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## Phylogeny of the Freshwater Crayfish Genus *Euastacus* and their Coevolution with Ectosymbiotic Temnocephalans

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*Euastacus* crayfish are endemic to the rain forests of the eastern coast of Australia. There are now 43 known species distributed throughout Queensland, New South Wales, and Victoria. Unfortunately many species inhabit areas where human growth, activity, and environmental modification threaten, limit or decrease their population size or natural range (Horwitz 1995; Merrick, 1997). The well being of these crayfish is important because many of these crayfish exist as keystone species in their community. Crayfish are polytrophic and therefore transform energy between a variety of levels and are important for the breakdown of debris (Turvey and Merrick, 1997; Parkyn et al. 1997). Because of their placement in the food chain, changes in population structure or density of crayfish species could have large effects on the structure of aquatic food chains and communities (Nyström, 2002; Parkyn et al. 1997). *Euastacus* is also a good bio-indicator of freshwater environment health (Merrick 1997).

The purpose of this project was to estimate the phylogenetic relationships between members of the genus *Euastacus*. A comprehensive phylogeny is important in studies of the biodiversity of *Euastacus* crayfish, a genus endemic to a narrow range of habitats where conservation is a key issue because of the far-reaching effects of human activity. An understanding of the phylogenetic history (the relationships between organisms) of each organism present in this area is necessary in order to protect the ecological diversity of this genus (Moritz, 2002).

This project is also part of a coevolutionary study between members of *Euastacus* and ectosymbiotic turbellarian worms of the genera *Temnohaswellia* and *Temnosewellia*. There is a question as to whether these symbionts have coevolved with the crayfish, resulting in a different species of temnocephalan for each different species of crayfish, or whether separate species of worms have switched from one host to another. This question can be answered by comparing the phylogenetic trees estimated from the DNA sequencing data of a variety of genes for both the crayfish and the worms.

### Materials and Methods

Samples were collected in Australia, and gill or leg tissue was fixed in 100% ethanol and sent to the Crandall lab. Locations of the samples were meticulously recorded to allow for distribution and diversity studies later on in the project and for future studies. DNA was extracted from the gill tissue using a cell lysis protocol (Crandall et al. 1999), and extractions stored in TLE buffer at -20°C. Aliquots of each extraction were diluted for PCR use. The Polymerase Chain Reaction (PCR) was used to obtain workable samples of mitochondrial genes (12S, 16S, CO1) and the variable region of the nuclear gene 28S. Products were checked on an agarose gel using ethidium bromide to verify a positive reaction and to check for contamination. PCR products were purified using a PCR clean up plate (Millipore). PCR products were sequenced using the ABI Big-dye Ready-Reaction kit, and sequences generated on the 3730 XL Automated

Sequencer. Nucleotide sequences were aligned using Clustal X and adjusted by eye (Thompson et al. 1997). Model optimization was performed using Modeltest 3.06 (Posada and Crandall, 1998). A Maximum Likelihood tree was estimated for each gene as well as for the combined data set using a Bayesian analysis.

## Results

This study is not yet finished but will be completed by early next year. All but four species have been collected and sequenced. The data sets for the 16s, 12s, and CO1 genes are nearly complete, and about half of the 28s sequences are finished. A big step came when genes were successfully amplified from tissue that had been fixed in formalin for many years, a process which fragments the DNA making it nearly impossible to sequence. We added one new species to our tree from this process. Preliminary trees show promising information. Previous morphological trees are all quite different from each other, so this new information has been useful in resolving those questionable relationships. Adding a nuclear gene (28s) to the data set will hopefully add more support to some of the branches. We have also found at least one instance where a named species does not comprise a monophyletic group with a common ancestor.

This study will be the first detailed estimation of the phylogenetic relationships between members of *Euastacus*. When completed, this phylogeny can be compared to morphological classifications and used to suggest any taxonomic revisions needed to the genus, as well as to help identify any misclassifications. It will be useful for further studies into the biogeographical distribution, evolutionary history, and conservation needs of this genus. The coevolution aspect of this study has not been started yet, but once both phylogenies have been satisfactorily estimated, analysis can be completed. An understanding of the coevolution of these two closely interacting organisms will provide information for the study of evolution as an adaptive process and to better understand the distribution of biodiversity.

## References

- Crandall, K. A., J. W. Fetzner Jr., S. H. Lawler, M. Kinnersley, C. M. Austin. 1999. Phylogenetic relationships among the Australian and New Zealand genera of freshwater crayfishes (Decapoda: Parastacidae). *Australian Journal of Zoology* 47:199-214.
- Horwitz, P. 1995. The Conservation Status of Australian Freshwater Crayfish: Review and Update. *Freshwater Crayfish* 10:70-80.
- Merrick, J. R. 1997. Conservation and Field Management of the Freshwater Crayfish, *Euastacus spinifer* (Decapoda: Parastacidae), in the Sydney Region, Australia. *Proc. Linn. Soc. NSW*, 118.
- Moritz, C. 2002. Strategies to Protect Biological Diversity and the Evolutionary Processes that Sustain It. *Syst. Biol.* 51:238-254.
- Nystrom, P. 2002. "Ecology" p. 192-235 in *Biology of Freshwater Crayfish*. Ed. David M. Holdich. Blackwell Science Ltd, London.
- Parkyn, S. M., C. F. Rabeni, K. J. Collier. 1997. Effects of crayfish (*Paranephrops planifrons*: Parastacidae) on in-stream processes and benthic faunas: A density manipulation experiment. *New Zealand Journal of Marine and Freshwater Research* 31:685-692.
- Posada, D. and Crandall, K.A. (1998). Modeltest: Testing the model of DNA substitution. *Bioinformatics* 14, 817-818.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24, 4876-4882.
- Turvey, P. and J. R. Merrick. 1997. Diet and Feeding in the Freshwater Crayfish, *Euastacus spinifer* (Decapoda: Parastacidae), from the Sydney Region, Australia. *Proc. Linn. Soc. NSW*. 118:175-185.