Evolution of African barbs from the Lake Victoria drainage system, Kenya (#29067)

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Evolution of African barbs from the Lake Victoria drainage system, Kenya

Violet M Ndeda [1, 2] , **Mariana Mateos** Corresp., [1] , **Luis A Hurtado** Corresp. [1]

[1] Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas, United States of America
[2] Department of Zoology, , Kenya, Maseno University, Maseno, Kenya

Corresponding Authors: Mariana Mateos, Luis A Hurtado
Email address: marianamateosh@gmail.com, lhurtado@tamu.edu

The Lake Victoria drainage basin (LVD) in Kenya is home to ten nominal species of small barbs (‘*Barbus*’) and one of large barbs (*Labeobarbus altianalis*). A recent molecular study genetically characterized small barbs in this region and found evidence of introgression between certain species, further complicating the taxonomy and species identification of these fishes. This study aimed to extend our understanding on the evolution of these fishes by: (1) examining the phylogenetic relationships of small barbs of the Kenyan LVD with those reported from other ichthyological provinces of Africa; (2) testing the sister relationship between *‘Barbus’ profundus*, endemic to Lake Victoria, and ‘*Barbus*’ *radiatus*, also found in Lake Victoria, which had been previously synonymized; (3) determining whether putatively pure individuals of *‘Barbus’ cercops* are found in the Kenyan LVD, as a previous study only found hybrid individuals of this species in this region; and (4) examining the phylogenetic relationships of *Labeobarbus altianalis* with other *Labeobarbus* species. To this end, we obtained mitochondrial Cytochrome b and nuclear Growth Hormone (GH) intron 2 gene sequences of nine ‘*Barbus*’ species from the LVD in Kenya, as well as cytochrome b sequences for *L. altianalis*. We conducted Maximum likelihood and Bayesian phylogenetic analyses to establish their evolutionary relationships in relation to many other barbs specimens from Africa. Phylogenetic analyses did not reveal instances of hybridization/introgression among the individuals sequenced by us. A sister relationship between ‘*B*’. *profundus* and ‘*B*’. *radiatus* was not found. This latter species shows instead a sister relationship with a lineage comprised of two species from West Africa. Other sister relationships between taxa from the East coast and other ecoregions from Africa are observed, suggesting that past drainage connections and vicariant events contributed to the diversification of this group. Finally, only a single haplotype was recovered among the *L. altianalis* individuals examined, which is most similar to a specimen from Lake Edward in Uganda.

EVOLUTION OF AFRICAN BARBS FROM THE LAKE VICTORIA DRAINAGE SYSTEM, KENYA

Violet M. Ndeda[1,2], Mariana Mateos[1], Luis A. Hurtado[1*]

[1] Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas

[2] Present address: Department of Zoology, Maseno University, Kenya

*Corresponding author:

Luis A. Hurtado, Department of Wildlife and Fisheries Sciences, Texas A&M University, 534
John Kimbrough Blvd., Wildlife Fisheries Ecological Sciences, Building #1537, Suite 278,
College Station, TX 77843-2258, USA.
Tel: +1-979-458-9878; Fax: +1-979-845-4096; E-mail: lhurtado@tamu.edu

ABSTRACT

The Lake Victoria drainage basin (LVD) in Kenya is home to ten nominal species of small barbs (‘*Barbus*’) and one of large barbs (*Labeobarbus altianalis*). A recent molecular study genetically characterized small barbs in this region and found evidence of introgression between certain species, further complicating the taxonomy and species identification of these fishes. This study aimed to extend our understanding on the evolution of these fishes by: (1) examining the phylogenetic relationships of small barbs of the Kenyan LVD with those reported from other ichthyological provinces of Africa; (2) testing the sister relationship between *‘Barbus’ profundus*, endemic to Lake Victoria, and ‘*Barbus*’ *radiatus*, also found in Lake Victoria, which had been previously synonymized; (3) determining whether putatively pure individuals of *‘Barbus’ cercops* are found in the Kenyan LVD, as a previous study only found hybrid individuals of this species in this region; and (4) examining the phylogenetic relationships of *Labeobarbus altianalis* with other *Labeobarbus* species. To this end, we obtained mitochondrial Cytochrome b and nuclear Growth Hormone (GH) intron 2 gene sequences of nine ‘*Barbus*’ species from the LVD in Kenya, as well as cytochrome b sequences for *L. altianalis*. We conducted Maximum likelihood and Bayesian phylogenetic analyses to establish their evolutionary relationships in relation to many other barbs specimens from Africa. Phylogenetic analyses did not reveal instances of hybridization/introgression among the individuals sequenced by us. A sister relationship between ‘*B*’. *profundus* and ‘*B*’. *radiatus* was not found. This latter species shows instead a sister relationship with a lineage comprised of two species from West Africa. Other sister relationships between taxa from the East coast and other ecoregions from Africa are observed, suggesting that past drainage connections and vicariant events contributed to the diversification of this group. Finally, only a single haplotype was recovered among the *L.*

altianalis individuals examined, which is most similar to a specimen from Lake Edward in Uganda.

1. INTRODUCTION

Barbs constitute a significant component of the freshwater fish fauna of Africa, and represent the most species-rich group of cyprinids in this continent (Hayes and Armbruster, 2017; Leveque and Daget, 1984; Ren and Mayden, 2016; Skelton, 1988; Skelton, 1993; Skelton et al., 1991). Molecular characterization of African barbs from different regions has greatly contributed to our understanding on the diversity and evolution of these fishes (Beshera et al., 2016; De Graaf et al., 2007; Hayes and Armbruster, 2017; Muwanika et al., 2012; Ren and Mayden, 2016; Schmidt et al., 2017; Yang et al., 2015). A large dataset of DNA sequences (particularly of the mitochondrial Cytochrome b gene) of African barbs from different regions has accrued, providing a resource for performing phylogenetic analyses across regions, which will enhance knowledge on the systematics, evolution, and biogeographic history of this important group.

Although they were treated as part of *Barbus* Cuvier and Cloquet, 1816, which included >800 species distributed across Eurasia and Africa (Berrebi et al., 1996; Skelton, 2012; Skelton et al., 1991), molecular phylogenetic studies have corroborated that this taxonomically complex and heterogeneous assemblage is a polyphyletic group (Ren and Mayden, 2016; Tsigenopoulos et al., 2002; Yang et al., 2015). The large hexaploid African barbs are now classified as *Labeobarbus* (tribe Torini; Tsigenopoulos et al., 2010; Yang et al., 2015), whereas the large tetraploid African barbs are classified as *Pseudobarbus* (tribe Smiliogastrini; Yang et al., 2015). The small, diploid, African barbs have also been assigned to the tribe Smiliogastrini, and Yang et al. (2015) proposed to include all of them within the genus *Enteromius*, the oldest available genus-group name for these fishes, even though they do not appear to correspond to a monophyletic group. This proposal is controversial, with some authors supporting it (Hayes and Armbruster, 2017), whereas others proposing that this group be referred to as '*Barbus*' to reflect its taxonomic uncertainty (Schmidt and Bart, 2015; Stiassny and Sakharova, 2016). To minimize confusion with a previous study that was conducted in the same area as the present study (Schmidt et al., 2017), hereafter we generally refer to them as '*Barbus*' in the main text. Nonetheless, in figures and references to figures, we generally use the genus and species name that was used by the original contributor of the corresponding sequence.

Barbs are an important biodiversity component of the Lake Victoria drainage Basin (LVD) in Kenya, and play a significant role in food security and socioeconomic development of the local community (Ochumba and Many Ala, 1992; Okeyo, 2014). A recent multilocus study (Schmidt et al., 2017), molecularly characterized most of the small barbs nominal species present in this region. Phylogenetic relationships among them were analyzed, and high levels of genetic divergence within some recognized species were uncovered. Further complicating the taxonomy and species identification within this group, this study revealed evidence of introgression involving five small barbs species. Several important phylogenetic questions, however, still remain to be answered for Kenyan LVD barbs.

First, phylogenetic relationships of small barbs from this region, which belong to the East Coast province, with those from other ichthyological provinces of Africa have not been examined. Numerous sequences, mostly of the Cytb gene, are available for other small barbs from the East Coast, as well as the Nilo-Sudan, Upper Guinea, Lower Guinea, Congo, and Southern provinces (as defined by Levêque et al., 2008; Roberts, 1975). The African continent has had a complex and dynamic geological history, in which past hydrological connections may have enabled exchange of taxa from different regions (Salzburger et al., 2014; Stewart, 2001).

Second, Schmidt et al. (2017) did not include *'B.' profundus* in their study, a species endemic to Lake Victoria, for which molecular analyses can help to clarify its evolutionary history and taxonomy. Greenwood (1970) originally described *‘B.’ profundus* as a subspecies of *‘B.’ radiatus*, another species found in Lake Victoria and other localities in Kenya. He considered *‘B.’ radiatus* was comprised of three subspecies: *B. radiatus profundus*, *B. radiatus radiatus* and *B. radiatus aurantiacus*. Stewart (1977), however, based on meristic and morphometric analyses concluded *‘B.’ profundus* is a separate species from *‘B.’ radiatus*; but did not find a basis for the separation of the other two subspecies. The two species occupy different depths in Lake Victoria; *‘B.’ profundus* is distributed at depths between 16 and 65 m (Greenwood, 1970), whereas *‘B.’ radiatus* occupies shallower waters (Stewart, 1977). Molecular analyses are thus needed to examine the relationship between *‘B.’ profundus* and *‘B.’ radiatus*.

Third, due to mitochondrial introgression from other species (i.e., *‘B.’ neumayeri* or ‘*B.’* c.f. *paludinosus* “Jipe”), Schmidt et al. (2017) were not able to obtain Cytb sequences that could be attributed to the *‘B.’ cercops* lineage. It is thus unclear whether pure populations of this species exist in the Kenyan LVD, which is important for conservation. Lack of Cytb sequences also limits examination of the evolutionary relationships of *‘B.’ cercops* with the other small African barbs for which Cytb sequences are available.

Finally, Cytb sequences for the large barb *Labeobarbus altianalis* in the Kenyan LVD have not been examined. This species has historically constituted an important fishery in this region (Whitehead, 1959), but overfishing has severely decimated its populations (Ochumba and Many Ala, 1992). Genetic diversity for this species in the Kenyan LVD has been studied with the mitochondrial control region, which revealed some population structure (Chemoiwa et al., 2013). Muwanika et al. (2012) examined partial Cytb sequences for *L. altianalis* from different localities in Uganda, including the Lake Victoria and Albertine basins. Therefore, obtaining Cytb sequences from this species in the Kenyan LVD will allow examination of differences among the populations from both countries. In addition, a large dataset of Cytb sequences exists for *Labeobarbus* from different regions in Africa, which has not been analyzed with this species (Beshera et al., 2016).

Herein, obtained Cytb sequences from eight species of small barbs and the large barb *L. altianalis* from different localities in the Kenyan LVD, and conducted phylogenetic analyses of these with a large dataset of reported sequences of small and large African barbs. We also obtained sequences of the nuclear GH intron and conducted phylogenetic analyses to help determine whether individuals were pure or exhibited evidence of hybridization/introgression (Schmidt et al., 2017). Our main objectives were to: (1) examine the phylogenetic relationships of small barbs of the Kenyan LVD with those reported from other ichthyological provinces of Africa; (2) test the sister relationship between *'B.' profundus* and *'B.' radiatus*; (3) determine whether putatively pure individuals of *'B.' cercops* are found in the Kenyan LVD; and (4) examine the phylogenetic relationships of *L. altianalis* with other *Labeobarbus* species.

2. MATERIALS AND METHODS

2.1 Tissue source for DNA

We used ethanol-preserved fin clips from nine species (*Labeobarbus altianalis, 'B.' apleurogramma, 'B.' profundus, 'B.' cercops, 'B.' nyanzae, 'B.' kerstenii, 'B.' jacksoni*, *'B.' neumayeri,* and *'B.' paludinosus*) loaned by the Kenya Marine and Fisheries Institute (KMFRI). The remainder of the specimens is stored in formalin at KMFRI. These specimens were originally identified by fish taxonomists from KMFRI using morphological identification keys according to Greenwood (1962). They were obtained from sixteen localities in the Lake Victoria drainage area (LVD) in Kenya, which included Lake Victoria, rivers draining to the lake and associated dams [represented by triangles in Fig. 1; circles indicate the approximate location of specimens from Schmidt et al. (2017) included in our analyses].

2.3 DNA isolation, PCR amplification and sequencing

Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN Inc.). The quality of extracted DNA was examined by visualization on a 1.5% agarose electrophoresis gel, and quantified with a NanoDrop® ND-1000 spectrophotometer. Fragments of one mitochondrial (Cytochrome *b*; Cytb; ~1140bp) and one nuclear (Growth Hormone Intron 2; GH; ~520bp) gene were PCR amplified from 1- 4 individuals per locality. PCR was performed in a 25 µl reaction containing 19.9 µl ultrapure water, 0.5 µl dNTP mix (2.5 mM), 2.5 µl of 10X buffer, 0.5 µl of each 10µM primer, 0.1 µl *Taq* polymerase (OneTaq, New England Biolabs, Inc), and 1 µl of DNA template. Cytb was amplified with primers Cytb L15267 (5'AATGACTTGAAGAACCACCGT3') and H16461 (5'CTTCGGATTACAAGACC3'), following Briolay et al. (1998). GH intron 2 was amplified using primers GH102F (5'TCGTGTACAACACCTGCACCAGC-3'), GH148R (5' TCCTTTCCGGTGGGTGCCTCA-3'), from Mayden et al. (2009). PCR amplification included a denaturation step of 2 min at 95˚C followed by 35 cycles of 1 min at 95˚C, 30 s at 58–60˚C (Cytb)/ 55˚C (GH) and 1 min at 72˚C followed in turn by a final extension of 6 min at 72˚C. Successful amplification was verified by running the PCR amplicons alongside a standard Lambda ladder on a 1.5% agarose gel stained with GelRed™ (Biotium Inc., Hayward, CA, USA). Products were sequenced bi-directionally using the amplification primers in an ABI 3730 capillary sequencer.

2.5 Sequence assembly and alignment

Nucleotide sequences were assembled and edited with Sequencher 4.8 (Gene Codes, Ann Arbor, MI, USA). Newly generated Cytb of '*Barbus'* were combined with publicly available sequences of African '*Barbus*' and their allies (*Systomus*, *Barboides*, *Clypeobarbus*, *Pseudobarbus, Labeobarbus*), including sequences from Schmidt et al. (2017). Sequences were aligned with MAAFT v.6.0 (Katoh and Toh, 2008). Cytb aligned sequences were translated into amino acids to verify the alignments and to rule out the occurrence of frameshifts and early stop codons that could be indicative of pseudogenes or sequencing errors. Species from the family Catostomidae, which represent a group of tetraploids thought to have arisen due to a hybridization event early (60 million years) in the history of the cypriniform fishes, were initially used as outgroups (Uyeno and Smith, 1974). Following preliminary analyses, the dataset was pruned to retain only taxa relevant to this study: the newly generated sequences; small barbs closely related to the taxa in this study; close relatives of *Labeobarbus altianalis*, and four appropriate outgroup taxa (*Pethia ticto*, *Hampala macrolepidota*, *Puntigrus tetrazona*, and *Systomus sarana*; following Schmidt et al. (2017)). The GH dataset included the newly generated sequences, representatives of seven *Barbus* species from the same region, and sequences of *Pethia* and *Garra* were used as outgroups.

2.6 Phylogenetic analyses

Phylogenetic analyses were performed using maximum likelihood (Stamatakis, 2014), and Bayesian inference (Huelsenbeck et al., 2001). Appropriate models of sequence evolution for these analyses were determined using PARTITIONFINDER v2.7 (Guindon et al., 2010; Lanfear et al., 2012; Lanfear et al., 2017) and JModeltest 2.1.10 (Darriba et al., 2012) under the Akaike Information Criterion (AIC), corrected AIC(c), and Bayesian Information Criterion (BIC) (Table 1).

Bayesian analyses were performed in MrBayes 3.2.6 (Ronquist et al., 2012) via the CIPRES Science Gateway (Miller et al., 2010). We used the model indicated by the BIC criterion of JModeltest or the closest more complex model available in MrBayes. The analysis was run for 10,000,000 generations consisting of four independent Markov Chain Monte Carlo (MCMC) chains sampled at every 1000 generations. TRACER v1.6 was used to assess MCMC stationarity and to ensure adequate effective sampling size values (>200) were achieved. The first 25% of the sampled trees were discarded as burn-in, whereas the remaining sampled trees were summarized with "sumt" command implemented in MrBayes.

Maximum Likelihood (ML) analysis was implemented in RaxML v 8.2.6 (Stamatakis, 2014) using rapid bootstrap and GTRGAMMA model via the CIPRES Science Gateway (Miller et al., 2010) to generate a maximum likelihood tree. Clade support was examined by a nonparametric bootstrap analysis of 200 replicates and summarized with 50% majority rule consensus tree computed using the SUMTREES script (v.3.3.1) (Sukumaran and Holder, 2010).

3. RESULTS AND DISCUSSION

We obtained new Cytb sequences for 48 specimens and new GH sequences for 34 specimens (overlap = 26; see Supporting Table S1; GenBank Accession Nos. MH484522-MH484603). Alignments are available as nexus files under Datasets S1 and S2. Phylogenetic reconstructions using GH and Cytb DNA sequences are shown in Figures 2 and 3–8, respectively. In our trees, we generally retained the genus and species name given by the original contributors of the sequences. With the exception of *'B.' cercops* Cytb sequences (see below), for the seven '*Barbus*' species that overlapped between our study and that of Schmidt et al. (2017) within the

LVD region, the Cytb haplotypes and GH alleles that we obtained were identical or very similar to those reported by Schmidt et al. (2017) (Figs. 2–8).

Comparison of Cytb and GH trees based on our sequences alone does not suggest instances of introgression or hybridization. All of the eight putative *‘B.’ cercops* specimens examined (localities 1, 5, 9; Fig. 1) had a GH sequence identical to the single allele reported by Schmidt et al. (2017) for 15 *‘B.’ cercops* specimens in the same area (i.e., localities 5, 16, 21, 23–25). The *‘B.’ cercops* GH allele is distinct from alleles found in specimens assigned to all other species examined to date. Based on GH, the *‘B.’ cercops* lineage (red branches in Fig. 2) forms a monophyletic group with *‘B.’ sp.* “Jipe” and a clade comprised of *‘B.’ jacksoni*, *‘B.’ trispilopleura*, and one specimen assigned to *B. trimaculatus* (Fig. 2).

The Cytb sequences of our ‘*B.’ cercops* specimens (n = 6) formed a distinct lineage (red branches in Fig. 4) that excluded the five ‘*B.’ cercops* specimens reported by Schmidt et al. (2017) and all other reported sequences to date [with the exception of GenBank record AF180841; identified as *Barbus nyanzae* from Kenya (Tsigenopoulos et al., 2002); discussed below]. Maximum Cytb divergence within this clade was 0.39% (K2P). This ‘*B.’ cercops* Cytb lineage was part of a larger clade (Clade A; Figs. 3 and 4) that included the closest relatives of ‘*B.’ cercops* according to the GH gene (see above), as well as additional lineages assigned to several other species. The Cytb sequence of the five putative ‘*B.’ cercops* specimens examined by Schmidt et al. (2017), including four for which they also obtained GH sequences, clustered with the Cytb sequences of other species. Four of their ‘*B.’ cercops* Cytb sequences clustered with a clade made up mostly of *‘B.’ neumayeri* specimens (brown branches; Figs. 3 and 5; found in localities 5 and 25; Fig. 1), whereas one of their ‘*B.’ cercops* Cytb sequences (from locality 23) clustered with individuals belonging to a subclade (i.e., “Jipe”) of specimens assigned to ‘*B.’*

cf. *paludinosus* (Clade D; green branches; Figs. 3 and 5). Therefore, whereas Schmidt et al. (2017) detected evidence consistent with mitochondrial introgression from other species (i.e., *'B.' neumayeri* and *'B.'* cf. *paludinosus*) into all five of the *'B.' cercops* specimens that they characterized for Cytb as well as for morphology or GH, we found no evidence of introgression among our specimens. Whether GenBank record AF180841 (Fig. 4) is an error or a result of introgression of *'B.' cercops* mitochondria into *'B.' nyanzae* cannot be determined [although Schmidt et al. (2017) did not detect such introgression], because information about the nuclear genetic background is not available for this specimen. Similarly, the identity of *'B.' cercops* voucher 1550 [blue taxon label in Fig. 2; from Schmidt et al. (2017)] is questionable, as its Cytb sequence (KX178183) is very divergent (not shown). A MegaBLAST search (Morgulis et al., 2008; Zhang et al., 2000) against the non-redundant NCBI nucleotide database indicates that this sequence, along with records KX178138 and KX178111 also labeled as *'B.' cercops*, belong to a different fish order (i.e., Siluriformes), implying a labeling or contamination error.

Schmidt et al. (2017) also detected a pattern suggestive of introgression in *'B.' kerstenii*. They assigned 30 specimens to *'B.' kerstenii* based on morphology, and characterized 16 of these for GH and 28 for Cytb (15 for both genes). The 16 GH sequences grouped into a distinct clade that excluded specimens assigned to other species (Fig. 2). In contrast, for Cytb, 11 grouped within the *'B.' neumayeri* clade (Fig. 5), one grouped with specimens assigned to *'B.' nyanzae* (purple in Fig. 6), and the remaining 17 formed a highly distinct clade that excluded specimens assigned to other species (magenta in Fig. 6). The latter presumably represent "pure" *'B.' kerstenii* specimens, as the phylogenetic position of this unique clade (i.e., as a close relative of *'B.' nyanzae*) is generally congruent between the two genes. Based on this criterion, the four

'*B.' kerstenii* specimens for which we obtained both GH and Cytb sequences represent "pure" individuals.

Our study is the first to report Cytb and GH DNA sequences from *'B.' profundus*, a species endemic to Lake Victoria (Greenwood, 1970). Each of the four specimens examined had a different Cytb haplotype (max. within clade divergence = 0.59% K2P), and formed a well supported clade (Fig. 7). The *'B.' profundus* lineage falls within Clade B, which contains mostly species distributed in the Nilo-Sudan (North) and Upper Guinea (West) provinces, as well as *'B.' radiatus*. A sister relationship between *B'. profundus* and *'B.' radiatus*, however, was not recovered in our Cytb or GH analyses, despite the fact that these species had been previously synonymized (Greenwood, 1970) and co-occur in Lake Victoria, albeit at different depths (Stewart, 1977). Instead, the Cytb phylogenetic reconstruction (Fig. 7) shows a sister relationship between *'B.' radiatus* and a lineage comprised of two species from West Africa (in the Lower Guinea ichthyological province): *'B.' aspilus* (from Cameroon) and *'B.'* cf. *guirali* (the sample is from Gabon; *'B.' guirali* is reported from Cameroon, Gabon and Congo). This is congruent with the results of Ren and Mayden (2016), who examined the same sequences for these taxa, but lacked *'B.' profundus*. Therefore, *'B.' profundus* and *'B.' radiatus* do not appear to constitute sister taxa, but phylogenetic analyses with additional taxa and markers are necessary to identify their closest relatives.

By including sequences from independent studies, our Cytb analyses revealed previously unknown relationships involving LVD species. '*B.' yongei* was sister (~12% K2P divergent) to a lineage comprised of two almost identical haplotypes from Guinea identified as *Enteromius sp.* and *Enteromius stigmatopygus* in Hayes and Armbruster (2017), which implies an East vs. West Africa divergence (Fig. 4). Another sister relationship involving LVD taxa uncovered by our analyzes was that of *'B.' nyanzae* (LVD) and '*B.*' *laticeps* (from Tanzania) (Fig. 6), which were ~9% divergent (K2P).

A single haplotype was recovered among the six *L. altianalis* individuals examined (Fig. 8), representing five localities. This haplotype is identical to GenBank record KX178106, submitted as part of the Schmidt et al. (2017) study PopSet (Accession No. 1187422681), but erroneously labeled as *'B.' cercops* (Fig. 8). The lack of Cytb diversity among our *L. altianalis* specimens sharply contrasts with a previous study of this species in this area that reports high haplotype diversity for the mitochondrial control region (Chemoiwa et al. (2013). Although our phylogenetic analyses provide little resolution within *Labeobarbus*, they suggest that our *L. altianalis* haplotype is most similar to GenBank record JN983691 (627 bp; K2P distance = 1.2%); a specimen from Uganda (Lake Edward; Albertine drainage; ~200 km West of Lake Victoria) contributed by Muwanika et al. (2012). The other *Labeobarbus* specimens examined by Muwanika et al. (2012) from LVD and the Albertine drainage in Uganda were 2.2–4.5% (K2P) divergent from our *L. altianalis* haplotype. Banister (1973) proposed, based on morphology, two groups within *Labeobarbus*: the *Labeobarbus intermedius* complex (*L. intermedius*, *L. altianalis*, *'Barbus' acuticeps*, and *'B.' ruasae*) and the *Labeobarbus bynni* complex (*L. bynni*, *L. gananensis*, *L. oxyrhynchus*, and *'B.' longifilis*). Cytb phylogenetic reconstructions in this study, however, do not support the separation of these two groups, which is congruent with the findings of a previous phylogenetic analyses that lacked *L. altianalis* sequences (Beshera et al., 2016). Nonetheless, Cytb may be too conserved to adequately assess these relationships.

Several interesting broad-scale phylogeographic patterns are apparent with the available Cytb data on African small barbs. Continental Africa is divided into nine ichthyological provinces (reviewed in Levêque et al., 2008). The Lake Victoria Drainage belongs to the East Coast province. From our analyses, the following East Coast vs. West splits can be inferred: (1) *'B.' radiatus* (LVD) vs. *'B.'* cf. *guirali* + *'B.' aspilus* (West: Lower Guinea province); (2) *'B.' profundus* (LVD) vs. one or more of the members of Clade B (all are from western Africa except *'B.' radiatus*); and (3) *'B.' yongei* (LVD) vs. *E. stigmatopygus* (West: Niger River). One or more additional East vs. West splits will be identified within clades A and C once relationships within these are resolved. Two East Coast vs. Southern ecoregion splits are inferred: (1) *'B.' trimaculatus* (South Africa) vs. *'B.' jacksoni* + *'B.' perince* + *'B.' trispilopleura* (including specimens assigned to *'B.' tanapelagius* and *'B.' humilis*; Fig. 4); and (2) the basal split within Clade D (*'B.'* cf. *paludinosus*). The multiple divergences between the East Coast and other provinces suggest that the dynamic and complex geological history of Africa provided opportunities, through hydrological connections, for exchange of lineages from different regions (Salzburger et al., 2014; Stewart, 2001).

4. CONCLUSION

The taxonomy and evolutionary history of the African barbs, including the role of hybridization, is far from resolved, and will require a much broader sampling of taxa, geographic locations, and genetic markers than what is presently available. Nonetheless, our analyses, which included most (if not all) available Cytb and GH sequences for this group, revealed several key insights. First, apparently pure *'B.' cercops* individuals do occur at the three localities where we obtained this species, including the Kendu Bay area (locality 5), where Schmidt et al. (2017) reported a *'B.' cercops* specimen harboring a *'B.' neumayeri* mitochondrion. Secondly, *'B.' radiatus* does not appear to be sister to *'B.' profundus*, with which it was previously synonymized. Thirdly, we found evidence of several sister relationships between taxa from the East Coast and other ecoregions of Africa, suggesting that past drainage connections and vicariant events contributed to the diversification of this group. Finally, only a single haplotype was recovered among the *L. altianalis* individuals examined, which is most similar to a specimen from Lake Edward than to specimens from other localities in Uganda.

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FIGURE LEGENDS

Figure 1. Localities of specimens sampled in this study (triangles). Circles represent localities within the Lake Victoria Drainage (LVD) sampled by Schmidt et al. (2017) that were not sampled in our study (locations are approximate based on their description of locality, as coordinates were not reported). The map was developed with ArcMap version 10.3—a part of the ESRI ArcGIS® Desktop suite. Localities where each species was sampled for our study are as follows: *'B.' apleurogramma* [2]; *'B.' cercops* [1, 5, 7, 9]; *'B.'* cf. *paludinosus* [13]; *'B.' jacksoni* [7]; *'B.' kerstenii* [1, 6, 14]; *'B.' neumayeri* [3]; *'B.' nyanzae* [7, 10]; *'B.' profundus* [8]; and *L. altianalis* [4, 5, 10, 11, 12].

Figure 2. Inferred relationships based on the GH gene. RaxML bootstrap consensus (60% majority rule) tree. Bold-faced taxon labels indicate sequences generated by the present study. Asterisks by taxon names indicate Cytb sequence was also generated in present study. Numbers in brackets correspond to localities in Fig. 1. Node support values from Bayesian (above) and ML below are given by nodes; an asterisk indicates support > 97% in all corresponding analyses. For visual clarity, several node support labels have been omitted. Red taxon labels indicate specimens identified by Schmidt et al. (2017) as introgressed. Blue taxon label indicates specimen whose cytb sequence is falls within the order Siluriformes. Clade labels and colors correspond to those in other figures. The dashed line of Clade A indicates those lineages that were not found monophyletic with the rest of Clade A members (as defined by the cytb tree; Figs. 3 and 4).

Figure 3. Inferred relationships among the major clades in this study based on the Cytb gene, and general distribution (East, West, or South Africa; where applicable). RaxML Bootstrap consensus (60% majority rule). Specific clades are expanded in Figs. 4–8). Numbers by nodes represent ML Bootstrap support values. For visual clarity, several node support labels have been omitted. Each taxon label contains the GenBank Accession Number and/or the citation and voucher ID.

Figure 4. Inferred relationships within Clade A (expanded from Fig. 3) based on the Cytb gene. RaxML bootstrap consensus (60% majority rule) tree. All taxa except those with grey shading are found in East Africa. Each taxon label contains the GenBank Accession Number and/or the citation and voucher ID, as well as locality label [if they were from the Lake Victoria Drainage (LVD); in bracket] corresponding to labels in Fig. 1. Bold-faced taxon labels indicate sequences generated by the present study. Asterisks by taxon names indicate GH sequence was also generated in present study. Asterisks by nodes represent support values > 97% for all analyses.

Figure 5. Inferred relationships within the clades of *‘B.’ neumayeri*, *‘B.’ apleurogramma*, and *‘B.’* cf. *paludinosus* (Clade D) (expanded from Fig. 3) based on the Cytb gene. RaxML bootstrap consensus (60% majority rule) tree. All taxa except the one with grey shading are found in East Africa. Bold-faced taxon labels indicate sequences generated by the present study. Asterisks by taxon names indicate GH sequence was also generated in present study. Numbers in brackets correspond to localities in Fig. 1. Node support values from Bayesian (above) and ML below are given by nodes; an asterisk indicates support > 97% in all corresponding analyses.

For visual clarity, several node support labels have been omitted. Red taxon label indicates putatively introgressed individuals from Schmidt et al. (2017).

Figure 6. Inferred relationships within Clade C (expanded from Fig. 3) based on the Cytb gene. RaxML bootstrap consensus (60% majority rule) tree. All taxa except those with grey shading are found in East Africa. Bold-faced taxon labels indicate sequences generated by the present study. Asterisks by taxon names indicate GH sequence was also generated in present study. Numbers in brackets correspond to localities in Fig. 1. Node support values from Bayesian (above) and ML below are given by nodes; an asterisk indicates support > 97% in all corresponding analyses. For visual clarity, several node support labels have been omitted. Red taxon label indicates specimen from Schmidt et al. (2017) assigned to ‘*B.’ kerstenii* based on morphology and GH sequence, but with a Cytb sequence that falls within ‘*B.’ nyanzae*.

Figure 7. Inferred relationships within Clade B (expanded from Fig. 3) based on the Cytb gene. RaxML bootstrap consensus (60% majority rule) tree. All taxa except those *‘B.’ radiatus* and ‘*B.’ profundus* (boldface taxon labels) are distributed in West Africa. Asterisks by taxon names indicate GH sequence was also generated in present study. Numbers in brackets correspond to localities in Fig. 1. Node support values from Bayesian (above) and ML below are given by nodes; an asterisk indicates support > 97% in all corresponding analyses. For visual clarity, several node support labels have been omitted.

Figure 8. Inferred relationships within the clades “*Labeobarbus* and allies” (expanded from Fig. 3) based on the Cytb gene. RaxML bootstrap consensus (60% majority rule) tree. Boldfaced

taxon labels correspond to our specimens of *L. altianalis*. Numbers in brackets correspond to localities in Fig. 1.

SUPPORTING INFORMATION DESCRIPTIONS

Table S1. Specimen information for samples sequenced in this study including geographic coordinates and GenBank Accession Numbers. Locality numbers match labels in Figures. Blue font indicates specimens for which both the Cytb and GH sequence were obtained.

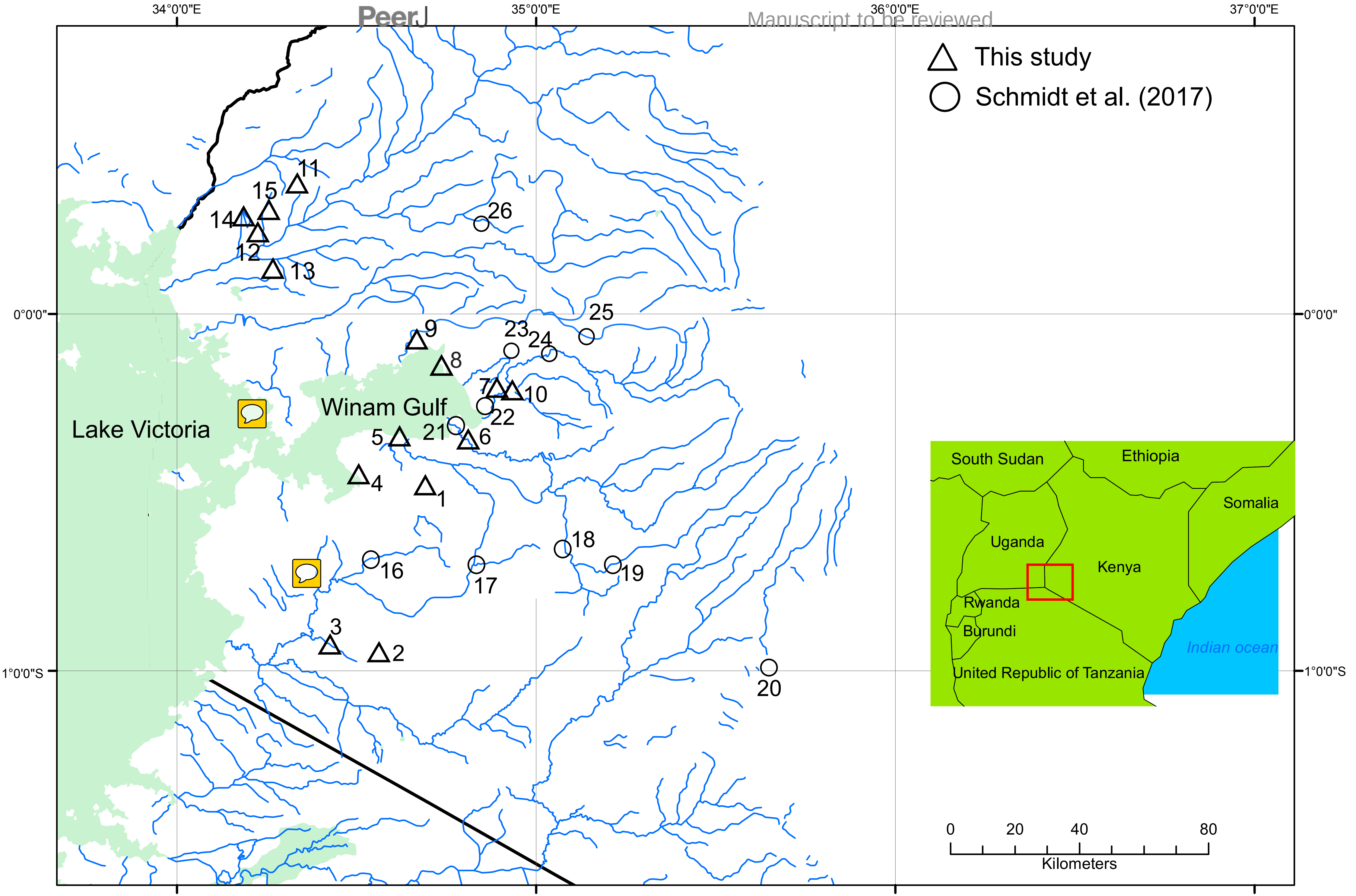
Dataset S1. Cytochrome b gene alignment. Annotated nexus-formatted file of the Cytochrome b gene sequence alignment used in this study.

Dataset S2. GH intron gene alignment. Annotated Nexus-formatted file of the GH gene sequence alignment used in this study.

Figure 1(on next page)

Localities of specimens sampled in this study (triangles).

Circles represent localities within the Lake Victoria Drainage (LVD) sampled by Schmidt et al. (2017) that were not sampled in our study (locations are approximate based on their description of locality, as coordinates were not reported). The map was developed with ArcMap version 10.3—a part of the ESRI ArcGIS® Desktop suite. Localities where each species was sampled for our study are as follows: *'B.' apleurogramma* [2]; *'B.' cercops* [1, 5, 7, 9]; *'B.'* cf. *paludinosus* [13]; *'B.' jacksoni* [7]; *'B.' kerstenii* [1, 6, 14]; *'B.' neumayeri* [3]; *'B.' nyanzae* [7, 10]; *'B.' profundus* [8]; and *L. altianalis* [4, 5, 10, 11, 12].


34°0'0"E
35°0'0"E
36°0'0"E
37°0'0"E
0°0'0"
1°0'0"S
This study
Schmidt et al. (2017)
Lake Victoria
Winam Gulf
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11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
South Sudan
Ethiopia
Somalia
Uganda
Kenya
Rwanda
Burundi
United Republic of Tanzania
Indian ocean
0
20
40
80
Kilometers

Figure 2(on next page)

Inferred relationships based on the GH gene.

RaxML bootstrap consensus (60% majority rule) tree. Bold-faced taxon labels indicate sequences generated by the present study. Asterisks by taxon names indicate Cytb sequence was also generated in present study. Numbers in brackets correspond to localities in Fig. 1. Node support values from Bayesian (above) and ML below are given by nodes; an asterisk indicates support > 97% in all corresponding analyses. For visual clarity, several node support labels have been omitted. Red taxon labels indicate specimens identified by Schmidt et al. (2017) as introgressed. Blue taxon label indicates specimen whose cytb sequence is falls within the order Siluriformes. Clade labels and colors correspond to those in other figures. The dashed line of Clade A indicates those lineages that were not found monophyletic with the rest of Clade A members (as defined by the cytb tree; Figs. 3 and 4).

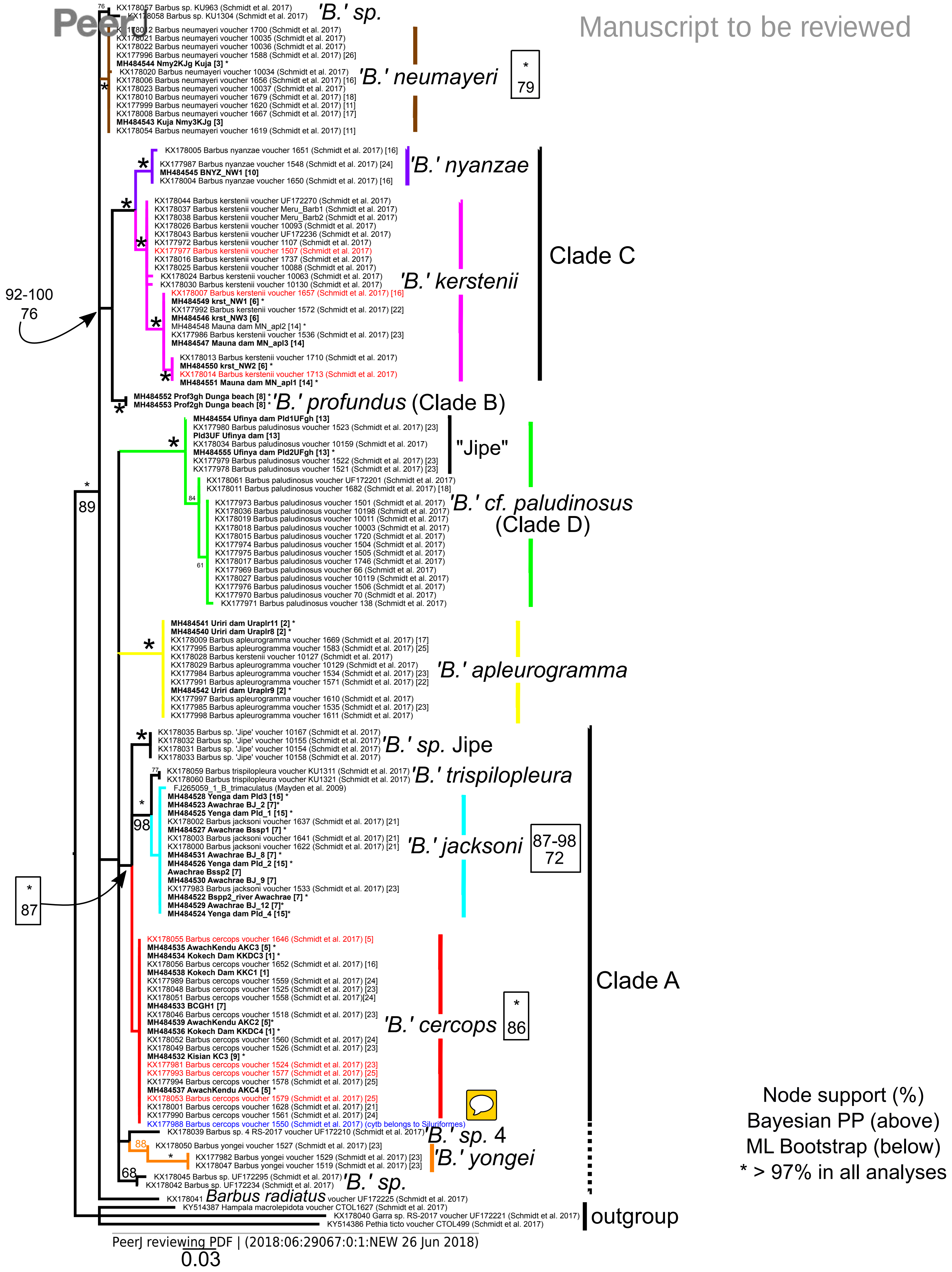
76
KX178057 Barbus sp. KU963 (Schmidt et al. 2017)
KX178058 Barbus sp. KU1304 (Schmidt et al. 2017)
'B.' sp.
KX178012 Barbus neumayeri voucher 1700 (Schmidt et al. 2017)
KX178021 Barbus neumayeri voucher 10035 (Schmidt et al. 2017)
KX178022 Barbus neumayeri voucher 10036 (Schmidt et al. 2017)
KX177996 Barbus neumayeri voucher 1588 (Schmidt et al. 2017) [26]
MH484544 Nmy2KJg Kuja [3] *
KX178020 Barbus neumayeri voucher 10034 (Schmidt et al. 2017)
KX178006 Barbus neumayeri voucher 1656 (Schmidt et al. 2017) [16]
KX178023 Barbus neumayeri voucher 10037 (Schmidt et al. 2017)
KX178010 Barbus neumayeri voucher 1679 (Schmidt et al. 2017) [18]
KX177999 Barbus neumayeri voucher 1620 (Schmidt et al. 2017) [11]
KX178008 Barbus neumayeri voucher 1667 (Schmidt et al. 2017) [17]
MH484543 Kuja Nmy3KJg [3]
KX178054 Barbus neumayeri voucher 1619 (Schmidt et al. 2017) [11]
'B.' neumayeri
*
79
*
KX178005 Barbus nyanzae voucher 1651 (Schmidt et al. 2017) [16]
KX177987 Barbus nyanzae voucher 1548 (Schmidt et al. 2017) [24]
MH484545 BNYZ_NW1 [10]
KX178004 Barbus nyanzae voucher 1650 (Schmidt et al. 2017) [16]
'B.' nyanzae
*
*
KX178044 Barbus kerstenii voucher UF172270 (Schmidt et al. 2017)
KX178037 Barbus kerstenii voucher Meru_Barb1 (Schmidt et al. 2017)
KX178038 Barbus kerstenii voucher Meru_Barb2 (Schmidt et al. 2017)
KX178026 Barbus kerstenii voucher 10093 (Schmidt et al. 2017)
KX178043 Barbus kerstenii voucher UF172236 (Schmidt et al. 2017)
KX177972 Barbus kerstenii voucher 1107 (Schmidt et al. 2017)
*
KX177977 Barbus kerstenii voucher 1507 (Schmidt et al. 2017)
KX178016 Barbus kerstenii voucher 1737 (Schmidt et al. 2017)
KX178025 Barbus kerstenii voucher 10088 (Schmidt et al. 2017)
KX178024 Barbus kerstenii voucher 10063 (Schmidt et al. 2017)
KX178030 Barbus kerstenii voucher 10130 (Schmidt et al. 2017)
KX178007 Barbus kerstenii voucher 1657 (Schmidt et al. 2017) [16]
MH484549 krst_NW1 [6] *
KX177992 Barbus kerstenii voucher 1572 (Schmidt et al. 2017) [22]
MH484546 krst_NW3 [6]
*
MH484548 Mauna dam MN_apl2 [14] *
KX177986 Barbus kerstenii voucher 1536 (Schmidt et al. 2017) [23]
MH484547 Mauna dam MN_apl3 [14]
KX178013 Barbus kerstenii voucher 1710 (Schmidt et al. 2017)
MH484550 krst_NW2 [6] *
KX178014 Barbus kerstenii voucher 1713 (Schmidt et al. 2017)
*
MH484551 Mauna dam MN_apl1 [14] *
'B.' kerstenii
Clade C
92-100
76
MH484552 Prof3gh Dunga beach [8] *
MH484553 Prof2gh Dunga beach [8] *
*
'B.' profundus (Clade B)
MH484554 Ufinya dam Pld1UFgh [13]
KX177980 Barbus paludinosus voucher 1523 (Schmidt et al. 2017) [23]
Pld3UF Ufinya dam [13]
*
KX178034 Barbus paludinosus voucher 10159 (Schmidt et al. 2017)
MH484555 Ufinya dam Pld2UFgh [13] *
KX177979 Barbus paludinosus voucher 1522 (Schmidt et al. 2017) [23]
KX177978 Barbus paludinosus voucher 1521 (Schmidt et al. 2017) [23]
"Jipe"
KX178061 Barbus paludinosus voucher UF172201 (Schmidt et al. 2017)
KX178011 Barbus paludinosus voucher 1682 (Schmidt et al. 2017) [18]
84
KX177973 Barbus paludinosus voucher 1501 (Schmidt et al. 2017)
KX178036 Barbus paludinosus voucher 10198 (Schmidt et al. 2017)
KX178019 Barbus paludinosus voucher 10011 (Schmidt et al. 2017)
KX178018 Barbus paludinosus voucher 10003 (Schmidt et al. 2017)
KX178015 Barbus paludinosus voucher 1720 (Schmidt et al. 2017)
KX177974 Barbus paludinosus voucher 1504 (Schmidt et al. 2017)
KX177975 Barbus paludinosus voucher 1505 (Schmidt et al. 2017)
KX178017 Barbus paludinosus voucher 1746 (Schmidt et al. 2017)
61
KX177969 Barbus paludinosus voucher 66 (Schmidt et al. 2017)
KX178027 Barbus paludinosus voucher 10119 (Schmidt et al. 2017)
KX177976 Barbus paludinosus voucher 1506 (Schmidt et al. 2017)
KX177970 Barbus paludinosus voucher 70 (Schmidt et al. 2017)
KX177971 Barbus paludinosus voucher 138 (Schmidt et al. 2017)
'B.' cf. paludinosus
(Clade D)
*
89
MH484541 Uriri dam Uraplr11 [2] *
MH484540 Uriri dam Uraplr8 [2] *
*
KX178009 Barbus apleurogramma voucher 1669 (Schmidt et al. 2017) [17]
KX177995 Barbus apleurogramma voucher 1583 (Schmidt et al. 2017) [25]
KX178028 Barbus kerstenii voucher 10127 (Schmidt et al. 2017)
KX178029 Barbus apleurogramma voucher 10129 (Schmidt et al. 2017)
KX177984 Barbus apleurogramma voucher 1534 (Schmidt et al. 2017) [23]
KX177991 Barbus apleurogramma voucher 1571 (Schmidt et al. 2017) [22]
MH484542 Uriri dam Uraplr9 [2] *
KX177997 Barbus apleurogramma voucher 1610 (Schmidt et al. 2017)
KX177985 Barbus apleurogramma voucher 1535 (Schmidt et al. 2017) [23]
KX177998 Barbus apleurogramma voucher 1611 (Schmidt et al. 2017)
'B.' apleurogramma
*
KX178035 Barbus sp. 'Jipe' voucher 10167 (Schmidt et al. 2017)
KX178032 Barbus sp. 'Jipe' voucher 10155 (Schmidt et al. 2017)
KX178031 Barbus sp. 'Jipe' voucher 10154 (Schmidt et al. 2017)
KX178033 Barbus sp. 'Jipe' voucher 10158 (Schmidt et al. 2017)
'B.' sp. Jipe
77
KX178059 Barbus trispilopleura voucher KU1311 (Schmidt et al. 2017)
KX178060 Barbus trispilopleura voucher KU1321 (Schmidt et al. 2017)
'B.' trispilopleura
FJ265059_1_B_trimaculatus (Mayden et al. 2009)
MH484528 Yenga dam Pld3 [15] *
*
MH484523 Awachrae BJ_2 [7]*
MH484525 Yenga dam Pld_1 [15] *
98
KX178002 Barbus jacksoni voucher 1637 (Schmidt et al. 2017) [21]
MH484527 Awachrae Bssp1 [7] *
KX178003 Barbus jacksoni voucher 1641 (Schmidt et al. 2017) [21]
KX178000 Barbus jacksoni voucher 1622 (Schmidt et al. 2017) [21]
MH484531 Awachrae BJ_8 [7] *
MH484526 Yenga dam Pld_2 [15] *
Awachrae Bssp2 [7]
MH484530 Awachrae BJ_9 [7]
KX177983 Barbus jacksoni voucher 1533 (Schmidt et al. 2017) [23]
MH484522 Bspp2_river Awachrae [7] *
MH484529 Awachrae BJ_12 [7]*
MH484524 Yenga dam Pld_4 [15]*
'B.' jacksoni
87-98
72
*
87
KX178055 Barbus cercops voucher 1646 (Schmidt et al. 2017) [5]
MH484535 AwachKendu AKC3 [5] *
MH484534 Kokech Dam KKDC3 [1] *
KX178056 Barbus cercops voucher 1652 (Schmidt et al. 2017) [16]
MH484538 Kokech Dam KKC1 [1]
KX177989 Barbus cercops voucher 1559 (Schmidt et al. 2017) [24]
KX178048 Barbus cercops voucher 1525 (Schmidt et al. 2017) [23]
KX178051 Barbus cercops voucher 1558 (Schmidt et al. 2017)[24]
MH484533 BCGH1 [7]
KX178046 Barbus cercops voucher 1518 (Schmidt et al. 2017) [23]
MH484539 AwachKendu AKC2 [5]*
MH484536 Kokech Dam KKDC4 [1] *
KX178052 Barbus cercops voucher 1560 (Schmidt et al. 2017) [24]
KX178049 Barbus cercops voucher 1526 (Schmidt et al. 2017) [23]
MH484532 Kisian KC3 [9] *
KX177981 Barbus cercops voucher 1524 (Schmidt et al. 2017) [23]
KX177993 Barbus cercops voucher 1577 (Schmidt et al. 2017) [25]
KX177994 Barbus cercops voucher 1578 (Schmidt et al. 2017) [25]
MH484537 AwachKendu AKC4 [5] *
KX178053 Barbus cercops voucher 1579 (Schmidt et al. 2017) [25]
KX178001 Barbus cercops voucher 1628 (Schmidt et al. 2017) [21]
KX177990 Barbus cercops voucher 1561 (Schmidt et al. 2017) [24]
KX177988 Barbus cercops voucher 1550 (Schmidt et al. 2017) (cytb belongs to Siluriformes)
'B.' cercops
*
86
Clade A
KX178039 Barbus sp. 4 RS-2017 voucher UF172210 (Schmidt et al. 2017)
'B.' sp. 4
88
KX178050 Barbus yongei voucher 1527 (Schmidt et al. 2017) [23]
*
KX177982 Barbus yongei voucher 1529 (Schmidt et al. 2017) [23]
KX178047 Barbus yongei voucher 1519 (Schmidt et al. 2017) [23]
'B.' yongei
68
KX178045 Barbus sp. UF172295 (Schmidt et al. 2017)
KX178042 Barbus sp. UF172234 (Schmidt et al. 2017)
'B.' sp.
KX178041 Barbus radiatus voucher UF172225 (Schmidt et al. 2017)
KY514387 Hampala macrolepidota voucher CTOL1627 (Schmidt et al. 2017)
KX178040 Garra sp. RS-2017 voucher UF172221 (Schmidt et al. 2017)
KY514386 Pethia ticto voucher CTOL499 (Schmidt et al. 2017)
outgroup
Node support (%)
Bayesian PP (above)
ML Bootstrap (below)
* > 97% in all analyses
0.03

Figure 3(on next page)

Inferred relationships among the major clades in this study based on the Cytb gene, and general distribution (East, West, or South Africa; where applicable).

RaxML Bootstrap consensus (60% majority rule). Specific clades are expanded in Figs. 4–8). Numbers by nodes represent ML Bootstrap support values. For visual clarity, several node support labels have been omitted. Each taxon label contains the GenBank Accession Number and/or the citation and voucher ID.

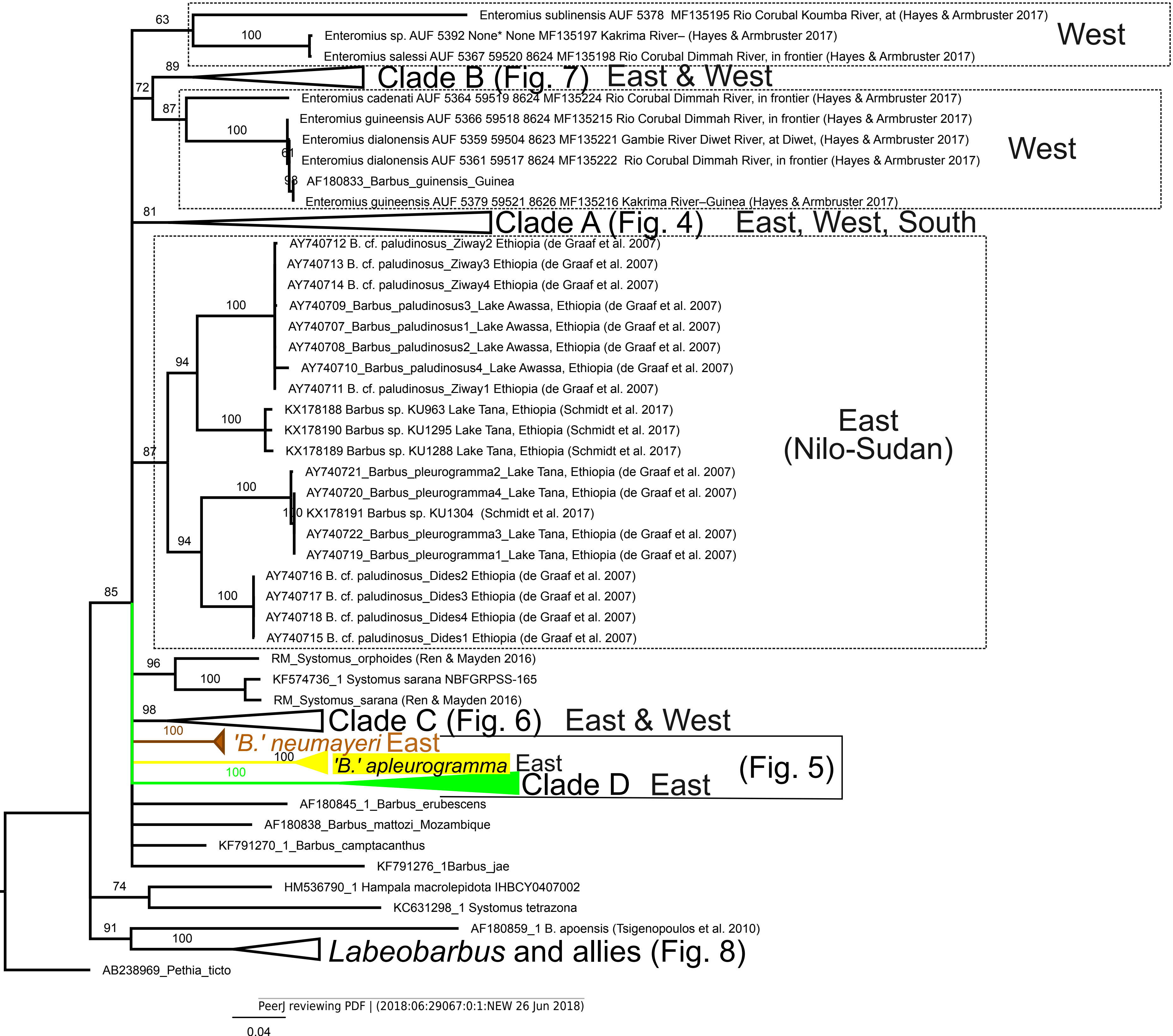
63
Enteromius sublinensis AUF 5378 MF135195 Rio Corubal Koumba River, at (Hayes & Armbruster 2017)
100
Enteromius sp. AUF 5392 None* None MF135197 Kakrima River– (Hayes & Armbruster 2017)
Enteromius salessi AUF 5367 59520 8624 MF135198 Rio Corubal Dimmah River, in frontier (Hayes & Armbruster 2017)
West
89
Clade B (Fig. 7) East & West
72
87
Enteromius cadenati AUF 5364 59519 8624 MF135224 Rio Corubal Dimmah River, in frontier (Hayes & Armbruster 2017)
100
Enteromius guineensis AUF 5366 59518 8624 MF135215 Rio Corubal Dimmah River, in frontier (Hayes & Armbruster 2017)
Enteromius dialonensis AUF 5359 59504 8623 MF135221 Gambie River Diwet River, at Diwet, (Hayes & Armbruster 2017)
61
Enteromius dialonensis AUF 5361 59517 8624 MF135222 Rio Corubal Dimmah River, in frontier (Hayes & Armbruster 2017)
98
AF180833_Barbus_guinensis_Guinea
Enteromius guineensis AUF 5379 59521 8626 MF135216 Kakrima River–Guinea (Hayes & Armbruster 2017)
West
81
Clade A (Fig. 4) East, West, South
AY740712 B. cf. paludinosus_Ziway2 Ethiopia (de Graaf et al. 2007)
AY740713 B. cf. paludinosus_Ziway3 Ethiopia (de Graaf et al. 2007)
AY740714 B. cf. paludinosus_Ziway4 Ethiopia (de Graaf et al. 2007)
100
AY740709_Barbus_paludinosus3_Lake Awassa, Ethiopia (de Graaf et al. 2007)
AY740707_Barbus_paludinosus1_Lake Awassa, Ethiopia (de Graaf et al. 2007)
AY740708_Barbus_paludinosus2_Lake Awassa, Ethiopia (de Graaf et al. 2007)
94
AY740710_Barbus_paludinosus4_Lake Awassa, Ethiopia (de Graaf et al. 2007)
AY740711 B. cf. paludinosus_Ziway1 Ethiopia (de Graaf et al. 2007)
KX178188 Barbus sp. KU963 Lake Tana, Ethiopia (Schmidt et al. 2017)
100
KX178190 Barbus sp. KU1295 Lake Tana, Ethiopia (Schmidt et al. 2017)
KX178189 Barbus sp. KU1288 Lake Tana, Ethiopia (Schmidt et al. 2017)
East
(Nilo-Sudan)
87
AY740721_Barbus_pleurogramma2_Lake Tana, Ethiopia (de Graaf et al. 2007)
100
AY740720_Barbus_pleurogramma4_Lake Tana, Ethiopia (de Graaf et al. 2007)
100
KX178191 Barbus sp. KU1304 (Schmidt et al. 2017)
AY740722_Barbus_pleurogramma3_Lake Tana, Ethiopia (de Graaf et al. 2007)
94
AY740719_Barbus_pleurogramma1_Lake Tana, Ethiopia (de Graaf et al. 2007)
AY740716 B. cf. paludinosus_Dides2 Ethiopia (de Graaf et al. 2007)
85
100
AY740717 B. cf. paludinosus_Dides3 Ethiopia (de Graaf et al. 2007)
AY740718 B. cf. paludinosus_Dides4 Ethiopia (de Graaf et al. 2007)
AY740715 B. cf. paludinosus_Dides1 Ethiopia (de Graaf et al. 2007)
96
RM_Systomus_orphoides (Ren & Mayden 2016)
100
KF574736_1 Systomus sarana NBFGRPSS-165
RM_Systomus_sarana (Ren & Mayden 2016)
98
Clade C (Fig. 6) East & West
100
'B.' neumayeri East
100
'B.' apleurogramma East
100
Clade D East
(Fig. 5)
AF180845_1_Barbus_erubescens
AF180838_Barbus_mattozi_Mozambique
KF791270_1_Barbus_camptacanthus
KF791276_1Barbus_jae
74
HM536790_1 Hampala macrolepidota IHBCY0407002
KC631298_1 Systomus tetrazona
91
AF180859_1 B. apoensis (Tsigenopoulos et al. 2010)
100
Labeobarbus and allies (Fig. 8)
AB238969_Pethia_ticto
0.04

Figure 4

Inferred relationships within Clade A (expanded from Fig. 3) based on the Cytb gene.

RaxML bootstrap consensus (60% majority rule) tree. All taxa except those with grey shading are found in East Africa. Each taxon label contains the GenBank Accession Number and/or the citation and voucher ID, as well as locality label [if they were from the Lake Victoria Drainage (LVD); in bracket] corresponding to labels in Fig. 1. Bold-faced taxon labels indicate sequences generated by the present study. Asterisks by taxon names indicate GH sequence was also generated in present study. Asterisks by nodes represent support values > 97% for all analyses.

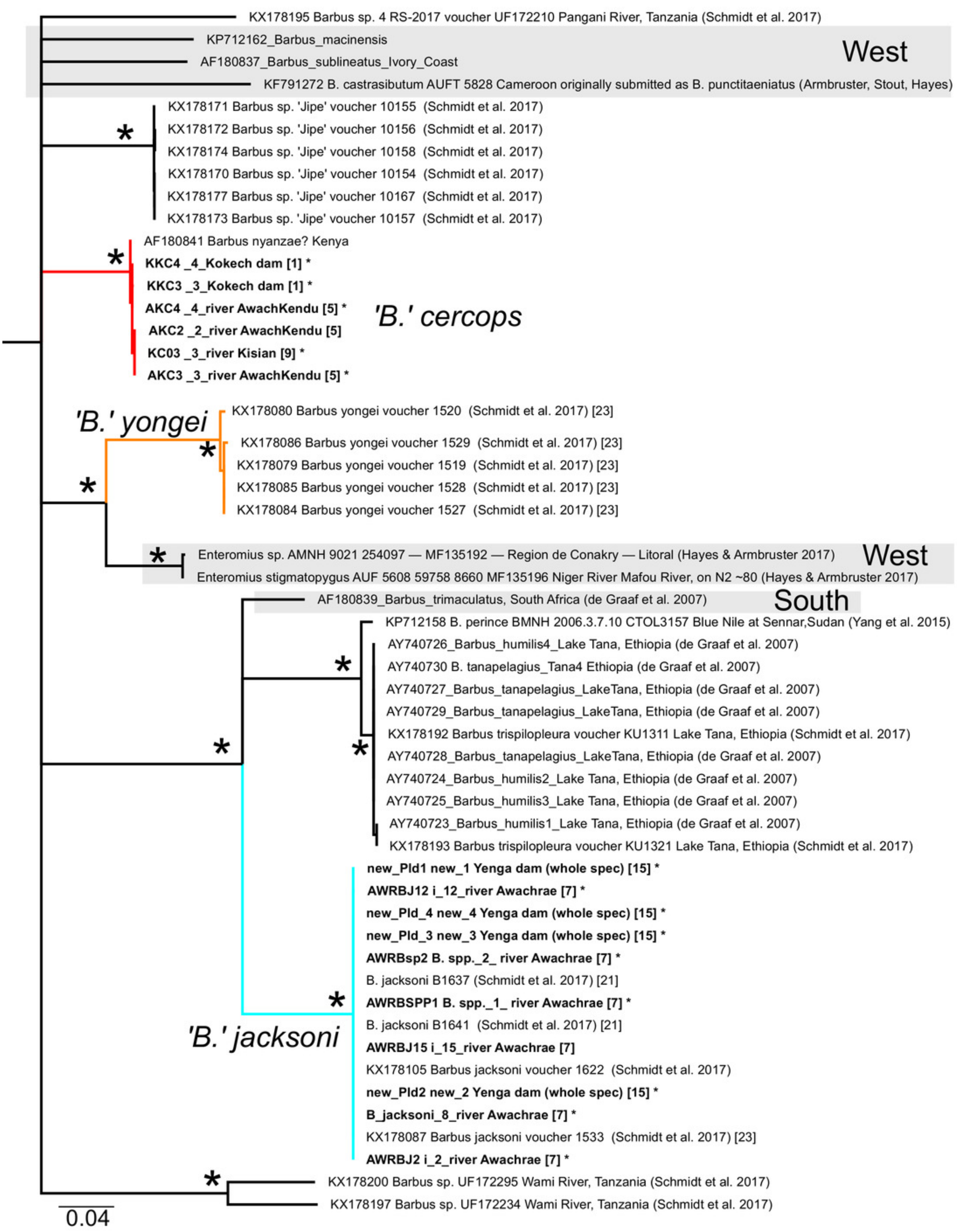
KX178195 Barbus sp. 4 RS-2017 voucher UF172210 Pangani River, Tanzania (Schmidt et al. 2017)
KP712162_Barbus_macinensis
AF180837_Barbus_sublineatus_Ivory_Coast
KF791272 B. castrasibutum AUFT 5828 Cameroon originally submitted as B. punctitaeniatus (Armbruster, Stout, Hayes)
West
*
KX178171 Barbus sp. 'Jipe' voucher 10155 (Schmidt et al. 2017)
KX178172 Barbus sp. 'Jipe' voucher 10156 (Schmidt et al. 2017)
KX178174 Barbus sp. 'Jipe' voucher 10158 (Schmidt et al. 2017)
KX178170 Barbus sp. 'Jipe' voucher 10154 (Schmidt et al. 2017)
KX178177 Barbus sp. 'Jipe' voucher 10167 (Schmidt et al. 2017)
KX178173 Barbus sp. 'Jipe' voucher 10157 (Schmidt et al. 2017)
*
AF180841 Barbus nyanzae? Kenya
KKC4 _4_Kokech dam [1] *
KKC3 _3_Kokech dam [1] *
AKC4 _4_river AwachKendu [5] *
AKC2 _2_river AwachKendu [5]
KC03 _3_river Kisian [9] *
AKC3 _3_river AwachKendu [5] *
'B.' cercops
'B.' yongei
*
*
KX178080 Barbus yongei voucher 1520 (Schmidt et al. 2017) [23]
KX178086 Barbus yongei voucher 1529 (Schmidt et al. 2017) [23]
KX178079 Barbus yongei voucher 1519 (Schmidt et al. 2017) [23]
KX178085 Barbus yongei voucher 1528 (Schmidt et al. 2017) [23]
KX178084 Barbus yongei voucher 1527 (Schmidt et al. 2017) [23]
*
Enteromius sp. AMNH 9021 254097 — MF135192 — Region de Conakry — Litoral (Hayes & Armbruster 2017)
Enteromius stigmatopygus AUF 5608 59758 8660 MF135196 Niger River Mafou River, on N2 ~80 (Hayes & Armbruster 2017)
West
AF180839_Barbus_trimaculatus, South Africa (de Graaf et al. 2007)
South
*
*
*
KP712158 B. perince BMNH 2006.3.7.10 CTOL3157 Blue Nile at Sennar,Sudan (Yang et al. 2015)
AY740726_Barbus_humilis4_Lake Tana, Ethiopia (de Graaf et al. 2007)
AY740730 B. tanapelagius_Tana4 Ethiopia (de Graaf et al. 2007)
AY740727_Barbus_tanapelagius_LakeTana, Ethiopia (de Graaf et al. 2007)
AY740729_Barbus_tanapelagius_LakeTana, Ethiopia (de Graaf et al. 2007)
KX178192 Barbus trispilopleura voucher KU1311 Lake Tana, Ethiopia (Schmidt et al. 2017)
AY740728_Barbus_tanapelagius_LakeTana, Ethiopia (de Graaf et al. 2007)
AY740724_Barbus_humilis2_Lake Tana, Ethiopia (de Graaf et al. 2007)
AY740725_Barbus_humilis3_Lake Tana, Ethiopia (de Graaf et al. 2007)
AY740723_Barbus_humilis1_Lake Tana, Ethiopia (de Graaf et al. 2007)
KX178193 Barbus trispilopleura voucher KU1321 Lake Tana, Ethiopia (Schmidt et al. 2017)
new_Pld1 new_1 Yenga dam (whole spec) [15] *
AWRBJ12 i_12_river Awachrae [7] *
new_Pld_4 new_4 Yenga dam (whole spec) [15] *
new_Pld_3 new_3 Yenga dam (whole spec) [15] *
AWRBsp2 B. spp._2_ river Awachrae [7] *
B. jacksoni B1637 (Schmidt et al. 2017) [21]
*
AWRBSPP1 B. spp._1_ river Awachrae [7] *
B. jacksoni B1641 (Schmidt et al. 2017) [21]
'B.' jacksoni
AWRBJ15 i_15_river Awachrae [7]
KX178105 Barbus jacksoni voucher 1622 (Schmidt et al. 2017)
new_Pld2 new_2 Yenga dam (whole spec) [15] *
B_jacksoni_8_river Awachrae [7] *
KX178087 Barbus jacksoni voucher 1533 (Schmidt et al. 2017) [23]
AWRBJ2 i_2_river Awachrae [7] *
*
KX178200 Barbus sp. UF172295 Wami River, Tanzania (Schmidt et al. 2017)
KX178197 Barbus sp. UF172234 Wami River, Tanzania (Schmidt et al. 2017)
0.04

Figure 5

Inferred relationships within the clades of *‘B.’ neumayeri*, *‘B.’ apleurogramma*, and *‘B.’* cf. *paludinosus* (Clade D) (expanded from Fig. 3) based on the Cytb gene.

RaxML bootstrap consensus (60% majority rule) tree. All taxa except the one with grey shading are found in East Africa. Bold-faced taxon labels indicate sequences generated by the present study. Asterisks by taxon names indicate GH sequence was also generated in present study. Numbers in brackets correspond to localities in Fig. 1. Node support values from Bayesian (above) and ML below are given by nodes; an asterisk indicates support > 97% in all corresponding analyses. For visual clarity, several node support labels have been omitted. Red taxon label indicates putatively introgressed individuals from Schmidt et al. (2017) .


KX178153 Barbus neumayeri voucher 10034 (Schmidt et al. 2017)
KX178156 Barbus neumayeri voucher 10037 (Schmidt et al. 2017)
KX178158 Barbus neumayeri voucher 10039 (Schmidt et al. 2017)
KX178157 Barbus neumayeri voucher 10038 (Schmidt et al. 2017)
KX178159 Barbus neumayeri voucher 10040 (Schmidt et al. 2017)
KX178155 Barbus neumayeri voucher 10036 (Schmidt et al. 2017)
KX178154 Barbus neumayeri voucher 10035 (Schmidt et al. 2017)
KX178100 Barbus neumayeri voucher 1587 Kakamega Forest, Kenya (Schmidt et al. 2017) [26]
KX178112 Barbus neumayeri voucher 1656 (Schmidt et al. 2017) [16]
KX178119 Barbus neumayeri voucher 1674 (Schmidt et al. 2017) [17]
KX178097 Barbus cercops voucher 1579 (Schmidt et al. 2017) [25]
KX178115 Barbus neumayeri voucher 1668 (Schmidt et al. 2017) [17]
KX178122 Barbus neumayeri voucher 1679 (Schmidt et al. 2017) [18]
KX178137 Barbus kerstenii voucher 1713 Mara River (Schmidt et al. 2017)
KX178184 Barbus neumayeri voucher 1619 (Schmidt et al. 2017) [11]
KX178143 Barbus neumayeri voucher 1734 (Schmidt et al. 2017)
KX178130 Barbus neumayeri voucher 1692 (Schmidt et al. 2017) [19]
*
'B.' neumayeri
Nmy2KJ [3] *
KX178185 Barbus neumayeri voucher 1680 (Schmidt et al. 2017)
Nmy1KJ [3]
KX178101 Barbus neumayeri voucher 1588 Kakamega Forest, Kenya (Schmidt et al. 2017) [26]
KX178108 Barbus cercops voucher 1647 (Schmidt et al. 2017) [5]
KX178134 Barbus neumayeri voucher 1708 (Schmidt et al. 2017)
KX178104 Barbus neumayeri voucher 1620 (Schmidt et al. 2017) [11]
KX178114 Barbus neumayeri voucher 1667 (Schmidt et al. 2017) [17]
KX178128 Barbus neumayeri voucher 1690 (Schmidt et al. 2017) [19]
KX178125 Barbus neumayeri voucher 1683 (Schmidt et al. 2017) [18]
KX178129 Barbus neumayeri voucher 1691 (Schmidt et al. 2017) [19]
KX178133 Barbus neumayeri voucher 1707 (Schmidt et al. 2017)
KX178096 Barbus cercops voucher 1577 (Schmidt et al. 2017) [25]
KX178107 Barbus cercops voucher 1646 (Schmidt et al. 2017) [5]
KX178126 Barbus neumayeri voucher 1688 (Schmidt et al. 2017) [19]
KX178118 Barbus neumayeri voucher 1673 (Schmidt et al. 2017) [17]
KX178131 Barbus neumayeri voucher 1700 (Schmidt et al. 2017)
KX178132 Barbus neumayeri voucher 1701 (Schmidt et al. 2017)
KX178127 Barbus neumayeri voucher 1689 (Schmidt et al. 2017) [19]
KX178148 Barbus kerstenii voucher 1753 Athi River (Schmidt et al. 2017)
KX178144 Barbus kerstenii voucher 1736 Athi River (Schmidt et al. 2017)
KX178149 Barbus kerstenii voucher 1754 Athi River (Schmidt et al. 2017)
KX178064 Barbus kerstenii voucher 26 (Schmidt et al. 2017)
KX178178 Barbus kerstenii voucher 10186 Athi River (Schmidt et al. 2017)
KX178162 Barbus kerstenii voucher 10111 Tana River (Schmidt et al. 2017)
KX178179 Barbus kerstenii voucher 10187 Athi River (Schmidt et al. 2017)
KX178071 Barbus kerstenii voucher 28 (Schmidt et al. 2017)
KX178078 Barbus kerstenii voucher 1507 (Schmidt et al. 2017)
KX178163 Barbus kerstenii voucher 10112 Tana River (Schmidt et al. 2017)
88/85/87
KX178167 Barbus apleurogramma voucher 10128 Athi River, Kenya (Schmidt et al. 2017)
KX178165 Barbus apleurogramma voucher 10122 Athi River, Kenya (Schmidt et al. 2017)
*
KX178166 Barbus apleurogramma voucher 10127 Athi River, Kenya (Schmidt et al. 2017)
KX178099 Barbus apleurogramma voucher 1586 Mbogo, Lake Victoria, Kenya (Schmidt et al. 2017) [25]
KX178098 Barbus apleurogramma voucher 1583 Mbogo, Lake Victoria, Kenya (Schmidt et al. 2017) [25]
KX178093 Barbus apleurogramma voucher 1570 Lake Victoria, Kenya (Schmidt et al. 2017) [22]
KX178089 Barbus apleurogramma voucher 1535 Nyando, Lake Victoria, Kenya (Schmidt et al. 2017) [23]
KX178088 Barbus apleurogramma voucher 1534 Nyando, Lake Victoria, Kenya (Schmidt et al. 2017) [23]
KX178094 Barbus apleurogramma voucher 1571 Ogenya, Lake Victoria, Kenya (Schmidt et al. 2017) [22]
*
KX178120 Barbus apleurogramma voucher 1675 Sondu, Lake Victoria, Kenya (Schmidt et al. 2017) [18]
KX178116 Barbus apleurogramma voucher 1669 Kuja, Lake Victoria, Kenya (Schmidt et al. 2017) [17]
KX178117 Barbus apleurogramma voucher 1670 Kuja, Lake Victoria, Kenya (Schmidt et al. 2017) [17]
KX178121 Barbus apleurogramma voucher 1676 Sondu, Lake Victoria, Kenya (Schmidt et al. 2017) [18]
'B.' apleurogramma
new_Uapl11ct new_11_Uriri dam [2] *
new_Urapl8ct new_8_Uriri dam [2] *
new_Urapl9ct new_9_Uriri dam [2] *
KX178103 Barbus apleurogramma voucher 1611 Lake Kanyaboli, Kenya (Schmidt et al. 2017)
KX178102 Barbus apleurogramma voucher 1610 Lake Kanyaboli, Kenya (Schmidt et al. 2017)
AF287437_1 B. paludinosus 2SK South Africa: Mooi: North West Province
South
KX178147 B. cf. paludinosus voucher 1747 (Schmidt et al. 2017)
KX178164 B. cf. paludinosus voucher 10119 (Schmidt et al. 2017)
KX178077 B. cf. paludinosus voucher 1506 (Schmidt et al. 2017)
KX178074 B. cf. paludinosus voucher 1501 (Schmidt et al. 2017)
KX178068 B. cf. paludinosus voucher 136 (Schmidt et al. 2017)
KX178066 B. cf. paludinosus voucher 84 (Schmidt et al. 2017)
KX178067 B. cf. paludinosus voucher 92 (Schmidt et al. 2017)
KX178180 B. cf. paludinosus voucher 10198 (Schmidt et al. 2017)
*
KX178069 B. cf. paludinosus voucher 138 (Schmidt et al. 2017)
KX178150 B. cf. paludinosus voucher 1755 (Schmidt et al. 2017)
KX178152 B. cf. paludinosus voucher 10011 (Schmidt et al. 2017)
KX178076 B. cf. paludinosus voucher 1505 (Schmidt et al. 2017)
KX178070 B. cf. paludinosus voucher 140 (Schmidt et al. 2017)
*
KX178065 B. cf. paludinosus voucher 70 (Schmidt et al. 2017)
KX178146 B. cf. paludinosus voucher 1746 (Schmidt et al. 2017)
*
KX178063 B. cf. paludinosus voucher 68 (Schmidt et al. 2017)
'B.' cf. paludinosus
(Clade D)
KX178073 B. cf. paludinosus voucher 1500 (Schmidt et al. 2017)
KX178075 B. cf. paludinosus voucher 1504 (Schmidt et al. 2017)
KX178062 B. cf. paludinosus voucher 66 (Schmidt et al. 2017)
KX178151 B. cf. paludinosus voucher 1756 (Schmidt et al. 2017)
KT199310_Barbus_paludinosus_1_Tanzania (Ren and Mayden 2016)
KX178194 Barbus cf. paludinosus voucher UF172201 Tanzania (Schmidt et al. 2017)
KT199311_Barbus_paludinosus_2_Tanzania (Ren and Mayden 2016)
Pld2UF Ufinya dam [13] *
*
93/84/83
76/82
B. paludinous Pld3UF Ufinya dam [13]
KX178141 B. cf. paludinosus voucher 1720 (Schmidt et al. 2017) [20]
KX178186 B. cf. paludinosus voucher 1729 (Schmidt et al. 2017)
KX178142 B. cf. paludinosus voucher 1728 (Schmidt et al. 2017)
KX178124 B. cf. paludinosus voucher 1682 (Schmidt et al. 2017) [18]
KX178123 B. cf. paludinosus voucher 1681 (Schmidt et al. 2017) [18]
0.04
KX178176 Barbus cf. paludinosus "Jipe" voucher 10160 (Schmidt et al. 2017)
*
KX178175 Barbus cf. paludinosus "Jipe" voucher 10159 (Schmidt et al. 2017)
KX178083 Barbus cf. paludinosus "Jipe" voucher 1523 (Schmidt et al. 2017) [23]
KY595071 Barbus cercops voucher 1524 (Schmidt et al. 2017) [23]
KX178081 Barbus cf. paludinosus "Jipe" voucher 1521 (Schmidt et al. 2017) [23]
KX178082 Barbus cf. paludinosus "Jipe" voucher 1522 (Schmidt et al. 2017) [23]

Figure 6(on next page)

Inferred relationships within Clade C (expanded from Fig. 3) based on the Cytb gene.

RaxML bootstrap consensus (60% majority rule) tree. All taxa except those with grey shading are found in East Africa. Bold-faced taxon labels indicate sequences generated by the present study. Asterisks by taxon names indicate GH sequence was also generated in present study. Numbers in brackets correspond to localities in Fig. 1. Node support values from Bayesian (above) and ML below are given by nodes; an asterisk indicates support > 97% in all corresponding analyses. For visual clarity, several node support labels have been omitted. Red taxon label indicates specimen from Schmidt et al. (2017) assigned to '*B.' kerstenii* based on morphology and GH sequence, but with a Cytb sequence that falls within '*B.' nyanzae*.

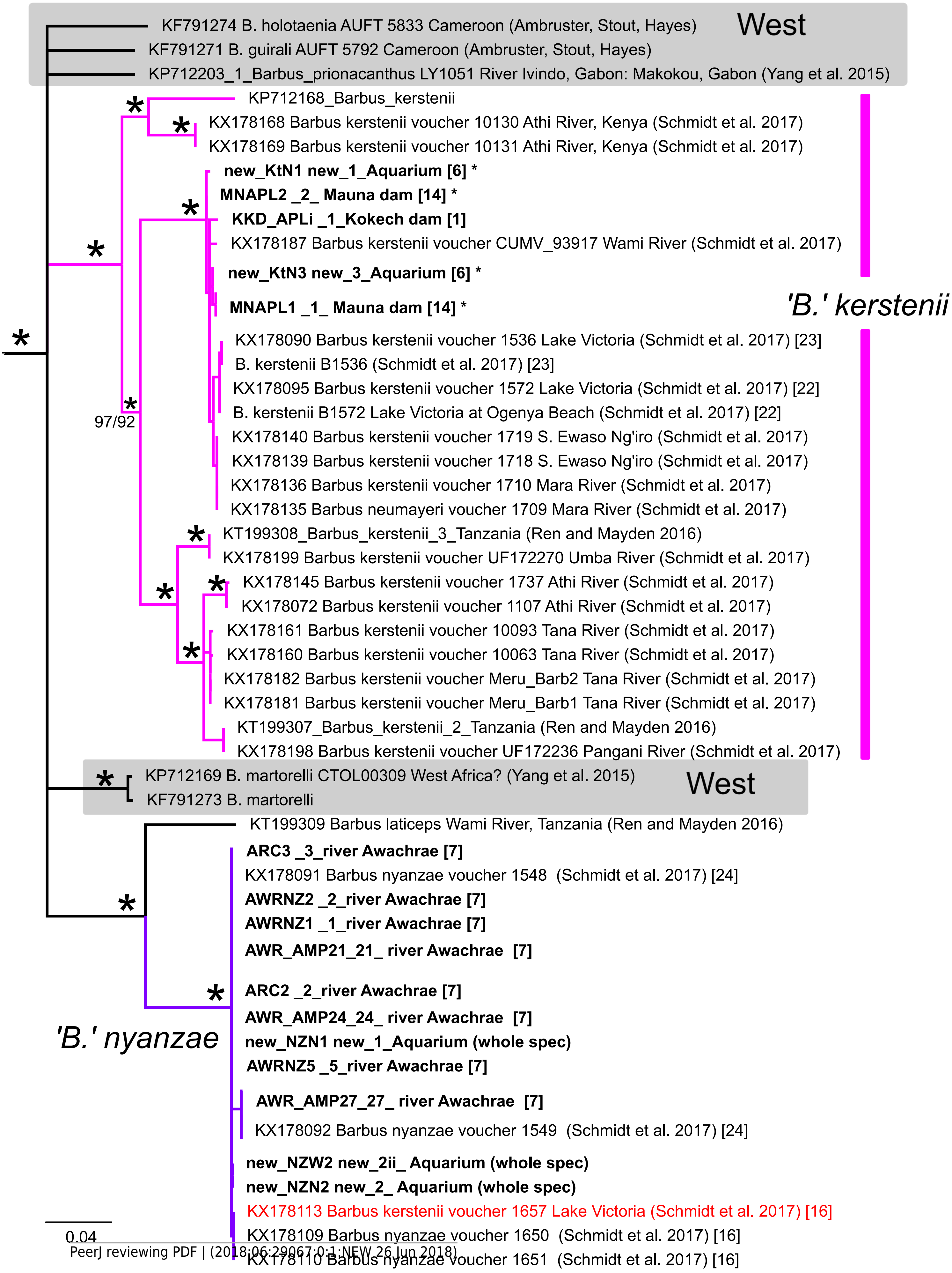
KF791274 B. holotaenia AUFT 5833 Cameroon (Ambruster, Stout, Hayes)
West
KF791271 B. guirali AUFT 5792 Cameroon (Ambruster, Stout, Hayes)
KP712203_1_Barbus_prionacanthus LY1051 River Ivindo, Gabon: Makokou, Gabon (Yang et al. 2015)
*
KP712168_Barbus_kerstenii
*
KX178168 Barbus kerstenii voucher 10130 Athi River, Kenya (Schmidt et al. 2017)
KX178169 Barbus kerstenii voucher 10131 Athi River, Kenya (Schmidt et al. 2017)
new_KtN1 new_1_Aquarium [6] *
MNAPL2 _2_ Mauna dam [14] *
*
KKD_APLi _1_Kokech dam [1]
*
KX178187 Barbus kerstenii voucher CUMV_93917 Wami River (Schmidt et al. 2017)
new_KtN3 new_3_Aquarium [6] *
MNAPL1 _1_ Mauna dam [14] *
'B.' kerstenii
*
KX178090 Barbus kerstenii voucher 1536 Lake Victoria (Schmidt et al. 2017) [23]
B. kerstenii B1536 (Schmidt et al. 2017) [23]
KX178095 Barbus kerstenii voucher 1572 Lake Victoria (Schmidt et al. 2017) [22]
*
97/92
B. kerstenii B1572 Lake Victoria at Ogenya Beach (Schmidt et al. 2017) [22]
KX178140 Barbus kerstenii voucher 1719 S. Ewaso Ng'iro (Schmidt et al. 2017)
KX178139 Barbus kerstenii voucher 1718 S. Ewaso Ng'iro (Schmidt et al. 2017)
KX178136 Barbus kerstenii voucher 1710 Mara River (Schmidt et al. 2017)
KX178135 Barbus neumayeri voucher 1709 Mara River (Schmidt et al. 2017)
*
KT199308_Barbus_kerstenii_3_Tanzania (Ren and Mayden 2016)
KX178199 Barbus kerstenii voucher UF172270 Umba River (Schmidt et al. 2017)
*
KX178145 Barbus kerstenii voucher 1737 Athi River (Schmidt et al. 2017)
*
KX178072 Barbus kerstenii voucher 1107 Athi River (Schmidt et al. 2017)
KX178161 Barbus kerstenii voucher 10093 Tana River (Schmidt et al. 2017)
*
KX178160 Barbus kerstenii voucher 10063 Tana River (Schmidt et al. 2017)
KX178182 Barbus kerstenii voucher Meru_Barb2 Tana River (Schmidt et al. 2017)
KX178181 Barbus kerstenii voucher Meru_Barb1 Tana River (Schmidt et al. 2017)
KT199307_Barbus_kerstenii_2_Tanzania (Ren and Mayden 2016)
KX178198 Barbus kerstenii voucher UF172236 Pangani River (Schmidt et al. 2017)
*
KP712169 B. martorelli CTOL00309 West Africa? (Yang et al. 2015)
West
KF791273 B. martorelli
KT199309 Barbus laticeps Wami River, Tanzania (Ren and Mayden 2016)
ARC3 _3_river Awachrae [7]
KX178091 Barbus nyanzae voucher 1548 (Schmidt et al. 2017) [24]
*
AWRNZ2 _2_river Awachrae [7]
AWRNZ1 _1_river Awachrae [7]
AWR_AMP21_21_ river Awachrae [7]
*
ARC2 _2_river Awachrae [7]
AWR_AMP24_24_ river Awachrae [7]
'B.' nyanzae
new_NZN1 new_1_Aquarium (whole spec)
AWRNZ5 _5_river Awachrae [7]
AWR_AMP27_27_ river Awachrae [7]
KX178092 Barbus nyanzae voucher 1549 (Schmidt et al. 2017) [24]
new_NZW2 new_2ii_ Aquarium (whole spec)
new_NZN2 new_2_ Aquarium (whole spec)
KX178113 Barbus kerstenii voucher 1657 Lake Victoria (Schmidt et al. 2017) [16]
0.04
KX178109 Barbus nyanzae voucher 1650 (Schmidt et al. 2017) [16]
KX178110 Barbus nyanzae voucher 1651 (Schmidt et al. 2017) [16]

Figure 7(on next page)

Inferred relationships within Clade B (expanded from Fig. 3) based on the Cytb gene.

RaxML bootstrap consensus (60% majority rule) tree. All taxa except those *'B.' radiatus* and '*B." profundus* (boldface taxon labels) are distributed in West Africa. Asterisks by taxon names indicate GH sequence was also generated in present study. Numbers in brackets correspond to localities in Fig. 1. Node support values from Bayesian (above) and ML below are given by nodes; an asterisk indicates support > 97% in all corresponding analyses. For visual clarity, several node support labels have been omitted.

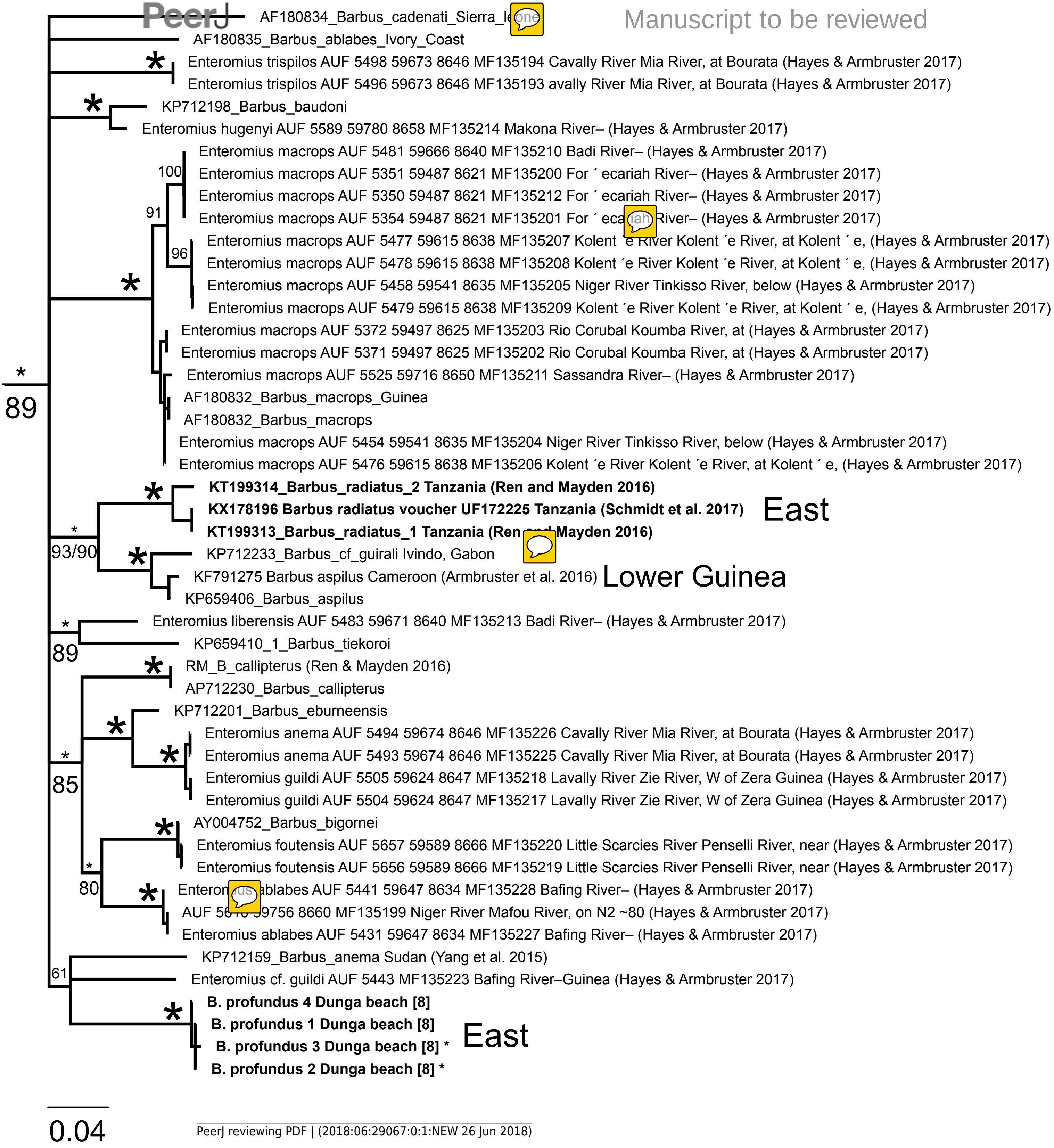
AF180834_Barbus_cadenati_Sierra_leone
AF180835_Barbus_ablabes_Ivory_Coast
*
Enteromius trispilos AUF 5498 59673 8646 MF135194 Cavally River Mia River, at Bourata (Hayes & Armbruster 2017)
Enteromius trispilos AUF 5496 59673 8646 MF135193 avally River Mia River, at Bourata (Hayes & Armbruster 2017)
*
KP712198_Barbus_baudoni
Enteromius hugenyi AUF 5589 59780 8658 MF135214 Makona River– (Hayes & Armbruster 2017)
Enteromius macrops AUF 5481 59666 8640 MF135210 Badi River– (Hayes & Armbruster 2017)
100
Enteromius macrops AUF 5351 59487 8621 MF135200 For ´ ecariah River– (Hayes & Armbruster 2017)
Enteromius macrops AUF 5350 59487 8621 MF135212 For ´ ecariah River– (Hayes & Armbruster 2017)
91
Enteromius macrops AUF 5354 59487 8621 MF135201 For ´ ecariah River– (Hayes & Armbruster 2017)
Enteromius macrops AUF 5477 59615 8638 MF135207 Kolent ´e River Kolent ´e River, at Kolent ´ e, (Hayes & Armbruster 2017)
96
Enteromius macrops AUF 5478 59615 8638 MF135208 Kolent ´e River Kolent ´e River, at Kolent ´ e, (Hayes & Armbruster 2017)
*
Enteromius macrops AUF 5458 59541 8635 MF135205 Niger River Tinkisso River, below (Hayes & Armbruster 2017)
Enteromius macrops AUF 5479 59615 8638 MF135209 Kolent ´e River Kolent ´e River, at Kolent ´ e, (Hayes & Armbruster 2017)
Enteromius macrops AUF 5372 59497 8625 MF135203 Rio Corubal Koumba River, at (Hayes & Armbruster 2017)
Enteromius macrops AUF 5371 59497 8625 MF135202 Rio Corubal Koumba River, at (Hayes & Armbruster 2017)
*
Enteromius macrops AUF 5525 59716 8650 MF135211 Sassandra River– (Hayes & Armbruster 2017)
89
AF180832_Barbus_macrops_Guinea
AF180832_Barbus_macrops
Enteromius macrops AUF 5454 59541 8635 MF135204 Niger River Tinkisso River, below (Hayes & Armbruster 2017)
Enteromius macrops AUF 5476 59615 8638 MF135206 Kolent ´e River Kolent ´e River, at Kolent ´ e, (Hayes & Armbruster 2017)
*
KT199314_Barbus_radiatus_2 Tanzania (Ren and Mayden 2016)
KX178196 Barbus radiatus voucher UF172225 Tanzania (Schmidt et al. 2017)
East
*
KT199313_Barbus_radiatus_1 Tanzania (Ren and Mayden 2016)
93/90
*
KP712233_Barbus_cf_guirali Ivindo, Gabon
KF791275 Barbus aspilus Cameroon (Armbruster et al. 2016)
Lower Guinea
KP659406_Barbus_aspilus
*
Enteromius liberensis AUF 5483 59671 8640 MF135213 Badi River– (Hayes & Armbruster 2017)
89
KP659410_1_Barbus_tiekoroi
*
RM_B_callipterus (Ren & Mayden 2016)
AP712230_Barbus_callipterus
KP712201_Barbus_eburneensis
*
Enteromius anema AUF 5494 59674 8646 MF135226 Cavally River Mia River, at Bourata (Hayes & Armbruster 2017)
*
*
Enteromius anema AUF 5493 59674 8646 MF135225 Cavally River Mia River, at Bourata (Hayes & Armbruster 2017)
85
Enteromius guildi AUF 5505 59624 8647 MF135218 Lavally River Zie River, W of Zera Guinea (Hayes & Armbruster 2017)
Enteromius guildi AUF 5504 59624 8647 MF135217 Lavally River Zie River, W of Zera Guinea (Hayes & Armbruster 2017)
*
AY004752_Barbus_bigornei
Enteromius foutensis AUF 5657 59589 8666 MF135220 Little Scarcies River Penselli River, near (Hayes & Armbruster 2017)
*
Enteromius foutensis AUF 5656 59589 8666 MF135219 Little Scarcies River Penselli River, near (Hayes & Armbruster 2017)
80
*
Enteromius ablabes AUF 5441 59647 8634 MF135228 Bafing River– (Hayes & Armbruster 2017)
AUF 5610 59756 8660 MF135199 Niger River Mafou River, on N2 ~80 (Hayes & Armbruster 2017)
Enteromius ablabes AUF 5431 59647 8634 MF135227 Bafing River– (Hayes & Armbruster 2017)
KP712159_Barbus_anema Sudan (Yang et al. 2015)
61
Enteromius cf. guildi AUF 5443 MF135223 Bafing River–Guinea (Hayes & Armbruster 2017)
*
B. profundus 4 Dunga beach [8]
B. profundus 1 Dunga beach [8]
East
B. profundus 3 Dunga beach [8] *
B. profundus 2 Dunga beach [8] *
0.04

Figure 8

Inferred relationships within the clades “*Labeobarbus* and allies” (expanded from Fig. 3) based on the Cytb gene.

RaxML bootstrap consensus (60% majority rule) tree. Boldfaced taxon labels correspond to our specimens of *L. altianalis.* Numbers in brackets correspond to localities in Fig. 1

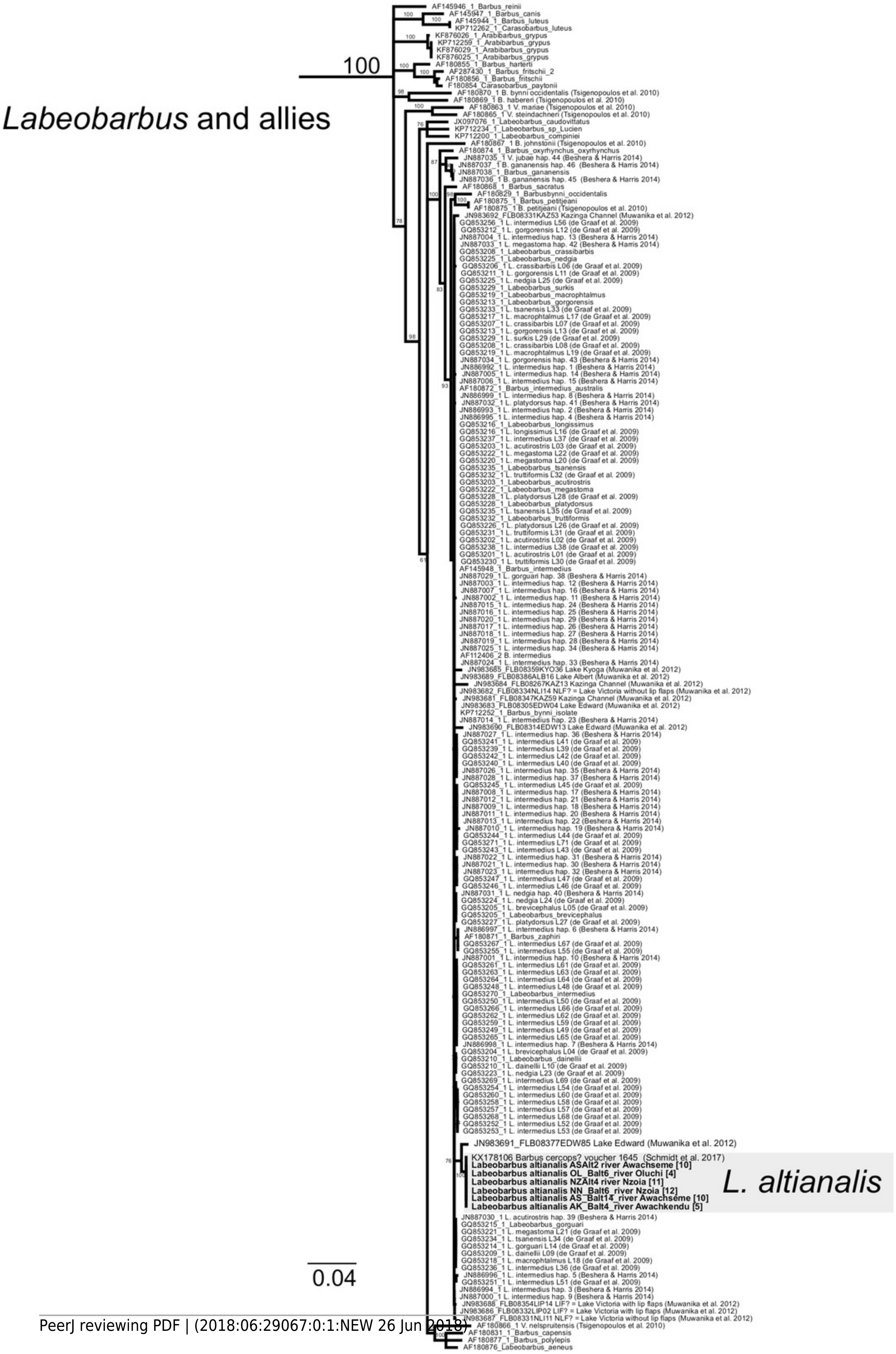
100
Labeobarbus and allies
AF145946_1_Barbus_reinii
100
AF145947_1_Barbus_canis
100
AF145944_1_Barbus_luteus
KP712262_1_Carasobarbus_luteus
100
KF876026_1_Arabibarbus_grypus
KP712259_1_Arabibarbus_grypus
KF876029_1_Arabibarbus_grypus
KF876025_1_Arabibarbus_grypus
100
AF180855_1_Barbus_harterti
100
AF287430_1_Barbus_fritschii_2
AF180856_1_Barbus_fritschii
F180854_Carasobarbus_paytonii
98
AF180870_1 B. bynni occidentalis (Tsigenopoulos et al. 2010)
AF180869_1 B. habereri (Tsigenopoulos et al. 2010)
100
AF180863_1 V. mariae (Tsigenopoulos et al. 2010)
AF180865_1 V. steindachneri (Tsigenopoulos et al. 2010)
76
JX097076_1_Labeobarbus_caudovittatus
KP712234_1_Labeobarbus_sp_Lucien
KP712200_1_Labeobarbus_compiniei
AF180867_1 B. johnstonii (Tsigenopoulos et al. 2010)
AF180874_1_Barbus_oxyrhynchus_oxyrhynchus
87
JN887035_1 V. jubae hap. 44 (Beshera & Harris 2014)
JN887037_1 B. gananensis hap. 46 (Beshera & Harris 2014)
JN887038_1_Barbus_gananensis
JN887036_1 B. gananensis hap. 45 (Beshera & Harris 2014)
AF180868_1_Barbus_sacratus
100
98
AF180829_1_Barbusbynni_occidentalis
100
AF180875_1_Barbus_petitjeani
AF180875_1 B. petitjeani (Tsigenopoulos et al. 2010)
JN983692_FLB08331KAZ53 Kazinga Channel (Muwanika et al. 2012)
78
GQ853256_1 L. intermedius L56 (de Graaf et al. 2009)
GQ853212_1 L. gorgorensis L12 (de Graaf et al. 2009)
JN887004_1 L. intermedius hap. 13 (Beshera & Harris 2014)
JN887033_1 L. megastoma hap. 42 (Beshera & Harris 2014)
GQ853208_1_Labeobarbus_crassibarbis
GQ853225_1_Labeobarbus_nedgia
GQ853206_1 L. crassibarbis L06 (de Graaf et al. 2009)
GQ853211_1 L. gorgorensis L11 (de Graaf et al. 2009)
GQ853225_1 L. nedgia L25 (de Graaf et al. 2009)
83
GQ853229_1_Labeobarbus_surkis
GQ853219_1_Labeobarbus_macrophtalmus
GQ853213_1_Labeobarbus_gorgorensis
GQ853233_1 L. tsanensis L33 (de Graaf et al. 2009)
GQ853217_1 L. macrophtalmus L17 (de Graaf et al. 2009)
GQ853207_1 L. crassibarbis L07 (de Graaf et al. 2009)
GQ853213_1 L. gorgorensis L13 (de Graaf et al. 2009)
GQ853229_1 L. surkis L29 (de Graaf et al. 2009)
98
GQ853208_1 L. crassibarbis L08 (de Graaf et al. 2009)
GQ853219_1 L. macrophtalmus L19 (de Graaf et al. 2009)
JN887034_1 L. gorgorensis hap. 43 (Beshera & Harris 2014)
JN886992_1 L. intermedius hap. 1 (Beshera & Harris 2014)
JN887005_1 L. intermedius hap. 14 (Beshera & Harris 2014)
JN887006_1 L. intermedius hap. 15 (Beshera & Harris 2014)
93
AF180872_1_Barbus_intermedius_australis
JN886999_1 L. intermedius hap. 8 (Beshera & Harris 2014)
JN887032_1 L. platydorsus hap. 41 (Beshera & Harris 2014)
JN886993_1 L. intermedius hap. 2 (Beshera & Harris 2014)
JN886995_1 L. intermedius hap. 4 (Beshera & Harris 2014)
GQ853216_1_Labeobarbus_longissimus
GQ853216_1 L. longissimus L16 (de Graaf et al. 2009)
GQ853237_1 L. intermedius L37 (de Graaf et al. 2009)
GQ853203_1 L. acutirostris L03 (de Graaf et al. 2009)
GQ853222_1 L. megastoma L22 (de Graaf et al. 2009)
GQ853220_1 L. megastoma L20 (de Graaf et al. 2009)
GQ853235_1_Labeobarbus_tsanensis
GQ853232_1 L. truttiformis L32 (de Graaf et al. 2009)
GQ853203_1_Labeobarbus_acutirostris
GQ853222_1_Labeobarbus_megastoma
GQ853228_1 L. platydorsus L28 (de Graaf et al. 2009)
GQ853228_1_Labeobarbus_platydorsus
GQ853235_1 L. tsanensis L35 (de Graaf et al. 2009)
GQ853232_1_Labeobarbus_truttiformis
GQ853226_1 L. platydorsus L26 (de Graaf et al. 2009)
GQ853231_1 L. truttiformis L31 (de Graaf et al. 2009)
GQ853202_1 L. acutirostris L02 (de Graaf et al. 2009)
GQ853238_1 L. intermedius L38 (de Graaf et al. 2009)
GQ853201_1 L. acutirostris L01 (de Graaf et al. 2009)
61
GQ853230_1 L. truttiformis L30 (de Graaf et al. 2009)
AF145948_1_Barbus_intermedius
JN887029_1 L. gorguari hap. 38 (Beshera & Harris 2014)
JN887003_1 L. intermedius hap. 12 (Beshera & Harris 2014)
JN887007_1 L. intermedius hap. 16 (Beshera & Harris 2014)
JN887002_1 L. intermedius hap. 11 (Beshera & Harris 2014)
JN887015_1 L. intermedius hap. 24 (Beshera & Harris 2014)
JN887016_1 L. intermedius hap. 25 (Beshera & Harris 2014)
JN887020_1 L. intermedius hap. 29 (Beshera & Harris 2014)
JN887017_1 L. intermedius hap. 26 (Beshera & Harris 2014)
JN887018_1 L. intermedius hap. 27 (Beshera & Harris 2014)
JN887019_1 L. intermedius hap. 28 (Beshera & Harris 2014)
JN887025_1 L. intermedius hap. 34 (Beshera & Harris 2014)
AF112406_2 B. intermedius
JN887024_1 L. intermedius hap. 33 (Beshera & Harris 2014)
JN983685_FLB08359KYO36 Lake Kyoga (Muwanika et al. 2012)
JN983689_FLB08386ALB16 Lake Albert (Muwanika et al. 2012)
JN983684_FLB08267KAZ13 Kazinga Channel (Muwanika et al. 2012)
JN983682_FLB08334NLI14 NLF? = Lake Victoria without lip flaps (Muwanika et al. 2012)
JN983681_FLB08347KAZ59 Kazinga Channel (Muwanika et al. 2012)
JN983683_FLB08305EDW04 Lake Edward (Muwanika et al. 2012)
KP712252_1_Barbus_bynni_isolate
JN887014_1 L. intermedius hap. 23 (Beshera & Harris 2014)
JN983690_FLB08314EDW13 Lake Edward (Muwanika et al. 2012)
JN887027_1 L. intermedius hap. 36 (Beshera & Harris 2014)
GQ853241_1 L. intermedius L41 (de Graaf et al. 2009)
GQ853239_1 L. intermedius L39 (de Graaf et al. 2009)
GQ853242_1 L. intermedius L42 (de Graaf et al. 2009)
GQ853240_1 L. intermedius L40 (de Graaf et al. 2009)
JN887026_1 L. intermedius hap. 35 (Beshera & Harris 2014)
JN887028_1 L. intermedius hap. 37 (Beshera & Harris 2014)
GQ853245_1 L. intermedius L45 (de Graaf et al. 2009)
JN887008_1 L. intermedius hap. 17 (Beshera & Harris 2014)
JN887012_1 L. intermedius hap. 21 (Beshera & Harris 2014)
JN887009_1 L. intermedius hap. 18 (Beshera & Harris 2014)
JN887011_1 L. intermedius hap. 20 (Beshera & Harris 2014)
JN887013_1 L. intermedius hap. 22 (Beshera & Harris 2014)
JN887010_1 L. intermedius hap. 19 (Beshera & Harris 2014)
GQ853244_1 L. intermedius L44 (de Graaf et al. 2009)
GQ853271_1 L. intermedius L71 (de Graaf et al. 2009)
GQ853243_1 L. intermedius L43 (de Graaf et al. 2009)
JN887022_1 L. intermedius hap. 31 (Beshera & Harris 2014)
JN887021_1 L. intermedius hap. 30 (Beshera & Harris 2014)
JN887023_1 L. intermedius hap. 32 (Beshera & Harris 2014)
GQ853247_1 L. intermedius L47 (de Graaf et al. 2009)
GQ853246_1 L. intermedius L46 (de Graaf et al. 2009)
JN887031_1 L. nedgia hap. 40 (Beshera & Harris 2014)
GQ853224_1 L. nedgia L24 (de Graaf et al. 2009)
GQ853205_1 L. brevicephalus L05 (de Graaf et al. 2009)
GQ853205_1_Labeobarbus_brevicephalus
GQ853227_1 L. platydorsus L27 (de Graaf et al. 2009)
JN886997_1 L. intermedius hap. 6 (Beshera & Harris 2014)
AF180871_1_Barbus_zaphiri
GQ853267_1 L. intermedius L67 (de Graaf et al. 2009)
GQ853255_1 L. intermedius L55 (de Graaf et al. 2009)
JN887001_1 L. intermedius hap. 10 (Beshera & Harris 2014)
GQ853261_1 L. intermedius L61 (de Graaf et al. 2009)
GQ853263_1 L. intermedius L63 (de Graaf et al. 2009)
GQ853264_1 L. intermedius L64 (de Graaf et al. 2009)
GQ853248_1 L. intermedius L48 (de Graaf et al. 2009)
GQ853270_1_Labeobarbus_intermedius
GQ853250_1 L. intermedius L50 (de Graaf et al. 2009)
GQ853266_1 L. intermedius L66 (de Graaf et al. 2009)
GQ853262_1 L. intermedius L62 (de Graaf et al. 2009)
GQ853259_1 L. intermedius L59 (de Graaf et al. 2009)
GQ853249_1 L. intermedius L49 (de Graaf et al. 2009)
GQ853265_1 L. intermedius L65 (de Graaf et al. 2009)
JN886998_1 L. intermedius hap. 7 (Beshera & Harris 2014)
GQ853204_1 L. brevicephalus L04 (de Graaf et al. 2009)
GQ853210_1_Labeobarbus_dainellii
GQ853210_1 L. dainellii L10 (de Graaf et al. 2009)
GQ853223_1 L. nedgia L23 (de Graaf et al. 2009)
GQ853269_1 L. intermedius L69 (de Graaf et al. 2009)
GQ853254_1 L. intermedius L54 (de Graaf et al. 2009)
GQ853260_1 L. intermedius L60 (de Graaf et al. 2009)
GQ853258_1 L. intermedius L58 (de Graaf et al. 2009)
GQ853257_1 L. intermedius L57 (de Graaf et al. 2009)
GQ853268_1 L. intermedius L68 (de Graaf et al. 2009)
GQ853252_1 L. intermedius L52 (de Graaf et al. 2009)
GQ853253_1 L. intermedius L53 (de Graaf et al. 2009)
JN983691_FLB08377EDW85 Lake Edward (Muwanika et al. 2012)
76
KX178106 Barbus cercops? voucher 1645 (Schmidt et al. 2017)
Labeobarbus altianalis ASAlt2 river Awachseme [10]
100
Labeobarbus altianalis OL_Balt6_river Oluchi [4]
Labeobarbus altianalis NZAlt4 river Nzoia [11]
Labeobarbus altianalis NN_Balt6_river Nzoia [12]
Labeobarbus altianalis AS_Balt14_river Awachseme [10]
Labeobarbus altianalis AK_Balt4_river Awachkendu [5]
L. altianalis
JN887030_1 L. acutirostris hap. 39 (Beshera & Harris 2014)
GQ853215_1_Labeobarbus_gorguari
GQ853221_1 L. megastoma L21 (de Graaf et al. 2009)
GQ853234_1 L. tsanensis L34 (de Graaf et al. 2009)
GQ853214_1 L. gorguari L14 (de Graaf et al. 2009)
GQ853209_1 L. dainellii L09 (de Graaf et al. 2009)
GQ853218_1 L. macrophtalmus L18 (de Graaf et al. 2009)
GQ853236_1 L. intermedius L36 (de Graaf et al. 2009)
0.04
JN886996_1 L. intermedius hap. 5 (Beshera & Harris 2014)
GQ853251_1 L. intermedius L51 (de Graaf et al. 2009)
JN886994_1 L. intermedius hap. 3 (Beshera & Harris 2014)
JN887000_1 L. intermedius hap. 9 (Beshera & Harris 2014)
JN983688_FLB08354LIP14 LIF? = Lake Victoria with lip flaps (Muwanika et al. 2012)
JN983686_FLB08332LIP02 LIF? = Lake Victoria with lip flaps (Muwanika et al. 2012)
JN983687_FLB08331NLI11 NLF? = Lake Victoria without lip flaps (Muwanika et al. 2012)
AF180866_1 V. nelspruitensis (Tsigenopoulos et al. 2010)
100
AF180831_1_Barbus_capensis
AF180877_1_Barbus_polylepis
AF180876_Labeobarbus_aeneus

Table 1(on next page)

Description of characters and substitution models identified by model selection analyses.

Best model selected by: (a) jModeltest 2.1.10 v20160303 (Darriba et al., 2012) according to each criterion (AICc, AIC, BIC) and its corresponding weight (on a fixed BioNJ tree); and (b) the best partitioning scheme according to the BIC implemented in PartitionFinder 2.7 (Guindon et al., 2010 ; Lanfear et al., 2012 ; Lanfear et al., 2017) : branchlengths = linked; and search = greedy.

Table 1. Description of characters and substitution models identified by model selection analyses. Best model selected by: (a) jModeltest 2.1.10 v20160303 (Darriba et al., 2012) according to each criterion (AICc, AIC, BIC) and its corresponding weight (on a fixed BioNJ tree); and (b) the best partitioning scheme according to the BIC implemented in PartitionFinder 2.7 (Guindon et al., 2010; Lanfear et al., 2012; Lanfear et al., 2017): branchlengths = linked; and search = greedy.

Gene	Non-redundant taxa	Characters used	Parsimony informative	Partitioning Scheme	AICc (weight)	AIC (weight)	BIC (weight)
GH	46	201	63	1	TPM2uf (0.36)	TVM (0.22)	TVM (0.24)
Cytb	291	1023	507	1	TIM2+I+G (0.99)	TIM2+I+G (0.61)	TIM2+I+G (0.99)
				3 (by codon)			
				Codon 1			SYM+I+G
				Codon 2			HKY+I+G
				Codon 3			GTR+G