# ResearchOnline@JCU

This file is part of the following reference:

Arango, Claudia Patricia (2002) Morphological and molecular phylogenetic analysis of the sea spiders (Arthropoda, Pycnogonida) and taxonomic study of tropical Australian forms. PhD thesis, James Cook University.

Access to this file is available from:

http://eprints.jcu.edu.au/24091/

The author has certified to JCU that they have made a reasonable effort to gain permission and acknowledge the owner of any third party copyright material included in this document. If you believe that this is not the case, please contact <u>ResearchOnline@jcu.edu.au</u> and quote <u>http://eprints.jcu.edu.au/24091/</u>



Morphological and Molecular Phylogenetic Analysis of the Sea Spiders (Arthropoda, Pycnogonida) and Taxonomic Study of Tropical Australian forms



Thesis submitted by Claudia Patricia Arango BSc

in February 2002

For the degree of Doctor of Philosophy School of Tropical Biology & School of Marine Biology and Aquaculture James Cook University

#### Statement of access

I, the undersigned, the author of this thesis, understand that James Cook University will make it available for use within the University library and, by microfilm or other means, allow access to users in other approved libraries. All users consulting this thesis will have to sign the following statement:

In consulting this thesis I agree not to copy or closely paraphrase it in whole or in part without the written consent of the author; and to make proper public written acknowledgment for any assistance that I have obtained from it.

Beyond this, I do not wish to place any restriction on access to this thesis.

Claudia P. Arango

23° May 2002

### ACKNOWLEDGEMENTS

First of all I thank my family for their immense love and support. I have no words to express all my gratefulness to them. To Guillermo, I owe him so much, without him, all this would have been ten thousand times harder; I am so glad we have shared all this together. I am very grateful to Drs. Richard Rowe and John Collins for their guidance and support, for sharing with me their enthusiasm for little creatures and for patiently reading many drafts. Thanks to Dr. David Blair for his expertise on molecular techniques and handling of data and all his comments and suggestions since the very beginning. Special thanks to Dr. Lynne van Herwerden for her kind help in the DNA lab and for discussion and comments; to my mates in the DNA lab, Selma, Julia and Line. Special thanks to all the people who helped me with the collection of material and field work, especially 'G' Díaz-Pulido, Jamal Jompa, Drs. Laurence McCook; Jurgen Otto and Warren Lee-Long, to Oli Floerl and 'Michelle' Lee and to Ms. Liz Turner from the Museum of Tasmania. Thanks to all the volunteers that looked for pycnogonids during their dives. Special thanks to overseas specialists, Drs Katsumi Miyazaki, Tomás Munilla and Karl-Heinz Tomaschko for kind donations of material, to Karl for running ecdysteroid essays with my samples. To Julianne Hancock, Barry Russell and Penny Berents for their assistance with loan of specimens. To David Staples for advice and loan of material. Thanks to Dr. George 'Buz' Wilson for his help with analysis and his support and friendliness during my visit to the Australian Museum. To Dr. Don Colgan for his kind help and his almost incredible support allowing me to be part of the Evolutionary Biology Unit (EBU Australian Museum) for a few weeks. I want to thank Dr. David Yeates for his help, advice and friendship. Thanks to Glenn Graham and Corinna Lange for helping me with molecular analyses. Special thanks to Dr. Allan Child (Smithsonian Institution) for his taxonomic expertise and his support since my early beginnings in pycnogonid taxonomy. To Dr. Roger Bamber (British Natural History Museum) for advice and good conversations, for comments on material collected. To N. Campbell and K. Wilson (Australian Institute of Marine Science) for helpful advice and aliquots. Thanks to Faye Christidis and David Slaney for sharing their experience in systematics with me. Thanks to all the JCU staff that made my life much easier during this process. Finally, I want to acknowledge the financial support of the School of Tropical Biology and the School of Marine Biology and Aquaculture James Cook University during these years.

# ABSTRACT

Pycnogonida is a subphylum of marine arthropods showing unique characteristics. Their position within the Arthropoda is not yet clear, but strong evidence has suggested they may be the extant sister taxon to all other arthropods. The phylogenetic affinities among the extant families of pycnogonids: Ammotheidae, Colossendeidae, Callipallenidae, Nymphonidae, Phoxichilidiidae, Pycnogonidae, Austrodecidae, Rhynchothoracidae, and the position of problematic genera such as Endeis, Pallenopsis and Tanystylum, are uncertain. Traditionally, it has been assumed that an evolutionary trend of gradual reduction of numbers of segments of the appendages, mainly involving chelifores, palps and ovigers (head appendages) has taken place within the group. Modern cladistic techniques have not been applied to resolve phylogenetic conflicts of the sea spiders. I approached the problem of the uncertain higherlevel phylogenetic affinities of pycnogonids to propose hypotheses of relationships based on cladistic analysis of morphological characters, thereby testing the hypothesis of a reduction trend. Additionally, I used a preliminary molecular approach to confront the morphological results. This is one of the first attempts to use molecular data in the study of systematics of pycnogonids. Phylogenetic relationships among the main lineages of extant sea spiders were studied using cladistic analysis of 36 morphological characters and 38 species from all the recognized families. A preliminary exemplar method was employed, and different assumptions of multistate character transformations were used to trace the evolution of the head appendages. Fragments of nuclear ribosomal DNA (18S and 28S) were sequenced to reconstruct the phylogenetic relationships among six higher taxa of sea spiders. Hypotheses of relationships were obtained from separate and combined analyses of these data sets under both maximum parsimony and maximum likelihood criteria. Trees derived from the molecular data set were compared with those from the set of 36 morphological characters previously analysed. Estimates of phylogeny were found to be significantly different between the molecular and the morphological data set and possible causes for incongruence, such as the coding of inapplicable characters in morphology and a very reduced set of taxa in the molecular analysis, are discussed. The position of Colossendeidae was a major cause of conflict, being supported as a relative of Ammotheidae by morphological characters but appearing closely related to Callipallenidae and Nymphonidae with DNA data. With the molecular characters, Austrodecus is identified as a basal taxon for the rest of the pycnogonids included, differing from its close relationship to ammotheids shown by morphology. Using morphological data, the family Ammotheidae appeared as paraphyletic as did Callipallenidae. Pallenopsis was related to Anoplodactylus according to DNA but not morphology. Although

no clear pattern of overall relationships among sea spiders is yet defined, several patterns useful for future systematic work have been noted. New sets of characters and compilation of data from all available sources will probably provide a better picture. Ontogenetic transformation could give some insights into character evolution, and knowledge of ecological traits is needed to complement morphological observations. A collection of fresh material of numerous species of sea spiders from the Great Barrier Reef and other localities of Queensland was useful for the phylogenetic analyses and also contributed to the knowledge of the marine fauna of Australia. Thirty-three species of tropical shallow-water sea spiders collected from the Queensland coast, the Great Barrier Reef and the Coral Sea are reported here. Among these were six undescribed species in the genera *Austrodecus, Anoplodactylus* and *Pycnogonum*, and other nine species, mostly of Indo-West pacific distribution not previously recorded for Australia.

v

Statement of access	ii
ACKNOWLEDGEMENTS	iii
ABSTRACT	iii
LIST OF PUBLICATIONS	xii
Statement on sources	xiii
CHAPTER ONE General Introduction	1
1.1 Overview	1
1.2 Morphology of Pycnogonida	2
1.3 Taxonomy and systematics of Pycnogonida	3
1.4 This study	4
CHAPTER TWO Sea spiders (Pycnogonida, Arthropoda) from the Great Barrier Reef and Nort	heastern
Australia: new species, new records and ecological annotations	7
2.1 Summary	7
2.2 Introduction	7
2.3 Materials and Methods	10
2.4 Results	12
2.5 Discussion	74
2.5.1 Observations on habitats studied	
2.5.2 Biogeographical comments	75
CHAPTER THREE Cladistic analysis of the Pycnogonida based on morphological characters	78
3.1 Summary	
3.2 Introduction	
3.3 Materials and Methods	80
3.3.1 Taxon sampling	
3.3.2 Characters	
3.3.3 Cladistic analysis	91
3.4 Results	94 vi

# TABLE OF CONTENTS

3.5 Discussion	95
3.5.1 Ammotheidae+Colossendeidae+Rhynchothoracidae+Austrodecidae	95
$\label{eq:stable} 3.5.2 \ (Nymphonidae+Callipallenidae+Pycnogonidae+Phoxichilidiidae)+Pallenopsis \dots $	
3.5.3 Character evolution	
3.5.4 Previous classifications	
3.6 Conclusions	

# 

4.1 Summary	
4.2 Introduction	
4.3 Materials and Methods	
4.3.1 Sampling of the molecular data	109
4.3.2 Sampling of the morphological data	110
4.3.3 Preparation of molecular samples	110
4.3.4 Phylogenetic analysis	111
Pycnogonida	112
4.4 Results	114
4.4.1 Alignment and nucleotide variation of 18S fragment	114
4.4.2 Phylogenetic analysis of 18S ribosomal DNA	115
4.4.3 Alignment and nucleotide variation of 28S fragment	116
4.4.4 Phylogenetic analysis of 28S ribosomal DNA	116
4.4.5 Combined analysis of the 18S and 28S ribosomal DNA	117
4.4.6 Combined Morphological and molecular data	117
4.5 Discussion	118
4.5.1 Outgroups	118
4.5.2 Ingroup relationships	
4.5.3 Additional data for 28S	124
4.5.4 Confronting morphology and DNA	124
4.5.5 Divergence times	125
GENERAL DISCUSSION	128
5.1 Overview and implications for future research	
5.2 Conclusions	131
References	133
Appendix 1	145
Key for genera of shallow-water pycnogonids from North Queensland collected in this study	145
	vii

Appendix 2	
Key for species of Anoplodactylus from North Queensland collected in this study:	
Appendix 3	
Species examined for the cladistic analysis of morphology (Chapter 3)	149
Appendix 4	
Diagrams of geometrical representation of the shapes of the proboscis in the Pycnogonida	152
Appendix 5	
i) Alignment of 506 sites of the V4 domain of 18S rDNA	153
ii) Alignment of 877 sites of the D4-D7 region of 28S rDNA	156
iii) Alignment of 533 sites of 16S mtDNA.	159

## LIST OF FIGURES

Figure 1.1. Diagram of a pycnogonid.	6
Figure 2.1. Ascorhynchus tenuirostris	14
Figure 2.2. Achelia assimilis.	18
Figure 2.3. Ammothella sp. 'slender form' .	21
Figure 2.4. Nymphopsis acinacispinata.	23
Figure 2.5. Tanystylum haswelli	25
Figure 2.6. Tanystylum rehderi	26
Figure 2.7. Austrodecus n. sp.	29
Figure 2.8. Rhopalorhynchus tenuissimum.	31
Figure 2.9. Nymphon micronesicum	33
Figure 2.10. Nymphon molleri	35
Figure 2.11. Pallenopsis hoeki.	37
Figure 2.12. Propallene saengeri	39
Figure 2.13. Callipallene n. sp.	41
Figure 2.14. Callipallene novaezealandiae	43
Figure 2.15. Parapallene famelica	45
Figure 2.16. Pigrogromitus timsanus.	48
Figure 2.17. Anoplodactylus batangensis	50
Figure 2.18. Anoplodactylus sp. A.	53
Figure 2.19. Anoplodactylus digitatus	55
Figure 2.20. Anoplodactylus glandulifer	56
Figure 2.21. Anoplodactylus longiceps.	58
Figure 2.22. Anoplodactylus n. sp. B.	62
Figure 2.23. Anoplodactylus tenuicorpus.	63
Figure 2.24. Anoplodactylus tubiferus.	64
Figure 2.25. Anoplodactylus versluysi.	66
Figure 2.26. Endeis biseriata.	69
Figure 2.27. Endeis flaccida	70
Figure 2.28. Endeis mollis.	72
Figure 2.29. Pycnogonum n. sp	73
Figure 3.1. Phylogenetic hypotheses of relationships among pycnogonid families	82
Figure 3.2 Scanning-electron microscopy images of some characters of pycnogonids	92
Figure 3.3 Reconstruction of the Devonian pycnogonid fossil Palaeoisopus problematicus Broili	93
Figure 3.4 Single most parsimonious polytomous tree obtained from the analysis of unordered characters	and
implied weights and presented as the preferred hypothesis of the pycnogonid phylogeny.	<b>9</b> 7
Figure 3.5. Single polytomous tree obtained with five multistate ordered characters (1, 5, 7, 8, 10)	98
Figure 3.6. Evolution of characters 1, 5, 7, 8 and 10 according to the proposed phylogeny presented in figure	; 3.4.
	.105
Figure 4.1 Phylogenetic tree of the Pycnogonida based on 18S	.119

ix

Figure 4.2 Phylogeny based on 18S after exclusion of outgroups	.120
Figure 4.3. Phylogeny based on 28S. Single MP tree rooted at midpoint	.121
Figure 4.4. Estimate of phylogeny based on 18S + 28S and morphology	.122

# LIST OF TABLES

Table 2.1 Collection sites of pycnogonids in Queensland and the Coral Sea, Australia	
Table 2.2. Distribution of the species of pycnogonids collected, in Australia and worldwide	77
Table 3.1 Species and coding of morphological characters.	83
Table 3.2. Character statistics from both unordered and ordered analyses in Pee-Wee	99
Table 3.3 Clades enforced according to previous classifications and their fit compared to the phylog	eny proposed
in this study.	106
Table 4.1. Species included in the study and the type of data used for each of them.	
Table 4.2 Summary of results obtained with each of the data sets used to infer phylogenetic aff	inities of the
Pycnogonida	126

Based on the work of this thesis, the following papers have been accepted or submitted to scientific journals for publication :

- Arango CP. 2001. Sea spiders (Pycnogonida) from the Great Barrier Reef, Australia, feed on fire corals and zoanthids. Memoirs of the Queensland Museum 46:656.
- Arango CP. 2000. Three species of sea spiders (Pycnogonida) from Santa Marta, Colombian Caribbean. Boletin de Investigaciones Marinas y Costeras 29:59-66.
- Arango CP. In press. Morphological phylogenetics of sea spiders (Arthropoda, Pycnogonida). Organisms Diversity and Evolution.
- Arango CP. In press. Sea spiders from the Great Barrier Reef area: New species, new records and ecological annotations. Journal of Natural History.
- Arango CP. Molecular approach to the phylogenetics of Pycnogonida (Arthropoda) using nuclear ribosomal DNA and morphology. Submitted to Molecular Phylogenetics and Evolution.
- Arango CP and Brodie GD. In press. Observations of predation on the tropical nudibranch Okenia sp. by the sea spider Anoplodactylus longiceps Williams (Pycnogonida, Arthropoda). The Veliger.
- Lee A. C. and Arango CP. Two new species and other records of sea spiders (Pycnogonida, Arthropoda) from tropical North Queensland, Australia. Submitted to Memoirs of the Queensland Museum.

#### Statement on sources

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Claudia P. Arango

23<sup>vd</sup> Moy 2002 Date