



18 **Abstract**

19 Adaptive radiation of fishes was long thought to be possible only in lacustrine environments.  
20 Recently, several studies have shown that also riverine and stream environments provide the  
21 ecological opportunity for adaptive radiation. In this study, we report on a riverine adaptive  
22 radiation of six ecomorphs of cyprinid hillstream fishes of the genus *Garra* in a river located in  
23 the Ethiopian Highlands in East Africa. *Garra* are predominantly highly specialized algae-  
24 scrapers with a wide distribution ranging from Southeastern Asia to Western Africa. However,  
25 adaptive phenotypic diversification in mouth type, sucking disc morphology, gut length and  
26 body shape have been found among these new species in a single Ethiopian river. Moreover, we  
27 found two novel phenotypes of *Garra* ('thick-lipped' and 'predatory') that were not described  
28 before in this species-rich genus (>160 species). Mitochondrial and genome-wide data suggest  
29 monophyletic, intra-basin evolution of *Garra* phenotypic diversity with signatures of gene flow  
30 from other local populations. Although sympatric ecomorphs are genetically distinct and can be  
31 considered to being young species as suggested by genome-wide SNP data, mtDNA was unable  
32 to identify any genetic structure suggesting a recent and rapid speciation event. Furthermore, we  
33 found evidence for a hybrid origin of the novel 'thick-lipped' phenotype, as being the result of  
34 the hybridization of two other sympatrically occurring species. Here we highlight how, driven by  
35 ecological opportunity, an ancestral trophically highly specialized lineage is likely to have  
36 rapidly adaptively radiated in a riverine environment, and that this radiation was promoted by the  
37 evolution of novel feeding strategies.

## 38 **Introduction**

39 Unravelling the mechanisms underpinning the biological diversity remains a major challenge in  
40 evolutionary biology. With more than 28,000 species, teleost fishes are the most diverse lineage  
41 of vertebrates, and thus an ideal system to address questions regarding diversification. The  
42 stunning phenotypic diversity of bony fishes has largely been produced through the process of  
43 adaptive radiation, the rapid proliferation of multiple ecologically distinct species from a  
44 common ancestor (Schluter, 2000). One of the most extraordinary examples of both adaptive  
45 radiation and explosive diversification is represented by the cichlid fishes inhabiting the East  
46 African Great Lakes (Kocher, 2004). According to Losos (2010) and Givnish (2015) adaptive  
47 radiation and explosive diversification are distinct phenomena: the former may or may not result  
48 in, or be accompanied by the latter. The evolutionary success of the cichlids, unmatched among  
49 vertebrates, has been promoted by a combination of different factors, where a dominant role has  
50 been played, for example, by limited dispersal (because of territoriality and mouth-brooding) and  
51 sexual selection for nuptial coloration and mating behavior (Henning & Meyer, 2014; Meyer,  
52 Kocher, Basasibwaki, & Wilson, 1990; Seehausen, 2000; Wagner, Harmon, & Seehausen, 2012).  
53 It has been suggested, however, that trophic radiation had preceded the diversification driven by  
54 other factors at least in cichlids of Lake Tanganyika (Muschick et al., 2014), a cradle of all other  
55 East African haplochromine radiations (Salzburger, Mack, Verheyen, E., & Meyer, 2005).  
56 Adaptive radiations and diversification bursts were found not only in cichlids, but also in other  
57 fish groups, even though in smaller scale, and often in a parallel manner - coregonids, Arctic  
58 charrs, and sticklebacks (e.g. Broderson, Post, & Seehausen, 2018; DeFaveri & Merila, 2013;  
59 Jacobs et al., 2020; McKinnon & Rundle, 2002; Præbel et al., 2013; Peichel et al., 2001;  
60 Schluter, 2000; Skúlason, 1999; Terekhanova et al., 2014) - some of the best known examples of  
61 intralacustrine radiations.

62 The most supported cases of monophyletic, closely related fish species that are believed to  
63 have arisen through an adaptive radiation event have been described from lakes rather than rivers

64 (Meyer et al. 1990; Seehausen, 2006; Sturmbauer, 1998; Taylor, 1999). For long time, riverine  
65 environment has not been considered suitable for adaptive radiation because of its unstable  
66 hydrological regimes, reduced habitat diversity and the commonly shallow and narrow  
67 watercourses that might facilitate gene flow (Seehausen & Wagner, 2014). However, during the  
68 last two decades, examples of fish adaptive radiations occurring in rivers have been reported  
69 (Burress et al., 2018; Dimmick, Berendzen, & Golubtsov, 2001; Levin, Simonov, Dgebuadze,  
70 Levina, & Golubtsov, 2020; Melnik, Markevich, Taylor, Loktyushkin, & Esin, 2020; Piálek,  
71 Říčan, Casciotta, Almirón, & Zrzavý, 2012; Schwarzer, Misof, Ifuta, & Schliewen, 2011;  
72 Whiteley, 2007). Although several cases of riverine diversification of cichlid fishes are  
73 considered as remnants of adaptive radiations occurred in the palaeo-Lake Makgadikgadi before  
74 it dried up back in the Holocene (Joyce et al., 2005), mounting evidence suggests that some fish  
75 species flocks of other species than cichlids have diversified within rivers (Burress et al., 2018;  
76 Levin et al., 2019; 2020; Melnik et al., 2020; Piálek et al., 2012)

77 In the present study, we investigate a highly diverse fish group that presumably adaptively  
78 radiated in riverine environments. The genus *Garra* is a species-rich lineage of labeonine  
79 cyprinids comprising more than 160 species and is distributed from Southeast Asia to West  
80 Africa (Fricke, Eschmeyer, & Van der Laan, 2021; Yang et al., 2012). *Garra* are mostly  
81 moderate-sized fish (usually less than 20 cm in length) with sucking gular disc that inhabit the  
82 rhithron zone of river systems (Kottelat, 2020). They are predominantly highly specialized algae  
83 scrapers that graze periphyton from rocks and stones using widened jaws equipped with horny  
84 scrapers. However, adaptations to still waters such as caves or lacustrine environment have been  
85 documented in the *Garra*, although rarely, accompanied by a reduction of the gular disc and a  
86 change of the foraging strategy from algae scraping to planktivory (Geremew, 2007; Kottelat,  
87 2020; Segherloo et al., 2018; Stiassny & Getahun, 2007; [www.briancoad.com](http://www.briancoad.com)).

88 The Ethiopian Highlands are recognized as a center of *Garra* diversity within Africa  
89 (Golubtsov, Dgebuadze, & Mina, 2002; Stiassny & Getahun, 2007), where 13 described species

90 out of the total 23 found in Africa are recorded (Moritz, El Dayem, Abdallah, & Neumann,  
91 2019). An assemblage of six *Garra* ecomorphs exhibiting extreme morphological diversity was  
92 recently discovered in the Sore River (the White Nile Basin) in southwestern Ethiopia during a  
93 survey of the Ethiopian fishes (Golubtsov, Cherenkov, & Tefera, 2012). In particular, two of the  
94 six forms display features not found elsewhere within the generic range: a form with a  
95 pronounced predatory morphology (large-sized, large-mouthed, with reduced sucking disk and a  
96 short gut that is equal to body length) and one with ‘rubber’ lips and prolonged snout region  
97 (Fig. 1, Table 1). The other four forms from the Ethiopian *Garra* assemblage drastically differ in  
98 mouth and gular disc morphology as well as in body shape (Fig. 1).

99 Fig. 1.

100 Our goals were twofold: i) to investigate the morpho-ecological relationships of six *Garra*  
101 sympatric ecomorphs from the Sore River, and ii) to test whether this assemblage has evolved  
102 sympatrically. In detail, we aimed at elucidating the population structure and evolutionary  
103 history of these ecomorphs using both mitochondrial DNA (mtDNA, cytochrome *b*) and  
104 genome-wide nuclear loci obtained with a double digest restriction-site associated DNA  
105 (ddRAD) approach.

106 Table 1.

107

## 108 **Materials and Methods**

### 109 *Study area*

110 The Sore River is a headwater tributary of the Baro-Akobo-Sobat drainage in the White Nile  
111 basin, (south-western Ethiopia, northern East Africa). It drains the Ethiopian Highlands close to  
112 the south-western escarpment. The region is covered by moist Afromontane forest that is  
113 drastically shrinking in the last decades due to agricultural development (Dibaba, Soromessa, &  
114 Workineh, 2019). The Sore is a rather little river with a length of *ca.* 160 km, its catchment area  
115 is *ca.* 2000 km<sup>2</sup> and characterized by substantial seasonal variation of rainfall (dry season from

116 December to March) (Kebede, Diekkrüger, & Moges, 2014). In comparison, the Italian Tiber  
117 River length is 406 km, its catchment area is 17375 km<sup>2</sup> (<https://en.wikipedia.org/wiki/Tiber>).  
118 Elevation difference between the Sore source (altitude of ca. 2215 m asl, above sea level) and its  
119 confluence with the Gabba (Geba) River (alt. 963 m asl) is 1.25 km. The Sore River basin shares  
120 drainage boundaries with two of six major watersheds of Ethiopia: Blue Nile in the north-east  
121 and Omo-Turkana in the south-east.

122 We sampled the middle reaches of the Sore River at two sites: (1) at the City of Metu  
123 (8°18'42" N 35°35'54" E, alt. 1550 m asl) and (2) ca. 35 km downstream along the river course  
124 (8°23'56" N 35°26'18" E, alt. 1310 m asl). The river width at the rapids sampled was 20-40 m at  
125 the beginning of the rainy season, depth <1 m, bottom consisted of rocks and large boulders. Fish  
126 fauna of the river segment under consideration includes (apart from *Garra* spp.) a species flock  
127 of *Labeobarbus* (Levin et al., 2020), *Enteromius* cf. *pleurogramma* (Boulenger 1902), *Labeo* cf.  
128 *cylindricus* Peters 1852, *Labeo forskalii* Rüppell 1835, *Chiloglanis* cf. *niloticus* Boulenger 1900  
129 (at the lower site only), and introduced *Coptodon zillii* (Gervais 1848). Presence of the stony  
130 loach (*Afronemacheilus*) reported by Getahun and Stiassny (1998) from the Sore River at Metu  
131 could no longer be confirmed (Melaku, Abebe Getahun, & Wakjira, 2017; Prokofiev &  
132 Golubtsov, 2013; present study). Attempts to re-sample a stony loach by intensive electrofishing  
133 in 2012 have resulted in the discovery of the enormous morphological *Garra* diversity in the  
134 Sore River (Golubtsov et al., 2012). A hundred kilometers westward, from the lowland part (alt.  
135 ca. 500 m asl) of the same river drainage >100 fish species are recorded (Golubtsov & Darkov,  
136 2008; Golubtsov, Darkov, Dgebuadze, 1995;) and >115 species from the Sudd and White Nile in  
137 Sudan and South Sudan (Moritz et al., 2019; Neumann, Obermaier, & Moritz, 2016;).

138

### 139 *Sampling*

140 *Garra* samples from the Sore River were collected using a battery driven electrofishing device  
141 (LR-24 Combo Backpack, Smith-Root, USA), cast and frame nets in June 2012 and April 2014.

142 In 2011-2014 comparative *Garra* samples were collected from nine sites in six main Ethiopian  
143 basins (Fig. 2, Table S1). Fish sampling was conducted under the umbrella of the Joint  
144 Ethiopian-Russian Biological Expedition (JERBE) with the permissions of National Fisheries  
145 and Aquatic Life Research Center (NFALRC) under Ethiopian Institute of Agricultural Research  
146 (EIAR) and Ethiopian Ministry of Science and Technology (presently Ministry of Innovation  
147 and Technology). Fish were killed with an overdose of an anesthetic MS-222, first preserved in  
148 10% formalin and then transferred to 70% ethanol. From each specimen fin tissue samples were  
149 fixed with 96% ethanol. Some fish specimens were pictured using a Canon EOS 50D camera.  
150 All specimens (Supplementary Table S1) are deposited at the A.N. Severtsov Institute of  
151 Ecology and Evolution, at the Russian Academy of Sciences, Moscow, under provisional labels  
152 of JERBE.

153

154 Fig. 2.

155

## 156 ***Morphological analysis***

### 157 *Morphometry*

158 The 28 morphometric characters from 107 individuals of all ecomorphs from the Sore River  
159 were examined following Hubbs and Lagler (1958) with additions from Menon (1964): standard  
160 length (SL), head length (HL), snout length (R), eye diameter (O), postorbital distance (PO),  
161 interorbital distance (IO), head width (HW), head height at nape (HH), head height at mid-of-eye  
162 (Hh), mouth width (MW), disc length (DL), disc width (DW), maximal body height (H),  
163 minimal body height at caudal peduncle (h), predorsal length (PL), postdorsal length (PDL),  
164 prepelvic length (PPL), preanal length (PAL), caudal peduncle length (CPD), dorsal fin base  
165 length (DFL), dorsal fin depth (DFP), anal fin base length (AFL), anal fin depth (AFD), pectoral  
166 fin length (PFL), ventral fin length (VFL), pectoral-ventral fin distance (PV), ventral-anal fin  
167 distance (VA), and distance between anal opening and anal fin (DAA). Measurements were done

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168 using a digital caliper (to nearest 0.1 mm). All measurements were performed by one operator  
169 for the purpose of consistency as recommended by Mina, Levin, and Mironovsky (2005).

170 Measured individuals had body length varied from 43.6 to 185.0 mm SL: ecomorph 1  
171 (71.5-151.0), ecomorph 2 (70.9-160.2), ecomorph 3 (49.3-100.6), ecomorph 4 (49.3-90.6),  
172 ecomorph 5 (43.6-81.0; one individual had outstanding length - 185.0), ecomorph 6 (118.4;  
173 139.4) (defined as in Fig. 1 and Table 1), intermediate phenotypes (59.3-105.2). The proportions  
174 of head and body were used for principal component analysis (PCA) - measurements of head  
175 parts were divided for head length and measurements of body parts were divided for standard  
176 length. Data was scaled. The gular disc in some specimens of ecomorph 5 was greatly reduced  
177 which hampered the detection of its borders. For the purpose of justification of the values of this  
178 character, the identical intermediate values were arbitrarily assigned for all specimens of this  
179 ecomorph. PCA was done using *prcomp* script implemented in R with a variance-covariance  
180 matrix.

181

### 182 *Gut length and preliminary assay of a diet*

183 Intestines were taken out from the body cavity of 62 preserved specimens of all ecomorphs  
184 except for no. 6 (represented by only two specimens), and measured using a ruler to the nearest 1  
185 mm. The sample size for each ecomorph is provided in Table 2. The standard length (SL) of  
186 examined individuals varied from 40 to 131 mm, one individual of ecomorph 5 had outstanding  
187 length - 185 mm. The ratio of gut length (GL) to SL was used for subsequent analyses. The  
188 Kruskal-Wallis test for multiple independent samples with Benjamini-Hochberg method of  
189 control of false discovery rate (FDR) (Benjamini & Hochberg, 1995) of *p*-value was applied to  
190 check a significance of differences at  $p < 0.05$ . The dependence of GL on SL was visualized using  
191 scatterplots and regressions. R-packages *ggplot2* and *PMCMR* were used to create plots and to  
192 test statistical significance of differences.

193 Diet was assessed for the same individuals, whose intestine length was measured. The  
194 main ecological and systematic groups were registered using stereo-microscope Micromed MC-  
195 2-ZOOM and microscope Olympus CX41. A composite measure of diet, an index of relative  
196 importance, IRI (Hart, Calver, & Dickman, 2002), was used to assess contribution of different  
197 components to a diet. The diet components were grouped in several items i) periphyton, ii)  
198 benthos, iii) macrophytes, and iv) others.

199

#### 200 *DNA sampling, extraction, amplification, and sequencing - mtDNA data*

201 DNA samples (n=107) were collected from *Garra* inhabiting the Sore River near the City of  
202 Metu in 2012 and 2014 from all six forms (see Table 2 for details). For comparison additional  
203 DNA samples (n=20) were collected from 8 *Garra* species inhabiting all main drainages of  
204 Ethiopia (10 localities – see map of sampling in Fig. 2). Total genomic DNA was extracted from  
205 ethanol-preserved fin tissues using the BioSprint 15 kit for tissue and blood (Qiagen). Sequences  
206 of the mitochondrial gene, cytochrome *b* (*cytb*) of 989 bp length, were amplified (see PCR  
207 conditions in Supplementary Material S2; Palumbi, 1996; Perdices & Doadrio, 2001). PCR  
208 products were visualized on 1% agarose gels, purified with ExoSAP-IT™ and sequenced at the  
209 Papanin Institute of Biology of Inland Waters (Russian Academy of Sciences) using an ABI  
210 3500 sequencer. All new sequences were deposited in GenBank (Accession Numbers: xxx -will  
211 be provided upon acceptance, see Supplementary Table S1).

212

213 Table 2.

214

#### 215 *Analysis of mtDNA data*

216 All sequences were aligned and edited using the MUSCLE algorithm (Edgar, 2004) as  
217 implemented in MEGA 6.0 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). A final set  
218 that includes also comparative material from Genbank (African and non-African *Garra* as well

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16

219 as outgroups) encompassed 143 *cytb* sequences (<https://www.ncbi.nlm.nih.gov>) (Table S1).  
220 *Akrokolioplax bicornis* and *Crossocheilus burmanicus* were included as outgroups according to  
221 previously published phylogenies (Yang et al., 2012).

222 Gene tree reconstruction was performed using both maximum-likelihood (ML) and  
223 Bayesian inference (BI) approaches. Prior to these analyses all sequences were collapsed into  
224 common haplotypes using ALTER software (Glez-Peña, Gómez-Blanco, Reboiro-Jato, Fdez-  
225 Riverola, & Posada, 2010). We determined the best fit models of nucleotide substitution for each  
226 codon position of *cytb* and optimal partitioning scheme using either ModelFinder (as  
227 implemented in IQ-TREE 1.6.12; Kalyaanamoorthy, Minh, Wong, Von Haeseler, & Jermin,  
228 2017; Nguyen, Schmidt, Von Haeseler, & Minh, 2015) or PartitionFinder 2.1.1 (Lanfear,  
229 Calcott, Ho, & Guindon, 2012) under Bayesian Information Criterion (BIC). The partition  
230 scheme selected by ModelFinder (codon position 1 - K2P+R2; codon position 2 - HKY+F+I;  
231 codon position 3 - TN+F+G4) was subsequently used in ML search with IQ-TREE, using 1 000  
232 bootstrap replicates.

233 Bayesian phylogenetic inference (BI) was carried out in MrBayes v. 3.2.6 (Ronquist et al.,  
234 2012). The selected partition scheme was following: codon position 1 with K80+I+G, codon  
235 position 2 with HKY+I, and codon position 3 with GTR+G. Two simultaneous analyses were  
236 run for  $10^7$  generations, each with four MCMC chains sampled every 500 generations.  
237 Convergence of runs was assessed by examination of the average standard deviation of split  
238 frequencies and the potential scale reduction factor. In addition, stationarity was confirmed by  
239 examining posterior probability, log likelihood, and all model parameters by the effective sample  
240 sizes (ESSs) in the program Tracer v1.6 (Rambaut, Suchard, Xie, & Drummond, 2014). The  
241 gene trees resulting in ML and BI analyses were visualized and edited using FigTree v.1.4.4  
242 (Rambaut, 2014). A haplotype network was constructed using the median joining algorithm  
243 (Bandelt, Forster, & Röhl, 1999) in PopArt 1.7 (Leigh & Bryant, 2015).

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245 *ddRAD-seq library preparation*

246 High molecular weight DNA was isolated from fin tissue preserved in ethanol using QIAamp  
247 DNA Mini Kit (Qiagen, Germany) or obtained by purification of salt method extracted DNA  
248 (Aljanabi & Martinez, 1997) using CleanUp Standard kit (Evrogen, Moscow). The dsDNA  
249 quantity was measured using dsDNA HS Assay Kit for fluorometer Qubit 3 (Life Technologies,  
250 USA). ddRAD-library was constructed following the quaddRAD protocol (Franchini, Monné  
251 Parera, Kautt, & Meyer, 2017) using restriction enzymes *Pst*I and *Msp*I. In total, 77 DNA  
252 samples of *Garra* ecomorphs from the Sore River (see Table 2) and 11 DNA samples from five  
253 other species of Ethiopian *Garra* from adjacent basins were sequenced by two independent runs  
254 of Illumina HiSeq2500 and Illumina X Ten (2 x 150 bp paired-end reads). The raw sequencing  
255 data were demultiplexed by the sequencing provider using outer Illumina TruSeq dual indexes.

256

257 *Processing of RAD-seq data*

258 The resulting reads were trimmed for remaining adapters and low quality reads Cutadapt  
259 implemented in the Trim Galore 0.4.5 package (<https://github.com/FelixKrueger/TrimGalore> -  
260 Martin, 2011). Read quality was assessed with FastQC 0.11.7 (Andrews & Krueger, 2010) and  
261 MultiQC 1.7 (Ewels, Magnusson, Lundin, & Källér, 2016) before and after trimming. Further  
262 demultiplexing of individually barcoded samples, construction and cataloging of RAD-loci, and  
263 SNP calling were done with STACKS 2.41 package (Catchen, Hohenlohe, Bassham, Amores, &  
264 Cresko, 2013). Identification and removal of PCR duplicates were done using the '*clone\_filter*'  
265 module of STACKS). STACKS module '*process\_radtags*' was used to demultiplex reads by the  
266 dual index inner barcodes and obtain separate fastq files for each individual. Samples that failed  
267 to produce more than 100 000 reads were excluded from further processing. To additionally  
268 evaluate data quality and identify possible contaminated samples, the reads were mapped to the  
269 reference genome of common carp *Cyprinus carpio* (GCF\_000951615.1) using bowtie2 2.3.5  
270 (Langmead & Salzberg, 2012) with '--local-sensitive' presettings. Then, only Read 1 (R1) files

271 were used for downstream processing and analyses. Prior to next steps, these R1 reads were  
272 trimmed at their 3' ends to a uniform length of 130 bp to reduce the influence of sequencing  
273 error (due to declined base quality at 3' end).

274 The *de novo* pipeline of STACKS was used to assemble loci and perform genotype calling.  
275 We selected optimal parameters using the approach suggested by Paris, Stevens, & Catchen  
276 (2017). Following the aforementioned procedure, we found that minimum stack depth (*-m*) of 5,  
277 distance allowed between stacks (*-M*) of 3, and the maximum distance required to merge catalog  
278 loci (*-n*) of 5 provided the best balance between data quality and quantity for our dataset (Fig.  
279 S1).

280

### 281 *Population genomic analyses*

282 Individual genotypes of sympatric *Garra* ecomorphs from the Sore River were exported to a vcf  
283 file using the 'populations' module of STACKS with the following settings: (i) loci genotyped in  
284 at least 90% of samples (*-r 0.90*) were kept; (ii) SNPs with a minor allele frequency (*--min-maf*)  
285 less than 0.04 and a maximum observed heterozygosity (*--max\_obs\_het*) above 0.99 were  
286 pruned; (iii) only single SNP per RAD locus was retained, to avoid inclusion of closely linked  
287 SNPs. We applied VCFtools 0.1.16 (Danecek et al., 2011) for further filtering of the dataset  
288 based on mean coverage and fraction of missing data for each sample. Samples with more than  
289 20% of missing data were blacklisted and excluded from further analyses. Thus, a high-quality  
290 dataset of 679 SNPs and 77 individuals was obtained and used for downstream population  
291 genetics analyses.

292 First, Principal Component Analysis (PCA) was performed using the 'glPca' function of  
293 the R-package *adegenet* 2.1.1 (Jombart, 2008; Jombart & Ahmed, 2011). Next, *rmaverick* 1.0.5  
294 (former MaverickK; Verity & Nichols, 2016) was used to infer population structure. This program  
295 estimates evidence for different numbers of populations (*K*), and different evolutionary models  
296 via generalised thermodynamic integration (GTI). A range of *K* values between 1 and 10 were

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297 explored, using 300 000 burn-in MCMC iterations and 10 000 sampling iterations. Convergence  
298 of MCMC was automatically tested every 1 000 burn-in iterations by activating option  
299 ‘auto\_converge’. This allows exit burn-in iterations when convergence is reached and  
300 immediately proceeds to sampling iterations. Parameter ‘rungs’ was set to 10 (number of  
301 multiple MCMC chains with different ‘temperature’ to run simultaneously). Both no admixture  
302 and admixture models were run, and compared by plotting values of the posterior distribution  
303 and overall model evidence in log space (log-evidence) (Fig. S2-S5). According to this  
304 comparison, the admixture model is decisively supported over the no admixture model, and used  
305 here to report the results. The same protocol was followed for consecutive hierarchical *rmaverick*  
306 runs for the identified clusters. Finally, global and pairwise Reich-Patterson  $F_{ST}$  values (Reich,  
307 Thangaraj, Patterson, Price, & Singh, 2009) with respective 95% confidence intervals for  
308 ecomorphs/genetic clusters were calculated using the R script from Junker et al. (2020). Basic  
309 genetic diversity statistics were calculated using the ‘populations’ module of STACKS.

310 To test for the gene flow between ecomorphs\genetic clusters, we used the Patterson’s D  
311 statistic (ABBA-BABA test), along with the  $f_4$ -ratio statistic (Patterson et al., 2012) and its  $f$ -  
312 branch metric (Malinsky et al., 2018), as implemented in Dsuite 0.4 software package (Malinsky,  
313 Matschiner, & Svardal, 2021). Patterson's D statistic is a widely used and robust tool to detect  
314 introgression between populations or closely related species, and to distinguish it from  
315 incomplete lineage sorting (ILS). The  $f_4$ -ratio statistic is a similar method aiming to estimate an  
316 admixture fraction. The  $f$ -branch metric is based on  $f_4$ -ratio results and serves to assign gene  
317 flow evidence to specific branches on a phylogeny. These tests were performed on a group  
318 containing ecomorphs\genetic clusters 2b, 3, 4, and 6, while the rest were used as outgroup (in  
319 accordance with the results of our phylogenomic analysis).

320

321 *Phylogenomic analyses*

322 IQ-TREE 2.0.5 (Minh et al., 2020) was used for ML phylogenetic analyses of RAD-seq data.  
323 First dataset included one to three specimens of each *Garra* ecomorph from the Sore river and  
324 other Ethiopian *Garra* species from adjacent basins. Multiple sequence alignments of all loci and  
325 respective partition files were created using the '--phylip-var-all' option of '*populations*' module  
326 of STACKS package. Heterozygous sites within each individual were encoded using IUPAC  
327 notation. During the analysis each RAD-locus was treated as a separate partition with  
328 independent best-fit substitution model. Node support values were obtained using ultrafast  
329 bootstrap procedure (Hoang, Chernomor, von Haeseler, Minh, & Vinh, 2018) with 1 000  
330 replicates. We also used SVDQuartets algorithm (Chifman & Kubatko, 2014) as implemented in  
331 PAUP\* 4.0a168 (Swofford, 2003) to perform species-tree inference under the multi-species  
332 coalescent model using 18,988 SNPs (single random SNP per locus, minor allele frequency  
333 cutoff 0.04, maximum observed heterozygosity cutoff: 0.99). Node support was estimated with 1  
334 000 bootstrap replicates.

335         The second dataset consisted of all genotyped specimens of sympatric *Garra* ecomorphs  
336 from the Sore River and a single, most closely related outgroup (*G. cf. dembeensis* from the  
337 Barokalu River, as revealed by the analysis of the first phylogenomic dataset that included  
338 samples from all the localities in Figure 2). It was analysed with IQ-TREE as described above,  
339 except for GTR+G substitution model was used for each partition. The phylogenetic trees were  
340 visualized and edited using FigTree 1.4.4 (Rambaut & Drummond, 2008).

341

342

## 343 **Results**

### 344 *Trophic Morphology*

345 PCA of head and body proportions of six sympatric ecomorphs from the Sore River revealed five  
346 well-defined clusters (Fig. 3A). Four clusters represent ecomorphs 3, 4, 5, and 6, while the fifth  
347 includes individuals from ecomorphs 1 and 2. The ecomorph 5 is the most divergent. PC1

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348 explained 72.3% of the total variance, while PC2 10.2%. The eigenvector with the highest  
349 eigenvalues for PC1 were head proportions - nine of ten most loaded ones (especially gular disc  
350 proportions, mouth width, interorbital distance, and snout length). The same pattern was detected  
351 for PC2 - nine of ten most loaded characters belonged to head proportions (mainly disc length,  
352 mouth width, height of head at nape and at eyes etc. - see Table S2 for details).

353 After excluding ecomorph 5, the ecomorphs 1 and 2 became more distinguishable with low  
354 overlapping (Fig. 3B). The PC1 explained 73.8% of variance, while PC2 8.1%. The most loaded  
355 eigenvectors of both PC1 and PC2 were from head proportions with few more contributions of  
356 some body proportion characters (see Table S3). The difference between ecomorphs 1 and 2  
357 revealed in PC2 is explained by height of head at both nape and eyes, interorbital distance, head  
358 width, body height as well as other characters (Table S3).

359 Fig. 3.

360

361

### 362 *Gut length and preliminary data on diet*

363 Gut length broadly varied consistently between ecomorphs (Fig. 3C). Shortest guts (107-160 %  
364 SL) were detected in ecomorph 5 suggested a predatory trophic type, while the longest guts were  
365 recorded in ecomorphs 1 (285-799 % SL) and 2 (354-555 % SL) that possessed the well-  
366 developed gular disc and therefore are specialized algal grazers, as also shown by their gut  
367 contents (see below). Other ecomorphs had intermediate values gut lengths: ecomorph 3 - 124-  
368 295 % SL, and ecomorph 4 - 175-513 % SL, respectively. Broad intra-group variation is  
369 explained by increase of gut length with body length detected in some ecomorphs (Fig. 3D).  
370 Nevertheless, the similar-sized individuals are divergent in gut length at the same manner that  
371 presented in Fig. 3C. Ecomorph 5 having the shortest gut displays even a slight decrease of gut  
372 length ontogenetically that was previously reported for piscivorous mode of feeding among  
373 African cyprinids (Levin et al., 2019).

27

28

374 The preliminary inspection of gut content revealed differences in the diet between some  
375 ecomorphs. Ecomorphs 1 and 2 had permanently filled intestines full of periphyton (diatom,  
376 green, and charophyte algae; IRI = 99.98% for ecomorph 1, and IRI = 97.99% for ecomorph 2)  
377 and, rarely other items (larvae of water insects - mayflies, chironomids, simuliids). The ecomorph  
378 3 had a half-filled gut with dominating periphyton (IRI = 86.3%) with a notable portion of insect  
379 larvae (7.62% - predominantly chironomids, also mayflies, and simuliids) and macrophytes  
380 (5.97%). Ecomorph 4 had fewer filled intestines compared to ecomorph 3 however with strongly  
381 dominating periphyton in diet (IRI = 99.49%). The gut of ecomorph 5 (shortest gut) frequently  
382 was empty including the largest individual (SL=185 mm). When guts were filled, benthos-  
383 associated prey was strongly prevalent (IRI = 99.31%; mayflies and chironomids).

384

#### 385 **Mitochondrial data**

386 Both BI and ML analyses of *cytb* revealed monophyly of the *Garra* from the Sore River (Fig.  
387 4A). The closest relative (and ancestor lineage) is from the Barokalu River, a tributary of the  
388 Baro River (White Nile drainage). Both Sore and Barokalu rivers share watershed in the Baro  
389 system and sampled localities are separated just ca. 50 km by land. Divergence between *Garra*  
390 populations from the Sore and Barokalu is low ( $p$ -distance =  $0.0105 \mp 0.0028$ ) and comparable  
391 with maximum intra-divergence in the Sore radiation ( $p$ -distance =  $0.0111 \mp 0.0033$ ). Being  
392 combined together White Nile lineage is a sister to the large clade of Ethiopian *Garra* from Blue  
393 Nile and Lake Tana, Atbara-Nile, Ethiopian Rift Valley, and Omo-Turkana basins.

394 At the same time, our phylogenetic analyses revealed that Ethiopian *Garra* are non-  
395 monophyletic (Fig. 4A). Some lineages are of more ancient origin and closer to Asian lineages  
396 (*G. tibanica* from Indian Ocean basin) or to lineages from West Africa (e.g. *G. vinciguerra* from  
397 Blue Nile basin). Matrilineal tree of Ethiopian *Garra* includes up to 12 lineages. Taking into  
398 account some species cluster together in one lineage like three species from Lake Tana or that

399 some species were unavailable, we conclude cladogenesis of *Garra* in Ethiopia Highlands has  
400 been more diversified than considered previously (Stiassney & Getahun, 2007).

401

402 Fig. 4.

403

404 The Sore lineage is composed of two sub-lineages or haplogroups highlighted by yellow  
405 and green (Fig. 4A-B). Haplotype net constructed on 107 *cytb* sequences confirms presence of  
406 two main haplogroups. The core haplotypes of these haplogroups are separated by 5  
407 substitutions. Four of six ecomorphs (2, 3, 4, and 5) share both haplogroups. The ‘green’  
408 haplogroup is prevalent in number of haplotypes (18), and number of individuals (88), and found  
409 in five ecomorphs. Ecomorph 1 is presented exclusively in this haplogroup. In contrast, the  
410 ‘yellow’ haplogroup (Fig. 4B) is smaller, with only different 9 haplotypes found in 19  
411 individuals (= 17.7 % of the individuals analyzed). One individual of ecomorph 4 is rather  
412 distant (6 substitutions) from the core haplotype of this haplogroup. ‘Yellow’ haplogroup  
413 consists of five ecomorphs as well. However, ecomorph 4 is much more frequently represented  
414 in this haplogroup (42 % of all individuals) compared to ‘green’ one (6.97 %).

415

#### 416 **RAD-seq data**

417 Raw reads statistics is given in Supplementary File S1.

418

#### 419 *Nuclear phylogeny*

420 The phylogeny of Ethiopian *Garra* based on a concatenated set of RAD-loci sequences (23,365  
421 partitions and 3,075,180 total sites with 0% missing data) is generally similar to that based on  
422 mtDNA data (Fig. 4) but it has more strongly supported nodes, as it is based on many more  
423 variable sites (Fig. 5A). Sympatric ecomorphs clustered together and form monophyletic  
424 lineages, sister to the population from the same riverine basin - Baro drainage in White Nile

31

16

32

425 system (Fig. 5A-B). Closest relative to *Garra* from White Nile system is *Garra* lineage in the *G.*  
426 *dembeensis* complex from neighbor drainage - Omo-Turkana system. The *G. vinciguerrae* from  
427 the Blue Nile (which recorded in Ethiopia for the first time in the current study) is ancestor  
428 lineage for both White Nile and Omo-Turkana lineages. The most divergent lineages, *G.*  
429 *makiensis* and *G. tibanica*, are from Ethiopian Rift Valley and Indian Ocean basins, respectively.

430

431 Fig. 5.

432

433 Compared to mitochondrial data, the nuclear phylogenomic tree shows much better  
434 segregation of *Garra* ecomorphs from the Sore River (Fig. 5A). Ecomorphs 3, 4, and 6 form  
435 monophyletic clusters, while other ecomorphs are divided into two (nos. 1 and 5) or even three  
436 (no. 2) clusters. We assign two distantly located branches of both ecomorph 1 (generalized) as  
437 1a/1b as well as ecomorph 2 (stream-lined) as 2a/2b according to population genomics analyses  
438 done below (Fig. 6-8). Ecomorphs 1 and 2 from one hand, and other ecomorphs from another  
439 hand form two clusters within Sore River adaptive radiation according to SVDQ species tree  
440 (Fig. 5B). Ecomorphs 3 (narrow-mouth), 4 (wide-mouth), and 6 (thick-lipped) are most recently  
441 diverged branches according to SVDQ-tree but the nodes are weakly supported (Fig. 5B).

442 Relationships among the Sore River sympatric ecomorphs based on analysis of all samples  
443 and full RAD-loci sequences (> 7000 loci and > 0.96 Mbp length sequences) are presented in  
444 Fig. 6. The ML analysis highly support the monophyly of each ecomorph except for ecomorph 2.  
445 The most basal lineage is ecomorph 2, which in turn, is paraphyletic, suggesting, possibly, that  
446 there is another 7<sup>th</sup> cryptic species that we could not distinguish phenotypically. Four individuals  
447 along with one individual of intermediate phenotype represent another lineage that we call 2b  
448 (Fig. 6). Lineage 2a is sister all other ecomorphs that are divided for two subclades - one  
449 includes only ecomorph 1 individuals (which, in turn is subdivided into what we call - 1a-1b),  
450 while another includes all other ecomorphs - 3, 4, 5, 6, and above mentioned 2b. That latter

33

34

451 lineage is composed of lineages, each containing samples of particular ecomorphs except for  
452 several samples which were intermediate in their phenotypes (Fig. 6). Ecomorph 6 (thick-lipped  
453 mouth) is resolved as sister to the 2b lineage albeit with an apparent rather deep last common  
454 ancestor. Generally, the placement of clade 2a as sister to all other *Garra* from the Sore River,  
455 that is characterized by a well-developed gular disc (type C), might suggest that this an ancestral  
456 condition of this radiation.

457

458 Fig. 6.

459

#### 460 ***Population genomics***

461 Principle component analyses of the 679 nuclear SNPs of sympatric ecomorphs revealed  
462 several well-defined clusters that correspond to the phenotypic differentiation (Fig. 7). Ecomorph  
463 1 (composed of two genetic sub-clusters 1a-1b), genetic cluster 2a, ecomorphs 3 and 4 are not  
464 overlapping, while clusters of 2b and ecomorph 5 broadly overlap. Thick-lipped ecomorph (6)  
465 interestingly (although it is difficult to place since we only found two individuals that we could  
466 include in this study) could not be identified by PCA as a distinct cluster.

467

468 Fig. 7.

469

470 The analysis of population structure with admixture revealed an optimum of three  
471 genomic clusters that correspond to the i) ecomorph 1 + 2a lineage, ii) ecomorphs 3 + 4, and iii)  
472 ecomorph 5 + 2b lineage (Fig. 8, Upper row, K3). Ecomorph 6 is characterized by admixture of  
473 two clusters from ecomorphs 3 and 4.

474

475 Fig. 8.

476

35

36

477 Subsequent analysis of each cluster (=lineage) revealed hierarchical subdivision. Thus  
478 ecomorph 1 and genetic lineage 2a each are also identified as cluster in the admixture analysis  
479 (Fig. 8 middle row, K=2). Although ecomorphs 3, 4, 5, and lineage 2b are supported as  
480 independent evolutionary units based on several types of genetic analyses, few individuals in all  
481 of these show signs of historical gene flow based on the admixture analysis (Fig. 8). While the  
482 two individuals from ecomorph 6 in our study seem most clearly be composed of genetic  
483 contributions by ecomorphs 3 (36.8-47.5%) and genetic lineage 2b (51.3-62.3%), possibly  
484 supporting a hybrid origin hypothesis. Interestingly, one more individual with combination of the  
485 same genomic clusters but with the opposite ratio (54.0% from ecomorph 3 and 43.9 % from  
486 lineage 2b) had no thick-lipped features (the main phenotypic diagnostic feature for ecomorph 6)  
487 and was phenotypically assigned to ecomorph 3 (Fig. 8). One more level of population  
488 subdivision was detected in ecomorph 1 (Fig. 8) with two genomic clusters (lineages 1a and 1b)  
489 of high degree of admixture. It suggests heterogeneous genomic structure of the generalized  
490 ecomorph as a result of secondary contact.

491 All Reich  $F_{ST}$  pairwise comparisons were statistically significant with values ranging from  
492 0.10 (lineages 1a vs. 1b) to 0.46 (ecomorphs 2b vs. 6) (Fig. 9). The ecomorph 6  $F_{ST}$  values were  
493 the highest (0.39-0.46).

494

495 Fig. 9.

496

497 As the *rmaverick* analysis suggested a notable level of admixture between lineage 2b and  
498 ecomorphs 3, 4, and 6 (Fig X), which form a single monophyletic cluster in our phylogenomic  
499 analysis (Fig 8), we performed a number of tests to distinguish between gene flow  
500 (introgression) and incomplete lineage sorting (ILS). The obtained D statistic was positive and  
501 significant for a number of comparisons (Table 3.). Visualization of  $f$ -branch metric (which is

502 based on  $f_4$ -ratio results) highlighting introgression between ecomorphs/genetic lineages 2b and  
503 3, 6 and 3, 5 and 3 (Fig 9).

504

505 Table 3.

506

507 The eighth genetic clusters possess from three (ecomorph 6) to 38 private alleles (ecomorph 4)  
508 (Table 4). The ecomorph 6 has also the lowest heterozygosity ( $H_o = 0.00058$ ) as well as  
509 nucleotide diversity ( $P_i = 0.00054$ ) compared to all other ecomorphs ( $H_o = 0.00104$ - $0.00128$ ;  $P_i$   
510  $= 0.00121$ - $0.00091$ ) (Table 4).

511

512 Table 4.

513

## 514 **Discussion**

515 Our study provides genetic support for the hypothesis of the evolution of an adaptive radiation in  
516 a riverine environment. By analyzing trophic features and sucking disc variation, as well as  
517 trophic ecology, we show morpho-ecological diversification of the cyprinid fish *Garra*  
518 *dembeensis* into six distinct ecomorphs. First, diversification of two novel phenotypes (thick-  
519 lipped and predatory) in the Sore River has evolved rapidly, an event that can be classified as  
520 burst of speciation sensu Givnish (2015). Second, adaptive radiation resulted in the origin of  
521 several highly specialized lineages of algae scrapers, i.e. specialized ancestor adaptively radiates  
522 giving rise to eco-morphological diverse lineages, that seem to be not only ecologically, but also  
523 reproductively isolated from each other and can be considered the new species.

524

### 525 ***Eco-morphological diversification and adaptive radiation of Garra***

526 The genus *Garra* is currently comprised of more than 160 species (Fricke et al., 2021; Yang et  
527 al., 2012). Only 23 of which occur in Africa (Moritz et al., 2019). So far, 13 described species

39

20

40

528 were reported from Ethiopia (Golubtsov et al., 2002; Stiassny & Getahun, 2007). In this study,  
529 we discovered six additional distinct ecomorphs that originated through adaptive radiation in the  
530 Sore River, and thus might warrant the description of five-six new African *Garra* species.

531 The ecomorphs of the Sore's *Garra* are exceptionally diverse in trophic and sucking disc  
532 morphology. Two novel phenotypes for the whole genus *Garra* – ‘thick-lipped’ and ‘predatory’ -  
533 have superficial similarities to Lake Tana large barbs species/morphotypes, e.g., thick-lipped  
534 barb *L. negdia* (Rüppell, 1836) and predatory *L. gorguari* (Rüppell, 1836) (Nagelkerke &  
535 Sibbing, 1997). This high degree of variation in the sucking disc in Sore's *Garra* can be  
536 observed - from well-developed disc with free posterior margin to complete absence. Such a  
537 degree of morphological diversity concentrated in one riverine spot of Ethiopian Highlands  
538 would seem to satisfy the requirements of a diversification burst (sensu Givnish, 2015).

539 Divergent feeding-related morphology and gut content analysis suggest trophic  
540 specialization of *Garra* sympatric forms. This is consistent with other cases of adaptive radiation  
541 among Ethiopian cyprinids, where trophic resource partitioning promoted diversification -  
542 *Labeobarbus* spp. in Lake Tana (Sibbing, Nagelkerke, Stet, & Osse, 1998) as well as in the  
543 Genale River (Levin et al., 2019). The most common foraging strategy among *Garra* is scraping  
544 of periphyton from stones and rocks (Hamidan, Jackson, & Britton, 2016; Matthes, 1963). This  
545 is predominant in Sore's *Garra* ecomorphs 1 and 2 that have long gut (4-5 times longer than  
546 body length) filled with periphyton and detritus. The ecomorphs 1 and 2 are divergent mainly in  
547 body shape. The latter has streamlined appearance and probably is adapted for life in more rapid  
548 flowing water. Ecomorph 3 has shorter gut length (ca. 2-times longer than body length) and a  
549 mixed diet with significant additions of benthic invertebrates. Ecomorph 5 has an extremely  
550 short gut, whose length is as long as the fish body. Short gut is a strong marker for  
551 predatory/piscivory feeding strategy in fishes, including cyprinids (Nagelkerke, 1997; Sibbing et  
552 al., 1998; Wagner, McIntyre, Buels, Gilbert, & Michel, 2009, Zandoná, Auer, Kilham, &  
553 Reznick, 2015). Predatory *Garra* from the Sore River have 4-5-times shorter gut length than

554 congeneric periphyton feeders and twice shorter gut than that of piscivory large-mouthed  
555 ecomorph of *Labeobarbus* from the Genale River, Ethiopia (Levin et al., 2019). We found an  
556 empty gut in many individuals of ecomorph 5, while small-sized fishes had gut filled with  
557 insects. Ecomorph 4 has a rather long intestine and predominantly periphyton in diet, but it is  
558 characterized by distinctly divergent mouth phenotype compared to ecomorphs 1 and 2 (Fig. 3).  
559 The gut of thick-lipped phenotype (ecomorph 6) was not analyzed because of the extreme rarity  
560 of samples. Hypertrophied lips (or ‘rubber lips’) of fishes is an adaptation to foraging on benthos  
561 hidden between rock crevices on pebble and rock fragments via increased sucking power by  
562 sealing cracks and grooves (Baumgarten, Machado-Schiaffino, Henning, & Meyer, 2015;  
563 Machado-Schiaffino, Henning, & Meyer, 2014; Matthes, 1963; Ribbink, Marsh, Marsh, &  
564 Sharp, 1983). This phenotype is widely distributed among other cyprinid fish, the *Labeobarbus*  
565 spp., inhabiting lakes and rivers of Ethiopian Highlands (Mina, Mironovsky, & Dgebuadze,  
566 1996; Mironovsky, Mina, & Dgebuadze, 2019; Nagelkerke, Sibbing, van den Boogaart,  
567 Lammens, & Osse, 1994) including the Sore River (Levin et al., 2020), but it was never detected  
568 among *Garra* species. Our study shows that the thick-lipped mouth phenotype represents an  
569 evolutionary novelty within the *Garra* lineage that most probably resulted from hybridization  
570 events between ecomorphs 2 (lineage 2b) and 3 because its genome had an admixture from these  
571 genetic lineages. Hybridogenic origin of the *Garra*’s thick-lipped phenotype may corroborate  
572 results of recent experimental study demonstrating the importance of hybridization in generating  
573 of functional novelty of ecological relevance in relation to trophic resources unavailable for  
574 parental species in cichlids (Selz & Seehausen, 2019). The origin of novel thick-lipped  
575 phenotype in the genus *Garra* is of particular interest in light of knowledge of non-hybrid origin  
576 of hypertrophied lips from ancestors with normally developed lips in cichlid fishes (Baumgarten  
577 et al., 2015; Machado-Schiaffino et al., 2017). Interestingly, there might only be a single locus  
578 involved in producing the hypertrophied cichlid phenotype (Kautt et al., 2020), the genomic  
579 basis of the lip phenotypes in *Garra* remains unknown.

580 Another novel phenotype for *Garra* detected in the Sore River is the “predatory” niche. A  
581 conspicuously piscivory trophic strategy is rare among Cypriniformes, presumably because they  
582 have a toothless jaw. Nevertheless, this feeding strategy is quite common among cyprinid fishes  
583 inhabiting water bodies of Ethiopian Highlands. For example, seven of the total 15 endemic  
584 *Labeobarbus* spp. found in Lake Tana are predatory on fish (Nagelkerke et al., 1994; Sibbing et  
585 al., 1998); that evolved multiple times among riverine populations of the genus *Labeobarbus*  
586 (Levin et al., 2020).

587 To our knowledge, only one sympatric diversification has previously suggested for *Garra*  
588 – the intralacustrine complex including three species inhabited Lake Tana in Ethiopia (Geremew,  
589 2007; Stiassny & Getahun, 2007). This diversification resulted in divergent phenotypes (gular  
590 discs varies from well-developed to reduced size) and ecology (one form is pelagic - *G. tana*)  
591 and can be considered as a recent speciation as suggested by the absence of mtDNA divergence  
592 among these species (Tang, Getahun, & Liu, 2009). Unfortunately, little is known about morpho-  
593 ecological and genetic diversity of this Lake Tana radiation. Sympatric divergence was also  
594 recently proposed as the most likely mechanisms for the origin of two blind *Garra* species, *G.*  
595 *typhlops* and *G. lorestanensis*, inhabited the same cave in Zagros Mountains, Iran (Segherloo et  
596 al., 2018).

597

#### 598 ***Possible scenarios of evolution of Garra’s adaptive radiation in the Sore River***

599 Both mtDNA and genome-wide SNPs data support monophyly of the Sore’s *Garra* as well as  
600 their recent speciation based on low genetic divergence between the nearest ancestor and Sore  
601 River’s ecomorphs. The closest relative and ancestor of the Sore River diversification inhabits  
602 the same subbasin of the White Nile in Ethiopia, therefore suggesting an intra-basin  
603 diversification of *Garra* there. On the one hand, mtDNA data might have failed to distinguish  
604 sympatric ecomorphs because of high level of shared genetic diversity caused by ILS and  
605 introgression, this latter highlighted by D-statistic calculated with the genome-wide nuclear data.

45

46

606 On the other hand, the SNP data support a reproductive isolation among closely-related  
607 ecomorphs despite few individuals having intermediate phenotypes and genetic admixture.  
608 Hybrid origin of intermediate phenotypes might suggest that reproductive isolation barriers are  
609 not complete yet.

610 Patterns of haplotype net (numerous haplotypes occurring in the same phenotypes) as well  
611 as SNP data (presence of more genetic clusters than phenotypes) could also suggest secondary  
612 contact of local sub-isolated populations. The riverine net of Ethiopian Highlands was  
613 significantly influenced by several episodes of dramatic volcanism and tectonism until the  
614 Quaternary (Ferguson et al., 2010; Hutchison et al., 2016; Prave et al., 2016). Thus, riverine net  
615 fragmentation, isolation or sub-isolation of some riverine parts, and captures of headwaters is a  
616 likely scenario given the geological history of Ethiopian Highlands (Mège, Purcell, Pochat, &  
617 Guidat, 2015), also supported by genetic studies on other Ethiopian fishes (Levin et al., 2019;  
618 2020). Concerning the Sore River, while waterfalls and rapids are rather frequent, no geological  
619 data that support its connection to other basins are known. In our view, the most reliable  
620 evolutionary scenario for the origin of the riverine adaptive radiation in the *Garra* species group  
621 draws upon a combination of allopatric and sympatric stages of speciation with hybridization and  
622 admixture. A comparable evolutionary history was detected in the *Labeobarbus* adaptive  
623 radiation in the Genale River (Ethiopia), which is part of the extended ancient riverine net in  
624 Juba-Wabe-Shebelle drainage (Levin et al., 2019).

625 Speciation with gene flow was detected in several studies (e.g. Feder, Egan, & Nosil, 2012;  
626 Fruciano, Franchini, Raffini, Fan, & Meyer, 2016; Kautt, Machado-Schiaffino, & Meyer, 2016;  
627 Kautt et al., 2018; Kautt et al., 2020; Machado-Schiaffino et al., 2017; Malinsky et al., 2018;  
628 Puebla, 2009; Rougeux, Bernatchez, & Gagnaire, 2017; Schwarzer et al., 2011; Smadja &  
629 Butlin, 2011; Zheng & Ge, 2010). Notably, it has been shown as genetic admixture between  
630 divergent populations/lineages may be a key factor in promoting rapid ecological speciation  
631 (Jacobs et al., 2020; Kautt et al., 2016; Kautt et al., 2020; Martin et al., 2015; Marques, Meier, &

632 Seehausen, 2019). Moreover, ancient hybridization is widely considered one of the most  
633 important factors driving the spectacular cichlid adaptive radiations in the Great African Lakes  
634 (Irissari et al., 2018; Meier et al., 2017; Verheyen, Salzburger, Snoeks, & Meyer, 2003).  
635 Seemingly, ancient introgressive hybridization could be a trigger for small-scaled repeated  
636 adaptive radiations among the Arctic charrs *Salvelinus* (Lecaudey et al., 2018). Furthermore,  
637 hybridization is the main mechanism generating polyploid lineages in fishes (tetraploid,  
638 hexaploid etc. - Braasch & Postlethwait, 2012), whose complex genomes constitute the raw  
639 material for the rapid origin of sympatric forms (e.g. *Schizothorax* in Central Asia - Berg, 1914;  
640 Burnashev, 1952; Terashima, 1984; *Labeobarbus* in Africa - Levin et al., 2020; Mina et al.,  
641 1996; Nagelkerke et al., 1994; Vreven, Musschoot, Snoeks, & Schlieuwen, 2016). Nevertheless,  
642 all described *Garra*, including the Ethiopian species, have diploid genomes (Krysanov &  
643 Golubtsov, 1993).

644

#### 645 ***Adaptive radiation in riverine environment***

646 Most adaptive radiations of fishes were reported from the lacustrine environment (e.g., Fryer &  
647 Iles 1972; Seehausen & Wagner, 2014). However, increasing evidence suggest that adaptive  
648 radiation can take place in other aquatic environments (e.g., marine, riverine) (Burruss et al.,  
649 2018; Dimmick et al., 2001; Feulner, Kirschbaum, & Tiedemann, 2008; Levin et al., 2019; 2020;  
650 Melnik et al., 2020; Matchiner, Hanel, & Salzburger, 2011; Piálek et al., 2012; Puebla, 2009;  
651 Whiteley, 2007). Several other cases of potential riverine adaptive radiations that includes  $\geq 3$   
652 sympatric ecomorphs exist, although they were not been tested with genetic methods yet - for  
653 instance, snow trout from Central Asia (Berg, 1914; Burnashev, 1952), barbs *Poropuntius* and  
654 *Neolissochilus* from Southeastern Asia (Roberts, 1998; Roberts & Khaironizam, 2008). Among  
655 cichlids, one of the first riverine adaptive radiations examined genetically were from Southern  
656 Africa (Joyce et al., 2005). However, the authors of this study suggested that the adaptive  
657 radiation occurred in the lacustrine environment in the palaeo lake Makgadikgadi that dried up in

658 the Holocene (Joyce et al., 2005). Other cichlid adaptive radiations from the rivers of Western  
659 Africa (Schwarzer et al., 2011), Southern America (Burruss et al., 2018; Piálek et al., 2012;) as  
660 well as four independently evolved riverine radiations of labeobarbs from East Africa (Levin et  
661 al., 2020), have instead took place in riverine drainages without known lacustrine conditions in  
662 the past.

663 The *Garra* lineage is adapted to fast and torrent waters. It possesses a morphological  
664 novelty - gular sucking disc - used to cling on the bottom of swift waters. This novelty allowed  
665 *Garra* to be distributed widely in highlands and montane zones from Southeastern China to  
666 Western Africa. Only a few species were found in the lacustrine environment (Lake Tana –  
667 Stiassny & Getahun, 2007) or in caves (e.g. Banister, 1984; Coad, 1996; Kruckenhauser, Haring,  
668 Seemann, & Sattmann, 2011; Mousavi-Sabet & Eagderi, 2016), indicating their potential to  
669 adapt to steady waters.

670 Despite the riverine network is generally considered more open to gene flow compared to  
671 landlocked water bodies, mountain and highland are an exception to this rule. The Ethiopian  
672 Highlands are a volcanic massif of flood and shield volcano basalts 0.5–3.0 km thick that form  
673 spectacular trap topography (1500–4500 m) flanking the Main Ethiopian Rift (Prave et al.,  
674 2016). The geological history of the Ethiopian Highlands was tectonically very dynamic and rich  
675 in volcanic episodes from Oligocene to Pleistocene time with very recent episodes (Prave et al.,  
676 2016). The volcanic activity has been severe enough to deleteriously affect the biota and cause  
677 major disruptions in ecosystems. This hypothesis found support in the inferred evolutionary  
678 history of the *Labeobarbus* in East Africa. The earliest fossil records of *Labeobarbus* were found  
679 in the Ethiopian Rift Valley and dated back to the late-Miocene (Stewart & Murray, 2017), but  
680 most of the Ethiopian lineages are younger (Pleistocene origin) (Beshera, Harris, & Mayden,  
681 2016; de Graaf, Megens, Samallo, & Sibbing, 2010; Levin et al., 2020). The tectonic activity of  
682 the region could have favored local isolation via the formation of waterfalls (e.g., 33 kya the  
683 Blue Nile basaltic blockade formed Tis-Isat waterfall - Prave et al., 2016) or river net

684 fragmentation (Juba-Wabe-Shebelle drainage Mège et al., 2015) along with climatic oscillations  
685 resulted to disconnection of water bodies during aridization (Benvenuti et al., 2002).  
686 Periodically, it resulted in vacant habitats and ecological opportunity (reviewed by Stroud &  
687 Losos 2018) for new species to exploit similar to islands or crater lakes (Burress et al., 2018).

688 The *Garra*'s diversification burst in the Sore River was detected in the riverine segment at  
689 an altitude range of 1310-1550 m asl, that is within the range of four riverine diversifications of  
690 the *Labeobarbus* detected throughout Ethiopian Highlands: 1050-1550 m (Levin et al., 2020).  
691 Despite the generally broader elevation gradient (175-2000 m asl - Levin et al., 2020) of the  
692 *Labeobarbus* species complex, the diversification bursts were only detected in mid-upper  
693 reaches. We believe that a combination of two factors might explain this observation: i) fauna in  
694 mid-upper reaches is poorer compared to lower reaches, where a more diversified fauna might  
695 have already filled the available ecological niches necessary for an adaptive radiation to unfold;  
696 ii) the biotopes are more diverse compared to the most upper reach, that means vacant niches are  
697 available.

698 Five endemic, and one introduced non-*Garra* species were recorded in the Sore River in  
699 the study area (data of this study). This is an extremely low number compared to more than 110  
700 fish species (Golubtsov & Darkov, 2008, and our data) recorded in the Baro River at Gambella at  
701 440m altitude (our data) to which the drainage of the Sore River belongs with a distance of  
702 ~150km between compared localities. The segment of the Sore River where *Garra*'s  
703 diversification was detected is rather rich in biotope complexity - pools are alternating pools  
704 slow currents, rift areas and rapids (Fig. S6). The depauperated fauna was suggested to provide  
705 the ecological opportunities for riverine adaptive radiations similar to the in Southeastern  
706 cyprinids of the genus *Poropuntius* (Roberts, 1998) and South America cichlids of the  
707 *Crenicichla* due to relaxed competition and vacant niches might have provided ecological  
708 opportunities for sympatric speciation by trophic specializations (Burress et al., 2018).

709 We discovered six new species within the genus *Garra* in the Sore River. Given that the  
710 same riverine segment is home for another riverine diversification of fishes represented by four  
711 phenotypically diverged ecomorphs of the genus *Labeobarbus* (Levin et al., 2020), we consider  
712 the Sore River to being a hot-spot of riverine diversification in the Ethiopian Highlands that  
713 requires conservation management. The Ethiopian Highlands are home for several young fish  
714 radiations - a large lacustrine diversification among cyprinids (15 species/morphotypes - Mina et  
715 al., 1996; Nagelkerke et al., 1994; Nagelkerke et al., 2015) as well as small-sized diversifications  
716 of *Garra* (three species – Stiassny & Getahun, 2007) and *Enteromius* (two species - de Graaf,  
717 Megens, Samallo, & Sibbing, 2007; Dejen et al., 2002) - all in Lake Tana, and five riverine  
718 adaptive radiations of cyprinids each including from four to seven species (Golubtsov, 2010;  
719 Golubtsov, Korostelev, & Levin, 2021; Levin et al., 2019; 2020; Mina, Mironovsky, Golubtsov,  
720 & Dgebuadze, 1998; current study), highlighting this region's importance as a hotspot for fish  
721 speciation that is in need of additional research on ecological speciation processes.

722

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731

### 732 **Author contributions**

733 BL, ES, PF, NM, AG, and AM designed and contributed to the original concept of the studies.

734 BL and AG collected most of the specimens and related data, BL and NM obtained mtDNA data

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735 and prepared DNA libraries for ddRAD, BL conducted morphologic analyses, ES conducted the  
736 most of bioinformatics, and BL, ES, PF, and AM finalized the manuscript. All authors partici-  
737 pated in project design, and read and approved the final manuscript.

#### 738 **Data availability statement**

739 Morphologic data (body proportions and gut lengths), mtDNA subsets (cytochrome *b*), and  
740 genotyping files (various sets of SNPs) have been uploaded to Dryad: [https://doi.org/10.5061/](https://doi.org/10.5061/dryad.j6q573ndp)  
741 [dryad.j6q573ndp](https://doi.org/10.5061/dryad.j6q573ndp)

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1194 2439–2454. [doi.org/10.1111/j.1365-294X.2010.04674.x](https://doi.org/10.1111/j.1365-294X.2010.04674.x)  
1195 <https://briancoad.com>  
1196 <https://en.wikipedia.org/wiki/Tiber>  
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1216 Table 1. Common names of the six ecomorphs of African *Garra* from the Sore River, and the  
1217 preliminary qualitative descriptions used in the field to identify each form.

Name used in the text	Basal description
No. 1, ‘generalized’	Well-developed round-shaped gular disc of type C with free posterior margin (disc classification follows Stiassny & Getahun, 2007). Body shape is generalized for <i>Garra</i> .
No. 2, ‘stream-lined’	Slender stream-line body with slim caudal peduncle and increased pectoral fins. Disc of type C.
No. 3, ‘narrow-mouth’	Disc is reduced in size, elongated, oval-shaped (closer to type A). Narrow mouth often with groove on lower jaw.
No. 4, ‘wide-mouth’	Disc is reduced in size, triangle-shaped. Wide mouth with significantly enlarged labellum (sensu Kottelat, 2020). Disc of type B in degree of development.
No. 5, ‘predator’	Completely or almost completely reduced gular disc (type A when presented). Wide head and mouth. This ecomorph

	achieves larger size compared to others. Largest individuals have nuchal hunch and almost terminal mouth with a bony projection on the lower jaw and matching incision on the upper jaw.
No. 6, 'thick-lipped'	Greatly developed lips, referred to as 'rubber lips' (Matthes, 1963). Intermediate lobe of the lower lip is ball-shaped and unattached. Gular disc is greatly reduced, oval-shaped (type A). Only two individuals recorded.

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1221 Table 2. DNA and morphology sample numbers of *Garra* ecomorphs from the Sore River.

Ecomorphs	Morphology		mtDNA	RAD-seq
	Measurements	Gut length and diet		
1	27	18	27	22
2	17	7	19	13
3	19	13	18	11
4	20	10	17	13
5	15	14	24	11
6	2	-	2	2
Intermediate phenotype	6	-	5	5
<b>Total</b>	<b>106</b>	<b>62</b>	<b>112</b>	<b>77</b>

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1226 Table 3. Results of Patterson's D statistic (ABBA-BABA test) and *f*<sub>4</sub>-ratio test on selected  
1227 genetic clusters of *Garra* from the Sore River.

P1	P2	P3	D statistic	Z-score	p-value	f <sub>4</sub> -ratio	BBAA	ABBA	BABA
4	3	6	0.1176	5.3829	<0.0001	0.1128	227.5	235.0	185.5
2b	3	5	0.0650	3.1078	0.0009	0.4226	253.5	246.5	216.4
2b	6	3	0.0646	2.3475	0.0095	0.2854	215.6	217.3	190.9

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4	3	2b	0.0624	3.8143	<0.0001	0.1237	264.6	241.4	213.0
4	3	5	0.0492	3.6742	0.0001	0.3277	276.2	247.4	224.2
2b	6	5	0.0327	1.4755	0.0700	0.2051	248.6	203.4	190.5
4	6	5	0.0304	1.5315	0.0628	0.2330	224.5	226.5	213.2
6	3	5	0.0199	0.9380	0.1741	0.1641	244.2	204.7	196.8
2b	4	5	0.0178	1.0774	0.1406	0.1134	245.9	246.3	237.7
2b	6	4	0.0040	0.1592	0.4368	0.0151	244.6	197.8	196.3

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1242 Table 4. Summary of the ecomorphs' genetic diversity indices averaged over 89 070 loci (both  
1243 variant and fixed).

Ecomorphs *	No. of private alleles, Np	No. of polymorphic loci, %	Heterozygosity		Coefficient of inbreeding (Fis) ± SE	Nucleotide diversity (Pi) ± SE
			Observed (Ho) ± SE	Expected (He) ± SE		
1a	19	0.42	0.00128±	0.00116±	-0.00014±0.0015	0.00121±

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			0.00008	0.00007		0.00007
1b	18	0.40	0.00128± 0.00008	0.00113± 0.00007	-0.00019±0.0011	0.00119± 0.00007
2a	27	0.41	0.00124± 0.00008	0.00114± 0.00007	-0.00007±0.0012	0.00120± 0.00007
2b	9	0.24	0.00104± 0.00008	0.00079± 0.00006	-0.00023±0.0012	0.00091± 0.00007
3	20	0.43	0.00127± 0.00008	0.00107± 0.00006	-0.00037±0.0013	0.00111± 0.00007
4	38	0.43	0.00109± 0.00007	0.001± 0.00006	-0.00008±0.0015	0.00104± 0.00006
5	33	0.44	0.00126± 0.00008	0.00115± 0.00007	-0.00011±0.0019	0.00120± 0.00007
6	3	0.10	0.00058± 0.00007	0.0004± 0.0000	-0.00006±0.0004	0.00054± 0.00006

1244 \* - letters 'a' and 'b' assign genetic lineages within ecomorphs 1 and 2.

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1255 Fig. 1A. *Garra* ecomorphs 1-3 from the Sore River: 1 - 'generalized': 136 mm SL; 2 -  
 1256 'stream-lined': 99 mm SL; 3 - 'narrow-mouth': 100 mm SL.

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1260 Fig. 1B. *Garra* ecomorphs 4-6 from the Sore River: 4 - 'wide-mouth': 100 mm SL; 5 -  
 1261 'predator': 193 mm SL; 6 - 'thick-lipped': 128 mm SL.

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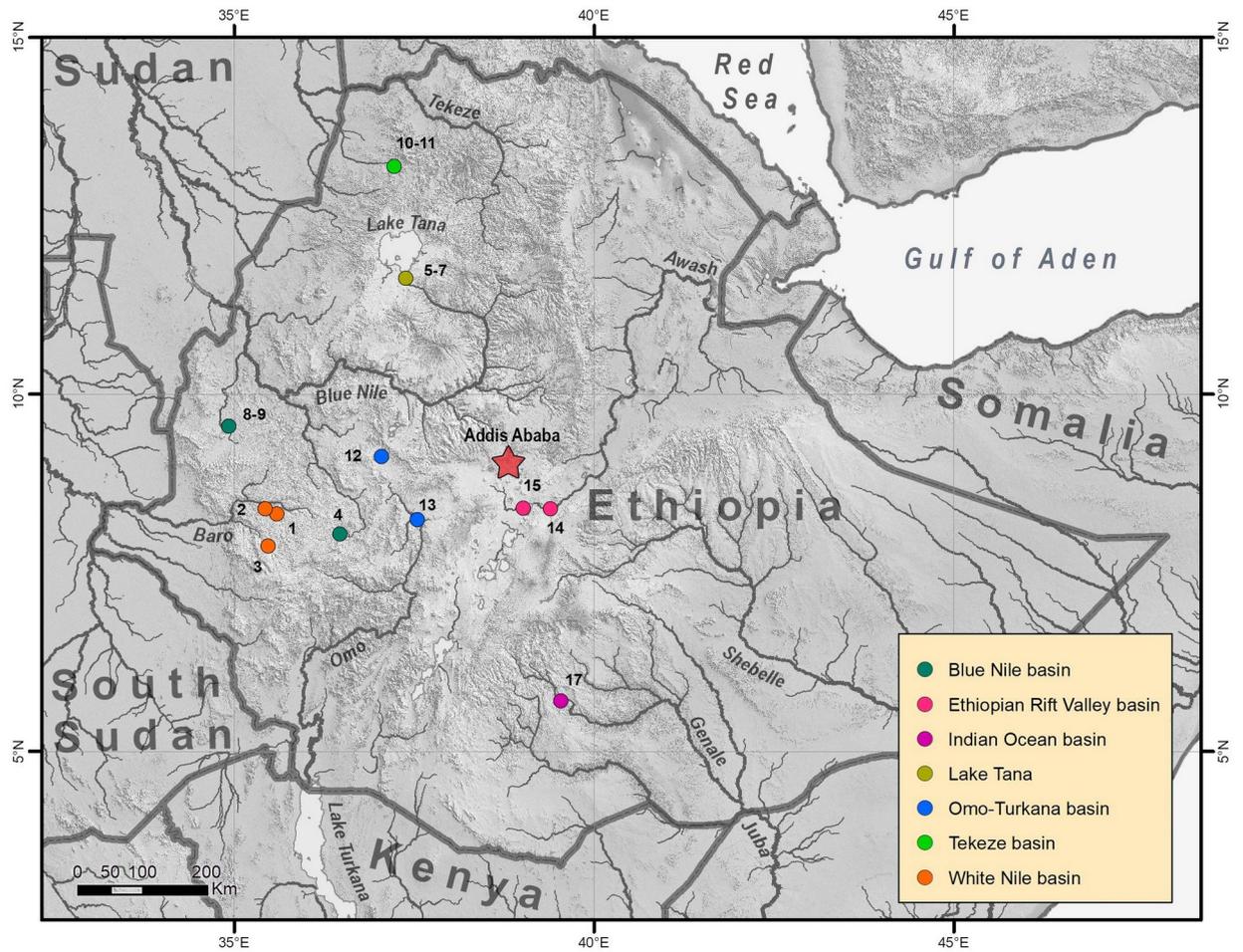
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1267 Fig. 2. Sampling sites of *Garra* in Ethiopian Highlands and Ethiopian Rift Valley; loc. 1-2 are in  
 1268 the Sore River.

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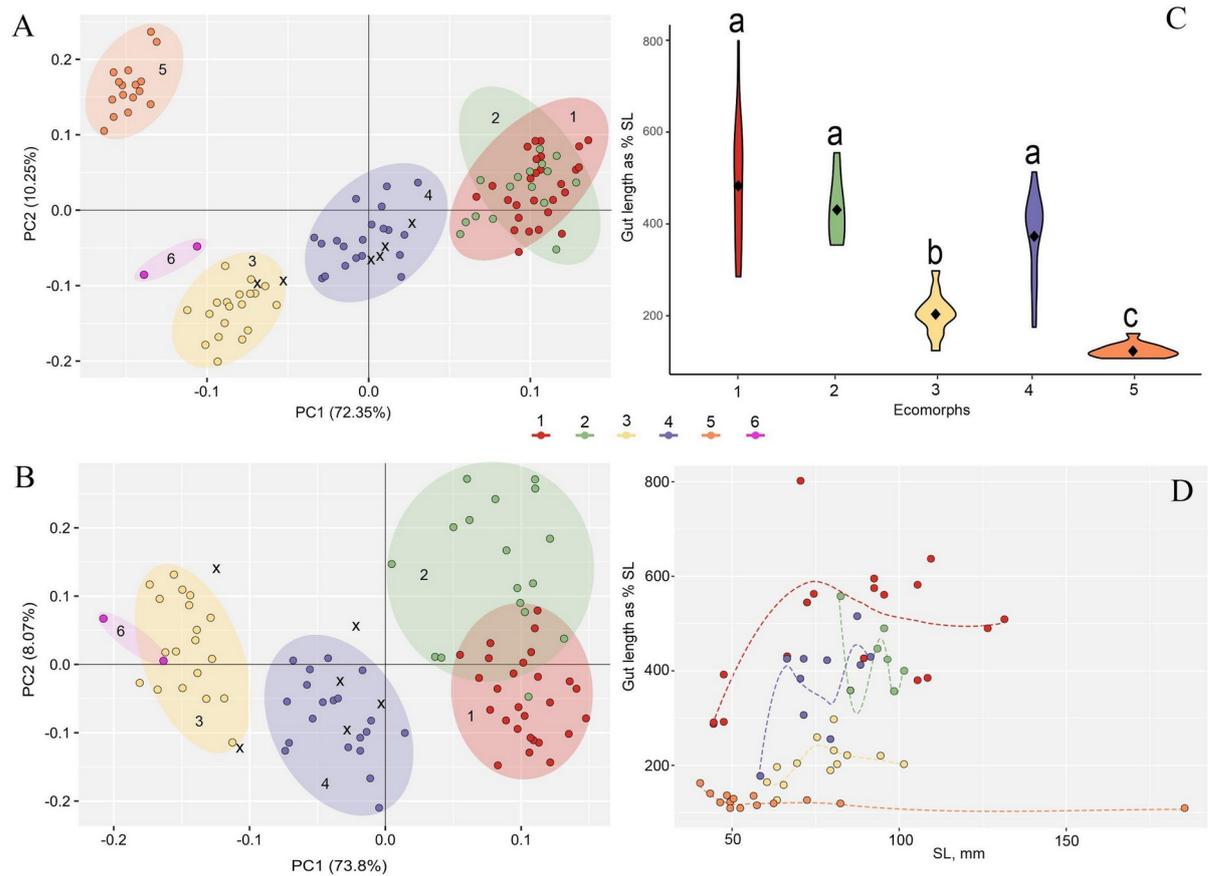
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1291 Fig. 3. (A) PCA of body and head proportions of six sympatric ecomorphs from the Sore River  
 1292 (n=107); (B) PCA of body and head proportions of five sympatric ecomorphs from the Sore  
 1293 River (n=90) excluding the most divergent sample, ecomorph 5. X designates intermediate  
 1294 phenotypes; (C) Gut length of five sympatric *Garra* ecomorphs from the Sore River represented  
 1295 as violin boxplots. Middle points are the means, and the box show the range respectively,  
 1296 samples are combined and each contains between 7 (ecomorph 2) and 18 (ecomorph 1)  
 1297 individuals, for a total of 62 individuals. Different lowercase letters above the boxplots indicate  
 1298 significant differences between ecomorphs ( $p < 0.05$ , Kruskal-Wallis test with BH adjustment of  
 1299  $p$ -value); (D) Dependence of gut length on body length in five *Garra* ecomorphs from the Sore  
 1300 River with smooth local regression lines (Loess regression).

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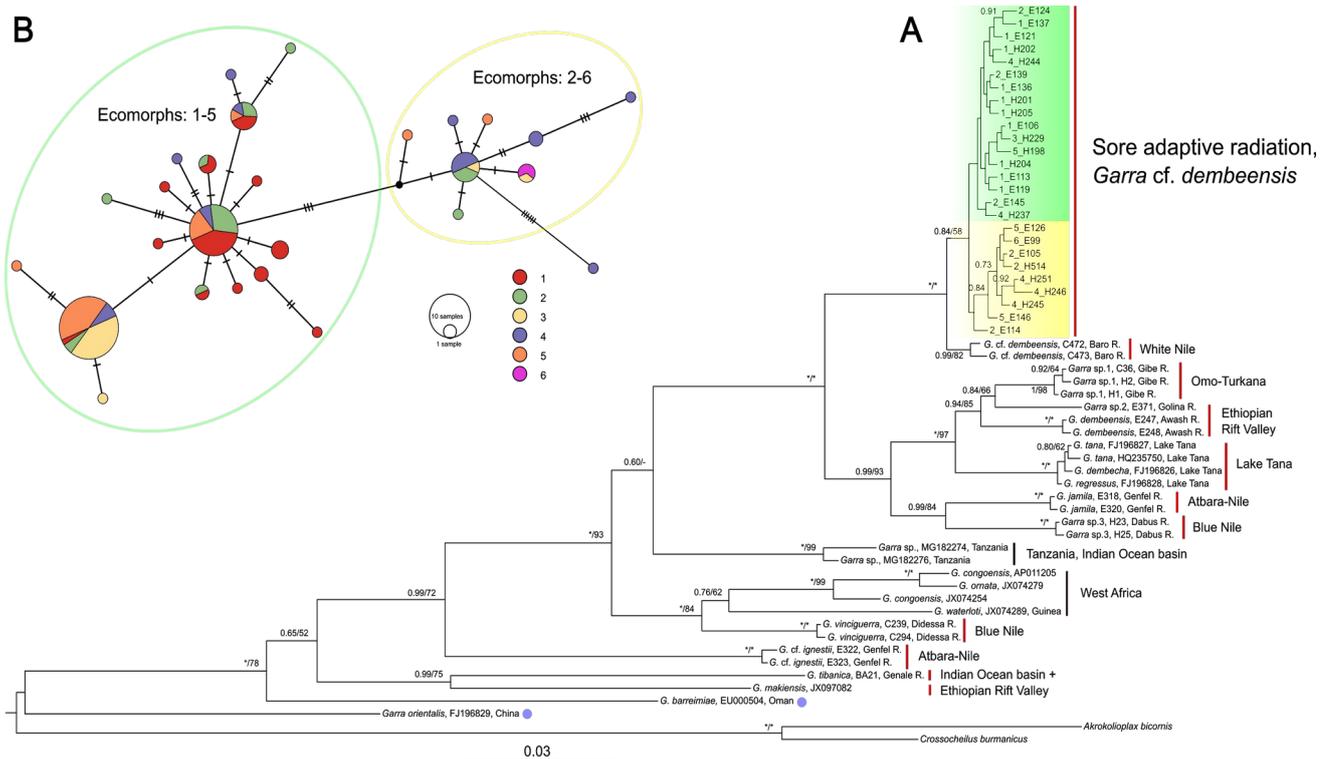
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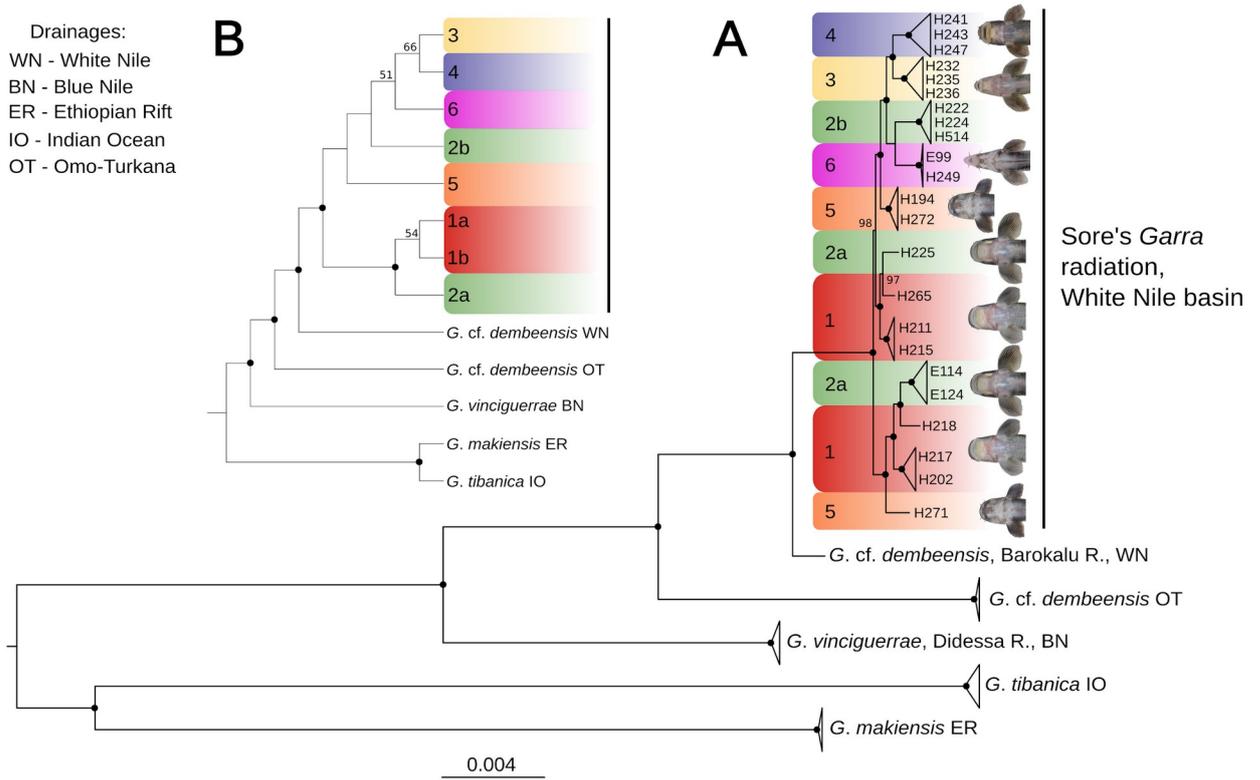
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1316 Fig. 4. (A) Consensus tree of relationships among the Ethiopian *Garra* from all main drainages  
 1317 based on *cytb* sequences. Bayesian posterior probabilities (before slash) from BI analysis and  
 1318 bootstrap values from ML analysis (after slash) above 0.5/50 are shown; asterisks represent  
 1319 posterior probabilities/bootstrap values of 1/100. Scale bar and branch lengths provide the  
 1320 expected substitutions per site. The green and yellow colors highlight two branches of *Garra* in  
 1321 the Sore River. (B) Median-joining haplotype network of the *Garra* from the Sore River, based  
 1322 on 107 *cytb* sequences (989 bp length). ‘Green’ haplogroup includes ecomorphs 1-5, while  
 1323 ‘yellow’ haplogroup includes ecomorphs 2-6. Black dots represent hypothetical intermediate  
 1324 haplotypes.

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1347 Fig. 5. (A) ML phylogenetic tree of Ethiopian *Garra* based on RAD-loci sequences - 23,365  
 1348 loci; 3,075,180 bp and (B) SVDQ species tree. Each locus was treated as a separate partition  
 1349 with GTR+G substitution model and heterozygous sites within each individual encoded using  
 1350 IUPAC notation. Black dots designate 100% bootstrap support, and only values above 50% are  
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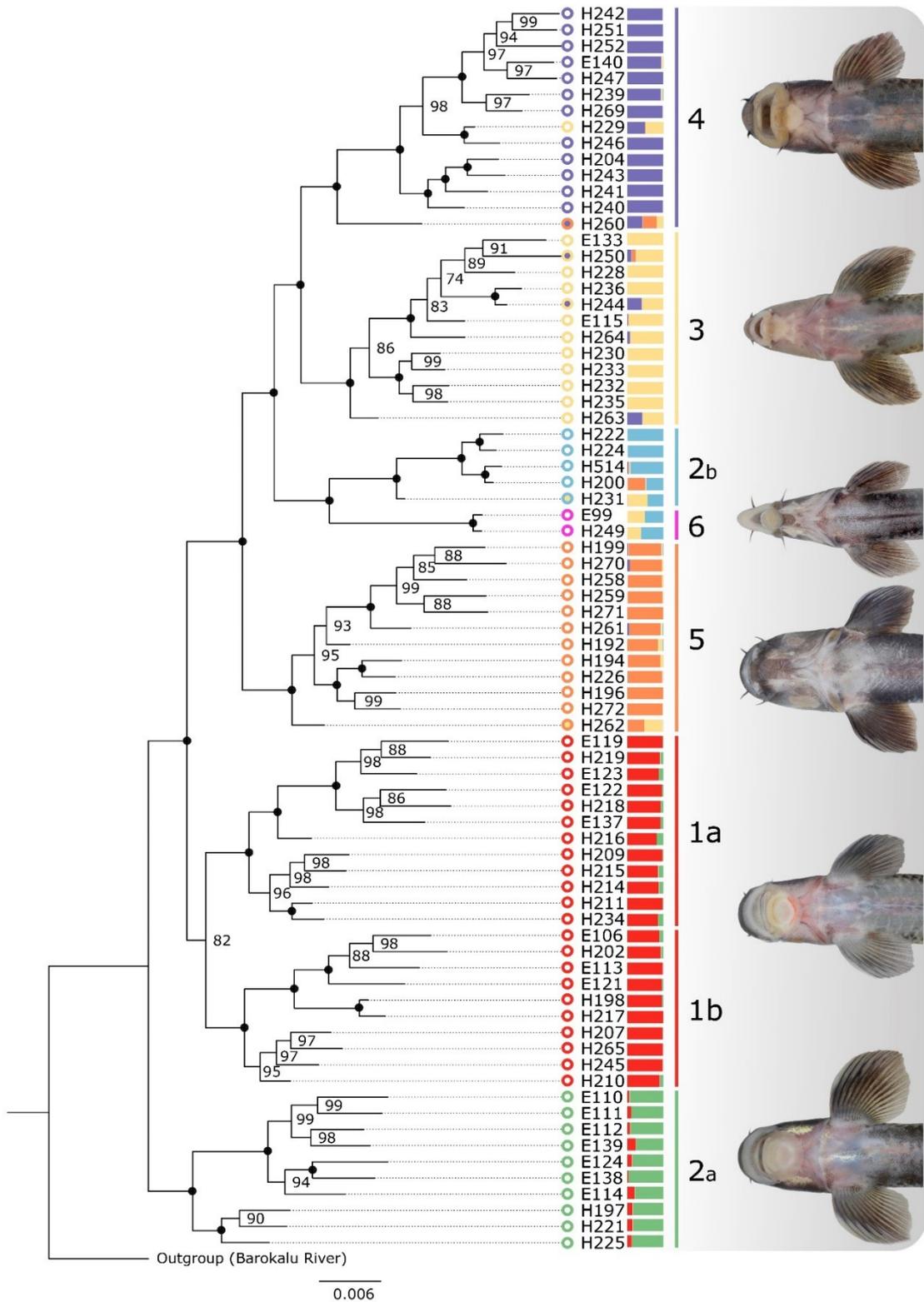
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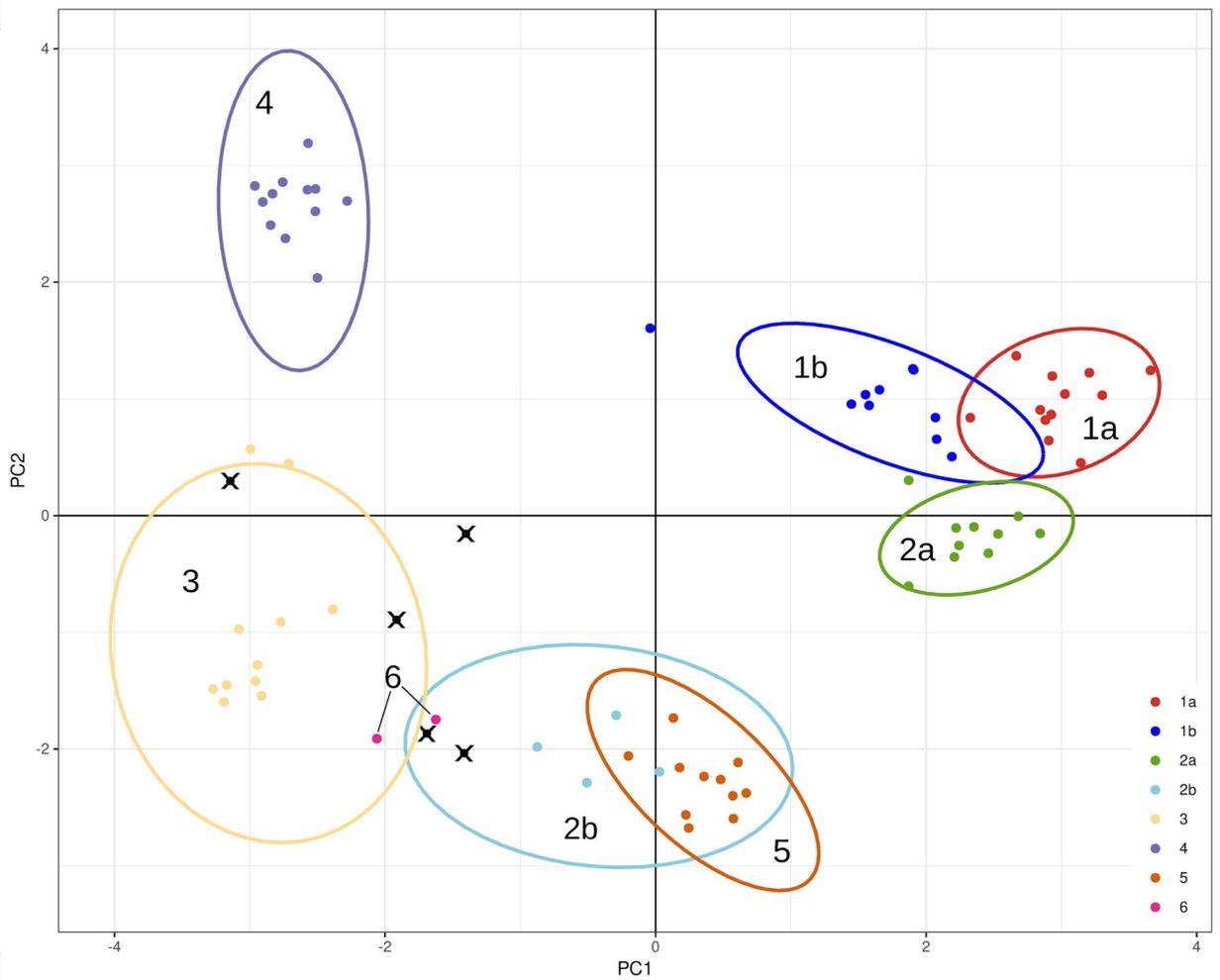


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1377 Fig. 6. ML phylogeny of sympatric *Garra* ecomorphs from the Sore River based on concatenated  
 1378 RAD-loci sequences (7,370 loci; 969,450 bp). Each locus was treated as a separate partition with  
 1379 GTR+G substitution model. Heterozygous sites within each individual encoded using IUPAC  
 1380 notation. The individual samples are colored based on the color scheme of Fig. 4 and  
 1381 intermediate (putative hybrids) phenotypes are depicted in another color. The genetic clusters  
 1382 proportions inferred by *rmaverick* analysis are shown to the right of sample numbers. Black  
 1383 points designate 100% bootstrap support.

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1385 Fig. 7. Principal Component Analysis (PCA) based on 679 nuclear SNPs of sympatric  
 1386 *Garra* ecomorphs from the Sore River. Points (individuals) and 95% confidence ellipses are  
 1387 colored by phenotype/genetic cluster. Crosses assign intermediate phenotypes.

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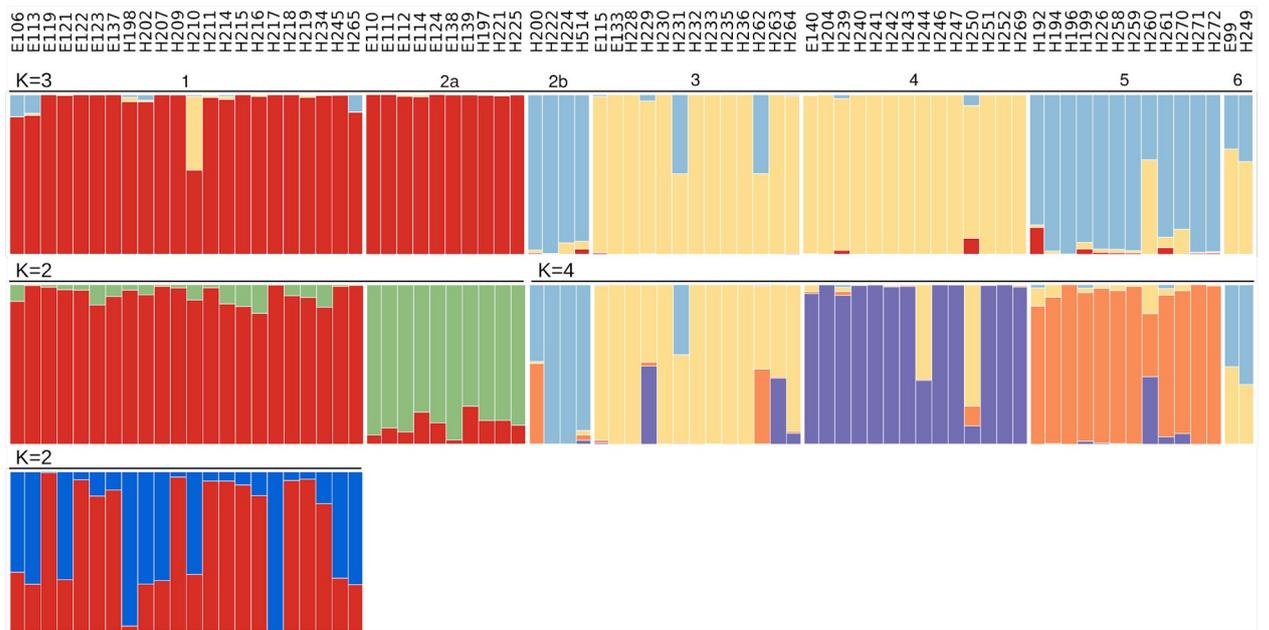
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1408 Fig. 8. Hierarchical *rmaverick* results for sympatric ecomorphs of *Garra* from the Sore River,  
 1409 based on 679 nuclear SNPs. Each column of the barplot shows individual assignments to one of  
 1410 the inferred genetic clusters. Independent runs of *rmaverick* are indicated by a solid black line  
 1411 above a plot, along with an inferred value of *K*.

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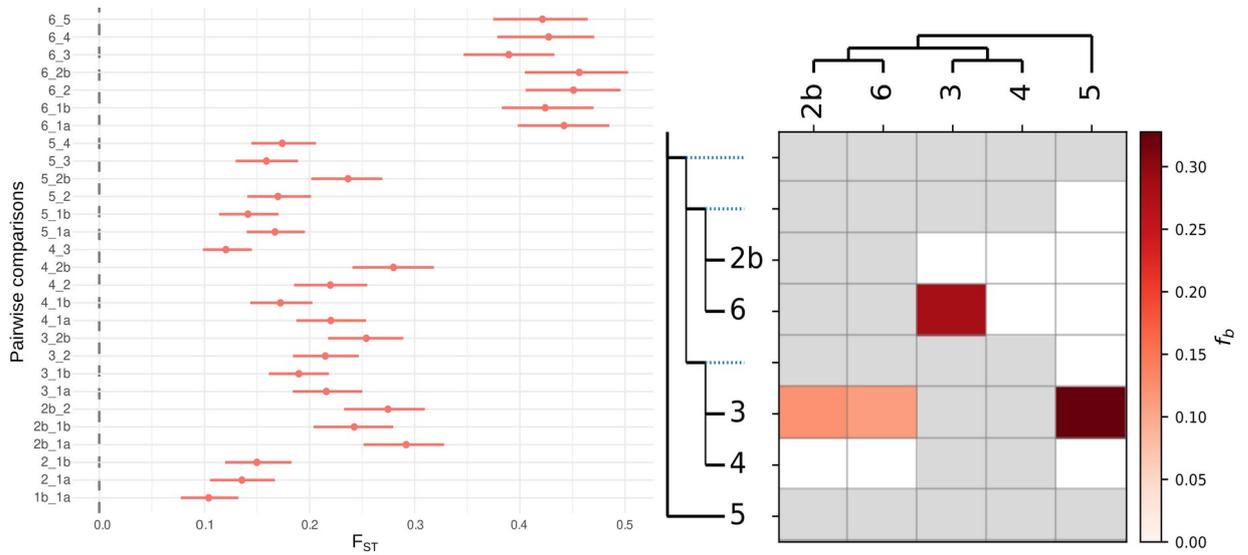
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1441 Fig. 9. Left - pairwise Reich  $F_{ST}$  values (points) with their respective 95% confidence  
 1442 intervals (horizontal lines) for *Garra* genetic lineages from the Sore River based on 679 SNPs.  
 1443 Right - heat map of  $f$ -branch metric for selected ecomorphs/lineages of the *Garra* Sore  
 1444 radiation. The used guide tree is shown along the x and y axes (in 'laddered' form along the y  
 1445 axis). The matrix shows the inferred  $f$ -branch metric, reflecting excess allele sharing between  
 1446 the branch of the 'laddered' tree on the y axis (relative to its sister branch) and the branches  
 1447 defined on the x axis.