SEEING STARS: A MOLECULAR AND MORPHOLOGICAL INVESTIGATION OF THE ODONTASTERIDAE (ASTEROIDEA)

by

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Abstract

Odontasterids (Asteroidea: Echinodermata) comprise a clade described by A.E. Verrill in 1899 and are placed within Valvatida, a derived assemblage of sea stars. Boasting a worldwide distribution, Odontasterids are found in the Southern, Atlantic, and Pacific Oceans, with a concentration in cold-water habitats, in high latitudes in the Southern Hemisphere. Most of species of Odontasteridae are from the lower shelf and upper bathyal region, though some have been collected in the tidal zone. Odontasteridae includes the following genera: *Acodontaster, Diabocilla, Diplodontias, Eurygonias, Hoplaster,* and *Odontaster*, which are typically characterized by two series of equal, opposite and usually conspicuous marginal plates without intermarginal channels. They also usually possess triangular mouths and two rows of tubefeet with suckers. This group of organisms occupies an important role in marine environments and is important to the understanding of marine systems. To date, the phylogenetic and evolutionary history within the Odontasteridae has not been rigorously examined. Here, a comprehensively sampled molecular and morphological phylogenetic analysis of the Odontasteridae to assess interrelationships among and between genera is presented. More specifically, the recent evolutionary history of the genus *Odontaster* throughout the Western Antarctic waters and on the South American shelf is examined. The mitochondrial 16S ribosomal and cytochrome *c* oxidase subunit I (COI) genes were sequenced from adult and larval specimens. Finally, at a finer scale, high resolution genetic markers (microsatellites) are used to look at the circumpolar population structure of *Odontaster validus.*

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CHAPTER 1. Introduction to Dissertation

1.1 GENERAL INTRODUCTION AND BACKGROUND ON ANTARCTIC BIOLOGY, ASTEROIDEA, AND TAXONOMIC HISTORY OF ODONTASTERIDAE

Asteroidea (phylum Echinodermata), commonly called sea stars, dates back to the Ordovician, and belongs to a major and diverse clade of benthic organisms (Figure 1). Sea stars display the distinctive echinoderm body plan and are characterized by an endoskeleton with a unique water vascular system. Typically they have a stellate body with five or more arms (Figure 2). Specifically, the arms are not set off from the central disc by distinct articulation, their mouth is directed towards the substrate, and they have ambulacral grooves containing tube feet with internal ampullae. Currently, seven orders of approximately 35 families, 300 genera and 1,800 extant species are described (Clark and Downey 1992, Hendler et al. 1995). These strictly marine invertebrates are an ecologically important group (e.g. Birkeland 1974) that often displays a close relationship between their biogeographic distributions and their phylogenetic history (Blake 1983, 1987, 1990). Phylogenetic relationships among asteroids remain unresolved, despite many molecular studies (Lafay et al. 1995, Wada et al. 1996, Knott and Wray 2000).

Within Asteroidea is a family of sea stars known as Odontasteridae, described by A.E. Verrill in 1899. Odontasteridae is grouped within Valvatida, a derived assemblage of sea stars characterized by two series of equal, opposite, and usually conspicuous marginal plates without intermarginal channels (Clark and Downey 1992). These stars also usually possess triangular mouths and two rows of tubefeet with suckers.

Specifically, Odontasteridae characteristically have five rays and are distinguishable by the hyaline-tipped spines surrounding the mouth. Their body form ranges from stellate to nearly

pentagonal. Pedicellariae are sometimes present. Although many species within Odontasteridae are familiar components in their ecosystems, little is known about their biology and ecology. Most species of Odontasteridae are from the lower shelf and upper bathyal region, though some have been collected in the tidal zone.

The diet and reproductive biology of only a few of the odontasterids have been studied. Specifically, Dearborn (1977) found that *Acodontaster conspicuus* and *A. hodgsoni* both feed on sponges. Dearborn also found that *Odontaster validus* is omnivorous, feeding on carrion bait, gastropods, crustaceans, echinoids (*Sterechinus*), smaller *Acodontaster* specimens, leafy algae, sponges, diatoms, and even seal feces. In addition, Pearse (1965) studied the reproductive periodicity of *Odontaster validus*. He concluded that it spawns in winter after a prolonged period of 18-24 months oocyte growth, giving rise to bipinnaria larvae. Comparable observations on other odontasterids remain to be made.

Taxonomic history of Odontasteridae has been quite complex over the past one hundred years. In total, there are currently six recognized genera and twenty-five accepted species within the Odontasteridae. Currently recognized genera include: *Acodontaster, Diabocilla, Diplodontias, Eurygonias, Hoplaster,* and *Odontaster*. *Acodontaster* is composed of five species, *A. capitatus, A. conspicuus, A. elongatus, A. hodgsoni,* and *A. marginatus.* This genus is found mainly in the Antarctic, with one subspecies *A. elongatus granuliferus* extending into the Atlantic. The deep-sea genus *Diabocilla* maybe a synonym of *Hoplaster*; however *Diabocilla clarki* was described by McKnight (2006). Despite the fact that the Odontasteridae is characteristically known for a recurved, hyaline-tipped glassy tooth, *Diabocilla,* along with *Hoplaster* are lacking the tooth. Otherwise, all other characters of *Hoplaster* are the same as the standard characters of the family. *Hoplaster* consists of the two species; *H. kupe* and *H. spinosus*.

Diplodontias and its four currently recognized species (*D. dilatatus, D. miliaris, D. robustus,* and *D. singularis*) were previously referred to as *Asterodon*. *Diplodontias* is uniquely characterized by a pair of large hyaline-tipped teeth at each jaw. The genus *Eurygonias* is comprised of a single species, *E. hyalacanthus,* described in 1913 by Farquhar. This monotypic genus is endemic to New Zealand. Lastly, the genus *Odontaster* is the largest and most conspicuous genus in the Odontasteridae and is found in many of the world's oceans, including the Atlantic, the Pacific, and the Southern Ocean. This genus is comprised of *O. aucklandensis, O. australis, O. benhami, O. crassus, O. hispidus, O. mediterraneus, O. meridionalis, O. penicillatus, O. rosagemmae, O. robustus, O. setosus,* and *O. validus.* Significant generic level characters include the number of recurved, hyaline-tipped teeth at the jaw apex and the extent of the papulae on the abactinal area.

One specifically interesting group of sea stars in Odontasteridae is in the genus *Odontaster*. Three members of this genus (*O. meridionalis, O. penicillatus, and O. validus*) are established throughout the Southern Ocean, from South America into the Antarctic, including the sub-Antarctic islands (Figure 3). These species occur at a wide rage of depths and in a variety of habitats (Fisher 1940). Also, this group of sea stars is quite conspicuous throughout the Southern Ocean and plays an important role in the ecosystem. Despite this, their biogeography, population structure, and evolutionary history are poorly understood.

Although pelagic larval stages are argued to be rare in polar asteroids and other benthic invertebrates, (Mileikovsky, 1971; Pearse & Bosch, 1994), the Antarctic asteroid genus, *Odontaster* (Verrill, 1880), has a planktotrophic larva that can stay in the water column for up to six months. In particular, *O. valius* spawns in the austral winter (Pearse, 1965; Tyler et al., 2003) probably to avoid pelagic predators associated with the summer phytoplankton bloom (Clarke,

1988). This life history strategy presumably allows for high dispersal (Pearse & Bosch, 1986), and is consistent with the circumpolar distribution of *O. validus*, extending into the sub-Antarctic. *Odontaster validus* is ubiquitous throughout the Southern Ocean and is also of ecological importance due to its influence on the benthic community. For example, Dayton et al. (1974) suggested that *O. validus* consumes benthic larvae and could be regulating invertebrate populations, specifically populations of important predators, *Acodontaster conspicuus* and *Doris mcmurdensis*, on space-dominating sponges. Thus, *O. validus* has been termed a keystone species in the Antarctic ecosystem (McClintock, 1988). Additionally, two other species, *O. meridionalis* and *O. penicillatus* are recognized as restricted to South American and circumpolar Antarctic distributions, respectively (Fisher, 1940).

The morphology of *Odontaster* in the Southern Ocean appears to be quite variable and consequently problematic in terms of taxonomic diagnosis. As an example, Fisher (1940) discusses five different morphotypes within *O. validus* and two within *O. penicillatus*. Morphological synapomorphies for *Odontaster* include the single recurved spine at each oral angle between the two associated plates, abactinal plates with a distinct tabulum crowned with spinelets of variable length, tabulate marginal plates with short spinelets, and actinal plates that are spinulose (Clark, 1962). *Odontaster meridionalis*, *O. penicillatus* and *O. validus* differ in external morphology in the number of spinelets on abactinal and actinal plates, the presence or absence of a border on marginal plates, and the occasional presence of pedicellariae (Clark, 1962); however, these differences have been reported to be variable, even within the same species (Fisher, 1940). The given distributions and variable morphology present the opportunity to explore the recent evolutionary history of Southern Ocean *Odontaster* in terms of dispersal

and population connectivity from both sides of the Drake Passage, separating South American and Antarctic waters.

Given the close proximity of the South American and Antarctic continental shelves, there is potential for *Odontaster* to disperse across the Antarctic Circumpolar Current (ACC) and Antarctic Polar Front (APF). Reported to be the strongest current in the world, the ACC is complex in terms of oceanographic dynamics (Stevens, 1997). In contrast, the APF is the steep temperature gradient where polar waters meet warmer temperate waters. Both the ACC and APF formed after the spreading of the sea floor opened the Drake Passage approximately 24-41 million years ago, and they (separately or in combination) have been hypothesized to promote isolation of Southern Ocean and Antarctic fauna (Crame, 1999; Pfuhl & McCave, 2005; Scher & Martin, 2006). Although endemism of many Antarctic organisms has been attributed to these potential barriers, 18% of echinoderm species including, Asteroidea, Ophiuroidea, and Echinoidea are found in both South American waters and the coastal waters of Antarctica (Ekman, 1953; Peck et al., 2005).

1.2. Research Objectives

To date, the phylogeography and evolutionary history of Odontasteridae has not been rigorously examined. This group of organisms occupies an important role in marine environments and is important to the understanding and interaction of marine systems. Specifically, in the Antarctic, *Odontaster validus* has been labeled a keystone species and is capable of applying considerable influence on the environment (McClintock 1988). Also, it is likely that other members of in the Odontasteridae play a similar role in their environments. Therefore, it is important to understand the evolutionary relationships within the Odontasteridae

and also is important to the overall understanding of ecosystems. To gain insight into the evolutionary history and diversification of this group over time, a combined morphological and molecular approach will be used.

In addition, little effort has been undertaken to assess the circumpolarity of benthic invertebrates in the Southern Ocean. Thus, a closer look will be taken by examining the phylogeography of the genus *Odontaster* throughout the Southern Ocean using molecular mitochondrial markers and morphological analyses. Even more specifically, the circumpolar population level analysis of *Odontaster validus* will be examined using high resolution genetic markers (microsatellites).

Studies of phylogenetic events in modern, post-Paleozoic asteroids, within the context of geological tectonic and macroevolutionary events provide significant perspective in understanding their present-day distributions. In particular, little is known in terms of phylogeography for this ecologically important group of invertebrates. A combined morphological and molecular approach of and within the Odontasteridae provides a unique opportunity to study biogeographic shifts in terms of evolutionary history in a group modern sea stars.

The overarching goal of this dissertation is to provide insight into the evolutionary relationships within Odontasteridae in a biogeographical and phylogenetic framework. Ideally, the methods used to carry out the following objectives present a model for addressing similar questions in other organisms. Specifically, research objectives are:

1) To explore the phylogenetic and relationships within in the Odontasteridae, inferred using molecular and morphological characters.

2) To asses the population structure (or lack thereof) in terms of evolutionary history, of the members of the genus *Odontaster* within the Southern Ocean using mitochondrial markers.
3) To explore the specific genetic connectivity of *Odontaster validus* using high-resolution genetic markers, throughout its range in Antarctic waters.

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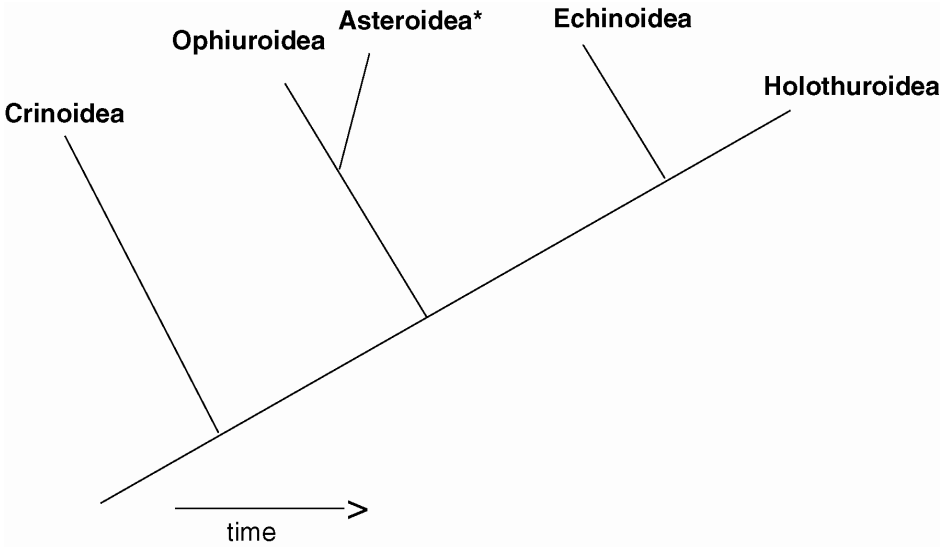

Figure 1. Echinoderm phylogenetic scheme depicting feather stars as the basal lineage, diverging early from the common ancestor of all echinoderms, and that urchins and sea cucumbers are more closely related to each other than to other echinoderms. * includes Concentricycloidea.

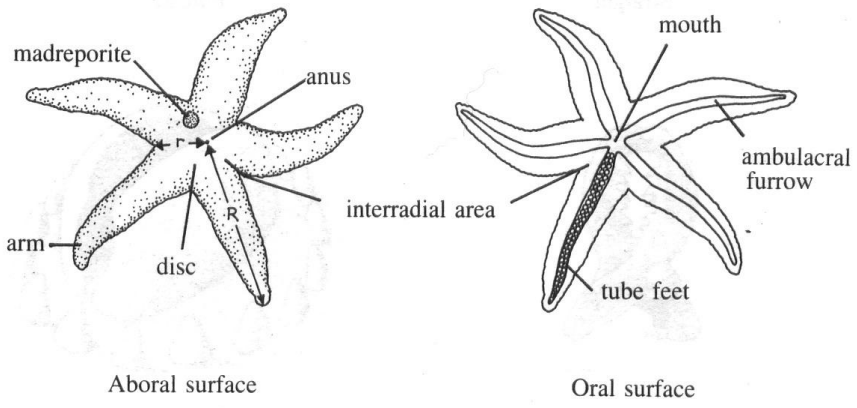

Figure 2. External features of a sea star. From Lambert, P. (1981) *The Sea Stars of British Columbia*. British Columbia Provincial Museum, Victoria

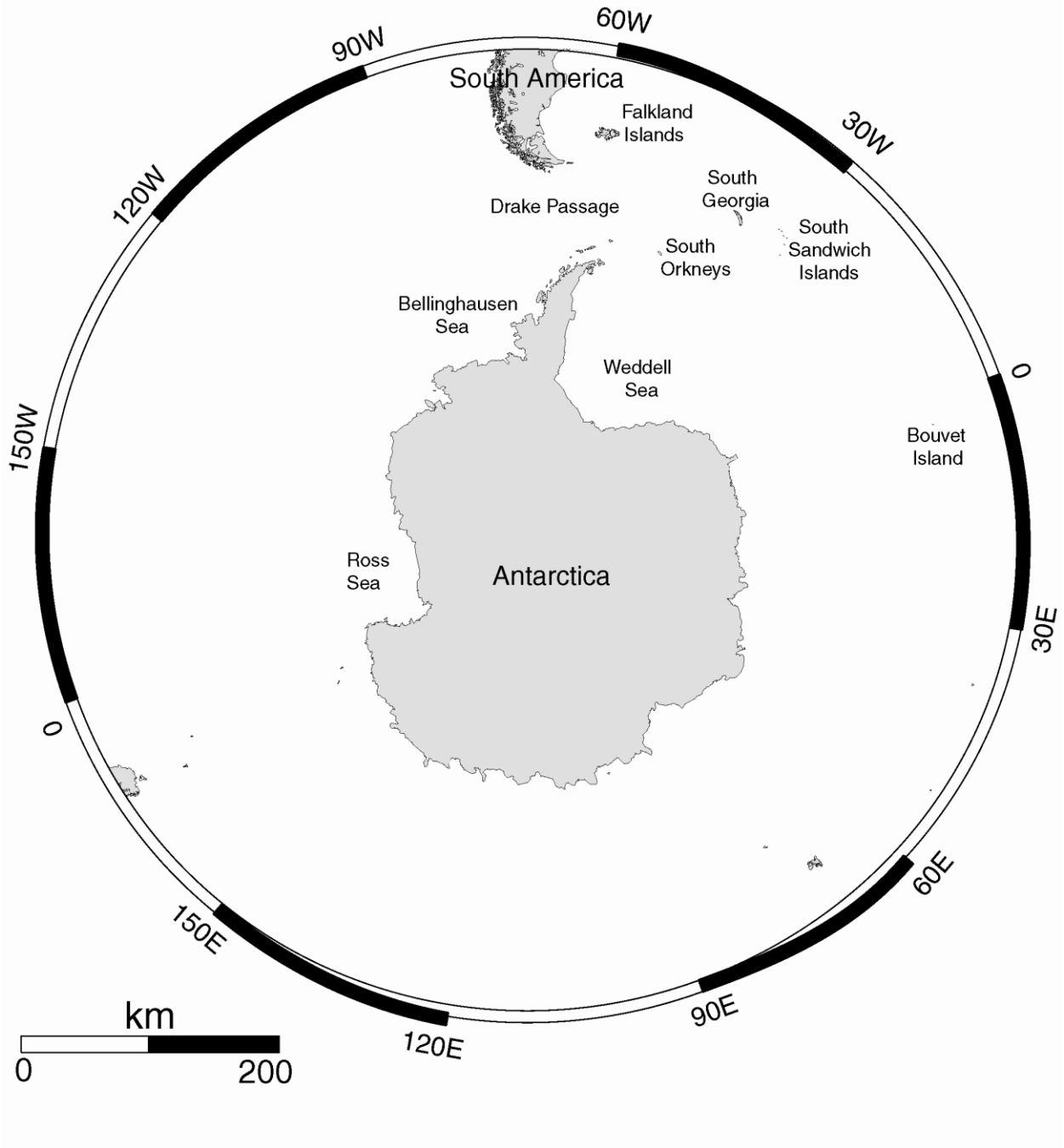

Figure 3. Map of significant geographic localities in the Southern Ocean.

CHAPTER 2. Combined morphological and molecular phylogeny of the evolutionary history of the Odontasteridae (Asteroidea)

2.1 ABSTRACT

Odontasteridae (Asteroidea: Echinodermata) comprise a clade described by Verrill 1899, and are placed within Valvatida, a derived assemblage of sea stars. Odontasterids are found in the Southern, Atlantic, and Pacific Oceans and are concentrated in high southern latitudes. To date, the phylogenetic and evolutionary history of Odontasteridae as a whole has not been rigorously examined. We conducted molecular and morphological phylogenetic analyses of Odontasteridae to assess interrelationships among and within recognized genera. Specifically, we used mitochondrial16S and COI molecular markers and 29 external morphological characters in an attempt to reconstruct the evolutionary history of the group. Generally, our results indicate that traditionally used external ossified characters are not representative of phylogenetic history of Odontasteridae. Still, we can conclude that species present in high latitudes in the Southern Hemisphere (i.e., Southern Ocean) are the most derived taxa. Additionally, mtDNA data suggests unrecognized lineages of odontasterids are present in high southern latitudes. A new species *Odontaster* nov. sp., is described from the Galapagos Islands.

2.2 INTRODUCTION

Odontasteridae Verrill 1899 is within the largest and most taxonomically diverse group of Asteroidea, Valvatacea (*sensu* Blake 1987). Odontasterids are a clade of sea stars typically found in lower shelf and upper bathyal regions, though some have been collected in the tidal and intertidal zones. Found in the Southern, Atlantic, and Pacific Oceans (Figure 1), Odontasteridae

is characterized by two series of equal, opposite, and usually conspicuous marginal plates without intermarginal channels, two rows of suckered tube-feet, and triangular mouths (Clark & Downey 1992). Odontasterid sea stars also characteristically have five rays, are most notably distinguishable by hyaline-tipped recurved spines surrounding the mouth (Figure 2), and range from stellate to almost pentagonal in shape.

Odontasteridae sea stars are exclusively benthic as adults and play an important role in marine ecosystems and food chains. For example, *Odontaster validus* has been labeled a keystone species in the Southern Ocean, capable of applying considerable influence on the environment through predation. It can be very abundant in shallow waters of high productivity where *O. validus* consumes a variety of organisms (McClintock 1988).

The taxonomic history of Odontasteridae has been complicated throughout the past two hundred years. Originally, Odontasteridae members were known as Gnathasteridae Perrier, 1894. The name Odontasteridae was first erected by Verrill in 1899 and as a result Gnathasteridae was invalidated when *Gnathaster* Sladen, 1889 was synonymized with *Odontaster* Verrill, 1880. Fisher (1940) and Clark and Downey (1992) have been the primary authorities in providing species descriptions and in sorting out Odontasteridae taxonomy. As currently recognized, there are six genera (*Acodontaster, Diabocilla, Diplodontias, Eurygonias, Hoplaster,* and *Odontaster*) and twenty-seven accepted species (Table 1). *Acodontaster* Verrill, 1899 is composed of five species, *A. capitatus, A. conspicuus, A. elongatus, A. hodgsoni,* and *A. marginatus* (Figure 3A-D); *Gnathaster elongatus* Sladen 1889 is the type species and is now regarded as *A. elongatus.* This genus is found mainly in the Antarctic, with one subspecies *A. elongatus granuliferus,* extending into the Atlantic. *Diabocilla* contains only one species, *D. clarki* McKnight, 2006, and is possibly a synonym of *Hoplaster* Perrier in Milne-Edwards, 1882

(*H. kupe* and *H. spinosus*: Figure 3F) (unpublished data; C. Mah pers. comm.). *Hoplaster spinosus* Perrier 1882, by monotypy is the type species. Despite the fact that Odontasteridae is characteristically known for hyaline-tipped glassy teeth, both the deep-sea *Diabocilla* and *Hoplaster* lack this character. *Diplodontias* Fisher, 1908, comprising *D. dilatatus, D. miliaris, D. robustus,* and *D. singularis* (Figure 4A-D), was previously referred to as *Asterodon* Perrier, 1891, and is uniquely characterized by a pair of large hyaline-tipped teeth at each jaw (Figure 2B). *Pentagonaster dilatatus* Perrier 1875 by monotypy in *Goniodon* Perrier 1894 is the type for *Dipladontias*. *Diplodontias* is revived from the synonymy of *Asterodon* Perrier, to which it was referred by Fell (1953) since both *Asterodon* and *Goniodon* are invalid junior homonyms. *Eurygonias* is monotypic; *E. hyalacanthus* Farquhar, 1913 (Figure 3E), has a single recurved spine on each oral plate and is endemic to New Zealand. Finally, *Odontaster* Verrill, 1880, comprised of 14 species, is the most speciose genus and is found in Atlantic, Pacific, and Southern Oceans. This genus includes *O. aucklandensis, O. australis, O. benhami, O. crassus, O. hispidus, O. mediterraneus, O. meridionalis, O. pearsei, O. penicillatus, O. rosagemmae, O. robustus, O. roseus, O. setosus,* and *O. validus* (Figures 5-6). *Odontaster hispidus* Verrill 1880 is described as the type species.

To date, the phylogenetic and evolutionary history within Odontasteridae has not been rigorously examined. Here, we use a combination of external morphological characters and molecular markers (16S ribosomal DNA and cytochrome c oxidase subunit I) to investigate their evolutionary history, providing insight into speciation and biogeographic patterns that may have shaped the evolution of Odontasteridae. In particular, we were interested in understanding if this group originated and radiated from the Southern Ocean to more northern latitudes or vice versa.

2.3 Materials and methods

Phylogenetic relationships within Odontasteridae were examined using molecular data (16S and COI). Then to further elucidate evolutionary trends, morphological characters were mapped onto the molecular topology. Specific methods are as follows.

2.3.1 Specimen Collection

Specimens were obtained from the Division of Echinoderms, Smithsonian Institution National Museum of Natural History (NMNH) in Washington, D.C., the Department of Invertebrate Zoology, California Academy of Sciences (CASIZ), San Francisco, California, and the National Institute of Water and Atmospheric Research (NIWA), New Zealand (Table 1). Most specimens were dried. Antarctic species were collected during two five-week research cruises aboard the *R/V Laurence M. Gould* in November/ December of 2004 and May/ June of 2006. Images of *Diabocilla clarki* were provided by NMNH.

2.3.2 Molecular data

Molecular methods follow Janosik et al. (2011). DNA extraction of specimens was performed using DNeasy® Tissue Kit (Qiagen). Two mitochondrial DNA markers (16S rDNA, COI mtDNA) were utilized to estimate the evolutionary history of Odontasteridae. Specifically, a 508 bp region of the mitochondrial 16S gene was amplified using the 16SarL and 16SbrL primers and protocols of Palumbi et al. (1991). For the same individuals, a 627 bp region of the COI gene was amplified using primers designed to work with *Odontaster* COI-Ast 22F (5' TTYTCNACNAAACAYAAGGA 3') and COI-Ast722R (5' GGRTGNCCRAARAAYCARAA 3') (Janosik et al. 2011). Amplified products were purified with either a Qiagen QIAquick® Gel

Extraction Kit (Qiagen Inc.) or using Montage PCR Filter Units (Millipore) according to the manufacturer's directions.Purified products were then sequenced bi-directionally on a Beckman CEQ 8000 Genetic Analysis System (Beckman Coulter). Sequences were edited and aligned using Sequencher 4.6 (Gene Codes Corporation) and Bioedit v.7.0.8 (Hall 1999). COI sequences were translated according to the echinoderm mitochondrial DNA code to aid in proofreading. Genbank accession numbers are listed in Table 2.

Based on current understandings of sea star relationships (Blake 1987, Mah & Foltz 2011), *Bathybiaster loripes*, *Chaetaster moorei*, *Crossaster papposus*, *Luidia foliolata*, *Mediaster aequalis*, and *Solaster stimpsoni* were chosen as the outgroup and sequences were downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank/)(Table 2).

Models of nucleotide substitution were selected with AIC criterion (GTR+G was calculated separately for the 16S and COI datasets) in MrModeltest ver. 2.2 (Nylander 2004). Separate analyses were performed on 16S and COI datasets followed by an analysis of the concatenated dataset. A Bayesian approach was used to infer phylogeny using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). Posterior probabilities were obtained by a Markov Chain Monte Carlo (MCMC) algorithm with two sets of one cold and three heated chains. Samples of trees and parameters were drawn every 100 steps from a total of 2 X 10^6 MCMC generations. The first 4,000 trees were discarded as the burnin (based on convergence of likelihood values) and the remaining trees were used to compute a consensus tree.

2.3.3 Morphological Data

Characters consist of external skeletal features and variation in accessory structures and spines. As many of the samples examined were loaned from museums, destruction for

examination of internal structures was not possible. For each species, multiple specimens were examined with the unaided eye and by stereomicroscope. Morphological characters were scored from published descriptions, photos, and/or museum specimens. Terminology follows Lambert (2000) and Clark and Downey (1992).Table 1 provides a list of species, references containing descriptions, and museum numbers employed herein. Additionally, *Odontaster* specimens collected from around the Galapagos Islands were also included in morphological character analyses. In 1998-9, the National Museum of Natural History (NMNH), Smithsonian Institution sponsored the making of a 3-D IMAX® film on the Galapagos Islands. Throughout filming, collecting submersible dives were completed using the *Johnson-Sea-Link* submersible. Pawson and Ahearn (2000) published a report of the echinoderms collected, including a new *Odontaster* species, which does not corroborate descriptions of known species. Morphological characters of these specimens were scored to quantify previously unrecognized biodiversity, but unfortunately the specimens proved not to be amendable to molecular analyses.

A data matrix consisting of 29 characters and 28 in-group taxa was constructed in NEXUS data editor 5.0 (Page 2001) (Appendix 1). Nine characters were scored as binary and 19 were coded as unordered multi-state. Morphological characters were mapped onto the recovered molecular tree to distinguish important external characters useful for phylogenetic analysis. Character transformations were evaluated and mapped on the molecular tree using a parsimony approach to show all most parsimonious states at each node using Mesquite ver. 2.74 (Maddison and Maddison 2010). First, the morphological character matrix was imported and followed by the combined 16S and COI Bayesian inference. Mesquite applies stochastic models of character state change and can explicitly accommodate uncertainty in ancestral states. Characters were mapped only for species present in the molecular tree.

2.4 RESULTS

Analyses of the concatenated 16S (GenBank accession number: XX-XX) and COI (GenBank accession numbers: XX-XX) molecular dataset consisted of 1135 bp, and provided greater resolution than topologies based on individual genes. Results for analyses based on individual genes (16S and COI) are shown in supplementary information (Appendices 2, 3). Discussion will focus on the concatenated gene topology (Figure 7). The recovered topology of the combined 16S and COI datasets using the GTR+G model had marginal support across the tree. Nonetheless a few key nodes had strong posterior probabilities, and they will be the focus of the discussion. For example, the node defining a monophyletic Odontasteridae had a posterior probability of 0.97 and monophyly of *Diplodontias* and *Odontaster* are well-supported (1.00, 0.97 respectively). *Acodontaster* is not recovered monophyletic as *A. elongatus* is recovered sister to *Odontaster* species, and *Hoplaster kupe* is recovered with the majority of *Acodontaster* species. *Eurygonias hyalacanthus* is recovered basal to *Acodontaster* and *Odontaster* species. *Diplodontias* is recovered as monophyletic and sister to all other Odontasteridae.

The morphological characters of Odontasteridae were mapped onto the Bayesian topology from the combined molecular markers (Appendix 4). Mapping was conducted using a parsimony criterion with the accelerated transformation in Mesquite ver. 2.74 (Maddison and Maddison 2010). Because we are interesting in understanding which characters are phylogenetically informative, characters with a Consistency Index of 0.50 or greater are shown in Figure 8. Such characters include: recurved spine(s) on oral plates (character 1), abactinal plate shape (character 2), number of spines per abactinal plate (character 3), presence or absence of glassy granules on abactinal plates (character 5), abactinal spine shape (character 6), distribution of papulae on abactinal surface (character 7), distinctness of marginal plate border

(character 8), length of spines on inferomarginal plates (character 14), and presence or absence of glassy granules on actinal plates (character 19). Monophyly of Odontasteridae is supported by recurved spine(s) on oral plates (character 1). Reversals were evident for four characters (Appendix 4: characters 1, 6, 7, 14). For example, shape of spines on inferomarginal and superomarginal plates (characters 12 & 14) is often used to distinguish between genera, but in terms of assessing phylogenetic relationships, these characters may not be useful due to repeated convergent evolution. Specific character changes, reversals, and losses are described in character descriptions below.

2.4.1 Morphological Character analysis of Odontasteridae:

1: Recurved spine on oral plates: 0= absent, 1= one spine, 2=two spines

Whether one or two recurved spines were present in the last common ancestor of Odontasteridae cannot be determined here. *Hoplaster* lacks a recurved spine, which is character reversal. *Acodontaster, Eurygonias, Odontaster* have one recurved, glassy spine per oral plate; *Diplodontias* has two recurved, glassy spines per oral plate, while the recurved, glassy spines are missing in *Diabocilla* and *Hoplaster.* Number of changes on tree (changes) = 2; Consistency Index (CI) = 1.0.

2: Abactinal plates: 0= tabulate, 1= paxillate, 2= highly paxillate

Tabulate abactinal plates are inferred as an ancestral character, with a change occurring in *Eurygonias hyalacanthus* and *Odontaster* species. A change to highly paxillate occurs in *O. validus. Acodontaster* has tabulate abactinal plates. *Diplodontias* has sub-tabulate abactinal plates. *Eurygonias* has abactinal plates that are paxillar and club-shaped. *Hoplaster* has abactinal plates that are tabulate. Overall, *Odontaster* has somewhat paxillate abactinal plates, although some species tend to have a more tabulate look. Changes = 3; C.I. = 0.67.

3: Abactinal spines per plate: 0= (5-10), 1= (11-15), 2= (16-20), 3= (21-25), 4= (26-30), 5= (30 and above)

Diplodontias and *Eurygonias* have the most spines, while a change occurs in *Diplodontias singularis* from a state 5 to 4. Changes = 10; C.I. = 0.45.

4: Abactinal spine: 0= smooth spines, 1= rough spines

Whether the last common ancestor of *Diplodontias* had either smooth or rough abactinal spines cannot be determined. *Acodontaster, Eurygonias, Hoplaster,* and *Odontaster* share a common ancestor that likely had rough abactinal spines.

Smooth or rough texture of spines on abactinal spines is not genus specific and varies greatly from species to species. Changes = 5; C.I. = 0.167.

5: Glassy granules on abactinal plate: 0= absent, 1= present

Glassy granules is a derived character present only in *Diplodontias miliaris, Diplodontias dilatatus, Hoplaster spinosus, O. aucklandensis, O. australis, O.* nov. sp. Changes = 2; C.I. = 0.5.

6: Abactinal spine shape: 0= granular, 1= short, stout, 2= short, slender, 3= clavate, 4= long, slender, 5= long, slender prominent spine in middle.

Granular abactinal spines are inferred as the most ancestral state. A change occurs in *Eurygonias hyalacanthus,* which has short, slender spines. A change also occurs at the base of the *Odontaster* clade to clavate spines. A reversal to granular spines occurs in *O. penicillatus.* A change occurs in *O. validus* and *O. robustus* to long, slender spines. *Acodontaster* and *Diplodontias* species all have granular abactinal spines. *Diabocilla* and *Hoplaster* species have clavate or club-shaped spines, while *Eurygonias* has short, slender spines. *Odontaster* species tend to have a variation of spine shapes. Changes = 7; C.I. = 0.71.

7: Papulae on abactinal surface: 0= restricted to arms and central disc, not found interradially, 1= absent from discs center and interradial area, 2= covering entire abactinal surface

Papulae are covering the entire abactinal surface is inferred as an ancestral character in *Eurygonias hyalacanthus* and *Diplodontias* species. *Odontaster* and *Acodontaster* species have papulae restricted to the arms and central disc. A reversal has occurred in *Hoplaster kupe,* which has papulae covering the entire abactinal surface. Changes = 3; C.I. = 0.67.

8: Marginal plate border: 0= plates form even border with abactinals, 1= plates form slightly raised border with abactinals, 2= plates form distinct border with abactinals

The inferred ancestral character is marginal plates that form a slightly raised border with the abactinals, while a change to forming an even border occurs at the base of the *Acodontaster* and *Odontaster* clade. A character reversal to a slightly raised border occurs in *O. crassus, O. meridionalis, O. pearsei,* and *O. roseus. Acodontaster hodgsoni* has marginal plates that form a distinct border with the abactinal plates. Changes = 4; C.I. = 0.50.

9: Grooves between marginal plates: 0= grooves not distinct, 1= deep grooves between plates

Diplodontias and *Eurygonias hyalacanthus* have deep grooves between plates. It is equally parsimonious that either deep grooves or grooves not distinct between marginal pates were present in the last common ancestor of *Acodontaster* and *Odontaster*. *Odontaster* species have deep grooves between marginal plates, while *Acodontaster* do not. Changes = 4; C.I. = 0.25.

10: Marginal plate shape: 0= wider than long, 1= square (block-like), 2= wedge-shaped, 3= rectangular with rounded corners

Square shaped marginal plates are inferred as ancestral, with change

occurring in *Acodontaster conspicuus* and *Eurygonias hyalacanthus* to rectangular with rounded corners shaped plates. *Diplodontias miliaris, O. crassus, O. benhami, O. meridionalis, O. pearsei, O. penicillatus, and O. roseus* have plates that are wider than long. A reversal occurs in *O. validus.* Changes = 7; C.I. = 0.43.

11: Superomarginal plates: 0= densely covered in spines of same length, 1= densely covered in spines, spines getting longer towards edge of plate

With the exception of *Hoplaster kupe, O. crassus,* and *O. hispidus*, all members of Odontasteridae have superomarginal plates densely covered in spines of the same length. *Acodontaster, Diplodontias, Eurygonias* species have superomarginal plates densely covered in spines of the same shape. *Diabocilla, Hoplaster,* and *Odontaster* species vary between densely covered in spines of the same shape and densely covered in spines getting longer towards the edge of the plate. Changes = 3; C.I. = 0.33.

12: Spines on superomarginal plates: 0= granules, 1= spinelets

Granules on the superomarginal plates are inferred as the ancestral character state. A change occurs at the base of the *Odontaster* clade to spinelets. Reversals occur in *Odontaster crassus, O. mediterraneus,* and *O. penicillatus. Acodontaster, Diplodontias,* and *Eurygonias* have granules for spines on the superomarginal plates. *Diabocilla* and *Hoplaster* have spinelets on the superomarginal plates, while *Odontaster* is varied, with some species having granules and some with spinelets. Changes = 5; C.I. = 0.20.

13: Inferomarginal plates: 0= densely covered in spines of the same length, 1= densely covered in spines getting longer toward the edge of the plate

With the exception of *Hoplaster kupe, O. crassus,* and *O. hispidus*, and *O. penicillatus*, all members of Odontasteridae have inferomarginal plates densely covered in spines of the same length. *Acodontaster*, *Diplodontias,* and *Eurygonias* have granules for spines on the inferomarginal plates. *Diabocilla* has spinelets on the superomarginal plates, while *Hoplaster* and *Odontaster* are varied, with some species having granules and some with spinelets. Changes = 4; C.I. = 0.25.

14: Spines on inferomarginal plates: 0= same as superomarginal plates, 1= longer than superomarginal plates, 2= granules, with one long, prominent spine towards outside edge of plate

Diplodontias, *Eurygonias hyalacanthus,* and *Acodontaster* have spines on inferomarginal plates that are the same as the superomarginal plates. A change to longer spines occurs in *O. meridionalis, O. pearsei, O. penicillatus,* and *O. roseus. Acodontaster marginatus* has one long, prominent spine towards the outside edge of the plate. Most *Acodontaster* species have spines that are the same length as the spines on the superomarginal plates. Changes = 4; C.I. = 0.50.

15: Glassy granules on superomarginal plates: 0= absent, 1= present, 2= present only on plates towards arm tips

The presence of glassy granules on the superomarginal plates is inferred as an ancestral character found in *Diplodontias* and *Eurygonias hyalacanthus*. Absence of glassy granules occurs at the base of *Acodontaster* and *Odontaster*, with changes in *O. benhami, O. crassus* and *O. mediterraneus. Odontaster hispidus* has glassy granules present only on the plates towards the arm tips. Changes = 5; C.I. = 0.40.

16: Glassy granules on inferomarginal plates: 0= absent, 1= present, 2= present only on plates towards arm tips

The ancestral state of the glassy granules on the inferomarginal plates cannot be determined. Glassy granules are present in *E. hyalacanthus, Diplodontias dilatatus, D. miliaris, Odontaster benhami, O. crassus, and O. mediterraneus. Odontaster hispidus* has glassy granules, but only on the plates towards the arm tips. Changes = 6; C.I. = 0.33.

17: Number of chevrons on actinal surface: 0= 3, 1= 4, 2= 5, 3= 6, 4= 7

The number of chevrons present on the actinal surface is quite variable across and within all genera (character not mapped on Figure 8).

18: Spines per plate on actinal surface: 0= (4-9), 1= (10-15), 2= (16-20), 3= (8-10 with one prominent longer spine)

The number of spines per plate on the actinal surface is varied across genera and species within genera. Sixteen to twenty spines per plate on the actinal surface was inferred as the ancestral state. Changes occur at the base of the *Diplodontias miliaris* and *D. dilatatus* clade and several times throughout *Acodontaster* and *Odontaster* clades. Changes = 10; C.I. = 0.10.

19: Glassy granules on actinal surface: 0= absent, 1= present

Acodontaster, Diabocilla, Diplodontias, Eurygonias, and *Hoplaster* are all lacking in glassy granules on the actinal surface. Only two members of *Odontaster* have glassy granules present on the actinal surface. Changes = 1; C.I. = 1.0.

20: Number of furrow spines: 0= (1-2), 1= (2-3), 2= (3-4), 3= (4-5)

The number of furrow spines is varied across and within taxa. The majority of taxa have 2-3 furrow spines. Changes = 9; C.I. = 0.33.

21: Furrow spine shape: 0= smooth, cylindrical, 1= smooth, pointy, 2= rough, cylindrical, 3= rough, pointy

Diplodontias species and *E. hyalacanthus* have smooth, cylindrical furrow spines. A change to smooth, pointy spines occurs at the base of the *Acodontaster* and *Odontaster* clade. Further changes occur within the *Odontaster* and *Acodontaster* clades. Character reversal to smooth, cylindrical furrow spines occurs in *O. benhami, O. hispidus,* and *O. penicillatus.* Changes = 14; C.I. = 0.21.

22: Adambulacral plate shape: 0= rectangle, 1= square

The ancestral character state is inferred as rectangular Adambulacral plates. Character state changes occur in lineages leading to *Acodontaster hodgsoni, A. marginatus, Hoplaster kupe, Odontaster penicillatus,* and *O. robustus.* Changes = 5; C.I. = 0.20.

23: Pedicellariae: 0= absent, 1= present

All *Acodontaster*, except *A. capitatus* have pedicellariae. *Diabocilla, Hoplaster,* and *Diplodontias* (except *D. singularis*) are lacking pedicellariae. *Eurygonias* and several members of *Odontaster* have pedicellariae. Type and appearance of pedicellariae are variable. Changes = 3; C.I. = 0.33.

24: Body shape outline: 0= pentagonal, 1= subpentagonal, 2= pentagonal-stellate, 3= substellate, 4= stellate

Determination of the body shape outline in the last common ancestor of Odontasteridae is not possible. Changes = 12; C.I. = 0.33.

25: Interradial arc: 0= rounded, 1= sublinear, 2= linear

A rounded interradial arc is the inferred ancestral character state. All *Acodontaster* and the majority of *Diplodontias*species have rounded interradial arcs. *Diabocilla* and *Hoplaster* have sublinear interradial arcs, while *Odontaster* have either rounded or sub-linear interradial arcs. *Eurygonias* has a completely linear interradial arc. Changes = 6; C.I. = 0.33.

26: Arm length: 0= short, 1= medium, 2= elongate

Acodontaster have elongate arms. *Diabocilla* has medium-length arms. *Diplodontias, Hoplaster,* and *Odontaster* have a variety of arm length, while *Eurygonias* have very short arms. Changes = 12; C.I. = 0.17.

27: Number of apical spines per oral plate: 0= 2, 1= 3, 2= 4, 3= 5, 4= 6

The majority of taxa have four apical spines per oral plate. Changes occur at terminal nodes within *Acodontaster* and *Diplodontias.* Several changes occur throughout the *Odontaster* clade (character not mapped).

28: Number of suboral spines per oral plate: 0= 2, 1= 3, 2= 4, 3= 5, 4= 6+

The character state of the last common ancestor of Odontasteridae is equivocal with either two or three suboral spines per oral plate. The number of suboral spines per oral plate is variable across and within genera (character not mapped).

29: Number of marginal spines per oral plate: 0= (2-3), 1= (3-4), 2= (4-5), 3= (5-6), 4= (6-7)

The number of marginal spines per oral plate is variable across and within genera (character not mapped).

The Galapagos *Odontaster* specimens (3 individuals) are morphologically distinguishable from all other known *Odontaster* species. Specifically, these *Odontaster* specimens can be

easily recognized by a longer, prominent spine in the middle of each abactinal plate (character 6). No other *Odontaster* species possess such a spine. Thus, Galapagos specimens represent distinct biodiversity and warrant full species status as *Odontaster* nov. sp. (see description below).

2.4.2 SYSTEMATICS

Order VALVATIDAPerrier, 1884
Family ODONTASTERIDAE Verrill, 1899

Genus ODONTASTER Verrill, 1880

Odontaster nov. sp.
(Figure 5E)

Material examined. *Holotype.* Equatorial Pacific Ocean; Galapagos Islands, Darwin Island, 01°42'N, 092° 00'W, 348- 435 meters in depth, specimen wet (alcohol) R = 2.3mm, r =1.4mm, USNM E51298, 18 July 1998, collected by D.L. Pawson and J. McCosker. *Paratypes:* USNM E51299 (two individuals R =1.8cm, r =1.2cm; R = 2.0cm, r = 1.3cm), Equatorial Pacific Ocean; Galapagos Islands, Darwin Island, 01°42'N, 092° 00'W, 348- 435 meters in depth, specimen wet (alcohol); CAS 115202 (one individual R=1.9cm , r=1.2cm), North Pacific Ocean; Galapagos Islands, Isla Espanola, 01°22.20'S, 089° 49.20'W, 353.5 meters in depth, specimen wet (alcohol).

Diagnosis. Arms 5. Generally: R= 2.03 mm; r= 1.30 mm; R/r= 1.56. Body form almost pentagonal; rays not distinguishable. Interradial margin slightly incurved. Overall, body laterally flat. Arm tips tilting slightly up. Characteristic recurved glassy-tip spine on each oral plate. All plates of this sea star are decorated with spines with one prominent, longer spine on abactinal plates.

Description. Abactinal plates small and almost flat. Abactinal plates hexagonal to rounded in shape and smaller in interradial regions. Radial regions swollen with interradial regions depressed. Each abactinal plate covered with 5-8 rough and slender spines, with one longer prominent spine in center of plate. All spines on abactinal plates taper from a thicker base to the tip, forming a point. Pedicellariae present on abactinal surface are mostly at boarder between abactinal plates and marginal plates. Pedicellariae are straight, with two to four hooks. Madreporite is rounded surrounded by abactinal plates with spines.

Approximately 14 marginal plates present interradially (arm tip to tip). Marginal plates form a strong rounded boarder with abactinal plates, but a smooth transition from marginals to actinal plates. Marginal plates are significantly larger compared to abactinal and actinal plates. Plates homogenous in size in interradial region, slightly smaller at tip of arm. Superomarginal plate surface covered in rough short spines with one significantly longer spine at edge of plate. Pedicellariae lining edges of superomarginal plate with a few randomly scattered on plates.

Inferomarginal plates similar in size to superomarginals. Plate surface covered in rough spines with two prominent rows of 3-5 longer spines at the edge of inferomarginal plate and superomarginal plate. Rows of medium spines also present before and after row of prominent longer spines. No pedicellariae present on inferomarginal plates.

Actinal plates arranged to form 3 complete chevrons with plates shaped polygonal to rectangular. Actinal plates towards arm tips are small compared to plates found interradially. Plates small and flat with spines for armature. All spines rough in appearance and taper, being more slender at tip compared to base. Approximately 6-9 spines per plate, with spines arranged in a circular fashion around one longer prominent spine situated in the center of the plate. Pedicellariae present on actinal plates positioned parallel to the Adambulacral plate series. One pedicellariae per plate, with two to four jaws. A few randomly scattered pedicellariae present on actinal plates. Furrow, sub-ambulacral regions crowded. Three to four rough, slender furrow spines with approximately 8 spines on each adambulacral. Oral plates covered with spines, with one dominating enlarged glassy-tipped recurved spine on each plate. Specifically, the armature on each plate consists of four spines lining each side of enlarged recurved spine. Oral plates triangular in shape. Ambulacral furrow shallow, lined with two rows of tube feet extending to arm tips. Wet preserved specimens, colored off-white.

Distribution. North Pacific Ocean, Galapagos Islands, Darwin Island; Isla Espanola, 349- 436 meters in depth.

Etymology. This sea star is named in honor of Cynthia Anne Gust Ahearn, Museum Specialist, Department of Invertebrate Zoology, USNM whose contributions greatly enriched echinoderm biology.

Remarks. This species is distinguishable by longer, prominent spine in the middle of each abactinal plate (character 6). The distribution of this species appears isolated and specimens have only been collected from around the Galapagos Islands. Additionally, based on morphological characters, *Odontaster* nov. sp. is most likely closely related to *O. crassus*.

2.5 Discussion

2.5.1 Phylogeny

Phylogenetic reconstruction based on mitochondrial genes suggests that a monophyletic Odontasteridae consists of three main clades. These clades roughly correspond to the three more speciose genera: *Acodontaster, Diplodontias,* and *Odontaster*. Whereas monophyly of *Odontaster* (PP=0.97) and *Diplodontias* (PP=1.00) are well supported by Bayesian Inference, support for a monophyletic *Acodontaster* is lacking. Moreover, *Diplodontias* is recovered as the basal-most clade (PP=0.97) and *Odontaster* as a derived clade within Odontasteridae.

Eurygonias hyalacanthus is recovered sister to *Acodontaster* and *Odontaster* species. *Hoplaster* appears to be related to main *Acodontaster* clade, which is consistent with depth distribution.

Monophyly of *Acodontaster* is called into question by the placement of *Acodontaster elongatus* as sister to *Odontaster* clade; although support for this placement is weak. Interestingly, *A. elongatus* has been previously referred to as *Odontaster cremeus* Ludwig, 1903. Fisher (1940) considered *Odontaster cremeus* a synonym of *A. elongatus.* Clark (1962) argued that *O. cremeus* was in fact a valid species but, according to Jangoux and Massin (1986) the *O. cremeus* type has been lost and thus made *O. cremeus* a junior synonym of *A. elongatus.* Based on our inspections herein, *A. elongatus* superficially looks more similar to *Odontaster* rather than *Acodontaster.* Specifically, *A. elongatus* has prominent marginal plates, a compressed flattened body, rather than the typical puffy *Acodontaster* appearance, and a body shape outline that is more similar to *Odontaster* species. Given the placement of *A. elongatus* as sister to *Odontaster*, Ludwig's original designation deserves reevaluation. Thus, taxonomic revision is likely necessary for *A. elongatus*, definitive revision should wait until the phylogenetic position of *A. elongatus* is better supported and additional samples *O. cremeus* and *A. elongatus* have been studied. With further investigation and possible location of the *Odontaster cremeus* type, taxonomic revisions are likely necessary for *A. elongatus.*

Biodiversity within Odontasteridae is greater than previously thought (see Janosik & Halanych 2010, chapter 3). Given previous hypotheses about Antarctic invertebrate endemicity and distributions spanning the Drake Passage (Dell 1972, Dayton et al. 1974, Arntz et al. 1994, Shaw et al. 2004, Hunter & Halanych 2008, Thornhill et al 2008), we included individuals of *Acodontaster capitatus* and *Odontaster meridionalis* collected from both the north and south sides of the Drake Passage in the Southern Ocean (i.e. South American waters and Antarctic

Peninsular waters). Molecular data show individuals from South American waters are different from individuals from Antarctic waters; a common trend in the Southern Ocean (reviewed in Janosik & Halanych 2010). Further phylogeographic analyses are needed especially for these species to determine if species diversity has been under recognized.

2.5.2 Morphological and taxonomic implications

Examination of the morphological characters in light of the molecular topology suggest some characters (i.e. number of recurved spine(s) (character 1); shape, texture, number of abactinal spines and plates (characters 2, 3, 6), and presence or absence of glassy granules (character 6)) are taxonomically useful when determining genera, species boundaries, or unrecognized biodiversity in Odontasteridae. Overall, the number of, or lack of, glassy recurved spines at the oral apex (character 1) appears to be an informative character when defining species of Odontasteridae and when distinguishing between genera (CI= 1.0, Figure 2A-B). Specifically, *Acodontaster, Eurygonias,* and *Odontaster* species all possess a glassy recurved spine on each set of oral plates, equaling a total of five per individual (Figure 2-A). *Diplodontias* species have two glassy recurved spines on each set of oral plates, for a total of ten spines at the jaw apex (Figure 2-B). Even though the number of recurved spines at the jaw apex is a strong generic level diagnostic character (Clark &Downey 1992), *Diabocilla* and *Hoplaster* both lack oral spines at the jaw apex. However, *Diabocilla* and *Hoplaster* maintain the other Odontasteridae taxonomically informative characters, such as abactinal plate shape (character 2), number of spines per abactinal plate (character 3), and marginal plate border (character 8). Whether the last common ancestor of Odontasteridae possessed one or two recurved glassy spines are equally parsimonious hypotheses (Figure 8). Additionally, a single recurved glassy spine at the jaw apex

(*Acodontaster, Odontaster)* is a more derived character, while no spine(s) at the jaw apex appears to have been lost at least once (*Hoplaster)*.

Other taxonomically informative characters include: paxillate or tabulate abactinal plates (character 2, CI= 0.67), number of abactinal spines per plate (character 3, CI= 0.5), and abactinal spine shape (character 6, CI=0.71). For example, *Odontaster* species have paxillate abactinal plates (character 2), while *Eurygonias hyalacanthus* has highly paxillate plates, both more derived character states than tabulate plates which are seen in most *Acodontaster* and *Diplodontias* individuals. Likewise, more basal genera have more abactinal spines per plate, while more derived genera have fewer. Abactinal spine shape (character 6, CI= 0.71) and presence or absence of glassy granules on the abactinal plates (character 5, CI= 0.50) is helpful in distinguishing species. Distribution of papulae on the abactinal surface (character 7, CI= 0.67) is also informative when distinguishing between ancestral and more derived genera. Overall, several characters on the abactinal plates appear to be important and informative for taxonomy and species designation, but the majority of external morphological characters are not as informative in reconstructing the evolutionary history of Odontasteridae.

Broadly, presence or absence of certain characters can be used to unite members of *Acodontaster*, *Diplodontias*, or *Hoplaster*, but not within *Odontaster*. Particularly, *Odontaster* seems to be the most variable and problematic genus, because finding morphological characters that unite this genus and/ or distinguish it from other members are lacking. As a general rule, *Odontaster* species have paxilliform abactinal plates (character 2). The great diversity of character variation within *Odontaster* may be due to the fact that there are fifteen currently recognized species in this genus. Other genera with Odontasteridae are far less speciose (*Acodontaster*= 5, *Diabocilla*= 1, *Diplodontias*= 4, *Hoplaster*= 2, *Eurygonias*= 1). Thus, given

that *Odontaster* has a world-wide distribution and is collected at a variety of depths, it is likely occupying different ecological niches possibly resulting in unique character evolution (Fisher 1940, Clark &Downey 1992, Clark &McKnight 2001). This variable and speciose genus may also be a result of human induced bias when defining and assigning species. Thus, although many morphological characters have been useful for taxonomic designations, several of the external ossified characters typically used in asteroid taxonomy are evolutionarily plastic. Because of this situation, more effort is need to understand which morphological features are appropriate for use in phylogenetic inference versus taxonomic designation and identification.

Unfortunately, we were not able to gain usable DNA from all Odontasteridae samples. Thus outstanding questions still remain; including, placement of *Odontaster* nov. sp. and *Diabocilla clarki*. As discussed below, some morphological characters are more representative of sea star phylogeny, as judged by the mtDNA tree, than others. By focusing on characters that show limited homoplasy, we are able to hypothesize that *Odontaster* nov. sp. is closely related to a derived *Odontaster* clade circumscribed by *O. mediterraneus* and *O. hispidus*. Similarly, *D. clarki* is likely associated with *Hoplaster*. McKnight (2006) states that *D. clarki* differs by having abactinal plates barely elevated and both abactinal and marginal plates that are covered with tubercles (granules) rather than spines (characters 2, 3). Characters uniting *Diabocilla* and *Hoplaster* include: missing recurved glassy spines at the jaw apex (character 1), clavate-shaped abactinal spines (character 6), and the marginal plates form an even border with the abactinal plates (character 8). Characters differences between *Diabocilla clarki* and *Hoplaster* include: more spines per abactinal plate (character 3), fewer spines per actinal plate (character 18), and papulae on the abactinal surface (character 7), although these characters are often variable within a genus.

2.5.3 BIOGEOGRAPHICAL IMPLICATIONS

Southern Ocean organisms are thought to have originated by introduction and subsequent diversification into Antarctic regions from adjacent regions or by origins in Antarctic waters with diversification into surrounding regions. Interestingly, diversity within Odontasteridae is highest in high southern latitudes. Our analyses show species occurring strictly in Antarctic waters (*Acodontaster* spp. and *Odontaster* spp. shown in blue on Figure 7) are among the most derived members, suggesting that patterns of diversification occurred into or within Antarctic regions. This scenario is plausible given that several odontasterids occur in areas adjacent to the Southern Ocean (i.e. southern tips of South America, and South Africa, New Zealand, Australia). Whereas this conclusion is consistent with other studies (e.g., Brandt 1992, Crame 1993, Gebruk 1994, Briggs 2003, Pawlowski et al. 2007, Brandt et al. 2007) that suggest the Southern Ocean to be a center of origin for deep-sea organisms, it contrasts with Strungell et al.'s (2008) findings that deep-sea octopods radiated out of the Antarctic during periods of diversification. Presumably, the presence of cold water is the common factor in both deep and polar waters that allow the organisms to survive, but patterns of diversification in the deep Southern Ocean may need to be treated on a taxon by taxon basis.

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2.6 LEGEND OF ILLUSTRATIONS

Figure 1. Worldwide distribution of Odontasteridae. Dots indicate select known collection localities.

Figure 2. Characteristic hyaline-tipped recurved spines surrounding the mouth. A. single recurved spine at jaw apex, B. double recurved spines at jaw apex, C. spines of *Odontaster* nov. sp.

Figure 3. Plate I: aboral view. A. *Acodontaster marginatus,* B. *Acodontaster elongatus,* C. *Acodontaster capitatus,* D. *Acodontaster conspicuus,* E. *Eurygonias hyalacanthus,* F. *Hoplaster kupe.*

Figure 4. Plate II: aboral view of *Diplodontias* species. A. *Diplodontias dilatatus,* B. *Diplodontias singularis,* C. *Diplodontias robustus,* D. *Diplodontias miliaris.*

Figure 5. Plate III: aboral view of *Odontaster* species. A. *Odontaster benhami,* B. *Odontaster crassus,* C. *Odontaster hispidus,* D. *Odontaster aucklandensis,* E. *Odontaster* nov. sp., F. *Odontaster meridionalis*

Figure 6. Plate IV: aboral view of *Odontaster* species. A. *Odontaster setosus,* B. *Odontaster validus,* C. *Odontaster robustus,* D. *Odontaster rosagemmae,* E. *Odontaster penicillatus,* F. *Odontaster roseus.*

Figure 7. Bayesian inference topology for combined 16S and COI sequence data. Analysis details are provided in text. Number next to node indicates Bayesian posterior probabilities. Color indicates species locality, see legend.

Figure 8. Morphological characters with Consistency Index of 0.50 or greater mapped on to Bayesian Inference tree. Characters include: recurved spine(s) on oral plates (character 1), abactinal plate shape (character 2), number of spines per abactinal plate (character 3), presence or absence of glassy granules on abactinal plates (character 5), abactinal spine shape (character 6), distribution of papulae on abactinal surface (character 7), distinctness of marginal plate border (character 8), length of spines on inferomarginal plates (character 14), and presence or absence of glassy granules on actinal plates (character 19). Analysis details are provided in text. Bars indicate where a change has occurred and squares indicate character reversals or loss of a character. Asterisks indicate character states that were equally parsimonious at a node.

Appendix 1. Matrix of morphological characters.

Appendix 2. Bayesian inference topology for 16S sequence data. Analysis details are provided in text. Number next to node indicates Bayesian posterior probabilities.

Appendix 3. Bayesian inference topology for COI sequence data. Analysis details are provided in text. Number next to node indicates Bayesian posterior probabilities.

Appendix 4. All morphological characters mapped on to Bayesian Inference tree. Analysis details are provided in text. Bars indicate where a change has occurred and squares indicate character reversals or loss of a character. Asterisks indicate character states that were equally parsimonious at a node.

Table 1. List of Odontasteridae species used in this study along with description reference, distribution, depth, and catalog numbers.

Taxon	Reference	Distribution	Depth	GenBank Accession Number	Museum/Catalog Number
Acodontaster					
capitatus	Clark 1962	Bellingshausen to Ross Sea	193-647m		USNM E53545, E53473
conspicuus	Fisher 1940	Adelie Land, Graham Land, South Georgia	46-647M		USNM E53236
elongatus	Fisher 1940	Sub-Antarctic, Palmer Archipelago, Patagonia, Falkland Islands	91-600m, 50-336m		USNM E13681, 1082875
hodgsoni	Clark 1962	Western Antarctic, South Georgia	4-457m		CASIZ 174674; USNM E43860
marginatus	Clarke & Johnson 2003	Graham Land and Queen Mary Land	250-291m		USNM 1082938
Diabocilla					
clarki	McKnight 2006	known only from near Chatham Rise, central New Zealand, on the hills Diabolical & Zombie	890-970m		N/A
Diplodontias					
dilatatus	Clark & McKnight 2001	Cook Strait southwards to Snares Island, New Zealand	0-70m		NIWA 43632, 43646; USNM E9986
miliaris	Clark & McKnight 2001	east coast of South Island, Kiakoura to Foveaux Strait, New Zealand	0-101m		NIWA 43636; USNM E10145
robustus	Clark & McKnight 2001	Auckland Islands, South of New Zealand	Intertidal		N/A
singularis	Clark & Downey 1992	Mar del Plata, N. Argentina to Tierra del Fuego, also from Chile	0-84m		USNM 1084439,
Eurygonias					
hyalacanthus	Clark & McKnight 2001	east coast of New Zealand from Cook Strait south to Snares Island	0-7m		NIWA 43624, 43622, 43618
Hoplaster					
kupe	Clark & McKnight	west of North Island, New Zealand, Fairway Trough, Bellona Gap, Lord Howe Rise	2000-2417m		NIWA 15439, 43647, 43631
spinosus	Clark & Downey 1992	Azores, west Ireland north up the Rockall Trough and far to the	1795-3310m		N/A

		south off Cape Town			
Odontaster					
aucklandensis	Clarke & McKnight 2001	Chatham Rise, Campbell Plateau, Bounty Platform, New Zealand	55-353m		NIWA 43626, 43629, 43637, 43688, 31216, 43640
australis	Clark & Downey 1992	west coast of South Africa	243-366m		
benhami	Clark & McKnight 2001	Hawke Bay southwards to S. New Zealand, Chatham Islands; New South Wales, Australia	0-549m, in Australia 468-549m		NIWA 43627, 43634, 43619, 43633, 43630, 28106, 43644; USNM E09755
crassus	Fisher 1911	Monterey Bay to San Diego, California	80-500m		CASIZ 113242; USNM 31828
nov. sp.	Pawson & Ahearn 2000	Galapagos Islands	105-925m		USNM E51299, E51298; CASIZ 115202
hispidus	Clark & Downey 1992	George's Band, NE of Cape Cod to Florida Strait	50-1160m		USNM E26326
mediterraneus	Clark & Downey 1992	Porcupine Seabight; SW of Ireland, Bay of Biscay; Mediterranean	414-1800m		USNM 030212
meridionalis	Fisher 1940	Antarctic, circumpolar; north to South Georgia, Marion Island, Kerguelen	0-646m		NIWA 43639USNM 1104652, E53413, 1091163
pearsei	Janosik & Halanych 2010	Antarctic Peninsula	132m		USNM 1127022
penicillatus	Clark & Downey 1992	around Cape Horn, Argentina, south to the Falkland-Magellan region; Chile	8-350m		NIWA 43628; USNM E47752, 1082945, 1104651, 1084431
rosagemmae	Clark & McKnight 2001	east of Chatham Island and off the east coast of North Island	445-1190m		NIWA 43623, 43635, 43621
roseus	Janosik & Halanych 2010	Antarctic Peninsula	132m		USNM 1127023
robustus	Clark & Downey 1992	S. of Cape Cod to Florida; northern Gulf of Mexico	160-675m		USNM E12910, E37332,
setosus	Clark & Downey 1992	from off Martha's Vineyard to Carolina coast	100-739m		USNM 1017559, 1017562
validus	Fisher 1940	Antarctic, circumpolar, north to South Georgia , Bouvet Island	0-653m		NIWA 43620, 43625, 27912, 27928; USNM E13408

Figure 1.

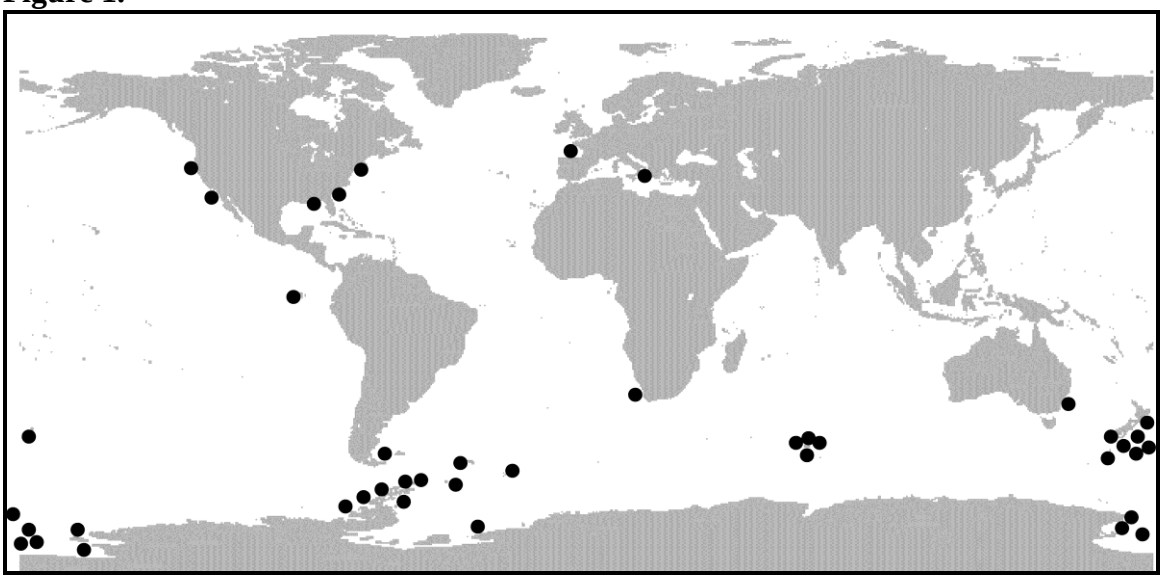


Figure 2.

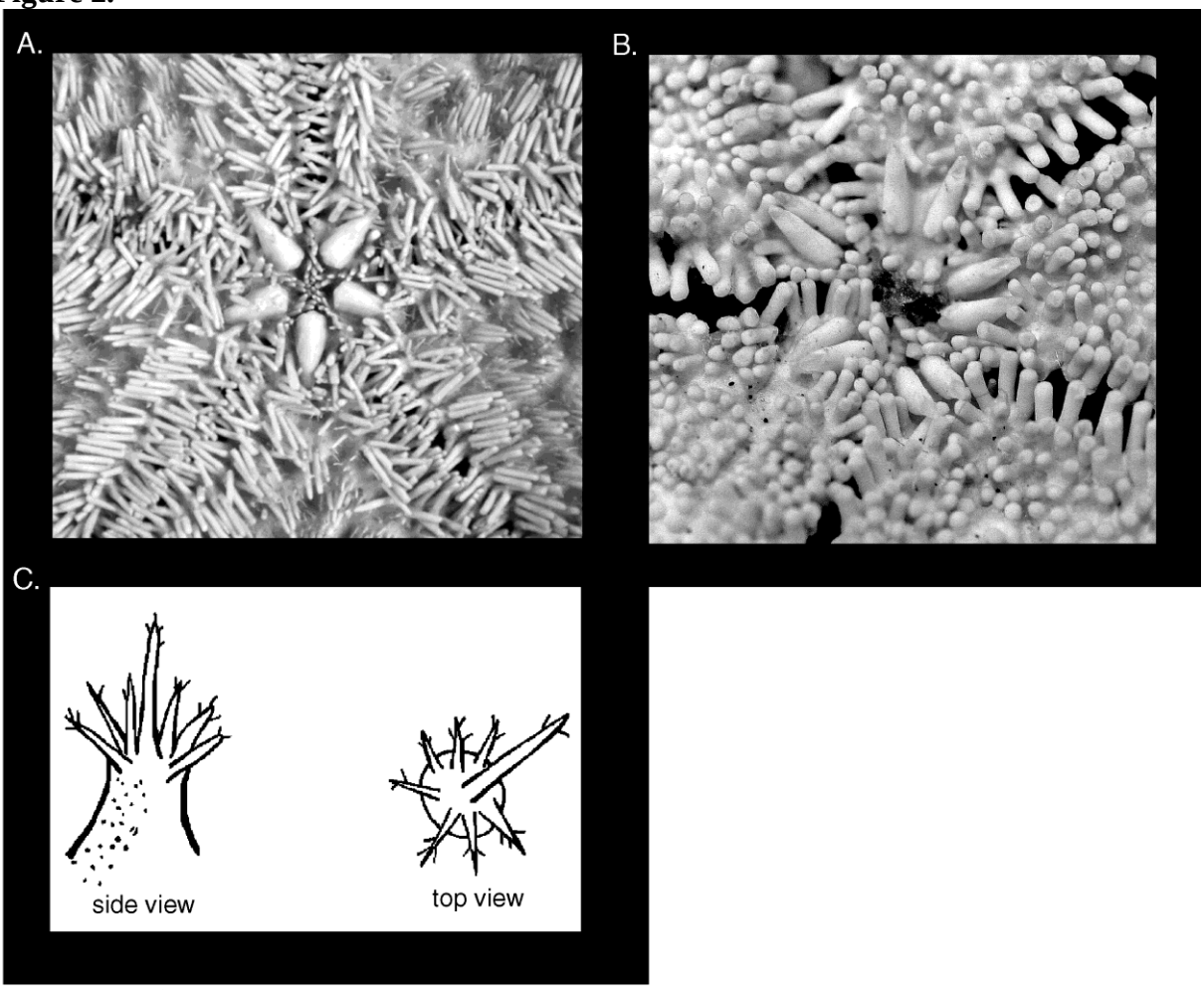


Figure 3.

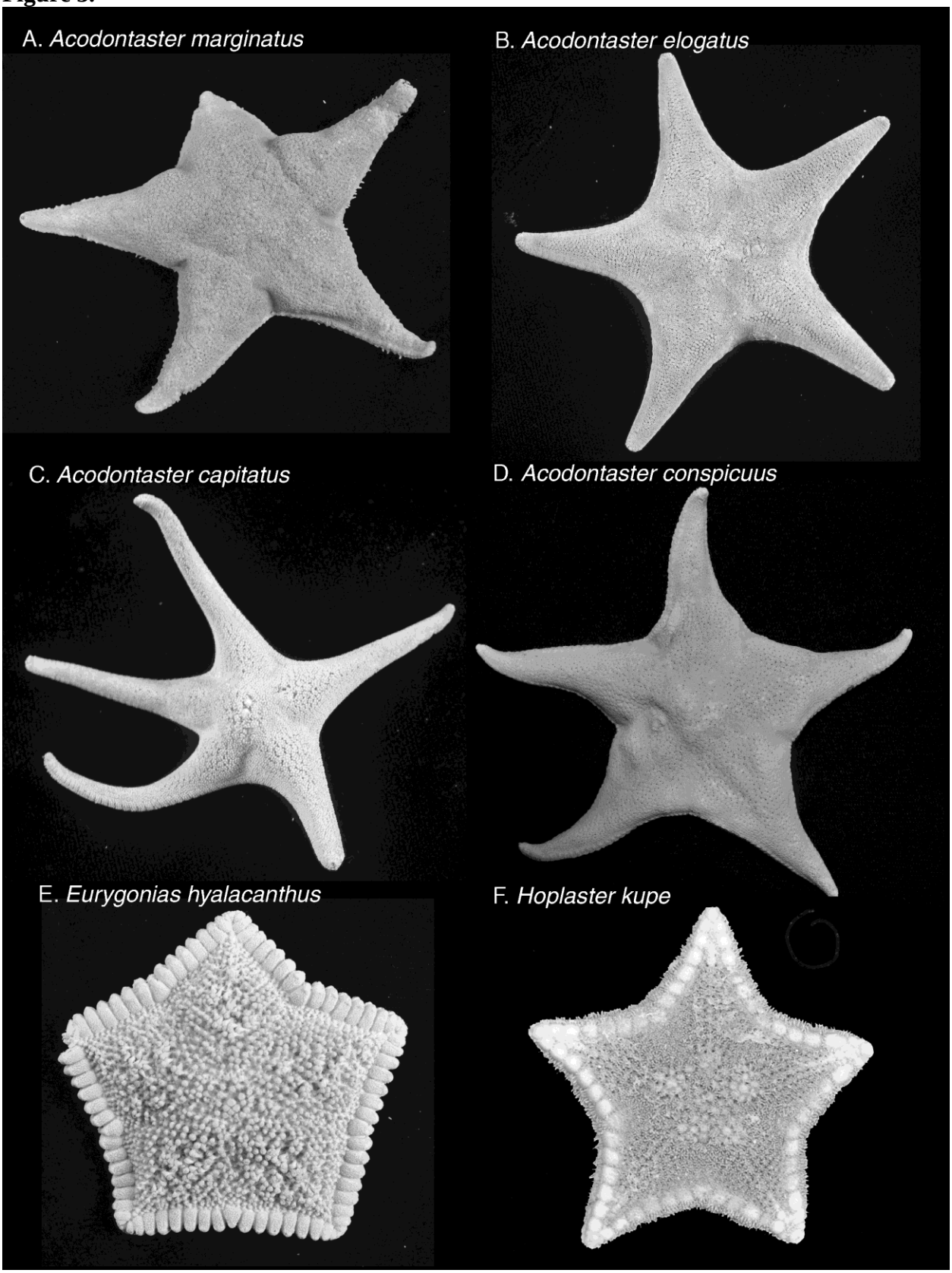

A. *Acodontaster marginatus*
B. *Acodontaster elogatus*
C. *Acodontaster capitatus*
D. *Acodontaster conspicuus*
E. *Eurygonias hyalacanthus*
F. *Hoplaster kupe*

Figure 4.

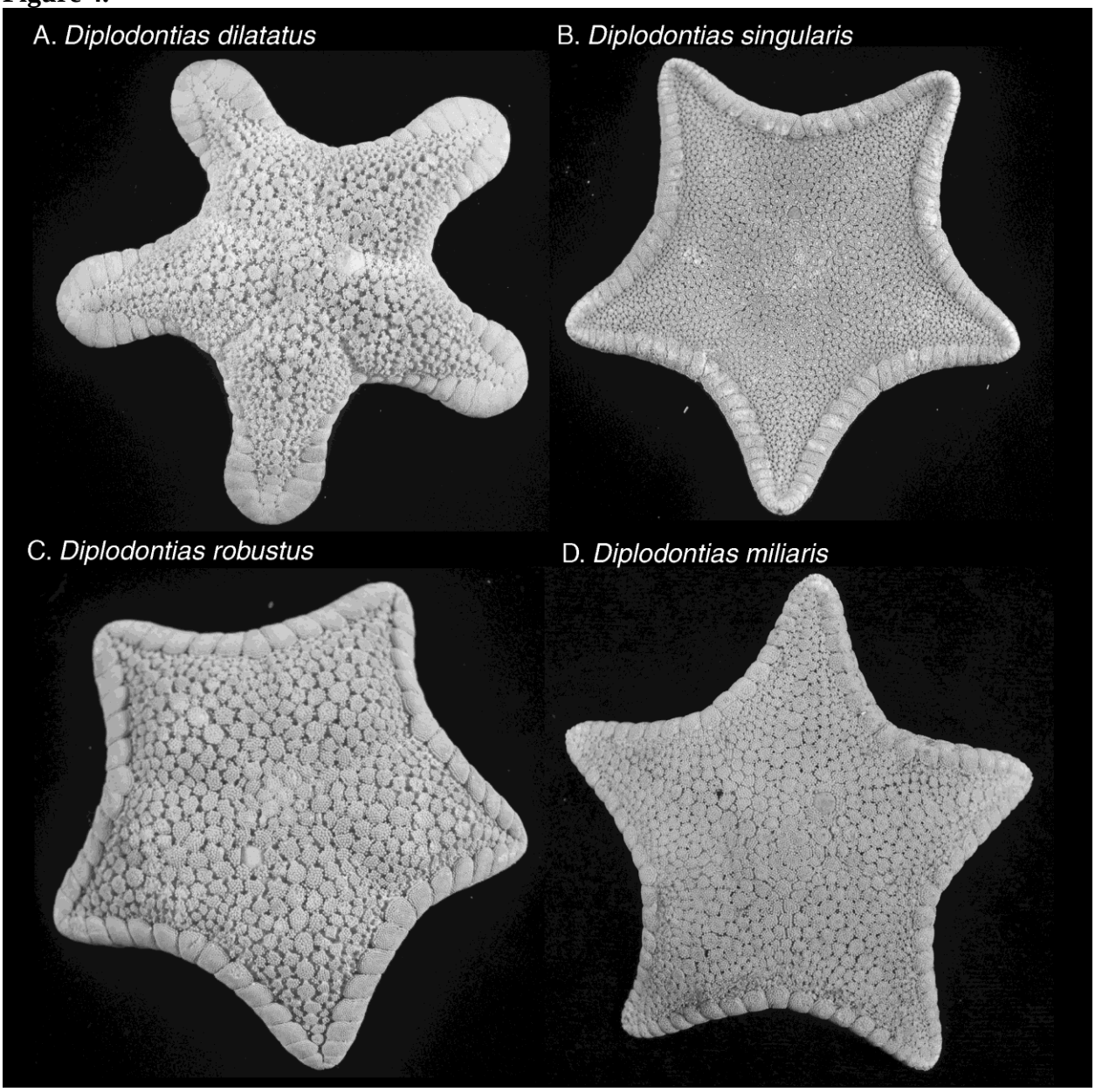

A. *Diplodontias dilatatus*
B. *Diplodontias singularis*
C. *Diplodontias robustus*
D. *Diplodontias miliaris*

Figure 5.

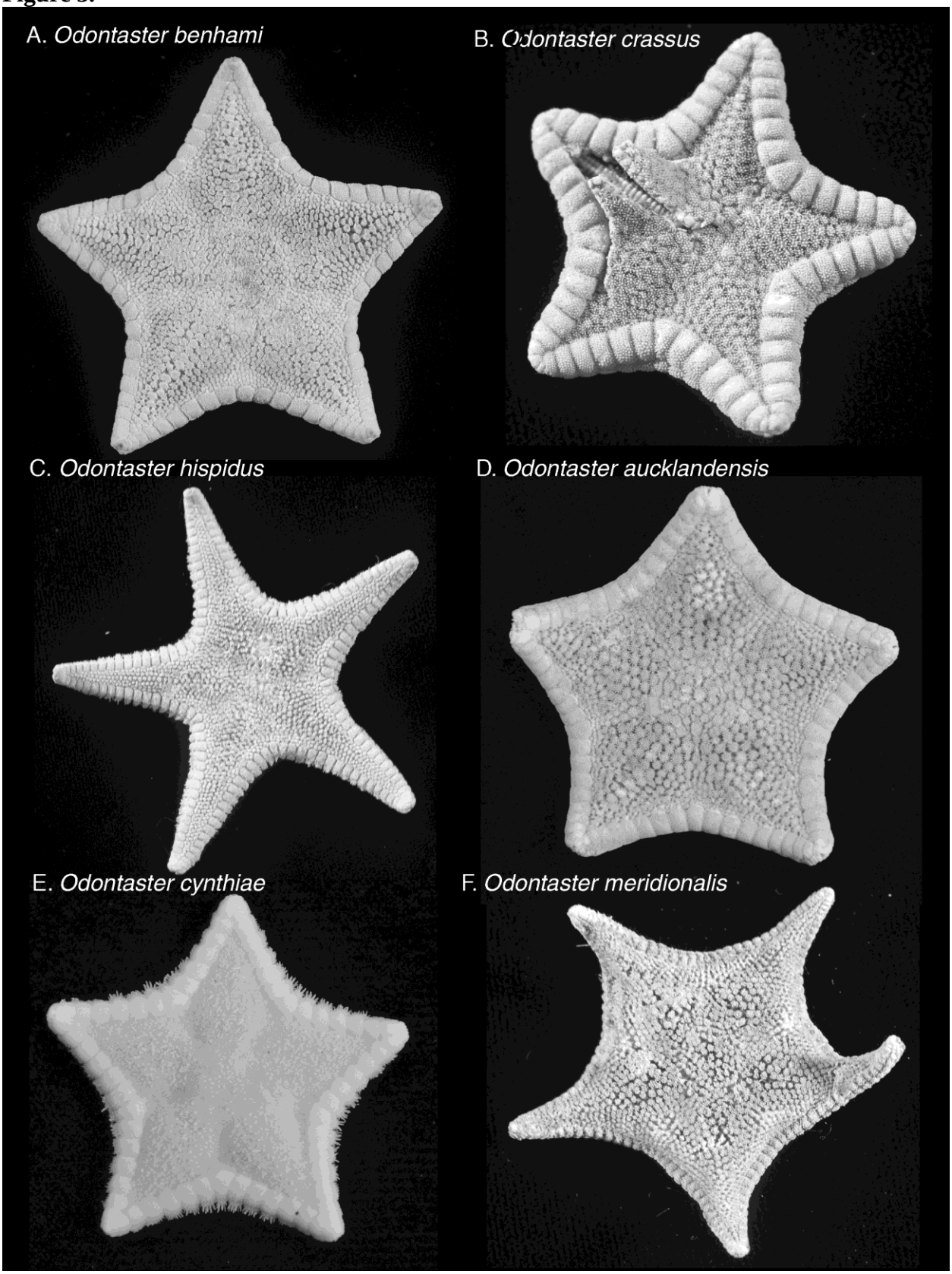

A. *Odontaster benhami*
B. *Odontaster crassus*
C. *Odontaster hispidus*
D. *Odontaster aucklandensis*
E. *Odontaster cynthiae*
F. *Odontaster meridionalis*

Figure 6.

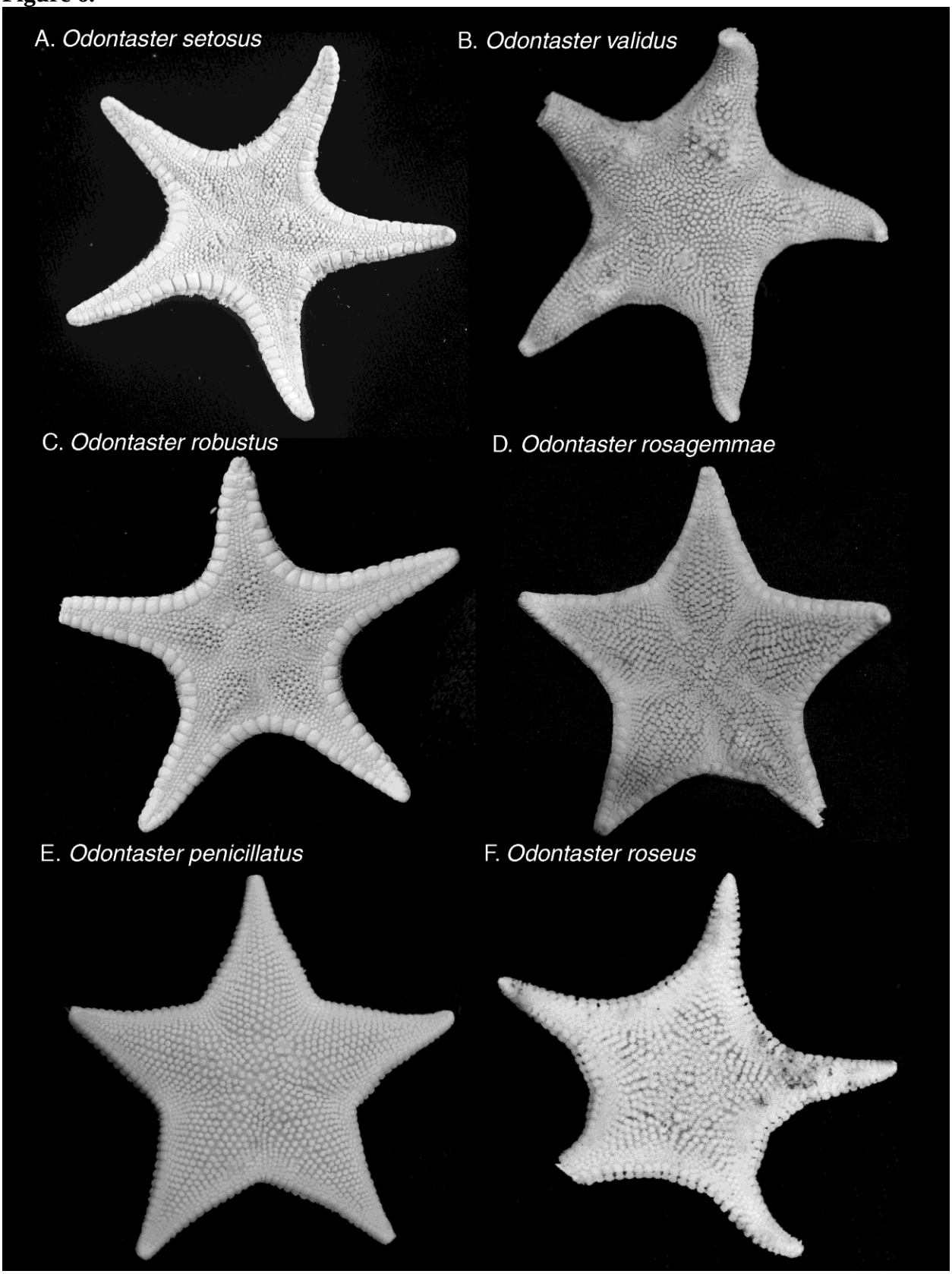

A. *Odontaster setosus*
B. *Odontaster validus*
C. *Odontaster robustus*
D. *Odontaster rosagemmae*
E. *Odontaster penicillatus*
F. *Odontaster roseus*

Figure 7.

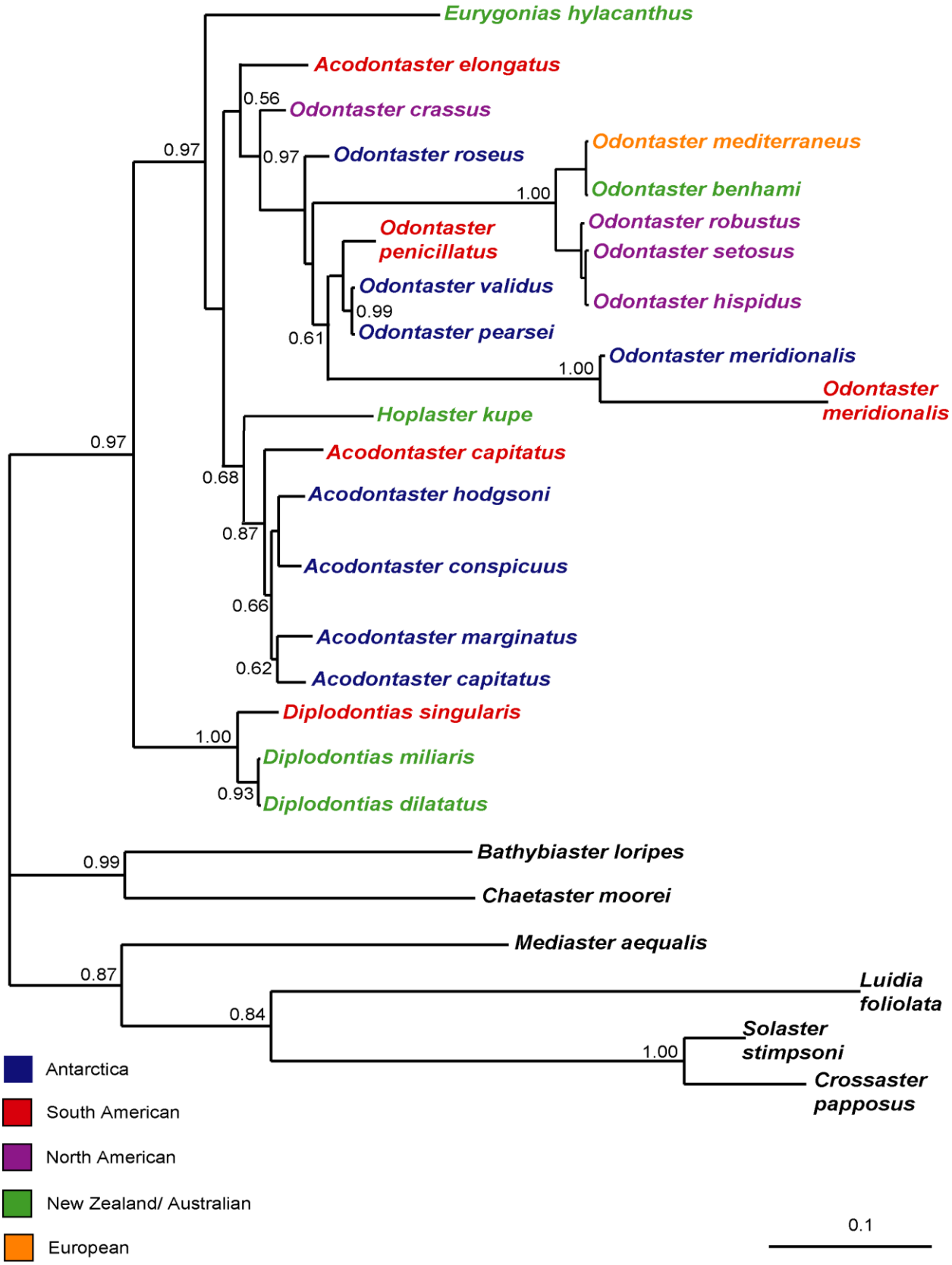
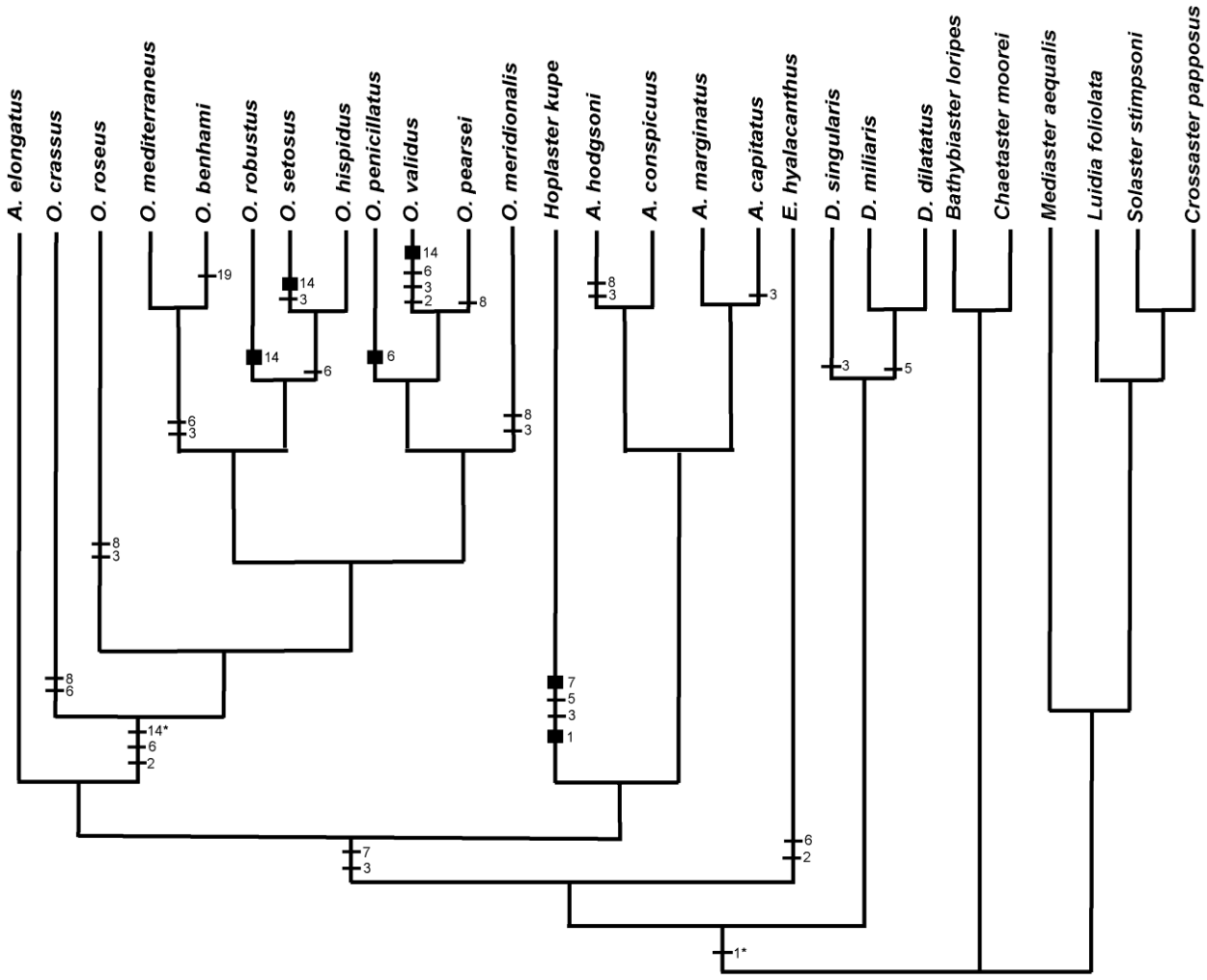

Eurygonias hylacanthus
Acodontaster elongatus
0.56
Odontaster crassus
0.97
0.97
Odontaster roseus
Odontaster mediterraneus
1.00
Odontaster benhami
Odontaster robustus
Odontaster penicillatus
Odontaster setosus
Odontaster validus
Odontaster hispidus
0.99
0.61
Odontaster pearsei
Odontaster meridionalis
1.00
Odontaster meridionalis
Hoplaster kupe
0.97
Acodontaster capitatus
0.68
Acodontaster hodgsoni
0.87
Acodontaster conspicuus
0.66
Acodontaster marginatus
0.62
Acodontaster capitatus
Diplodontias singularis
1.00
Diplodontias miliaris
0.93
Diplodontias dilatatus
0.99
Bathybiaster loripes
Chaetaster moorei
Mediaster aequalis
0.87
Luidia foliolata
0.84
Solaster stimpsoni
1.00
Crossaster papposus
Antarctica
South American
North American
New Zealand/ Australian
European
0.1

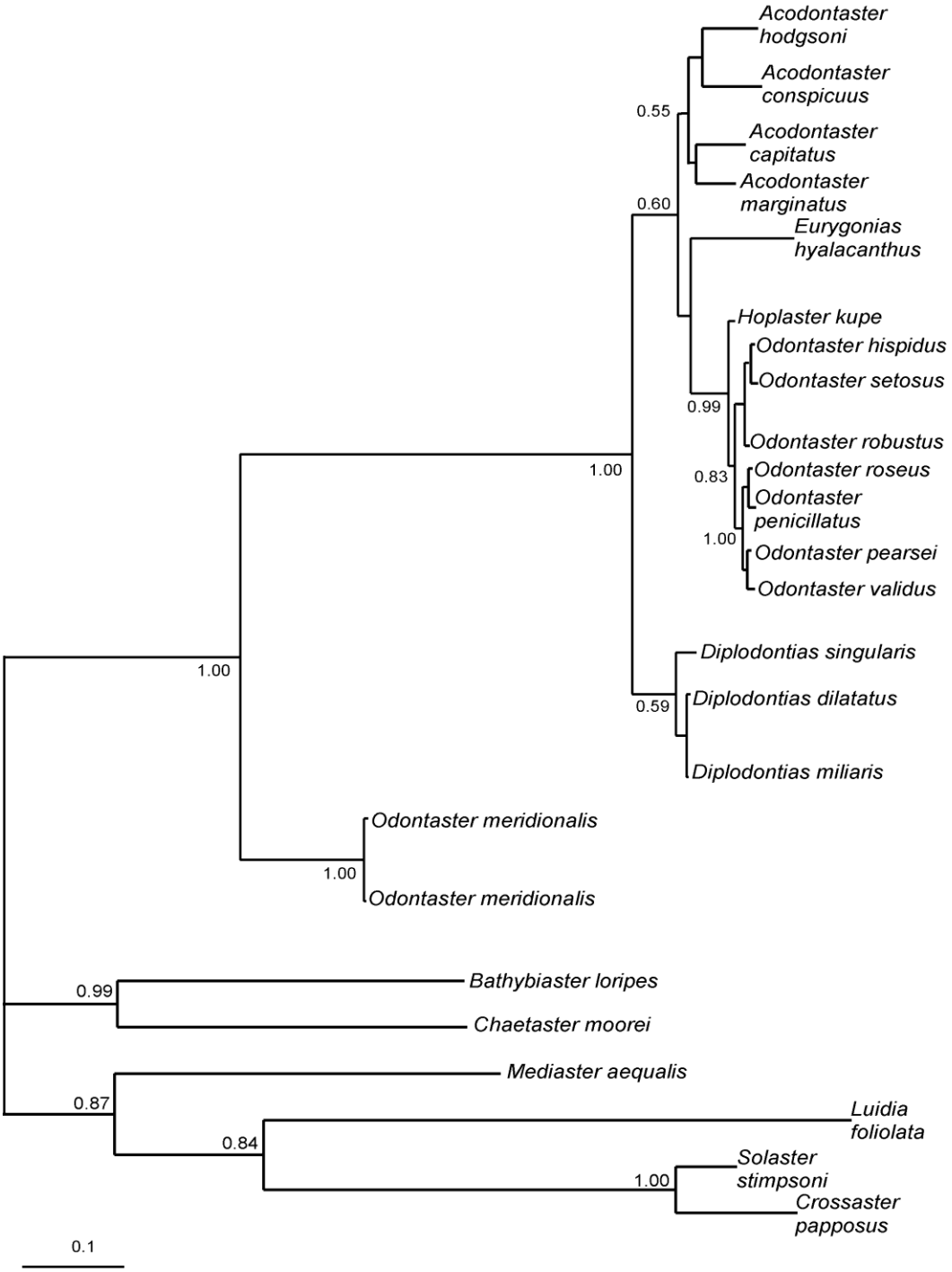
Figure 8.


A. elongatus
O. crassus
O. roseus
O. mediterraneus
O. benhami
O. robustus
O. setosus
O. hispidus
O. penicillatus
O. validus
O. pearsei
O. meridionalis
Hoplaster kupe
A. hodgsoni
A. conspicuus
A. marginatus
A. capitatus
E. hyalacanthus
D. singularis
D. miliaris
D. dilatatus
Bathybiaster loripes
Chaetaster moorei
Mediaster aequalis
Luidia foliolata
Solaster stimpsoni
Crossaster papposus

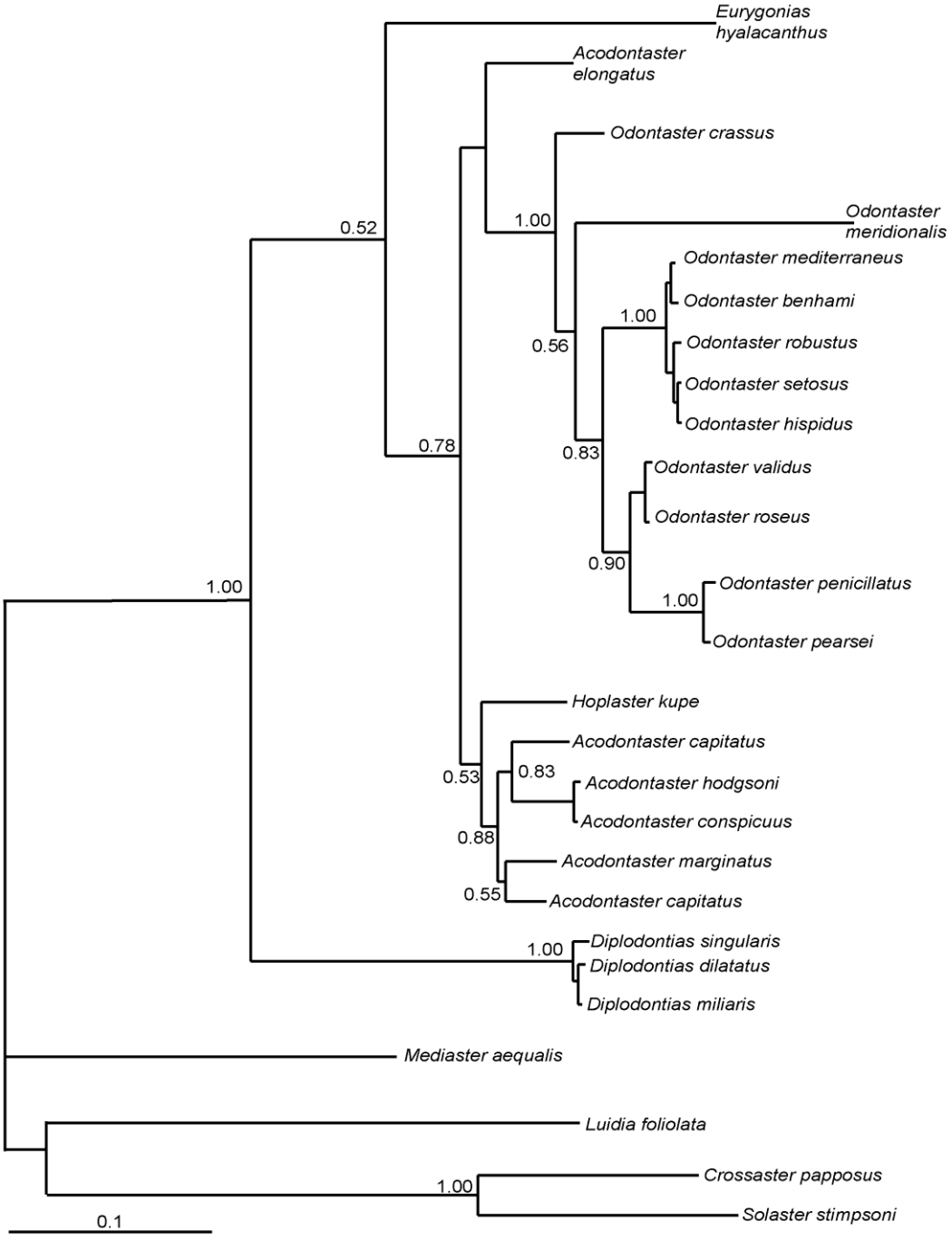
2.8 APPENDIX 1: MATRIX OF MORPHOLOGICAL CHARACTERS

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
A. capitatus	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	2	0	0	4	0	2	2	3	3
A. conspicuus	1	0	2	0	0	0	0	0	0	3	0	0	0	0	0	0	4	3	0	1	1	0	1	4	0	2	1	2	2
A. elongatus	1	0	2	1	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	1	1	0	1	4	0	2	2	0	2
A. hodgsoni	1	0	1	0	0	0	0	2	0	1	0	0	0	0	0	0	2	1	0	0	0	1	1	4	0	2	2	1	2
A. marginatus	1	0	2	1	0	0	0	0	0	1	0	0	0	2	0	0	4	1	0	0	1	1	1	4	0	2	0	4	2
D. clarki	0	0	3	0	1	3	1	0	0	0	1	1	1	0	1	1	2	0	0	1	0	0	0	2	1	1	?	4	3
D. dilatus	2	0	5	0	1	0	2	1	1	1	0	0	0	0	1	1	2	1	0	1	0	0	0	2	0	1	3	0	2
D. miliaris	2	0	5	1	1	0	2	0	1	0	0	0	0	0	1	1	3	1	0	1	0	0	0	3	0	2	2	1	2
D. robustus	2	0	5	0	0	0	2	1	1	0	0	0	0	0	0	0	2	2	0	1	?	1	0	2	0	2	?	?	?
D. singularis	2	0	4	0	0	0	2	1	1	1	0	0	0	0	1	0	4	2	0	0	0	0	1	1	1	1	0	0	3
E. hylacanthus	1	1	5	1	0	2	2	1	1	3	0	0	0	0	1	1	3	2	0	1	0	0	1	0	2	0	2	0	3
H. kupe	0	0	1	1	0	3	2	0	0	2	1	1	1	1	0	0	2	2	0	2	3	1	0	1	1	0	0	4	4
H. spinosus	0	0	2	?	1	3	0	0	0	1	0	1	0	1	1	1	?	2	0	1	0	?	0	1	1	1	0	3	4
O. aucklandensis	1	1	5	1	0	2	0	1	1	0	0	1	0	0	1	0	3	2	0	2	0	0	0	1	1	0	1	2	3
O. australis	1	1	2	1	1	3	0	0	1	1	0	1	0	1	1	0	1	?	0	?	2	1	1	3	0	0	?	?	?
O. benhami	1	0	4	1	1	1	0	0	1	0	0	1	0	1	1	1	1	2	1	2	0	0	0	3	1	1	1	3	3
O. crassus	1	0	2	1	0	1	0	1	1	0	1	0	1	1	1	1	2	2	0	1	1	0	0	1	1	0	2	2	3
O. sp. Galapagos	1	0	0	1	1	5	0	1	1	1	1	1	1	1	1	1	1	0	1	2	3	0	1	1	1	0	2	2	2
O. hispidus	1	1	2	1	0	4	0	0	1	1	1	1	1	1	2	0	2	1	0	0	0	0	0	4	0	2	1	1	4
O. mediterraneus	1	0	4	0	0	1	0	0	1	1	0	0	0	1	1	1	1	2	0	2	1	0	0	4	0	2	2	1	2
O. meridionalis	1	1	4	1	0	3	0	1	1	0	0	1	0	1	0	0	4	2	0	2	1	0	0	3	0	1	1	1	3
O. pearsei	1	1	2	1	0	3	0	1	1	0	0	1	0	1	0	0	0	0	0	2	3	0	0	3	0	2	?	?	?
O. penicillatus	1	1	2	0	0	0	0	0	1	0	0	0	1	1	0	0	2	1	0	1	0	1	1	2	1	1	2	1	2
O. rosagemmae	1	1	5	1	0	2	2	0	1	0	0	1	0	1	2	0	4	2	0	2	1	0	0	3	1	1	2	1	4
O. roseus	1	1	1	1	0	3	0	1	1	0	0	1	0	1	0	0	1	3	0	?	3	0	0	4	0	2	?	?	?
O. robustus	1	1	2	1	0	4	0	0	1	1	0	1	0	0	2	0	0	2	0	3	2	1	0	4	0	2	1	2	3
O. setosus	1	0	1	1	0	4	0	0	1	1	0	1	0	0	0	0	1	2	0	2	3	0	0	4	0	2	2	0	2
O. validus	1	2	1	0	0	4	0	0	1	1	0	1	0	0	0	0	3	0	0	1	3	0	0	4	0	2	0	1	2

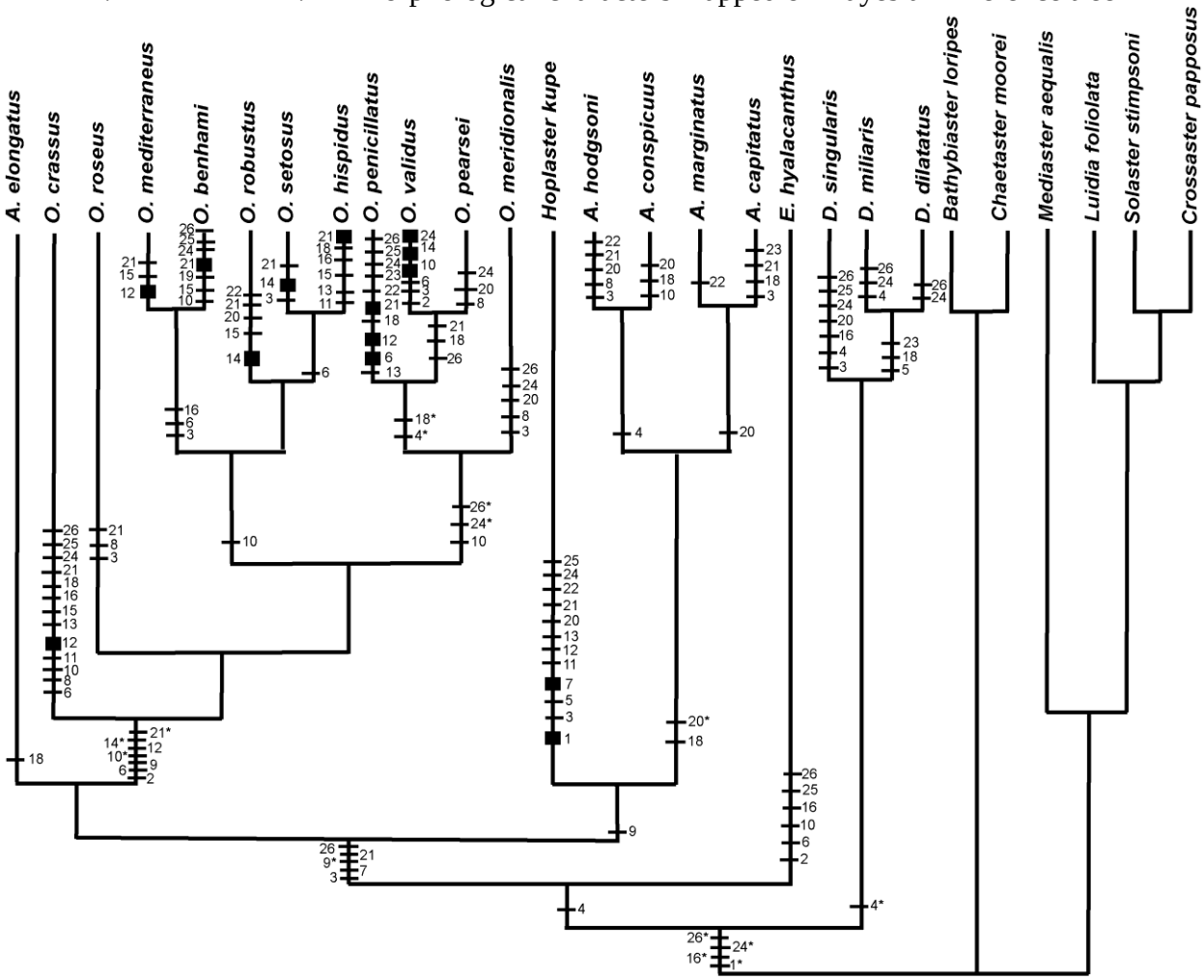
2.9 Appendix 2: Bayesian inference topology for 16S sequence data



2.10 APPENDIX 3: BAYESIAN INFERENCE TOPOLOGY FOR COI SEQUENCE DATA



2.11 APPENDIX 4: All morphological characters mapped on Bayesian Inference tree



CHAPTER 3. Unrecognized Antarctic biodiversity: a case study with *Odontaster* (Odontasteridae; Asteroidea)

3.1 SYNOPSIS

Antarctica has a complex and multifaceted geologic and oceanographic history that has influenced and shaped patterns of marine invertebrate diversity. This evolutionary history consists of major events on a wide range of time scales such as the formation of the Antarctic Polar Front (25–41 million years ago) to repeated glacial cycles during the past million years. These factors variably influenced genetic connectivity of fauna to produce a highly unique, but incredibly diverse marine community. Use of molecular phylogeographic methods is creating the need to revise our understanding of Antarctic patterns of biodiversity. In particular, almost every phylogeographic study carried out to date, suggests that the biodiversity of Antarctic marine shelf fauna is considerably underestimated. In discovering this diversity, some lineages (i.e., cryptic lineages) show no diagnostic morphological differences whereas others (i.e., unrecognized species) show differences that were unknown to science. The sea star genus *Odontaster* is among the best-studied of Antarctic invertebrate groups. Nonetheless, two unrecognized lineages were recently discovered along the Antarctic Peninsula, which is one of the best-studied regions in Antarctica. Herein, we elucidate the molecular and morphological uniqueness of these species and name them *O. roseus* and *O. pearsei*. The latter is in honor of John Pearse, an Antarctic biologist, as well as past President and long-time member of the Society of Integrative and Comparative Biology.

3.2 INTRODUCTION

Despite a cold and harsh environment, the Southern Ocean hosts amazing organismal diversity. In particular, levels of endemism and overall diversity observed in the marine fauna are high south of the Antarctic Polar Front (APF) (Ekman 1953, Hempel 1985, Arntz et al. 1997). Understanding factors that promote, maintain, and influence the uniqueness and level of diversity is of great interest. Specifically, biologists, geologists and oceanographers are trying to better understand biotic and abiotic variables of the system. Although we currently have a relatively limited understanding, recent efforts have radically been reshaping our views of Antarctic marine invertebrate endemism and diversity. Genetic analyses of mitochondrial DNA, among others, have revealed a wealth of hitherto unknown organismal lineages found in, and around, Antarctic waters.

In this contribution, we compile literature demonstrating that Antarctic biodiversity is underestimated, outline some of the key elements that influence this diversity, and provide an example indicating that molecular tools are forcing us to critically re-examine morphological taxonomy. Moreover, we argue that there is a need for using more precise scientific language when discussing whether lineages of distinct species are difficult to detect (i.e., cryptic) or merely have not been noticed (i.e., unrecognized). For the purposes of this article, we will include species that are distributed south of the APF, including the sub-Antarctic islands, which mostly fall south of the APF boundary.

3.3 GEOLOGICAL SETTING

The APF is a steep temperature boundary in the Southern Ocean limiting north–south surface water exchange. Dramatic changes can be seen in surface water temperatures and are often detectable to depths down to 1000 m. In addition, the Antarctic Circumpolar Current

(ACC), known as the strongest current in the world, swirls around the continent and is driven by the world's mightiest westerly winds (Orsi et al. 1995). Both the APF and the ACC formed upon the opening of the Drake Passage, ~ 24–41 million years ago (MYA), after the South American and Antarctic continents separated. Earlier, on the opposite side of the Earth, Australia and Antarctica pulled apart creating the Tasmania Gateway (50–41 MYA), which also greatly contributed to the formation of the ACC (Wei 2004). These complex oceanographic features (separately or in combination) have been hypothesized to act as strong biogeographical barriers to most marine organisms, aside from migratory marine mammals and seabirds (Crame 1999; Pfuhl and McCave 2005; Scher and Martin 2006). Thus, fauna on either side of the APF are hypothesized to have a long (424 MYA) evolutionary history of separation.

Whereas formation of the ACC and AFP are usually implicated in generating Antarctica endemic fauna, glaciations during the most recent glacial period are credited with shaping much of the recent faunal distribution of Antarctic marine invertebrates (Thatje et al. 2005). Traditionally, authors (e.g., Brey et al. 1996) have argued that during glacial maxima, continental shelf fauna were largely extirpated and forced down on to the continental slope due to lack of habitat. However, in recent years, asynchronous glacial cycles and the presence of refugia on the continental shelf has been emerging as an explanation as to how Antarctic continental shelf fauna survived extensive glaciations (Thatje et al. 2005, 2008). Potential genetic outcomes of such refugia are two-fold. On one hand, reduced effective population size decreases genetic diversity ''within'' organismal lineages. In contrast, small population size in combination with multiple refugia can allow substantial genetic differences to quickly accumulate ''between'' lineages due to genetic drift. This latter scenario can result in genetically isolated lineages that are morphologically very similar as they recently diverged from a common ancestor.

An accurate assessment of the timing of geological or oceanographic events is critical for understanding the cause and effect they have on organismal lineages. Unfortunately, accurately matching biological pattern to environmental underpinnings can be difficult (see Marko and Moran 2009, Marko et al. 2010). In the case of Antarctic fauna, factors that promoted isolation and endemism (e.g., APF), may be different from factors promoting diversity (recent glacial cycles) or genetic connectivity (e.g., life history, rafting). Recognition of differences in such events and timing can often be obscured. For example, establishment of the ACC has been hypothesized to be both a cause of isolation resulting in endemism (Arntz et al. 1997) and a factor promoting dispersal and establishment of circumpolar species (Fell 1961, Dell 1972, Fevolden & Schneppenheim 1989, Arntz et al. 1994, Thornhill et al. 2008, Waters 2008). Yet the timescales of ACC formation (25–41 MYA) and current connectivity among species (roughly the past million years) are vastly different. Thus, hypotheses seeking to explain Antarctic endemism and diversity need to carefully consider evolutionary timing within an appropriate geological or oceanographic construct.

3.4 BIOGEOGRAPHY AND GENETICS

Biogeographic understanding of Antarctic marine fauna, as in other regions, is based on observations of species collected in specific geographic regions. Because of this simple fact, our best understanding of species diversity is in regions with established research stations—e.g., along the Antarctic Peninsula and in the Ross Sea. When the ‘‘same’’ organism has been found in two disparate regions, most researchers have traditionally assumed them to be present between those sampling localities as well. Unfortunately, and in part due to logistical constraints such as ice cover and operating costs of ships, several regions of Antarctic waters are very poorly known

and biologically explored (e.g., Amundsen Sea, much of the Eastern Antarctic, see Grant and Linse 2009), and taxonomists have often had a limited number of representatives with which to assess variation and delimit species. Likewise, we have very little information as to whether a given species occupies several disjunct locations, or has one large range that is occupied throughout. Many species, nonetheless, are currently regarded as having a circumpolar distribution (e.g., *Odontaster validus, Sterechinus antarcticus, Parborlasia corrugatus, Lissarca notorcadensis*, Fisher 1940, Pawson 1969, Gibson 1983, Dell 1990; also see Brey et al. 1996, Arntz et al. 1997, Clarke and Johnston 2003, Thatje et al. 2005, Griffiths et al. 2009).

With the advent of molecular tools, taxonomic hypotheses can be tested with essentially independent data. Whereas some of the first phylogeographic studies on Antarctic species focused on cetaceans (Wada and Numachi 1991, Palsbøll et al. 1995, Hoelzel 1998, Pastene et al. 2005), pinnipeds (Gales et al. 1989, Slade et al. 1998, Wynen et al. 2000) and penguins (Lambert et al. 2002, Ritchie et al. 2004), more complete aspects of Antarctic flora and fauna are now being tested using molecular approaches (see Rogers 2007). Fortunately, for the study of the marine continental shelf fauna, molecular phylogeographic techniques can be used to assess similarity and disparity between organismal populations, even with discontinuous sampling. These techniques have been producing interesting results, challenging some of our notions about Antarctic marine invertebrate biogeography and diversity.

Table 1 shows a compilation of Antarctic marine shelf fauna examined to date using molecular phylogeographic methods. The most striking feature of these studies is that previously unrecognized lineages were discovered in almost every case. Given that these studies are relatively restricted geographically (mainly Antarctic Peninsula and Weddell Sea), the data lend strong support to the notion that Antarctic marine biodiversity is underestimated (Clarke and

Johnston 2003, Mahon et al. 2010). Having an accurate estimation of Antarctic biodiversity is critical for numerous reasons: for example, understanding organismal roles in ecosystem function; accurately monitoring effects of climatic change on fauna in the most rapidly warming region on Earth (Vaughan et al. 2003); inferring how geological and oceanographic processes have shaped organismal evolution in the region; assessing whether the same genetic lineage is repeatedly being used in biological experimentation; etc.

3.5 CRYPTIC VERSUS UNRECOGNIZED DIVERISTY

In documenting this unknown biodiversity, elucidating both the diversity of lineages and the disparity between lineages is important. The two concepts often become blurred in the phylogeographic literature. For example, the term ‘‘cryptic species’’ is often invoked to document additional genetic lineages that have been discovered. This situation is true in the Antarctic literature as well (e.g., Brierley et al. 1993, Held 2003, 2005; Raupach & Wagele 2006). However, the word ‘‘cryptic’’ implies that the diversity was hidden or hard to find. Such has been the case with the isopod *Ceratoserolis trilobitoides* (Held 2003) and with the brittle star *Astrotoma agassizii* (Hunter and Halanych 2008) which shows multiple genetic lineages but fail to display morphological characters that can be used to confidently assign them to distinct lineages.

In such cases, other features, such as diet or behavior may allow discrimination between lineages (de Aranzamendi et al. 2008). However, in the context of current Antarctic research, assigning taxa based on such features is not practical because we know so little about the biology of all but a few of invertebrate taxa, and we usually do not have the ability to observe them within their environment. Additionally, as mentioned above, we do not currently have a good

understanding of the ranges of organisms due to very limited sampling; this limits our ability to assign names to taxa on geographic grounds. For all intents and purposes, species designations and taxonomic nomenclature is limited to our understanding of species boundaries based on morphological or molecular genetic tools.

In many cases, different genetic lineages may be associated with distinct morphology that was unrecognized. Cryptic or sibling species are defined when speciation occurs without detectable morphological change, yet the species are genetically distinct and often exhibit overlapping geographic ranges (Lomolino et al. 2006). In contrast to ‘‘cryptic’’ lineages, which display no obvious morphological differences, ‘‘unrecognized’’ species do have clear diagnostic morphology that has escaped detection. When novel genetic lineages are uncovered by use of molecular tools, verifying the similarity or disparity in morphology is informative and aids in improving the accuracy of taxonomic hypotheses. By extension, this improves accuracy and understanding of patterns of biodiversity.

Recognition of cryptic species complexes should be derived from a solid list of evidence, similar to that which Held (2003) and Held and Wagele (2005) described for serolid isopods. Specifically Held stated that there should be (1) bimodal distribution of pairwise distance measures with no intermediate values, (2) differentiation at a level known for this gene from other undisputed species pairs closely related to the studied species, and (3) persistence of high levels of genetic differentiation in sympatry. Combining haplotype networks and phylogenetic reconstruction with morphological characters, provides a sound foundation for testing species boundaries (Brandao 2010).

3.6 CASE STUDY: *ODONTASTER* SPECIES

The sea star genus *Odontaster* Verrill 1880, provides an interesting case study of unrecognized species diversity in Antarctic waters. This sea star, described in 1906 by Kohler, was hypothesized to have a circumpolar distribution (Fisher 1940) and is an important component of the Antarctic ecosystem. *Odontaster validus* spawns in the austral winter and boasts a planktotrophic larva with high dispersal ability (Pearse 1965, Pearse and Bosch 1986) and occurs over a wide range of depths and in a variety of habitats (Fisher 1940). Moreover, it is arguably one of the earliest known and best-studied marine invertebrate organisms in the Southern Ocean (see Pearse 1965, 1966, 1967, 1969, Belman and Giese 1974, Dayton et al. 1974, Pearse and Bosch 1986, 2002, Olson et al. 1987, McClintock et al. 1988, Bosch et al. 1990, Stanwell-Smith et al. 1998, Kidawa 2001, Peck and Prothero-Thomas 2002, Tyler et al. 2003, Janecki and Rakusa-Suszczewski 2004, Grange et al. 2007, McClintock et al. 2008, Peck et al. 2008).

While exploring phylogeographic structure of this species in the Western Antarctic, A.M. Janosik et al. (2011) discovered at least five deeply branched genetic lineages corresponding to individuals belonging to three recognized morphological species (*O. validus*, *O. penicillatus*, *O. meridionalis*). Using a combination of haplotypes networks and phylogenetic reconstruction based on mitochondrial sequence data, Janosik et al. showed that all of the *Odontaster* lineages formed monophyletic clades and produced individual parsimony based networks. As such, they warrant the status of full species because of their genetic uniqueness (CO1 divergence values from 3.5 to 10%) *sensu* Hart et al. (2006). Interestingly, both novel lineages occurred along the Antarctic Peninsula, a very well sampled area.

Moreover, further morphological investigation revealed that the species were not cryptic, but merely unrecognized. Diagnostic differences were found in the spines and plates of all taxa.

Because diversity can remain unknown unless species are formally described (Oliver et al. 2009), the two previously unrecognized species of *Odontaster* from Antarctic waters (collected during two 5-week Antarctic research cruises aboard the R/V Laurence M. Gould in November/December of 2004 and May/June of 2006; using a Blake trawl, wire dredge, or epibenthic sled) are described below and compared with the other currently recognized species of *Odontaster* in Figs. 1, 2, and 3. In addition, a diagnostic key of the Southern Ocean of *Odontaster* species is presented. Terminology follows Lambert (2000) and Clark and Downey (1992).

Family ODONTASTERIDAE Verrill, 1899

Genus *ODONTASTER* Verrill, 1880

***Odontaster roseus* nov. sp.** (Figs. 1d, 2d, and 3d)

Holotype: Antarctica, 63º24.9610S, 61º50.4840W, 132 m in depth, specimen wet (alcohol) R¼1.3 cm, r¼0.6 cm (Fig. 1), USNM 1127023. Collected by K. M. Halanych and A. M. Janosik.

Paratypes: Two specimens were morphologically examined. Antarctica, 62º56.0040S, 61º28.7510W, R¼2.5 cm, r¼0.9 cm.

Etymology: The descriptor roseus describes the rosy to drab red and tan color of this species.

Diagnosis: A species with an almost pentagonal outline, rough spinelets on abactinal plates, four chevrons of plates on the actinal side, superomarginal and inferomarginal plates densely covered in slender, smooth spines with deep grooves between plates.

Description: Body relatively flattened with a stellate outline. Abactinal plates with distinct tabulum crowned with truncate paxillae, comprised of 10–12 spinelets per plate. Spinelets tapering and are of variable lengths, with small spines at end of spinelets (i.e., spinelets rough in

appearance). Glassy granules absent on abactinal plates. Papulae on abactinal surface are restricted to the central disc and radial areas. Marginal plates form a distinct border with the abactinal and actinal plates, with deep grooves between plates. Specifically, superomarginal plates are paxillate, densely covered with ~18 spinelets per plate. Inferomarginal plates also paxillate, densely covered with 15–18 spinelets per plate. Spinelets on lateral side of inferomarginal plates are considerably longer than superomarginal spinelets and are rough in appearance. Oral surface possesses the characteristic *Odontaster* recurved, glassy tipped spine on each mouth plate. Actinal plates with four complete chevrons, including 8–10 spinelets per plate, with one prominent longer spine. Spinelet circumference even from base to tip. Glassy granules also absent from actinal plates. Adambulacral plates with long armament. Spines tapering and rough in appearance. Approximately three to four furrow spines present along the ambulacral plates. Pedicellariae absent.

DNA: Two adults and two larvae were molecularly characterized. Unique diagnostic sequences from the mitochondrial COI and 16S rDNA genes are deposited to GenBank under the following accession numbers: COI—GQ29359 Holotype; GQ29489, GQ29490 Paratypes; 16S—GQ294413 Holotype; GQ294414, GQ29447 Paratypes.

Color note: Live color rosy to drab red and tan compared to the typical bright red of *Odontaster validus*, but still brighter red than *O. pearsei* nov. sp.

Distribution: Northern Antarctic Peninsular, South Shetland Islands, collected at 132–188 m.

***Odontaster pearsei* nov. sp.** (Figs. 1e, 2e, and 3e)

Holotype: Antarctic Peninsula 54º29.0710S, 62º12.8570W 132 m in depth, specimen wet (alcohol) R ¼ 2.4 cm, r¼1.3 cm. USNM 1127022. Collected by K. M. Halanych and A. M. Janosik.

Paratypes: Eight individuals were morphologically examined. Antarctic Peninsula 64824.620S, 64830.130W, R¼1.7 cm, r¼1.0 cm, Antarctic Peninsula 67º43.6070S, 69º18.1030W, R¼0.9 cm, r¼0.5 cm, Antarctic Peninsula 63º24.9610S, 61º50.4840W, R¼1.8 cm, r¼0.9 cm, Antarctic Peninsula (two individuals) 65º39.8430S, 68º02.2240W, R¼1.6 cm, r¼0.8 cm, R¼1.6 cm, r¼0.8 cm, Antarctic Peninsula 67º44.4200S, 69º17.3790W, R¼1.5 cm, r¼0.7 cm, sub-Antarctic 54º380S, 3º500W, R¼1.2 cm, r¼0.6 cm.

Etymology: This species is named for Dr John S. Pearse in honor of his numerous contributions to Antarctic marine invertebrate ecology and asteroid biology.

Diagnosis: A species with a stellate outline, rough spinelets on abactinal plates, three chevrons of plates on the actinal side, superomarginal and inferomarginal plates densely covered in rough spines with deep grooves between plates.

Description: Body relatively flattened, with a stellate to sub-pentagonal outline. Abactinal plates with distinct tabulum crowned with truncate paxillae, comprised of 16–20 spinelets per plate. Spinelets taper towards base, with small spines at end of each spine (i.e., rough in appearance). Glassy granules absent on abactinal plates. Papulae on abactinal surface also restricted to the central disc and radial areas. Marginal plates form a distinct border with the abactinal and actinal plates, with deep grooves between plates. Specifically, superomarginal plates are paxillate, densely covered with ~15 spinelets per plate and are rough in appearance. Inferomarginal plates also paxillate, densely covered with 10–12 spinelets per plate. Spinelets on lateral side of inferomarginal plates considerably longer than superomarginal spinelets and rough in appearance

with small spines at end of spinelet. Oral surface possesses the characteristic *Odontaster* recurved, glassy tipped spine on each mouth-plate. Actinal plates with three complete chevrons, including five to eight spinelets per plate. Spinelets taper from tip to base, being more slender at the base. Glassy granules also absent from actinal plates. Adambulacral plates with long armament, with spines tapering and rough in appearance. Three furrow spines present along the ambulacral plates. Pedicellariae absent.

DNA: Eight adults were characterized molecularly. Unique diagnostic sequences from the mitochondrial COI and 16S rDNA genes are deposited to GenBank under the following accession numbers: COI— GQ294358 Holotype; GQ294357, GQ294358, GQ294372, GQ294383, Paratypes; 16S—GQ294412; Holotype, GQ294411, GQ294412, GQ294415, GQ294423, GQ294426, GQ294439 Paratypes.

Color note: Color of live specimen orange to tan, more drab than *O. roseus* or the typical bright red of *Odontaster validus*.

Distribution: Northern Antarctic Peninsula, South Shetland Islands, Anvers Island, Beer Island, and Marguerite Bay, collected at 132–282 m.

Key to the *Odontaster* species of the Southern Ocean

The genus *Odontaster* is characterized by a recurved, glassy-tipped spine on each mouth-plate, two side-by-side, at each mouth angle. In addition, *Odontaster* spp. have abactinal plates with a distinct tabulum crowned with short to rather long spinelets; marginal plates small, to well-developed, more or less tabulate, spinulose; actinal area densely spinulose.

(1) Radial paxillae with 20–30 spinelets, the middle ones markedly clavate (Figs. 2a and 3a); actinal plates also with numerous radiating spines, up to 15, central ones more clavate than the peripheral (Antarctic, circumpolar, including South Georgia, Marion Island, and Kerguelen): . . . . . ***Odontaster meridionalis***

Radial paxillae with fewer than 20 spinelets: 2

(2) Outline more pentagonal than stellate, marginal plates obvious with short, usually granuliform spinelets, barely longer than wide; abactinal spinelets as in Figs. 2b and 3b; dorsal side flat or slightly convex (Patagonia, Falkland Plateau): . . .. ***Odontaster penicillatus***

Outline more stellate; spinelets of marginal plates otherwise: 3

(3) Radial paxillae with about a dozen spinelets that are smooth, slender and tapering (Figs. 2c and 3c); five actinal plate chevrons, actinal plates with up to seven similar, slender spinelets that are even from base to tip, two to three furrow spines (Antarctic, circumpolar, including South Georgia and Bouvet Island): . . . . ***Odontaster validus***

Radial paxillae with rough, tapering spinelets with little spines at the tips; two to four (commonly three)
furrow spines: 4

(4) Radial paxillae with 10–12 spinelets (Figs. 2d and 3d); four complete actinal plate chevrons, actinal plates with spines of different lengths (8–10), specifically with one prominent spine in the middle, (Antarctic Peninsula): . . . . ***Odontaster roseus***

Radial paxillae with 16–20 spinelets (Figs. 2e and 3e); three complete actinal plate chevrons, actinal plate with slender tapering (from tip to base) spines of equal length (five to eight) (Antarctic Peninsula): .. . . . . ***Odontaster pearsei***

3.7 Current and future directions

Although many studies attempt to estimate biodiversity in the Antarctic, assessing how many species are present is not simple. Specifically, Arntz et al. (1997) estimated 5,200 species and Clarke and Johnston (2003) estimate 4100 species, while Gutt et al. (2004) estimated anywhere from 11,000 to 17,000 macrozoobenthic species using statistical methods. Based on the phylogeographic studies listed in Table 1, considerable diversity in the Antarctic remains to be discovered and distinguished as either cryptic or unrecognized. Using the publications in Table 1 as a guide, we can approximate how much additional marine benthic diversity has yet to be discovered in the Antarctic. For example, there are currently ~400 species of echinoderms recognized in the Antarctic (Arntz et al. 1997). Specifically, four nominal echinoderm species

from the Antarctic have been examined by phylogeographic studies and at least ten unrecognized lineages were discovered. Thus, there are 3.5 times as many echinoderms species than expected, leading to an estimate of a total of 1400 echinoderm species in the Antarctic. Of note, these studies were also conducted in the well-examined areas. By the same logic, there are currently 900 species of crustaceans recognized, but preliminary genetic evidence suggests that this group may be underestimated by three-fold. Similarly, although 500 species of pycnogonids are known, estimates based on under-representation observed in phylogeographic analyses suggest as many as 2250 spp. in the Antarctic. These numbers are at best very rough estimates, and as more data are gathered probably will be refined. Clearly, we have much more to discover in the waters around Antarctica.

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Table 1. Phylogeographic studies on Antarctic fauna using molecular tools.

Clade	Species	Region	# previously unrecognized genetic lineages	Marker(s)	Authors
Arthropoda					
Isopoda	*Ceratoserolis trilobitoides*	King George Island, Weddell Sea, Antarctic Peninsula	1	16S	Held 2003
	Glyptonotus antarcticus	Weddell Sea, Antarctic Peninsula, Ross Sea	3	16S	Held and Wagele 2005
	Acanthaspidia drygalskii	Weddell Sea	2	16S	Raupach and Wagele 2006
	Ceratoserolis trilobitoides	Weddell Sea, Antarctic Peninsula	1	Microsat.	Leese and Held 2008
	Macroscapha opaca *Macroscapha tensa*	Sub-Antarctic Weddell Sea, Ross Sea	8	COI, ITS	Brandao et al. 2010
Euphausiidae	*Euphausia superba*	Subantarctic, Antarctic Peninsula, Ross Sea	1	ND1	Zane et al. 1998
Pycnogonida	*Colossendeis megalonyx*	Subantarctic, Antarctic Peninsula	5	COI	Krabbe et al. 2010

	Nymphon australe	Antarctic Peninsula	2	COI, 16S	Mahon et al. 2008
Mollusca					
Bivalvia	*Lissarca notocadensis*	Subantarctic, Weddell Sea, Antarctic Peninsula, Ross Sea	1	COI	Linse et al. 2007
Cephalopoda	*Pareledone turqueti*	Subantarctic	1	isozymes	Allcock et al. 1997
Cephalopoda	*Martilia hyadesi*	Antarctic Peninsula	1	isozymes	Brierley et al. 1993
Gastropoda	*Doris kergulensis*	Subantarctic, Weddell Sea, Antarctic Peninsula, Ross Sea	28	COI,	Wilson et al. 2009
	Nacella concinna	Antarctic Peninsula	1	ISSR-PCR	de Aranzamendi et al. 2008
Nemertea					
	Parborlasia corrugatus	Subantarctic, Antarctic Peninsula, Ross Sea	1	COI,	Thornhill et al. 2008
	multiple species	Antarctic Peninsula	19	16S	Mahon et al. 2010
Echinodermata					
Crinoidea	*Promachocrinus kerguelensis*	Subantarctic, Antarctic Peninsula	5	COI, CytB	Wilson et al. 2007
Ophiuroidea	*Ophionotus victoriae*	Subantarctic, Antarctic Peninsula	1	COI, 16S	Hunter and Halanych 2010

	Astrotoma agassizii	South America, Antarctic Peninsula	2	COII, 16S rRNA	Hunter and Halanych 2008
Asteroidea	*Odontaster validus*	Subantarctic, Antarctic Peninsula, Ross Sea	2	COI, 16S	Janosik et al. *submitted*
Echinoidea	*Sterechinus antarcticus/ agassizi*	South America, Subantarctic, Antarctic Peninsula	-1 (two species the same)	CO1, 16S	Cox et al. *submitted*

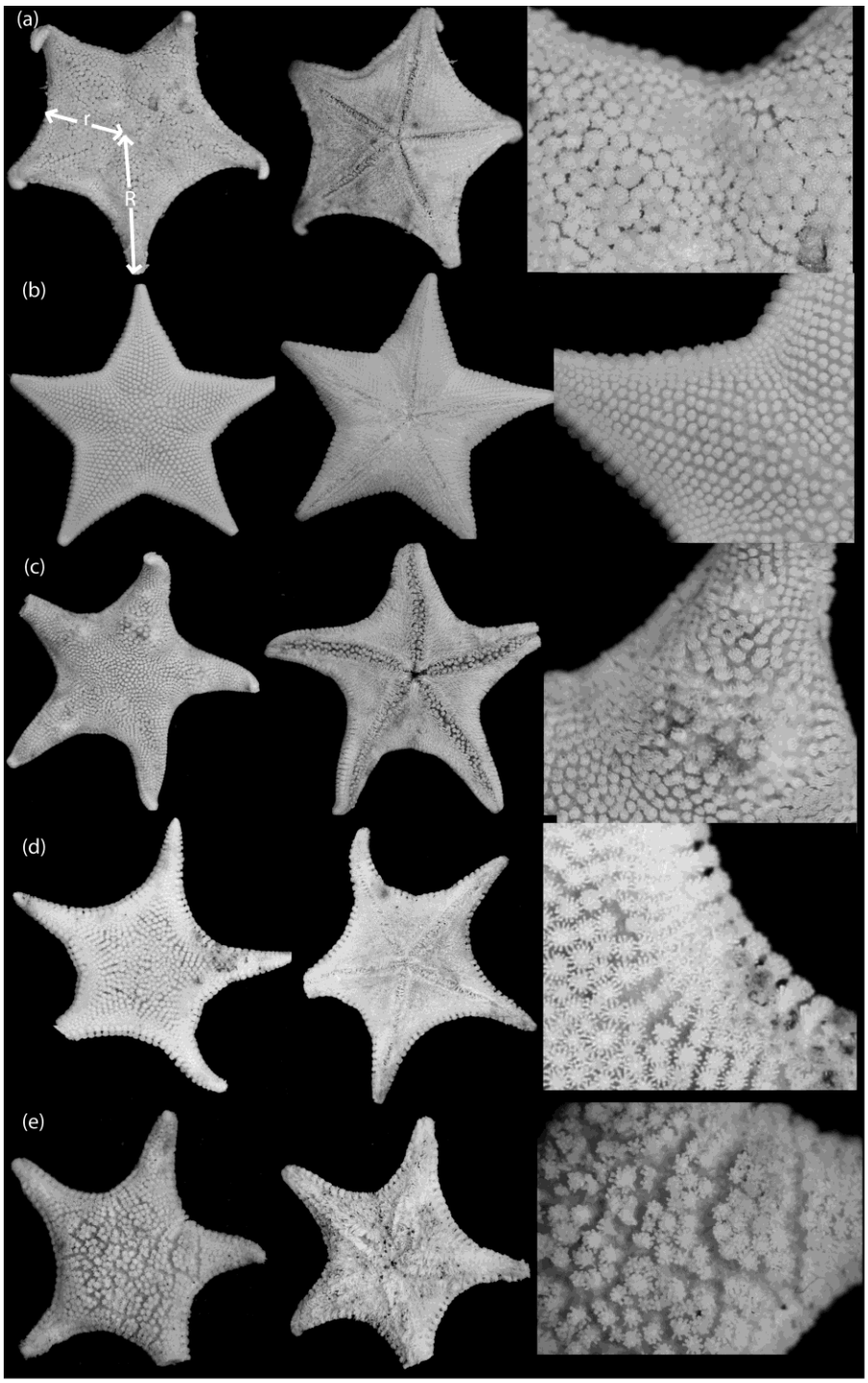

Figure 1. *Odontaster* morphology. Aboral, oral, and close-up of aboral side are pictured, respectively for: (a) *Odontaster meridionalis*, (b) *Odontaster penicillatus*, (c) *Odontaster validus*, (d) *Odontaster roseus* nov. sp., (e) *Odontaster pearsei* nov. sp.

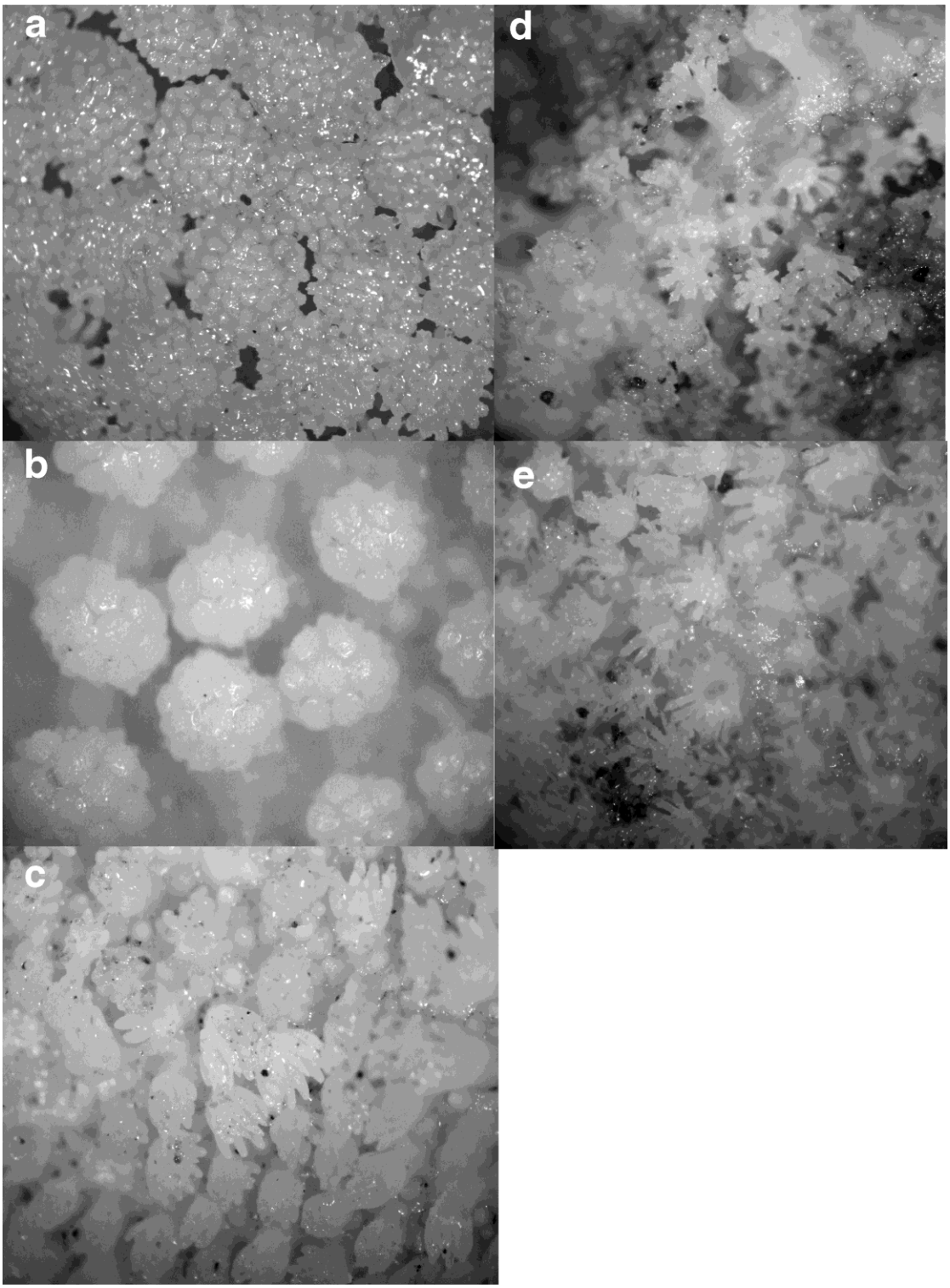

Figure 2. Photograph of the spine morphology of *Odontaster*. Spine on paxillae are pictured for: (a) *Odontaster meridionalis*, (b) *Odontaster penicillatus*, (c) *Odontaster validus*, (d) *Odontaster roseus* nov. sp., (e) *Odontaster pearsei* nov. sp.

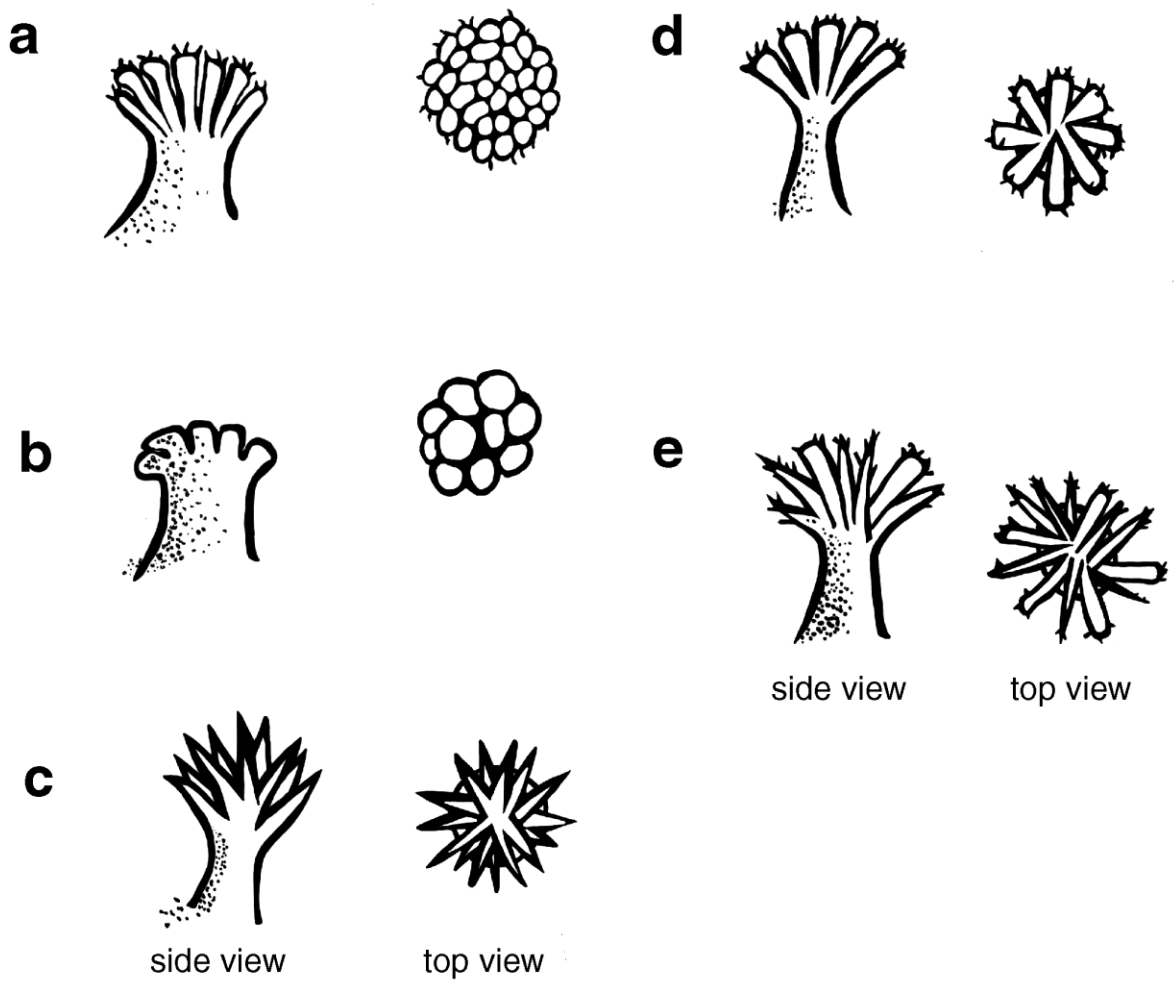

Figure 3. Drawing of the spine morphology of *Odontaster*. Spine on paxillae are pictured for: (a) *Odontaster meridionalis*, (b) *Odontaster penicillatus*, (c) *Odontaster validus*, (d) *Odontaster roseus* nov. sp., (e) *Odontaster pearsei* nov. sp.

CHAPTER 4. Evolutionary history of Southern Ocean *Odontaster* sea star species (Odontasteridae; Asteroidea)

4.1 ABSTRACT

We investigated the recent evolutionary history of demersal sea stars in the genus *Odontaster* throughout the Western Antarctic waters and on the South American shelf. The mitochondrial 16S ribosomal and cytochrome *c* oxidase subunit I (COI) genes were sequenced from adult and larval specimens. TCS parsimony network analysis and Bayesian inference were used to examine evolutionary history. Hierarchical AMOVA and mitochondrial DNA diversity statistics were also computed. Additionally, morphological characters were used. In assessing *O. validus*, we discovered morphological and range descriptions of *Odontaster* species to be inaccurate and include other *Odontaster* species in the Southern Ocean. We found *O. meridionalis* on both sides of the Antarctic Circumpolar Current (ACC) and Antarctic Polar Front (APF), whereas *O. validus* and *O. penicillatus* do not appear to have permeated these oceanographic features. Additionally, we discovered previously unrecognized species of *Odontaster*. Subsequent examination revealed diagnostic morphological differences in the number of spinelets on the abactinal and actinal plates. Mitochondrial characterization of *Odontaster* species suggest their recent history has been influenced by the APF and ACC in different ways. With the exception of *O. meridionalis, Odontaster* species are restricted to either side of the Drake Passage. *O. validus* shows genetic connectivity throughout sampled Antarctic waters.

4.2 INTRODUCTION

Southern Ocean marine invertebrate fauna provide a unique opportunity to study evolutionary history, especially when considering possible circum-Antarctic distributions and high levels of endemism. Given the close proximity of the South American and Antarctic continental shelves, there has been potential for species to disperse, on various evolutionary time scales, across the Antarctic Circumpolar Current (ACC) and Antarctic Polar Front (APF). The ACC is reported to be the strongest current in the world, driven by the world's mightiest westerly winds, contains three distinct circumpolar fronts (i.e. sub-Antarctic (SAF) and Polar Front (PF), and a third deep-reaching front observed persistently as part of the ACC to the south of the PF at the Drake Passage) (Orsi et al. 1995), and is complex in terms of oceanographic dynamics (Stevens and Ivchenko 1997). The APF is the steep temperature gradient where polar waters meet warmer temperate waters. Both the ACC and the APF formed after the spreading of the sea floor opened the Drake Passage approximately 24-41 million years ago, and they (separately or in combination) have been hypothesized to be contributing to the isolation of Southern Ocean and Antarctic fauna since that geological event (Crame 1999; Pfuhl and McCave 2005; Scher and Martin 2006). Although endemism of many Antarctic organisms has been attributed to these potential barriers, 18% of echinoderm species including, Asteroidea, Ophiuroidea, and Echinoidea are reported in both South American waters and coastal waters of Antarctica (Ekman 1953; Arntz et al. 1997). However, prevalence of endemism and occurrence of biogeographic barriers in the Southern Ocean has only recently been tested with molecular tools (e.g. Patarnello et al. 1996; Page and Linse 2002; Linse et al. 2007; Mahon et al. 2008; Thornhill et al. 2008; Wilson et al. 2009).

We set out to explore the evolutionary history of the Southern Ocean sea stars in the genus *Odontaster,* in terms of dispersal ability and population connectivity using molecular analyses to examine specimens from waters on both sides of the Drake Passage. Specifically, *Odontaster validus* has a circumpolar distribution extending into the sub-Antarctic. Two other *Odontaster* species, *O. meridionalis* Smith 1876, and *O. penicillatus* Philippi 1870, are recognized as restricted to South American and circumpolar Antarctic distributions, respectively (Fisher 1940). Surprisingly, we also discovered two unrecognized species (*O. pearsei and O. roseus*) in the Peninsula region.

Planktotrophic larval stages are argued to be rare in polar asteroids and other Antarctic benthic invertebrates, given the benthic diversity of Antarctic waters (Mileikovsky 1971; Pearse and Bosch 1994, Thatje et al. 2005). However, the Antarctic asteroid genus *Odontaster* Verrill, 1880, has a planktotrophic larva that can stay in the water column for up to six months. In particular, *O. validus* Kohler 1906, spawns in the austral winter (Pearse 1965; Tyler et al. 2003) probably to avoid pelagic predators associated with the summer phytoplankton bloom (Clarke 1988). This life history strategy presumably allows high dispersal ability (Pearse and Bosch 1986), and is consistent with *O. validus*'s circumpolar distribution. *Odontaster validus* is ubiquitous throughout the Southern Ocean and occurs over a wide depth range in a variety of habitats (Fisher 1940). In general, species of *Odontaster* are of ecological importance due to their influence on the benthic community. For example, Dayton et al. (1974) suggested that *O. validus* consumes benthic larvae and could be regulating invertebrate populations, specifically populations of important predators, *Acodontaster conspicuus* and *Doris mcmurdensis*, on space-dominating sponges. Also, *O. validus* is thought to feed in a number of ways, including active predation, scavenging, filter feeding, and grazing based on opportunity of available prey items

(Pearse 1965; Dayton et al. 1974), and for these reasons has been suggested to be a keystone species because the impact of *O. validus* extends beyond that predicted by its biomass or abundance (McClintock et al. 1988; 2008).

Morphology of *Odontaster* species in the Southern Ocean appears to be quite variable and consequently problematic in terms of taxonomic diagnosis. As an example, Fisher (1940) discusses five different forms within *O. validus* and two within *O. penicillatus*. Morphological synapomorphies for *Odontaster* include the single glassy-tipped, recurved spine at each oral angle between the two associated plates, abactinal plates with a distinct tabulum crowned with spinelets of variable length, tabulate marginal plates with short spinelets, and actinal plates that are spinulose (Clark 1962). *Odontaster meridionalis, O. pearsei, O. penicillatus, O. roseus,* and *O. validus* differ in external morphology in the number of spinelets on abactinal and actinal plates, the presence or absence of a border on marginal plates, and the occasional presence of pedicellariae (Clark 1962). However, these differences have been reported to be variable, even within the same species (Fisher 1940).

In order to understand the recent evolutionary history of *Odontaster* species, we used mitochondrial 16S ribosomal and cytochrome *c* oxidase subunit I (COI) gene sequence data, as well as, morphological characters from South American, sub-Antarctic, Antarctic Peninsula, and Ross Sea *Odontaster* adults. This allowed reassessment of our current understanding of species ranges. Larval specimens from the Antarctic Peninsula were also examined and we focused on the phylogeographic structure of *O. validus* within Antarctic waters. Both morphological characters and molecular data were employed to determine the presence of unrecognized *Odontaster* species in the relatively well-studied Antarctic Peninsula region

4.3 MATERIALS AND METHODS

4.3.1 SAMPLING AND INDENTIFICATION

Individuals from South American and Antarctic waters were collected during two five-week Antarctic research cruises aboard the *R/V Laurence M. Gould* in November/ December of 2004 and May/ June of 2006. Numbers, localities, and depth of specimen occurrence are given in Table 1 and Figure 1. Benthic samples were collected using a Blake trawl, wire dredge, or epibenthic sled. Depth of sampled sites ranged from 116-1170m. Upon collection, samples were immediately identified, photographed, and preserved. *Odontaster* specimens, or parts thereof, were then either frozen or placed in ethanol (95%) for molecular analyses. Morphological voucher specimens were originally preserved in formalin and then transferred to 70% ethanol and deposited to the Smithsonian Institution Natural History Museum (USNM 1127016-1127025). Larval specimens were also collected from the Antarctic Peninsula, during *R/V Gould* cruises using a conical net ¾ meter at its mouth, with a 250 micron mesh. The net was slowly towed for twenty minutes at an oblique angle to a depth of approximately 180m and then returned to the surface in a similar manner. Adult specimens from South Sandwich Island, South Georgia, and Bouvet Island were collected aboard the *R/V Nathaniel Palmer* during the ICEFISH 2004 cruise (provided by S. Lockhart and W. Detrich) and were fixed in ethanol. Ross Sea specimens were collected by SCUBA and provided by Stacy Kim.

Adult individuals were identified based on external morphology (Fisher 1940) by AMJ and kindly verified by Christopher Mah (Smithsonian Institution, National Museum of Natural History, NMNH, Washington D.C., USA). Larvae were identified as asteroid brachiolaria or

bipinnaria and were assigned to species based on phylogenetic analysis as in Janosik et al. (2008).

4.3.2 DNA EXTRACTION, PCR, AND SEQUENCING

DNA extraction of adult specimens was performed using DNeasy® Tissue Kit (Qiagen). Single larvae were subjected to whole genome amplification using a GenomiPhi kit following manufacturer's recommendations (GE Healthcare) without a separate DNA extraction step (the first heating step of the protocol lyses cells; Janosik et al. 2008). For 88 adult and 22 larvae (110 total individuals), a 508 bp region of the mitochondrial 16S gene was amplified using the 16SarL and 16SbrL primers following Palumbi et al. (1991). For the same individuals, a 627 bp region of the COI gene was amplified using *Odontaster*-specific primers designed for this study: COI-Ast 22F (5' TTYTCNACNAAACAYAAGGA 3') and COI-Ast722R (5' GGRTGNCCRAARAAYCARAA 3'). The 16S amplified products were purified using a gel-freeze excision method (i.e. excise DNA fragment from 1.5% TAE gel (3/4 TAE low melting temperature agarose), dice band up, place in 1.5ml tube, freeze for 20 minutes, centrifuge at max speed for 10 minutes, draw off liquid and place in new collection tube, vacufuge down if necessary to ~20µl) and COI amplified products were purified with either a Qiagen QIAquick® Gel Extraction Kit (Qiagen Inc.) or Montage PCR Filter Units (Millipore) according to the manufacturer's directions.

Purified products were then sequenced in both directions on a Beckman CEQ 8000 Genetic Analysis System (Beckman Coulter). Sequences were edited using Sequencher 4.6 (Gene Codes Corporation) and aligned with Clustal X algorithm and manually corrected by eye

using Bioedit v.7.0.8 (Hall 1999). COI sequences were translated according to the echinoderm mitochondrial DNA code to aid in proof-reading.

4.3.3 ANALYSES

Both a phylogeographic and a phylogenetic approach were used to assess species boundaries. Additionally, population-level approaches were carried out on *O. validus* and to some degree *O. penicillatus*. Sample numbers in other lineages were too small to include such approaches. The16S and COI genes were analyzed both separately and combined with TCS ver.1.21 (Clement et al. 2000) to visually examine mtDNA haplotype relationships in a parsimony network with a 95% connection (default value) limit between haplotypes. Gaps were treated as missing data. Genetic distances (uncorrected, *p*-distance values) were calculated using MEGA4 (Tamura et al. 2007). Nucleotide (π) and haplotype (h) diversities for *O. validus* and *O. penicillatus* were calculated using Dnasp v4.1 (Rozas et al. 2003). Tajima's D (Tajima 1989) and Fu's F_s (Fu 1997) tests were also calculated in Dnasp to evaluate selective neutrality of *O. validus* mtDNA sequences.

For *O. validus,* A hierarchical AMOVA (analysis of molecular variance) was carried out for the combined dataset using Arlequin ver. 3.1 (Excoffier et al. 2005) to evaluate the likely population structure and geographical subdivisions in the sub-Antarctic and the Antarctic. Variance was partitioned into three hierarchical levels: among regions (Φct; i.e. sub-Antarctic, Antarctic Peninsula, and the Ross Sea) among sampling stations within regions (Φsc), and finally within stations (Φst). Additionally, pairwise Φst tests were computed between all collection stations for the combined 16S and COI dataset of *O. validus* by using Arlequin with 10,000

permutations using the Tamura- Nei model (Tamura and Nei 1993) to determine the genetic structure of these populations across the sampled range.

A Bayesian approach was used to infer phylogeny. MrModeltest ver. 2.2 (Nylander 2004) was used for both COI and 16S datasets to select models of nucleotide substitution with AIC criterion (GTR+G was calculated for both). After separate analyses and due to limited variation in 16S (see Results), 16S and COI datasets were concatenated for analyses in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), and were treated as unlinked partitions. Based on current understanding of sea star relationships (Blake 1987), *Acodontaster conspicuus* (Genbank accession number: 16S: DQ297071, COI: DQ380237) was chosen as the outgroup. Posterior probabilities were obtained by a Markov Chain Monte Carlo (MCMC) algorithm with two independent runs of one cold and three heated chains. Samples of trees and parameters were drawn every 100 steps from a total of 2 X 10^6 MCMC generations. The first 4,000 trees were discarded as the burnin (based on convergence of likelihood values) and the remaining trees were used to compute a consensus tree.

4.4 RESULTS

The combined 16S (Genbank accession numbers: GQ294397-GQ294457) and COI (Genbank accession numbers: GQ294339-GQ294396) dataset consisted of 1135 bp from 110 individuals (30 South America, 21 sub-Antarctic, and 59 Antarctic). These sequences had 241 polymorphic sites and 64 unique haplotypes. Alignments have been submitted to TreeBase (www.TreeBase.org).

4.4.1 POPULATION STRUCTURE AND GENETIC DISTANCES

TCS analyses of combined data and individual genes produced consistent results. Therefore, in the interest of brevity, we present the combined results below and provide results based on the separate COI and 16S data in Supplementary Materials. Whereas the COI data recovered the same pattern of networks as the combined data, the 16S produced only 3 networks with the most divergent lineages (*Odontaster meridionalis*, see below), forming separate networks. These differential results can be attributed to a slower rate of evolution of the 16S gene, when compared to the COI gene (e.g., Edmands et al. 1996; Hart et al. 1997; Lessios et al. 1999; Thornhill et al. 2008; Wilson et al. 2009).

The combined 16S and COI statistical parsimony (TCS) analysis recovers six discrete haplotype networks (Figure 2). The largest network (clade I) included 67 individuals from Antarctic and sub-Antarctic waters that morphologically corresponded to *Odontaster validus*. Clade II, *O. penicillatus*, consisted of 26 individuals from South American waters and one individual from the sub-Antarctic. Clade III (4 individuals) and clade IV (8 individuals) are networks comprised of Antarctic individuals, which are morphologically distinct from *O. validus* (discussed below). Individuals in clades V, 4 individuals, and VI, 1 individual, correspond to the morphological definition of *O. meridionalis*. Notably, clade V includes individuals from South American and Antarctic waters. Mean uncorrected (*p*) distance values for the combined dataset are shown in Table 2. Sequence divergence comparing clades V and VI to all other clades ranges from 15.1-15.8%, where as the divergence when comparing clades I, II, III, and IV ranges from 2.5-4.3%. (Values for 16S and COI dataset presented in Supporting Information, online resources 1-4.)

Due to the number of individuals sampled within clades, we focused population level analyses on clade I, *O. validus*. For the combined16S and COI dataset, nucleotide (π) and

haplotype (*h*) diversities were 0.00223 and 0.769, respectively. Neutrality test results for Tajima's *D* (-2.47458, P > 0.01) and Fu's F_s (-22.951, P > 0.05) were significantly negative suggesting recent population expansion (Fu 1997). Significantly negative values for Tajima's *D* and Fu's F_s indicate an excess of rare polymorphisms in a population, which could be a result of purifying selection or a recent population expansion (Wares 2009). AMOVA results for *O. validus* for all three levels of variance were highly significant and indicated that most observed variation was within sampling stations (97.02%; Table 4). Pairwise Φst results indicate that some station localities from the sub-Antarctic, Antarctic Peninsula, and the Ross Sea are genetically indistinguishable from one another, whereas others are significantly distinct (Table 5). Specifically, station 06-33 is significantly different from stations 04-33, 04-45P, and 04-68P, but the latter three are not significantly different from one another.

4.4.2 PHYLOGENETIC RELATIONSHIPS

Results from the Bayesian analysis correspond to TCS network results. The Bayesian topology based on combined data (Figure 3) reveals multiple distinct groups corresponding to recognized species of *Odontaster* and the recovered TCS clades. Clade I (posterior probability value of 1.00) is solely comprised of individuals with morphology that is characterized by the definition of *O. validus,* from Bouvet Island and South Sandwich islands in the sub-Antarctic, the Antarctic Peninsula Region, and the Ross Sea. Clade II (posterior probability = 0.93) is comprised of *O. penicillatus* individuals from South American waters, with the exception of one individual from the sub-Antarctic region. *Odontaster meridionalis* individuals (clades V and VI, posterior probability = 1.00) fall basal to other *Odontaster* lineages sampled herein. Clades III and IV from the TCS analysis were recovered as distinct lineages in the Bayesian analysis. Clade IV

(posterior probability = 0.93), composed of Antarctic individuals, is sister to *O. penicillatus*, the South American species. Clade III (posterior probability = 0.99), again exclusively Antarctic, falls out sister to *O. validus* (Clade I)/ *O. penicillatus* (Clade II)/Clade IV clade.

4.4.3 MORPHOLOGICAL CHARACTERS

Based on molecular results, we re-examined available specimens for morphological differences. Morphological characters consist of external endoskeletal features, variation in accessory structures and spines, which were quantified among *Odontaster* species. Published descriptions and keys from Fisher (1940) and Clark (1962) of *O. meridionalis, O. penicillatus,* and *O. validus* were used. These characters revealed diagnostic morphologies associated with Clades III and IV. Due to the presence of both unique morphological and molecular characters, previously unrecognized species for Clades III and IV are examined and described in Janosik and Halanych (2010, chapter 3). Clade III is named *Odontaster roseus* and Clade IV is *Odontaster pearsei***.**

4.5 DISCUSSION

4.5.1 UNDERESTIMATED BIODIVERSITY OF *ODONTASTER*

Phylogenetic analyses and morphological characters reveal that the Southern Ocean sea star genus *Odontaster* was more species rich than previously known, similar to previous Antarctic faunal studies showing unrecognized species diversity (Hedgepeth 1969; Dell 1972; Held and Leese 2007; Wilson et al. 2007; Hunter and Halanych 2008; Mahon et al. 2008; Thornhill et al. 2008; Wilson et al. 2009, Mahon et al. 2009; Krabbe et al. 2010). Morphological

characters and molecular data indicate the presence of at least two previously unrecognized *Odontaster* species in the Antarctic Peninsula region. The differences in morphological and molecular characters are sufficient to consider *O. roseus* and *O. pearsei* (corresponding to clades III and IV, respectively) as unrecognized biodiversity (with clear diagnostic morphology that has escaped detection) rather than cryptic species (without detectable morphological difference, yet genetically distinct) or a species complex (Janosik and Halanych *in press*). Additionally, molecular data support current morphological definitions of *O. meridionalis, O. penicillatus* and *O. validus* (Figure 3). All *Odontaster* individuals examined here possess the characteristic *Odontaster* glassy tipped, recurved, spine on each mouth plate and a tabulum crowned with spinelets, diagnostic of the genus (Fisher 1940). Rough morphological differences can be seen in the number of spines on abactinal plates and spine length, as well as differences in marginal plates and marginal spines (complete descriptions and key in Janosik and Halanych *in press*). In other words, distinct clades exhibit clear differences in external morphology and form reciprocally monophyletic lineages.

Interclade (Clades I –IV) uncorrected genetic distances for the COI dataset (3.5-6.9%) are comparable to the 5-7% typically found between echinoderm species (Foltz 1997; Hart et al. 1997; Lessios et al. 2001; Waters and Roy 2003). Exceptionally, *O. meridionalis* (Clades V and VI) displays considerably higher genetic distances (approx. 15%) when compared to all other clades. Although samples size is limited, genetic distance between *O. meridionalis* clades V and VI is small (0.09%), indicating they are likely a single trans-Drake species; however further sampling is necessary to verify these conclusions. Evolutionary history of *Odontaster* inferred from Bayesian analysis suggests dispersal from the Antarctic to South American waters, a pattern also observed for southern ocean octopus (Strugnell et al. 2008). Specifically, tree

topology suggests that *O. penicillatus* originated within a clade that included Antarctic and sub-Antarctic representatives, yet no representatives from South American waters, indicating that *O. penicillatus* ancestors dispersed from waters south of the APF.

4.5.2 PHYLOGEOGRAPHY OF *ODONTASTER*

Combined mitochondrial data show the majority of *Odontaster* species are not actively dispersing across the Drake Passage (i.e., the APF or ACC), with one possible exception. Specifically, populations of *O. validus,* along with *O. pearsei* and *O. roseus*, appear to be currently isolated to the Antarctic side of the Drake Passage, while *O. penicillatus* currently resides on the South American side. Comparatively, despite limited sampling, *O. meridionalis,* which also possesses planktotrophic larvae, could be a trans-Drake species (occurring on both sides of the Drake Passage), showing possible recent genetic connectivity between South American populations and Antarctic Peninsula populations. At the very least, we can conclude that the sampled *O. meridionalis* specimens descended from a recent common ancestor and that at least one recent dispersal event across the Drake Passage has taken place in this species. Although low in sample size, *O. roseus* and *O. pearsei* were only observed along the Antarctic Peninsula (locations indicated on Figure 1), although no immediate geographic pattern corresponding to currents or landmasses emerges. *Odontaster roseus* and *O. pearsei* appear sympatric with *O. validus* at a broad geographic level, but a finer resolution of the specific ecological habitat is needed. This pattern along the peninsula is comparable to that seen in the crinoid *Promachocrinus kerguelensis* (Wilson et al. 2007).

The mitochondrial data show *O. validus* to be geographically isolated to Antarctic and sub-Antarctic waters despite a planktotrophic mode of development with the capabilities for vast

dispersal. *Odontaster validus* is likely currently constrained to Antarctic waters perhaps by the combination of physiological constraints and physical barriers encircling the Antarctic continent and the sub-Antarctic islands. In particular, the APF may be facilitating speciation, acting as a barrier between the two geographic regions, and restricting the north-south exchange of organisms (Clarke et al. 2005). The APF has long been assumed to be a barrier to dispersal and subsequently gene flow across the Drake Passage (Crame 1999; Bargelloni et al. 2000; Clarke et al. 2005). For example, the nemertean worm *Parborlasia corrugatus* is capable of vast dispersal, but displays no population connectivity between the Antarctic and South American waters (Thornhill et al. 2008). Populations of krill, species also regarded as capable of long-distance dispersal, appear to be genetically isolated to either side of the Drake Passage (Patarnello et al. 1996). Additionally, brooding brittle star, *Astrotoma agassizii,* has genetically distinct populations in South America and Antarctica (Hunter and Halanych 2008). Lastly, in Held (2000) a biogeographic study on serolid isopods, show molecular evidence of separation of species between South America and Antarctica at a higher taxonomic level.

In the case of *Odontaster*, as with other organisms (e.g. Page and Linse 2002; Hunter and Halanych 2008; Thornhill et al. 2008, Wilson et al. 2007), the barrier to gene flow is far younger than the formation of the APF, which is presumed to be well over 20MYA (Pfuhl and McCave 2005; Scher and Martin 2006). Like *Astrotoma agassizii*, if we employ a standard echinoderm mtDNA divergence rate of 3.1-3.5% per million years (Lessios et al. 1999; McCartney et al. 2000), then *O. penicillatus* and *Odontaster pearsei* separated approximately 1MYA. The uncorrected distance of 3.5% between these taxa is a slight underestimate, as a distance measure that corrects for multiple substitutions will suggest more divergence. Nonetheless, the time of separation of these taxa is more than an order of magnitude different from the opening of the

Drake Passage. Furthermore, for some species, the APF appears to be permeable on recent time scales. A possible mechanism for permeability may be correlated with the last glacial maximum (LGM) (approximately 21,000 years ago) and the fact that the boundary of the APF is changing and moving, as shown by Gersonde et al. (2005). Specifically, although today the APF is both a physical water mass boundary and an ecological boundary, at the last glacial maximum the two boundaries likely became uncoupled allowing the ecological boundary between polar and nonpolar species to shift north of the APF (Moore et al. 2000). For example, *O. meridionalis* and *Sterechinus antarcticus* (pers. comm. L.N. Cox, KMH) appear to be trans-Drake species, showing similar genetic signatures on both sides of the APF. Clearly, population connectivity across the Drake Passage in the Southern Ocean is species-specific and influenced by many factors that may include life history, larval duration, ocean current patterns, as well as thermal, physiological, and ecological tolerances.

While the APF may act as a barrier between South America and the Antarctic, the ACC appears to be homogenizing genetic structure of marine organisms within Antarctic waters. Specifically, some organisms exhibit long-distance population connectivity, with the same haplotype found from the Atlantic sector to the Ross Sea (i.e. Raupach et al. 2010). The long-distance genetic similarity observed here with *O. validus*, mirrors the results of *Parborlasia corrugatus* (Thornhill et al. 2008), which shows the same haplotype from the Ross Sea to Bouvet Island in the sub-Antarctic (approximately 8000km). Our data suggests connectivity in *O. validus* for populations from the sub-Antarctic, Antarctic Peninsula and Ross Sea, and demonstrates historical connectivity between all of these populations. Additionally, results from the analysis of molecular variance (AMOVA) show insignificant genetic differentiation of *O. validus* throughout Antarctic populations; reported percentage of variation is highest within

stations (97.02%), and low fixation indices indicate little to no genetic structure at all three hierarchical levels of variance. These results are not surprising given the dispersal ability of *O. validus.* Although these molecular data suggest *O. validus* likely has a broad, and perhaps circumpolar, distribution, this result is not a given as the crinoid, *Promachocrinus kerguelensis,* appears to have clades which are geographically isolated within Antarctic waters despite a long-lived dispersal larval form (Wilson et al. 2007). However, although the Drake Passage spans approximately 900 km and reaches ~5000 m depths in some places (Whitworth et al. 1982), *O. validus* appears to exhibit genetic similarity from the sub-Antarctic to the Ross Sea, a distance of at least 3000 km. Thus, the ACC and the APF, rather than geographic distance, are likely acting as a barrier to gene flow between South America and Antarctica.

Life history strategy and larval dispersal ability can play an important role in population structure of marine organisms. The use of molecular markers to investigate genetic homogeneity has shown that phylogeographic patterns are not always readily predictable based on dispersal potential (Waters and Roy 2003; Hunter and Halanych 2008; Mahon et al. 2008; Thornhill et al. 2008; Hayes and Karl 2009). Factors such as larval duration, habitat preferences, ocean currents, and palaeogeographic conditions also need to be considered. The most obvious mechanism for the observed genetic similarity over long-distances in *O. validus* is passive transport during the planktonic larval phase (Smith 1997; Figure 1, insert). *O. validus* produces larvae in the austral winter, when productivity is low, developmental rates are slow, and availability of food is reduced, however larvae are able to overcome these obstacles and exhibit success with a winter spawning strategy, apparent through their wide distribution and abundance (Stanwell-Smith and Peck 1998). Currents such as the ACC, the gyres surrounding the Antarctic, and the Antarctic coastal current (a.k.a., East Wind Drift, Phillpot 1985) are likely propelling larvae around the

continent, as well as into the sub-Antarctic. Dispersal could also be occurring by migration of adults along the sea floor, specifically along the Scotia Arc south to the Peninsula Region or vice versa. Rafting of adults has been suggested for adult dispersal (Helmuth et al. 1994), but this explanation seems less likely given the biology of these sea stars. Specifically, these sea stars have not been recorded from the Drake Passage and also don't possess the correct anatomical attachment features for rafting on kelp or driftwood.

Based on mitochondrial gene diversity and morphological characters populations of *O. validus* appear to be connected over great geographic distances south of the APF in Antarctic and sub-Antarctic waters, yet isolated from South American populations, and present data for two previously undescribed species (described in Janosik and Halanych 2011). Therefore, the APF appears to be a barrier to recent dispersal in *O. validus* across the Drake Passage. Unrecognized diversity and long-distance genetic homogeneity appear to be common trends when investigating Antarctic organisms, but factors influencing population structure and distribution seem to be species specific. Further work and sampling are necessary to wholly understand *Odontaster* populations in the Southern Ocean. With an understanding of the systematics and population structure, better conclusions can be drawn about the driving forces behind the evolutionary history in *Odontaster* and surrounding species that make up the complex, isolated Antarctic ecosystem.

4.6 References

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Table 1. *Odontaster* collection data. Station numbers correspond to Figure 2, N refers to the number of individuals sequenced for 16S and COI, and A/L refers to whether the specimen is a larva or an adult.

Geographic Region	Station Number	N	A/L	Depth (m)	Sample Name	Latitude	Longitude
Argentina	LMG 04-02	1	A	78	As 1	S 53°24’	W66°57’
Argentina	LMG 04-07	12	A	108	As 2, 3, 4, 5, 6, 35, 36, 37, 38, 77, 78, 79	S 54°27’	W63°52’
Argentina	LMG 04-09	2	A	321	As 39, 40	S 54°28’	W 62°12’
Argentina	LMG 06-04	7	A	170	As 21, 22, 55, 56, 57, 61, 81, 88	S 53°47’	W 60°42’
Argentina	LMG 04-14	1	A	207	As 42	S 54°41’	W 59°23’
Argentina	LMG 06-08	1	A	274	As 59	S 54°22’	W 61°52’
Argentina	LMG 06-09	2	A	318	As 23, 60	S 54°29’	W 62°12’
Argentina	LMG 16-19	1	A	114	As 49	S 52°60’	W 59°01’
Argentina	LMG 06-01	2	A	96	As 53, 54	S 53°16’	W 66°23’
Argentina	LMG 06-07	1	A	125	As 58	S 54°20’	W 60°59’
sub-Antarctic	57-32	6	A	130	As 8, 20, 44 52, 75, 76	S 57°05’	W26°75’
sub-Antarctic	38-18	1	A	46	As 41,47,48,80	S 53°55’	W 37°00’

sub-Antarctic	66-57	2	A	180	As 30, 66	S 54°38’	W3 °50’
sub-Antarctic	51-35	7	A	335	As 17, 18, 19, 50, 51, 73, 74	S 58°47’	W26°19’
sub-Antarctic	52-43	2	A	-	As 7, 43	S 58°56’	W26°28’
Antarctic Peninsula	LMG 06-13	3	A	132	As 24, 25, 26	S 63°24’	W 61°50’
Antarctic Peninsula	LMG 06-14	1	A	188	As 27	S 62°56’	W 61°28’
Antarctic Peninsula	LMG 04-33	8	A	117	As 9, 10, 11, 12, 13, 14, 15, 45	S 61°09’	W55°51’
Antarctic Peninsula	LMG 06-33	4	A	122	As 28, 62, 63, 82	S 67°44’	W 69°17’
Antarctic Peninsula	LMG 04-38	1	A	207	As 16	S 62°44’	W56°44’
Antarctic Peninsula	LMG 06-45	1	A	195	As 29	S 67°43’	W 69°18’
Antarctic Peninsula	LMG 06-21	1	A	194	As 61	S 63°08’	W 57°07’
Antarctic Peninsula	LMG 06-52	3	A	282	As 64, 65, 83	S 65°39’	W 68°24’
Antarctic Peninsula	LMG 04-74	1	A	202	As 46	S 64°24’	W64°30’
Antarctica	89-56	5	A	-	As 31, 32, 67, 84, 85	S 62º16’	W 60º46’
Ross Sea	CA-OV	8	A	-	As 33, 68, 69, 70, 71, 86, 87, 88	S 77º51’	W 166º39’

Ross Sea	TR-OV	2	A	-	As 34, 72	S 77º44’	W 166º46’
Antarctic Peninsula	LMG 06-10P	1	L	0-180	As 89	S 64º23’	W 62º59’
Antarctic Peninsula	LMG 06-30P	1	L	0-180	As90	S 65º50’	W 62º59’
Antarctic Peninsula	LMG 04-47P	4	L	0-180	As 91, 92, 93, 94	S 62º51’	W 59º27’
Antarctic Peninsula	LMG 04-45P	7	L	0-180	As 95, 96, 97, 98, 99, 100, 101	S 62º15’	W58º16’
Antarctic Peninsula	LMG 04-68P	4	L	0-180	As 102, 103, 104, 105	S 63º28’	W62º23’
Antarctic Peninsula	LMG 04-60P	5	L	0-180	As 106, 107, 108, 109, 110	S 62º58’	W61º35’

Table 2. Uncorrected (*p)* distance values between clades ± standard error for combined data.

	Clade I	**Clade II**	**Clade III**	**Clade IV**	**Clade V**	**Clade VI**
Clade I *O. validus*	-					
Clade II *O. penicillatus*	0.031 ± 0.010	-				
Clade III *Odontaster roseus*	0.024 ± 0.010	0.025 ± 0.013	-			
Clade IV *Odontaster pearsei*	0.043 ± 0.008	0.034 ± 0.008	0.033 ± 0.010	-		
Clade V *O. meridionalis*	0.152 ± 0.069	0.157 ± 0.072	0.151 ± 0.069	0.153 ± 0.070	-	
Clade VI *O. meridionalis*	0.154 ± 0.070	0.158 ± 0.073	0.152 ± 0.069	0.154 ± 0.071	0.006 ± 0.004	-

Table 3. Genetic statistics for combined 16S and COI data within clades, N refers to the number of individuals, H refers to the number of haplotypes, π refers to nucleotide diversity, and *h* is haplotype diversity.

Geographic region	Clade	N	H	π	*h*
Antarctica	I	67	27	0.00222	0.75433
South America	II	26	11	0.00369	0.79692
Antarctica	III	4	2	0.00236	0.66667
Antarctica	IV	8	6	0.00227	0.89286
South America/Antarctica	V, VI	5	4	0.00471	0.90000

Table 4. Pairwise ϕ_{ST} values for collection stations for *O. validus* in Antarctic waters.

Source of Variation	Degrees of Freedom	Sum of Squares	Variance Component	Percentage of Variation	Fixation Indices
Within stations	49	129.470	2.64224Vc	97.02	ϕ_{SC}= 0.03813
Among stations within a region	8	25.457	0.10475Vb	3.85	ϕ_{ST}= 0.02978
Among regions	2	5.756	-0.02365 Va	-0.87	ϕ_{CT}= -0.00868
Total	59	160.683	2.72335		

Table 5. Population pairwise ϕ_{ST} values for *O. validus*. Bold indicates $P< 0.05$. Negative values changed to a dash (-).

	Station	St.38-18	St.57-32	St.51-35	St04-33	St.04-45P	St.04-60P	St.04-68P	St.89-56	St.06-33	St.CA-OV	St.TR-OV
Geographic region												
sub-Antarctica	**St.38-18**	-										
	St.57-32	0.05939	-									
	St.51-35	-	-	-								
Antarctic Peninsula	**St04-33**	0.05691	-	0.0407	-							
	St.04-45P	0.07911	-	0.0191	-	-						
	St.04-60P	-	0.02299	0.0576	0.05707	0.06143	-					
	St.04-68P	0.19298	0.05376	0.0133	0.00565	0.03838	-	-				
	St.89-56	-	-	0.0050	-	-	0.00346	0.07133	-			
	St.06-33	0.21588	0.30000	0.1842	**0.24879**	**0.31944**	-	**0.37778**	0.21868	-		
Ross Sea	**St.CA-OV**	0.05934	0.04684	-	0.05588	0.05294	0.06085	0.03653	0.0000	0.18424	-	
	St.TR-OV	0.20530	0.01031	-	-	-	-	0.04000	0.04110	0.35514	-	-

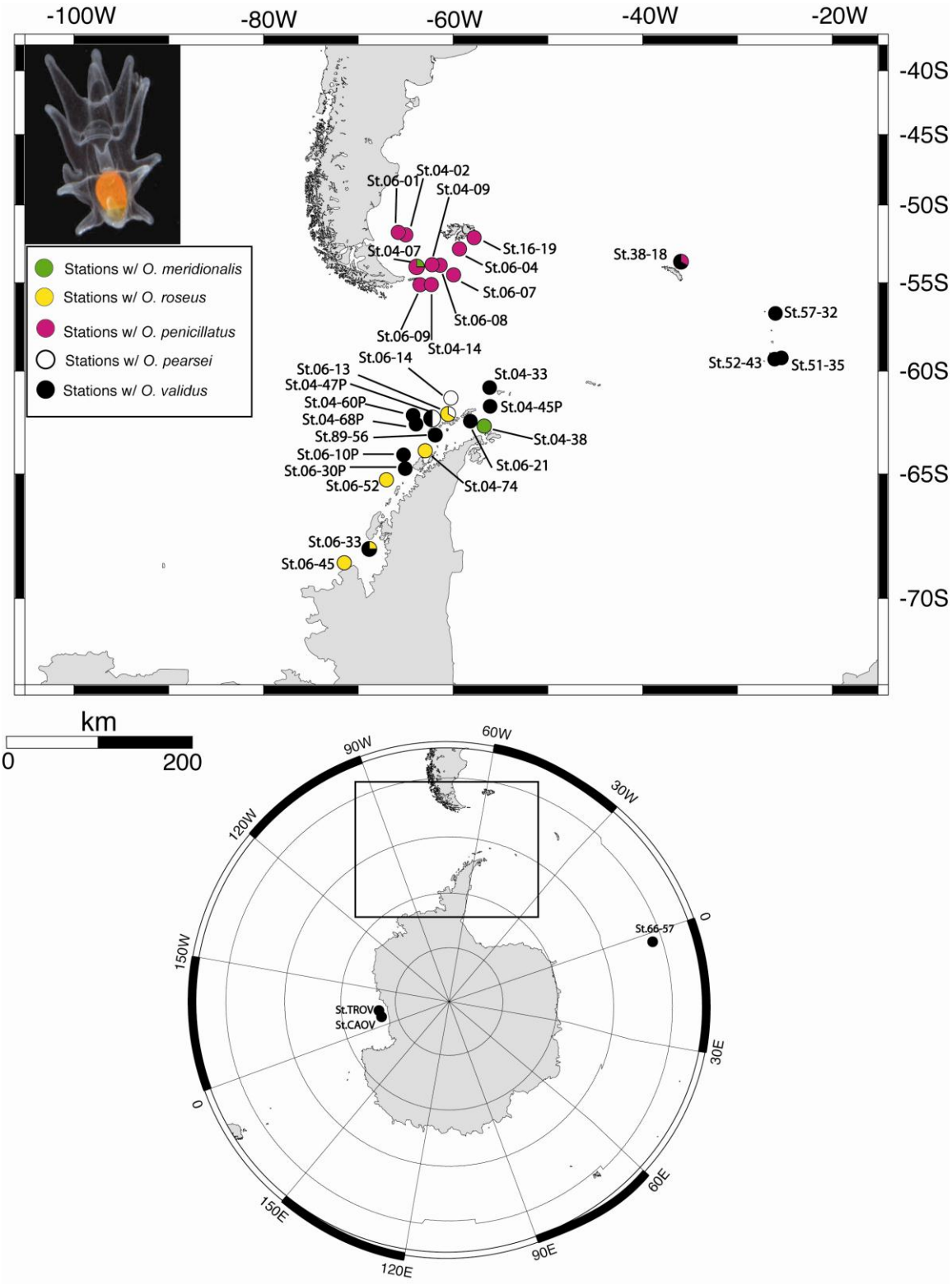


Figure 1. Map showing collection localities for *Odontaster* from South American and Antarctic waters, P denotes larval station locality (see picture insert).

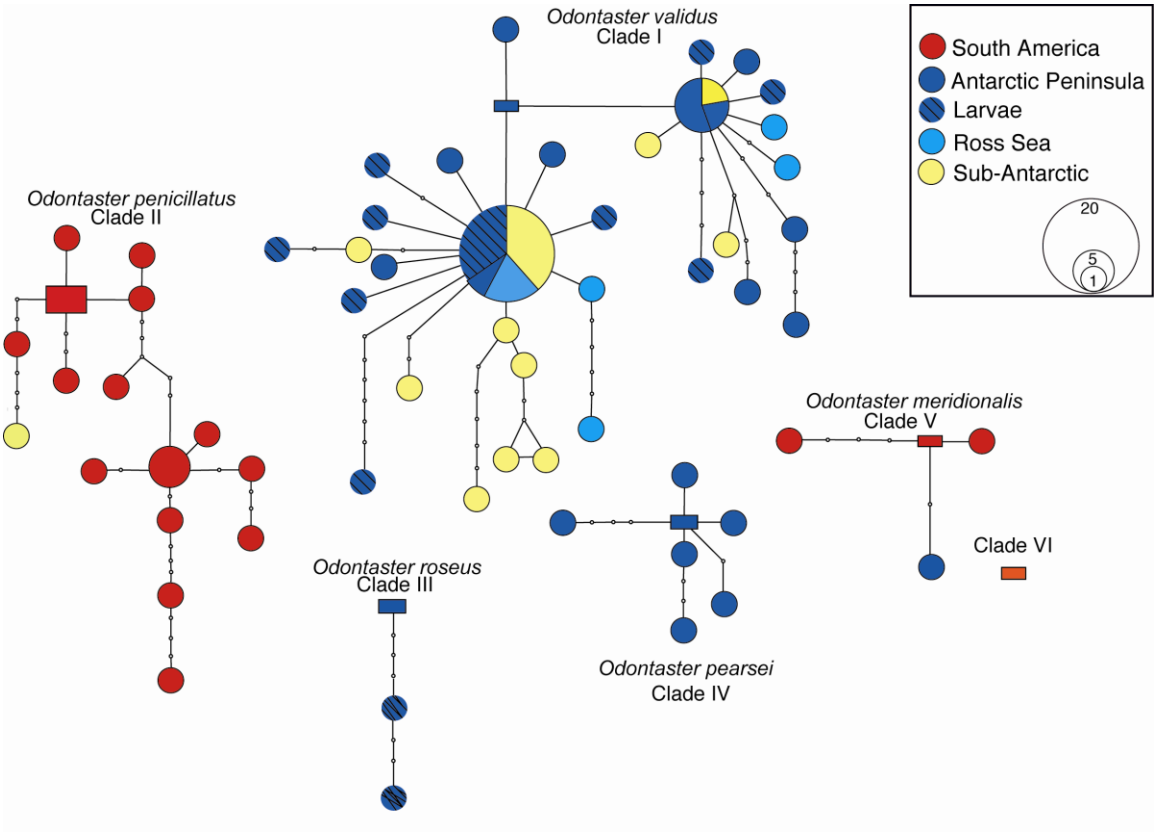


Figure 2. Network representation for 16S and COI combined data. Networks are coded by geographic locality. Haplotypes are sized according to abundance and missing haplotypes are denoted by small, closed circles. Rectangles denote presumed ancestral haplotypes

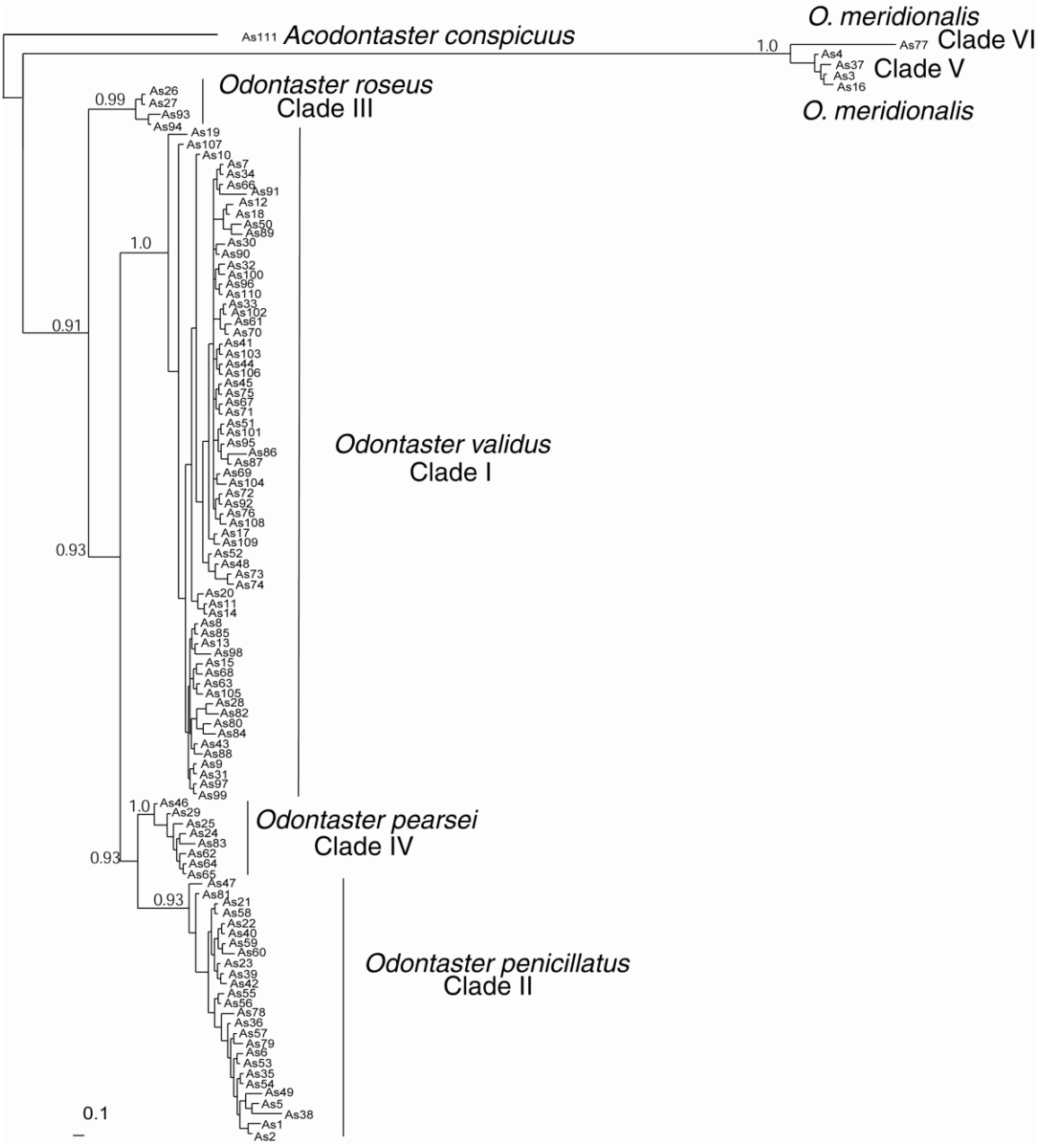
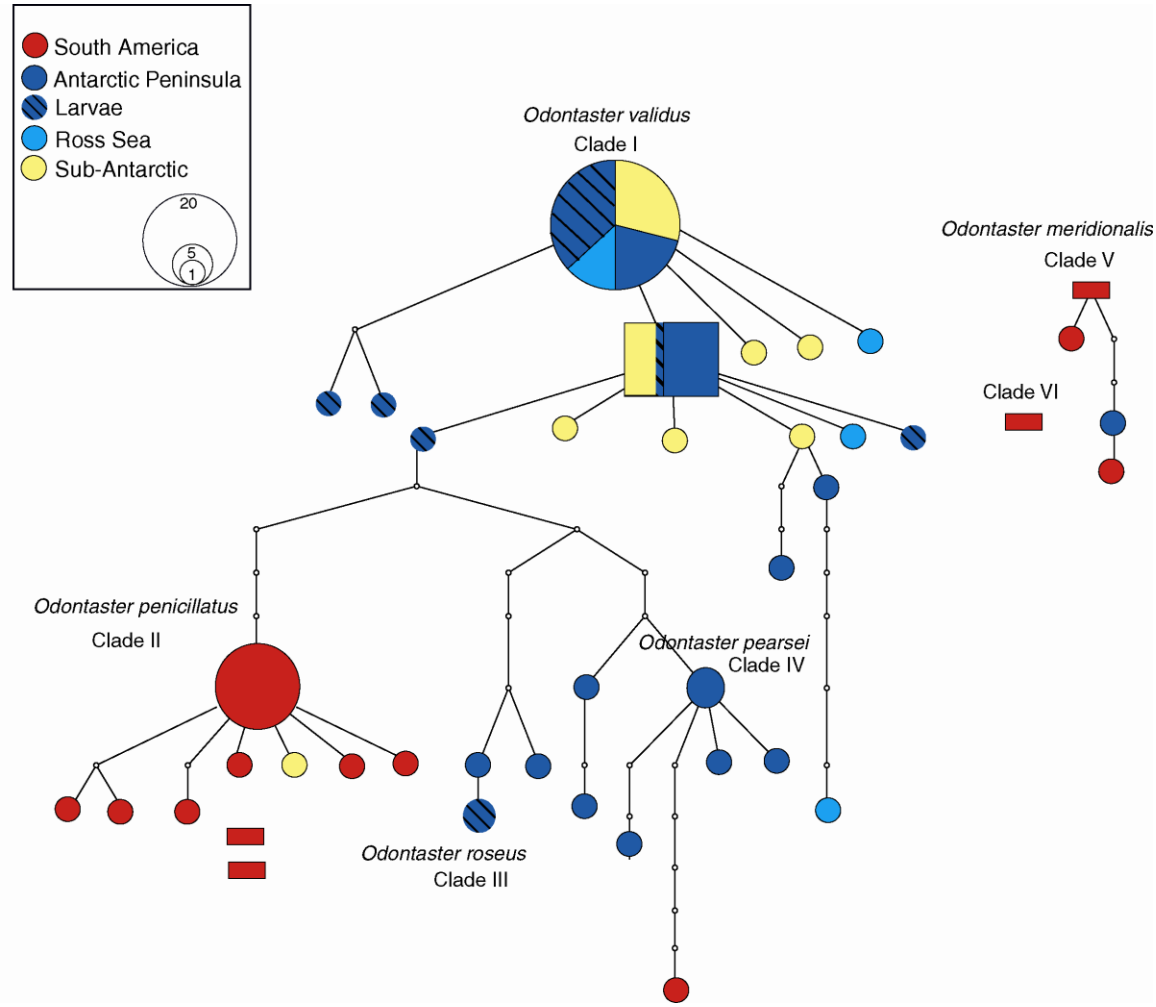
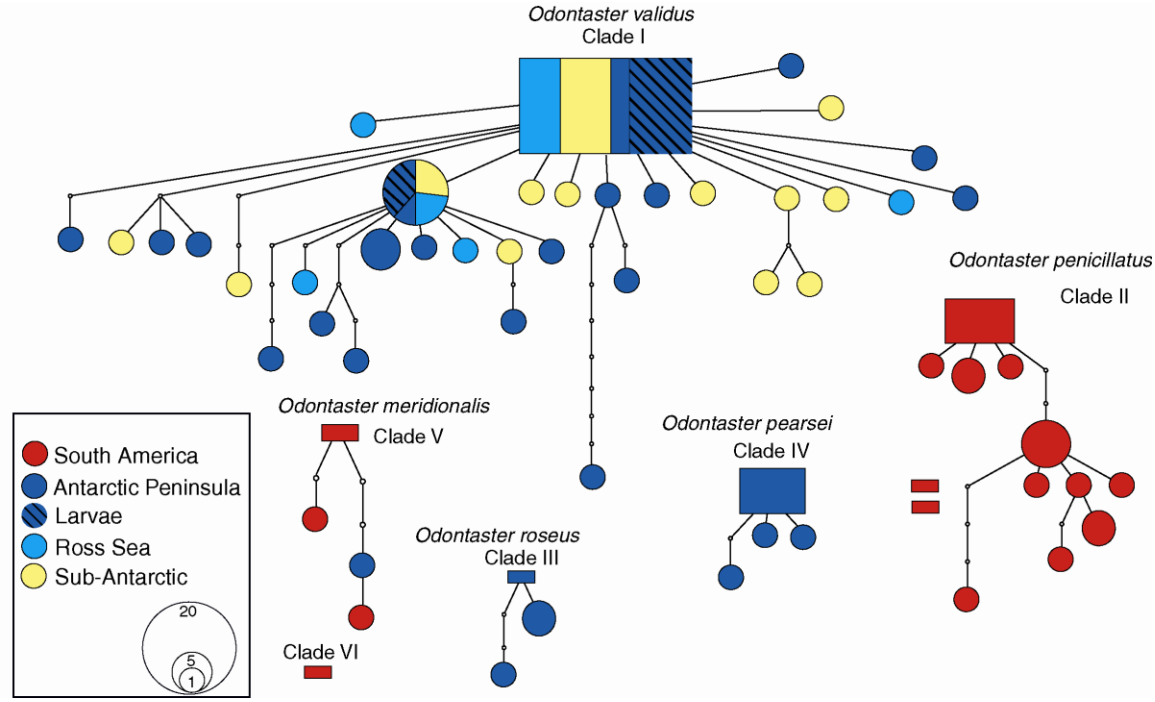


Figure 3. Bayesian inference topology for combined 16S and COI data of South American, sub-Antarctic, and Antarctic *Odontaster* spp. Analysis details are given in the text. Number next to nodes indicates Bayesian posterior probabilities. Alphanumeric names correspond to individual numbers from Table 1. Tree rooted with *Acodontaster conspicuus*.



Online Resource 1 Network representation for 16S data.



Online Resource 2 Network representation for COI data.

Online Resource 3. Uncorrected (*p)* distance values between clades ± standard error for 16S data.

	Clade I	**Clade II**	**Clade III**	**Clade IV**	**Clade V**	**Clade VI**
Clade I *O. validus*	-					
Clade II *O. penicillatus*	0.031 ± 0.010	-				
Clade III *Odontaster* roseus	0.018 ± 0.002	0.038 ± 0.010	-			
Clade IV *Odontaster* pearsei	0.015 ± 0.001	0.026 ± 0.010	0.025 ± 0.003	-		
Clade V *O. meridionalis*	0.199 ± 0.018	0.219 ± 0.033	0.185 ± 0.065	0.195 ± 0.051	-	
Clade VI *Odontaster meridionalis*	0.198 ± 0.027	0.230 ± 0.054	0.200 ± 0.10	0.196 ± 0.075	0.005 ± 0.023	-

Online Resource 4. Uncorrected (*p)* distance values between clades ± standard error for COI data.

	Clade I	**Clade II**	**Clade III**	**Clade IV**	**Clade V**	**Clade VI**
Clade I *O. validus*	-					
Clade II *O. penicillatus*	0.050 ± 0.006	-				
Clade III *Odontaster* roseus	0.048 ± 0.004	0.069 ± 0.011	-			
Clade IV *Odontaster* pearsei	0.035 ± 0.002	0.039 ± 0.008	0.045 ± 0.010	-		
Clade V *O. meridionalis*	0.103 ± 0.047	0.109 ± 0.049	0.116 ± 0.056	0.103 ± 0.050	-	
Clade VI *O. meridionalis*	0.186 ± 0.025	0.188 ± 0.041	0.179 ± 0.086	0.174 ± 0.065	0.009 ± 0.083	-

CHAPTER 5. Exploring population connectivity of *Odontaster validus* in the Southern Ocean using high-resolution molecular markers

5.1 ABSTRACT

Phylogeographic studies on Antarctic invertebrates, especially those using microsatellite markers, are limited. Here, we investigate the potential role of oceanographic features and historical events in shaping population connectivity of a common Antarctic sea star *Odontaster validus* across the Western Antarctic. In this study, we use microsatellite markers, which have greater resolution than previous mtDNA studies. Additionally, we examine individuals across a large scale (i.e. ~8,000km), for which studies on Antarctic benthic invertebrates are lacking. Analyses of six microsatellite markers from 59 individuals, revealed little genetic structure between populations from the sub-Antarctic over to the Ross Sea sector and are similar to mtDNA results previous work. Congruence between marker systems shows a lack of genetic differentiation throughout the Western Antarctic. Microsatellites demonstrate population connectivity since the Last Glacial Maximum and connectivity is attributed to long-lived planktotrophic larvae, driven by strong currents moving around the Antarctic in the Southern Ocean.

5.2 INTRODUCTION

Current species' distributions and spatial patterns are shaped by biogeographic processes. For example, combinations of historical events, such as glaciations and climate change; as well as oceanographic features and organismal interactions with the biotic environment play a role in

influencing marine species distributions. For example, ocean currents and glaciation can influence dispersal, colonization rates, and habitat availability, among other factors. As such, gene flow in Southern Ocean marine communities has been hypothesized to be influenced by several historical and biotic factors.

In particular, historical factors influencing Antarctic benthos include marine geological and geophysical data that suggest during repeated glaciations, grounded ice masses advanced across the Antarctic continental shelf and erased much of the shallow benthic fauna (Brey et al. 1996, Thatje et al. 2005). Grounded ice, however, likely did not advance to the shelf edge on some parts of the Eastern Antarctic (Thatje et al. 2005). Thus, such shelf areas could have acted as refugia, where benthic fauna might have survived and migrated between dynamic shelters. Additionally, during the last glacial period, deglaciation of the Antarctic shelf likely occurred diachronously (Anderson et al. 2002). This process may have resulted in shallow-water niches, prone to recolonization by pioneering species with planktotrophic life cycles that could disperse and radiate around Antarctica during the following interglacial period (Thatje 2005). In terms of genetic structure in benthic invertebrates, glaciation events generally result in two hypothesized outcomes: either organisms are expected to have a circumpolar distribution due to genetic homogenization from dispersal between refugia, or organisms become isolated and display population structure, i.e. several lineages isolated around the continent.

Additionally, Antarctica has been isolated both geographically and thermally for millions of years by distance, deep water, oceanographic currents, and sharp temperature boundaries (Pfuhl & McCave 2005, Scher & Martin 2006). Specifically, the Antarctic Polar Front (APF) acts as a sharp boundary between warmer salty waters and fresher, colder polar waters and extends to depths of approximately 1000 meters (Kock 1992, Eastman 1993). The Antarctic

Circumpolar Current (ACC) is the world's largest ocean current, flows around the continent as a series of eastward moving currents, and extends from the sea surface to 4000 meters deep (Klinck & Nowlin 2001, Lawver & Gahagan 2003). Although the APF has often been proposed as a barrier to dispersal contributing to extreme Antarctic isolation; the ACC combined with prolonged larval duration has been described as a mechanism for circum-Antarctic larval transport and subsequent organismal distribution (Arntz et al. 1997). As a result of historical processes and environmental factors, the Antarctic benthos is one of the most isolated and faunistically unique ecosystems on the planet (Arntz et al. 1994).

Some Antarctic species distributions have historically been thought of as circumpolar, i.e. a single-connected population around the isolated Antarctic continent (Thatje et al. 2005). Recently, through phylogeographic investigations utilizing molecular tools, two general themes about benthic invertebrates have emerged. First, some studies have provided evidence for distinct population structure and unrecognized lineages which represent underestimated biodiversity in Antarctic waters (see Brierley et al. 1993, Allcock et al. 1997, Held 2003, Held & Wagele 2005, Raupach & Wagele 2006, Linse et al. 2007, Leese & Held 2008, Hunter & Halanych 2010, Janosik & Halanych 2010, Mahon et al. 2010). Comparatively, other studies show long-distance connectivity between and among populations distributed over long distances (Zane et al. 1998, Hunter & Halanych 2008, Thornhill et al. 2008, Wilson et al. 2007, Wilson et al. 2009, Hemery et al. 2012). Although these studies provide great insight into Antarctic species distributions, because they mainly rely on mitochondrial DNA, it is difficult to describe present-day population structure as a result of historical demographic events and processes.

Even though the majority of the Antarctic ecosystem is composed of benthic invertebrates (Gutt et al. 2004), phylogeographic studies using more quickly evolving

microsatellite markers in the Antarctic have mostly been focused towards vertebrates (Davis et al. 2002, Hoelzel et al. 2002, Reilly & Ward 1999, Roeder et al. 2001, Shaw et al. 2002, Valsecchi et al. 1997, Van Houdt et al. 2006) and terrestrial organisms (e.g. Convey et al. 2008, McGaughran et al. 2010). To our knowledge, only a handful of studies have used microsatellite markers to examine intraspecific patterns of DNA polymorphisms and gene flow of Southern Ocean invertebrates (Held & Leese 2007, Leese & Held 2008, Strugnell et al. 2009). Microsatellite markers display higher rates of change when compared to mtDNA and thus are better at measuring population genetic parameters (see Held & Leese 2007). Thus, intraspecific genetic structure in Antarctic benthic organisms is poorly understood and there is a need for genetic research in this region (Thatje et al. 2005, Held & Leese 2007, Hoffman et al. 2010).

The species of interest in this study is the red sea star, *Odontaster validus* Koehler 1906, a conspicuous icon of Antarctic benthic communities and has been a major player in many Antarctic benthic studies throughout the past 50 years (e.g. Fisher 1940, Clark 1962, Pearse 1965, Pearse & Bosch 1986, Clark et al 2008, Kidawa et al. 2010). More specifically, *O. validus* has been a model organism for understanding ecological and physiological biology in the Antarctic, which is currently a rapidly changing ecosystem (Clark et al. 2007, Barnes & Peck 2008). *Odontaster validus* has a long-lived feeding larval stage (planktotrophic) and should therefore have the ability for passive dispersal across long distances (Pearse & Bosch 1994, Janosik et al. 2011, chapter 4). An Antarctic circumpolar distribution (including the sub-Antarctic islands South Georgia and Bouvet Island) has been hypothesized for *O. validus.* In *O. validus*'s case, larval transport is likely augmented by the Antarctic Circumpolar Current (ACC), gyres surrounding the Antarctic continent, and the Antarctic coastal current (a.k.a., Eastwind Drift, Phillpot 1985, see Janosik et al. 2011 (chapter 4), Raupach et al. 2010, Thornhill et al.

2008). Using mitochondrial sequence data, Janosik et al. (2011) showed a single Antarctic population from sub-Antarctic, Antarctic Peninsula, and Rose Sea localities, finding the same haplotype from the Atlantic sector over to the Ross Sea (~8000km).Whereas the mtDNA data shows variability for *Odontaster* species after the opening of the Drake Passage, more quickly evolving markers are needed to understand the recent phylogeographic history when the availability of the Antarctic shelf was variable due to glaciations. Given these factors, a circumpolar Antarctic, sub-Antarctic distribution, and planktotrophic larvae, *O. validus* is an ideal candidate for evaluating population structure using microsatellite markers in Antarctic waters.

With the introduction of high-throughput DNA sequencing techniques, genomic tools for phylogeography have come more accessible. Using traditional methods to build a microsatellite library can be quite costly and time consuming. In this study, we used 454 genomic data to develop microsatellite markers to explore specific genetic connectivity and to assess intra-specific genetic polymorphisms within *Odontaster validus* throughout sampled Antarctic waters. Specifically, we build on previous mtDNA work with *O. validus* (Janosik et al 2011). We used six microsatellite markers to look for genetic diversity and spatial partitioning within 59 *O. validus* individuals. We compared these results with previous mitochondrial data (Janosik et al. 2011), and looked for population genetic structure and patterns of recent population expansions as a result of recent or historical events (i.e. ice scouring, glacial retreat), which may have led to the current distribution of *Odontaster validus*.

5.3 MATIERALS AND METHODS

5.3.1 SAMPLING, COLLECTION LOCALITIES, AND DNA EXTRACTIONS

Collecting benthic organisms in the Antarctic is logistically challenging given ship costs and capabilities, geographic isolation, and extreme climatic conditions (Griffiths 2010). Given these challenges, numbers of individuals in this study are limited, but geographic sampling ranges from the Ross Sea sector to Bouvet Island. Individuals from Antarctic waters were collected during two five-week Antarctic research cruises aboard the *R/V Laurence M. Gould* in November/ December of 2004 and May/ June of 2006. Specimens from South Sandwich Island, South Georgia, and Bouvet Island were collected aboard the *R/V Nathanial Palmer* during the ICEFISH 2004 cruise (provided by S. Lockhart and W. Detrich) and were fixed in ethanol. Samples were collected using a Blake trawl, wire dredge, or epibenthic sled. Ross Sea specimens were collected by SCUBA and provided by Stacy Kim. Individuals were identified based on external morphology (Fisher 1940) by AMJ and kindly verified by Christopher Mah (Smithsonian Institution, National Museum of Natural History, NMNH, Washington D.C., USA). Numbers, localities, and depth of specimen occurrence are given in Table 1 and Figure 1. *Odontaster validus* specimens, or parts thereof, were then either frozen or placed in ethanol (95%) for molecular analyses. Morphological voucher specimens were originally preserved in formalin and then transferred to 70% ethanol and deposited to the Smithsonian Institution Natural History Museum (USNM 1127016-1127025, Janosik et al. 2011). DNA extraction of specimens was performed using DNeasy® Tissue Kit (Qiagen). Sample sizes for each population are: 22 individuals from the Antarctic Peninsula (AP), 25 individuals from the sub-Antarctic (SUB), and 12 individuals from the Ross Sea (ROSS).

5.3.2 MICROSATELLITE PRIMER DEVELOPMENT

Microsatellite primers were developed from genomic 454 sequencing data (Macrogen) that was enriched using the reference genome method (Leese et al. 2008). First, raw sequence reads were searched for microsatellite loci using SPUTNIK (downloaded Oct. 2012, available at http://www.cbib.u-bordeaux2.fr/pise/sputnik.html). Then the SPUTNIK output was searched for microsatellite loci that were flanked by non-repetitive sequences at least 50 nucleotides in length. PRIMER3 (Rozen & Skaletsky 2000) was used to design primers for flanking regions of the tandem repeats. After completing the PRIMER3 run, robust primer pairs for repeat regions were chosen for tri- and tetra-repeats. Primers with a Tm of 95> ºC, but greater than 72 ºC were chosen.

In total, 30 primer sets (Supplementary Information Table 1) were tested and PCR products were obtained for all sets. Six robust microsatellite loci with clear and consistent patterns of amplification specific to *O. validus*, and which showed appropriate levels of variation, were chosen for this study. Microsatellite amplifications were performed in 10 μL reactions containing 10x Taq buffer advanced, 25 mM $Mg(OAc)_2$, 200 μL dNTP, 0.5 U *Taq* polymerase, 0.15 μM WellRED D2, D3, or D4 fluorescent-labeled M-13 primer (Sigma-Proligo), 0.5 μM forward primer, 0.5 μM reverse primer, and approximately 10ng of template DNA. Nineteen nucleotides (5' CACGACGTTG TAAAACGAC-3') were added to the 5' end of the reverse primers to allow attachment of the M-13 fluorescent-labeled primer into PCR products. Thermocycling conditions were: initial denaturation 3 min at 94 ºC; 40 cycles of denaturation at 94 ºC for 45 s, annealing 45 ºC for 1 min, and extension at 72 ºC for 1 min, followed by a final extension of 5 min at 72 ºC and held at 6 ºC.

Allele size determination was performed on a capillary-based Beckman CEQ 8000 Genetic Analysis System (Beckman Coulter) under default fragment analysis parameters.

Specifically, each well contained 2-4μL of PCR product, 20 μL sample loading solution, and 0.5 μL 400 bp DNA size ladder (Beckman Coulter). Microsatellite scoring was automatically conducted using the fragment analysis software bundled with the Beckman-Coulter CEQ 8,000 Genetic Analysis System (Beckman Coulter) and manually inspected for corrections. Allele size was scored and reported according to their true allele size by excluding the 5' nucleotides from the fluorescent-labeled M13 primers. Allele scoring using fragment analysis software was repeated to verify calls and to remove the possibility of variation induced by PCR artifacts.

5.3.3. Microsatellite Analysis

Variability of the six loci was evaluated with the EXCEL MICROSATELLITE TOOLKIT (Park 2001) which detects invalid alleles and aided file formatting. MICRO-CHECKER (Van Oosterhout et al 2004) was used to estimate or account for deviations from Hardy-Weinberg equilibrium, specifically by estimating null allele frequencies and detecting for an excess of homozygotes. To assess genetic variability and geographic subdivisions within populations and regions, ARLEQUIN ver. 3.1 (Excoffier et al., 2005) was used to perform hierarchical analysis of molecular variance (AMOVA). Variance was partitioned into three hierarchical levels: among geographic regions (Antarctic Peninsula, sub-Antarctic, Ross Sea), among populations within geographic regions, and finally within populations (see Figure 1). We also calculated pairwise population coefficients (F_{ST}) and pairwise differences based on allele-size based estimates (R_{ST}) using ARLEQUIN. To infer the presence of distinct populations, assign individuals to populations, identify possible migrants, and to estimate population allele frequencies, the program STRUCTURE 2.3.2 (Pritchard et al. 2000) was used. STRUCTURE is a model-based clustering method used for inferring population structure using genotype data.

The model assumes linkage disequilibrium within in subpopulations, thus markers cannot be close together. STRUCTURE assigns individual genotypes to populations and calculates the likelihood of the genotype dataset for a given number of populations (K).

The program BOTTLENECK (Piry et al. 1999) was used to test for historical bottlenecks. Specifically, BOTTLENECK is based on the hypothesis that if populations have experienced recent reductions in size, then reduced allelic richness and heterozygosity will be observed. If populations are expanding, we might expect to see an increased number of alleles compared to heterozygosity. For each locus, BOTTLENECK computes expected heterozygosity (H_E) from the number of observed alleles (N_A), given the sample size (n) under the assumption of the mutation-drift equilibrium. This is computed through simulating the coalescent process of *n* genes under three possible mutation models, e.g. 1) the Infinite Allele Model, 2) the Two-Phase Model, and 3) the Stepwise-Mutation Model. The statistical significance of parameters was inferred by applying a Sign-test and a Wilcoxon-rank-test (Cornuet & Luikart 1996, Luikart et al. 1998, Piry et al. 1999).

5.4 RESULTS

Six microsatellite loci were successfully collected for 57 of the 59 specimens allocated into three populations. Despite multiple attempts, we were unable to collect locus P2 for two individuals from the sub-Antarctic population. The number of alleles per locus ranged from 4 to 10, with an average of 7.5 alleles per locus per population. Table 2 shows allelic distribution for all loci according to geographic region. Observed heterozygosity for all populations by locus ranged from 0.041 to 0.583 (Table 2). Total observed heterozygosities for populations are AP= 0.1212, SUB= 0.2435, ROSS= 0.3333 (Table 3).

AMOVA results indicate that most variation was observed is among individuals (Table 4). Among populations within regions contributed 5.05% of the total variation, and the variation between regions was negligible. Genetic differentiation results from ARLEQUIN indicate that the geographic divisions of sub-Antarctic, Antarctic Peninsula, and Ross Sea are genetically indistinguishable from one another. Specifically, population pairwise Fst values between the sub-Antarctic and Antarctic Peninsula (0.03739) and between the Antarctic Peninsula and the Ross Sea (0.04692) are low. Values between the sub-Antarctic and the Ross Sea are also negligible (0.01184). Similarly, Rst estimates reflect Fst estimates, but were lower, indicating little population structure (Table 5). Rst estimates are appropriate for this study because Rst values are expected to be larger if a significant amount of differentiation between populations is not only caused by drift, but by independent mutations in the different, isolated populations according to the stepwise mutation model (SMM). Thus, low Rst values indicate minimal differentiation between geographic regions.

Using STRUCTURE, with the admixture model (allowing individuals to have mixed ancestry), the most likely number of populations was inferred without known geographic location of individuals. The number of MCMC steps needed to reach convergence was first estimated by comparing run lengths between 10,000 and 1,000,000 steps. Convergence was generally reached in less than 5,000. STRUCTURE assigned all individuals to one population (K=1). Specifically, when analyzing for the number of populations, Ln Pr (D|K) was highest for K=1 (Figure 2). Additionally, if we defined K=3 and asked STRUCTURE to assign individuals to AP, SUB, or ROSS population, it assigned approximately an equal portion of individuals from all three localities to each population. This result is also indicative of one population (Figure 3).

When testing for recent demographic changes (i.e. contractions or expansions) using BOTTLENECK, with different mutation models, we found significant heterozygosity deficiency under the step-wise mutation model. Specifically, under the SMM, there was a significant heterozygosity deficiency for the sub-Antarctic population (Table 6). This provides evidence for recent population expansion.

5.5 Discussion

5.5.1 Genetic homogeneity in *Odontaster validus*

Based on microsatellite data and similar to mtDNA data (Janosik et al. 2011) we find little population genetic structure among *Odontaster validus* over ~8,000km. Despite the large geographical distance between sampled regions, the sub-Antarctic, Antarctic Peninsula, and Ross Sea regions are genetically indistinguishable from one another. No fixed population specific differences are observed in microsatellites. Additionally, while some significant values are indicated, low Fst and Rst differentiation values also demonstrate little to no population structure. Results from the hierarchical analysis of molecular variance (AMOVA) indicate that the majority of variation is present among individuals. The majority of genetic variation among individuals within a population indicates that individuals maintain gene flow over a large scale.

The pattern found in *O. validus* from microsatellite data is congruent with the pattern found from mtDNA data (figure 4). Janosik et al. (2011) recovered a single (59 individuals) TCS parsimony network (Clement et al. 2000) with a 95% connection limit between haplotypes using mtDNA (16S & COI genes). Absence of population structure between the sub-Antarctic, Antarctic Peninsula and Ross Sea regions is supported by lack of genetic differentiation in quickly evolving nuclear markers and by congruence with mitochondrial data, again, demonstrating a single population.

Given that microsatellite markers are more quickly evolving when compared to mtDNA, they were chosen to examine more recent historical events that may have contributed to current phylogeographic patterns. Results from BOTTLENECK only indicate weakly significant heterozygote deficiency for the sub-Antarctic region. The Antarctic Peninsula and Ross Sea populations show no significant indication for recent population expansion. Given events during the last glacial maximum (LGM ~10,000 years ago) and deglaciation of the Antarctic shelf, we can conclude that mixing across the region has occurred since. In other words, if glacial and interglacial cycles caused disruption of population connectivity of *O. validus*, since that time, genetic mixing has occurred between geographically distant populations. Lasting effects from the LGM likely vary depending on the dispersal ability of a species. In some cases, after the LGM, lack of dispersal ability may result in hidden lineages (i.e. cryptic species or previously unrecognized biodiversity; see Janosik & Halanych 2010) a result of isolating glaciation events and thus, lack of population connectivity since. Contrastingly, genetic signal from past glaciation events may be masked in species with high dispersal ability, resulting in long-distance population connectivity, as is the case with *O. validus.*

5.5.2 CIRCUMPOLARITY

Both genetic markers are consistent with the hypothesis that the current distribution of *Odontaster validus* is circumpolar. Specifically, lack of population genetic structure in *O. validus* between three major geographic regions is indicative of a single Antarctic population. Collection and inclusion of more samples from the Eastern Antarctic are needed to test this conclusion. The Antarctic Circumpolar Current (ACC), an easterly flowing current, and the East Wind Drift (Philpot 1985), a weaker counter current that circulates close to the Antarctic coast,

are likely the driving forces for a circum-Antarctic *O. validus* population. Specifically, the long-lived planktotrophic larval form is likely passively dispersing around the continent as well as into the Sub-Antarctic by open ocean circulation. Specifically, propulsion of larvae by the ACC is plausible given the speed of the current (0.25- 0.4m s^{-1}; Whitworth et al. 1982, Klinck & Nowland 2001) and the water column duration of larvae (up to 6 months; Pearse 1965, Pearse et al. 1985, Pearse & Bosch 1986). Distances between the Ross Sea and Bouvet Island (~8,000km) at a speed ranging from 0.25- 0.4m s^{-1} could be covered in a 0.634 - 1.010 years. Thus, given the speed of the ACC and extended larval duration of *O. validus*, distances from the Ross Sea sector, the Antarctic Peninsula, and the sub-Antarctic could easily be covered by larval dispersal.

Dispersal around the continent with the ACC and the East Wind Drift has been long been presumed for other Antarctic organisms (Fell 1962; Arntz et al. 1994). Several molecular studies report long-distance population connectivity. For example, using seven microsatellites and one mtDNA gene, Leese et al. (2010) demonstrate long-distance dispersal in the Southern Ocean brooding isopod, *Septemserolis septemcarinata*. This benthic isopod lacks a pelagic larval stage and the ability to swim. Leese et al. (2010) find 1.1% genetic distance between haplotypes from the mtDNA and suggest directional dispersal on kelp by the ACC from South Georgia, Bouvet Island, and Marion Island in the sub-Antarctic based on results from the microsatellites. Likewise, Matschiner et al. (2009) used eight microsatellite markers, mtDNA d-loop sequences, and satellite-tracked drifting buoys to investigate population connectivity of the benthic humped rockcod, *Gobionotothen gibberifrons.* They explain the lack of genetic differentiation among samples from around the Scotia Sea by suggesting ongoing gene flow between sampling sites and by passive unidirectional larval dispersal following the direction with the ACC. Matschiner et al. (2009) also find that the drifters cross the Scotia Sea between the

Antarctic Peninsula and South Georgia in less than four months. Thus, they suggest ample time for larval dispersal by the ACC. Contrastingly, Baird et al. (2012) use seven microsatellite markers to assess two populations in the widespread, brooding amphipod, *Orchomenella franklini*, in the East Antarctic at Casey and Davis station. They report significant population structure and genetic differentiation in a stepping-stone model and suggest insufficient gene flow for this brooding species. Given these examples, patterns of genetic population structure of Antarctic fauna seem to be species specific and possibly dependent on life history strategy, dispersal ability, and other environmental factors. Further investigation of Antarctic benthic species using high resolution markers is necessary for understanding the correlation between life history strategy and current population genetic structure.

5.5.3 Conclusions

In summary, we present congruent results from both microsatellite and mtDNA markers, both of which indicate that *O. validus* is genetically homogenous throughout the sampled range suggesting a lack of a genetic barrier for this species. We propose that gene flow has occurred since the LGM and is likely occurring through larval dispersal by the ACC and the East Wind Drift. Further, we suggest that population connectivity of *O. validus* occurs around the entire continent. Further analyses with more samples, especially from the Eastern Antarctic are needed to fully assess circumpolarity, population changes, and genetic diversity in *O. validus*. Future research on the Antarctic benthos should aim to examine population connectivity over several spatial scales and look closely at life history strategy and dispersal ability.

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Figure Legend

Figure 1. Sampling stations for *O. validus*.

Figure 2. Plot of likelihood (lnPr (D|K) of the genotype dataset for a given number of populations (K).

Figure 3. Results of analyses performed with Structure. The map displays *Odontaster validus* individuals assigned to a population if we define K=3. Each pie graph contains individuals from AP= Antarctic Peninsula, SUB= sub-Antarctic, and ROSS= Ross Sea populations.

Figure 4. Statistical parsimony network of mtDNA haplotypes of *O. validus* adapted from Janosik et al. (2011). Haplotypes are coded by geographic region (AP= Antarctic Peninsula, ROSS= Ross Sea, SUB= sub-Antarctic), sized according to abundance, and missing haplotypes are denoted by small, closed circles. Rectangles denote presumed ancestral haplotypes. Picture insert is of planktotrophic larval form.

Table Legend

Table 1. *Odontaster validus* collection and sample data.

Table 2. Total number of individuals scored for each locus (N_S), number of different alleles (N_A), observed heterozygosity (H_O), and expected heterozygosity (H_E) for six microsatellites and three populations of *Odontaster validus.*

Table 3. Mean number of alleles (N_A), observed heterozygosity (H_O), and expected heterozygosity per population (H_E).

Table 4. Hierarchical analysis of molecular variance (AMOVA) within and between regions using 6 microsatellite markers.

Table 5. Genetic Differentiation among populations for *O. Validus* from the Antarctic Peninsula (AP), the sub-Antarctic (SUB), and the Ross Sea (ROSS), F-statistics (F_{ST} lower diagonal) and R-statistics (R_{ST} upper diagonal), based on six polymorphic microsatellite loci.). * indicates $P<0.05$.

Table 6. Results for significant heterozygosity (*H*) excess or deficiency in three *O. validus* populations assuming two different mutation models (IAM, SMM). AP= Antarctic Peninsula, SUB= sub-Antarctic, ROSS= Ross Sea. P-values are based on 1000 permutations. Significant values are printed in bold.

Table 1. *Odontaster validus* collection and sample data.

Sample Name	Station Number	Latitude	Longitude	Geographic Region	Depth (m)
AP63.2c.3	06-33	S61°09.843'	W55°51.625'	Antarctic Peninsula	117m
AP63.2c.5	06-33	S61°09.843'	W55°51.625'	Antarctic Peninsula	117m
AP63.2c.7	06-33	S61°09.843'	W55°51.625'	Antarctic Peninsula	117m
AP63.2c.9	06-33	S61°09.843'	W55°51.625'	Antarctic Peninsula	117m
AP63.2c.11	06-33	S61°09.843'	W55°51.625'	Antarctic Peninsula	117m
AP63.2c.12	06-33	S61°09.843'	W55°51.625'	Antarctic Peninsula	117m
AP63.3c.1	06-33	S61°09.843'	W55°51.625'	Antarctic Peninsula	117m
AP63.3c.2	06-33	S61°09.843'	W55°51.625'	Antarctic Peninsula	117m
AP63.4c.2	06-33	S61°09.843'	W55°51.625'	Antarctic Peninsula	117m
AP63.4c.9	06-33	S61°09.843'	W55°51.625'	Antarctic Peninsula	117m
AP298.3c.2	06-13	S 63°24.961'	W 61°50.484'	AntarcticPenninsula	132m
AP298.3c.3	06-13	S 63°24.961'	W 61°50.484'	AntarcticPenninsula	132m
AP319.2c	06-14	S 62°56.004'	W 61°28.751'	AntarcticPenninsula	188m
AP345.2c.1	06-21	S 63°08.838'	W 57°07.441'	AntarcticPenninsula	146m
AP345.2c.2	06-21	S 63°08.838'	W 57°07.441'	AntarcticPenninsula	146m
AP363.2c.1	06-29	S 64°08.321'	W 62°45.603'	AntarcticPenninsula	156m
AP363.2c.2	06-29	S 64°08.321'	W 62°45.603'	AntarcticPenninsula	156m
AP363.2c.5	06-29	S 64°08.321'	W 62°45.603'	AntarcticPenninsula	156m
AP363.2c.6	06-29	S 64°08.321'	W 62°45.603'	AntarcticPenninsula	156m
AP384.3c.5	06-33	S 67°44.420'	W 69°17.379'	AntarcticPenninsula	122m
AP448.1c.1	06-52	S 65°39.843'	W 68°02.224'	AntarcticPenninsula	282m
AP448.1c.3	06-52	S 65°39.843'	W 68°02.224'	AntarcticPenninsula	282m
SUB482.1e.1	66-57	S 54°38’	W3 °50’	Sub-Antarctic	180m
SUB482.1e.2	66-57	S 54°38’	W3 °50’	Sub-Antarctic	180m
SUB217.1e.7	38-18	S 53°55’	W 37°00’	Sub-Antarctic	46m
SUB219.1e.2	38-18	S 53°55’	W 37°00’	Sub-Antarctic	46m
SUB219.1e.4	38-18	S 53°55’	W 37°00’	Sub-Antarctic	46m
SUB219.1e.9	38-18	S 53°55’	W 37°00’	Sub-Antarctic	46m
SUB219.1e.10	38-18	S 53°55’	W 37°00’	Sub-Antarctic	46m
SUB219.1e.12	38-18	S 53°55’	W 37°00’	Sub-Antarctic	46m
SUB219.1e.8	38-18	S 53°55’	W 37°00’	Sub-Antarctic	46m
SUB221.1e.1	57-32	S 57°05’	W26°75’	Sub-Antarctic	130m
SUB221.1e.2	57-32	S 57°05’	W26°75’	Sub-Antarctic	130m
SUB221.1e.3	57-32	S 57°05’	W26°75’	Sub-Antarctic	130m
SUB221.1e.4	57-32	S 57°05’	W26°75’	Sub-Antarctic	130m
SUB221.1e.5	57-32	S 57°05’	W26°75’	Sub-Antarctic	130m
SUB35OT32.1	35-32	S 53°46’	W38°17’	Sub-Antarctic	201m
SUB35OT32.2	35-32	S 53°46’	W38°17’	Sub-Antarctic	201m
SUB38BT18.1	38-18	S 53°55’	W37°00’	Sub-Antarctic	46m
SUB38BT18.2	38-18	S 53°55’	W37°00’	Sub-Antarctic	46m
SUB52OT43.1	52-43	S 58°56’	W26°28’	Sub-Antarctic	120m
SUB52OT43.2	52-43	S 58°56’	W26°28’	Sub-Antarctic	120m
SUB57BT32.1	57-32	S 57°05’	W26°75’	Sub-Antarctic	130m
SUB57BT32.2	57-32	S 57°05’	W26°75’	Sub-Antarctic	130m
SUB80BT42.1	80-42	S 54°23’	W3°28’	Sub-Antarctic	159m
SUB80BT42.2	80-42	S 54°23’	W3°28’	Sub-Antarctic	159m
rossCAOV1	CAOV	S 77°51’	W166°39’	Ross Sea	-
rossCAOV5	CAOV	S 77°51’	W166°39’	Ross Sea	-
rossCAOV6	CAOV	S 77°51’	W166°39’	Ross Sea	-

rossCAVO7	CAOV	S 77°51’	W166°39’	Ross Sea	-
rossCAOV8	CAOV	S 77°51’	W166°39’	Ross Sea	-
rossCAOV9	CAOV	S 77°51’	W166°39’	Ross Sea	-
rossCAOV11	CAOV	S 77°51’	W166°39’	Ross Sea	-
rossCAOV12	CAOV	S 77°51’	W166°39’	Ross Sea	-
rossCAOV14	CAOV	S 77°51’	W166°39’	Ross Sea	-
rossCAOV15	CAOV	S 77°51’	W166°39’	Ross Sea	-
rossTROV2	TROV	S 77°44’	W166°46’	Ross Sea	-
rossTROV1	TROV	S 77°44’	W166°46’	Ross Sea	-

Table 2. Total number of individuals scored for each locus (N_S), number of different alleles (N_A), observed heterozygosity (H_O), and expected heterozygosity (H_E) for six microsatellites and three populations of *Odontaster validus.*

	AP	SUB	ROSS	Mean N_A/locus
Locus P2				
N_S	22	23	12	
N_A	11	6	6	7.667
H_O	0.318	0.261	0.417	
H_E	0.830	0.743	0.841	
Locus P5				
N_S	22	23	12	
N_A	9	7	6	7.333
H_O	0.091	0.320	0.583	
H_E	0.873	0.687	0.757	
Locus P6				
N_S	22	23	12	
N_A	8	9	5	7.333
H_O	0.041	0.320	0.417	
H_E	0.841	0.735	0.7134	
Locus P19				
N_S	22	23	12	
N_A	9	6	2	5.667
H_O	0.136	0.200	0.417	
H_E	0.832	0.714	0.518	
Locus P23				
N_S	22	23	12	
N_A	8	5	3	5.333
H_O	0.045	0.120	0.083	
H_E	0.589	0.384	0.163	
Locus P24				
N_S	22	23	12	
N_A	12	14	7	11.0
H_O	0.136	0.240	0.083	
H_E	0.902	0.894	0.699	

Table 3. Mean number of alleles (N_A), observed heterozygosity (H_O), and expected heterozygosity per population (H_E).

	AP	SUB	ROSS	Mean N_A /locus
Mean N_A per location	9.50	7.83	4.83	7.38
Mean H_O	0.1212	0.2435	0.3333	
Mean H_E	0.8111	0.6928	0.6153	

Table 4. Hierarchical analysis of molecular variance (AMOVA) within and between regions using 6 microsatellite markers.

Source of variation	*df*	Sum of squares	Variance component	Percentage of variation	Fixation indices
Among regions	1	4.373	-0.04709 Va	-2.13	Φct= 0.02132
Among populations within regions	1	5.1715	0.11159 Vb	5.05	Φsc= 0.04946
Among individuals within populations	115	246.615	2.14448 Vc	97.08	Φst= 0.0290
Total	117	256.703	2.20898		

Table 5. Genetic Differentiation among populations for *O. Validus* from the Antarctic Peninsula (AP), the sub-Antarctic (SUB), and the Ross Sea (ROSS), F-statistics (F_{ST} lower diagonal) and R-statistics (R_{ST} upper diagonal), based on six polymorphic microsatellite loci.). * indicates *P<0.05.*

	AP	**SUB**	**ROSS**
AP	-	0.0372	0.00414
SUB	0.03739*	-	0.11047*
ROSS	0.04692*	0.01184	-

Table 6. Results for significant heterozygosity (*H*) excess or deficiency in three *O. validus* populations assuming two different mutation models (IAM, SMM). AP= Antarctic Peninsula, SUB= sub-Antarctic, ROSS= Ross Sea. P-values are based on 1000 permutations. Significant values are printed in bold.

			Sign Test			**Wilcoxon Test**
Population	Model	Expected # of loci with heterozygosity excess	Observed # of loci with heterozygosity excess	Observed # of loci with heterozygosity deficiency	P	P (one tailed for heterozygosity deficiency)
AP	IAM	3.59	4	2	0.5423	0.78125
	SMM	3.52	2	4	0.1988	0.05469
SUB	IAM	3.58	3	3	0.4623	0.65625
	SMM	3.53	1	5	**0.0462**	**0.01563**
ROSS	IAM	3.40	4	2	0.4752	0.65625
	SMM	3.43	2	4	0.2186	0.28125

Figure 1. Sampling stations of *O. validus*.

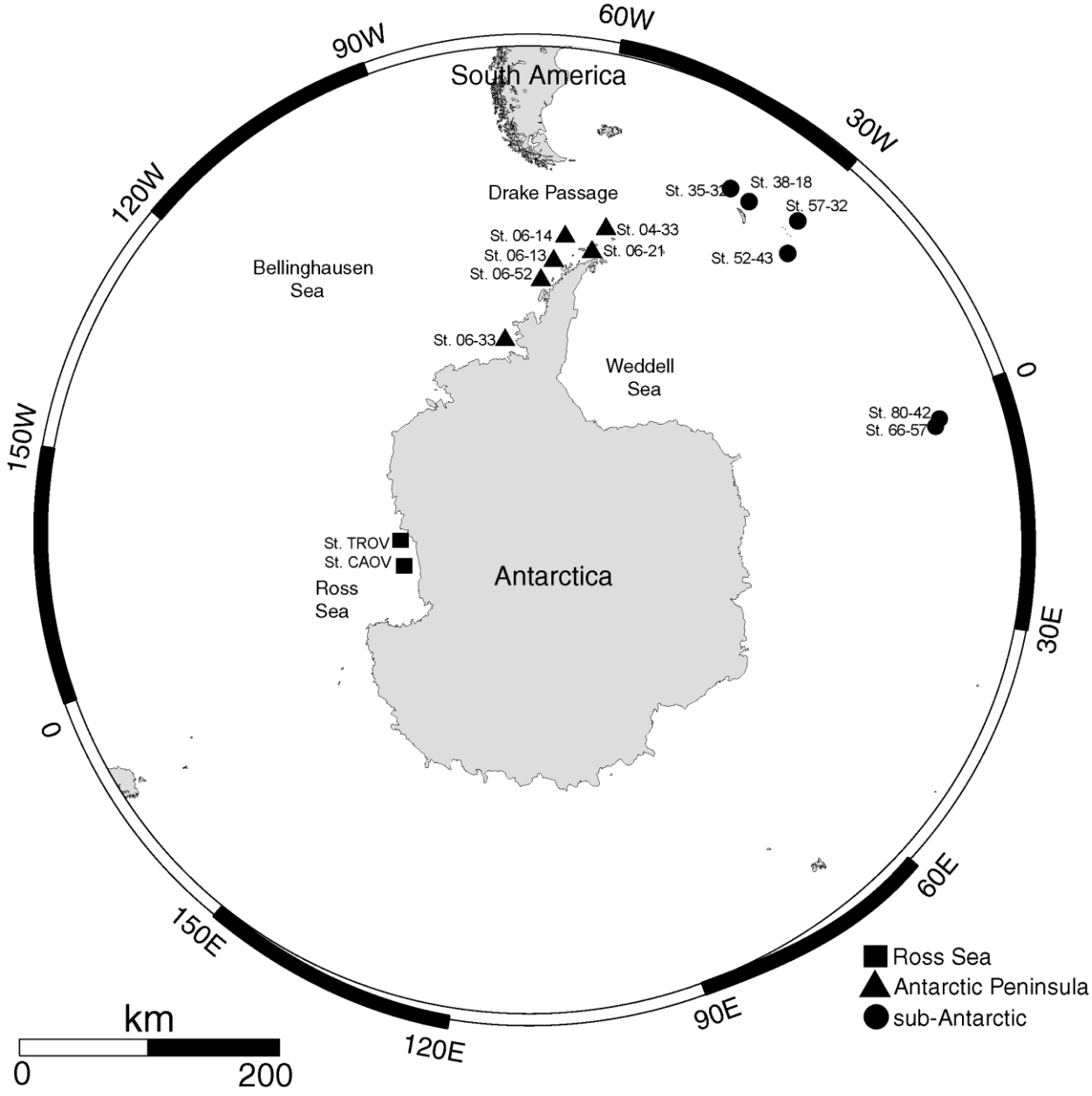


Figure 2. Plot of likelihood (lnPr (D|K) of the genotype dataset for a given number of populations (K).

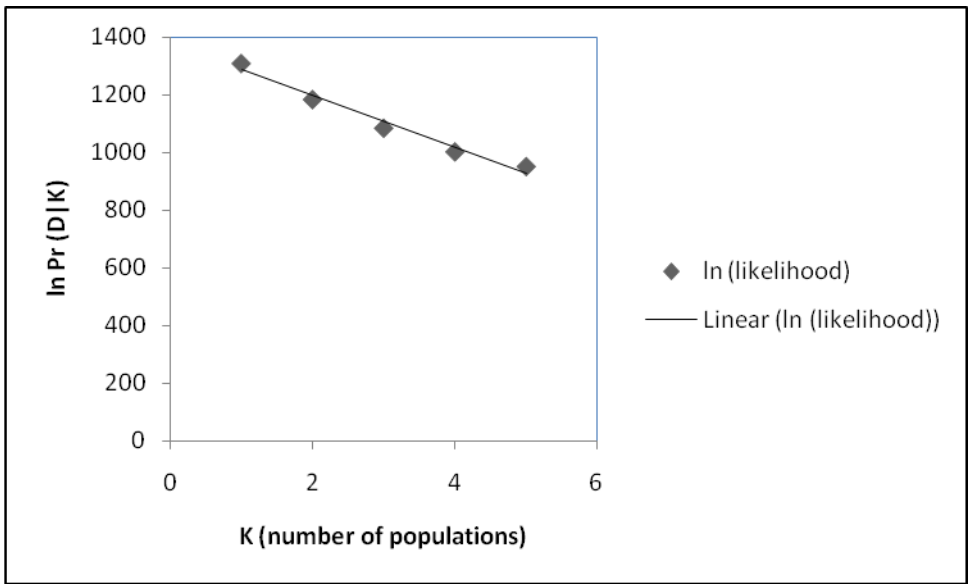


Figure 3. Results of analyses performed with Structure. The map displays *Odontaster validus* individuals assigned to a population if we define K=3. Each pie graph contains individuals from AP= Antarctic Peninsula, SUB= sub-Antarctic, and ROSS= Ross Sea populations.

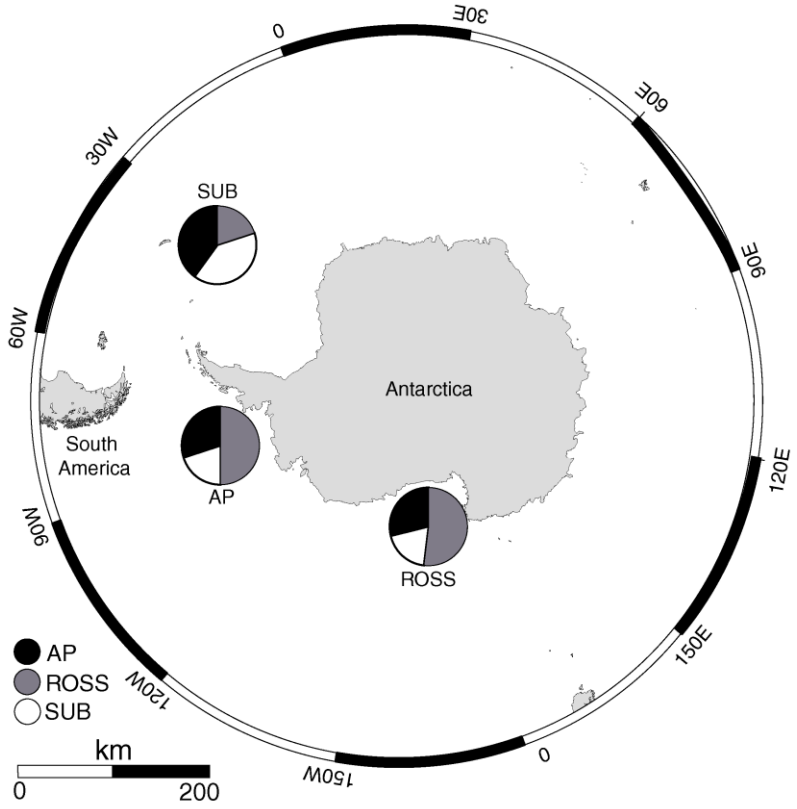
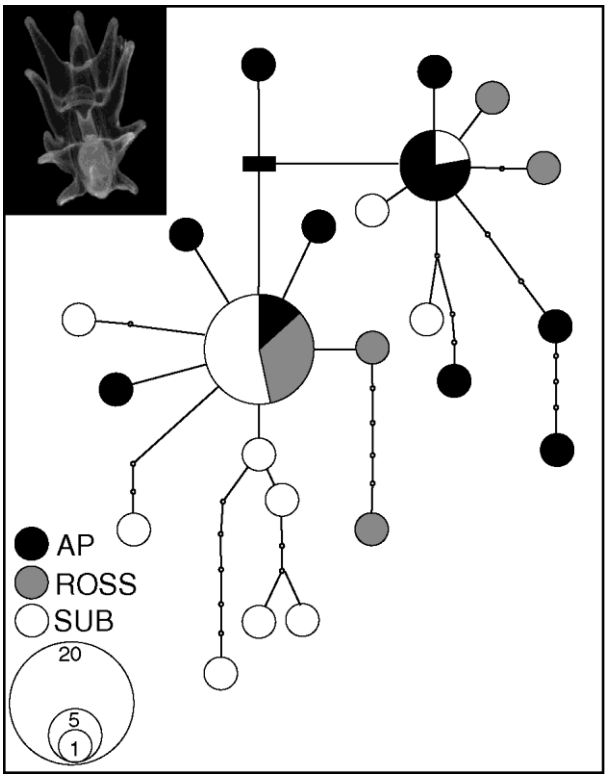


Figure 4. Statistical parsimony network of mtDNA haplotypes of *O. validus* adapted from Janosik et al. (2011). Haplotypes are coded by geographic region (AP= Antarctic Peninsula, ROSS= Ross Sea, SUB= sub-Antarctic), sized according to abundance, and missing haplotypes are denoted by small, closed circles. Rectangles denote presumed ancestral haplotypes. Picture insert is of planktotrophic larval form.



CHAPTER 6. Conclusions

6.1 CONCLUSIONS

Current distributions of Southern Ocean benthic organisms have been shaped from a combination of geological, oceanographic and biological elements over time. Despite a harsh and cold environment, the Southern Ocean hosts a diverse and rich community that is heavily dominated by invertebrate organisms. In particular, Asteroidea (sea stars or starfishes) are important members of the Antarctic benthic community and their diversity is exemplified by varied morphological forms, wide range of habitat and depth, and complex developmental life histories. Understanding what factors have contributed, shaped, and maintained uniqueness and diversity in Antarctic is of great interest.

The purpose of this research was to examine the evolutionary history and phylogeography of an ecologically important group of sea stars with a high concentration of species in the Southern Ocean. Specifically, the evolutionary history and diversification of Odontasteridae was investigated using both morphological and molecular characters. Phylogeography and diversity of the Southern Ocean genus *Odontaster* was then explored using mitochondrial DNA. Finally, further investigation and comparison was done using faster-evolving microsatellite markers on the Antarctic species *Odontaster validus.*

First, by examining morphological characters and mtDNA (16S and COI) of odontasterids, I have demonstrated that commonly used external ossified morphological characters alone prove insufficient for phylogenetic inference. These characters however, are still useful and informative when making taxonomic designations and in recognizing undescribed biodiversity. Although mitochondrial DNA provides weak phylogenetic signal, some relationships were well-supported. Specifically, mitochondrial data support monophyly of

Odontasteridae. I also conclude that species of Odontasteridae present in high latitudes in the Southern Hemisphere (i.e., Southern Ocean) are the most derived taxa. Additionally, mtDNA data suggests unrecognized lineages of odontasterids are present in high southern latitudes. Finally, based on morphological characters, a new species, *Odontaster* nov. sp., was described from the Galapagos Islands. Overall, biodiversity of Odontasteridae was underestimated and I conclude that morphological characters prove to still be useful when challenging taxonomic boundaries or designating new species. Further investigation through the use of molecular tools and morphological characters is necessary to infer a well-supported phylogenetic history of this group.

Southern Ocean members of *Odontaster* (*O. meridionalis, O. penicillatus,* and *O. validus*) were examined using mtDNA (16S and COI) in a phylogeographic framework. This study revealed that the Antarctic Polar Front (APF) is likely the force separating the majority (with one possible exception: *O. meridionalis*) of *Odontaster* species, keeping them from dispersing across the Drake Passage. Specifically, *O. validus* and *O. penicillatus* appear to be isolated to the Antarctic side and the South American side, respectively. Additionally, two previously unrecognized species, a common Antarctic trend (Held & Leese 2007; Wilson et al. 2007; Hunter & Halanych 2008; Mahon et al. 2008; Thornhill et al. 2008; Wilson et al. 2009; Mahon et al. 2010; Krabbe et al. 2010), *O. pearsei* and *O. roseus* are described and are also isolated to the Antarctic side of the Drake Passage. Though limited in number of samples, investigation of *O. meridionalis* shows possible recent genetic connectivity between South American and Antarctic Peninsula populations, indicating the possibility of a trans-Drake species. Thus, it is likely that some species of *Odontaster* are endemic to the Antarctic. While the APF appears to be an isolating force for some species, it appears to have been permeable

since its formation for Southern Ocean *Odontaster* species. Thus, we can conclude that population connectivity across the Drake Passage is species-specific and probably influenced by several other factors that may include life history, larval duration, ocean current patters and as well as thermal, physiological and ecological tolerances.

Lastly, using high-resolution microsatellite markers patterns of speciation and distribution based on historical glaciations and oceanographic currents were further examined population structure of the conspicuous Antarctic sea star *Odontaster validus*. This is one of the first studies that has utilized microsatellites to study the phylogeography of benthic invertebrates in the Antarctic on such a broad geographic scale. Based on lack of genetic structure from six microsatellite loci, I conclude that individuals from the sub-Antarctic are genetically indistinguishable from individuals from the Antarctic Peninsula and from the Ross Sea. Microsatellite and mtDNA data are in congruence. Current distribution of *O. validus* is likely a result of a combination of historical glaciation events and oceanographic features. Currently, *O. validus* likely has a circumpolar distribution with long-distance connectivity. Specifically, based on the fast-evolving nature of microsatellite markers and that enough lifecycle generations have passed, changes in population demographics would be detected since the Last Glacial Maximum (LGM). Thus, mixing of populations has occurred since the LGM. Moreover, I conclude that there is a high dispersal rate for *O. validus* that is likely facilitated by its long-lived planktotrophic larval stage. The Antarctic Circumpolar Current (ACC), along with gyres surrounding the continent and the Antarctic coastal current (a.k.a. East Wind Drift, Phillpot 1985), are likely propelling the larvae of *O. validus* around the continent as well as up into the sub-Antarctic, resulting in genetic homogenization (Stein & Heywood 1994). In summary, while the APF is likely acting as a barrier to gene flow for some Antarctic species, the ocean currents

flowing around the continent appear to be facilitating gene flow, whether by a planktotrophic larval stage or by rafting on marine debris (e.g. kelp, driftwood).

Overall, this work provided insight into the forces that have shaped Southern Ocean organismal distributions and thus, some recommendations in studying Southern Ocean invertebrate phylogeography can be made. Importantly, this work suggests that phylogeography and organismal distributions in the Southern Ocean are species-specific. For example, while predictions for *O. validus* based on life history strategy were valid, a combination of other factors such as currents, fronts, and glaciations has clearly also had an effect on the current distribution of this benthic species. Several molecular studies have shown that some organisms are truly circumpolar (Zane et al. 1998, Hunter and Halanych 2008, Rauphach et al. 2010, Thornhill et al. 2008, Wilson et al. 2007, Wilson et al. 2009, Hemery et al. 2012), while others are comprised of multiple lineages around the continent (see Janosik and Halanych 2010; Brierley et al. 1993, Allcock et al. 1997, Zane et al. 1998, Held 2003, Held & Wagele 2005, Raupach and Wagele 2006, Linse et al. 2007, Leese & Held 2008, Hunter & Halanych 2010). Clear patterns in the Southern Ocean between observed or assumed mobility based on life history mode and actual gene flow are not always readily apparent. Generalizations and predictions of current species distributions based solely on taxonomic group or life history should be avoided. Instead, historical events (i.e. glaciations and climate change), oceanographic features (i.e. currents and circulation), and life history strategy all must be considered when using molecular tools to study population genetic connectivity and distributions of Southern Ocean benthic animals.

Notably, this work has also demonstrated that biodiversity in the Antarctic is currently underestimated. Although the sea star *Odontaster validus* is among the best studies organisms in

the Antarctic, two unrecognized lineages were discovered along the Antarctic Peninsula. Additionally, by compiling molecular phylogeographic studies on organisms in the Southern Ocean, we demonstrated that nearly every study to date suggests biodiversity is considerably underestimated. Thus, a sizable amount of diversity in the Antarctic remains to be discovered and distinguished as either cryptic or unrecognized. We also caution future researchers to use precise scientific language when discussing whether newly found lineages of distinct species either fail to display morphological characters that can be used to confidently assign them to distinct lineages (i.e., cryptic) or whether distinctness merely has escaped detection (i.e., unrecognized).

In the future, addition of samples from under surveyed regions of the Southern Ocean, investigation with multiple marker systems with varied rates of evolution, and investigation at several spatial scales will help verify the conclusions made herein and our understanding of speciation and gene flow the Antarctic. As research continues our understanding and view of Antarctic diversity and marine organismal history will undoubtedly change.

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APPENDIX 1. Life history of the Antarctic sea star *Labidiaster annulatus* (Asteroidea: Labidiasteridae) revealed by DNA barcoding

Labidiaster annulatus, Sladen (1889) is a multi-rayed (9–50) voracious Antarctic sea star with numerous, large, conspicuous crossed pedicellariae. An active and opportunistic predator it commonly preys upon euphausiids, amphipods, and small fish in the water column (Dearborn et al. 1991). *Labidiaster annulatus* is distributed around the Antarctic, Kerguelen, South Orkney, South Sandwich Islands, South Georgia, and Shag Rocks, at recorded depths of 30–440 m (Fisher 1940, unpublished data).

Nothing is reported on the mode of reproduction in *Labidiaster*. Furthermore the recognized family Labidiasteridae, composed of *Labidiaster*, *Corobaster*, *Rathbunaster*, and *Plazaster*, is unlikely to be monophyletic, and the closest extant relative to *Labidiaster* remains unknown (Foltz et al. 2007). In such a case larval identification by barcoding can be an important tool for examining life history. Here we use DNA barcoding techniques on partial mitochondrial 16S sequences, which serendipitously matched adults of *L. annulatus* to unknown asteroid larvae collected along the western Antarctic Peninsula and Bransfield Strait region.

Larvae and adult specimens were collected during two, five-week Antarctic voyages aboard the RV *Laurence M. Gould* from 23 November–22 December 2004 and 12 May–13 June 2006 (Table I). Larval specimens were collected using a conical 75 cm plankton net and with a 250 micron mesh towed for 20 min in a slow oblique decent to a depth of c. 180 m and then similarly returned to the surface. Benthic samples were collected using a Blake trawl, wire

dredge, or epibenthic sled. Adult voucher specimens have been deposited at The Smithsonian Institution National Museum of Natural History (USNM 1115369 and 1115370).

Individual asteroid larvae (19 bipinnaria and eight brachiolaria) were subjected to whole genome amplification using GenomiPhi Kit following the manufacturer's recommendations (GE Healthcare) without prior DNA extraction because the protocol's first heating step lyses cells. DNA of adult specimens was extracted using the DNeasy Tissue Kit (Qiagen). An approximately 500 bp region of the 16S gene was amplified using the 16SarL and 16SbrH primers following Palumbi (1991). Purified products were sequenced in both directions on a CEQ 8000 Genetic Analysis System (Beckman Coulter). Novel sequences are deposited under Genbank accession numbers EU248958-EU248964. Edited sequences were compared to Genbank sequences using blastn (Altschul et al. 1990). Genetic distances (uncorrected p-distance values) were calculated using PAUP*4.0 (Swofford 2003). To objectively confirm that all the sequences probably represented a single species, sequences were analysed using TCS 1.21 (gaps treated as missing) to create a parsimony network with a 95% connection limit between haplotypes (Clement et al. 2000).

Of the 27 larvae examined, four (three bipinnaria and one brachiolaria) from the 2004 voyage showed > 99% sequence similarity to the *L. annulatus* sequence reported by Foltz et al. (2007; Genbank accession AY706154). All other larvae sampled were *Odontaster* forms. To confirm the result, we sequenced three adult *L. annulatus* and found uncorrected p-distance of > 0.378% when compared to larval samples and the Foltz et al. sample (representing four unique haplotypes). The parsimony network found all samples to be within a single network with a maximum distance of three changes in the network (data not shown).

Thus, *Labidiaster annulatus* has an indirect mode of development with planktonic bipinnaria and brachiolaria larvae. The less than 0.4% uncorrected distance values recovered among *L. annulatus* individuals are considerably lower than the 5–7% interspecific mtDNA sequence divergences generally found in echinoderms (Foltz 1997, Hart et al. 1997, Waters & Roy 2003, Waters et al. 2004). Furthermore, 16S sequence data are known to be informative and variable in intraspecific studies for Antarctic marine invertebrates (Raupach & Wagele 2006, Wilson et al. 2007, Hunter & Halanych 2008, Mahon et al. 2008) as well as within asteroids (Waters et al. 2004).

Unfortunately, we cannot determine with certainty the morphology of the *L. annulatus* larvae. Larval samples were destroyed in data collection and no photographs could be taken of live larvae. Larvae were examined under a dissecting microscope and *L. annulatus* larvae seem to be superficially similar to those of *Odontaster*. Substantial morphological differences were not immediately obvious, and as such it is most likely that these are feeding (planktotrophic) larvae, but future research may further explore this issue.

Linking larval and adult forms together via DNA barcoding raises some interesting issues about *L. annulatus*. We now know that this organism has planktonic development, but the duration of larvae in the water column is unknown because length of larval development can vary greatly and there can be a long delay in settlement after reaching competence if cues for metamorphosis are lacking (e.g. Strathmann & Strathmann 2007). Moreover, studies of modes of reproduction in Antarctic marine invertebrates indicate that larvae often spend very long periods of time in the plankton (Pearse et al. 1991). Larvae of *L. annulatus* were present in the summer 2004 but not in May/June 2006. Although our numbers are low, the fact that more bipinnaria were found suggest that larvae were in the water column well past the May/June time frame

during which we sampled. Our results suggest that the South American sister species, *Labidiaster radiosus* may also have planktonic larval development. Future efforts should expand the temporal and spatial sampling of larvae so that a better understanding of the life history of this conspicuous predatory sea star can be obtained.

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Table 1 Collection information for *Labidiaster annulatus* and *Odontaster validus.*

Species	Station Number	Adult or Larva	Depth (m)	Latitude	Longitude	Number of Individuals
L. annulatus	LMG 06-13	A	132	S63°24.96’	W61°50.48’	1
	LMG 06-14	A	132	S62°56.004’	W61°28.751’	1
	LMG 06-45	A	195	S67°43.60’	W69°18.10’	1
	LMG 04-68	L	0-180	S63°28.02’	W62°23.97’	1
	LMG 04-47	L	0-180	S62° 51.00’	W59°27.07’	3
	-	A	-	S60°58.08’	W55°6.85’	1[a]
O. validus[b]	LMG 04-45	L	0-180	S62º15.80’	W58º16.70’	7
	LMG 04-47	L	0-180	S62º51.00’	W59º27.07’	4
	LMG 04-60	L	0-180	S62º58.07’	W61º35.46’	5
	LMG 04-68	L	0-180	S63º28.02’	W62º23.97’	5
	LMG 06-10	L	0-180	S64º23.54’	W62º59.82’	1
	LMG 06-30	L	0-180	S65º50.51’	W66º59.83’	1

a- from Foltz et al. 2007

b- from Janosik et al. in prep.