Extensive genetic diversity and endemism across the global range of the oceanic copepod

Pleuromamma abdominalis

Junya Hirai ^{*1}, Atsushi Tsuda ², Erica Goetze ³

¹ National Research Institute of Fisheries Science, Fisheries Research Agency, 2-12-4 Fukuura, Kanazawa, Yokohama, Kanagawa 236-8648, Japan

² Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8564, Japan

³ Department of Oceanography, University of Hawaii at Manoa, Honolulu, HI 96822, USA

* Corresponding author: Junya Hirai

E-mail: jhirai@affrc.go.jp

Tel.: + 81 45 788 7819

ABSTRACT

Many oceanic zooplankton species have been described as cosmopolitan in distribution; however, recent molecular work has detected species complexity with highly divergent genetic lineages within several of these taxa. To further resolve the species complexity within these ecologically-important and widespread species, we performed both molecular and morphological analyses of the oceanic copepod *Pleuromamma abdominalis* using a comprehensive collection of material from 944 individuals collected at 46 sites across the global ocean. Phylogenetic analyses of mitochondrial cytochrome oxidase subunit I (mtCOI) sequences detected eighteen divergent evolutionary lineages within *P. abdominalis*, with an additional four singleton specimens that were also genetically divergent. Two phylogenetically distinct groups, PLAB1 and PLAB2, were supported by concordant sequence variation in the nuclear large subunit ribosomal RNA gene (nLSU). Within PLAB1, two mtCOI clades, 1a-1 and 1b-1 were observed, and each clade contained geographically distinct sub-clades 1a-2 and 1b-2. PLAB2 was composed of sixteen well-supported mtCOI clades (2a–2p) as well as four singletons. High genetic divergence among the mtCOI lineages within both PLAB1 and PLAB2, ranging between 9.2–11.2% and 4.3–18.9% K2P distances respectively, suggests the presence of additional species within these groups. Significant differences were observed in the presence and shape of antennule spines of adult females between sympatric clades with genetic distances greater than 5.7–7.0% (K2P). The biogeographic distributions of mtCOI clades indicated greater specialization to particular oceanographic provinces than observed in the nominal species *P. abdominalis*, with mtCOI clades ranging from antitropical in subtropical waters of all three ocean basins (Atlantic, Pacific and Indian; clade 1b-1 and 2a) to taxa that are endemic to a particular ocean region, for example restricted to equatorial waters of the Atlantic Ocean (clade 1b-2 and 2b). We hypothesize that many of these mtCOI clades are likely distinct, and currently undescribed species. The well-known high dominance of *P. abdominalis* across a range of pelagic habitats may occur as a spatial composite of genetically-distinct species with more restricted distributions and greater

ecological specialization to particular marine habitats than was previously recognized.

Keywords:

Species complex; marine biogeography; cytochrome oxidase subunit I; *Pleuromamma abdominalis*

1. Introduction

Many oceanic zooplankton are recognized as broadly-distributed species across the global ocean, with high dispersal potential and tolerance to a range of oceanographic environments (van der Spoel and Heyman, 1983; Angel, 1993; Norris, 2000). Recent population genetic studies, however, have uncovered limited gene flow among populations across the distributional range in a number of oceanic species (e.g., Unal and Bucklin, 2010; Norton and Goetze, 2013; Andrews et al., 2014). In addition, divergent genetic lineages have been observed both in allopatry and in sympatry within a single nominal species for several planktonic groups, including species of chaetognaths (Peijnenburg et al., 2004, 2006; Miyamoto et al., 2010, 2012), copepods (Bucklin et al., 1996; Goetze, 2003, 2005, 2010; Blanco-Bercial et al. 2014; Cornils and Held, 2014), euphausiids (Bucklin et al., 2007), molluscs (Jennings et al., 2010; Burridge et al., 2015), and cnidarians (Schroth et al., 2002). These divergent lineages demonstrate that the genetic structure of cosmopolitan zooplankton species is more complex than previously thought in many cases, with several nominal species now known to consist of a mosaic of distinct evolutionary lineages. Relatively high undescribed metazoan diversity also has been reported in metabarcoding studies of oceanic assemblages (Lindeque et al., 2013; de Vargas et al., 2015; Hirai and Tsuda, in press), indicating that many described species may harbor these divergent lineages. In contrast to coastal waters, where there can be strong geographic isolation (Lee, 2000; Chen and Hare, 2011), it is unclear how genetic diversification of zooplankton occurs in open ocean environments. Zooplankton are abundant and serve important roles in marine food webs and biogeochemical cycles in the open ocean; therefore, it is important to understand the

mechanisms driving diversification in this faunal group. Documenting the genetic diversity present within cosmopolitan taxa is also important to better understanding the individual ecological roles of their constituent species.

The oceanic copepod *Pleuromamma abdominalis* (Lubbock, 1856) is a good model species for global-scale genetic studies of oceanic zooplankton, due to its cosmopolitan distribution (Steuer 1932; Wilson, 1932; Razouls et al., 2005–2015), high abundance across a range of marine ecosystems (Steinberg et al., 2000; Al-Mutairi and Laundry, 2001; Andersen et al., 2004), and known intraspecific variation (both morphological and genetic: Fornshell and Ferrari, 2010; Hirai et al., 2013). First described as *Diaptomus abdominalis* by Lubbock (1856), this species was then placed under the name *Pleuromma abdominale* by Claus (1863). The species name was finally established as *Pleuromamma abdominalis* after Giesbrecht and Schmeil (1898). There are currently 11 described species in the genus *Pleuromamma*, and all are characterized by the presence of a black spot, or ‘pigment knob’, on the right or left side of the lateral prosome (Blades-Eckelbarger and Youngbluth, 1988; Boxshall and Halsey, 2004). *Pleuromamma abdominalis* is of intermediate body size relative to all species in the genus, and distributed worldwide primarily in tropical and subtropical oceans. This nominal species is highly abundant in the upper mesopelagic zone (Longhurst, 1985; Hays et al., 1997; Andersen et al., 2004), and serves as important, and even preferred, prey to larger predatory crustaceans and fish (Foxton and Roe, 1974; Lancraft et al., 1988; Bacha and Amara, 2009). *Pleuromamma abdominalis* also undergoes an extensive vertical migration to the surface layer at night, acting as a transporter of organic matter to the mesopelagic zone, along with other vertically migrating zooplankton (Hays et al., 1997; Steinberg et al., 2000).

Intraspecific morphological variation has been reported within *P. abdominalis*, with three currently recognized forms (reviewed in Fornshell and Ferrari, 2010): *P. abdominalis* forma *abdominalis* (forma *typica*), *P. abdominalis* forma *abyssalis*, and *P. abdominalis* forma *edentata*. Forma *abyssalis* is known only in males, and forma *edentata* is described only in females. Forma

edentata is discriminated from forma *abdominalis* (*typica*) by the absence of a large curved spine on the proximal segment of the antennule (A1 spine) in the female (Steuer, 1932). However, it is also known that the shapes of A1 spines in adult female *P. abdominalis* are more variable than implied by description of these forms within the nominal species and forms (Fornshell and Ferrari, 2010). In males, forma *abdominalis* (*typica*) shows a strongly asymmetrical urosome and simple lateral seta on the left caudal ramus, in comparison to forma *abyssalis*, which is more symmetrical in body form (Steuer, 1932; Fornshell and Ferrari, 2010). Several prior studies have reported that the vertical and horizontal distributions of these forms are not obviously distinct or not fully characterized (Steuer, 1932; Sewell, 1947; Fornshell and Ferrari, 2010). However, different distribution patterns also have been observed between forma *typica* and forma *edentata* in the eastern North Pacific Ocean (Longhurst, 1985; Haury, 1988). Collectively, these studies provide prior indication that the diversity within *P. abdominalis* is not fully characterized in the current systematic literature.

The genus *Pleuromamma* is also known to harbor cryptic species complexes, with a number of extant species comprised of several evolutionary lineages. At least four genetic clades exist within *P. xiphias* and these clades exhibit both geographically distinct distributions across the global subtropics and tropics, as well as in some cases significantly different body size (Goetze, 2011). A global study of *P. gracilis* and *P. piseki* also revealed that these morphological species did not form monophyletic groups, and twelve genetically-divergent mitochondrial clades were observed (Halbert et al., 2013, also see Blanco-Bercial et al. 2014). Although there have been no prior comprehensive genetic studies of *P. abdominalis*, in the western subtropical Pacific this nominal species was comprised of at least two genetic groups (0.2% K2P divergence), based on combined sequences of the slowly-evolving nuclear internal transcribed spacer 2 and the large subunit ribosomal RNA genes (nLSU), with some evidence for differences in body size between these populations (Hirai et al., 2013). High sequence differentiation also was observed in the mitochondrial cytochrome oxidase subunit I (mtCOI) sequences that were previously reported for DNA barcoding of *P. abdominalis* in

GenBank (see placement of these sequences in Fig. 3). These genetic separations also suggest that there may be several distinct species within *P. abdominalis*.

In this study, we conducted a global molecular and morphological survey of the evolutionary lineages present within the nominal species *P. abdominalis*. Our goals were to detect evolutionarily distinct lineages within the described species, resolve ambiguities raised by the intraspecific morphological and genetic variation reported within *P. abdominalis*, and provide a novel synthesis of the diversity and biogeography of the lineages present within this dominant, ecologically-important and cosmopolitan copepod species. We recognize the value of integrative taxonomic studies and multiple data sources in effectively detecting species boundaries (de Queiroz, 1998, 2007; McManus and Katz, 2009; Toews and Brelsford, 2012; Carstens et al., 2013; Pante et al., 2015), and have therefore attempted to obtain diverse information on the phenotypic, genetic, and biogeographic variation present across the global distribution of the species. Embracing the view that species are hypotheses (de Queiroz, 2007; Carstens et al., 2013; Pante et al. 2015), we present our results as partial evidence toward formal systematic revision of the species. Using a comprehensive collection of material from across the global ocean (46 sites; Atlantic, Pacific, and Indian Oceans), we survey genetic diversity within *P. abdominalis* using sequences from the mitochondrial gene cytochrome oxidase subunit I (mtCOI), and then investigate support for species-level divergence among mitochondrial clades using sequences from the nuclear large subunit ribosomal RNA gene (nLSU). Given known variation in the presence, shape, and the extent of development of antennule spines within adults of this genus, we also examined adult females for variation in these traits, in order to test for concordance with mitochondrial results. Finally, we report on the global distributions of evolutionary lineages newly discovered within this nominal species. There are currently very few global molecular studies of oceanic plankton, and many zooplankton species have been recognized as cosmopolitan, with occurrence across a range of pelagic environments. This study provides important and novel insight into the undescribed diversity and basin-scale biogeography of lineages

present within cosmopolitan plankton species.

2. Materials and methods

2.1. Samples and specimens

Samples were collected at a total of 46 sites: 14 in the Atlantic Ocean, 7 in the Indian Ocean, and 25 in the Pacific Ocean (Fig. 1). Detailed information on the number of individuals at each site, sampling location, date, cruise, and sampling gear are reported in Table 1. Bulk plankton samples were preserved in 95-99% ethanol during cruises in the Pacific and Indian Oceans (VANC10MV, KT-10-21, SY-11-05, KH-11-10, KH-12-01, KH-13-01, and KH-13-02). Ethanol was replaced within 24 h of initial preservation, and samples were kept at -20°C for long-term storage. On the Atlantic cruise (AMT22), *P. abdominalis* specimens were sorted live at sea, preserved immediately in RNAlater Stabilization Solution (Life technologies), flash frozen in liquid nitrogen, and stored at -80°C. A total of 944 individuals of *P. abdominalis* were included in this study. Adult females were selected primarily, however, males and copepodites also were used in cases of insufficient numbers of adult females in the Pacific Ocean material. *Pleuromamma abdominalis* is intermediate in body size, and easily distinguished by size from the smaller species *P. gracialis*, *P. piseki*, and *P. borealis*, as well as from the larger species *P. xiphias* (Chihara and Murano, 1997; Bradford-Grieve 1999). Adult females of *P. abdominalis* are distinguishable from *P. quadrangulata* by the presence of single, rather than paired curved spines on each antennule (A1 spines), and from *P. robusta* by the central positioning of the genital swelling on the genital segment in lateral view (rather than in an anterior position; Bradford-Grieve, 1999). *Pleuromamma indica* was identified by its relatively small body size in comparison to *P. abdominalis*, as well as by the straight A1 spines, whose termini were oriented towards the distal portion of the antennule (Chihara and Murano, 1997). The majority of our *P. abdominalis* specimens showed a large curved A1 spine. However, we also observed *P. abdominalis* specimens with straight A1 spines; in these cases, they were distinguished from *P.*

indica by body size and by the direction of the A1 spine termini, which were oriented toward the proximal part, rather than the distal part, of the antennule (see Fig. 2C). Morphological observations were made only on adult females of *P. abdominalis*, with 808 of the 944 individuals included in morphological observations for major mitochondrial groups. The following characters were recorded: prosome length (PL), location of the pigment knob on the lateral prosome (right/left), and the presence, shape, and extent of development of the A1 spines. The shape of the A1 spines were classified into three categories: (1) curved, (2) straight, and (3) reduced in size (as shown in Fig. 2). Four specimens of *P. indica* and one specimen of *P. robusta* also were included in phylogenetic analyses, and the collection sites for these specimens are reported in Table 1.

2.2. DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (QIAGEN), and the DNA was kept at -20 or -80°C. A 441-bp fragment of the mitochondrial gene cytochrome oxidase subunit I (mtCOI) was used for phylogenetic inference, with polymerase chain reaction (PCR) amplification using the primer pair L1384-COI (5'-GGTCATGTAATCATAAAGATATTGG-3'; Machida et al., 2004) and PLAB-R (5'-GTKGTAAAATATGCYCGTGTGTC-3'; this study). PCR also was carried out for a 406-bp fragment of the nuclear large subunit ribosomal RNA gene (nLSU), in order to assess concordance with mtCOI results, using primers LSU Cop-D1F (5'-GCGGAGGAAAAGAAAACAAC-3') and LSU Cop-D3R (5'-CGATTAGTCTTTGCCCCCT-3'; Hirai et al., 2015). PCR amplification for samples in the Pacific was carried out using TaKaRa Z-Taq polymerase (Takara) in 15 µL reaction mixtures containing 7.62 µL of distilled water, 1.5 µL 10X buffer, 1.2 µL dNTPs (2.5 µM each), 1.8 µL of each primer (5 µM), 0.08 µL Z-Taq, and 1 µL of template DNA. PCR cycling included denaturation at 94°C for 2 min, followed by 35 cycles of 5 s denaturation at 94°C, 5 s annealing at 45°C (mtCOI) or 55°C (nLSU), and 10 s extension at 72°C, with a final extension set at 72°C for 2 min. For samples in the Atlantic and Indian Ocean,

MangoMix (BIOLINE) was used for PCR in 10 μ L reaction mixtures containing 2.5 μ L distilled water, 5 μ L 2 $\times$ Reaction Mix containing MangoTaq, 0.25 μ L of each primer (10 μ M), and 2 μ L template DNA, with PCR cycling as follows: 95°C for 30 s, followed by 40 cycles of 40 s denaturation at 95°C, 30 s annealing at 45°C (mtCOI) or 55°C (nLSU), and 1 min extension at 72°C, with a final extension at 72°C for 4 min. All PCR products were purified with ExoSap-IT (GE Health BioScience), prepared for sequencing using the BigDye Terminator v3.1 (Applied Biosystems), and analyzed on a 3130 DNA Sequencer (Applied Biosystems).

2.3. Phylogenetic analyses

MtCOI sequences were obtained from all 944 individuals, and manually confirmed and edited by comparing aligned sequences for both forward and reverse strands using Geneious R7 (Biomatters). Consensus sequences from all individuals were aligned using MUSCLE (Edgar, 2004). There were no indels (insertions or deletions) in the final mtCOI sequence alignment, and no stop codons in the amino acid translation. Phylogenetic analyses were performed using both Bayesian inference (BI) and maximum likelihood (ML) for mtCOI sequence data. The GTR+I+G model was selected as the best-fit nucleotide substitution model based on the Akaike Information Criterion in MrModeltest 2.3 (Nylander et al., 2004). Bayesian analyses were performed in MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003). The Markov Chain Monte Carlo chains were run for 5.0×10^7 generations, and trees were sampled every 1,000 generations. The first 25% of the generated trees were eliminated as burn-in. Maximum likelihood analyses were performed with the GTR+G model and 1,000 bootstrap replicates to assess nodal support, using RAxML v.8.0.0 (Stamatakis, 2014). We included six *P. abdominalis* mtCOI sequences from GenBank [accession numbers: AB379989, North Pacific around Japan (Machida et al., unpublished data); GU171314, Sargasso Sea (Bucklin et al., 2010); HM045329 and HM045400–045402, Chinese coastal waters (Sun et al., unpublished data)] in our phylogenetic analyses, in order to place these prior reference specimens in context relative to the

global diversity within the species. We also obtained mtCOI sequences of sympatric, intermediate body-sized species *P. robusta* (LC050731) and *P. indica* (LC050732–LC050733), and used these species as outgroups in our phylogenetic analyses. Using results of phylogenetic analyses, mtCOI sequences were classified into genetic clades based on the following criteria: (i) sequences within a monophyletic group with > 0.9 Bayesian posterior probability; (ii) the group contained at least two individuals; and (iii) the group was characterized by less than 3% pairwise nucleotide differences for intra-clade sequence comparisons. We defined this threshold (3%) based on the observed pairwise nucleotide differences for all mtCOI sequences in the study. This value also was supported by results from the R-package SPIDER (Brown et al., 2012), which yielded a 3.1% pairwise distance as the optimal threshold to distinguish operational taxonomic units (OTUs) for our data. Note that we observed other divergent sequences (2.2–4.4% average K2P distance from the closest genetic clade) that occurred in only single specimens; these sequences are described throughout as ‘singletons’. Genetic distances between and within mtCOI clades were calculated using Kimura’s two-parameter (K2P) nucleotide substitution model (Kimura, 1980) using ARLEQUIN 3.5 (Excoffier and Lischer, 2010). Observations on morphological variation across clades, as well as sequence variation at the nuclear marker nLSU, were used to assess support for species-level divergence among mtCOI clades. Prosome lengths for each clade were compared using the non-parametric Kruskal-Wallis test and Dunn’s test using SPSS 21.0 (IBM Corporation).

2.4. Clade distributions

We investigated the composition of genetic clades at each site and described the global biogeography of each mtCOI clade. In the Atlantic Ocean, we also compared clade distributions to ocean temperature and chlorophyll-*a* (chl-*a*) (0-300m), in order to investigate how environmental variables co-vary with clade distributions. Vertical profiles of temperature and chl-*a* concentration on the Atlantic Meridional Transect Cruise 22 (AMT22) were obtained using a Sea-Bird Electronics

3P Temperature Sensor and Chelsea MKIII Aquatracka Fluorometer, with data calibrated and archived by the British Oceanographic Data Center (BODC; https://www.bodc.ac.uk/data/online_delivery/nodb/search/).

3. Results

3.1. Phylogenetics

A total of 430 haplotypes were sampled among the 944 mtCOI sequences. Amino-acid substitutions were observed at eight sites, and four substitution sites were present in only a single individual. The mtCOI phylogeny revealed eighteen mtCOI clades and four genetically divergent singletons that were well supported by BI posterior probability and ML bootstrap values (Fig. 3). All 944 mtCOI sequences were submitted and are publicly available as a PopSet under GenBank accession numbers KT319926–KT320869, with information on collection site, mtCOI clade, and haplotype for each individual.

Two genetically very divergent clades, PLAB1 and PLAB2, were reciprocally monophyletic, with high posterior probability in Bayesian analyses (1.00), but with lower bootstrap support for PLAB2 in ML analyses (< 70% bootstrap support). The inter-clade K2P distance between PLAB1 and PLAB2 was very high, at 16.6–22.7% (Table 2). PLAB2 was more diverse than PLAB1, and was composed of sixteen clades (2a–2p) and four singletons. PLAB1 included two major clades 1a-1 and 1b-1, with additional sub-clades 1a-2 and 1b-2, respectively. The sub-clades 1a-2 and 1b-2 were analyzed separately, due to relatively high genetic distances from 1a-1 and 1b-1 and their distinct biogeography (see Fig. 5 below). Genetic divergence among mtCOI clades within PLAB1 ranged from 9.2% (K2P) between sub-clades 1a-1 and 1b-1 up to 11.2% between 1a-2 and 1b-2. Within PLAB2, clades 2a–2d and singletons S1–S3 were placed within a well-supported group (Fig. 3), with these lineages 4.3–7.0% divergent from one another (Table 2). Clades 2a, 2b, and 2c were reciprocally monophyletic relative to one another, and clade 2d was placed in a polytomy with the

other clades within this group. Clades 2e–2i were earlier diverging lineages relative to clades 2a–2d, with moderate inter-clade distances of 7.3–8.9% (K2P, Table 2) between the closely related clades. The most basal lineages within PLAB2 were clades 2j–2p, and these lineages showed high genetic distances (>10%), even between the most closely related clades, with the exception of sister clades 2k and 2l (5.2% K2P distance) and 2m and 2n (4.3% K2P distance). Within PLAB2, the maximum inter-clade genetic distance was high for all clades, ranging from 16.6% to 18.9%. Singletons (S1–S4) were not highly divergent genetically from their closest clades (2.2–4.4 %; Fig. 3, Table 2), but also did not fall within well-defined groups (< 3% intra-clade genetic distance). Therefore, they are reported as singletons due to their ambiguous status. Intra-clade K2P distances were 0–1.9%, with the highest value observed in clade 2e (1.9%). In comparison to other described congeners within *Pleuromamma*, clades within PLAB1 showed 22.1–24.8% K2P distances to *P. robusta* and 22.1–24.4% to *P. indica*. Within PLAB2, the lowest K2P distance to *P. indica* was observed in clade 2n (17.4%), which was nearly equivalent to some of the inter-clade K2P distances observed within PLAB2. *Pleuromamma abdominalis* reference sequences from earlier DNA barcoding studies were placed into genetic clades 1b-1 (HM045401, Chinese coastal seas), 2a (GU171314, Sargasso Sea, and HM045329, HM045400, Chinese coastal seas), 2e (HM045402, Chinese coastal seas), and 2f (AB379989, North Pacific, Japanese waters) (Fig. 3).

nLSU sequences were obtained from nearly all individuals in the Pacific Ocean (445 sequences), and from selected individuals in the Atlantic and Indian Oceans (52 sequences, Table 3). Three types of nLSU sequence were observed, based on four diagnostic nucleotide sites (Fig. S1): nLSU type 1 (GenBank accession: LC050728), type 2 (LC050729) and type 3 (LC050730). Type 1 was only observed in PLAB1 and type 2 only in PLAB2. Both PLAB1 and PLAB2 contained individuals with type 3 sequences. There were individuals with an admixture of sequence types, which appeared as nearly equivalent double base calls (see Fig. S1). These double base calls were observed between types 1 and 3 (type 1+3) in PLAB1, and between types 2 and 3 (type 2+3) in

PLAB2. nLSU type 3 was primarily observed in the Atlantic and Indian Oceans, with few individuals of nLSU type 3 in the Pacific (Table 3). There were no individuals with admixtures of sequence types 1 and 2: We consider this sufficient evidence to infer that PLAB1 and PLAB2 must be divergent at the species level (or above, see *Results* below).

3.2. Morphological variation

Average prosome length was significantly different among clades, both within PLAB1 (1.99–2.13 mm, Kruskal-Wallis test, $p < 0.05$) and within PLAB2 (1.83–2.4 mm, Kruskal-Wallis test, $p < 0.05$) (Fig. 4A). In PLAB1, clade 1a-2 was significantly larger than 1a-1 and 1b-2, but not 1b-1 (Dunn's test, $p < 0.05$). Note that the large size of clade 1a-2 might occur as a result of its cold-water distribution in temperate regions (see next section). In PLAB2, statistical analysis was only performed for clades with sufficient sample sizes (> 10 individuals), including clades 2a, 2b, 2c, 2d, 2e, 2i, 2j, and 2m. Clade 2i (2.4 mm) was significantly larger than all other clades (Dunn's test, $p < 0.05$), despite its tropical distribution and high seawater temperature oceanographic conditions. Clades 2a, 2b and 2e were significantly larger than clades 2c, 2d, 2j and 2m (Dunn's test, $p < 0.05$).

The shape of antennule spines in adult females was closely related to mtCOI clade membership, although curved A1 spines were the most common across all clades (Fig. 4B). The majority of clades showed fixed character states for A1 spine shape characters (defined as $< 5\%$ variation in the trait; Wiens and Servedio, 2000). Clades 1a-2, 1b-2, 2a, 2b, 2c, 2e, 2i, and 2j all were effectively invariant in having curved A1 spines, with curved spines likely the ancestral character state for *P. abdominalis*. Clades 2d and 2m were close to invariant, with 2d having 95% of 57 specimens with straight A1 spines (N=3 exceptions), and 2m with 93% of specimens with reduced A1 spines. Clade 2d, therefore, showed significant differences to closely related clades 2a, 2b and 2c all of which are fixed in having curved A1 spines (5.7–7.0% K2P distances, Fig. 4B). Of the remaining clades with sample sizes larger than 10 individuals (clades 1a-1, 1b-1), 1a-1 in particular

was variable in A1 shape characters, with 71% of animals having reduced spines and 29% of animals with curved spines. Although sample sizes were low, some differences in A1 spines were also observed between sister clades 2k (reduced spines, N=4) and 2l (curved spine, N=4).

The pigment knob was observed on both sides of the lateral prosome in all mtCOI clades with sample sizes > 5 (Fig. 4C). Although the proportions of right or left side animals were different across each clade, and ranged from being approximately equivalently common to being highly biased to one side, no clear relationship was observed between the location of the pigment knob and mtCOI clade membership.

3.3. Global biogeography of mtCOI clades

The global distributions of all mtCOI clades are shown in Fig. 5 and summarized in Table 3. Categorical biogeographic regions observed in this study are illustrated in Fig. S2. As described below, some clades were endemic to a specific biogeographic region (see Fig. S2): In contrast, others had wide distributions across the global ocean. MtCOI clade range limits in the Atlantic Ocean co-varied with ocean seawater temperature and chlorophyll *a*, with major oceanographic transitions also marked by changes in the dominant mtCOI lineages (Fig. 6).

Of the PLAB1 lineages, clade 1a-1 occurred in the equatorial Indo-Pacific, with its distribution extending northward into the western subtropical North Pacific. This clade was the only *P. abdominalis* lineage occurring in the easternmost equatorial Pacific (sites 40 and 41). The sub-clade 1a-2 was restricted to temperate regions of the Atlantic and Indian Oceans with low sea surface temperature and high chl-*a*, and extended into the northern edge of the subtropical gyre in the northern hemisphere. Clade 1b-1 was globally observed across the Pacific, Indian and Atlantic Oceans in subtropical waters, but mainly found in the subtropical western North Pacific. Closely-related sub-clade 1b-2 was restricted to the equatorial Atlantic with higher chl-*a* concentrations and shallower thermocline depth, in comparison to the subtropical gyres. The range

margins of clade 1b-2 agreed with the equatorward margins of the subtropical gyres, marked by the transition to high sea surface temperatures, low chl-*a* concentrations and a deep chl-*a* maximum within the subtropics.

Of the PLAB2 lineages, clades 2a, 2c, and 2d were distributed in subtropical waters. Clade 2a was globally distributed, and dominant in the North Pacific and North Atlantic subtropical gyres, while also present in the South Pacific and Indian Ocean subtropical gyres. Clades 2c and 2d were observed in the South Pacific subtropical gyre, with a few specimens of clade 2c also found in the North Pacific subtropics at sites 22 and 28. Clades 2a, 2c, and 2d co-occurred in the South Pacific subtropical gyre at sites 44–46. Clade 2a was widely distributed across the Atlantic transect and dominant in the North Atlantic subtropical gyre, but the dominant clade was replaced by clade 2b in the equatorial Atlantic. Clade 2b was an endemic clade to the Atlantic Ocean, with a distribution closely matching that of sub-clade 1b-2. Clade 2j dominated in the highly oligotrophic subtropical gyre in the South Atlantic, and its distribution extended into the western part of the Indian Ocean. Clade 2p was observed in the temperate North Pacific and Atlantic (sites 1 and 14). Other major mtCOI clades ($N > 10$), including clades 2e, 2i, and 2m, had distributions restricted to equatorial waters of the Pacific and Indian Oceans, mainly in the equatorial Indo-Pacific (regions no. 7 and 9 in Fig. S2), including the eastern Indian Ocean (sites 19-21) and the western Pacific Ocean (sites 29-33). The distribution of these clades also extended northward into the Kuroshio region in most cases. Clades 2f and 2k occurred only in the central equatorial Pacific (sites 36–39). The biogeography of other clades (2g, 2h, 2l, 2n, and 2o) and the singletons were poorly characterized, due to their rarity and insufficient sample sizes. Clade 2g was observed in the equatorial Atlantic and Indian temperate zones and clade 2l in Atlantic and Indian subtropical waters (Table 1). Clades 2h, 2n and 2o were collected in the eastern Indian or western Pacific Oceans. Singletons were observed at sites 28 (S1), 25 (S2), 29 (S3), and 18 (S4) across the Pacific and Indian Oceans.

4. Discussion

Cosmopolitan distributions across global ranges are common in marine species under conventional morphological taxonomy; however, molecular analyses have uncovered several genetic lineages in a number of marine taxa, suggesting the presence of undescribed species (e.g., Miya and Nishida, 1997; Knowlton, 2000; Pérez-Portela et al., 2013). Although appropriate recognition of species is important for the conservation of species diversity and monitoring of marine ecosystems, the numbers of marine species are significantly underestimated at present (Appeltans et al., 2012; de Vargas et al., 2015). For marine zooplankton, cosmopolitan distributions are accurate in some cases, and have been validated by molecular results (e.g., shared haplotypes across ocean basins; Goetze, 2005). In other cases, nominal cosmopolitan zooplankton are comprised of several evolutionarily distinct lineages (Goetze, 2011; Miyamoto et al., 2012; Weiner et al., 2012; Halbert et al., 2013; Cornils and Held, 2014; BurrIDGE et al., 2015). Because sibling species often show distinct distributional patterns and ecology in oceanic copepods (e.g., *Clausocalanus farrani* and *C. jobei* with 9% mtCOI variation; Bucklin et al., 2003), it is important to reveal species boundaries and broader evolutionary patterns of ecologically important cosmopolitan species that show high abundance under a variety of oceanographic conditions. In this study, extensive genetic diversity was observed within the cosmopolitan copepod *Pleuromamma abdominalis*, with clade-specific morphological differences and biogeographic distributions. First, we evaluate the possibility that these divergent genetic clades are distinct, and currently undescribed species. Then we discuss the biogeography of genetic clades, and mechanisms that may drive diversification and speciation in the group.

4.1. Are these MtCOI clades distinct species?

The global mtCOI phylogeny revealed a total of eighteen well-supported mtCOI clades and four singletons within *P. abdominalis*, making this nominal species one of the most genetically

diverse oceanic copepods reported on to date (e.g., Goetze, 2010; Cornils and Held, 2014). Several other cosmopolitan species within the genus *Pleuromamma* are also known to be comprised of several evolutionary lineages, including *P. xiphias* with four genetic clades (Goetze, 2011) and *P. gracilis/piseki* with twelve genetic clades (Halbert et al., 2013), suggesting that cryptic or pseudocryptic species complexes may be common to the group and the genus is in need of significant systematic revision. The mitochondrial lineages observed within *P. abdominalis* could reflect either: (1) valid distinct species that were previously undetected within the group, or (2) ancient mitochondrial lineages present within animals that are capable of interbreeding. Genetic analyses in this study support the inference of at least two distinct species, PLAB1 and PLAB2, that were supported by concordant results between independently-inherited mtCOI and nLSU. However, there was high mtCOI divergence within both PLAB1 (9.2–11.2% K2P distance) and PLAB2 (4.3–18.9% K2P distance), indicating the possibility of additional species within these major clades. MtCOI divergence between clades was above typical intraspecific variation observed in crustaceans, including copepods, and most genetic distances (> 10%) were higher than those seen between crustacean sibling species (Bucklin et al., 2003; Costa et al., 2007). Some divergent clades within PLAB2 were nearly equivalent in genetic distance to that between *P. abdominalis* and *P. indica* (Table 2), supporting the inference that these *P. abdominalis* mtCOI clades are likely valid species. nLSU is a more slowly-evolving gene than mtCOI in copepods (Machida and Tsuda, 2010; Blanco-Bercial et al., 2011), and nLSU was less informative than mtCOI for detecting species boundaries within *P. abdominalis*. Nonetheless, differences in sequence variation were observed among major clades, providing support that at least some of these lineages are almost certainly distinct species. Multi-locus genetic analyses are necessary for molecular species delimitation (Boissin et al., 2008; Casteleyn et al., 2010; Chen and Hare, 2011; Gaither et al., 2011), however, it has been found that global molecular surveys using mitochondrial DNA can be very effective for initial detection of species boundaries in oceanic copepods (Goetze, 2010). Andrews et al. (2014)

recently reported on two cryptic species within the subtropical copepod *Haloptilus longicornis* with genetic clades that were only 1.6% divergent at mitochondrial marker mtCOII, using nuclear microsatellite markers to confirm that these close mitochondrial lineages were valid species. Therefore, the relatively high sequence divergences detected in this study suggest that many of the mtCOI lineages within *P. abdominalis* may be reproductively-isolated species under natural conditions.

Significant morphological differentiation in prosome length and A1 spine characters also was observed among mtCOI clades, across a range of levels of genetic divergence. Clade 2d was nearly fixed for straight A1 spines, in contrast to the closely-related and sympatric clades 2a and 2c (curved spines), and K2P distances between these clades (5.7% between 2c and 2d, and 7% between 2a and 2d) were close to the minimum inter-clade distance observed in this study (4.3%) (Table 2). We also observed differentiation in body size among clades that was not easily interpreted as environmentally driven. Differences in body size are an easily recognized character, and different body sizes have been reported between sibling species of oceanic copepods (Chihara and Murano, 1997; Bradford-Grieve, 1999), as well as between undescribed genetic lineages within *Nannocalanus minor* (Bucklin et al. 1996), *Neocalanus flemingeri* (Machida and Tsuda, 2010), *P. xiphias* (Goetze, 2011) and *P. gracilis/piseki* (Halbert et al., 2013). In summary, morphological variation among clades supports our hypothesis that several of the mtCOI clades within both PLAB1 and PLAB2 likely correspond to distinct species. The location of the pigment knob on the lateral prosome of adult females showed no clear relationship with genetic variation, as was also observed in *P. xiphias* (Goetze, 2011) and *P. gracilis/piseki* (Halbert et al., 2013).

4.2. Mechanisms determining the biogeography of mtCOI clades

The cosmopolitan distribution of the nominal species *P. abdominalis* is shown here to result from a spatial composite of mtCOI clades with more restricted distributions. The nominal species *P.*

abdominalis is an ecosystem dominant across a wide range of oceanographic regions (Steinberg et al., 2000; Al-Mutairi and Laundry, 2001; Andersen et al., 2004), and we infer that this likely occurs as a result of the diversity of genetic clades being adapted to particular oceanographic environments. Some mtCOI lineages remain fairly widespread in subtropical waters, and occupy a large proportion of the original described range for *P. abdominalis* (e.g., clades 1b-1 and 2a) (Fig. 5). However, some lineages show an unexpected level of endemism, for example, clades 1b-2 and 2b were restricted to equatorial waters of the Atlantic Ocean. While the Indo-Pacific equatorial distribution shown by clades 2i, 2e, 2m and 1a-1 is well known and occurs in many other pelagic species (Brinton, 1962; Lang, 1965; Nishida, 1985), the occurrence of Atlantic equatorial endemics has been rarely reported in prior work (but see *C. cancapae*, Burrridge et al., 2015). Equatorial mtCOI clades do not extend their distributions into the oligotrophic subtropical gyres, a biogeographic pattern that is typical for described equatorial plankton species that appear to be adapted to more eutrophic conditions (e.g., McGowan, 1971). In some cases, the distributions of equatorial clades extended into the Kuroshio region in the Pacific, possibly due to transport in the Kuroshio Current and favorable oceanographic environments, which are not as oligotrophic as at the center of the subtropical gyre (Longhurst, 2007). The absence of PLAB2 clades and the presence of clade 1a-1 in the eastern tropical Pacific (ETP) indicates that clade 1a-1 may be more adapted to relatively cool water from the Humboldt Current, equatorial upwelling, and the well-pronounced oxygen minimum zone in this region (Fiedler and Talley, 2006). Finally, equatorial regions also may act as dispersal barriers for subtropical mtCOI clades whose primary distributions are within oligotrophic gyres (as in Norton and Goetze, 2013, Andrews et. al. 2014), as a few of these clades show a similar pattern of being widespread in subtropical waters and present but in low abundance within equatorial regions (e.g., clades 2a, 1b-1).

Several subtropical mtCOI clades also were restricted in distribution to oligotrophic gyres of the southern hemisphere (e.g., clades 2d, 2j; Figs. 4 and 5), and show little overlap with the northern

hemisphere populations. For example, in the South Pacific subtropical gyre, clade 2d appeared to be adapted to extremely oligotrophic environments (region no. 12 in Fig. S2), in comparison to the closely-related subtropical clades 2a and 2c that were more widespread in both the North and South Pacific gyres. This pattern has been previously reported in very few cases; *P. gracilis*/*P. piseki* also appears to have one clade that is largely restricted to the oligotrophic South Pacific (Halbert et al. 2013). As shown in the Atlantic Ocean transect (Fig. 6), the boundaries of biogeographic distributions for mtCOI clades often corresponded to regions of oceanographic transition, for example at the edges of subtropical gyre systems where seawater temperature and chlorophyll concentrations changed significantly. In summary, given the distributions of mtCOI clades, we hypothesize that they are adapted to distinct oceanographic conditions, with the extensive genetic diversity leading to a biogeographic mosaic with more geographically-restricted lineages making up the cosmopolitan distribution of the nominal species *P. abdominalis*.

Considering the high genetic divergence and complex biogeographic patterns of lineages within the nominal species *P. abdominalis*, the current genetic structure likely formed over a long evolutionary time period. In the pelagic realm, both physical barriers, such as land masses or ocean currents, and environmental barriers, including oceanographic gradients in the horizontal or vertical dimensions, have been suggested to be primary factors driving diversification of planktonic species (Peijnenburg et al., 2006; Miyamoto et al., 2012; Weiner et al., 2012; Norton and Goetze, 2013; Andrews et al., 2014). The present-day sympatric distributions of several genetic lineages may result from range expansion and secondary contact, if allopatric speciation occurred in the past. Another hypothesis is that diversification occurred through adaptive divergence even in the presence of gene flow, as zooplankton may have high capacity to respond to environmental selective pressures (Peijnenburg and Goetze, 2013). Applying a common crustacean mtCOI mutation rate of 1.4% per million years (Knowlton and Weigt, 1998), PLAB1 and PLAB2 share a common ancestor dating back to the Miocene (11.6–16.0 million years ago) or before. A global-scale cooling event began in

the middle Miocene (Zachos et al., 2001), and both the ocean environment and geography have changed towards the present, including the formation of the Isthmus of Panama (Schmittner et al., 2004) and increased primary productivity in equatorial regions (Barron, 1985; Rea et al., 1991). Although the specific mechanisms driving diversification and speciation in the group are unknown, these oceanographic changes likely drove the evolution of high genetic diversity and complex biogeography observed within the extant, nominal species *P. abdominalis*.

5. Conclusions

Global-scale molecular and morphological analyses revealed eighteen divergent genetic lineages within *P. abdominalis* that we hypothesize are valid species. Concordant molecular results from mitochondrial and nuclear loci support the inference of reproductive isolation between the globally-distributed clades PLAB1 and PLAB2, with high genetic divergence also observed among mtCOI clades within both PLAB1 and PLAB2. Morphological variation in antennule spine characters and body size support inference of species level rank for several clades within PLAB2 (at least 5.7% K2P distance). The biogeographic distributions of mtCOI clades indicate far greater ecological specialization to particular oceanographic conditions than the cosmopolitan nominal species *P. abdominalis*, with several mtCOI clades endemic to equatorial waters (Atlantic, Indo-Pacific) or the highly oligotrophic gyres of the southern hemisphere. These mtCOI genetic lineages may have evolved through adaption to local oceanographic environments within each ocean basin. We collected a comprehensive set of material from across all three major ocean basins; however, further sampling may reveal additional evolutionary lineages and better characterization of the biogeography of minor genetic clades and singletons. Accurate delimitation of species is required for robust ecological studies of marine zooplankton, and further integrative taxonomic work on cosmopolitan species is needed to assess the true diversity and distribution of these groups.

Acknowledgements

This work was supported by JSPS KAKENHI Grant Number 261192 to JH, JSPS KAKENHI Grant Number 24121004 to AT, and National Science Foundation grants OCE-1029478 and OCE-1338959 to EG. We thank M. Moeller for assistance in the lab, and S. Nishida, K. Takahashi, K. T. C. A. Peijnenburg and two anonymous reviewers for suggestions on the manuscript. We also thank J. Aoyama for collecting samples during the KH-13-01 and KH-13-02 cruises. This is contribution no. 270 of the Atlantic Meridional Transect (AMT) Programme.

References

- Al-Mutairi, H., Landry, M.R., 2001. Active export of carbon and nitrogen at Station ALOHA by diel migrant zooplankton. *Deep-Sea Research Part II: Topical Studies in Oceanography* 48, 2083–2103.
- Andersen, V., Devey, C., Gubanova, A., Picheral, M., Melnikov, V., Tsarin, S., Prieur, L., 2004. Vertical distributions of zooplankton across the Almeria–Oran frontal zone (Mediterranean Sea), *Journal of Plankton Research* 26, 275–293.
- Andrews, K.R., Norton, E., Fernandez-Silva, I., Portner, E., Goetze, E., 2014. Multilocus evidence for globally-distributed cryptic species and distinct populations across ocean gyres in a mesopelagic copepod. *Molecular Ecology* 23, 5462–5479.
- Angel, M.V., 1993. Biodiversity of the Pelagic Ocean. *Conservation Biology* 7, 760–772.
- Appeltans, W., Ah Yong, S.T., Anderson, G., Angel, M.V., Artois, T., Bailly, N., Bamber, R., Barber, A., Bartsch, I., Berta, A., Błażewicz-Paszkowycz, M., Bock, P., Boxshall, G., Boyko, C.B., Brandão, S.N., Bray, R.A., Bruce, N.L., Cairns, S.D., Chan, T.Y., Chan, L., Collins, A.G., Cribb, T., Curini-Galletti, M., Dahdouh-Guebas, F., Davie, P.J.F., Dawson, M.N., De Clerck, O., De Cock, W., De Grave, S., de Voogd, N.J., Domning, D.P., Emig, C.C., Erséus, C., Eschmeyer, W., Fauchald, K., Fautin, D.G., Feist, S.W., Fransen, C.H.J.M., Furuya, H.,

Garcia-Alvarez, O., Gerken, S., Gibson, D., Gittenberger, A., Gofas, S., Gómez-Daglio, L., Gordon, D.P., Guiry, M.D., Hoeksema, B.W., Hopcroft, R., Jaume, D., Kirk, P., Koedam, N., Koenemann, S., Kolb, J.B., Kristensen, R.M., Kroh, A., Lambert, G., Lazarus, D.B., Lemaitre, R., Longshaw, M., Lowry, J., Macpherson, E., Madin, L.P., Mah, C., Mapstone, G., McLaughlin, P., Meland, K.L., Messing, C.G., Mills, C.E., Molodtsova, T.N., Mooi, R., Neuhaus, B., Ng, P.K.L., Nielsen, C., Norenburg, J., Opresko, D.M., Osawa, M., Paulay, G., Perrin, W., Pilger, J.F., Poore, G.C.B., Pugh, P., Read, G.B., Reimer, J.D., Rius, M., Rocha, R.M., Rosenberg, G., Saiz-Salinas, J.I., Scarabino, V., Schierwater, B., Schmidt-Rhaesa, A., Schnabel, K.E., Schotte, M., Schuchert, P., Schwabe, E., Segers, H., Self-Sullivan, C., Shenkar, N., Siegel, V., Sterrer, W., Stöhr, S., Swalla, B., Tasker, M.L., Thuesen, E.V., Timm, T., Todaro, A., Turon, X., Tyler, S., Uetz, P., van der Land, J., van Ofwegen, L.P., van Soest, R.W.M., Vanaverbeke, J., Vanhoorne, B., Walker-Smith, G., Walter, T.C., Warren, A., Williams, G., Wilson, S.P., Hernandez, F., Mees, J., Costello, M.J., 2012. The magnitude of global marine species diversity. *Current Biology* 22, 1–14.

Bacha, M., Amara, R., 2009. Spatial, temporal and ontogenetic variation in diet of anchovy (*Engraulis encrasicolus*) on the Algerian coast (SW Mediterranean). *Estuarine. Coastal and Shelf Science* 85, 257–264.

Barron, J.A., 1985. Diatom paleoceanography and paleoclimatology of the central and eastern equatorial Pacific between 18 and 6.2 Ma. *Initial Reports, Deep Sea Drilling Project* 85, 935–945.

Blades-Eckelbarger, P.I., Youngbluth, M.J., 1988. Ultrastructure of the “pigment knob” of *Pleuromamma* spp. (Copepoda: Calanoida). *Journal of Morphology* 197, 315–326.

Blanco-Bercial, L., Álvarez-Marqués, F., Bucklin, A., 2011. Comparative phylogeography and connectivity of sibling species of the marine copepod *Clausocalanus* (Calanoida). *Journal of Experimental Marine Biology and Ecology* 404, 108–115.

- Blanco-Bercial, L., Cornils, A., Copley, N., Bucklin, A., 2014. DNA barcoding of marine copepods: assessment of analytical approaches to species identification. PLOS Currents Tree of Life. 2014 June 23. Edition 1. <http://dx.doi.org/10.1371/currents.tol.cdf8b74881f87e3b01d56b43791626d2>.
- Boissin, E., Hoareau, T.B., Féral, J.P., Chenuil, A., 2008. Extreme selfing rates in the cosmopolitan brittle star species complex *Amphipholis squamata*: data from progeny-array and heterozygote deficiency. Marine Ecology Progress Series 361, 151–159.
- Boxshall, G.A., Halsey, S.H., 2004. An Introduction to Copepod Diversity. The Ray Society, London.
- Bradford-Grieve, J., 1999. The Marine Fauna of New Zealand: Pelagic Calanoid Copepoda: Bathypontiidae, Arietellidae, Augaptilidae, Heterorhabdidae, Lucicutiidae, Metridinidae, Phyllopodidae, Centropagidae, Pseudodiaptomidae, Temoridae, Candaciidae, Pontellidae, Sulcanidae, Acartiidae, Tortanidae. NIWA Biodiversity Memoir 111, 1–268.
- Brinton, E., 1962. The distribution of Pacific euphausiids. Bulletin of the Scripps Institute of Oceanography 8, 51–270.
- Brown, S.D.J., Collins, R.A., Boyer, S., Lefort, M-C., Malumbres-Olarte, J., Vink, C.J., Cruickshank, R.H., 2012. Spider: An R package for the analysis of species identity and evolution, with particular reference to DNA barcoding. Molecular Ecology Resources 12, 562–565.
- Bucklin, A., LaJeunesse, T.C., Curry, E., Wallinga, J., Garrison, K., 1996. Molecular diversity of the copepod, *Nannocalanus minor*: Genetic evidence of species and population structure in the North Atlantic Ocean. Journal of Marine Research 54, 285–310.
- Bucklin, A., Frost, B.W., Bradford-Grieve, J., Allen, L.D., Copley, N.J., 2003. Molecular systematics and phylogenetic assessment of 34 calanoid copepod species of the Calanidae and Clausocalanidae. Marine Biology 142, 333–343.
- Bucklin, A., Wiebe, P.H., Smolenack, S.B., Copley, N.J., Beaudet, J.G., Bonner, K.G., Färber-Lorda,

- J., Pierson, J.J., 2007. DNA barcodes for species identification of euphausiids (Euphausiacea, Crustacea). *Journal of Plankton Research* 29, 483–493.
- Bucklin, A., Ortman, B.D., Jennings, R.M., Nigro, L.M., Sweetman, C.J., Copley, N.J., Sutton, T., Wiebe, P.H., 2010. A “Rosetta Stone” for metazoan zooplankton: DNA barcode analysis of species diversity of the Sargasso Sea (Northwest Atlantic Ocean). *Deep Sea Research Part II: Topical Studies in Oceanography* 57, 2234–2247.
- Burridge, A.K., Goetze, E., Raes, N., Huisman, J., Peijnenburg, K.T.C.A., 2015. Global biogeography and evolution of *Cuvierina* pteropods. *BMC Evolutionary Biology* 15, 39.
- Carstens, B.C., Pelletier, T.A., Reid, N.M., Satler, J.D., 2013. How to fail at species delimitation. *Molecular Ecology* 22, 4369–4383.
- Casteleyn, G., Leliaert, F., Backeljau, T., Debeer, A.-E., Kotaki, Y., Rhodes, L., Lundholm, N., Sabbe, K., Vyverman, W., 2010. Limits to gene flow in a cosmopolitan marine planktonic diatom. *Proceedings of the National Academy of Sciences of the United States of America* 107, 12952–12957.
- Chen, G., Hare, M.P., 2011. Cryptic diversity and comparative phylogeography of the estuarine copepod *Acartia tonsa* on the US Atlantic coast. *Molecular ecology* 20, 2425–2441.
- Chihara, M., Murano, M., 1997. An illustrated guide to marine plankton in Japan. Tokai University Press, Tokyo.
- Claus, C., 1863. Die frei lebenden Copepoden mit besonderer Berücksichtigung der Fauna Deutschlands, der Nordsee und des Mittelmeeres. Verlag von Wilhelm Englemann, Leipzig.
- Cornils, A., Held, C., 2014. Evidence of cryptic and pseudocryptic speciation in the *Paracalanus parvus* species complex (Crustacea, Copepoda, Calanoida). *Frontiers in Zoology* 11, 19.
- Costa, F.O., deWaard, J.R., Boutillier, J., Ratnasingham, S., Dooh, R.T., Hajibabaei, M., Hebert, P.D.N., 2007. Biological identifications through DNA barcodes: the case of the Crustacea. *Canadian Journal of Fisheries and Aquatic Sciences* 64, 272–295.

- de Queiroz, K., 1998. The general lineage concept of species, species criteria, and the process of speciation: A conceptual unification and terminological recommendations. In: Howard, D.J., Berlocher, S.H. (Eds.), *Endless forms: Species and speciation*. Oxford University Press, New York, pp. 57–75.
- de Queiroz, K., 2007. Species concepts and species delimitation. *Systematic Biology* 56, 879–886.
- de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., Lara, E., Berney, C., Le Bescot, N., Probert, I., Carmichael, M., Poulain, J., Romac, S., Colin, S., Aury, J., Bittner, L., Chaffron, S., Dunthorn, M., Engelen, S., Flegontova, O., Guidi, L., Horák, A., Jaillon, O., Lima-Mendez, G., Lukeš, J., Malviya, S., Morard, R., Mulot, M., Scalco, E., Siano, R., Vincent, F., Zingone, A., Dimier, C., Picheral, M., Searson, S., Kandels-Lewis, S., Tara Oceans Coordinators, Acinas, S.G., Bork, P., Bowler, C., Gorsky, G., Grimsley, N., Hingamp, P., Iudicone, D., Not, F., Ogata, H., Pesant, S., Raes, J., Sieracki, M.E., Speich, S., Stemmann, L., Sunagawa, S., Weissenbach, J., Wincker, P., Karsenti, E., 2015. Eukaryotic plankton diversity in the sunlit ocean. *Science* 348, 1261605.
- Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32, 1792–1797.
- Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10, 564–567.
- Fiedler, P.C., Talley, L.D., 2006. Hydrography of the eastern tropical Pacific: a review. *Progress in Oceanography* 69, 143–180.
- Fornshell, J., Ferrari, F., 2010. Morphological variability of *Pleuromamma abdominalis* (Copepoda, Calanoida, Metridinidae) along two latitudinal transects in the eastern North Pacific Ocean. *Crustaceana* 83, 753–765.
- Foxton, P., Roe, H.S.J., 1974. Observations on the nocturnal feeding of some mesopelagic decapod

crustacea. Marine Biology 28, 37–49.

- Gaither, M.R., Bowen, B.W., Bordenave, T.-R., Rocha, L.A., Newman, S.J., Gomez, J.A., van Herwerden, L., Craig, M.T., 2011. Phylogeography of the reef fish *Cephalopholis argus* (Epinephelidae) indicates Pleistocene isolation across the Indo-Pacific barrier with contemporary overlap in the coral triangle. BMC Evolutionary Biology 11, 189.
- Giesbrecht, W., Schmeil, O., 1898. Copepoda. I. Gymnoplea. Das Tierreich 6, 1–169.
- Goetze, E., 2003. Cryptic speciation on the high seas; global phylogenetics of the copepod family Eucalanidae. Proceedings of the Royal Society B 270, 2321–2331.
- Goetze, E., 2005. Global population genetic structure and biogeography of the oceanic copepods *Eucalanus hyalinus* and *E. spinifer*. Evolution 59, 2378–2398.
- Goetze, E., 2010. Species discovery in marine planktonic invertebrates through global molecular screening. Molecular Ecology 19, 952–967.
- Goetze, E., 2011. Population differentiation in the open sea: Insights from the Pelagic Copepod *Pleuromamma xiphius*. Integrative and Comparative Biology 51, 580–597.
- Halbert, K.M.K., Goetze, E., 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.
- Haury, L., 1988. Vertical distribution of *Pleuromamma* (Copepoda: Metridinidae) across the eastern North Pacific Ocean. In: Boxshall, G., Schminke, H.K. (Eds.), Biology of Copepods. Springer, Netherlands, pp. 335–342.
- Hays, G.C., Harris, R.P., Head, R.N., 1997. The vertical nitrogen flux caused by zooplankton diel vertical migration. Marine Ecology Progress Series 160, 57–62.
- Hirai, J., Tsuda, A., in press. Metagenetic community analysis of epipelagic planktonic copepods in the tropical and subtropical Pacific. Marine Ecology Progress Series.
- Hirai, J., Shimode, S., Tsuda, A., 2013. Evaluation of ITS2-28S as a molecular marker for identification of calanoid copepods in the subtropical western North Pacific. Journal of

Plankton Research 35, 644–656.

Hirai, J., Kuriyama, M., Ichikawa, T., Hidaka, K., Tsuda, A., 2015. A metagenetic approach for revealing community structure of marine planktonic copepods. *Molecular Ecology Resources* 15, 68–80.

Jennings, R.M., Bucklin, A., Ossenbrügger, H., 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.

Knowlton, N., 2000. Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia* 420, 73–90.

Knowlton, N., Weigt, L.A., 1998. New dates and new rates for divergence across the Isthmus of Panama. *Proceedings of the Royal Society B* 265, 2257–2263.

Lancraft, T.M., Hopkins, T.L., Torres, J.J., 1988. Aspects of the ecology of the mesopelagic fish *Gonostoma elongatum* (Gonostomatidae, Stomiiformes) in the eastern Gulf of Mexico, *Marine Ecology Progress Series* 49, 27–40.

Lang, B.T., 1965. Taxonomic review and geographical survey of the copepod genera *Eucalanus* and *Rhincalanus* in the Pacific Ocean. Ph.D. Thesis, University of California, San Diego.

Lee, C.E., 2000. Global phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate "populations". *Evolution* 54, 2014–2027.

Lindeque, P.K., Parry, H.E., Harmer, R.A., Somerfield, P.J., Atkinson, A., 2013. Next generation sequencing reveals the hidden diversity of zooplankton assemblages. *Plos One* 8, e81327.

Longhurst, A.R., 1985. Relationship between diversity and the vertical structure of the upper ocean. *Deep Sea Research Part A: Oceanographic Research Papers* 32, 1535–1570.

Longhurst, A.R., 2007. *Ecological Geography of the Sea*. Academic Press, London.

- Lubbock, J., 1856. On some Entomostraca collected by Dr. Sutherland in the Atlantic Oceans. Transactions of the Entomological Society of London, New Series 4, 8–37.
- Machida, R.J., Tsuda, A., 2010. Dissimilarity of species and forms of planktonic *Neocalanus* copepods using mitochondrial *COI*, *12S*, nuclear *ITS*, and *28S* gene sequences. Plos One 5, e10278.
- Machida, R.J., Miya, M.U., Nishida, M., Nishida, S., 2004. Large-scale gene rearrangements in the mitochondrial genomes of two calanoid copepods *Eucalanus bungii* and *Neocalanus cristatus* (Crustacea), with notes on new versatile primers for the srRNA and COI genes. Gene 332, 71–78.
- McGowan, J.A., 1971. Oceanic biogeography of the Pacific. In: Funnell, B.M., Riedel, W.R. (Eds.), The micropaleontology of the oceans. Cambridge University Press, Cambridge, pp. 3–74.
- McManus, G.B., Katz, L.A., 2009. Molecular and morphological methods for identifying plankton: what makes a successful marriage? Journal of Plankton Research 31, 1119–1129.
- Miya, M., Nishida, M., 1997. Speciation in the open ocean. Nature 389, 803–804.
- Miyamoto, H., Machida, R.J., Nishida, S., 2010. Genetic diversity and cryptic speciation of the deep sea chaetognath *Caecosagitta macrocephala* (Fowler, 1904). Deep Sea Research Part II 57, 2211–2219.
- Miyamoto, H., Machida, R.J., Nishida, S., 2012. Global phylogeography of the deep-sea pelagic chaetognath *Eukrohnia hamata*. Progress in Oceanography 104, 99–109.
- Nishida, S., 1985. Taxonomy and distribution of the family Oithonidae (Copepoda, Cyclopoida) in the Pacific and Indian oceans. Bulletin of the Ocean Research Institute, University of Tokyo. Tokyo 20, 1–167.
- Norris, R.D., 2000. Pelagic species diversity, biogeography, and evolution. Paleobiology 26, 236–258.
- Norton, E.L., Goetze, E., 2013. Equatorial dispersal barriers and limited population connectivity

- among oceans in a planktonic copepod. *Limnology and Oceanography* 58, 1581–1596.
- Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P., Nieves-Aldrey, J., 2004. Bayesian Phylogenetic Analysis of Combined Data. *Systematic Biology* 53, 47–67.
- Pérez-Portela, R., Arranz, V., Rius, M., Turon, X., 2013. Cryptic speciation or global spread? The case of a cosmopolitan marine invertebrate with limited dispersal capabilities. *Scientific Reports* 3, 3197.
- Peijnenburg, K.T.C.A., Goetze, E., 2013. High evolutionary potential of marine zooplankton. *Ecology and Evolution* 3, 2765–2781.
- Peijnenburg, K.T.C.A., Breeuwer, J.A.J., Pierrot-Bults, A.C., Menken, S.B.J., 2004. Phylogeography of the planktonic chaetognath *Sagitta setosa* reveals isolation in European seas. *Evolution* 58, 1472–1487.
- Peijnenburg, K.T.C.A., Fauvelot, C., Breeuwer, J.A.J., Menken, S.B.J., 2006. Spatial and temporal genetic structure of the planktonic *Sagitta setosa* (Chaetognatha) in European seas as revealed by mitochondrial and nuclear DNA markers. *Molecular Ecology* 15, 3319–3338.
- Razouls, C., de Bovée, F., Kouwenberg, J., et Desreumaux, N., 2005–2015. Diversity and Geographic Distribution of Marine Planktonic Copepods. Available at <http://copepodes.obs-banyuls.fr/en> [Accessed March 16, 2015].
- Rea, D.K., Pisias, N.G., Newberry, T., 1991. Late Pleistocene paleoclimatology of the central equatorial Pacific: flux patterns of biogenic sediments. *Paleoceanography* 6, 227–244.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.
- Schmittner, A., Sarnthein, M., Kinkel, H., Bartoli, G., Bickert, T., Crucifix, M., Crudeli, M., Groeneveld, J., Kösters, F., Mikolajewicz, U., Millo, C., Reijmer, C., Schäfer, P., Schmidt, D., Schneider, B., Schulz, M., Steph, S., Tiedemann, R., Weinelt, M., Zuvela, M., 2004. Global impact of the Panamanian seaway closure. *EOS* 85, 526.

- Schroth, W., Jarms, G., Streit, B., Schierwater, B., 2002. Speciation and phylogeography in the cosmopolitan marine moon jelly, *Aurelia* sp. BMC Evolutionary Biology 2, 1.
- Sewell, R.B.S., 1947. Free swimming planktonic Copepoda. Scientific Reports of John Murray Expedition 8, 1–303.
- Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.
- Steinberg, D.K., Carlson, C.A., Bates, N.R., Goldthwait, S.A., Madin, L.P., Michaels, A.F., 2000. Zooplankton vertical migration and the active transport of dissolved organic and inorganic carbon in the Sargasso Sea. Deep Sea Research Part 47, 137–158.
- Steuer, A., 1932. Copepoda 6: *Pleuromamma* Giesbr. 1898 der Deutschen Tiefsee-Expedition. Wissenschaftliche Ergebnisse der Deutschen Tifsee-Expedition 1898–1899 24, 1–128.
- Toews, D.P.L., Brelsford, A., 2012. The biogeography of mitochondrial and nuclear discordance in animals. Molecular Ecology 21, 3907–3930.
- Unal, E., Bucklin, A., 2010. Basin-scale population genetic structure of the planktonic copepod *Calanus finmarchicus* in the North Atlantic Ocean. Progress in Oceanography 87, 175–185.
- van der Spoel, S., Heyman, R.P., 1983. A comparative atlas of zooplankton. Springer-Verlag, Berlin.
- Weiner, A., Aurahs, R., Kurasawa, A., Kitazato, H., Kucera, M., 2012. Vertical niche partitioning between cryptic sibling species of a cosmopolitan marine planktonic Protist. Molecular Ecology 21, 4063–4073.
- Wiens, J.J., Servedio, M.R., 2000. Species delimitation in systematics: inferring diagnostic differences between species. Proceedings of the Royal Society B 267, 631–636.
- Wilson, C.B., 1932. The copepods of the Woods Hole region, Massachusetts. Bulletin of the United States National Museum 158, 1–635.
- Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, Rhythms, and Aberrations in Global Climate 65 Ma to Present. Science 292, 686–693.

Figure legends

Fig. 1. Sampling locations of this study.

Samples were collected at 46 sites in the Atlantic (1–15), Indian (16–21), and Pacific Oceans (22–46).

Detailed collection information is reported in Table 1.

Fig. 2. Morphological characters surveyed in adult female *Pleuromamma abdominalis* specimens.

(A) An adult female *Pleuromamma abdominalis*. Pigment knob is indicated by the arrow, approximate location of the antennule spines (A1 spines), as shown. A1 spines were classified as either: (B) curved, (C) straight, or (D) reduced in size.

Fig. 3. Phylogenetic relationships among 944 mtCOI *P. abdominalis* sequences inferred using Bayesian methods.

Numbers at the nodes report Bayesian posterior probability values (upper) and bootstrap values from maximum likelihood analysis (lower). Support values are reported for >0.70 posterior probability, and >70% for bootstrap values. The scale bar indicates expected substitutions per site. Two primary mtCOI clades, PLAB1 and PLAB2, are concordant with sequence variation at nLSU. In PLAB2, sixteen well-supported mtCOI clades are labeled by letter (2a–2p) and color. In PLAB1, two mtCOI groups are labeled 1a-1 and 1b-1, with two sub-clades marked 1a-2 and 1b-2, respectively. The tree includes six *P. abdominalis* mtCOI sequences from GenBank (accession numbers shown), as well as sequences from congeners *P. robusta* and *P. indica* (from this study). *Pleuromamma robusta* was used to root the tree in this analysis.

Fig. 4. Morphological variation within and among mtCOI clades.

(A) Prosome length. Average prosome lengths are plotted for each mtCOI clade (mm), with standard deviations shown for mtCOI clades with > 3 individuals. * indicates a significant difference by

Kruskal-Wallis and Dunn's tests. Sub-clade 1a-2 was significantly larger than other clades within PLAB1, with the exception of 1b-1. In PLAB2, clade 2i was significantly larger than other clades with sufficient sample sizes (clades 2a, 2b, 2c, 2d, 2e, 2i, 2j, and 2m). (B) Shape of the antennule spines (A1 spine). A1 spines were categorized into curved, straight, or reduced (see Fig 2). * indicates clades that are fixed for one A1 spine character state ($\geq 95\%$ occurrence of the trait, for clades with $N > 10$; Wiens and Servedio, 2000). (C) The lateral position of the pigment knob (right or left). Note that only adult females were examined for morphological characters and the numbers in parentheses indicate the number of individuals used for morphological analyses within each mtCOI clade.

Fig. 5. Global distribution of the mtCOI clades within (A) PLAB1 and (B) PLAB2.

The proportion of mtCOI clades is plotted at each site with three or more individuals. In (A), * indicates a site with less than three individuals of PLAB1, as the majority of animals at that site were of PLAB2. In (B), mtCOI clades with less than four individuals, as well as singletons, are included in 'Others' (grey).

Fig. 6. Distribution of mtCOI clades across ocean regions in the Atlantic Ocean.

Proportions of eight mtCOI clades and sub-clades at 14 sites plotted for PLAB1 in (A) and PLAB2 in (B), with color for each clade as indicated in the legend (at right). Ocean section plots of chlorophyll *a* (C) and seawater temperature (D) are used to illustrate ocean regions and mtCOI clade distributions across the Atlantic Ocean (data from Atlantic Meridional Transect cruise 22). Regions are labeled as North Atlantic subtropical gyre (NA gyre), Equatorial Atlantic (Equatorial), and South Atlantic subtropical gyre (SA gyre).

Tables

Table 1. Metadata for plankton samples, including the number of individuals (N), collection latitude, longitude, date, cruise, original station number, sampling gear, as well as the primary data for the distribution of mtCOI clades. Sampling gear is as follows: bongo (oblique bongo tow), RMT1 (rectangular midwater trawl 1m), VMPS (Vertical Multiple Plankton Sampler), NORPAC (North Pacific Standard Plankton net), IKMT (Isaacs-Kidd midwater trawl), ORI net. PLAB indicates *P. abdominalis*, PLRO *P. robusta*, and PLIN *P. indica*.

(see separate file)

Table 1. Metadata for plankton samples, including the number of individuals (N), collection latitude, longitude, date, cruise, original station number, sampling gear, as well as the primary data for the distribution of mtCOI clades. Sampling gear is as follows: bongo (oblique bongo tow), RMT1 (rectangular midwater trawl 1m), VMPS (Vertical Multiple Plankton Sampler), NORPAC (North Pacific Standard Plankton net), IKMT (Isaacs-Kidd midwater trawl), ORI net. PLAB indicates *P. abdominalis*, PLRO *P. robusta*, and PLIN *P. indica*.

Table 2. Average Kimura 2-parameter (K2P) genetic distance (%) between (non-bold) and within (bold) mtCOI clades.

K2P distances within clade PLAB1 are shaded gray, and K2P distances between clades PLAB1 and PLAB2 are enclosed by black lines. K2P distances were also calculated between *P. abdominalis* and described congeners *P. robusta* (PLRO) and *P. indica* (PLIN); these comparisons are marked by underlined values. Note that sub-clades 1a-2 and 1b-2 were analyzed independently from clades 1a-1 and 1b-1.

	PLRO	PLIN	1a-1	1a-2	1b-1	1b-2	2a	2b	2c	2d	2e	2f	2g	2h	2i	2j	2k	2l	2m	2n	2o	2p
1a-1	<u>22.1</u>	<u>22.6</u>	1.0																			
1a-2	<u>22.9</u>	<u>22.1</u>	5.0	0.5																		
1b-1	<u>24.8</u>	<u>23.5</u>	9.2	11.1	1.1																	
1b-2	<u>24.7</u>	<u>24.4</u>	9.6	11.2	2.7	0.6																
2a	<u>26.5</u>	<u>22.0</u>	19.6	19.3	21.1	20.7	1.2															
2b	<u>25.9</u>	<u>22.5</u>	20.2	21.1	22.7	22.1	4.4	0.9														
2c	<u>28.6</u>	<u>24.8</u>	20.7	21.3	21.3	20.9	6.1	4.3	0.7													
2d	<u>26.5</u>	<u>23.2</u>	20.4	20.9	21.3	20.7	7.0	6.2	5.7	1.7												
2e	<u>26.3</u>	<u>18.9</u>	21.9	22.1	22.2	22.2	13.1	12.7	14.1	13.5	1.9											
2f	<u>24.8</u>	<u>22.4</u>	19.8	21.2	18.3	18.3	14.3	13.7	14.3	15.4	11.5	0.5										
2g	<u>24.5</u>	<u>20.1</u>	18.7	20.0	18.3	18.6	15.3	14.4	13.6	15.6	12.0	7.3	0.9									
2h	<u>28.5</u>	<u>22.5</u>	19.7	20.9	19.6	20.2	12.4	12.5	11.9	12.7	8.9	7.5	8.9	0.0								
2i	<u>26.6</u>	<u>18.9</u>	21.0	21.7	20.6	20.6	12.6	13.7	14.1	14.1	8.9	9.9	11.1	8.5	0.6							
2j	<u>26.1</u>	<u>23.2</u>	18.2	20.4	17.4	18.0	17.0	17.2	17.3	18.1	15.8	16.8	17.4	15.8	14.2	0.4						
2k	<u>24.3</u>	<u>19.4</u>	18.3	18.0	19.2	18.7	14.7	14.6	15.7	16.6	12.5	14.7	13.7	14.5	11.1	15.3	0.6					
2l	<u>26.3</u>	<u>21.5</u>	19.7	19.0	19.7	19.1	17.4	16.5	18.1	18.4	16.2	17.1	14.9	16.5	14.2	16.7	5.2	0.0				
2m	<u>22.2</u>	<u>19.0</u>	16.6	17.5	18.5	18.0	17.5	18.3	17.4	16.2	15.6	16.1	16.4	16.2	14.3	16.9	13.1	16.1	0.5			
2n	<u>23.0</u>	<u>17.4</u>	18.0	19.5	20.6	19.7	17.2	17.2	17.1	16.5	15.2	15.8	16.7	16.7	14.6	18.4	13.2	16.1	4.3	0.9		
2o	<u>22.0</u>	<u>21.3</u>	18.7	19.4	20.1	19.2	18.9	18.3	17.2	17.0	18.5	18.5	16.2	18.3	17.5	17.1	14.8	17.4	10.8	11.2	0.2	
2p	<u>22.4</u>	<u>18.8</u>	17.4	16.8	19.2	18.9	16.2	15.3	16.0	17.8	15.9	14.7	13.8	14.0	15.1	17.4	12.9	16.3	13.1	15.9	13.6	0.2

Table 3. Summary of genetic diversity and approximate biogeographic distribution of each mtCOI clade.

The number of haplotypes (H) is listed for each mtCOI clade. nLSU sequence types are listed within each mtCOI clade, and examples of each nLSU type are provided in Fig. S1. nLSU sequences were obtained from nearly all individuals in the Pacific Ocean, and from selected individuals in the Atlantic and Indian Oceans. See figure S2 for an illustration of biogeographic regions observed in this study.

Clade	mtCOI sequences		nLSU sequences				Biogeographic distribution
			Atlantic and Indian Ocean		Pacific Ocean		
	N	H	N	Type (N)	N	Type (N)	
1a-1	118	38	2	1+3 (2)	90	1 (89), 1+3 (1)	Tropical–subtropical, Indian and Pacific Oceans, especially in equatorial waters
1a-2	37	10	6	1+3 (2), 3 (4)	–	–	Temperate, Atlantic and Indian Oceans, transition zone
1b-1	62	36	6	1+3 (2), 3 (4)	48	1 (48)	Tropical–subtropical, circumglobal, especially at the margins of subtropical gyres
1b-2	48	22	6	1+3 (1), 3 (5)	–	–	Equatorial Atlantic
2a	258	119	12	2+3 (9), 3 (3)	96	2 (95), 2+3 (1)	Tropical–temperate, circumglobal, especially in subtropical gyres
2b	49	20	6	2+3 (2), 3 (4)	–	–	Equatorial Atlantic
2c	40	24	–	–	39	2 (39)	Pacific, at margins of the subtropical gyres
2d	79	50	–	–	77	2 (75), 2+3 (1), 3 (1)	South Pacific in oligotrophic central gyre water
2e	47	20	3	2+3 (1), 3 (2)	33	2 (33)	Tropical, Indo-Pacific, extending into Kuroshio
2f	18	11	–	–	18	2 (18)	Equatorial Pacific
2g	2	2	1	2+3 (1)	–	–	Poorly characterized; observed in the equatorial Atlantic and Indian temperate zones
2h	3	1	–	–	3	2 (3)	Poorly characterized; observed in the western subtropical Pacific
2i	39	19	3	2+3 (1), 3 (2)	20	2 (20)	Tropical, Indo-Pacific Oceans, equatorial waters
2j	85	30	3	2+3 (1), 3 (2)	–	–	Subtropical–temperate, South Atlantic and Indian Oceans especially in subtropical gyres
2k	7	5	–	–	7	2 (7)	Equatorial Pacific
2l	4	1	2	3 (2)	–	–	Poorly characterized; observed in Atlantic and Indian subtropical waters
2m	27	9	–	–	3	2 (3)	Tropical, Indo-Pacific, extending into Kuroshio
2n	3	3	–	–	–	–	Poorly characterized; observed in the eastern Indian Ocean
2o	2	2	–	–	2	2 (2)	Poorly characterized; observed in the western equatorial Pacific
2p	12	4	2	2+3 (1), 3 (1)	9	2 (9)	Subtropical–temperate, Atlantic and Pacific Oceans, poorly characterized

Figure 1

Figure 2

Figure 3

Figure 4

(A) Prosome length

(B) A1 spine

(C) Pigment knob

Figure 6