Available online at www.sciencedirect.com
ScienceDirect
Molecular Phylogenetics and Evolution 43 (2007) 645–659
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

An island in the stream: Australia's place in the cosmopolitan world of Indo-West Pacific freshwater shrimp (Decapoda: Atyidae: *Caridina*)

Timothy J. Page [a,*], Kristina von Rintelen [b], Jane M. Hughes [a]

[a] *Australian Rivers Institute, Faculty of Environmental Sciences, Griffith University, Nathan Campus, Qld., 4111, Australia*
[b] *Museum of Natural History, Humboldt-University Berlin, Invalidenstrasse 43, D-10115, Berlin, Germany*

Received 3 May 2006; revised 5 August 2006; accepted 8 August 2006
Available online 18 August 2006

Abstract

Mitochondrial DNA sequences were used to investigate phylogenetic and biogeographic relationships among Australian freshwater shrimp from the genus *Caridina* H. Milne Edwards, 1837 (Atyidae) and congeners from potential source populations throughout the Indo-West Pacific region. Numerous Australian taxa have close evolutionary relationships with non-Australian taxa from locations throughout the region, indicating a diverse origin of the Australian freshwater fauna. This implies many colonisations to or from Australia over a long period, and thus highlights the surprising adeptness of freshwater shrimp in dispersal across ocean barriers and the unity of much of the region's freshwater biota. Interestingly, a study on Australia's other main genus of atyid shrimp, *Paratya* Miers, 1882, inferred only a single colonisation. A number of potential species radiations within Australia were also identified. This agrees with patterns detected for a large number of Australian freshwater taxa, and so implies a vicariant explanation due to the development of colder, dryer climates during the late Miocene/early Pliocene.

Keywords: *Caridina*; Atyidae; Decapoda; 16S; COI; mtDNA; Dispersal; Colonisation; Biogeography; Phylogenetics

1. Introduction

> "... it is necessary to state the relations and affinities [of taxa] to each other in order to get an idea of the peculiarities of the geographical distribution and to find out their cause."
> Ortmann (1894), *A study of the systematic and geographical distribution of the decapod family Atyidae Kingsley*

In the introduction to Ortmann's classic work on Atyidae (Crustacea: Decapoda), the author identifies the key role of phylogeny in both the systematics and biogeography of freshwater shrimp. The presumed dispersal limitations of freshwater organisms can prove useful when investigating biogeographic relationships between landmasses. In particular, we were interested in the formation of the Australian freshwater biota. Freshwater crustaceans in Australia are likely to have colonised from Asia as the Sahul shelf met the Sunda shelf in the Miocene (Bishop, 1967; Griffin and Yaldwyn, 1968; Williams, 1981; Williams and Allen, 1987; Bănărescu, 1995). We were interested in whether, how often, when and how this may have happened. This has been investigated for a few decapod genera in Australia, but with differing conclusions. Murphy and Austin (2004) (*Macrobrachium* Bate, 1868) and Gopurenko and Davie (unpublished) (*Austrothelphusa* Bott, 1969) both found multiple colonisations of Australia.

Previous studies have used either genera with limited distributions or else have not sampled widely. We chose a pantropical, common and speciose genus of freshwater shrimp, *Caridina* H. Milne Edwards, 1837 (Atyidae), because it would provide an interesting comparison to another atyid genus, *Paratya* Miers, 1882, which, in contrast to the other decapod studies above, has apparently

* Corresponding author. Fax: +61 7 3735 7615.
E-mail address: t.page@griffith.edu.au (T.J. Page).

1055-7903/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2006.08.007

only colonised Australia once (Page et al., 2005a). *Caridina* has proven to be a very useful model organism for experimental biology due to its ubiquity and variation in biology, ecology and life history (Jalihal et al., 1994). It has been used as a genetic model for systematic, ecological and biogeographic studies at smaller scales within the Indo-West Pacific (Australia: Chenoweth and Hughes, 2003; Page et al., 2005b; Hong Kong: Yam and Dudgeon, 2005; Sulawesi: von Rintelen et al., submitted for publication). In particular, *Caridina* was one of a number of taxa in the biogeographic study of Sri Lanka and the Western Ghats of India (Bossuyt et al., 2004).

Caridina is represented by many species in Australia, including both cosmopolitan and endemic species (Davie, 2002) and thus provides an excellent model for the study of colonisation and species radiation at multiple scales within the geologically complex region of the Indo-West Pacific (Hall, 2002). Molecular phylogenetics has become common in crustacean biogeographic studies; including within the Atyidae in Europe (Zakšek et al., 2007), and within other shrimp familes at the global scale (Lavery et al., 2004), in the Americas (Harrison, 2004) and in the Indo-West Pacific (de Bruyn et al., 2004). The aim of this study is to use mtDNA to infer the colonisation history of *Caridina* and to reveal how the Australian freshwater biota relates to that of the Indo-West Pacific (IWP).

2. Materials and methods

2.1. Specimen collection

Specimens of *Caridina* from Australia, Indonesia, Malaysia and Vanuatu were collected by the authors and preserved in 70–100% EtOH. Many colleagues from institutions around the world provided specimens from throughout the Indo-West Pacific (Table 1; Fig. 1). We attempted to limit the confounding effects of incomplete taxon sampling by including as many taxa and geographic areas as possible (Graybeal, 1998). We included 11 of the 13 *Caridina* taxa described in Australia (11 species, 2 subspecies; Davie, 2002), as well as a further 11 as yet undescribed Australian species (confirmed by morphology and molecules) which were discovered in either this study, Page et al. (2005b) or Chenoweth and Hughes (2003). The Australian specimens hailed from 77 sites in 58 river basins, representing all seven drainage divisions (defined by Geoscience Australia: www.ga.gov.au) from which *Caridina* are reported (Fig. 1).

Non-Australian specimens were selected from as wide an area as possible to encompass the entire worldwide distribution of *Caridina*; from South Africa and Madagascar in the west, to Iraq and Taiwan in the north, to Moorea in the east. A particular effort was made to include shrimp from nearby landmasses most likely to be a source for Australian populations, namely Papua New Guinea, various Indonesian islands, New Caledonia, Vanuatu and the Solomon Islands.

Where possible, species designations were assigned by relevant museums and taxonomists. For unidentified non-Australian specimens, a cut-off of 3% corrected 16S rDNA and 11% uncorrected COI sequence divergence was used as a rough "molecular threshold" (Lefébure et al., 2006) of taxon boundaries (derived from a combined morphological/molecular *Caridina* study, Page et al., 2005b).

2.2. DNA extraction and sequencing

Genomic DNA was extracted using a modified version of a CTAB-phenol/chloroform extraction (Doyle and Doyle, 1987). Fragments of mitochondrial (mtDNA) genes for the 3′-portion of 16S ribosomal DNA (16S rDNA) and the 3′-portion of cytochrome oxidase subunit I (COI) were amplified using the polymerase chain reaction (PCR). This is a popular and powerful combination for decapod phylogenetics at the generic level (e.g. Harrison, 2004; Lavery et al., 2004; Zakšek et al., 2007). Forward primers for the 16S PCR were 16S-F-Car (5′-TGCCTGTTTATCA AAAACATGTC-3′; von Rintelen et al., submitted for publication) or 16Sar (Palumbi et al., 1991), and reverse primers 16S-R-Car (5′-AGATAGAAACCAACCTGGCTC-3′), 16S-R-Car1 (5′- GAAAGATAGAAACTAACCTGGCT-3′; both von Rintelen et al., submitted for publication) or 16Sbr (Palumbi et al., 1991). Forward primers for the COI PCR were CDC0.La (Page et al., 2005b) or COI.f (Palumbi et al., 1991), with COIa.H (Palumbi et al., 1991) as the reverse. Cycling conditions were; 16S primer sets: 40 cycles (30 s/94 °C, 30 s/50 °C, and 30 s/72 °C); COI: 15 cycles (30 s/94 °C, 30 s/40 °C, and 60 s/72 °C) then 25 cycles (30 s/94 °C, 30 s/55 °C, and 60 s/72 °C). All individuals were sequenced in both directions with 16S primers, and a sub-set was also sequenced in both directions for COI. 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. Sequences were edited and primers removed using Sequencher 4.1.2 (Gene Codes Corporation).

2.3. Dataset construction

One hundred and twenty one specimens of *Caridina* were sequenced for 16S (54 Australian and 67 non-Australian), producing sequences of 489–545 base pairs (bp) depending on primer set (16S Genbank accession numbers from this study DQ478483–DQ478569, and DQ486900; see Table 1 for all accession numbers). To these were added 19 Australian haplotypes (from 32 specimens) (Page et al., 2005a,b) and 14 Indian and Sri Lankan sequences (Bossuyt et al., 2004) (Table 1). Four species of the atyid genus *Paratya* from Australia, Lord Howe Island, New Caledonia and the Ryukyu Islands served as an outgroup. Sequences were trimmed to include all specimens and this resulted in

Table 1
Caridina specimens and sequences listed by country with 16S rDNA and COI Genbank accession numbers and provenance of specimens

Country/area	Species	Site (Aus. Basin, Aus. State)—Provenance	Australian drainage division	GenBank Acession Nos. 16S rDNA	COI	Specimen voucher[b]
Australia						
	Caridina confusa	Molo Creek, Atherton Tableland (Johnstone, QLD)[A]	NEC	DQ478495[a]	DQ478450	QM-W22107
	Caridina gracilirostris	North Johnstone River (Johnstone, QLD)[A]	NEC	DQ478497[a]	DQ478452	QM-W19747
	Caridina indistincta A	Williams Creek (Bribie Island, QLD)[B]	NEC	DQ478498		GU-001
		Oxley Creek (Brisbane, QLD)[C]	NEC	AY795036[1]		GU-028
		Waraba Creek (Caboolture, QLD)[D]	NEC	AY795036[1]		GU-079,080
		Gerowweea Creek (Fraser Island, QLD)[C]	NEC	AY795038[1]		GU-119
		Lake Bowarrady (Fraser Island, QLD)[C]	NEC	AY795039[1a]	DQ478453	GU-608
		Ocean Lake (Fraser Island, QLD)[C]	NEC	DQ478499		GU-605
		Tallebudgera Creek (Gold Coast, QLD)[D]	NEC	AY795037[1]		GU-124
	Caridina indistincta B	Creightons Creek (Goulburn-Broken, VIC)[E]	MD	AY795041[1]		GU-P4
		Stony Creek (Goulburn-Broken, VIC)[E]	MD	AY795041[1]		GU-349,350
		8 km east of Murrays Bridge (Lower Murray River, SA)[F]	MD	DQ478500		GU-844
		Kin Kin Creek (Noosa, QLD)[B]	NEC	AY795040[1]		GU-047
		Sandy Creek, Byfield (Waterpark, QLD)[G]	NEC	AY795043[1]		GU-313,329
		Marshalls Creek (Brunswick, NSW)[B]	SEC	DQ478501		GU-345
		Macleay River, Sherwood (Macleay, NSW)[C]	SEC	AY795042[1a]	DQ478454	GU-190
	Caridina indistincta C	Rocky Creek (Fraser Island, QLD)[C]	NEC	DQ478503		GU-966
		Honeyeater Lake (Moreton Island, QLD)[C]	NEC	DQ478502[a]	DQ478455	GU-273
		Spitfire Creek (Moreton Island, QLD)[B]	NEC	AY795044[1]		GU-034
		North Pine River (Pine, QLD)[D]	NEC	AY795046[1]		GU-081
		Blue Lake (Stradbroke Island, QLD)[C]	NEC	AY795047[1]		GU-210
		Campembah Creek (Stradbroke Island, QLD)[B]	NEC	AY795045[1]		GU-116
		Bellinger River, Bellingen (Bellinger, NSW)[C]	SEC	AY795050[1a]	DQ478456	GU-471
		Nana Creek, Nana Glen (Clarence, NSW)[C]	SEC	AY795050[1]		GU-184
		Kings Creek, Bago Rd. (Hastings, NSW)[C]	SEC	AY795048[1]		GU-188
		Byron Creek, Binna Burra (Richmond, NSW)[C]	SEC	AY795049[1]		GU-418
	Caridina longirostris	Thompson Creek Tributary (Daintree, QLD)[H]	NEC	DQ478507		GU-758
		Cape Melville National Park (Jeannie, QLD)[A]	NEC	DQ478506[a]	DQ478458	QM-W25825
		Hartleys Creek (Mossman, QLD)[B]	NEC	DQ478506		GU-524
	Caridina serratirostris	Arnot Creek, N. of Ingham (Herbert, QLD)[A]	NEC	DQ478515[a]	DQ478461	QM-W22142
	Caridina sp. D	Washpool Waterhole (Flinders, QLD)[I]	GOC	DQ478525		GU-995
		Little River, Inournie (Gilbert, QLD)[B]	GOC	DQ478525		GU-842
		Alexandra River, Talawanta Crossing (Leichhardt, QLD)[B]	GOC	DQ478524		GU-794
		Gregory River, Riversleigh (Nicholson, QLD)[C]	GOC	DQ478519		GU-989
		Lawn Hill Creek, Adel's Grove (Nicholson, QLD)[C]	GOC	DQ478520		GU-990
		O'Shannassy River (Nicholson, QLD)[B]	GOC	DQ478518[a]	DQ478442	GU-951
		Staaten River, Dorunda (Staaten, QLD)[B]	GOC	DQ478525		GU-547
		Balonne River, St. George (Condamine-Balonne, QLD)[C]	MD	AY795053[1]		GU-396
		Buckinbah Weir (Condamine-Balonne, QLD)[C]	MD	AY795052[1]		GU-387
		Elbow Valley (Condamine-Balonne, QLD)[B]	MD	AY795052[1]		GU-038
		Pallal Creek (Gwydir, NSW)[C]	MD	DQ478521		GU-475
		RobinVale, Murray River (Mallee, VIC)[F]	MD	DQ478521		GU-857
		Moonie River (Moonie, QLD)[C]	MD	DQ478521		GU-405
		Lockyer Creek, Helidon (Brisbane, QLD)[B]	NEC	DQ478521		GU-150
		Burdekin River, Blue Range (Burdekin, QLD)[B]	NEC	DQ478522		GU-572

(*continued on next page*)

Table 1 (*continued*)

Country/area	Species	Site (Aus. Basin, Aus. State)—Provenance	Australian drainage division	GenBank Acession Nos. 16S rDNA	GenBank Acession Nos. COI	Specimen voucher[b]
		Reid River (Haughton, QLD)[A]	NEC	DQ478522		QM-W18264
		Rocky Dam (Plane, QLD)[B]	NEC	DQ478523[a]	DQ478462	GU-544
	Caridina sp. E	Clarence River, Cowper (Clarence, NSW)[C]	SEC	AY795051[1a]	DQ478463	GU-181
		Hastings River, Letterewe (Hastings, NSW)[C]	SEC	AY795051[1]		GU-186
		Manning River, Wingham (Manning, NSW)[C]	SEC	AY795051[1]		GU-171
		Warrell Creek (Nambucca, NSW)[C]	SEC	DQ478526		GU-195,196
		Richmond River, Woodburn (Richmond, NSW)[C]	SEC	AY795051[1]		GU-200,201
		Tweed River, Murwillumbah (Tweed, NSW)[C]	SEC	AY795051[1]		GU-425
	Caridina sp. Gulf 1	Archer River at telegraph line (Archer, QLD)[B]	GOC	DQ478530		GU-567
		Williams Waterhole, Cloncurry River (Flinders, QLD)[I]	GOC	DQ478532		GU-996
		Wenlock River, Stones Crossing (Wenlock, QLD)[B]	GOC	DQ478531		GU-584
		Coomalie Creek (Finniss, NT)[J]	TS	DQ478533[a]	DQ478464	ZMB-29240
	Caridina sp. LE	Algebuckina Waterhole, Neales River (Lake Frome, SA)[K]	LE	DQ478534[a]	DQ478465	GU-726,727
	Caridina sp. NT 1	Melville Island (Melville Island, NT)[L]	TS	DQ478537[a]	DQ478467	SAM-GU291-3
	Caridina sp. NT nilotica	Roper River at Bar (Roper, NT)[J]	GOC	DQ478510[a]	DQ478468	ZMB-29199
		Bamboo Creek (Daly, NT)[J]	TS	DQ478509		ZMB-29191
		Holmes Jungle (Finniss, NT)[M]	TS	DQ478508		NTM-CR9399
	Caridina sp. WA 1	Lower Charnley (Isdell, WA)[N]	TS	DQ478548		GU-308
		Anne Creek, Mt. Hart (Lennard, WA)[N]	TS	DQ478549[a]		GU-536
	Caridina sp. WA 2	Johnson Confluence (Drysdale, WA)[N]	TS	DQ478551[a]	DQ478474	GU-533
		Camp Creek (King Edward, WA)[N]	TS	DQ478550		GU-339
	Caridina sp. WA 3	Gnieraoora Pool (Onslow Coast, WA)[N]	IO	DQ478552		GU-307
		Millstream, Yarraloola Road (Onslow Coast, WA)[N]	IO	DQ478553[a]	DQ478475	GU-309
	Caridina sp. WA 4	Calder Yard (Fitzroy, WA)[N]	TS	DQ478554		GU-333,334
		Geike Gorge (Fitzroy, WA)[N]	TS	DQ478554		GU-337
		Mantinea Flats (Ord, WA)[N]	TS	DQ478555[a]		GU-537
	Caridina spinula	McIlwraith Range (Lockart, QLD)[B]	NEC	DQ478527[a]		GU-CI1
	Caridina thermophila	Edgbaston Station, N. of Aramac (Cooper Creek, QLD)[A]	LE	DQ478556[a]		QM-W15964
	Caridina typus	Bluewater Creek (Black, QLD)[B]	NEC	DQ478561		GU-571
		Coconut Beach (Lizard Island, QLD)[O]	NEC	DQ478562		AM-P21882
		Brandy Creek (Proserpine, QLD)[A]	NEC	DQ478562[a]	DQ478478	QM-W22202
	Caridina zebra	Davidson Creek (Tully, QLD)[P]	NEC	AY661486[2a]		GU-Z1
French Polynesia						
	Caridina cf. weberi	Opunolu River, Moorea[Q]		DQ478493[a]		GU-993
Hong Kong						
	Caridina cantonensis	Lam Truen River, Hong Kong Island[R]		DQ478487[a]	DQ478445	GU-818
	Caridina serrata	Lung Fu Shan Stream, Hong Kong Island[R]		DQ478512[a]		GU-747
	Caridina trifasciata	Task Yue Wu, New Territories[R]		DQ478557[a]	DQ478476	GU-750
India						
	Caridina sp. FB-03	Near Kanjirappalli, Kerala[S]		AY708100[3a]		FB-2264Ind
	Caridina sp. FB-19	Vikom, Kerala[S]		AY708124[3a]		FB-2328Ind
	Caridina sp. FB-20	near Sanchipuram, Tamil Nadu[S]		AY708108[3a]		FB-2281Ind
	Caridina sp. FB-21	Unknown[S]		AY708099[3a]		FB-2263Ind
	Caridina sp. FB-24	Kattakada, Kerala[S]		AY708096[3a]		FB-2260Ind

Indonesia				
Caridina brachydactyla	Kangkang, NW of Denpasar, Bali[J]	DQ478528[a]		ZMB-29346
Caridina brevicarpalis	Sangatta River, E. Kalimantan, Borneo[T]	DQ478484[a]		GU-891
Caridina cf. gracilirostris	Yehembang River, South Bali[J]	DQ478529[a]		ZMB-29099
Caridina sp. Borneo	Sangatta River, E. Kalimantan, Borneo[T]	DQ478490[a]	DQ478448	GU-892
Caridina sp. Sum 1	Martabe, Silolga, N.W. Sumatra[T]	DQ478546[a]	DQ478472	GU-938
Caridina wyckii	Tondano catchment, North Sulawesi[C]	DQ478494[a]		ZMB-29274
Iraq				
Caridina babaulti basrensis	Khur-Traad Creek, Garmat Ali River, Shatt Al-Arab[U]	DQ486900		GU-1007
Madagascar				
Caridina japonica	Namorona River, Ranomafana[A]	DQ478504[a]		QM-W25634
Malaysia				
Caridina celebensis	Sg Keliling, Pulau Tioman Island[S]	AY708127[3a]		FB-2333Mal
Caridina sp. Borneo	Besako River, Sarawak, Borneo[V]	DQ478491		ZMB-KZ241
Micronesia				
Caridina weberi	Mahnd River, Pohnpei[A]	DQ478564[a]		QM-W25035
New Caledonia				
Caridina cf. imitatrix	Déversoir[W]	DQ478489[a]	DQ478447	GU-NCI1,894
Caridina cf. nilotica	Dumbéa River (Northern Branch)[W]	DQ478492[a]	DQ478449	GU-467,468
	Pouembout[W]	DQ478492		GU-915
	Voh[W]	DQ478492		GU-917
Caridina serratirostris	Pouembout[W]	DQ478516[a]		GU-916
	Voh[W]	DQ478516		GU-918
Caridina sp. Ncal A	Coco[W]	DQ478535[a]	DQ478466	GU-908
	Pandanus[W]	DQ478536		GU-914
Caridina typus	Cap Ndoua[X]	DQ478559[a]	DQ478477	GU-871
	Coco[W]	DQ478558		GU-893
	Foret d'Ougne[X]	DQ478558		GU-868
	Pandanus[W]	DQ478558		GU-913
	Voh[W]	DQ478558		GU-919
Papua New Guinea				
Caridina cf. blancoi	Ramu River, Sepik[T]	DQ478488[a]	DQ478446	GU-929
Caridina sp. PNG A	Laloki River, Port Moresby[Y]	DQ478538[a]		GU-356-7,528
Caridina sp. PNG B	Laloki River, Port Moresby[Y]	DQ478539-40[a]	DQ478469	GU-358,529-30
Caridina sp. PNG C	Laloki River, Port Moresby[L]	DQ478541-42[a]	DQ478470	SAM-GU296-7
Philippines				
Caridina serratirostris	near Toledo City, Cebu[T]	DQ478513-14[a]	DQ478460	GU-887,888
Ryukyu Is.				
Caridina serratirostris	Ada River west of Ada, Okinawa[Z]	DQ478517[a]		GU-985
Caridina typus	River near Ibu, Okinawa[Z]	DQ478560[a]		GU-895
Solomon Is.				
Caridina brevicarpalis	Matepono, Guadalcanal[T]	DQ478485[a]		GU-881
Caridina sp. Sol 1	Lower Kwara, Guadalcanal[T]	DQ478544		GU-886
	Tinahula, Guadalcanal[T]	DQ478543[a]		GU-879
Caridina sp. Sol 2	Matepono, Guadalcanal[T]	DQ478545[a]	DQ478471	GU-880

(*continued on next page*)

Table 1 (*continued*)

Country/area	Species	Site (Aus. Basin, Aus. State)—Provenance	Australian drainage division	GenBank Acession Nos. 16S rDNA	GenBank Acession Nos. COI	Specimen voucher[b]
South Africa						
	Caridina africana	Carbis Road stream, Hayfields, Pietermaritzburg[AA]		DQ478483[a]	DQ478443	GU-699,700
Sri Lanka						
	Caridina cruzi	Imaduwa, Southern Province[S]		AY708107[3a]		FB-2279SL
	Caridina cumariae	Rozella, Central Province[S]		AY708115[3a]		FB-2303SL
	Caridina pristis	Peradeniya, Central Province[S]		AY708114[3a]		FB-2301SL
	Caridina propinqua	Ratgama Lake, Southern Province[S]		AY708117[3a]		FB-2309SL
	Caridina singhalensis	Galpalama, Central Province[S]		AY708123[3a]		FB-2325SL
	Caridina sp.FB-18	Modera, Western Province[S]		AY708095[3a]		FB-2259SL
	Caridina sp.FB-27	Kottawa, Southern Province[S]		AY708119[3a]		FB-2313SL
	Caridina typus	Rumassala, Southern Province[S]		AY708118[3a]		FB-2311SL
Taiwan						
	Caridina formosanae	Datuan River, Danshuei, Taipei County[BB]		DQ478496[a]	DQ478451	GU-987
	Caridina pseudodenticulata	Sihjiaolin, Dongshih, Taichung County[BB]		DQ478511[a]	DQ478459	GU-986
	Caridina sp. Taiwan1	Nanrenshan, Kending, Pingtung County[BB]		DQ478505[a]	DQ478457	GU-988
Vanuatu						
	Caridina brevicarpalis	Mele, Efate Island[C]		DQ478486[a]	DQ478444	GU-722,784
	Caridina typus	Mele, Efate Island[C]		DQ478563[a]		GU-721
	Caridina weberi	Valesdir, Epi Island[C]		DQ478565[a]	DQ478479	GU-720,725
Vietnam						
	Caridina sp. Viet 1	Duc Trong, Lam Dong Province[CC]		DQ478547[a]	DQ478473	GU-508-10
Outgroups						
	Paratya australiensis	Lake Crescent, Derwent, Tasmania, Australia[DD]		DQ478566[a]	DQ478480	GU-997
	Paratya howensis	First Creek, Lord Howe Island[EE]		DQ478569[a]	DQ478482	GU-FIR1
	Paratya cf. intermedia	Dumbéa River, New Caledonia[W]		DQ478567[a]	DQ478481	GU-912
	Paratya c. compressa	Okuma River, Okinawa, Ryukyu Is.[FF]		DQ478568[a]		GU-444

Specimen sources: [A]P. Davie (QLD Museum); [B]S. Choy (Natural Resources Dept. QLD); [C] Authors; [D]EHMP/Griffith University(GU); [E]B. Cook (GU); [F]J. Fawcett (GU); [G]A. Toon (GU); [H]K. Durrant (GU); [I]C. Leigh (GU); [J]M. Glaubrecht/T.von Rintelen (Berlin Museum, ZMB); [K]S. Barter (SA Water); [L]T. Laperousaz (SA Museum); [M]G. Dally (NT Museum); [N]M. Scanlon (CALM); [O]P. Berents (Australian Museum); [P]D. Hurwood (GU); [Q]R. Mazor (U.Cal.); [R]R. Yam/D. Dudgeon (U.HongKong); [S]Sequences from Bossuyt et al. (2004); [T]R. Smith (Hydrobiology); [U]M. Nasser (U.Basra); [V]F. Koehler (Berlin Museum, ZMB); [W]C. Pöllabauer (ERBIO); [X]P. Grimbacher/G.Monteith (GU/ QLD Museum); [Y]D. Robert; [Z]C. Rogers(EcoAnalaysts); [AA]R. Hart(U.KwaZulu-Natal); [BB]Hsi-Te Shih (Chung Hsing U.); [CC]D. Pham (Inst. Tropical Biology); [DD]A. Wheatley (GU); [EE]T. Moulton (U.Estado do Rio de Janeiro); [FF]Y. Cai (Raffles Museum).

Australian States: NSW, New South Wales; NT, Northern Territory; QLD, Queensland; SA, South Australia; TAS, Tasmania; VIC, Victoria; and WA, Western Australia.

Australian Drainage Divisions: GOC, Gulf of Carpentaria; LE, Lake Eyre; IO, Indian Ocean; MD, Murray-Darling; NEC, Northeast Coast; SEC, Southeast Coast; TAS, Tasmania; and TS, Timor Sea.

Sources of additional sequences: [1]Page et al. (2005b); [2]Page et al. (2005a); [3]Bossuyt et al. (2004).

[a] Included in subsequent 16S rDNA analyses.

[b] Specimen voucher locations: AM, Australian Museum; FB, Bossuyt et al. (2004); GU, Griffith University; NTM, Northern Territory Museum; QM, Queensland Museum; SAM, South Australian Museum; ZMB, Berlin Museum.

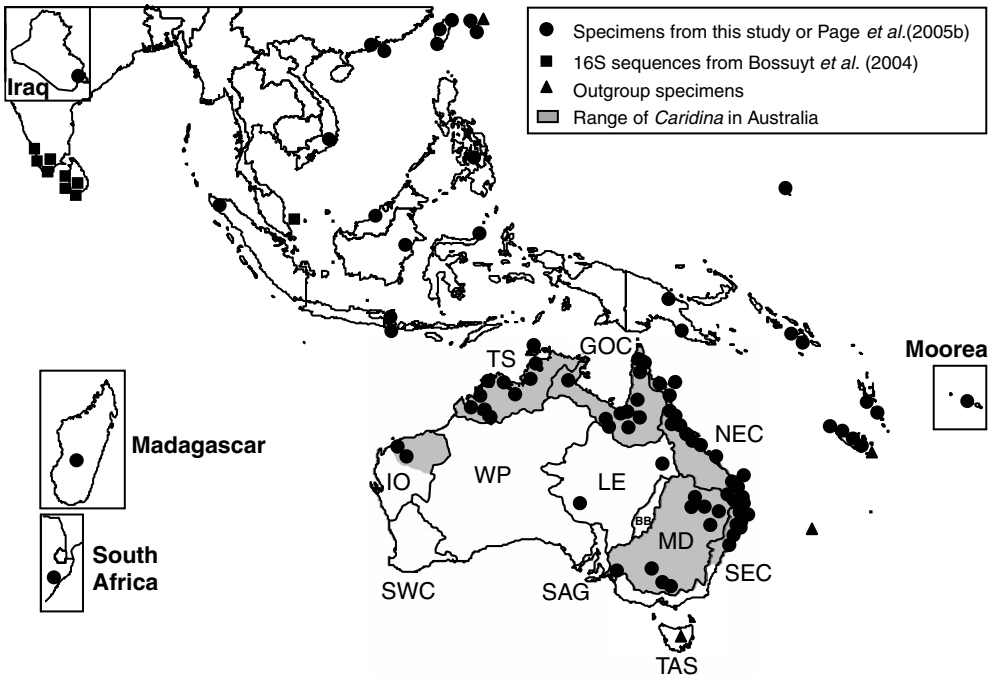

Fig. 1. Sampling locations of *Caridina* from throughout the Indo-West Pacific and Australia (see Table 1 for site details) (Australian drainage divisions: BB, Bulloo-Bancannia; GOC, Gulf of Carpentaria; LE, Lake Eyre; IO, Indian Ocean; MD, Murray-Darling; NEC, Northeast Coast; SAG, South Australian Gulf; SEC, Southeast Coast; SWC, Southwest Coast; TAS, Tasmania; TS, Timor Sea; and WP, Western Plateau).

a total of 112 unique *Caridina* and four *Paratya* 16S haplotypes.

To remove redundancy and speed subsequent analyses, a preliminary Minimum Evolution analysis of all haplotypes was performed in PAUP* version 4.0 b10 (Swofford, 2002) using Modeltest-derived model distances (Akaike Information Criterion-AIC). In the event that sequences were less than 2% divergent, came from the same landmass and formed a strong clade (>80%, most 95–100%, from 500 bootstrap pseudoreplicates), one was chosen as an exemplar for subsequent analyses as it was deemed to be part of the same evolutionary lineage in each area and would thus serve as a proxy. The final post-cull 16S dataset consisted of 72 *Caridina* and 4 *Paratya* sequences.

For the COI dataset, a sub-set of 18 Australian and 20 non-Australian specimens were sequenced and aligned with 3 outgroup *Paratya* species into a gap-free dataset of 41 unique haplotypes of 557bp (Accession Nos. DQ478442–DQ478482).

2.4. 16S rDNA sequence alignment

16S sequences from this study were aligned using ClustalX (Thompson et al., 1997) at all combinations of gap opening and extension values of 1, 6, 10 and 15. These all led to nearly identical datasets of 478 or 479bp. Default values of 15/6 for gap opening/extension were chosen giving an aligned dataset of 479 bp.

Blocks of poorly aligned sites were identified using Gblocks version 0.91b (Castresana, 2000) (Parameters = minimum number sequences for conserved position: 57, minimum number sequences for flanking position: 57, maximum number contiguous non-conserved positions: 8, minimum length of block: 6, allowed gap positions: with half). Gblocks identified 40 positions (9%) as being problematic and so these were not included in later analyses due to their suspect nature. The main areas identified by Gblocks are clustered together in the region of Helix 75, which is also the most "problematic" for phylogenetics (Buckley et al., 2000).

2.5. Phylogenetic analyses

The two datasets included in this study (16S, CO1) were analysed in the same manner but separately, because of the difference in the completeness of the datasets and the different phylogenetic levels at which the different genes are informative (as found in Harrison, 2004). The more conserved 16S dataset is more likely to establish relationships between more distant taxa, because the COI dataset has a higher likelihood of saturation (Lefébure et al., 2006). But given the aim of this paper is to establish strong relationships at the tips rather than to investigate deeper nodes, both datasets are effective. To delve into the deeper nodes would likely require the addition of nuclear genes.

Modeltest version 3.06 (Posada and Crandall, 1998) was used to select the best-fit model of evolution for both datasets (AIC). PAUP* was used to perform two analyses for each dataset: Parsimony Analysis (PA; heuristic, random addition sequence, 100 replicates) and Minimum Evolution (ME). PHYML version 2.4.4 (Guindon and Gascuel, 2003) was used for Maximum Likelihood analyses (ML). Each

dataset was bootstrapped 1000 times (PA and ME: heuristic, random addition sequence, 10 replicates). Bremer support values (BSV) were calculated for both PA analyses in TreeRot v.2 (Sorenson, 1999).

3. Results

3.1. Model selection

Within the ingroup 16S sequences (after the exclusion of the Gblocks positions), 191 bases were variable, with 157 parsimony informative. A χ^2 test of homogeneity of base frequencies across taxa found no significant difference ($P = 1.000$). Modeltest (AIC) selected the Tamura–Nei Model with a proportion of invariable sites and a Γ distribution of site-to-site variation (TrN+I+G; Γ distribution shape: 0.848; Proportion of invariable sites: 0.506; base frequencies = A:0.330, C:0.085, G:0.209, T:0.376; Rmat = 1.000, 7.951, 1.000, 1.000, 11.769).

Within the ingroup COI sequences, 239 bases were variable, with 220 parsimony informative (14% in the 1st codon position, 3% in 2nd, 83% in 3rd). χ^2 base frequencies were not significant ($P = 0.997$). For the COI dataset, Modeltest selected the General Time Reversible model (GTR+I+G; Γ distribution shape: 0.355; Proportion of invariable sites: 0.483; base frequencies = A:0.367, C:0.222, G:0.110, T:0.301; Rmat = 0.381, 20.444, 0.700, 4.633, 32.044).

3.2. Placement of Australian species in 16S and COI tree topologies

PA (best trees = 1234 steps), ME (best tree = 3.810) and ML (best tree = −6059.316) analyses for the 16S dataset were largely congruent in the strong tip clades (Fig. 2, Table 2). Fig. 2 displays a 16S PA phylogram of one of 250 trees of 1234 steps (CI = 0.288, RI = 0.618, RC = 0.178). A strict consensus of the 250 trees only collapses deeper nodes, leaving clades of interest untouched.

The relevant COI clades were congruent with those from the 16S dataset that had representatives in both datasets (Fig. 3, Table 2). Fig. 3 shows a COI PA phylogram of four trees of 2168 steps (CI = 0.206, RI = 0.367, RC = 0.076)(best ME tree = 22.604; best ML tree = −8375.205).

Australian species of *Caridina* were recovered in many independent and congruent clades (Fig. 2) by the various tree-building methods (see Table 2 for all bootstrap and BSV values for relevant clades in all analyses). Some clades were well supported (>80%) and some weakly. These include clades of solely Australian species (and so possible Australian radiations) and clades including both Australian and non-Australian specimens, making plain the cosmopolitan nature of these Australian taxa.

The fact that some of the stronger clades in the 16S dataset include both Australian and non-Australian taxa is not entirely surprising as the Australian specimen is from widespread Indo-West Pacific species (Davie, 2002) (Fig. 2); for example the "Typus" clade (Australia, Vanuatu, New Caledonia, Okinawa, Sri Lanka), "Gracilirostris" (Australia, Sulawesi, India) and "Serratirostris" (Australia, Malaysia, Okinawa, New Caledonia, Philippines). Surprisingly, strong clades containing Australian and non-Australian taxa were also found for recently uncovered cryptic species considered to be endemic (Page et al., 2005b), such as the "Sp. D" and "Sp. E" clades, as well as for undescribed species, such as "Gulf1" and *C. sp. WA4* (part of "Serratirostris").

Potential Australian radiations of species of *Caridina* were also identified in clades of Australian-only species, but these tend to have lower support than the Australian/non-Australian clades (Fig. 2; Table 2). The two strongest of these, "Indistincta" and "NT-WA" also receive support in the COI dataset (Table 2) and thus may reflect ancient and geographically widespread radiations.

Although not the focus of this paper, there are also clades evident that do not include Australian representatives (Table 2; Fig. 2). These include widespread species on different landmasses, such as *C. weberi* (Micronesia, Vanuatu) and *C. brevicarpalis* (Solomon Islands, Vanuatu, Borneo) and different species on different landmasses, such as *C. sp. PNGA*/*C. sp. Sum1* (Papua New Guinea, Sumatra) and *C. sp. Borneo*/*C.cruzi* (Borneo, Sri Lanka); and localised radiations, such as *C. serrata*/*C.trifasciata* (Hong Kong). A strong clade of note (from the preliminary 16S analysis only) includes *C. babaulti* from southern Iraq and *Caridina sp. FB-03* from southern India (99% bootstrap; 1.03% corrected 16S divergence).

4. Discussion

4.1. Biotic exchange between Australia and her neighbours

Freshwater species, theoretically at least, have severely limited dispersal abilities, because both land and sea should be effective barriers. Despite this, it is evident from this study that there has been a potentially large number of independent colonisation events of (or from) Australia by *Caridina* over the course of many millions of years. Australia appears not to be merely a source or sink, but is an integrated part of a much wider Indo-West Pacific freshwater decapod biota. This highlights the surprisingly good dispersal abilities of small, freshwater shrimp and the important role of dispersal in the biogeographic patterns of much of the Indo-West Pacific freshwater biota (de Queiroz, 2005).

Australian and various non-Australian *Caridina* taxa form many separate clades, both shallow and deep (Fig. 2), suggesting both recent and ancient colonisation events. The relationships between Australian and non-Australian *Caridina* can be characterised in two ways. Firstly, cosmopolitan IWP clades containing Australian representatives, which presumably disperse readily, easily and often. The shallow and strong clades "Typus" and

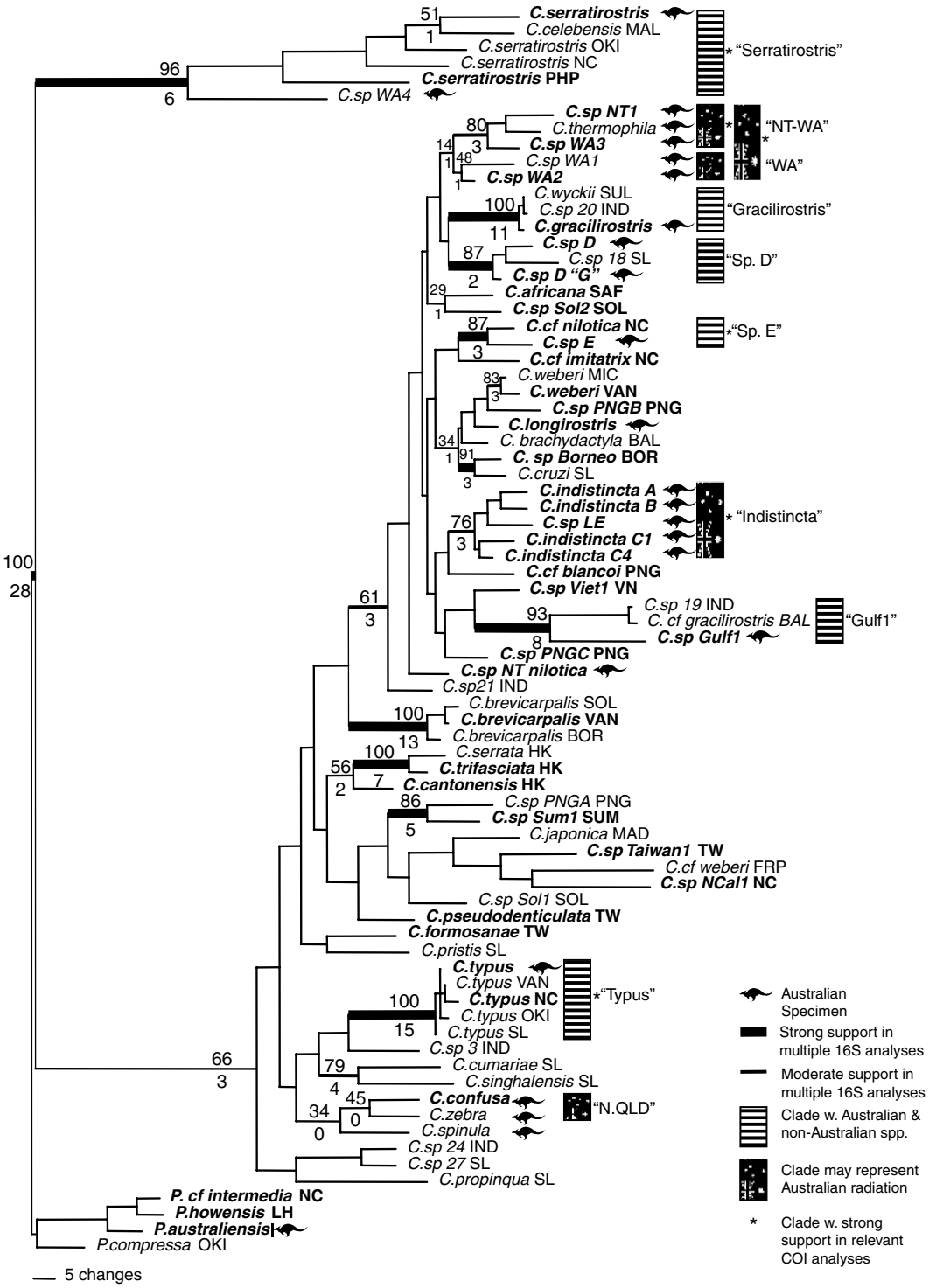

Fig. 2. Parsimony analysis (PA) phylogram of 16S rDNA dataset, showing specimen geographic origin, selected clades and support levels from other analyses (bootstrap values above node and Bremer support values below) (Specimens in bold also in COI analysis) (geographic codes: , Australia; BAL, Bali; BOR; Borneo; FRP, French Polynesia; HK, Hong Kong; IND, India; MAD, Madagascar; MAL, Malaysia; MIC, Micronesia; NC, New Caledonia; OKI, Okinawa, PHP, Philippines; PNG, Papua New Guinea; SAF, South Africa; SL, Sri Lanka; SOL, Solomons; SUL, Sulawesi; SUM, Sumatra; TW, Taiwan; VAN, Vanuatu; and VN, Vietnam).

"Gracilirostris" are typical of this type. Members of these clades are closely related despite their huge ranges (Australia, Vanuatu, New Caledonia, Okinawa, Sri Lanka and Australia, Sulawesi, India respectively). Their Australian presence probably dates to colonisation events during the Pleistocene, or may even be as a result of ongoing gene flow. These two cases are unsurprising given their spread throughout the Indo-West Pacific and a tolerance of brackish conditions (Choy and Marshall, 1997; Davie, 2002).

Table 2
Bootstrap and Bremer support values for selected clades in all analyses

Clade	16S rDNA				COI			
	MP (%)	BSV	ME (%)	ML (%)	MP	BSV	ME	ML
Colonisations								
"Gracilirostris"	100	11	100	100	N/A	N/A	N/A	N/A
"Gulf1"	93	8	96	94	N/A	N/A	N/A	N/A
"Serratirostris"	96	6	97	99	90%	9	96%	99%
"Sp. D"	87	2	94	94	N/A	N/A	N/A	N/A
"Sp. E"	87	3	92	91	97%	10	99%	99%
"Typus"	100	15	100	100	100%	27	100%	100%
Australian radiations								
"Indistincta"	76	3	77	86	82%	7	86%	89%
"N.QLD"	45	0	52	64	N/A	N/A	N/A	N/A
"NT-WA"	80	3	77	84	68%	4	75%	74%
"WA"	48	1	43	58	N/A	N/A	N/A	N/A
"NT-WA All"	14	1	0	21	52%	4	92%	83%
Non-Australian clades								
"C.brevicarpalis"	100	13	100	100	N/A	N/A	N/A	N/A
"Csp.Borneo/cruzi"	91	3	82	93%	N/A	N/A	N/A	N/A
"Csp.PNGA/Sum1"	86	5	86	90	N/A	N/A	N/A	N/A
"C.serrata/trifasciata"	100	7	99	100	N/A	N/A	N/A	N/A
"C.weberi"	83	3	55	86	N/A	N/A	N/A	N/A

PA, parsimony analysis; BSV, bremer support value; ME, minimum evolution; and ML, maximum likelihood.

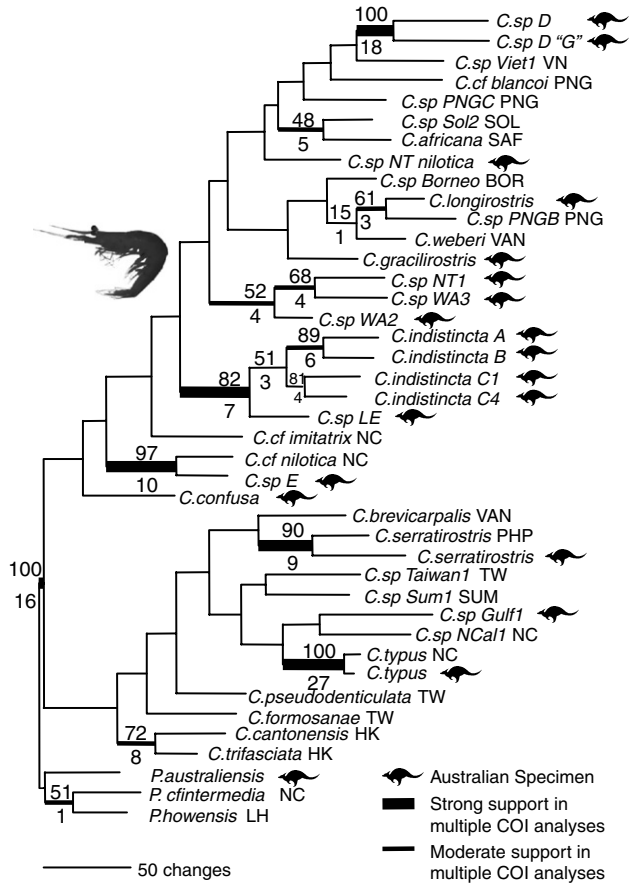

Fig. 3. Parsimony analysis (PA) phylogram of COI dataset, showing specimen geographic origin, and clade support levels (only for clades with Bremer support values >1) (bootstrap values above node and BSV below) (see Fig. 2 legend for geographic codes).

The other form of relationship apparent is that of an Australian representative of a widespread, ancient lineage. The two deepest of these are "Gulf1" (Australia, Bali, India) and "Serratirostris" (Australia, Malaysia, Okinawa, New Caledonia, Philippines), with "Serratirostris" probably representing two separate Australian colonisations. *Caridina serratirostris* is also widely reported in the IWP, but appears to be much more highly differentiated on each landmass than *C. typus* or *C. gracilirostris*.

"Sp. D" (Australia, Sri Lanka) and "Sp. E" (Australia, New Caledonia) represent clades with an intermediate level of divergence. Williams (1981) considered that there were two primary routes by which *Caridina* may have entered Australia; from Southeast Asia to the north (as per most of the above examples) or from the Pacific to the east (as per "Sp. E"). Australian and New Caledonian *Paratya* coalesce between $3\frac{1}{2}$ and 8 million years ago (Page et al., 2005a) and an Australian *Macrobrachium* also forms a clade with a New Caledonian species (Murphy and Austin, 2004). Turner et al. (2001) pointed out that taxa with a Pacific origin further confused the attempt to tease out the interchange of taxa between Australia and Asia.

There is a strong relationship evident within the "Sp. D" clade of two Australian taxa and one from Sri Lanka. The clades identified between these areas most likely represent widespread lineages that have unsampled members within the vast, speciose area between Australia and South Asia.

Many authors have split the Australian freshwater decapod fauna into those groups with Gondwanan affinities (e.g. parastacid crayfish) and those with Indo-West Pacific/Asian affinities (Bishop, 1967; Griffin and Yaldwyn, 1968; Williams, 1981; Williams and Allen, 1987; Bănărescu,

1995). Three groups in particular are often mentioned as likely to have colonised Australia from the north as the Australian Sahul shelf crashed into the Asian Sunda Shelf (starting 25 MYA; Hall, 2002), namely the Palaemonidae (e.g. *Macrobrachium*), Atyidae (e.g. *Paratya* and *Caridina*) and Parathelphusidae (e.g. *Austrothelphusa*) (Bishop, 1967; Williams and Allen, 1987).

The original dispersal of these taxa was from outside Australia to inside, but subsequent colonisations could as easily have progressed from Australia towards her new neighbours. A molecular study of *Macrobrachium rosenbergii* showed gene flow from Australia to the Philippines (de Bruyn et al., 2004) and a parsimony analysis of endemism of many terrestrial taxa in this complex contact area between two continental faunas identified dispersal in both directions (Turner et al., 2001).

Molecular studies have confirmed the polyphyletic nature of Australian *Macrobrachium* (see Murphy and Austin, 2004), *Caridina* (this study) and *Austrothelphusa* (see Gopurenko and Davie, unpublished). Probably the most interesting comparison for *Caridina* is with another atyid genus, *Paratya*. This genus is found throughout eastern Australia, often in sympatry with *Caridina*, and is morphologically very similar. Despite this, it appears to have a very different biogeographic history in that there was only a single colonisation of the Australian continent during the late Miocene/Early Pliocene (Page et al., 2005a).

4.2. Radiation of Caridina within Australia

Upon reaching Australia, there are a number of competing factors constraining the likelihood of speciation and radiation of *Caridina* taxa. Fostering speciation is Australia's large landmass and great variety of ecological niches (Williams and Allen, 1987; Short, 2004). Australia's extreme and variable aridity over large portions of the continent, especially in the interior, could make successful movement of a freshwater species problematic. But given a successful colonisation of a new area, aridity would also lead to isolation and possible allopatric speciation (Ponder, 1986; Williams and Allen, 1987; Unmack, 2001). In contrast, the very low topographical relief of most of the landscape could lead to a relative ease of movement and genetic homogenisation (Williams and Allen, 1987; Short, 2004).

Assuming there has been colonisation and radiation in Australia, the first question must be where did each lineage enter Australia? We saw above that most of the colonisations by *Caridina* came from the north, which would make northern Australia the most likely entrance point; from the Pilbara in the west (Indian Ocean Division, south of Bali), through the Kimberleys and coastal Northern Territory (Timor Sea Division, south of Timor and West Papua respectively), east to Cape York (Gulf of Carpentaria and Northeast Coast Divisions, south of Papua New Guinea) (Fig. 4).

Short (2004) suggested that many *Macrobrachium* lineages entered Australia via Cape York because the

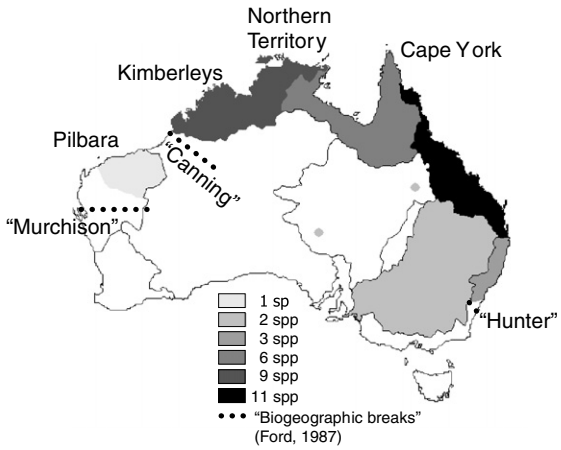

Fig. 4. Species richness of Australian *Caridina* by drainage division, important geographic regions and recognised biogeographic breaks (Ford, 1987). Includes all species from Davie (2002), this study, and two further undescribed species not included in this study (one from GOC and one from TS).

northeast has the highest species diversity. Freshwater fish (Unmack, 2001) also have high species diversity in this area, as well as in coastal Northern Territory. Both authors also highlight that as one moves south and west away from potential source populations and into many arid areas, that there is lower diversity. This pattern also holds for *Caridina* (Fig. 4), which has the highest diversity in the Northeast Coast division, and also high diversity in the other northern drainage divisions, except for Indian Ocean (Pilbara). It is most likely that there were successful colonisations of *Caridina* throughout northern Australia to the east of the Kimberleys. Of course, differences in latitudinal species richness and environments are also likely to play a role in geographical variation in species diversity.

The Australian members of the "colonisation clades" above, with the exception of *C. sp. E* (which has a New Caledonian affinity), are found almost exclusively in northern Australia: *C. serratirostris* (Northeast Coast: NEC), *C. typus* (NEC), *C. sp. D* (NEC, Gulf of Carpentaria: GOC, Murray-Darling: MD), *C. gracilirostris* (NEC, Timor Sea: TS), *C. sp. Gulf1* (GOC) and *C. sp. WA4* (TS), further suggesting a colonisation from the north.

There are a number of possible Australian radiations identified in this study (Fig. 2). These should be treated with some caution because some are not well-supported and it is possible that unsampled non-Australian specimens could interpose between specimens of a "radiation." Despite this, a few potential radiations warrant our attention. A particularly interesting example is the "NT-WA" clade. This includes (1) a heat-adapted species, *C. thermophila*, whose entire range encompasses some springs in the arid Lake Eyre Basin (Riek, 1953); (2) *C. sp. NT1*, a species from Melville Island off the northwest coast of the Northern Territory (Timor Sea); (3) and the only species so far found in the Indian Ocean Division, *C. sp. WA3*. This intriguing relationship covers a large

geographical area but is well-supported in both datasets. *Caridina thermophila* did not colonise the Lake Eyre Basin via subterranean pathways as its eyes are well developed (Riek, 1953). Most of the area between them is currently very dry. The presumption must be that *C. thermophila* somehow colonised the interior many millions of years ago under a more benign climate regime and was subsequently isolated, as has been shown for spring-associated snails (Perez et al., 2005).

The Western Plateau Division bisects "NT-WA", with *C. sp. WA3* the only known species of *Caridina* to the west. This area has almost no permanent surface water (Williams and Allen, 1987). It hosts a well known biogeographic break known as the "Canning" (Ford, 1987) (Fig. 4), which is also the furthest west reached by *Macrobrachium* (Short, 2004). Unmack (2001) stresses the uniqueness of the Pilbara freshwater fish fauna and hypothesises that this is due to the onset of aridity.

It is currently unclear precisely where *Caridina*'s range ends in the southwest, but it appears to be somewhere to the north of the "Murchison" biogeographic break (Ford, 1987) (Fig. 4). The far southwest has a distinct and depauperate freshwater fauna which is probably due to the isolating effect of aridity (Bunn and Davies, 1990).

Another interesting radiation is "Indistincta" in eastern Australia, made up of (1) a widespread species, found in three drainage divisions (*C. sp. B* in NEC, SEC and MD drainages); (2) a very diverse species (in molecular terms) found in two divisions that may host cryptic species within it (*C. sp. C* in southern NEC and northern SEC); (3) a restricted species (*C. sp. A* in southern NEC); (4) and a highly disjunct clade member in an extremely arid area in the southwest of the Lake Eyre Basin (*C. sp. LE*, first mentioned in Mitchell, 1985). *Caridina sp. LE* is found in the Lake Eyre supergroup of springs to the west of Lake Eyre (Ponder, 1986). The other Lake Eyre Basin *Caridina*, *C. thermophila,* is found far to the north and east of *C. sp. LE* in the Barcaldine spring supergroup (Ponder, 1986). They are not closely related, and so the Lake Eyre basin has been colonised more than once, as is the case for spring snails (Perez et al., 2005).

The other members of "Indistincta" are found in the eastern drainages, but curiously they extend much further south in the Murray-Darling division than in the Southeast Coast (SEC) division. Their exact southernmost boundary on the east coast is unclear, but it is probably somewhere to the north of the "Hunter" biogeographic break identified in Ford (1987) (Fig. 4). This is the area where Unmack (2001) placed a border between two freshwater fish regions and also where the range of *Macrobrachium* ceases (Short, 2004), possibly being climate related. The more cold- tolerant *Paratya* dominate from here south. *Macrobrachium*, along with *Austrothelphusa*, also continues much farther south within the neighbouring Murray-Darling Division than along the coast (Bishop, 1967).

A further possible radiation is located in northern NEC. The "N.QLD" clade (Fig. 2; possibly also including *C.spinula*) is only weakly supported genetically, but these taxa do regularly group together in multiple analyses. They are all morphologically similar and are geographically restricted to rainforest and upland areas of tropical north Queensland (Choy and Marshall, 1997) and so may represent either an ancient radiation or may descend from a common stock of independent colonists from Papua New Guinea.

4.3. Processes in biogeography of Caridina

The most evident process that has lead to the phylogenetic and biogeographic patterns visible in this study in terms of large-scale biogeography (i.e. between landmasses) is the primacy of dispersal over vicariance. Because Australia (Sahul) and Southeast Asia (Sunda) have been isolated from each other since time immemorial, and because there are numerous clades of wildly differing ages between these areas, the explanation must be a series colonisations via dispersal rather than vicariance. Biswas and Pawar (2006) point out that topology alone can not differentiate dispersal from vicariance, but the degree of differentiation between taxa can make one more likely than the other. Long (and short) distance trans-oceanic dispersal (reviewed in de Queiroz, 2005 and Nathan, 2005) is not merely stochastic "noise," as some vicariance biogeographers have claimed (Cowie and Holland, 2006). It appears to have been responsible for the formation of much of the Australian continental freshwater biota (Murphy and Austin, 2004; Page et al., 2005a; this study), and not just obscure taxa on remote oceanic islands.

That still leaves the problem of how this dispersal process was achieved. Although hard to prove, a number of reasonable methods are evident. Firstly, many atyids (including many *Caridina*) are amphidromous with salt-tolerant marine planktonic larvae (and adults)(Benzie, 1982; discussed in Page et al., 2005a). The salinity tolerance of freshwater taxa frequently best explains their ability to disperse and their current distribution (*Macrobrachium*: Short, 2004; fish: McDowall, 2002). Another oft-posed dispersal vector are birds. Recently this seemingly unlikely dispersal method has been studied carefully and shown to be a distinct possibility for crustaceans (Figuerola et al., 2005).

A different emphasis is evident when one looks at the patterns of *Caridina* within Australia. Obviously there must have been dispersal around Australia when different *Caridina* lineages first arrived, but many of these appear to have been sundered and therefore the result of vicariance. Many of the *Caridina* divergences within Australia may converge on the late Miocene/early Pliocene. This would agree with a host of other Australian freshwater crustacean studies (*Caridina*: Chenoweth and Hughes, 2003; *Paratya*: Cook et al., 2006; *Macrobrachium*: de Bruyn et al., 2004; Murphy and Austin, 2004; *Euastacus* Clark, 1936: Ponniah and Hughes, 2004; *Cherax* Erichson, 1846: Munasinghe et al., 2004; *Austrothelphusa*: Gopurenko and Davie, unpublished), as well as other Australian

freshwater taxa (mussels: Baker et al., 2003; fish: Unmack, 2001; Page et al., 2004; waterpennies: Wheatley et al., unpublished).

The Miocene was largely wet and warm, and therefore likely to be ideal conditions for a tropical freshwater shrimp. By the late Miocene/early Pliocene, the climate had become colder and drier, and deserts spread, thus isolating freshwater populations and fostering allopatric speciation (Williams and Allen, 1987; Unmack, 2001; Munasinghe et al., 2004; Perez et al., 2005). Despite the obvious handicap of a lack of water, other factors may have contributed to the distributional restrictions of *Caridina* (and other freshwater taxa). These include competition (Short, 2004), predation (Leberer and Nelson, 2001) and, importantly, temperature (de Silva and de Silva, 1994; Bănărescu, 1990, 1995; Unmack, 2001; Ponniah and Hughes, 2004). While there are cases of high temperatures limiting the distributions of both *Paratya* (Fawcett et al., unpublished) and *Caridina* (de Silva and de Silva, 1994), for *Caridina* mostly lower temperatures seem to be a key factor in limiting this tropically-derived genus (Ortmann, 1894; de Silva and de Silva, 1994).

4.4. The influence of scale on biogeographic studies

The variety of range-limiting elements above highlights the complex interaction of factors that can explain distributions. Poff (1997) described these as "filters" constraining dispersal and recruitment in freshwater taxa. Importantly these are scale-dependent and nested, as are freshwater systems (pool, reach, river, basin, drainage, landmass; Poff, 1997; Bohonak and Jenkins, 2003). This means that the suite of traits possessed by a particular species (de Queiroz, 2005) in combination with the local and regional conditions will determine its range.

This is important as each species has slightly different needs and therefore may react differently, and so can make larger scale biogeographic patterns difficult to discern (Turner et al., 2001; Unmack, 2001; Bohonak and Jenkins, 2003; Bossuyt et al., 2004; McDowall, 2004; Biswas and Pawar, 2006). This means that the big-scale picture is made up of many small-scale individual stories.

These scales can be both geographic and temporal (Beever et al., 2006 and references within). For instance, at the longest time-scale, dispersal appears more important than vicariance in explaining *Caridina*'s presence in Australia, but, within the last few million years, vicariance has dictated many of the observable patterns within Australia. Dispersal and vicariance have different levels of prevalence depending on the temporal scale being considered (e.g. "ecological", "geomorphological" or "geological time"; Michaux, 2001). No doubt both vicariance and dispersal continue to influence patterns at the smaller temporal and geographic scales at the population level. This time scale is relative. Is the colonisation of Australia every million years or so by *Caridina* or the Hawaiian Islands by snails (Cowie and Holland, 2006) common or rare?

Bossuyt et al. (2004) used many taxa, including *Caridina*, to study the biotic relationships within a single biodiversity hotspot (Sri Lanka and the Western Ghats of India). Their study highlights the distinctness of the Sri Lankan fauna, making a single biogeographic unit problematic, but also recognises an Indian origin of much of the fauna. When we added *Caridina* sampled from throughout the Indo-Pacific, we found numerous species interposed within various Indian and Sri Lankan taxa from Australia, Iraq, Bali, Borneo, Vanuatu, New Caledonia, Okinawa and possibly Taiwan. At least for *Caridina*, Sri Lanka and India are parts of a larger Indo-West Pacific biota and not a distinct biogeographic region. This leads to the inevitable and complex problem of different bioregions for different taxa depending on scale.

And finally, when small-scale genetic studies of *Caridina* relationships in the Brisbane area of eastern Australia were carried out, it became obvious that there are multiple cryptic species (Chenoweth and Hughes, 2003; Page et al., 2005b). Was this a localised speciation? To test this, one needs to sample throughout Australia, and one would then discover, surprisingly, that a species in the far-off deserts of South Australia is related. Is this an Australian radiation? To test that, one would need to sample throughout the range of *Caridina*, and one would find that a species from Sri Lanka and one from New Caledonia are related to two separate local Brisbane cryptic species. Therefore, to understand the biogeographic history of a single localised "species" in one small area, one would need to expand the geographic scale to be considered as far west as Sri Lanka and as far east as New Caledonia. Appropriate scaling is vital in biogeographic and phylogenetic studies, otherwise a strongly supported, and yet incomplete, conclusion is entirely possible.

Acknowledgments

This study was only possible because many people from around the world kindly provided specimens to us (see bottom of Table 1). We thank Kaye Stuart, Ben Cook, James Fawcett and Joel Huey (Griffith University) for help in the field. We also thank Matthias Glaubrecht, Thomas von Rintelen and Frank Koehler (ZMB, Germany) for collecting material and their kind support. We are grateful to Daisy Wowor and Ristiyanti Marwoto (MZB, Java) for their helpful logistic support in Indonesia and to LIPI (Indonesian Institute of Sciences) for providing research permits. Satish Choy (Queensland Department of Natural Resources) helped with species identifications. David Gopurenko (Purdue University), Mark Ponniah and Ben Cook (Griffith University) and three anonymous reviewers made insightful comments on this work. Funding was provided by the CRC for Freshwater Ecology, Australian Postgraduate Award and Tangalooma Marine & Research Foundation. This is dedicated to the memory of Patricia Page, who continually and significantly supported this work.

References

Baker, A.M., Bartlett, C., Bunn, S.E., Goudkamp, K., Sheldon, F., Hughes, J.M., 2003. Cryptic species and morphological plasticity in long-lived bivalves (Unionoida: Hyriidae) from inland Australia. Mol. Ecol. 12, 2707–2717.

Bănărescu, P., 1990. Zoogeography of Freshwaters Vol. 1. General Distribution and Dispersal of Freshwater Animals. Aula-Verlag, Wiesbaden.

Bănărescu, P., 1995. Zoogeography of Freshwaters Vol. 3. Distribution and dispersal of freshwater animals in Africa, Pacific areas and South America. Aula-Verlag, Wiesbaden.

Beever, E.A., Swihart, R.K., Bestelmeyer, B.T., 2006. Linking the concept of scale to studies of biological diversity: evolving approaches and tools. Divers. Distrib. 12, 229–235.

Benzie, J.A.H., 1982. The complete larval development of *Caridina mccullochi* Roux, 1926 (Decapoda, Atyidae) reared in the laboratory. J. Crust. Biol. 2, 493–513.

Bishop, J.A., 1967. The zoogeography of the Australian freshwater decapod Crustacea. In: Weatherly, A.H. (Ed.), Australian Inland Waters and Their Fauna. Australian National University, Canberra, pp. 107–122.

Biswas, S., Pawar, S.S., 2006. Phylogenetic tests of distribution patterns in South Asia: towards an integrative approach. J. Biosci. 31, 95–113.

Bohonak, A.J., Jenkins, D.G., 2003. Ecological and evolutionary significance of dispersal by freshwater invertebrates. Ecol. Lett. 6, 783–796.

Bossuyt, F., Meegaskumbura, M., Beenaerts, N., Gower, D.J., Pethiyagoda, R., Roelants, K., Mannaert, A., Wilkinson, M., Bahir, M.M., Manamendra-Arachchi, K., Ng, P.K.L., Schneider, C.J., Oommen, O.V., Milinkovitch, M.C., 2004. Local endemism within the western Ghats-Sri Lanka biodiversity hotspot. Science 306, 479–481.

Buckley, T.R., Simon, C., Flook, P.K., Misof, B., 2000. Secondary structure and conserved motifs of the frequently sequenced domains IV and V of the insect mitochondrial large subunit rRNA gene. Insect Mol. Biol. 9, 565–580.

Bunn, S.E., Davies, P.M., 1990. Why is the stream fauna of south-western Australia so impoverished? Hydrobiologia 194, 169–176.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17, 540–552.

Chenoweth, S.F., Hughes, J.M., 2003. Speciation and phylogeography in *Caridina indistincta*, a complex of freshwater shrimps from Australian heathland streams. Mar. Freshwater Res. 54, 807–812.

Choy, S., Marshall, J., 1997. Two new species of freshwater atyid shrimps (Crustacea: Decapoda: Atyidae) from northern Queensland and the distributional ecology of the *Caridina typus* species-group in Australia. Mem. Qld. Mus. 42, 25–36.

Cook, B.D., Baker, A.W., Page, T.J., Grant, S.C., Fawcett, J.H., Hurwood, D.A., Hughes, J.M., 2006. Biogeographic history of an Australian freshwater shrimp, *Paratya australiensis* (Atyidae): the role life history transition in phylogeographic diversification. Mol. Ecol. 15, 1083–1093.

Cowie, R.H., Holland, B.S., 2006. Dispersal is fundamental to biogeography and the evolution of biodiversity on oceanic islands. J. Biogeogr. 33, 193–198.

Davie, P.J.F., 2002. Atyidae. In: Davie, P.J.F. (Ed.), Crustacea: Malacostraca: Phyllocarida, Hoplocarida, Eucarida (Part 1) Vol. 19.3A. CSIRO, Melbourne, pp. 222–229.

de Bruyn, M., Wilson, J.A., Mather, P.B., 2004. Huxley's line demarcates extensive genetic divergence between eastern and western forms of the giant freshwater prawn, *Macrobrachium rosenbergii*. Mol. Phylogenet. Evol. 30, 251–257.

de Queiroz, A., 2005. The resurrection of oceanic dispersal in historical biogeography. Trends Ecol. Evol. 20, 68–73.

de Silva, P.K., de Silva, K.H.G.M., 1994. Geographic distribution and probable evolutionary trends of Atyidae (Decapoda, Natantia) of inland waters of Sri Lanka. Verh. Internat. Verein. Limnol. 25, 2455–2459.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of leaf tissue. Phytochem. Bull. 19, 11–15.

Figuerola, J., Green, A.J., Michot, T.C., 2005. Invertebrate eggs can fly: evidence of waterfowl-mediated gene flow in aquatic invertebrates. Am. Nat. 165, 274–280.

Ford, J., 1987. Hybrid zones in Australian birds. Emu 87, 158–178.

Graybeal, A., 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? Syst. Biol. 47, 9–17.

Griffin, D.J.G., Yaldwyn, J.C., 1968. The constitution, distribution and relationships of the Australian decapod Crustacea. Proc. Linn. Soc. N.S.W. 93, 164–183.

Guindon, S., Gascuel, O., 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Hall, R., 2002. Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions, model and animations. J. Asian Earth Sci. 20, 353–431.

Harrison, J.S., 2004. Evolution, biogeography, and the utility of mitochondrial 16s and COI genes in phylogenetic analysis of the crab genus *Austinixa* (Decapoda : Pinnotheridae). Mol. Phylogenet. Evol. 30, 743–754.

Jalihal, D.R., Almelkar, G.B., Sankolli, K.N., 1994. Atyid Shrimps of the Genus *Caridina* Edwards, H., Milne, 1837—potential Crustacean material for experimental biology. Crustaceana 66, 178–183.

Lavery, S., Chan, T.Y., Tam, Y.K., Chu, K.H., 2004. Phylogenetic relationships and evolutionary history of the shrimp genus *Penaeus* s.l. derived from mitochondrial DNA. Mol. Phylogenet. Evol. 31, 39–49.

Leberer, T., Nelson, S.G., 2001. Factors affecting the distribution of Atyid shrimps in two tropical insular rivers. Pac. Sci. 55, 389–398.

Lefébure, T., Douady, C.J., Gibert, J., 2006. Relationship between morphological taxonomy and molecular divergence within Crustacea: proposal of a molecular threshold to help species delimitation. Mol. Phylogenet. Evol. 40, 435–447.

McDowall, R.M., 2002. Accumulating evidence for a dispersal biogeography of southern cool temperate freshwater fishes. J. Biogeogr. 29, 207–219.

McDowall, R.M., 2004. What biogeography is: a place for process. J. Biogeogr. 31, 345–351.

Michaux, B., 2001. Dispersal versus vicariance, artifice rather than contest. In: Metcalfe, I. (Ed.), Faunal And Floral Migrations And Evolution In SE Asia Australasia. A.A. Balkema, Lisse, pp. 311–318.

Mitchell, B.D., 1985. Limnology of mound springs and temporary pools south and west of Lake Eyre. In: Greenslade, J., Joseph, L., Reeves, A. (Eds.), South Australia's Mound Springs. Nature Conservation Society of South Australia, Adelaide, pp. 51–63.

Munasinghe, D.H.N., Burridge, C.P., Austin, C.M., 2004. Molecular phylogeny and zoogeography of the freshwater crayfish genus *Cherax* Erichson (Decapoda : Parastacidae) in Australia. Biol. J. Linn. Soc. 81, 553–563.

Murphy, N.P., Austin, C.M., 2004. Multiple origins of the endemic Australian *Macrobrachium* (Decapoda : Palaemonidae) based on 16S rRNA mitochondrial sequences. Aust. J. Zool. 52, 549–559.

Nathan, R., 2005. Long-distance dispersal research: building a network of yellow brick roads. Divers. Distrib. 11, 125–130.

Ortmann, A.E., 1894. A study of the systematic and geographical distribution of the decapod family Atyidae Kingsley. Proc. Acad. Natl. Sci. Phila. 1894, 397–416.

Page, T.J., Sharma, S., Hughes, J.M., 2004. Deep phylogenetic structure has conservation implications for ornate rainbowfish (Melanotaeniidae: *Rhadinocentrus ornatus*) in Queensland, eastern Australia. Mar. Freshwater Res. 55, 165–172.

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

Page, T.J., Choy, S.C., Hughes, J.M., 2005b. The Taxonomic Feedback Loop: symbiosis of morphology & molecules. Biol. Lett. 1, 139–142.

Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G., 1991. A Simple Fool's Guide to PCR. University of Hawaii Press, Honolulu.

Perez, K.E., Ponder, W.F., Colgan, D.J., Clark, S.A., Lydeard, C., 2005. Molecular phylogeny and biogeography of spring-associated hydrobiid snails of the Great Artesian Basin, Australia. Mol. Phylogenet. Evol. 34, 545–556.

Poff, N.L., 1997. Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. J. N. Am. Benthol. Soc. 16, 391–409.

Ponder, W.F., 1986. Mound springs of the Great Artesian Basin. In: De Dekker, P., Williams, W.D. (Eds.), Limnology in Australia. CSIRO, Melbourne, pp. 403–420.

Ponniah, M., Hughes, J.M., 2004. The evolution of Queensland spiny mountain crayfish of the genus *Euastacus*. I. Testing vicariance and dispersal with interspecific mitochondrial DNA. Evolution 58, 1073–1085.

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

Riek, E.F., 1953. The Australian freshwater prawns of the family Atyidae. Aust. J. Mar. Freshwater Res. 23, 111–121.

Short, J.W., 2004. A revision of Australian river prawns, *Macrobrachium* (Crustacea: Decapoda: Palaemonidae). Hydrobiologia 525, 1–100.

Sorenson, M.D., 1999. TreeRot Version 2. Boston University, Boston, MA.

Swofford, D.L., 2002. PAUP* Version 4.0b5—Phylogenetic Analysis Using Parsimony (* and other Methods). Sinauer Associates, Sunderland, MA.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876–4882.

Turner, H., Hovenkamp, P., van Welzen, P.C., 2001. Biogeography of Southeast Asia and the West Pacific. J. Biogeogr. 28, 217–230.

Unmack, P.J., 2001. Biogeography of Australian freshwater fishes. J. Biogeogr. 28, 1053–1089.

von Rintelen, K., von Rintelen, T., Glaubrecht, M., submitted for publication. Diversification without in situ radiation in an ancient lake species-flock of the shrimp *Caridina* on Sulawesi, Indonesia. Biol. Lett.

Williams, W.D., 1981. The Crustacea of Australian Inland Waters. In: Keast, A. (Ed.), Ecological Biogeography of Australia—The Inland Fresh Waters, vol. 3. Dr. W. Junk, The Hague, pp. 1103–1138.

Williams, W.D., Allen, G.R., 1987. Origins and adaptations of the fauna of inland waters. In: Dyne, G.R., Walton, D.W. (Eds.), Fauna of Australia: General Articles, vol. 1A. CSIRO, Canberra, pp. 184–201.

Yam, R.S.W., Dudgeon, D., 2005. Genetic differentiation of *Caridina cantonensis* (Decapoda: Atyidae) in Hong Kong streams. J. N. Am. Benthol. Soc. 24, 845–857.

Zakšek, V., Sket, B., Trontelj, P., 2007. Phylogeny of the cave shrimp *Troglocaris*: evidence of a young connection between Balkans and Caucasus. Mol. Phylogenet. Evol. 42, 223–235.