

Gene flow at the margin of Lake Matano's adaptive sailfin silverside radiation: *Telmatherinidae* of River Petea in Sulawesi

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Abstract Classical speciation concepts focus almost exclusively on the evolution of strict reproductive isolation as a prerequisite for speciation. However, there is a growing body of evidence indicating that speciation is possible despite or even triggered by gene flow among populations or species. Previous findings indicate that introgressive hybridization is a dominant phenomenon in the adaptive radiation of sailfin silversides (*Telmatherinidae*) endemic to Lake Matano (Sulawesi). In this study, we investigate patterns of genotypic and phenotypic variation of “sharpfin” sailfin silversides in the outlet area of L. Matano and six locations along River Petea, which is the only connection between L.

Matano and other lakes and streams of the Malili Lakes system. Fieldwork revealed no hints for a previously cited major waterfall in River Petea, which was thought to separate L. Matano's sailfin silverside radiation from the diversity of the downstream lake drainages. Likewise, genomic (AFLP) and morphometric data suggest high levels of gene flow between upper and lower stretches of this river, as well as between riverine Petea and lacustrine Matano populations. Increasing levels of genotypic and phenotypic dissimilarity are correlated with distance over a remarkably short geographic range.

Keywords Malili Lakes · *Telmatherinidae* · Introgression · Clinal differentiation · Isolation by distance · AFLP · Geometric morphometrics

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Introduction

Allopatric speciation through the complete cessation of gene flow in geographically fully separated areas has been the dominant view of speciation over the past six decades (Turelli et al., 2001). Recently, concepts of sympatric or parapatric speciation in the presence of gene flow have gained more consideration (Coyne & Orr, 2004). This discussion is accompanied by the idea that introgression and gene flow among recently diverged populations could affect single genes or genomic regions rather than

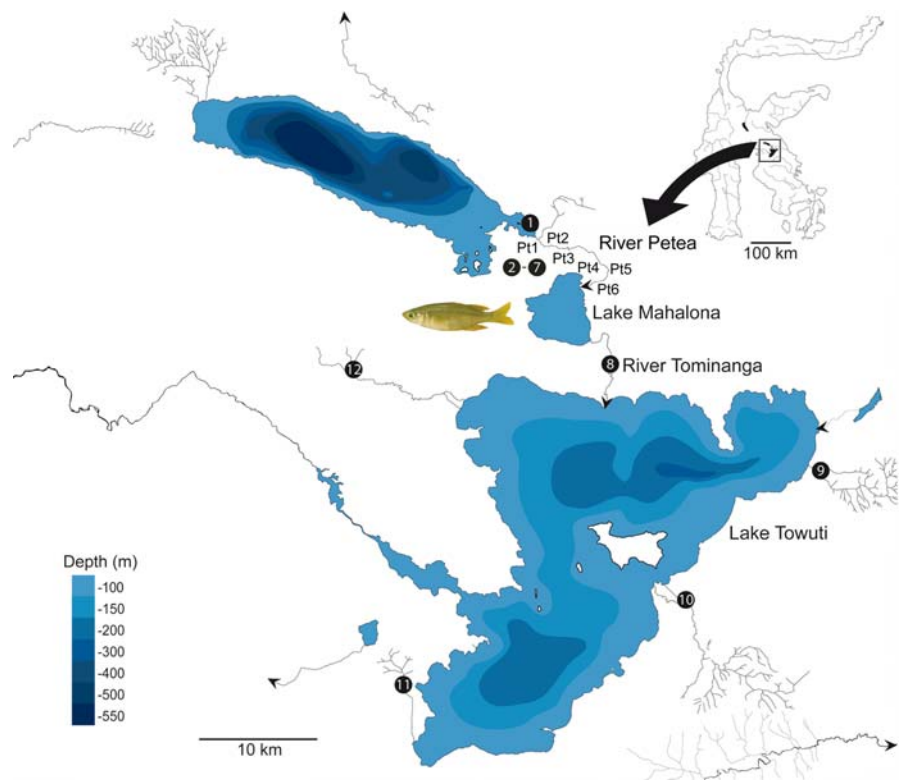
the whole genome (Templeton, 1981; Feder, 1998). This, in turn, suggests that adaptive speciation might be restricted to those traits directly affected by distinct selective regimes, without the prerequisite of strict reproductive isolation (Wu, 2001; Seehausen, 2004; Mallet, 2007). The idea of “porous genomes” (Shaw & Danley, 2003) meets mounting support from field studies suggesting that speciation has proceeded despite ongoing gene flow or even triggered by reticulate processes (e.g. Shaw et al., 2000; Salzburger et al., 2002; Schliewen & Klee, 2004; Nolte & Sheets, 2005; Schelly et al., 2006).

Introgressive hybridization has been demonstrated to be a major phenomenon in the species flock of sailfin silversides (Teleostei: Atheriniformes: Telmatherinidae) endemic to the ancient (Brooks, 1950) Malili Lakes in Central Sulawesi, Indonesia (Herder et al., 2006a, Fig. 1). The three major lakes of the system, Lakes Matano, Mahalona and Towuti, are deep tropical lakes of tectonic origin (Abendanon, 1915; Haffner et al., 2001). They form a cascade interconnected by rivers (Fig. 1), inhabited by endemic radiations of various freshwater organisms, including gastropods (von Rintelen et al., 2004; von Rintelen &

Glaubrecht, 2005), bivalves (von Rintelen & Glaubrecht, 2006), crustaceans (Fernandez-Leborans et al., 2006; Zitzler & Cai, 2006) and fish (Kottelat, 1990a, b, 1991; Whitten et al., 2002; Roy et al., 2004; Gray & McKinnon, 2006; Herder et al., 2006a, b).

The lacustrine ichthyofauna of the Malili Lakes is characterized by local endemism either to L. Matano (Ahmad, 1977; Haffner et al., 2001) or to Lakes Mahalona/Towuti (Kottelat, 1990a, b, 1991; Herder et al., 2006b). L. Matano, the uppermost and with approx. 590 m the deepest lake of this drainage (Ahmad, 1977; Haffner et al., 2001), harbours an endemic adaptive radiation of Telmatherinidae (Herder et al., 2006a, in press). According to nuclear markers and consistent to morphological concepts (Kottelat, 1991; Herder et al., 2006b), two major phylogenetic clades are well supported within the L. Matano radiation, “roundfins” and “sharpfins” (Herder et al., 2006a). In contrast, analyses of mitochondrial markers reveal three likewise well-supported clades, dividing the clade of “sharpfins” into two (Roy et al., 2004, 2007a, b; Herder et al., 2006a). Interestingly, inclusion of stream populations inhabiting most permanently flowing waters of the

Fig. 1 The Malili Lakes system with sample sites included in this study. L. Matano is a very deep (590 m), graben lake, drained by the steep R. Petea to L. Mahalona, from where the flow continues to L. Towuti, which drained to the sea. Sampling locations are indicated as dots, with numbers referring to the following location names: 1, L. Matano Outlet; 2–7, R. Petea sample sites Pt1 to Pt6; 8, R. Tominanga; 9–12, L. Towuti streams. Map by T. & K. von Rintelen, modified with permission



Malili Lakes system (Herder et al., 2006a) revealed that parts of the stream individuals share the lacustrine “sharpfin” haplotype, strongly suggesting introgressive hybridization from stream to “sharpfin” populations (Herder et al., 2006a). R. Petea is the only connection of L. Matano with the remaining lakes of the Malili Lakes system. Its potential as a barrier for the dispersal of freshwater organisms has been the object of discussions and speculations in the recent past (Roy et al., 2004, 2006, 2007a, b; Herder et al., 2006a, b). This short river drops 72 m of elevation along its only approx. 9 km length, initiating speculations about a major waterfall (Roy et al., 2004). Conspicuously, stream-inhabiting individuals from R. Petea are the only known river-dwelling “sharpfins”, sharing a clade with lacustrine “sharpfins” in the nuclear multilocus dataset (see Herder et al., 2006a).

In the present study, we focus on sailfin silversides inhabiting R. Petea. We use individual-based population assignment tests based on Amplified Fragment Length Polymorphism (AFLP) data (Duchesne & Bernatchez, 2002; Campbell et al., 2003) to test for potential gene flow between lacustrine and river-dwelling “sharpfins”, based on individuals inhabiting different sections of the river itself and a representative “sharpfin” sample of the lake’s outlet area. We hypothesize that genetic contact between river and lake populations is a strong or even ongoing phenomenon, which should be detectable within a multilocus nuclear dataset. For this purpose, we collected individuals on a fine scale, based on six sampling locations along R. Petea and the river’s transition area to L. Matano. Consequently, we estimate the level of genetic and morphometric differentiation between lacustrine and riverine “sharpfins” and test for isolation by distance.

Materials and methods

Study site and sampling

Samples were collected between February and April 2004 at the Malili Lakes system in Central Sulawesi (Indonesia) at six sampling sites along R. Petea, named downstream Pt1 to Pt6, with 19 to 23 individuals taken from each site (total $N = 123$, Fig. 2). In addition, 450 “sharpfin” specimens were collected at the transition area of L. Matano and

R. Petea, from which a pooled sample of 133 specimens was randomly taken (“L. Matano Outlet”). The random sampling approach was applied because phenotypic variation present in the outlet area did not allow unequivocal assignment of all individuals to “sharpfins” known from L. Matano (see Kottelat, 1990a, b, 1991; Herder et al., 2006b). Finally, riverine specimens from rivers Beau, Lemolemo, Wawondula and Tominanga as well as from the southern part of L. Towuti ($N = 16$, see Fig. 1) were included for a genetic comparison as these populations partly share mtDNA-haplotypes with “sharpfins” of R. Petea and L. Matano (rivers Wawondula and Tominanga; Herder et al., 2006a). In contrast, lacustrine species from L. Mahalona and L. Towuti were not included, because previous phylogenetic analyses (Herder et al., 2006a) as well as high F_{ST} values (between 0.2 and 0.3, JS unpublished data) suggest that they are genetically highly distinct from R. Petea and L. Matano “sharpfins”. In total, 272 specimens were used for AFLP analysis and 103 specimens were used for morphometric analyses (101 for landmarks, 103 for head width parameters).

Genetic analysis

DNA extraction was performed using the DNeasy Tissue Kit (Qiagen) according to the manufacturer’s protocol. Genomic DNA was extracted from the right pectoral fin, including muscle tissue. AFLP analysis was carried out following the slightly modified protocol of the AFLP method (Vos et al., 1995), described in Schlieven & Klee (2004). Restriction and ligation were carried out in a single step in a thermocycler (2 h at 37°C, 8 h at 16°C). Genomic DNA was digested with restriction enzymes *MseI* (1 unit) and *EcoRI* (5 units). Polymerase chain reaction (PCR) adaptors specific to the cutting sites were ligated using DNA-Ligase (1 unit; all enzymes from New England Biolabs). Preselective PCR was performed with one selective base on each primer (*MseI*-C and *EcoRI*-A). In selective amplifications, two additional bases were added to each 3’ end of six primer pairs (*MseI/EcoRI*^{DYE}): AA-CT^{FAM}, AA-GG^{JOE}, AT-CT^{FAM}, AT-GG^{JOE}, TA-CT^{FAM} and TA-CT^{JOE}. DNA quality and concentration was checked before restriction-ligation, PCR product quality after preselective amplification. The fragments

were separated with 6% LongRanger polyacrylamid gels (FMC Bioproducts) on an ABI PRISM™ 377 automated sequencer, adding internal size standard to each lane (GS-500 ROX; Applied Biosystems). Signal detection was carried out by GeneScan version 3.1 software (Applied Biosystems).

Fragment analysis and subsequent quality check of the AFLP profiles were performed as specified in Herder et al. (2006a); fragment categories were created according to fragment distribution. Scoring for the presence/absence of the peaks was conducted between 100 and 499 bases with threshold set to 100 normalized units. Bin (category) width set to 0.6 bases reduces repeated scoring of the same fragment. Using spreadsheet routines bins differing by less than 0.35 bases were excluded, corresponding to the double standard deviation of the sequencer (Lazaruk et al., 1998). Repeated analysis of four samples was used to exclude bins without full reproducibility (<5%), resulting in the recognition of 446 polymorphic AFLP loci. AFLP data were analysed using AMOVA (Analysis of Molecular Variance) implemented in the Arlequin software package version 2.0 (Schneider et al., 2000). This procedure partitions the genotypic variance, resulting in a F_{ST} that refers to the correlation of genotypes rather than to the correlation of genes as in the classical F_{ST} (Excoffier et al., 1992). Assuming that the mating pattern is identical in all studied populations, based on prior knowledge that *Telmatherina* show a very conserved mating behaviour (Gray & McKinnon, 2006), this measure is informative with respect to the differentiation between populations (Schneider et al., 2000). Pairwise F_{ST} values for each combination of populations were determined and the significance of F_{ST} values was calculated from 1,000 permutations using the non-parametric permutation approach of Excoffier et al. (1992). Afterwards P -values were Bonferroni corrected based on the number of pairwise comparisons. Simple Mantel tests for “isolation by distance” (Wright, 1943, 1946) were performed to test for correlations between distance matrices. The software zt (Bonnet & Van de Peer, 2002) was used to test for pairwise relationships between the natural log of geographic distance (km) and genetic differentiation ($F_{ST}/1 - F_{ST}$). Shoreline distances were measured on a detailed geographical map (1:50,000, Lembar 2113-34 und 2213-13, 1:50,000, Peta Rupabumi Indonesia), verified by own ground checks.

These units for distance and genetic differentiation were chosen as recommended in Rousset (1997) for a two-dimensional system. Each Mantel test was based on 10^7 permutations.

AFLP-based assignment tests

The probability of a composite AFLP genotype belonging to either the population (=sampling site) of its origin or one of the other populations was calculated for each individual from R. Petea, L. Matano Outlet and L. Towuti streams according to Paetkau et al. (1995), adjusted for dominant markers as implemented in AFLPOP version 1.0 (Duchesne & Bernatchez, 2002). Specimens were reassigned on the basis of log-likelihood values, which were computed for each population. By this means, differences in reassignment probability, represented through log-likelihood values, could be visualized on an individual level for each population. Differences between these values provided an estimate of the power of the assignments. Low log-likelihood differences (LD) may represent inaccurate assignments, as individuals could not be clearly allocated to one population. In this study, individuals were reassigned to a given population on the basis of the highest likelihood of occurrence (Potvin & Bernatchez, 2001). No threshold for reassignment was chosen, based on a series of simulations run with different values for the minimum likelihood difference for assignment (MLD, Duchesne & Bernatchez, 2002), resulting in $MLD = 0$ as optimal value. The log-likelihood differences and the proportion of reassignment were used as an estimator for genetic identity of sampled populations.

Assuming that only non-introgressed genotypes can be clearly assigned (meaning with a high log-likelihood difference to the second likely population) to their original population whereas mixed genotypes are not clearly assignable in this approach (Manel et al., 2005) as they represent a composite of at least two populations (Arnold, 1997), the minimum likelihood difference for AFLPOP assignment (MLD) was increased from $z = 0$ to $z = 1$ in a second assignment run. Thereby, only specimens with genotypes 10 times more likely allocated to one population than to the second likely one were assigned (Duchesne & Bernatchez, 2002). We use this approach as simple estimate of the amount of “non-introgressed”

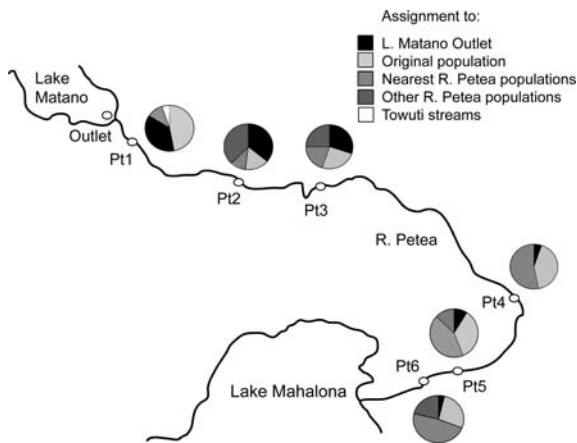


Fig. 2 Clinal differentiation in genetic traits along R. Petea “sharpfins”. Riverine specimens collected closer to L. Matano (Pt1 ($N = 19$), Pt2 ($N = 19$) to Pt3 ($N = 20$)), were more similar to lacustrine specimens than those in greater distance to L. Mahalona (Pt4 ($N = 19$), Pt5 ($N = 23$) to Pt6 ($N = 23$)). The pie charts above R. Petea sample locations represent the assignment patterns of each local population

genotypes in different sections of R. Petea and in L. Matano Outlet.

Anticipating low local reassignment to each sample site, and to evaluate more general patterns, populations Pt1, Pt2 and Pt3 were in a following step merged into “upper R. Petea” and Pt4, Pt5 and Pt6 into “lower R. Petea” (see also Fig. 2). By this means, each specimen was assigned either to “Lower R. Petea”, “Upper R. Petea”, “L. Matano Outlet”, “Towuti streams” or to “None”. We used the proportion of clearly assigned specimens per population as an estimator for the proportion of non-introgressed lacustrine or riverine specimens in our dataset.

Morphometric analyses

To characterize potential differences in body shape, lacustrine specimens from L. Matano Outlet and four riverine R. Petea populations (Pt2, Pt3, Pt5 and Pt6) were morphometrically analysed. As head width has previously been used as taxonomical character to distinguish stream-dwelling sailfin silversides (Kottelat, 1991), this parameter was included as an addition to the two-dimensional shape analyses. Because of a strong sexual dimorphism present in telmatherinids (Kottelat, 1990a, 1991; Herder et al., 2006b) only adult male specimens were used in the

morphometric approach. However, this resulted in the exclusion of sample sites Pt1 and Pt4, as few adult males were available from these sites. Ultimately, the following samples were included in the analysis: L. Matano Outlet ($N_{\text{Landmarks}} = 73$, $N_{\text{Head}} = 74$), R. Petea populations Pt2 + Pt3 ($N_{\text{Head}} = 20$), Pt2 ($N_{\text{Landmarks}} = 4$), Pt3 ($N_{\text{Landmarks}} = 15$) and Pt5 + Pt6 ($N_{\text{Landmarks/Head}} = 9$).

Pre-analyses of the trait “interorbital width” revealed no significant differences between Pt2 and Pt3 (Mann–Whitney U test, $N_1 = 16$, $N_2 = 4$, $Z = -1.134$, $P = 0.257$) and between Pt5 and Pt6 (Mann–Whitney U test, $N_1 = 6$, $N_2 = 4$, $Z = -0.640$, $P = 0.522$). On the basis of geographical proximity and small sample size, specimens from Pt5 and Pt6 and Pt2 and Pt3 were combined in the classical morphometric analysis. Goodall’s F test based on 2,500 bootstrap replicates also revealed no significant difference in body shape between the above-mentioned groups (2,500 repeats, Pt5/6: $F = 0.97$, $P = 0.43$; Pt2/3: $F = 1.91$, $P = 0.06$). However, due to the marginally significant p-value between specimens from Pt2 and Pt3, we chose a conservative approach and treated them separately in the geometric morphometric approach.

A landmark-based geometric morphometric (GM) approach (Rohlf & Marcus, 1993) based on the x , y coordinates of 20 homologous landmarks was used to quantify differences in body shape between lacustrine and riverine populations (Fig. 3). Data acquisition was conducted using the software packages Tpsutil and Tpsdig (Rohlf, 2004a, b). All subsequent morphometric analyses were employed using the IMP software package (Sheets, 2002). To remove non-shape variation, a Generalized Procrustes Analysis (Rohlf, 1990; Rohlf & Slice, 1990) was performed which translates all specimens to a

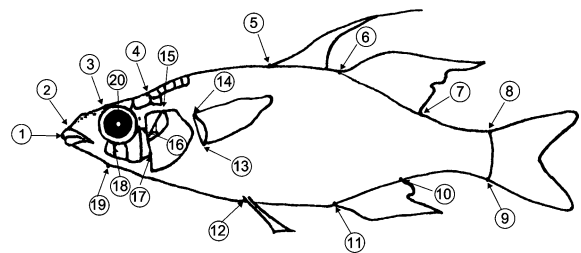


Fig. 3 Position of the landmarks used in the geometric morphometric approach. Twenty landmarks were chosen to analyse variability in body shape

Table 1 Characterization of all calliper-based measurements to determine the head and body width

Measurement	Description
Interorbital width	Distance of the dorsal between bony margin on the left and right orbit
Body width	Distance between the upper margins of pectoral fins
Head width	Distance between the dorsal end of opercular openings
Snout width	Distance from the right to the left corner of the mouth
Standard length	Distance from upper jaw symphysis to caudal margin of hypuralia

The measured sections were chosen following Kottelat (1990a). All measurements are highly correlated and reflect the same differentiation pattern between the tested populations

common location, scales them to a unit size, and rotates them until corresponding landmarks line up as closely as possible. After superimposition, the data were translated into Partial Warps using the thin-plate spline model (Bookstein, 1989). These linearly transformed variables are suitable for use with standard multivariate statistical methods, since they carry the same number of variables as degrees of freedom (Zelditch et al., 2004). PCA reduction was conducted to scale down the number of variables in the dataset using the program CVA-Gen60. Consequently, instead of using landmark coordinates or partial warp scores in the subsequent analysis, PCA axis scores of the specimens were used (Sheets, 2002). A Canonical Variates Analysis (CVA) based on the reduced dataset (consisting of eight PC Axes) was accomplished. The CVA was used to describe the potential morphological discrimination along predefined groups, displaying the greatest variance between groups relative to the variance within groups.

As a two-dimensional landmark approach was conducted, we additionally took five calliper-based measurements characterizing head and body width of each specimen (interorbital width, head width, body width, snout width and standard length, Table 1) using a digital calliper to the nearest of 0.1 mm. As the values for all measurement sections were highly correlated (Pearson correlation, $P < 0.001$, coefficient of correlation: all $r > 0.702$), only the results for interorbital width are presented. However, pre-analyses revealed that all measured sections reflect the same result. Residuals were calculated by regression analysis with “standard length” as the independent and “interorbital width” as the dependent variable. Differences in residuals between riverine and lacustrine groups were analysed using one-way ANOVA and Post hoc tests.

Results

Genetic differentiation

F_{ST} values ranged from 0.01 to 0.09 between L. Matano’s and R. Petea’s “sharpfins” and from 0 to 0.08 between individual R. Petea populations (Table 2). The riverine populations from streams near L. Towuti were differentiated from R. Petea and L. Matano “sharpfins” with F_{ST} values between 0.14 and 0.21, showing the highest differentiation from lacustrine sharpfins (Table 2). The Mantel test revealed significant correlation between geographic and genetic distances of populations from L. Matano Outlet and R. Petea sample sites ($N = 7$, $r = 0.55$, $P = 0.02$), indicating that genetic differentiation of the riverine Petea “sharpfin” populations to L. Matano Outlet “sharpfins” increases with higher geographical distance from L. Matano.

The likelihood-based assignment revealed that 71% of L. Matano Outlet “sharpfins” and 87.5% of riverine populations from streams surrounding L. Towuti were reassigned to their original population. Reassignment success of R. Petea populations (Pt1 to Pt6) was comparatively low, ranging from 16% (Pt2) to 42% (Pt4) (Table 3). Log-likelihood distance to the next likely population was overall comparatively low (Table 3).

Assignment of R. Petea specimens to L. Matano Outlet ranged from 4.5% (Pt6) to 47% (Pt1, Fig. 2). Twenty-seven percent of lacustrine Matano “sharpfins” were assigned to R. Petea sample sites. The assignment to the lacustrine Matano population differed significantly between “upper R. Petea” populations (Pt1, Pt2 and Pt3, assignments between 32% and 47%) and “lower R. Petea” populations (Pt4, Pt5 and Pt6, assignments between 4% and 8%; χ^2 -test, $\chi^2 = 22.34$, $P < 0.001$; Fig. 2).

Table 2 Pairwise F_{ST} estimates across all loci (below diagonal in bold letters)

Sample location	L. Matano Outlet	R. Petea						L. Towuti streams
		Pt1	Pt2	Pt3	Pt4	Pt5	Pt6	
L. Matano Outlet	–	0.36	1.80	3.30	6.30	7.90	8.20	19.50
Pt1	0.01^{n.s.}	–	1.50	3.00	6.00	7.60	7.90	19.20
Pt2	0.03*	0.03*	–	1.50	4.50	6.10	6.40	17.70
Pt3	0.04*	0.03*	0.00^{n.s.}	–	3.00	4.60	4.90	16.20
Pt4	0.09*	0.08*	0.06*	0.06*	–	1.60	1.90	13.20
Pt5	0.02*	0.02^{n.s.}	0.02*	0.02^{n.s.}	0.04*	–	0.30	11.60
Pt6	0.05*	0.04*	0.02^{n.s.}	0.04*	0.01^{n.s.}	0.01^{n.s.}	–	11.30
L. Towuti streams	0.21*	0.16*	0.15*	0.13*	0.15*	0.14*	0.14*	–

Significance was Bonferroni corrected resulting in: $\alpha < 0.002$, *, significant; n.s., not significant

Geographic distances between sample locations (km) are shown above diagonal

Table 3 Assignment and log-likelihood differences with MLD $z = 0$

Sample location	L. Matano Outlet	R. Petea						L. Towuti streams
		Pt1	Pt2	Pt3	Pt4	Pt5	Pt6	
L. Matano Outlet	97 (72%)	9 (47%)	7 (37%)	6 (30%)	1 (5%)	2 (9%)	1 (4.5%)	1 (10%)
Pt1	5 (4%)	7 (37%)	1 (5%)	1 (5%)	0	2 (9%)	1 (4.5%)	0
Pt2	8 (6%)	0	3 (16%)	3 (15%)	0	0	3 (13%)	0
Pt3	2 (1.5%)	0	2 (10.5%)	5 (25%)	0	1 (4.5%)	1 (4.5%)	0
Pt4	3 (2.3%)	0	1 (5%)	0	8 (42%)	4 (17%)	5 (22%)	0
Pt5	17 (13%)	3 (16%)	2 (10.5%)	5 (25%)	3 (16%)	8 (35%)	6 (26%)	0
Pt6	0	0	3 (16%)	0	7 (37%)	6 (26%)	6 (26%)	0
Towuti streams	0	0	0	0	0	0	0	9 (90%)
Log-likelihood difference (median)	1.69	0.8	1.12	0.78	0.85	0.96	1.23	4.6
± s.d.	2.07	0.85	0.96	0.70	0.72	1.34	1.06	3.14

Total number of assigned specimens, percentage values of assignment (brackets) and median MLD for the assignment of each population are shown

The rejection level for assignment was increased from the lowest MLD $z = 0$ to the next higher level $z = 1$, which forces assignment of specimens to a population only when there is a 10 or more times difference between the most likely and the second most likely population. This had a pronounced impact on the number of non-classified individuals (Table 4). The reassignment success differed between L. Matano and R. Petea “sharpfins”, with 49.2% of the specimens correctly reallocated to L. Matano Outlet, 57% to lower R. Petea sample sites (Pt4, Pt5 and Pt6) but only 7% to “upper R. Petea” sample sites (Pt1, Pt2 and Pt3).

Forty-six percent of the specimens from L. Matano Outlet and 72.4% from “upper R. Petea” (Pt1, Pt2

and Pt3) were not unambiguously allocated, whereas 37% from “lower R. Petea” sample sites (Pt4, Pt5 and Pt6) could not be clearly allocated (Table 4). Based on this test, the amount of “non-introgressed” genotypes is highest in L. Matano Outlet and “lower R. Petea” sample sites whereas it is comparatively low in the “upper R. Petea” sample sites.

Morphometric analyses

The CVA based on eight PC axes revealed three axes explaining significant variation between the predefined groups. All tested groups form clusters along the first two CV axes, which display 77% of the total variance between the populations relative to the

Table 4 Assignment to populations with MLD of $z = 1$

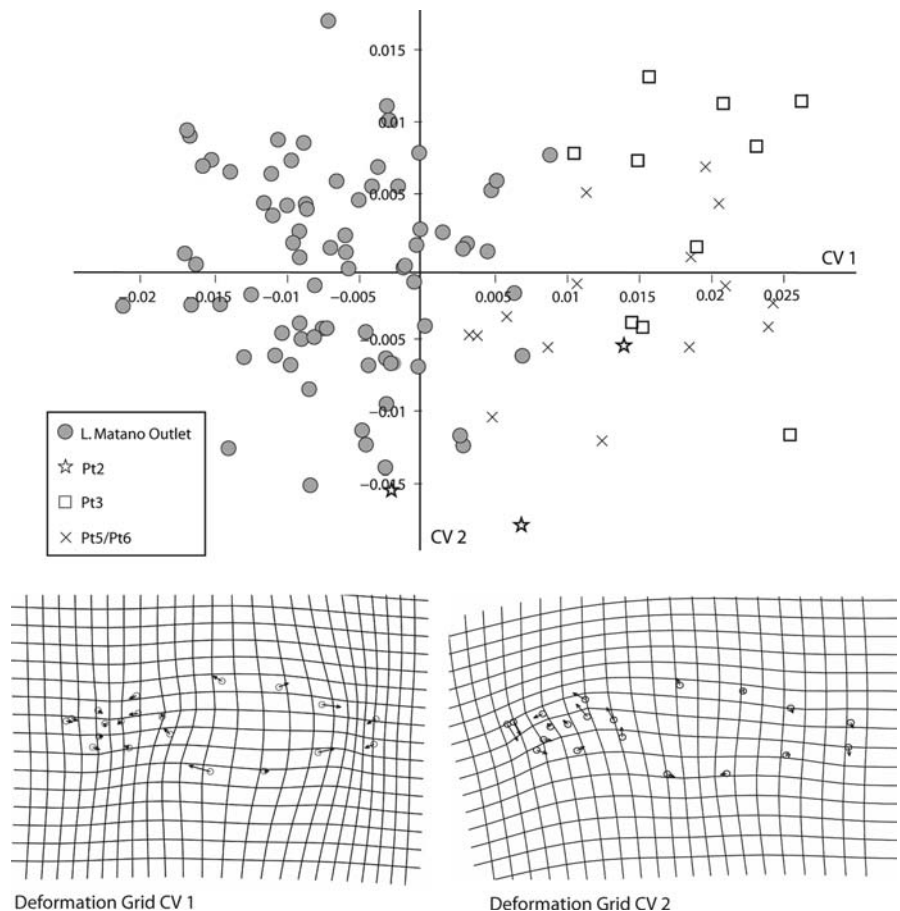
Sample location	L. Matano Outlet	R. Petea		L. Towuti streams
		Pt1/Pt2/Pt3	Pt4/Pt5/Pt6	
L. Matano Outlet	65 (49.2%)	8 (13.8%)	1 (1.6%)	0
Pt1/Pt2/Pt3	4 (3%)	4 (7%)	3 (4.7%)	0
Pt4/Pt5/Pt6	2 (1.5%)	4 (7%)	37 (57%)	0
L. Towuti streams	(0%)	0	0	8 (80%)
None	61 (46%)	42 (72.4%)	24 (37%)	2 (20%)

R. Petea populations were merged in to two groups “upper Petea” (Pt1/2/3) and “lower Petea” (Pt4/5/6)

variance within the populations (Axis 1: Lambda = 0.20, $\chi^2 = 156.74$, $df = 12$, $P < 0.01$, explains 77% of total variance; Axis 2: Lambda = 0.63, $\chi^2 = 44.34$, $df = 6$, $P < 0.01$, explains 11% of total variance; Axis 3: Lambda = 0.85, $\chi^2 = 15.19$, $df = 2$, $P < 0.01$, explains 11% of total variance; Fig. 4). Overlaps were present between all of the tested groups. The main factors responsible for the

morphometric differentiation of the groups are present in the head region, the orientation of the snout and in the length of the caudal peduncle (see deformation grids in Fig. 4). To test for a potential influence of imbalanced sampling on the observed differentiation, CVA scores and centroid sizes were correlated. As this revealed no significant correlation (Spearman signed rank correlation: CV1 vs. Size: $r_s = -0.107$,

Fig. 4 Results of the Canonical variates analyses. Along the first two CV axes the tested populations are weakly separated, showing overlaps. Deformation grids with relative displacement vectors for each landmark display shape changes captured by CV axes 1 and 2. The main shape changes between lacustrine and riverine populations are present in the position of the dorsal and anal fins, the caudal peduncle length and the head region. All tested populations did not differ significantly in standard length (One way Anova, $P = 0.121$), so an allometric effect on the observed result can be most likely excluded



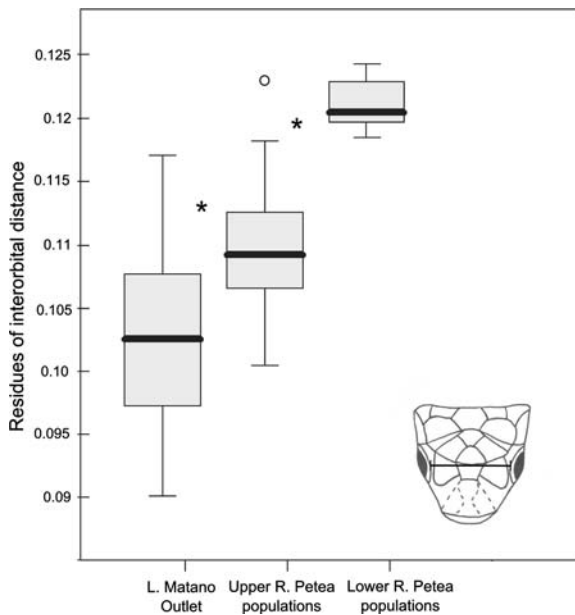


Fig. 5 Differentiation in interorbital width between lacustrine and riverine sharpfin specimens from L. Matano ($N = 74$), upper ($N = 20$) and lower ($N = 9$) R. Petea sampling sites. Significant differences were present between all tested populations (Tukey HSD, all $P < 0.001$, marked by an asterisk). Interorbital width increases in riverine Petea populations with increasing distance to L. Matano

$P = 0.09$, CV2 vs. Size: $r_s = -0.014$, $P = 0.831$), an impact of body size on the observed morphometric differentiation can be excluded.

Analysis of “Interorbital Width” revealed significant differences between “upper R. Petea”, “lower R. Petea” and L. Matano populations (one-way ANOVA; $P < 0.001$, Tukey HSD Test, $P < 0.001$) associated with an increasing interorbital width with higher distance to L. Matano (Fig. 5).

Discussion

Hybridization and introgression are currently discussed to potentially increase rather than necessarily retard the generation of biodiversity, especially with regard to adaptive radiations (Seehausen, 2004; Mallet, 2007). Significant signal of introgression by stream-dwelling sailfin silversides is coupled with conspicuously high phenotypic diversity in the radiation of “sharpfin” sailfin silversides of L. Matano, suggesting that introgression might be involved in the generation of diversity in this radiation (Herder et al.,

2006a). In this context we hypothesized that substantial gene flow is present between riverine and lacustrine “sharpfins” at the interface between L. Matano and R. Petea.

Our results suggest ongoing gene flow between L. Matano’s and R. Petea’s “sharpfins”, because (1) pairwise population comparisons show comparatively low levels of genetic differentiation (F_{ST}), indicating moderate to high levels of migration (Hartl & Clark, 1997) between all sampled populations (Table 2); (2) R. Petea populations exhibit increasing morphometric (Fig. 5) and genetic similarity (Table 2, Fig. 2) to lacustrine “sharpfins” of the L. Matano Outlet area with decreasing distance to L. Matano (Fig. 2); and (3) the proportion of unassignable riverine Petea individuals increases closer to L. Matano (Table 4).

Presence of unassigned individuals when applying the strict rejection level ($z = 1$) can be explained in two ways. Genomic similarity of these individuals to more than one potential source population could prevent clear assignment, indicative of ongoing gene flow. Alternatively, origin from an unsampled source population would also result in non-assignment under stringent (MLD $z = 1$) conditions and increased the likelihood of assignment by chance under MLD $z = 0$ (Manel et al., 2005). This would imply that those unassigned specimens are of unknown origin, i.e. from an unsampled source population. We consider this as rather unlikely, as extensive explorations in L. Matano, the whole Malili Lakes system and along R. Petea (own surveys in 2002, 2004, 2006; survey by Kottelat in the early 1990th (Kottelat, 1990a, b, 1991)) revealed no hints for important additional neighbouring populations. Moreover, phylogenetic analyses of *Telmatherina*-relatives (Herder et al., 2006a) as well as preliminary morphometric and AFLP-based assignment tests (JS, unpublished data) including all known lacustrine and riverine “sharpfin” specimens of L. Matano (see Herder et al., 2006b) render it comparatively unlikely that other known “sharpfin” populations had an impact on the observed assignment pattern. In conclusion, the observed differences in assignment, based on geographic origin, are most likely related to a decreasing amount of gene flow or a decreasing relatedness between the populations, especially seen against the background of the morphometric results.

Clinal variation and isolation by distance

Our results suggest clinal variation in genetic traits (Fig. 2) and isolation by distance along R. Petea. Remarkably, this pattern is obvious over the very short distance of only about 9 km, and despite an expected high mobility of these agile, non-territorial fish (Gray & McKinnon, 2006). With decreasing distance from the lake, R. Petea populations become genetically more similar to lacustrine “sharpfins” of L. Matano Outlet (Fig. 3, Table 2). This is partially reflected in morphometric characters, too (Fig. 5). Whereas no clinal variation is obvious in body shape (Fig. 4), head width variation also reflects a clinal pattern, with more bulky headed specimens further away from L. Matano. Generally, we observed that riverine specimens develop wider heads than lacustrine L. Matano Outlet specimens (Fig. 5), indicating that general adaptations to lake and stream habitats might be present. This corresponds to previous studies, demonstrating adaptive divergence between lake- and stream-dwelling fish: Hendry et al. (2002) found stream sticklebacks (*Gasterosteus* spp.) to have deeper bodies and fewer gill rakers than lake sticklebacks; McGuigan et al. (2003) reported adaptive differences for rainbow fish (Melanotaeniidae) which are closely related to telmatherinids (Sparks & Smith, 2004), with lacustrine rainbow fish having more posteriorly positioned first dorsal and pelvic fins than stream-inhabiting ones. However, although we do assume general adaptations to riverine and lacustrine habitats in “sharpfins”, it appears unlikely that fine-scale local adaptations to each single R. Petea sample site have evolved, given very high levels of gene flow and the extremely small geographic distances with no obvious habitat changes along the river.

Secondary contact or ongoing divergence?

Two alternative scenarios appear plausible to explain the observed pattern of morphometric and genetic clinal variation of “sharpfins” within R. Petea. Secondary gene flow due to incomplete reproductive isolation between hybridizing but originally separated populations may be one. Following this idea, balance between dispersal and selection against hybrids as explained in the tension-zone model (Barton & Hewitt, 1985) might cause a clinal pattern like the

one observed here. Alternatively, ongoing evolution of increasingly limited gene flow between parapatrically diverging populations of L. Matano and R. Petea “sharpfins” would lead to decreasing similarity.

Under the idea of secondary contact, genomic exchange between riverine and lacustrine sailfin silverside populations could potentially contribute to genomic enrichment of the highly diverse lacustrine “sharpfins” of L. Matano with riverine alleles, as suggested for stream-dwelling *T. bonti* by previous analyses (Herder et al., 2006a). Also, the alternative scenario, parapatrically diverging populations of L. Matano and R. Petea, could allow for introgression of alleles adapted to stream conditions into the lacustrine flock. Increasing dissimilarity with increasing geographic distance, as indicated here, would be a result of ongoing divergence. In a recent model, Gavrilets & Vose (2005) point out that adaptive radiation can take place extremely quickly in the presence of ongoing gene flow. Hence, population divergence should potentially proceed without initial evolution of reproductive isolation, and result in a high amount of similarities between diverging populations with few adaptive differences between them. This corresponds to the patterns observed in case of L. Matano and R. Petea “sharpfins”, with different eco-phenotypes in lacustrine and riverine habitats.

However, a full reconstruction of the L. Matano introgression/parapatric speciation scenario is beyond the scope of the present study. A larger-scale approach, including population genetic sampling of all riverine populations of the L. Matano-catchments as well as all “sharpfin”-populations of L. Matano, is required to test for the potential impact of introgression by R. Petea’s “sharpfins” into the Matano-flock.

A barrier within River Petea?

R. Petea and its potential role as barrier within the Malili Lakes system have recently gained substantial interest. The isolation of L. Matano could play a significant role in its use as a model system to address questions regarding the evolution of species flocks (Roy et al., 2004, 2007a, b; Herder et al., 2006a, b). It has previously been assumed that L. Matano is “separated from downstream lakes by a 72 m waterfall ...” (Roy et al., 2004, p. 1269), citing Brooks (1950) and Kottelat (1990b). In contrast to these

assumptions, our fieldwork as well as population genetic analysis unambiguously showed that there is no major waterfall and no major population genetic barrier for stream-dwelling “sharpfins” in R. Petea. During sampling it became evident that R. Petea is very steep and mostly extremely fast-flowing, but does not contain waterfalls which can be regarded as complete upstream barriers for stream-dwelling fishes. Indeed, we observed R. Petea’s “sharpfins” directly in strong rapids. Given this observation, low levels of genetic differentiation of sailfin silversides along R. Petea appear highly plausible and are congruent with the lack of a major waterfall. This result is only seemingly in contrast with population genetic studies of *Caridina* freshwater shrimps that supported the “barrier hypothesis” by finding strong distinction of semi-lacustrine *Caridina*-populations from above and below the river course of R. Petea (Roy et al., 2006). In contrast to this study, the analysis presented here is the first one investigating material sampled from the river itself and not comparing lacustrine populations of different lakes separated by strongly riverine habitats. Our results highlight the importance of incorporating both lacustrine and riverine populations when comparing lacustrine populations within the Malili Lakes drainage system.

Implications for conservation

Present results indicate that R. Petea is not an impassable barrier for endemic freshwater fish and correspond to previous results suggesting natural hybridization between stream- and lake-inhabiting populations (Herder et al., 2006a). This has direct implications for conservation issues: besides the lakes, also the rivers connecting them, namely Rivers Petea and Tominanga (connecting L. Mahalona and L. Towuti), should be taken into consideration when attempting to sustainably preserve the worldwide unique ecosystem of “Wallace’s Dreamponds” (Herder et al., 2006a). The Malili Lakes area is object to massively increasing surface nickel mining and smelting, with considerable loss or modification of natural habitats and high demands for electric energy, supplied mainly by hydroelectric damming. Given the arguments listed above and the presence of an only recently discovered diversity of fish inhabiting most running waters of the Malili Lakes

drainage (Herder et al., 2006b), we strongly recommend to include streams and rivers, especially those connecting the lakes, into management decisions.

Conclusions

The present study suggests ongoing gene flow between “sharpfins” of L. Matano and R. Petea, with decreasing genotypic and phenotypic similarity of stream- to lake fish along this remarkably short river. Although our data do not have the power to differentiate between different scenarios explaining the observed gene flow patterns, the results are one important step beyond previous findings. They indicate strongly that genetic exchange between the lacustrine and riverine “sharpfins” takes place, potentially allowing introgression into L. Matano from lower lakes and streams. However, the significance of the presented results for the evolution of complex diversity patterns in L. Matano remains to be tested. We stress that there is no evidence for a major physical barrier within R. Petea and that overall gene flow of stream “sharpfins” along the river appears high. Implications of the fact that L. Matano’s adaptive sailfin silverside radiation is not physically isolated from the downstream telmatherinid diversity should be taken into consideration when analysing evolution of L. Matano’s spectacular diversity.

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