

Genetic and morphological data supporting the hypothesis of adaptive radiation in the endemic fish of Lake Matano

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Abstract

Adaptive radiation resulting from differential selection acting on functional features is believed to be an important source of biodiversity. In this study, morphometric measures and mitochondrial DNA are used to test for adaptive radiation within four fish genera (*Glossogobius*, *Oryzias*, *Dermogenys* and *Telmatherina*) endemic to an ancient island lake (Lake Matano, Sulawesi, Indonesia), using the framework proposed by Schluter (*The Ecology of Adaptive Radiation*, Oxford University Press, 2000). We demonstrate common ancestry and rapid divergence in one genus (*Telmatherina*) based on 560 bp of 16S sequence data. We found higher levels of variation in feeding-related traits ($N = 8$) for *Telmatherina* relative to the other genera, while no differences were found for sexual display traits ($N = 8$) or neutral morphological traits ($N = 8$). *Telmatherina* also had the highest number of distinct colouration patterns among the four genera. These data, combined with the very low productivity of the lake, are indicative of selection driving adaptive radiation. The morphometric divergence in the *Telmatherina* likely results from selection acting on feeding traits in this low productivity lake, leading to trophic specialization among closely related morphotypes. These results provide indirect but compelling data supporting the adaptive radiation of *Telmatherina* in this system.

Introduction

An elevated rate of divergence (or level of variation) both within and among taxa is common to most examples of species radiation. Elevated divergence can either reflect drift acting in a nondirectional manner, or be a response to selection acting directionally on particular traits. Radiation due to drift is characterized by the even, slow accumulation of variation within and among taxa. In contrast, radiation as a result of selection accumulates variation relatively quickly, is manifest in critical traits, and will be taxon biased. Rapid divergence among taxa in response to selection pressures is adaptive radiation

and is an important mechanism driving biodiversity (Schluter, 1995).

The identification of adaptive radiation in natural systems has been complicated by the lack of a cohesive concept or distinguishing criteria used to detect its presence (Schluter, 2000; Losos & Miles, 2002). Even recent studies describing adaptive radiation events often assume its occurrence without demonstrating a potentially adaptive component within the focal taxon (e.g. von Rintelen & Glaubrecht, 2003). Schluter (2000) recommends that taxa exhibiting adaptive radiation should adhere to four distinct criteria; the focal taxon should (i) have common ancestry, (ii) exhibit varying phenotypes corresponding to their divergent environments, (iii) be differentiated by traits that are functional (or utilitarian) in nature, and (iv) exhibit rapid divergence, or 'burst' speciation (Schluter, 2000). Together, Schluter's criteria provide a framework by which taxa and phylogenies can be systematically evaluated for

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evidence of adaptive radiation. Presently, few published examples of adaptive radiation have been demonstrated to meet these criteria. There is a dearth of studies identifying natural systems where the influence of selection on taxa radiation can be easily recognized and investigated.

Ancient lakes are increasingly recognized as important study systems for evolution and speciation (Rossiter & Kawanabee, 2000; von Rintelen & Glaubrecht, 2003). Such closed and aged environments provide a high potential for radiation events, especially among early invading fauna and flora. Consequently, ancient lakes typically support high levels of endemism such that divergence among taxa can be evaluated in terms of a response to drift or selection. The most notable examples of species radiation in ancient lakes are those described for the cichlids from the East African Rift valley lakes (e.g. Liem, 1974; Meyer *et al.*, 1990; Danley & Kocher, 2001; Jordan *et al.*, 2003). These complex assemblages of fish have made important contributions to our understanding of the theory of adaptive radiation and speciation (e.g. Sturmbauer, 1998; Seehausen, 2000). Yet, even these well-known examples of adaptive radiation have not been shown to fulfil the requirements of Schluter's criteria. Furthermore, inferences of radiation and speciation in the East African Rift valley lakes are complicated by the tremendous number of species and species flocks still under scrutiny (e.g. Smith *et al.*, 2003), and by the wide scope of factors purported to explain their divergences (Verheyen *et al.*, 1996; Sturmbauer, 1998; Markert *et al.*, 1999; Seehausen, 2000; Jordan *et al.*, 2003; see Kornfield & Smith, 2000). The African Great Lakes have also been highly perturbed by continued anthropogenic stress, and are currently undergoing a rapid loss of biodiversity (Seehausen *et al.*, 1997). To quantify the relative importance of adaptive radiation vs. drift, there is a need to locate a system where the influence of selection on radiation among taxa can be readily identified. The ideal system would support a relatively simple assemblage of taxa to allow clarification of functional mechanisms driving phylogenetic radiation.

Lake Matano, located on the island of Sulawesi in the Indonesian archipelago (Fig. 1), is situated along an active fault line, forms the hydrological head of the Malili Lakes watershed, and is separated from downstream lakes by a 72-m waterfall (Brooks, 1950; Kottelat, 1990b). It is estimated to be at least 4 million years old, to have levels of endemism greater than 80%, and has been identified as the principal seeding source of endemic species within the Malili Lakes watershed (Brooks, 1950; Haffner *et al.*, 2001). This relatively small graben lake (surface area approximately 164 km²) has very little littoral area surrounding a very deep basin (maximum depth of 590 m). Lake Matano contains very clear water (Secchi depth ~23 m) and does not support a top predator, indicating very low levels of primary

productivity (Haffner *et al.*, 2001). Matano also possesses a comparatively simple fish community with few endemic genera (Kottelat, 1990a, b, c, 1991; Kottelat *et al.*, 1993). Because of its isolation, Lake Matano has been subjected to few anthropogenic stressors and remains relatively pristine, and is therefore an excellent 'natural experiment' where the importance of drift vs. adaptive radiation can be evaluated. Brooks (1950), concluded that intralacustrine speciation among native taxa must be occurring, as the long isolation of Matano provided an extremely limited potential for multiple invasions. More recent investigations by Kottelat (Kottelat, 1990a, b, c, 1991; Kottelat *et al.*, 1993) described multiple species endemic to Lake Matano belonging to four genera; all of which are of marine origin and thought to have colonized the lake at similar times (Brooks, 1950; Kottelat, 1990a, b, c, 1991; Aarn *et al.*, 1998).

The purpose of the present study is to evaluate the evidence for adaptive radiation within the endemic fish community of this ancient tropical island lake using Schluter's (2000) criterion. We evaluate Schluter's first criterion using 16S rDNA gene fragment sequence to determine the ancestry of the four most common genera endemic to this lake. We then test for Schluter's fourth criteria by estimating relative rates of divergence among the four focal genera using genetic variability within each genus. Finally, Schluter's second and third criteria are evaluated using three categories of morphological traits to determine if potential radiations within this fish community have adaptive components. The results of these diverse approaches provide compelling data in support of adaptive radiation within one endemic genus from this ancient lake system.

Materials and methods

Approximately 50–100 fish were collected by beach seine from each of 11 locations around Lake Matano, Sulawesi Island, Indonesia (Fig. 1), and were identified to genus (*Glossogobius*, *Oryzias*, *Dermogenys* and *Telmatherina*) according to Kottelat (Kottelat, 1990a, b, c, 1991; Kottelat *et al.*, 1993). We observed no spatial bias in the abundance of fish from the four genera. Fish belonging to the genus *Telmatherina* were further separated into five morphotypes previously described as species by Kottelat (1991), and four new morphotypes based on colouration and other phenotypic characters used by Kottelat (1990c, 1991). Selected specimens from each genus were anaesthetized in clove oil, digitally photographed, fin clipped, and allowed to recover in oxygenated water before being released back into the lake. Collected fin tissue was stored in 95% ethanol. A smaller number of whole fish samples were also collected for morphometric analysis and were stored in 10% formalin and 95% ethanol ($N = 40$).

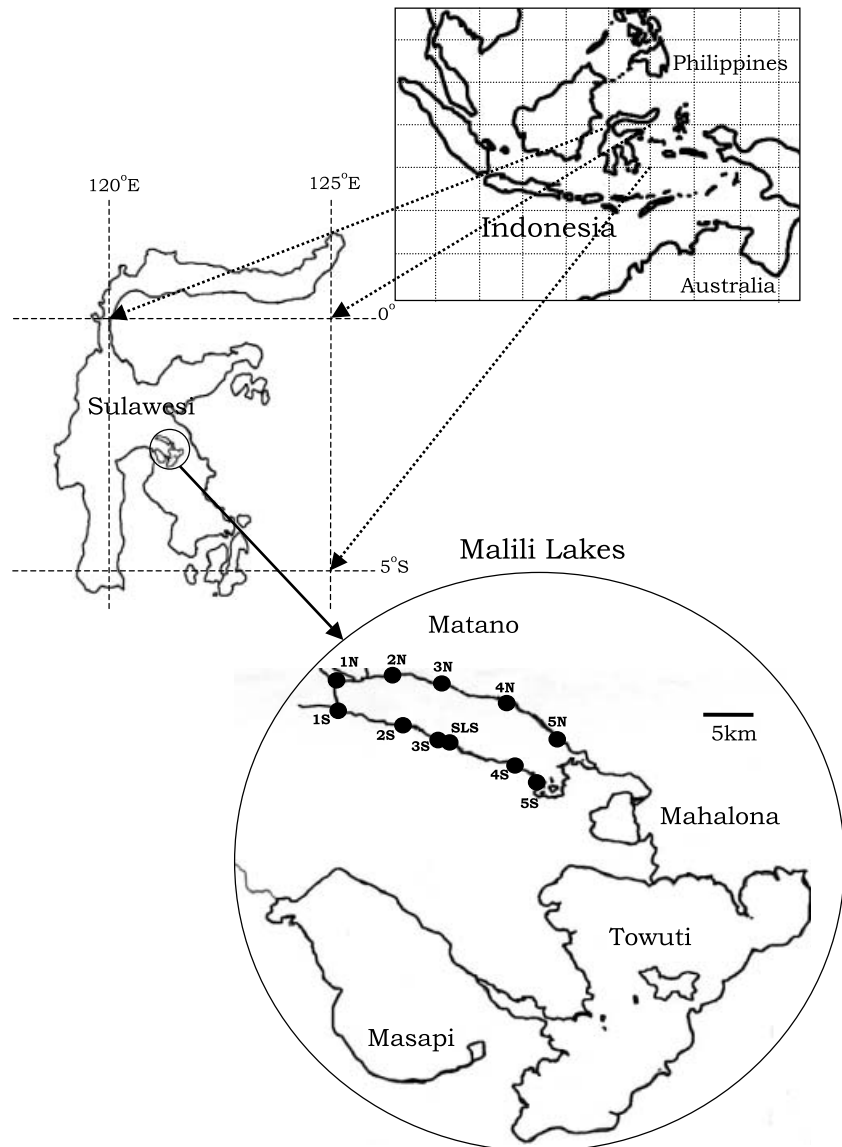


Fig. 1 Location of Lake Matano on the continental island of Sulawesi (inset) in the Indonesian archipelago. Beach seining locations where fish samples were collected around the lake are indicated with dark circles and alphanumeric values. *Glossogobius* collected from 1N, 1S, 3N, 3S, 4N and 5S. *Oryzias* collected from 1N, 2N, 2S, 3S, 4N and 5S. *Dermogenys* collected from 1S, 2N, 3N and 4S. *Telmatherina* used for initial DNA analysis collected from 1S, 2N, 2S, 3S, 4N and 5N. Additional *Telmatherina* were collected from all sampled beaches.

Genetic analysis

Genomic DNA was extracted from fin tissue of 10 fish from each of the four genera (nine for *Glossogobius*), as well as 68 additional *Telmatherina* selected to include all identified colouration morphotypes (Kottelat, 1991), using the Promega WIZARD® DNA extraction kit (Promega, Madison, WI, USA) following the standard 'isolation from animal tissue' protocol. Polymerase chain reaction (PCR) amplification of approximately 600 bp of the mitochondrial 16S rRNA gene was accomplished using published primers [16Sar-L (5'-CGCCTG TTTATCAAAAACAT-3') and 16Sbr (5'-CCGGTCTGAAC TCAGATCACG-3'); Palumbi, 1996]. The 16S rRNA gene was selected as most appropriate for the highly

genetically diverse endemic genera of Lake Matano. Individual 25- μ L reactions contained 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM MgCl₂, 0.2 mM of each dNTP, 0.3 μ M of each primer, 0.5 U of *Taq* DNA polymerase (Invitrogen, Burlington, Canada) and 1.0 μ L of a 10 : 1 dilution of extracted genomic DNA. Reactions were run for 35 cycles consisting of denaturation at 96 °C for 1 min, primer annealing at 50 °C for 1 min, and extension at 72 °C for 1.5 min; the 35 cycles were followed by a final 5-min 72 °C extension. PCR products from a total of 107 fish were sequenced using the DTCS Quick Start cycle sequencing kit (Beckman Coulter, Fullerton, CA, USA) and CEQ8000 automated sequencer, following manufacturer's instructions.

Common ancestry

To determine ancestry of the genera sampled, derived sequences were aligned using the CLUSTAL W algorithm with a gap open penalty of 10, a gap extension of 5 and a DNA transition weight of 0.5 (Omega 1.2, Oxford Molecular Ltd, Oxford, UK). Sequences were then analysed using the Molecular Evolutionary Genetics Analysis program version 2.1 (Kumar *et al.*, 2001). Pairwise genetic distances were calculated and corrected for transition bias using the Kimura 2-parameter, Tamura-Nei, and the Jukes-Cantor distance models assuming a gamma distribution for base substitution (Kocher & Carleton, 1997). Insertions and deletions (indels) were treated equally as complete deletions, while tests for transition saturation were negative and therefore allowed both transitions and transversions to be used in distance estimates (Kocher & Carleton, 1997). A phylogenetic relationship based on corrected distances among the 107 fish was estimated using the Neighbour-joining algorithm supported by a confidence assessment of 10 000 bootstrap permutations (Saitou & Nei, 1987).

Lineage divergence rate

Because speciation rate is difficult to determine without complete phylogenies (Nee *et al.*, 1994; Schluter, 2000), and because the phylogenies of the endemic genera from Lake Matano at the species level are not currently available, we used mean lineage divergence as a proxy for speciation rate. Lineage divergence within individual genera was estimated by calculating pairwise genetic distances among 10 (nine for *Glossogobius*) fish from each genus based on the 16S sequences (see above). We analysed the distribution of the pairwise genetic distance estimates among genera using a Kruskal–Wallis test and *post-hoc* tested for differences between pairs of genera using a Mann–Whitney *U*-test. To verify anomalously high levels of divergence observed within *Telmatherina* relative to the other genera, mean pairwise genetic distance within this genus was re-calculated 50 times using 10 randomly chosen individuals from a pool of 78 individuals (with replacement). This was carried out to determine whether the level of divergence observed within this genus was simply due to chance. Ultimately, we included all available sequence data ($N = 78$) to increase the accuracy of the lineage divergence estimate for this genus.

Morphometric trait assessment

Although fish colouration patterns can be subjective and difficult to quantify, they have nevertheless been shown to be key morphological characters on which fish can cue and segregate (Seehausen, 2000). A chi-square (χ^2) analysis was performed to determine if any particular

genus had a greater number of colour morphotypes than any other, based on in-field colour morphotype determination. Ten fish from each of the four genera were also analysed using morphometric parameters described in Kottelat (1990b, c) along with five additional parameters ($N = 32$; Table 1). Morphometric traits were separated into three categories; feeding, sexual display, and neutral traits (see Table 1). Feeding traits were those commonly reported in the literature as associated with mouth morphology and food acquisition (Humphries, 1993; Gysels *et al.*, 1997; Hyndes *et al.*, 1997). Sexual display traits were those typically associated with attracting a mate or sequestering reproductive opportunity (Jennions *et al.*, 2001; Karino & Haijima, 2001). Neutral traits consisted of traits not included in previous categories, and on which selection has not been demonstrated, but are conventionally used as taxonomically informative traits.

All measured traits were normalized using standard length to account for differences in body size, whereas data for meristic traits were kept as counts. Coefficients of variation for each trait, within a genus, were calculated and z-score corrected across traits within categories to fit a Gaussian distribution. z-Corrections applied to the coefficients of variation also standardized variances among traits within categories allowing parametric analyses (Sokal & Rohlf, 1998). Analyses of variance were then performed on corrected data to assess if there were significant differences in the amount of variation within categories among the four genera. *Post-hoc* Tukey tests identified specific genera which significantly differed from others (Sokal & Rohlf, 1998). All statistical analyses were completed using either SYSTAT statistical software package (SYSTAT v. 8.0 1998, SPSS Inc., Chicago, IL, USA) or specifically designed programs written in IDL (Interactive Data Language v. 5.3.1 2000, Research Systems Inc., Boulder, CO, USA) and evaluated at the $P < 0.05$ level of significance.

Results

Genetics

The 16S rDNA sequences generated in this study are available in GenBank under accession numbers AY62534–AY62538 and AY545822–AY545875. Aligned sequences comprised 560 bp; 170 sites (31%) were variable, 147 (26%) of which were phylogenetically informative. All distance models (listed above) yielded the same or very similar distance matrices. We report only the Kimura 2-parameter results, as this model is most commonly used for assessing fish mtDNA phylogenetics (Kocher & Carleton, 1997), and thus allows broad comparison to other work. Overall, sequence differences were characterized by 15 indels and an average of 15.5 and 14.9 transition and transversion substitutions, respectively.

Table 1 Coefficient of variation calculated for several morphometric traits, separated into three trait categories (feeding, sexual display and neutral), in the four endemic genera of Lake Matano. The classification of these traits into feeding or sexual display traits is based on published descriptions (see text). Neutral traits are those typically used as diagnostic features delineating taxa, but on which selection has not been demonstrated.

Trait	<i>Glossogobius</i>	<i>Oryzias</i>	<i>Dermogenys</i>	<i>Telmatherina</i>
Feeding traits				
Snout length	9.63	3.77	9.80	14.94
Head depth	4.12	5.33	6.26	7.25
Mouth position	22.82	0.00	14.21	80.76
Mouth protruberosity	1.28	0.78	2.31	0.77
Gape width	7.80	7.45	11.20	14.95
Gape Height	8.16	10.17	16.54	13.07
Maxillary length	23.42	17.11	17.97	19.78
No. of gill rakers on 1st gill arch	9.39	3.42	5.21	18.18
Sexual display traits				
1st dorsal fin length	20.56	7.33	7.34	38.58
Caudal fin length	8.20	8.33	10.93	8.55
Caudal fin depth	7.88	3.91	1.94	4.19
Head length	5.98	5.03	6.27	3.03
Anal fin length	6.12	4.60	5.96	12.43
Body depth	9.08	3.50	4.79	10.18
Pre-anal length	9.00	3.26	1.95	3.79
Pelvic fin length	8.89	6.71	7.10	13.36
Neutral traits				
No. of lateral scales	6.31	3.99	6.35	3.10
No. of transverse scales	14.62	6.73	4.71	7.41
No. of caudal rays	0.03	0.04	0.03	0.09
No. of pectoral rays	1.87	4.52	2.66	8.37
No. of anal rays	0.00	2.85	1.96	11.48
No. of dorsal rays	0.00	0.00	0.00	5.21
Inner orbital width	19.61	6.47	10.06	8.67
No. of pelvic rays	0.00	0.00	0.00	5.36

Common ancestry

The Neighbour-joining algorithm segregated sequences into four distinct monophyletic clusters corresponding to the four endemic genera (Fig. 2). We present all unique haplotypes as branch tips, despite low bootstrap support, to avoid misleading genus-level biases. Nodes separating genera were supported by >90% of bootstrapping permutations. Equally shallow terminal branching exhibited within each genus indicates approximately equivalent divergence time from a common ancestor (Fig. 2). The *Telmatherina* genus showed high sequence variation; however, the identified topology did not correspond to previously identified species. The *Glossogobius* genus was subdivided into two groups with strong bootstrap support (98%). *Dermogenys* also demonstrated some topography with moderate bootstrap support, while *Oryzias* showed little intragenetic sequence divergence, with nine identical 16S sequences.

Lineage divergence rate

Frequency distributions of the pairwise genetic distances within each genus demonstrated that the *Telmatherina*

genus had higher lineage divergence and genetic variation relative to the other genera endemic to the lake (Fig. 3). *Glossogobius* and *Dermogenys* showed intermediate levels of divergence and variation, while there was essentially no variation within the *Oryzias* genus (Fig. 3). The levels of genetic divergence among genera were highly significantly heterogeneous ($P < 0.0001$), and *post-hoc* Mann–Whitney *U*-tests results confirmed that all comparisons among pairs of genera were significantly different ($P < 0.001$). All 50 bootstrap iterations used to re-calculate mean sequence divergence within *Telmatherina* were greater than the mean sequence divergence calculated within *Glossogobius*, the genus with the next highest mean sequence divergence (Fig. 4a). Including more individuals in the estimation of the *Telmatherina* mean sequence divergence marginally lowered the estimate and also reduced the standard error (Fig. 4b).

Morphometric trait assessment

A significant difference was observed in the number of colour morphotypes identified within each genus (Fig. 5a). The *Telmatherina* genus was characterized by nine colour types, while the other genera were described

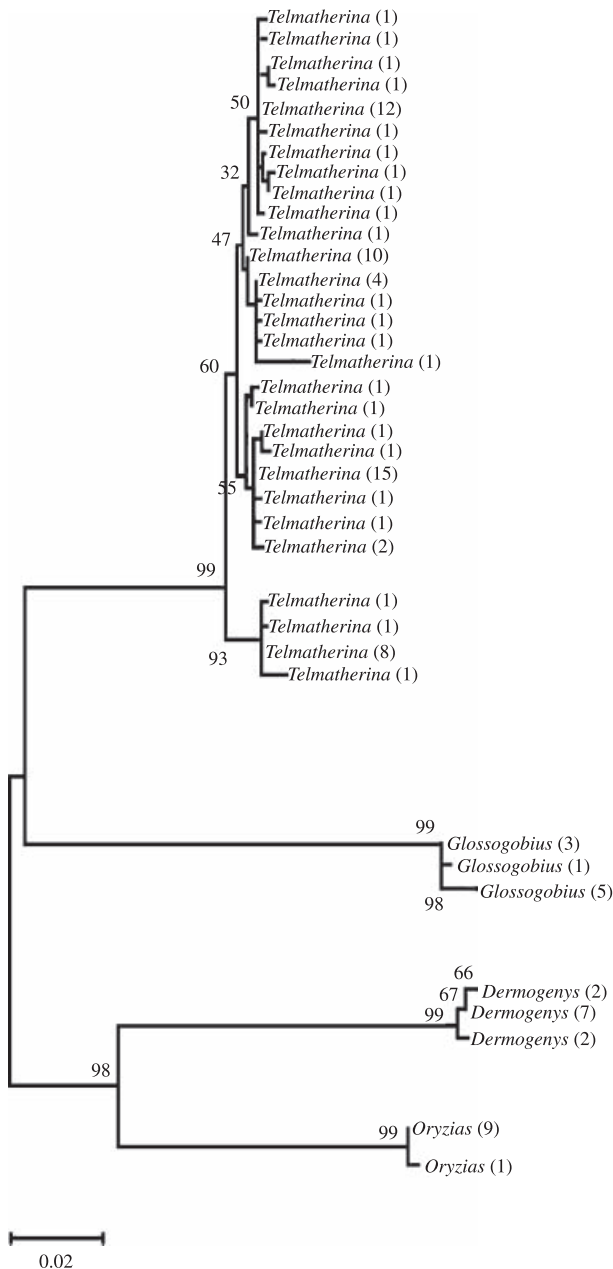


Fig. 2 Genetic relationship within and among the four endemic fish genera (*Glossogobius*, *Dermogenys*, *Oryzias* and *Telmatherina*) of Lake Matano, constructed using Neighbour-joining analysis with the Kimura 2-parameter distance model assuming a gamma distribution for base substitution. Bootstrap support is indicated at all nodes. Numbers beside taxon name indicate the number of individuals sharing the same sequence, all labelled branch tips represent unique sequence data.

by three or fewer. Significant differences were also observed in mean standardized, z-score corrected coefficients of variation calculated for eight feeding traits among the different genera (Fig. 5b). Adjusted *post-hoc*

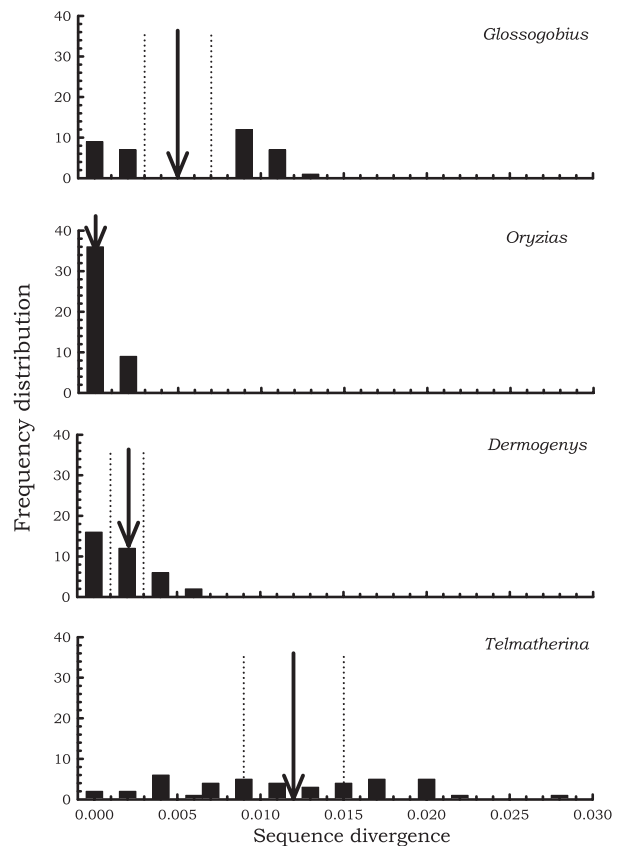


Fig. 3 Frequency distribution of pairwise genetic distances (a proxy for genetic divergence rate) calculated within each genus. Pairwise distances were corrected for transition bias using the Kimura 2-parameter distance model assuming a gamma distribution for base substitution. Arrows indicate mean sequence divergence within genus and dotted lines show ± 1 SE (calculated using 10 000 bootstrap permutations). All four distributions differed significantly in mean genetic divergence (Mann-Whitney *U*-test; $P < 0.001$).

analysis revealed *Telmatherina* to be more variable in feeding traits than either the *Glossogobius* or *Oryzias* genera, but not the *Dermogenys* genus. No significant differences were found in the amount of variation in sexual display traits estimated among the endemic genera sampled from this lake (Fig. 5c). The variation observed in neutral traits was greatest within the *Telmatherina* and lowest within the *Oryzias* genus; however, even differences using these extremes were not significant (Fig. 5d).

Discussion

Studies of adaptive radiation contribute to our understanding of the fundamental principles regulating speciation as it serves to rapidly increased variation, in response to selective pressures. In this study, we provide data supporting the hypothesis of adaptive radiation in the *Telmatherina* genus in Lake Matano, based on the

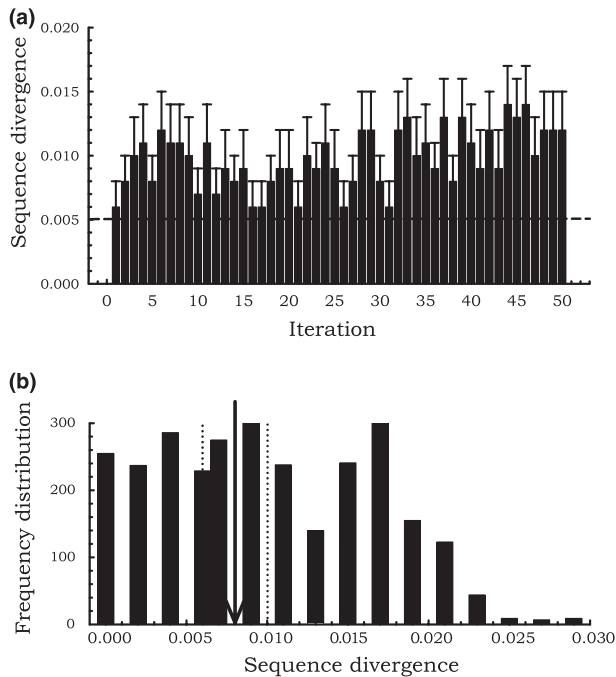


Fig. 4 (a) Mean sequence divergence (± 1 SE) calculated through 50 iterations within the *Telmatherina* genus using 10 randomly selected individuals from a pool of 78 (with replacement). All estimates of the mean were performed using 10 000 bootstrap permutations of the Kimura 2-parameter distance model assuming a gamma distribution for base substitution. All 50 iterations demonstrated greater sequence divergence within *Telmatherina* than that estimated within *Glossogobius*, the genus with the next highest divergence (indicated with dashed line). (b) Frequency distribution of pairwise genetic distances using all 78 individual *Telmatherina*. Arrow indicates mean sequence divergence and dotted lines show ± 1 SE (calculated using 10 000 bootstrap permutations).

framework provided by Schluter (2000). The phylogenetic relationships generated using the 16S rDNA sequences demonstrate common ancestry for each of the endemic genera in Lake Matano, while elevated levels of sequence divergence within the *Telmatherina* and the *Glossogobius* genera were evident. These data satisfy the first and fourth criteria of Schluter's adaptive radiation framework, and indicate that both the *Telmatherina* and, to a lesser extent, *Glossogobius* are potentially radiating in this system.

Estimates of morphological variation in colouration patterns and feeding traits, meanwhile, imply adaptive components to the *Telmatherina* radiation that are not evident in *Glossogobius*. The larger number of colouration patterns exhibited, and the greater variation in feeding traits (Fig. 5a,b) demonstrate adaptive components to the *Telmatherina* radiation which address the second and third criteria of Schluter's (2000) framework. Although we demonstrated no direct association between *Telmatherina* phenotypes and explicit environment or habitat

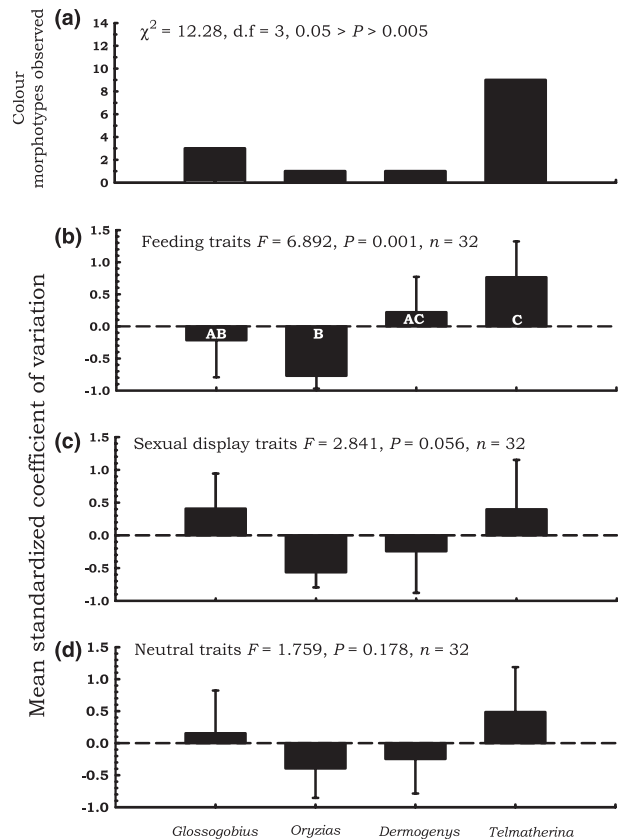


Fig. 5 Comparison of morphological variation in the four fish genera endemic to Lake Matano, Sulawesi Island, Indonesia: (a) number of colouration patterns observed within each genus, and mean z-score corrected coefficient of variation (± 2 SE.) among: (b) feeding traits, (c) sexual display traits, and (d) neutral traits for each genus. Trait groups that showed significant variation among genera were tested for pairwise differences among genera using a Tukey's *post-hoc* test: shared letters indicate nonsignificant differences.

differences, the evolution of variation in feeding morphology has been widely demonstrated for fish in low-resource environments (Humphries, 1993; Schluter, 1995; Ruzzante *et al.*, 1998; Rüber *et al.*, 1999; Sibbing & Nagelkerke, 2001). In such cases, closely related species exhibit divergent phenotypic variation in feeding morphologies associated with variation in the food resource (Liem, 1974; Schluter & MacPhail, 1993; Ruzzante *et al.*, 1998; Rüber *et al.*, 1999; Schluter, 2000; Sibbing & Nagelkerke, 2001). The low level of primary productivity in Lake Matano is reflected throughout the food chain as indicated by the lack of piscivorous fish and the large Secchi depth (Haffner *et al.*, 2001). Greater variation in feeding traits observed in both *Telmatherina* and *Dermogenys* suggests that both these genera are under selective pressure in response to a limited feeding resource. In particular, the feeding traits demonstrating the greatest variation in *Telmatherina* are those typically

reported as associated with trophic specialisation (i.e. snout length, mouth position, gape height, gill rakers – see Table 1; Sibbing & Nagelkerke, 2001). The *Dermogenys* have mouth morphology and spatial distribution consistent with obligate surface feeding (elongated lower jaws, and near surface distribution). The *Telmatherina* and *Oryzias* are both pelagic, however the *Oryzias* have the lowest variation in feeding morphology, indicative of feeding specialization.

Greater morphological variation in the *Telmatherina* also satisfies Schluter's third criterion. The larger number of colouration patterns and greater variation in feeding, but not sexual display or neutral traits, supports the directed differential divergence in *Telmatherina* resulting from selection acting on functional (or utilitarian) features. Selection acting on colouration and/or feeding traits in the *Telmatherina* has the potential to create mating barriers that accelerate drift at neutral genetic markers (i.e. 16S rDNA) within reproductively isolated lines. This work thus establishes a link between genetic divergence and functional traits on which selection may be acting within the *Telmatherina*, and implicates trophic specialization as the most likely mechanism driving adaptive radiation.

Our data do not support assortative mating among colour morphotypes as a major contributing factor to the radiation observed in *Telmatherina*. Sexual selection, especially with strong assortative mating among colour types, will create barriers to gene flow and hence segregation among closely related taxa, even in sympatry (Seehausen *et al.*, 1997; Seehausen, 2000). Hence, a consequence of speciation driven by sexual selection is high variability in sexual display traits among closely related taxa (Seehausen, 2000). Sexual selection of this nature has been proposed as a mechanism regulating the relatively recent explosive radiation of haplochromine and other cichlid fishes in the African Rift lakes (Seehausen *et al.*, 1997; Kornfield & Smith, 2000; Seehausen, 2000). Although we observed a significantly greater number of colouration patterns in *Telmatherina* relative to the other Lake Matano genera, we found no significant difference in the level of variation in other sexual traits (including dorsal and anal fins noted in the field to be used during displays).

An alternative explanation of the differences in genetic divergence observed among Lake Matano endemic genera is variation in colonization history. If the *Telmatherina* colonized the lake earlier than the other genera, or colonized the lake in multiple-colonizing events, the *Telmatherina* would be expected to exhibit greater genetic variation, independent of selective effects. Pre-existing divergence expected from multiple colonists within a particular genus, however, should manifest greater intragenetic genetic divergence at the 16S locus (deeper branching within genus phylogeny) than was observed here. Moreover, similar (but not identical) shallow terminal branch lengths among all genera suggests a common colonization time for all genera in this system.

Common colonization of endemic genera in Lake Matano is consistent with the published reports available addressing the evolution of the fish community in this system (Brooks, 1950; Kottelat, 1990a, b, c; Kottelat *et al.*, 1993; Aarn *et al.*, 1998). Brooks (1950) suggests that the tectonic origin of Lake Matano, its great depth, and location along an active fault are indicative of an ancient origin predating that of the other Malili lakes. Brooks (1950), Kottelat (1990b) and Aarn *et al.* (1998), all use local topology (Matano separated from downstream lakes by a 72-m drop) to support the current isolated nature of this lake relative to others in this watershed. The isolated nature of Lake Matano and the paucity of shared species with downstream lakes are not consistent with multiple colonization of this system (Brooks, 1950; Kottelat, 1990c, 1991; Kottelat *et al.*, 1993; Aarn *et al.*, 1998). Therefore, we conclude that the endemic species of Lake Matano are the consequence of intralacustrine speciation from original, genus-specific, common ancestors which colonized the lake at similar times and subsequently provided propagules for downstream varieties (Brooks, 1950; Aarn *et al.*, 1998).

The most parsimonious explanation for the higher genetic diversity and the curious pattern of morphological variation of *Telmatherina* in Lake Matano, relative to the three other endemic fish genera, is differential selection acting on functional feeding traits. This study supports adaptive radiation in this genus as a consequence of divergence in resource acquisition in a resource poor environment. The adaptive radiation hypothesis for the *Telmatherina* in Lake Matano provides specific, testable predictions for studies of resource use specialization and phylogenetic relationships within the endemic genera of this lake. Furthermore, the *Telmatherina* in this simple, isolated ancient lake represent a particularly valuable study system for the evaluation of the fundamental mechanisms driving the creation of biodiversity in tropical freshwater aquatic ecosystems.

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