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# The Molecular Phylogenetics of Antarctic Sea Spiders (Pycnogonida)

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A dissertation submitted in partial fulfilment of the requirements for the degree of  
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## **Dedication**

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til mine besteforældre, Mormor og Morfar

## Abstract

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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>).

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## List of Abbreviations

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A	Adenine
ABI	Applied Biosystems Incorporated
AIC	Akaike Information Criterion
BioRoss	Biodiversity of the Ross Sea
bp	base pair
BR	BioRoss
°C	degrees Celsius
C	Cytosine
CI	Chloroform/Isoamyl
COI	Cytochrome <i>c</i> Oxidase 1
CSB	Cell Suspension Buffer
ddH <sub>2</sub> O	double distilled water
DNA	Deoxyribonucleic Acid
dNTP	2'-deoxynucleotide 5'-triphosphate
EDTA	Ethylenediamine-Tetra-Acetic-Acid
EtOH	Ethanol
FFEP	Formalin-Fixed Ethanol-Preserved
G	Guanine
GTR+I+G	General Time Reversible plus Gamma
hLRT	hierarchical Likelihood Ratio Test
ILD	Incongruence Length Distance
kb	kilobase
LML	Low Mass Ladder
<i>-ln L</i>	log likelihood
M	Molar
MFish	Ministry of Fisheries
mg	milligram
μL	microlitre
mL	millilitre
ML	Maximum Likelihood
mm	millimetre
mM	millimolar
mtDNA	Mitochondrial DNA

NaCl	Sodium Chloride
NCBI	National Centre for Biotechnology Information
ng	nanogram
NH <sub>4</sub> OAC	Ammonium Acetate
NIWA	National Institute of Water and Atmospheric Research
NJ	Neighbour Joining
nm	nanometre
OTU	Operational Taxonomic Unit
PAUP*	Phylogenetic Analysis Using Parsimony (*and other methods)
PCI	Phenol/Chloroform/Isoamyl
PCR	Polymerase Chain Reaction
pH	measure of acidity
pmol	picomole
rDNA	Ribosomal Deoxyribonucleic Acid
rpm	revolutions per minute
rRNA	Ribosomal Ribonucleic Acid
RSB	Reticulolysate Standard Buffer
S	Svedburg unit
S-H	Shimodaira-Hasegawa
spp.	species
SDS	Sodium Dodecyl Sulfate
T	Thymine
<i>Taq</i>	<i>Thermus aquaticus</i>
TBE	Tris-Borate-EDTA
TBR	Tree Bisection-Reconnection
TE	Tris-EDTA
TrNef+I+G	Tamura-Nei plus Gamma
TVM+I+G	Transversion Model plus Gamma
U	enzyme unit
UV	Ultraviolet Light
V	Volt