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Biogeographical and phylogeographical relationships of the bathyal ophiuroid fauna of the Macquarie Ridge, Southern Ocean

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Abstract There are relatively few studies examining the latitudinal distribution of polar, subantarctic and temperate faunas on the bathyal seafloor across the Southern Ocean. Here, we investigate the relationship between the subantarctic Macquarie Ridge and adjacent regions of Antarctica (including the Ross Sea) and temperate Australia and New Zealand at depths of 200–2,500 m. We study the fauna at two levels of classification (1) morpho-species (MSPs) accepted by taxonomists and (2) evolutionary significant units defined as reciprocally monophyletic clades derived from phylogenies of mitochondrial DNA. The ophiuroid fauna on the Macquarie Ridge has a predominantly temperate origin, with far more MSPs shared with south-eastern Australia (78 % of species) and southern New Zealand (83 %) than neighbouring Antarctic regions (33 %). However, this asymmetry also reflects the relative species richness of these regions. Many species that are shared between Antarctica and the Macquarie Ridge have diverged into distinct mtDNA lineages indicative of a recent barrier to gene flow.

Keywords Subantarctic · Ophiuroidea · COI · Biogeography · Phylogeography

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Introduction

A key challenge for deep-sea biogeographical research is to investigate patterns of species distribution across the vast oceanic basins. While the fauna of the continental slope (or 'bathyal' seafloor, 200–3,500 m depth) has been extensively researched in some regions, most notably around the North Atlantic and North Pacific Oceans, other areas, including much of the Southern Ocean, are relatively unexplored. The large cost of deep-sea research expeditions and divergent national interests have typically precluded substantial trans-oceanic studies.

To overcome these obstacles, the Census of Marine Life (CoML, 2000–2010) was funded by the Sloan Foundation to foster international marine research. Two CoML collaborative projects have contributed substantially to seafloor samples and data for the Southern Ocean between Australia, New Zealand and Antarctica. The Global Census of Marine Life on Seamounts (CenSeam) supported expeditions to the Macquarie Ridge (TAN0803), off southern Tasmania (SS02/2007) and around New Zealand (e.g., TAN0604) which contributed to a re-evaluation of the macroecology of seamounts (Clark et al. 2010). The Census of Antarctic Marine Life (CaML) supported several

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benthic research voyages as part of the International Polar Year (2006–2008), including the Australian–French expedition to eastern Antarctica (CEAMARC, Hosie et al. 2011) and the New Zealand expedition to the Ross Sea, Admiralty and Scott seamounts (TAN0802, Bowden et al. 2011). Among the collected marine invertebrates, ophiuroids (brittle-stars) have become useful model organisms for large-scale biogeographical research because of their abundant and frequent occurrence across a range of benthic habitats, from intertidal to hadal depths, from equatorial to polar regions, and from rocky to muddy substrata (O'Hara et al. 2011).

An initial biogeographical analysis, derived from habitat suitability models of 267 ophiuroid species, identified broad faunal bands across the Australian/New Zealand and eastern Antarctic region at both littoral (0–200 m) and upper bathyal (200–2,000 m) depths (O'Hara et al. 2011). These included a 'temperate' fauna and a 'polar' fauna with a broad transition zone at subantarctic latitudes around Macquarie Island. However, it is unclear what drives the formation of this transition zone, which also occurs between the Antarctic Peninsula and South America (Griffiths et al. 2009). While limits to species ranges are usually considered in terms of environmental or dispersal constraints (McClain and Hardy 2010), it is also possible that patterns are driven by sampling artefacts or an inadequate taxonomic understanding of species boundaries. Artefacts can arise through sampling different habitats or bathymetric ranges in different regions, for example the Macquarie Ridge is largely composed of rocky seamounts, ridge outcrops and scree slopes (Butler et al. 2000), whereas the Ross Sea is mainly sedimentary plains (Anderson et al. 1984). Many molecular studies of echinoderms have documented previously unrecognised species frequently leading to the taxonomic reassessment of species boundaries (e.g., O'Hara et al. 2004). On the other hand, nominal species described from different regions have later proved to be synonyms (O'Hara and Stöhr 2006).

Consequently, in this paper, we investigate the subantarctic faunal transition through an analysis of biogeographical relationships of the Macquarie Ridge ophiuroid fauna at two levels of classification, morpho-species (MSPs) accepted by current taxonomists and evolutionary significant units (ESUs) derived from phylogenetic reconstructions of mitochondrial COI sequences. We use ESUs (1) to refine the taxonomy of our species because preliminary genetic data raised obvious limitations in the nomenclature such as polyphyletic taxa and (2) as a measure of within-species historical connectivity. The designation of ESUs is a simple qualitative summary of phylogeographical patterns; the limited and uneven sampling precluding the use of more quantitative approaches. We do not assume these units will be eventually considered as separate biological species or deserving of active conservation management (Moritz 1994).

We use the COI gene to define ESUs because a large number of sequences have become available through the sponsorship of CaML and the International BarCode of Life programme. Although the use of COI for phylogenetic reconstructions can be problematic, for example through introgression (Balloux 2010), in general, phylogeographical structure derived from nuclear genes does not contradict that from mitochondrial data (Zink and Barrowclough 2008), including for ophiuroids (Naughton and O'Hara unpublished data). COI 'barcode' sequences typically discriminate echinoderm morpho-species (Ward et al. 2008; Uthicke et al. 2009; Hemery et al. 2012), although a few cases are known where forms with distinct morphologies or reproductive habits have not achieved reciprocal monophyly, that is, when sequences within each taxon are genealogically closer to one another than to those in the other taxon (e.g., Williams 2000; Stöhr et al. 2009; Hoareau and Boissin 2010). To refine our designation of ESUs, we included comparative material from western Antarctica in our phylogenetic analyses.

To avoid habitat sampling artefacts, we have included specimens collected from hard-substrata habitats, typical of the Macquarie Ridge, from neighbouring regions, including the recently surveyed Balleny, Admiralty and Scott Islands/Seamounts adjacent to the Ross Sea. Finally, we have assessed the congruence of the latitudinal and bathymetric distribution of the MSPs and ESUs with environmental data.

Methods

The study area is a sector of the eastern Southern Ocean between 40°–75°S and 135°E–170°W at seafloor depths of 200–2,500 m. The study area was divided into six regions on the basis of the latitudinal gradient and spread of samples (Fig. 1): two temperate regions (south-eastern Australia and New Zealand), the Macquarie Ridge, and three Antarctic regions (Terre Adélie/King George V Land; Balleny/Admiralty/Scott seamounts and Islands; and the Ross Sea). Species distributional data were extracted from a database of ophiuroid distributional records that originated from a synthesis of known museum records and the literature data from throughout the region (O'Hara et al. 2011). The presence/absence of MSPs in regions across the study area was visualised through multidimensional scaling (MDS) ordination and the Bray-Curtis dissimilarity coefficient using the software PRIMER-E v6 (Clarke and Warwick 2001).

Tissue samples for molecular analysis were extracted from the collections of (a) National Institute of Water &

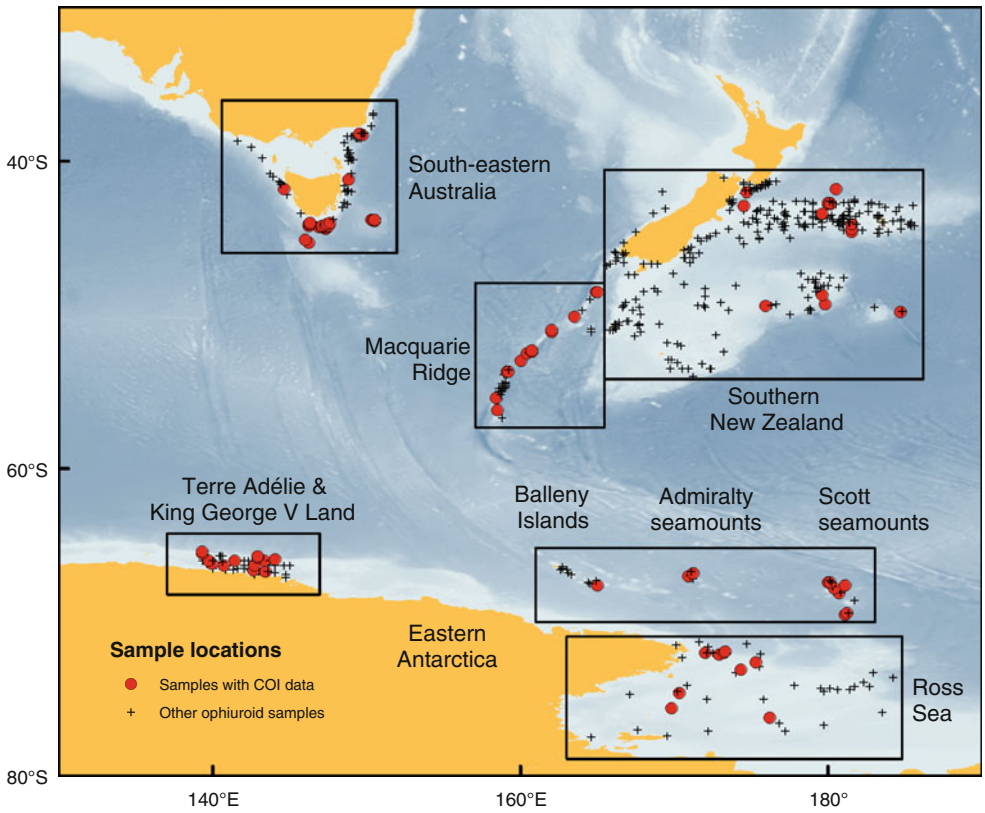

Fig. 1 Map of sample sites and regions used in the analysis

Atmospheric Research Wellington (including samples collected from the voyages TAN0803 to the Macquarie Ridge, TAN0802 to the Ross Sea/Admiralty/Scott seamounts and TAN0604 to the Graveyard seamounts on the Chatham Rise), (b) Museum Victoria (SS01/99 to the Macquarie Ridge, SS02/2007 to seamounts south of Tasmania, TN228 sampling Tasmanian deep-sea coral communities, Icefish 2004 to the Southern Atlantic Ocean and the US-AMLR 2009 voyage to South Georgia), (c) Muséum National d'Histoire Naturelle (CEAMARC to eastern Antarctica) and (d) California Academy of Sciences (Andeep 2002 to the western Southern Ocean).

A small piece of muscle tissue was lysed in 45 µl cetyltrimethylammonium bromide (CTAB) lysis buffer solution (Ivanova et al. 2008) plus 5 µl proteinase K. These samples were incubated at 56 °C for 12–18 h, and DNA was then extracted using the manual protocol of Ivanova et al. (2008) with a 3 µm PALL Acroprep glass fibre plate and re-suspended in 50 µl of ddH_2O. The 650 bp barcode region of COI was amplified under the following thermal conditions: 1 min at 94 °C; 5 cycles of 94 °C for 40 s, 45 °C for 40 s and 72 °C for 1 min, followed by 35 cycles at 94 °C for 40 s, 40 s at 51 °C, and 1 min at 72 °C; and a final step of 72 °C for 1 min. The 12.5 µl PCR mixes included 6.25 µl of 10 % trehalose, 2.00 µl of ultrapure water, 1.25 µl 10X PCR buffer [200 mM Tris–HCl (pH 8.4), 500 mM KCl], 0.625 µl $MgCl_2$ (50 mM), 0.125 µl of each primer [0.01 mM, using LCOech1aF1 (5′-TTTTTTCTACTAAACACAAGGATATTGG-3′, Doug Eernisse unpublished) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′, Folmer et al. 1994)], 0.062 µl of each dNTP (10 mM), 0.060 µl of Platinum® Taq Polymerase (Invitrogen) and 2.0 µl of DNA template. PCR amplicons were visualised on a 1.2 % agarose gel E-Gel® (Invitrogen) and bidirectionally sequenced using the primers listed above and the BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) on an ABI 3730 capillary sequencer following manufacturer's instructions. Bidirectional sequences were assembled and edited using CodonCode Aligner (CodonCode Corporation). Specimen and collection data, sequences, images and trace files are available from the Barcode of Life database, BOLD (Ratnasingham and Hebert 2007) in the public data set "Ophiuroids of the Southern Ocean (OPSOUTH1)" and from GenBank.

We selected the GTR+Γ+I model of molecular evolution for our COI sequences based on AIC tests within the software MrModelTest 2.3 (Nylander 2008). Bayesian phylogenetic analyses were performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) with the selected evolutionary model (nset = 6, rates = invgamma). We ran the MCMC search on two runs of four chains for 1 million generations (sampled every 100) until the final average standard deviation of split frequencies was lower than 0.01.

Twenty-five per cent of the initial trees were discarded as Burn-in. Maximum Likelihood (ML) trees were obtained using the program RAxML 7.0.3 for Windows using the GTR+Γ (GTRGAMMA) model of evolution (Stamatakis 2006) and 1,000 pseudo-replicates to calculate bootstrap support values (Stamatakis et al. 2008).

Haplotype networks, showing regional patterns of mutational steps, were created using the Median-joining algorithm in the software NETWORK 4.5.1.6 (Bandelt et al. 1999). We excluded missing data (otherwise treated as a fifth state) by trimming remaining sequences to 499–633 bp.

We recognised separate ESUs within an MSP (a) if they formed separate regionally discrete reciprocally monophyletic clades of two or more specimens on a COI phylogeny (e.g., Fig. 3d) or (b) if lineages of one or more specimens were separated on a phylogeny by another MSP, that is, the original MSP was para- or polyphyletic (e.g., Fig. 3e) (Fraser and Bernatchez 2001). In addition, we note that within some clades, there was evidence of regional differentiation of sequences on haplotype networks or phylogenies but not enough samples to satisfy our criteria for recognising ESUs (e.g., Figs. 3b, 4c). We labelled these sequences as regional outliers, suggestive of some barrier to gene flow, but requiring more sequences before being confirmed as belonging to distinct ESUs. Biogeographical analyses with and without these outliers were compared.

Annual mean seafloor temperature (°C) and salinity (‰) data were derived from the CARS2009 data set, which contains mean values and seasonal coefficients (annual/semi-annual sine/cosine values) created by averaging/interpolating available oceanographic cast data for 79 depth layers at a resolution of 0.5° latitude/longitude (Dunn and Ridgway 2002) (http://www.marine.csiro.au/~dunn/cars2009/). Particulate organic carbon (POC) was derived from a global NPZD (nutrient–phytoplankton–zooplankton–detritus) model at 1.0° resolution and 66 depth strata (Yool et al. 2007; Álvarez et al. 2009). All data sets were then averaged over the longitudinal span of the study area (140°E–170°W) to form two-dimensional latitude/depth matrices and graphed using the R v2.14.0 function filled.contour() (R Development Core Team 2011).

Results

Distribution of morpho-species

Forty morpho-species of ophiuroid from 8 families and 23 genera are recorded from the Macquarie Ridge at depths between 200 and 2,500 m (Table 1), including 29 described species and 11 well-characterised species awaiting description (O'Hara unpublished data). None of these species are known to be endemic to the Ridge. The total of forty is an underestimate as there are juvenile or damaged specimens of seven genera (*Amphiura*, *Ophiacantha*, *Ophiopristis*, *Ophioparva*, *Ophioscolex*, *Ophiozonella* and *Ophioplinthus*) that cannot be currently assigned with confidence to a known species either using morphology or COI barcode, and so are not listed in Table 1. The list also excludes the Antarctic species (a) *Amphiura angularis*, as the specimens recorded from Macquarie Island (McKnight 1984) and the Chatham Rise (Fell 1960) are juveniles and not reliably identifiable to species, and (b) *Amphiura joubini*, as specimens recorded from New Zealand by Fell (1958), McKnight (1967) are mis-identified, being *A. correcta* and *Amphioplus ctenacantha*, respectively (Mills and O'Hara, in review).

Of the 40 Macquarie species, 35 (88 %) have also been recorded at similar depths from continental slopes and seamounts around south-eastern Australia and/or New Zealand, while only 14 (35 %) have been recorded from eastern Antarctica, the Ross Sea, Balleny Islands and/or the Admiralty and Scott seamounts. Consequently, the community composition is more similar to Australia/New Zealand than Antarctica (Fig. 2). However, the number of shared species reflects the biodiversity known from these surrounding regions, with far more species being found off Australia ($n = 129$) and New Zealand (116) than the Antarctic regions (31–47). The percentage of species contributed by surrounding regions to the Macquarie ophiuroid fauna was more variable from Antarctic regions (Terre Adelie/King George V—25 %, Ross Sea—19 %, Balleny/Admiralty/Scott—35 %, mean 27 %) than from temperate regions (south-eastern Australia—24 %, southern New Zealand—28 %, mean 26 %). Seven species were recorded as occurring across the study area.

The MSPs recorded around the Balleny Islands and on the Admiralty and Scott seamounts ($n = 31$) are a subset of the fauna known from eastern Antarctica and the Ross Sea (56 in total) and lack several well-known Antarctic genera such as *Ophiosteira* and *Euvondrea*. Only one species, the undescribed *Ophiura* sp. MoV 4526, was recorded from these islands/seamounts but not from the Antarctic continental margin.

Molecular and morphological congruence

COI sequences ($n = 316$) were obtained for 36 of these morpho-species, although not consistently across all regions (Table 1 and Fig S1). Phylogenetic analyses of these sequences, in combination with others from western Antarctica (ANDEEP, US-AMLR 2009) and the southern Atlantic (ICEFISH), showed congruency with some existing species boundaries (e.g., *Ophiocamax* spp.) but in others, it became evident that existing taxonomy was

Table 1 Species found on the Macquarie Ridge that also occur around Antarctica (including offshore islands and seamounts), off south-eastern Australia or New Zealand

Family	Species name	No. of COI sequences	Antarctic			Sub-antarctic	Temperate	
			Terre Adélie/King George V	Ross Sea	Balleny/ Admiralty Scott	Macquarie Ridge	SE Australia	New Zealand
Amphiuridae	*Amphioplus* sp. MoV 2722	5				*	A	*
	Amphipholis squamata	–				*	*	*
	Amphiura magellanica	10				A	B	*
Gorgonocephalidae	*Gorgonocephalus chilensis*	7			A	B		B+
Ophiacanthidae	*Ophiacantha otagoensis*	5				A		*
	Ophiacantha rosea	14				A+	A, B, C, D	A
	Ophiacantha sp. MoV 4532	5				A	B	*
	Ophiacantha sp. MoV 5488	3				*	A	*
	Ophiacantha spectabilis	6				A	A	*
	Ophiacantha vilis	–				*		*
	Ophiacantha vivipara/pentactis	40	A	A	A	A, B, C	C	C
	Ophiacantha yaldwyni	6				A	A	*
	Ophiocamax gigas/applicatus	17			A	B	*	B
	Ophiolebes sp. MoV 3581	1				A	*	*
	Ophiolimna antarctica	7	A	*	*	A, B	A	*
	Ophiomitrella conferta	13	A	*		A+	B	*
	Ophiomitrella sp. MoV 2732	3				*	A	*
	Ophioplinthaca plicata	3				*	A	*
	Ophiurothamnus clausa	5				A	B	*
Ophiactidae	*Ophiactis abyssicola*	11				A	A	A
	Ophiactis hirta	2				*	A	*
Ophiolepididae	*Ophioplocus incipiens*	9	B	A		*		
	Ophiozonella stellata	1				A		*
Ophioleucidae	*Ophioleuce regulare*	4	*	*	*	*	A	*
Ophiomyxidae	*Ophiologimus prolifer*	3				A	A, B	*
	Ophiomyxa sp. MoV 5486	14				A	A	A
	Ophioscolex sp. MoV 2721	4				A	A	*
Ophiuridae	*Ophiocten* sp. MoV 2733	4				A	A	
	Ophiocten cryptum	1				*	A	*
	Ophiocten sp. MoV 5674	7				A	A	
	Ophiopleura inermis	13	A		A	B	B	*
	Ophioplinthus gelida/brevirima	42	A	A	B	B		
	Ophioplinthus accomodata	2				A	*	*
	Ophiura carinifera/lymani	28	A, C	C	C, D	D+		
	Ophiura irrorata	8		A		B+	B	B, C
	Ophiura meridionalis	7	A	A	A	A+		
	Ophiura rugosa	–				*	*	*
	Ophiura sp. MoV 2728	14				A	A	*
	Ophiura sp. MoV 4526	5			A	A+		
	Stegophiura elevata/singletoni	4	A		A	*	A+	*
Number of species			10	9	11	40	31	33
	Total across latitudinal groups		14			40	35	

Species with a MoV number are undescribed. Letters (A, B, C, etc.) designate ESUs (see Figs. 3, 4 and S1)

n number of sequences, + regional outliers, * indicates that the species has been recorded from that region but no molecular sequences obtained

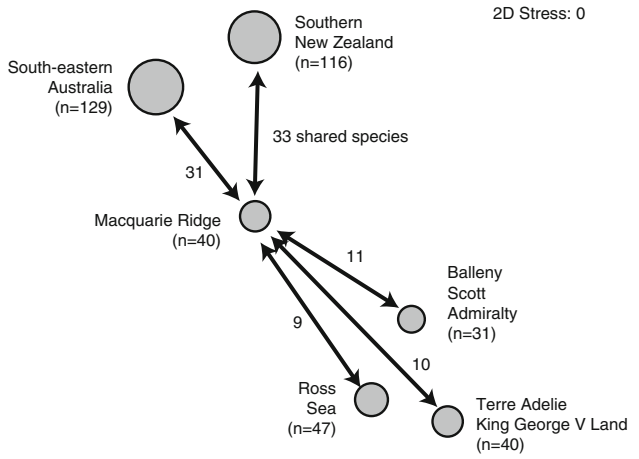

Fig. 2 MDS ordination of the presence/absence of morpho-species (MSPs) across the study regions using the Bray-Curtis dissimilarity coefficient. The size of the ordination symbol is scaled to the number of MSPs in each region. The numbers next to the *arrows* refer to the number of shared MSPs between regions

inadequate, either because these 'species' contained deep-lineages suggestive of cryptic speciation (e.g., *Ophiacantha vivipara* complex, *Ophiura carinifera/lymani*, *O. irrorata*) or because specimens were frequently misidentified (e.g., *Ophioplinthus gelida/brevirima* complex).

Some examples comparing existing taxonomy and the new sequence data are illustrative. *Ophiocamax* species are morphologically very polymorphic and difficult to separate (O'Hara and Stöhr 2006). However, our molecular sequences were congruent with the nominal species *Ophiocamax applicatus* (Australia, New Zealand and Macquarie Island), *O. gigas* (Antarctic, above 2,000 m) and *O. drygalskii* (Antarctic, below 2,000 m) (Fig. 3a). In contrast, the *Ophiacantha vivipara* complex formed numerous clades (Fig. 3b) that do not reflect existing taxonomy. Six-armed specimens (*O.* 'vivipara') were present in three clades, including one from Australia/New Zealand and the Macquarie Ridge, and two other clades mainly from Antarctica. One of the latter clades (A) also appeared to include five-armed specimens (*O.* 'pentactis'). A haplotype network of this clade (Fig. 4) showed that six-armed specimens formed multiple distinct groups around a diverse five-armed network, suggesting that 6-armed lineages have repeatedly evolved from 5-armed ones. No haplotypes were shared between five- and six-armed forms. Specimens of *Ophiura irrorata* also formed numerous deep-lineages (Fig S1), suggesting that the many synonyms currently included in this complex should be re-evaluated. Our taxonomic understanding of the *Ophioplinthus gelida/brevirima/martensi* complex appeared inadequate (Fig S1). The molecular data indicated that there were at least 12 clades (not shown, as most were from other parts of Antarctica), many of which contained specimens identified as two or more species.

Spatial distribution of molecular ESUs

Fewer ESUs than morpho-species were shared between regions (Table 1). ESUs tended to be allopatric rather than sympatric. However, this affected the regional relationships asymmetrically (Table 2), with relatively more shared morpho-species definitely or possibly forming distinct clades between Antarctica and the Macquarie Ridge (57 %) compared to those shared between the Macquarie Ridge and Australia/New Zealand (20 %). Only three morpho-species contained clades shared between Antarctica and the Macquarie Ridge, compared to 13 between the Macquarie Ridge and Australia/New Zealand. Only two MSPs contained more than one clade from the Macquarie Ridge (Table 1) but no MSP shared more than one clade with an adjacent region (although *Ophiacantha vivipara* shared a second clade B with the Burwood Bank off western Antarctica, see Fig. 3e).

Environmental signature of ESUs

ESUs located on the continental slope of Antarctica and the Macquarie Ridge inhabit different water masses: Antarctic Bottom Water around the continent (excluding the summits of the shallowest seamounts) and Antarctic Intermediate Water arising from the subantarctic convergence, respectively (Fig. 5a). While salinity levels tend to be similar (Fig. 5a), temperature profiles are quite distinct, for example the temperature range for the Antarctic *Ophiocamax gigas* is 0.5–1 °C, while it is 2.5–6.1 °C for *O. applicatus* (Fig. 5b). Annual carbon flux shallows with latitude south of 40°S, so ESUs inhabiting similar depth profiles experience less carbon flux around Antarctica, a situation accentuated for ESUs inhabiting deeper water around Antarctica (Fig. 5c). Nevertheless, some ESUs, including *Ophiacantha vivipara* A (Fig. 4a) and *Ophioplinthus gelida* B (Fig. 4b) occur both around Antarctica and the Macquarie Ridge.

Sympatric ESUs within a morpho-species sometimes inhabited different bathymetric ranges. For example, clade A of *Ophiolimna antarctica* was recorded from 1,318 to 2,945 m compared with 501–998 m for clade B (Fig S1). Some of the *Ophiacantha rosea* ESUs also had different depth profiles (Fig. 3e).

Discussion

The presence of so many ESUs within recognised ophiuroid MSPs suggests that our current taxonomic understanding of

some species complexes needs to be reviewed. In particular, the *Ophioplinthus gelida/brevirima/martensi*, *Ophiura carinifera/lymani*, *Ophiura irrorata* and *Ophiacantha vivipara/pentactis/rosea/densispina* and *Ophiolimna antarctica* complexes require re-examination. This is similar to the situation in better studied shallow-water species such as

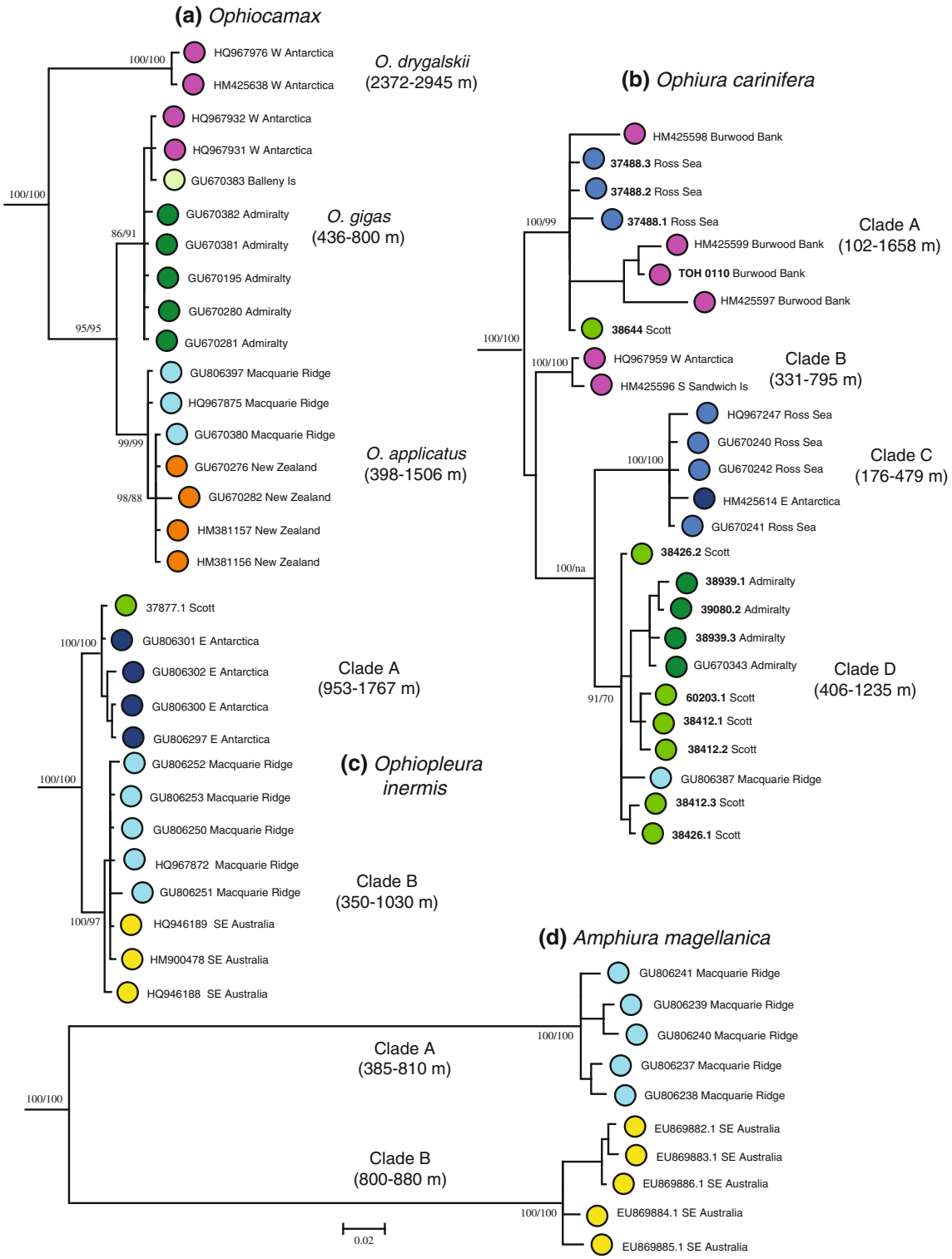

Fig. 3 Bayesian consensus haplotype trees of selected species complexes produced using the GTR+Γ+I model in MrBayes v3.1.2. Trees include comparative specimens from western Antarctic regions. Values associated with selected nodes are Bayesian posterior probabilities followed by bootstrap support (10,000 replicates) from a Maximum Likelihood tree constructed using the GTR+Γ model in RAxML v7.0.4

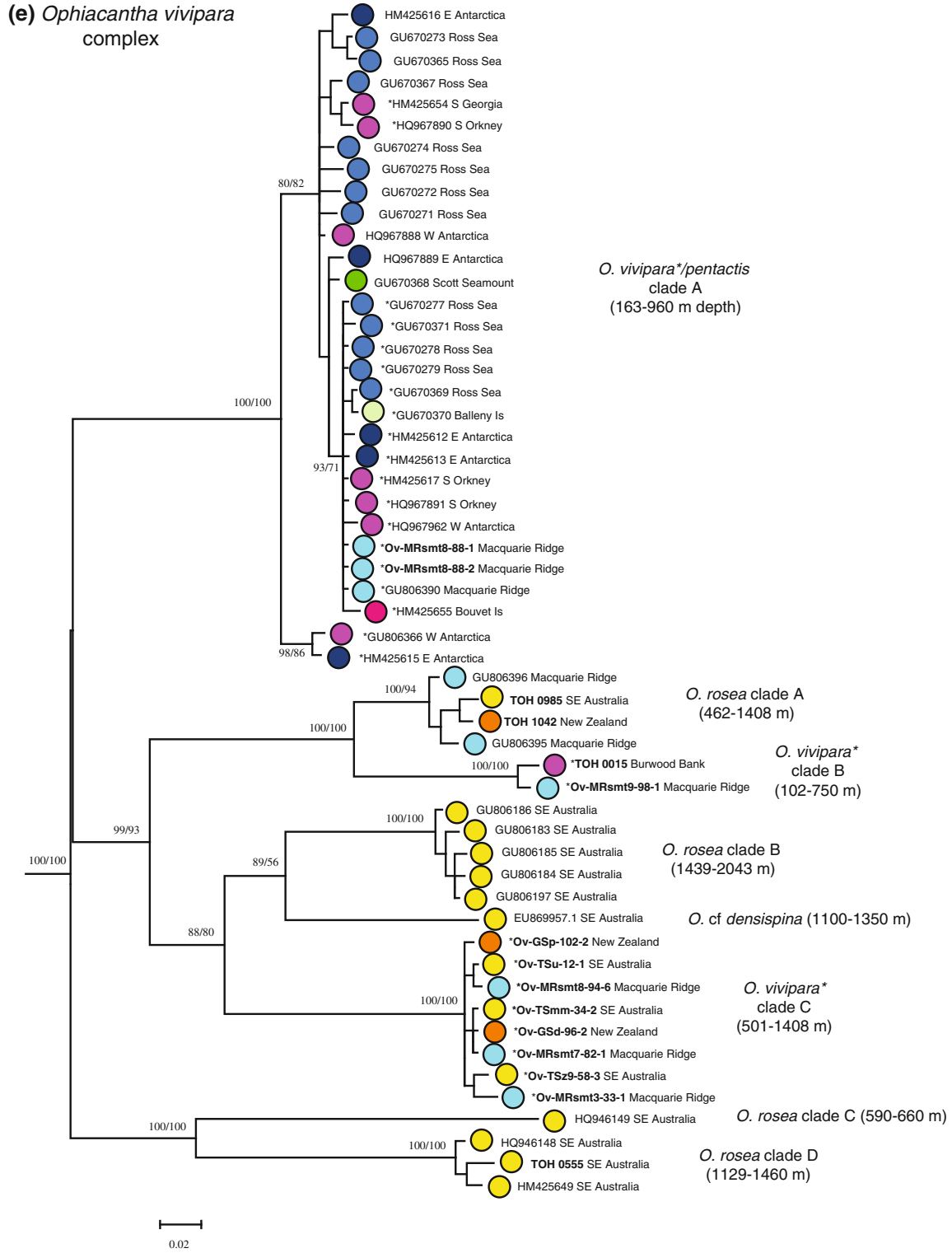

Fig. 3 continued

Amphipholis squamata (Boissin et al. 2008) and *Acrocnida brachiata* (Muths et al. 2006). While some of this genetic differentiation may be driven by oceanographic barriers to gene flow, some echinoderms have been found to have rapidly evolving reproductive systems. Sister species, distinguishable by brooding/non-brooding or fissiparous/non-fissiparous reproduction, can be difficult (Hart et al. 2003) or impossible (Williams 2000) to separate using COI

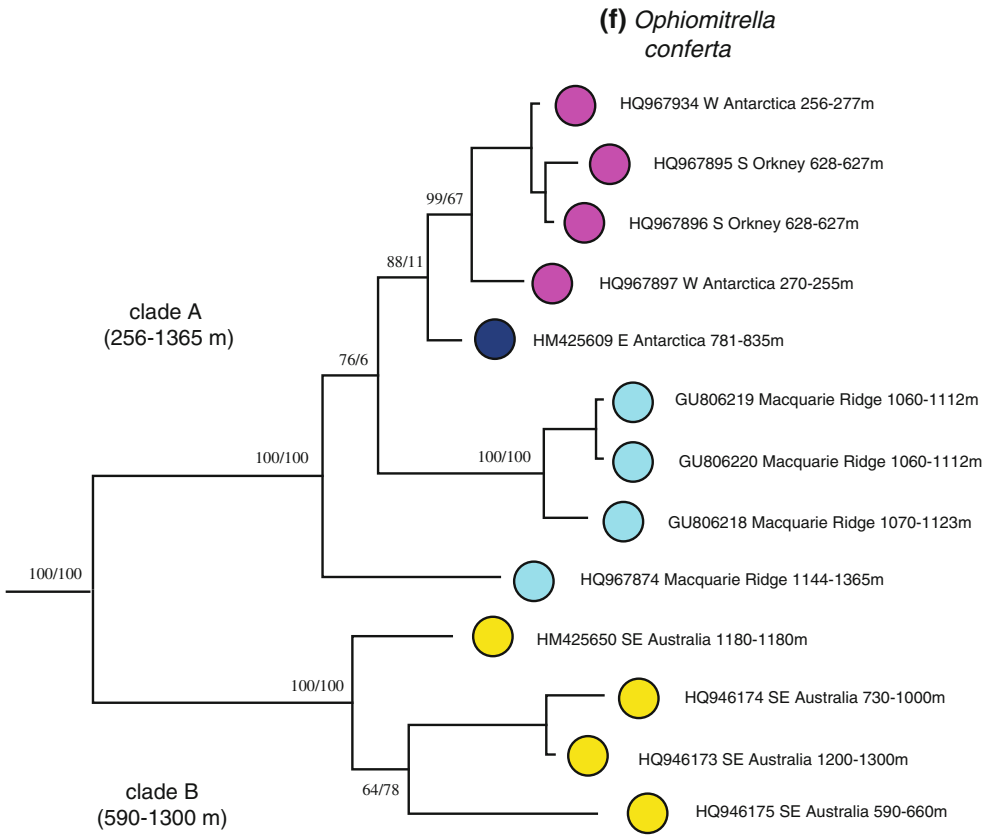

Fig. 3 continued

mtDNA. For our study region and taxa, previously, research has suggested that *Ophiacantha vilis* specimens from New Zealand are probably a separate species to those at Macquarie Island based on observed reproductive characters (O'Hara 1998b). Martín-Ledo et al. (2012) have recently described another brooding species in the *Ophiacantha vivipara* complex from South Georgia. Conversely, identification is complicated within the *Ophioplinthus* complex by the presence of the parasitic sponge *Iophon* which can change the shape of the epidermal plates, a crucial suite of characters in ophiuroid taxonomy (Smirnov unpublished data).

One of the purposes of our study was to test whether biogeographical patterns differed between MSPs and ESUs defined from mtDNA. For MSPs, the Macquarie Ridge ophiuroid fauna is closely aligned to Australia and New Zealand (35 shared species) with less than half that of number species (14) shared with Antarctica. This includes those recorded from Antarctic seamounts or islands (Balleny, Admiralty and Scott) at similar depths to samples collected off the Macquarie Ridge, which leads us to reject the hypothesis that differences in community composition was due to sampling artefacts alone. There are no known endemic ophiuroid MSPs recorded from the ridge, although some juvenile or damaged specimens cannot be currently assigned to known species and may be endemic. Of the 36 ESUs for which we have some genetic data, 13 are spread between the Macquarie Ridge and Australia and/or New Zealand, but only 3 are definitely shared between Antarctica and the Macquarie Ridge. Of the 14 MSPs believed to have been shared between Antarctica and the Macquarie Ridge, five were found to consist of allopatric ESUs, three others show some sequence divergence between regions, and for three, we have no information. Three of the MSPs shared between Macquarie and Australia/New Zealand resolved into distinct monophyletic ESUs, and all (*Amphiura magellanica*, *Ophiomitrella conferta*, *Ophiacantha* sp. MoV 4532) are brooders (Mortensen 1936; O'Hara unpublished data), presumably with limited dispersal capability.

Thus, the bathyal ophiuroid fauna on the Macquarie Ridge appears to have a predominantly temperate origin; effectively, it is a subset of the diverse faunas around south-eastern Australia (129 species) and southern New Zealand (116 species). This result is not biased by the proximity of New Zealand and the northern Macquarie Ridge (sharing 33 MSPs); only slightly fewer species are shared between south-eastern Australia and the Macquarie Ridge (31 MSPs), separated by 1,500 km of abyssal plain. The Antarctic Intermediate Water Mass extends across all these

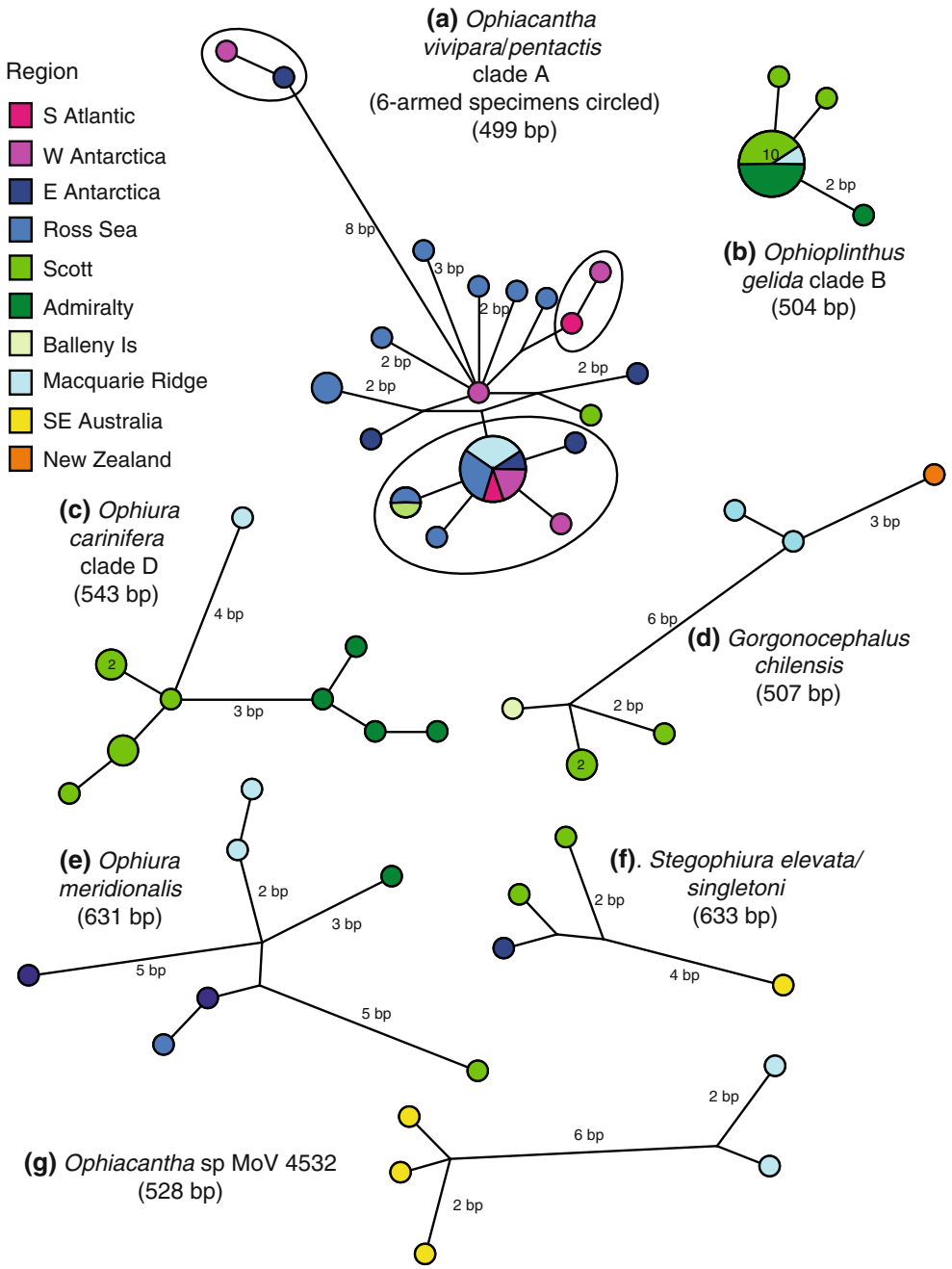

Fig. 4 Median-joining haplotype networks of mtDNA COI sequences for selected species. The number of specimens is superimposed onto some of the more abundant haplotypes. Branch lengths are to scale. Networks include comparative specimens from western Antarctic regions

regions at upper bathyal depths, and they share broadly similar environmental regimes. However, if the shared species are considered as a proportion of the surrounding regional faunas, individual Antarctic and temperate regions have contributed similar percentages of species to the Macquarie fauna (mean of 27 and 26 %, respectively). The Balleny/Admiralty/Scott seamounts and islands contributed the highest proportion of their species (35 %).

The genetic analyses suggest a recent biogeographical barrier between eastern Antarctica and the Macquarie Ridge. Although 14 MSPs were shared between these regions, more than half of these had ESU distributions indicative of a barrier to dispersal. With a few exceptions, gene flow appears to have been historical rather than contemporary. The expanded ice-sheet and position of the polar front at the height of Pleistocene glaciations (Gersonde et al. 2005) is

Table 2 Breakdown of phylogenetic relationships between populations of morpho-species sequenced from Macquarie Ridge, compared to Antarctica and Australia/New Zealand

Relation between regional populations within morpho-species	Macquarie Ridge and			
	Antarctica/ Balleny/ Scott/ Admiralty	%	Australia/ New Zealand	%
Reciprocally monophyletic ESUs	5	36	3	9
Regional outliers (possibly separate ESUs)	3	21	4	11
Shared ESUs	3	21	13	37
Insufficient information	3	21	15	43
Total	14	100	35	100

likely to have pushed biotas northwards (Fraser et al. 2009). Thus, some Antarctic species may have become established on the Macquarie Ridge and then persisted and adapted to warmer conditions during interglacial periods. Conversely, southern populations following the retreating ice-sheets may become progressively isolated from northern populations. Whether this isolation has arisen from sheer distance between suitable habitat (O'Hara 1998a), from oceanographic conditions (e.g., the strong west to east currents or presence of the polar front) or from diverging environmental conditions (especially temperature) cannot be determined from our data. Our uncertainty is compounded by a sampling gap between the Admiralty/Scott seamounts and the Macquarie Ridge. More sampling on the isolated shallow features on the Pacific–Antarctic Ridge in this area (Fig. 1) would

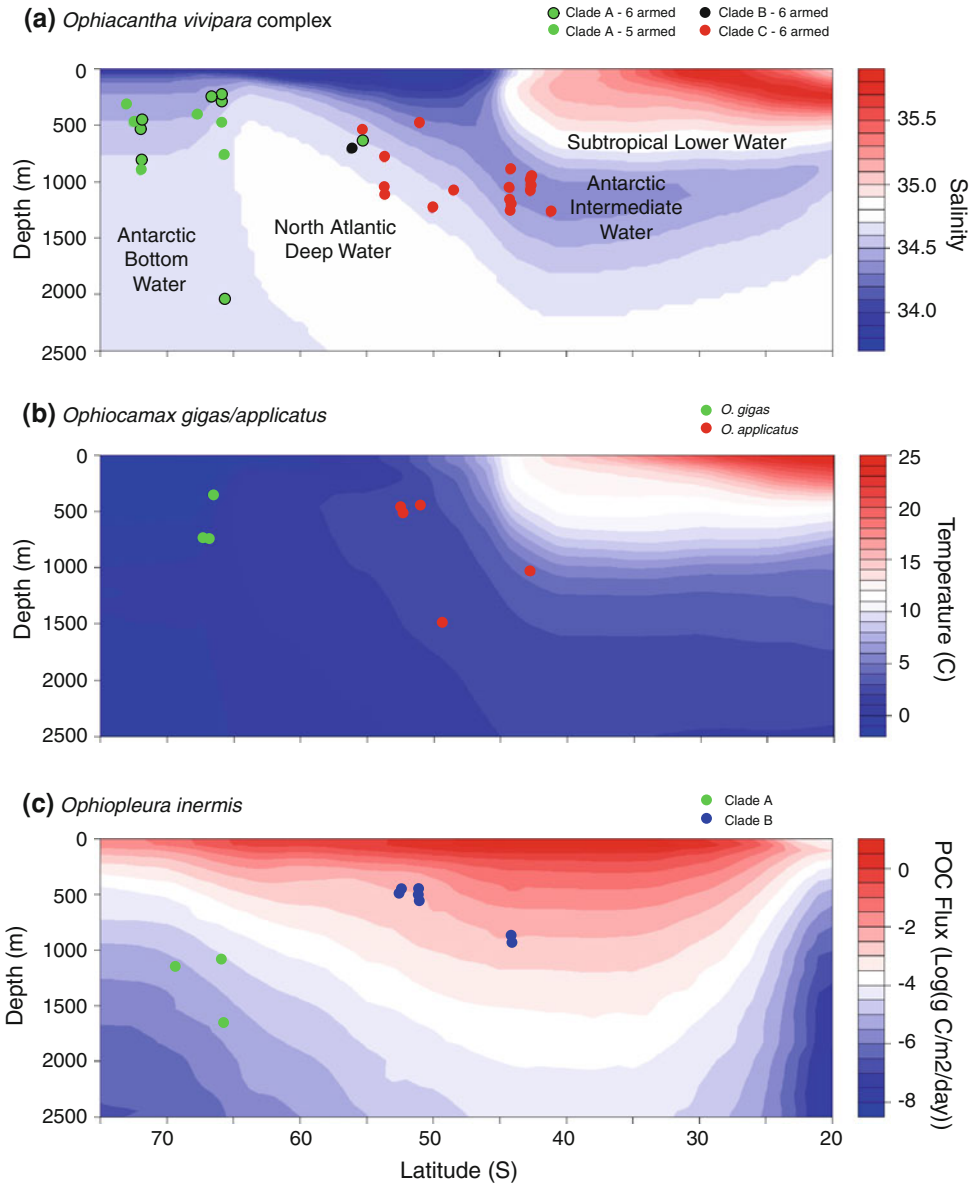

Fig. 5 Latitudinal and bathymetric location of selected ESU specimens superimposed onto environmental contours: **a** salinity, as an indicator of water mass, **b** seafloor temperature, and **c** particulate organic carbon flux

improve our understanding of the position and magnitude of this biogeographical transition zone.

The Antarctic/subantarctic transition differs between regions. Many Antarctic species extend into southern South America below 40°S (Mortensen 1936; Smirnov and Ahearn 2009), although multivariate community analyses generally differentiate the South American and Antarctic faunas (Griffiths et al. 2009; Sands et al. 2012). At the molecular level, a phylogeographical study of a widespread ophiuroid, *Astrotoma agassizii*, found that two South American lineages clustered separately from an Antarctic one (Hunter and Halanych 2008) indicating that, at least for this species, a geographical gap of 500 km is sufficient to be a barrier to dispersal. On the other hand, Kerguelen and Heard islands (approx 70°E) have a bathyal ophiuroid fauna composed of Antarctic and endemic species, lacking a temperate component. Only one species is shared with the South African margin (*Astrophiura permira*) and one with the temperate St Paul/Amsterdam Islands in the central Indian Ocean (*Ophiolimna antarctica*) (O'Hara unpublished data). Both Kerguelen and Heard islands sit on a fragment of continental crust that has been isolated from South Africa for over 120 million years (Gaina et al. 2007).

These ophiuroid patterns are similar to those found in other echinoderm classes. O'Loughlin et al. (2011) found that the ranges of at least seven holothuroidea morphospecies (among 39 with genetic data) crossed the Antarctic Convergence. Five of these have distinct ESUs either side of the convergence indicating past dispersal followed by differentiation, while the other two showed little genetic structure indicating more recent gene flow. The genetic affinities of the Kerguelen population of the crinoid *Promachocrinus kerguelenesis* are more similar to those on Bouvet, South Sandwich and South Shetland Islands than populations on the adjacent Antarctic continent (Hemery et al. 2012). Moreover, ecological niche modelling has shown that some areas north of the Convergence are suitable habitat for some Antarctic echinoid species (Pierrat et al. 2012).

In conclusion, the bathyal ophiuroid fauna of the Macquarie Ridge has affinities with both neighbouring temperate and Antarctic regions. It shares a higher number of MSPs with the nearby temperate Australia and New Zealand ($n = 35$) than Antarctica (14), but a broadly similar percentage of the fauna (26–27 %) when the latitudinal decline in species richness across the study area is taken into consideration. Differentiated ESUs are more likely to occur between Antarctica and the Macquarie Ridge than between the temperate regions and the Macquarie Ridge, indicating that dispersal is more intermittent across the Antarctic–Macquarie oceanic barrier than along the northern Macquarie Ridge.

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