

New Zealand Aquatic
Environment and Biodiversity
Report No. 3
2006
ISSN 1176-9440

Whose larva is that?
Molecular identification of planktonic larvae
of the Ross Sea

M. A. Sewell
S. Lavery
C. S. Baker

Whose larva is that?
Molecular identification of planktonic larvae
of the Ross Sea

M. A. Sewell
S. Lavery
C. S. Baker

School of Biological Sciences
University of Auckland
Private Bag 92019
Auckland

**Published by Ministry of Fisheries
Wellington
2006**

ISSN 1176-9440

©
**Ministry of Fisheries
2006**

Citation:
Sewell, M.A.; Lavery, S.; Baker, C.S. (2006).
Whose larva is that? Molecular identification of planktonic larvae of the Ross Sea.
New Zealand Aquatic Environment and Biodiversity Report No. 3. 57 p.

This series continues the
Marine Biodiversity Biosecurity Report series
which ceased with No. 7 in February 2005.

EXECUTIVE SUMMARY

Sewell, M.A.; Lavery, S.; Baker, C.S. (2006). Whose larva is that? Molecular identification of planktonic larvae of the Ross Sea. *New Zealand Aquatic Environment and Biodiversity Report No. 3*. 57 p.

- 1) This research has investigated the potential for combining morphological and molecular analysis of wild-caught specimens of benthic adults and planktonic larvae to resolve this problem.
- 2) Field collections were made in McMurdo Sound during November and December 2002 of both planktonic larvae and the dominant species of benthic invertebrates. Larvae were digitally photographed before preservation for further morphological and DNA analysis. Adults were photographed where possible before tissue samples were preserved for DNA analysis. All samples were transported to the University of Auckland for molecular analysis and further morphological description. In total, over 1000 individual plankton (including holoplankton and meroplankton) and over 100 samples from individual adults were collected for analysis.
- 3) A relatively diverse meroplankton community was seen in McMurdo Sound. On each sampling day we identified an average of 24 marine invertebrate larvae, comprising about 13 morphological larval forms. Polychaete nectochaetes were the most abundant larval form collected, with high numbers of nudibranch veligers and nemertean pilidia also present. Echinoderm larvae were rarely collected. Large numbers of eggs and embryos were also collected throughout the sampling period. Multivariate analysis of the meroplankton community and principal co-ordinates analysis showed a generally similar meroplankton community during November and December.
- 4) DNA sequences have been obtained from 77 adults of 24 species of Antarctic marine invertebrates, most for the first time. These data has provided an invaluable adult reference data set with which to compare all planktonic larvae and is a major advance in our description of Ross Sea benthic invertebrate biodiversity. All specimens, photographs and DNA extractions and sequences are being archived, and will form the basis of a new reference collection describing the molecular diversity of Ross Sea benthic invertebrates.
- 5) Successful techniques have been developed for rapid DNA extraction, PCR amplification for more than two gene loci, and subsequent DNA sequencing of individual meroplankton. Using these techniques, it is now feasible and potentially economical to use these molecular techniques for species-specific discrimination and identification of Ross Sea meroplankton. The addition of further adult DNA sequence information in the future will greatly increase our ability to identify a broader range of meroplankton species.
- 6) Over 500 individual planktonic larvae have been molecularly analysed through DNA extraction and PCR amplification of two gene loci producing over 550 DNA sequences. All sequences were used to classify and identify these larvae where possible. A subset of these provide the best data for molecular and morphological species comparison. Complete data is supplied as electronic appendices.
- 7) Using a DNA sequencing approach to larval identification we have identified over 24 “species” of larvae from the McMurdo Sound meroplankton, including 9 polychaetes, 6 echinoderms, 5 nemerteans and 4 gastropods. From these we have matched eggs/embryos and larvae to known adult Antarctic species, ascribed many larvae to higher taxonomic levels than species, and clarified identifications in previous research on Antarctic meroplankton.
- 8) This research has shown that it is both possible to molecularly identify individual planktonic larvae from the Ross Sea, and also feasible to do this in a mass-screening manner. Together with the accumulating morphological descriptions and increasing molecular and image databases, this now offers the ability to approach broader ecological and biodiversity questions about Ross Sea benthic invertebrate communities.

1. INTRODUCTION

1.1 Overview

Our understanding of the diversity of marine organisms in the Antarctic is gradually increasing, due largely to the increased efforts being made in sampling from ships (e.g., the MFish-sponsored Ross Sea Voyage 2004) and particularly in sampling and observations from scuba diving (e.g., NIWA project – Ecology of coastal benthic communities in Antarctica – MFish project ZBD2002/01, Norkko et al. 2002). However, a considerable bottleneck exists that greatly inhibits our abilities to adequately describe Antarctic marine biodiversity, and to answer broader ecological questions about how that diversity is sustained. Even though there is a long history of collecting and describing the larvae of marine organisms in the Antarctic, we are still unable to positively identify to species most planktonic larvae collected from Antarctic waters. Most current descriptions of larvae identify only “morphotypes”, and ascribe them to a higher taxonomic level, such as family (e.g., Stanwell-Smith et al. 1999). This is because either the larvae of closely related species are very difficult to distinguish morphologically, or, more importantly, there is no knowledge of the morphological appearance of the larvae of most species. Therefore, at present, there is almost no way of linking our understanding of the abundance and distributions of adult marine organisms in the Antarctic (Bradford-Grieve & Fenwick 2001, Waterhouse 2001, Page et al. 2002) with that of larval abundance and distribution (Bhaud et al. 1999). This is a major constraint to furthering our understanding of the biodiversity and ecology of marine communities (Fenwick & Bradford-Grieve 2002). Our goal in this study was to use a coordinated approach of morphological and molecular description of both larvae and adults in order to identify to species the common marine larvae of the Ross Sea, and hence allow Antarctic biodiversity research to move ahead more rapidly. It is only once the species of an ecosystem can be adequately identified that more detailed examinations of biological interactions and processes can be properly undertaken. It is impossible to determine the processes sustaining biological diversity if the diversity itself cannot be adequately described. It is this fundamental knowledge of the biology of the Ross Sea that this study addresses.

1.2 Background

Marine biologists working in Antarctica in the 19th century made two general observations regarding the reproductive biology of marine invertebrates. Firstly, that there was a general scarcity of invertebrate larvae in the plankton (Murray 1895), and, secondly, that there was a high incidence of brooding (Thomson 1878, 1885). These ideas were further developed by later workers (reviewed by Shreeve & Peck (1995)), and received wider attention when Thorson (1936, 1950) noted that there was a trend for a reduction in pelagic stages with increasing latitude in both the Arctic and Antarctic Oceans. This hypothesis, which has become known as “Thorson’s Rule” (Mileikovsky 1971), initially received general support (reviewed by Clarke (1992)), but a detailed re-examination of this hypothesis (Pearse et al. 1991, Pearse 1994) has instead shown that ‘for many groups the proportion of shallow-water species with pelagic development is similar to that found elsewhere’ (Pearse et al. 1991, p. 70).

Despite the recognition that there is a high proportion of Antarctic species with pelagic development, few studies have focused on invertebrate and fish larvae in the plankton (the meroplankton). Earlier studies associated with the early Antarctic expeditions described and tentatively suggested the adult species for larvae of several marine invertebrates and fish (e.g., MacBride & Simpson 1908, MacBride 1920, Regan 1916). More recent work on the meroplankton of the Antarctic has similarly described larvae only to the higher taxonomic levels: phyla, class, and occasionally order or family. Stanwell-Smith et al. (1999), for example, found 131 larval OTUs (operational taxonomic units; morphotypes or “species”) at Signy Island in the maritime Antarctic. Similarly, 16 larval “types” (identified to phylum or class level) were found in a 2-month survey in the Bellinghausen Sea (Shreeve & Peck 1995), and recent work has shown at least 18 “types” of planktonic polychaete larvae (identified to the family level) from Terre Adélie and the Ross Sea (Bhaud et al. 1999). These studies all strongly suggest that

reports of the ‘almost total absence of pelagic larvae belonging to benthonic organisms’ (Murray 1895, p. 1459) may be a sampling artefact, but advance our knowledge no further as to the species that actually produce these larvae.

Three approaches can be used to identify planktonic larvae to the species level. The first involves the collection of adults from the field during the appropriate spawning seasons, spawning of adult male and female specimens in the laboratory, and detailed description of the morphology of the larval forms. With this approach the species of origin for the larvae is known with certainty, but there are a number of disadvantages. In particular it is labour intensive, and sometimes problematic, to obtain and maintain larval cultures, especially in providing a suitable source of food during development. The second approach is to collect larval forms from the plankton and, as described above, raise these in the laboratory until they settle as juveniles. This approach also requires time consuming and labour-intensive culture of larval forms; in Antarctic species this is increasingly problematic due to the extended developmental times of many species (e.g., Peck 1993, Shilling & Manahan 1994). The third approach, recently developed in temperate habitats, is to use the polymerase chain reaction (PCR) and DNA sequence variation to match larval and adult sequences (Olson et al. 1991). In the original use of this technique, a portion of the 16s ribosomal RNA gene was sequenced from the collected sea cucumber larvae and, based on larval morphology, the assumed adult source (Olson et al. 1991, Medeiros-Bergen et al. 1995). Instead, the larvae were identified as being derived from a species from an entirely different order of sea cucumber. Subsequently PCR-based molecular techniques have been widely used to identify larvae from a variety of species and habitats. Examples include invasive zebra mussels (Baldwin et al. 1996), mussels (Comtet et al. 2000) and crabs (Epifanio et al. 1999), temperate oysters (Li & Hedgecock 1998), bivalves (Hare et al. 2000), mussels (Martel et al. 2000), and tropical goboid fish (Lindstrom 1999). The benefits of this type of approach are numerous. There are clear savings in time and resources in being able to make final identifications, and for many species this approach is likely to be the only feasible method for larval identification. This study provides the first use of this technique for the identification of Antarctic larvae.

1.3 Molecular markers for species identification

For over 40 years, molecular methods have been applied to species identification in marine systems. Among the first molecular markers that were used in discriminating and identifying species were allozyme loci, which differentiate based on the migration of alleles of water-soluble proteins (enzymes) in a charged electrophoretic gel (reviewed by Thorpe & Solé-Cava (1994)). Although allozymes can differentiate between species, there are disadvantages with this technique. These include the need for fresh or frozen tissue to be used to avoid enzyme degradation, that comparison cannot be made with type specimens fixed in ethanol or formalin, and that electrophoretic data are difficult to compare between laboratories due to differences in extraction methods and electrophoretic procedures (Thorpe & Solé-Cava 1994). As a consequence, in the last 10 years the molecular technologies used for species identification have shifted from the higher structural level of proteins to the fundamental information code in the cell, DNA.

The advantage of using an organism’s DNA for species identification is that it is invariant with age, stage, or physiological state, yet it varies among different taxonomic groups (Hillis et al. 1996). With this approach, a species is first identified by a taxonomic expert or from a voucher specimen. DNA is extracted and isolated from this species and one or more genes of interest amplified using the PCR. After DNA amplification, the molecular approach used in species identification depends on many factors, not the least being the particular question that is being asked. Some of the alternative approaches include RFLP (restriction fragment length polymorphism) analysis, SSCP (single-stranded conformation polymorphism) analysis, microsatellite analysis and DNA sequencing (see Hillis et al. (1996) for more technical details of these and other techniques). RFLP, SSCP, and microsatellite analyses, although they can be used to discriminate among species (e.g., Comtet et al. 2000, Hare et al. 2000), are most powerful in comparisons of closely related species or within-species comparisons, and often require direct comparison against reference samples at the same time in the same laboratory. As a consequence, these techniques tend to be most used in studies examining parentage, relatedness, or

population level questions, or when differentiation among only a few species in a group is required, e.g., among sea cucumbers, or among bivalves (see review by Garland & Zimmer (2002)).

With the advent of automated DNA sequencing, in recent years there has been a shift to direct DNA sequencing for many applications. Mitochondrial DNA (mtDNA) and nuclear ribosomal DNA (rDNA) have been the main targets in animal studies, due largely to their high copy number per cell, their ease of amplification, and the relatively high levels of variation among species. Some studies in which species identification is the goal have used taxon-specific PCR amplification primers (e.g., Hare et al. 2000). This can be useful if only a few related species are to be discriminated, but if a wide variety of taxa are to be analysed at the same time, much more “universal” primers (i.e., those which can amplify the same gene from a very wide range of animal taxa) must be used.

Thus each of the different molecular methods has advantages and disadvantages, depending on the exact nature of the questions, the available expertise and equipment, and the need for wide comparison with the results from other laboratories. Some of the most crucial aspects of this project were, firstly, to be able to use the extensive genetic data already available on related marine species and, secondly, to make our data available to future workers in the most usable form. These criteria strongly favoured the collection of DNA sequence data, for sequences are the most easily and reliably shared without need for lengthy additional methodological standardisation. DNA sequences also have the advantage of providing the highest level of discriminatory power for any given locus. Here, because we were interested in matching particular larval morphologies to an adult sequence, the best approach to use is direct DNA sequencing.

In a project such as this, there are an almost infinite number of possible genetic loci from which to obtain DNA sequence. However, there are many criteria that quickly reduce the number of feasible candidates. We wished to amplify and sequence species-specific loci from large numbers of very small specimens from diverse taxa and compare them, where possible, to previous reference sequences. As such, we required strong and robust PCR amplifications of relatively commonly used loci exhibiting high among-species variability, preferably using a limited number of PCR primers that would work on a variety of taxa. We also aimed to collect data from both mitochondrial (mt) and nuclear (n) DNA loci, which can aid in species discrimination. To meet these criteria, we trialled a number of loci, including mitochondrial 12s and 16s ribosomal RNA, cytochrome oxidase I (COI), and cytochrome b (cytB) genes, and nuclear 18s and 28s ribosomal RNA and elongation factor 2 α genes. As part of this project we aimed to determine the most appropriate and reliable gene or genes for use in species identification of the larval forms.

An important aspect of our study was the need to compare our sequences with those already determined from related species, and available on the public databases (e.g., GENBANK). Different gene sequences vary in their prevalence in the public databases. The mitochondrial (12s and 16s) and nuclear (18s and 28s) ribosomal genes tend to be the genes most commonly available across most taxonomic groups, particularly among invertebrates. For invertebrate species, the mitochondrial locus COI is the next most commonly represented, but at present falls far behind the ribosomal genes. However, there are currently major efforts world-wide to utilise COI as a “universal” gene for molecular taxonomy, as part of the “DNA barcoding” programme (Hebert et al. 2003). Thus in the near future we will expect many more COI sequences to become available. At the time of preparation of this report, by far the best coverage across taxa was still the ribosomal genes, in particular the 16s locus, and was therefore a likely prime focus for our research.

In general, there were two stages to the molecular sequence analysis of the Antarctic larvae in this study. First, because of the small size of the larvae, and the potential problems in PCR and sequencing, we wanted to be able to confirm that the DNA sequence we obtained from any one sample belonged to the phylum/taxon with which it could be morphologically identified; i.e., that the sequence had genetic similarity to other species from that broad taxonomic group. Following this confirmation, we then wanted to match the DNA sequence to that of one of the adult sequences that we determined or were in the public databases. If there was no exact match, we wished to determine as precisely as possible the taxonomic affinities of the specimen.

In this report we taxonomically identify a range of benthic invertebrate larvae using molecular and morphological techniques, and match Antarctic larval forms to adult forms for the first time.

1.4 Objectives

The overall objective for this research project was to describe the biodiversity of the marine invertebrate and fish larvae of the Ross Sea planktonic community using morphology and to use molecular identification to assign these larval forms to the species-level where possible. There were three specific objectives:

- To describe, using photographs and/or line drawings, the common planktonic organisms of the Ross Sea (larvae of benthic marine invertebrates and fish).
- To produce a database of molecular sequence information from suitable nuclear and mitochondrial markers for the aforementioned planktonic organisms.
- To produce a database of the same sequence information from common benthic organisms and fish of the Ross Sea.

This report quantifies the meroplankton community of McMurdo Sound during the Nov-Dec. period based on a single site near McMurdo Station. We present high quality digital images of the larval forms and provide molecular sequence matching for those larval forms and adult marine invertebrates from which we could obtain confirmed DNA sequences. As no larval fish were found during the sampling, this report considers only marine invertebrate larvae.

2. METHODS

2.1 Plankton collections and sorting

Meroplankton samples were collected from a single 25 cm ice-hole in an area adjacent to the Ice Runway Road from McMurdo Station to the airfield; henceforth called the McMurdo Runway site (Figure 1: 77°51.1'N, 166°38.9'E; water depth 62 m). The ice hole was drilled through the 3.5 m of sea-ice with a Jiffy drill and the hole cleared of ice debris. An Endura tent was then erected above the plankton sample hole and hot water poured down the hole each day after sampling had been completed; the increased seawater temperature prevented extensive freezing and resulted in a significant time-saving in maintenance of the sampling hole. This also meant that all quantitative plankton sampling was conducted at a single location for the entire field season.

Plankton samples were collected from 21 November to 14 December 2002, on all days except Sundays (excludes 24 November and 1 and 8 December) resulting in a total of 21 sampling points. On each day a total of four replicate plankton hauls were taken using a collapsible plankton net based on that described by Kirkwood & Burton (1987). The plankton net attached to the umbrella-frame was constructed from 100 μ m mesh and with the same design and dimensions as used by Kirkwood & Burton (1987) and Kirkwood (1993). The net consisted of a 2 m conical portion with a square mouth (28x28 cm: 784 cm²) and, assuming 100% efficiency, each 50 m plankton tow sampled 3.92 m³ of seawater (3920 litres). This design, with a straight section at the mouth of the plankton net, was found to have improved filtration efficiency by reducing clogging by phytoplankton (Kirkwood 1993).

For each tow, the plankton net was passively lowered to a depth of 50 m using the weight of the umbrella net and the codend. The net did not “fish” on its descent as the weight of the stainless steel umbrella ensured that this end descended first (Sewell 2005). At 50 m depth, which was within 10–12 m of the bottom, the primary hauling rope was held taut, opening the net, and the rope hauled to the surface by hand at a rate of about 2.4 m/s (2 min per tow). The net was closed just below the ice-surface with the secondary rope so that the net could be pulled through the 3.5 m of sea-ice covering the water surface.

The plankton sample was transferred to a 600 ml sample jar using the tap fitted to the codend. Four replicate samples were taken on each day over a total sampling period of about 15 min. Samples were returned to Scott Base in an insulated container and the first three samples collected were freshly sorted as described below. The fourth sample was preserved in the formalin-based Steedman's fix (Steedman 1974) and returned unsorted to the University of Auckland as a reference sample.

In addition to the plankton samples collected from the McMurdo Runway site we also obtained from Peter Wederall (Scott Base engineer) the filter bag from the seawater intake to the reverse-osmosis (RO) water plant (Figure 1: 77°51.002'N, 166°46.136'E; ca 30 m from the Summer Laboratory, Scott Base; water depth 2 m). This filter was the same mesh size as the plankton net (100 µm), but filtered approximately 90 000 l per day (compared to the 11 760 litres from the three plankton net tows). The Scott Base water intake sample was sorted if time was available after completion of the McMurdo Runway site sorting and processing. However, due to the large volume of plankton collected per day, the entire sample could not be sorted in the time available and larval numbers could not be quantified. Any larvae sorted from the Scott Base water intake were, however, processed for morphological or DNA analysis.

Plankton samples were sorted fresh under a dissecting microscope in the Summer Laboratory. Individual larvae were separated from the sample using a pipette, and digital photographs taken of clean, undamaged specimens on clean depression slides using an Olympus C4040 camera at either x40 or x100 magnification. Larvae for DNA sequencing were then transferred by pipette to a clean glass slide, rinsed in distilled water, and transferred to an individual well of a 96-well PCR plate with minimal water; 50–100 µl of 95% ethanol was then added, and the well sealed with a silicon rubber cap. Sample plates were stored at room temperature in the Summer Laboratory until the end of the season. They were then transported at 4 °C to the University of Auckland where they were stored at –20 °C until analysis. Additional specimens for morphology were similarly transferred to an individual well of a 96-well cell culture plate, preserved in 4% formalin in sea water, sealed with silicon rubber sealers, and transported back to the University of Auckland.

2.2 Adult collections

Adult Antarctic marine invertebrates were collected for DNA analysis through sampling programmes already underway in Antarctica. Specimens were provided by the NIWA group undertaking the other BioRoss funded project in the Ross Sea (Dr. A. Norkko), through incidental bycatch from fish-traps (Dr. Gretchen Hofmann, University of California, Santa Barbara), by removing a few tube-feet from starfish collected by scientists from the University of Southern California for spawning experiments (Dr. Donal Manahan, PI; D. Ginsberg, diver) and through collections made by the Italian benthic programme at Terra Nova Bay (Dr. Mariachiara Chiantore). Depending on size, after photographing, adults were either stored whole in 95% ethanol, or first a tissue subsample was taken before storing in ethanol. Subsequent storage and transport to the University of Auckland was as per the plankton samples. These specimens will be the start of the adult Antarctic database.

2.3 DNA Sequence Analysis

Several methods were trialled during this project. Only the final, optimal, methods used are reported here.

2.3.1 DNA extraction

2.3.1.1 Adult DNA extraction

Whole genomic DNA extraction was undertaken using standard proteinase-K, phenol/chloroform techniques, and checked for quality and concentration by electrophoresis on 0.6% agarose gels. Final

purified DNA was stored in TE buffer (10 mM Tris, 1mM EDTA) at -20°C . Reference DNA is archived at -70°C .

2.3.1.2 Larval DNA extraction

Ethanol was first evaporated from each plate of samples by placing in a thermocycler at 94°C for approx. 30 min. 50 μl of a solution of 10 mg/ml proteinase K and 5% chelex was then added to each well. Samples were then incubated at 55°C for 30 min., followed by 94°C for 30 min, and cooled to 4°C for 1 min. Each sample tray was then frozen either at -20°C overnight or at -70°C for 10 min, before again being incubated at 94°C for 10 min.

2.3.2 Polymerase Chain Reaction (PCR)

2.3.2.1 Adult PCR

Genomic DNA extractions were diluted 1:10 or 1:100 in 1/10 TE (depending on DNA concentration) for use as template in PCR reactions. 1 μl of one or both of these dilutions used in a single 25 μl PCR reaction. A variety of genes were trialled for amplification, including mitochondrial 12s and 16s ribosomal RNA, cytochrome oxidase I (COI) and cytochrome b (cytB), and nuclear 18s and 28s ribosomal RNA and elongation factor 2 α . The final genes used were the mt large subunit 16s rRNA (16s) and cytochrome oxidase subunit I (COI). Primers used were the conserved 16sAR and 16sBR (Palumbi 1996), and COI 1490 and COI 2498 (Folmer et al. 1994). PCRs used 10 mM Tris, 1 mM KCl, 2.5 mM Mg, 0.25 mM dNTPs, 200 pM of each primer, 0.01units/ μl of ABI AmpliTaq DNA polymerase and about 20 ng of DNA template. The PCR profile was: initial denaturation of 94°C , 3 min, followed by 35 cycles of 94°C 30 s, 45°C 1 min, 72°C 2 min, and a final extension of 72°C , 3 min. Annealing temperatures were raised above 45°C where possible to reduce the production of additional non-specific products.

2.3.2.2 Larval PCR

Plankton DNA extractions were generally used without dilution in PCR reactions. Either 1 μl or 4 μl were used per reaction. The same primer pairs were used for plankton as for adults. Identical reaction conditions were used, except that the number of PCR cycles was increased to 40, and a more sensitive and specific Hotstart polymerase (Invitrogen Platinum Taq) was required to ensure consistent amplification.

2.3.3 DNA Sequencing

All PCR products were treated to “purification” using the Shrimp Alkaline Phosphatase (SAP) / Exonuclease I (Exo) method (Werle et al. 1994). This removes carry-over dNTPs and oligonucleotides from the sequencing reaction. Direct cycle sequencing was performed using ABI BigDye reagents and one of the original PCR primers. Products were analysed on an ABI 3100 automated sequencer. Many individuals were sequenced more than once for each locus to either improve the final sequence or confirm the original sequence. This involved a combination of repeat sequencing, reverse direction sequencing, and sequencing from additional PCR products, depending on initial results.

2.3.4 Bioinformatics

All sequences were initially screened for quality and erroneous base calls using Sequencher software. Species comparisons and identification were then carried out in a variety of ways. Initially, sequences were BLASTed against the GenBank DNA database to check for exact or close matches with species

already in the public databases. Using those preliminary results, sequences were then aligned (using ClustalX) with sequences of the same phylum from other plankton and reference adults from this project, as well as all relevant reference sequences from GenBank. 16S alignments used the reference European Ribosomal Database alignments based on secondary structure (Wuyts et al. 2004). Kimura 2-parameter nucleotide distances, neighbour-joining trees and bootstrapping were then undertaken with PAUP*.

2.3.5 Databases

The final databases comprise both photo files and DNA sequence files (see listing of electronic appendices in Appendix). The photo description files are Excel files that list the date, photograph number, larval type and sample from which it was sorted, magnification and other notes, and are cross-referenced with the DNA sequences (see Excel file for detailed descriptions). All photos are provided as jpeg files with identifying codes. The DNA sequence summary files (one for reference adults and one for meroplankton) are Excel files that list all identified (and unidentified) individuals, both adult and larval, their sampling details, and identifying characteristics. These characteristics include the DNA sequence loci, the match probabilities with the allocated taxon, and genetic similarity from the most closely related sequenced species. All DNA sequences (including all reference public database sequences) used in the analyses are provided in a series of files of aligned sequences within taxon groups. All sequence files are text files, readable and interpretable in any text editor (e.g., Word). All databases are included in the electronic appendices.

The electronic appendices and databases are provided on 5 accompanying CDs: a “Database” CD and four Photo file CDs. The details of all files and which CD they appear on, as well as the number of records and the data fields are provided in the appendix. The key summary files are Excel files that list all individuals and their details for morphological or sequence analyses. Each of these files has as its first worksheet an explanation of the structure of that database. Each adult or plankton individual has a unique identifying number that can be cross-referenced among all databases and all phylogenetic trees presented in this report. For example, each adult has an identifying number (ID#) of the form “A02.01” and each meroplankton individual an ID# of the form “D5.A1”. The DNA sequencing, photographs and morphological analysis for any specific adult or plankton individual can be found within each database by searching for this unique ID#, and all databases are cross-referenced by this ID#. For example, if one wished to find all sequences and photos of plankton individual “D7.L5” – the first brittle star plankta found on the tree in Fig. 7 – one would look in file “plankton sample+results.xls” for sequence information on sheet D7 on row L5; for photos one would look in “PhotosCrossRefDNA.xls” and search for D7.L5, where all photos taken of this individual would be listed with their file names.

2.4 Statistical analysis

Statistical analysis could be performed only on the larval samples collected from the McMurdo Runway site. At this site we had a balanced design with the larval numbers quantified from three plankton samples on each of the 21 sampling days. For statistical analysis the sorted larvae were combined into 13 morphological larval “types” to reduce the number of zero cells in the data matrix. The sampling design used here is a simple 1-way ANOVA with the fixed factor Day and three plankton sample replicates per day. Abundances of all larval types were first analysed in a non-parametric MANOVA using the Fortran program NPMANOVA with a $\ln(x+1)$ transformation and using the Bray-Curtis dissimilarities and 9999 permutations (Anderson 2001, 2003, McArdle & Anderson 2001). Parametric univariate analyses were also performed on abundant larval types (defined as larvae that were present in more than 30% of the plankton samples) using SAS v.9.0. Natural log transformations $[\ln(x+1)]$ were made before analysis and the assumptions of analysis of variance checked with Brown-Forsythe (homogeneity of variance) and Shapiro-Wilk’s (normality) tests as outlined in the SAS documentation. Further analysis of the larval abundance data was by a

principal co-ordinate analysis on the $\ln(x+1)$ transformed abundances of the larval types using the Fortran programme PCOORD (Anderson 2000).

3. RESULTS

3.1 Meroplankton larval types from the McMurdo Runway site

After excluding the holoplankton fraction that was dominated by copepods, 499 marine invertebrate larvae were collected during the 21 days of sampling at the McMurdo Runway site (Figure 2A). Except on 25 November, the mean number of larval forms per sample was greater than 4; with a maximum of 21 larvae per sample (Figure 2B). No larval fish were collected, perhaps due to the slow rate of hauling of the plankton net. There was no significant difference in total larval numbers between sampling days (1-way ANOVA: $F = 1.16$, d.f. = 20,42; NS) and only one of the 63 samples collected had no larvae present.

The most abundant larval “type” collected was from the Phylum Annelida, comprising 54.9% ($n = 274$) of the collected larvae (Figure 2). These larvae were all from the Class Polychaeta and included a range of developmental stages (metatrochophores; Plate 10A, 10B, ≤ 2 setiger larvae, Plate 1E, and nectochaetes; Plate 10C, 10D, 10F–H, Plate, 11B–D, 11G, 11H). Over 90% of the nectochaetes were initially identified as spionids ($N = 121$), although there were at least three obvious morphological “types” of nectochaetes that differed in the length of the setae. The nectochaete category was often very abundant (Figure 3: 2 December); however, as with the total numbers, there was no significant difference in mean nectochaete numbers throughout the period of sampling (1-way ANOVA: $F = 1.33$, d.f. = 20,42; NS). In contrast, the earlier developmental stages (metatrochophores and ≤ 2 setiger larvae combined) showed significant differences in numbers throughout the sampling period (1-way ANOVA: $F = 4.26$, d.f. = 20,42; $p < 0.001$). No ≤ 2 setiger larvae were seen before 27 November and they remained in low numbers after this date, peaking with a mean of 5.67 per sample on the final sampling day (Figure 3).

About 17% ($N = 85$) of the larvae collected were from the Phylum Mollusca (see Figure 2). These included large numbers of a nudibranch veliger (60/499 larvae; Plate 9E–H), small unidentified veligers (Plate 1F), a small gastropod, and larger shelled gastropod veligers (echinospira) of two sizes (Figure 3; Plate 8). The small veligers (Plate 1F) were observed in late November and then again in the last week of sampling, but the echinospira were found only in December (Figure 3). In contrast, nudibranch veligers were found on nearly all of the sample days (Figure 3) with no significant difference in numbers with time (1-way ANOVA: $F = 1.17$, d.f. = 20, 42; NS). The nudibranch veligers all appeared to be of the same morphological type.

A further 8.8% ($N = 44$) of the larvae were pilidia from the Phylum Nemertea (ribbon worms; see Figure 2). Three larval “types” were observed with different size and morphology (Plates 5–7), and one specimen was found with a metamorphosing juvenile. With the exception of the first two days, pilidia were found throughout the sampling period and there was no significant difference in mean numbers of pilidia with sample day (1-way ANOVA: $F = 0.28$, d.f. = 20,42; NS). Occasional nemertean juveniles (Plate 5A) and planuliform larvae (Plate 5B) were also seen in the plankton samples.

Four percent of the larvae ($N = 20$) collected were representatives of the Phylum Echinodermata (see Figure 2). Larvae of brittle stars (ophiopluteus; Plate 4D–F) and starfish (bipinnaria; Plate 3K–M) were seen in the samples (see Figure 3). Echinoderm larvae were found throughout the sampling period, but were always rare representatives of the meroplankton.

About 15% ($N = 76$) of the larvae observed have been grouped into a category egg/embryo (Figure 2). These include a range of developmental stages including unfertilised eggs, fertilised embryos, and early developmental stages (examples shown in Plate 1A–D, 1H). This category of the meroplankton was present in high numbers because of the use of a smaller mesh plankton net than in previous zooplankton sampling in McMurdo Sound. There was an overall significant difference in the numbers

of eggs/embryos throughout the sampling period (1-way ANOVA: $F = 1.95$, d.f. = 20,42; $p < 0.05$; Figure 3). However, *a posteriori* testing showed no particular days with statistically higher numbers of this category (Tukey test $\alpha = 0.05$).

3.2 Meroplankton community at the McMurdo Runway site

Multivariate analysis of variance of the meroplankton data collected from the McMurdo Runway site showed that there was an overall significant difference in the meroplankton community between days ($F = 1.7851$, $df = 20, 42$, $P = 0.0003$). However, subsequent pair-wise *a posteriori* comparisons showed no significant differences ($p < 0.05$) between any of the possible day combinations. Principal co-ordinates analysis was used to determine if there was any pattern of the larval numbers in two-dimensional space (Figure 4). Replicate plankton samples collected on the same calendar day varied widely in their distribution in multivariate space (Figure 4). There was no obvious clustering between consecutive days or any obvious trend through time. These results suggest that there is a generally similar meroplankton community in McMurdo Sound in the period sampled.

3.3 DNA sequence analysis

To meet the project criteria, we trialled a number of loci, including mitochondrial 12s and 16s ribosomal RNA, cytochrome oxidase I (COI) and cytochrome b (cytB) genes, and nuclear 18s and 28s ribosomal RNA and elongation factor 2 α genes. All these loci were amplified, but to different extents and reliability. Ultimately, the only loci that amplified sufficiently robustly to ensure a high proportion of success among the meroplankton were the loci 16s and COI. For comparative purposes, these were also very good choices, as these loci are the most represented in the public databases among invertebrates, and COI has recently been proposed as a universal “bar-coding” locus (Hebert et al. 2003). Although it was unfortunate that none of the nuclear loci proved to be as reliable as 16s and COI, this does not weaken our species discrimination or identification in the analyses. These two mitochondrial loci have proved to be at least as reliable (and probably more so) as any nuclear loci (Hebert et al. 2003).

Successful DNA sequences (of either 16s or COI) have been obtained from 47 adult Antarctic benthic marine invertebrates, representing 24 species (Table 1). Most species are represented by both 16s and COI sequences (see Table 1). Of the meroplanktonic larvae collected for DNA analysis from both the McMurdo Runway site and the Scott Base water intake, about 500 individuals had DNA extracted and were subjected to PCR of both 16s and COI loci (Table 2). About 350 of these individuals produced clean PCR amplifications of the expected size (suitable for sequencing) for one or both loci during the single-pass PCR mass-screening protocol, resulting in a grand total of about 500 DNA sequences of various quality and length (Table 2)(For details, see electronic appendix).

We had some difficulty obtaining suitable PCR amplification and DNA sequence from some of the larval samples collected early in the season (November). This is most likely a result of the additional time spent in larval handling (including removal from the plankton sample, preparation for digital photography, cleaning contaminating material) before preservation of the larva in ethanol. Handling routines were improved during the course of field work as we adapted to the relatively primitive laboratory conditions of the Summer Laboratory, and we also introduced steps to keep the specimens at low temperatures during the handling process. Although not apparent at the time, larval samples collected later in the season resulted in more reliable final PCR amplifications, suggesting that minimising both handling time and storage temperature before preservation assists in maximising the proportion of successful results from meroplankton samples.

The primary results used for larval identification (all of which are included in the phylogenetic trees of Figures 5–12) were the complete set of 16s and COI DNA sequences that were suitable for high-volume bioinformatic analysis; that is, they were very clean, required little post-processing, and provided unambiguous results. Overall, the 16s locus provided suitable PCR amplification and DNA

sequences from a higher proportion of plankton, and ultimately required less bioinformatic processing to permit taxonomic identification. DNA sequence from the COI locus was largely obtained from only a subset of the individuals for which 16s sequence was obtained. No individuals from which both loci have been examined have yielded contradictory taxonomic classifications. Obviously the specificity of taxonomic identification varies between loci for different taxonomic groups due to their degree of representation in databases such as GenBank. The 16s sequences always provided more detailed species identification and phylogenetic relationships for the specimens in our collection, due to the greater number of 16s sequences in the public databases (GenBank). The more limited reference data for the COI gene (from both our adults and databases) meant that species relationships and identification were not as accurate as those determined by the 16s data, although no conflicts were detected. The considerably fewer reference COI sequences meant that the phylogenetic trees constructed from COI data may provide a misleading (and incorrect) image that there are conflicts between 16s and COI datasets. As such, only the more accurate 16s phylogenetic trees are provided in this report, although all sequence data and trees for both loci are provided in the accompanying databases (see electronic appendices). Ultimately, the main value of the COI data was to confirm the distinction of MOTUs (molecular operational taxonomic units – i.e., presumed species), and in some cases to confirm species identification. Thus, all identifications are based on both genes where the data exist. Even where identifications are based on only one gene, we do not consider this to be problematic. Each gene has enough identification power on its own to ensure species identification certainty (Hebert et al. 2003). The primary advantage accruing from the use of multiple genes comes from the benefit of multiple independent tests where possible (data from each gene provide an independent test of the identification of each specimen), that is, the use of multiple genes provides a check on any possible systemic errors that may occur (e.g., sequence mis-labelling).

The initial expectation, based on previous work in McMurdo Sound (Sewell, unpublished data), was that we would collect a number of characteristic larval types from the plankton samples and that representative specimens would be preserved for either DNA or morphology (in ethanol and formalin respectively). When the initial DNA sequencing was completed we found that in some groups, especially the polychaetes and the nemertean pilidia, that the same DNA sequence could be represented by a number of morphological forms. This meant that we could not assume that a particular DNA sequence always represented only a single morphological/larval type. It also made it impossible to obtain both a DNA sequence (from ethanol preserved specimen) and line drawings/SEM (from formalin preserved specimen), as anticipated in the original proposal, from the same larval form. We were, however, able to provide high-quality digital photographs of the same specimens that were used in the DNA analyses, which is a significant advantage. Although all reliable DNA sequences are reported in the phylogenetic trees, in our discussion we can make the strongest conclusions about those specimens for which we have both a good high resolution digital photograph (clear, in focus, at appropriate magnification), and a reliable DNA sequence. It is only for these 161 specimens that we can be 100% certain of our matching of morphological type and DNA, and subsequently of our species-matching.

3.3.1 Phylum Echinodermata

3.3.1.1 Class Asteroidea

Adult DNA sequences were obtained from 12 species of starfish (Figure 5), *Macroptychaster accrescens*, *Porania antarctica*, *Porania* sp., *Psilaster charcoti*, *Pteraster affinis*, *Perknaster fuscus*, *Lophaster gaini*, *Diplasterias brucei*, *Acodontaster hodgsonii*, *Acodontaster conspicuous*, *Odontaster validus*, and *Odontaster meridionalis* (see Table 1). In most cases both 16s and COI sequences were obtained (see Table 1). Samples of tube feet were taken from two adults from McMurdo Sound that could not be identified to species at the time by asteroid experts. It was believed that they were different, or perhaps new, species. We obtained sequences from these two unknown adults, and they were identified as *Lophaster gaini* and *Diplasterias brucei* (Figure 5). This is a clear example of how molecular taxonomic identification can be extremely useful when within-species morphological polymorphism clouds conventional taxonomic identification.

We obtained 24 DNA sequences from 21 individuals from the plankton samples which fell into two asteroid groupings (Table 2, Figure 5).

(i) DNA sequences obtained from three eggs of an unknown species of asteroid. These sequences did not match any of the Antarctic adult starfish sequenced during this study. The only sequences in GenBank with any similarity are from the Family Ophidiasteridae, which are primarily found in the tropical Atlantic and Pacific. It is expected, therefore, that these eggs might be derived from another family within the Order Valvatida. Two of the eggs obtained from the McMurdo Runway sampling site were within fertilisation membranes and were 445 μm in diameter (Plate 1A, 1B). The other egg was obtained from the Scott Base water intake and was unfertilised and with a smaller diameter (233 μm , Plate 1C).

(ii) Various embryo and larval stages of the common Antarctic asteroid *Odontaster validus* were observed in samples from the McMurdo Runway site (N = 2) and from the Scott Base water intake (N = 16; total N = 18 individuals, represented by 21 sequences). These fell into the following categories:

- (a) Embryos were collected in the first two weeks of December. Stages included eggs within fertilisation membranes (Plate 2A–F), 4- and 8-cell stages (Plate 2G, 2I) and gastrula (Plate 2H, 2J). All embryos were collected from the Scott Base water intake (N = 16). Early stage embryos of *Odontaster* were unexpected during December as this species has a spawning season in McMurdo Sound of June to mid September (Pearse 1965). However, it cannot be confirmed whether these were viable embryos or eggs remaining in apparently fresh condition from spawning during the previous winter.
- (b) Bipinnaria – an early stage bipinnaria was collected on 30 November (Plate 2K, 2M), and a more advanced bipinnaria was collected on the 3 December (Plate 2L), both at the McMurdo Runway sampling site.

3.3.1.2 Class Echinoidea

DNA sequence was obtained from a single adult species of echinoid, the common Antarctic sea urchin *Sterechinus neumayeri*. Sequences were obtained from both 16s and COI (see Table 1). We obtained nine individual DNA sequences from the plankton samples that were identified as echinoids. These fell into two groupings (Table 2, Figure 6).

(i) A large unfertilised egg, 585 μm in diameter (Plate 1D), that was not closely related to any sea urchin sequence recorded in GenBank. The most closely related sequences were from regular sea urchins, and, because of its large size, this egg is likely to be derived from a regular sea urchin with lecithotrophic development.

(ii) Eight embryos of *Sterechinus neumayeri* (Plate 3). These embryos were collected between 29 November and 3 December at both the McMurdo Runway site (N = 4) and from the Scott Base water intake (N = 4). Stages collected included blastula (Plate 3A–3D) and multicell embryos (Plate 3E–3G). Collection of these embryos is within the same time period as Bosch et al. (1987) reported early embryological stages of *S. neumayeri* in the plankton (late November to early December). No larval stages (echinoplutei) of *S. neumayeri* were found.

3.3.1.3 Class Ophiuroidea

DNA sequence was obtained from adults of two species of ophiuroid, the euryalinid *Astrotoma agassizii*, and an immature ophiurid, tentatively identified as *Ophionotus victoriae* (see Table 1). We obtained 11 DNA sequences from 8 individuals from the plankton samples that were identified as ophiuroids. These fell into two groupings (Table 2, Figure 7).

(i) Three large unfertilised eggs of *Astrotoma agassizii* (Plate 4A–C), represented by five sequences. These eggs were collected in the late November – early December period, with one egg from the McMurdo Runway site (Plate 4C) and two eggs from the Scott Base water intake (Plate 4A, 4B). The eggs were large (over 450 μm) and orange.

(ii) Five ophioplutei (represented by six sequences) of an unknown species of ophiuroid in two developmental stages: early (Plate 4D, 4E) and later stage ophioplutei (Plate 4F). There was some sequence similarity between these ophioplutei and the immature ophiurid described above, but the closest, though not identical, species match was with the *Ophiura signata* sequence in GenBank (ca. 1% difference in sequence in 16s). It is therefore likely that these ophioplutei are derived from one of the three *Ophiura* species in the Ross Sea (Fell 1961). The ophioplutei were all collected from the McMurdo Runway site and were small. The posterolateral arm length of the later stage ophioplutei was about 135 μm .

3.3.2 Phylum Nemertea

DNA sequence was obtained from a single adult species of nemertean, the large and numerically abundant *Parborlasia corrugatus*. This sequence was confirmed using the recent addition of a *Parborlasia* sequence to the GenBank database during the period of study and we obtained both 16s and COI sequences from a number of individuals (see Table 1). We obtained 49 individual DNA sequences from the plankton samples that were identified as nemerteans. These were collected from both the McMurdo Runway site and from the Scott Base water intake and fell into five groupings (Table 2, Figure 8).

(i) A nemertean found in the water column at the McMurdo Runway site on 29 November was identified as *Parborlasia corrugatus* (Plate 5A). As *P. corrugatus* has an indirect life cycle with a pilidia stage (Peck 1993), this is probably a metamorphosed juvenile. Nemertean juveniles have a nondescript morphology and were extremely difficult to photograph as they were not anaesthetised in the alcohol which was necessary for DNA preservation. This individual was about 430 μm long with few distinguishing features. No pilidia of *P. corrugatus* were collected in the plankton samples.

(ii) A nemertean with a high degree of sequence similarity to the palaeonemertean *Procephalothrix* sp. based on sequence in the GenBank database (Plate 5B) was collected on 3 December at the McMurdo Runway site. In morphology this individual is similar to the direct developing planuliform larvae of other palaeo- and hoplonemerteans (Norenburg & Stricker 2002), with a distinctive central gut and 578 μm long.

(iii) Eight individual pilidia that are related to the heteronemertean genus *Parvicirrus* based on sequence in the GenBank database. These specimens were collected at both the McMurdo Runway site and from the Scott Base water intake in late November and early December. They had a prominent apical tuft and there appeared to be two developmental stages present. The smallest pilidia, and presumably the earliest developmental stages, were less than 600 μm in size (Plate 5D–5F). A single pilidia, 760 μm in body height, had more extended development of the lateral lobes (5G).

(iv) Two individual pilidia that are related to the heteronemertean genus *Cerebratulus* based on sequence in the GenBank database. These specimens were both collected at the McMurdo Runway site in late November and early December. The anterior lobe of the pilidia, (Plate 5C, left) is extended, and the pilidia are about 580 μm in body height.

(v) Thirty seven individual pilidia from a single species that is related to the heteronemertean genus *Lineus geniculatus* based on sequence in the GenBank database. These specimens were collected at both the McMurdo Runway site and from the Scott Base water intake in late November and early December. Again there were a number of distinct developmental stages (Plates 6B–H and 7A–H). Pilidia in this grouping were the largest seen during the period of sampling, regularly being over 1 mm long. The shape and size of the anterior lobe differed between specimens, at times resembling an

elephant's trunk. However, there was no obvious morphological feature that distinguished pilidia in this grouping that could be used for consistent "species" identification.

The 47 pilidia collected with affinities to heteronemertean sequences in GenBank (*Parvicirrus*, *Cerebratulus*, and *Lineus*) are all in the Family Lineidae that contains *Parborlasia corrugatus* (Tholleson & Norenburg 2003). This is an abundant nemertean family in Antarctica, including 21 species south of latitude 50 °S (Gibson 1985).

3.3.3 Phylum Mollusca

DNA sequence was obtained from six adult molluscs: the scallop *Adamussium colbecki*, the bivalve *Laternula* sp., three shelled gastropod species (*Neobuccinum eatoni*, *Tritonella* sp., *Marseniopsis* sp.) and a nudibranch (*Austrodoris* sp.). For all species we obtained both 16s and COI sequence, with the exception of *Laternula* sp. where no COI sequence was obtained (see Table 1). Additionally we sequenced two species of planktonic pteropod mollusc (*Clione antarctica* and *Limacina* sp.; Figure 9). Due to the small size of the shelled pteropod *Limacina* sp., we processed this species as per the plankton samples and obtained both 16s and COI sequence (see Table 1). We obtained 41 individual DNA sequences from the plankton samples that were identified as molluscs – all were gastropods (Table 2). Three phylogenetic trees are presented showing these larval sequences in relation to all mollusc sequences on GenBank (Figure 9), with two more detailed trees including all the gastropod sequences from the Suborder Hypsogastropoda (Figure 10) and all the nudibranch sequences in GenBank (Figure 11).

3.3.3.1 Class Gastropoda

The 41 DNA sequences obtained from the plankton samples fell into four groupings (Figure 9, 10, 11).

(i) A species of gastropod represented by four individual sequences that clearly belongs to the Suborder Hypsogastropoda, but is not closely related to any of the sequences from this Suborder in GenBank (Figure 9, 10), or from the adult collections (see Table 1). These larvae have a characteristic form where the larval shell, which eventually develops into that of the adult, is covered by a secondary shell (Plate 8A–8D) which is known as echinospira or scaphoconch (Lebour 1935, McCloskey 1973). In this species the echinospira appeared smooth and was usually clear of debris (Plate 8A–8D). The dimensions of the shell within the egg case were 560 µm (Plate 8A), 600 µm (Plate 8B), 1.22 mm (Plate 8C), and 725 µm (Plate 8D); perhaps different developmental stages of the same species. These individuals were all collected from the Scott Base water intake in early December. The source of these larvae is currently unknown. However, as echinospira are found in some, but not all, members of the superfamilies Lamellariacea, Cypraeacea, and Calyptraeacea (McCloskey 1973), these larvae are likely derived from an Antarctic species of one of these families.

(ii) Seven individuals of a second species of gastropod (represented by nine DNA sequences) that clearly belongs to the Suborder Hypsogastropoda, but is not closely related to any of the sequences from this Suborder in GenBank (Figure 9, 10), or from the adult collections (see Table 1). As described above, the larval shell was covered by a secondary shell, but, in contrast, to gastropod species (i) the exterior of this egg case was often covered with debris and the larval shell edge had sculpture (Plate 8E–8H). The dimensions of the shell within the egg case were again a range of sizes (Plate 8). Individuals of this species were collected from both the McMurdo Runway site and the Scott Base water intake in early December. The source of these larvae is currently unknown.

(iii) Six individual (represented by nine DNA sequences) of a molluscan trochophore of an unknown species (Plate 9A–D) with no close sequence similarity to any of the gastropod sequences in GenBank (Figure 9, 10), or from the adult collections (see Table 1). All these trochophores were obtained from the Scott Base water intake during early December.

(iv) Nineteen individual sequences of a nudibranch veliger (Plate 9E–H) that was related to the aeolid nudibranch *Eubranchus* (Figure 9, 11). The nudibranch larval sequence was not that of the nudibranch sequenced in the adult samples (*Austrodoris*; Figure 12). The length of the shell was about 325–350 μm and they were distinguished by two distinct eyespots, the extended larval velum and foot, and prominent lipid globules at the posterior (Plate 9E–H). Nudibranch veliger sequence was obtained from samples taken at the McMurdo Runway site and the Scott Base water intake in late November and early December.

3.3.3.2 Class Bivalvia

A single bivalve juvenile was collected from the Scott Base water intake on 2 December (Plate 1G). Repeated attempts to obtain DNA sequence were unsuccessful, perhaps because of the delay in sample preservation due to an extended period of digital photography. This juvenile appears similar to one of the bivalves depicted by Stanwell-Smith et al. (1997, p. 82).

3.3.4 Phylum Annelida

3.3.4.1 Class Polychaeta

The polychaetes were the most problematic group in terms of the objectives to be attained in this research. For DNA sequencing the samples needed to be preserved in ethanol, which did not sufficiently anaesthetise the polychaetes for digital photography. Thus, often there was some physical damage to the polychaete and for many specimens only poor quality photographs were obtained. No adult polychaetes were sequenced in this study. We obtained 27 sequences from polychaete embryos and larvae and found nine groups of DNA sequences. None of these sequences were closely related to any sequences from GenBank. This phylum has particularly diverse 16s sequences and is so far poorly represented in the public DNA databases. These sequences should help resolve those unknown species. The nine groups of sequences we found among our plankton samples (which are likely to represent distinct species) are shown in Figure 12.

(i) Two individual sequences from polychaete trochophores (Plate 10A, 10B). One of the individuals was 289 μm long with no setae (Plate 10A). The second, more advanced, metatrochophore was 311 μm long and had distinct setae up to 150 μm long (Plate 10B). Both individuals were collected from the Scott Base water intake.

(ii) A grouping of two nectochaetes with a very distinctive morphology (Plate 10C, 10D). Both were collected from the Scott Base water intake, but on two separate sampling days in December. These nectochaetes had larger parapodia with bunches of setae and extended palp processes which are a feature of the Family Spionidae. One of the individuals had its proboscis extended (Plate 10D).

(iii) A single sequence derived from a polychaete embryo collected from the McMurdo Runway site on 30 November (Plate 10E).

(iv) Two individuals (represented by three sequences) obtained from a nectochaete as shown in Plate 10F. This species was distinguished by the prominent parapodia (ca. 150 μm length) with very long (over 650 μm) setae. The presence of palps meant that these nectochaetes were also from the Family Spionidae. Both nectochaetes were collected at the McMurdo Runway site in mid December.

(v) A single individual (represented by two sequences) obtained from a spionid nectochaete as shown in Plate 10G. As in the previous species, the nectochaete had enlarged parapodia (ca. 65 μm) with large bunched setae up to 780 μm long. This species was collected at the McMurdo Runway site on 7 December.

(vi) Three sequences obtained from a 2-setiger polychaete (not shown) and two nectochaetes (Plate 10H). The parapodia were about 50 μm long with short setae up to 160 μm long (Plate 10H). This species was collected at the McMurdo Runway site and from the Scott Base water intake in early December.

(vii) Eight sequences obtained from an embryo (Plate 11A) and seven nectochaetes (Plate 11B–D). The embryo (120 μm in length) was inside an egg case 224 μm in diameter (Plate 11A). The nectochaetes had setae up to 300 μm long (Plate 11B) and possessed palps (Plate 11D) and thus were spionids. This species was collected at both the McMurdo Runway site and from the Scott Base water intake in early December.

(viii) Three sequences obtained from polychaete embryos. The embryos (Plate 11E, 11F) were from a number of developmental stages and were distinguished by the sculptured egg case and green embryo. The earliest embryo was 123 μm in diameter within an egg case 287 μm in diameter (Plate 11E). The more advanced embryo was 166 μm long within an egg case 234 μm in diameter (Plate 11F). All three embryos were collected from the McMurdo Runway site in late November.

(ix) Three sequences obtained from polychaete nectochaetes with long setae (Plate 11G, 11H). The setae were easily broken (missing in Plate 11H) and about 950 μm long (Plate 11G). They were collected from both the McMurdo Runway site and from the Scott Base water intake in early December.

3.3.5 Phylum Chordata

3.3.5.1 Class Osteichthyes

Although no larval fish were collected from the plankton samples, we have compiled a list of DNA sequences from all available Antarctic fish species. This list is included in our database of reference sequences (see electronic appendices).

4. DISCUSSION

We have clearly shown that there is a high degree of taxonomic biodiversity in the meroplankton component of the McMurdo Sound plankton. We found that the meroplankton community commonly includes representatives from at least four invertebrate phyla, with the polychaete annelids being numerically the most abundant. Additionally, in a single sample we often found polychaete embryos and later stages (2-setigers and nectochaetes), nemertean pilidia, nudibranch veligers and, more rarely, echinoderm and mollusc larvae. The meroplanktonic community, in general, mirrors that observed by Foster (1987, 1989a, 1989b) in McMurdo Sound with high numbers of polychaetes and nemerteans and low numbers of asteroid larvae. However, some larval forms collected by Foster (1987, 1989a) were not found in the current sampling. Specifically, this sampling did not collect the rarely found cirripede larvae (Foster 1987, 1989b), decapod crustacean larvae (Foster 1987, 1989a), or hydrozoan actinulae (Foster 1989a). Several previously undescribed members of the meroplankton community in McMurdo Sound during November–December (Foster 1987, 1989a) have, however, been quantified in the present study. These include a number of smaller larval forms (nudibranch veliger, ophioplutei, 2-setiger polychaetes, eggs/embryos), that are most likely present due to the use of a smaller mesh plankton net (100 μm) than in previous zooplankton studies (reviewed by Sewell 2005).

Although the plankton sampling was conducted at a single site near McMurdo Station, a southward flowing current along the shore of Ross Island brings oceanic water into McMurdo Sound (see Figure 1: defined as the portion of the Ross Sea between Ross Island and the Victoria Land Coast; Knox (1981)) from the southeastern Ross Sea (Littlepage 1965, Barry & Dayton 1988). At Cape Armitage, near Scott Base (see Figure 1), the mean current speed in November–December is reported as 9.9 cm/s (Littlepage 1965) and 4.3 cm/s in the summer (Barry & Dayton 1988). Although there are diurnal and

tidally based currents in this area (Littlepage 1965, Barry & Dayton 1988) if, for simplicity, we assume that this is a constant and unidirectional southward current at an intermediate value of 7.1 cm/s, then the water mass being sampled at a single location would have been about 6 km to the north 24 hours previously. Thus, during the 24 days of sampling we are likely to have been sampling waters derived from up to 150 km away, well beyond the geographical limits of McMurdo Sound (see Figure 1). Consequently, even though sampling was restricted to a single location, two lines of evidence point to this meroplankton sampling being representative of McMurdo Sound during the early summer. Firstly, as described above, a different water mass was being sampled each day; and secondly, there are general similarities in the meroplankton community to that described from other parts of McMurdo Sound (Cape Evans: Foster 1989a, Sewell 2005; Cape Royds: Foster 1989a, see Figure 1).

The total numbers of larvae in the meroplankton community of McMurdo Sound remained fairly stable throughout the November-December sampling period (see Figure 3), though the community representatives changed through time (e.g., increased numbers of 2-setiger larvae in December). Thus, our overall conclusion is that there is a well developed meroplankton community in McMurdo Sound during the early summer, supporting previous observations of taxonomically diverse meroplankton communities in the maritime Antarctic (Stanwell-Smith et al. 1999) and on the Antarctic Peninsula (Shreeve & Peck 1995), and providing further evidence for a high proportion of Antarctic marine invertebrates having pelagic development.

The absence of larval fish in the meroplankton collected in this study is likely related to a combination of the slow rate of hauling of the plankton net (ca. 2.4 m/s) and the low ichthyoplankton concentrations in McMurdo Sound (only a single fish larvae collected in ca 200 samples at Cape Armitage near Scott Base by Foster (1987); no fish larvae collected from the high volume of seawater sampled in the Scott Base water intake). Previous studies of the ichthyoplankton in the Ross Sea have used an EZ-NET BIONESS sampler consisting of 10 nets each with a mouth opening of 0.25 m² (Guglielmo et al. 1998), which can reduce the likelihood of net avoidance by larval fish. This type of net can be used only in ice-free conditions, which are not found in McMurdo Sound during the summer months (Knox 1981). The collapsible plankton net used in sampling had been previously field-tested in McMurdo Sound and had caught several larval fish (Sewell, unpublished data); thus we did not anticipate that there would be a sampling problem. However, it was important that for the time-series samples taken at the McMurdo Runway site that we maintained a constant hauling speed of the plankton net for two reasons: firstly, so that we could quantify larval numbers using the same sampling protocol and, secondly, because by increasing the rate of hauling of the plankton net we would have potentially damaged many of the delicate marine invertebrate larvae present. As we were interested in clean, undamaged, specimens to permit both clear morphological and molecular description, this study has therefore focused on the biodiversity of the invertebrate meroplankton.

The approach taken here using DNA sequencing for detailed study of the McMurdo Sound invertebrate meroplankton community has proved extremely fruitful. We have been able to discriminate a number of species, and to identify these either to species or a higher taxonomic level to a degree not possible from morphology alone. A major achievement of this study has been the taxonomic breadth of identifications. Although a number of previous studies have used molecular methods to identify marine species, only a handful have successfully managed to identify invertebrate planktonic larvae (Olson et al. 1991, Coffroth & Mulawka 1995, Bell & Grassle 1998, Evans et al. 1998, MaKinster et al. 1999, Martel et al. 2000, Hare et al. 2000, Comtet et al. 2000, Deagle et al. 2003). These studies have been largely successful in their identification aims, although their aims were fairly limited. Evans et al. (1998) and Deagle et al. (2003) used PCR techniques to detect the invasive northern Pacific seastar, *Asterias amurensis*, and discriminate it from its co-geners in Tasmanian waters and ballast water. Bell & Grassle (1998), MaKinster et al. (1999), Martel et al. (2000), Hare et al. (2000) and Comtet et al. (2000) all used a variety of DNA-based techniques (including DNA hybridisation, detection of correct-size PCR fragments, or RFLP (restriction fragment length polymorphism) comparison of PCR products) in order to detect and/or identify a single species. Olson et al. (1991) used DNA sequencing to discriminate two species of sea cucumber. Coffroth & Mulawka (1995) used RAPDs (random amplified polymorphic DNAs) to discriminate among five species of coral. Hare et al. (2000) developed species-specific PCR amplification primers to discriminate five

bivalve species. Each of these studies had a primary aim of identifying large numbers of plankton, but their aims were all of limited taxonomic breadth. Of these studies, all have used techniques to either detect the presence of one species, or to discriminate among a few already-characterised species. The present study, however, has been able to achieve molecular identifications across a very wide taxonomic range of invertebrates, and at the same time still manage to analyse a large number of specimens. This has been made possible by using high-throughput DNA sequencing techniques. Although these techniques have previously proved too expensive and time-consuming for routine large-scale analysis, increases in cost-efficiency and sensitivity have now made this a feasible approach. At present the costs are still comparatively high, but these are steadily decreasing, and given the considerably greater power of identification possible through a DNA sequence, the benefit/cost ratio is very favourable.

Further developments of these techniques could be undertaken for more specific goals in the Antarctic. For example, if it was required to merely identify the presence of a particular species, then presence/absence detection techniques could be developed (e.g., as in Martel et al. (2000) and MaKinster et al. (1999)). These could be streamlined in their efficiency in order to analyse very large numbers (thousands) of samples. Alternatively, if there was a need to identify and discriminate a few closely related species (e.g., as in Hare et al. (2000) and Deagle et al. (2003)), techniques that amplify different sized PCR products are likely to prove the most efficient for large numbers. It is possible now, particularly with the advent of robust quantitative-PCR protocols and instrumentation, that these types of molecular identifications could be given a quantitative element. That is, the relative numbers (or proportions) of different species could potentially be estimated by bulk analysis of crude, unsorted plankton samples, in a manner similar to that used for quantifying microbial loads (de Monbrison et al. 2003). Combining these techniques with automated plankton sampling, perhaps at different depths, may provide a very good comparison of the distributions and abundances of species over area, depth and time.

We encountered some early difficulties in the PCR amplification and DNA sequencing. The final protocols developed here and minor modifications to the field preservation of larval material will be implemented in future research on Ross Sea meroplankton. Specifically, it appears that one of the most critical aspects for DNA analyses of Antarctic meroplankton is the initial handling conditions of the larval specimens. In the relatively crude laboratory facilities available, it was not possible to undertake all usual standards of molecular biology techniques, or to rigorously test alternative protocols. However, it became clear that steps to minimise the handling time during photography and keeping the samples on ice for as much of the sampling process as possible appear to be critical factors in reliable PCR amplification and subsequent DNA sequencing.

Using a DNA sequencing approach to larval identification we have achieved three key results. Firstly, we have been able to match eggs/embryos and larvae to known adult Antarctic species. Examples include the embryos of the sea urchin *Sterechinus neumayeri*, the embryos and larvae of the starfish *Odontaster validus*, and the previously undescribed juvenile (Peck 1993) of the nemertean *Parborlasia corrugatus*. Secondly, we have been able to ascribe many larvae to higher taxonomic levels than species. Examples include the ophiopluteus that had a strong degree of similarity with the genus *Ophiura*, and the nudibranch veliger that is likely derived from an aeolid nudibranch. Thirdly, we have also been able to clarify identifications in previous research on Antarctic meroplankton. For example, some of the planula depicted by Stanwell-Smith et al. (1997; pp. 3–14) may in fact be the direct developing planuliform nemertean larvae shown in Plate 5B.

We have made significant progress in identifying the early summer meroplankton community of McMurdo Sound. The use of a smaller mesh-size plankton net than in previous studies resulted in the collection of high numbers of eggs and embryos (15% of the meroplankton at the McMurdo Runway site). Some of these have been identified to the species level. Others, such as the polychaete embryos, which are similar to that depicted by Stanwell-Smith et al. (1997) as “miscellaneous zooplankton” (p. 123) have been here identified to the class level. Using DNA sequencing to identify the source of eggs and embryos is, because of the problems involved in larval culture of Antarctic larvae with extended developmental times, a rapid, cost-effective way of determining which Antarctic marine invertebrates

have a pelagic larval stage, and gaining a better understanding of the timing of reproduction and spawning of individual species. For example, in the present study was the identification of *Odontaster validus* embryos in early December, outside the spawning season of June to September (Pearse 1965), or when *Odontaster validus* embryos are found in the plankton (June-September: Pearse & Bosch 1986, Stanwell-Smith & Clarke 1998).

The aim of the present research was to match embryo and larval forms collected from the plankton to adult benthic marine invertebrates. However, the observation that there can be numerous morphologically distinct larval forms, with the same DNA sequence, present in the plankton within a short time period makes this problematic. This problem is exemplified by the diverse range in both size and morphology of nemertean pilidia species (v) (Plates 6, 7). Extended plankton sampling and DNA sequencing is required to record all developmental stages of a single species. A different problem was encountered with the polychaete nectochaetes which were difficult to photograph before preservation for DNA sequencing. Groupings by DNA sequencing were then difficult to match to morphology. We, therefore, recommend that large nectochaetes be split after sorting: the head and half the body would be preserved for morphological examination in formalin and the remainder of the body preserved in ethanol for DNA sequencing.

An obvious constraint was the few Antarctic marine invertebrate sequences contained within GenBank and the difficulty of an extended sampling for adult marine invertebrates during a short research period in McMurdo Sound. It is recommended that future research on biodiversity of the Ross Sea should focus on collection and sequencing of the adults of marine invertebrates from which we found high larval diversity, particularly the Phylum Annelida, Class Polychaeta, and the Phylum Nemertea. Future research on the biodiversity of the Ross Sea meroplankton should also consider other time periods, particularly spring and late summer, and extend sampling into more oceanic parts of the Ross Sea. The current methods lends themselves to automated sampling protocols which could prove extremely useful in extending sampling in both space and time with minimal cost.

Finally, it should be noted that the methods developed here to allow PCR amplification and DNA sequencing of larval forms with no additional handling after sample collection can be readily adapted to other related applications, such as the study of marine larval diversity in temperate New Zealand waters. A further obvious application of these techniques is to biosecurity concerns. A major difficulty in marine biosecurity control is the timely identification of foreign larvae among the many organisms that can be carried in the ballast water and sea chests of vessels entering from foreign ports. These molecular techniques could easily be adapted to rapidly screen large numbers of organisms filtered from substantial volumes of water. The techniques could be adapted to target the specific problems of greatest concern, be they the detection of particular species, or the screening for broad taxa of concern.

In summary, this research has shown that it is both possible to molecularly identify individual planktonic larvae from the Ross Sea, and also feasible to do this in a mass-screening manner. Together with the accumulating morphological descriptions and increasing molecular and image databases, this now offers the ability to approach broader ecological and biodiversity questions about Ross Sea benthic invertebrate communities.

5. ACKNOWLEDGMENTS

Thanks are especially due to Marcus Cameron and Jennifer Jury for their cheerful assistance in the field and the laboratory, and to Antarctica New Zealand and the staff at Scott Base for logistics support and field assistance. Special thanks to Peter Wederall (ANZ, Scott Base engineer) for his enthusiastic support of the objectives of this research. Thanks to our colleagues for generously providing the adult specimens, and particularly Vonda Cummings, NIWA, who collected a wide range of invertebrate forms. DNA sequencing work was completed in the Molecular Ecology Laboratory, School of Biological Sciences, and in the School of Biological Sciences DNA Sequencing unit; thanks to K. Boxen and D. Steel for their assistance in the use of these facilities. This research was funded

under the New Zealand Biodiversity Strategy through the Ministry of Fisheries Biodiversity of the Ross Sea Programme (Contract ZBD2002-02).

6. REFERENCES

- Anderson, M.J. (2000). PCOORD: a FORTRAN computer program for principal coordinate analysis. Department of Statistics, University of Auckland.
- Anderson, M.J. (2001). A new method for non-parametric multivariate analysis of variance. *Australian Ecology* 26: 32–46.
- Anderson, M.J. (2003). NPMANOVA: a FORTRAN computer program for non-parametric multivariate analysis of variance (for any two-factor ANOVA design) using permutation tests. Department of Statistics, University of Auckland.
- Baldwin, B.S.; Black, M.; Sanjour, O.; Gustafson, R.; Lutz, R.A.; Vrijenhoek, R.C. (1996). A diagnostic molecular marker for zebra mussels (*Dreissena polymorpha*) and potentially co-occurring bivalves. *Molecular Marine Biology and Biotechnology* 5: 9–14.
- Barry, J.P.; Dayton, P.K. (1988). Current patterns in McMurdo Sound, Antarctica and their relationship to local biotic communities. *Polar Biology* 8: 367–376.
- Bell, J.L.; Grassle, J.P. (1998). A DNA probe for identification of larvae of the commercial surfclam (*Spisula solidissima*). *Molecular Marine Biology and Biotechnology* 7: 127–137.
- Bhaud, M.; Koubbi, P.; Razouls, S.; Tachon, O.; Accornero, A. (1999). Description of planktonic polychaete larvae from Terre Adélie and the Ross Sea (Antarctica). *Polar Biology* 22: 329–340.
- Bosch, I.; Beauchamp, K.A.; Steele, M.E.; Pearse, J.S. (1987). Development, metamorphosis, and seasonal abundance of embryos and larvae of the Antarctic sea urchin *Sterechinus neumayeri*. *Biological Bulletin* 173: 126–135.
- Bradford-Grieve J.M.; Fenwick, G. (2001). A review of the current knowledge describing the biodiversity of the Ross Sea region, p. 94. MFish ZBD 2000/01, Wellington.
- Clarke, A. (1992). Reproduction in the cold: Thorson revisited. *Invertebrate Reproduction and Development* 22: 175–184.
- Coffroth, M.A.; Mulawka, J.M. (1995). Identification of marine invertebrate larvae by means of PCR-RAPD species-specific markers. *Limnology and Oceanography* 40: 181–189.
- Comtet, T.; Jollivet, D.; Khripounoff, A.; Segonzac, M.; Dixon, D.R. (2000). Molecular and morphological identification of settlement-stage vent mussel larvae, *Bathymodiolus azoricus* (Bivalvia: Mytilidae), preserved *in situ* at active vent fields on the Mid-Atlantic Ridge. *Limnology and Oceanography* 45: 1655–1661.
- Deagle, B.E.; Bax, N.; Patil, J.G. (2003). Development and evaluation of a PCR-based test for detection of *Asterias* (Echinodermata : Asteroidea) larvae in Australian plankton samples from ballast water. *Marine and Freshwater Research* 54: 709–719.
- Epifanio, C.E.; Perovich, G.; Dittel, A.I.; Cary, S.C. (1999). Development and behavior of megalopa larvae and juveniles of the hydrothermal vent crab *Bythograea thermydron*. *Marine Ecology Progress Series* 185: 147–154.
- Evans, B.S.; White, R.W.G.; Ward, R.D. (1998). Genetic identification of asteroid larvae from Tasmania, Australia, by PCR-RFLP. *Molecular Ecology* 7: 1077–1082.
- Fell, H.B. (1961). The fauna of the Ross Sea. Part 1. Ophiuroidea. New Zealand Department of Scientific & Industrial Research Bulletin 42. 79p.
- Fenwick, G.; Bradford-Grieve, J.M. (2002). Recommendations for future directed research to describe the biodiversity of the Ross Sea region, p. 41. MFish ZBD 2000/01 Objective 2, Wellington. (Unpublished report held by Ministry of Fisheries, Wellington.)
- Folmer, O.; Black, M.; Hoeh, W.; Lutz, R.; Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.
- Foster, B.A. (1987). Composition and abundance of zooplankton under the spring sea-ice of McMurdo Sound. *Polar Biology* 8: 41–48.
- Foster, B.A. (1989a). Time and depth comparisons of sub-ice zooplankton in McMurdo Sound. *Polar Biology* 9: 431–435.

- Foster, B.A. (1989b). Balanomorph barnacle larvae in the plankton at McMurdo Sound. *Polar Biology* 10: 175–177.
- Garland E.D.; Zimmer, C.A. (2002). Techniques for the identification of bivalve larvae. *Marine Ecology Progress Series* 225: 299–310.
- Gibson, R. (1985). Antarctic nemerteans. Heteronemertea – descriptions of new taxa, reappraisals of the systematic status of the existing species and a key to the heteronemerteans recorded south of latitude 50 °S. *Zoological Journal of the Linnean Society* 83: 95–227.
- Guglielmo, L.; Granata, A.; Greco, S. (1998). Distribution and abundance of postlarval and juvenile *Pleuragramma antarcticum* (Pisces, Nototheniidae) off Terra Nova Bay (Ross Sea, Antarctica). *Polar Biology* 19: 37–51.
- Hare, M.P.; Palumbi, S.R.; Butman, C.A. (2000). Single-step species identification of bivalve larvae using multiplex polymerase chain reaction. *Marine Biology* 137: 953–961.
- Hebert, P.D.N.; Cywinska, A.; Ball, S.L.; deWaard, J.R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London, Series B* 270: 313–321.
- Hillis, D.; Moritz, C.; Mable, B. (1996). Molecular Systematics. Sinauer Associates, Inc., Sunderland, Massachusetts. 655p.
- Kirkwood, J.M. (1993). Zooplankton community dynamics and diel vertical migration in Ellis Fjord, Vestfold Hills, Antarctica. Unpublished PhD thesis, Monash University, Melbourne. 351p.
- Kirkwood, J.M.; Burton, H.R. (1987). Three new zooplankton nets designed for under-ice sampling; with preliminary results of collections made from Ellis Fjord, Antarctica during 1985. *Proceedings of the NIPR. Symposium on Polar Biology. 1*: 112–122.
- Knox, G.A. (1981). Biological oceanography of the Ross Sea (Extended abstract). *Journal of the Royal Society of New Zealand* 11: 341–347.
- Lebour, M.V. (1935). The echinospira larvae (Mollusca) of Plymouth. *Proceedings of the Zoological Society, London 1935*: 163–174.
- Li, G.; Hedgecock, D. (1998). Genetic heterogeneity, detected by PCR-SSCP, among samples of larval Pacific oysters (*Crassostrea gigas*) supports the hypothesis of large variance in reproductive success. *Canadian Journal of Fisheries and Aquatic Science* 55: 1025–1033.
- Lindstrom, D.P. (1999). Molecular species identification of newly hatched Hawaiian amphidromous goboid larvae. *Marine Biotechnology* 1: 167–174.
- Littlepage, J.L. (1965). Oceanographic investigations in McMurdo Sound, Antarctica. In: Llano, G. (ed.), Biology of Antarctic Seas, vol. II, *Antarctic Research Series* 2: 1–37.
- Martel, A.L.; Auffrey, L.M.; Robles, C.D.; Honda, B.M. (2000). Identification of settling and early postlarval stages of mussels (*Mytilus* spp.) from the Pacific coast of North America, using prodissoconch morphology and genomic DNA. *Marine Biology* 137: 811–818.
- MacBride, E.W. (1920). Echinoderma (Part II.) and Enteropneusta. Larvae of Echinoderma and Enteropneusta. *Natural History Report of the British Antarctic “Terra Nova” Expedition 1910 Zoology* 3:83–94.
- MacBride, E.W.; Simpson, J.C. (1908). Echinoderma. II. Echinoderm Larvae. *National Antarctic Expedition 1901-1904 (Natural History)* 4: 1–9.
- MaKinster, J.G.; Roberts, J.E.; Felder, D.L.; Chlan, C.A.; Boudreaux, M.; Bilodeau, A.L.; Neigel, J.E. (1999). PCR amplification of a middle repetitive element detects larval stone crabs (Crustacea: Decapoda: Menippidae) in estuarine plankton samples. *Marine Ecology Progress Series* 188: 161–168.
- McArdle, B.H.; Anderson, M.J. (2001). Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* 82: 290–297.
- McCloskey, L.R. (1973). Development and ecological aspects of the echinospira shell of *Lamellaria rhombica* Dall (Prosobranchia: Mesogastropoda). *Ophelia* 10: 155–168.
- Medeiros-Bergen, D.E.; Olson, R.R.; Conroy, J.A.; Kocher, T.D. (1995). Distribution of holothurian larvae determined with species-specific genetic probes. *Limnology and Oceanography* 40: 1225–1235.
- Mileikovsky, S.A. (1971). Types of larval development in marine invertebrates, their distribution and ecological significance: a re-evaluation. *Marine Biology* 10: 193–213.
- de Monbrison, F.; Angei, C.; Staal, A.; Kaiser, K.; Picot, S. (2003). Simultaneous identification of the four human *Plasmodium* species and quantification of *Plasmodium* DNA load in human blood

- by real-time polymerase chain reaction. *Transactions of the Royal Society of Tropical Medicine Hygiene* 97: 387–390.
- Murray, J. (1895). General observations on the distribution of marine organisms. Report of the Scientific Research Voyage of the H.M.S. Challenger. A summary of scientific results. pp. 1431–1462.
- Norkko, A.; Andrew, N.; Thrush, S.; Cummings, V.; Schwarz, A.-M.; Hawes, I.; Mercer, S.; Budd, R.; Gibbs, M.; Funnell, G.; Hewitt, J.; Goring, D. (2002). Ecology and biodiversity of coastal benthic communities in McMurdo Sound, Ross Sea: development of sampling protocols and initial results, p. 113. MFish ZBD2001/02 Objectives 1, 2 and 3, Wellington. (Unpublished report held by Ministry of Fisheries, Wellington.)
- Norenburg, J.L.; Stricker, S.A. (2002). Phylum Nemertea. In: Young, C.M.; Sewell, M.A.; Rice, M.E. (eds.), *Atlas of marine invertebrate larvae*, pp. 163–177. Academic Press, London.
- Olson, R.R.; Runstadler, J.A.; Kocher, T.D. (1991). Whose larvae? *Nature* 351: 357–358.
- Page, M.J.; Alcock, N.; Gordon, D.; Kelly-Shanks, M.; Nelson, W.; Neill, K.; Watson, J. (2002). Preliminary assessment of the biodiversity of benthic macrobiota of the western Ross Sea, Antarctica, p. 47. MFish ZBD 2000/02, Wellington. (Unpublished report held by Ministry of Fisheries, Wellington.)
- Palumbi, S. (1996). Nucleic acids II: the polymerase chain reaction. In: Hillis, D.M.; Moritz, C.; Mable, B.K. (eds.). *Molecular systematics*, pp. 205–246. Sinauer Associates, Inc., Sunderland, Massachusetts.
- Pearse, J.S. (1965). Reproductive periodicities in several contrasting populations of *Odontaster validus* Koehler, a common Antarctic asteroid. *Antarctic Research Series* 5: 39–85.
- Pearse, J.S. (1994). Cold-water echinoderms break ‘Thorson’s rule’. In: Eckelbarger, K. J.; Young, C.M. (eds.). *Reproduction, larval biology and recruitment in the deep-sea benthos*, pp. 26–43. Columbia University Press, New York.
- Pearse, J.S.; Bosch, I.S. (1986). Are the feeding larvae of the commonest Antarctic asteroid really demersal? *Bulletin of Marine Science* 39: 477–484.
- Pearse, J.S.; McClintock, J.B.; Bosch, I.S. (1991). Reproduction of Antarctic benthic marine invertebrates: tempos, modes and timing. *American Zoology* 31: 65–80.
- Peck, L.S. (1993). Larval development in the Antarctic nemertean *Parborlasia corrugatus* (Heteronemertea: Lineidae). *Marine Biology* 116: 301–310.
- Regan, C.T. (1916). Larval and postlarval fishes. 1. Antarctic and subantarctic fishes. *Natural History Report of the British Antarctic “Terra Nova” Expedition 1910 Zoology I*: 125–156.
- Sewell, M.A. (2005). Examination of the meroplankton community in the south-western Ross Sea, Antarctica, using a collapsible plankton net. *Polar Biology* 28: 119–131.
- Shreeve, R.S.; Peck, L.S. (1995). Distribution of pelagic larvae of benthic marine invertebrates in the Bellinghausen Sea. *Polar Biology* 15: 369–374.
- Shilling, F.M.; Manahan, D.T. (1994). Energy metabolism and amino acid transport during early development of Antarctic and temperate echinoderms. *Biological Bulletin* 187: 398–407.
- Stanwell-Smith, D.; Hood, A.; Peck, L. (1997). A field guide to the pelagic invertebrate larvae of the maritime Antarctic. British Antarctic Survey, Cambridge. 152p.
- Stanwell-Smith, D.; Clarke, A. (1998). Seasonality of reproduction in the cushion star *Odontaster validus* at Signy Island, Antarctica. *Marine Biology* 131: 479–487.
- Stanwell-Smith, D.; Peck, L.S.; Clarke, A.; Murray, A.W.A.; Todd, C.D. (1999). The distribution, abundance and seasonality of pelagic marine invertebrate larvae in the maritime Antarctic. *Philosophical Transactions of the Royal Society of London B* 354: 471–484.
- Steedman, H.F. (1974). Laboratory methods in the study of marine zooplankton. *Journal du Conseil, Conseil International Pour l’Exploration de la Mer* 35: 351–358.
- Tholleson, M.; Norenburg, J.L. (2003). Ribbon worm relationships: a phylogeny of the phylum Nemertea. *Proceedings of the Royal Society of London B* 270: 407–415.
- Thomson, C.W. (1878). Notice of some peculiarities on the mode of propagation of certain echinoderms in the southern seas. *Journal of the Linnean Society of London - Zoology*. 13: 55–79.
- Thomson, C.W. (1885). Notes on the reproduction of certain echinoderms of the southern oceans. *Report of the Scientific Research Voyage of the H.M.S. Challenger*. Vol. 1, Narrative, pp. 379–396.

- Thorpe, J.P.; Solé-Cava, A.M. (1994). The use of allozyme electrophoresis in invertebrate systematics. *Zoologica Scripta* 23: 3–18.
- Thorson, G. (1936). The larval development, growth and metabolism of Arctic marine bottom invertebrates compared with those of other seas. *C. A. Reitzels Forlag, Kobenhavn, Meddelelser om Gronland udgivne af kommissionen for videnskabelige undersogelser i Gronland, Bd. 100, Nr. 6, Treaarsexpeditionentil Christian Den X's Land 1931-34 under Ledelse af Lauge Koch. 100: 1–155.*
- Thorson, G. (1950). Reproduction and larval ecology of marine bottom invertebrates. *Biological Review* 25: 1–45.
- Waterhouse, E.J. (2001). Ross Sea Region 2001: A state of the environment report for the Ross Sea region of Antarctica, p. 115. New Zealand Antarctic Institute, Christchurch.
- Werle, E.; Schneider, C.; Renner, M.; Volker, M.; Fiehn, W. (1994). Convenient single-step, one tube purification of PCR products for direct sequencing. *Nucleic Acids Research* 22: 4354–4355.
- Wuyts, J.; Perriere, G.; de Peer, Y.V. (2004). The European ribosomal RNA database. *Nucleic Acids Research* 32: D101–D103.

Table 1: Number of DNA sequences and individuals sequenced for 16s and COI from adult Antarctic benthic marine invertebrates. Species are represented in the table in the order that they are discussed in the text.

	Total no. individs. collected	No. final sequences	16s No. individs. sequenced	No. final sequences	COI No. individs. sequenced
PHYLUM					
ECHINODERMATA					
Class Asteroidea					
<i>Macroptychaster accrescens</i>	1	2	1	1	1
<i>Porania antarctica</i>	1	0	0	0	1
<i>Porania</i> sp.	1	2	1	0	0
<i>Psilaster charcoti</i>	2	4	2	2	1
<i>Pteraster affinis</i>	1	2	1	1	1
<i>Perknaster fuscus</i>	1	2	1	1	1
<i>Lophaster gaini</i>	2	4	2	1	1
<i>Diplasterias brucei</i>	4	8	4	0	0
<i>Acodontaster hodgsonii</i>	1	2	1	1	1
<i>Acodontaster conspicuous</i>	1	2	1	1	1
<i>Odontaster validus</i>	7	13	7	3	3
<i>Odontaster meridionalis</i>	1	2	1	1	1
Class Echinoidea					
<i>Sterechinus neumayeri</i>	2	4	2	2	2
Class Ophiuroidea					
<i>Astrotoma agassizii</i>	1	2	1	0	0
<i>Ophionotus victoriae</i> (?)	1	2	1	1	1
Total Echinodermata	26	51	26	15	15
NEMERTEA					
<i>Parborlasia corrugatus</i>	6	9	6	9	6
MOLLUSCA					
Class Bivalvia					
<i>Adamussium colbecki</i>	15	20	15	1	1
<i>Laternula</i> sp.	4	6	4	0	0
Class Gastropoda					
<i>Neobuccinum eatoni</i>	11	13	9	17	9
<i>Tritonella</i> sp.	2	4	2	4	2
<i>Marseniopsis</i> sp.	1	2	1	2	1
<i>Austrodoris</i> sp.	2	2	1	2	1
<i>Clione antarctica</i>	1	1	1	2	1
<i>Limacina</i> sp.	12	12	12	0	6
Total Mollusca	48	60	46	28	21
Total	80	120	46	52	42

Table 2: Numbers of DNA sequences of 16s and COI from meroplankton of Antarctic benthic marine invertebrates. Species are represented in the table in the order that they are discussed in the text.
 (* - N.B. Many individual plankton could not be initially classified to phylum because they were unidentifiable eggs or embryos, but could subsequently be identified by DNA sequence. Hence the “No. of individuals sequenced” can be greater than the “No. morphologically identified individuals collected” for any one phylum.)

PHYLUM	FAMILY	SPECIES	Photo ref. (plates)	No. morphologically identified individuals collected*	16s		COI	
					Total no. sequences	No. individuals sequenced	Total no. sequences	No. individuals sequenced
ECHINODERMATA								
Class Asteroidea	Ophiasteridae	unknown	1 A-C		3	3	5	1
	Odontasteridae	<i>Odontaster validus</i>	2		21	18	29	15
Class Echinoidea	?	unknown	1 D		1	1	0	0
	Echinidae	<i>Sterechinus neumayeri</i>	3		8	8	7	4
Class Ophiuroidea	Gorgonocephalidae	<i>Astrotoma agassizii</i>	4 A-C		5	3	11	3
	Ophiuridae	<i>Ophiura</i> sp.	4 D-F		6	5	15	4
		Total Echinodermata		20	44	38	67	27
NEMERTEA								
	Lineidae	<i>Parborlasia corrugatus</i>	5 A		1	1	0	0
	Cephalothrichidae	<i>Procephalothrix</i> sp.	5 B		1	1	0	0
	Lineidae	<i>Parvicirrus</i> sp.	5 D-G		8	8	6	4
	Lineidae	<i>Cerebratulus</i> sp.	5 C		2	2	2	1
	Lineidae	<i>Lineus</i> sp.	6 + 7		37	37	52	23
		Total Nemertea		44	49	49	61	29
MOLLUSCA								
Class Gastropoda	Suborder Hypsogastropoda	unknown	8 A-D		4	4	9	2
	Suborder Hypsogastropoda	unknown	8 E-H		9	7	9	5
	?	unknown trochophore	9 A-D		9	6	0	0
	Suborder Aeolidina?	<i>Eubranchius</i> sp.?	9 E-H		19	19	21	10
		Total Mollusca		85	41	36	39	17

PHYLUM	FAMILY	SPECIES	Photo ref. (plates)	No. morphologically identified individuals collected*	Total no. sequences	16s No. individs. sequenced	Total no. sequences	COI No. individs. sequenced
ANNELIDA								
Class Polychaeta								
	?	Unknown 1	10 A-B		2	2	2	1
	Family Spionidae	Unknown 2	10 C-D		2	2	0	0
	?	Unknown 3	10 E		1	1	0	0
	?	Unknown 4	10 F		3	2	2	1
	Family Spionidae	Unknown 5	10 G		2	1	2	1
	?	Unknown 6	10 H		3	3	2	1
	Family Spionidae	Unknown 7	11A-D		8	8	6	4
	?	Unknown 8	11E-F		3	3	0	0
	?	Unknown 9	11G-H		3	3	2	1
		Total Polychaeta		274	27	25	16	9
				76				
				499	161	148	183	82
UNKNOWN EGGS/EMBRYOS								
Totals								

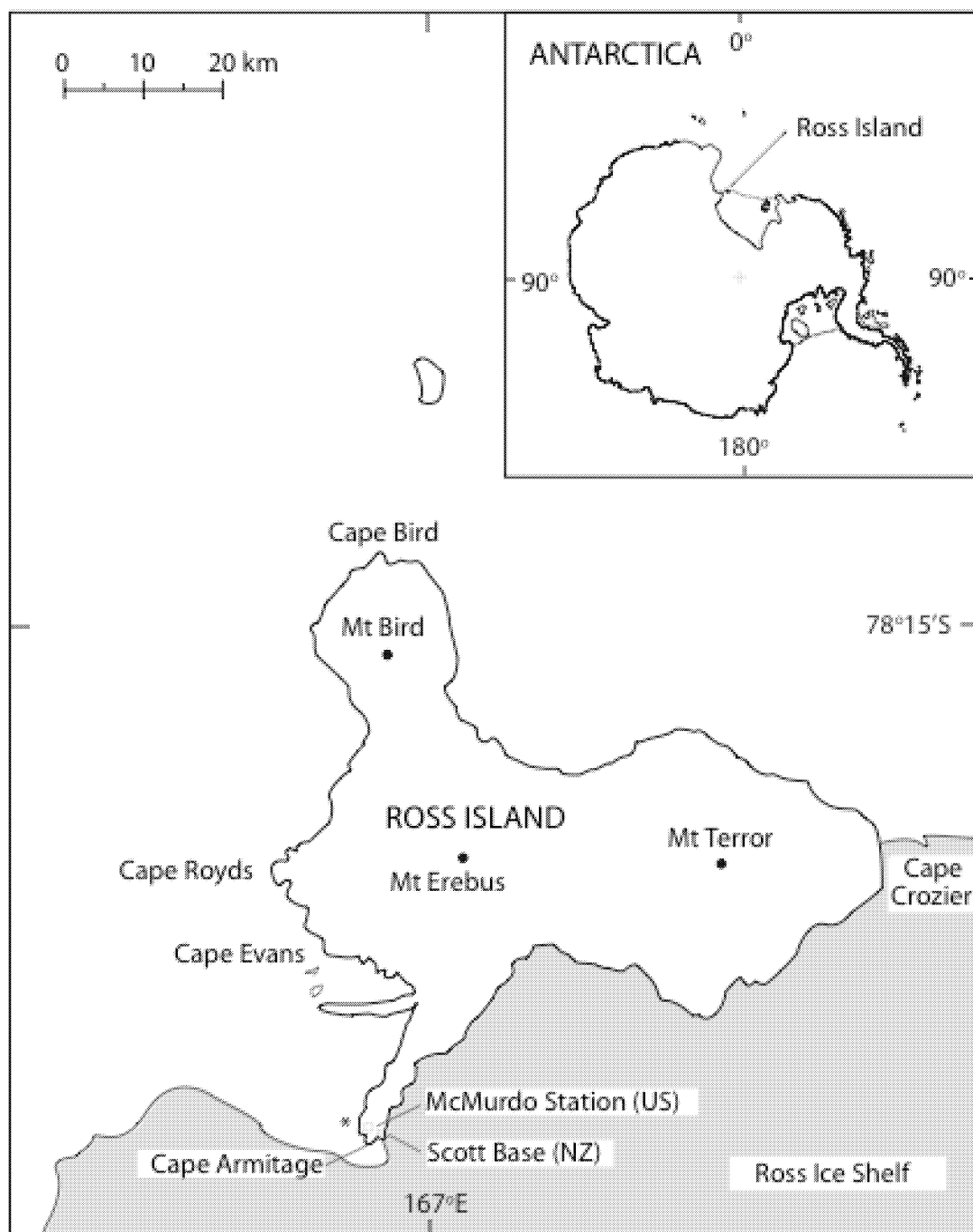


Figure 1: Location map showing Ross Island, McMurdo Sound, McMurdo Station and Scott Base in the southwestern Ross Sea. Dotted line running westwards from Cape Bird marks the northern boundary of McMurdo Sound. Star shows approximate location of the McMurdo Runway Site. Scott Base water intake is part of the Scott Base Station.

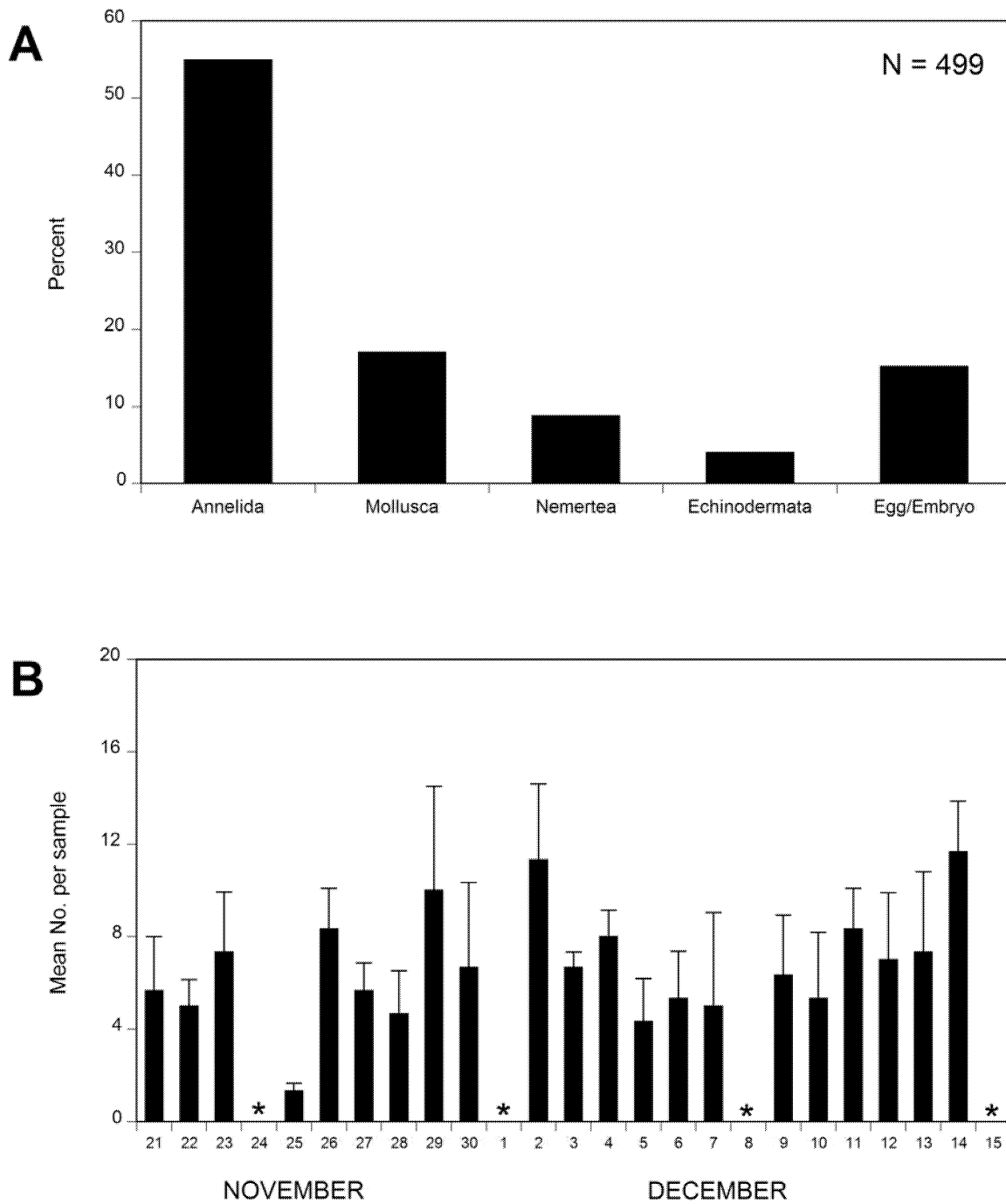


Figure 2: A. Percent of larvae collected at the McMurdo Runway site by phyla. Phylum Annelida includes the larval types polychaete embryo, <2-setiger and nectochaete (N = 274); Phylum Mollusca includes nudibranch veliger, veliger, small and large gastropod (N = 85); Phylum Nemertea includes pilidia and planuliform larvae (N = 44); Phylum Echinodermata includes bipinnaria and ophiopluteus (N = 20); Egg/embryo includes eggs of unidentified phyla (N = 76).
B. Mean total larvae collected per day at the McMurdo Runway site between 21 November and 14 December 2002 \pm SE (N = 3). * days with no sampling, thus values are not zero.

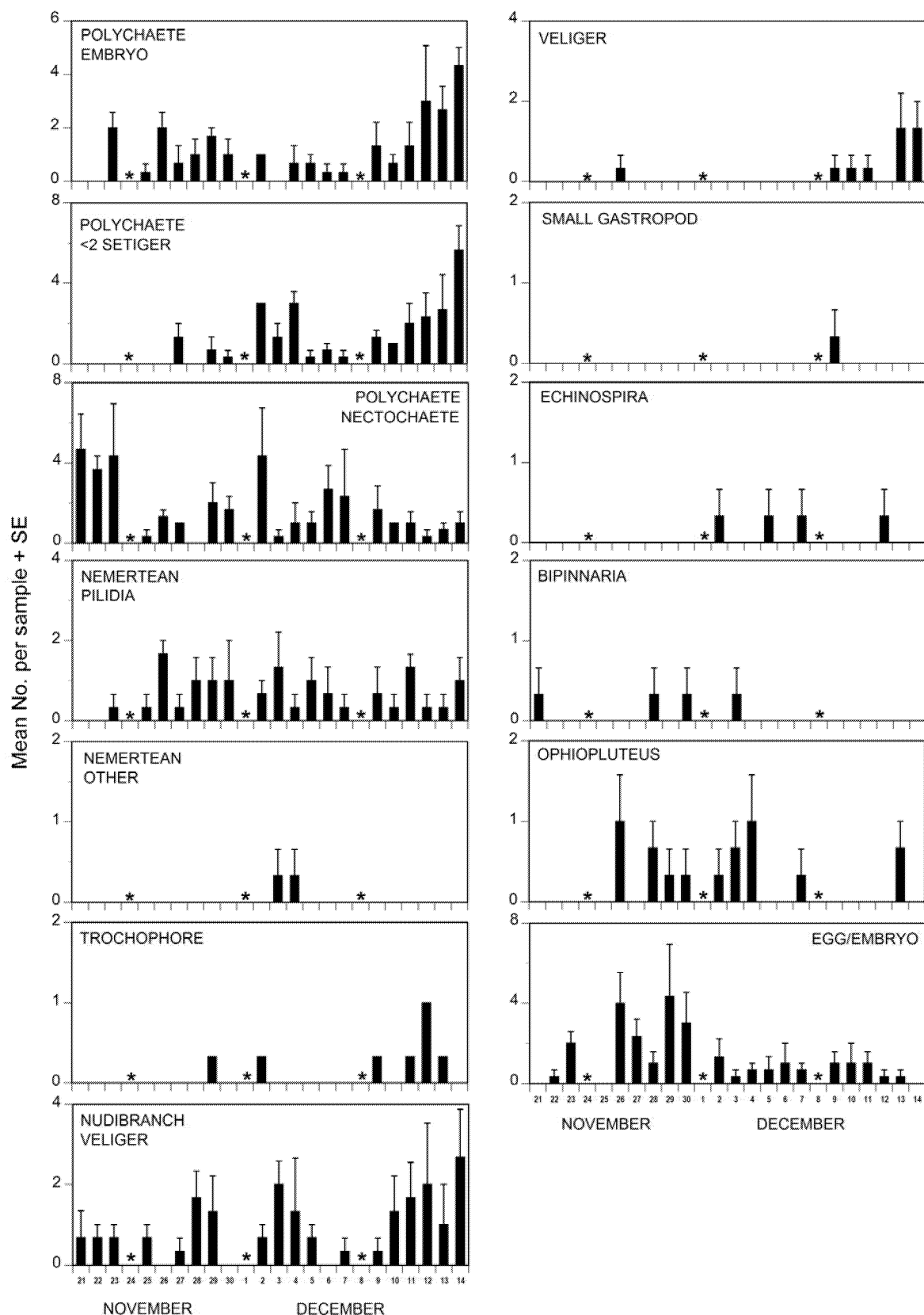


Figure 3: Mean numbers of the 13 larval "types" collected at the McMurdo Runway site between 21 November and 14 December 2002 \pm SE (N = 3). * days with no sampling, thus values are not zero. Nectochaete, advanced polychaete larvae; <2 setiger includes metatrochophores and 2-setiger polychaetes. Nemertean includes nemertean juvenile and planuliform larvae.

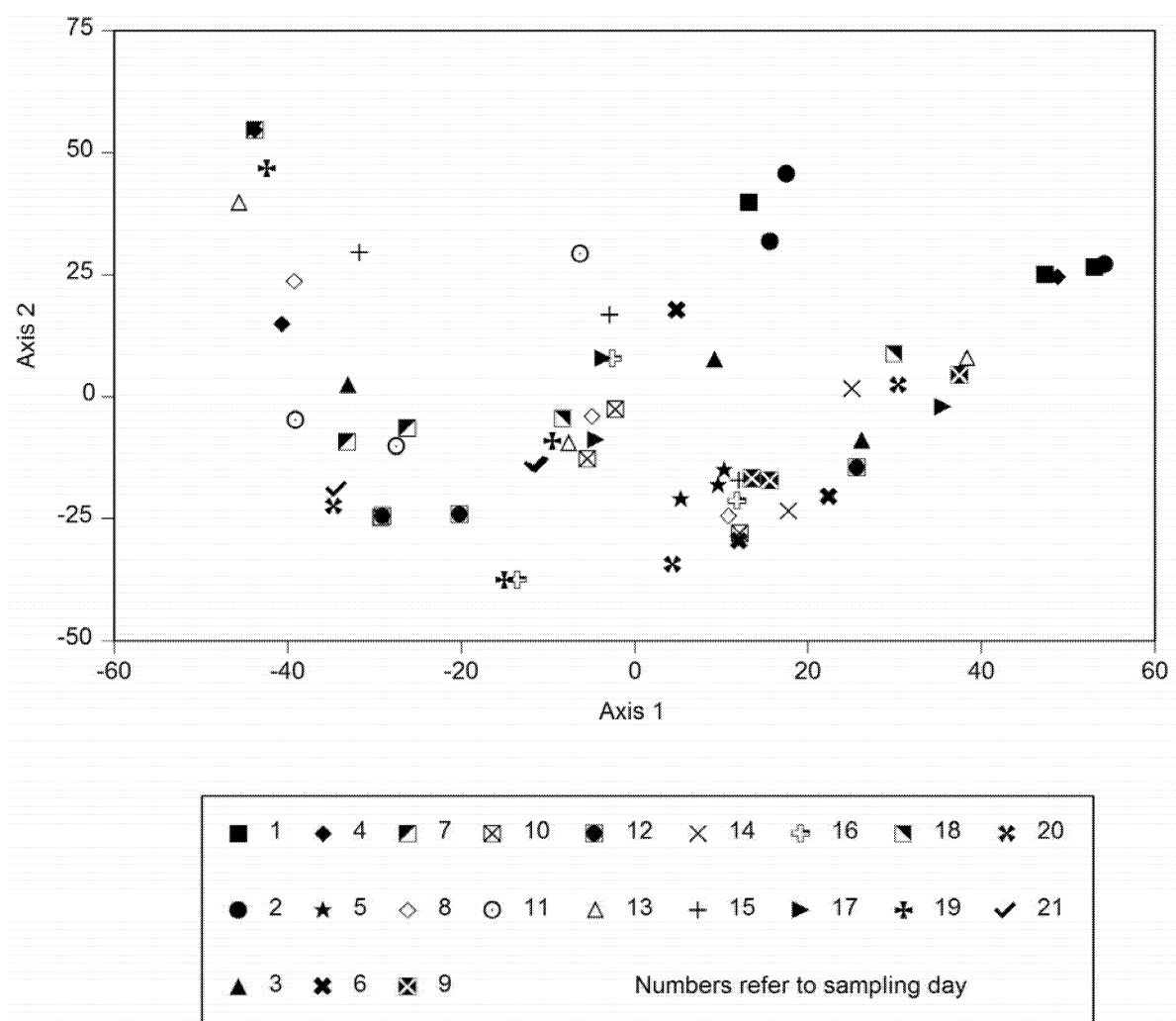


Figure 4: Results of PCORD showing distribution of the 63 sample points from the McMurdo Runway site (21 days x 3 replicate samples per day) in two-dimensional space. Different symbols refer to different sampling days: number 1 on 21 November to 21 on 14 December 2002.

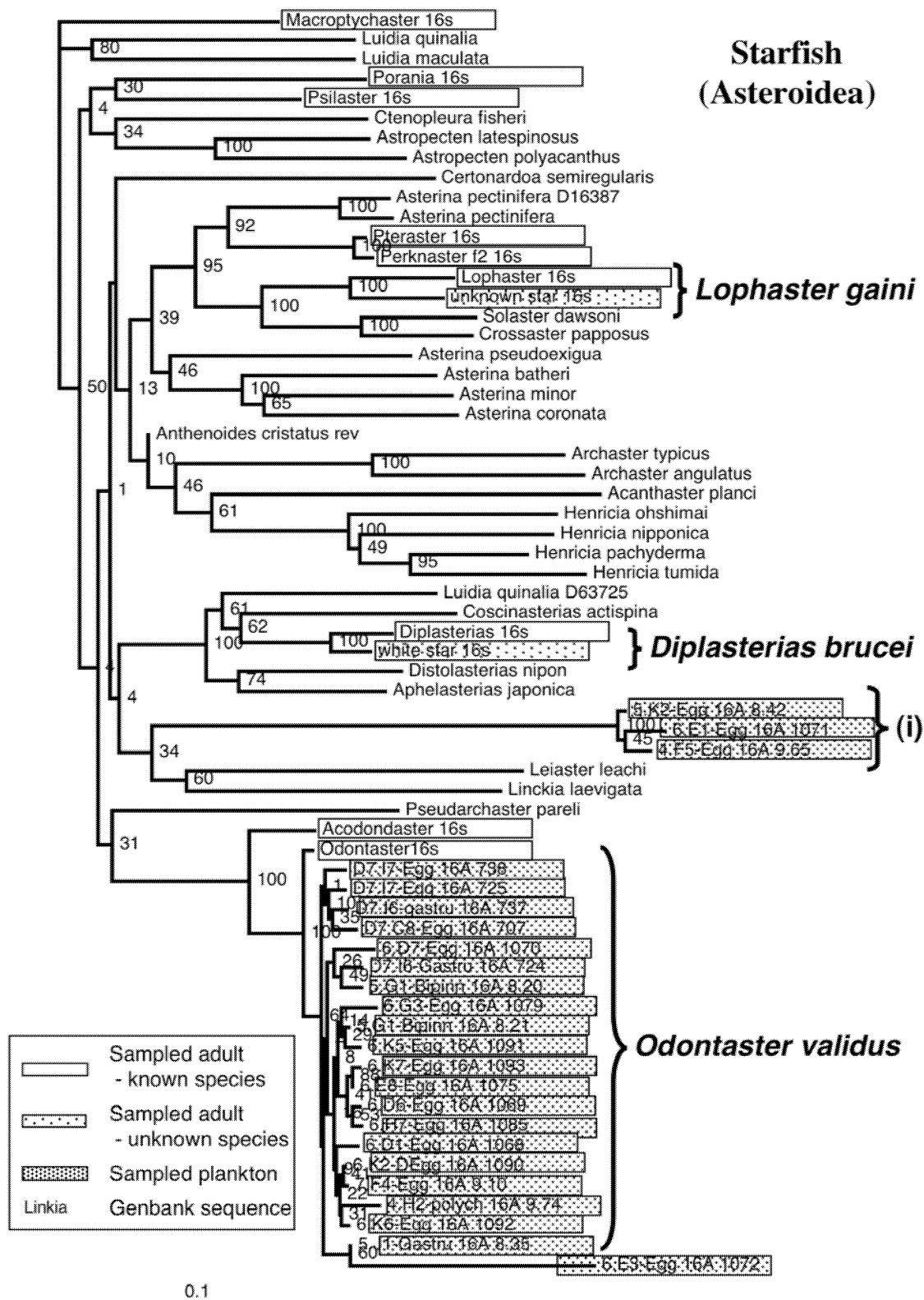


Figure 5: Phylogenetic tree of asteroid mtDNA 16s sequences from adult and plankton samples from the Ross Sea. All related sequences available in GenBank are included. (Sequences from this study shown are: adults 11 species., plankton 24 sequences from 21 individuals. Remaining sequences are from GenBank). (Scale shows nucleotide divergence). (This and all subsequent trees calculated using the neighbour-joining method on pairwise sequence divergences, and show percentage bootstrap support for all nodes.)

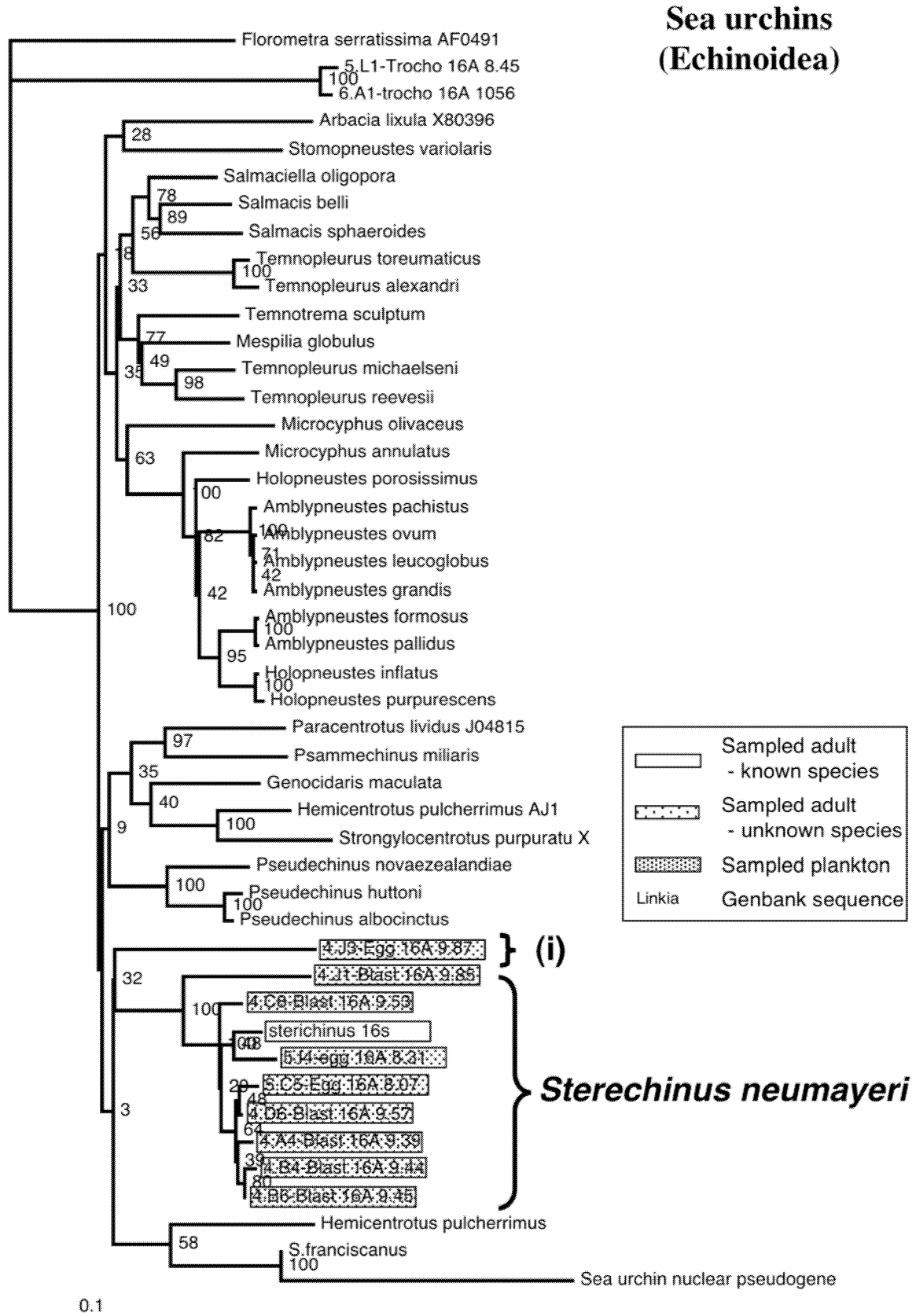


Figure 6: Phylogenetic tree of echinoid mtDNA 16s sequences from adult and plankton samples from the Ross Sea. All related sequences available in GenBank are included. (Sequences from this study shown are: adults 1 species, plankton 9 sequences from 9 individuals. Remaining sequences are from Genbank.) (Scale shows nucleotide divergence).

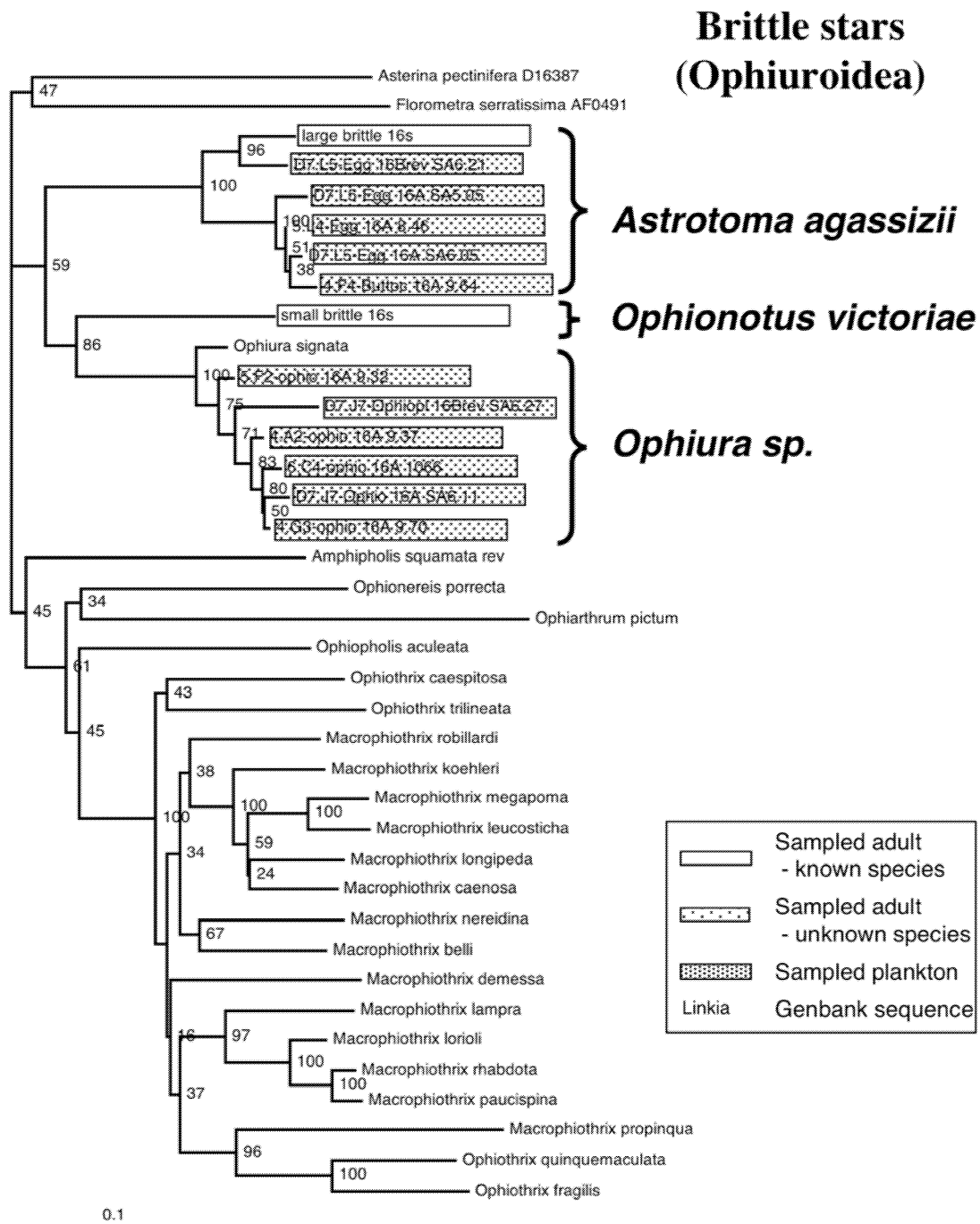


Figure 7: Phylogenetic tree of ophiuroid mtDNA 16s sequences from adult and plankton samples from the Ross Sea. All related sequences available in GenBank are included. (Sequences from this study shown are: adults 2 species, plankton 11 sequences from 8 individuals. Remaining sequences are from GenBank.) (Scale shows nucleotide divergence).

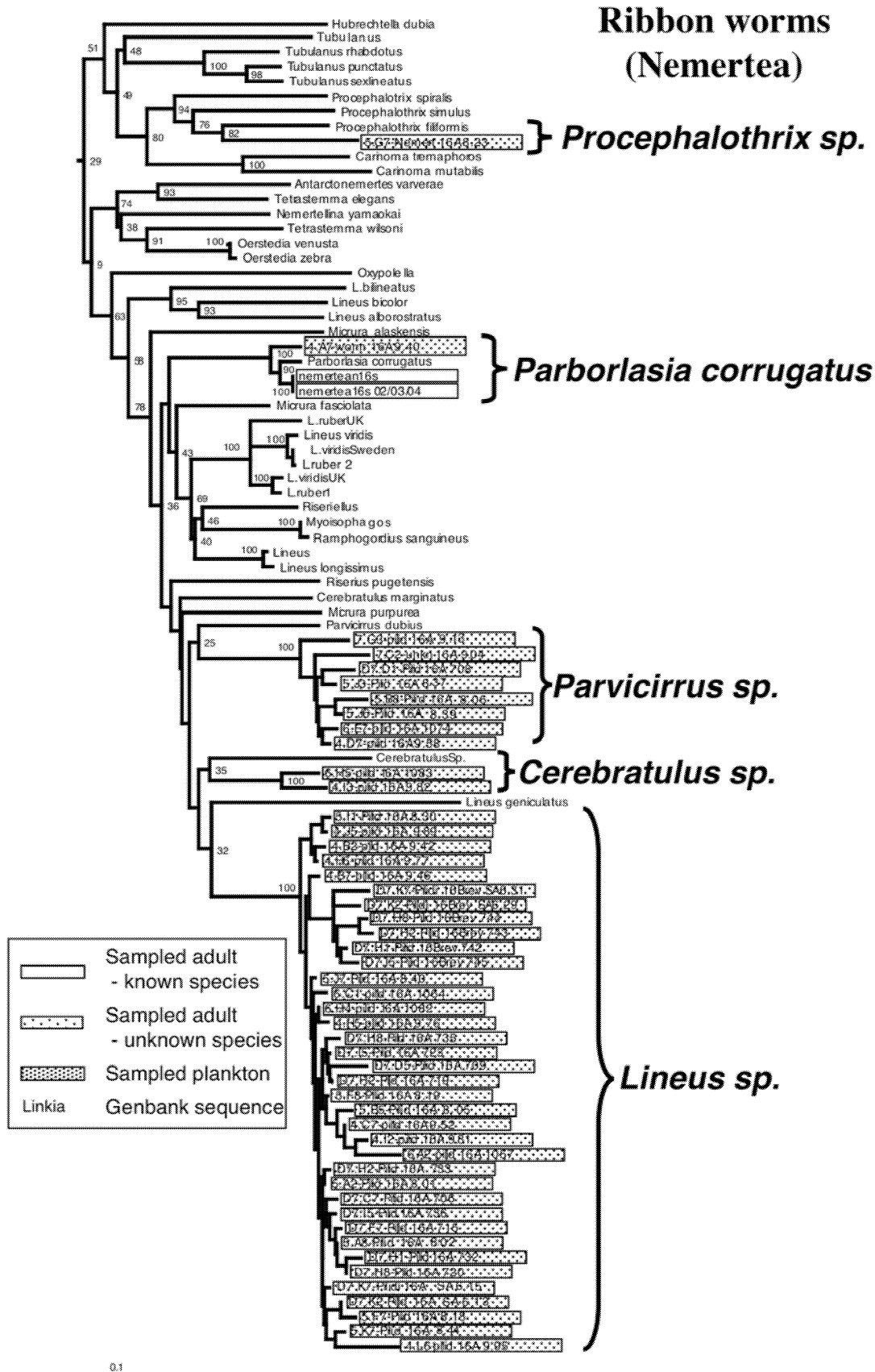


Figure 8: Phylogenetic tree of nemertean mtDNA 16s sequences from adult and plankton samples from the Ross Sea. All related sequences available in GenBank are included. (Sequences from this study shown are: adults 2 species, plankton 49 sequences from 49 individuals. Remaining sequences are from GenBank.) (Scale shows nucleotide divergence).

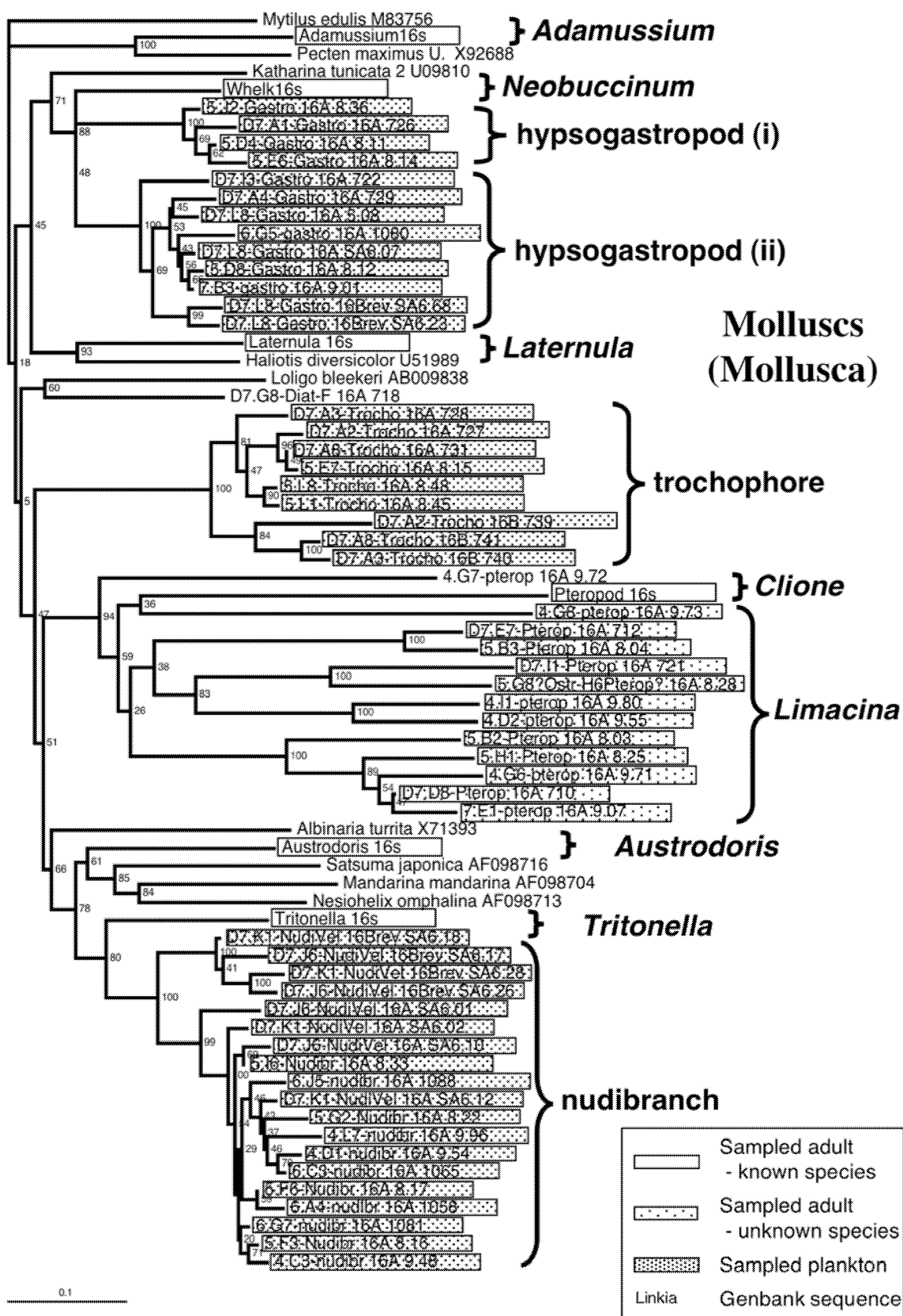


Figure 9: Phylogenetic tree of mollusc mtDNA 16s sequences from adult and plankton samples from the Ross Sea. All related sequences available in GenBank are included. (Sequences from this study shown are: adults 6 species, plankton 41 sequences from 36 individuals. Remaining sequences are from GenBank.) (Scale shows nucleotide divergence).

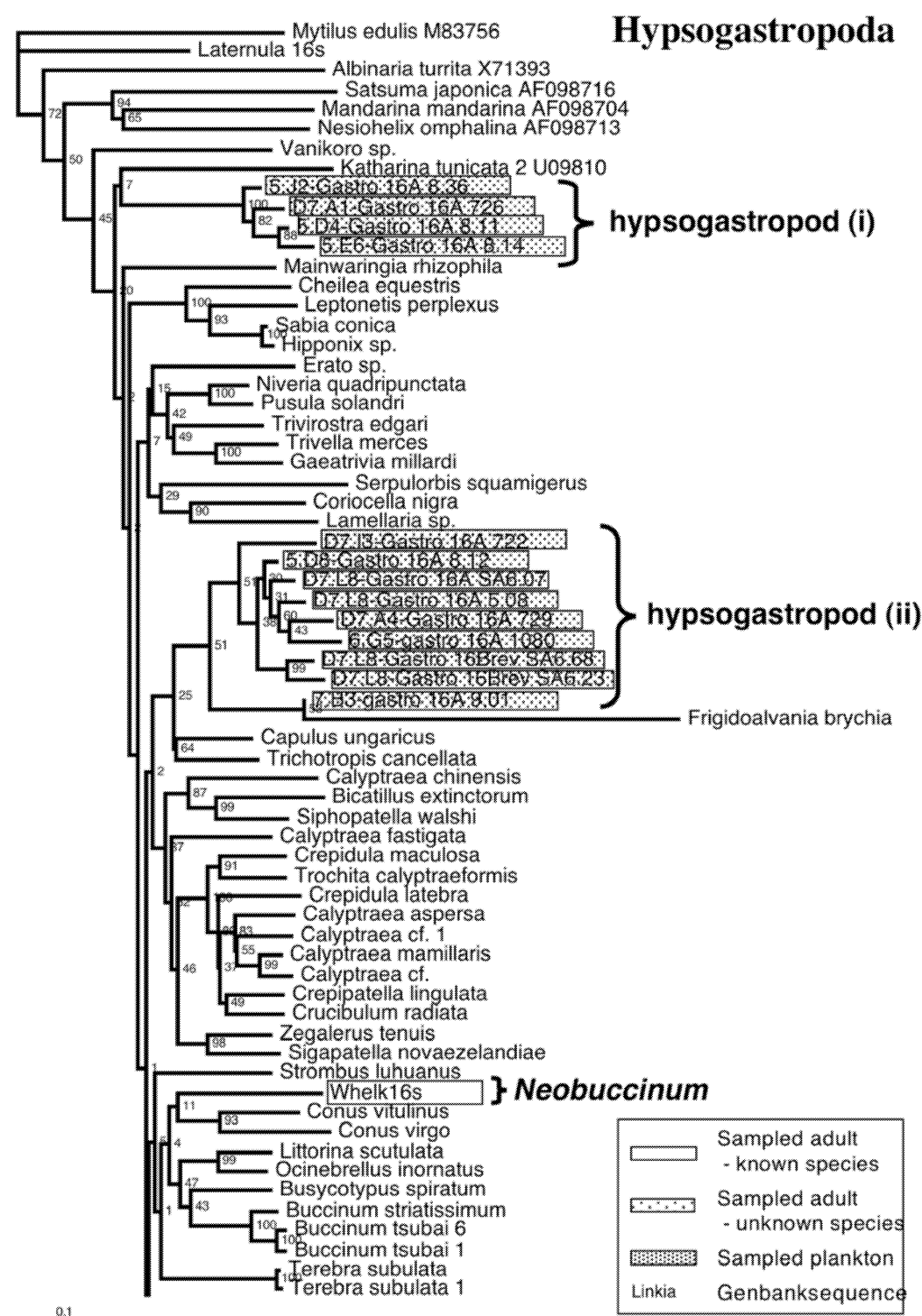


Figure 10: Phylogenetic tree of hypsogastropod mtDNA 16s sequences from adult and plankton samples from the Ross Sea. All related sequences available in GenBank are included. (Sequences from this study shown are: adults 1 species, plankton 13 sequences from 11 individuals. Remaining sequences are from GenBank.) (Scale shows nucleotide divergence).

Nudibranchs

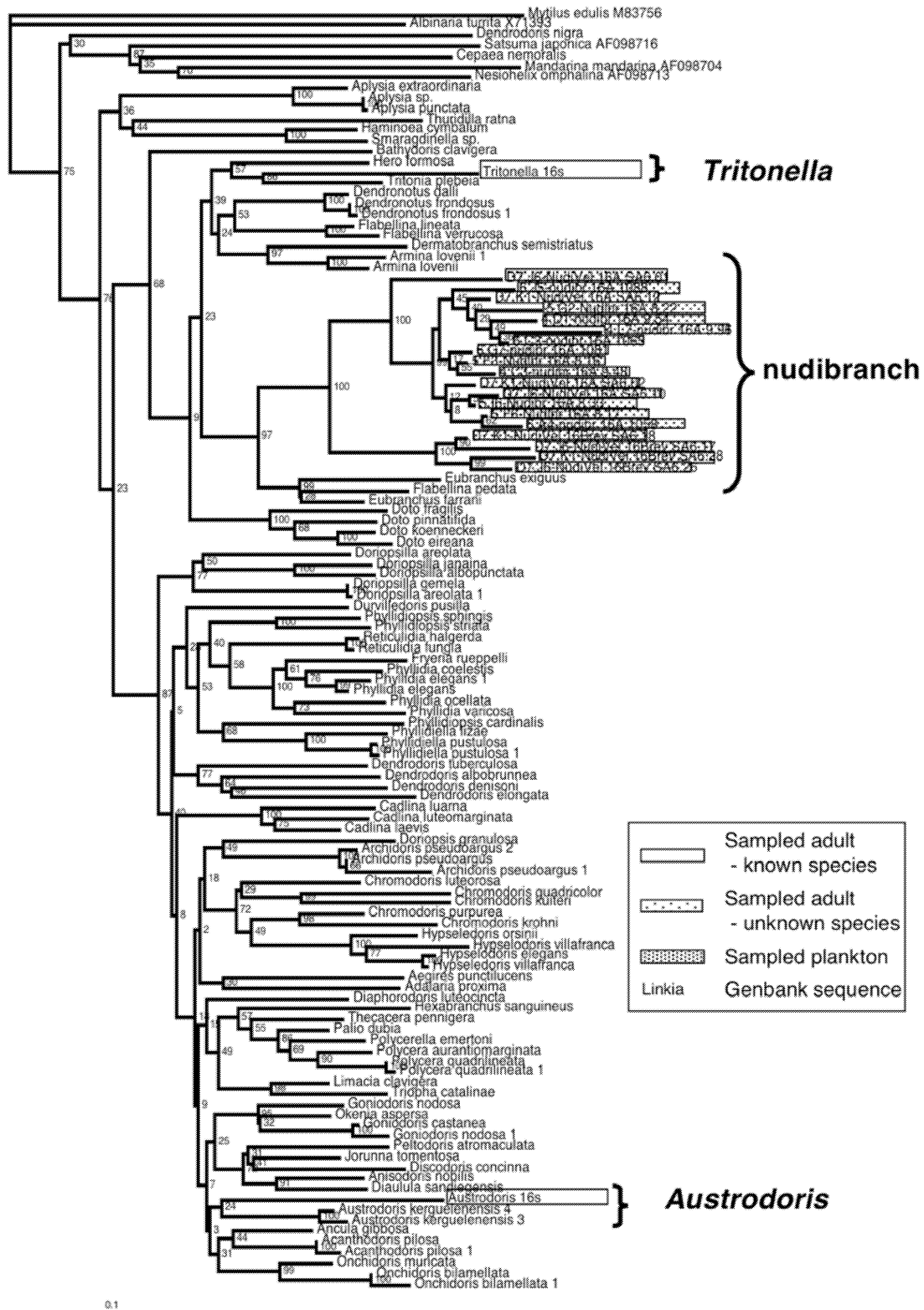


Figure 11: Phylogenetic tree of nudibranch mtDNA 16s sequences from adult and plankton samples from the Ross Sea. All related sequences available in GenBank are included. (Sequences from this study shown are: adults 2 species, plankton 19 sequences from 19 individuals. Remaining sequences are from GenBank.) (Scale shows nucleotide divergence).

Plate 1. Unidentified eggs, larvae, and juveniles collected in McMurdo Sound, Nov-Dec. 2002. Scale bars: A, B, D, G, H, 100 µm; C, E, F, 50 µm.

- A, B, egg of an unknown asteroid; not matched to any asteroids in adult database using DNA sequence.
- C, egg of an unknown asteroid; not matched to any asteroids in adult database using DNA sequence.
- D, unfertilised egg of an unknown echinoid; not matched to any echinoid in adult database using DNA sequence.
- E, 2-setiger polychaete found abundantly in plankton samples.
- F, small molluscan veliger found rarely in plankton samples.
- G, bivalve juvenile collected from Scott Base water intake. No DNA sequence could be obtained.
- H, large orange egg of unidentified species.

Plate 2: Eggs, embryos, and bipinnaria identified using DNA sequencing to be from the common Antarctic starfish *Odontaster validus*. Scale bars: A–J, 50 µm; K–M, 100 µm.

- A–F, egg within fertilization membrane.
- G, 4-cell embryo within fertilization membrane.
- H, J, gastrula.
- I, 8-cell embryo within fertilization membrane.
- K–M, bipinnaria. K, side view; L, M, dorsal view.

Plate 3: Eggs and embryos identified using DNA sequencing to be from the common Antarctic sea urchin *Sterechinus neumayeri*. Scale bars: 50 µm.

- A–D, blastula within fertilization membrane.
- E–G, multicell embryo. G, within fertilisation membrane.

Plate 4: Eggs and ophioplutei identified using DNA sequencing as ophiuroids. Scale bars: A–C, 100 µm; D–F, 25 µm.

- A, B, unfertilized orange egg of *Astrotoma agassizii*.
- C, cleaving embryo of *Astrotoma agassizii*.
- D–F, ophiopluteus of ophiurid. D, E, early stage; F, later stage.

Plate 5: Larval stages and juveniles identified using DNA sequencing as nemerteans. Body height measured from base of apical tuft to bottom of lateral lobes. Scale bars: A, 50 µm; B–G, 100 µm.

- A, metamorphosed juvenile of *Parborlasia corrugatus*.
- B, direct developing planuliform larvae of unknown nemertean species (ii).
- C, pilidia of unknown nemertean distantly related to *Cerebratulus*; nemertean species (iv).
- D–G, pilidia of unknown nemertean distantly related to *Parvicirrus*; nemertean species (iii).

Plate 6. Pilidia of unknown nemertean species, identified using DNA sequencing. A, nemertean species (iii), distantly related to *Parvicirrus*. B–H, nemertean species (v), distantly related to *Lineus*. Additional pilidia are shown in Plate 7. Body height measured from base of apical tuft to bottom of lateral lobes. A–E, side view; F–H, top view. Scale bars: A, 100 µm; B–H, 200 µm.

Plate 7. Side view of pilidia of unknown nemertean species (v), identified using DNA sequencing as distantly related to *Lineus*. Additional pilidia are shown in Plate 6. Body height measured from base of apical tuft to bottom of lateral lobes. Scale bars: A, B, D–H, 200 µm; C, 100 µm.

Plate 8. Larval stages identified using DNA sequencing as gastropod molluscs. Scale bars: A, B, D, 100 µm; C, 200 µm; E–H, 50 µm.

- A–D, unidentified gastropod species (i) within egg case.
- E–H, Unidentified gastropod species (ii) within egg case.

Plate 9. Larval stages identified using DNA sequencing as gastropod molluscs. Scale bars: 100 µm.

- A–D, trochophore of unidentified gastropod (iii).
- A. E–H, nudibranch veliger of unidentified gastropod (iv) distantly related to the genus *Eubranchus*.

Plate 10. Larval stages identified using DNA sequencing as annelids. Scale bars: A, B, 50 µm; C, H, 100 µm; D, F, G, 200 µm; E, 25 µm.

- A, trochophore of unidentified polychaete species (i).
- B, metatrochophore of unidentified polychaete species (i).
- C, D, nectochaete of unidentified polychaete species (ii).

- E, embryo of unidentified polychaete species (iii).
F, nectochaete of unidentified polychaete species (iv).
G, nectochaete of unidentified polychaete species (v).
H, nectochaete of unidentified polychaete species (vi).

Plate 11. Larval stages identified using DNA sequencing as annelids. Scale bars: A, E, F, 25 μm ; B, D, G, H, 100 μm ; C, 200 μm .

- A, embryo of unidentified polychaete species (vii).
B–D, nectochaete of unidentified polychaete species (vii). Note that head is missing in B.
E, F, embryo of unidentified polychaete species (viii).
G, H, nectochaete of unidentified polychaete species (ix). Note missing long setae on H.

Electronic Appendices (directory structure & file names)

The electronic appendices and databases are provided on 5 accompanying CDs: a “Database” CD and four Photo file CDs. The details of all files and which CD they appear on are provided below. The key summary files are Excel files that list all individuals and their details for morphological or sequence analyses. Each of these files has as its first worksheet an explanation of the structure of that database. Each adult or plankton individual has a unique identifying number that can be cross-referenced among all databases and all phylogenetic trees presented in this report. For example, each adult has an identifying number (ID#) of the form “A02.01” and each plankton individual an ID# of the form “D5.A1”. The DNA sequencing, photographs and morphological analysis for any specific adult or plankton individual can be found within each database by searching for this unique ID#, and all databases are cross-referenced by this ID#. For example, if one wished to find all sequences and photos of plankton individual “D7.L5” – the first brittle star plankta found on the tree in Fig. 7 – one would look in file “plankton sample+results14.xls” for sequence information on sheet D7 on row L5; for photos one would look in “PhotosCrossRefDNA” and search for D7.L5, where all photos taken of this individual would be listed with their file names.

Raw data:

Type	Location	Folder	File names
Field notebook	CD - Database	DataSheets	FieldNotes.pdf
Larval data	CD - Database	DataSheets	[date].pdf
Morphological data	CD - Database	-	MorphData.xls
Photographic Records	CD - Database	-	PhotosCrossRefDNA.xls

Processed data:

Type	Location	Folder	File names
Larval counts	CD – Database	-	LarvalData.xls

Photographs:

Type	Location	Folder	File names
Adults	CD - Database	AdultPhotos	[number].jpg
Plankton	CD – Photo Files Disc 1	[date]	[number].jpg
	CD – Photo Files Disc 2	[date]	[number].jpg
	CD – Photo Files Disc 3	[date]	[number].jpg
	CD – Photo Files Disc 4	[date]	[number].jpg

Molecular results:

(a) Samples and results:

Type	Location	Folder	File names
Adults	CD - Database	-	adult sample+results.xls
Plankton	CD - Database	-	plankton sample+results.xls

(b) DNA sequences:

(N.B. All sequence files are text files, readable and interpretable in any text editor (e.g. Word). File suffixes indicate the specific file format for further analysis: fasta – fasta format; aln – Clustal format; nxs – nexus format)

(i) 16s

Type	Location	Folder	No. sequences	File names
Adults	CD - Database	16s	30	adult 16s.fasta
Asteroids	CD - Database	16s	64	GB stars-6.aln
Echinoids	CD - Database	16s	46	GB urchins ed.aln
Ophiuroids	CD - Database	16s	38	GB ophios-3.aln
Nemerteans	CD - Database	16s	120	SA10 pilid.aln
Molluscs	CD - Database	16s	70	SA10 mollusc.aln
Hypsogastropods	CD - Database	16s	156	Hypsogastropoda genera ed2.aln
Nudibranchs	CD - Database	16s	121	GB nudibr ed.fasta.aln
Polychaetes	CD - Database	16s	101	polychaete 16s.aln

(ii) COI

Type	Location	Folder	No. sequences	File names
Adults	CD - Database	COI	22 154	adult COI.fasta, allCOI+adult.nxs
Asteroids	CD - Database	COI	96 12	SA2 seqns.fasta, SA3 16B+CO2rev.fasta
Echinoids	CD - Database	COI	95	SA4 seqns.fasta
Ophiuroids	CD - Database	COI	15	SA5 COI.nex.fasta
Nemerteans	CD - Database	COI	11 22	SA6 COI 1.nex.fasta, SA6 COI 2.nex.fasta
Molluscs	CD - Database	COI	96	SA7.fasta
Hypsogastropods	CD - Database	COI	44	SA8 COI.fastaPC
Nudibranchs	CD - Database	COI	19 44	SA9-CO2.fasta, SA10-CO2 rev.nex
Polychaetes	CD - Database	COI	66 42	SA11-CO2.fasta, SA12-COIa.fasta

(iii) Fish

Type	Location	Folder	No. sequences	File names
12s	CD - Database	fish	15 25	AntFish12s.fasta, AntFish12s-R.fasta
16s	CD - Database	fish	15 23	AntFish16s.fasta, AntFish16s-R.fasta
ND2	CD - Database	fish	20	AntFishND2.fasta

MorphData.xls

This file consists of 192 records with the following data fields.

F1, F2 = Refers to Morphology Plate 1, Morphology Plate 2.

Code = Refers to the well within the plate to which the sample was placed (each plate has A-G, 1-12)

Larval form = Larval type

Sample information = Sample date, Sorter Name, Source of Larvae (Jar No. being sorted; see also LarvalData Database. WATER refers to Scott Base Water Intake), Code [Letter refers to sorter (J= Jenn, M= Marcus, S= Shane), number to a particular form that day]

Photos = Photo No. if taken, * = no photo

PhotosCrossRefDNA.xls

This file consists of 1162 records with the following data fields.

Date = sampling date

Card No. = Refers to the download of files from the memory card of the digital camera to the computer.

Photo No. = Refers to the photo within that particular card. In later records this is sequential.

Larval type/ Sorter/ Jar No./ J1 = Larval type = Larval form being photographed; Sorter = Person who sorted the larvae; see also LarvalData Database; Jar No. = The Jar No. being sorted; see also LarvalData Database. WATER refers to Scott Base Water Intake; J1 = Letter refers to sorter (J= Jenn, M= Marcus, S= Shane), number to a particular form that day.

Objective = Whether 40x or 100x objective used for photograph.

Destination = Coded for DNA (D) or Morphology (F). For example D3.F1 refers to the third DNA plate, well F1 (each plate has A-G, 1-12). Note that these are the ORIGINAL codings.

DNA sequence = Refers to DNA sequence recorded in Excel Spreadsheet "plankton sample + results"

Adult sample+results.xls

This file consists of 192 records with the following data fields.

“samples” sheet:

Species

common name

name for tube

abbrev. for tube

photo?

source

location

(Initial) sample #

Allocated sample #

(DNA) extraction?

DNA quantition /quality (where available)

tissue

sample jar/tube

“results” sheet:

Species

common name

ID # = identification number

ID? = final species identification

Closest seqn = most closely related DNA sequence overall

DNA = was DNA extracted? (“x” = yes; same for all following fields)

For each of 16s and COI separately:
PCR = was PCR carried out?
Good = was there good PCR product?
f-seq = was forward sequence obtained?
r-seq = was reverse sequence obtained?
Closest seqn = taxon of most closely related DNA sequence
Blast closest seqn = species of most closely related DNA sequence from Blast search
Taxonomy = taxonomic hierarchy of above
% sim. = % similarity of DNA sequence
length = length in base pairs of DNA sequence similarity

Plankton sample+results.xls

This file consists of 672 records with the following data fields on each sheet. Each worksheet contains the records of one 96-well plate of samples preserved for DNA analysis, and is named from "D1" to "D11"

ID # = identification number
sample description = how the sample was described
ID? = the preliminary identification
Closest seqn = most closely related DNA sequence overall
DNA = was DNA extracted? ("x" = yes; same for all following fields)
For each of 16s and COI separately:
PCR = was PCR carried out?
Good = was there good PCR product?
f-seq = was forward sequence obtained?
r-seq = was reverse sequence obtained?
Closest seqn = taxon of most closely related DNA sequence
Blast closest seqn = species of most closely related DNA sequence from Blast search
Taxonomy = taxonomic hierarchy of above
% sim. = % similarity of DNA sequence
length = length in base pairs of DNA sequence similarity

