Comparative Analysis of the Structure of Differences between Some Morphotypes of Large African Barbs of Genus Barbus (Labeobarbus auctorum) from Lake Tana, Ethiopia

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Abstract—It is shown that the phyletic relationships of the morphotypes of large African barbs Barbus (Labeobarbus auctorum) may be assessed by the results of a comparative analysis of the structure of morphological differences between them. At the same time, the structure of differences between two samples is characterized by the distribution of eigenvector loadings of characters on the principle component, according to which their most clear separation is revealed; and the similarity of the structure of differences between pairs of samples is assessed by comparing the structure of differences between samples in the first and second pairs. The hypothesis, according to which the phenetic hierarchy of morphotypes of large African barbs from Lake Tana arose as a result of independent branching of ontogenetic channels from the channel of the generalized form is confirmed. The similarity of the structure of differences between morphotypes is characterized by the similarity of eigenvector loadings in pairwise comparisons and the similarity of the location of characters ranked by the magnitude of their eigenvector loadings.

Keywords: Barbus (Labeobarbus auctorum), phenetic diversity, hierarchical organization, structure of differences, morphotypes, generalized form, characters eigenvector loadings, independent divergences, sympatric origination of forms

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INTRODUCTION

Extreme phenetic diversity is a characteristic of large African barbs of the g. Barbus (Labeobarbus auctorum), inhabiting Lake Tana (Ethiopia). Adult individuals, similar in appearance, form discrete or not quite discrete groupings. Banister (1973) called these groups ecophenotypes, while Nagelkerke et al. (1994) first defined them as morphotypes but later as species (Nagelkerke and Sibbing, 2000). Whether all or only some morphotypes may be considered species is a controversial issue, the discussion of which is beyond the scope of this study.

Quite many studies have been published devoted to the analysis of phenetic relationships between the morphotypes of large African barbs, estimated by the magnitude of differences between them both in terms of external and skull characters (Nagelkerke et al., 1994; Mina et al., 1996a; Nagelkerke and Sibbing 2000; Mina et al., 2011). However, differences of the same magnitude may have different origins, and the phenetic relationships of morphotypes may not reflect phyletic ones.

The goal of the present paper is to assess the phyletic relationships of morphotypes of large African barbs using a previously unused comparative analysis of the structure of differences between morphotypes. The assessment is based on a multivariate analysis of morphological characters. The structure of differences between two morphotypes (samples) is characterized by the distribution of eigenvector loadings of characters on the principle component, according to which their most clear separation is revealed, while the similarity of the structure of differences between morphotypes of one pair and morphotypes of another pair is estimated by comparing the structure of differences in these pairs.

MATERIALS AND METHODS

The materials we used for the study was taken from the collection of the Joint Russian–Ethiopian Biological Expedition of the Russian Academy of Sciences sampled in 1992–2010.

The morphotypes, i.e., the groups of adult specimens identified according to external morphological characters (Nagelkerke et al., 1994), served as operational units. As it was shown previously these groups also differ in skull characters (Mina et al., 1996a, 1996b; Mina et al., 2011).

The barbs exhibiting morphological traits of feeding specialization (de Graaf et al., 2008) were considered as representatives of specialized morphotypes; not having such traits, as representatives of the gener-
alized form\(^1\) (hereinafter GF). In our materials, GF from the Lake Tana basin is represented by samples from the lake itself—119 individuals (Tn) and from the Gelda River inflowing the lake—38 individuals (Ge). The specialized morphotypes are represented by samples of piscivorous forms: acute (ac)—70 individuals, bigmouth big-eye (be)—51 individuals, bigmouth small-eye (se)—49 individuals, dark (da)—32 individuals, truite-like (tr)—32 individuals, white hunch (wh)—33 individuals, and a sample of the benthivorous barbel (ba)—33 individuals (Fig. 1). In addition, a sample (za) was used that included five large-mouth (piscivorous) individuals from the Didessa River, inflowing the Blue Nile below Lake Tana. This sample represents the morphotype described as the species *Barbus zaphiri* Boulenger, 1906.

A total of 14 measurements of the skull bones were analyzed (Fig. 2). In the calculations, the values of the indices, the ratio of the absolute values of the measurements to the basal length of the skull (BL) were used. Further in the discussion, when we mention this or that feature, we mean its index, and not the measurement itself. Index values were converted to natural logarithms to normalize distributions.

\(^1\) Initially, generalized barbs from Lake Tana were described as the intermedius morphotype (Nagelkerke et al., 1994); in what follows, we use the terms “GF from the Lake Tana basin” and “intermedius morphotype” as synonyms.

The data were processed statistically applying NTSYS 2.02k (Rohlf, 1998) and Statistica 6 software packages. At the principal components analysis (PCA), eigenvectors were calculated based on the correlation matrix; vector length was taken as 1.

**Calculation Algorithm**

1. PCA of pairwise combinations of samples representing morphotypes \(M_1, M_2, M_3 \ldots M_n\) is carried out (comparison of two morphotypes is denoted as \(M_i - M_j\)), eigenvector loadings of characters are estimated. Since the morphotypes in pairwise comparisons most clearly diverge according to PC1, the differences structure between them is characterized by the distribution of eigenvector loadings of characters on this component.

2. The eigenvector loadings of characters on PC1 are compared in different pairwise comparisons by plotting a two-dimensional distribution, where the loadings estimates for one pair of morphotypes are plotted along the abscissa; for the other pair, along the ordinate. In this case, pairs are compared, either including different samples (\(M_i - M_j\) and \(M_k - M_l\)), or the same sample is included in both pairs (\(M_i - M_j\) and \(M_i - M_k\)). The structure of differences between the samples in 30 pairs was estimated and 134 comparisons of the distributions of eigenvector loadings of characters between different pairs were made:

![Representatives of the generalized form (GF) (a) and specialized morphotypes (b–h) of barbs of the *Barbus intermedius* sensu Banister (1973) complex from Lake Tana: b—bigmouth big-eye (be), c—bigmouth small-eye (se), d—troutlike (tr), e—acute (ac), f—barbel (ba), g—white hunch (wh), h—dark (da); names of specialized morphotypes are given according to Nagelkerke et al., 1994.](image-url)
eight comparisons of the $M_i$–$Tn$ vs $Tn$–$Ge$ series, 21 comparisons of the $M_j$–$Tn$ vs $M_i$–$Tn$ series, and 105 comparisons of the $M_j$–$M_i$ vs $M_g$–$M_i$ series.

3. The similarity of the structure of differences between morphotypes represented by samples in different pairs is characterized by the shape of the scatter and by the value of the rank correlation coefficient of Spearman ($r_S$). The similarity is the greater, the more clearly a linear relationship of variables is traced in the two-dimensional distribution. Previously, the Spearman rank correlation coefficient was used in the analysis of the structure of differences between samples of Altai osmans of the g. *Oreoleuciscus* from the lakes of Mongolia (Dgebuidze et al., 2008). Andreev and Reshetnikov (1978) used an approach based on the assessment of the interrelations of vector loadings of characters to determine the directions of intraspecific differentiation of the whitefish *Coregonus lavaretus*. These authors visually assessed the position of the scatter (two-dimensional distribution).

As an indicator of the generalized phenetic distance of similarity between samples of specialized morphotypes and GF, we used the generalized taxonomic $D$ distance (Rohlf, 1998). Cluster analysis of the distance matrix was performed using the unweighted pair-group method (UPGMA).

**RESULTS**

The data analysis revealed that the similarity of the structure of differences between morphotypes (scatter shape and $r_S$) varies greatly. In some cases, the structure of differences between the specialized morphotype and GF from Lake Tana is close to that observed between the GF samples from Lake Tana and the
Fig. 3. The structure of differences in skull indices between specialized morphotypes and the Tn morphotype in comparison with that between the generalized morphotypes in barbs of the *Barbus intermedius* complex from Lake Tana and the Gelda River (Tn−Ge): a—da−Tn, $r_S = 0.92$ (here and hereafter $p < 0.001$); b—se−Tn, $r_S = 0.92$; c—be−Tn, $r_S = 0.85$; d—wh−Tn, $r_S = 0.82$; e—ac−Tn, $r_S = 0.86$; f—za−Tn, $r_S = 0.97$. Parameter loads: (●)—jaw acr.: Pmx, Mx, De; (▲) —operculum: Pop and Op, (●) —Iop; (○) —neurocranium: $B_1, B_2, B_3, B_4, HS_1, HS_2$; (■) —Hm. (za)—piscivorous form from the Didessa River basin; other designations as in Figs. 1 and 2.
Fig. 4. Comparison of the structure of differences in skull indices in barbs of the *Barbus intermedius* complex from Lake Tana between pairs that include different specialized morphotypes and a generalized form (Tn): a—tr–Tn and da–Tn, $r_S = 0.92$ (here and hereafter $p < 0.001$); b—se–Tn vs da–Tn, $r_S = 0.87$; c—be–Tn vs wh–Tn, $r_S = 0.81$; d—da–Tn vs be–Tn, $r_S = 0.80$; e—ac–Tn vs wh–Tn, $r_S = 0.83$; f—ac–Tn vs ba–Tn, $r_S = 0.83$. Designations as in Figs. 1−3.

Gelda River (M−Tn and Tn−Ge) (Figs. 3a−3d); the two-dimensional distribution trend is described by a straight line; only in the case of the ac morphotype the trend is curved (Fig. 3e). In all cases there is strong ranks correlation ($r_S > 0.80$). Somewhat unexpectedly, the highest similarity in the structure of differences is observed in comparison of a pair including the piscivorous form za from the Didessa River basin and Tn
Fig. 5. Comparison of the structure of differences in skull indices in barbs of the *Barbus intermedius* complex from Lake Tana between pairs in which all morphotypes are specialized, and the same morphotype is included in both pairs: a—se–da vs se–ba, $r_S = 0.94, p < 0.001$; b—be–tr vs be–wh, $r_S = 0.77, p < 0.01$; c—da–tr vs da–wh, $r_S = 0.65, p < 0.05$; d—se–be vs se–wh, $r_S = 0.57, p < 0.05$; e—tr–se vs tr–wh, $r_S = –0.006, p > 0.05$; f—wh–tr vs wh–se, $r_S = –0.52, p > 0.05$. Designations as in Figs. 1–3.
with a pair of GF (Tn–Ge) samples from the Lake Tana basin (Fig. 3f) ($r_s = 0.97$, the trend is linear).

The structure of differences between pairs that include different specialized morphotypes and Tn is also similar: $M_i$–Tn vs $M_j$–Tn (Figs. 4a–4d). The exception is comparisons with the $ac$–Tn pair (Figs. 4e, 4f). In these cases, there is no single trend, but the correlation of ranks is strong ($r_s \geq 0.80$). The structure of differences between the $M_j$–$M_i$ and $M_z$–$M_i$ pairs, in which all morphotypes are specialized (and the same morphotype is included in both pairs), is extremely diverse, and in most cases there is no similarity in the differences structure (Fig. 5). However, in comparison of $se$–$ba$ and $se$–$da$ (Fig. 5a: linear trend and $r_s = 0.94$), the picture is the same as in comparisons of $M_j$–Tn and $M_z$–Tn (Fig. 4). However, if other morphotypes are analyzed instead of $ba$ and $da$, then there is no such similarity in the differences structure (Figs. 5b–5f).

**DISCUSSION**

There are no doubts that the specialized morphotypes of large barbs that exist or existed in the rivers and lakes of Africa, including Lake Tana, originated from a generalized form, but the ways and means of their origin could be different.

The phenetic diversity of Lake Tana barbs is hierarchically organized (Fig. 6). If we assume that the given dendrogram reflects the phyletic scheme of diversification, then we must conclude that at first the ac morphotype separated from a common generalized ancestor similar to the Tn and Ge morphotypes presented in our materials, then the common ancestor of the be and se morphotypes (or one of these morphotypes, from which another one then descended), and then the common ancestor of the wh, da, ba, and tr morphotypes diverged from the ancestral GF.

According to an alternative hypothesis, morphotypes separated from generalized ancestors independently of each other, just as it happens in ontogeny. Previously, the analysis of the morphological changes in Lake Tana barbs, has shown (Mina et al., 1996b) that during ontogenesis a specialized morphotype is formed in the terminal section of the ontogenetic canal, with canals of different specialized morphotypes departing from the GF channel) at different sizes of fish, and earlier than others, the channel of the ac morphotype branches off. This hypothesis is confirmed by the present study. The strong similarity of the structure of the Tn–Ge differences with the pairs including the specialized morphotype and the Tn morphotype suggests that the divergence of each specialized morphotype from GF was similar to the separation of Tn and Ge, whose phyletic closeness is undeniable.

The independence of the divergences of specialized morphotypes is evidenced by the similarity of the structure of differences between each of them and the intermedius morphotype ($M_j$–Tn and $M_z$–Tn, where $M_j$ and $M_z$ are specialized morphotypes) (Fig. 4), while the similarity of the structure of differences between pairs of specialized morphotypes, when the same morphotype is included in both pairs ($M_i$–$M_j$ and $M_i$–$M_z$), it is not described by a single scheme, and in most cases there is no or little similarity in the differences structure (Fig. 5). We tend to regard the similarity of the differences structure in the comparison of $se$–$ba$ and $se$–$da$ as an accidental coincidence of the differences structure in the compared pairs, i.e. as an realization of one of many possible options.

Note that the ancestral GF could be not identical to Tn or Ge. This is evidenced by the great similarity of the differences structure between za–Tn and Tn–Ge (Fig. 3f). Since za originates from the Didessa River, far from Lake Tana (although also in the Blue Nile Basin), it is unlikely that the generalized morphotype from which za diverged could be identical to some morphotype from Lake Tana. Consequently, we cannot state that all Tana morphotypes are descended from one GF morphotype, from Tn or Ge in particular.

It is worth noting that during the sampling period, individuals of all studied morphotypes, except for da, spawned simultaneously, entering the same rivers, and there was no complete reproductive isolation between them (Nagelkerke and Sibbing, 1996; Dgebuadze et al., 1999; Dzerzhinsky et al., 2007). If such a situa-

![Fig. 6. Similarity dendrogram (UPGMA) of morphotypes of barbs of the Barbus intermedius complex from Lake Tana and the Gelda River: Tn, Ge—generalized form from the lake and river; names of other morphotypes are given in Fig. 1.](image-url)
tion also existed during the formation of morphotypes, then it must be concluded that they did not originate from reproductively isolated GF populations. However, it is highly probable that the situation in the past was different from the present one, and, possibly, the morphotypes arose from different reproductively isolated populations that existed at that time and came into contact, already having a predisposition to interbreeding.

Judging by our data, in the process of divergence of a specialized morphotype from GF, the structure of differences between them changes. The structure of differences between ac and Tn looks different than between other specialized morphotypes and Tn. The earlier separation of ac from generalized ancestors (as evidenced by the largest phenetic distance between ac and Tn (Fig. 6)) may explain this. It is noteworthy that in the process of divergence, a close rank correlation of the differences structure between the specialized and generalized morphotypes is preserved despite changes in the shape of the scatter, i.e. the order of the characters in a row of vector loadings ranked by their magnitude changes little.

It is the task of further research to find out to what extent the method proposed by us may be useful in studying the phentetic diversity of fish. Hopefully, with its help it will be possible to identify cases where the hierarchy of phenotypes arose as a result of independent divergences from a common tree.

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