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Open-ocean barriers to dispersal: a test case with the Antarctic Polar Front and the ribbon worm *Parborlasia corrugatus* (Nemertea: Lineidae)

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Abstract

Open-ocean environments provide few obvious barriers to the dispersal of marine organisms. Major currents and/or environmental gradients potentially impede gene flow. One system hypothesized to form an open-ocean dispersal barrier is the Antarctic Polar Front, an area characterized by marked temperature change, deep water, and the high-flow Antarctic Circumpolar current. Despite these potential isolating factors, several invertebrate species occur in both regions, including the broadcast-spawning nemertean worm *Parborlasia corrugatus*. To empirically test for the presence of an open-ocean dispersal barrier, we sampled *P. corrugatus* and other nemerteans from southern South America, Antarctica, and the sub-Antarctic islands. Diversity was assessed by analyzing mitochondrial 16S rRNA and cytochrome *c* oxidase subunit I sequence data with Bayesian inference and TCS haplotype network analysis. Appropriate neutrality tests were also employed. Although our results indicate a single well-mixed lineage in Antarctica and the sub-Antarctic, no evidence for recent gene flow was detected between this population and South American *P. corrugatus*. Thus, even though *P. corrugatus* can disperse over large geographical distances, physical oceanographic barriers (i.e. Antarctic Polar Front and Antarctic Circumpolar Current) between continents have likely restricted dispersal over evolutionary time. Genetic distances and haplotype network analysis between South American and Antarctic/sub-Antarctic *P. corrugatus* suggest that these two populations are possibly two cryptic species.

Keywords: 16S, Antarctic Circumpolar Current, Antarctic Polar Front, Antarctica, COI, cryptic species

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Introduction

Geographical and hydrological barriers that limit gene flow are well documented in terrestrial and freshwater environments. However, in marine systems such barriers are often less apparent (e.g. Palumbi 1992, 1994) and have been postulated to be absent in many cases (e.g. Norris 2000). Although some holoplanktonic metazoans exhibit little genetic differentiation over large (> 1000 km) spatial scales (e.g. Fevolden & Scheppenheim 1989; Bucklin & Kocher 1996; Bucklin *et al*. 1996, 1997, 2000; Zane *et al*. 1998; Zane & Patarnello 2000; Jarman *et al*. 2002; Sands *et al*. 2003), many benthic and demersal organisms do not realize their full dispersal potential (e.g. Burton & Feldman 1981; Knowlton & Keller 1986; Cowen *et al*. 2006; Lessios *et al*. 2001; Baums *et al*. 2005, 2006; Jones *et al*. 2005; Severance & Karl 2006; Bird *et al*. 2007). Limited dispersal can be caused by ecological factors (e.g. physiological tolerances, competition, or life-history traits) and/or extrinsic biogeographical barriers. In marine environments, known biogeographical barriers include: (i) land masses, such as the Isthmus of Panama (Bermingham & Lessios 1993); (ii) land barriers that emerge during periods of low sea-surface level, such as the Indonesian channels separating the Indian and Pacific Oceans (Benzie 1999; Barber *et al*. 2000); (iii) latitudinal changes in climate, such as the California transition zone (Dawson 2001); and/or (iv) oceanic

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currents, such as the Mona Passage separating the islands of Hispaniola and Puerto Rico (Baums *et al*. 2005, 2006). Examples of biogeographical barriers in open-ocean environments are less well understood. Several examples have been proposed, including the East Pacific Barrier (Ekman 1953) and the Antarctic Polar Front (APF).

The Antarctic Polar Front is an area surrounding the Antarctic continent characterized by both intense current and a 3–4 °C temperature cline (Eastman 1993). The APF formed following the break up of the South American and Antarctic continents that led to the creation of a seaway, the Drake Passage, and the establishment of the Antarctic Circumpolar Current (ACC; Lawver & Gahagan 2003; Pfuhl & McCave 2005; Scher & Martin 2006). Biologically, the hypothetical result of this separation was the isolation of Antarctic fauna for between 20 million years and 41 million years (Lawver *et al*. 1992; Lawver & Gahagan 2003; Scher & Martin 2006; Rogers 2007), creating a high degree of endemism in Antarctica (Arntz *et al*. 1997). Additionally, the Drake Passage is apparently too deep to allow dispersal of shelf organisms along the benthos (e.g. Shaw *et al*. 2004; Hunter & Halanych 2008), despite eurybathy in many organisms (Gutt 1991; Brey *et al*. 1996). However, certain invertebrate groups reportedly include species that occur on both sides of the Drake Passage (e.g. Ekman 1953; Hempel 1985), including the nemertean *Parborlasia corrugatus* (McIntosh 1876). Such a distribution implies some level of gene flow must be occurring, a remarkable phenomenon given the extremes of distance, depth, temperature clines, and prevailing currents that organisms must overcome to move between South America and Antarctica (Whitworth *et al*. 1982; Eastman 1993; Dayton *et al*. 1994; Klink & Nowlin 2001).

Parborlasia corrugatus is a conspicuous heteronemertean reported to range from intertidal to 3950 m in depth across South American, sub-Antarctic, and Antarctic regions (Gibson 1983). This nemertean can be quite abundant, with reported densities ranging from 0.3 m^{-2} in McMurdo Sound (Heine *et al*. 1991) to 26.2 m^{-2} around Signy Island (Clarke & Prothero-Thomas 1997). Additionally, *P. corrugatus* is relatively well known due to its large size (up to 2 m in length and a wet mass of over 140 g; Knox 1970; Gibson 1983, 1985; Heine *et al*. 1991). This avid scavenger and predator is reported to have a diverse diet including detritus, diatoms, sponges, anemones, polychaetes, gastropods, amphipods, isopods, and/or vertebrate carrion (Gibson 1983; Clarke & Prothero-Thomas 1997). However, like many nemerteans, *P. corrugatus* is likely distasteful to other organisms as there are no reports of predation upon them (Heine *et al*. 1991). Two congeners of *P. corrugatus* have been described from the region — *Parborlasia fueguina* Serna de Esteban & Moretto, 1968, from the Bay of Ushuaia but reported from additional sites by Gibson (1985), and *Parborlasia landrumae* (Gibson 1985), from one site off Wilkes Land, on the South Indian Ocean side of Antarctica.

The dioecious *P. corrugatus* broadcast spawns, presumably throughout the year (Pearse *et al*. 1991; Shreeve & Peck 1995; Stanwell-Smith *et al*. 1997), producing pilidium larvae that are long-lived in the water column (possibly up to 150 days; Peck 1993). Consistent with these observations, heteronemertean pilidia (presumably including *P. corrugatus*) can be found in the water column around Antarctica throughout the year (Pearse *et al*. 1991; Stanwell-Smith *et al*. 1999; K. M. Halanych, personal observation.). Therefore, *P. corrugatus* likely has the capacity to disperse across long distances. Additionally, metamorphosed juvenile *P. corrugatus* can be pelagic for a time and have been reported further offshore and in deeper waters than pilidia (Shreeve & Peck 1995). Finally, adult *P. corrugatus* have been found attached to large macroalgae (Gibson 1983) and may be capable of rafting (Highsmith 1985), but the importance of such dispersal to historical genetic patterns is unclear.

To date, genetic patterns of *P. corrugatus* have only been explored around the South Orkney Islands (~600 km northeast of the Antarctic Peninsula). Using allozyme markers, Rogers *et al*. (1998) detected high levels of genetic identity, but also noted the lack of heterozygotes at the sampled localities. Here, we build on this previous work by examining populations of *Parborlasia* spp. from southern South America, sub-Antarctic islands, and Antarctica to determine the level of genetic differentiation that exists between populations on either side of the Drake Passage and around the Antarctic continent. More specifically, patterns of gene flow may elucidate if and how the ACC and the APF are promoting or hindering gene flow in this species. Sequence data from 16S rRNA and cytochrome *c* oxidase subunit I (COI) mitochondrial genes were used in a Bayesian phylogenetic and coalescent context to infer evolutionary history. In addition, museum specimens of whole and sectioned *P. corrugatus*, *P. fueguina*, and *P. landrumae* were examined in order to compare molecular results with morphological characteristics based on descriptions of *Parborlasia* species diversity.

Materials and methods

Sample collection

Parborlasia corrugatus individuals, *Parborlasia*-like worms, and all other available nemerteans were collected from South America and Antarctica during two Antarctic expeditions aboard the *R/V Laurence M. Gould* in November–December of 2004 and May–June of 2006. Benthic samples were collected using a Blake trawl, wire dredge, or epibenthic sled. Larval nemertean specimens were collected using a 250 μm mesh conical net with a 0.75-m diameter mouth, by towing for 20 min in a slow oblique descent to a depth of approximately 200 m followed by a similar return to the surface. Additionally, H. W. Deitrich (via S. J. Lockhart)

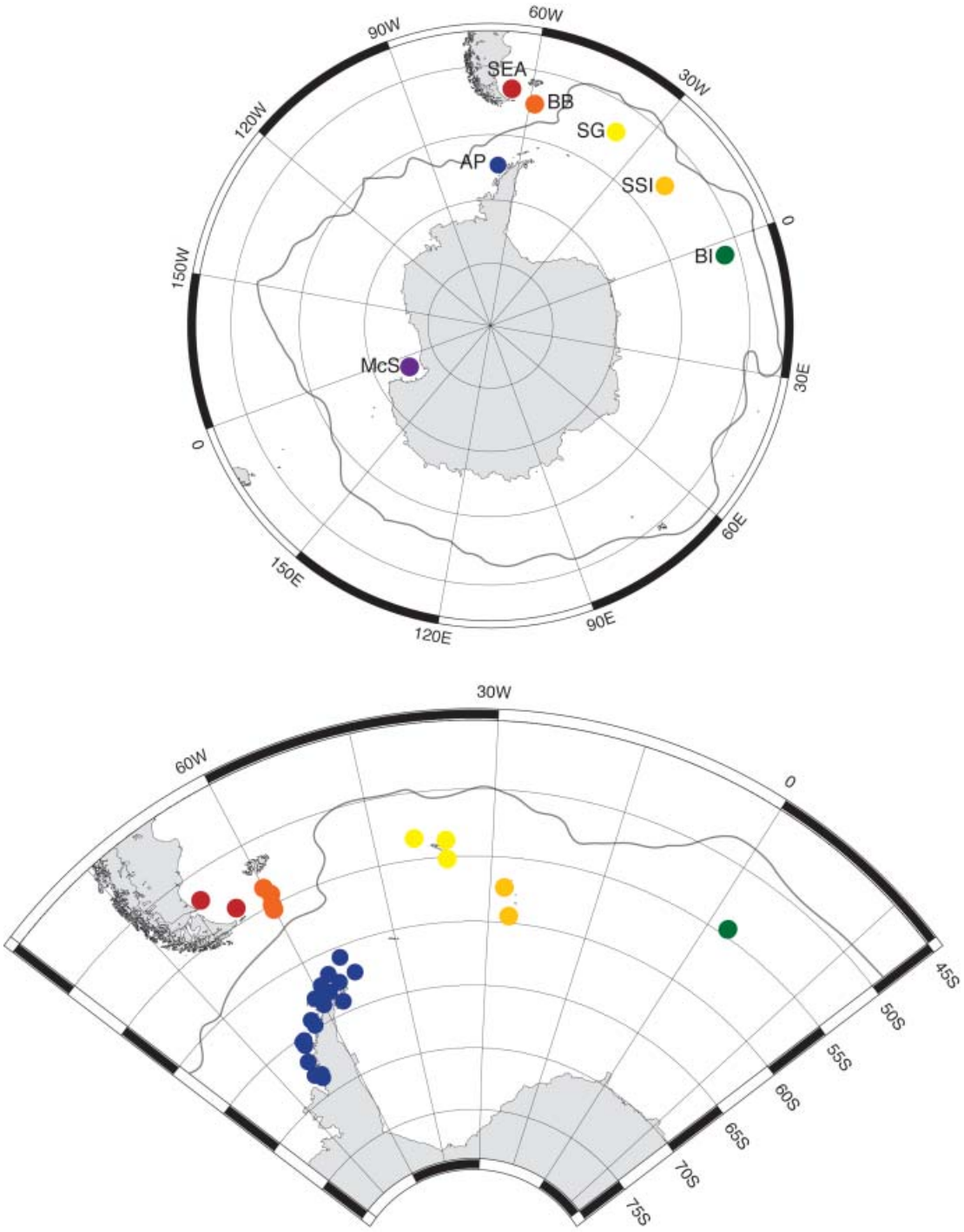

Fig. 1 Collection localities of *Parborlasia corrugatus*. (a) Southern Ocean region depicted with regional geographical designations used in study. Abbreviations: AP, Antarctic Peninsula; BB, Burdwood Bank; BI, Bouvet Island; McS, McMurdo Sound; SEA, southeastern Argentina; SG, South Georgia Island/Shag Rocks; SSI, South Sandwich Islands. Grey line represents approximate boundary of the Antarctic Polar Front (APF), based on Orsi *et al*. (1995). (b) Close up the Antarctic Peninsula and southern South America showing more detail of collection localities (see Table S1 for coordinates of sample localities).

kindly provided samples from a May–June 2004 expedition to South America and the sub-Antarctic islands on the *R/V Nathanial Palmer*. Sample size was limited by the difficulty and expense of collecting Antarctic fauna. Collection locality and mitochondrial haplotype names for *Parborlasia* spp. nemerteans are given in Fig. 1 and Table S1, Supporting information. Collection information for non-*Parborlasia* spp. nemerteans is provided in Table S2, Supporting

information. Collection depths ranged from 5–440 m (Tables S1–S2). Upon collection, adult and larval samples were immediately sorted and photographed. Morphological voucher specimens were preserved in buffered formalin, transferred to ~70% ethanol, and deposited to the Smithsonian Institution Natural History Museum (USNM 1111744–1111771). Samples to be used for the molecular analysis were frozen at –80 °C or preserved in ~85% ethanol.

Molecular analysis

Genomic DNA was extracted using the DNeasy Tissue Kit (QIAGEN Inc.). For all nemerteans, an approximately 520-bp fragment of the mitochondrial 16S rRNA gene was amplified using the primers 16SarL (5′-CGCCTGTTTATCAAAAACAT-3′) and 16SbrH (5′-CCGGTCTGAACTCAGATCACGT-3′) (Palumbi *et al*. 1991). For *Parborlasia* spp. worms, an approximately 620-bp fragment of the cytochrome *c* oxidase subunit I (COI) gene was also amplified using the primers HCO (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) and LCO (5′-GGTCAACAAATCATAAAGATATTGG-3′) (Folmer *et al*. 1994). All polymerase chain reaction (PCR) cycling conditions were as follows: initial denaturation at 94 °C for 2 min; 35 cycles of denaturation at 94 °C for 30 s; annealing at 50 °C for 30 s; extension at 72 °C for 1 min; and final extension at 72 °C for 7 min. PCR products were verified by 1× sodium borate agarose gel electrophoresis. Because these 16S and COI primers work with variable efficiency across invertebrate taxa, QIAquick Gel Extraction Kits (QIAGEN Inc.) were used when bands did not have crisp edges to improve the quality of the template for sequencing. Products were bidirectionally sequenced using Genome Lab Quick Start Mix (Beckman Coulter) on a Beckman CEQ 8000 Genetic Analysis System (Beckman Coulter). Sequences were deposited in GenBank (accession nos EU194791–EU194826, EU718358, EU718362–EU718429, EU718431, EU718433–EU718448, EU718450–EU718469; Table S1–S2). *Parborlasia corrugatus* 16S and COI sequences from Thollesson & Norenburg (2003) were also included in this analysis (accession nos AJ436829 and AJ436939). Seven nucleotides from AJ436829 and six nucleotides from AJ436939 were truncated from the 3′ terminus of these sequences in order to avoid ambiguities.

Edited sequences were aligned using Clustal X (Thompson *et al*. 1997) and manually corrected by eye using SEAL version 2.0a11 (available at http://tree.bio.ed.ac.uk/software/seal/) and MacClade version 4.06 (Sinauer & Associates) software. COI sequences were translated (*Drosophila* code) in MacClade version 4.06 to ensure that stop codons were not present. Each unique *Parborlasia* spp. haplotype sequence was designated by a letter for COI, number for 16S, or alphanumeric name for the concatenated data (representing the combination of COI and 16S haplotype names).

A partitioned homogeneity test in PAUP*4.0b10 (Swofford 2001) was conducted on the *Parborlasia* spp. data with 1000 replicates, heuristic searches limited to 100 000 rearrangements, tree-bisection–reconnection (TBR) branch swapping, and 10 random sequence additions. For all analyses conducted herein, 16S and COI data were examined separately and as a concatenated data set.

Topologies of *Parborlasia* spp. data were constructed under Bayesian inference using MrBayes version 3.12 (Huelsenbeck & Ronquist 2001) implementing the Hasegawa–Kishino–Yano (HKY) + Γ model of substitution, as suggested by MrModelTest version 2 (Nylander 2002) for both 16S and COI. Based on the results of Thollesson & Norenburg (2003), *Parvicirrus dubius* sequences (accession nos AJ436940 and AJ436840) were used to root phylogenetic trees. Two sets of four chains (three hot, one cold) were run for 1×10^7 generations and sampled every 1000 generations. In all cases, the chains converged within 1.5×10^6 generations. Therefore, the first 1500 trees (1.5×10^6 generations) were discarded as burn-in and a 50% majority-rule consensus tree was calculated from the resulting 8500 trees. Nodal support was reported as posterior probabilities. Maximum parsimony bootstrap analyses were also performed in PAUP*4.0b10 (Swofford 2001) on the Bayesian topologies using 1000 replicates.

To explore the more recent history of *P. corrugatus*, haplotype networks were constructed with TCS version 1.21 (Clement *et al*. 2000) using default settings and assumptions, with indels treated as missing data. Plausible branch connections between haplotypes were tested at both 90% and 95% connection limits. A reticulation between haplotypes D1, G1, and F1 was resolved following Crandall *et al*. (1994). Additionally, uncorrected genetic distances (p) between haplotypes were calculated using PAUP*4.0b10 (Swofford 2001).

Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997) neutrality tests were conducted using DnaSP 4.0 (Rozas *et al*. 2003) for *P. corrugatus*. Significantly negative values for Tajima's D and Fu's F_S reflect an excess of rare polymorphisms in a population, which indicates either positive selection or a recent increase in population size (Aris-Brosou & Excoffier 1996). Nucleotide diversity was also calculated using DnaSP 4.0.

Morphological analysis

For samples collected in this study, morphology of whole specimens was assessed to determine whether any morphological characters correlated with geographical location, mitochondrial haplotype (see below), or other attributes. Examples of the range of morphological diversity encountered in *Parborlasia* sp. are presented in Fig. S1, Supporting information.

In addition, morphology of whole and sectioned specimens of *Parborlasia fueguina* (USNM 081538–081557), *Parborlasia landrumae* (USNM 081595–081597) and *P. corrugatus* (USNM 069919–069948, 086472–086597) already in the Smithsonian collections were reexamined to assess the reliability of morphological features to distinguish these described species. Specimens were originally preserved in formalin, later transferred to 70% ethyl alcohol, and subsequently identified by Gibson (1985) and later by J. Norenburg.

Results

Genetic diversity of Parborlasia corrugatus *from the Southern Ocean*

Of the 86 *Parborlasia corrugatus* individuals (83 adults and 3 larvae) examined from 30 sampling stations, results of 16S analyses were more conservative (i.e. less variable) and less informative than COI (Figs 2 and 3). The greater divergence, and therefore more rapid evolution, in COI compared to 16S has been noted in other studies (e.g. Hebert *et al*. 2003; Govindarajan *et al*. 2005; Mueller 2006). A partition homogeneity test on a limited data set was significant. However, generally similar patterns (i.e. a major genetic break between continents) were found between Antarctica and South America for both genes and both genes are on a single locus, the mitochondrial genome. Thus, the data were analysed as both individual genes and as a concatenated data set for results presented herein.

The combined *P. corrugatus* COI and 16S data set consisted of 1104 nucleotide positions, all of which (100%) could be unambiguously aligned. Ninety-four characters (8.5%) were variable and 77 (7.0%) were parsimony informative. Sequences were adenine and thymine (A-T) rich (63.3%) compared to mean guanine and cytosine (G-C) content (36.7%). The maximum uncorrected genetic distance (p) between *P. corrugatus* individuals was 0.0656. The aligned data set is available from TreeBase (www.Treebase.org study accession no. S2178; matrix accession no. M4125–4126).

Thirty-two separate COI/16S haplotypes were detected in the 86 samples examined in this study (this included 25 unique COI sequences and 9 unique 16S sequences). Two haplotypes (i.e. A1 and F1) accounted for the majority of samples (A1 $n = 30$ adults and 3 larvae; F1 $n = 18$ adults). These two abundant haplotypes were distributed around Antarctica and sub-Antarctic islands. The remaining 30 haplotypes occurred at low abundance, with only one or two representatives of each detected in the data set.

Phylogenetic analysis of P. corrugatus *in the Southern Ocean*

Bayesian inference phylogenetic reconstruction of concatenated and COI data (Fig. 2a, b) indicated that *P. corrugatus* formed two well-supported clades, separated by geography. The first clade included all samples from Antarctica and sub-Antarctic islands. Sequences within this clade showed limited nucleotide diversity ($\pi = 0.00118$) and a maximum uncorrected distance value of $p = 0.00815$ (Table 1). A second distinct clade comprised entirely of South American *P. corrugatus* was also well supported in these analyses. Interestingly, this clade contained a higher level of sequence divergence (maximum uncorrected $p = 0.01902$, $\pi = 0.01014$, Table 1) despite the considerably smaller sample size ($n = 12$ for South American samples vs. $n = 74$ for Antarctic samples). The average uncorrected p between South America and Antarctica/sub-Antarctic islands was 0.0549 for the concatenated data set (0.0155 for 16S; 0.0941 for COI). No obvious morphological characters were identified that correlated with a particular clade or region (see Fig. S1).

Additionally, Bayesian topologies based on COI and concatenated genes indicated the existence of two subclades of South American *Parborlasia* (posterior probability/boostrap vales of 0.81/100, respectively, for concatenated data, 0.79/100 for COI alone). These South American subclades were geographically partitioned, with one clade from Southeast Argentina and a second clade from Burdwood Bank.

Bayesian analysis of the 16S rRNA gene sequence data alone produced a somewhat different result (Fig. 2c). Here, the South American samples formed a monophyletic clade nested within a polytomy consisting of the low diversity Antarctic and sub-Antarctic samples. Furthermore, no phylogeographical genetic break was detected within South American samples.

Haplotype networks and demography for P. corrugatus *in the Southern Ocean*

Statistical parsimony (TCS) analysis, using a 95% connection limit, of the COI and concatenated COI/16S data sets resulted in three distinct haplotype networks (Fig. 3a, b). One haplotype network consisted of all Antarctic and sub-Antarctic *P. corrugatus*, whereas the remaining two networks were comprised by the South American *P. corrugatus* specimens. In all cases, there were no shared haplotypes across the Drake Passage or APF, indicating a lack of gene flow between continents.

Within the Antarctic/sub-Antarctic COI and concatenated COI/16S networks, 73 out of 74 samples differed by only 0 to 2-bp changes from the inferred ancestral haplotype (Fig. 3a, b). Only one sample from South Georgia Island showed greater divergence (7-bp differences from the ancestral haplotype). No geographical partitioning of haplotypes was observed within the Antarctic/sub-Antarctic network and the ancestral haplotype was found distributed across the Antarctic Peninsula, McMurdo

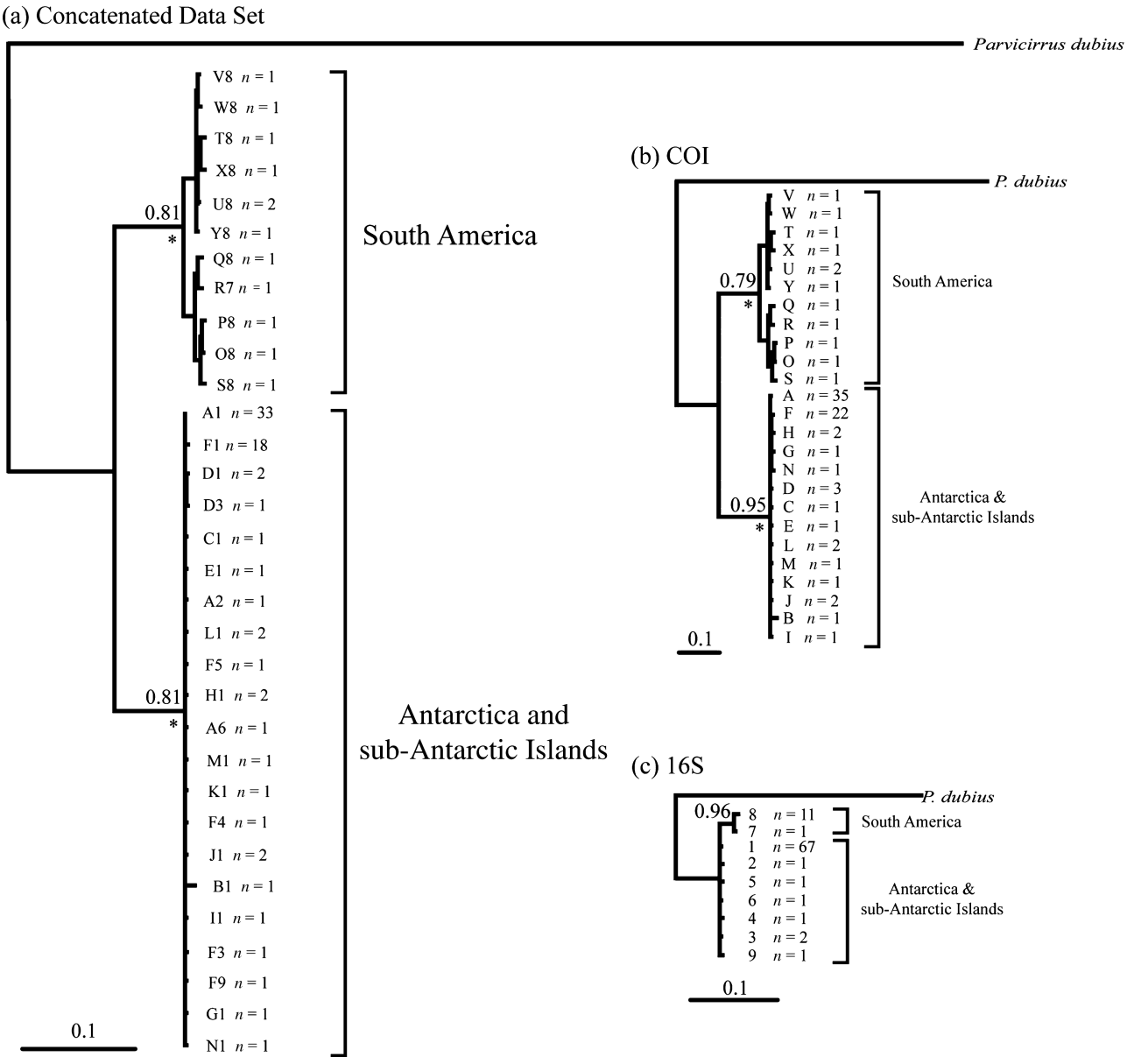

Fig. 2 Bayesian inference topology of South American, sub-Antarcti, and Antarctica *Parborlasia* spp. Nodal support indicated as both posterior probabilities (numerical values) and maximum parsimony boostrap values (an asterisk denotes 100% support) next to the relevant node. (a) Topology based on concatenated 16S and COI mtDNA genes. (b) Topology based solely on COI mtDNA. (c) Topology based solely on 16S mtDNA. Alphanumeric names (designated by letters corresponding to COI haplotype and numbers corresponding to 16S haplotype) and number of replicates (designated as '*n* = ') are provided for each haplotype.

Sound, Bouvet Island, and South Georgia Island, despite thousands of kilometre distance between these locations.

Despite the genetic similarity of the Antarctic population, no shared haplotypes were observed between the South American and Antarctic clades of *P. corrugatus*. In both the COI and concatenated COI/16S TCS analyses, South American *P. corrugatus* comprised two haplotype networks that were further geographically partitioned between the western region of the Argentine continental shelf and Burdwood Bank. However, lowering the connection limit to 90% joined these two South American populations into a single haplotype network (Fig. 3a, b). This genetic break within South American *P. corrugatus* was also observed in the Bayesian analyses above (Fig. 2).

In contrast to results presented above, parsimony analysis of 16S rRNA gene sequence data alone resulted in a single haplotype network (Fig. 3c). This single network likely reflects the slower evolutionary rate, and therefore less

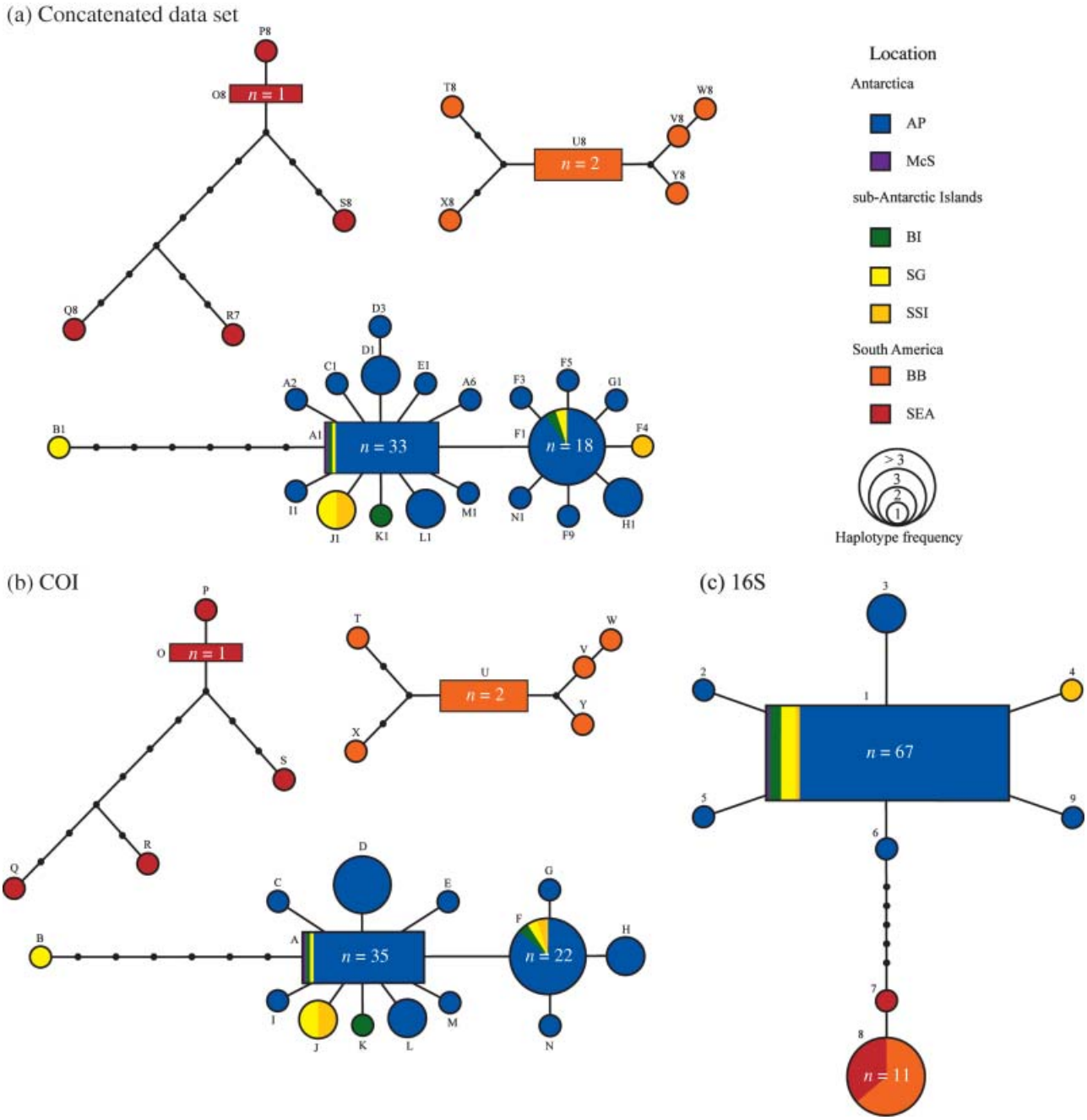

Fig. 3 TCS networks of South American, sub-Antarctic, and Antarctic *Parborlasia* spp. using the 95% connectivity level. (a) Networks based on concatenated 16S and COI mtDNA genes. (b) Networks based solely on COI mtDNA. (c) Network based solely on 16S mtDNA. Alphanumeric names (designated by letters corresponding to COI sequence and numbers corresponding to 16S sequence) are provided for each haplotype. Sampled haplotypes are indicated by coloured circles or rectangles; missing or unsampled haplotypes are indicated by black dots. Rectangles depict the haplotype with the highest ancestral probability. Each branch indicates a single mutational difference. Circle size is proportional to observed haplotype frequency; observed number of ancestral haplotypes is indicated by '*n* = ' (the number of times that haplotype was obtained). Haplotypes are coloured according to the geographical region from which the sample was collected (see legend).

divergence, in 16S vs. COI mtDNA, similar to the results seen in other studies (e.g. Hebert *et al*. 2003; Govindarajan *et al*. 2005; Mueller 2006). Despite this, there was no indication of gene flow across the APF in the 16S networks; Antarctic and sub-Antarctic *P. corrugatus* clustered together while South American *P. corrugatus* clustered together. Finally, there was no indication of a genetic break between sites in South America within the 16S data.

Table 1 Genetic distance statistics and neutrality tests for populations of *Parborlasia corrugatus*

Geographical region	*n*	*nh*	π	*h*	Tajima's *D*	Fu's F_S
South America	12	11	0.01014 (0.00125)	0.985 (0.040)	–0.02482	–2.520
sub-Antarctic Islands	9	6	0.00262 (0.00100)	0.917 (0.073)	–1.34518	–1.252
Antarctica	65	17	0.00098 (0.00011)	0.735 (0.047)	–1.87098*	–15.597*
Antarctica and sub-Antarctic Islands	74	21	0.00118 (0.00019)	0.762 (0.042)	–2.28188*	–20.007*
All regions	86	32	0.01566 (0.00324)	0.826 (0.034)	–0.27537	–0.292

n, number of sampled individuals; *nh*, number of haplotypes detected; π, nucleotide diversity (and standard deviation); *h*, haplotype diversity (and standard deviation); *$P < 0.05$.

Tests of neutrality using Tajima's *D* and Fu's F_S indicated significantly negative values for the Antarctic *P. corrugatus*, which also influence the combined Antarctic and sub-Antarctic values (Table 1). In contrast, the South American population and the entire data set did not show significant deviation from neutrality (Table 1).

Lack of molecular and morphological evidence for the existence of Parborlasia fueguina *and* Parborlasia landrumae

The *P. corrugatus* 16S rRNA gene sequence data presented above was also compared to 16S rDNA data from a wider sampling of 109 additional Southern Ocean larval and adult nemertean specimens (deposited to GenBank under accession nos. EU718358, EU718362–EU718429, EU718431, EU718433–EU718448, and EU718450–EU718469). No evidence of additional sister clades to *P. corrugatus* was detected in this analysis. All additional Southern Ocean nemerteans differed from *P. corrugatus* by ≥ 16.9% (0.169 uncorrected *p*) at 16S. The closest available lineage to *P. corrugatus* was *Parvicirrus dubius* (average total uncorrected $p = 0.137$), a temperate nemertean from the east coast of North America (Thollesson & Norenburg 2003).

Our findings were contrary to what was expected; sister taxa should have been detected for the congeneric species *Parborlasia fueguina* and *Parborlasia landrumae* if these taxa represent valid biological species and were sampled. For instance, Gibson (1985) recorded thirty *P. fueguina* specimens from near Anvers (2), South Shetland (25), South Georgia (2), and South Sandwich Islands (1), all south of the APF. An additional 48 *P. fueguina* were recorded by Gibson (1985) from locations north of the APF — near Falkland Islands (6) and Tierra del Fuego (42). This implies that this species should be relatively common south of the APF in the areas we sampled; however, no second genotype was found among the *Parborlasia*-like worms from this region. We cannot exclude at this point the possibility that one of the South American genotypes is *P. fueguina*, but specimens sampled for DNA from the South American clade had variable cephalic pigment patterns. (In life, *P. fueguina* is recognized by a yellow transverse cephalic band.) *Parborlasia corrugatus* has been recorded from the vicinity of Wilkes Land (the type locality of *P. landrumae*), but this is at the opposite side of Antarctica from the specimens sampled here for DNA. Therefore, due to the lack of molecular evidence for *P. fueguina* and *P. landrumae* from the South America to Antarctica surveys, a re-examination of the morphological evidence for these species was conducted on preserved whole specimens from the Smithsonian collections (see Appendix S1, Supporting information for details of this analysis).

Discussion

Molecular evidence of an open-ocean barrier to gene flow

Genetic structure observed in *Parborlasia corrugatus* was consistent with the hypothesis that the APF and ACC act as open-ocean barriers to gene flow over evolutionary time (e.g. Clarke *et al*. 2005). Here, *P. corrugatus* exhibited genetic disparity across the Drake Passage, despite its reportedly broad species distribution. Furthermore, the lack of recent gene flow across this waterway cannot be explained by an inability to disperse. Given the single haplotype network found for Antarctic and sub-Antarctic *P. corrugatus*, gene flow may be sufficient to maintain genetic connectivity throughout the western Antarctic Peninsular and sub-Antarctic islands, a distance of at least 8000 km. Alternatively, the genetic similarity could be the result of a colonization event followed by range expansion as suggested by the neutrality measures. Admittedly, more sampling is needed to confirm the status of *P. corrugatus* in the Ross Sea area ($n = 1$), but presence of a common and ancestral haplotype A1 suggests recent connectivity.

Parborlasia corrugatus's dispersal capacity is perhaps explainable in the context of its larval biology. Pilidia are known to survive long periods (up to 150 days) in the Antarctic and can be found in the water column throughout the year (Pearse *et al*. 1991; Peck 1993; Stanwell-Smith *et al*. 1999). The rapid flow ($100–150 \times 10^6$ m^3/s; Klink & Nowlin

2001) of the ACC could facilitate dispersal of *P. corrugatus* across such vast distances (Fevolden & Schneppenheim 1989). Of note, there is a less substantial circum-Antarctic current that flows between Antarctica and the ACC (known as the east-wind drift) which may also aid larval dispersal near the continent (Phillpot 1985). Thus, this substantial dispersal capacity makes *P. corrugatus* an appropriate study system to test the strength of the APF as an open-ocean barrier.

Consideration of the approximate time of separation between Antarctic and South American *P. corrugatus* would be helpful in determining the strength of the APF as a barrier to gene flow. Unfortunately, this endeavour is hindered by the lack of fossil records or molecular clocks in nemerteans. Based on estimations of substitution rate of third position sites in COI from other non-anthozoan invertebrate groups (see Wares & Cunningham 2001; Govindarajan *et al*. 2005; Table S3, Supporting information), the level of genetic differentiation between the South American and Antarctic/sub-Antarctic populations is consistent with the isolation of these clades for ~4.2 to 14.5 million years. However, examining 16S or all codon positions of COI results in even more recent estimates of divergence (see Govindarajan *et al*. 2005; Table S3). Interestingly, this level of divergence is not as great as would be expected if they had been separated since the initial establishment of the ACC, which most studies place at greater than 20 million years ago (Lawver & Gahagan 2003; Barker & Thomas 2004; Pfuhl & McCave 2005; Scher & Martin 2006). Thus, it is unlikely that the APF and ACC have acted as continuous or impermeable barriers to gene flow since their initial formation.

In order to migrate across the APF, organisms must travel great distances (> 850 km), over considerable depths (> 4000 m), across temperature clines of 3–4 °C, and against strong prevailing currents (Whitworth *et al*. 1982; Eastman 1993; Clarke *et al*. 2005). Recent molecular studies in other taxonomic groups (i.e. bivalves, Page & Linse 2002; brittle stars, Hunter & Halanych 2008; krill, Patarnello *et al*. 1996; notothenioid fish, Bargelloni *et al*. 2000; patagonian toothfish, Shaw *et al*. 2004; *Phaeocystis* spp. colonial alga, Medlin *et al*. 1994) similarly observed genetic breaks between South America and Antarctica. Perhaps the most parsimonious explanation for this phenomenon is that the APF acts as an open-ocean barrier, restricting dispersal in multiple groups of animals. However, in many cases, the level of divergence between South American and Antarctic taxa indicate a date of separation well after the formation of the APF (Bargelloni *et al*. 2000; Page & Linse 2002; Hunter & Halanych 2008; but see Medlin *et al*. 1994; Patarnello *et al*. 1996). Estimates for the timing of APF establishment vary considerably; however, even the most conservative estimates place the formation of the APF at greater than 20 million years ago (Lawver & Gahagan 2003; Pfuhl & McCave 2005; Scher & Martin 2006). The pattern observed here and in other studies suggests that the APF has been an active dispersal barrier over evolutionary time, but this barrier was not impermeable for its entire history in all taxonomic groups. Alternatively, the low levels of divergence between clades on either side of the APF could be the result of slower mtDNA evolutionary rates in cold environments (but see Held 2001).

One unresolved question is whether there is synchronous timing of the genetic break across the APF among multiple taxonomic groups. Evidence for concurrent timing of connectivity in multiple taxa would indicate a physical oceanographic event, such as a northward migration of the APF boundary or ebbing of the ACC, that permitted dispersal between continents. Interestingly, there was a marked cooling event during the Middle Miocene Climatic Transition (approximately 12–14 million years ago) during which the ACC intensified (Rogers 2007). This event led to diversification in penguins (Baker *et al*. 2006) and may also correspond to cladogenesis in *Parborlasia* and other Antarctic fauna. Conversely, incongruent timing in the various taxa would imply independent dispersal across the ACC resulting in autonomous speciation events. Additional studies on the timing of these genetic breaks are warranted to address this issue.

In an allozyme study of *P. corrugatus* from the South Orkney Islands, Rogers *et al*. (1998) found high genetic identities in their samples, consistent with our findings of a single Antarctic/sub-Antarctic species. Rogers *et al*. (1998) also reported a lack of heterozygotes in *P. corrugatus*, which was primarily attributed to variability in prevailing currents potentially providing variation in the origin of recruits from two genetically distinct populations. Although the South Orkney Islands were not sampled in this study, our mitochondrial data indicated little genetic diversity in Antarctic and sub-Antarctic *P. corrugatus* over much larger geographical scales (1000s of kilometres). Possible explanations for this difference could include slow rates of mitochondrial gene evolution in heteronemerteans (J. Norenburg and M. Schwartz, personal observation), cold environments (Bargelloni *et al*. 1994; although this seems unlikely, see Held 2001), and/or genetic sweeps of haplotypes through a population (Gillespie 2000a, b, 2001), among others. One additional possibility discussed by Rogers *et al*. (1998) was the sampling of cryptic *Parborlasia* species. The oceanography of the Southern Ocean is complex and mesoscale eddies provide one potential mechanism of transport across the APF and ACC (Joyce *et al*. 1981; Clarke *et al*. 2005; Barnes *et al*. 2006; R. S. Scheltema, personal communication). Therefore, it is possible that the South Orkney Islands are a localized contact zone between the Antarctic and South American clades and/or other haplotypes that were not sampled in the current study. Such localized contact zones have been observed in other taxa

from the Antarctic (e.g. Ritchie *et al*. 2004; Ashford *et al*. 2008).

Molecular diversity in P. corrugatus

Parborlasia corrugatus's genetic diversity generally decreases with increasing latitude and Antarctic localities showed the lowest nucleotide and haplotypic diversity despite their larger sample sizes. This trend has been noted in the brittle star *Astrotoma agassizii* (Hunter & Halanych 2008) and several other invertebrate groups in both hemispheres (e.g. Dahlgren *et al*. 2000; Wares & Cunningham 2001). Reduced haplotype diversity, combined with the negative values for Tajima's D and Fu's F_S, most likely indicate a recent expansion in population size (Aris-Brosou & Excoffier 1996). Alternatively, these negative values might signal positive selection (Tajima 1989; Fu 1997). Population expansion presumably resulted from the retreat of glaciers which released benthic habitat on the continental shelf (Thatje *et al*. 2005). Furthermore, the glacial history of the Antarctic (i.e. diachronous deglaciation) is hypothesized to have promoted the circumpolar distribution of species with colonization potential, such as nemerteans with long-lived larvae (Thatje *et al*. 2005).

Recently, species boundaries have been inferred on the basis of parsimony networks, wherein multiple haplotype networks are interpreted as separate species (e.g. Tarjuelo *et al*. 2004; Uthicke *et al*. 2004; Addison & Hart 2005; Jolly *et al*. 2005). The network analysis of this study suggests that there are potentially two cryptic species that were previously considered *P. corrugatus*: one putative species in Antarctica and the sub-Antarctic islands and a second putative species in southern South America. Additionally, South American *P. corrugatus* were further partitioned into two haplotype networks, one along the western region of the Argentinean continental shelf and a second along Burdwood Bank. Similar phylogeographical patterns have also been reported in other taxa (e.g. Hunter & Halanych 2008). Unfortunately, these *Parborlasia* populations were difficult to differentiate morphologically, a problem common to many nemertean taxa (Envall & Sundberg 1998; Schwartz & Norenburg 2001; Turbeville 2002; Strand & Sundberg 2005). For instance, one putatively diagnostic character for *P. corrugatus* is a pale to white coloured margin along the anterior one-half to two-thirds of the cephalic slits, at the end of which the whitish pigment turns mediad, forming either pale triangles in the dorsal pigment or elongating to form a dorsal line across the head. However, this character was extremely variable both within and between geographical regions (Fig. S1), making it a poor correlate to the clades and haplotype networks detected using mitochondrial gene sequences. No obvious external morphological character was apparent that, in our estimation, distinguished the populations of *P. corrugatus* found on either side of the Drake Passage. The presence of potentially cryptic species raises taxonomic issues concerning which lineage should retain the original name. In the case of *P. corrugatus*, the type specimen originated from the Kerguelen archipelago in the sub-Antarctic (McIntosh 1876) which is outside of the area sampled in this study. Thus, this issue needs further sampling before it can be resolved.

Lack of molecular and morphological evidence for Parborlasia fueguina *and* Parborlasia landrumae

Two additional congeneric *Parborlasia* species have been described from the Southern Ocean, *P. fueguina* and *P. landrumae*. Although our survey attempted to collect all available nemerteans, including adults and larvae, we detected no sister clades or other taxa closely related to *P. corrugatus*. This lack of molecular evidence for *P. fueguina* and *P. landrumae* could be explained by three possible scenarios: (i) we simply did not manage to collect any *P. fueguina* or *P. landrumae*, (ii) these species were originally misclassified as belonging to the genus *Parborlasia* and instead should have been placed in another genus (i.e. *Parborlasia* is polyphyletic), or (iii) *P. fueguina* and *P. landrumae* are not true biological taxa and therefore these nominal species were based upon erroneously identified samples. Gibson (1985) apparently distinguished specimens of putative *P. fueguina* and *P. landrumae* as different from the hundreds of specimens otherwise assumed to be *P. corrugatus* after observing presumed diagnostic differences in histological sections. In order to further evaluate the second and third possibilities, we re-assessed the morphology of whole and sectioned specimens from the Smithsonian collections and found no evidence that these species can be morphologically distinguished. Although no definitive statement on the taxonomic status of *P. fueguina* or *P. landrumae* can be made at this time, the lack of molecular evidence and questionable diagnostic morphological features give reason for revaluating these nominal taxa.

Conclusions

This analysis of mitochondrial gene diversity indicates that a single, broadly distributed population of *Parborlasia corrugatus* is found around large areas of Antarctica and the sub-Antarctic islands. Despite this, no evidence of genetic connectivity, and therefore recent gene flow, was found between this population and individuals measured in southern South America. Therefore, the APF represents a considerable barrier to open-ocean dispersal over evolutionary time, although dispersal events have occurred since the establishment of the APF greater than 20 million years ago. According to the phylogenetic species concept, the genetic differences between Antarctic and South American *Parborlasia* populations are possibly sufficient to

consider these populations as two putative cryptic species. Similar patterns were recently reported in other studies of Antarctic invertebrates and these combined results may have interesting implications for other invertebrate fauna occurring on both sides of the Drake Passage. Additional studies further characterizing the diversity of *P. corrugatus*, the diversity of other Antarctic invertebrates, and the timing of separation across the Drake Passage will continue to improve our understanding of the processes driving the ecology and evolution in organisms from this isolated region.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1 Examples of the morphological variation encountered in *Parborlasia* samples from the Antarctic Peninsula (a through d; haplotypes L1, A1, D1, and A2 respectively), Burdwood Bank (e; haplotype U8), and southeastern Argentina (f; haplotype O8). All of the specimens depicted here were included in the molecular analysis. Note the plasticity in pigmentation and the anterior banding pattern (i.e., the light coloured 'collar') between specimens.

No obvious morphological differences were identified that corresponded with geography or the genetic differences reported in this study. Images are not to scale relative to one another.

Table S1 Haplotype name, collection information, and GenBank Accession nos for *Parborlasia* spp. samples from South America, Antarctica and the sub-Antarctic Islands. Station numbers 04-xx and 06-xx were collected on the 2004 and 2006 cruises aboard the R/V Lawrence M. Gould, respectively. Others were either obtained from the Icefish cruises (via S. J. Lockhart) or directly from GenBank (i.e., the McMurdo Sound sample).

Table S2 Collection information, age class, and GenBank Accession nos for nemerteans other than *Parborlasia corrugatus* from South America, Antarctica and the sub-Antarctic Islands. Station numbers 04-xx and 06-xx were collected on the 2004 and 2006 cruises aboard the R/V Lawrence M. Gould, respectively. Others were either obtained from the Icefish cruises (via S. J. Lockhart).

Table S3 Genetic-distance statistics for *Parborlasia corrugatus* mtDNA genes (16S, COI, and the third positions of COI).

Appendix S1 Supplementary results and discussion

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