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Genetic differentiation over small spatial scales in the absence of physical barriers in an Australian rainforest stream fish

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This study investigated the genetic population structure of the regionally endemic rainforest fish *Cairnsichthys rhombosomoides* by analysing sequence variation in the mitochondrial control region. The results indicated that individual populations, even those located in close proximity (5 km), were highly distinct. The pattern of genetic structure cannot be explained by physical barriers to gene flow and was not consistent with models based on separation of populations by distance or any hierarchical structuring within the riverine network. The management implications of the observed genetic structure are discussed.

Key words: divergence; freshwater fish; gene flow; mitochondrial DNA; rainforest streams.

INTRODUCTION

Investigations into the mechanisms creating population genetic structure in stream organisms, especially fish, have mostly focused on physical barriers that reduce gene flow between populations. Examples of these processes include fluctuating Pleistocene sea levels that result in intermittent seawater barriers (Hänfling *et al.*, 2002; Near *et al.*, 2003; Perdices *et al.*, 2003), volcanism leading to drainage rearrangements (McGlashan & Hughes, 2000; Stewart, 2001) and barriers to migration within drainages such as waterfalls (Currens *et al.*, 1990; Carlsson & Nilsson, 1999; Taylor *et al.*, 2003). The influence of physical barriers on gene flow generally results in a genetic signature where populations within the same tributaries or sub-catchments are more genetically similar than those in adjacent catchments (Meffe & Vrijenhoek, 1988; Keenan, 1994; Hernandez-Martich & Smith, 1997).

Biological barriers to gene flow (*e.g.* intrinsic behaviour such as philopatry or interspecific interactions such as competition or predation) can also play a role

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in the genetic structure of stream organisms. For instance, strong natal homing for spawning sites, combined with low frequency of straying, can create patterns of genetic differentiation in salmonid populations (Stabell, 1984; Elliott, 1994; Ferguson *et al.*, 1995). However, examples of strong genetic structuring, as a result of biological barriers to gene flow, remain poorly studied compared with those that cite mechanisms of a physical nature.

Cairnsichthys rhombosomoides (Nichols & Raven, 1928) is a conservationally significant, geographically restricted and regionally endemic rainbowfish of the Australian Wet Tropics, which may offer an unique opportunity to investigate the potential for biological barriers to produce patterns of genetic population structure in streams. This species has an intriguing pattern of distribution in that although it is typically restricted to and most common in small streams with an intact riparian canopy (mean catchment area <6 km^2, mean stream width <6 m and mean riparian cover 56·5%), such small streams may occur over a comparatively wide elevation range (0–100 m a.s.l.) and include headwater streams as well as short lowland adventitious tributary streams (Pusey *et al.*, 2004). Accordingly, the types of habitats in which it occurs range from headwater cascade/step pool habitats with high gradients (maximum 7·33%) to low gradient (0·02%) streams meandering through lowland coastal swamps. The type of substrate found across this range varies from being dominated by bedrock and rocks with very little organic matter through to mud and sand substrates covered in detritus and leaf litter. Average water velocities in these habitats are typically low ($<0·15$ m sec^{-1}) but may be as high as 0·5 m sec^{-1}. Heterogeneity in physico-chemical parameters is common across the range of streams inhabited by *C. rhombosomoides* to the extent that this variation encompasses all values of these same parameters observed at lower order sites where *C. rhombosomoides* is consistently absent (Pusey *et al.*, 2004). Despite there being no obvious physical barriers to dispersal, extensive sampling over the full range of stream habitats present within rivers of the region has never revealed the presence of *C. rhombosomoides* in lower order stream channels (*i.e.* those streams connecting the spatially disparate streams described) (Pusey & Kennard, 1996; Pusey *et al.*, 2004).

The conservation status of *C. rhombosomoides* has been variously listed as vulnerable or rare (Pusey *et al.*, 2004). It has been suggested that the spatially segregated distribution of *C. rhombosomoides* renders local populations susceptible to local extirpation (Pusey *et al.*, 2004, 2008) if dispersal between populations is limited. The present investigation is aimed at determining whether local populations do indeed show distinct genetic structure indicative of reduced gene flow and to suggest possible mechanisms for restricted gene flow in the absence of physical barriers.

METHODS

STUDY AREA, SITE SELECTION AND GENETIC SAMPLING

Eighty-nine individuals of *C. rhombosomoides* were collected by dip-netting during the dry season (June to August 2004) from 10 stream sites within drainages spread across most of the species' distributional range in the Queensland Wet Tropics Bioregion (Fig. 1). Sites were selected to allow three replicated distance comparisons among

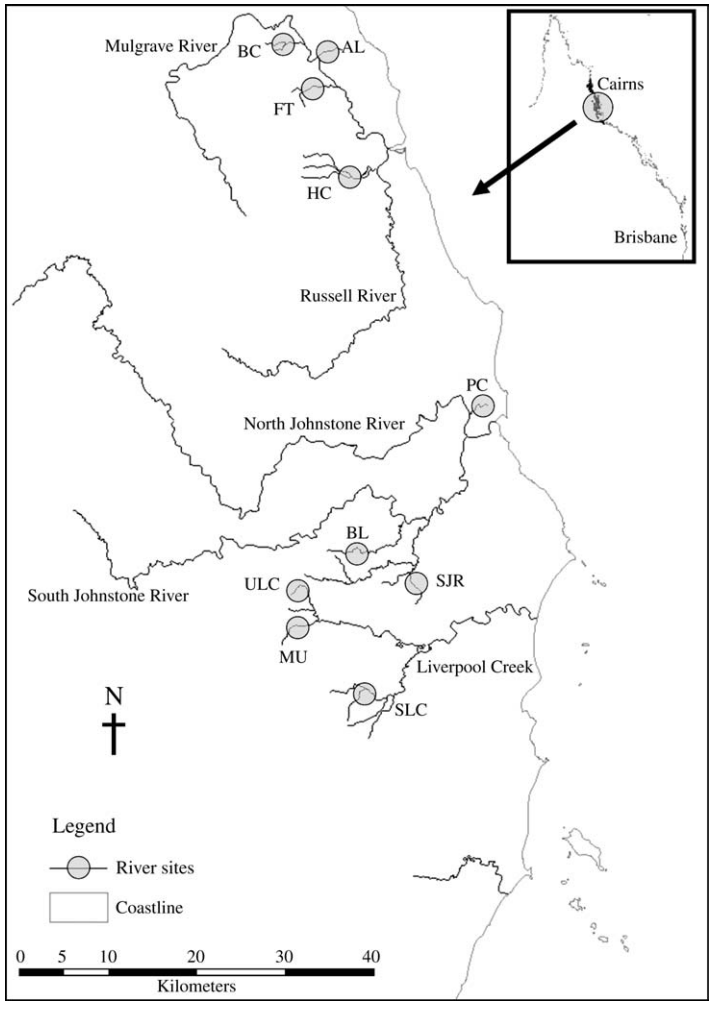

FIG. 1. Map illustrating sample sites for genetic analysis of *Cairnsichthys rhombosomoides*. Site abbreviations are as follows: Behana Creek (BC), unnamed creek at Aloomba (AL), Figtree Creek (FT) and Harvey Creek (HC); three within the South Johnstone system, Polly Creek (PC), unnamed South Johnstone Creek (SJR) and Boolabah Creek (BL); and three within the Liverpool system, Meuribah Creek (MU), Upper Liverpool Creek (ULC) and South Liverpool Creek headwaters (SLC).

populations with: (1) no saltwater barriers to movement (permanent freshwater linkage), (2) intermittent saltwater barriers to movement (estuarine confluence) and (3) full saltwater barriers to movement (inter-drainage populations). A small (*c.* 0·3 cm^2) caudal fin clip was taken from each fish. Fish were returned to the stream after completion of sampling at each site.

DNA MARKER SELECTION, DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

Total genomic DNA was extracted using a Qiagen™ DNA-Easy extraction kit and resuspended in 200 μl of elution buffer. A 400 base pair (bp) fragment of domain 1

of the mtDNA control region was amplified using the polymerase chain reaction (PCR) and two oligonucleotide primers. The heavy strand primer (MT16498H; 5′ CCT GAA GTA GGA ACC AGA TG 3′; Meyer *et al.*, 1990) is located in the conserved central domain of the control region. The light-strand primer [L19; 5′ ACC ACT AGC ACC CAA AGC TA 3′ modified from (Bernatchez & Danzmann, 1993)] occurs in the proline tRNA gene, which flanks the control region. PCR reactions contained 1·25 μl dNTPS (combined, 25 mM), 1·25 μl of 50 mM MgCl, 1·25 μl of 10× buffer ($MgCl_2$), 1·25 μl of 1 mmol of each primer, 0·125 μl Taq, 15–20 mg genomic DNA in a 12·5 μl reaction volume. Thermocycling protocols were as follows: initial denaturing 94° C for 1 min, then 30 cycles of 94° C denaturing for 30 s; 50° C annealing for 30 s; 72° C extension for 1 min and a final extension step of 72° C for 5 min; PCR product was subject to spin column purification (UltraClean™, MO BIO Inc.) following manufacturer specifications and sequenced using Big Dye™ incorporation (Applied BIOSYSTEMS, Foster City, CA, U.S.A.). Screening and purification of sequencing products occurred in an ABI Prism™ 377 automated DNA SEQUENCER (Applied BIOSYSTEMS). Bidirectional sequences were aligned by eye and edited using PROSEQ (version 2.91; Filatov 2002).

GENETIC ANALYSIS: ANALYSIS OF MOLECULAR VARIANCE

An analysis of molecular variance (AMOVA) was performed on mtDNA control region (domain 1) sequences to indirectly estimate contemporary levels of gene flow based on the current distribution of genetic diversity among populations (Excoffier *et al.*, 1992). Three different hierarchical models were considered: model A grouped populations into present drainage lines separated by oceanic saltwater barriers: *i.e.* Mulgrave/Russel, South/North Johnstone and Liverpool catchment; model B placed all lowland populations within a single group across all drainages. This model hypothesis gene flow across drainages for lowland populations during peak wet season discharge periods when freshwater extends out to sea but retains isolation of upland habitats; and model C left all populations as independent units. The relative effectiveness of the barrier proposed in each model was tested by comparing the alternative *a priori* groupings to the most parsimonious hierarchical structure by AMOVA. This analysis generates Φ_{ST}, an analogue of Wright's F_{ST} that estimates the level of genetic divergence among predefined population groupings using both the frequency and similarity of haplotypes in different populations. The significance of Φ_{ST} values was determined using 5000 permutations of a Markov chain analysis as described by Raymond & Rouset (1995) in ARLEQUIN, version 2.00 (Schneider *et al.*, 2000). The most parsimonious structure was defined as that which maximized the genetic variation among predefined groups while minimizing the variation among populations within those groups (Congdon *et al.*, 2002). When using AMOVA to assess contemporary gene flow, it is assumed that effective population sizes are at equilibrium and have not undergone recent bottlenecks or subsequent expansions (Excoffier *et al.*, 1992). This assumption was tested using a mismatch analysis (discussed below).

GENETIC ANALYSIS: ISOLATION BY DISTANCE

In addition to the predefined hierarchical group definitions, observed patterns of population genetic structure may also be explained by an inter-population distance effect. To test for such a distance relationship, the instream geographic distances (km) between all *C. rhombosomoides* populations (calculated using 1:50 000 topgraphic maps) were compared with the equivalent pair-wise genetic distances (linearized Φ_{ST} from the AMOVA) using the statistical programme isolation by distance (IBD; Bohonak 2002). A Mantel test (Manly, 1994) was used to test for significance.

GENETIC ANALYSIS: MISMATCH DISTRIBUTIONS AND RAPID POPULATION EXPANSIONS

A mismatch analysis was used to test for a recent population expansion by comparing the frequency distribution of pair-wise DNA sequence differences (mismatch distribution) between all individuals to that expected under expansion or equilibrium models (Rogers & Harpending, 1992; Congdon *et al.*, 2000; Peck & Congdon, 2004). For a population that has rapidly expanded recently, the mismatch distribution is unimodal and approximates a Poisson curve, in contrast to the multi-modal mismatch distribution of stable populations at equilibrium. Parametric bootstrapping tested observed mismatch distributions against those expected under the sudden expansion model (Excoffier & Schneider, 1999).

GENETIC ANALYSIS: TESTS OF NEUTRALITY

The population history of *C. rhombosomoides* and the potential for selection to influence the findings of the AMOVA were assessed using an array of neutrality statistics that are capable of detecting the genetic traces of selection, population growth, decline or stability. Tajima's *D* test assumes that under neutrality, a random sample of sequences (π) should have nucleotide differences that are equal to the number of differences between the segregating (polymorphic) sites only (θ). A population expansion commonly causes a significant, negative departure from zero for Tajima's *D* (Tajima, 1989*a*, *b*), while selection causes significant positive values of Tajima's *D*.

Fu's F_S tests have also proven useful for detecting population growth (Fu, 1997), by determining if a population has an excess of low frequency alleles, as expected for an expanding population. To use Fu's F_S in this way, Fu's and Li's F^* and D^* statistics were estimated. Such a comparison is necessary as a population expansion can be distinguished from the effects of background selection by the pattern produced from F_S, F^* and D^* (Fu, 1997). A significant F_S and non-significant F^* and D^* indicates a range expansion, while the inverse suggests the pattern is driven by selection (Fu, 1997; Joseph *et al.*, 2002). All mismatch and neutrality statistics were performed in DnaSP version 3, (Rozas & Rozas, 1999). An 'expansion coefficient' (S/d) was employed to assess differences between contemporary and historical population sizes, where 'S' represents the ratio of variable sequence positions, relative to the mean number of pair-wise nucleotide differences 'd' between haplotypes. A population that has recently expanded is represented by a large value, while populations that have remained stable and at equilibrium are denoted by small values (von Haeseler *et al.*, 1996).

RESULTS

MTDNA VARIATION

From the 400 bp of mtDNA control region assayed, 19 haplotypes were identified with a total of 14 variable sites (Table I) (sequences submitted to GenBank, accession number AY736195). The majority of haplotypes differed by transitional substitutions, although several differences involved transversions and three involved indels (insertions or deletions). One multibase (TA or thymine-adenine) indel found in seven haplotypes appeared to be the result of a replication error that has added or removed a single repeat unit of a small microsatellite sequence. Therefore, this indel was treated as a single mutation in further analysis.

TABLE I. Variable sites of 19 haplotypes detected within mtDNA control region (domain 1) for *Cairnsichthys rhombosomoides*. Dots indicate homology to h1, while dashes indicate indels. Drainage and site is as indicated along with numbers of haplotypes that occur at each site. HC, Harvey Creek; AL, Aloomba; BC, Behana Creek; PC, Polly Creek; SJR, unnamed creek in South Johnstone; BL, Boolabah Creek; MU, Meuribah Creek; ULC, Upper Liverpool Creek headwaters; SLC, South Liverpool Creek headwaters

Haplotype	Number of individuals per locality											Nucleotide site													
	Mulgrave/Russell River				South/North Johnstone River			Liverpool River																	
	HC	AL	BC	FT	PC	SJR	BL	MU	ULC	SLC	Total	1 5	2 3	3 2	4 8	6 6	1 3 0	1 3 1	1 3 2	1 4 6	1 5 6	1 6 0	2 6 1	2 6 2	3 5 4
h1		4	1	2	7			1		1	16	TA	C	A	T	A	T	-	T	T	G	A	C	T	A
h2	6										6	-	.	.	.	.	.	C	.	.	.	.	.	.	.
h3		5		1				5		6	17	-	.	.	.	.	.	.	.	.	.	.	.	.	.
h4		2								1	3	-	.	.	C	.	.	.	.	.	.	.	.	.	.
h5							8				8	-	.	.	.	.	.	.	.	.	.	.	.	C	.
h6									1		1	.	.	G	.	.	.	.	.	.	A	.	.	.	.
h7										1	1	-	.	.	.	.	C	.	.	C	.	.	.	.	.
h8						10		1			11	-	G	.	.	.	.	.	.	.	.	.	.	.	G
h9								1			1	-	T	.	.	.	.	.	.	.	.	.	.	.	.
h10			4	1							5	.	.	.	.	.	.	.	.	.	.	.	.	.	G
h11			2								2	.	.	.	.	G	.	.	.	.	.	.	.	.	.
h12	2			4							6	-	.	.	.	.	.	.	.	.	.	.	.	.	G
h13				2							2	.	.	.	.	.	.	C	.	.	.	.	.	.	.
h14			2								2	-	.	.	.	.	.	.	.	.	.	G	.	.	.
h15								1	2		3	-	G	.	.	.	.	.	.	.	.	.	T	.	.
h16									2		2	-	G	.	.	.	.	.	.	.	.	.	.	.	.
h17									1		1	-	.	G	.	.	.	.	.	A	.	.	.	.	.
h18										1	1	.	G	.	.	.	.	.	.	.	.	.	.	.	.
h19										1	1	.	A	.	.	.	.	.	.	.	.	.	.	.	.

AMOVA: TESTS OF GENETIC STRUCTURE

None of the three basic *a priori* hierarchical models provided the most parsimonious grouping of populations. In all three models, variation among populations within groups was significant, while variation among groups was low and non-significant (Table II). The most parsimonious structure explaining the patterns of genetic variation was obtained when all populations were left

as independent units. This analysis identified 50·45% of the variation as among-group variation (Table II). The pattern of population genetic structure among sites indicates that it was not possible to predict the level of genetic divergence between two populations based on the geographic distance that separates them ($r^2 = 0{\cdot}02$, $P > 0{\cdot}05$) (Fig. 2). Significant levels of genetic divergence occurred between pairs of freshwater-linked populations over stream distances as little as 5 km [FT (Figtree Creek)–AL (Aloomba); $\Phi_{ST} = 0{\cdot}17$; Fig. 2], with fixed differences among haplotype frequencies (no shared haplotypes) being observed between freshwater-linked populations at 39 km [BL (Boolabah Creek)–SJR (unnamed South Johnstone Creek)); $\Phi_{ST} = 1{\cdot}00$; Fig. 2]. Unique, site-specific haplotypes occurred at 13 sites (Table I), while one headwater site (BL) was fixed for a unique allele.

All within-drainage populations separated by an intermittent estuarine confluence were significantly divergent from each other (Fig. 2). The smallest in-stream spatial scale at which this occurred was 15 km (FT–HC), with both pair-wise comparisons across estuarine confluences at distances of >50 km displaying fixed differences (PC–BL and SJR–PC; FST ¼100; Fig. 3). Interestingly, two pair-wise comparisons of populations between drainages showed these populations were not significantly different from each other (AL–MU and AL–SLC). To ensure that fixed populations in the South Johnstone River did not bias the overall pattern of genetic structuring, all AMOVA and IBD analyses were repeated with the South Johnstone River populations removed. Their removal did not change the overall results obtained (data not shown).

TABLE II. AMOVA results for different hierarchical models of *Cairnsichthys rhombosomoides* populations from mtDNA control region (domain 1) sequences. *P*-values were generated using 5000 permutations. Models placed populations into groups as follows, (A) placed all populations into respective drainage lines, (B) inland headwater populations into respective drainage lines *v.* coastal lowland populations within their drainage lines and (C) same as (B) except lowland coastal populations are grouped as a single unit across all drainages

Hierarchical models	Variance component	% total variance	*F*-statistics	*P*
All populations independent	Among groups	50·45	0·51	<0·001
	Within populations	49·55	n/a	<0·001
(A) Drainage	Among groups	3·86	0·04	0·14
	Among populations within groups	42·25	0·44	<0·001
	Within populations	53·89	0·46	<0·001
(B) Inland headwater isolation/coastal lowland isolation	Among groups	9·64	0·10	0·02
	Among populations within groups	37·51	0·42	<0·001
	Within populations	52·85	0·47	<0·001
(C) Inland headwater isolation/coastal connectivity	Among groups	14·8	0·15	0·04
	Among populations within groups	32·03	0·38	<0·001
	Within populations	53·17	0·47	<0·001

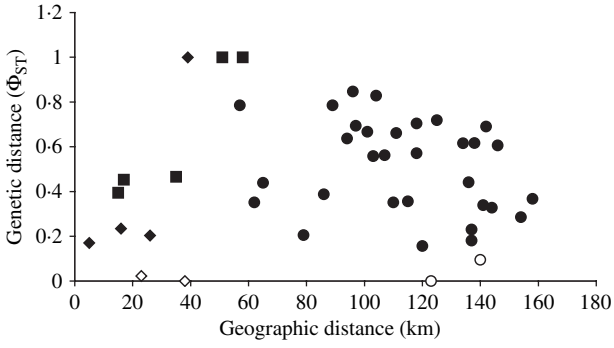

FIG. 2. Scatter plot of genetic distance (Φ_{ST}) *v.* in-stream geographic distance for *Cairnsichthys rhombosomoides*. Each symbol represents a pair-wise population comparison in one of three categories; freshwater linked populations (◆), populations with an intermittent saltwater barrier (■) and populations with permanent saltwater barriers (●). Significantly divergent population pairs ($P \leq 0{\cdot}05$) are denoted by solid symbols, while genetically non-divergent populations are denoted by open symbols.

MISMATCH DISTRIBUTIONS AND TESTS OF NEUTRALITY

A minimum spanning haplotype network of base pair changes (Fig. 3) showed a star-shaped pattern of relationships with most haplotypes being only one or two base pair changes apart. This pattern is consistent with *C. rhombosomoides* having undergone a recent and rapid population expansion (Slatkin & Hudson, 1991). Seven populations had unique site-specific haplotypes (Table I). When all *C. rhombosomoides* mtDNA sequences were pooled, the observed mismatch distribution was a close fit to that expected under a model of population expansion [Fig. 4(a)]. This same distribution departs significantly from that expected under a stable population model at equilibrium [Fig. 4(b)].

No evidence of selection in the mtDNA control region sequence used in this study was detected by the neutrality statistics: Fu's F_S statistic ($F_S = -15{\cdot}00$, $P < 0{\cdot}001$) was significant and negative, indicating an excess of recent mutations and consistent with a recent expansion (Fu, 1997). Similarly, the expansion event is supported by non-significant results for Fu's F^* and D^* (Table III). A significant and negative Tajima's D value also indicates population expansion D (Tajima, 1989*a*, *b*).

DISCUSSION

Populations of *C. rhombosomoides* examined in this study are genetically isolated and show significant spatial genetic structuring that is unrelated to geographic distance between populations or the hierarchical way in which study locations were placed in the riverine landscape. However, the results of the mismatch analysis indicate that *C. rhombosomoides* has undergone a rapid population expansion. Such a population expansion implies that the observed associations among populations may not solely reflect contemporary relationships, but that there may also be an historic signature imprinted on the population genetic structure and this possibility is supported by the anomalous finding that some

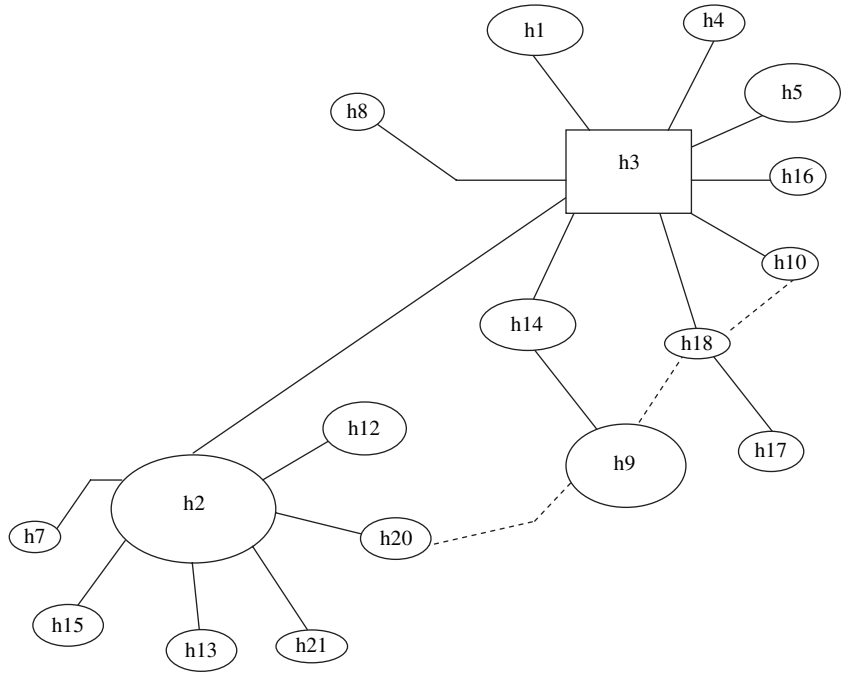

FIG. 3. Parsimony network for mtDNA control region (domain 1) for *Cairnsichthys rhombosomoides* sequences from the Wet Tropics. The size of the ovals is proportional to the number of individuals sharing a particular haplotype, while the square (h3) is shared by the most individuals. Each straight line represents one base pair change and dotted lines indicate inferred homoplasies (alternative connections).

populations, located at large inter-drainage distances across full saltwater barriers, are not significantly divergent (AL–MU and AL–SLC). Given the level of genetic divergence observed within drainages, it is unlikely that even small-scale movements are made within drainages, let alone between drainages across a saltwater marine barrier. Therefore, a feasible explanation for the observed lack of genetic differentiation between these sites is the retention of ancestral haplotypes from a period of historic connection.

Although genetic structure was not primarily associated with any *a priori* hierarchical groupings, significant divergence was common across both continuous and intermittent saltwater barriers. This agrees with studies of other freshwater fish in the region that have demonstrated the importance of oceanic salt barriers (Musyl & Keenan, 1992; Jerry, 1997; Wong *et al.*, 2004) and intermittent estuarine barriers (McGlashan & Hughes, 2000) to the creation of population genetic structure. Importantly, *C. rhombosomoides* populations with complete freshwater linkages were also significantly divergent at a level equivalent, both spatially and in terms of sequence divergence, to that of populations having physical saltwater barriers between them (Fig. 2).

That physical barriers are absent between divergent freshwater-linked sites within drainages is unequivocal. For example, many tributaries of the lower Mulgrave River, including two sampled in this study (AL and FT), are <5 km apart

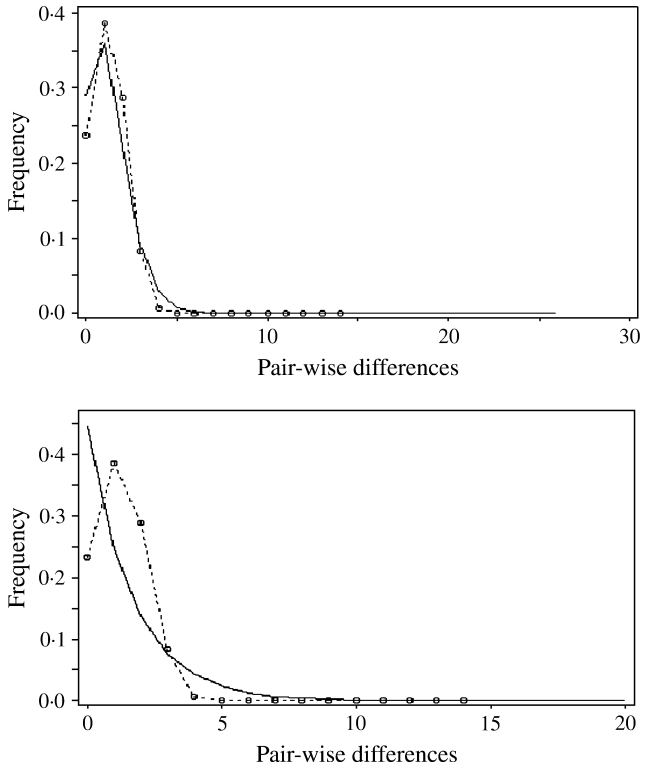

FIG. 4. Observed (——) and expected (-----) mismatch distributions for *Cairnsichthys rhombosomoides* mtDNA control region (domain 1) sequences under both (a) population expansion (upper graph) and (b) stable (lower graph) expectations.

and have populations of *C. rhombosomoides* extending to within a 100 m of the main river channel (pers. obs.). Considering the dispersal potential of other closely related freshwater fishes (Hadfield *et al.*, 1979; Wong *et al.*, 2004) physical barriers to gene flow could not be invoked to explain significant genetic structure over such small distances. This strong pattern of genetic structure, over such

TABLE III. Neutrality and population expansion indices for *Cairnsichthys rhombosomoides* calculated from mtDNA control region sequences. Significant ($P < 0{\cdot}05$) values are given in bold

	Control region (domain 1)	Expectation under	
		Selection	Range expansion
Nucleotide diversity (%)	0·12	Low	Low
Expansion coefficent (S/d)	11·33		High
Tajima's *D*	−1·24	Significant (+)	Significant (−)
Fu and Li's (1993) *F**	−2·02	Significant	NS
Fu and Li's (1993) *D**	−1·79	Significant	NS
Fu's (1997) F_S	**−15·00**	NS	Significant

small distances within catchments, is in contrast to most other investigations of freshwater fish in the region, where populations are generally differentiated between sub-catchments and/or drainages. Recent investigations have shown that two species of dryland river catfish [*Porochilus argenteus* (Zietz, 1896) and *Neosilurus hyrtlii* (Steindachner, 1867)] demonstrate population structuring independent of barriers within a drainage (Huey *et al.*, 2006). Here, the authors cite irregular flood events that spread genes across the catchment, of which only a small and potentially fixed sub-set will eventually persist in isolated waterholes once floodwaters recede, as an explanation for genetic differentiation within drainages.

It was beyond the scope of this study to determine the exact nature of putative non-physical barriers to gene flow. However, the authors suggest that there are three potential, non-mutually exclusive, mechanisms for reducing gene flow in this species:

(1) Competition: Competitive interactions with the closely related eastern rainbowfish [*Melanotaenia splendida splendida* (Peters, 1866)], an inhabitant of large streams and lowland rivers may act to reduce gene flow among *C. rhombosomoides* populations. The two species infrequently occur together and when they do, zones of sympatry are typically very narrow (Pusey *et al.*, 2004). It has been proposed that *C. rhombosomoides* is the more ancestral of the two species (McGuigan *et al.*, 2000; Sparks & Smith, 2004), implying that it is the original rainbowfish of the Wet Tropics and potentially more widely distributed within individual river systems (Pusey *et al.*, 2004). If so, it is possible that subsequent relatively recent invasion by *M. s. splendida* (Hurwood & Hughes, 2001) has acted to reduce *C. rhombosomoides* distribution through competitive interactions (Pusey *et al.*, 2004).
(2) Predation: Downstream reaches of streams inhabited by *C. rhombosomoides* have a diverse assemblage of piscivorus fishes that are mostly absent from headwaters (Pusey & Kennard, 1996). If *C. rhombosomoides* experiences strong predatory or competitive interactions within main river channels and large tributaries, it may behaviourally shift to preferentially use predator or competitor-free smaller tributaries and upper headwaters (Fraser *et al.*, 1995). If so, barriers to gene flow could develop between these upstream populations through strong selection against individuals that attempt to migrate into larger stream areas or between tributary streams.
(3) Philopatry: Finally, populations of *C. rhombosomoides* may be constrained by philopatry, an intrinsic behavioural/biological process in which fish remain in their natal or optimal habitat. Philopatry in *C. rhombosomoides* could result from preferences associated with a historic period of adaptation in isolation with the current observed distribution resulting from 'stepping stone' dispersal (Kimura & Weiss, 1964) following isolation. Alternatively, philopatry could result from the development of *in situ* preferences due to character displacement associated with either the competition or predation pressures described above. Philopatry is often invoked to explain population structuring in salmonids among drainages.

Whatever the mechanisms preventing gene flow between populations, they operate so effectively, and at such spatially restricted scales, that almost all

examined populations of *C. rhombosomoides* can be considered separate 'management units' (Moritz, 1994). Such a result has significant management implications. This species is of high conservation significance (Pusey *et al.*, 2004), and while many, but not all, upland populations reside in National Parks or within the estate of the World Heritage Area, lowland populations are not so well protected. The lowlands of the Wet Tropics Bioregion are largely given over to sugar cane cropping and urban development, resulting in significant implications for the health and integrity of aquatic systems. Moreover, lowland populations of this species are potentially threatened by alien fish species also (Pusey *et al.*, 2008). Given the small size of the streams in which *C. rhombosomoides* occurs, effective population size of isolated population units is likely to be small and inherently prone to extinction in the absence of colonization from elsewhere.

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