildekamp, R. H. 1978. Redescription of *Nothobranchius brieni* Poll, 1938 and the description of three new *Nothobranchius* species (Pisces, Cyprinodontidae) from the province of Shaba, Zaire. Revue de Zoologie Africaine, 92: 341–354.
- 2004. A world of killies. Atlas of the oviparous cyprinodontiform fishes of the world. Volume IV. American Killifish Association, Mishawaka, 368 pp.
- Wildekamp, R. H. & J. Rosenstock. 1989. Anmerkungen zu den *Nothobranchius*-Arten Sambias mit der Beschreibung von *Nothobranchius kafuensis* spec. nov. (Cyprinodontiformes; Nothobranchiinae). Die Aquarien und Terrarien Zeitschrift, 42: 413–419.
- Wildekamp, R. H., K. M. Shidlovskiy & B. R. Watters. 2009. Systematics of the *Nothobranchius melanospilus* species group (Cyprinodontiformes: Nothobranchiidae) with description of two new species from Tanzania and Mozambique. Ichthyological Exploration of Freshwaters, 20: 237–254.
- Wood, T. 2001. A search for nothos' in Zambia – 1997. British Killifish Association, Killi-News, 433: 125–135.
- Worthington, E. B. 1933. The fishes (other than Cichlidae) of Lake Bangweulu and adjoining regions, including descriptions of three new species. Annals and Magazine of Natural History, Ser. 10, 12: 34–52.
- Zar, J. H. 2010. Biostatistical analysis. Pearson Prentice-Hall, Upper Saddle River, N. J., 960 pp.
- Zelditch, M. L., D. L. Swiderski & H. D. Sheets. 2012. Geometric morphometrics for biologists: a primer. Academic Press, London, 478 pp.
- Zuur, A. F., E. N. Ieno & G. M. Smith. 2007. Analysing ecological data (statistics for biology and health). Springer, New York, 698 pp.

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*Liniparhomaloptera macrostoma* (Photograph by Jiahu Lan)  
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