An investigation into Australian freshwater zooplankton with particular reference to *Ceriodaphnia* species (Cladocera: Daphniidae)

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Abstract

The taxonomy of *Ceriodaphnia* Dana, 1853 has long been uncertain, and their species richness often underestimated due to a lack of good morphological taxonomy. Modern molecular techniques such as genetic barcoding allow the detection of cryptic species. In this study DNA sequences from two mitochondrial DNA (COI and 16s) and one nuclear DNA (28s) gene fragments, were used to investigate *Ceriodaphnia* diversity in Australia, and is the first molecular analysis of this genus from Australia.

A preliminary study using morphology and DNA barcoding of zooplankton diversity, particularly *Ceriodaphnia* from two South Australian reservoirs: Myponga and South Para and supplemented by specimens from water bodies across Australia, revealed cryptic speciation for *Ceriodaphnia*. Derived sequences support the recognition of three beaked species in *Ceriodaphnia cornuta* Sars (1885) complex, *C*. sp. nov. 1 and *C*. sp. nov. 2, and three non-beaked species *C. dubia* Richard, 1894, *C. spinata* Henry, 1919 and *C*. sp. nov. 3.

We also examined the Folmer primer (LCO 1490 and HCO 2198) region, sourced from 725 complete mitochondrial genomes across 17 animal phyla is universal. The correlation between the frequency of base pair differences in the primer region at different taxonomic levels were analysed using 1-way ANOVA and Fishers Post Hoc analysis. A significant difference in the means of base pair differences from Order to Species and Class to Kingdom levels (ANOVA, $F= 8.193$, $p = 0.00$) for LCO 1490 and at the level of Genus (ANOVA, $F= 2.538$, $p = 0.027$) for HCO 2198 was detected.

This study has demonstrated the potential of DNA barcoding in identifying species in a taxonomically uncertain group like *Ceriodaphnia*. However, the success of DNA barcoding relies on sequence databases that have been verified taxonomically. This research highlights the importance of an integrative approach required to clarify the taxonomic status of *Ceriodaphnia* from Australia. It also shows that automated primer sites can be generated for species which have an insufficient numbers of sequences in online genetic databases for which the Universal primers (LCO 1490 and HCO 2198) have been unsuccessful in amplifying the COI gene.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Pranay Sharma and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Published Articles:

Sharma P & Kotov A 2013. Molecular approach to identify sibling species of the *Ceriodaphnia cornuta* complex (Cladocera: Daphniidae) from Australia with notes on the continental endemism of this group. Zootaxa 3702: 079–089.

Sharma, P and Kobayashi, T 2014. Are "Universal" DNA primers really universal? Journal of Applied Genetics, DOI: 10.1007/s13353-014-0218-9.

Sharma, P; Elias-Gutierrez, M and Kobayashi, T 2014. The identification of common Cladocerans and Calanoids in two South Australian reservoirs using DNA Barcoding and morphological analysis: An Integrative Approach. Crustaceana 87 (7): 834–855.

Pranay Sharma

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Preamble

As most of this thesis has either been prepared for publication or published each chapter has been written as a journal article or compilation of journal articles. Consequently there is some repetition between the general introduction and conclusion with the introduction and conclusion of each chapter. To keep this to a minimum, the General Introduction (Chapter 1) and General Conclusion (Chapter 7) have been kept brief, focussing on broader issues. There is also some repetition with regards to methodology used among the chapters. The title page of each chapter details publication information, along with authorship contributions. All references for each chapter have been compiled separately, following the particular journal's style.