# Molecular and conservation biogeography of freshwater caridean shrimps in north-western Australia

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ABSTRACT

Rivers in north-western Australia have been long isolated systems and several of them, including those in the central north Kimberley (CNK) and Pilbara (PIL) regions, are known for their endemism in freshwater fishes. Furthermore, some species of freshwater fish are shared between rivers of the south-western Kimberley (SWK) and rivers of the western Arafura-Carpentarian (WAC) regions to the exclusion of CNK, despite CNK being geographically intermediate to these regions. This suggests that the freshwater biota of the CNK may have an independent evolutionary history with respect to the SWK and the WAC, and that the latter two may have relatively strong biogeographic affinities. This pattern warrants further examination using phylogeographic analyses of freshwater biota, such as freshwater caridean shrimp. In this study we used mtDNA data and conservation biogeographic approaches to assess molecular patterns of biodiversity within freshwater shrimp, genus *Caridina* (Atyidae), in north-western Australia using reported biogeographic patterns in freshwater fishes as a hypothesis. Specifically, we expected CNK and PIL to have higher endemism and phylogenetic diversity ($PD$) within the *Caridina* than SWK and WAC, and shrimps of SWK and WAC to have stronger biogeographic affinities with one another than either one has with CNK. Results showed high endemism within genus *Caridina* from the CNK and PIL, although $PD$ and species richness was lowest for PIL and highest for CNK. Two lineages within *Caridina* were shared between the SWK and the WAC to the exclusion of the CNK. The results of this study for *Caridina* shrimp are thus strikingly similar to previous analyses of freshwater fishes, and support earlier studies that define PIL as a bioregion. We suggest that the incorporation of phylogeographic data for both fishes and freshwater macroinvertebrates (i.e., shrimps and molluscs) in future analyses may also identify CNK as a bioregion.

## 1 INTRODUCTION

Rivers are highly isolated ecological systems, somewhat akin to oceanic islands (MacArthur & Wilson 1967); thus they have long interested biogeographers (Rauchenberger 1988; Banarescu 1990) and phylogeographers (Bermingham & Avise 1986). Island biogeography theory predicts that highly isolated habitats (e.g., islands, rivers, lakes, mountain tops) contain a high proportion of endemic species because opportunities for biotic exchange between unconnected habitats are very limited (MacArthur & Wilson 1967; Whittaker et al. 2008). Examples of isolated freshwater systems with high endemism include various lake systems around the world (e.g., Africa's Rift Valley lakes, Marijnissen et al. 2009; Lake Poso, Indonesia, von Rintelen et al. 2007), desert springs of the

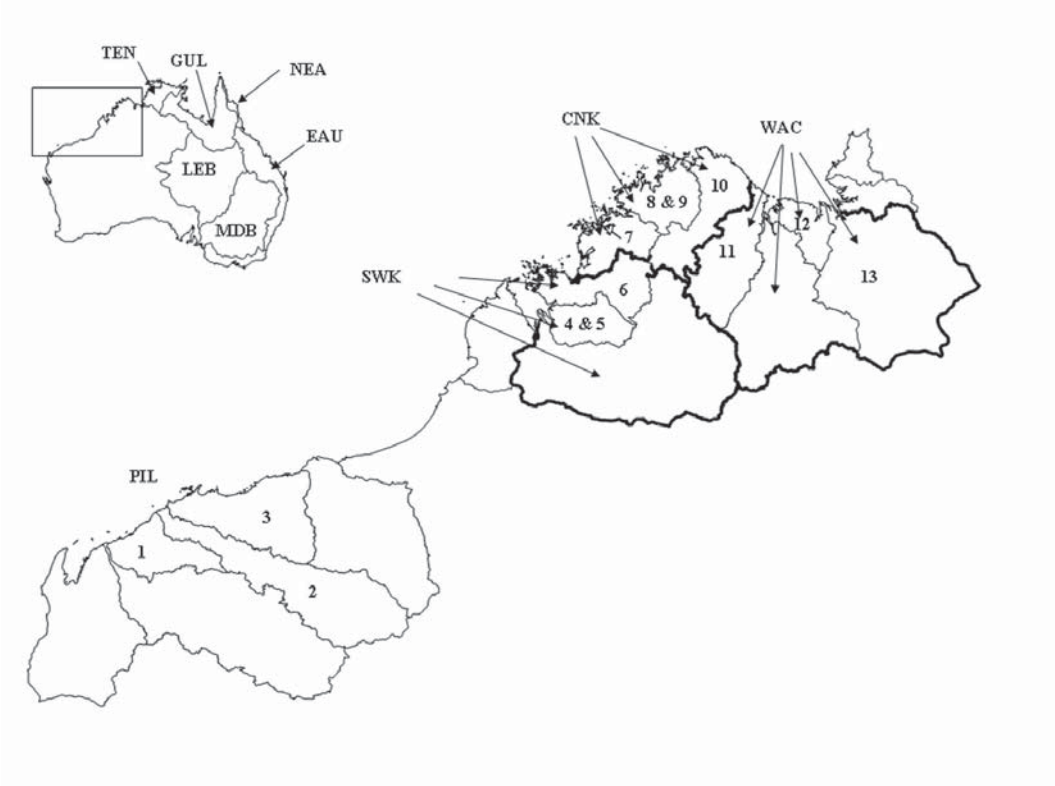


**Figure 1.** Map of north-western Australia showing the rivers and regions considered in this study. The north-western regions depicted are as follows: PIL: Pilbara; SWK: south-western Kimberly; CNK: central-northern Kimberley; WAC: western Arafura-Carpentaria. Numbers refer to rivers as presented in Table 1. Regions from elsewhere in Australia are shown on the inset, as follows: TEN: Top End of Australia; GUL: rivers draining to the Gulf of Carpentaria; NEA: North-eastern Australia; EAU: Eastern Australia; MDB: Murray-Darling Basin; LEB: Lake Eyre Basin.

United States of America, Mexico, and central Australia (Colgan & Ponder 2000; Kodric-Brown & Brown 2007), and various stygobiont ecosystems, globally (e.g., Edward & Harvey 2008).

Endemic taxa may also be relicts, i.e., geographically restricted taxa that are phylogenetically distinct from their closest relatives (Erwin 1991). Thus, the places they occur have high endemism and high phylogenetic diversity ($PD$, Faith 1992). Endemism and $PD$ are therefore complementary metrics that can be used to assess the conservation value of places and the historical biogeography of biota in isolated habitats. Habitats with a high proportion of relict species would have high conservation value and indicate that the long-term isolation of "place" is a key driver of concerted and strong biogeographic patterns across the species, including the long-term stability of species assemblages (Zink 2002; Lapointe & Rissler 2005). Use of these metrics in molecular conservation biogeographic analyses may therefore identify key patterns of freshwater biodiversity for freshwater bioregionalization and conservation.

North-western Australia (Figure 1) is renowned for its ancient (i.e., Proterozoic) geology and geographic isolation, particularly the many sandstone escarpments and gorges (Ollier et al. 1988; Bowman et al. 2010). Consequently, the rivers of the Kimberley and Pilbara regions are highly

isolated ecological systems and most remained discrete basins during historical periods of lowered sea levels when many other rivers of northern Australia in other regions were repeatedly connected by ancient confluences (Voris 2000; Harris et al. 2005). The freshwater fish fauna of the Kimberley and Pilbara regions therefore contain a high proportion of endemic species (Unmack 2001; Allen et al. 2002; Morgan et al. 2009) and both are recognized freshwater bioregions on account of their unique freshwater fish faunas (Unmack 2001; Abell et al. 2008). Patterns of endemism in freshwater biota in the Kimberley region are especially pronounced for rivers draining the sandstone plateau country of the central and northern Kimberley (CNK) (e.g., Morgan et al. 2009; Figure 1). Indeed, riverine biota from the south-western Kimberley (SWK) region may have stronger biogeographic affinities with the biota of the western-most rivers in the Arafura-Carpentaria bioregion (Western Arafura-Carpentaria, WAC; Figure 1) than with the CNK. For example, Unmack (2001) reports that several species of freshwater fish are shared between the SWK and WAC, and are absent from CNK. The biogeographic distinctiveness of the CNK warrants further examination using phylogeographic analyses of key freshwater groups.

One group that is widespread in northern Australia is the freshwater caridean shrimp, genus *Caridina* (Decapoda: Atyidae), that has been used to demonstrate strong freshwater biogeographic and phylogeographic patterns elsewhere in Australia (Page & Hughes 2007; Cook et al. 2008). In the present study we used mtDNA data and conservation biogeographic approaches to assess molecular patterns of biodiversity within the freshwater shrimp, genus *Caridina* (Atyidae), in north-western Australia using reported biogeographic patterns in freshwater fishes as a hypothesis. Specifically, we expected CNK and PIL to have higher endemism and phylogenetic diversity within *Caridina* than SWK and WAC, and shrimps of SWK and WAC to have stronger biogeographic affinities with one another than either one has with CNK. Support for these predictions would suggest that CNK should be considered a distinct freshwater bioregion.

## 2 MATERIALS AND METHODS

### 2.1 Samples used and genotyping method

A total of 58 individual shrimps from 13 rivers (23 sites) from the four regions were sequenced for a 453 basepair fragment of the cytochrome c oxidase subunit I (COI) mtDNA gene (Table 1; Appendix, Tables A1 and A2). Total genomic DNA was extracted from each individual using the CTAB/phenol chloroform method and a fragment of the COI mtDNA gene was amplified using polymerase chain reaction (PCR) and the CDC0.La (5'-CCN GGG TTY GGR ATA ATT TCT C-3'; Page et al. 2005a) and COIa (5'- AGT ATA AGC GTC TGG GTA GTC-3'; Palumbi et al. 1991) primers. PCR cycling conditions were as follows: 3 min at 94 °C; 15 cycles of 30 s at 94 °C, 30 s at 40 °C, 60 s at 72 °C; then 25 cycles of 30 s at 94 °C, 30 s at 55 °C, 60 s at 72 °C; 7 min at 72 °C, and finally held at 4 °C. PCR product was purified with the exonucleoase I-shrimp alkaline phosphatase method, using 2.5 $\mu$l PCR product, 2.0 $\mu$l shrimp alkaline phosphatase (Promega) and 0.5 $\mu$l exonucleoase I (Fermentas), and a two-step thermocycling profile: 35 min at 37 °C, 20 min at 80 °C. Ten micro-liter sequencing reactions contained 0.5 $\mu$l purified product, 0.32 $\mu$l forward primer (3.5 pmol/$\mu$l), 2 $\mu$l BigDye v1.1 (Applied Biosystems) and 2 $\mu$l BigDye, 5× sequencing buffer (Applied Biosystems), and standard thermal cycling conditions were used: 1 min at 96 °C; 30 cycles of 10 s at 96 °C, 5 s at 50 °C, 4 min at 60 °C; and a hold period of 4 °C. Two exemplars of each lineage were sequenced using the reverse primer to verify basepair composition. Sequencing was conducted on a 3130xl Capillary Electrophoresis Genetic Analyser (Applied Biosystems) and sequences were aligned and edited using SEQUENCHER version 4.1.2 (Gene Codes), yielding 453 unambiguous basepairs, and deposited in GenBank. To test for the possibility of nuclear copies of mitochondrial DNA (NUMTs) in the data and other nucleotide anomalies in the COI mtDNA data,

**Table 1.** Origin of freshwater shrimp of *Caridina* used for this study with regions and rivers sampled and sample sizes for each river (number of sites and number of individuals). River numbers correspond to numbers used in Figure 1.

| Region | River | No. of sites | No. of individuals |
|---|---|---|---|
| PIL | 1. Onslow Coast | 3 | 5 |
| PIL | 2. Fortescue River | 3 | 4 |
| PIL | 3. Port Headland Coast | 1 | 3 |
| SWK | 4. May River | 2 | 8 |
| SWK | 5. Robinson River | 1 | 5 |
| SWK | 6. Isdell River | 1 | 1 |
| CNK | 7. Prince Regent River | 1 | 1 |
| CNK | 8. Mitchell River | 3 | 7 |
| CNK | 9. King Edward River | 3 | 9 |
| CNK | 10. Drysdale River | 2 | 5 |
| WAC | 11. Pentecost River | 1 | 4 |
| WAC | 12. Keep River | 1 | 3 |
| WAC | 13. Victoria River | 1 | 3 |

which may artificially produce false genetic lineages in phylogenetic analyses (Song et al. 2008), nucleotide sequences were translated to amino acid sequences using the invertebrate mtDNA code as implemented in GENEDOC version 2.7.000 (Nicholas & Nicholas 1997). The presence of one or more stop codons in the amino acid sequence would suggest the possibility of NUMTs in the data. Exemplars of each COI lineage were sequenced for a 455 base-pair fragment of the 16S mtDNA gene (methods described in von Rintelen et al. 2007; primer sequences: 16S-F-Car: 5'-TGC CTG TTT ATC AAA AAC ATG TC-3', 16S-R-Car: 5'-AGA TAG AAA CCA ACC TGG CTC-3') and aligned with published 16S sequences of other Australian *Caridina* from GenBank (Appendix, Table A1). All new sequences (COI and 16S) were submitted to Genbank and can be retrieved under accession numbers JN012514–JN012565.

## 2.2 Data analysis

Phylogenetic analyses for both the COI and 16S datasets were performed using 1,000 bootstrap pseudoreplicates of the Maximum Likelihood (ML) method incorporating the GTR model as implemented in PHYML (Guindon & Gascuel 2003). We used *Caridina serratirostris* (GenBank Accession number DQ478515) from north-eastern Australia as an outgroup for the COI phylogeny, and *Paratya australiensis* (GenBank Accession number DQ78566) as an outgroup for the 16S phylogeny. The gene trees with best log likelihood scores were used in analysis of endemism and phylogenetic diversity after nodes with less than 50% bootstrap support were collapsed using MESQUITE (Maddison & Maddison 2000).

Endemism was calculated for each river basin and each region using the Site Endemism Index ($SEI$, Rebelo & Siegfried 1992) and the identified COI lineages as taxa, with $SEI = \sum k/a_{\mathrm{i}}$, where $k$ is the total number of sites (i.e., river basin or region) and $a_{\mathrm{i}}$ is the number of sites at which species i occurs. $SEI$ ranges from one, where all taxa at a location have broad geographical ranges, through to infinity, with larger values indicating the presence of an increasing number of geographically restricted taxa. $SEI$ therefore reflects the richness of endemic species. Phylogenetic diversity ($PD$, Faith 1992) was then calculated for each river basin and each region for the COI

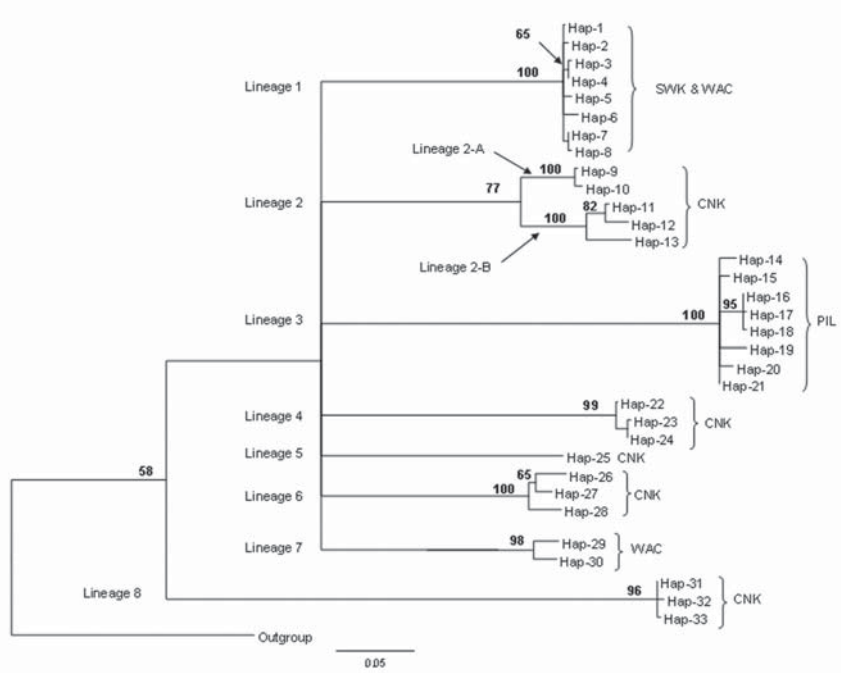


**Figure 2.** Maximum likelihood COI mtDNA gene tree (ln -2715.56) for *Caridina* spp. sampled from north-western Australia, showing bootstrap values (after 1,000 pseudorelilcates) along branches. Nodes with less than 50% bootstrap support were collapsed and the scale bar indicates five percent difference in basepair composition. The distributions of the lineages with respect to the regions are indicated on the right-hand side of the tree.

mtDNA ML phylogeny, with branch lengths calculated using MESQUITE and summed for each region and river, respectively. $PD$ is a measure that integrates phylogeny and complementarity (Faith 1992) and ranges from zero, where a location has low endemism and complementarity with respect to the total phylogeny of the taxa, through to infinity, with larger values indicating that the location contains taxa that incorporate increasing proportions of the phylogeny that are not present elsewhere in the landscape. Because some regions had relatively few sites sampled, we tested for correlations between the number of sites and the diversity metrics (i.e., endemism and $PD$) using Pearson's correlation coefficient in SPSS.

Finally, we used the 16S phylogeny and regional-scale analyses of $SEI$ and $PD$ to compare patterns of molecular biodiversity within *Caridina* in north-western Australia with other regions throughout Australia (see Figure 1 and Appendix, Table A1 for description of these regions). Whereas lineages were used to calculate COI $SEI$ and $PD$, several of the 16S lineages were comprised of sublineages, many relating to described or previously distinguished taxa (see Results). Thus, taxa (rather than lineages) were used in analyses of 16S endemism and $PD$.

## 3 RESULTS

Stop codons and double peaks were not detected in the COI data, reducing the possibility of including NUMTs in our analyses. Eight divergent COI mtDNA lineages were recovered by ML analyses (Figure 2), six of which received high bootstrap support (i.e., Lineages 1, 3, 4, 6, 7, and 8; all boot-

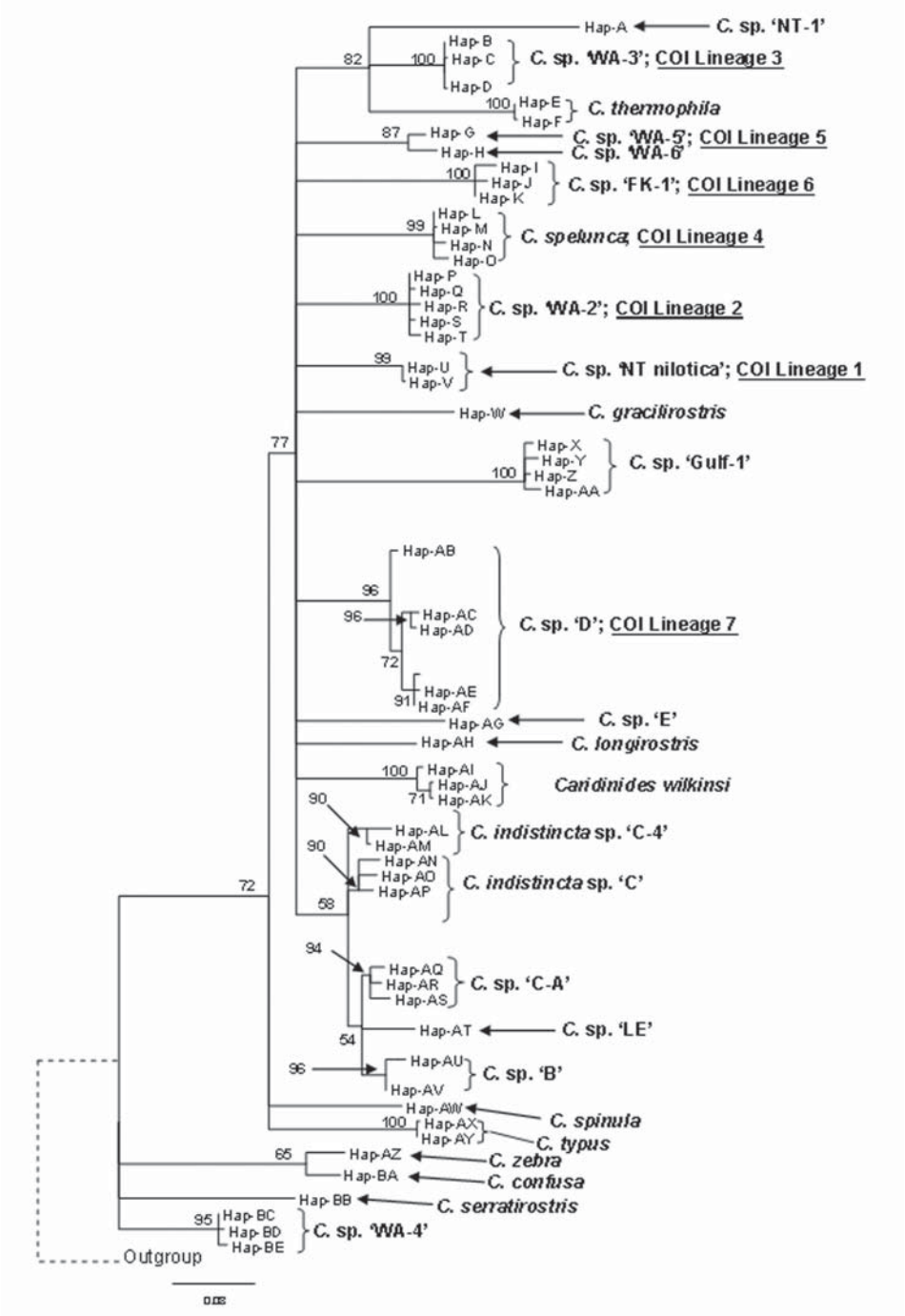


**Figure 3.** Maximum likelihood 16S mtDNA gene tree (ln -3801.86) for *Caridina* spp. sampled from throughout Australia, showing bootstrap values (after 1,000 pseudoreplicates) along branches. Nodes with less than 50% bootstrap support were collapsed and the scale bar indicates eight percent difference in basepair composition. The COI lineages corresponding to the 16S lineages are indicated on the right-hand side of the tree in underlined font. The distributions of 16S taxa from throughout Australia are presented in Appendix, Table A1.

strap values $> 95\%$), one (i.e., Lineage 2), which had good bootstrap support (i.e., 77%), and one (i.e., Lineage 5) which was unable to be assessed by bootstrapping because it was represented by only a single haplotype. Lineage 2 contained two strongly supported sublineages (i.e., 2-A and 2-B), and Lineage 8 appears to be monophyletic to all other lineages, although this lineage had relatively low bootstrap support. The phylogenetic relationships between Lineages 1 to 7 could not be determined due to poor bootstrap support at several of the deeper nodes, which were collapsed into a soft polytomy.

ML analysis of the 16S data recovered 20 lineages (Figure 3), although several of these were comprised of sublineages, many relating to described or previously distinguished taxa. Five of the COI lineages related to previously reported 16S taxa, as follows: Lineage 1 relates to *Caridina* sp. "NT *nilotica*" (Page et al. 2007a); Lineage 2 relates to *Caridina* sp. "WA-2" (Page et al. 2007a); Lineage 3 relates to *Caridina* sp. "WA-3" (Page et al. 2007a); Lineage 4 relates to *Caridina spelunca*; and Lineage 7 relates to *Caridina* sp. "D" (Page et al. 2005a). Two COI lineages (i.e., Lineages 5 and 6) do not relate to previously reported 16S taxa and COI Lineage 8 was not represented in the 16S phylogeny because only a very short 16S fragment (i.e., $< 300$ bp) could be amplified for this taxon. We note here that this short fragment does not align with previously reported 16S taxa, suggesting that these three COI lineages (i.e., Lineages 5, 6, and 8) represent new taxa. We included an additional 16S lineage shared between SWK and WAC for which we did not have COI data (i.e., WA-4; Figure 3); thus, two 16S lineages were shared between SWK and WAC to the exclusion of CNK.

The geographic distribution of the COI mtDNA lineages varied, with five lineages restricted to CNK (i.e., Lineages 2, 4, 5, 6, and 8), one lineage restricted to PIL (i.e., Lineage 3), one lineage restricted to the WAC (i.e., Lineage 7), and one lineage shared among regions (i.e. Lineage 1 shared by WAC and SWK). These striking patterns of distribution resulted in similarly impressive patterns of endemism at both river and region scales, with the King Edward River and CNK regions having the highest levels of endemism, respectively (Figure 4). Endemism was lowest for the May, Robinson, Pentecost and Keep Rivers, and the SWK region, respectively. Endemism was not correlated with the number of sample sites in a drainage basin (Pearson's correlation coefficient = 0.418, $P = 0.177$).

Phylogenetic Diversity ($PD$, Faith 1992) as assessed for the COI mtDNA ML phylogeny was highest in the King Edward River and CNK region, respectively (Figure 5). The Mitchell and Drysdale Rivers also had high $PD$ at the river basin scale. Rivers of the SWK and WAC regions had the lowest $PD$ at the river basin scale, whereas PIL had the lowest $PD$ at the region scale. $PD$ was correlated with the number of sampled sites in a drainage basin (Pearson's correlation coefficient = 0.772, $P = 0.003$).

The 16S data indicated that north-eastern Australia is the region with the greatest endemism within *Caridina*, and that CNK was ranked as sharing the second-highest levels of endemism with eastern Australia, closely followed by the Top End region (Figure 6). PIL had a low score for $SEI$, but the single 16S lineage detected from this region is endemic. SWK, WAC, and Gulf of Carpentaria region (GUL) all have relatively low levels of endemism as most taxa from these regions are common to at least one other region. North-eastern Australia also received the highest score for $PD$, with CNK being ranked as equal second highest with the Top End region. Eastern Australia had third-highest $PD$, although unlike endemism this value was not much greater than for most other regions.

## 4 DISCUSSION

The molecular biogeographic analyses of *Caridina* shrimp from north-western Australia indicate striking phylogeographic structuring and endemism, including three previously unreported taxa.

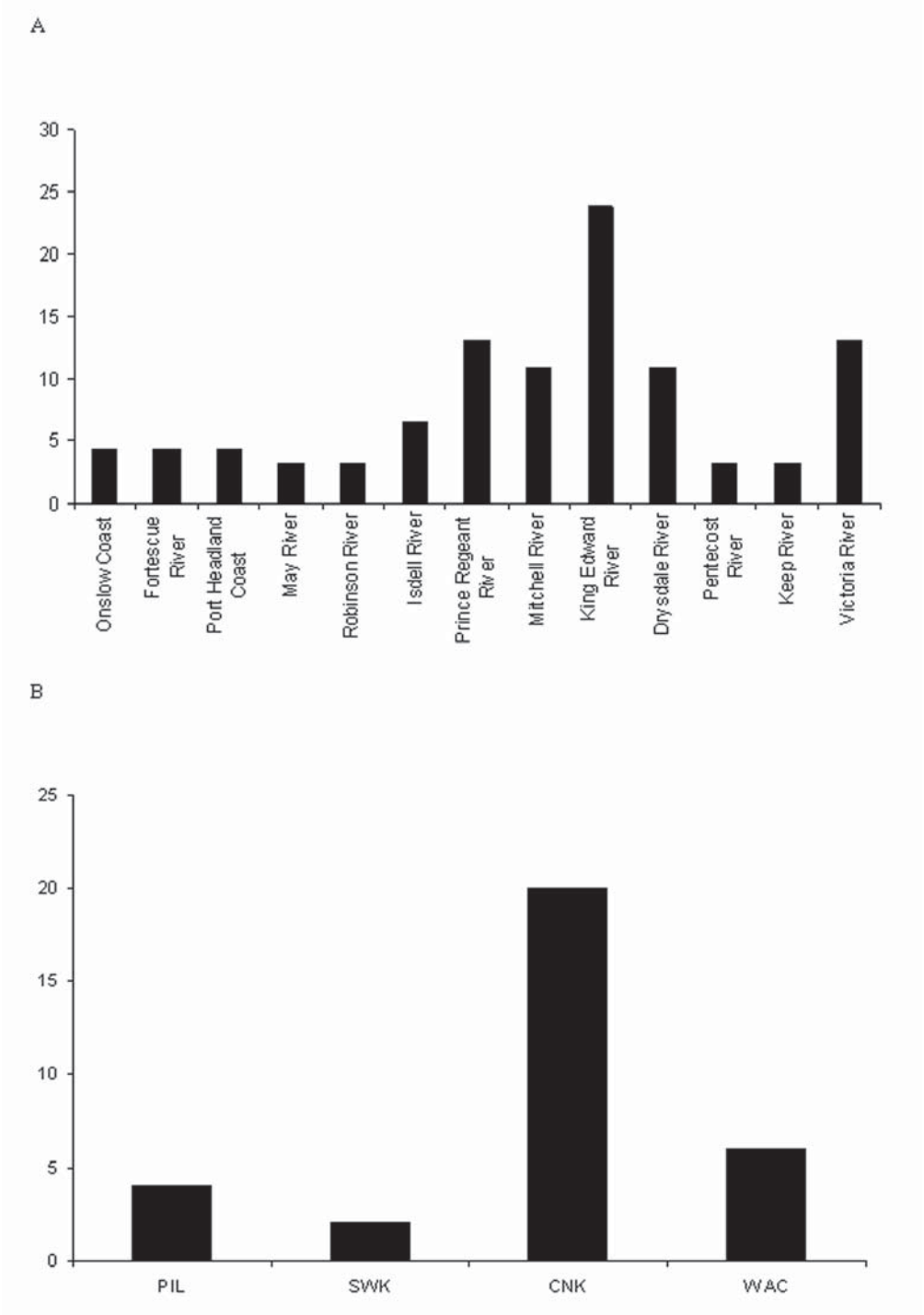


**Figure 4.** Histogram of COI mtDNA endemism as assessed using the site endemism index ($SEI$) within the genus *Caridina* from north-western Australia for A: rivers, and B: regions.

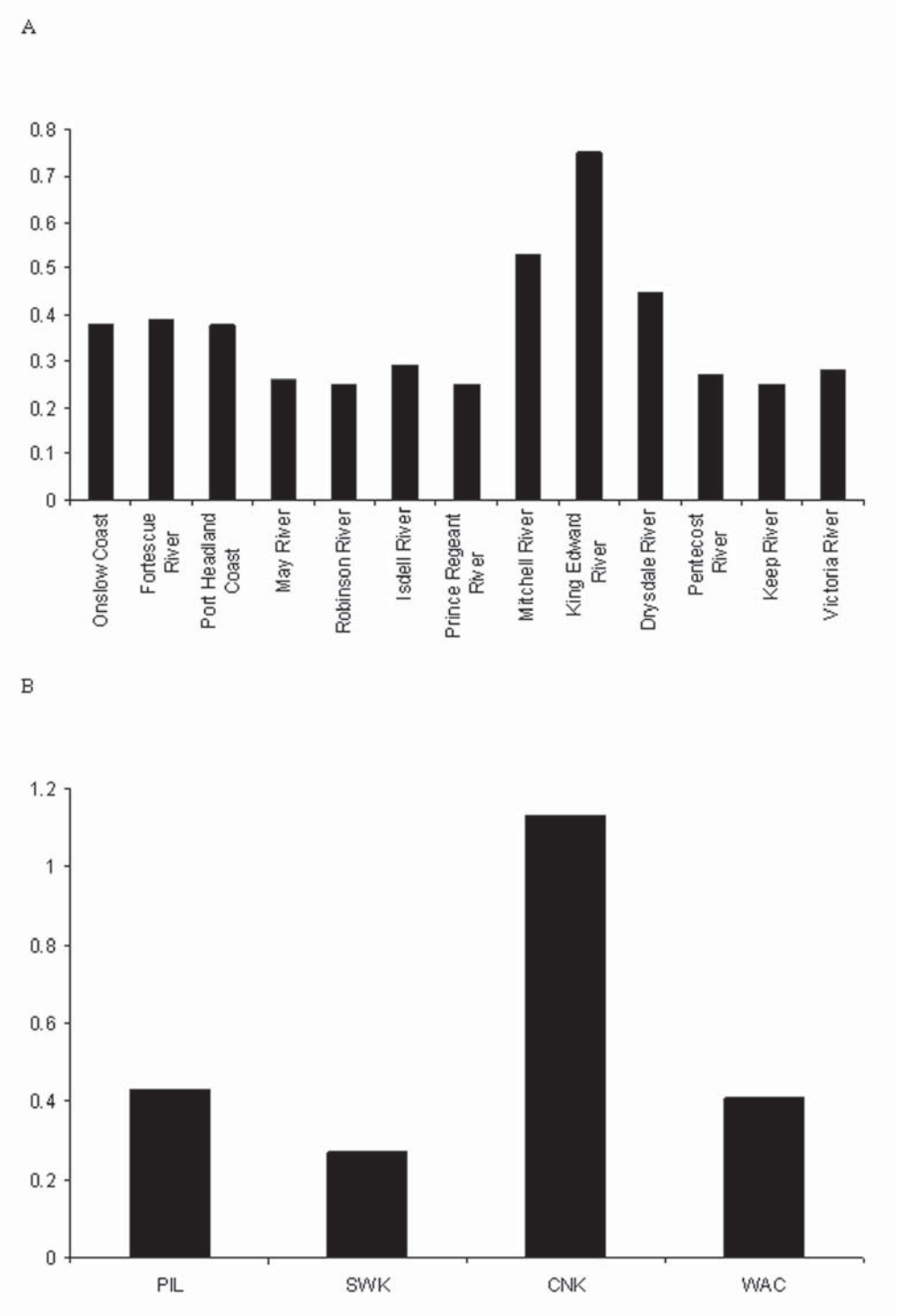


**Figure 5.** Histogram of COI mtDNA phylogenetic diversity ($PD$) within the genus *Caridina* from north-western Australia for A: rivers, and B: regions.

Biogeographic patterns of high species richness and endemism in freshwater fishes from the CNK (Allen et al. 2002; Unmack 2001; Morgan et al. 2009) were also found within *Caridina* (this study). The region has remained geographically isolated for both freshwater fishes and shrimps over evolutionary time scales and the many gorges in the region have acted as refugia, enabling the long-term persistence of freshwater species (Unmack 2001). Interestingly, freshwater crayfish are absent from this region, although they are common in most other regions of Australia (Whiting et al. 2000). Overall, these data indicate biogeographic distinctiveness of the freshwater biota of this region, similar to that shown for freshwater biodiversity of South Africa's Cape floristic region (Wishart & Day 2002) and patterns of diversity within *Caridina* from other isolated habitats (e.g., Lake Poso, von Rintelen et al. 2007). Whilst the phylogenetic relationships between most taxa within the Australian *Caridina* could not be established, it is clear from the 16S data that at least some taxa from north-western Australia are more closely related to species in more eastern regions of Australia than to other taxa from the west. A very similar pattern was found for species within the freshwater fish genus *Craterocephalus* (i.e., *C. helenae*, endemic to the CNK, and *C. lentigenosus*, distributed across SWK, CNK, and WAC), which were more closely related to geographically distant species than to each other (Unmack & Dowling 2010). Indeed, some Australian species of *Caridina* are known to be more closely related to species from Asia than to other species from Australia (Page et al. 2007a).

Endemism and phylogenetic diversity are complimentary approaches for assessing diversity and bioregional patterns in biota. For the COI analyses, both metrics identified the same river (i.e., King Edward River) and region (i.e., CNK) as having the highest diversity. These results indicate the presence of a high proportion of endemic taxa, which contrasts patterns found in the shrimp genus *Paratya* from eastern Australia in which high diversity at the river basin scale was due to the co-occurrence of occasionally detected endemic taxa with widespread taxa (Cook et al. 2008). We note, however, that $PD$ was correlated with the number of sites sampled in a river basin; thus, our results for $PD$ in this study must be considered a prediction which we hope to test in the near future using more comprehensive sampling. For the continent-wide 16S analyses, $SEI$ and $PD$ both identified north-eastern Australia as the region with the highest diversity in Australia within *Caridina*, and both metrics ranked CNK as having equal second highest diversity, indicating this region has significant freshwater shrimp biodiversity at the national scale. However, CNK was equal second with eastern Australia for endemism, and equal second with the Top End region for $PD$. These contrasting patterns demonstrate the utility of applying both these metrics in biodiversity assessments; eastern Australia had high endemism due to a large number of cryptic taxa within the *C. indistincta* complex (Page et al. 2005a) but had lower $PD$ because these species were very closely related relative to the fewer but more distantly related taxa present in the Top End. Similarly, PIL had low $SEI$ within *Cardinia* relative to $PD$. However, as $SEI$ increases within increasing richness of endemics, places with low species richness will have low $SEI$, even if the few species are endemic. The single taxon we detected within *Caridina* from PIL was endemic (i.e., PIL has 100% endemism for *Caridina*), suggesting perhaps that both $SEI$ and percent endemism should be assessed in conservation biogeographic analyses. The pattern of low richness and high endemism in freshwater biodiversity from PIL is reflected in freshwater fishes at both species (e.g., *Craterocephalus cuneips* and *Leiopotherapon aheneus*) and genus (i.e., *Milyeringa veritas*) levels, as well as in caridean shrimp, including species within *Caridina* (i.e., COI Lineage 3) and for the endemic shrimp genus *Stygiocaris* (Atyidae) (Page et al. 2008a). Low species richness and high endemism is a classical expectation of island biogeographic theory for highly isolated habits (MacArthur & Wilson 1967).

Previous biogeographic analyses report the shared distribution of several species of freshwater fish (e.g., *Anodontiglanis dahli*, *Arius midgleyi*, and *Craterocephalus stramineus*) between the SWK and WAC, with these species being absent from CNK despite the CNK being geographically

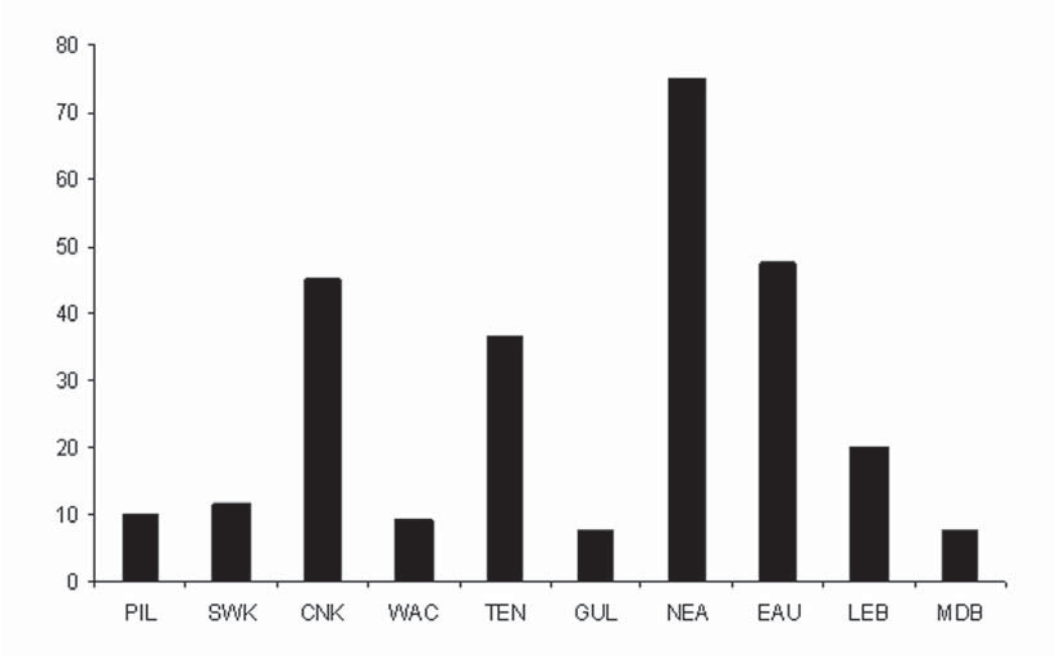


**Figure 6.** Histogram of regional levels of 16S endemism within the genus *Caridina* in Australia. The regions considered are: TEN: Top End of Australia; GUL: rivers draining into the Gulf of Carpentaria; NEA: North-eastern Australia; EAU: Eastern Australia; MDB: Murray-Darling Basin; LEB: Lake Eyre Basin.

intermediate to SWK and WAC (Unmack 2001). Our analyses of *Caridina* show a similar pattern, with two 16S lineages shared between these two regions, to the exclusion of CNK, suggesting close biogeographic affinities between these two regions, probably reflecting past inland drainage connections. Past offshore riverine connections mediated by palaeoeustatic processes likely had an important influence on freshwater biogeographic patterns in other regions of northern Australia, such as the Gulf of Carpentaria (e.g., de Bruyn et al. 2004; Cook & Hughes 2010), although drainages of north-western Australia remained discrete basins during glacial phases (see Harris et al. 2005). The offshore and extensive palaeo-hydrosystems to the west of the Kimberly and Pilbara predicted by Harris et al. (2005) were therefore extremely unlikely conduits for past connectivity for *Caridina* shrimp between WAC and SWK, to the exclusion of CNK. We are presently investigating the biogeographic affinities of WAC and SWK, to the exclusion of the distinctive and diverse CNK, using phylogeographic methods in both freshwater fishes and other invertebrates (e.g., other genera of caridean shrimp and molluscs).

We note the need for more comprehensive sampling of *Caridina* from throughout the study area, particularly considering that $PD$ was correlated with the number of sampled sites in a river basin, to demarcate freshwater bioregional units in north-western Australia. Phylogeographic patterns in other freshwater species, including fish and other invertebrate groups, would also contribute greatly to developing a freshwater bioregionalization for north-western Australia. Whilst unrelated taxa may not have shared evolutionary histories and may therefore not share the same bioregional boundaries (e.g., Growns 2009), the striking concordance in diversity patterns within freshwater fish and *Caridina* shrimp suggests a strong degree of congruence in biogeographic history. Abell et al. (2008) suggest that periodic reviews of the bioregional boundaries they propose should incorpo-

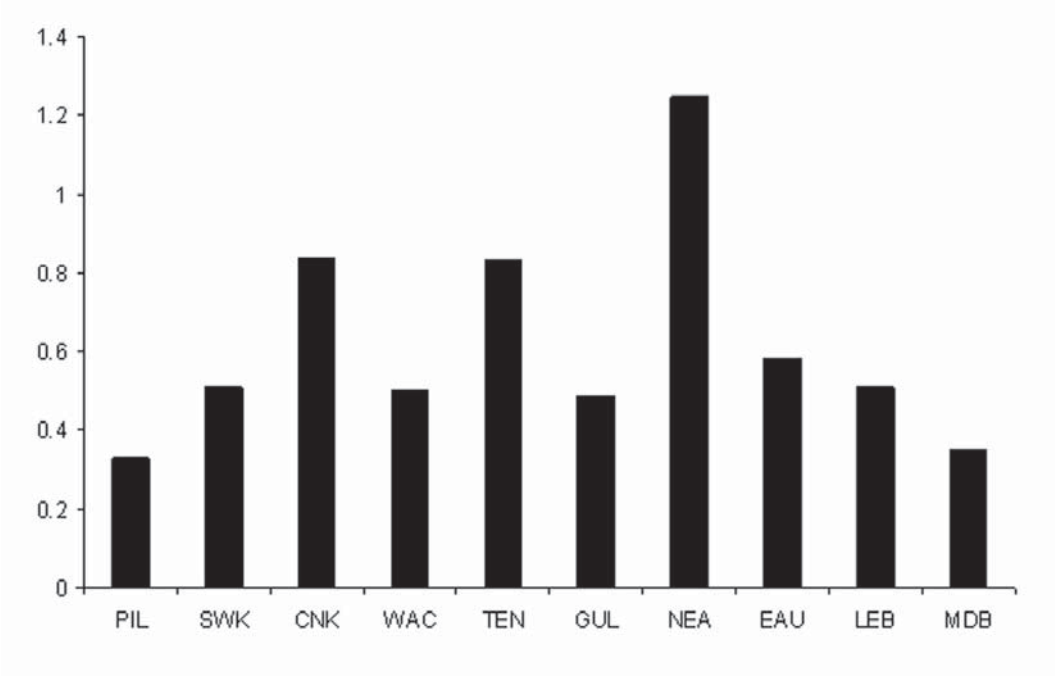


**Figure 7.** Histogram of regional levels of 16S phylogenetic diversity within genus *Caridina* in Australia. The regions considered are: TEN: Top End of Australia; GUL: rivers draining to the Gulf of Carpentaria; NEA: North-eastern Australia; EAU: Eastern Australia; MDB: Murray-Darling Basin; LEB: Lake Eyre Basin.

rate updated data, including fish and taxa other than fish. They also state that obligate freshwater macroinvertebrates respond to localized ecological and evolutionary factors that are too small to be meaningful for ecoregion delineation. However, conservation biogeographic analyses of caridean shrimp in eastern Australia were very similar to cryptic biodiversity patterns in freshwater fishes (Cook et al. 2008), as shown for fishes and shrimps in this study. It would be interesting to assess phylogeographic patterns in other obligate freshwater macroinvertebrates (e.g., molluscs) throughout north-western Australia, many of which do have very localised population structures (e.g., Carini & Hughes 2006; Colgan & Ponder 2000), to determine if similar biogeographic patterns are found in these taxa.

The delineation of boundaries between bioregional units is a debated issue (e.g., Unmack 2001; Filipe et al. 2009), with discrepancies concerning the use of single versus multiple taxonomic groups (as noted in the previous paragraph) and criteria for boundaries, such as the use of alpha or beta components of biodiversity. Alpha diversity criteria include species richness or percent endemism, although the percentage of endemism that should be used to separate biogeographic units is not established in biogeographic theory (Unmack 2001). Beta diversity criteria may include geographical points of turnover of multiple species' distributions and turnover points of divergent genetic lineages within species, although no rule of thumb exists concerning the proportion of taxa needed to exhibit turnover at either species or genetic levels to constitute a "unit of biodiversity with a shared evolutionary history" which is a commonly used definition of "bioregion" and its synonyms (e.g., "province" and "ecoregion", Unmack 2001; Spalding et al. 2007; Abell et al. 2008). In practice, however, the criteria used to delineate bioregional boundaries will be determined by data availability (Richardson & Whittaker 2010). Our analysis of endemism and $PD$, which are both components

of alpha biodiversity, showed markedly different patterns among the four regions of north-western Australia, notably with both metrics indicating higher within-region diversity for CNK. High endemism also suggests substantial differences in the composition of biodiversity between biogeographic units (i.e., beta diversity), although we did not explicitly test biotic dissimilarity within *Caridina* among the regions. A conservation biogeographic study of caridean shrimp in eastern Australia showed that the composition of cryptic biodiversity within shrimp of the genera *Caridina* and *Paratya* can be significant among river basins (Cook et al. 2008), and we suggest that such analyses based on more comprehensive sampling would likely indicate that CNK and PIL are both distinct bioregional units. In contrast, it is probable that SWK and WAC would be shown to have relatively high similarity in alpha and beta components of biodiversity within *Caridina*. Thus, our analyses of *Caridina* support previous analysis of freshwater fishes that define PIL as a freshwater bioregion (Unmack 2001; Abell et al. 2008) and indicate that CNK could also be considered a distinct bioregion. This study demonstrates that molecular-based bioregional studies of freshwater shrimps can indicate strong patterns of freshwater biodiversity and identify units for freshwater conservation management, as also shown in other regions of Australia and for other freshwater crustacean groups (e.g., Whiting et al. 2000; Cook et al. 2008; Bentley et al. 2010).

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APPENDIX

**Table A1.** 16S mtDNA data used, with Genbank numbers, source publication and geographic distribution represented by the sequence. TEN: Top End of Australia; GUL: rivers draining to the Gulf of Carpentaria; NEA: North-eastern Australia; EAU: Eastern Australia; MDB: Murray-Darling Basin; LEB: Lake Eyre Basin.

| **Species** | **Distribution** | **GenBank number** | **Reference** |
|---|---|---|---|
| *Caridina confusa* | NEA | DQ478495 | Page et al. (2007a) |
| *Caridina gracilirostris* | NEA | DQ478452 | Page et al. (2007a) |
| *Caridina longirostris* | NEA | DQ478507 | Page et al. (2007a) |
| *Caridina serratirostris* | NEA | DQ478515 | Page et al. (2007a) |
| *Caridina* sp. D | GUL, EAU, MDB | AY795052 | Page et al. (2005a) |
| *Caridina* sp. D | GUL, EAU, MDB | DQ478523 | Page et al. (2007a) |
| *Caridina* sp. D | WAC | | this study |
| *Caridina* sp. DG | GUL | DQ478519 | Page et al. (2007a) |
| *Caridina* sp. DG | GUL | DQ478520 | Page et al. (2007a) |
| *Caridina* sp. E | EAU | AY795051 | Page et al. (2005a) |
| *Caridina* sp. FK1 | CNK | | this study |
| *Caridina* sp. FK1 | CNK | | this study |
| *Caridina* sp. FK1 | CNK | | this study |
| *Caridina* sp. Gulf 1 | TEN, GUL | | this study |
| *Caridina* sp. Gulf 1 | TEN, GUL | | this study |
| *Caridina* sp. Gulf 1 | TEN, GUL | DQ478531 | Page et al. (2007a) |
| *Caridina* sp. Gulf 1 | TEN, GUL | DQ478533 | Page et al. (2007a) |
| *Caridina* sp. *indistincta* A | EAU | | Page et al. (2007a) |
| *Caridina* sp. *indistincta* A | EAU | DQ478499 | Page et al. (2007a) |
| *Caridina* sp. *indistincta* A | EAU | AY795039 | Page et al. (2005a) |
| *Caridina* sp. *indistincta* B | EAU, MDB | AY795040 | Page et al. (2005a) |
| *Caridina* sp. *indistincta* B | EAU, MDB | AY795043 | Page et al. (2005a) |
| *Caridina* sp. *indistincta* C | EAU | AY795046 | Page et al. (2005a) |
| *Caridina* sp. *indistincta* C | EAU | AY795045 | Page et al. (2005a) |
| *Caridina* sp. *indistincta* C | EAU | DQ478503 | Page et al. (2007a) |
| *Caridina* sp. *indistincta* C4 | EAU | AY795048 | Page et al. (2005a) |
| *Caridina* sp. *indistincta* C4 | EAU | AY795049 | Page et al. (2005a) |
| *Caridina* sp. LE | LEB | DQ478534 | Page et al. (2007a) |
| *Caridina* sp. NT 1 | TEN | DQ478537 | Page et al. (2007a) |
| *Caridina* sp. NT *nilotica* | SWK, WAC, NT | | this study |
| *Caridina* sp. NT *nilotica* | SWK, WAC, NT | DQ478510 | Page et al. (2007a) |
| *Caridina* sp. WA 2 | CNK | | this study |
| *Caridina* sp. WA 2 | CNK | | this study |
| *Caridina* sp. WA 2 | CNK | | this study |
| *Caridina* sp. WA 2 | CNK | DQ478550 | Page et al. (2007a) |
| *Caridina* sp. WA 2 | CNK | DQ478551 | Page et al. (2007a) |
| *Caridina* sp. WA 3 | PIL | DQ478552 | Page et al. (2007a) |
| *Caridina* sp. WA 3 | PIL | | this study |
| *Caridina* sp. WA 3 | PIL | | this study |
| *Caridina* sp. WA 4 | SWK, WAC, TEN | | this study |
| *Caridina* sp. WA 4 | SWK, WAC, TEN | DQ478554 | Page et al. (2007a) |
| *Caridina* sp. WA 4 | SWK, WAC, TEN | DQ478555 | Page et al. (2007a) |

**Table A1.** Continuation.

| Species | Distribution | GenBank number | Reference |
|---|---|---|---|
| *Caridina* sp. WA 5 | CNK | | this study |
| *Caridina* sp. WA 6 | CNK | | this study |
| *Caridina spelunca* | CNK, SWK | | this study |
| *Caridina spelunca* | CNK, SWK | EU123845 | Page et al. (2008a) |
| *Caridina spelunca* | CNK, SWK | DQ478548 | Page et al. (2007a) |
| *Caridina spelunca* | CNK, SWK | DQ478549 | Page et al. (2007a) |
| *Caridina spinula* | NEA | DQ478527 | Page et al. (2007a) |
| *Caridina thermophila* | LEB | EU123846 | Page et al. (2008a) |
| *Caridina thermophila* | LEB | DQ478556 | Page et al. (2007a) |
| *Caridina typus* | NEA | DQ478561 | Page et al. (2007a) |
| *Caridina typus* | NEA | DQ478562 | Page et al. (2007a) |
| *Caridina zebra* | NEA | AY661486 | Page et al. (2005b) |
| *Caridinides wilkinsi* | TEN, NEA | | this study |
| *Caridinides wilkinsi* | TEN, NEA | DQ681272 | Page et al. (2008b) |
| *Caridinides wilkinsi* | TEN, NEA | DQ681273 | Page et al. (2007b) |

**Table A2.** Collection accession numbers of specimens and species from this study. AM: Australian Museum; GU: Griffith University; QM: Queensland Museum; VM: Museum Victoria; WAM: West Australian Museum; ZMB: Museum fr Naturkunde Berlin.

| Species | Institution | Specimen number |
|---|---|---|
| **Specimens from this study** | | |
| *Caridinides wilkinsi* | GU | CAR99 |
| *Caridina* sp. FK1 | GU | CAR54-6, CAR128, TP777 |
| *Caridina* sp. FK2 | GU | CAR121-6 |
| *Caridina* sp. Gulf 1 | GU | CAR68, CAR103 |
| *Caridina* sp. NT *nilotica* | GU | CAR36-7, CAR39, CAR79-96 |
| *Caridina* sp. D | GU | CAR70, CAR73, CAR75, TP540 |
| *Caridina spelunca*/sp. WA1 | GU | CAR62-3, TP308 |
| *Caridina* sp. WA 2 | GU | CAR24-5, CAR61, CAR64, CAR132, CAR156, TP533 |
| *Caridina* sp. WA 3 | GU | TP306-7, TP309, TP335-6, TP539, TP563, TP763-4, TP833, TP862 |
| *Caridina* sp. WA 4 | GU | TP1257 |
| *Caridina* sp. WA 5 | GU | TP1196 |
| *Caridina* sp. WA 6 | GU | TP1198 |
| **Same taxa in other institutions** | | |
| *Caridinides wilkinsi* | QM | W22083 |
| *Caridina* sp. Gulf 1 | ZMB | 29.24 |
| *Caridina* sp. NT *nilotica* | ZMB | 29.191 |
| *Caridina* sp. D | VM | J 53098 |
| *Caridina spelunca* | AM | P38512 |
| *Caridina* sp. WA 5 | WAM | C38998 |
| *Caridina* sp. WA 6 | WAM | C39000 |