

1 **Parallel trophic diversifications in polyploid cyprinid fish from East**  
2 **Africa: from preadaptive polymorphism to trophic specialization**

3

4

5 Boris A. Levin<sup>1,2#</sup>, Aleksandra S. Komarova<sup>1,2</sup>, Alexei V. Tiunov<sup>2</sup>, Alexander S. Golubtsov<sup>2</sup>

6

7

8 <sup>1</sup>Papanin Institute for Biology of Inland Waters of Russian Academy of Sciences - Borok,  
9 Russia

10 <sup>2</sup>A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences -  
11 Moscow, Russia

12

13 <sup>#</sup>Corresponding Author: 152742 Borok, Yaroslavl Region, Russia.

14 E-mail: [borislyovin@gmail.com](mailto:borislyovin@gmail.com). Tel. +74854724349

15

16 Foundation item: This work was supported by the Russian Science Foundation (grant no. 19-  
17 14-00218)

18

19

20

21

22

23

24

25

26

27

## Abstract

28 Trophic diversification is one of the main mechanisms driving the adaptive radiation. The  
29 polyploid lineage of the cyprinid genus *Labeobarbus* represent an excellent model for  
30 studying the trophically-based adaptive radiation in either lacustrine or riverine  
31 environments. Recently discovered four diversifications in rivers of the Ethiopian Highlands  
32 (East Africa) demonstrate independently evolved repeated mouth polymorphisms each  
33 represented by four core mouth phenotypes: (i) generalized, (ii) thick-lipped, (iii) scraping,  
34 and iv) large-mouthed. Mouth phenotypes in some radiations can be further divided to  
35 subtypes representing from four to eight sympatric ecomorphs. Using the stable isotope and  
36 gut content analyses we tested hypothesis on trophic resource partitioning within each  
37 radiation, revealed disparity in degree of diversification between radiations and tried to  
38 reconstruct the process of trophic diversification. Three of four radiations demonstrated  
39 partitioning of trophic resources within five trophic niches: i) detritophagy, ii)  
40 macrophytophagy, iii) invertivorous benthophagy, iv) periphyton feeding, and v) piscivory.  
41 The studied riverine radiations were likely at the different stages of the diversification. One  
42 radiation having a similar set of mouth phenotypes was not trophically divergent displaying a  
43 remarkable decouple of form and function. A unique case of ecologically non-functional  
44 mouth polymorphism at an incipient stage of trophic diversification supports a concept of the  
45 plasticity-first evolution. This phenomenon stems from the pre-existing genomic templates of  
46 mouth polymorphism ancestrally inherited upon the allopolyploid origin of the *Labeobarbus*  
47 lineage. The predetermined and preadaptive mouth polymorphism can be considered a key  
48 innovation of the *Labeobarbus* that promoted to resource-based diversification via adaptive  
49 radiation.

50

51 Running Title: Preadaptive polymorphism and trophic specialization

52

53 **Key words:** adaptive radiation, polymorphism, trophic resource partitioning, ecological  
54 speciation, vertebrates

55

## 56 **1. Introduction**

57 Trophic divergence is one of the main drivers of ecological speciation and adaptive radiation  
58 (Schluter, 2000; Seehausen & Wagner, 2014; Martin & Richards, 2019). The fishes being the  
59 most diverse group among vertebrates display numerous examples of trophic diversification  
60 during adaptive radiation - not only among textbook examples from cichlids, whitefish,  
61 Arctic charrs (Seehausen & Wagner, 2014) - but also in other taxa. Despite tremendous  
62 amount of studies focused on the mechanisms underpinning the rapid diversification during  
63 adaptive radiation (and achieved progress – e.g., Brawand *et al.*, 2014; McGee *et al.*, 2020;  
64 Ronco *et al.*, 2021), the phenomenon is still poorly understood (Martin & Richards, 2019;  
65 Gillespie *et al.*, 2020). One of the greatest findings of the last decade – a contribution of the  
66 heterogenous genome (usually evolved via past hybridization) into accelerated rates of  
67 ecological diversification and speciation (Nosil *et al.*, 2009; Genner & Turner, 2012; Meier *et*  
68 *al.*, 2017; Irisarri *et al.*, 2018; Marques *et al.*, 2019; Svardal *et al.*, 2020). One of the  
69 mechanisms to increase genetic heterogeneity is a polyploidization. After a round of fish-  
70 specific genome duplication (Meyer & Van de Peer, 2005) occurred ca. 350 mya in the ray-  
71 finned fish (Actinopterygii) lineage, the numerous further polyploidization events happened  
72 within and resulted in >1000 species mostly within Cypriniformes, Salmoniformes and  
73 Acipenseriformes (Yang *et al.*, 2022). Contrary to plants, the animal polyploidization is

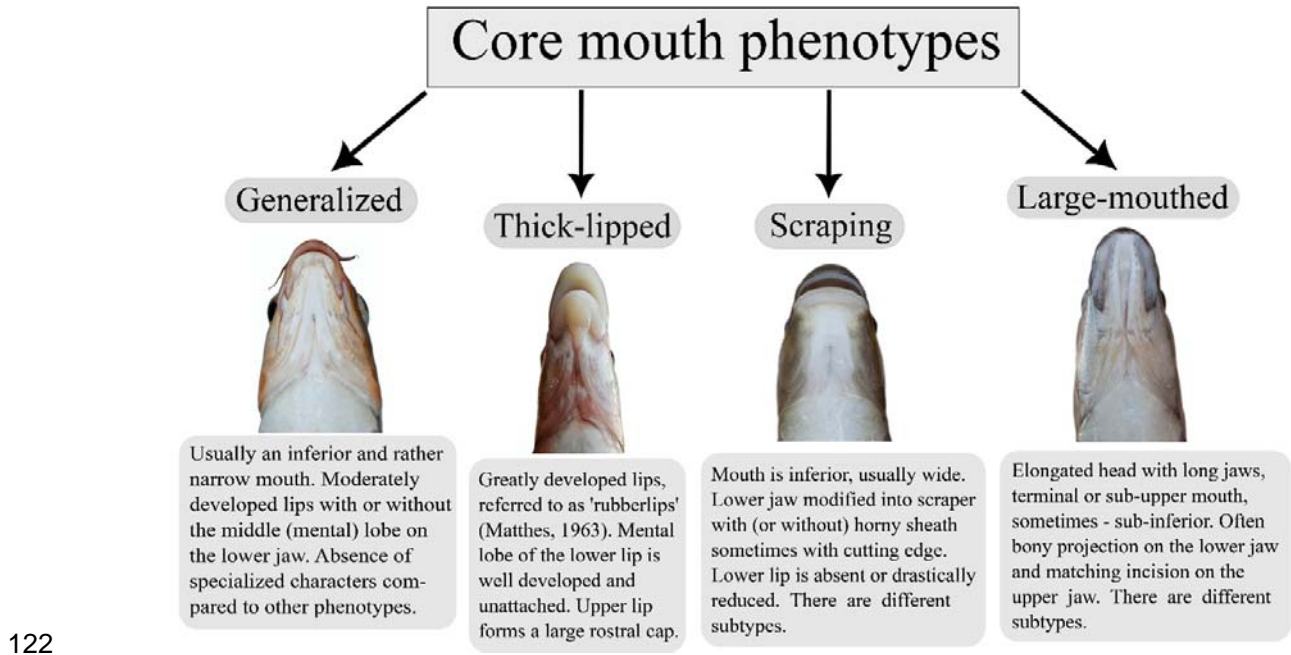
74 realized predominantly *via* allopolyploidization (Gregory & Mable, 2005) that means the  
75 most of fish polyploid lineages contains the different subgenomes. Therefore, the polyploid  
76 lineages look as perfect candidates to test a hypothesis on role of complex genome in further  
77 diversification during adaptive radiation. Herein a polyploidy apart prospective gains also has  
78 many restrictions on fish biology that can dismiss the expected benefits (Gregory & Mable,  
79 2005; Van de Peer *et al.*, 2017). Nevertheless, the most researchers agreed that polyploid taxa  
80 are characterized by increased ecological and phenotypic plasticity that (i) facilitate to  
81 overcome the stressful and harsh environment and (ii) can promote to further diversification  
82 and speciation that is within ‘flexible stem’ hypothesis (West-Eberhard, 2003; Wund *et al.*,  
83 2008; Li & Guo, 2020; Van de Peer *et al.*, 2021). Some polyploid lineages, for instance –  
84 Salmonidae and Coregonidae – represent nice examples of phenotypic and ecological  
85 plasticity along with possibility to evolve small-scale adaptive radiation especially within  
86 genera *Salvelinus*, *Salmo*, and *Coregonus* (Reshetnikov, 1980; Bernatchez *et al.*, 1999;  
87 Alekseyev *et al.*, 2002; Markevich *et al.*, 2018; Segherloo *et al.*, 2021; Levin *et al.*, 2022).

88 The cyprinid fishes (fam. Cyprinidae sensu Tan & Armbruster, 2018) being one of the  
89 most diverse families of teleostean fishes (> 1780 species - Fricke *et al.*, 2023) are the  
90 champions in the number of the polyploid species (ca. 600 species – Yang *et al.*, 2022). Some  
91 of cyprinid polyploid lineages display bright examples of adaptive radiation based on trophic  
92 polymorphism (Nagelkerke *et al.*, 1994; Roberts & Khaironizam, 2008; Levin *et al.*, 2019;  
93 2021; Qiao *et al.*, 2020; Komarova *et al.*, 2021). Among these, the most distinguished one in  
94 terms of the morpho-ecological diversification is the cyprinid genus *Labeobarbus*  
95 representing species-rich African hexaploid lineage ( $2n=150$  – Oellerman & Skelton, 1990;  
96 Golubtsov & Krysanov, 1993) that comprises >130 species (Vreven *et al.*, 2016; Fricke *et al.*,  
97 2023) and displays numerous trophically-based adaptive radiations (Nagelkerke *et al.*, 1994;  
98 2015; Mina *et al.*, 1996; Sibbing & Nagelkerke, 2000; Dimmick *et al.*, 2001; Golubtsov,

99 2010; Shkil *et al.*, 2015; Levin *et al.*, 2020). The *Labeobarbus* is widespread in Africa and  
100 presents in each of the ten African ichthyofaunal provinces (Vreven *et al.*, 2016). Based on  
101 the recent data on phenotypic, ecological, and genetic data, the taxonomic diversity of the  
102 *Labeobarbus* might be greatly underestimated (Levin *et al.*, 2019, 2020; Decru *et al.*, 2022).

103 The Ethiopian Highlands is remarkably distinguished as a hotspot for multiple adaptive  
104 radiations of the *Labeobarbus* discovered during last 30 years (see Golubtsov *et al.*, 2021).  
105 The most famous example of rapid adaptive radiation among non-cichlid fishes belongs to  
106 the lacustrine radiation of the *Labeobarbus* in Lake Tana, Ethiopia, where up to 15  
107 species/ecomorphs were described that partitioned trophic resources (Nagelkerke *et al.*, 1994;  
108 Mina *et al.*, 1996; Zworykin *et al.*, 2006; Shkil *et al.*, 2015). Apart from the lacustrine  
109 radiation strongly predominating among fishes (e.g. Seehausen & Wagner, 2014), there are  
110 striking examples of the riverine adaptive radiations in non-cyprinid (Turner *et al.*, 1985;  
111 Piálek *et al.*, 2012; Esin *et al.*, 2021; Říčan *et al.*, 2021; Burress *et al.*, 2022) as well as  
112 cyprinid fishes - *Labeobarbus*, in particular (Levin *et al.*, 2019; 2020).

113 The most conspicuous phenotypic difference between sympatric ecomorphs of the  
114 *Labeobarbus* refers to mouth type variation. The core of mouth polymorphism is composed  
115 of four main phenotypes (Figure 1) that were recorded throughout Africa (Worthington,  
116 1929; 1933; Matthes, 1963; Banister, 1973; Skelton *et al.*, 1991; Nagelkerke *et al.*, 1994): i)  
117 generalized (typical for barbs), ii) thick-lipped ('rubberlip'), iii) scraping ('chiselmouth'), and  
118 iv) large-mouthed. Mouth phenotypes are subjected to further sub-diversification and can  
119 include subtypes or variants that are recruited for occupying the new ecological niches (Levin  
120 *et al.*, 2021b). Moreover, there are intermediate phenotypes possibly of hybrid origin  
121 (Banister, 1972; Vreven *et al.*, 2019) that does not exclude their further specialization.



122

123 Figure 1. Pictorial scheme of core mouth phenotypes of the *Labeobarbus*. Photograph  
124 examples referred to the *Labeobarbus* from the rivers of the Ethiopian Highlands.

125

126 This outstanding mouth polymorphism of the *Labeobarbus* likely stems from ancestral  
127 inherited genetic templates since both parental lineages involved in the allopolyploidization  
128 event (Yang *et al.*, 2015; 2022) belong to extant lineages and represent different mouth types.  
129 The contemporary representatives of the maternal lineage are characterized by generalized  
130 and thick-lipped mouth polymorphism (tetraploid Torinae,  $2n = 100$  - Roberts &  
131 Khaironizam, 2008; Walton *et al.*, 2017; Coad, 2021) while paternal diploid lineage  
132 (*Cyprinion*,  $2n = 50$ , Ünlü *et al.*, 1997; Esmaeili & Piravar, 2006) is represented by scraping  
133 mouth phenotype (Coad, 2021). Hence, mouth polymorphism of the *Labeobarbus* is  
134 ancestrally heritable, likely preadaptive and might re-evolve.

135           The parallel diversifications of the *Labeobarbus* in the riverine environment of the  
136 Ethiopian Highlands were recently discovered within the four geographically isolated  
137 drainages - i) the Didessa River in Blue Nile basin; ii) the Sore River in White Nile basin; iii)  
138 the Gojeb River in Omo-Turkana basin; and iv) the Genale River in Juba-Wabe-Shebelle  
139 basin, Indian Ocean catchment (Mina *et al.*, 1998; Golubtsov, 2010; Levin *et al.*, 2019; 2020;  
140 Golubtsov *et al.*, 2021). Each diversification includes from four to eight sympatric ecomorphs  
141 divergent in mouth phenotype (Table 1) that presumably involves trophic adaptations. The  
142 genetic studies showed that all four riverine radiations of *Labeobarbus* were independently  
143 evolved from different ancestors although they are very closely-related based on mtDNA data  
144 that suggests recent and rapid diversification (Levin *et al.*, 2013; 2019; 2020). Moreover,  
145 existing data suggests these radiations might be at various stages of the diversification (Levin  
146 *et al.*, 2020; 2023 - in press). Of special interest is that these radiations could repeatedly  
147 emerge in the riverine environment that has been considered to be inappropriate for adaptive  
148 radiation due to heterogeneous and unpredictable conditions for a long time (reviewed in  
149 Burress *et al.*, 2023).

150           A few studies on trophic ecology showed that sympatric riverine ecomorphs of the  
151 *Labeobarbus* bearing different mouth phenotypes have partitioned trophic resources - in  
152 particular, in the Genale River (Levin *et al.*, 2019). Other riverine radiations of the  
153 *Labeobarbus* were not studied comprehensively from the point of trophic divergence. The  
154 recent but independent origin of similar diversifications in mouth phenotypes raises several  
155 questions. Do all diversifications partition trophic resources according to homologous mouth  
156 phenotypes? What is trophic specialization (if present) of various sympatric ecomorphs?  
157 Whether riverine diversifications comprising different numbers of ecomorphs / mouth  
158 (sub)phenotypes are at various stages of the process? If so, could patterns of the trophic  
159 diversification be sorted from more primitive to advanced aiming reconstruction of the

160 trophic radiation? We try to answer these questions in a study of trophic diversification of  
 161 sympatric ecomorphs in each of four *Labeobarbus* riverine radiations using the stable isotope  
 162 composition and gut content.

163 Table 1. The composition of riverine sympatric ecomorphs of *Labeobarbus* spp. in  
 164 isolated riverine basins in the Ethiopian Highlands (summarized from Golubtsov, 2010;  
 165 Levin *et al.*, 2019; 2020; 2021; Mina *et al.*, 1998 and our unpublished data). The photographs  
 166 of the fish ecomorphs are given in Figure 2.

Species, river and basin	Ecomorphs	Source
<i>Labeobarbus gananensis</i> complex, Genale River, Wabe-Shebelle basin, Indian Ocean catchment.	1. Generalized A (common) 2. Thick-lipped 3. V-scraeper <sup>1</sup> ( <i>L. jubae</i> ) 4. S-scraeper <sup>2</sup> 5. i-Scraeper <sup>3</sup> 6. Short <sup>4</sup> 7. Large-mouthed A <sup>5</sup>	Golubtsov, 1993; Mina <i>et al.</i> , 1998; Dimmick <i>et al.</i> , 2001; Levin <i>et al.</i> , 2019; Golubtsov <i>et al.</i> , 2021
<i>Labeobarbus intermedius</i> complex, <i>L. zaphiri</i> , <i>L. beso</i> . Didessa River, Nile basin, Atlantic Ocean catchment.	1. Generalized A 2. Generalized B <sup>6</sup> 3. Thick-lipped 4. Semi-thicklipped <sup>7</sup> 5. V-scraeper ( <i>L. beso</i> ) 6. Large-mouthed A1 ( <i>L.</i>	Golubtsov, 2010; Mina <i>et al.</i> , 1998; Levin <i>et al.</i> , 2020; 2021; original observations



---

<i>zaphiri</i>		
7. Large-mouthed	A2	
<i>(Labeobarbus sp.1)</i> <sup>8</sup>		
8. Large-mouthed	B <sup>9</sup>	
<i>(Labeobarbus sp.2)</i>		

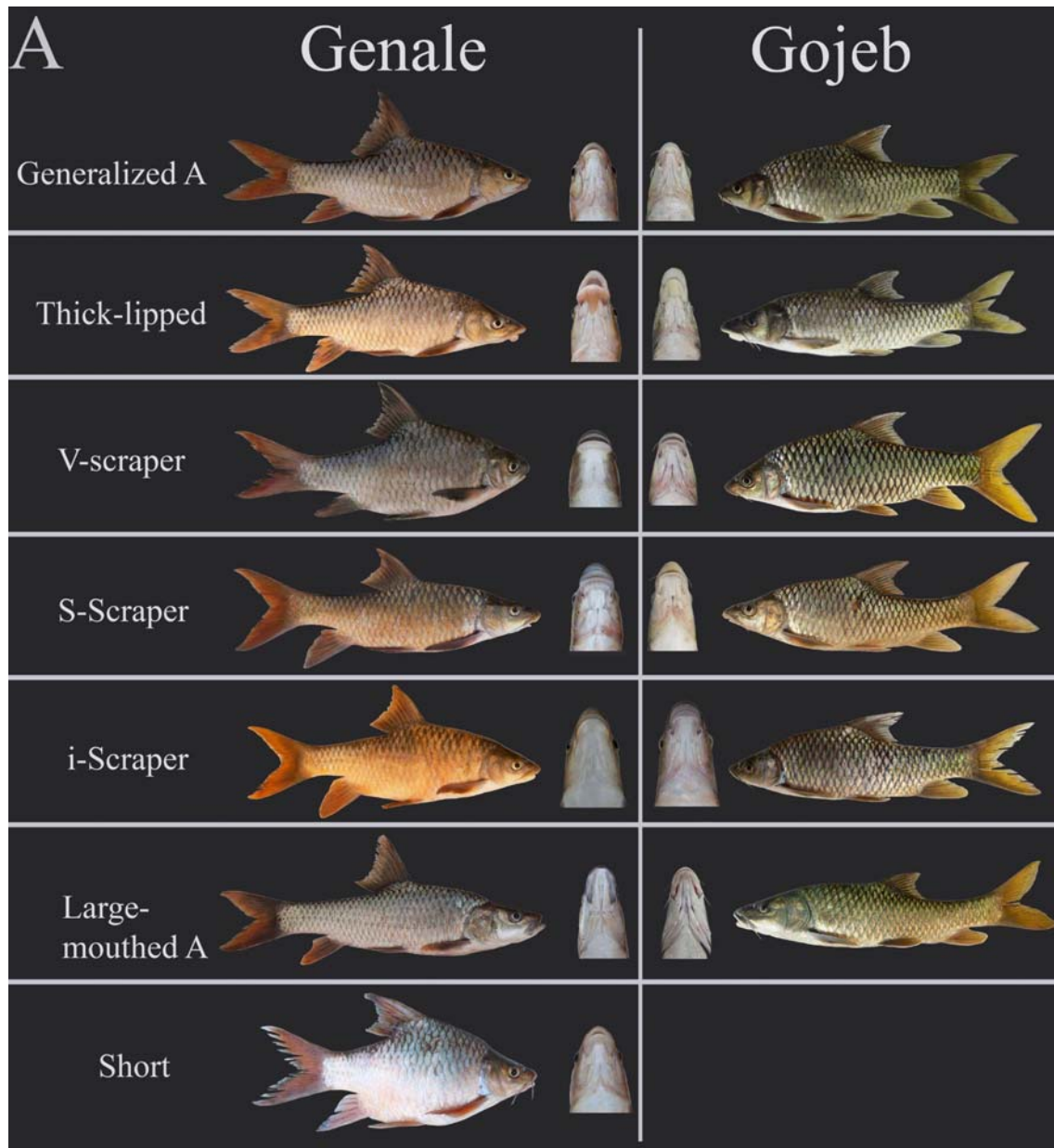
---

<i>Labeobarbus</i>	1. Generalized A	Golubtsov, 2010; Levin <i>et al.</i> ,
<i>intermedius</i> complex.	2. Thick-lipped	2020; 2021; original
Gojeb River, Omo-	3. V-scraper	observations
Turkana basin, inland	4. S-scraper	
drainage.	5. i-Scraper	
	6. Large-mouthed A	

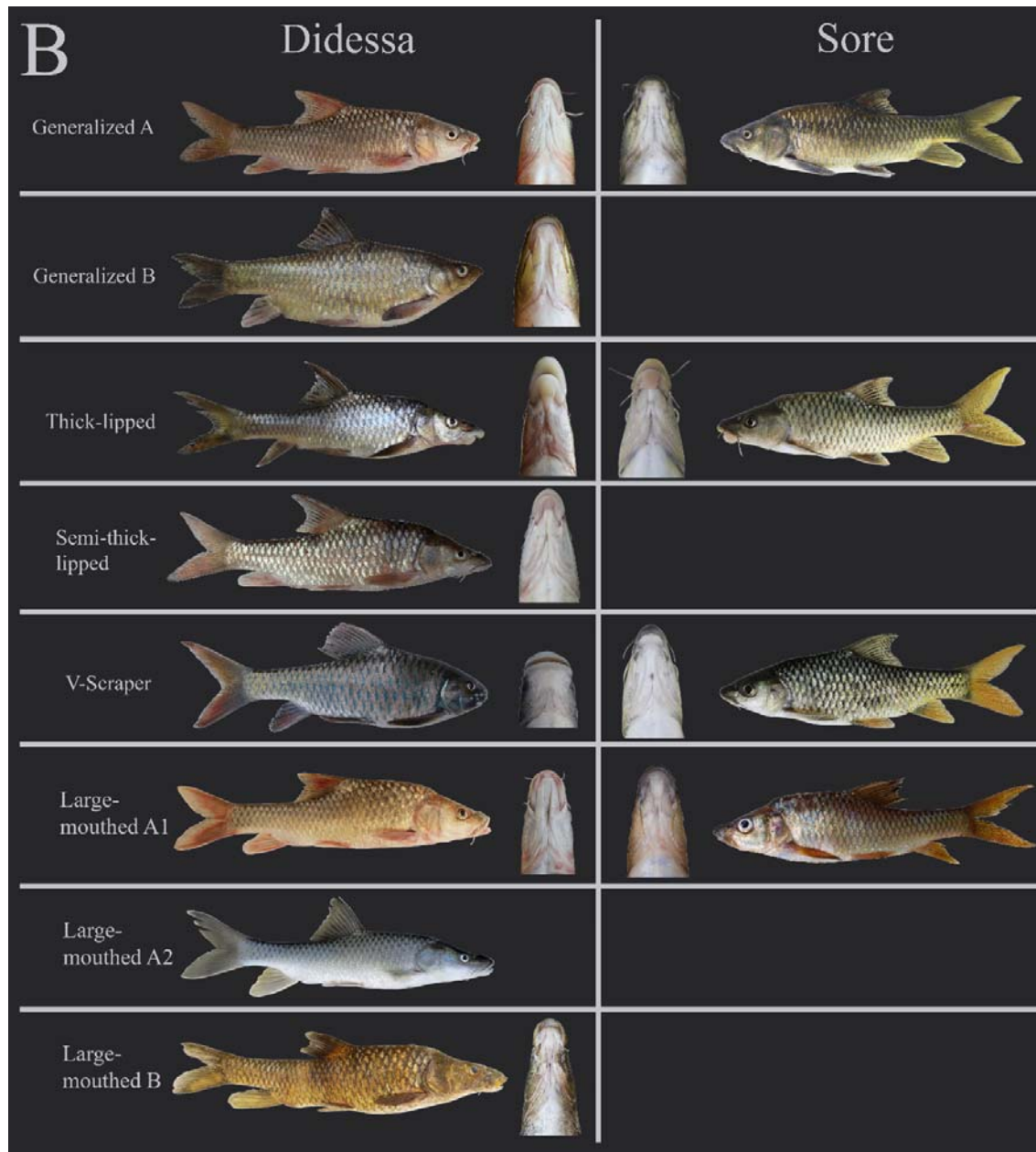
---

<i>Labeobarbus</i>	- Generalized A	Golubtsov 2010; Levin <i>et al.</i> ,
<i>intermedius</i> complex.	- Thick-lipped	2020; original observations
Sore River, White Nile	- V-scraper	
basin, Atlantic Ocean	- Large-mouthed <sup>9</sup>	
catchment.		

---



168



169

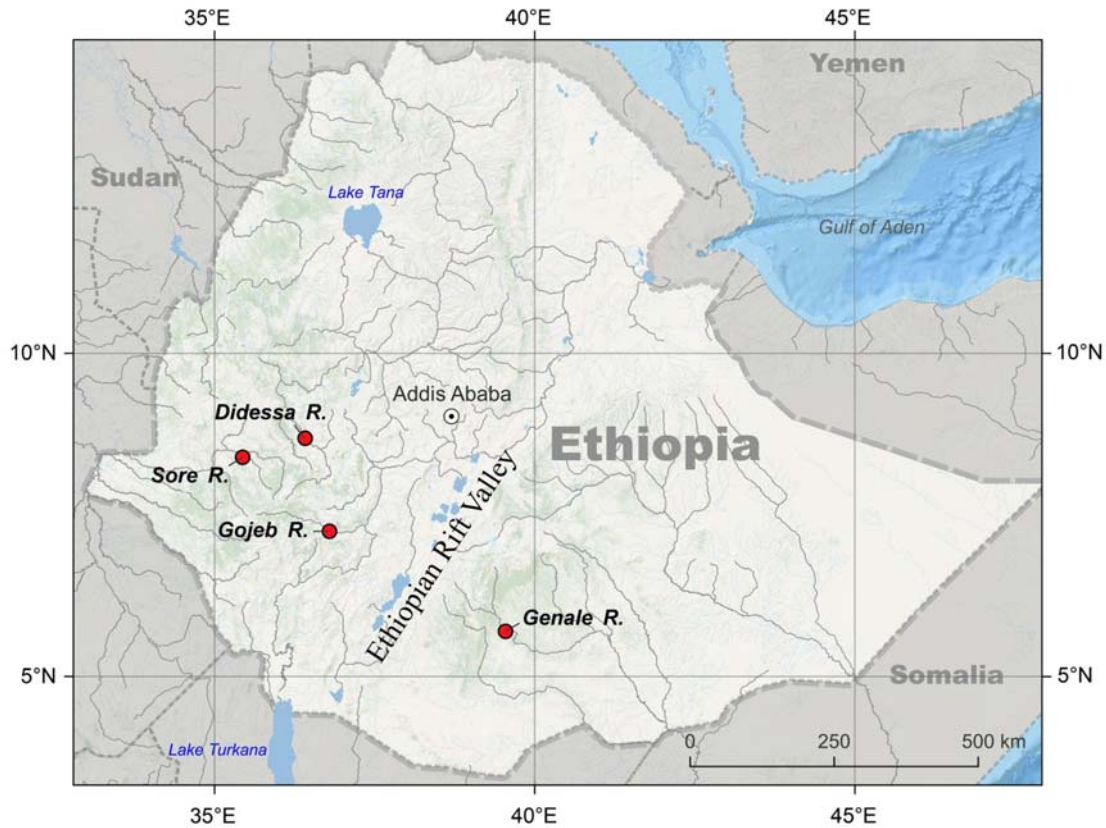
170 Figure 2. Appearance and mouth phenotypes of the ecomorphs of the *Labeobarbus* in  
171 different rivers of the Ethiopian Highlands: (A) the Genale and Gojeb Rivers; (B) the Didessa  
172 and Sore Rivers. An exceptionally rare representative of the Large-mouthed A2 ecomorph  
173 from the Didessa River is presented only from the lateral side.

174

175 **2. Material and Methods**

176 *Ethical approval.* All applicable international, national, and institutional guidelines for the  
177 care and use of animals were strictly followed. All animal sample collection protocols  
178 complied with the current laws of Russian Federation and Federal Democratic Republic  
179 of Ethiopia.

180 *Study sites and material studied.* Samples were collected under the framework of the Joint  
181 Ethiopian-Russian Biological Expedition (JERBE) in four rivers draining the Ethiopian  
182 Highlands and belonging to four major river basins (Fig. 2): (1) the Juba-Wabe-Shebelle  
183 basin in the Indian Ocean catchment – the Genale R. – 5.7025° N 39.5446° E; (2) the Blue  
184 Nile basin – the Didessa R., a tributary of the Blue Nile – 8.6921° N 36.4144° E; (3) the  
185 Omo-Turkana enclosed basin – the Gojeb R., a tributary of the Omo R. – 7.2539° N 36.7943°  
186 E; (4) the White Nile basin – the Sore R. ~35 km downstream City Metu – 8.3987° N,  
187 35.4378° E. Fish were caught by gill and cast nets in February–March 2011 (Didessa),  
188 March–April 2009, 2019 (Genale), February 2011 (Gojeb), April 2014 (Sore). Fish were  
189 killed with an overdose of MS-222 anaesthetic and then photographed using a Canon EOS  
190 50D camera (Canon Inc. Tokyo, Japan).



191

192 Figure 3. Map of sampling sites of the four riverine radiations of the Ethiopian *Labeobarbus*.

193

194 Standard length (SL, mm) and gut length (GL, mm) were measured with a ruler. Fish were  
195 preserved first in 10% formalin and then transferred to 70 % ethanol. Gut length was studied  
196 in 461 individuals. All specimens were deposited at A.N. Severtsov Institute of Ecology and  
197 Evolution, the Russian Academy of Sciences, Moscow under provisional labels of JERBE.  
198 The detailed information on material studied (gut length, stable isotope composition and gut  
199 content) is given in Supporting Table S2.

200 *Stable isotopes composition.* For stable isotope (SI) analyses, white muscle tissue from the  
201 dorsal side of the body under the dorsal fin was sampled from freshly collected specimens.  
202 White muscle samples were dried at 60 °C. The samples were weighed using a Mettler

203 Toledo MX5 microbalance (Mettler Toledo, Columbus, OH, United States) with 2  $\mu\text{g}$   
204 accuracy, and wrapped in tin capsules. The weight of the fish tissue samples varied from 250  
205 to 500  $\mu\text{g}$ . SI analysis was conducted at the Joint Usage Center of the A.N. Severtsov Institute  
206 of Ecology and Evolution RAS, Moscow. Briefly, a Thermo Delta V Plus continuous-flow  
207 IRMS was coupled to an elemental analyzer (Flash 1112) equipped with a Thermo No-Blank  
208 device. The isotopic composition of N and C was expressed in the  $\delta$  notation relative to the  
209 international standards (atmospheric nitrogen and VPDB, respectively):  $\delta X (\text{‰}) =$   
210  $[(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , where R is the molar ratio of the heavier and lighter  
211 isotopes. The samples were analyzed with a reference gas calibrated against the International  
212 Atomic Energy Agency (IAEA) reference materials USGS 40 and USGS 41 (glutamic acid).  
213 The measurement accuracy was  $\pm 0.2 \text{ ‰}$ . Along with the isotopic analysis, the nitrogen and  
214 carbon content (as %) and C/N ratios were determined. In total, 400 white-muscle samples  
215 were analyzed.

216 *Diet analysis.* Following the best practice for estimation of trophic niches the in tropic rivers  
217 (e.g., Davis *et al.*, 2012) we studied gut content in addition to SI composition. Gut content  
218 was extracted from preserved specimens, dried on filter paper and weighed using a Pioneer  
219 PX84/E balance with 0.0001 g accuracy. The diet particles were identified using Olympus  
220 CX41 microscope (100–1000 $\times$  magnification) and Motic DMW-143-N2GG  
221 stereomicroscope (100–400 $\times$  magnification). The diet components were grouped into: (i)  
222 detritus, (ii) invertebrates, (iii) macrophytes, (iv) periphyton, (v) filamentous algae, (vi) fish  
223 (body remnants and scales), and (vii) mineral ground. The group ‘Invertebrates’ included  
224 mainly the larvae and imago of amphibiotic insects and their fragments as well as imago of  
225 aerial insects (Coleoptera, Hymenoptera); rarely Cladocera. The group ‘Macrophytes’  
226 included any fragments of helophytic and semi-aquatic plants - such as leaves, stems or  
227 seeds. A composite measure of diet, an index of relative importance (IR) [Natarajan &

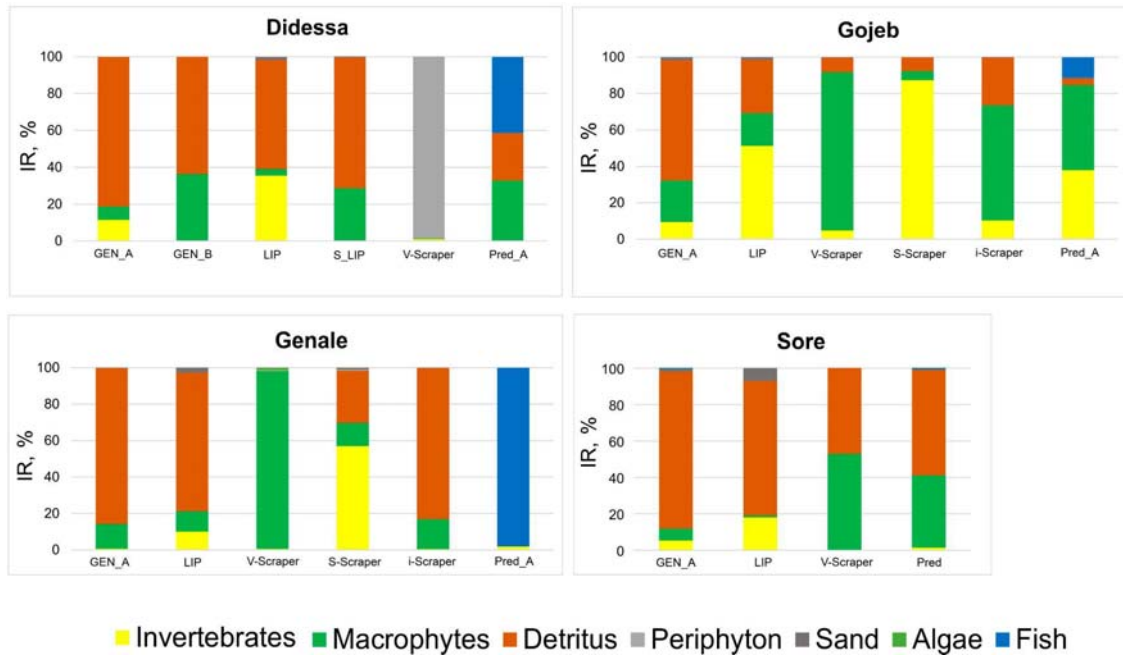


228 Jhingran, 1961; Popova & Reshetnikov, 2011], was used to assess the contribution of  
229 different components to the diet. The IR index was calculated as follows:  $IR = (F_i \times$   
230  $P_i) / (\sum (F_i \times P_i)) \times 100\%$ , where  $F_i$  = the frequency of occurrence of each food group, and  $P_i$   
231 = its part by weight; the value of  $i$  itself changes from 1 to  $n$  ( $n$  = the part of food organisms  
232 in the food bolus). In total, 195 food boluses were analyzed for gut content (Supplementary  
233 Table S2).

234 *Statistical analyses.* Several R packages and functions were used for the statistical analyses  
235 and plot construction. Basal descriptive statistics was obtained using the *summarytools*  
236 library [Comtois, 2022]. Central tendencies in the text are presented as means and 1SD. The  
237 Kruskal-Wallis test was applied for comparisons of the values of gut length GL and SI  
238 composition using the function *kruskalTest* in each radiation (in library *PMCMRplus*) with  
239 subsequent pairwise comparisons using post-hoc Dunn's all-pairs test with Bonferoni  
240 adjustment - function *kwAllPairsDunnTest* [R Core Team, 2023]. The violin boxplots were  
241 obtained using the *ggplot2* library [Wickham, 2022]. The package SIBER v.2.1.6 [Jackson *et*  
242 *al.*, 2011] was used to assess the differences in the isotopic trophic niche features. The total  
243 convex hull areas (TA), core trophic niche breadths, and sample size-corrected standard  
244 ellipse area (SEAc) were estimated. The trophic overlap for 95% TA was estimated using  
245 nicheROVER [Lysy *et al.*, 2021], a method that is insensitive to the sample size and  
246 incorporates statistical uncertainty using Bayesian approach [Swanson *et al.*, 2015].

### 247 **3. Results**

248 *3.1. Diet.* Food spectra of *Labeobarbus* spp. were rather diverse. Feeding of sympatric  
249 ecomorphs in all four rivers was divergent but at various degrees as estimated by index of  
250 relative importance (Figure 4). Detailed description of the diet for each ecomorph is given in  
251 Supplementary material S3.



252

■ Invertebrates ■ Macrophytes ■ Detritus ■ Periphyton ■ Sand ■ Algae ■ Fish

253

Figure 4. Food spectra (IR: the index of relative importance) of the sympatric ecomorphs of

254

the *Labeobarbus* spp. from the Didessa, Gojeb, Genale, and Sore rivers. Abbreviations of the

255

ecomorphs: GEN\_A - Generalized A; GEN\_B - Generalized B; LIP - Thick-lipped; S\_LIP -

256

Semi-thicklipped; Pred\_A - Large-mouthed A; Pred\_B - Large-mouthed B.

257

258

Based on the food spectra, up to five trophic specializations can be detected in the

259

Didessa River: i) detritivore (Generalized A), ii) detritivore-macrophytophage (Generalized B

260

and Semi-thicklipped), iii) detritivore-invertivore (Lipped), iv) periphyton feeder (V-scraper),

261

and v) piscivore-omnivore (Large-mouthed A and B). Notably, detritus seems to be a core

262

food for many ecomorphs except for the V-Scraper and Large-mouthed. A sub-specialization

263

within the piscivory strategy is possible taking into account the drastic difference in mouth

264

structure (Figure 2B) and diet between the Large-mouthed ecomorphs. Seemingly, up to five

265

feeding strategies can be distinguished in the Gojeb River: i) detritivore (Generalized A), ii)

266

invertivore-detritivore (Lipped); iii) macrophytophagous (V-Scraper and i-Scraper), iv)



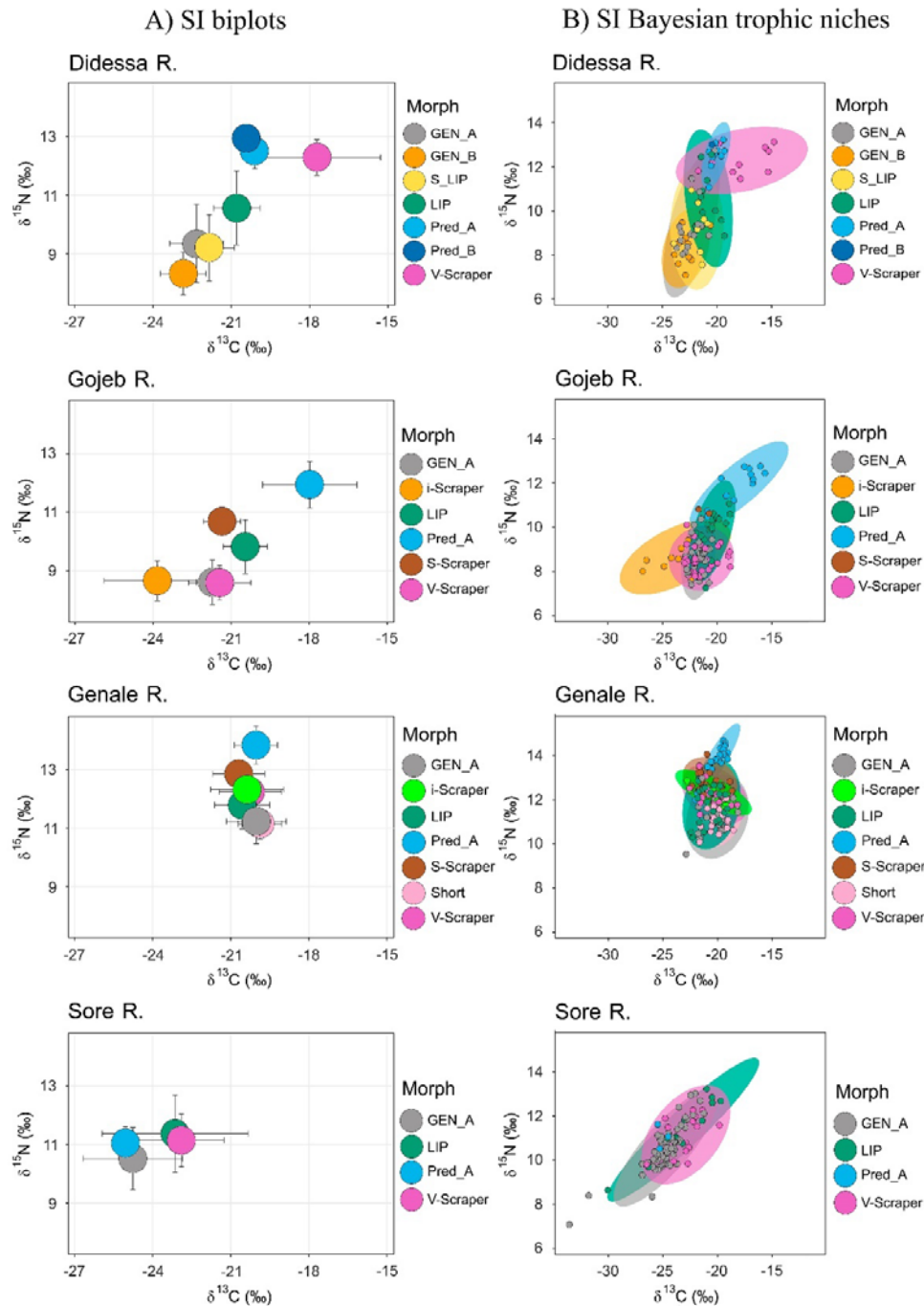
267 invertivore (S-scraper), and v) omnivore-piscivore (Large-mouthed A). Compared to the  
268 Didessa, a detritus was a core food only for the Generalized A ecomorph. Large-mouthed  
269 ecomorph is not predominantly piscivorous but rather omnivorous with a small portion of  
270 fish food. In the Genale River, up to four feeding strategies can be detected – i) detritivore  
271 (Generalized A, Lipped, and i-Scraper), ii) macrophytophagous (V-Scraper), iii) invertivore  
272 (S-scraper), and iv) piscivore (Large-mouthed A). Remarkably, a detritus was a core food for  
273 three ecomorphs in the Gojeb River, while the Large-mouthed ecomorph is a strongly  
274 piscivorous specialist. In contrast, little diversification in food spectra was detected in the  
275 Sore River. Briefly, two feeding strategies can be recognized: i) detritivore (Generalized A,  
276 Lipped, and i-Scraper), and ii) macrophytophagous-detritivore (V-Scraper and Large-  
277 mouthed). Remarkably detritus was a core food for three of four ecomorphs although a  
278 notable portion (almost 20 %) of benthic invertebrates was detected in the Lipped ecomorph.  
279 Large-mouthed ecomorph is not a piscivorous specialist in the Sore River.

280 *3.2. Stable Isotope Composition.* Basic statistics for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values and data on  
281 the total area (TA), standard ellipse area (SEA), and corrected standard ellipse area (SEAc)  
282 are given in Supplementary Materials S4. Detailed results are presented in subsequent  
283 sections.

284 *3.2.1. Didessa Radiation.* Among seven ecomorphs, several were significantly divergent in SI  
285 values (six pairwise comparisons in both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  - Table S4). The largest values of  
286  $\delta^{15}\text{N}$  were detected in two Large-mouthed (piscivory) ecomorphs ( $12.5\pm 0.6$  and  $12.9\%$ ) as  
287 well as in highly specialized V-scraper *L. beso* ( $12.3\pm 0.6\%$ ). The V-scraper was also most  
288 enriched in  $^{13}\text{C}$  ( $\delta^{13}\text{C} -17.7\pm 2.4\%$ ) (Figure 5). The lowest values of both  $\delta^{15}\text{N}$  ( $8.33\pm 0.7\%$ )  
289 and  $\delta^{13}\text{C}$  ( $-22.8\pm 0.9\%$ ) were in the Generalized B ecomorph. Other ecomorphs, Generalized  
290 A, Thick-lipped, and Semi-thicklipped, had intermediate  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values, although

291 Thick-lipped ecomorph had somewhat higher  $\delta^{15}\text{N}$  values ( $10.6\pm 1.3\text{‰}$ ) compared to both  
292 Generalized A and Semi-thicklipped ( $8.3\pm 1.3$  and  $9.2\pm 1.1\text{‰}$ , respectively). The maximum  
293 difference in mean  $\delta^{15}\text{N}$  values between ecomorphs in the Didessa was 4.61‰. The highest  
294 difference between ecomorphs in the mean  $\delta^{13}\text{C}$  values was 5.10‰. The isotopic niches (as  
295 assessed by the standard ellipses) were almost fully separated between Generalized B and V-  
296 Scraper (overlap <1%), between Generalized B and Large-mouthed A (overlap <1%), and  
297 weakly overlapped between Semi-Thicklipped and V-scraper, Semi-Thicklipped and Large-  
298 mouthed A, and Generalized A and V-Scraper (overlap <6%, <10 %, and <11%,  
299 respectively) (see details in Supplementary File S4). The most overlapping trophic niches  
300 were between Generalized A and B (57 and 90%) and between Generalized A and Semi-  
301 Thicklipped (77 and 79%) (Supplementary File S4).

302 *3.2.2. Gojeb Radiation.* Among six ecomorphs, some were significantly divergent from  
303 others in SI values (five and six pairwise comparisons in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values, respectively;  
304 Table S4). The largest values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were found in Large-mouthed (piscivory)  
305 ecomorph ( $11.94\pm 0.8\text{‰}$  and  $-17.98 \pm 1.8\text{‰}$ , respectively) that was notably (sometimes  
306 significantly - Figure 5, Table S4) higher than in all other sympatric ecomorphs. The minimal  
307 values of  $\delta^{15}\text{N}$  were detected in the V-scraper ( $8.60\pm 0.6\text{‰}$ ) and Generalized ( $8.61\pm 0.8\text{‰}$ )  
308 ecomorphs while in the i-Scraper ecomorph the  $\delta^{13}\text{C}$  value was at the minimum ( $-23.84\pm 2.0$   
309 ‰). The total divergence between ecomorphs was lower in  $\delta^{15}\text{N}$  but higher in  $\delta^{13}\text{C}$  values  
310 compared to the Didessa radiation. In particular, the highest difference between ecomorphs in  
311 the mean  $\delta^{15}\text{N}$  values achieved 3.34‰, while the same for the mean  $\delta^{13}\text{C}$  values was 5.86‰  
312 (Figure 5, Table S4). The isotopic niches were almost fully separated between Large-  
313 mouthed and V-Scraper (overlap <2%) and between Generalized and Large-mouthed (<4%)  
314 while the most overlapping trophic niches were between Generalized and V-Scraper (overlap  
315 70 and 91%) (Supplementary File S4).



316

317 Figure. 5. Stable isotope biplots (A) showing mean values and 1 SD, and Bayesian ellipses  
 318 showing trophic niche widths and overlaps (B) in sympatric ecomorphs of the *Labeobarbus*  
 319 spp. from the Didessa, Gojeb, Genale, and Sore Rivers. Ellipses with 95% confidence  
 320 intervals are based on standard ellipses corrected for small sample sizes (SEAc; isotopic  
 321 niche metrics; SIBER package). Each point corresponds to the individual isotopic value.

322 Abbreviations of the ecomorphs: GEN\_A - Generalized A; GEN\_B - Generalized B; LIP -  
323 Thick-lipped; S\_LIP - Semi-thicklipped; Pred\_A - Large-mouthed A; Pred\_B - Large-  
324 mouthed B.

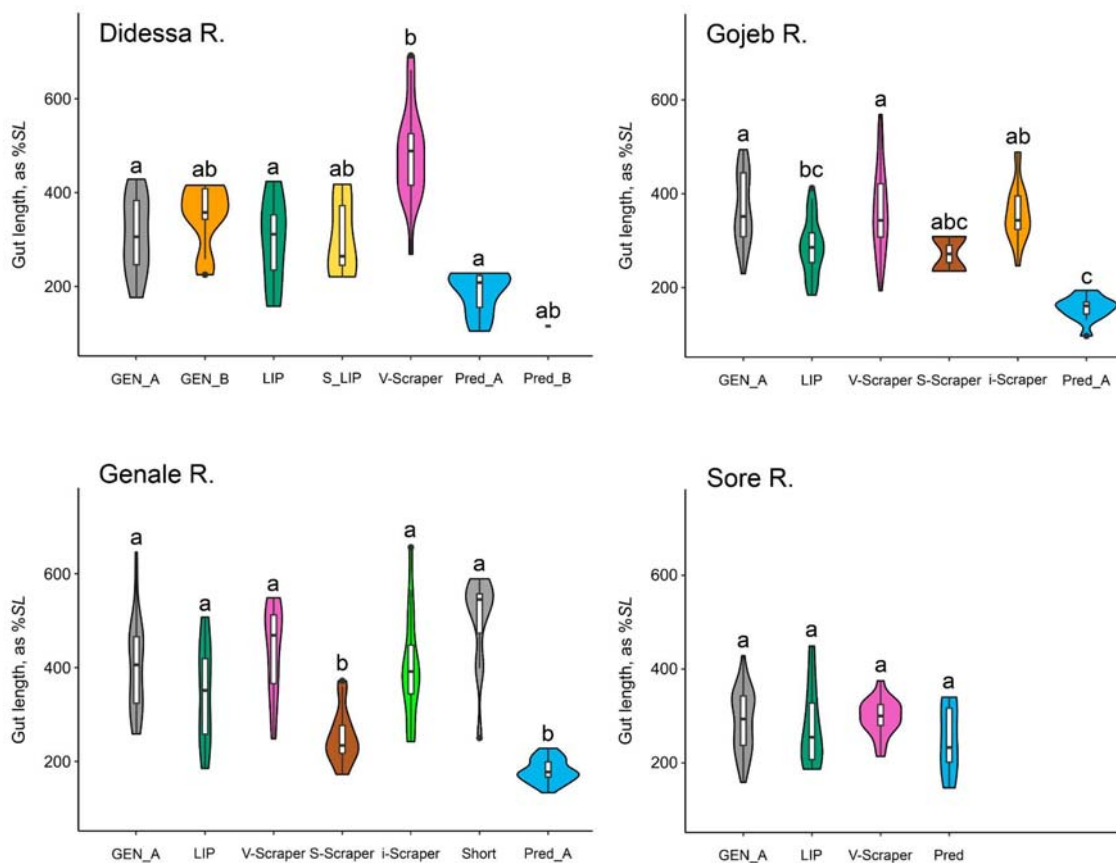
325

326 *3.2.3. Genale Radiation.* Among seven ecomorphs, some were significantly divergent from  
327 others in  $\delta^{15}\text{N}$  values (eight pairwise comparisons), but not in  $\delta^{13}\text{C}$  values (Table S4). The  
328 largest value of  $\delta^{15}\text{N}$  was found in the Large-mouthed (piscivory) ecomorph ( $13.83 \pm 0.7$  ‰)  
329 (Figure 5, Table S4). The minimal values of  $\delta^{15}\text{N}$  were detected in the Short and Generalized  
330 ecomorphs ( $11.15 \pm 0.5$  ‰ and  $11.23 \pm 0.8$  ‰, respectively). The range between largest and  
331 lowest mean  $\delta^{13}\text{C}$  values was small, from  $-19.87 \pm 0.8$  ‰ (Short) to  $-20.69 \pm 1.0$  ‰ (S-Scraper).  
332 The total divergence between ecomorphs was lower in  $\delta^{15}\text{N}$  values than in both the Didessa  
333 and Gojeb radiations. In particular, the highest difference between ecomorphs in mean  $\delta^{15}\text{N}$   
334 values was 2.73‰, and only 0.82‰ in  $\delta^{13}\text{C}$  values. In the isotopic niches the most divergent  
335 ecomorph was the Large-Mouthed, which overlap with other ecomorphs ranged from 0.06%  
336 (vs. Short) to 38 and 43% (vs. benthophagous S-Scraper). Among other comparisons a low  
337 overlap was detected between S-Scraper and Short (7 and 8%) (Supplementary File S4).

338 *3.2.4. Sore Radiation.* Among four ecomorphs, a few were significantly divergent in  $\delta^{13}\text{C}$   
339 values only (two pairwise comparisons - Generalized vs. Thick-Lipped and Generalized vs.  
340 V-Scraper; Table S4). The highest difference between ecomorphs in  $\delta^{15}\text{N}$  values was 0.85‰  
341 only, while in the mean  $\delta^{13}\text{C}$  values it was 1.86‰. The isotopic niches greatly overlapped  
342 (67...82%) in all comparisons (Supplementary File S4).

343 *3.3. Gut Length.* The relative gut length varied significantly within each radiation except for  
344 the Sore River (Figure 6). In three of four radiations, there were significant differences  
345 between some ecomorphs (Figure 6; Supplementary File S5). Longest guts (up to 548-692%

346 SL) were detected among V-scrapers (in the Didessa, Genale, and Gojeb Rivers). The large-  
347 mouthed ecomorphs showed shortest guts (highest values were up 194-228% SL) except for  
348 the Sore River, where it reached  $251 \pm 75\%$ . Other ecomorphs had intermediate gut length;  
349 sometimes they differed from each other like Generalized and Thick-lipped ecomorphs in the  
350 Gojeb River (Figure 6). In spite of diverse mouth phenotypes in the Sore River, the  
351 ecomorphs were not divergent from each other representing the middle length of the gut  
352 (means varied within 251-290% SL).



353

354 Figure 6. Violin plots of relative gut length distribution in sympatric ecomorphs from the four  
355 riverine radiations of the *Labeobarbus*. Min-max values (whiskers), 1st and 3rd quartiles  
356 (white vertical bars), median values (black horizontal bars), and outliers (black points) are  
357 indicated. Letters above the violin plots indicate significant differences between ecomorphs

358 (p < 0.05, Kruskal–Wallis test with Dunn’s post hoc test). Abbreviations of the ecomorphs:  
359 GEN\_A - Generalized A; GEN\_B - Generalized B; LIP - Thick-lipped; S\_LIP - Semi-  
360 thicklipped; Pred\_A - Large-mouthed A; Pred\_B - Large-mouthed B.

361

## 362 **4. Discussion**

363 Our results show that parallel trophic diversifications occurred within the polyploid lineage,  
364 the genus *Labeobarbus*. These diversifications differ in degrees of specialization within the  
365 same set of core mouth phenotypes (generalized, thick-lipped, scraping, and large-mouthed),  
366 indicating various stages of the process. We discuss the results obtained in the context of  
367 trophic specializations of certain mouth phenotypes, the preadaptive nature of the ancestral  
368 discrete mouth polymorphism, which likely originated via allopolyploidization of the  
369 *Labeobarbus* lineage, and attempt to reconstruct the process of trophic diversification by  
370 analyzing four repeated cases discovered in the riverine environment of the Ethiopian  
371 Highlands.

### 372 *4.1. Trophic resource partitioning and trophic specializations.*

373 Our study revealed that differences in diet between ecomorphs of the *Labeobarbus* in each  
374 river were generally confirmed by SI analysis. For instance, the large-mouthed ecomorphs  
375 which had piscivorous diet were also significantly enriched in  $\delta^{15}\text{N}$  values (Figures 4 and 5).  
376 In contrast, the ecomorphs with detritivorous mode of feeding had lowest  $\delta^{15}\text{N}$  values. The  
377 periphyton feeder that have been recorded in the Didessa River had a high level of  $\delta^{15}\text{N}$   
378 values comparable with such in piscivorous ecomorphs but was characterized by enriched  
379  $\delta^{13}\text{C}$  values (Figure 5). The invertivorous ecomorphs whose diet was based on the larvae of  
380 amphibiotic insects had intermediate levels of  $\delta^{15}\text{N}$  values between detritivores and

381 piscivores. The data on gut length were less informative although the piscivorous ecomorphs  
 382 had significantly shortened gut (Figure 6) that is in line with literature data for both  
 383 *Labeobarbus* (Nagelkerke et al., 1994; Levin et al., 2019) and other fishes (e.g., Hugueny &  
 384 Pouilly, 1999; Wagner et al., 2009). We summarized obtained data on the trophic  
 385 specialization of sympatric ecomorphs from all rivers based on the diet, gut length, and SI  
 386 composition in Table 2.

387

388 Table 2. Correlation between mouth phenotype and trophic specialization in the *Labeobarbus*  
 389 spp. from four riverine radiations. The expected trophic niche is based on prediction from the  
 390 mouth phenotype and published data on trophic ecology\*. Observed trophic niche - data  
 391 obtained in this study.

Mouth phenotype (core/subtype)	Expected trophic niche	River and observed trophic niches			
		Didessa	Gojeb	Genale	Sore
1. Generalized A	Omnivore	Detritivore	Detritivore	Detritivore	Detritivore
2. Generalized B	Omnivore	Detritivore- macrophyto phage	-	-	-
3. Thick-lipped	Benthic invertivore	Detritivore- invertivore	Invertivore- detritivore	Detritivore	Detritivore
4. Thick-lipped / Semi-thicklipped	Invertivore ?	Detritivore- macrophyto	-	-	-

	phag				
5. Scraping / V- Scraper	Periphyton feeder	Periphyton feeder	Macrophyto phage	Macrophyto phage	Detritivore- macrophyto phage
6. Scraping / S- Scraper	Periphyton feeder	-	Benthic invertivore	Benthic invertivore	-
7. Scraping / i- Scraper	Periphyton feeder?	-	Macrophyto phage	Detritivore	-
8. Large-mouthed A	Piscivore	Piscivore- omnivore	Omnivore- piscivore	Piscivore	Detritivore- macrophyto phage
9. Large-mouthed B	Piscivore	Piscivore?	-	-	-
No. trophic niches	-	5	5	4	2

392 \* References used: Matthes, 1963; Nagelkerke et al., 1994; Golubtsov, 2010; Levin et al., 2019; Teshome et al.,  
393 2023; Levin et al., [in press](#).

394

395 Results of this study suggest five main trophic strategies among sympatric  
396 ecomorphs of the *Labeobarbus*: i) detritivory, ii) macrophytophagy, iii) invertivorous  
397 benthophagy, iv) periphyton feeding, and v) piscivory. Apart those, some ecomorphs had  
398 mixed modes of feeding - e.g., detritivory-macrophytophagy, detritivory-invertivory, and  
399 piscivory-omnivory (also with inclusion of a noted portion of detritus). Their more flexible



400 trophic specialization might be an adaptive strategy for living in mountain rivers with  
401 unstable hydrological regimes (Jepsen & Winemiller, 2002). Although the core of mouth  
402 phenotypes (generalized, thick-lipped, scraping, and large-mouthed) was the same in four  
403 riverine radiations of the *Labeobarbus*, some phenotypes display more diversified sets of  
404 ecologically relevant mouth subtypes. Scraping mouth phenotype was represented by three  
405 subtypes, which occupy four different trophic niches (periphyton feeding, detritophagy,  
406 invertivorous benthophagy, and macrophytophagy). It is an outstanding example of trophic  
407 diversification of scraping mouth phenotype that previously was considered as adapted for  
408 feeding via scraping the algal periphyton from stones and rocks (Matthes, 1963; see also  
409 Vreven et al., 2016). Recent studies uncovered similar patterns of diversifications within  
410 other phylogenetically distant cyprinid lineages bearing the scraping mouth phenotype - the  
411 *Garra* and *Schyzopygopsis*, in particular (Komarova et al., 2021; 2022; Levin et al., 2021a).  
412 Contrary to the *Labeobarbus* radiations that likely initiated from the generalized ancestor as  
413 most ubiquitous throughout the generic range, the highly specialized scraper lineages (*Garra*  
414 and *Schyzopygopsis*) could give a trophic radiation outside the ancestral narrow  
415 specialization.

416         The Large-mouthed phenotype of the *Labeobarbus* demonstrated further sub-  
417 specialization within the adaptive zone of the piscivory in the Didessa River while in other  
418 rivers this phenotype had only one feeding strategy that varied from obligate piscivory  
419 (Genale) via piscivory-omnivory (Gojeb) to non-piscivory mode of feeding (Sore). The sub-  
420 specialization of the Large-mouthed phenotype in the riverine environment is similar in some  
421 extent to the more diversified set of specializations discovered in Lake Tana where up to  
422 seven sympatric large-mouthed species/ecomorphs were described (Nagelkerke et al., 1994),  
423 whose piscivory specialization subdivided in benthic and pelagic zones (de Graaf et al.,  
424 2010).

425           A large incongruence between mouth phenotype and expected trophic niche is noted  
426 not only for the trophic specialists. The Generalized ecomorph, including Ethiopian species  
427 and populations, was considered omnivorous (Matthes, 1963; Teshome et al., 2023), but  
428 according to our data it is detritivorous in all studied rivers (Levin et al., 2019; 2023 - **in**  
429 **press**; this study). At the same time further diversification within Generalized mouth  
430 phenotype (A and B subtypes) have been found. It is the emergence of an ecomorph with  
431 horseshoe shape of the lower jaw without mental lobe and deep body in the Didessa River  
432 (Generalized B – Figure 2B). This ecomorph had lowest both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values among  
433 other sympatric ecomorphs occupying a niche of detritivore-macrophytophage (Figures 4-5).

434           Noticeably, a detritus was the main food not only for generalists but often for thick-  
435 lipped phenotype and also for all ecomorphs from the Sore River regardless their mouth  
436 phenotypes. We consider detritus as the most available and permanent trophic resource in  
437 riverine ecosystems of the Ethiopian Highlands with an unstable hydrological regime. In fact,  
438 it is a core food for the Generalized ecomorph and an important food resource for other  
439 ecomorphs.

440           Apart from the core mouth phenotypes, the intermediate ones were also found. For  
441 instance, i-Scraper phenotype is intermediate between the Scraping and Generalized. Its  
442 hybrid origin in the Genale River (by S-scraper and Generalized) was confirmed by genetic  
443 data (Levin et al., 2019). Isotopic niche of i-Scraper from the Genale was between parental  
444 species (Figure 5) but the gut content was similar to sympatric Generalized ecomorph.  
445 Supposedly, i-Scraper intermediate phenotype might have its own trophic sub-niche. Another  
446 example of intermediate mouth phenotype that seemingly occupies its own sub-niche refers  
447 to the Semi-thicklipped ecomorph (see Figures 4-5) in the Didessa River. Obtained results are  
448 consistent with the syngameon hypothesis, proposing that hybridization between members of

449 the radiation can promote further niche expansion and diversification (see Seehausen, 2004;  
450 Frei et al., 2022). Intermediate mouth phenotypes were reported among the *Labeobarbus* –  
451 between scraping ‘*Varicorhinus*’-like and generalized phenotypes by Banister (1972) and  
452 Nagelkerke and Sibbing (1996) as well as between scraping and thick-lipped mouth  
453 phenotypes by Vreven et al. (2019). In the last case the intermediate phenotype resembled the  
454 generalized one (Generalized B in our study). Authors revealed a hybrid nature of this  
455 phenotype by genetics (Vreven et al., 2019) but there was no data on trophic ecology or  
456 specialization. Actually, the recruiting of novel trophic niches via hybridization is an  
457 intriguing issue that is yet weakly studied (but see experimental study of Selz and Seehausen,  
458 2019).

459 In summary, the *Labeobarbus* trophic radiations involve a mixture of pelagic  
460 predator (piscivore) and a wide assortment of benthic-oriented specialists. The benthic-  
461 pelagic habitat axis is most frequent in cyprinid and cichlid adaptive radiations in both lakes  
462 and rivers (Nagelkerke et al., 1994; Cooper et al. 2010; Levin et al., 2019; Burress et al.,  
463 2023).

#### 464 4.2. *Decoupled form and function – preadaptive phenotypes and their functionalization.*

465 Our results show that the same mouth phenotypes in the *Labeobarbus* may occupy different  
466 trophic niches. A mismatch between expected and observed trophic specializations may have  
467 different nature: i) specialized phenotype has unspecialized (generalized or omnivorous)  
468 feeding mode that is known as Liem’s paradox (Liem, 1980; Robinson et al., 1998), and ii)  
469 specialized phenotype has unexpected (biased) trophic specialization possibly due to  
470 insufficient knowledge on trophic specializations in certain lineages. We focus in this study  
471 on the first phenomenon. Liem’s paradox was established for cichlid fishes (e.g., Liem, 1980;  
472 Sturmbauer et al., 1992; Wagner et al., 2009; Binning et al., 2009; Torres-Dowdall & Meyer,

473 2021) but it was also detected in other taxonomic groups (e.g., in cyprinids – Lammens et al.,  
474 1991; Levin et al., 2021a; Komarova et al., 2022). The mismatch between form and function  
475 might be i) a temporary phenomenon for proper specialist explained by plasticity of its diet in  
476 some circumstances (ecological release that may be a base for evolutionary re-specialization  
477 – see above-mentioned example of highly specialized scraping periphyton feeders of the  
478 genus *Garra* that could re-specialize in other trophic specializations – Komarova et al., 2022)  
479 or ii) heterochronous decouple of form and function when the emerging phenotype is not yet  
480 functionalized, i.e. phenotype is preadaptive (expectation on its trophic adaptation based on  
481 phenotypic features) but not yet involved in trophic resource partitioning. This is exactly the  
482 case of the mouth diversity in the Sore River, where detritus was a main food for all mouth  
483 phenotypes including the Large-mouthed, i.e. mouth divergence was uncorrelated with the  
484 use of food resources. In other words, mouth polymorphism in the Sore River is ecologically  
485 almost non-functional compared to other riverine diversifications. This contradicts the  
486 ‘ecological theory’ of adaptive radiation in neglecting the ‘habitat first rule’ in particular (see  
487 Schluter, 2000) but fits the flexible stem hypothesis (Wund et al., 2008; Gibert, 2017) or its  
488 variant known as the ‘plasticity-first’ evolution (Levis & Pfennig, 2016; 2019). According to  
489 this hypothesis, an adaptive phenotypic plasticity in an ancestral population could precede  
490 adaptation to a new environment through the process of genetic assimilation. Remarkably, the  
491 case of decoupled form and function in the *Labeobarbus* is shared to some extent with such  
492 in riverine adaptive radiations in the pike cichlids, genus *Crenicichla* (Burress et al., 2023)  
493 that may be a general feature for young radiations in riverine environments.

494 As already mentioned, the repeated mouth polymorphisms of the *Labeobarbus* may  
495 be predetermined due to its polyploid origin (Yang et al., 2022). Its maternal lineage is  
496 characterized by generalized / thick-lipped mouth polymorphism widely distributed among  
497 Torinae in the genera *Tor* and *Neolissochilus* (Hoang et al., 2015; Walton et al., 2017) – so

498 the generalized/thick-lipped polymorphism is persistent within a Torinae lineage. Paternal  
499 lineage referred to contemporary genus *Cyprinion* is represented by scraping mouth  
500 phenotype (Coad, 2021). Hence, mouth polymorphism of the *Labeobarbus* is ancestrally  
501 heritable and re-evolved under particular ecological circumstances. Given this, the thick-  
502 lipped and scraping mouthed phenotypes of the *Labeobarbus* in the Sore River are seemingly  
503 ‘preadaptive’ upon emergence *de-novo*.

504 Modular-assembling genome of the *Labeobarbus* consisted of the different  
505 subgenomes (Yang et al., 2022) that is able to produce discrete mouth phenotypes inherent to  
506 parental lineages is formally still within ‘flexible stem hypothesis’. However, from another  
507 hand, it might be also in frame of ‘transporter process’ (Schluter & Conte, 2009; Marques et  
508 al., 2019; Martin & Richards, 2019) meaning that the adaptive alleles and genetic  
509 architectures differentiating each species within a rapid radiation are older than the radiation  
510 itself. The oldest paleorecords of the *Labeobarbus* in East Africa are dated by Late Miocene  
511 (Stewart & Murray, 2017). Some trophic specialists, for instance, with scraping phenotype  
512 are remarkably older than the recently emerged repeated radiations in the Ethiopian  
513 Highlands under consideration. Their age is dated as Pleistocene according to molecular  
514 clocks (Beshera et al., 2016). It suggests that similar mouth diversification might evolved  
515 repeatedly before the diversifications we observe in this study. Therefore, the genomes of the  
516 modern *Labeobarbus* might be rather experienced, i.e. may have the standing genetic  
517 variation contributing to the ecological speciation and diversification. Regardless which is the  
518 certain prerequisite for adaptive radiation of the *Labeobarbus* – ‘flexible stem’ or ‘transporter  
519 process’ (or their combination) – it might significantly facilitate the diversification.

520 *4.3. Possible scenario of evolution of trophic radiation: from incipient to matured*

521 Our results demonstrate that four riverine assemblages of the *Labeobarbus* in the Ethiopian  
522 Highlands being similar in the core mouth phenotypes and direction of trophic diversification  
523 are at various stages of the evolution. The number of mouth phenotypes together with  
524 subtypes and their ecologic functionality in the *Labeobarbus* varied significantly from river  
525 to river. We tried to range the cases from simplest to most matured based on the obtained  
526 results – diversity of ecomorphs/mouth phenotypes and their functionality (as estimated by  
527 the gut content and isotopic niche) within each radiation (Table 3; Figures 4-5).

528 Table 3. Ranking the diversifications by number of ecological niches and other aspects of  
529 trophic differentiation. Number of points in rows 3 and 4 corresponds to the number of  
530 significant differences in pairwise comparisons between sympatric ecomorphs (taken from  
531 Supplementary File S4).

Nos.	Parameters	Sore	Genale	Gojeb	Didessa
1	Nos. mouth phenotypes	4	6	6	7 <sup>1</sup>
2	Nos. trophic niches <sup>2</sup>	2	4	5	5
3	Nos. dif. in $\delta^{15}\text{N}$	0	8	5	6
4	Nos. dif. in $\delta^{13}\text{C}$	2	0	6	6
5	Nos. trophic levels <sup>3</sup>	0.36	1.18	1.45	2.0

532 <sup>1</sup>Eight ecomorphs were detected in the Didessa but only seven were included in the study due to absence of data  
533 on the exceptionally rare Large-mouthed ecomorph. <sup>2</sup>Number of trophic niches is taken from Table 2. <sup>3</sup>One  
534 trophic level is arbitrary taken as 2.3‰ between mean  $\delta^{15}\text{N}$  values according to McCutchan et al. (2003) and  
535 Vanderklift & Ponsard (2003): the number of trophic levels was counted by dividing the difference between  
536 minimal and maximal mean  $\delta^{15}\text{N}$  values within a pool of sympatric ecomorphs by 2.3.  
537

538 The trophic diversifications could be ranged from the most undifferentiated in the Sore River  
539 via the Genale and Gojeb Rivers to the most diversified in the Didessa River (Table 3, Figure  
540 7). Next stages of the trophic diversification can be provisionally outlined (see scheme on  
541 Figure 7):

542 (1) Burst of mouth polymorphism. A set of core mouth phenotypes quickly emerges but  
543 their ecological functionalization is immature (preadaptive phenotypes).  
544 Morphological phenotypes are trophically irrelevant or there is a weak phenotype-  
545 trophic correlation (Liem's paradox). At this stage phenotypic diversity strongly  
546 dominates over the ecological diversity - see the case of the Sore River (four  
547 phenotypes vs. two trophic niches).

548 (2) Filling the trophic niches (ecological accommodation). Preadaptive phenotypes began  
549 to be functionalized by occupation of the trophic niches best matched to the mouth  
550 type. This process might be named as ecological accommodation of phenotypic trait –  
551 analogously to term 'phenotypic accommodation' sensu West-Eberhard (2003) The  
552 numbers of phenotypes and trophic niches are almost equal. Probably it is a very  
553 rapidly realizing process else it is hard to explain what are reasons to sustain non-  
554 functional mouth polymorphism.

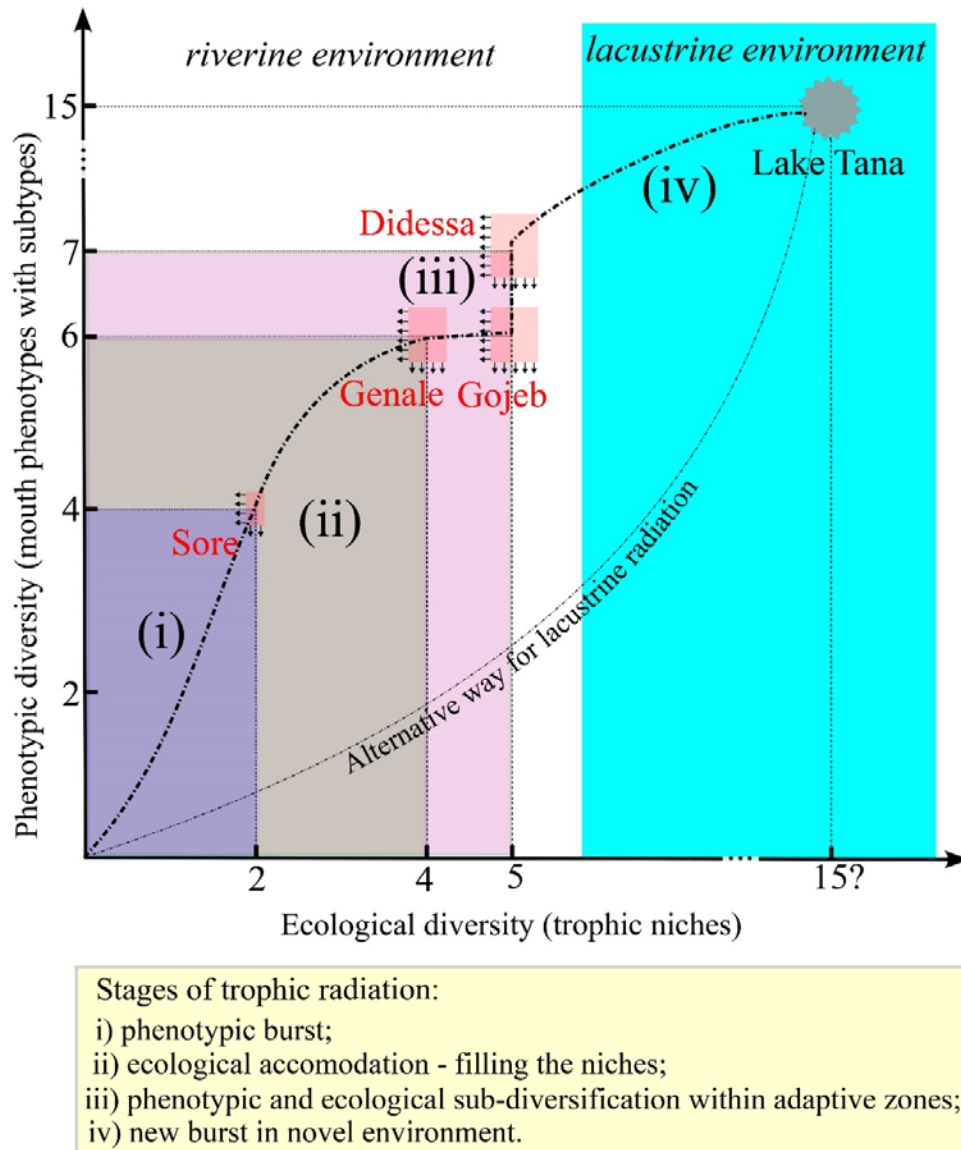
555 (3) Continued diversification within the core mouth phenotypes with corresponding  
556 increase of the ecological diversity. Upon filling the most evident trophic niches, a  
557 further sub-diversification occurs in some radiations. It might go by various scenarios  
558 - *via* ecological sub-specialization of certain phenotypes or *via* hybridization and  
559 functionalization of intermediate phenotypes. At matured stages of the diversification,  
560 the biotic interactions may serve as an additional ecological opportunity ('diversity  
561 begets diversity' – Martin & Richards, 2019). Significant increase of the populations  
562 size during morpho-ecological diversification (due to utilization of various trophic

563 resources in a river) makes the piscivory strategy more reliable that may result in sub-  
564 diversification within this adaptive zone - like in the Didessa River (see also  
565 cascading speciation - Broderson et al., 2017; Bracewell et al., 2018).

566 (4) A new diversification burst provoked by colonization of the novel environment might  
567 occur as exemplified from the lacustrine burst of the *Labeobarbus* in Lake Tana  
568 (Ethiopia) resulted in evolution of 15-16 species/ecomorphs (Nagelkerke et al., 1994;  
569 Mina et al., 1996; Sibbing, Nagelkerke, 1998; Nagelkerke et al., 2015; Beshera and  
570 Harris, 2023). It is still unknown whether Lake Tana radiation evolved from a single  
571 generalized ancestor or stemmed from already diversified set of riverine radiation.

572





573

574 Figure 7. Schematic reconstruction of process of morpho-ecological diversification of the  
 575 *Labeobarbus* in the Ethiopian Highlands. The number of little arrows at each box correspond  
 576 to the numbers of mouth phenotypes (horizontal arrows) and numbers of trophic niches  
 577 (vertical arrows).

578

579 The most immature diversification has been detected in the Sore River where a form  
 580 (mouth phenotype) and its ecological function were largely decoupled. The ecologically non-

581 functional or non-matured mouth polymorphism in the Sore River might be explained by  
582 several reasons. First, it might be due to a very young age of this radiation (at stage of  
583 incipient diversification) that is supported by low genetic diversity and absence of mtDNA  
584 haplotype sorting (Levin et al., 2020). Another reason refers to insufficient ecological  
585 opportunities in the river to realize the existing potential (mouth polymorphism).  
586 Heterogeneous and unpredictable environment in the rivers (flow regimes, turbidity, depth,  
587 water chemistry, temperature, food availability, etc.) may weaken the divergent selection  
588 necessary to drive specialization, instead favoring opportunistic ecological roles such as  
589 detritivory-omnivory (Jepsen and Winemiller, 2002; Burress et al., 2023). But the Sore River  
590 is a home for another bright adaptive radiation of hillstream cyprinid fish of the genus *Garra*  
591 that is represented by six genetically, morphologically and trophically divergent young  
592 species (Golubtsov et al., 2012; Levin et al., 2021a; Komarova et al., 2022). Possible  
593 overlapping in the trophic specializations (predator, periphyton feeding, benthic invertivore)  
594 with more advantageous cyprinid radiation in shared environment can acutely suppress or  
595 constrain the diversification process in the Sore population of the *Labeobarbus* resulting in  
596 freezing the non-functional mouth polymorphism stage for a while. Simultaneously, other  
597 studied riverine populations of the *Labeobarbus* have represented more advanced trophic  
598 diversification stages in absence of other sympatric radiations (as far as we know) and  
599 characterized by matured or ecologically functional mouth phenotypes.

600

## Conclusions

601 Using the polyploid African barbs of the genus *Labeobarbus* as model, we showed the  
602 parallel morpho-ecological diversifications being similar in this phenomenon with other  
603 prominent examples of adaptive radiation among vertebrate animals like cichlids (Seehausen,  
604 2015; Torres-Dowdall & Meyer, 2021; Burress et al., 2023) and Caribbean *Anolis* (Losos,

605 2009; Stroud & Losos, 2020). The most striking difference from above-mentioned examples  
606 is the sharply preadaptive nature of inherited mouth polymorphism as a starter of radiation.  
607 Likely, the diversification of the *Labeobarbus* starts from the onset of genomic pre-existing  
608 templates of mouth polymorphism. The emerged mouth phenotypes are preadaptive but not  
609 (or weakly) functional at the incipient stage of trophic diversification demonstrating  
610 decoupled form and function. Enhancement of ecological functions of preadaptive  
611 phenotypes (ecological accommodation) increases upon filling the trophic niches and result  
612 in maturation of trophic specialization that is often continued by further sub-diversification of  
613 mouth types and sub-specialization within previously occupied adaptive zones. The  
614 preadaptive mouth polymorphism can be considered a key innovation of the *Labeobarbus*  
615 that makes the bridge between phenotypic diversification and ecological opportunities  
616 remarkably shorter that might accelerate diversification rates. It apparently explains why this  
617 lineage overcomes the obstacles in unstable riverine environments unfavored for adaptive  
618 radiations by other fish lineages.

619

## 620 **Acknowledgements**

621 We are grateful to all members of the Joint Ethiopian–Russian Biological Expedition  
622 (JERBE), who participated in our field operations (S. E. Cherenkov, Y. Y. Dgebuadze,  
623 Genanaw Tesfaye, Fekadu Tefera, M. V. Mina, and I. S. Razgon), and especially to JERBE  
624 coordinator Dr. A. A. Darkov for his permanent and invaluable aid. We are grateful to S. E.  
625 Cherenkov for photographing the fish as well as to Y. Y. Dgebuadze and M. V. Mina for  
626 discussion of unpublished results. The study was supported by the Russian Science  
627 Foundation (grant no. 19-14-00218).

628

629 **Scientific field survey permission information.** All fish samples were collected under the  
630 framework of Joint Ethiopian-Russian Biological Expedition using the permits of the  
631 Ministry of Innovation and Technology, Addis Ababa, Ethiopia provided to A.S.G and  
632 B.A.L. by National Fishery and Other Aquatic Life Research Center of the Ethiopian Institute  
633 of Agricultural Research - EIAR, Sebeta.

634 **Data availability.** Raw isotopic data are available as Supplementary data.

635 **Supplementary data.** Supplementary data to this article can be found online.

636 **Competing interests.** The authors declare that they have no competing interests.

637 **Authors' contributions.** B.A.L. and A.S.G. conceived and designed the research, conducted  
638 field surveys and collected samples; A.S.K. and A.V.T. performed the stable isotope samples  
639 preparation and their analysis; A.S.K. performed diet analysis; All authors wrote the  
640 manuscript. All authors read and approved the final version of the manuscript.

641

642

## References

643 Alekseyev, S. S., Samusenok, V. P., Matveev, A. N. et al. (2002). Diversification, sympatric  
644 speciation, and trophic polymorphism of Arctic charr, *Salvelinus alpinus* complex, in  
645 Transbaikalia. *Environmental Biology of Fishes*, 64, 97-114.

646 Banister KE. 1972. On the cyprinid fish *Barbus alluaudi* Pellegrin: a possible intergeneric  
647 hybrid from Africa. Studies on African Cyprinidae. Part I. Bulletin of the British Museum  
648 (Natural History), Zoology 24: 261–290.

649 Banister, K.E. (1973). A revision of the large *Barbus* (Pisces, Cyprinidae) of East and Central  
650 Africa. Studies of African Cyprinidae. Part II. *Bulletin of the British Museum (Natural*  
651 *History) Zoology*, 26, 3–148. <https://doi.org/10.5962/bhl.part.204>

- 652 Bernatchez, L., Chouinard, A., & Lu, G. (1999). Integrating molecular genetics and ecology  
653 in studies of adaptive radiation: whitefish, *Coregonus* sp., as a case study. *Biological Journal*  
654 *of the Linnean Society*, 68(1-2), 173-194.
- 655 Beshera, K. A., Harris, P. M., & Michael, S. K. (2023). Relative Genetic Homogeneity within  
656 a Phenotypically Diverse group: The Case of Lake Tana *Labeobarbus* (Cyprinidae) Species  
657 Flock, Ethiopia. *Zootaxa*, 5301(2), 182-198.
- 658 Beshera, K. A., Harris, P. M., & Mayden, R. L. (2016). Novel evolutionary lineages in  
659 *Labeobarbus* (Cypriniformes; Cyprinidae) based on phylogenetic analyses of mtDNA  
660 sequences. *Zootaxa*, 4093(3), 363-381.
- 661 Binning, S.A., Chapman, L.J., & Cosandey-Godin, A. (2009). Specialized morphology for a  
662 generalist diet: evidence for Liem's paradox in a cichlid fish. *Journal of Fish Biology*, 75,  
663 1683–1699. <https://doi.org/10.1111/j.1095-8649.2009.02421.x>
- 664 Bracewell, R. R., Vanderpool, D., Good, J. M. et al. (2018). Cascading speciation among  
665 mutualists and antagonists in a tree-beetle-fungi interaction. *Proceedings of the Royal*  
666 *Society B*, 285(1881), 20180694.
- 667 Brawand, D., Wagner, C. E., Li, et al. (2014). The genomic substrate for adaptive radiation in  
668 African cichlid fish. *Nature*, 513(7518), 375-381.
- 669 Brodersen, J., Post, D. M., & Seehausen, O. (2018). Upward adaptive radiation cascades:  
670 predator diversification induced by prey diversification. *Trends in Ecology &*  
671 *Evolution*, 33(1), 59-70.
- 672 Burress, E. D., & Muñoz, M. M. (2022). Ecological opportunity from innovation, not islands,  
673 drove the anole lizard adaptive radiation. *Systematic Biology*, 71(1), 93-104.
- 674 Burress, E. D., Piálek, L., Casciotta, J. et al. (2023). Rapid parallel morphological and  
675 mechanical diversification of South American pike cichlids (*Crenicichla*). *Systematic*  
676 *Biology*, 72(1), 120-133.
- 677 Coad, B. Freshwater Fishes of Iran. Retrieved from  
678 <http://www.briancoad.com/Species%20Accounts/Contents%20new.htm> (2021).
- 679 Comtois, D. (2022). Summarytools: Tools to Quickly and Neatly Summarize Data. R  
680 Package Version 0.8. 72018. Available online: [https://CRAN.R-](https://CRAN.R-project.org/package=summarytools)  
681 [project.org/package=summarytools](https://CRAN.R-project.org/package=summarytools) (accessed on 26 July 2022)

- 682 Cooper, W. J., Parsons, K., McIntyre, A. et al. (2010). Benthopelagic divergence of cichlid  
683 feeding architecture was prodigious and consistent during multiple adaptive radiations within  
684 African rift-lakes. *PLoS one*, 5(3), e9551.
- 685 Decru, E., Snoeks, J., Walanga, A. et al. (2022). Disentangling the diversity of the  
686 *Labeobarbus* taxa (Cypriniformes: Cyprinidae) from the Epulu Basin (DR Congo, Africa).  
687 *Diversity*, 14, 1022. <https://doi.org/10.3390/d14121022>
- 688 Davis, A. M., Blanchette, M. L., Pusey, B. J. et al. (2012). Gut content and stable isotope  
689 analyses provide complementary understanding of ontogenetic dietary shifts and trophic  
690 relationships among fishes in a tropical river. *Freshwater Biology*, 57(10), 2156-2172.
- 691 De Graaf, M., van de Weerd, G. H., Osse, J. W. et al. (2010). Diversification of prey capture  
692 techniques among the piscivores in Lake Tana's (Ethiopia) *Labeobarbus* species flock  
693 (Cyprinidae). *African Zoology*, 45(1), 32-40.
- 694 Dimmick, W. W., Berendzen, P. B., & Golubtsov, A. S. (2001). Genetic comparison of three  
695 *Barbus* (Cyprinidae) morphotypes from the Genale River, Ethiopia. *Copeia*, 2001(4), 1123-  
696 1129.
- 697 Esin, E.V., Markevich, G.N., Melnik, N.O. et al. (2021). Natural toxic impact and thyroid  
698 signalling interplay orchestrates riverine adaptive divergence of salmonid fish. *Journal of*  
699 *Animal Ecology*, 90, 1004–1019. <https://doi.org/10.1111/1365-2656.13429>
- 700 Esmaili, H. R., & Piravar, Z. (2006). On the karyotype of *Cyprinion tenuiradius* Heckel,  
701 1849 (Pisces: Cyprinidae) from the Southwest of Iran. *Zoology in the Middle East*, 39(1), 75-  
702 80.
- 703 Frei, D., Reichlin, P., Seehausen, O. et al. (2023). Introgression from extinct species  
704 facilitates adaptation to its vacated niche. *Molecular Ecology*, 32(4), 841-853.
- 705 Fricke, R., Eschmeyer, W.N., & van der Laan, R. (eds.). (2023). Eschmeyer's Catalog of  
706 Fishes: Genera, Species, References. Available online:  
707 <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>  
708 (accessed on 12 June 2023).
- 709 Genner, M. J., & Turner, G. F. (2012). Ancient hybridization and phenotypic novelty within  
710 Lake Malawi's cichlid fish radiation. *Molecular Biology and Evolution*, 29(1), 195-206.
- 711 Gibert, J.-M. (2017). The flexible stem hypothesis: evidence from genetic data. *Development*  
712 *Genes and Evolution*, 227, 297–307. <https://doi.org/10.1007/s00427-017-0589-0>

- 713 Gillespie, R. G., Benjamin, S. P., Brewer, M. S. et al. (2018). Repeated diversification of  
714 ecomorphs in Hawaiian stick spiders. *Current Biology*, 28(6), 941-947.
- 715 Gregory, T. R., & Mable, B. K. (2005). Polyploidy in animals. In *The evolution of the*  
716 *genome* (pp. 427-517). Academic Press.
- 717 Golubtsov, A. S. (1993). Biogéographie des ‘grands *Barbus*’ d’Éthiopie avec référence  
718 spéciale à des formes à statuts taxinomiques incertains. *Cah. Ethol*, 13(2), 227-230.
- 719 Golubtsov, A. S. (2010). Fish ‘Species Flocks’ in Rivers and Lakes: Sympatric Divergence in  
720 Poor Fauna Fish Communities as Particular Modus of Evolution in *Relevant Problems of*  
721 *Contemporary Ichthyology* (ed. Pavlov, D. S., Dgebuadze, Y. Y., & Shatunovsky, M. I.) 96-  
722 123.
- 723 Golubtsov, A.S., & Krysanov, E.Y. (1993). Karyological study of some cyprinid species from  
724 Ethiopia. The ploidy differences between large and small *Barbus* of Africa. *Journal of Fish*  
725 *Biology*, 42, 445–455. <https://doi.org/10.1111/j.1095-8649.1993.tb00347.x>
- 726 Golubtsov, A.S., Korostelev, N.B., & Levin, B.A. (2021). Monsters with a shortened  
727 vertebral column: A population phenomenon in radiating fish *Labeobarbus* (Cyprinidae).  
728 *PLoS ONE*, 16, e0239639. <https://doi.org/10.1371/journal.pone.0239639>
- 729 Hashemzadeh Segherloo, I., Najafi Chaloshory, S., Naser, M. D. et al. (2022). Sympatric  
730 morphotypes of the restricted-range Tashan Cave *Garra*: distinct species or a case of  
731 phenotypic plasticity?. *Environmental Biology of Fishes*, 105(9), 1251-1260.
- 732 Hoang, H. D., Pham, H. M., Durand, J. D. et al. (2015). Mahseers genera *Tor* and  
733 *Neolissochilus* (Teleostei: cyprinidae) from southern Vietnam. *Zootaxa*, 4006(3), 551-568.
- 734 Hugueny, B., & Pouilly, M. (1999). Morphological correlates of diet in an assemblage of  
735 West African freshwater fishes. *Journal of Fish Biology*, 54(6), 1310-1325.
- 736 Jackson, A.L., Parnell, A.C., Inger, R. et al. (2011). Comparing isotopic niche widths among  
737 and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. *Journal of Animal*  
738 *Ecology*, 80, 595–602. <https://doi.org/10.1111/j.1365-2656.2011.01806.x>
- 739 Jepsen, D. B., & Winemiller, K. O. (2002). Structure of tropical river food webs revealed by  
740 stable isotope ratios. *Oikos*, 96(1), 46-55.



- 741 Irisarri, I., Singh, P., Koblmüller, S. et al. (2018). Phylogenomics uncovers early  
742 hybridization and adaptive loci shaping the radiation of Lake Tanganyika cichlid  
743 fishes. *Nature communications*, 9(1), 3159.
- 744 Komarova A.S., Rozanova O.L., & Levin B.A. (2021). Trophic resource partitioning by  
745 sympatric ecomorphs of *Schizopygopsis* (Cyprinidae) in a young Pamir Mountain lake:  
746 preliminary results. *Ichthyological Research*, 68, 191–197. [https://doi.org/10.1007/s10228-](https://doi.org/10.1007/s10228-020-00773-3)  
747 020-00773-3
- 748 Komarova, A.S., Golubtsov, A.S., & Levin, B.A. (2022). Trophic diversification out of  
749 ancestral specialization: an example from a radiating African cyprinid fish (genus *Garra*).  
750 *Diversity*, 14, 629. <https://doi.org/10.3390/d14080629>
- 751 Lammens, E. H. R. R., & Hoogenboezem, W. (1991). Diets and feeding behaviour.  
752 In *Cyprinid fishes: systematics, biology and exploitation* (pp. 353-376). Dordrecht: Springer  
753 Netherlands.
- 754 Levin, B. A. (2012). New data on morphology of the African scraping feeder *Varicorhinus*  
755 *beso* (Osteichthyes: Cyprinidae) with the special reference to specialized traits. *Journal of*  
756 *Ichthyology*, 52, 908-923.
- 757 Levin, B., Simonov, E., Franchini, P. et al. (2021a). Rapid adaptive radiation in a hillstream  
758 cyprinid fish in the East African White Nile River basin. *Molecular Ecology*, 30, 5530–5550.  
759 <https://doi.org/10.1111/mec.16130>
- 760 Levin, B.A., Komarova, A.S., Rozanova, O.L. et al. (2021b). Unexpected diversity of feeding  
761 modes among chisel-mouthed Ethiopian *Labeobarbus* (Cyprinidae). *Water*, 13, 2345.  
762 <https://doi.org/10.3390/w13172345>
- 763 Levin, B.A., Casal-López, M., Simonov, E. et al. (2019). Adaptive radiation of barbels of the  
764 genus *Labeobarbus* (Cyprinidae) in an East African river. *Freshwater Biology*, 64, 1721–  
765 1736. <https://doi.org/10.1111/fwb.13364>
- 766 Levin, B.A., Golubtsov, A.S., Dgebuadze, Y.Y. et al. (2013). New evidence of homoplasy  
767 within the African genus *Varicorhinus* (Cyprinidae): An independent origin of specialized  
768 scraping forms in the adjacent drainage systems of Ethiopia inferred from mtDNA analysis.  
769 *African Zoology*, 48, 400–406. <https://doi.org/10.1080/15627020.2013.11407609>



- 770 Levin, B., Komarova, A., Simonov, E. et al. (2023). A speciation continuum within repeated  
771 origin of hypertrophied lips in cyprinid fish from East Africa. *Ecology and Evolution* – in  
772 press.
- 773 Levin, B.A., Simonov, E., Dgebuadze, Y.Y. et al. (2020). In the rivers: Multiple adaptive  
774 radiations of cyprinid fishes (*Labeobarbus*) in Ethiopian Highlands. *Scientific Reports*, *10*,  
775 7192. <https://doi.org/10.1038/s41598-020-64350-4>.
- 776 Levis, N. A., & Pfennig, D. W. (2016). Evaluating ‘plasticity-first’ evolution in nature: key  
777 criteria and empirical approaches. *Trends in ecology & evolution*, *31*(7), 563-574.
- 778 Levis, N. A., & Pfennig, D. W. (2019). Plasticity-led evolution: evaluating the key prediction  
779 of frequency-dependent adaptation. *Proceedings of the Royal Society B*, *286*(1897),  
780 20182754.
- 781 Li, X., & Guo, B. (2020). Substantially adaptive potential in polyploid cyprinid fishes:  
782 evidence from biogeographic, phylogenetic and genomic studies. *Proceedings of the Royal*  
783 *Society B*, *287*(1920), 20193008.
- 784 Liem, K.F. (1980). Adaptive significance of intra- and interspecific differences in the feeding  
785 repertoires of cichlid fishes. *American Zoologist*, *20*, 295–314.  
786 <https://doi.org/10.1093/icb/20.1.295>
- 787 Losos J.B. 2009. Lizards in an evolutionary tree: ecology and adaptive radiation of anoles  
788 (Vol. 10). Los Angeles: University of California Press.
- 789 Lysy, M., Stasko, A.D., & Swanson, H.K. (2021). nicheROVER: Niche Region and Niche  
790 Overlap Metrics for Multidimensional Ecological Niches. R package version 1.1.0. 2021.  
791 Available online: <https://CRAN.R-project.org/package=nicheROVER> (accessed on 26 July  
792 2022).
- 793 Markevich, G., Esin, E., & Anisimova, L. (2018). Basic description and some notes on the  
794 evolution of seven sympatric morphs of Dolly Varden *Salvelinus malma* from the Lake  
795 Kronotskoe Basin. *Ecology and Evolution*, *8*(5), 2554-2567.
- 796 Marques, D. A., Meier, J. I., & Seehausen, O. (2019). A combinatorial view on speciation  
797 and adaptive radiation. *Trends in ecology & evolution*, *34*(6), 531-544.
- 798 Matthes, H. (1963). A comparative study of the feeding mechanisms of some African  
799 Cyprinidae (Pisces, Cypriniformes). *Bijdragen tot de Dierkunde*, *33*, 3–35.  
800 <https://doi.org/10.1163/26660644-03301001>

- 801 Martin, C. H., & Richards, E. J. (2019). The paradox behind the pattern of rapid adaptive  
802 radiation: how can the speciation process sustain itself through an early burst?. *Annual review*  
803 *of ecology, evolution, and systematics*, 50, 569-593.
- 804 McCutchan Jr, J. H., Lewis Jr, W. M., Kendall, C. et al. (2003). Variation in trophic shift for  
805 stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, 102(2), 378-390.
- 806 McGee, M. D., Borstein, S. R., Meier et al. (2020). The ecological and genomic basis of  
807 explosive adaptive radiation. *Nature*, 586(7827), 75-79.
- 808 Meier, J. I., Marques, D. A., Mwaiko, S. et al. (2017). Ancient hybridization fuels rapid  
809 cichlid fish adaptive radiations. *Nature communications*, 8(1), 14363.
- 810 Meyer, A., & Van de Peer, Y. (2005). From 2R to 3R: evidence for a fish-specific genome  
811 duplication (FSGD). *Bioessays*, 27(9), 937-945.
- 812 Mina, M.V., Mironovsky, A.N., Dgebuadze, Y. (1996). Lake Tana large barbs: phenetics,  
813 growth and diversification. *Journal of Fish Biology*, 48, 383-404.  
814 <https://doi.org/10.1111/j.1095-8649.1996.tb01435.x>.
- 815 Mina, M.V., Mironovsky, A.N., Golubtsov, A.S. et al. (1998). II – Morphological diversity of  
816 "large barbs" from Lake Tana and neighbouring areas: Homoplasies or synapomorphies?  
817 *Italian Journal of Zoology*, 65(S1), 9-14. <https://doi.org/10.1080/11250009809386789>
- 818 Nagelkerke, L. A., Mina, M. V., Wudneh, T. et al. (1995). In Lake Tana, a unique fish fauna  
819 needs protection. *Bioscience*, 772-775.
- 820 Nagelkerke, L. A. J., & Sibbing, F. A. (1996). Reproductive segregation among the *Barbus*  
821 *intermedius* complex of Lake Tana, Ethiopia. An example of intralacustrine  
822 speciation?. *Journal of Fish Biology*, 49(6), 1244-1266.
- 823 Nagelkerke, L.A.J., Sibbing, F.A., van den Boogaart, J.G.M. et al. (1994). The barbs (*Barbus*  
824 spp.) of Lake Tana: a forgotten species flock? *Environmental Biology of Fishes*, 39, 1-22.  
825 <https://doi.org/10.1007/BF00004751>
- 826 Natarajan, A.V., & Jhingran, A.G. (1961). Index of preponderance – A method of grading the  
827 food elements in the stomach analysis of fishes. *Indian Journal of Fisheries*, 8, 54-59.
- 828 Nosil, P., Harmon, L. J., & Seehausen, O. (2009). Ecological explanations for (incomplete)  
829 speciation. *Trends in ecology & evolution*, 24(3), 145-156.

- 830 Piálek, L., Říčan, O., Casciotta, J. et al. (2012). Multilocus phylogeny of *Crenicichla*  
831 (Teleostei: Cichlidae), with biogeography of the *C. lacustris* group: species flocks as a model  
832 for sympatric speciation in rivers. *Molecular Phylogenetics and Evolution*, 62(1), 46-61.
- 833 Popova, O.A., & Reshetnikov, Y.S. (2011). On complex indices in investigation of fish  
834 feeding. *Journal of Ichthyology*, 51, 686–691. <https://doi.org/10.1134/S0032945211050171>
- 835 Říčan, O., Říčanová, Š., Almirón, A. et al. (2021). Parallel evolution of a new sympatric  
836 species pair of *Crenicichla* (Teleostei: Cichlidae) from Misiones, Argentina with a review of  
837 biogeography and mitonuclear discordance in the *C. mandelburgeri* species complex. *Journal*  
838 *of Zoological Systematics and Evolutionary Research*, 59(8), 2134-2149.
- 839 Roberts, T.R., & Khaironizam, M.Z. (2008). Trophic polymorphism in the Malaysian fish  
840 *Neolissochilus soroides* and other Old World Barbs (Teleostei, Cyprinidae). *Natural History*  
841 *Bulletin of the Siam Society*, 56, 25–53.
- 842 Robinson, B.W., Wilson, D.S. (1998). Optimal foraging, specialization, and a solution to  
843 Liem's Paradox. *The American Naturalist*, 151, 223–235. <https://doi.org/10.1086/286113>
- 844 Ronco, F., Matschiner, M., Böhne et al. (2021). Drivers and dynamics of a massive adaptive  
845 radiation in cichlid fishes. *Nature*, 589(7840), 76-81.
- 846 RStudio Team. (2023). *RStudio: Integrated Development for R*; RStudio, PBC: Boston, MA,  
847 USA. Available online: <http://www.rstudio.com> (accessed on 26 July 2022).
- 848 Schluter, D. (2000). The ecology of adaptive radiation. New York: Oxford University Press.  
849 300 p.
- 850 Schluter, D., & Conte, G. L. (2009). Genetics and ecological speciation. *Proceedings of the*  
851 *National Academy of Sciences*, 106(supplement\_1), 9955-9962.
- 852 Seehausen, O. (2004). Hybridization and adaptive radiation. *Trends in ecology & evolution*,  
853 19(4), 198-207.
- 854 Seehausen, O. (2015). Process and pattern in cichlid radiations—inferences for understanding  
855 unusually high rates of evolutionary diversification. *New Phytologist*, 207(2), 304-312.
- 856 Seehausen, O., & Wagner, C.E. (2014). Speciation in Freshwater Fishes. *Annual Review of*  
857 *Ecology, Evolution, and Systematics*, 45, 621–651. [https://doi.org/10.1146/annurev-ecolsys-](https://doi.org/10.1146/annurev-ecolsys-120213-091818)  
858 [120213-091818](https://doi.org/10.1146/annurev-ecolsys-120213-091818)

- 859 Selz, O. M., & Seehausen, O. (2019). Interspecific hybridization can generate functional  
860 novelty in cichlid fish. *Proceedings of the Royal Society B*, 286(1913), 20191621.
- 861 Shkil, F. N., Lazebnyi, O. E., Kapitanova, D. V. et al. (2015). Ontogenetic mechanisms of  
862 explosive morphological divergence in the Lake Tana (Ethiopia) species flock of large  
863 African barbs (*Labeobarbus*; Cyprinidae; Teleostei). *Russian journal of developmental*  
864 *biology*, 46, 294-306.
- 865 Sibbing, F.A., & Nagelkerke, L.A.J. (2000). Resource partitioning by Lake Tana barbs  
866 predicted from fish morphometrics and prey characteristics. *Reviews in Fish Biology and*  
867 *Fisheries*, 10, 393–437. <https://doi.org/10.1023/A:1012270422092>
- 868 Skelton, P. H., Tweddle, D., & Jackson, P. B. N. (1991). Cyprinids of Africa. *Cyprinid fishes:*  
869 *systematics, biology and exploitation*, 211-239.
- 870 Stewart, K. M., & Murray, A. M. (2017). Biogeographic implications of fossil fishes from the  
871 Awash River, Ethiopia. *Journal of Vertebrate Paleontology*, 37(1), e1269115.
- 872 Stroud, J. T., & Losos, J. B. (2020). Bridging the process-pattern divide to understand the  
873 origins and early stages of adaptive radiation: a review of approaches with insights from  
874 studies of Anolis lizards. *Journal of Heredity*, 111(1), 33-42.
- 875 Sturmbauer, C., Mark, W., & Dallinger, R. (1992). Ecophysiology of Aufwuchs-eating  
876 cichlids in Lake Tanganyika: Niche separation by trophic specialization. *Environmental*  
877 *Biology of Fishes*, 35, 283–290. <https://doi.org/10.1007/BF00001895>
- 878 Svardal, H., Quah, F. X., Malinsky, M. et al. (2020). Ancestral hybridization facilitated  
879 species diversification in the Lake Malawi cichlid fish adaptive radiation. *Molecular Biology*  
880 *and Evolution*, 37(4), 1100-1113.
- 881 Swanson, H.K., Lysy, M., Power, M. et al. (2015). A new probabilistic method for  
882 quantifying n-dimensional ecological niches and niche overlap. *Ecology*, 96, 318–324.  
883 <https://doi.org/10.1890/14-0235.1>
- 884 Tan, M., & Armbruster, J. W. (2018). Phylogenetic classification of extant genera of fishes of  
885 the order Cypriniformes (Teleostei: Ostariophysi). *Zootaxa*, 4476(1), 6-39.
- 886 Teshome, M., Mingist, M., Dadebo, E. et al. (2023). Food and feeding habits of the large  
887 cyprinid fishes in the upper Blue Nile River, Ethiopia. *Fisheries and Aquatic Sciences*, 26(5),  
888 344-354.

- 889 Torres-Dowdall, J., & Meyer, A. (2021). Sympatric and Allopatric Diversification in the  
890 Adaptive Radiations of Midas Cichlids in Nicaraguan Lakes. In M.E. Abate & D.L. Noakes  
891 (Eds.), *The Behavior, Ecology and Evolution of Cichlid Fishes. Fish & Fisheries Series*, vol  
892 40. (pp. 175–216). Springer, Dordrecht. [https://doi.org/10.1007/978-94-024-2080-7\\_6](https://doi.org/10.1007/978-94-024-2080-7_6)
- 893 Turner, B. J., Grudzien, T. A., Adkisson, K. P. et al. (1985). Extensive chromosomal  
894 divergence within a single river basin in the goodeid fish, *Ilyodon*  
895 *furcoidens*. *Evolution*, 39(1), 122-134.
- 896 Qiao, J., Hu, J., Xia, Q. et al. (2020). Pelagic–benthic resource polymorphism in  
897 *Schizopygopsis thermalis* Herzenstein 1891 (Pisces, Cyprinidae) in a headwater lake in the  
898 Salween River system on the Tibetan Plateau. *Ecology and evolution*, 10(14), 7431-7444.
- 899 Van de Peer, Y., Ashman, T. L., Soltis, P. S. et al. (2021). Polyploidy: an evolutionary and  
900 ecological force in stressful times. *The Plant Cell*, 33(1), 11-26.
- 901 Van de Peer, Y., Mizrachi, E., & Marchal, K. (2017). The evolutionary significance of  
902 polyploidy. *Nature Reviews Genetics*, 18(7), 411-424.
- 903 Vanderkluft, M. A., & Ponsard, S. (2003). Sources of variation in consumer-diet  $\delta^{15}\text{N}$   
904 enrichment: a meta-analysis. *Oecologia*, 136, 169-182.
- 905 Vreven, E. J., Musschoot, T., Decru, E. et al. (2019). The complex origins of mouth  
906 polymorphism in the *Labeobarbus* (Cypriniformes: Cyprinidae) of the Inkisi River basin  
907 (Lower Congo, DRC, Africa): Insights from an integrative approach. *Zoological Journal of*  
908 *the Linnean Society*, 186(2), 414-482.
- 909 Vreven, E.J., Musschoot, T., Snoeks, J. et al. (2016). The African hexaploid Torini  
910 (Cypriniformes: Cyprinidae): Review of a tumultuous history. *Zoological Journal of the*  
911 *Linnean Society*, 177, 231–305. <https://doi.org/10.1111/zoj.12366>
- 912 Wagner, C.E., McIntyre, P.B., Buels, K.S. et al. (2009). Diet predicts intestine length in Lake  
913 Tanganyika's cichlid fishes. *Functional Ecology*, 23, 1122–1131.  
914 <https://doi.org/10.1111/j.1365-2435.2009.01589.x>
- 915 Walton, S. E., Gan, H. M., Raghavan, R. et al. (2017). Disentangling the taxonomy of the  
916 mahseers (*Tor* spp.) of Malaysia: An integrated approach using morphology, genetics and  
917 historical records. *Reviews in Fisheries Science & Aquaculture*, 25(3), 171-183.
- 918 West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University  
919 Press.

- 920 Wickham, H. (2022). *ggplot2: Elegant Graphics for Data Analysis*. <http://ggplot2.org>
- 921 Worthington, E.B. (1929). New species of fish from the Albert Nyanza and Lake Kioga.  
922 *Proceedings of the Zoological Society of London* 1929: 429–440.
- 923 Wund, M.A., Baker, J.A., Clancy et al. (2008). A Test of the “Flexible Stem” Model of  
924 Evolution: Ancestral Plasticity, Genetic Accommodation, and Morphological Divergence in  
925 the Threespine Stickleback Radiation. *The American Naturalist*, 172, 449–462.  
926 <https://doi.org/10.1086/590966>
- 927 Yang, L., Naylor, G.J.P., & Mayden, R.L. (2022). Deciphering reticulate evolution of the  
928 largest group of polyploid vertebrates, the subfamily Cyprininae (Teleostei: Cypriniformes).  
929 *Molecular Phylogenetics and Evolution*, 166, 107323.  
930 <https://doi.org/10.1016/j.ympev.2021.107323>
- 931 Yang, L., Sado, T., Hirt, M.V. et al. (2015). Phylogeny and polyploidy: Resolving the  
932 classification of cyprinine fishes (Teleostei: Cypriniformes). *Molecular Phylogenetics and*  
933 *Evolution*, 85, 97–116. <https://doi.org/10.1016/j.ympev.2015.01.014>
- 934 Zworykin, D. D., Budaev, S. V., Darkov, A. A. et al. (2006). Assessment of the role of  
935 chemoreception in the mate choice in barbs of the *Barbus intermedius* complex from Lake  
936 Tana, Ethiopia. *Journal of Ichthyology*, 46, 661-667.
- 937
- 938