

**AN EVIDENCE OF PAST INTROGRESSIVE HYBRIDIZATION BETWEEN
LABEOBARBUS ETHIOPICUS AND *L. INTERMEDIUS* IN THE ETHIOPIAN RIFT
VALLEY, EAST AFRICA**

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ABSTRACT: Analyses of nucleotide sequences of mitochondrial DNA (cytochrome *b* fragment) in 30 common barbs (*Labeobarbus intermedius*) from Lake Langano in the Ethiopian Rift Valley revealed the genetic heterogeneity of the samples. Whereas 29 individuals shared the same haplotype similar to those found in the poorly structured assemblage of *L. intermedius* sensu lato inhabiting the Rift Valley and basins of western Ethiopia, a single individual possessed a haplotype found exclusively in small-scaled Ethiopian barb *L. ethiopicus*. The latter species is phylogenetically distant from *L. intermedius* from any Ethiopian basins and differs from it by the substantially increased lateral line scale counts. *Labeobarbus ethiopicus* is the narrow range endemic of the Lake Zway basin. Presently barb migration from Zway to Langano is impossible because of the high water mineralization of Lake Abijata located on route. Past introgressive hybridization between two barb species could be enhanced by higher water level in the Zway-Langano-Abijata-Shala lake basin during humid periods in the course of paleoclimatic oscillations. Additionally, the increased lateral line scale counts were found in the Langano barbs compared to other barb population from the central part of the Ethiopian Rift valley, while the barbs from this region exhibited the increased values of this character compared to any other *L. intermedius* populations from Ethiopia and Kenya. These increased values could be a result of gene flow from *L. ethiopicus* to *L. intermedius*. The endangered status of *L. ethiopicus* as well as *L. intermedius* population from lakes Zway and Langano is highlighted.

Key words/phrases: *Labeobarbus*, Introgressive hybridization, Paleoclimatic oscillations.

INTRODUCTION

The endorrheic system of the four lakes (Zway, Langano, Abijata and Shala) is located in the central part of the Ethiopian Rift Valley. Lakes Zway and Langano discharge into Lake Abijata via the Bulbula and Hurokalu

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(Horocallo) rivers, respectively, while Lake Shala can be occasionally connected with Lake Abijata via the Gidu River (Baxter, 2002). There is clear evidence that these four lakes were once united into one large lake, which overflowed into the Awash River in the Late Pleistocene. Early Holocene, and in that time the fish fauna of this palaeobasin included species now absent in the region (Grove *et al.*, 1975).

The fish fauna of this lake system is now extremely depauperated and includes only nine indigenous fish species (Golubtsov *et al.*, 2002). The apparent reasons for this are isolation from the large river systems, general desiccation of the region at the present stage of climatic oscillations and resulting increased water mineralization in all the four lakes under consideration, especially in Abijata and Shala, where fish can survive exclusively in the mouths of tributaries. Moreover, during last decades, fish communities in Zway and Langanos lakes were greatly transformed by overfishing and impact of invasive fish species, mostly common and crucian carps, *Cyprinus carpio* and *Carrasius* sp.

Before the human-caused alterations, the large African barbs of the genus *Labeobarbus* dominated the fish communities in the many Ethiopian lakes and rivers including lakes Hawassa, Langanos and Zway in the central part of the Ethiopian Rift Valley and, therefore, were important for local fisheries (Demeke Admassu and Elias Dadebo, 1997). Presently, invasive carps severely compete for resources with the local *Labeobarbus* and practically substituted them in commercial fish catches at lakes Langanos and Zway, but not yet in Lake Hawassa (Lemma Abera *et al.*, 2014; our personal observations).

The small-scaled Ethiopian barb *Labeobarbus ethiopicus* is described by Zolezzi (1939) from Lake Zway (Fig. 1). It differs from all other Ethiopian members of the genus by increased number of scales in lateral line (46–52 versus 20–40 scales in other Ethiopian species) (Banister, 1973; Alekseyev, 1994; Golubtsov *et al.*, 2002; 2004). Three other *Labeobarbus* species – *L. macmillani* (Boulenger, 1906), *L. macronema* (Boulenger, 1902) and *L. zuaicus* (Boulenger, 1906) described from the Lake Zway basin, as well as dozens of *Labeobarbus* taxa described from other parts of Ethiopia and northern Kenya – were recognized as synonyms of *L. intermedius* (Rüppell, 1836) by Banister (1973), based on morphological characters (particularly, on the number of scales in lateral line continuously ranging from 26 to 34). There are still neither morphological nor genetic data that contradict Banister's synonymy, excluding those for the Lake Tana *Labeobarbus*

species flock (Nagelkerke and Sibbing, 1997; 2000; but see de Graaf *et al.*, 2010; Beshera and Harris, 2014 for genetics).

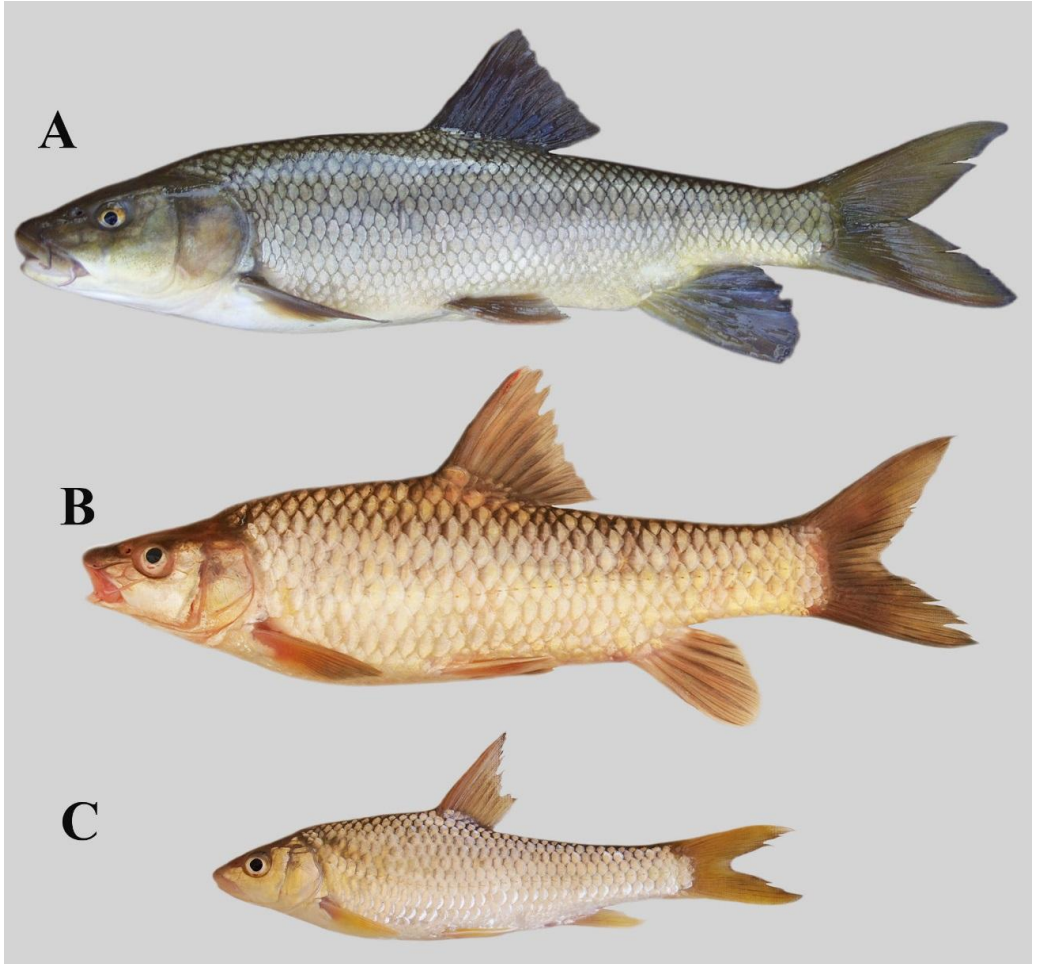


Fig. 1. Lateral view of freshly caught individuals of the large African barbs: A) Ethiopian barb *L. ethiopicus* (SL = 320 mm) from the Meki R., a tributary of Lake Zway; B) common barb *L. intermedius* (SL = 246 mm) from Lake Langan; C) juvenile of *L. intermedius* (SL = 125 mm) from Lake Langan with mtDNA haplotype of *L. ethiopicus*.

The fifth *Labeobarbus* species described from Lake Zway and still known from the holotype only is *L. microterolepis* (Boulenger, 1902). It has 40 scales in the lateral line. Banister (1973, p. 91) did not recognize it as a junior synonym of *L. intermedius*, but suggested that it could be “a hybrid between the small-scaled *Barbus ethiopicus* of Lake Zway and *Barbus intermedius*”. Since no more specimens with such morphology were found,

Golubtsov *et al.* (2002) finally recognized *L. microterolepis* as a hybrid between the two above mentioned species.

Labeobarbus ethiopicus is endemic to Lake Zway and its tributaries in Ethiopian Rift Valley (Golubtsov *et al.*, 2002; 2004; Redeat Habteselassie and Kassahun Asaminew, 2008). Despite many erroneous indications of this species for the Tana Lake basin in the Blue Nile system (Tsigenopoulos *et al.*, 2002; Borckenhagen, 2014; Vreven *et al.*, 2016), there is no species with 46–52 scales in lateral line among local large barbs (Nagelkerke and Sibbing, 1997; 2000; de Graaf *et al.*, 2008). Karyological and phylogenetic studies showed that *L. ethiopicus* is a member of the evolutionary lineage of the large hexaploid African barbs (Golubtsov and Krysanov, 1993; Tsigenopoulos *et al.*, 2002; 2010). *Labeobarbus ethiopicus* is a basal clade in radiation of the large barbs of Ethiopia which form in general a monophyletic group as demonstrated by the phylogenetic studies based on mtDNA markers (Levin *et al.*, 2013; Yang *et al.*, 2015; Beshera *et al.*, 2016). According to a recent taxonomic review, *L. ethiopicus* belongs to the African hexaploid Torini lineage of barbs (Vreven *et al.*, 2016).

The only large barb taxon described from Lake Langano is *Barbus platystomus* var. *vatovae* by Zolezzi (1939). This barb has 39–40 scales in lateral line. *Barbus platystomus* was described by Boulenger (1902) from Lake Tana. Both taxa are currently recognized as junior synonyms of *L. intermedius* (Banister, 1973; Golubtsov *et al.*, 2002; Vreven *et al.*, 2016; Eschmeyer *et al.*, 2017). We have to note that *Varicorhinus platystomus* Pappenheim, 1914 is currently valid as *L. platystomus* (De Vos *et al.*, 2001; Eschmeyer *et al.*, 2017). It inhabits the Mukungwa River in Rwanda and is quite distantly related to the large barbs from the Ethiopian Rift Valley.

This study is aimed at genetic and morphological comparison of the barbs from Lake Langano with *Labeobarbus* from the Ethiopian Rift Valley and other Ethiopian basins with particular reference to a specimen from Lake Langano exhibiting outstanding mitochondrial DNA haplotype.

MATERIALS AND METHODS

Barbs from the Lake Langano basin for molecular study (30 specimens) were collected at four localities in 2009: the lake itself (07°33' N, 38°41' E and 07°31' N, 37°45' E) and its southern tributaries, the Lepis (07°31' N, 37°45' E) and Huluka (07°30' N, 37°44' E) rivers. Two specimens of *Labeobarbus ethiopicus* were collected from the Meki River (8°16' N, 38°31' E), a tributary of Lake Zway, in 2008. Comparative genetic data were obtained from GenBank (<https://www.ncbi.nlm.nih.gov>).

DNA was extracted from a small piece of the fin using the salt method (Aljanabi and Martinez, 1997). For all individuals studied, a fragment of the mtDNA, cytochrome *b* (*cyt-b*) gene (964 bp) was amplified by PCR using the primers GluDg: 5'-TGACTTGAARAACCA YCGTTG-3' (Palumbi, 1996) and H16460: 5'-CGAYCTTCGGATTAACAAGACCG-3' (Perdices and Doadrio, 2001). In most cases, each PCR product was sequenced using the two mentioned amplification primers and sometimes using two other primers GluF: 5'-AACCACCGTTGTATTCAACTACAA-3' and ThrR: 5'-ACCTCCGATCTTCGGATTACAAGACCG-3' (Machordom and Doadrio, 2001). Double-stranded DNA was amplified in 25–50 μ l reactions [1 \times buffer, 1.5 μ M MgCl₂, 0.5 mM of each primer, 0.2 μ M of each dNTP, 1 μ l template DNA, and 1U Taq polymerase (Sileks, Moscow)]. PCR was performed at 94°C of initial denaturation for 2 min, followed by 30 cycles at 94°C (45 s), 48°C (1 min), 72°C (1 min 30 s), and a final extension at 72°C (5 min). PCR products were visualized on 1.5% agarose gels and later purified using ethanol. Both strands were sequenced on the Applied Biosystems 3500 DNA sequencer following the manufacturer's instructions.

Nucleotide sequences were initially aligned using Clustal X (Thompson *et al.*, 1997) and then adjusted manually against previously published *cyt-b* of *Labeobarbus* (Tsigenopoulos *et al.*, 2010). The tetraploid European barbel *Barbus meridionalis* (AF045997) was selected as outgroups.

A subset of the *Labeobarbus cyt-b* sequences from the study of Beshera and Harris (2014) was used as a comparative material. Combined set of our and GenBank sequences comprised 108 individuals including one sequence of an outgroup. Only unique haplotypes were used for subsequent analysis (67 sequences), which were detected using soft DnaSP v.5.10 (Librado and Rozas, 2009). The transition (ti)/transversion (tv) rate for set of unique haplotypes was estimated using a maximum-likelihood approach (*cyt b* ti/tv = 7.89). Base frequencies were as follows: A = 0.299, C = 0.282, G = 0.132, and T = 0.287. There was no evidence of saturation for data set of sequences.

Set of the unique haplotypes (n = 67) with no missed data was used for phylogenetic analysis. We determined the best-fit models of nucleotide substitution using jModelTest 2.1.7 (Posada, 2009) by corrected Akaike Information Criterion (AICc). Bayesian phylogenetic inference (BI) was performed using MrBayes v.3.2.6 (Ronquist and Huelsenbeck, 2003). Two simultaneous runs with four Markov chains each were run under the TIM1+I+G model for 2.5×10^6 generations and sampled every 500

generations. The first 25% of generations were discarded as burn-in. Convergence of runs was assessed by examination of the average standard deviation of split frequencies and the potential scale reduction factor. The phylogenetic tree resulting in BI analysis was visualized and edited using FigTree v.1.4 (Rambaut, 2008).

Morphological samples from Lake Langano were obtained in 2004, 2008 and 2009. Comparative morphological material was collected from the following localities in 2004-2012: the Awash River at Sodore (8°24' N, 39°24' E); the Didessa River, a tributary of the Blue Nile (07°30' N 37°44' E); the Gojeb River, a tributary of the Omo (7°15' N, 36°48' E); Lake Hawassa (fish market at the City of Hawassa); the Raya River, a tributary of Lake Abaya (6°28' N, 37°44' E); the Sile (Seli) River, a tributary of Lake Chamo (5°54' N, 37°30' E); the Sago (Sego) River, a tributary of Lake Chamo (5°53' N, 37°25' E). Fish were caught with cast and gill nets or purchased from local fishermen. Following Banister (1973, p. 5), “the lateral line scale count was taken from the first pore-bearing scale behind the head to the scale lying lateral to the end of the hypurals”. Additional comparative morphological data were taken from Banister’s (1973, fig. 52) histograms of distribution of the lateral line scale counts in nine populations of *L. intermedius*.

Statistical analysis of the data was performed using Statistica® software (Statsoft Inc., ver. 8.0). Due to small or uneven sample sizes, non-parametric Mann-Whitney U-test was used to analyze differences in lateral line scale counts.

RESULTS

Bayesian analysis of cytochrome *b* sequences clearly demonstrates that two specimens of *L. ethiopicus* from the Meki R., a tributary of Zway Lake, as well as one specimen of *L. intermedius* from Lake Langano cluster together with GenBank sequence of *L. ethiopicus* (Fig. 2). Moreover, they share the same haplotype. We have to stress that a cytochrome *b* sequence of *L. ethiopicus* deposited to GenBank (Accession number AF180828) by Tsigenopoulos *et al.* (2002) is not from Lake Tana as indicated by authors of this publication. This is a result of mislabeling. We believe that this sample was collected by one of the authors of present study (ASG) from the Meki River, a tributary of Lake Zway in March 1996 and passed to Dr. V. Šlechta (Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic). Further handling of this sample was out of our responsibility.

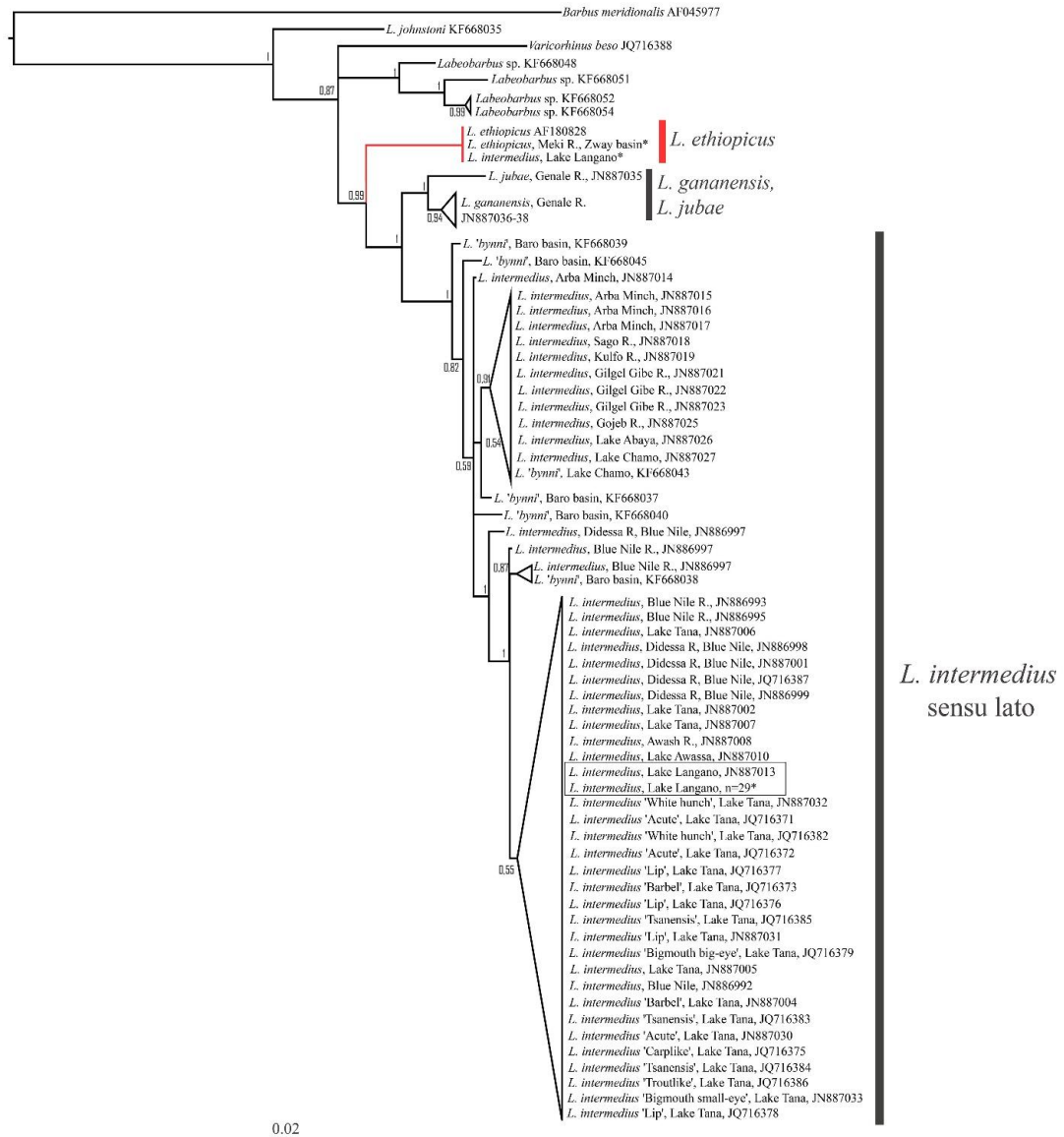


Fig. 2. Phylogenetic tree of the large African barbs from Ethiopia rendered by Bayesian analysis of the mitochondrial cytochrome *b* data set (964 base pairs). Numbers above branches mean posterior probabilities of Bayesian Inference (only values larger than 0.50 are shown). Our samples are marked by asterisk. Specimens from Lake Langano are nested inside the poorly structured assemblage of *L. intermedius* sensu Banister (1973) and bordered by square. One specimen of *L. intermedius* from Lake Langano is clustered together with *L. ethiopicus* from Lake Zway basin. The scale bar represents 2% estimated sequence divergence.

The remaining 29 specimens of *L. intermedius* from Lake Langano share the same cytochrome *b* haplotype and cluster within the poorly solved assemblage including *L. intermedius* from different Ethiopian populations and some *L. 'bynni'* sensu Banister (1973). Despite poor resolution of its internal structure, the monophyly of this barb assemblage from the Ethiopian Rift Valley and western Ethiopia is well supported (posterior probability = 1). The sister group of this assemblage are the barbs from south-eastern Ethiopia (the Indian Ocean basin) including *L. jubae* formerly considered within the genus *Varicorhinus* by Banister (1984). Its affiliation with the monophyletic assemblage of *Labeobarbus* from south-eastern Ethiopia (the Indian Ocean basin) has been recently demonstrated by Levin *et al.* (2013). *Labeobarbus ethiopicus* plus the unique haplotype of *L. intermedius* from Lake Langano is a sister group to combined western and south-eastern assemblages of *Labeobarbus* of Ethiopia (Fig. 2).

DISCUSSION

Presence of the *L. ethiopicus* mtDNA haplotype in Lake Langano is an evidence of introgressive hybridization between *L. intermedius*, the widely distributed and highly variable species (Banister, 1973), and the narrow range endemic *L. ethiopicus*. Unfortunately, we had no possibility directly to trace the effects of this hybridization on the nuclear hexaploid genome because corresponding markers for *L. ethiopicus* were not yet developed. We may look, however, at the morphological data. It is noteworthy, that the specimen with the unique *L. ethiopicus* haplotype from Lake Langano has increased number of the lateral line scales (36 and 35 at the left and right sides, respectively) compared to the average value (32.57) in other Langano barbs (Table 1). In general, the Langano barbs have highest average number of the lateral line scales among 18 *L. intermedius* populations from Ethiopia and Kenya presented in Table 1 and ranged from the highest to lowest values of the character. The upper limit (38 scales) is also highest in our sample from Lake Langano compared to remaining 17 populations presented in Table 1. Moreover, we have to note that Zolezzi (1939) reported 39–40 scales in his *Barbus platystomus* var. *vatovae* from Lake Langano, but his method of scale counting (the total number of pore-bearing scales) should give the values higher for 2–3 scales compared to Banister's (1973) method used by us. Thus, the Langano barbs are characterized by increased average and maximum values of the lateral line scale counts compared to all other *L. intermedius* populations under this study.

Table 1. Variation of the lateral line scale counts in the Ethiopian and Kenyan populations of *Labeobarbus intermedius* sensu Banister (1973); n – sample size.

Nos	Location	n	Mean \pm SD	Limits	Source
1	L. Langano	54	32.57 \pm 1.61	29-38	This study
2	L. Koka (R. Awash basin)	12	31.75 \pm 1.36	30-34	This study
3	L. Hawassa	35	31.69 \pm 1.21	29-34	This study
4	L. Zway	30	31.61 \pm 1.33	29-35	Banister, 1973
5	R. Awash	10	30.30 \pm 1.34	28-32	This study
6	R. Raya (L. Abaya basin)	17	30.29 \pm 1.83	27-34	This study
7	R. Awash	32	30.19 \pm 1.55	27-34	Banister, 1973
8	L. Tana	181	30.18 \pm 1.67	26-34	Banister, 1973
9	R. Wabi Shebele	23	29.87 \pm 1.14	27-32	Banister, 1973
10	R. Sile (L. Chamo basin)	10	29.56 \pm 1.94	27-32	This study
11	L. Baringo	53	29.51 \pm 1.55	27-33	Banister, 1973
12	R. Didessa (a tributary of the Blue Nile)	27	29.44 \pm 1.67	27-34	This study
13	R. Blue Nile	28	29.21 \pm 1.57	26-36	Banister, 1973
14	L. Chew Bahir	30	29.20 \pm 1.54	26-33	Banister, 1973
15	L. Abaya	46	28.74 \pm 1.41	26-32	Banister, 1973
16	R. Gojeb (a tributary of R. Omo)	50	27.90 \pm 1.36	25-32	This study
17	R. Omo	17	27.76 \pm 1.15	26-30	Banister, 1973
18	R. Sago (L. Chamo basin)	10	26.90 \pm 2.42	24-32	This study

It is interesting that in Lake Zway, where the population of *L. intermedius* occurs sympatric with *L. ethiopicus* and where hybridization between these species was possible at least a century ago (judging from the presence of *Barbus microterolepis* described by Boulenger in 1902), the average value of lateral line scale counts is moderate compared to other *L. intermedius* populations from the central part of the Ethiopian Rift Valley. The difference between our Langano sample (n = 54) and the combined samples from the central part of the Ethiopian Rift Valley including the Hawassa, Zway, Koka and two Awash samples (ours and Banister's ones) (n = 120) is highly significant (Mann-Whitney U test $Z = 5.11$, $p < 0.0001$). In its turn, the difference between these combined samples from the central part of the Ethiopian Rift Valley excluding the Langano sample (n = 120) and the remaining Ethiopian and Kenyan samples of *L. intermedius* (n = 491) is also highly significant (Mann-Whitney U test $Z = 9.23$, $p < 0.0001$). Let us examine to what extent the observed geographic differences in lateral line scale counts may be explained by the introgressive hybridization between *L. ethiopicus* and some populations of *L. intermedius*. First, however, we need to return to modern geography and paleogeography of the central part of the Ethiopian Rift Valley.

Crow flight distance between Lake Zway and Langano is about 16 km, but there is the volcanic mountain Alutu between them. The Bulbula River (about 40 km in length) flows from Lake Zway into Lake Abijata, while

Lake Langano discharges into the same lake via the Hurokalu stream (about 12 km in length). Lake Abijata is saline and alkaline (salinity > 18 g/l and pH = 9.85) according to Elizabeth Kebede *et al.* (1994) and Dagnachew Legesse *et al.* (2004). Barb migration through this lake is apparently impossible under the current conditions. However, four other lakes (Zway, Langano, Abijata and Shala) are remnants of a large fresh-water lake that existed several times during the Late Pleistocene/Early Holocene humid periods (Grove *et al.*, 1975; Gasse and Street, 1978). During these periods, including the last one (about 5000–2500 years B.P., Benvenuti *et al.*, 2002), the range of *L. ethiopicus* (currently endemic to the Lake Zway basin) could have been wider including the whole large palaeolake basin and possibly the upper reaches of the Awash drainage. The separation of Lake Zway from southern lakes occurred 2500–2000 years B.P. (Benvenuti *et al.*, 2002). The gene flow between the barb populations of lakes Zway and Langano might exist much longer, until the formation of extreme water salinity in Lake Abijata. Thus, we suggest the past introgressive hybridization between *L. ethiopicus* and *L. intermedius* evidenced by the presence of the unique mtDNA haplotype in Lake Langano. Localization of the hybridization event is impossible, but it is clear that the palaeoclimatic oscillations could enhance the gene flow between the Langano and Zway basins.

It is likely that the increased number of lateral line scales in the Langano population of *L. intermedius* is a result of nuclear gene flow from *L. ethiopicus* to the former species. The increased number of lateral line scales in the Langano population of *L. intermedius* compared to the Zway population of this species (for which the hybridization with *L. ethiopicus* is currently possible) could be explained in the following way. Prezygotic isolation between the two barb species still exist in the Zway basin that allows a separate reproduction of the two species. At the same time, the genetic swamping provoked by a reduction of the population number of *L. ethiopicus* (as it is demonstrated for the European barbels - Lajbner *et al.*, 2009; Meraner *et al.*, 2013) might take place somewhere in the large paleolake basin after the decrease of water level and formation of semi-isolated smaller lake basins, particularly the Langano protobasin.

The increased number of lateral line scales in the *L. intermedius* populations from the central part of the Ethiopian Rift Valley, in general, may also be a result of the gene exchange with *L. ethiopicus*. In light of lack of direct genetic evidence of hybridization, however, the other reasons (such as environmentally mediated selection by increased water mineralization or peculiarities of phylogeny and ancestor genetics) could be equally plausible

explanations for this phenomenon.

The introgressive hybridization between the European evolutionary tetraploid ($2n = 100$) barbels (the genus *Barbus* sensu stricto) is rather well studied. The introgression was reported between *B. meridionalis* and *B. haasi* in Spain (Machordom *et al.*, 1990), *B. barbatus* and *B. meridionalis* in France (Berrebi *et al.*, 1993), *B. barbatus* and *B. carpathicus* in Slovakia (Lajbner *et al.*, 2009), *B. barbatus* and *B. plebejus* in Italy (Meraner *et al.*, 2013). In some cases, the asymmetrical hybridization with disproportionate contribution of *B. barbatus* mothers was detected (Lajbner *et al.*, 2009; Meraner *et al.*, 2013). Moreover, in case of *B. barbatus* and *B. carpathicus*, no F2 progeny or backcrosses to *B. carpathicus* were found (Lajbner *et al.*, 2009).

We detected matrilineal DNA of *L. ethiopicus* in phenotype of *L. Intermedius*, that means that ‘mother species’ for the remote hybridization event was *L. ethiopicus*. However, the larger species among the European barbels more often become ‘mother species’ (Meraner *et al.*, 2013). Previously, the maximum standard length recorded for *L. ethiopicus* was 269 mm (Golubtsov *et al.*, 2002). In December 24 of 2005, nine live specimens for the National Fishery and Other Aquatic Life Research Center in Sebeta were collected from the Meki River at the bridge along the road Addis Ababa-Butajira, their standard length ranged from 158 to 320 mm. Even with new recorded maximum length (320 mm), *L. ethiopicus* is smaller compared to *L. intermedius* (maximum standard length more than 500 mm). In the European barbels, females are larger than males and spawning fish form small groups including a female and several males (Poncin *et al.*, 1994). The same is true for the Ethiopian barbels (Alekseyev *et al.*, 1996; Dzerzhinskii *et al.*, 2007). The larger female of *L. intermedius* might accept small *L. ethiopicus* males, while a large *L. intermedius* male might be rejected by a small *L. ethiopicus* female. Additional reason for asymmetrical hybridization is unequal female fertility of hybridizing species. Larger cyprinid species usually have larger fecundity. The fecundity of *L. ethiopicus* is unknown, while the mean fecundity of *L. intermedius* from tributaries of Lake Tana was assessed as 3588 eggs (ranged between 1761 and 8367 eggs) per female (Wassie Anteneh *et al.*, 2007). For both these reasons, in the case of hybridization between *L. ethiopicus* and *L. intermedius* the nuclear gene input of the former species (via males) in hybrid progeny should be higher than it is indicated by mtDNA exchange.

Available data on the hybridization between the two *Labeobarbus* species are very limited. Nevertheless, we obtained the first genetic evidence of hybridization between the large African barbs in the course of normal bisexual reproduction (but see Yang *et al.*, 2015 for the cases of hybrid speciation with changes of ploidy level). Unfortunately, we have no genetic materials from the Lake Zway barbs that may elucidate the issue of hybridization between the two local *Labeobarbus* species. Decline of the barb population numbers hampers obtaining such materials. The small-scaled Ethiopian barb *L. ethiopicus* is already an endangered species (Golubtsov *et al.*, 2004; <http://www.iucnredlist.org>). The genetic swamping with *L. intermedius* might be an additional threat to this species, as suggested for some European barbels (Lajbner *et al.*, 2009; Meraner *et al.*, 2013). Even the situation with the Langanos barbs is troublesome. On March 31, 2016, while visiting the landing sites at the north-eastern shore of Lake Langanos, we did not find any large barbs in the catch. According to information from the local fishermen, *Labeobarbus* have practically disappeared from this part of the lake.

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REFERENCES

- Alekseyev, S.S. (1994). The role of heterochrony in the formation of morphological differences of “large” and “small” African barbs (*Barbus*, Cyprinidae) (Russian-Ethiopian investigations of Ethiopian fauna). *J. Ichthyol.* **34**: 119–141.
- Alekseyev, S.S., Mina, M.V., Dgebuadze, Y. and Mironovski, A.N. (1996). Small “large barbs” spawning in tributaries of Lake Tana: What are they? *Folia Zool.* **45**

- (suppl.1): 85–96.
- Aljanabi, S.M. and Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res.* **25**(22): 4692–4693.
- Banister, K.E. (1973). A revision of the large *Barbus* (Pisces, Cyprinidae) of East and Central Africa. Part II. *Bull. Br. Mus. Nat. Hist. (Zool.)* **26**: 1–148.
- Banister, K.E. (1984). Three new species of *Varicorhinus* (Pisces, Cyprinidae) from Africa. *Bull. Br. Mus. Nat. Hist. (Zool.)* **47**: 273–282.
- Baxter, R.M. (2002). Lake morphology and chemistry. In: **Ethiopian Rift Valley Lakes**, pp. 45–60 (Tudorancea, C. and Taylor, W.D., eds.). Backhuys Publishers, Leiden.
- Benvenuti, M., Carnicelli, S., Belluomini, G., Dainelli, N., Di Grazia, S., Ferrari, G.A., Iasio, C., Sagri, M., Ventra, D., Balemwald, A. and Kebede, S. (2002). The Ziway–Shala lake basin (main Ethiopian rift, Ethiopia): a revision of basin evolution with special reference to the Late Quaternary. *J. East Afr. Earth Sci.* **35**: 247–269.
- Berrebi, P., Cattaneo-Berrebi, G. and Le Brun, N. (1993). Natural hybridization of two species of tetraploid barbels: *Barbus meridionalis* and *Barbus barbus* (Osteichthyes, Cyprinidae) in southern France. *Biol. J. Linn. Soc.* **48**: 319–333.
- Beshera, K.A. and Harris, P.M. (2014). Mitochondrial DNA phylogeography of *Labeobarbus intermedius* complex (Pisces, Cyprinidae) from Ethiopia. *J. Fish Biol.* **85**: 228–245.
- Beshera, K.A., Harris, P.M. and Mayden R.L. (2016). Novel evolutionary lineages in *Labeobarbus* (Cypriniformes; Cyprinidae) based on phylogenetic analyses of mtDNA sequences. *Zootaxa* **4093**(3): 363–381.
- Borkenhagen, K. (2014). A new genus and species of cyprinid fish (Actinopterygii, Cyprinidae) from the Arabian Peninsula, and its phylogenetic and zoogeographic affinities. *Environ. Biol. Fish.* **97**: 1179–1195.
- Boulenger, G.A. (1902). Description of new fishes from collection made by Mr. E. Degen in Abyssinia. *Ann. Mag. Nat. Hist. (Ser. 7)* **10** (60): 421–437.
- Boulenger, G.A. (1906). On a collection of fishes from Gallaland. *Ann. Mag. Nat. Hist. (Ser. 7)* **17** (102): 557–566.
- Dagnachew Legesse, Vallet-Coulomb, C. and Gasse, F. (2004). Analysis of the hydrological response of a tropical terminal lake, Lake Abiyata (main Ethiopian Rift Valley) to changes in climate and human activities. *Hydrol. Process.* **18**: 487–504.
- de Graaf, M., Eshete Dejen, Osse, J.W.M. and Sibbing, F.A. (2008). Adaptive radiation of Lake Tana's (Ethiopia) *Labeobarbus* species flock (Pisces, Cyprinidae). *Mar. Freshwater Res.* **59**: 391–407.
- de Graaf, M., Megens, H.-J., Samallo, J. and Sibbing, F. (2010). Preliminary insight into the age and origin of the *Labeobarbus* fish species flock from Lake Tana (Ethiopia) using the mtDNA cytochrome *b* gene. *Mol. Phylogenet. Evol.* **54**: 336–343.
- Demeke Admassu and Elias Dadebo (1997). Diet composition, length-weight relationship and condition factor of *Barbus* species Rüppell, 1836 (Pisces: Cyprinidae) in Lake Hawassa, Ethiopia. *SINET: Ethiop. J. Sci.* **13**: 83–96.
- De Vos, L., Snoeks, J. and Thys van den Audenaerde, D.F.E. (2001). An annotated checklist of the fishes of Rwanda (East Central Africa), with historical data on introductions of commercially important species. *J. E. Afr. Nat. Hist.* **90**: 41–68.
- Dzerzhinskii, K.F., Shkil, F.N., Belay Abdissa, Wondie Zelalem and Mina, M.V. (2007).

- Spawning of large *Barbus* (*Barbus intermedius* complex) in a small river of the Lake Tana basin (Ethiopia) and relationships of some putative species. *J. Ichthyol.* **47**: 639–646.
- Elizabeth Kebede, Zinabu Gebremariam and Ahlgren, I. (1994). The Ethiopian Rift Valley lakes: chemical characteristics of a salinity-alkalinity series. *Hydrobiologia* **288**: 1–12.
- Eschmeyer, W.N., Fricke, R. and Van der Laan, R. (eds.). (2017). Catalogue of Fishes: Genera, species, references. (<http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>). Electronic version updated 30 March 2017.
- Gasse, E. and Street, F.A. (1978). Late Quaternary lake-level fluctuations and environments of the northern Rift Valley and Afar region (Ethiopia and Djibouti). *Palaeogeogr. Palaeoclim.* **24**: 279–325.
- Golubtsov, A.S., Dgebuadze, Y.Y. and Mina, M.V. (2002). Fishes of the Ethiopian Rift Valley. In: **Ethiopian Rift Valley Lakes**, pp. 167–258 (Tudorancea, C. and Taylor, W.D., eds.). Backhuys Publishers, Leiden.
- Golubtsov, A.S., Dimmick, W.W. and Redeat Habteselassie (2004). Threatened fishes of the world: *Barbus ethiopicus* Zolezzi, 1939 (Cyprinidae). *Env. Biol. Fish.* **70**: 66.
- Golubtsov, A.S. and Krysanov, E.Y. (1993). Karyological study of some cyprinid species from Ethiopia. The ploidy differences between large and small *Barbus* of Africa. *J. Fish Biol.* **42**: 445–455.
- Grove, A.T., Alayne Street, F. and Goudie, A.S. (1975). Former lake levels and climatic change in the Rift Valley of southern Ethiopia. *Geogr. J.* **141**: 177–202.
- Lajbner, Z., Šlechtová, V., Šlechtá, V., Švátora, M., Berrebi, P. and Kotlík, P. (2009). Rare and asymmetrical hybridization of the endemic *Barbus carpathicus* with its widespread congener *Barbus barbus*. *J. Fish Biol.* **74**: 418–436.
- Lemma Abera, Abebe Getahun and Brook Lemma (2014). Composition of commercially important fish species and some perspectives into the biology of the African catfish *Clarias gariepinus* (Burchell), Lake Ziway, Ethiopia. *Int. J. Adv. Res.* **2**: 864–871.
- Levin, B.A., Golubtsov, A.S., Dgebuadze, Y.Y. and Mugue, N.S. (2013). New evidence of homoplasmy within the African genus *Varicorhinus* (Cyprinidae): an independent origin of specialized scraping forms in the adjacent drainage systems of Ethiopia inferred from mtDNA analysis. *Afr. Zool.* **48**: 400–406.
- Librado, P. and Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451–1452.
- Machordom, A., Berrebi, P. and Doadrio, I. (1990). Spanish barbel hybridization detected using enzymatic markers: *Barbus meridionalis* Risso × *Barbus haasi* Mertens (Osteichthyes, Cyprinidae). *Aquat. Living Resour.* **3**: 295–303.
- Machordom, A. and Doadrio, I. (2001). Evolutionary history and speciation modes in the cyprinid genus *Barbus*. *Proc. R. Soc. Lond. B: Biol. Sci.* **268**: 1297–1306.
- Meraner, A.S., Venturi, A., Ficaretola, G.F., Rossi, S., Candiotta, A. and Gandolfi, A. (2013). Massive invasion of exotic *Barbus barbus* and introgressive hybridization with endemic *Barbus plebejus* in Northern Italy: where, how and why? *Mol. Ecol.* **22**: 5295–5312.
- Nagelkerke, L.A.J. and Sibbing, F.A. (1997). A revision of the large barbs (*Barbus* spp., Cyprinidae, Teleostei) of Lake Tana, Ethiopia, with a description of seven new species. In: **The Barbs of Lake Tana, Ethiopia: Morphological Diversity and its Implications for Taxonomy, Trophic Resource Partitioning, and Fisheries**.

- (Nagelkerke, L.A.J., ed.). Published Doctoral Dissertation. Wageningen Agricultural University, Wageningen, The Netherlands.
- Nagelkerke, L.A.J. and Sibbing, F.A. (2000). The large barbs (*Barbus* spp., Cyprinidae, Teleostei) of Lake Tana (Ethiopia), with a description of a new species, *Barbus osseensis*. *Neth. J. Zool.* **50**: 179–214.
- Palumbi, S.R. (1996). Nucleic acids II: the polymerase chain reaction. *Mol. Syst.* **2**: 205–247.
- Perdices, A. and Doadrio, I. (2001). The molecular systematics and biogeography of the European cobitids based on mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* **19**: 468–478.
- Poncin, P., Jeandarme, J. and Berrebi, P. (1994) A behavioural study of hybridization between *Barbus barbus* and *Barbus meridionalis*. *J. Fish Biol.* **45**: 447–451.
- Posada, D. (2009). Selection of models of DNA evolution with jModeltest. In: **Bioinformatics for DNA sequence analysis**, pp. 93–112 (Posada, D., ed.). Humana Press, New York.
- Rambaut, A. (2008). FigTree v1.4: tree figure drawing tool [available at <http://tree.bio.ed.ac.uk/software/figtree/>]
- Redeat Habteselassie and Kassahun Asaminew (2008). The current status of an endemic fish species (*Barbus ethiopicus*, Zolezzi 1939) in Lake Ziway basin, Ethiopia. In: **Ecological and Faunistic Studies in Ethiopia**. Proceedings of Jubilee Meeting ‘Joint Ethio-Russian Biological Expedition: 20 Years of Scientific Cooperation’, Addis Ababa, February 21–23, 2007, pp. 20–29 (Pavlov, D.S., Dgebuadze, Y.Y., Darkov, A.A., Golubtsov, A.S. and Mina, M.V., eds.). KMK Scientific Press, Moscow.
- Ronquist, F. and Huelsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- Rüppell, E. (1836). “Neuer Nachtrag von Beschreibungen und Abbildungen neuer Fische im Nil entdeckt”, Frankfurt am Main, Mus. Senckenberg., Abhandlungen aus dem Gebiete der beschreibenden Naturgeschichte, **2**(1): 1–28.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**: 4876–4882.
- Tsigenopoulos, C.S., Kasapidis, P. and Berrebi, P. (2010). Phylogenetic relationships of hexaploid large-sized barbs (genus *Labeobarbus*, Cyprinidae) based on mtDNA data. *Mol. Phylogenet. Evol.* **56**: 851–856.
- Tsigenopoulos, C.S., Ráb, P., Naran, D. and Berrebi, P. (2002). Multiple origins of polyploidy in the phylogeny of southern African barbs (Cyprinidae) as inferred from mtDNA markers. *Heredity* **88**: 466–473.
- Vreven, E.J., Musschoot, T., Snoeks, J. and Schlieven, U.K. (2016). The African hexaploid Torini (Cypriniformes: Cyprinidae): review of a tumultuous history. *Zool. J. Linn. Soc.* **177**: 231–305.
- Wassie Anteneh, Abebe Getahun and Eshete Dejen (2007). Reproductive biology of the fish *Labeobarbus intermedius* in the Dirma and Megech tributary rivers of Lake Tana (Ethiopia). *Eth. J. Sci. Tech.* **4**: 13–26.
- Yang, L., Sado, T., Hirt, M.V., Pasco-Viel, E., Arunachalam, M., Li, J., Wang, X., Freyhof, J., Saitoh, K., Simons, A.M., Miya, M., He, S. and Mayden, R.L. (2015). Phylogeny and polyploidy: Resolving the classification of cyprinine fishes (Teleostei: Cypriniformes). *Mol. Phylogenet. Evol.* **85**: 97–116.

Zolezzi, G. (1939). Descrizione di tre nuovi Ciprinidi raccolti dalla Missione Ittiologica in A.O.I. Boll. *Pesca Piscic. Idrobiol.* **15**: 369-373.