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Rapid adaptive radiation in a hillstream cyprinid fish in the East African White Nile River basin

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Abstract

Adaptive radiation of freshwater fishes was long thought to be possible only in lacustrine environments. Recently, several studies have shown that riverine and stream environments also provide the ecological opportunity for adaptive radiation. In this study, we report on a riverine adaptive radiation of six ecomorphs of cyprinid hillstream fishes of the genus *Garra* in a river located in the Ethiopian Highlands in East Africa. *Garra* are predominantly highly specialized algae-scrapers with a wide distribution ranging from Southeast Asia to West Africa. However, adaptive phenotypic diversification in mouth type, sucking disc morphology, gut length and body shape have probably been found among these ecomorphs in a single Ethiopian river. Moreover, we found two novel phenotypes of *Garra* ("thick-lipped" and "predatory") that had not been discovered before in this species-rich genus (>160 species). Mitochondrial and genome-wide data suggest monophyletic, intrabasin evolution of *Garra* phenotypic diversity with signatures of gene flow from other local populations. Although sympatric ecomorphs are genetically distinct and can be considered to being young species as suggested by genome-wide single nucleotide polymorphism data, mitochondrial DNA was unable to identify any genetic structure suggesting recent and rapid speciation events. Some data suggest a hybrid origin of the novel "thick-lipped" ecomorph. Here we highlight how, driven by ecological opportunity, an ancestral trophically highly specialized lineage is likely to have rapidly radiated in a riverine environment promoted by the evolution of novel feeding strategies.

KEYWORDS
adaptive radiation, African fishes, *Garra*, mouth polymorphism, speciation

1 | INTRODUCTION

Unravelling the mechanisms that drive biological diversity remains a major challenge in evolutionary biology. With more than 28,000 species, teleost fishes are the most diverse lineage of vertebrates, and thus an ideal system to address questions regarding mechanisms and geographical settings of diversification. A large portion of the phenotypic diversity of bony fishes has been produced through the process of adaptive radiation, the rapid proliferation of multiple ecologically distinct species from a common ancestor (Schluter, 2000). One of the most extraordinary examples of both adaptive radiation and explosive diversification is represented by the cichlid fishes inhabiting the East African Great Lakes (Meyer, 1993). The evolutionary success of the cichlids, unmatched among vertebrates, has been promoted by a combination of different factors, such as limited dispersal, habitat specialization, and sexual selection for nuptial

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coloration and mating behaviour (Meyer et al., 1990; Sturmbauer et al., 2008; Verheyen et al., 1996). It has been suggested, however, that trophic radiation had preceded the diversification driven by other factors at least in cichlids of Lake Tanganyika (Muschick et al., 2014; Rüber et al., 1999), a cradle of all other East African haplochromine radiations (Irisarri et al., 2018; Ronco et al., 2021). Adaptive radiations and diversification bursts were found not only in cichlids, but also in other fish groups, even though at smaller scale, and often in a parallel manner—coregonids, Arctic charrs and sticklebacks (e.g., Brodersen et al., 2018; DeFaveri and Merilä, 2013; Jacobs et al., 2020; McKinnon & Rundle, 2002; Præbel et al., 2013; Peichel et al., 2001; Schluter, 2000; Skúlason, 1999; Terekhanova et al., 2014) are some of the best known examples of intralacustrine radiations.

The most well-supported cases of monophyletic, closely related fish species that are believed to have arisen through an adaptive radiation event have been described from lakes rather than rivers (Meyer et al., 1990; Seehausen, 2006; Sturmbauer, 1998; Taylor, 1999). Riverine environments had long been considered to be not suitable for adaptive radiation because of its unstable hydrological regimes, reduced habitat diversity, and the commonly shallow and narrow watercourses that might facilitate gene flow (Seehausen & Wagner, 2014). However, during the last two decades, several examples of fish adaptive radiations occurring in rivers have been reported (Burress et al., 2018; Dimmick et al., 2001; Levin et al., 2020; Melnik et al., 2020; Piálek et al., 2012; Schwarzer et al., 2011; Whiteley, 2007). Although several cases of riverine diversification of cichlid fishes are considered as remnants of adaptive radiations that occurred in palaeo-Lake Makgadikgadi before it dried up in the Holocene (Joyce et al., 2005), mounting evidence suggests that some fish species flocks of species other than cichlids have diversified within rivers (Levin et al., 2019, 2020; Melnik et al., 2020; Roberts, 1998; Roberts & Khaironizam, 2008).

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

The Ethiopian Highlands are recognized as a centre of *Garra* diversity within Africa (Golubtsov et al., 2002; Stiassny & Getahun, 2007), where 13 described species out of the total 23 found in Africa are recorded (Moritz et al., 2019). An assemblage of six *Garra* ecomorphs (ecomorph *sensu* Williams, 2013) exhibiting extreme morphological diversity was recently discovered in the Sore River (the White Nile Basin) in southwestern Ethiopia during a survey of Ethiopian fishes (Golubtsov et al., 2012). In particular, two of the six ecomorphs display features not found elsewhere within the genus: an ecomorph with a pronounced predatory morphology (large-sized, large-mouthed, with reduced sucking disc and a short gut that is equal to body length) and one with "rubber" lips and prolonged snout region (Figure 1, Table 1). The other four ecomorphs from the Ethiopian *Garra* assemblage drastically differ in mouth and gular disc morphology as well as in body shape (Figure 1).

Our goals were two-fold: (i) to investigate the morpho-ecological relationships of six *Garra* sympatric ecomorphs from the Sore River, and (ii) to test whether this assemblage has evolved sympatrically. In an effort to elucidate the population structure and evolutionary history of these ecomorphs we used both mitochondrial DNA (mtDNA, cytochrome *b*) and a genome-wide nuclear approach based on loci obtained by double digest restriction-site associated DNA (ddRAD).

2 | MATERIALS AND METHODS

2.1 | Study area

The Sore River is a headwater tributary of the Baro-Akobo-Sobat drainage in the White Nile basin (southwestern Ethiopia, northern East Africa). It drains the Ethiopian Highlands close to the southwestern escarpment. The region is covered by moist Afromontane forest that has been drastically shrinking in recent decades due to agricultural development (Dibaba et al., 2019). The Sore is quitre with a length of ~160 km, its catchment area is ~2000 km^2 and

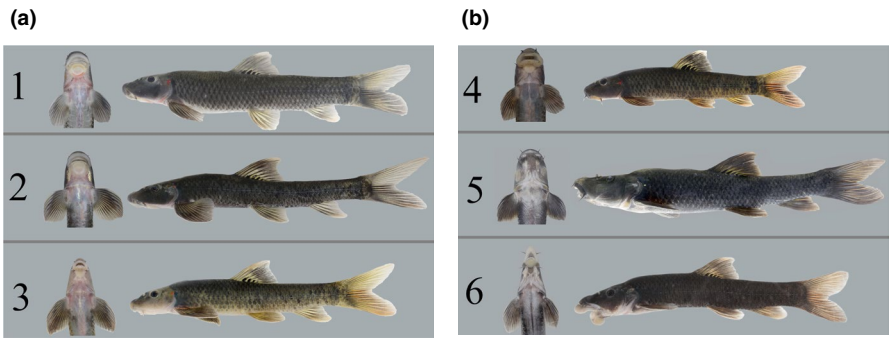

FIGURE 1 (a) *Garra* ecomorphs 1–3 from the Sore River: 1, "generalized": 136 mm SL; 2, "stream-lined": 99 mm SL; 3, "narrow-mouth": 100 mm SL. (b) *Garra* ecomorphs 4–6 from the Sore River: 4, "wide-mouth": 100 mm SL; 5, "predator": 193 mm SL; 6, "thick-lipped": 128 mm SL [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Common names of the six ecomorphs of African *Garra* from the Sore River, and the preliminary qualitative descriptions used in the field to identify each form

Name used in the text	General description
No. 1, "generalized"	Well-developed round gular disc of type C with free posterior margin (disc classification follows Stiassny & Getahun, 2007). Body shape is generalized for *Garra*.
No. 2, "stream-lined"	Slender streamlined 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 the other ecomorphs. Largest individuals have a 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.

characterized by substantial seasonal variation of rainfall (dry season from December to March) (Kebede et al., 2014). The elevation difference between the Sore source (altitude of ~2215 m asl, above sea level) and its confluence with the Gabba (Geba) River (alt. 963 m asl) is 1.25 km. The Sore River basin shares drainage boundaries with two of six major watersheds of Ethiopia: Blue Nile in the northeast and Omo-Turkana in the southeast.

We sampled the middle reaches of the Sore River at two sites: (i) at the City of Metu (8°18′42″N, 35°35′54″E, alt. 1550 m asl) and (ii) ~35 km downstream along the river course (8°23′56″N, 35°26′18″E, alt. 1310 m asl). The river width at the rapids sampled was 20–40 m at the beginning of the rainy season, depth <1 m, and the bottom consisted of rocks and large boulders. The fish fauna of the river segment under consideration includes (apart from *Garra* spp.) a species flock of *Labeobarbus* (Levin et al., 2020), *Enteromius* cf. *pleurogramma* (Boulenger 1902), *Labeo* cf. *cylindricus* Peters 1852, *Labeo forskalii* Rüppell 1835, *Chiloglanis* cf. *niloticus* Boulenger 1900 (at the lower site only) and introduced *Coptodon zillii* (Gervais 1848). The presence of the stony loach (*Afronemacheilus*) reported by Getahun and Stiassny (1998) from the Sore River at Metu could not be confirmed (Melaku et al., 2017; Prokofiev & Golubtsov, 2013; present study). One hundred kilometres west, from the lowland part (alt. ~500 m asl) of the same river drainage, >100 fish species are recorded (Golubtsov & Darkov, 2008; Golubtsov et al., 1995) and >115 species from the Sudd and White Nile in Sudan and South Sudan (Moritz et al., 2019; Neumann et al., 2016).

2.2 | Sampling

Garra samples from the Sore River were collected using a battery-driven electrofishing device (LR-24 Combo Backpack, Smith-Root), cast and frame nets in June 2012 and April 2014. In 2011–2014 comparative *Garra* samples were collected from nine sites in six main Ethiopian basins (Figure 2; Table S1). Fish sampling was conducted under the umbrella of the Joint Ethiopian–Russian Biological Expedition (JERBE) with the permissions of National Fisheries and Aquatic Life Research Center (NFALRC) under Ethiopian Institute of Agricultural Research (EIAR) and Ethiopian Ministry of Science and Technology (presently Ministry of Innovation and Technology). Fish were killed with an overdose of MS-222 anaesthetic, first preserved in 10% formalin and then transferred to 70% ethanol. From each specimen fin tissue samples were fixed with 96% ethanol. A subset of the fish samples was photographed using a Canon EOS 50D camera. All specimens (Table S1) are deposited at the A.N. Severtsov Institute of Ecology and Evolution, at the Russian Academy of Sciences, Moscow, under provisional labels of JERBE.

2.3 | Morphological analysis

2.3.1 | Morphometry

In total, 28 morphometric characters from 107 individuals of all ecomorphs from the Sore River were examined following Hubbs and Lagler (1958) with additions from Menon (1964): standard length (SL), head length (HL), snout length (R), eye diameter (O), postorbital distance (PO), interorbital distance (IO), head width (HW), head height at nape (HH), head height at mid-of-eye (Hh), mouth width (MW), disc length (DL), disc width (DW), maximal body height (H), minimal body height at caudal peduncle (h), predorsal length (PL), postdorsal length (PDL), prepelvic length (PPL), preanal length (PAL), caudal peduncle length (CPD), dorsal fin base length (DFL), dorsal fin depth (DFP), anal fin base length (AFL), anal fin depth (AFD), pectoral

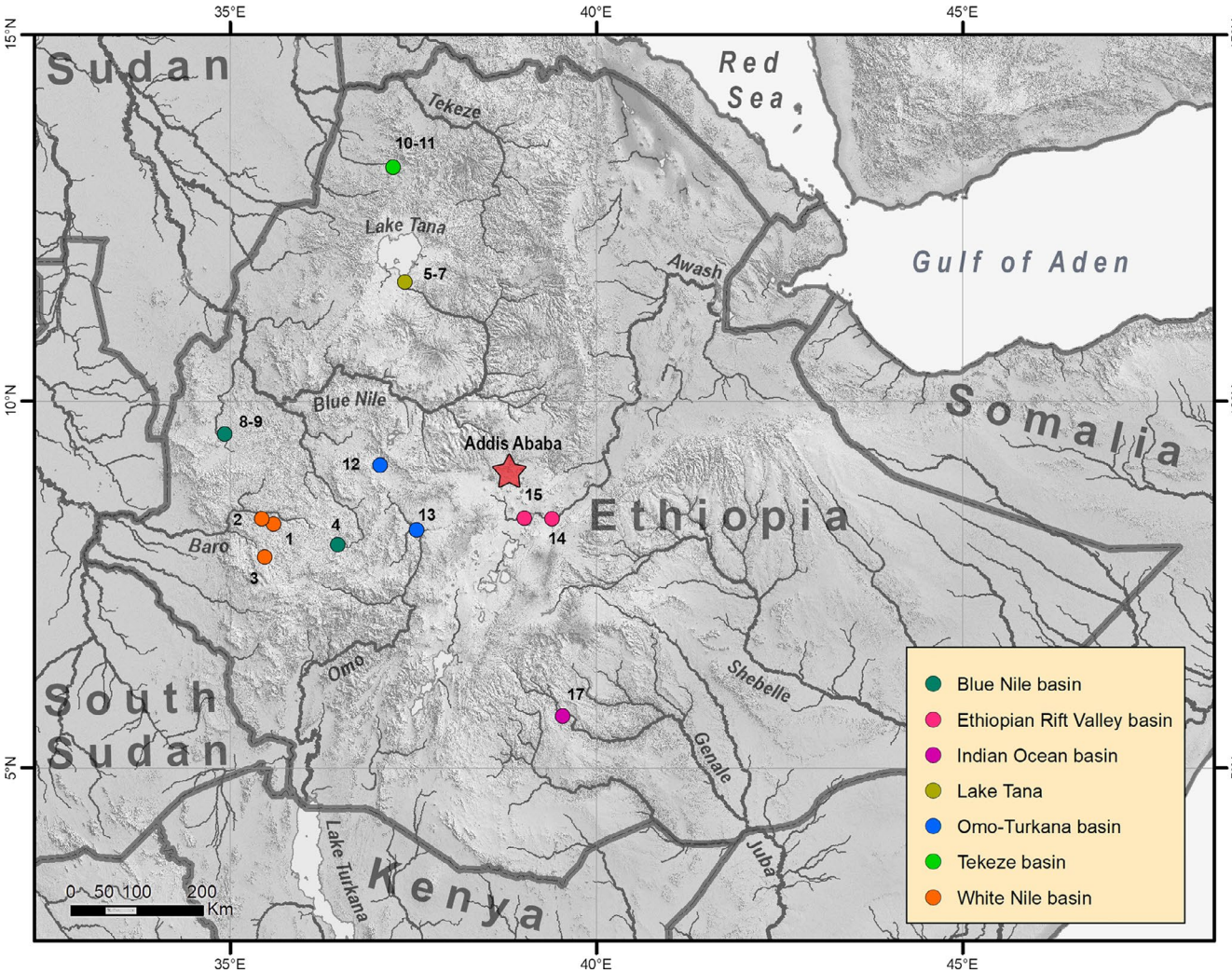

FIGURE 2 Sampling sites of *Garra* in the Ethiopian Highlands and Ethiopian Rift Valley; locations 1 and 2 are in the Sore River [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 DNA and morphology sample numbers of *Garra* ecomorphs from the Sore River

	Morphology			
Ecomorphs	Measurements (standard length range, mm)	Gut length and diet	mtDNA	RAD-seq
1	27 (71.5–151.0)	18	27	22
2	17 (70.9–160.2)	7	17	13
3	19 (49.3–100.6)	13	17	11
4	20 (49.3–90.6)	10	16	13
5	15 (43.6–185)	14	23	11
6	2 (118.4; 139.4)	–	2	2
Intermediate phenotype	6 (59.3–105.2)	–	5	5
Total	106	62	107	77

fin length (PFL), ventral fin length (VFL), pectoral–ventral fin distance (PV), ventral–anal fin distance (VA), and distance between anal opening and anal fin (DAA). Measurements were made using a digital caliper (to the nearest 0.1 mm). All measurements were performed by one operator for the purpose of consistency as recommended by Mina et al. (2005).

Measured individuals had body length ranging from 43.6 to 185.0 mm standard length (SL) (Table 2). The proportions of head

and body were used for principal component analysis (PCA); measurements of head parts were divided for head length, and measurements of body parts were divided for SL. The gular disc in some specimens of the "predator" ecomorph was greatly reduced which hampered the detection of its borders. To justify the values of this character, identical intermediate values were arbitrarily assigned for all specimens of this ecomorph. Input data were first standardized using the scaling procedure implemented in the *prcomp* R package, and then the PCA was performed on the variance–covariance matrix.

2.3.2 | Gut length and preliminary assay of a diet

Intestines were taken from the body cavity of 62 preserved specimens of all ecomorphs except for "thick-lipped" (represented by only two specimens), and measured using a ruler to the nearest 1 mm. The sample size for each ecomorph is provided in Table 2. The SL of examined individuals varied from 40 to 131 mm; one individual of ecomorph 5 had an extreme length of 185 mm. The ratio of gut length (GL) to SL was used for subsequent analyses. A Kruskal–Wallis test with Dunn's post hoc test was applied to check for differences between the groups with adjustment $p < .05$ after controlling for multiple testing with the false discovery rate (FDR) (Benjamini & Hochberg, 1995). The dependence of GL on SL was visualized using scatterplots and regressions. The R-packages *ggplot2* and *PMCMR* were used to create plots and to test whether the observed differences were statistically significant.

Diet was assessed for the same individuals whose intestine length was measured. The main ecological and systematic groups were identified using a Micromed MC-2-ZOOM stereomicroscope and Olympus CX41 microscope. A composite measure of diet, an index of relative importance, IRI (Hart et al., 2002), was used to assess the contribution of different components to a diet. The diet components were grouped into several items: (i) periphyton, (ii) benthos, (iii) macrophytes and (iv) others.

2.3.3 | DNA sampling, extraction, amplification and sequencing—mtDNA data

DNA samples (n = 107) were collected from *Garra* inhabiting the Sore River near the City of Metu in 2012 and 2014 from all six forms (Table 2 for details). For comparison, additional DNA samples (n = 20) were collected from eight *Garra* species inhabiting all main drainages of Ethiopia (10 localities—see map of sampling in Figure 2). Total genomic DNA was extracted from ethanol-preserved fin tissues using the BioSprint 15 kit for tissue and blood (Qiagen). Sequences of the mitochondrial gene cytochrome *b* (*cytb*), 989 bp in length, were amplified (see polymerase chain reaction [PCR] conditions in Material S2; Palumbi, 1996; Perdices & Doadrio, 2001). PCR products were visualized on 1% agarose gels, purified with ExoSAP-IT and sequenced at the Papanin Institute of Biology of Inland Waters (Russian Academy of Sciences) using an ABI 3500 sequencer. All new sequences were deposited in GenBank (accession nos.: MZ570972-MZ571096 and MZ66554-MZ665542—Table S1 for details).

2.4 | Analysis of mtDNA data

All sequences were aligned and edited using the MUSCLE algorithm (Edgar, 2004) as implemented in MEGA 6.0 (Tamura et al., 2013). A final set that also includes comparative material from GenBank (African and non-African *Garra* as well as outgroups) encompassed 143 *cytb* sequences (accession nos. are provided in Table S1). *Akrokolioplax bicornis* and *Crossocheilus burmanicus* were included as outgroups according to previously published phylogenies (Yang et al., 2012).

Gene tree reconstruction was performed using both maximum-likelihood (ML) and Bayesian inference (BI) approaches. Prior to these analyses, all sequences were collapsed into common haplotypes using ALTER (Glez-Peña et al., 2010). We determined the best fit models of nucleotide substitution for each codon position of *cytb* and optimal partitioning scheme using MODELFINDER (as implemented in IQ-TREE 1.6.12; Kalyaanamoorthy et al., 2017; Nguyen et al., 2015) for ML inference or PARTITIONFINDER 2.1.1 (Lanfear et al., 2012) for (BI under the Bayesian Information Criterion (BIC). The partition scheme selected by MODELFINDER (codon position 1, K2P+R2; codon position 2, HKY+F+I; codon position 3, TN+F+G4) was subsequently used in ML searches with IQ-TREE, using 1,000 bootstrap replicates.

BI was carried out in MRBAYES version 3.2.6 (Ronquist et al., 2012). The selected partition scheme was as follows: codon position 1 with K80+I+G, codon position 2 with HKY+I, and codon position 3 with GTR+G. Two simultaneous analyses were run for 10^7 generations, each with four Monte Carlo Markov chains (MCMC) sampled every 500 generations. Convergence of runs was assessed by examination of the average standard deviation of split frequencies and the potential scale reduction factor. In addition, stationarity was confirmed by examining posterior probability, log likelihood, and all model parameters by the effective sample sizes (ESSs) in the program TRACER version 1.6 (Rambaut et al., 2014). The gene trees resulting in ML and BI analyses were visualized and edited using FIGTREE version 1.4.4 (Rambaut, 2014). A haplotype network was constructed using the median joining algorithm (Bandelt et al., 1999) in POPART 1.7 (Leigh & Bryant, 2015) with the default value of epsilon (0).

2.5 | ddRAD-seq library preparation

High-molecular-weight DNA was isolated from fin tissue preserved in ethanol using a QIAamp DNA Mini Kit (Qiagen) or obtained with a salt-based DNA extraction method (Aljanabi & Martinez, 1997) followed by purification using a CleanUp Standard kit (Evrogen). The quantity of dsDNA was measured using a dsDNA HS Assay Kit for fluorometer Qubit 3 (Life Technologies). A ddRAD-library was constructed following the quaddRAD protocol (Franchini et al., 2017) using restriction enzymes *Pst*I and *Msp*I. In total, 77 DNA samples of *Garra* ecomorphs from the Sore River (see Table 2) and 11

DNA samples from five other species of Ethiopian *Garra* from adjacent basins were sequenced by two independent runs of Illumina HiSeq2500 and Illumina X Ten (2 × 150 bp paired-end reads). The raw sequencing data were demultiplexed by the sequencing provider using outer Illumina TruSeq dual indexes.

2.6 | Processing of RAD-seq data

The resulting reads were trimmed for remaining adapters and low-quality reads using CUTADAPT implemented in the TRIM GALORE 0.4.5 package (https://github.com/FelixKrueger/TrimGalore - Martin, 2011). Read quality was assessed with FASTQC 0.11.7 (Andrews & Krueger, 2010) and MULTIQC 1.7 (Ewels et al., 2016) before and after trimming. Further demultiplexing of individually barcoded samples, construction and cataloguing of RAD-loci and single nucleotide polymorphism (SNP) calling were done with STACKS 2.41 (Rochette et al., 2019). Identification and removal of PCR duplicates were done using the *"clone_filter"* module of STACKS. The STACKS module *"process_radtags"* was used to demultiplex reads by the dual index inner barcodes and obtain separate fastq files for each individual. Samples that failed to produce more than 100,000 reads were excluded from further processing. To additionally evaluate data quality and identify possible contaminated samples, the reads were mapped to the reference genome of the common carp *Cyprinus carpio* (GCF_000951615.1) using BOWTIE2 2.3.5 (Langmead & Salzberg, 2012) with "--local-sensitive" presettings. Only Read 1 (R1) fastq files were used for downstream processing and analyses. Finally, R1 reads were trimmed at their 3′ ends to a uniform length of 130 bp to reduce the influence of sequencing error (due to decreased base quality at the 3′ end).

The de novo pipeline of STACKS was used to assemble loci and perform genotype calling. We selected optimal parameters using the approach suggested by Paris et al. (2017). Following the aforementioned procedure, we found that a minimum stack depth (-*m*) of 5, distance allowed between stacks (-*M*) of 3 and maximum distance required to merge catalogue loci (-*n*) of 5 provided the best balance between data quality and quantity for our data set (Figure S1).

2.7 | Population genomic analyses

Individual genotypes of sympatric *Garra* ecomorphs from the Sore River were exported to a vcf file using the "*populations*" module of STACKS with the following settings: (i) loci genotyped in at least 90% of samples (-r 0.90) were kept; (ii) SNPs with a minor allele frequency (--min-maf) less than 0.04 and a maximum observed heterozygosity (--max_obs_het) above 0.99 were pruned; and (iii) to avoid inclusion of closely linked SNPs, only a single SNP per RAD locus was retained. VCFTOOLS 0.1.16 (Danecek et al., 2011) was applied for further filtering of the data set based on mean coverage and fraction of missing data for each sample. Samples with more than 20% of missing data were removed from further analyses. A high-quality data set of 679 SNPs and 77 individuals was used for downstream population genetics analyses.

First, PCA was performed using the *"glPca"* function of the R-package ADEGENET 2.1.1 (Jombart, 2008; Jombart & Ahmed, 2011). Next, RMAVERICK 1.0.5 (former MavericK; Verity & Nichols, 2016) was used to infer population structure. This program estimates evidence for different numbers of populations (*K*), and different evolutionary models via generalized thermodynamic integration (GTI). A range of *K* values between 1 and 10 were explored, using 300 000 burn-in MCMC iterations and 10,000 sampling iterations. Convergence of MCMC was automatically tested every 1,000 burn-in iterations by activating option "auto_converge." This allows exit burn-in iterations when convergence is reached and immediately proceeds to sampling iterations. Parameter "rungs" was set to 10 (number of multiple MCMC chains with different "temperature" to run simultaneously). Both "no admixture" and "admixture" models were run and compared by plotting values of the posterior distribution and overall model evidence in log space (log-evidence) (Figures S2–S5). According to this comparison, the admixture model is decisively supported over the no admixture model and used here to report the results. The same protocol was followed for consecutive hierarchical RMAVERICK runs for the identified clusters. Finally, global and pairwise Reich–Patterson F_{ST} values (Reich et al., 2009) with respective 95% confidence intervals for ecomorphs/genetic clusters were calculated using the R script from Junker et al. (2020). Basic genetic diversity statistics (heterozygosity, nucleotide diversity, number of private alleles, etc.) were calculated using the "*populations*" module of STACKS.

To test for gene flow between ecomorphs/genetic clusters, we used Patterson's D statistic (ABBA-BABA test), along with the f_4-ratio statistic (Patterson et al., 2012) and its *f*-branch metric (Malinsky et al., 2018), as implemented in the DSUITE 0.4 software package (Malinsky et al., 2021). Patterson's D statistic is a widely used and robust tool to detect introgression between populations or closely related species, and to distinguish it from incomplete lineage sorting (ILS). The f_4-ratio statistic is a similar method aiming to estimate an admixture fraction. The *f*-branch metric is based on $_4$-ratio results and serves to assign gene flow evidence to specific branches on a phylogeny. These tests were performed on a group containing ecomorphs/genetic clusters 2b, 3, 4 and 6, while the rest were used as an outgroup (in accordance with the results of our phylogenomic analysis).

2.8 | Phylogenomic analyses

IQ-TREE 2.0.5 (Minh et al., 2020) was used for ML phylogenetic analyses of RAD-seq data. The first data set included one to three specimens of each *Garra* ecomorph from the Sore River and other Ethiopian *Garra* species from adjacent basins. Multiple sequence alignments of all loci and respective partition files were created using the "--phylip-var-all" option of the "*populations*" module of STACKS. Heterozygous sites within each individual were encoded using IUPAC notation. During the analysis, each RAD-locus was treated as

a separate partition with an independent best-fit substitution model. Node support values were obtained using an ultrafast bootstrap procedure (Hoang et al., 2018) with 1,000 replicates. We also used the SVDQuartets algorithm (Chifman & Kubatko, 2014) as implemented in PAUP* 4.0a168 (Swofford, 2003) to perform species-tree inference under the multispecies coalescent model using 18,988 SNPs (single random SNP per locus, minor allele frequency cutoff 0.04, maximum observed heterozygosity cutoff 0.99). Node support was estimated with 1,000 bootstrap replicates.

The second data set consisted of all genotyped specimens of sympatric *Garra* ecomorphs from the Sore River and a single, most closely related outgroup (*G.* cf. *dembeensis* from the Barokalu River, as revealed by the analysis of the first phylogenomic data set that included samples from all the localities in Figure 2). This data set was analysed with IQ-TREE as described above, except for the GTR+G substitution model that was used for each partition. The phylogenetic trees were visualized and edited using FIGTREE 1.4.4 (Rambaut, 2014).

3 | RESULTS

3.1 | Trophic morphology

PCA of head and body proportions of six sympatric ecomorphs from the Sore River revealed five well-defined clusters (Figure 3a). Four clusters represent "narrow-mouth," "wide-mouth," "predator" and "thick-lipped" ecomorphs, while the fifth includes individuals from the "generalized" and "stream-lined" ecomorphs. The "predator" ecomorph is the most divergent. PC1 and PC2 explained 72.3% and 10.2% of the total variance, respectively. The eigenvectors with the highest eigenvalues for PC1 were head proportions—nine of the 10 most loaded (especially gular disc proportions, mouth width, interorbital distance and snout length). The same pattern was detected for PC2—nine of the 10 most loaded characters belonged to head proportions (mainly disc length, mouth width, height of head at nape and at eyes, etc.; Table S2 for details).

After excluding the "predator" ecomorph, the "generalized" and "stream-lined" ecomorphs became more distinguishable with low overlap (Figure 3b). PC1 and PC2 explained 73.8% and 8.1% of the total variance, respectively. The most loaded eigenvectors of both PC1 and PC2 were from head proportions with few more contributions of body proportion characters (Table S3). The difference between the "generalized" and "stream-lined" ecomorphs revealed in PC2 is explained by height of the head at both nape and eyes, interorbital distance, head width, body height as well as other characters (Table S3).

3.2 | Gut length and preliminary data on diet

Gut length varied consistently between ecomorphs (Figure 3c). Shortest guts (107–160% SL) were detected in the "predator" ecomorph suggesting a predatory trophic type, while the longest guts were recorded in the "generalized" (285–799% SL) and "stream-lined" (354–555% SL) ecomorphs that possessed the well-developed gular disc and therefore are specialized algal grazers, as also shown by their gut contents (see below). Other ecomorphs had intermediate values of gut length: "narrow-mouth" ecomorph, 124%–295% SL' and "wide-mouth" ecomorph, 175%–513% SL, respectively. Broad intragroup variation is explained by an increase of gut length with body length detected in some ecomorphs (Figure 3d). Nevertheless, the similar-sized individuals are divergent in gut length at the same manner shown in Figure 3c. The "predator" ecomorph having the shortest gut even displays a slight decrease of gut length ontogenetically that was previously reported for a piscivorous mode of feeding among African cyprinids (Levin et al., 2019).

The preliminary inspection of gut contents revealed differences in the diet between some ecomorphs. The "generalized" and "stream-lined" ecomorphs had permanently filled intestines full of periphyton (diatoms, green and charophyte algae; IRI = 99.98% for "generalized" and 97.99% for "stream-lined" ecomorphs, respectively) and rarely other items (larvae of water insects: mayflies, chironomids, simulids). The "narrow-mouth" ecomorph had a half-filled gut with dominating periphyton (IRI = 86.3%) with a notable portion of insect larvae (7.62%: predominantly chironomids, also mayflies, and simulids) and macrophytes (5.97%). The "wide-mouth" ecomorph had fewer filled intestines compared to the "narrow-mouth" ectomorph, but with strongly dominating periphyton in the diet (IRI = 99.49%). The gut of the "predator" ecomorph (shortest gut) frequently was empty, including the largest individual (SL = 185 mm). When guts were filled, benthos-associated prey was strongly prevalent (IRI = 99.31%; mayflies and chironomids).

3.3 | Mitochondrial data

Both BI and ML analyses of *cytb* revealed monophyly of the genus *Garra* from the Sore River (Figure 4a). The closest relative (and ancestor lineage) is from the Barokalu River, a tributary of the Baro River (White Nile drainage). Both Sore and Barokalu rivers share a watershed in the Baro system and sampled localities are separated by only ~50 km. Divergence between *Garra* populations from the Sore and Barokalu is low (*p*-distance =0.0105 ± 0.0028) and comparable with maximum intradivergence in the Sore radiation (*p*-distance =0.0111 ± 0.0033). The White Nile lineage is sister to the large clade of Ethiopian *Garra* from the Blue Nile and Lake Tana, Atbara-Nile, Ethiopian Rift Valley, and Omo-Turkana basins.

Phylogenetic analyses revealed that Ethiopian *Garra* are non-monophyletic (Figure 4a). These results are in line with a recent study of Englmaier et al. (2020) that demonstrated paraphyly of Ethiopian *Garra*. Some lineages are of more ancient origin and closer to Asian lineages (*G. tibanica* from the Indian Ocean basin) or to lineages from West Africa (e.g., *G. vinciguerra* from the Blue Nile basin). The matrilineal tree of Ethiopian *Garra* includes up to 12 lineages. Taking into account that some species cluster together in one lineage (e.g., three

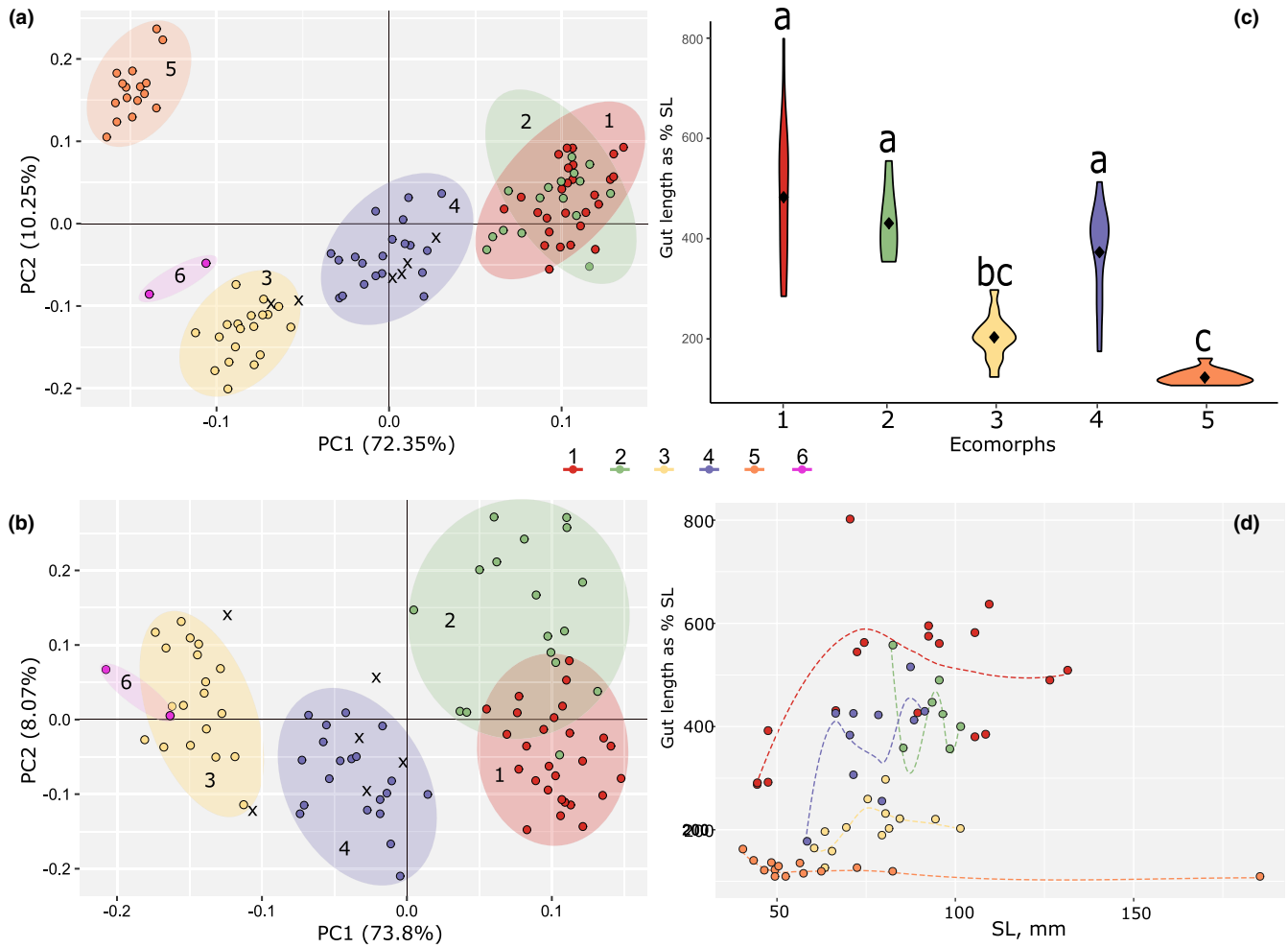

FIGURE 3 (a) PCA of body and head proportions of six sympatric ecomorphs from the Sore River ($n = 107$). (b) PCA of body and head proportions of five sympatric ecomorphs from the Sore River ($n = 90$) excluding the most divergent sample of the "predator" ecomorph. X designates intermediate phenotypes. (c) Gut length of five sympatric *Garra* ecomorphs from the Sore River represented as violin boxplots. Middle points are the means, and the box shows the range respectively; samples are combined and each contains between seven ("stream-lined" ecomorph) and 18 ("generalized") individuals, for a total of 62 individuals. Different lowercase letters above the boxplots indicate significant differences between ecomorphs ($p < .05$, Kruskal–Wallis test with Dunn's post-hoc test and BH adjustment of the p-value—see descriptive statistics in File S1). (d) Dependence of gut length on body length in five *Garra* ecomorphs from the Sore River with smooth local regression lines (Loess regression). Designations of ecomorphs: 1, "generalized"; 2, "stream-lined"; 3, "narrow-mouth"; 4, "wide-mouth"; 5, "predator"; 6, "thick-lipped" [Colour figure can be viewed at wileyonlinelibrary.com]

species from Lake Tana) or that some species were unavailable, we suggest that *Garra* from the Ethiopia Highlands is more diversified than previously recognized (Stiassny and Getahun, 2007).

The Sore River lineage is composed of two sublineages or haplogroups (highlighted by yellow and green in Figure 4a,b). A haplotype net constructed on 107 *cytb* sequences confirms the presence of two main haplogroups. The core haplotypes of these haplogroups are separated by five substitutions. Four of six ecomorphs ("stream-lined," "narrow-mouth," "wide-mouth" and "predator") share both haplogroups. The "green" haplogroup is prevalent in a number of haplotypes (18), and number of individuals (88), and found in five ecomorphs. The "generalized" ecomorph is presented exclusively in this haplogroup. In contrast, the "yellow" haplogroup (Figure 4b) is less frequent in our sample, with only eight different haplotypes found in 19 individuals (17.7% of the individuals analysed). The "yellow" haplogroup consists of five ecomorphs as well. However, the "wide-mouth" ecomorph is much more common in this haplogroup (42% of all individuals) compared to the "green" one (6.97%).

3.4 | RAD-seq data

3.4.1 | Nuclear phylogeny

The phylogeny of Ethiopian *Garra* based on a concatenated set of RAD locus sequences (23,365 partitions and 3,075,180 total sites with 0% missing data; raw reads statistics is provided in File S2) is generally similar to that based on mtDNA data (Figure 4), but it has more strongly supported nodes (Figure 5a). Sympatric ecomorphs

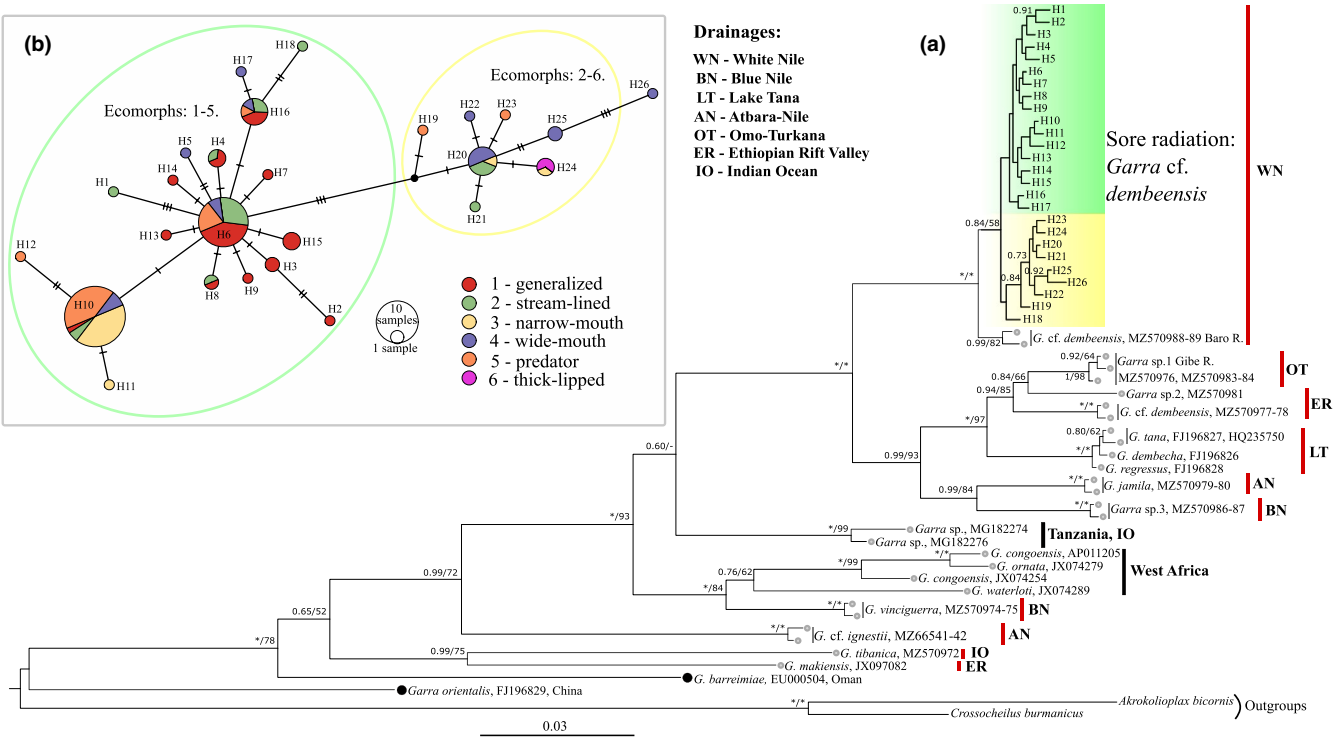

FIGURE 4 (a) Consensus tree of relationships among Ethiopian *Garra* from all main drainages based on *cytb* sequences. Bayesian posterior probabilities (before slash) from BI analysis and bootstrap values from ML analysis (after slash) above 0.5/50 are shown; asterisks represent posterior probabilities/bootstrap values of 1/100. Red vertical bars indicate Ethiopian *Garra* while black bars indicate African *Garra* from other regions; black circles indicate Asian *Garra*. Sore River *Garra* are named by haplotype numbers. Scale bar and branch lengths provide the expected substitutions per site. The green and yellow colours highlight two branches of *Garra* in the Sore River. (b) Median-joining haplotype network of *Garra* from the Sore River, based on 107 *cytb* sequences (989 bp in length). The "green" haplogroup includes ecomorphs 1–5, while the "yellow" haplogroup includes ecomorphs 2–6. Black dots represent hypothetical intermediate haplotypes [Colour figure can be viewed at wileyonlinelibrary.com]

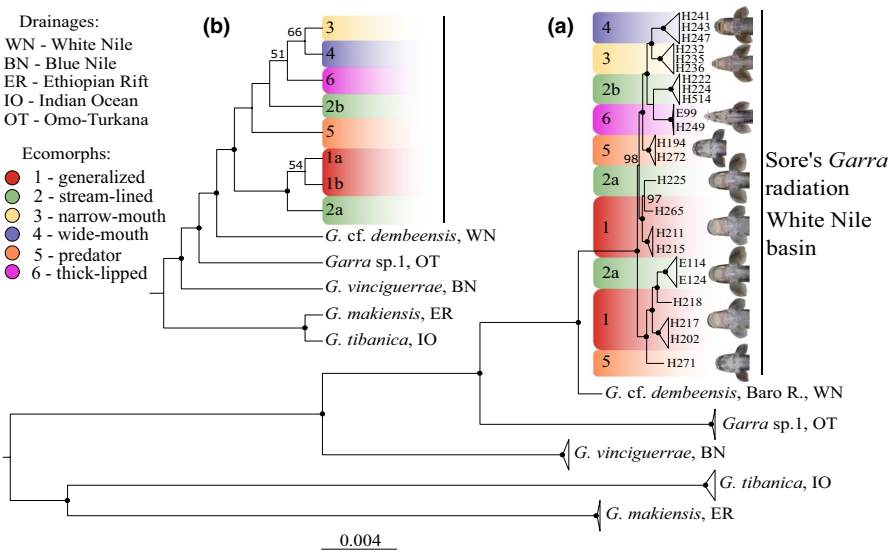

FIGURE 5 (a) ML phylogenetic tree of Ethiopian *Garra* based on RAD-loci sequences: 23,365 loci; 3,075,180 bp, and (b) SVDQ species tree. Each locus was treated as a separate partition with the GTR+G substitution model and heterozygous sites within each individual encoded using IUPAC notation. Black dots designate 100% bootstrap support, and only values above 50% are given. Sore River *Garra* individuals are labelled by their voucher numbers listed in BioProject ID PRJNA749254 (https://www.ncbi.nlm.nih.gov/) [Colour figure can be viewed at wileyonlinelibrary.com]

clustered together and form a monophyletic lineage, sister to the population from the same riverine basin—Baro drainage in the White Nile system (Figure 5a,b). The closest relative to *Garra* from the White Nile system is the *Garra* lineage in the *G. dembeensis* complex from the neighbouring drainage—the Omo-Turkana system. *Garra vinciguerrae* from the Blue Nile (which was recorded in Ethiopia for the first time in this study) is the sister lineage of both the White Nile and Omo-Turkana lineages. The most divergent lineages, *G. makiensis* and *G. tibanica*, are from the Ethiopian Rift Valley and Indian Ocean basins, respectively (Figure 5).

Compared to mitochondrial data, the nuclear phylogenomic tree shows much better segregation of *Garra* ecomorphs from

the Sore River (Figure 5a). The "narrow-mouth," "wide-mouth" and "thick-lipped" ecomorphs form monophyletic clusters, while other ecomorphs are divided into two ("generalized" and "predator") or even three ("stream-lined") clusters. We assign two distantly located branches of the "generalized" ecomorph as 1a/1b, and "stream-lined" ecomorph as 2a/2b according to population genomics analyses described below (Figures 6–8). The "generalized" and "stream-lined" ecomorphs on the one hand, and other ecomorphs on the other form two clusters within the Sore River adaptive radiation according to the SVDQ species tree (Figure 5b). The "narrow-mouth," "wide-mouth" and "thick-lipped" ecomorphs are the most recently diverged branches according to the SVDQ-tree, but the nodes are only weakly supported (Figure 5b).

Relationships among the Sore River sympatric ecomorphs based on analysis of all samples and full RAD-loci sequences (>7,000 loci and >0.96 Mbp length sequences) are presented in Figure 6. ML analysis gives high support to the monophyly of each ecomorph except for the "stream-lined" ecomorph. The lineage of the "stream-lined" ecomorph is paraphyletic, possibly suggesting that there is another seventh cryptic species that we could not distinguish phenotypically. Four individuals of the "stream-lined" ecomorph along with one individual of intermediate phenotype represent another lineage that we call 2b (Figure 6). Lineage 2a is sister to all other ecomorphs that are divided into two subclades—one includes only "generalized" ecomorph individuals (which, in turn is subdivided into what we call 1a–1b), while another includes all other ecomorphs: "narrow-mouth," "wide-mouth," "predator," "thick-lipped" and above

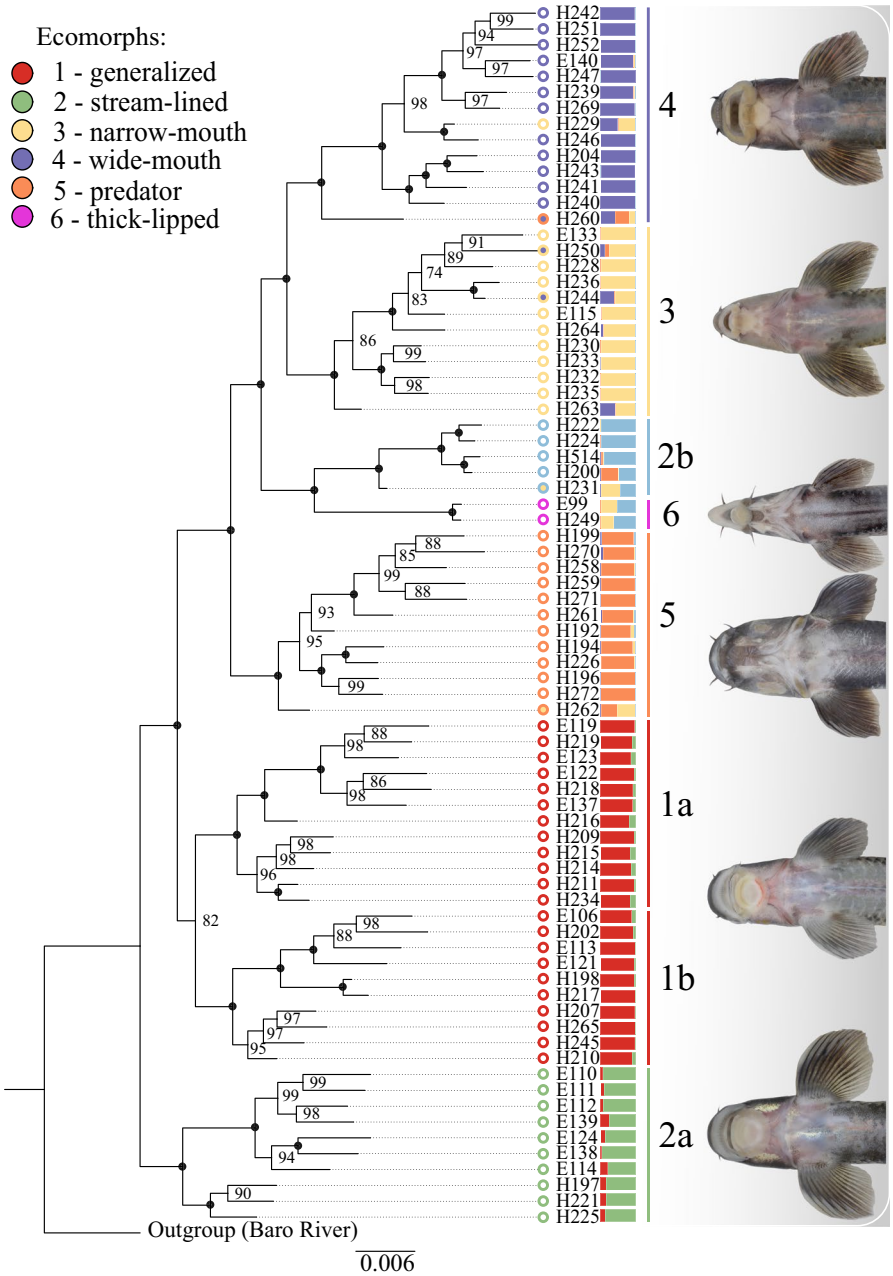

FIGURE 6 ML phylogeny of sympatric *Garra* ecomorphs from the Sore River based on concatenated RAD-loci sequences (7,370 loci; 969,450 bp). Each locus was treated as a separate partition with the GTR+G substitution model. Heterozygous sites within each individual encoded using IUPAC notation. The individual samples are coloured based on the colour scheme of Figure 4 and intermediate (putative hybrids) phenotypes are depicted in another colour. The genetic cluster proportions inferred by RMAVERICK analysis are shown to the right of sample numbers. Black points designate 100% bootstrap support. Sore River *Garra* individuals are labelled by their voucher numbers listed in BioProject ID PRJNA749254 (https://www.ncbi.nlm.nih.gov/) [Colour figure can be viewed at wileyonlinelibrary.com]

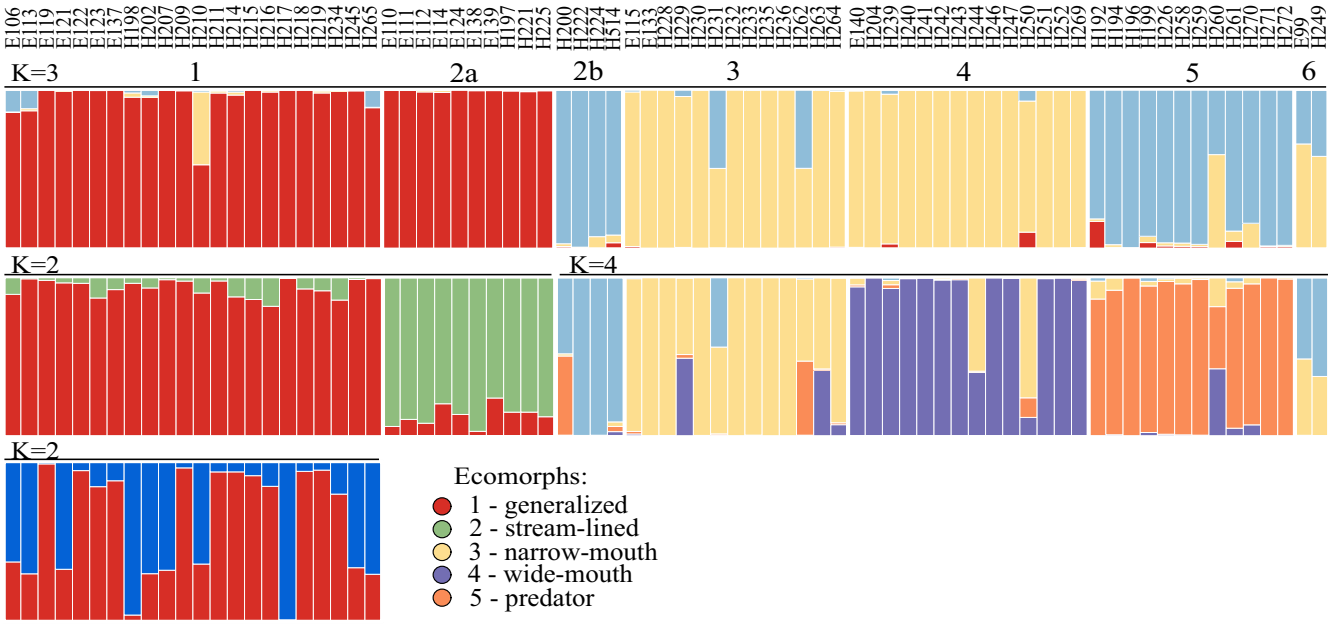

FIGURE 7 Hierarchical RMAVERICK results for sympatric ecomorphs of *Garra* from the Sore River, based on 679 nuclear SNPs. Each column of the barplot shows individual assignments to one of the inferred genetic clusters. Independent runs of RMAVERICK are indicated by a solid black line above a plot, along with an inferred value of *K*. Sore River *Garra* individuals are labelled by their voucher numbers listed in BioProject ID PRJNA749254 (https://www.ncbi.nlm.nih.gov/) [Colour figure can be viewed at wileyonlinelibrary.com]

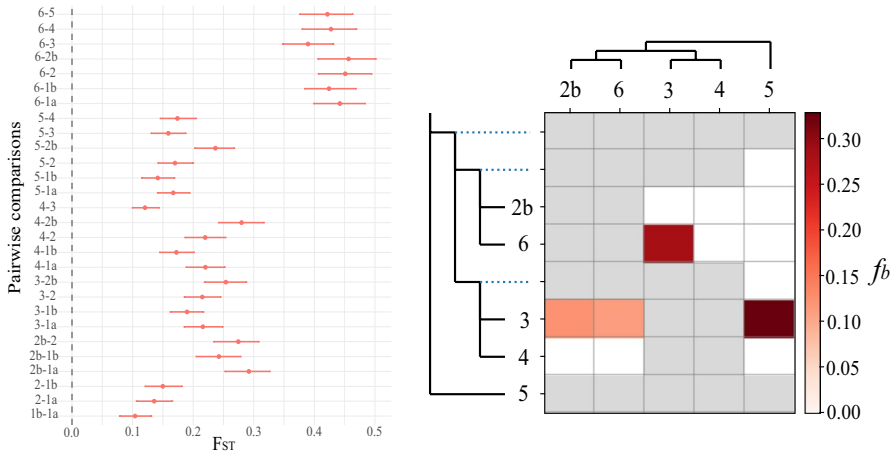

FIGURE 8 Left: pairwise Reich F_{ST} values (points) with their respective 95% confidence intervals (horizontal lines) for *Garra* genetic lineages from the Sore River based on 679 SNPs. Right: heat map of the *f*-branch metric for selected ecomorphs/lineages of the *Garra* Sore radiation. The used guide tree is shown along the *x*- and *y*-axes (in "laddered" form along the *y*-axis). The matrix shows the inferred *f*-branch metric, reflecting excess allele sharing between the branch of the "laddered" tree on the *y*-axis (relative to its sister branch) and the branches defined on the *x*-axis. Designations of ecomorphs: 1, "generalized"; 2, "stream-lined"; 3, "narrow-mouth"; 4, "wide-mouth"; 5, "predator"; 6, "thick-lipped" [Colour figure can be viewed at wileyonlinelibrary.com]

mentioned "stream-lined" 2b. The latter subclade is composed of lineages, each containing samples of particular ecomorphs except for several samples which were intermediate in their phenotypes (Figure 6). The "thick-lipped" ecomorph was found to be sister to the 2b lineage albeit with an apparent rather deep last common ancestor. Generally, the placement of clade 2a as sister to all other *Garra* from the Sore River, which is characterized by a well-developed gular disc (type C), might suggest that this morphology is an ancestral condition of this radiation.

3.4.2 | Population genomics

PCAs of the 679 nuclear SNPs of sympatric ecomorphs revealed several well-defined clusters that mirror their phenotypic differentiation (Figure S6). The "generalized" ecomorph (composed of two genetic subclusters 1a and 1b), genetic cluster 2a of the "stream-lined" ecomorph as well as the "narrow-mouth" and "wide-mouth" ecomorphs form well-distinguished groups, while cluster 2b of the "stream-lined" and "predator" ecomorphs broadly overlap. Two

individuals of the "thick-lipped" ecomorph were placed between the cluster of the "narrow-mouth" ecomorph and cluster 2a of the "stream-lined" ecomorph.

Analysis of population structure carried out with admixture revealed an optimum of three (*K*) genomic clusters that correspond to the (i) "generalized" + "stream-lined" (2a lineage) ecomorphs, (ii) "narrow-mouth" + "wide-mouth" ecomorphs, and (iii) "predator" + "stream-lined" (2b lineage) ecomorphs (Figure 7, upper row, $K = 3$). The "thick-lipped" ecomorph is characterized by an admixture of two clusters from the "narrow-mouth" and "stream-lined" (2b lineage) ecomorphs.

Subsequent analysis of each cluster (= lineage) revealed hierarchical subdivision. Thus the "generalized" and "stream-lined" (2a lineage) ecomorphs each are also identified as distinct clusters in the admixture analysis (Figure 7, middle row, $K = 2$). Although the "narrow-mouth," "wide-mouth," "predator" and "stream-lined" (lineage 2b) ecomorphs are supported as independent evolutionary units based on several types of genetic analyses, few individuals in all of these show signs of historical gene flow based on admixture analysis (Figure 7). The two individuals from the "thick-lipped" ecomorph showed a high level of admixture with the "narrow-mouth" (36.8%–47.5%) and "stream-lined" (lineage 2b) (51.3%–62.3%) ecomorphs, possibly supporting a hybrid origin hypothesis. One additional level of population subdivision was detected in the "generalized" ecomorph (Figure 7) with two genomic clusters (lineages 1a and 1b) with a high degree of admixture. This suggests heterogeneous genomic structure of the "generalized" ecomorph as a result of secondary contact.

All Reich F_{ST} pairwise comparisons were statistically significant with values ranging from 0.10 ("generalized" ecomorph: lineages 1a vs. 1b) to 0.46 ("thick-lipped" ecomorph vs. lineage 2b of "stream-lined" ecomorph) (Figure 8). Although the "thick-lipped" ecomorph had the highest F_{ST} values (0.39–0.46), it should be treated cautiously because of low sample size.

As the RMAVERICK analysis suggested a notable level of admixture between lineage 2b of the "stream-lined" ecomorph and "narrow-mouth," "wide-mouth" and "thick-lipped" ecomorphs (Figure 8), which form a single monophyletic cluster in our phylogenomic analysis (Figure 7), we performed a number of tests to distinguish between gene flow (introgression) and ILS. Patterson's D statistic was positive and significant for a number of comparisons (Table 3). Visualization of the *f*-branch metric (which is based on f_4-ratio results) highlighted introgression between the "stream-lined" (lineage 2b) and "narrow-mouth" ecomorphs, "thick-lipped" and "narrow-mouth" ecomorphs, and "predator" and "narrow-mouth" ecomorphs (Figure 8).

The eighth genetic clusters possess from three ("thick-lipped" ecomorph) to 38 private alleles ("wide-mouth" ecomorph) (Table 4). The "thick-lipped" ecomorph has also the lowest heterozygosity ($H_O = 0.00058$) as well as nucleotide diversity ($\pi = 0.00054$) compared to all other ecomorphs (H_O =0.00104–0.00128; $\pi = 0.00121$–0.00091) (Table 4).

4 | DISCUSSION

Our study provides genetic support for the hypothesis of the evolution of a (potentially adaptive) radiation in a riverine environment. By analysing trophic features and sucking disc variation, as well as trophic ecology, we show morpho-ecological diversification of the cyprinid fish *Garra dembeensis* into six distinct ecomorphs. First, diversification of two novel phenotypes ("thick-lipped" and "predator") in the Sore River has evolved rapidly, an event that can be classified as a burst of speciation *sensu* Givnish (2015). Second, this radiation resulted in the origin of several highly specialized lineages of algae scrapers; that is, a specialized ancestor adaptively radiated giving rise to ecomorphologically diverse (and adapted to their particular niches) lineages that seem to be not only ecologically but also reproductively isolated from each other.

4.1 | Ecomorphological diversification

The genus *Garra* currently comprises more than 160 species (Fricke et al., 2021; Yang et al., 2012), only 23 of which occur in Africa (Moritz et al., 2019). So far, only 13 described species have been reported from Ethiopia (Golubtsov et al., 2002; Stiassny & Getahun,

TABLE 3 Results of Patterson's *D* statistic (ABBA-BABA test) and f_4-ratio test on selected genetic clusters of *Garra* from the Sore River

P1	P2	P3	*D* statistic	*Z*-score	*p*-value	f4-ratio	BBAA	ABBA	BABA
4	3	6	0.1176	5.3829	**<.0001**	0.1128	227.5	235.0	185.5
2b	3	5	0.0650	3.1078	**.0009**	0.4226	253.5	246.5	216.4
2b	6	3	0.0646	2.3475	**.0095**	0.2854	215.6	217.3	190.9
4	3	2b	0.0624	3.8143	**<.0001**	0.1237	264.6	241.4	213.0
4	3	5	0.0492	3.6742	**.0001**	0.3277	276.2	247.4	224.2
2b	6	5	0.0327	1.4755	.0700	0.2051	248.6	203.4	190.5
4	6	5	0.0304	1.5315	.0628	0.2330	224.5	226.5	213.2
6	3	5	0.0199	0.9380	.1741	0.1641	244.2	204.7	196.8
2b	4	5	0.0178	1.0774	.1406	0.1134	245.9	246.3	237.7
2b	6	4	0.0040	0.1592	.4368	0.0151	244.6	197.8	196.3

TABLE 4 Summary of the ecomorphs' genetic diversity indices averaged over 89,070 loci (both variant and fixed)

Ecomorph*	No. of private alleles, *Np*	No. of polymorphic loci (%)	Heterozygosity		Coefficient of inbreeding (F_{IS}) ± *SE*	Nucleotide diversity (π) ± *SE*
			Observed (H_O) ± *SE*	Expected (H_E) ± *SE*		
1a	19	0.42	0.00128 ± 0.00008	0.00116 ± 0.00007	−0.00014 ± 0.0015	0.00121 ± 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

*Letters "a" and "b" assign genetic lineages within ecomorphs 1 and 2.

2007). In this study, we discovered six additional distinct ecomorphs in the Sore River, and thus might warrant the description of five to six new species.

The ecomorphs of the Sore's *Garra* are exceptionally diverse in trophic and sucking disc morphology. Two novel phenotypes that had not been discovered before for this genus, "thick-lipped" and "predator,"have superficial similarities to Lake Tana large barbs species/morphotypes, such as thick-lipped barb *L. negdia* (Rüppell, 1836) and predatory *L. gorguari* (Rüppell, 1836) (Nagelkerke & Sibbing, 1997). The high degree of variation in the sucking disc in Sore's *Garra* can be observed—from a well-developed disc with free posterior margin to complete absence. Such a degree of morphological diversity in a single river of the Ethiopian Highlands is remarkable.

Divergent feeding-related morphology and gut content analysis suggest trophic specialization of *Garra* sympatric forms. This is consistent with other cases of apparent adaptive diversification among Ethiopian cyprinids, where trophic resource partitioning promoted diversification: *Labeobarbus* spp. in Lake Tana (Sibbing et al., 1998) as well as in the Genale River (Levin et al., 2019). The most common foraging strategy among *Garra* is scraping of periphyton from stones and rocks (Hamidan et al., 2016; Matthes, 1963). This is predominant in the "generalized" and "stream-lined" ecomorphs that have a long gut (four to five times longer than body length) filled with periphyton and detritus. The "generalized" and "stream-lined" ecomorphs are divergent mainly in body shape. The latter has a streamlined appearance and is probably adapted for life in more rapid flowing water. The "narrow-mouth" ecomorph has a shorter gut length (about two times longer than body length) and a mixed diet with significant additions of benthic invertebrates. The "predator" ecomorph has an extremely short gut, whose length is as long as the fish body. A short gut is a strong marker for a predatory/piscivory feeding strategy in fishes, including cyprinids (Nagelkerke, 1997; Sibbing et al., 1998; Wagner et al., 2009, Zandoná, Auer, Kilham, & Reznick, 2015). Predatory *Garra* from the Sore River have four to five times shorter gut length than congeneric periphyton feeders and a two-fold shorter gut than that of piscivory the large-mouthed ecomorph of *Labeobarbus* from the Genale River, Ethiopia (Levin et al., 2019). We found empty guts in many individuals of the "predator" ecomorph, while small fishes had gut filled with insects. The "wide-mouth" ecomorph has a rather long intestine and predominantly periphyton in its diet, but it is characterized by a distinctly divergent mouth phenotype compared to the "generalized" and "stream-lined" ecomorphs (Figure 3). The gut of the "thick-lipped" ecomorph was not analysed because of the extreme rarity of samples. Hypertrophied lips (or "rubber lips") of fishes is an adaptation to foraging on benthos hidden between rock crevices on pebble and rock fragments via increased sucking power by sealing cracks and grooves (Baumgarten et al., 2015; Matthes, 1963; Ribbink et al., 1983). The thick-lipped phenotype is widely distributed among other cyprinid fish, *Labeobarbus* spp., inhabiting lakes and rivers of the Ethiopian Highlands (Mina et al., 1996; Mironovsky et al., 2019; Nagelkerke et al., 1994) including the Sore River (Levin

et al., 2020), but it was not detected among *Garra* species. Our study shows that the thick-lipped mouth phenotype represents an evolutionary novelty within the *Garra* lineage that probably resulted from hybridization events between the "stream-lined" (lineage 2b) and "narrow-mouth" ecomorphs because its genome had an admixture from these genetic lineages. The hybrid origin of the *Garra's* thick-lipped phenotype is in line with results of a recent experimental study demonstrating the importance of hybridization in generating functional novelty of ecological relevance in relation to trophic resources unavailable for parental species in cichlid fishes (Selz & Seehausen, 2019). At the same time, the origin of the novel thick-lipped phenotype in the genus *Garra* is of particular interest in light of knowledge of the nonhybrid origin of hypertrophied lips from ancestors with normally developed lips in cichlids (Baumgarten et al., 2015; Machado-Schiaffino et al., 2017). Interestingly, there might only be a single locus involved in producing the hypertrophied cichlid phenotype (Kautt et al., 2020), and the genomic basis of the lip phenotypes in *Garra* remains unknown.

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

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

4.2 | Possible scenarios of evolution of Garra's adaptive radiation in the Sore River

Both mtDNA and genome-wide SNP data support the monophyly of the Sore's *Garra* as well as their recent speciation based on low genetic divergence between the nearest ancestor and Sore River ecomorphs and low SNP numbers. The closest relative and ancestor of the Sore River diversification inhabits the same subbasin of the White Nile in Ethiopia, therefore suggesting an intrabasin diversification of *Garra* there. On the one hand, mtDNA data might have failed to distinguish sympatric ecomorphs because of a high level of shared genetic diversity caused by ILS and introgression, this latter highlighted by the D-statistic calculated with the genome-wide nuclear data. On the other hand, the SNP data support a reproductive isolation among closely related ecomorphs despite few individuals having intermediate phenotypes and genetic admixture. A hybrid origin of intermediate phenotypes might suggest that reproductive isolation barriers are not yet complete.

Patterns of haplotype net (numerous haplotypes occurring in the same phenotypes) as well as SNP data (presence of more genetic clusters than phenotypes, such as within the "generalized" and "stream-lined ecomorps") could also suggest secondary contact of local subisolated populations. The riverine net of the Ethiopian Highlands was significantly influenced by several episodes of dramatic volcanism and tectonism until the Quaternary (Ferguson et al., 2010; Hutchison et al., 2016; Prave et al., 2016). Thus, riverine net fragmentation, isolation or subisolation of some riverine parts, and captures of headwaters is a likely scenario given the geological history of the Ethiopian Highlands (Mège et al., 2015), as also supported by genetic studies on other Ethiopian fishes (Levin et al., 2019, 2020). Concerning the Sore River, while waterfalls and rapids are frequent, no geological data that support its connection or headwater capture to other basins are known. In our view, the most reliable evolutionary scenario for the origin of the riverine adaptive radiation in the *Garra* species group draws upon a combination of allopatric and sympatric stages of speciation with hybridization and admixture. A comparable evolutionary history was detected in the *Labeobarbus* adaptive radiation in the Genale River (Ethiopia), which is part of the extended ancient riverine net in the Juba–Wabe–Shebelle drainage (Levin et al., 2019).

Speciation with gene flow was detected in several studies (e.g., Feder et al., 2012; Fruciano et al., 2016; Kautt et al., 2018; Kautt et al., 2020; Machado-Schiaffino et al., 2017; Malinsky et al., 2018; Puebla, 2009; Rougeux et al., 2017; Schwarzer et al., 2011; Smadja & Butlin, 2011; Zheng & Ge, 2010). Notably, it has been shown that genetic admixture between divergent populations/lineages may be a key factor in promoting rapid ecological speciation (Jacobs et al., 2020; Kautt et al., 2020; Marques et al., 2019; Martin et al., 2015). Moreover, ancient hybridization is widely considered one of the most important factors driving the spectacular cichlid adaptive radiations in the African Great Lakes (Irisarri et al., 2018; Meier et al., 2017; Verheyen et al., 2003). Seemingly, ancient introgressive hybridization could be a trigger for small-scale repeated adaptive radiations among the Arctic charrs (*Salvelinus*) (Lecaudey et al., 2018). Furthermore, hybridization is the main mechanism generating polyploid lineages in fishes (tetraploid, hexaploidy, etc.; Braasch & Postlethwait, 2012), whose complex genomes constitute the raw material for the rapid origin of sympatric forms (e.g., *Schizothorax* and *Schizopygopsis* in Central Asia: Berg, 1914; Burnashev, 1952; Terashima, 1984; Savvaitova et al., 1988; Komarova et al., 2021; *Labeobarbus* in Africa: Levin et al., 2020; Mina et al., 1996; Nagelkerke et al., 1994; Vreven et al., 2016). Nevertheless, all described *Garra*, including the Ethiopian species, have diploid genomes (Krysanov & Golubtsov, 1993).

4.3 | Adaptive radiation in riverine environment

Most adaptive radiations of freshwater fishes have been reported from the lacustrine environments (e.g., Fryer & Iles, 1972; Seehausen & Wagner, 2014; Verheyen et al., 2003). However, increasing evidence suggests that adaptive radiation can take place in other aquatic environments (e.g., marine, riverine) (Burress et al., 2018; Dimmick et al., 2001; Feulner et al., 2008; Levin et al., 2019, 2020; Melnik et al., 2020; Matschiner et al., 2011; Piálek et al., 2012; Puebla, 2009; Whiteley, 2007). Several other cases of potential riverine adaptive radiations that include three or more sympatric ecomorphs exist, although they have not yet been tested with genetic methods: for instance, snow trout from Central Asia (Berg, 1914; Burnashev, 1952), and *Poropuntius* and *Neolissochilus* barbs from Southeast Asia (Roberts, 1998; Roberts & Khaironizam, 2008). Among cichlids, one of the first riverine adaptive radiations examined genetically was from southern Africa (Joyce et al., 2005). However, the authors of that study suggested that the adaptive radiation occurred in the lacustrine environment in the palaeo-lake Makgadikgadi that dried up during the Holocene (Joyce et al., 2005). Other cichlid adaptive radiations from the rivers of West Africa (Schwarzer et al., 2011), South America (Burress et al., 2018; Piálek et al., 2012) as well as four independently evolved riverine radiations of labeobarbs from East Africa (Levin et al., 2020) instead took place in riverine drainages without known lacustrine conditions in the past.

The *Garra* lineage is adapted to fast and torrent waters. It possesses a morphological novelty (gular sucking disc) used to cling on the bottom of fast-moving waters. This novelty allowed *Garra* to be distributed widely in highlands and montane zones from Southeast China to West Africa. Only a few species were found in the lacustrine environment (Lake Tana: Stiassny & Getahun, 2007) or in caves (e.g., Banister, 1984; Coad, 1996; Kruckenhauser et al., 2011; Mousavi-Sabet & Eagderi, 2016), indicating their potential to adapt to steady water flows.

Although the riverine network is generally considered more open to gene flow compared to landlocked water bodies, mountain and highland areas are an exception to this rule. The Ethiopian Highlands are a volcanic massif of flood and shield volcano basalts 0.5–3.0 km thick that form a spectacular trap topography (1,500–4,500 m) flanking the Main Ethiopian Rift (Prave et al., 2016). The geological history of the Ethiopian Highlands was tectonically very dynamic and rich in volcanic episodes from the Oligocene to Pleistocene with very recent episodes (Prave et al., 2016). The volcanic activity has been severe enough to deleteriously affect the biota and cause major disruptions in ecosystems. This hypothesis has found support in the inferred evolutionary history of the genus *Labeobarbus* in East Africa. The earliest fossil records of *Labeobarbus* were found in the Ethiopian Rift Valley and dated back to the late Miocene (Stewart & Murray, 2017), but most of the Ethiopian lineages are younger (Pleistocene origin) (Beshera et al., 2016; de Graaf et al., 2010; Levin et al., 2020). The tectonic activity of the region could have favoured local isolation via the formation of waterfalls (e.g., 33,000 years ago the Blue Nile basaltic blockade formed Tis-Isat waterfall; Prave et al., 2016) or river net fragmentation (Juba-Wabe-Shebelle drainage; Mège et al., 2015) along with climatic oscillations that resulted in disconnection of water bodies during aridization (Benvenuti et al., 2002). Periodically, it resulted in vacant habitats and ecological opportunity (reviewed by Stroud & Losos 2018) for new species to exploit, similar to islands or crater lakes (Burress et al., 2018).

The diversification burst in *Garra* in the Sore River was detected in the riverine segment at an altitude range of 1,310–1,550 m asl, that is within the range of four riverine diversifications of *Labeobarbus* detected throughout the Ethiopian Highlands: 1,050–1,550 m (Levin et al., 2020). Despite the generally broader elevation gradient (175–2,000 m asl; Levin et al., 2020) of the *Labeobarbus* species complex, the diversification bursts were only detected in mid- to upper reaches. We believe that a combination of two factors might explain this observation: (i) fauna in mid- to upper reaches is poorer compared to lower reaches, where a more diversified fauna might have already filled the available ecological niches necessary for an adaptive radiation to unfold; and (ii) the biotopes are more diverse compared to the most upper reach, such that vacant niches are available.

Five endemic, and one introduced non-*Garra* species have been recorded in the Sore River in the study area (data of this study). This is an extremely low number compared to more than 110 fish species (Golubtsov & Darkov, 2008; our data) recorded in the Baro River at Gambella at 440 m altitude (our data) to which the drainage of the Sore River belongs, with a distance of ~150 km between localities. The segment of the Sore River where the diversification of *Garra* was detected is rather rich in biotope complexity: pools with slow currents alternate with rift areas and rapids (Figure S7). The depauperated fauna was suggested to provide the ecological opportunities for riverine adaptive radiations similar to that in southeastern cyprinids of the genus *Poropuntius* (Roberts, 1998) and South America cichlids of *Crenicichla* as relaxed competition and vacant niches might have provided ecological opportunities for sympatric speciation by trophic specializations (Burress et al., 2018).

We discovered six genetically distinct ecomorphs within the genus *Garra* in the Sore River that can be considered young or incipient species. Given that the same riverine segment is home to another riverine diversification of fishes represented by four phenotypically diverged ecomorphs of the genus *Labeobarbus* (Levin et al., 2020), we consider the Sore River to be a hotspot of riverine diversification in the Ethiopian Highlands that requires conservation management. The Ethiopian Highlands are home to several young fish radiations—a large lacustrine diversification among cyprinids (15 species/morphotypes: Mina et al., 1996; Nagelkerke et al., 1994, 2015) as well as small diversifications of *Garra* (three species: Stiassny & Getahun, 2007) and *Enteromius* (two species: Dejen et al., 2002; de Graaf et al., 2007)—all in Lake Tana, and five riverine adaptive radiations of cyprinids each including from four to seven species (Golubtsov, 2010; Golubtsov et al., 2021; Levin et al., 2019, 2020; Mina et al., 1998; current study), highlighting this region's importance as a hotspot for fish speciation that is in need of additional research on ecological speciation processes.

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AUTHOR CONTRIBUTIONS

BL, ES, PF, NM, AG and AM designed and contributed to the original concept of the studies. BL and AG collected most of the specimens and related data, BL and NM obtained mtDNA data and prepared DNA libraries for ddRAD, BL conducted morphological analyses, ES conducted the most of bioinformatics, and BL, ES, PF and AM finalized the manuscript. All authors participated in project design, and read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Morphological data (body proportions and gut lengths), mtDNA subsets (cytochrome *b*) and genotyping files (various sets of SNPs) have been uploaded to Dryad: https://doi.org/10.5061/dryad.j6q573ndp. Genetic (cytochrome *b* sequences) and genomic data (raw reads) were deposited to GenBank under Accession nos. MZ570972–MZ571096, MZ665541–MZ665542 and Bioproject ID PRJNA749254, respectively.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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