Deep-sea Research Part I-oceanographic Research Papers
February 2014, Volume 84 Pages 18-28
https://doi.org/10.1016/j.dsr.2013.09.009
https://archimer.ifremer.fr/doc/00760/87200/

Limited phylogeographic structure for five bathyal ophiuroids at continental scales

O'Hara Timothy D. [1, *], England Phillip R. [2], Gunasekera Rasanthi M. [2], Naughton Kate M. [1]

[1] Museum Victoria, Melbourne, Vic 3001, Australia.
[2] CSIRO Wealth Oceans Flagship, Hobart, Tas 7001, Australia.

* Corresponding author : Timothy D. O'Hara, email address : tohara@museum.vic.gov.au

Abstract :

There have been comparatively few large-scale studies on spatial genetic structure of bathyal seafloor fauna, despite the importance of these data to the successful management of the world's oceans. We use a comparative analysis of mitochondrial DNA from five bathyal (200-3500 m) species of brittle-stars (Ophiuroidea) to assess phylogeographic structure along an extensive (8000 km) longitudinal gradient at temperate latitudes (28-56 degrees S) from south-west Australia (113 degrees E) to seamounts east of New Zealand (175 degrees W). We found no evidence of a genetic discontinuity between Australia and New Zealand, either across the temperate Tasman Sea or across the Southern Ocean between the South Tasman Rise and the Macquarie Ridge. However, there were latitudinal phylogeographical breaks between tropical, temperate and polar regions; longitudinal breaks across the eastern Indian Ocean; and a bathymetric break at approximately 1700 m. Although there was limited regional structure in the frequency of haplotype distributions within the major clades, and no clade appeared to be strictly panmictic, the regional structure in general was not concordant with a simple isolation-by-distance model. Demographic structure varied with three clades having a simplified haplotype network, low effective population sizes and no evidence of significant population expansion, and two clades having a high diversity of haplotypes, relatively high effective population sizes and signs of recent population expansion. These results are discussed with respect to putative dispersal strategies. We hypothesise that the 'brooding' species produce both brooded young and pelagic larvae, allowing for both the maintenance of local populations and long-distance dispersal.

Highlights

► Limited longitudinal phylogeographic structure within five bathyal ophiuroids inhabiting southern Australia and New Zealand. ► Phylogeographic breaks recorded across the eastern Indian Ocean, between tropical, temperate and polar regions, and bathymetrically at 1700 m. ► Brooding species appear to be able to disperse to remote habitat fragments suggesting that they produce at least some pelagic larvae.

Keywords : Continental slope, Seamounts, Australia, New Zealand, Mitochondrial DNA, Planktotrophy, Lecithotrophy, Viviparity

1. Introduction

Our knowledge of deep-sea macro-ecological patterns and processes is deficient. Investigating large-scale patterns of biodiversity remains a logistical challenge. Data are difficult and expensive to collect and many studies are limited in temporal and spatial scope. Nevertheless, an understanding of the spatial extent of interconnected populations is fundamental to our successful management of the world's oceans, including the mitigation of the effects of fisheries and mining, and the design of successful marine protected areas systems (UNESCO, 2009).

Assessing the spatial extent of deep-sea benthic organisms has been problematic. Although many morphologically similar forms appear to have widespread distributions, cryptic species are common (e.g., Jörger *et al*. 2012), and recent ecological research has shown that deep-sea habitats can be patchily distributed at various spatial scales (McClain and Hardy, 2010). There have been relatively few large-scale phylogeographical studies in the deep sea due to the degraded state of specimen DNA in historical collections, the high cost of collecting new material from remote locations and the difficulty of working across a seascape fragmented into separate national jurisdictions. Many of the studies that have been performed are from specialised chemosynthetic habitats on mid-ocean ridges (e.g., Plouviez *et al.*, 2009) or from seamounts (e.g., Cho and Shank, 2010; Miller *et al.*, 2010) and may not be representative of the deep sea in general.

The South-western Pacific has emerged as a key region for bathyal (200-3500 m) macro-ecological research because of recent concurrent programs of marine biodiversity discovery around Australia, New Zealand, New Caledonia and surrounding seafloor (O'Hara *et al.*, 2011). Large collections of

marine invertebrates are available from continental slope and seamount habitats. Biogeographic analyses of marine species (defined by morphology) across this region show differing patterns for continental shelf (0-200 m) compared to continental slope (200-3500 m) biomes. Southern Australia and New Zealand shallow-water marine assemblages form distinct subregions, including south-western and south-eastern Australia and northern and southern New Zealand (Spalding *et al.*, 2007). However, analyses of bathyal assemblages at most distinguish south-western Australia (Schnabel *et al.*, 2011) or combine the entire region in an extensive latitudinal band bordered by a tropical region to the north and polar region to the south (O'Hara *et al.*, 2011). With limited exceptions (Miller *et al.*, 2010), these extensive bathyal ranges have not been tested with genetic data. It is unclear whether bathyal animals (particularly brooding megafauna) can disperse across the wide abyssal plain of the Tasman Basin between southern Australia and New Zealand.

Among benthic invertebrates, brittle-stars (Ophiuroidea) have become useful model organisms for phylogeographic and biogeographic studies. They are abundant and frequently occur across a range of habitats, from intertidal to hadal depths, from equatorial to polar regions, and from rocky to muddy substrata (Cho and Shank, 2010; O'Hara *et al.*, 2011; O'Hara *et al.*, 2013). Moreover, ophiuroids display a variety of dispersal strategies that affect population connectivity, including planktotrophic (feeding) larvae, lecithotrophic (non-feeding) larvae, viviparity (brooding) and asexual fissiparous reproduction (Byrne and Selvakumaraswamy, 2002).

In this study we use a comparative analysis of mitochondrial DNA from five bathyal (200-3500 m) species of ophiuroids to assess phylogeographic structure along an extensive (8,000 km) longitudinal gradient at temperate latitudes (28-56°S) from south-west Australia (113°E) to seamounts east of New Zealand (175°W). We compare the phylogenetic relationships of these populations to those in other regions. Specifically, we examine whether the phylogeographic patterns are 1) indicative of longitudinal dispersal across the region via continuous oceanic currents or 2) whether there is evidence of one or more long-term barriers to gene flow. In particular, we investigate the potential dispersal barrier for bathyal species across the abyssal plain (>3500 m deep) between southern Australia and New Zealand/Macquarie Ridge. We interpret these results in the context of the species' variable dispersal strategies and differences in latitudinal and bathymetric range.

2. Materials and methods

2.1. Tissue sampling

We obtained specimens from numerous deep-sea expeditions (1999-2012) that have been deposited in museums in Australia and New Zealand (Table S1).We selected five ophiuroid species for molecular analysis on the basis of their widespread occurrence in Indo-Pacific temperate bathyal waters and diversity of habitat requirements or life history strategies. Because ophiuroid morpho-species have been shown to frequently contain cryptic species (Boissin *et al.* 2011; O'Hara *et al*. 2013; Hoareau *et al.* 2013), we investigated species boundaries by including some similar specimens from other regions as putative outgroups in the phylogenetic analyses. Comparative material was sourced from the ICEFISH 2004 (South Atlantic); Poseidon 252, 292, Vital 2002, and Meteor 61/1 (North Atlantic); Akademik Ioffe 29 (South Atlantic Ridge); and James Cook 66 (SW Indian Ocean Ridge) expeditions.

Ophiomyxa vivipara is a dioecious brooding species that has been reported from the Magellanic region of South America, off the island of Tristan da Cunha in the South Atlantic and South Africa (as the subspecies *capensis*) in depths of 75-400 m (Mortensen, 1936). We have found similar specimens on seamounts and continental slopes (300-1100 m) of southern Australia, New Zealand and the Macquarie Ridge (Fig. 1) (O'Hara *et al.*, 2008 as Ophiomyxa sp MoV 5486). A similar species, *O. serpentaria*, which has large yolky eggs but is apparently non-brooding (Mortensen, 1933), has been described from the North Atlantic Ocean. *Ophiacantha vivipara* is a circum-polar Antarctic species (0-1100 m) that has also been recorded from seamounts off Tasmania, the Graveyard complex on the Chatham Rise, and the Macquarie Ridge (400-1700 m) (O'Hara *et al.*, 2008). It is inferred to be a proterogynic hermaphrodite that broods its young in the bursae (Mortensen, 1936).

Ophiactis abyssicola is known from the North and South Atlantic, including around South Africa in depths of 125-4700 m (Paterson, 1985). It is variable morphologically and currently considered to include several junior synonyms described throughout its extensive range (Paterson, 1985). In the SW Pacific several other similar species or subspecies have been recorded including *Ophiactis abyssicola cuspidata* from the Kermadec Ridge, New Zealand and off Tasmania (800-1772 m), and *Ophiactis amator* from 2340 m off eastern Tasmania (Rowe and Gates, 1995). These species live in cryptic habitats, such as gastropod shells or cavities in corals and sponges, and filter-feed by extending mucus-covered arms into the water column (Pearson and Gage, 1984). *Ophiothrix aristulata* is known from southern Africa to the western Pacific on the upper continental slope (O'Hara, 1998a). It is shallower than the other species across our study area (50-650 m) and does not

occur south of 45°S. It is usually associated with sponges and can occur in great abundance. Finally, *Ophiura ooplax* is known from soft sediment habitats on the outer continental shelf and upper slope (100-1100 m) of Japan, Philippines, Southern Australia and New Zealand. A morphologically similar species, *O. trimeni*, has been described from South Africa. Reproduction within these three species complexes has not been previously investigated.

2.2. Examination of gonads

We used a light dissecting microscope to examine gonads from numerous specimens (at least 20 from each species) from the most numerous clade of all five target species, collected at different times of the year (see Table S1), to gain an approximate estimate of maximum egg-size. These resulting measurements are likely to be slight underestimates of actual egg sizes due to shrinkage associated with ethanol preserved tissue.

2.3. Sequencing

The mitochondrial COI gene is considered an effective 'barcoding' gene for echinoderm species diagnosis (Ward *et al.*, 2008) and has been used to demonstrate phylogeographic structure for many ophiuroids (e.g., Cho and Shank, 2010; Hunter and Halanych, 2008; Stöhr *et al.*, 2009). We selected the 'barcode' region of COI in order to include additional ingroup and outgroup sequences generated from several International Barcode of Life (iBOL) projects (O'Hara *et al.*, 2013; Ward *et al.*, 2008). We extracted DNA from arm tissue of ethanol-preserved whole animals. Genomic DNA extractions were performed using Qiagen DNeasy Blood & Tissue DNA extraction kit (QIAGEN Inc) according to the supplier's instructions but with an extended period of lysis (overnight at 56°C) to ensure complete digestion of tissues.

We designed a new set of degenerate primers corresponding to the universal primer region (Folmer *et al.*, 1994) and alignment of available in-house and GenBank ophiuroid sequences. The new degenerate primers are: Oph-CO1-F 5' TTTCAACTAATCAYAAGGAYATWGG 3' and Oph-CO1-R 5' CTTCAGGRTGWCCRAARAAYCA 3'. This primer pair successfully amplified the COI loci of four of the selected ophiuroid species and outgroups. We performed PCR amplifications using AmpliTaq Gold 360 Master Mix following supplier's instructions (Applied Biosystems, USA). All the amplifications were done in 25 µl volumes containing 12.5 µl of 2X AmpliTaq Gold 360 Master Mix, 0.4 µM of each primer, 0.5 µl of 360 GC Enhancer or 2.5 µl of 10X BSA and 1 µl DNA (around 10ng). Thermal cycling comprised 10 mins at 95°C, followed by 40 cycles of 95°C for 30 secs, 45-55°C annealing for 60 secs, and 72°C extension for 60 secs and then a final 7 mins extension at 72°C. PCR products were purified

using the QIAquick PCR purification kit (Qiagen Inc) and sent to the Australian Genomic Research Facility (AGRF) at Brisbane for cycle sequencing using forward (Oph-COI-F) and reverse (Oph-COI-R) primers separately. Electropherograms were checked, edited and nucleotides aligned using DNASTAR Lasergene8 software package for each individual. We did not find any sequence differences between pair (forward & reverse) reads of any individuals. Subsequently the verified sequences were aligned using Crustal-W software (Thompson *et al.*, 1994) and checked by eye. These five species consistently yielded a 658bp fragment of the COI gene.

The last species (*Ophiura ooplax*) was amplified using the consensus clamp primers of Hoareau & Boissin (2010) and, in the case of degraded DNA, novel internal primers designed specifically for this species. These internal primers allowed individuals with degraded DNA to be amplified in up to four overlapping fragments (primers available on request from the authors) resulting in a 580 bp fragment of COI.

2.4. Molecular analyses

Because of the ubiquitous occurrence of cryptic species in the marine environment (Knowlton, 1993) we have adopted a stepwise approach to the de-novo analysis of the target species. Firstly, we performed phylogenetic analyses to identify the major clade or lineage within each species complex that occurs within our study area. These analyses also include comparative material from regions external to our study area in order to determine the spatial extent of the selected clades and the most appropriate nomenclature. Secondly, we used haplotype networks and tests of spatial genetic structure across the selected clades in order to detect interconnected populations and intra-specific barriers to gene flow. Thirdly, we performed some demographic analyses on putative interbreeding populations to explore the effects of depth and life history.

2.4.1. Clade selection

The selection of suitable clades for population genetics within each morpho-species was determined from phylogenetic analyses. Unique haplotypes were identified using the Collapse v1.2 software (Posada, 2004) without counting start and end missing data as sequence differences. The longest sequence of each haplotype was used in subsequent analyses. 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 MyBayes 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 one (*Ophiomyxa*, *Ophiura*), three (*Ophiacantha*,

Ophiothrix) and five (*Ophiactis*) million generations (sampled every 100), until final average standard deviation of split frequencies were lower than 0.01. Twenty-five percent of the initial trees was 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 1000 pseudo-replicates to calculate bootstrap support values (Stamatakis *et al.*, 2008).

2.4.2. Clade regional structure

Haplotype networks, created to show patterns of mutational steps and their regional distribution and frequencies, 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 sequences to 588 bp (*Ophiomyxa*, *Ophiacantha*, *Ophiactis*, *Ophiothrix*) and 580 bp (*Ophiura*).

Genetic differentiation between regional populations was assessed using AMOVA (Excoffier *et al.*, 1992) in Arlequin v3.5.1.2. Regions were defined using the discrete spatial aggregation of samples (Fig. 1). Nested AMOVA analyses (individuals, locations, regions) proved problematic due to low sample sizes at many locations (Fitzpatrick, 2009), consequently we restricted analyses to one-way AMOVAs, partitioning genetic variation between and within regions. The Hudson, Boos and Kaplan (1992) permutation test was used to detect regional structure based on the K*s measure of allele frequencies and 10000 replicates using the webware MPWEB (http://wwwabi.snv.jussieu.fr/public/mpweb/) (Achaz *et al.*, 2004). Isolation by distance between regions was tested using the Rousett (1997) statistic (untransformed $ø_{ST}/1- ø_{ST}$), the Kimura-2 genetic distance measure and 1000 replicates using the webware IBDWS 3.23 (http://ibdws.sdsu.edu/~ibdws/) (Jensen *et al.*, 2005).

2.4.3. Demographic analyses

For the most abundant clades in each species complex, we calculated standard measures of genetic diversity (haplotype diversity h and nucleotide diversity π) using the software Arlequin v3.5.1.2 (Excoffier and Lischer, 2010). We used statistics that have the ability to detect signatures of recent population expansion including Tajima's D, Fu's Fs and Ramos' R_2 (Fu, 1997; Ramos-Onsins and Rozas, 2002; Tajima, 1989) using the software Arlequin v3.5.1.2 and the R-package Pegas v0.3-1 for R_2 (Paradis, 2010). We also used a coalescent approach to estimating effective population sizes over time using Bayesian skyline plots (Drummond *et al.*, 2006) in BEAST v1.7.1 (Drummond and Rambaut, 2007). To ensure adequate ESS (>200) for all estimated parameters, we used a simple model of nucleotide substitution (HKY), empirically determined base frequencies, no-partitioning, a

strict clock and long MCMC chain lengths (50,000,000). These assumptions were reasonable given that the vast majority of mutations exhibited in the data were in the third codon position and silent. Without sequence mutation rates to calibrate a time-based plot, we also restricted our Skyline analyses to two groups of coalescent intervals, essentially testing for population expansion.

3. Results

3.1. Clade selection, range and nomenclature

A total of 562 sequences of COI was obtained (Table S1). All of the target species showed significant phylogenetic structure suggestive of the presence of cryptic species (Fig. 2). The specimens of *Ophiomyxa*, provisionally identified as *O. vivipara*, formed three main clades (Fig. 2a) (2.9 to 3.7% Kimura 2-parameter (K2P) genetic distance). Clade A included 102 specimens, 95 from the study region (SE Australia, the Macquarie Ridge, several Graveyard seamounts north of the Chatham Rise) and seven from the South Atlantic Ocean (off Tristan da Cunha and the Falkland Islands) and the SW Indian Ridge. Clade C consisted of a sub-clade from SW Australia and clade B specimens from Nukuhou seamount off NE New Zealand and Morgue Seamount on the Chatham Rise. There appear to be life history differences within clade A. Specimens from Tristan da Cunha and the Falkland Islands clearly have brooded young in the bursal sacs. However, with a single exception, we found no evidence of brooding in any specimens from Australia, New Zealand or the Macquarie Ridge (collected throughout the year). Instead, female specimens had large yolky eggs (to 0.5 mm diameter, ethanol preserved) consistent with a lecithotrophic larva (Byrne and Selvakumaraswamy, 2002). The exception was one large (19 mm disc diameter) female from the Macquarie Ridge (sample TOH_0441) that had a tiny brooded juvenile in the gonad. We observed no other morphological or live colour differences (orange-red from on-board images) between any of these populations.

The 72 specimens of *Ophiacantha vivipara* formed three distinct clades (Fig. 2b) (14.1-16.7% K2P). Clade A (n=9) was found only in polar regions, from the Antarctic Peninsula, South Georgia, Bouvet Island, eastern Antarctica and the southern Macquarie Ridge. Clade B (n=4) was recorded from seamounts on the Macquarie Ridge and the Burdwood Bank near the Falkland Islands. Clade C (n=60) occurred across south-eastern Australia, New Zealand and the northern Macquarie Ridge. We confirmed that both clade A and C brood young in the bursae. Clade C gonads had eggs up to 0.6 mm in diameter. The three clades were sympatric only on seamount ‘8’ on the southern Macquarie Ridge (55.4°S, 158.4°E, 539-648 m). The holotype of *Ophiacantha vivipara* was likely to have come from

the Falkland Islands (Fell, 1961), thus possibly falling within clade A or B. There appeared to be no available name for clade C. The only other six-armed species (*O. anomala* and *O. nodosa*) have been collected from the North Atlantic Ocean. In particular, *Ophiacantha anomala* is similar to clade C. Both are brooders, of relatively small body size (compared to *O. vivipara*) and occur at upper bathyal depths; however, we had no sequences from the Atlantic to confirm the relationship.

Ophiura ooplax formed two deep clades (Fig. 2c); one (n=73 samples) restricted to southern Australia and New Zealand, and the other (n=4) to north-western Australia, suggesting that there could be a temperate-tropical species pair (22.0 % K2P divergence). We did not secure samples from Japan or the Philippines (or South Africa for the possible synonym *O. trimeni*) to fully establish the potential range of these clades. In the interim, we use the trinomial *Ophiura ooplax chathamensis* for the southern Australasian clade. This clade was also dioecious; female gonads contained numerous eggs to 0.06 mm in diameter.

The specimens in the *Ophiactis abyssicola* complex fell into two major clades that exhibit different bathymetric ranges (Fig. 2d). The larger clade (*O. abyssicola*, n=160) can be characterised as upper bathyal, occurring in depths of 174-1801 m (only one specimen > 1700 m), from the Atlantic Ocean, SW Indian Ocean, SE Australia, around New Zealand, the Tasman Sea, and the Macquarie Ridge. The smaller of the two clades (here recognised as *O. amator*, n=14) had a lower bathyal distribution (1142-2640 m, with only one specimen < 1700 m) from around SE Australia and northern New Zealand. Within *O. abyssicola*, the 11 sequences from the Atlantic and western Indian Oceans formed a discrete group (here regarded as *Ophiactis abyssicola abyssicola*) allopatric from those from the SW Pacific (*Ophiactis abyssicola cuspidata*, n=149) (6.7% K2P). This clade was dioecious; female gonads contained numerous eggs to 0.10 mm in diameter.

Although the 82 sequences of *Ophiothrix aristulata* from Australia (including tropical NW Australia) and New Zealand formed a single clade with shallow structure, two sequences from Madagascar were divergent (22.9% K2P), suggestive of cryptic speciation (Fig. 2e). Since the holotype of *O. aristulata* was from South Africa, we used the available name *Ophiothrix aristulata megaloplax* for the Australian/New Zealand clade. This clade was dioecious; female gonads contained numerous eggs to 0.10 mm in diameter.

3.2. Population structure and demographics

We restricted the demographic analyses to the largest clade (n≥60 specimens) within each species-complex that predominated across southern Australia and New Zealand: *Ophiomyxa vivipara* A, *Ophiacantha vivipara* C, *Ophiactis abyssicola cuspidata*, *Ophiothrix aristulata megaloplax* and *Ophiura ooplax chathamensis* (Table 1). Thus we excluded divergent clades, which may represent cryptic species, in order to maximise the likelihood that included populations could potentially interbreed. A few specimens within these larger clades were also excluded from the analyses because they fell outside the study area (*Ophiomyxa* from the South Atlantic and SW Indian Oceans – see above) or because sample numbers were too low in one of the designated regions (*Ophiothrix* from NW Australia).

Two broad patterns were evident in these clades. Group 1, with *Ophiomyxa vivipara* clade A, *Ophiacantha vivipara* clade C, and *Ophiura ooplax chathamensis*, were characterised by relatively low haplotype and nucleotide diversity (Table 2), with a few widespread (across three or more regions) haplotypes dominating the median-joining networks (Fig. 3). Group 2, with *Ophiactis abyssicola cuspidata* and *Ophiothrix aristulata*, had higher haplotype and nucleotide diversity, and complex haplotype networks with many singletons. The two-way AMOVA regional analyses (Table 1) were significantly different for group 1 species (9-28% inter-regional variance) but not for group 2 (1-2%). Hudson et al.'s (1992) test for regional differentiation was significant, and thus hypotheses of panmixia rejected for each species. However, the absolute value of K*s (log average within-region similarity between sequences) was higher for group 2 (>1.8) than 1 (<0.9). Isolation by distance between regions was significant only for *Ophiactis* explaining 48 % of the variance in the data, although the slope was negligible (1.0).

The signature of population expansion differed between indices (Table 2). Fu's Fs was significant for all species, although more negative in value for group 2 (approximately -25) than group 1 (-6). Tajima's D was significant for all species except *Ophiacantha* and Ramos-Onsins-Rozas's R2 was significant for *Ophiomyxa*, *Ophiura* and *Ophiothrix*. The absolute values of D and R2 did not reflect the two groups. The BEAST Skyline plots showed recent population expansion for Group 2 with non-overlapping confidence-bounds for the recent versus past skyline time-groups. Group 1 plots showed little or no population expansion (Fig. 4).

4. Discussion

4.1. Population structure across the Australia/New Zealand region

This study used mitochondrial DNA from five bathyal species of ophiuroids to assess phylogeographic structure along an extensive longitudinal gradient from south-west Australia to seamounts east of New Zealand. Although the use of mitochondrial DNA for phylogenetic analyses can be problematic, for example because of introgression (Balloux 2010), typically phylogeographic structure derived from nuclear genes is congruent with that from mitochondrial data (Zink and Barrowclough 2008), including for ophiuroids (Naughton and O'Hara unpublished data). Our approach is to look for congruent patterns through a comparative analysis of phylogenetically-distant species.

All five ophiuroids studied here contained a widespread mtDNA clade that extended across southern Australia and New Zealand, from the subtropical Kermadec Ridge to the subantarctic Macquarie Ridge (depending on their latitudinal extent). All these clades contained widespread haplotypes spread across three or more regions. There was no evidence of a biogeographical break between the two continental fragments, either across the temperate Tasman Sea or across the Southern Ocean near the Macquarie Ridge. Other studies have also found a lack of differentiation for benthic invertebrate populations across Australia and New Zealand. Miller *et al.* (2010) found that two-thirds of seamount coral species studied showed no evidence of regional substructure across Australia and New Zealand (although sample numbers were low for some species and need confirmation). This included the important habitat-forming colonial corals *Solenosmilia*, *Enallopsammium* and *Madrepora*. One scleractinian *Desmophyllum dianthus* showed some divergence between Tasmanian and Macquarie Ridge seamount populations, although this result was confounded by available samples coming from different depth strata. Two antipatharians showed some regional structure between Lord Howe, Norfolk and Kermadec Ridges to the north of New Zealand.

All the major ophiuroid clades included samples from seamounts and the continental slope. Recent studies have emphasised the connectivity of bathyal communities along seamount chains and with continental slopes (Castelin *et al.*, 2010; Castelin *et al.*, 2012; Clague *et al.*, 2012; Samadi *et al.*, 2006) including for ophiuroids (Cho and Shank, 2010). Initial reports of highly endemic faunas on seamounts (e.g., Richer de Forges *et al.*, 2000) have been modified by more comprehensive sampling and taxonomic analysis (Castelin *et al.*, 2011; O'Hara, 2007; Samadi *et al.*, 2006). The finding that assemblages occur at similar depth ranges at remote locations suggests that these assemblages

were generated from dispersal (O'Hara *et al.*, 2008). Long distance dispersal also has been found to occur between isolated deep-sea hydrothermal vent communities (Bachraty *et al.*, 2009).

The evidence of widespread connectivity for bathyal species contrasts with many shallow-water invertebrates that have diverged to form species pairs across the Tasman Sea. This includes the ophiuroids (Mills and O'Hara, 2013) where sufficient numbers of shallow-water (0-200 m) species were restricted to either Australia or New Zealand to form separate biogeographic assemblages (O'Hara *et al.*, 2011). There are exceptions, such as the cool-temperate clade of the seastar *Coscinasterias muricata* (Waters and Roy, 2003b) and the rock lobster *Jasus edwardsii* (Ovenden *et al.*, 1997), which have atypical long-lived larvae. For ophiuroids, however, there appear to be different biogeographic processes operating for shallow-water and bathyal species.

The latitudinal range of our five clades suggests two routes of dispersal between Australia and New Zealand, a northerly route across the Tasman Sea via the Lord Howe Rise and Norfolk Ridge at approximately 32-35°S and a southerly route from Tasmania to the Macquarie Ridge across the Southern Ocean at 45-55°S. *Ophiothrix aristulata megaloplax* and *Ophiura ooplax chathamensis* occur on the Lord Howe Rise and Norfolk Ridge but not on the Macquarie Ridge (Fig. 1) suggesting a trans-Tasman dispersal. Currents do not flow strongly in an east-west direction across the northern Tasman Sea at 1000 m (Miller *et al.*, 2010; Przeslawski *et al.*, 2011). However, the maximum dispersal distance between known populations on the Australian continental slope and seamounts on the western rim of the Lord Howe Rise is only approximately 500 km (which would be reduced to <290 km if populations are discovered on the intermediate Tasmantid seamounts). Alternatively, both clades occur on the outer shelf and may be able to disperse via the strong west-to-east flow and eddies of the shallow-water East Australian current after it diverges from the Australian mainland to cross the Coral and Tasman Seas (Ridgway and Dunn, 2003). Neither has been found by the extensive French collecting effort around New Caledonia and the Coral Sea suggesting that a potential third dispersal route, from the north along the Lord Howe Rise or Norfolk Ridge to New Zealand, is unlikely (at least under current environmental conditions). *Ophiomyxa vivipara* A and *Ophiacantha vivipara* C have a more southerly distribution and the shortest distribution route would be via the Macquarie Ridge where a propagule has to cross a maximum of 1200 km of abyssal plain between known populations on the Cascade Seamount (SE of Tasmania) and the central Macquarie Ridge. This gap may only be 750 km if populations are eventually discovered on the under-sampled South Tasman Rise, south of Tasmania. Currents at 1000 m flow strongly (~10 cm/s) from the South Tasman Rise to the Macquarie Ridge and then around the Campbell Plateau northwards to the

Chatham Rise (Miller *et al.*, 2010), thus supporting this hypothesis. The last clade (*Ophiactis abyssicola cuspidata*) has been found both across the Tasman Sea and along the Macquarie Ridge and could possibly disperse via either route.

Multiple independent genetic markers are required to satisfactorily estimate migration rates from coalescent analyses (Kuhner, 2009). However, some broad generalisations can be made regarding connectivity of our species from our mitochondrial data. This can be summarised as follows: although there was limited regional structure in the frequency of haplotype distributions across all examined clades, and no clades appeared to be strictly panmictic, the regional structure in general was not concordant with a simple isolation-by-distance model. An explanation for these data is that currents between populations are spatially and temporally variable and not necessarily correlated with geographic distance between populations. These findings are broadly concordant with the only other phylogeographic study on bathyal ophiuroids (Cho and Shank, 2010).

4.2. Oceanic and bathymetric barriers to distribution

Although our focus in this study was on the temperate Australian/New Zealand region, we obtained some relevant samples from tropical north-western Australia, the south-western Indian Ocean, the Atlantic Ocean and Antarctica. Although these were relatively few in number and hence the results preliminary (almost certainly not all lineages have been sampled which could affect inferred phylogenetic relationships), they showed some interesting biogeographic and bathymetric patterns.

In contrast to the general lack of longitudinal structure across temperate southern Australia and New Zealand, there was evidence of significant phylogeographic breaks between tropical, temperate and polar clades, and for temperate clades across the Indian Ocean. For example, COI sequences from *Ophiura ooplax* specimens off tropical Australia were markedly divergent from those across southern Australia, the Tasman Sea and New Zealand. While the main clade (A) of the *Ophiomyxa vivipara* complex occurred around the Southern Ocean from the Falkland Islands to New Zealand, distinct subtropical clades were found around north-east New Zealand (clade B) and south-west Australia (clade B), although clades A and B partially overlapped on the Chatham Rise. To the south, Antarctic populations in the *Ophiacantha vivipara* complex formed distinct clades (A & B) relative to populations across south-east Australia and New Zealand (clade C), overlapping only on the southern Macquarie Ridge (O'Hara *et al.*, 2013). The exception appears to be *Ophiothrix aristulata*, where our single specimen from north-west Australia fell within the main Australian/New Zealand clade, but

clearly more sequences are required to resolve the distribution limits within this complex. The distinction between tropical, temperate and polar clades is largely concordant with the biogeographic analysis of O'Hara et al. (2011), who found that many bathyal and shelf morpho-species are distributed into similar latitudinal bands across the region.

A phylogeographic discontinuity across the eastern Indian Ocean occurs within *Ophiactis abyssicola*, which contained one clade from the northern Atlantic to the south-western Indian Ocean Ridge (33°S, 57°E), and another (*cuspidata*) from south-eastern Australia (144°E) to New Zealand (174°W). We failed to get sequences from dried museum specimens from around St Paul and Amsterdam Islands (approx. 38°S, 77°E) in the mid Indian Ocean (O'Hara unpublished data), but its presence on the nearby Atlantis Bank, and the complete absence of any specimens collected from off the well-sampled south-western coast of Australia, suggests that these would belong to the Atlantic clade. In summary, we have found two allopatric clades (6.7% K2 genetic distance) separated by at least 7,500 km across the south-eastern Indian Ocean. The two sequences we obtained of *Ophiothrix aristulata* from off Madagascar were considerably divergent (22.9%) from Australian/New Zealand material. Conversely, for other species, sequences from populations on the south-western Indian Ocean Ridge and off south-eastern Australia diverged by 0.4% (*Ophiomyxa vivipara* clade A, Fig. 2a) and 0.3% K2 distance (*Ophiactis profundi*, an outgroup in our study, Fig. 2b).

In shallow water, invertebrates at temperate latitudes are generally separated into distinct African, Australian and sometimes mid-ocean (e.g., Amsterdam Island) clades, which may (e.g., Waters and Roy, 2004) or may not (e.g., Ovenden *et al.*, 1997; Waters and Roy, 2003a) have a sister relationship. To the north, some shallow-water species apparently disperse across the equatorial Indian Ocean. The "southern Indian" clade of the *Acanthaster planci* (Crown-of-Thorns Seastar) complex shows little regional genetic structure in mitochondrial DNA between Cocos Keeling Island (off Indonesia), Chagos Island (central Indian Ocean) and the eastern African coast (Vogler *et al.*, 2012). Further to the south, long distance dispersal on the Antarctic Circumpolar Current (ACC, ~50-60°S) has been well studied, particularly with reference to rocky shore animals rafting on the brown algae *Macrocystis* and *Durvillaea* from one subantarctic island to another (Nikula *et al.*, 2010; Nikula *et al.*, 2012). Some bathyal species also appear able to disperse widely around the Southern Ocean. The same COI haplotype of the seastar *Hippasteria phrygiana* has been recorded from off Chile, Bouvet, Kerguelen, and New Zealand (Foltz *et al*. 2013).

We have been unable to locate comparable studies examining the genetic structure or larval connectivity of bathyal benthic invertebrates across the temperate Indian Ocean. However, topography and currents differ between surface and bathyal depths, potentially affecting dispersal capacity. There are areas of seafloor shallower than 2000 m on mid oceanic ridges and on volcanic seamounts scattered through the area (Yesson *et al.*, 2011), allowing more opportunities for stepping-stone dispersal than in shallow water (Brewin *et al.*, 2007). A plausible vector for long distance dispersal of bathyal animals in the southern hemisphere is the so-called 'supergyre', a nested system of subtropical boundary currents that flow across the southern Indian and Pacific Oceans at approximately 1000 m depth (Ridgway and Dunn, 2007). These currents flow towards the west across the Tasman Sea, around Tasmania, along the coast of southern Australia and across the Indian Ocean to Madagascar at approximately 20°S. The flow then turns southwards along south-west Africa and then back east across the Southern Ocean at approximately 40-50°S, crossing the Macquarie Ridge and flowing around the Campbell Plateau south-east of New Zealand. The distribution of the ophiuroid clades suggests that temperate bathyal animals are more likely to use the southern route from west to east. The absence of *Ophiactis abyssicola* and *Ophiacantha vivipara*, and the presence of a divergent clade (C) of *Ophiomyxa vivipara*, off south-western Australia are indicative of a barrier to dispersal or colonisation to that region. The two species with clades that do occur across southern Australia (*Ophiothrix aristulata* and *Ophiura ooplax*) both have a shallower bathymetric range, including the outer shelf (>100 m depth), and also have closely related clades around the tropical Indo-Pacific across to South Africa. Hence they are likely to have a distinct phylogeographic history. We were unable to obtain sequences from *Ophiura trimemi* (the analogue of *O. ooplax* off South Africa), but as mentioned above, sequences of *Ophiothrix aristulata* from south-eastern Africa were divergent from Australian populations and not concordant with a recent east to west dispersal route across the subtropical Indian Ocean.

Finally, we found that two sister species within *Ophiactis*, *O. abyssicola* and *O. amator*, occupied distinct bathymetric rather than geographic ranges. Speciation events based on bathymetry may be common for marine fauna (Imgram, 2011) and are hypothesised to arise through ecological specialisation along the depth gradient, followed by divergent natural selection and assortative mating (Doebeli and Dieckmann, 2003). We were unable to obtain recently collected specimens of *O. abyssicola* from lower bathyal depths in the Atlantic Ocean, and consequently it is unclear whether the speciation event is likely to have occurred in the south-western Pacific or Atlantic Oceans.

4.3. Life history strategies

Demographic structure varied across our target clades. Group 1 (*Ophiomyxa vivipara* clade A, *Ophiacantha vivipara* clade C, and *Ophiura ooplax chathamensis*) had simplified haplotype networks with 1-3 dominant widespread haplotypes and a relatively low diversity of rare haplotypes, low effective population sizes, regional subdivision, and little evidence of significant population expansion. This combination suggests a relatively recent population bottleneck or speciation event followed by limited migration and the beginnings of regional divergence. The cause of such population bottlenecks is usually credited to glacial climatic cycles (Allcock and Strugnell, 2012). Group 2 (*Ophiactis abyssicola cuspidata* and *Ophiothrix aristulata megaloplax*) was characterised by a high diversity of haplotypes, relatively high effective population sizes, little population structure and signs of recent population expansion.

The two groups were not distinguished by depth or latitudinal range (*Ophiothrix* and *Ophiura* occur in shallower water than the others and are absent from the Macquarie Ridge) or egg size. While group 2 had small eggs (0.1 mm diameter), group 1 possessed small (*Ophiura*, to 0.06 mm) to large eggs (*Ophiomyxa*, *Ophiacantha*, to 0.5-0.6 mm). Large eggs are indicative of lecithotrophic (non-feeding) larvae and/or brooded young (Hendler, 1991; Sewell and Young, 1997). Although small eggs (<0.15 mm) in ophiuroids are typically indicative of planktotrophic feeding larvae, they can also develop into abbreviated larvae (e.g., reduced pleutei), or even occur in brooders (Hendler, 1991).

Most deep-sea ophiuroids appear to have pelagic lecithotrophic larvae (Young, 2003). Unlike in shallow water, where lecithotrophic larvae are relatively short lived, they appear to be able to survive for long periods in cold water (Young *et al.*, 1997) and potentially could disperse large distances in deep sea currents. However, lecithotrophic species have relatively few eggs compared to planktotrophs and it is unclear how frequently long distance dispersal occurs. Echinoderms with planktotrophic larvae are generally highly fecund and the larval duration can extend to months for shallow-water species (Uthicke *et al.*, 2009). But planktotrophy is not a common strategy for deep-sea ophiuroids (Young, 2003). Planktotrophic larvae are constrained by the availability of food, which is either concentrated near the sea surface, at depths where there is high detrital export from the sea surface, or near the seafloor where there are high levels of resuspended organic matter (Young, 2003).

The presence of brooded juveniles in group 1 is perplexing. The brooding *Ophiacantha vivipara* clade C was found to share haplotypes between isolated populations on seamount clusters around

Tasmania, on the Chatham Rise and down the Macquarie Ridge. Cho and Shank (2010) argued that two widely distributed species of *Ophioplinthaca* may have been brooders because brooding species occur in the same family (Ophiacanthidae). However, there was no direct evidence for this mode of reproduction in their species. Our study therefore presents the first evidence of long distance dispersal in a deep-sea ophiuroid brooder. It is unclear how animals without a dispersive phase can migrate long distances across unsuitable habitat (the abyssal plain) to reach isolated seamounts. Although rafting on macroalgae is a plausible mechanism for the long distance dispersal of brooding echinoderms that live on shallow-water rocky reefs (O'Hara, 1998b), there is no obvious vector for deep sea brooders.

It is possible that these animals can vary their reproductive strategy from pelagic larvae to brooded young over time or across their range. Divergence between pelagic and brooding echinoderms can be very rapid, dated to only 6,000 years for a lecithotroph-brooding species-pair in *Cryptasterina* (Puritz *et al.*, 2012) where the brooded embryos appear to be derived from lecithotrophic larvae that are retained internally in the parent past metamorphosis (Byrne *et al.*, 2003). The Antarctic ophiuroid *Astrotoma agassizii* is known to have some allopatric populations that brood and others releasing pelagic larvae (Heimeier *et al.*, 2010). In our study there appear to be both brooding and non-brooding populations within *Ophiomyxa vivipara* A. However, we would argue that, for *Ophiomyxa* at least, there is unlikely to have been a single evolutionary shift from lecithotrophy to brooding. The species distribution and demographic structure are indicative of recent dispersal across the Southern Atlantic and Indian Oceans between isolated habitat patches. Obligate brooding would have to have evolved multiple times at distant locations.

An alternative is that brooding is facultative. The brooding seastars *Pteraster militaris* and *Henricia lisa* have been observed releasing a small proportion of their clutch as free-swimming larvae which developed as normal (McClary and Mladenov, 1988; Mercier and Hamel, 2008). These echinoderms potentially have a reproductive strategy analogous to many fissiparous species, which use asexual reproduction to generate large local populations in favourable habitats but also maintain a low level of sexual reproduction and larval dispersal in order to colonise new areas (Mladenov and Emson, 1984). The prevalence of brooding may be regulated by external factors (poecilogony). This process has been invoked to explain variation in brooding behaviour within other *Ophiomyxa* species (Hendler, 1991). In this context we note that brooding populations of *Ophiomyxa vivipara* A from the southern Atlantic Ocean occur at much shallower depths (therefore different niches) than

Australian/New Zealand populations. More molecular and life history data are required to unravel the dispersal processes of these challenging animals.

Acknowledgements

This project is an output from the Marine Biodiversity Research Hub, funded through the Commonwealth National Environmental Research Program (NERP), and administered through the Australian Government's Department of Sustainability, Environment, Water, Population and Communities. Additional funding was also provided to TOH by the Census of Marine Life field programme CenSeam (a global census of marine life on seamounts) to collect tissues from museum samples in New Zealand and France. We thank the many museum curators and collection mangers who have facilitated access to their collections of ophiuroids, particularly Sadie Mills (National Institute of Water & Atmospheric Research, Wellington), Nadia Améziane and Marc Eléaume (Muséum national d'Histoire naturelle Paris), Sabine Stöhr (Swedish Museum of Natural History, Stockholm), Alexander Martynov (Moscow State Museum) and Margaret Packer (Natural History Museum London). We also thank Susanne Lockhart (NOAA Southwest Fisheries Science Center) for providing the ICEFISH ophiuroid collection from the South Atlantic; and Alex Rogers (Oxford University) and David Staples (Museum Victoria) for collecting material on the SW Indian Ridge. We also thank Andrew Hugall (Museum Victoria) and three anonymous reviewers for suggesting improvements to draft manuscripts.

References

Achaz, G., Palmer, S., Kearney , M., Maldarelli , F., Mellors, J.W., Coffin, J.M., Wakeley , J., 2004. A Robust Measure of HIV-1 Population Turnover Within Chronically Infected Individuals. Mol. Biol. Evol. 21, 1902-1912.

Allcock, A.L., Strugnell, J.M., 2012. Southern Ocean diversity: new paradigms from molecular ecology. Trends Ecol. Evol. 27, 520-528.

Bachraty, C., Legendre, P., Desbruyères, D., 2009. Biogeographic relationships among deep sea hydrothermal vent faunas at global scale. Deep Sea Res. Part I 56, 1371-1378.

Bandelt, H.-J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37-48.

Boissin, E., Stöhr, S., Chenuil, A., 2011. Did vicariance and adaptation drive cryptic speciation and evolution of brooding in *Ophioderma longicauda* (Echinodermata: Ophiuroidea), a common Atlanto-Mediterranean ophiuroid? Mol. Ecol. 22, 4737-4755.

Brewin, P.E., Stocks, K.I., Menzies, G., 2007. A history of seamount research. In: Pitcher, T.J., Morato, T., Hart, P.J.B., Clark, M.R., Haggen, N., Santos, R.S. (Eds.), Seamounts: Ecology, Fisheries and Conservation. Blackwell, Oxford, pp. 41-62.

Byrne, M., Hart, M.W., Cerra, A., Cisternas, P., 2003. Reproduction and larval morphology of broadcasting and viviparous species in the *Cryptasterina* species complex. Biol. Bull. 205, 285-294.

Byrne, M., Selvakumaraswamy, P., 2002. Phylum Echinodermata: Ophiuroidea. In: Young, C.M., Sewell, M.E. (Eds.), Atlas of Marine Invertebrate Larvae. Academic Press, London, pp. 483-498.

Castelin, M., Lambourdiere, J., Boisselier, M.-C., Lozouet, P., Couloux, A., Cruaud, C., Samadi, S., 2010. Hidden diversity and endemism on seamounts: focus on poorly dispersive neogastropods. Biol. J. Linn. Soc. Lond. 100, 420-438.

Castelin, M., Lorion, J., Brisset, J., Cruaud, C., Maestrati, P., Utge, J., Samadi, S., 2012. Speciation patterns in gastropods with long-lived larvae from deep sea seamounts. Mol. Ecol. 21, 4828–4853.

Castelin, M., Puillandre, N., Lozouet, P., Sysoev, A., Richer de Forges, B., Samadi, S., 2011. Molluskan species richness and endemism on New Caledonian seamounts: Are they enhanced compared to adjacent slopes? Deep Sea Res. Part I 58, 637–646.

Cho, W., Shank, T.M., 2010. Incongruent patterns of genetic connectivity among four ophiuroid species with differing coral host specificity on North Atlantic seamounts. Mar. Ecol. 31, 121-143.

Clague, G.E., Jones, W.J., Paduan, J.B., Clague, D.A., Vrijenhoek, R.C., 2012. Phylogeography of *Acesta* clams from submarine seamounts and escarpments along the western margin of North America. Mar. Ecol. 33, 1-13.

Doebeli, M., Dieckmann, U., 2003. Speciation along environmental gradients. Nature 421, 259–264.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7:214, 1-8.

Drummond, A.J., Rambaut, A., Shapiro, B., Pybus, O.G., 2006. Bayesian Coalescent Inference of Past Population Dynamics from Molecular Sequences. Mol. Biol. Evol. 22, 1185-1192.

Excoffier, L., Lischer, H., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Res. 10, 564-567.

Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479-491.

Fell, H.B., 1961. The fauna of the Ross Sea. Part 1 Ophiuroidea. Mem. N. Z. Oceanogr. Inst. 18, 9-79, 19 pls.

Fitzpatrick, B.M., 2009. Power and sample size for nested analysis of molecular variance. Mol. Ecol. 18, 3961-3966.

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. Biotechnol. 5, 294-299.

Foltz, D.W., Fatland, S.D., Eléaume, M., Markello, K., Howell, K.L., Neill, K. & Mah, C.L., 2013. Global population divergence of the sea star *Hippasteria phrygiana* corresponds to the onset of the last glacial period of the Pleistocene. Mar. Biol. 160, 1285–1296.

Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147, 915-925.

Heimeier, D., Lavery, S., Sewell, M.A., 2010. Molecular Species Identification of *Astrotoma agassizii* from Planktonic Embryos: Further Evidence for a Cryptic Species Complex. J. Hered. 101, 775-779.

Hendler, G., 1991. Echinodermata: Ophiuroidea. In: Giese, A.C., Pearse, J.S., Pearse, V.B. (Eds.), Reproduction of Marine Invertebrates: Vol. 6 Echinoderms and Lophophorates. Boxwood Press, Pacific Grove, pp. 355-511.

Hoareau, T.B., Boissin, E., 2010. Design of phylum-specific hybrid primers for DNA barcoding: addressing the need for efficient COI amplification in the Echinodermata. Mol. Ecol. Res. 10, 960-967.

Hoareau, T.B., Boissin, E., Paulay, G., Bruggemann, J.H., 2013. The Southwestern Indian Ocean as a potential marine evolutionary hotspot: perspectives from comparative phylogeography of reef brittle-stars. J. Biogeogr. DOI: 10.1111/jbi.12155.

Hudson, R.R., Boos, D.D., Kaplan, N.L., 1992. A statistical test for detecting geographic subdivision. Mol. Biol. Evol. 9, 138-151.

Hunter, R.L., Halanych, K.M., 2008. Evaluating Connectivity in the Brooding Brittle Star *Astrotoma agassizii* across the Drake Passage in the Southern Ocean. J. Hered. 99, 137-148.

Imgram, T., 2011. Speciation along a depth gradient in a marine adaptive radiation. Proc. R. Soc. B 278, 613-618.

Jensen, J.L., Bohonak, A.J., Kelley, S.T., 2005. Isolation by distance, web service. BMC Genet. 6, 1-6.

Jörger, K.M., Norenburg, J.L., Wilson, N.G., Schrödl, M., 2012. Barcoding against a paradox? Combined molecular species delineations reveal multiple cryptic lineages in elusive meiofaunal sea slugs. BMC Evol. Biol. 12, 245, 1-18.

Knowlton, N., 1993. Sibling species in the sea. Ann. Rev. Ecol. Syst. 24, 189-216.

Kuhner, M.K., 2009. LAMARC: Estimating population genetic parameters from molecular data. In: Lemey, P., Salemi, M., Vandamme, A.-M. (Eds.), The Phylogenetic Handbook: A Practical Approach to Phylogenetic Analysis and Hypothesis Testing. Cambridge University Press, Cambridge, pp. 592-612.

McClain, C.R., Hardy, S.M., 2010. The dynamics of biogeographic ranges in the deep-sea. Proc. R. Soc. B 277, 3533-3546.

McClary, D.J., Mladenov, P.V., 1988. Brood and broadcast: a novel mode of reproduction in the sea star *Pteraster militaris*. In: Burke, R.D., Mladenov, P.V., Lambert, P., Parsley, R.L. (Eds.), Echinoderm Biology: Proceedings of the Sixth International Echinoderm Conference, Victoria, 23-28 August 1987. Balkema, Rotterdam, pp. 163-168.

Mercier, A., Hamel, J.-F., 2008. Depth-related shift in life history strategies of a brooding and broadcasting deep sea asteroid. Mar. Biol. 156, 205-223.

Miller, K., Williams, A., Rowden, A.A., Knowles, C., Dunshea, G., 2010. Conflicting estimates of connectivity among deep sea coral populations. Mar. Ecol. 31 (Suppl. 1), 144–157

Mills, V.S., O'Hara, T.D., 2013. Ophiuroids (Echinodermata; Ophiuroidea) of biogenic habitats on the continental shelf of New Zealand. Zootaxa 3613, 401-444.

Mladenov, P., V., Emson, R., H., 1984. Divide and broadcast: sexual reproduction in the West Indian brittle star *Ophiocomella ophiactoides* and its relationship to fissiparity. Mar. Biol. 81, 273-282.

Mortensen, T., 1933. Ophiuroidea. Danish Ingolf Exped. 4 (8), 1-120, 123 pls.

Mortensen, T., 1936. Echinoidea and Ophiuroidea. Discovery Rep. 12, 199-348, 199 pls.

Nikula, R., Fraser, C.I., Spencer, H.G., Waters, J.M., 2010. Circumpolar dispersal by rafting in two subantarctic kelp-dwelling crustaceans. Mar. Ecol. Prog. Ser. 405, 221–230.

Nikula, R., Spencer, H.G., Waters, J.M., 2012. Passive rafting is a powerful driver of transoceanic gene flow. Biol. Lett. 9, 20120821.

Nylander, J., 2008. MrModeltest v. 2.3. Department of Systematic Zoology, Uppsala University, Sweden.

O'Hara, T.D., 1998a. Mortensen's Pacific Expedition: Re-identification of ophiuroids (Echinodermata) from southern Australia. Steenstrupia 24, 37-50.

O'Hara, T.D., 1998b. Origin of Macquarie Island echinoderms. Pol. Biol. 20, 143-151.

O'Hara, T.D., 2007. Seamounts: Centres of endemism or species-richness for ophiuroids? Glob. Ecol. Biogeogr. 16, 720-732.

O'Hara, T.D., Rowden, A.A., Bax, N.J., 2011. A southern hemisphere bathyal fauna is distributed in latitudinal bands. Curr. Biol. 21, 226-230.

O'Hara, T.D., Rowden, A.A., Williams, A., 2008. Cold-water coral habitats on seamounts: do they have a specialist fauna? Divers. Distrib. 14, 925-934.

O'Hara, T.D., Smith, P.J., Mills, V.S., Smirnov, A.V., Steinke, D., 2013. Biogeographical and phylogeographical relationships of the bathyal ophiuroid fauna of the Macquarie Ridge, Southern Ocean. Pol. Biol. 36, 321-333.

Ovenden, J.R., Booth, J.D., Smolenski, A.J., 1997. Mitochondrial DNA phylogeny of red and green rock lobsters (genus *Jasus*). Mar. Freshw. Res. 48, 1131–1136.

Paradis, E., 2010. Pegas: an R package for population genetics with an integrated modular approach. Bioinformatics 26, 419-420.

Paterson, G.L.J., 1985. The deep sea Ophiuroidea of the North Atlantic Ocean. Bull. Brit. Mus. (Nat. Hist.) Zool. 49, 1-162.

Pearson, M., Gage, J.D., 1984. Diets of some deep sea brittle stars in the Rockall Trough. Mar. Biol. 82, 247-258.

Plouviez, S., Shank, T.M., Faure, B., Daguin-Thiebaut, C., Viard, F., Lallier, F.H., Jollivet, D., 2009. Comparative phylogeography among hydrothermal vent species along the East Pacific Rise reveals vicariant processes and population expansion in the South. Mol. Ecol. 18, 3903-3917.

Posada, D., 2004. COLLAPSE 1.2 Free Software Foundation Incorporation, Boston.

Przeslawski, R., Williams, A., Nichol, S.L., Hughes, M.G., Anderson, T.J., Althaus, F., 2011. Biogeography of the Lord Howe Rise region, Tasman Sea. Deep Sea Res. Part II 58, 959-969.

Puritz, J.B., Keever, C.C., Addison, J.A., Byrne, M., Hart, M.W., Grosberg, R.K., Toonen, R.J., 2012. Extraordinarily rapid life-history divergence between *Cryptasterina* sea star species. Proc. R. Soc. B 279, 3914-3922.

Ramos-Onsins, S.E., Rozas, J., 2002. Statistical properties of new neutrality tests against population growth. Mol. Biol. Evol. 19, 2092-2100.

Richer de Forges, B., Koslow, J.A., Poore, G.C.B., 2000. Diversity and endemism of the benthic seamount fauna in the southwest Pacific. Nature 405, 944-947.

Ridgway, K.R., Dunn, J.R., 2003. Mesoscale structure of the mean East Australian Current System and its relationship with topography. Prog. Oceanogr. 56, 189–222.

Ridgway, K.R., Dunn, J.R., 2007. Observational evidence for a Southern Hemisphere oceanic supergyre. Geophys. Res. Lett. 34, L13612, Doi:13610.11029/12007gl030392.

Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572-1574.

Rousset, F., 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145, 1219-1228.

Rowe, F.W.E., Gates, J., 1995. Echinodermata. In: Wells, A. (Ed.), Zoological Catalogue of Australia. Australian Government Printing Service, Canberra, pp. 1-510.

Samadi, S., Bottan, L., Macpherson, E., Richer de Forges, B., Boisselier, M.-C., 2006. Seamount endemism questioned by the geographic distribution and population genetic structure of marine invertebrates. Mar. Biol. 149, 1463–1475.

Schnabel, K.E., Cabezas, P., McCallum, A., Macpherson, E., Ahyong, S., Baba, K., 2011. Worldwide patters of distribution of squat lobsters (Galatheidae and Chirostylidae: Anomura). In: Poore, G.C.B., Ahyong, S.T., Taylor, J. (Eds.), Squat Lobster Biology. CSIRO Publishing, Melbourne, pp. 149-182.

Sewell, M.A., Young, C.M., 1997. Are echinoderm egg size distributions bimodal? Biol. Bull. 193, 297-305.

Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdaña, Z.A., Finlayson, M., Halpern, B.S., Jorge, M.A., Lombana, A., Lourie, S.A., Martin, K.D., Mcmanus, E., Molnar, J., Recchia, C.A., Robertson, J., 2007. Marine ecoregions of the world: A bioregionalization of coastal and shelf areas. BioScience 57, 573-583.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688-2690.

Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web servers. Syst. Biol. 57, 758-771.

Stöhr, S., Boissin, E., Chenuil, A., 2009. Potential cryptic speciation in Mediterranean populations of *Ophioderma* (Echinodermata: Ophiuroidea). Zootaxa 2071, 1-20.

Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585-595.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673-4680.

UNESCO, 2009. Global Open Oceans and Deep Seabed (GOODS) – Biogeographic Classification. IOC Technical Series, 84. UNESCO-IOC., Paris.

Uthicke, S., Schaffelke, B., Byrne, M., 2009. A boom and bust phylum? Ecological and evolutionary consequences of large population density variations in echinoderms. Ecol. Monogr. 79, 3-24.

Vogler, C., Benzie, J., Barber, P.H., Erdmann, M.V., Ambariyanto, Sheppard, C., Tenggardjaja, K., Gérard, K., Wörheide, G., 2012. Phylogeography of the Crown-of-Thorns Starfish in the Indian Ocean. PLoS ONE 7 (8), e43499.

Ward, R.D., Holmes, B.H., O'Hara, T.D., 2008. DNA barcoding discriminates echinoderm species. Mol. Ecol. Res. 8, 1202-1211.

Waters, J.M., Roy, M.S., 2003a. Global phylogeography of the fissiparous sea-star genus *Coscinasterias*. Mar. Biol. 142, 185-191.

Waters, J.M., Roy, M.S., 2003b. Marine biogeography of southern Australia: phylogeographical structure in a temperate sea-star. J. Biogeogr. 30, 1787-1796.

Waters, J.M., Roy, M.S., 2004. Out of Africa: The Slow Train to Australasia. Syst. Biol. 53, 18-24.

Yesson, C., Clark, M.R., Taylor, M., Rogers, A.D., 2011. The global distribution of seamounts based on 30-second bathymetry data. Deep Sea Res. Part I 58, 442-453.

Young, C.M., 2003. Reproduction, development and life histort traits. In: Tyler, P.A. (Ed.), Ecosystems of the Deep Oceans. Elsevier, Amsterdam, pp. 381-426.

Young, C.M., Sewell, M.A., Tyler, P.A., Metaxas, A., 1997. Biogeographic and bathymetric ranges of Atlantic deep sea echinoderms and ascidians: the role of larval dispersal. Biodivers. Conserv. 6, 1507-1522.

Tables

Table 1. Population statistics for sequences of five ophiuroids across seven Australian and New Zealand regions: NW=North-western Australia, SW=South-western Australia, S=Southern Australia, SE=South-eastern Australia, T=Tasman Sea (Lord Howe Rise and Norfolk Ridge), MQ=Macquarie Ridge, NZ= New Zealand (including the Chatham Rise and Campbell Plateau) (see Fig. 1). *Excluded from the analyses due to insufficient number of samples.

	Number of samples per region							AMOVA between regions			Hudson et al's (1992) test for geographical subdivision		Isolation by distance ($ø_{ST}/1- ø_{ST}$ vs km)		
Species	NW	SW	S	SE	T	MQ	NZ	% variance	F statistic	p	K*s	p	Slope ($x10^2$)	P	R^2
Ophiomyxa vivipara clade A				56		21	18	28.42	0.284	**0.000**	0.20	**0.000**	0.04	0.17	0.86
Ophiacantha vivipara clade C				23		19	18	9.2	0.092	**0.001**	0.86	**0.001**	0.01	0.165	0.94
Ophiura ooplax chathamensis	18	31		7	10		7	12.32	0.123	**0.005**	0.29	**0.001**	-0.02	0.501	0.00
Ophiactis abyssicola cuspidata				22	11	44	72	1.69	0.017	0.059	1.87	**0.002**	0.01	**0.046**	0.48
Ophiothrix aristulata megaloplax	1*	6	18	42	11		4	1.83	0.018	0.144	1.82	**0.048**	-0.02	0.829	0.63

Table 2. Summary statistics and demographic analyses for the largest clade within each species-complex across the study region (NW Australia to New Zealand), including *n*=number of individuals, *h*=haplotype diversity, π =nucleotide diversity, results of Beast Skyline plots (Fig. 4), Fu's F_S, Tajima's *D*, and Ramos-Onsins-Rozas' R_2 (significant values at 0.05 are in bold).

Species	*N*	no of Haplo-types	*H*	*Π*	Beast Skyline Plot	F_S	D	R_2
Ophiomyxa vivipara clade A	95	8	0.31 ± 0.06	0.0006 ± 0.0006	No expansion	**-6.83**	**-1.87**	**0.039**
Ophiacantha vivipara clade C	60	15	0.85 ± 0.03	0.0031 ± 0.0020	No expansion	**-6.45**	-1.39	0.056
Ophiura ooplax chathamensis	73	9	0.34 ± 0.07	0.0011 ± 0.0009	No expansion	**-6.01**	**-1.99**	**0.032**
Ophiactis abyssicola cuspidata	149	78	0.97 ± 0.01	0.0125 ± 0.0065	expansion	**-24.72**	**-1.68**	0.039
Ophiothrix aristulata megaloplax	82	66	0.98 ± 0.01	0.0103 ± 0.0055	expansion	**-25.20**	**-2.13**	**0.027**

Figures

Fig. 1. Map of sample sites and analysis regions for the five species complexes.

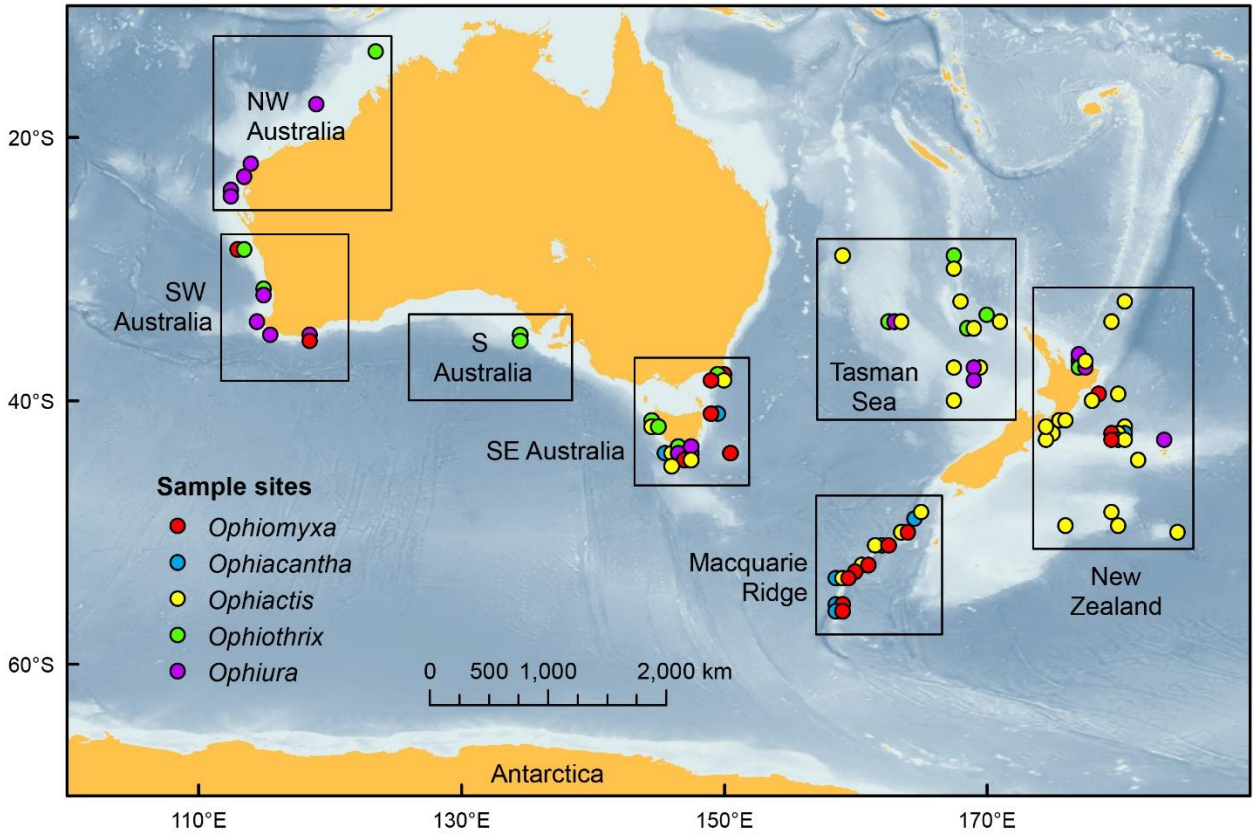


Fig. 2. Bayesian consensus haplotype trees produced using the GTR + Γ + I model in MrBayes v3.1.2 for the five target species-complexes and associated outgroups. Values associated with selected nodes are bayesian posterior probabilities followed by bootstrap support (10000 replicates) from the Maximum Likelihood tree constructed using the GTR + Γ model in RAxML v7.0.4.

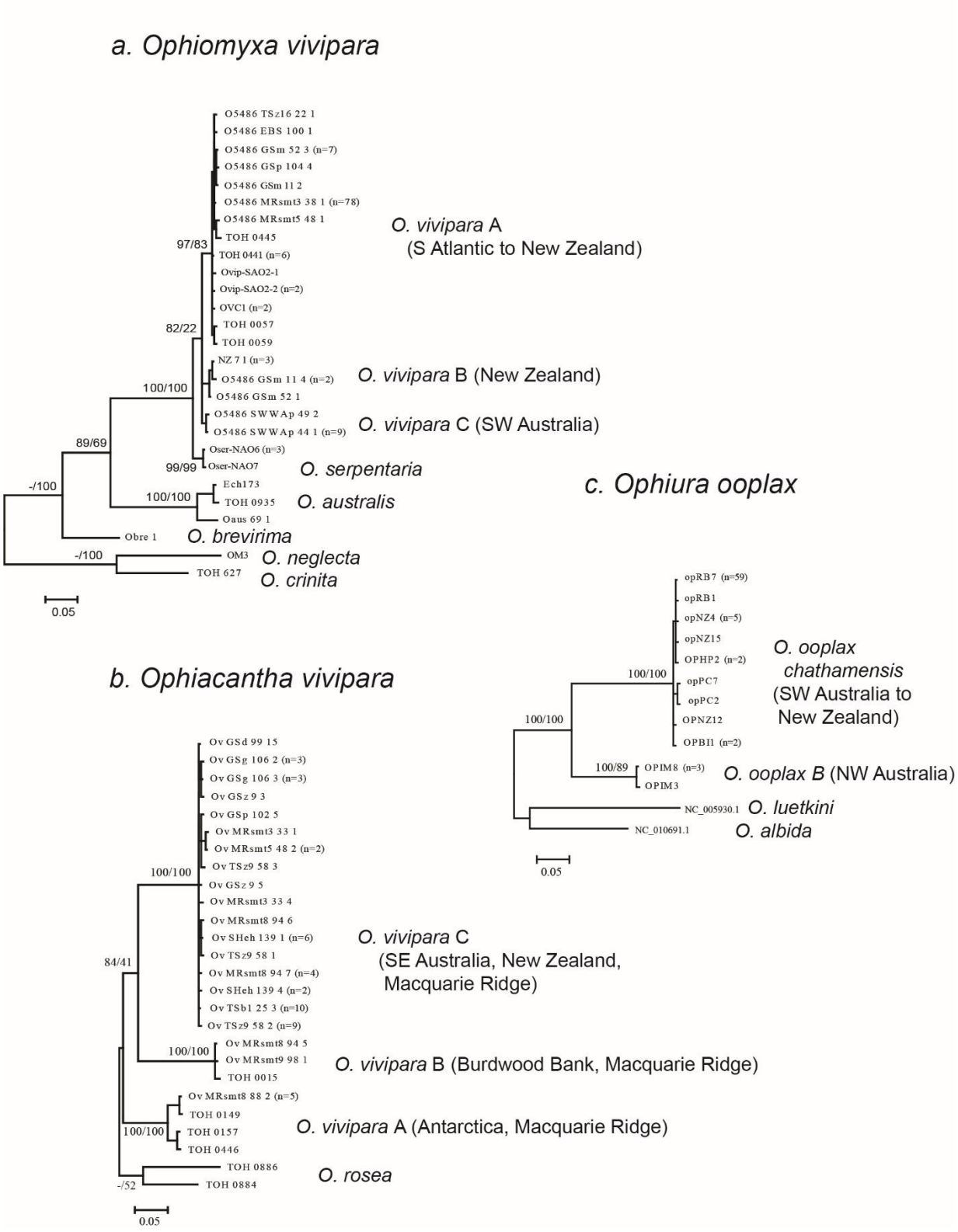


Fig. 2b. (Fig. 2 continued)

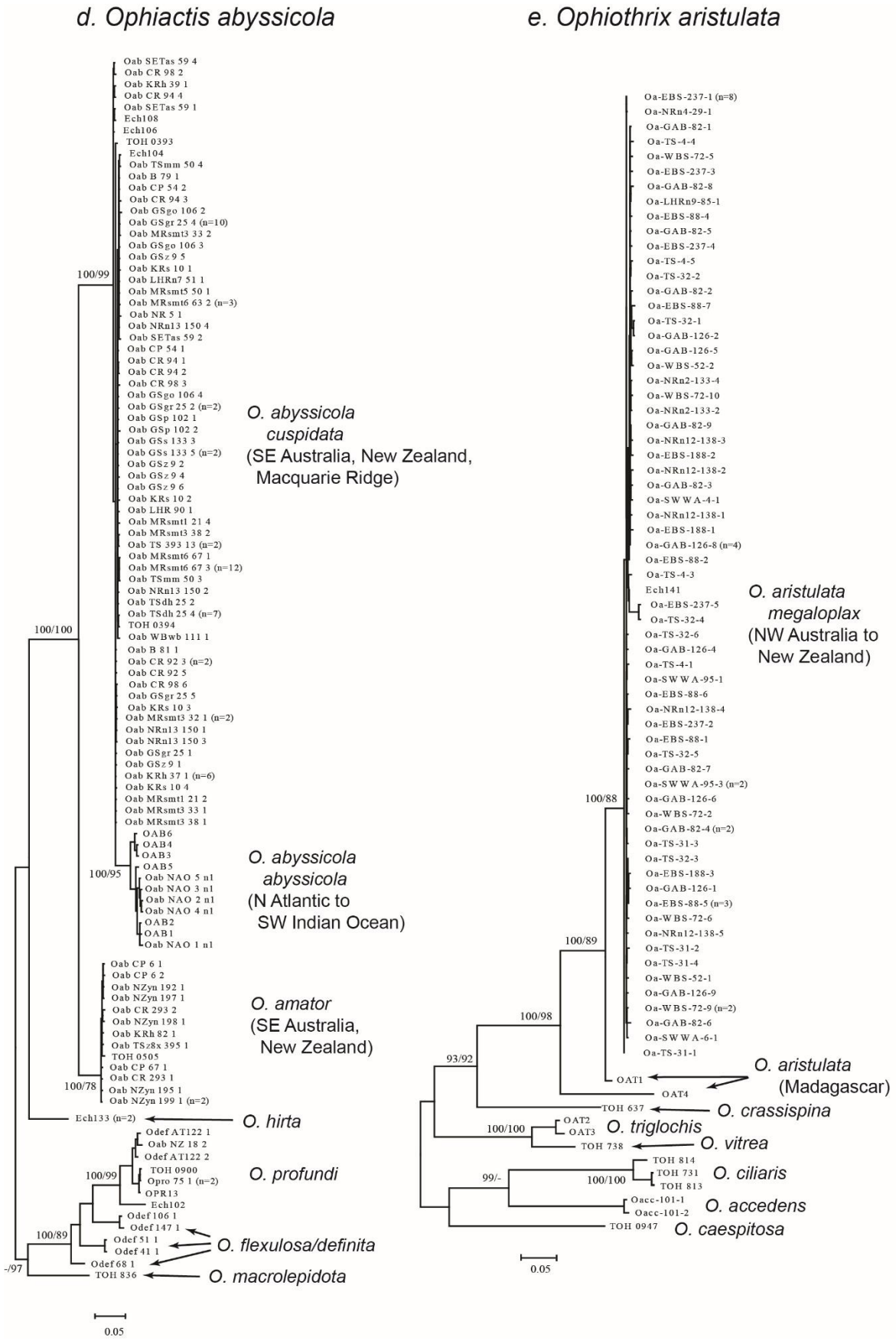


Fig. 3. Median-joining haplotype networks of mtDNA COI sequences for five of the most abundant clades (588 bp except for *O. ooplax*, 580 bp). The number of specimens is superimposed onto the more abundant haplotypes.

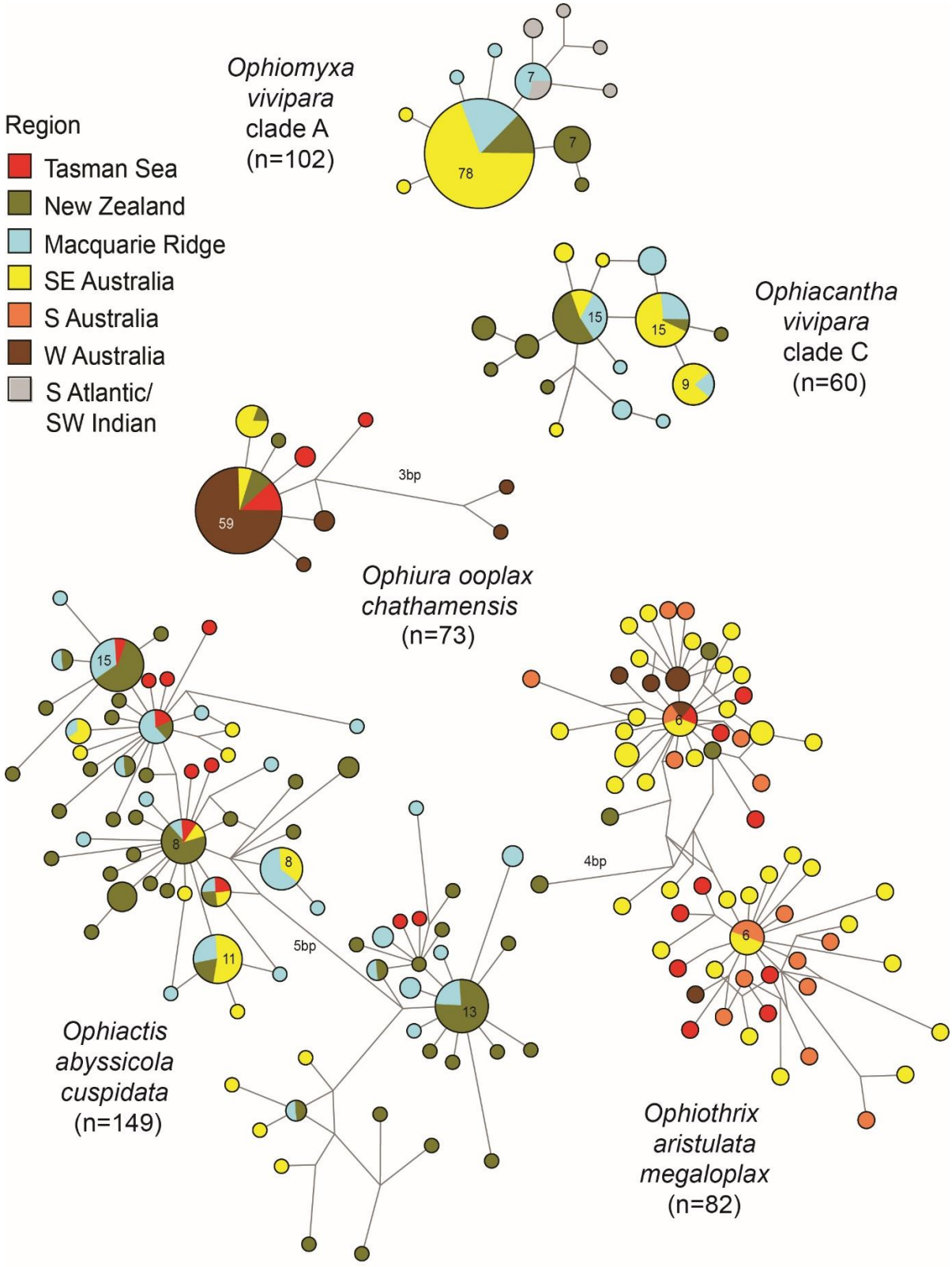
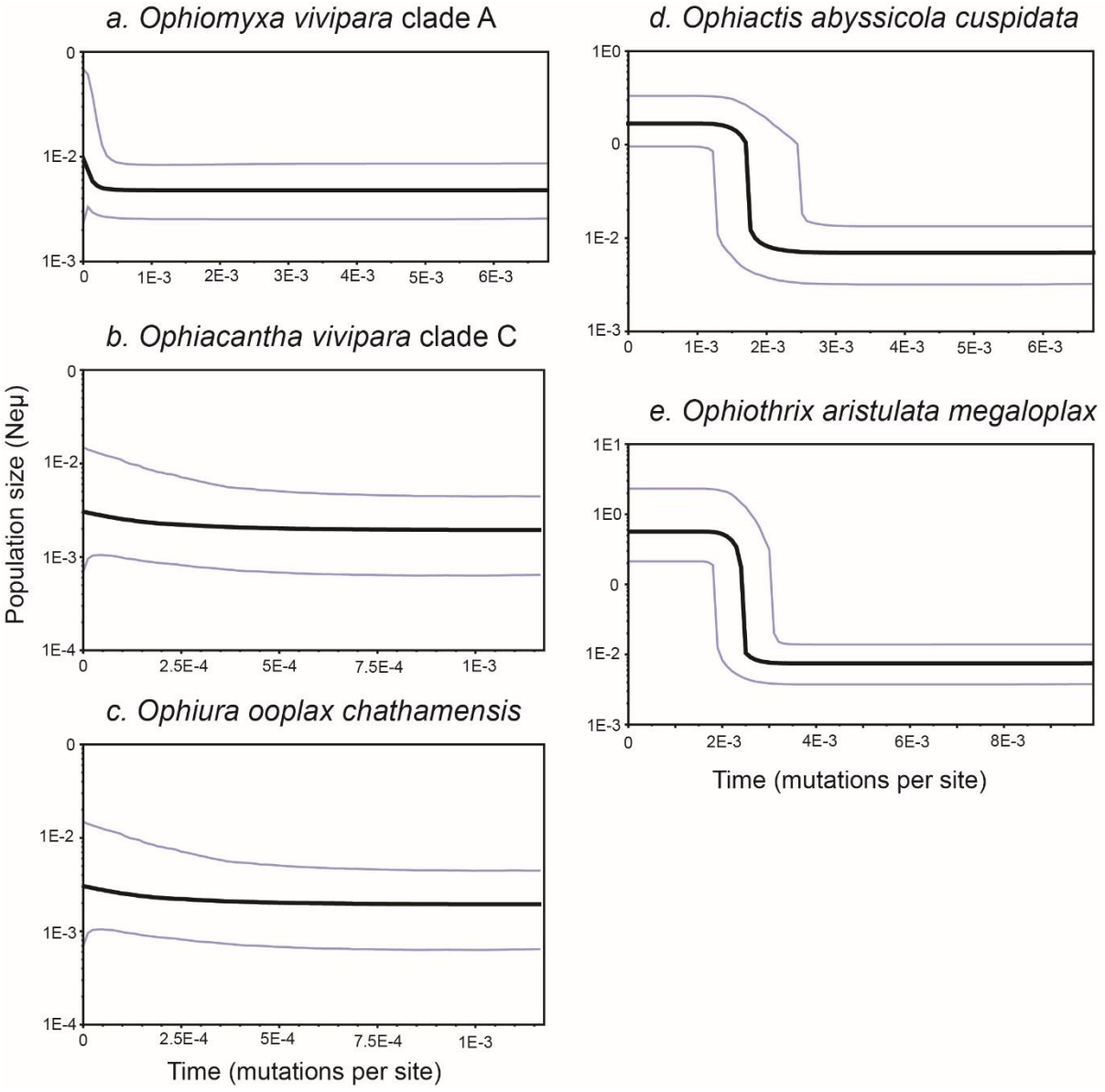


Fig. 4. Bayesian skyline plots showing population changes over time. The analysis was restricted to two time groups (effectively 'past' and 'present') to ensure coalescence with the single available locus (COI). The y axis is the product of the effective population size and nucleotide mutation rate. Time is expressed in terms of mutations per site as the COI mutation rate for these species is unknown.



Supplementary table captions

Table S1. List of material sequenced for this study, including the haplotype and clade identity derived from sequence and phylogenetic analyses, and the aligned sequence data. The haplotype name was derived from the longest synonymous sequence.

Table S2. A list of ophiuroid species listed in this paper along with their authorities and references.