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Description of a new endemic *Enteromius* (Teleostei: Cyprinidae) from the upper Malagarazi in Burundi: Lessons for a protected area under implementation

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Abstract

Recent collecting efforts in the upper Malagarazi basin (2013–2022) allowed for an integrative study based on qualitative (colour), quantitative (meristic and metric), and barcoding gene [mtDNA, cytochrome c oxidase (COI)] data of specimens similar to *Enteromius* sp. 'ascutelatus', being a previously identified, potentially, new species. Based on these data, the present study confirms its identification as a new species for science, which is here formally described as *Enteromius nzigidaherai* sp. nov. This new species belongs to the group of *Enteromius* species for which the last unbranched ray of the dorsal fin is flexible and devoid of serrations along its posterior edge. This species has a horizontal series of black spots at the midlateral level of the sides. Three congeneric species, known from the Congo basin sensu lato, with two of them also found in the upper Malagarazi basin, are most similar to it. However, *E. nzigidaherai* sp. nov. is distinguished from the two sympatric upper Malagarazi species, that is, *E. quadrilineatus* and *E. lineomaculatus*, at least by two meristics and two morphometrics. It is also distinguished from *E. urostigma*, known from the upper Congo basin, by two meristics and one, apparently related, morphometric. In addition, a barcoding (mtDNA, COI) study revealed that the specimens of *E. nzigidaherai* sp. nov. form a well-supported, separate lineage, with a K2P genetic distance of more than 10% with specimens identified as *E. quadrilineatus* and *E. lineomaculatus*, both

originating from the upper Malagarazi basin and for which tissue samples were available. Finally, the new species was found to be endemic to the upper reaches of two left bank affluents of the upper Malagarazi basin: the Muyovozi and the Kinwa. However, both affluents are threatened by human activities, which seem to have resulted in its local disappearance as recent intensive collecting efforts in the latter affluent have remained unsuccessful. The species should thus be considered Critically Endangered (CR) according to IUCN criteria B1ab(ii,iv)c(i,iii). Therefore, it is hoped that the present description draws renewed attention to the importance of aquatic protection in the region by highlighting the need for the effective establishment of the Malagarazi Nature Reserve and concern for its optimal delimitation to efficiently protect the entire ichthyofauna of the upper Malagarazi, without excluding the fish species confined to its affluent rivers.

KEYWORDS
aquatic protection, COI barcoding, colour pattern, *Enteromius nzigidaherai* sp. nov., Malagarazi Nature Reserve, Nkoma Massif

1 | INTRODUCTION

With 1788 valid species attributed to 156 genera (Fricke et al., 2024), the Cyprinidae are one the largest freshwater fish family in the world. Globally, the family is only surpassed in number of valid species (1975 species) by the Gobiidae, of which the majority, however, are marine (Fricke et al., 2024; Nelson et al., 2016). Cyprinids are found in Eurasia, Africa, and North America (Berra, 2007; De Weirdt et al., 2007; Nelson et al., 2016; Seegers, 1996). Members of the family are characterised by a body generally covered with cycloid scales; a bare head; pelvic fins inserted in the middle of the abdomen; the mouth being devoid of teeth, with the upper jaw usually protrusible, bordered only by the premaxilla, often one or two pairs of barbels, rarely none, and well-developed pharyngeal bones, these last with one to three rows of teeth and a maximum of eight teeth per row (Berra, 2007; Nelson et al., 2016).

For the African fresh waters, with 483 valid species distributed over 20 genera (De Weirdt et al., 2007; Froese & Pauly, 2023; Skelton et al., 1991), the family Cyprinidae is surpassed by the Cichlidae only in numbers of valid species and genera, with 1100 species distributed over around 150 genera (Froese & Pauly, 2023). Among these African representatives, a total of 195 valid cyprinid species, distributed over nine genera, are known from the Congo basin sensu lato (s.l.) (RMCA, unpublished data), that is, the Congo basin, including Lake Tanganyika with its affluent river basins such as the Malagarazi and Rusizi, and thus also including Lake Kivu for the latter (Kashindye Benedicto et al., 2021; Katemo Manda, Snoeks, Decru, et al., 2023; Katemo Manda, Snoeks, Chocha Manda, et al., 2023; Mukweze Mulelenu et al., 2020; Roberts, 1975; Snoeks et al., 2011). However, species richness in certain African genera of the family, such as the widespread small African barbs or minnows of the genus *Enteromius* Cope 1867, has long been underestimated (Schmidt et al., 2017; Van Ginneken et al., 2017; Yang et al., 2015). Due to its large species diversity (Maetens et al., 2020; Schmidt et al., 2019), many species still remain undescribed, awaiting formal description for scientific recognition. In contrast, already described species have been synonymised (Seegers, 1996; Van Ginneken et al., 2017; Schmidt et al., 2018). These two, apparently opposite, alpha-taxonomic activities — species descriptions and synonymizations — however, seem to be linked to their great overall morphological similarity (Lévêque, 1989). Therefore, the use of an integrative approach, based on a combined use of qualitative (colour), other morphological (meristics and metrics), and molecular data, can be helpful in elucidating species identification and/or delineation problems in the genus (Hubert et al., 2008; Skelton, 2016). This has been exemplified, for instance, in the Congo basin through a case study on the *Enteromius miolepis*/*Enteromius eutaenia* species complex of the northeast part of the basin (Decru et al., 2016), as well as in a more encompassing part of the basin (Van Ginneken et al., 2017). Both of these studies, based on an integrated morphological and cytochrome c oxidase (COI) gene barcoding approach, revealed that *E. miolepis*/*E. eutaenia* species complex might be a waste bin appellation covering a total of, at least, 13 distinct species.

During recent field explorations (A. Bigirimana, 2013–2022), *Enteromius* specimens were collected in the upper Malagarazi basin (Figure 1), defined in this study as covering the basin from its source in south-eastern Burundi, at about 1600 m of altitude, and reaching downstream up to the Kibuyo Rapids situated in the mountainous area north of the Kigoma Region in Tanzania (Banyankimbona et al., 2012a; De Vos et al., 2001). These specimens turned out to be conspecific to *Enteromius* sp. 'ascutelatus', an undescribed species first collected by L. De Vos and L. Taverne (in 1991) and L. De Vos and P. Weiler (in 1991) and subsequently first identified by L. De Vos (in 1992) and later also reported by De Vos et al. (2001). However, based on the study of 9 of the 30 specimens of *E.* sp. 'ascutelatus' housed in the fish collection of the Royal Museum for Central Africa (RMCA), Banyankimbona et al. (2012a) concluded that these specimens were conspecific to *Enteromius lineomaculatus* (Boulenger 1903).

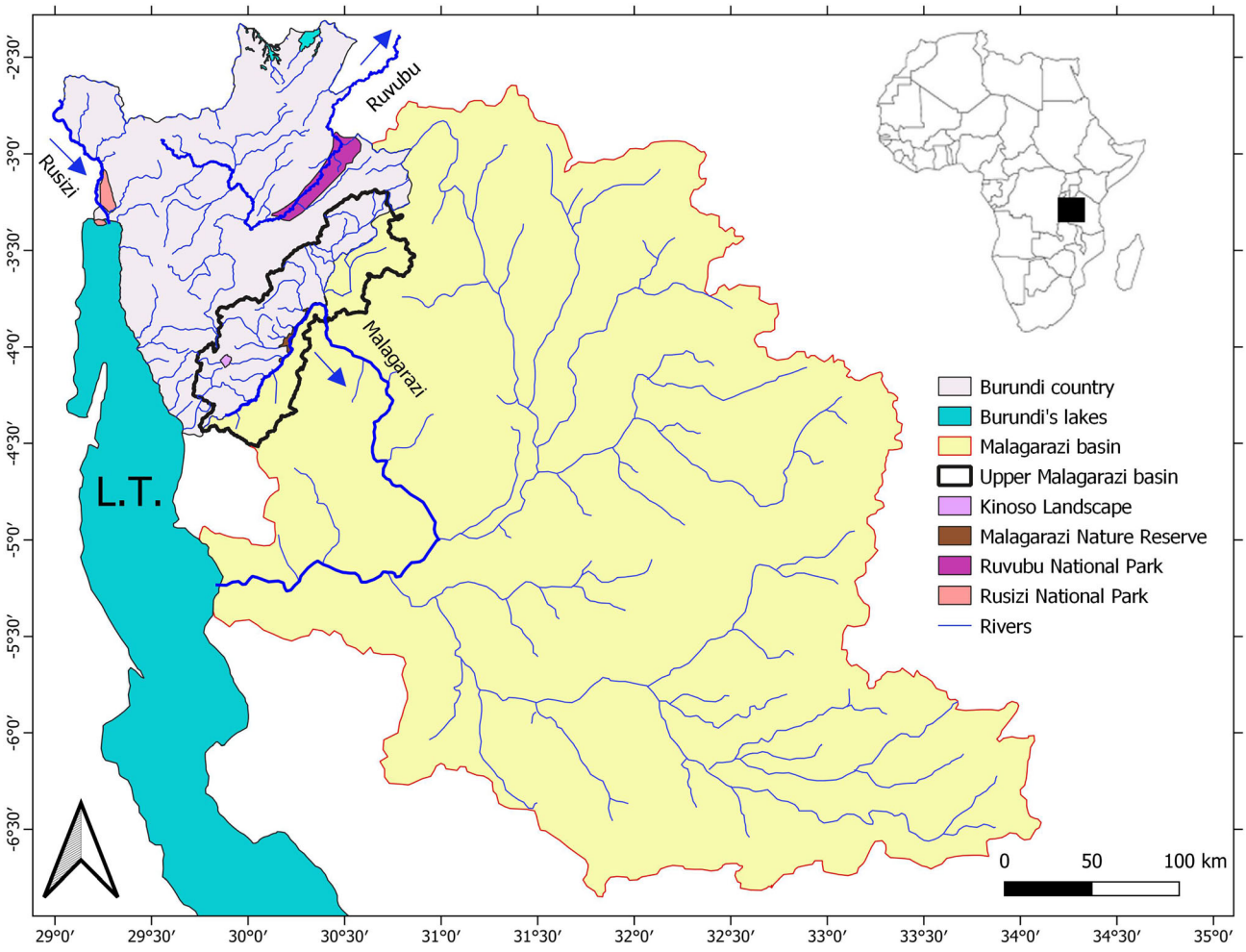

FIGURE 1 The upper Malagarazi (uM) basin in Burundi. L.T.: Lake Tanganyika. The flow direction of the rivers is indicated by a blue arrow. Malagarazi: Malagarazi basin; Ruvubu: Ruvubu basin, and Rusizi: Rusizi basin

Nevertheless, *E. lineomaculatus* had previously been identified as the senior synonym of three nominal species (Lévêque & Daget, 1984), with the other two being *Enteromius quadrilineatus* (David 1937) [junior synonym (j.s.) following Poll, 1946] and *Enteromius lornae* (Ricardo-Bertram 1943) (j.s. following Jackson, 1961). However, *E. lineomaculatus* occurs sympatrically in the upper Malagarazi basin, but only with *E. quadrilineatus*, and also has a much larger distribution (De Vos et al., 2001; Poll, 1933; Skelton, 2001). In contrast, *E. lornae* is known only from Luapula-Mweru and the Bangweulu-Chambeshi in Zambia, specifically in the upper part of the upper Congo basin only (Ricardo-Bertram, 1943; Seegers, 1996; Van Steenberge et al., 2014). As such, *E. lineomaculatus* might be considered a species complex, which comprises specimens and species that are similar in having the last unbranched dorsal-fin ray flexible and devoid of serrations along its posterior edge; and a horizontal series of black spots on the flank, these situated above the lateral line (LL), at the level of the dorsal fin especially (Seegers, 1996). The newly collected specimens were investigated in detail, using an integrative approach, combining colour (qualitative), other morphological (meristics and metrics) and molecular, that is, DNA barcoding (COI), data. These results confirmed those specimens are, indeed, conspecific with *E.* sp. 'ascutelatus' and distinct from all other currently known species and thus represent a new species for science. As such, it is here formally described and named *Enteromius nzigidaherai* sp. nov. This study also highlights the various human threats identified in the two affluents rivers where the new species was collected. In addition, based on this discovery, the present findings underline the importance of the protection and conservation of the aquatic diversity in the upper Malagarazi basin and thus the formal recognition of the Malagarazi Nature Reserve (MNR). However, considering the too-distant hydrographic occurrence of the new species with regard to the actual delimitation of the MNR, other, more optimal, conservation initiatives need to be envisioned as well.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

The specimens of the new species are either part of the historical collection of the RMCA or have recently (2013–2022) been collected in the upper Malagarazi, Burundi. Collecting for scientific purpose was authorised by the Burundian Office for Environmental Protection [Office

Burundais pour la Protection de l'Environnement (OBPE)] following Article 36 of the law no. 1/17 of November 30, 2016, regulating the fisheries and aquaculture in Burundi. Live specimens were anaesthetised using clove oil, and this following the Annex IV of the European Directive 2010/63/EU on the protection of animals used for scientific purposes.

2.2 | Collections

Specimens used in this study are from both historical and recent (2013–2022) collections. The latter were made in the upper Malagarazi in Burundi and contain specimens identified as *E. lineomaculatus*, *E. quadrilineatus*, and the new species, with the previously given cheironym *E.* sp. 'ascutelatus' and here formally described as *E. nzigidaherai* sp. nov. During these recent sampling campaigns, complementary fishing techniques were used, such as gill-, cast-, fyke-, and dip-nets. For each specimen selected for fin clip sampling, photographs, of the left side of the fish, were taken in a photarium also. The entire, or part of the, right pelvic fin (fin clip), and eventually also the right pectoral fin, was taken and stored in 96% ethanol for molecular studies. The specimens themselves were fixed in 10% formalin before being transferred to 70% ethanol for long-term preservation at the RMCA. Specimens from which a fin clip has been taken have also been provided with a tag with a unique number linking the fin clip to that specific specimen. Other specimens used in this study, such as those of *Enteromius urostigma* (Boulenger 1917), and for which no fresh specimens were collected, are part of the historical collections of the RMCA and the Natural History Museum, London, United Kingdom (NHM).

Sampling dates, for both recent and historical collections, were linked to the seasons of the year. For Burundi, two major seasons per year are identified, that is, a dry season, from June to September, and a main rainy season, from October to May. However, the rainy season is made up of two parts, a shorter rainy season from October to December and a longer rainy season from mid-February to May. In between both, from January to February, there is usually a short dry season, with frequent spells and local differences (Nzigidahera, 2006; MATTE, 2007).

Finally, all locality data have been translated into English. According to the new RMCA policy, the collection numbers RMCA A0-A9 are listed as RMCA 2000–2009 and RMCA B0-B6 as RMCA 2010–2016. Coordinates are also provided for the type locality(ies) and are based on information from the original description existing on the museum label(s) or obtained from other sources. In the latter case, '±' was added to the coordinates as well as their reference source. Coordinates always follow the geographical unit to which they refer.

2.3 | Morphological data

A total of 110 specimens of *Enteromius* were examined: 30 specimens of *E. lineomaculatus*, comprising the lectotype, the paralectotype, and 28 additional specimens; 30 specimens of *E. quadrilineatus*, including 10 (out of the 19) syntypes and 20 additional specimens; 20 specimens of *E. urostigma*, containing 14 (out of the 107) syntypes and 6 additional specimens; and 30 specimens of *E.* sp. 'ascutelatus' (= *E. nzigidaherai* sp. nov.) comprising the holotype and 16 paratypes, as identified here and all originating from the Muyovozi River, a left bank affluent of the upper Malagarazi (Burundi), and 13 additional specimens. All these additional specimens, identified as belonging to the new species also, are from historical collections made in the Kinwa River, another left bank affluent of Malagarazi (Burundi). Therefore, considering its clear discontinuous distribution, the latter specimens were not included in the type series of the new species.

In addition, other specimens examined were, first, 6 out of 11 type specimens of *E. lornae*, including the lectotype (BMNH 1943.7.27:178; 51.6 mm L_S) and four paralectotypes (BMNH 1943.7.27:179–187; studied specimens all <35.0 mm L_S). Indeed, only the lectotype of *E. lornae* is somewhat larger, whereas all paralectotypes are small. Second, the three (supposed) syntypes of *Enteromius taeniopleura* (Boulenger 1917), including one syntype, still identified as *E. taeniopleura* (RMCA P-11540), and two others (RMCA P-11541) reidentified by Lore David in 1936 (see: RMCA original handwritten catalogue) as *E. lineomaculatus* were also studied. *Enteromius taeniopleura* was described based on at least two specimens (see Boulenger, 1917), considering the ranges he provided for several characters listed (maximum size: 80 mm L_T), and all these originated from Kasarala brook at Gongwe Village, Lake Tanganyika basin (DRC). Unfortunately, none of the so-called syntypes listed by Daget et al. (1984) reaches this size, and one of them (BMNH 1919.1.16:31) did not even originate from the type locality (ICZN, 1999: Art 76). Indeed, this specimen (BMNH 1919.1.16:31: 53 mm L_T) is from the Kalemie River [the left bank outlet of Lake Tanganyika (DRC)] [presented by Mr. Dhont-De Bie (see BMNH fish collection catalogue), instead of Dr. L. Stappers, as given by Boulenger, 1917 himself]. In addition, it was not registered as a syntype in the original handwritten BMNH fish collection catalogue. Finally, it was registered only in 1919, which is well after the publication of *E. taeniopleura* by Boulenger in 1917. Therefore, this BMNH specimen is not considered a syntype of *E. taeniopleura*. Both other syntype lots [RMCA P-11540 (24.6 mm L_S) and RMCA P-11541 (two specimens: 22.6 & 24.0 mm L_S)] are from the type locality as given by Boulenger (1917). However, RMCA P-11541 currently contains two specimens of almost equal size, instead of one as to be expected considering the unique collection number given. Therefore, the syntype status of both these specimens remains, at least, unclear as does their conspecificity with the other RMCA syntype of *E. taeniopleura*.

As a result, the available and studied type specimens of both, *E. lornae* and *E. taeniopleura*, are (i) few, that is six and three (?) specimens, respectively, and also (ii) mostly small-sized. Therefore, these were not included in the subsequent multi- and univariates analyses on the meristics nor the measurements (see Kocovsky et al., 2009). Nevertheless, in addition to the colour pattern differences provided first (see Results), differentiating and thus, potentially, diagnostic meristics and morphometrics were both added to the diagnosis.

Ten counts and 21 point-to-point measurements [see Supplementary Information (SI): Figure S1] were taken on the left-hand side of each specimen according to Bamba et al. (2011), except in cases where damage necessitated the use of the right-hand side. Counts

and measurements were taken under a Nikon binocular with a digital caliper to the nearest 0.01 mm. However, data were rounded off to 0.1 mm. Three counts, that is, the number of scales from dorsal fin to the lateral line (LL), and the number of pelvic and anal-fin rays were invariable, and thus were not included in the multivariate analyses.

Principal component analyses (PCAs) were performed to explore the multivariate data matrixes and to reduce a large number of variables into a few more easily interpretable significant axes (Bookstein et al., 1985; Humphries et al., 1981). For the counts, the raw data were used, whereas measurements were transformed, either in logarithms or in percentages of head length (L_H) or standard length (L_S), for head and body measurements, respectively. The PCAs on the meristics were performed using the correlation matrix, with scores on the first (PC1) and second axes (PC2) taken into account. The PCAs on the measurements were carried out on the variance-covariance matrix with (1) the data transformed into logarithms, and thus plotting the scores of the second axis (PC2) against those of the third axis (PC3), because the first axis largely represents size (Bookstein et al., 1985); and (2) the data transformed into percentages, but then plotting the scores of the first axis (PC1) against those of the second axis (PC2). In both cases, PC scores were also plotted against L_S (mm) to check for the effects of size on the scores (SI: Figures S5 and S7-S8). Depending on which of the both presented the best results, in terms of their interpretation, the PCA results on the log-transformed or those on the percentage data, instead, are presented here (see also Decru et al., 2012, 2013, 2016, 2019; Evans et al., 1998; Kasongo Ilunga et al., 2020; Vreven et al., 2019; Zengeya et al., 2011).

Univariate comparisons of the raw meristics and percentages of the measurements were performed using Mann–Whitney *U* tests (MWU). Significance levels were corrected for multiple comparisons using the Bonferroni correction (Rice, 1989). Only samples with more than four studied specimens were compared (see Vreven & Teugels, 2005); this was done to restrict MWU tests to more representative samples (Snoeks, 2004). Furthermore, to avoid size interference, only samples with non-significantly different standard lengths (L_S: $p > 0.5$) were compared (Vranken et al., 2020). Therefore, specimens of the same size class, (i) $37.3 \leq L_S \leq 64.3$ for *E. nzigidaherai* vs. *E. lineomaculatus*; (ii) $32.5 \leq L_S \leq 48.0$ for *E. nzigidaherai* vs. *E. quadrilineatus*, and (iii) $33.0 \leq L_S \leq 58.0$ for *E. nzigidaherai* vs. *E. urostigma*, were compared regarding the measurements. As no allometric meristics were studied, these size restrictions were not applied to those.

Finally, the most usable variables for diagnostic purposes were identified among those that were (highly) significantly different based on the MWU tests by using scatterplots. Variables (in percentages) were plotted against standard length or head length (in millimeters), for body and head measurements, respectively, to visualise the overlap between specimens of the new species, *E. nzigidaherai* sp. nov., against other comparative species studied, and to visualise the influence of size effects on the data as well. For the statistical analyses, both STATISTICA 12 (Statsoft, 2004) and Past 3.26 software (Hammer et al., 2001) were used.

2.4 | Molecular data

A total of 24 sequences of the partial COI gene were generated from newly available *Enteromius* samples from both the upper Malagarazi and the Rusizi basins. DNA was extracted from fin clips by using the Nucleospin Tissue Kit (Macherey-Nagel, Germany), following the manufacture's protocol. After the extraction, the COI region was amplified using a PCR. This PCR had a total reaction volume of 25 μL for each sample on the plate. The reaction mix contained 2.5 μL of PCR buffer (10×), 2.5 μL of dNTP (2 mM), 1.25 μL of primer cocktail (2 μM), 0.2 μL of Taq DNA Polymerase (5 units per μL), 17.75 μL of mQ-H_2O, and 1.0 μL of extracted DNA. The COI gene was amplified using the universal M13-tailed primer cocktail for fish DNA barcoding (Ivanova et al., 2007). The PCR profile started with an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 51°C for 40 s and extension at 72°C for 1 min 30 s, and concluded with a final extension at 72°C for 10 min (Decru et al., 2016). The product was visualised and checked by using 1.5% agarose gels with Midori Green direct (FastGene). If the amplification of a sample failed, the original extracted DNA sample was amplified once more. The PCR product was purified using the ExoSAP-IT PCR Cleanup kit (Affymetrix, Inc., USA) following the manufacture's protocol. After the purification, the Sanger sequencing was conducted by an external company, Macrogen (Macrogen, Inc., South Korea). At Macrogen, the samples were sequenced bidirectionally by using an ABI 3730XL capillary sequencer. The sequences were quality checked, de novo assembled, and cleaned in Geneious 10.2.6 (Kearse et al., 2012).

The newly generated sequences ($n = 24$) were combined with 304 partial COI sequences of *Enteromius* downloaded from GenBank to generate an initial general maximum likelihood (ML) phylogenetic tree (see SI: Figure S2). Three sequences of *Systomus sarana* (Hamilton 1822), a small Asian barbel and sister group of *Enteromius*, were included as out-group to root the tree (Yang et al., 2015: figure 2d,e; Katemo Manda et al., 2019). Only 33 *Enteromius* sequences were retained in the final tree presented based on the distribution of preselected species, defined by morphological evidence, on the initial tree. Thus, the final subset of sequences included sequences from the upper Malagarazi species included in the morphological explorations and sequences of *Enteromius atromaculatus* (Nichols & Griscom 1917), based on a well-supported sister group relationships with *E. lineomaculatus*, a population from the upper Malagarazi previously included. Other species were excluded, although some of them were sister groups to one of those included, but with poor bootstraps (BS < 70) only. Sequence alignments were performed using MEGA 10.0 (Kumar et al., 2018). The model test indicated GTR + G as the optimal model using the Akaike Information Criterium. The sequences, including the holotype and six paratypes of the new species, following the nomenclature of Chakrabarty (2010), were deposited in GenBank for future reference (SI: Table S1).

Among the species names linked to the COI sequences downloaded from GenBank, some of those associated voucher specimens

needed re-examination and possible reidentification. This holds true for two specimens of *E.* cf. *urostigma* (RMCA 2011-009-P-099 and RMCA 2011-009-P-0100), one specimen of *Enteromius stanleyi* (Poll & Gosse 1974) (RMCA 2011-009-P-0101), and one specimen of *Enteromius* sp. '10cps' (RMCA 2011-009-P-0023), which together form a well-supported clade in the initial ML phylogenetic tree (see SI: Figure S2). Based on the following morphological characters: (i) 23 scales along the LL (vs. 25–30 in *E. urostigma*), (ii) eight scales along the pre-dorsal (vs. 12 in *E. urostigma*), (iii) 10 scales around the caudal peduncle (vs. 12 in *E. urostigma*), and (iv) the presence of the three large black spots on the flanks, the present identification of the two *E.* cf. *urostigma* specimens and the *E. stanleyi* specimen could not be confirmed. Instead, these specimens are similar to *Enteromius candens* (Nichols & Griscom 1917), with 23 scales along LL, 8 pre-dorsal scales, 10 scales around the caudal peduncle, and the presence of three large black spots on the flanks. This, indeed, holds true also for the *E. stanleyi* identified specimen, previously reidentified as *Enteromius camptacanthus* (Bleeker 1863) by Decru et al. (2016). However, this specimen has 11 scales around the peduncle caudal, which might reveal some intraspecific variation for this character. Despite the similarities noted, these specimens differ from *E. candens* by the number of barbels, that is, two pairs of barbels [vs. one pair of barbels in *E. candens* (see Nichols & Griscom, 1917: 701, figure 15)]. Therefore, pending an in-depth study, these specimens are here referred to as *E.* cf. *candens*. The last specimen, *E.* sp. '10cps', was not re-examined because it was not found during our stay at the RMCA, but should have 10 scales around the caudal peduncle according to its naming. As such, its present identification has been kept. Consequently, as none of these specimens seem morphologically similar to *E. urostigma*, those four specimens have not been included in the final tree. In addition, two more members of the *E. lineomaculatus* species complex, *E. lornae* and *E. taeniopleura*, were also not included due to the lack of available sequences and tissue samples.

The average distance based on Kimura 2-parameters (K2P) (Kimura, 1980) and *p*-distance (uncorrected *p*-distance) (Srivathsan & Meier, 2012) values between and within species were also calculated in MEGA 10.0. These two-distance metrics are frequently reported in DNA barcoding studies and thus enable direct comparison with other studies (see also Bektas et al., 2018; Levin et al., 2018). Both values are reported, because *p*-distances are preferable with short sequences derived from closely related species, whereas K2P distances tend to better perform species delimitation when based on sequences with large interspecific differences (Srivathsan & Meier, 2012).

2.5 | Nomenclatural acts

According to the International Code of Zoological Nomenclature (ICZN) as currently amended, the name of the new species described herein is available under this code from the electronic edition of this article. This published work and the nomenclatural acts it contains have therefore been registered in the ICZN online registration system, ZooBank. The ZooBank Life Science Identifier (LSID) is available at https://zoobank.org/urn:lsid:zoobank.org:pub:C641723E-7BF3-4411-855D-1A78EA5C6310.

2.6 | Abbreviations

Abbreviations for museums: AMNH: American Museum of Natural History, New York, USA; BMNH: British Museum of Natural History, London, United Kingdom (for the fish collection); NHM: Natural History Museum, London, United Kingdom (for the institution); RMCA: Royal Museum for Central Africa, Tervuren, Belgium; and ZSM: Zoologische Staatssammlung München, Munich, Germany.

Other abbreviations: affl.: affluent; B.: basin; asc: *E.* sp. 'ascutelatus'; AOO: area of occupancy; CRSNE: Centre de Recherche en Sciences Naturelles et de l'Environnement (Centre of Research in Natural and Environmental Sciences), Bujumbura, Burundi; DGD: Directorate-General Development Cooperation and Humanitarian Aid, Belgium; DRC: Democratic Republic of the Congo; EMB: Eastern Massif of Burundi; EOO: extent of occurrence; GD: genetic distance; H, height of spots/band; HS: highly significant; ht: holotype; ICZN: International Code of Zoological Nomenclature; kil: Kilimanjaro; K2P: Kimura 2-parameters; LDF: level of the dorsal fin; L_H: head length; lin: *E. lineomaculatus*; LL: lateral line; log: decimal logarithm; L_S: standard length; LSID: Life Science Identifier; lt: lectotype; L. Tang.: Lake Tanganyika; mal: Malagarazi; mtDNA: mitochondrial DNA; M&M: Materials and Methods; MNR: Malagarazi Nature Reserve; MWU test: Mann–Whitney *U* test; MYA: million years ago; NS: no significant; OBPE: Office Burundais pour la Protection de l'Environnement, Bujumbura, Burundi; Past: Paleontological statistics; PC: principal component; PCA: principal component analysis; plt: paralectotype; pts: paratypes; qua: *E. quadrilineatus*; R.: River; S: significantly different; SI: Supplementary Information; sts: syntypes; UB: Université du Burundi, Bujumbura, Burundi; ul: upper Lualaba; uM basin: upper Malagarazi basin in Burundi (unless explicitly mentioned to be otherwise); un. p: uncorrected *p*-distance; uro: *E. urostigma*; and VHS: very highly significant.

3 | RESULTS

3.1 | An *E. lineomaculatus* species complex: What might it entail?

Currently, three species are known to be part of what might be referred to as the *E. lineomaculatus* species complex. As such, besides *E. lineomaculatus* itself, it includes two more species, *E. lornae* and *E. quadrilineatus*, which both have been junior synonyms of *E. lineomaculatus* (see Jackson, 1961; Poll, 1946, respectively), but have been revalidated, by Seegers (1996), since. In addition, two syntypes of *E. urostigma* (i.e., RMCA P-8689 and RMCA P-14036 according to Poll, 1946) and one (in fact two specimens also: see M&M) of the syntype series of *E. taeniopleura* (Boulenger, 1917) (i.e. RMCA

P-11541 according to David in 1936 as stated in the RMCA database) were reidentified as *E. lineomaculatus*, thus illustrating the overall similarity between these five species.

Among these five potential members of the *E. lineomaculatus* species complex, two species are currently known to occur in the upper Malagarazi (uM), that is, *E. lineomaculatus* itself and *E. quadrilineatus*, the latter endemic to the basin (Banyankimbona et al., 2012a; De Vos et al., 2001; Seegers, 1996). In addition, an undescribed species, *E.* sp. 'ascutelatus', has been reported from this part of the basin (De Vos et al., 2001), but has later been equated with *E. lineomaculatus* (Banyankimbona et al., 2012a), the nominal species of this species complex.

3.2 | Colour pattern differentiation between the different (potential) species of the *E. lineomaculatus* species complex

In terms of colour, *E.* sp. 'ascutelatus' (Figures 2a and SI: S3a,b illustrating some intraspecific variation) differs from both these sympatric species by four out of the five colour pattern characteristics documented (Table 1). Indeed, the potential new species has unpigmented scales on the LL [vs. pigmented in *E. lineomaculatus* (Figure 2b) and *E. quadrilineatus* (Figure 2c)]. Likewise, it also differs from these species by (i) the shape and size of the horizontal spots present on the flanks, (ii) the shape of the spot present at the base of the caudal fin, and (iii) the colour of a horizontal band present on the flanks (Table 1). However, despite these mentioned differences in colour pattern, as these species occur sympatrically, they were both included in subsequent morphological analyses.

Compared to the other species of the *E. lineomaculatus* species complex, the potential new species differs from *E. lornae* by four out of the five colour pattern characteristics documented (Table 1). Indeed, the potential new species shows the absence of a black spot at the base of the anal fin [vs. spot always present in *E. lornae* (Figures 2e and SI: S3d)]. In addition, it differs from *E. lornae* by (i) the shape of the horizontal spots present on the flanks, (ii) the shape of the spot present at the base of the caudal fin, and (iii) the colour and size of a horizontal band present on the flanks.

Furthermore, the potential new species differs from *E. taeniopleura* by three out of five colour pattern characteristics documented (Table 1), that is, (i) the presence of a series of horizontal black spots on the flanks [vs. spots absent in *E. taeniopleura* (Figure 2f)], (ii) the faint, small diamond-shaped black spot at the base of the caudal [vs. large roundish black spot (Figure 2f)], and (iii) the yellowish horizontal band on the flanks [vs. black horizontal band on the flanks (Figure 2f)] (Table 1). Nevertheless, the current colour of the only specimen that can be positively identified as a syntype of *E. taeniopleura* (RMCA P-11540) presents some inconsistencies with the original description (see Boulanger, 1917, page 364); for example, it presents a black spot at the base of the caudal fin, which is not mentioned in the original description, and a segmented longitudinal band, whereas it was reported to be continued in the original description

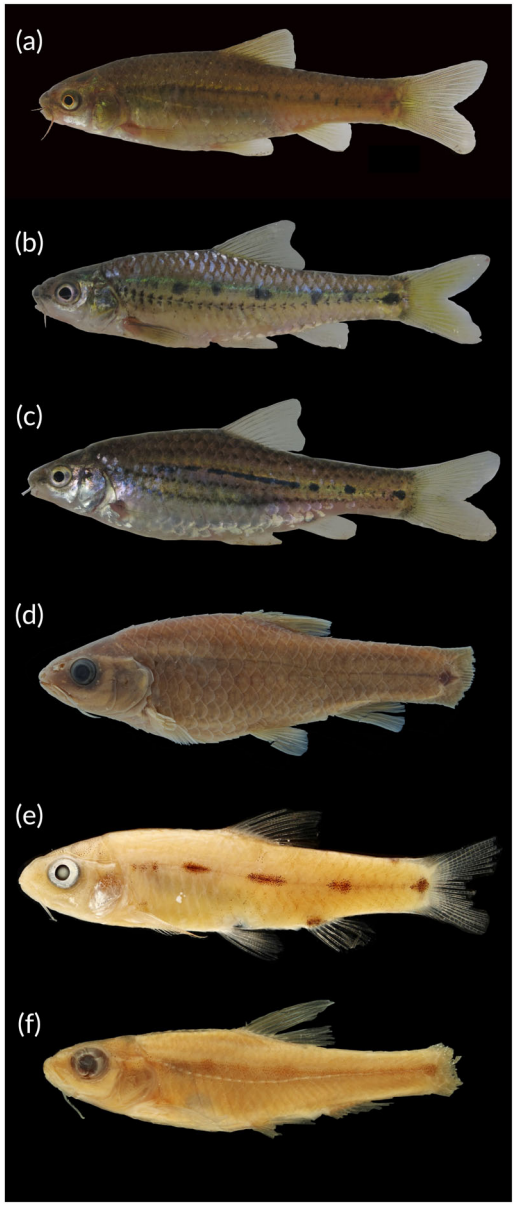

FIGURE 2 Photographs of the lateral view of living (when available) or preserved specimens of *Enteromius* sp. 'ascutelatus' (= *E. nzigidaherai* sp. nov.) and its most similar congeners from the upper Malagarazi (uM) basin and the Congo basin s.l.: (a) *E.* sp. 'ascutelatus' (= *E. nzigidaherai* sp. nov.) [RMCA 2018-034-P-0130 (holotype, tag no. 922/29): 61.0 mm L_S], live specimen; (b) *E. lineomaculatus* [RMCA 2018-034-P-0146 (tag no. 921/44): 51.3 mm L_S], live specimen; (c) *E. quadrilineatus* [RMCA 2018-034-P-0135 (tag no. 920/03): 43.3 mm L_S], live specimen; all from the upper Malagarazi basin in Burundi; (d) *E. urostigma* [BMNH 1920.5.25.52–61 (syntype): 49.5 mm L_S] from the Kampinda River, Lake Tanganyika basin in the Democratic Republic of the Congo (DRC), preserved specimen; (e) *E. lornae* [BMNH 1943-7-27-179–187 (paralectotype): 26.5 mm L_S] from Lake Young and Mansya River, Bangweulu Region, Zambia, preserved specimen; (f) *E. taeniopleura* [RMCA P-11540 (syntype): 24.5 mm L_S] from the Kasarala brook at Gongwe, Lake Tanganyika in the DRC, preserved specimen. For *E. lornae*, a photograph of a paralectotype is provided because the lectotype is curved and the black spot at the base of the anal fin is somewhat faded

TABLE 1 Characterisation of five major colour pattern elements as found in the different species of the *Enteromius lineomaculatus* species complex

Species	Type locality	(i) LL scale pigmentation	(ii) Anal-fin spot	(iii) Horizontal spots on flanks	(iv) Caudal-fin spot	(v) Band(s) present on flanks
Sympatric species						
Enteromius sp. 'ascutelatus' (= *Enteromius nzigidaherai* sp. nov.) (Figures 2a and SI: S3a,b)	Muyovozi R., affl. of uM B. (Burundi)	Absent	Absent	A series of ill-defined, horizontally orientated, diamond-shaped, small black spots (H ≤ one scale), sometimes partially confluent, and situated ~1½ scale above LL at LDF	Faint, small, diamond-shaped, black spot	(a) Yellowish longitudinal band (H ≤ one scale), situated ~2 scales above LL at LDF, and (b) Band overhanging horizontal the series of black spots (see element iii)
Enteromius lineomaculatus (Figure 2b) (Malagarazi basin) **	Lumi R., affl. of Lake Jipi (Tanzania)	Black chevron-shaped spots	Absent	A series of well-defined, horizontally orientated, roundish, large black spots (H ≤ one scale), never confluent, and situated ~½ scale above LL at LDF	Well-marked, large, roundish, black spot	(a) Greenish longitudinal band (H ≤ one scale), situated ~1 scale above LL at LDF, and (b) Band interconnected or not by a series of horizontal spots
Enteromius quadrilineatus (Figure 2c)	uM B. (Burundi)	Dark-greyish roundish spots	Absent	1st series of well-defined, horizontally orientated, roundish, small black spots (H ≤ one scale), often confluent forming small stripes, and situated ~2 scales above LL at LDF; 2nd series of ill-defined and small black spots (H ≤ ½ scale), often confluent and situated ~½ scale below LL at LDF	Well-marked, medium-sized, roundish, black spot	(a) Light yellowish longitudinal band (H ≤ one scale), situated ~2½ scales above LL at LDF, and (b) Band overhanging the 1st series of horizontal black spots (see element iii)
Other species						
Enteromius lornae (Figures 2e and SI: S3d) *	Lake Young and Mansya R., Bangweulu Region, Upper Congo B. (Zambia)	Absent	Present	A series of well-defined, horizontally orientated, roundish, small black spots (H ≤ one scale), often confluent and forming small stripes, and situated ~1 scale above LL at LDF	Well-marked, medium, roundish, black spot	(a) Black longitudinal band (H ≤ ½ scale), situated ~1 scale above LL at LDF, and (b) Band interconnect by a series of horizontal black spots
Enteromius taeniopleura (Figure 2f) *	Kasarala brook at Gongwe Village, L. Tang. B. (DRC)	Absent	Absent	Absent ***	Well-marked, large, roundish black spot	(a) Black longitudinal band not well-discernible (H ≤ 1½ scale), segmented in two large spots (b) No other spots
Enteromius urostigma (Figure 2d) *	Brooks at Mazonde, Kibondwe, and Gongwe villages, L. Tang. B. (DRC)	Absent	Absent	Absent	Well-marked, small, diamond-shaped black spot	(a) Black longitudinal band well marked (H ≤ ½ scale), from LDF to base of caudal fin, situated ~1½ scale above LL at LDF (b) No other spots

TABLE 1 (Continued)

Species	Type locality	(i) LL scale pigmentation	(ii) Anal-fin spot	(iii) Horizontal spots on flanks	(iv) Caudal-fin spot	(v) Band(s) present on flanks
Other species						
E. urostigma (SI: Figure S3c) ** (lower Lufira)	Munte R. and Kayombwa-Muye R., two right bank affl. lower Lufira (upper Lualaba) (DRC)	Absent	Absent	A series of, ill-defined, horizontally oriented, roundish, small black spots (H ≤ 1 scale), never confluent, and situated ~1 scale above the LL at the LDF	Well-marked, medium-sized, roundish black spot	(a) Black longitudinal band (H ≤ ½ scale), from LDF to base of caudal fin, situated ~1 scale above LL at LDF, and (b) Band interconnected by a series of horizontal black spots

Note: The characteristics are listed in the order of their importance. *, on preserved type specimen(s); **, on preserved non-type specimens, and ***, colour pattern of type specimen as given in the original description itself, as the current colour pattern of the type(s) seem(s) partially faded away.

Abbreviations: affl., affluent; B., basin; DRC, Democratic Republic of the Congo; H, height of spots/band; LDF, level of the dorsal fin; LL, lateral line; L. Tang., Lake Tanganyika; R., River; uM B., upper Malagarazi basin

(see Table 1). These inconsistencies, however, might well be, at least partially, the result of its long-time post-mortem conservation.

In addition, the RMCA P-11541 syntype lot of *E. taeniopleura*, which has subsequently been reidentified as *E. lineomaculatus* by Lore David in 1936 (see RMCA original catalogue, unpublished data), contains two specimens. As such it remains unclear if both or which of the both was originally part of the syntype series of *E. taeniopleura*. Moreover, both have a somewhat different colour pattern. The smallest of both specimens (22.6 mm L_S) presents three spots on the flanks, that is, a first post-opercular one, a second at the level of dorsal fin, and the last one at the base of the caudal fine. The second specimen (24.0 mm L_S), instead, presents a less clear longitudinal band, which seems to be divided into two streaks at the level of dorsal fin. Nevertheless, both specimens differ morphometrically from *E. lineomaculatus* by a short post-dorsal I distance, 51.3–51.6% L_S (vs. 53.5–57.2% L_S in *E. lineomaculatus*) and a short dorsal-fin base length, 11.3–13.8% L_S (vs. 14.1–17.1% L_S). As a result, these differences do not allow us to confirm their conspecificity with *E. lineomaculatus*. Therefore, questions with regard to the true colour pattern of *E. taeniopleura* cannot be solved without a collection of fresh topotypic specimens and an extensive re-examination of *E. taeniopleura* identified museum specimens from all over its distribution. However, both these questions fall outside the framework of the present study, which follows the colouration of the original description. Therefore, as *E. lornae* and *E. taeniopleura*, at least for the confirmed syntype, showed fewer colour pattern similarities with the potential new species, the two allopatric species (Boulenger, 1917; Seegers, 1996) were not included in the subsequent analyses. However, further evidence for their differentiation with regard to the potential new species has been provided later on (see diagnosis of the new species).

Finally, *E.* sp. 'ascutelatus' differs from the syntypes of *E. urostigma* by only two out of five colour pattern characteristics documented (Table 1). Indeed, it has a series of horizontal black spots on the flank [vs. spot absent in *E. urostigma* (Figure 2d)], and it also differs from the latter by the colour of a horizontal band present on the sides (Table 1). However, its original description noted the absence of this longitudinal band, indicated that the scales on the flanks are often edged with dark brown or blackish at their base, and reported a round black spot at the base of the caudal fin (Boulenger, 1917). Thus, the colour pattern description, as provided in the original description, seems to be more similar to that observed on the non-type specimens (SI: Figure S3c) examined. They, indeed, present a series of horizontal black spots on the flank interconnected by a thin black band, with a roundish spot present at the base of caudal fin (Table 1). Therefore, although the currently observed colour pattern on preserved specimens compared to the one as provided in the original description seems to be somewhat confused, it is still more similar to the colour pattern of the potential new species by the presence of a well-marked, diamond-shaped black spot anterior to the base of the caudal fin and the presence of a series of horizontal black spots observed on the non type specimens. Therefore, although known to occur only allopatrically (Boulenger, 1917), *E. urostigma* was included in the subsequent morphological analyses, that is, those on the meristics and morphometric.

3.3 | Other morphological explorations

3.3.1 | Analyses of meristics

A first PCA was performed on seven meristics ($n = 110$) (Figure 3). The most important loadings on PC1 are for the number of scales from lateral line (LL) to mid-way of belly and the number of pre-dorsal scales. The most important loadings on PC2 are for the number of scales on the LL and the number of dorsal-fin rays (Table 2a). Size effects (L_S) on PCs were explored using scatterplots (see SI: Figure S4).

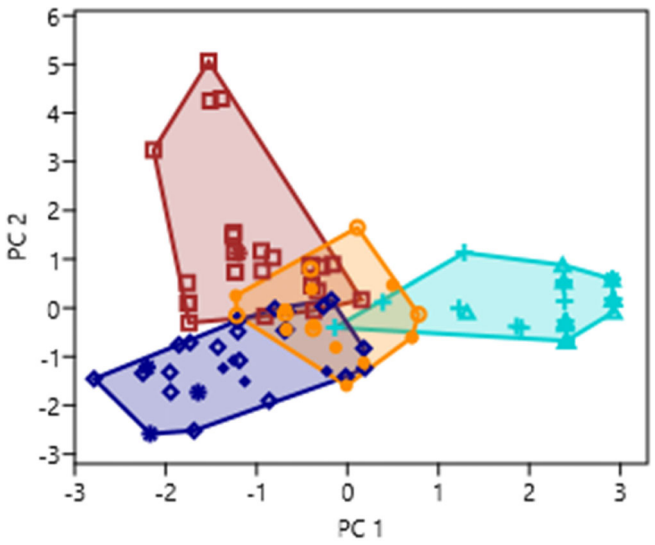

FIGURE 3 Scatterplot of PC1 vs. PC2 for a principal component analysis (PCA) on seven meristics of all *Enteromius* specimens examined ($n = 110$). *Enteromius lineomaculatus*: ✱ lectotype, □ other specimens; *E. quadrilineatus*: ✱ syntypes, ◇ other specimens; *E. urostigma*: ● syntypes (affluent rivers of the Lake Tanganyika basin), ○ specimens from the upper Lualaba; and *Enteromius* sp. 'ascutelatus' (= *E. nzigidaherai* sp. nov.): ✱ holotype, △ specimens from the Muyovozi River, and + specimens from the Kinwa River

A scatterplot of PC1 vs. PC2 of the PCA results shows that three of the four predefined groups are almost entirely separated from each other and that the fourth group overlaps, significantly or minimally, with the other three (Figure 3). A first group, which is part of the three well-separated groups, is situated largely on the negative side of PC1 and largely on the positive side of PC2 and corresponds to *E. lineomaculatus*. A second group lies also largely on the negative side of PC1 but on the negative side of PC2, and corresponds to *E. quadrilineatus*. A third group, instead, lies almost entirely on positive side of PC1 and around the 0 point of PC2 and corresponds to the specimens attributed to the potential new species *E.* sp. 'ascutelatus' The fourth group, here identified as *E. urostigma*, is situated around the 0 point of both axes and overlaps importantly with the specimens of *E. quadrilineatus* and *E. lineomaculatus*, but rather minimally with that of the specimens attributed to the potential new species *E.* sp. 'ascutelatus'.

Mann-Whitney *U* tests with sequential Bonferroni correction were performed on these seven meristics (Table 2b). These tests revealed that for the comparisons of *E.* sp. 'ascutelatus' with: (1) *E. lineomaculatus*, six of the seven meristics are significantly different, among which two are highly and three are very highly significant; (2) *E. quadrilineatus*, five of the seven meristics are significantly different, among which four are very highly significant; and (3) *E. urostigma*, four of the seven meristics are significantly different, among which three are very highly significant.

Among the very highly significant meristics, *E.* sp. 'ascutelatus', the potential new species can be diagnosed from the three most similar species studied, at least for some of the specimens attributed to

TABLE 2 (a) Variable loadings of the first two axes of a PCA performed on seven meristics for all *Enteromius* specimens examined ($n = 110$). The most important loadings are in bold. (b) MWU tests performed on these seven meristics for *E.* sp. 'ascutelatus' (= *Enteromius nzigidaherai* sp. nov.) (asc: 30) against *E. lineomaculatus* (lin: 30), *E. quadrilineatus* (qua: 30), and *E. urostigma* (uro: 20)

	a. PC loadings		b. MWU tests		
			asc vs. lin	asc vs. qua	asc vs. uro
	PC1	PC2	*p*-level	*p*-level	*p*-level
Caudal peduncle scales	0.34539	0.41730	S	VHS	NS
Pre-dorsal scales	**0.53207**	0.03708	VHS	VHS	VHS
Scales on lateral line	−0.01533	**0.65051**	HS	S	NS
Scales from lateral line to pelvic fin	0.38417	0.07037	HS	VHS	HS
Scales from lateral line to mid-way of belly	**0.53432**	−0.04723	VHS	VHS	VHS
Dorsal-fin rays	−0.12973	**0.59339**	NS	NS	NS
Pectoral-fin rays	0.38401	−0.20497	VHS	NS	VHS
PC % variance contribution	35.9	20.0			

Note: NS, no significant difference ($p > 0.05$); S, significant difference ($p < 0.05$); HS, highly significant difference ($p < 0.01$); and VHS, very highly significant difference ($p < 0.001$). asc: *E.* sp. 'ascutelatus'; lin: *E. lineomaculatus*; qua: *E. quadrilineatus*; and uro: *E. urostigma*. The most important loadings are in bold.

Abbreviations: MWU test, Mann–Whitney *U* test; PCA, principal component analysis

those, by: its high number of pre-dorsal scales, 12–13 (median: 12) [vs. lower, 10–11 (10) in *E. lineomaculatus* and *E. quadrilineatus* and usually lower, 10–12 (11) in *E. urostigma*] (SI: Table S2); and its high number of scales between the LL and the middle of the belly, 4.5–5.5 (5.5) [vs. usually lower, 4.5 in *E. lineomaculatus* and 4.5–5.5 (4.5) in *E. quadrilineatus* and *E. urostigma*] (SI: Table S2). Likewise, it can also be diagnosed from at least some of them by: its high number of pectoral-fin rays, 14–16 (16) [vs. usually lower, 14–16 (15) in *E. lineomaculatus* and *E. urostigma*], and its high number of scales around the caudal peduncle, 12 [vs. usually lower, 10–12 (11) in *E. quadrilineatus*] (Table 2b and SI: Table S2).

3.3.2 | Analyses of measurements

A second PCA was performed on 21 log-transformed measurements (Figure 4a) ($n = 110$). The most important loadings on PC2 are for dorsal-fin base length, dorsal-fin height, snout length, and inter-orbital width. The most important loadings on PC3 are for the eye diameter and body depth II (Table 3a: PCAa). Size effects (L_S) on PCs were explored using scatterplots (see SI: Figure S5).

A scatterplot of PC2 vs. PC3 of this PCA shows that two of the four predefined groups are mostly separated from the other two, which, instead, largely overlap (Figure 4a). A first group of specimens is located entirely on the positive side of PC2 and largely on the positive side of PC3 and corresponds to *E. lineomaculatus*. A second group of specimens is also found largely on the positive side of PC2, but, instead, largely on the negative side of PC3, and corresponds to *E. quadrilineatus*. A third group is largely situated on the negative side of PC2 and around the 0 point of PC3 and includes two of the four predefined groups, that is, a first group that is mainly situated on the negative side of PC2, and corresponds to *E. urostigma*, and a second one, which is entirely situated on the negative side of PC2 and corresponds to the potential new species, *E.* sp. 'ascutelatus'.

As the PCA on the log-transformed measurements showed an important overlap between the potential new species and *E. urostigma*, a second PCA between those two species only ($n = 50$) was also undertaken. However, a PCA on the log-transformed measurements of both these groups only ($n = 50$) [SI: Figure S6 and Table S3; for size effects (L_S) on PCs see SI: Figure S7] did not reveal well-interpretable results in terms of tentative species diagnosability. Instead, a PCA on the measurements in percentages (Figure 4b) ($n = 50$) revealed to be more interpretative. The most important loading on PC1 is for the inter-orbital width. The most important loadings on PC2 are for the pre-occipital length and pre-dorsal distance (Table 3a: PCAb). Size effects (L_S) on PCs were explored using scatterplots (see SI: Figure S8).

A scatterplot of PC1 vs. PC2 of this PCA shows those two predefined groups are well separated with a minimal overlap between both. Both are situated around 0 point of PC1 although in a more condensed way for *E. urostigma*, and with a more important extension on the positive part of the axis for the group of specimens identified as the potential new species, *E.* sp. 'ascutelatus'. As such, there is no separation on PC1. However, whereas a first group is entirely situated on the positive side of PC2 and corresponds to *E. urostigma*, the second one is largely situated on the negative side of PC2 and corresponds to the tentative new species, *E.* sp. 'ascutelatus'.

Mann-Whitney *U* tests with sequential Bonferroni correction were carried out on the 21 measurements (Table 3b). These tests revealed that for the comparisons of *E.* sp. 'ascutelatus' with (1) *E. lineomaculatus*, 10 out of the 21 measurements are very highly significantly different; (2) *E. quadrilineatus*, 13 out of the 21 measurements are significantly different, among which four are highly significant and eight are very highly significant; and (3) *E. urostigma*, eight out of the 21 measurements are significantly different, among which four are very highly significant.

Scatterplots of the measurements, in percentages, show that the tentative new species, *E.* sp. 'ascutelatus', differs from the three most

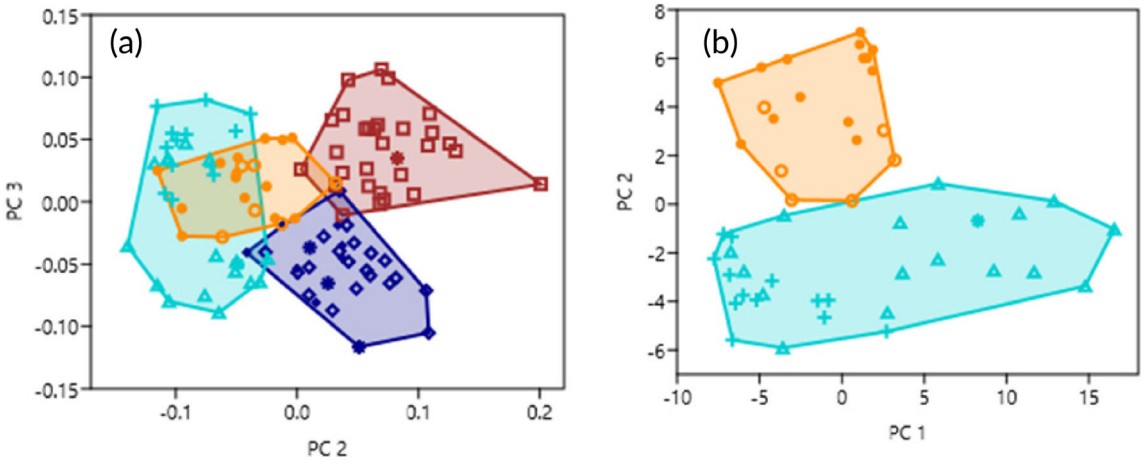

FIGURE 4 Scatterplot of: (a) PC2 vs. PC3 for a principal component analysis (PCA) on 21 log-transformed measurements of all *Enteromius* specimens examined ($n = 110$); (b) PC1 vs. PC2 for a PCA on 21 measurements in percentages after excluding the two previously best separated species, that is, *E. lineomaculatus* and *E. quadrilineatus* ($n = 50$). *Enteromius lineomaculatus*: ✱ lectotype, □ other specimens; *E. quadrilineatus*: ✱ syntypes, ◇ other specimens; *E. urostigma*: ● syntypes (affluent rivers of the Lake Tanganyika basin), ○ specimens from the upper Lualaba; and *E.* sp. 'ascutelatus' (= *E. nzigidaherai* sp. nov.): ✱ holotype, △ specimens from the Muyovozi River, and + specimens from the Kinwa River

TABLE 3 (a) Variable loadings of the PC2 and PC3 of a first PCA (PCAa) performed on 21 log-transformed measurements for all *Enteromius* species examined ($n = 110$) and variable loadings of the first two PCs of a second PCA (PCAb) performed on 21 measurements expressed in percentages after excluding the two previously best separated species, that is, *E. lineomaculatus* and *E. quadrilineatus* ($n = 50$). The most important loadings are in bold. (b) The MWU tests performed on 21 log-transformed measurements between *E.* sp. 'ascutelatus' against *E. lineomaculatus*, *E. quadrilineatus*, and *E. urostigma*

	a. PC loadings				b. MWU tests		
	PCAa		PCAb		asc vs. lin (*n*: 23 vs. 30)	asc vs. qua (*n*: 15 vs. 24)	asc vs. uro (*n*: 22 vs. 17)
	PC2	PC3	PC1	PC2	*p*-level	*p*-level	*p*-level
Standard length	0.03408	0.02125	-	-	NS	NS	NS
Head length	−0.27563	0.13144	−0.06536	−0.23939	VHS	VHS	VHS
Pre-occipital length	−0.11170	0.22598	−0.19313	**0.59710**	VHS	HS	VHS
Eye diameter	0.19093	**0.46659**	−0.29395	0.26487	VHS	HS	NS
Snout length	**−0.29661**	0.13524	0.28099	0.17509	NS	NS	NS
Pre-opercular distance	−0.26429	0.18427	0.06244	0.29785	NS	NS	S
Inter-orbital width	**−0.29359**	−0.22855	**0.84545**	0.14815	NS	VHS	NS
Pre-dorsal distance	−0.15717	−0.03382	−0.01501	**−0.40172**	VHS	VHS	VHS
Post-dorsal distance I	0.17778	0.07563	−0.07690	0.19427	VHS	S	NS
Post-dorsal distance II	0.09623	0.03092	0.00424	0.16202	NS	NS	NS
Dorsal-fin base length	**0.44481**	0.26717	−0.05435	0.04749	VHS	HS	NS
Dorsal-fin height	**0.38936**	0.16460	−0.16177	0.21605	VHS	VHS	NS
Pre-pectoral distance	−0.23699	0.24643	−0.10507	−0.23456	VHS	VHS	VHS
Pre-pelvic distance	0.03261	0.01185	0.01322	−0.02278	NS	NS	NS
Pre-anal distance	0.02768	0.04286	0.05459	0.01195	NS	NS	NS
Post-anal distance I	0.13710	−0.04163	−0.00146	−0.05859	NS	NS	NS
Post-anal distance II	0.29744	−0.14087	−0.01726	−0.05244	VHS	HS	NS
Body depth I	−0.04687	−0.21643	0.03041	−0.18195	VHS	NS	S
Body depth II	0.17375	**−0.41285**	0.10660	0.07020	NS	VHS	NS
Maximum caudal peduncle depth	0.13118	−0.33034	0.08568	0.01103	NS	VHS	S
Minimum caudal peduncle depth	0.00636	−0.30336	0.07055	0.01915	NS	VHS	S
PC % variance contribution	2.6	1.3	52.4	19.9			

Note: NS, no significant difference ($p > 0.05$); S, significant difference ($p < 0.05$); HS, highly significant difference ($p < 0.01$); and VHS, very highly significant difference ($p < 0.001$).
Abbreviations: MWU test, Mann–Whitney *U* test; PCA, principal component analysis

similar species studied by: its long head, 27.0–30.9 (mean: 28.5)% L_S [vs. 22.9–26.7 (25.3)% L_S in *E. lineomaculatus* and 23.4–26.7 (25.3)% L_S in *E. quadrilineatus*] (SI: Figure S9); long pre-dorsal distance, 52.2–56.3 (53.8)% L_S [vs. 46.8–51.7 (49.0)% L_S in *E. lineomaculatus* and 49.2–51.8 (50.6)% L_S in *E. urostigma*] (SI: Figure S10); short post-dorsal distance, 48.8–53.4 (51.4)% L_S [vs. 53.5–57.2 (54.8)% L_S in *E. lineomaculatus*] (SI: Figure S11); short dorsal-fin base length, 11.6–14.2 (13.0)% L_S [vs. 14.0–17.1 (15.6)% L_S in *E. lineomaculatus*] (Figure 5a); and short dorsal-fin height, 17.8–23.6 (21.1)% L_S [vs. 23.7–28.2 (25.7)% L_S in *E. lineomaculatus*] (SI: Figure S12); and long pre-pectoral distance, 27.1–31.3 (29.2)% L_S [vs. 24.3–27.0 (25.6)% L_S in *E. quadrilineatus*] (SI: Figure S13). Finally, it differs from *E. urostigma* by its wide inter-orbital width (Figure 5b), but this difference is only for larger-sized specimens, that is, >55 mm L_S, due to its positive allometry in the tentative new species.

3.3.3 | Mitochondrial COI DNA sequence analyses

The initial general ML phylogenetic tree (see SI: Figure S2), comprising all available COI barcoding sequences for *Enteromius*, underscores that the specimens identified as *E.* sp. 'ascutelatus' (= *E. nzigidaherai* sp. nov.) form a well-supported (BS: 100) clade. The new lineage, *E.* sp. 'ascutelatus', revealed to be distantly related to *E. lineomaculatus* and *E. quadrilineatus*, both morphologically most similar to the new species included in the tree and also occurring in the upper Malagarazi basin. The second ML phylogenetic tree (n: 33), based on the available studied populations/species and their well-supported sister group species, is presented below (Figure 6).

The K2P genetic distances (GDs) (Table 4) indicate that the intra-group K2P GDs vary from 0.0% to 0.2% between the sequences from the same clade, that is, species, and this only when both hydrographic *E. lineomaculatus* clades are taken separately. The *E.* sp. 'ascutelatus'

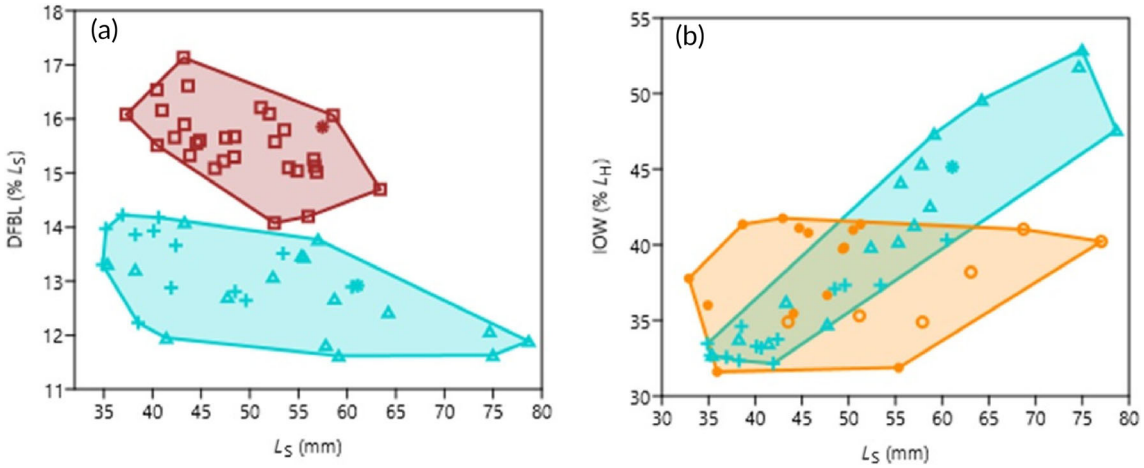

FIGURE 5 Scatterplots of standard length (L_S) (in millimeters) vs. (a) dorsal-fin base length (DFBL) (in % L_S); and (b) inter-orbital width (IOW) (in % L_H). *Enteromius lineomaculatus*: lectotype, other specimens; *E. urostigma*: syntypes (affluent rivers of the Lake Tanganyika basin), specimen from the upper Lualaba; and *E.* sp. 'ascutelatus' (*E. nzigidaherai* sp. nov.): holotype, specimens from the Muyovozi River, and specimens from the Kinwa River

clade separates from the other *Enteromius* clades/species, included in the second tree, by a K2P GDs of more than 10.0%. Finally, the *E. lineomaculatus* (Rus) clade, from Rusizi basin, separates from that from Malagarazi (Mal) by a GD of 4.9% only.

3.3.4 | Summary of the results of the integrative approach

The results obtained after the study of the (i) colour pattern, (ii) meristic, (iii) morphometric, and (iv) COI (mtDNA) genetic data, for both the most similar upper Malagarazi basin species, *E. lineomaculatus* and *E. quadrilineatus*, and the (i) colour pattern, (ii) meristic, and (iii) morphometric data for *E. urostigma* indicate altogether that *E.* sp. 'ascutelatus' is different from its most similar congeners and therefore constitutes solid integrative evidence supporting the validation of it as a new species for science. The species is therefore described below and named *E. nzigidaherai* sp. nov.

3.4 | *Enteromius nzigidaherai* sp. nov.

Figures 2a and 7 and Table 5.

Barbus sp. 'ascutelatus' (Banyankimbona, 2012; De Vos et al., 2001).

3.4.1 | Holotype

RMCA 2018-034-P-0130, 61.0 mm L_S, DNA tag no. 922-29, Burundi, upper Malagarazi basin, Nyankende River (right bank affluent of Muyovozi River), at Nyenzuki Village, 10 km before Rutana City, Ijenda-Gitega bifurcation, 03°52′49.2″S, 29°58′12.7″E, coll. A. Bigirimana & G. Banyankimbona, 28/11/2018.

3.4.2 | Paratypes

All from Burundi, upper Malagarazi. AMNH 276985 (former RMCA 91-030-P-0038), one specimen, 43.3 mm L_S; BMNH 2022.6.9.1 (former RMCA 91-030-P-0037), one specimen, 35.4 mm L_S; RMCA 91-030-P-0034-0035, two specimens, 38.2–47.7 mm L_S; and ZSM-48367 (former RMCA 91-030-P-0036), one specimen, 41.4 mm L_S, Sagara River (right bank affluent of Muyovozi River), at a bridge on provincial road 74, 03°53′S, 29°57′E, coll. L. De Vos & L. Taverne, 03/04/1991. AMNH 276986 (former RMCA 2018-034-P-0005), one specimen, 57.0 mm L_S; BMNH 2022.60.9.2 (former RMCA 2018-034-P-0004), one specimen, 57.0 mm L_S; RMCA 2018-034-P-0001-0002, two specimens, 52.3 & 58.7 mm L_S; RMCA 2018-034-P-0124-0129, six specimens, 57.3–78.5 mm L_S, DNA tag no. 922-20, -22, -24, -26, -27, -30; and ZSM-48366 (former RMCA 2018-034-P-0003), one specimen, 55.6 mm L_S, same locality as for holotype, coll. A. Bigirimana & G. Banyankimbona, 28/11/2018.

3.4.3 | Additional non type specimens

All from Burundi, upper Malagarazi. Specimens included in the analyses: RMCA 91-030-P-0047-0063, 34.8–60.4 mm L_S, 12 out of 17 specimens and RMCA 91-030-P-0046, 53.4 mm L_S, one specimen, Kinwa River (left bank affluent of Malagarazi River), at a bridge on provincial road 85, at Buyaga locality, 03°55′S, 30°10′E, coll. L. De Vos & L. Taverne, 04/04/1991. Specimens not included in analyses: RMCA 2019-023-P-0001-0003, three specimens, Sagara River (right bank affluent of Muyovozi River), at Matuta, 03°51′25.8″S,

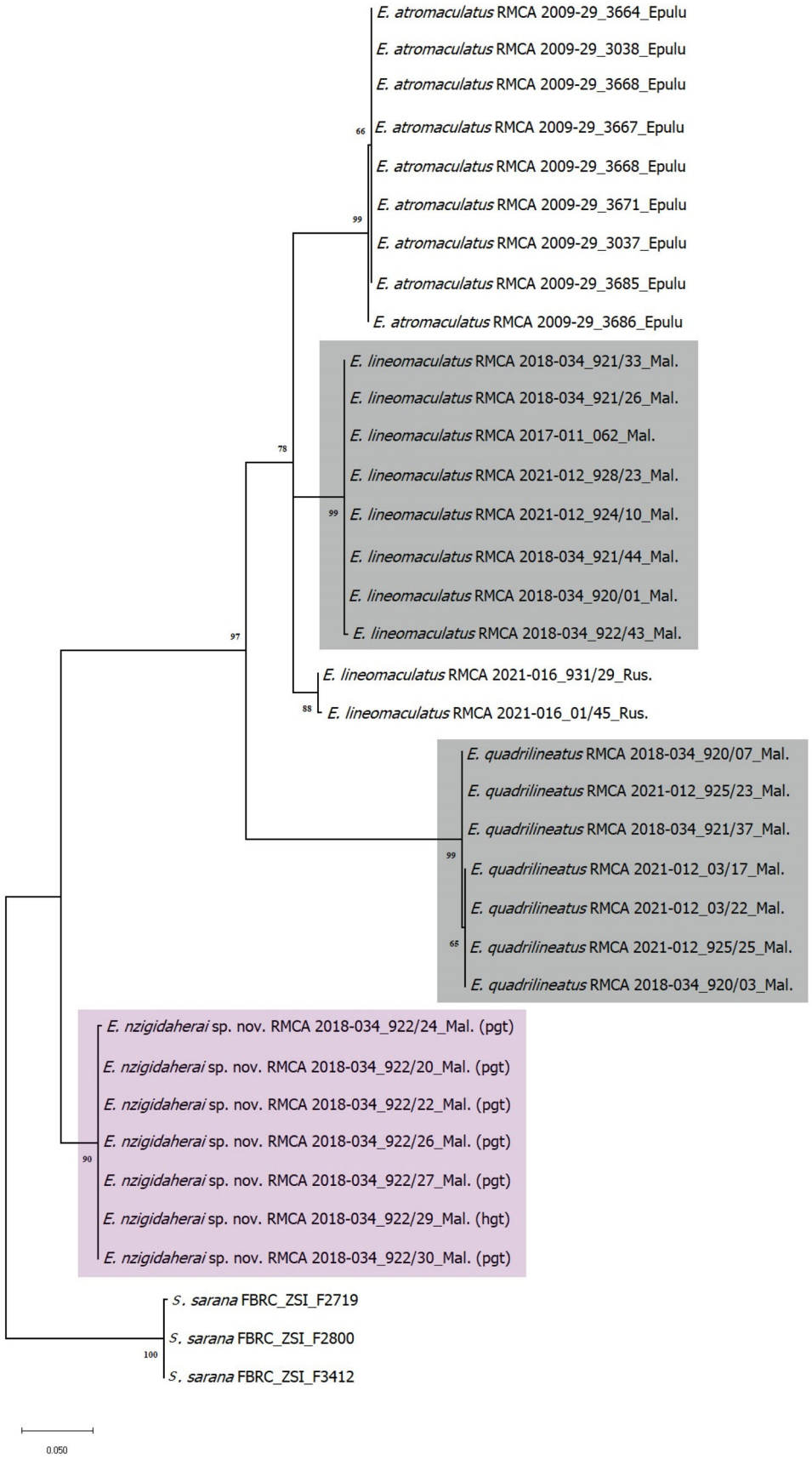

FIGURE 6 Maximum likelihood phylogenetic tree (33 specimens) of a set of selected *Enteromius* populations/species (see M&M section) based on partial cytochrome oxidase subunit I (COI) sequences (495 bp alignment length). Bootstrap approach (500 iterations) is illustrated. The clades formed by specimens from the upper Malagarazi are shaded: light mauve for *Enteromius* sp. 'ascutelatus' (= *E. nzigidaherai* sp. nov.) and dark grey for *E. lineomaculatus* and *E. quadrilineatus*. hgt: hologenetype; Mal.: Malagarazi; pgt: paragenetype; Rus.: Rusizi

TABLE 4 Average genetic distance (%) generated from the COI sequences, between and within, a set of selected *Enteromius* populations/species

		Between groups					Within groups	
		1	2	3	4	5	K2P	Un. p
1	*E. quadrilineatus*		10.2	10.9	10.1	11.8	0–0.2	0–0.2
2	*E. atromaculatus*	11.5		5.4	6.4	09.9	0–0.2	0–0.2
3	*E. lineomaculatus* Rus.	12.4	5.7		4.6	10.5	0–0.2	0–0.2
4	*E. lineomaculatus* Mal.	11.4	6.9	4.9		10.6	0–0.2	0–0.2
5	*E. nzigidaherai* sp. nov.	13.5	11.0	11.8	12.0		0–0.2	0–0.2

Note: K2P distance and uncorrected *p*-distance between species are separated by the diagonal with K2P given above the diagonal and uncorrected *p*-distances given below.
Abbreviations: K2P, Kimura 2-parameters; Mal., Malagarazi; Rus., Rusizi.; Un. p: uncorrected *p*-distance

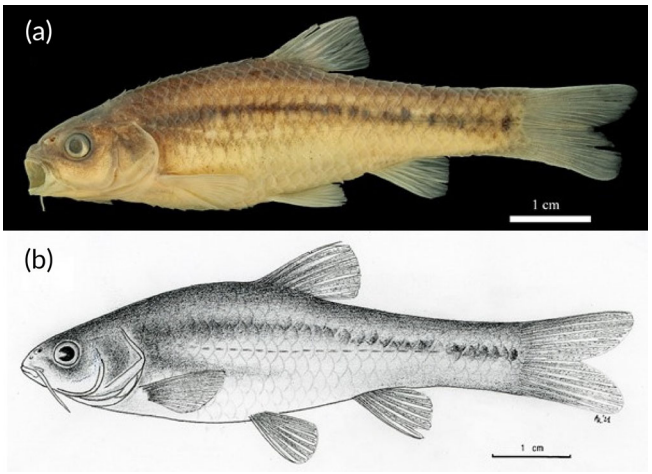

FIGURE 7 *Enteromius nzigidaherai* sp. nov., RMCA 2018-034-P-0130, 61.0 mm L_S, holotype, Burundi, upper Malagarazi basin, Nyankende River (right bank affluent of the Muyovozi River). (a) laboratory photograph of the preserved specimen; (b) drawing of the preserved specimen with closed mouth by Alain Reygel (RMCA)

29°56′20.9″E, coll. A. Bigirimana & G. Banyankimbona, 10/11/2019. RMCA 2019-023-P-0004-0007, four specimens, DNA tag no. 918-06, -08, -10, -12, -13, Muyovozi River, Ramvya locality, 03°50′41.1″S, 29°56′26.7″E, coll. A. Bigirimana & G. Banyankimbona, 10/11/2019. RMCA 2019-023-P-0008-0010, three specimens, DNA tag no. 918-06, -11, -14, Muyovozi River, at Ramvya locality, 3°50′41.1″S, 29°56′26.7″E, coll. A. Bigirimana & G. Banyankimbona, 10/11/2019. RMCA 2019-023-P-0011-0055, 45 specimens, Muyovozi River (left bank affluent of Malagarazi River), at Ramvya locality, 03°50′41.1″S, 29°56′26.7″E, coll. A. Bigirimana & G. Banyankimbona, 10/11/2019. RMCA 2018-034-P-0006-0123, 118 specimens, 52.3–58.7 mm L_S, same locality as for holotype, coll. A. Bigirimana & G. Banyankimbona, 28/11/2018. RMCA 1991-030-P-0039, one specimen, Sagara River (right affluent of Muyovozi River), at a bridge on provincial road 74, coll. L. De Vos & L. Taverne, 03/04/1991. RMCA 91-030-P-0040-0045, five specimens, Sagara River (right affluent of Muyovozi River), at a bridge on provincial road 74, 03°53′S, 29°57′E, coll. L. De Vos & L. Taverne, 03/04/1991. RMCA 1991-062-P-1844-1849, six specimens, 10.7–20.4 mm L_S, channels at 10 km before Rutana, near bifurcation Ijenda-Gitega, 03°52′S, 29°58′E, coll. L. De Vos & P. Weiler, 03/08/1991.

3.4.4 | Diagnosis

Enteromius nzigidaherai sp. nov. is one of the 60 *Enteromius* species of the Congo basin s.l. for which the last unbranched ray of the dorsal fin is flexible and devoid of serrations along its posterior edge. *Enteromius nzigidaherai* sp. nov. is distinguished from its congeners by the presence of two pairs of barbels [vs. no barbels in *E. aspilus* (Boulenger 1907), *E. baudoni* (Boulenger 1918), *E. brazzai* (Pellegrin 1901), *E. carens* (Boulenger 1912), *E. erythrozonus* (Poll & Lambert 1959), *E. haasianus* (David 1936), *E. jae* (Boulenger 1903), *E. stigmatopygus* (Boulenger 1903), *E. toppini* (Boulenger 1916), and *E. trispilomimus* (Boulenger 1907); or vs. one pair of barbels only in *E. brevidorsalis* (Boulenger 1915), *E. candens* (Nichols & Griscom 1917), *E. hulstaerti* (Poll 1945), *E. nigrifilis* (Nichols 1928), *E. owenae* (Ricardo-Bertram 1943), *E. papilio* (Banister & Bailey 1979), and *E. syntrechalepis* (Fowler 1949)]; a complete lateral line (LL) [vs. incomplete in *E. brachygramma* (Boulenger 1915) and *E. lujae* (Boulenger 1913)]; 26–29 scales in the LL [vs. fewer, i.e., 21 in *E. okae* (Fowler 1949), 20–22 in *E. stigmasemion* (Fowler 1936), 21–22 in *E. pygmaeus* (Poll & Gosse 1963), and 21–23 in *E. camptacanthus* (Bleeker 1863); or vs. more scales in the LL, that is 31–33 in *E. poechii* (Steindachner 1911), 32–33 *E. deguidei* (Matthes 1964), *E. motebensis* (Steindachner 1894), and *E. mocoensis* (Trewavas 1936), and 31–35 in *E. trimaculatus* (Peters 1852)]; 12 scales around the caudal peduncle [vs. fewer, i.e., 8 in *E. lufukiensis* (Boulenger 1917), 8–10 in *E. amanpoae* (Lambert 1961), 10 in *E. humeralis* (Boulenger 1902) and *E. mediosquamatus* (Poll 1967), and 11 in *E. stanleyi* (Poll & Gosse 1974)]; 12–13 pre-dorsal scales [vs. fewer, 9 in *E. tomiensis* (Fowler 1936), 10 in *E. bifrenatus* (Fowler 1935) and *E. castrasibutum* (Fowler 1936)]; III,7 dorsal-fin rays [vs. more, III,8 in *E. atromaculatus*, *E. lukusiensis* (David & Poll 1937), *E. marmoratus* (David & Poll 1937), *E. taeniurus* (Boulenger 1903), and *E. radiatus* (Peters 1853)]; the lack of spots on the operculum [vs. presence of spots on the operculum in *E. ablabes* (Bleeker 1863) and *E. lamani* (Lönnberg & Rendahl 1920)]; presence of a longitudinal series of black spots, sometimes confluent, on the flank above the LL [vs. lack of spots in *E. innocens* (Pfeffer 1896); one or two black spots above the LL in *E. barotseensis* (Pellegrin 1920), *E. caudosignatus* (Poll 1967); two

TABLE 5 Measurements and meristics for the new species, *Enteromius nzigidaherai* sp. nov., and its most similar congeners from Congo basin s.l.: *E. lineomaculatus*, *E. quadrilineatus*, and *E. urostigma*

	E. nzigidaherai sp. nov.					*E. lineomaculatus*			*E. quadrilineatus*				*E. urostigma*			
	ht	ht+ pts (17)	mean	range* (30)	mean	lt (kil)	lt+ plt + mal (30)	mean	sts range (10)	mean	range (mal: 30)	mean	sts range (14)	mean	sts + ul (20)	mean
Standard length	61.0	35.3–78.7	56.2	34.9–78.7	50.5	57.4	37.3–63.4	49.3	29.6–40.4	33.6	29.3–47.2	38.2	32.9–55.4	44.5	32.9–77.0	49.2
Measurements %LS																
Head length	27.3	27.0–29.0	28.0	27.9–31.0	28.1	25.1	22.9–26.7	25.3	25.2–26.7	25.9	23.4–26.7	25.3	25.7–27.9	26.8	25.7–27.9	26.9
Pre-dorsal distance	52.7	52.3–55.5	53.2	52.3–56.3	53.8	51.7	46.8–51.7	49.0	48.9–52.1	50.3	48.3–53.8	50.7	49.3–51.8	50.7	49.3–51.8	50.6
Post-dorsal distance I	52.4	48.8–53.0	51.3	48.8–53.4	51.4	54.6	53.5–57.2	54.8	49.4–54.0	52.3	49.4–56.0	53.3	50.7–55.1	53.1	50.5–55.1	52.9
Post-dorsal distance II	38.0	36.8–40.1	38.7	36.3–40.1	38.2	39.0	36.4–41.6	39.3	35.6–40.6	37.8	35.6–41.0	38.5	37.2–41.7	39.3	37.2–41.7	39.4
Dorsal-fin base length	12.9	11.6–14.1	12.7	11.6–14.2	13.6	15.8	14.1–17.1	15.6	12.8–15.1	14.0	12.8–15.4	14.3	12.1–14.6	13.6	11.4–14.6	13.2
Dorsal-fin height	20.6	17.8–23.6	20.8	17.8–23.6	21.1	-	23.7–28.2	25.7	-	-	23.8–27.8	25.3	22.4–25.6	23.6	19.7–25.6	23.0
Pre-pectoral distance	27.1	27.1–31.2	28.5	27.1–31.3	29.2	23.6	23.7–30.1	26.5	24.6–26.9	25.7	24.3–27.0	25.6	25.9–28.5	27.4	25.9–29.3	27.7
Pre-pelvic distance	49.3	49.3–52.7	50.6	48.5–52.7	50.6	51.5	48.3–53.3	50.9	48.6–51.5	49.9	47.9–53.2	50.3	47.4–52.3	50.5	47.4–52.6	51.0
Pre-anal distance	68.4	68.4–73.4	70.9	68.7–73.4	70.8	71.7	67.8–73.2	71.3	68.1–71.2	69.1	68.1–72.9	69.9	68.7–73.2	70.8	68.7–73.7	71.5
Post-anal distance I	29.8	27.2–30.3	28.8	26.7–30.3	28.8	30.5	26.0–31.3	29.3	29.1–32.7	30.7	26.4–32.7	29.9	26.5–31.2	28.4	25.3–31.2	27.9
Post-anal distance II	21.3	17.9–21.8	20.0	17.9–22.0	20.1	21.3	18.2–23.3	21.3	20.6–23.7	22.2	18.4–24.6	21.9	17.4–22.1	19.6	17.4–22.1	19.3
Body depth I	23.0	22.7–24.9	23.8	22.0–25.4	24.0	21.5	20.0–24.3	22.5	22.4–25.8	23.7	20.6–25.9	23.8	20.7–23.9	22.3	20.7–23.9	22.5
Body depth II	28.7	25.2–30.4	28.3	24.6–30.4	27.5	29.7	23.9–32.2	28.0	26.2–31.4	28.9	25.8–34.8	29.7	25.2–30.3	27.7	25.2–30.3	27.8
Maximum caudal peduncle depth	14.4	12.9–15.7	14.5	12.2–15.7	13.6	13.9	13.3–16.2	14.4	13.7–16.5	15.2	13.7–16.5	15.1	13.2–14.8	13.9	13.2–15.8	14.2
Minimum caudal peduncle depth	13.8	12.0–14.7	13.5	11.8–14.7	13.1	12.3	11.7–14.5	12.8	12.9–14.4	13.9	12.6–14.7	13.6	11.8–13.6	13.0	11.8–14.5	13.3
Measurements %LH																
Pre-occipital length	70.4	68.4–74.8	71.2	68.4–75.6	71.8	80.4	71.2–84.9	78.4	74.6–82.6	77.9	72.2–82.6	76.7	73.3–79.0	76.6	73.1–79.0	76.0
Eye diameter	19.6	17.4–24.9	20.3	17.4–24.9	21.6	24.5	24.3–33.9	27.2	23.2–29.0	26.0	23.2–29.2	25.9	22.1–26.6	24.3	20.4–26.6	23.7
Snout length	32.9	26.8–35.8	30.9	25.7–35.8	27.8	31.6	26.7–35.8	30.1	24.9–31.7	28.7	24.9–31.8	28.4	29.2–32.3	30.4	27.9–32.3	30.0
Pre-opercular distance	69.7	66.0–71.0	68.2	66.0–71.0	68.0	69.4	64.3–72.0	68.9	65.8–70.3	68.2	64.1–70.7	67.6	68.9–72.2	70.4	67.6–72.2	69.9
Inter-orbital width	45.1	32.7–52.9	42.3	32.2–52.9	39.0	35.4	31.8–43.8	38.2	33.0–40.8	36.9	33.0–43.8	37.6	31.6–41.8	38.3	31.6–41.8	38.0
Meristics			median		median			median		median		median		median		median
Dorsal-fin ray	III 7	III 7	III 7	III 7	III 7	III 7	III 7-III 8	III 7	III 7	III 7	III 7	III 7	III 7	III 7	III 7	III 7
Anal-fin ray	III 5	III 5	III 5	III 5	III 5	III 5	III 5	III 5	III 5	III 5	III 5	III 5	III 5	III 5	III 5	III 5
Pelvic-fin ray	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Pectoral-fin ray	16	14–16	16	14–16	16	-	14–16	15	-	-	-	-	14–16	15	14–16	15
Scales on lateral line	29	26–29	27	26–29	27.5	30	28–31	29	24–27	26	24–28	26	24–28	26	24–30	26
Scales from dorsal to lateral line	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Scales from lateral line to pelvic	3.5	3.5	3.5	2.5–3.5	3.5	2.5	2.5–3.5	3	2.5–3.5	2.5	2.5–3.5	2.5	2.5–3.5	2.5	2.5–3.5	2.5
Pre-dorsal scales	13	12–13	12	12–13	12	10	10–11	10	10	10	10–11	10	10–12	11	10–12	11
Circumpeduncular scales	12	12	12	12	12	12	11–12	12	10–11	10	10–12	11	11–12	12	11–12	12
Scales from lateral line to mid-way of belly	5.5	5.5	5.5	4.5–5.5	5.5	4.5	4.5	4.5	4.5	4.5	4.5–5.5	4.5	4.5–5.5	4.5	4.5–5.5	4.5

Abbreviations: ht, holotype; kil, Kilimanjaro; lt, lectotype; mal, Malagarazi; plt, paralectotype; pts, paratypes; sts, syntypes; ul, upper Lualaba; range*, including not only types from the Muyovozi basin but also the specimens from the Kinwa basin

black spots above the LL in *E. tetrastigma* (Boulenger 1913); an indistinct dark lateral band sometimes present above LL in *E. kessleri* (Steindachner 1866) and *E. luluae* (Fowler 1930); a continuous dark greyish-black above LL in *E. citrinus* (Boulenger 1920) and *E. kamalondoensis* (Poll 1938); several parallel longitudinal black bands on the flanks in *E. neefi* (Greenwood 1962); a dark vertical band behind the gill opening in *E. fasciolatus* (Günther 1868) and *E. thespesios* Katemo Manda, Snoeks, Decru, Bills & Vreven 2020].

Finally, *E. nzigidaherai* sp. nov. is distinguished from the other four species contained in the morphological *E. lineomaculatus* species complex by its usually high number of pre-dorsal scales, 12–13 (median: 12) [vs. lower, 10–11 (10) in *E. lineomaculatus* and *E. lornae*; 10 in *E. taeniopleura*; 10–11 (10) in *E. quadrilineatus* and usually lower, 10–12 (11) in *E. urostigma*] (SI: Tables S2 and S4); its usually high number of scales between the LL and the middle of the belly, 4.5–5.5 (5.5) [vs. usually lower, 4.5 in *E. lineomaculatus* and 4.5–5.5 (4.5) in *E. quadrilineatus* and *E. urostigma*] (SI: Table S2); its long head, 27.0–31.0 (mean: 28.5)% L_H [vs. shorter 22.9–26.7 (25.3)% L_S in *E. lineomaculatus* and 23.4–26.7 (25.3)% L_S in *E. quadrilineatus* and usually shorter, 25.3–28.0 (26.8)% L_S in *E. lornae*] (SI: Figure S9 and Table S4); its long pre-dorsal distance, 52.3–56.3 (mean: 53.8)% L_S [vs. shorter, 46.8–51.7 (49.0)% L_S in *E. lineomaculatus*; 49.6% in *E. taeniopleura*; 49.3–51.8 (50.7)% L_S for the syntypes of *E. urostigma* and 49.7–51.4 (50.4)% L_S for the specimens of *E. urostigma* from the upper Lualaba; and usually shorter, 47.0–53.6 (50.9)% L_S in *E. lornae* and 48.3–53.8 (50.7)% L_S in *E. quadrilineatus*] (SI: Figure S10 and Table S4); its short dorsal-fin base length, 11.6–14.2 (13.0)% L_S [vs. usually longer, 14.0–17.1 (15.6)% L_S in *E. lineomaculatus*] (Figure 5a); its long pre-pectoral distance 27.1–31.3 (29.2)% L_S [vs. shorter, 24.3–27.0 (25.7)% L_S in *E. quadrilineatus*] (SI: Figure S13); its short dorsal-fin height, 17.8–23.6 (21.1) L_S [vs. longer, 23.8–27.8 (25.3)% L_S in *E. quadrilineatus*] (SI: Figure S12); its short post-anal I distance, 26.7–30.3 (28.8) [vs. longer 31.0–33.4 (32.5)% L_S in *E. lornae*] (SI: Figure S14 and Table S4); and its broad inter-orbital width, mainly for larger-sized specimens (>55 mm L_S), due to its positive allometry, 40.2–52.9 (45.7)% L_H [vs. usually narrower, 31.9% L_H for the single larger-sized syntype of *E. urostigma* and 34.9–41.0 (37.2)% L_H for the larger-sized specimens of *E. urostigma* from the upper Lualaba] (Figure 5b).

3.4.5 | Description

Description is based on holotype and 16 paratypes, all from Muyovozi River, left bank affluent of Malagarazi River, and its affluents and 13 additional non-type specimens from the Kinwa River, another left bank affluent of Malagarazi. Indeed, meristics and morphometric data are given in Table 5. Rather small-sized species of *Enteromius*, with maximum recorded size of 78.7 mm. General body shape fusiform. Slightly elongated body with concave profile dorsally; body depth greatest anterior to dorsal fin; maximum height of body less than length of head; long head; length of snout as long or longer than diameter of eye; snout and lower mouth rounded; two pairs of barbels, anterior one shorter than posterior one; and anterior barbel always touching anterior edge of eye but never exceeding middle of diameter of eye, whereas posterior one exceeding posterior border of eye but never reaching posterior border of preopercular bone. Eye relatively small, located laterally; that is, visible from both above and below, closer to tip of snout than to posterior region of operculum. Inter-orbital profile flat. Distal edge of dorsal fin almost straight to convex with its anterior origin located at midbody above anterior origin of pelvic fin; first branched dorsal-fin ray longest, posterior rays decreasing progressively in size. Distal profile of pectoral fin slightly convex, not reaching anterior base of pelvic fin. Distal profile of pelvic fin convex. Distinct axillary pelvic scale usually absent or present but reduced (Figure 7a). Caudal fin forked with more or less rounded lobes. Cycloid scales rounded and striate. Lateral line slightly curved downwards over abdomen but running straight along middle of caudal peduncle and ending at base of caudal fin. Specimens with 26–29 on LL; 4½ between LL and origin of the dorsal fin; 2½–3½ between LL and origin of the pelvic; 4½–5½ between LL and middle of belly.

3.4.6 | Life colouration

In life (Figure 2a), specimens are light brown dorsally on head and body, yellowish laterally with a somewhat iridescent gold operculum and silvery-white to whitish ventrally. Above LL, there is a series of horizontally elongated black spots and this from posterior level of operculum to caudal-fin base. Sometimes the series is somewhat confluent, or spots are interconnected by a narrow, ill-defined, and lighter brown line. As such, this series of spots forms a continuous band in most specimens up to level of origin of dorsal fin, where series itself is situated at its maximum height, being about one scale above LL. Most posterior spots, instead, are found at level of LL itself. In addition, laterally, there is often a narrow, ill-defined, and discontinuous light-yellowish band overhanging this series of spots. As such, this light-yellowish band is situated about two scales above LL at level of dorsal fin and only about one scale above LL on caudal peduncle. In larger specimens (usually >60 mm L_S), two more series of dark brown spots are present. One series situated about three and half scales above LL at level of dorsal fin, whereas second one situated on dorsal midline. Most often, fin rays are pale grey for their proximal 1/3. The remaining part of fins is translucent and even transparent. Iris of eye is black and surrounded by wide iridescent gold band. Upper part of the pupil is light brown, whereas its ventral part is whitish. Barbels are all greyish.

3.4.7 | Colouration in alcohol

Preserved specimens (Figure 7a) are blackish brown on sides and their back, whereas belly is pale grey. The series of mediolateral black spots originating behind operculum and extending to origin of caudal fin is still present as are those on back. All fins are whitish. Yellowish band

above series of mediolateral situated black spots, the latter itself already situated above LL, and iridescent gold band that surrounds iris both disappears. Barbels remain light greyish.

3.4.8 | Distribution

The distribution of *E. nzigidaherai* sp. nov. is limited to the upper Malagarazi in Burundi and more specifically to the upper part of two of its left bank affluents, that is the Muyovozi River, main course, and two of its right bank affluents, the Nyankende and Sagara Rivers, all situated above the upper Nyaganza Falls (Figure 8: no. 4), and the Kinwa River (Figure 8: no. 5). As such, it seems to be endemic to these affluents of the upper Malagarazi, as first noted by L. De Vos and L. Taverne (in 1991), L. De Vos and P. Weiler (in 1991), and De Vos et al. (2001). Furthermore, as a result of this, it is known to have a discontinuous distribution (see also discussion: The present discontinuous distribution of *E. nzigidaherai*). It is to be noted also, that both the

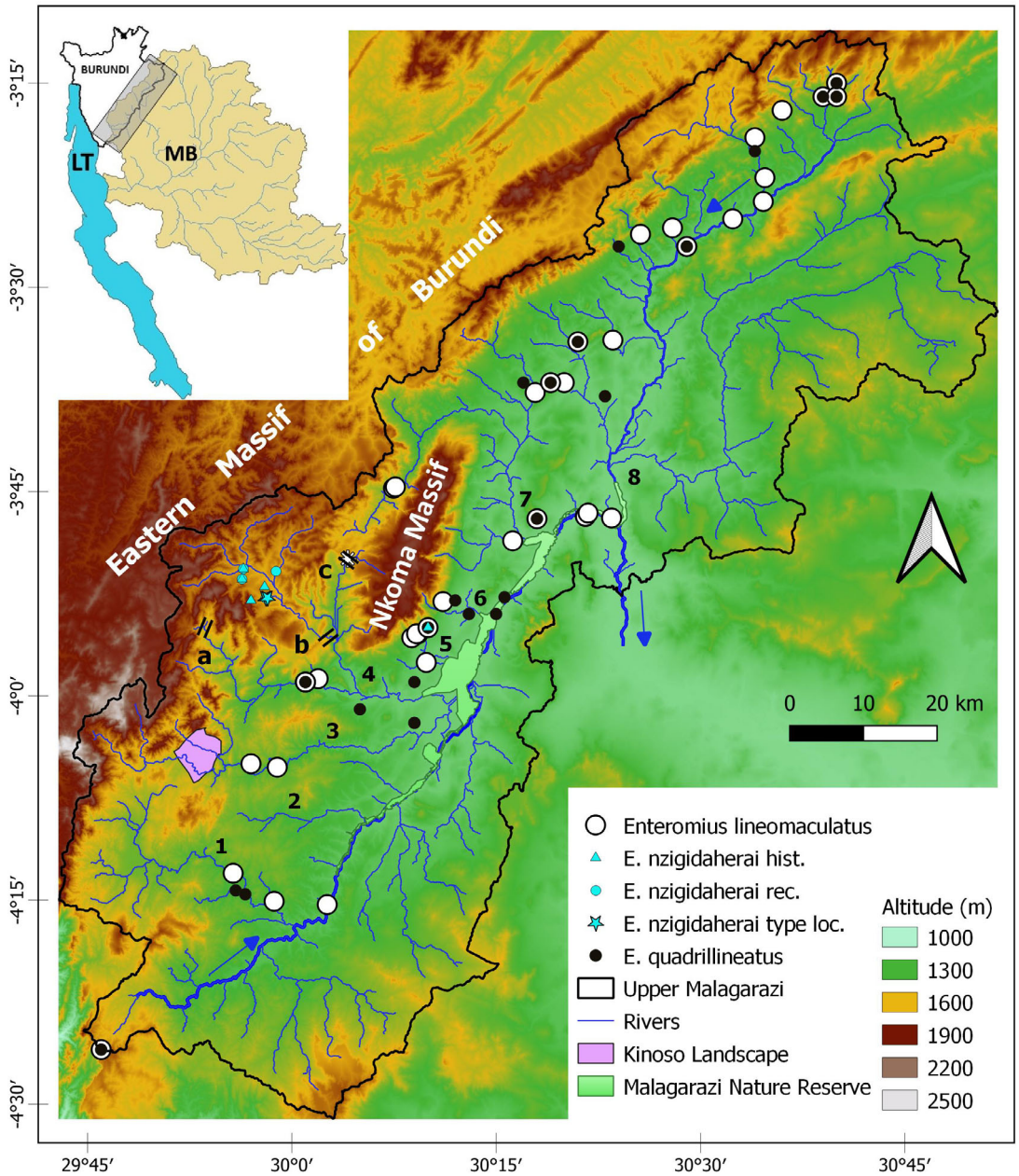

FIGURE 8 Distribution of *Enteromius nzigidaherai* sp. nov. (type loc.: type locality; hist.: historical sampling before 2008; rec.: recent sampling from 2008 onwards) and of its two most similar congeners also present in upper Malagarazi (uM) in Burundi, that is, *E. lineomaculatus* and *E. quadrilineatus*. The flow direction of the rivers is indicated by a blue arrow. Main affluents of the uM in Burundi (from up- to downstream): 1. Rukoziri, 2. Nyakabanda, 3. Mutsindozi, 4. Muyovozi, 5. Kinwa, 6. Mazimero, 7. Mukazye, and 8. Rumpungwe. Double bars indicate major falls: (a) Cikinga Falls (Musasa River, right bank affluent of the Muyovozi River), (b) Nyaganza Falls (Muyovozi River), and (c) Karera Falls (Karera River, left bank affluent of the Muyovozi River). LT, Lake Tanganyika, and MB, Malagarazi basin

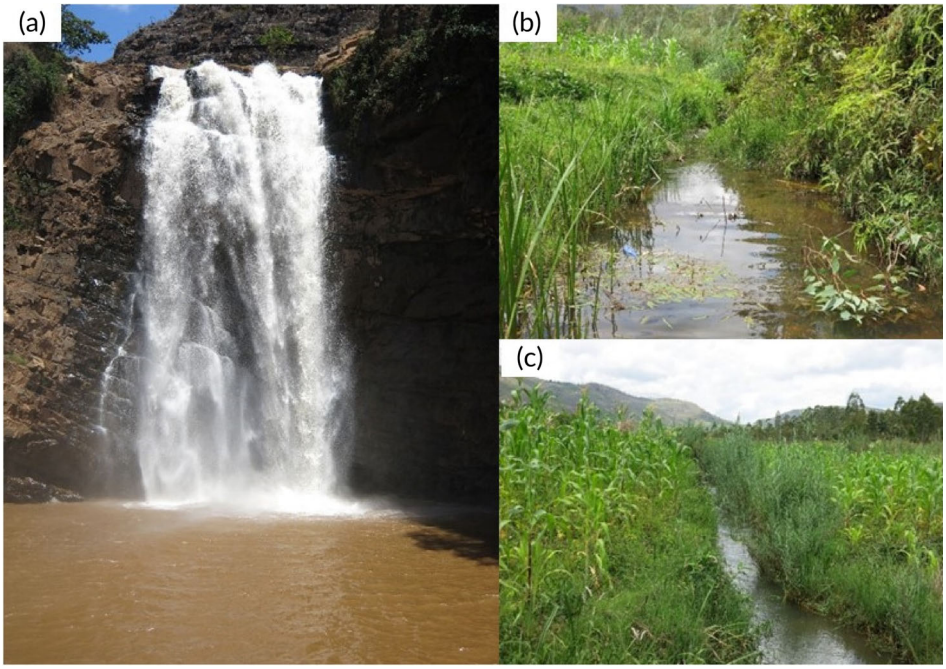

FIGURE 9 Photographs of (a) the Nyaganza I Falls on the Muyovozi River, near Kibinzi Village (03°55′35.3″S, 30°03′21.6″E) (23/08/2021); (b) a sampling locality of the new species on the Nyankende River, a right bank affluent of the Muyovozi River, the type locality (03°52′49.0″S, 29°58′05.1″E) (09/11/2019); (c) a sampling locality of the new species on the main course of the Muyovozi River at Ramvya Village (03°50′41.1″S, 29°56′26.7″E) (10/11/2019)

upper parts of these affluent river systems are situated outside the borders of the MNR as presently conceived.

3.4.9 | Ecology

During recent field expeditions in the upper Malagarazi basin (2013–2022), new specimens of *E. nzigidaherai* sp. nov. were collected from the upper stretch of the Muyovozi River, that is, above the Nyaganza (I and II) Falls (see Figure 9a), a left bank affluent of the uM basin. These localities were characterised by a moderately weak water flow, sandy substrate, sometimes mixed with gravel; and grass banks with or without *Phragmites* (Poaceae) (Figure 9b,c). At two of the three localities sampled, specimens were collected in medium to large schools (∼50 and 130 specimens) (see SI: Table S5). In addition, both the other sympatric species of the *E. lineomaculatus* species complex, that is, *E. lineomaculatus* or *E. quadrilineatus*, are absent from the upper stretch of this affluent (Figure 8; SI: Table S5).

Furthermore, historical samples (L. De Vos and L. Taverne in 1991; L. De Vos and P. Weiler in 1991) of *E. nzigidaherai* sp. nov. were reported, not only from the Muyovozi River above the Nyaganza (I and II) Falls but also from the upper stretch of the Kinwa River (SI: Table S5), another, more downstream, left bank affluent of the uM basin (see Figure 8). As fishing details are missing for these collecting efforts, it remains difficult to know if the preserved samples represent the entire school caught or were originally caught as part of larger-sized schools of specimens. Indeed, the existing historical samples for both the Muyovozi (≤11 specimens) and Kinwa (≤17 specimens) affluent rivers (SI: Table S5) are well below the size of the large-sized schools recently collected in the Muyovozi and its affluent rivers. For the historical sampling efforts on the Muyovozi River above the Nyaganza (I and II) Falls, both sympatric species, that is, *E. lineomaculatus* and *E. quadrilineatus*, were not collected. However, there are syntopic collection records for both other species from the upper stretch of the Kinwa River.

It is worthwhile to note that, despite important collecting efforts, with gillnets and dip-nets, the new species has not been recently collected in the Kinwa River (A. Bigirimana, personal observation, 2013–2022). Moreover, the available data regarding school size in relation to the seasons (50–130 specimens), although preliminary, seem to suggest that specimens of the new species seem to regroup, at least, through the rainy season [October to November and March to April (see Nzigidahera, 2006; MATTE, 2007)], usually considered the breeding period (Nhiwatiwa et al., 2017). However, further detailed observations will be needed to confirm this.

The recent field data and available historical samples belonging to both most similar and sympatric *Enteromius* species, that is, *E. lineomaculatus* and *E. quadrilineatus*, revealed that both, compared to the new species, have a much wider distribution in the uM basin (see Figure 8). Furthermore, both these species were often sampled syntopically and collected as small schools (≤10 specimens) or single individuals and during both the dry and rainy seasons, alike (SI: Table S5). Thus, although preliminary, these data seem to suggest that, unlike for the new species, both the species live as single individuals or small-sized schools year-round. However, also here, further detailed observations will be needed to confirm this.

3.4.10 | IUCN conservation assessment proposition for the new species

Enteromius nzigidaherai sp. nov. is endemic to uM in Burundi. Its distribution is limited to two affluents of the uM, that is the upper

Muyovozi and Kinwa Rivers (Figure 8: nos. 4 and 5, respectively). Recent sampling (2008–2022) only confirmed its occurrence in the former river. Indeed, despite intensive sampling in the Kinwa River, where the species was collected in the 1990s (De Vos et al., 2001), the species has no longer been found there (Figure 8). When including the historical sampling sites, the overall extent of occurrence (EOO) of *E. nzigidaherai* sp. nov. covered about 54 km^2, whereas its current area of occupancy (AOO) was 24 km^2. Currently, *E. nzigidaherai* sp. nov., restricted to the upstream of the Muyovozi River, above the Nyaganza Falls, presents an EOO of about 11 km^2 and an AOO of 16 km^2. As such, its population size has certainly suffered a substantially decrease over this time period. The EOO is below the IUCN thresholds (according to criterion B1, EOO is <100 km^2), the number of locations is ≤5, a continued decline of AOO, and an extreme fluctuation of the EOO is observed (see IUCN Standards and Petitions Committee, 2022). Therefore, according to the categories and criteria of the IUCN (IUCN Standards and Petitions Committee, 2022), *E. nzigidaherai* sp nov. should be considered as Critically Endangered (CR) B1ab(ii,iv)c(i,iii).

3.4.11 | Etymology

It had been reported that *E.* sp. 'ascutelatus', here named *E. nzigidaherai* sp. nov., differs from the other species of the *E. lineomaculatus* species complex of the upper Malagarazi by the absence of a long scale, called scutella, at the base of the pelvic fin (L. De Vos and L. Taverne in 1991; L. De Vos and P. Weiler in 1991). However, it has been observed that, instead, at least some specimens have a scutellum, even if it is not well developed. Therefore, despite its current cheironym, we prefer not to describe the species under that name.

This species is dedicated to the late Benoit Nzigidahera (1964–2018), a Burundian environmentalist who was an expert of the OBPE from 1996 to 2018. He is also one of the principal Burundian personalities who campaigned for the protection of the environment in Burundi, in general, and for the creation of the Malagarazi Nature Reserve (MNR), in particular.

3.5 | DISCUSSION

3.5.1 | *Enteromius lineomaculatus* in the upper Malagarazi: A morphological species complex

A new species of *Enteromius*, here named *E. nzigidaherai*, is described from the upper Malagarazi (uM) basin in Burundi. This species is to be added to the list of 73 fish species previously reported from the uM, among which 14 species are still in need of further taxonomic attention (Banyankimbona et al., 2012a; Bigirimana, et al., 2024). Thus, the new species is also to be added to the 12 *Enteromius* species (16% of 73 fish species presently known from the uM basin) currently known from this part of the basin, among which two of them, *Enteromius* cf. *apleurogramma* (Boulenger 1911) (Kisekelwa et al., 2020; D. Muzumani Risasi et al., unpublished data) and *Enteromius* cf. *cercops* (Whitehead 1960) (Banyankimbona et al., 2012a; Bigirimana et al., 2024; De Vos et al., 2001), are still in need of further taxonomic attention. Thus, with 13 species known, to date, from the uM basin the genus *Enteromius* is the most diverse, preceding *Labeo* (with six valid species), and *Clarias* and *Synodontis* (each with four valid species).

Three out of the five known species of the *E. lineomaculatus* species complex, namely *E. lineomaculatus*, *E. quadrilineatus* and the new species, *E. nzigidaherai*, occur in sympatry in the uM basin including both its left bank affluents, the Muyovozi and Kinwa rivers, where historical and current records confirmed its presence (Figure 8: river nos. 4 and 5). In the past, however, they have also been found to occur syntopically in the Kinwa River, but not in the Muyovozi River. Thus, besides their overall morphological similarity, this co-occurrence clearly illustrates the relevance of comparing both species, *E. lineomaculatus* and *E. quadrilineatus*, with the new species through an integrative approach (see above). This included also the use of mitochondrial COI (mtDNA) barcoding.

Nevertheless, although the species of the *E. lineomaculatus* species complex are morphologically similar, at least in some colour pattern characteristics, the COI mtDNA barcoding results did not reveal the existence of a *E. lineomaculatus* species complex. Indeed, all three species of the species complex included in the extended ML COI barcoding tree (SI: Figure S2), *E. lineomaculatus*, *E. quadrilineatus*, and *E. nzigidaherai*, seem to be more distantly related, because they do not form a well-supported monophyletic lineage, but instead were scattered over the overall phylogenetic tree.

It would have been interesting, indeed, to also include *E. urostigma* in the COI barcoding tree. However, all sequences available on GenBank for *E.* cf. *urostigma* revealed to be based on misidentifications as all were reidentified *E.* cf. *candens*. Nevertheless, the morphological differences documented between the new species and *E. urostigma*, that is, at least two independent characters—(i) the number of scales between the LL and the middle of the belly, (ii) the number of pre-dorsal scales, with the pre-dorsal distance (% L_S) being related to it, and (iii) the distinctively positive allometric eye diameter, at least for the larger specimens—support their differentiation as two distinct species (see Diagnosis for more details). Indeed, in general, the occurrence of two independent morphological characters has been considered sufficient evidence for species distinction (Turner, 1999; Wiens, 2007). Furthermore, some previous studies on *Enteromius* have also denoted that new species can be differentiated based on at least two independent morphological diagnostic characters, including qualitative ones, such as the dorsal spine morphology or the presence/absence of a sheath of prominent scales at the base of the dorsal fin (see Banyankimbona et al., 2012b; Kisekelwa et al., 2022).

In addition, in both the extended (SI: Figure S2) and selected COI barcoding trees (Figure 6), *E. lineomaculatus* revealed to be composed of two, well-supported, distinct lineages, with specimens only from the uM basin ($n = 8$) and with specimens only from the Rusizi basin ($n = 2$). Apparently, morphologically, the specimens from upper

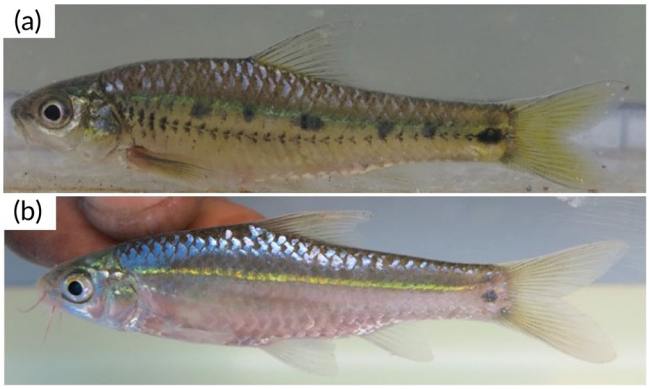

FIGURE 10 Photographs of *Enteromius lineomaculatus*: (a) live specimen from the upper Malagarazi (uM) basin (RMCA 2018-034-P-0146; tag no. 921/44), at Buyaga Village, Kinwa River (02/12/2018); (b) live specimen from the Rusizi basin (RMCA 2021-016-P-0133-0139; tag no. 886–10) at confluence of the Muhira River (left bank affluent of the Rusizi River) (08/08/2021)

Malagarazi differ from those of the Rusizi by their life colouration as well. Fresh specimens from the upper Malagarazi (Figure 10a) present a horizontal series of about six well-defined and more or less roundish black spots along the flanks, never confluent, situated about half a scale above the LL at the level of the dorsal fin. In addition, toward the upper border of this series of black spots, there is a thin greenish longitudinal band, which itself is situated about one scale above the LL at the level of the dorsal fin. Furthermore, those specimens bear distinctive black chevron-shaped markings on the LL scales from behind the operculum up to the origin of caudal fin and a round, well-visible, black spot toward the anterior base of the caudal. Instead, fresh specimens from the Rusizi (Figure 10b) present a yellowish longitudinal band, starting above the posterior half of the eye, continuing above the operculum and along the upper mid-lateral side. This yellowish band is situated about a half scale above the LL at the level of the dorsal fin. In addition, the LL scales, usually, lack the chevron-shaped black markings. A minority of specimens indeed present, a few, tiny, chevron-shaped black markings on the LL scales. Nevertheless, the round, well-visible, black spot toward the base of the caudal is also present, although usually smaller (SI: Figure S15). Although the studied meristics revealed no differences between the specimens from the two basins, two measurements differences were noted. These are: a longer preopercular distance (POD) for those from the Rusizi (L_S: 71.8 and 76.6 mm; 2 specimens), 74.0–75.2% L_H [vs. shorter for those from uM basin (L_S: 37.3–63.4 mm; 28 specimens), 64.3–72.0% L_H] (SI: Figure S16a), and a larger inter-orbital distance (IOW), 44.3–45.9% L_H (vs. 33.4–43.8% L_H) (SI: Figure S16b). However, considering the size differences (L_S) between the specimens from both basins, the first measurement seems rather isometric for all the comparative species and could therefore indeed indicate a real possible difference between the two. The second measurement, however, is clearly positive allometric, at least for the new species. Therefore, more specimens will be needed to further clarify the nature of the documented morphometric differences.

A K2P GD of 4.9% separates both *E. lineomaculatus* populations (Table 3). As such, instead of (hydro)geographic intraspecific variation between two hydrographic populations, the Malagarazi vs. Ruzizi basins, this might point to the possible occurrence of two distinct, but otherwise morphologically similar, species. Nevertheless. especially due to the small number of Rusizi specimens studied and analysed at present, the question remains open and is currently under further study.

3.5.2 | The present discontinuous distribution of *E. nzigidaherai*

In the Muyovozi River, the new species has been collected only from its upper course, above the Nyaganza Falls (Figure 8: river no. 4, falls b) on the Nkoma Massif, the latter made up of erosion-resistant quarzitic rocks but with numerous north-east to south-west oriented cracks (Laghmouch et al., 2019; Marques et al., 2020) (SI: Figure S17). The river exits the Nkoma Massif southwards through major cracks. The species has also been reported to occur in the upper Kinwa River (L. De Vos and L. Taverne in 1991), draining the south-eastern margin of the Nkoma Massif (Figure 8: river no. 5). This raises questions about the origin of the present discontinuous distribution of the new species.

The Nkoma Massif is located on the south-eastern side of the Eastern Massif of Burundi (EMB) (Figure 8). Two major rivers bypass the Nkoma Massif, separating it from the EMB. First, the Mukazye River (Figure 8: river no. 7), which bypasses it in the north with a south-west to north-east oriented headwater, this until the northern edge of the Nkoma Massif, where it follows by south-eastwards getting around (SI: Figure S17). The Mukazye River lays, mostly, on shist rocks (Laghmouch et al., 2019), more sensitive to erosion (Marques et al., 2020), explaining the presence of a deep valley in-between Nkoma Massif and the EMB. Secondly, the Karera River, a left bank affluent of the Muyovozi River, bypasses the Nkoma Massif in the south with a north-east to south-west oriented headwater until its confluence with the Muyovozi River above the Nyaganza Falls (Figure 8: river no. 4, falls b).

The disappearance of the new species from the downstream part of both the Muyovozi and the Kinwa rivers, as well as the entire connecting section of the upper Malagarazi River itself, seems unlikely. Thus, the actual discontinuous distribution of the new species (Figure 8) seems to point to the possibility that the Muyovozi River once was the headwater stream of the Kinwa River. This probable interruption of the outlet of the Muyovozi River could have been caused by tectonic movements in the Nkoma Region, among which the most recent would be during the Cenozoic age (Tack et al., 1992). This would have resulted in the uplift of the eastern side of Nkoma Massif of about 500 m, altogether with a succession of westwards downfaulted blocks toward the Malagarazi River plain (Tack et al., 1992). Therefore, this deformation would have caused the interruption of the main course of the Muyovozi River, and both sections of the river newly created would follow the line of the great slopes (L. Habonimana, personal observation, 2022). Therefore, the headwater of the Muyovozi River, situated south-west of the Nkoma Massif, flows from the Nyaganza Falls toward the plain of Malagarazi River by following the major fault lines

(see SI: Figure S17). The absolute age of this event, that is, if this has happened at the time of the creation of Western and Eastern Massifs of Burundi (known from the Cenozoic age with both massifs linked to the creation of the Lake Tanganyika ditch, dated around 20 MYA) (Cazenave-Piarrot et al., 2015; De Vos et al., 2001; Roberts, 1975; Waleffe, 1965). Considering the entrance of *Enteromius* (diploid Smiliogastrinae) in Africa, through the Arabian Peninsula, was already dated at around 20 MYA (Stewart & Murray, 2017), this seems to indicate that the rise of the Nkoma Massif would have been a more recent event compared to the creation of the Lake Tanganyika ditch.

3.5.3 | Anthropogenic impacts and protection in the upper Malagarazi (Burundi)

The currently known distribution of *E. nzigidaherai* is limited to two left bank affluents of the upper Malagarazi: the Muyovozi River (Figure 8: river no. 4), and both its right bank affluents, the Nyankende and Sagara; and the Kinwa River (Figure 8: river no. 5). This poses specific challenges to the efficient protection of this new species.

The ecoregion of the Malagarasi-Muyovosi (ecoregion no. 543 in Abell et al., 2008; ecoregion no. 11 in Thieme et al., 2005), which encompasses the entire upper Malagarazi, was considered as relatively intact and stable (Abell et al., 2008; Thieme et al., 2005). However, the aquatic habitats are threatened by anthropogenic impacts, especially by the growing human populations in the region using destructive fishing techniques resulting in overfishing. In addition, water pollution due to the industrial waste, that is, from the SOSUMO sugar refinery, discharged into the Malagarazi River itself (Banyankimbona et al., 2012a), and agricultural chemicals, that is, pesticides and fertilisers, also showed to be a problem by modifying critical water parameters such as dissolved oxygen and conductivity (Mateo-Sagasta et al., 2017, 2018). Finally, at present, the cutting down of the gallery forests along the riverbanks and the use of water for agro-pastoral purposes also showed to be a problem as it leads to the destruction of some microhabitats through, for instance, the reduction or overall removal of the canopy closure (A. Bigirimana, personal observation, 2013–2022).

First, despite historical (L. De Vos and L. Taverne in 1991; L. De Vos and P. Weiler in 1991) and recent sampling efforts (A. Bigirimana, 2013–2022), below the Nyaganza Falls on the Muyovozi River, no specimens of the new species have been collected, confirming it is apparently absent in this river stretch (Figure 8: river no. 5). Furthermore, at present, this downstream part of the Muyovozi River, that is, below the Nyaganza Falls, seems intensely disturbed. Interviews to fishermen have reported illegal fishing by using an ichthyotoxin called Ubuhunwa, in the local Kirundi language (A. Bigirimana and G. Banyankimbona, personal observations, 2013–2022). This ichthyotoxin is made mainly from leaves, roots, and/or fruits of local plants, such as *Tephrosia vogelii* Hook.f. (Umutaruhunwa or Umuruka in Kirundi) and *Albizia versicolor* Welw. ex Oliv. (Ingongo, Ihwa, or Umuramba) (both Fabaceae), and *Solanum aculeastrum* Dunal (Umutobotobo) (Solanaceae), or non-native plant species, such as

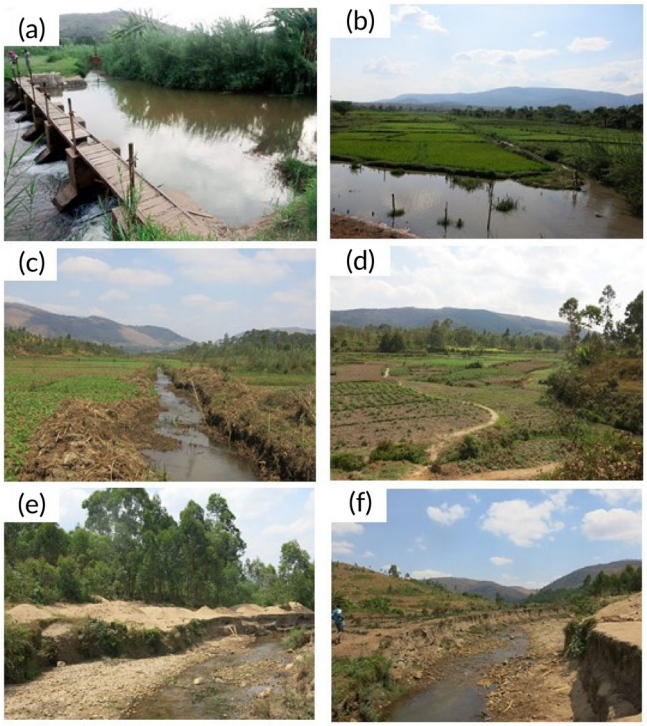

FIGURE 11 Photographs of: (a) irrigation dam built on the main course of the Muyovozi River at Rugwe Village (03°58′54″S, 30°01′01″E) (19/08/2021); (b) rice crop fields along the Kinwa River at Buyaga Village (03°57′34.8″S, 30°09′54.1″E) (22/08/2021); (c) destruction of the overall vegetation in the valley of the Muyovozi River at Ramvya Village (03°50′41.1″S, 29°56′26.7″E) (17/08/2021); (d) overall deforestation of the Nyankende Valley (right bank affluent of the Muyovozi River) at Nyankende Village (03°52′49.0″S, 29°58′05.1″E) (17/08/2021); (e) river bank destruction on the Sagara River (right bank affluent of Muyovozi River) by sand exploitation, for construction purposes; and (f) resulting erosion, at Sagara Village (03°51′25.8″S, 29°56′20.9″E) (17/08/2021)

Capsicum annuum L. (Agapiripiri) (Solanaceae) (Banyankimbona, 2012). The use of artisanal toxins is illegal by the Decree Law no. 1/17 of November 30, 2016, on the organization of fishing and aquaculture in Burundi. Moreover, downstream of the Nyaganza Falls, near the village of Rugwe, a concrete irrigation dam (Figure 11a) blocks the river and changes its water flow by forming a small lake, altering the natural upstream river habitat. As such, the combination of the present anthropogenic impacts, downstream of the Nyaganza Falls, do not seem to offer good conditions to most native species, in general, and *E. nzigidaherai*, in particular.

Second, based on historical collecting efforts, the new species is reported to occur in the Kinwa River (Figure 8: river no. 5), which takes its source at the base of the south-eastern edge of the Nkoma Massif (Fofo, 2007). However, no specimens of the new species were collected during recent field collecting efforts (2008–2012; 2013–2022) on the Kinwa River, likely resulting from habitat degradation (SI: Figure S18). The main threats to the Kinwa River are the destruction of its riverbanks by deforestation, the installation of an artisanal dam for irrigation, and the use of agricultural chemicals (Figure 11b),

that is, pesticides and fertilisers, for food crops such as rice, beans, maize, and potatoes (A. Bigirimana, personal observation, 2013–2022). Indeed, recent collecting efforts have remained unsuccessful (A. Bigirimana, personal observation, 2022) and thus further substantiate the possible local extinction of the new species in this affluent of the uM basin in Burundi.

The farmers surrounding the Muyovozi basin entered its valley and cleared the vegetation and the gallery forest along its banks (Figure 11c,d) for their cultivation of food crops. This farmers' migration followed the degradation of the soil's fertility on the hillside fields due to their intensive agricultural (over)use. In addition, the accentuation of the flow of water due to the important runoffs and important materials in suspension, essentially in rainy season, and the removal of important amounts of sand, as construction material (Figure 11e), induce an increased erosion of the riverbanks (Figure 11f) and resulted in the overall siltation of Muyovozi River and its affluents.

3.5.4 | The Malagarazi Nature Reserve (MNR): a protected area under implementation

For more than a decade now, a project to implement the creation of the Malagarazi Nature Reserve (MNR) in Burundi has been underway (Nzigidahera & Nindorera, 2009). However, its currently presented boundaries mostly include only the main course of the uM basin and, as such, do not cover the presently known distribution area of the new fish species described in the present paper (Figure 8). The same holds true for 13 more fish species also known from the uM basin, including 5 endemics, 3 of which are still in need of a formal description, *Chiloglanis* sp. 'musasae', *Clariallabes mutsindoziensis* Taverne & De Vos 1998, *Clariallabes* sp. 'nyaruhandazi', *Mastacembelus* sp. 'devosi', and *Orthochromis mazimeroensis* De Vos & Seegers 1998.

Moreover, based on their current distribution in the rivers of Burundi, five out of the eight non-endemic species are known, in Burundi, only from the uM basin (Banyankimbona et al., 2012a). Two of these five species have a disrupted and restricted distribution in this part of the basin. It concerns, first, *Enteromius pseudotoppini* (Seegers 1996), known only from two left bank affluents of the upper Malagarazi, being the Mutsindozi and Mukazye. This species has originally been described from the Piti River, a left bank affluent of Rungwa River, Mbeya Region, in the eastern area of the Lake Rukwa drainage (Seegers, 1996). Second, this holds true also for specimens similar to *Zaireichthys rotundiceps* (Hilgendorf 1905), identified from the Mutsindozi River, a major left bank affluent of the uM basin. This species has originally been described from the Bubu River, endorheic system, at Irangi (Kondoa) in central Tanzania (Eccles et al., 2011). However, it is known to have a widespread distribution from the east, that is Lake Victoria, Rufiji and Lake Rukwa systems (Seegers, 2008) to the south, that is the upper and middle Congo, Okavango, Zambezi, Pungwe, Buzi, Save river systems as well as the Lake Malawi catchment (Seegers, 2008; Skelton, 1993).

The remaining three species are, nevertheless, widespread in the Malagarazi River basin as a whole. It concerns *Amphilius pedunculus* Thomson & Pages 2015, *Amphilius* sp. 'mutsindozi' (Bigirimana et al., 2024), and *Chiloglanis kazumbei* Friel & Vigliotta 2011. Finally, the other three of the eight non-endemic species are shared with the Rusizi basin in Burundi, instead (Figure 1): *Enteromius pellegrini* (Poll 1939), *Labeobarbus somereni* (Boulenger 1911), and *Clarias liocephalus* Boulenger 1898, but with the last one not occurring in the Ruzizi National Park in Burundi (Figure 1) (D. Muzumani Risasi, unpublished data). In addition, the last two, that is, *L. somereni* and *C. liocephalus*, are shared with the Ruvubu basin, and both are present in Ruvubu National Parc as well (Banyankimbona et al., 2012a; De Vos, 1991).

A higher fish species diversity is to be expected in lower and middle river stretches (Lévêque & Paugy, 2017; Winemiller et al., 2008), with, instead, although less species rich, a highly adapted (endemic) ichthyofauna in the higher altitude stretches of these (Jacobsen, 2008). Therefore, the partial differentiation of the ichthyofaunal composition observed between the upper Malagarazi main river and its affluent rivers could be explained by the presence of distinct microhabitats occurring only in these affluents (see Banyankimbona, 2012; Meyer et al., 2007). Their faster water flow, being situated at higher altitude relatively to the main course of the upper Malagarazi (Figure 8) might by itself, as for other hydrographic elements, such as waterfalls, be physical barriers isolating the affluents from the main river (see Jacobsen, 2008; Negi & Mamgain, 2013; Soo et al., 2021). This seems true, for instance, for the occurrence of the small rapid habitats that are found in the affluent rivers only (A. Bigirimana, personal observation, 2013–2022).

Therefore, it is hoped that the present description of a new fish species endemic to the uM basin brings to the attention of the national decision and policies makers and aquatic conservation institutes or non-government organization alike the importance of accomplishing, as much as possible, an all-encompassing fish diversity protection of the uM basin. This objective will, undoubtedly, need a review of the current delimitation of the MNR as to include, as much as possible, all endemic fish species known to date. Furthermore, recent sampling confirms the presence of the new species in one of both the affluents from which it has historically been reported, that is the upper stretches of Muyovozi River. However, the adjustments needed to include the upper stretches of this affluent to the present delimitation of MNR would require a surface extension of about 50% to the present 80 km^2 (MEEATU, 2014) covered by it. Such an extension seems most unlikely. Therefore, the creation of new protected area in the upper Muyovozi River was proposed (Bigirimana et al., 2024). In addition, because little work has been done for Africa, studies for a better understanding of spatiotemporal patterns should be undertaken to identify the most important environmental gradients that condition the distribution of fish and drive fish community diversity in the main course of the upper Malagarazi and its affluents. Therefore, this could further contribute to the elaboration of effective, fact-based, fish conservation plans, particularly in Burundi and globally in Africa.

3.5.5 | Comparative material

Enteromius lineomaculatus

All types from Kenya. BMNH 1902-11-8-22 (lectotype), 57.5 mm L_S, Lumi River, Kilimanjaro Region (Lumwe River: ±03°31′S, 37°43′E; USBGN, 1964a), donor A. Percival. BMNH 1902-11-8-23 (paralectotype), 40.9 mm L_S (not in good condition), same data as lectotype.

Additional specimens. From Burundi. RMCA 2009-013-P-0250-0254, four specimens, 43.8–63.4 mm L_S, Nyagafunzo River (right bank affluent of Mukazye River), Nyagafunzo swamp, at Bubumba Village, upper Malagarazi basin, 03°48′37.4″S, 30°16′13.1″E, coll. G. Banyankimbona, 19/02/2009. RMCA 2012-007-P-0372, one specimen, 58.5 mm L_S, Mazimero River (left bank affluent of Malagarazi River), right the dam on bridge Rutana-Giharo Road, upper Malagarazi basin, 03°53′05.3″S, 30°11′06.0″E, coll. G. Banyankimbona & D. Muzumani Risasi, 23/02/2012. RMCA 2018-034-P-0151-0156, 6 of 11 specimens, 40.5–54.8 mm L_S, Kinwa River (left bank affluent of Malagarazi River), at Buyaga Village, upper Malagarazi River, 03°57′36.1″S, 30°10′40.3″E, coll. A. Bigirimana & G. Banyankimbona, 02/12/2018. RMCA 2018-034-P-0158-0160, three of four specimens, 53.9–56.8 mm L_S, Nyarugunga River (right bank affluent of Rumpungwe River), at Migende Village, upper Malagarazi River, 03°25′54.6″S, 30°25′31.4″E, coll. A. Bigirimana & G. Banyankimbona, 03/12/2018. RMCA 2018-034-P-0139-0145, six of seven specimens, 42.2–53.5 mm L_S, Mutsindozi River (left bank affluent of Malagarazi River), at Kabizi Village, upper Malagarazi River, 04°05′19.4″S, 29°59′19.2″E, coll. A. Bigirimana & G. Banyankimbona, 01/12/2018. RMCA 2021-016-P-0131, one specimen, 76.6 mm LS, Kaganga River (left bank affluent of the Rusizi River), at Mbaza-Miduha Village, 02°47′37.1″S, 29°01′12.1″E, coll. A. Bigirimana, 21/12/2017. RMCA 2021-016-P-0132, one specimen, 71.8 mm LS, Rusizi River, at Kuryinyo Village (on Avenue n°6 of Buganda township), 03°01′02.6″S, 29°11′51.3″E, coll. A. Bigirimana, 12/01/2018.

From Tanzania. RMCA 94-034-P-0186-0187, one of two specimens, 52.5 mm L_S, Luika-pools below the falls (±08°24′S, 32°52′E; USBGN, 1965), south-east of Malesa (±08°12′S, 30°58′E; USBGN, 1965), near Mbangala, coll. L. Seegers, 19/07/1993. RMCA 94-048-P-0647-0738, 7 of 91 specimens, 37.3–56.5 mm L_S, Nyamgongo brook (affluent Rusigi, sub-affluent Malagarazi River), on road from Uvinza to Kasulu, ±30 km before Kasulu, 04°48′S, 30°14′E, coll. L. De Vos, 22/06/1994.

Enteromius lornae

All from Zambia, BMNH 1943.7.27.178 (lectotype), one specimen, 51.6 mm L_S, Lake Young (±11°14′S, 31°43′E; USBGN, 1972) and Mansya River (±10°58′S, 31°06′E; USBGN, 1972), Chambezi basin, probably Katete River (±11°10′S, 31°45′E; USBGN, 1972) for the lectotype (see Seegers, 1996: 111) [Fricke et al., 2024; Ricardo-Bertram, 1943: station list (no. 70)], coll. R.J. Owen & C.K. Ricardo-Bertram, 1936. BMNH 1943.7.27.179-187 (paralectotype), five of nine specimens, 25.9–34.3 mm L_S, Lake Young (±11°14′S, 31°43′E; USBGN, 1972) and Mansya River (±10°58′S, 31°06′E; USBGN, 1972), Chambezi basin (Fricke et al., 2024; Ricardo-Bertram, 1943: 204; Seegers, 1996: 111), coll. R.J. Owen & C.K. Ricardo-Bertram 1936–1937.

Enteromius quadrilineatus

From Burundi. BMNH 1964-3-2-1-3 (syntypes), three specimens, 29.9–38.4 mm L_S, Malagarazi River (±03°57′S, 30°12′E; USBGN, 1964b), and its affluents, Buriri Terr., Tanganyika. RMCA P-47279 (syntype), one specimen, 40.3 mm L_S, Malagarazi River (±03°57′S, 30°12′E; USBGN, 1964b) and its affluents, Bururi Terr., coll. A. Lestrade, 1936. RMCA P-47280-294 (syntypes), 6 of 15 specimens, 30.0–40.3 mm L_S, Malagarazi River (±03°57′S, 30°12′E; USBGN, 1964b), and its affluents, Bururi Terr., coll. A. Lestrade, 1936.

Additional specimens. All from Burundi. RMCA 91-62-P-702-717, 5 of 16 specimens, 29.3–40.5 mm L_S, Gatare brook (right bank affluent of Mwambu River, right sub-affluent of Rumpungwe River), on road Cendajuru-Gasenyi, 03°16′S, 30°39′E, coll. L. De Vos & P. Weiler, 07/08/1991. RMCA 2011-003-P-0161-0164, two of four specimens, 43.1 & 44.9 mm L_S, Rubarandwa brook (right bank affluent Rukoziri, left affluent of Malagarazi River), near confluence Rukoziri River, Malagarazi basin, upper Malagarazi River, 04°14′35.4″S, 29°56′35″E, coll. G. Banyankimbona, 21/11/2010. RMCA 91-62-P-688-690, one of three specimens, 41.3 mm L_S, Musasa River (right bank affluent of Muyovozi River) on ± of Rutana. 03°59′S, 30°01′E, coll. L. De Vos & P. Weiler, 03/08/1991. RMCA 91-30-P-179-184, two of six specimens, 32.5 & 36.3 mm L_S, Kinwa River (left bank affluent of Malagarazi River), up the bridge on the provincial road 85, at Buyaga Village, 03°55′S, 30°10′E, coll. L. De Vos & L. Taverne, 04/04/1991. RMCA 2018-034-P-0133-0134, two specimens, and RMCA 2018-034-P-0135-0136, two specimens, 35.8–43.3 mm L_S, at Migende Village, Nyarugunga River (right bank affluent of Rumpungwe River), upper Malagarazi River, 03°25′54.6″S, 30°25′31.4″E, coll. A. Bigirimana & G. Banyankimbona, 03/02/2018. RMCA 2018-034-P-0131, one specimen, 40.6 mm L_S, Buyaga Village, Kinwa River (left bank affluent of Malagarazi River), upper Malagarazi River, 03°57′36.1″S, 30°10′40.3″E, coll. A. Bigirimana & G. Banyankimbona, 02/12/2018. RMCA 2019-023-P-0056-0059, three specimens, 45.1–47.1 mm L_S, at Migende Village. Nyarugunga River (right bank affluent of Rumpungwe River), upper Malagarazi River, 03°26′04.3″S, 30°25′34.9″E, coll. A. Bigirimana & G. Banyankimbona, 16/11/2019.

Enteromius taeniopleura

From DRC. RMCA P-11540 (syntype), one specimen, 24.6 mm L_S; and RMCA P-11541 (syntypes?), two specimens, 22.6 & 24.0 mm L_S, Kasarala brook at Gongwe (±06°45′S, 29°20′E; USBGN, 1964c), Lake Tanganyika, Katanga, coll. L. Stappers, 04/09/1912.

Enteromius urostigma

From DRC. RMCA P-14076-14105 (syntypes), 5 of 30, 38.6–55.3 mm L_S, Kampinda River to Mazonde brook (±07°09′S, 29°39′E; RMCA database), coll. L. Stappers, 23/07/1912. RMCA P-8546-8551 (syntypes), three of five specimens, 35.9–47.7 mm L_S, Lake Tanganyika, Gongwe brook, 06°45′S, 29°20′E, coll. L. Stappers. RMCA P-8687-8688 (syntypes), two specimens, 32.9–42.9 mm L_S, Kibondwe brook, 07°5′S, 29°26′E, coll. L. Stappers, 30/08/1912. BMNH 1920-5-25-52-61 (syntypes), 4 of 10 specimens, 34.9–49.5 mm L_S, Kampinda River, Katanga, Lake Tanganyika.

Additional specimens: From DRC. RMCA 79-1-P-3392-3420, 5 of 29 specimens, 43.5–77.0 mm L_S, Katongo (±08°49′S, 27°00′E; USBGN, 1964c) (left affluent of Mubale River and first affluent left of Munte River, sub-affluent of Lufira River), alt. 1750 m, coll. G.F. De Witte, 19/04/1948.

AUTHOR CONTRIBUTIONS

Anatole Bigirimana and Gaspard Banyankimbona were responsible for the fieldwork and the preliminary fish identification. Anatole Bigirimana, Tchalondawa Kisekelwa, and Emmanuel J.W.M.N. Vreven participated in the implementation of the methodological approach and design of the study and wrote jointly the first and subsequent revised versions of the manuscript. Charlotte E.T. Huyghe and Tchalondawa Kisekelwa were mainly responsible for molecular work, sequence data analysis, and writing and supervision of the paragraphs related to this part of the paper. Luis M. da Costa contributed extensively to the final writing and editing of the manuscript in collaboration with Anatole Bigirimana and Emmanuel J.W.M.N. Vreven. Emmanuel J.W.M.N. Vreven and Gaspard Banyankimbona supervised the overall work. All authors contributed to useful discussions, read and revised the manuscript, and approved its final version.

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REFERENCES

Abell, R., Thieme, M. L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., Coad, B., Mandrak, N., Balderas, S. C., Bussing, W., Stiassny, M. L. J., Skelton, P., Allen, G. R., Unmack, P., Naseka, A., Ng, R., Sindorf, N., Robertson, J., Armijo, E., … Petry, P. (2008). Freshwater ecoregions of the world: A new map of biogeographic units for freshwater biodiversity conservation. *Bioscience*, *58*(5), 403–414.

Bamba, M., Vreven, E. J., & Snoeks, J. (2011). Description of *Barbus teugelsi* sp. nov. (Cypriniformes: Cyprinidae) from the little Scarcies Basin in Guinea, Africa. *Zootaxa*, *2998*, 48–65.

Banyankimbona, G. (2012). The Fish diversity of the Burundese Rivers and the impact of humain activites on this ichthyofauna. PhD thesis. KU Leuven. https://limo.libis.be/primo-explore/fulldisplay?docid=LIRIAS1705617&context=L&vid=Lirias&search_scope=Lirias&tab=default_tab&lang=en_US

Banyankimbona, G., Vreven, E. J., Ntakimazi, G., & Snoeks, J. (2012a). The riverine fishes of Burundi (East Central Africa): An annotated checklist. *Ichthyological Exploration of Freshwaters*, *23*(3), 193–288.

Banyankimbona, G., Vreven, E., & Snoeks, J. (2012b). '*Barbus*' *devosi*, new species from the Malagarazi River basin in Burundi and Tanzania, East Africa (Cypriniformes: Cyprinidae). *Ichthyological Exploration of Freshwaters*, *23*(2), 181–192.

Bektas, Y., Aksu, I., Kaya, C., & Turan, D. (2018). DNA barcoding of the genus *Capoeta* (Actinopterygii: Cyprinidae) from Anatolia. *Turkish Journal of Fisheries and Aquatic Sciences*, *19*, 739–752.

Berra, T. M. (2007). *Freshwater fish distribution*. The University of Chicago press.

Bigirimana, A., Kisekelwa, T., da Costa, L.M., Muzumani, D.R., Mukweze Mulelenu, C., Abwe, E., Banyankimbona, G., & Vreven, E. (2024). An Updated Checklist of the Fishes from the Upper Malagarazi (Lake Tanganyika Basin) in Burundi: Implications for an under Implementation Malagarazi Nature Reserve. *Diversity*, *16*(7), 417. https://doi.org/10.3390/d16070417

Bookstein, F. L., Chernoff, B. L., Elder, R. L., Humphries, J. M., Smith, G. R., & Strauss, R. E. (1985). *Morphometrics in evolutionary biology: The geometry of size and shape change, with examples from fishes*.

Special publication (Vol. 15, pp. 1–277). Academy of Natural Sciences of Philadelphia.

Boulenger, G. A. (1903). Description of four new species of *Barbus* discovered by Mr A. Blayney Percival in East Africa. *Annals and Magazine of Natural History (Serie 7)*, *XI*, 52–53.

Boulenger, G. A. (1917). Descriptions of new fishes from Lake Tanganyika forming part of the collection made by the late Dr. L. Stappers for the Belgian Government. *Annals and Magazine of Natural History (Serie 8)*, *20*(119), 363–368.

Cazenave-Piarrot, A., Ndayirukiye, S., & Valton, C. (2015). *Atlas des Pays du Nord-Tanganyika*. Marseille.

Chakrabarty, P. (2010). Genetypes: A concept to help integrate molecular phylogenetics and taxonomy. *Zootaxa*, *2632*, 67–68.

Daget, J., Gosse, J.-P., & Thys van den Audenaerde, D. F. E. (1984). Check-List of the Freshwater Fishes of Africa. ORSTOM, Paris; MRAC, Tervuren. Vol. I. https://doi.org/10.2307/1445588

De Weirdt, D., Getahun, A., Tshibwabwa, S., & Teugels, G. G. (2007). Cyprinidae. In M. L. J. Stiassny, G. G. Teugels, & C. D. Hopkins (Eds.), *Poissons d'eaux douces et saumâtres de basse Guinée, Ouest de l'Afrique Centrale/The fresh and brackish water fishes of lower Guinea, west-Central Africa* (Vol. 1, pp. 467-572). MRAC Tervuren 572. http://horizon.documentation.ird.fr/exl-doc/pleins_textes/ed-06-08/010044384.pdf

De Vos, L. (1991). Les poissons du parc national de la Ruvubu. *Annales du Musée Royal de l'Afrique Centrale, Sciences Zoologiques*, *265*, 1–25.

De Vos, L., Seegers, L., Taverne, L., Van Don, T., & Audenarde, D. F. E. (2001). L'ichtyofaune du bassin de la Malagarasi (système du lac Tanganyika): une synthèse de la connaissance actuelle. *Annales du. Musée Royal de l'Afrique Centrale, Sciences Zoologiques*, *285*, 117–135.

Decru, E., Moelants, T., De Gelas, K., Vreven, E., Verheyen, E., & Snoeks, J. (2016). Taxonomic challenges in freshwater fishes: A mismatch between morphology and DNA barcoding in fish of the north-eastern part of The Congo Basin. *Molecular Ecology Resources*, *16*(1), 342–352.

Decru, E., Snoeks, J., & Vreven, E. (2013). The true identity of the holotype of *Hepsetus odoe* and the names of the two West African species of *Hepsetus* (Teleostei: Hepsetidae). *Ichthyological Exploration of Freshwaters*, *24*(2), 187–192.

Decru, E., Sullivan, J. P., & Vreven, E. (2019). *Marcusenius wamuinii* (Teleostei: Mormyridae), a new elephantfish from the Mangroves National Park, Democratic Republic of the Congo. *Ichthyological Exploration of Freshwaters*, *29*(3), 223–237.

Decru, E., Vreven, E., & Snoeks, J. (2012). A revision of the West African *Hepsetus* (Characiformes: Hepsetidae) with a description of *Hepsetus akawo* sp. nov. and a redescription of *Hepsetus odoe* (Bloch, 1794). *Journal of Natural History*, *46*(1–2), 1–23.

Eccles, D. H., Tweddle, D., & Skelton, P. H. (2011). Eight new species in the dwarf catfish genus *Zaireichthys* (Siluriformes: Amphiliidae). *Smithiana Bulletin*, *13*, 3–28.

Evans, B. J., Morales, J. C., Picker, M. D., Melnick, D. J., & Kelley, D. B. (1998). Absence of extensive introgression between *Xenopus gzlli* and *Xenopus laevis laevis* (Anura: Pipidae) in Southwestern Cape Province, South Africa. *Copeia*, *2*, 504–509.

Fofo, A. (2007). Etude systématique de l'ichtyofaune de la rivière Kinwa du bassin de la Malagarazi. *Bulletin Scientifique de l'Institut National Pour l'Environnement et la Conservation de la Nature*, *2*, 32–38.

Fricke, R., Eschmeyer, W. N., & Van der Laan, R. (Eds.) (2024). *Eschmeyer's catalog of fishes: Genera*. Species, References. Electronic version https://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp

Froese, R., & Pauly, D. (Eds.). (2023). *FishBase*. World Wide Web Electronic Publication. www.fishbase.org

Hammer, Ø., Harper, D. A., & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, *4*(1), 9.

Hubert, N., Hanner, R., Holm, E., Mandrak, N. E., Taylor, E., Burridge, M., Watkinson, D., Dumont, P., Curry, A., Bentzen, P., Zhang, J., April, J., & Bernatchez, L. (2008). Identifying Canadian freshwater fishes through DNA barcodes. *PLoS One*, *3*(6), 1–8.

Humphries, J. M., Bookstein, F. L., Chernoff, C., Smith, G. R., Elder, R. L., & Poss, S. G. (1981). Multivariate discrimination by shape in relation to size. *Systematic Zoology*, *30*, 291–308.

International Code of Zoological Nomenclature (ICZN). (1999). *Adopted by the International Union of Biological Sciences* (4th ed.). International Trust for Zoological Nomenclature. https://www.iczn.org/the-code/the-code-online/

IUCN Standards and Petitions Committee. (2022). Guidelines for using the IUCN red list categories and criteria: Version 15. Prepared by the Standards and Petitions Committee. Downloadable from https://www.iucnredlist.org/documents/RedListGuidelines.pdf

Ivanova, N. V., Zemlak, T. S., Hanner, R. H., & Hebert, P. D. N. (2007). Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, *7*(4), 544–548.

Jackson, P. B. N. (1961). *The fishes of northern Rhodesia: A checklist of indigenous species*. Lusaka. Govt. Printer.

Jacobsen, D. (2008). Tropical high-altitude streams. In D. Dudgeon (Ed.), *Tropical stream ecology* (pp. 219–256). Academic Press.

Kashindye Benedicto, B., Katemo Manda, B., Friel, J. P., Chakona, A., & Vreven, E. (2021). *Chiloglanis msirii*, a new species of African suckermouth catfish (Teleostei: Mochokidae), from the Upper Congo basin. *Ichthyological Exploration of Freshwaters*, *22*(IEF-1158), 1–14. https://doi.org/10.23788/IEF-1158

Kasongo Ilunga, M., Abwe, E., Decru, E., Chocha Manda, A., & Vreven, E. (2020). Description of a new small-sized *Synodontis* species (Siluriformes: Mochokidae) that is important for local subsistence fisheries in the middle Lufira (Upper Congo River, DR Congo). *Journal of Fish Biology*, *96*(5), 1142–1159. https://doi.org/10.1111/jfb.14076

Katemo Manda, B., Snoeks, J., Chocha Manda, A., Abwe, E., Mukweze Mulelenu, C., Ilunga Kayaba, M. K., Kiwele Mutambala, P., Ngoy Kalumba, L., & Vreven, E. J. W. M. N. (2023). The Upemba National Park (Upper Congo Basin, DR Congo): An updated checklist confirming its status as an African fish biodiversity hotspot. *Diversity*, *15*(9), 966. https://doi.org/10.3390/d15090966

Katemo Manda, B., Snoeks, J., Decru, E., Bills, R., & Vreven, E. (2019). *Enteromius thespesios* (Teleostei: Cyprinidae): A new minnow species with a remarkable sexual dimorphism from the south-eastern part of the Upper Congo River. *Journal of Fish Biology*, *96*(5), 1160–1175.

Katemo Manda, B., Snoeks, J., Decru, E., Brecko, J., & Vreven, E. J. W. M. N. (2023). Revision of *Nannocharax luapulae* Boulenger, 1915 (Characiformes: Distichodontidae) from the Upper Congo basin: Evidence for a species pair. *Journal of Fish Biology*, *103*(3), 557–573. /10.1111/jfb.15400

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), 1647–1649.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*, 111–120.

Kisekelwa, T., Snoeks, J., Decru, E., Schedel, F. B. D., Isumbisho, M., & Vreven, E. (2022). A mismatch between morphological and molecular data in lineages of *Enteromius* (Cypriniformes: Cyprinidae) from the Lowa basin (East Democratic Republic of the Congo: DRC) with the description of a new species. *Systematics and Biodiversity*, *20*(1), 1–22. https://doi.org/10.1080/14772000.2022.2135630

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

Kocovsky, P. M., Adams, J. V., & Bronte, C. R. (2009). The effect of sample size on the stability of principal components analysis of the effect of sample size on the stability of principal components analysis of truss-based fish morphometrics. *Transactions of the American Fisheries Society*, *138*, 487–496. https://doi.org/10.1577/T08-091.1

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*, 1547–1549.

Laghmouch, M., Nimpagaritse, G., Mudende, L., Minani, M., Ndereyimana, J., Icitegetse, I., Nahimana, A., Ndarihonyoye, P., Niyongabo, J. B., Fernandez, M., Baudet, D., Tack, L., & Kervyn, F. (2019). *Carte géologique du Burundi. Ministère de l'Hydraulique, de l'Energie et des Mines, Echelle 1/250000*. Africa Museum.

Lévêque, C. (1989). Remarques taxonomiques sur quelques petits *Barbus* (Pisces. Cprinidae) d'Afrique de l'ouest (Première Partie). *Cybium*, *13*(1), 165–180.

Lévêque, C., & Daget, J. (1984). Cyprinidae. In J. Daget, J.-P. Gosse, & D. F. E. Thys van den Audenaerde (Eds.), *Check-list of the freshwater fishes of Africa. CLOFFA. Volume 1* (pp. 217–342). ORSTOM Paris, MARC Tervuren.

Lévêque, C., & Paugy, D. (2017). Fish communities in river systems and associated biotopes. In D. Paugy, C. Lévêque, & O. Otero (Eds.), *The inland water fishes of Africa: Diversity, ecology and human use* (pp. 349–360). IRD Éditions.

Levin, B. A., Simonov, E., Matveyev, M. P., Artaev, O. N., Mustafayev, N. J., Pashkov, A. N., & Roubenyan, H. R. (2018). DNA barcoding of the fishes of the genus *Alburnoides* (Actinopterygii. Cyprinidae) from Caucasus. *Mitochondrial DNA Part A DNA Mapping, Sequencing, and Analysis*, *29*, 49–55.

Maetens, H., Van Steenberge, M., Snoeks, J., & Decru, E. (2020). Revalidation of *Enteromius alberti* and presence of *Enteromius* cf. *mimus* (cypriniformes: Cyprinidae) in the lake Edward system, East Africa. *European Journal of Taxonomy*, *700*, 1–28.

Marques, E. A. G., Vargas, E. d. A., Jr., & Leão, M. F. (2020). Weathering of rocks in Brazil. In M. Kanji, M. He, & L. Ribeiro e Sousa (Eds.), *Soft rock mechanics and engineering* (pp. 251–290). Springer.

Mateo-Sagasta, J., Marjani Zadeh, S., & Turral, H. (2017). *Water pollution from agriculture:* A global review. Executive summary (p. 35). FAO Rome & IWMI Colombo.

Mateo-Sagasta, J., Marjani Zadeh, S., & Turral, H. (2018). *More people, more food, worse water? A global review of water pollution from agriculture.* (p. 224). FAO Rome & IWMI Colombo.

MATTE (Ministère de l'Aménagement du Territoire du Tourisme et de l'Environnement). (2007). *Plan d'action national d'adaptation aux changements climatiques "PANA"*. Bujumbura. https://unfccc.int/resource/docs/napa/bdi01f.pdf

Meyer, J. L., Strayer, D. L., Wallace, J. B., Eggert, S. L., Helfman, G. S., & Leonard, N. E. (2007). The contribution of headwater streams to biodiversity in river networks. *Journal of the American Water Resources Association*, *43*(1), 86–103. https://doi.org/10.1111/j.1752-1688.2007.00008.x

Mukweze Mulelenu, C., Katemo Manda, B., Decru, E., Chocha Manda, A., & Vreven, E. (2020). The *Cyphomyrus* Myers 1960 (Osteoglossiformes: Mormyridae) of the Lufira basin (Upper Lualaba: DR Congo): A generic reassignment and the description of a new species. *Journal of Fish Biology*, *96*(5), 1123–1141. https://doi.org/10.1111/jfb.14237

Negi, R. K., & Mamgain, S. (2013). Species diversity, abundance and distribution of fish community and conservation status of tons river of Uttarakhand State, India. *Journal of Fisheries and Aquatic Science*, *8*(5), 617–626. https://doi.org/10.3923/jfas.2013.617.626

Nelson, J. S., Grande, T. C., & Wilson, M. V. H. (2016). *Fishes of the world* (Fifth ed.). John Wiley & Sons.

Nhiwatiwa, T., Maseko, Z., & Dalu, T. (2017). Fish communities in small subtropical reservoirs subject to extensive drawdowns, with focus on the biology of *Enteromius paludinosus* (Peters, 1852) and *Clarias gariepinus* (Burchell, 1822). *Ecological Research*, *32*(6), 971–982. https://doi.org/10.1007/s11284-017-1504-x

Nichols, J. T., & Griscom, L. (1917). Fresh-water fishes of The Congo basin obtained by the American Museum Congo expedition, 1909–1915. *Bulletin of the American Museum of Natural History*, *37*, 653–756.

Nzigidahera, B. (2006). *Etude de vulnérabilité et d'adaptation aux changements climatiques au Burundi: Ecosystèmes terrestres*. Bujumbura. http://www.abctaxa.be/burundi/implementation/questions-transectorielles/changements-climatiques-et-biodiversite/etude-de-vulnerabilite-et-dadaptation-aux/Ecosystemes__terrestres_et_changements_climatiques1.pdf/download/fr/1/Ecosystemes__terrestres_et_changements_climatiques1.pdf

Nzigidahera, B., & Nindorera, D. (2009). *Plan de gestion et d'amenagement de la Réserve Naturelle de la Malagarazi*. Bujumbura. https://bi.chm-cbd.net/sites/bi/files/2020-04/plan-gestio-malagarazi.pdf

Poll, M. (1933). Contribution à la faune ichthyologique du Katanga. *Annales du Musée du Congo Belge. Zoologie*, *3*(3), 101–152.

Poll, M. (1946). Révision de la faune ichthyologique du lac Tanganyika. *Annales du Musée du Congo Belge. Zoologie*, *4*, 141–364.

Ricardo-Bertram, C. K. (1943). The fishes of the Bangweulu region. *Journal of the Linnean Society of London, Zoology*, *41*(278), 183–217.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution*, *3*(1), 223–225.

Roberts, T. R. (1975). Geographical distribution of African freshwater fishes. *Zoological Journal of the Linnean Society*, *57*(4), 249–319.

Schmidt, R. C., Bart, H. L., & Nyingi, W. D. (2017). Multi-locus phylogeny reveals instances of mitochondrial introgression and unrecognized diversity in Kenyan barbs (Cyprininae: Smiliogastrini). *Molecular Phylogenetics and Evolution*, *111*, 35–43.

Schmidt, R. C., Dillon, M. N., Kuhn, N. M., Bart, H. L., & Pezold, F. (2019). Unrecognized and Imperilled Diversity in an Endemic Barb (Smiliogastrini, *Enteromius*) from the Fouta Djallon Highlands. *Zoologica Scripta*, *48*(5), 605–613. https://doi.org/10.1111/zsc.12362

Schmidt, R. C., Bart Jr, H. L., & Nyingi, W. D. (2018). Integrative taxonomy of the Red-Finned Barb, *Enteromius apleurogramma* (Cyprininae: Smiliogastrini) from Kenya, supports recognition of *E. amboseli* as a valid species. *Zootaxa*, *4482*, 566–578. https://doi.org/10.11646/zootaxa.4482.3.8

Seegers, L. (1996). The fishes of the lac Rukwa drainage. In *Annales du Musée Royal d'Afrique central, Sciences Zoologiques* (p. 287). Belgium.

Seegers, L. (2008). *The catfishes of Africa: A handbook for identification and maintenance*. Aqualog Verlag A.C.S.

Skelton, P. H. (1993). *A complete guide to the freshwater fishes of southern Africa*. Southern Book Publishers.

Skelton, P. H. (2001). *A complete guide to the freshwater fishes of southern Africa*. Struik Publishers.

Skelton, P. H. (2016). Name changes and additions to the Southern African freshwater fish fauna. *African Journal of Aquatic Science*, *41*(3), 345–351.

Skelton, P. H., Tweddle, D., & Jackson, P. B. N. (1991). Cyprinid of Africa. In I. J. Winfield & J. S. Nelson (Eds.), *Cyprinid fishes. Systematics, biology and exploitation* (pp. 211–239). Springer Dordrecht.

Snoeks, J. (2004). *The cichlid diversity of Lake Malawi/Nyasa/Niassa: Identification, distribution and taxonomy*. Cichlid Press.

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: Under water, under threat. An analysis of the status and distribution of freshwater species throughout mainland Africa* (pp. 42–91). IUCN.

Soo, C. L., Nyanti, L., Idris, N. E., Ling, T. Y., Sim, S. F., Grinang, J., Ganyai, T., & Lee, K.-S.-P. (2021). Fish biodiversity and assemblages along the altitudinal gradients of tropical mountainous forest streams.

Scientific Reports, *11*(1), 1–11. https://doi.org/10.1038/s41598-021-96253-3

Srivathsan, A., & Meier, R. (2012). On the inappropriate use of Kimura-2-Parameter (K2P) divergences in the DNA-barcoding literature. *Cladistics*, *28*(2), 190–194.

Statsoft Inc. (2004). STATISTICA (data analysis software system). Version 7.

Stewart, K. M., & Murray, A. M. (2017). Biogeographic implications of fossil fishes from the Awash River, Ethiopia. *Journal of Vertebrate Paleontology*, *37*(1), 1–16. https://doi.org/10.1080/02724634.2017.1269115

Tack, L., Sindayihebura, A., & Cimpaye, D. (1992). The Nkoma (SE Burundi): An episodically reactivated lower Burundian (Middle Proterozoic) siliciclastic sequence, locally overlain by a Malagarasian (Upper Proterozoic) sedimentary breccia. In W. Pohl & J. Klerkx (Eds.), *IGCP Projet 255 'Metallogeny of the Kibara Belt, Central Africa'* (Vol. 4, pp. 31–43). Newsletter/Bulletin.

Thieme, M. L., Abell, R., Stiassny, M. L. J., Skelton, P. H., Lehner, B., Teugels, G. G., Dinerstein, E., Toham, A. K., Burgess, N. D., & Olson, D. (2005). *Freshwater ecoregions of Africa and Madagascar: A conservation assessment*. Island Press.

Turner, G. F. (1999). What is a fish species? *Reviews in Fish Biology and Fisheries*, *9*, 281–297.

USBGN. (1964a). *Kenya: official standard names approved by the United States Board on Geographic Names* (p. 78). Office of Geography, Department of the Interior, Gazetteer no.

USBGN. (1964b). *Burundi: official standard names approved by the United States Board on Geographic Names* (p. 84). Office of Geography, Department of the Interior, Gazetteer no.

USBGN. (1964c). *Republic of the Congo (Léopoldville): official standard names approved by the United States Board on Geographic Names* (p. 80). Office of Geography, Department of the Interior, Gazetteer no.

USBGN. (1965). *Tanzania: official standard names approved by the United States Board on Geographic Names* (p. 92). Office of Geography, Department of the Interior, Gazetteer no.

USBGN. (1972). *Zambia: official standard names approved by the United States Board on Geographic Names* (p. 120). Geographic Names Division, U.S. Army Topographic Command, Gazetteer no.

Van Ginneken, M., Decru, E., Verheyen, E., & Snoeks, J. (2017). Morphometry and DNA barcoding reveal cryptic diversity in the genus *Enteromius* (Cypriniformes: Cyprinidae) from The Congo Basin, Africa. *European Journal of Taxonomy*, *310*, 1–32.

Van Steenberge, M., Vreven, E., & Snoeks, J. (2014). The fishes of the Upper Luapula area (Congo basin): A fauna of mixed origin. *Ichthyological Exploration of Freshwaters*, *24*(4), 329–345.

Vranken, N., Van Steenberge, M., & Snoeks, J. (2020). Similar ecology, different morphology: Three new species of oral-Mollusc shellers from Lake Edward. *Journal of Fish Biology*, *96*(5), 1202–1217. https://doi.org/10.1111/jfb.14107

Vreven, E. J., & Teugels, G. G. (2005). Redescription of *Mastacembelus liberiensis* Boulenger, 1898 and description of a new west African spiny-eel (Synbranchiformes: Mastacembelidae) from the Konkoure River Basin, Guinea. *Journal of Fish Biology*, *67*(2), 332–369. https://doi.org/10.1111/j.0022-1112.2005.00729.x

Vreven, E. J. W. M. N., Musschoot, T., Decru, E., Wamuini Lunkayilakio, S., Obiero, K., Cerwenka, A. F., & Schliewen, U. K. (2019). 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*(2), 414–482. https://doi.org/10.1093/zoolinnean/zly049

Waleffe, A. (1965). Etude géologique du Sud-Est du Burundi (Régions du Mosso et du Nkoma). Musée Royal de l'Afrique Centrale, Annales, Série n° 8, Sciences Géologique, 48.

Wiens, J. J. (2007). Species delimitation: New approaches for discovering diversity. *Systematic Biology*, *56*(6), 875–878.

Winemiller, K. O., Agostinho, A. A., & Caramaschi, E. P. (2008). Fish ecology in tropical streams. In D. Dudgeon (Ed.), *Tropical stream ecology* (pp. 107–146). Academic Press.

Yang, L., Sado, T., Hirt, M. V., Pasco-Viel, E., Arunachalam, M., Li, J., Wang, X., Freyhof, J., Saitoh, K., Simons, A. M., Miya, M., He, S., & Mayden, R. L. (2015). Phylogeny and polyploidy: Resolving the classification of cyprinine fishes (Teleostei: Cypriniformes). *Molecular Phylogenetics and Evolution*, *85*, 97–116. https://doi.org/10.1016/j.ympev.2015.01.014

Zengeya, T., Decru, E., & Vreven, E. (2011). Revalidation of *Hepsetus cuvieri* (Castelnau, 1861) (Characiformes: Hepsetidae) from the Quanza, Zambezi and Southern part of The Congo ichthyofaunal provinces. *Journal of Natural History*, *45*(27–28), 1723–1744. https://doi.org/10.1080/00222933.2011.560724

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