diversity

MDPI

Article

Two New Shellear Species (Gonorhynchiformes: Kneriidae), from the Luansa River (Upper Congo Basin): Hidden Diversity Revealed by Integrative Taxonomy

Lewis Ngoy Kalumba [1], Emmanuel Abwe [1,2,3], Frederic D. B. Schedel [4,5,6], Auguste Chocha Manda [1], Ulrich K. Schliewen [6,*] and Emmanuel J. W. M. N. Vreven [2,3,7]

1 Unité des Recherches en Biodiversité, Gestion et Exploitation Durable des Zones Humides, Faculté des Sciences Agronomique, Université de Lubumbashi, Lubumbashi BP 1825, Democratic Republic of the Congo; lewiskalumba1@gmail.com (L.N.K.); Abwe.emmanuel@Unilu.ac.cd (E.A.); augustechocha@gmail.com (A.C.M.)
2 Ichthyology, Vertebrate Section, Royal Museum for Central Africa, Leuvensesteenweg 13, B-3080 Tervuren, Belgium; emmanuel.vreven@africamuseum.be or emmanuel.vreven@kuleuven.be
3 Research Group of Fish Diversity and Conservation, Biology Department, KU Leuven, Charles Deberiotstraat 32, B-3000 Leuven, Belgium
4 Zoological Institute, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland; frederic.schedel@unibas.ch or schedelfred@hotmail.de
5 Division of Evolutionary Biology, Faculty of Biology, Ludwig-Maximilians-Universität Munich, Großhaderner Straße 2, 82152 Planegg-Martinsried, Germany
6 Department of Ichthyology, Bavarian State Collection of Zoology, Staatliche Naturwissenschaftliche Sammlungen Bayerns, Münchhausenstr. 21, 81247 München, Germany
7 National Research Foundation-South African Institute for Aquatic Biodiversity (NRF-SAIAB), Makhanda P.O. Box 94, South Africa
* Correspondence: schliewen@snsb.de

Citation: Kalumba, L.N.; Abwe, E.; Schedel, F.D.B.; Chocha Manda, A.; Schliewen, U.K.; Vreven, E.J.W.M.N. Two New Shellear Species (Gonorhynchiformes: Kneriidae), from the Luansa River (Upper Congo Basin): Hidden Diversity Revealed by Integrative Taxonomy. *Diversity* **2023**, *15*, 1044. https://doi.org/10.3390/d15101044

Academic Editors: Phillip Lobel and Luis Moreira Da Costa

Received: 11 April 2023
Revised: 8 August 2023
Accepted: 13 August 2023
Published: 28 September 2023

Abstract: Two new *Kneria* species, *K. luansaensis* sp. nov. and *K. maxi* sp. nov., are described from the Luansa River, a left bank tributary of the lower Luapula in the Bangweulu–Mweru ecoregion, based on an integrative approach using morphological and COI barcoding evidence. While *K. luansaensis* sp. nov. occurs from the source of the Luansa further downstream to above the last of the three Sanshifolo Falls, *K. maxi* sp. nov. only occurs downstream of all these three major falls. In *Kneria*, males of about ≥33 mm L_S have an opercular and a postopercular organ. The number of lamellae on the latter seems to contain some alpha-taxonomic information, although this requires further study as allometric changes occur at about ≤45 mm L_S. Additional external morphological characters differ between sexes, i.e., the (i) pectoral fin width (wider in males than females), (ii) dorsal fin height (longer in males than females), and (iii) length of the longest ray of the lower caudal fin lobe (longer in males than females). Agriculture, fishing with ichthyotoxines, and logging are the most pressing threats on the Luansa and thus to both the new species. Their discovery in one of the rivers of the Kundelungu Plateau and its surroundings located outside the present-day boundaries of the Kundelungu National Park highlights the need for a refined and improved protection strategy for this freshwater key biodiversity area.

Keywords: COI barcoding; *Kneria luansaensis* sp. nov.; *K. maxi* sp. nov.; kundelungu national park; kundelungu plateau; morphological approach; opercular/postopercular organ; protection; sexual dimorphism; waterfalls

1. Introduction

The Kneriidae are freshwater fishes endemic to Africa [1–4]. Based on their external morphology, members of this family are characterised by having a subterminale mouth, a protruding upper jaw, a lateral line, and cycloid scales present in some of its representatives only [5–7]. To date, the family contains 31 valid species divided over the four present-day

genera [8]. The genus *Kneria* Steindachner, 1866 (13 species) [Supplementary Materials (SM): Table S1] is the second most species–rich genus after *Parakneria* Poll, 1965 (15 species), which are both clearly more species–rich than the two remaining genera, *Cromeria* Boulenger, 1901 (two species), and *Grasseichthys* Géry, 1964 (monospecific at present; but, possibly containing a second species) [9].

The genus *Kneria* was erected in the original description of *Kneria angolensis* Steindachner, 1866 from Angola, and the species itself was designated type species of the genus by monotypy [10], i.e., it being the only species attributed to this new genus. As the description of *K. angolensis* was based on a single female specimen, it lacks both the opercular and postopercular organs [11]. Instead, based on the presence of both the opercular and postopercular organs, Pellegrin [12] described the genus *Xenopomichthys*, with *K. auriculata* from the Muza River, a left bank subtributary of the Buzi, the latter a coastal basin draining to the Indian Ocean (Mozambique), as type species; this by monotypy as well. Both of these organs were, based on observations made by Poll [13], identified as sexual dimorphic and occurring in males only, thus rendering the genus *Xenopomichthys* a junior synonym of the genus *Kneria* [14]. The opercular organ consists of a circular adhesive disc located on the opercular bone and having a well-developed reinforcement in adult males [11]. The postopercular organ, instead, is an oval, striated organ, formed by a dermal thickening bearing differentiated scales constituting a series of lamellae [15]. Males apparently use this apparatus to attach themselves to females, at least during prespawning behaviour. However, its exact function, such as its possible use during the spawning act, remains unknown [16].

Kneria have a slightly depressed body [17]. The dorsal fin is situated behind or above the pelvic fins and the anal fin is closer to the pelvic fins than to the caudal [18,19] or intermediate to both [20]. The head is depressed and wide, and the snout narrow and pointed. The mouth is inferior with a cutaneous fold, forming a wide lower lip with a keratinised sharp rim. The eyes are in a lateral position, being visible both from above as well as from below the head [15]. The lateral line is curving downwards behind the operculum at the pectoral level in species of the genus *Kneria*, a character distinguishing it from the species of the genus *Parakneria*, whose lateral line is more or less straight [16,21].

To date, nine valid species [22] are known from the Congo Basin sensu lato (s.l.) (SM: Table S1), i.e., including Lake Tanganyika and its tributary rivers as well as the Lake Kivu Basin [23]. Only four of these species were originally described from the Congo Basin s.l. (SM: Table S1), i.e., *K. katangae* Poll, 1976, *K. paucisquamata* Poll and Stewart, 1975, *K. stappersii* Boulenger, 1915, and *K. wittei* Poll, 1944. Further, four more species, i.e., *K. angolensis*, *K. ansorgii*, *K. auriculata,* and *K. polli* were reported from the Congo Basin. However, in 1976, Poll [20] already reported that none of these four species are to be found in the Congo Basin s.l., while in 1984, he stipulated that *K. auriculata* is [2]. Nevertheless, later, Seegers [16] and Skelton [19] even questioned the presence of that last one in the Congo Basin s.l.

Kneria wittei was described from the Lukuga Basin, the outlet of Lake Tanganyika, at Makala Village [18]. It was also reported from some rivers draining the Kundelungu Plateau (KP), including some of the Kundelungu National Park (KNP) and from the Biano and Kibara plateaus in the Upemba National Park (UNP) [20]. However, in 1976, Malaisse [24–26] and Poll [20] indicated that, all *Kneria* populations from the Luansa River above and below the waterfalls are *K. wittei.* This makes *K. wittei* the only species reported from the lower Luapula, i.e., from below the Mambilima Rapids [27].

Different recent expeditions (2012–2018) were organised to study the fish diversity of the KNP (Figure 1). In 2014, 2016, and 2017, a total of six *Kneria* populations were sampled in the Luansa River (Luansa is the phonetically correct spelling following its appellation in Lamba, a Bantu language spoken by the autochthonous inhabitants of Kabyashya, although referred to as Luanza by Malaisse [24]).

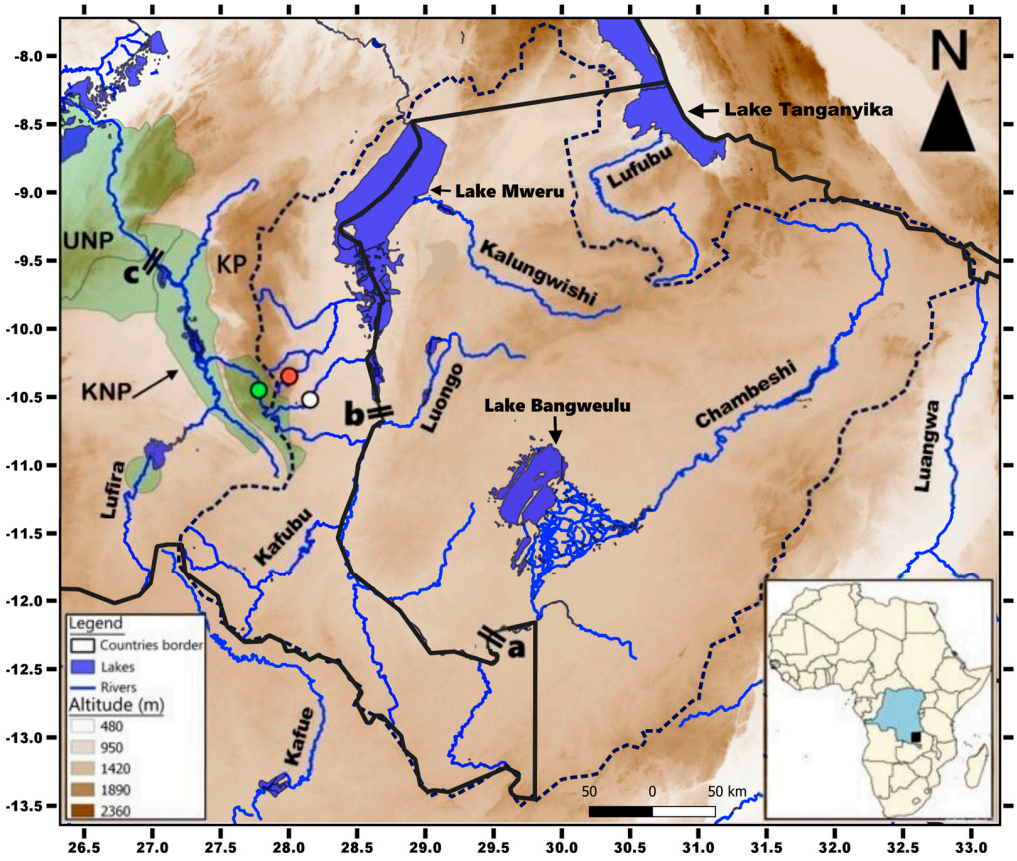

Figure 1. The Bangweulu–Mweru (B–M) ecoregion (ECR) (dotted line) showing ◉, the Luansa River [(eastern side of the Kundelungu Plateau (KP)] amongst its neighbouring basins in the ecoregion such as ◉, the Lofoi River (western side of the KP), and ○ the Lutshipuka River (eastern side of the KP). (a) Mumbatuta Falls, (b) Mambilima Rapids, and (c) Kyubo Falls. KNP: Kundelungu National Park, KP: Kundelungu Plateau, and UNP: Upemba National Park.

However, the different colour patterns of specimens originating from six different, recently sampled, localities along the Luansa do not correspond to those described for *K. wittei* [28]. Indeed, an integrative study, based on colour and colour pattern (qualitative), meristic and measurement (quantitative), and genetic (COI mtDNA) data, confirmed that none of these populations can be assigned to *K. wittei*, nor to any other known *Kneria* species from the Bangweulu–Mweru (B–M) and the Upper Lualaba (UL) ecoregions (ECR; sensu Abell et al. [29]). Instead, all six sampled populations correspond to two new species for science, with the specimens from the five most upstream populations here named *K. luansaensis* sp. nov., and those from the most downstream populations only here named *K. maxi* sp. nov. As a result, the need for improved aquatic conservation/protection in the region is discussed as the Luansa River, draining part of the KP and its surroundings, is affected by several anthropogenic impacts. These are worrying, as they not only threaten both new species, but its aquatic fauna as a whole, mainly because the major part of the Luansa Basin is located outside the KNP.

2. Material and Methods

2.1. Sampling Methods

Specimens were collected during expeditions organised in 2014, 2016, and 2017. International and national recommendations for fish handling and ethical standards were considered [30]. Fish were caught using two fishing methods: (i) rotenone, as authorised for scientific sampling based on article 20 of the Democratic Republic of the Congo (DRC) of the nature conservation law of February 2014, while referring also to article 30 of the DRC ordinance of 18 January 1958, and (ii) hand net fishing. All specimens were anaesthetised using clove oil before identification, photography, and fixation in accordance to the European Directive 2010/63/EU following the guidelines from the Animal Ethics Committee of the KU Leuven (Belgium). Fish were tagged and a fin clip of the pelvic, or, sometimes, of the pectoral fin, was taken from the right side and kept in alcohol 96% for genetic analysis. Thereafter, fish were fixed in 10% formaldehyde before being transferred to 70% ethanol for long-term storage at the Royal Museum for Central Africa (RMCA) or the SNSB-Bavarian State Collection of Zoology (ZSM).

2.2. Morphological Approach

A total of 167 specimens were examined in full detail. These include 131 newly collected specimens from six populations along the Luansa River (Figures 1 and 2). From up-to downstream, these are: 37 specimens [19 males (M) and 18 females (F)] from the main channel, on the Kundelungu Plateau (KP), above Kasompola Falls; 30 (15 M and 15 F) from the Milembwe River, a left bank tributary of the Luansa, on the KP, above Kasompola Falls; 10 (5 M and 5 F) from the main channel above the first Sanshifolo Falls; 25 (12 M and 13 F) from the main channel above the second Sanshifolo Falls; 10 (5 M and 5 F) from the main channel above the third Sanshifolo Falls; and finally, 19 (10 M and 9 F) from the main channel downstream of all falls. Further, these also include 27 specimens (11 M and 16 F) of *K. wittei* collected from the rivers of the Lukuga Basin near Makala Village, type locality of *K. wittei* (SM: Table S1), and include the holotype (F). Finally, these also include 9 specimens (3 M and 6 F) of *K. stappersii* collected from the Lubumbashi River and its tributaries. This species, originally described and known from the middle Luapula (SM: Table S2), is morphologically highly similar to the species found downstream of all the falls on the Luansa River but geographically separated by the Mambilima Rapids, delimitating the middle from the lower Luapula [27]. For more details, see comparative material studied and SM Table S2. All locality data were translated into English. Following the current RMCA policy, collection numbers were renamed with former RMCA numbers A0 up to A9 now being listed as RMCA 2000–2009 and B0 up to B6 as RMCA 2010–2016.

In addition, for 101 more males, the standard length (L_S), the tubercle/lamellar structure of the opercular organ, and the number of lamellae in the postopercular organ were documented as to further critically evaluate the species specificity of the last two characters. These specimens included: (i) 37 *K.* sp. 'lua-upstream' (=*K. luansaensis* sp. nov.), of which five of *K.* sp. 'lua-upstream' IntP1, seven of *K.* sp. 'lua-upstream' IntP2, and 25 of *K.* sp. 'lua-upstream' IntP3; (ii) one of *K.* sp. 'lua downstream' (=*K. maxi* sp. nov.); (iii) 13 of *K. stappersii*; and (iv) 50 of *K. wittei*.

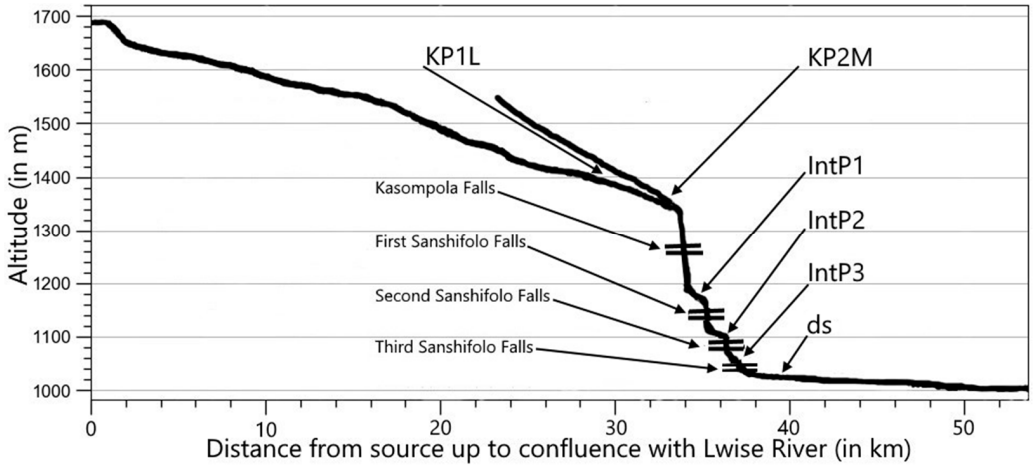

Figure 2. Longitudinal profile of the Luansa River, a right bank tributary of the Lwise, up to its confluence with the latter. (i) The major falls, with their names (left side), and (ii) the six sampling sections, with their acronym (right side) from up-to downstream, are indicated as follows: KP1L (section i): first locality on the Kundelungu Plateau (KP), in the Luansa River (L) itself; KP2M: second locality on the KP, in the Milembwe River (M), its left bank tributary; IntP1: intermediate plateau 1; IntP2: intermediate plateau 2; IntP3: intermediate plateau 3; and ds: downstream of all falls.

For both new species, only specimens studied in full detail were selected as part of the type series; all other specimens are listed as "additional non-type material examined", instead. Further, for the holotype of both new species, a male specimen was chosen, even if the holotype of *K. wittei* is a female. The main reason for this is that both the opercular and postopercular organs are present in males only, and bear additional meristic and morphometric characters that are possibly diagnostic, i.e., potentially separating species.

Ten meristic counts were taken following Poll [20]. Among these, nine were taken from both sexes, except for the number of lamellae in the postopercular organ, as only males have this organ.

Thirty-four measurements were also taken, 31 from both sexes, and three additional ones related to the presence of the opercular organ, from males only. The last ones, as described by Abwe [28], are: (i) the outer diameter of the opercular organ; (ii) the inner diameter of the opercular organ; and (iii) the postopercular organ length (SM: Figure S1). For the remaining measurements, eight or ten were taken on the head of female and male specimens, respectively; and 23 and 24, respectively, from the body (SM: Figure S1). Sixteen of these measurements were taken as described by Poll [20] (SM: Figure S1a: n°4, 6, 8, and 21; SM: Figure S1b: n°13, and 23–26; and SM Figure S1c: n°1–3, 5, 7, 9, 20, and 25), and three as by Seegers [16] (SM: Figure S1c: n°17, 18, and 22). Finally, the 15 remaining measurements were taken as described by Abwe [28] (SM: Figure S1b: n°10, 11, 12, 14, 16; SM: Figure S1c: n°19, 27, 28, 29, 31, 32, 30, 33, and 34). All measurements were taken with digital callipers to the nearest 0.01 mm on the left side of the specimens, except if this side was damaged. Counts and measurements were carried out under a stereomicroscope.

Data were explored and analysed using principal component analyses (PCAs). Meristics and measurements were analysed separately, with the correlation matrix used for the PCA on the raw meristics and the covariance matrix for the PCA on the log-transformed measurements [31,32]. Statistical analyses were executed using Past Software (Paleontological statistics) 3.10 [33] and Statistica 8.0, for PCA as a multivariate approach, and Mann-Whitney U (MWU) tests as a univariate approach, respectively. Being invariable, the numbers of simple and branched dorsal fin rays, simple and branched caudal fin rays, and simple anal fin rays, were not included in the PCAs. For the PCAs of meristics, the scores on the first (PCI) and the second axis (PCII) were considered. For the PCAs of the log-transformed measurements, however, the first axis (PCI) of the PCA can be interpreted

as a proxy for size, and therefore, the second (PCII) and third axes (PCIII) were used to explore presumably size-independent variation [32].

Further, non-parametric MWU-tests were used for the univariate comparison of raw meristics and relative measurements (percentages) [34–36], with sequential Bonferroni corrections applied to correct for multiple comparisons [37]. MWU-tests were only performed on sample sizes of n > 4 specimens. Further, only specimens of a similar length class (L_S: p value > 0.5) were used to minimise allometric effects [38]. Finally, the diagnostic value of the studied characters was inferred by using simple scatterplots of the measurements, in percentage, against head length (L_H), for measurements taken on the head, or against standard length (L_S), for measurements taken on the body.

To explore for possible sexual dimorphism, meristics and measurements were first analysed separately for females and males, and only subsequently for both sexes together. As such, females were first compared among each other, because the holotype of *K. wittei* is a female, and so far, *K. wittei* is the only *Kneria* species reported from the lower Luapula Basin to which the Luansa belongs. Thus, in a first PCA, sexes were analysed separately to search for species-specific differences, and in the second PCA, counts and measurements were analysed for both sexes together to identify patterns of secondary sexual dimorphism beyond the presence/absence of the (post)opercular organ. To enable direct comparison between sexes, only nine counts and 31 measurements were used in the second PCA approach on the meristics and the measurements, respectively.

To explore the possible influence of seasonality on the development of the lamellae in the opercular and the postopercular organ, sampling dates were partitioned according to five different seasons originally defined based on phenological observations of the vegetation [39]: cold dry season (C–DS: May–July), hot dry season (H–DS: August–September), early rainy season (October–November), peak of the rainy season (P–RS: December–February), and late rainy season (L–RS: March–April).

2.3. Genetic Approach

2.3.1. Taxonomic and Nucleotide Sampling

The genetic analyses conducted in this study are based on complete mitochondrial cytochrome c oxidase I (COI) sequences extracted from partial mitochondrial genomes (SM: Table S3).

In total, 94 COI sequences were obtained; these from 83 *Kneria* specimens (10 from the Luansa River) and 11 additional ones representing the three more extant genera of the family Kneriidae sensu stricto (s.s.) (see [40]), and two other Gonorhynchiformes genera as out-groups (SM: Table S3). *Phractolemus* and *Chanos* were chosen as out-group taxa based on previous studies, as the former is the sister group of Kneriidae s.s. and the latter is the sister group of Kneriidae s.l., e.g., [3,7,41] (for details see: SM: Table S3).

The newly sequenced *Kneria* specimens include three valid species, i.e., *K. stappersii* (n = 5) and *K. wittei* (n = 2), both from the Congo Basin and from their type localities, and *K. uluguru* (n = 2) from its type basin in eastern Africa. The two valid Congo Basin species included are those (i) that are morphologically most similar to the Luansa populations, and (ii) that are also the ones for which genetic samples were readily available.

In addition to specimens from the Luansa River, specimens from (i) neighbouring rivers on the KP, such as the Lofoi and the Lutshipuka (see Figure 1), as well as the Masansa and Musipasi, both tributaries of the latter; and (ii) a large number of populations from the rivers of the upper Congo (UC) Basin [42], were also sequenced. All newly generated sequences were deposited in GenBank (GenBank accession numbers MN594176 to MN594258; SM Table S3). Paragenetype sequences were labelled following the definition of Chakrabarty [43]. Finally, eleven COI sequences extracted from full mitogenome sequences were downloaded from GenBank (see SM Table S3). These include sequences from six different genera, i.e., *Kneria* (n = 5), *Cromeria* (n = 2), *Chanos* (n = 1), *Grasseichthys* (n = 1), *Parakneria* (n = 1), and *Phractolemus* (n = 1).

2.3.2. Molecular Methods

The DNA extractions were conducted at the molecular laboratory of the SNSB-Bavarian State Collection of Zoology (ZSM) in Germany. These were carried out using the standard cetyl-trimethyl-ammonium-bromid (CTAB) extraction protocol including an RNAase treatment step [44]. DNA concentrations of the resulting extraction products were measured using a spectrophotometer (NanoDrop ND-1000; Thermo Scientific, Waltham, MA, USA) and subsequently adjusted to a total volume of 25 ng/µL. The following pair of long-range PCR primers: KneriaA1985F: 5′-GGC AAA CAC CTA AAG CCT CTG TTT ACC AAA AAC-3′ (front primer) and GeneralBLR-30R: 5′-CCT TCG ATC TCC GGT TTA CAA GAC TGG TGC-3′ (back primer) were used for amplification of a large fraction [~13,000 base pairs (bp)] of the mitochondrial genome. These primers were designed in the Geneious v.10.2.2 program [45] based on the aforementioned mitochondrial genome sequences obtained from GenBank. The COI sequences used in this study were extracted from the large ~13,000 bp mitochondrial fragment. Long-range PCR, purification of amplification products, preparation of the Illumina MiSeq libraries, individual library assembly, and sequence editing (quality control, and cutting and assembly of the adapters) followed [46]. Assembled sequences (average sequence length: 13,000 bp) were annotated in Geneious v.7.05 [46], using one annotated reference sequence of *Kneria* with a minimum reference similarity of 75%.

2.3.3. DNA–Sequence Edition and Phylogenetic Analysis

Since only the COI gene was used for downstream phylogenetic analysis because of its widespread application and comparative DNA–sequence availability in fish DNA–barcoding studies, this gene was extracted from the full set of 13 protein coding genes of the mitochondrial genome prior to the in-silico extraction of COI, and the mitogenomic data were processed following different steps explained below.

The total length of the COI alignment, of 94 specimens, was 1551 bp. The COI–alignment was exported in (i) relaxed Phylip format and (ii) Nexus format for downstream phylogenetic analysis.

A maximum likelihood (ML) analysis was performed on the COI alignment using the RAxML v.8.0.24 program [47] on the CIPRES V.3.3 science platform [48] and the GTR + I + Γ model as implemented on CIPRES. Prior to the analyses, the surrogate model that best matched the data was estimated using the jModeltest v.2.1.5 + program [49] using the Akaike Information Criterion (AIC). Bootstrap replications were stopped automatically after 500 RAxML replications (using the majority rule criterion). In addition, the dataset was partitioned into first, second, and third codon position. *Chanos chanos*, *Phractolemus ansorgii*, *Cromeria nilotica*, *Cromeria occidentalis*, *Grasseichthys gabonensis*, and *Parakneria cameronensis* were defined as out-group taxa based on previous studies e.g., [3,7,41].

Genetic distances were calculated in MEGA 7.0.26 [50] as Kimura-2-parameter (K2P) distances [51] and as uncorrected p-distances [52], and are in %. The use of K2P- and uncorrected p-distances were necessary as K2P-distances tend to exaggerate species delimitation based on closely related sequences, whereas the p-distance does not account for multiple mutation hits in strongly divergent sequences (see [52]). Nevertheless, since both distance values are routinely reported in DNA–barcoding studies, both are presented to enable direct comparison with other studies (see also [53,54]). The number of base substitutions per site averaged over all sequence pairs within each population and between sequences is shown. This analysis involved 61 sequences. Codon positions included were 1st + 2nd + 3rd. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There are a total of 1551 bp in the final dataset.

2.4. Nomenclatural Acts

According to the International Code of Zoological Nomenclature (ICZN) as currently amended, the names of the two new species herein described are available under this code from the electronic edition of this article. The published work and its nomenclatural acts were therefore registered in the ICZN online registration system: ZooBank. The ZooBank Life Science Identifier (LSID) is available and the associated information can be accessed through any standard web browser by appending the LSID to the prefix http://zoobank.org/, accessed on 16 March 2023. Therefore, the LSID of the present publication is: urn: lsid: zoobank.org: pub: D4E41B0B-FF46-4B94-A2C8-C0A58E9B6C19.

3. Results

3.1. Morphological Explorations

3.1.1. Variation in the Lamellar Structure of the Opercular (OP) and Postopercular (POP) Organ in Males

The tubercles or lamellae of the opercular (T/LOP) organ occur as three different types (SM: Figure S2): (1) without tubercles or lamellae; (2) with tubercles towards its outer edge; and (3) with lamellae towards its outer edge. Visualisations of their occurrence in the six studied populations from the Luansa and for two comparative species are given from about 32.0 mm L_S onwards, which is the first size at which both the opercular and postopercular organs appear, in males only (Figure 3; and SM Table S4).

Two observations are noteworthy with regard to presence/absence of tubercles or lamellae in the opercular organ (Figure 3a–g; and SM Table S4). First, tubercles or lamellae presence/absence apparently reflects seasonal variation: there are none during the peak and late rainy season (P- and L-RS) (Figure 3g), but they rather appear during the hot dry season (H-DS) (Figure 3a,f). Thus, it is likely that the hot dry season is the reproduction one (see discussion below). Second, if these structures are present, there is nevertheless an overall large variation in the development of these structures detectable, even in specimens of similar size (e.g., Figure 3a,d,f,g: H-DS).

Further, three additional major observations can be made regarding the number of lamellae of the postopercular organ (Figure 4a–h; and SM Table S4). First, lamellae are always present in the postopercular organ from a certain size onwards, i.e., from 37.5 mm L_S in *K. stappersii* (Figure 4g), from 32.2 mm L_S in *K. wittei* (Figure 4h), and from 35.7 mm L_S in the populations of the Luansa (Figure 4). Thus, their presence/absence does not depend upon the season, but rather upon the size (age) of the studied specimens (Figure 4a–h). Second, the number of lamellae in the postopercular organ increases with size (positive allometric) in some populations, this within a restricted size range only. From a certain size on, the numbers seem to be stable (see Figure 4a,f,h). Third, in some populations, this positive allometry seems absent. This might be an artefact of the limited size class of individuals available for study (Figure 4c). However, this does not seem to be the case for two of the six studied Luansa populations either (IntP2 and 3: Figure 4d,e), which have sufficiently large size classes.

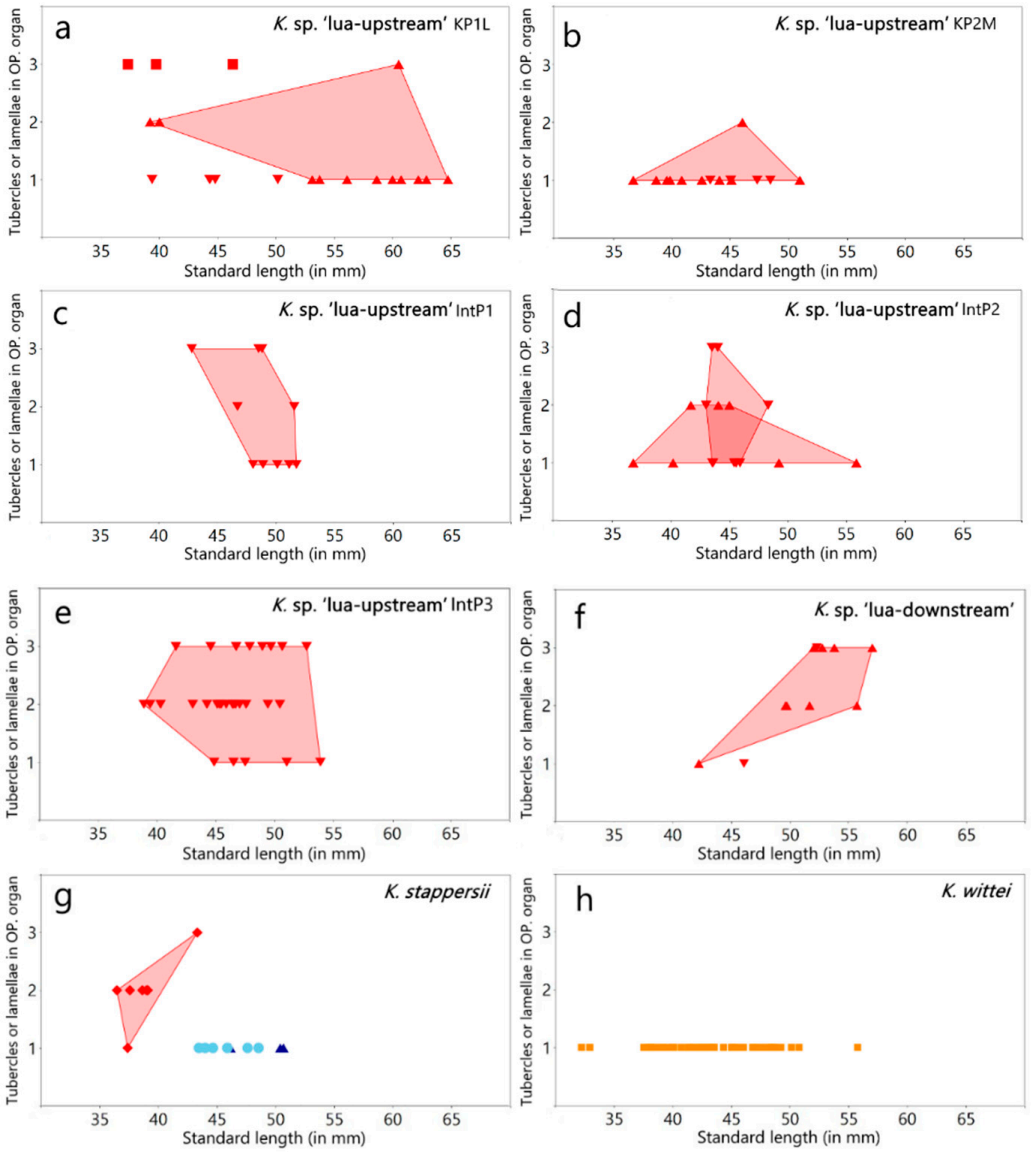

Figure 3. Scatterplot of the coded presence of tubercles or lamellae in the opercular organ (1: opercular organ smooth, 2: with tubercles, and 3: with lamellae, see SM Figure S2 and SM Table S4), against standard length (in mm), and this for six populations from the Luansa (**a**–**f**): up-to downstream) and both comparative species, *K. stappersii* and *K. wittei* (**g**–**h**), and according to collecting season. *Kneria* population of the Luansa River sections (**a**) *K.* sp. 'lua-upstream' KP1L (=*K. luansaensis* sp. nov.): ■ (H-DS: 19 September 2014), ▲ (H-DS: 20 August 2016), and ▼ (H-DS: 21 September 2017); (**b**) *K.* sp. 'lua-upstream' KP2M (=*K. luansaensis* sp. nov.): ▲ (H-DS: 20August 2016), and ▼ (H-DS: 21 September 2017); (**c**) *K.* sp. 'lua-upstream' IntP1 (=*K. luansaensis* sp. nov.): ▼ (H-DS: 20 September 2017); (**d**) *K.* sp. 'lua-upstream' IntP2 (=*K. luansaensis* sp. nov.): ▲ (H-DS: 23 August 2016), and ▼ (H-DS: 20 September 2017); (**e**) *K.* sp. 'lua-upstream' IntP3 (=*K. luansaensis* sp. nov.): ▼ (H-DS: 20 September 2017); (**f**) *K.* sp. 'lua-downstream' (=*K. maxi* sp. nov.): ▲ (H-DS: 22 August 2016), and ▼ (H-DS: 19 and 21 September 2017). Of the two comparative species (**g**) *K. stappersii*: ◆ (H-DS: August 1935), ▲ (P-RS: 2 January 2016), and ● (L-RS: 4 March 2020); and (**h**) *K. wittei*: ■ (C-DS: 07 June and 30 June 2015). Luansa populations studied (up-to downstream) (see also Figure 2): KP1L = Luansa River on KP 1; KP2M = Milembwe tributary river on KP 2; IntP1 = intermediate plateau 1; IntP2 = intermediate plateau 2; and IntP3 = intermediate plateau 3. Seasonality: ds = dry season; and rs = rainy season; c = cold ds (orange symbols); h = hot ds (red); l = late rs (light blue); and P = peak rs (dark blue).

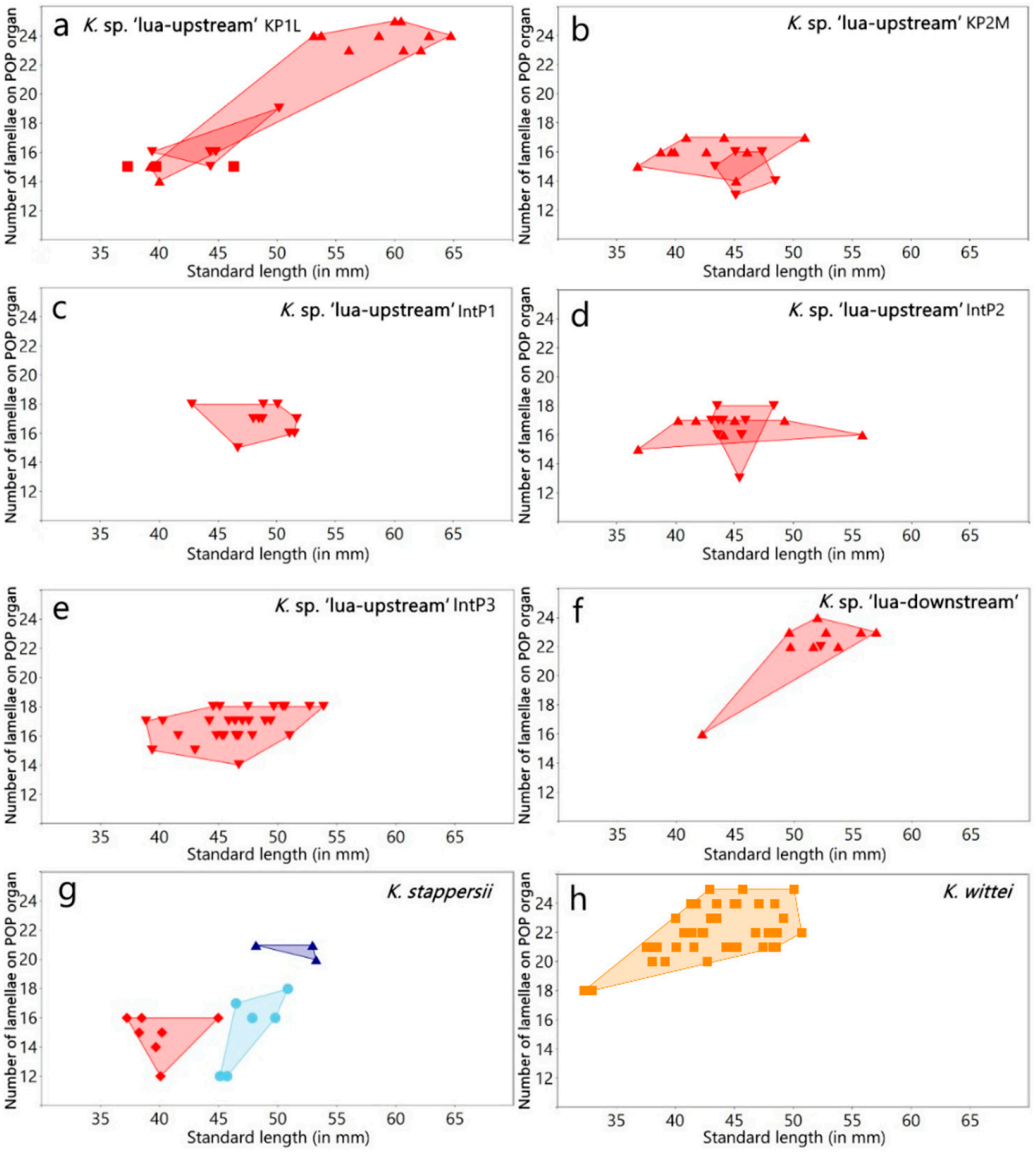

Figure 4. Scatterplot of the number of lamellae on the post opercular (POP) organ against standard length (in mm), and this for the six populations of the Luansa (a–f: up-to downstream) and both comparative species, *K. stappersii* and *K. wittei*, and according to collecting season. The *Kneria* population of the Luansa River sections (**a**) *K.* sp. 'lua-upstream' KP1L (=*K. luansaensis* sp. nov.): ■ (H–DS: 19 September 2014), ▲ (H–DS: 20 August 2016), and ▼ (H–DS: 21 September 2017); (**b**) *K.* sp. 'lua-upstream' KP2M (=*K. luansaensis* sp. nov.): ▲ (H–DS: 20 August 2016), and ▼ (H–DS: 21 September 2017); (**c**) *K.* sp. 'lua-upstream' IntP1 (=*K. luansaensis* sp. nov.): ▼ (H–DS: 20 September 2017); (**d**) *K.* sp. 'lua-upstream' IntP2 (=*K. luansaensis* sp. nov.): ▲ (H–DS: 23 August 2016), and ▼ (H–DS: 20 September 2017); (**e**) *K.* sp. 'lua-upstream' IntP3 (=*K. luansaensis* sp. nov.): ▼ (H–DS: 20 September /2017); (**f**) *K.* sp. 'lua-downstream' (=*K. maxi* sp. nov.): ▲ (H–DS: 22 August 2016), and ▼ (H–DS: 19 and 21 September 2017); and of the two comparative species (**g**) *K. stappersii*: ◆ (H–DS: August 1935), ▲ (P–RS: 02/01/2016), and ● (L–RS: 04 March 2020); and (**h**) *K. wittei*: ■ (C–DS: 7/ June and 30 June 2015). Luansa populations studied (up-to downstream) (see also Figure 2): KP1L = Luansa River on KP 1; KP2M = Milembwe tributary river on KP 2; IntP1 = intermediate plateau 1; IntP2 = intermediate plateau 2; and IntP3 = intermediate plateau 3. Seasonality: ds= dry season; and rs = rainy season; c= cold ds (orange symbols); h = hot ds (red); l = late rs (light blue); and P = peak rs (dark blue).

3.1.2. Qualitative Observations

The colour pattern of the Luansa populations and of the two comparative species was studied, in particular with regard to: (i) variation/differences in colour pattern within the same population/species, i.e., between sexes and stages of sexual maturity, and (ii) differences in colour pattern between the different (potential) species studied.

Colouration and Colour Pattern Variation within a Single Population

The case of the *Kneria* population of *K.* sp. 'lua-upstream' IntP2, one of the two most upstream populations, is presented here because (i) specimens are available for two consecutive years (2016–2017), and (ii) some intra-population colouration and colour pattern variation were observed for the specimens collected in September 2017 (end of the dry season just before the onset of the first rains of the rainy season: l–ds), at least (Figure 5). However, (i) no important colouration and colour pattern differences were observed among specimens collected in August 2016 (mid dry season, well before the first rains of the rainy season: m–ds). Rather, all are characterised, in life, by a light sandy–brown background colour and mainly two to five horizontal dark brown or even black bands, one being a broad medio-dorsal one, and two lateral ones at the level of the lateral line and below this level; this in specimens of both sexes (Figure 5: a1, male, and a2, female). In contrast, (ii) a more yellowish background colour characterises one male and two females collected in September 2017 (Figure 5: b1 vs. b2–b3). Only the gravid female had horizontal dark brown or even black bands (Figure 5: b2). As a result, the gravid female appears much darker in overall colour as compared to the two other specimens (Figure 5 b1 vs. b3). It is to be noted that these lighter versus darker (for gravid females only) colour patterns were observed on both live and preserved specimens. Gravid females also feature two prominent, dark brown, oblique bands each one at the base of the two caudal fin lobes, as is also the case with non-gravid females.

The specimens of the five upstream populations of the Luansa, i.e., from upstream of the third Sanshifolo Falls and equally caught in September 2017, all feature a similar colour pattern variation (see SM Figure S3 for preserved specimens of the *K.* sp. 'lua-upstream' KP1L). The other populations/species studied, i.e., the downstream population of the Luansa, and those of *K. wittei* and *K. stappersii*, do not present this intra-populational colour pattern variation. However, this might be due to the fact that no gravid females were collected from those.

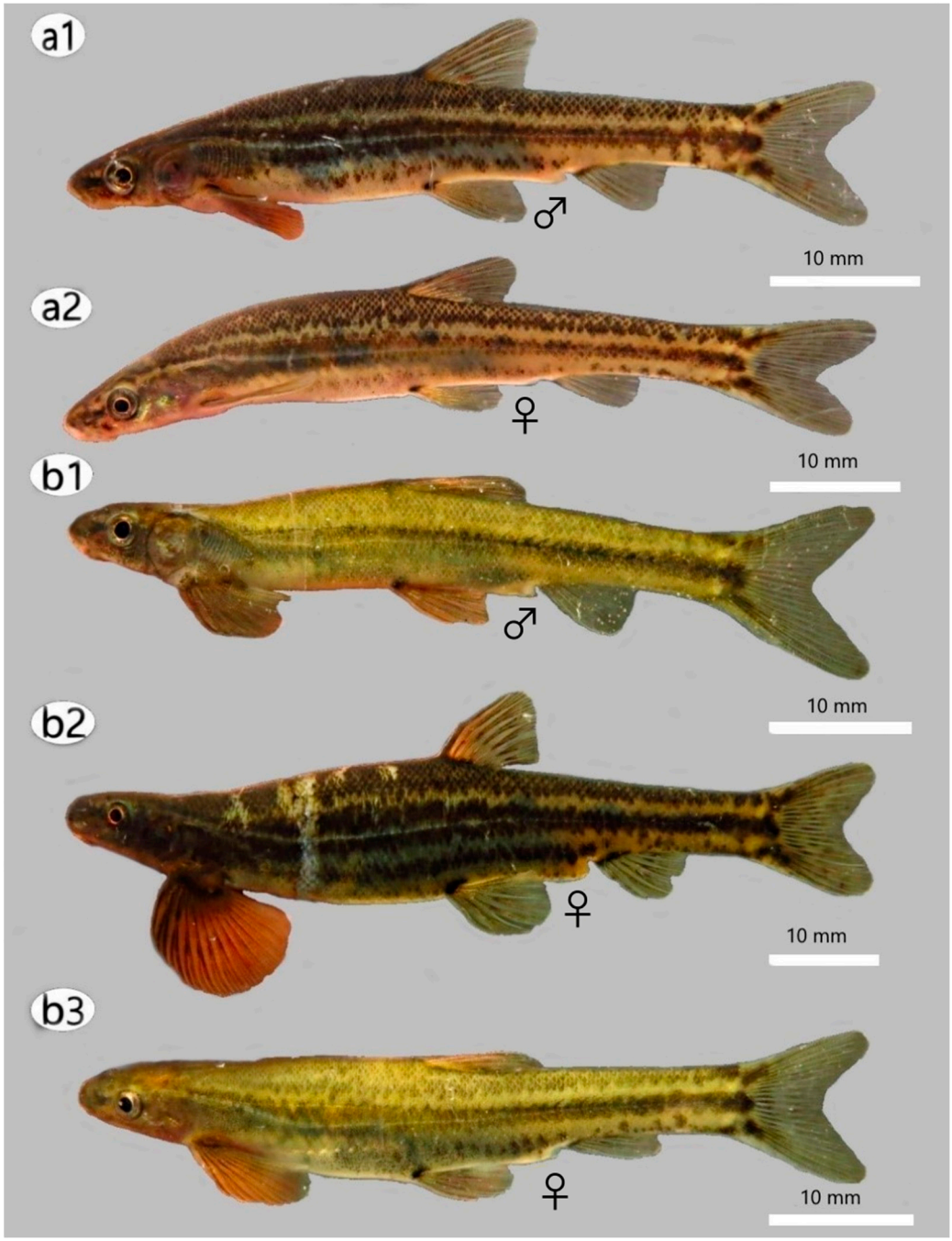

Figure 5. Life colour pattern variation in the *Kneria* population of *K.* sp. 'lua-upstream' IntP2 (=*K. luansaensis* IntP2 for males (**a1**,**b1**) and females (**a2**,**b2**,**b3**) of two consecutive years (2016–2017). (**a1**) ♂ (RMCA 2016-038-P-0218; DNA tag 088, 45.0 mm L_S), (M-DS: 23 August 2016); (**a2**) ♀ (RMCA 2016-038-P-0201-0203; DNA tag 083, 52.8 mm L_S), (M-DS: 23 August 2016); (**b1**) ♂ (RMCA 2018,020,P,0021-0026; DNA tag 40, 45.6 mm L_S), (L-DS: 20 September 2017); (**b2**) ♀ (gravid), (RMCA 2018,020,P,0016-0020; DNA tag 36, 65.8 mm L_S), (L-DS: 20 September 2017); and (**b3**) ♀ (RMCA 2018,020,P,0016-0020; DNA tag 39, 48.8 mm L_S), (L-DS: 20 September 2017). The red/orange colour of the pectoral fins (**a1**,**b2**,**b3**) is an artefact of a human hand keeping the specimen in place.

Colouration and Colour Pattern Differences between (Potential) Species

Colouration and colour pattern similarities and differences, independent of sex and reproductive state, were studied and documented: (i) between the two putative Luansa species, thus pooling the Luansa specimens of the different populations considered to be (potentially) conspecific (SM: Figure S3), and (ii) between the two putative Luansa species and *K. wittei* and *K. stappersii*. As no important colouration differences were observed between all five most upstream *Kneria* populations, these are treated together [i.e., populations PK1L, PK2M (Figure 6: a1 male, vs. a2 female), PInt1, PInt2 (Figure 5: a1 and b1 males, vs. a2 and b2–b3 females), and PInt3] (see also SM Figure S3).

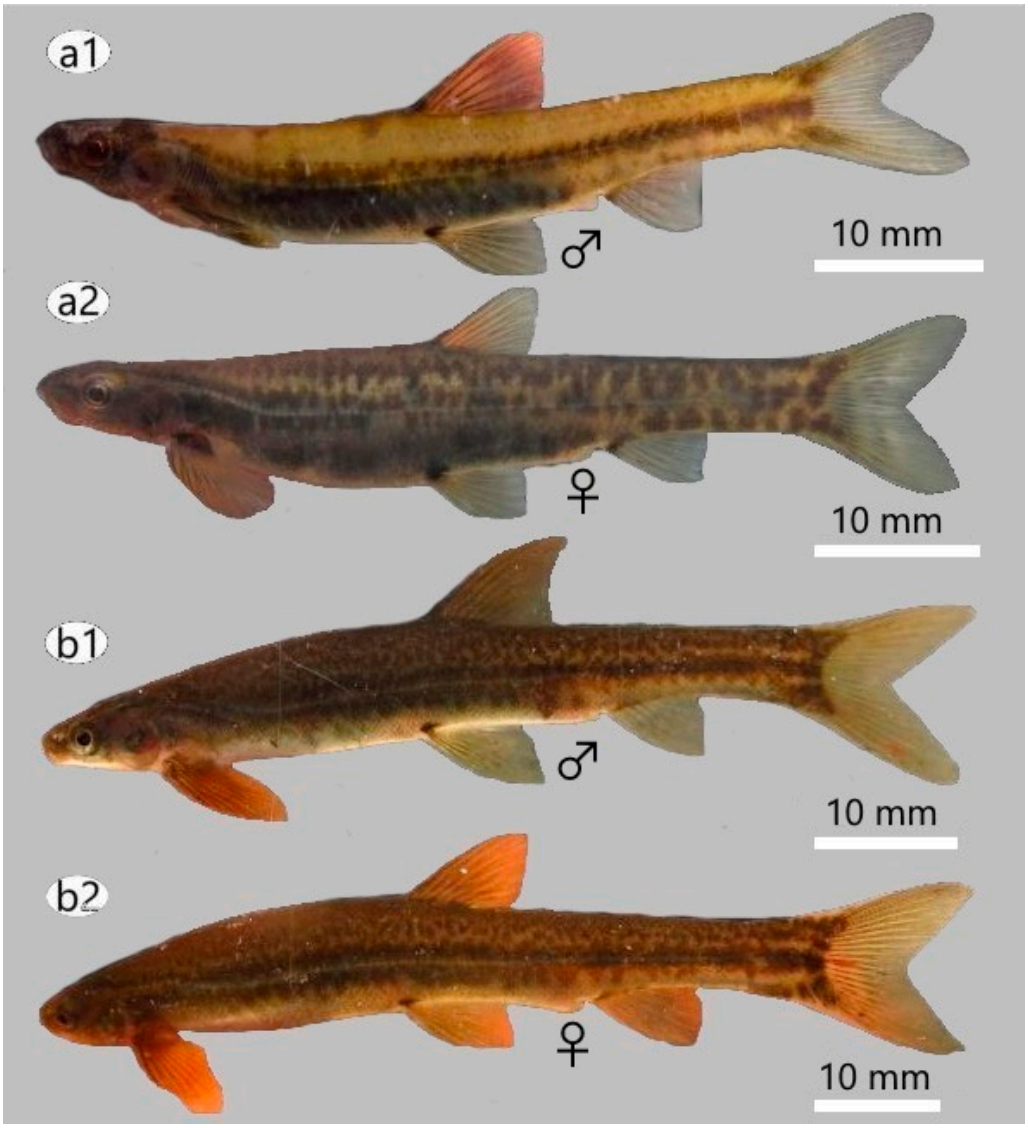

Figure 6. *Cont.*

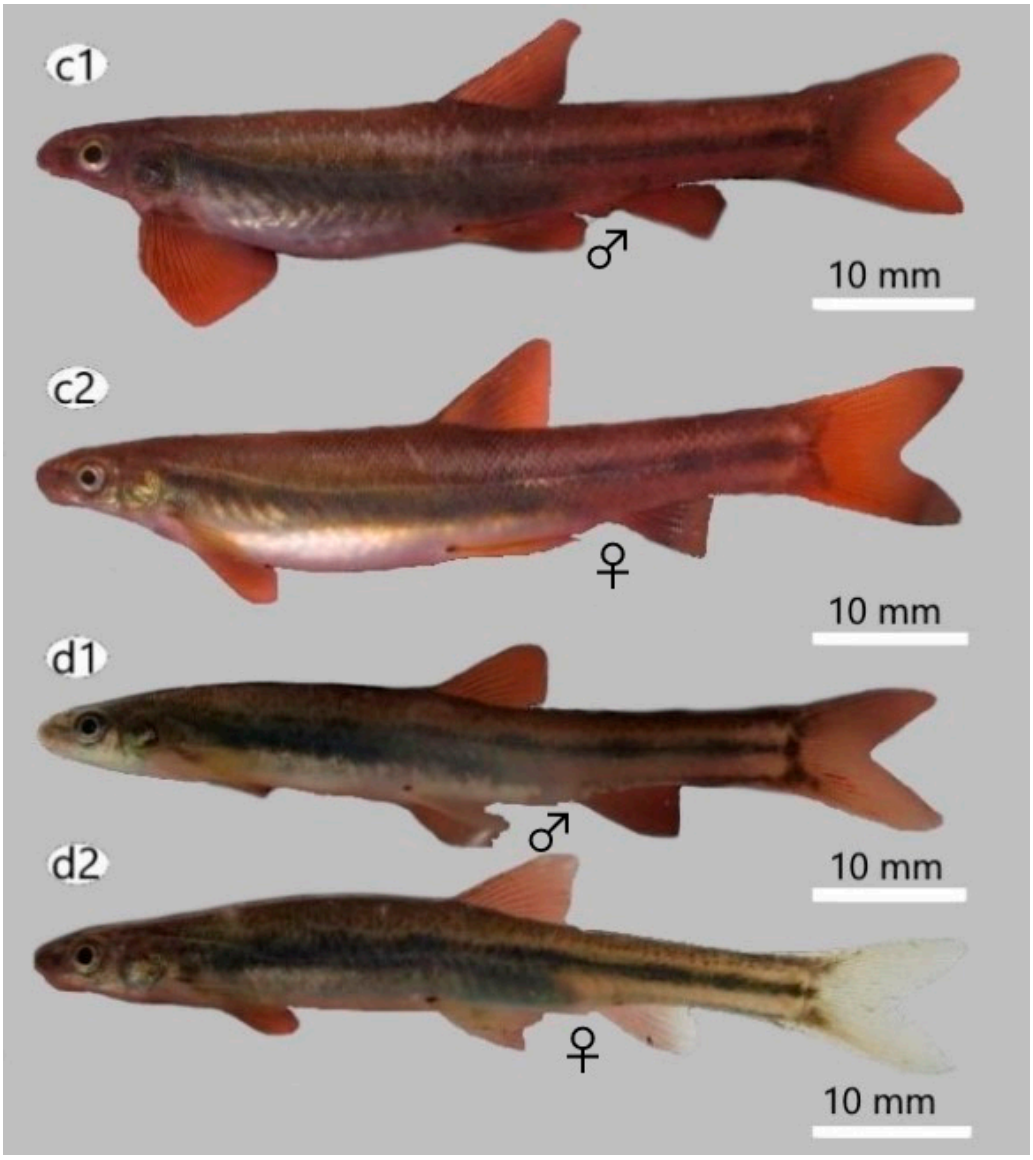

Figure 6. Life photographs of the two putative Luansa species and both comparative species, i.e., *K. stappersii* and *K. wittei*: (**a1**) *K.* sp. 'lua-upstream' KP2M (=*K. luansaensis* KP2M) ♂(RMCA 2018-020-P-0072-0076; DNA tag 79, 47.4 mm L_S), (H-DS: 21 September 2017); (**a2**) *K.* sp. 'lua-upstream' KP2M (=*K. luansaensis* sp. nov.) ♀ (gravid), (RMCA 2018-020-P-0067-0071; DNA tag 75, 49.1 mm L_S), (H-DS: 21 September 2017); (**b1**) *K.* sp. 'lua-downstream' (=*K. maxi* sp. nov.) ♂ (RMCA 2016-038-P-0310; DNA tag 056, 55.7 mm L_S), (H-DS: 22 August 2016); and (**b2**) *K.* sp. 'lua-downstream' (=*K. maxi* sp. nov.) ♀ (non-gravid) (RMCA 2016-038-P-0311-0314; DNA tag 051, 62.0 mm L_S), (H-DS: 22 August 2016). The red/orange colour of the fins [pectoral (**b1**,**b2**), dorsal (**a1**,**b2**) anal, and pelvic (**b1**)] is an artefact of a human hand keeping the specimen in place. (**c1**) *K. wittei* ♂ (RMCA 2015-07-P-uncat; DNA tag MK13, 48.7 mm L_S), (C-DS: 07 June 2015); and (**c2**) *K. wittei* ♀ (RMCA 2015-07-P-uncat; DNA tag MK11, 50.2 mm L_S), (C-DS: 07 June 2015); (**d1**) *K. stappersii* ♂ (RMCA 2021-021-P-0005-0010; Stap02, No DNA tag, 49.8 mm L_S), (L-RS: 04 March 2020); and (**d2**) *K. stappersii* ♀ (RMCA 2021-021-P-00011-0014; Stap0, No DNA tag, 50.1 mm L_S), (L-RS: 4 March 2020). The red/orange colour of fins [pectoral (**c1**,**c2**,**d2**), dorsal (**c1**,**c2**,**d1**,**d2**), pelvic (**c1**), anal (**c1**,**c2**,**d1**), and caudal (**c1**,**c2**,**d1**)] is an artefact of a human hand keeping the specimen in place.

Specimens from the five upstream Luansa River populations can be distinguished from those from the most downstream population by generally having, (i) a light sandy background in non-breeding and a yellowish sandy colouration in breeding specimens; (ii) a yellowish-grey-white caudal fin often with two oblique, well-demarcated, dark brown bands at the base of both lobes in gravid females, but less visible in non-gravid females and all males; and (iii) pinkish pectoral fins. This contrasts with the downstream population, which has (i) a more brownish sandy background colouration, (ii) a light grey caudal fin without well-differentiated, dark brown bands at the base of both lobes, and (iii) yellowish grey (proximal half) to light grey (distal half) pectoral fins. Finally, the up-and downstream populations can be distinguished from the two most similar species, *K. stappersii* and *K. wittei*, as follows: *K. wittei* specimens have (i) an overall light brown background colouration with a broad greyish horizontal band located mainly just below the lateral line in its predorsal part, followed by a series of discrete postdorsal brown spots along the lateral line, (ii) a dark brown caudal fin base without two well-differentiated oblique bands at the base of both lobes in all specimens; and (iii) dorsal, pectoral, pelvic, and anal fins (proximal part), dark brown and light brown (distal part) (Figure 6(c1,c2)); *K. stappersii* specimens have (i) a more greyish background colouration, often with a horizontal black band or a series of well or only weakly pronounced black spots along the lateral line, or an unspotted lateral band in small-sized unsexed specimens < 37 mm L_S; (ii) a light grey caudal fin without two well-demarcated oblique bands at the base of both lobes in all specimens; and (iii) unpaired fins grey, and pectoral and pelvic-fins greyish–brown (proximal part) and light grey (distal part) (Figure 6(d1,d2)).

3.1.3. Meristic Variation

Considering the occurrence of morphological sexual dimorphism beyond the presence of opercular and a postopercular organs in males (see below), and to enable assessment of morphometric differences between species and not only between sexes, analyses were first performed on single–sex datasets. Results are first presented for the females because the holotype of *K. wittei*, the only species reported from the lower Luapula to date, is a female.

Finally, for the six Luansa River populations studied (Figure 2), MWU-test results consistently reveal (i) no or almost no significant differences between same-sex specimens of the five upstream populations, i.e., the *K.* sp. 'lua-upstream' populations, but (ii) significant differences between those and the sixth most downstream, i.e., the *K.* sp. 'lua-downstream', population. Therefore, results are only briefly mentioned in the text itself and reference is made to the Supplementary Materials (SM): Table S5–S9 and Table SM text material SM1–3, as well, for more details. Thus, the MWU-test results discussed in the text are only those between the two (putative) species identified and the two most similar ones.

Meristic Variation in Females

A first PCA was performed on the only four variable meristics of all examined females (n = 87) (Figure 7). The highest loading on PCI is for the number of lateral line scales. The highest loading on PCII is for the total number of pectoral fin rays (SM: Table S9a).

A scatterplot of PCI against PCII shows two distinct and non-overlapping groups (Figure 7). A first group is situated predominantly on the negative side of PCI and comprises specimens of *K. wittei* and the five most upstream populations of the Luansa (see Figure 2), collectively referred to as *K.* sp. 'lua-upstream'. The second group is situated entirely on the positive side of PCI and comprises specimens of both *K. stappersii* and the most downstream population of the Luansa (see Figure 2), referred to as *K.* sp. 'lua-downstream'.

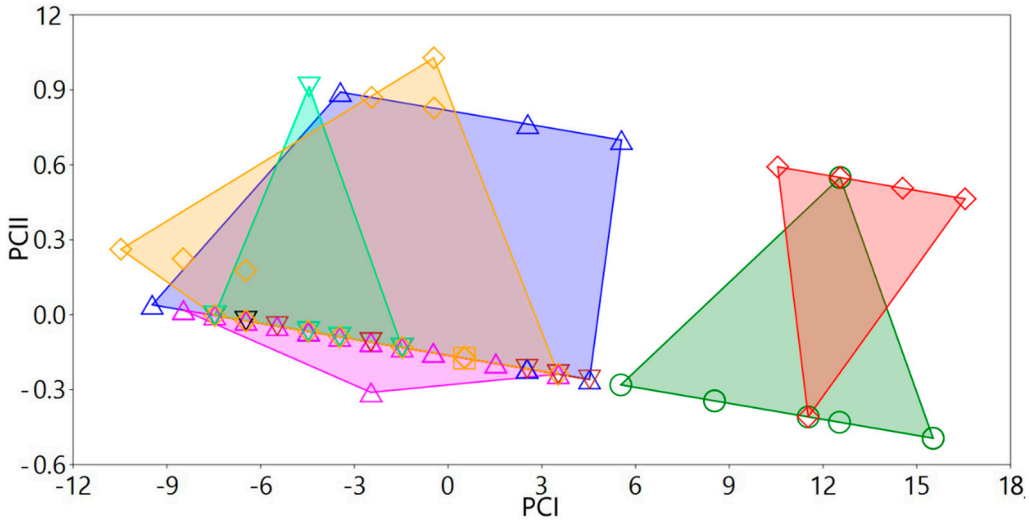

Figure 7. Scatterplot of PCI against PCII for a principal component analysis on four counts including all examined females of *Kneria* spp. (n = 87). *Kneria* sp. 'lua-upstream' (=*K. luansaensis*), populations from up-to downstream: △, KP1L; △, KP2M; ▽, IntP1; ▽, IntP2; and ▽, IntP3. *Kneria* sp. 'lua-downstream' (=*K. maxi*): ○, below the last Sanshifolo Falls. *Kneria wittei*: □, holotype; ◇, no type specimens. *Kneria stappersii*: ◇, no type specimens. All Luansa populations, from up-to downstream: KP1L: Luansa River on the Kundelungu Plateau (KP), loc 1; KP2M: Milembwe River on the KP, loc 2; IntP1: intermediate plateau 1; IntP2: intermediate plateau 2; and IntP3: intermediate plateau 3. Symbols: triangle (upstream Kasompola Falls); inverse triangle (downstream Kasompola Falls); circle (downstream of all falls); and primary types (square).

Two subsequent PCAs were then performed on the counts. The first restricted to *K. wittei* and *K.* sp. 'lua-upstream' (n = 72), and the second to *K. stappersii* and *K.* sp. 'lua-downstream' (n = 15), solely. However, neither of both these PCAs provided any further insight into the data (not illustrated).

The results of the MWU-tests between each of the six studied Luansa River populations reveal that all five upstream populations are highly similar to each other and well distinct from the downstream population [see SM Tables S6 and S7 and associated text (see Text SM1: MWU-tests meristic variation in female populations)].

Further, MWU-test for the meristic character variation between all four (putative) species, i.e., for all meristic characters included in the first PCA (SM: Table S9c), were also studied. They showed that *K.* sp. 'lua-upstream', composed of the five most upstream populations of the Luansa, is significantly different ($p \leq 0.05$) from (i) *K. wittei* for the total pelvic fin ray counts; from (ii) *K. stappersii* for the lateral line scales and total pectoral fin rays counts, the last being highly significantly different; and from (iii) *K.* sp. 'lua-downstream' for the lateral line scales count, which is also highly significantly different. Further, these tests also showed that *K.* sp. 'lua-downstream', the most downstream population of the Luansa, is highly significantly different from (i) *K. wittei* for the lateral line scale count, but (ii) no count was found to significantly differentiate between *K.* sp. 'lua-downstream' and *K. stappersii* (SM: Table S9c).

The total number of lateral line scales fully separates *K.* sp. 'lua-upstream' from *K. stappersii* (65–80 vs. 85–91) and *K.* sp. 'lua-downstream' from *K. wittei* (80–90 vs. 64–78) (see SM Figure S4 for females only; see also below under heading: meristic variation in both and for all populations/species). The same holds true, to a lesser degree, to separate *K.* sp. 'lua-upstream' from *K.* sp. 'lua-downstream' (65–80 vs. 80–90), as there is a slight overlap, of one scale only, between those two.

Meristic Variation in Males

A second PCA was performed on the four variable meristics for all examined males only, including the number of lamellae on the POP organ and excluding six invariable counts (n = 80) (Figure 8a). The highest loading on PCI is for the number of lateral line scales. The highest loading on PCII is for the number of lamellae in the POP organ (SM: Table S9b).

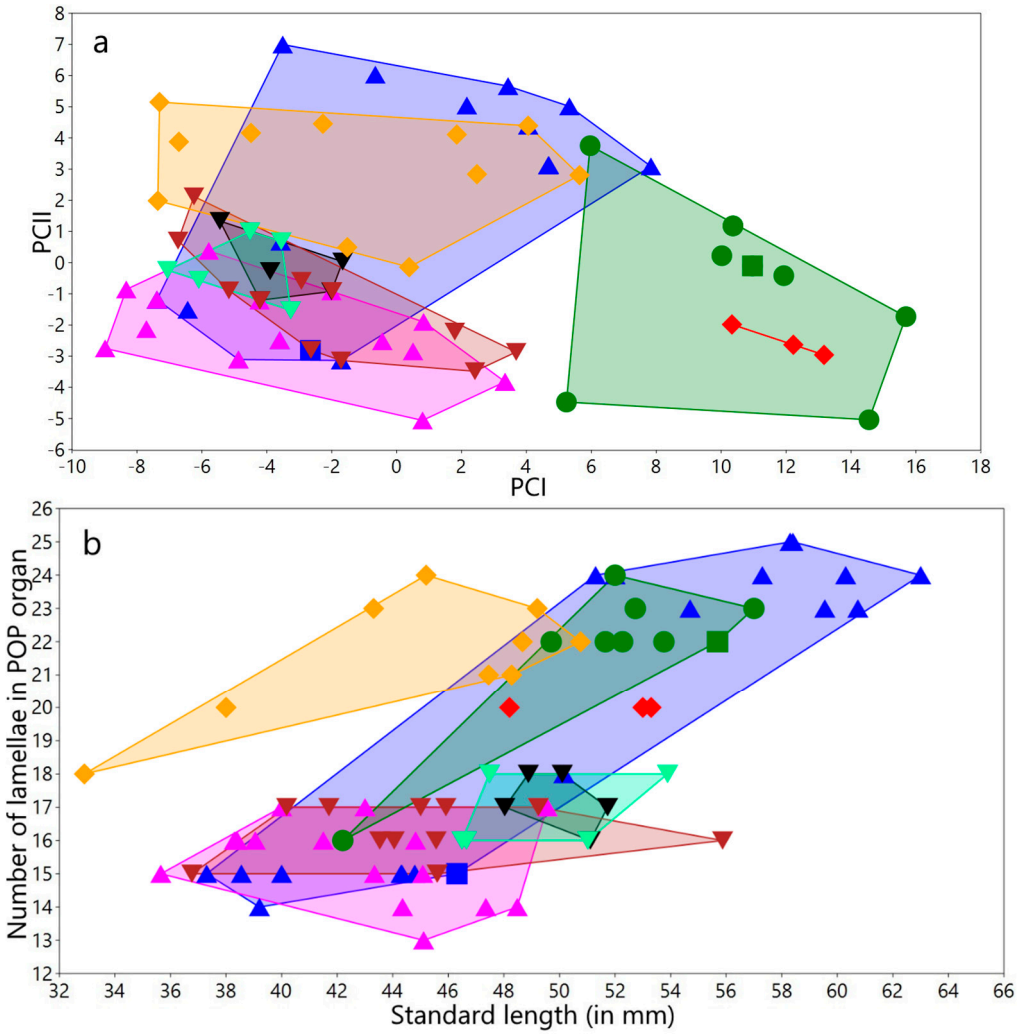

Figure 8. Scatterplot of (**a**) PCI against PCII for a principal component analysis on four counts including all examined males of *Kneria* spp. (n = 80), and (**b**) the number of lamellae in the postopercular (POP) organ against the standard length (L_S) (in mm). *Kneria* sp. 'lua-upstream' (=*K. luansaensis*), populations from up-to downstream: ■, holotype, ▲, specimens from KP1L; ▲, KP2M; ▼, IntP1; ▼, IntP2; and ▼, IntP3. *Kneria* sp. 'lua-downstream' (=*K. maxi*): ■, holotype; ●, specimens from below the last Sanshifolo falls. *Kneria wittei*: ◆, specimens. *Kneria stappersii*: ◆, specimens. All Luansa populations, from up-to downstream: KP1L: Luansa River on the KP, loc 1; KP2M: Milembwe River on the KP, loc 2; IntP1: intermediate plateau 1; IntP2: intermediate plateau 2; and IntP3: intermediate plateau 3. Symbols: triangle (upstream Kasompola Falls); inverse triangle (downstream Kasompola Falls); and primary types (square).

A scatterplot of PCI against PCII shows two main groups (Figure 8a). A first group is entirely situated on the positive side of PCI and contains the specimens of *K. stappersii*, superimposing those of the most downstream population from the Luansa River, *K.* sp. 'lua-downstream'. The second group is mainly situated on the negative side of the PCI and contains the specimens of *K. wittei* and those of the five upstream populations on the Luansa River, *K.* sp. 'lua-upstream', with only a very limited overlap with *K.* sp. 'lua-downstream'; this with the most upstream population of *K.* sp. 'lua-upstream' KP1L only.

Two subsequent PCAs were then performed on the counts. The first restricted to *K. wittei* and *K.* sp. 'lua-upstream' (n = 69), and the second to *K. stappersii* and *K.* sp. 'lua-downstream' (n = 11), solely. However, neither of both these PCAs provided any further insight into the data (not illustrated).

The results of the MWU-tests between each of the six studied Luansa River populations reveal the five upstream populations to be highly similar among themselves, but well distinct from the downstream population [see SM Tables S6 and S8 and associated text (see Text SM2: MWU-tests meristic variation in male populations)].

Further, MWU-tests, between the two putative species and the two most similar ones, were also performed on all four meristic characters included in the second PCA (SM: Table S9d). They revealed only one single significantly different count, i.e., the total number of lamellae on the POP organ between *K.* sp. 'lua-upstream' and *K. wittei*. However, high intra-population and intra-specific variation in the total number of lamellae on the POP was found between one of the most upstream populations of *K.* sp. 'lua-upstream' and the other populations. Based on the results obtained with our yet limited data set, an allometric transition towards more lamellae takes place already at a size of ~33.0–39.0 mm L_S for *K. wittei*, but at more than ~42.0 mm L_S in *K.* sp. 'lua-downstream', or slightly above ~39.0–46.0 mm L_S in *K.* sp. 'lua-upstream' (Figure 8b; see also SM: Table S9c). Further, *K.* sp. 'lua-upstream' is significantly different ($p \leq 0.05$) from (i) *K. wittei* in the lamellae count of the POP organ and for the total pelvic fin ray count; and from (ii) *K.* sp. 'lua-downstream' in the lamellae count of the POP organ also and for the lateral line scale count, the last being highly significantly different (SM: Table S9b), as well. Second, *K.* sp. 'lua-downstream', the most downstream population of the Luansa, is significantly different from *K. wittei* for the lateral line scale count, but this remains undocumented for *K. stappersii* because of the small sample size (SM: Table S6).

The total number of lateral line scales fully separates *K.* sp. 'lua-upstream' from *K. stappersii* (66–80 vs. 84–87) (SM: Figure S5 for males only; see also Figure 9b for both females and males). The same holds true to a lesser degree for *K.* sp. 'lua-downstream' vs. *K. wittei* (78–89 vs. 61–78), and for *K.* sp. 'lua-upstream' vs. *K.* sp. 'lua-downstream' (66–80 vs. 78–89), as there is only a slight overlap of one up to three scales between the (tentative) species compared.

Meristic Variation in Both Sexes and for All Populations/Species

A third PCA was performed on four meristics, i.e., excluding five invariable ones, for all examined specimens (n = 167, 87 females and 80 males; Figure 9a). The highest loading on PCI is for the number of lateral line scales. The highest loading on PCII is for the total number of pectoral fin rays (SM: Table S5a).

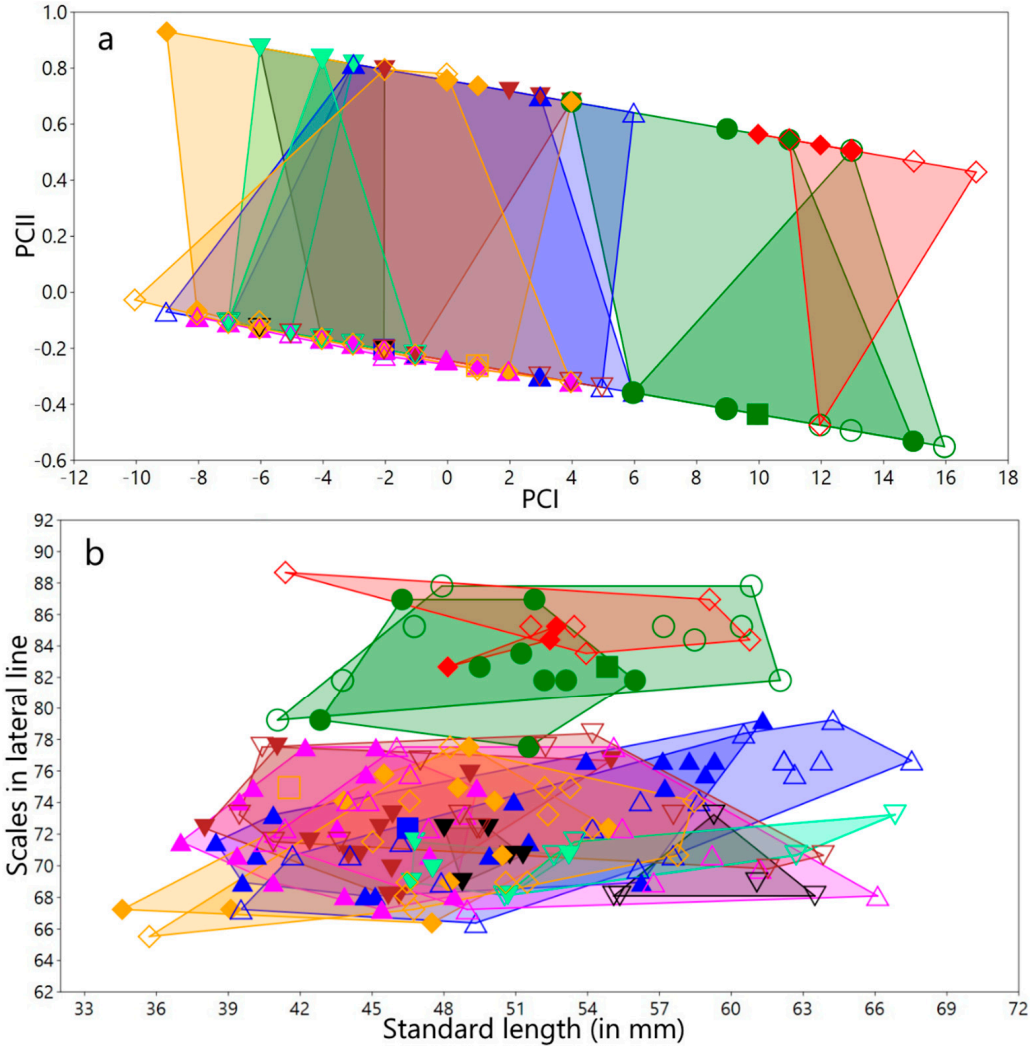

Figure 9. Scatterplot of (**a**) PCI against PCII for a principal component analysis on four counts including all examined specimens of *Kneria* spp. (n = 167: 87 females and 80 males) and (**b**) the total number of lateral line scales against standard length (L_S) (in mm). *Kneria* sp. 'lua-upstream' (=*K. luansaensis* sp. nov.) populations from up-to downstream: ■, holotype (male), ▲, males and △, females from KP1L; ▲, males and △, females from KP2M; ▼, males and ▽, females from IntP1; ▼, males and ▽, females from IntP2; and ▼, males and ▽, females from IntP3. *Kneria* sp. 'lua-downstream' (=*K. maxi* sp. nov.): ■, holotype (male); ●, males and ○, females from below the last Sanshifolo falls. *Kneria wittei*: □, holotype (female); ◆, males and ◇, females. *Kneria stappersii*: ◆, males, and ◇, females. All Luansa populations from up-to downstream: KP1L: Luansa River on the Kundelungu Plateau (KP), loc 1; KP2M: Milembwe River on the KP, loc 2; IntP1: intermediate plateau 1; IntP2: intermediate plateau 2; and IntP3: intermediate plateau 3; and ds: downstream, us: upstream. Symbols: triangle (upstream Kasompola Falls); inverse triangle (downstream Kasompola Falls); male (full symbols); female (open symbols); and primary types (square).

A scatterplot of PCI against PCII does not show any clearly distinct groups (Figure 9a). However, whereas the specimens of *K. stappersii* are entirely situated on the positive side of PCI, the specimens of *K. wittei* are mostly situated on the negative side of PCI. The same holds true for populations from the Luansa River, with specimens of the five most upstream populations mainly situated on the negative side of PCI, and the specimens of the single most downstream population being located entirely on the positive side of PCI.

Two subsequent PCAs were also performed on the counts. The first restricted to *K. wittei* and *K.* sp. 'lua-upstream' (n = 141), and the second to *K. stappersii* and *K.* sp. 'lua-downstream' (n = 28). Neither of both these PCAs provided any further insight into the data (thus not illustrated).

The results of sex-differentiated MWU-test for differentiation of the six studied Luansa River populations, again reveal the five upstream populations to be highly similar among each other, but clearly differentiated from the downstream population [see SM Tables S7–S10 and S12b and associated text (see Text SM1 and SM2 but also SM3: MWU-test meristic variation between both sexes and for all populations)].

Further, MWU-tests, for the two putative species and the two most similar ones, were performed only on the four meristics included in the third PCA to explore for differences between males and females of those four (putative) species (SM: Table S5b). They showed that only one meristic value, i.e., the total number of pectoral fin rays, was significantly different ($p \leq 0.05$) between males and females of *K.* sp. 'lua-upstream' (SM: Table S5c). None of the four meristics were significantly different ($p \leq 0.05$) between males and females of *K.* sp. 'lua-downstream' and *K. wittei* (SM: Table S5c). Nevertheless, this remains undocumented for *K. stappersii* as its sample size too limited (SM: Table S6).

3.1.4. Variation in Measurements

Female Variation in Measurements

A fourth PCA (Figure 10a) was performed on 31 log-transformed measurements for all examined females (n = 87). The highest loadings on PCII are for body height, mouth width, anal fin base width, and lower caudal fin lobe. The highest loadings on PCIII are for pectoral fin base width, anal fin base width, body height, pelvic anal distance, and mouth width (SM: Table S11a).

A scatterplot of PCII against PCIII revealed three groups (Figure 10a). A first group is situated entirely on the positive side of PCII and PCIII and is composed only of specimens of *K. wittei*. A second group is also situated entirely on the positive side of PCII, but on the negative side of PCIII, instead, and is composed of specimens of *K. stappersii* and *K.* sp. 'downstream'. Finally, the third group is situated entirely on the negative side of PCII and around the zero point of PCIII and is composed of all five most upstream populations of the Luansa River here collectively referred to as *K.* sp. 'lua-upstream'.

As *K.* sp. 'lua-downstream' and *K. stappersii* fully overlap each other in the previous PCA, a fifth PCA was performed on 31 log-transformed measurements on all females of both these (putative) species only (n = 15). The highest loadings on PCII are for body height, anal fin base width, eye diameter, and snout length. The highest loadings on PCIII are for dorsal height, snout length, mouth width, pectoral length, pectoral fin base width, upper caudal fin lobe, and head width (SM: Table S11b).

The resulting scatterplot of PCII against PCIII revealed two groups (Figure 10b). A first group is situated entirely on the negative side of PCII and mainly on the positive side of PCIII and composed of specimens here collectively referred to *K. stappersii*. The second group is situated mainly on the positive side of PCII and on both sides of PCIII and composed of specimens here collectively referred to as *K.* sp. 'lua-downstream'.

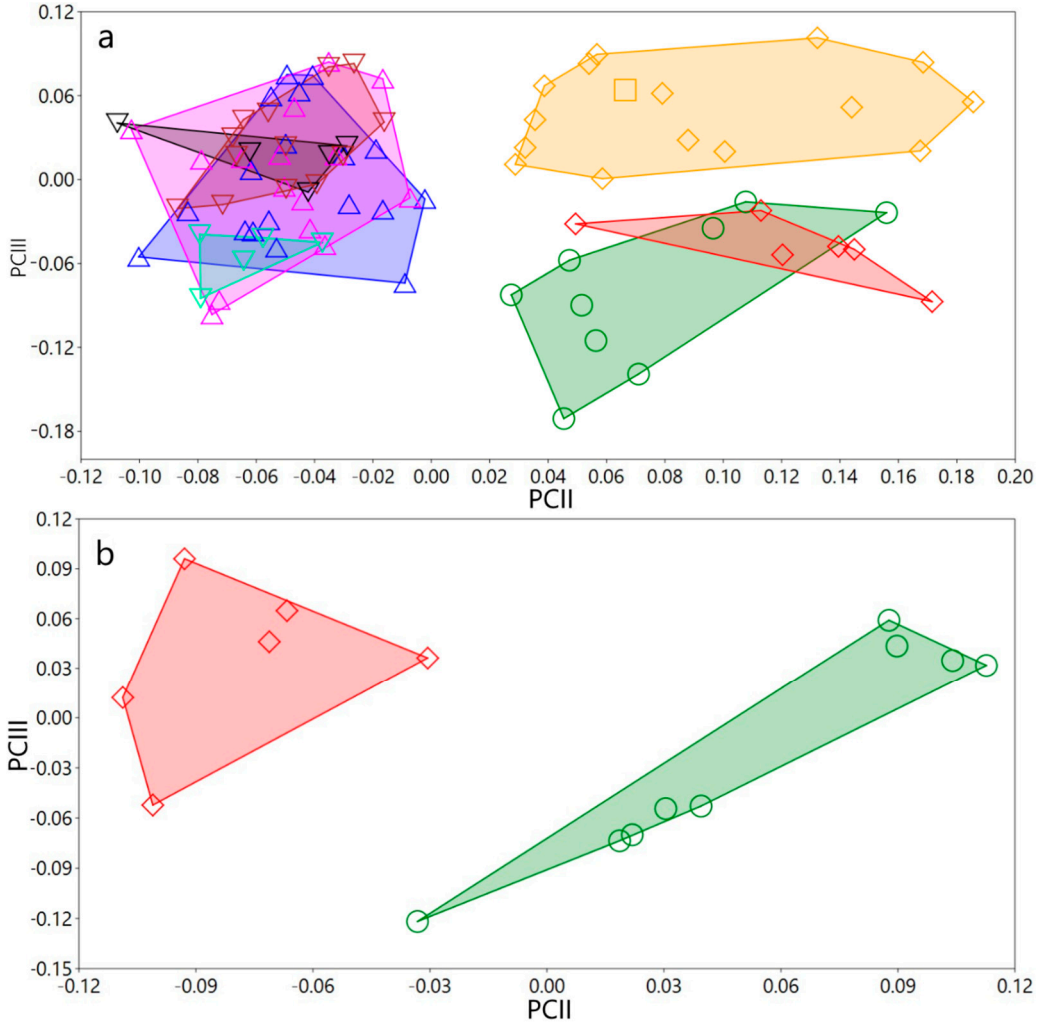

Figure 10. Scatterplot of: (**a**) PCII against PCIII for a principal component analysis on 31 log-transformed measurements of all examined females of *Kneria* spp. (n = 87); and (**b**) PCII against PCIII for a principal component analysis on 31 log-transformed measurements of the females of *K. stappersii* and *K.* sp. 'lua-downstream' (=*K. maxi* sp. nov.) (n = 15). *Kneria* sp. 'lua-upstream' (=*K. luansaensis* sp. nov.), populations from up-to downstream: △, KP1L; △, KP2M; ▽, IntP1; ▽, IntP2; ▽, IntP3. *Kneria* sp. 'lua-downstream' (=*K. maxi* sp. nov.): ○, below the last Sanshifolo Falls. *K. wittei*: □, holotype; and ◇, specimens. *K. stappersii*: ◇, specimens. All Luansa populations, from up-to downstream: KP1L: Luansa River on the Kundelungu Plateau, loc 1; KP2M: Milembwe River on the Kundelungu Plateau, loc 2; IntP1: intermediate plateau 1; IntP2: intermediate plateau 2; and IntP3: intermediate plateau 3; ds: downstream, and us: upstream. Symbols: triangle (upstream Kasompola Falls); inverse triangle (downstream Kasompola Falls); circle (downstream of all falls); and primary types (square).

Finally, another PCA on the specimens of the five upstream populations of the Luansa River separately (SM: Figure S6), here all collectively referred to as belonging to a single (potential) species *K.* sp. 'lua-upstream', did not reveal any differentiation between these studied populations, and is thus not further discussed (not illustrated).

The results of the MWU-tests between each of the six studied Luansa River populations reveal the five upstream populations to be highly similar among each other but clearly differentiated from the most downstream population [see SM Tables S6 and S12 and associated text (see Text SM4: MWU-tests variation in measurements in female populations)].

Further, MWU-tests between the two (putative) species were also performed on the females on all measurements included in the last PCA (SM: Table S11c) except on one comparison, i.e., between the females of *K.* sp. 'lua-downstream' vs. *K. stappersii*, due to

significant size class differences (SM: Table S10). The results of those tests show that ten out of the 31 measurements were significantly different ($p \leq 0.05$), among which three are highly significant ($p \leq 0.001$) between *K.* sp. 'lua-upstream' and *K. wittei*. However, if taken alone, none of these ten measurements could discriminate these two species without overlap. Nevertheless, a combination of two of those, i.e., mouth width against the body height (both highly significantly different), enabled the ability to diagnose the females of both (Figure 11a) (see also SM Figure S7a,b for a scatterplot of both these measurements against, respectively, L_S and L_H). Five measurements were significantly different between *K.* sp. 'lua-upstream' and *K. stappersii.* However, only body width diagnostically distinguishes both species (9.8–11.6% L_S for *K.* sp. 'lua-upstream' vs. 8.6–9.1% for *K. stappersii*) (SM: Figure S8). Four measurements were significantly different, among which three were highly significant, between *K.* sp. 'lua-upstream' and *K.* sp. 'lua-downstream'. However, only body width distinguishes both (9.8–11.6% L_S for *K.* sp. 'lua-upstream' vs. 8.0–9.3% for *K.* sp. 'lua-downstream') (SM: Figure S8). Finally, body width significantly differentiated between *K.* sp. 'lua-downstream' and *K. wittei* and also distinguished both species from each other (8.0–9.3% L_S for *K.* sp. 'lua-downstream' vs. 9.6–11.7% for *K. wittei*) (SM: Figure S8).

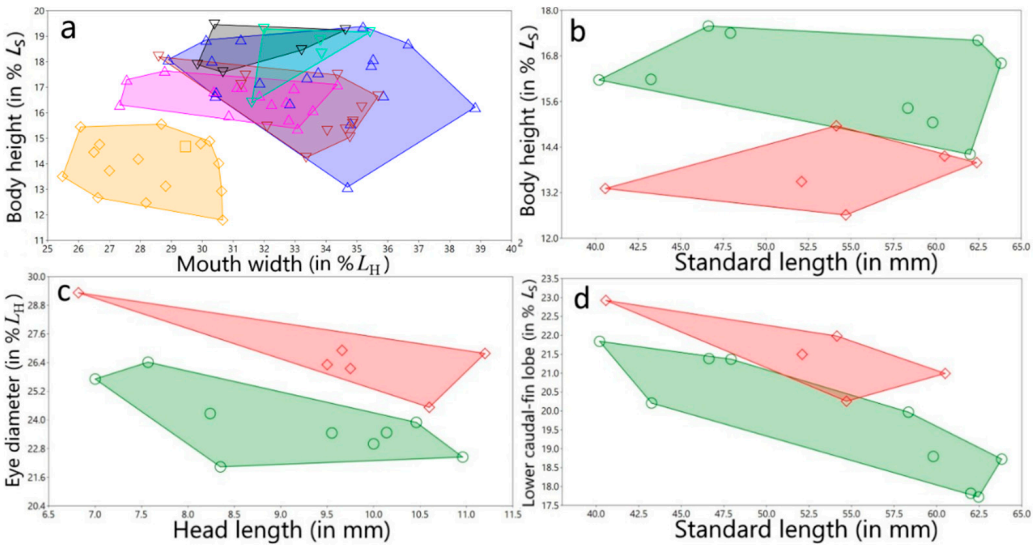

Figure 11. Scatterplot of: (**a**) body height (in % L_S) against mouth width (in % L_H) for females only, (**b**) body height (in % L_S) against standard length (L_S) in mm for females only, (**c**) eye diameter (in % L_H) against head length (L_H) in mm for females only, and (**d**) lower caudal fin lobe (in % L_S) against standard length (L_S) in mm for females only. *Kneria* sp. 'lua-upstream' (=*K. luansaensis* sp. nov.), populations from up-to downstream: △, KP1L; △, KP2M; ▽, IntP1; ▽, IntP2; and ▽, IntP3. *K.* sp. 'lua-downstream' (=*K. maxi* sp. nov.): ○, below the last Sanshifolo Falls. *K. wittei*: □, holotype; ◇, non-type specimens. *K. stappersii*: ◇, non-type specimens. All Luansa populations, from up-to downstream: KP1L: Luansa River on the Kundelungu Plateau, loc 1; KP2M: Milembwe River on the Kundelungu Plateau, loc 2; IntP1: intermediate plateau 1; IntP2: intermediate plateau 2; and IntP3: intermediate plateau 3; ds: downstream, and us: upstream. Symbols: triangle (upstream Kasompola Falls); inverse triangle (downstream Kasompola Falls); circle (downstream of all falls); circle (downstream of all falls); and primary types (square).

Although based on small sample sizes and partially overlapping values between *K.* sp. 'lua-downstream' and *K. stappersii*, (i) body height (in % L_S) against standard length (L_S) in mm (Figure 11b), (ii) eye diameter (in % L_H) (negative allometric) against head length (L_H) in mm (Figure 11c), and (iii) the lower caudal fin lobe (in % L_S) (negative allometric) against standard length (L_S) in mm (Figure 11d) are most discriminative between both species, although more specimens are needed to confirm these preliminary observations (see Table provided under Section 3.4 Species Descriptions, for ranges).

Male Variation in Measurements

A sixth PCA (Figure 12) was performed on 34 log-transformed measurements, including the OP and POP organ-related measurements, for all examined males (n = 80) (Figure 12). The highest loadings on PCII are for mouth width, eye diameter, dorsal fin base width, body width, and pelvic fin base width. The highest loadings on PCIII are for post-opercular organ length, eye diameter, interorbital distance, mouth width, pelvic fin base width, and caudal peduncle height (SM: Table S13a).

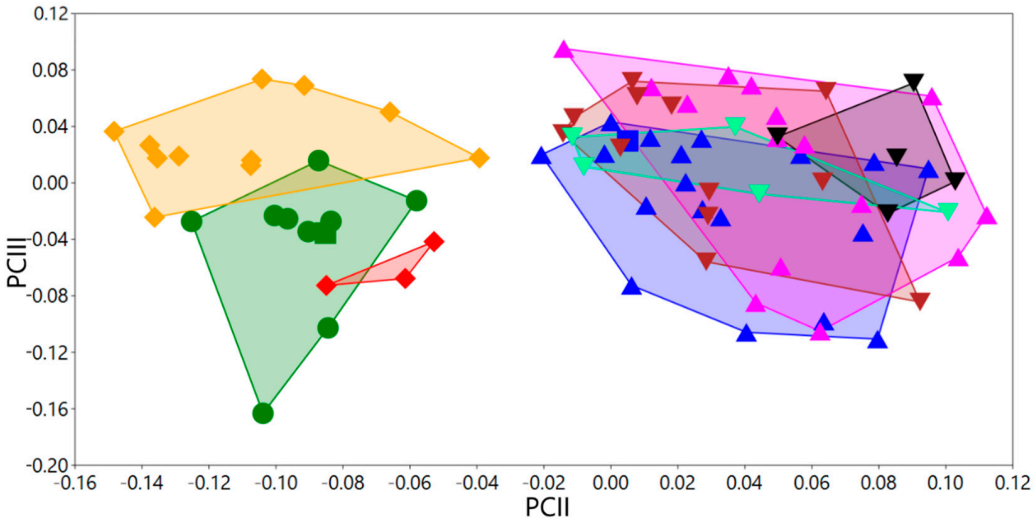

Figure 12. Scatterplot of PCII against PCIII for a principal component analysis on 34 log-transformed measurements of all examined males of *Kneria* spp. (n = 80). *Kneria* sp. 'lua-upstream' (=*K. luansaensis* sp. nov.), populations from up-to downstream: ■, holotype, ▲, specimens from KP1L; ▲, KP2M; ▼, IntP1; ▼, IntP2; and ▼, IntP3; *K.* sp. 'lua-downstream' (=*K. maxi* sp. nov.) from below the last Sanshifolo Falls: ■, holotype; ●, specimens; *K. wittei*: ◆, specimens; and *K. stappersii*: ◆, specimens. All Luansa populations, from up-to downstream: P1L: plateau one on the Luansa River, loc1; P2M: plateau two on the Milembwe River, loc2; IntP1: intermediate plateau 1, IntP2: intermediate plateau 2, IntP3: intermediate plateau 3, ds: downstream, and us: upstream. Symbols: triangle (upstream Kasompola Falls); inverse triangle (downstream Kasompola Falls); circle (downstream of all falls); circle (downstream of all falls); and primary types (square).

A scatterplot of PCII against PCIII revealed two main groups (Figure 12). A first group is situated entirely on the negative side of PCII and on both sides of PCIII. This group is composed of specimens of *K. wittei*, mainly situated on the positive side of PCIII, and of *K. stappersii* and *K.* sp. 'lua-downstream' situated, respectively, entirely and mostly on the negative side of PCIII. The second group is situated mainly on the positive side of PCII and around the zero point of PCIII. This group is composed of the five most upstream populations of the Luansa River here collectively referred to as *K.* sp. 'lua-upstream' (Figure 12).

Further, another PCA was performed on the 34 log-transformed measurements for all examined males of *K. wittei*, *K. stappersii*, and *K.* sp. 'lua-downstream' only (n = 24). A scatterplot of PCII against PCIII revealed three well-separated groups (SM: Figure S9) corresponding to the three (potential) species but did not reveal any new insights and is thus not further discussed (not illustrated).

Finally, another PCA was performed on the 34 log-transformed measurements for all examined males of the five upstream populations of the Luansa River separately, here collectively referred to *K.* sp. 'lua-upstream'. This PCA did not reveal any differentiation between the different studied populations (SM: Figure S10) and is thus not further discussed (not illustrated).

The results of the MWU-tests between each of the six Luansa River populations reveal the five upstream populations to be highly similar among each other but clearly distinct from the downstream population [see SM Tables S6 and S13b and associated text (see Text SM5: MWU-test variation in measurements in male populations].

Further, MWU-tests, between the two putative species identified and the two most similar ones, were also performed on all measurements, including in the latter PCA but only between the following entities: *K.* sp. 'lua-upstream' vs. *K. wittei* and vs. *K.* sp. 'lua-downstream' (SM: Table S13c). They were not performed between *K.* sp. 'lua-downstream' vs. *K. stappersii* (n: 10 vs. 3) and *K.* sp. 'lua-upstream' vs. *K. stappersii* (n: 56 vs. 3) due to few studied specimens for *K. stappersii* (n = 3 < 5); and between *K.* sp. 'lua-downstream' vs. *K. wittei* due to significant differences in standard lengths (SM: Table S10). The results of those tests show seven out of 31 measurements to be significantly different ($p \leq 0.05$), among which one is highly significant ($p \leq 0.001$), between *K.* sp. 'lua-upstream' and *K. wittei*. However, none of these measurements, nor a possible combination of two of these, could effectively distinguish these two species. Further, four out of 31 measurements were significantly different, among which one was highly significant between *K.* sp. 'lua-upstream' and *K.* sp. 'lua-downstream'. Nevertheless, among these only body width revealed to be a good discriminating character (9.5–11.7% L_S for *K.* sp. 'lua-upstream' vs. 8.7–9.4% for *K.* sp. 'downstream') (SM: Figure S11).

Further, although based on a very limited sample size for at least one of the two compared species (SM: Table S10), *K.* sp. 'lua-upstream' (n = 56) vs. *K. stappersii* (n = 3) seem also distinguishable from each other by the body width (9.5–11.7% L_S for *K.* sp. 'lua-upstream' vs. 8.4–9.2% for *K. stappersii*) (SM: Figure S11); *K.* sp. 'lua-downstream' (n = 10) vs. *K. wittei* (n = 11), also by the body width (8.7–9.4% L_S for *K.* sp. 'lua-downstream' vs. 9.6–10.8% for *K. wittei*) (SM: Figure S11); and *K.* sp. 'lua-downstream' (n = 10) vs. *K. stappersii* (n = 3) by both (i) the lower caudal fin lobe (19.7–22.3% L_S for *K.* sp. 'lua-downstream' vs. 24.3–24.9% L_S for *K. stappersii*) and the eye diameter (22.0–26.5% L_S for *K.* sp. 'lua-downstream' vs. 24.5–29.3% for *K. stappersii*) (SM: Figure S12a,b).

Variation in Measurements of Both Sexes and for All Populations/Species Studied

A seventh PCA was performed on 31 log-transformed measurements for all specimens examined, regardless of sex (n = 167; 87 females and 80 males; Figure 13). The highest loadings on PCII are for pectoral fin base width, lower caudal fin lobe length, dorsal height, anal fin base width, head width, dorsal fin base width, and snout length. The highest loadings on PCIII are for pectoral fin base width, mouth width, pelvic fin base width, post–orbital distance, and body height (SM: Table S14a).

A scatterplot of PCII against PCIII showed that (Figure 13): (i) males are entirely, or mostly, situated on the positive side of PCII while, instead, females are entirely, or mostly, situated on the negative side of PCII; (ii) females and males of the five most upstream populations on the Luansa River, i.e., all except those from the most downstream section of the river, are situated entirely, or mostly, on the positive side of PCIII, whereas the females and males of the most downstream section of the river, and of both *K. stappersii* and *K. wittei*, are situated entirely on the negative side of PCIII.

The results of the MWU-test between the males and females of the six Luansa populations reveal all of them to be highly similar among each other [see SM Tables S6 and S14b and associated text (see Text SM6: MWU-test variation in measurements between both sexes and for all populations].

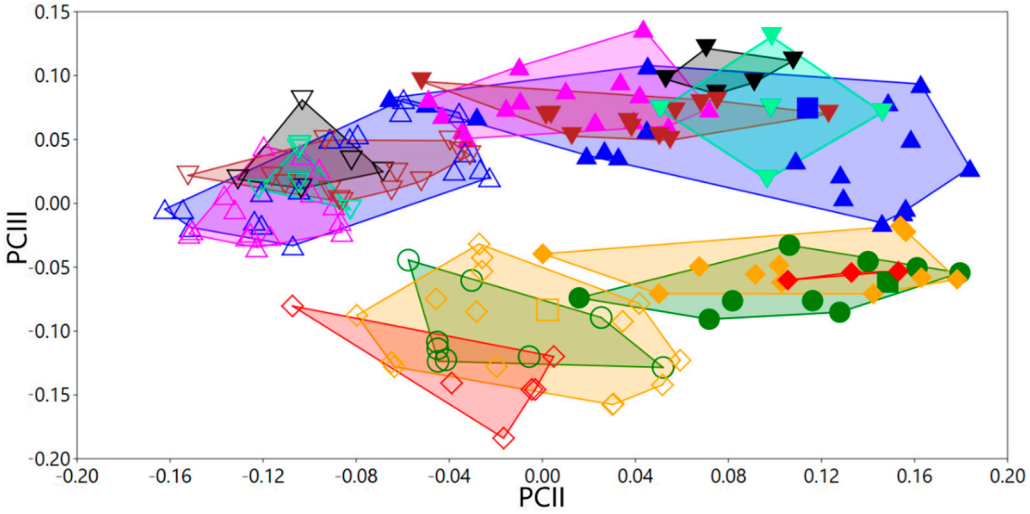

Figure 13. Scatterplot of PCII against PCIII for a principal component analysis on 31 log-transformed measurements of *Kneria* spp. (n = 167). *Kneria* sp. 'lua-upstream' (=*K. luansaensis* sp. nov.), populations from up-to downstream: ■, holotype (male), ▲, males, and △, females from KP1L; ▲, males, and △, females from KP2M; ▼, males, and ▽, females from IntP1; ▼, males, and ▽, females from IntP2; and ▼, males and ▽, females from IntP3. *Kneria* sp. 'lua-downstream' (=*K. maxi* sp. nov.): ■, holotype (male); ●, males, and ○, females from below the last Sanshifolo falls. *Kneria wittei*: □, holotype (female); and ◆, male and ◇, females. *Kneria stappersii*: ◆, males, and ◇, females. All Luansa populations, from up-to downstream: KP1L: Luansa River on the KP, loc 1; KP2M: Milembwe River on the KP, loc 2; IntP1: intermediate plateau 1; IntP2: intermediate plateau 2; and IntP3: intermediate plateau 3; and ds: downstream, us: upstream. Symbols: triangle (upstream Kasompola Falls); inverse triangle (downstream Kasompola Falls); circle (downstream of all falls); circle (downstream of all falls); and primary types (square).

Further, MWU-tests, for the two putative species identified and the two most similar ones, were performed on all measurements included in the PCA (SM: Table S14c), except for *K. stappersii* due to small sample size (see M&M section), to explore for differences between males and females of the four (putative) species (SM: Table S10). These tests (SM: Table S14c) showed 21 measurements to be significantly different ($p \leq 0.05$), among which 14 were highly significantly different ($p \leq 0.001$), between the males and the females in *K.* sp. 'lua-upstream'. Four measurements were significantly different between males and females in *K.* sp. 'lua-downstream', and three were significantly different between males and females in *K. wittei*. Three of these, at most, are shared with at least one of the other Luansa upstream populations studied. However, differences in sexual dimorphism between populations seem, at least partially, sample size related, as small sample sizes often tend to result in non-significant differences, and this even for variables that revealed to be sexual dimorphic in populations for which sufficient specimens were available. Two measurements, namely, (i) the pectoral fin base width (Figure 14a), and (ii) the dorsal fin height (Figure 14b), are sexually dimorphic for all population/species comparisons studied (SM: Table S14b,c). Nevertheless, this remains undocumented for *K. stappersii* because of its small sample size (SM: Table S10). Other measurements are, however, sexually dimorphic in only some species. This seems true for the length of the lower caudal fin lobe in *K.* sp. 'lua-upstream', *K. stappersii* and *K. wittei*, but not in *K.* sp. 'lua-downstream' (SM: Figure S13). The same holds true for both the predorsal distance, sexually dimorphic in *K.* sp. 'lua-upstream' and *K.* sp. 'lua-downstream', and the dorsal fin base width, sexually dimorphic in *K.* sp. 'lua-upstream' and *K. wittei*, but both not in the other two (putative) species studied (not shown). Sexual dimorphism in other measurements even seems species–specific, because it only occurs in one species. This seems true for the prepectoral distance, which seems sexually dimorphic in *K.* sp. 'lua-upstream' only. This question of a

general vs. a less inclusive or even species–specific sexual dimorphism is further detailed in a study by Abwe [28], treating populations from the KP and its surroundings as a whole.

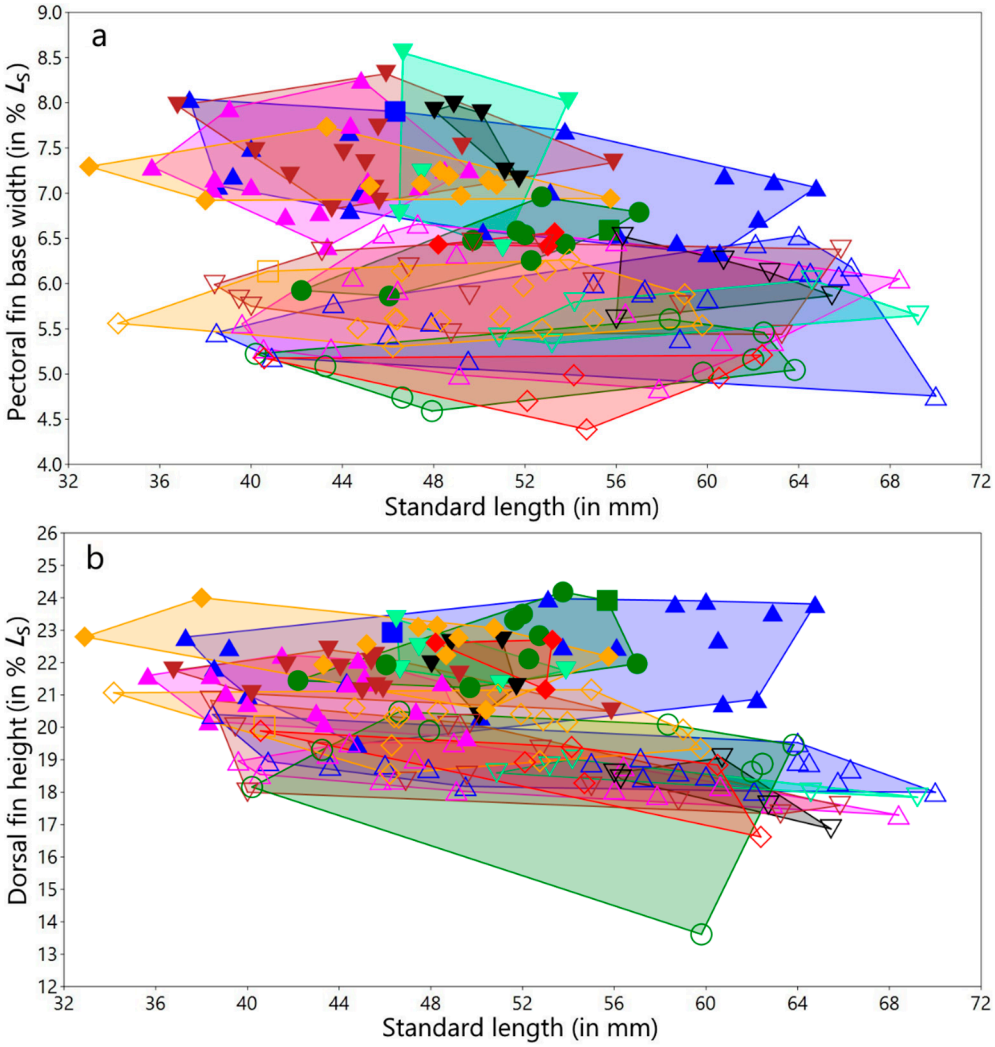

Figure 14. Scatterplot of (**a**) pectoral fin base width (in % standard length) against standard length (L_S) in mm; and (**b**) dorsal fin height (in % standard length) against standard length (L_S) in mm. *Kneria* sp. 'lua-upstream' (=*K. luansaensis* sp. nov.), populations from up-to downstream: ■, holotype (male), ▲, males and △, females from KP1L; ▲, males, and △, females from KP2M; ▼, males, and ▽, females from IntP1; ▼, males, and ▽, females from IntP2; and ▼, males, and ▽, females from IntP3. *Kneria* sp. 'lua-downstream' (=*K. maxi* sp. nov.): ■, holotype (male); ●, males, and ○, females from below the last Sanshifolo falls. *Kneria wittei*: □, holotype (female); and ◆, males, and ◇, females. *Kneria stappersii*: ◆, males, and ◇, females. All Luansa populations, from up-to downstream: KP1L: Luansa River on the KP, loc 1; KP2M: Milembwe River on the KP, loc 2; IntP1: intermediate plateau 1; IntP2: intermediate plateau 2; and IntP3: intermediate plateau 3; ds: downstream, and us: upstream. Symbols: triangle (upstream Kasompola Falls); inverse triangle (downstream Kasompola Falls); circle (downstream of all falls); circle (downstream of all falls); and primary types (square).

3.2. Phylogenetic Exploration: Mitochondrial COI DNA–Sequence Analyses

A ML tree based on the mitochondrial COI gene revealed the presence of 11 well-supported *Kneria* clades for the included *Kneria* samples, including four described and, at least, seven candidate species (Figure 15). The two putatively new species of the Luansa River form two well-distinct clades. Both are also clearly distinct from both the clades of *K. stappersii* and *K. wittei*, respectively (Figure 15).

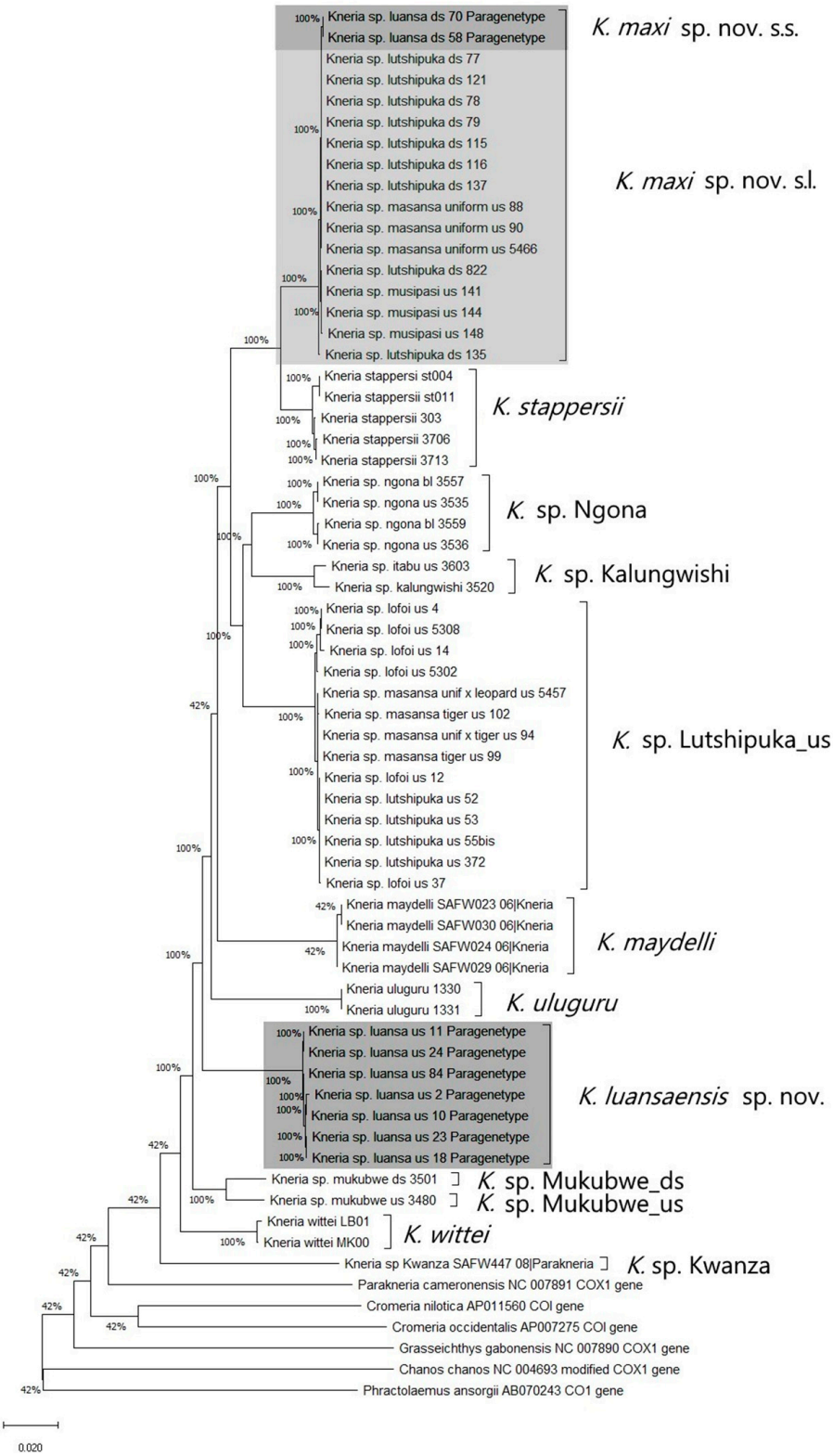

Figure 15. Maximum likelihood phylogenetic tree (67 specimens) based on COI sequences (1551 bp alignment length). Statistical node supports (500 bootstrap replications) are illustrated. s.l.: sensu lato; s.s.: sensu stricto. *Kneria* sp. 'lua-upstream' (=*Kneria* sp. luansa us = *K. luansaensis* sp. nov.) (n = 8) and *K.* sp. 'lua-downstream' s.s. (=*Kneria* sp. luansa ds = *K. maxi* sp. nov. s.s.) (n = 2) are shaded dark grey, while *K.* sp. 'lua-downstream' s.l. (*Kneria* sp. lutshipuka ds, *K.* sp. masansa uniform and *K.* sp. musipasi = *K. maxi* sp. nov. s.l.) (n = 15) is shaded light grey, but also includes both *K.* sp. 'lua-downstream' s.s. specimens.

The most downstream population, *K.* sp. 'lua-downstream' from the Luansa, is here named *K.* sp. 'lua-downstream' sensu stricto (s.s.) (see Figure 15 = *K. maxi* sp. nov. s.s.). This putative species is part of a larger clade, *K.* sp. 'lua-downstream' sensu lato (s.l.) (see Figure 15 = *K. maxi* sp. nov. s.l.), which contains also populations from the Lutshipuka Basin. Indeed, it is part of a larger clade consisting of specimens from the main Lutshipuka River (downstream section) and from its left bank tributaries Masansa and Musipasi (upstream sections), which hydrographically belong to the lower Luapula Basin. This finding might indicate that this new species might also occur outside the Luansa River Basin. This question is currently under further study and will be subject of a separate paper (Abwe et al., in prep). *Kneria* sp. 'lua-downstream' s.l. (see Figure 15 = *K. maxi* sp. nov. s.l.) is sister group to the well-supported (BS = 100) clade of *K. stappersii*. As such, *K.* sp. 'lua-downstream' s.l. is clearly distinct from both *K. stappersii* and *K. wittei* and diverges from those by a K2P genetic distance (GD) of 2.9 and 8.4%, respectively (Table 1).

The five upstream populations (*K.* sp. 'lua-upstream' KP1L, KP2M, IntP1, IntP2, and IntP3) form a single well-supported clade (BS = 100). Here, *K.* sp. 'lua-upstream' is the sister group to a clade containing *K. uluguru* from the upper Ruvu drainage, Sombesi River, draining the eastern slopes of Uluguru Mountains in Tanzania, *K. maydelli* from the Cunene River in Angola, and the entire Bangweulu–Mweru (B–M) ecoregion (ECR) species assemblage, which is composed of all populations/species from the B-M ECR, except for the clade composed of the two Mukubwe River populations (i.e., up-and downstream of Lupupa Falls), a southern bank tributary river of Lake Mweru–Wantipa. *Kneria* sp. 'lua-upstream' diverges from the most downstream population, *K.* sp. 'lua-downstream' s.s. by a K2P GD of 7.9% (Table 1). Finally, it is also clearly distinct from both morphologically highly similar species *K. stappersii* and *K. wittei* by a K2P GD of 7.6% and 8.8%, respectively (Table 1).

3.3. Taxonomic Decisions Based on the Integrative Approach

Based on an integrative approach, integrating the results obtained for the colour pattern, meristic, morphometric, and COI mtDNA genetic data, two new species for the science are identified from the Luansa River. One is composed of the five populations from the most upstream part on the Luansa River, i.e., above the Sanshifolo Falls III (KP1L, KP2M, IntP1, IntP2, and IntP3), and is here named *Kneria luansaensis* sp. nov. The other contains only the most downstream population on the Luansa River, i.e., below the Sanshifolo Falls, and is here named *K. maxi* sp. nov. The last, however, seems to be more widespread based on our genetic analysis of additional samples of neighbouring drainage systems (see Figure 15).

Table 1. Estimated average K2P and uncorrected p distances of evolutionary divergence over sequence pairs: (**a**) inter-group distance: lower triangle = K2P distances, and upper triangle = uncorrected p; and (**b**) intra-group distance. New species in bold; n/c for species with only one specimen included. *Kneria maxi* sp. nov. comprises both; all specimens of the clade named *K. maxi* sp. nov. s.l. as well as those, from the Luansa Basin, named *K. maxi* sp. nov. s.s.

Populations/Species		**a. Inter−Group Distance**												**b. Intra−Group Distance**	
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**K2P**	**Uncor P**
***K. luansaensis* sp. nov.**	**1**		7.0	8.8	8.2	9.5	7.9	6.6	6.7	7.5	8.2	8.5	7.1	0.2	0.2
***K. maxi* sp. nov.**	**2**	7.9		7.8	6.5	11.6	6.2	6.9	7.2	6.2	2.8	8.2	7.7	0.1	0.1
K. maydelli	**3**	9.6	8.3		9.0	11.3	8.1	7.4	8.1	7.4	7.5	8.5	7.8	0.1	0.1
K. sp. kalungwishi	**4**	8.7	7.0	9.8		10.7	5.6	6.8	6.4	5.0	6.1	7.8	6.7	1.0	1.0
K. sp. kwanza	**5**	10.5	12.9	12.5	11.8		10.8	10.4	10.1	10.7	11.0	11.2	8.7	n/c	n/c
K. sp. lutshipuka	**6**	8.6	6.6	8.7	5.9	12.0		6.7	6.1	5.3	6.1	7.1	7.1	0.2	0.2
K. sp. mukubwe ds	**7**	7.1	7.4	8.0	7.2	11.5	7.2		2.8	6.8	6.5	7.4	5.2	n/c	n/c
K. sp. mukubwe us	**8**	7.2	7.7	8.7	6.8	11.1	6.5	2.9		6.3	6.7	7.4	5.2	n/c	n/c
K. sp. ngona	**9**	8.1	6.5	7.9	5.2	11.8	5.6	7.3	6.8		6.1	7.7	6.7	0.3	0.3
K. stappersii	**10**	8.8	2.8	8.0	6.5	12.1	6.5	6.9	7.2	6.4		8.3	7.7	0.3	0.3
K. uluguru	**11**	9.2	8.9	9.2	8.4	12.4	7.5	7.9	7.9	8.3	9.0		8.2	0	0
K. wittei	**12**	7.6	8.3	8.4	7.2	9.4	7.6	5.4	5.4	7.2	8.3	8.8		0.3	0.3

3.4. Species Descriptions

3.4.1. *Kneria luansaensis* sp. nov.

Figure 16 & Table 2

Holotype: RMCA 2015-006-P-0731, male, 46.3 mm L_S, Luansa River, upstream of Kasompola Falls on the KP, upstream Kabyashya Village (10°18′15.9″ S 28°03′30.5″ E); Alt. 1389 m a.s.l.; DNA tag 747; KNP Expedition 2014, 19 September 2014.

Paratypes: RMCA 2015-006-P-0732-0733, two males, 37.3–39.7 mm L_S, same data as holotype DNA tags 749–750; KNP Expedition 2014, 19 September 2014. RMCA 2015-006-P-0734, one female, 38.5 mm L_S, Luansa River upstream of Kasompola Falls below the KP, upstream of Kabyashya Village (10°18′15.9″ S 28°03′30.5″ E); Alt. 1389 m a.s.l.; DNA tags 751; KNP Expedition 2014, 19 September 2014. RMCA 2016-038-P-0186-0187, two males, 39.2–40.0 mm L_S, Luansa River, upstream of Kasompola Falls below the KP, upstream of Kabyashya Village (10°18′15.9″ S 28°03′30.5″ E); Alt. 1389 m a.s.l.; DNA tag 001-002; KNP Expedition 2016, 20 August 2016. RMCA 2016-038-P-0186-0187, two females, 40.9–46.0 mm L_S, Luansa River, upstream of Kasompola Falls under the KP, upstream of Kabyashya Village (10°18′15.9″ S 28°03′ 30.5″ E); Alt. 1389 m a.s.l.; DNA tag 011-00; KNP Expedition 2016, 20 August 2016. RMCA 2016-038-P-0197-0199, three males, 49.2–55.9 mm L_S, Luansa River upstream of the second Sanshifolo (Kyanga) Falls from up to downstream, up the Kabyashya Village, below the KP (10°18′15.8″ S 28°04′42.5″ E); Alt. 1078 m a.s.l.; DNA tag 86, 87, 89; KNP Expedition 2016, 23 August 2016. RMCA 2016-038-P-0200, one male, 40.2 mm L_S, Luansa River upstream of the second Sanshifolo (Kyanga) Falls from up to downstream, up the Kabyashya Village, below the KP (10°18′15.8″ S 28°04′42.5″ E); Alt. 1078 m a.s.l.; KNP Expedition 2016, 23 August 2016. RMCA 2016-038-P-0201-0203, three females, 49.2–55.9 mm L_S, Luansa River upstream of the second Sanshifolo (Kyanga) Falls from up to downstream, up the Kabyashya Village, below the KP (10°18′15.8″ S 28°04′42.5″ E); Alt. 1078 m a.s.l.; DNA tag 84 and 2 without tag; KNP Expedition 2016, 23 August 2016. RMCA 2018-020-P-0016-0020, five females, 48.8–65.8 m L_S, Luansa River upstream of the second Sanshifolo (Kyanga) Falls from up to downstream (10°18′15.8″ S 28°04′42.5″ E); Alt. 1078 m a.s.l.; DNA tag 35–39 KNP Expedition 2017, 20 September 2017. RMCA 2018-020-P-0021-0026, six males, 43.5–45.9 m L_S, Luansa River upstream of the second Sanshifolo (Kyanga) Falls from up to downstream (10°18′15.8″ S 28°04′42.5″ E); Alt. 1078 m a.s.l.; DNA tag 40–45 KNP Expedition 2017, 20 September 2017. RMCA 2018-020-P-0037-0041, five males, 46.5–53.9 m L_S, Luansa River upstream of the third Sanshifolo (Kyanga) Falls from up to downstream (10°18′15.8″ S 28°04′42.5″ E); Alt. ~1078 m a.s.l.; DNA tag 46–49 and 54; KNP Expedition 2017, 20 September 2017. RMCA 2018-020-P-0042-0046, five females, 50.9–69.2 m L_S, Luansa River upstream of the third Sanshifolo (Kyanga) Falls from up to downstream ~(10°18′15.8″ S 28°04′42.5″ E); Alt. ~1078 m a.s.l.; DNA tag 50–53 and 55; KNP Expedition 2017, 20 September 2017. RMCA 2018-020-P-0047-0051, five females, 56.0–65.5 m L_S, Luansa River upstream of the first Sanshifolo (Kyanga) Falls from up to downstream (10°18′00.6″ S 28°04′38.8″ E); Alt. 1155 m a.s.l.; DNA tag 64–68; KNP Expedition 2017, 20 September 2017. RMCA 2018-020-P-0042-0056, five males, 48.0–51.7 m L_S, Luansa River upstream of the first Sanshifolo (Kyanga) Falls from up to downstream (10°18′00.6″ S 28°04′38.8″ E); Alt. 1155 m a.s.l.; DNA tag 69–73; KNP Expedition 2017, 20 September 2017. RMCA 2018-020-P-0067-0071, five females, 49.1–68.4 m L_S, Milembwe River, left tributary of the Luansa, ±50 m from the confluence, upstream of Kasompola Falls (10°18′10.8″ S 28°03′26.7″ E); Alt. 1336 m a.s.l.; DNA tag 75–77 and 80–81; KNP Expedition 2017, 21 September 2017. RMCA 2018-020-P-0072-0076, five males, 43.3–48.8 m L_S, Milembwe River, left tributary of the Luansa, ±50 m from the confluence, upstream of Kasompola Falls (10°18′10.8″ S 28°03′26.7″ E); Alt. 1336 m a.s.l.; DNA tag 78–79 and 82–84; KNP Expedition 2017, 21 September 2017. RMCA 2018-020-P-0077-0081, five females, 43.6–58.8 m L_S, Luansa River, about 50 m upstream of the confluence with the Milembwe, upstream of Kasompola Falls (10°18′16.7″ S 28°03′17.5″ E); Alt. 1394 m a.s.l.; DNA tag 88–92; KNP Expedition 2017, 21 September 2017. RMCA

2018-020-P-0082-0086, five males, 38.6–50.2 m L_S, Luansa River, about 50 m upstream of the confluence with the Milembwe, upstream of Kasompola Falls (10°18′16.7″ S 28°03′17.5″ E); Alt. 1394 m a.s.l.; DNA tag 93–97; KNP Expedition 2017, 21 September 2017.

Additional non-type material examined: RMCA 2015-006-P-0735-0738, 4 unsexed (not measured as used for visual observations only), same data as the holotype. RMCA 2016-038-P-0190-0192, three females, 40.9–46.0 mm L_S, Luansa River upstream of Kasompola Falls (Luansa) below the KP, upstream of Kabyashya Village (10°18′15.9″ S 28°03′30.5″ E); Alt. 1389 m a.s.l.; DNA tag 012-014; KNP Expedition 2016, 20 August 2016. RMCA 2016-038-P-0193-0195, three males (not measured as used for visual observations only), Luansa River upstream of Kasompola Falls (Luansa) on the KP, upstream of Kabyashya Village (10°18′15.9″ S 28°03′30.5″ E); Alt. 1389 m a.s.l.; DNA tag 009 and two without tag; KNP Expedition 2016, 20 August 2016. RMCA 2016-038-P-0196, one male (not measured as used for visual observations only), same data as 2016-038-P-0193-0195. RMCA 2016-038-P-0204, one female (not measured as used for visual observations only), Luansa River upstream of the second Sanshifolo (Kyanga) Falls from up to downstream, upstream the Kabyashya Village, on the KP (10°18′15.8″ S 28°04′42.5″ E); Alt. 1078 m a.s.l.; KNP Expedition 2016, 23 August 2016. RMCA 2016-038-P-0205-0211, seven females (not measured as used for visual observations only), Luansa River upstream of the second Sanshifolo (Kyanga) Falls from up to downstream, upstream the Kabyashya Village, on the KP (10°18′15.8″ S 28°04′42.5″ E); Alt. 1078 m a.s.l.; KNP Expedition 2016, 23 August 2016. RMCA 2016-038-P-0212-0217, six males (not measured as used for visual observations only), Luansa River upstream of the second Sanshifolo (Kyanga) Falls from up to downstream, up the Kabyashya Village, on the KP (10°18′15.8″ S 28°04′42.5″ E); Alt. 1078 m a.s.l.; KNP Expedition 2016, 23 August 2016. RMCA 2016-038-P-0218, one male (not measured as used for visual observations only), Luansa River upstream of the second Sanshifolo (Kyanga) Falls from up to downstream, up the Kabyashya village, on the KP (10°18′15.8″ S 28°04′42.5″ E); Alt. 1078 m a.s.l.; KNP Expedition 2016, 23 August 2016. RMCA 2016-038-P-0219-0234, 10 males, 51.3–60.0 mm L_S, (six not measured as used for visual observations only), Luansa River on the confluence with Milembwe, upstream of Kasompola Falls (Luansa) below the KP, upstream of Kabyashya Village; KNP Expedition 2016, 20 August 2016. RMCA 2016-038-P-0235-0260, 10 females 55.0–70.0 mm L_S, (16 not measured as used for visual observations only), Luansa River on the confluence with Milembwe, upstream of Kasompola Falls under the KP, upstream of Kabyashya Village; KNP Expedition 2016, 20 August 2016. RMCA 2016-038-P-0261-0280, 10 females 39.6–56.4 mm L_S (10 not measured as used for visual observations only), Milembwe River, left-bank tributary of the Luansa River upstream of Kasompola Falls (Luansa) on the KP, upstream of the village of Kabyashya (10°18′16.7″ S 28°3′17.5″ E); Alt. 1394 m a.s.l.; KNP Expedition 2016, 20 August 2016. RMCA 2016-038-P-0261-0280, 10 males 35.7–49.6 mm L_S, (10 not measured as used for visual observations only), Milembwe River, left-bank tributary of the Luansa River upstream of Kasompola Falls on the KP, upstream of the village of Kabyashya (10°18′16.7″ S 28°3′17.5″ E); Alt. 1394 m a.s.l.; KNP Expedition 2016, 20 August 2016. RMCA 2018-020-P-0027-0031, five females, (not measured as used for visual observations only), Luansa River upstream of the second Sanshifolo (Kyanga) Falls from up to downstream (10°18′15.8″ S 28°04′ 42.5″ E); Alt. 1078 m a.s.l.; KNP Expedition 2017, 20 September 2017. RMCA 2018-020-P-0032-0036, five males, 43.0–48.3 m L_S, Luansa River upstream of the second Sanshifolo (Kyanga) Falls from up to downstream (10°18′15.8″ S 28°04′ 42.5″ E); Alt. 1078 m a.s.l.; KNP Expedition 2017, 20 September 2017. RMCA 2018-020-P-0057-0061, five females (not measured as used for visual observations only), Luansa River upstream of the first Sanshifolo (Kyanga) Falls from up to downstream (10°18′00.6″ S 28°04′38.8″ E); Alt. 1155 m a.s.l.; KNP Expedition 2017, 20 September 2017. RMCA 2018-020-P-0062-0066, five males (not measured as used for visual observations only), Luansa River upstream of the first Sanshifolo (Kyanga) Falls from up to downstream (10°18′00.6″ S 28°04′38.8″ E); Alt. 1155 m a.s.l.; KNP Expedition 2017, 20 September 2017. RMCA 2018-020-P-0087-0111, 25 males, 38.9–52.7 m L_S, Luansa

River upstream of the third Sanshifolo (Kyanga) Falls from up to downstream (~10°18′15.8″ S 28°04′42.5″ E); Alt. ~1078 m a.s.l.; KNP Expedition 2017, 20 September 2017.

Diagnosis: In the Congo Basin s.l., *K. luansaensis* sp. nov. differs from their UC Basin congeners *K. paucisquamata* and *K. katangae* by its higher number of lateral line scales, 65–80 (vs. 58–63 for *K. paucisquamata*; and 55–59 for *K. katangae*), its higher number of pectoral fin rays, 15–16 (vs. 12–13 for *K. paucisquamata*; and 13 for *K. katangae*), its shallower body depth, 13.3–19.5% L_S (vs. 20.3–22.2% for *K. paucisquamata*; and 20.1–21.5 for *K. katangae*); from *K. katangae*, alone, by its wider mouth, 27.1–40.0% L_H (vs. 25.1–26.7% for *K. katangae*). It differs from *K. stappersii* by its wide body width, 9.8–11.6% L_S (vs. 8.6–9.4% L_S for *K. stappersii*), its relatively wide mouth, 27.1–40.0%, mean 33.4% L_H (vs. 22.6–28.6%, mean 27.0% for *K. stappersii*); from *K. wittei* by its life colour pattern, i.e., its general yellowish overall background colour covered with dark brown markings, the latter becoming more intense in gravid females, and most often without, as being present in few specimens only, a series of discrete black spots along the lateral line, a yellowish-grey-whitish caudal fin with often two marked oblique bands on the basal half of the fin on gravid females and less visible on non-gravid females and males, anal, dorsal, and light grey pelvic fins; and pectoral fin rose-whitish (vs. light brown overall background colour, with a series of discrete post-dorsal brown spots along the lateral line, a caudal fin of light brown colour without marked oblique bands at the base of both lobes in all specimens, dorsal, anal, and pelvic fins of light brown colour for *K. wittei*); and its relatively wide mouth, 27.1–40.0, mean 33.4% L_H (vs. 25.4–31.2, mean 28.4% for *K. wittei*). Further, in the Luansa River itself, it differs from its downstream congener, *K. maxi* sp. nov., by its wider body width, 9.8–11.6%L_S (vs. 8.0–9.1% for *K. maxi* sp. nov.), its relatively wide mouth, 27.1–40.0, mean 33.4% L_H (vs. 23.5–30.8, mean 28.1% for *K. maxi* sp. nov.), and by its relatively low total number of lateral line scales, 65–80, median 71 (vs. 75–93, median 84, for *K. maxi* sp. nov.).

Finally, *K. luansaensis* sp. nov. can be distinguished from the four other *Kneria* species reported, but not yet confirmed, for the Congo Basin s.l. as follows: from *K. angolensis*, *K. ansorgii* and *K. polli* by its higher number of pectoral fin rays, 15–16 (vs. 13–14 for *K. angolensis* and *K. ansorgii*; and 13 only for *K. polli*); from both *K. angolensis* and *K. ansorgii* by its lower number of soft anal fin rays, 6 (vs. 8 for *K. angolensis* and *K. ansorgii*); from *K. angolensis* only, by its lower number of lateral line scales, 65–80 (vs. 100 for *K. angolensis*); from *K. polli* by its higher number of dorsal fin rays, 7 (vs. 6 for *K. polli*); and from *K. auriculata* by its shorter dorsal fin height, 19.5–24.0 mean 21.7% L_S (vs. 24.4–25.6 mean 24.9% L_S for *K. auriculata*), its relatively short pre-dorsal distance, 45.4–51.4; and mean 48.6% L_S (vs. 50.4–51.0, mean 50.7% for *K. auriculata*), by its relatively longer predorsal distance 49.8–54.7, mean 52.5% L_S (vs. 48.1–50.6, mean 49.7% for *K. auriculata*)

Description: Representative male and female specimens are shown in Figures 5a–e and 16a,b. The counts and measurements are given in Table 2. The observed maximum size is 55.9 mm L_S in males and 70.0 mm L_S in females.

The body is elongated with the head length greater than the body height. The head is often slightly longer than wide, and its height is comparable to its width. The dorsal fin is located above the pelvic fins. The anterior base of the anal fin is closer to the origin of the pelvic than the caudal fin.

Sexual dimorphism: In females, the head width, head length, pre-dorsal distance, pre-pelvic distance, pre-anal distance, pre-pectoral distance, body height and pectoral fin length are longer, and the pelvic fin base width is wider or longer than in males (Table 2: F vs. M). Upper and lower lobe of the caudal fin is of the same size.

In males, the mouth width, eye diameter, post-dorsal distance, caudal peduncle height, caudal peduncle length, dorsal height, pelvic length, upper caudal fin lobe, lower caudal fin lobe, dorsal fin base width, pectoral fin base width, anal fin base width, and anal-caudal distance are wider or longer than in females (Table 2: M vs. F). Upper lobe of caudal fin is smaller than lower lobe.

Coloration in life: The coloration is variable according to (i) sampling period and (ii) sex and maturity stage for this species (see Figure 5).

In males (Figure 16a) and non-gravid females (Figure 5b3), colour pattern characterised by a general yellowish background colour covered by numerous small dark brown markings containing a less pronounced, dark brown, lateral band starting about midway between head and anterior base of dorsal fin; upper half of body with a wide yellowish band situated midway between dorsal fin and lateral line, starting above level of the opercular organ and reaching until base of caudal fin insertion. Caudal, anal, and pelvic fin are light grey; caudal fin is without two faint, brownish, vertical bands on basal half of both lobes. Pectoral fin is without black spot on its base; pelvic fin is always with a black spot at base, and anal fin is often with another one along its base. Eyes have a black pupil, surrounded by an orange rim and a dark brownish iris.

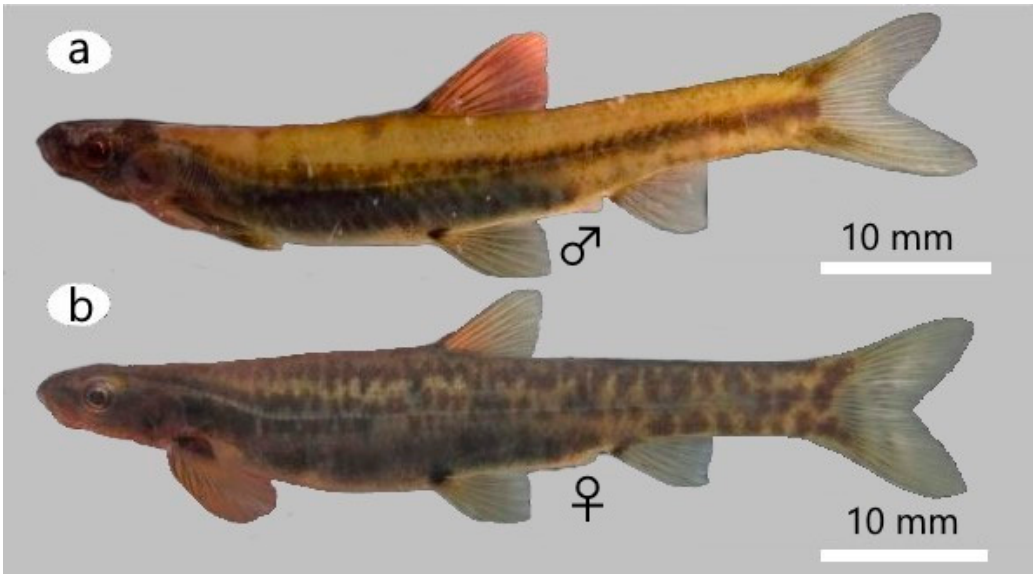

Figure 16. Life photographs of *K. luansaensis* sp. nov.: (**a**) RMCA 2018,020,P,0072-0076; ♂ 47.4 mm L_S, DNA tag 79; and (**b**) RMCA 2018,020,P,0067-0071; ♀ 49.1 mm L_S, DNA tag 75; Milembwe River, a left-bank tributary of the Luansa River upstream of Kasompola Falls (Luansa) on the KP, upstream of Kabyashya Village. 21 September 2017. The pink colour of the dorsal fin in the first specimen (**a**) is an artefact of the human hand keeping the specimen in place.

In gravid females (Figure 16b), instead, the colour pattern is characterised by a general yellowish-green background colour covered by numerous big dark brown, often irregularly shaped, markings, resulting in an overall far more dark brown appearance of the upper half of the body than in males and non-gravid females. Moreover, an often well-pronounced and continuous dark brown mid-lateral band starting at the level of a non-developed opercular organ is visible on gravid females (Figure 5b2); however it is sometimes less pronounced and far less continuous (Figure 16b). The upper half of the body with a narrow yellowish-green band situated about midway between dorsal fin and lateral line, starting at the level of the opercular organ and reaching until the base of the caudal fin. The yellowish-grey-whitish caudal fin often has two faint, brownish, vertical bars on basal half of both lobes. Pectoral and pelvic fins always have a black spot at their base and anal fin often with another one along its base. Eyes, as in males, have a black pupil surrounded by an orange rim and a dark brownish iris.

Coloration in alcohol: In alcohol, specimens lose their vivid yellowish-greenish colour, these parts becoming pale whitish, while nevertheless retaining their overall colour pattern, thus reinforcing the differences between the different pre-existing colour pattern elements.

Distribution and habitat: The species is currently only known from the five most upstream stretches sampled on the Luansa River above the Sanshifolo (Kyanga) Falls and its left bankd affluent the Milembwe River (Figures 2 and 17).

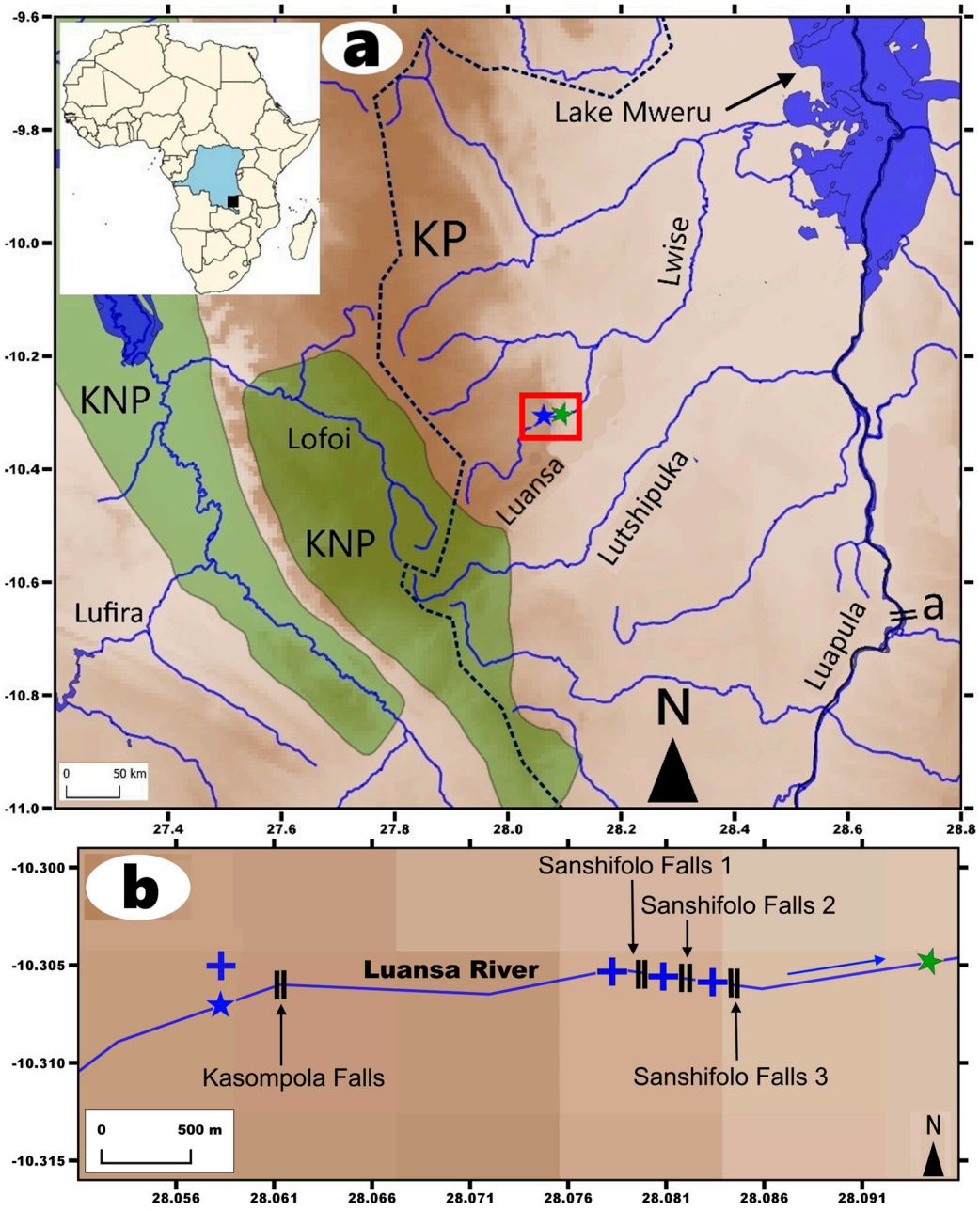

Figure 17. (**a**) Overall situation of the current distribution of both new species, i.e., *Kneria luansaensis* sp. nov., and *K. maxi* sp. nov. (**b**) Detailed areal view on all localities sampled (see red rectangle area on map a) for both new species. *Kneria luansaensis* sp. nov., ★ type locality and + other localities and *Kneria maxi* sp. nov., ★ type locality. Luansa River and its neighbouring basins, a. Mambilima Rapids, KP: Kundelungu Plateau. B-M ECR: Bangweulu–Mweru Ecoregion (right hand side of dotted line); UL ECR: Upper Lualaba Ecoregion (left hand side of dotted line); and Kundelungu National Park (KNP) (green areas).

The water level was low but there was a moderate current above the rocky substrate at some localities (Figure 18) and stagnate or slow moving water in the pool situated on the Milembwe River (SM: Figure S14). The depth of the water was variable (21 September 2017), about ≤50 cm above the rocky substrate and about ≤30 cm in the pool situated on the Milembwe River.

Figure 18. Habitat of *K. luansaensis* sp. nov., Luansa River ~50 m upstream of Kasompola Falls (Luansa) on the KP, upstream of Kabyashya Village (10°18′15.9″ S 28°03′30.5″ E); Alt. 1389 m a.s.l., 21 September 2017.

Physico-chemical parameters of the water at the type locality on the upper (on the KP) and middle (the three intermediate plateaux) Luansa River on 20–21 September 2017 between 9 a.m. and 4 p.m., where it is surrounded by gallery forest, taken during the sampling period: temperature: 17.7–22.7 °C; conductivity: 23.8–143.0 (µS); dissolved oxygen: 4.6–8.4 (mg/L); and pH: 6.0–6.7.

Etymology: The specific name, *luansaensis*, is a Latin adjective referring to the Luansa River to which the new species seems endemic.

Table 2. Proportional measurements and meristic characters of males and females for *Kneria luansaensis* sp. nov.; *K. maxi* sp. nov., *K. wittei*, and *K. stappersii*. h: holotype, m: mean, and me: median. Ranges for males or females include the holotype of the same sex, M: male, and F: female.

Measurements	*K. luansaensis* sp. nov.					*K. maxi* sp. nov.					*K. wittei*					*K. stappersii*				
	h (M)	Males (n = 56)		Females (n = 57)		h (M)	Males (n = 10)		Females (n = 9)		h (F)	Males (n = 8)		Females (n = 16)		Males (n = 3)		Females (n = 6)	
		Range	m	Range	m		Range	m	Range	m		Range	m	Range	m	Range	m	Range	m
Standard length L_S (mm)	46.3	35.6–63.0	47.1	35.0–70.0	53.7	55.7	42.2–57.0	51.3	40.2–63.8	53.8	40.7	32.9–55.8	46.4	34.2–59.8		48.2–53.3	51.5	40.6–62.4	54.1
Head length (L_H)	7.7	6.7–9.3	7.9	5.7–12.3	9.2	9.2	7.2–9.2	8.5	7.0–11.0	9.1	7.7	6.8–9.3	8.32	7.0–10.7	9.0	8.3–8.9	8.7	6.8–11.2	9.6
Head measurements (%L_H)																			
Post-orbital distance	43.2	35.1–47.6	42.1	37.6–48.3	43.1	44.9	41.9–48.8	45.6	40.2–50.0	45.6	41.8	39.7–50.7	44.4	39.7–49.5	45.0	42.2–45.2	43.7	44.2–47.4	45.5
Interorbital distance	42.8	28.9–47.1	38.5	35.9–48.9	40.7	37.2	31.9–41.0	38.2	35.8–41.0	38.9	40.5	36.8–40.0	38	36.8	39.1	37.4–42.1	40.2	37.2–39.6	38.9
Head height	61.0	51.8–67.1	57.6	51.5–66.2	57.3	56.7	51.4–62.3	58.2	52.6–64.2	58.2	55.6	52.9–66.8	58.3	51.9–62.3	57.3	54.0–55.9	54.7	51.1–61.3	54.7
Head width	60.7	51.3–67.6	59.0	57.0–71.2	62.7	55.2	50.0–60.3	56.7	49.1–60.3	55.9	58.2	51.8–62.9	56.6	52.1–67.3	58.8	50.6–54.2	52.5	42.6–56.5	52.0
Snout length	29.5	26.2–38.6	32.2	26.3–37.7	32.5	29.2	27.5–29.9	28.9	24.8–31.6	28.6	28.4	23.7–32.9	28.9	24.3–34.2	29.2	29.0–30.1	29.7	29.3–35.2	31.6
Mouth width	36.2	27.1–40.0	34.1	27.0–38.8	32.7	29.0	24.1–30.8	28.5	23.5–30.0	27.7	29.4	26.4–31.3	28.5	25.4–30.6	28.3	27.1–27.7	27.4	22.6–28.6	26.5
Eye diameter	24.6	22.0–34.0	27.1	21.9–31.0	25.0	24.0	22.3–26.5	24.2	22.0–26.4	23.7	26.1	20.6–27.9	23.8	21.0–26.1	22.7	27.1–27.7	27.4	24.5–29.3	26.7
Outer opercular organ diameter	38.0	28.8–48.2	40.8	–	–	43.6	34.0–47.6	41.3	–	–		32.0–43.2	38.3	–	–	34.9–38.6	36.6	–	–
Inner opercular organ diameter	22.0	16.1–23.7	20.5	–	–	19.6	18.3–21.9	20.2	–	–		16.2–25.0	21.1	–	–	19.8–22.9	21.4	–	–
Body measurements (%L_S)																			
Head length	16.7	15.4–19.9	17.1	15.3–19.7	17.5	16.5	15.4–18.2	16.6	15.4–17.9	17.1	18.8	16.4–20.7	17.9	17.1–20.3	18.3	16.5–17.2	16.8	16.8–18.2	17.7
Postopercular organ length	11.5	9.1–13.2	11.5	–	–	10.8	10.7–12.7	11.4	–	–		10.2–13.7	12.4	–	–	10.4–11.3	11.0		
Pre-dorsal distance	49.3	45.4–51.4	48.6	48.1–54.1	50.2	49.0	48.2–50.3	49.0	50.4–53.5	51.4	50.8	47.2–50.2	48.8	48.4–50.8	49.6	48.5–49.8	49.3	50.4–53.8	51.6
Pectoral-pelvic distance	34.7	32.3–37.7	34.5	31.5–37.9	34.4	30.6	30.6–34.7	32.1	30.4–34.5	32.2	34.1	31.1–33.9	33.0	31.1–34.1	32.8	31.5–32.5	32.0	31.4–35.0	32.3
Pectoral-anal distance	57.7	54.2–62.1	58.5	54.5–61.8	58.4	56.5	55.6–59.2	56.9	54.7–59.1	57.2	55.7	54.8–57.7	56.0	54.5–57.4	56.1	55.4–57.0	56.4	53.8–58.7	56.3
Post-dorsal distance	52.3	49.8–54.7	52.5	47.8–52.9	51.1	52.8	50.2–52.8	51.3	48.5–51.0	49.6	49.1	50.0–53.5	51.6	49.1–52.4	51.1	50.2–52.3	51.3	48.1–50.7	49.3
Pre-pelvic distance	49.3	45.5–51.7	48.4	46.8–52.0	49.4	46.6	45.7–50.2	47.2	45.3–49.4	47.9	51.6	48.1–50.3	49.1	47.1–51.6	49.4	46.3–47.3	46.9	46.5–50.5	48.2
Pre-anal distance	72.0	68.1–75.0	71.6	70.2–75.7	72.5	71.9	71.2–73.5	72.0	70.5–73.4	72.0	72.9	69.8–72.6	71.1	70.9–72.9	71.9	70.8–72.8	71.6	71.5–73.3	72.2
Pre-pectoral distance	15.5	13.1–18.2	14.9	14.54–18.1	16.0	14.5	13.6–16.9	14.9	14.4–16.3	15.5	18.9	14.5–17.7	16.0	15.5–18.9	17.0	15.1–16.1	15.5	16.2–18.5	17.1
Body height	15.7	13.4–19.2	16.0	13.1–19.6	17.1	14.7	14.7–16.8	15.3	14.2–17.6	16.2	14.7	13.0–15.7	14.4	11.8–15.6	14.0	13.7–15.4	14.6	12.6–15.0	13.8
Body width	10.2	9.7–12.3	10.6	9.9–12.0	10.7	8.0	8.0–9.0	8.8	8.7–9.1	8.8	10.6	9.6–10.8	10.1	9.7–11.7	10.4	8.4–9.2	8.9	8.6–9.1	8.9
Caudal peduncle height	9.9	8.1–11.7	9.8	8.1–12.1	9.6	10.9	9.5–10.9	10.2	8.8–10.4	9.6	10.1	9.5–12.0	10.8	9.2–11.5	10.2	10.3–10.9	10.6	9.2–10.6	10.0
Caudal peduncle length	22.5	18.7–22.5	20.9	18.2–22.9	20.2	19.03	18.3–20.2	19.3	18.2–21.4	19.2	18.4	18.2–20.6	19.4	18.2–20.7	19.7	20.8–21.5	21.0	19.6–22.1	20.6
Dorsal fin height	22.9	19.5–24.0	21.7	16.9–20.8	18.7	23.9	21.2–24.2	22.6	13.6–20.5	18.7	20.0	20.5–24.0	22.6	18.6–21.2	20.1	21.2–22.7	22.2	16.6–19.9	18.7
Pelvic fin length	21.0	15.5–21.0	18.5	15.3–19.1	17.0	17.7	15.8–18.9	17.7	14.8–19.1	17.1	18.7	17.9–20.9	19.1	16.1–20.5	18.3	18.1–18.8	18.5	15.4–18.1	16.9
Pectoral fin length	16.5	15.7–21.1	18.0	15.7–21.8	18.9	16.2	13.3–18.2	15.9	14.4–20.1	17.4	20.9	15.9–20.2	18.1	17.7–20.9	19.0	16.0–17.2	16.6	16.2–18.0	17.3
Upper caudal fin lobe	22.3	17.2–3.2	20.9	16.8–22.0	19.3	19.6	18.6–22.5	20.2	17.7–21.6	19.7	–	18.3–23.0	21.0	15.1–21.7	19.3	20.8–21.4	21.0	14.3–21.2	19.3
Lower caudal fin lobe	24.8	19.6–26.0	22.8	14.2–22.0	18.7	20.1	19.7–22.3	21.1	17.7–21.8	19.8	–	22.1–24.8	23.1	17.6–21.8	19.7	24.3–24.9	24.6	20.3–22.9	21.5
Dorsal fin base width	9.7	7.7–10.2	9.0	7.3–9.4	8.4	9.9	8.5–10.2	9.4	8.2–9.8	8.8	9.8	9.2–11.6	10.4	8.5–10.4	9.4	9.4–9.6	9.5	7.9–8.7	8.3
Pelvic fin base width	2.9	2.6–3.5	3.1	2.5–3.7	3.1	3.6	3.3–3.7	3.6	3.1–3.6	3.4	3.1	3.0–3.7	3.3	2.9–3.7	3.44	3.3–3.4	3.4	3.1–3.7	3.5
Pectoral fin base width	7.9	6.3–8.6	7.3	4.8–6.7	5.8	6.6	5.9–7.0	6.5	4.6–5.6	5.1	6.1	6.9–7.7	7.2	5.3–6.3	5.8	6.9–7.0	7.0	4.4–5.2	4.9
Anal fin base width	6.9	5.8–9.6	7.7	6.0–8.6	7.1	7.4	6.8–8.8	7.7	6.10–8.06	6.8	8.6	7.7–8.7	8.2	6.3–8.8	8.0	7.4–7.9	7.7	7.2–8.4	7.7
Pelvic-anal distance	24.2	22.1–28.2	25.0	21.1–27.3	24.9	24.6	23.9–26.0	25.0	22.6–27.9	25.3	22.1	22.1–24.5	23.0	22.1–25.1	23.4	25.2–26.3	25.7	24.0–26.5	25.2
Anal-caudal distance	30.5	26.2–31.8	29.5	26.4–30.9	28.3	28.7	26.8–29.2	28.5	27.2–29.0	28.1	26.8	27.1–30.8	29.0	26.8–29.8	28.4	28.8–30.2	29.5	27.3–29.9	28.7

Table 2. *Cont.*

Measurements	*K. luansaensis* sp. nov.					*K. maxi* sp. nov.					*K. wittei*					*K. stappersii*				
	h (M)	Males (*n* = 56)		Females (*n* = 57)		h (M)	Males (*n* = 10)		Females (*n* = 9)		h (F)	Males (*n* = 8)		Females (*n* = 16)		Males (*n* = 3)		Females (*n* = 6)		
		Range	m	Range	m		Range	m	Range	m		Range	m	Range	m	Range	m	Range	m	
Meristics	**h**	**range**	**me**	**range**	**me**	**h**	**range**	**me**	**range**	**me**	**h**	**range**	**me**	**range**	**me**	**range**	**me**	**range**	**me**	
Simple dorsal fin rays	3	3	3	3	3	3	3	3	3	3	–	3	3	3	3	3	3	3	3	
Branched dorsal fin rays	7	7	7	7	7	7	7	7	7	7	–	7	7	7	7	7	7	7	7	
Simple caudal fin	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
Branched caudal fin rays	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	
Simple anal fin rays	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
Branched anal fin rays	6	6	6	6	6	6	6	6	6	6	–	6	6	5.0–6	6	6	6	6	6	
Total pelvic fin rays	8	8	8	8	8	8	8	8	8	8	8	7.0–8	8	7.0–8	8	8	8	8	8	
Total pelvic fin rays	15	15–16	15	15–16	15	15	15–16	15	15–16	15	15	16	16	15–16	15	16	16	15–16	16	
Lateral line scales	72	66–80	71	65–80	72	84	78–89	83.5	80–90	87	75	61–78	72	64–78	71.5	84–87	86	85–91	87	
Lamellae in the postopercular organ	15	13–25	16	–	–	22	22–24	22	–	–	–	18–24	22	–	–	20	20	–	–	

3.4.2. *Kneria maxi* sp. nov.

Figure 19 & Table 2

Holotype: RMCA 2016-038-P-0310, male, 55.7 mm L_S, Luansa River, Wasalangana Oxbow arm, downstream of all falls, upstream of Kabyashya Village (10°18′05.6″ S 28°05′25.7″ E); Alt. 1062 m a.s.l.; DNA tag 056; KNP Expedition 2016, 22 August 2016.

Paratypes: RMCA 2016-038-P-0311-0314, four males, 48.7–57.0 mm L_S, same data as holotype. DNA tags 054, 055 (LSa7ABKW), 057, and 058; KNP Expedition 2016, 22 August 2016. RMCA 2016-038-P-0315-0318, four females, 43.3–62.0 mm L_S, same data as holotype. DNA tag 050-053; KNP Expedition 2016, 22 August 2016. RMCA 2018-020-P-0008-0011, four females, 46.6–63.8 mm L_S, Luansa River, Wasalangana downstream of all falls, upstream of Kabyashya Village (10°18′1.3″ S 28°5′37″ E); Alt. 1036 m a.s.l.; DNA tag 98, 99, 101, and 102; KNP Expedition 2017, 21 September 2017. RMCA 2018-020-P-0012, one male, 46.1 mm L_S, Luansa River Wasalangana site, downstream of all falls, upstream of Kabyashya Village (10°18′1.3″ S 28°5′37″ E); Alt. 1036 m a.s.l.; DNA tag 100; KNP Expedition 2017, 21 September 2017. RMCA 2018-020-P-0015, one male, 52.3 mm, same data as 2018-020-P-0008-0011.

Additional non-type material examined: RMCA 2016-038-P-0319-0323, five unsexed specimens (not measured as used for visual observations only), same data as holotype. DNA tags LSa1ABKW, 2ABKW, 3ABKW, 4ABKW, and 4BAKW; KNP Expedition 2016, 22 August 2016. RMCA 2016-038-P-0324-0328, five unsexed specimens (not measured as used for visual observations only), same data as holotype. Tag LSa6-10BAKW; KNP Expedition 2016, 22 August 2016. RMCA 2016-038-P-0329-0355, 27 unsexed specimens (not measured as used for visual observations only), same data as holotype; KNP Expedition 2016, 22 August 2016. RMCA 2018-020-P-0013-0014, two unsexed specimens (not measured as used for visual observations only), Luansa River, Wasalangana site, downstream of all falls, upstream of Kabyashya Village (10°18′1.3″ S 28°5′37″ E); Alt. 1036 m a.s.l.; KNP Expedition 2017, 21 September 2017.

Diagnosis: In the Congo Basin s.l., *K. maxi* sp. nov. differs from their UC Basin congeners *K. paucisquamata* and *K. katangae* by its higher number of lateral line scales, 78–90 (vs. lower, 58–63 for *K. paucisquamata*; and 55–59 for *K. katangae*), its higher number of pectoral fin rays, 15–16 (vs. lower, 12–13 for *K. paucisquamata*; and 13 for *K. katangae*), and its shallower body height, 14.2–17.6% L_S (vs. deeper, 20.3–22.2% for *K. paucisquamata*; and 20.1–21.5% for *K. katangae*); from *K. stappersii* by its general yellowish-brown background, with no spots on the lateral line, and with dorsal, caudal, anal, and pelvic fins of greyish-brown colour (vs. general brown background, often with eight pronounced black spots along lateral line, and with dorsal, caudal, anal, and pelvic fins of light -brown colour in *K. stappersii*), by its smaller eye diameter, 22.3–26.5% L_S (vs. 27.1–27.7% for *K. stappersii*), and by its short lower caudal fin lobe, 19.7–22.3 (vs. 22.1–24.8 for *K. stappersii*) in males; and from *K. wittei* by its narrower body width, 8.0–9.4% L_S (vs. 9.6–11.7% for *K. wittei*); and its yellowish-brown background, with a black dorsal region, caudal, anal, dorsal, and its pelvic fins being light grey, and pectoral fin being yellowish (vs. a light brown background, with a dark-brown dorsal region, mostly with a series of discrete, post-dorsal, brown spots along the lateral line, light brown dorsal, caudal, anal, and pelvic fins and orange pectoral fins in *K. wittei*).

Further, in the Luansa River itself, it differs from its upstream congener, *K. luansaensis* sp. nov., by its narrower body width, 8.0–9.1% L_S (vs. 9.8–11.6% for *K. luansaensis* sp. nov.), its relatively narrow mouth, 23.5–30.8, mean 28.1% L_H (vs. 27.1–40.0, mean 33.4% for *K. luansaensis* sp. nov.), and by its relatively high total number of lateral line scales, 78–90, median 80 (vs. 65–80, median 71, for *K. luansaensis* sp. nov.) (Figures 7 and 9).

Finally, *K. maxi* sp. nov. can be distinguished from the four other *Kneria* species reported, but r not yet confirmed, for the Congo Basin s.l. as follows: from *K. angolensis*, *K. ansorgii* and *K. polli* by its higher number of pectoral fin rays, 15–16 (vs. 13–14 for *K. angolensis* and *K. ansorgii*; and 13 only for *K. polli*); from both *K. angolensis* and *K. ansorgii* also by its lower number of soft anal fin rays, 6 (vs. 8 for *K. angolensis* and *K. ansorgii*);

from *K. angolensis*, only, also by its lower number of lateral line scale, 78–90 (vs. 100 for *K. angolensis*); from *K. polli* also by its higher number of dorsal fin rays, 7 (vs. 6 for *K. polli*); from *K. auriculata* by its smaller eye diameter, 22.0–26.0% L_H (vs. 32.0–32.8% for *K. auriculata*), its shallower body height, 14.2–17.6% L_S (vs. 18.2–18.7% for *K. auriculata*), and its narrower body width, 8.0–9.1% L_S (vs. 9.6–10.2% for *K. auriculata*).

Description: The holotype (male) and a representative female are shown in Figure 19. The counts and measures are given in Table 2. The observed maximum size is 57.0 mm L_S in males and 63.8 mm L_S in females.

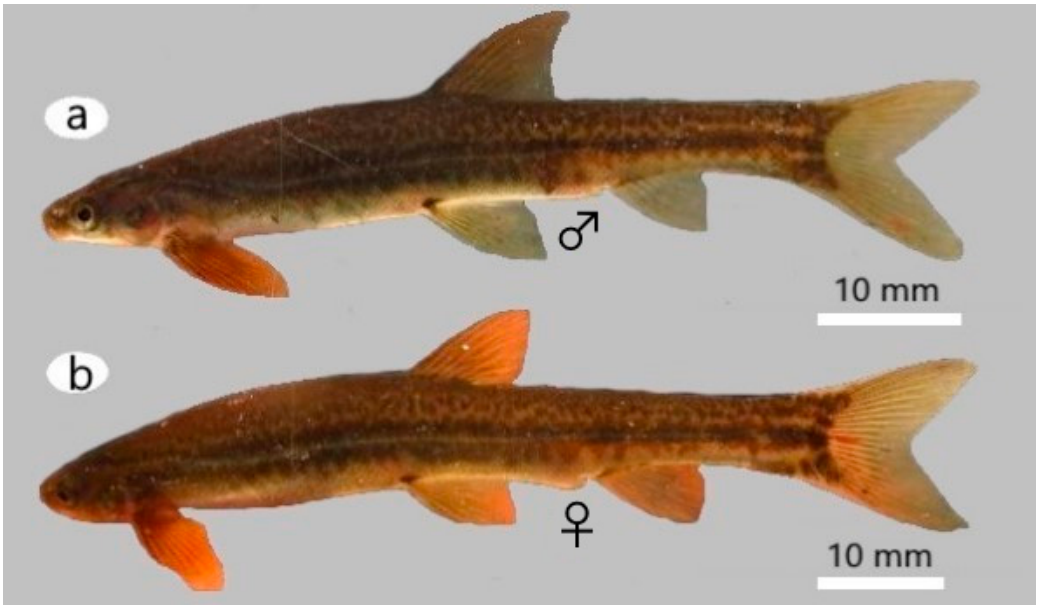

Figure 19. Life photographs of *Kneria maxi* sp. nov. (**a**) RMCA 2016-038-P-0310, ♂ 55.7 mm L_S DNA tag 056; (**b**) RMCA 2016-038-P-0311-0314, ♀ 62.0 mm L_S DNA tag 051; 22 August 2016; Luansa River, Wasalangana oxbow arm downstream of all the falls, and upstream of Kabyashya Village. The red/orange and pink colour of some of the fins is an artefact of a human hand keeping the specimen in place.

The body is elongated with the head length greater than the body height. The head is often slightly longer than wide, and its height is comparable to its width. The mouth is inferior, and of a narrow width. The snout is pointed. The body is narrow. The dorsal fin is located above the pelvic fins or slightly behind. The anterior base of the anal fin is closer to the origin of the pelvic than the caudal fin. The caudal is indented, with the longest caudal fin ray length of both the upper and lower lobe being of comparable length.

Sexual dimorphism: In females, the pre-dorsal distance and pectoral fin length are both longer than in males (Table 2: F). The dorsal fin origin is often closer to the base of the caudal fin than to the anterior tip of snout.

In males, the post-dorsal distance, dorsal fin height, and pectoral fin base width is longer, higher, and wider than in females (Table 2: M). Dorsal fin origin is often closer to the anterior tip of snout that to base of caudal fin. When present, in most specimens, the opercular and postopercular organs are relatively well developed (42.2–57.0 mm L_S).

Coloration in life: This species does not present any sexual dichromatism. Note that the absence of sexual variation in colour pattern for this species might be due to the fact that, up to date, only non-gravid females where sampled.

Males and non-gravid females, at least (Figure 19), have the same live colour, characterised by a general yellowish-brown background and a less pronounced continuous dark brown band, situated at the level of the lateral line, starting above the anterior level of the region of the opercular organ and reaching the caudal fin base (see specimens in Figure 19). The upper half of the body has a narrow yellowish-green band situated about

midway in-between the dorsal fin and lateral line, generally starting behind level of the region of the opercular organ, but most pronounced near the anterior base of the dorsal and pelvic fin until the base of the caudal fin and often more pronounced posteriorly, from dorsal fin level towards caudal fin base. The dorsal region of the head and body are darker brown, mottled, and the ventral region of the head and body, uniformly, is yellowish-white. The dorsal midline is often marked with 4–6 pre-dorsal and 4–6 post-dorsal, flared, dark brown, spots in large-sized specimens (≥~40.0 mm SL), but is not punctuated as such in smaller-sized ones. Caudal, anal, dorsal, pectoral, and pelvic fins are light grey. The pelvic fin is less visible with a black, ellipsoid spot at its anterior base and anal fin with a more elongated one at its mid-base. Eyes have a black pupil, surrounded by a yellowish-orange rim, with lower quarter of iris whitish, and the remaining part is darker brown.

Coloration in alcohol: In alcohol, the specimens lose their vivid yellowish/greenish colour and these parts become pale whitish, while nevertheless retaining their overall colour pattern, thus reinforcing the differences between the different colour pattern elements.

Distribution and habitat: The species sensu stricto is currently only known from its type locality on the Luansa River, which is situated downstream of the last Sanshifolo (Kyanga) Falls (Figure 17). Based on COI mtDNA evidence (Figure 15), the species is also possibly occurring in the lower Lutshipuka River and two of its tributaries, another left bank tributary of the lower Luapula Basin. However, this needs further confirmation.

The water level was low with a moderate current above the rocky substrate in some riverine localities, while for the water in the pool of the Wasalangana Oxbow arm (Figure 20), the depth of the water was variable; about ≤50 cm on the rocky substrate of the riverine localities and about ≥50 cm in the pool of the Wasalangana Oxbow arm was slow moving to stagnant (Figure 20).

Figure 20. Pool habitat of *K. maxi* sp. nov., Luansa River, Wasalangana Oxbow arm, downstream of the last Sanshifolo (Kyanga) Falls, upstream of Kabyashya Village (10°18′05.6″ S 28°05′25.7″ E); Alt. 1062 m a.s.l., 22 August 2016.

Physico-chemical variables of the water at the type locality on the lower Luansa River, i.e., the Wasalangana Oxbow arm, downstream of all falls, where it is surrounded by gallery forest and grassy savannah, taken during the sampling period, in the morning at 10 a.m. on 21 September 2017: 22.5–22.3 °C; conductivity, 53.2 (µS); dissolved oxygen, 7.4 (mg/L); and pH 6.2.

Etymology: This species is named in honour of Professor Max Poll (1908–†1991), a Belgian ichthyologist, for his extensive studies in African ichthyology, in general, and on the genus [13,18,20,23], in particular, which serves, to this very day, as a basis for the identification of species of the genus, and who was the first to identify *Kneria* specimens collected from the Luansa River. As *Kneria polli* Trewavas, 1936 was already dedicated to him, the species name here refers to his first name, Max.

4. Discussion

4.1. The External Morphological Characterisation of Sexual Maturity

4.1.1. The Opercular Organ

The presence of tubercles/lamellae on the opercular organ and their placement was reported to be of diagnostic value for species identification in the genus *Kneria* [16]. However, Peters [55] already pointed out that questions remained regarding the systematic value of this kind of morphological difference, as laboratory results reveal that the development of both the opercular and postopercular organs depends on the liberation of sex hormones, such as testosterone, and that it correlates with gonad (testes) maturation status. He inferred that only adult males can be used for systematic purposes, since reproductive state might affect the shape of the lamellae in the opercular organ, and that the ecological conditions, e.g., temperature, might further influence the development of both organs and the postopercular organ. From this, he postulated that the morphological characteristics of the opercular organ provided in the description of some *Kneria* species might be far less diagnostic than postulated, because this characterisation was often derived from a single sample from a single locality.

In the present study, comparatively large sample sizes were available, particularly for the two new species described herein, and these samples were collected in different seasons and from different localities. Thus, this sampling enabled us to show that even in a sample of specimens caught the same day, some males had tubercles/lamellae in the opercular organ, while others lacked them completely; and further, that even within the same population, tubercles/lamellae expression patterns may vary seasonally.

Nevertheless, the case of *K. luansaensis* sp. nov. exemplifies that size alone apparently does not determine lamellae presence in the opercular organ, as, e.g., 21 males ranging between 37.3 and 60.5 mm L_S (Figure 3a–e) feature lamellae, whereas 46 others of a roughly similar size (36.8–64.8 mm L_S) (Figure 3a–e) lack them. Some of these specimens were even collected at the same locality and on the same day. This suggests that not only seasonality and ecological conditions appear to determine the presence of these tubercles/lamellae in selected specimens, but possibly their maturity status or other factors, too. In contrast to the aforementioned case, virtually all *K. maxi* sp. nov. specimens ranging between 49.6 and 57.0 mm L_S, collected from the most downstream section of the Luansa River, featured lamellae in the opercular organ (Figure 3f). Only the two smallest studied specimens of 42.2 mm and 46.1 mm L_S, respectively (Figure 3f), lacked them completely. Thus, the development of lamellae in the opercular organ seems, somehow, apparently size and/or age related also—at least in some *Kneria* species.

4.1.2. The Postopercular Organ

The total number of lamellae in the postopercular organ was reported to be of diagnostic value in genus *Kneria*, too [16]. However, in some of the examined population/species, their number is positively allometric up to a certain size, i.e., to a size, above which the count does not further increase (Figure 4a,f,h). Nevertheless, in some population/species,

such a positive allometry could possibly be absent (Figure 4b–e), although this might be partially due to a limited sample of the respective size class (Figure 4c: population IntP1).

In *K. luansaensis* sp. nov. (Figure 4a–e), all specimens sampled in the same season, at the same site, and of similar size (35.7–55.8 mm L_S) had a rather consistent number of lamellae in the postopercular organ, i.e., 13–19 lamellae; this except for a subsample of ten out of twelve specimens from the uppermost locality of the Luansa above Kasompola Falls, which were somewhat larger (51.3–63.5 mm L_S) and had a higher count, i.e., 23–25 lamellae (Figure 4a; SM: Table S4). These ten specimens were collected on the same day (SM: Table S4; 8 August 2016: H–DS), together with two specimens having a lower number of 15 lamellae, and which were of a smaller size (39.2 and 40.0 mm L_S), thus being in the range as reported for the other localities for specimens of the same size range and collected during the same season. As such, the number of lamellae in the postopercular organ seems to be size dependent in this species.

In *K. maxi* sp. nov. (Figure 4f), the specimens ranging between 49.6 and 57.0 mm L_S had a high number of lamellae in the postopercular organ (22–24), and only the two smallest specimens (42.2 and 46.1 mm L_S) had either a lower number (16 for the 42.2 mm L_S specimen) or lacked it altogether (46.1 mm L_S specimen). Thus, the number of lamellae in the postopercular organ seems to be a function of size/maturation in this species, too.

The same holds true for *K. wittei*, since small-sized specimens had lower numbers of lamellae on the postopercular organ than large-sized ones (Figure 4h): the size of the five studied populations of this species, all collected during the cold dry season, ranged between 37.5 and 55.8 mm L_S, and these had a comparable number of lamellae in the postopercular organ (20–25), whereas only the two smallest specimens (32.2 and 32.9 mm L_S) had no more than 18 lamellae.

For the studied *K. stappersii* specimens, the data are less clear with regard to an allometric increase in the number of lamellae (Figure 4g): three large males (48.2–53.3 mm L_S) collected during the mid-rainy season (SM: Table S4) had a high number of 20 lamellae in the postopercular organ, whereas seven small specimens (37.3–45.0 mm L_S) collected during the hot dry season and from another locality had only 12–16 lamellae. Another four specimens of rather large size (46.5–50.9 mm L_S) collected during the late rainy season and from yet another locality had 16–18 lamellae, and two rather intermediate specimens (45.2 and 45.8 mm L_S) had only 12.

4.1.3. Body Tubercles on Males

The development of body tubercles might possibly be influenced by season, because, in general, the presence of body tubercles is considered a seasonal character of sexual maturity in several ostariophysan families, such as the Phractolaemidae and Kneriidae (order Gonorhynchiformes) or Cyprinidae (order Cypriniformes) [56–59]. Tubercles might serve, e.g., as epithelium protection [60,61], agonistic territoriality [59]), or contact facilitation during the spawning act [56]. As such, it is suspected, somehow, that also in *Kneria* spp., the development of tubercles might go hand in hand with the development of tubercles/lamellae (T/L–OP) in the opercular organ of males and that both are related to their reproductive activity, even if for the former, their actual function remains unknown. To test this hypothesis, an additional qualitative character, being the development of tubercles on the body, i.e., on the head and/or trunk, in relation to seasonality, i.e., main reproduction season (see below) or not, was also documented (SM: Table S15).

In general, according to our data, indeed, the presence of tubercles in the body correlates with the presence of tubercles/lamellae in the opercular organ of males. First, the complete absence of body tubercles in all *K. wittei* specimens presenting an opercular organ (T/L–OP) in stage 1 (see Figure 3h and SM Table S15), and almost all specimens of other populations/species in this stage, seems to confirm this hypothesis. Further, T/L–OP stage 2 appears to be a transitional one, as those specimens either lack or have body tubercles. Finally, T/L–OP stage 3 corresponds with the greatest development of body tubercles, and this all over the body.

Moreover, in two of the five populations of *K. luansaensis* sp. nov., being IntP2 and IntP3, the only ones for which the males have well-developed tubercles on the opercular organ, many of the syntopically and synchronically collected females are found to be gravid and vice versa. These observations seem, indirectly, to suggest that the development of tubercles and lamellae in the opercular organ of the males are both related to their reproductive activity.

The fact that in most males of *K. maxi* sp. nov., collected during the middle dry season and those of *K. stappersii*, also collected during the mid-dry season, have lamellae in the opercular organ but lack conical tubercles on both the head and body might possibly be since the specimens were collected after the rainy season (see SM Table S15). As the early and the late rainy season are, somehow (see below), likely the reproductive season, it is possible that, afterwards, the tubercles disappear faster than the lamellae in the opercular organ. Wild-caught specimens of *K. stappersii* caught by us in the peak of the rainy season (48.2–53.3 mm L_S), i.e., mid rainy season, neither have tubercles/lamellae in the opercular organ nor tubercles on the head or body, which might point to the fact that they are not in their reproductive period. Indeed, the lack of lamellae on the opercular organ, the absence of tubercles on the head or body, and also the absence of ripe eggs in females of *K. stappersii* collected by us during the peak of the rainy season suggest that reproduction is halted during this period. This situation appears comparable to *K. auriculata* from Mpumalanga, South Africa, for which the breeding season seems to correlate either with the early or with the late rainy season (September/October to April/May), thus avoiding the peak of the rainy season [62]. Nevertheless, field monitoring studies will be indispensable to document the actual extent of the reproduction period in *Kneria* species.

4.1.4. Sexual Dichromatism

Sexual dichromatism was observed in all populations of *K. luansaensis* sp. nov. Indeed, the colouration is clearly different between males and non-gravid females vs. gravid females collected during the early rainy season (localities: IntP2 and IntP3), but not for males and females collected during the peak of the dry season (albeit only documented for InP2). Thus, sexual dichromatism occurring at the start of the rainy season, a period characterised by high temperatures, might be expressed during the reproductive period only. This coincides with *K. luansaensis* males having well-developed lamellae on the opercular organ and well-developed conical tubercles on the body; the latter generally known to be related to breeding season and reproductive activities in fish [16,56]. Furthermore, most females with supposed breeding coloration were gravid, whereas females without supposed breeding coloration were confirmed as not being gravid (presence of eggs was tested by applying slight pressure on the abdomen to observe extrusion of eggs). Interestingly, none of the specimens of the studied *K. maxi* sp. nov., *K. stappersii* and *K. wittei* populations were sexually dichromatic and none contained gravid females. This may indicate that these populations were not sampled during the breeding season of those species, but this needs further, year-round collection of specimens.

4.2. Sexual Dimorphism beyond the Presence/Absence of the Opercular and Postopercular Organ

Sexual dimorphism (SD) in the genus *Kneria* was long known only by the presence of an opercular and a postopercular organ in males [13,14,16]. However, a detailed study of the sexual dimorphism in the *Kneria* of the KP and its surroundings revealed that it is not restricted to these organs, but that some counts and measurements are also sexually dimorphic ([28,63]; Abwe pers. data and in prep.). Particularly, certain sexually dimorphic differences in measurements seem to be present in all four studied species. These include the pectoral fin base width, being larger in males than in females (Figure 14a), and the dorsal fin height, being larger in males than in females as well (Figure 14b). A different set of measurements seems, however, only to occur in three out of the four studied species, i.e., dorsal fin base width, being larger in males than in females, and pre-pectoral distance, being larger in females than in males, and this for both measurements in *K. luansaensis*

sp. nov., *K. stappersii* and *K. wittei*. The same holds true for: the pre-dorsal and pre-pelvic distance, both larger in females than in males, in *K. luansaensis* sp. nov., *K. maxi* sp. nov., and *K. stappersii*. Furthermore, in another set of measurements, i.e., the post-dorsal distance, and pelvic length, both longer in males than in females, sexual dimorphism is only present in two of the four studied species, *K. luansaensis* sp. nov. and *K. maxi* sp. nov. The same holds true for the length of the lower caudal fin lobe and the caudal peduncle length, both longer in males than in females, and for which sexual dimorphism is thus present in both, *K. luansaensis* sp. nov. and *K. stappersii* only; and the head width, being larger in females than in males, and the caudal peduncle height, being larger in males than in females, and for which sexual dimorphism is thus present in both, *K. luansaensis* sp. nov. and *K. wittei* only. Finally, some sexually dimorphic measurements are even unique to a single, out of the four studied, species. This is true, for instance, for mouth width, eye diameter, head length, pre-anal distance, body height, pectoral length, upper caudal fin lobe, anal fin base width, and anal-caudal distance found to be sexually dimorphic in *K. luansaensis* sp. nov., and for which the largest sample size is available. The same holds true for snout length, which was found to be sexually dimorphic in *K. maxi* sp. nov. only (see Tables 2 and S7). The implications of these results for *Kneria* were and will be discussed elsewhere ([28]; Abwe, pers. data and in prep.), but they needed to be highlighted here in order to enable a sound description of both new species as provided herein.

4.3. Species Diversity in Kneria: A Concise Overview of an Underestimated Phenomenon

The genus *Kneria* is known to occur in six of the ten African ichthyogeographical provinces, i.e., the Angolan, Cape, Congolese, Eastern, Great Lakes of East Africa, and Zambezi provinces (sensu Levêque and Paugy [64]: figure 5.1). New species were described from each of these, except for the Cape (SM: Table S1).

Some species, such as *K. angolensis* and *K. ansorgii*, originally described from the Angolan Province, *K. auriculata* and *K. polli* originally described from the Zambezi Province, and *K. wittei*, originally described from the Congo Province, are currently considered to be widespread [2,8,22]. However, other species, such as *K. katangae*, *K. paucisquamata*, and *K. stappersii*, originally described from the Congo province, *K. rukwaensis*, originally described from the Great Lakes of East Africa Province and a nearby Lake Tanganyika tributary of the Congo Province, *K. ruaha* and *K. uluguru*, both originally described from the East Coast Province, and finally *K. maydelli* and *K. sjolandersi*, both originally described from the Zambezi Province, have more restricted distributions, as being only known from their type locality, a few nearby localities, or known as single river basin endemic species (SM: Table S1).

Seegers [16] concluded that collecting efforts were concentrated to certain regions, such as the Angolan and Congolese provinces; and that the taxonomic allocation of certain populations to presumably widespread species is mainly due to the lack of detailed studies on the alpha-taxonomy of the genus. Thus, it seems likely that the large distribution areas reported for some species simply reflect more intense (historical) collection activity in these areas (see SM Table S1). Illustrative of this situation are the four species originally described from beyond the borders of the Congo ichthyogeographical Province (SM: Table S1), but whose occurrence in the Congo Province was reported in the past: (1) *K. angolensis* reported by Boulenger [65]; (2) *K. ansorgii* reported by Poll [66]; (3) *K. auriculata* reported by [13]; and (4) *K. polli* reported by David and Poll [67]. Nevertheless, Poll [20] did only include two species, *K. auriculata* and *K. polli*, in his list of the Congo Basin, although he considered their occurrence in the basin as doubtful. In addition, in the CLOFFA, Poll [2] also only retained *K. auriculata* as present in the Congo Basin. However, Seegers [16] and Skelton [19] further questioned the presence of *K. auriculata*, originally described from the Zambezi Province, in the Congo Basin. This renders the status of these four species in the Congo Province unclear. Furthermore, several known populations of *Kneria* in Zambia and Zimbabwe are likely to represent new species for science [16]. Therefore, Seegers [16] also stipulated that more detailed studies on *Kneria* may alter the known distribution patterns, as shown by his study

on the Ruvu, Rukwa, upper Kalambo, and Ruaha Basins (see SM Table S1), which resulted in the description of three new *Kneria* species from the East Coast and Congo provinces.

Based on this inference, only the five species originally described from the Congo Basin s.l., i.e., *K. katangae*, *K. paucisquamata*, *K. rukwaensis*, *K. stappersii*, and *K. wittei* can safely be considered to occur in the Congo Province. Among these, *K. wittei* is known to have a widespread distribution, covering three ecoregions (sensu Thieme et al. [68]), including the upper Congo and the upper Lualaba ecoregions, with important *K. wittei* collections from the Upemba National Park [20], and the Bangweulu–Mweru ecoregion, with important *K. wittei* collections from the neighbourhood of the Kundelungu National Park [20,24,26].

The two new *Kneria* species described from the Luansa River are found to occur in what was previously reported to be part of the distribution area of *K. wittei* [2,20,24]. Both species are, however, quite different from this species since they are clearly distinguished from *K. wittei* by their distinct colour pattern (Figure 6). Further, the two new species are genetically well-distinct from *K. wittei*, and this with a K2P genetic distance of 7.1–7.7% for the COI mitochondrial gene (Table 1). In contrast, however, they are morphologically quite similar to *K. wittei*, explaining, in part, the misidentifications made by Seegers [20], which only used a morphological approach.

4.4. On the Possible Influence of Waterfalls on the Species Diversity in Kneria

Several authors stipulated that geographical segregation is the main factor of speciation in continental waters, particularly for Africa [64,69,70]. They further pointed out that the simplest mode of segregation is physical, this by impassable geographic barriers, i.e., waterfalls, or separation of watersheds. In addition, rapids may present strong ecological barriers, at least for poorly swimming fish species, as are, vice versa, swamps or lakes for some rheophilic species [69]. The Congo Basin [71], including some of its tributaries, such as the Chambeshi-Luapula-Luvua System (see [27]), contains numerous waterfalls that are important barriers, at least, to upstream fish migration [71].

Between the four described and valid *Kneria* species occurring in the upper Congo Basin sensu Roberts and Stewart [71], two belong to the Bangweulu–Mweru ecoregion, which is mainly covered by the Chambeshi-Luapula-Luvua system. It is divided into three sections, the upper, middle, and lower Luapula, respectively, by the ~15 m-high Mumbatuta Falls, more upstream, and the Mambilima Rapids (a 5 km stretch), more downstream [72,73]. Two valid *Kneria* species were originally described from the Luapula Basin, *K. stappersii*, known from the left bank tributaries of the middle Luapula [2,16]; and *K. paucisquamata*, known from the headwaters of the Luongo River [22], a right bank tributary of the middle Luapula [74,75]. These two species are, however, also well-separated from each other by the upstream Mumbuluma Falls (~54 m height) and, more downstream, Musonda Falls (~33 m height), both situated on the Luongo River itself [75]. Both these falls and rapids constitute barriers to fish migration between the middle Luapula, including lower Luongo against that of the middle and upper Luongo.

In addition to these two valid species, Seegers [16] reported the presence of an undescribed *Kneria* species in the upper Gumba (Ngumba), a small sub-tributary of the upper part of the Chambeshi River (Zambia) in the Bangweulu–Mweru ecoregion, not attributed to either of the two known species in the Chambeshi-Luapula-Luvua system or to any other congeneric species known to date. This river section, situated upstream of Lake Bangweulu, is separated by both the Mumbatuta Falls (~15 m height), and further downstream by the Mambilima Rapids, from more downstream river sections and *Kneria* populations/species. Indeed, this undescribed species seems to occur considerably farther upstream in the Bangweulu–Mweru ecoregion than both the *Kneria* species of the Luansa River studied here, thus further suggesting the importance of waterfalls, and possible rapids as well, in constraining kneriid species distribution and, possibly, speciation processes.

Considering the results of the present study, there are two new species to be added for the lower Luapula. These two new species were both found in the Luansa River, which is partitioned by two groups of falls, namely the Kasompola (Luansa) Falls (~170 m height) and the series of the three Sanshifolo (Kyanga) Falls (totalling ~75 m height) (Figure 2) [24,25]. However, the two studied populations from above the Kasompola (Luansa) Falls on the Kundelungu Plateau and the three from above the three Sanshifolo (Kyanga) Falls constitute a single species. The last of the three Sanshifolo (Kyanga) Falls, with a height of only ~25 m, constitutes the actual border between the distribution of the two species occurring in the Luansa River, being *K. luansaensis* sp. nov. and *K. maxi* sp. nov., with the latter only occurring downstream of these falls. Based on COI mtDNA barcoding data, *K. luansaensis* sp. nov. is not the sister species to its downstream congener, *K. maxi* sp. nov. This seems to imply independent colonisation of the up-and downstream stretch of the basin. Most likely, *K. luansaensis* sp. nov. first occupied the uppermost part of the Luansa on the KP and then, subsequently, occupied the different downstream sections, all upstream of the third Sanshifolo (Kyanga) Falls by downstream colonisation. However, it does not seem to have been able to colonise the most downstream part of the Luansa River, this below the third of the Sanshifolo (Kyanga) Falls, where *K. maxi* sp. nov. occur nowadays and thus possibly prevented further downstream colonisation due to competition [76–78]. In addition, ecological factors such as temperature, conductivity, dissolved oxygen, pH, etc., are also different up- and downstream in all three sections of the Sanshifolo (Kyanga) Falls [24], and thus also further hampered downstream colonisation. Moreover, *Kneria maxi* sp. nov. is the sister species of *K. stappersii*, known from the middle Luapula Basin [78], by diverging with a K2P GD of only 3.0%. Nevertheless, both are separated from each other by an ecological and/or physical barrier, namely the flood zones of the lower Luapula and the Mambilima Rapids on the Luapula mainstream.

The overall current situation might be explained by the past colonisation of the Luapula River. The presence of *Kneria* species at high altitudes might predate the uplift of the southern African highlands as a whole [16], which occurred in two successive episodes, in the later Neogene (~20 Mya) and during the Miocene (~5 Mya) [79], and the Katanga Plateaus, comprising the KP, which also uplifted in two successive episodes, the late Neocene (~20 Mya) and the early Quaternary (~2 Mya) [79–81]). This is much more recent than the estimated minimum age of *Kneria*, dated at 46 Mya based on fossil and genetic (i.e., both mt and nDNA) evidence [82,83].

Interestingly, the distribution of these two new *Kneria* species conforms to the general conclusion that the last (third) Sanshifolo (Kyanga) Falls (~25 m height) is a clear (hydro)physiographic barrier for most of the fishes [24], including *K. maxi* sp. nov. Indeed, it was pointed out that it is the first major waterfall encountered by fishes, thus hampering their further upstream migration on the Luansa River [24].

4.5. Protection of the Fishes of the Luansa River, the KP, and Its Surroundings

The Bangweulu–Mweru ecoregion was classified as vulnerable [68]. The major general anthropogenic threats identified are the rapidly growing human population and its encroachment, resulting in overfishing, overhunting, and deforestation of the surrounding areas [68,84,85]. When taking this ecoregion as a whole, there are three major anthropogenic threats to the Luansa River drainage. First, agricultural practices, such as slash and burn agriculture, that alter the riverbanks. These practices are carried out, for instance, by the inhabitants of the Katshupa guard post on the Kundelungu Plateau inside the national park, and by inhabitants of Kabyashya Village, the latter situated downstream of all the falls ([24]; L.N.K., pers. obs. 2017). Second, collective fishing by poisoning with "Buba", the local Bantu name for *Tephrosia vogelii* (family: Fabaceae) in the Lamba language, is practiced all along the Luansa River ([25]; L.N.K., pers. obs. 2017). Finally, logging for charcoal production results in deforestation and riverbank destruction, as due to the proximity of the major town of Lubumbashi, a charcoal trade developed at Kabyashya Village (L.N.K., pers. obs. 2017). Therefore, to quantify the current status of the Bangweulu–Mweru Ecoregion,

it was suggested that research should be carried out on the protection status of some of the threatened fish species, such as the cichlids of Lake Bangweulu [68] and the annual killifishes, i.e., *Nothobranchius symoensi* and *N. rosenstocki*, both endemic to the upper Luapula system, assessed as endangered and vulnerable, respectively [85–87]. It was further suggested to assess the impact of the fisheries in this ecoregion in general [68]. Therefore, such research on existing anthropogenic impacts and status should not be carried out for at least one of both new *Kneria* species only, i.e., the one with an apparently narrow range in the Luansa, but for the ichthyofauna of the Luansa River system as a whole. The Luansa Basin drains part of the Kundelungu Plateau and its surroundings, which was identified as a key biodiversity area, defined as sites contributing significantly to the global persistence of biodiversity (see [88,89]), and this despite its overall fish fauna remaining poorly known ([28,63]; and Abwe et al., in prep.). Unfortunately, as of today, no specific freshwater species protection measures are in place neither for the Kundelungu Plateau, nor the Kundelungu National Park and its surroundings.

The Luansa River itself is identified as of scientific interest due to its diversity in biotopes, covering springs, marshes, gallery forests, waterfalls, etc., and the occurrence of species with restricted distributions and thus, most likely, also a place for the occurrence of new species [90]. As such, the area was retained in the inventory of continental waters worthy of being proposed for conservation [90]. The discovery of two new *Kneria* species for science in the Luansa River, one endemic to the Luansa on the Kundelungu Plateau and its eastern flank, and the other described from the Luansa downstream of the falls in the plains zone, but possibly more widespread in the lower Luapula Basin (see [28]), supports its proclaimed scientific and conservation importance as well as its potential to hold new species for science. However, although both new species are occurring on and/or around the Kundelungu Plateau, both are only home to the buffer zone of the Kundelungu National Park, defined as within a 50 km range outside the borders of the park (DRC-Ord. n° 75-097 of 1 March 1975), and thus more prone to conservation issues of all kinds. Therefore, it is hoped that their discovery further highlights the urgent need for a better protection of this freshwater key biodiversity area and its surroundings as a whole.

5. Comparative Material Examined

5.1. Kneria ansorgii

Angola. Luculla Basin, BMNH 1910.11.28. 58–59, syntypes, 2, 55.6–58.0 mm L_S (males), Luculla River at Luculla, Angola.

5.2. Kneria auriculata

Mozambique. MNHN 1905-0119, lectotype, 1, 35.7 mm L_S (male), Muza River. MNHN B-2554 [ex MNHN 1905-0119], paralectotype, 1, 34.3 mm L_S (male), Muza River. RMCA 154710-711, paralectotype, 1, 36.3 mm L_S (male), Muza River.

5.3. Kneria katangae

DR Congo. Lower Lufira Basin, RMCA 79-1-P-1274, holotype, 1, 36.5 mm L_S (male), Mubale, confluent area Mubale-Munte, tributary of the Lufira River. RMCA 79-1-P-1276-314, paratypes, 3, 32.6–38.5 mm L_S (males), Mubale, confluent area Mubale-Munte, tributary of the Lufira River.

5.4. Kneria paucisquamata

Zambia. RMCA 74-83-P-1-2, paratypes, 2, 33.3–41.7 mm L_S (males), headwaters of Luongo River, 30 kilometres west of Luwingu. RMCA 74-38-P-10-14, paratypes, 5, 45.3–50.6 mm L_S (females), Headwaters of Luongo River, 30 kilometres west of Luwingu.

5.5. Kneria polli

Angola. Cuvo River system, BMNH 1935.3.20.52-56, syntypes, 2 of 5, 40.3–48.5 mm L_S (males), Mount Moco, Cuvo River system.

5.6. Kneria stappersii

DR Congo, middle Luapula Basin, Lubumbashi River sub-basin, RMCA 11,836–11,838, syntypes, 1 of 3, 29.0 mm L_S (unsexed), Lubumbashi River, 5–8 kilometres downstream from Elisabethville [Lubumbashi]. RMCA-2015-09-P-uncat, 1, 62.3 mm L_S (female), fish pond number 8, BEZHU, fish-breeding site at the Lubumbashi Zoo (Tilapia Fishing). RMCA 2016-038-P-uncat, 3, 40.6–60.5 mm L_S (females), Kamatete River, tributary of the Lubumbashi River, up and downstream of the culvert on the Kassapa Road. RMCA 2016-038-P-uncat, 3, 48.2–53.5 mm L_S (females), Kamatete River, tributary of the Lubumbashi River, up and downstream of the culvert on the Kassapa Road.

5.7. Kneria wittei

DR Congo. Lukuga Basin, IRSNB 70, holotype, 40.7 mm L_S, (female), Makala, near Albertville [Kalemie]. RMCA 2015-07-uncat, 2, 48.3–50.4 mm L_S, (males), Kyasombo River, near Makala village. RMCA 2015-07-P-uncat, 2, 44.7–48.3 mm L_S (females), Kyasombo River, near Makala village. RMCA 2015-07-P-uncat, 6, 43.3–55.8 mm L_S (males), Kamikuwa River, near Makala village. RMCA 2015-07-uncat, 11, 46.2–59.8 mm L_S (females), Kamikuwa River, near Makala village. RMCA-2015-07-uncat, 2, 32.9–38.0 mm L_S (males), Kaongo River, near Makala village. RMCA-2015-07-uncat, 2, 34.2–46.4 mm L_S (females), Kaongo River, near Makala village. RMCA-2015-07-uncat, 17, 38.2–48.6 mm L_S (female), Lubuye River, near Makala village.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15101044/s1.

Author Contributions: L.N.K., E.A. and E.J.W.M.N.V. were responsible for the fieldwork, fish identification and study design, and wrote the first and subsequent revised versions of the manuscript. In addition, E.J.W.M.N.V. supervised the research and critically revised the subsequent versions and the final version of the manuscript. A.C.M. collected the specimens of *K. wittei* from the surroundings of Makala Village, Lukuga Basin. U.K.S. and F.D.B.S. were mainly responsible for the molecular work, sequence data analyses participated in the writing and redactional supervision of the paragraphs related to the genetic work, and the whole manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was made possible by a DEA scholarship (2017–2019) to the first author, L.N.K., awarded by the SNSB-ZSM project "Exploiting the Genomic Record of Living Biota to Reconstruct the Landscape Evolution of South Central Africa" 2015–2019, and funded by the Volkswagen Foundation. It was also made possible by the Mbisa Congo I project (2013–2018), financed through a framework agreement project between the RMCA and the Directorate-General for Development Cooperation and Humanitarian Aid (DGD), which enabled the collection of the type specimens of the two new species. This study was completed thanks to two study visits (2017 and 2019) to the RMCA and the ZSM by L.N.K. within the framework of his DEA. These were, mostly, funded, respectively, by the RMCA ABIC program (2017) and the Mbisa Congo project II (2019–2023), a follow-up framework agreement project of the RMCA, both also funded by the DGD, with additional support of the "Exploiting the Genomic Record of Living Biota to Reconstruct the Landscape Evolution of South Central Africa" SNSB-ZSM project. Finally, the Mbisa Congo I project also enabled for the participation of the first author, L.N.K., to the PAFFA 2018 congress in Mangochi (Malawi) where this study's results were first presented.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We like to thank Nathalie Andries (RMCA), Muriel Van Nuffel (RMCA) and Eva November (RMCA) for their help regarding the organisation of the study visits of the first author, L.N.K. (2017 and 2019), to the RMCA. Further, we like to thank, Miguël Parrent (RMCA), Baudouin Willy (RMCA), and Dirk Neumann (ZSM) for cataloguing the newly made collections and their logistic assistance in finding specimens in the RMCA and ZSM collections respectively. We are grateful to Olivier Pauwels (IRSNB), Sébastien Bruaux (IRSNB), Zouhaira Gabsi (MNHN), Jonathan Pfliger (MNHN), Patrice Pruvost (MNHN) and James Maclaine (BMNH) for the loan of specimens under their care. Finally, we are also grateful to Felix Mbayo Lukasu wa Kabulo, Provincial Director of ICCN/Katanga and Jean-Pierre Jobogo Mirindi, former senior warden of the KNP, Evariste Mwape Musenge, the sector chief of Bakunda in the territory of Kasenga/Haut-Katanga, for the 2014–2017 permissions to collect fish in the KNP and Diakene Mankaila, a fisherman from the Kabyashya Village who helped us with our fish collecting efforts on the Luansa.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Abbreviations

Institutional abbreviations: AMNH, American Museum of Natural History, New York USA; BEZHU, unité de recherche en Biodiversité gestion et Exploitation durable des Zones Humides, UNILU, Lubumbashi, DRC; BMNH, British Museum of Natural History, London, UK (for the fish collections of the NHM); NHM, Natural History Museum, London, UK; RMCA, Royal Museum of Central Africa, Tervuren, Belgium; UNILU, Université de Lubumbashi, Lubumbashi, DRC; and ZSM, SNSB-Bavarian state collection of Zoology, Munchen, Germany. Other abbreviations: a.s.l., altitude above sea level; B–M, Bangweulu–Mweru ecoregion; C–DS, cold dry Season; DGD, Directorate-General for Development Cooperation and Humanitarian Aid; DRC, Democratic Republic of the Congo; IP, ichthyogeographical province; ECR, ecoregion; KNP, Kundelungu National Park; H–DS, hot dry season; L_H, head length; L_S, standard length; LPOP, lamellae in postopercular organ; L–RS, late rainy season; P–RS, peak rainy season; T/LOP, tubercles or lamellae of the opercular organ; MBISA CONGO, MBIsi SAmaki ya CONGO; MWU-test, Mann-Whitney U-test; OP, opercular organ; PAFFA, Pan African Fish and Fisheries Association; PC, principal component; and PCA, principal component analysis.

References

1. Poll, M. Nombre et distribution géographique des poissons d'eau douce africains. *Bull. Muséum Natl. D'histoire Nat.* **1973**, *150*, 113–128.
2. Poll, M. Kneriidae (129–133), Cromeriidae (p. 134), Grasseichthyidae (p. 135). In *Check List of the Freshwater Fishes of Africa*; Daget, J., Gosse, J.P., van den Audenaerde, D.F.E.T., Eds.; Royal Museum for Central Africa: Tervuren, Belgium, 1984; Volume I, p. 410.
3. Lavoué, S.; Miya, M.; Inoue, J.G.; Saitoh, K.; Ishiguro, N.B.; Nishida, M. Molecular systematics of the gonorynchiform fishes (Teleostei) based on whole mitogenome sequences: Implications for higher-level relationships within the Otocephala. *Mol. Phylogenetics E* **2005**, *37*, 165–177. [CrossRef] [PubMed]
4. Poyato-Ariza, F.J.; Grande, T.; Diogo, R. Gonorynchiform interrelationships: Historic overview, analysis, and revised systematics of the group. In *Origin and Phylogenetic Interrelationships of Teleosts. Honoring Gloria Arratia*; Nelson, J.S., Wilson, H.P., Schultze, M.V.H., Eds.; CRC Press: München, Germany, 2010; pp. 269–293.
5. Berra, T.M. *Freshwater Fish Distribution*; University of Chicago Press: Chicago, IL, USA, 2007.
6. Britz, R.; Moritz, T. Reinvestigation of the osteology of the miniature African freshwater fishes Cromeria and Grasseichthys (Teleostei, Gonorynchiformes, Kneriidae), with comments on kneriid relationships. *Zoosystematics Evol.* **2007**, *83*, 3–42. [CrossRef]
7. Lavoué, S.; Miya, M.; Moritz, T.; Nishida, M. A molecular timescale for the evolution of the African freshwater fish family Kneriidae (Teleostei: Gonorynchiformes). *Ichthyol. Res.* **2011**, *59*, 104–112. [CrossRef]
8. Fricke, R.; Eschmeyer, W.; Fong, J. *Catalog of Fishes: Genera, Species, References*; California Academy of Sciences: San Francisco, CA, USA, 2017.
9. Schelly, R.C. KNERIIDAE. *Poisson. D'eaux Douces Saumâtres Basse Guinée Ouest L'afrique Cent.* **2007**, *1*, 335–342.
10. Steindachner, F. Ichthyologische Mittheilungen. (IX.). *Verh. Zool. Bot. Ges. Wien* **1866**, *16*, 761–771.
11. Giltay, L. Notes ichthyologiques. IX. Contribution à l'étude du genre Xenopomatichthys (Kneriidae). *Bull. Musée R. D'histoire Nat. De Belg.* **1934**, *10*, 1–22.
12. Pellegrin, J. Poissons nouveaux du Mozambique Bull. *Muséum Natl. D'histoire Nat.* **1905**, *11*, 145–146.

13. Poll, M. Contribution à la Faune Ichthyologique du Katanga. *Ann. Musée Congo Belg.* **1933**, *1*, 52.
14. Trewavas, E. DR. KARL JORDAN'S Expedition to South-West Africa and Angola: The fresh-water fishes. *Novit. Zool.* **1936**, *40*, 63–74.
15. Lenglet, G. Contribution à l'étude de l'anatomie viscérale des Kneriidae. *Ann. Société R. Zool. Belg.* **1973**, *103*, 239–270.
16. Seegers, L. Revision of the Kneriidae of Tanzania with description of three new Kneria-species (Teleostei: Gonorhynchiformes). *Ichthyol. Explor. Freshw.* **1995**, *6*, 97–128.
17. Lenglet, G. Note sur la morphologie des Kneriidae (Pisces, Gonorhynchiformes). *Les Nat. Belg.* **1973**, *54*, 170–174.
18. Poll, M. Descriptions de poissons nouveaux recueillis dans la région d'Albertville (Congo Belge) par le Dr G. Pojer. *Bull. Du Musée R. D'histoire Nat. Belg.* **1944**, *20*, 1–12.
19. Norris, S.M.; Skelton, P.H. A Complete Guide to the Freshwater Fishes of Southern Africa. *Copeia* **1996**, *1996*, 755. [CrossRef]
20. Poll, M. *Exploration du Parc National de l'Upemba*; Poissons. Fondation pour favoriser les Recherches Scientifiques en Afrique, Bruxelles: Brussels, Belgium, 1976; p. 127.
21. Poll, M.; Gosse, J.P. Genera des poissons d'eau douce de l'Afrique. Mémoire de la Classe des Sciences. *Acad. R. Belg.* **1995**, *9*, 324p.
22. Froese, R.; Pauly, D. FishBase. Available online: www.fishbase.org (accessed on 16 March 2023).
23. Runge, J. The Congo River, Central Africa. In *Large Rivers: Geomorphology and Management*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2008; pp. 293–309.
24. Malaisse, F. *Ecologie de la Rivière Luanza = Ecology of the Luanza River*; Cercle Hydrobiologique de Bruxelles; Séries Exploration Hydrobiologique du lac Bangweolu et du Luapula: Brussels, Belgium, 1976; p. 151.
25. Malaisse, F. La pêche collective par empoisonnement au 'Buba' (Tephrosia vogelii Hook). Son utilisation dans l'étude des populations de poissons. *Nat. Belg. Bull. Mens.* **1969**, *50*, 481–500.
26. Malaisse, F. *Se Nourrir en Foret Claire Africaine: Approche Écologique et Nutritionnelle*; CTA: Chicago, IL, USA, 1997.
27. Van Steenberge, M.; Vreven, E.; Snoeks, J. The fishes of the Upper Luapula area (Congo basin): A fauna of mixed origin. *Ichthyol. Explor. Freshw.* **2014**, *24*, 329–345.
28. Abwe, E. *The Fish Fauna of the Kundelungu National Park (DR Congo): Diversity and Conservation*; KU Leuven: Leuven, Belgium, 2022.
29. Abel, R.; Thieme, M.L.; Revenga, C.; Bryer, M.; Kottelat, M.; Bogutskaya, N.; Coad, B.; Mandrak, N.; Balderas, S.C.; Bussing, W.; et al. Freshwater ecoregions of the world: A new map of biogeographic units for freshwater biodiversity conservation. *BioScience* **2008**, *58*, 403–414. [CrossRef]
30. Neumann, D.A. Kinesiology of the Hip: A Focus on Muscular Actions. *J. Orthop. Sports Phys. Ther.* **2010**, *40*, 82–94. [CrossRef] [PubMed]
31. Somers, K.M. Multivariate Allometry and Removal of Size with Principal Components Analysis. *Syst. Zool.* **1986**, *35*, 359. [CrossRef]
32. Bookstein, F.L.; Chernoff, B.; Elder, R.L. *Morphometrics in Evolutionary Biology: The Geometry of Size and Shape Change, with Examples from Fishes*; Academy of Natural Sciences of Philadelphia: Philadelphia, PA, USA, 1985; p. 15.
33. Hammer. *PAleontological STatistics (PAST), Version 3.10, Reference Manual*; Natural History Museum, University of Oslo: Oslo, Norway, 2015.
34. Snoeks, J. *The Cichlid Diversity of Lake Malawi/Nyasa/Niassa: Identification, Distribution and Taxonomy*; Cichlid Press: El Paso, TX, USA, 2004; p. 360.
35. Vreven, E.J.; Teugels, G.G. Redescription of Mastacembelus liberiensis Boulenger, 1898 and description of a new West African spiny-eel (Synbranchiformes: Mastacembelidae) from the Konkoure River basin, Guinea. *J. Fish Biol.* **2005**, *67*, 332–369. [CrossRef]
36. Vreven, E.J.; Snoeks, J. A new mastacembelid species from Lake Tanganyika: A case of complex evolutionary history. *J. Fish Biol.* **2009**, *75*, 1018–1047. [CrossRef] [PubMed]
37. Rice, W.R. Analyzing Tables of Statistical Tests. *Evolution* **1989**, *43*, 223. [CrossRef]
38. Moelants, T.; Mbadu, Z.V.; Snoeks, J.; Vreven, E. A review of the Distichodus antonii assemblage (Characiformes: Distichodontidae) from the Congo basin. *J. Nat. Hist.* **2014**, *48*, 1707–1735. [CrossRef]
39. UNESCO. The Miombo ecosystem. In *Tropical Forest Ecosystems, a State of Knowledge*; UNESCO: Paris, France, 1978.
40. Mutambala, P.K.; Abwe, E.; Schedel, F.D.B.; Manda, A.C.; Schliewen, U.K.; Vreven, E.J.W.M.N. A new Parakneria Poll 1965 (Gonorhynchiformes: Kneriidae), 'Mikinkidi' from the Upper Lufira Basin (Upper Congo: DRC): Evidence from a morphologic and DNA barcoding integrative approach. *J. Fish Biol.* **2022**, *102*, 4–26. [CrossRef]
41. Near, T.J.; Dornburg, A.; Friedman, M. Phylogenetic relationships and timing of diversification in gonorynchiform fishes inferred using nuclear gene DNA sequences (Teleostei: Ostariophysi). *Mol. Phylogenetics E* **2014**, *80*, 297–307. [CrossRef] [PubMed]
42. Roberts, T.R. Geographical distribution of African freshwater fishes. *Zoöl. J. Linn. Soc.* **1975**, *57*, 249–319. [CrossRef]
43. Chakrabarty, P. Genetypes: A concept to help integrate molecular phylogenetics and taxonomy. *Zootaxa* **2010**, *2632*, 67–68. [CrossRef]
44. Knebelsberger, T.; Stöger, I. DNA Extraction, Preservation, and Amplification. In *DNA Barcodes: Methods and Protocols*; Kress, J.W., Erickson, D.L., Eds.; Springer: Berlin/Heidelberg, Germany, 2012; pp. 311–338.
45. Drummond, A.J.; Ashton, B.; Buxton, S.; Cheung, M.; Cooper, A.; Heled, J.; Kearse, M.; Moir, R.; Stones-Havas, S.; Sturrock, S.; et al. 2010 Geneious v5.5. Available online: http://www.geneious.com (accessed on 16 March 2023).
46. Schedel, F.D.B.; Musilova, Z.; Schliewen, U.K. East African cichlid lineages (Teleostei: Cichlidae) might be older than their ancient host lakes: New divergence estimates for the east African cichlid radiation. *BMC E Biol.* **2019**, *19*, 94. [CrossRef] [PubMed]

47. Stamatakis, A. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **2014**, *30*, 1312–1313. [CrossRef] [PubMed]
48. Miller, M.A.; Pfeiffer, W.; Schwartz, T. Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. In Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA, USA, 14 November 2010.
49. Guindon, S.; Gascuel, O. A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. *Syst. Biol.* **2003**, *52*, 696–704. [CrossRef]
50. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. E* **2018**, *35*, 1547–1549. [CrossRef] [PubMed]
51. Kimura, M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. E* **1980**, *16*, 111–120. [CrossRef]
52. Srivathsan, A.; Meier, R. On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. *Cladistics* **2011**, *28*, 190–194. [CrossRef] [PubMed]
53. Bektas, O.; Jones, J.A.; Sankararaman, S.; Roychoudhury, I.; Goebel, K. Reconstructing secondary test database from PHM08 challenge data set. *Data Brief* **2018**, *21*, 2464–2469. [CrossRef] [PubMed]
54. Levin, B.A.; Simonov, E.; Matveyev, M.P.; Artaev, O.N.; Mustafayev, N.J.; Pashkov, A.N.; Roubenyan, H.R. DNA barcoding of the fishes of the genus Alburnoides (Actinopterygii, Cyprinidae) from Caucasus. *Mitochondrial DNA Part A DNA Mapp. Seq. Anal.* **2018**, *29*, 49–55. [CrossRef]
55. Peters, N. Opercular-und Postoperculararorgan (Occipitalorgan) der Gattung Kneria (Kneriidae, Pisces) und ein Vergleich mit verwandten Strukturen. *Z. Für Morphol. Und Okol. Der Tiere* **1967**, *59*, 381–435. [CrossRef]
56. Wiley, M.L.; Collette, B.B. Breeding tubercles and contact organs in fishes: Their occurrence, structure, and significance. *Bull. Am. Mus. Nat. Hist.* **1970**, *143*, 145–216.
57. Mittal, A.K.; Whitear, M. Keratinization of fish skin with special reference to the catfish Bagarius bagarius. *Cell Tissue Res.* **1979**, *202*, 213–230. [CrossRef]
58. Roberts, T.R. Unculi (Horny Projections Arising from Single Cells), an Adaptive Feature of the Epidermis of Ostariophysan Fishes. *Zoöl. Scr.* **1982**, *11*, 55–76. [CrossRef]
59. Manda, B.K.; Snoeks, J.; Decru, E.; Bills, R.; Vreven, E. *Enteromius thespesios* (Teleostei: Cyprinidae): A new minnow species with a remarkable sexual dimorphism from the south-eastern part of the Upper Congo River. *J. Fish Biol.* **2019**, *96*, 1160–1175. [CrossRef] [PubMed]
60. Ahnelt, H.; Keckeis, H. Breeding tubercles and spawning behavior in *Chondrostoma nasus* (Teleostei: Cyprinidae): A correlation? *Ichthyol. Explor. Freshw.* **1994**, *5*, 321–330.
61. Kortet, R.; Taskinen, J.; Sinisalo, T.; Jokinen, I. Breeding-related seasonal changes in immunocompetence, health state and condition of the cyprinid fish, *Rutilus rutilus* L. *Biol. J. Linn. Soc.* **2003**, *78*, 117–127. [CrossRef]
62. Kleynhans, C.J. Aspects of the ecology of kneria auriculata (Pellegrin, 1905) (pisces: Kneriidae) from the eastern transvaal, south africa. *J. Limnol. Soc. South Afr.* **1988**, *14*, 108–118.
63. Kalumba, L.N.; Abwe, E.; Frederic, S.; Manda, A.C.; Schliewen, U.; Vreven, E.J.W.M.N. Exploring the Kneria Steindachner, 1866 (Gonorhynchiformes: Kneriidae) «Miringiri» diversity of the Luanza and the Luongo rivers (Bangweulu–Mweru basin; DR Congo & Zambia). In *Proceedings of the Sixth International Conference of the Pan African Fish and Fisheries, PAFFA, Malawi, 24–28 September 2018, African Fish and Fisheries: Diversity, Conservation and Sustainable Management*, 6th ed.; PAFFA: Mangochi, Malawi, 2018; pp. 28–29.
64. Levêque, C.; Paugy, D. Geographical distribution and affinities of African freshwater fishes. In *The Inland Water Fishes of Africa: Diversity, Ecology and Human Use*; Paugy, D., Levêque, C., Otero, O., Eds.; RMCA: Tervuren, Belgium, 2017; pp. 97–114.
65. Boulenger, G.A. *Catalogue of the Fresh-Water Fishes of Africa in the British Museum (Natural History)*; Taylor and Francis: London, UK, 1909.
66. Poll, M. Revision des Characidae nains Africans. *Ann. Mus. R. De L'afrique Cent. Zool.* **1967**, *8*, 158p.
67. David, L.R.; Poll, M. Contribution à la faune ichthyologique du Congo Belge collections du Dr. H. Schouteden (1924–1926). *Ann. Musée Congo Belg. Zool.* **1937**, *3*, 189–294.
68. Thieme, M.L.; Abell, R.; Burgess, N.; World Wildlife Fund; Lehner, B.; Dinerstein, E.; Olson, D.; Teugels, G.; Kamdem-Toham, A.; Stiassny, M.L.J.S.; et al. *Freshwater Ecoregions of Africa and Madagascar: A Conservation Assessment*; Mendeley: Washington, DC, USA, 2005.
69. Daget, J. Evolution et spéciation dans les milieux autres que les grands lacs. In *Biologie et Écologie des Poissons D'eau Douce Africains*; Leveque, C., Bruton, M.N., Ssentongo, G.W., Eds.; ORSTOM: Paris, France, 1988; pp. 53–63.
70. Lévêque, C.; Agnèse, J.-F. La diversité des poissons africains: L'héritage de l'évolution. In *Les Poissons des Eaux Continentales Africaines: Diversité, Écologie, Utilisation par L'homme*; Lévêque, C., Paugy, D., Eds.; IRD: Paris, France, 2006; pp. 89–104.
71. Roberts, T.R.; Stewart, D.J. An Ecological and Systematic Survey of Fishes in the Rapids of the Lower Zaire or Congo River. *Bull. Mus. Comp. Zool. Harv. Coll.* **1976**, *147*, 239–317.
72. Worthington, E.B., III. The fishes (other than Cichlidæ) of Lake Bangweulu and adjoining regions, including descriptions of three new species. *Ann. Mag. Nat. Hist.* **1933**, *12*, 34–52. [CrossRef]
73. PJackson, B.N. *The Fishes of Northern Rhodesia: A Check List of Indigenous Species*; Lusaka, Govt.: Printer, Zambia, 1961.

74. Poll, M.; Stewart, D. Un Mochocidae et un Kneriidae nouveaux de la rivière Luongo (Zambia), affluent du bassin du Congo (Pisces). *Rev. Zool. Afr.* **1975**, *89*, 151–159.
75. Balon, E.K.; Stewart, D.J. Fish assemblages in a river with unusual gradient (Luongo, Africa-Zaire system), reflections on river zonation, and description of another new species. *Environ. Biol. Fishes* **1983**, *9*, 225–252. [CrossRef]
76. Romero, A.; Paulson, K.M. It's a wonderful hypogean life: A guide to the troglomorphic fishes of the world. *Biol. Hypogean Fishes* **2001**, *62*, 13–41. [CrossRef]
77. White, T.A.; Perkins, S.E. The ecoimmunology of invasive species. *Funct. Ecol.* **2012**, *26*, 1313–1323. [CrossRef]
78. Boulenger, G.A. Mission Stappers au Tanganika-Moero. Diagnoses de poissons nouveaux. II. Mormyrides, Kneriides, Characinides, Cyprinides, Silurides. *Rev. Zool. Afr.* **1915**, *4*, 162–171. [CrossRef]
79. Daly, M.C.; Green, P.; Watts, A.B.; Davies, O.; Chibesakunda, F.; Walker, R. Tectonics and Landscape of the Central African Plateau and their Implications for a Propagating Southwestern Rift in Africa. *Geochem. Geophys. Geosystems* **2020**, *21*, e2019GC008746. [CrossRef]
80. De Dapper, M. The micro-relief of the sand-covered plateaux near Kolwezi (Shaba, Zaire). 1. The micro-relief of the over-all dilungu. *Rev. Int. Géologie Géographie D'écologie Trop.* **1979**, *3*, 1–18.
81. Cotterill, F.P.D. The Upemba lechwe, Kobus anselli: An antelope new to science emphasizes the conservation importance of Katanga, Democratic Republic of Congo. *J. Zool.* **2005**, *265*, 113–132. [CrossRef]
82. Davis, M.P.; Arratia, G.; Kaiser, T.M. The first fossil shellear and its implications for the evolution and divergence of the Kneriidae (Teleostei: Gonorynchiformes). *Mesoz. Fishes 5-Glob. Divers. Evol.* **2013**, 325–362.
83. Lavoué, S. Origins of Afrotropical freshwater fishes. *Zool. J. Linn. Soc.* **2019**, *188*, 345–411. [CrossRef]
84. Chabwela, H.N. Current threats to the wetlands of Zimbabwe. In *Wetlands Ecology and Priorities for Conservation in Zimbabwe: Proceedings of a Seminar on Wetlands Ecology and Priorities for Conservation in Zimbabwe*; Matiza, T., Crafter, S.A., Programme, I.W., Eds.; IUCN: Gland, Switzerland, 1994; pp. 13–15.
85. Toham, A.K. *A Vision for Biodiversity Conservation in Central Africa: Biological Priorities for Conservation in the Guinean-Congolian Forest and Freshwater Region*; World Wildlife Fund: Gland, Switzerland, 2006.
86. Brooks, E.G.E.; Allen, D.J.; Darwall, W.R.T. *The Status and Distribution of Freshwater Biodiversity in Central Africa*; IUCN (International Union for the Conservation of Nature and Natural Resources): Gland, Switzerland; Cambridge, UK, 2011.
87. Brooks, R.P.; Serfass, T.L. Aquatic Habitat Classifications: Implications for Otter Conservation. In Proceedings of the 14th International Otter Congress: Sichuan, China, 8–19 April 2019; Volume 31, pp. 3–12.
88. Holland, R.A.; Garcia, N.; Brooks, E.G.E.; Juffe, D. Synthesis for all taxa. In *The diversity of Life in African Freshwaters: Underwater, Underthreat. An Analysis of the Status and Distribution of Freshwater Species throughout Mainland Africa*; Darwall, W., Smith, K., Allen, D., Holland, R., Brooks, I., Harrison, E., Eds.; IUCN: Gland, Switzerland; Cambridge, UK, 2011; pp. 228–269.
89. Brooks, P.D.; Chorover, J.; Fan, Y.; Godsey, S.E.; Maxwell, R.M.; McNamara, J.P.; Tague, C. Hydrological partitioning in the critical zone: Recent advances and opportunities for developing transferable understanding of water cycle dynamics. *Water Resour. Res.* **2015**, *51*, 6973–6987. [CrossRef]
90. Luther, H.; Rzo, J. *Project Aqua: A Source Book of Inland Waters Proposed for Conservation*; Blackwell Scientific: Oxford, UK, 1971.