Mitochondrial sequence data expose the putative cosmopolitan polychaete Scoloplos armiger (Annelida, Orbiniidae) as a species complex

Christoph Bleidorn*1,2, Inken Kruse3, Sylvia Albrecht1,4 and Thomas Bartolomaeus1

Address: 1Animal Systematics and Evolution, Institute for Biology, Zoology, Free University Berlin, Koenigin-Luise-Str. 1-3, D-14195 Berlin, Germany, 2Unit of Evolutionary Biology/Systematic Zoology, Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Strasse 24-25, Haus 26, D-14476 Potsdam-Golm, Germany, 3Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, Florida, USA and 4Institute for Zoo and Wildlife Research, 10252 Berlin, Germany

Email: Christoph Bleidorn* - bleidorn@uni-potsdam.de; Inken Kruse - inkenkruse@web.de; Sylvia Albrecht - sylvia-albrecht@arcor.de; Thomas Bartolomaeus - tbartol@zoosyst-berlin.de

* Corresponding author

Abstract

Background: Polychaetes assigned as Scoloplos armiger (Orbiniidae) show a cosmopolitan distribution and have been encountered in all zoogeographic regions. Sibling S. armiger-like species have been revealed by recent studies using RAPDs and AFLP genetic data. We sequenced a ~12 kb fragment of the Scoloplos cf. armiger mitochondrial genome and developed primers for variable regions including the 3' end of the cox3 gene, trnQ, and most of nad6. A phylogenetic analysis of this 528-nucleotide fragment was carried out for S. armiger-like individuals from the Eastern North Atlantic as well as Pacific regions. The aim of this study is to test the cosmopolitan status, as well as to clarify the systematics of this species complex in the Eastern North Atlantic, while using a few specimens from the Pacific Ocean for comparison.

Results: Phylogenetic analysis of the cox3-trnQ-nad6 data set recovered five different clades of Scoloplos cf. armiger. The fragment of the mitochondrial genome of Scoloplos cf. armiger is 12,042 bp long and contains 13 protein coding genes, 15 of the 22 expected tRNAs, and the large ribosomal subunit (rrnL).

Conclusion: The sequenced cox3-trnQ-nad6 fragment proved to be very useful in phylogenetic analyses of Scoloplos cf. armiger. Due to its larger sampling scale this study goes beyond previous analyses which used RAPD and AFLP markers. The results of this study clearly supports that Scoloplos armiger represents a species complex and not a cosmopolitan species. We find at least two S. armiger-like species within the Pacific region and three different S. armiger-like species in the North Atlantic. Implications for the taxonomy and the impact on ecological studies are discussed.
respects one of the dominant macrofauna species in a Norwegian fjord [1], in a Portuguese estuary [2], and in the Peter the Great Bay in the Sea of Japan [3]. Besides its wide ranging distribution, S. armiger also plays a more or less important role in recent ecological studies. It has been identified as a possible intermediate host for the flatfish nematode Cucullanus heterochrous [4] and the population dynamics of S. armiger and its predator Nephtys hombergii (Nephtyidae) on intertidal flats in the Netherlands’ part of the Wadden Sea are well studied [5].

Scoloplos armiger (Orbiniidae) has been reported to show a cosmopolitan distribution and has been encountered in all zoogeographic regions [6,7] where it is present from the intertidal to the subtidal [8,9]. In the North Sea region it is one of the most common polychaetes and a direct development in egg cocoons was observed at many intertidal flats [8]. In additional to these well known and eye-catching cocoons, free swimming pelagic larvae of these worms have been reported from the North Sea near the island of Helgoland, Germany [10].

For many marine invertebrate species a worldwide distribution has been reported. At least four hypotheses can reasonably explain such a distribution pattern; (a) truly cosmopolitan species, (b) cosmopolitan morphospecies which correspond to genetically distinct species [11,12], (c) poor taxonomic understanding of a taxon, causing "the cosmopolitan syndrome" [13-15] and (d) cosmopolitan species where the current range distribution is the result of human introductions. An example for the latter is the reef-building serpulid Ficopomatus enigmaticus which can be found in brackish waters of warm-temperate regions all over the world, and which is supposed to be distributed through human shipping [16]. However, most reports of cosmopolitan distributional ranges of marine invertebrate species after application of molecular methods turned out to be the result of an over-conservative taxonomy [17-19].

Among marine invertebrates, polychaete annelids have a high frequency of cosmopolitan species [20,21]. Polychaetes like Owenia fusiformis (Oweniidae), Sertaspis scutata (Sertaspidae) and Scoloplos armiger (Orbiniidae) are recorded from all oceans in different depths and nearly all temperate regions [7,15,22]. However O. fusiformis later has been found to consist of more than one species [23].

Only a few genetic studies investigated such "cosmopolitan" polychaetes and most of them did not use discrete nucleotide data. RAPDs and ITS sequence data confirmed the amphi-Atlantic distribution pattern of the ctenodrilid Ctenodrilus serratus [24]. The worldwide distributed Pettitia amphipathalma (Syllidae) has been investigated with RAPD markers [25,26] which do not support the cosmopolitan status of this taxon. The phylogeography of the invasive sabellid Sabella spallanzanii was investigated using nuclear markers [27] and human introduction to Australia due to ballast water has been suggested for this polychaete. The cosmopolitan status of Hesionides arenaria (Hesionidae) was confirmed using RAPD markers [28], nevertheless it cannot be ruled out that lack of differences in the band pattern of RAPDs is due to primer choice. In contrast to this, the cosmopolitan status of another hesionid (Hesionides gohari) was not supported by RAPD data [29].

Interestingly, none of these studies used mitochondrial markers which are commonly used for phylogeographic studies in other animal groups [30]. This might be due to the lack of suitable primers for the amplification of variable regions of the mitochondrial genome. Although many polychaetes are recorded from different zoogeographic regions, truly cosmopolitan species seem to be rare, and in many cases taxonomy is unable to distinguish between morphologically similar taxa [31]. It is supposed that widely distributed species are frequently being found to consist of distinguishable subspecies or siblings when examined in sufficient detail [12].

In a series of papers it has been shown with RAPD data, AFLPs, cross-breeding experiments and investigation of the sperm morphology, that different developmental traits of Scoloplos armiger collected near the island of Sylt (Germany) belong to two distinct Scoloplos species [32-34]. This means that two sympatric sibling species of Scoloplos cf. armiger occur in the North Sea: one living in the intertidal with egg cocoons and one living subtidally with pelagic larvae.

The aim of the present study is to investigate the status of different Scoloplos cf. armiger populations in the Northern East Atlantic (see Fig. 1 for collection sites) and the Northern East Pacific using mitochondrial markers. For this purpose we sequenced a 12 kb fragment of the mitochondrial genome of Scoloplos cf. armiger to develop primers for a variable mitochondrial region. Our present study gives no support for a cosmopolitan distribution of Scoloplos cf. armiger and phylogenetic analyses of the investigated populations reveal five distinct reciprocal monophyletic clades of Scoloplos cf. armiger.

Results

Genome organisation, base composition, and codon usage of the mitochondrial genome of Scoloplos cf. armiger

The fragment of the mitochondrial genome of Scoloplos cf. armiger individual SL14 is 12,042 bp long and contains 13 protein coding genes, 16 of the 22 expected tRNAs, and the large ribosomal subunit (rrnL). As in the case for all annelids so far studied all genes are transcribed from the same strand. One difference found in the gene arrange-
ment of *Scoloplos cf. armiger* when compared with the other known orbiniid mitochondrial genome of *Orbinia latreillii* [35] is that the gene trnG is missing within the so far sequenced portion of *Scoloplos cf. armiger* (Fig. 2). The mitochondrial genome is AT-rich (63.66%), and the base frequencies are A = 0.31, C = 0.24, G = 0.12, and T = 0.33. All 13 protein-coding genes typically found in metazoan mtDNA [36] are identified for *Scoloplos cf. armiger*. In 12 of these AUG is used as a start codon. The exception is *cox3*, for which sequence alignment comparison with other annelids reveals the use of GUU as alternative start codon. An alternative start codon is also found for the
same gene in *Orbinia* [35]. Except for *nad1* complete stop codons (eight times UAA and three times UAG) are found in all other protein coding genes in *Scoloplos cf. armiger* (with the exception that the 3’ end of *nad4* is not completely sequenced).

Phylogenetic analysis of the **cox3**–**trnQ**–**nad6** data set

Within 55 individuals we found 25 unique sequence haplotypes for which we produced an alignment spanning 528 characters. Of these 528 characters, 245 characters are constant, 105 characters are variable but parsimony uninformative, and 178 are parsimony informative. The nucleotide composition is AT biased, as is it common for polychaete mitochondria [35,37] and the empirical base frequencies are A = 0.318, C = 0.227, G = 0.113, and T = 0.342. The chi-square test of homogeneity of base frequencies across taxa resulted in no significant P-values (chi-square = 25.28, df = 72, P = 0.999).

The application of the different phylogenetic methods yielded different tree topologies (Fig. 3), but the same major clades are recovered by all. The MP approach yielded 40 equally parsimonious trees (each with 593 steps) which are presented as a strict consensus tree (Fig. 3b). Five different reciprocal monophyletic clades of *Scoloplos cf. armiger* are recovered: a clade containing the individuals from Malibu ('Malibu clade'), one containing the individuals from San Diego ('San Diego clade'), one containing the intertidal specimens from Roscoff, Low Newton by the Sea, and Sylt ('intertidal clade'), one containing individuals from Sylt and Fehrmanns Belt which were collected from the subtidal ('subtidal clade'), and one also containing individuals from the latter two locations, as well as individuals from Trondheim and Kristiansand. The sample site in Kristiansand is located near the type locality and so this clade is named the 'type locality clade'. The 'Malibu clade' is represented by two identical sequences and the monophyly of the other clades is well supported through bootstrap values and Bayesian posterior probabilities (BPP) (Fig. 3). The relationship between these clades remains unclear, but no analysis recovered a monophyletic *Scoloplos cf. armiger* clade. ML and Bayesian inference indicates that the 'Malibu clade' is closely related to *Leitoscoloplos pugettensis*, but this relationship is only poorly supported through BPP (0.87), as well as that there is a sistergroup relationship between the 'San Diego clade' and *Scoloplos* sp. The relationships between the three clades from European waters are also not clear. Whereas MP recovers a sister group relationship between the 'type locality clade' and the 'intertidal clade' (Fig. 3b), a sister group relationship between the 'subtidal clade' and the 'type locality clade' is suggested by the most likely tree (Fig. 3c, 3d). The majority rule tree of the Bayesian inference does not resolve this issue (Fig. 3a).

As expected from this phylogenetic analyses, comparison of average nucleotide diversity between different *Scoloplos cf. armiger* clades shows that variation between clades (Table 2) are much higher than within clades (Table 3).

There are several amino acid changes within the *nad6* gene (Fig. 4). Within the *Scoloplos cf. armiger* group unique amino acid substitutions are present for the 'Malibu clade', the 'San Diego clade', the 'subtidal clade', and the 'type locality clade'.

**trnQ secondary structures**

Proposed *trnQ* secondary structures for all clades/taxa are given in Figure 5 and all possess the common cloverleaf structure with an acceptor stem, TPAC stem and loop, anticodon stem and loop, and DHU stem and loop (clockwise in Fig. 5). Secondary structures are identical within each clade/taxon and therefore only structures of one individual are shown. The secondary structure predicted for the 'San Diego clade' differs from the other *Scoloplos cf. armiger* taxa in possessing 5 bp instead of 4 bp in the TPAC...
Figure 3
Phylogenetic relationships of *Scoloplos*. (A–C) Topologies generated by different phylogenetic analyses: (A) Majority-rule consensus of the Bayesian analysis (GTR+I+Γ) with posterior probabilities at the nodes; (B) Strict consensus of the 40 equally parsimonious trees generated by maximum parsimony (MP) analyses with bootstrap values at the nodes; (C) Maximum Likelihood topology (GTR+I+Γ) with bootstrap values at the node; (D) Maximum Likelihood tree with branch lengths.
loop, 4 paired bases instead of 5 paired bases in the anticodon stem, and 9 bp instead of 7 bp in the anticodon loop.

**Discussion**

**Relationships of different Scoloplos cf. armiger populations**

The results of the phylogenetic analyses of the cox3-trnQ-trnA nad6 data set clearly supports that Scoloplos armiger represents a species complex and not a cosmopolitan species. We find at least two S. armiger-like genetic clades within the Pacific region and these are more closely related to other Pacific species (Leitoscoloplos pugettensis, Scoloplos sp. from Morro Bay, CA) than to the European Scoloplos cf. armiger clades. Under the phylogenetic species concept sensu Mishler and Theriot [38] it is parsimonious to assume that these clades represent distinct evolutionary lineages which should be considered as species distantly related to S. armiger. We identified three well supported reciprocal monophyletic clades within European Scoloplos cf. armiger clades. Applying the phylogenetic species concept sensu Mishler and Theriot [38] to our data, we hypothesize the presence of at least three different species (subtidal clade, type locality clade, intertidal clade) formerly referred to as S. armiger in the North Atlantic.

The inference of a monophyletic clade containing all intertidally collected individuals from European waters confirms the results that individuals that are producing egg-cocoons and live on intertidal flats represent a distinct evolutionary lineage rather than part of a S. armiger species with different developmental modes [32]. Surprisingly the results show that in subtidal areas of the North Sea and Baltic Sea there are two clearly separated genetic clades of Scoloplos cf. armiger, which are also distinct in amino acid data: the 'subtidal clade' and the 'type locality clade'. Whereas we found both genetic types in the North Sea and Baltic Sea samples in sympatry, only one of these clades seems to be present in the Norwegian samples, which include the type locality. As pointed out before, we consider it likely that these two genetic clades represent two different species. However, at this point the possibility must be considered that processes unrelated to speciation have generated reciprocal monophyletic mtDNA haplotype lineages [39], especially for the separation of the 'subtidal clade' and the 'type locality clade'. This hypothesis should be tested with additional data, e.g. by application of independent nuclear markers.

Differences in sperm morphology and in the length of anal cirri of benthic juveniles between intertidal and

---

### Table 1: Sampling sites, sequenced individuals, and GenBank accession numbers of analysed taxa.

| Taxon Location | Individuals | Accession-Nr. |
|----------------|-------------|---------------|
| Orbinia lateilli | Roscoff, France | AY961084 |
| Leitoscoloplos fragilis | Little Buttermilk Bay, MA, USA | DQ408432 |
| Leitoscoloplos pugettensis | Friday Harbor, WA, USA | DQ408433 |
| Leitoscoloplos pugettensis | Santa Monica, CA, USA | DQ408434 |
| Scoloplos sp. | Morro Bay, CA, USA | DQ408435 |
| Scoloplos cf. armiger | Malibu Beach (CA, USA), intertidal | DQ408436-DQ408437 |
| Scoloplos cf. armiger | San Diego (CA, USA), subtidal | DQ408438-DQ408440 |
| Scoloplos cf. armiger | Buholmsanden, Kristiansand (Norway) | DQ408441-DQ408445 |
| Scoloplos cf. armiger | Sletvik, Agdenes, Trondheimsfjord, (Norway) | DQ408446-DQ408451 |
| Scoloplos cf. armiger | Fehrmanns Belt, Baltic Sea (Germany), subtidal | DQ408477-DQ408484 |
| Scoloplos cf. armiger | Sylt (Germany), intertidal | DQ408452-DQ408455 |
| Scoloplos cf. armiger | Sylt (Germany), subtidal | DQ408456-DQ408470 |
| Scoloplos cf. armiger | Roscoff (France), intertidal | DQ408473-DQ408476 |
| Scoloplos cf. armiger | Low Newton by the Sea (Great Britain), intertidal | DQ408471-DQ408472 |

---

### Table 2: Average Kimura Two-Parameter distances calculated from Transition and Transversion changes within different Scoloplos cf. armiger clades

| Location | type locality | Sylt intertidal | Sylt subtidal |
|----------|---------------|----------------|--------------|
| San Diego | 0.238 | 0.209 | 0.237 | 0.231 |
| type locality | 0.132 | 0.136 | 0.149 | 0.088 |
| Sylt intertidal | 0.053 | 0.088 | 0.095 | 0.005 |
| Sylt subtidal | not calculated | | | |

---

### Table 3: Average Kimura Two-Parameter distances calculated from Transition and Transversion changes within different Scoloplos cf. armiger clades

| Location | type locality | Sylt intertidal | Sylt subtidal |
|----------|---------------|----------------|--------------|
| San Diego | 0.009 | 0.008 | 0.013 | 0.002 |
| type locality | | | | |
| Sylt intertidal | | | | |
| Sylt subtidal | | | | |

---
Figure 4
Amino acid alignment of the nad6 gene. Differences within the Scoloplos cf. armiger group marked in bold.
Figure 5
Proposed secondary structure of the *trnQ* gene of different *Scoloplos cf. armiger* clades. There are no different secondary structures within the clades and as such only secondary structure of *trnQ* one representative of each clade is shown. Discussed feature are marked with an arrow.
subtidal populations have been reported by Kruse & Reise [33], but they did not find any such differences or variation within the ‘subtidal clade’. The same holds true for chaetal characters. Furcate chaetae are present in abdominal segments of individuals of the subtidal populations, but these are lacking in intertidal individuals [40]. These characters were also compared with individuals from the type locality (Kristiansand, Norway), but no differences to the subtidal individuals from the Sylt population are found. However, our results explain why significantly higher genetic diversity has been found in a RAPD study within the subtidal populations [32]. It is very likely that this has been caused by mixing of two cryptic species which together contribute to an ostensible high variability.

The question emerges if there are ecological differences between the two cryptic subtidal species. Study of the ecological background of the two Scoloplos cf. armiger species in the Wadden Sea (North Sea, Germany) has revealed that there is a higher tolerance against sulphide and hypoxia for intertidal individuals, which can be interpreted as an adaptation to intertidal habitats being cut off from supply with oxygenated sea water during low tide. However, no unusual high variation of the physiological tolerance of subtidal Scoloplos cf. armiger individuals is revealed by these physiological studies [34].

Interestingly, it can be observed that intertidal and subtidal populations both spawn their egg cocoons and pelagic larvae respectively in spring and additionally pelagic larvae from subtidal populations were present in autumn. Spawning asynchrony is typical for marine sibling species living in sympatry [12,41] and in the future it needs to be tested if this is realized in the two clades with subtidal Scoloplos cf. armiger: one spawning in autumn and one in spring.

Implications for Scoloplos taxonomy
It is obvious from this analysis, as well as from molecular study of phylogenetic relationships of Orbiniidae [42], that the genus Scoloplos is not monophyletic. Characters currently used for genus diagnoses in orbinids are highly variable within this group and are not suitable for cladistic analysis [42]. The status of the worldwide distributed Scoloplos armiger was doubted by some authors before [43]. S. armiger is a species with variable morphological characters. Descriptions of this species differ so widely that more than one species may have been confused [44].

From the present analysis it becomes clear that at least three additional Scoloplos species should be erected within the species complex currently referred to as Scoloplos armiger: the Malibu clade, San Diego clade, and the intertidal clade. Additional to this, according to our data the existence of sibling species within the subtidal populations of the Eastern North Atlantic is highly likely (type locality clade, subtidal clade).

Whereas the descriptions for the Malibu clade and the intertidal clade are in preparation, the two other clades need further investigation before formal description. In future, the name Scoloplos armiger should be restricted to the type locality clade.

With the present molecular analysis at hand it is very likely that different species have been mixed in previous ecological studies. Whereas it seems reasonable that European Scoloplos armiger-like individuals from tidal flats can be assigned to the intertidal clade, the status of subtidal populations remains more ambiguous. In the future reports of S. armiger from non-European waters should be treated with caution. The analysis of the few included pacific individuals clearly indicates that these represent different species, which appear to be distantly related to European Scoloplos species. It would be interesting to include Mediterranean as well as subtidal species from the Sea of Japan, White Sea, and British waters in future studies to clarify the species status of different S. armiger-like populations and to understand their distribution.

Scoloplos mitochondrial genome data
This is the first attempt to use mitochondrial data to distinguish between Scoloplos species which has proven to be very powerful. We present the first nearly complete mitochondrial genome (ca. 12 kb) for this genus including all protein coding genes. Long-PCR's ranging from nad4 to 16S were not successful. Problems with amplifying the part of the mitochondrial genome including the putative control region have also been reported by others [37]. Compared with the mitochondrial genome of the orbiniid Orbinia latreillii [35] two translocations of tRNA genes must be assumed. This shows that gene rearrangements might be more frequent in annelids than previously assumed [35,37].

We analysed a fragment of the mitochondrial genome starting from the 3’-end of cox3, continuing over the complete trnQ, and finishing after a large part of nad6. Analysis of the secondary structure of the trnQ genes in our data set show the typical functional cloverleaf structure, which indicates that we most likely did not encounter mitochondrial pseudogenes, so called numts [45]. The sequenced fragment in this study proved to be very useful in phylogenetic analyses for the distinction of different clades. Due to its larger sampling scale this study goes beyond previous analyses which used RAPD and AFLP markers [32].
Conclusion
The phylogenetic analysis of mitochondrial sequence data (cox3-trnQ-nad6 fragment) reported here revealed that Scoloplos armiger represents a species complex and not a cosmopolitan species. We find at least two S. armiger-like species within the Pacific region and two or three different S. armiger-like species in the North Atlantic. One of these species is represented by the intertidal clade, for which previous studies clearly had supported species status. Further morphological as well as genetical investigations of S. armiger-like individuals from the subtidal and the type locality clade will shed additional light on a cryptic speciation within Scoloplos. It appears likely that inclusion of more Scoloplos cf. armiger specimens from different parts of the world would add more species to this complex.

Methods
Samples, identification, and DNA extraction
Individuals of Scoloplos cf. armiger and other orbiniids were collected at different sample locations (Table 1, see Fig. 1 for collection sites of the European Scoloplos samples) and preserved in 99% ethanol. Pacific Scoloplos species of the Malibu clade have been collected in the intertidal area of Malibu Beach (Los Angeles, USA) and were determined using taxonomic keys for the Californian Fauna [46,47] and afterwards this identification was checked by Leslie H. Harris (LACM Los Angeles). Specimens from San Diego were provided by Rick Rowe (San Diego) and have been collected in 25 m depth. European Scoloplos species have been all identified using the key from Hartmann-Schröder [6]. Voucher specimens for the Malibu clade, San Diego clade, type locality clade, and intertidal clade have been deposited in the collection "Vermes" of the Museum für Naturkunde der Humboldt-Universität zu Berlin (Germany) under the numbers 11213–11216. See Table 1 for sampling locations of European Scoloplos. DNA extraction was performed using the Qiagen DNeasy™ Tissue Kit (Qiagen, Germany) according to the manufacturer’s instructions.

mtDNA sequencing of Scoloplos cf. armiger individual SS14
To develop new genetic markers a 12 kb fragment including all coding genes was amplified from an individual of Scoloplos cf. armiger. The individual was collected subtidally near Sylt (Germany). In the first step small fractions of the rml, cox1, cob, and nad4 genes were amplified using conserved primers as described in Bleidorn et al. [35]. All products were purified with the Qiaquick PCR Purification Kit (Qiagen). Sequencing reactions were performed using the PCR primers with a dye terminator procedure and loaded on capillary automatic sequencer CEQ™ 8000 (Beckman Coulter, Fullerton CA, USA) according to the recommendations of the manufacturer.

In a second step the determined sequences were used to design three additional PCR primer pairs (Table 2) bridging the gaps between rml-cox1, cox1-cob, and cob-nad4. A long PCR approach using these primer pairs was performed using the Takara LA-Taq (MoBiTech). The 50 μl reaction volumes were set up as follows: 26.25 μl sterilized distilled water, 7 μl 10× reaction buffer, 7 μl MgCl₂ solution, 3.5 μl dNTP mix, 2 μl primer mix (10 μM each), 2 μl DNA template, 0.25 μl (1 u) Takara LA-Taq polymerase. A touchdown PCR approach was used for these fragments: 94°C for 3 min; 7 cycles with 94°C for 1 min, 63°C for 1 min (-0.5°C in every step), and 70°C for 8 min; 35 cycles with 94°C for 1 min, 60°C for 1 min 30 seconds, and 70°C for 8 min; final extension at 70°C for 10 min. PCR products were inspected under UV transillumination and a PCR purification of these four approximately 4 kb fragments was done using the PCR Gel extraction kit (Qiagen). Sequences were determined using direct sequencing from the ends of these fragments, then internally by primer walking.

cox3-trnQ-nad6 amplification and sequencing
Using the mitochondrial genome data a primer pair spanning a ca. 600 bp region corresponding to the 3’ end of cox3, trnQ, and most of the nad6 was designed (see Fig. 1 for priming sites on the genome, NAD6F: GCC TCW ACW TTC TTC GTA GCA CY, NAD6R: TTT TAC TGA RGC GAT TAR TGT TAG). All amplifications were carried out on a Mastercycler and Mastercycler gradient (Eppendorf). The PCR temperature reaction for this fragment was 94°C for 2 min; 34 cycles with 94°C for 30 seconds, 50°C for 45 seconds, and 70°C for 1 min; final extension at 70°C for 7 min.

All products were purified with the Qiaquick PCR Purification Kit (Qiagen). Sequencing reactions were performed with a dye terminator procedure and loaded on capillary automatic sequencer CEQ™ 8000 (Beckman Coulter, Fullerton CA, USA) according to the recommendations of the manufacturer. The trailing ends were trimmed, so that all sequences that were submitted to GenBank (for accession numbers see Table 1) are 528 bp in length.

Gene annotation
Protein-coding genes and ribosomal RNA genes were identified by blasting on NCBI entrez databases and by comparing with other annelid mitochondrial genomes using DOGMA [48]. Boundaries of nc (the largest non-coding region) and the ribosomal genes could not be identified by sequence homology alone and were inferred from the boundaries of flanking genes. Transfer RNA genes were identified by their potential secondary structures using the tRNAscan-SE Search Server [49]. Transfer-RNA identity was specified by its anticodon sequence.
The sequence of the mitochondrial genome of *Scoloplos cf. armiger* individual SS14 has been submitted to GenBank (DQ517436).

**Phylogenetic analysis**

Individuals possessing identical sequences were combined into a single operational taxonomic unit (OTU). Sequences were aligned with CLUSTAL W [50] using the default parameters for gap opening and gap penalty. Alignment of the protein coding regions was unambiguous, a few gap positions are only found within a non-coding region between *cox3* and *trnQ* and within the transfer RNA. The alignment is available in treebase [51].

Phylogenetic analyses were carried out using PAUP* version 4.0b10 [52] and MrBayes 3.0B4 [53]. According to the hypothesis of orbiniid phylogeny by Bleidorn [42] we used *Orbinia latreillii* as outgroup and this taxon served to root all trees. A chi-square test of homogeneity of base frequencies across taxa was used to estimate the frequency distribution of observed number of substitutional changes per character for each gene.

It is suggested that the Akaike Information Criterion (AIC) is superior to the hierarchical likelihood ratio test [54] and so we used this criterion for model selection as implemented in the program Modeltest 3.7 [55,56]. Average sequence distances were calculated using MEGA 2.1 [57].

Maximum likelihood analysis was performed under the likelihood settings suggested for the given dataset by the result of the modeltest using the heuristic search option with Tree Bisection Reconnection (TBR) branch swapping and 100 random sequence addition replicates. AIC indicates that GTR+I+Γ represents the optimal model in respect to the dataset (GTR = general time reversible, I = invariant sites, Γ = among-site rate variation modeled to fit a discrete gamma distribution).

Bootstrap values were determined from 1,000 replicates subject to full heuristic searches with simple addition sequence and NNI branch swapping to provide measures of relative clade support.

Bayesian analyses were conducted using MrBayes 3.0B4 [53]. All priors were set according to the chosen model (Isset nst = 6 rates = invgamma; pset RevMatPr = dirichlet(1,0,1,0,1,0,1,0,1,0) StateFreqPr = dirichlet(1,1,1,1) ShapePr = uniform(0.05,50.0) PinVarPr = uniform(0.0,1.0)). Two times four Markov chains in parallel, three heated and one cold, were started from a random tree and all eight chains ran simultaneously for 1,000,000 generations, with trees being sampled every 500 generations for a total of 2,001 trees. After the likelihood of the trees of each chain converged, the first 101 trees were discarded as burn in. The majority-rule consensus tree containing the posterior probabilities of the phylogeny was determined from 1,900 trees.

An equally weighted maximum parsimony search was run with 1,000 random addition replicates, heuristic search option with TBR branch swapping, holding one tree per step, and keeping all most-parsimonious trees. Clade support was assessed with nonparametric bootstrap as implemented in PAUP* (heuristic search, 1,000 replicates, TBR branch swapping, and simple addition sequence).

**Abbreviations**

AFLP, amplified fragment length polymorphism; *atp6* and 8, ATP synthase subunit 6 and 8; C, cytosine; *cox1-3*, cytochrome *c* oxidase subunits 1–3; *cob*, cytochrome *b* apoenzyme; *nad1-6* and 4L, NADH dehydrogenase subunits 1–6 and 4L, noncoding region; *L1* and *L2*, *rrnL*(CUIN) and *trnL*(UUR); RAPD, random amplified polymorphic DNA; *rrnS* and *rrnL*, small and large ribosomal RNA subunit; *S1* and *S2*, *trnS*(AGN) and *trnS*(UCN); tRNA and *trn* transfer RNA

**Authors' contributions**

CB and IK planned the study. CB, IK, and SA carried out field samples. CB did most of the molecular genetic work, analysed the data and drafted the manuscript. CB, IK, and SA assisted in the molecular genetic work. TB supervised the work. All authors read and approved the final manuscript.

**Acknowledgements**

We are grateful to T. Bakken (Norwegian Biodiversity Information Centre), R. Bastrop (University of Rostock), A.S.Y. Mackie (National Museum Cardiff), and R. Rowe (San Diego) for providing specimens for this study. We also thank L.H. Harris (Natural History Museum of Los Angeles County) and E. Wrânes (Agder University College) for assistance in the field. Raphael Ritson-Williams (Smithsonian Marine Station) proofread the manuscript. We thank two anonymous reviewers for their comments. This study was supported by a grant of the Deutsche Forschungsgemeinschaft (BL787/1-1) to CB.

**References**

1. Holte B: The macrofauna and main functional interactions in the sill basin sediments of he pristine Holandsford, Northern Norway, with auteological reviews for some species. Sarsia 1998, 83:55-68.
2. Carvalho S, Ravara A, Quintino V, Rodrigues AM: Macrobenthic community characterisation of an estuary from the western coast of Portugal (Sado estuary) prior to dredging operations. Bol Inst Esp Oceanogr 2001, 17:179-190.
3. Belan T: Benthos abundance pattern and species composition in conditions of pollution in Amursky Bay (the Peter the Great Bay, the Sea of Japan). Mar Poll Bull 2003, 46:1111-1119.
4. Koie M: The life-cycle of the flatfish nematode *Cucullanus heterochrous*. J Helminthol 2000, 74:323-328.
5. van der Meer J, Beukema JJ, Dekker R: Population dynamics of two marine polychaetes: the relative role of density dependence, predation, and winter conditions. ICES J Mar Sci 2000, 57:1488-1494.
6. Hartmann-Schröder G: Polychaeta Stuttgart: G. Fischer; 1996.
16. Allen FE: Population ecology and genetics of the polychaete Scapolostrum armiger (Orbiniidae). Jber Pol Mar Biol Rijeka 2003, 43:31-38.
17. Gibbs PE: Observations on the population of Scapolostrum armiger at Whistable. J Mar Bio Ass UK 1968, 48:225-254.
18. Holte B, Gulliksen B: Common macrofauna dominant species in the sediments of some north Norwegian and Svalbard glacial fjords. Mar Biol 1998, 137:375-382.
19. Plate S, Husemann E: An alternative mode of larval development in Scapolostrum armiger (F. O. Müller, 1776) (Polychaeta, Orbiniidae). Helgel Mar Ecosyst 1991, 45:487-492.
20. Grassle JF, Grassle JP: Sibling species in the marine pollution indicator Capitella (Polychaeta). Science 1976, 192:567-569.
21. Knowlton N: Sibling species in the sea. Ann Rev Ecol Syst 1993, 24:189-216.
22. Williams SJ: The status of Terebellides stromi (Polychaeta, Tychobranchiidae) as a cosmopolitan species on a worldwide morphological survey, including description of a new species. In Proceedings of 1st international polychaete conference Volume 984. Edited by: Hutchings PA, Linn Soc NSW, Sydney; 118-142.
23. Hutchings PA, Glasby CJ: Phylogenetic implications of the biogeography of Australian Terebellidae (Polychaeta). Ophelia 1993, 38:55-572.
24. Daunin JC, Thiebaut E: Is Oenina fusiformis Delle Chiave a a cosmopolitan species? Actes Mem Mus Nat Hist Nat 1994, 162:383-404.
25. Allen FE: Distribution of marine invertebrates by ships. Austr J Mar Freshwater Res 1953, 4:307-316.
26. Knowlton N: Molecular genetic analyses of species boundaries in the sea. In Marine Genetics Edited by: Thorpe JP, Dordrecht: Kluwer Academic Publishing; 2000:73-90.
27. Thorpe JP, Sole,Cava AM: The use of allozyme electrophoresis in invertebrate systematics. Zool Scripta 1994, 23:3-18.
28. Klautau M, Russo CAM, Lazoski C, Bury-Esnault N, Thorpe JP, Sole-Cava AM: Does cosinoptism result from overconservative systematics? A case study using the marine sponge Chondrina nucula. Evolution 1999, 53:1414-1422.
29. Day JH: A monograph on the Polychaeta of Southern Africa London: Trustees of the British Museum (Natural History); 1967.
30. Glasby CJ, Alvarez B: Distribution patterns and biogeographic analysis of Austral Polychaeta (Annelida). J Biogeogr 1999, 26:307-314.
31. Fiege D, Butfering B: SEM-investigations on the morphology and anatomy of Sternaspis scutata (Ranzani, 1817) (Annelida: Polychaeta). Bull Mar Sci 2000, 67:652-663.
32. Koh BS, Bland M: Description of Owenia gomsoni N. SP. (Oweniidae, Annelida: Polychaeta) from the Yellow Sea and evidence that Owenia fusiformis is not a cosmopolitan species. Vie et Milieu 2001, 51:77-86.
33. Westheide W, Haji-Cordes E, Krakus M, Muller M: Ctenodrilus serratus (Polychaeta: Ctenodrilidae) is a truly amphi-atlantic meiofauna species – evidence from molecular data. Mar Biol 2003, 142:637-642.
34. Soosten C, Schmidt H, Westheide W: Genetic variability and relationships among geographically widely separated populations of Pettia amphiphthalma (Polychaeta: Syllidae). Results from RAPD-PCR investigations. Mar Biol 1998, 131:659-669.
35. Westheide W, Hass-Cordes E: Molecular taxonomy: description of a cryptic Pettia species (Polychaeta: Syllidae) from the island of Mahe (Seychelles, Indian Ocean) using RAPD markers and ITS2 sequences. J Zool Syst Evol Res 2001, 39:103-111.
36. Patti FP, Gambi MC: Phylogeography of the invasive polychaete Sabella spallanzani (Sabellidae) based on the nucleotide sequence of internal transcribed spacer 2 (ITS2) of nuclear ribosomal DNA. Mar Ecol Prog Ser 2001, 219:169-177.
37. Schmidt H, Westheide W: Are the meiofaunal polychaetes Hesionides arenaria and Stygocapitella subterranea true cosmopolitan species? – results of RAPD-PCR investigations. Zool Scr 2000, 29:17-27.
38. Schmidt H, Westheide W: Genetic relationships (RAPD-PCR) between geographically separated populations of the “cosmopolitan” intertidal polychaete Hesionides gohari (Hesionidae) and the evolutionary origin of the freshwater species Hesionides riegerorum. Biol Bull 1999, 196:216-226.
39. Avise JC: Phylogeography Cambridge: Cambridge University Press; 2000.
40. Driscoll HR: Diversity and zoogeography of marine Tubificidae (Annelida, Oligochaeta) with notes on variation in widespread species. Hydrobiologia 1984, 115:191-196.
41. Kruse I, Reusch TBH, Schneider MV: Sibling species or poecilogony in the polychaete Scapolostrum armiger? Mar Biol 2003, 143:937-947.
42. Kruse I, Reise K: Reproductive isolation between intertidal and subtidal Scapolostrum armiger (Polychaeta, Orbiniidae) indicates sibling species in the North Sea. Mar Biol 2003, 143:511-517.
43. Kruse I, Strasser M, Thiermann F: The role of ecological divergence in speciation between intertidal and subtidal Scapolostrum armiger (Polychaeta, Orbiniidae). J Sea Res 2004, 51:53-62.
44. Bleidorn C, Poisson-Debray L, Bartolomeaus T: The complete mitochon- drial genome of the polychaete Orbina latreillii (Annelida, Orbiniidae) – a novel gene order for Annelida and vertebrates. Nucleic Acids Res 1999, 27:1767-1780.
45. Jensens RM, Halanych KM: Mitochondrial genomes of Clonemelina torquata (Maldanidae) and Riftia pachyptila (Sibilo- chondridae). Evidence for conserved gene order in Annelida. Mol Biol Evol 2005, 22:210-222.
46. Mishler BD, Theriot EC: The phylogenetic species concept (sensu Mishler and Theriot): Monophyly, apomorphy, and phylogenetic species concepts. In Species concepts and phylogene- tic theory: A debate Edited by: Wheeler QD, Meier R. Columbia University Press, New York; 2000:44-54.
47. Avise JC, Shapiro JF, Daniel SW, Aquadro CF, Lansman RA: Mitochon- drid DNA differentiation during the speciation process in Peromus. Mol Biol Evol 1983, 1:38-56.
48. Albrecht S: Vergleichende Morphologie eu- und sublitoraler Polychaeta der Gattung Scapolostrum in der Nordsee. Diploma thesis, Philipps University Marburg 2004.
49. Giangrande A: Polychaete reproductive patterns, life cycles and life histories: an overview. Oceangraph Mar Biol Ann Rev 1997, 35:323-386.
50. Bleidorn C: Phylogenetic relationships and evolution of Orbi- niidae (Annelida, Polychaeta) based on molecular data. Zool J Linn Soc 2005, 144:59-73.
51. Beasley PL, Ross GJB, Glasby CJ: Polychaetes and Allies: The southern Synthesis. Fauna of Australia. Vol. 4A: Polychaeta, Myzostomida, Pogonophora, Echiura, Spiculina Melbourne: CSIRO Publishing; 2000.
52. Day JH: A review of the Australian and New Zealand Orbiniidae. In In memory of Dr. Olga Hartman. Essays on polychaetous annelids Edited by: Reish DJ, Fauchald K. Los Angeles: Allan Hancock Foundation Special Publication; 1977:217-246.
53. Bensasson D, Zhang DX, Hard DL, Hewitt GM: Mitochondrial pseudogenes: evolution’s misplaced witnesses. Trends Ecol Evol 2001, 16:314-321.
54. Blake JA: Family Orbiniidae Hartman, 1942. In Taxonomic Atlas of the Benthic Fauna of the Santa Maria Basin and Western Santa Barbara Channel, The Annelida, Part 3: Polychaeta: Orbiniidae to Cassidulidae Volume 6. Edited by: Blake JA, Hillbig B, Scott PH. Santa Barbara Museum of Natural History, Santa Barbara, CA; 1996:1-26.
55. Hartman O: Atlas of the sedentarie polychaetous annelids from Califor- nia Los Angeles: Allan Hancock Foundation, University of Southern California; 1969.
56. Wyman SK, Jansen RK, Boone JL: Automatic annotation of organellar genomes with DOGMA. Bioinformatics 2004, 20:3252-3255.
57. Lowe TM, Eddy SR: tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 1997, 25:953-964.
58. Swoford DL: PAUP**: Phylogenetic analysis using parsimony, version 4.0b8 Sinauer, Sunderland, MA, 2001.
59. Hulsulienek JP, Ronquist FR, MrBayes: Bayesian inference of phylogeny. Bioinformatics 2001, 17:754-755.
60. Posada D, Buckley TR: Model selection and model averaging in phylogenetics: advantages of Akaike Information criterion
and Bayesian approaches over likelihood ratio tests. Syst Biol 2004, 53:793-808.

55. Posada D, Crandall KA: Modeltest: testing the model of DNA substitution. Bioinformatics 1998, 14:917-918.

56. Posada D, Crandall KA: Selecting the best-fit model of nucleotide substitution. Syst Biol 2001, 50:580-601.

57. Kumar S, Tamura K, Jakobsen IB, Nei M: MEGA2: molecular evolutionary genetics analysis software. Bioinformatics 2001, 17:1244-1245.