

Evaluating Connectivity in the Brooding Brittle Star *Astrotoma agassizii* across the Drake Passage in the Southern Ocean

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Abstract

Studies examining population 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, 2 mitochondrial DNA 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 3 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.

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 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 and 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 (Paternello 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 CO₂ concentration (DeConto and Pollard 2003), is thought to have driven the gradual cooling and glaciation that began approximately 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).

Whereas 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 mollusk conspecifics have been reported on both Antarctic and South American continental shelves, and a well-recognized faunal affinity exists between these 2 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, Marcato et al. 2000; Bargelloni, Zane et al. 2000; Stankovic et al. 2002), krill (Paternello et al. 1996; Bargelloni, Zane et al. 2000), and mollusks (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 13 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; Smirnov IS, personal communication) 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 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 mitochondrial DNA (mtDNA) gene fragments were employed to evaluate the effects of geographic

distance, depth, and the polar front on population structure and connectivity in this conspicuous Southern Ocean species.

Materials and Methods

Data Collection

Astrotoma agassizii samples were collected during 2 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 to 22 December 2004 and the second from 12 May to 13 June 2006. In total, 207 individuals were collected from 11 stations in South American waters and 30 individuals were collected from 6 Antarctic stations (Figure 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 -80°C or preserved in approximately 85% ethanol.

DNA was extracted using the DNeasy[®] Tissue Kit (Qiagen, Valencia, CA) following manufacturer's protocol. Two mitochondrial gene fragments, 16S rDNA (16S) and cytochrome oxidase subunit II (COII), were amplified using standard polymerase chain reaction (PCR) protocols. 16SarL (5'-CGCCTGTTTATCAAAAACAT-3') and 16SbrH (5'-CCGGTCTGAACTCAGATCAGT-3') (Palumbi et al. 1991) amplify an approximately 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'-TCSGARCA-TTGSCCATARAA-3') amplify an approximately 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, Bedford, MA). Purified PCR products were bidirectionally sequenced using a CEQ8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA). All *A. agassizii* haplotypes were deposited in GenBank and correspond to accession numbers EF565745–EF565820 (see Supplementary Material).

Population Structure Analyses

Sequences were edited in SeqMan (DNA* LASERGENE, Madison, WI) and aligned with ClustalW (Thompson et al. 1994) in MegAlign (DNA* LASERGENE). Alignments were examined visually in MACCLADE v4.0 (Maddison WP and Maddison DR 2000), and COII sequences were translated to ensure that stop codons were not present. 16S and COII alignments are available in TreeBASE. Repetitive sequences were collapsed into representative haplotypes in COLLAPSE v1.2 (<http://darwin.uvigo.es/>). Preliminary analyses of 16S and COII indicated that 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%

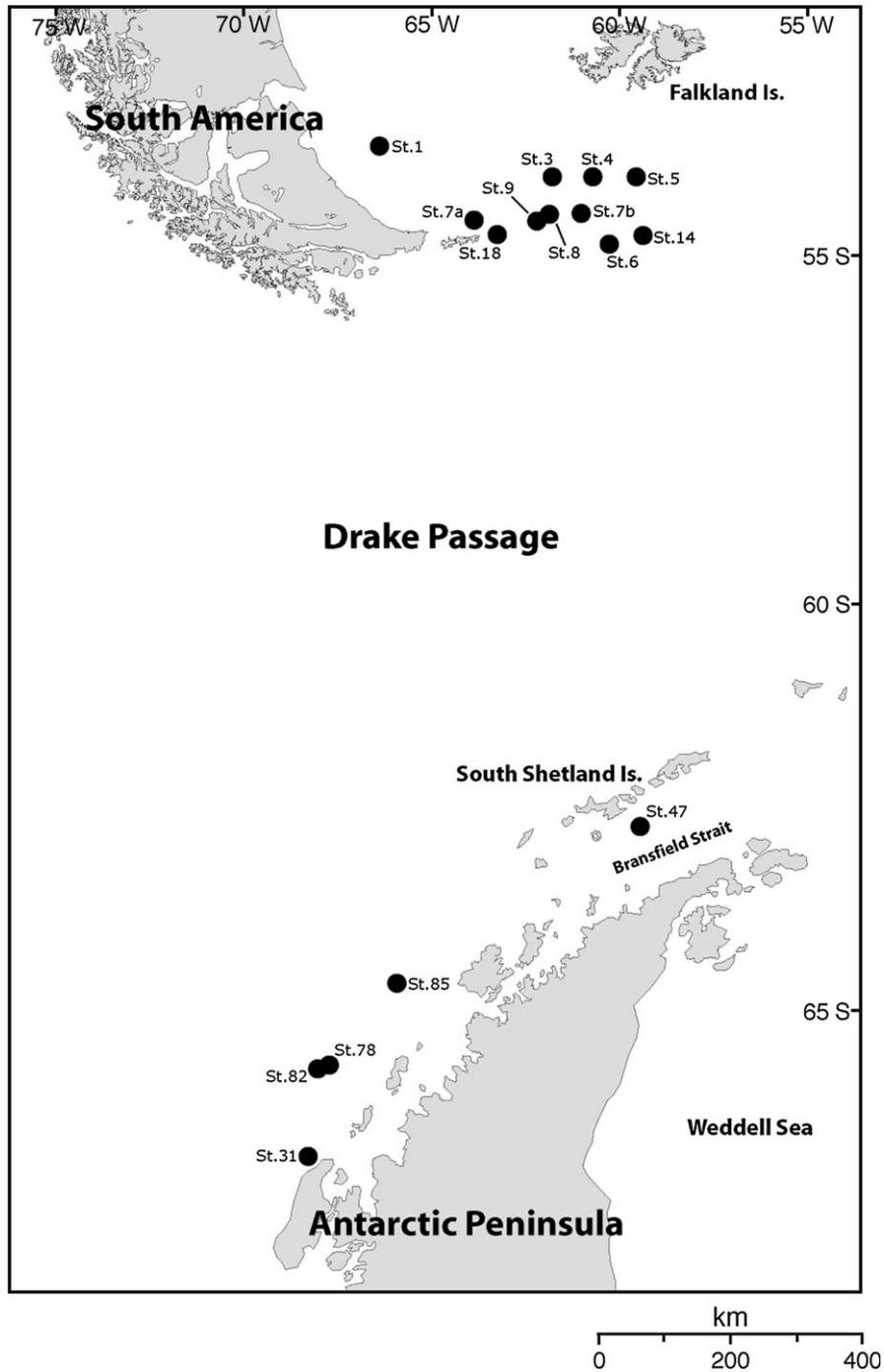


Figure 1. Map showing collection localities for *Astrotoma agassizii* from South America and Antarctica.

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 3 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 data set with 10 000 permutations

Table 1. Collection information for *Astrotoma agassizii* in South America and Antarctica

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

Collection station numbers correspond with Figure 1 and *N* refers to number of individuals sequenced for 16S and COII.

and the Tamura–Nei model (Tamura and Nei 1993) with among-site rate variation were used.

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 4 or more individuals were sampled.

In order to evaluate levels of migration between pairs of populations, a Markov chain Monte Carlo approach was taken as implemented in the program MDIV (Nielsen and Wakeley 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 Hasegawa, Kishino, and Yano (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.

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, whereas for COII, the GTR + Γ model was chosen. Conditions for analysis were uniform prior distribution of parameters and 2 sets of 4 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 transversal model with gamma shape parameter ($\alpha = 0.778$) and proportion of invariable sites (0.7696) was selected.

Results

In total, 118 individuals were sequenced for 16S (490 bp) and COII (493 bp), resulting in a 983-bp concatenated data set. The combined data set 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 was required for alignment.

Population Structure

Parsimony network analysis resulted in 3 haplotype networks, whether mtDNA gene fragments were analyzed separately (data not shown) or combined (Figure 2). For the combined data, network 1 (=clade 1) included 38 haplotypes from 58 individuals from South America, network 2 (=clade 2) was composed 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

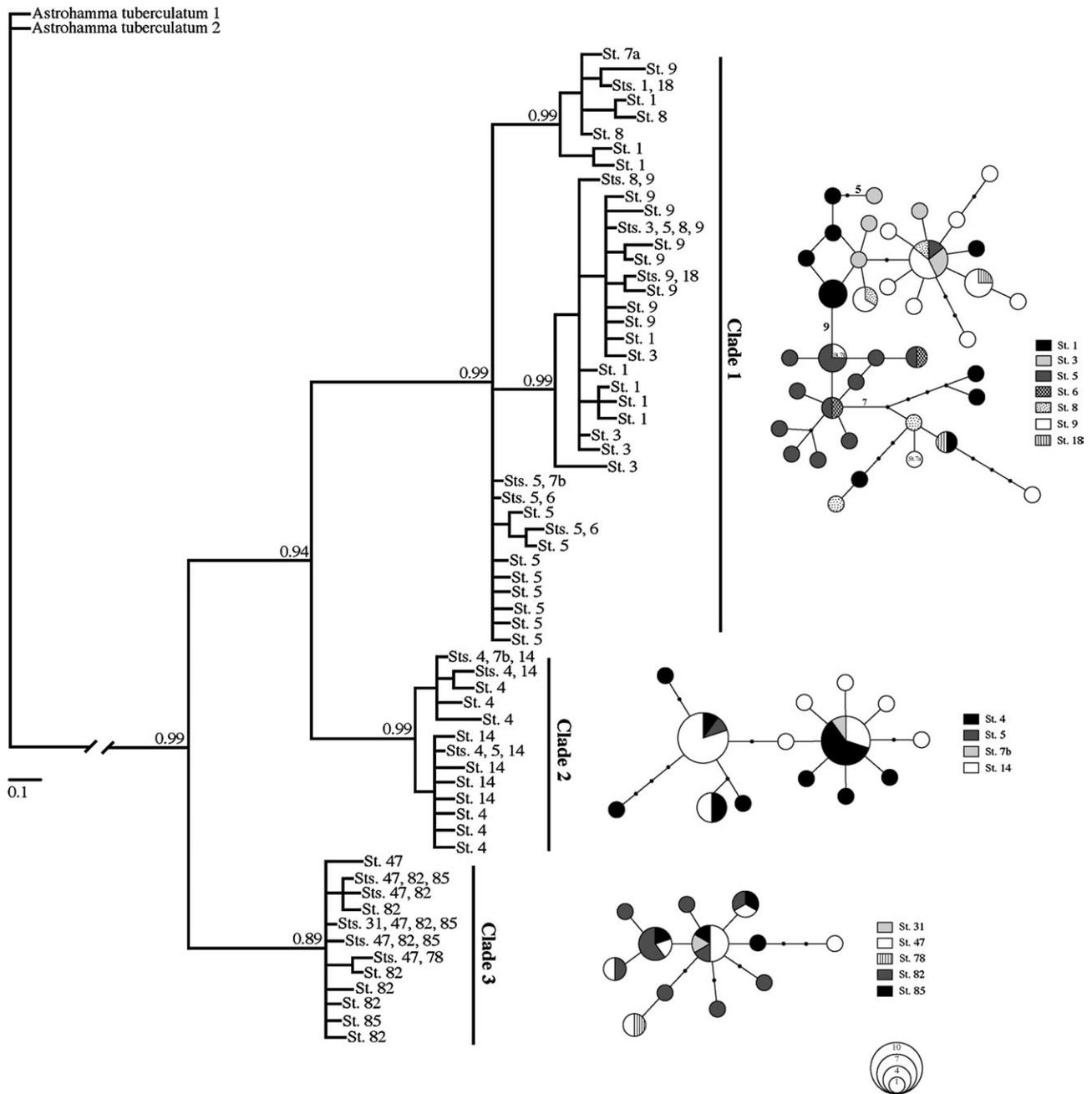


Figure 2. Bayesian tree of unique 16S + COII mtDNA haplotypes, with corresponding haplotype networks for each of 3 phylogenetic clades. Numbers next to nodes indicate Bayesian posterior probabilities. On the Bayesian tree, haplotypes are labeled according to station. In networks, circles are coded by 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.

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 3 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, whereas only 5% ($P < 0.0001$) was attributable to that between collection stations within a clade.

Table 2. Pairwise Φ_{ST} for each 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 $P < 0.05$. St., station.

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 with clade 1. Results of the Mantel test, performed only on collection stations within clades 1 and 3 given that clade 2 contained only 2 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 nonsignificant for 16S, whereas 3 populations (station 5, station 9, and stations 4/14) had significantly negative values for COII (Figure 3), indicative of past population expansion (Aris-Brosou and Excoffier 1996). The shape of the 16S and COII mismatch distributions was unimodal for clades 2 and 3 (Figure 3), suggesting past population expansion. Clade 1 distributions were primarily ragged and multimodal (Figure 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 2 stations.

Migration analyses revealed stations 47 and 82 (clade 3) from Antarctica to be experiencing the highest levels of gene flow. These 2 collection stations were 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).

Phylogenetic Relationships and Genetic Distances

Phylogenetic analysis of mtDNA haplotypes revealed 3 distinct lineages within *A. agassizii*, 2 in South America and 1 in Antarctica (Figure 2). These 3 clades correspond to the 3 networks recovered in the parsimony network analysis. The 2 South American clades (clades 1 and 2) were recovered as sister clades with a posterior probability of 0.94, whereas 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), whereas 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.

Table 3. Hierarchical AMOVA for South American and Antarctic populations of *Astrotoma agassizii*

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

Discussion

Cryptic Species in *A. 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;

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
Antarctica	2	Sts. 4, 14	33	3	12	0.0003 ± 0.0002	0.0037 ± 0.0006	0.12 ± 0.08	0.82 ± 0.05
	3	Sts. 47, 82, 85	23	5	10	0.0009 ± 0.0004	0.0037 ± 0.0005	0.32 ± 0.12	0.88 ± 0.04

St., station.

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 3 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 3 distinct lineages. Further, mtDNA genetic distances between the 3 clades ranged from 4.8% to 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 1 and likely criterion 2. 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. Although 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 *A. agassizii* is a complex of at least 3 cryptic species in the Southern Ocean.

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 approximately 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 et al. 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 of *A. agassizii* 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.

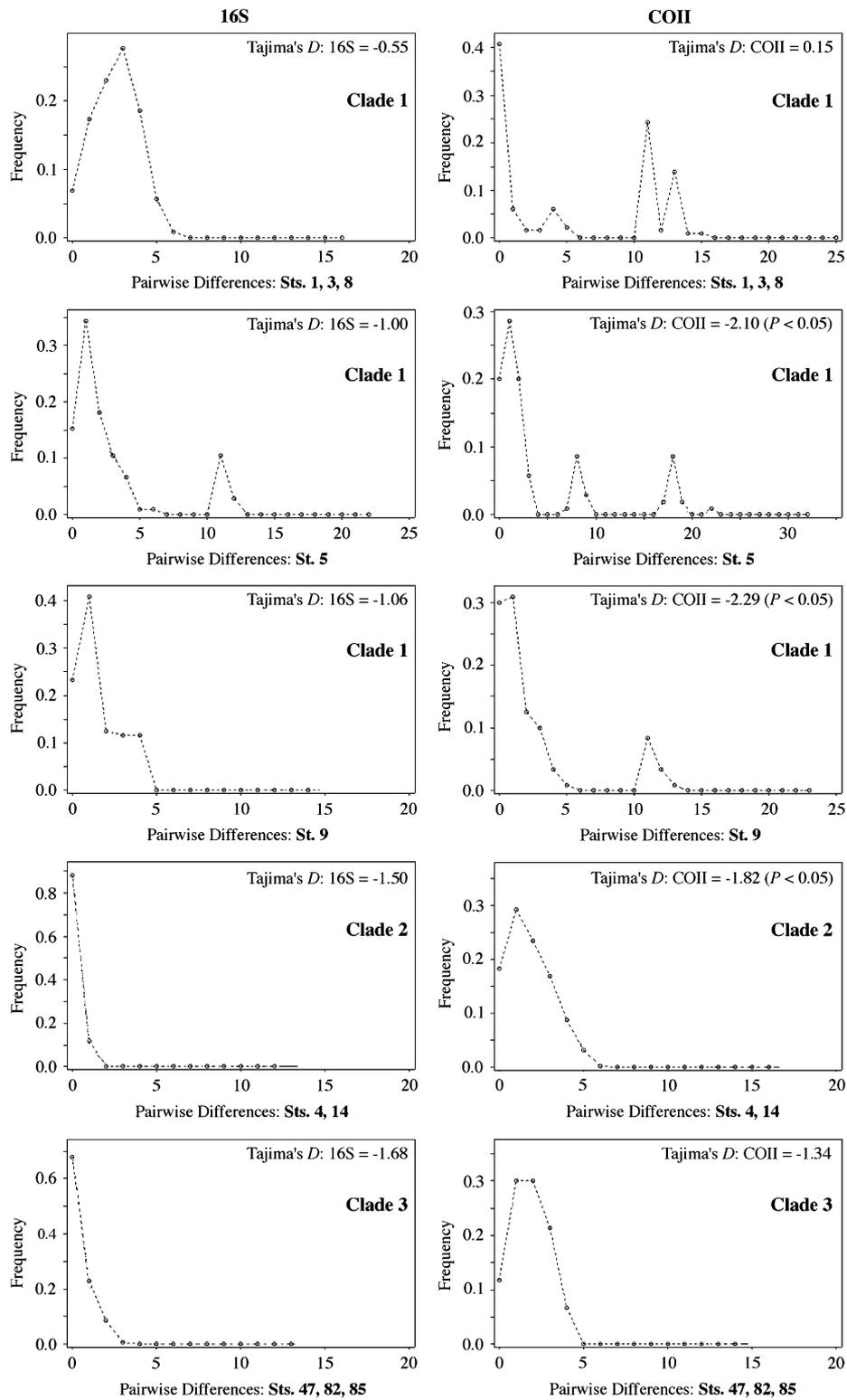


Figure 3. Mismatch distributions and Tajima's *D* statistic for pooled *Astrotoma agassizii* collection stations. Significant Tajima's *D* values are indicated by $P < 0.05$.

Restricted gene flow was also evident within the South American continental shelf. The 2 South American clades had lower interclade genetic distances when compared with the Antarctic clade. However, genetic distances were similar for clades 1–2 and clades 2–3 comparisons, indicating that the South American clades split soon after diverging from the common ancestor of all 3 clades. Comparisons of physical characteristics between collection stations belonging to the 2 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).

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 (Spöner and Roy 2002; Richards et al. 2007). Given that *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 (Spöner 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 (Figure 1) to the southern peninsula, and vice versa. A surface current circulates counterclockwise around the Antarctic coast (Phillipot 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 clades 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 2 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 2 instances of divergent haplotypes co-occurring at the same collection station. Station 5 was composed 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 clades 1 and 2 to be existing sympatrically at this locale. Similarly, station 7b, where only 2 individuals were sampled, was characterized by a single clade 1 haplotype and a single clade 2 haplotype.

In summary, *A. 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.

Supplementary Material

Supplementary material can be found at <http://www.jhered.oxfordjournals.org/>.

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