Evidence of small-scale vicariance in *Caridina lanceolata* (Decapoda: Atyidae) from the Malili Lakes, Sulawesi

Denis Roy,[1]* David W. Kelly,[1] Charles H.J.M. Fransen,[2] Daniel D. Heath[1] and G. Douglas Haffner[1]

[1]*Great Lakes Institute for Environmental Research, University of Windsor, 401 Sunset Avenue, Windsor, Ontario N9B 3P4, Canada and* [2]*Nationaal Natuurhistorisch Museum – Naturalis, PO Box 9517, 2300 RA Leiden, The Netherlands*

ABSTRACT

Question: Are dispersal ability and vicariance influential factors regulating population divergence and the formation of new species?

Hypothesis: Barriers can lead to dramatic population divergence even over small geographic distances, especially when such barriers prevent dispersal and ultimately gene flow among closely related populations.

Organism: *Caridina lanceolata* Woltereck, 1937.

Field site: Lake Matano (Sulawesi, Indonesia) is the hydrological head of the Malili Lakes and is separated from downstream lakes by the Petea River, which negotiates a 72-m change in elevation, part of which consists of highly flowing rapids.

Methods: We use morphological traits and genetic data to assess relationships among populations that occur above and below this barrier.

Conclusions: The lack of morphological and phylogenetic differentiation among populations within lakes indicates no barriers to gene flow within lake systems. Elevated phylogentic differences between lakes, however, coupled with subtle morphological discontinuities indicates strong differentiation across the Petea River. Thus, dispersal and gene flow of *C. lanceolata* is restricted by the Petea River, leading to significant population structure among sampled populations. The magnitude of the cross-river genetic differentiation coupled with overall general morphological similarities among populations is consistent with cryptic sibling species.

Keywords: cryptic species, dispersal, ecological significant unit, Lake Matano, Malili Lakes, population divergence, vicariance.

INTRODUCTION

Speciation is dependent on variation among individuals and divergence between populations (Darwin, 1859; Futuyma, 1986). While divergence may result from selection on

---

functional traits such as behaviour or physiology, the effects of selection are most apparent in morphological differences that are consequently used in species identification. Alternatively, vicariance, or limitations to dispersal, may produce allopatric populations that diverge through genetic drift but where balancing selection may limit changes in morphology (Slatkin, 1985; Futuyma, 1986). This leads to the formation of cryptic species and indicates that drift plays an important role in the speciation process.

Cryptic species often confound traditional species definitions in that, if they differ genetically but are morphologically very similar, do they represent different species (Mayr, 1963; Coyne and Orr, 2004)? This is dependent on the choice of species concept, which can be viewed from phylogenetic or morphological perspectives, among many others (Hey, 2001; Schluter, 2001; Agapow *et al.*, 2004; Coyne and Orr, 2004). Nevertheless, clarifying mechanisms building variation among populations is critical to understanding processes leading to species formation, but is often clouded by an inability to discriminate effects of drift from those of selection (Moritz and Faith, 1998; Agapow *et al.*, 2004). Studies of allopatric cryptic sibling species may address this problem and allow an assessment of dispersal limitations and the time span over which neutral processes shape divergence free of the confounding effects of selection (Knowlton *et al.*, 1993; Knowlton and Weigt, 1998; Cristescu *et al.*, 2003; Page *et al.*, 2005).

Sulawesi Island, Indonesia, has been isolated from other land masses since its formation 12–14 million years ago (mya) and exhibits elevated patterns of species diversity and endemism (Brooks, 1950; Whitten *et al.*, 2000; Evans *et al.*, 2003). Such patterns may be explained by its isolation, unusual geological history, and variety of unique ecosystems (Brooks, 1950; Myers *et al.*, 2000; Whitten *et al.*, 2000). The Malili Lakes are a series of interconnected tropical lakes centrally located on Sulawesi with Lake Matano constituting the hydrological head (Brooks, 1950; Kottelat, 1990a, 1990b, 1991; Haffner *et al.*, 2001). Although the age of these lakes is uncertain, Brooks (1950) estimated an origin 4 mya on the basis of local geology and Matano's substantial depth (maximum depth = 598 m). In addition, Hamilton (1979) estimated a similar age (2–4 million years old) based on displacement along both the Matano and the Palu Koro faults purported to have been the formative agents of these aquatic systems. Investigations of sedimentary cores taken from various areas around Sulawesi and in parts of Matano proper suggest relatively constant water levels since its formation (Haffner *et al.*, 2001; Hope, 2001; Visser *et al.*, 2004).

Lake Matano, located along the active Matano fault and at the head of the Malili Lakes, has likely provided source propagules for colonization of downstream lakes (Brooks, 1950). However, it remains effectively isolated by a 72-m change in elevation, consisting of cascading rapids within the connecting Petea River (Brooks, 1950; Kottelat, 1990a) (see Fig. 1). This barrier has likely limited the dispersal of aquatic species between Matano and the other Malili Lakes. The Malili Lakes thus provide an opportunity to assess the influence of physical barriers on population divergence and evolutionary processes in tropical freshwater systems.

Using molecular and morphological data, we assess the small-scale phylogeographic structure of the endemic crustacean *Caridina lanceolata* (Decapoda: Atyidae) by comparing populations above and below the Petea River. Given that other Atyid species exhibit extensive population structure among different river drainages separated by inhospitable environments (Woolschot *et al.*, 1999; Hurwood and Hughes, 2001; Baker *et al.*, 2004), we expect that populations will be divergent across the Petea River.
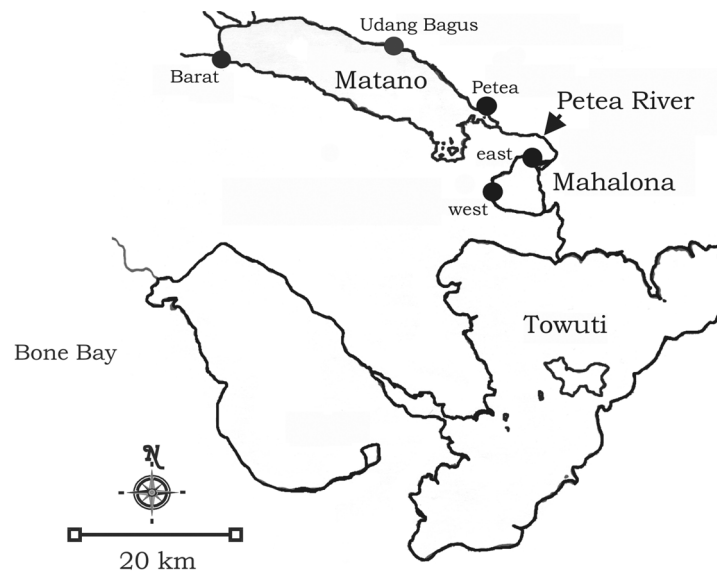
Fig. 1. Map of the Malili Lakes located in the south central portion of Sulawesi Island, Indonesia. Solid circles indicate the geographic location of each beach from where *Caridina lanceolata* specimens were collected. Populations in Matano are separated from those in Mahalona by the Petea River, which flows down a 72-m change in elevation between the lakes (see Brooks, 1950).

METHODS AND MATERIALS

Approximately 53 *C. lanceolata* were collected by both seine and dip nets from five beaches in the Malili Lakes. Three beaches, located in Matano, consisted of Barat and Udang Bagus separated by 19.3 km, and Petea separated from Udang Bagus and Barat by 12.1 and 30.3 km, respectively. Two beaches were selected below the Petea River in Lake Mahalona and included Mahalona East, located at the Petea River inflow, and Mahalona West, located 7.2 km southwest of Mahalona East (Fig. 1). Mahalona East is located 8.5 km, 20.6 km, and 36.9 km away from Petea, Udang Bagus, and Barat respectively, while Mahalona West is located 9.6 km, 19.8 km, and 34 km respectively from these same beaches. Sub-samples from each beach were registered and deposited at the National Museum of Natural History Naturalis, Leiden, The Netherlands, under the specimen voucher numbers RMNH D 51165–51178.

DNA was extracted from 10 individuals per beach, using the WIZARD® DNA kit following the standard protocol (Promega, Madison, WI, USA). Universal primers (Folmer *et al.*, 1994) amplified COI in individuals from Mahalona but not those from any of the Matano beaches. Consequently, the Mahalona sequences were aligned with other Atyid sequences from GenBank, and new primers were designed within highly conserved regions. Newly developed primers (CLW-F 5′-TYCGAGCAGAACTAGGHCAAC-3′, and CLW-R 5′-CGATCTGTTARTAGTATAGTA-3′) were used with 1 μl of raw genomic DNA in standard PCR reactions, described elsewhere (Roy *et al.*, 2004), amplifying a 480-bp region of the COI mitochondrial genome. Reactions were run for five cycles with a denaturation of 96°C for 1 min, primer annealing at 45°C for 1 min, and an extension at 72°C for 1.5 min. This was followed by 30 cycles with an annealing temperature of 50°C and a final

termination by extension for 5 min at 72°C. The PCR products were then sequenced as in Roy *et al.* (2004) and aligned using the clustal W algorithm with a gap open penalty of 10, gap extension of 5, and a DNA transition weight of 0.5 (Omiga, 1.2, Oxford Molecular Ltd, Oxford, UK). Aligned sequences were entered into PAUP version 4.0 (Swofford, 1998) for phylogenetic analyses. Both transition and transversion substitutions were used in the analyses, since transition saturation tests were negative (Kocher and Carleton, 1997). Insertions and deletions (indels) were treated as complete deletions, and pair-wise distances were calculated using the GTR + G substitution model determined from an Akaike information criterion (AIC) survey of 56 substitution models implemented by MODELTEST version 3.7 in PAUP (Posada and Crandall, 1998; Swofford, 1998). Patterns of phylogeographic divergence in the distance matrix were assessed using Neighbour-Joining with a gamma parameter of 0.4149, and nodal support was estimated using 10,000 bootstrap permutations (Kocher and Carleton, 1997). An additional test of phylogenetic structure was also conducted by Maximum Parsimony (MP) heuristic searches with Close Neighbour Interchange of 1 and a Random Addition Trees of 10 replicates supported by 1000 permutations. Sequences of *Paratya australiensis* Kemp 1917, an Atyid species from the Indo-Pacific region, were obtained from GenBank for use as an outgroup (Baker *et al.*, 2004).

Patterns of population structure both among and within lakes were assessed using an analysis of molecular variance using populations within lakes as a nested factor [AMOVA (Excoffier *et al.*, 1992)] and pair-wise F_{ST} based on derived distances, implemented in ARLEQUIN version 3.0 (Excoffier, 2005). Global significance was determined at an alpha level of 0.05 using 10,000 permutations, and pair-wise differences were evaluated with an alpha value corrected for multiple comparisons ($\alpha = 0.005$). To determine if patterns of genetic variation were attributable to neutral mutations (drift) or exhibited signs of directional selection, population expansion, or recent bottlenecks at the COI locus, Tajima's (1989) *D* statistic was calculated across all populations.

Morphometric measurements were taken (following Woltereck, 1937a, 1937b) from the remaining individuals collected from each population to determine morphological population differences. Morphological differences were analysed using a multivariate analysis of covariance (MANCOVA) across all traits using pre-orbital carapace length as a covariate and populations within lakes as a nested factor. The multivariate test was followed by a series of univariate analyses of covariance (ANCOVA; corrected for type I errors using sequential Bonferroni adjustment) using populations within lakes as a nested factor to determine if populations among and within lakes differed in specific traits. *Post-hoc* comparisons (also corrected using sequential Bonferroni) were performed to determine which populations within lakes were different (STATISTICA v. 6.0, Statsoft Inc., 2001, Tulsa, OK).

RESULTS

COI mtDNA sequences generated in this study are available on GenBank under the accession numbers DQ155568–DQ155590. Of the 480-bp fragment of the COI gene, 103 sites were polymorphic, 91 of which were phylogenetically informative. There were no indels and we found an average transition : transversion ratio of 3.376. Similar topologies were recovered using both distance- and parsimony-based analyses, and both demonstrated substantial distances to the outgroup. As such, distances to the outgroup were truncated for display purposes (Fig. 2). *Caridina lanceolata* sampled from the Malili Lakes were strongly
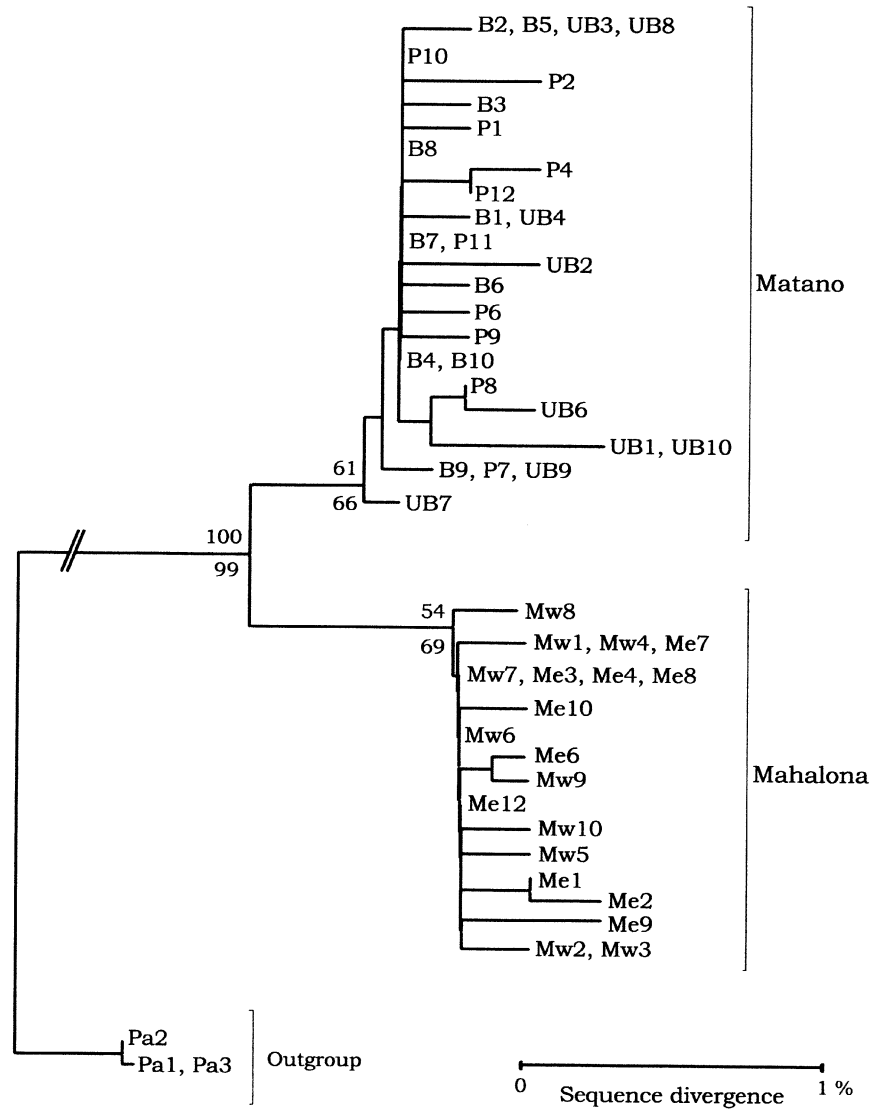
Fig. 2. Phylogenetic relationship determined among five populations of *Caridina lanceolata* (Decapoda: Atyidae) endemic to the Malili Lakes, Sulawesi Island, Indonesia. Bootstrap 50% majority rule consensus tree constructed by Neighbour-Joining using a GTR + G distance model with a gamma distribution for base substitution ($\lambda = 0.4149$). Phylogeny was recovered using Maximum Parsimony (MP) heuristic searches with Close Neighbour Interchange of 1 and a Random Addition Trees of 10 replicates recovered identical general topology. Bootstrap support for distances (from 10,000 permutation) and MP (1000 permutations) indicated above and below the nodes, respectively. Each specimen is identified in the phylogeny by an alphanumeric code using beach designation (see Fig. 1) and specimen number. Lake of origin is given to the right of the tree.

spatially structured, revealing two distinct clades with strong bootstrap support (Fig. 2). The first, designated 'Matano', encompassed 20 different haplotypes of individuals from all three Matano beaches, but without any structure with respect to beach (Fig. 2). Two Matano haplotypes were present at both Barat and Udang Bagus, while another was present at all three sampled Matano beaches. The second clade, designated 'Mahalona', consisted of 14 haplotypes, two of which were shared among Mahalona beaches (Fig. 2). The sharing of haplotypes among various beaches within individual lakes indicates no impediment to within-lake dispersal (Figs. 1 and 2). The Matano and Mahalona clades, however, did not share any haplotypes, indicating a lack of gene flow between the two lakes.

Limited gene flow between lakes was also indicated by similar mean sequence divergence within the Matano and Mahalona clades (0.403%, standard error = 0.0013% and 0.31%, standard error = 0.0013% respectively), whereas inter-clade divergence was high (1.5%, standard error = 0.0048%). AMOVA revealed that most of the genetic variation among sampled beaches was attributable to differences between lakes (Table 1), with pair-wise population comparisons showing that the Mahalona populations were significantly different from all those in Matano (Table 2). No population structure, however, was observed either within the Matano or Mahalona populations (Table 2). Tajima's D was not significant across populations, suggesting neutral mutations rather than selection were responsible for the observed variation among populations at the COI locus ($D = -0.1131$, $P > 0.05$).

Table 1. Results of an analysis of molecular variance (Excoffier *et al.*, 1992) performed on five populations of *Caridina lanceolata* collected from the Malili Lakes, Sulawesi Island, Indonesia

Source of variation	Degrees of freedom	Sum of squares	Variance component	Percentage of variation
Among lakes	1	60.817	2.490ct	73.19
Among populations within lakes	3	3.183	0.017sc	0.49
Within populations	45	40.300	0.896st	26.32

Note: The analysis was performed using COI sequence data and calculated pair-wise differences using the GTR + G ($\gamma = 0.4149$) base pair substitution model, and the algorithm was permutated 1023 times.
P-values estimated from permutations as follows: F_{ST}: P(rand. values < obs. values) = 0.0000; F_{sc}: P(rand. values < obs. values) = 0.1740; F_{ct}: P(rand. values < obs. values) = 0.0000.

Table 2. Pairwise F_{ST}-values and associated *P*-values (in parentheses) determined among five populations of *Caridina lanceolata* (Decapoda: Atyidae) sampled from the Malili Lakes

	Barat		Udang Bagus		Petea		Mahalona East	
Barat	—	—						
Udang Bagus	0.007	(0.473)	—					
Petea	0.016	(0.490)	0.030	(0.265)	—	—		
Mahalona East	**0.788**	**(0.0001)**	**0.698**	**(0.0001)**	**0.741**	**(0.0001)**	—	—
Mahalona West	**0.791**	**(0.0001)**	**0.699**	**(0.0001)**	**0.744**	**(0.0001)**	0.011	(0.376)

Note: *P*-values generated from 1023 permutations of the AMOVA algorithm performed in ARLEQUIN version 3.0 (Excoffier, 2005). **Bold** entries indicate significant genetic differentiation among compared populations with the α-value corrected to 0.005 for multiple comparisons.

MANCOVA revealed significant differences both between lakes and among populations within lakes in morphometric measurements (Wilks' $\lambda = 0.098$, $F_{18,28} = 14.25$, $P < 0.0001$; Wilks' $\lambda = 0.049$, $F_{54,84.25} = 2.73$, $P < 0.0001$, respectively). Univariate ANCOVAs between lakes demonstrated that six traits related to the rostrum and the chelae differed between individuals in each lake (Table 3). Individuals in Mahalona were characterized by a longer rostrum with fewer ventral but more dorsal teeth. Matano individuals also had significantly fewer rostral postorbital dorsal teeth and shorter first and second chelae lengths than individuals in Mahalona (Table 3). Once univariate tests were corrected for sample sizes ($N = 10$ per population) and for multiple comparisons, significant differences among populations were only observed in those traits that differed between lakes (Table 3). Within these traits, Mahalona populations were not significantly different from each other, but were significantly different from all Matano populations.

DISCUSSION

Assessing biological diversity often depends on accurate identification of species that are usually recognized by clear morphological criteria (Barton, 1988). A lack of obvious morphological differences among groups, however, may overlook genetic population structure and possibly speciation due to vicariance barriers to dispersal (Slatkin, 1985; Palumbi et al., 1997; Collin, 2005). The data presented here provide an example of this phenomenon, whereby dispersal appears restricted across the Petea River, leading to divergence between populations of *C. lanceolata* in Lakes Matano and Mahalona. Lake Matano populations showed substantial dispersal ability over at least 30 km, as evidenced by haplotype sharing between populations, and intermixing of individuals from both populations within the Matano clade. This is consistent with the lack of morphological differentiation between the three Matano and the two Mahalona populations, and the relatively low level of genetic divergence within both clades (Tables 1 and 2; Table 3; Fig. 2). Low genetic divergences observed within each lake, and the small F_{ST} among both the Matano and Mahalona populations, are consistent with studies describing high dispersal and gene flow among other Atyids, including *Caridina* species, within contiguous systems such as river catchments and drainage basins (Woolschot et al., 1999; Hurwood and Hughes, 2001; Baker et al., 2004; Page et al., 2005).

The Matano and Mahalona populations, however, are separated by physical distances less than that separating the two most distant Matano populations (Fig. 1). Thus, physical distances alone are not a significant hindrance to dispersal of this species, but rather the Petea River is likely an unsuitable environment, preventing dispersal. Whether the river itself or some specific feature within the river (i.e. cascading rapids) is responsible for the discontinuity in genetic structure is difficult to determine. However, *C. lanceolata* specimens recovered from both the inflow and outflow of the Petea River suggest that the river alone is not the dispersal barrier (Figs. 1 and 2). This is consistent with studies describing dispersal of other Atyid and *Caridina* species across a variety of habitats ranging from lakes and riverine systems to estuaries and small terrestrial divides (Woolschot et al., 1999; Hurwood and Hughes, 2001; Baker et al., 2004; Page et al., 2005). These studies also demonstrate significant population structure across substantial barriers, such as divided watersheds and large marine expanses (Woolschot et al., 1999; Hurwood and Hughes, 2001; Baker et al., 2004). Yet, the cascading rapids and fast flowing water of the Petea River are likely an obstruction to *C. lanceolata* dispersal in the Malili Lakes system. Kottelat (1990a, 1990b, 1991) described a similar pattern in the fish

Table 3. Results of univariate nested ANCOVAs performed on morphological features measured (following Woltereck, 1937a, 1937b) in five populations of *Caridina lanceolata* (Decapoda: Atyidae) collected from the Malili Lakes

Trait		Udang Bagus	Barat	Petea	Mahalona West	Mahalona East
Rostrum						
	length	5.43 ± 2.57^A	5.02 ± 1.38^A	5.52 ± 1.49^A	7.03 ± 3.16^B	7.43 ± 1.52^B
	# dorsal teeth	14.00 ± 3.43^A	14.22 ± 1.33^A	15.50 ± 3.92^A	11.90 ± 2.20^B	12.82 ± 2.50^B
	# postorbital dorsal teeth	2.00 ± 0.63^A	2.44 ± 1.05^A	2.40 ± 1.03^A	1.70 ± 0.97^B	1.55 ± 1.04^B
	dorsal subdistal	1.00 ± 0.84^A	1.89 ± 0.67^A	1.60 ± 1.03^A	1.70 ± 1.35^A	2.18 ± 0.81^A
	# ventral teeth	6.00 ± 1.35^A	5.44 ± 1.76^A	6.80 ± 3.24^A	8.10 ± 2.57^B	8.18 ± 2.34^B
Diaeresis						
	left	8.00 ± 1.05^A	8.25 ± 2.18^A	8.20 ± 1.26^A	6.80 ± 3.24^A	8.09 ± 2.09^A
	right	8.00 ± 1.75^A	7.13 ± 1.20^A	8.10 ± 1.48^A	6.80 ± 1.58^A	8.00 ± 1.55^A
Telson						
	# dorsal spines left	4.00 ± 1.03^A	3.44 ± 1.05^A	3.00 ± 0.00^A	3.50 ± 1.41^A	3.82 ± 0.81^A
	# dorsal spines right	4.00 ± 1.05^A	3.22 ± 0.88^A	3.20 ± 0.84^A	3.60 ± 1.03^A	3.82 ± 1.21^A

Sixth abdominal segment		2.95 ± 1.13^A	2.82 ± 0.59^A	2.95 ± 0.50^A	2.96 ± 0.87^A	3.33 ± 0.61^A
P1 carpus	length	0.77 ± 0.26^A	0.73 ± 0.18^A	0.83 ± 0.17^A	0.73 ± 0.23^A	0.86 ± 0.23^A
	height	0.37 ± 0.05^A	0.20 ± 0.09^A	0.19 ± 0.06^A	0.21 ± 0.04^A	0.25 ± 0.06^A
P1 chela	**length**	$\mathbf{0.82 \pm 0.25}^A$	$\mathbf{0.77 \pm 0.12}^A$	$\mathbf{0.80 \pm 0.15}^A$	$\mathbf{0.84 \pm 0.15}^B$	$\mathbf{0.94 \pm 0.18}^B$
	height	0.37 ± 0.09^A	0.33 ± 0.07^A	0.29 ± 0.08^A	0.33 ± 0.05^A	0.39 ± 0.10^A
P2 carpus	length	1.43 ± 0.42^A	1.40 ± 0.23^A	1.45 ± 0.35^A	1.44 ± 0.54^A	1.60 ± 0.35^A
	height	0.16 ± 0.04^A	0.15 ± 0.03^A	0.15 ± 0.02^A	0.15 ± 0.03^A	0.18 ± 0.04^A
P2 chela	**length**	$\mathbf{0.86 \pm 0.26}^A$	$\mathbf{0.83 \pm 0.17}^A$	$\mathbf{0.89 \pm 0.18}^A$	$\mathbf{0.91 \pm 0.25}^B$	$\mathbf{1.02 \pm 0.22}^B$
	height	0.28 ± 0.06^A	0.26 ± 0.04^A	0.25 ± 0.06^A	0.29 ± 0.04^A	0.31 ± 0.07^A

Note: Significance for univariate tests was assessed by sequential Bonferroni correction (starting at $\alpha = 0.0027$ between lakes and *post-hoc* tests adjusted to $\alpha = 0.00027$ among population comparisons). **Bold** entries and superscripts indicate significant differences both among lakes and populations for particular traits.

species from Lake Matano, which are endemic and restricted to it. More recently, von Rintelen *et al.* (2004) demonstrated a segregation of gastropod genetic clades wherein the Matano clade was present in all lakes but lower lake clades were absent from Matano. Together, these data suggest that the Petea River is a substantial barrier to dispersal for *C. lanceolata* and probably so for many other species in the Malili Lakes.

Although the formation of such geological discontinuities can occur rather quickly, the high levels of divergence observed in *C. lanceolata* between the two lakes suggests that this barrier is not recent. Invoking established rates of divergence (1.4–2.6% per million years) among other Caridean lineages separated by the uplift of the Isthmus of Panama set by Knowlton *et al.* (1993) and Knowlton and Weigt (1998), and assuming a constant rate of evolution, the data indicate a separation dating back to the Pleistocene. Hence, the approximate age of the Petea River obstruction is 0.6–1.0 mya. The divergence reported here is comparable to that observed in other invertebrates occurring throughout the world, but is manifest over a much smaller scale of less than 10 km (Knowlton *et al.*, 1993; Knowlton and Weigt, 1998; Woolschot *et al.*, 1999; Critescu *et al.*, 2003).

In this study, we report differences in both rostral and chelae features (see Table 3) (Woltereck, 1937b). These morphological differences may be due to either differential selective pressures acting in the two different lakes, and is a response to selection, or may be neutral traits allowed to drift unchecked by balancing selection. Indeed, Woltereck (1937b) suggested that these traits do not provide adaptive advantages to particular environments and thus are unlikely to be under selection. The morphological differences reported here are consistent with other studies of allopatric cryptic species demonstrating subtle yet distinct differences in various phenotypic traits among closely related sibling species (reviewed by Mayr, 1963; see also Fernandez *et al.*, 2006; Sanders *et al.*, 2006).

In fact, the genetic divergence observed in this study is also accompanied by relatively few morphological differentiations between the *C. lanceolata* populations in the two lakes. The overall morphological similarities between these populations despite the absence of gene flow may be maintained by balancing selection. Such balancing selection has been invoked to explain deep population- and species-level divergence without accompanied similar levels of morphological divergence in other cryptic species (e.g. Knowlton *et al.*, 1993; Palumbi *et al.*, 1997; Collin, 2005).

Nevertheless, the few obvious morphological differences coupled with insignificant levels of selection on the COI locus (Tajima's *D* statistic) indicates that the divergence observed in the *C. lanceolata* clades is predominantly an effect of drift, and provides one of the clearest examples of drift-building variation among closely related populations. Elevated levels of divergence between the two clades further suggests the presence of cryptic, or at the very least incipient, species using the phylogenetic species concept, resulting from the separation of the populations by the Petea River.

The formation of such cryptic species highlights the need to identify species from a variety of perspectives, which can provide a more reliable and tangible consensus of an ecological/evolutionarily significant unit. Such consensus units would be far more practical and useful in conservation and mechanistic-based studies (Moritz and Faith, 1998; Hey, 2001; Agapow *et al.*, 2004).

This results of this study demonstrate the importance of physical topography and resulting vicariance for gene flow patterns in *C. lanceolata*, and perhaps other species in the Malili Lakes. Local and broader scale geography has had a significant impact on the generation of faunal and floral endemism in the Malili Lakes region, and

Sulawesi Island in general (Whitten *et al.*, 2000; Evans *et al.*, 2003). These data add to a growing body of evidence demonstrating the unique evolutionary history of this isolated continental island.

ACKNOWLEDGEMENTS

REFERENCES

Agapow, P., Bininda-Emonds, O.R.P., Crandall, K.A., Gittleman, J.L., Mace, G.M., Marshall, J.C. *et al.* 2004. The impact of species concept on biodiversity studies. *Q. Rev. Biol.*, **79**: 161–178.

Baker, A.M., Hurwood, D.A., Krogh, M. and Hughes, J.M. 2004. Mitochondrial DNA structure of restricted gene flow within divergent lineages of an atyid shrimp (*Paratya australiensis*). *Heredity*, **93**: 196–207.

Barton, N.H. 1988. Speciation. In *Analytical Biogeography: An Integrated Approach to the Study of Animal and Plant Distributions* (A.A. Myers and P.S. Geller, eds.), pp. 185–218. London: Chapman & Hall.

Brooks, J.L. 1950. Speciation in ancient lakes (concluded). *Q. Rev. Biol.*, **25**: 131–176.

Collin, R. 2005. Development, phylogeny, and taxonomy of *Bostrycapulus* (Caenogastropoda: Calyptraeidae), an ancient cryptic radiation. *Zool. J. Linn. Soc.*, **144**: 75–101.

Coyne, J.A. and Orr, H.A. 2004. *Speciation*. Sunderland, MA: Sinauer Associates.

Cristescu, M.E.A., Hebert, P.D.N. and Oncui, T.M. 2003. Phylogeography of Ponto-Caspian crustaceans: a benthic–planktonic comparison. *Mol. Ecol.*, **12**: 985–996.

Darwin, C. 1859. *The Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. London: John Murray.

Evans, B.J., Supriatna, J., Andayani, N., Setiadi, M.I., Cannatella, D.C. and Melnick, D.J. 2003. Monkeys and toads define areas of endemism on Sulawesi. *Evolution*, **57**: 1436–1443.

Excoffier, L. 2005. *Arlequin, Version 3.0b: A Software for Genetic Data Analysis*. Geneva: Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Excoffier, L., Smouse, P.E. and Quattro, J.M. 1992. Analysis of molecular variance from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**: 479–491.

Fernandez, C.C., Shevock, J.R., Glazer, A.N. and Thompson, J.N. 2006. Cryptic species within the cosmopolitan desiccation-tolerant moss *Grimmia laevigata*. *Proc. Natl. Acad. Sci USA*, **103**: 637–642.

Folmer, O., Black, M., Hoeth, W., Lutz, R. and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotech.*, **3**: 294–299.

Futuyma, D. 1986. *Evolutionary Biology*, 2nd edn. Sunderland, MA: Sinauer Associates.

Haffner, G.D., Hehanussa, P.E. and Hartoto, D. 2001. The biology and physical processes of large lakes of Indonesia. In *The Great Lakes of the World: Food-web Health and Integrity* (M. Munawar and R.E. Hecky, eds.), pp. 183–194. Leiden: Backhuys.

Hamilton, W. 1979. *Tectonics of the Indonesian Region*. Geological Survey Professional Paper #1078. Washington, DC: US Government Printing Office.

Hey, J. 2001. The mind of the species problem. *Trends Ecol. Evol.*, **16**: 326–329.

Hope, G. 2001. Environmental changes in the late Pleistocene and later Holocene at the Wanda site, Soroako, South Sulawesi, Indonesia. *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, **171**: 129–145.

Hurwood, D.A. and Hughes, J.M. 2001. Nested clade analysis of the freshwater shrimp, *Caridina zebra* (Decapoda: Atyidae), from north-eastern Australia. *Mol. Ecol.*, **10**: 113–125.

Knowlton, N. and Weigt, L.A. 1998. New dates and new rates for divergence across the Isthmus of Panama. *Proc. R. Soc. Lond B*, **265**: 2257–2263.

Knowlton, N., Weigt, L.A., Solorzano, L.A., Mills, D.K. and Bermingham, E. 1993. Divergence of proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. *Science*, **260**: 1629–1631.

Kocher, T.D. and Carleton, K.L. 1997. Base substitution in fish mitochondrial DNA: patterns and rates. In *Molecular Systematics of Fishes* (T.D. Kocher and C.A. Stepien, eds.), pp. 13–24. London: Academic Press.

Kottelat, M. 1990a. The ricefishes (Oryziidae) of the Malili Lakes, Sulawesi, Indonesia, with the description of a new species. *Ichthyol. Explor. Freshwat.*, **1**: 151–166.

Kottelat, M. 1990b. Sailfin silversides (Pisces: Telmatherinidae) of Lakes Towuti, Mahalona and Wawontoa (Sulawesi, Indonesia) with descriptions of two new genera and two new species. *Ichthyol. Explor. Freshwat.*, **1**: 35–54.

Kottelat, M. 1991. Sailfin silversides (Pisces: Telmatherinidae) of Lake Matano, Sulawesi, Indonesia, with the description of six new species. *Ichthyol. Explor. Freshwat.*, **1**: 321–344.

Mayr, E. 1963. *Animal Species and Evolution*. Cambridge, MA: Harvard University Press.

Moritz, C. and Faith, D.P. 1998. Comparative phylogeography and the identification of genetically divergent areas for conservation. *Mol. Ecol.*, **7**: 419–429.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. and Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature*, **403**: 853–858.

Page, T.J., Baker, A.M., Cook, B.D. and Hughes, J.M. 2005. Historical transoceanic dispersal of a shrimp: the colonization of the South Pacific by the genus *Paratya* (Atyidae). *J. Biogeogr.*, **32**: 581–593.

Palumbi, S.R., Grabowsky, G., Duda, T., Geyer, L. and Tchino, N. 1997. Speciation and population genetic structure in tropical pacific sea urchins. *Evolution*, **51**: 1506–1517.

Posada, D. and Crandall, K.A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**: 817–818.

Roy, D., Docker, M.F., Hehanussa, P., Heath, D.D. and Haffner, G.D. 2004. Genetic and morphological data supporting the hypothesis of adaptive radiation in the endemic fish of Lake Matano. *J. Evol. Biol.*, **17**: 1268–1276.

Sanders, K.L., Malhota, A. and Thorpe, R.S. 2006. Combining molecular, morphological and ecological data to infer boundaries in a cryptic pitviper. *Biol. J. Linn Soc.*, **87**: 343–364.

Schluter, D., 2001. Ecology and the origin of species. *Trends Ecol. Evol.*, **16**: 372–380.

Slatkin, M. 1985. Gene flow in natural populations. *Annu. Rev. Ecol. Syst.*, **16**: 393–430.

Swofford, D.L. 1998. *PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods)*, Version 4.0. Sunderland, MA: Sinauer Associates.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**: 585–595.

Visser, K., Thundell, R. and Gozi, M.A. 2004. Glacial–interglacial organic carbon record from the Makassar Strait Indonesia: implications for regional changes in continental vegetation. *Quaternary Sci. Rev.*, **23**: 17–27.

von Rintelen, T., Wilson, A.B., Meyer, A. and Glaubrecht, M. 2004. Escalation and trophic specialization drive adaptive radiation of freshwater gastropods in ancient lakes on Sulawesi, Indonesia. *Proc. R. Soc. Lond B*, **271**: 2541–2549.

Whitten, T., Mustafa, M. and Henderson, G.S. 2000. *The Ecology of Sulawesi*. The Ecology of Indonesia Series Vol. IV. Jakarta, Indonesia: Periplus.

Woltereck, E. 1937a. Systematisch – variationanalytische Untersuchungen über die Rassen – und Artbildung bei Süßwassergarneelen aus der Gattung *Caridina* (Decapoda, Atyidae). *Intern. Rev. Ges. Hydrobiol. Hydrogr.*, **34**: 208–262.

Woltereck, E. 1937b. Zur Systematik und geographischen Verbreitung der Caridinen. *Intern. Rev. Ges. Hydrobiol. Hydrogr.*, **34**: 294–330.

Woolschot, L., Hughes, J.M. and Bunn, S.E. 1999. Dispersal among populations of *Caridina* sp. (Decapoda: Atyidae) in coastal lowland streams, south-eastern Queensland, Australia. *Mar. Freshwat. Res.*, **50**: 681–688.