

# Resource-based adaptive divergence in the freshwater fish *Telmatherina* from Lake Matano, Indonesia

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## Abstract

Adaptive radiations are an important source of biodiversity, but resolving which ecological pressures seed these processes in natural systems remains difficult. Here the adaptive radiation among *Telmatherina*, a genus of freshwater fish endemic to an ancient lake in central Sulawesi, Indonesia, was examined to determine its causal root. We demonstrate that all *Telmatherina* in this lake can be categorized into three lineages each possessing specialized skull shapes and pharyngeal jaw bones allowing them to exploit different resources. These data demonstrate a natural example of how resource partitioning has likely initiated adaptive radiation in a resource limited environment.

*Keywords:* adaptive radiation, ecological pressures, Lake Matano, resource limitation, *Telmatherina*, trophic specialization

Received 3 June 2006; revision accepted 25 July 2006

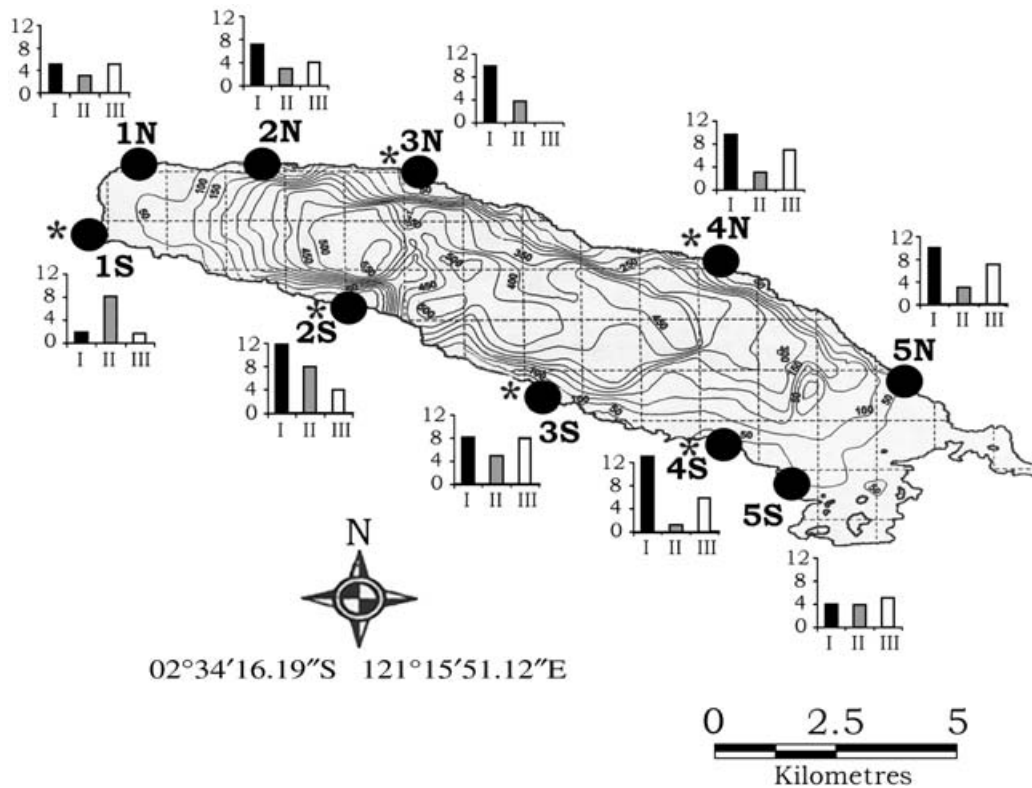
## Introduction

Adaptive radiations are an important source of biodiversity, and understanding factors initiating these processes is critical in a world of ever-increasing species loss (Seehausen *et al.* 1997; Myers *et al.* 2000; Sodhi *et al.* 2004). Mounting evidence suggests that adaptive radiations are seeded by ecological selection among taxa that subsequently diverge via other selective forces related to inter and/or intraspecific communication (Danley & Kocher 2001; Streelman & Danley 2003; Kocher 2004). Ecological pressures such as competition and predation have been predicted to play significant initiating roles in adaptive radiations and their implication in many notable systems supports this hypothesis (Schluter 2000; Danley & Kocher 2001; Doebeli & Dieckmann 2003; McKinnon *et al.* 2004; Rundle & Nosil 2005). Determining the relative importance of the various ecological pressures responsible for initial divergence remains challenging largely because such pressures likely act synergistically to produce adaptive radiations (Schluter 2000; Streelman & Danley 2003; Kocher 2004).

Some excellent studies have identified putative causal mechanisms in specific adaptive radiations and have demonstrated that resource polymorphism can play an initiating role in sympatric divergence. For example, Skúlason *et al.* (1993), Schluter (1994) and Bernatchez (2004) all demonstrated that resource limitation in Icelandic charr, sticklebacks and whitefish was likely the causative mechanism driving divergence leading to resource specialization among sympatric morphotypes. Such studies suggest that stable resource-limited environments can often promote the evolution of resource specialists from polymorphic generalist ancestors (MacArthur & Pianka 1966; Skúlason *et al.* 1993; Schluter 1994; Bernatchez 2004). More recent theory on adaptive dynamics, however, proposes that the colonization of new environments by resource specialists can initiate niche expansion and subsequent contraction leading to the formation of many closely related resource-specific species (Waxman & Gavrillets 2005). A general trend supporting either the generalists to specialist or the adaptive dynamic hypothesis in documented cases of adaptive radiations, however, remains elusive (Schluter *et al.* 1997).

The Indonesian island of Sulawesi has long been recognized as a centre of biodiversity due to the high degree of endemism among its native fauna (Wallace 1892; Myers *et al.* 2000; Whitten *et al.* 2002). Most studies characterizing

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**Fig. 1** Location of beaches from where *Telmatherhina* used in this study were collected. Bar graphs at each beach indicate the number of individuals (on the *y*-axis) from each genetic clade (on the *x*-axis: I, II and III) included in the samples. Clades refer to phylogenetic clusters derived in the text (Fig. 2A). Beaches indicated with stars coincide with those from where previous sampled specimens were collected (Kottelat 1991).

evolutionary relationships among Sulawesi biota come from terrestrial systems (Wallace 1892; Whitten *et al.* 2002; Evans *et al.* 2003; Kingston & Rossiter 2004), but equally intriguing results are emerging from investigations of Sulawesi's unique aquatic systems (Brooks 1950; Kottelat 1990a, b, c, 1991; Roy *et al.* 2004; Rintelen & Glaubrecht 2005).

Lake Matano is an ancient fault lake forming the headwaters of the Malili Lakes on Sulawesi (Brooks 1950; Kottelat 1990a; Haffner *et al.* 2001), and is isolated from downstream systems by physical barriers to aquatic dispersal (Brooks 1950; Kottelat 1990a; Rintelen *et al.* 2004; Roy *et al.* 2006). It is estimated to have an origin dating 2–4 million years ago (Ma) based on the geology of the surrounding area and on displacement measurements taken along the active Matano and Palu Koro faults, thought to be the formative agents of these island lakes (Brooks 1950; Hamilton 1979). The fishes occurring in this lake have been little studied and have all been purported to have a marine origin (Kottelat 1991; Haffner *et al.* 2001; Roy *et al.* 2004). However, more recent evidence suggests that most (*Telmatherhina* in particular) owe their current distribution to sequential colonization of freshwater systems such as Lake Matano, made available by the breakup of Gondwana and

continued tectonic action in the Indonesian archipelago (Wilson & Moss 1999; Sparks & Smith 2004). Lake Matano is a relatively small (164 km<sup>2</sup>) but very deep (~600 m) fault lake with extremely steep sides and is surrounded by limited littoral areas (Fig. 1). The fish community in this lake is sparse and most members are found mainly within littoral areas, with a marked absence of fish beyond the 30–40 m lake contour (Roy, unpublished acoustic data). Lake Matano has very clear water (Secchi depths > 23 m) and low phytoplankton biomass (< 52 µg/L wet weight) composed predominantly of cyanobacteria. Such conditions do not maintain exclusively piscivorous top predators and indicate a highly oligotrophic state (Brooks 1950; Roy *et al.* 2004; Sabo 2006; Heath personal communication).

Previous surveys of the fishes in this system reported several endemic species belonging to four principal genera, namely *Glossogobius*, *Oryzias*, *Dermogenys* and *Telmatherhina* (Kottelat 1990a, b, 1991; Larson & Kottelat 1992; Kottelat *et al.* 1993; Aarn & Kottelat 1998). A more recent study (Roy *et al.* 2004) used published criteria (Schluter 2000) to demonstrate that of the fish genera present in Matano, the *Telmatherhina* show evidence of adaptive radiation as a consequence of either sexual selection among brightly

coloured morphotypes, or adaptive feeding structures which may be important features (Liem 1974; Galis & Drucker 1996) allowing these fish to segregate along resource-based adaptive peaks (Schluter 2000; Doebeli & Dieckmann 2003; Bernatchez 2004). Here we present evidence that resource partitioning is the critical ecological pressure likely responsible for the initial divergence of *Telmatherina* in this system.

## Materials and methods

### Genetics

To determine the phylogenetic structure within *Telmatherina*, fish were collected by seine from 10 locations around the lake (Fig. 1) and sexed anatomically. Only male specimens were subsequently used in this study. Genomic DNA was extracted from fin tissue using the Promega WIZARD DNA extraction kit following the standard 'isolation from animal tissue' protocol (Promega). Amplification of 490 bp of the 16S rDNA and 600 bp of the cytochrome *b* gene was performed using published and specifically designed primers [16Sar-L (5'-CGCCTGTTTATCAAAAACAT-3') and 16Sbr (5'-CCGGTCTGAACTCAGATCACG-3') (Palumbi 1996)] and [GLUDG-5 (5'-TGACTTGAARAACCACCGTTG-3') (Palumbi 1996) and CBtelm-R (GTGGAGGAGGGGTAC-GACTA-3') developed here]. Polymerase chain reactions (PCRs) were performed as described in Roy *et al.* (2004) using an annealing temperature of 50 °C and 60 °C for the 16S and cytochrome *b* reactions, respectively. PCR products of 82 *Matano Telmatherina*, and five *Marosatherina lagdesi*, a closely related *Telmatherina* species from South Sulawesi used as an outgroup, were cleaned and sequenced using the DTCS Quick Start cycle sequencing kit (Beckman Coulter) and CEQ8000 automated sequencer following the manufacturer's instructions.

Resulting sequences from both genes were aligned separately using the CLUSTAL W algorithm (Omega 1.2, Oxford Molecular) and verified by eye. Sequences from both cytochrome *b* and 16S regions were imported into PAUP version 4.0b10 (Swofford 2001) and an incongruent length difference test (ILD; Farris *et al.* 1994) was performed to determine if sequences from the two regions could be combined. The ILD test used parsimony based heuristic searches employing the tree-bisection-reconnection (TBR) algorithm and was replicated 1000 times. Although significant ILD tests do not strictly imply incongruence among gene partitions, they do serve as a general gauge pointing to major inconsistencies among them (Cunningham 1997). ILD test results demonstrated congruence between 16S and cytochrome *b* gene partitions ( $P = 0.0714$ ) allowing the two sequences to be concatenated to form a single 1090 bp sequence per individual. A phylogenetic relationship among sequences was constructed by neighbour-joining (NJ) using

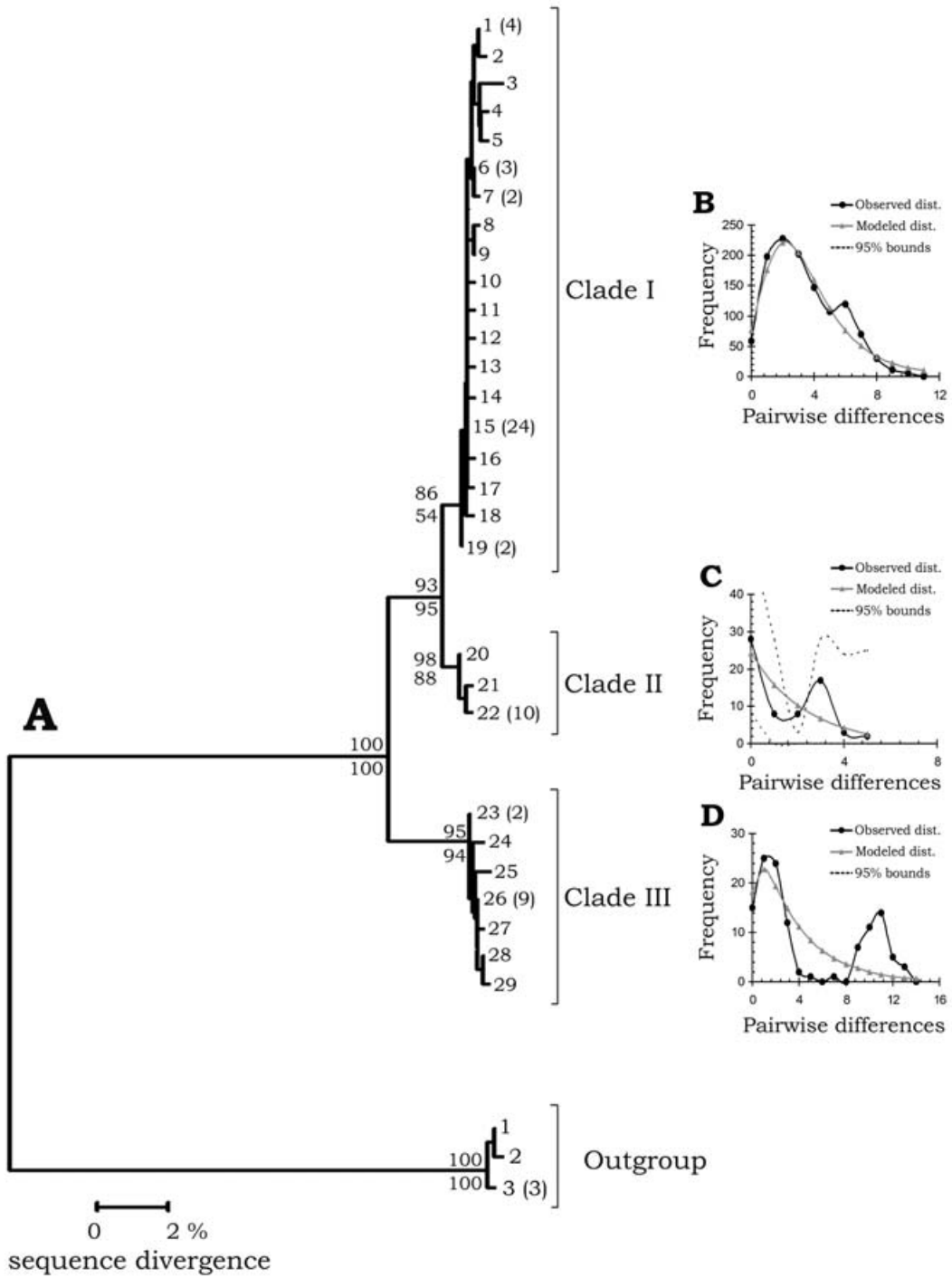
**Table 1** Restriction fragment length polymorphisms of the cytochrome *b* PCR product identifying individual *Telmatherina* to specific genetic clades determined from Fig. 1

	Uncut cytochrome <i>b</i> fragment	<i>FokI</i> fragments (5'...GGATG(N) <sub>9</sub> /...3')	<i>SphI</i> fragments (5'...GCATTG/C...3')
Clade I	1036	990/46	859/177
Clade II	1036	990/46	593/443
Clade III	1036	539/497	920/116

/indicates cut site within recognition sequence.

Kimura 3-parameter with unequal frequencies in base composition, a proportion of invariant sites (0.5517), and a gamma shape parameter for base substitution (0.6715). This substitution model was determined by the Akaike information criterion (AIC) survey of 56 different models implemented by MODELTEST version 3.7 (Posada & Crandall 1998). Ten thousand bootstrap iterations of the distance-based NJ algorithm were performed to establish nodal confidence and a 50% majority rule consensus tree was generated (Fig. 2A). As an additional test, phylogeny was also recovered from a maximum-parsimony heuristic search with close neighbour interchange of 1 and random addition trees of 10 replicates, supported by 1000 bootstrap permutations. A mismatch analysis of the sequences was performed to determine the demographic history of the derived lineages produced from the phylogeny. Mismatch analysis can be used to reveal if and when a series of haplotypes within a population have undergone sudden or stepwise demographic expansion (Rogers & Harpending 1992; Graven *et al.* 1995; Schneider & Excoffier 1999). The mismatch analysis attempts to fit the observed data with models of population expansion using both a generalized least squares procedure (Schneider & Excoffier 1999) and a test of raggedness (Rogers & Harpending 1992) both of which were iterated 1000 times in a bootstrap parametric approach to determine significance ( $\alpha = 0.05$ ). All demographic analyses were performed in ARLEQUIN version 3.01 (Excoffier 2005).

Two restriction endonucleases *FokI* and *SphI* (New England Biolabs) provided diagnostic restriction fragment length polymorphisms of the cytochrome *b* fragment that identified *Telmatherina* to specific clades (see Table 1 and Fig. 2). Genomic DNA was subsequently extracted from more than 300 individuals and approximately 1000 bp of cytochrome *b* was amplified using PCR and primers described above. Resulting PCR products were digested with the two enzymes following manufacturer's instruction, to determine clade membership and 32 individuals belonging to each *Telmatherina* clade were selected for all remaining analyses with no spatial bias in regards to



**Fig. 2** (A) Phylogenetic relationship among sampled *Telmatheterina* (n = 82) from Lake Matano, Sulawesi, Indonesia, using both 16S and cytochrome *b* mitochondrial sequence data. Numbers above and below internal nodes represent bootstrap support from both distance and Maximum-parsimony-based analyses, respectively. Number of haplotypes and individuals sharing them (in brackets) indicated at terminal nodes. (B) Frequency distribution of pairwise differences (mismatch) among individuals within Clade I (C) Clade II and (D) Clade III. Circles represent observed data while triangles represent best fit model to the distribution using least squares estimate (ARLEQUIN version 3.01; Excoffier 2005). Dashed lines represent the 95% confidence bounds on the model and are only present in Clade II as this was the only Clade whose mismatch distribution was successfully fitted by the model.

distribution around the lake (Fig. 1). Subsequent to initial phylogenetic reconstruction, the same individual fish were used in all analyses, minimizing the number of specimens destroyed and providing strong support for relationships derived among various results. Samples of fish from the other three endemic genera (*Glossogobius*, *Dermogenys* and *Oryzias*) were also collected in a similar manner and processed for stable isotopes ( $n = 7$ ; see below).

### Geometric morphometrics

To explore putative morphological feeding differences within *Telmatherina* lineages, the heads of 32 fish from each genetic clade were X-rayed using an SDS X-ray imaging system with PCCR 812 HS OREX digital scanner. The upper and lower pharyngeal jaw bones were excised and imaged using an FEI XL 30 ESEM scanning electron microscope using 60–65 KV. Angular displacements of imaged structures were standardized and corrected using digital micro-adjustments to both the scanning plate and microscope stand. Resulting images of skulls and upper and lower left pharyngeal jaw bones were analysed using geometric morphometrics (GM), a landmark-based analysis of shape (Rohlf 1999; Rüber & Adams 2001; Zelditch *et al.* 2004).

Homologous landmarks on skull radiographs and electron scans were chosen based on standard landmark descriptions (Bookstein 1991; Rohlf 1999; Zelditch *et al.* 2004) and on identifiable features that have important functions in feeding and food acquisition, as has been previously described in centrarchids and cichlids (Galis & Drucker 1996), in the generalized cichlid and perciform (Liem 1974; Barel *et al.* 1976), in actinopterygii (Grubich 2001) in atheriniforms (Dyer & Chernoff 1996) and other fish groups (Grubich 2003). Our interpretations of osteological function and mechanics were derived as much as possible from atheriniforms (which include *Telmatherina*). When such information was unavailable our interpretations were based on results from the aforementioned studies in other fish groups. This is justified, in part, by the fact that both the upper and lower pharyngeal jaws in *Telmatherina* share many features in common with those described in the generalized cichlid form.

Both the lower (5th ceratobranchials) and upper jaws (1st, 2nd, 3rd and 4th pharyngobranchials) form the caudal-ventral and caudal-dorsal ends of the pharyngeal basket, respectively. Although not fused together, the lower jaws has pronounced dorso-caudal hinges or horns and lateral ventral keels that taper at the rostral end towards their insertion into the central pharyngeal axis (Liem 1974; Barel *et al.* 1976). Only the largest, the 3rd pharyngobranchial, was used to represent the upper jaw in this study. This structure is characterized on its dorsal side by a neurocraniad articulation facet allowing it to rotate around a bony process on the underside of the skull (apophysis) by

muscle contraction (Liem 1974; Barel *et al.* 1976; Galis & Drucker 1996).

Landmarks from all specimens were superimposed using the generalized least squares Procrustes superimposition analysis (GPA, Rohlf 1999) (TPSrelw; Rohlf 2003) generating an overall consensus configuration of landmarks for each analysed structure. Thin-plate splines were used to calculate interpolation functions (principal warps) among the landmarks of the consensus configuration (Rohlf *et al.* 1996; Rohlf 1999; Rüber & Adams 2001; Zelditch *et al.* 2004) and to superimpose each specimen's corrected landmark configuration onto that of the consensus. Deviations of each specimen's landmarks from those in the consensus configuration generated shape variables (partial warps) which were then used in multivariate analysis (Rohlf *et al.* 1996; Caldecutt & Adams 1998; Rohlf 1999; Rüber & Adams 2001; Zelditch *et al.* 2004). Discriminate function analyses (DFA) on the partial warps was used to determine whether significant differences existed in shape variables among genetically defined clades, and differences among clades were visualized using various ordination axes in a canonical variates analyses (CVA) (Rohlf *et al.* 1996; Caldecutt & Adams 1998; Rüber & Adams 2001; Cavalcanti 2004; Zelditch *et al.* 2004). Post hoc pairwise comparisons among *Telmatherina* clades  $F$ -values, derived from generalized Mahalanobis distances, determined which clade(s) significantly differed in shape. Experiment-wise error was kept at 0.05 by Bonferroni correction of  $\alpha$  values to 0.0166.

Principal components analyses (PCAs) were performed on the partial warps of the analysed *Telmatherina* structures generating a series of relative warps (principal components) outlining the most important and biologically meaningful shape differences among the genetically identified clades (Rohlf 1999; Cavalcanti 2004; Zelditch *et al.* 2004). PCA results were used to generate deformation grids for each clade (TPSrelw; Rohlf 2003) using their mean shapes defined along the two most important relative warps (Cavalcanti 2004; Zelditch *et al.* 2004). Each structure (skull, lower left pharyngeal jaw and upper left pharyngeal jaw) was analysed separately, and mean shapes defined along relative warps were exaggerated to emphasize differences (skull 3 $\times$ ; lower jaw 2 $\times$ ; upper jaw 2 $\times$ ).

### Diet data

To determine whether feeding strategies of the *Telmatherina* clades matched those predicted from their morphology, we analysed clade-specific gut content. The dietary tract from each selected *Telmatherina* was removed and contents were identified to general prey categories (see Genner *et al.* 1999, 2003; Barluenga *et al.* 2006). Gut contents of each fish were analysed using a variant on the points method (Hynes 1950) as explicitly described in Gysels *et al.* (1997), and in Genner *et al.* (1999 and 2003).

Briefly, the points method allocates points to items in the dietary tract depending on their relative volumetric proportions, in descending order (i.e. items taking up the most volume are attributed the highest points). The percentage volume of each component ( $\alpha$ ) within the gut of each specimen ( $X$ ) is calculated using the following formula (see Genner *et al.* 1999).

$$\% \text{ volume of } \alpha \text{ in } X = \frac{\# \text{ points allocated to } \alpha \text{ in } X}{\Sigma \text{ points allocated to } X}$$

The percentage volume of each component for each individual was entered into the program PRIMER and converted to a similarity matrix among all individuals using the Bray-Curtis similarity algorithm. The converted data was used in a nonparametric one-way analysis of similarity (ANOSIM) determining an index of similarity among genetic clades using 10 000 bootstrap iterations (Clarke & Warwick 2001). Similarity indices were also calculated in pairwise comparison among clades with adjusted experiment-wise significance kept at  $\alpha = 0.05$  by Bonferroni correction. Similarity indices using ANOSIM ( $R$ ) are bound by  $-1$  and  $1$ , where an  $R = 0$  indicates that as much similarity exist both among and within groups. An  $R = 1$  indicate a large similarity within, but little similarity among groups, while an  $R = -1$  indicates a greater similarity among rather than within groups (Clarke & Warwick 2001). Both Bray-Curtis similarity conversion and ANOSIM analyses were performed in PRIMER version 5 (Primer-E 2001).

### Stable isotopes

Each fish selected for diet analysis was also dried at  $100^\circ\text{C}$  for 48 h after which whole specimens were macerated into a fine powder using mortar and pestle. A 1 mg subsample of the homogenate powder was weighed into a standardized tin capsule, sealed and processed for the stable isotopes of nitrogen (N) and carbon (C) (see Methods in Paterson *et al.* 2006). Samples were analysed using a continuous flow VG Micromass 903E isotope ratio mass spectrometer at the Environmental Isotope Laboratory (Department of Earth Sciences, University of Waterloo, Waterloo, Ontario, Canada). Stable isotope ratios were given as deviations from standard materials (Pee Bee belemnite limestone for C, or  $\delta^{13}\text{C}$ ; and atmospheric nitrogen for N, or  $\delta^{15}\text{N}$ ).

## Results and Discussion

### Genetics

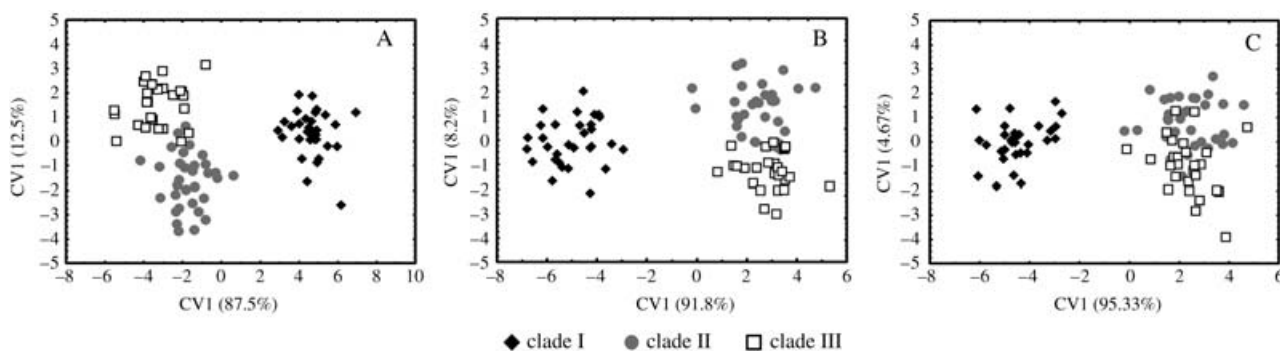
Sequences obtained in this study are available on GenBank (Accession nos AY62534–AY62538, AY545822–AY545875, DQ023624–DQ023667, DQ002506–DQ002547, and DQ054795–DQ054796). Concatenated sequences were

**Table 2** Average pairwise genetic distances within and among clades determined using Kimura 3-parameter with unequal frequencies in base composition, proportion of invariant sites (0.5517), and gamma shape parameter for base substitution (0.6715)

	Clade I	Clade II	Clade III
Clade I	0.32%		
Clade II	1.9%	0.12%	
Clade III	3.9%	3.5%	0.41%
Outgroup	18.0%	18.2%	17.7%

characterized by 207 variable sites, 187 of which were phylogenetically informative. Sequences were also characterized by three insertions (related to the outgroup) and an average transition:transversion ratio of 3.2617. Transition saturation tests were negative allowing both transition and transversion substitutions to be included in phylogenetic analyses. Both distance- and character-based phylogenetic reconstructions separated *Telmatherina* into three distinct clades with strong bootstrap support (Fig. 2). A predominant number of individuals in clade I were more slender in shape and were characterized by rounded second dorsal and anal fins. These characteristics seemed to match morphological descriptions of the *Telmatherina antoniae* species defined by Kottelat (1991), but individuals in both clades II and III had no obvious discernable pattern allowing them to be resolved according to previous morphological descriptions (Kottelat 1991; Kottelat *et al.* 1993). All three *Telmatherina* clades were highly divergent from each other consistent with the presence of three distinct species (Table 1, Avise & Walker 1999). However, because these results were generated with mitochondrial DNA (mtDNA) partitions alone, we subsequently refer to them as different clades or lineages. Although the use of molecular clocks has been largely debated, we cautiously invoke established distance-based divergence rates of the cytochrome *b* and other mtDNA partitions among other fish and vertebrate species of 1–2% per million years (Avise & Walker 1999; Hebert *et al.* 2003) to make an approximation of divergence times among clades (Barluenga *et al.* 2006). If we assume a constant rate of evolution, and use pairwise distances calculated from the substitution model determined by MODELTEST version 3.7 (Table 2), we estimate a 1.85–3.7 million-year-old initial divergence time between clade III and clades I and II, in keeping with the estimated age of the system (see above, Avise & Walker 1999; Hebert *et al.* 2003). Clades I and II, however, appear more recently diverged relative to clade III with an estimated divergence time of 0.95–1.9 Ma.

The mismatch analysis performed on each clade demonstrated that none of them supported a typical bell-shaped curve in their mismatch distributions, a characteristic pattern found in populations that have passed through an



**Fig. 3** Canonical variates plots based on partial warps analysis generated from independent geometric morphometrics analysis of various head structures in *Telmatherina* clades sampled from Lake Matano. (A) Skull; (B) Lower pharyngeal jaw; (C) 3rd Pharyngobranchial (upper jaw). Values inside brackets indicate proportion of total variation described along each axes. All three structures show statistically significant separation among clades except in the case of the left upper jaw (C) where no differences were observed between clades II and III.

important demographic expansion (Fig. 2B; Graven *et al.* 1995; Sotka *et al.* 2005). Rather, each clade exhibited distributions with several peaks, more typical of populations having had stable demographic histories (Graven *et al.* 1995; Sotka *et al.* 2005). Only clade II could be fitted with an expansion model (SSD = 0.04,  $P = 0.4$ , Rag = 0.15,  $P = 0.6$ ) which showed minimal growth in effective population size over the estimated expansion time ( $\theta_0 = 0.00$  and  $\theta_1 = 1.744$ ). These data suggest that all *Telmatherina* clades in Lake Matano have had fairly stable effective populations in this system for quite some time. Thus, the genetic data presented support the presence of three distinct *Telmatherina* lineages in Lake Matano that have likely diverged from each other some time ago within the confines of this very old system.

#### Geometric morphometrics

Discriminate function analyses (DFA) performed on the shape variables among genetically determined lineages revealed significant differences for all structures analysed using GM (Skull: Wilk's  $\Lambda = 0.027$ ,  $F_{26,140} = 19.24$ ,  $P \ll 0.0001$ , body size covariate; Lower jaw: Wilk's  $\Lambda = 0.037$ ,  $F_{44,130} = 12.28$ ,  $P \ll 0.0001$ , body size covariate; Upper jaw: Wilk's  $\Lambda = 0.054$ ,  $F_{44,132} = 9.86$ ,  $P \ll 0.0001$ ). Robustness of DFA results were confirmed by performing 100 iterations of the DFA where the clade assignment of each individual was randomized (see Fig. S5, Supplementary material). Post hoc comparisons among *Telmatherina* indicated that all genetic clades significantly differed in skull shapes (clade I vs. II  $F_{18,70} = 31.25$ ,  $P < 0.0001$ ; clade I vs. III,  $F_{18,70} = 41.64$ ,  $P < 0.0001$ ; clade II vs. III,  $F_{18,70} = 8.19$ ,  $P < 0.0001$ ) and lower jaw shapes (clade I vs. II  $F_{22,66} = 31.30$ ,  $P < 0.0001$ ; clade I vs. III  $F_{22,66} = 31.62$ ,  $P < 0.0001$ ; clade II vs. III,  $F_{22,66} = 3.70$ ,  $P < 0.0001$ ). Upper jaw shapes were also significantly different between clades I and II ( $F_{22,66} = 26.01$ ,  $P < 0.0001$ ) and clades I and III ( $F_{22,66} = 23.67$ ,  $P < 0.0001$ ), but not between clades II and III ( $F_{22,66} = 1.61$ ,  $P = 0.071$ ).

Overall, clades II and III were much more similar in shape to each other than either was to clade I (Fig. 3). Morphological data nevertheless revealed significant differences among the three *Telmatherina* lineages in structures associated with the acquisition and processing of food.

The first two relative warps derived from the analysis of skull shapes accounted for 52.64% of the variation in skulls among the three clades. Differences were attributable to the relative position and size of the eye, the ethmoid and the lower limb of the preopercle (Fig. 4, skull). The smaller and more elevated eye, the alignment of the ethmoid with the premaxilla insertion, and the lengthened lower preopercle limb with its rostral tip dorsally rotated, in clades II and III relative to clade I, are features allowing the lateral expansion of the buccal cavity in these fish (Liem 1974; Barel *et al.* 1976; Dyer & Chernoff 1996; Galis & Drucker 1996; Grubich 2001). The large lower preopercle limb also allows attachment of larger muscles which can produce powerful negative pressure required to dislodge substrate-associated prey in a vacuum-like manner (Galis & Drucker 1996; Dyer & Chernoff 1996; Grubich 2001). These features, more pronounced in clade III relative to II (Fig. 4, skull), indicate a greater ability for suction-based feeding. These same skull features dramatically differ in clade I where the eye is larger and more sunken into the head. The ethmoid is anteriorly extended and the lower limb of the preopercle is smaller, pushed more anteriorly with its rostral tip ventrally rotated (Fig. 4, skull). These characterize an elongated, cylindrical buccal cavity in clade I relative to the other clades. The larger eyes and a tube-like buccal cavity are consistent with tracking and ram feeding on smaller, more pelagic prey (Galis & Drucker 1996; Grubich 2001).

The relative warps of the lower jaws accounted for 68.03% of the variation in this structure among the three clades. The presence of large bony processes or 'hinges' on the dorso-caudal end of the lower jaw (Fig. 4, lower jaw) in *Telmatherina* likely allows the strong occlusion between

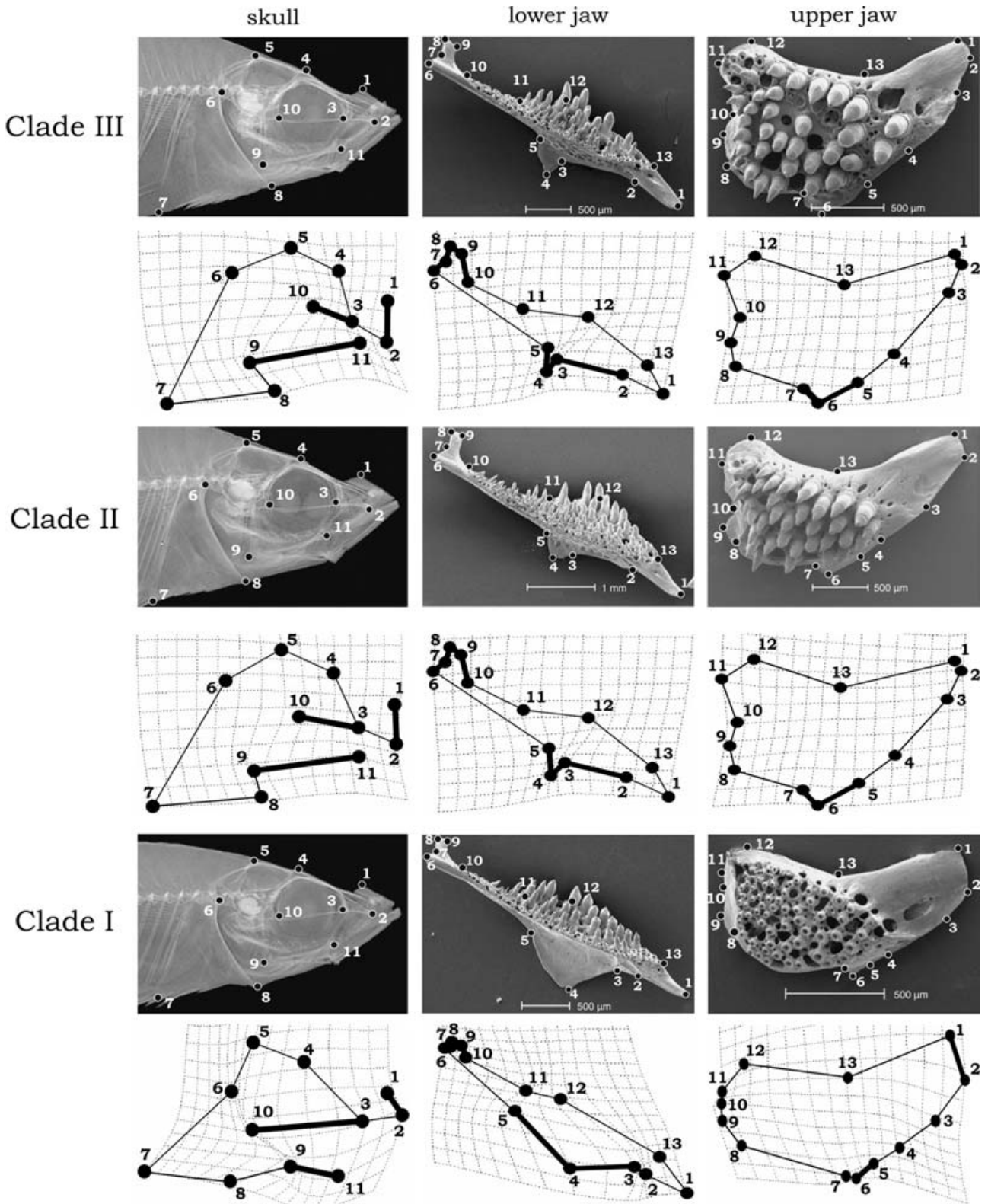
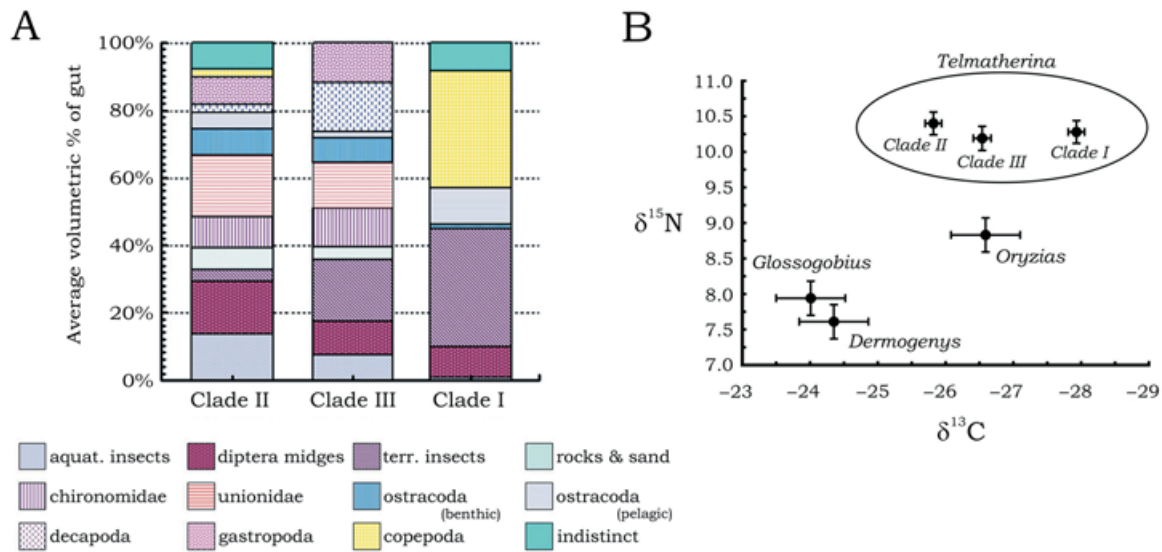


Fig. 4 Images and deformation grids outlining biologically meaningful shape differences in structures analysed using geometric morphometrics among the three *Telmatherina* clades from Lake Matano. Deformation grids show shape change using the first two relative warps which account for 52.6%, 68.0% and 60.1% of the variation in skull, lower jaw, and 3rd pharyngobranchial, respectively. Salient divergent features in structures outlined in bold. Skull: 3–10, eye diameter and position; 1–2, premaxilla tip to rostral tip of ethmoid; and 9–11, size and position of preopercle lower limb. Lower jaw: 6–10, hinge; 3–5, keel. Upper jaw: 1–3, frayed zone; 5–7, ventral medial process.





**Fig. 5** *Telmatherina* diet analyses. (A) Mean volumetric percentage of various items in the gut of three *Telmatherina* clades in Lake Matano, Sulawesi, Indonesia ( $n = 32$ ). Clade I has a significantly different diet from both clade II and III (ANOSIM;  $R = 0.504$ ,  $P < 0.001$  and  $R = 0.444$ ,  $P < 0.001$ , respectively), consisting of pelagic and surface prey such as copepods and terrestrial insects. Although clades II and III have many food items of a littoral nature in common, they nevertheless also have significantly different diets (ANOSIM;  $R = 0.110$ ,  $P < 0.001$ ). (B) Average carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotope signature of the endemic fish from Lake Matano.

upper and lower pharyngeal teeth (Liem 1974; Barel *et al.* 1976; Galis & Drucker 1996; Grubich 2003). The exaggerated hinge observed in both clades II and III (Fig. 4, lower jaw) suggests that these clades feed on hard shelled organisms where crushing is critical to process prey as has been shown in cichlids, centrarchids and in general perciforms (Liem 1974; Barel *et al.* 1976; Galis & Drucker 1996; Grubich 2003). The relatively reduced hinge observed in clade I results in a substantial decrease in biting power. The extended keel of the lower jaw in clade I, however, provides an anchor point for larger muscles (Liem 1974; Barel *et al.* 1976; Galis & Drucker 1996; Grubich 2003) connecting it to the cleithrum, the hyoid and the 4th ceratobranchial (Fig. 4, lower jaw). Larger muscles can move the lower jaw more efficiently (Liem 1974; Galis & Drucker 1996) making it a more effective masticating apparatus for clade I, a feature consistent with pelagic feeding (Liem 1974; Barel *et al.* 1976; Galis & Drucker 1996; Grubich 2001, 2003).

The relative warps outlining shape differences in the upper jaws accounted for 60.12% of the variation in this structure among *Telmatherina* clades. The frayed zone on this bone connects it to the 1st and 2nd pharyngobranchials (Barel *et al.* 1976). In clades II and III this region is smaller and anteriorly narrowed to a point, whereas in clade I it is broadened (Fig. 4, upper jaw). The larger and broadened frayed zone in clade I likely attaches larger 1st and 2nd pharyngobranchials providing larger chewing surfaces for softer, more malleable prey (Barel *et al.* 1976; Galis & Drucker 1996). Both clades II and III are also characterized

by a ventral medial process, virtually absent in clade I (Fig. 4, upper jaw). This enlarged process permits larger muscles to attach to the upper jaw providing greater pressure during jaw occlusion (Liem 1974; Barel *et al.* 1976; Galis & Drucker 1996) allowing the crushing and breaking of harder prey. Thus, GM recovers significant morphological differentiation within each *Telmatherina* genetic clades. If these morphological differences initiated adaptive radiation, then we expect predictably different feeding strategies among these three clades (Genner *et al.* 1999; Schluter 2000; Doebeli & Dieckmann 2003; Grubich 2003).

#### Diet analysis

The three clades differed significantly in their diets (Fig. 5A); clade I consumed mostly soft pelagic items and terrestrial insects, consistent with the types of prey predicted from the osteological data (Fig. 5A), while clades II and III fed mostly on littoral prey with items also matching predicted diets (Fig. 4A). Despite some overlap, the diets of clades II and III also significantly differed, primarily due to a substantial proportion of terrestrial insects in the clade III diet (Fig. 5A). This is consistent with skull differences between clade II and III, where greater suction in clade III would allow the capture of terrestrial insects from the surface. Terrestrial insects constituted 3%, 16% and 34% of the clade II, clade III, clade I diets, respectively, suggesting that clade III has a generalist feeding strategy combining items from both clades I and II diets.

### Stable isotopes

Diet analysis offers only a 'snapshot' of recent feeding activity (France 1995; Genner *et al.* 1999; Post 2002; Paterson *et al.* 2006). The stable isotopes of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ), however, provide time integrated measures of relative trophic position and energy sources of specific consumers within food-webs (France 1995; Genner *et al.* 1999; Post 2002; Paterson *et al.* 2006). In aquatic systems,  $\delta^{15}\text{N}$  increases with trophic position while  $\delta^{13}\text{C}$  tends to become less negative as a consumer relies less on pelagic and more on littoral-benthic food sources (France 1995; Post 2002; Paterson *et al.* 2006). The  $\delta^{15}\text{N}$  signatures of all *Telmatherina* clades were significantly enriched relative to other fish genera in Lake Matano ( $F_{3,24} = 24.82, P < 0.0001$ ), but not relative to each other ( $F_{2,104} = 0.674, P = 0.512$ ; Fig. 5B).  $\delta^{13}\text{C}$  signatures, however, were all significantly different among clades ( $F_{2,104} = 45.82, P < 0.0001$ ) (Fig. 5B). These data suggest that although all *Telmatherina* were caught at the same beach sites, clade I and II partition these sites. Clade I feeds on pelagic items while clade II feeds on littoral items, thus representing pelagic and littoral extremes of *Telmatherina* energy sources, respectively. The intermediate  $\delta^{13}\text{C}$  signature of clade III further supports its generalist feeding strategy resulting from an integration of both extremes of prey isotopic signatures (France 1995; Post 2002; Paterson *et al.* 2006). These differences are consistent and comparable with other studies using  $\delta^{13}\text{C}$  values demonstrating littoral and pelagic resource partitioning among centrarchid life-history stages in temperate lakes (Paterson *et al.* 2006) and cichlid fishes of East Africa (France 1995; Genner *et al.* 2003). The development of resource specialists acquiring narrower feeding niches in resource-limited environments is also consistent with studies of European whitefish wherein systems harbouring two morphs often develop resource specialists vs. those with a single generalist morph (Amundsen *et al.* 2004). In this study, however, all three morphs (or types; pelagic and littoral specialists and a generalist) occur in the same system and at the same sites. Nevertheless, both diet and stable isotope data confirm clear partitioning of resources within the *Telmatherina* trophic position.

### Divergence scenarios

Determining which morphological features are associated with divergence among closely related taxa can identify putative traits on which selection and drift have acted to allow adaptation to environmental fitness peaks leading to speciation (Coyne & Orr 2004). In this study, we demonstrate that the *Telmatherina* sampled from Lake Matano can be categorized into three distinct genetic lineages and that these lineages exhibit diet differences associated with their osteological differences. These data provide compelling

evidence that the divergence within the *Telmatherina* genus in Lake Matano was initiated by resource partitioning in this trophically limited environment. These results are consistent with other studies describing resource-based divergence and speciation in fish from different systems including Arctic charr in Icelandic lakes (Skúlason *et al.* 1993), sticklebacks in North temperate coastal lakes (Schluter 1994), coregonids in both Canadian and Norwegian lakes (Amundsen *et al.* 2004; Bernatchez 2004; Østbye *et al.* 2005), cichlids in Lake Malawi (Albertson *et al.* 2005), and neotropical cichlids in Nicaragua (Barluenga *et al.* 2006). A striking departure of this divergence from that described in the other examples, however, is the production of three distinct lineages where the magnitudes of the genetic and morphological divergences are incongruent. Genetically, clade III is the most divergent from both clades I and II (Fig. 2), yet morphologically, clades II and III are much more similar and share more food resources (Figs 3, 4 & 5).

The disparity between genetic and morphological divergence can be attributed to three possible scenarios. First, while individuals from all clades were caught using similar methods at the sampling sites (Fig. 1), the three clades may partition these sites and maintain site fidelity on a much finer scale than what was sampled, suggestive of microallopatric divergence. The large genetic divergence between clades I and II, as well as between clades I and III, may be related to their adaptations to more pelagic and more littoral areas of a site, respectively. The divergence between clades II and III (both more littoral types) may be related to even more extreme philopatric behaviours resulting in micro-allopatric divergence occurring over the scale of just a few metres. Such highly developed philopatric behaviour driving species divergence has been demonstrated in other systems (Case & Taper 2000; Rico & Turner 2002). This scenario seems unlikely in the current study, however, given the morphological similarities between clades II and III. Morphologically similar sibling species relegated to small proximate habitats do not generally maintain strict assortative mating, especially when they share common resources (Schluter 2000; Doebeli & Dieckmann 2003; Rundle & Nosil 2005). Yet, if assortative mating within these clades is linked to other factors such as female preference for male coloration (i.e. by sexual selection), then such a condition may evolve as has been described in the African Lakes cichlids (Seehausen *et al.* 1997; Kocher 2004; Salzburger *et al.* 2006). Such a scenario would be consistent with the theory that many factors contribute synergistically to the process of adaptive radiation, and that such interplaying factors have varied degrees of influence over time (Schluter 2000; Kocher 2004). Coloration-pattern associations within and among the three *Telmatherina* lineages have not been tested and warrant further investigation, but is beyond the scope of the present study.

A second scenario involves incomplete lineage sampling and the potential for hybridization among described lineages and possible unidentified types of *Telmatherina*. Variable susceptibility to our capture techniques or habitats other than beach sites may have excluded certain lineages from this analysis. Clades I and II are genetically distinct (Fig. 2A) and exhibit morphological features (Fig. 4) consistent with pelagic and littoral existences, respectively (Fig. 5). Clade III is also genetically distinct but its morphological features, though different, more closely resemble those of clade II (Figs 3 and 4) with which it shares many resources (Fig. 5A). If another lineage not included in this study (for example a riverine or extreme shallow form) hybridizes with the littoral form (clade II), then the hybrid may exhibit morphological features similar to, and share many resources with, the littoral form, but could be very different from the littoral and the pelagic clades in its maternal genetic signal (lineage). Such a scenario cannot be confirmed or refuted with the data presented here, but warrants further investigation.

A third explanation for the genetic-morphological disparity, consistent with a recent model of adaptive radiation (Streelman & Danley 2003; Kocher 2004), is that the generalist lineage (clade III) is the most recent common ancestor (Fig. 2) of the newly derived specialist lineages (clades I and II) and that the divergence among *Telmatherina* is ongoing (Roy *et al.* 2004). This scenario supports the hypothesis that adaptive radiations initiated by resource limitation typically give rise to specialists from more generalist ancestors (MacArthur & Pianka 1966). Although this has been demonstrated in simulations and in manipulated conditions (Cooper & Lenski 2000; Chow *et al.* 2004), few clear examples have been documented in natural systems. Schluter *et al.* (1997) reviewed notable cases of adaptive radiation testing their statistical likelihood of either a generalist or specialist origin. Schluter *et al.* (1997) and subsequent investigations were inconclusive as to the general applicability of the generalist-to-specialist trend (Pagel 1999; Nosil & Mooers 2005). However, many investigations do show a propensity toward generalist ancestry when such radiations were based on resource limitation (e.g. food and prey types; see Schluter *et al.* 1997; Schluter 2000, pp. 44–45). In the present case, the littoral specialist (clade II) shows slight yet distinct modifications in specific features consistent with sharing many resources with the generalist. These features (e.g. eye size or pharyngeal keel size) are the very same that are most divergent in clade I (Fig. 4), allowing it to exploit completely different resources (pelagic specialist). Consistent differences among the same features in the three distinct genetic lineages support the theory suggesting that morphological divergence is accomplished by simple modifications of pre-existing forms (modification by descent; Darwin 1859; Goodwin 2001; Lenski *et al.* 2003).

The relatively limited number of *Telmatherina* 'species' in Lake Matano may be regarded as the product of both genetic and/or environmental constraints that may prevent the explosive divergence of this genus in this system (Streelman & Danley 2003; Kassen *et al.* 2004). It must be borne in mind, however, that the lineages described in this study reflect ancient divergences and that these lineages may or may not continue to segregate. The morphologically distinct characters within clades, however, suggests that segregation among them continues as otherwise clade-specific morphology would have quickly eroded through hybridization (Coyne & Orr 2004, pp. 36–37). Moreover, and as outlined in the 'radiation in stages' model (Danley & Kocher 2001; Streelman & Danley 2003; Kocher 2004), more recent divergence within *Telmatherina* clades cannot be ruled out and may be occurring based on communicatory features, such as coloration (Seehausen *et al.* 1997; Salzburger *et al.* 2006). This is especially likely considering the highly variable coloration patterns described in these fish (Kottelat 1991; Roy *et al.* 2004). Recent divergence (< 20 000 years) is beyond the resolution of our genetic analysis, and thus we may have underestimated the number of *Telmatherina* species present. Therefore, arguments pointing to the apparent constrained divergence of *Telmatherina* in Lake Matano ought to consider that more recently developed reproductive isolation among coloration groups within each clade may increase *Telmatherina* taxa number.

Overall, this study provides genetic, morphological, diet and trophic data that are consistent with the ecological seeding of adaptive radiation among the *Telmatherina* in Lake Matano and provides an independent data set, separate from more noted systems (Galapagos finches, African cichlids, Hawaiian *Drosophila*), demonstrating the ecological basis of this process. Clearly, ecological pressures such as resource limitation are potent initiators of adaptive radiations in natural systems.

### Acknowledgements

We thank D. Morris, D. Schluter, C. Mora, M. McKinney, J. Leney, K. Østbye and three anonymous reviewers for discussions and critiques on improving this manuscript. D. Tase, P. Hehanussa, P. Sampetoding, A. Bramburger and B. Roy were instrumental in providing field assistance and logistic support during data collection. This work was funded by the Natural Science and Engineering Research Council of Canada, by INCO Ltd. and Pt. INCO Tbk.

### Supplementary material

The supplementary material is available from <http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC3106/MEC3106sm.htm>

**Table S1** Results of an analysis of molecular variance (Excoffier *et al.* 1992) performed among the three *Telmatherina* clades collected

from various locations in Lake Matano, Sulawesi Island, Indonesia. Analysis performed using 490 bp of the 16S and 600 bp of the cytochrome *b* mtDNA partitions and calculated pairwise differences using the K81uf + G + I ( $\gamma = 0.6715$ ,  $I = 0.5517$ ) base pair substitution model, and algorithm permuted 1023 times implemented in ARLEQUIN version 3.01 (Excoffier 2005). *P* values estimated from permutations provided as footnote.

**Table S2** Pairwise  $F_{ST}$  values and associated *P* values (in brackets) determined among the three *Telmatherina* clades sampled from Lake Matano, Sulawesi Island, Indonesia. *P* values generated from 1023 permutations of AMOVA algorithm performed in ARLEQUIN version 3.01 (Excoffier 2005). Bold entries indicate significant genetic differentiation among compared clades with  $\alpha$  values corrected to 0.01667 for multiple comparisons.

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D. Roy completed his PhD at the Great Lakes Institute for Environmental Research (GLIER) in 2006 and is interested in understanding ecological factors acting during species evolution. This work is part of a collaboration among GLIER, PT INCO Tbk, and The Science Institute of Indonesia to emphasize the unique patterns and processes responsible for the evolution of the endemic fauna and flora of the Malili Lakes and conserve it for the future. G. Paterson's research uses contaminants as trophic markers in aquatic systems. P. Hamilton is curator of aquatic invertebrates at the Canada Museum of Nature. D. Heath studies the evolutionary ecology of various fishes and D. Haffner is interested in the conservation of biodiversity in ancient lakes.

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