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The identity of juvenile Polynoidae (Annelida) in the Southern Ocean revealed by DNA taxonomy, with notes on the status of *Herdmanella gracilis* Ehlers *sensu* Augener

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Abstract

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Using molecular data (CO1, 16S and H3 genes), we provide evidence for a long-held view that Southern Ocean scaleworms (Polynoidae) morphologically agreeing with *Herdmanella gracilis* sensu Augener, 1929 Ehlers sensu Augener are in fact juveniles of another species. The problematic genus *Herdmanella* is declared a *nomen dubium*. Importantly, at least two species were identified; one adult counterpart is a common circumpolar species, *Austrolaenilla antarctica* Bergström, 1916, and the other is of an as yet unknown identity. More adult counterparts are likely to be discovered with greater sequencing effort and larger taxon coverage. We have discovered a great genetic diversity within the *A. antarctica* clade in the CO1 gene, and future studies may elucidate if this represents a cryptic species. Currently, we adopt a conservative approach and suggest that given low diversity in mt16S and complete identity in H3 genes, this clade represents a single species, with only the specimen from South Georgia likely deserving the status of a cryptic species, as shown by haplotype network analysis. High mtDNA diversity in populations of Antarctic scaleworms may be linked to habitat fragmentation during recent glacial periods. Our study also highlights the importance of identifying juvenile specimens correctly in order to understand ecological processes such as the apparent high productivity in the Amundsen Sea region.

Keywords

Antarctica, Polychaete, DNA barcoding, marine diversity, population genetics, deep sea, cryptic species

Introduction

Exploration of our still largely unknown oceans continues to yield new taxonomic discoveries, mostly resulting in descriptions of new species. However, increased collecting effort, combined with new molecular tools, also provides an opportunity to address longstanding problems in taxonomy. Molecular taxonomy in general, and 'DNA barcoding' (Hebert et al., 2003) in particular, has grown quickly as a discipline in the past decade. Despite important problems with this approach (e.g. Meier et al. 2008; Collins and Cruickshank, 2012; Bergsten et al., 2012; Srivathsan and Meier, 2012), DNA barcoding has become an important tool in a diverse range of biological disciplines. In taxonomy, it has been primarily implemented for problems of species identification and species delimitation (see e.g. Monaghan et al., 2006; Vogler and Monaghan, 2007; Hamilton et al. 2011). Additionally, DNA barcoding has proved successful in linking different developmental stages of the same species in a wide range of animal taxa, such as shrimps (Shank et al., 1998), beetles (Ahrens et al., 2007), marine invertebrates (Webb et al., 2006; Heimeier et al., 2010; Bracken-Grissom et al., 2012) and fish (Pegg et al., 2006; Valdez-Moreno et al., 2010).

In the marine environment, plankton and nekton collections commonly include larval and juvenile developmental stages that differ from their adult counterparts in their morphology. Early taxonomists, often unaware of the existence of the larval stages, sometimes misidentified these morphologies as independent adult lineages (Bracken-Grissom et al., 2012). Past approaches to these problems included rearing experiments in aquaria (Richards and Saksena, 1980; Haynes, 1982) or *in situ* (Haynes, 1979). However, this approach is not always practical and requires a collection of live larvae. Often, if specimens were primarily collected for other types of studies, such as biodiversity surveys, they may not have been collected live and a molecular approach therefore represents an alternative tool for identification of larval stages.

For over 100 years, the polynoid species *Herdmanella gracilis* Ehlers, 1908 presented a problem for polychaete taxonomists. Ehlers (1908) described this species upon examination of a number of specimens collected from deep water (1500–2000 m) off the coast of East Africa (in the Indian Ocean) during the *Valdivia* expedition 1898–99. It is not clear if a type specimen of this species was deposited, but no types are known to exist (Pettibone, 1976). Given the small size of this species (1.5 mm long, 15 segments, 8 pairs of elytra), it has long been suggested that it represents a juvenile form of an otherwise benthic species. Ehlers (1908) expressed some reservations: "… it is not impossible (even though in my opinion improbable) that it is a juvenile of a known species …", but proceeded with formal description anyway. His decision to assign this species to the genus *Herdmanella* Darboux, 1899 was never properly explained. Later, Monro (1930) suggested *H. gracilis* could be a juvenile stage of the polynoid *Antinoë pelagica*, now known as *Austrolaenilla pelagica* Monro (1930) from the Southern Ocean. Hartmann-Schröder (1974) considered *Herdmanella gracilis* to be the juvenile of a species related to the polynoid genus *Harmothoe*, a view supported by Pettibone (1976), who considered *H. gracilis* to be a doubtful species belonging to the subfamily Harmothoinae (now Polynoinae, see Muir, 1982). Once recognised as a valid species, Augener (1929) identified small polynoids from the Weddell Sea as *Herdmanella gracilis,* and in doing so expanded its range to the Southern Ocean, proposing that it could be a very widespread form that can presumably live in the tropical deep sea as well as the shallow high-latitude regions. This resulted in *H. gracilis* being listed in *Polychaeta Errantia of Antarctica*, an atlas compiled by Hartman (1964).

Further, the new species *Herdmanella aequatorialis* Støp-Bowitz, 1991 has been described from off West Africa (equatorial Atlantic Ocean) at 300 m depth. Støp-Bowitz (1991) recognised that, given the small size of the single, damaged specimen, it may be a juvenile, but unable to assign it to any other known genera, and given its similarity to *H. gracilis*, he proceeded with erecting a new species in the genus *Herdmanella*. The two species *H. gracilis* and *H. aequatorialis* are currently regarded as the only valid species in *Herdmanella*, although this itself is a problematic genus (its status is addressed in the Discussion section of this paper). The other species previously referred to this genus, *Herdmanella nigra* Hartman, 1967, has been transferred to *Bathyeliasona* by Pettibone 1976.

As part of the 2005 BIOPEARL I expedition to the Scotia Sea (Linse, 2008) and the 2008 BIOPEARL II expedition to the Amundsen Sea in west Antarctica (fig. 1, table 1), a large number of polychaete worms were collected (Linse et al., 2013; Neal et al., in prep.). Polynoidae were particularly abundant in the Amundsen Sea ($n > 5000$) and were represented by 23 species. Currently there are about 66 recognised polynoid species in the Southern Ocean (WoRMS, 2013), and hence the Amundsen Sea collection represents a reasonable coverage of the polynoid diversity of this region. A large number (>2000) of these individuals were small polynoids either considered to be indeterminable juveniles or to be representatives of the small-sized species *Herdmanella gracilis* (based on locality we consider these *Herdmanella gracilis* Ehlers sensu Augener, 1929, rather than Ehlers, 1908). As specimens were preserved both for molecular studies in ethanol and for morphological studies in formaldehyde, there was an opportunity to use molecular taxonomy methods to analyse the validity of the species determination of *H. gracilis*, to examine the genetic heterogeneity in populations, and to commence investigation of the ecological significance of this abundance of juvenile Polynoidae.

Methods

Field methods

The macrobenthic samples were collected by epibenthic sledge (EBS) during the BIOPEARL I and II expeditions with RRS *James Clark Ross* (JR144 and JR179) (fig. 1, table 1). The EBS (for a detailed description see Brenke (2005)) consists of an epi- (lower) and a supra- (upper) net, each with an opening of 100-cm width and 33-cm height, 500-μm mesh size on the sides and ending in cod ends with a mesh size of 300 μm. The EBS was hauled over the seabed at 1 knot for 10 min. On deck, the samples of the first 1000-m and 1500-m and the first two 500-m EBS hauls per station were immediately fixed in 96% pre-cooled ethanol and kept for 48 h in –20°C for later DNA extraction, while the samples of the remaining 500-m, 1000-m and 1500-m EBS hauls per station were fixed in 4% formaldehyde for morphological analysis.

Morphological investigation

Where possible, live specimens were examined aboard ship, with sorted samples being preserved individually and images taken on ship with a Nikon Coolpix camera mounted on a Leica stereo microscope. In the laboratory, Leica MZ6 and DM5000 stereo and compound microscopes were used to further identify polynoid specimens. Images of these specimens were captured using a Zeiss V.20 and AxioCam HRc, and a Leica DFC 480 dedicated camera system connected to the DM5000.

Molecular work

In total, DNA was extracted from 33 ingroup specimens. Eleven specimens morphologically agreed with *Herdmanella gracilis* Ehlers sensu Augener, 1929, 19 with *Austrolaenilla antarctica*, three with *A. pelagica* (table 2). Eight outgroup sequences were included (table 2.) Five outgroup sequences were obtained from GenBank and three were generated as a part of wider polynoid study (Wiklund et al., in prep.). Based on the availability of sequences, we included other species currently in the subfamily Polynoinae (*Harmothoe fuligineum* (Baird, 1865), *Harmothoe oculinarum* (Storm, 1879), *Bylgides groenlandicus* (Malmgren, 1867), *Antarctinoe ferox* (Baird, 1865), *Malmgrenia mcintoshi* (Tebble & Chambers, 1982)), as well representatives of three other subfamilies (*Macellicephala violacea* (Levinsen, 1887), *Eulagisca gigantea* Monro, 1939, and *Lepidasthenia berkeleyae* Pettibone, 1948).

DNA was extracted from parapodia with a DNAeasy Tissue Kit (Qiagen) following the protocol supplied by the manufacturer.

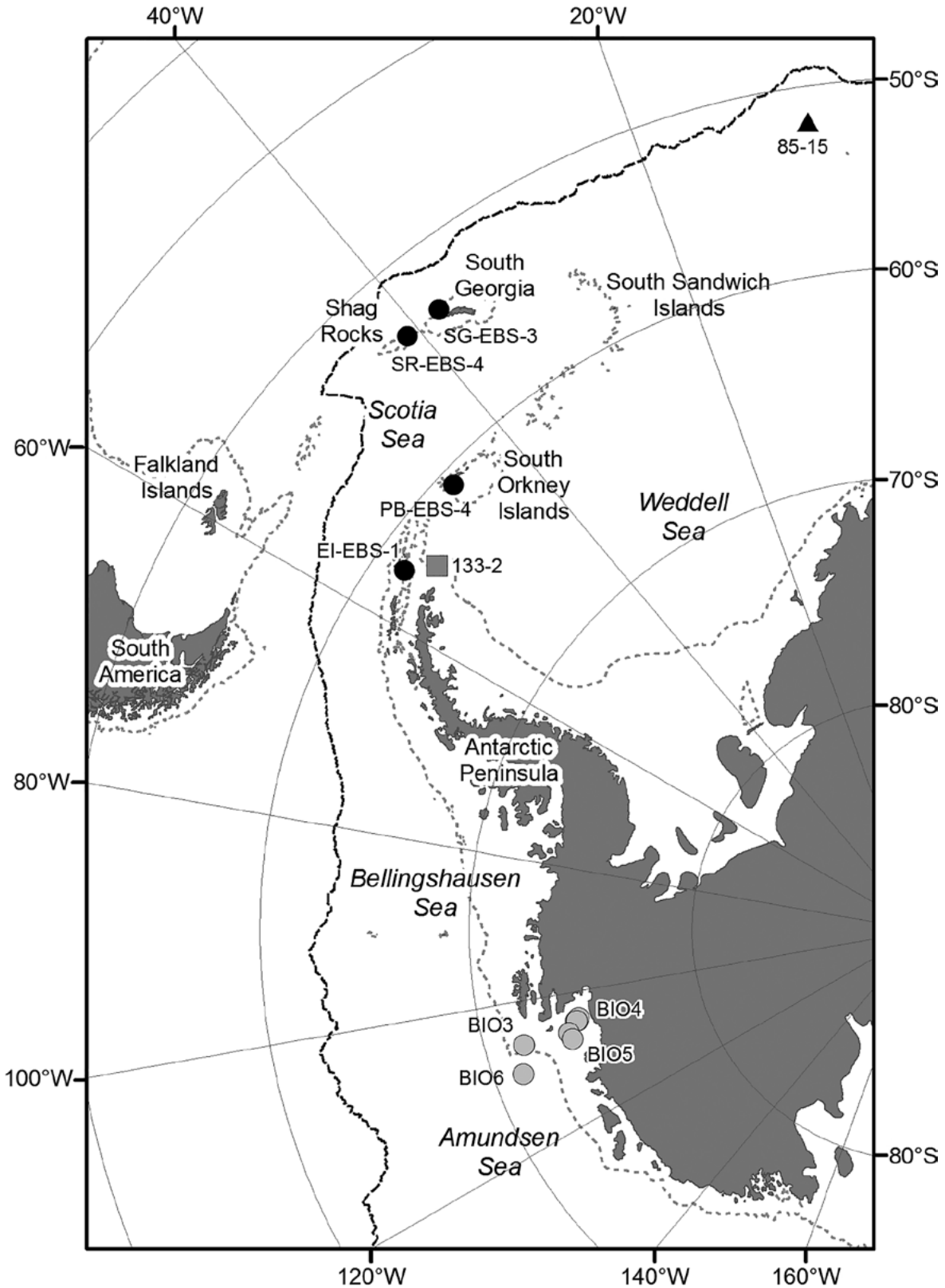

Figure 1. Map showing sampling localities. Black circles refer to BIOPEARL I samples, grey circles to BIOPEARL II samples, grey square to ANDEEP III samples and black triangle to ANDEEP-SYSTCO samples.

Table 1. Details of sampling stations used in this study. Latitude and longitude are provided for the ship's location when the sampling device first landed on the seafloor.

Cruise	Locality	Station	Depth (m)	Latitude	Longitude
ANDEEP III	Weddell Sea (WS)	133	1580	62° 46' 44" S	53° 2' 34" W
ANDEEP - SYSTCO	Weddell Sea (WS)	85-15	2752	52° 0' 28" S	8° 0' 13" W
BIOPEARL I	South Georgia (SG)	SG-EBS-3	500	53° 35' 51" S	37° 54' 11" W
BIOPEARL I	Elephant Island (EI)	EI-EBS-1	1500	61° 36' 43" S	55° 13' 3" W
BIOPEARL I	Powell Basin (PB)	PB-EBS-4	500	60° 49' 18" S	46° 29' 6" W
BIOPEARL I	Shag Rocks (SR)	SR-EBS-4	200	53° 37' 41" S	40° 54' 28" W
BIOPEARL II	Amundsen Sea BIO3	BIO3-EBS-1B	500	71° 47' 29" S	106° 12' 50" W
BIOPEARL II	Amundsen Sea BIO3	BIO3-EBS-1C	500	71° 47' 9" S	106° 12'27" W
BIOPEARL II	Amundsen Sea BIO4	BIO4-AGT-1B	1500	74° 21' 28" S	104° 43' 50" W
BIOPEARL II	Amundsen Sea BIO4	BI04-EBS-3E	500	74° 23' 46" S	104° 45' 28" W
BIOPEARL II	Amundsen Sea BIO4	BIO4-EBS-1A	1500	74° 21' 35" S	104° 44' 45" W
BIOPEARL II	Amundsen Sea BIO4	BIO4-EBS-2B	500	74° 29' 16" S	104° 19' 58" W
BIOPEARL II	Amundsen Sea BIO4	BIO4-EBS-3D	500	74° 23' 27" S	104° 46' 2" W
BIOPEARL II	Amundsen Sea BIO5	BIO5-EBS-2A	1000	73° 52' 49" S	106° 18' 59" W
BIOPEARL II	Amundsen Sea BIO5	BIO5-EBS-3A	500	73° 58' 19" S	107° 25' 22" W
BIOPEARL II	Amundsen Sea BIO6	BIO6-EBS-3D	500	71° 20' 56" S	109° 57' 53" W

Three genes were targeted: the 'barcoding' mitochondrial (mt) protein-coding gene CO1, the mt non-coding 16S and the nuclear (n) protein-coding H3 gene. About 650 bp or 350 bp of CO1, 500 bp of 16S and 300 bp of H3 were amplified using primers listed in table 3. PCR mixtures contained 1 μl of each primer (10μM), 2 μl template DNA and 21 μl Red Taq DNA Polymerase 1.1X MasterMix (VWR) in a mixture totalling 25 μl. The temperature profile was as follows: 96°C for 240 s, followed by (94°C for 30 s, 48°C for 30 s then 72°C/60 s)*35 cycles, followed by 72°C for 480 s. PCR purification was performed using a Millipore Multiscreen 96-well PCR Purification System, and sequencing was performed on an ABI 3730XL DNA Analyser (Applied Biosystems) at the Natural History Museum Sequencing Facility, using the primers mentioned above. Overlapping sequence fragments were merged into consensus sequences using Geneious (Drummond et al., 2007). CO1 and H3 sequences were aligned using MUSCLE (Edgar, 2004) with default settings, while 16S sequences were aligned using MAFFT (Katoh et al., 2002) with default settings, both programs provided as plug-ins in Geneious. The program jModelTest (Posada, 2008) was used to assess the best model for each partition (CO1, 16S and H3) with BIC, which suggested GTR+I+G as the best model for all of the genes. The data was partitioned into the three parts (16S, H3, COI), the evolutionary model mentioned above was applied to each partition and corresponding codon position. The parameters used for the partitions were unlinked. Bayesian phylogenetic analyses (BAs) were conducted with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Analyses were run three times with the CO1 separate dataset, and with the CO1, H3 and 16S combined dataset, for 10,000,000 generations. Of these, 2,500 000 generations were discarded as burn-in. Haplotype networks using statistical parsimony (Templeton, 1992) were constructed with the program TCS (Clement et al., 2000). In total, 28 CO1 sequences of *Austrolaenilla antarctica* and *Herdmanella gracilis* were cut to the same length of 320 bp. As part of data exploration, different statistical limits ranging from 90% (the lowest limit available in TCS) to 95% were employed. The distances among CO1 sequences were calculated in MEGA version 5.0 (Tamura et al., 2011) and expressed as K2P distance (uncorrected p-distances were also calculated, but the results were very similar) for the purpose of comparison with other studies.

Results

Morphology

During the morphological investigation, a large number of (>2000) small polynoid specimens (fig. 2a and b) were found and at first considered to be indeterminable juveniles (fig. 2c). Upon closer examination, two morphotypes were distinguished: those with cephalic peaks (fig. 3a) and those without cephalic peaks (fig. 3b). No other morphological differences were found between these two morphotypes using light microscopy. The cephalic peaks were not reported in the descriptions either by Ehlers (1908) or Augener (1929); therefore, only specimens without cephalic peaks were assigned to *Herdmanella gracilis*.

Table 2. Taxa studied, outgroups, DNA identification, collection sites, haplotype ID, Clade ID and NCBI GenBank accession numbers. Voucher numbers are available through the GenBank website.

Morphology ID	DNA ID	Locality	Site	Depth (m)	Haplotype #	Clade	COI	16S	H3
Austrolaenilla antarctica	*A. antarctica*	Amundsen Sea	BIO3-1	500	14	2	KJ676619	n/a	n/a
"	"	"	"	"	19	2	KJ676624	n/a	n/a
"	"	"	BIO4-1	1500	18	2	KJ676623	n/a	n/a
"	"	"	BIO4-3	500	1	3	KJ676612	KJ676606	KJ676637
"	"	"	"	"	1	3	"	n/a	n/a
"	"	"	"	"	1	3	"	n/a	n/a
"	"	"	"	"	1	3	"	n/a	n/a
"	"	"	"	"	1	3	"	n/a	n/a
"	"	"	"	"	1	3	"	n/a	n/a
"	"	"	"	"	3	3	KJ676613	n/a	n/a
"	"	"	"	"	4	3	KJ676614	n/a	n/a
"	"	"	"	"	5	3	KJ676615	n/a	n/a
"	"	"	"	"	6	3	KJ676616	n/a	n/a
"	"	"	BIO5-2	1000	16	2	KJ676621	KJ676606	KJ676637
"	"	"	BIO5-2	1000	17	2	KJ676622	n/a	n/a
"	"	Elephant Is.	EI-EBS-1	1500	15	2	KJ676620	KJ676606	KJ676637
"	"	South Georgia	SG-EBS-4	500	SG	1	KJ676631	n/a	n/a
"	"	Weddell Sea	138	1580	13	2	KJ676618	n/a	n/a
"	"	"	85-15	2752	13	2	"	n/a	n/a
Herdmanella gracilis	*A. antarctica*	Amundsen Sea	BIO4-3	500	2	3	KJ676625	n/a	n/a
"	"	"	"	"	1	3	KJ676612	KJ676606	KJ676637
"	"	"	"	"	1	3	"	n/a	n/a
"	"	"	"	"	7	3	KJ676626	n/a	n/a
"	"	"	"	"	8	3	KJ676627	n/a	n/a
"	"	"	"	"	9	3	KJ676617	n/a	n/a
"	"	"	"	"	10	3	KJ676628	n/a	n/a
"	"	"	"	"	11	3	KJ676629	n/a	n/a
"	"	"	"	"	12	3	KJ676630	KJ676606	KJ676637
"	juvenile indet.	Powell Basin	PB-EBS-3	500	n/a	B	KJ676636	KJ676610	KJ676641
"	juvenile indet.	Shag Rocks	SR-EBS-4	500	n/a	B	"	n/a	n/a
Austrolaenilla pelagica	*A. pelagica*	Amundsen Sea	BIO4-3	500	n/a	A	KJ676632	KJ676607	KJ676638
"	"	"	BIO5-3	500	n/a	A	"	n/a	n/a
"	"	"	"	"	n/a	A	"	n/a	n/a
Antarctinoe ferox	outgroup				n/a	outgroup	KJ676611	n/a	n/a
Bylgides groenlandicus	outgroup	GenBank			n/a	outgroup	HQ024272	n/a	n/a
Eulagisca gigantea	outgroup	Amundsen Sea			n/a	outgroup	KJ676633	KJ676608	KJ676639
Harmothoe fuligineum	outgroup	Amundsen Sea			n/a	outgroup	KJ676634	KJ676609	KJ676640
Harmothoe oculinarum	outgroup	GenBank			n/a	outgroup	AY894314	n/a	n/a
Lepidasthenia berkeleyae	outgroup	GenBank			n/a	outgroup	HM473443	n/a	n/a
Macellicephala violacea	outgroup	GenBank			n/a	outgroup	JX119016	n/a	n/a
Malmgrenia mcintoshi	outgroup	GenBank			n/a	outgroup	JN852935	n/a	n/a

Table 3. List of primers used in this study.

Primer	Sequence 5'–3'	References
16SF	CGCCTGTTTATCAAAAACAT	Palumbi (1996)
16SbrH	CCGGTCTGAACTCAGATCACGT	Palumbi (1996)
H3F	ATGGCTCGTACCAAGCAGACVGC	Colgan et al. (2000)
H3R	ATATCCTTRGGCATRATRGTGAC	Colgan et al. (2000)
LCO	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
HCO	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)
355R	GGGTAAACTGTTCATCCTGTTCC	Nylander et al. (1999)

Table 4. Within- and between-clade distances as measured by K2P, expressed as mean % (range in brackets).

	Within-clade distance	Between-clade distance					
		A	B	C	C1	C2	C3
A	0	–	–	–	–	–	–
B	0.45 (0.3–0.6)	15.4 (14.3–16)	–	–	–	–	–
C	2.9 (0–7.3)	18.3 (14.8–20.9)	14.4 (12.4–16.3)	–	–	–	–
C1	–	–	–	–	–	–	–
C2	2.5 (0.3–4.1)	–	–	–	6.7 (5.4–7.3)	–	–
C3	1 (0–3.5)	–	–	–	6.4 (5.4–7.1)	4.3 (2.9–5.1)	–

No morphological differences were found among individuals lacking cephalic peaks assigned to *H. gracilis*, and these were therefore assumed to belong to a single species, based on morphology. A short description of the juveniles initially thought to be *H. gracilis* is provided here.

Systematics

Polynoidae Malmgren, 1867

Juvenile, indeterminable

Figures 2, 3.

Material examined. Over 2000 specimens, from BIOPEARL I and II expeditions to the Amundsen Sea, Antarctic, in March 2006 and March 2008, cruise numbers JR144 and 179, station numbers listed in table 1, depth 500 m.

Description. Voucher material. Length excluding palps 1.5 mm, 14–15 segments, 8 pairs of elytra. Colour of preserved specimen white to creamy yellow, in live specimens anterior body translucent, the posterior body bright yellow to orange. Prostomium bilobed, rhomboid to oval, anterior lobes rounded but without cephalic peaks; 2 pairs of small, black, subdermal eyes present, anterior pair positioned medially at widest part of prostomium. 3 antennae; median antenna often missing and only large antennophore present, inserted distally on prostomium, two lateral antennae inserted anteroventrally on prostomium, styles short, slender, papillated. Pair of long (twice length of prostomium), thick, smooth palps present, narrowing distally. Proboscis when extended with 2 pairs of amber-coloured jaws and 9 pairs of small, equal-sized triangular papillae on the rim. Two pairs of tentacular cirri present, lateral to prostomium, styles slender, papillated, tentaculophores of similar size, tentacular segment with notochaetae, few, stout, serrated. Parapodia biramous, notopodia smaller than neuropodia with long, slender, papillated dorsal cirrus; notochaetae present in moderate numbers, stout, straw-like in colour, serrated, much shorter than neurochaetae; neuropodia with long, slender ventral cirrus inserted proximally; neurochaetae numerous, extremely long, thin, almost capillary-like, all unidentate. Elytra often missing, when present small, ovoid, translucent with rough surface, with sparse microtubercules only, some elongated papillae irregularly present on surface and fringe. Pygidium conical, anal cirri not observed.

Remarks. The specimens morphologically agree with the description of *Herdmanella gracilis* Ehlers, 1908; however, it was decided not to assign them to this species without a molecular assessment considering that the specimens are likely to be juveniles, and the type locality (East Africa) is far distant from the Amundsen Sea.

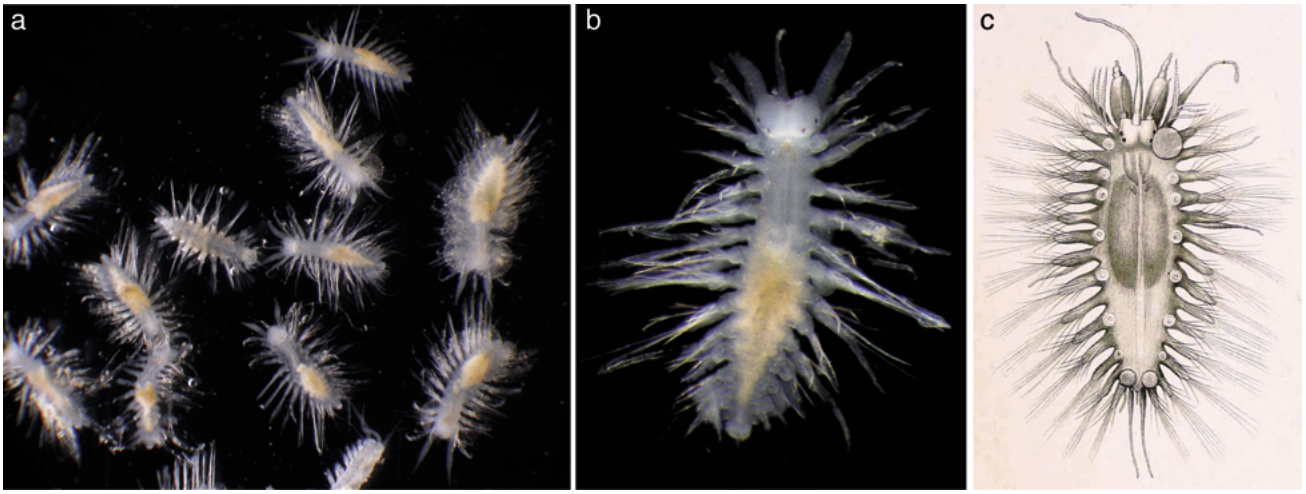

Figure 2. Juvenile Polynoidae: a, image of live specimens agreeing morphologically with *Herdmanella gracilis* Ehlers, 1908; b, detail of specimen; c, drawing of *H. gracilis* from the orginal description published by Ehlers (1908).

Molecular data

The results from the molecular phylogenetic methods based on the CO1 gene only (fig. 4) and on the combined analysis of CO1, 16S and H3 genes (fig. 5) suggest that *Herdmanella gracilis*-like specimens from the Southern Ocean represent juvenile stages of at least two species (clade B and C in fig. 4a). The identity of the juvenile specimens collected in the Powell Basin and at Shag Rocks (clade B in fig. 4a) remains unresolved; however, *Herdmanella gracilis*-like specimens from the Amundsen Sea (clade C) were a close match with adult *Austrolaenilla antarctica* Bergström, 1916, forming a well-supported monophyletic group. The *A. antarctica* clade forms three subclades (C1, C2 and C3), and CO1 diversity is high, with an average K2P distance of 2.9% (range 0–7.3%) (table 4). The changes were found in the third codon position and did not result in changes to amino sequences once translated. Additionally, mt16S and nH3 sequences were obtained for representatives of clades C2 (n = 2) and C3 (n = 3). In 16S, the genetic distance within clade C was reduced to <1% for all specimens, and in nuclear *H3* genes, their sequences were identical.

The 28 specimens in clade C, morphologically assigned to *Austrolaenilla antarctica* and *Herdmanella gracilis* represented 20 haplotypes (table 2). Only seven specimens (five of *A. antarctica* and two of *H. gracilis*) belonged to the same haplotype (no. 1), and all of these were from the Amundsen Sea station BIO4, 500 m depth (table 2). Two specimens from the deep Weddell Sea shared one haplotype, no. 13. The rest of the specimens were all unique haplotypes. A single haplotype network was not recovered using a 90% connectivity limit (11 steps), the lowest limit available in the TCS program. These settings in TCS resulted in the South Georgian haplotype not connecting to the main network formed by all other haplotypes, no. 1–19 (fig. 6a). The same result was obtained using a 91% connectivity limit (results not shown). Ultimately, increasing the parsimony limit to 95% (seven steps) resulted in a breakdown into five haplotype networks (fig. 6b). Three of

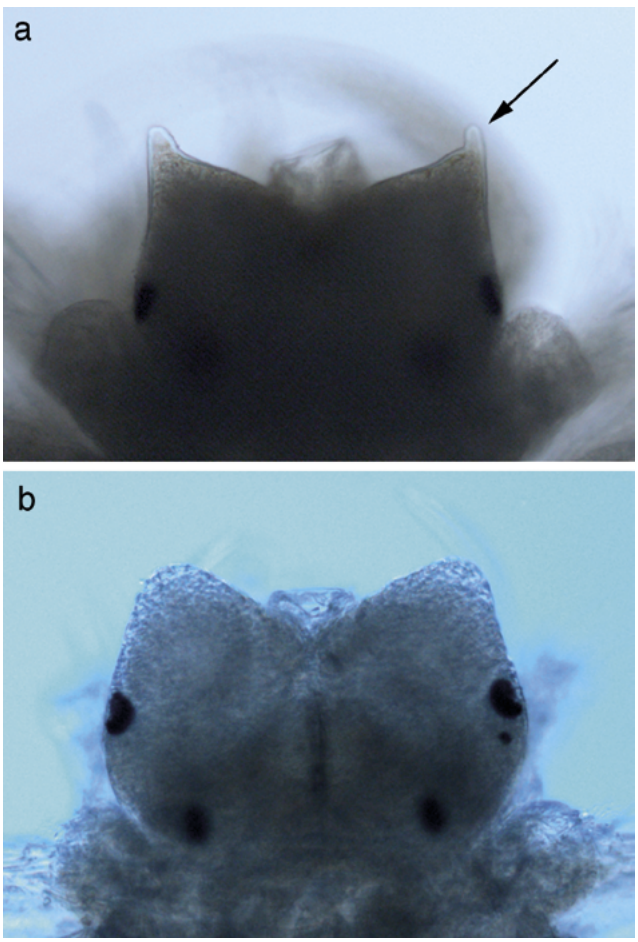

Figure 3. Presence of cephalic peaks in juvenile polynoids: a, type 1 juvenile of *Harmothoe fuligineum*—cephalic peaks clearly present (arrowed); b, type 2 juvenile—cephalic peaks absent, consistent with *H. gracilis* Ehlers, 1908.

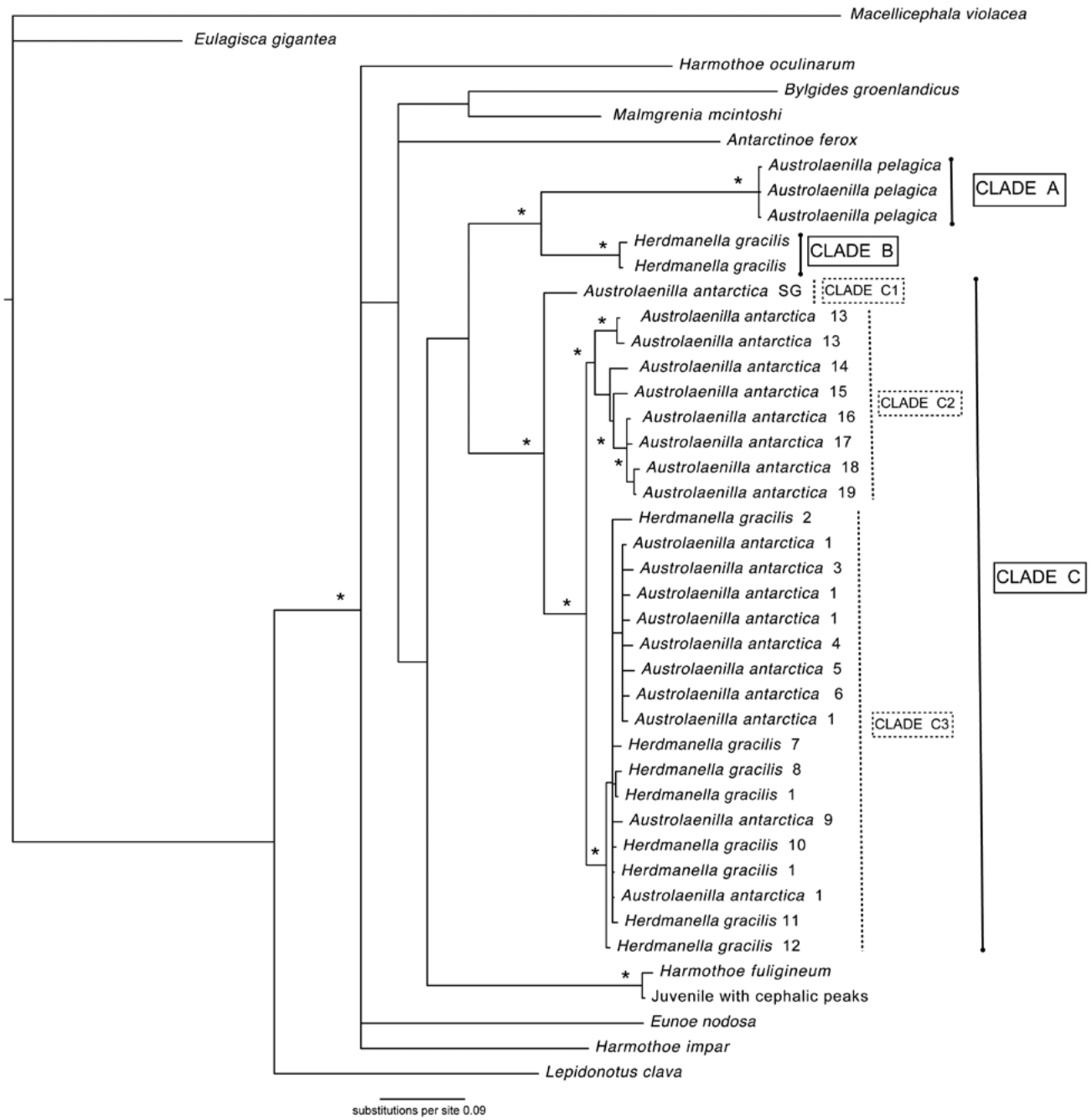

Figure 4. Phylogenetic tree from Bayesian consenus analysis based on CO1 (mtDNA) only. Stars represent significant node values (≥95%) for Bayesian posterior probabilities. Clade numbers and letters refer to table 2 and the main text.

these - South Georgian (SG), Amundsen Sea Station BIO3 (no. 14), and Weddell Sea (no. 13) were represented by single haplotypes only. Four haplotypes from various sampling stations and depths in the Amundsen Sea (nos 16–19) formed a separate network. The largest network was composed of haplotypes of *A. antarctica* and *H. gracilis* from the Amundsen Sea station BIO4, 500 m (haplotypes no. 1 -12) with the addition of a single haplotype from Elephant Island (haplotype no. 15).

Discussion

Taxonomy and genetic diversity: Herdmanella gracilis *in the Southern Ocean*

Ever since Augener (1929) first identified small polynoid specimens from the Southern Ocean as *Herdmanella gracilis* Ehlers, 1908, this species was considered to have an Antarctic, as well as Indian Ocean (type locality) distribution. However, given

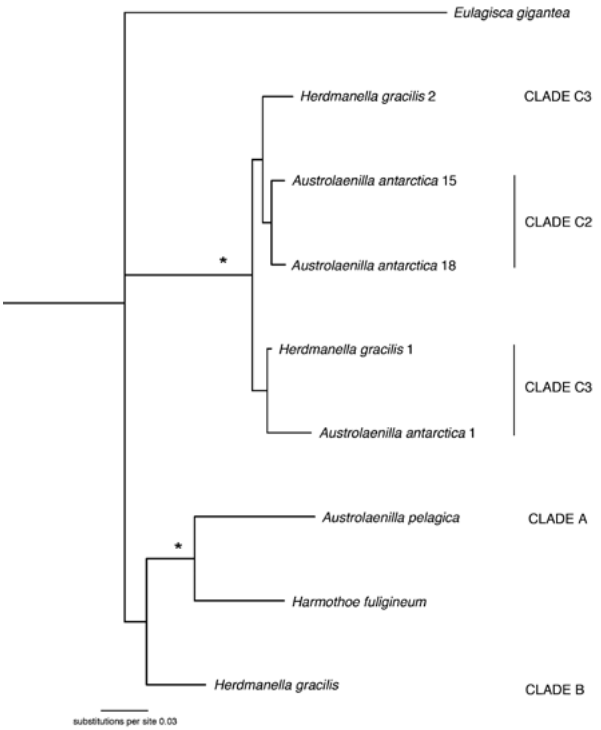

Figure 5. Phylogenetic tree from Bayesian consenus analysis based on the CO1, 16S (mtDNA) and *H3* (nDNA) combined dataset of selected specimens. Stars represent significant node values (≥95%) for Bayesian posterior probabilities.

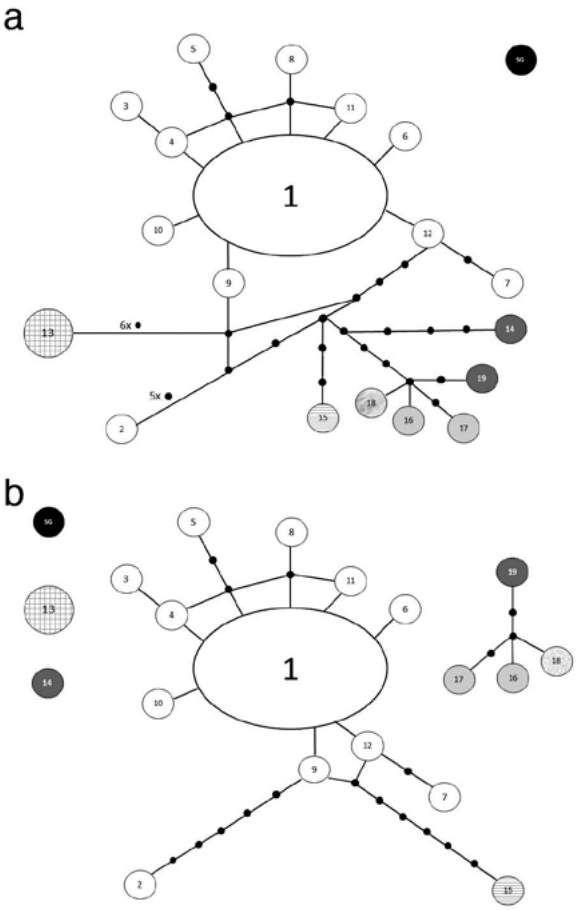

Figure 6. Haplotype network of 20 haplotypes based on *Austrolaenilla antarctica* and *Herdmanella gracilis* mtCO1 data: a, representing 91% connection limit; b, representing 95% limit. Small black circles represent unsampled haplotypes, large circles represent the sampled haplotypes with their size proportional to the frequency of the haplotype (n = 1, 2 and 7). Numbers in the shapes correspond to haplotype identification numbers (see table 2). Different geographical locations are coded—white circles are Amundsen Sea BIO4, 500-m depth; light grey circles are BIO4, 1500-m depth; dark grey circles are BIO3; cross-hatched circles are Weddell Sea; horizontal hatched circles are Elephant Island; and black circles are South Georgia.

the small size of the specimens, the longstanding suggestion by many authors was that they were juveniles, with links suggested to the genus *Austrolaenilla* (*Antinoe* in Monro, 1930) or *Harmothoe* Kinberg, 1855 (Augener, 1929; Hartmann-Schröder, 1974; Pettibone, 1976. During the morphological investigation of a large number of these small polynoids in our study, two morphotypes were distinguished: those with cephalic peaks (a feature not reported in *Herdmanella gracilis*) (fig. 3a) and without cephalic peaks (fig. 3b) (consistent with *H. gracilis*). The small specimens with cephalic peaks are not the subject of this paper, but molecular methods employed in a wider phylogenetic study of Southern Ocean Polynoidae have identified these as juveniles of *Harmothoe fuligineum* (Baird, 1865) (Wiklund et al., in prep.). However, when identifiers are presented with a large number of these small worms (thousands in this study), the two morphotypes can be confused, as the cephalic peaks can be easily overlooked.

The molecular methods based on the CO1 gene only and on combined analysis of the CO1, 16S and H3 genes suggest that *Herdmanella gracilis*-like specimens from the Southern Ocean, which are morphologically indistinguishable, do in fact represent juvenile stages of at least two species (clades B and C in fig. 4). It is very likely that greater sequencing effort would identify other species within the *Herdmanella gracilis*-like juveniles. The identity of the adult stage for *H. gracilis*-like juveniles from the Scotia Sea (clade B in fig. 4) remains unresolved. *Herdmanella gracilis*-like specimens from the Amundsen Sea are a close match with the adult stage of *Austrolaenilla antarctica* Bergström, 1916, a common species with wide circumpolar distribution, with *Austrolaenilla antarctica* and *H. gracilis* constituting a well-supported monophyletic group (clade C in fig. 4). In CO1, the genetic diversity in clade C was rather high (see further discussion below); the smallest distance between *A. antarctica* and *H. gracilis*-like specimens was 0%, but the average distance was approximately 4%. However, the gap between 'good' species, e.g. between *A. antarctica* and *A. pelagica* was on average 18.3 (range 14.8–20.9) (table 4), which suggests that an average of 4% distance may constitute potential intraspecific variation. Given this relatively large 'barcoding gap', even for specimens sampled from the same locality, 16S and H3 sequences were obtained for

a small number of representative specimens with high CO1 divergences. In 16S, the genetic distance was reduced to <1%, and in nuclear H3 genes, these sequences were identical.

Additionally, haplotype networks with a 95% connectivity limit provide a useful tool for species delimitation (e.g. Hart and Sunday, 2007; Monaghan et al., 2006). For the purposes of clarifying the identity of the *Herdmanella gracilis*-like specimens from the Southern Ocean, the haplotype networks confirmed the identity of many of these specimens as juveniles of *Austrolaenilla antarctica*, even under the strict 95% connectivity limit (fig. 6b, table 2). However, the status of specimens morphologically agreeing with *A. antarctica* (from various locations in the Southern Ocean) as a single species can be questioned. The single specimen from South Georgia (corresponding to clade C1 in fig. 4) could be justified as a different species since the single haplotype network could not be recovered, even under a 90% limit (fig. 6a). Specimens from clades C2 and C3 formed a single haplotype network under 90 and 91% limits (fig. 6a), but failed under the more conservative 95% limit (fig. 6b). However, the network of the haplotype numbers 16–19 fails to be connected to the main network by a single step.

As with the 'barcoding gap', the coveted 95% connectivity limit may not work well for *Austrolaenilla antarctica*, although the presence of cryptic species cannot be discounted. A larger sampling effort, covering other locations and including a greater number of specimens of *A. antarctica*, would be needed to clarify its status as a single or cryptic species. Several studies using DNA to delimit Southern Ocean taxa based on mitochondrial sequence data suggested the discovery of new (often cryptic) species, e.g. the polychaete *Glycera kerguelensis* (Schüller, 2010), the pycnogonids *Nymphon australe* (Mahon et al., 2008) and *Colossendeis megalonyx* (Krabbe et al., 2010), amphipods (Loerz et al., 2009; Baird et al., 2011), nudibranch *Doris kerguelensis* (Wilson et al., 2009), crinoid *Promachocrinus kerguelensis* (Wilson et al., 2007), ostracods (Brandão et al., 2010) or octopus (Allcock et al., 2011), while fewer studies supported a true circumpolar distribution (e.g. Raupach et al., 2010; Arango et al., 2011). A summary of the extent of the barcoding studies in the Southern Ocean has been provided by Grant et al. (2011) and Allcock and Strugnell (2012). Nygren (2013) provides an in depth review of cryptic diversity in polychaetes.

The problem of species delimitation and species concepts has a long history in biology (e.g. de Queiroz, 2007; Wiens, 2007; Wilkins, 2011). In recent years, the molecular approach has been added to the toolbox for both species identification and species delimitation. As already mentioned above, a common approach, which we also adopted here, is to search for discontinuities in DNA sequence variation either through the statistical parsimony method (Templeton et al., 1992), which is used to build haplotype networks, or by the discovery of a barcoding gap (Hebert et al., 2003). This gap is supposed to represent the difference between the highest genetic distance found within species and the lowest genetic distance found between species. The most common part of DNA used in animal barcoding studies is the CO1 gene of mtDNA. However, this choice of marker, as well as the concept of the barcoding gap itself, has been subject to fierce criticism ever since it was proposed by Hebert et al. (2003). In their review of mitochondrial DNA, Galtier et al. (2009) concluded that, given the heterogeneous evolutionary rate of mtDNA and processes such as hybrid introgression or balancing selection, the universal distance-based 'gaps' for delineation of taxa into species do not exist. This view has been supported by many other workers, adding problems of small sample sizes or narrow geographical coverage, which may affect the size of the 'gap', resulting in extensive literature concerning the use of mtDNA barcodes (e.g. Moritz and Cicero, 2004; Meyer and Paulay, 2005; Meyer et al., 2008; Bergsten et al., 2012; Collins and Cruickshank, 2012). The increasingly accepted approach to species delimitation will rely on examination of mtDNA sequence distance, variation at multiple (including nuclear) genetic loci in a phylogenetic context, careful morphological examination, as well as ecological and biological observations (see references in Nygren, 2013). However, to obtain all these lines of evidence is not always possible and certainly takes time.

The most comprehensive work on barcoding of polychaetes to date was completed by Carr et al. (2011) on Arctic polychaetes. Their analysis of 1876 specimens, representing 333 provisional species, revealed 40 times more between-species sequence divergence in CO1 as opposed to within species (16.5% versus 0.38%), as estimated by Kimura 2 parameter (K2P) distance measure. In Carr et al. (2011), the CO1 barcodes have high discriminatory power for polychaetes because the average observed within-clade divergence in their study was 3.8% (highest within-species divergence was 5.7%), indicating that barcodes naturally form tight clades with low variation. A smaller regional barcoding study on Chilean polychaetes conducted by Maturana et al. (2011) corroborated the results of Carr et al. (2011) by finding that mean pairwise sequence distance comparisons, based on K2P within-species, ranged from 0.2 to 0.4%, while interspecific comparisons were much higher and ranged between 18 and 47%.

Results from our study approach the interspecific differences observed in other research on polychaetes (e.g. Schüller, 2010; Maturana et al., 2011; Carr et al., 2011), with the average K2P distance in CO1 found to range from 14.4 to 18.3% (table 4). However, variable results were obtained for within-species diversity in CO1. There was no divergence in *Austrolaenilla pelagica* clade A (fig. 4) in specimens from various sampling sites in the Amundsen Sea (greatest distance ca. 500 km apart). Similarly, the two juveniles of unknown identity forming clade B (fig. 4a) were separated by less than 1% in CO1, despite the fact that these specimens came from the geographically distant sites of the Powell Basin and Shag Rocks. Additionally, unpublished work by Ramon (pers. comm.) on Antarctic marine larvae revealed a close match to the unknown species in clade B in CO1 and 16S sequences with unidentified polynoid larvae from the Ross Sea. The low level of divergence within these clades (A and B), which also correspond to morphological species, is in agreement with the results of other studies mentioned above. Large distribution areas have often been accepted for marine fauna in the past, but this assumption has been questioned (e.g. Hellberg, 2009). Further, the discovery of cryptic species also challenges this idea (Nygren, 2013). However, wide distributions have been

shown for polychaetes by Schüller and Hutchings (2012), who demonstrated long-distance dispersal in *Terebellides gingko* using 16S rDNA sequences.

In contrast, a very high level of diversity was observed in the *Austrolaenilla antarctica* clade A, with the greatest distance being 7.3%. The specimens in the *A. antarctica* clade were collected from several locations in the Southern Ocean (table 1), covering a total geographical distance of ca. 6000 km (if following the shelf of islands in the Scotia Arc). Reflecting this, the *A. antarctica* clade itself is formed of three clades (C1, C2 and C3). The most divergent specimen from South Georgia (clade C1) may possibly represent a cryptic species, a notion also supported by the haplotype network analysis. Clade C2 is comprised entirely of adults that agreed morphologically with *A. antarctica*, with an average distance of approximately 2.5% (range 0.03–4.1% (table 4)). These specimens were mostly collected at locations from across the Amundsen Sea at varying depths, but the clade also contains one specimen from Elephant Island, 1500 m (BIOPEARL I collection), and the most divergent specimen is from the abyssal Weddell Sea (ANDEEP collection). Clade C3 includes exclusively juveniles and adults collected from a single sampling station on inner shelf BIO4 at 500 m. Individuals from a single station were selected to establish the identity of the juveniles previously identified as *Herdmanella gracilis* in order to reduce the diversity introduced by factors such as geographical or bathymetrical distance. Even though the average distance was low at 1% within this clade, the highest CO1 distance was 3.5% (table 4). In this study, only a small number of individuals were sequenced from >1000 specimens collected at this particular site, and potential future work specifically aimed at population genetics may provide further insights.

In a large-scale study of lumbricid earthworms in Britain, King et al. (2008) found that two morphs of *Allolobophora chlorotica* (with over 14% divergence at CO1) are interbreeding and therefore represent a single taxon. This conclusion was further supported by amplified fragment length polymorphism (AFLP) markers. In their overall survey of CO1 sequence diversity of nine species of British earthworms, represented by 71 individuals, they found that sequence divergences within species varied from 0.35% to 12.35%, highlighting yet again the lack of a universal threshold for the barcoding gap, even within closely related species, which is similar to our results for two recognised and one suspected species of *Austrolaenilla*. Similar results were obtained in a recent study by Achurra and Erséus (2013) examining population structure of the aquatic oligochaete *Stylodrilus heringianus* Claparède, 1862, covering its range on a European scale (Estonia to Spain) using sequences of the mt CO1 and two nuclear genes: internal transcribed spacer region and histone 3. The authors also found a large CO1 diversity, with a maximum distance of 7.7%, as measured by K2P. Nevertheless, nuclear genes failed to confirm any lineage separation, and it was concluded that the sampled specimens all belong to the same species, asserting that the mitochondrial single-locus approach can be problematic for the accurate delimitation of species.

Of several hypotheses proposed to explain high diversity in mitochondrial sequence data and its discordance with nuclear genes (see e.g. King et al., 2008), that of incomplete speciation following isolation in distinct glacial refugia is of particular relevance to the Southern Ocean fauna. The Earth's climatic history is marked by alternating glacial and interglacial periods. During the ice ages, populations of plants and animals have shown primarily two survival strategies: migration or survival *in situ*, helped by the existence of glacial-free refugia. Populations in different refugia will diverge from one another through genetic drift, which may lead to reproductive isolation of those populations. The recent insights into the history of glaciation in Antarctica have shown that at glacial maxima, grounded ice sheets extended over much of the Antarctic continental shelf (Thatje et al., 2005). As a result, most (if not all) available habitat for marine benthos was destroyed, making this group particularly vulnerable to extinction. Earlier workers such as Dell (1972) proposed that the continental shelf fauna was completely eradicated by glacial cycles and recolonised from the deep sea. Others suggested that some fauna survived on the continental shelf itself in ice-free refugia (Clarke et al., 2004). These ice-free regions existed on a range of temporal and spatial scales, and not all areas around the continent have been glaciated at the same time (Anderson et al., 2002). As such, isolation of historic populations in Cenozoic glacial refugia could provide some explanation for the high mtDNA diversity in our modern Antarctic polychaete populations. The shelf of the Amundsen Sea, the site of collection of most specimens used in this study, is particularly complex in its bathymetry as a result of past as well as present day glacial activity (Lowe and Anderson, 2002).

Status of the genus Herdmanella

Darboux, 1899, erected the genus *Herdmanella* for the species *Polynoe* (?) *ascidioides* McIntosh, 1885, which was found at station 160 of the Challenger Expedition in the Southern Ocean (42°42'S 134°10'E, south of Australia) in the branchial chamber of an ascidian on red clay at 4755m depth. This is therefore the type species of the genus by monotypy. McIntosh, 1885, himself mentioned some similarity between his species *Polynoe* (?) *ascidioides* and *Polynoe (Macellicephala) mirabilis* McIntosh, 1885. Uschakov (1971) also compared this species to the genus *Macellicephala*. However, Hartmann-Schröder (1974) referred to it as *Macellicephala (Macellicephala) ascidioides* (McIntosh, 1885), saying that it is incompletely known, but explicitly making *Herdmanella* a junior synonym of *Macellicephala* McIntosh, 1885. More recently, Pettibone (1976) referred to *Polynoe* (?) *ascidioides* as a 'doubtful Polynoidae'. The holotype is apparently the only specimen of this species that has been reported. This holotype has been re-examined by Muir (1982), who found it to be in bad condition and lacking a head, so it cannot with certainty be referred to any polynoid subfamily. It is clear, therefore, that *Polynoe* (?) *ascidioides* McIntosh, 1885 must be regarded as a *nomen dubium* (a name of unknown or doubtful application). If the name of the type species of a genus (*Polynoe* (?) *ascidioides*) is a *nomen dubium*, it follows that the generic name *Herdmanella* must also be a *nomen dubium*. It is not clear why previous authors did not arrive at this conclusion.

Although our study provides clear evidence that specimens from the Southern Ocean morphologically consistent with

Herdmanella gracilis are in fact juveniles of at least two species in the polynoid genus *Austrolaenilla*, we cannot come to a definite conclusion about the identity of *H. gracilis* from the type locality in the equatorial Indian Ocean. We have, however, certainly strengthened the longstanding suggestion that it is a juvenile, and the results from the Southern Ocean point towards the genus *Austrolaenilla* as adult counterparts, but no adults of *Austrolaenilla* species are known from the equatorial Indian Ocean. Similarly *Herdmanella aequatorialis* from the Gulf of Guinea, currently regarded as the other valid species in the genus *Herdmanella*, is also likely to be a juvenile of an, as yet unknown, polynoid. *Austrolaenilla meteorae* (originally placed in *Harmothoe*) was described by Hartmann-Schröder (1982) from the equatorial Atlantic Ocean off West Africa and may possibly be the adult of *H. aequatorialis*. As the genus *Herdmanella* is now regarded as a *nomen dubium*, the two species *H. gracilis* and *H. aequatorialis* are now without a generic placement, and as they probably represent juveniles of other species they are also best regarded as *nomina dubia*.

Ecology of polynoid juveniles

Larval ecology is important to understanding patterns and processes influencing marine populations, communities and ecosystems. However, one of the limitations to the study of community ecology is the ability to correctly identify marine larvae and juveniles. As this study shows, not only is it problematic to attempt to distinguish juveniles of related polynoid species on the basis of morphology alone, but juveniles may well have been considered different species in the past. This of course will have repercussions for subsequent analysis of community structure. It is indeed rather arbitrary how to treat a large collection of juveniles in an ecological analysis if their identity is spurious. Without being able to identify these correctly, it may be sensible to exclude indeterminable juveniles from the analysis. However, the distribution and abundance patterns of juveniles are of great interest, given that very little is known about this subject.

The large number of juveniles (>2000) belonging to several polynoid species collected in the Amundsen Sea is suggestive of recent spawning. The samples were collected towards the end of the austral summer in early March of 2008. This points to a potentially synchronised summer spawning event of at least two polynoid species—*Austrolaenilla antarctica* and *Harmothoe fuligineum*. The largest numbers were found at 500 m depth (the shallowest horizon sampled during the BIOPEARL II cruise), and they were exceptionally abundant at two inner-shelf stations in Pine Island Bay. Although recent studies indicated the existence of so-called food banks available to benthos throughout the year (Smith et al., 2002; Glover et al., 2008), it is likely that recruitment predominantly occurs during the summer months and is linked to high food availability. In addition, the inner-shelf BIOPEARL II stations are characterised by the presence of polynyas, areas known for high productivity (Arrigo and Van Dijken, 2003). The high number of polynoid juveniles in this region is likely linked to this. The analysis of biodiversity and ecology of polychaetes from Amundsen Sea is currently underway (Neal et al., in prep.).

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References

Achurra, A., and Erséus, C. 2013. DNA barcoding and species delimitation: the *Stylodrilus heringianus* case (Annelida: Clitellata: Lumbriculidae). *Invertebrate Systematics* 27(1): 118–128.

Ahrens, D., Monaghan, M.T., and Vogler, A.P. 2007. DNA-based taxonomy for associating adults and larvae in multi-species assemblages of chafers (Coleoptera: Scarabaeidae). *Molecular Phylogenetics and Evolution* 44(1): 436–449.

Allcock, A.L., Barratt, I., Eléaume, M., Linse, K., Norman, M.D., Smith, P.J., Steinke, D., Stevens, D.W., and Strugnell, J.M. 2011. Cryptic speciation and the circumpolarity debate: a case study on endemic Southern Ocean octopuses using the COI barcode of life. *Deep-Sea Research Part II: Topical Studies in Oceanography* 58(1): 242–249.

Allcock, A.L., and Strugnell, J.M. 2012. Southern Ocean diversity: new paradigms from molecular ecology. *Trends in Ecology and Evolution* 27(9): 520–528.

Anderson, J.B., Shipp, S.S., Lowe, A.L., Wellner, J.S., and Mosola, A.B. 2002. The Antarctic Ice Sheet during the Last Glacial Maximum and its subsequent retreat history: a review. *Quaternary Science Reviews* 21(1): 49–70.

Arango, C.P., Soler-Membrives, A., and Miller, K.J. 2011. Genetic differentiation in the circum-Antarctic sea spider *Nymphon australe* (Pycnogonida: Nymphonidae). *Deep-Sea Research Part II: Topical Studies in Oceanography* 58(1): 212–219.

Arrigo, K.R., and Van Dijken, G.L. 2003. Phytoplankton dynamics within 37 Antarctic coastal polynyas, *Journal of Geophysical Research* 108(C8): 3271. 18 pp.

Augener, H. 1929. Beitrage zur Planktonbevolkerung der Weddellsee nach dem Ergebnissen der Deutschen Antarktischen Expedition 1911–1912. V. Die Polychaeten der Weddellsee. *Internationale Revue der Gesamten Hydrobiologie und Hydrographie* 22: 273–312.

Baird, W. 1865. Contributions towards a monograph of the species of Annelides belonging to the Aproditacea, containing a list of the known species, and a description of some new species contained in the National

Collection of the British Museum. *Journal of the Linnean Society of London, Zoology* 8:172–202.

Baird, H.P., Miller, K.J., and Stark, J.S. 2011. Evidence of hidden biodiversity, ongoing speciation and diverse patterns of genetic structure in giant Antarctic amphipods. *Molecular Ecology* 20(16): 3439–3454.

Bergsten, J., Bilton, D.T., Fujisawa, T., Elliott, M., Monaghan, M.T., Balke, M., Hendrich, L., Geijer, J., Herrmann, J., Foster, G.N., Ribera, I., Nilsson, A.N., Barraclough, T.G., and Vogler, A.P. 2012. The effect of geographical scale of sampling on DNA barcoding. *Systematic Biology* 61(5): 851–869.

Bracken-Grissom, H.D., Felder, D.L., Vollmer, N.L., Martin, J.W., and Crandall, K. 2012. Phylogenetics links monster larva to deep-sea shrimp. *Ecology and Evolution* 2(10): 2367–2373.

Brandão, S.N., Sauer, J., and Schön, I. 2010. Circumantarctic distribution in Southern Ocean benthos? A genetic test using the genus *Macroscapha* (Crustacea, Ostracoda) as a model. *Molecular Phylogenetics and Evolution* 55(3): 1055–1069.

Brenke, N. 2005. An epibenthic sledge for operations on marine soft bottom and bedrock. *Journal of the Marine Technology Society* 39(2): 10–19.

Carr, C.M., Hardy, S.M., Brown, T.M., Macdonald, T., and Hebert, P.D.N. 2011. A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PloS ONE* 6(7): e22232.

Clarke, A., Aronson, R.B., Crame, J.A., Gili, J.-M., and Blake, D.B. 2004. Evolution and diversity of the benthic fauna of the Southern Ocean continental shelf. *Antarctic Science* 16(4): 559–568.

Clement, M., Posada, D.C.K.A., and Crandall, K.A. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9(10): 1657–1659.

Colgan, D., Ponder, W.F., and Eggler, P.E. 2000. Gastropod evolutionary rates and phylogenetic relationships assessed using partial 28S rDNA and histone *H3* sequences. *Zoologica Scripta*, 29(1): 29–63.

Collins, R.A, and Cruickshank, R.H. 2012. The seven deadly sins of DNA barcoding. *Molecular Ecology Resources* 13: 969–975.

Darboux, J.G. 1899. Recherches sur les aphroditiens. *Travaux de l'Institut de Zoologie de l'Université de Montpellier et de la Station Maritime (Zoologique) de Cette* 6: 1–276.

Dell, R.K. 1972. Antarctic benthos. *Advances in Marine Biology* 10: 1–216.

De Queiroz, K. 2007. Species concepts and species delimitation. *Systematic Biology* 56(6): 879–886.

Drummond, A.J., Ashton, B., Cheung, M., Heled, J., Kearse, M., Moir, R., and Stones-Havas S. 2007. Geneious ver. 3. 0. Available at http://www.geneious.com

Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5): 1792–1797.

Ehlers, E. 1908. Die Bodensässigen Anneliden aus den Sammlungen der deutschen Tiefsee-Expedition. *Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer Valdivia 1898–1899* 16(1): 1–168.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.

Galtier, N., Nabholz, B., Glémin, S., and Hurst, G.D.D. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology* 18(22): 4541–4550.

Glover, A.G., Smith, C.R., Mincks, S.L., Sumida, P.Y.G., and Thurber, A.R. 2008. Macrofaunal abundance and composition on the West Antarctic Peninsula continental shelf: evidence for a sediment 'food bank' and similarities to deep-sea habitats. *Deep-Sea Research II* 55: 2491–2501.

Grant, R.A., Griffiths, H.J., Steinke, D., Wadley, V., and Linse K. 2011. Antarctic DNA barcoding; a drop in the ocean? *Polar Biology* 34(5): 775–780.

Hamilton, C., Formanowicz, D.R., and Bond, J.E. 2011. Species delimitation and phylogeography of *Aphonopelma hentzi* (Araneae, Mygalomorphae, Theraphosidae): cryptic diversity in North American tarantulas. *PloS ONE* 6(10): e26207.

Hart, M.W., and Sunday, J. 2007. Things fall apart: biological species form unconnected parsimony networks. *Biology Letters* 3(5): 509–512.

Hartman, O. 1964. Polychaeta Errantia of Antarctica. *Antarctic Research Series* 3: 1–131.

Hartman, O. 1967. Polychaetous annelids collected by the USNS *Eltanin* and *Staten Island* cruises, chiefly from Antarctic seas. *Allan Hancock Monographs in Marine Biology* 2: 1–387.

Hartmann-Schröder, G. 1974. Die Unterfamilie Macellicephalinae Hartmann-Schröder, 1971. *Mittheilungen aus dem Hamburgischen Zoologischen Museum und Institut* 71: 75–85.

Hartmann-Schröder, G. 1982. Die Polychaeten der Fahrt 36 (1975) von F.S. 'Meteor' in das Auftriebsgebiet vor Nordwest-Afrika. *METEOR Forschungsergebnisse* D(35): 1–20.

Haynes, E. 1979. Description of larvae of the northern shrimp, *Pandalus borealis*, reared *in situ* in Kachemak Bay, Alaska. *Fisheries Bulletin* 77(1): 157–173.

Haynes, E.B. 1982. Description of larvae of the golden king crab, *Lithodes aequispina*, reared in the laboratory. *Fisheries Bulletin* 80(2): 305–313.

Hebert, P.D.N., Ratnasingham, S., and de Waard, J.R. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 270 Supplement 1: S96–S99.

Heimeier, D., Lavery, S., and Sewell, M. 2010. Using DNA barcoding and phylogenetics to identify Antarctic invertebrate larvae: lessons from a large scale study. *Marine Genomics* 3(3–4): 165–177.

Hellberg, M.E. 2009. Gene flow and isolation among populations of marine animals. *Annual Review of Ecology, Evolution, and Systematics* 40: 291–310.

Katoh, K., Misawa, K., Kuma, K., and Miyata, T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30(14): 3059–3066.

King, R.A., Tibble, A.L., and Symondson, W.O.C. 2008. Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. *Molecular Ecology* 17(21): 4684–4698.

Krabbe, K., Leese, F., Mayer, C., Tollrian, R., and Held, C. 2010. Cryptic mitochondrial lineages in the widespread pycnogonid *Colossendeis megalonyx* Hoek, 1881 from Antarctic and Subantarctic waters. *Polar Biology* 33(3): 281–292.

Linse, K. 2008. The BIOPEARL expedition to the Scotia Sea in 2006. *Antarctic Science* 20(3): 211–212.

Linse, K., Griffiths, H.J., Barnes, D.K., Brandt, A., Davey, N., David, B., DeGrave, S., d'Udekem d'Acoz, C., Eleaume, M., Glover, A.G., Hemery, L., Mah, C., Martín-Ledo, R., Munilla, T., O'Loughlin, M., Pierrat, B., Saucede, T., Sands, C., Strugnell, J.M., and Enderlein, P. 2013. The macro- and megabenthic fauna on the continental shelf of the eastern Amundsen Sea, Antarctica. *Continental Shelf Research* 68: 80–90.

Loerz, A., Maas, E., Linse, K., and Coleman, C.O. 2009. Do circum-Antarctic species exist in peracarid Amphipoda? A case study in the genus *Epimeria* Costa, 1851 (Crustacea, Peracarida, Epimeriidae). *ZooKeys* 18: 91–128.

Lowe, A.L., and Anderson, J.B. 2002. Reconstruction of the West Antarctic ice sheet in Pine Island Bay during the Last Glacial Maximum and its subsequent retreat history. *Quaternary Science Reviews* 21:1879–1897.

Mahon, A.R., Arango, C.P., and Halanych, K.M. 2008. Genetic diversity of Nymphon (Arthropoda: Pycnogonida: Nymphonidae) along the Antarctic Peninsula with a focus on Nymphon australe Hodgson 1902. *Marine Biology* 155(3): 315–323.

Maturana, C.S., Moreno, R.A., Labra, F.A., González, C.A., Rozbaczylo, N., Carrasco, F.D., and Poulin, E. 2011. DNA barcoding of marine polychaetes species of southern Patagonian fjords. *Revista de Biología Marina y Oceanografía* 46(1): 35–42.

McIntosh, W.C. 1885. Report on the Annelida Polychaeta collected by H.M.S. Challenger during the years 1873–76. *Challenger Reports* 12: 1–554.

Meier, R., Zhang, G., and Ali, F. 2008. The use of mean instead of smallest interspecific distances exaggerates the size of the "barcoding gap" and leads to misidentification. *Systematic Biology* 57(5): 809–813.

Meyer, C.P., and Paulay, G. 2005. DNA barcoding: error rates based on comprehensive sampling. *PLoS Biology* 3(12): e422.

Monaghan, M.T., Balke, M., Pons, J., and Vogler, A.P. 2006. Beyond barcodes: complex DNA taxonomy of a South Pacific Island radiation. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 273(1588): 887–893.

Monro, C.C.A. 1930. Polychaete worms. *Discovery Reports* 2: 1–222.

Moritz, C., and Cicero, C. 2004. DNA barcoding: promise and pitfalls. *PLoS Biology* 2: 1529–1531.

Muir, A.I. 1982. Generic characters in the Polynoinae (Annelida, Polychaeta), with notes on the higher classification of scale-worms (Aphroditacea). *Bulletin of the British Museum (Natural History). Zoology Series* 43(3): 153–177.

Nygren, A. 2013. Cryptic polychaete diversity: a review. *Zoologica Scripta* 43: 172–183.

Nylander, J.A.A., Erséus, C., and Källersjö, M. 1999. A test of monophyly of the gutless Phallodrilinae (Oligochaeta, Tubificidae) and the use of a 573 bp region of the mitochondrial cytochrome oxidase I gene in analysis of annelid phylogeny. *Zoologica Scripta* 28: 467–482.

Palumbi, S.R. 1996. Nucleic acid II: the polymerase chain reaction. Pp. 205–247 in: Hillis, D.M., Moritz, G. and Mable, B.K. (eds), *Molecular systematics*. Sinauer Associates: Sunderland, Maryland.

Pegg, G.G., Sinclair, B., Briskey, L., and Aspden, W.J. 2006. MtDNA barcode identification of fish larvae in the southern Great Barrier Reef, Australia. *Scientia Marina* 70(S2): 7–12.

Pettibone, M.H. 1976. Revision of the genus *Macellicephala* McIntosh and the subfamily Macellicephalinae Hartmann-Schröder (Polychaeta, Polynoidae). *Smithsonian Contributions to Zoology* 229: 1–71.

Posada, D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25(7): 1253–1256.

Raupach, M.J., Thatje, S., Dambach, J., Rehm, P., Misof, B., and Leese, F. 2010. Genetic homogeneity and circum-Antarctic distribution of two benthic shrimp species of the Southern Ocean, *Chorismus antarcticus* and *Nematocarcinus lanceopes*. *Marine Biology* 157(8): 1783–1797.

Richards, W.J., and Saksena, V.P. 1980. Description of larvae and early juveniles of laboratory-reared gray snapper, *Lutjanus griseus* (Linnaeus) (Pisces, Lutjanaidae). *Bulletin of Marine Science* 30(2): 515–521.

Ronquist, F. and Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Schüller, M. 2010. Evidence for a role of bathymetry and emergence in speciation in the genus *Glycera* (Glyceridae, Polychaeta) from the deep Eastern Weddell Sea. *Polar Biology* 34(4): 549–564.

Schüller, M., and Hutchings, P.A. 2012. New species of *Terebellides* (Polychaeta: Trichobranchidae) indicate long-distance dispersal between western South Atlantic deep-sea basins. *Zootaxa* 2395: 1–16.

Shank, T.M., Lutz, R.A., and Vrijenhoek, R.C. 1998. Molecular systematics of shrimp (Decapoda: Bresiliidae) from deep-sea hydrothermal vents: enigmatic "small orange" shrimp from the Mid-Atlantic Ridge are juvenile *Rimicaris exoculata*. *Molecular Marine Biology and Biotechnology* 7(2): 88–96.

Smith, C.R., Mincks, S.L., Glover, A.G., DeMaster, D.J., and Sumida, P.Y.G. 2002. FOODBANCS on the Antarctic Peninsula shelf: the benthic food bank hypothesis and the seasonal deposition pulse. *American Geophysical Union, Ocean Sciences Meeting, Honolulu, HI. EOS* 83.

Srivathsan, A., and Meier, R. 2012. On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. *Cladistics* 28(2): 190–194.

Støp-Bowitz, C. 1991. Some new or rare species of pelagic polychaetes from the Gulf of Guinea. *Ophelia* Supplement 5: 261–270.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28(10): 2731–2739.

Templeton, A.R., Crandall, K.A., and Sing, C.F. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132(2): 619–633.

Thatje, S., Hillenbrand, C.-D., and Larter, R. 2005. On the origin of Antarctic marine benthic community structure. *Trends in Ecology and Evolution* 20(10): 534–540.

Uschakov, P.V. 1971. On a new abyssal species of *Macellicephala* McIntosh (Polychaeta, Errantia) from the Aleutian Trench. *Trudy Instituta Okeanologii Akademiia Nauk* SSSR 92: 36–40.

Valdez-Moreno, M., Vásquez-Yeomans, L., Elías-Gutiérrez, M., Ivanova, N.V., and Hebert, P.D.N. 2010. Using DNA barcodes to connect adults and early life stages of marine fishes from the Yucatan Peninsula, Mexico: potential in fisheries management. *Marine and Freshwater Research* 61(6): 655–671.

Vogler, A.P., and Monaghan, M.T. 2007. Recent advances in DNA taxonomy. *Journal of Zoological Systematics and Evolutionary Research* 45(1): 1–10.

Webb, K.E., Barnes, D.K., Clark, M.S., and Bowden, D. 2006. DNA barcoding: a molecular tool to identify Antarctic marine larvae. *Deep-Sea Research Part II: Topical Studies in Oceanography* 53(8–10): 1053–1060.

Wiens, J.J. 2007. Species delimitation: new approaches for discovering diversity. *Systematic Biology* 56(6): 875–878.

Wilkins, J.S. 2011. Philosophically speaking, how many species concepts are there? *Zootaxa* 2765: 58–60.

Wilson, N.G., Hunter, R.L., Lockhart, S.J., and Halanych, K.M. 2007. Multiple lineages and absence of panmixia in the "circumpolar" crinoid *Promachocrinus kerguelensis* from the Atlantic sector of Antarctica. *Marine Biology* 152(4): 895–904.

Wilson, N,G,, Schrödl, M., Halanych, K.M. 2009. Ocean barriers and glaciation: evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelenensis* (Mollusca, Nudibranchia). *Molecular Ecology* 18:965–984.

WoRMS Editorial Board. 2013. World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ.