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ORIGINAL PAPER

Is the Scotia Sea a centre of Antarctic marine diversification? Some evidence of cryptic speciation in the circum-Antarctic bivalve *Lissarca notorcadensis* (Arcoidea: Philobryidae)

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Abstract The bivalve *Lissarca notorcadensis* is one of the most abundant species in Antarctic waters and has colonised the entire Antarctic shelf and Scotia Sea Islands. Its brooding reproduction, low dispersal capabilities and epizoic lifestyle predict limited gene flow between geographically isolated populations. Relationships between specimens from seven regions in the Southern Ocean and outgroups were assessed with nuclear 28S rDNA and mitochondrial cytochrome oxidase subunit I (COI) genes. The 28S dataset indicate that while *Lissarca* appears to be a monophyletic genus, there is polyphyly between the Limopsidae and Philobryidae. Thirteen CO1 haplotypes were found, mostly unique to the sample regions, and two distinct lineages were distinguished. Specimens from the Weddell and Ross Sea form one lineage while individuals from the banks and islands of the Scotia Sea form the other. Within each lineage, further vicariance was observed forming six regionally isolated groups. Our results provide initial evidence for reproductively isolated populations of *L. notorcadensis*. The islands of the Scotia Sea appear to act as centres of speciation in the Southern Ocean.

Keywords *Lissarca notorcadensis* · Bivalvia · Antarctic · Cytochrome oxidase I · Cryptic species

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Introduction

Antarctica represents an unrivalled laboratory for undertaking evolutionary studies of the origins and maintenance of biodiversity (Clarke 2000). The Antarctic continent and islands are separated from neighbouring landmasses by sheer distance and in the marine realm the Polar Front acts as a barrier to dispersal. Within Antarctica, marine and terrestrial habitats can be isolated by geological (e.g. distance, fragmented landscapes), physical (e.g. oceanic gyres, ice sheets/shelves, temperature) or ecological (e.g. niche variety, food availability) barriers. Habitat fragmentation can hinder dispersal among populations, limit gene flow and lead to allopatric speciation (e.g. Avise 2004; Frankham et al. 2004; Wagner and Liebherr 1992). The Antarctic fauna provides an excellent opportunity to study colonisation and gene flow between separated habitats, along environmental gradients and over evolutionary time-scales. Previously, the marine and terrestrial Antarctic invertebrate fauna had been considered to be low in species richness but having a high level of endemism (e.g. Boenigk et al. 2006; Clarke and Johnston 2003; Gutt et al. 2000; Maslen and Convey 2006; Stary and Block 1998). Recent studies using molecular genetics have revealed that the species richness in Antarctica is higher than previously suggested (Allcock et al. 1997; Freckman and Virginia 1997; Held and Waegele 2005; Lörz et al. 2006; Pawlowski et al. 2002; Stevens et al. 2006). On land, glacial events are important in structuring the genetic diversity of terrestrial arthropods (Allegrucci et al. 2006; Frati et al. 2001; Stevens and Hogg, 2003, 2006). In the sea, ice-sheet cycles, covering the continental shelf, and separation of water masses are of

great consequence in the make-up of marine biodiversity and the radiation of species (Held 2000; Held and Wägele 2005; Page and Linse 2002; Raupach et al. 2004; Raupach and Wägele 2006). Few studies have analysed the intraspecific genetic variability in Antarctic marine invertebrates (Held and Leese 2006; Held and Wägele 2005; Raupach and Wägele 2006) and none so far have focussed on molluscan species.

Since the early days of exploration of the Southern Ocean, molluscs were a major subject of scientific investigations (Dell 1990; Hain 1990; Powell 1951). Overall the bivalve and gastropod fauna of Antarctic waters is probably described more completely than any other group of benthic marine invertebrates (Clarke and Johnston 2003). Current biogeographic results using SOMBASE, the Southern Ocean Mollusc Database (Griffiths et al. 2003), showed that the Scotia-Arc and Peninsula region appears to be a hot spot of species richness (Linse et al. 2006). In order to address fundamental evolutionary questions pertaining to the origins and diversification of Antarctic marine fauna (such as why the Scotia Arc region is so species-rich, where the origin of Antarctic species is, how the isolation of water masses supports radiation and what are the roles of islands and shallow shelves as possible stepping stones of colonisation and radiation in Antarctica?), we queried SOMBASE for a suitable taxon/species-complex. The ideal target species or group had to have a circum-Antarctic and sub-Antarctic distribution. This might give the possibility to link the evolutionary history of the species with plate tectonics (Barker and Burrell 1977; Lawver and Gahagan 2003) and oceanographic current systems such as the northbound currents along the western Antarctic Peninsula towards the Scotia Arc islands (Hofmann et al. 1998), its counter current in the Bransfield Strait (Stein and Heywood 1994; Whitworth et al. 1994), and water mass circulation in the Weddell Sea (Fahrbach et al. 1992, 1994; Orsi et al. 1993).

Among the species found to be suitable for this study the small philobryid bivalve *Lissarca notorcadensis* Melvill and Standen 1907 stood out because of its wide distributional range (Dell 1990; Hain 1990), reproductive mode (Prezant et al. 1992), ecological success (Brey and Hain 1992; Brey et al. 1993) and regional morphological differences (Cope and Linse 2006). The species is attached by byssus threads to the spines of cidaroid sea urchins reaching densities of 350 individuals/cidaroid (Brey and Clarke 1993; Brey et al. 1993; Hain 1990). Morphometric population studies on *L. notorcadensis* indicated that large-shelled adults live on the top end of the spines while the juveniles live on the more protected bases of the spines and lead to the hypothesis that parents and offspring live on the same cidaroid (Brey et al. 1993). Following this hypothesis gene flow within and between populations would be limited and genetic differences should be detectable with molecular techniques.

The aim of this study is to test three hypotheses using *L. notorcadensis* as the model species: (1) The current species richness of bivalves in the Antarctic and sub-Antarctic regions is higher than suggested because of existence of cryptic species, (2) The isolated Scotia Arc Islands have acted as centres for speciation in the Scotia Sea region, and (3) the Weddell Sea Gyre isolates Weddell Sea populations from populations of the western Antarctic Peninsula and Scotia Arc Islands.

Materials and methods

Specimen collection

Individuals of *L. notorcadensis* were collected during research expeditions of RV "Polarstern" to the Scotia Sea (ANT XIX 3–5: ANDEEP I/II & LAMPOS), to the eastern Weddell Sea (ANT XXI-2: BENDEX) and RV "Tangaroa" to the Ross Sea (TAN0402/BIOROSS 2004) (Fig. 1). The sampling included Shag Rocks (SR), Saunders and Southern Thule of the South Sandwich Islands (SSI), Herdman Bank (HB), Discovery Bank (DB), South Orkney Islands (SO), Elephant Island (EI), the eastern Weddell Sea (WS) and the northeastern Ross Sea (RS). When the samples reached the deck, invertebrates were sorted by hand with focus on *L. notorcadensis*. Specimens were immediately fixed in pre-cooled 96% ethanol, kept at –20°C for at least 48 h and then refrigerated for later molecular analysis. Additionally specimens of *Lissarca miliaris* (Philippi 1845) were collected in the intertidal of Signy Island/South Orkney islands.

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was isolated from the entire specimen in small individuals. DNA was extracted with the DNeasy Tissue Extraction Kit (Qiagen, Crawley, West Sussex, United Kingdom) as directed by the manufacturer.

Partial 28S rDNA (domain 2) was amplified using the primers LSU 5 and LSU 3 (Littlewood 1994). The fragment contains the domans 1–3. PCR amplification was performed in a 50 µl reaction volume, containing the following: 50–100 ng of template DNA, 200 µM of

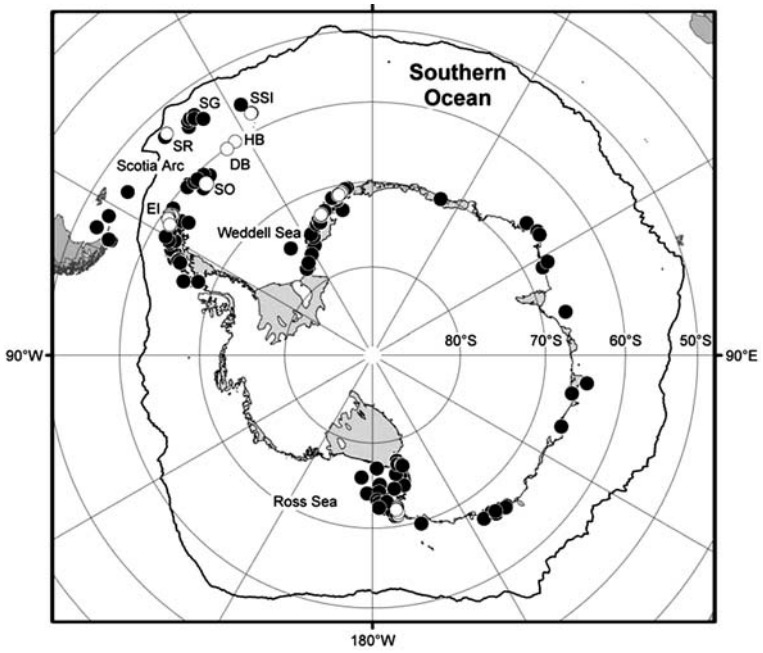

Fig. 1 Distribution records and sample sites of *L. notorcadensis*. *White circles* sample sites, *black circles* distribution records, *black line* Polar Front DB Discovery Bank, *EI* Elephant Island, *HB* Herdman Bank, *SG* South Georgia, *SO* South Orkney Islands, *SR* Shag Rocks, *SSI* South Sandwich Islands (Figure modified from Cope and Linse 2006)

each dNTP, 0.5 μM of each primer, 10 × Qiagen PCR Buffer and 5 × Q-solution. Thermal cycling began with a single denaturation step at 94°C for 5 min. Then 40 cycles were performed consisting of denaturation at 94°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 60 s. A single extension step at 72°C for 7 min was used to complete extension of DNA fragments.

A fragment of the coding COI region was amplified using the primers HCO 1490 and LCO 2198 (Folmer et al. 1994). Thermal cycle conditions were 95°C for 4 min and then 5 cycles of 94°C for 60 s, 45°C for 90 s, and 72°C for 9 s followed by 35 cycles of 94°C for 60 s, 52°C for 60 s, and 72°C for 60 s and finished with 72°C for 5 min.

PCR products were purified with QIAquick Spin Columns (Qiagen). Approximately 200 ng of double stranded PCR product was used in cycle sequencing reactions following the protocol outlined in the DYEnamic ET Dye Terminator Cycle Sequencing kit for MegaBACE DNA (Amersham Biosciences, Little Chalfont, Buckinghamshire, United Kingdom). Unincorporated dyes were removed from sequencing reaction products by using DyeEX 96 Kit (Qiagen) following the manufacturer's instructions. Reaction products were visualised on a MegaBACE 500 automated DNA sequencer (Amersham Pharmacia, Little Chalfont, Buckinghamshire, United Kingdom).

The sequences were aligned and proofread in CodonCode Aligner Version 1.5.2 (CodonCode Coporation 2006). Additional 28S and COI sequences from GenBank were added as outgroups to the aligned dataset, selected from the sister-taxon *Cosa* (Philobryidae) and the related families Limopsidae (*Empleconia* and *Limopsis*) and Arcidae (*Barbatia* and *Scapharca*) (Table 1).

Ambiguous alignment positions associated with gaps in the 28S sequences were omitted from the dataset.

Phylogenetic analysis

Phylogenetic reconstruction was conducted using maximum parsimony (MP) optimality criterion and maximum likelihood (ML) in PAUP*b10 (Swofford 2002). A heuristic search was conducted with maximum trees held set to 1,000, 50 random additions, tree bisection reconnection (TBR) branch swapping, with five trees held at each step and with "MulTrees" turned off. One thousand bootstrap pseudoreplicates were conducted to assess the reliability of the reconstructed nodes. We used an iterative optimisation approach to find an appropriate substitution model for ML analyses: the data were used to construct a neighbour joining tree in PAUP*b10. From this tree, parameters were estimated and used for likelihood analysis (heuristic search, one random addition, TBR). Parameters were re-estimated and the process repeated until the likelihood scores converged. The tree topology was assessed by a likelihood search with 500 bootstrap pseudo-replicates. Bayesian phylogenetic reconstruction was conducted using Mr Bayes v3.1.2 (Ronquist and Huelsenbeck 2003, 2005). A six parameter model was used with a gamma distribution and proportion of invariant sites

Table 1 Individual codes, GenBank accession numbers and sample location for examined *Lissarca* specimens

	Spec no.	GenBank accession no.		Haplotype/ H group	Locality
		28S rRNA	COI		
Philobryidae					
Lissarca notorcadensis	EI_59_1		EF162480	h5/HG3	PS61/059–1, 61°25,42′S/056°08,90′W-61°26,30′S/056°06,21′W, 298–326 m
	EI_73_1I1	EF162535			PS61/073-1, 61°00,10′S/055°55,96′W-61°01,68′S/055°56,49′W, 277–298 m
	EI_73_1I2	EF162536			PS61/073-1, 61°00,10′S/055°55,96′W-61°01,68′S/055°56,49′W, 277–298 m
	EI_251_3	EF162537			PS61/251-1, 61°23,79′S/055°26,87′W-61°23,75′S/055°26,71′W, 292–290 m
	EI_251_4		EF162479	h5/H3	PS61/251-1, 61°23,79′S/055°26,87′W-61°23,75′S/055°26,71′W, 292–290 m
	SR_607_2		EF162484	h6/HG4	PS61/164-1, 53°23,80′S/042°42,03′W-53°23,82′S/042°42,46′W, 312–321 m
	SR_607_3	EF162525			PS61/164-1, 53°23,80′S/042°42,03′W-53°23,82′S/042°42,46′W, 312–321 m
	SR_607_4	EF162527			PS61/164-1, 53°23,80′S/042°42,03′W-53°23,82′S/042°42,46′W, 312–321 m
	SR_607_6	EF162526			PS61/164-1, 53°23,80′S/042°42,03′W-53°23,82′S/042°42,46′W, 312–321 m
	SR_729_4	EF162529			PS61/164-1, 53°23,80′S/042°42,03′W-53°23,82′S/042°42,46′W, 312–321 m
	SR_729_5	EF162530			PS61/164-1, 53°23,80′S/042°42,03′W-53°23,82′S/042°42,46′W, 312–321 m
	SR_606_1		EF162483	h7/HG4	PS61/169-1, 53°22,94′S/042°41,37′W-53°22,89′S/042°41,50′W, 284–292 m
	SR_606_2	EF162524			PS61/169-1, 53°22,94′S/042°41,37′W-53°22,89′S/042°41,50′W, 284–292 m
	SR_606_3	EF162528			PS61/169-1, 53°22,94′S/042°41,37′W-53°22,89′S/042°41,50′W, 284–292 m
	SSI_758_2	EF162514	EF162487	h3/HG1	PS61/194-1, 57°40,55'S/026°25,14'W-57°40,66'S/026°25,90'W, 308–289 m
	SSI_761_2	EF162513			PS61/200-1, 57°40,78′S/026°24,91′W-57°40,84′S/026°24,98′W, 270–263 m
	SSI_214_1	EF162511	EF162485	h1/HG1	PS61/214-1, 59°42,55′S/027°57,02′W-59°42,62′S/027°57,68′W, 333–341 m
	SSI_214_2	EF162510			PS61/214-1, 59°42,55′S/027°57,02′W-59°42,62′S/027°57,68′W, 333–341 m
	SSI_214_4	EF162512	EF162486	h3/HG1	PS61/214-1, 59°42,55′S/027°57,02′W-59°42,62′S/027°57,68′W, 333–341 m
	HB_674_3		EF162481	h2/HG1	PS61/217-1, 59°54,98′S/032°28,33′W-59°54,79′S/032°28,93′W, 521–518 m
	HB_674_4	EF162523			PS61/217-1, 59°54,98′S/032°28,33′W-59°54,79′S/032°28,93′W, 521–518 m
	DB_801_1	EF162532	EF162477	h3/HG1	PS61/223-1, 60°08,16′S/034°55,59′W-60°08,39′S/034°54,96′W, 375–379 m
	DB_801_2	EF162531			PS61/223-1, 60°08,16′S/034°55,59′W-60°08,39′S/034°54,96′W, 375–379 m
	DB_801_4		EF162478	h3/HG1	PS61/223-1, 60°08,16′S/034°55,59′W-60°08,39′S/034°54,96′W, 375–379 m
	DB_801_5	EF162533			PS61/223-1, 60°08,16′S/034°55,59′W-60°08,39′S/034°54,96′W, 375–379 m
	DB_801_6	EF162534			PS61/223-1, 60°08,16′S/034°55,59′W-60°08,39′S/034°54,96′W, 375–379 m
	SO_820_2	EF162508			PS61/231-1, 60°59,19′S/043°27,42′W-60°58,89′S/043°26,71′W, 402–399 m
	SO_820_3	EF162509			PS61/231-1, 60°59,19′S/043°27,42′W-60°58,89′S/043°26,71′W, 402–399 m
	SO_677_1	EF162506	EF162482	h4/HG2	PS61/241-1, 61°11,15′S/045°43,84′W-61°10,95′S/045°41,42′W, 323–322 m
	SO_677_2	EF162507			PS61/241-1, 61°11,15′S/045°43,84′W-61°10,95′S/045°41,42′W, 323–322 m
	SO_677_3	EF162505			PS61/241-1, 61°11,15′S/045°43,84′W-61°10,95′S/045°41,42′W, 323–322 m
	WS_198_5	EF162515			PS65/090-1, 70°56,14′S/010°31,70′W-70°55,92′S/010°32,37′W, 274–288 m
	WS_198_6	EF162516			PS65/090-1, 70°56,14′S/010°31,70′W-70°55,92′S/010°32,37′W, 274–288 m
	WS_260_1	EF162518			PS65/132-1, 70°55,86′S/010°30,15′W-70°55,78′S/010°29,96′W, 243–238 m
	WS_260_4	EF162517			PS65/132-1, 70°55,86′S/010°30,15′W-70°55,78′S/010°29,96′W, 243–238 m
	WS_260_9	EF162519			PS65/132-1, 70°55,86′S/010°30,15′W-70°55,78′S/010°29,96′W, 243–238 m
	WS_522_11	EF162521			PS65/253-1, 71°04,89′S/011°32,21′W-71°04,30′S/011°33,92′W, 295–309 m
	WS_570_5		EF162489	h8/HG5	PS65/274-1, 70°52,16′S/010°43,69′W-70°52,15′S/010°42,16′W, 291–288 m
	WS_570_6		EF162490	h9/HG5	PS65/274-1, 70°52,16′S/010°43,69′W-70°52,15′S/010°42,16′W, 291—288 m
	WS_570_10		EF162488	h8/HG5	PS65/274-1, 70°52,16′S/010°43,69′W-70°52,15′S/010°42,16′W, 291–288 m
	WS_682_2		EF162493	h8/HG5	PS65/292-1, 72°51,43′S/019°38,62′W-72°51,84′S/019°39,41′W, 598–577 m
	WS_682_3	EF162522	EF162494	h8/HG5	PS65/292-1, 72°51,43′S/019°38,62′W-72°51,84′S/019°39,41′W, 598–577 m
	WS_682_5		EF162495	h9/HG5	PS65/292-1, 72°51,43′S/019°38,62′W-72°51,84′S/019°39,41′W, 598–577 m
	WS_682_9		EF162496	h9/HG5	PS65/292-1, 72°51,43′S/019°38,62′W-72°51,84′S/019°39,41′W, 598–577 m
	WS_682_10		EF162491	h9/HG5	PS65/292-1, 72°51,43'S/019°38,62'W-72°51,84'S/019°39,41'W, 598–577 m
	WS_682_11		EF162492	h9/HG5	PS65/292-1, 72°51,43′S/019°38,62′W-72°51,84′S/019°39,41′W, 598–577 m
	WS_787_5		EF162498	h9/HG5	PS65/308-1, 72°50,18′S/019°35,94′W-72°50,09′S/019°35,82′W, 622–616 m
	WS_787_8		EF162499	h10/HG5	PS65/308-1, 72°50,18′S/019°35,94′W-72°50,09′S/019°35,82′W, 622–616 m
	WS_787_11		EF162497	h9/HG5	PS65/308-1, 72°50,18′S/019°35,94′W-72°50,09′S/019°35,82′W, 622–616 m
	WS_787_12	EF162520			PS65/308-1, 72°50,18′S/019°35,94′W-72°50,09′S/019°35,82′W, 622–616 m
	RS_k8		EF162500	h11/HG6	TAN0204/63, 72°19,30′S/170°28,72′E-72°19,30′S/170°28,72′E, 303-293 m
	RS_k7_2		EF162503	h13/HG6	TAN0204/63, 72°19,30′S/170°28,72′E-72°19,30′S/170°28,72′E, 303–293 m
	RS_118_1		EF162501	h11/HG6	TAN0204/118, 71°17,95′S/170°32,19′E-71°17,95′S/170°32,19′E, 312-323 m
	RS_118_2	EF162538			TAN0204/118, 71°17,95′S/170°32,19′E-71°17,95′S/170°32,19′E, 312–323 m
	RS_118_5	EF162541			TAN0204/118, 71°17,95′S/170°32,19′E-71°17,95′S/170°32,19′E, 312-323 m
	RS_118_7	EF162539			TAN0204/118, 71°17,95′S/170°32,19′E-71°17,95′S/170°32,19′E, 312–323 m
	RS_140_2		EF162502	h12/HG6	TAN0204/140, 70°00,81′S/170°46,47′E-70°00,81′S/170°46,47′E, 231-240 m
	RS_140_8	EF162540			TAN0204/140, 70°00,81′S/170°46,47′E-70°00,81′S/170°46,47′E, 231–240 m
Lissarca miliaris	SO_834_4	EF162504			PS61/Signy, 60°41S/045°34W-60°41S/045°34W, 1 m
Cosa waikikia		**AB101614**	**AB084107**		
Limopsidae					
Limopsis enderbyensis		**AY321301**			
Limopsis marionensis		**AY319791**			
Limopsis lilliei		**AY319806**			

Table 1 continued

	Spec no.	GenBank accession no.		Haplotype/ H group	Locality
		28S rRNA	COI		
Empleconia cumingii		**AB101610**	**AB076930**		
Arcidae					
Barbatia barbata			**AF120845**		
Barbatia virescens		**AB105356**			
Scapharca globosa			AB254194		

In bold are GenBank accession number of the outgroup taxa

h haplotype, *HG* haplotype group, *spec no.* specimen number

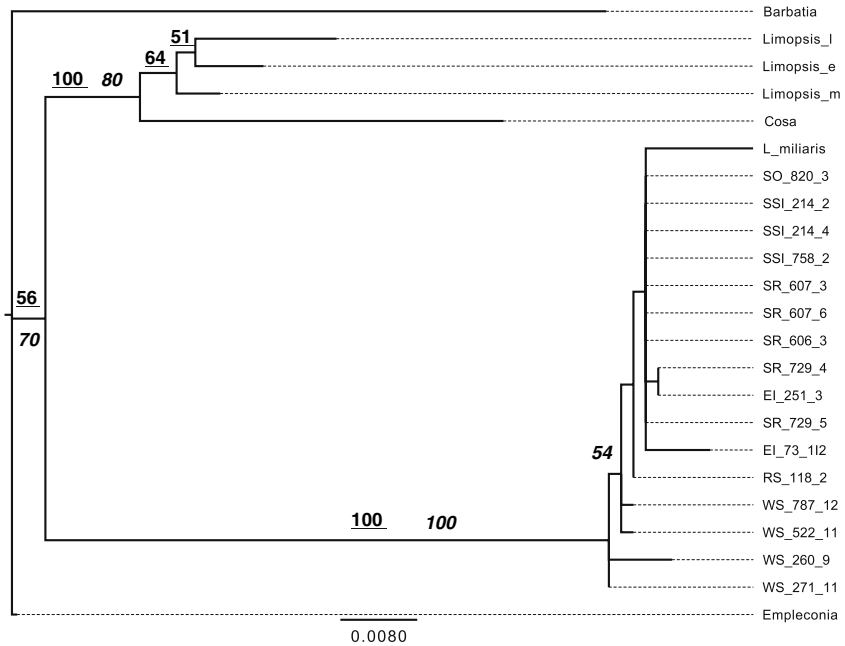

Fig. 2 Molecular phylogeny of single gene analysis of the 28S rDNA gene for the reduced *L. notorcadensis* sequence set. Tree was produced by ML analysis. Support values in italics are ML bootstrap values of 1,000 replicates. Bayesian support values (posterior probabilities) are underlined

(GTR + I + Γ) with flat priors. 50,000,000 generations with four chains were run, sampling every 50th generation.

Pair-wise distances were calculated in MEGA v 3 (Kumar et al. 2004). The evolutionary relationships among COI haplotypes were reconstructed using statistical parsimony in TCS v1.21 (Clement et al. 2000). In order to explore protein evolution, sequences were translated into amino acids and phylogenies reconstructed in MEGA.

Results

Partial sequences of the 28S rDNA and COI genes were generated to examine the phylogenetic relationships and population structure of *L. notorcadensis*. For the first 28S rDNA gene segment we determined sequences of 36 *L. notorcadensis* and one *L. miliaris* and added Genbank sequences of one phylobryid, four limopsids and one arcid as outgroup taxa. The aligned 28S rDNA dataset comprised 806 characters of which 106 were variable and 55 were parsimony informative. Within *L. notorcadensis* only 16 characters varied. Average base frequencies were pi(A)21.5%, pi(C) = 24.3%, pi(G) = 33.2% and pi(T) = 20.9%.

Within *Lissarca* there was not enough resolution to identify any structure using ML, MP or Bayesian analyses. However, between *Lissarca* and the included phylobryids and limopsids, the three analysis techniques yielded congruent results.

The ML Trees were rooted with the arcid *Barbatia* to reveal the phylogenetic position of *Lissarca* within the Limopsidae and Phylobryidae (Fig. 2). The topology of the tree shows that neither of the sister families is monophyletic but supports *Lissarca* as a monophyletic taxon.

For the COI segment we obtained sequences of 27 *L. notorcadensis* and added Genbank sequences of one phylobryid, one limopsid and two arcids as outgroup taxa. The length of the aligned COI dataset consisted of 337 nucleotides of which 169 were variable and 93

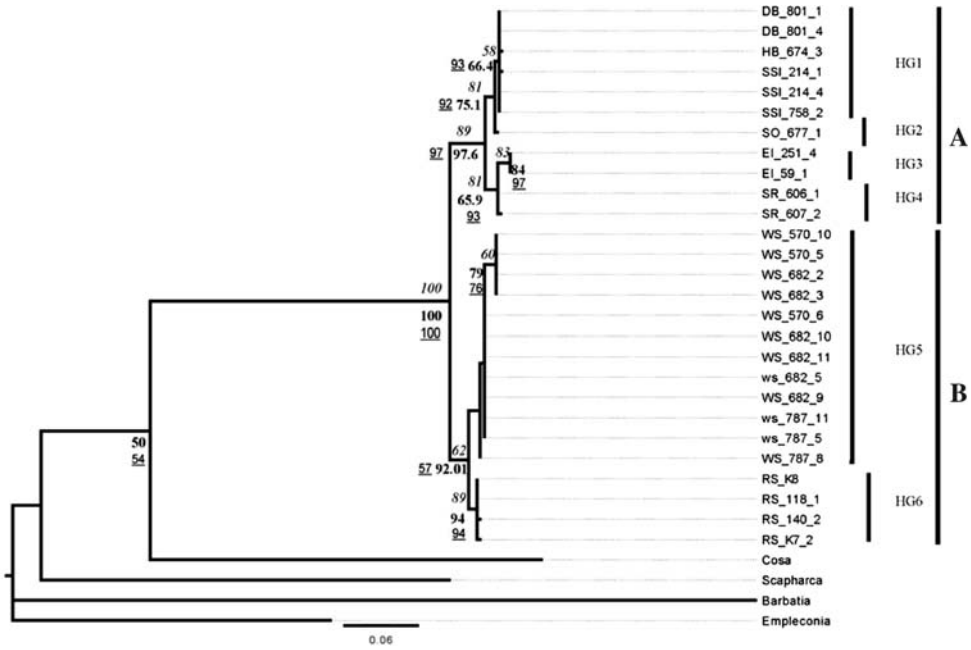

Fig. 3 Molecular phylogeny of single gene analysis of the COI mtDNA gene for *L. notorcadensis*. Tree was produced by ML analysis. ML and MP support values are bootstrap values of 1,000 replicates, MP values are in *bold*, ML values are in *italics*. Bayesian support values (posterior probabilities) are *underlined*. A lineage A, B lineage B, HG haplotype group

were parsimony informative. Within *L. notorcadensis*, the COI fragment revealed 27 variable sites and thirteen haplotypes forming six haplotype groups (Table 1). Of the 27 detected nucleotide substitutions, 21 were third codon, 5 second codon and 1 first codon. The unusual ratio, due to a comparatively high number of second codon changes, prompted us to investigate the geographic associations of second position variation. Of the five second position changes, three of them separate WS and RS from the SA. Within the Weddell Sea/Ross Sea group there were ten variable sites, all third positions. Within the Scotia arc group there were nine variable sites; one first position, two second position and six third position changes. The second position changes form two groups; ESA and SO form one group while SR and EI form a second.

Tree topologies from MP, ML and Bayesian analyses were congruent and revealed two major, highly supported lineages in *L. notorcadensis*; lineage A comprising individuals from the banks and islands of the Scotia Sea, and lineage B individuals from the Weddell and Ross Seas (Fig. 3). Within Lineage A, four well supported haplotype groups (HG) were identified: HG1 includes individuals from around the South Sandwich Islands, Discovery Bank and Herdman Bank. HG2 contains a single haplotype from South Orkneys, HG3 contains a single haplotype from Elephant Island, and HG4 contains two haplotypes from Shag Rocks near South Georgia. Lineage B consists of two strongly supported haplotype groups, Weddell Sea and Ross Sea with three haplotypes each. Pair-wise genetic differences highlight within-lineage variabilities (Fig. 4). The East Scotia Arc (ESA) group is clearly different from the Elephant Island and Shag Rock groups. The statistical parsimony networks in Fig. 5 graphically display the genetic and geographic associations of haplotypes (Fig. 5).

Discussion

The work presented here has detected population structure within *L. notorcadensis* and explored unresolved issues in the systematics of the Philobryidae and Limopsidae. Based on shell and soft part morphology, Tevez (1977) suggested the ancestry of the Philobryidae to be traced to the Limopsidae. On a molecular level, the relationships between these two families have not been adequately studied. Matsumoto (2003), in his phylogeny of the Pteriomorpha using the CO1 gene, cited *Cosa waikikia* (Dall, Bartsch and Rehder 1939) and the limopsid *Empleconia cumingii* (Adams 1863) as examined species in his dataset but only the latter was used in his analysis. The Matsumoto 28S and CO1 sequences for *Cosa* and *Empleconia* were included in our analyses. As the position of the philobryid *Cosa* within the Limopsidae questions the monophyly of these families, only further analyses including more species of the philobryid genera, like *Adacnarca*, *Philobrya* and *Cratis*, will resolve this question.

Our primary goal was to establish a model to address the three a priori hypotheses relating to (1) the underestimation of biodiversity in Antarctic and sub-Antarctic Mollusca, (2) the Scotia Arc is a source of

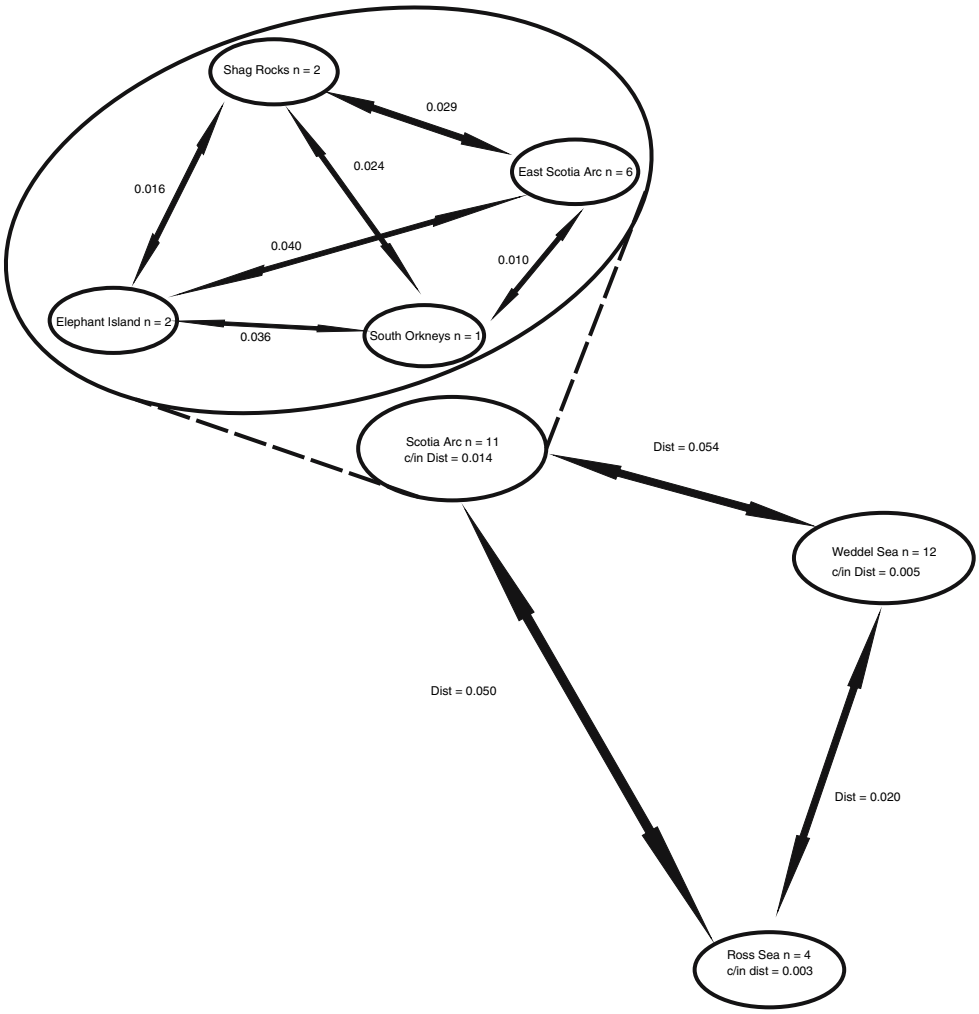

Fig. 4 Pair-wise genetic differences within and between the lineages A and B of COI haploype groups of *L. notorcadensis*. *n* number of specimens

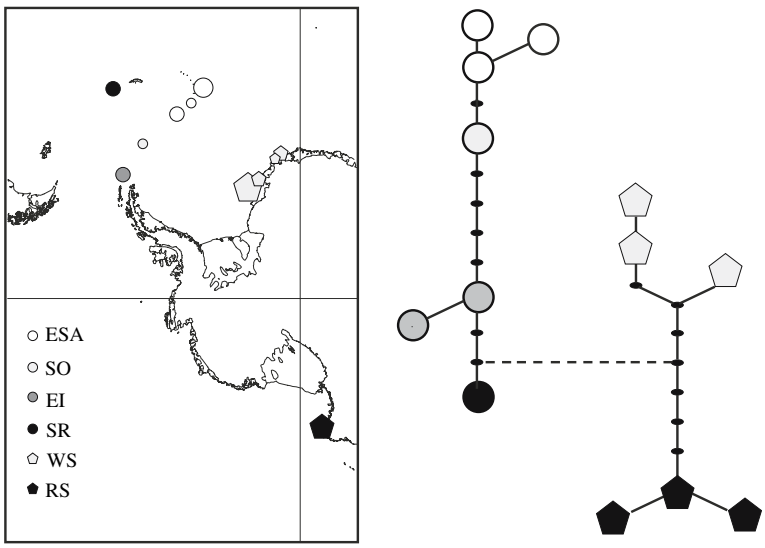

Fig. 5 Statistical parsimony networks displaying the genetic and geographic associations of haplotypes. *Dashes* 18 steps separating networks, *EI* Elephant Island, *ESA* East Scotia Arc, *SO* South Orkney Islands, *SR* Shag Rocks, *WS* Weddell Sea, *RS* Ross Sea

biodiversity, and (3). the Weddell Sea gyre isolates Weddell Sea populations from those of the Scotia Sea.

The 28S data set was not informative enough to confidently distinguish geographic differences, the CO1 results clearly identified two deeply diverged lineages separating continental Antarctic from Scotia Sea samples, and six geographically explicit haplotype groups indicating discrete populations, one each in the

Weddell and Ross Seas, and four in the Scotia Sea. These lineages are also supported by phenotypic data. Cope and Linse (2006) found significant difference in the shell length/height ratios between Scotia Sea specimens and Weddell/Ross Sea specimens. Whether or not the populations from the Weddell Sea and Ross Sea are typical of a linked circum-Antarctic distribution is not tested here. Even so, the Antarctic Peninsula appears to be associated with a barrier for the Antarctic lineage sampled, so the samples we examined from the Weddell Sea and the Ross Sea are likely to be at the extremes of the range of this population and thus should reflect the greatest genetic distance. However, the variation within the Scotia Sea lineage exceeds that within the continental Antarctic Lineage (Fig. 4). A study on the isopod *Glyptonotus antarcticus* Eights, 1852 found a strikingly similar pattern regarding the genetic versus the geographic distances between Ross Sea, Weddell Sea and Antarctic Peninsula populations (Held and Wägele 2005). Using mitochondrial 16S sequences from specimens collected in the eastern Weddell Sea, at Elephant Island, Adelaide Island/Antarctic Peninsula and in the Ross Sea, Held and Wägele (2005) found 11 unique haplotypes forming four haplotype groups. One group contained the haplotypes from Elephant Island and Adelaide Island and another the Ross Sea individuals while two haplotype groups occurred in the Weddell Sea. The pairwise genetic differences between the Ross Sea group and the Weddell Sea groups were smaller than those between the former groups and the Elephant/Adelaide Island group. As suggested by Held and Wägele (2005) in the case of the isopod, we find it likely that the continental Antarctic and Scotia Sea populations may in fact denote cryptic species and supports the premise of the first hypothesis.

Within the Scotia Sea lineage genetic variation is congruent with the general morphological differences found by Cope and Linse (2006) between Shag Rock and other Scotia Sea specimens. The depth of the divergence between these groups lends support for the second hypothesis. The diversity present among different island groups of the Scotia Arc may be strongly affected by deep water separating habitats and preventing migration. This was found to be the case with the octopod *Pareledone turqueti* where deep water between South Georgia and Shag Rocks presented a barrier to geneflow resulting in profound population structure (Allcock et al. 2007). As *L. notorcadensis* is a brooding species and restricted to shelf upper slope waters (Cope and Linse 2006), the deep water is likely to prevent or substantially restrict migration between shallow water habitats. It is interesting to note the pattern of divergence, with Elephant Island specimens more similar to Shag Rock than South Orkney and East Scotia Arc specimens despite greater geographic distance of deep waters separating Elephant Island and Shag Rock relative to that between South Orkney. To further investigate the patterns, processes and depth of divergence between these putative populations, a more intense geographical sampling regime is required together with a multilocus genetic analysis approach.

Our third hypothesis stated that the Weddell Sea gyre partially acted as a barrier to geneflow between Eastern Weddell Sea populations and those of the Antarctic Peninsula and the Scotia arc. Using mitochondrial 16S sequence data Raupach and Wägele (2006) found strong differentiation between Eastern and Western Weddell Sea samples in the isopod *Acanthaspidia drygalski* Vanhöffen, 1914. This pattern is similar to that of the isopod *Glyptonotus antarcticus* mentioned above (Held and Wägele 2005) which does indeed indicate restricted geneflow between the east and west Weddell Sea. Interestingly, divergence between groups within the Weddell Sea was greater than that between the Weddell Sea and the Ross Sea. Our results clearly indicate that Weddell Sea samples are genetically distinct from those of the Scotia arc. However, as there is a similar difference between the Ross Sea and Scotia arc we find no specific evidence supporting the premise that the Weddell Sea gyre is the sole mechanism preventing geneflow.

An interesting question relates to the genetic differences between haplotypes from the geographically well-separated Weddell and Ross Seas that are smaller than those between the neighbouring and connected Weddell and Scotia Seas in our study and in that of Held and Wägele (2005). Not only molecular data show close relations between the Weddell and the Ross Seas. The most recent biogeographic analysis of bivalves and shelled gastropods at various systematic levels showed that the relationships between these two seas are closer than either ‘s relationship to the Scotia Sea islands (Linse et al. 2006). The processes behind these patterns are yet unknown. Possible explanations lie either in the past of the Antarctic continent or in its more recent oceanography. A linking seaway between the Weddell and Ross Seas may have been present during the Eocene to mid-Miocene (50–15 Ma BP), connecting the two and separating them from the West Antarctic, microcontinental fragments that later on formed the Antarctic Peninsula and Scotia arc islands (Lawver and Gahagan 2003). Scherer et al. (1998) proposed a Pleistocene collapse of the West Antarctic Ice Sheet providing seaways between the Weddell and Ross Seas which may have facilitated geneflow

between these populations. The present large-scale features in Antarctic oceanography are the clockwise Antarctic circumpolar current (ACC) system, separating the warmer water masses north of the Polar Front from the colder Southern Ocean, the continental, anticlockwise counter current and the Weddell Gyre (Fahrbach et al. 1994; Orsi et al. 1993, 1995). The continental counter current used as a migration tool could explain the lower genetic variability between the Weddell and Ross Seas under the assumption that related haplotypes occur along the East Antarctic coastline. The strong flowing ACC, initiated in the Early Oligocene (30 Ma BP) (Lawver and Gahagan 2003), is likely to be important for the closer haplotype relationships within the Scotia Sea.

In this study we have identified strong genetic structure in the brooding bivalve *L. notorcadensis* between Antarctic and sub-Antarctic groups, and within the sub-Antarctic specimens sampled throughout the Scotia arc. We have found evidence for cryptic species and it may be that more detailed phylogeographic studies with intensive sampling and multiple single copy loci could uncover the true nature of the genetic structure and identify the processes underlying the patterns.

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References

Allcock AL, Breirley AS, Thorpe JP, Rodhouse PG (1997) Restricted geneflow and evoltuionary divergence between geographically separated populations of the Antarctic octopus *Pareledone turqueti*. Mar Biol 129:97–102

Allcock AL, Strugnell JM, Prodoehl P, Piatkowski U, Vecchione M (2007) A new species of *Pareledone* (Cephalopoda: Octopodidae) from the Antarctic Peninsula. Polar Biol doi:10.1007/s00300-006-0248-9

Allegrucci G, Carchini G, Todisco V, Convey P, Sbordoni V (2006) A molecular phylogeny of Antarctic chironomidae and its implications for biogeographical history. Polar Biol 29:320–326

Avise JC (2004) Molecular markers. Sinauer, Sunderland, p 541

Barker PF, Burrell J (1977) The opening of the Drake passage. Mar Geol 25:15–34

Boenigk J, Pfandl K, Garstecki T, Harms H, Novarino G, Chatzinotas A (2006) Evidence for geographic isolation and signs of endemism within a protistan morphospecies. Appl Environ Microbiol 72(8):5159–5164

Brey T, Hain S (1992) Growth, reproduction and production of *Lissarca notorcadensis* (Bivalvia, Philobryidae) in the Weddell Sea, Antarctica. Mar Ecol Prog Ser 82:219–226

Brey T, Clarke A (1993) Population dynamics of marine benthic invertebrates in Antarctic and subantarctic enviroments: are there unique adaptaions? Antarct Sci 5(3):253–266

Brey T, Starmans A, Magiera U, Hain S (1993) *Lissarca notorcadensis* (Bivalvia: Philobryidae) living on I sp. (Echinoidea:Cidaridae):population dynamics in limited space. Polar Biol 13:89–95

Clarke A (2000) Evolution in the cold. Antarct Sci 112:257

Clarke A, Johnston NM (2003) Antarctic marine benthic diversity. Oceanogr Mar Biol 41:47–114

Clement M, Posada D, Crandall K (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9(10):1657–1660

Cope T, Linse K (2006) Morphological differences *in Lissarca notorcadenis* Melvill and Standen, 1907 from the Scotia, Weddell and Ross Seas. Deep-Sea Res II 53:903–911

Dell RK (1990) Antarctic Mollusca: with special reference to the fauna of the Ross Sea. Bull Roy Soc NZ 27:1–311

Fahrbach E, Rohardt G, Krause G (1992) The Antarctic Coastal Current in the southeastern Weddell Sea. Polar Biol 12:171–182

Fahrbach E, Rohardt G, Schroeder M, Strass V (1994) Transport and structure of the Weddell Gyre. Annales Geophysicae 12:840–855

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech 3:294–299

Frankham R, Ballou JD, Briscoe DA (2004) A Primer of conservation genetics. University Press, Cambridge pp 234

Frati F, Spinsant G, Dallai R (2001) Genetic variation of mtCOII gene sequences in the collembolan *Isotoma klovstadi* from Victoria Land, Antarctica: evidence of population differentiation. Polar Biol 12:934–940

Freckman DW, Virginia RA (1997) Low-diversity Antarctic soil nematode communities: distribution and response to disturbance. Ecology 78(2):363–369

Griffiths HJ, Linse K, Crame JA (2003) SOMBASE—Southern Ocean Mollusc Database: a tool for biogeographic analysis in diversity and ecology. Org Divers Evol 3(3):207–213

Gutt J, Sirenko BI, Arntz WE, Smirnov IS, De Broyer C (2000) Biodiversity of the Weddell Sea: macrozoobenthic species (demrsal fish included) sampled during the expedition ANT XIII/3 (EASIZ I) with RV 'Polarstern'. Ber Polarforsch 372

Hain S (1990) Die beschalten benthischen Mollusken (Gastropoda und Bivalvia) des Weddellmeeres, Antarktis. Berichte zur Polarforschung 70:1–181

Held C (2000) Phylogeny and biogeography of serolid isopods (Crustacea, Isdopoda, Serolidae) and the use of ribosomal expension segments in molecular systematics. Mol Phylogenet Evol 15(2):165–178

Held C, Wägele JW (2005) Cryptic speciation in the giant Antarctic isopod *Glyptonotus antarcticus* (Isopoda: Valvifera: Chaertiliidae). Scientia Marina 69(2):175–181

Held C, Leese F (2006) The utility of fast evolving molecular markers for studying speciation in the Antarctic benthos. Polar Biol 30:513–521

Hofmann EE, Klinck JM, Locarnini RA, Fach B, Murphy E (1998) Krill transport in the Scotia Sea and environs. Antarct Sci 10:406–415

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163

Lawver LA, Gahagan LM (2003) Evolution of Cenozoic seaways in the circum-Antarctic region. Paleogeogr Paleoclimatol Paleoecol 198(1–2):11–37

Linse K, Griffiths HJ, Barnes DKA, Clarke A (2006) Biodiversuty and biogeography of Antarctic and sub-Antarctic Mollusca. Deep-Sea Res II 53:985–1008

Littlewood DTJ (1994) Molecular phylogenetics of cupped oysters based on partial 28S rDNA gene sequences. Mol Phylogenet Evol 3:221–229

Lörz AN, Maas EW, Linse K, Fenwick GD (2007) *Epimeria schiaparelli* sp. nov., an amphipod crustacean (family Epimeriidae) from the Ross Sea, Antarctica, with molecular characterisation of the species complex. Zootaxa 1402:23–37

Maslen NR, Convey P (2006) Nematode diversity and distribution in the southern maritime Antarctic—clues to history? Soil Biol Biochem 38(10):3141–3151

Matsumoto M (2003) Phylogenetic analysis of the subclass. Pteriomorphia (Bivalvia) from mtDNA COI sequences. Mol Phylogenet Evol27(33):429–440

Orsi AH, Nowlin WD, Whitworth T (1993) On the circulation and stratification of the Weddell Gyre. Deep-Sea Res 40:169–203

Orsi AH, Whitworth T, Nowlin WD (1995) On the meridional extent and fronts of the Antarctic Circumpolar Current. Deep-Sea Res Part I-Oceanogr Res Pap 42(5):641–673

Page TJ, Linse K (2002) More evidence of speciation and dispersal across the Antarctic Polar Front through molecular systematics of Southern Ocean *Limatula* (Bivalvia: Limidae). Polar Biol 25:818–826

Pawlowski J, Fahrni JF, Brykczynska U, Habura A, Bowser SS (2002) Molecular data reveal high taxonomic diversity of allogromiid Foraminifera in Explorers Cove (McMurdo Sound, Antarctica). Polar Biol 25(2):96–105

Powell AWP (1951) Antarctic and sub-Antarctic Mollusca: Pelecypoda and Gastropoda. Discov Rep 26:49–196

Prezant RS, Showers M, Winstead RL, Cleveland C (1992) Reproductive ecology of the Antarctic bivalve *Lissarca notorcadensis* (Philobryidae). Am Malacol Bull 9(2):173–186

Raupach MJ, Wägele JW (2006) Distinguishing cryptic species in Antarctic Asellota (Crustacea: Isopoda)—a preliminary study of mitochondrial DNA in *Acanthaspidia drygalskii*. Antarct Sci 18:191–198

Raupach MJ, Held C, Wägele JW (2004) Multiple colonization of the deep sea by the Asellota (Crustacea: Peracarida: Isopoda). Deep-SEa Res II 51:1787–1795

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinform 19:1572–1574

Ronquist F, Huelsenbeck JP (2005) Bayesian analysis of molecular evolution using MrBayes. In: Nielsen R (eds) Statistical methods in molecular evolution. Springer, New York

Scherer RP, Aldahan A, Tulaczyk S, Possnert G, Engelhardt H, Kamb B (1998) Pleistocene collapse of the West Antarctic Ice sheet. Science 281:82–85

Stary J, Block W (1998) Distribution and biogeography of oribatid mites (Acari: Oribatida) in Antarctica, the sub-Antarctic islands and nearby land areas. J Nat Hist 32(6):861–894

Stein M, Heywood RB (1994) Antarctic environment—physical oceanography: the Antarctic Peninsula and Southwest Atlantic region of the Southern Ocean. In: El-Sayed SZ (eds) Southern Ocean ecology. CPU, Cambridge pp 11–24

Stevens MI, Hogg ID (2003) Long-term isolation and recent range expansion revealed for the endemic springtail Gomphiocephalus hodgsoni from southern Victoria Land, Antarctica. Mol Ecol 12:2357–2369

Stevens MI, Hogg ID (2006) Contrasting levels of mitochondrial DNA variability between mites (Penthalodidae) and springtails (Hypogastruridae) from the Trans-Antarctic Mountains suggest long-term effects of glaciation an dlife history on substitution rates, and speciation processes. Soil Biol Biochem 38:3171–3180

Stevens MI, Greenslade P, Hogg ID, Sunnucks P (2006) Southern hemisphere springtails: could any have survived glaciation of Antarctica? Mol Biol Evol 23:874–882

Swofford D (2002) Paup*. Phylogenetic Analysis using Parsimony (*and other methods). (Version 4.10beta). Sinauer Associates, Sunderland, Massachusetts

Tevesz MJS (1977) Taxonomy and ecology of the Philobryidae and Limopsidae (Mollusca: Pelecypoda). Postilla 171:1–64

Wagner DL, Liebherr JK (1992) Flightlessnes in insects. Trends Ecol Evol 7:216–220

Whitworth T, Nowlin WD, Orsi AH, Locarnini RA, Smith SG (1994) Weddell Sea Shelf Water in the Bransfield Strait and Weddell-Scotia Confluence. Deep-Sea Res Part I-Oceanogr Res Pap 41(4):629–641