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Molecular data reveal a highly diverse species flock within the munnopsoid deep-sea isopod *Betamorpha fusiformis* (Barnard, 1920) (Crustacea: Isopoda: Asellota) in the Southern Ocean

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Abstract

Based on our current knowledge about population genetics, phylogeography and speciation, we begin to understand that the deep sea harbours more species than suggested in the past. Deep-sea soft-sediment environment in particular hosts a diverse and highly endemic invertebrate fauna. Very little is known about evolutionary processes that generate this remarkable species richness, the genetic variability and spatial distribution of deep-sea animals. In this study, phylogeographic patterns and the genetic variability among eight populations of the abundant and widespread deep-sea isopod morphospecies *Betamorpha fusiformis* [Barnard, K.H., 1920. Contributions to the crustacean fauna of South Africa. 6. Further additions to the list of marine isopods. Annals of the South African Museum 17, 319–438] were examined. A fragment of the mitochondrial 16S rRNA gene of 50 specimens and the complete nuclear 18S rRNA gene of 7 specimens were sequenced. The molecular data reveal high levels of genetic variability of both genes between populations, giving evidence for distinct monophyletic groups of haplotypes with average *p*-distances ranging from 0.0470 to 0.1440 (*d*-distances: 0.0592–0.2850) of the 16S rDNA, and 18S rDNA *p*-distances ranging between 0.0032 and 0.0174 (*d*-distances: 0.0033–0.0195). Intermediate values are absent. Our results show that widely distributed benthic deep-sea organisms of a homogeneous phenotype can be differentiated into genetically highly divergent populations. Sympatry of some genotypes indicates the existence of cryptic speciation. Flocks of closely related but genetically distinct species probably exist in other widespread benthic deep-sea asellotes and other Peracarida. Based on existing data we hypothesize that many widespread morphospecies are complexes of cryptic biological species (patchwork hypothesis).

Keywords: Biogeography; Cryptic species; Patchwork hypothesis; Phylogeography; 16S rDNA; 18S rDNA

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1. Introduction

Two-thirds of the Earth's surface is covered by oceans, with the majority lying deeper than 1000 m. Nevertheless, our knowledge about the phylogeny, speciation, radiation and evolution of the animals inhabiting the deep sea is poor. Although large number of specimens have been brought back from many deep-sea expeditions, it has often been assumed that the deep-sea environment is homogenous, with communities largely comprised of opportunistic habitat generalists (Wolff, 1970; Menzies et al., 1973; Vinogradowa, 1997). During the 1960s and 1970s it became apparent that species diversity was much greater than originally expected (Sanders et al., 1965; Hessler and Sanders, 1967; Rex, 1981; Rex et al., 1993; Gray, 2002). Grassle and Maciolek (1992) estimated the total species pool of benthic deep-sea organisms at 10 million, while Lambshead (1993) suggested that such number might account only for the benthic macrofaunal species and would increase if meiofaunal taxa were included. While such estimates of deep-sea diversity are contentious (Briggs, 1991), it is clear that the deep-sea benthos harbours many more species than previously thought and may compare to other species-rich habitats such as coral reefs and tropical rain forest.

Today, we know that the biodiversity of the deep sea can vary regionally and locally (Grassle and Maciolek, 1992). Since the mid-1970s, numerous studies have been undertaken to determine the genetic population structure of deep-sea animals using a variety of biochemical and molecular techniques, especially allozymes electrophoreses and DNA sequencing. However, most studies have been carried out on vertebrates, especially Teleostei (e.g., Creasey and Rogers, 1999; Rogers, 2003; Aboim et al., 2005), and only a few studies analysing DNA sequences of invertebrates exist. For example, molecular studies on amphipods of the species *Eurythenes gryllus* reveal two distinct populations at different depths (France and Kocher, 1996). Chase et al. (1998) analysed specimens of the deep-sea protobranch bivalve *Deminucula atacellana* of the North Atlantic, showing that continental slope (<2500 m) and rise (>2500 m) populations are genetically distinct. The same is true for populations of different deep-sea basins (Zardus et al., 2006). High degrees of genetic differentiation also have been observed between abyssal populations of other molluscs (Etter et al., 1999, 2005; Peek et al., 2000; Quattro et al., 2001), the decapod *Chaceon quinquedon* (Weinberg et al., 2003) and some corals (France et al., 1996; Le Goff-Vitry, 2004a, b). However, more detailed studies have focused on the invertebrate fauna of hydrothermal vents, including tube worms (Vestimentifera) (e.g., Halanych et al., 2001; Goffredi et al., 2003; Hurtado et al., 2004) molluscs (e.g., Schwarzpaul and Beck, 2002; Won et al., 2003; Smith et al., 2004; Johnson et al., 2006) and bresiliid shrimps (Shank et al., 1999; Tokuda et al., 2006).

Despite the fact that asellote isopods are one of the most abundant taxons of the deep-sea fauna (Hessler and Wilson, 1983; Paterson and Lambshead, 1995; Howell et al., 2002), only two studies analysing the genetic variability within deep-sea asellotes exist until now (Raupach and Wägele, 2006; Brökeland and Raupach, 2007). However, both studies give striking evidence for high genetic variability and the existence of cryptic species. Asellote isopods are morphologically diverse small animals (less than 10 mm length) of reduced mobility, that have colonized the deep sea in several lineages (Wägele, 1989; Raupach et al., 2004). Even though munnopsoid species can swim temporarily (Hessler and Strömberg, 1989; Marshall and Diebel, 1995), Asellota are benthic animals. Their dispersal capacities may be restricted since isopods possess a marsupium (brood pouch) where the embryos undergo their entire larval development to juveniles (mancas). All these aspects probably reduce gene flow and increase the probability of speciation events.

Within the deep-sea Asellota, *Betamorpha fusiformis* (Barnard, 1920) represents an abundant and widespread munnopsoid of the deep Atlantic and Southern Oceans (Thistle and Hessler, 1977). Currently 10 different species of *Betamorpha* are known, which show subtle variations on a few features: the shape of the body, head and mouthfield are nearly identical, but the species are clearly separable on subtle differences of vertex shape, the position of uropod insertion and orientation of tergite margins (Thistle and Hessler, 1977). However, in spite of minor or subtle morphological traits, the identification of phenotypic plasticity and species-specific morphological traits will remain difficult. In this study mitochondrial 16S rDNA of 50 specimens of *B. fusiformis* were analysed to investigate the genetic variability within an abundant deep-sea munnopsoid morphospecies of the Southern Ocean. Beside this we analysed the

Table 1
Individual codes, haplotype codes, GenBank accession numbers for DNA sequences and sample locality of the analysed *Betamorpha fusiformis* specimens of this study

Individual codes	Haplotype group	Accession no.16S rDNA	Accession no.18S rDNA	Sample locality
BF7	A	EF116524		Cape Basin (16-10): 41°07′S/9°54′E; 4687–4669 m
BF9	A	EF116525		Cape Basin (16-10): 41°07′S/9°54′E; 4687–4669 m
BF10	A	EF116526		Cape Basin (16-10): 41°07′S/9°54′E; 4687–4669 m
BF11	A	EF116527	EF116546	Cape Basin (16-10): 41°07′S/9°54′E; 4687–4669 m
BF12	A	EF116528		Cape Basin (16-10): 41°07′S/9°54′E; 4687–4669 m
BF138	C	EF116535		Cape Basin (16-10): 41°07′S/9°54′E; 4687–4669 m
BF139	F	EF116523		Cape Basin (16-10): 41°07′S/9°54′E; 4687–4669 m
BF20	F	EF116520		Angulhas Basin (21-7): 47°38′S/4°15′E; 4555–4552 m
BF21	F	EF116521	EF116542	Angulhas Basin (21-7): 47°38′S/4°15′E; 4555–4552 m
BF22	F	EF116522		Angulhas Basin (21-7): 47°38′S/4°15′E; 4555–4552 m
BF57	B	EF116529		Kapp Norvegia (74-6): 71°18′S/13°57′W; 1030–1040 m
BF58	B	EF116530	EF116547	Kapp Norvegia (74-6): 71°18′S/13°57′W; 1030–1040 m
BF59	B	EF116531		Kapp Norvegia (74-6): 71°18′S/13°57′W; 1030–1040 m
BF60	B	EF116532		Kapp Norvegia (74-6): 71°18′S/13°57′W; 1030–1040 m
BF91	D	EF116533	EF116548	Explora Esc. (80-9): 70°39′S/14°43′W; 3103–3102 m
BF94	D	EF116534		Explora Esc. (80-9): 70°39′S/14°43′W; 3103–3102 m
BF81	E	EF116541		Weddell Sea (94-11): 66°38′S/27°05′W; 4893–4894 m
BF160	G	EF116501		Weddell Sea (94-11): 66°38′S/27°05′W; 4893–4894 m
BF162	G	EF116502		Weddell Sea (94-11): 66°38′S/27°05′W; 4893–4894 m
BF163	E	EF116539		Weddell Sea (94-11): 66°38′S/27°05′W; 4893–4894 m
BF164	E	EF116540		Weddell Sea (94-11): 66°38′S/27°05′W; 4893–4894 m
BF186	G	EF116510		Weddell Sea (102-13): 65°34′S/36°31′W; 4805–4803 m
BF187	G	EF116511		Weddell Sea (102-13): 65°34′S/36°31′W; 4805–4803 m
BF188	G	EF116512		Weddell Sea (102-13): 65°34′S/36°31′W; 4805–4803 m
BF189	G	EF116514		Weddell Sea (102-13): 65°34′S/36°31′W; 4805–4803 m
BF190	G	EF116513		Weddell Sea (102-13): 65°34′S/36°31′W; 4805–4803 m
BF191	G	EF116515		Weddell Sea (102-13): 65°34′S/36°31′W; 4805–4803 m
BF192	G	EF116516	EF116543	Weddell Sea (102-13): 65°34′S/36°31′W; 4805–4803 m
BF193	G	EF116517		Weddell Sea (102-13): 65°34′S/36°31′W; 4805–4803 m
BF194	G	EF116518		Weddell Sea (102-13): 65°34′S/36°31′W; 4805–4803 m
BF195	G	EF116519		Weddell Sea (102-13): 65°34′S/36°31′W; 4805–4803 m
BF196	G	EF116493		Weddell Sea (102-13): 65°34′S/36°31′W; 4805–4803 m
BF197	G	EF116509		Weddell Sea (102-13): 65°34′S/36°31′W; 4805–4803 m
BF225	G	EF116508		Weddell Sea (102-13): 65°34′S/36°31′W; 4805–4803 m
BF204	G	EF116495		Weddell Sea (110-8): 65°0′S/43°02′W; 4698–4696 m
BF205	G	EF116498		Weddell Sea (110-8): 65°0′S/43°02′W; 4698–4696 m
BF206	G	EF116494		Weddell Sea (110-8): 65°0′S/43°02′W; 4698–4696 m
BF208	G	EF116496	EF116544	Weddell Sea (110-8): 65°0′S/43°02′W; 4698–4696 m
BF210	E	EF116537		Weddell Sea (110-8): 65°0′S/43°02′W; 4698–4696 m
BF211	G	EF116499		Weddell Sea (110-8): 65°0′S/43°02′W; 4698–4696 m
BF216	E	EF116538		Weddell Sea (110-8): 65°0′S/43°02′W; 4698–4696 m
BF229	G	EF116503		Weddell Sea (110-8): 65°0′S/43°02′W; 4698–4696 m
BF239	G	EF116504		Weddell Sea (110-8): 65°0′S/43°02′W; 4698–4696 m
BF246	G	EF116500	EF116545	Weddell Sea (110-8): 65°0′S/43°02′W; 4698–4696 m
BF250	G	EF116505		Weddell Sea (110-8): 65°0′S/43°02′W; 4698–4696 m
BF251	G	EF116506		Weddell Sea (110-8): 65°0′S/43°02′W; 4698–4696 m
BF252	G	EF116497		Weddell Sea (110-8): 65°0′S/43°02′W; 4698–4696 m
BF253	G	EF116507		Weddell Sea (110-8): 65°0′S/43°02′W; 4698–4696 m
BF254	E	EF116536		Weddell Sea (110-8): 65°0′S/43°02′W; 4698–4696 m
BF287	G	EF116492		Weddell Sea (121-11): 63°37′S/50°38′W; 2663–2659 m

complete nuclear 18S rRNA gene of seven selected specimens from different sites.

2. Material and methods

2.1. Specimens, sampling and DNA isolation

Sample localities, individual codes and accession numbers are listed in Table 1. All analysed specimens of *B. fusiformis* were collected in the Southern Ocean during the ANDEEP III expedition (ANT XXII/3) in 2005. Animals were caught using an epibenthic sledge or Agassiz trawl deployed from RV *Polarstern* (see Fahrbach, 2006). The outgroup taxon *Ilyarachna* sp. was collected during the expedition ANT XXI/2 in 2003/2004 (station 284-1, for more details see Arntz and Brey, 2005). Due to the fact that isopods display highly active nucleases, which digest DNA quickly (Dreyer and Wägele, 2001, 2002), a fast fixing with precooled ethanol (0 °C) was essential. After collection, samples were stored for at least 36 h at 0 °C. DNA was extracted on board from several dissected legs of the specimens, using the QIAmp© Tissue Kit (Qiagen GmbH), following the extraction protocol. Specimens have been deposited in the collection of the Zoological Institute and Museum of Hamburg, Germany.

2.2. PCR amplification and DNA sequencing

The polymerase chain reaction (PCR, Saiki et al., 1988) was used to amplify a homologous region of the mitochondrial 16S rRNA gene, ranging from 513 to 520 base pairs (bp) in 50 specimens of *B. fusiformis* (see Table 1) and one specimen of the genus *Ilyarachna* (accession no. EF116491). Amplifications were performed in 25-μl reactions containing 2.5 μl 10 × Qiagen PCR buffer, 2.5 μl dNTPs (2 mmol/μl), 0.3 μl of each primer (forward primer 5′-CGC CTG TTT ATC AAA AAC AT-3′, reverse primer 5′-CCG GTC TGA ACT CAG ATC ACG-3′, both 50 pmol/μl (Palumbi et al., 1991)), 1–2 μl of DNA template, 5 μl Q-Solution©, 0.2 μl Qiagen Taq (5 U/μl), filled up to 25 μl with sterile H_2O, on a Progene Thermocycler (Techne Ltd.). The temperature profile of the PCR consisted of an initial denaturation of 94 °C (5 min), followed by 38 cycles of 94 °C (45 s), 44 °C (45 s) and 72 °C (70 s). Negative and positive controls were included with each round of reactions. Three μl of amplified product were controlled by electrophoresis in a 1% agarose gel with ethidium bromide using DNA size standards, while the remaining PCR product was purified with the QIAquick© PCR Purification Kit (Qiagen GmbH). Purified PCR products were cycle sequenced (Sanger et al., 1977) from both directions with the same primers used for amplification using a Thermo-Sequenase Fluorescent Labelled Primer Cycle Sequencing Kit (Amersham Pharmacia Biotech) on a Primus96^{plus} Thermocycler (MWG-Biotech AG). Cycle sequencing conditions were: 2 min at 94 °C (initial denaturation), followed by 30 cycles of denaturation at 94 °C for 25 s, annealing at 56–58 °C for 25 s, and extending at 70 °C for 35 s. A LI-COR 4000 (LI-COR Inc.) was used for automated sequencing.

In addition to the mitochondrial gene sequences, complete 18S rDNA sequences of seven specimens of *B. fusiformis* (see Table 1) and one specimen of *Ilyarachna* sp. (EF116549) were amplified and sequenced. Detailed information about PCR primers, PCR conditions and sequencing primers are given in Dreyer and Wägele (2001). Sequencing gels were proof-read using the image analysis software of the automated sequencer. Double stranded sequences were assembled with the program AlignIR v1.2; identities of all new sequences were confirmed with BLAST searches (Altschul et al., 1997). All sequences were deposited in GenBank (EF116491-EF116549, see Table 1).

2.3. Sequence alignment and phylogenetic analyses of sequence data

Sequences were aligned using MUSCLE Version 3.6 (Edgar, 2004) with default settings, generating an alignment of 534 bp with 129 parsimony-informative characters (16S rDNA) and 2260 bp with 40 parsimony-informative characters (18S rDNA), respectively, which can be obtained at request from the first author. All variable positions of the 16S rDNA are spread across the sequenced part, but most variations of the 18S rDNA sequences are located within the expansion segments V4 and V7. Both alignments were tested for nucleotide bias using a chi-square test of base composition homogeneity across taxa implemented in PAUP*4.0b10 (Swofford, 2002). The appropriate best-fit substitution model of DNA evolution for both data sets was determined using the Akaike information criterion (AIC) (Akaike, 1974) implemented in MODELTEST 3.7 (Posada and Crandall, 1998). Bayesian analyses were run for both data sets in MrBayes v3.1 (Huelsenbeck and Ronquist, 2001),

using the model and parameters selected by MODELTEST. Two parallel Markov chain Monte Carlo analyses were run for 20,000,000 generations starting with using random trees. Trees were sampled every 100 cycles, yielding 398,002 (16S rDNA) and 398,502 (18S rDNA) samples of the Markov chains after "burn ins" of 1000 (16S rDNA alignment) and 750 (18S rDNA alignment) generations. In addition, PAUP*4.0b10 was used for distance calculations and performing maximum parsimony analyses under the TBR branch swapping algorithm. To assess statistical support for hypothesized clades, 5000 (16S rDNA) and 10,000 bootstrap replicates (18S rDNA) were calculated.

3. Results

MODELTEST 3.7 indicated that the TrN + I + G model (for 16S rDNA) and the GTR + I model (for 18S rDNA), were the appropriate nucleotide substitution models with the following parameters: TrN + I + G: nucleotide frequencies (A: 0.3688, C: 0.1328, G: 0.1723, T: 0.3262), substitution rates (R[AC]: 1.00, R[AG]: 3.40, R[AT]: 1.00, R[CG]: 1.00, R[CT]: 7.19, R[GT]: 1.00), gamma distribution shape = 0.5082 and a proportion of invariable sites = 0.3454; GTR + I: nucleotide frequencies (A: 0.2318, C: 0.2301, G: 0.2759, T: 02622), substitution rates (R[AC]: 0.22, R[AG]: 0.51, R[AT]: 0.39, R[CG]: 0.27, R[CT]: 1.93, R[GT]: 1.00) and a proportion of invariable sites = 0.8511. Both alignments show no significant differences in base composition (chi-square test 16S rDNA [18S rDNA]: df = 150 [21], $p = 1.00$ [1.00]).

Fig. 1 shows the 50% majority rule consensus tree of the 16S rDNA sequences recovered using the Bayesian approach, while Fig. 2 provides the Bayesian results of the 18S rDNA data set. Bootstrap values of maximum parsimony analyses were plotted in addition to the posterior probabilities. The phylogenetic tree of the 16S rDNA sequences is characterized by seven major divergent monophyletic clades of haplotypes (haplotype groups A–G) within the analysed *B. fusiformis* specimens, all supported by high posterior probabilities (0.92–1.00) and bootstrap values (94–100). In contrast to this, most relationships between these clades are poorly supported. However, both analysed data sets give evidence for a close relationship of the haplotype groups B, C, D and E.

Average uncorrected (*p*-distances) and corrected distances (*d*-distances) of both data sets are listed in Tables 2 and 3. Values between the seven haplotype groups range from 0.0470 to 0.1440 (*p*-distances) across all 16S rDNA sequences (*d*-distances: 0.0592–0.2850) and from 0.0032–0.0174 (*d*-distances: 0.0033–0.0195) for the complete 18S rDNA sequences. However, there are no intermediate values between the seven 16S rDNA haplotype groups. Fig. 3 shows the geographic distribution of all 16S rDNA haplotype groups, revealing relationships between biogeography, sympatric occurrence and genetic variation. For example, four haplotype groups (A, B, C and D) were found only at one locality, while all other groups at least occur at two sample regions. Haplotype groups A and C are only found in the Cape Basin (station 16), group B on the Antarctic shelf (station 74) and haplotype group D in the deep eastern Weddell Sea (station 80). In contrast to these restricted distributions, specimens bearing haplotype group G were found at four stations (station 94, 102, 110 and 121) across the Weddell Abyssal Plain, group E at two stations on the central Weddell Abyssal Plain and group F in the Cape and Angulhas Basins.

4. Discussion

The use of mitochondrial DNA to study the phylogeography or genetic variability of populations has become quite popular in the last years (e.g., Avise, 2000; Hewitt, 2001). First studies with a focus on deep-sea organisms revealed a high genetic variability within species (Chase et al., 1998; Quattro et al., 2001; Le Goff-Vitry et al., 2004b; Etter et al., 2005; Zardus et al., 2006), but there are only a few examples for 16S rDNA distances between populations of deep-sea crustaceans. While France and Kocher (1996) found a maximum of 0.112 for *p*-distances within bathymetrically separated populations of the lysianassid amphipod *E. gryllus*, Weinberg et al. (2003) detected values up to 0.029 between different populations of the deep-sea crab *C. quinquedens*. The first case study of deep-sea Asellota (Raupach and Wägele, 2006) found ML distances of up to 0.1689 (minimum: 0.1115) between three distinct populations of the *Acanthaspidia drygalskii* species complex in the Southern Ocean. However, the only use of mitochondrial DNA can be problematic. Polymorphisms may be caused by introgression or incomplete lineage sorting (e.g., Mason et al., 1995; Gießler et al., 1999; Sota et al., 2001; Wares, 2001) and suggest speciation when there is none. In these cases

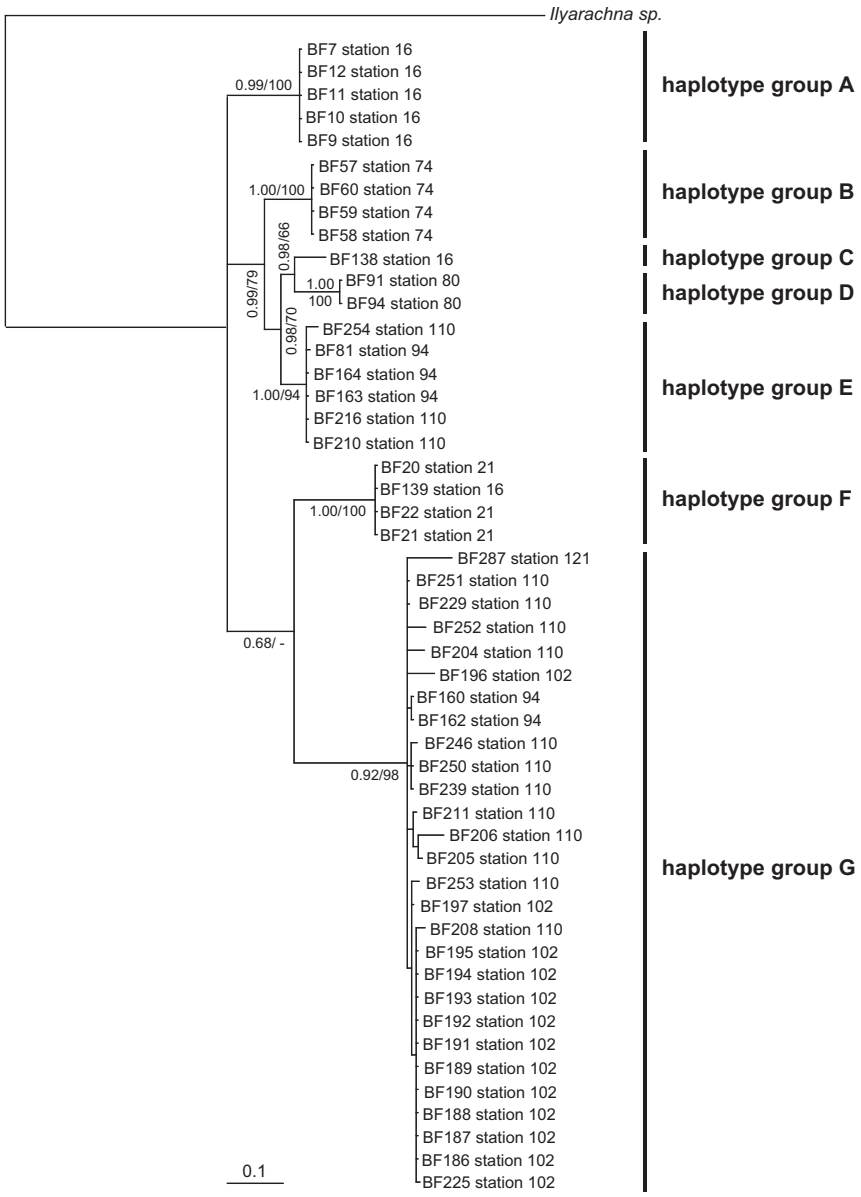

Fig. 1. Bayesian 50% majority rule consensus tree of the 16S rDNA data set. Model choice based on the AIC: TrN model with gamma distributed rates (alpha = 0.5082) and a proportion of invariant sites (0.3454). Numbers at the nodes represent posterior probabilities (left; values below 0.50 not shown), and bootstrap values of an additional maximum parsimony analysis (right; values below 50% not plotted). For more details see text. The tree is characterized by seven major divergent monophyletic haplotype clades (A–G), all supported by high posterior probabilities and bootstrap values.

the incongruence between mitochondrial and nuclear gene phylogenies (Chen et al., 2004) will indicate that the mitochondrial phylogeny is not the one of the phenotype. The additional analysis of nuclear genes represents a good method solving this problem. This has been demonstrated by Brökeland and Raupach (2007), who found *p*-distances of at least 0.0732 (*d*-distances: 0.0914) for the 16S rDNA and 0.0140 (*d*-distances: 0.0143) for the 18S rDNA between morphologically very similar species of the deep-sea asellote genus *Haploniscus*.

In our present study, *p*-distances range from 0.0470 (*d*-distances: 0.0592) to 0.1440 (0.2850) across all 16S rDNA sequences (see Table 2), while distances for the complete 18S rDNA have values of 0.0032–0.0174 (*d*-distances: 0.0033–0.0195;

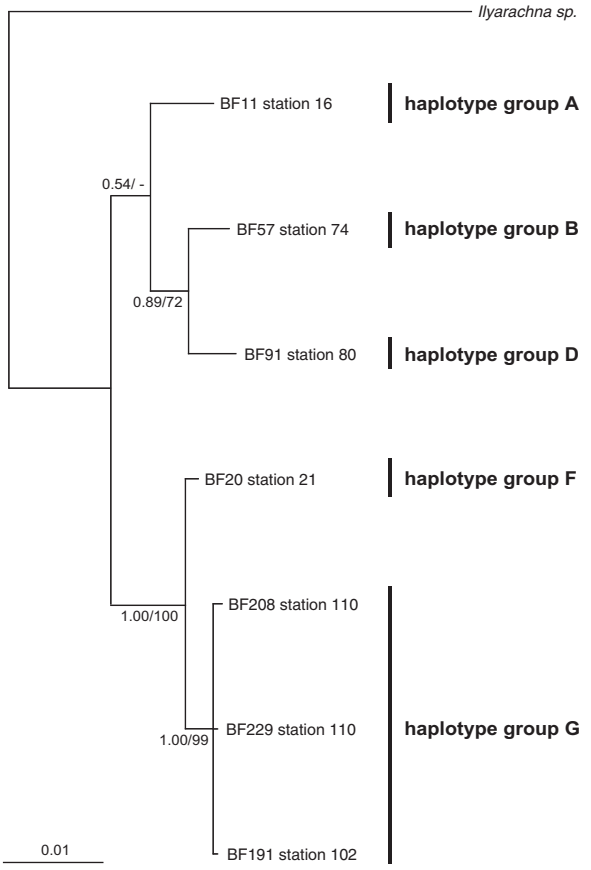

Fig. 2. Bayesian 50% majority rule consensus tree of the 18S rDNA data set, with a model choice based on the AIC: GTR model with a proportion of invariant sites (0.8511). Numbers at the nodes represent support by posterior probabilities (left) and bootstrap values of an additional maximum parsimony analysis (right). Posterior probabilities with values below 0.50 and bootstrap values below 50% are not shown. For more details see text.

see Table 3). Some 16S rDNA haplotype groups (C, D and E) have lower distances than those found by Brökeland and Raupach (2007), and 18S rDNA distances between group A, B and D also have lower values. However, it should be mentioned that the genus *Betamorpha* does not belong to the Haploniscidae, and therefore even lower distance values may indicate distinct species. On the other hand, 16S rDNA distances between haplotype groups F and G (0.1176/0.1884) are high, but low for 18S rDNA distances (*p*-distances: 0.0032–0.0037, *d*-distances: 0.0033–0.0038). This result may be caused by ancient polymorphisms in the mitochondrial DNA (see above) or caused by different rates of evolution between mitochondrial and nuclear genes. Of course, the best method to test if gene flow between haplotype groups is still possible are crossbreeding experiments, but such studies handling deep-sea species are currently impossible. Nevertheless, it is interesting to see that some haplotype groups are widespread and sample locations are separated by hundreds of kilometres (e.g. specimens of haplotype G were sampled across the Weddell Abyssal Plain, see Fig. 3).

Our study reveals the existence of closely related but distinct species within an abundant and widespread asellote isopod, raising an important question: do widespread benthic deep-sea Asellota really exist at all? Many asellote species are known to have a wide distribution, e.g., *Bathyopsurus nybelini* (Wolff, 1962) or *Acanthocope galathea* (Schmid et al., 2002). These observations are only based upon morphology, and the genetic divergence remains unknown. However, there are two detailed morphological studies dedicated to variations in widespread asellote species. First, *Eurycope complanata*, once thought to have a wide distribution in the North Atlantic, has been shown to be a complex of at least 12 species, each with a restricted distribution (Wilson, 1983a). On the other hand, clinal variations in the form of the cephalic rostrum have been observed in *Eurycope iphthima* (Wilson, 1983b), allowing to identify distinct populations.

All available data, both molecular and morphological, reveal an unexpected high variability within deep-sea asellotes, which has crucial consequences for studies of taxonomy and biodiversity. We hypothesize that most, if not all, widespread asellote species and many other Peracarida with benthic life styles, for example Tanaidacea, represent are in reality widespread groups of closely related but distinct species that also can appear in sympatry. We name this hypothesis the "patchwork theory". Factors affecting speciation and generation of biodiversity in the deep sea are almost unknown (Wilson and Hessler, 1987), and most hypotheses on speciation processes are mainly based on the study of zoogeographic patterns (Gage and Tyler, 1991; Creasey and Rogers, 1999). On the other hand, environmental gradients, for example the deep-sea sediment structure, texture and composition, deep-sea currents, benthic storms or different rates of nutrient input from surface production, are perceived as most important in providing the opportunities for the selective pressures that drive speciation (Gage and Tyler, 1991; Gage, 1996; Rex et al., 2005). It is also not clear if asellote isopods or other

Table 2
Average uncorrected (*p*-distances, upper values) and maximum-likelihood estimates (*d*-distances, lower values) of the seven haplotype groups of mitochondrial 16S rDNA sequences of *Betamorpha fusiformis*

	Haplotype group A ($n = 5$)	Haplotype group B ($n = 4$)	Haplotype group C ($n = 1$)	Haplotype group D ($n = 2$)	Haplotype group E ($n = 6$)	Haplotype group F ($n = 4$)	Haplotype group G ($n = 28$)
Haplotype group A ($n = 5$)	0						
	0						
Haplotype group B ($n = 4$)	0.0943	0					
	0.1430	0					
Haplotype group C ($n = 1$)	0.1002	0.0605	0				
	0.1642	0.0853	0				
Haplotype group D (n = 2)	0.1100	0.0625	0.0507	0			
	0.1918	0.0858	0.0664	0			
Haplotype group E ($n = 6$)	0.0905	0.0573	0.0470	0.0536	0.0028		
	0.1428	0.0752	0.0592	0.0720	0.0035		
Haplotype group F ($n = 4$)	0.1338	0.1230	0.1175	0.1193	0.1069	0	
	0.2489	0.2196	0.2032	0.2132	0.1744	0	
Haplotype group G ($n = 28$)	0.1356	0.1440	0.1352	0.1391	0.1392	0.1176	0.0102
	0.2653	0.2850	0.2575	0.2670	0.2655	0.1884	0.0234

For more details about the calculation of *d*-distances see text. With n = number of specimens.

Table 3
Uncorrected (lower triangle, upper values) and maximum-likelihood estimates (lower triangle, lower values) of pairwise genetic distances of seven complete 18S rDNA sequences of *Betamorpha fusiformis*

	Haplotype group A (BF11)	Haplotype group B (BF57)	Haplotype group D (BF91)	Haplotype group F (BF20)	Haplotype group G (BF208)	Haplotype group G (BF191)	Haplotype group G (BF229)
Haplotype group A (BF11)	–	11	10	18	16	16	16
		17	17	17	20	19	19
Haplotype group B (BF57)	0.0126		11	16	17	17	17
	0.0135	–	6	16	19	18	18
Haplotype group D (BF91)	0.0122	0.0077		17	18	18	18
	0.0131	0.0080	–	17	20	19	19
Haplotype group F (BF20)	0.0160	0.0146	0.0155		5	5	5
	0.0175	0.0162	0.0172	–	3	2	2
Haplotype group G (BF208)	0.0165	0.0165	0.0174	0.0037		0	0
	0.0183	0.0186	0.0195	0.0038	–	1	1
Haplotype group G (BF191)	0.0160	0.0160	0.0169	0.0032	0.0005		0
	0.0176	0.0178	0.0188	0.0033	0.0005	–	0
Haplotype group G (BF229)	0.0160	0.0160	0.0169	0.0032	0.0005	0.0000	
	0.0176	0.0178	0.0188	0.0033	0.0005	0.0000	–

Upper triangle: number of observed genetic distances (transitions versus transversions).

deep-sea animals are examples of a source-sink hypothesis, which has been demonstrated for abyssal molluscs (Rex et al., 2005). However, Knowlton (1993, 2000) argued that marine habitats are filled with cryptic and sibling species even though they are rarely identified due to our limited access to marine habitats and to the fact that speciation processes may be less coupled to morphology than to other phenotypic aspects, notably chemical recognition systems.

We have just begun to understand that the remarkable diversity of the deep-sea fauna is

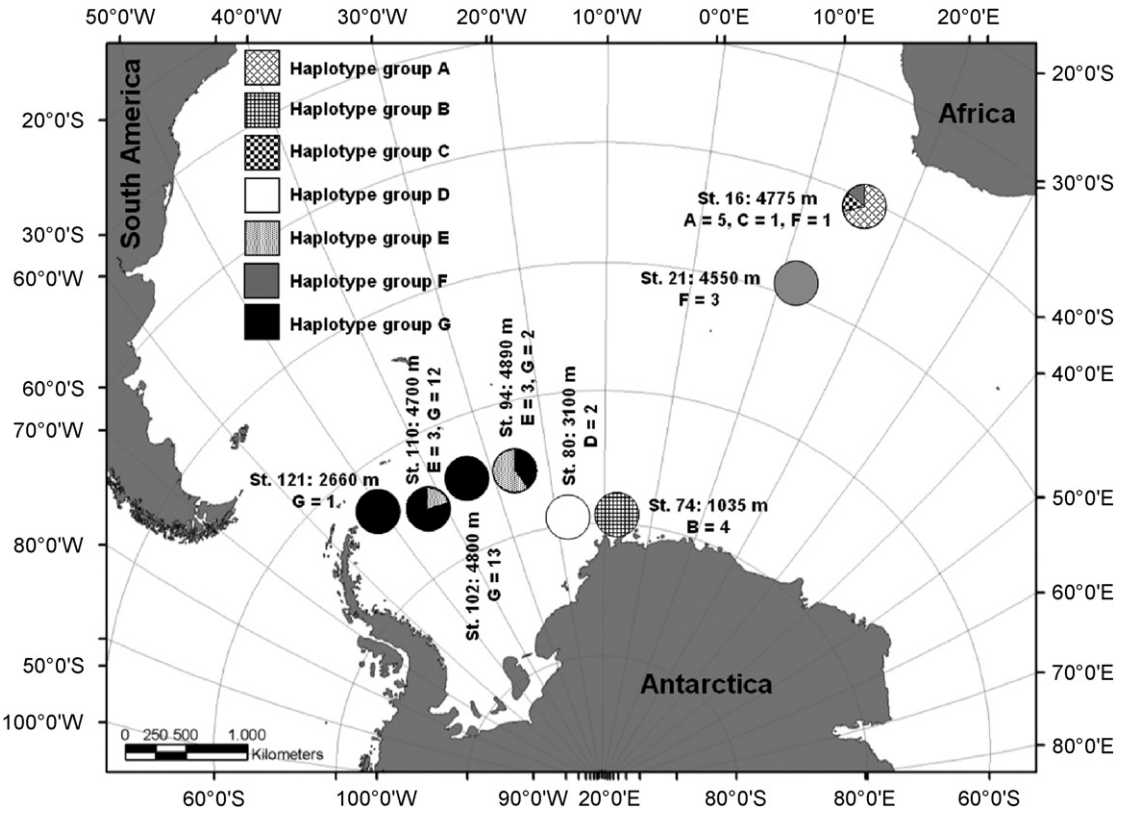

Fig. 3. Geographic distribution of the seven sampled 16S rDNA haplotype groups (A–G) of *Betamorpha fusiformis* in the Southern Ocean.

difficult to explain and continues to challenge contemporary ecological and evolutionary theory (Etter et al., 2005), but it is obvious that molecular methods will become essential to understand the diversity, speciation processes and the evolutionary origin of the deep-sea fauna.

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