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Disentangling the Diversity of the *Labeobarbus* Taxa (Cypriniformes: Cyprinidae) from the Epulu Basin (DR Congo, Africa)

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Abstract: In an attempt to disentangle the complex taxonomy of the *Labeobarbus* species of the Epulu River, a right bank headwater affluent of the Aruwimi, Central Congo basin, a morphological study was undertaken on 221 specimens from the Epulu and 32 type specimens. As a result, five different species have been distinguished, including four so-called rubberlips, *L. caudovittatus*, *L. macroceps*, *L. mawambiensis*, and *L.* sp. 'thick lip', and one chiselmouth, *L. longidorsalis*. While rubberlips have a curved mouth with well-developed lips and often a mental lobe, chiselmouths have a straight mouth with a keratinised cutting edge on the lower jaw. Among the specimens examined, several presented an intermediate mouth morphology between *L. mawambiensis* and *L. longidorsalis*, either with one or two pairs of barbels. One specimen exhibited an intermediate morphology between *L. mawambiensis* and *L. macroceps*. This morphological study, complemented with a molecular study of the mitochondrial gene cytochrome *b* (cyt *b*), suggests that these intermediates are probably hybrid specimens. The Epulu case is reminiscent to a case of possible hybridisation recently discovered in the Inkisi River (Lower Congo basin), but differs in having a lower relative abundance of hybrid specimens in the population, and in phylogenetic patterns.

Keywords: chiselmouths; cyt *b*; Epulu; hybridisation; *Labeobarbus*; mouth phenotypes; morphology; rubberlips; *Varicorhinus*

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1. Introduction

The large-sized hexaploid African cyprinids belong to the tribe Torini [1]. The Afrotropical Torini include the genus *Labeobarbus* Rüppell, 1835 and its junior synonym *Varicorhinus* Rüppell, 1835 [1–3], and the monospecific genera *Acapoeta* Cockerell, 1910 and *Sanagia* Holly, 1926, of which the latter belongs to the *Labeobarbus* lineage based on COI (mtDNA) evidence [1]. Species of the former genus *Varicorhinus* are called chiselmouths, while those of *Labeobarbus*, excluding *Varicorhinus*, are called rubberlips. Typically, rubberlips and chiselmouths differ from each other in their mouth morphology, with rubberlips having a curved mouth with well-developed or even hypertrophied lips and often also a well-developed mental lobe on the lower lip, while chiselmouths have a straight mouth with a characteristic keratinised cutting edge on their lower jaw. In-between both extremes, a whole range of different mouth phenotypes has been identified. In addition, some unique mouth phenotypes exist such as one with papillated lips or with a prognathous lower jaw. A first overall classification of these mouth phenotypes was presented in the review by Vreven et al. [4]. Although typical rubberlips and chiselmouths are easily distinguished from each

other based on these characteristics, their taxonomic status has since long been unclear, not least, due to the occurrence of such individuals with an intermediate mouth morphology (see [4] for a historical overview). The synonymisation of *Varicorhinus* with *Labeobarbus* was suggested based on a molecular analysis of the mitochondrial cytochrome *b* gene [2]. Tsigenopoulos et al. [2] found that *Varicorhinus* is not monophyletic and that lineages of *Varicorhinus beso* and several other species, formerly included in *Varicorhinus*, clustered with *Labeobarbus*. The synonymy of *Varicorhinus* with *Labeobarbus* has been accepted in several reviews [3–5] and also in the present study. Although this synonymy was not implemented in Yang et al. [1], their results also support this hypothesis.

Specimens with an intermediate mouth morphology are known since Boulenger (1911) [6], but Banister [7,8] was the first to convincingly illustrate that at least some of these specimens—which were intermediate for other characters as well—should be considered as hybrids between species of *Labeobarbus* and *Varicorhinus*. Various species, already 27 in the Congo basin s.l. (i.e., including lakes Tanganyika and Kivu and the affluents Malagarazi and Ruzizi), have been described on specimens with an intermediate mouth morphology, which could be hybrids between typical rubberlips and chiselmouths. To date, for the whole African continent, three nominal species have been considered to be of hybrid origin [4]. An extensive review of the African Torini revealed that 125 valid African species of *Labeobarbus* were recognized, 39 of which occurring in the Congo basin s.l. [4]. Additionally, a major hybrid complex, comprising two new species for science, has been documented from the Inkisi River (Lower Congo basin) [9].

The Congo basin is the second largest river basin of the world. With over 1250 valid freshwater fish species and still many more left to discover, it is also the second most species-rich river on earth [10]. However, for many parts of the basin, the ichthyofauna is still poorly known. The Aruwimi (Figure 1) is an important right bank affluent of the upper stretch of the extensive Cuvette Centrale (Middle Congo basin) [11]. The headwaters of the Aruwimi are known as the Ituri, with the Epulu River as one of its main right bank affluents [12,13]. The Aruwimi flows across a number of rapids and waterfalls before joining the Congo [11]. Important waterfalls with a height of ca. 15 m, called the Arabia Falls, are situated on the Epulu, just upstream of its confluence with the Ituri (Google Earth and W.M. Ilodiri, pers. obs. 2022). These falls isolate the Epulu from the rest of the Ituri River and most probably form an important physical barrier, at least to upstream fish dispersal, resulting in a somewhat specific ichthyofauna in the former [14]. A small part of the Ituri and most of the Epulu catchment area lay within the Okapi Wildlife Reserve (OWR) [15]. In 1992, the Okapi Conservation Project established the OWR mainly in order to protect the numerous mammals, birds and plants in this area, many of which are endemic and/or threatened. The OWR covers an area of over 13,000 km2 and occupies about one-fifth of the Ituri Forest. In 1998, the reserve was placed on the list of World Heritage in Danger because of, amongst others, the large-scale invasion and habitat destruction by miners, militias and refugees [16].

To date, 41 species of *Labeobarbus* are known from the Congo basin s.s. Many species of *Labeobarbus*, but especially those from the Congo basin, are only known from their original description, except those studied by Banister in his revision of the large *Barbus* of East and Central Africa [17]. In the present study, the diversity of *Labeobarbus* from the Epulu River, upstream of the Arabia Falls, has been examined. Two explorative field surveys have been undertaken to this region (2009 & 2011), which provided important new collections that allowed us to re-assess the ichthyo-diversity of the Epulu, a river that was until recently only poorly studied [14]. We attempted to disentangle the species diversity of the genus based on morphological analyses and mtDNA (cyt *b*) results. The species of *Labeobarbus* currently known from the Epulu are *: L. caudovittatus*, *L. mawambiensis* and *L. macroceps*. *Labeobarbus caudovittatus* is a widespread species, *L. mawambiensis* is known from the Ituri and the Dja, though its presence in the Dja is questioned [18], and *L macroceps* is an Epulu endemic [14]. The inclusion of *L. longidorsalis* in the species list of [14] is already based on the preliminary results of the present study. Previously, *L. longidorsalis* was only known

from the Luhoho River, a left bank affluent of the Lowa Basin (Upper Congo). In addition, three species are known only from their type localities in the Ituri: *L. humphri*, *L. iturii* and *L. mirabilis*. Among the specimens from the Epulu sampled in 2009 and 2011, several specimens with an intermediate mouth morphology were found, which is indicative for the occurrence of hybrids. Therefore, differences between the species recognized and the putative hybrids are discussed in detail, and their morphological features documented.

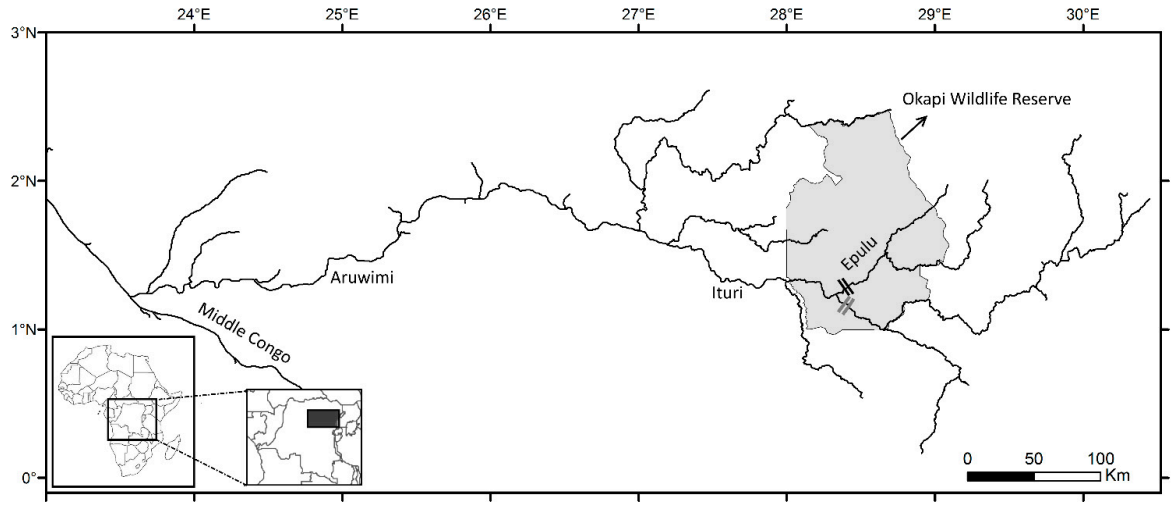

Figure 1. Map of the study area within the Upper Aruwimi, i.e., the Ituri and Epulu, and part of the upper stretch of the Middle Congo basin. The parallel lines indicate the position of the waterfalls: in black the Arabia Falls on the Epulu, in grey the Ngoy Falls on the Ituri. The grey area indicates the Okapi Wildlife Reserve (OWR). Insert maps shows the positioning of the study region in Africa and the Democratic Republic of the Congo, respectively.

2. Materials and Methods

We used a pragmatic approach to the species concept vs. species delimitation problem. To date, at least 25 species concepts exist, which have been much debated in the past. However, a unified species concept has been presented [19], which is followed in our present study. This conceptualization of the species, nevertheless, needs to be separated from the practical approach to the species delimitation problem [19]. Therefore, to try to elucidate the species in the *Labeobarbus* from the Epulu River, we used an integrative approach, combining morphology and genetics [20].

2.1. Morphology

A total of 221 *Labeobarbus* specimens from the Epulu River have been examined. Only specimens from the 2009 expedition (formalin fixed and alcohol preserved) have been examined for analysis as those of 2011 were in bad shape, probably due to fixation problems in the field. Additionally, 32 type specimens, belonging to eight nominal species, have been included (see Appendix A. list of specimens examined) based on the type locality being situated in the Ituri basin or the overall similarity with some of the Epulu specimens in mouth morphology, number of barbels, dorsal spine morphology and/or number of lateral line scales. These type specimens include: the nine syntypes of *L. mawambiensis* (Steindachner, 1911), the holotype and 10 of the 11 paratypes of *L. humphri* (Banister, 1976), the two syntypes of *L. caudovittatus* (Boulenger, 1902), the holotype of *L. fasolt* (Pappenheim, 1914), the holotype of *L. mirabilis* (Pappenheim, 1914), the holotype of *L. mawambi* (Pappenheim; 1914), the holotype of *L. longidorsalis* (Pellegrin, 1935), and six of the eight syntypes of *L. macrolepidotus* (Pellegrin, 1928). The holotype of *L. macroceps* (Fowler, 1936) was not available for loan due to the loan policy of the host institute. Therefore, the most important diagnostic characters were checked on photographs of the preserved type specimens. We could not examine the holotype of *L. iturii* (Holly, 1929) which is considered lost [4,21]. The type specimens

of the five junior synonyms of *L. caudovittatus*, and its revalidated junior synonym *L. pojeri* (Poll, 1944) [22], have not been included in the analyses as their type locality is not located in the Ituri basin and *L. caudovittatus* seems to represent a separate species complex. A complete revision of the *L. caudovittatus* species complex is outside the scope of the present study, and is currently being executed [23].

Based on some morphological key characteristics: the mouth morphology as characterised in [4], the number of barbels and the ossification of the last unbranched dorsal fin ray, a first classification into morphotypes was done for the specimens from the Epulu River.

Next, we explored whether and how these morphotypes can be further distinguished based on morphometric and genetic data (cyt *b*), to assess the species status of the different morphotypes.

On each specimen, 20 counts and 31 measurements were taken following [9]. Principal Component Analyses (PCAs) were used to explore the multivariate data matrix and to reduce the large number of variables into a few meaningful axes [24,25]. Meristics and measurements were analysed separately. For the meristics, the raw data were used. The number of unbranched dorsal fin rays, branched and unbranched anal fin rays, caudal fin rays, and caudal peduncle scales were invariable and thus not included in the PCAs. Measurements were log-transformed (for PCAs) or expressed as percentages (for scatterplots and tables), with body measurements as a percentage of standard length (SL) and head measurements as a percentage of head length (HL). For a PCA on log-transformed measurements, PC1 is a proxy of size [24,25]. Possible differences between groups were visualised in plots of PC2 vs. PC3. As PC1 does not correct for all size aspects, possible remaining size effects were evaluated by individual PC plots of PC2 or PC3 against SL and discussed when necessary. The height of the segmented part of the dorsal fin was not included in the PCAs since data were missing for a large part of the studied specimens due to damage of the distal tip of the dorsal fin. In the examined chiselmouths, the anterior barbels were absent and the posterior barbels consisted of small protuberances, too small to be measured, and a premaxillary pedicel is lacking [7]. Therefore, barbel and premaxillary pedicel lengths were also not included in the PCAs. Specimens for which information on a certain variable was lacking, were case-wise deleted from the analyses. Possible differences of individual variables between groups were explored with non-parametric Mann–Whitney U (MWU) tests corrected with sequential Bonferroni [26]. For measurements, specimens of similar length classes, i.e., ranges for which the SL did not differ significantly ($p \geq 0.5$) are needed to prevent interference of allometric growth effects. However, as for several groups only a low number of specimens was left after size restriction, the MWU results were often not suitable for interpretation, and therefore not discussed.

2.2. Genetics

DNA was extracted from fin clips or muscle tissue using the NucleoSpin® Tissue kit (Macherey-Nagel) following the standard protocol provided by the manufacturer. A region spanning the complete mitochondrial cytochrome *b* (cyt *b*) gene (1141 bp) was amplified using primers L15267 (5′-AAT GAC TTG AAG AAC CAC CGT-3′) and H16461 (5′-CTT CGG ATT ACA AGA CC-3′) [27]. Cyt *b* was chosen because it possesses highly variable as well as conservative regions [28], which results in good phylogenetic resolution in Cyprinidae [2,29], and because it allows integration of other datasets of *Labeobarbus* using the same marker (e.g., [1,2]). Amplifications were performed according to [30] in 10 µL volumes containing 5 µL Multiplex Mix (Qiagen), 1 µL genomic DNA, 0.8 µL of each Primer (2.5 nmol), 1 µL Q-Solution (Qiagen) and 1.4 µL water. Amplifications were carried out in 41 cycles according to the temperature profile: 15 min at 94 °C (initial denaturation), 1 min at 94 °C, 45 s at 60 °C, 1 min at 72 °C (one cycle); 1 min at 94 °C, 45 s at 60–55 °C (−0.5 °C touchdown each cycle), 1 min at 72 °C (ten cycles); 1 min at 94 °C, 45 s at 55 °C, 1 min at 72 °C (30 cycles) and finally 10 min at 72 °C. PCR products were purified with ExoSAP-IT (Fermentas) and diluted with 10–20 µL HPLC water, depending on product concentration. In each run, negative PCR controls with no template DNA were used.

Sequencing was performed according to standard methods, using Big Dye 3.1 terminator (Applied Biosystems). DNA sequences were read using an ABI 3130XL DNA sequencer (Applied Biosystems). Electropherograms and sequences were edited, aligned and analysed using BioEdit 7.2.5 [31], after using ClustalW (default settings) for a preliminary alignment.

In addition to the newly generated sequences, sequences from GenBank were added to the alignment for the outgroups. As outgroups we selected available sequences of representatives of the two other lineages within the *Labeobarbus* clade (sensu [1]): *L. habereri* and *Pterocapoeta maroccana* from the *Pterocapoeta* lineage and *'Labeobarbus' reinii* (see [1] for the taxonomic status), *Arabibarbus grypus*, *Carasobarbus harteri* and *C. canis*, and *Mesopotamichthys sharpeyi* from the *Carasobarbus* lineage. An overview of all newly generated sequences and comparative sequences from GenBank is given in the Supplementary Material (Table S1).

Genetic data analyses were all performed in MEGA 6.06. The appropriate model was evaluated using Modeltest and the model GTR+G+I revealed to be the most suitable for the data using the Akaike Information Criterion. Maximum Likelihood (ML) and Neighbour-Joining (NJ) trees with 100 Bootstrap (BS) replications were constructed. As both trees gave similar branching patterns, only the ML tree is illustrated, but with statistical node support (BS values) of both trees.

2.3. Abbreviations

BS, bootstrap; COI, cytochrome c oxidase I; cyt *b*, cytochrome *b*; DRC, Democratic Republic of the Congo; HL, head length; mtDNA, mitochondrial DNA; MWU test, Mann–Whitney U test; ML, Maximum Likelihood; nDNA, nuclear DNA; NJ, Neighbour Joining; PC: Principal Component; PCA, Principal Component Analysis; SL, standard length. Institutional abbreviations follow [32].

All localities have been translated in English. The collection numbers of the RMCA have been adapted to the new system for collection years (e.g., A0 = 2000, B0 = 2010). When coordinates were not specified for the museum specimens, approximate coordinates were taken from the Gazetteer of the Democratic Republic of the Congo [33].

3. Results

3.1. The Epulu Specimens: A Phenotypic Classification

Based on the morphological key characters, eight different morphotypes were recognized within the Epulu *Labeobarbus* specimens (Table 1a and Figure 2). They were assigned a working name referring to their most representative morphological key characters. "Lab-like" refers to the rubberlip morphology but is different from the real *Lab.*-mouth phenotypes (sensu [4]) in that in the Epulu morphotype, the mental lobe is often attached instead of detached from the lower lip, hence the name "Lab-like". A single specimen was found that also had an attached lobe, though with a flexible dorsal spine: "flex". Several specimens had a flexible dorsal spine and clearly hypertrophied lips with a free mental lobe: "thick lip". Another morphotype had a clearly prognathous mouth: "prog". One chiselmouth morphotype was found which was given the name "Var" referring to the former genus *Varicorhinus*. Several specimens were found with a mouth morphology intermediate between "Lab-like" and "Var". They lacked a lobe and the mouth was less curved than in "Lab-like", though still more curved then in "Var", and they also lacked the keratinised cutting edge. Some of these specimens had one pair of barbels, while others had two. Hence, the names "inter1" and "inter2". Finally, one specimen seemed to have an intermediate mouth morphology in-between "Lab like" and "prog", having a lower jaw that is slightly longer than the upper one, and with a dorsal spine as in "Lab-like". The given work name for this specimen is "Lab-prog".

Through multivariate morphometric analyses and comparisons with types, we further assessed the taxonomic status of these morphotypes. These results are presented in Section 3.2.

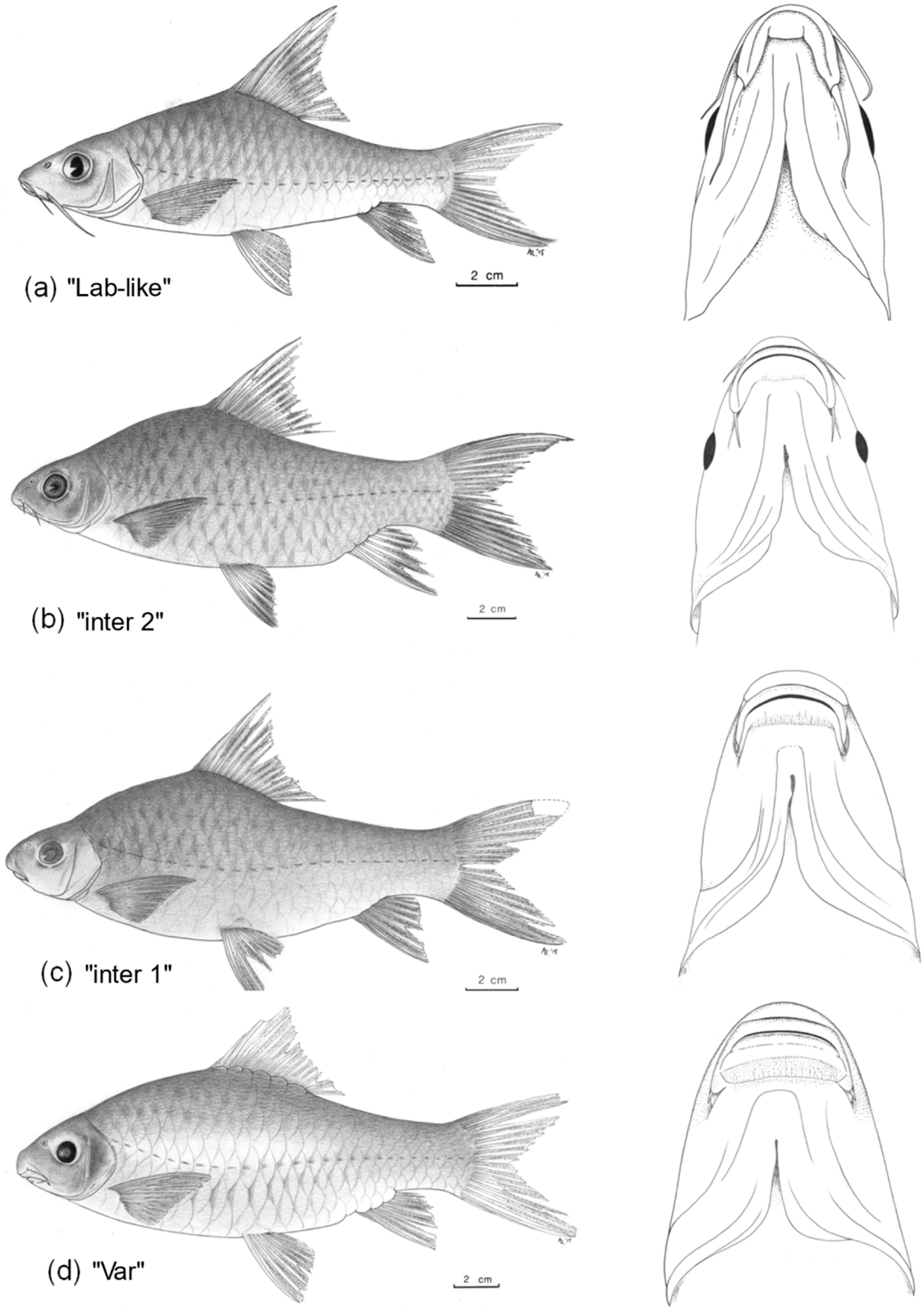

Figure 2. *Cont.*

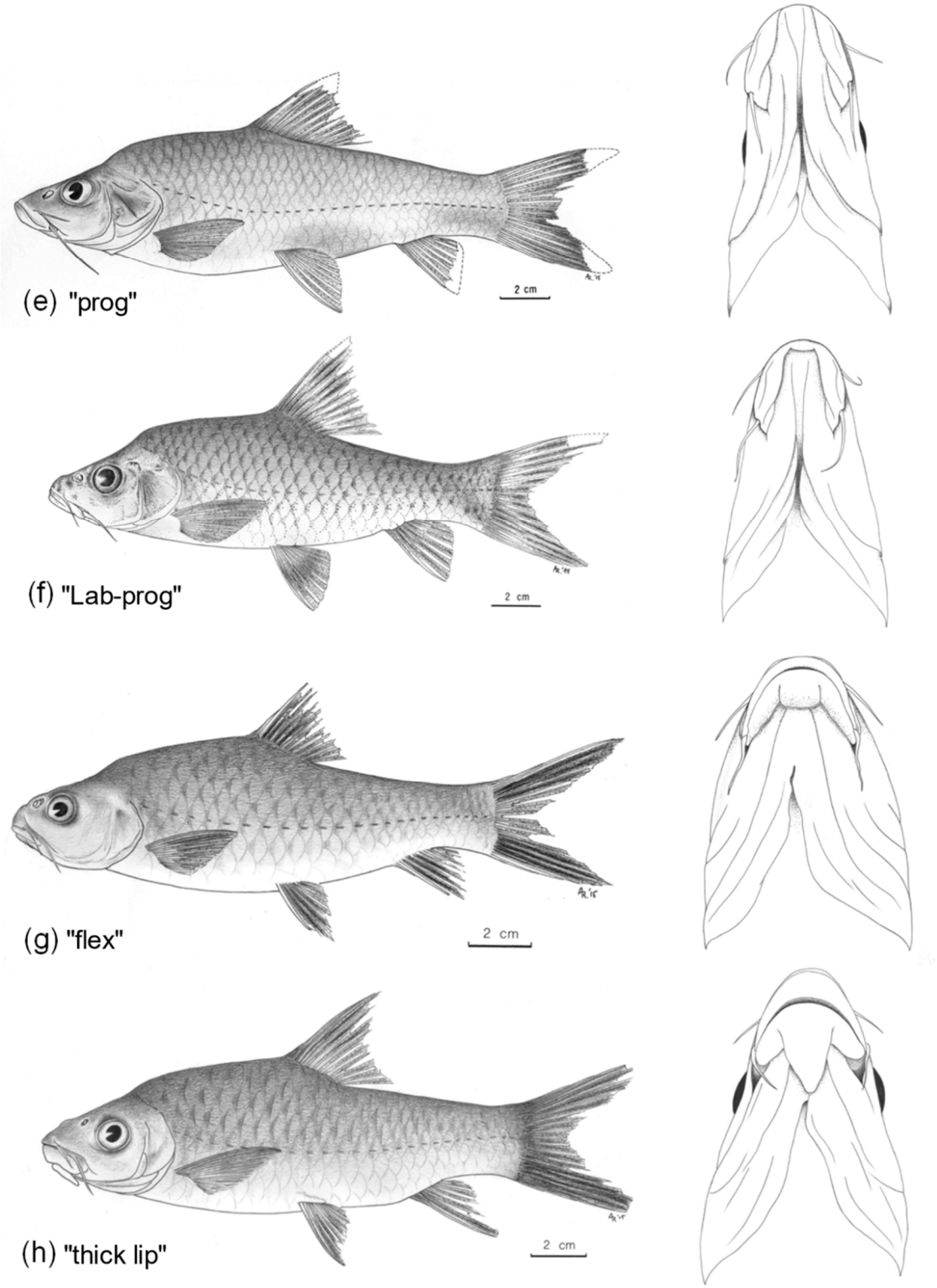

Figure 2. Illustrations of the lateral view (left) and ventral view of the head (right) of the different morphotypes occurring in the Epulu River: (**a**) "Lab-like" (*L. mawambiensis*) (RMCA 2009-29-P-0370); (**b**) "inter 2" (putative *L. mawambiensis* x *longidorsalis* hybrid with two pairs of barbels) (RMCA 2009-29-P-0290); (**c**) "inter 1" (putative *L. mawambiensis* x *longidorsalis* hybrid with one pair of barbels) (RMCA 2009-29-P-0287); (**d**) "Var" *L. longidorsalis* (RMCA 2009-29-P-0288); (**e**) "prog" *L. macroceps* (RMCA 2009-29-P-0310); (**f**) "Lab-prog" (putative *L. macroceps* x *L. mawambiensis* hybrid) (RMCA 2009-29-DNA3516); (**g**) "flex" (*L. caudovittatus*) (MRAC 2009-29-P-0347,); and (**h**) *L.* sp. 'thick lip' (RMCA 2009-29-P-0346).

Table 1. Overview of the morphological key characters of the eight morphotypes recognized in the Epulu River (**a**) and the nominal species studied for comparison (**b**). Morphotype specification follows terminology of Vreven et al. [4]. Abbreviations: a= attached lobe; ce = cutting edge; f = free lobe; h = hypertrophied; inter.= intermediate; *Lab.* = *Labeobarbus;* LLS = lateral line scales; *long* = *longidorsalis*; *mac* = *macroceps; maw* = *mawambiensis;* n = no lobe; prog = prognathous (lower jaw clearly longer than upper); ±prog = slightly prognathous (lower jaw slightly longer than upper); inf= inferior (upper jaw clearly longer than lower); *Var.* = *Varicorhinus*; 1p = 1pair; and 2p = 2 pairs. The 'x' refers to the putative hybrid status.

(a)

	Mouth Phenotypes	Mouth Position	Barbels	Dorsal Spine	LLS	Identification
Epulu morphotypes						
"Lab-like"	f or a	inf	2p	spine	21–28	*L. mawambiensis*
"inter2"	n	inf	2p	inter	24–28	*L. maw* x *L. long* (2p)
"inter1"	n	inf	1p	inter	24–27	*L. maw* x *L. long* (1p)
"Var"	n & ce	inf	1p	inter	23–27	*L. longidorsalis*
"prog"	n	prog	2p	flexible	31–36	*L. macroceps*
"Lab-prog"	n	supra	2p	spine	29	*L. mac* x *maw*
"flex"	a	inf	2p	flexible	24	*L. caudovittatus*
"thick lip"	h & f	inf	2p	flexible	24–27	*L.* sp. 'thick lip'

(b)

	Mouth Phenotypes	Mouth Position	Barbels	Dorsal Spine	LLS	Status
types						
L. caudovittatus	a	inf	2p	flexible	26	valid
L. fasolt	a	inf	2p	flexible	26	=*L. caudovittatus*
L. humphrii	a	inf	2p	spine	24–28	valid
L. iturii	a	inf	2p	flexible	29	valid
L. longidorsalis	n & ce	inf	1p	inter	29	valid
L. macroceps	n	inf	2p	flexible	32	valid
L. macrolepidotus	n	inf	1p	flexible	25–27	valid
L. mawambi	n	inf	2p	flexible	28	=*L. mirabilis*
L. mawambiensis	f or a	inf	2p	spine	23–26	valid
L. mirabilis	n	inf	2p	inter	31	valid

3.2. Morphological Analyses

Based on a unique combination of mouth morphology (no lobe, mouth inferior for both), dorsal fin spine morphology (respectively flexible and partially flexible), and the number of lateral line scales (respectively 28 and 31) (see Table 1b) the holotypes of *L. mawambi* and *L. mirabilis* could be separated from all other specimens. Therefore, these two holotypes were not included in further analyses.

3.2.1. Meristics

Based on a higher number of lateral line scales (31–36 vs. 21–28) and lower number of gill rakers on the first gill arch (9–11 vs. 13–23), "prog" could be separated from all other specimens. The single specimen of "Lab-prog" had an intermediate number of lateral line scales between "prog" and the remainder specimens (29 vs. 31–36 and 21–28), and also had an intermediate position on a PCA of the meristics on all the specimens (not illustrated).

A PCA (n = 232) excluding "prog" and "Lab-prog" was performed (Figure 3). The most important loadings on PC1 were for the number of branched dorsal fin rays, the number of gill rakers on the upper branch, and the total number of gill rakers of the first gill arch, and the number of lateral line scales between the anterior dorsal- and pelvic-fin base.

The most important loadings on PC2 were for the number of gill rakers on the lower branch of the first gill arch and the number of scales between the dorsal and caudal fin (Table 2). The scatterplot of PC2 against PC1, revealed "thick lip", situated entirely on the negative part of PC1 and the positive part of PC2, to be separated from the other specimens, mainly due to its higher number of gill rakers on the first gill arch (19–23 vs. 13–19). The single specimen of "flex" fell separately from the rest of the Epulu specimens, on the negative part of PC2 and positive part of PC1, mainly due to a rather low number of gill rakers on the first gill arch (9) (Figure 3).

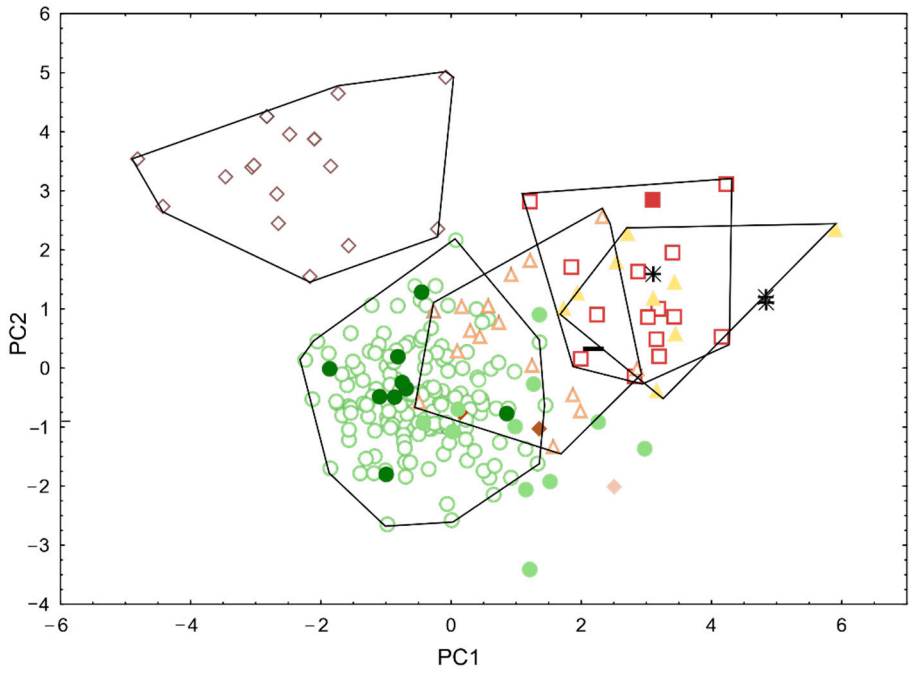

Figure 3. Scatterplot of PC2 against PC1 for a PCA carried out on 16 meristics (n = 232). Polygons visualize the different groups present in the Epulu River. ♦: "flex", ○: "Lab-like", △: "inter2", ▲: "inter1", □: "Var", ◇: "thick lip". Type specimens: - holotype of *L. fasolt*, ■: holotype of *L. longidorsalis*, ●: syntypes of *L. mawambiensis*, ●: holotype and paratypes of *L. humphri*, ✳: syntypes of *L. macrolepidotus*, ♦: syntypes of *L. caudovittatus*.

The remaining specimens were largely situated on the positive part of PC1 and the negative part of PC2, and contain "Lab-like", "Var", "inter1" and "inter2", and all type specimens. "Lab-like" and "Var" were clearly separated from each other, mainly on PC1, as they differed in the numbers of branched dorsal fin rays (8–10, exceptionally 11 vs. 11–12), and lateral line scales between anterior dorsal- and pelvic-fin base (0.5–2.5 vs. 2.0–4.0). The intermediate morphotypes with two pairs of barbels ("inter2") mainly occupied an intermediate position in-between "Lab-like" and "Var", with some specimens situated within the polygon "Lab-like" and only two in the "Var" polygon. The morphotype with only one pair of barbels ("inter1"), instead, overlapped almost completely with "Var". The holotype of *L. longidorsalis* was situated within the polygon of "Var"; one of the syntypes of *L. macrolepidotus* was situated in the overlapping area between "Var" and "inter1", while two of the syntypes were located in the polygon of "inter1". The three remaining syntypes of *L. macrolepidotus* were not included due to lacking data on the gill rakers. The polygon of "Lab-like" comprised the syntypes of *L. mawambiensis* and *L. caudovittatus*, and overlapped partially with the syntypes of *L. humprii*. The holotype of *L. fasolt* was situated among the specimens of "Var" and "inter1". A PCA excluding "thick lip" (not illustrated) did not reveal any other meaningful patterns for the remaining specimens.

Based on the analyses of the meristics, "prog" and "thick lip" are clearly separated from the rest, and also the single specimens of "Lab-prog" and "flex" fell separately on

the PCAs. Although "Lab-like" and "Var" are clearly distinguished from each other, the specimens with intermediate morphology, "inter1" and "inter2", overlapped, respectively partially with "Var" and "Lab-like", and with each other. Below, we further refer to the grouping of "Lab-like", "Var", "inter1" and "inter2" as the "Lab/Var"-complex.

Table 2. PC loadings and percentage of total variance explained for the first two axis of a PCA on 15 meristics; a: including all specimens except types of *L. mawambi* and *L. mirabilis* and also excluding the morphotypes "prog" and "Lab prog" (n = 232, Figure 3). Most important loadings are in bold.

	PCI (20.7%)	**PCII (12.7%)**
Total number of lateral line scales	0.141	0.250
Number of predorsal scales	−0.127	−0.069
Number of scales above the lateral line	0.203	0.101
Lateral line-pelvic scales	0.232	0.050
Lateral line–ventral midline scales	0.032	−0.125
Number of dorsal-fin base scales	0.272	0.057
Number of anal-fin base scales	−0.013	0.029
Number of branched dorsal fin rays	**0.439**	0.249
Number of branched pectoral fin rays	−0.227	−0.278
Number of branched pelvic fin rays	0.108	0.212
Number of scales between dorsal and caudal fin	−0.136	**0.328**
Number of lateral line scales between anterior dorsal- and pelvic-fin base	**0.397**	0.145
Number of gill rakers on lower branch of first gill arch	−0.204	**0.591**
Number of gill rakers on upper branch of first gill arch	**−0.404**	−0.044
Total number of gill rakers on the first gill arch	**−0.397**	**0.485**

3.2.2. Measurements

A PCA on 27 log-transformed measurements (n = 249) was performed. The most important loading on PC2 was for the unsegmented dorsal fin height. The most important loadings on PC3 were for the lower jaw length, the dorsal fin base length, the pre-operculum length and the head length (Table 3a).

On the scatterplot of PC3 against PC2 (Figure 4), "prog" and "thick lip" were clearly separated on the positive part of PC3 and on the negative part of PC2, with "prog" having the highest values on PC3. The holotype of *L. fasolt* fell within the group of "thick lip", but on an additional PCA on these specimens alone (not illustrated), they clearly separated.

The morphotypes "Var" and "inter1" were situated on the most negative part of PC3, and were almost completely separated from the rest, but "inter1" fell almost entirely within the polygon of "Var". In contrast to the results of the PCA on the meristics, on this PCA, "Var" and "inter1" were entirely separated from the syntypes of *L. macrolepidotus*, but a subsequent scatterplot of PC2 against PC1 (not illustrated) of the same analysis revealed that this was due to their smaller size. The holotype of *L. longidorsalis* was not included in this analysis as the dorsal fin height could not be measured due to damage. An additional PCA on the measurements without dorsal fin height (not illustrated) showed that the holotype of *L. longidorsalis* indeed falls within the polygons of "Var" and "inter1".

The morphotype "Lab-like" was situated mainly on the positive part of both axes and overlapped largely with the types of *L. mawambiensis* and those of *L. humphri*. Specimens of "inter2" were situated in-between "Lab-like" and the overlapping groups of "Var" and "inter1". The single specimen of "Lab-prog" was located within the group of "Lab-like", but on the edge of this polygon. The one specimen of "flex" was separated from all other Epulu specimens, and situated near the two syntypes of *L. caudovittatus*.

The "Lab/Var"-complex displayed similar patterns as for the meristics: "Lab-like" and "Var" were completely separated (on PC3); "inter2" occupied a position in-between both, while "inter1" overlapped almost entirely with "Var".

The morphotypes are, to a large extent, based on mouth phenotype differences and these were, most often, reflected in differences of measurements made on the head as well.

Table 3. PC loadings and percentage of total variance explained for the first three axis of two PCAs on 27 (a) and 18 (b) log-transformed measurements; (**a**): including all specimens except types of *L. mawambi* and *L. mirabilis* (n = 249, Figure 4); (**b**): including specimens from the "Lab/Var"-complex only and excluding head measurements (n = 216, Figure 5). Most important loadings for PC2 and 3 are in bold.

	(a)			(b)		
	PC1 (91.1%)	**PC2 (3.8%)**	**PC3 (2.1%)**	**PC1 (93.1%)**	**PC2 (4.0%)**	**PC3 (0.9%)**
Standard length	−0.189	−0.039	0.038	−0.231	0.002	0.180
Body depth	−0.204	−0.009	−0.236	−0.259	0.143	−0.274
Predorsal length	−0.191	−0.007	0.140	−0.231	−0.094	0.166
Dorsal fin base length	−0.189	0.051	**−0.326**	−0.247	0.177	−0.186
Dorsal fin height	−0.149	0.281	−0.036	−0.188	−0.271	**−0.418**
Unsegmented dorsal fin height	−0.126	**0.902**	0.039	−0.179	**−0.864**	−0.115
Post-dorsal length	−0.194	−0.064	−0.016	−0.241	0.039	0.327
Dorsal-pelvic length	−0.211	−0.022	−0.257	−0.271	0.163	−0.263
Prepectoral length	−0.179	−0.015	0.264	−0.208	−0.158	0.276
Pectoral fin length	−0.186	0.056	−0.084	−0.232	0.009	−0.029
Prepelvic length	−0.191	−0.071	0.072	−0.229	0.015	0.156
Pelvic fin length	−0.183	−0.010	−0.138	−0.229	0.094	−0.111
Anal fin base length	−0.203	0.002	−0.195	−0.257	0.133	0.178
Anal fin height	−0.182	0.084	−0.134	−0.235	−0.003	−0.140
Caudal peduncle length	−0.200	−0.042	0.106	−0.242	−0.038	**0.435**
Maximum caudal peduncle height	−0.195	−0.056	−0.182	−0.246	0.153	−0.201
Minimum caudal peduncle height	−0.202	−0.054	−0.152	−0.253	0.126	−0.187
Pre-anal length	−0.199	−0.046	0.026	−0.245	0.017	0.188
Head length	−0.181	−0.019	**0.300**	-	-	-
Pre-operculum length	−0.179	−0.002	**0.313**	-	-	-
Head width	−0.199	−0.005	0.029	-	-	-
Inter-orbital distance	−0.230	−0.117	−0.179	-	-	-
Lower jaw length	−0.195	−0.043	**0.443**	-	-	-
Mouth width	−0.238	−0.176	−0.011	-	-	-
Eye diameter	−0.110	0.112	0.181	-	-	-
Inter-nasal distance	−0.225	−0.067	−0.072	-	-	-
Snout length	−0.217	−0.099	0.259	-	-	-

Therefore, a subsequent PCA was done on only the body measurements of the specimens of the "Lab/Var"-complex, to assess whether additional morphological differences, not related to mouth morphology, could be found (n = 216). The most important loading on PC2 was for the unsegmented dorsal fin height (Table 3b); no separation between morphotypes was found on PC3, and PC1 is a proxy for size. Even when excluding head measurements, "Lab-like" and "Var" were still clearly separated from each other (Figure 5), mainly based on the unsegmented dorsal fin height which is smaller in "Var" than in "Lab-like". Although the position of "inter1" and "inter2" was similar to that obtained in the previous PCA (Figure 4), "inter2" now largely overlapped with "Lab-like", illustrating that their earlier separation from "Lab-like" is mainly due to differences in head morphology.

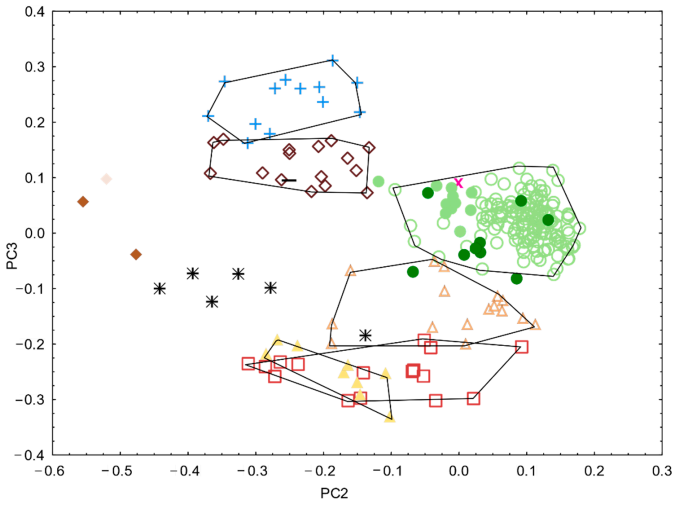

Figure 4. Scatterplot of PC3 against PC2 for a PCA carried out on 27 log-transformed measurements (n = 249). Polygons visualise the different groups present in the Epulu River. ♦: "flex", +: "prog", X: "Lab-prog", ○: "Lab-like", △: "inter2", ▲: "inter1", □: "Var", ◇: "thick lip". Type specimens: - holotype of *L. fasolt*, ■: holotype of *L. longidorsalis*, ●: syntypes of *L. mawambiensis*, ●:holotype and paratypes of *L. humphri*, ✳: syntypes of *L. macrolepidotus*, ♦: syntypes of *L. caudovittatus*.

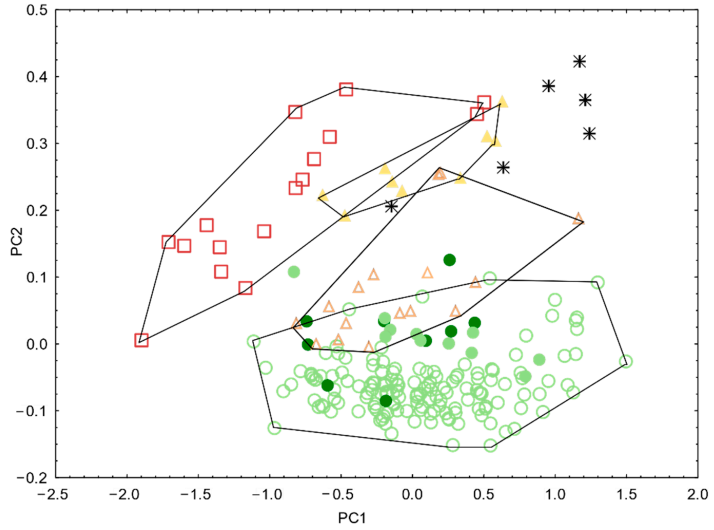

Figure 5. Scatterplot of PC2 against PC1 carried out on 18 log-transformed measurements (excluding head measurements) (n= 216). Polygons visualise the different groups present in the Epulu River. ○: "Lab-like", △: "inter2", ▲: "inter1", □: "Var". Type specimens: ●: syntypes of *L. mawambiensis*, ●: holotype and paratypes of *L. humphri*, ✳: syntypes of *L. macrolepidotus*.

3.3. Genetics

In a ML tree based on the mitochondrial cyt *b* gene of the Epulu specimens five well-supported genetic clades (Bootstrap ≥ 98) are present (Figure 6A–E), largely representing the main morphotypes. However, most of these clades did not only contain specimens belonging to one particular morphotype, but also specimens belonging to one or several of the remaining morphotypes identified. Clade A contains all specimens of "thick lip" and the single specimen of "flex". Clade B contains only specimens of "Lab-like", though one specimen of "Lab-like" had a rather unexpected position as it forms a separate lineage, though not well supported (BS: 44). Clade C contains all specimens of "prog" and the one specimen of "Lab-prog". Clades D and E are subclades of the Clade F with a genetic divergence between them of 1.4%. The larger Clade F contains all specimens of "Var" and both morphotypes with intermediate mouth phenotypes: "inter1" and "inter2". Clade E

contains all specimens of "Var" and some specimens of both "inter1" and "inter2", while Clade D contained the other specimens of "inter1" and "inter2".

This ML tree suggest a non-monophyly of *Labeobarbus* due to the position of *L. habereri*, though with low statistical support. In a ML tree with multiple other outgroups available from GenBank (Supplementary Material, Figure S1), all species of *Labeobarbus*, including *L. habereri*, form a monophyletic clade.

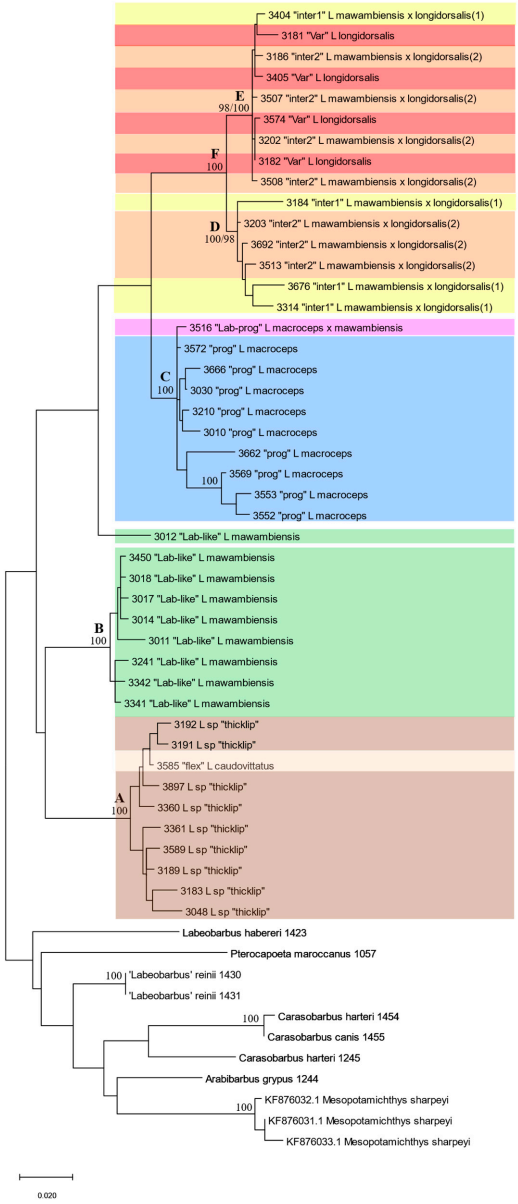

Figure 6. Maximum Likelihood tree with 100 bootstrap replications on the cyt *b* gene (1130 bp) of *Labeobarbus* species from the Epulu and some additional outgroups. Statistical node support is shown as ML bootstrap/NJ bootstrap, or as a single number when both are identical; only bootstrap values > 95 % are shown. Branch lengths indicate the number of substitutions per site. Taxon names include both the names of the morphotypes and the eventual identifications. Different colours are given to the different morphotypes from the Epulu. Five well-supported genetic clades (Bootstrap $\geq$ 98) for the samples of the Epulu are indicated with letters A–F.

3.4. Integrative Synthesis

Based on the PCAs of the meristics and the measurements, most of the initially recognized morphotypes from the Epulu River (Table 1a,b) could be distinguished from each other, and

some of them formed distinct clades on the ML tree (Figure 6). The morphotype "prog" was clearly distinct. The holotype of *L. macroceps* was not available for loan, though the morphological characteristics and variables of "prog" matched those on the photographs of the holotype at our disposal and the original description of this species. Hence, "prog", the morphotype with the prognathous mouth, is considered as conspecific with *L. macroceps.* The morphotype "thick lip", instead, did not seem conspecific with any of the type species and is considered a species new to science, which we call *L.* sp. 'thick lip'.

The morphotypes "Lab-like" and "Var" were clearly distinguished from each other based on the PCAs. They had different cyt *b* haplotypes, with "Lab-like" forming a well-supported clade on the ML tree, though "Var" clustering together with "inter1" and "inter2". On the PCAs, the specimens of "Lab-like" always fell together with the syntypes of *L. mawambiensis*. The specimens also overlapped with the syntypes of *L. humphri,* though the latter had a more divergent position due to a generally smaller body depth, caudal peduncle depth and dorsal fin height. Therefore, "Lab-like" is identified as *L. mawambiensis.* On the PCAs, the "Var" morphotypes and "inter1" always clustered together and with the holotype of *L. longidorsalis.* In meristics (Figure 3) they also corresponded well to the syntypes of *L. macrolepidotus*. Even though on a PCA on the measurements (Figure 4) they did not cluster with the syntypes of *L. macrolepidotus*, a subsequent scatterplot of PC2 against PC1 (not illustrated) of the same analysis revealed that this was due to their smaller size. The holotype of *L. longidorsalis* had a keratinised cutting edge on the lower jaw, while the syntypes of *L. macrolepidotus* lacked this feature and had an intermediate-mouth phenotype. Therefore, the "Var" morphotype is here identified as *L. longidorsalis.* "inter1" clearly had an intermediate morphology in-between "Var" and "Lab-like" (i.e., *L. longidorsalis* and *L. mawambiensis*), though intermediacy in other characteristics than mouth morphology was not found in the PCAs. As they lacked a keratinised cutting edge, they thus resemble *L. macrolepidotus*. However, the fact that the haplotypes of "inter1" cluster with those of *L. longidorsalis* on the ML tree (Figure 6), rather supports the hypothesis of this morphotype being a hybrid between *L. longidorsalis* and *L. mawambiensis*, instead of a distinct species (*L. macrolepidotus).* This issue is further discussed in the discussion section.

"Inter 2" had an intermediate position between *L. mawambiensis* and *L. longidorsalis* in all PCAs, even when excluding head measurements (Figure 5). This morphotype however seemed more similar in meristics to *L. mawambiensis* (Figure 3), while based on genetics, it clustered with *L. longidorsalis.* Based on the morphological and genetic results, this morphotype seems also a putative hybrid between *L. mawambiensis* and *L. longidorsalis.*

A single specimen, "Lab-prog", was found with an intermediate overall morphology between *L. macroceps* and *L. mawambiensis*. Additionally, in a PCA on the meristics (not illustrated), this specimen had a position in-between *L. macroceps* and *L. mawambiensis,* mainly due to an intermediate number of lateral line scales (29 vs. 31–36 and 21–28). In a PCA on measurements (Figure 4), it was located within the polygon of *L. mawambiensis*, but near to its margin. Based on the morphological results, this morphotype seems a putative hybrid between *L. macroceps* and *L. mawambiensis*. The clustering of this specimen with the clade of *L. macroceps* on the ML tree, then would indicate it having the maternal DNA of *L. macroceps* (Figure 6).

The single specimen of "flex" could be separated from all Epulu groups based on meristics and measurements (Figures 3 and 4), but always fell near the syntypes of *L. caudovittatus,* a species to which it also resembled in overall morphology (Table 1a,b). Additionally, it also displayed the two black bands along the distal end of both caudal fin lobes, which are considered characteristic for *L. caudovittatus.* On the ML tree (Figure 6), this specimen, however clustered with *L.* sp. 'thick lip', from which it is clearly morphologically different, not only in mouth phenotype, but also by its lower number of gill rakers,. We thus consider "flex" a specimen of *L. caudovittatus.* The clustering with *L.* sp. 'thick lip' is further addressed in the discussion section.

An overview of the meristics and measurements of all species and possible hybrids from the Epulu River, and of all measured types is given in Table 4a,b and Table 5a,b.

Table 4. Values ranges of the measurements (**a**) and meristics (**b**) of the *Labeobarbus* species and putative hybrids from the Epulu River. *L. maw* x *long* 2 and 1 = putative hybrids between *L. mawambiensis* and *L. longidorsalis* with, respectively 2 and 1 pair(s) of barbels. *L. mac* x *maw* = putative hybrid between *L. macroceps* and *L. mawambiensis*.

	L. mawambiensis	***L. longidorsalis***	***L. maw* x *long* 2**	***L. maw* x *long* 1**	***L. macroceps***	***L. mac* x *maw***	***L.* sp. 'thick lip'**	***L. caudovittatus***
(a)	***n* = 149**	***n* = 16**	***n* = 16**	***n* = 9**	***n* = 13**	***n* = 1**	***n* = 16**	***n* = 1**
Standard length (mm)	53.3–220.0	93.4–322.0	63.0–180.0	85.9–164.6	123.2–232.2	170.5	93.0–190.1	145.4
Measurements in %SL								
Body depth	22.7–37.8	33.4–39.6	31.2–39.4	32.4–37.0	24.4–29.5	30.1	25.3–31.2	30.3
Predorsal length	47.1–57.3	45.6–50.2	46.0–51.0	45.2–49.0	51.6–55.8	52.7	50.2–54.3	52.6
Dorsal fin base length	13.7–19.8	19.9–23.4	17.5–19.7	18.5–21.5	12.8–15.9	17.2	11.9–15.0	12.0
Dorsal fin height	24.8–43.9	21.8–33.0	25.9–34.3	25.2–28.2	18.9–24.2	28.3	25.6–30.0	17.7
Unsegmented dorsal fin height	17.7–35.8	10.4–19.2	14.4–24.2	12.3–17.0	8.8–13.9	18.9	10.0–15.7	7.1
Segmented dorsal fin height	7.5–34.0	26.0–46.1	9.9–45.5	24.1–37.5	28.6–48.8		37.7–58.6	35.0
Post-dorsal length	28.3–38.8	29.2–38.6	34.9–39.0	31.8–38.7	27.6–35.8	34.5	33.0–42.1	37.0
Dorsal-pelvic length	24.6–35.7	32.3–37.4	28.2–36.4	30.3–36.8	23.2–28.8	29.6	23.3–30.9	26.1
Pre-pectoral length	25.1–33.1	21.4–25.3	23.2–28.6	22.2–25.4	27.7–31.8	29.1	26.7–31.6	30.3
Pectoral fin length	19.2–28.2	21.4–24.7	22.2–24.5	21.4–24.1	17.7–22.0	22.2	18.3–22.9	20.6
Pre-pelvic length	50.1–57.7	50.5–56.9	49.6–56.3	50.5–56.9	53.4–59.8	54.5	51.7–57.3	55.3
Pelvic fin length	16.8–22.9	19.1–24.0	19.4–22.4	19.9–23.3	16.0–19.1	19.8	16.8–20.1	17.1
Anal fin base length	5.4–9.7	7.2–11.1	6.8–8.3	7.2–9.0	6.1–8.1	7.6	5.7–7.2	6.6
Anal fin height	18.8–26.9	18.2–26.9	20.9–24.7	21.0–25.2	14.2–19.3	22.1	18.2–22.9	18.4
Caudal peduncle length	11.9–19.9	13.5–17.0	12.5–19.1	12.4–16.7	12.7–18.5	14.8	12.9–18.3	13.5
Maximum caudal peduncle height	12.7–17.9	15.4–18.3	14.9–17.6	15.9–17.8	12.1–14.4	15.6	13.0–16.1	15.2
Minimum caudal peduncle height	10.7–15.0	13.2–15.0	12.6–14.1	12.8–14.3	10.3–12.5	13.2	11.4–13.6	12.0
Pre-anal length	69.7–81.2	74.8–81.6	73.5–79.1	71.3–77.8	73.1–79.0	80.2	71.6–79.0	74.6
Head length	25.8–32.3	22.0–25.4	23.3–27.5	22.8–24.6	29.3–34.2	30.1	28.1–30.9	29.1
Measurements in %HL								
Pre-operculum length	69.7–80.6	63.8–77.8	71.6–79.3	70.5–75.4	69.5–74.9	73.9	69.9–83.9	71.2
Head width	49.7–62.8	61.1–73.9	53.2–63.0	55.8–67.6	40.4–47.8	49.8	46.0–61.4	57.9
Inter-orbital distance	22.9–35.7	36.4–57.7	30.7–41.0	35.0–45.5	19.8–23.7	29.2	27.2–38.8	35.9
Lower jaw length	30.4–42.7	24.7–48.8	30.9–40.9	27.7–35.6	43.1–48.7	44.2	31.4–45.5	36.2
Mouth width	14.2–30.0	21.6–43.8	16.9–29.2	20.4–33.2	17.0–26.1	18.5	15.7–27.8	24.8
Eye diameter	21.6–41.9	18.6–31.6	25.9–37.8	26.1–32.7	17.8–23.2	24.5	22.5–38.3	23.4
Inter-nasal distance	13.5–23.1	22.1–31.2	14.7–22.6	17.1–25.0	9.9–16.1	18.1	14.6–23.5	19.4
Snout length	26.8–43.5	32.0–47.7	31.4–44.0	31.1–39.4	30.5–35.7	34.2	37.3–45.2	36.9
Anterior barbel length	16.1–40.7		7.8–12.6		10.4–23.6	21.8	14.4–21.5	15.8
Posterior barbel length	19.9–42.9	1.5–7.2	5.1–17.1	3.6–9.2	15.0–27.0	27.0	17.1–25.4	19.6
Premaxillary pedicel length	9.0–23.6		15.3–32.4		15.8–22.4	20.2	20.7–31.3	21.0

Table 4. *Cont.*

	L. mawambiensis	*L. longidorsalis*	*L. mawx long* **2**	*L. mawx long* **1**	*L. macroceps*	*L. macx maw*	*L.***sp. 'thick lip'**	*L. caudovittatus*
(b)	***n*= 149**	***n*= 16**	***n*= 16**	***n*= 9**	***n*= 13**	***n*= 1**	***n*= 16**	***n*= 1**
Total number of lateral line scales	21–28	23–27	24–28	24–27	31–36	29	24–27	24
Number of predorsal scales	7–11	8–10	8–11	8–9	10–14	10	7–10	8
Number of scales above the lateral line	3.5–5.5	4.5–5.5	4.5–5.5	4.5–5.5	5.5–5.5	5.5	3.5–4.5	4.5
Lateral line-pelvic scales	1.5–2.5	2–2.5	2–2	2–3	2–3	2.5	1.5–2	2.5
Lateral line–ventral midline scales	3.5–5.5	3.5–4.5	3.5–4.5	4.5–4.5	4.5–5.5	4.5	3.5–4.5	4.5
Caudal peduncle scales	12	12	12	12	12	12	12	12
Number of dorsal-fin base scales	5–10	6–10	6–10	7–11	6–10	8	5–8	8
Number of anal-fin base scales	1–5	2–4	3–4	3–4	3–5	4	3–5	3
Number of unbranched dorsal fin rays	4	4	4	4	4	4	4	4
Number of branched dorsal fin rays	8–11	11–12	10–12	11–12	10–11	10	9–10	10
Number of unbranched anal fin rays	3	3	3	3	3	3	3	3
Number of branched anal fin rays	6	6	6	6	6	6	6	6
Number of branched pectoral fin rays	14–17	13–15	14–16	13–15	13–15	14	14–16	15
Number of branched pelvic fin rays	7–9	8–9	8–9	8–9	8–8	8	8–9	8
Number of caudal fin rays	17	17	17	17	17	17	17	17
Number of scales between dorsal and caudal fin	9–15	11–13	11–14	10–13	12–18	13	11–15	11
Number of lateral line scales between anterior dorsal- and pelvic-fin base	0.5–2.5	2–3.5	1–2.5	2–2.5	1–2	1.5	1–2	2
Number of gill rakers on lower branch of first gill arch	9–15	10–14	10–13	10–13	5–7	9	13–17	9
Number of gill rakers on upper branch of first gill arch	2–6	1–4	2–6	2–4	2–3	4	3–6	4
Total number of gill rakers on the first gill arch	14–19	14–18	14–18	14–18	9–11	14	19–23	14

Table 5. Value ranges for measurements (**a**) and meristics (**b**) of the examined type specimens of the nominal species.

	L. caudovittatus	*L. fasolt*	*L. humphrii*	*L. longidorsalis*	*L. macrolepidotus*	*L. mawambi*	*L. mawambiensis*	*L. mirabilis*
(a)	**2 syntypes**	**holotype**	**holotype & 10 paratypes**	**holotype**	**6 syntypes**	**holotype**	**9 syntypes**	**holotype**
Standard length (mm)	71.2–74.7	464.0	143.2 & 80.1–207.7	234.3	62.7–129.4	61.7	92.3–173.9	334.0
Measurements (in % SL)								
Body depth	25.3–26.5	31.8	27.2 & 22.6–29.6	34.2	29.0–34.8	28.6	28.9–34.4	31.7
Predorsal length	52.2–52.9	53.2	48.8 & 47.2–51.1	46.6	46.0–50.1	53.3	50.0–55.6	54.5
Dorsal fin base length	13.6–16.5	13.7	16.1 & 15.2–17.3	23.6	15.3–22.1	14.0	12.7–19.2	18.1
Dorsal fin height	19.8–20.5	18.9	22.1 & 18.3–29		23.5–28.6	20.6	23.6–32.0	16.0
Unsegmented dorsal fin height	8.1–9.9	8.4	20.7 & 15–27.6	17.8	10.7–15.6	12.8	19.2–26.4	11.5
Segmented dorsal fin height		35.5			37.4–44.2	27.2	34.7–34.7	14.7
Post-dorsal length	34.8–36.3	35.3	39 & 31.9–41.3	35.5	29.8–34.9	33.7	31.4–37.8	31.3
Dorsal-pelvic length	24.6–27.8	29.3	26.5 & 22.1–26.4	32.0	25.0–32.3	28.4	28.2–33.0	32.1
Pre-pectoral length	27.4–28.9	30.1	26 & 26.3–29.8	20.7	24.6–30.0	29.0	26.8–32.4	28.0
Pectoral fin length	16.7–19.7	21.2	21.6 & 19.5–21.7	21.7	22.5–25.0	19.9	21.0–25.5	21.5
Pre-pelvic length	54.4–55.0	57.0	51.7 & 50.7–54	53.7	52.9–56.7	54.7	50.8–55.4	56.2
Pelvic fin length	18.7–19.5	17.0	18.7 & 16.2–22	21.8	19.0–23.3	19.0	17.8–21.5	17.1
Anal fin base length	6.2–7.4	8.4	8.4 & 6.8–8.3	10.0	6.9–8.3	8.5	6.4–8.9	7.2
Anal fin height	17.7–20.1	15.0	19.7 & 18.8–22	24.1	20.8–23.2	18.5	19.9–25.7	19.4
Caudal peduncle length	12.8–17.0	15.2	13.8 & 13.7–17.9	16.0	12.7–17.0	13.5	13.4–17.3	14.0
Maximum caudal peduncle height	11.8–16.1	13.2	13.8 & 12–13.4	15.8	14.5–15.9	15.1	13.3–18.1	14.2
Minimum caudal peduncle height	9.8–13.8	12.6	9.9 & 10–11.4	12.8	12.3–13.7	12.8	11.6–14.9	12.6
Pre-anal length	74.4–77.7	78.0	76.5 & 69–78.5	77.6	72.6–75.8	78.6	73.6–80.9	81.8
Head length	27.5–27.7	28.5	26.2 & 27.6–29.8	20.8	25.0–30.0	28.8	26.8–29.6	26.2
Measurements (in % HL)								
Pre-operculum length	72.0–74.0	73.0	72 & 69.4–74	71.0	72.5–78.9	75.5	70.8–76.8	74.2
Head width	50.7–53.6	61.7	55.2 & 50.3–55.8	73.8	50.0–62.2	46.8	51.3–58.8	59.2
Inter-orbital distance	32.1–33.8	46.1	30.4 & 26.3–34.2	53.0	31.4–37.2	25.3	26.5–36.0	41.2
Lower jaw length	34.3–35.7	34.6	35.2 & 32.3–39.1	36.1	29.6–34.1	42.4	32.8–38.2	36.3
Mouth width	20.8–22.4	39.9	19.2 & 17.9–21.9	44.6	20.7–26.9	21.1	19.1–23.7	29.2
Eye diameter	31.1–32.4	18.7	26.9 & 20.5–32.5	29.2	28.5–32.4	33.7	24.4–33.3	24.8
Inter-nasal distance	17.9–19.9	27.5	17.1 & 15.8–20	25.6	16.5–24.8	12.1	14.5–19.6	22.2
Snout length	30.6–32.9	36.2	34.4 & 21–38.5	32.3	32.4–38.2	31.7	28.8–35.7	35.0
Anterior barbel length	18.4–21.7	17.1	23.5 & 14.8–26.3			13.4	20.9–31.9	19.0
Posterior barbel length	15.5–28.1	22.1	28.8 & 24–30.7	3.3	3.7–9.4	21.6	22.1–37.4	22.3

Table 5. *Cont.*

	L. caudovittatus	*L. fasolt*	*L. humphrii*	*L. longidorsalis*	*L. macrolepidotus*	*L. mawambi*	*L. mawambiensis*	*L.mirabilis*
(b)	**2 syntypes**	**holotype**	**holotype + 10 paratypes**	**holotype**	**6 syntypes**	**holotype**	**9 syntypes**	**holotype**
Total number of lateral line scales	26	26	26 & 24–28	29	25–27	28	23–26	31
Number of predorsal scales	10	8.5	8 & 8–10	10	7–8	10	8–10	14
Number of scales above the lateral line	4.5	4.5	4.5 & 4.5–4.5	4.5	4.5–5.5	5.5	4.5–4.5	5.5
Lateral line–pelvic scales	1.5–2.0	3	2 & 2–2.5	2	2.0–2.5	2.0	2.0	3.0
Lateral line–ventral midline scales	4.5	4.5	4.5 & 4.5–5.5	4.5	4.5	4.5	4.5–5.5	5.5
Caudal peduncle scales	12.	12	12 & 12–12	12	12	12	12	12
Number of dorsal-fin base scales	7	6	8 & 5–9	6	6–11	5	5–8	7
Number of anal-fin base scales	2–3	3	5 & 3–4	4	3–4	4	3–4	4
Number of unbranched dorsal fin rays	4	4	4	4	4	4	4	4
Number of branched dorsal fin rays	10	10	10 & 9–10	13	10–12	11	10	11
Number of unbranched anal fin rays	3	3	3	3	3	3	3	3
Number of branched anal fin rays	6	6	6 & 6–6	6	6	6	6	6
Number of branched pectoral fin rays	14–15	15	16 & 15–17	14	13–16	15	14–17	15
Number of branched pelvic fin rays	8	8	8 & 8–8	8	8	8	8	8
Number of caudal fin rays	17	17	17 & 17–17	17	17	17	17	17
Number of scales between dorsal and caudal fin	10–14	14	13 & 9–14	11.5	10–12	13	12–13	12
Number of lateral line scales between anterior dorsal- and pelvic-fin base	2.0–2.5	1.5	2 & 1.5–2.5	3	2.5–3	1.5	1.0–2.5	1
Number of gill rakers on lower branch of first gill arch	9–11	11	11 & 9–12	13	12–13	8	11–13	10
Number of gill rakers on upper branch of first gill arch	4	3	3 & 2–4	3	2–3	3	3–4	2
Total number of gill rakers on the first gill arch	14–16	15	15 & 13–16	17	15–16	12	15–18	13

4. Discussion

4.1. Which Labeobarbus Species Are Present in the Epulu Basin?

Disentangling the *Labeobarbus* diversity in the Epulu has proven to be a complex task, especially since, besides some well-delineated species, morphotypes with an intermediate-mouth phenotype also occurred. Using an integrative approach [20], combining morphological and genetic approaches was indispensable. We followed the reasoning of [34] that evidence for species status is not required in all approaches. For each of the morphotypes identified, an explanation to possible discordances between approaches should be attempted in a most parsimonious way and using an evolutionary perspective. We did so in the discussions below. Additionally, putting a name on the recognized species was not always straightforward. The decisions made on the taxonomic status of each of the groups, are listed in Table 1b. Furthermore, the measurements and counts of all morphotypes from the Epulu and all types of the nominal species examined are summarised in Table 4a,b and Table 5a,b, respectively. Finally, an identification key to the *Labeobarbus* species of the Epulu River is provided.

Morphological and genetic (mtDNA: cyt *b*) analyses indicated that at least five *Labeobarbus* species are present in the Epulu River: *L. longidorsalis L. macroceps, L. mawambiensis, L.* sp. 'thick lip' and *L. caudovittatus.*

Labeobarbus sp. 'thick lip' is the only species having a real *Lab.*-mouth phenotype following the classification of [4]. It could not be assigned to any of the currently valid species and thus probably represents a new species for science. Although it is morphologically most similar to *L. caudovittatus* and genetically clustered with the one specimen identified as *L. caudovittatus*¸ marked morphological differences were found. Besides having much more hypertrophied lips than *L. caudovittatus, L.* sp. 'thick lip' also had a higher number of gill rakers on the first gill arch [19–23 (median: 21) vs. 14–16], which is an independent meristic characteristic. The haplotypes of *L. caudovittatus* and *L.* sp. 'thick lip' clustering together could be explained by, e.g., introgression or incomplete lineage sorting, as conspecificity is very unlikely in this case due to the large and independent (i.e., mouth phenotype, meristic and colour pattern), morphological differences. *Labeobarbus caudovittatus* is a very widespread species with a high amount of intraspecific morphological variation [17]. Interestingly, both *L. caudovittatus* and *L.* sp. 'thick lip' (as *L.* cf. *caudovittatus* in [35]) have recently been found in the Lowa basin, a right bank affluent of the Upper Congo or Lualaba (K. Tchalondawa, pers. comm.), illustrating that the undescribed species has a more widespread occurrence. *Labeobarbus caudovittatus* has five junior synonyms, among which one with a *Var.*-mouth phenotype, *Labeobarbus stappersii* (Boulenger, 1917). The species is widespread, and a high variety in (mouth) morphology is observed within its distribution range. Therefore, this *L. caudovittatus* species-complex, will be further examined in a follow-up study [23]. In this study, *L.* sp. 'thick lip' will be formally described after a detailed comparison with specimens from the whole distribution range of *L. caudovittatus* and its junior synonyms as well as of the recently revalidated *L. pojeri*.

Labeobarbus longidorsalis is the only species in the Epulu with a *Var.*-mouth phenotype. *Labeobarbus macroceps* is the only species in the Epulu with a prognathous lower jaw, and is an Epulu endemic.

The specimens of *L. mawambiensis* have an attached lobe or, occasionally, a free lobe, and non-hypertrophied lips, and have thus a *Lab.*-like and sometimes even a *Lab.*-mouth phenotype following the classification of [4]. One specimen of *L. mawambiensis* formed a separate, but not well-supported lineage on the ML tree (cyt *b*, mtDNA). This could point to the presence of yet another species, but since the specimen was morphologically not distinguishable from the other *L. mawambienis* specimens, this is highly unlikely. Other hypotheses to explain this unexpected position, such as incomplete lineage sorting or introgression after hybridisation, cannot be ruled out, but to further evaluate these, nuclear DNA data are needed.

In addition to the species recognized, three intermediate morphotypes were found in the Epulu basin. They are discussed below in Section 4.2.

The morphotypes that occur in the Epulu are similar to the morphotypes that occur in the 'species flocks' from the Ethiopian highlands [36,37]. In the Epulu, we also discovered a lipped form (*L.* sp. 'thick lip'), a generalist form (*L. mawambiensis*), a scraper form (*L. longidorsalis*) and a large-mouthed form (*L. macroceps*). Levin et al. [36,37] found that in the Ethiopian highlands, several of these 'species flocks' occurred in riverine environments, being the result of adaptive radiations to different ecological niches. In a riverine environment, depauperate fish faunas of isolated upper reaches, like the Epulu, can facilitate trophic polymorphisms [38]. However, although the presence of similar morphotypes is obvious in the Epulu, there may be a difference on the genetic level compared to the species flocks in the Ethiopian highlands. A NJ tree of our cyt *b* sequences and those of *Labeobarbus* found on GenBank (Supplementary Material, Figure S2) revealed that the species from the Epulu do not form a monophyletic group. These results could be influenced by saturation due to the inclusion of distantly related species as only a few, additional, sequences from the Congo basin are available on GenBank. However, the rather young age of the *Labeobarbus* clade (Late Miocene) [2] seems to preclude such an interpretation. In addition, preliminary results already revealed that several morphotypes of the Epulu also occur downstream of the Arabia Waterfall in the Ituri, while endemicity is one of the prerequisites of species flocks [36]. Furthermore, *L.* sp. 'thick lip', is also found in the Lowa River (Upper Congo), which was also confirmed with genetic results (Kisekelwa, pers. data). The same holds true for *L. longidorsalis*, a scraper form, which was originally described from the Luhoho (Lowa Basin: Upper Congo), and is also found in the Epulu River (Kisekelwa, pers. data). As monophyly and endemicity do not apply for the species in the Epulu, these species most probably do not constitute a species flock. Nevertheless, further genetic/genomic studies, complemented with ecological studies (e.g., stable isotope analysis) to study trophic specialisation are needed to fully tackle the species flock hypothesis.

4.2. The Labeobarbus mawambiensis/longidorsalis Hybrid Complex

Thirteen percent (25/190 specimens) of the examined specimens of the *L. mawambiensis/longidorsalis* complex (i.e., the "Lab/Var"-complex) had a mouth phenotype intermediate between that of *L. mawambiensis* and *L. longidorsalis*. These specimens lack the mental lobe, typical for the *Lab.*(like)-mouth phenotype of *L. mawambiensis*, but also lack the typical keratinised cutting edge of the *Var.*-mouth phenotype of *L. longidorsalis*. They have a harder lower lip and straighter mouth than in the typical *Lab.*- and *Lab.*-like mouth phenotypes, but more curved than in the *Var.*-mouth phenotype. Based on the number of barbels, two or one pair, two different kinds of such morphotypes have been distinguished within this complex ("inter2" and "inter1").

Based on the synthesis of the morphological results above, the specimens of "inter2" were morphologically clearly intermediate between *L. mawambiensis* and *L. longidorsalis*. This could point to intraspecific variation or plasticity in mouth morphology, with an intraspecific range of morphotypes from "Lab-like" over "inter 1" and "inter 2" to "Var". However, in the cyt *b* analysis, the two extreme morphotypes "Lab-like" and "Var" form two clearly distinct clades, indicating the presence of distinct species. All intermediate morphotypes clustered with *L. longidorsalis.* Hence, they are considered putative hybrids between *L. mawambiensis* and *L. longidorsalis,* containing the maternal DNA of *L. longidorsalis.* The presence of *Labeobarbus* hybrids in the Epulu would be in line with several other indications of possible hybridisation events within this genus [4] and the recent discovery of another hybridisation complex in the Inkisi River, Lower Congo basin [9].

The status of the intermediate mouth phenotype specimens with one pair of barbels is more difficult to interpret. In addition to the fact that they strongly resembled *L. longidorsalis*, they shared the same cyt *b* haplotype with *L. longidorsalis*. The only characteristic in which they thus differ from *L. longidordalis* is the lack of the cutting edge on the lower lip. An alternative for the hypothesis of hybridisation is the presence of intraspecific variation assuming specimens with and without a cutting edge within *L. longidorsalis*. However, in a similar case in the Inkisi River, specimens with a comparable phenotype, lacking the typical

Var.-mouth phenotype cutting edge, were most parsimoniously interpreted as interspecific hybrids, based on AFLP data [9]. Adding to the complexity, "inter1" always clustered with the syntypes of *L. macrolepidotus*, a species which also lacks the keratinised cutting edge. However, *L. macrolepidotus* is currently only known from the Kasai system and the Lower Congo [39], hence, the conspecificity of "inter1" with *L. macrolepidutus* is rather unlikely. In addition, although the putative hybrids have morphologically been classified into two categories based on the number of barbels, variability in mouth morphology still exist within these groups, rather displaying a kind of continuum in barbel lengths and the curviness of the mouth, with some leaning more towards *Lab.*-like phenotypes and others more towards *Var.*-mouth phenotypes. Furthermore, both "inter2" and "inter1" clustered with *L. longidorsalis* on the ML tree (Figure 6). It would thus not be parsimonious to consider "inter1" to be a distinct species (*L. macrolepidotus),* while "inter2" is considered a putative hybrid between *L. longidorsalis* and *L. mawambiensis*. They are thus both considered putative hybrids between *L. longidorsalis* and *L. mawambiensis*.

Another issue is that two different cyt *b* haplotypes were present in the putative hybrids, which were not concordant with the two different morphotypes, nor was there a clear geographical pattern. In contrast, one of the putative parental species, *L. longidorsalis*, was only present in one of these subclades. These results point to the need for further genetic analyses beyond cyt *b* mtDNA genotyping.

Our results seem to confirm that hybridisation between species with a *Lab.*- and a *Var.*-mouth phenotype, and between *Labeobarbus* species in general, is not exceptional (see, e.g., [7,40]), and is, most probably, a widespread phenomenon [4]. The hybridisation complex found in the Inkisi River displays, however, is different from the Epulu complex in several aspects. In the present study, only 25 of the 190 specimens of the *L. mawambiensis/longidorsalis* complex were identified as putative hybrids. In the Inkisi, however, the major part of the *Labeobarbus* specimens were considered to be hybrids [9]. In addition, the phylogenetic patterns are different between the Epulu and the Inkisi complexes. While in the Epulu both parental species formed two well-defined mtDNA (cyt *b*) lineages and both groups of putative hybrids belonged to only one of these, in the Inkisi, in contrast, both parental species and their hybrid specimens formed a single mtDNA lineage (COI).

The fact that in the Epulu both groups of putative hybrids clustered with *L. longidorsalis* on the cyt *b* tree (Figure 6), indicates that all putative hybrids have the maternal mtDNA of only one parent species, i.e., *L. longidorsalis.* This could be explained by, e.g., genomic incompatibilities, selection of certain mtDNA genotypes, or random extinction of hybrids containing the mtDNA of the other parent due to genetic drift. Based on the existing collections, *L. longidorsalis* is much more rarely found in the Epulu than *L. mawambiensis.* Studies on *Labeobarbus* species from Lake Tana demonstrated that species of *Labeobarbus* are group spawners [41,42]. Although spawning behaviour is mentioned to be non-specific [43], segregation in spatial and temporal spawning has been found between morphotypes of *L. intermedius* in Lake Tana [44]. Experiments on specimens of the *Labeobarbus intermedius* complex from Lake Tana examined the possibility of mate choice by males through chemical signalisation [45], though no significant preference for the same morphotype was found in any of their eight setups. Mate choice and spawning behaviour have not been studied yet for the Epulu species. A lack of mate choice and segregation in spawning behaviour might explain the results that all putative hybrids have the maternal DNA of *L. longidorsalis.* As in the Epulu, *L. longidorsalis* is far less abundant, the eggs of this species may indeed accidentally be fertilized by non-conspecific males (*L. mawambiensis*) during group spawning, producing hybrid offspring and leading to the hybrids having the maternal DNA of *L. longidorsalis.* If no post-zygotic isolation mechanisms exist, then this group spawning behaviour may have facilitated widespread hybridisation in *Labeobarbus*.

In addition to the *L. caudovittatus* species-complex currently under revision, and the *L. mawambiensis/longidorsalis* hybridisation complex discussed above, another case of uncertain taxonomic status has been identified. A single putative hybrid specimen ("Labprog") with intermediate mouth morphology between *L. mawambiensis* and *L. macroceps*

was found. While the mouth phenotype was more similar to the one of *L. macroceps*, the dorsal spine was characteristic for *L. mawambiensis.* A PCA on the meristics (not illustrated) confirmed its intermediate position between both species. Furthermore, on the ML tree, this specimen clustered within the *L. macroceps* lineage (Figure 6). We thus consider this specimen to be a putative hybrid between *L. mawambiensis* and *L. macroceps.*

4.3. Additional Nomenclatorial Decisions

Based on the results of the presented study, some additional decisions with nomenclatorial implications have been made.

(1) *Labeobarbus mawambi* and *L. mirabilis* are both only known by their holotype, collected from the Ituri River at Mawambi (~1°17′21″ N 28°25′37″ E). Based on their general morphology and morphological analyses (PCAs not illustrated), both could be distinguished from all other groups (Table 5a,b), but not from each other. According to the original descriptions and subsequent observations [4], both holotypes have the same mouth phenotype with an interrupted lower lip, but differ in dorsal spine morphology, i.e., a flexible vs. a bony spine. However, we observed the dorsal spine of *L. mirabilis* to be only weakly bony. In addition, the fact that the holotype of *L. mirabilis* has a weakly bony spine (instead of flexible) might be size-related as this specimen is quite large (334.0 mm SL vs. 61.7 mm SL in *L. mawambi*). As no further morphological differences could be found between the holotypes of these two nominal species, which moreover are described from the same locality, *L. mawambi* is hereby formally synonymized with *L. mirabilis*, as already tentatively suggested by Bannister [17].

(2) *Labeobarbus iturii* has originally been described based on one specimen from the Ituri River; which is considered lost (H. Wellendorf, pers. comm. 2014). Based on its original description, the species does not match with any of the types of the other nominal species examined, nor with the other specimens examined (Table 1a,b). The description of the species stipulates the presence of well-developed uninterrupted lips with a small mental lobe and two pairs of barbels, which matches the mouth morphology of both *L. mawambiensis* and *L. caudovittatus*, though it is not specified whether the mental lobe of *L. iturii* is posteriorly attached or not. However, according to its description, *L. iturii* has a higher number of lateral line scales (29 vs. 21–28 and 24–26, respectively), and a flexible dorsal fin spine, while *L. mawambiensis* has a strongly ossified dorsal spine. The well-developed lips, two pairs of barbels, and flexible dorsal spine also matches the general morphology of *L.* sp. 'thick lip' (Table 1a,b), but since the mental lobe of *L. iturii* is described as small, it is most probably different from the large, posteriorly free mental lobe of *L.* sp. 'thick lip'. Additionally, *L.* sp. 'thick lip' has fewer lateral line scales (24–27). Hence, *L. iturii* has not been found in the Epulu. Since no other specimens are available of *L. iturii*, a neotype for this species could not be designated.

(3) Based on the results of the morphological analyses, the "Lab-like" morphotype was identified as *L. mawambienis* (Table 1a,b). However, the specimens of this morphotype were also similar to *L. humphri*, a species only known from its type series from the Tabie River (~0°15′44″ N 29°27′30″ E), a small headwater stream of the Ituri River near the Congo/Nile divide. These types differ slightly from the Epulu specimens and the type series of *L. mawambiensis* by a generally lower number of gill rakers on the first gill arch (13–16 vs. 14–19), a shallower body and caudal peduncle, a lower dorsal fin, and a smaller eye diameter (see Table 5a,b). Because of these differences, and awaiting further studies on specimens from the headwaters of the Ituri, *L. humphri* is still considered a valid species, absent from the Epulu River.

For *L. iturii, L. mirabilis, L. humphri,* which are all described from the Ituri headwaters, no additional specimens besides the types have been found. The fact that species from the Ituri headwaters were not encountered in the Epulu, could be due to the presence of waterfalls and rapids in the area. The Arabia Falls on the Epulu just upstream of its confluence with the Ituri may account for the endemism of *L. macroceps* in the Epulu. However, on the Ituri itself, just upstream of the Epulu/Ituri confluence, there is also a

waterfall, named the Ngoy Falls (Figure 1), which could contribute to the fact that certain species only occur in the Ituri headwaters. This illustrates the need for additional sampling in the Ituri.

(4) *Labeobarbus mawambiensis* was originally described as *Barbus hindii mawambiensis* (Steindachner, 1911) based on seven specimens from the Ituri River, and a year later elevated to the species level [46]. Later on, Steindachner [47] reported three additional specimens from the Dja River (Cameroon), and the Ituri. Currently nine specimens, all housed at the NMW, are listed as syntypes of *L. mawambiensis:* NMW 54177 (2), 54286 (3), 54287 (2) and 54288 (2) (see [48]). The current NMW catalog does not contain any *L. mawambiensis* specimen from the Dja (A. Palandacic, pers. comm. 2017). As stipulated by Steindachner [47] (p. 25), the largest of these Dja specimens has been illustrated, though the illustration has probably been mixed up with the illustration of *L. habereri* [4]. According to the drawing that represents *L. mawambiensis*, the illustrated specimen from the Dja has a size of about 100 mm SL and 130 mm TL, which seems to correspond to the smallest of the three specimens listed by Steindachner [47]. Currently, there is one NMW sample holding three *L. mawambiensis* specimens (i.e., NMW 54286), which could thus contain the three additional specimens reported from the Dja and Ituri [47]. Although labelled as originating from the Ituri, several elements cast doubt on the correct labeling of these specimens: (i) none of the current labels seem to be original; (ii) for NMW 54286, but not for the other lots, the label stipulates "syntypes?" confirming uncertainties about the type status of these specimens; and (iii) the standard and total lengths do not correspond well with those provided by Steindachner [47]. As a result and in view of: (i) the fact that nine specimens are currently labelled as syntypes of *L. mawambiensis*, whereas the original description only reported seven; (ii) the uncertainties with regard to the syntype status of NMW 54286; (iii) the fact that some specimens currently indicated as syntypes possibly originate from the Dja and not from the type locality, the Ituri; and (iv) to avoid further confusion; the largest of the syntypes (NMW 54177: 170.9 mm SL), which is in very good state of preservation, is here designated as the lectotype of *L. mawambiensis*.

4.4. Hybridisation: A Widespread and Variable Phenomenon in Labeobarbus

Hybridisation among *Labeobarbus* species has been documented for the first time by Banister [7,8], and several other cases have been reported since (e.g., [40,44,49], and see [4] for a historical overview). Our study and other cases, e.g., [9], already pointed to the frequent occurrence of hybridisation within the genus. The fact that within the hybridisation complex a kind of continuum of mouth morphology is noticed, is another indication that these specimens are the result of various hybridisation processes (from F1 hybrids to subsequent hybrids over multiple generations with possibly backcrosses with one or both parent species). The multitude of indications of hybridisation in the African Torini points to the absence of assortative mating and hence incomplete prezygotic isolation [50]. The fact that the species are probably group spawners likely contributes to the lack of prezygotic isolation.

Hybridisation events (both hybridisation into the ancestral lineage and genetic exchange between diverging lineages) are known to facilitate speciation events [51]. This is well documented in the intensively studied adaptive cichlid radiations of the East African lakes. These studies provided evidence that hybridisation events, varying in scale from hybrid individuals, over introgressed populations, to species and even lineages of hybrid origin, have largely influenced the evolution of these cichlid lineages (e.g., [30,52–54]). To which extent the evolutionary history of species of *Labeobarbus* is influenced by such hybridisation events is currently not known. Yet, it has recently been found that the origin of the hexaploid genus *Labeobarbus* itself is the result of ancient hybridisation events [1]. In addition, an adaptive radiation of *Labeobarbus* species is known from Lake Tana, where hybridisation might have facilitated ecological diversification [55], resulting in a syngameon (sensu [56]).

Considering the evolutionary complexity of *Labeobarbus*, characterised possibly by multiple hybridisation events and their hexaploidy, a genomic approach should be envisaged to further study the evolutionary history of its constituting species. Within the Cyprinidae, hybridisation has also been detected in other genera, e.g., *Enteromius* [57], *Capoeta* and *Carasobarbus* [3,4,58]. In fact, hybridisation has been reported in several freshwater fish and other animal taxa [56]. The notion of hybridisation and introgression forces us to reconsider the existing views on species delineation and species boundaries, where species are still too often seen as diagnosable distinct and isolated entities [59], but should perhaps more be seen as evolving, i.e., dynamic, entities [19].

4.5. Identification Key to the Labeobarbus Species and Possible Hybrids of the Epulu Basin

An identification key to the species and the different putative hybrids is provided based on the studied specimens of the basin. Illustrations of the species and putative hybrids are presented in Figure 2.

(1) Well-defined keratinised cutting edge on lower jaw (Figure 2d)	*L. longidorsalis*
No keratinised cutting edge on lower jaw (Figure 2a–c,e–h)	2
(2) Lower jaw slightly to clearly prognathous (Figure 2e,f); 29–36 (median: 33) lateral line scales; 9–14 gill rakers on first gill arch	3
Mouth inferior; 21–29 (25) lateral line scales; 14–23 rakers on first gill arch	4
(3) 31–36 lateral line scales; 9–11 gill rakers on first gill arch; last unbranched dorsal fin ray flexible (38.0–61.6% of the dorsal fin height unsegmented) (Figure 2e)	*L. macroceps*
29 lateral line scales; 14 gill rakers on first gill arch; last unbranched dorsal fin ray a well-ossified spine (66.8% of the dorsal fin height unsegmented; strongly ossified) (Figure 2f)	*L. macroceps* x *mawambiensis* hybrid
(4) 19–23 (median: 21) gill rakers on first gill arch; lower lip with a large, posteriorly detached, median lobe (Figure 2h)	*L.* sp. 'thick lip'
14–19 (17) gill rakers on first arch; lower lip with or without a mental lobe; if present, mostly posteriorly attached (Figure 2a–c,g)	5
(5)One pair of short posterior barbels (Figure 2c)	putative *L. longidorsalis* x *mawambiensis* hybrid with one pair of barbels
Two pairs of barbels	6
(6) Barbels short; anterior barbels 7.8–12.6%HL and posterior barbels 5.1–17.1%HL; 10–12 (median: 11) branched dorsal fin rays; no mental lobe (Figure 2b)	putative *L. longidorsalis* x *mawambiensis* hybrid with two pairs of barbels
Barbels long; anterior barbels 15.8–40.7%HL, posterior barbels 19.6–42.9%HL; 8–11 (10) branched dorsal fin rays; mental lobe present, mostly posteriorly attached, sometimes free (Figure 2a,g)	7
(7) Last unbranched dorsal fin ray flexible (weakly ossified proximal part: 39.9%); dark grey to black band along the distal part of upper and lower caudal-fin lobes (Figure 2g)	*L. caudovittatus*
Last unbranched dorsal fin ray a well ossified spine (strongly ossified proximal part: 57.6–98.6%); upper and lower caudal-fin lobes uniform yellowish to grey (Figure 2a)	*L. mawambiensis*

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14121022/s1, Table S1: Overview of all newly generated cyt *b* sequences and comparative sequences downloaded from GenBank; Figure S1: Maximum Likelihood tree with 100 bootstrap replications on the cyt *b* gene (1130 bp) of *Labeobarbus* species from the Epulu and multiple additional outgroups. Statistical node support is shown as ML bootstrap/NJ bootstrap, or as a single number when both are identical; only bootstrap values > 95 % are shown. Branch lengths indicate the number of substitutions per site. Taxon names include both the names of the morphotypes and the eventual identifications. Different colours are given to the different morphotypes from the Epulu; Figure S2: Neighbor Joining tree with 100 bootstrap replications on the cyt *b* gene of all sequences from Figure 6, with addition of all available cyt *b* sequences of *Labeobarbus* from Genbank.

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writing—original draft preparation, E.D.; writing—review and editing, E.J.W.M.N.V. and J.S.; visualization, E.D. and E.J.W.M.N.V.; supervision, E.J.W.M.N.V. and J.S.; project administration, E.J.W.M.N.V. and J.S.; funding acquisition, E.J.W.M.N.V. and J.S. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All sequences and corresponding voucher numbers are uploaded in Genbank (See SI, Table S1). An alignment and morphological data can be provided upon request.

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Appendix A. Specimens Examined

Measurements given for the specimens examens refer to the SL.

Type specimens

Labeobarbus caudovittatus: RMCA1168 (syntype), 1, 74.7 mm, Banzyville, ±4°18′ N 21°10′ E, Royaux, 1901; BMNH 1901.12.26.26 (syntype), 1, 71.2 mm, Ubangi, DRC, 4°18′ N; 21°11′ E, Capt. Royaux, unknown collecting date.

Labeobarbus fasolt: ZMB 19061 (holotype), 1, 464.0 mm, Ituri River at Irumu, DRC, ~1°29′ N 29°51′ E, Schubotz.

Labeobarbus humphri: RBINS 559 (holotype), 1, 143.2 mm, Tabie River, about 25 km south of Beni, North Kivu District, DRC, ~0°30′ N 29°28′ E; RBINS 564 (paratypes), 10, 80.1–207.7 mm, same data as for holotype.

Labeobarbus longidorsalis: MNHN 1935–0065 (holotype), 1, 234.3 mm, Kanséhété River, tributary to Luhoho River, Kivu region, DRC, ~2°05′S 28°30′ E.

Labeobarbus macrolepidotus (syntypes): RMCA 19945, 1, 65.6 mm, Luluabourg, Kasai River, ~ 05°53′S 22°25′ E, Callewaert, 13 Feb 1930; RMCA 138767, 1, 72.1 mm, same data as for other syntypes; NMB 3983, 1, 129.4 mm, same data as for other syntypes; NMB 3985, 1, 87.0 mm, same data as for other syntypes; NMB 3988, 1, 66.7 mm, same data as for other syntypes; NMB 3989, 1, 62.7 mm, same data as for other syntypes.

Labeobarbus mawambi: ZMB 19062 (holotype), 1, 61.7 mm, Ituri River at Mawambi, DRC, ~1°03′ N 28°36′ E

Labeobarbus mawambiensis (syntypes)*:* NMW 54177, 2, 165.8–170.9 mm, Ituri River at Mawambi, DRC, ~1°03′ N 28°36′ E, Grauer, 1901; NMW 54286–54288, 7, 92.3–173.9 mm, same data as for other syntypes.

Labeobarbus mirabilis: ZMB 19059 (holotype), 1, 334.0 mm, Ituri River at Mawambi, DRC, ~1°03′ N 28°36′ E.

Specimens from the Epulu River

Labeobarbus caudovittatus: RMCA 2009–029–P–0347, 1, 145.4 mm, Edoro River, upstream of the bridge, near the research camp at Edoro–Afarama, affluent of Epulu River; 1°33′01,2″N 28°30′40,6″ E; Okapi Reserve Expedition, 11 July 2009.

Labeobarbus longidorsalis: RMCA 90–30–P–1272–1279, 7, 160.3–290.3 mm, Epulu River at Epulu, ca. 2 km upstream of the bridge near the Okapi station, no coordinates, 23–25 February 1990; RMCA 2009–029–P–0279, 1, 165.7 mm, Epulu River at Epulu, ca. 250 m upstream of the bridge, before the GIC building, 1°24′07,6″N 28°34′43,6″E, Okapi Reserve Expedition, 22 June 2009; RMCA 2009–029–P–0283–0284, 2, 152.0–206.9 mm, Epulu River at Bandisende, 30 km from the RFO station; 1°24′47,1″ N 28°44′21,5″E; Okapi Reserve Expedition, 01 July 2009; RMCA 2009–029–P–0285, 1, 93.4 mm, Epulu River at Bandisende, 30 km from the RFO station, 1°24′47,1″N 28°44′21,5″E, Okapi Reserve Expedition, 30 June 2009; RMCA 2009–029–P–286, 1, 183.8 mm, Epulu River at Epulu, ca. 250 m upstream of the bridge, before the GIC building, 1°24′07,6″N 28°34′43,6″E, Okapi Reserve Expedition, 22 June 2009; RMCA 2009–029–P–0288–0289, 2, 174.2–187.6 mm, Epulu River at Epulu, right bank, downstream of the bridge, 1°24′00,6″ N 28°34′31,7″ E, Okapi Reserve Expedition, 01 July 2009; and 2 more uncatalogued specimens housed at the RMCA.

Labeobarbus macroceps: RMCA 2009–029–P–0297, 1, 232.2 mm, Epulu River at Epulu, right bank, upstream of the bridge, across the chimpanzee island; 1°24′34,7″ N 28°35′06,5″ E, Okapi Reserve Expedition, 04 June 2009; RMCA 2009–029–P–0302–0307, 6, 145.6–208.9 mm, Edoro River, upstream of the bridge, near the research camp at Edoro–Afarama, affluent of Epulu River; 1°33′01,2″N 28°30′40,6″ E; Okapi Reserve Expedition, 13 July 2009; RMCA 2009–29–P–0308, 1, 144.6 mm, Nduye River at Nduye, upstream of the bridge, behind the police camp, affluent of Epulu River; 1°49′56,0″ N 28°58′40,1″ E, Okapi Reserve Expedition, 25 July 2009; RMCA 2009–029–P–0309, 1, 123.2 mm, Nduye River at Nduye, upstream of the bridge, behind the police camp, affluent of Epulu River; 1°49′56,0″ N 28°58′40,1″ E, Okapi Reserve Expedition, 25 July 2009; RMCA 2009–029–P–0310, 1, 188.3 mm, Nduye River at Nduye, upstream of the bridge, behind the police camp, affluent of Epulu River; 1°49′56,0″ N 28°58′40,1″ E, Okapi Reserve Expedition, 25 July 2009; and 3 more uncatalogued specimens housed at the RMCA.

Labeobarbus mawambiensis: RMCA 2009–029–P–0278, 1, 128.6 mm, Epulu River at Epulu, right bank, upstream of the bridge, 1°24′18,5″ N 28°35′00,7″ E, Okapi Reserve Expedition, 03 June 2009; RMCA 2009–029–P–0313, 1, 139.4 mm, Epulu River at Epulu, right bank, downstream of the bridge, 1°24′00,6″ N 28°34′31,7″ E, Okapi Reserve Expedition, 25 May 2009; RMCA 2009–029–P–0318, 1, 182.2 mm, Epulu River at Epulu, right bank, downstream of the bridge, 1°24′00,6″ N 28°34′31,7″ E, Okapi Reserve Expedition, 01 June 2009; RMCA 2009–29–P–0319–0321, 2, 110–134.2 mm, Epulu River at Epulu, right bank, upstream of the bridge, 1°24′18,5″ N 28°35′00,7″ E, Okapi Reserve Expedition, 02 June 2009; RMCA 2009–029–P–0341, 1, 118.1 mm, Epulu River at Epulu, ca. 250 m upstream of the bridge, before the GIC building, 1°24′07,6″ N 28°34′43,6″ E, Okapi Reserve Expedition, 25 June 2009; RMCA 2009–029–P–0342, 1, 150.4 mm, Epulu River at Epulu, right bank, upstream of the bridge, across the chimpanzee island; 1°24′34,7″ N 28°35′06,5″E, Okapi Reserve Expedition, 27 June 2009; RMCA 2009–29–P–0345–0346, 2, 150.4–178.2 mm, Edoro River, upstream of the bridge, near the research camp at Edoro–Afarama, affluent of Epulu River; 1°33′01,2″ N 28°30′40,6″ E; Okapi Reserve Expedition, 03 July 2009; RMCA 2009–029–P–0348–0351, 4, 102.5–180.7 mm, Epulu River at Epulu, right bank, downstream of the bridge, 1°24′00,6″ N 28°34′31,7″ E, Okapi Reserve Expedition, 24 May 2009; 2009–029–P–0365, 1, 134.2 mm, Epulu River at Epulu, right bank, upstream of the bridge, across the chimpanzee island; 1°24′34,7″ N 28°35′06,5″ E, Okapi Reserve Expedition, 04 June 2009;

RMCA 2009–029–P–0370, 1, 135.5 mm, Epulu River at Epulu, ca. 250 m upstream of the bridge, before the GIC building, 1°24′07,6″ N 28°34′43,6″ E, Okapi Reserve Expedition, 24 June 2009; RMCA 2009–029–P–0392–0393, 2, 138.4–153.5 mm, Edoro River, upstream of the bridge, near the research camp at Edoro–Afarama, affluent of Epulu River; 1°33′01,2″ N 28°30′40,6″ E; Okapi Reserve Expedition, 11 July 2009; RMCA 2009–P–029–P–0401–0402, 2, 160.8–162.1 mm, Afarama River, affluent of Edoro River, affluent of Epulu River, 1°33′05,5″ N 28°30′16,8″ E, Okapi Reserve Expedition, 12 July 2009; RMCA 2009–029–P–0403–0405, 3, 112.7–140.3 mm, Nduye River at Nduye, upstream of the bridge, behind the police camp, affluent of Epulu River; 1°49′56,0″ N 28°58′40,1″ E, Okapi Reserve Expedition, 24 July 2009; RMCA 2009–029–P–0412–0413, 2, 125.3–174.3 mm, Epulu River at Epulu, right bank, downstream of the bridge, 1°24′00,6″ N 28°34′31,7″ E, Okapi Reserve Expedition, 24 May 2009; RMCA 2009–029–P–0414–0415, 2, 86.7–166.6 mm, Epulu River at Epulu, right bank, downstream of the bridge, 1°24′00,6″ N 28°34′31,7″ E, Okapi Reserve Expedition, 25 May 2009; RMCA 2009–029–P–0440, 1, 182.7 mm, Afarama River, affluent of Edoro River, affluent of Epulu River, 1°33′05,5″N 28°30′16,8″E, Okapi Reserve Expedition, 12 July 2009; RMCA 2009–029–P–0494–0497, 4, 124.9–135.5 mm, Lelo River, agricultural area of the Epulu centre, affluent of Epulu River, in primary forest, 1°25′52,7″N 28°34′27,4″E, Okapi Reserve Expedition, 24 June 2009; RMCA 2009–029–P–0498–0502, 5, 92.1–164.0 mm, Lelo River, agricultural area of the Epulu centre, affluent of Epulu River, in primary forest, 1°25′52,7″N 28°34′27,4″E, Okapi Reserve Expedition, 28 Jun 2009; RMCA 2009–029–P–1152, 1, 136.0 mm, Epulu River at Bandisende, 30 km from the RFO station, 1°24′47,1″N 28°44′21,5″E, Okapi Reserve Expedition, 01 July 2009; and 112 more uncatalogued specimens housed at the RMCA.

Labeobarbus mawambiensis x *macroceps* hybrid: RMCA 2009–029–P–1153, 1, 170.5 mm, Epulu River at Bandisende, 30 km from the RFO station, 1°24′47,1″N 28°44′21,5″E, Okapi Reserve Expedition, 01 July 2009.

Labeobarbus mawambiensis x *longidorsalis* hybrid with two pairs of barbels: RMCA 2009–029–P–0271, 1, 163.8 mm, Epulu River at Epulu, right bank, upstream of the bridge, across the chimpanzee island; 1°24′34,7″ N 28°35′06,5″ E, Okapi Reserve Expedition, 26 June 2009; RMCA 2009–029–P–0272–0274, 3, 93.3–148.1 mm, Epulu River at Bandisende, 30 km from the RFO station, 1°24′47,1″ N 28°44′21,5″ E, Okapi Reserve Expedition, 30 June 2009; RMCA 2009–029–P–0275–0277, 3, 107.5–144.2 mm, Epulu River at Epulu, right bank, upstream of the bridge, 1°24′18,5″ N 28°35′00,7″ E, Okapi Reserve Expedition, 03 June 2009; RMCA 2009–029–P–0290–0291, 2, 170.6–180.0 mm, Epulu River at Bandisende, 30 km from the RFO station, 1°24′47,1″ N 28°44′21,5″ E, Okapi Reserve Expedition, 30 Jun 2009; RMCA 2009–029–P–0475, 1, 111.8 mm, Epulu River at Epulu, right bank, upstream of the bridge, across the chimpanzee island; 1°24′34,7″ N 28°35′06,5″ E, Okapi Reserve Expedition, 03 June 2009; and 6 more uncatalogued specimens housed at the RMCA.

Labeobarbus mawambiensis x *longidorsalis* hybrid with one pair of barbels: RMCA 2009–029–P–0280–0281, 2, 96.6–133.5 mm, Epulu River at Epulu, ca. 250 m upstream of the bridge, before the GIC building, 1°24′07,6″ N 28°34′43,6″ E, Okapi Reserve Expedition, 22 June 2009; RMCA 2009–029–P–0282, 1, 150.9 mm, Nduye River at Nduye, upstream of the bridge, behind the police camp, affluent of Epulu River; 1°49′56,0″ N 28°58′40,1″ E, Okapi Reserve Expedition, 26 July 2009; RMCA 2009–029–P–287, 1, 164.6 mm, Epulu River at Epulu, ca. 250 m upstream of the bridge, before the GIC building, 1°24′07,6″ N 28°34′43,6″ E, Okapi Reserve Expedition, 22 June 2009; and 5 more uncatalogued specimens housed at the RMCA.

Labeobarbus sp. 'thick lip': RMCA 2009–029–P–0312, 1, 190.1 mm, Epulu River at Epulu, right bank, downstream of the bridge, 1°24′00,6″ N 28°34′31,7″ E, Okapi Reserve Expedition, 24 May 2009; RMCA 2009–029–P–0322–0323, 2, 129.3–181.2 mm, Epulu River at Epulu, right bank, upstream of the bridge, 1°24′18,5″ N 28°35′00,7″ E, Okapi Reserve Expedition, 03 June 2009; RMCA 2009–029–P–0334, 1, 133.5 mm, Lelo River, agricultural area of the Epulu centre, affluent of Epulu River, in primary forest, 1°25′52,7″ N 28°34′27,4″ E, Okapi Reserve Expedition, 27 June 2009; RMCA 2009–029–P–0339–0340, 2, 95.2–176.1 mm, Epulu River at Epulu, ca. 250 m upstream of the bridge, before the GIC

building, 1°24′07,6″ N 28°34′43,6″ E, Okapi Reserve Expedition, 22 June 2009; RMCA 2009–029–P–0343, 1, 167.5 mm, Edoro River, upstream of the bridge, near the research camp at Edoro–Afarama, affluent of Epulu River; 1°33′01,2″ N 28°30′40,6″ E; Okapi Reserve Expedition, 11 July 2009; RMCA 2009–029–P–0344, 1, 170.5 mm, Nduye River at Nduye, downstream of the bridge, affluent of Epulu River, 1°50′00,9″ N 28°59′30,5″ E, Okapi Reserve Expedition, 30 July 2009; and 8 more uncatalogued specimens housed at the RMCA.

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