

#### RESEARCHSPACE@AUCKLAND

#### http://researchspace.auckland.ac.nz

#### ResearchSpace@Auckland

#### **Copyright Statement**

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

To request permissions please use the Feedback form on our webpage. <a href="http://researchspace.auckland.ac.nz/feedback">http://researchspace.auckland.ac.nz/feedback</a>

#### General copyright and disclaimer

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form.

# THE ORDER HADROMERIDA (PORIFERA:DEMOSPONGIAE), TAXONOMY AND RELATIONSHIPS OF THE MAJOR FAMILIES

## by Michelle Kelly-Borges

A thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Zoology at the University of Auckland,

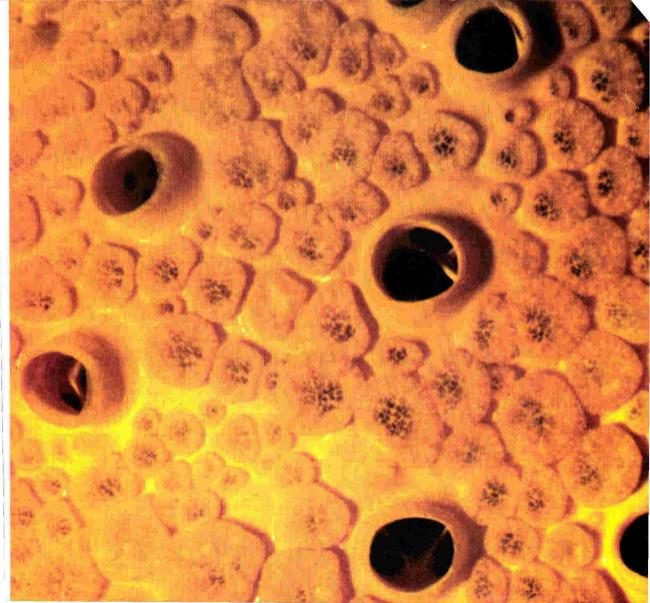
Auckland,

New Zealand

University of Auckland, 1991

UNIVERSITY OF AUCKLAND LIBRARY BIOLOGY THESIS

91-173



# THE ORDER HADROMERIDA (PORIFERA:DEMOSPONGIAE), TAXONOMY AND RELATIONSHIPS OF THE MAJOR FAMILIES

FRONTISPIECE: CLIONA CELATA (FAMILY CLIONIDAE) TABLE OF CONTENTS
ACKNOWLEDGEMENTS ABSTRACT ABBREVIATIONS
FIGURES, TABLES AND PLATES
I GENERAL INTRODUCTION

I GENERAL INTRODUCTION	1
II SYSTEMATICS	4
INTRODUCTION	
INTRODUCTION	
MATERIALS AND METHODOLOGY	
Collections Specimen preparation Taxonomic character definition and analysis Terminology Phylogenetic analysis	11 12 12 13 13
SYSTEMATICS	
ORDER HADROMERIDA TOPSENT FAMILY TETHYIDAE GRAY GENUS TETHYA LAMARCK Tethya aurantium (Pallas) Tethya sp 1 Tethya sp 2 Tethya sp 3 Tethya sp 4 Tethya sp 5 Tethya sp 6 Tethya sp 6 Tethya seychellensis (Wright) Tethya sp 7 Tethya robusta Bowerbank Tethya sp 8 Tethya sp 9 Tethya sp 10 Tethya sp 11	18 19 22 25 27 30 33 35 37 39 42 45 47 49 51
DISCUSSION	54

FAMILY SUBERITIDAE SCHMIDT GENUS SUBERITES NARDO Suberites sp nov GENUS AAPTOS GRAY Aaptos aaptos (Schmidt) Aaptos sp 1 Aaptos sp 2 Aaptos sp 3 Aaptos sp 4 Aaptos sp 5	69 69 72 75 77 80 82 85 87
DISCUSSION	93
FAMILY POLYMASTIIDAE GRAY GENUS POLYMASTIA BOWERBANK Polymastia fusca Bergquist Polymastia sp 1 Polymastia hirsuta Bergquist Polymastia sp 2 Polymastia sp 3 Polymastia sp 4 Polymastia crassa Carter Polymastia sp cf massilis Carter Polymastia sp cf bicolor Carter Polymastia sp 5	102 105 108 111 116 120 122 125 128 132 136
GENUS <i>PROTELEIA</i> DENDY AND RIDLEY <i>Proteleia</i> sp 1 <i>Proteleia</i> sp 2	140 142
DISCUSSION	145
III MOLECULAR SYSTEMATICS	
INTRODUCTION Morphology and molecules Which molecule? Assumptions Reconstructing evolutionary trees	158 158 159 161 162
MATERIALS AND METHODS	164
EXTRACTION OF TOTAL RIBOSOMAL RNA Field Collections Laboratory preparation of fresh specimens Laboratory preparation of frozen specimens Extraction of total rRNA Electrophoresis of total rRNA Storage and working use of rRNA	164 165 166 166 167 171

18S rRNA SEQUENCING 18S rRNA sequencing Oligonucleotides Reverse transcriptase sequencing Electrophoresis and autoradiography Sequence data acquisition	172 174 174 175 176
PHYLOGENETIC ANALYSIS Sequence alignment Data preparation for analysis Parsimony analysis Distance methods	177 178 180 182
RESULTS	
EXTRACTION OF TOTAL rRNA	184
18S rRNA SEQUENCING RNA template contamination Reverse transcriptase sequencing	185 186
PHYLOGENETIC ANALYSIS 18S rRNA sequence data Variability of sequence data Phylogenetic analysis	187 187 189
DISCUSSION	192
IV GENERAL DISCUSSION	195
V REFERENCES	198
VI APPENDICES	

APPENDIX 1 Preparation of sponge tissues for taxonomy

APPENDIX 2 Precautions against ribonucleases.

APPENDIX 3 Solutions, chemicals and methodology

APPENDIX 4 Gel electrophoresis of nucleic acids and sequencing products.

APPENDIX 5 Problems with reverse transcriptase sequencing and autoradiograph interpretation.

APPENDIX 6 The Polymerase Chain Reaction

APPENDIX 7 Character data and change lists for phylogenetic analyses.

#### VII PUBLICATIONS

### ACKNOWLEDGEMENTS

I thank my supervisors, Professors Patricia and Peter Bergquist who are a constant source of inspiration and motivation to me. It is difficult to express the depth of my gratitude to such people as Klaus Borges, my mother Lorraine, sisters Fiona and Therese, and Hannah Lobb, for the support and encouragement which was given freely throughout these years of research. Without the help of the University Grants Committee and DSIR in the form of scholarship funding, this period would have been more difficult than it was.

In the Zoology Department, I sincerely thank Allen Rodrigo and Roderick Page for their assistance with phylogenetic analyses. Iain MacDonald and Raewyn Eager contributed greatly to my work with their skills in photography and scanning electron microscopy. Thank you also David Todd, Beryl Davy, Victoria Williams and Laurel Walker, for your assistance and patience.

For guidance through the mysteries of nucleic acid extraction and sequencing, I thank in particular my colleagues in the Department of Cellular and Molecular Biology, Moreland Gibbs, Liam Williams, David Saul, Ellen Podivinsky, Ronnie McHale, Pat Stapleton and Judy McAnulty.

#### ABSTRACT

Despite advances of recent years no stable higher order classification of the Porifera has yet emerged. To address this problem, relationships at various taxonomic levels within the Order Hadromerida have been evaluated. Descriptions of new species of *Tethya*, *Aaptos* and *Polymastia* from northern New Zealand are given in conjunction with a review and redefinition of specific diagnostic characters for these genera. A range of species, genera and families within the Hadromerida have been subjected to 18S rRNA sequencing. Using morphological and molecular sequence data together in phylogenetic analysis, the existing familial groups of the Hadromerida are confirmed and some rearrangement of genera is indicated following sequence alignment and comparison. These data serve as a baseline for molecular approaches to resolving relationships between other sponge groups.

### **ABBREVIATIONS**

B-MCE B-mercaptoethanol

DEPC diethylpyrocarbonate

DNase deoxyribonuclease

DNA deoxyribonucleic acid

A adenosine
T thymine
G guanine

C cytosine

cDNA complementary DNA

CTAB cetyltrimethylammoniumbromide

dA/T/G/C/TP deoxy A/T/G/C/ triphosphate ddA/T/G/C/TP dideoxy A/T/G/C/ triphosphate

DTT dithiothreitol

EDTA ethylenediamine-tetra-acetic acid

MOPS 3-(N-morpholino)propane-sulphonic acid PAUP phylogenetic analysis using parsimony

rRNA ribisomal ribonucleic acid

Sarkosyl N-lauroylsarcosine

SDS sodium dodecyl sulphate

Taq Thermus aquaticus DNA (polymerase)

TBE Tris/borate electrophoresis buffer

TE Tris/EDTA (buffer)

TEMED N,N,N',N'-tetramethylethylenediamine
Tris tris(hydroxymethyl)aminomethane

UWGCG University of Wisconsin Genetics Computor

Group

#### FIGURES, TABLES AND PLATES

#### PHOTOGRAPHIC PLATES

- Figure 2. (Photographic Plate 1). Microscleres of *Tethya*: 1. long-rayed conical oxyspherasters; 2. short-rayed conical oxyspherasters; 3. concave oxyspherasters; 4. mammilate (convex) oxyspherasters; 5. unbranched oxyasters; 6. branched oxyasters.
- Figure 3. (Photographic Plate 2). Microscleres of *Tethya*: 1.acanthotylasters; 2. acanthostrongylasters; 3. acanthochiasters; 4. acanthooxyspherasters; 5. microoxyspherasters; 6. microoxyspherasters;
- **Figure 4.** (Photographic Plate 3). 1-2. Polyrhabds of *Tethya* sp 11; 3-4. *Tethya* sp 1; 5. *Tethya* sp 2; 6. *Tethya* sp 3.
- Figure 9 (Photographic plate 4). Preserved specimens of *Tethya*; 1. *Tethya* sp 4; 2. *Tethya* sp 5; 3. *Tethya* sp 6; 4. *Tethya seychellensis*; 5. *Tethya* sp 7; 6. *Tethya* sp 8.
- Figure 17. (Photographic Plate 5). Preserved specimens and spicules of *Tethya*:1.

  Tethya sp 9; 2. Tethya sp 9, spicules; 3. Tethya sp 10; 4. Tethya sp 11.
- Figure 23. (Photographic plate 6) Preserved specimens of Suberites and Aaptos:
  1. Suberites sp nov; 2. Aaptos sp 1; 3.Aaptos sp 2; 4.Aaptos sp 3; 5. Aaptos sp 4; 6.Aaptos sp 5.
- Figure 32. (Photographic Plate 7). Preserved specimens of *Polymastia*: 1. *Polymastia fusca* Bergquist; 2. *Polymastia* sp 1; 3. *Polymastia hirsuta* Bergquist; 4. *Polymastia* sp 2; 5. *Polymastia* sp 3; 6. *Polymastia* sp 4.
- Figure 39. (Photographic Plate 8). Preserved specimens of *Polymastia* sp cf massilis Carter; 3. *Polymastia* sp cf bicolor Carter; 4. Exotyles of *Proteleia*; 5. *Proteleia* sp 1; 6. *Proteleia* sp 2.
- Figure 50. (Photographic Plate 9) 1. Sponge total rRNA; 2. Effects of concentration of contaminants on reverse transcriptase sequencing; 3. The quantity of rRNA eluted from QIAGEN-Tip20 after decontamination compared to the original volume. 4. The positive effect of QIAGEN on reverse transcriptase sequencing.
- Figure 54. (Photographic plate 10). Autapomorphic and synapomorphic characters in sequence data; 1. Autapomorphic positions in Primer A; 2. Autapomorphic positions in Primer C; 3.Synapomorphic characters in Primer B; 4. Synapomorphic characters in Primer C.

#### COLOUR PLATES

Plate I a. Tethya sp 1, in situ. b. Tethya sp 4, in situ.

Plate II a. Tethya sp 5, in situ. b. Tethya sp 9, in situ.

Plate III a. *Polymastia*. sp 2, in situ b. *Polymastia crassa* Carter, in situ.

#### APPENDIX PLATE 1

#### Appendix 2 and 6.

- 1. Total ribosomal RNA from sponges.
- 2. Ribosomal RNA from *Tethya* aurantium; extraction by two methods.
- 3. Genomic DNA from sponges.
- 4. PCR amplification of sponge DNA samples.

#### APPENDIX PLATE 2

- 1. Crossbanding in sequencing gel autoradiographs.
- 2. Fading in sequencing gel autoradiographs.
- 3. Apparent insertions into sequencing gel autoradiographs.

#### TEXT FIGURES

Text Figure 1. Collection localities.

Text Figure 5. General skeletal arrangement of Tethya aurantium.

**Text Figure 6.** General skeletal arrangement of *Tethya sp 1*.

Text Figure 7. General skeletal arrangement of Tethya sp 2.

**Text Figure 8.** General skeletal arrangement of *Tethya sp 3*.

Text Figure 10. General skeletal arrangement of *Tethya* sp 4.

Text Figure 11. General skeletal arrangement of *Tethya* sp 5.

Text Figure 12. General skeletal arrangement of *Tethya* sp 6.

Text Figure 13. General skeletal arrangement of Tethya seychellensis

**Text Figure 14.** General skeletal arrangement of *Tethya* sp 7.

Text Figure 15. General skeletal arrangement of Tethya robusta.

Text Figure 16. General skeletal arrangement *Tethya* sp 8.

Text Figure 18. General skeletal arrangement of *Tethya* sp 9.

Text Figure 19. General skeletal arrangement of *Tethya* sp 10.

Text Figure 20. General skeletal arrangement of *Tethya* sp 11.

Text Figure 21. Characters and character states of Tethya.

**Text Figure 22.** Hypothesised relationships within the genus *Tethya*.

Text Figure 23. General skeletal arrangement of Suberities sp nov.

Text Figure 24. General skeletal arrangement of Aaptos aaptos (Schmidt).

Text Figure 25. General skeletal arrangement of *Aaptos* sp 1.

Text Figure 26. General skeletal arrangement of *Aaptos* sp 2.

Text Figure 27. General skeletal arrangement of Aaptos sp 3.

Text Figure 28. General skeletal arrangement of Aaptos sp 4.

Text Figure 29. General skeletal arrangement of Aaptos sp 5.

Text Figure 30. General skeletal arrangement of Trachya pernucleata Carter.

Text Figure 31. Hypothesised relationships between *Aaptos* and *Suberites*.

Text Figure 33. General Skeletal arrangement of Polymastia fusca Bergquist.

Text Figure 34. General skeletal arrangement of Polymastia sp 1.

Text Figure 35.General skeletal arrangement of *Polymastia hirsuta* Bergquist.

Text Figure 36. General skeletal arrangement of *Polymastia* sp 2.

**Text Figure 37.** General skeletal arrangement of *Polymastia* sp 3.

Text Figure 38. General skeletal arrangement of Polymastia sp 4.

Text Figure 40. General skeletal arrangement of *Polymastia crassa* Carter.

Text Figure 41. General skeletal arrangement of Polymastia sp. cf. massilis

**Text Figure 42.** General skeletal arrangement of *Polymastia* sp cf *bicolor* Carter, and *Polymastia* sp 5..

Text Figure 43. General skeletal arrangement of Proteleia sp 1.

Text Figure 44. General skeletal arrangement of Proteleia sp 2.

Text Figure 45. Cortex architecture in *Polymastia* and *Proteleia*.

**Text Figure 46.** Hypothesised relationships within the genus *Polymastia* and *Proteleia*.

**Text Figure 47.** The position of sequencing primers on a secondary structure model for eukaryotic small subunit rRNA.

Text Figure 48. Ambiguous sequence alignment in Primer C sequence data.

Text Figure 49. Positions 1556-1558 in 18SrRNA sequences from various phyla.

Text Figure 51. Primer A partial 18S rRNA sequence data.

Text Figure 52. Primer B partial 18S rRNA sequence data.

Text Figure 53. Primer C partial 18S rRNA sequence data.

Text Figure 55. Parsimony analysis of partial 18S in rRNA sequence data.

**Text Figure 56.** Hypothesised phylogenetic relationships of sponges (Orders Hadromerida and Dictyoceratida) from rRNA sequence data.

Text Figure 57. Neighbour-joining analysis of partial ribosomal 18SrRNA sequence data

#### TABLES

Table 1. Classification of the Hadromerida.

**Table 2.** Megascleres of *Tethya robusta* Bowerbank.

Table 3. Character-taxon matrix for Tethya.

Table 4. Megascleres of Aaptos sp 1.

Table 5. Megascleres of Aaptos sp 3.

Table 6. Skeletal features of Aaptos and Trachya.

Table 7. Characters and character states for Aaptos.

**Table 8.** Character-taxon matrix for *Aaptos*.

**Table 9.** Character comparison between *Tethya*, *Suberites* and *Aaptos*.

Table 10. Megascleres of Polymastia hirsuta Bergquist.

- Table 11. Megascleres of Polymastia sp 2.
- Table 12. Megascleres of Polymastia Carter.
- **Table 13.** Megascleres of *Polymastia* sp cf massilis Carter.
- **Table 14.** Megascleres of *Polymastia* sp cf bicolor Carter.
- Table 15. Megascleres of Polymastia sp 5.
- Table 16. Character-taxon matrix for Polymastia and Proteleia.
- Table 17. Sponges collected for rRNA sequencing.
- **Table 18.** Oligonucleotide primer sequences for reverse transcriptase sequencing.
- **Table 19.** A method of coding nucleotides from several gels to obtain a consensus sequence.
- Table 20. Variation in sponge sequence data.
- Table 21. Character taxon matrix for rRNA sequence data.