

Phylogenetic and biogeographic relationships of subterranean and surface genera of Australian Atyidae (Crustacea: Decapoda: Caridea) inferred with mitochondrial DNA

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Abstract. The biogeographic and phylogenetic relationships of six of the eight Australian genera of freshwater shrimp from the family Atyidae were investigated using mitochondrial 16S rDNA and cytochrome oxidase I sequences. Previous studies on two of the epigeal genera (*Caridina*, *Paratya*) indicate that Australian species have strong links to congeners from outside, with Australian members of *Paratya* being monophyletic and *Caridina* polyphyletic. The present study found that the endemic Australian epigeal genus *Australatya* forms a strong clade with Pacific ‘*Atya*-like’ genera (*Atyoida*, *Atyopsis*), and that the endemic Australian epigeal genus *Caridinides* falls within a clade containing *Caridina* species from the Australian ‘*indistincta*’ group. The two hypogean genera included in this study (*Parisia*, *Pycnisia*) form a strong clade in all analyses, implying an Australian subterranean speciation. The possibility of a relationship between *Parisia*/*Pycnisia* and an Australian *Caridina* species may have implications for the monophyly of the highly disjunct genus *Parisia* (Australia, Madagascar, Philippines). *Parisia* may descend from local *Caridina* species and represent convergent morphologies.

Introduction

The Atyidae is an ancient family of freshwater shrimps with a very wide distribution on all continents, bar Antarctica. Australian atyids are relatively small (<35 mm, except the genus *Australatya* McCulloch & McNeill, 1923, with species reaching ~60 mm: Davie 2002) and are largely found in freshwater creeks, lakes and caves, with some using estuaries for breeding (Davie 2002). A particular feature of the Atyidae is the brush of setae at the ends of their chelae (claws) (Bruce 1992).

Despite the salinity tolerance of some species, there are no known fully marine atyids (Huxley 1880; Fryer 1977), in contrast to the other family of common freshwater shrimps in Australia, Palaemonidae (e.g. the genus *Macrobrachium* Bate, 1868), which has many marine representatives (Short and Doumenq 2003). The atyids have been in freshwater environments for an extremely long time, as evidenced by the occurrence of fossil atyids in freshwater deposits (Tertiary, Brazil; Oligocene, France: Glaessner 1969), as well as a complex morphology adapted to fluvial conditions (Fryer 1977). Furthermore, there are many hypogean (subterranean) atyid genera and species around the world, including Australia (Holthuis 1986), with specialised, and presumably very old, adaptations to living in the underworld.

Although particularly speciose in the tropics (Bănărescu 1990), some atyid taxa display an anti-tropical distribution (Page *et al.* 2005a). This extremely wide distributional range is likely the result of a combination of vicariance, because of their

great age, and dispersal (both small-scale and transoceanic), because of variable levels of salinity tolerance in some taxa (Bănărescu 1990). The wide range in geographic distributions and complex evolutionary history, both ancient and recent, makes the Atyidae of particular interest for biogeography.

There are currently eight genera of Atyidae recognised in Australia (Choy and Horwitz 1995; Davie 2002). Four of these genera are epigeal (surface): *Caridina* H. Milne Edwards, 1837 (widespread and speciose in the Indo-Pacific: Page *et al.* in press); *Paratya* Miers, 1882 (anti-tropical: Page *et al.* 2005a); *Caridinides* Calman, 1926 (endemic to northern Australia: Smith and Williams 1982); and *Australatya* (endemic to eastern Australia: Chace 1983). The remaining four genera are hypogean: *Parisia* Holthuis, 1956 (Madagascar, Philippines, Northern Territory (Australia): Cai and Anker 2004); *Pycnisia* Bruce, 1992 (endemic to northern Australia: Suzuki and Davie 2003); *Pycneus* Holthuis, 1986 (endemic to Western Australia: Holthuis 1986); and *Stygocaris* Holthuis, 1960 (endemic to Western Australia: Williams 1964).

The biogeographic and phylogenetic relationships between these Australian taxa and non-Australian taxa are likely to be complex. Much of the Australian freshwater biota is thought to have colonised the continent from south-east Asia in the north as the Sahul plate met the Sunda plate during the Miocene (Bishop 1967; Williams and Allen 1987), but some species have vicariant Gondwanan heritage, such as parastacid crayfish

(Williams and Allen 1987). Australian atyids and freshwater palaemonids, in particular, are thought to have come from south-east Asia (Bishop 1967; Williams and Allen 1987). This has been partially tested within a phylogenetic framework, in that both *Caridina* (Page *et al.* in press) and *Macrobrachium* (Murphy and Austin 2004) from Australia have multiple independent phylogenetic links with taxa from south-east Asia and further afield, implying multiple dispersal events in the distant past up to the present day. In contrast, Australian species of *Paratya* (Page *et al.* 2005a) are monophyletic and so a single colonisation is likely.

The relationships of the remaining six Australian atyid genera, of which five are endemic and four hypogean, are unclear. Of particular interest are the subterranean taxa, which can present a biogeographic conundrum. The occurrence of *Parisia* in Madagascar as well as Australia may argue for Gondwanan ancestry, whereas the presumed close relationship of *Parisia*, *Pycneus* and *Pycnisia* (Bruce 1992) may imply an Australian subterranean radiation. In fact, Australia hosts a very rich and diverse subterranean fauna (Cooper *et al.* 2002; Humphreys 2006) of which 82% of the stygobitic (obligate groundwater) families are crustaceans (Humphreys 2006).

The inference of phylogenetic relationships from subterranean fauna can be especially challenging because of morphological homoplasy owing to the particular requirements of cave life (Cooper *et al.* 2002; Proudlove and Wood 2003; Lefébure *et al.* 2006). For this reason, we use molecular data to test the morphology-based taxonomy to clarify the systematic and biogeographic relationships of the Australian atyid genera, as has been done effectively for various freshwater crustacean groups (e.g. Murphy and Austin 2002; Munasinghe *et al.* 2004; Zakšek *et al.* 2007). Molecular data should aid morphological study, and vice versa, in an 'integrative' taxonomy (Dayrat 2005; Page *et al.* 2005b), because a more-or-less complete view of evolutionary history can only really be attained through accessing the relative strengths of both morphological characters and molecules (Hillis and Wiens 2000; Lee 2004).

Materials and methods

Specimen collection

Specimens of six of the eight genera of Atyidae reported in Australia (Choy and Horwitz 1995; Davie 2002) were analysed in this study. Those included were all the epigean genera (*Australatya*, *Caridinides*, *Caridina*, *Paratya*), the first two of which are Australian endemics; and two of the four hypogean genera (*Pycnisia*, *Parisia*), the first of which is an endemic. The Western Australian endemic subterranean genera *Pycneus* and *Stygiocaris* were not included owing to a difficulty in obtaining specimens. Various other atyid genera were included from throughout the Indo-Pacific (*Atyoida* Randall, 1840, *Atyopsis* Chace, 1983, *Halocaridina* Holthuis, 1963, *Neocaridina* Kubo, 1938), as well as non-Australian species of *Caridina* and *Paratya*, to provide biogeographic context for Australian specimens. Further atyid genera were added from the Americas and Europe (*Typhlatya* Creaser, 1936, *Atyaephyra* de Brito Capello, 1867) to make a total of 31 species from 12 atyid genera, representing all four subfamilies of the Atyidae (*sensu* Holthuis 1993, based on Bouvier 1925 and Holthuis 1986) (Table 1).

Specimens were kindly provided by many museums, institutions and individuals from around the world (Table 1).

DNA extraction and sequencing

Genomic DNA was extracted and fragments of the most widely used genes in crustacean studies (Lefébure *et al.* 2006) were amplified, namely the mitochondrial genes for the large subunit 16S rDNA (16S) and cytochrome oxidase subunit I (COI). These were amplified and sequenced as per Page *et al.* (2005a). For the 16S polymerase chain reaction (PCR), forward primers were either 16S-F-Car (von Rintelen *et al.* in press; see Page *et al.* in press for primer sequences) or 16Sar (Palumbi *et al.* 1991). The reverse primers were 16S-R-Car, 16S-R-Car1 (von Rintelen *et al.* in press or 16Sbr (Palumbi *et al.* 1991). For the COI PCR, forward primers were CDC0.La (Page *et al.* 2005b) or COI.f (Palumbi *et al.* 1991), and COIa.H (Palumbi *et al.* 1991) was the reverse. Genetic material from all individuals was sequenced in both directions with 16S and COI primers. We used BigDye version 3.1 Terminator (Applied Biosystems, Foster City, CA, USA.) 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 primer regions removed using Sequencher 4.1.2 (Gene Codes Corporation, Ann Arbor, MI, USA.).

Sequence alignment and dataset construction

16S sequences were aligned using ClustalX (Thompson *et al.* 1997) at a variety of gap opening/extension values (1, 6, 10, 15, 50), resulting in similar datasets of between 489 and 498 base pairs (bp). The default settings of 15/6 (gap opening/extension) was chosen to generate an aligned dataset of 493 bp, corresponding to positions 11434–11908 of the atyid *Halocaridina rubra* Holthuis, 1963 mitochondrial genome (GenBank accession number NC008413, online at: <http://www.ncbi.nlm.nih.gov/>; Ivey and Santos in press). Poorly aligned sites (8%) were excluded (as recommended by Lefébure *et al.* 2006) after being identified with Gblocks version 0.91b (Castresana 2000) (Parameters = minimum number sequences for conserved position: 30; minimum number sequences for flanking position: 30; maximum number contiguous non-conserved positions: 6; minimum length of block: 4; allowed gap positions: with half). We compared our sequences with the 16S secondary-structure model of the brine shrimp *Artemia salina* (Linnaeus, 1758) (3' half, Domain V, available at www.rna.icmb.utexas.edu [verified 21 May 2007]; Cannone *et al.* 2002). Most of the Gblocks-identified sites are located in the region of helices 75 and 84, which are difficult to align for many taxa (Buckley *et al.* 2000). The COI dataset contained no gaps and was aligned to 532 bp, corresponding to positions 763–1294 of the *Halocaridina rubra* mitochondrial genome. The two mitochondrial genes were combined into a single dataset of 1025 bp after a partition homogeneity test was carried out in PAUP* version 4.0 b10 (Swofford 2002).

Seventeen new 16S sequences from nine atyid genera were generated by this study (GenBank accession numbers DQ681268–DQ681291; all accession numbers are listed in Table 1). Seventeen 16S sequences of *Australatya*, *Caridina* and *Paratya* were included from Page *et al.* (2005b, in press) and two *Typhlatya* sequences from Webb (2003). For the COI

Table 1. Atyid specimens and sequences listed by area and genus, with 16S and COI GenBank accession numbers and specimen provenance

Genus	Species	Subfamily [#]	Sample site (Australian basin, Australian state)	GenBank accession nos.	
				16S rDNA	COI
Australian atyid taxa					
<i>Australatya</i>	<i>striolata</i>	Aty	Johnstons Ck, Appletree Flat (Manning, NSW) ^A	AY795035 ¹	DQ681261
<i>Caridina</i>	<i>confusa</i>	Aty	Molo Ck, Atherton Tableland (Johnstone, QLD) ^B	DQ478495 ²	DQ478450 ²
	<i>indistincta</i> A	Aty	Lake Bowarrady (Fraser Island, QLD) ^A	AY795039 ¹	DQ478453 ²
	<i>indistincta</i> B	Aty	Murray River 8km E. of Murrays Bridge (Lower Murray, SA) ^C	DQ478500 ²	DQ681248
	<i>indistincta</i> C1	Aty	Honeyeater Lake (Moreton Island, QLD) ^A	DQ478502 ²	DQ478455 ²
	<i>indistincta</i> C4	Aty	Bellinger R, Bellingen (Bellinger, NSW) ^A	AY795050 ¹	DQ478456 ²
	<i>serratirostris</i>	Aty	Arnot Ck, N. of Ingham (Herbert, QLD) ^B	DQ478515 ²	DQ478461 ²
	sp. D	Aty	Little R, Inourne (Gilbert, QLD) ^D	DQ478525 ²	DQ681250
	sp. LE	Aty	Algebuckina Waterhole, Neales River (Lake Frome, SA) ^E	DQ478534 ²	DQ478465 ²
<i>Caridinides</i>	<i>wilkinsi</i>	Car	Ninds Ck (W. branch) at highway crossing (Johnstone, QLD) ^B	DQ681273	DQ681247
	<i>wilkinsi</i>	Car	One Mile Ck, W. Innisfail (Johnstone, QLD) ^B	DQ681273	DQ681247
<i>Paratya</i>	<i>australiensis</i>	Par	Lake Crescent nr boat ramp (Derwent, TAS) ^F	DQ478566 ²	DQ478480 ²
<i>Parisia</i>	<i>unguis</i> *	Car	Cutta Cutta Caves nr Katherine (Fitzmaurice, NT) ^G	DQ681288	DQ681251
	<i>unguis</i> *	Car	Cutta Cutta Caves nr Katherine (Fitzmaurice, NT) ^H	DQ681289	DQ681251
<i>Pycnisia</i>	<i>raptor</i> *	Car	Grants Caves nr Katherine (Fitzmaurice, NT) ^G	DQ681271	DQ681252
Indo-Pacific atyid taxa					
<i>Atyoida</i>	<i>bisulcata</i>	Aty	Waiau Stream, Hawaii, Hawaiian Is. ^I	DQ681278	DQ681257
	<i>pilipes</i>	Aty	Putoa Falls, nr Afareaitu, Moorea, French Polynesia ^J	DQ681279	DQ681255
	<i>pilipes</i>	Aty	Yela R, Kosrae, Micronesia ^B	DQ681276	DQ681256
	<i>pilipes</i>	Aty	Mele, Efate Island, Vanuatu ^A	DQ681277	DQ681258
<i>Atyopsis</i>	<i>moluccensis</i>	Aty	Petshop in Germany (origin in Indo-Pacific Region) ^A	DQ681281	DQ681246
	<i>spinipes</i>	Aty	Tinahula, Guadalcanal, Solomon Is. ^K	DQ681282	DQ681260
<i>Caridina</i>	<i>cantonensis</i>	Aty	Lam Truen River, Hong Kong Is. ^L	DQ478487 ²	DQ478445 ²
	<i>trifasciata</i>	Aty	Task Yue Wu, New Territories, Hong Kong ^L	DQ478557 ²	DQ478476 ²
	<i>steineri</i> *	Aty	Lakata Zafera, Madagascar ^D	DQ681274	DQ681249
	<i>serratirostris</i>	Aty	nr Toledo City, Cebu, Philippines ^K	DQ478513 ²	DQ478460 ²
	sp. Sol 2	Aty	Matepono, Guadalcanal, Solomon Is. ^K	DQ478545 ²	DQ478471 ²
	<i>africana</i>	Aty	Carbis Road stream, Pietermaritzburg, South Africa ^M	DQ478483 ²	DQ478443 ²
<i>Halocaridina</i>	<i>rubra</i> *	Car	Halape Iki, Hawaii Volcano National Park, Hawaiian Is. ^I	DQ681287	DQ681262
<i>Neocaridina</i>	<i>denticulata</i>	Aty	Lake Biwa, Shiga prefecture, Honshu, Japan ^N	DQ681268	DQ681253
	sp. TW	Aty	Longchuanwoshan, Longci, Tainan County, Taiwan ^O	DQ681270	DQ681254
<i>Paratya</i>	cf. <i>intermedia</i>	Par	Dumbéa River, New Caledonia ^P	DQ478567 ²	DQ478481 ²
	<i>howensis</i>	Par	First Ck, Lord Howe Is. ^Q	DQ478569 ²	DQ478482 ²
Other atyid taxa					
<i>Atyaephyra</i>	<i>desmarestii</i>	Par	Rio Coghinas, Sardinia, Italy ^R	DQ681285	DQ681263
	<i>desmarestii</i>	Par	Arroyo María, Guadalhorce, Spain ^S	DQ681286	DQ681264
<i>Typhlatya</i>	<i>mittelli</i> *	Typ	Yucatán Peninsula, Mexico ^T	AF513538 ³	AF513523 ³
	<i>pearsi</i> *	Typ	Yucatán Peninsula, Mexico ^T	AY115539 ³	AY115534 ³
Outgroups					
<i>Macrobrachium</i>	sp.		Laloki River, Pt. Moresby, Papua New Guinea ^U	DQ681290	DQ681266
	<i>tolmerum</i>		Flinders Beach Swamp (Stradbroke Island, QLD), Australia ^A	DQ681291	DQ681267
<i>Metapenaeus</i>	<i>affinis</i>		Coast of Xiamen in the East China Sea, China ^V	AY264886 ⁴	AY264904 ⁴
	sp.		Brunswick R, Mullumbimby (Brunswick, NSW), Australia ^A	DQ681283	DQ681265

* Subterranean.

Atyid subfamilies *sensu* Holthuis (1993): Aty, Atyinae; Car, Caridellinae; Par, Paratyinae; Typ, Typhlatyinae.Specimen sources: ^AAuthors; ^BP. Davie (Queensland Museum); ^CJ. Fawcett (Griffith University); ^DS. Choy (Natural Resources Department of Queensland);^ES. Barter (SA Water); ^FA. Wheatley (Griffith University); ^GG. Dally (Northern Territory Museum); ^HM. Titellius (Western Australian Museum); ^IK. Hopkins(University of Hawaii); ^JR. Mazon (University of California); ^KR. Smith (Hydrobiology); ^LR. Yam/D. Dudgeon (University of Hong Kong); ^MR. Hart(University of KwaZulu-Natal); ^NK. Nishi (Ecology & Civil Engineering Society); ^OHsi-Te Shih (Chung Hsing University); ^PC. Pöllabauer (Etudes etRecherches biologiques (ERBIO)); ^QT. Moulton (Universidade do Estado do Rio de Janeiro); ^RM. Jowers (University of Glasgow); ^SC. Sánchez (Centro deEstudios Hidrográficos); ^TSequence from Webb(2003); ^UD. Robert; ^VSequence from Quan *et al.*(2004).

Australian states: NSW, New South Wales; NT, Northern Territory; QLD, Queensland; SA, South Australia; TAS, Tasmania.

Sources of additional sequences: ¹Page *et al.*(2005b); ²Page *et al.*(in press); ³Webb (2003); ⁴Quan *et al.*(2004).

dataset, 20 new COI sequences were produced for this study (GenBank accession numbers DQ681246–DQ681267) and were added to 14 sequences from Page *et al.* (in press) and two from Webb (2003) (see Table 1).

Thomas Huxley (1880) noted that the Atyidae had no obvious marine ancestor or close relatives. Christoffersen (1986) placed seven families in Atyoidea, whereas Chace (1992) and Holthuis (1993) placed Atyidae alone in Atyoidea. For this reason, more than one outgroup was included because incorrect rooting can lead to spurious results (Hillis and Wiens 2000). Two shrimp species from the genus *Macrobrachium* (Palaemonidae; from the same infraorder as Atyidae: Caridea) were sequenced for both genes for this study (GenBank accession numbers DQ681266–DQ681267, DQ681290–DQ681291), as well as two species from the more distant genus *Metapenaeus* Wood-Mason, 1891 (Penaeidae, superorder Dendrobranchiata) (one species from this study, DQ681265, DQ681283; one from Quan *et al.* 2004). As demonstrated by Porter *et al.* (2005), the Atyidae have ancient divergences within the family and so a full investigation of the deeper nodes (i.e. subfamily level) would require nuclear genes as well.

Phylogenetic analyses

We used Modeltest version 3.06 (Posada and Crandall 1998) to select the best-fit model of evolution for each dataset (16S, COI and concatenated) (Akaike information criterion, as recommended by Posada and Buckley 2004). Three methods of phylogenetic inference were employed in this study. These were Bayesian analysis (BA) in MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001), maximum likelihood (ML) in PHYML version 2.4.4 (Guindon and Gascuel 2003) and parsimony analysis (PA) in PAUP (full heuristic with 100 random repetitions). MrBayes parameters were as follows: 2 million generations, trees sampled every 100 cycles, dataset partitioned by gene, 25% burn-in, two runs of four chains heated to 0.2. For the ML analyses, a single model of evolution was selected for the concatenated dataset. The dataset was bootstrapped 1000 times for ML and PA. TreeRot version 2 (Sorenson 1999) was used to calculate Bremer support values for the PA analyses. Two phylogenetic hypotheses (monophyly of genera and subfamilies) were investigated using the Shimodaira–Hasegawa (S–H) test in PAUP (1000 replicates of RELL (resampling of estimated log-likelihoods) test distribution).

Results

The partition homogeneity test showed no significant difference in phylogenetic signal between the two mitochondrial genes ($P = 0.510$), so they were analysed together. There were 224 variable bases within the ingroup 16S sequences (after the removal of the Gblocks positions), with 198 parsimony-informative sites. A χ^2 test of homogeneity of base frequencies across taxa found no significant difference ($P = 1.000$). For the 16S portion of the dataset, Modeltest 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: 0.598; proportion of invariable sites: 0.405). For the ingroup COI sequences, 239 bases were variable, with 228 parsimony informative (19% in the 1st codon position, 5% in 2nd, 76% in 3rd). χ^2 base frequencies were not significant ($P = 0.615$). For the COI portion, Modeltest selected the general time reversible

model (GTR+I+G; Γ distribution shape: 0.574; proportion of invariable sites: 0.515). For the ML analyses, Modeltest selected a single model of evolution for the concatenated dataset (GTR+I+G; Γ distribution shape: 0.637; proportion of invariable sites: 0.473).

The Bayesian consensus topology of the two runs (Bayes factor harmonic mean = $-12\,886.66$), with posterior probabilities (converted to ‘clade credibility values’ as percentages), is displayed in Fig. 1. ML analysis recovered one tree (log-likelihood score = $-12\,937.167$) and PA analyses recovered three trees of 2986 steps (consistency index = 0.286, retention index = 0.509, rescaled consistency = 0.145). ML and PA bootstraps and PA Bremer support values are displayed in Fig. 1.

Placement of Australian taxa

Australian genera were spread throughout the topologies (Fig. 1). As shown in Page *et al.* (2005a), the Australian species of *Paratya* are recovered in a strong clade with non-Australian congeners. At a higher level, *Paratya* forms a clade with the disparate and divergent genera *Atyaephyra*, *Halocaridina* and *Typhlatya*. It is currently unclear whether this ‘non-Atyinae’ clade is a result of long-branch attraction (Bergsten 2005) or common ancestry.

The Australian endemic genus *Australatya* forms a clade with specimens of *Atyopsis* and, at a higher level, falls within a clade containing *Atyoida*, which equates to the ‘*Atya*-like shrimps of the Indo-Pacific region’ (Chace 1983).

The northern Australian stygobitic genera *Parisia* (Northern Territory) and *Pycnisia* (endemic; Northern Territory, Queensland) form a strong clade in all analyses. This ‘NT Cave’ group consistently forms a clade with a *Caridina* species from northern Queensland (*Caridina confusa* Choy & Marshall, 1997). The Australian species of *Caridina* are polyphyletic and very diverse (Page *et al.* in press), as exemplified by the ‘*Serratirostris*’ clade of Australian and non-Australian *Caridina*, which is divergent.

The Australian endemic, monotypic genus *Caridinides* forms a clade with Australian *Caridina* species of the ‘*indistincta*’ species-complex. This ‘*indistincta*’ group falls within a varied group of *Caridina* species that includes a widespread Australian species (*Caridina* sp. D *sensu* Page *et al.* 2005b), and some from Africa, Madagascar and the Solomon Islands.

The Asian genus *Neocaridina* also may have common ancestry with geographically proximate species of *Caridina*. With the notable exception of *Caridina*, the atyid genera proved to be monophyletic in all analyses. When all genera are constrained to monophyly (including *Caridina*), the resulting topology is significantly worse than the unconstrained topology (S–H test: $P < 0.001$). Although subfamily level may be too deep for these genes, subfamily-constrained trees are significantly worse than unconstrained (S–H test: $P < 0.001$).

Discussion

Australian epigeal genera

Earlier studies on the most common and widespread epigeal genera in Australia (*Caridina*, *Paratya*) have shown a complex web of relationships between Australian and non-Australian

species at both shallow and deep levels (Page *et al.* 2005a, in press), with Australian species of *Paratya* monophyletic and *Caridina* polyphyletic. The other widespread Australian epigean genus, *Australatya* (east Australian coast), is the Australian representative of the larger-bodied 'Atya-like' group of species from the Pacific region (Chace 1983), which is recovered as a monophyletic group.

Australia's fourth epigean genus, *Caridinides*, is also monotypic and endemic and is found in freshwater and brackish coastal creeks in northern Australia (Queensland and Northern Territory) (Smith and Williams 1982; Davie 2002). Calman (1926) designated it a separate genus based on the presence of a 'well developed exopod on the first pair of chelipeds'. Our analysis, however, finds that *Caridinides* falls within *Caridina*, and has a firm relationship with the Australian 'indistincta' complex. This should not be especially surprising because most authors have noted a strong resemblance between *Caridina* and *Caridinides*, apart from the exopods (Calman 1926; Johnson 1961; Smith and Williams 1982).

The presence of exopods has been considered a hallmark of a primitive taxon within Decapoda (Johnson 1961) and, along with the presence of a supraorbital spine, defines the valid monophyletic genus *Paratya* (Choy and Horwitz 1995). However, it may be an unsafe assumption because, as Calman (1926) pointed out when erecting the genus *Caridinides*, exopods are sometimes retained in larvae and can be present in adults of unrelated genera. This paedomorphic explanation is backed up by the finding of exopods on some specimens of *Caridina thermophila* Riek, 1953 (Riek 1953; Choy and Horowitz 1995) and the occasional appearance of a reduced exopod on the second pair of chelipeds in *Caridinides* (Smith and Williams 1982). Martin and Wicksten (2004) reported a difference in exopod number between two otherwise very similar congeneric atyid species. Even Calman (1926) considered the generic delineation of *Caridinides* as a 'practical convenience' and did not consider it was 'phylogenetically primitive'. It may be more useful as a character for identification rather than phylogenetic inference. Despite Smith and Williams (1982)

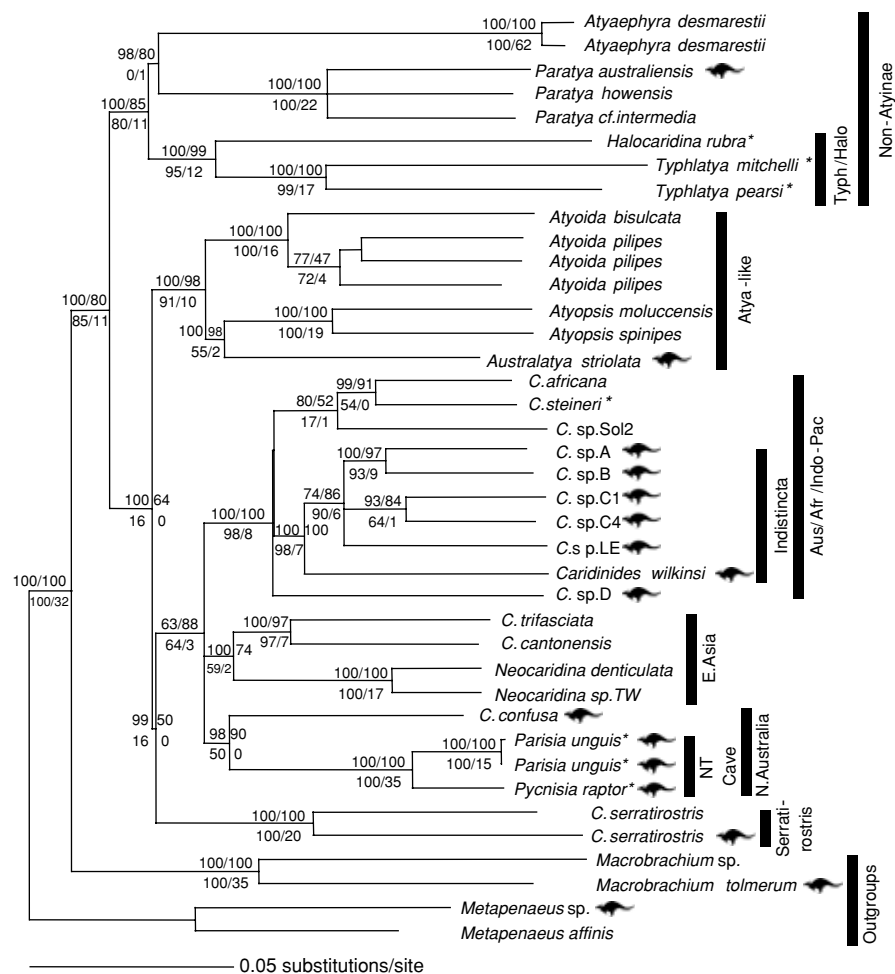


Fig. 1. Bayesian (BA) consensus topology of combined 16S/COI dataset, with informal taxon group names. BA posterior probabilities / maximum likelihood bootstrap values above node and parsimony bootstrap / Bremer support values below. 🐞 = Australian specimen.

considering *Caridinides* a distinct genus, it would appear to make phylogenetic, biogeographic and systematic sense to consider the *Caridinides* species as an unusual *Caridina* (as Johnson 1961 recommended); and an Australian one at that, rather than related to New Guinea species as suggested by Smith and Williams (1982).

The potential relationship between *Caridinides* (and *Neocaridina*) and geographically proximate *Caridina* species highlights the weakness and difficulty in presuming *Caridina* is a single entity (Gurney 1984).

Australian hypogean genera

Half of the Australian atyid genera inhabit the subterranean world (*Parisia*, *Pycneus*, *Pycnisia*, *Stygiocaris*). The two representatives in our analyses, *Parisia unguis* Williams, 1964 and *Pycnisia raptor* Bruce, 1992, are recovered in a strong clade in all analyses. This is in line with expectations, because *Parisia*, *Pycnisia* and *Pycneus* (not included in this study) are considered to be closely related; to the extent that the name *Pycnisia* is a combination of the names of the other two genera (Bruce 1992), although the relationship of all three genera has not yet been tested with molecular data. There are two species of *Parisia* in Australia (sympatric in caves near Katherine, Northern Territory), which are highly differentiated from each other in their morphologies (Williams 1964; Bruce 1992). Because of this, the precise relationship of these two *Parisia* species would also benefit from molecular analyses. *Parisia* is also found in caves in Madagascar (four species: Gurney 1984) and the Philippines (one species: Cai and Anker 2004). The Australian endemic monotypic genus *Pycneus* has only been reported from caves in the Gibson Desert of central Western Australia (Holthuis 1986), whereas the endemic *Pycnisia* was originally described from caves near Katherine (sympatric with *Parisia unguis*: Bruce 1992). A second species of *Pycnisia* has recently been described from caves in north-western Queensland (Lawn Hill: Suzuki and Davie 2003).

The fourth hypogean genus, *Stygiocaris* (not included in this study) is also endemic and has only been found in the North-west Cape in Western Australia (two sympatric species from freshwater wells with tidal influence) (Davie 2002). Although *Stygiocaris* is not a close relative of the other Australian subterranean atyids, it also is considered to have links with the Malagasy fauna, in that the genus *Typhlopatsa* Holthuis, 1956 (endemic to caves in Madagascar) is considered its closest relation (Williams 1964; Short and Doumenq 2003).

Evolution of Australian subterranean atyids

The phylogenetic relationships outlined above raise some interesting questions regarding the evolutionary and geographic pathways these taxa may have taken to arrive among the diverse Australian stygofauna (Humphreys 2006). *Pycnisia raptor* is an aggressive predator with enlarged 'raptorial' pereopods (walking legs) (Bruce 1992). The morphology of *Pycnisia* is in sharp contrast to all other atyids, which use their brush-like setae to sweep food into their mouths (Fryer 1977; Bruce 1992). This derived adaptation of *Pycnisia* would have evolved after its divergence from *Parisia* and may have occurred in response to cave conditions, because these environments are low in energy (Humphreys 2006). Interestingly, there is often a shift from an

epigean predatory lifestyle to hypogean omnivory in many taxa (Humphreys 2006); i.e. precisely the opposite direction taken by *Pycnisia raptor*. Given their shared stygobitic lifestyles, *Pycnisia* and *Parisia* presumably speciated after adapting to the subterranean world, and somewhere in north-western Australia.

This raises the difficult and oft-discussed issue of the affinity between the subterranean atyids of Australia and Madagascar (Bishop 1967; Bănărescu 1990). If *Parisia*/*Pycnisia* represents a radiation of subterranean taxa within Australia, how is it that *Parisia* is also present in Madagascar (and the Philippines)? One possibility is that *Parisia* (and *Stygiocaris*) represents a very ancient vicariant Gondwanan hypogean relict (Williams and Allen 1987), from which *Pycnisia* (and possibly *Pycneus*) speciated within Australia. After all, disjunct vicariant relationships are not uncommon for subterranean fauna (Humphreys 2006). A related hypothesis is that the common Gondwanan ancestors of *Parisia* and *Stygiocaris*/*Typhlopatsa* were epigean in habit, and the various related taxa acclimatised to the underworld while their surface cousins subsequently became extinct (Smith and Williams 1981), but whether subterranean environments were colonised pre- or post-continental break-up is unclear. The disjunct ranges of two species of cave atyids in the eastern USA (the genus *Palaemonias* Hay, 1901) have been explained with a similar epigean to hypogean move and subsequent surface extinction (Hobbs 1994). Of course, the Gondwanan explanation would not explain the presence of *Parisia* in the Philippines.

Another possibility is that the ancestors of Australia and Madagascar's hypogean fauna were present in the marine environment of the southern arm of the Tethys Sea when the landmasses were not so remote (Bănărescu 1990; Humphreys 2006), with cave adaptation and surface extinction as above. This Tethyan explanation has also been invoked to explain the widely disjunct distribution of the northern hemisphere hypogean atyid, *Typhlatya* (Bănărescu 1990; Sanz and Platvoet 1995), which is said to have attained its wide range before obtaining its cave-suited morphologies (Sanz and Platvoet 1995) ('troglolobiosis': reduced eyes and pigmentation, etc.; Humphreys 2006).

An epigean ancestor, whether marine or freshwater, implies that the suite of troglomorphic traits may be the result of convergence, which is a common feature of crustaceans in subterranean environments (Hobbs 1994; Proudlove and Wood 2003; Lefébure *et al.* 2006), as well as among many other stygofauna (Cooper *et al.* 2002; Humphreys 2006). This is one of the strongest arguments for using molecules as well as morphology in teasing apart the relative influences of common selective pressures and common ancestry on phylogenetic inference (Proudlove and Wood 2003; Lee 2004; Lefébure *et al.* 2006). However, Smith and Williams (1981) felt this parallel evolution was an unlikely proposition for *Parisia*, *Stygiocaris* and *Typhlatya*, in particular because the presence of sympatric *Parisia* species with differing morphologies argued against it. They were not working from within a phylogenetic framework, and did not consider secondary contact, which Webb (2003) has demonstrated in sympatric *Typhlatya* species from Mexico through the medium of subterranean connections.

If a widespread hypogean taxon, such as *Parisia*, has epigean ancestors, these may be descended from localised epigean

species rather than a single widespread ancestor, as has been shown for European cave atyids (Zakšek *et al.* 2007), Western Australian stygobitic water beetles (Cooper *et al.* 2002) and subterranean decapods in the Americas (Hobbs 1994). This pattern may be visible in our data, because *Parisia* and *Pycnisia* (from northern Australia) form a clade with a *Caridina* from northern Australia. If this relationship is real, it is not recent. Epigean and hypogean sister-taxa among Australian water beetles diverged 9–12.5 million years ago, presumably in underground refugia in response to aridity (Cooper *et al.* 2002), and this may be consistent with some atyid underground colonisations. The relationship between *Caridina* and *Parisia* was hinted at by Bishop (1967), who suggested that the presence of *Parisia* in both Madagascar and Australia may not be of ‘great zoological significance’ because *Parisia* and *Caridina* were closely related and both were present in Australia and Madagascar. One should not be surprised by a close relationship between *Parisia* and *Caridina*, as this is well established. When Williams (1964) described the two Australian species of *Parisia*, he said it was ‘most prudent’ to include them in the genus *Parisia* (at that time only known from Madagascar), and noted that it ‘strongly resembles’ *Caridina*. Bruce (1992) also noted the similarity between *Caridina* and *Pycnisia* mouthparts.

Cai and Shokita (2006) have questioned the validity of the separation from *Caridina* of several hypogean genera, including *Parisia* and *Paracaridina* Liang & Guo, 1999, because some of the characters on which they were based appear to vary even between closely related species of *Caridina*. The close relationship between atyid hypogean and epigean genera is common, for example the epigean genus *Lancaris* Cai & Bahir, 2005 is morphologically most similar to the hypogean genera *Parisia*, *Pycnisia*, *Pycneus* and *Paracaridina* (Cai and Bahir 2005). This has been reinforced by a recent molecular phylogenetic study that showed that the nearest relation to one species of the European atyid cave genus *Troglocaris* Dormitzer, 1853 is a geographically proximate epigean species of another genus, rather than other ‘congeneric’ hypogean species (Zakšek *et al.* 2007).

Caridina is largely an epigean genus (Short and Doumenq 2003), as we treat it above. However, it might also be a reasonable choice as a sister-group of some hypogean taxa because of its presence in many subterranean locations (Short and Doumenq 2003). These include the facultative use of these environments (‘stygophile’: Humphreys 2006); for example the well-named Western Australian species *Caridina spelunca* Choy, 1996 (Choy 1996) and central Queensland *Caridina thermophila*, neither of which displays troglomorphies (Williams 1964; Choy 1996). A complete range of morphologies from the epigean to the troglobitic is visible in different *Caridina* species within caves from the same countries that also host *Parisia*. Madagascar has at least seven species of cavernicolous *Caridina*, three of which are troglobitic (Cai 2005). This includes the cave species *Caridina steineri* Cai, 2005, which appears in the analysis above and forms a clade with an epigean *Caridina* species from the African mainland. There are also numerous *Caridina* species in caves in the Philippines, at least one of which is a troglobite (Cai and Anker 2004). It may be that *Parisia* species are descended from local *Caridina* taxa that adapted to the subterranean world in the more distant past and, further, that the proto-stygofauna may not necessarily have

entered the underground world during the same epoch in each place (Humphreys 2006). These hypotheses could be tested relatively easily using phylogenetic techniques were specimens of subterranean and surface taxa available from all the relevant areas. The correct delineation of relationships, which will be aided by future nuclear DNA studies, is important, because hypotheses based on unreal groups must also be unreal.

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