Resolving a taxonomic ambiguity: Variation in the pycnogonid

Pseudopallene ambiguа
(Stock 1956)

Dave Stevenson BSc (Tas)

This thesis is submitted in partial fulfilment for the degree of Bachelor of Science with Honours in Marine, Freshwater and Antarctic Biology at the University of Tasmania.

November 2003
1 Abstract

A combination of molecular and morphological techniques was applied to resolve a nearly 50-year-old ambiguity in the Australian pycnogonid *Pseudopallene ambigua* (Stock 1956). Stock was hesitant to initially describe *P. ambigua* due to confusion with a sister species *P. pachychiera*. More recent work has clarified the distinction between these taxa but *P. ambigua* remains enigmatic with the type series suspected to contain several species.

For this study, *P. ambigua* were collected from a 10-11 kilometre section of the Tasmanian east coast around Eaglehawk Neck, SE Tasmania. The 56 specimens of *P. ambigua* were examined and the data used to generate morphological groups within this species. Samples from these morphological groups were then sequenced at two rapidly evolving gene regions, the mitochondrial 16S ribosomal gene and the mitochondrial cytochrome c oxidase I protein coding gene.

Comparison of the sequence data for both 16S and cytochrome c oxidase determined that two species were present in this collection. These genetically identified species were consistent with the morphological data. Morphological characters including proboscis shape, oviger spine number, tibiae surface texture, heel spine pattern and propodus width-height ratio were found to confirm the molecular 16S and CO1 sequences separating a consistent group of specimens. Examination of the morphological data provided significant evidence for the characters proboscis shape and propodus ratio that will enable the new species to be readily identified from *P. ambigua*. 
## CONTENTS

1 **ABSTRACT**  

2 **LIST OF PLATES, TABLES, FIGURES AND MAPS**  

   2.1 **PLATES**  
   2.2 **TABLES**  
   2.3 **FIGURES**  
   2.4 **Maps**  

3 **ACKNOWLEDGEMENTS**  

4 **INTRODUCTION**  

   3.1 Taxonomy – the basis of biological science  
   3.2 Modern taxonomic work  
   3.3 Project background  
   3.4 **Aim**  

5 **METHODS**  

   4.1 **Collection**  
   4.2 **Morphological Methods**  
   4.3 **Molecular Methods**  
   4.4 **Environmental factors correlated with colour pattern?**  

6 **RESULTS**  

   5.1 **Morphological results**
Morphological character analysis

5.2 Molecular results

6 DISCUSSION

6.1 Overview

6.2 Morphology

6.3 Molecular sequence data

6.4 Why red stripes?

6.5 Further work

7 CONCLUSION

8 GLOSSARY

9 APPENDIX A:

9.1 Additional extraction protocols tested
2 List of Plates, Tables, Figures and Maps

All plates, tables, figures and maps are the work of the author unless otherwise acknowledged.

2.1 PLATES

Titlepage: *P. ambigua* (Yellow colour form) [Plate 1a: Yellow form *P. ambigua*. (Male carrying eggs and larvae.)]........18

Plate 1b: Red stripe form of *P. ambigua*.
.................................................................................................18

Plate 2a: Anterior view of Yellow 3 form of *P. ambigua* showing constricted scapes and proboscis.
.................................................................................................19

Plate 2b: Anterior view of Yellow 1 form of *P. ambigua*. Proboscis and scapes unconstricted.
.................................................................................................19

Plate 3a: Red stripe colour form of *P. ambigua* on host bryozoan.
.................................................................................................20

Plate 3b: Yellow colour form of *P. ambigua* on host bryozoan.
.................................................................................................20

2.2 TABLES

Table 1: Summary of morphological characters
.................................................................................................26

Table 2: Summary of morphological characters by type
.................................................................................................28

Table 3: Primers tested on pycnogonid extracts
.................................................................................................46

Table 4: PCR product amounts after cleaning
.................................................................................................48

Table 5: Results of an ANOVA on each morphological character and *post hoc* Tukey test results to identify taxa significantly different at each character.
Table 6: Summary of sequence results for both gene regions

Table 7: Intraspecific and interspecific nucleotide substitutions for 16S sequences

Table 8: Intraspecific and interspecific nucleotide substitutions for COI sequences

2.3 FIGURES

Figure 1: General overview of pycnogonid external morphology (Child 1979)

Figure 2: Abdomen lengths relative to 4th lateral process

Figure 3: Proboscis shapes

Figure 4: Oviger terminal claw

Figure 5: Fifth oviger segment distal projection male and female view.

Figure 6: Dorsal view of *Pseudopallene ambigua*, showing measurements and major anatomical segments.

Figure 7: Anterior view of *Pseudopallene* sp showing scapes and chelifores

Figure 8: Side view of second leg segments of *P. ambigua* showing lengths measured

Figure 9: Chelifore lengths, FF – Fixed Finger, MF – Movable Finger

Figure 10: Scape shapes in male and female *Pseudopallene* sp and *Stylopallene longicauda*

Figure 11: Heel spine patterns in *Pseudopallene* sp and *Stylopallene longicauda*
Figure 12: Lateral process angle

Figure 13: Ocular tubercle height

Figure 14: Female second coxa and genital aperture

Figure 15: Dorsal surface texture patterns on femur and tibiae

Figure 16: Second leg of *Pseudopallene pachychiera* showing constrictions

Figure 17: Dorsal view of *Stylopallene longicauda*

Figure 18: Cluster analysis for all untransformed morphological character data (UPGMA = -0.1)

Figure 19: Cluster analysis of untransformed morphological characters with sex and age based characters excluded (UPGMA = -0.1)

Figure 20: Canonical Discriminant Analysis plot of continuous morphometric characters.

Figure 21: Cluster analysis of transformed morphological characters with sex and age based characters excluded (UPGMA = -0.1)

Figure 22: The nucleotide sequence of a part of the mitochondrial 16S region for the 3 morphological groups of *Pseudopallene ambigua* and one *Stylopallene longicauda* outgroup.

Figure 23: Phylogenetic tree of 16S sequence data.

Figure 24: The nucleotide sequence of a part of the Cytochrome Oxidase I region for the 3 morphological groups of *Pseudopallene ambigua*.
Figure 25: Phylogenetic tree of COI sequence data.

Figure 26: Algal overgrowth compared to algal coverage of host Bryozoa for each form of *P. ambigua*.

Figure 27: Comparison of substrate aspect for the Red Stripe colour forms of *P. ambigua*.

Figure 28: Comparison of substrate aspect for the pure yellow colour forms of *P. ambigua*.

2.4 Maps

Map 1: Study site showing positions where pycnogonids were collected in January and February 2003.
Acknowledgements

I would like to express my sincere gratitude to the people who assisted me during the course of my Honours program. In particular, I would like to thank:

Dr Karen Miller and Associate Professor Alastair Richardson for their enlightening supervision and encouragement throughout the year.

Karen Gowlett-Holmes for teaching me underwater photography which stimulated anew my love of the sea. Gary Myers and Mic Baron for getting me out to the best dive sites and back again!

My fellow honours students for tolerating an “over achieving mature age student” in their midst all year; especially Clare Lawrence and Anthony Reid for their positive feedback, timely advice and willingness to listen.

All the members of the Zoology administrative, technical and academic staff, especially Adam Smolenski for his invaluable help in the Molecular Genetics Laboratory.