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Cryptic speciation and the circumpolarity debate: A case study on endemic Southern Ocean octopuses using the COI barcode of life

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ABSTRACT

Three hundred and fifty specimens of the endemic Southern Ocean octopus genus *Pareledone*, were sequenced for the barcoding gene COI. Geographic coverage comprised the South Shetland Islands, the Ross Sea, Adélie Land, George V Land, the Weddell Sea, under the site of the former Larsen B ice shelf, Prydz Bay, the South Orkney Islands and the Amundsen Sea. The greatest number of specimens was captured at the three first-mentioned localities. At least 11 species were represented in the samples and the analyses revealed cryptic species. Six species were found to have extended distributions. Circumpolarity is supported for at least one species. Evidence is presented for a barrier to gene flow to the west of the Antarctic Peninsula, with haplotypes of *P. aequipapillae* becoming progressively more diverse in a clockwise direction from the South Shetland Islands to the Amundsen Sea. This pattern is akin to that seen in ring species, although we suggest that comparatively warm bottom water acts as a physical barrier preventing completion of the ring.

1. Introduction

The Census of Antarctic Marine Life (CAML) and the focus provided by the International Polar Year (IPY) (2007–2008) have provided unparalleled opportunities for collection of zoological specimens from the Southern Ocean. At the same time, the Barcode of Life project has initiated programmes that allow high throughput sequencing of the mitochondrial gene cytochrome *c* oxidase subunit I (COI). The Marine Barcode of Life project (MarBOL) is an international collaboration with several goals, one of which is to develop marine barcoding as a research tool in taxonomy. Recent co-operation between CAML and MarBOL has allowed systematists working on Southern Ocean species to investigate the usefulness of the barcode gene as a taxonomic tool.

Potential uses of barcoding include identifying the full range of known species, highlighting previously overlooked species and enabling identifications through molecular methods (Hebert et al., 2003, 2004a; Ward et al., 2005; Bucklin et al., 2007; Smith et al., 2008; Steinke et al., 2009a). Several studies illustrate the potential usefulness of the barcode gene in other groups. For example, Ward et al. (2009) estimated that barcodes based on a 648 base pair (bp) region of the mitochondrial COI gene separate 98% of all marine fish and Zemlak et al. (2009) used barcodes to conclude that more than 300 fish species thought to bridge South African and Australian waters might in fact represent pairs of sister species. These estimates are necessarily based on the use of a divergence threshold for distinguishing between intra- and interspecific sequence variation. Valentini et al. (2009) briefly reviewed the problems associated with use of a divergence threshold, which include low taxon coverage (Meyer and Paulay, 2005) and low numbers of individuals per species (Matz and Nielsen, 2005; Nielsen and Matz, 2006). The level of intra- and interspecific sequence divergence varies between taxa. For example, mean intra- and interspecific distances (*D*, Kimura's

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two-parameter; Kimura, 1980) were estimated to be 0.35 and 8.11 for fish (Ward et al., 2009) and 0.0075 and 0.2705 for copepods (Bucklin et al., 2010). Estimates are not always directly comparable from the literature because of the different distance measures utilised. However, the key to successful identification using barcodes is a lack of, or very reduced, overlap between the ranges of intra- and interspecific distances (Hebert et al., 2004b; Zemlak et al., 2009).

Research on the morphological characteristics of Southern Ocean octopuses has provided evidence of cryptic speciation and endemic radiation (Allcock, 2005; Allcock et al., 2007), with one genus, *Pareledone*, proving to be particularly diverse. Indeed *Pareledone* meets many of the criteria for marine species flocks, including monophyly, diversity and endemism, as defined by Eastman and McCune (2000).

The genus *Pareledone* contains two clades, one containing smooth-skinned species such as *P. turqueti* Joubin, 1905, the other comprising some 12 valid papillated species (Table 1), nine of which occur sympatrically on the continental shelf at Elephant Island, South Shetland Islands (Allcock et al., 2008). The visual difference between the smooth and papillated *Pareledone* is stark (Fig. 1).

Circumpolarity has been proposed for several Southern Ocean octopus species (e.g., Dell, 1972), but the evidence is equivocal. Taxonomic workshops have tended to question circumpolarity, but a paucity of specimens from areas other than the South Shetland Islands, where a CCAMLR (Committee for the Conservation of Antarctic Marine Living Resources) sponsored groundfish survey conducted every few years regularly yields an abundance of octopuses, has hampered progress. Octopus taxonomy is difficult because of the lack of hard characters and the subtlety of informative characters (Allcock, 2005). Systematics is therefore reliant on soft characters whose plasticity needs to be assessed and which can vary simply as a result of fixation techniques. Because of their large size relative to most invertebrates, it has been impossible to amass sufficient number of octopus specimens with adequate geographic coverage to assess the morphological variability comprehensively and therefore elucidate the ranges of species using traditional taxonomic techniques.

In this collaborative study, we have used sequences of the mitochondrial COI gene (the designated Barcode of Life gene for animals) from 350 specimens of *Pareledone* from a range of locations throughout the Southern Ocean. Targeting material from IPY cruises aboard the research vessels *James Clark Ross*, *Tangaroa*, *Aurora Australis* and *Polarstern*, we have obtained collections from the South Shetland Islands, the South Orkney Islands, the eastern Weddell Sea, the coast of Adélie Land, the coast of George V Land, the Ross Sea and the Amundsen Sea (Fig. 2). Combining our genetic data with the extensive morphological systematics knowledge of *Pareledone* around Elephant Island (Allcock, 2005; Allcock et al., 2007) gained from the frequent and intensive trawl surveys in this area allows us to estimate the usefulness of the barcode gene in this group and to address three pertinent questions:

(1) Are barcodes of COI useful for species identification in this group?
(2) What are the ranges of species of *Pareledone* and are any species circumpolar in their distribution?
(3) Are there any hitherto undiscovered species?

Table 1
Species of the genus *Pareledone* currently considered valid together with type locality.

Species	Type locality
Pareledone aequipapillae Allcock, 2005	South Shetland Islands
Pareledone albimaculata Allcock, 2005	South Shetland Islands
Pareledone aurata Allcock, 2005	South Shetland Islands
Pareledone aurorae Berry, 1917	Queen Maud Land
Pareledone charcoti Joubin, 1905	Graham Land
Pareledone cornuta Allcock, 2005	South Shetland Islands
Pareledone felix Allcock et al., 2007	South Shetland Islands
Pareledone framensis Lu and Stranks, 1994	Prydz Bay
Pareledone panchroma Allcock, 2005	South Shetland Islands
Pareledone prydzensis Lu and Stranks, 1994	Prydz Bay
Pareledone serperastrata Allcock, 2005	South Shetland Islands
Pareledone subtilis Allcock, 2005	South Shetland Islands

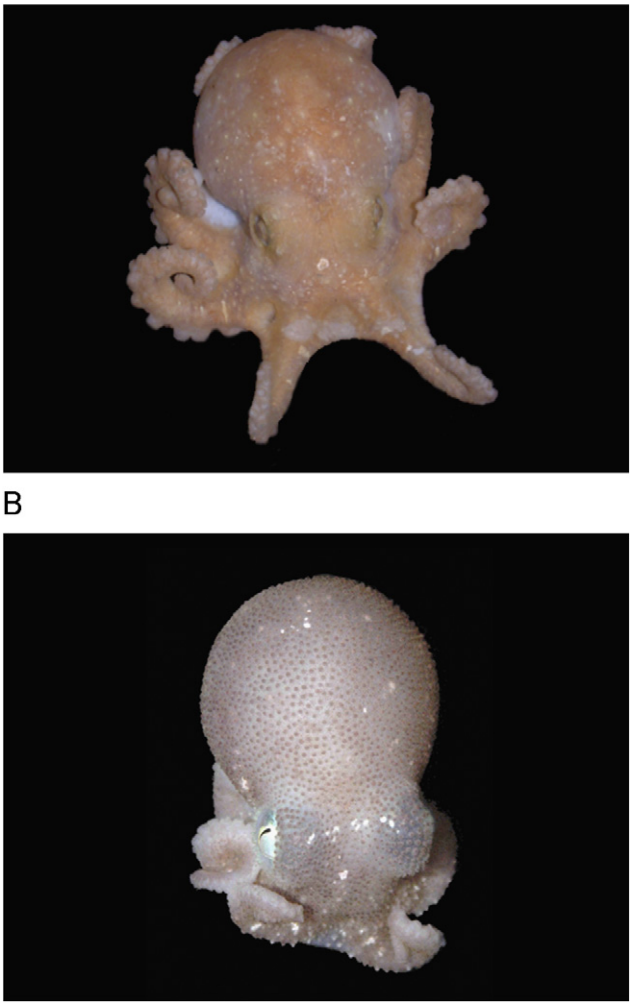

Fig. 1. (A) *Pareledone turqueti*, a 'smooth-skinned' species and (B) *Pareledone aequipapillae*, a 'papillated' species Part B reproduced with permission of Wiley-Blackwell from Allcock, 2005.

2. Methods

2.1. Specimen collection

Specimens were collected from 14 cruises over a period of 12 years, with particular collection effort during the International Polar Year (Table 2). Specimens were collected from eight general areas abbreviated as follows: SSh, the seabed around the South Shetland Islands including Elephant Island; SO, the South Orkney Islands; LB, the area formerly under the Larsen B ice shelf; WS; the eastern Weddell Sea; PB, Prydz Bay; AL, Adélie Land and George V

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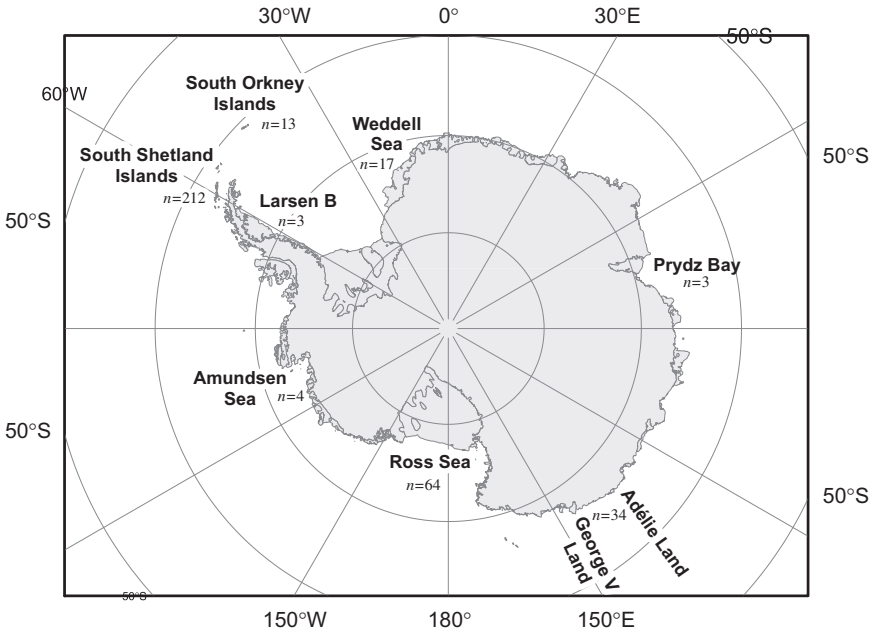

Fig. 2. Map of Antarctica showing general areas from which samples were collected during this study and number of samples collected.

Table 2
Details of cruises from which octopods were collected for this study.

Vessel	Cruise	Code	Location	Dates	Reference
Aurora Australis	CEAMARC: Collaborative East Antarctic Marine Census	2007/08 V3	Adélie Land, Eastern Antarctica	December 2007–January 2008	Beaman & O'Brien (2009)
Polarstern	EASIZ I: Ecology of the Antarctic Sea Ice Zone	ANT XIII/3	Weddell Sea	January 1996–March 1996	Arntz and Gutt (1997)
Polarstern	CCAMLR fish survey	ANT XIV/2	Elephant Island and South Shetland Islands	November 1996–January 1997	Kattner (1998)
Polarstern	EASIZ III: Ecology of the Antarctic Sea Ice Zone	ANT XVII/3	Weddell Sea and South Shetland Islands	February 2000–April 2000	Arntz and Brey (2001)
Polarstern	CCAMLR fish survey	ANT XIX/3	Elephant Island and South Shetland Islands	January 2002–April 2002	Fütterer et al. (2003)
Polarstern	LAMPOS (Latin American *Polarstern* Study)	ANT XIX/5	Scotia Sea and Magellan Region	April 2002–May 2002	Arntz and Brey (2003)
Polarstern	BENDEX: Benthic Disturbance Experiment	ANT XXI/2	Eastern Weddell Sea	November 2003–January 2004	Arntz and Brey (2005)
Polarstern	ANDEEP III: Antarctic Benthic Deep Sea Biodiversity	ANT XXII/3	Weddell Sea	January 2005–April 2005	Linse et al. (2007)
Polarstern	CCAMLR fish survey	ANT XXIII/8	South Shetland Islands	November 2006–January 2007	Gutt (2008)
James Clark Ross	BIOPEARL I (Biodiversity, Phylogeny, Evolution and Adaptive Radiation of Life in Antarctica)	JR147	Scotia Sea	February 2006–April 2006	Linse (2008)
James Clark Ross	BIOPEARL II (Biodiversity, Phylogeny, Evolution and Adaptive Radiation of Life in Antarctica)	JR179	Amundsen Sea	February 2008–April 2008	
Tangaroa	New Zealand IPY-CAML survey of the Ross Sea region, Antarctica		Ross Sea	February 2008–March 2008	
Tangaroa	Hydrographic and Biodiversity Survey	TAN0402	Western Ross Sea	January 2004–March 2004	
Aurora Australis	KROCK		Prydz Bay	January 2001	

Land; RS, Ross Sea and AS, Amundsen Sea (Fig. 1). Capture effort varied widely between locations. Three locations, the South Shetland Islands, the Ross Sea and Adélie Land, were intensively sampled with large trawls. At other locations, gears less suitable for octopod capture such as Agassiz trawls and epibenthic sleds were used, and octopod collection was more opportunistic. Handling of specimens varied depending on the cruise but generally a small piece of muscle tissue was removed from the octopus mantle and placed in 70–100% ethanol whilst the animal was either fixed immediately in 4% formalin, or frozen and later transferred to 4% formalin. These voucher specimens have been placed in a variety of museums and institutes. Full details of the repository of each specimen and station data associated with it can be found in the Barcode of Life Data System (BOLD, http://www.boldsystems.org), see (Ratnasingham and Hebert, 2007) listed in the project folder "Papillated Pareledone".

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2.2. Specimen identification

Specimens from cruises ANT XIII/3, ANT IV/2, ANT XVII/3, ANT XIX/3, ANT XXIII/8, JCR149 and JCR 179 were identified by the first author who is the sole recognised taxonomic authority on the group (see Allcock, 2005; Allcock et al., 2007). Geographic coverage of these cruises extended from the Weddell Sea in the east, across the Scotia Sea to the South Orkney Islands and South Shetland Islands and as far west as the Amundsen Sea.

2.3. Barcoding methodology

Laboratory procedures were conducted at the barcoding facility at Guelph. A sample of muscle tissue from each specimen was extracted using an automated Glass Fiber protocol (Ivanova et al., 2006). The 650 bp barcode region of COI was subsequently 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 reaction mixes included 6.25 μl of 10% trehalose, 2.00 μl of ultrapure water, 1.25 μl 10× 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 LCO1490/HCO2198 (Folmer et al., 1994) with M13 tails], 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 visualized on a 1.2% agarose gel E-Gel® (Invitrogen) and bidirectionally sequenced using sequencing primers M13F or M13R and the BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) on an ABI 3730 capillary sequencer following manufacturer's instructions.

Sequence data are available on both BOLD and GenBank (Accession nos. GQ843835–GQ844230). Sequences, and trace files are listed in the project folder "Papillated Pareledone" on BOLD.

2.4. Data analysis

To avoid the problems associated with missing data, alignments were trimmed and those sequences shorter than 540 base pairs were deleted. The resulting data matrix comprised 350 sequences of 541 base pairs of 69 different haplotypes. Average nucleotide frequencies were C (16.8%), T (38.0%), A (30.8%) and G (14.3%).

Sequences were tested for saturation by the number of substitutions (transversions and transitions) against corrected sequence divergence (Kimura two-parameter).

Haplotypes were assigned to a species group by reference to known haplotypes (where the voucher specimen was identified by the first author) and by reference to clades supported by neighbour-joining (NJ) analysis (not shown) and network analysis (see below).

A haplotype network was generated using TCS 1.21 (Clement et al., 2000). The network was constructed using statistical parsimony. The probability threshold that infers that character changes defining connections are due to a single mutation was increased from the default setting of 95% up to 99% in one percent increments.

Kimura two-parameter (K2P) distances were calculated for pairwise comparisons of sequence divergence within species and between species. The mean and range of K2P distances for intraspecific comparisons of each species and for interspecific comparisons were calculated.

For *P. aequipapillae*, the most abundant species (88 specimens) with the greatest number of haplotypes (12), occurring at the most locations (6), mean K2P distances were calculated between locations. Two matrices approximating to geographic distance between locations were also constructed. Marine distances are not simple as they are affected by topology, currents, etc., and the shortest linear distance may involve land. Therefore we used the number of degrees longitude between locations as a proxy for distance. In the first matrix, the shortest distance was recorded. In the second matrix, the shortest distance between two locations that did not involve passing through the warmer water (Clarke et al., 2009) to the west of the Antarctic Peninsula was recorded. Mantel tests based on ranks (Legendre and Lapointe, 2004) were used to test for significant relationships between mean genetic distance and the two geographic distance measures. Mantel tests were carried out in PRIMER (Clarke and Warwick, 1994), a computer package that calculates rank correlations between distance matrices as a measure of association (in the RELATE subroutine; Somerfield et al., 2002).

3. Results

Most haplotypes could be assigned to known species with some confidence, in part due to the large number of specimens identified by the first author. All specimens from the South Shetland Islands, South Orkney Islands, Weddell Sea, Larsen Ice Shelf and Amundsen Sea, comprising 249 out of 350 specimens in total, were identified by the first author. This included all specimens assigned to *P. charcoti*, *P. albimaculata*, *P. serperastrata*, *P. subtilis* and *P. felix*. In the network analysis, specimens identified as *P. felix* formed two separate clusters. The COI sequence of the holotype of this species (GenBank Acc no. EF102183; see Allcock et al., 2007) was used to identify *P. felix* sensu stricto. There is apparently previously unrecognized diversity within the taxon currently known as *P. felix*. Specimens from each haplotype of *P. cornuta* were identified by the first author. Haplotypes assigned to *P. panchroma* are widely spaced in the network analysis. Specimens from the South Orkney Islands (haplotype 61) and South Shetland islands (haplotype 58) were identified by the first author and other haplotypes were assigned to *P. panchroma* because of their association with haplotypes 58 and 61 in the network analysis (Fig. 3). The haplotypes assigned to *P. ?prydzensis* are also quite widely spaced and this grouping may comprise more than one species. Only the Weddell Sea specimens assigned to this species were examined by the first author. They are morphologically distinct from, but bear some resemblance to, the type specimen of *P. prydzensis*. These and the haplotypes that fall between them in the network analysis (Fig. 3) are tentatively grouped together. Also widely spaced are the haplotypes assigned to *P. aequipapillae*. However 36 of these specimens were identified by the first author including the most peripheral haplotypes and we are confident in this grouping. The identities of haplotypes 39, 44, 50 and 54 are uncertain. They were not identified by the first author, they do not cluster closely with known haplotypes in the network analysis, nor do they fall in highly supported clades with other haplotypes in an NJ tree (not shown).

Network analysis revealed these closely related species comprised a single network under the default 95% probability threshold (Fig. 3). With a 96–98% probability threshold, the network split into three parts. One network comprised *P. felix*, *P. charcoti* and *P. aequipapillae*. The South Orkney Island haplotype of *P. panchroma* was separate from all other haplotypes. The largest network comprised all other species/haplotypes. With a 99% probability threshold, the network split into 18 parts. The connections that broke under this scenario are illustrated with dashed lines in Fig. 3. There is some correspondence with species boundaries (e.g., for *P. cornuta*, *P. charcoti* and *P. subtilis*).

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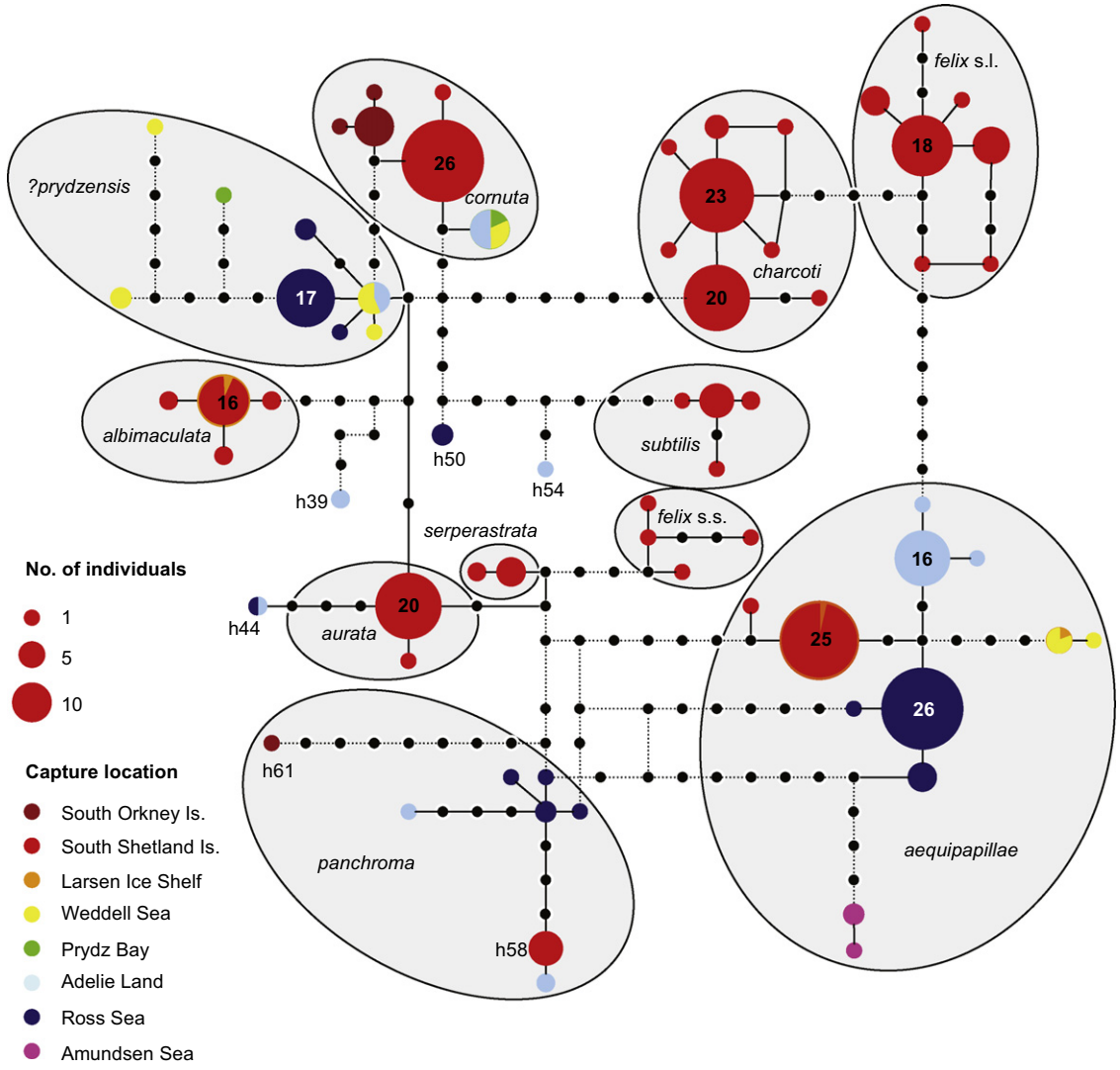

Fig. 3. A haplotype network generated using maximum parsimony in TCS 1.21 (Clement et al., 2000) with default settings including a 95% probability threshold that infers that character changes defining connections are due to a single mutation. Dashed lines indicate network connections that break under a 99% probability threshold (see text). Small black circles indicate absent haplotypes. Size of circles indicates number of specimens of each haplotype. Abundances greater than ten are given within the circle. Haplotypes referred to in text indicated by *hx*, where *x* is the haplotype number.

The network reveals no overlap of haplotypes between species. Except in a few cases, haplotypes tended to be restricted to a particular geographic location. Where haplotypes were present at two or more locations, these locations were always adjacent. Additionally, haplotypes from any one location for a given species tended to cluster together in the network. These findings indicate restricted geneflow between locations. Only three specimens from the area of the Larsen B ice shelf were barcoded. None of the haplotypes was unique to this area. Two were shared with the South Shetland Islands and the third was shared with the Weddell Sea.

Weddell Sea haplotypes of the *P. ?prydzensis* grouping are widely divergent which suggests there may be more than one species present in this grouping.

Ten of the eleven species identified occurred around the South Shetland Islands. Six species, *P. aurata*, *P. charcoti*, *P. felix*, *P.* cf. *felix*, *P. serperastrata* and *P. subtilis*, were found only here, while the somewhat disparate grouping tentatively identified as *P. ?prydzensis* was not found here. The remaining four species all appeared to have extended geographic distributions.

The divergence of haplotypes within those species with a broad geographic spread (e.g., *P. aequipapillae*) is as great as the apparent divergence between some species pairs. This is emphasized by the large overlap between intra- and interspecific distances in the genus *Pareledone* (Fig. 4). However, on average, interspecific distances are approximately eight times greater than intraspecific distances.

For *P. aequipapillae*, Mantel tests revealed no significant congruence between mean genetic distances (K2P) between locations and the shortest distance between locations ($rho=0.099$ and $P=0.392$). There was, however, significant congruence between mean genetic distance between locations and the shortest distance between them avoiding the warm water to the west of the Antarctic Peninsula ($rho=0.498$ and $P=0.017$).

4. Discussion

The present study represents one of the largest barcoding studies conducted on a Southern Ocean genus to date. COI, a relatively fast evolving gene, revealed a network of closely related species, suggesting recent, rapid cladogenesis. Cryptic speciation, extended ranges and a circumpolar distribution of at least one species is detected. The study shows that DNA barcoding can be a

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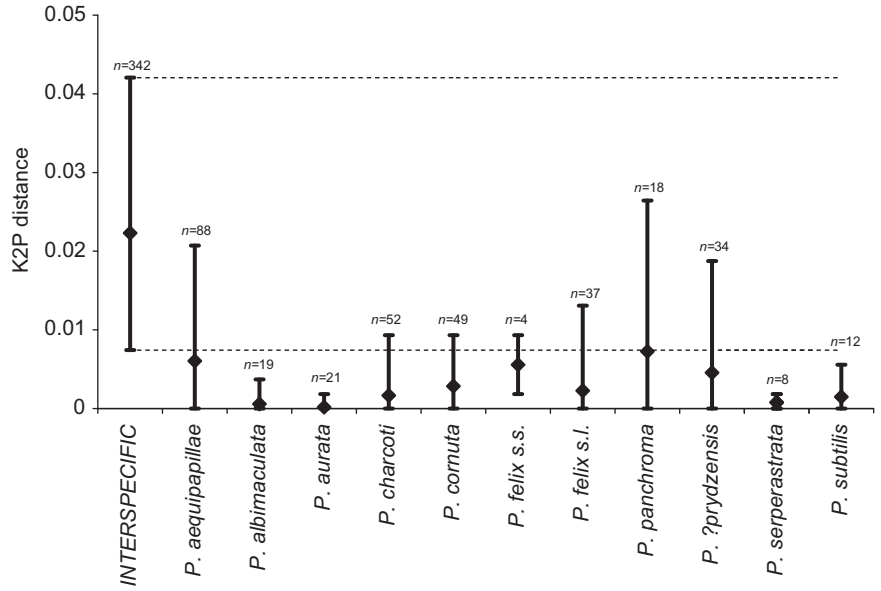

Fig. 4. Intraspecific versus interspecific variation in *Pareledone*. Mean and range of *D*, Kimura two parameter distance (Kimura, 1980). Four haplotypes unassigned to species were excluded.

useful identification tool for *Pareledone* species when a reference set of sequences is available, yet is limited in its use to help determine species boundary definitions.

Interspecific variation in COI sequences of *Pareledone* is small (mean 2.2% K2P, min. 0.7% and max. 4.2%) compared to that in decapod crustaceans (mean 17.16%, min. 4.92% and max 23.66%) (Costa et al., 2007), the amphipod genus *Gammarus* (mean 25.33, min. 5.58% and max. 31.39%), (Costa et al., 2007) and fish (mean 8.11%, min. 9% and max. 24.18%) (Ward et al., 2009). However, recent studies on the rockfish genus, *Sebastes* (Steinke et al., 2009b), also show very low levels of interspecific variation in COI (mean 3.4%), with different sympatric species sharing very similar or identical barcodes. Steinke et al. (2009b) suggested incomplete lineage sorting and introgressive hybridisation as potential reasons for this observation.

Intraspecific variation in *Pareledone* is also very small, with mean values between 0.08% (*P. serperastrata*) and 0.77% (*P. subtilis*), yet in all cases members of each species group together in the haplotype network. Similarly, Ward et al. (2005) showed that each tuna (*Thunnus*) species clustered as a separate assemblage (no individuals were misplaced), but genetic differences were also very small within species with a mean K2P distance of 0.11%. Both *Thunnus* and *Sebastes* have been suggested to have diverged relatively recently (Elliot and Ward, 1995; Steinke et al., 2009b) and this may also be the case for *Pareledone* which clearly consists of closely related species (Allcock, 2005).

With a nearly 10-fold difference between mean interspecific and mean intraspecific variation in *Pareledone*, barcoding should theoretically prove useful for identification in this genus (Hebert et al., 2004b; Zemlak et al., 2009), and indeed all species examined in this study possess COI sequences that permit their separation from any other taxon included in this study. However, the range of intraspecific variation overlaps greatly with the range of interspecific variation and varies greatly among species (Fig. 4). This means that where the intraspecific diversity of a species is not known, it cannot be implied from data for other species and, clearly, the current common practice in large-scale barcode sampling to collect five specimens per species is insufficient to assess the intraspecific diversity within this genus. Nonetheless, the barcoding technique has revealed hidden diversity in this group and is therefore proving to be extremely useful as a taxonomic tool.

Cryptic speciation was detected within *P. felix*. Further traditional taxonomic work will now be required to elucidate morphological characters than distinguish *P. felix* s.s. and *P.* cf. *felix*. Cryptic speciation based on genetic evidence has recently been reported for a number of Antarctic invertebrate species, including the pycnogonid *Colossendeis megalonyx* (Krabbe et al., 2010), the crinoid *Promachocrinus kerguelensis* (Wilson et al., 2007), the isopod *Glyptonotus antarcticus* (Held and Wägele, 2005), the brittle star *Astrotoma agassizii* (Hunter and Halanych, 2008) and asellote isopods (Raupach and Wägele, 2006). Although some studies deal with vicariance due to geographic barriers (e.g., Hunter and Halanych, 2008), others have revealed sympatric cryptic species to be present in the Weddell Sea (Held and Wägele, 2005; Raupach and Wägele, 2006), at Bouvet Island (Krabbe et al., 2010), and at the South Sandwich Islands and South Shetland Islands (Wilson et al., 2007; Krabbe et al., 2010). In a study of the sea slug *Doris kerguelensis*, Wilson et al. (2009) recovered 29 separate haplotype networks based on a parsimony analysis of COI sequences. Many of these 'species' occurred sympatrically, the most speciose area being the Bransfield Straight (between the South Shetland Islands and the Antarctic Peninsula) which yielded haplotypes from 17 of these clades. The authors suggest that their results represent a recent explosive radiation.

The present study suggests that the South Shetland Islands is the most speciose region for the genus *Pareledone*. Of the three heavily sampled areas, the South Shetland Islands boast nine species, Adélie Land seven and the Ross Sea five. Several authors (Allcock et al., 2001; Thatje et al., 2005) have suggested glacial cycles may have promoted speciation in Antarctica, whereby certain areas have acted as refugia during glacial maxima. Since most *Pareledone* species are restricted to limited depth ranges (Allcock, 2005), the fragmented nature of available habitats around the South Shetland Islands archipelago off the Antarctic Peninsula might also act to favour speciation via population fragmentation. However, sampling effort has not been equal in our study. It is not known how the efficiencies of the capture gears used at each location compare, but it appears that the

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CCAMLR fish surveys around Elephant Island in the South Shetland Islands yield more specimens (Kattner, 1998; Fütterer et al., 2003; Gutt, 2008). It is therefore much more likely that rarer species have been captured from the South Shetland Islands than from other areas. Also, some species, e.g. *P. charcoti* are restricted to shallow waters (mostly less than 100 m around the South Shetland Islands). These depths have been hardly sampled at other areas so the absence of *P. charcoti* from other areas might be an artifact of sampling. Similarly, Griffiths et al. (this issue) shows that the South Shetland Islands region contains the highest number of species for many Southern Ocean taxa, but also demonstrates that this area is also often sampled more heavily than other regions around Antarctica. Clearly, despite the progress made by the Census of Antarctic Marine Life, further survey work is required to fully understand the distributions of these species.

The presence of some *Pareledone* species in multiple locations is indicative of circumpolarity. This particularly appears to be the case for *P. aequipapillae* (collected from Adélie Land, the Ross Sea, the South Shetland Islands, the Amundsen Sea, the Weddell Sea and the Larsen Ice Shelf). It must also be noted that true absences of species in a particular area are very difficult to confirm without an extremely comprehensive sampling program. Hemery and Eléaume (unpublished data) found that at least four of the clades of the crinoid *Promachocrinus kerguelensis* (clades C, D, E and F) described by Wilson et al. (2007) from the South Shetland Islands, are also present in the Adélie Land—George V Land area. This quasi circumpolarity in *Promachocrinus* is congruent with *Pareledone* results. Circumpolar distributions have often been listed as a characteristic of the Southern ocean fauna (Arntz et al. 1997), yet apparent circumpolar species are not often genetically homogenous (Rogers, 2007). Similarly, restricted geneflow was detected between locations within species in the present study (Fig. 3). Restricted geneflow is not unexpected, given that all these *Pareledone* species have large (10–20 mm) eggs (see summary table in Barratt et al., 2008).

Although most *Pareledone* species were not shown to have a circumpolar distribution this study has increased the known range for several species. Ignoring the cluster of haplotypes (Fig. 3) tentatively identified as *P. ?prydzensis*, since it is uncertain whether these comprise a single species, several other species have extended distributions.

Distinct morphological variation is apparent between specimens from different locations in some species. *Pareledone cornuta* was identified in samples from the South Orkney Islands during cruise JR149 (Table 2) of the *RRS James Clark Ross* (Strugnell and Allcock, unpublished data) and, although recognizable as *P. cornuta*, specimens had much larger and more distinct papillae than specimens from the South Shetland Islands. Prior to this study, *P. cornuta* was also recognised as being present in the Weddell Sea using molecular markers (Strugnell, unpublished data), and these specimens showed even greater morphological differences to those at the South Shetland Islands (Allcock, unpublished data) to the extent that they were initially thought to comprise a new species. This raises questions as to the validity of species described from widely spaced geographic locations around the Antarctic continent. None of our clades was identified as *P. aurorae* or *P. framensis*. Are these species from Eastern Antarctica conspecific with (but morphologically distinguishable from) specimens from Western Antarctica? Unfortunately, the paucity of specimens from their type localities means that this question cannot be answered by the present study.

Not all species show large variations in their morphology across their range, however. Specimens of *P. aequipapillae* were examined morphologically from the South Shetland Islands, the area previously under the Larsen B ice shelf, the Weddell Sea and the Amundsen Sea, and all specimens were remarkably similar. This is even more striking when the wide diversity of haplotypes is considered (Fig. 3). Similarly, specimens of *P. panchroma* from the South Shetland Islands and from the South Orkney Islands had no obvious morphological differences.

Analysis of the haplotype diversity of *P. aequipapillae* revealed congruence between the genetic dataset and a hypothesized scenarios whereby warmer waters around the west of the Antarctic Peninsula act as a barrier to gene flow in this species. In the area south of the South Shetland Islands extending towards the Bellinghausen and Amundsen Seas, the sea bed is warmed by Circumpolar Deep Water and is as much as 2° warmer than the sea-bed temperatures found in the Weddell and Ross Seas and 1.5° warmer than elsewhere around the continent (Clarke et al., 2009). It is possible that papillated *Pareledone* do not tolerate these conditions. Indeed, papillated *Pareledone* are absent from South Georgia, where the sea bed is the warmest in the Southern Ocean, despite the presence there of other octopus species endemic to the Southern Ocean (Collins et al., 2004). In their absence, there would be no gene flow since these species do not have a planktonic dispersal phase. The apparent lack of gene flow between the South Shetland Islands and the Amundsen Sea suggests that some species of *Pareledone* may be exhibiting evolutionary patterns similar to those seen in ring species (e.g., Irwin et al., 2005). Whilst the barrier to geneflow at the ends of the ring might be physical, specimens of *P. aequipapillae* from the Amundsen Sea and South Shetland Islands might be sufficiently diverse to be reproductively isolated. Certainly there is greater separation of these haplotypes in the parsimony network (Fig. 3) than there is between the closest haplotypes of some pairs of sympatric species.

5. Conclusion

The evolutionary pathways of *Pareledone* are complex and barcoding has revealed insights into potential speciation processes that could not have otherwise have been discerned. High species diversity in the South Shetland Islands indicates that this area might be a hotspot for speciation. The South Shetland Islands harbour *Pareledone* species that appear to be endemic to these islands as well as species with circumpolar distributions and species with intermediate geographic ranges. Evidence of cryptic speciation has also been found in this area.

This study has established that most *Pareledone* species possess a tightly cohesive array of barcode sequences distinct from that of any other species representing a starting point for an all cephalopod barcoding effort as recently proposed (Strugnell and Lindgren, 2007).

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