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The taxonomic feedback loop: symbiosis of morphology and molecules

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Here, we relate the ongoing taxonomic story of a species complex of problematic, cryptic Australian freshwater shrimp (Atyidae; *Caridina*) to highlight the relative strength and utility of different taxonomic methods in assessing species boundaries. We used popular 'DNA barcode' gene fragments cytochrome *c* oxidase 1 and 16S ribosomal DNA. We then assessed the morphological attributes of these specimens and developed an identification key to complement the molecular results, and conclude that, despite occasionally strident arguments in favour of either molecular or morphological taxonomy, the two are inseparably linked and form parts of a greater whole.

Keywords: molecular; morphological; taxonomy; DNA barcode; cryptic taxa; *Caridina*

1. INTRODUCTION

Many recent papers have argued strongly for placing molecular data at the centre of taxonomy (e.g. Hebert *et al.* 2003a; Tautz *et al.* 2003; Blaxter 2004). They argue that 'molecular taxonomy' can use 'DNA barcodes' to identify and classify nearly all animal life. Others have countered that this is a 'caricature of real taxonomy' (Lipscomb *et al.* 2003), arguing that morphology must continue to play the central role (Dunn 2003; Will & Rubinoff 2004). They point out that assigning species boundaries with molecular data is no easier than with morphology (Will & Rubinoff 2004). Molecules have been effective in discovering cryptic taxa (Proudlove & Wood 2003), but this has not always proved useful for field biologists unable to use this information without a DNA laboratory.

The freshwater shrimp genus *Caridina* is a challenge to taxonomists of all hues because of the confusion between intra- and interspecific variation of characters (Riek 1953; Smith & Williams 1980). Two species and one subspecies are currently recognized in southeastern Australia: *Caridina indistincta* (northern distribution), *C.i. sobrina* (one location, Fraser Island) and *Caridina mccullochi* (south; Riek 1953). The neat taxonomic and geographical delineation of these taxa has long been suspect, as

morphological taxonomists have noted similarities of specimens from disparate locations (and taxa), while also noting extreme variation in sympatric specimens. *Caridina* would thus appear to lend itself to study using molecular taxonomic techniques.

Allozyme analysis on specimens of *Caridina* from a limited area identified three natural groupings (Woolschot *et al.* 1999; J. M. Hughes, unpublished data). These correspond exactly to mitochondrial DNA (mtDNA) clades (J. M. Hughes, unpublished), with a fourth added from previously unsampled areas. Three of these groups (*Caridina* 'species A, B and C') were included in a mtDNA study (Chenoweth & Hughes 2003), which revealed surprisingly deep divergences and an anomalous group (SC1) that fell between the others.

We felt that this confused situation could benefit from the unique insights available through molecular taxonomy. We used specimens from throughout southeastern Australia. We chose to employ the two markers proposed as most informative in delimiting species boundaries with DNA barcodes, namely cytochrome *c* oxidase 1 (COI; Hebert *et al.* 2003a) and 16S ribosomal DNA (16S; Blaxter 2004). Any resolved genetic groupings would then be validated and 'ground-truthed' with morphology to see if they told a similar story, and to see if we could bring this new genetic information back into the purview of traditional taxonomy by creating morphological keys that could accurately characterize the previously unappreciated level of biodiversity.

2. MATERIAL AND METHODS

To uncover accurate species boundaries, populations were sampled from throughout the known range (54 sites in 24 river basins, Electronic Appendix table 2), as recommended by Bond (2004). DNA extraction, gene amplification and 16S primers were as per Baker *et al.* (2004). The forward COI primer was a modification of that in Chenoweth & Hughes (2003) (see Electronic Appendix) and the reverse primer was CO1a.H (Palumbi *et al.* 1991). A total of 107 shrimps were sequenced for COI and added to 19 sequences from Chenoweth & Hughes (2003) to form a dataset of 65 unique haplotypes (450 bp). Thirty-one shrimps, from each COI clade, were also sequenced for 16S and aligned to 19 unique haplotypes (508 bp). The atyid shrimp, *Australatya striolata*, was sequenced for both fragments as an outgroup for the combined analysis (all accession numbers AY794988–AY795053).

Nilsson *et al.* (2004) show that phylogenetic analyses will outperform phenetic analyses in correctly identifying sequences. Will & Rubinoff (2004) also highlight the phenetic analyses of Hebert *et al.* (2003a) as a weakness, and thus we incorporated both cladistic/phylogenetic analyses (maximum parsimony, MP) and phenetic/distance analyses (minimum evolution, ME; multi-dimensional scaling, MDS).

MODELTEST (Posada & Crandall 1998) was used to select the appropriate model of nucleotide substitution for four separate datasets: (1a) COI with no outgroup; (1b) COI *Caridina* species A–C; (1c) 16S *Caridina* species A–C; (1d) combined dataset of COI and 16S with outgroup (958 bp). MP and ME analyses were performed for datasets 1a and 1d in PAUP* (Swofford 2002), bootstrapped 1000 times, and decay indices calculated (TREEROT; Sorenson 1999). Distance matrices were calculated in PAUP* using the MODELTEST model for datasets 1b and 1c and used for MDS plots in PRIMER (Primer-E 2001).

Morphological study and photography were subsequently carried out on over 100 specimens from all major genetic clades using a Leica MS5 stereo-microscope and mounted Nikon Coolpix digital camera. A standard suite of morphological and meristic characters (Smith & Williams 1980) of each specimen was analysed and a dichotomous key developed.

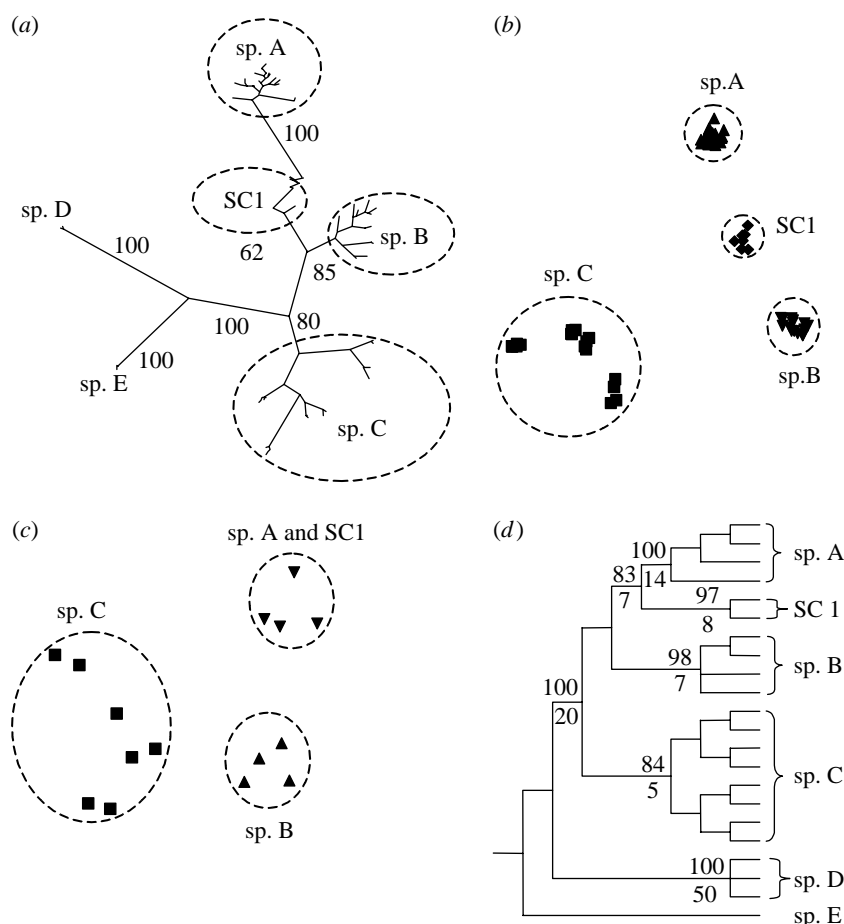


Figure 1. Molecular taxonomic analysis of *Caridina* spp. (a) Unrooted MP phylogram of entire *Caridina* COI dataset (with selected bootstrap values). (b) MDS plot of COI distance matrix for species A–C (stress=0.06). (c) MDS plot of 16S distance matrix for species A–C (stress=0.08). (d) MP strict consensus cladogram of entire combined COI/16S dataset (bootstrap values above, decay indices below, outgroup not displayed).

3. RESULTS

COI MP and ME analyses (MP, figure 1a; ME not displayed) recovered the same four monophyletic clades identified in earlier allozyme and mitochondrial studies (species A–D) and an anomalous group (SC1; Chenoweth & Hughes 2003). A new fifth clade (species E) was identified from coastal basins in New South Wales. Species D and species E are very divergent from the others (14–19% uncorrected *p*-distance (proportion of sites different); Electronic Appendix table 3) and from each other (17%). Species A–C (including SC1) form a strong clade (100% bootstrap) and only these were included in the MDS of dataset 1b (figure 1b), which also displays the separation between species A–C, with SC1 falling between species A and species B. The MDS of dataset 1c (16S species A–C) recapitulates the tripartite split of the previous dataset, but places SC1 firmly within species A (SC1 specimens share species A haplotypes) and highlights the high level of variation within species C. The MP and ME analyses of combined COI and 16S datasets (MP bootstrap cladogram, figure 1d) recovered the five clades, with SC1 strongly associated with species A.

The previously clear delineation of the described taxa (Riek 1953) has proved complicated (Electronic Appendix table 2). The southern *C. mccullochi* was identified as species B, and also located in the north of the range. The northern *C. indistincta* proved to be

species A, B, C and D. *Caridina indistincta sobrina* from Fraser Island was species C and was also located on other offshore islands, as well as the mainland. Fraser Island proved to host not only species C, but also at least two groups of species A. Species E was never found sympatric with other clades, whereas SC1 was always sympatric with species A. Four out of the six possible pairwise combinations of sympatry for species A–D have been located (Electronic Appendix).

Genetically identified specimens were then reassessed for identifying characters, and a key created and blind tested to reflect the genetic clades (table 1). Morphological identification at the ‘species’ level was relatively straightforward once a genetic framework was available and followed the genetics closely. Morphologically, SC1 keyed out as species A, mirroring the 16S rather than the COI results.

4. DISCUSSION

Both of the recommended DNA barcodes (COI and 16S) performed well in the splitting of the *Caridina* specimens into broadly congruent genetic groupings. Many questions still remain, such as (i) how many species are there within these *Caridina*? (ii) What happens when the datasets disagree? (iii) Do these groups reflect real biological entities? (iv) Could morphology have completed the same job?

Table 1. Species-level dichotomous identification key to *Caridina* of southeastern Australia.

1a	anterior dorsal part of the rostrum not naked (i.e. teeth placed all along the dorsal rostrum); eggs greater than 0.5 mm in length and less than 100 in number, endopod of first pleopod of males without appendix interna	2
1b	anterior dorsal part of the rostrum naked but with a sub-apical tooth; eggs less than 0.5 mm in length and greater than 100 in number, endopod of first pleopod of males with appendix interna	4
2a	rostrum elongate, straight or sigmoid in shape, not very deep (lance-like); eggs less than 1.5 mm in length and greater than 50 in number	3
2b	rostrum stout, straight and deep (dagger-like); eggs greater than 1.5 mm in length and less than 50 in number	sp. C
3a	rostrum relatively deep, straight, downward or upward pointing but never strongly sigmoid in shape, 7–11 ventral teeth	sp. A
3b	rostrum not deep (slender and elongate) and generally sigmoid, 4–8 ventral teeth	sp. B
4a	anterior dorsal part of the rostrum less than a quarter naked (no teeth), less than 15 ventral rostral teeth	sp. D
4b	anterior dorsal part of the rostrum greater than a quarter naked, greater than 15 ventral rostral teeth	sp. E

Both datasets agree that *Caridina* species D and species E are very divergent and well differentiated, and that species A, B and C (including SC1) form a strong clade. The phrase ‘species complex’ is apt, as species A–C (and SC1) could be split in a number of ways. The COI dataset (figure 1*b*) would suggest four taxa, whereas the 16S dataset (figure 1*c*) would suggest three. This could be owing to a COI barcode of 450 bp being too short for a resolved phylogenetic signal (Blaxter 2004), or to the 16S fragment being too conserved to reveal a potentially significant divergence (SC1).

One way of delimiting species boundaries is to define a cut-off point of sequence divergence (a molecular operational taxonomic unit, MOTU; Blaxter 2004). This leads to the problem of rate variation across taxa and the question of how different is different enough? It is probable that there are as many answers as there are taxa. Hebert *et al.* (2003*b*) found the mean COI sequence divergence between congeneric Crustacea to be 15.4% (*p*-distance), which, if applied to these *Caridina* divergences (Electronic Appendix table 3) as a species-level definition, would combine species A–C (and SC1) into a single species, with species D and E both separate species. Plainly, a considerable amount of information would need to be gathered before appropriate taxon-specific levels could be set (Hebert *et al.* 2003*a*), and any conclusions would be plastic, depending on the species concept adopted (Mallet & Willmott 2003; Lee 2004), but mtDNA divergences do provide a ‘necessary first step’ (Baker *et al.* 2004). Where possible, allozyme analysis of sympatric specimens

may provide evidence of reproductive isolation, after which genetic divergence could be used as a proxy (Lee 2004). Sympatric, divergent morphotypes with equally divergent genotypes (as in the present study) could also serve as a proxy for reproductive isolation and therefore biological species.

For these *Caridina*, morphology agrees with molecular results, and therefore the MOTU identified appear to reflect significant biological differences (Blaxter 2004). Morphology comes to the rescue in the confusion over the status of SC1, which corresponds with species A. In contrast, genetics provides some clarity with respect to unclear taxonomic characters. For example, egg size has been long recognized as highly variable within Australian *Caridina* (Riek 1953), but this can now be used (with caution) as an identifying feature (table 1) as a result of molecular derived groupings. The high level of polymorphism within species C (figure 1*b,c*; Electronic Appendix table 3), meaning the possibility of further cryptic species, is visible within both molecular and morphological frames of reference. This is unsurprising, as both morphological and molecular variation reflects the distinctive evolutionary history of a taxon. Morphology informs and validates (Blaxter *et al.* 2004) these molecular identifications of *Caridina*, but the morphological differences were only brought into sharp focus after a framework of relationships was provided by molecular taxonomy. This framework was then brought back into the world of traditional taxonomy, which can now produce identification keys useful to the wider world, far beyond the narrow interests of either the geneticist or taxonomist. This approach of morphologically ‘validated’ molecular taxonomy can be useful for any taxa, as demonstrated in Bond (2004; spiders) and Dalebout *et al.* (2004; whales).

A ‘chicken and egg’ situation ensues and a recursive feedback loop develops between morphological and molecular taxonomy. Each informs and refines the other, as the process of discovery is a continuing and heuristic one (Blaxter 2004). This feedback loop reaches beyond taxonomy, which informs and is informed by a multitude of disciplines that fall within and without biology.

Interesting underlying processes that may explain incongruent molecular and morphological datasets (e.g. morphological and ecological plasticity, selection, convergence, cryptic taxa, gene transfer) will only be evident if there are multiple sources of data (morphology, genetics, behaviour, ecology) to place inconsistencies in context (Lipscomb *et al.* 2003). Molecular-only studies are effective for rapid biodiversity assessment by non-taxonomists, but not particularly meaningful in isolation (Lipscomb *et al.* 2003). But when molecules work in concert with other ‘disciplines’ in a ‘total evidence’ approach (Mallet & Willmott 2003; Bond 2004; Lee 2004), a whole greater than the sum of its parts emerges and all sources of data combine to help describe the diversity of life.

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- Baker, A. M., Hughes, J. M., Dean, J. C. & Bunn, S. E. 2004 Mitochondrial DNA reveals phylogenetic structuring and cryptic diversity in Australian freshwater macroinvertebrate assemblages. *Mar. Freshw. Res.* **55**, 629–640. (doi:10.1071/MF04050)
- Blaxter, M. L. 2004 The promise of a DNA taxonomy. *Phil. Trans. R. Soc. B* **359**, 669–679. (doi:10.1098/rstb.2003.1447)
- Blaxter, M., Elsworth, B. & Daub, J. 2004 DNA taxonomy of a neglected animal phylum: an unexpected diversity of tardigrades. *Proc. R. Soc. B* **271**(Suppl. 4), S189–S192. (doi:10.1098/rsbl.2003.0130)
- Bond, J. E. 2004 Systematics of the Californian euctenizine spider genus *Apomastus* (Araneae: Mygalomorphae: Cyrtaucheniidae): the relationship between molecular and morphological taxonomy. *Invertebr. Syst.* **18**, 361–376. (doi:10.1071/IS04008)
- Chenoweth, S. F. & Hughes, J. M. 2003 Speciation and phylogeography in *Caridina indistincta*, a complex of freshwater shrimps from Australian heathland streams. *Mar. Freshw. Res.* **54**, 807–812. (doi:10.1071/MF02168)
- Dalebout, M. L., Baker, C. S., Mead, J. G., Cockcroft, V. G. & Yamada, T. K. 2004 A comprehensive and validated molecular taxonomy of beaked whales, family Ziphiidae. *J. Hered.* **95**, 459–473. (doi:10.1093/jhered/esh054)
- Dunn, C. P. 2003 Keeping taxonomy based in morphology. *Trends Ecol. Evol.* **18**, 270–271. (doi:10.1016/S0169-5347(03)00094-6)
- Hebert, P. D. N., Cywinska, A., Ball, S. L. & DeWaard, J. R. 2003a Biological identifications through DNA barcodes. *Proc. R. Soc. B* **270**, 313–321. (doi:10.1098/rspb.2002.2218)
- Hebert, P. D. N., Ratnasingham, S. & deWaard, J. R. 2003b Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. B* **270**(Suppl. 1), S96–S99. (doi:10.1098/rsbl.2003.0025)
- Lee, M. S. Y. 2004 The molecularisation of taxonomy. *Invertebr. Syst.* **18**, 1–6. (doi:10.1071/IS03021)
- Lipscomb, D., Platnick, N. & Wheeler, Q. 2003 The intellectual content of taxonomy: a comment on DNA taxonomy. *Trends Ecol. Evol.* **18**, 65–66. (doi:10.1016/S0169-5347(02)00060-5)
- Mallet, J. & Willmott, K. 2003 Taxonomy: renaissance or Tower of Babel? *Trends Ecol. Evol.* **18**, 57–59. (doi:10.1016/S0169-5347(02)00061-7)
- Nilsson, R. H., Larsson, K. H. & Ursing, B. M. 2004 Galaxie—CGI scripts for sequence identification through automated phylogenetic analysis. *Bioinformatics* **20**, 1447–1452. (doi:10.1093/bioinformatics/bth119)
- Palumbi, S. R., Martin, A., Romano, S., McMillan, W. O., Stice, L. & Grabowski, G. 1991 *A simple fool's guide to PCR*. Honolulu: University of Hawaii Press.
- Posada, D. & Crandall, K. A. 1998 MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818. (doi:10.1093/bioinformatics/14.9.817)
- Primer-E 2001 *PRIMER version 5.2.8*. Plymouth: Primer-E Ltd.
- Proudlove, G. & Wood, P. J. 2003 The blind leading the blind: cryptic subterranean species and DNA taxonomy. *Trends Ecol. Evol.* **18**, 272–273. (doi:10.1016/S0169-5347(03)00095-8)
- Riek, E. F. 1953 The Australian freshwater prawns of the family Atyidae. *Rec. Aust. Mus.* **23**, 111–121.
- Smith, M. J. & Williams, W. D. 1980 Intraspecific variation within the Atyidae: a study of morphological variation within a population of *Paratya australiensis* (Crustacea: Decapoda). *Aust. J. Mar. Freshwat. Res.* **31**, 397–407.
- Sorenson, M. D. 1999 *TREEROT version 2*. Boston, MA: Boston University.
- Swofford, D. L. 2002 *PAUP* 4.b10*. Sunderland, MA: Sinauer.
- Tautz, D., Arctander, P., Minelli, A., Thomas, R. H. & Vogler, A. P. 2003 A plea for DNA taxonomy. *Trends Ecol. Evol.* **18**, 70–74. (doi:10.1016/S0169-5347(02)00041-1)
- Will, K. W. & Rubinoff, D. 2004 Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics* **20**, 47–55. (doi:10.1111/j.1096-0031.2003.00008.x)
- Woolschot, L., Hughes, J. M. & Bunn, S. E. 1999 Dispersal among populations of *Caridina* sp. (Decapoda: Atyidae) in coastal lowland streams, south-eastern Queensland, Australia. *Mar. Freshw. Res.* **50**, 681–688. (doi:10.1071/MF99011)

The supplementary Electronic Appendix is available at <http://dx.doi.org/10.1098/rsbl.2005.0298> or via <http://www.journals.royalsoc.ac.uk>.