PHYLOGEOGRAPHY AND POPULATION STRUCTURE OF ANTARCTIC OPHIUROIDS: EFFECTS OF LIFE HISTORY, OCEANOGRAPHY AND PALEOCLIMATOLOGY

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PHYLOGEOGRAPHY AND POPULATION STRUCTURE OF ANTARCTIC OPHIUROIDS: EFFECTS OF LIFE HISTORY, OCEANOGRAPHY AND PALEOCLIMATOLOGY

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DISSERTATION ABSTRACT

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The Antarctic landmass and surrounding continental shelf have been isolated for over 24 million years. Geographic and thermal isolation have resulted in a highly endemic, diverse benthic marine fauna. This fauna has been relatively well characterized morphologically, but little is known about the evolutionary history, genetic diversity and population genetic structure of Antarctic benthic marine invertebrates. Several important questions remain largely unanswered, including 1) are circumpolar species genetically homogeneous throughout their range, 2) are non-endemic species maintaining

connectivity between populations distributed in Antarctica and South America, and 3) how does early life history influence dispersal throughout Antarctica?

This research examined phylogeographic patterns within four Antarctic brittle stars (ophiuroids). Ophiuroid species were chosen that differed in their mode of development (i.e., presence/absence of a pelagic larval stage) and geographic distribution. A non-endemic brooding species, *Astrotoma agassizii*, was evaluated in order to assess connectivity across a major oceanographic barrier separating Antarctic and South American populations. Three Antarctic endemics, *Ophiurolepis gelida, O. brevirima* and *Ophionotus victoriae*, all possessing some form of pelagic larvae, were obtained from various locations around Antarctica in order to characterize their population structure and levels of gene flow throughout the Antarctic continental shelf.

Analysis of intraspecific mitochondrial sequence data revealed several interesting patterns. First, all species showed evidence of restricted connectivity between major geographic regions in the Antarctic, subantarctic and/or South America (depending on sampling), regardless of developmental mode. Additionally, cryptic divergence and/or speciation were recovered in all cases. Second, the brooding species, *A. agassizii*, displayed evidence of greater connectivity within geographic regions compared to species with pelagic larvae. These results suggest that phylogeographic patterns are not easily predicted for Antarctic brittle stars, and ophiuroid diversity is underestimated in the Southern Ocean. These generalizations likely apply to other Antarctic marine invertebrates, and suggest that much more research will be required to quantify Southern Ocean biodiversity and fully understand the contemporary and historical processes driving evolution in this region.

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CHAPTER 1. Introduction to dissertation

1.1 INTRODUCTION

Understanding factors contributing to distributional patterns of organisms in the marine environment has been a focus of numerous recent studies (e.g., Palumbi *et al*. 1997; Lessios *et al*. 1998; McCartney *et al*. 2000; Waters *et al*. 2000; Muss *et al*. 2001). Oceanography, life history and behavior have all been shown to affect dispersal of marine organisms, especially invertebrates (Palumbi 1994). As a result of these studies, several barriers to dispersal have been identified based on concordant phylogeographic patterns in a wide variety of taxa. The most prominent of these include the Eastern Pacific Barrier, a ~5000 km deep-water gap between the central and eastern Pacific Ocean, the southern tip of South Africa, due to cold water upwelling, the deep-water separation of the eastern and western Atlantic (Lessios *et al*. 2003), and the Polar Front in the Southern Ocean (Clarke *et al*. 2005). While effects of oceanographic barriers and life history constraints have been evaluated for many geographic regions, one of the more distinctive biogeographical regions, the Southern Ocean, is not well understood in this context. The Antarctic Polar Front (APF), other oceanographic features, and certain life history traits affecting dispersal of marine organisms in this part of the world require further investigation. As such, Antarctica and the surrounding Southern Ocean are ideal localities for further study of the physical and biological factors driving distributional patterns and population genetic structure in marine invertebrates.

Isolation of the Antarctic continent has been a driving evolutionary force for Antarctic fauna for 24-41 million years (Lawver & Gahagan 2003; Pfuhl & McCave 2005; Scher & Martin 2006). Separation of Antarctica from South America and the ensuing onset of the Antarctic Circumpolar Current (ACC) are presumed to have been primary forces driving speciation in many Southern Ocean marine taxa. ACC formation is thought to have driven gradual cooling and glaciation that began ~34 mya in the Antarctic (Zachos *et al*. 2001). This cooling and long period of isolation have led to a diverse and abundant benthic fauna that are typically stenothermal, eurybathic and endemic to the Antarctic (Hempel 1985). Endemism is particularly high in certain groups such as fish (95%), amphipods (95%), pycnogonids (90%), isopods (87%), and echinoderms (73%) (Knox & Lowry 1977; Brandt 1991; Jazdzewski *et al.* 1991). Antarctic benthos are believed to have originated from three sources: 1) remnant Gondwana forms, including forms originating in Antarctica, 2) forms from the surrounding deep sea, and 3) forms from South America that migrated to Antarctica (Dell 1972; Hempel 1985; Watling & Thurston 1989; Dayton *et al*. 1994). Many present-day fauna conform to certain biogeographic patterns. The most common of these is circumpolarity, largely attributed to passive dispersal via the ACC (Fell *et al*. 1969). Other fauna are restricted to west Antarctica (Antarctic Peninsula, Weddell Sea and Bellingshausen Sea) or the subantarctic islands (Dayton *et al*. 1994) (Fig. 1). Several groups have undergone extensive radiations upon isolation in Antarctica, these include pycnogonids, echinoderms, gastropods, ascidians, some isopods and notothenioid fish (Dell 1972; Arntz & Gallardo 1993).

While many Antarctic taxa exhibit high levels of endemism, others show much lower levels despite geographic and thermal isolating factors. For example, polychaete, echinoderm and mollusc conspecifics have been reported on the continental shelves of both Antarctica and South America, and a well-recognized faunal affinity exists between these two regions (Dell 1972; Arntz *et al*. 1994; Dayton *et al*. 1994). A lack of endemism in these species indicates some level of recent gene flow between populations separated by the ACC and APF. Several gene flow mechanisms have been proposed, including human-mediated transport, migration of benthic adults, larval dispersal and rafting. Human-mediated transport into Antarctica is possible, though unlikely, as fouling and ballast water organisms would have to be pre-adapted to the extreme environmental conditions in the Antarctic in order to survive (Thatje 2005). Migration of adults has long been assumed to occur across the Scotia Arc, a submerged ridge with a series of emergent islands including South Georgia, South Sandwich Islands and the South Orkneys. The Scotia Arc forms a "stepping-stone" connection between the Antarctic Peninsula and South America (Fell *et al*. 1969). Dispersal of larvae or rafting adults/juveniles would most likely occur across the Drake Passage, the portion of the ACC separating Antarctica and South America representing the shortest distance between Antarctica and any other continent. Mechanistically, dispersal could occur via warm- and cold-core rings, mesoscale eddies known to transport larvae and rafting organisms into and out of Antarctica across the ACC (Clarke *et al*. 2005). The recent findings of Antarctic krill in Chilean fjords, and South American anomuran and brachyuran crab larvae in the Antarctic Peninsula highlight the penetrability of this current (Antezana 1999; Thatje & Fuentes 2003; Clarke *et al*. 2005). Dispersal of rafting adults from South America to

subantarctic South Georgia has been shown in a brooding bivalve species (Helmuth *et al*. 1994).

1.1.1 Larval dispersal

Reproduction in polar marine invertebrates has been the focus of much speculation and discussion in the literature for more than a century. The paradigm known as 'Thorson's Rule', coined by Mileikovsky (1971), resulted from few observations that led to the idea that polar and deep-sea species were almost exclusively brooders (e.g., Thomson 1878, 1885; Murray 1885; Thorson 1936). Applicability of this 'rule' is now known to be limited for many groups, especially echinoderms and bivalves (Clarke 1992; Pearse 1994). New paradigms concerning reproductive trends in the Antarctic, based on larger amounts of data, are emerging. These data have shown that over 70% of Antarctic echinoderm species have a planktonic larval stage (Pearse 1994). Pearse (1994) summarized previous research (Bosch & Pearse 1990; Pearse *et al.* 1991) on echinoderm reproduction in McMurdo Sound, Ross Sea, Antarctica, and determined that 23% of echinoderms had a feeding pelagic (planktotrophic) larval form, 50% had a non-feeding pelagic (lecithotrophic) form and 27% had either a non-feeding demersal larvae or were brooders.

These prevailing modes of development can be used to infer dispersal potential, as determined by pelagic larval duration, of Antarctic benthic invertebrates. Studies on a variety of marine invertebrates have shown that species with longer pelagic larval period often show less population differentiation than those with abbreviated larval development (Berger 1973; Crisp 1978; Janson 1987; McMillan *et al.* 1992; Duffy 1993; Hunt 1993;

Hellberg 1996; Hoskin 1997; Arndt & Smith 1998). However, this relationship can vary greatly, and some marine invertebrates show high levels of population differentiation despite a long-lived pelagic larvae (e.g., Tracey *et al.* 1975; Burton 1986). In addition, brooding species can be more widespread than planktotrophic species (Johannesson 1988).

The enhanced dispersal ability of Antarctic marine invertebrates with pelagic larvae has been invoked to explain their ability to quickly recolonize shelf habitat recently disturbed by ice scour (Poulin *et al.* 2002). Historically, disturbances caused by expansion and contraction of the Antarctic ice sheet during Pleistocene glacial periods are thought to be responsible for the circumpolar distribution typical of many Antarctic benthic invertebrates (Thatje *et al.* 2005). Thatje et al. (2005) suggested that the shallow continental shelf that opened up during periods of diachronous deglaciation (Anderson *et al.* 2002) would be recolonized first by species with highly dispersive larvae. These species could then recolonize shelf habitat around the continent of Antarctica as it became available during interglacial periods, resulting in a circumpolar distribution (Thatje *et al.* 2005). Conversely, diachronous deglaciation could also drive cryptic speciation in species with low dispersal potential, such as brooders, due to isolation in shelf refugia during glacial periods (Thatje *et al*. 2005). This type of speciation has been shown in Antarctic brooding isopods and a brooding sea slug (Held 2003; Held & Wägele 2005; Wilson *et al*. 2009), as well as a notothenioid fish (Janko *et al*. 2007).

1.1.2 Molecular studies on Antarctic fauna

Studies investigating the evolutionary history of Antarctic fauna using molecular tools have emerged in recent years. These studies have focused on notothenioid fish, known for their adaptive radiation in Antarctica over the past 2-12 million years (Bargelloni *et al*. 2000a, 2000b; Stankovic *et al*. 2002; Patarnello *et al*. 2003; Janko *et al.* 2007), krill (Patarnello *et al*. 1996; Zane *et al*. 1998; Bargelloni *et al*. 2000b), isopods (Held 2003; Held & Wägele 2005; Raupach & Wägele 2006; Leese & Held 2008), molluscs (Beaumont & Wei 1991; Allcock *et al*. 1997; Page & Linse 2002; Linse *et al.* 2007; Wilson *et al.* 2009), nemerteans (Thornhill *et al*. 2008), and pycnogonids (Mahon *et al*. 2008). Many studies have concentrated on the ACC's role in promoting speciation and divergence in the Southern Ocean. For example, Page and Linse (2002) concluded that bivalve sister species split after the formation of the ACC, indicating that initially it was not a barrier to dispersal in this group (see also Thornhill *et al*. 2008). Conversely, Patarnello et al. (1996) found evidence of vicariant speciation for Antarctic and subantarctic krill caused by formation of the ACC. In Antarctica, notothenioid fish show genetic discontinuity among benthic populations distributed around the continent (Bargelloni *et al.* 2000b). The Antarctic krill *Euphausia superba* exhibits surprising differentiation between populations from subantarctic South Georgia and from the Weddell Sea, given the high dispersal potential of this planktonic organism (Zane *et al*. 1998; Bargelloni *et al.* 2000b). The circumpolar crinoid *Promachocrinus kerguelensis* is represented by at least six cryptic lineages, despite having a pelagic larval stage in its development (Wilson *et al*. 2007), while the planktotrophic nemertean *Parborlasia corrugatus* and brooding pycnogonid *Nymphon australe* are genetically homogenenous

over large distances throughout the Antarctic Peninsula (Mahon *et al*. 2008; Thornhill *et al*. 2008).

1.1.3 Research objectives

Despite the above examples, many Antarctic benthic groups are unstudied in terms of their evolutionary history, population connectivity and biogeography. A conspicuous example is the ophiuroid echinoderms, one of the most common and abundant Antarctic benthic groups (Fell *et al.* 1969). Ophiuroids represent an important ecological component of the benthic community; certain species have been reported with local densities up to 10^7 individuals/ km^2 (Fell *et al.* 1969). Many Antarctic ophiuroids have a circumpolar distribution, attributed to dispersal by the ACC, or for deeper forms, by traversing deep ocean floors. The Antarctic Peninsula region has the highest species diversity for ophiuroids, probably because of faunal exchange with South America (Fell *et al*. 1969).

Given that ophiuroids are an important component of the Antarctic benthic community and have received little attention, the primary goal of this research was to evaluate genetic population structure and levels of connectivity using molecular tools. Where homogenous population genetic structure is identified, dispersal of larvae and/or adults will be proposed. In cases where heterogeneous population genetic structure results, limited dispersal and/or physical barriers to dispersal will be evaluated. Several ophiuroid species with different reproductive developmental modes will be examined and hypotheses will be developed based on differing dispersal capacities that correspond to expected levels of population connectivity. Pearse (1994) summarized approximate

persistence times for lecithotrophic and planktotrophic larval forms in the Antarctic. Pelagic lecithotrophic forms have been recorded spending 2-3 months in the plankton (based on asteroid data from Bosch & Pearse 1990), whereas pelagic planktotrophic forms are suspected to spend 5-6 months (based on echinoid and asteroid data from Pearse & Bosch 1986 and Bosch *et al*. 1987).

Specific research objectives are:

1) Determine phylogenetic relationships between species in the Southern Ocean genus *Ophiurolepis* using morphological data
2) Evaluate population genetic structure and connectivity across the Drake Passage for *Astrotoma agassizii*, a brooding Antarctic species
3) Examine population genetic structure and connectivity in *Ophionotus victoriae* in the Antarctic Peninsula and subantarctic islands, a planktotrophic Antarctic species
4) Determine population genetic structure and levels of connectivity for *Ophiurolepis brevirima* in the Antarctic Peninsula and Weddell Sea, and for *Ophiurolepis gelida* in the Ross Sea to Bouvet Island, both probable lecithotrophic Antarctic species

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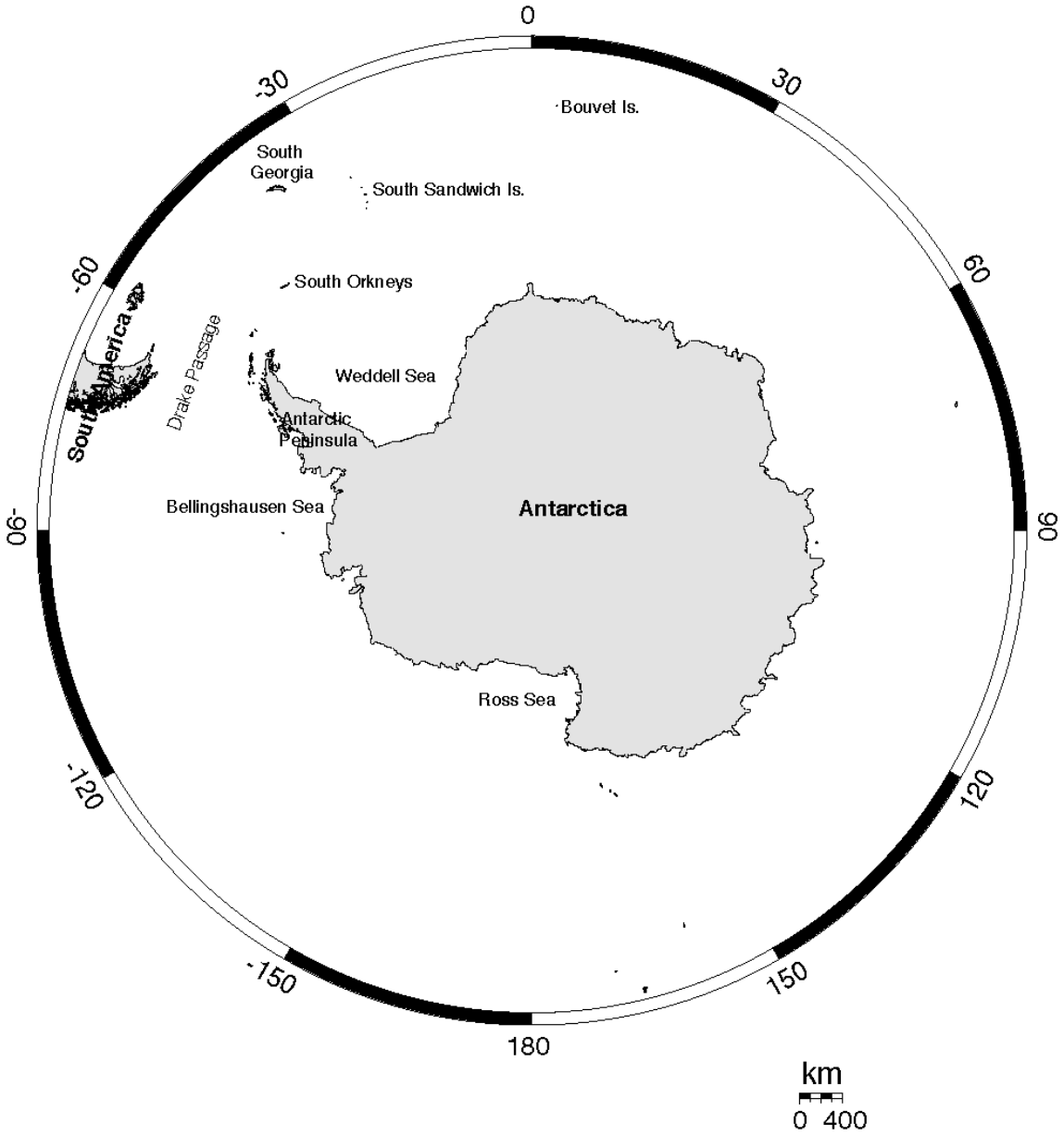

Figure 1 Relevant geographic localities in Antarctica and South America.

CHAPTER 2: Morphological cladistic analysis of *Ophiurolepis* Matsumoto, 1915 (Ophiurida: Ophiuridae) from the Southern Ocean

2.1 ABSTRACT

A phylogenetic analysis using morphological characters was done on the Antarctic ophiuroid genus *Ophiurolepis* Matsumoto, 1915. Species within the genus *Ophiurolepis* are abundant and ecologically dominant in the Antarctic and surrounding Southern Ocean. Maximum parsimony was used to infer phylogenetic relationships. Although strongly supported nodes were not recovered for most groupings within *Ophiurolepis*, this first attempt at a phylogeny revealed the presence of three tentative clades. Two of the three *Ophiurolepis* clades included species currently assigned to other genera, but closely allied with *Ophiurolepis* in the taxonomic literature. This indicates that *Ophiurolepis* as currently defined is not a monophyletic group. Additional forms of data, namely molecular, are needed to more definitively resolve relationships within this group.

2.2 INTRODUCTION

The brittle star genus *Ophiurolepis* Matsumoto, 1915 is one of the more speciose ophiuroid genera in the Southern Ocean (Fell *et al.* 1969). Nine of the fourteen species are endemic to the Antarctic/subantarctic region and four species have a circumpolar

distribution (Table 1). A circumpolar distribution has been proposed as the general distributional pattern for Antarctic ophiuroids, presumably influenced by the west-wind drift (Fell 1962; Pawson 1993). Of the five non-endemic species, *O. martensi* (Studer, 1885) and *O. scissa* (Koehler, 1908) extend from Antarctica to southernmost South America, and *O. turgida* Mortensen, 1936 has been found only from the southernmost South American shelf (Mortensen 1936). In addition to Antarctica, *O. mordax* Koehler, 1922 has been reported from the abyssal region of the Mozambique Channel (Vadon & Guille 1984; Guille & Vadon 1986). Recently, *O. carinata* (Studer, 1876), previously known only from the subantarctic Kerguelen and South Sandwich Islands, was collected close to methane seeps off Concepción Bay, Chile at approximately 36°S latitude (Sellanes & Krylova 2005).

Little is known about the life history of any species of *Ophiurolepis*. Lifespan was calculated for *O. brevirima* Mortensen, 1936 and *O. gelida* (Koehler, 1901) as 25 and 33 years, respectively, by analyzing vertebral ossicle growth bands (Dahm 1996). In his monograph on the echinoids and ophiuroids of the Antarctic/subantarctic region, Mortensen (1936) speculated about the reproductive biology of several *Ophiurolepis* and other closely related species. He postulated that six species (*O. brevirima, O. gelida, O. tuberosa* (Mortensen, 1936)*, Theodoria partita* (Koehler, 1908)*, T. wallini* (Mortensen, 1925)*,* and *Homalophiura inornata* (Lyman, 1878)) were dioecious with some form of direct development, but were not viviparous. Egg size was reported as ~0.3 mm diameter in these species. This is well within the oocyte diameter range (0.1-1.0 mm) known for brooding ophiuroids (Hendler 1991), but also within range for those with non-feeding pelagic lecithotrophic larvae, the dominant larval type in Antarctic echinoderms (Pearse

1994). Two exceptions noted by Mortensen (1936) were *O. martensi*, assumed to be viviparous based on the presence of five to six juveniles per bursa in some specimens, and *O. carinata*, thought to develop via the typical planktotrophic ophiopluteus larvae due to the small size and high number of eggs.

Several *Ophiurolepis* species are well known because of the dominant role they play in the Antarctic benthic community. For example, *O. brevirima*, *O. gelida* and *O. martensi* are among the dominant asterozoan species in Weddell Sea benthic assemblages (Piepenburg *et al.* 1997). *Ophiurolepis brevirima* and *O. gelida* are considered "permanent species" (CixDi% > 5; where Ci is a measure of frequency of occurrence and Di is a measure of numerical dominance) throughout the South Shetland Islands of the Antarctic Peninsula (Manjón-Cabeza & Ramos 2003). *Ophiurolepis martensi* is the most numerous ophiuroid around Marion Island, present in abundances of up to 232 m^{-2} (Beckley & Branch 1992). In contrast, other *Ophiurolepis* species are known from only a few localities where they have been collected in low numbers. Both *O. accomodata* Koehler, 1922 and *O. granulifera* Bernasconi and D'Agostino, 1973 were described from three or fewer specimens and have been reported only from one or two localities (Koehler 1922; Madsen 1955; Bernasconi & D'Agostino 1973). One species, *O. turgida*, has been described from a single specimen (Mortensen 1936).

2.2.1 Taxonomic history

Ophiurolepis was erected by Matsumoto (1915) to accommodate a single species that had been independently described by two taxonomists and placed in different genera. In 1876, Studer described *Ophiolepis carinata* from Kerguelen Island while two years

later Lyman (1878) described *Ophioglypha deshayesi* from Kerguelen Island. The two names were synonymized and transferred to *Ophiurolepis*, with *O. carinata* as the type specimen. Matsumoto (1915) diagnosed *Ophiurolepis* as having a disk with large rounded plates surrounded by belts of smaller plates, well-developed dorsal arm plates, triangular ventral arm plates, one minute arm spine and three tentacle scales, both teeth and oral papillae present, and with the second oral tentacle pores opening outside the mouth.

Within *Ophiurolepis* several species have been shuffled among closely related genera owing to morphological similarities with congeners and a lack of sufficient synapomorphies characterizing some genera. For example, *O. martensi*, originally described as *Ophioglypha martensi* (Studer, 1885), was transferred to *Ophiozona* (Bell 1902), then back to *Ophioglypha* (Koehler 1911), and then to *Homalophiura* and subsequently *Amphiophiura* (Clark 1915), prior to being assigned to *Ophiurolepis* (Koehler 1922). Two other species, *O. gelida* and *O. scissa*, were at one time placed in *Homalophiura*, and *O. anceps* (Koehler, 1908) was provisionally placed in *Amphiophiura* (Clark 1915). Additionally, Fell (1961) excluded two taxa from *Ophiurolepis* on the basis of three external skeletal characters shared by *O. wallini* and *O. partita*, thereby restricting the definition of *Ophiurolepis*. A new genus, *Theodoria* Fell 1961, was established for these two species and one other species, *Amphiophiura relegata* (Koehler, 1922).

The genus *Homalophiura* is closely related to *Ophiurolepis* and includes some species that might be referable to *Ophiurolepis*. The type species for this genus, *H. inornata*, has been suggested to belong within *Ophiurolepis*. Several workers (Hertz

1926; Mortensen 1936; Bartsch 1982) have stated that *Homalophiura* and *Ophiurolepis* are not easily distinguished and that *Homalophiura* probably cannot be maintained, despite the fact that Clark (1915) claimed *Homalophiura* was a "well-characterized and homogeneous group". Clark (1915) diagnosed *Homalophiura* as having large plates mingled with smaller plates on the disk, with a reduced arm comb and small tentacle pores, few minute arm spines, and second oral tentacle pores opening outside the mouth. In the taxonomic literature (Madsen 1951, 1967; Tommasi 1968; Bernasconi & D'Agostino 1971, 1973, 1977), the application of *H. inornata* has been inconsistent. Paterson (1985) proposed reconciling the genus by suggesting reassignments for the nineteen currently recognized species in *Homalophiura*. *Homalophiura inornata, H. brucei* (Koehler, 1908) and *H. confragosa* (Lyman, 1878) were reassigned to *Ophiurolepis*; however, no formal revision of the genus has since been made.

Little information exists on phylogenetic relationships within *Ophiurolepis*. Mortensen (1936) stated that *O. gelida* and *O. brevirima* were closely related, based on overall morphological similarity. However, no phylogenetic relationships have been suggested for the group apart from subjective assessments due to morphological similarity. Given uncertain relationships within *Ophiurolepis* and uncertain affinity with several *Theodoria* and *Homalophiura* species, a formal assessment of phylogenetic relationships is needed. Using morphological characters in a cladistic framework, this study explores the evolutionary relationships among these important Southern Ocean species and will provide phylogenetic hypotheses that can be tested with additional data. Gaining insight into the evolutionary history of *Ophiurolepis* in Antarctica will allow

further understanding of the relevant biogeographic processes and speciation mechanisms that have contributed to biodiversity in this area.

2.3 MATERIALS AND METHODS

2.3.1 Data matrix

In order to test phylogenetic relationships of species of *Ophiurolepis*, a data matrix of 36 morphological characters and 24 ingroup species was constructed in MacClade 4.0 (Maddison & Maddison 2000) (Appendix). The following species were included (Table 1 and Fig. 1): the fourteen species currently recognized in *Ophiurolepis*, *Theodoria wallini*, *T. partita*, *T. relegata*, *T. madseni* Tommasi, 1976, *Homalophiura inornata*, *H. brucei*, *H. confragosa*, *H. intorta* (Lyman, 1878), *H. euryplax* Clark, 1939 and *H. clasta* (Clark, 1911). *Theodoria* species were included to determine the relationship of *Theodoria* to *Ophiurolepis*, since two of these species were at one time placed in *Ophiurolepis* (*T. wallini* and *T. partita*). Several *Homalophiura* species were included to help elucidate the relationship of *Homalophiura* and *Ophiurolepis*. *Homalophiura inornata*, *H. brucei* and *H. confragosa* were suggested by Paterson (1985) to belong in *Ophiurolepis* while *H. euryplax* and *H. intorta* were allied with *Ophiura* and *H. clasta* was suggested to belong in *Homophiura* (Paterson 1985). Additionally, a second analysis was done excluding *Theodoria relegata* and *T. madseni*, since these two species have reportedly never been considered as having a close affinity to *Ophiurolepis*, but are congeneric with two species that were once placed in *Ophiurolepis*. Two species from the same subfamily (Ophiurinae) as *Ophiurolepis*, *Ophiuroglypha carinata* (Koehler, 1901) and *Ophiuroglypha lymani* (Ljungman, 1870), were used as outgroups.

2.3.2 Character scoring

Of the 36 characters used in analyses, 34 were ossicular, one was soft tissue and one was ecological. Characters were scored using published descriptions (Studer 1876; Lyman 1878; Koehler 1902, 1922; Clark 1911; Matsumoto 1915; Mortensen 1925, 1936; Madsen 1955, 1967; Fell 1961; Cherbonnier 1962; Bernasconi & D'Agostino 1973; Bartsch 1982; Paterson 1985) and verified using museum specimens when possible. Samples were obtained from the Smithsonian Institution National Museum of Natural History and the South Australian Museum, and collected during a 2004 Antarctic cruise. Twenty-six characters were scored as binary and 10 were scored as multistate. Character descriptions and justification of scoring are given in the Appendix and select characters are photographically illustrated.

2.3.3 Phylogenetic analysis

Data were analyzed in PAUP 4.0b10 (Swofford 2002) using maximum parsimony (MP) based reconstructions. All characters were treated as unordered and equally weighted. For the analysis including *T. relegata* and *T. madseni*, a heuristic search with 1000 random addition replicates and tree bisection reconnection (TBR) branch-swapping was performed. For the analysis excluding *T. relegata* and *T. madseni*, a branch and bound search was done. Character-state optimization used the accelerated transformation (ACCTRAN) option in PAUP. To test the robustness of the MP trees, 1000 bootstrap replicates were done and decay indices (Bremer 1988) were calculated in TreeRot.v2 (Sorenson 1999). Character transformation was evaluated in MacClade 4.0.

2.4 RESULTS

Analysis of morphological data yielded 817 most parsimonious trees (strict consensus shown in Fig. 2) with a tree length (TL) = 78, consistency index (CI) = 0.60 and retention index (RI) = 0.77. Bootstrapping proved to be too computationally intensive for searches including *T. relegata* and *T. madseni*, therefore only decay indices were calculated. Searches excluding *T. relegata* and *T. madseni* yielded six most parsimonious trees (strict consensus shown in Fig. 3) with TL = 71, CI = 0.65 and RI = 0.79.

2.4.1 Analysis including *T. relegata* and *T. madseni*

The most parsimonious trees from searches including *T. relegata* and *T. madseni* required seven additional steps and were supported by lower CI and RI values when compared to searches excluding these taxa. The strict consensus of these trees is not well resolved (Fig. 2), however, two clades, denoted A and C, are present. Resolution among all other species in the tree is lacking. These are denoted as clade B in order to make comparisons with the analysis that excluded *T. relegata* and *T. madseni*. This lack of resolution is probably due to the confounding effect of adding these two taxa, which share similarities to the other two *Theodoria* species (*T. wallini* and *T. partita*), which in turn share similarities to *Ophiurolepis* species. Characters shared between the four *Theodoria* species are primarily those used in diagnosing the genus: deeply excavate jaws and conspicuous basal tentacle pores.

2.4.2 Analysis excluding *T. relegata* and *T. madseni*

Owing to the confounding effect of *T. relegata* and *T. madseni*, I focus instead on relationships recovered when these two taxa were removed. Morphological data suggest that two, possibly three, major clades (Fig. 3) are present within *Ophiurolepis*. Two of these clades, clades A and C, are present also in the analysis that includes *T. relegata* and *T. madseni*, whereas Clade B collapses when these two taxa are included. Clade A, a ten species clade, contained only species currently recognized as *Ophiurolepis*. Clade B contained three *Ophiurolepis* species as well as *Theodoria wallini, T. partita* and *Homalophiura brucei*. *Homalophiura brucei* was previously suggested to have a greater affinity with *Ophiurolepis* than with *Homalophiura* (Paterson 1985). Within clade B, *T. partita* and *T. wallini* were supported as sister taxa. The third clade, clade C, was basal to A and B and included three *Homalophiura* species (*H. clasta, H. confragosa* and *H. inornata*) and *O. scissa*. As previously stated, *H. inornata* and *H. confragosa* have been suggested as belonging within *Ophiurolepis*, while *H. clasta* was allied with *Homophiura* (Paterson 1985). *Ophiurolepis scissa* had at one time been placed within *Homalophiura* (Clark 1915), reflecting a previously recognized affinity between this species and some members of *Homalophiura*. *Homalophiura intorta* and *H. euryplax* were basal to all *Homalophiura* and *Ophiurolepis* species. This was not surprising given that Paterson (1985) grouped these two species with *Ophiura* in his revision of the *Homalophiura*, and not *Ophiurolepis*. Both bootstrap proportions and decay indices indicated low support for relationships within and between clades A-C, but moderate support for grouping these clades to the exclusion of *H. euryplax* and *H. intorta*.

2.5 DISCUSSION

This first attempt to elucidate phylogenetic relationships within *Ophiurolepis* has revealed two major clades, and tentatively a third. Many species within *Ophiurolepis* are distinguished by only a few morphological characters, some of which vary widely within these species. This condition seemingly creates a continuum among certain species that are difficult to separate from one another based on morphological character data. For example, *O. olstadi* Madsen, 1955 was noted by Madsen (1955) as resembling *O. gelida* in "overall appearance", but distinguished by a more thickened skeleton and short genital slits. It could be that skeleton thickness is a variable morphological character and that this species is actually a form of *O. gelida* with short genital slits. Another species, *O. brevirima*, differs from *O. olstadi* only in having broader jaws and adoral plates and in having fewer arm spines (Madsen 1955). These distinctions may represent morphological variability in these characters and not species boundaries. Therefore, the lack of strong support for relationships within *Ophiurolepis* is not surprising.

Twenty-three morphological character states differed between *Ophiurolepis* clades A/B and the outgroup *Ophiuroglypha* (Fig. 4), suggesting that considerable morphological evolution has occurred between these two genera. In stark contrast, only two synapomorphies separate clades A/B from clade C, composed primarily of *Homalophiura* species. Clades A/B are distinguished by their disc elevation (character 1) while members of clade C possess fragmented dorsal arm plates (character 31). However, this latter synapomorphy has convergently arisen in both *O. tuberosa* and *T. partita* of clade B, casting doubt on its phylogenetic utility.

The question remains whether *Homalophiura* is a valid genus, or if *H. inornata* should be transferred to *Ophiurolepis*, invalidating the genus. The present analysis indicates that three *Homalophiura* species (*H. inornata*, *H. confragosa* and *H. clasta*), one of which is the type species, are scarcely distinguishable from other *Ophiurolepis* species. One *Homalophiura* species, *H. brucei*, even falls within an *Ophiurolepis* clade. Also of importance is that an *Ophiurolepis* species, *O. scissa*, was recovered as sister to *H. inornata*, the type species. Therefore, it seems practical to consider clade C as a basal *Ophiurolepis* clade, sister to the two derived clades A and B. This placement has been proposed by others (Hertz 1926; Mortensen 1936; Bartsch 1982), most explicitly by Paterson (1985). Conversely, *H. intorta* and *H. euryplax* are distinguished by a greater number of synapomorphies and separate from the ingroup species with moderate support. This indicates, as previously recognized (Paterson 1985), that at least these two *Homalophiura* species do not belong within *Ophiurolepis*.

The two derived *Ophiurolepis* clades are differentiated by the gonad character (character 34) of clade B, and by the characteristic thickening of the disc plates (character 2) in clade A (Fig. 4). It was not surprising that *Theodoria partita* and *T. wallini* fell within *Ophiurolepis*. In the original description, Fell (1961) stated that *Theodoria* was most closely related to *Ophiurolepis*, differing only by deeply excavate jaws, three conspicuous tentacle pores on the proximal arm joints, and relatively smaller and often fragmented oral shields. However, four *Ophiurolepis* species are known to have fragmented oral shields, as does *H. inornata*. Furthermore, the size of the oral shields appears to be comparable among the two genera when scaled for disc size (personal observation). Therefore, only two diagnostic morphological characters separate *T. partita*

and *T. wallini* from *Ophiurolepis* species. These characters could be easily explained if *T. partita* and *T. wallini* were derived from within clade B. Potentially, *T. relegata* and *T. madseni* also are derived taxa within *Ophiurolepis*, but more data are needed to unambiguously determine the relationship of these two species to other *Ophiurolepis* and *Theodoria* species. However, it is likely also that these two taxa are allied with *T. partita* and *T. wallini* only on the basis of the characters discussed above. Some of these characters, such as size and fragmentation of the oral shields, have been shown to be convergent and may not reflect any real phylogenetic relationship among these two taxonomic groups.

2.5.1 Morphological characters

The utility of morphological characters used in this study was examined by analyzing their pattern on the resulting phylogeny. Convergence or reversals were evident for fifteen characters, most of which are diagnostic characters used in ophiuroid taxonomy. For example, the length of the genital slit often is used to separate closely related species, such as *O. gelida* and *O. brevirima*. However, this study has shown that this character may not be useful in assessing phylogenetic relationships among these ophiuroid species, due to repeated convergent evolution. Similarly, fragmentation of the oral shields has arisen independently in several lineages. The convergent nature of many characters used currently in ophiuroid taxonomy seems to limit their effectiveness in reconstructing phylogenies, especially of closely related species. This is reflected in the paucity of morphological cladistic studies that exist for ophiuroids at all taxonomic levels (e.g., Smith *et al.* 1995).

2.5.2 Taxonomic revision of *Ophiurolepis*

Although a systematic revision of *Ophiurolepis*, *Homalophiura* and *Theodoria* is beyond the scope of this study, preliminary recommendations may facilitate a more formal revision in the future. Morphological evidence points to the inclusion of *H. inornata*, *H. clasta*, *H. confragosa* and *H. brucei* in *Ophiurolepis*. Following this recommendation, *Ophiurolepis* species would be diagnosable by three synapomorphies: rudimentary arm combs, distalmost arm spine separated from the other arm spine and tentacle scales by a gap, and crescent-shaped genital plates. Remaining *Homalophiura* species would consequently be invalidated due to re-assignment of the type species, *H. inornata*. Further examination of these species, including two used in this study, *H. intorta* and *H. euryplax*, is necessary to determine whether re-assignments into existing genera are appropriate or if a new genus/genera is needed to accommodate these species. For the genus *Theodoria*, *T. partita* and *T. wallini* share one synapomorphy, jaws excavate on the midline, that separates them from other *Ophiurolepis* species. However, these two species also share two character reversals, evenly spaced arm spines across the distal margin of the lateral arm plate and conspicuous basal tentacle pores. *Theodoria relegata* exhibits these character states, but the position of this species remains unclear from the phylogenetic analysis conducted herein. Therefore, given that *T. partita* and *T. wallini* are distinct within *Ophiurolepis*, it is recommended that a more thorough investigation of these two taxa, with *T. relegata* and *T. madseni*, be carried out.

2.5.3 Biogeographic considerations

With some understanding of the phylogenetic relationships within *Ophiurolepis*, it is possible to evaluate hypotheses on the biogeographic history of this group. The topology depicted in Figure 3 suggests that clades A and B arose from a geographically widespread ancestor, based on the wide distribution of its sister group, clade C. Subsequently, part of this ancestral stock occurring around Antarctica could have then become isolated as Antarctica separated from Australia and then South America (~41 mya), and become further isolated by the onset of the Antarctic Circumpolar Current in the late Eocene (Scher & Martin 2006). Given that the greatest diversity of the genus is in the Antarctic and that all but one species occur there, it is likely that speciation occurred after isolation, resulting in the present day clades A and B. Some species then subsequently spread into southern South America and in the case of *O. mordax* and *O. carinata*, spread to ocean basins based on the supposed ability of deeper water forms to traverse ocean floors (Fell *et al.* 1969). This seems feasible given that *O. mordax* has been collected as deep as 2500 m. In fact, this particular species could be a rare example of "polar emergence", in which cold stenothermal forms are found in the same Antarctic water layer, the Antarctic Bottom Water, but at different latitudes (Vadon & Guille 1984).

Faunal exchange between South America and the Antarctic Peninsula and South Georgia region has long been presumed and is reflected in the twenty-five ophiuroid species shared between these two areas (Fell *et al.* 1969). Faunal affinities between these regions have been determined for a number of Antarctic taxa (see Bargelloni *et al.* 2000a; Bargelloni *et al.* 2000b; Page & Linse 2002; Stankovic *et al.* 2002). Therefore, it is

highly probable that several *Ophiurolepis* species independently established populations in southern South America. One of these species, *O. turgida*, may have even become extinct in the Antarctic, now being endemic to southern South America.

Why is *Ophiurolepis* so speciose in the Antarctic? One possibility is reflected in the small degree of morphological difference between many *Ophiurolepis* species and could be the result of "taxonomic splitting". This effect can lead to many taxonomic species within a group that do not represent biological species. Future molecular and ecological data could begin to answer these questions that remain unresolved at the morphological level. Second, at least seven species, *O. brevirima*, *O. gelida*, *O. martensi*, *O. tuberosa*, *T. partita*, *T. wallini* and *H. inornata* are probably brooders, while for the rest the reproductive mode is unknown (Mortensen 1936; Hendler 1991). Thus, it is possible that speciation in this group was enhanced or even driven by the lowered dispersal capabilities of brooding species, especially if brooding was the ancestral state. This pattern has been discussed for Antarctic asteroids (Pearse & Bosch 1993) and noted for Antarctic brooding echinoids, which are more speciose than their relatives who undergo planktotrophic development (Pearse *et al.* 1991).

Examination of morphological traits in *Ophiurolepis* has shed some light on phylogenetic relationships within this genus. However, since these and other ophiuroid species appear to be plagued by convergent morphology, other types of data, namely molecular, are necessary to resolve relationships among these ecologically important and abundant animals. Despite this, systematic determination of the convergent or homologous nature of specific morphological characters remains important, since it can

provide great insight into the utility of certain characters used in ophiuroid taxonomy and phylogeny.

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Table 1 Distributions, depth ranges and catalog numbers for species of *Ophiurolepis*, *Theodoria* and *Homalophiura* used in this study.

Taxon	Distribution	Depth Range	USNM Catalog No.	Reference
Ophiurolepis				
accomodata	Marion Is.	1300 m		Koehler 1922
anceps	Ross Sea, Weddell Sea	700-2580 m	1014081	Koehler 1908; USNM[1]
banzarei	east Antarctica	190-300 m	SAM[2]: K1206-1208, K1210	Madsen 1967
brevirima	circumpolar, South Georgia	200-750 m	E43649, E43679	Lyman 1878; Mortensen 1936; Fell 1961; Madsen 1967; Bernasconi & D'Agostino 1975; USNM
carinata	Chile, South Sandwich Is., Kerguelen Is.	50-760 m		Studer 1876; Lyman 1878; Mortensen 1936; Sellanes & Krylova 2005
gelida	circumpolar Antarctic/subantarctic	40-650 m	E43606, E43634	Koehler 1902, 1922, 1923; Mortensen 1936; Clark 1951; Fell 1961; Madsen 1967
granulifera	Petermann Is., Petrel Base	250-400 m		Bernasconi & D'Agostino 1973
martensi	circumpolar Antarctic/subantarctic, Cape Horn	20-310 m	1078489, E45077, E10583, E52048	Koehler 1922, 1923; Mortensen 1936; Clark 1951; Fell 1961; Madsen 1967; Rowe & Clark 1975; USNM
mordax	east Antarctica, South Sandwich Is., Mozambique Channel	220-2500 m	1078474	Koehler 1922; Vadon & Guille 1984; USNM
olstadi	Antarctic Peninsula, Ross Sea	600 m	E52417, E52834	Madsen 1955; USNM
scissa	Weddell Sea, Falkland Is.	650-2560 m	E46838	Koehler 1908; USNM
tuberosa	Antarctic Peninsula, Ross Sea	200-750 m	E43785, E43789	Mortensen 1936; Madsen 1955; Fell 1961; USNM
tumescens	circumpolar	200-2450 m		Koehler 1922; Madsen 1955, 1967; Bernasconi & D'Agostino 1975
turgida	Falkland Is.	340 m		Mortensen 1936
Theodoria				
madseni	Laguna Grande Peru	3883-4004 m	E11375	Tommasi 1976
partita	Antarctic Peninsula, Ross Sea, South Georgia	130-3250 m	E44698, E46807	Mortensen 1936; USNM

relegata	circumpolar	110-550 m	E43592, E44602	Fell 1961; USNM
wallini	circumpolar	130-640 m	E43786, E44701	Mortensen 1925, 1936; Madsen 1967
Homalophiura				
brucei	west Antarctica	3000-4440 m	1019242, 1019630	Koehler 1908; USNM
clasta	Japan	930-1680 m	25547	Clark 1911
confragosa	north Atlantic, Patagonia–Buenos Aires	100-1100 m	9848, 26267	Lyman 1878; USNM
euryplax	Gulf of Aden, Oman Sea, Maldives	1270 m		Clark 1939; Vadon & Guille 1984
inornata	cosmopolitan	50-3380 m	E52508	Lyman 1878; Koehler 1904, 1906, 1914, 1922; Clark 1915; Mortensen 1936; Madsen 1967; Bernasconi & D'Agostino 1971, 1977; USNM
intorta	Marion Is.	90-140 m		Lyman 1878

[1]refers to distribution records of catalogued specimens from the Smithsonian Institution National Museum of Natural History (USNM)
[2]South Australian Museum

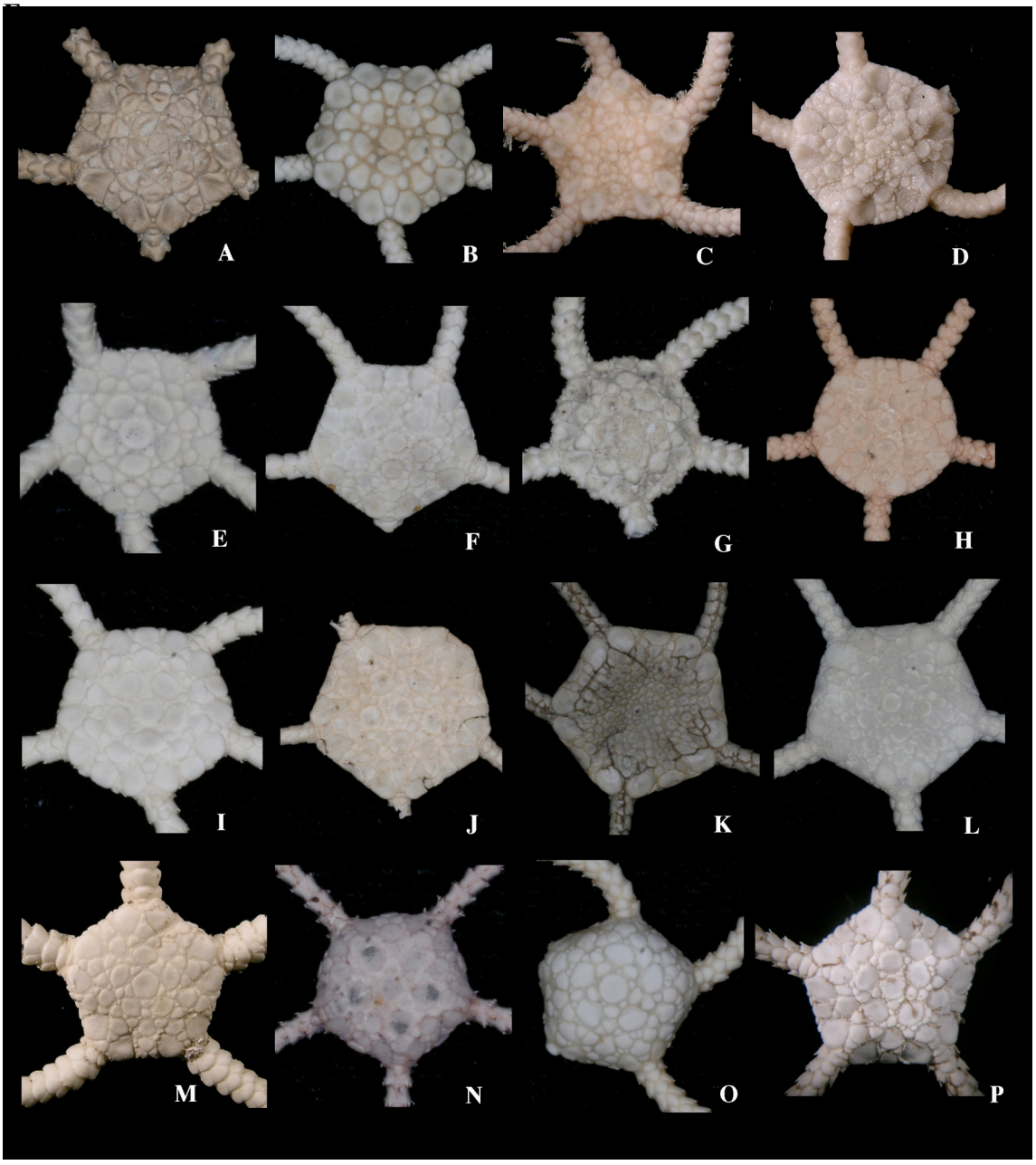

Figure 1 Plate showing aboral disc view of various ophiuroid species used in this study. A. *Ophiurolepis anceps,* B. *O. banzarei,* C. *O. brevirima,* D. *O. gelida,* E. *O. martensi,* F. *O. mordax*, G. *O. olstadi*, H. *O. scissa*, I. *O. tuberosa*, J. *Homalophiura brucei*, K. *H. clasta*, L. *H. confragosa*, M. *H. inornata*, N. *Theodoria partita*, O. *T. relegata*, P. *T. wallini*.

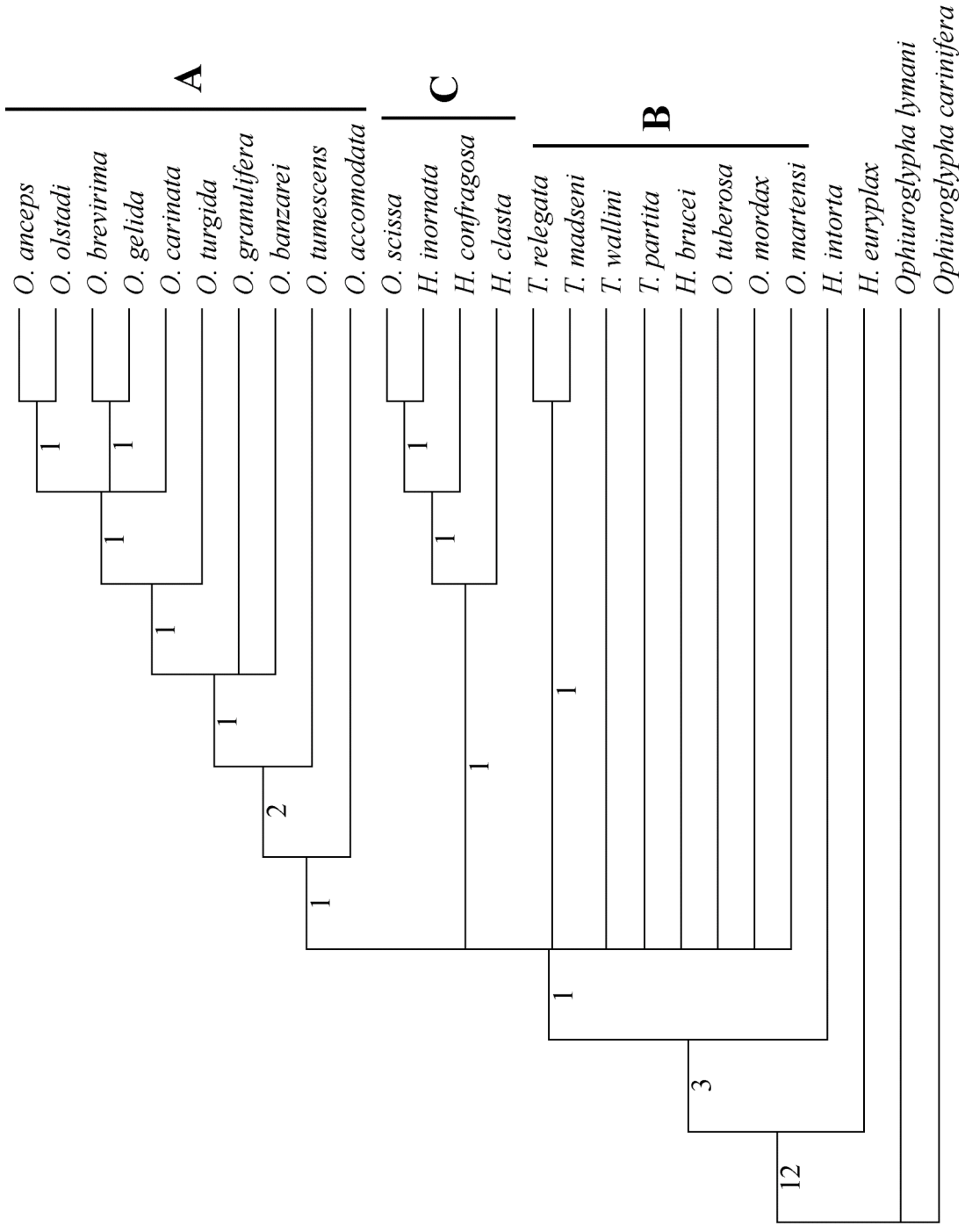

Figure 2 MP strict consensus tree for data including *T. relegata* and *T. madseni.* Numbers below branches represent decay indices.

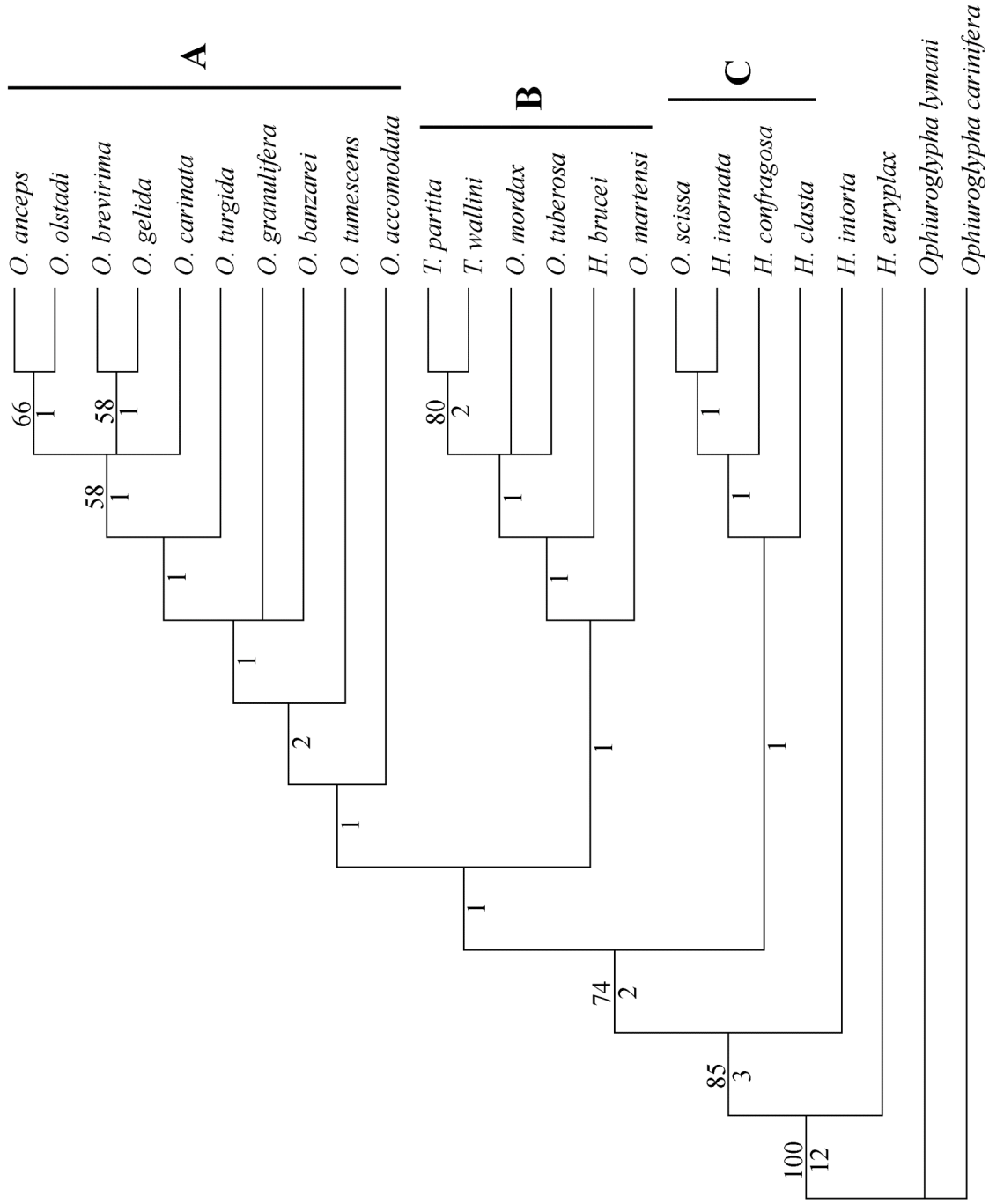

Figure 3 MP strict consensus tree for data excluding *T. relegata* and *T. madseni*. Numbers above branches represent bootstrap proportions and numbers below represent decay indices.

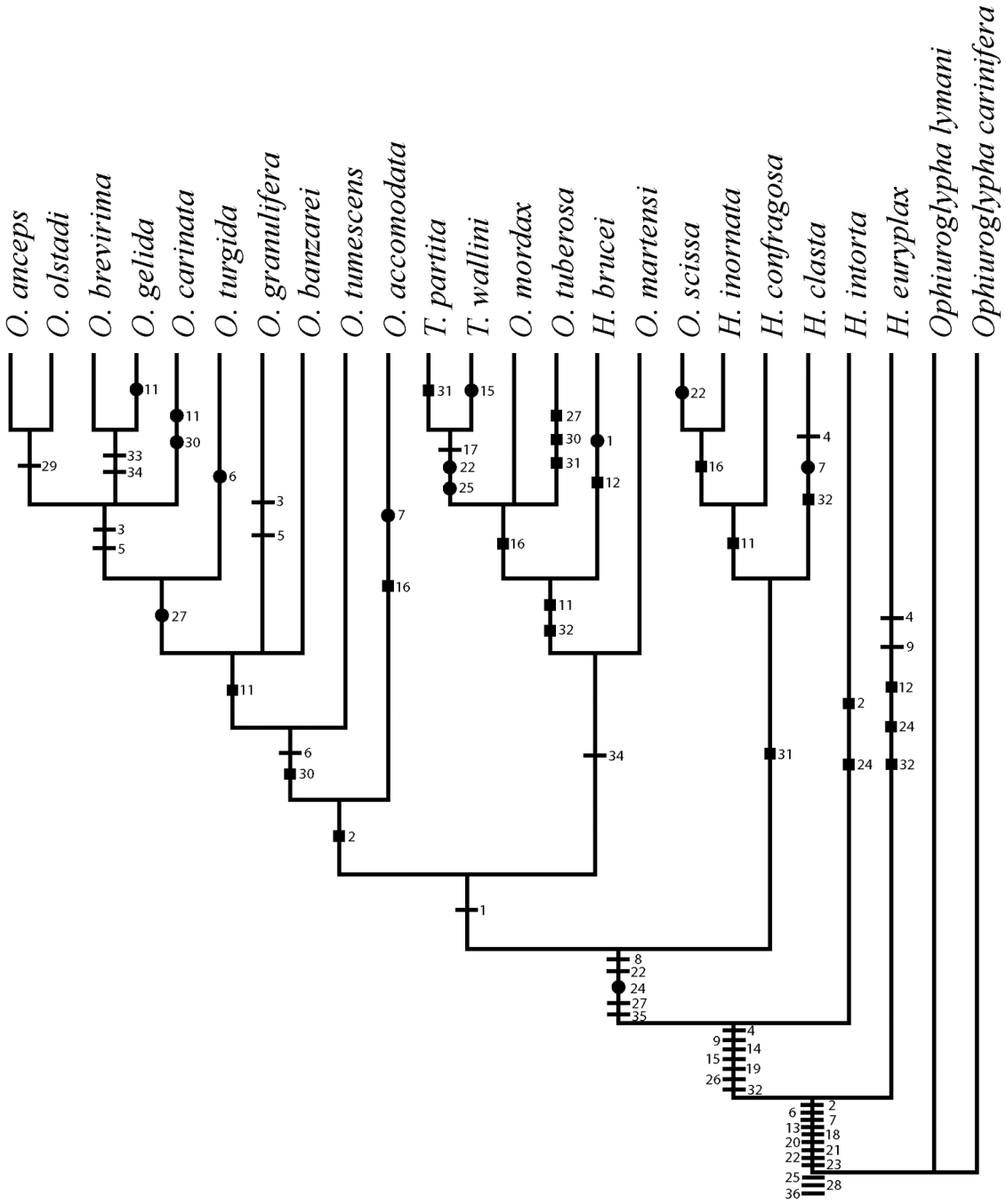

Figure 4 Morphological characters mapped onto the strict consensus MP tree for data excluding *T. relegata* and *T. madseni*. Character numbers correspond with Appendix. Bars indicate where a change has occurred, circles indicate reversals and squares indicate convergent character changes.

CHAPTER 3: Evaluating connectivity in the brooding brittle star *Astrotoma agassizii* across the Drake Passage in the Southern Ocean

3.1 ABSTRACT

Studies examining population genetic structure and genetic diversity of benthic marine invertebrates in the Southern Ocean have emerged in recent years. However, many taxonomic groups remain largely unstudied, echinoderms being one conspicuous example. The brittle star *Astrotoma agassizii* is distributed widely throughout Antarctica and southern South America. This species is a brooding echinoderm and therefore may have limited dispersal capacity. In order to determine the effect of hypothesized isolating barriers in the Southern Ocean, such as depth, geographic distance and the Polar Front, two mtDNA markers were used to compare populations from the South American and Antarctic continental shelves. *Astrotoma agassizii* was shown to be genetically discontinuous across the Polar Front. In fact, populations previously assumed to be panmictic instead represent three separate lineages that lack morphological distinction. However, within lineages, genetic continuity was displayed across a large geographic range (> 500 km). Therefore, despite lacking a pelagic larval stage, *A. agassizii* can disperse across substantial geographic distance within continental shelf regions. These results indicate that geographic distance alone may not be a barrier to dispersal, but rather

the combined effects of distance, depth and the Polar Front act to prevent gene flow between *A. agassizii* populations in the Southern Ocean.

3.2 INTRODUCTION

Oceanographic current patterns and life history traits, such as reproductive strategy, have been shown to directly affect dispersal of organisms in the marine environment (reviewed in Palumbi 1994), thereby profoundly influencing distributional patterns in the world's oceans. These factors and their influence on population genetic structure of marine organisms have been studied across a wide range of taxa, allowing generalizations to be established on which hypotheses can be based. For example, studies have shown that marine invertebrate species with longer pelagic larval duration often show less population differentiation than those with abbreviated larval development (Berger 1973; Crisp 1978; Janson 1987; McMillan *et al.* 1992; Duffy 1993; Hunt 1993; Hellberg 1996; Hoskin 1997; Arndt and Smith 1998). However, this relationship can vary greatly, with some species showing high levels of differentiation despite long-lived pelagic larvae (e.g., Tracey *et al.* 1975; Burton 1986; Hare and Avise 1996). Effects of oceanographic barriers and life-history constraints have been evaluated across many geographic regions, however one particularly distinctive biogeographic region, the Southern Ocean, is not well understood in this context. The roles of the Polar Front, small-scale gyres and life history traits that affect dispersal of marine organisms in the Southern Ocean are only beginning to be understood.

Isolation of the Antarctic continent is hypothesized to have been a driving evolutionary force for Antarctic fauna. Separation of Antarctica from South America and

the ensuing onset of the Antarctic Circumpolar Current (ACC), dated to between 24–41 mya (Lawver and Gahagan 2003; Pfuhl and McCave 2005; Scher and Martin 2006), are presumed to have been primary forces promoting speciation in Southern Ocean taxa (Patarnello *et al*. 1996; Page and Linse 2002; Clarke *et al.* 2005). The Polar Front, the region of the ACC marked by a 3-4°C temperature change and high-flow velocity (Eastman 1993), is a particularly strong physical barrier (Clarke *et al.* 2005). ACC formation, coupled with decreasing atmospheric CO_2 concentration (DeConto and Pollard 2003), is thought to have driven the gradual cooling and glaciation that began ~34 mya in the Antarctic (Zachos *et al*. 2001). This cooling and long period of isolation have led to a diverse and abundant benthic fauna that is typically stenothermal, eurybathic and endemic to Antarctica (Ekman 1953; Hempel 1985). Endemism is particularly high in certain groups including fish (95%), amphipods (95%), pycnogonids (90%), isopods (87%), and certain echinoderm classes (73%) (Knox and Lowry 1977; Brandt 1991; Jazdzewski *et al*. 1991).

While many Antarctic benthic organisms exhibit high levels of endemism, others show much lower levels despite apparent geographic and thermal isolation. For example, polychaete, echinoderm and mollusc conspecifics have been reported on both Antarctic and South American continental shelves, and a well-recognized faunal affinity exists between these two geographic regions (Dell 1972; Arntz *et al*. 1994; Dayton *et al*. 1994). Lack of endemism in these species suggests some level of recent or ongoing gene flow between populations separated by the ACC. Several gene flow mechanisms have been proposed, including migration of benthic adults, larval dispersal and rafting. Migration of adults is thought to occur along the Scotia Arc, a submerged ridge with a series of

emergent islands that form a "stepping-stone" connection between the Antarctic Peninsula and South America (Fell *et al*. 1969). Dispersal of larvae or rafting adults/juveniles would most likely occur across the Drake Passage, the portion of the ACC separating Antarctica and South America representing the shortest distance between Antarctica and any other continent. Mechanistically, dispersal could occur across the ACC via warm- and cold-core rings (Clarke *et al*. 2005), mesoscale eddies known to transport larvae and rafting organisms (Robinson 1983; Scheltema 1986).

Studies investigating the evolutionary history of Antarctic fauna using molecular tools have emerged in recent years. These studies have focused primarily on groups such as notothenioid fish (Bargelloni *et al*. 2000a, 2000b; Stankovic *et al*. 2002), krill (Patarnello *et al*. 1996; Bargelloni *et al*. 2000b), and molluscs (Brierley *et al*. 1993; Allcock *et al*. 1997; Page and Linse 2002), and concentrate on the ACC's role in promoting speciation and divergence in the Southern Ocean. Many Antarctic benthic organisms remain unstudied in terms of their evolutionary history, population connectivity and biogeography, and only a few studies exist evaluating population connectivity of Southern Ocean species within South America (e.g., Brierley *et al*. 1993; Shaw *et al*. 2004). A conspicuous example of an unstudied taxon is the Ophiuroidea, abundant and ecologically important components of the Antarctic benthic community.

Astrotoma agassizii is one of thirteen ophiuroid species shared between Antarctica and South America (Fell *et al.* 1969). This species has a circumpolar Antarctic/subantarctic distribution and occurs throughout the southern part of South America, in depths of 80–1200 m (Bartsch 1982). *Astrotoma agassizii* broods its embryos (Bernasconi 1965; De La Serna De Estaban 1966; Bartsch 1982; IS Smirnov, pers.

comm.) and therefore lacks a dispersive larval stage. *Astrotoma agassizii* is recognized as a morphologically uniform species throughout Antarctica and South America. However, given potential for significant population genetic structure owing to presumed limited dispersal capacity, we wanted to determine whether morphological uniformity corresponds also with genetic uniformity in this geographically and bathymetrically widespread species. Two mtDNA gene fragments were employed to evaluate the effects of geographic distance, depth and the Polar Front on population genetic structure and connectivity in this conspicuous Southern Ocean species.

3.3 MATERIALS AND METHODS

3.3.1 Data collection

Astrotoma agassizii samples were collected during two cruises to the southern tip of South America and Antarctic Peninsula aboard the *R/V Laurence M. Gould*. The first cruise took place from 23 November – 22 December 2004 and the second from 12 May – 13 June 2006. In total, 207 individuals were collected from eleven stations in South American waters and 30 individuals were collected from six Antarctic stations (Fig. 1 and Table 1). Benthic samples were collected with an epibenthic sled, Blake trawl, or rock dredge. Samples intended for DNA analysis were either frozen upon collection at -80ºC or preserved in ~85% ethanol.

DNA was extracted using the DNeasy® Tissue Kit (QIAGEN) following manufacturer's protocol. Two mitochondrial gene fragments, 16S rDNA (16S) and cytochrome oxidase subunit II (COII), were amplified using standard PCR protocols. 16SarL (5′-CGCCTGTTTATCAAAAACAT-3′) and 16SbrH

(5′-CCGGTCTGAACTCAGATCACGT-3′) (Palumbi *et al.* 1991) amplify a ~500 bp fragment from the middle of 16S. For COII, primers were designed based on a COII alignment spanning the diversity of extant echinoderms. The novel primers CO2_23AF (5′-MCARCTWGGWTTWCAAGA-3′) and CO2_577R (5′-TCSGARCATTGSCCATARAA-3′) amplify a ~550 bp fragment from the 5′ end of the gene. Double-stranded PCR products were purified using either a gel-freeze method or Montage™ PCR Filter Units (Millipore). Purified PCR products were bi-directionally sequenced using a CEQ8000 Genetic Analysis System (Beckman Coulter). All *A. agassizii* haplotypes were deposited in GenBank and correspond to accession numbers EF565745–EF565820.

3.3.2 Population structure analyses

Sequences were edited in SeqMan (DNA* LASERGENE) and aligned with Clustal W (Thompson *et al.* 1994) in MegAlign (DNA* LASERGENE). Alignments were examined visually in MACCLADE v4.0 (Maddison and Maddison 2000) and COII sequences were translated to ensure stop codons were not present. 16S and COII alignments are available in TreeBASE (accession number M3740). Repetitive sequences were collapsed into representative haplotypes in COLLAPSE v1.2 (http://darwin.uvigo.es/). Preliminary analyses of 16S and COII indicated they were congruent and were subsequently combined for select analyses.

Parsimony networks were constructed using mtDNA haplotypes in TCS v1.18 (Clement *et al.* 2000), with a 95% connection limit between haplotypes. Gaps were treated as missing data. To determine the number of genetic populations present across

the sampled range of *A. agassizii*, pairwise Φ_{ST} were computed between all collection stations in ARLEQUIN v3.1 (Excoffier *et al.* 2005). Accordingly, collection stations where pairwise comparisons were not significantly different from zero were pooled in subsequent analyses. ARLEQUIN was used to perform an analysis of molecular variance (AMOVA) on mtDNA sequences to assess how haplotypic variation is partitioned geographically. For the AMOVA, variance was partitioned into three hierarchical components: within collection stations (Φ_{ST}), among collection stations within a clade (Φ_{SC}), and among clades (Φ_{CT}), where clades were determined by phylogenetic analysis (see below). For both pairwise Φ_{ST} and AMOVA, a 16S+COII concatenated dataset was used with 10,000 permutations and the Tamura-Nei model (Tamura and Nei 1993) with among site rate variation.

Nucleotide (π) and haplotype (h) diversities were calculated in DNASP v4.1 (Rozas *et al.* 2003). Isolation by distance among collection stations was tested using a Mantel test (Mantel 1967) in ARLEQUIN with 1000 permutations. For the Mantel test, 16S+COII pairwise Φ_{ST} values were used for genetic distances, and the linear distance between collection stations was $\log_{10}$ transformed and used for geographic distances.

Tajima's *D* (Tajima 1989) test statistic was calculated in DNASP to evaluate the assumption of selective neutrality of mtDNA sequences. Mismatch analyses were done in DNASP by comparing the observed versus expected distribution of pairwise nucleotide differences between 16S and COII haplotypes, to determine if population expansion had occurred in the history of *A. agassizii*. Population expansion was also evaluated by the neutrality test, because values significantly different from zero can be indicative not only of selection, but also of demographic patterns such as past population expansion (Aris-

Brosou and Excoffier 1996). With the exception of the haplotype network analysis, population-level analyses were performed only with collection stations where four or more individuals were sampled.

In order to evaluate levels of migration between pairs of populations, an MCMC approach was taken as implemented in the program MDIV (Nielsen and Wakely 2001; http://cbsuapps.tc.cornell.edu/mdiv.aspx). Initial runs were done with all population pairs (where $N \geq 6$) to obtain an upper limit of the scaled migration rate (M_{max}) for subsequent runs. Three independent runs with different random number seeds were completed for each comparison and results averaged. For these analyses, the finite-sites model (HKY) was used, with Markov chain length = 5×10^6, 10% burn-in and M_{max} = 10, 25, 50 or 100. The migration rate per generation was determined by the *M* value with highest posterior probability.

3.3.3 Phylogenetic and genetic distance analyses

Phylogenetic relationships among mtDNA haplotypes were estimated using Bayesian methods in MRBAYES v3.1 (Huelsenbeck and Ronquist 2001). For Bayesian analysis, 16S and COII were treated as unlinked partitions and MRMODELTEST v2.2 (Nylander 2004) was used to determine the best-fit model for each partition under the Akaike information criterion (AIC). For 16S, the HKY+I+Γ model was selected, while for COII the GTR+Γ model was chosen. Conditions for analysis were uniform prior distribution of parameters and two sets of four simultaneous chains run for 1×10^6 generations with trees sampled every 100 generations. Stationarity was evaluated by examining log-likelihood values per generation. Burn-in trees were discarded before

computing a 50% majority-rule consensus tree with nodal support given by the posterior probability of each recovered clade. Resulting topologies were rooted with the outgroup species *Astrohamma tuberculatum*.

Genetic distances were calculated using 16S+COII combined data in PAUP* v4.0 (Swofford 2002) in order to evaluate levels of divergence within *A. agassizii*. MODELTEST v3.7 (Posada and Crandall 1998) was used to determine the best-fit model of sequence evolution under the AIC for the corrected genetic distances. The transversional (TVM) model with gamma shape parameter ($\alpha = 0.778$) and proportion of invariable sites (0.7696) was selected.

3.4 RESULTS

In total, 118 individuals were sequenced for 16S (490 bp) and COII (493 bp), resulting in a 983 bp concatenated dataset. The combined dataset included 64 mitochondrial haplotypes, representing 25 individuals from Antarctica and 93 from South America. No insertions, deletions or stop codons were observed among the 118 individuals for COII. For 16S, the inclusion of a few gaps were required for alignment.

3.4.1 Population structure

Parsimony network analysis resulted in three haplotype networks whether mtDNA gene fragments were analyzed separately (data not shown) or combined (Fig. 2). For the combined data, network 1 (= clade 1) included 38 haplotypes from 58 individuals from South America, network 2 (= clade 2) was comprised of 14 haplotypes

from 35 individuals from South America, and network 3 (= clade 3) included 12 haplotypes from 25 individuals from Antarctica.

Pairwise Φ_{ST} (Table 2) indicated that some collection stations within South America were genetically indistinct from one another, as were all collection stations within Antarctica. South American stations 1, 3 and 8 from clade 1 were pooled and stations 4 and 14 from clade 2 were pooled. In Antarctica, all collection stations (where N > 4) were pooled (stations 47, 82 and 85). Within clade 1, Φ_{ST} values were significant for every pairwise comparison that included station 5, suggesting that this station is genetically isolated from other clade 1 stations. As expected, between-clade Φ_{ST} values approached 1.0 due to the three clades being fixed for alternate mtDNA haplotypes. AMOVA results (Table 3) further confirmed genetic isolation between clades as the greatest proportion of variance (84%, $P = 0.0001$) was attributable to that between clades. The second largest variance proportion (11%, $P < 0.0001$) was that within collection stations while only 5% ($P < 0.0001$) was attributable to that between collection stations within a clade.

Corroborating parsimony network results, nucleotide (π) and haplotype (h) diversity values indicated that clade 1 was more genetically diverse than clades 2 or 3 (Table 4; 16S and COII analyzed separately). These latter clades exhibited much lower levels of nucleotide and haplotype diversities, with the exception of COII haplotype diversity, which was slightly higher in clades 2 and 3 compared to clade 1. Results of the Mantel test, performed only on collection stations within clades 1 and 3 given that clade 2 contained only two geographic localities, did not support isolation by distance for stations within clade 1 ($r = –0.03$; $P = 0.49$) or clade 3 ($r = 0.02$; $P = 0.51$).

Tajima's *D* was negative but non-significant for 16S, whereas three populations (St. 5; St. 9; Sts. 4, 14) had significantly negative values for COII (Fig. 3), indicative of past population expansion (Aris-Brosou and Excoffier 1996). The shape of the 16S and COII mismatch distributions were unimodal for clade 2 and 3 (Fig. 3), suggesting past population expansion. Clade 1 distributions were primarily ragged and multimodal (Fig. 3), suggesting stable population size (Harpending *et al*. 1998). However, stations 5 and 9 COII mismatch distributions were characterized by a high frequency peak corresponding to low pairwise differences and a secondary low frequency peak corresponding to higher number of pairwise differences, potentially explaining the significantly negative Tajima's *D* value for these two stations.

Migration analyses revealed stations 47 and 82 (clade 3) from Antarctica to be experiencing the highest levels of gene flow. These two collection stations are the most geographically distant populations sampled in Antarctica (with sufficient numbers to perform analyses), therefore it is presumed that geographically intermediate stations are experiencing equivalent if not higher levels of gene flow. For stations 47 and 82, the posterior probability distribution plateaued beyond $M = 30$ migrants per generation, with its highest value attained at $M = 66$. Although lower than clade 3, relatively high levels of gene flow were also estimated for clade 2. The best estimate of the number of migrants per generation between stations 4 and 14 was $M = 9.5$. For clade 1, migration rates between station 5 and both stations 1 and 3 (the furthest and closest stations to station 5, respectively) suggested little to no gene flow, as highest posterior probability values corresponded with less than 1 migrant per generation ($M = 0.30, 0.32$). Lack of gene flow is similarly reflected in significant pairwise Φ_{ST} values for station 5. Between other clade

1 populations, migration rates were also low, albeit slightly higher than station 5 comparisons. Migration estimates between stations 1, 3 and 9 were around 1 migrant per generation (0.62–1.7).

3.4.2 Phylogenetic relationships and genetic distances

Phylogenetic analysis of mtDNA haplotypes revealed three distinct lineages within *Astrotoma agassizii*, two in South America and one in Antarctica (Fig. 2). These three clades correspond to the three networks recovered in the parsimony network analysis. The two South American clades (clades 1 and 2) were recovered as sister clades with a posterior probability of 0.94, while the Antarctic clade (clade 3) was supported as sister to the South American clades with a posterior probability of 0.99. Intraclade genetic distances were low, averaging from 0.34% (clade 3) to 1.13% (clade 1), while interclade distances were substantially higher, 4.8% between clades 1 and 2, 5.1% between clades 2 and 3, and 6.8% between clades 1 and 3.

3.5 DISCUSSION

3.5.1 Cryptic species in *Astrotoma agassizii*

Astrotoma agassizii is not a genetically contiguous, panmictic species, but rather characterized by substantial levels of cryptic diversity. Parsimony network based approaches to recognizing species boundaries have been advocated in recent years (Lee and ÓFoighil 2004; Tarjuelo *et al*. 2004; Uthicke *et al*. 2004; Addison and Hart 2005; Baratti *et al*. 2005; Jolly *et al*. 2005; Hart *et al.* 2006), where multiple haplotype networks have been interpreted as multiple species. According to these criteria, *A*. *agassizii* as

currently defined constitutes at least three putative species. Three networks were recovered at the 95% connection limit, and each network can tentatively be inferred as corresponding to a separate species. Phylogenetic analysis, pairwise Φ_{ST} and AMOVA provide additional support for the existence of three distinct lineages. Further, mtDNA genetic distances between the three clades ranged from 4.8%–6.8%, and distances of this magnitude (5%–7%) are typically found between echinoderm species easily distinguished by phenotypic or behavioral differences (Foltz 1997; Hart *et al*. 1997; Lessios *et al*. 2001; O'Loughlin *et al*. 2003; Uthicke and Benzie 2003; Waters and Roy 2003; Uthicke *et al*. 2004; Waters *et al*. 2004; Hart and Podolsky 2005).

Cryptic speciation has been documented extensively in the marine environment and reported for the Southern Ocean as well (Beaumont and Wei 1991; Held 2003; Held and Wägele 2005; Raupach and Wägele 2006; Wilson *et al*. 2007). Held (2003) discussed several criteria for establishing cryptic species. First, species in question should have a bimodal distribution of pairwise genetic distances with no intermediate values. Second, genetic divergence must be found at a level known to exist between sister species that are closely related to the species in question. Third, this level of divergence should be found among sympatric populations, although this last criterion was suggested to be less crucial. *Astrotoma agassizii* fulfills criterion one and likely criterion two. Although no studies exist examining sister species closely related to *A. agassizii*, genetic divergences among other echinoderms are consistent with the hypothesis of separate species. While genetically divergent sympatric populations were not sampled for this study, degree of spatial continuity between collection sites is unknown and divergent populations were found at sites separated by less than 80 km. Furthermore, examination of diagnostic

morphological characters did not reveal fixed differences between clades. Namely, genital slit length, arm spine number, shape of teeth, and disc and arm granulation were uniform among voucher specimens from each collection station. Taken together, these data provide compelling evidence that *Astrotoma agassizii* is a complex of at least three cryptic species in the Southern Ocean.

3.5.2 Connectivity between Antarctica and South America

Data from this study indicate that *A. agassizii* in Antarctica is genetically distinct and geographically isolated from *A. agassizii* in South America. Gene flow is not occurring between Antarctic and South American populations. The Drake Passage separating Antarctica and South America spans approximately 900 km and reaches ~4500 m depths in some places (Whitworth *et al*. 1982), potentially beyond the range of dispersal or migration for *A. agassizii*. However, genetic homogeneity was found across distances greater than 500 km within the Antarctic continental shelf and across distances greater than 300 km within the South American continental shelf. Therefore, it is likely that the Polar Front and/or deep-water passages, but not sheer geographic distance, act as barriers to gene flow between Antarctica and South America. A similar pattern has been found in Antarctic demersal fish where genetic homogeneity is maintained within continental shelves but breaks down in populations separated by distances greater than 1000 km or deep-water troughs (Shaw *et al*. 2004). Restricted gene flow between shelf areas separated by great depths (1000–1750 m) has also been shown for the Antarctic octopus *Pareledone turqueti* (Allcock 1997).

Studies investigating the role of the Polar Front in structuring Southern Ocean populations have shown it to be a barrier even for organisms with high dispersal potential, such as krill (Patarnello *et al*. 1996). However, other studies have shown the Polar Front to be penetrable. For example, bivalve sister species were inferred to have diverged after formation of the Polar Front, indicating that at one time individuals were able to disperse across this water mass (Page and Linse 2002). Using an approximate echinoderm mtDNA divergence rate of 3.1%–3.5%/my (Lessios *et al*. 1999; McCartney *et al*. 2000), separation of Antarctic and South American populations can be dated roughly at 1.4–1.6 mya, well after Polar Front formation 24–41 mya. These dates suggest that Antarctic and South American populations split well after the environmental factors that isolate Antarctica were established. In the case of *A. agassizii*, the Polar Front may have prevented high levels of gene flow, but historically was not an absolute barrier. Even though these dates are crude estimates, the conclusion that dispersal occurred across the Polar Front at least once is a robust conclusion even if we assume a vastly slower echinoderm mtDNA molecular clock.

Restricted gene flow was also evident within the South American continental shelf. The two South American clades had lower interclade genetic distances when compared to the Antarctic clade. However, genetic distances were similar for clade 1–2 and clade 2–3 comparisons, indicating that the South American clades split soon after diverging from the common ancestor of all three clades. Comparisons of physical characteristics between collection stations belonging to the two South American clades revealed no obvious differences in depth distribution or faunal assemblages. Interestingly, samples from station 5 (clade 1), which were significantly differentiated from all other

stations, were collected from substantially deeper depths (850 m) than any other South American samples. Conversely, station 47 in Antarctica was 900 m and showed no significant differentiation with any other Antarctic collection locality. Bathymetry may be an isolating force for South American populations of *A. agassizii* but not Antarctic populations; not surprising given Antarctic fauna are typically eurybathic (Hempel 1985).

3.5.3 Intraclade population structure

The Mantel test showed no evidence for increasing genetic differentiation with geographic distance. Within Antarctica, high levels of gene flow resulting in mtDNA homogeneity were demonstrated across a 518 km range throughout the Antarctic Peninsula, an unexpected result given the brooding nature of this species. Genetic homogeneity spanning large geographic distances has been reported for brooding marine invertebrates (Sponer and Roy 2002; Richards *et al*. 2007). Given *A. agassizii* lacks a pelagic larval stage, this species must rely on dispersal of benthic adults or juveniles to maintain population connectivity. Passive transport of rafting adults has been suggested as a means of dispersal for brittle stars (Sponer and Roy 2002). *Astrotoma agassizii* is known to tightly wrap its coiled arms around gorgonians and hydrocorals (Bartsch 1982) and has been recorded climbing up rocks, sponges, bryozoans and other sessile organisms projecting off the seafloor (Dearborn *et al.* 1986; Ferrari and Dearborn 1989). The epifaunal propensity of this species could provide numerous opportunities for passive transport of dislodged organisms. Furthermore, *A. agassizii* has been reported to occur in dense aggregations clinging to octocorals (Dearborn *et al.* 1986; Ferrari and Dearborn 1989), increasing the likelihood that individuals could be dislodged and carried by ocean

currents to other geographic localities. Juveniles and small adults probably have a greater chance for passive dispersal due to the large size attained by full-grown adults (≤ 60 mm disc diameter; Mortensen 1936).

Migration of adults/juveniles along the Antarctic continental shelf could also maintain connectivity across these distances. Benthic migration seems less plausible, however, because *A. agassizii* is irregularly distributed throughout Antarctica, occurring primarily in locally abundant patches (Dearborn *et al*. 1986). Therefore, connectivity via movement of adults/juveniles along the continental shelf is less likely and dispersal probably occurs by occasional uprooting of small adults or juveniles attached to sessile substrate and passively dispersed by ocean currents. Passive transport between *A. agassizii* patches throughout the Antarctic Peninsula could occur from Bransfield Strait (Fig. 1) to the southern peninsula, and vice versa. A surface current circulates counterclockwise around the Antarctic coast (Phillpot 1985) and could promote dispersal from Bransfield Strait southward. Conversely, water from the Bellingshausen Sea in the southern peninsula flows north into the Bransfield Strait (Wilson *et al*. 1999) and could allow for northern transport.

In South America, significant genetic differentiation was absent within clade 1 across distances as great as 320 km, despite low levels of ongoing gene flow. Conversely, high migration was shown for clade 2, however, clade 2 populations are separated by only 131 km. The fact that clade 1 and 2 individuals are separated by 72–484 km suggests that dispersal ability may not be the primary factor driving isolation between these clades. Instead, present-day population structure in South America may be explained by allopatric divergence of clade 1 and 2. For example, station 14 of clade 2,

situated at the easternmost margin of the sampled range of *A. agassizii*, or a similar unsampled population, could have at one time been sufficiently peripheral for allopatric divergence to occur, resulting in two South American clades. Subsequently, this once isolated population could have undergone population expansion resulting in a wider distribution. Past population expansion is supported for clade 2 based on the negative Tajima's *D* value, unimodal mismatch distribution and shape of the haplotype network. The demographic history of clade 1, the more widely sampled and genetically diverse clade, was characterized by stable population size for some populations whereas others showed signatures of past population expansion.

Interestingly, within South America there were two instances of divergent haplotypes co-occurring at the same collection station. Station 5 was comprised exclusively of clade 1 haplotypes with the exception of a single individual possessing a clade 2 haplotype. This occurrence could be the result of a rare migration event between stations 5 and 14 (where the majority of that clade 2 haplotype were sampled), or additional sampling could reveal clade 1 and 2 to be existing sympatrically at this locale. Similarly, station 7b, where only two individuals were sampled, was characterized by a single clade 1 haplotype and a single clade 2 haplotype.

In summary, *Astrotoma agassizii* is characterized by unexpected levels of genetic diversity and represents a complex of cryptic species. Populations of *A. agassizii* separated by the Polar Front are genetically isolated and belong to separate lineages. Paradoxically, this "species" was also found to have unexpected levels of genetic continuity for a brooding invertebrate over large geographic distances within continental shelf regions. Similar levels of genetic diversity and divergence likely exist within many

other Southern Ocean benthic invertebrates. Additional work is needed to further document biodiversity in this isolated biogeographic region in order to more fully understand the dynamic physical processes and extreme environmental conditions driving this diversity.

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Table 1 Collection information for *Astrotoma agassizii* in South America and Antarctica. Collection station numbers correspond with Figure 1 and N refers to number of individuals sequenced for 16S and COII.

Geographic region	Collection station	N	Latitude	Longitude	Depth
South America	1	12	S 53°16'	W 66°23'	96 m
	3	6	S 53°47'	W 61°48'	403 m
	4	15	S 53°47'	W 60°42'	170 m
	5	15	S 53°47'	W 59°33'	854 m
	6	2	S 54°49'	W 60°16'	110 m
	7a	1	S 54°27'	W 63°53'	108 m
	7b	2	S 54°21'	W 60°60'	125 m
	8	4	S 54°23'	W 61°53'	274 m
	9	16	S 54°28'	W 62°12'	321 m
	14	18	S 54°41'	W 59°24'	207 m
	18	2	S 54°41'	W 63°14'	254 m
Antarctica	31	1	S 66°37'	W 68°19'	261 m
	47	8	S 62°51'	W 59°27'	900 m
	78	1	S 65°37'	W 67°47'	217 m
	82	11	S 65°40'	W 68°02'	278 m
	85	4	S 64°41'	W 65°56'	368 m

Table 2 Pairwise Φ_{ST} for each *Astrotoma agassizii* collection station in South America and Antarctica.

Geographic region	Clade	Collection station	St. 1	St. 3	St. 5	St. 8	St. 9	St. 4	St. 14	St. 47	St. 82	St. 85
South America	1	St. 1	–									
	1	St. 3	0.142	–								
	1	St. 5	**0.347**	**0.497**	–							
	1	St. 8	-0.043	0.212	**0.326**	–						
	1	St. 9	**0.264**	0.029	**0.561**	**0.303**	–					
	2	St. 4	**0.851**	**0.908**	**0.827**	**0.873**	**0.905**	–				
	2	St. 14	**0.873**	**0.930**	**0.851**	**0.902**	**0.920**	-0.010	–			
Antarctica	3	St. 47	**0.869**	**0.936**	**0.870**	**0.891**	**0.927**	**0.926**	**0.944**	–		
	3	St. 82	**0.882**	**0.943**	**0.882**	**0.909**	**0.932**	**0.930**	**0.946**	-0.038	–	
	3	St. 85	**0.849**	**0.933**	**0.855**	**0.861**	**0.923**	**0.926**	**0.947**	-0.153	-0.073	–

Bold indicates $P < 0.05$

Table 3 Hierarchical analysis of molecular variance (AMOVA) for South American and Antarctic populations of *Astrotoma agassizii*.

Source of variation	% variation	Φ statistic	*P* value
Among clades	83.65	Φ_{CT}=0.84	0.0001
Among collection stations within a clade	5.36	Φ_{SC}=0.33	*
Within collection stations	10.99	Φ_{ST}=0.89	*

*$P < 0.0001$

Table 4 Genetic diversity statistics for pooled *Astrotoma agassizii* collection stations, N refers to number of individuals, H is the number of haplotypes, π refers to nucleotide diversity and *h* is haplotype diversity.

Geographic region	Clade	Collection station	N	H		π		*h*	
				16S	COII	16S	COII	16S	COII
South America	1	Sts. 1, 3, 8	22	13	6	0.0054 ± 0.0006	0.0113 ± 0.0024	0.93 ± 0.04	0.59 ± 0.12
		St. 5	14	7	8	0.0033 ± 0.0008	0.0044 ± 0.0018	0.82 ± 0.08	0.77 ± 0.12
		St. 9	16	6	8	0.0030 ± 0.0008	0.0050 ± 0.0023	0.77 ± 0.08	0.70 ± 0.13
	2	Sts. 4, 14	33	3	12	0.0003 ± 0.0002	0.0037 ± 0.0006	0.12 ± 0.08	0.82 ± 0.05
Antarctica	3	Sts. 47, 82, 85	23	5	10	0.0009 ± 0.0004	0.0037 ± 0.0005	0.32 ± 0.12	0.88 ± 0.04

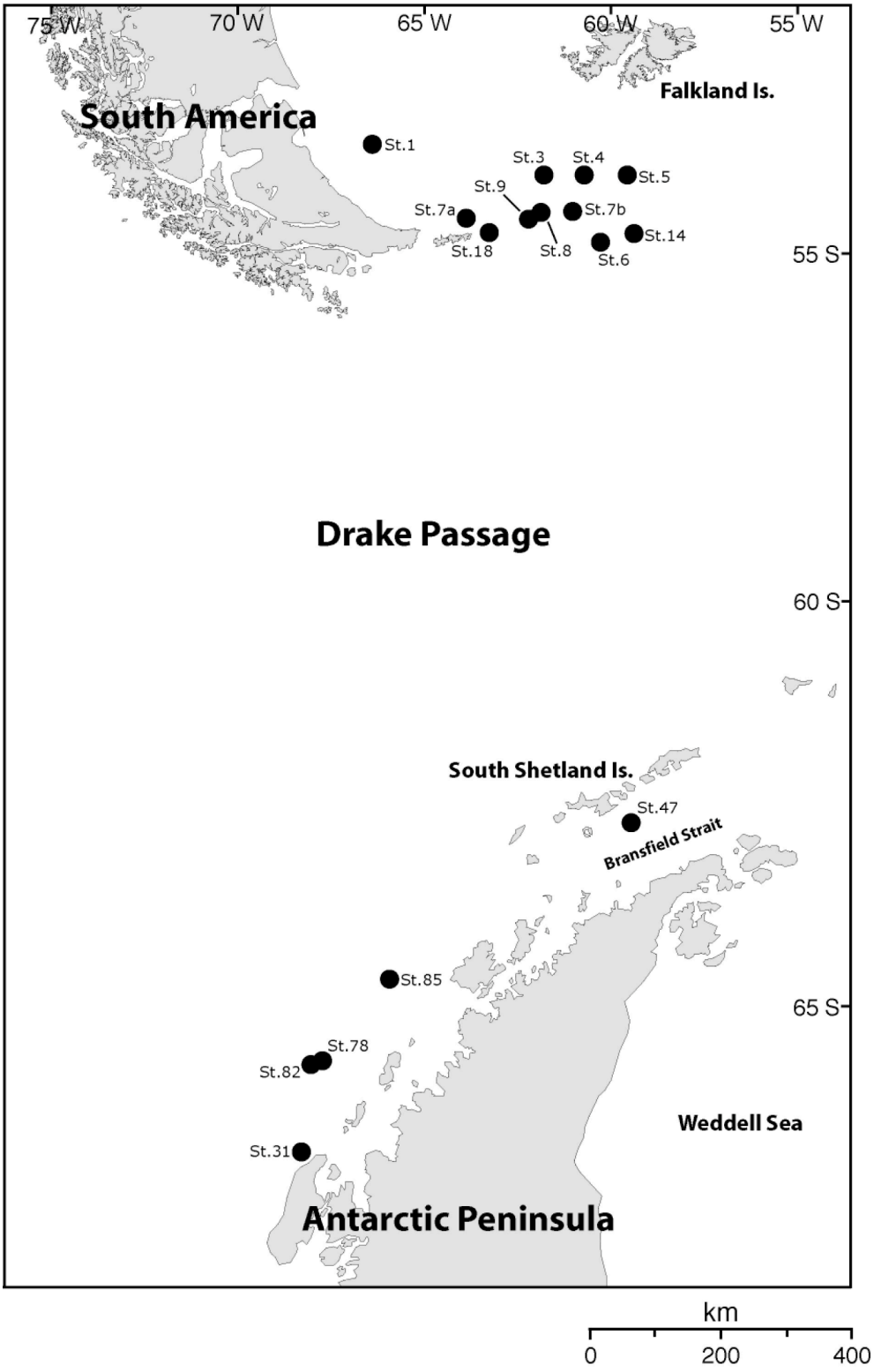

Figure 1 Map showing collection localities for *Astrotoma agassizii* from South America and Antarctica.

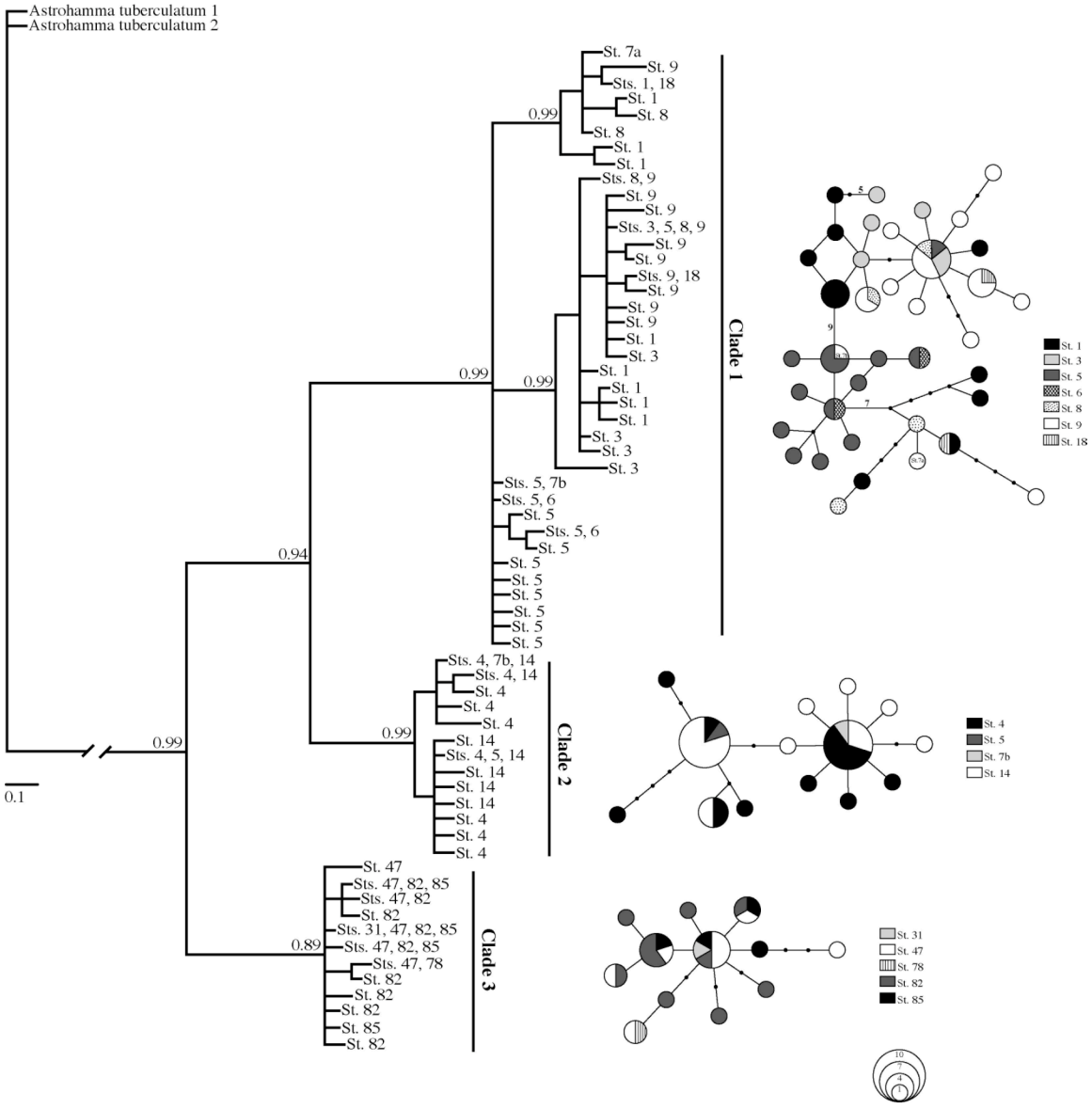

Figure 2 Bayesian tree of unique 16S+COII mtDNA haplotypes, with corresponding haplotype networks for each of three phylogenetic clades. Numbers next to nodes indicate Bayesian posterior probabilities. On the Bayesian tree, haplotypes are labeled according to station. In networks, circles are coded by station and a unique key is given for each clade. Coding does not overlap between clades. Haplotypes are sized according to relative abundance and missing haplotypes are denoted by small, closed black circles.

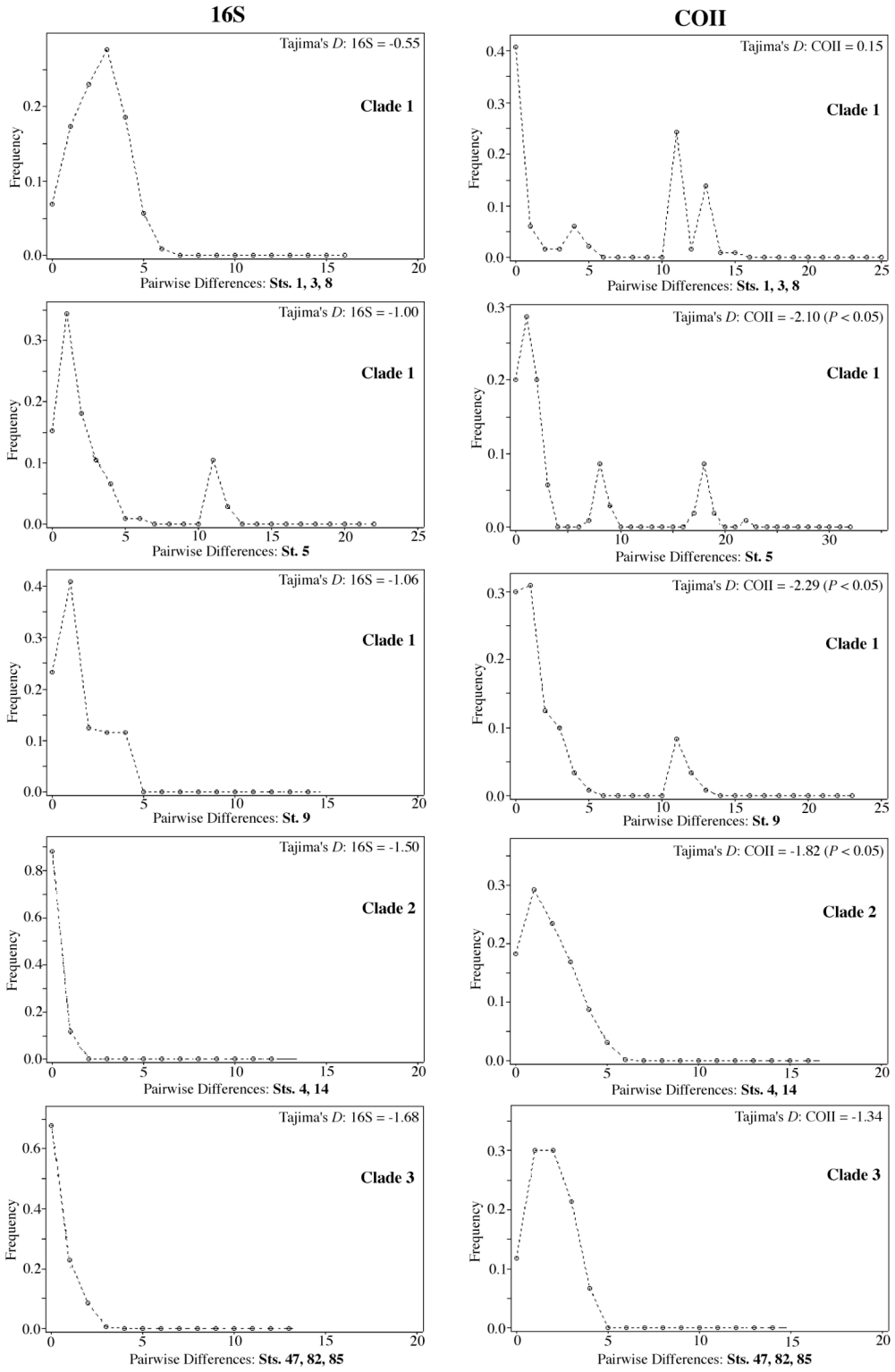

Figure 3 Mismatch distributions and Tajima's *D* statistic for pooled *Astrotoma agassizii* collection stations. Significant Tajima's *D* values are indicated by ($P < 0.05$).

CHAPTER 4: Phylogeography of the Antarctic planktotrophic brittle star *Ophionotus victoriae* reveals genetic structure inconsistent with early life history

4.1 ABSTRACT

In the marine environment, connectivity is influenced by physical oceanography as well as life history and behavioral traits, which in combination with historical geologic/climatic events, determine population genetic structure. The Antarctic brittle star *Ophionotus victoriae* develops via a feeding planktonic larval stage, and therefore has potential for long-distance dispersal throughout its Antarctic/subantarctic range. To evaluate this hypothesis, the phylogeography of this ecologically dominant species was elucidated by sequence analyses of two mtDNA genes from individuals collected throughout the Antarctic Peninsula and from two subantarctic islands. Counter to expectations of genetic homogeneity, mtDNA data revealed substantial levels of genetic differentiation as well as diversity. While there were some genetically homogeneous populations, such as those throughout Bransfield Strait, we found *O. victoriae* to have significant population genetic structure throughout much of the Antarctic Peninsula, with evidence of potential cryptic speciation between the western and eastern Antarctic Peninsula. Furthermore, Antarctic Peninsula populations were genetically distinct from subantarctic island populations. The low levels of connectivity implied for *O. victoriae*

contrast with those found for many other Antarctic benthic taxa, suggesting a complex interplay between oceanography, recent climate history and larval ecology.

4.2 INTRODUCTION

The genetic composition of a population is influenced by biological properties as well as physical environmental factors and historical processes. Biological properties include dispersal ability, which is expected to correlate with species range size (Jablonski and Lutz 1983; Jablonski 1986; Jeffery and Emlet 2003) and often predicts genetic structure (Burton 1982; Palumbi 1994; Ward et al. 2004). For benthic marine organisms, adults typically have low mobility and dispersal occurs predominantly during a planktonic larval stage (Kinlan and Gaines 2003; Gerber and Heppell 2004; Paulay and Meyer 2006). Theoretically, species with longer planktonic larval duration should disperse greater distances than brooders or those with a brief larval period (Scheltema 1986; Kinlan and Gaines 2003; Shanks et al. 2003), although some marine organisms are known to raft long distances as larvae and/or adults (Highsmith 1985; Helmuth et al. 1994; O'Foighil et al. 1999). Accordingly, many studies have shown that benthic organisms with long-lived planktonic larvae have less population differentiation than those with abbreviated larval development (Berger 1973; Crisp 1978; Janson 1987; McMillan et al. 1992; Duffy 1993; Hunt 1993; Hellberg 1996; Hoskin 1997; Arndt and Smith 1998). However, mounting evidence suggests the relationship between early life history and dispersal is anything but straightforward and predictable. Recent studies have shown that in many cases, marine species with planktonic larvae display genetic patterns

consistent with low levels of connectivity and geographic subdivision (Hare and Avise 1996; Palumbi 1996; Taylor and Hellberg 2003; Sotka et al. 2004; Galarza et al. 2009).

Physical barriers in the marine environment can limit or prevent connectivity in species with otherwise high dispersal potential, resulting in decreased gene flow and increased population differentiation. Examples of such barriers include oceanographic processes such as fronts, eddies, prevailing currents (Hedgecock 1986; Scheltema 1986; Cowen et al. 1993; Hare and Cowen 1996; Gaylord and Gaines 2000) and varying temperature/salinity composition of adjacent water masses (Hutchins 1947; Valentine 1966; Gaines et al. 2007), as well as land barriers (Lessios 1981; Knowlton 1993). Historically, Pleistocene glacial cycles subjected benthic fauna to fluctuating sea-levels and cyclic expansions/contractions of large ice sheets in both the Northern and Southern hemispheres (Hewitt 2000), with a cumulative effect of increasing divergence and speciation rates while reducing genetic diversity in a number of marine and terrestrial species (Hewitt 2000).

Antarctica and the surrounding Southern Ocean provide an unparalleled system in which to study biological and physical factors affecting marine connectivity. Antarctica has been geographically and thermally isolated for 28-41 million years (Lawver and Gahagan 2003; Pfuhl and McCave 2005; Scher and Martin 2006), resulting in high endemism (Knox and Lowry 1977; Brandt 1991; Jazdzewski et al. 1991) and stenothermy, with many species unable to survive even slight increases in water temperature (Peck and Conway 2000; Peck et al. 2004; Peck et al. 2009). Dispersal ability of many Antarctic benthic invertebrates is enhanced by the slow development of their larvae in the cold Southern Ocean, allowing longer persistence times in the water

column (Bosch et al. 1987; Stanwell-Smith and Peck 1998). For example, Antarctic echinoderm planktotrophic (i.e., feeding) larvae are estimated to spend 5-6 months in the water column (Pearse and Bosch 1986; Bosch et al. 1987), and lecithotrophs (i.e., non-feeding) may persist for up to 2-3 months (Bosch and Pearse 1990).

Circumpolar currents in the Southern Ocean have long been considered to influence the distribution of Antarctic marine organisms (Fell 1962; Dell 1972; Arntz et al. 1994; Waters 2008). These include the Antarctic Circumpolar Current (ACC), a powerful, easterly-flowing current, and the Eastwind Drift, a weaker countercurrent that circulates around the Antarctic coast (Phillpot 1985; Stein and Heywood 1994). The ACC and Eastwind Drift are presumed to have a homogenizing effect on populations by transporting larvae and/or adults around the Antarctic continent (Fell 1962; Dell 1972; Arntz et al. 1994; Waters 2008). Large cyclonic gyres in the Weddell and Ross Seas are also thought to play a role in determining levels of connectivity among Antarctic marine organisms (Patarnello et al. 1996). For example, the Weddell gyre has been implicated in restricting gene flow between populations occurring on either side of the gyre (Bargelloni et al. 2000). Additionally, the Antarctic benthos have been heavily impacted by the expanding/contracting polar ice sheet throughout the Pleistocene, and the continental slope may have acted as a refugium for shelf fauna during glacial maxima when most of the continental shelf was covered by glaciers (Clarke and Crame 1989, 1992; Thatje et al. 2005; Clarke 2008).

Studies investigating the evolutionary history of Antarctic marine organisms have, in many cases, recovered greater genetic divergence among populations than would be expected based on dispersal potential (e.g., Bargelloni et al. 2000; Wilson et al. 2007;

Thornhill et al. 2008). Brittle stars (Ophiuroidea) are abundant throughout Antarctica, yet have received little attention in terms of their evolutionary history. *Ophionotus victoriae* is a widely distributed and abundant ophiuroid throughout the Antarctic/subantarctic region, typically being the dominant brittle star in Antarctic benthic assemblages (Dahm 1996). This species has a circumpolar Antarctic/subantarctic distribution and is found inhabiting a variety of substrates ranging from mud to rocky bottoms at depths of 5-1300 m (Fell 1961; Fratt and Dearborn 1984). *Ophionotus victoriae* produces a typical ophiopluteus planktotrophic larvae, and spawns annually during November-December, coincident with the austral summer phytoplankton bloom (Grange et al. 2004). Given the ecological and biogeographic importance of this species, combined with its potential for long-distance dispersal, *O. victoriae* is an ideal candidate for evaluating the effects of life history, oceanography and historical processes on population genetic structure in Antarctic invertebrates. We sequenced and analyzed two mitochondrial genes (16S rDNA and COI) to determine whether this species conforms to the hypothesized pattern of genetic homogeneity across populations, or instead shows evidence of limited connectivity owing to contemporary and/or historical forces.

4.3 MATERIALS AND METHODS

4.3.1 Data collection

Ophionotus victoriae individuals from the Antarctic Peninsula were collected during two cruises aboard the *R/V Laurence M. Gould* during November-December 2004 and May-June 2006. Benthic samples were collected using an epibenthic sled, Blake trawl, or rock dredge. Samples from the subantarctic South Sandwich and Bouvet Islands

were collected during the 2004 ICEFISH expedition aboard the *R/V Nathaniel B. Palmer* using Blake or otter trawls. Samples for DNA analysis were either frozen upon collection at -80ºC or preserved in ~85% ethanol. Detailed sampling information is provided in Table 1 and Figure 1.

DNA was extracted using the DNeasy® Tissue Kit (QIAGEN) following manufacturer's protocol. Two mitochondrial gene fragments, 16S rDNA (16S) and cytochrome *c* oxidase subunit I (COI), were amplified using standard PCR protocols. Primers 16SarL (5′-CGCCTGTTTATCAAAAACAT-3′) and 16SbrH (5′-CCGGTCTGAACTCAGATCACGT-3′) (Palumbi et al. 1991) amplify a ~500 bp fragment from the middle of the 16S gene. For COI, the primer set LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994) was used to amplify a ~560 bp fragment from the 5′ end of the COI gene. Double-stranded PCR products were purified using the QIAquick® Gel Extraction Kit (QIAGEN) following manufacturer's protocol. Purified PCR products were bi-directionally sequenced using a CEQ8000 Genetic Analysis System (Beckman Coulter). All *O. victoriae* haplotypes were deposited in GenBank under accession numbers FJ917290-FJ917354.

Sequences were edited in SeqMan (DNA* LASERGENE) and aligned with Clustal W (Thompson et al. 1994) in MegAlign (DNA* LASERGENE). Alignments were examined by eye in MacClade v4.0 (Maddison and Maddison 2000) and COI sequences were translated to ensure stop codons were not present. No gaps were required for the 16S alignment. 16S and COI alignments are available in TreeBASE (www.treebase.org; accession nos. XXXX). Given that mitochondrial genes represent a

single, non-recombining locus and as such have a single evolutionary history (Avise 2004), 16S and COI gene fragments were concatenated for data analysis.

4.3.2 Population structure analyses

Parsimony networks were constructed using mtDNA haplotypes in TCS v1.18 (Clement et al. 2000) with a 95% connection limit between haplotypes. To assess levels of genetic differentiation between sampling stations, pairwise Φ_{ST}'s were computed in Arlequin v3.1 (Excoffier et al. 2005). Arlequin was used to perform an analysis of molecular variance (AMOVA) on mtDNA sequences to assess how haplotypic variation was geographically partitioned. For the AMOVA, variance was partitioned into three hierarchical components: within sampling stations (Φ_{ST}), among sampling stations within a geographic region (Φ_{SC}), and among geographic regions (Φ_{CT}). For these analyses, geographic regions were Northern Peninsula, Southern Peninsula and Subantarctic Islands. These regions were determined *a priori* based on oceanographic discontinuities between the Northern and Southern Peninsula and because of the >1800 km separating South Sandwich and Bouvet Islands from the Antarctic Peninsula. For both the pairwise Φ_{ST} and AMOVA analyses, the 16S+COI dataset was used with 10,000 permutations, and the Tamura-Nei model (Tamura and Nei 1993) with among site rate variation was chosen because it most closely approximated the model of sequence evolution selected by Modeltest v3.7 (Posada and Crandall 1998).

4.3.3 Historical demography and migration analyses

Nucleotide (π) and haplotype (h) diversities were calculated in DnaSP v4.1 (Rozas et al. 2003) to quantify levels of genetic diversity within *O. victoriae*. Tajima's D (Tajima 1989) test statistic was calculated in DnaSP to evaluate the assumptions of selective neutrality of mtDNA sequences as well as population equilibrium. Fu's F_S (Fu 1997) neutrality statistic, shown to be particularly sensitive to population demographic expansion as indicated by large, negative F_S values (Fu 1997), was calculated in DnaSP and significance assessed by 10,000 permutations. Genetic distances were measured as uncorrected p values in PAUP* v4.0 (Swofford 2002) to determine levels of divergence between *O. victoriae* haplotypes

In order to estimate levels of migration throughout the Antarctic Peninsula and between the Peninsula and subantarctic islands, an MCMC approach was taken as implemented in the program MDIV (Nielsen and Wakeley 2001; http://cbsuapps.tc.cornell.edu/mdiv.aspx). Three independent runs with different random number seeds, but otherwise identical run conditions, were completed for each comparison and results averaged. For these analyses, the finite-sites model (HKY) was used, with Markov chain length = 5×10^6, 10% burn-in and M_{max} = 10, 30 or 50. The migration rate per generation was determined by the M value with highest posterior probability.

4.4 RESULTS

Mitochondrial 16S (496 bp) and COI (563 bp) data were collected from 134 individuals from 15 sampling stations, resulting in a 1059 bp combined 16S and COI

dataset that yielded 60 unique mtDNA haplotypes. The majority of haplotypes (39, 65%) were singletons. Of the remaining 21 haplotypes, 17 were collected from more than one sampling station.

4.4.1 Population structure

Parsimony network analysis resulted in two networks (Fig. 2) at the 95% connection limit, which allowed a maximum of 14 mutational steps. One network (= Clade 1) consisted of the majority of *O. victoriae* individuals (127 individuals, 58 haplotypes), while the second network (= Clade 2; average 1.8% sequence divergence between Clades 1 and 2) consisted of seven individuals corresponding to two haplotypes. Six of these seven individuals were collected near Eagle Island in the Weddell Sea (St. 40), while one individual was obtained at Station (St.) 21 in the northeast corner of Bransfield Strait (see Fig. 1). Given that individuals collected at St. 40 belonged to two separate clades, this station was divided into 40a (Clade 1 individuals; N = 5) and 40b (Clade 2 individuals; N = 6) for subsequent analyses. Additionally, the single Clade 2 individual collected from St. 21 was not included in analyses involving this station. Clades 1 and 2 could be joined into a single network when the connection limit was lowered to 93%, where Clade 2 haplotypes were separated from Clade 1 haplotypes by 16 mutational steps.

The Clade 1 network revealed substantial genetic diversity and divergence in *O. victoriae*. A majority of individuals possessed unique mtDNA haplotypes, and a divergent group of haplotypes within Clade 1 was identified. Within Clade 1a, hereafter used to refer to the majority of Clade 1 (see Fig. 2), individuals from the subantarctic

islands did not share haplotypes with Antarctic Peninsula individuals, with one exception. Clade 1b, hereafter used to refer to the divergent Clade 1 group, was separated by a minimum of 11 mutational steps from Clade 1a (average 1.5% sequence divergence between Clades 1a and 1b). Clade 1b was comprised almost exclusively of individuals from three sampling stations from the Antarctic Peninsula, Sts. 17, 58 and D8. Clade 1b formed a separate network at the 97% connection limit, which allowed a maximum of 10 mutational steps.

Pairwise Φ_{ST} values (Table 2) indicated that Stations 21, 51, D5 and D6 from the Northern Peninsula are genetically homogeneous. Furthermore, the two southernmost Antarctic Peninsula stations (Sts. 33 and 47) are genetically homogeneous with the above-mentioned Northern Peninsula populations. Individuals collected from Deception Island (St. 64) in the Bransfield Strait are genetically distinct from all other populations except Bransfield Strait St. 51, the most geographically proximate station. Also in the Northern Peninsula, St. 17, situated just outside Bransfield Strait northwest of the South Shetland Islands, had large and significant Φ_{ST} values when compared to other Northern Peninsula stations. However, St. 17 was genetically homogenous with Sts. 58 and D8 in the Southern Peninsula. These three populations largely comprised Clade 1b.

Pairwise Φ_{ST} values between the subantarctic islands and Antarctic Peninsula were significant and often large. However, Φ_{ST} values were lower (and in some cases not significant) between South Sandwich Island stations and Clade 1a stations. Individuals sampled from the South Sandwich Islands showed significant genetic differentiation when compared to those collected from Bouvet Island. Not surprisingly, Φ_{ST} values from comparisons with Clade 2 were high (≥ 0.59) and significant in all cases.

The AMOVA (Table 3) confirmed that the greatest proportion of genetic variance (47%, $P < 0.0001$) was attributable to that among stations within a geographic region. An almost equivalent amount of genetic variation was found within stations (45%, $P < 0.0001$), while only 8% ($P = 0.100$) was attributable to between geographic regions.

4.4.2 Historical demography

Nucleotide diversity indices (Table 1) were similar in magnitude for all populations with the exception of Station 40b from the Weddell Sea, which exhibited lower nucleotide diversity. Similarly, haplotype diversities (Table 1) were typically high, with the exception of Station 40b. Tajima's *D*, while negative for many populations, was not significant in any case (Table 1). Fu's F_S was also negative for the majority of stations, and significant for four populations (Table 1), indicating that recent expansion is not supported for most *O. victoriae* populations. When Clade 1 and Clade 1a were analyzed, significant negative Fu's F_S values resulted, possibly because these clades include genetically distinct populations (Skibinski 2000).

To assess migration between Bransfield Strait and other geographic regions, 17 individuals from three homogenous populations within Bransfield Strait (Sts. 21, 51 and D6) were randomly chosen and pooled to form a composite Bransfield Strait population. Gene flow between Bransfield Strait and St. 33 in the southern Peninsula was high, as the posterior probability distribution plateaued around $M = 10$ migrants per generation (Fig. 3). In contrast, migration between Bransfield Strait and St. 17, situated just 100 km northwest of Bransfield Strait, was low ($M = 0.34$), as was migration between Bransfield Strait and the two Anvers Island populations ($M = 0.3$) (the two homogenous Anvers

Island populations, Sts. 58 and D8, were pooled for migration analyses). Gene flow between the Anvers Island populations and St. 17 was moderate, around three migrants per generation. In order to estimate the number of migrants between the Antarctic Peninsula and the subantarctic islands, migration analyses were performed using the composite Bransfield Strait population and a pooled South Sandwich Islands population, comprised of all individuals sampled from the South Sandwich Islands (Sts. 51 and 57). The two Bouvet Island populations (Sts. 76 and 81) were similarly pooled. Levels of migration between Bransfield Strait and the South Sandwich Islands were very low, less than one migrant per generation ($M = 0.2$), and were comparable to migration rates between Bransfield Strait and Bouvet Island ($M = 0.3$).

4.5 DISCUSSION

In this study, a planktotrophic brittle star, *Ophionotus victoriae*, showed evidence of genetic divergence between some closely situated populations, while other more distant populations were genetically homogeneous. The genetic structure of *O. victoriae* is complex, with no clear concordance between genealogy and geography (Fig. 4). Instead, physical oceanography, bottom topography, recent geologic history and larval ecology appear to have shaped the modern-day population genetic structure of this ecologically dominant Antarctic echinoderm.

4.5.1 Interclade genetic relationships

Mitochondrial data show that *Ophionotus victoriae* has been subject to some degree of contemporary or historical isolation throughout the Antarctic Peninsula.

Evidence from statistical parsimony indicates at least two distinct genetic lineages occur in this region. One lineage, Clade 1, appears widespread throughout the Antarctic Peninsula and occurs around South Sandwich and Bouvet Islands. In contrast, the second lineage, Clade 2, was sampled from only two stations (Sts. 21 and 40). The restricted geographic region from which Clade 2 haplotypes were sampled encompasses the northern and southern margins of the Antarctic Sound (Fig. 1), the body of water flowing between the northeast end of the Antarctic Peninsula and Joinville Island. The Antarctic Sound is approximately 48 km and connects Bransfield Strait on the western side of the Antarctic Peninsula to the Weddell Sea on the eastern side of the Antarctic Peninsula. Clades 1 and 2 have haplotypes distributed on either side of the Antarctic Sound, suggesting recent or ongoing gene flow within clades across this body of water. Gene flow could occur via larval transport in Antarctic Surface Water, which flows from the Weddell Sea into Bransfield Strait through the Antarctic Sound, and is therefore likely unidirectional (Stein and Heywood 1994).

Given the majority of Clade 2 individuals were collected from the Weddell Sea, it is possible that Weddell Sea populations of *O. victoriae* are genetically distinct from western Antarctic Peninsula and subantarctic populations. This pattern has been recovered for other Antarctic taxa, including several notothenioid fish, the Antarctic krill *Euphausia superba*, and the giant Antarctic isopod *Glyptonotus antarcticus*. These taxa similarly show significant genetic differentiation between populations from the western Antarctic Peninsula and/or subantarctic islands compared to the Weddell Sea, despite substantial dispersal potential (Zane et al. 1998; Bargelloni et al. 2000; Patarnello et al. 2003; Held and Wägele 2005). The Weddell Gyre, which separates much of the Weddell

Sea from surrounding waters, and the Weddell-Scotia Confluence, where the ACC and Weddell Gyre interact, have been implicated in limiting gene flow in and out of the Weddell Sea (Zane et al. 1998; Bargelloni et al. 2000; Díez et al. 2004).

In the case of *O. victoriae*, migration across the Antarctic Sound may have been restricted during Pleistocene glacial periods, due to lowered sea levels and ice sheet expansion (Thatje et al. 2005). Isolation of western and eastern Antarctic Peninsula populations during glacial periods could explain present-day divergent lineages. Clades 1 and 2 may have only recently established secondary contact, with contemporary oceanographic barriers reinforcing isolation of Clade 2 in the Weddell Sea. Using an approximate echinoderm mtDNA divergence rate of 3.1%-3.5%/my (Lessios et al. 1999; McCartney et al. 2000), separation of Clades 1 and 2 can be dated around 510,000-580,000 years ago, during the mid-Pleistocene. Interestingly, this timing roughly coincides with the split between the two most closely related cryptic lineages of the Antarctic crinoid *Promachocrinus kerguelensis* (Wilson et al. 2007). Further sampling throughout the Weddell Sea could reveal whether the population at St. 40 represents an anomalous divergence, or is typical of *O. victoriae* populations in the Weddell Sea.

Whether Clades 1 and 2 represent cryptic species not previously recognized on the basis of morphology requires further sampling throughout the Weddell Sea and eastern Antarctic. However, examination of external morphological characters did not reveal any fixed differences between clades. Given the prevalence of cryptic speciation among Antarctic benthic marine invertebrates (e.g., Beaumont and Wei 1991; Held 2003; Held and Wägele 2005; Raupach and Wägele 2006; Linse et al. 2007; Wilson et al. 2007; Hunter and Halanych 2008; Leese and Held 2008; Mahon et al. 2008; Thornhill et al.

2008; Wilson et al. 2009), it would not be surprising if additional collections revealed *O. victoriae*, as currently recognized, to be comprised of two or more cryptic species.

4.5.2 Clade 1 relationships: the Antarctic Peninsula

Clade 1 is comprised of two genetically divergent subclades. Based on our sampling, Clade 1a occurs throughout the Bransfield Strait and more southern regions of the Antarctic Peninsula, as well as around South Sandwich and Bouvet Islands. Clade 1b was collected primarily from two geographically proximate stations near Anvers Island, and from a third station approximately 100 km northwest of the South Shetland Islands. Anvers Island is situated between Bransfield Strait and the two southernmost sampling sites along the Antarctic Peninsula. Given that the Antarctic Peninsula continental shelf has been open since the end of the last glacial maxima ~19,000 years ago (Gersonde et al. 2005), it is likely that present-day mechanisms, rather than historical effects, are responsible for this distributional pattern. Although a well-established oceanographic barrier does not exist that could explain this distribution of mtDNA haplotypes, water masses of different origins flowing at different depths over complex topography throughout the Antarctic Peninsula may be involved. For example, water from the ACC, Eastwind Drift, and Weddell Sea flows throughout the Antarctic Peninsula as Antarctic Surface Water. In contrast, Antarctic Bottom Water, formed primarily in the Weddell Sea, flows north at deep depths along the continental shelf and slope, while Circumpolar Deep Water flows south and upward towards the continent at intermediate depths (El-Sayed 1985; Stein and Heywood 1994).

In general, it is thought that limited water exchange occurs along the Antarctic Peninsula continental shelf, due to narrow shelf topography and water mass structure (Smith et al. 1999). Additionally, the shallow topography of the southwest entrance to Bransfield Strait likely prevents southern Antarctic Peninsula water from entering Bransfield Strait (Smith et al. 1999). However, some ACC water flowing from the southwest does enter Bransfield Strait through a deep gap between two islands at the southwest margin (Zhou et al. 2002). Given this, planktonic larvae of *O. victoriae* from the southern Peninsula could potentially become entrained in the ACC, and occasionally transported into Bransfield Strait in Antarctic Surface Water. This transport mechanism could account for gene flow occurring between Sts. 33 and 47 in the southern Antarctic Peninsula and Bransfield Strait. The distinct genetic signature of the populations near Anvers Island implies that oceanographic patterns in this region differ from those farther south along the Antarctic Peninsula, or that larvae are being transported in different water masses. Larvae in this region may be transported offshore in Antarctic Surface Water as it flows northward (El-Sayed 1985), and enter the ACC beyond the point where some ACC water enters Bransfield Strait. Genetic homogeneity and moderate gene flow between the Anvers Island populations and the station north of the South Shetland Islands (St. 17) support this hypothesis, given that St. 17 is offshore and directly in the path of the ACC.

In contrast to *O. victoriae*, other studies of Antarctic planktotrophic marine invertebrates have recovered identical mtDNA haplotypes across large distances throughout the Antarctic Peninsula (Thornhill et al. 2008; LN Cox pers. comm.; AM Janosik pers. comm.), and even Antarctic brooders show evidence of extensive

connectivity throughout the Antarctic Peninsula (Hunter and Halanych 2008; Mahon et al. 2008). One notable exception is the lecithotrophic crinoid *Promachocrinus kerguelensis*, characterized by multiple cryptic lineages, several of which are not shared between Bransfield Strait and the southern Antarctic Peninsula (Wilson et al. 2007). While little is known about *O. victoriae* ophioplutei, it is possible that larval ecology is limiting dispersal between certain geographic regions. For example, vertical migration behavior may expose *O. victoriae* ophioplutei to different water masses compared to other planktotrophic larvae. Additionally, temporal differences in spawning may influence dispersal. Two planktotroph species that display extensive genetic connectivity throughout the Antarctic Peninsula (i.e., *Odontaster validus* and *Parborlasia corrugatus*) spawn at different times of the year compared to *O. victoriae* (Pearse et al. 1991; Shreeve and Peck 1995; Stanwell-Smith and Peck 1998), which may expose their larvae to different oceanographic and environmental conditions. Indeed, Antarctic zooplankton tend to be more abundant in coastal waters in the summer, and in deeper, open-ocean waters in the winter (El-Sayed 1985).

4.5.3 Clade 1 relationships: Subantarctic Islands

Gene flow between the subantarctic South Sandwich and Bouvet Islands and the Antarctic Peninsula appears to be infrequent. Only a single haplotype was shared between these regions and significant genetic structure was recovered. The South Sandwich Islands are situated approximately 1800 km from Bransfield Strait, whereas Bouvet Island is approximately 3300 km from Bransfield Strait. Significant genetic structure was also recovered between the South Sandwich Islands and Bouvet Island,

separated by about 1500 km. These results contrast with phylogeographic patterns found for several other Antarctic taxa, where multiple mtDNA haplotypes were shared between the Antarctic Peninsula and these subantarctic islands (Thornhill et al. 2008; LN Cox pers. comm.; AM Janosik pers. comm.; but see Wilson et al. 2007).

Due to the eastward circumpolar flow of the ACC, any gene flow occurring between the Antarctic Peninsula and subantarctic islands would most likely be unidirectional. Larvae could be transported in Antarctic Surface Water as it exits the northeast margin of the Bransfield Strait, passes by Elephant Island, and merges with the ACC, which then flows past the subantarctic islands (Stein and Heywood 1994). While planktonic larval duration has not been measured in *O. victoriae*, the Antarctic planktotroph echinoderms *Sterechinus neumayeri* and *Odontaster validus* have larval durations of around 115 days and 165 days, respectively (Pearse and Bosch 1986; Bosch et al. 1987). Thus, the duration of *O. victoriae* ophioplutei development is likely within the same range. The ACC has surface speeds ranging from 0.25-0.4 m s^{-1} (Klinck and Nowland 2001). Flow of that magnitude, coupled with a development time potentially greater than 100 days, would allow ample time for larvae to disperse from the Antarctic Peninsula to the subantarctic islands. Again, larval behavior may explain the apparent low levels of genetic connectivity if ophioplutei regularly migrate to deeper depths where current speeds decline to only a few centimeters per second near the bottom (Klinck and Nowland 2001).

4.5.4 Clade 1 relationships: Deception Island

Individuals from St. 64 in the Bransfield Strait were collected from Deception Island, an active volcano with a sunken, water-filled caldera inhabited by marine organisms. Three large eruption events occurred from 1967-1970 (Lovell and Trego 2003), resulting in a mass mortality event thought to have largely eradicated the benthic flora and fauna inhabiting the drowned caldera (Gallardo and Castillo 1968, 1970). In a recent survey of community structure at Deception Island, *O. victoriae* was determined to be the dominant epibenthic organism (Gallardo and Castillo 1968, 1970; Arnaud et al. 1998; Lovell and Trego 2003) and the only ophiuroid species occurring in this highly disturbed locality (Manjón-Cabeza and Ramos 2003). The Deception Island population showed significant genetic differentiation to all other populations except for the most geographic proximate population, St. 51, separated by only 57 km. Due to the recent volcanic disturbance, individuals inhabiting Deception Island might have recently immigrated from other existing *O. victoriae* populations, despite limited water exchange between Deception Island and the rest of Bransfield Strait (Lovell and Trego 2003). Along with this, a recent expansion is suggested for this population, as evident by significantly negative values of Fu's F_S. Negative values for Fu's F_S can be explained by purifying selection, population expansion immediately following a bottleneck, or by a low level of migration from a genetically distinct population(s) (Skibinski 2000). Given the recent geologic history of Deception Island, combined with genetic homogeneity with St. 51, the *O. victoriae* population at Deception Island is likely experiencing spatial expansion following re-colonization by closely-related individuals from St. 51 in Bransfield Strait.

In summary, *Ophionotus victoriae* is characterized by previously unrecognized levels of genetic diversity and divergence, and is not genetically homogeneous throughout the sampled range. It should be emphasized that this study sampled only a portion of the supposedly circumpolar distribution of this species, yet revealed levels of geographic subdivision that appear to be atypical among Antarctic benthic marine invertebrates. Thus, additional sampling around the Antarctic continent would surely reveal even greater genetic diversity and likely cryptic speciation. Given the almost non-existent ability of this organism to adapt to changing environmental conditions (Peck et al. 2009), thoroughly assessing the extent of genetic variation and connectivity in this keystone Antarctic species is vital.

4.6 REFERENCES

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Table 1 Population summary statistics for *Ophionotus victoriae* (derived from the 16S+COI concatenated dataset): N refers to number of individuals, H is the number of haplotypes, π refers to nucleotide diversity, and h is haplotype diversity. Tajima's D and Fu's F_S refer to results of neutrality tests. Station numbers correspond with Figure 1.

Region Station	Lat/Long	Depth	N	H	$\pi \pm$ SD	$h \pm$ SD	Tajima's D	Fu's F_S
Northern Peninsula								
17 – South Shetland Is.	S 62°19', W 61°45'	334 m	8	5	0.0048 ± 0.0023	0.86 ± 0.11	−1.421	−0.807
21 – Bransfield Strait	S 63°09', W 57°07'	192 m	5	4	0.0019 ± 0.0008	0.83 ± 0.22	−0.780	0.134
40a – Eagle Is.	S 63°40', W 57°20'	335 m	5	5	0.0044 ± 0.0009	1.00 ± 0.13	0.461	−1.481
40b – Eagle Is.	S 63°40', W 57°20'	335 m	6	2	0.0003 ± 0.0002	0.33 ± 0.22	−0.933	−0.003
51 – Bransfield Strait	S 63°23', W 60°03'	227 m	15	11	0.0040 ± 0.0005	0.95 ± 0.04	−0.366	−4.086*
64 – Deception Is.	S 62°57', W 60°39'	161 m	10	7	0.0026 ± 0.0006	0.91 ± 0.08	−0.977	−2.291*
D5 – Elephant Is.	S 61°12', W 54°44'	239 m	6	6	0.0078 ± 0.0022	1.00 ± 0.10	−0.633	−1.258
D6 – South Shetland Is.	S 62°17', W 58°27'	192 m	10	10	0.0041 ± 0.0005	1.00 ± 0.05	−0.861	−6.556***
Southern Peninsula								
33 – Adelaide Is.	S 67°44', W 69°17'	122 m	11	7	0.0037 ± 0.0007	0.87 ± 0.09	0.113	−1.001
47 – Adelaide Is.	S 67°40', W 68°15'	170 m	10	6	0.0062 ± 0.0020	0.89 ± 0.08	0.141	1.039
58 – Petermann Is.	S 65°11', W 64°15'	285 m	10	5	0.0016 ± 0.0003	0.76 ± 0.13	−0.229	−1.021
D8 – near Anvers Is.	S 64°53', W 62°54'	187 m	5	3	0.0065 ± 0.0031	0.80 ± 0.16	−0.840	2.942
Subantarctic Islands								
51 – South Sandwich Is.	S 58°29', W 26°12'	270 m	9	8	0.0036 ± 0.0004	0.97 ± 0.06	−0.249	−3.517*
57 – South Sandwich Is.	S 57°03', W 26°45'	130 m	6	5	0.0036 ± 0.0007	0.93 ± 0.12	0.499	−0.839
76 – Bouvet Is.	S 54°38', W 03°18'	648 m	8	5	0.0020 ± 0.0007	0.79 ± 0.15	−1.045	−1.037
81 – Bouvet Is.	S 54°29', W 03°18'	169 m	10	5	0.0027 ± 0.0003	0.84 ± 0.08	0.710	0.235
All								
Clade 1a			103	49	0.0047 ± 0.0002	0.97 ± 0.01	−1.094	−39.158***
Clade 1b			24	8	0.0015 ± 0.0002	0.74 ± 0.06	−0.826	−2.582
Clade 1 Total			127	57	0.0076 ± 0.0004	0.97 ± 0.01	−0.458	−31.733***
Clade 2 Total			7	3	0.0049 ± 0.0030	0.67 ± 0.16	0.559	0.589

*$0.05 \geq P \geq 0.01$; **$0.01 > P \geq 0.001$; ***$P < 0.001$

Table 2 Pairwise Φ_{ST} values between *Ophionotus victoriae* Antarctic and subantarctic stations. Station numbers correspond with Figure 1.

	Northern Peninsula								Southern Peninsula				Subantarctic Islands			
	17	21	40a	40b	51	64	D5	D6	33	47	58	D8	51	57	76	81
Northern Peninsula																
17	–															
21	**0.67****	–														
40a	**0.63***	−0.10	–													
40b	**0.68*****	**0.70***	**0.66***	–												
51	**0.68*****	0.02	−0.00	**0.72*****	–											
64	**0.73*****	**0.29***	**0.22***	**0.75*****	0.06	–										
D5	**0.48***	−0.05	−0.10	**0.59****	0.00	**0.12***	–									
D6	**0.67*****	−0.05	−0.05	**0.70*****	0.00	**0.19****	−0.04	–								
Southern Peninsula																
33	**0.70*****	0.17	0.07	**0.73*****	0.09	**0.34****	0.01	0.04	–							
47	**0.50****	0.06	0.01	**0.62*****	**0.09***	**0.11***	−0.04	**0.10***	**0.24****	–						
58	0.06	**0.88****	**0.84*****	**0.80*****	**0.81*****	**0.86*****	**0.70*****	**0.81*****	**0.83*****	**0.69*****	–					
D8	−0.12	**0.59***	**0.54***	**0.64****	**0.64*****	**0.69*****	**0.37***	**0.61*****	**0.65*****	**0.42****	0.06	–				
Subantarctic Islands																
51	**0.67*****	**0.17***	0.08	**0.70*****	**0.27*****	**0.40*****	**0.18****	**0.27*****	**0.37*****	**0.24*****	**0.82*****	**0.61*****	–			
57	**0.66****	0.20	0.11	**0.68****	**0.28*****	**0.41*****	**0.17****	**0.28*****	**0.41*****	**0.21***	**0.84*****	**0.60****	−0.10	–		
76	**0.73*****	**0.59****	**0.51****	**0.76*****	**0.53*****	**0.65*****	**0.42*****	**0.54*****	**0.60*****	**0.45*****	**0.87*****	**0.69****	**0.43*****	**0.47****	–	
81	**0.73*****	**0.48****	**0.44*****	**0.75*****	**0.47*****	**0.60*****	**0.39*****	**0.47*****	**0.54*****	**0.43*****	**0.86*****	**0.68*****	**0.42*****	**0.44*****	0.13	–

*$0.05 \geq P \geq 0.01$; **$0.01 > P \geq 0.001$; ***$P < 0.001$

Table 3 Hierarchical analysis of molecular variance (AMOVA) for Antarctic and subantarctic populations of *Ophionotus victoriae*.

Source of variation	% variation	Φ statistic	*P* value
Among regions	8.21	Φ_{CT}=0.08	0.100
Among stations within a region	47.24	Φ_{SC}=0.51	*
Within stations	44.54	Φ_{ST}=0.55	*

*$P < 0.0001$

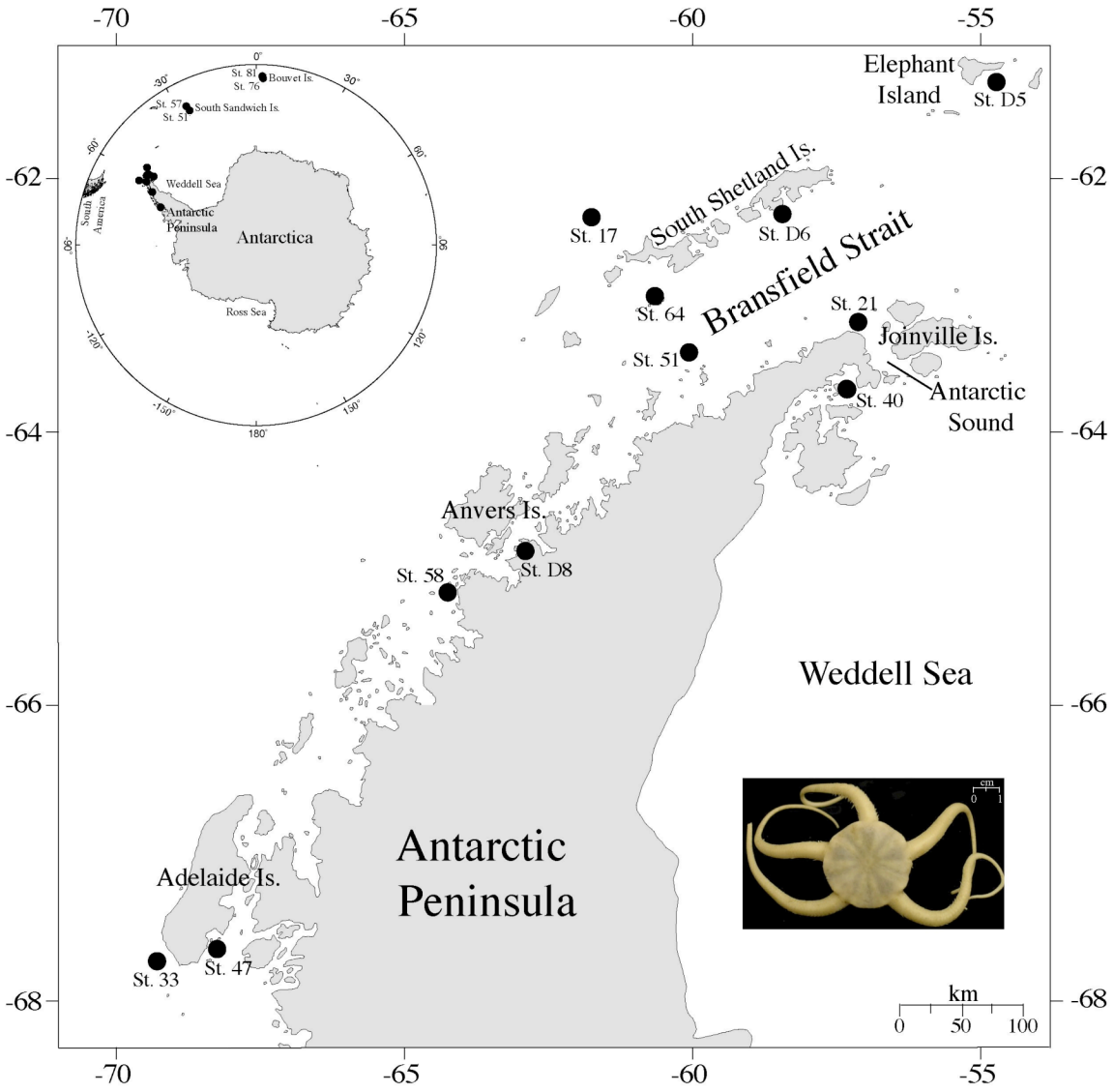

Figure 1 Map showing collection localities for *Ophionotus victoriae* from the Antarctic Peninsula and subantarctic South Sandwich Islands and Bouvet Island (inset).

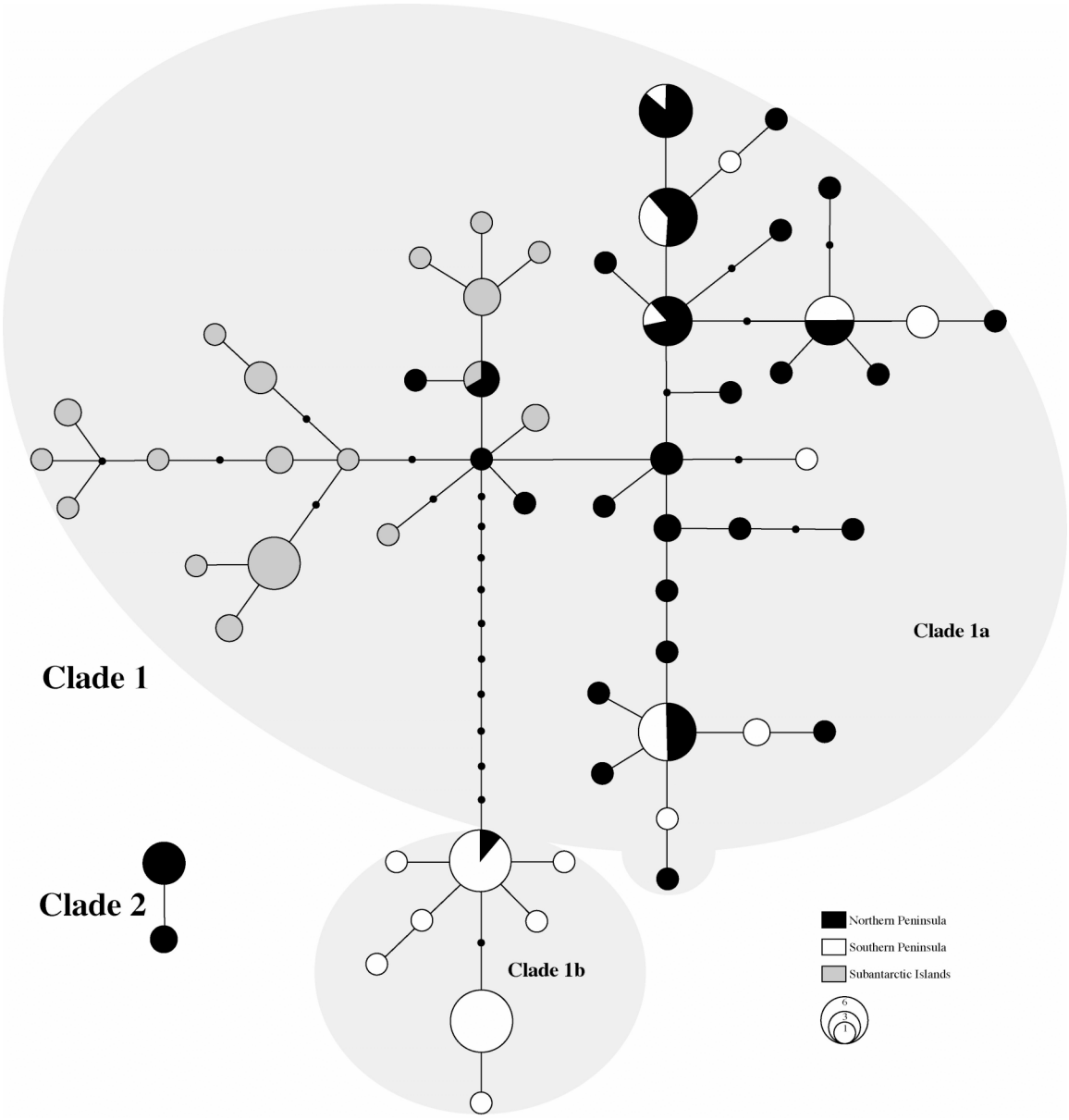

Figure 2 16S + COI parsimony networks of 134 *O. victoriae* individuals collected throughout the Antarctic Peninsula and subantarctic islands. Haplotype circles are coded by geographic region and sized according to relative abundance. Missing (unsampled) haplotypes are denoted by small black circles.

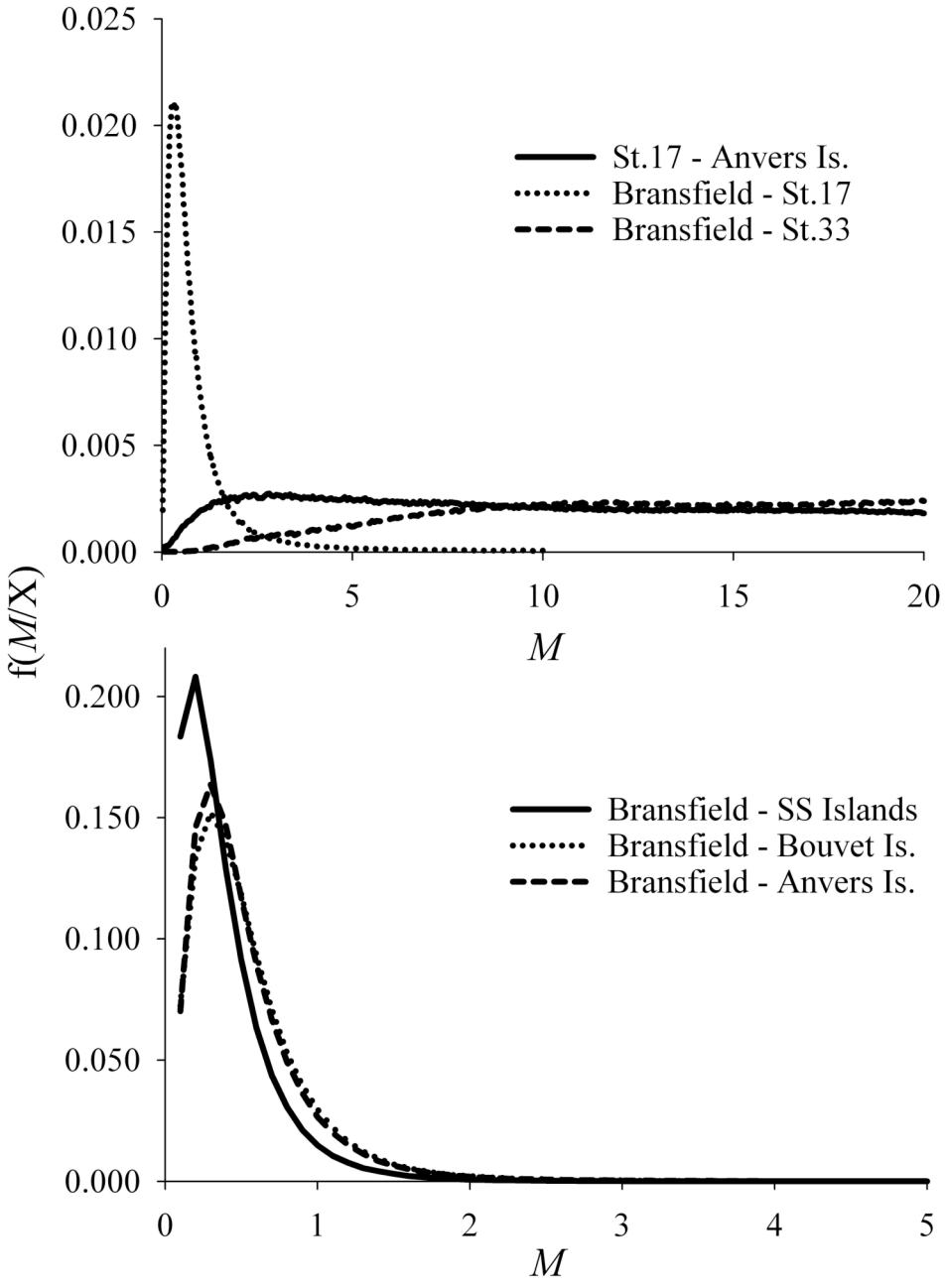

Figure 3 Posterior probability distributions for the number of migrants per generation (*M*) between certain *O. victoriae* stations/regions.

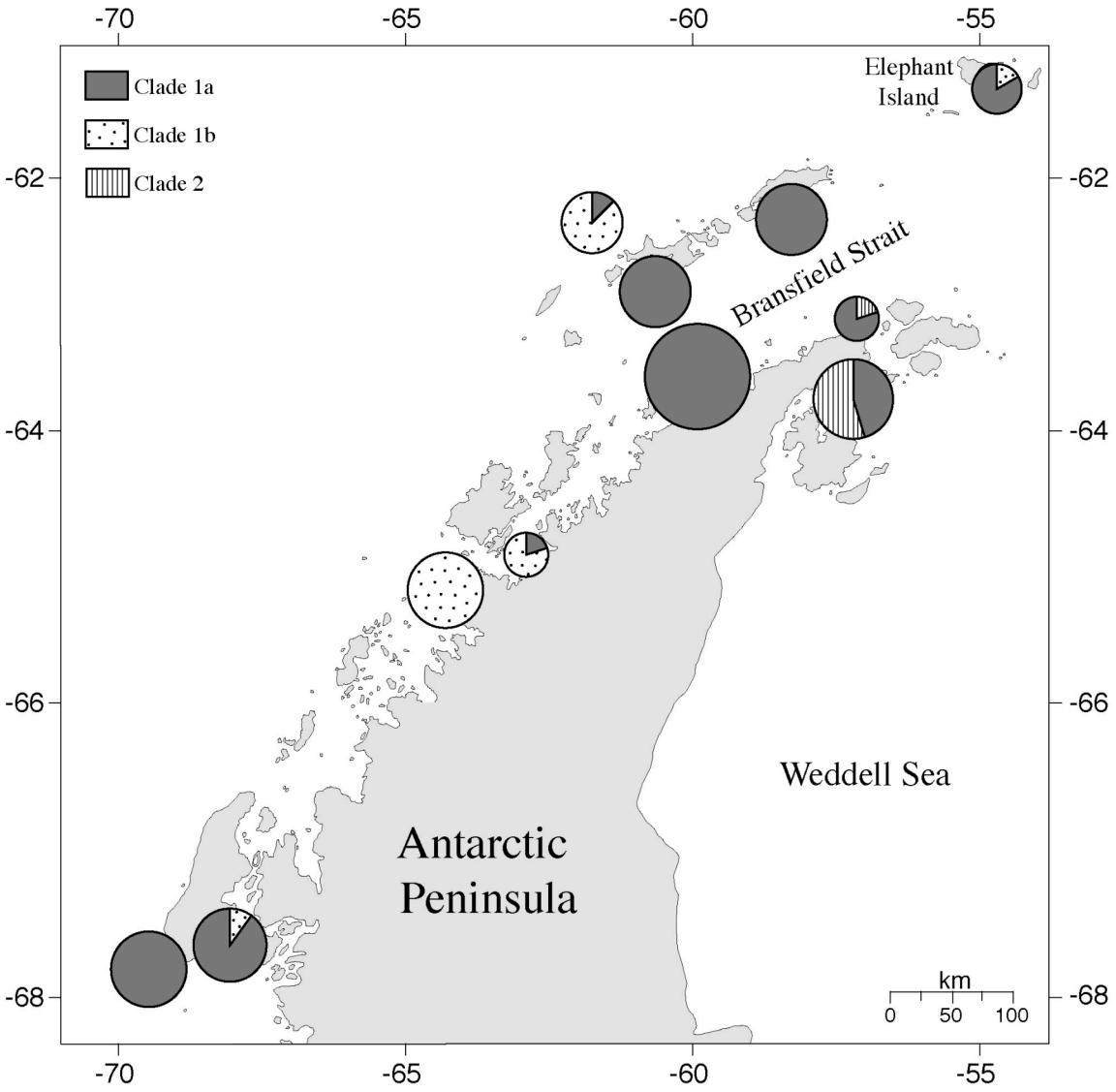

Figure 4 Map showing the distribution of *O. victoriae* clades throughout the Antarctic Peninsula. Given that all subantarctic populations belonged to Clade 1a, these geographic localities were not included.

CHAPTER 5: Geographical subdivision and demographic history in two Antarctic ophiuroids: the role of Pleistocene glacial cycles and contemporary oceanography

5.1 ABSTRACT

Two common ophiuroid species were collected from a portion of their range in order to assess their population connectivity and genetic diversity. *Ophiurolepis gelida* was sampled from the Antarctic Peninsula, Ross Sea, Weddell Sea and several subantarctic islands, and *O. brevirima* was sampled from the northern Antarctic Peninsula (Bransfield Strait) and Weddell Sea. Both species are thought to produce lecithotrophic larvae and therefore have potential for larval dispersal. Mitochondrial (16S rDNA) data were analyzed using coalescent and frequency-based methods. These data showed evidence of significant population genetic structure in both species between major geographic regions. Within regions however, population connectivity was evident, and parsimony network analyses and neutrality tests indicated a recent population expansion in Bransfield Strait for both *O. gelida* and *O. brevirima*. These results suggest that populations within distinct geographic regions have a unique genetic signature, highlighting the potential for cryptic speciation and underestimation of genetic diversity and divergence in Antarctic benthic invertebrates.

5.2 INTRODUCTION

Connectivity and distributional patterns of benthic organisms inhabiting the Antarctic continental shelf are influenced by contemporaneous factors including oceanography and life history, as well as historical forces such as palaeoclimatology (e.g., Pleistocene glacial cycles). For example, the Antarctic Circumpolar Current (ACC), a fast-flowing easterly current, is thought to play an important role in dispersing marine organisms around Antarctica (Fell 1962; Dell 1972; Arntz et al. 1994; Thornhill et al. 2008; Waters 2008), while the Antarctic Polar Front (APF), a frontal region associated with the ACC, is considered to prevent exchange between Antarctic and temperate populations (Patarnello et al. 1996; Shaw et al. 2004; Clarke et al. 2005; Hunter and Halanych 2008; Thornhill et al. 2008). Coastal currents (e.g., Eastwind Drift) and large oceanic gyres (e.g., Weddell and Ross Sea gyres) in the Southern Ocean also act to structure populations (Patarnello et al. 1996; Bargelloni et al. 2000; Linse et al. 2007).

The Southern Hemisphere has experienced four major episodes of ice sheet expansion during the past 1.2 my (Elliot 1985), with the most recent glacial maximum occurring roughly 17 kya to 21 kya (Gersonde et al. 2005). During glacial periods, grounded ice sheets covered the majority of the Antarctic continental shelf (Anderson et al. 2002; Huybrechts 2002; Hodgson et al. 2003), and any open shelf regions were frequently disturbed by iceberg scour (Beaman and Harris 2003). Expanding ice sheets displaced or eradicated continental shelf fauna, forcing species onto the continental slope and/or deep sea, or into isolated shelf refugia (Thatje et al. 2005). During interglacial periods, benthic organisms with dispersal ability could recolonize continental shelf habitat as it opened up around Antarctica (Poulin et al. 2002; Thatje et al. 2005). The

extent to which glacial/interglacial cycles impacted the genetic signature of Antarctic benthos is not fully understood, but increased genetic diversity and radiations in several taxonomic groups have been attributed to Pleistocene climatic processes (Eastman and Clarke 1998; Held 2000; Allcock 2005; Raupach et al. 2007; Wilson et al. 2007; Wilson et al. 2009).

Life history traits such as presence/absence of a dispersive larval stage interact with physical forces in Antarctica to determine population connectivity. Historically, cold-water invertebrates were considered to be almost exclusively brooders (Thomson 1878, 1885; Murray 1885; Thorson 1936), however it is currently recognized that many Antarctic taxa produce pelagic larvae (Pearse et al. 1991; Clarke 1992; Pearse 1994). For example, over 70% of Antarctic echinoderms have a planktonic larval stage (Pearse 1994). Additionally, Antarctic organisms have delayed development in the cold Southern Ocean, which increases persistence times of their larvae in the water column, presumably enhancing dispersal ability (Bosch et al. 1987; Stanwell-Smith and Peck 1998).

Many taxonomic groups in Antarctica remain relatively unstudied in terms of their biogeography, levels of population connectivity and genetic diversity. Ophiuroids, commonly known as brittle stars, the most diverse group of echinoderms with over 90 species recognized in Antarctic/subantarctic waters (Smirnov 1994), are one conspicuous example. *Ophiurolepis gelida* and *Ophiurolepis brevirima* are closely-related ophiuroids that are abundant and dominant throughout the Antarctic benthos (Dahm 1996; Piepenburg et al. 1997; Manjón-Cabeza and Ramos 2003). *Ophiurolepis gelida* occurs throughout the Antarctic/subantarctic (Mortensen 1936; Fell 1961; Madsen 1967), whereas *O. brevirima* is restricted to Antarctica. Both *O. gelida* and *O. brevirima* are

reported to have circumpolar distributions, a common hypothesized distributional pattern among Antarctic benthic organisms (Fell et al. 1969; Dayton et al. 1990; Dell 1990). The reproductive biology of these two species is not definitively known, but Mortensen (1936) postulated that neither species were brooders, based on an absence of brooded embryos and the presence of numerous eggs of moderate size (~0.3 mm) (RL Hunter, pers. obs.). He suggested that they were lecithotrophic, producing a non-feeding planktonic larval form that could persist in the plankton for up to 2-3 months (Bosch and Pearse 1990).

Ophiurolepis gelida and *O. brevirima* occur sympatrically in some regions of Antarctica, including the Antarctic Peninsula (Mortensen 1936). Given the similar life history of these species, we compared sympatric populations to evaluate the effect of Pleistocene glacial cycles on population structure and genetic diversity within each species, and to assess the role of oceanography in structuring present-day populations. Additionally, *O. gelida* was collected from a wide range around Antarctica to determine if *O. gelida* larvae are realizing their dispersal potential, or whether ongoing and/or historical factors have limited circumpolar gene flow in this widespread species. For *O. brevirima*, in addition to evaluating connectivity within the Antarctic Peninsula, a population from the Weddell Sea was sampled to assess whether the Weddell Sea gyre is restricting gene flow between Antarctic Peninsula and Weddell Sea populations.

5.3 MATERIALS AND METHODS

5.3.1 Data collection

Ophiurolepis gelida and *O. brevirima* were collected during cruises to the Antarctic Peninsula in 2004 and 2006 aboard the *R/V Laurence M. Gould*. Benthic samples were collected with an epibenthic sled, Blake trawl, or rock dredge. *O. gelida* was collected from the subantarctic South Sandwich and Bouvet Islands during the 2004 ICEFISH expedition, and from the Ross Sea by SCUBA. Both species were collected from the Weddell Sea during the 2005 ANDEEP-3 cruise. Samples for DNA analysis were either frozen upon collection at -80ºC or preserved in ~85% ethanol. Sampling information is provided in Table 1 and Figure 1.

DNA was extracted using the DNeasy® Tissue Kit (QIAGEN) following manufacturer's protocol. The mitochondrial gene fragment 16S rDNA (16S) was amplified using standard PCR protocols. Primers 16SarL (5′-CGCCTGTTTATCAAAAACAT-3′) and 16SbrH (5′-CCGGTCTGAACTCAGATCACGT-3′) (Palumbi et al. 1991) amplify a ~500 bp fragment from the middle of 16S. Double-stranded PCR products were purified using the QIAquick® Gel Extraction Kit (QIAGEN) following manufacturer's protocol. Purified PCR products were bi-directionally sequenced using a CEQ8000 Genetic Analysis System (Beckman Coulter). All *O. gelida* and *O. brevirima* haplotypes will be deposited in GenBank.

Sequences were edited in SeqMan (DNA* Lasergene) and aligned with Clustal W (Thompson et al. 1994) in MegAlign (DNA* Lasergene). For *O. gelida*, a small number of gaps were required for alignment, and no gaps were necessary to align *O. brevirima*

sequences. Alignments were examined visually in MacClade v4.0 (Maddison and Maddison 2000) and will be available in TreeBASE (www.treebase.org).

5.3.2 Population structure analyses

Parsimony networks were constructed using mtDNA haplotypes in TCS v1.18 (Clement et al. 2000) with a 95% connection limit between haplotypes. Gaps were treated as missing data. To assess levels of genetic differentiation between sampling stations (Table 1), pairwise Φ_{ST}'s were computed (where $N \geq 4$) in Arlequin v3.1 (Excoffier et al. 2005). Given the close proximity of the two South Sandwich Islands stations, and the three Bouvet Island stations, sites within these regions were pooled for pairwise analysis.

Arlequin was used to perform an analysis of molecular variance (AMOVA) to evaluate the relationship between haplotype variation and geography in *O. gelida*. An AMOVA was not carried out for *O. brevirima* due to sampling limitations (only a single population was sampled from the Weddell Sea region). For the AMOVA, variance was partitioned into three hierarchical components: within sampling stations (Φ_{ST}), among sampling stations within a geographic region (Φ_{SC}), and among geographic regions (Φ_{CT}). For *O. gelida*, geographic regions were Bransfield Strait, the Southern Peninsula (stations south of Low Is.) and Subantarctic Islands, and were determined *a priori* based on oceanographic discontinuities in the Antarctic Peninsula (Zhou et al. 2002) and due to the large distance (>1800 km) separating the subantarctic islands from the Antarctic Peninsula. For both pairwise Φ_{ST}'s and the AMOVA, the Tamura-Nei model (Tamura and Nei 1993) was selected in Arlequin as it most closely approximated the model of sequence evolution chosen by Modeltest v3.7 (TrN + I for *O. gelida*; TIM for *O.*

brevirima) (Posada and Crandall 1998), and 10,000 permutations were carried out to assess significance. For subsequent analyses, genetically homogenous populations (determined by non-significant pairwise Φ_{ST}'s) within a geographic region were pooled. With the exception of the haplotype network analysis, analyses were done only using collection stations or pooled regions where four or more individuals were sampled.

5.3.3 Historical demography and divergence dating

In order to quantify genetic diversity within *O. gelida* and *O. brevirima*, nucleotide (π) and haplotype (h) diversities were calculated in DnaSP v4.1 (Rozas et al. 2003). Tajima's D (Tajima 1989) test statistic was calculated in DnaSP to evaluate the assumption of selective neutrality of 16S sequences as well as population equilibrium. Fu's F_S (Fu 1997) neutrality statistic, shown to be particularly sensitive to population demographic expansion as indicated by large, negative F_S values (Fu 1997), was computed in DnaSP and significance assessed by 10,000 data permutations. Mismatch distributions for each species were examined in Arlequin, and 10,000 coalescent simulations, based on parameters estimated from a sudden demographic expansion, were done to assess significance of test statistics. The mismatch distribution compares the observed versus expected distribution of pairwise nucleotide differences and was used to determine if population expansion had occurred in the history of *O. gelida* or *O. brevirima*.

Divergence dates were computed in BEAST v1.4.8 (Drummond and Rambaut 2007), which uses a coalescent-based MCMC approach to estimate time to the most recent common ancestor (tMRCA). BEAST was used to calculate coalescense times

within lineages and to date splitting events between lineages recovered from statistical parsimony. In BEAST, the HKY + I model of sequence evolution was used and a strict clock was employed with runs conducted under two different starting conditions. In the first set of runs, the mean mutation rate per site per year was set to 2.5×10^{-9}, and in the second set of runs, 1.5×10^{-8} was used. These values span the typical range of average pairwise sequence divergences reported for the 16S gene for invertebrates (0.5-3.0%/my; Held 2001; Govindarajan et al. 2005; Johnson 2005). Fifteen million MCMC generations were run to ensure effective sampling sizes exceeded 200 (Drummond et al. 2007). MCMC chains were sampled every 500 iterations and 10% were discarded as burn-in. Dating times and confidence intervals were filtered using Tracer v1.4 (Rambaut and Drummond 2007).

5.4 RESULTS

DNA sequences from a portion (503 bp) of the mitochondrial 16S rDNA gene were obtained from 118 *Ophiurolepis gelida* individuals and 87 *O. brevirima* individuals. Within both species, the majority of 16S haplotypes were distributed across multiple sampling localities. In *O. gelida*, 118 individuals yielded 29 unique haplotypes, and in *O. brevirima*, 87 individuals yielded 17 haplotypes, one of which was found at every collection site except the Weddell Sea.

5.4.1 Population structure

Statistical parsimony analysis of *O. gelida* sequences indicated at least four geographic "subclades" are present within this species, corresponding to Bransfield Strait

(plus Southern Peninsula St. 29), Southern Peninsula, Subantarctic Islands and Ross Sea/Weddell Sea (Fig. 2). *O. gelida* and *O. brevirima* were comparable in the portion of their haplotype networks representing individuals collected from the Bransfield Strait, the geographic region where their distributions overlapped. In this region, both species were characterized by a numerically dominant, geographically widespread 16S haplotype, with a few additional haplotypes differing by only one or two substitutions. Also in both species, individuals collected from St. 29 in the Southern Peninsula, situated immediately southwest of the west entrance to Bransfield Strait, clustered with Bransfield Strait individuals, as did *O. brevirima* St. 72 individuals (in close proximity to St. 29). None of the five *O. brevirima* individuals from the eastern Weddell Sea shared haplotypes with Antarctic Peninsula individuals, nor was the single *O. gelida* individual from the Weddell Sea identical to any other *O. gelida* individuals.

For *O. gelida*, genetic subdivision was recovered between all major geographic regions where sample sizes were sufficient to conduct analyses (Table 2a). Φ_{ST} values between populations in Bransfield Strait (including St. 29), the Southern Peninsula, and subantarctic islands were large (≥ 0.54) and significant in all cases. Conversely, within geographic regions, Φ_{ST} values were typically low and non-significant. For *O. brevirima*, populations within and immediately southwest of Bransfield Strait were genetically homogenous (Table 2b), with one exception (St. 51). Genetic differentiation between the Antarctic Peninsula and Weddell Sea was supported by large, significant Φ_{ST} values (≥ 0.72) in all pairwise comparisons, owing to a lack of shared mtDNA haplotypes.

AMOVA based on *a priori* geographic regions resulted in similar levels of genetic variation among geographic regions (41%, $P < 0.05$) and among stations within a

geographic region (34%, $P < 0.0001$) in *O. gelida*. Less genetic variation (25%, $P < 0.0001$) was attributed to within collection stations. Because pairwise Φ_{ST} values were low and non-significant between Bransfield Strait populations and one Southern Peninsula population, an AMOVA was performed that included St. 29 in the Bransfield Strait region. Under these conditions, the majority of genetic variation existed among geographic regions (79%, $P < 0.01$), whereas almost no variation was present among stations within a geographic region (0.1%, $P > 0.05$). The remainder of the variation was at the within collection station level (20%, $P < 0.0001$).

5.4.2 Historical demography and divergence

Nucleotide and haplotype diversity estimates were low ($\pi \leq 0.0011$, $h \leq 0.45$; Table 3a) for most geographic regions for both species, with the exception of *O. gelida* in the Southern Peninsula ($\pi = 0.0076$, $h = 0.76$), which was moderately diverse. Both Tajima's *D* and Fu's F_S were significantly negative for the Bransfield Strait region in both species, as well as for the subantarctic island region for *O. gelida*. Significant negative values for neutrality statistics can be explained by purifying selection, a selective sweep, or by a population expansion immediately following a bottleneck (Skibinski 2000). The distribution of pairwise differences between haplotypes within a geographic region closely modeled the expected distribution under a sudden demographic expansion in all cases except *O. gelida* in the Southern Peninsula (Fig. 3). The sum of square deviations (*SSD*) between the observed and expected distribution of a sudden demographic expansion, and raggedness statistic (*r*) (Harpending 1994) for the mismatch distribution were not statistically significant (Table 3b), indicating that the sudden

expansion model could not be rejected for either species in any geographic region (Harpending 1994; Schneider and Excoffier 1999).

Estimation of divergence dates for *O. gelida* and *O. brevirima* lineages resulted in means with large confidence intervals (Table 4), possibly due to using a single locus with too few informative sites. For *O. gelida*, the Bransfield Strait-Southern Peninsula split was dated to the early Pliocene, prior to the onset of Pliocene-Pleistocene glacial cycles, using the slower mutation rate (0.5%/MY), while the faster mutation rate (3%/MY) recovered a much ealier split during the Miocene. The Bransfield Strait-Subantarctic separation was estimated to have occurred during the late-Pliocene to mid-Pleistocene. Estimation of coalescence times within lineages suggested that *O. gelida* lineages were typically of Pleistocene origin, or originated just prior to the Pleistocene. Similar date estimations were recovered for *O. brevirima*. The split between Bransfield Strait and Weddell Sea lineages was dated as late-Pliocene to mid-Pleistocene, and the age of the Bransfield Strait clade fell within the same range.

5.5 DISCUSSION

Phylogeographic analyses of mtDNA data from *Ophiurolepis gelida* and *O. brevirima* resulted in a clear pattern of genetic heterogeneity between geographic regions in contrast to genetic homogeneity within geographic regions. In the case of *O. gelida*, genetic differentiation was found between populations in Bransfield Strait (+ St. 29), the Southern Peninsula and the subantarctic South Sandwich and Bouvet Islands. Parsimony analysis suggested a distinct Ross Sea/Weddell Sea population as well, although limited sampling in these regions prevents robust conclusions. For *O. brevirima*, populations

from the Antarctic Peninsula and eastern Weddell Sea appear to be limited in their connectivity. Overall, these results call into question the previously reported circumpolarity of these species.

5.5.1 *O. gelida*

A north-south phylogeographic break exists within *O. gelida* along the Antarctic Peninsula, separating Bransfield Strait individuals from those southwest of Brabant Island (see Fig. 1). There were only four cases (3.4% of individuals) where "Bransfield Strait" haplotypes were collected south of Brabant Island. This north-south break could be attributed to the mostly shallow bottom topography of the entrance to Bransfield Strait, which restricts intermediate and deep water exchange between Bransfield Strait and the Southern Peninsula (Zhou et al. 2002). Limited water flow between these regions would reduce or effectively eliminate larval transport as a mechanism for maintaining connectivity. Differentiation between Bransfield Strait populations and at least some Southern Peninsula populations has been found in the ophiuroid *Ophionotus victoriae* (Hunter and Halanych submitted) and crinoid *Promachocrinus kerguelensis* (Wilson et al. 2007). However, this pattern contrasts with results from other phylogeographic studies in which populations from Bransfield Strait and the Southern Peninsula were genetically homogeneous (Hunter and Halanych 2008; Mahon et al. 2008; Thornhill et al. 2008; LN Cox pers. comm.; AM Janosik pers. comm.).

Divergence estimation suggested that the Bransfield Strait and Southern Peninsula lineages may have split prior to the onset of Pleistocene glacial cycles, which began around 1.2 mya in the Southern Hemisphere (Elliot 1985). However, 95% confidence

intervals from both slow and fast mutation rate estimates include the early-mid Pleistocene. Therefore, Pleistocene glacial cycles cannot be excluded as a potential mechanism driving the divergence of these lineages. Populations in the northern and southern peninsula may have been isolated in separate refugia during early glacial periods, and subsequently accumulated sufficient genetic differences to prevent mixing during interglacial periods. Fragmentation into separate glacial refugia has been invoked to explain present-day population structure in a notothenioid fish (Janko et al. 2007) and Antarctic sea slug (Wilson et al. 2009), as well as in a number of Northern Hemisphere taxa (Hewitt 1996). Given limited water exchange throughout the Antarctic Peninsula, genetic structure of *O. gelida* in this region may be the result of historical isolation combined with low potential for contemporary dispersal. However, there was some evidence of ongoing or historical gene flow over large distances throughout the Antarctic Peninsula. The fact that three "Bransfield Strait" haplotypes were collected from one of the southernmost sampled populations (Sts. 33/45) suggests low levels of ongoing or historical transport from Bransfield southwest along the peninsula.

Weddell and Ross Sea mtDNA haplotypes were less divergent from Southern Peninsula haplotypes than from those collected from Bransfield Strait. However, there were no shared haplotypes between Weddell Sea, Ross Sea or Southern Peninsula individuals. The single Weddell Sea individual was separated by only a single mutation from a Ross Sea individual, and other studies have similarly uncovered a closer genetic relationship between Ross Sea and Weddell Sea samples compared to Antarctic Peninsula samples (Held and Wägele 2005; Linse et al. 2007). A hypothesized historical seaway linking the Ross and Weddell Seas (Lawver and Gahagan 2003) is one

explanation for this phenomenon (Linse et al. 2007). Additional sampling from the Ross and Weddell Seas will aid in determining levels of connectivity between these geographic regions, and with the Antarctic Peninsula.

Genetic discontinuity was also recovered between Antarctic Peninsula and subantarctic island populations of *O. gelida*. Even though the Antarctic Peninsula and subantarctic islands are linked by the ACC, approximately 1800 km separates the South Sandwich Islands from the Antarctic Peninsula, while a 3300 km distance separates the peninsula from Bouvet Island. Despite *O. gelida*'s dispersal potential, long-distance connectivity may be limited by a combination of high larval mortality and low probability of larvae reaching suitable shelf habitat on the subantarctic islands. Nonetheless, other studies have recovered multiple shared mtDNA haplotypes between the subantarctic islands and Antarctic Peninsula (Thornhill et al. 2008; LN Cox pers. comm.; AM Janosik pers. comm.).

The South Sandwich and Bouvet Island populations of *O. gelida* may be the result of a single colonization event from the Antarctic continental shelf during the Pleistocene. Some Antarctic invertebrates with pelagic larvae may have found refuge on the subantarctic islands during glacial periods (Thatje et al. 2005). *O. gelida* could have dispersed to one or more of these islands as glacial expansion impacted the Antarctic continental shelf, and following colonization, underwent a range expansion. The presumed ancestral haplotype of the subantarctic island subclade was found equally distributed among both island localities, therefore it was not possible to determine the direction in which colonization occurred. However, given that the ACC flows from the

South Sandwich Islands to Bouvet Island, dispersal likely occurred in an eastward direction.

5.5.2 *O. brevirima*

For *O. brevirima*, 16S data reveal that little to no connectivity has occurred between Antarctic Peninsula populations and the eastern Weddell Sea population most likely since the Pleistocene. Antarctic Peninsula and Weddell Sea populations may have been isolated in separate glacial refugia in their respective geographic regions. Additionally, oceanographic features such as the Weddell Gyre, separating the Weddell Sea from surrounding waters, and/or the Weddell-Scotia Confluence, where the ACC and Weddell Gyre interact, may have played a role in historical and/or contemporary isolation of Weddell Sea populations. Temperature and salinity differences between Weddell Sea and Antarctic Peninsula water masses (Stein and Heywood 1994) may have further reduced mixing between these regions. Genetic divergence between western Antarctic Peninsula and Weddell Sea populations has been found in several other Antarctic taxa (Zane et al. 1998; Bargelloni et al. 2000; Patarnello et al. 2003; Held and Wägele 2005; Hunter and Halanych submitted).

Another potential isolating factor is the depth from which Weddell Sea individuals were sampled. *O. brevirima* was collected from ≤ 500 m depths from the Antarctic Peninsula, while the depth of the single Weddell Sea station was 1017 m. Eurybathy is a common phenomenon among Antarctic marine invertebrates (Hempel 1985), but the degree to which populations are homogeneous over large depth ranges has not been well studied. There are, however, preliminary indications that depth is not

typically a limiting factor for dispersal in the Antarctic, and species distributed over moderately wide depth ranges (e.g., 100-1200 m) have been shown to be genetically homogenous (Wilson et al. 2007; Hunter and Halanych 2008). Given that we have only characterized a single Weddell Sea population with few individuals, additional sampling is necessary to fully document the extent of genetic divergence, and to determine if depth plays any role in structuring *O. brevirima* populations in the Weddell Sea and throughout the Antarctic. Additionally, although *O. brevirima* has been recorded as being circumpolar (Madsen 1967), we did not find this species south of Brabrant Island.

5.5.3 Phylogeography within Bransfield Strait

Where sampling of *O. gelida* and *O. brevirima* overlapped, similar phylogeographic patterns were recovered. Both species were genetically homogenous throughout Bransfield Strait, with the exception of one *O. brevirima* population. Furthermore, stations situated just outside the western margin of Bransfield Strait (St. 29 for *O. gelida*; Sts. 29 and 72 for *O. brevirima*) were genetically homogenous with Bransfield Strait populations. Even though Bransfield Strait is semi-enclosed, some water does enter through a deep gap between Brabant and Smith Islands (Zhou et al. 2002), right in the vicinity of stations 29 and 72. Clearly, these populations are "Bransfield Strait" in composition, and not allied with other Southern Peninsula populations.

The low levels of genetic variation and significantly negative neutrality statistics recovered for *O. gelida* and *O. brevirima* in Bransfield Strait can be explained by either a recent range expansion or selection acting on the mitochondrial genome (Skibinski 2000). Selection, while not ruled out, seems less likely given that it would have had to similarly

affect both species. A more likely explanation for the correspondence in geography and genetics between these species is that both species have recently experienced range expansions throughout Bransfield Strait, as suggested by mismatch distributions.

The Antarctic continental shelf and slope are considered to have been the most heavily impacted environments on earth during Pleistocene glacial cycles, and were largely uninhabitable during glacial periods (Thatje et al. 2005). Presently, Antarctic benthic habitats recently disturbed by iceberg scour are recolonized first by benthic invertebrates possessing a pelagic larval stage (Thatje 2005; Thatje et al. 2005). Accordingly, Antarctic invertebrates with planktonic larvae may have been among the few to survive on the continental shelf during glacial periods, persisting by migrating from refugia to refugia. Antarctic species with such dispersal potential would have been the first to re-colonize from deep sea/slope habitats during interglacial periods as well (Thatje 2005; Thatje et al. 2005). Given that *O. gelida* and *O. brevirima* likely produce a lecithotrophic larvae, both species could have persisted in isolated refugia in the Bransfield Strait, or on the continental slope/deep sea, during a glacial period and subsequently expanded during an interglacial period. The age of these lineages appears to pre-date the last glacial period in Antarctica (70 kya-10 kya; Anderson et al. 2002), suggesting that both species underwent population expansions prior to the last glacial maximum (17 kya to 21 kya; Gersonde et al. 2005). Krill and notothenioid fish have similarly been shown to have undergone population expansions prior to the last glacial maximum (Zane et al. 1998; Zane et al. 2006).

While our circumpolar sampling is not complete, especially for *O. brevirima*, data from this study suggest that neither *O. gelida* nor *O. brevirima* are genetically

homogeneous circumpolar species. Circumpolar populations within each species may have been isolated during glacial periods, with contemporary connectivity restricted by oceanography. While phylogeographic patterns have been evaluated using a single mtDNA region, 16S is one of the slowest evolving regions of the animal mitochondrial genome (Brown 1985). Recovery of phylogeographic breaks with 16S data indicates that only greater levels of genetic structure and divergence would be revealed using additional mtDNA genes. Ongoing work with microsatellite markers will provide more precise timing estimates for divergences and population expansions (Carstens and Knowles 2007), and will allow any fine-scale structure existing within geographic regions to be uncovered.

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Table 1 Collection information for *Ophiurolepis gelida* and *O. brevirima* from Antarctica. Station numbers correspond with Figure 1.

Region/Station	Lat/Long	Depth	N	H
Ophiurolepis gelida				
Bransfield Strait				
Low Is. – St. 13	S 63°25', W 61°51'	132 m	14	3
near Livingston Is. – St. 14	S 62°56', W 61°29'	188 m	3	2
Trinity Peninsula – St. 21	S 63°09', W 57°07'	192 m	14	2
near D'Urville Is. – St. 38	S 62°45', W 56°45'	207 m	10	2
mid-Bransfield – St. 51	S 63°23', W 60°03'	277 m	4	3
mid-Bransfield – St. D2	S 63°21', W 60°00'	246 m	3	2
Elephant Is. – St. D5	S 61°12', W 54°44'	239 m	3	1
Southern Peninsula				
Brabant Is. – St. 29	S 64°08', W 62°46'	156 m	13	5
Adelaide Is. – St. 31	S 66°37', W 68°19'	261 m	3	1
Adelaide Is. – Sts. 33/45	S 67°44', W 69°17'	122/195 m	15	8
Marguerite Bay – St. 37	S 68°11', W 67°36'	232 m	2	1
Renaud Is. – St. 53	S 65°56', W 66°54'	183 m	3	3
near Adelaide Is. – St. 80	S 66°32', W 69°59'	500 m	1	1
near Renaud Is. – St. 82	S 65°40', W 68°02'	278 m	1	1
near Anvers Is. – St. 85	S 64°41', W 65°56'	368 m	6	2
near Anvers Is. – St. 89	S 64°46', W 62°43'	445 m	1	1
near Anvers Is. – St. D8	S 64°53', W 62°54'	187 m	2	2
Subantarctic Islands				
South Sandwich Is. – St. 51	S 58°29', W 26°12'	270 m	4	2
South Sandwich Is. – St. 52	S 58°56', W 26°30'	120 m	4	2
Bouvet Is. – St. 71	S 54°22', W 03°23'	200 m	3	2
Bouvet Is. – St. 80	S 54°30', W 03°28'	159 m	2	2
Bouvet Is. – St. 81	S 54°29', W 03°18'	169 m	3	1
Ross Sea			3	3
Weddell Sea – St. 74_2	S 71°10', W 13°34'	1017 m	1	1
Total			118	29
Ophiurolepis brevirima				
Bransfield Strait				
Low Is. – St. 13	S 63°25', W 61°51'	132 m	17	3
near Livingston Is. – St. 14	S 62°56', W 61°29'	188 m	11	5
Trinity Peninsula – St. 21	S 63°09', W 57°07'	192 m	16	5
near D'Urville Is. – St. 38	S 62°45', W 56°45'	207 m	8	1
mid-Bransfield – St. 49	S 63°14', W 58°45'	87 m	3	2
mid-Bransfield – St. 51	S 63°23', W 60°03'	277 m	7	3
Southern Peninsula				
Brabant Is. – St. 29	S 64°08', W 62°46'	156 m	15	3
Brabant Is. – St. 72	S 63°51', W 62°38'	256 m	5	3
Weddell Sea – St. 74_2	S 71°10', W 13°34'	1017 m	5	2
Total			87	17

N number of individuals, H number of haplotypes

Table 2a Pairwise Φ_{ST} values between *O. gelida* stations (where N ≥ 4) from the Antarctic Peninsula and Subantarctic Islands. Station numbers correspond with Figure 1 and Table 1.

	Bransfield Strait				Southern Peninsula			Subantarctic Islands	
Bransfield Strait	St. 13	St. 21	St. 38	St. 51	St. 29	Sts. 33/45	St. 85	SSI	BI
St. 13	–								
St. 21	0.00	–							
St. 38	–0.01	0.01	–						
St. 51	0.14	0.21	0.14	–					
Southern Peninsula									
St. 29	0.01	**0.08***	0.05	0.02	–				
Sts. 33/45	**0.66*****	**0.67*****	**0.63*****	**0.54****	**0.63*****	–			
St. 85	**0.96*****	**0.97*****	**0.96*****	**0.92****	**0.92*****	0.02	–		
Subantarctic Islands									
SSI	**0.85*****	**0.88*****	**0.86*****	**0.76****	**0.73*****	**0.65*****	**0.95*****	–	
BI	**0.85*****	**0.88*****	**0.86*****	**0.76****	**0.73*****	**0.64*****	**0.95*****	0.00	–

*0.05 ≥ *P* ≥ 0.01; **0.01 > *P* ≥ 0.001; ***$P < 0.001$

Significant values ($P < 0.05$) are in boldface type

SSI pooled South Sandwich Islands population, BI pooled Bouvet Island population

Table 2b Pairwise Φ_{ST} values between *O. brevirima* stations (where N ≥ 4) from the Antarctic Peninsula and Weddell Sea.

	Bransfield Strait					Southern Peninsula		Weddell Sea
Bransfield Strait	St. 13	St. 14	St. 21	St. 38	St. 51	St. 29	St. 72	WS
St. 13	–							
St. 14	0.01	–						
St. 21	0.02	0.02	–					
St. 38	–0.05	–0.03	–0.01	–				
St. 51	**0.30****	**0.14***	**0.18****	**0.28***	–			
Southern Peninsula								
St. 29	0.03	0.03	0.04	–0.01	**0.25****	–		
St. 72	0.05	–0.05	0.00	0.10	0.15	0.08	–	
Weddell Sea								
WS	**0.88*****	**0.72*****	**0.75*****	**0.93*****	**0.76****	**0.84*****	**0.77****	–

*0.05 ≥ P ≥ 0.01; **0.01 > P ≥ 0.001; ***P < 0.001
Significant values (P < 0.05) are in boldface type

Table 3a Results of diversity estimates and neutrality statistics for *Ophiurolepis gelida* and *O. brevirima*.

			Diversity Estimates		Neutrality Tests	
	N	H	$\pi \pm$ SD	$h \pm$ SD	Tajima's D	Fu's F_S
Ophiurolepis gelida						
Bransfield Strait (including St. 29)	55	9	0.0009 ± 0.0002	0.36 ± 0.08	**–2.026***	**–8.819****
Southern Peninsula (excluding St. 29)	21	8	0.0076 ± 0.0021	0.76 ± 0.07	–0.818	–0.281
Subantarctic Islands	16	5	0.0010 ± 0.0004	0.45 ± 0.15	**–1.831***	**–3.314****
All Individuals	118	24	0.0086 ± 0.0007	0.75 ± 0.04	–1.127	**–7.421***
Ophiurolepis brevirima						
Bransfield Strait (excluding St. 51) + Southern Peninsula	72	12	0.0010 ± 0.0002	0.40 ± 0.07	**–2.230****	**–13.06****
St. 51	4	3	0.0020 ± 0.0007	0.83 ± 0.22	**–0.710**	–0.887
Weddell Sea	5	2	0.0008 ± 0.0005	0.40 ± 0.24	–0.817	0.090
All Individuals	87	17	0.0015 ± 0.0003	0.51 ± 0.07	**–2.281****	**–18.05****

*$0.05 \geq P \geq 0.01$; **$0.01 > P \geq 0.001$; ***$P < 0.001$

Significant values ($P < 0.05$) are in boldface type

N number of individuals, H number of haplotypes, π nucleotide diversity, h haplotype diversity

Table 3b Results of mismatch distribution for *Ophiurolepis gelida* and *O. brevirima*.

	Mismatch Distribution			
	θ_0 (95% CI)	θ_1 (95% CI)	*SSD*	*r*
Ophiurolepis gelida				
Bransfield Strait (including St. 29)	0.000 (0.000–0.004)	0.543 (0.000–99999)	0.014	0.251
Southern Peninsula (excluding St. 29)	0.000 (0.000–0.900)	2.772 (1.250–99999)	0.052	0.089
Subantarctic Islands	0.000 (0.000–0.054)	99999 (41.89–99999)	0.007	0.148
All Individuals	0.000 (0.000–0.319)	33334 (14.38–99999)	0.024	0.162
Ophiurolepis brevirima				
Bransfield Strait (excluding St. 51) + Southern Peninsula	0.000 (0.000–0.033)	99999 (15.29–99999)	0.000	0.146
St. 51	0.002 (0.000–0.012)	99999 (24.08–99999)	0.048	0.286
Weddell Sea	0.005 (0.000–0.009)	99999 (2.540–99999)	0.007	0.200
All Individuals	0.002 (0.000–0.018)	99999 (13.97–99999)	0.018	0.211

*$0.05 \geq P \geq 0.01$; **$0.01 > P \geq 0.001$; ***$P < 0.001$

$\theta_0 = 2\mu N_0$ and $\theta_1 = 2\mu N_1$ where N_0 and N_1 are the population sizes before and after expansion, *SSD* sum of squared deviations, *r* raggedness statistic

Table 4 Time of the most recent common ancestor for *O. gelida* and *O. brevirima* lineages. Top date was obtained using a 3%/MY mutation rate, and the bottom number resulted from a slower mutation rate of 0.5%/MY. 95% CI given in parentheses.

O. gelida	Bransfield Strait	Southern Peninsula	Subantarctic
Bransfield Strait	0.26 MY (0.06–0.57)	_	
	2.93 MY (0.07–7.30)		
Southern Peninsula	2.17 MY (0.35–5.36)	0.48 MY (0.11–1.01)	_
	13.1 MY (0.42–32.4)	5.63 MY (0.14–13.7)	
Subantarctic	0.31 MY (0.07–0.67)		0.09 MY (0.01–0.22)
	0.66 MY (0.07–1.74)		1.04 MY (0.02–2.51)
O. brevirima	Bransfield Strait		
Bransfield Strait	0.28 MY (0.07–0.59)		
	2.42 MY (0.08–6.42)		
Weddell Sea	0.31 MY (0.07–0.64)		
	2.69 MY (0.09–7.24)		

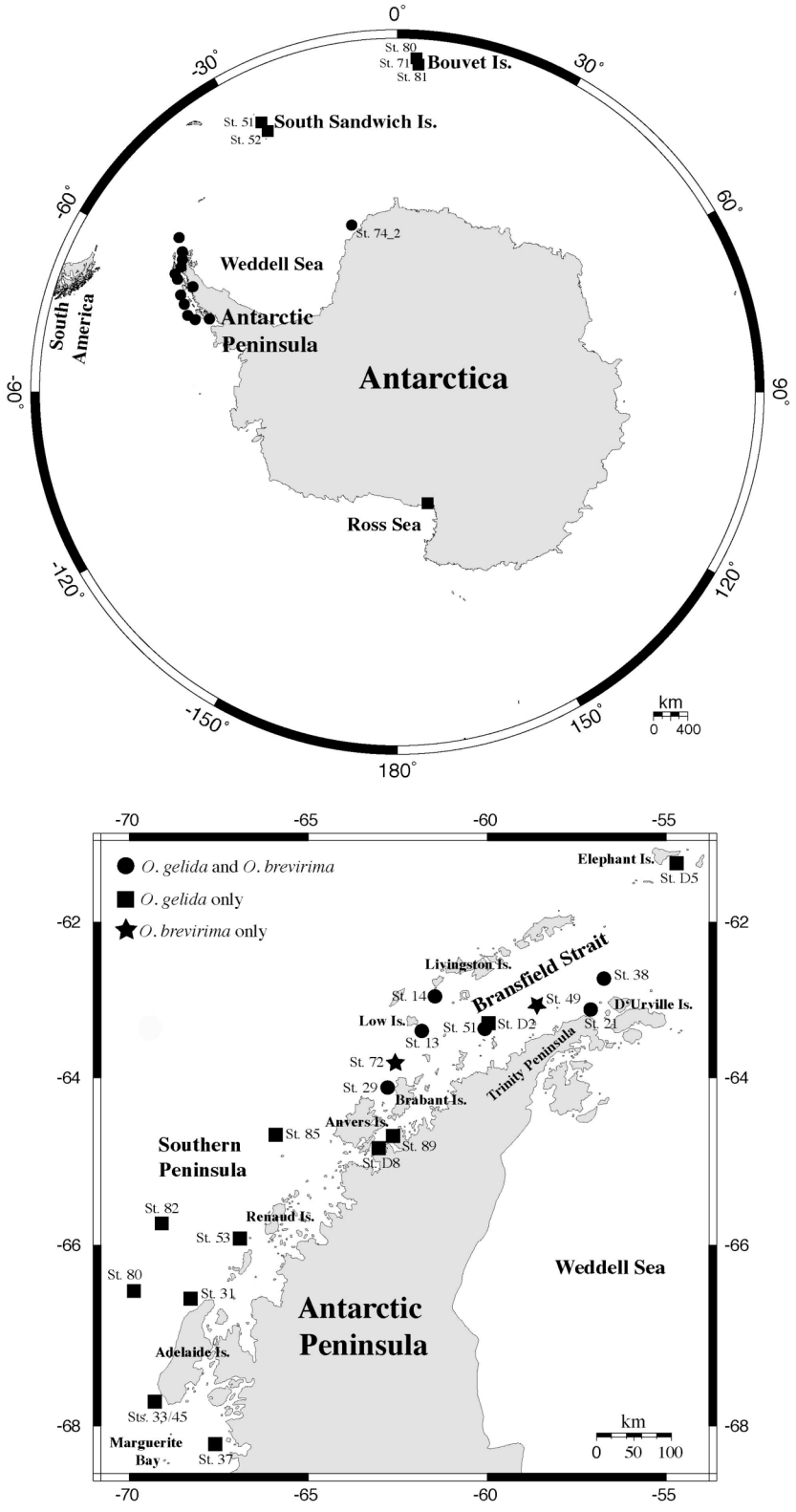

Figure 1 Map showing collection localities for *Ophiurolepis gelida* and *O. brevirima* from Antarctica and subantarctic South Sandwich Islands and Bouvet Island.

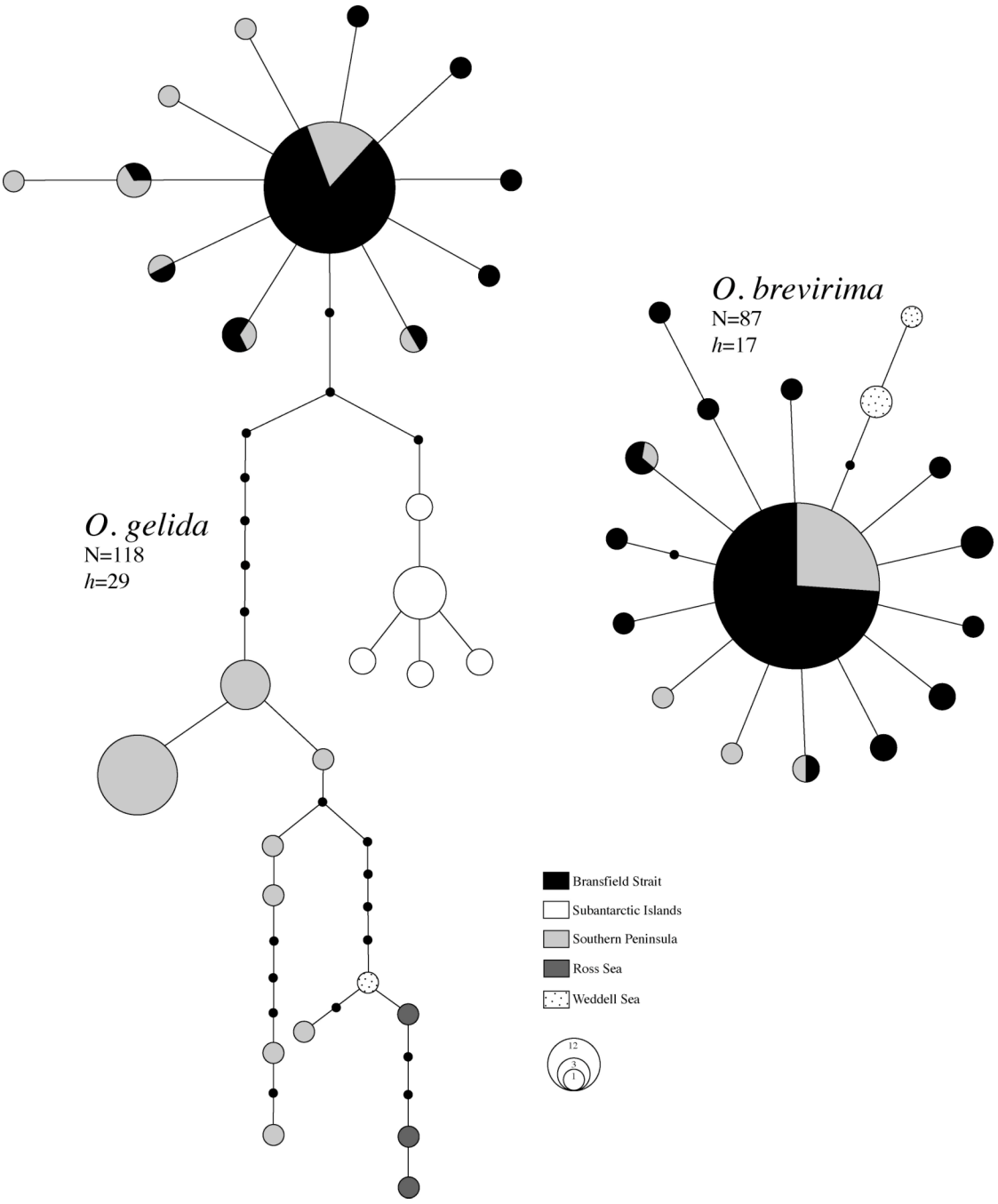

Figure 2 16S parsimony networks of 118 *O. gelida* individuals collected throughout Antarctica and from two subantarctic islands; and 87 *O. brevirima* individuals collected from the Antarctic Peninsula and Weddell Sea. Haplotype circles are coded by geographic region and sized according to relative abundance. Missing (unsampled) haplotypes are denoted by small black circles.

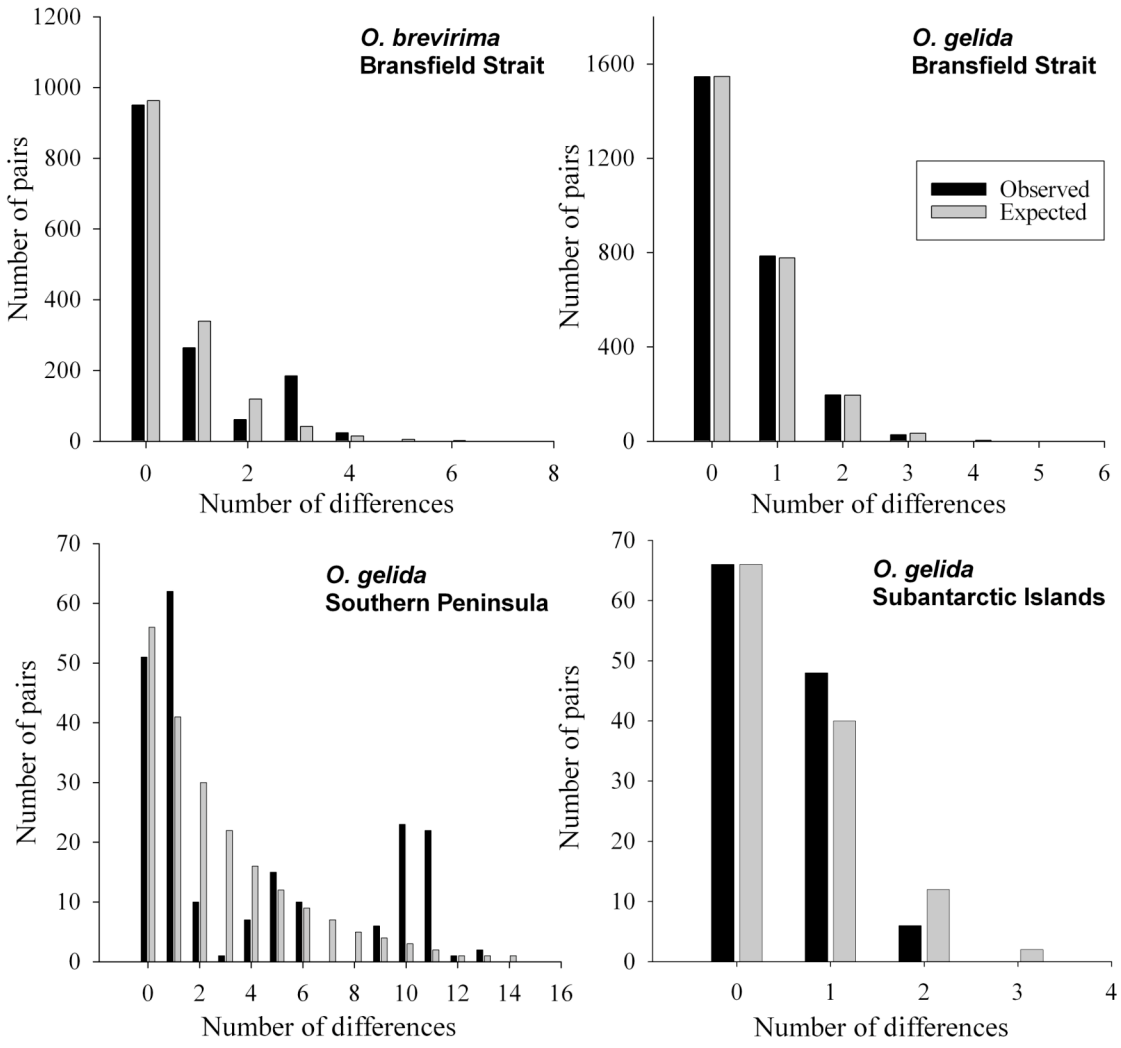

Figure 3 Observed and expected mismatch distributions for pooled *O. gelida* and *O. brevirima* geographic regions.

6.1 CONCLUSIONS

The primary goal of phylogeography studies is to understand the relationship between genealogy and geography within and among closely related species (Avise 2009). Specifically, these studies aim to determine the relative roles of contemporary and historical processes affecting the distribution and genetic composition of populations (Avise 2004). In the marine environment, phylogeography is influenced by oceanography (Wares 2002; Palumbi 2004), organismal biology and ecology (Vermeij 1989; Lindberg 1991; Duffy 1996; Hellberg 1996; Wares and Cunningham 2001; Uthicke and Benzie 2003), land barriers (Knowlton 1993), and paleoclimatology (Hewitt 2000). Major conclusions of marine phylogeographic studies to date include: 1) many species with high dispersal potential are characterized by substantial levels of population genetic structure (Avise 1994; Palumbi 1997; Benzie 1999a), 2) concordant phylogeographic breaks in multiple taxa correspond to major biogeographic boundaries in the ocean (Reeb and Avise 1990; Burton 1998; Benzie 1999a, b; Dawson 2001), and 3) cryptic speciation appears to be common (Knowlton 1993).

While comparatively few phylogeography studies have focused on Antarctic/ Southern Ocean marine organisms, recent work has revealed numerous cases of intraspecific geographic subdivision and cryptic speciation (e.g., Held 2003; Held and Wägele 2005; Linse et al. 2007; Wilson et al. 2007; Wilson et al. 2009). However, examples of long-distance connectivity and genetic homogeneity across major

biogeographic barriers in the Southern Ocean exist as well (e.g., Hunter and Halanych 2008; Mahon et al. 2008; Thornhill et al. 2008; LN Cox pers. comm.; AM Janosik pers. comm.). The research presented herein examined phylogeographic patterns in four Antarctic brittle star (ophiuroid) species, which span the range of invertebrate developmental modes and have been subject to similar oceanographic and historical climatic conditions, at least where their ranges overlap.

Although sampling in the Southern Ocean varied across species, a concordant pattern of restricted connectivity between major geographic regions emerged. South American and Antarctic populations of the brooding brittle star *Astrotoma agassizii* are genetically distinct, and gene flow is not occurring across the Drake Passage. Levels of mtDNA genetic divergence between geographically restricted clades were sufficiently high to suggest cryptic speciation in *A. agassizii*. Despite possessing planktonic larvae, both *Ophiurolepis gelida* and *Ophionotus victoriae* showed evidence of limited connectivity between Antarctic Peninsula populations and those from the subantarctic South Sandwich and Bouvet Islands. Furthermore, divergent Weddell Sea lineages were recovered in *Ophiurolepis brevirima*, *O. gelida* and *Ophionotus victoriae*. Additionally, *O. gelida* individuals in the Ross Sea are genetically distinct from Antarctic Peninsula and subantarctic individuals. These results suggest that brittle stars in the Southern Ocean often do not disperse in accordance with predictions based on life history, and mtDNA data have provided strong evidence of cryptic divergence and/or speciation for all ophiuroids studied to date. In some instances, brittle stars have lower levels of connectivity compared with other Antarctic marine invertebrates with similar life histories (see Thornhill et al. 2008; LN Cox pers. comm.; AM Janosik pers. comm.).

Until more is known about the life history, ecology and behavior of these ecologically important members of the Antarctic benthos, explanations remain tentative.

A more varied picture resulted from analysis of intraspecific mtDNA data within major geographic regions. My data indicated that the brooding *A. agassizii* was the only ophiuroid examined with genetically homogeneous populations throughout the Antarctic Peninsula (over at least 500 km). In contrast, the planktonic developing *O. gelida* and *O. victoriae* exhibited genetic subdivision between northern and southern Antarctic Peninsula populations, although some *O. victoriae* northern and southern populations were genetically similar. This seemingly paradoxical result, where a brooder displays evidence of greater connectivity than species with pelagic larvae, may be explained by the lifetime dispersal potential of these species, not just the capacity for dispersal during development. *A. agassizii*'s habit of wrapping its curled arms around epifauna subject to being dislodged (Bartsch 1982; Dearborn et al. 1986; Ferrari and Dearborn 1989), may confer greater dispersal ability to adult and juvenile *A. agassizii* individuals compared to *O. gelida* and *O. victoriae*. Accordingly, evidence is emerging that suggests the relationship between dispersal ability, based on presence/absence of a pelagic larval stage, and genetic structure is complex and not easily predicted (Benzie 1999a, 2000; Hellberg et al. 2002). Realized dispersal distances likely depend on multiple interacting biotic factors including spawning characteristics, larval behavior, interaction with conspecifics, and specific habitat requirements (Shulman and Bermingham 1995; Barber et al. 2002; Kirkendale and Meyer 2004; Imron et al. 2007), often unknown parameters for marine organisms living in extreme environments.

O. gelida and *O. victoriae* possess planktonic larvae and theoretically should be capable of long-distance dispersal (but see above). Despite their life history, both species showed restricted connectivity between northern and southern regions of the Antarctic Peninsula. However, the pattern of geographic subdivision was discordant between these species, and different phylogeographic breaks emerged. *O. gelida* is characterized by a sharp north-south phylogeographic break, separating Bransfield Strait and southern peninsula populations. In *O. victoriae*, genetic structure exists between some north-south populations, while others are genetically homogeneous. The longer-lived planktotrophic larvae of *O. victoriae* may facilitate connectivity between distant northern and southern populations (separated by ~800 km), while local oceanographic conditions may be contributing to the genetic divergence recovered between others. For *O. gelida*, multiple lines of evidence supported divergence between northern-southern populations sometime during the early Pleistocene, and populations within both regions showed evidence of fluctuations corresponding to Pleistocene glacial oscillations. While oceanography presumably plays a role in influencing contemporary levels of gene flow in *O. gelida*, the genetic signature of this species has likely been shaped primarily by recent climate conditions in Antarctica.

Overall, this work suggests that life history alone cannot be used to predict connectivity and genetic structure in Antarctic marine invertebrates. Rather, a complex interplay of life history, regional oceanography and recent climate history has likely shaped the genetic composition of most Antarctic invertebrates, and cryptic divergence/speciation is probably commonplace. This research should caution against the use of sweeping generalizations when characterizing patterns of connectivity and

diversity in the Southern Ocean, as these do not seem to be easily predictable. However, one generalization is likely to hold, future studies in the Antarctic will continue to reveal biodiversity far exceeding previous expectations.

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APPENDIX

Morphological characters used in phylogenetic analyses, with photographic illustrations of select characters and morphology data matrix.

1. Disc elevation: 0 = not higher than arms; 1 = higher than arms

Ophiurolepis and *Theodoria* are distinguished from the closely related genus *Ophiuroglypha* by two morphological characters. One character relates to the arm spines while the other is a disc that is conspicuously higher than the arms in the former two genera (Hertz 1926; Fell 1961). *Homalophiura* is intermediate to these two groups in having the arm spine character of *Ophiurolepis* and *Theodoria* but the low disc character of *Ophiuroglypha* (Fell 1960).

2. Disc plates: 0 = tumid to varying degrees; 1 = irregularly thickened; 2 = flattened

The dorsal and ventral disc plates in *Ophiuroglypha* are evenly swollen to varying degrees. Among *Ophiurolepis*, some species exhibit an uneven thickening of the disc plates while other species have flattened disc plates, as is characteristic of *Homalophiura* and *Theodoria*.

3. Disc plate texture: 0 = smooth; 1 = concentric growth lines; 2 = granular

Homalophiura, *Theodoria* and *Ophiuroglypha* typically have smooth disc plating. Species within *Ophiurolepis* have smooth disc plates or plates with conspicuous circular growth lines, with the exception of *O. granulifera* which has fine granules covering the disc (Bernasconi & D'Agostino 1973).

4. Aboral disc pattern: 0 = six primary plates conspicuous but small and widely separated by even smaller plates; 1 = six primary plates conspicuous and separated by smaller plates usually in single or double rows; 2 = rounded plates of varying size separated by a thickened skin; 3 = six primary plates form a compact pentagon in the center of the disc

The characteristic disc pattern of *Ophiurolepis* and *Theodoria* is one primary plate located centrally with five surrounding primary plates in a rosette pattern. These six primaries are separated by smaller accessory plates in belts of one or more rows. A similar pattern is found in *Ophiuroglypha*, but the six primaries are much smaller relative to the overall disc size and more widely separated by accessory plates.

5. Radial shield texture: 0 = smooth; 1 = concentric growth lines; 2 = granular

Homalophiura, *Theodoria* and *Ophiuroglypha* typically have a smooth appearance to the radial shields. Within *Ophiurolepis*, species have either a smooth

appearance or conspicuous growth lines, with the exception of *O. granulifera* which has fine granules covering the radial shields (Bernasconi & D'Agostino 1973).

6. Radial shields: 0 = tumid to varying degrees; 1 = flat; 2 = depressed

The radials shields in *Ophiuroglypha* are swollen to varying degrees in a similar manner to the other disc plates. In *Theodoria* and *Homalophiura*, the radial shields are flattened, while in *Ophiurolepis* they are either flattened or slightly concave.

7. Radial shield size: 0 = 3x or larger than the primary plates; 1 = same size to 1.5x size of primary plates

The radial shields are approximately the same size or slightly larger than the six primary plates in *Ophiurolepis*, *Theodoria* and *Homalophiura*. The radial shields are three times or greater the size of the primary plates in *Ophiuroglypha*.

8. Arm comb: 0 = well-developed; 1 = rudimentary

The genera *Ophiurolepis*, *Theodoria* and *Homalophiura* were originally described as having reduced or rudimentary arm combs. However, some *Homalophiura* species, such as *H. euryplax* and *H. intorta*, have well-developed arm combs. This character conflict as well as several others has been used in an attempt to revise the genus *Homalophiura* (Paterson 1985).

9. Ventral interradius: 0 = occupied by numerous imbricating small plates; 1 = occupied by the oral shield and several other plates of similar or smaller size; 2 = occupied by large thin scales

The ventral interradius is similar among *Ophiurolepis*, *Homalophiura* and *Theodoria* species. It is characterized by a conspicuous oral shield and several other plates of varying size, one which is similar in size to the oral shield. In contrast, in *Ophiuroglypha* the ventral interradius is characterized by many overlapping scales of similar size and a small oral shield.

10. Ventral disc plates separated by deep grooves: 0 = absent; 1 = present

One of the characters typically used to separate *Theodoria relegata* from the other two Antarctic *Theodoria* species, *T. wallini* and *T. partita*, is the presence of ventral disc plates separated by deep grooves (Fell 1961). *Homalophiura intorta* also displays this character (Lyman 1878).

11. Length of genital slit: 0 = extending to ambitus; 1 = not longer than basal arm joint

The length of the genital slit is a common diagnostic character used to separate several closely related *Ophiurolepis* species. In the species included in this study, the genital slit extends either to the edge of the disc or just to the end of the first arm joint.

12. Genital slit ornamentation: 0 = papillae along interradial margin; 1 = naked

Whether the genital slit extends to the edge of the disc or the first arm joint, its interradial edge is usually adorned with small papillae (Matsumoto 1917). These papillae often continue to the aboral surface to form a rudimentary arm comb.

13. Number of oral papillae: 0 = 7-10; 1 = 3-6

The number of oral papillae on one side of the jaw ranges from three to a maximum of six in the ingroup taxa, more typically being four or five. In *Ophiuroglypha*, the oral papillae are more numerous.

14. Shape of oral papillae: 0 = conical within and more blunt towards outside; 1 = block-like

The oral papillae are block-like and appear soldered together in *Ophiurolepis*, *Homalophiura* and *Theodoria*, whereas in *Ophiuroglypha* they are spiniform.

15. Shape of the infradental papilla: 0 = spiniform; 1 = diamond-shaped

The species used in this study typically have one infradental papilla at the apex of the jaw. This papilla is diamond to triangular-shaped in the ingroup species and spiniform in *Ophiuroglypha*.

16. Oral shield fragmentation: 0 = absent; 1 = present

Fragmentation of the oral shields is present in several species of *Theodoria*, *Ophiurolepis* and *Homalophiura*. *Theodoria partita* and *T. wallini* are distinguished in part from *T. relegata* by the presence of fragmented oral shields (Fell 1961). *Homalophiura inornata* is also known to have fragmented oral shields (Mortensen 1936). Among *Ophiurolepis*, *O. tuberosa*, *O. scissa* and *O. mordax* commonly exhibit fragmented oral shields (Koehler 1908, 1922; Mortensen 1936).

17. Jaw excavate on midline: 0 = absent; 1 = present

The genus *Theodoria*, most closely related to *Ophiurolepis*, was erected to accommodate species that had jaws that were excavate along the midline and that had conspicuous tentacle pores on the three basal arm joints (Fell 1961). The three species placed in *Theodoria* were originally described as *Amphiophiura relegata* (Koehler 1922), *Ophiurolepis wallini* (Mortensen 1925) and *Ophioglypha partita* (Koehler 1908). There are currently four species in the genus, *T. relegata*, *T. wallini*, *T. partita* and *T. madseni*.

18. Shape of arm segments in cross section: 0 = sharply triangular; 1 = circular

Ophiuroglypha species have a sharp dorsal crest in cross section (Bartsch 1982) whereas the ingroup taxa have circular arm segments in cross section.

19. Size of arm spines: 0 = one-third length of arm joint; 1 = less than one-third length of arm joint

In addition to the elevation of the disc, the other morphological character that distinguishes *Ophiurolepis* and *Theodoria* from *Ophiuroglypha* is arm spine size. *Ophiurolepis*, *Homalophiura* and *Theodoria* have minute arm spines while *Ophiuroglypha* has conspicuous arm spines that are approximately one-third the length of one arm joint (Fell 1960, 1961).

20. Number of arm spines: 0 = 3; 1 = 2

The genus *Ophiurolepis* was originally described as having two minute, peg-like arm spines and two similar tentacle scales (Matsumoto 1915). The same character state occurs in *Homalophiura* (Mortensen 1936; Paterson 1985) but differs in *Theodoria* which has three arm spines (Fell 1961).

21. Shape of arm spines: 0 = blunt; 1 = conical

Ophiurolepis was originally described as having peg-like arm spines (Matsumoto 1915). The same character state occurs in both *Homalophiura* (Mortensen 1936; Paterson 1985) and *Theodoria* (Fell 1961).

22. Arm spine arrangement: 0 = contiguous; 1 = widely but evenly spaced; 2 = dorsal-most spine separated from other arm spine and tentacle scales by a gap

Most *Ophiurolepis* species have a dorsal arm spine that is separated from the other arm spine and tentacle scales by a gap. Several *Homalophiura* species share this character. *Theodoria* and *Ophiuroglypha* species have arm spines that are evenly spaced across the distal margin of the lateral plate.

23. Middle arm spine formed into a hyaline hook: 0 = present; 1 = absent

The genus *Ophiuroglypha* characteristically has the middle arm spine transformed into a hyaline hooklet in the distal part of the arms (Hertz 1926; Fell 1961).

24. Distal arm spine modified into a hook: 0 = absent; 1 = present

Homalophiura euryplax and *H. intorta* belong to the group of *Homalophiura* species that are referable to *Ophiura*, according to Paterson (1985). This group includes *Homalophiura* species that share several morphological characters, one of which is the distalmost arm spine being transformed into a hook.

25. Basal tentacle pores: 0 = conspicuous; 1 = not conspicuous

The diagnostic morphological characters for *Theodoria* are conspicuous tentacle pores on the three basal arm joints and jaws that are excavate along the midline of each jaw. *Ophiurolepis* has inconspicuous tentacle pores throughout the length of the arm (Fell 1961).

26. Number of tentacle scales beyond proximal joints: 0 = 1; 1 = 2

Ophiurolepis was originally described as having two minute, peg-like arm spines and two similar tentacle scales (Matsumoto 1915). The same character state occurs in *Homalophiura* (Mortensen 1936; Paterson 1985) and *Theodoria* (Fell 1961).

27. Dorsal arm plates: 0 = contiguous throughout most of arm length; 1 = contiguous only proximally; 2 = not contiguous

The dorsal arm plates in *Ophiurolepis* vary between being contiguous throughout the entire length of the arm to being contiguous only in the proximal arm joints. In *Theodoria*, the dorsal arm plates are proximally contiguous, while amongst *Homalophiura* they can be contiguous throughout, only proximally contiguous or discontinuous throughout.

28. Shape of dorsal arm plates beyond proximal joints: 0 = rectangular; 1 = lozenge shaped

The dorsal arm plates become lozenge shaped after the first several arm joints in *Ophiurolepis, Theodoria* and *Homalophiura*. In *Ophiuroglypha*, they become markedly rectangular.

29. Dorsal arm plates modified into hook-like knobs: 0 = absent; 1 = present

Ophiurolepis olstadi and *O. anceps* are similar morphologically to *O. gelida* and *O. brevirima*, but can be distinguished in part by their conspicuous dorsal arm plates, which form hook-like knobs.

30. Dorsal arm plates with distal tubercle: 0 = absent; 1 = present

Among *Ophiurolepis*, *O. brevirima*, *O. gelida*, *O. tuberosa*, *O. turgida, O. olstadi* and *O. anceps* are distinct in having the distal edge of the dorsal arm plates raised into a tubercle (Mortensen 1936).

31. Dorsal arm plate fragmentation: 0 = absent; 1 = present

This feature of the dorsal arm plates is found in several *Theodoria*, *Ophiurolepis* and *Homalophiura* species. *Theodoria partita* is distinguished from *T. wallini* by its fragmented dorsal arm plates (Fell 1961). *Homalophiura inornata* is known to have fragmented dorsal arm plates (Mortensen 1936) as is *H. clasta* (Clark 1911). Among *Ophiurolepis*, *O. tuberosa* and *O. scissa* commonly show this feature (Koehler 1908; Mortensen 1936).

32. Ventral arm plates: 0 = broadly contiguous throughout; 1= contiguous only proximally; 2 = not contiguous

The ventral arm plates are contiguous on the proximal arm joints only or discontinuous throughout in *Ophiurolepis* and *Homalophiura*. In *Theodoria*, the ventral arm plates are most commonly discontinuous on all arm joints.

33. *Iophon* known to parasitize: 0 = absent; 1 = present

The parasitic sponge, *Iophon radiatus*, is known to infest only *Ophiurolepis gelida* and *O. brevirima*. Fell (1961) suggested that the degree of infestation could even be used to distinguish between *O. gelida* and *O. brevirima*, since *O. gelida* was more heavily infested than *O. brevirima* in specimens examined. It is unknown why this sponge species infests only two *Ophiurolepis* species when several other species are common (Dearborn *et al.* 1973).

34. Gonads: 0 = numerous gonads along adradial and interradial side of genital slits; 1 = two to four gonads along adradial and interradial side of genital slits; 2 = two gonads at interradial side of genital slit with one to two gonads at the adradial side of genital slit

Mortensen (1936) commented on the gonad character of the *Ophiurolepis, Theodoria, Homalophiura* and *Ophiuroglypha* species he examined. *Ophiurolepis* species are variable for this character, with some species having numerous gonads and others only a few.

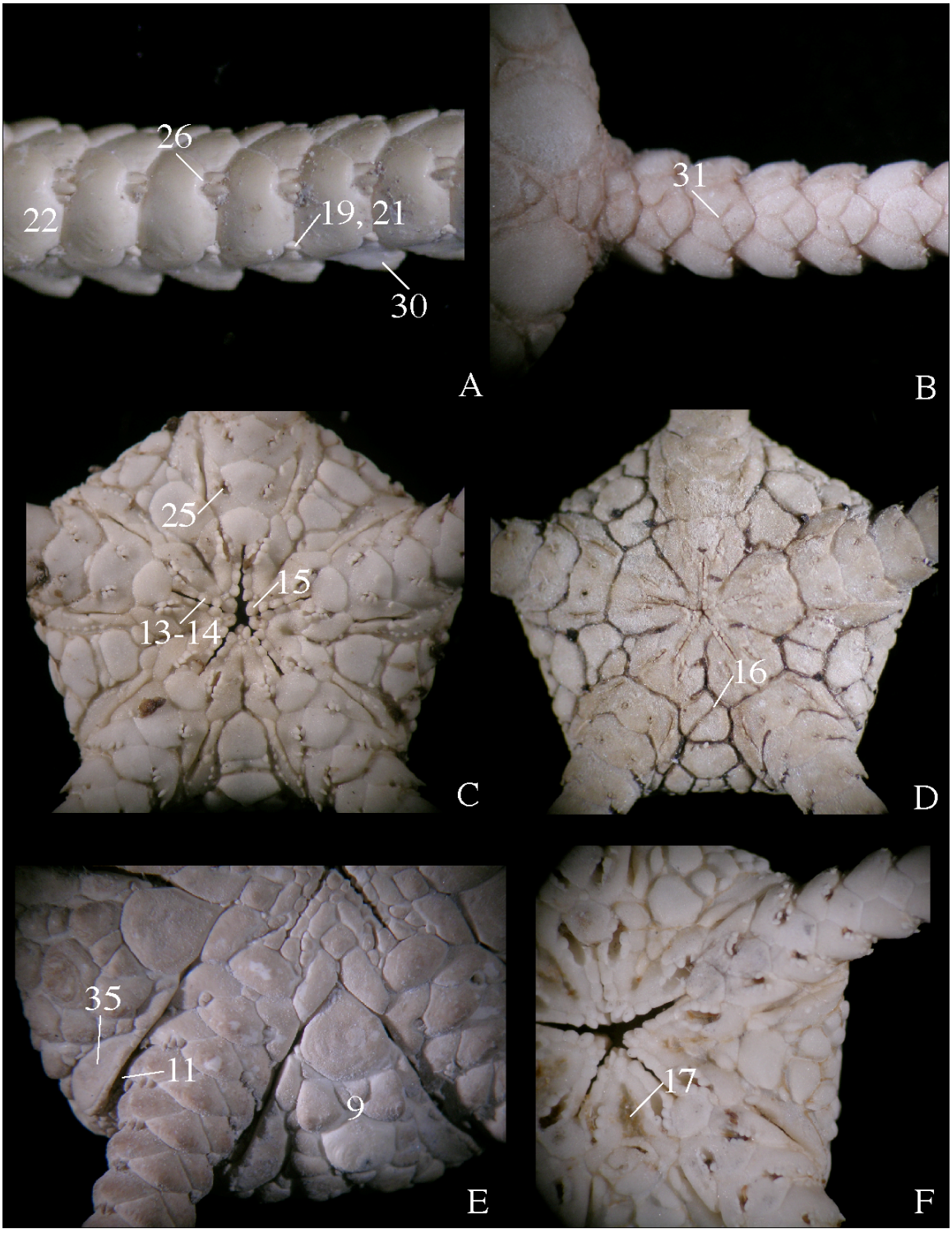
35. Genital plate shape: 0 = narrow and bar-like; 1 = half-moon shape

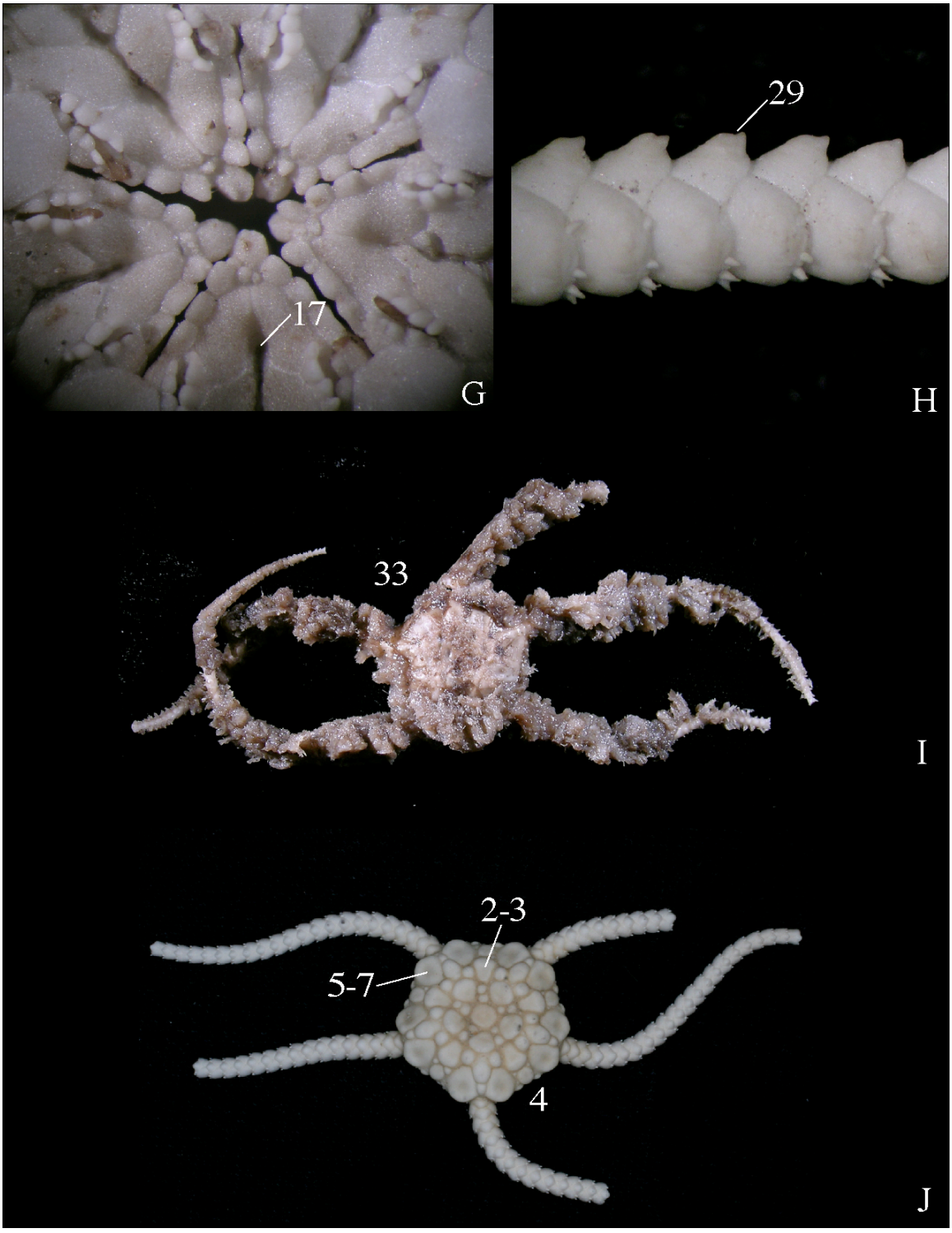
The genital plate has a wide crescent shape in *Ophiurolepis* species and in *T. partita* and *T. wallini*. This character is variable in *Homalophiura*, with some species possessing a narrow, straight genital plate and others the crescent shape typical of *Ophiurolepis*.

36. Second oral tentacle pore opens into oral opening: 0 = present; 1 = absent

In *Ophiuroglypha*, the second oral tentacle pore opening is positioned towards and opens into the oral opening. In *Ophiurolepis, Theodoria* and *Homalophiura*, the second oral tentacle pore is separate from the oral opening.

Select morphological characters used in analysis. Character numbers correspond with Appendix. A. *O. gelida* lateral arm view. B. *O. scissa* dorsal arm view. C. *T. wallini* oral view. D. *O. tuberosa* oral view. E. *O. gelida* oral view. F. *T. relegata* oral view. G. *T. wallini* oral view. H. *O. olstadi* lateral arm view. I. *O. gelida* aboral view. J. *O. banzarei* aboral view.




17
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Matrix of morphology characters

	00000000011111111112222222223333333 12345678901234567890123456789012345 6
O. accomodata	11010101100011110111121011110001 0?11
O. anceps	11111211101011100111121011?111010?11
O. banzarei	11010211101011100111121011110101 0?11
O. brevirima	11111211101011100111121011010101 1111
O. carinata	11111211100011100111121011010001 0011
O. gelida	11111211100011100111121011010101 1111
O. granulifera	11212211101011100111121011110101 0?11
O. martensi	12010111100011100111121011110001 0211
O. mordax	12010111101011101111121011110002 0?11
O. olstadi	11111211101011100111121011011101 0?11
O. scissa	02010111101011101111110111100110?11
O. tuberosa	12010111101011101111121011210112 0?11
O. tumescens	11010211100011100111121011110101 0?11
O. turgida	11010111101011100111121011010101 0?11
H. brucei	02010111101111100111?101111000 20?11
H. confragosa	02010111101011100111121011110011 0?11
H. inornata	02010111101011101111121011110011 0011
H. clasta	02020101100011100111121011110012 0?11
H. euryplax	02030110200110000101111110210002 0?01
H. intorta	01010110110011100111111111??00010?01
T. partita	12010111101011111111111001110012 0211
T. relegata	12010111110011101111111001110002 0?01
T. wallini	12010111101011011111111001110002 0211
T. madseni	?111?11111??1110111?1?100???0?020???
Ophiuroglypha carinifera	00000000000000000000000000000000000 0
Ophiuroglphya lymani	00000000000000000000000000000000000 0