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The Molecular Phylogenetics of Antarctic Sea Spiders (Pycnogonida)

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Supervisor: Dr. Shane Lavery

A dissertation submitted in partial fulfilment of the requirements for the degree of Bachelor of Science (Honours) in Biological Science

The University of Auckland
December 2005
Dedication

til mine besteforældre, Mormor og Morfar
Abstract

Sea spiders, or pycnogonids, are a unique group of exclusively marine invertebrates that are found worldwide. A scarcity of pycnogonid research is reflected in the unclear position of this group with regards to the phylum Arthropoda and lack of certainty in their family-level phylogeny. Traditionally, the pycnogonid phylogeny has relied on the external morphological characters of temperate, shallow water species. The Antarctic sea spider fauna displays a high degree of endemism and a number of species have the potential to address several long-standing questions regarding the pycnogonid evolution. This research uses new sequence data from Antarctic species to provide the most complete molecular phylogenetic reconstructions of the Pycnogonida, and is the first study to formally test a number of alternative hypotheses on the interfamilial relationships of this group of organisms.

The BioRoss 2004 pycnogonid collection was classified into 18 different OTUs (5 families & 10 genera) and used, in combination with publicly accessible sequences, to provide samples for this study. Partial regions of the nuclear 18S and 28S rDNA, mitochondrial 12S and 16S rDNA and protein coding COI loci were sequenced for each dataset, and the concatenated data tested for incongruence using the Partition of Homogeneity test. The distance based Neighbour Joining and character based Maximum Likelihood tree-building algorithms were used to reconstruct the pycnogonid phylogeny for each locus independently and as a concatenated dataset. A series of alternative evolutionary hypotheses based on previous studies were examined via the Shimodaira-Hasegawa test. The primary hypothesis examined was the cephalic appendage reductive trend, which implies that ancestral sea spider taxa possess the greatest complexity of anterior appendages.

On all the individual locus trees the family Nymphonidae were the earliest diverged lineage of pycnogonids, although low resolution at the roots of the trees implies that the data are not strong enough to reject an alternative hypothesis of a basal Ammotheidae group. Pycnogonidae is not the most recently derived sea spider family and the cephalic appendage loss hypothesis is thus rejected. None of the phylogenies supported a close relationship between the Colossendeidae and Nymphonidae families and doubt is raised over the true identification of several GenBank sequences. Polymerous species do not form a combined, ancestral group but are instead more likely to represent recent divergences from three separate families. Strong evidence supports the placement of the transient Austropallene genus (Callipallenidae) at the base of the Nymphonidae family.

This study, and ongoing work, has generated large amounts of new sequence data. This can be used in future pycnogonid phylogenetic research and/or in investigations on the highly contentious position of the Pycnogonida with regards to the phylum Arthropoda. A DNA Surveillance website has been created to assist in the molecular identification of pycnogonids from future benthic bio-discovery expeditions (http://www.dna-surveillance.auckland.ac.nz).
Acknowledgments

I would first and foremost like to thank my supervisor, Dr. Shane Lavery, for all his time, advice and the endless amount of support that he has generously given me throughout this year. He not only provided me with a number of new academic learning opportunities, but has also inspired me through his patient and encouraging approach to research.

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Data for this research project was enhanced by publicly accessible DNA sequences and, in particular, previous work conducted by Dr. Claudia Arango and I appreciate having been able to incorporate these sequences into this dissertation.

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<th>Description</th>
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<tbody>
<tr>
<td>A</td>
<td>Adenine</td>
</tr>
<tr>
<td>ABI</td>
<td>Applied Biosystems Incorporated</td>
</tr>
<tr>
<td>AIC</td>
<td>Akaike Information Criterion</td>
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<tr>
<td>BioRoss</td>
<td>Biodiversity of the Ross Sea</td>
</tr>
<tr>
<td>bp</td>
<td>base pair</td>
</tr>
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<td>BR</td>
<td>BioRoss</td>
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<td>°C</td>
<td>degrees Celsius</td>
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<td>C</td>
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<td>CI</td>
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<td>COI</td>
<td>Cytochrome c Oxidase 1</td>
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<tr>
<td>CSB</td>
<td>Cell Suspension Buffer</td>
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<td>ddH₂O</td>
<td>double distilled water</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>dNTP</td>
<td>2’-deoxynucleotide 5’-triphosphate</td>
</tr>
<tr>
<td>EDTA</td>
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<tr>
<td>EtOH</td>
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<tr>
<td>FFEP</td>
<td>Formalin-Fixed Ethanol-Preserved</td>
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<td>G</td>
<td>Guanine</td>
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<td>GTR+I+G</td>
<td>General Time Reversible plus Gamma</td>
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<tr>
<td>hLRT</td>
<td>hierarchical Likelihood Ratio Test</td>
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<td>kilobase</td>
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