

Radically different scales of phylogeographic structuring within cryptic species of freshwater shrimp (Atyidae: *Caridina*)

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Abstract

We compared the phylogeographic structures of four cryptic species of freshwater shrimp from the *Caridina indistincta* complex (Atyidae) in eastern Australia using sequences of the mitochondrial gene cytochrome oxidase subunit I. We found very large differences between the species in the scales of overall geographic distribution, intraspecific divergence, and population structure. These species were characterized as either: (1) species with large ranges, low intraspecific divergence, and limited phylogeographic structuring (sp. D); (2) species with large ranges, high intraspecific divergence, and a high level of phylogeographic structuring (sp. B); (3) species with limited ranges, low intraspecific divergence, and no phylogeographic structuring (sp. E); or (4) species with limited ranges, high intraspecific divergences, and a high level of phylogeographic structuring (sp. A and sp. C [from another study]). A single haplotype of sp. D has a much larger distribution than other entire species, which have divergent intraspecific phylogroups isolated from each other at very small geographic scales. These patterns likely reflect a combination of large-scale factors, such as landscape structure and climate change, and small-scale factors, such as species-specific tolerances to local conditions and differing dispersal capabilities. Life history variation (egg size) between *Caridina* species may be linked with differing dispersal abilities. Species in this study with the smallest eggs have the least intraspecific divergence and largest distribution, whereas those with the biggest eggs have the most divergence and smallest distribution, with medium-sized egg species in between.

The landscape that freshwater creatures inhabit is highly structured (e.g., river, lake) (Poff 1997; Bohonak and Jenkins 2003) and so dispersal should be highly constrained. The isolation between river basins (catchments) and major drainage divisions (watersheds) is considered a major factor in the population structuring of many freshwater invertebrates (Hurwood and Hughes 2001; Bohonak and Jenkins 2003; Hebert et al. 2003) and fish (Avice 2000), and may even be responsible for the differentiation of Australian aboriginal languages, which also often equate to major drainage divisions (Peterson 1976). Landscape structure and long-term climate change are examples of large-scale “extrinsic”/“regional” factors (McMillen-Jackson and Bert 2003; Havel and Shurin 2004). Given the peculiar requirements of freshwater animals, such extrinsic factors (“filters” sensu Poff 1997) should severely constrain dispersal equally among taxa, and yet

some freshwater taxa are found widely. This anomaly is likely explained by species-specific responses to local environments and different life history traits (“functional species traits” sensu Poff 1997). These are examples of small-scale “intrinsic”/“local” factors (McMillen-Jackson and Bert 2003; Havel and Shurin 2004). Thus, the interplay between extrinsic and intrinsic factors will determine distributions, within-species genetic structure, and community structure (Nix 1982; Poff 1997; Havel and Shurin 2004).

The comparison between the geographic structuring of different taxa (comparative phylogeography; Avice 2000) presents an opportunity to understand some of the processes, in particular dispersal, involved in generating patterns of biodiversity. Dispersal is important because the amount of gene flow between populations should be directly related to their differentiation (Havel and Shurin 2004). If patterns are concordant between taxa, then extrinsic factors are likely to be more influential than intrinsic factors, whereas the opposite may be true should patterns be significantly different between taxa. These patterns are clearer and more meaningful when they are common (or different) between sympatric taxa (McMillen-Jackson and Bert 2003). Single-species studies cannot compare the relative importance of dispersal, and multi-species studies are even more meaningful when the taxa involved are sister taxa (Bohonak 1999), and thus a series of comparisons is possible within and between species and within and between intraspecific phylogroups. The use of population genetic techniques has allowed the presence and potential mechanisms of dispersal and recruitment to be inferred and quantified at multiple scales (Bunn and Hughes 1997; Bohonak and Jenkins 2003; Havel and Shurin 2004). The hierarchical and nested geographical structure of aquatic habitats (Poff 1997; Bohonak and

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Jenkins 2003) lends itself well to hierarchical phylogeographic analyses.

Assuming that dispersal is equivalent to gene flow (i.e., successful colonization), then highly dispersive taxa should have relatively less population divergence and more genetic homogeneity distributed across a large area. In contrast, the enforced allopatry resulting from a poor dispersal ability should lead to deep divergences, via genetic drift, which are highly geographically structured (Havel and Shurin 2004). This has been confirmed in large-scale comparative studies. Ward et al. (1994) found that marine fish had significantly less subpopulation differentiation than freshwater fish (with anadromous fish in between), and related this to the relative absence of barriers to dispersal in the sea. Bohonak (1999) compared over 300 species from varied environments (including freshwater invertebrates), with each species ranked according to inferred dispersal ability. A reduced dispersal ability was negatively correlated with population structure (i.e., more private alleles, higher F_{ST} ; F_{ST} is a measure of population structure that represents the total amount of genetic variance that occurs between populations).

A particular life history trait that has proven to be key in determining fitness for aquatic invertebrates (Hancock et al. 1998), and highly influential for both dispersal ability and population subdivision, is egg variation. Freshwater zooplankton, such as cladocerans, display differences in egg structure between species, with some possessing diapausing or barbed eggs (Havel and Shurin 2004). These eggs have increased resistance to desiccation, which should facilitate dispersal, at least at the small scale (Zeller et al. 2006).

The freshwater shrimp taxon *Caridina indistincta* Calman, 1926 (Decapoda: Atyidae) represents a complex of species and lineages, as do many cladocerans (Hebert et al. 2003), with some sympatric and some not (Chenoweth and Hughes 2003; Page et al. 2005). The presence of cryptic species is rife in Australian freshwater taxa (Page et al. in press), including within another Australian atyid shrimp, *Paratya australiensis* Kemp, 1917 (see Cook et al. 2006). *C. indistincta* is found widely in eastern Australia in rivers and lakes from the far north to the deep south. Based on a combined phylogenetic–morphological study (Page et al. 2005), there are at least five *Caridina* species (sp. A, sp. B, sp. C, sp. D, and sp. E) that, until recently, were all considered *C. indistincta* (or *C. mccullochi* J. Roux, 1926, which is a junior synonym). Surprisingly, these five species have diverse origins, with three forming a strong clade (sp. A, sp. B, and sp. C) that probably represents an ancient Australian radiation, whereas the other two both have phylogenetic ties to non-Australian taxa and probably represent two further separate colonizations of the continent (Page et al. in press).

Atyid shrimps also display a large range of different egg-related life history strategies: (1) small-sized eggs and planktonic larvae with high salinity tolerance, often estuarine; (2) medium eggs, intermediate number of larval stages, and intermediate salinity tolerance, often lowland; and (3) large eggs, direct (abbreviated) development, and low salinity tolerance, often upland (Hayashi and Hamano

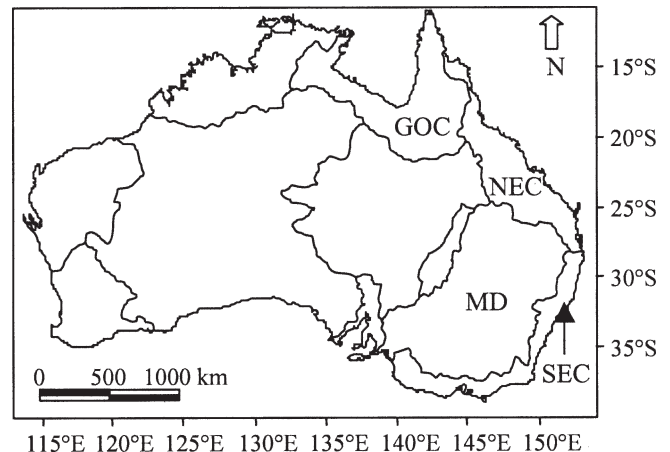


Fig. 1. Australian drainage divisions, with those sampled in this study labeled (GOC, Gulf of Carpentaria; MD, Murray-Darling; NEC, Northeast Coast; SEC, Southeast Coast).

1984; Hancock et al. 1998). Species with smaller eggs and planktonic larvae with wide tolerances should disperse more readily (Hancock et al. 1998). Egg size is highly heritable within atyids, whereas egg number may be environmentally mediated (Hancock et al. 1998). Egg size differences are evident not only between recognized species, but also between cryptic lineages and species of atyids, for both Australian *Paratya* (Hancock et al. 1998; Cook et al. 2006) and *Caridina* (Benzie 1982; Page et al. 2005). All three life history strategies are found within the five *Caridina* species relevant to this study: small eggs (sp. D and sp. E), medium eggs (sp. A and sp. B), and large eggs (sp. C) (Benzie 1982; Page et al. 2005).

The presence of multiple taxa, some sisters and some sympatric, makes this species complex an ideal model for comparative phylogeography, because many phylogeographic structure comparisons are possible at different geographic and phylogenetic levels. This means that we can attempt to assess the relative roles of intrinsic and extrinsic factors. Should patterns be common between *Caridina* taxa, then extrinsic factors should be largely responsible. In contrast, should the phylogeographic structuring vary, then intrinsic factors should predominate, and, further, relative dispersal abilities can be inferred from the scale, depth, and breadth of the observed variation. Any dispersal differences can be further broken down if the potentially important life history differences between *Caridina* taxa in egg size correlate with phylogeographic structure.

Methods

Sample collection—*C. indistincta* sensu lato were sampled from a wide area for this study (84 sites from 34 basins within four drainage divisions). This sample area, when combined with sites from published sequences, encompasses a total of 122 sites from 40 basins within four drainage divisions (Figs. 1, 2) that stretches for 2,105 km from north to south and 1,430 km from east to west (Table 1 for list by basin; Web Appendix 1 for list by site: www.aslo.org/lo/toc/vol_52/issue_3/1055a1.pdf). River ba-

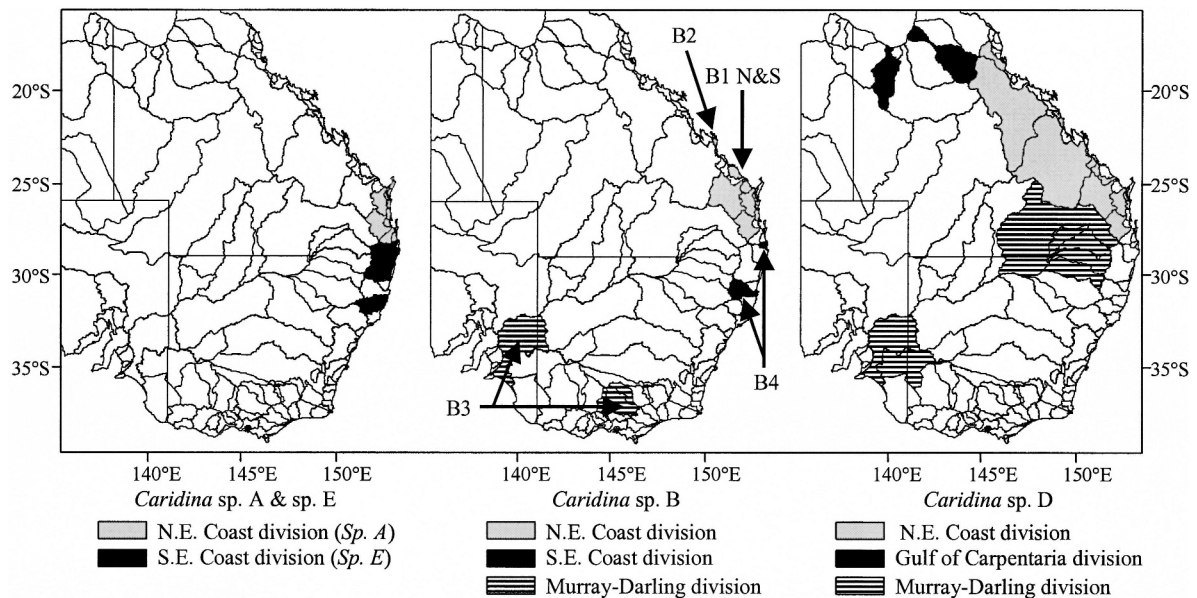


Fig. 2. River basins (and drainage divisions) from which various *Caridina* taxa were included in this study.

sins are nested within larger drainage divisions as defined by Geoscience Australia (available at: www.ga.gov.au/meta). Shrimp were caught with seines or dip nets and preserved in liquid nitrogen or 95% ethanol.

Laboratory techniques—Total deoxyribonucleic acid (DNA) was extracted and a fragment of the 3' portion of the mitochondrial cytochrome oxidase subunit I gene (COI) was amplified with the polymerase chain reaction and sequenced as per Page et al. (2005). All specimens were sequenced with the forward primer (CDC0.La; Page et al. 2005) and a quarter of specimens also with the reverse primer COIa.H (Palumbi et al. 1991). We used BigDye v.3.1 Terminator (Applied Biosystems) for the sequencing reaction, and all sequences were produced on an Applied Biosystems 3130xl Genetic Analyser at the DNA Sequencing Facility at Griffith University.

Dataset Construction—A total of 187 *C. indistincta* COI sequences were produced by this study (new Genbank accession numbers for unique haplotypes DQ788732–DQ788791). These were combined with 110 COI sequences from other phylogenetic studies (see Web Appendix 1) and aligned into a dataset of 450 base pairs. As the focus of this study is intraspecific phylogeography, the dataset was split into four sections corresponding to different *Caridina* species (sensu Chenoweth and Hughes 2003; Page et al. 2005), namely sp. A (96 sequences), sp. B (70), sp. D (108), and sp. E (23) (Table 1; Web Appendix 1). Statistics derived from sequences of a fifth *Caridina* species, sp. C (Page and Hughes in press), are included to provide context.

Genetic analyses—The best-fit model of molecular evolution, based on the Akaike Information Criterion, was selected separately for each dataset using Modeltest version 3.06 (Posada and Crandall 1998) in PAUP* version

4.0 b10 (Swofford) Distance matrices between sequences, using the relevant models, were exported from PAUP* and used for multidimensional scaling (MDS) plots in Primer version 5.2.8 (Primer-E) with 10,000 restarts, allowing visualization of phenetic groupings within each species.

Haplotype networks were constructed individually for sp. A, sp. B, and sp. D in TCS version 1.21 (Clement et al. 2000) using statistical parsimony (95% probability cutoff) to explore the genealogical relationships between specific haplotypes.

The relationships between egg size of the different species and three diversity measures ([1] within-species mean genetic divergence, [2] overall Φ_{ST} , and [3] mean number of haplotypes per basin) were assessed with a scatter plot and a Spearman nonparametric correlation in SPSS version 11.0.1 (SPSS Inc.). Egg sizes used were: sp. D and sp. E, 0.4 mm; sp. A and sp. B, 0.5–1.5 mm (mean 1.0 mm); and sp. C, 1.6 mm (Page et al. 2005).

Phylogeographic analyses—Nested clade analysis (NCA; Templeton et al. 1995) and analysis of molecular variance (AMOVA; Excoffier et al. 1992) were used to infer relationships between genetic structure and geography at the species level and within certain subspecific phylogroups. The relative contribution of individual river basin delineations and groups of basins to the partitioning of genetic variation was explored using Arlequin version 2.0 (<http://lgb.unige.ch/arlequin/>) to calculate Φ -statistics given various nested geographical hierarchies in an AMOVA (10,000 permutations for significance testing). Φ -statistics are analogous to F-statistics (e.g., F_{ST}), but instead of dealing solely with haplotype frequencies, Φ -statistics also incorporate the sequence divergence between haplotypes (Excoffier et al. 1992).

Haplotype nestings, using the rules of Templeton et al. (1987) and Templeton and Sing (1993), and latitude and longitude for each sampling site were entered into Geodis

Table 1. *Caridina* sampling areas (by basin; north to south within taxon) and DNA sequences listed by species and phylogroup with Genbank accession numbers.

Taxon	Division	Basin	Basin code	State*	Site <i>n</i>	Specimen <i>n</i>	Genbank accession numbers (haplotype <i>n</i>)
Sp. A							
CCR	NEC	Noosa†‡	NO	QLD	4	8	AF155477(1), AF155485(1), AF155491(1), DQ788748(1), DQ788749(1), DQ788750(1), DQ788752(1), DQ788753(1)
		Maroochy-Mooloolah†‡	MM	QLD	5	7	AF155482(1), AF155488(1), AF155495(1), DQ788747(2), DQ788748(2)
FIa	NEC	Gold Coast§	GC	QLD	1	1	AY794992(1)
FIb	NEC	Fraser Island§	FI	QLD	2	7	AY794995(7)
GHM	NEC	Fraser Island§	FI	QLD	2	6	AY794993(4), AY794994(1), DQ656435(1)
		Maroochy-Mooloolah†	MM	QLD	1	7	DQ788736(7)
		Glasshouse Mtns.†‡§	GM	QLD	7	16	AF155476(1), AF155483(1), AF155484(1), AF155486(5), AF155489(1), AF155490(1), AF155494(1), AY794997(1), DQ788735(3), DQ788741(1)
		Bribie Island	BI	QLD	2	2	DQ656433(1), DQ656434(1)
		Caboolture/Pine†§	CP	QLD	3	6	AY794998(1), DQ788737(1), DQ788739(3), DQ788740(1)
		Brisbane†§	BR	QLD	4	9	AY794996(3), DQ788734(3), DQ788738(2), DQ788742(1)
		Logan-Albert†	LA	QLD	1	2	DQ788732(2)
		Gold Coast§	GC	QLD	1	1	AY794999(1)
TCB	NEC	Fraser Island§	FI	QLD	2	4	AY795000(2), DQ656436(2)
		Tin Can Bay†‡	TC	QLD	9	17	AF155478(2), AF155479(1), AF155481(1), AF155487(1), AF155492(1), AF155493(1), DQ788733(4), DQ788743(1), DQ788744(1), DQ788745(2), DQ788746(1), DQ788751(1)
		Mary†‡	MA	QLD	1	2	AF155480(1), DQ788754(1)
		Glasshouse Mtns.‡	GM	QLD	1	1	AF155475(1)
Sp. B							
B1N	NEC	Baffle§	BA	QLD	1	4	AY795004(2), DQ788763(1), DQ788765(1)
		Burrum†	BM	QLD	6	13	DQ788755(2), DQ788756(1), DQ788757(1), DQ788759(1), DQ788760(1), DQ788762(1), DQ788764(1), DQ788766(2), DQ788770(1), DQ788771(1), DQ788777(1)
		Burnett†§	BN	QLD	3	4	AY795002(1), AY795003(1), DQ788758(2)
		Mary‡§	MA	QLD	4	5	AF155497(1), AY795005(1), AY795006(1), AY795007(1), DQ788761(1)
B1S	NEC	Mary‡§	MA	QLD	2	4	AF155501(1), AF155502(1), AY795001(2)
		Noosa§	NO	QLD	1	3	AY795001(3)
		Maroochy-Mooloolah‡	MM	QLD	1	1	AF155500(1)
		Brisbane†	BR	QLD	2	4	DQ788767(1), DQ788768(1), DQ788769(2)
B2	NEC	Waterpark§	WP	QLD	1	4	AY795013(4)
B3	MD	Lower Murray†¶	LM	SA	2	3	DQ681248(2), DQ788774(1)
		Campaspe†	CA	VIC	1	2	DQ788775(1), DQ788776(1)
		Goulburn-Broken§	GB	VIC	6	10	AY795008(5), AY795009(5)
B4	SEC	Tweed§	TW	NSW	1	3	AY795012(3)
		Brunswick†	BW	NSW	2	5	AY795012(3), DQ788773(2)
		Macleay§	MC	NSW	1	5	AY795010(3), AY795011(1), DQ788772(1)
Sp. D							
	GOC	Gilbert¶	GI	QLD	1	1	DQ681250(1)
		Leichhardt†	LE	QLD	1	1	DQ788790(1)
	MD	Condamine-Balonne†§	CB	QLD	6	27	AY795029(1), AY795030(16), AY795031(3), DQ788780(5), DQ788781(1), DQ788783(1)
		Moonie†	MO	QLD	1	5	AY795030(4), DQ788789(1)
		Border Rivers†	BO	QLD	1	6	DQ788782(5), DQ788782(1)
		Gwydir†	GW	NSW	3	17	AY795030(17)
		Mallee†	ML	VIC	2	3	DQ788786(3)
		Lower Murray†	LM	SA	4	7	AY795030(2), AY795031(1), DQ788782(1), DQ788786(3)

Table 1. Continued.

Taxon	Division	Basin	Basin code	State*	Site <i>n</i>	Specimen <i>n</i>	Genbank accession numbers (haplotype <i>n</i>)
	NEC	Herbert†	HE	QLD	1	2	DQ788787(2)
		Burdekin†	BU	QLD	1	1	DQ788787(1)
		Plane#	PL	QLD	1	1	DQ478462(1)
		Fitzroy-Dawson†	FD	QLD	3	7	DQ788778(3), DQ788787(4)
		Calliope†	CO	QLD	2	5	DQ788788(4), DQ788791(1)
		Boyne (coastal)†	BY	QLD	2	5	AY795030(4), DQ788779(1)
		Kolan†	KO	QLD	2	4	DQ788787(4)
		Burnett†§	BN	QLD	3	5	AY795030(5)
		Mary†	MA	QLD	2	2	DQ788784(2)
		Brisbane†	BR	QLD	5	9	AY795029(3), AY795030(6)
Sp. E	SEC	Tweed†§	TW	NSW	2	5	AY795032(5)
		Richmond§	RI	NSW	1	4	AY795032(3), AY795033(1)
		Clarence§	CL	NSW	1	4	AY795032(4)
		Nambucca§	NA	NSW	1	2	AY795032(2)
		Hastings§	HA	NSW	1	4	AY795032(4)
		Manning§	MN	NSW	1	4	AY795032(4)

* NSW, New South Wales; QLD, Queensland; SA, South Australia; VIC, Victoria.

† This study.

‡ Chenoweth and Hughes (2003).

§ Page et al. (2005).

|| Page and Hughes (in press)

¶ Page et al. (unpubl. data).

Page et al. (in press).

version 2.4 (Posada et al. 2000) to test for significant associations between geography and genetic structure. We used the November 2005 NCA Inference Key (available at: Darwin.uvigo.es/download/geodisKey_11Nov05.pdf) to attempt to differentiate historical from contemporary processes for the significant clades from the Geodis output.

Molecular divergence calculations—Two methods of calculating molecular divergences between intraspecific phylogroups were used. First, the percentage divergence between phylogroups within the MDS distance matrices was calculated including a correction for within-clade polymorphism (\pm SE). A molecular clock was applied to these divergences using a Caridean shrimp COI divergence rate of 1.4% per million yrs (Knowlton and Weigt 1998). Second, the coalescent method of Nielsen and Wakeley (2001) was used to calculate the timing of population divergences. This was implemented in MDIV (Nielsen and Wakeley 2001; available at: cbsuapps.tc.cornell.edu/mdiv.aspx) using the following parameters (model = Finite sites [HKY], cycles = 5,000,000, burn in = 10%, Mmax & Tmax = various). The highest likelihood value for scaled time was converted into years (Nielsen and Wakeley 2001) with the above COI rate. One should be wary of these sorts of calculations, in particular because the divergence rate used is from phylogenetically distant taxa. Notwithstanding, molecular clock estimates can put divergences in a relative context and can place the timings of clade divergences in a broad geological framework.

Results

Geographical ranges of species—The four different species display hugely different distributions (Table 2), from a north-to-south range of 341 km (sp. A) up to 1,907 km (sp. D); and an east-to-west range of 80 km (sp. A) up to 1,406 km (sp. B). Sp. A was found only in a restricted area of 11 basins (including one island) (Table 1), within the southernmost portion of the North-east Coast division (NEC) in southeastern Queensland (Fig. 2). Sp. E was also found in a relatively small area of six basins (Table 1) in the northernmost part of the Southeast Coast division (SEC) in northeastern New South Wales (Fig. 2). In contrast, sp. B was located over a large area in 14 basins within three divisions (Table 1), including one basin in central NEC, seven basins in the southern portion of NEC, three basins in the northern part of SEC, and a further three basins in the southern part of the inland flowing Murray-Darling division (MD) in the states of Victoria and South Australia (Fig. 2). Sp. D displays the largest range of all, being located in 18 basins within three divisions (Table 1). This includes the far north, in 2 basins of the Gulf of Carpentaria division (GOC); 10 basins from the north to the south of NEC; and 6 basins from the north and south of MD (Fig. 2). In fact, a single sp. D haplotype (AY795030) is found over a larger range (1,195 km north to south, 1,316 km east to west, within seven basins in two divisions) than most of the other entire species. For comparison, a fifth *Caridina* species (sp. C) from Page and Hughes (in press) has a very restricted distribution,

Table 2. Genetic diversity measures and geographic ranges by species and phylogroup. N.S.=not significant; N/A = not applicable.

Taxon	Division <i>n</i>	Basin <i>n</i>	Site <i>n</i>	Specimen <i>n</i>	Haplotype <i>n</i>	Intrataxon mean divergence (%) (SE)	Overall Φ_{ST} (all sig.)	Mean haplotypes/ basin	Haplotypes/ specimen <i>n</i>	N-S range (km)	E-W range (km)	Haplotypes/ N-S 100 km	Haplotypes/ E-W 100 km
Sp. A	1	11	45	96	57	2.71(0.04)	0.8970	3.63	0.59	341	80	16.72	71.25
CCR	1	3	10	16	13	1.03(0.05)	0.6292	4.67	0.81	237	48	5.49	27.08
F1a	1	1	2	7	1	0.00(0.00)	N.S.	1.00	0.14	4	2	25.00	50.00
F1b	1	1	2	6	3	0.30(0.08)	N.S.	3.00	0.50	35	16	8.57	18.75
GHM	1	7	19	43	23	1.11(0.04)	0.7434	3.29	0.53	155	80	14.84	28.75
TCB	1	4	13	24	17	1.13(0.04)	0.5098	4.25	0.71	150	47	11.33	36.17
Sp. B	3	14	34	70	40	3.90(0.08)	0.9420	2.87	0.57	1540	1406	2.60	2.84
B1 all	1	7	20	38	28	2.19(0.06)	0.8277	3.75	0.74	265	189	10.57	14.81
B1 N	1	4	14	26	22	1.56(0.05)	0.6872	5.50	0.85	233	166	9.44	13.25
B1 S	1	4	6	12	6	1.42(0.13)	0.7682	2.00	0.50	116	107	5.17	5.61
B2	1	1	1	4	1	0.00(0.00)	N.S.	1.00	0.25	N/A	N/A	N/A	N/A
B3	1	3	9	15	6	0.45(0.06)	0.7134	2.00	0.40	281	671	2.14	0.89
B4	1	3	4	13	5	0.79(0.13)	0.8541	2.00	0.38	300	78	1.67	6.41
Sp. D	3	18	40	108	19	0.93(0.06)	0.6116	1.78	0.18	1907	1349	1.00	1.41
GOC	1	2	2	2	2	0.45(0.00)	N.S.	1.00	1.00	35	241	5.71	0.83
NEC- MD	2	16	38	106	17	0.54(0.01)	0.4788	1.88	0.16	1907	1349	0.89	1.26
Sp. E	1	6	7	23	2	0.22(0.00)	N.S.	1.17	0.09	396	104	0.51	1.92
Sp. C*	1	7	27	136	28	7.31(0.26)	0.9904	4.43	0.21	278	74	10.07	37.84
C1	1	2	11	72	14	0.75(0.04)	0.8337	8.00	0.19	57	10	24.56	140.00
C2	1	5	9	45	6	0.54(0.06)	0.9627	1.40	0.13	176	77	3.41	7.79
C3	1	1	7	19	8	1.89(0.27)	0.9288	8.00	0.42	42	16	19.05	50.00

* Sequences from Page and Hughes (in press).

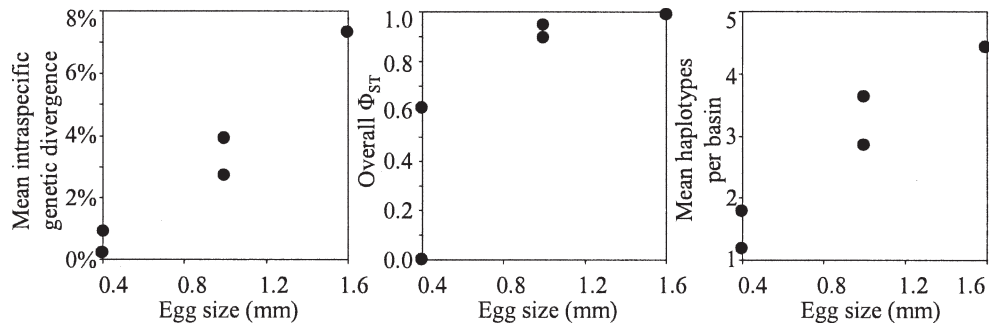


Fig. 3. Scatter plots of correlations between egg size and three diversity measures (Spearman's rho correlation coefficients for all comparisons = 0.949, $n = 5$, Sig. = 0.007).

located in seven basins (three of which are islands) in the south of NEC (278 km north to south, 74 km east to west).

The correlations between egg sizes of the different species and three diversity measures were all significant (egg size scatter plots; Fig. 3) (Spearman's rho correlation coefficients for all comparisons = 0.949, $n = 5$, significance = 0.007).

Phylogeography—Sp. A: The cryptic species display great differences in levels of intraspecific genetic divergence and diversity (Table 2), which is visible in the MDS plots (Fig. 4). Sp. A is genetically diverse and falls into five reasonably well-defined phenetic groupings, namely CCR, GHM, and TCB (all three were originally highlighted in Chenoweth and Hughes 2003 and are quite closely related to each other) and two further independent, divergent groups, F1a and F1b (Fig. 4). These phylogroups are highly geographically structured, with the divergent “F1a” and “F1b” found only on Fraser Island. GHM, TCB, and CCR are found almost entirely in different groups of contiguous basins in the south of the NEC (Table 1; Web Appendix 1). This equates to a Category I phylogeographic pattern (deep gene tree, major lineages allopatric; sensu Avise 2000). Haplotype networks were constructed for sp. A, but TCS will not join clades into a network that are too divergent to assign plausible connections, and so the output for sp. A (not displayed) was divided into three unconnected subnetworks (GHM/TCB/CCR, F1a, and F1b). An NCA was not

possible for sp. A because of a high level of homoplasy within the GHM haplotype network.

When an AMOVA was done for all the sp. A sequences with sites grouped into basins (Web Appendix 1), 41.2% of the variation was partitioned by basin ($\Phi_{CT} = 0.412$, $p < 0.001$). When a “by basin” AMOVA was done with the divergent sequences from F1a and F1b removed (so only GHM/TCB/CCR included), 47.92% of the variation was partitioned by basin ($\Phi_{CT} = 0.479$, $p < 0.001$). These same sequences were used in another AMOVA with sites grouped not by individual basins but into one of three groups of contiguous basins (north, middle, or south of sp. A range; north: FI, MA, TC; middle: MM and NO; and south: BI, BR, CP, GC, GM, and LA; basin codes in Table 1), and 50.30% of the variation was partitioned by these groups of basins ($\Phi_{CT} = 0.503$, $p < 0.001$). Therefore, the previous “by basin” partitioning for these sp. A sequences is best explained by groups of basins rather than by individual basins, largely reflecting the ranges of the intraspecific phylogroups (GHM, TCB, CCR). When sites were grouped into basins separately for each intraspecific phylogroup, Φ_{CT} values were significant only within GHM ($\Phi_{CT} = 0.234$, $p = 0.011$).

Molecular clock estimates using two different methods date the divergences between the F1a and F1b phylogroups at 4.08–4.26 million yrs ago (mya), and between each of these phylogroups and GHM/CCR/TCB at 2.60–3.11 mya. Divergences among the more closely related sp. A

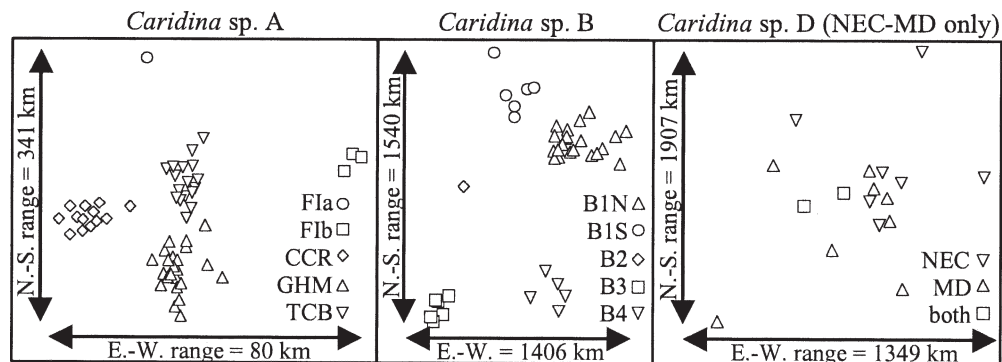


Fig. 4. Comparative MDS plots of pairwise sequence distances for sp. A, sp. B, and sp. D (NEC and MD only for sp. D), showing intraspecific phylogroups and geographical distribution sizes for each species (stress = 0.14, 0.09, and 0.18 respectively).

Table 3. Intraspecific genetic divergences between phylogroups. mya = millions of years ago.

Taxon	Corrected mean genetic divergence (SE) (%)	Divergence in mya (SE)	MDIV (mya)
Sp. A			
GHM vs. TCB	1.47(0.04)	1.05(0.03)	1.44
GHM vs. CCR	2.04(0.04)	1.45(0.03)	1.72
CCR vs. TCB	2.15(0.04)	1.53(0.03)	1.50
F1a vs. F1b	5.84(0.13)	4.17(0.09)	4.16
GHM/TCB/CCR vs. F1a	3.73(0.10)	2.67(0.07)	2.94
GHM/TCB/CCR vs. F1b	4.29(0.06)	3.07(0.04)	2.92
Sp. B			
B1 vs. B2	3.33(0.12)	2.38(0.09)	2.51
B1 vs. B3	5.49(0.06)	3.92(0.04)	3.24
B1 vs. B4	3.89(0.08)	2.78(0.06)	2.52
B1N vs. B1S	1.90(0.07)	1.36(0.05)	1.81
B2 vs. B3	4.63(0.13)	3.31(0.09)	2.94
B2 vs. B4	4.11(0.22)	2.94(0.16)	2.43
B3 vs. B4	3.69(0.06)	2.63(0.04)	1.98
Sp. D			
GOC vs. NEC-MD	2.01(0.05)	1.44(0.03)	1.11
MD vs. NEC			0.10
Sp. C*			
C1 vs. C2	12.49(0.10)	8.92(0.07)	
C1 vs. C3	9.55(0.09)	6.82(0.06)	
C2 vs. C3	5.32(0.15)	3.80(0.10)	

* Sequences from Page and Hughes (in press).

phylogroups (CCR, GHM, and TCB) range between 1.02 and 1.72 mya (Table 3).

In summary, sp. A, which has medium-sized eggs, displays deep, allopatric divergences over a small area.

Sp. B: Sp. B also has distinct phylogroups (Fig. 4; Table 2), although over a much larger geographic range than sp. A, namely B1 (further divided into B1S and B1N), B2, B3, and B4. The sp. B haplotype network also consists of divergent unconnected subnetworks, which correspond to the MDS groups B1, B2, B3, and B4 (B1 network in Fig. 5). The network for sp. B1 (Fig. 5) consists of two further phylogroups, B1N and B1S. The intraspecific phylogroups within sp. B are all isolated from each other geographically (Fig. 2), with B1 and B2 in the NEC division, B3 in MD, and B4 in SEC, although this apparent isolation may be partially explained by intervening unsampled areas. Like sp. A, this equates to a Category I phylogeographic pattern. A “by basin” AMOVA for all sp. B is significant ($\Phi_{CT} = 0.701$, $p < 0.001$), but, as for sp. A above, this is best explained by the intraspecific phylogroups (AMOVA by groups of basins hosting B1–B4: $\Phi_{CT} = 0.704$, $p < 0.001$). Pairwise molecular clock estimates of coalescence between the sp. B intraspecific phylogroups are 1.98–3.96 mya (Table 3).

There also appears to be significant genetic structure within B1 (Figs. 4, 5). This structure is divided into northern (B1N) and southern (B1S) basins within the relatively small area occupied by B1, with a single basin hosting both (i.e., the Mary basin; see Fig. 5). This can also be described as a Category I pattern at a small scale. A “by basin” Φ_{CT} for all B1’s is 0.236 ($p = 0.019$), but as above, groups of basins (north: BA, BM, BN, and MA; south: NO, MM, and BR; basin codes in Table 1), roughly equating to intrataxon phylogroup areas, partition a greater proportion of the variation than individual basins alone ($\Phi_{CT} = 0.365$, $p = 0.001$). “By basin” Φ_{CT} values within B1N and B1S individually are not significant. The lower level clades within a nested clade analysis of B1 are all insignificant, but two three-step clades are significant: 3-4 ($p = 0.001$; inference key = No. 19 “allopatric fragmentation”) and 3-5 ($p = 0.045$; No. 9 “allopatric fragmentation”). The inference for the Total Cladogram ($p < 0.001$) is “allopatric fragmentation” and equates to the split between B1N (clade 4-2, $p < 0.001$, No. 4 “restricted gene flow with isolation by distance”) and B1S (clade 4-1, $p = 0.020$, No. 12 “contiguous range expansion”). Molecular clock divergences between B1N and B1S range from 1.31 to 1.81 mya (Table 3).

Therefore, sp. B (medium-sized eggs) has deep, allopatric divergences over a large geographic area, and also contains deep, allopatric divergences within at least one phylogroup over a small area.

Sp. D: This species displays much lower diversity (Table 2) and less geographic structure than sp. A or sp. B, with two main phylogroups, NEC-MD (Figs. 4, 5) and GOC (Fig. 5; not included in Fig. 4). GOC represents the northern GOC and NEC-MD the southern and eastern NEC and MD (Fig. 2). The intraspecific variation is divided geographically between the GOC and the NEC-MD (Fig. 5), with a significant Φ_{CT} value between these geographic areas (0.894, $p = 0.001$), which, as for sp. A and sp. B, partitions more of the variation than a simple “by basin” ($\Phi_{CT} = 0.628$, $p < 0.001$) or even “by division” hierarchy ($\Phi_{CT} = 0.429$, $p < 0.001$). The GOC versus NEC-MD split is reflected in the NCA Total Cladogram ($p = 0.021$, no. 19 “allopatric fragmentation”). The split between the GOC and NEC-MD dates to about 1.11–1.47 mya (Table 3) and is also a Category I pattern.

Although the divergences and diversity within NEC-MD are low (Figs. 4, 5), only two haplotypes are shared between the divisions. This includes a central, presumably ancestral, haplotype that was found in half of all the sp. D sequenced and is within two mutational steps of every specimen of sp. D from the NEC and MD (Fig. 5). Within a nested clade analysis (Fig. 5), clade 1-6 ($p < 0.001$, no. 12 “contiguous range expansion”) represents the ancestral haplotype and those within a single step. The Φ_{CT} value between the divisions is significant (0.086, $p = 0.001$) and the MDIV estimated population divergence is 100,000 yrs (Table 3). This implies restricted gene flow and is equivalent to a Category V phylogeographic pattern (shallow gene tree, lineage distributions varied; Avise 2000), despite being on such a large geographic scale.

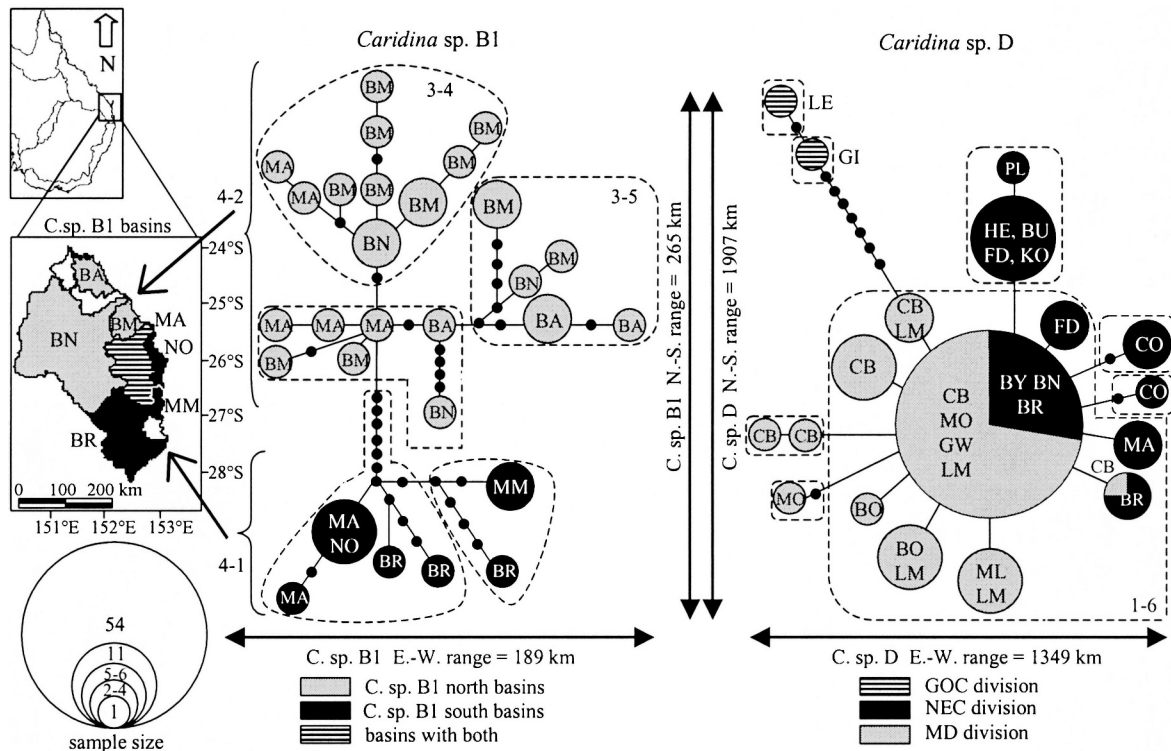


Fig. 5. Comparative haplotype networks for sp. B1 and sp. D placed in geographic context with geographical distribution sizes and basin names (see Table 1 for basin codes). Nested clade analysis clade numbers are displayed for significant clades only.

On one level, sp. D (small eggs) displays a deep divergence over a large area when one includes the GOC. However, when one considers sp. D over the overwhelming majority of its range (NEC, MD), it displays shallow divergences and limited geographic structure over a large area.

Sp. E: This species has the least diversity and no real geographic structure (Category IV; shallow gene tree, lineages sympatric; Avise 2000), implying continuing or very recent gene flow. Nearly every specimen has an identical haplotype, which is spread over six unconnected, but relatively close, coastal basins (Fig. 2). A single individual has a different haplotype, which is only one base pair divergent, and is sympatric with the common haplotype.

Sp. C: For comparison, sp. C displays the highest diversity (Table 2), with very deep divergences (Table 3) between allopatric phylogroups over a small geographic area (Page and Hughes in press), which is equivalent to a Category I pattern.

Discussion

The results from this study clearly show that the scales of geographic distribution, intraspecific diversity, and population structuring are radically different between cryptic species of very similar, and often sympatric, freshwater shrimp. Other *Caridina* studies, on much smaller geo-

graphic scales, have also found differences between taxa. For example, a study of a single species of *Caridina* (*C. zebra*) from northeastern Australia found deep intraspecific divergences, with differing ranges and diversity levels between clades (Hurwood and Hughes 2001). Yam and Dudgeon (2005) found that intraspecific divergences within one species of *Caridina* (*C. cantonensis*) from Hong Kong were on a par with interspecific divergences amongst other sympatric species. A similar situation, with varying patterns of phylogeographic structure among related, sympatric crustacean species, has been observed in mysids (Audzijonyte et al. 2006) and marine penaeid shrimp (McMillen-Jackson and Bert 2003). McMillen-Jackson and Bert (2003) report that one species shows no structure (Category IV; Avise 2000), whereas the other displays a great deal of phylogenetic structure (Category II: deep gene tree, major lineages broadly sympatric).

Extrinsic factors—Many of the “by basin” AMOVAs in the present study are significant, but when the effect of intrataxon groupings is factored out, individual river basin delineation is less important. Generally it is a group of contiguous basins that partitions the most variation, thus implying allopatric differentiation in a single basin and subsequent local recolonization of nearby basins, and the presence of a small number of major barriers, such as the Great Dividing Range for sp. A and sp. B (see below).

The methods of dispersal between basins can be the result of an event such as river capture (Hurwood and Hughes 2001; Burrige et al. 2006), temporary connections

resulting from floods (Yam and Dudgeon 2005), or even the intervention of a third party such as humans (Havel and Shurin 2004; Audzijonyte et al. 2006) or birds (Hebert et al. 2003; Figuerola et al. 2005). The presence of the common haplotype of sp. D and two phylogroups of sp. B in both the MD and the NEC (i.e., on both sides of the Great Dividing Range) implies dispersal, by whatever means, across this ancient biogeographic barrier. Similarly, lineages and sub-lineages of *Paratya australiensis* are also shared across the range (Cook et al. 2006), as are many freshwater fish (Unmack 2001).

Similar patterns between different taxa imply a common “extrinsic” historical event (comparative phylogeography; Avise 2000), but this is less likely if either the patterns are only superficially similar, or the levels of divergence very different (as within sp. B and sp. D). This situation might be explained by similar events occurring in the same location (i.e., the Great Dividing Range), but at different times (“oscillations”; Avise 2000), as has been shown within North American *Daphnia* (Hebert et al. 2003). Climatic oscillations can also influence suites of taxa at different times. For example, increasing cold and aridity during the Miocene (sp. C), Pliocene (sp. B), and Pleistocene (sp. A) probably structured allopatric populations (Table 2).

Intrinsic factors—Despite the opportunities and challenges afforded by large-scale events, such as the river capture described in BurrIDGE et al. (2006), climate change described in McMillen-Jackson and Bert (2003) or migratory bird pathways described in Figuerola et al. (2005), not every taxon responds in the same manner. This may be because of pure chance, in that a particular taxon may have not been in the right place at the right time, as may be the case for one of the fish in BurrIDGE et al. (2006), and could also explain sp. A’s absence from the MD. Another, possibly more common, explanation is that each species (and individual) responds and interacts subtly differently with its local environment (Nix 1982; Poff 1997). Thus, a potential dispersal does not necessarily equate to a successful colonization if the colonists do not survive and breed (Bohonak and Jenkins 2003). One important limiting factor for distributions is temperature tolerance, which likely defines the range limits of many freshwater fish (Unmack 2001) and *Caridina* (de Silva and de Silva 1988), and may even be a selective pressure on egg size (Hancock et al. 1998). Other local factors that have been suggested as limiting *Caridina* distributions are hydrography (Richardson et al. 2004; Cook et al. unpubl. data) and competition with other species (Poff 1997; Richardson et al. 2004). The responses displayed by a particular species to such factors can depend on the scale being investigated (i.e., stream vs. landscape; Poff 1997; Cook et al. unpubl. data). Another important variable may be salinity tolerance (de Silva and de Silva 1988; Audzijonyte et al. 2006; Cook et al. 2006). Differing levels of salinity tolerance among species may be responsible for the different phylogeographic patterns observed among mysid crustaceans (Audzijonyte et al. 2006). Thus far sp. E has been identified only in the lowland, partially saline portions of large rivers just above

the estuary, and may be hemmed in by too high salinity on one side (common for most other *Caridina*) and too low on the other. Australian *Paratya* also displays a range of salinity tolerances, and the multiple, independent life history switches from amphidromous to fully freshwater by different lineages probably explains their varying distributions (Cook et al. 2006).

Genetic consequences of life history variation—Of the three cladocerans studied in Figuerola et al. (2005), the least successful disperser was also the one with eggs least tolerant to desiccation, and further it displayed deep genetic divergence between lineages. This, and the *Paratya* mentioned above, highlights the key role of life history differences in determining the likelihood of dispersal and successful colonization (Poff 1997; Havel and Shurin 2004). There may be a link between the dispersal ability of different *Caridina* species and egg size variation, in that the species from this study with the largest distribution (sp. D) has small eggs, whereas the species with the smallest distribution (sp. C) has large eggs (Table 2). This correlation between a large egg size and a small distribution is also visible in *Caridina* elsewhere, as large-egged species are presumed to have limited dispersal abilities in Hong Kong (Yam and Dudgeon 2005) and a very limited distribution in Sri Lanka (de Silva and de Silva 1988).

If a taxon is an effective disperser, as sp. D appears to be, then one might expect a complete absence of structure, as reported for one species of marine shrimp in McMillen-Jackson and Bert (2003). Although sp. D is obviously an effective disperser, it is still highly constrained by its channelized landscape in a way that a marine shrimp is not, and so the geographic divergences within sp. D are not surprising (Fig. 5). Computer simulations on the effects of different forms of dispersal have confirmed that taxa employing long-distance dispersal have low localized divergence and yet can have remote, differentiated, populations that survive for a long time ahead of the “invasion front” (Ibrahim et al. 1996). The interplay between large-scale processes, like dispersal, and small-scale processes, like local adaptation, recruitment, and competition, will be influenced not just by the form of dispersal, but also by its rate (Bunn and Hughes 1997; Bohonak and Jenkins 2003).

It must be noted that the dispersal rate, ability, and form for each of the taxa from this study (and many of those in the comparative studies above) is not known via direct observation but only by inference. There does appear to be a relationship for these species between egg size and intraspecific divergence, haplotypes per basin, overall Φ_{ST} (all Fig. 3), distribution (Table 2), and population subdivision (Figs. 3–5). A complicating factor may be that the egg size measurements have been pooled by species and do not represent all populations (Page et al. 2005). Ongoing morphological study should refine these measurements further. For instance, the egg size we have used for both sp. A and sp. B is 0.5–1.5 mm (mean 1.0 mm), but we suspect that sp. A is likely in the top half of this range (thereby possibly explaining its small distribution), whereas sp. B is probably in the bottom half of this range. This is backed up

by Benzie's (1982) average egg measurement of *C. mccullochi* (probably equivalent to sp. B) of 0.77 mm, and so its smaller eggs may go some way to explaining sp. B's large range.

Life history or evolutionary history?—Although differences in a life history trait may result in varied population structures, it is also possible that varied population structures are the simple result of a difference in the amount of time each taxon has been associated with the relevant landscape. For example, an ancient taxon would have had a great deal of time, and many opportunities, to diverge, whereas a more recently derived (or arrived) taxon would not, and so evolutionary history rather than life history may be responsible for some of the different patterns displayed by *Caridina*. The precise ages of particular taxa are difficult to gauge, especially in the absence of fossils, but relative ages can be inferred from a phylogeny. For instance, within the clade formed by sp. A, sp. B, and sp. C, sp. C is basal and sp. A and sp. B are sister taxa (Page et al. 2005; Page et al. in press), and so sp. C is older, and sp. A and sp. B are the same age. Sp. D and sp. E are less clear, because they are divergent from both sp. A, sp. B, and sp. C and each other, and because their sister taxa are non-Australian (Sri Lankan and New Caledonian respectively) (Page et al. in press). Most likely they are more recent arrivals in Australia, and so the inferred order of species ages in Australia from youngest to oldest is likely sp. D and sp. E, sp. A and sp. B, and sp. C.

When one correlates relative species ages against the same diversity measures used for egg size in Fig. 3, the results are identical to using egg size, meaning that species age is as good an explanation of these particular diversity measures. One possibility is that the egg size of *Caridina* is related to of the amount of time a taxon has been in Australia. This is because dispersive, salt-tolerant species (i.e., small-egged species) are more likely to be the original colonizers of each lineage into Australia from the north (Page et al. in press). Adaptation to fully freshwater conditions (including the gaining of large eggs) would only have come considerably later, and so large-egged species are more likely to have a long Australian pedigree, whereas small-egged species could be either an ancient Australian species or a more recent arrival. Therefore, the average large-egged Australian species of *Caridina* should be older than the average small-egged species.

It may be true that an older taxon would have more time to diverge, but it should also be true that an older taxon would have more time to disperse widely as well, and yet the oldest species (sp. C) has the smallest range, whereas the youngest species (sp. D) has the largest. Conversely, it is possible that older species may have been confined to their present distributions by numerous large-scale events through time, to which more recent arrivals have not yet been subjected. Thus, a strictly deterministic explanation based on age is unlikely to tell the whole story.

The points raised above suggest that although one life history variable may explain a great deal of the variation between species (in range, diversity, and structure), there can be multiple competing factors. Extrinsic factors

(landscape structure and climate) set the stage upon which the different taxa must act in their own individual ways (differing tolerances, dispersal abilities, etc.). Evidently, a knowledge of both biological attributes and phylogenetic history is required to unravel comparative population structures. Fortunately, we have a reasonable understanding of the phylogenetic relationships of these taxa, and are beginning to understand their biology more fully. The radically different patterns in geographic ranges and phylogeographic structures for sympatric, cryptic species that are reported here certainly suggest the presence of at least one highly significant life history trait that varies between species. This is most likely related to egg size, and highlights the extreme differences that may result from only slight variation between species (McMillen-Jackson and Bert 2003).

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