New evidence of homoplasy within the African genus *Varicorhinus* (Cyprinidae): an independent origin of specialized scraping forms in the adjacent drainage systems of Ethiopia inferred from mtDNA analysis

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Received 9 April 2013. Accepted 4 August 2013

Interrelationships of the two specialized scraping periphyton-feeders, *Varicorhinus beso* and *V. jubae*, and some large African barbs, *Labeobarbus* spp., inhabiting three main regions of Ethiopia (the Western and Eastern Plateaus, and the Rift Valley separating them) were investigated using the sequence analysis of a fragment (609 base pairs) of the mtDNA control region. The two scraping forms in question appeared to be phylogenetically distant: *V. beso* had branched off before the main radiation of the Ethiopian *Labeobarbus* took place, whereas *V. jubae* is a sister group of the *Labeobarbus gananensis* complex sympatrically occurring with it in the south of the Eastern Plateau. For geographical reasons, among the congeneric species, *V. jubae* could be considered as the most closely related to *V. beso*, the type species of the genus, but judging from the available data this genus seems to be monotypic, while *jubae* should be classified as a member of *Labeobarbus*.

Key words: Cyprinidae, parallel evolution, scraping feeder, polyphyly, *Varicorhinus*, Africa.

The scraping periphyton-feeders with specialized morphological features (the widened straight mouth, horny edge of the lower jaw and some others) evolved in several cyprinid lineages. In some of them (such as the Asian genera *Capoeta*, *Cyprinion*, *Onychostoma*, *Scaphiodonichthys,* and *Semiplotus*), all the species are specialized periphyton-feeders, in others (such as the Asian *Diptychus*, *Schizocypris*, *Schizopygopsis, Schizothorax, Poropuntius*, and African *Labeobarbus*) only some species exhibit this specialization. It is clear that these numerous cases of trophic and morphological similarity resulted from the parallel evolution.

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For a long time, the chiselmouths of the genus *Varicorhinus* Rüppell (1835) were considered as an assemblage of African scraping periphyton-feeders occurring in different parts of the continent but sharing the trophic specialization and corresponding morphological adaptations based on a common ancestry (Boulenger 1909–1916; Lévêque & Daget 1984; Poll & Gosse 1995; Tweddle & Skelton 1998). Some morphological observations, however, call into question the monophyletic nature of the group (Banister 1972, 1984; Banister & Bailey 1979), while recent molecular data (Tsigenopoulos *et al*. 2010) have clearly demonstrated an independent origin of at least some '*Varicorhinus*' species from South, West and North Africa. At the same time, these molecular data support the karyological findings (Golubtsov & Krysanov 1993; Guégan *et al*. 1995; Krysanov & Golubtsov 1996) that at least some species affiliated with the genus *Varicorhinus* belong to the lineage of African evolutionary hexaploid barbins composed mostly of the large African barbs currently assigned to the genus *Labeobarbus* (Skelton 2001).

The genus *Varicorhinus,* established by Rüppell (1835) for *V. beso* (Fig. 1a) from the upper reaches of the Blue Nile system, includes up to 36 valid species according to *CLOFFA 1* (Lévêque & Daget 1984) and the *Catalog of Fishes* (Eschmeyer 1998, updated version http://researcharchive.calacademy.org/research/Ichthyology/catalog/fishcatmain.asp). *Varicorhinus jubae* (Fig. 1b) was described by Banister (1984) from the Juba River system in southeastern Ethiopia (the catchment area of the Indian Ocean). Among the congeneric species, it has the range

Fig. 1. **a**, *Varicorhinus beso*, SL 206 mm, from the Didessa River drainage, the Blue Nile system in the Atlantic Ocean catchment; **b**, '*Varicorhinus' jubae*, SL 243 mm, from the Genale River, the Juba system in the Indian Ocean catchment: lateral view of the freshly caught specimens and bottom view of their heads with the horny cutting edge on the lower jaw (arrowed).

nearest to that of the type species of the genus. No other *Varicorhinus* species currently recognized as valid (Lévêque 1990; Tsigenopoulos *et al*. 2010) are known in the Nilo-Sudan ichthyofaunal province as defined by Roberts (1975). It is reasonable to assume that if there is a clade represented by *V. beso* and other related species, the most probable sister group of *V. beso* is *V. jubae*.

The present work was aimed at clarification of the phylogenetic interrelationships of *V. beso*, *V. jubae* and other Ethiopian evolutionary hexaploid barbins using mtDNA sequence data and using the European barbel *Barbus meridionalis* Risso, 1827, the evolutionary tetraploid, as an outgroup.

Material was collected in the three main geographical regions of Ethiopia (Fig. 2) in 2008–2012: *V. beso* and *Labeobarbus intermedius* (Rüppell 1835) sensu Banister (1973) from the Western Plateau and Ethiopian Rift Valley; *V. jubae* and *Labeobarbus gananensis* (Vinciguerra 1895) complex from the Eastern Plateau; and *Labeobarbus ethiopicus* (Zolezzi 1939), endemic to the Lake Ziway basin, from the Ethiopian Rift Valley (Fig. 2). Sampling localities and the GenBank accession numbers for DNA sequences are indicated in Table 1. Voucher specimens were deposited at the Zoological Museum of Moscow State University (ZMMU), Moscow. DNA samples from 146 specimens of *Varicorhinus* and *Labeobarbus* were sequenced, while the data on a specimen of *Barbus meridionalis* (Gilles *et al.* 2001, No. AJ388416) and eight specimens of *L. gananensis* complex (Dimmick *et al*. 2001) were taken from the GenBank sequence database.

DNA was extracted from a small piece of the fin or muscle using the salt method (Aljanabi & Martinez 1997). For all individuals studied, a fragment of the mtDNA control region was PCR amplified with the primers LProF (5′-AACTCTCA CCCCTAGCTCCCAAAG-3′) (Meyer *et al.* 1994) and DL623 (5′-GGAATAGATATGTTATGC ACTTG-3′) (designed for this study). Double-stranded DNA was amplified in 25 μl reactions (2 μl 1x Hot buffer, 2 μM $MgCl_2$, 0.25 mm of each primer, 0.2 μM dNTP of each nucleotide, 18.5 μl ddH_20, 1 μl template DNA, and 1U Hot Taq polymerase (Sileks, Moscow). The PCR process was 95°C (10 min), 30 cycles at 95°C (20 s), 52°C (30 s), 72°C (1 min), and a final extension at 72°C (5 min 30 s). The PCR products were visualized by mini-gel electrophoresis using ethidium bromide staining and 1.5% agarose gels. The PCR products were purified with double volume of ethanol 96% and sodium acetate (3M) precipitated after 10 min centrifugation at 13200 rpm, and centrifuged twice with 70% alcohol. Both strands were sequenced on Applied Biosystems 3100 DNA sequencer following the manufacturer's instructions. The

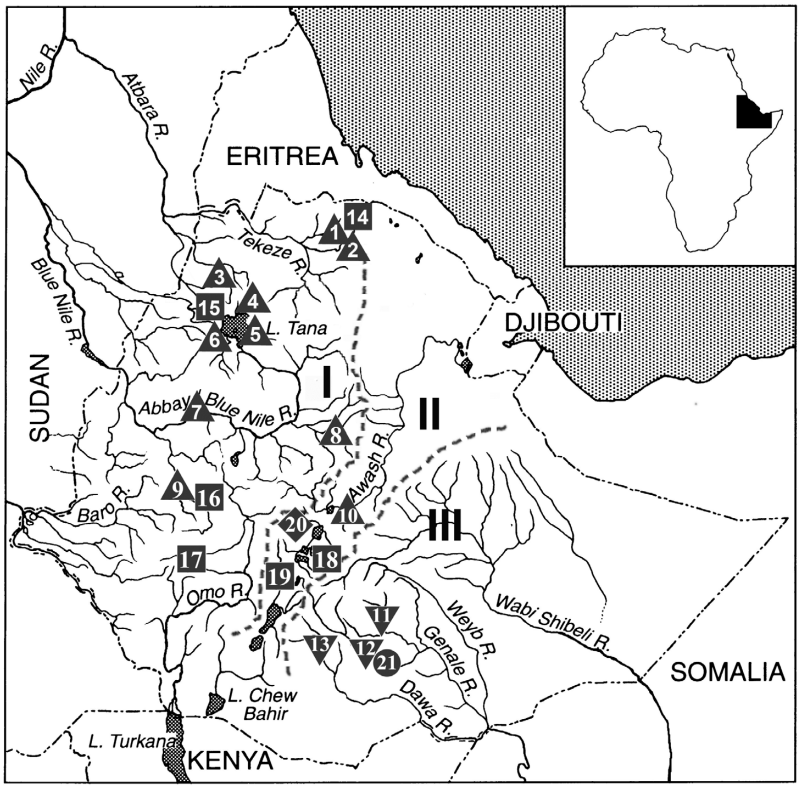

Fig. 2. Schematic drawing of hydrographic network of Ethiopia with three main regions designated: Western Plateau (I), Rift Valley (II, its edges shown with the dashed lines) and Eastern Plateau (III). Sampling localities for *Varicorhinus beso* (▲),'*Varicorhinus*' *jubae* (▼), *Labeobarbus intermedius* complex (■), *Labeobarbus ethiopicus* (◆), and *Labeobarbus gananensis* complex (●) numbered with the same sample codes as in Table 1.

DNA sequences were deposited in GenBank (accession numbers are indicated in Table 1).

Homologous regions were aligned manually against previously published control region sequences of the large African barbs (Dimmick *et al.* 2001). Chromatograms and alignments were visually checked and verified; there were no gaps in the resulting part of control region gene alignment. The Mediterranean barbel *Barbus meridionalis*, a representative of the Eurasian lineage of evolutionary tetraploid barbs (Doadrio *et al.* 2002), was used as an outgroup. The Akaike Information Criterion (AIC) implemented in jModelTest 0.1.1 (Posada 2008) was used to determine the evolutionary model that best fits the data set. The model selected was used for subsequent analyses. Bayesian inference (BI) performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) by simulating two simultaneous Markov chain analyses (MCMC) for 2 700 000 generations each, to estimate the posterior probabilities distribution. The first 4500 trees were discarded as burn-in. Topologies were sampled every 100 generations and a majority-rule consensus tree was estimated after eliminating the first 10^5 generations in each analysis. The tree was constructed using FigTree v.1.2.3.

The part of control region gene (609 base pairs) was sequenced. Base frequencies were A = 0.35, C = 0.17, G = 0.13, T = 0.35. For this marker, 223 characters were variable, 144 sites were parsimony informative (23.65%). The best-fit model selected following the Akaike Information Criterion (AIC) was the TrN+G. Rate matrix was as follows: R(a)[A-C] = 1.15, R(b)[A-G] = 14.77, R(c)[A-T] = 0.98, R(d)[C-G] = 0.94, R(e)[C-T] = 11.56, R(f)[G-T] = 1.00. Among-site variation was approached by the gamma distribution shape parameter (a) = 0.262.

As follows from the phylogram obtained (Fig. 3), *Varicorhinus beso* and '*Varicorhinus*' *jubae* belong to distinctly different lineages. The haplotypes of *V. beso* formed the cluster basal to all other Ethiopian hexaploids studied. Phylogenetic position of this species in relation to other African hexaploids

Table 1. Materials studied from Ethiopia.

Sample no., locality	Number of specimens studied	ZMMU catalogue numbers	GenBank Accession numbers
Varicorhinus beso			
1, Genfel River, Atbara-Tekeze system, 13°46′N, 39°35′E	4	P-23448	KC576931
2, Agula River, Atbara-Tekeze system, 13°41′N, 39°35′E	3	P-23449	KC576932-33
3, Bebew River, tributary of Angereb River, Atbara-Tekeze system,13°10′N, 37°14′E	5	P-23450	KC576930
4, Ribb River, Lake Tana tributary, 11°47′N, 37°41′E	1	P-23451	JQ701748
5, Gumara River, Lake Tana tributary, 11°47′N, 37°41′E	2	P-21990	JQ701744–45
6, Lake Tana at Bahir-Dar, 11°35′N, 37°23′E	3	P-19747	JQ701746–47
7, Dura River, Blue Nile system, 10°59′N, 36°25′E	1	P-23452	JQ701755
8, Chancho (Gorfo) River, Blue Nile system, 9°24′N, 38°50′E	4	P-23453	JQ701750
9, Didessa River, Blue Nile system, 8°41′N, 36°25′E	6	P-21991	JQ701751–54
10, Awash River, Rift Valley, 8°24′N, 39°25′E	4	P-23454	JQ701749
'Varicorhinus' jubae			
11, Welmel River, Juba River system, 6°14′N, 39°49′E	2	P-23455	JQ701761–62
12, Genale River, Juba River system, 5°42′N, 39°32′E	8	P-23456	JQ701763–70
13, Awata River, Juba River system, 5°47′N, 38°56′E	3	P-23457	JQ701758–60
***Labeobarbus intermedius* complex**			
14, Genfel River, Atbara-Tekeze system, 13°46′N, 39°35′E	7	P-23458	JQ701784–85
15, Lake Tana at Bahir-Dar, 11°35′N, 37°23′E	71	ZMMU uncat.	JQ701788–93
16, Didessa River, Blue Nile system, 8°24′N, 36°17′E	4	P-23460	JQ701778–79, JQ701787
17, Gojeb River, Omo-Turkana basin, 7°15′N, 36°48′E	4	P-23461	JQ701774–77
18, Lake Langano, Rift Valley, 7°33′N, 38°41′E	2	P-23462	JQ701786
19, Lake Awassa, Rift Valley, 7°05′N, 38°28′E	2	P-23463	JQ701782–83
Labeobarbus ethiopicus			
20, Meki River, Lake Ziway tributary, Rift Valley, 8°16′N, 38°31′E	5	P-23464	JQ701780–81
***Labeobarbus gananensis* complex**			
21, Genale River, Juba River system, 5°42′N, 39°32′E			
generalized and lipped forms	10	P-23466	AF352842*, AF352843*, AF352858*, AF352859*, AF352860*, JQ701771–73
large-mouthed form	8	P-23467	AF352861*, AF352862*, AF352864*, JQ701756–57

*Obtained from GenBank.

(at the wider geographic scale) was investigated by Tsigenopoulos *et al.* (2010) using the cytochrome *b* sequences. They showed that it was rather ancient but not basal derivate on a tree of the African hexaploids. For the sake of the present consideration, we note merely that *V. beso* had branched off before the radiation of other Ethiopian hexaploids (including the very morphologically distinct *Labeobarbus ethiopicus*) took place.

All haplotypes of '*Varicorhinus*' *jubae* sampled from the three different tributaries of the Juba River in the Eastern Plateau (Fig. 3, Table 1) clustered together. It is noteworthy that previously this species was known only from the type locality, the Genale River (Banister 1984). The *jubae* cluster was a sister group of the *Labeobarbus gananensis* complex reported from the Genale River (Golubtsov 1993; Mina *et al.* 1998; Dimmick *et al.* 2001). Thus, in our tree the hexaploids from the Eastern Plateau appeared to be opposed to all hexaploid barbs studied from the Western Plateau and Ethiopian Rift Valley. Previously discovered divergence between the generalized and lipped forms on the one hand, and the large-mouthed predatory form on the other, within the *L. gananensis* complex (Dimmick *et al.* 2001) was confirmed in the present study with four and five new individuals analysed for the former and latter groups, respectively.

The haplotypes of *Labeobarbus intermedius* sensu Banister (1973) from the endorheic drainages of the Rift Valley, the Omo-Turkana and Nile systems draining the Western Plateau, as well as the representatives of the large barb species flock from Lake

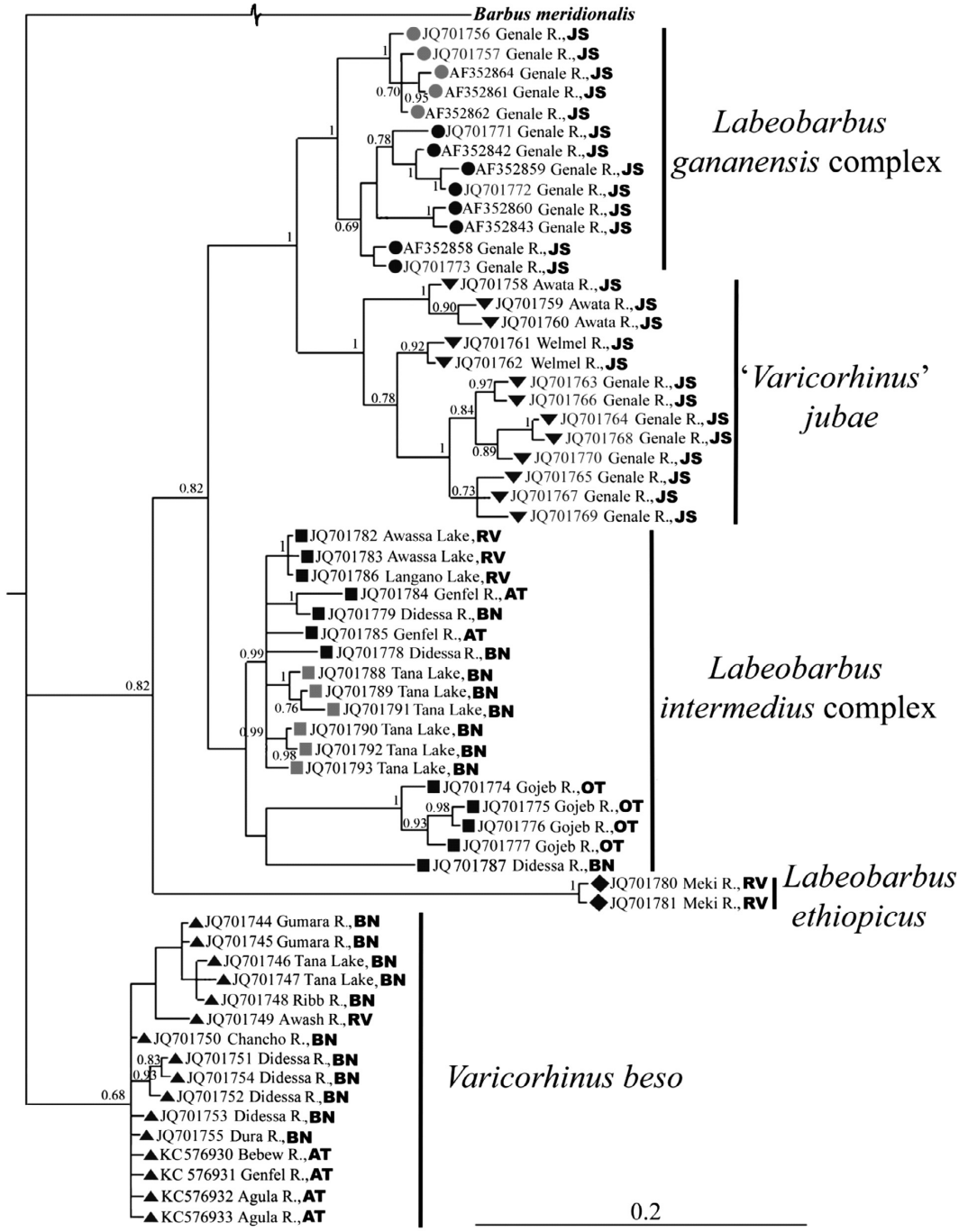

Fig. 3. Phylogenetic tree rendered by Bayesian analysis of the mitochondrial control region data set (609 base pairs). Numbers above branches mean posterior probabilities of Bayesian Inference (only values larger than 0.60 are shown). Terminal branches are marked with pictorial codes of the taxa same as in Fig. 2; after the GenBank number of particular haplotype, the name of source river or lake is given supplemented with the basin code: JS, Juba system (Eastern Plateau); RV, Rift Valley; AT, Atbara-Tekeze; BN, Blue Nile; OT, Omo-Turkana systems (the last three in the Western Plateau). Large-mouthed form within the *Labeobarbus gananensis* complex and different Lake Tana morphotypes within the *Labeobarbus intermedius* complex are indicated with grey symbols. The scale bar represents 20% estimated sequence divergence.

Tana (Nagelkerke *et al.* 1994; Mina *et al.* 1996), produced a cluster whose structure was not completely resolved with the data available. Six mtDNA haplotypes of *Labeobarbus* from Lake Tana mentioned in Table 1 were found in 71 specimens representing *L. acutirostris* (Bini 1940) or 'Acute' morphotype, *L. crassibarbis* (Nagelkerke & Sibbing 1997) or 'Barbel' morphotype, *L. macrophtalmus* (Bini 1940) or 'Bigmouth big-eye' morphotype and not-identifiable 'shore complex' in terms of Nagelkerke & Sibbing (1997) including *Labeobarbus intermedius* (Rüppell 1835). The mtDNA haplotypes were randomly distributed among the Lake Tana species/morphotypes as it was previously demonstrated (De Graaf et al. 2010) for cytochrome *b*. Thorough consideration of the relationships within the *L. intermedius* complex is beyond the scope of this paper. Generally, there was correspondence between the mtDNA sequence similarity and geographic origin of the samples, but it was not complete. The distinct position of *Labeobarbus ethiopicus* matching to its peculiar morphology was already mentioned.

Based on the phylogeny revealed with mtDNA sequences, the scraping form from the Juba River system should be classified as *Labeobarbus jubae* (Banister 1984). Taking into account the independent origin of specialized scraping forms in the adjacent drainage systems of Ethiopia, as well as the morphological (Banister 1972, 1984; Banister & Bailey 1979) and genetic (Tsigenopoulos *et al*. 2010) evidences of independent origin of such forms in the other parts of the African continent, it is reasonable to suggest a monotypy of the genus *Varicorhinus*. It is interesting to contrast the evolutionary history of the apparently polyphyletic African scraping feeders (considered previously as '*Varicorhinus*') to the West-Asian scraping feeders of the genus *Capoeta* comprising about 20 species of monophyletic origin; at least some members of this lineage are evolutionary hexaploids, while the whole group is nested within the evolutionary tetraploid *Luciobarbus* (Levin *et al.* 2012). Morphological comparison of the Ethiopian scraping feeders to Asian *Capoeta* showed that in a number of characters related to the feeding mode (jaw modification, widening of the head, gut elongation, reduction of barbels) *V. beso* exhibited the level of transformation similar to the most specialized *Capoeta*, while *L. jubae* manifested less specialized traits than most *Capoeta* species (Karaman 1969; Levin *et al.* 2005; Levin 2012; Levin *et al.* 2012).

Finally, we have to draw attention to *Capoeta bingeri* Pellegrin 1905, a scraping feeder described from the Wabi Shibeli River system neighboring the Juba system. It is known only from a holotype and later classified as a large African *Barbus* (= *Labeobarbus*) by Lévêque & Daget (1984). This taxon may be a senior synonym of *L. jubae* or present another example of parallel evolution of a scraping form among the Ethiopian barbins.

Material for this study was collected within the scope of the Joint Ethio-Russian Biological Expedition (JERBE-III). We thank A.A. Darkov for providing logistic support; Fekadu Tefera, Redeat Habteselassie and F.N. Shkil for assistance in material collecting and sharing field operations; S.E. Cherenkov for photography and M.V. Mina for the critical comments on the manuscript. This study was funded in part by the Russian Foundation for Basic Research (Project Nos 11-04-00109, 11-04-01252, and 11-04-02056, and 13-04-01319) and the Programs of Presidium of Russian Academy of Sciences 'Biodiversity' and 'Problems of Life Origin and Biosphere Establishment'.

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Responsible Editor: O.L.F. Weyl