ICES Journal of Marine Science

ICES Journal of Marine Science (2017), 74(7), 1875–1884. doi:10.1093/icesjms/fsw255

Contribution to the Symposium: '*6th Zooplankton Production Symposium*'

Original Article

Genetic and morphological diversity of the cosmopolitan chaetognath *Pseudosagitta maxima* (Conant, 1896) in the Atlantic Ocean and its relationship with the congeneric species

Dmitry N. Kulagin[1]* and Tatiana V. Neretina[2,3]

[1]*P.P. Shirshov Institute of Oceanology, Russian Academy of Science, Moscow 117997, Russia*
[2]*N.A.Pertsov White Sea Biological Station, Biological faculty, M.V. Lomonosov Moscow State University, Moscow 119234, Russia*
[3]*Pirogov Russian National Research Medical University, Moscow 117997, Russia*

**Corresponding author: tel: +7 4991247749; fax: +7 4991245983; e-mail: kulagin.dima@gmail.com*

Kulagin, D. N. and Neretina, T. V. 2017. Genetic and morphological diversity of the cosmopolitan chaetognath *Pseudosagitta maxima* (Conant, 1896) in the Atlantic Ocean and its relationship with the congeneric species. – ICES Journal of Marine Science, 74: 1875–1884.

Received 28 July 2016; revised 23 December 2016; accepted 28 December 2016; advance access publication 28 January 2017.

Until recently many oceanic zooplankton species have been considered as cosmopolitan organisms. At present it became evident that some of them comprise many distinct molecular operational taxonomic units (MOTUs) that often are regarded as cryptic species. As they can significantly change our perceptions of large-scale biogeographic patterns, it is important to characterize the true diversity within common and ecologically important groups. We have analysed the molecular and morphological diversity of the cosmopolitan mesopelagic chaetognath *Pseudosagitta maxima* throughout the Atlantic Ocean from 60° S to 85° N and its position within the genus *Pseudosagitta*. Three distinct mitochondrial clades within *P. maxima* were revealed with phylogenetic analyses (Maximum Likelihood, Bayesian Inference) and were geographically separated. The subsequent analyses of nuclear markers (H3, ITS1) have shown that *P. maxima* most likely comprises two distinct MOTUs, tropical and bipolar, that also have some morphological differences. The latter MOTU consists of two genetically slightly divergent populations: southern and northern. The morphological examination allowed the determination of a character (type of hook coloration) that accurately distinguishes juveniles of the *P. maxima* complex from the other congeneric species. Molecular data have shown that evolutionary *P. lyra* and *P. gazellae* are more closely related to each other than to *P. maxima*. Number of hooks, number of anterior and posterior teeth and the arrangement of ova in the ovary were proposed to be the most useful morphological characters to distinguish between tropical and bipolar MOTUs within the *P. maxima* complex. The first three characters should be determined for each maturity stage separately.

Keywords: bipolar species, Chaetognatha, cryptic species, marine biodiversity, phylogeography, Pseudosagitta maxima, Pseudosagitta phylogeny, zooplankton.

Introduction

In recent decades, the number of studies on phylogeography and the genetic structure of the zooplankton species from several taxa have increased [e.g. copepods (Goetze, 2010; Halbert *et al.*, 2013), pteropods (Jennings *et al.*, 2010; Burridge *et al.*, 2015), chaetognaths (Peijnenburg *et al.*, 2004; Miyamoto *et al.*, 2012; Kulagin *et al.*, 2014), cnidarians (Holland *et al.*, 2004; Ramšak *et al.*, 2012)]. The main aims of nearly all of these investigations are to reveal real biodiversity and the evolutionary mechanisms that drive biological diversification in holoplanktonic organisms. In some cases, these studies are finding that species initially described to be widely distributed, with ranges spanning multiple ocean basins, in fact consist of genetically divergent populations or even cryptic species that can be restricted in distribution to particular pelagic habitats. Because cryptic species can heavily bias our perceptions of large-scale biogeographic patterns (Bickford *et al.*, 2007; Hubert *et al.*, 2012), it is important to characterize, where possible, the true diversity within common and ecologically important groups (Halbert *et al.*, 2013).

Chaetognaths are regular and typical components of zooplanktonic assemblages that are found from the coastal waters to the open ocean. They are often highly abundant and are functionally very important in marine food webs (Longhurst, 1985; Bone *et al.*, 1991). *Pseudosagitta maxima* (Conant, 1896) is one of the largest members of the phylum: up to 90 mm in length (Casanova, 1999). The horizontal distribution of this species is wide, ranging from the Arctic basin to the Antarctic zone of the Southern Ocean (Alvariño, 1964; David, 1965; Pierrot-Bults and Nair, 1991; Kosobokova *et al.*, 2011). In the subarctic Atlantic Ocean and in the Subantarctic zone of the Southern Ocean *P. maxima* is one of the most abundant chaetognath species (David, 1965; Samemoto, 1987; Pierrot-Bults, 2008). *P. maxima* inhabits mainly the mesopelagic layer (200–1000 m) but its vertical distribution changes with latitude: in the polar and subpolar regions this species occurs predominantly in the layer 100–600 m, whereas in the lower latitudes (tropical and subtropical regions) the species is found at depths over 400 m (Alvariño, 1964; David, 1965; Cheney, 1985b). Therefore, ecological and hydrological patterns of environments that *P. maxima* inhabits, differ in the various parts of the World Ocean. Whether there is still a connection between Arctic and Antarctic populations of this species is a matter of debate (Pierrot-Bults, 1976; Pierrot-Bults and Nair, 2010). That makes this species a good subject with which to study the extent of gene flow within the Atlantic Ocean.

The genus *Pseudosagitta* within the family Sagittidae was subdivided by Bieri (1991) and consists of 4 or 3 species: *P. lyra* (Krohn, 1853), *P. maxima, P. gazellae* (Ritter-Záhony, 1909) and *P. scrippsae* (Alvariño, 1962); the latest one is accepted by some authors as a junior synonym of *P. lyra. P. lyra* in its distribution is restricted to the tropical and subtropical regions of all three oceans, *P. gazellae* occupies the Southern Ocean and *P. scrippsae* is found in the North Pacific transitional waters (Pierrot-Bults and Nair, 1991). The monophyly and validity of the genus *Pseudosagitta* are well supported by recent phylogenetic studies that used the nuclear markers (Telford and Holland, 1997; Gasmi *et al.*, 2014).

In the present work, we investigated the genetic diversity of *P. maxima* based on mitochondrial [cytochrome *c* oxidase subunit I (COI)] and nuclear marker genes [histone h3 (H3) and ribosomal internal transcribed spacer 1 (nITS1)]. The specimens for the study were collected from the Arctic basin, North, Equatorial and Southwest Atlantic, and from the Antarctic and Subantarctic zones in the Drake Passage. *P. lyra* and *P. gazellae* specimens from the same samples were included in this study as an outgroup and to clarify phylogenetic relationships within the genus. We aim to define population subdivision of *P. maxima*, to elucidate their geographic boundaries and to discuss the possible mechanism that forms the present genetic structure of this species. We also examined some morphological characters of all analyzed *P. maxima* specimens to reveal some differences between populations.

Material and methods

Sample collection and morphological examination

Zooplankton were collected in bulk plankton samples from 34 stations in the Atlantic (32 stations) and Arctic (2 stations) Oceans (Figure 1) from 60°S to 85°N in 6 cruises that took place from 2011 to 2015. Bulk plankton were preserved immediately in 96% ethanol with a change in ethanol after 24 h of the initial fixation, and maintained at −20 °C to minimize degradation of DNA.

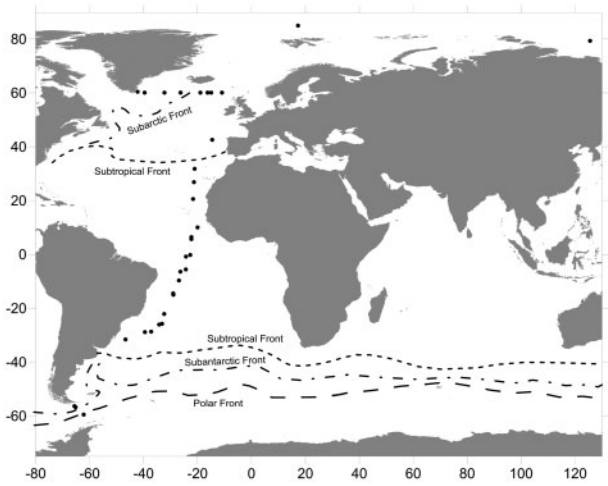

Figure 1. Distribution of sampling locations. Fronts are marked in accordance with Tomczak and Godfrey (1994) and Pingree *et al.* (1999).

Specimens were removed from bulk samples in the laboratory and identified to the species level according to Casanova (1999). Due to the rare occurrence of mature individuals in our collections, we also used juvenile and immature animals for the present study. For the following morphological analysis the maturity stage of the animals was determined using the method described by Kramp (1939). Stage I animals had faint development with no visible ovaries and no sign of testes; stage II had signs of testes development and well developed ovaries without mature eggs; and stage III had the sperm totally/partly evacuated, ovaries without mature eggs, and seminal receptacles filled with the sperm; stage IV had the sperm totally evacuated and ovaries filled with ripe eggs; and in stage V, the sperm was evacuated as were the eggs. For the morphological characterization of *P. maxima* populations, the following characters were used: the number of hooks, number of anterior and posterior teeth, pigmentation, size of the eyes, and the arrangement of ova in the ovary.

In general, 33 specimens of *P. maxima* were collected at 18 locations (Table 1). Specimens of two other congeneric species (*P. lyra* and *P. gazellae*) were also collected and were used as outgroups for the molecular analysis. These three species are usually distinguished by two characters: (1) the relative position of the origin of anterior fin (AF) to ventral ganglion (VG) (in *P. maxima* AF beginning at level of VG, in *P. lyra*—a short distance from VG and in *P. gazellae*—far from the VG) and (2) the relative length of the tail segment (in adult *P. maxima* the tail segment comprise 19–29% of the body length, in *P. lyra*—14–18% and in *P. maxima*—<15%).

DNA extraction and PCR amplification

The Wizard SV Genomic DNA Purification Kit (Promega) was used for tissue lysis and DNA purification. DNA concentration and purification efficiency were determined by the electrophoresis on a 1.2% agarose gel. The mitochondrial gene cytochrome *c* oxidase subunit I (COI) was chosen as the primary genetic marker for this study. Polymerase chain reaction (PCR) amplification of

Table 1. Collection data for *Pseudosagitta spp.* specimens analysed in this study.

Geographic region and station	Latitude	Longitude	Date	Cruise	Sampling gear, (sampling layer, m)	Number of specimens *P. maxima/lyra/gazellae*
Arctic						
PS80/388-1	84.37N	17.56E	28.09.2012	ARC-XXVII-3	Bongo net (0–700)	5/-/-
AT-76	78.71N	125.86E	20.09.2015	NABOS-2015	Bongo net (0–130)	1/-/-
North Atlantic						
2974	59.50N	10.67W	06.07.2013	AI-41	Juday net (0–700)	1/-/-
2980	59.50N	14.67W	07.07.2013	AI-41	Juday net (0–300)	-/1/-
2982	59.50N	16.00W	08.07.2013	AI-41	Juday net (0–300)	-/1/-
2986	59.50N	18.67W	08.07.2013	AI-41	Juday net (0–300)	1/-/-
2997	59.50N	26.00W	10.07.2013	AI-41	Juday net (0–300)	1/-/-
3006	59.50N	32.00W	12.07.2013	AI-41	Juday net (100–300)	1/-/-
3017	59.50N	39.33W	14.07.2013	AI-41	Juday net (0–300)	1/-/-
3024	59.81N	41.93W	15.07.2013	AI-41	Juday net (0–300)	1/-/-
NE Atlantic						
2500	41.97N	14.28W	22.09.2013	ASV-37	Bogorov-Rass net (1180–3000)	1/-/-
2504	31.20N	20.80W	27.09.2013	ASV-37	Bogorov-Rass net (190–500)	-/1/-
2505	26.23N	21.05W	28.09.2013	ASV-37	Bogorov-Rass net (200–500)	-/1/-
North Central Atlantic						
2506	19.98N	21.37W	30.09.2013	ASV-37	Bogorov-Rass net (0–200, 200–500)	-/4/-
Equatorial Atlantic						
2507	5.08N	22.0W	03.10.2013	ASV-37	Bogorov-Rass net (200–500)	4/2/-
2518	1.42S	24.00W	09.10.2013	ASV-37	Bogorov-Rass net (200–500)	1/-/-
2519	7.02S	26.07W	11.10.2013	ASV-37	Bogorov-Rass net (200–500)	1/-/-
2474	9.42N	19.73W	24.10.2012	ASV-36	Bogorov-Rass net (200–600, 600–1200)	4/-/-
2483	0.83S	22.43W	28.10.2012	ASV-36	Bogorov-Rass net (200–500)	2/-/-
SW Atlantic						
2528	29.28S	36.93W	21.10.2013	ASV-37	Bogorov-Rass net (500–1200)	-/1/-
2499	32.18S	46.44W	10.11.2012	ASV-36	Bogorov-Rass net (0–3000)	2/2/-
Subantarctic						
2601	56.93S	65.38W	03.11.2011	AI-36	Juday net (0–300)	1/-/-
2598	57.35S	64.93W	03.11.2011	AI-36	Juday net (0–300)	4/-/2
Antarctic						
2579	60.08S	61.93W	30.10.2011	AI-36	Juday net (0–300)	1/-/-

the COI gene was performed with the universal primers: jgLCO1490-tailed (5′-TGTAAAACGACGGCCAGTTNTCNACN AAYCAYAARGAYATTGG-3′) and jgHCO2198-tailed (5′-CA GGAAACAGCTATGACTANACYTCNGGRTGNCCRAARAAYC A-3′) (Geller *et al.*, 2013). Since the analysis of the COI sequences indicated three distinct clades within the *P. maxima* populations, we further analysed the H3 and ITS1 regions to determine whether these clades represented reproductively isolated populations. PCR amplification of the H3 gene fragment was performed with the primers H3af (5′-ATGGCTCGTACCAAGCAGACVGC-3′) and H3ar (5′-ACATATCCTTRGGCATRATRGTGAC-3′) (Colgan *et al.*, 1998); 18S-ITS1-5.8S fragment—with the primers SR6R (5′-AAGTAAAAGTCGTAACAAGG-3′) and 5.8S (5′-CGCTGCGTTCTTCATCG-3′) (Vilgalys and Hester, 1990). Loci were amplified using the Encyclo PCR kit (Evrogen). Amplifications were made in a total volume of 25 µl reaction mix that contained 1 × PCR buffer, 1 µl of 10 µM of primer pair mix, 1 µl of template, 0.2 mM of each dNTP, and 0.5 units of Taq polymerase. Reactions mixtures were heated to 94 °C for 120 s; followed first by 35 cycles of 15 s at 94 °C, 30 s at specific annealing temperature, and 60 s at 72 °C; and then by a final extension of 7 min at 72 °C on Veriti® Thermal Cycler. Annealing temperature was set to 45 °C for COI primer pair jgLCOI 1490 and jgHCOI 2198, 50 °C for ITS pair primers 5.8S and SR6R and 53 °C for histone H3 primer pair H3af and H3ar. The PCR Purification Kit protocol (Promega) was used to purify amplification products of COI and histone fragments. ITS1 fragments were separated by agarose gel electrophoresis; the amplified band of approximately 500 bp was purified, ligated into pGEM-T vector, and transformed into competent E. coli–strain DH5a. Two or three clones were sequenced from most samples to account for polymorphisms or errors introduced by Taq DNA polymerase; however, if initial clones differed at only 2% of sites or fewer, these were treated as ambiguities and coded appropriately in phylogenetic analyses. These products were sequenced in both directions. Each sequencing reaction mixture, including 1 µl of BigDye 3.1 (Applied Biosystems), 1 µl of 1 µM primer, and 1 µl of purified PCR template, was run for 40 cycles of 96 °C (15 s), 50 °C (30 s), and 60 °C (4 min). Sequences were purified by ethanol precipitation in order to remove unincorporated primers and dyes. Products were re-suspended in 12 µl formamide and electrophoresed in ABI Prism3500 sequencer (Applied Biosystems).

Sequence data analysis

Sequences were assembled into contigs, aligned and edited using CodonCode Aligner 3.7.1, and base calls were confirmed by aligning both strands. Four COI sequences named *Chaetognatha* sp. from the NCBI GenBank database (Accession Numbers: KF931008–KF931011) were 99–100% similar to some of our *P. maxima* sequences and they evidently represented the same species. Only two of them with the similarity of 99% were included

in the present study (Table 1), while the other two had small length and were identical with our sequences. Multiple alignments within each dataset were performed using the ClustalW algorithm (Wang and Jiang, 1994). For the phylogenetic analyses, the aligned sequences were trimmed to the shortest sequence within each contig. The obtained sequences were submitted to the NCBI GenBank database (KT309090–KT309128, KT351853–KT351861, and KY027072–KY027099).

To assess phylogenetic relationships among specimens, Maximum Likelihood (ML) and Bayesian phylogenetic trees were inferred. We selected substitution models using the Akaike information criterion (AIC), as implemented in MrModeltest 2.3 (Nylander, 2004). The best-fit model was the General time-reversible model with a gamma distribution and invariable sites (GTR + G+I) for COI, the GTR + I model for H3 and Hasegawa-Kishino-Yanomodel (HKY) for ITS1 data set. The ML analysis was performed in RAxML GUI 1.3 (Stamatakis, 2006). To assign support to branches in the ML tree bootstrap resampling with 1000 replicates was conducted using the thorough bootstrap procedure. Bayesian phylogenetic analysis was made with the use of MrBayes 3.2.1 (Ronquist and Hulsenbeck, 2003). The Markov Chain Monte Carlo (MCMC) analysis was further used with the following settings: (1) for COI—7 million generations, trees sampled every 5000 generation, and the first 350 trees discarded, (2) for H3—4 million generations, trees sampled every 5000 generation, and the first 200 trees discarded. The average standard deviation of split frequencies between two runs of MCMC was less than 1% for each analysis, thus indicating convergence.

To quantify genetic distances between clades, the divergence over sequence pairs between and within clades was averaged using the Kimura 2-parameter model (Kimura, 1980) in MEGA 5 (Tamura *et al.*, 2011). We calculated estimates of genetic variation for all the determined clades as haplotype diversity (HD) and nucleotide diversity (p) (Nei, 1987) using DNAsp 5.10.1 (Librado and Rozas, 2009). Tajima's tests of selective neutrality (Tajima, 1989) were performed also in DNAsp.

Results

Genetic diversity and molecular phylogeny

COI gene. The final alignment of the 591 bp COI gene fragment included 35 specimens of *P. maxima*, 4 specimens of *P. lyra* and 2 specimens of *P. gazellae*. Amino acid sequences translated from the nucleotide sequences had no stop codons within the open reading frame using the invertebrate mitochondrial code, and a majority of substitutions were in the third codon position. The numbers of synonymous and non-synonymous changes were 156 and 10, respectively.

Three genetically divergent mitochondrial clades (A, B and C) appeared within *P. maxima* and were well supported both in Bayesian (posterior probabilities ≥ 0.99) and ML (bootstrap $> 75\%$) phylogenetic analyses (Figure 2). The haplotype and nucleotide diversities within each of the *P. maxima* clades were relatively low (Table 2). Tajima's D was negative and non-significant for clades A, B, C, and *P. lyra*, but positive and significant for pooled *P. maxima* specimens.

Average genetic K2P distances between *P. maxima* and other congeneric species ranged from 43.2% to 45.0% (with *P. lyra* and *P. gazellae*, respectively). Within *P. maxima* average genetic distances between clades ranged from 5.5% to 22% (Table 3); the largest differences were found between clades A and C and the lowest—between clades B and C. No marked genetic diversity appeared within clades A, B, and C.

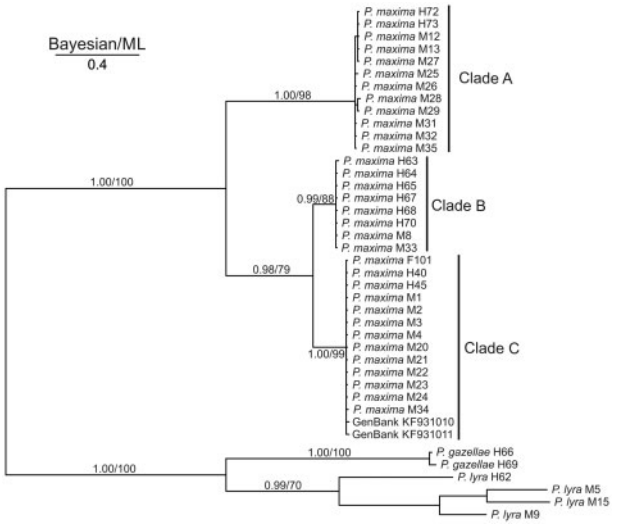

Figure 2. Bayesian tree of the *Pseudosagitta* species inferred from the COI gene fragment (591 bp). The horizontal scale bar marks the number of expected substitutions per site. Statistical support indicated as Bayesian posterior probabilities (left values) and ML bootstrap analysis with 1000 replicates (right values).

H3 gene. Two specimens were randomly selected from each of the three COI clades of *P. maxima* as well as from *P. lyra* and *P. gazellae*, and used for the H3 analysis. The length of these sequences was 394 bp. No stop codons were observed in H3 sequences. Within the final alignment, there were 27 variable sites, all of which were parsimony-informative. We did not observe any differentiation between *P. maxima* COI clades; specimens within each of these three clades had identical sequences with the heterozygotes in the same positions. K2P distances between *P. maxima* and two congeneric species were equal and reached 6.1%. Divergence between *P. lyra* and *P. gazellae* was markedly lower and reached only 1.4%.

ITS1 region. The same individuals of *P. maxima* used in H3 analysis and additional specimens from clades B and C were screened for ITS1 variability. Five specimens had multiple forms of ITS1 and a total of 18 sequences were determined from 9 individuals. The final alignment had a complete ITS1 region with the length of 512 bp. Two lineages were determined by ML method with a high bootstrap value (100%) (Figure 3). The average K2P distance between these two lineages was 2.4%, and diversity within each lineage did not exceed 0.2%. One lineage comprised the sequences obtained from the specimens of mitochondrial clade A and the other lineage from the specimens of mitochondrial clades B and C. No obvious phylogenetic structure was found within the second lineage.

Morphology

We examined the morphology of all 33 *P. maxima* specimens used in molecular analyses. Most were in maturity stage I (Table 4). We compared morphological characters within each maturity stage as the size of *P. maxima* varies depending on the region. Arctic adults are as long as 90 mm, whereas those in the Antarctic are 55 mm in length (David, 1958). In tropical waters the length

Table 2. Molecular diversity data of each species and *Pseudosagitta* COI gene clades.

	Number of specimens	Number of haplotypes	Haplotype diversity (Hd ± s.d.)	Nucleotide diversity (π ± s.d.)	Tajima's *D*
P. maxima	35	11	0.83 ± 0.04	0.10 ± 0.01	3.00*
Clade A	12	5	0.76 ± 0.09	0.00 ± 0.00	−0.42
Clade B	8	2	0.25 ± 0.18	0.00 ± 0.00	−1.05
Clade C	15	4	0.37 ± 0.15	0.00 ± 0.00	−1.69
P. lyra	4	4	1.00 ± 0.18	0.15 ± 0.03	−0.53
P. gazellae	2	2	1.00 ± 0.50	0.01 ± 0.00	–

*$p < 0.01$.

Table 3. Genetic diversity (in diagonal) within and distances (lower triangle) between clades of *Pseudosagitta maxima*, *P. lyra*, and *P. gazellae*.

	P. maxima				
	Clade A	Clade B	Clade C	*P. lyra*	*P. gazellae*
P. maxima					
Clade A	0.2				
Clade B	21.1	0			
Clade C	22.0	5.5	0.1		
P. lyra	44.9	42.4	42.2	17.3	
P. gazellae	45.4	44.4	45	31.2	0.7

Distances were calculated using the K2P model and averaged over all sequence pairs between clades and within clades. Values are given in percentages.

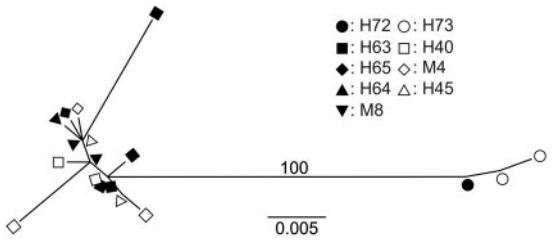

Figure 3. ML tree of ITS1 sequences of *Pseudosagitta maxima* specimens (512 bp). Statistical support indicated as ML bootstrap values with 1000 replicates. Symbols located at the end of each branch and correspond to each individual. Black and white circles represent mitochondrial clade A specimens (voucher numbers are given to the right of the symbols), other black symbols correspond to clade B specimens and other white symbols—to clade C specimens.

of the adults does not exceed 36–43 mm (Ducret and Casanova, 1968; Srinivasan, 1971). In our collection, some stage I individuals from Arctic and North Atlantic were larger than fully mature individuals from the Equatorial and Southwest Atlantic (approximately 40 mm vs. 30 mm).

We did not observe differences among specimens from all three clades in the size of the eyes, in pigmentation and size of the pigment spot. Some differences appeared between *P. maxima* clades in the numbers of hooks, and anterior (AT) and posterior (PT) teeth. Within each maturity stage, specimens from clade A carried a smaller number of hooks, AT and PT than specimens from clades B and C (Table 4), though at maturity stage I these morphological differences were unclear. With growth and maturation, the number of hooks in all three clades decreased. At maturity stages II and IV (specimens in maturity stage III were absent in our collection) we observed clear differences in the arrangement of ova in the ovary between specimens from clade A on the one hand and clades B and C on the other (Table 4). We found no differences in shape and coloration of hooks and teeth between all three clades.

We examined 14 *P. lyra* specimens from our collections to search for additional morphological differences with *P. maxima* not mentioned in the identification key (Casanova, 1999). The characters used in the key are for the adult specimens but cannot be used for juveniles. The COI gene fragment was sequenced for all analysed specimens to confirm morphological identification (GenBank: KT309092–KT309105). We found a character that unambiguously distinguished one species from another, even among juveniles 8 mm in length. All *P. maxima* specimens had hooks fully coloured in brown (Supplementary Figure S1a), while all *P. lyra* specimens had only the outer margin of hooks coloured brown, with the rest remaining transparent (Supplementary Figure S1b).

Discussion

Morphological and genetic differentiation between *Pseudosagitta* species

Three *Pseudosagitta* species that inhabit the Atlantic Ocean can be clearly distinguished from one another by the relative position of the origin of the anterior fin to the ventral ganglion (Casanova, 1999). Unfortunately, this character is almost impossible to discern in juveniles, which comprise the majority of individuals in populations. Casanova (1999) suggested that in *P. maxima* the teeth and hooks are brown, while clear amber in the two other species; but this is not entirely correct. In juveniles of *P. maxima* (with the length <15 mm), the teeth are transparent, as well as in the two other species. More adult *P. maxima* and *P. gazellae* specimens (personal observations) have more or less brown teeth. Adults (maturity stage II and higher) of both species have brown teeth that clearly distinguish them from *P. lyra*. Hooks of *P. maxima* are fully coloured brown, even in juveniles. In *P. lyra*, only the outer margin of hooks is brown while the rest part of hooks remains transparent. *P. maxima* and *P. lyra* occur throughout most of the Atlantic Ocean; we propose to distinguish them by the coloration of hooks that can be easily seen even in juveniles. Juveniles of *P. gazellae* have a coloration of hooks like in *P. lyra* (Supplementary Figure S1b, c), and adults—as in *P. maxima*. At the same time, even juveniles of *P. maxima* can be easily distinguished from *P. gazellae* by the relative length of the tail segment [19–29% in *P. maxima* and less than 15% in *P. gazellae* (Casanova, 1999)].

All three *Pseudosagitta* species are clearly distinguished by the COI barcoding region. The averaged K2P genetic distances

Table 4. Morphological differences of *P. maxima* specimens from clades A, B, and C.

	Number of specimens	Number of hooks, min–max	Number of anterior teeth, min–max	Number of posterior teeth, min–max	Arrangement of ova in the ovary, number of rows
Stage I					
Clade A	9	7–8(9[a])	2–3	1–4	–
Clade B	6	9	3–5	3–5	–
Clade C	12	(8[a])9–11	4–7	2–8	5–8
Stage II					
Clade A	2	4–5	3–4	4	3–4
Clade C	1	7–8	6	7–8	5–6
Stage IV					
Clade A	1	4	3	2	3
Clade B	1	6	4–5	5	5
Stage V					
Clade B	1	5	4–5	4–5	–

[a]Only one individual in clade A and two individuals in clade C had 8 hooks on the one side and 9 hooks on the other side of the head (morphologically overlapped), but the same individuals in clade C had more anterior teeth (4–6 vs. 3 in clade A) and posterior teeth (5–6 vs. 3).

among species (31–45%) are comparable with the results obtained for another genus, *Eukrohnia* (HKY distances 17–45%) (Miyamoto *et al.*, 2012). Both mitochondrial and nuclear markers show that evolutionarily *P. lyra* and *P. gazellae* are more closely related to each other than to *P. maxima*.

Differentiation and distribution of *P. maxima* populations

It is believed that *P. maxima* is a cosmopolitan species in the Atlantic Ocean and is distributed over the entire area (Pierrot-Bults and Nair, 1991; Casanova, 1999). This species displays a phenomenon called tropical submergence when it can be found only at depth in the tropical Atlantic while also at the surface in colder regions (David, 1963). The maximum abundance of *P. maxima* occurs in the Subarctic zone of the North Atlantic between approximately 52°N and 65°N (Samemoto, 1987; Pierrot-Bults, 2008). Northward, it is found in smaller numbers but regularly throughout the Arctic basin (Kosobokova *et al.*, 2011). Southward from Subarctic Atlantic, the abundance of *P. maxima* also decreases. This species was found in low abundance along the North American coast to the south up to 34°N (Grant, 1991; Coston-Clements *et al.*, 2009) and in the Central Northwest Atlantic (32°–38°N, 62°–73°W) at depths 500–3500 m (Pierrot-Bults, 1982; Cheney, 1985b). However, *P. maxima* was not found in the upper 1000 m in the Northwest Atlantic (the Sargasso Sea) between 33°N, 70°W and 14°N, 54°W (Pierrot-Bults and Nair, 2010). In small numbers, but regularly, *P. maxima* specimens were sampled in the Caribbean Sea (16°–11°N, 86°–75°W), in the Tropical Central Atlantic close to the Brazilian coast (3°–6°S, 32°–35°W) and in the Eastern Central Atlantic (4°–8°N, 26°–23°W) (Colman, 1959). Also a few juvenile specimens were found in the East Atlantic between 11°N, 20°W and 3°N, 15°W in the layer 600–1000 m (Pierrot-Bults and Nair, 2010). Southward, *P. maxima* is abundant along African coast between 0° and 35°S and is associated with the Benguela Current (David, 1965; Ducret and Casanova, 1968). In the South Atlantic, *P. maxima* is mostly abundant in the Subantarctic zone (David, 1965), but in small numbers it is also found in the Antarctic zone (Kruse *et al.*, 2009). Thus, available data show a gap in latitudinal distribution of the species between 32°N and 16°N. Ducret and Casanova (1968), followed by Cheney (1985a), suggested that *P. maxima* does not have independent populations in central and equatorial waters but is instead limited to polar, subpolar and transition waters and perhaps to upwelling regions on the eastern margins of the oceans.

The present molecular analysis recognized three well supported COI clades within *P. maxima* in the Atlantic Ocean. According to our data all three clades have distinct geographical distribution (Figure 4). Clade A is distributed in the Equatorial Atlantic at depths of 200–1200 m. We suggest that clade B occurs in the Subantarctic and Antarctic zones of the Southern Ocean from the surface to depth but it can also be found in the north to at least 32°S along South America to the Subtropical zone in the Antarctic Intermediate Water. Clade C is distributed throughout the Arctic and North Atlantic to the north of the Subtropical front at depths from the surface to at least 1200 m. The geographic distribution of clade C haplotypes in the North Atlantic indicated high gene flow through the whole region, as the same haplotype was found in many locations between 42°N and 85°N. Clade C is likely and evolutionarily young clade and has a high dispersal potential.

Tajima's D test was significant (and positive) only for *P. maxima* when three clades were analyzed all together. Positive values of Tajima's D arise from an excess of intermediate frequency alleles (Tajima, 1989). Although the Tajima's D statistic is commonly employed as a test for natural selection, this is confounded by the influence of population history. For instance, balancing selection may yield a positive value for Tajima's D. However, demographic processes such as population reduction, a recent bottleneck (Maruyama and Fuerst, 1985), population subdivision, or migration can also produce this result (Schmidt and Pool, 2002). In our case significant, positive value of Tajima's D most likely predicts a population subdivision in the distant past.

Results from the analysis of COI and ITS1 sequences indicate that *P. maxima* includes at least two distinct, reproductively isolated populations: tropical (clade A) and bipolar (clades B and C). This differentiation was not observed in the more conservative H3 gene. Nevertheless, we suggest that these populations should be considered as two distinct evolutionarily young species as some morphological differences between them were observed.

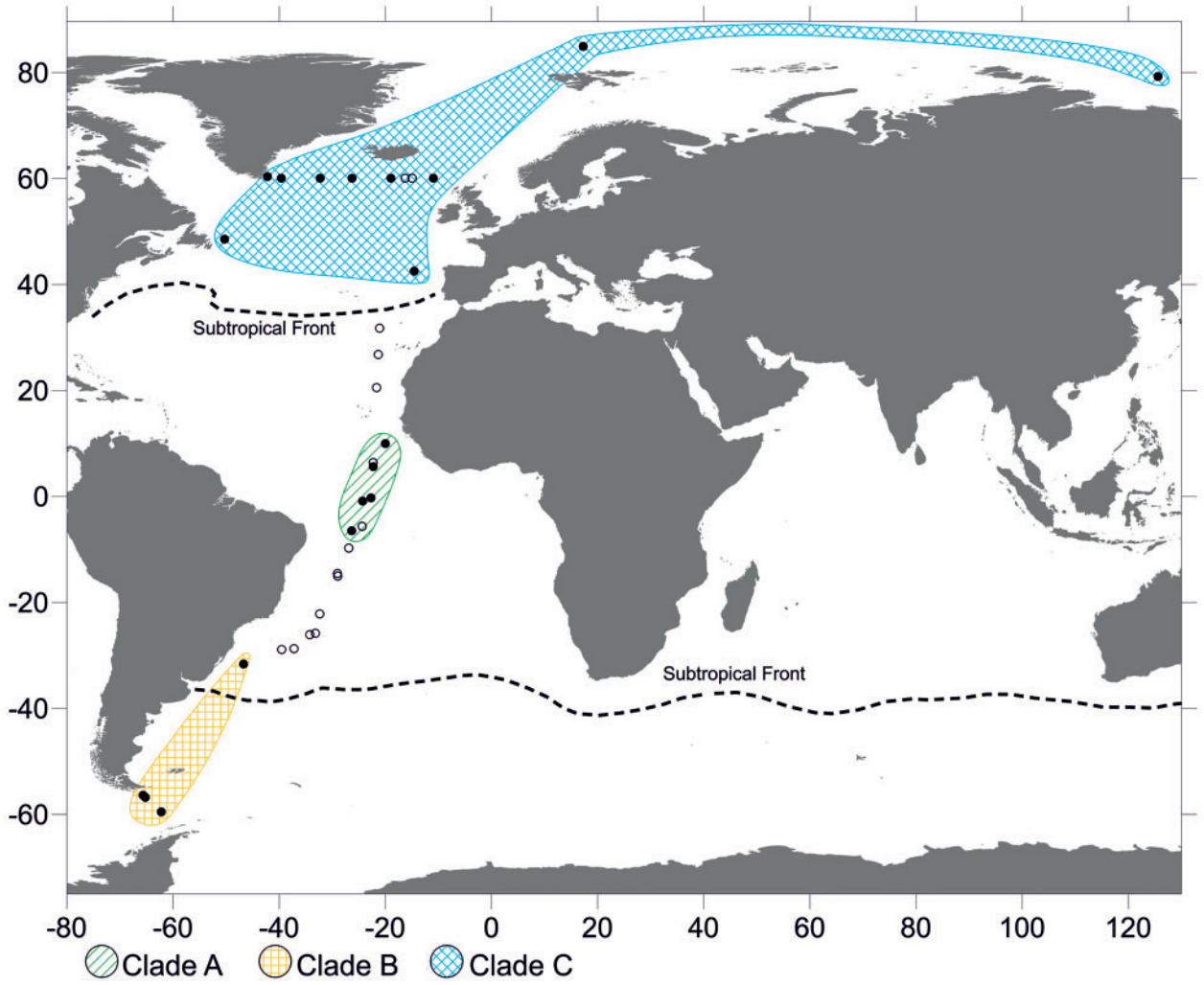

Figure 4. Geographical distribution of *Pseudosagitta maxima* mitochondrial clades. Small, open circles indicate sample locations where no specimens of this species were found.

For the present study, only a small number of *P. maxima* specimens was available and was insufficient to perform a robust morphological analysis. Nonetheless, we were able to compare our own observations with those in the literature to characterize *P. maxima* specimens from different parts of the Atlantic Ocean. David (1958) first described morphological differences between southern (attributed to our clade B) and northern (attributed to our clade C) *P. maxima* specimens which differ only in maximum body length. Arctic specimens are as long as 90 mm, whereas those in the Antarctic are 55 mm in length. At the same time, Colman (1959) conducted a more detailed morphological comparison of 167 specimens collected near south-west Greenland (clade C) and in tropical central Atlantic including the Caribbean Sea (probably clade A). He showed a gap in body length of adult specimens (stages II–IV); northern specimens ranged in size from 42 to 59 mm, whereas tropical specimens ranged from 16 to 34.6 mm. In addition, northern adults had larger numbers of hooks (H) and posterior teeth (PT) compared to those from the tropics (H6-8, PT5-8, and H3-5, PT2-5, respectively). Later Ducret and Casanova (1968) compared 325 specimens collected near Newfoundland (clade C) and along the coast of Angola in the Benguela Current. They found differences in the maximal size (58 mm—near Newfoundland and 43 mm—near Angola) in the number of hooks and posterior teeth (H7-8, PT4-5 vs. H4-5, PT5-6) and in the position of seminal vesicles. Differences in the relative tail length and in the relative position of the origin of anterior fin were unclear. Ghirardelli (1997) examined immature specimens (stages I–III) with body lengths up to 43 mm in the Magellan Strait, Subantarctic Zone (clade B). The number of hooks (8–10) was greater among the specimens 10–14 mm in length than in larger specimens, which had 5–6 hooks. The number of AT and PT were 2–4 and 3–5, respectively, and ova in their ovaries were arranged in 4–5 rows. This morphological characterization coincides with our clade B specimens.

Adult specimens that were analysed in the present study had damaged seminal vesicles and we were unable to compare them. Also we did not compare the body length as this character is variable and often depends on the environmental conditions (McLaren, 1963; Sameoto, 1971). Nevertheless, fully mature specimens from clade B (the Southern Ocean) had numbers of H, AT, and PT that were within the range observed by Colman for the northern population (attributed to our clade C). In stage I

juveniles, we also did not find clear differences in the numbers of hooks and teeth between clades B and C. Thus, both our results and previous results did not reveal any morphological differences between clades B and C, except for the maximum body length. The morphological characters of our clade A (tropical) specimens coincided with those in tropical Atlantic described by Colman (1959). Moreover, the adult clade A specimens from our samples were morphologically almost identical to those described by Srinivasan (1971) in the tropical Indian Ocean (10°N, 75°E). They had body lengths ranging from 33 to 36 mm; H4; AT2 and PT2-3; ova were arranged in two rows. We suggest that clade A has a wide tropical distribution in the oceans.

We could not clearly attribute the sample from the Benguela Current to one of our clades. In number of hooks, it is more closely related to clade A (tropical species), but the number of posterior teeth is similar to clade B (subantarctic) specimens.

Mature *P. maxima* specimens from the polar and subpolar regions have ova arranged in 4–6 rows instead of 2–3 rows in tropical waters. Thus, the arrangement of ova in the ovary might be a good character to distinguish between tropical (clade A) and bipolar (clades B and C) species within the *P. maxima* complex.

Numbers of hooks, posterior and anterior teeth are also proposed to be useful morphological characters to distinguish between tropical and bipolar species. These characters should be determined for each maturity stage separately. This is crucially important as otherwise they will be shared between both species without clear differences. Our morphological findings are preliminary; further morphological investigations will determine which characters can be used to distinguish between species within the *P. maxima* complex.

Based on the phylogenetic and geographic structure of the *P. maxima* species complex, the diversification within the *P. maxima* complex most likely took place in tropical waters. It can be assumed that clade B + C differentiated from their tropical ancestor due to colonization of the southern or northern subpolar zones and adaptation to biotopes with rich, but seasonally variable recourses. Later, splitting of B + C and establishment of a bipolar distribution could took as a result of the northward/southward migration through the Central Atlantic. Geological and paleontological evidence does not support vicariance as a process to create bipolar distributions (Lindberg, 1991) but favours biotic interchange, both southward and northward, between the hemispheres. These events are probably related to a plurality of mechanisms (i.e. regional tectonic events and Pleistocene glaciations) rather than a single event (Stepanjants *et al.*, 2006). Trans-equatorial dispersals are likely to have increased during cooling periods associated with glacial cycling in the Plio-Pleistocene; e.g. low-latitude sedimentary records indicate the presence of subpolar planktonic foraminifera within the equatorial zone during these cooling cycles (McIntyre *et al.*, 1989).

Trans-equatorial migration of clade B or C in the Atlantic Ocean could occur by one of the two ways: (1) passage through the tropics via deep water currents, (2) along the African coast with the cold currents and through the upwelling systems. The first way seems less probable due to scarcity of *P. maxima* in tropical and subtropical waters. The second way is more likely due to relatively higher abundance of *P. maxima* along the African coast at least from the South Africa to equator. Furthermore, during the last glacial maximum (18 000 years before present) in the tropical region, increased upwelling off Africa's west coast and intensification of the cool-water boundary currents (the Canary and Benguela), combined with the drop in global temperatures, produced cooler and narrower tropical water masses than those found today (McIntyre *et al.*, 1976; Mix and Morey, 1996). This would have increased the potential for trans-equatorial dispersals to higher latitudes. Darling *et al.* (2000) discussed this mechanism, associated with the cool boundary currents and the cool seasonal upwelling zones off west Africa, as a possible way for genetic exchange between the subpolar populations of planktonic foraminifers in the past and possibly at present. Moreover, for the copepod *Calanoides carinatus s. l.* that inhabit upwelling systems along the West Africa, recently it was observed unhampered gene flow along the African coast from 34°S to the Bay of Biscay (Viñas *et al.*, 2015). The uncertain morphological attribution of the Benguela Current population (Ducret and Casanova, 1968) to one of the two species in the *P. maxima* complex may indicate that this region is inhabited by both species. Further sampling in tropical waters and along the African coast are needed to resolve this question. Due to the absence of the molecular clock's estimates for chaetognaths, we are unable to establish the timelines of diversification of the clades within the *P. maxima* complex.

The absence of differences in nuclear markers between clades B and C implies that gene flow is occurring at present or occurred until recently between the polar regions. The latter is more likely, as there were no shared COI haplotypes between the Southeast and North Atlantic populations. Observed sequence divergences between individuals in Clades B and C (5.5%) are markedly lower than those usually observed in chaetognatha, even between cryptic species (Jennings *et al.*, 2010; Miyamoto *et al.*, 2010, 2012). Since we did not find any differences in morphology and nuclear markers (H3 and ITS1) between clades B and C, these clades at present should be considered as two populations (or subspecies) within a single bipolar species.

Future morphological and molecular investigations of the *P. maxima* complex should clarify differences between two young species (tropical and bipolar) within each stage of maturity to formally validate them as distinct species.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

Acknowledgements

We acknowledge the assistance of the Captains and crew of the R/V *Akademik Ioffe* (AI-30), R/V *Akademik Sergey Vavilov* (ASV-31, ASV-36), R/V *Polarstern* (ANT XXVII/2, ARC-XXVII-3) and R/V *Akademik Tryoshnikov* (NABOS-2015). Some specimens were kindly collected by Carmen David, Elena Rybakova and Galina Abyzova on their cruises on *Polarstern* and *ASV*. We are also very grateful for the kind help of Alexander Vereshchaka, Anastasia Lunina, Vladimir Gagarin and Elizaveta Ershova in collecting the samples. We gratefully acknowledge the support of the Russian Scientific Foundation, project №14-50-00029. The laboratory work was funded by the Russian Foundation for Basic Research, grants №15-29-02447, №16-04-00375 and №17-05-00725.

References

Alvariño, A. 1964. Bathymetric distribution of chaetognaths. Pacific Science, 18: 64–82.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K., Meier, R., Winker, K., Ingram, K. K. *et al.* 2007. Cryptic species as a window

on diversity and conservation. Trends in Ecology and Evolution, 22: 148–155.

Bieri, R. 1991. Systematics of the Chaetognatha. *In* The biology of Chaetognaths, pp. l22–136. Ed. by Q. Bone, H. Kapp, and A. Pierrot-Bults. Oxford Press, Oxford. 173 pp.

Bone, Q., Kapp, H., and Pierrot-Bults, A. C. 1991. Introduction and relationships of the group. *In* The biology of Chaetognaths, pp. 1–4. Ed. by Q. Bone, H. Kapp, and A. Pierrot-Bults. Oxford Press, Oxford. 173 pp.

Burridge, A. K., Goetze, E., Raes, N., Huisman, J., and Peijnenburg, K. T. 2015. Global biogeography and evolution of Cuvierina pteropods. BMC Evolutionary Biology, 15: 1.

Casanova, J. P. 1999. Chaetognatha. *In* South Atlantic Zooplankton, pp. 1353–1374. Ed. by D. Boltovskoy. Backhuys Publishers, Leiden. 1706 pp.

Cheney, J. 1985a. Spatial and temporal abundance patterns of oceanic chaetognaths in the western North Atlantic—I. Hydrographic and seasonal abundance patterns. Deep Sea Research Part A. Oceanographic Research Papers, 32: 1041–1059.

Cheney, J. 1985b. Spatial and temporal abundance patterns of oceanic chaetognaths in the western North Atlantic—II. Vertical distributions and migrations. Deep Sea Research Part A. Oceanographic Research Papers, 32: 1061–1075.

Colgan, D. J., McLauchlan, A., Wilson, G. D. F., Livingston, S., Macaranas, J., Edgecombe, G. D., Cassis, G., *et al.* 1998. Molecular phylogenetics of the Arthropoda: relationships based on histone H3 and U2 snRNA DNA sequences. Australian Journal of Zoology, 46: 419–437.

Colman, J. S. 1959. The Rosaura Expedition 1937-38. Chaetognatha. Bulletin of the British Museum, Zoology, 5: 219–253.

Coston-Clements, L., Waggett, R. J., and Tester, P. A. 2009. Chaetognaths of the United States South Atlantic Bight: distribution, abundance and potential interactions with newly spawned larval fish. Journal of Experimental Marine Biology and Ecology, 373: 111–123.

Darling, K. F., Wade, C. M., Stewart, I. A., Kroon, D., Dingle, R., and Brown, A. J. L. 2000. Molecular evidence for genetic mixing of Arctic and Antarctic subpolar populations of planktonic foraminifers. Nature, 405: 43–47.

David, P. M. 1958. The distribution of the chaetognatha of the Southern Ocean. Discovery Reports, 29: 199–228.

David, P. M. 1963. Some aspects of speciation in the Chaetognatha. *In:* Speciation in the Sea, pp. 129–143. Ed. by J. Harding, and N. Tebble. The Systematics Association, London. 199 pp.

David, P. M. 1965. The Chaetognatha of the Southern Ocean. *In* Biogeography and ecology in Antarctica, pp. 296–323. Ed. by J. Mieghen, and P. Oye. Junk Press, Hague. 762 pp.

Ducret, F., and Casanova, J. P. 1968. Variations géographiques chez le chaetognathe *Sagitta maxima* Conanat, 1896. Conseil International pourl'Exploration de la Mer. Comité Du Plankton, 1–3.

Gasmi, S., Nève, G., Pech, N., Tekaya, S., Gilles, A., and Perez, Y. 2014. Evolutionary history of Chaetognatha inferred from molecular and morphological data: a case study for body plan simplification. Frontiers in Zoology, 11: 84.

Geller, J., Meyer, C., Parker, M., and Hawk, H. 2013. Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. Molecular Ecology Resources, 13: 851–861.

Ghirardelli, E. 1997.Chaetognaths. *In:* Atlas of marine zooplankton, Straits of Magellan: Amphipods, Euphausiids, Mysids, Ostracods, and Chaetognaths, pp. 245–275. Ed. by L. Guglielmo, and A. Ianora. Springer-Verlag, Berlin. 275 pp.

Goetze, E. 2010. Species discovery through large-scale molecular screening in the planktonic copepod family Eucalanidae. Molecular Ecology, 19: 952–967.

Grant, G. C. 1991. Chaetognatha from the central and southern Middle Atlantic Bight: Species composition, temperature-salinity relationships, and interspecific associations. Fishery Bulletin, 89: 33–40.

Halbert, K. M., Goetze, E., and Carlon, D. B. 2013. High cryptic diversity across the global range of the migratory planktonic copepods *Pleuromamma piseki* and *P. gracilis*. PLoS One, 8: e77011.

Holland, B. S., Dawson, M. N., Crow, G. L., and Hofmann, D. K. 2004. Global phylogeography of *Cassiopea* (Scyphozoa: Rhizostomeae): molecular evidence for cryptic species and multiple invasions of the Hawaiian Islands. Marine Biology, 145: 1119–1128.

Hubert, N., Meyer, C. P., Bruggemann, H. J., Guerin, F., Komeno, R. J., Espiau, B., Causse, R., *et al.* 2012. Cryptic diversity in Indo-Pacific coral-reef fishes revealed by DNA-barcoding provides new support to the centre-of-overlap hypothesis. PLoS One, 7: e28987.

Jennings, R. M., Bucklin, A., and Pierrot-Bults, A. 2010. Barcoding of arrow worms (Phylum Chaetognatha) from three oceans: genetic diversity and evolution within an enigmatic phylum. PLoS One, 5: 1–7.

Jennings, R. M., Bucklin, A., Ossenbrügger, H., and Hopcroft, R. R. 2010. Species diversity of planktonic gastropods (Pteropoda and Heteropoda) from six ocean regions based on DNA barcode analysis. Deep Sea Research Part II: Topical Studies in Oceanography, 57: 2199–2210.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16: 111–120.

Kosobokova, K. N., Hopcroft, R. R., and Hirche, H. J. 2011. Patterns of zooplankton diversity through the depths of the Arctic's central basins. Marine Biodiversity, 41: 29–50.

Kramp, P. L. 1939. The Godthaab Expedition 1928. Chaetognatha. Meddelelser Om Grønland, 80: 3–40.

Kruse, S., Bathmann, U., and Brey, T. 2009. Meso-and bathypelagic distribution and abundance of chaetognaths in the Atlantic sector of the Southern Ocean. Polar Biology, 32: 1359–1376.

Kulagin, D. N., Stupnikova, A. N., Neretina, T. V., and Mugue, N. S. 2014. Spatial genetic heterogeneity of the cosmopolitan chaetognath *Eukrohnia hamata* (Möbius, 1875) revealed by mitochondrial DNA. Hydrobiologia, 721: 197–207.

Librado, P., and Rozas, J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25: 1451–1452.

Lindberg, D. R. 1991. Marine biotic interchange between the northern and southern hemispheres. Paleobiology, 17: 308–324.

Longhurst, A. R. 1985. The structure and evolution of plankton communities. Progress in Oceanography, 15: 1–35.

Maruyama, T., and Fuerst, P. A. 1985. Population bottlenecks and non-equilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. Genetics, 111: 675–689.

McIntyre, A., Kipp, N. G., Bé, A. W., Crowley, T., Kellogg, T., Gardner, J. V., Prell, W., *et al.* 1976. Glacial North Atlantic 18,000 years ago: a CLIMAP reconstruction. Geological Society of America Memoirs, 145: 43–76.

McIntyre, A., Ruddiman, W. F., Karlin, K., and Mix, A. C. 1989. Surface water response of the equatorial Atlantic Ocean to orbital forcing. Paleoceanography, 4: 19–55.

McLaren, I. A. 1963. Effects of temperature on growth of zooplankton, and the adaptive value of vertical migration. Journal of the Fisheries Board of Canada, 20: 685–727.

Mix, A. C., and Morey, A. E. 1996. Climate feedback and Pleistocene variations in the Atlantic south equatorial current. *In* The South Atlantic: Present and Past Circulation, pp. 503–525. Ed. by G. Wefer, W.H. Berger, G. Siedler, and D.J. Webb. Springer, Berlin, 644 pp.

Miyamoto, H., Machida, R. J., and Nishida, S. 2010. Genetic diversity and cryptic speciation of the deep sea chaetognath *Caecosagitta*

macrocephala (Fowler, 1904). Deep Sea Research Part II: Topical Studies in Oceanography, 57: 2211–2219.

Miyamoto, H., Machida, R. J., and Nishida, S. 2012. Global phylogeography of the deep-sea pelagic chaetognath *Eukrohnia hamata*. Progress in Oceanography, 104: 99–109.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York. 512 pp.

Nylander, J. A. A. 2004. MrModeltest ver. 2.3. Program distributed by the author. Evolutionary Centre, Uppsala University.

Peijnenburg, K. T., Breeuwer, J. A., Pierrot-Bults, A. C., and Menken, S. B. 2004. Phylogeography of the planktonic chaetognath *Sagitta setosa* reveals isolation in European seas. Evolution, 58: 1472–1487.

Pierrot-Bults, A. C. 1976. Zoogeographic patterns in chaetognaths and some other planktonic organisms. Bulletin Zoologisch Museum, 5: 59–67.

Pierrot-Bults, A. C. 1982. Vertical distribution of Chaetognatha in the central Northwest Atlantic near Bermuda. Biological Oceanography, 2: 31–61.

Pierrot-Bults, A. C. 2008. A short note on the biogeographic patterns of the Chaetognatha fauna in the North Atlantic. Deep Sea Research Part II: Topical Studies in Oceanography, 55: 137–141.

Pierrot-Bults, A. C., and Nair, V. R. 1991. Distribution patterns in Chaetognatha. *In* The biology of Chaetognaths, pp. 86–116. Ed. by Q. Bone, H. Kapp, and A. Pierrot-Bults. Oxford press, Oxford. 173 pp.

Pierrot-Bults, A. C., and Nair, V. R. 2010. Horizontal and vertical distribution of Chaetognatha in the upper 1000m of the western Sargasso Sea and the Central and South-east Atlantic. Deep Sea Research Part II: Topical Studies in Oceanography, 57: 2189–2198.

Pingree, R. D., Garcia-Soto, C., and Sinha, B. 1999. Position and structure of the Subtropical/Azores Front region from combined Lagrangian and remote sensing (IR/altimeter/SeaWiFS) measurements. Journal of the Marine Biological Association of the UK, 79: 769–792.

Ramšak, A., Stopar, K., and Malej, A. 2012. Comparative phylogeography of meroplanktonic species, *Aurelia* spp. and *Rhizostoma pulmo* (Cnidaria: Scyphozoa) in European Seas. Hydrobiologia, 690: 69–80.

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

Samemoto, D. D. 1987. Vertical distribution and ecological significance of chaetognaths in the arctic environment of Baffin Bay. Polar Biology, 7: 317–328.

Sameoto, D. D. 1971. Life history, ecological production, and an empirical mathematical model of the population of *Sagitta elegans* in St. Margaret's Bay, Nova Scotia. Journal of the Fisheries Board of Canada, 287: 971–985.

Schmidt, D., and Pool, J. 2002. The Effect of Population History on the Distribution of the Tajima's D Statistic. Cornell University Press, New York.

Srinivasan, M. 1971. Two new records of meso-and bathy-planktonic chaetognaths from the Indian seas. Journal of the Marine Biological Association of India, 13: 130–132.

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

Stepanjants, S. D., Cortese, G., Kruglikova, S. B., and Bjørklund, K. R. 2006. A review of bipolarity concepts: history and examples from Radiolaria and Medusozoa (Cnidaria). Marine Biology Research, 2: 200–241.

Tajima, F. 1989. Statistical methods to test for nucleotide mutation hypothesis by DNA polymorphism. Genetics, 123: 585–595.

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: 2731–2739.

Telford, M. J., and Holland, P. W. 1997. Evolution of 28S ribosomal DNA in chaetognaths: duplicate genes and molecular phylogeny. Journal of Molecular Evolution, 44: 135–144.

Tomczak, M., and Godfrey, J. S. 1994. Regional Oceanography: An Introduction. Pergamon, Oxford. 422 pp.

Vilgalys, R., and Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology, 172: 4238–4246.

Viñas, M. D., Blanco-Bercial, L., Bucklin, A., Verheye, H., Bersano, J. G. F., and Ceballos, S. 2015. Phylogeography of the copepod *Calanoides carinatus s.l.* (Krøyer) reveals cryptic species and delimits *C. carinatus s.s.* distribution in SW Atlantic Ocean. Journal of Experimental Marine Biology and Ecology, 468: 97–104.

Wang, L., and Jiang, T. 1994. On the complexity of multiple sequence alignment. Journal of Computational Biology, 1: 337–348.

Handling editor: Stewart Grant