Check for updates

Received: 9 December 2020 | Accepted: 2 March 2021

DOI: 10.1111/jfb.14719

REGULAR PAPER

JOURNAL OF FISH BIOLOGY fsbi

Association between *Labeobarbus* spp. (Teleostei: Cyprinidae) and environmental variables in the Luhoho basin (Eastern Congo River basin; DRC)

Tchalondawa Kisekelwa[1,2,3] | Jos Snoeks[2,3] | Armel Ibala Zamba[4] | Gaston Sefu Amzati[5] | Mwapu Isumbisho[1] | Pascal Mulungula Masilya[1,6] | Pieter Lemmens[7] | Emmanuel Vreven[2,3]

[1]Unité d'Enseignement et de Recherche en Hydrobiologie Appliquée, Département de Biologie-Chimie, ISP/Bukavu, Bukavu, Democratic Republic of the Congo

[2]Section Vertebrates, Ichthyology, Royal Museum for Central Africa, Tervuren, Belgium

[3]Laboratory of Biodiversity and Evolutionary Genomics, KU Leuven, Leuven, Belgium

[4]Ecole Nationale Supérieure d'Agronomie et de Foresterie, Université Marien Ngouabi, Brazzaville, Democratic Republic of the Congo

[5]Faculty of Agricultural and Environmental Sciences, Université Evangélique en Afrique, Research Unit of Veterinary Epidemiology and Biostatistics, Bukavu, Democratic Republic of the Congo

[6]Département de Biologie, Centre de Recherche en Hydrobiologie (CRH) d'Uvira, Uvira, Democratic Republic of the Congo

[7]Laboratory of Aquatic Ecology, Evolution & Conservation, KU Leuven, Leuven, Belgium

Correspondence
Tchalondawa Kisekelwa, Unit of Teaching and Research in Applied Hydrobiology (UERHA), Biology-Chemistry Department, Institut Supérieur Pedagogique of Bukavu, BP 854 Bukavu, Democratic Republic of the Congo and Research Centre of Biodiversity, Ecology, Evolution and Conservation (CRBEC).
Email: kisengoja@yahoo.fr

Funding information
International Foundation for Science, Grant/Award Number: IFS n°A5787-1; MBISA Congo; Belgian Development Cooperation

Abstract

The African cyprinid genus *Labeobarbus* is characterized by a high polymorphism in mouth morphology. The association between four species of this genus (two chiselmouths, one rubberlip with a well-developed fleshy mental lobe on the lower jaw, *i.e.*, a fleshy appendage on the chin, and one usually with a poorly developed one) and 26 environmental variables was investigated in four affluents of the Luhoho River in the Albertine Highlands of the Upper Congo basin. A combination of univariate and multivariate analyses suggested that differences exist in habitat composition at localities where different *Labeobarbus* species were reported, and within different mouth phenotypes. The association of *L. longidorsalis* with the presence of cobbles and pebbles and of *L. brauni* with the presence of boulders suggests that the occurrence of chiselmouths phenotype is associated with stony habitats, whereas phenotypes with a mental lobe, *i.e.*, *Labeobarbus longifilis* and *L. paucisquamatus*, mostly occupy habitats without stones. Differences in the distribution between these two species appear to be linked to physico-chemical parameters such as electrical conductivity and water temperature rather than substrate type. The findings of this study are relevant for developing guidelines that aim effective local preservation and conservation of these *Labeobarbus* species.

KEYWORDS
chiselmouth, habitat niche, rubberlip, segregation, Torini

* Pieter Lemmens and Emmanuel Vreven contributed equally to this study.

 © 2021 Fisheries Society of the British Isles.

1 | INTRODUCTION

The cyprinid genus *Labeobarbus* has long been considered part of the genus *Barbus* Daudin, 1805. Subsequently it has been recognized as a separate genus because of its hexaploidy, whereas members of the genus *Barbus sensu stricto* are tetraploid (Berrebi, 1981; Berrebi, 1995; Guégan *et al*., 1995; Oellermann & Skelton, 1990; Skelton, 2001). In addition, *Labeobarbus* species are usually characterized by a distinct scale pattern (parallel *vs*. striated in *Barbus*). These characters supported the decision to resurrect the name *Labeobarbus* (Skelton, 2001). Machordom and Doadrio (2001) and Tsigenopoulos *et al*. (2010) confirmed the monophyly of the genus *Labeobarbus*, but only with the inclusion of the genus *Varicorhinus*. As a consequence, the synonymy of *Varicorhinus* with *Labeobarbus* has been proposed (Tsigenopoulos *et al*., 2010) and implemented by Levin *et al*. (2013), Berrebi *et al*. (2014) and Vreven *et al*. (2016). As such, the genus *Labeobarbus* now includes 125 valid species with a tumultuous taxonomy (Vreven *et al*., 2016). Some of these species have strongly developed or hypertrophied lips and a fleshy mental lobe on the lower jaw (referred to as the *Lab*.-mouth phenotype) (Figure 1a1,b1), and are called rubberlips, whereas others with a sharp cornified cutting edge on the anterior edge of their lower jaw (the *Var*.-mouth phenotype) (Figure 1f1,g1) are called chiselmouths. Species with an intermediate mouth phenotype also occur (Kisekelwa *et al*., 2020; Vreven *et al*., 2016, 2018). In addition, some species exhibit a highly variable intraspecific mouth morphology. As such, it remains unknown how many of the originally described species only represent distinct, mouth phenotype, morphs of one or a few polymorphic species or, instead, indeed represent distinct species.

The occurrence of mouth polymorphism in the genus is known for more than a century, and its taxonomic implications have since long been debated (Crass, 1964; Vreven *et al*., 2016). Nonetheless, in the Congo basin, this morphological variability has never been investigated in an ecological context. This also holds for other regions in Africa, although, *e.g*. Crass (1964) already noticed that no one had tried to correlate the lip variation in rubberlips with the species' habitats in Southern African ecosystems. One exception is Lake Tana in Ethiopia where an incipient species flock of *Labeobarbus* occurs that has been intensively studied (Nagelkerke & Sibbing, 1997), especially with regard to trophic segregation related to different environmental conditions (Sibbing & Nagelkerke, 2000). These studies also linked the morphological differentiation of the mouth within rubberlips with differences in jaw protrusion (de Graaf *et al*., 2010), mouth width and the position of the lower jaw relative to the upper jaw (Nagelkerke *et al*., 1994). These ecological results have been critical in confirming the existence of, at least, 15 *Labeobarbus* species in Lake Tana (Nagelkerke *et al*., 1994; Sibbing & Nagelkerke, 2000).

In various Ethiopian rivers, *Labeobarbus* forms with divergent mouth morphologies were found to differ in stable isotope signals, indicating different feeding strategies (Levin *et al*., 2019). An additional study uncovered recurrent patterns of ecological diversification in mouth phenotypes in four Ethiopian highland rivers, indicative of parallel adaptive radiation (Levin *et al*., 2020). In these studies, the taxonomic status of the ecomorphs could, nonetheless, not be determined, though some of them may represent species based on the divergence, genetic distances, found between mtDNA sequence data.

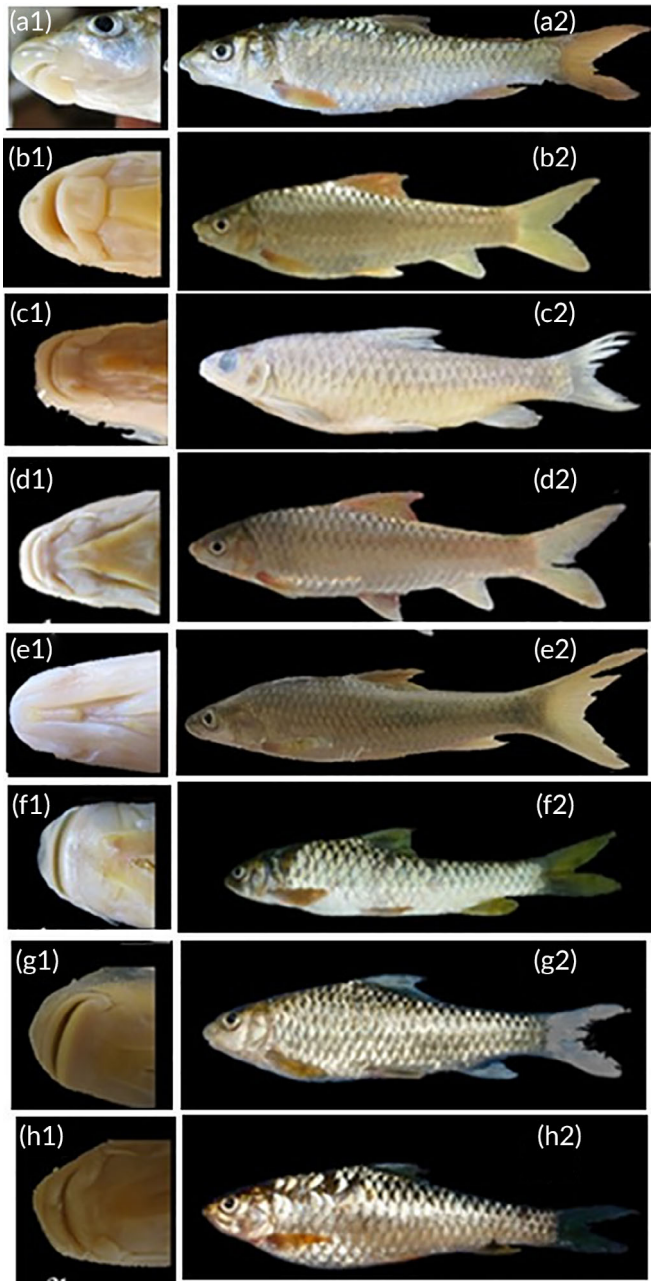


FIGURE 1 Mouth morphology (1) and habitus (2) of *Labeobarbus* species from the Middle Luhoho. (a) *Labeobarbus* cf. *caudovittatus*; (b) *Labeobarbus longifilis*; (c) *Labeobarbus paucisquamatus*, (d) *Labeobarbus paucisquamatus*, intermediate specimen without mental lobe on the lower jaw; (e) *Labeobarbus* sp. "isangi"; (f) *Labeobarbus brauni*; (g) *Labeobarbus longidorsalis* and (h) *L. brauni*, intermediate specimen with a sharp cornified cutting edge but with fleshy mouth corners (size: 100 ≤ 200 mm SL). Only taxa (a), (b), (c), (f) and (g) were included in this study because the intermediates (d) and (h) and species (e) were very rare in the samples of this study for inclusion. Nevertheless, they have been illustrated to provide a complete picture of the variation in mouth morphology and habitus in the Middle Luhoho

The Congo basin harbours a large diversity of *Labeobarbus* species with a complex array of mouth phenotypes within some lineages (Katemo Manda, 2020; Kisekelwa, 2019; Kisekelwa *et al*., 2020; Vreven *et al*., 2018). The observed mouth polymorphism within rubbermouths, chiselmouths and their intermediates might result from two non-exclusive phenomena: (a) hybridization between species with a *Lab*.- and *Var*.-mouth morphology (Banister, 1972, 1976; Vreven *et al*., 2018), or (b) an adaptation to different feeding ecologies involving differential habitat use (Oellermann & Skelton, 1990; Sibbing & Nagelkerke, 2000). Indeed, Gaigher (1975), referring to Jubb (1967), emphasized that *Varicorhinus* and *Labeobarbus* forms are not genetically determined, but rather the result of differential habitat use. Anecdotical evidence seems to support this hypothesis as a specimen of *L. holubi* (Steindachner, 1894) [junior synonym of *L. aeneus* (Burchell, 1822)], with thick lips caught in fast-flowing water, lost its rubber lips and fleshy mental lobe on the lower jaw almost completely after living in a pond with a muddy bottom for 1 year (Groenewald, 1958). Hexaploidy in *Labeobarbus* is thought to increase heterozygosity (Oellermann & Skelton, 1990), which can facilitate adaptation to different environmental conditions by generating different phenotypes (Levin *et al*., 2020). Phenotypic plasticity and selection might underpin adaptation to local environmental conditions (Grenier *et al*., 2016). In the Congo basin, the observed phenotypic variation in mouth morphology in some species seems to be linked to hybridization events (Kisekelwa, 2019; Vreven *et al*., 2018). Nonetheless, more studies are needed to disentangle hybridization from plasticity, ecological speciation (without hybridization) or a complex co-occurrence of several of these processes as a possible source of this phenotypic variation.

The Middle Luhoho provides a unique setting to investigate the extent to which the different *Lab*.- and *Var*.-mouth phenotype species occurring in the basin also differ in habitat use. In this basin, six *Labeobarbus* species with different mouth phenotypes occur. *Labeobarbus* cf. *caudovittatus* (Boulenger, 1902) and *L. longifilis* (Pellegrin, 1935) have thick lips with a well-developed free fleshy mental lobe on the lower jaw (Figure 1a,b). *Labeobarbus paucisquamatus* (Pellegrin, 1935) specimens possess a poorly developed, free and fleshy mental lobe (Figure 1c) or an intermediate mouth phenotype (Figure 1d,h). In contrast, *Labeobarbus* sp. "isangi" totally lacks a mental lobe and has a distinctly prognathous mouth (Figure 1e), whereas *L. brauni* (Pellegrin, 1935) (Figure 1f) and *L. longidorsalis* (Pellegrin, 1935) (Figure 1g) both have a cornified cutting edge on their lower jaw. Meristics and body measurements of *L. longifilis* and *L. paucisquamatus* overlap (Banister, 1973; Kisekelwa, 2019), but both species can be distinguished based on differences in mouth and dorsal spine morphology. The former species can be identified by its well-developed fleshy mental lobe and well-ossified and long dorsal spine, whereas the latter generally has a minute, slightly free fleshy lobe and a shorter less-developed dorsal spine (Banister, 1973). Morphological and genetic mtDNA results indicate that *L. paucisquamatus* probably resulted from hybridization events between *L. longifilis* and *L. brauni* (Kisekelwa, 2019).

The Middle Luhoho is characterized by a strong habitat heterogeneity, exemplified by the presence of riffles and pools, the occurrence of different types of substrate and a large variation in hydrodynamic factors including current, velocity, depth and river width, some of which have been identified as important determinants of fish community characteristics in rivers (Crosa *et al*., 2002; Erős *et al*., 2003; Humphries *et al*., 2014; Kawanishi *et al*., 2010; Moyle & Cech Jr., 2004). As such, *Labeobarbus* with different mouth phenotypes may occur in localities with different habitats for feeding. Thus, *Labeobarbus* species with a similar mouth morphology are supposed to frequent mostly similar habitats. Furthermore, in this study, the authors adopted the following two premises: (a) *Labeobarbus* species are powerful swimmers, as illustrated by the capacity of some species at least, to migrate during spawning (Anteneh *et al*., 2012; de Graaf *et al*., 2005; Gebremedhin *et al*., 2012). As such, (b) they are considered capable to disperse, at least, over the lower part of the four explored affluents of the Middle Luhoho, as the distance between these affluents and between the different stations within each affluent can be considered small in relation to their dispersal capacity. Thereby, *Labeobarbus* species in the Luhoho might not be segregated in relation to environmental variables across localities where they occur.

The present study aims to explore to what extent the differences in mouth morphology are linked to differential habitat use by the different *Labeobarbus* species occurring in the Middle Luhoho. Using data on fish species occurrences and local environmental conditions from a large number of localities distributed over four affluents in the Luhoho River, the authors first evaluate the occurrence of the species in the four affluents and subsequently describe the characteristics of the Middle Luhoho affluents where the different *Labeobarbus* species occur. Second, the authors link local habitat conditions to variation in species composition, and subsequently evaluate the importance of individual habitat variables in influencing the distribution of these species in the Middle Luhoho. Such information can be relevant for the development of guidelines for an effective conservation of the different Labeobarbus species.

2 | MATERIALS AND METHODS

2.1 | Study area

This study was conducted in the larger framework of a fish fauna survey of the river systems draining the Kahuzi Biega National Park (KBNP) and its neighbourhood (Kisekelwa, 2019), such as the Luhoho River (Figure 2). The authors sampled the *Labeobarbus* community and quantified the major environmental variables at multiple localities in four left bank affluents of the Luhoho River (the Heke, the Lwana, the Nyamunene and the Tchinganda River, from down- to upstream) during two subsequent dry seasons (2015 and 2016). The Luhoho is a left bank affluent of the Lowa, which itself is one of the major right bank affluents of the Upper Congo or Lualaba, which drains the region from the western summit of the Albertine highlands (Brown & Abell, 2005). The Middle Luhoho and the four explored rivers drain a steep slope covered by a transitional rainforest (*c*. 800–1000 m) between the mountain rainforest (>1000 m) and the typical equatorial rainforest

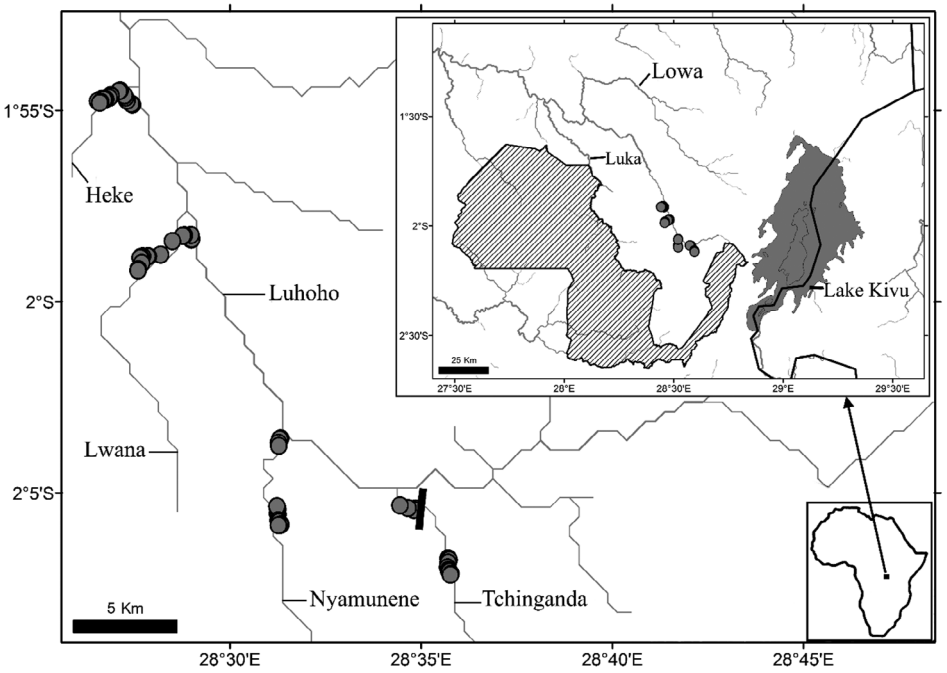


FIGURE 2 The sampling sites in the Middle Luhoho. Striped area represents the Kahuzi-Biega National Park; and (▮) Tchinganda Falls at Bulambika

(<700 m). This study was conducted at a small spatial scale level, with only *c*. 21 km between the two most distant river mouths (Heke and Tchinganda). The fish and environmental data were collected, due to a special collecting permit (document n°038/ICCN/DIRSITE-PNKB/RECH./2013), which was delivered to enable T.K. to conduct research on the fish diversity within and around the KBNP during his PhD programme (2013–2017).

2.2 | Field data collection

Within each river, *Labeobarbus* specimens were collected in the upstream direction as suggested by Grossman and Freeman (1987) over a distance of 5 km from the confluence with the Luhoho River. Because of logistic constraints, 4 days were spent at each of the four selected rivers during each expedition. The *Labeobarbus* community in each affluent was sampled qualitatively at each location during daytime using a cast-net (Bonar *et al*., 2009; Lévêque, 1997; Sheaves *et al*., 2010) at the different habitats. A cast-net can be used across a large range of habitats (Johnston & Sheaves, 2008). Thus, the authors preferred the use of cast-nets over gillnets as the four rivers have a swift current and a bottom often covered with stones and pebbles. Castings have been arbitrarily conducted while walking upstream in each of the affluents. Environmental data were collected at those localities where a specimen was found in the cast-net (see below). A total of 105 localities were included in the data set of this study, from which at least one *Labeobarbus* specimen was caught during the samplings organized in dry season (66 in July 2015 and 39 in July 2016), of which 27 in the Heke, 28 in the Lwana, 22 in the Nyamunene and 28 in the Tchinganda. Specimens sampled with a cast net were unconscious on retrieval. Nevertheless, all live-caught specimens were anaesthetized using clove oil before identification and fixation in accordance to the European Directive 2010/63/EU following the guidelines from the Animal Ethics Committee of the KU Leuven (Belgium).

A set of 26 environmental parameters were measured at each catch locality following Broad *et al*. (2001) and Yamazaki *et al*. (2006) (Table 1). Each locality was visually identified as a pool [(0), (0.20–0.75 m s^{-1}; average 0.48 m s^{-1})], intermediate [(0.5), (0.18–0.75 m s^{-1}; average 0.48 m s^{-1})] or riffle [(1), (0.21–1.60 m s^{-1}; average 0.61 m s^{-1})] according to the water current at the sampling locality. Depth measurements were taken with a depth sounder (PlastimoEchotest II, 59588) at the sampling locality. Depth was calculated as the average of nine depth measurements, taken near the sampling point within a distance of 1–5 m (two upstream and two downstream, and two to the right and two to the left of the capture point, as well as at the centre). Velocity (m s^{-1}) was measured as the average of three times at each location over a distance of 4 m, using a floating ball. Substrate types and percentage of surface cover of each substrate (over a surface of *c*. 100 m^2) were visually estimated following Yamazaki *et al*. (2006) and Seilheimer and Fisher (2010) and include clay/silt, sand, gravel, pebble, cobble, boulder and bedrock. Water temperature (°C), pH and electrical conductivity (μS cm^{-1}) were measured directly in the field using a field multi-probe (Thermo Scientific Orion). The authors coded stations with a high turbidity as 0 and transparent waters as 1. The percentage of canopy closure at the sampling location was estimated visually. The distance of the sampling location to the nearest river bank was measured and subsequently converted as a ratio of the width of the stream at this location, both measured with a laser (Leica Disto Tm D210). The remaining variables (see Table 1) were coded as present (1) or absent (0).

2.3 | Data analysis

The data from 2015 and 2016 were pooled because of the relatively low number of specimens per species caught during each individual year.

Three series of data analyses, combining univariates and multivariate approaches, were used to explore (a) the differences in the community composition of *Labeobarbus* between rivers, (b) the differences in environmental conditions between rivers and (c) the association between environmental variables and the occurrences of *Labeobarbus* species.

Goodness of fit tests (chi-squared: χ^2) were used to test for differences in occurrences of *Labeobarbus* species between streams. An ordination plot of a principal component analysis (PCA) was used to visualize the association between the environmental variables in relation to the rivers. Differences in depth and velocity between rivers were tested by ANOVA, whereas Kruskal–Wallis (KW) tests followed by Mann–Whitney *U*-tests were used for the remaining variables. A modified sequential Bonferroni correction following Rice (1989) was applied to minimize Type I errors (Garcia, 2004; Moran, 2003).

Two approaches were used to explore the association between the *Labeobarbus* species and environmental variables in the Luhoho: (a) Redundancy Analysis (RDA) and (b) logistic regression (LR). The RDA (*a priori* Detrended Canonical Correspondence Analysis indicated a gradient length of <3) was used to test for an effect of the entire set of environmental variables on the variation in *Labeobarbus* community composition between sampling localities. Significant environmental variables were identified using a forward selection procedure following Blanchet *et al*. (2008). Given that the Tchinganda Falls (*c*. 15 m height) might act as a dispersal barrier for *Labeobarbus* and that this might affect the results of this study, two separate RDAs were conducted. The first one included all 105 sampling localities, whereas the second one was done based on 85 sampling localities, excluding 20 localities upstream of the Tchinganda Falls. The significance of the RDA models was assessed with Monte Carlo permutations (*n*= 999).

Because RDA explores all variables and communities simultaneously, some variables can be discarded in the model because of the reciprocal effect between variables and individual species-environmental effects. Therefore, the authors conducted complementary LR to link each species individually to the environmental variables (see Carmona *et al*., 1999; Eikaas *et al*., 2005). In contrast to the RDA model, the LR tests the effect of each environmental variable on each

TABLE 1 List of the environmental variables that have been quantified at the sampling localities

Variable	Abbreviations	Category or scale of measurement	Methodology
Geographic coordinates	No	degree-min-s	GPS
Categorization as riffle, pool or intermediate	No	1, 0, 0.5	Observation
Stream depth	Mdept	cm	Echosounder
Velocity	Vel	m s^{-1}	Float method
Silt	No	Percentage	Estimation
Sand	No	Percentage	Estimation
Gravel	Grav	Percentage	Estimation
Pebble	Peb	Percentage	Estimation
Cobble	Cob	Percentage	Estimation
Boulder	Boul	Percentage	Estimation
Bedrock	Dedr	Percentage	Estimation
Water temperature	Temp	°C	Multiprobe
Electrical conductivity	Cond	µScm	Multiprobe
pH	No	Scales of pH	Multiprobe
Transparency	Transp	Transparent (1) or turbid (0)	Observation
Time of sampling	No	Hour	Clock
Canopy closure	Canop	Percentage	Estimation
Distance from a bank	Disbank	m	Laser Leica
Stream width	Width	Meters	Echosounder
Grass on bank	No	Absent (0)/present (1)	Observation
Shrub on bank	No	Absent (0)/present (1)	Observation
Tree on bank	Tre	Absent (0)/present (1)	Observation
Oil palm on bank	Palm	Absent (0)/present (1)	Observation
Secondary forest	Fal	Absent (0)/present (1)	Observation
Primary forest	Pfore	Absent (0)/present (1)	Observation
Root macrophytes	Root	Absent (0)/present (1)	Observation

Note. Abbreviations are supplied for the variable names as shortened in the multivariate analyses.

of the *Labeobarbus* species separately. A stepwise approach was used to select the significant predictor variables that explain the occurrence of each *Labeobarbus* species. All variables with $P > 0.2$ were removed in subsequent analyses (Sanharawi & Naudet, 2013). A chi-square automatic interaction was used to establish the role of the environmental variables on the occurrence of the *Labeobarbus* species individually. In principle, a variation inflation factor (VIF) may affect multiple LR, because of interference between the predictor variables (Thompson *et al*., 2017). Because of the potential relevance of each variable for the characterization of the habitat conditions of each *Labeobarbus* species, and given that none of these variables has an *a priori* assumption to determine their occurrence, the authors used multiple LR involving all 15 variables. After reducing the number of variables with the stepwise approach, simple LRs were conducted on the remaining variables to test for their significance on occurrence/non-occurrence at the threshold of $P < 0.05$. This approach was preferred above testing the VIF in line with Cohen (1977), Craney and Surles (2002) and Thompson *et al*. (2017). Variables for which the sign of the coefficient changed from positive to negative or inversely between the multiple (see Thompson *et al*., 2017) and simple LRs were not retained among the important variables for the corresponding species. The general logit equation is: $\pi(\chi) = e^{g(\chi)}/(1 + e^{g(\chi)})$ (Hosmer & Lemeshow, 2000; Keng & Rahman, 2012; Luoto & Seppälä, 2002).

Before statistical analysis, multiple data transformations were conducted to improve assumptions of normality. Temperature and electrical conductivity data were ln transformed (Kamdem & Teugels, 1998), whereas river depth and width were log-transformed (Erős *et al*., 2003; Pettit *et al*., 2012). The ratio of substrate and canopy cover in percentage was square-root arcsine transformed to reduce the large range of differences between raw data (Erős *et al*., 2003; Grossman & Freeman, 1987). All statistical analyses were performed in CANOCO for Windows 4.5, R 3.6.1 packages, STATA 11 and STATISTICA 12.

Labeobarbus sp. "isangi" (Figure 1e) was excluded from all analyses as only one specimen was collected over the entire set of sampling localities. The specimens with an intermediate mouth identified as *L. paucisquamatus* (intermediate) (Figure 1d) and *L. brauni* (intermediate) (Figure 1h) were also not included in RDA and LR analyses because of low numbers ($n < 5$).

For the RDAs, the final data set was composed of *Labeobarbus brauni*, *L. longidorsalis*, *L. longifilis*, *L. paucisquamatus* and *L.* cf. *caudovittatus*; for LRs the latter species was excluded because of the low occurrence ($n < 10$).

3 | RESULTS

3.1 | Occurrence of *Labeobarbus* species within the four rivers of the Luhoho basin

A total of 161 specimens were collected during this study. The pattern of distribution in terms of abundance differed across the basin

TABLE 2 Number of specimens and occurrence (absolute and relative) of the *Labeobarbus* species in the four investigated rivers of the Middle Luhoho

Taxa/affluent	*Labeobarbus longifilis*			*Labeobarbus paucisquamatus*			*Labeobarbus* cf. *caudovittatus*			*Labeobarbus brauni*			*Labeobarbus longidorsalis*			Total specimens	Total localities
	Ns	O	%X/%Y	Ns	O	%X/%Y	Ns	O	%X/%Y	Ns	O	%X/%Y	Ns	O	%X/%Y		
Heke	19	17	63/50	2	3	11/9	4	3	11/60	31	11	41/30	1	1	3/8	57	27
Lwana	13	12	43/35	3	3	11/9	2	2	7/40	2	3	11/8	13	12	43/92	33	28
Nyamunene	6	5	23/15	9	8	36/24	0	0	0/0	17	13	59/35	0	0	0/0	32	22
Tchinganda	0	0	0/0	25	20	71/59	0	0	0/0	14	10	36/27	0	0	0/0	39	28
Total	38	34		39	34		6	5		64	37		14	13		161	105

Note. Ns is the number of *Labeobarbus* specimens. O is the number of localities at which each species was encountered within each affluent. For the percentages (%), the numerator represents the relative percentage related to the total number of localities within an affluent (X); the denominator corresponds to the relative percentage related to the total number of localities for a species (Y). The total number of localities (last column) for a given river may be lower than the cumulative number for the individual species because of the presence of multiple species at a same locality.

and between rivers (Table 2). The occurrence of the *Labeobarbus* species differed considerably between sampling localities (Table 2). The most frequently caught species was *L. brauni*, which was found at 37 of the 105 sampled localities, followed by *L. longifilis* and *L. paucisquamatus* both at 34 localities, whereas *L. longidorsalis* was only found at 13 localities and *L.* cf. *caudovittatus* at five. Chi-square tests revealed significant differences in the presence of these species between the four rivers (χ^2 tests, *d.f.*: 21, $P < 0.001$) (Table 2). *L. longifilis* was most frequently found in the Heke and Lwana, but only sporadically in the Nyamunene, and was absent in the Tchinganda. In contrast, *L. paucisquamatus* was found frequently in the Nyamunene and Tchinganda, but only occasionally in the Heke and Lwana. *L. brauni* was caught frequently in the Heke, Nyamunene and Tchinganda, but only occasionally in the Lwana. With the exception of one individual in the Heke, the remaining specimens of *L. longidorsalis* were found in the Lwana. *Labeobarbus* cf. *caudovittatus*, conversely, was rare and only found in the Heke and Lwana.

3.2 | Environmental variables in relation to rivers

A PCA was performed on 24 environmental variables, excluding the coordinates and the time of sampling, to identify the links between environmental variables and localities. In a projection of the two first principal components (Supporting Information Figure S1 and Figure 3), no distinct clustering of localities per river was observed based on the environmental variables included. Nonetheless, the localities on the Heke and the Lwana are more similar to each other than to those of the Nyamunene and Tchinganda which are mostly situated on the opposite side of the orthogonal projection. It appears that the Heke and Lwana rivers are situated in a large forest with more trees and shrubs than the Nyamunene and Tchinganda. Furthermore, high values of pH and electrical conductivity and a low temperature seem to characterize the localities in the Nyamunene and Tchinganda, *vs.* those of the Heke and the Lwana. Silt and sand are inversely related to cobble, pebble and velocity. Sand dominated in the Heke, Tchinganda and Nyamunene with, respectively, 35, 34 and 27%, whereas in the Lwana, cobble represented 41% (Supporting Information Table S1). Indeed most of the localities of the Lwana are situated in the area of the PCA plot determined by the presence of cobble.

Mann–Whitney *U*-tests after KW ($P < 0.05$) revealed significant differences between affluents for electrical conductivity ($P < 0.001$), pH ($P < 0.01$), water temperature ($P < 0.001$) and river width ($P < 0.05$) (Figure 3). The electrical conductivity was significantly higher in Nyamunene and Tchinganda than in Heke and Lwana. Temperature was highest in Heke and Lwana, lowest in Tchinganda and significantly different between Tchinganda and Heke; and Lwana and Nyamunene. pH differed significantly between Heke and Nyamunene, and Tchinganda, and between Lwana and Nyamunene. River width was significantly larger in Lwana compared to Heke. Instead, the authors did not observe significant differences between rivers for river depth and river velocity. For substrates (not in figures), silt cover was similar over the four rivers, whereas sand cover was significantly different between Heke and Lwana and gravel cover was highly significantly different between the same rivers. Pebble cover was highly significantly different between Heke and Lwana, highly significantly different between Lwana and Nyamunene and significantly different between Lwana and Tchinganda. For the cobble cover, Heke and Lwana, and also Lwana and Nyamunene were significantly different. Boulder cover was similar over all rivers. The bedrock cover was significantly different between Lwana and Tchinganda.

3.3 | Association between *Labeobarbus* species and environmental variables

As all binary variables were insignificant in the RDA model and in the LR model ($P > 0.05$), they were removed from the analyses. RDA analysis revealed a significant effect of the entire set of environmental variables on the variation in the *Labeobarbus* community composition ($P = 0.002$, *d.f.* 5 and r^2 adjusted 0.17). Forward selection identified water depth, river width, canopy cover, silt, water temperature and electrical conductivity as significant environmental variables. The first and the second axes accounted for, respectively, 12.5 and 6.1% of the total variance in community composition.

In a plot of the first *vs.* the second RDA axis (Figure 4a), the localities from the Heke and Lwana cluster together and are distinct from the cluster of localities from the Nyamunene and Tchinganda, because of a lower electrical conductivity, and a higher river width, water temperature and canopy cover. There is a higher probability for the occurrence of *L. longifilis* in the former cluster and a higher probability for *L. paucisquamatus* in the latter.

In a second RDA, the localities upstream of the Tchinganda Falls and the corresponding *Labeobarbus* communities were excluded. Water temperature and silt were not significant and therefore removed from the analysis. Instead, pebble revealed to be significant and was included. The first and the second axes accounted for, respectively, 10.1 and 7.5% of the total variance in the community composition. The model was significant on all axes ($P = 0.001$ with *d.f.* 4 and r^2 adjusted 0.18). The general pattern is comparable with that of the first RDA (Figure 4b) in terms of clustering of localities, explaining variables (except water temperature) and associations with species. In addition, the occurrences of *L. brauni* and *L. longidorsalis* seem to be positively associated with the abundance of pebble and negatively with depth.

For the four major *Labeobarbus* species occurring in the Luhoho basin, an LR was applied using the environmental variables included in the PCA and the RDA for all samples and species, to explore the extent to which each individual variable is associated with the presence/absence of the individual *Labeobarbus* species (Supporting Information Table S2). The LR performed on *L. longidorsalis* revealed the goodness of fit (χ^2 probability) to be significant ($P < 0.001$) (Supporting Information Table S2a). After consecutive stepwise analyses ($P > 0.2$) with backward deletion and forward selection of the variables, pH, river width, the percentages of pebble and cobble, and electrical conductivity revealed to be the principal variables that predicted the occurrence of *L. longidorsalis* in the rivers studied. For

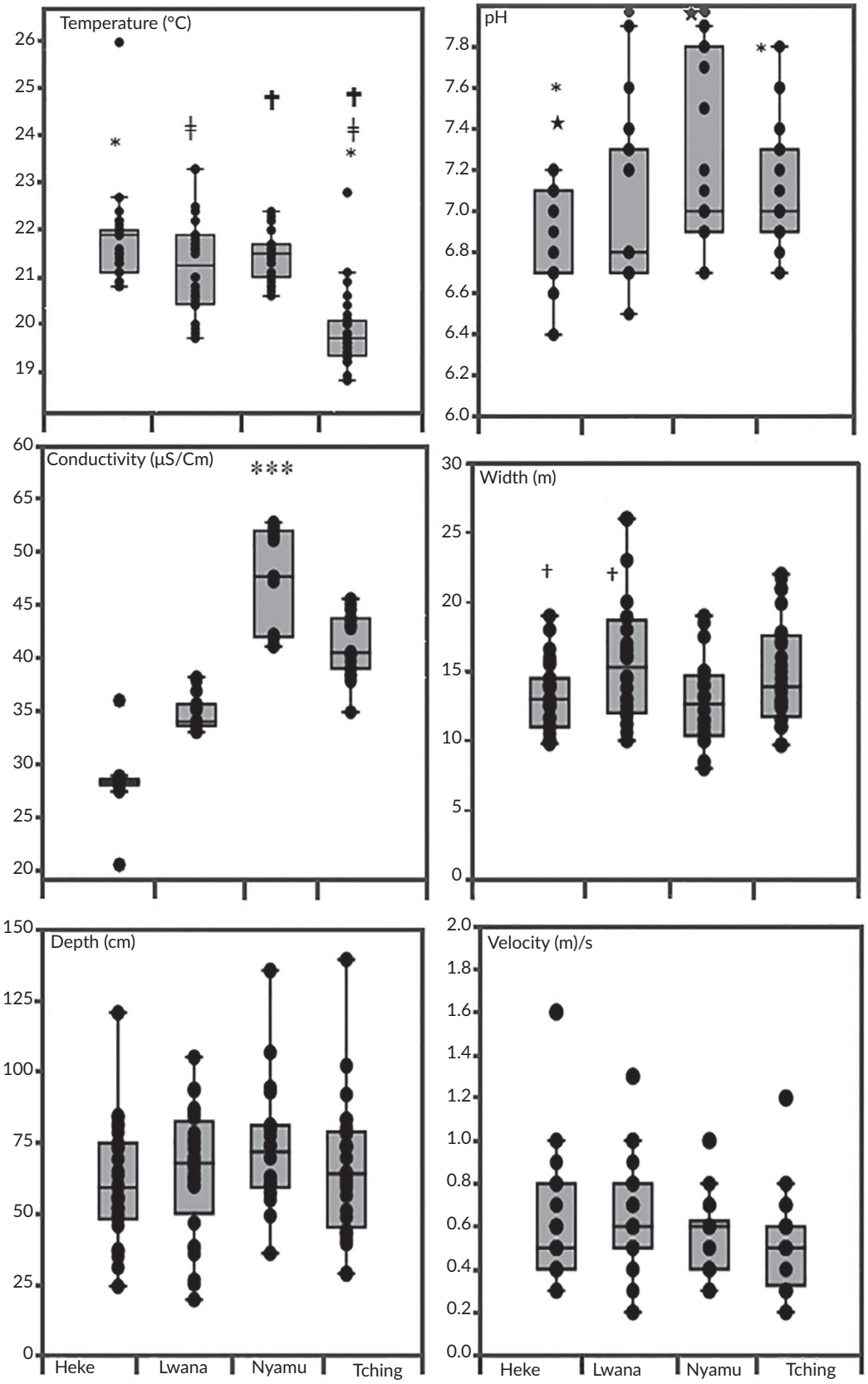


FIGURE 3 Boxplots with mean, S.D. and minimum and maximum values of the physico-chemical and hydrological parameters within the four studied rivers of the Middle Luhoho: Heke, Lwana, Nyamunene (Nyam) and Tchinganda (Tching). Symbols refer to a significant difference between: (⁎) Heke *vs*. Tchinganda; (ǂ) Lwana *vs*. Tchinganda; (✝) Nyamunene *vs*. Tchinganda; (★) Heke *vs*. Nyamunene; (◆) Lwana *vs*. Nyamunene; (†) Heke *vs*. Lwana; and (⁎⁎⁎) all tests between rivers were significantly different (for electrical conductivity)

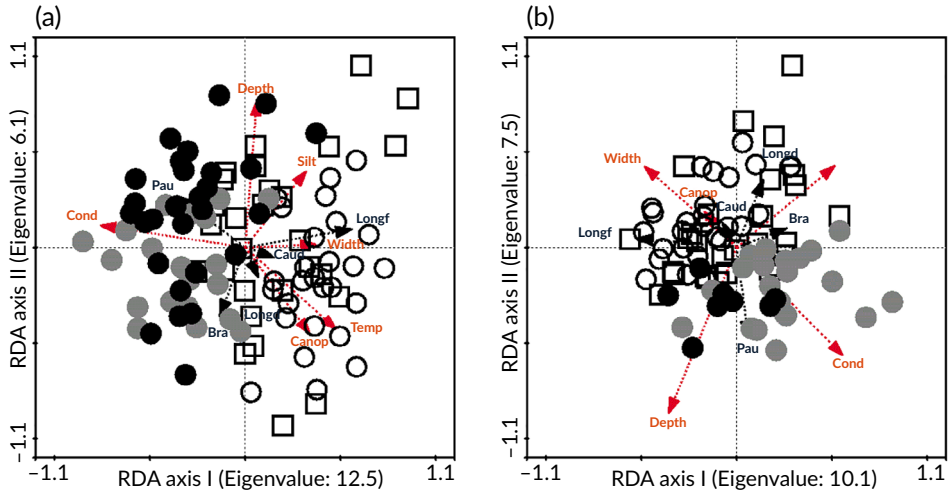


FIGURE 4 Orthogonal projection resulting from a Redundancy Analysis of the sampling stations in relation to: (a) six environmental variables statistically significant to explain the *Labeobarbus* assemblage in the 105 sampling localities, and (b) five environmental variables statistically significant to explain the *Labeobarbus* assemblage after removing the 20 stations from upstream of the Tchinganda Falls. Heke, (○); Lwana, (□); Nyamunene, (●) and Tchinganda, (●). Species vectors added: *L. brauni* (Bra), *L.* cf. *caudovittatus* (Caud), *L. longidorsalis* (Longd), *L. longifilis* (Longf) and *L. paucisquamatus* (Pau). Abbreviations used for the environmental variables are explained in Table 1

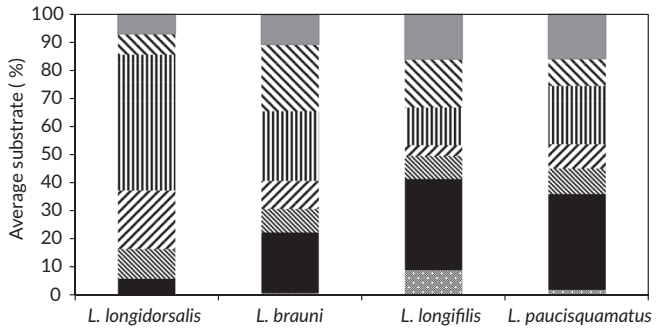


FIGURE 5 Average percentage of coverage of each individual substrate type per species

L. brauni, the probability associated with the goodness of fit in the LR model was weak ($P < 0.05$) (Supporting Information Table S2b). After consecutive stepwise analyses, boulder and electrical conductivity were the significant variables with potential for predicting the occurrence of *L. brauni* (Supporting Information Table S2b). For *L. longifilis*, the probability associated with the goodness of fit was also significant ($P < 0.01$) (Supporting Information Table S2c). Only electrical conductivity was significant as a predicting variable. Finally, the probability associated with the goodness of fit was significant ($P < 0.001$) for *L. paucisquamatus* (Supporting Information Table S2d). The model indicated that water temperature and electrical conductivity were significant predictors of the occurrence of this species.

3.4 | Patterns of substrate coverage by *Labeobarbus* species in the four rivers

The average composition of the substrates across the different sampling localities for the major four studied *Labeobarbus* species in the Luhoho revealed the heterogeneous composition of the substrate irrespective of the mouth phenotype (Figure 5). The average composition of substrate joins the results obtained through the RDA and LR approaches. Although *L. longidorsalis* and *L. brauni* share a scraping mouth phenotype, cobble (48%) and pebble (21%) are roughly dominant for the former, whereas the substrate composition of the latter is fairly represented by cobble (25%), boulder (24%) and sand (22%). Based on the substrate composition, *L. longidorsalis* hints to be rigid on the requirement of the hard substrate, whereas *L. brauni* uses a large range of substrates. Furthermore, the similarity in substrate composition is underscored for the species with a fleshy mental lobe. Indeed, the sand coverage represents 32 and 34% for *L. longifilis* and *L. paucisquamatus*, respectively. It appears that *L. longifilis* and *L. paucisquamatus* do not particularly prefer large hard substrates.

4 | DISCUSSION

4.1 | Environmental studies of fishes at the species level in the Congo basin are scarce

Ecological studies on the fish fauna of the Congo basin have rarely been undertaken and the existing studies were performed on a community-based level rather than at the species level. The results of the most recent studies are only available as part of PhD manuscripts (Banyankimbona, 2012; Decru, 2015; Moelants, 2015). Nonetheless, the first data about the links between fish communities and environmental variables for a major part of the Congolese section of the Inkisi River, the largest of the Lower Congo affluents, were provided by Wamuini Lunkayilakio *et al.* (2010) as for the Lefini basin published by Ibala Zamba *et al.* (2019).

The present study investigated, for the first time, the link between environmental variables and *Labeobarbus* species occurrences at a small geographical scale. Furthermore, it has been concentrating on an assemblage of *Labeobarbus* species and the relationships between their occurrence and a set of studied environmental variables. Exploring the Lowa basin has been severely hampered because of the presence of armed groups throughout most of the basin and the inaccessibility of the area because of the lack of roads. The occurrence of *Labeobarbus* species in four affluent rivers of the Luhoho, along a stretch covering *c*. 21 km, was an unprecedented opportunity to conduct such a study.

4.2 | Insight into habitat segregation between and within *Var*.- and *Lab*.-mouth phenotypes

The findings of this study reveal different habitat preferences between and to some extent within the two major mouth phenotypes. *L. brauni* and *L. longidorsalis*, both with a cornified cutting edge on the lower jaw (*Var*.-mouth phenotype; chiselmouths), seemed to have similar environmental preferences, illustrated by an adjacent position of their vectors in both RDAs. For example, in the second RDA, pebble revealed to be an important substrate for both species. In the LR, in contrast, the occurrence of both species appears to be predicted by a different set of variables. In the latter approach, cobble and pebble were important substrates for *L. longidorsalis*, whereas boulder revealed to be the most important substrate for *L. brauni*. The rejection of pebble in the LR for *L. brauni* might be because of the little proportion of this substrate in various localities where *L. brauni* was collected compared to those where *L. longidorsalis* was caught (Figure 5).

The differences in substrate composition at the localities where *L. longidorsalis* and *L. brauni* were found (cobble and pebble *vs*. boulder: Supporting Information Table S2a,b) provide a novel insight into the understanding of the ecology of *Labeobarbus* populations with a *Var*.-mouth phenotype and illustrates that having a similar *Var*.-mouth phenotype does not necessarily imply a similar use of hard rocky substrate types (see also Levin *et al*., 2019). In general, nonetheless, the results point to the association of the *Var*-mouth species with some forms of hard rocky substrate. In addition, both species with a scraping mouth were not found syntopically. In the Middle Luhoho, *L. longidorsalis* is mostly limited to the Lwana affluent and was only once caught in the Heke. The river bed of the Lwana is dominated by a large amount of cobble and pebble and only a small amount of boulders. Instead, in the Heke, Nyamunene and Tchinganda, the proportion of both pebble and cobble is lower.

L. brauni is widespread in the basin. It has been reported in the four investigated affluents and was syntopic at 10 stations with *L. longifilis*, with a fleshy mental lobe on the lower jaw (*Lab*.-mouth phenotype: rubberlip). Instead, *L. longidorsalis* was only once syntopic with *L. longifilis* in the Lwana. The authors therefore presume that although both have a scraping mouth, the feeding habits of *L. brauni* and *L. longidorsalis* are slightly different.

L. longifilis and *L. paucisquamatus* both have a fleshy mental lobe on the lower jaw. In the former, it is well developed, and in the latter, it is small, sometimes attached, or even completely absent. Nonetheless, both species appear to be linked to substrates without pebbles, cobbles and boulders (Figure 4 and Supporting Information Table S2). Nevertheless, both the RDAs and the LR indicated that they differ in other ecological parameters, not related to substrate. As such, *L. longifilis* occurred in warmer localities with a relatively low electrical conductivity, whereas *L. paucisquamatus* occurred at localities with a lower temperature and a higher electrical conductivity (Supporting Information Table S2c,d). The Heke and Lwana are situated in the lowlands (810–876 and 834–890 m asl, respectively), along a vegetation of the equatorial forest type. Instead, the Nyamunene and Tchinganda affluents are situated more into the highlands (876–938 and 1007–1103 m asl, respectively) within a transitional forest vegetation. The temperature difference is related to the difference in altitude.

The lack of a strong association of *L. longifilis* and *L. paucisquamatus* with pebble and/or cobble seems to be in contrast with Matthes' (1963: 29) statement that a developed lobe results from an adaptation to a feeding mode over stony habitats. It appears that relatively little ecological fieldwork was conducted within the framework of Matthes' (1963) study and that, instead, the eco-functional meaning of the mouth phenotype in *Labeobarbus* with a fleshy lobe was inferred from indirect observations, such as the study of gut contents and the observation of live fish behaviour in aquaria (see Matthes, 1963: 3). Nevertheless, Matthes' (1963) conclusion is supported in case he had considered as rubberlips a phenotype with hypertrophied lips and a mental lobe on the basis of the figure of *L. nedgia* Rüppell, 1835 that he had shown (plate ix a). Across the investigated rivers of the Luhoho this phenotype is scarce, but two specimens of similar phenotype were sampled in the basin during the fieldwork of Kisekelwa *et al*. (2020) only over localities covered by muddy substrate. Matthes' conclusion seems to be supported by anecdotical evidence on a thick lipped specimen (developed mental lobe) of *Labeobarbus holubi*, now *L. aeneus*, from a fast-flowing stretch of Vaal River that changed into a normal-lipped specimen after having been kept in a pond with muddy substrate for over a year (Groenewald, 1958). Groenewald (1958) thought that the degree of mental lobe development in *Labeobarbus* was not species specific. Nonetheless, in the Luhoho, variation in the development of the mental lobe has been found to be related to multiple hybridization events between a rubberlip and chiselmouth species (Kisekelwa, 2019). As such, further investigations are needed to address the questions regarding the development of the mental lobe in *Labeobarbus* and its possible ecological significance.

At present, it is difficult to postulate about the reasons for the differences between both *Labeobarbus* species with a fleshy mental lobe. They may be related to differential preferences in food items. Marlier (1960) mentioned that in river systems of the Kivu region, several aquatic invertebrates are seemingly influenced by chemical characteristics of the water. In Brazilian rivers, electrical conductivity was found important to determine the macroinvertebrate community (Godoy *et al*., 2017). Possibly, the differential distribution of the prey items

explains the differences in the distribution of the two *Labeobarbus* species.

In general, the electrical conductivity of the rivers of the Lowa system is relatively low (Kisekelwa, 2012), because of the low dissolution of the bedrock and the overall chemical characteristics of the region (Marlier, 1954). The higher electrical conductivity in the Nyamunene and the Tchinganda could result from changes in land use, such as agriculture and mining activities, resulting in an increased influx of chemicals into the water. Unfortunately, historical data on the electrical conductivity are lacking for the Middle Luhoho (Marlier, 1954). In addition, the Tchinganda drains a densely populated agglomeration, which may also contribute to a higher level of dissolved substances. Nevertheless, the authors refrain from attributing the difference in electrical conductivity between rivers solely to human activities, as inter-basin differences occur throughout the entire Middle Luhoho basin. Unfortunately, at present, geological data that may explain these differences are lacking. The absence of *L. longifilis* from the Tchinganda may not singularly be attributed to low temperature and high electrical conductivity but could also be related to the presence of the Tchinganda Falls (15 m height) near the city of Bulambika (Figure 2). In general, waterfalls could act as a barrier against the dispersal of *Labeobarbus* species (Lévêque, 2006). Nonetheless, as other species, *i.e. L. paucisquamatus* and *L. brauni*, are present in stretches both up- and downstream of these falls, it is difficult to explain why *L. longifilis* is blocked by the same hydro-geographic structure.

The results of this study provide the basis for future studies on the ecology of the riverine *Labeobarbus* species in the Congo basin upon which evolutionary understandings might be documented. One possible avenue for further research is to investigate the ecological segregation observed by radio-tag telemetry in combination with invertebrate community differences along the occupied river stretches.

4.3 | Need for the protection of the *Labeobarbus* in the Luhoho basin

Numerous human activities such as intensified agriculture, including palm oil exploitation in the watershed (Brown & Abell, 2005), mining and overfishing using explosives and ichthyotoxins, are threatening the *Labeobarbus* species in the Luhoho basin (Kisekelwa *et al*., 2020). The more so as agriculture activities will further need to intensify considering the African human population is expected to double by 2050 (Darwall *et al*., 2011). As such, the African continent will experience an unprecedented increase in agricultural activities (Dudgeon *et al*., 2011) that will further emphasize the integrity of the biological diversity in many aquatic ecosystems (Snoeks *et al*., 2011). Substrate may be removed along the bank and habitat availability reduced as well as increase in electrical conductivity because of intense agriculture activities and dumping of wastes from small palm oil plants (Brown & Abell, 2005; Helfman, 2007; Kasangaki *et al*., 2008; Magierowski *et al*., 2012). Furthermore, fishing with dynamite also seriously alters substrate availability directly on the riverbed across the Luhoho basin (Kisekelwa, 2019). The present study provides important information for the development programmes that aim an effective local conservation of the different *Labeobarbus* species in the Middle Luhoho. It can be done by increasing population awareness on the use of appropriate agriculture far away from the river banks and the dynamite should be vehemently prohibited. As such, *L. brauni* and *L. paucisquamatus* can be preserved, the restricted endemic species to the Luhoho basin, whereas *L. longifilis* is probably also present in the Luka basin, another left bank affluent of the Lowa basin (Figure 2). *L. longidorsalis* seems even more widespread as also present in the Luka (Kisekelwa *et al*., 2020), but in the Aruwimi/Ituri basin, a right bank affluent of the upper stretch of the Middle Congo, as well (Decru *et al*., 2017).

ACKNOWLEDGEMENTS

Heri Muzungu, Akonkwa Kahasha, La Vie Buterezi, Abdoul Shehe and Itaji Waishire Baba Solo are warmly thanked for their assistance in collecting the data. We thank Grite Nelson (Animal Ecology, Global Change and Sustainable Development, KU Leuven, Belgium and Department of Water, Environmental Sciences and Engineering, Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania) for having kindly accepted to generate the r^2 adjusted and d.f. of the RDA in R packages and Boniface Nkombe (Division of Soil and Water, KU Leuven, Belgium & Life Sciences and Bio-Engineering, Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania) for correcting the spelling mistakes in an earlier version of the manuscript. The data for this study were collected and analysed within the framework of the PhD of the first author financially supported by the Mbisa Congo I (2013–2018) and the Mbisa Congo II (2019–2023) projects, both financed through a framework agreement between the Royal Museum of Central Africa and the Belgian Development Cooperation. The first author also obtained a grant from the International Foundation for Science (IFS n°A5787-1 (2015–2017)) which partially covered the fieldwork of this study.

AUTHOR CONTRIBUTIONS

T.K. designed the study, developed the sampling method, designed the statistical approach, analysed the data and wrote the manuscript. J.S. participated in the discussion on the study design and on data analysis, and in manuscript writing. A.I.Z. and G.A.S. helped, respectively, in discussion on RDA and logistic regression implementation. P.M.M. and I.M. participated in text writing. P.L. participated in discussions on data analysis (RDA) and manuscript writing. E.V. participated in discussions on problem design and in manuscript writing.

ORCID

Tchalondawa Kisekelwa https://orcid.org/0000-0003-4730-5285
Armel Ibala Zamba https://orcid.org/0000-0002-9899-0780
Gaston Sefu Amzati https://orcid.org/0000-0002-9019-224X
Pascal Mulungula Masilya https://orcid.org/0000-0002-6205-2004
Pieter Lemmens https://orcid.org/0000-0002-3135-9724
Emmanuel Vreven https://orcid.org/0000-0002-2503-4712

REFERENCES

Anteneh, W., Getahun, A., Dejen, E., Sibbing, F. A., Nagelkerke, L. A. J., de Graaf, M., … Palstra, A. P. (2012). Spawning migrations of the endemic *Labeobarbus* (Cyprinidae, Teleostei) species of Lake tana, Ethiopia: Status and threats. *Journal of Fish Biology*, *81*, 750–765. https://doi.org/10.1111/j.1095–8649.2012.03362.x.

Banister, K. E. (1972). On the cyprinid fish *Barbus alluaudi* Pellegrin: A possible intergeneric hybrid from Africa. *Bulletin of the British Museum of Natural History (Zoology)*, *24*, 261–290.

Banister, K. E. (1973). A revision of the large *Barbus* (Pisces, Cyprinidae) of east and Central Africa, studies on African Cyprinidae part II. *Bulletin of the British Museum Natural history*, *23*, 1–148.

Banister, K. E. (1976). A possible intergeneric cyprinid hybrid from Lake Tanganyika. *Bulletin of the British Museum Natural History (Zoology)*, *30*, 171–189.

Banyankimbona, G. (2012). *The fish diversity of the burundese rivers and the impact of human activities on this ichtyofauna* (PhD thesis). KU Leuven, Leuven, Belgium.

Berrebi, P. (1981). Contribution à l'étude du sous-genre *Labeobarbus* (genre *Barbus*, Poissons Cyprindiés) au Maroc. *Bulletin de l'Institut Scientifique, Rabat*, *198*(5), 59–72.

Berrebi, P. (1995). Speciation of the genus *Barbus* in the north Mediterannean Basin: Recent advances from biochemical genetics. *Biological Conservation*, *72*, 237–249.

Berrebi, P., Chenuil, A., Kotlik, P., Machordom, A., & Tsigenopoulos, C. S. (2014). Disentangling the evolutionary history of the genus *Barbus sensu lato*, a twenty years adventure. In M. J. Alves, A. Cartaxana, A. M. Correia, & L. F. Lopes (Eds.), *Professor Carlos Almaça (1934–2010) - Estado da Arte em Áreas Cientificas que Desenvolveu* (pp. 29–55). Lisboa, Portugal: Museu Nacional de História Natural e da Ciência.

Blanchet, G. F., Legendre, P., & Borcard, D. (2008). Forward selection of explanatory variables. *Ecology*, *89*(9), 2623–2632.

Bonar, S. A., Contreras-Balderas, S., & Iles, A. C. (2009). An introduction to standardized sampling. In S. A. Bonar, W. A. Hubert, & D. W. Wills (Eds.), *Standard methods for sampling north American freshwater fishes* (pp. 1–11). Bethesda, MD: American Fisheries Society.

Broad, T. L., Townsend, C. R., Closs, G. P., & Jellyman, D. J. (2001). Microhabitat use by longfin eels in New Zealand streams with contrasting riparian vegetation. *Journal of Fish Biology*, *59*, 1385–1400.

Brown, A., & Abell, R. (2005). Albertine Highlands. In L. M. Thieme, R. Abell, M. L. J. Stiassny, P. Skelton, B. Lehner, G. G. Teugels, et al. (Eds.), *Freshwater ecoregions of Africa and Madagascar. A conservation assessment* (pp. 244–246). Washington, DC: Island Press.

Carmona, J. A., Doadrio, I., Márquez, A. L., Real, R., Hugueny, B., & Vargas, J. M. (1999). Distribution patterns of indigenous freshwater fishes in the Tagus River basin, Spain. *Environmental Biology of Fishes*, *54*, 371–387.

Cohen, J. (1977). *Statistical power analysis for the behavioral sciences*. New York, NY: Academic Press.

Craney, T. A., & Surles, J. G. (2002). Model-dependent variance inflation factor cutoff values. *Quality Engineering*, *14*(3), 391–403. https://doi.org/10.1081/QEN-120001878.

Crass, R. S. (1964). *Freshwater fishes of Natal*. Pietermaritzburg, South Africa: Shuter & Shooter.

Crosa, G., Villa, S., & Cotta-Ramusino, M. (2002). Local *versus* longitudinal biological variability in a high gradient stream. *Hydrobiologia*, *477*, 107–114.

Darwall, W., Smith, K., Allen, D., Holland, R., Harrison, I., & Brooks, E. (Eds.). (2011). *The diversity of life in African freshwaters: Underwater, under threat; an analysis of the status and distribution of freshwater species throughout mainland Africa*. Gland, Switzerland and Cambridge, UK: IUCN Red List.

de Graaf, M., Nentwich, E. D., Osse, J. W. M., & Sibbing, F. A. (2005). Lacustrine spawning: Is this a new reproductive strategy among 'large' African cyprinid fishes? *Journal of Fish Biology*, *66*, 1214–1236. https://doi.org/10.1111/j.1095-8649.2005.00671.x.

de Graaf, M., van de Weerd, G. H., Osse, J. W. M., & Sibbing, F. A. (2010). Diversification of prey capture techniques among the piscivores in Lake Tana's (Ethiopia) *Labeobarbus* species flock (Cyprinidae). *African Zoology*, *45*(1), 32–40.

Decru, E. (2015). *The ichthyofauna of the Central Congo basin: Diversity and distribution in the north-eastern tributaries* (PhD thesis). KU Leuven, Leuven, Belgium.

Decru, E., Vreven, E., Danadu, C., Walanga, A., Mambo, T., & Snoeks, J. (2017). The ichthyofauna of the Itimbiri, Aruwimi and Lindi/Tshopo Rivers (Congo basin): Diversity and distribution patterns. *Acta Ichthyologica et Piscatoria*, *47*(3), 225–247.

Dudgeon, D., Paugy, D., Lévêque, C., Rebelo, L. M., & McCartney, M. P. (2011). Background. In W. Darwall, K. Smith, D. Allen, R. Holland, I. Harrison, & E. Brooks (Eds.), *The diversity of life in African freshwaters: Underwater, under threat; an analysis of the status and distribution of freshwater species throughout mainland Africa* (pp. 4–28). Gland, Switzerland and Cambridge, UK: IUCN Red List.

Eikaas, H. S., Kliskey, A. D., & Mcintosh, A. R. (2005). Spatial modeling and habitat quantification for two diadromous fish in New Zealand streams: A GIS-based approach with application for conservation management. *Environnemental Management*, *36*(5), 726–740.

Erős, T., Botta-Dukát, Z., & Grossman, G. D. (2003). Assemblage structure and habitat use of fishes in a central European submontane stream: A patch-based approach. *Ecology of Freshwater Fish*, *12*, 141–150.

Gaigher, I. G. (1975). The occurrence of '*Varicorhinus*' and 'rubberlip' mouth forms in the small scaled yellowfish, *Barbus polylepis*. *Piscator*, *92*, 162–163.

Garcia, L. V. (2004). Escaping the Bonferroni iron claw in ecological studies. *Oikos*, *10*, 657–663.

Gebremedhin, S., Mingist, M., Getahun, A., & Anteneh, W. (2012). Spawning migration of *Labeobarbus* spp. (pisces: Cyprinidae) of lake tana to Arno-Garno River, lake tana sub-basin, Ethiopia. *Ethiopian Journal of Sciences*, *35*(2), 95–106.

Godoy, B. S., Queiroz, L. L., Lodi, S., & Oliveira, L. G. (2017). Environment and spatial influences on aquatic insect communities in Cerrado streams: The relative importance of conductivity, altitude, and conservation areas. *Neotropical Entomology*, *46*, 151–158. https://doi.org/10.1007/s13744-016-0452-4.

Grenier, S., Barre, P., & Litrico, I. (2016). Phenotypic plasticity and selection: Nonexclusive mechanisms of adaptation. *Scientifica*, *2016*, 7021701.

Grossman, G. D., & Freeman, M. C. (1987). Microhabitat use in a stream fish assemblage. *Journal of Zoology (London)*, *212*, 151–176.

Groenewald, A. A. V. J. (1958). A revision of the genera *Barbus* and *Varicorhinus* in Transvaal. *Annals of the Transvaal Museum*, *23*, 263–330.

Guégan, J. F., Rab, P., Machordom, A., & Doadrio, I. (1995). New evidence of hexaploidy in 'large' African *Barbus* with some consideration on the origin of hexaploidy. *Journal of Fish Biology*, *47*, 192–198.

Hosmer, D. W., & Lemeshow, S. (2000). *Applied logistic regression*. New York, US, Chichester, UK, Weinheim, DE, Brisbane, AU, Singapore, SG, Toronto, CA: A Wiley-Interscience Publication.

Helfman, G. S. (2007). *Fish conservation: A guide to understanding and restoring. Global aquatic biodiversity and fishery resources*. Washington, Covelo, USA, London, UK: Islands Press.

Humphries, P., Keckeis, H., & Finlayson, B. (2014). The river wave concept: Integrating River ecosystem models. *Bioscience*, *64*(10), 870–882.

Ibala Zamba, A., Vreven, E., Mamonekene, V., & Snoeks, J. (2019). Fish community assemblages in relation to environmental variables in the Lefini River, middle Congo River basin (republic of Congo). *Cybium*, *43*(1), 83–95.

Johnston, R., & Sheaves, M. (2008). Cross-channel distribution of small fish in tropical and subtropical coastal wetlands is trophic-, taxonomic-,

and wetland depth-dependent. *Marine Ecology Progress Series*, *357*, 255–270.

Jubb, R. A. (1967). *Freshwater fishes of southern Africa*. Cape Town, South Africa: A. A. Balkema.

Kamdem, A. T., & Teugels, G. (1998). Diversity patterns of fish assemblages in the lower Ntem River basin (Cameroon), with notes on potential effects of deforestation. *Archiv für Hybobiologia*, *141*, 421–446.

Kasangaki, A., Chapman, L. J., & Balirwa, J. (2008). Land-use and the ecology of benthic macroinvertebrate assemblages of high-altitude rainforest streams in Uganda. *Freshwater Biology*, *53*, 681–697. https://doi.org/10.1111/J.1365-2427.2007.01925.X.

Katemo Manda, B. (2020). *Ichtyofaune du Parc National de l'Upemba (PNU/RD Congo): diversité, écologie, conservation et gestion durable* (PhD thesis). KU Leuven, Leuven, Belgium.

Kawanishi, R., Inoue, M., Takagi, M., Miyake, Y., & Shimizu, T. (2010). Habitat factors affecting the distribution and abundance of the spinous loach *Cobitis shikokuensis* in southwestern Japan. *Ichthyological Resources*, *58* (3), 202–208. https://doi.org/10.1007/s10228-011-0208-4.

Keng, W. M., & Rahman, H. (2012). Logistic regression to predict termite occurrences with environmental variables in primary Forest and oil palm ecosystem: The case study in Sabah, Malaysia. *Biological & Environmental Engineering Society*, *APCBEE Procedia*, *4*, 53–57.

Kisekelwa, T. 2012. *Contribution à la connaissance de l'ichtyofaune des rivières Lowa et Elila à l'Est de la RDC* (Master thesis). University of Burundi.

Kisekelwa, T. (2019). *The fish diversity of the river systems of the Kahuzi-Biega National Park (East DRC)* (PhD thesis). KU Leuven, Leuven, Belgium.

Kisekelwa, T., Snoeks, J., & Vreven, E. (2020). An annotated checklist of the fish fauna of the river systems draining the Kahuzi-Biega National Park (eastern DR Congo). *Journal of Fish Biology*, *96*(3), 700–721.

Lévêque, C. (1997). *Biodiversity dynamics and conservation. The freshwater fish of tropical Africa*. New York, NY: Cambridge University Press.

Lévêque, C. (2006). Biogéographie et mise en place des faunes ichtyologiques actuelles. In C. Lévêque & D. Paugy (Eds.), *les poissons des eaux continentales africaines: diversité, écologie et utilisation par l'homme* (pp. 75–88). Paris, France: IRD.

Levin, A. B., Golubtsov, A. S., Dgebuadze, Y. Y., & Mugue, N. S. (2013). New evidence of homoplasy within the African genus *Varicorhinus* (Cyprinidae): An independent origin of specialized scraping forms in the adjacent drainage systems of Ethiopia inferred from mtDNA analysis. *African Zoology*, *48*(2), 400–406.

Levin, A. B., Casal-López, M., Simonov, E., Dgebuadze, Y. Y., Mugue, N. S., Tiunov, A. V., … Golubtsov, A. S. (2019). Adaptive radiation of barbs of the genus *Labeobarbus* (Cyprinidae) in an east African river. *Freshwater Biology*, *64*, 1721–1736. https://doi.org/10.1111/fwb.13364.

Levin, A. B., Simonov, E., Dgebuadze, Y. Y., Levina, M., & Golubtsov, A. S. (2020). In the rivers: Multiple adaptative radiations of cyprinid fishes (*Labeobarbus*) in Ethiopian highlands. *Nature*, *10*, 7192. https://doi.org/10.1038/s41598-020-64350-4.

Luoto, M., & Seppälä, M. (2002). Modelling the distribution of Palsas in Finnish Lapland with logistic regression and GIS. *Permafrost Periglacial Process*, *13*, 17–28. https://doi.org/10.1002/ppp.404.

Machordom, A., & Doadrio, I. (2001). Evolutionary history and speciation modes in the cyprinid genus *Barbus*. *Proceedings of the Royal Society*, *268*, 1297–1306.

Magierowski, R. H., Davies, P. E., Read, S. M., & Horrigan, N. (2012). Impacts of land use on the structure of river macroinvertebrate communities across Tasmania, Australia: Spatial scales and thresholds. *Marine and Freshwater Research*, *63*, 762–776. https://doi.org/10.1071/MF11267.

Marlier, G. (1954). Recherches hydrobiologiques dans les rivières du Congo orientale. *Hydrobiologia*, *6*, 225–264.

Marlier, G. (1960). Les rivières du Kivu. *Extrait de 'les Naturalistes Belges'*, *41*, 427–437.

Matthes, H. (1963). A comparative study of the feeding mechanisms of some African Cyprinidae (Pisces, Cypriniformes). *Bijdragen tot de Dierkunde*, *3*, 3–35.

Moelants, T. (2015). *Diversity and ecology of the ichthyofauna of the middle and upper Congo basin: A case–study in the region of the Wagenia falls (Democratic Republic of Congo)* (PhD thesis). KU Leuven, Leuven, Belgium.

Moyle, P. B., & Cech, J. J., Jr. (2004). *Fishes. An introduction to ichthyology* (5th ed.). Upper Saddle River, NJ, USA: Pearson Prentice Hall.

Moran, M. D. (2003). Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos*, *100*(2), 403–405.

Nagelkerke, L. A. J., Sibbing, F. A., van den Boogart, J. G. M., Lammens, E. H. R. R., & Osse, J. W. M. (1994). The barbs (*Barbus* spp.) of Lake tana: A forgotten species flock? *Environmental Biology of Fishes*, *39*, 1–22.

Nagelkerke, L. A. J., & Sibbing, F. A. (1997). A revision of the large barbs (*Barbus* spp., Cyprinidae, Teleostei) of Lake tana, Ethiopia, with a description of seven new species. In L. A. J. Nagelkerke (Ed.), *The barbs of Lake tana, Ethiopia: Morphological diversity and its implications for taxonomy, trophic resource partitioning, and fisheries* (pp. 105–170). Wageningen, The Netherlands: Wageningen Agricultural University.

Oellermann, L. K., & Skelton, P. H. (1990). Hexaploidy in yellowfish species (*Barbus* Pisces, Cyprinidae) from southern Africa. *Journal of Fish Biology*, *37*, 105–115.

Pettit, N. E., Warfe, D. M., Kennard, M. J., Pusey, B. J., Davies, P. M., & Douglas, M. M. (2012). Dynamics of in–stream wood and its importance as fish habitat in a large tropical floodplain river. *River Research and Applications*, *29*, 864–875.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution*, *43*, 223–225.

Sanharawi, M. E., & Naudet, F. (2013). Comprendre la régression logistique. *Journal Français d'Ophtamologie*, *36*, 710–715.

Seilheimer, T. S., & Fisher, W. L. (2010). Habitat use by groundwater–dependent streams of southern Oklahoma. *American Midland Naturalist Journal*, *164*, 201–216.

Sheaves, M., Johnston, R., & Connolly, R. M. (2010). Temporal dynamics of fish assemblages of natural and artificial tropical estuaries. *Marine Ecology Progress Series*, *410*, 143–157. https://doi.org/10.3354/meps08655.

Sibbing, F. A., & Nagelkerke, L. A. J. (2000). Resource partitioning by Lake tana barbs predicted from fish morphometrics and prey characteristics. *Reviews in Fish Biology and Fisheries*, *10*, 393–437.

Skelton, P. H. (2001). *A complete guide to the freshwater fishes of southern Africa*. Cap Town, SA: Stuik Publishers.

Snoeks, J., Harrison, I. J., & Stiassny, M. L. J. (2011). The status and distribution of freshwater fishes. In W. Darwall, K. Smith, D. Allen, R. Holland, I. Harrison, & E. Brooks (Eds.), *The diversity of life in African freshwaters: Underwater, under threat; an analysis of the status and distribution of freshwater species throughout mainland Africa* (pp. 42–91). Gland, Switzerland and Cambridge, UK: IUCN Red List.

Thompson, C. G., Kim, R. S., Aloe, A. M., & Betsy, J. B. (2017). Extracting the variance inflation factor and other multicollinearity diagnostics from typical regression results. *Basic and Applied Social Psychology*, *39* (2), 81–90. https://doi.org/10.1080/01973533.2016.1277529.

Tsigenopoulos, C. S., Kasapidis, P., & Berrebi, P. (2010). Phylogenetic relationships of hexaploid large–sized barbs (genus *Labeobarbus*, Cyprinidae) based on mtDNA data. *Molecular Phylogenetics and Evolution*, *56*, 851–856.

Vreven, E. J. W. M. N., Musschoot, T., Snoeks, J., & Schliewen, U. K. (2016). The African hexaploid Torini (Cypriniformes: Cyprinidae): Review of a tumultuous history. *Zoological Journal of Linnean Society*, *177*, 231–305.

Vreven, E. J. W. M. N., Musschoot, T., Decru, E., Wamuini Lunkayilakio, S., Obiero, K., Cerwenka, A. F., & Schliewen, U. K. (2018). The complex

origins of mouth polymorphism in the *Labeobarbus* (Cypriniformes: Cyprinidae) of the Inkisi River basin (lower Congo, DRC, Africa): Insights from an integrative approach. *Zoological Journal of the Linnean Society*, *186*, 414–482.

Wamuini Lunkayilakio, S., Vreven, E., Vandewalle, P., Mutambue, S., & Snoeks, J. (2010). Contribution à la connaissance de l'ichtyofaune de l'Inkisi au Bas–Congo / RDC. *Cybium*, *34*(1), 83–91.

Yamazaki, Y., Haramoto, S., & Fukasawa, T. (2006). Habitat uses of freshwater fishes on the scale of reach system provided in small streams. *Environmental Biology of Fishes*, *75*, 333–341. https://doi.org/10.1007/s10641-006-0032-2.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Kisekelwa T, Snoeks J, Zamba Al, et al. Association between *Labeobarbus* spp. (Teleostei: Cyprinidae) and environmental variables in the Luhoho basin (Eastern Congo River basin; DRC). *J Fish Biol*. 2021;1–14. https://doi.org/10.1111/jfb.14719