

Supplementary Materials

SM. Details on the problematic taxonomic cases

SM: Kneriidae 1. *Kneria wittei* Poll, 1944 species complex

Kneria wittei differs from the 10 taxa identified from the Kundelungu Plateau (KP) and its surroundings by its live colour pattern (Figure 3al). In addition, it differs from five of these taxa (*K. sp.* 'katwei-ab', *K. sp.* 'seegersi-lofoi-mwena-bl', *K. sp.* 'luijiensis-ab', *K. sp.* 'restrictus-luiji-bl', and *K. sp.* 'luansa-bl') by its longer head length. Finally, it differs from the five remaining taxa either by its high number of lamellae in the post opercular organ (for *K. sp.* 'kasangaensis', *K. sp.* 'luijiensis' and *K. sp.* 'masansa-ab-uniform'), or morphometric characters such as its short pectoral-fin length (for *K. sp.* 'musipasi-ab') or its long post-dorsal distance (for *K. sp.* 'lutshipuka-bl') [56]. *Kneria stappersii* is different from seven of these 10 taxa by its live colour pattern and/or several other morphological characters. Besides that it is similar in morphology, and eventually in live colour pattern as well, to the three remaining species from the KP and its surroundings, to such extent that it can only be effectively distinguished by DNA barcoding [56]. This is the case for *K. sp.* 'lutshipuka-bl', *K. sp.* 'luansa-bl' and *K. sp.* 'seegersii-bl'. In fact, *K. stappersii* differs from the last two species only in live colour pattern, and not a single character was found to distinguish *K. stappersii* and *K. sp.* 'lutshipuka-bl'. Nevertheless *K. stappersii* diverges from these three species by a K2P GD of 2.6-3.2% on *COI* and 4.0-4.1% on *Cytb* [56].

SM: Kneriidae 2 *Parakneria thysi* Poll, 1965 complex

The newly collected specimens, here identified as *Parakneria thysi*, have slightly different life colour patterns when compared to topotypic and type specimens depending on the tributaries in which they were collected. They usually have three to five transverse bands on the caudal fin, a number which increases with the size of the fish (recorded size ranges 36.5-99.0 mm SL) [43], instead of three bands reported for *P. thysi* [41]. Furthermore, they also have four pre-dorsal spots and a dozen of medio-lateral spots. In fact, Poll (1965) [41] also reported such a series of medio-lateral spots, but did not report pre-dorsal spots. Their presence could not be verified on the types as they have lost their melanin pattern. An ongoing study is looking into this intra-specific variation [43]. Awaiting its results, however, no distinct species status is attributed to the different populations, although, what is currently regarded as *P. thysi* is to be considered a species complex.

SM: Kneriidae 3. *Parakneria malaissei* Poll, 1969 complex

Two populations have been collected at an altitude between 1005 and 1024 m. None of these specimens, however, belong to *P. malaissei* because of a higher number of lateral line scales, 95-104 (vs. 89-91). In addition, their colour pattern is somewhat different (Figures A3k-l and Figure 1 in [42]). Indeed, although the melanin pattern of the types of *P. malaissei* has faded, eight to nine medio-lateral spots forming a more or less continuous band and a series of well-marked black saddle-like blotches, especially on the post-dorsal midline, are still visible. The specimens from the Luisé River, in contrast, have a seemingly continuous band of more or less uniform width on the flanks (Figure A3o) and those from the Lutshipuka have numerous irregular, discontinuous black spots on the flanks (Figure A3p), especially on the post-dorsal midline. Detailed morphological and mitogenomic studies to establish the taxonomic status of these populations are ongoing. Awaiting these results, no distinct species status is attributed to these two populations, although, what is currently regarded as *P. malaissei* is considered a species complex.

SM: Cyprinidae 1. *Enteromius* of the chubby head barb species complex

Specimens of the chubby head barb species complex (CBSC) from the rivers of the Kundelungu Plateau (KP) were found to be different from *E. m. motebensis* and *E. m. kamaiaie* [48] by their lower number of lateral line scales, 28-32 (vs. 33-35 and 33-34 respectively), and lower number of scales between the lateral line and the dorsal-fin origin, 5 ½ (vs 6 ½ for both). No morphological differences were found

between the Lofoi population and those from river basins on the Eastern side of the KP. However, colour differences exist between the various populations, with some populations being light yellow without mid-lateral black stripe, while others are dark yellow with a discrete mid-lateral black band. Nevertheless, these populations are regarded as belonging to a single species referred to as *E. sp. 'kundelungu'*. This conclusion was supported by a *COI* mtDNA barcoding study [48]. *Enteromius sp. 'kundelungu'* was found to be the sister clade of a clade (*E. sp. 'luiji'*) containing the CBSC populations from the Luiji River (middle Lufira, Western side of the KP). The CBSC population from the Lutshipuka (*E. sp. 'lutshipuka-bl'*) belongs to a clearly different clade, not related to populations from the KP itself. Furthermore, this population is composed of more slender fishes compared to those from the KP (Figures A4a, b, c, vs. d) [48].

Although *Enteromius sp. 'luiji'* forms a single *COI* clade and is considered a single species, some differences were observed between the up- and downstream populations. The specimens of *E. sp. 'luiji'* from above the falls have a longer head and a shallower body than the specimens from below the falls (Figures A4a vs. A4b). In-depth morphological analyses are ongoing [48] to quantify the observed differences and further clarify the taxonomic status of these two populations.

SM: Cyprinidae 2. *Labeobarbus trachypterus* (Boulenger, 1915) complex

These three different phenotypes of *Labeobarbus* were found in specimens of all sizes, which seems to confirm the hypothesis of Vreven *et al.* (2016) [50] that also in this case the mouth phenotype is not size related as previously suggested by Banister (1973) [49]. However, in some rivers a dominance of one phenotype over the other was found. For example, specimens with a chiselmouth are most abundant in the Lofoi River, while those with rubber lips are most abundant in the Luiji River, although both are right bank tributaries of the mLf and seem to have a similar general habitat type. Only two specimens of *L. trachypterus*, both with a chiselmouth were collected in the Luisé, in the lower Luapula basin. However, no chiselmouth *Labeobarbus* species is currently known from the lower Luapula and Lake Moero from which *L. trachypterus* was described [50]. The intermediate-mouth phenotype specimens have a poorly developed or no mental lobe (Figure A4f). This group may be regarded as possible hybrids between a rubber lip and a chiselmouth species [51]. Hybridisation between a rubber mouth and a chiselmouth phenotype species has been reported from the Inkisi River (lower Congo), which led to a wide variation in intermediate-mouth phenotypes [51]. Nevertheless, some valid species include only specimens with an intermediate lip phenotype [49,50], such as *L. gestetneri* from the Kalumengongo River, a right bank affluent of the upper Lualaba, above its major falls on the Kibara Plateau.

SM: Cyprinidae 3. *Labeo rosae* Steindachner, 1894 problem

According to Van Steenberge *et al.* (2014) [53], considering the large discontinuity with its known natural distribution, this species could have been accidentally introduced into the mLf. Both specimens were identified as *L. rosae* based on three important counts that are within the range of this species, i.e. 20 circumpeduncular scales, 38 + 2 lateral line scales and 34 vertebrae. The counts of a third specimen (RMCA P-87516), previously identified as *Labeo sp.* are also within the same range and appears to be conspecific. Unfortunately, Van Steenberge *et al.* (2014) [53] did not compare the morphometrics of the *Labeo* specimens of the mLf with that of *L. rosae*. Comparison of principal measurements according to Reid (1985 [52]: Table 8 with both length classes combined, i.e. 50-250 mm SL) shows that the specimens of the mLf differ from *L. rosae* in their longer caudal peduncle, 110.2-133.0 (vs. 71.8-88.2 %SL in *L. rosae*), their shorter pre-dorsal distance, 42.2-44.7 (vs. 46.0-49.1 %SL), and their shallow body, 25.2-28.2 (vs. 32.5-39.1 %SL).

SM: Danionidae

Based on their counts, the specimens *Chelaethiops congicus* of the mLf are similar to *C. congicus* from the middle Congo. However, based on their measurements, the specimens of the mLf differ by their shallower caudal peduncle, which is 2 1/5 times to 2 1/3 times longer than deep (vs. only 1 1/2 times longer than deep in *C. congicus* [see 14,23,54]).

SM: Amphiliidae 1. *Amphilius uranoscopus* (Pfeffer, 1889) group of the KP and its surroundings

Among the five *Amphilius* new species, three are clearly different in colour pattern and/or caudal fin shape from all previously reported species in the region (Figures A5a, c-e). However, the specimens collected from the Masansa River above the falls and the Lutshipuka River downstream of the falls, here named *A. sp. 'murielae-masansa-ab/bl'* are similar to *A. cryptobullatus* mainly in their mottled colour pattern and in caudal their fin shape (Figure A5b). It differs, however, in one qualitative character, having a normally developed bilateral bony swim bladder capsule (vs. an extremely hypertrophied one in *A. cryptobullatus*) and three quantitative characters, i.e., 3, rarely 4, pre-dorsal vertebrae (vs. 2, very rarely 1 or 3) and a short 15.3-18.3 (vs. 20.8-23.8 %SL) and shallow adipose fin 2.1-3.3 (vs. 3.5-4.1 %SL) [56]. The specimens from the Luansa River, here named *A. sp. 'luansaensis'*, are more similar to *A. grandis* in overall morphology and colour pattern. It differs from *A. grandis* in one quantitative character, i.e. a large post-adipose distance, 11.0-14.5 (vs. 9.6-11.1 %SL in *A. grandis*) and a qualitative character, the colour pattern in life (Figure A5c vs. Figure 4-1. in [57]). Thus, both, *A. sp. 'murielae-masansa-ab/bl'* and *A. sp. 'luansaensis-ab'*, are also considered new species for science [56]. A study on three mitochondrial genes (*Cytb*, *COI* and *ND2*) supported the specific status of four KNP species including *A. sp. 'luansaensis-ab'*, which forms a distinct mtDNA genetic lineage from *A. grandis*. However, the specimens of *A. sp. 'murielae-masansa-ab/bl'* cluster with specimens of *A. cryptobullatus* from downstream of the Luongo Falls, whereas the *A. cryptobullatus* specimens from above the Luongo Falls (the type locality), fall in a well-separated clade [56].

SM: Amphiliidae 2. *Amphilius frieli* Thomson & Page, 2015 of the surroundings of the KP

The Lutshipuka specimens of *A. frieli* have a shorter adipose-fin length than specimens of *A. frieli* from other populations 12.7-16.7 (vs. 16.4-23.2 for *A. frieli* from the Lofoi, and 18.6-22.9 %SL for *A. frieli sensu* Thomson *et al.* (2015) [32]) and a shorter dorsal-fin base 8.0-10.0 (vs. 9.6-11 for *A. frieli* from the Lofoi, and 9.6-12.7 %SL for *A. frieli sensu* Thomson *et al.* (2015) [32]).

SM: Amphiliidae 3. *Zaireichthys* of the surroundings of the KP

Zaireichthys sp. 'luiji' differs from the populations identified as *Z. brevis* in its colour pattern. Indeed, *Z. sp. 'luiji'* is characterised by a large diffuse blackish mottling on the body, a uniformly greyish head, and a transverse black band on the caudal fin (vs. large irregular brownish spots on the body, a head with small black spots, and one to two transverse black bands on the caudal fin in *Z. brevis* [58,59]). In addition, the two species differ in their humeral process which is broad and short and not situated close to the interneural bone in *Z. sp. 'luiji'* (vs. thin, long, and almost touching the interneural bone in *Z. brevis* [58,59]).

SM: Mochokidae 1. *Euchilichthys royauxi* Boulenger, 1902 of the Lufira River

Two *Euchilichthys* specimens, a male and a female, both collected from the Kyubo Falls on the Lufira River by H.J. Bredo, have been identified as *E. royauxi* by Poll in 1946. Unfortunately, the locality data "Kyubo Falls, Lufira River, near Sampwe" does not provide any precision whether these specimens were collected from above or below the Kyubo Falls. In addition, Sampwe (9°21'11.32"S-27°25'59.09"E) is a city on the Luvua Drainage, a right bank affluent of the middle Lufira, about 50 Km far from the Kyubo Falls. As such the locality information remains very confusing. During our surveys, no new *Euchilichthys* specimens were collected from above the Kyubo Falls nor in the mLf tributaries and rapids. However, a single specimen of *Euchilichthys* was collected just below the Kyubo Falls by Bauchet Katemo Manda in 2017 (Katemo Manda, 2019, pers. data). It therefore seems likely that the two

Euchilichthys specimens collected by Bredo come from downstream the Kyubo Falls, since Poll (1976) [8] already reported seven specimens of this species from the Kilwezi River, a right bank tributary of the lower Lufira. As such the species has not been retained as part of the mLf basin's ichthyofauna.

SM: Mochokidae 2. *Synodontis denticulatus* Kasongo Ilunga, Abwe, Decru, Chocha Manda & Vreven, 2020 new species

A second species, also restricted to the mLf as *S. lufirae*, was collected during our expeditions. This *Synodontis* species had already previously been collected in 1960 (RMCA P-183345-183362) by D.F.E. Thys van den Audenaerde who identified it as *S. nebulosus* Peters, 1852 in the RMCA collections. However, *S. nebulosus* is only known from the middle and lower Zambezi systems [37]. Comparison of the specimens of the mLf with *S. nebulosus* revealed that they indeed differ from *S. nebulosus*, and represent a new species described by Kasongo Ilunga et al. (2019) [85].

SM: Mochokidae 3. *Chiloglanis* sp. 'luansa-luisé' vs. *C. congcicus* Boulenger, 1920

Chiloglanis sp. 'luansa-luisé' differs from *C. congcicus* in several features: the mandibular teeth, which are organised in one or two well-spaced lateral rows (vs. mandibular teeth not organized in distinct rows, but in a bouquet-like arrangement for *C. congcicus*), a short pre-anal distance, 64.9-72.6 (vs. 73.0-78.3 %SL), a short pre-pelvic distance, 53.3-58.6 (vs. 60.0-62.6 %SL) and a long pectoral spine, 13.9-17.0 (vs. 10.8-14.6 %SL). The two populations of *C. congcicus*, also differ from each other. Long external mandibular barbels are lacking in the topotypic specimens, while they are present in the specimens from the Kinsuka/Kintambo Rapids ([61]: Figure 16; [62]: Figure 1B) and the topotypic specimens have relatively short pelvic fins, 11.3-14.2 (vs. 13.2-14.2 %SL). Given these differences and the geographical distance between these two populations of *C. congcicus*, a more in depth study is necessary.

SM: Mochokidae 4. *Chiloglanis elisabethianus* (Boulenger, 1915) complex

The specimens of the *Chiloglanis elisabethianus* complex from the KNP s.l. differ from *Chiloglanis elisabethianus* by their: large pelvic fin length, 14.2-16.1 (vs. 12.1-14.1%SL for *C. elisabethianus*), and large anal-fin height, 18.3-21.7 (vs. 17.0-17.3%SL). In addition, the *Chiloglanis* specimens from the mLf only differ from those of the lower Luapula and *C. elisabethianus* in two additional characters: their long pre-anal length, 74.0-77.4 (vs. respectively 65.1-71.0 for *C. elisabethianus* and 69.4-71.7 for the lower Luapula *Chiloglanis*), and their long pre-pelvic length, 60.8-62.9 (vs. respectively 54.5-58.1 and 52.4-57.8 %SL). Due to these differences, the *Chiloglanis* specimens of the mLf have been identified as an undescribed species, here referred to as *C. sp. 'lufira'* (Figures A6c, c') and those from the lower Luapula subbasin are here referred to as *C. cf. elisabethianus* (Figures A6d, d'), pending an in-depth study. *Chiloglanis* sp. 'lufira' differs from *C. lufirae*, known from the Muye and Kilwezi rivers (lower Lufira) [8], by its fewer mandibular teeth aligned into a single row, 7-9 (vs. 10-14 teeth arranged in a bouquet-like for *C. lufirae*) and its forked caudal fin (vs. a truncated or emarginated caudal fin for *C. lufirae*).

SM: Auchenoglanididae

The specimens of *Parauchenoglanis* of mLf have a caudal peduncle that is higher than long following the definition as presented by Geerinckx et al. (2004 [29]: Figures 1 & 3); and a yellowish overall background colouration with small black spots arranged in 6 to 8 vertical bands along the flanks (Figure A5i). Using these two characters, the mLf specimens fall in the *P. punctatus* and *P. ngamensis* (Boulenger, 1911) species group (Geerinckx et al., 2004) [29]. The specimens of the mLf have a slightly serrated proximal base of the anterior margin of the pectoral spine, and two to three well-distinct serrations on the distal end of its anterior margin with the in-between margin being smooth; for about 1/3 to 2/3 of its entire length. Geerinckx et al. (2004 [29]; 2007 [64]) reported that both the anterior and the posterior margin of the pectoral spine are coarsely serrated in both *P. punctatus* and *P. ngamensis*. However, serrations on the anterior margin of the pectoral spine are not reported in *P. punctatus* nor in *P. ngamensis* by other authors [37,65]. An examination of the holotype of *P. ngamensis*, a rather large specimen of 184.1 mm SL, confirms the absence of serrations on the anterior side of the pectoral spine except for a single one

on its distal end. Conversely, an examination of the holotype of *P. punctatus*, a small-sized specimen of 64.0 mm SL, revealed the anterior margin of its pectoral spine bears small-sized and closely set serrations over its entire length.

Given the small size of the holotype of *P. punctatus*, a comparison was undertaken between *P. punctatus* specimens and some newly collected specimens from the mLf of the similar size classes (92.5-126.6 mm SL, n=4 and 58.2-115.8 mm SL, n=5, respectively). The *P. punctatus* specimens included the holotype from the Lindi River, a right bank tributary of Tshopo (middle Congo), one specimen from the Tshopo River (RMCA 1990-030-P-1191: 126.7 mm SL), both referred to as the Tshopo specimens, and three specimens from the Epulu River (Ituri basin) (RMCA 1990-030-P-1163-1181: 92.5-107.6 mm SL) from which *P. iturii* (Steindachner, 1911), an actual junior synonym of *P. punctatus* was described [29].

Given the isolation of the Epulu River from the Ituri main stream, conspecificity of specimens originating from both and of the Tshopo is to be verified. Therefore, the middle Lufira specimens were compared with the Tshopo and the Epulu specimens separately. The middle Lufira (mLf) specimens have, a high number of serrations on the posterior margin of the pectoral spine, 9-12 (vs. 7 in *P. punctatus* from the Tshopo), short maxillary barbels, 47.7-66.1 (vs. 92.0-94.9 % HL), short mandibular barbels, 83.9-97.1 (vs. 131.6-150.0 % HL), and short inner mandibular barbels, 33.8-45.8 (vs. 57.6-58.0 % HL). The mLf specimens are more similar to those of the Epulu than to those of the Tshopo. In fact, only slight differences were found between the mLf and the Epulu specimens, such as the smaller minimum height of the caudal peduncle, 10.6-11.7 (vs. 11.8-12.4 %SL for the Epulu specimens), the relatively short caudal peduncle length, 12.8-14.9 (vs. 15.0-15.8 %SL) and the shorter pectoral spine, 15.6-17.1 (vs. 17.2 -19.6% SL).

SM: Procatopodidae

Lacustricola sp. 'lofoi' can be differentiated from all its congeners by the presence of a dark blotch on pectoral fin insertion and on the end of the caudal peduncle, whereas *Lacustricola* sp. 'luiji' (Figure A7b) can be distinguished from all its congeners, by the presence of a deeper body and a bright yellow dorsal fin. *Lacustricola* sp. 'luiji' is similar to the deep body *Lacustricola* species that were previously placed in *Hypsopanchax* Myers 1924: *L. jubbi* (Poll & Lambert, 1965), *L. jobaerti* (Poll & Lambert, 1965) and *L. stiassnyae* (Van der Zee, Sonnenberg & Mayi Munene, 2015) [66]; (Bragança, unpublished data).

SM: Cichlidae 1. *Sargochromis mellandi* (Boulenger, 1905) complex

The number of scales around the caudal peduncle is a stable character in *Sargochromis*, as five out of the eight valid species [*S. coulteri* (Bell-Cross, 1975), *S. greenwoodi* (Bell-Cross, 1975), *S. mellandi*, *S. mortimeri* (Bell-Cross, 1975), and *S. thysi* (Poll, 1967)], always have 16 circumpeduncular scales. The three remaining species, i.e. *S. carlottae* (Boulenger, 1905), *S. codringtoni* (Boulenger, 1908) and *S. giardi* (Pellegrin, 1903), often have 16, but rarely may have up to 18 circumpeduncular scales [70]. *Sargochromis* sp. 'lufira', instead, is currently the sole *Sargochromis* species with 18 to 20 circumpeduncular scales. In addition, *Sargochromis* sp. 'lufira' differs from *S. mellandi*, by its shallow caudal peduncle depth, 11.5-13.1 (vs. 13.5-15.4 %SL for *S. mellandi*), its shallow body at anus, 24.5-33.3 [vs. 32.1-39.0 %SL for *S. mellandi* (overlap due to positive allometry)]; and its long caudal peduncle length, 15.4-18.8 (vs. 13.9-15.8 %SL for *S. mellandi*).

In addition to the number of circumpeduncular scales, *S. sp. 'lufira'* can also be distinguished from the three Zambezi species, being *S. carlottae*, *S. codringtoni* and *S. giardi*, by its lower body depth at pelvic fin insertion, 30.0-36.5 (vs. respectively 40.0-48.0 in *S. carlottae*, 37.3-50.5 in *S. codringtoni* and 38.7-48.0 %SL in *S. giardi*).

When identifying *S. sp. 'lufira'*, five populations of *S. mellandi* were examined as well, three populations, one from each of the three parts of the BM (the upper, middle and lower Luapula *sensu* Van Steenberge *et al.*, 2014 [19]), one from the upper Lufira (uLf) basin, upstream of Mwadingusha Dam, and one from the uLf, upstream of the N'zilo Dam. Their examination seems to show that *S. mellandi* might represent a species complex, since some measurements appear to differ between them. For example, the population of the uLf has a lower caudal peduncle height, 11.0-13.0 (vs. 14.0-15.0 %SL

for the *S. mellandi* from Lake Bangweulu and the lower Luapula). Furthermore, adult males of this population lack an extended filament on the pelvic fin (vs. with an extended filament on the pelvic fin reaching or sometimes even exceeding the level of the anus in adult males of Lake Bangweulu and the lower Luapula). Finally, the colour pattern also differs among the populations (Figures A8b-f). Therefore, an in-depth integrative study is needed to clarify the taxonomic status of these five populations.

SM: Cichlidae 2. *Pseudocrenilabrus* sp. 'lufira'

In adult males of *P. sp. 'lufira'*, the longest pelvic rays reach the anal fin insertion; a character state only known from *P. nicholsi* [79,81]. Nevertheless, adult males of *P. sp. 'lufira'* differ from *P. nicholsi* in their life colouration on the body, the dorsal and anal fins (Figure A8b, h). Also, for the comparison between *P. sp. 'lufira'* and *P. philander*, beside the pelvic fin length in adult males, the life colour pattern is also different between their adult males (Figures A8b, j). However, juveniles and females of *P. sp. 'lufira'* are more or less similar to those of *P. philander* and are therefore quite difficult to distinguish from each other.

SM: Cichlidae 3. *Coptodon/Tilapia* sp. 'pungwe'

The specimen of *Coptodon/Tilapia* sp. 'pungwe', differs from *C. rendalli* by the absence of vertical black stripes on the flanks (vs. 5-7 vertical black stripes in *C. rendalli* [37]), and the whitish ventral surface from the tip of the snout to the caudal-fin insertion (vs. red or orange ventral surface in *C. rendalli*) (Figures A9a & c). All basic counts and measurements taken fall within the range of *C. rendalli*, except for the premaxillary length which is longer in *Coptodon/Tilapia* sp. 'pungwe', 36.5 (vs. 29.0-32.6%HL in *C. rendalli*). It also differs by its colour pattern from *T. sparrmanii* and *T. ruweti* (Figure A9a). In addition, it has a high number of dorsal fin rays, 16 spines and 12 soft rays (vs. 14 and 10-11 spines and 14-15 and 10-12 soft rays respectively in *T. sparrmanii* and *T. ruweti*), a high number of perforated scales on the lower lateral line, 13 (vs. 10-11, rarely 12), a long pectoral fin, 39.9 (vs. respectively 26.0-32.0 and 22.0-23.0 %SL for both species) and an emarginated caudal fin (vs. rounded).