

Global-scale biogeography of the pelagic polychaete family Tomopteridae (Annelida) revealed by DNA barcoding and genome-wide single nucleotide polymorphisms

Kanako Amei^{a,*}, Junya Hirai^a, Erica Goetze^b, Yuichiro Nishibe^a

^a Atmosphere and Ocean Research Institute, The University of Tokyo, Kashiwa-shi, Chiba 277-8564, Japan

^b Department of Oceanography, The University of Hawai'i at Mānoa, Honolulu, HI 96822, USA

ARTICLE INFO

Keywords:

Biogeography
Annelida
Tomopteridae
Genome-wide SNPs
Barcoding
Potential species diversity
Holopelagic

ABSTRACT

Zooplankton are essential members of marine ecosystems that respond sensitively to environmental changes. While the distribution patterns of zooplankton have been extensively investigated in the global ocean, pelagic polychaetes, particularly the Tomopteridae family, are underexplored in diversity and biogeography, due to their fragile bodies and subtle morphological traits. In the current study, mitochondrial cytochrome *c* oxidase subunit I (mtCOI), nuclear 28S rRNA, and genome-wide single-nucleotide polymorphisms (SNPs) were analyzed to characterize the diversity and distribution of tomopterid species globally. Phylogenetic analyses of mtCOI were conducted for 603 specimens collected from 54 stations across the global ocean. Concordance was detected between 14 mitochondrial lineages and their genome-wide SNPs, while species-level operational taxonomic units (OTUs) were defined based on mtCOI sequence similarity ($\geq 2.4\%$) supported by genome-wide SNPs. The comprising the global dataset contained 35 OTUs and 17 singletons, representing at least 52 tomopterid species. The number of species we assumed in this study exceeds previous morphological reports from certain ocean regions, including the eastern and western North Pacific Ocean (18 OTUs:11 species and 9 OTUs:7 species, respectively), suggesting the existence of previously unrecognized species. No specimens in this study were classified to species by morphotaxonomy. Tomopterids exhibited distribution patterns that corresponding to the general biogeography of other zooplankton, including subarctic/boreal, subarctic/bipolar, temperate-subtropical/circumglobal, temperate-subtropical/Indo-Pacific, equatorial, and upwelling region distributions. As this is the first species-level study of the global-scale biogeography of Tomopteridae, the findings provide fundamental information for future ecological and evolutionary studies on tomopterids.

1. Introduction

Marine zooplankton play a key role in pelagic food webs by linking lower and higher trophic levels, responding sensitively to changes in marine environments, and serving as bioindicators of marine ecosystems (Hays et al., 2005; Richardson, 2008). Due to their ecological importance, extensive research has been conducted on the spatial and temporal distributions of major zooplankton taxa in global oceans, relying primarily on morphological classifications by taxonomic experts. These investigations have mentioned the presence of numerous cosmopolitan species with high dispersal potential across ocean basins (Van der Spoel and Heyman, 1983; Norris, 2000; Knowlton, 2000). Given the

challenges in classifying sibling and cryptic zooplankton species through conventional microscopic observations, DNA barcoding techniques have been adopted. This approach utilizes sequence variances, primarily based on mitochondrial markers (e.g., mitochondrial cytochrome *c* oxidase subunit I: mtCOI) (Bucklin et al., 2011; Bucklin et al., 2021). DNA barcoding can reveal the hidden diversity within zooplankton and facilitates the large-scale biogeography of species-level genetic lineages, such for copepods (Goetze, 2003; Goetze and Ohman, 2010; Cornils and Held, 2014; Hirai et al., 2015), euphausiids (Bucklin et al., 2007), chaetognaths (Jennings et al., 2010; Miyamoto et al., 2012), gastropods (Burrige et al., 2015), and cnidarians (Schroth et al., 2002). However, molecular methods have not been applied to the full range of highly

Abbreviations: OTU, Operational taxonomic unit; mtCOI, Mitochondrial cytochrome oxidase I; ML, Maximum Likelihood; SNPs, single nucleotide polymorphisms; K2P, kimura 2-parameter; Hd, haplotype diversity; WoRMs, world register of marine species; HTS: high-throughput sequencing; ITS, internal transcribed spacer.

* Corresponding author at: current address: Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo, Japan.

E-mail address: amei@g.ecc.u-tokyo.ac.jp (K. Amei).

<https://doi.org/10.1016/j.pocean.2026.103689>

Received 22 October 2024; Received in revised form 7 November 2025; Accepted 30 January 2026

Available online 5 February 2026

0079-6611/© 2026 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

diverse zooplankton groups, making it challenging to determine the global evolution of their diversity and biogeography.

Among zooplanktonic taxa, pelagic polychaetes represent an underexplored group regarding their diversity and biogeography. The holoplanktonic polychaetes comprise nine families belonging to multiple genetic clades alongside benthic species within the orders Phyllodocida and Flabelligerida (Annelida) (Struck et al., 2007; Osborn and Rouse, 2008; Tilic et al., 2022). Previous studies have provided partial insights into the biogeography of pelagic polychaetes based on morphological identification in the Pacific (Tebble, 1962), the northern Atlantic (Støp-Bowitz, 1948), and the Pacific and Atlantic (Treadwell, 1943) Oceans. Additionally, the community structures of pelagic polychaetes have been examined in various regions, including the subarctic and subtropical western North Pacific (Amei et al., 2021; Amei et al., 2023), California Current (Fernández-Alamo et al., 2003), Southern Ocean (Siciński, 1998), South Adriatic Sea (Guglielmo et al., 2019), and Strait of Magellan in southern America (Guglielmo et al., 2014), through morphological identification to the lowest possible taxonomic level. Despite these efforts, investigations into the species diversity and biogeography of pelagic polychaetes remain limited, even for the predominant family, Tomopteridae (Order Phyllodocida), which is ubiquitously distributed from tropical to polar regions and from shallow to deep ocean layers. The transparent soft bodies with paddle-like parapodia of tomopterid polychaetes are considered adaptive to the pelagic water column as they facilitate metachronous swimming (Daniels et al., 2021) and support bioluminescent communication (Gouveaneux and Mallefet, 2013; Gouveaneux et al., 2017; Gouveaneux, 2016; Francis et al., 2016; Kin et al., 2022). Additionally, tomopterid species exhibit ultrastructural variations in the chemosensory nuchal organ, which may be linked to their pelagic lifestyle (Purschke and Helm, 2023). Although 52 nominal species have been accepted based on conventional morphological classifications, their validity is questionable due to vague descriptions based on poorly preserved specimens (Kolbasova et al., 2023). Same as other gelatinous zooplankton, fragile tomopterids are easily damaged during net sampling, fixation, and preservation, complicating morphological identification of specimens and linking species names to DNA barcodes. For instance, despite 270 mtCOI sequences in public databases such as GenBank (Benson et al., 2013) and Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert, 2007), only six species-level records exist, including *T. helgolandica*, *T. planktonis*, and *Enapteris euchaeta* (accessed May 25, 2024; Supplementary Table S1).

DNA barcoding, particularly based on mtCOI, is a powerful tool for studying the diversity of fragile animals, including Tomopteridae. However, species boundaries must be carefully evaluated using both nuclear and mitochondrial markers (Avise, 2000; Avise, 2004) due to potential incongruence, as observed in the carnivorous chaetognaths *Spadella cephaloptera* and *Parasagitta elegans* (Marlétaz et al., 2017). Common nuclear markers (e.g., internal transcribed spacer [ITS] and 18S/28S rRNA genes) mutate markedly more slowly than mtDNA markers (Machida and Tsuda, 2010), limiting their ability to support mtCOI lineages. Nevertheless, genome-wide approaches effectively identify species boundaries among closely related zooplankton species. Andrews et al. (Andrews et al., 2014) successfully demonstrated the presence of cryptic species in the mesopelagic copepod *Haloptilus longicornis* using mtCOI and microsatellite markers. Rapid developments in high-throughput sequencing (HTS) technology have facilitated the provision of genome single-nucleotide polymorphisms (SNPs) for various species, aiding species-delimitation in copepods (*Pleuromamma abdominalis* (Hirai, 2020) and four *Calanus* species (*C. helgolandicus*, *C. finmarchicus*, *C. glacialis*, and *C. hyperboreus* (Choquet et al., 2023)), as well as ctenophores (*Bolinopsis microptera* (Johnson et al., 2022)) and gastropods (*Limacina bulimoides* (Choo et al., 2023)). The hidden diversity of Tomopteridae has been revealed in specific ocean regions using mtCOI and 28S off the Kii Peninsula, Japan (Kin et al., 2022), and using 18S/28S in the Southern Ocean (Kolbasova et al., 2023). However,

genome-wide SNPs are needed in order to assess mtDNA groups within Tomopteridae and evaluate their potential species diversity and biogeography across ocean basins.

The aim of the current study is to characterize the diversity and biogeography of tomopterid species across the global ocean using molecular approaches. We first examined mtCOI and morphological diversity across all major oceans and conducted phylogenetic analyses using available tomopterid mtCOI data from public databases. Next, we calculated the minimum mtCOI genetic distance for species boundaries based on genome-wide SNP analyses with the aid of 28S sequences. Additionally, the genetic distance is then applied to all available data to evaluate the global-scale potential species diversity of Tomopteridae. Finally, the global distribution patterns of Tomopteridae species are defined, and possible biogeographic formation processes in pelagic environments are discussed.

2. Material and methods

2.1. Sample collection and DNA extraction

Sampling was conducted at 37 sites across three oceans: 11 in the Atlantic, 11 in the Indian, and 15 in the Pacific (Fig. S1, Table S2). Zooplankton samples were collected using oblique tows with either a 1.6 m diameter ORI net or a 0.71 m diameter bongo net, employing mesh sizes of 200–335 µm during ten cruises (Table S2). All tomopterid specimens were sorted from each bulk sample, which were preserved in 95% or 99% ethanol and stored at -20°C. Body length (BL) and segment numbers were recorded from 200 specimens under a Leica M165C stereomicroscope. The mean ratio of BL to segmental numbers was calculated to calibrate the bias of the body length range at different growth stages by the segment numbers. Additional traits such as presence/absence of tentacular cirri and tail, were recorded alongside photographs focusing on the head and entire body as feasible. Notably, it should be noted that the morphological traits such as body length described in this study are based on the ethanol-fixed specimens, which often lose glandular structures within the ramus and rosette glands on the parapodial trunk due to damage caused by net sampling or ethanol preservation. Genomic DNA was extracted from whole-body specimens in most cases using the DNeasy Blood & Tissue Kit (QIAGEN). Environmental data were collected for each sampling station, including seawater temperature and salinity, measured with a CTD system, SBE-911 Plus; Sea-Bird Electronics, and chlorophyll *a* concentration (measured with a Seapoint Chlorophyll Fluorometer). For the Atlantic Meridional Transect Cruise 24 (AMT24), CTD data were archived using profiles from the British Oceanographic Data Center (BODC: JR20140922 [AMT24, JR303] CTD profiles).

2.2. Polymerase chain reaction (PCR)-amplification and sequencing for mtCOI and 28S

MtCOI was amplified via PCR using the primer pairs polyLCO (5'-GAYTATWTTCAACAAATCATAAAGATATTGG-3') and polyHCO (5'-TAMACTTCWGGGTGACCAARAATCA-3') (Carr et al., 2011). Additionally, LCO1490TOSP2 (5'-GTTGATTATATTCTACAAATCATAAAGATATTGG-3'; this study) and HCO1490TOSP (5'-TAMACYTCWGGATGACCAAAAAATCA-3'; this study) were employed. The 28S ribosomal RNA gene was amplified to establish closely-related groups for subsequent SNP analysis using primers F63.2 (5'-ACCGCTGAAYTTAAGCATAT-3'; (Passamaneck et al., 2004) and 28R6 (5'-CAACTTTCCTCAGGTAAGTCTG-3'; (Passamaneck et al., 2004). PCRs were conducted in 15 µL mixtures comprised of 2.5 µL of distilled water, 1.5 µL of each primer (5 µM), 7.5 µL of KOD One® PCR Master Mix (Toyobo), and 2 µL of template DNA. The PCR cycling conditions included an initial denaturation at 94°C for 1 min, followed by 35 or 40 cycles of denaturation at 98°C for 10 s, annealing at 48°C (mtCOI) or 52°C (28S) for 5 s, and extension at 68°C for 5 s, concluding with a final extension at 68°C

for 1 min. Purification of all PCR products was performed using ExoSap-IT (GE Health BioScience), followed by Sanger sequencing preparation with the BigDye Terminator v3.1 (Applied Biosystems). Sequence analysis was conducted using a 3130xL Genetic Analyzer or 3500xL Genetic Analyzer (Applied Biosystems).

2.3. Phylogenetic analyses based on mtCOI and 28S

Sequences obtained in this study were manually confirmed and edited after alignment of forward and reverse strands using Geneious Prime 2023.2 (Biomatters). The edited sequences were aligned with MUSCLE (Edgar, 2004) and visually verified to ensure that mtCOI sequences contained no indels or stop codons in the amino acid translation. In this study, 28S sequences were obtained to select closely related specimens for MIG-seq. Sequences were trimmed to 559-bp for mtCOI and 214-bp for 28S using MEGA11 (Tamura et al., 2021). The 604 mtCOI sequences included 349 specimens newly acquired in this study. An additional 255 sequences from GenBank and BOLD were compiled from 17 bulk samples collected across 15 sites (Table S1). In total, 111 28S sequences were analyzed, including 20 from GenBank, with only those sequences of sufficient length included in phylogenetic analysis (≥ 559 bp for mtCOI; ≥ 214 bp for 28S). The mtCOI sequence from *Tomopteris planktonis* (KY701741; GenBank) was excluded, as it was deemed a doubtful sequence (Kin et al., 2022).

Model selection and maximum-likelihood (ML) phylogenetic analyses were conducted for mtCOI and 28S rRNA. Based on the Akaike Information Criterion (AIC), TVM + I + G (mtCOI) and GTR + G4 (28S) were selected as the best-fitting nucleotide substitution models utilizing ModelTest-NG (Darriba et al., 2019). Phylogenetic analyses were executed using maximum likelihood methods via RAXML-NG v1.2.0 (Kozlov et al., 2019), including a bootstrap analysis with 1,000 replicates. For phylogenetic analyses, mtCOI sequences from GenBank belonging to Glyceriformia, a group closely related to Tomopteridae (Tilic et al., 2022; Weigert et al., 2014), were utilized as outgroups.

2.4. Library preparation and high-throughput sequencing

To ensure robust lineage delimitation of mtCOI, genome-wide SNPs data were used, has been widely reported in analogous studies (e.g., Hirai, 2020). For genome-wide SNP analysis, multiplexed inter-simple sequence repeat (ISSR) genotyping by sequencing (MIG-seq) was employed (Suyama and Matsuki, 2015). MIG-seq is a PCR-based method to amplify genome-wide ISSR regions including nuclear DNA, and it is applicable to small organisms or degraded DNA and does not necessitate restriction enzyme digestion steps. Eighty-nine specimens were selected for genome-wide SNP analysis using MIG-seq, encompassing the mtCOI lineages detected in this study, as much as we could (Table S3). Library preparation for MIG-seq followed the protocol by Suyama et al. (Suyama et al., 2022), with slight modifications (Suyama and Matsuki, 2015). Size selection before high-throughput sequencing (HTS) involved the addition of 0.2–1.8 AMPure XP (Beckman Coulter) after performing the second PCR for 12 cycles. Paired-end sequence reads of 2×150 bp were obtained utilizing Illumina HiSeq or NovaSeq platforms. All library preparations and HTS runs were carried out in GENEWIZ from Azenta Life Sciences, yielding between 385,925 and 8,025,939 raw sequence reads (mean: 2,026,604 reads) per individual.

2.5. De novo assembly, variant calling, and phylogenetic analysis

Quality filtering of raw sequences was executed with Trimmomatic v. 0.36 (Bolger et al., 2014). In the MIG-seq process, 17-bp primer sites, including ISSR and anchor regions, were removed. 133-bp sequences with an average quality score ≥ 25 over every 10-bp were retained to maintain depth and quality. SNPs were detected from quality-filtered paired-end sequences using Stacks v. 2.3 (Catchen et al., 2013). Parameters for Stacks were determined based on Paris et al. (Paris et al.,

2017) and preliminary data analyses, set as follows: m (minimum stack depth) = 3, M (distance allowed between stacks) = 2, and n (distance allowed between catalog loci) = 2. Six analyses were conducted separately based on the 28S results to identify all SNP variant sites, selecting those observed in 30–50% of individuals. ML phylogenetic analysis was performed utilizing SNP data, as previously detailed for mtCOI and 28S analyses.

2.6. Defining operational taxonomic units (OTUs)

The lineages detected through mtCOI and genome-wide SNPs were compared to ascertain the minimum genetic distance of mtCOI as a criterion for species boundaries. Since SNP data were not available for the mtCOI lineages from publicly available sequences (Genbank, BOLD), the established criteria were used to define mtCOI lineages as OTUs for these groups. The OTUs identified in this study were species-level mtCOI groups within Tomopteridae, characterized by a minimum nearest-neighbor distance between OTUs supported by SNP data, with each OTU containing at least two individuals. Genetic distances between and within OTUs were calculated using Kimura's two-parameter method with MEGA11 (Tamura et al., 2021). Within each OTU, the haplotype diversity (H_d) and nucleotide diversity (π) were obtained using DnaSP v6 software (Rozas et al., 2017). The total tomopterid abundance can reflect the peak at the surface layer (Amei et al., 2021; Amei et al., 2023), varying by ocean region and season. To characterize OTU biogeographies, the composition of OTUs was mapped at each sampling site and OTU occurrence was compared with surface seawater temperature (mean value at 0–50 m), surface chlorophyll *a* concentration (mean value at 0–50 m), and the maximum chlorophyll *a* concentration in the upper water column. Plots and linear figures were generated using R Studio (R Core Team, 2023).

3. Results

3.1. OTU delimitation and phylogenetics

A total of 196 unique mtCOI haplotypes were detected among 586 specimens. In the mtCOI ML phylogenetic tree, 29 monophyletic mucoid clades with bootstrap values exceeding 75% were identified, excluding clades COI28 (67%) and IN1 (60%) (Fig. 1a, Table 1). Some lineages formed single nested-clades, including COI2 nested within COI1, COI29 nested within COI22, and COI15-1, COI15-2, and COI15-KC nested within COI15-AMT. These complex clades were further analyzed using unrooted ML phylogenetic analyses, resulting in clearly separated mtCOI lineages (Fig. 1b–e). The conserved 28S phylogenetic tree revealed 15 groups, with 6 groups integrating with specimens from certain mtCOI lineages or singletons: 28S-1 (COI5, M3), 28S-2 (COI15-1, COI15-2), 28S-5 (IN1, S4, S5), 28S-7 (COI12, IN2, COI27, COI26, COI28, S6, S7), 28S-10 (COI22, S9), 28S-13 (M1, COI18), and 28S-15 (COI1, COI2, S11, S12) (Fig. S2). Additionally, two OTUs contained sequences with species names in public databases. The mtCOI sequence of M2 corresponded to the BOLD sequences of *T. helgolandica* (accessions MT03457, MT03459, RMNH.5101351, and RMNH.5101503). COI15-AMT (28S-3) belonged to the same group as the GenBank sequence of *T. septentrionalis* (accession OP626280; Kolbasova et al., 2023) in the 28S phylogenetic tree (Fig. S2).

MIG-seq method is not suitable for comparing genetically distinct materials because genetically different genomes share fewer SNPs (Suyama et al., 2022). To delimit mtCOI clades into OTUs, genome-wide SNPs from specimens belonging to six clades containing closely-related mtCOI lineages were analyzed (Fig. 2a–f, Table S3). Between 1594 and 24,595 SNPs were obtained for each clade. ML trees based on the SNPs clearly supported the 14 mtCOI lineages (Fig. 2a–f), with a minimum mtCOI genetic distance of 2.4% between COI15-1 and COI15-2 (Table S4). Singleton specimens based on mtCOI sequences (Tom668 S5, Tom401 S7, Tom98 S9, and Tom629 S12) were also distinct from

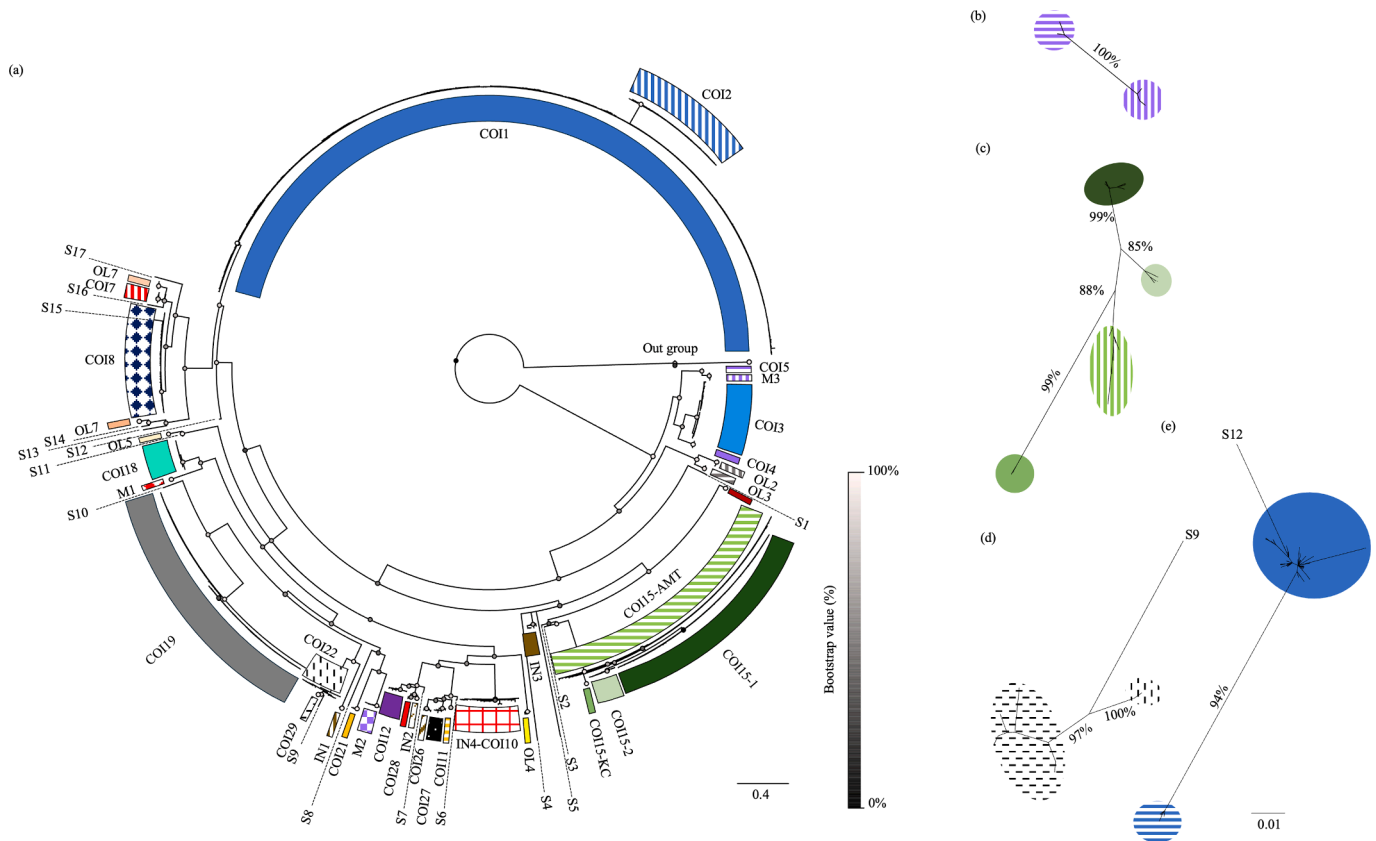


Fig. 1. Maximum likelihood (ML) tree based on mtCOI. (a) ML tree of all tomopterid specimens in this study, constructed using the TVM + I + G model with 1000 bootstrap replicates; circle color represent bootstrap values for the color in the resend. Detailed bootstrap values for each mtCOI lineage are listed in Table 2. Unrooted ML trees for clades with complex mtCOI lineages: (b) COI5 + M3 using the TIM1 model, (c) COI15-1 + COI15-2 + COI15-AMT + COI15-KC using the HKY + G4 model, (d) COI22 + COI29 using the TIM1 + I model, (e) COI1 + COI2 using the TIM3 + I + G4 model. Scale bar indicates genetic distance.

closely related OTUs in the SNP analyses (Fig. 2a-c, f). Therefore, a mtCOI genetic distance of 2.4% was established as the species boundary for OTUs for which SNP data were not available. A clear barcoding gap was observed for most OTUs, with 0–2.8% intraspecific and 2.2–26% interspecific K2P distances (Table 1, Fig. S3). The 2.2% K2P inter-OTU distance was noted only between sample ID Tom15 of COI15-1 and OR379243.1 of COI15-2, which was smaller than all other specimens between COI15-1 and COI15-2 (2.4–2.8%). No SNP data were available for accession OR379243.1 from GenBank; however, these two individuals (Tom15 and OR379243.1) were distinctly separated within the COI15-1 and COI15-2 mtCOI lineages. In total, 35 OTUs (21 OTUs supported with 28S/SNPs) and 17 singletons (7 singletons supported with 28S/SNPs) were identified (Fig. 1).

3.2. Morphological information

Various morphological traits are commonly evaluated to guide their species identification so far: (1) presence/absence of tentacular cirri, (2) presence/absence and location of glands (chromophile glands, hyaline glands, and rosette glands) in a ramus and the parapodial trunk, and (3) presence/absence of a tail. Besides, many references mentioned presence and characteristics of eyes, including its colors in species diagnoses (Rosa, 1908; Stop-Bowitz, 1948). In this study, no tomopterids were classified morphologically due to the absence of glandular structures inside the ramus and rosette glands on the parapodial trunk. The photos of their entire body and head were shown in Fig. 3. Only COI5, COI22, and COI29 displayed tail regions, indicating their classification within

the subgenus *Johnstonella* (Table 2). No other specimens had tails. The dominant OTU, COI1, comprised 236 specimens (39% of the total). The closely related COI15-1 and COI15-AMT (Fig. 3c-d) had overlapping body lengths, segment numbers, and mean BL/segment numbers (Table 2). COI19 displayed the largest body size (35–41 mm), while tentacular cirri and tail region were not observed (Table 2, Fig. 3f).

3.3. Global biogeography

Global investigations of OTU distribution revealed broad patterns across ocean basins and endemic distributions in specific regions (Fig. 4, Table 1). COI1, COI2, and COI18 were detected in all ocean basins, with the most abundant COI1, found in temperate, subtropical, and tropical regions. COI2 displayed an antitropical distribution in the Pacific (occurred worldwide), while COI18 was observed in tropical and subtropical regions. Only IN4-COI10 was shared between the equatorial Indian Ocean and the subtropical Pacific Ocean, including the Kuroshio Current region. IN4-COI10 was also a significant species at site 26 in the South Pacific Ocean.

Endemic OTUs were primarily observed at high latitudes, including the subarctic regions of the Atlantic and Pacific Oceans as well as the Southern Ocean (Fig. 4). The Atlantic-endemic OTUs COI15-AMT, COI26, COI27, COI28, and COI29 were restricted to sites 1, 2, 4, 10, and 11, respectively with COI15-AMT and COI29 distributed in both northern and southern hemispheres. In the Pacific Ocean, COI15-1 and COI19 were restricted to the subarctic region at sites 29, 30, and 35, as well as Monterey Bay. COI15-KC and OL4 were exclusively detected in

Table 1

List of bootstrap values and intra- and inter-OTU Kimura 2-parameter (K2P) distances, genetic diversity (haplotype- and nucleotide diversity), biogeographic distribution for each mtCOI lineage, and accession ID of specimens belonging to each OTU. MtCOI clades are as indicated in Fig. 1. Singletons are listed following OTUs in this table and included in the calculation of inter-OTU distances. The lineages consisted with multiple OTUs were highlighted rows as gray. [†] in the Biogeographic distribution column indicates poorly characterized distributions. Fig. 1 (b)–(e).

OTU or singleton	Fig. 1 (a)	Fig. 1 (b)–(e)	K2P distance (%)		Hd	π	Biogeographic distribution	accession ID of specimens
	bootstrap-value (%)		intra OTU	inter OTU				
COI1	94	94	0–2.7	3.3–24	0.93	0.0043	Circumglobal/ temperate-tropical	MW125113–MW125122, MW125164–MW125166, MW125170–MW125173, LC847324–LC847327, LC847336–LC847337, LC847339, LC847345–LC847346, LC847349–LC847353, LC847355–LC847360, LC847362, LC847364–LC847367, LC847369, LC847374, LC847377, LC847400–LC847402, LC847404–LC847405, LC847408, LC847411, LC847413–LC847417, LC847428–LC847431, LC847433–LC847437, LC847439–LC847447, LC847450–LC847463, LC847465–LC847475, LC847479, LC847481–LC847484, LC847487, LC847489–LC847491, LC847493–LC847494, LC847498, LC847500, LC847502–LC847511, LC847519–LC847527, LC847534–LC847536, LC847538–LC847540, LC847544–LC847570, LC847573–LC847574, LC847577, LC847584, LC847586–LC847588, LC847590–LC847591, LC847594–LC847595, LC847597–LC847598, LC847601–LC847602, LC847607, LC847612, LC847615–LC847627, LC847629–LC847645, LC847647–LC847648, LC847650, LC847652–LC847661, LC847663, LC895814, OL504868–OL504878
COI2		94	0–0.72	6.8–25	0.71	0.0018	Circumglobal/ antitropical?	MW125105–MW125108, OR379313, LC847328–LC847329, LC847331, LC847333, LC847335, LC847338, LC847341, LC847344, LC847368, LC847371–LC847373, LC847375–LC847376, LC847379, LC847398–LC847399, LC847403, LC847406–LC847407, LC847409, LC847486, LC847492, LC847529–LC847532, LC847541, LC847600, LC847628, LC847646
COI3	82		0–2.2	6.2–25	0.71	0.0099	eastern North Pacific Ocean	OL504885, OL504925–OL504950, OK340107
COI4	99		0.18–0.54	6.2–26	1	0.003	Monterey Bay and Oregon [†]	OL504923–OL504925, OK340129
COI5	88	100	0–0.54	2.6–23	0.83	0.003	North Pacific Ocean [†]	OL504921–OL504922, LC847425, LC847448
M3		100	0.54	2.6–24	0.67	0.0024	North Pacific Ocean [†]	OR379237, LC847363
COI7	96		0–0.36	5.4–23	0.89	0.0043	eastern North Pacific Ocean	OK209634, OL504886, LC533821
COI8	90		0–1.3	3.5–23	1	0.0036	Monterey Bay [†]	OK209619–OK209623, OK209625–OK209633, OL504889–OL504898, OL504900, OL504902–OL504905, OK340104
COI11	81		0.36	4.6–21	1	0.011	western North Pacific Ocean [†]	OK209618, OL504784
COI12	77		0.18–1.6	3.7–24	0	0	South Pacific Ocean	LC533812–LC533814, LC533817–LC533818, LC847410, LC847599
COI13	98		0	3.7–23	0.41	0.00092	North Pacific Ocean/ subarctic	LC847334, LC847343
COI15-1	95	99	0–0.54	2.2–21	0.69	0.0035	North Pacific and North Atlantic Ocean/ subtropical	QHAK-01153 –QHAK-01156 [BOLD], ZHAK-01903 [BOLD], OK209616–OK209617, LC847342, LC847381–LC847383, LC847385–LC847391, LC847393–LC847397, LC847418–LC847423, LC847477–LC847478, LC847593, OL504838–OL504867
COI15-2		85	0.36	4.1–22	0.2	0.00143	Circumglobal/ antitropical?	LC533816, LC533819, OR378929, OR379243, LC847424, LC847596, LC847609, LC847611
COI15-AMT		88	0–0.72	2.2–22	0.92	0.0065	Atlantic Ocean/ bipolar?	LC847315, LC847318, LC847320–LC847323, LC847488, LC847495–LC847496, LC847501, LC847514–LC847515
COI15-KC		99	0–1.8	2.4–22	1	0.0036	Southern Ocean	KC754377, KC754378
COI18	83		0–0.72	3.7–22	0.69	0.0073	North Pacific Ocean	LC847330, LC847332, LC847378, LC847380, LC847476, LC847480, LC847485, LC847497, LC847542, LC847576
COI19	95		0–1.6	8.5–22	0.67	0.0012	Pacific Ocean/ equatorial	ZHAK-01919 [BOLD], LC533828, OL504790–OL504797, OL504799–OL504821, OL504824–OL504828, OL504830–OL504831, OL504833, MZ848429–MZ848430, MZ848432–MZ848443, MZ960314, MZ562525, OL504834–OL504837, OK340105–OK340106, LC847384, LC847392
COI21	99		0–0.18	11–20	0.89	0.0097	Pacific Ocean [†]	LC847347–LC847348, LC847354
COI26	82		1.1–2.2	5.0–24	1	0.017	South Atlantic Ocean	LC847316–LC847317, LC847319
COI27	81		0.9–2.8	4.2–24	1	0.018	South Atlantic Ocean	LC847513, LC847516–LC847518
COI28	67		1.1	2.9–23	1	0.011	Atlantic Ocean [†]	MW125102, LC847537

(continued on next page)

Table 1 (continued)

OTU or singleton	Fig. 1 (a)	Fig. 1 (b)–(e)	K2P distance (%)		Hd	π	Biogeographic distribution	accession ID of specimens
	bootstrap-value (%)		intra OTU	inter OTU				
COI22	99	97	0–1.8	2.2–20	1	0.0018	Atlantic Ocean/ subtropical	OL504789, LC533823–LC533827, LC847340, LC847604–LC847605
COI29		100	0.18	2.2–20	0.83	0.018	Indo-Pacific Ocean/ subtropical	LC847512, LC847533
IN1	60		0–2.4	6.7–23	1	0.0072	Indian Ocean [†]	LC847572, LC847575, LC847606, LC847662
IN2	95		0.72	2.9–24	1	0.018	Indian Ocean [†]	LC847578–LC847579
IN3	98		0.18	18–26	0.67	0.0072	Indo-Pacific Ocean/ subtropical	LC847581, LC847649
IN4-COI10	99		0–3.1	11–25	0.5	0.0009	Monterey Bay	LC847412, LC847426, LC847427, LC847432, LC847438, LC847449, LC847580, LC847582–LC847583, LC847585, LC847589, LC847592, LC847603, LC847608, LC847610, LC847613–LC847614, LC847651
OL2	96		0–0.18	8.6–24	0.67	0.0024	Monterey Bay and Gulf of California	OL504917–OL504920
OL3	93		0–0.36	8.6–25	1	0.0096	Southern Ocean, Weddell Sea	OL504915–OL504916
OL4	97		0.36–1.3	13–22	1	0.0036	Monterey Bay and Gulf of California	OL504785–OL504787
OL5	97		0.36	7.4–24	1	0.0027	Monterey Bay and Gulf of California	OL504781–OL504782
OL6	83		0.18–0.36	5.2–23	1	0.0018	Monterey Bay and Gulf of California	OL504884, OL504887–OL504888
OL7	98		0.18	4.8–24	1	0.0072	Cape Verde and the South Pacific Ocean [†]	OL504880–OL504881
M1	94		0.72	9.9–23	0.9	0.0022	North Sea	OR379114, LC847370
M2	94		0–0.36	10–19	1	0.0054	Cape Verde and the South Pacific Ocean [†]	MT03457 [BOLD], MT03459 [BOLD], RMNH.5101351 [BOLD], RMNH.5101503 [BOLD], OL504788
S1	–		–	10–26				LC847464
S2	–		–	1.6–23				OR379149
S3	–		–	1.6–23				OK209576
S4	–		–	2.4–23				LC847606
S5	–		–	2.8–23				LC847571
S6	–		–	4.2–23				OL504783
S7	–		–	3.7–23				LC847499
S8	–		–	17–23				KF977359
S9	–		–	3.9–20				LC847361
S10	–		–	8.5–21				LC533815
S11	–		–	7.4–26				LC533820
S12	–		–	3.3–23				LC533815
S13	–		–	7.7–22				OL504879
S14	–		–	4.8–23				OL504882
S15	–		–	3.5–22				OR378927
S16	–		–	5.4–23				LC533822
S17	–		–	8.7–25				OL504883

the Antarctic Ocean (5 specimens). Additionally, endemic OTUs were identified in the equatorial region, including IN2 and IN3 (site 13) in the Indian Ocean and COI21 (site 23) in the Pacific Ocean, although our sampling effort was insufficient for in-depth discussions of their endemism.

The distribution of COI1, the predominant species, spanned various habitats with a wide range of surface seawater temperatures from 14.3 to 29.0°C (Fig 0.5). COI1 also exhibited high genetic diversity, with 63 haplotypes observed in this study ($Hd = 0.93$; $\pi = 0.0043$; Table 1). In contrast, the circumglobal OTUs COI2 and COI18 displayed narrower distribution ranges, which are characterized by the ocean region with surface seawater temperatures of 19.7–28.1°C and 19.7–27.0°C, respectively, along with fewer haplotypes (nine for COI2: $Hd = 0.71$; $\pi = 0.0018$, two for COI18: $Hd = 0.20$; $\pi = 0.00143$). Certain OTUs (e.g., COI19, COI26, COI27, COI15-1, and COI15-AMT) were found exclusively at high latitudes, characterized by seawater temperature, with upper limits near 20°C. Chlorophyll *a* concentrations ranging from 4.8×10^{-3} to 1.6 mg/m^3 (mean at 0–50 m) and 1.1×10^{-1} to 2.7 mg/m^3 (maximum) were observed at each station (Fig. 5), with relatively high values recorded at high latitudes (sites 1, 2, 10, 11, 29, 30, and 35) and minor peaks in the equatorial regions (sites 7, 13, and 23).

4. Discussion

This study examines the species-level genetic diversity and biogeography of pelagic polychaete Tomopteridae across multiple ocean basins using integrated molecular approaches for the first time. Identifying specimens to species and investigating the large-scale distributions of tomopterids is challenging with conventional morphological observations. Thus, the results obtained in this study represent new insights into understanding the biogeography of tomopterids across the global ocean. Our research results provide a basic framework for tomopterid species as OTUs. In this study, we discuss their biogeography as extensively as possible using the valuable and comprehensive dataset, while biases of sample sizes and different sampling depths should be considered.

4.1. Species delimitation

The phylogenetic tree based on mtCOI was supported by genome-wide SNPs, suggesting that the identified mtCOI lineages and singletons represent reproductively isolated species. The nearest neighbor distance, ranged from 2.4% to 7.4% between mtCOI lineages or singletons. Similar genetic distance values have been reported for cryptic species supported by genome-wide SNPs in other marine zooplankton,

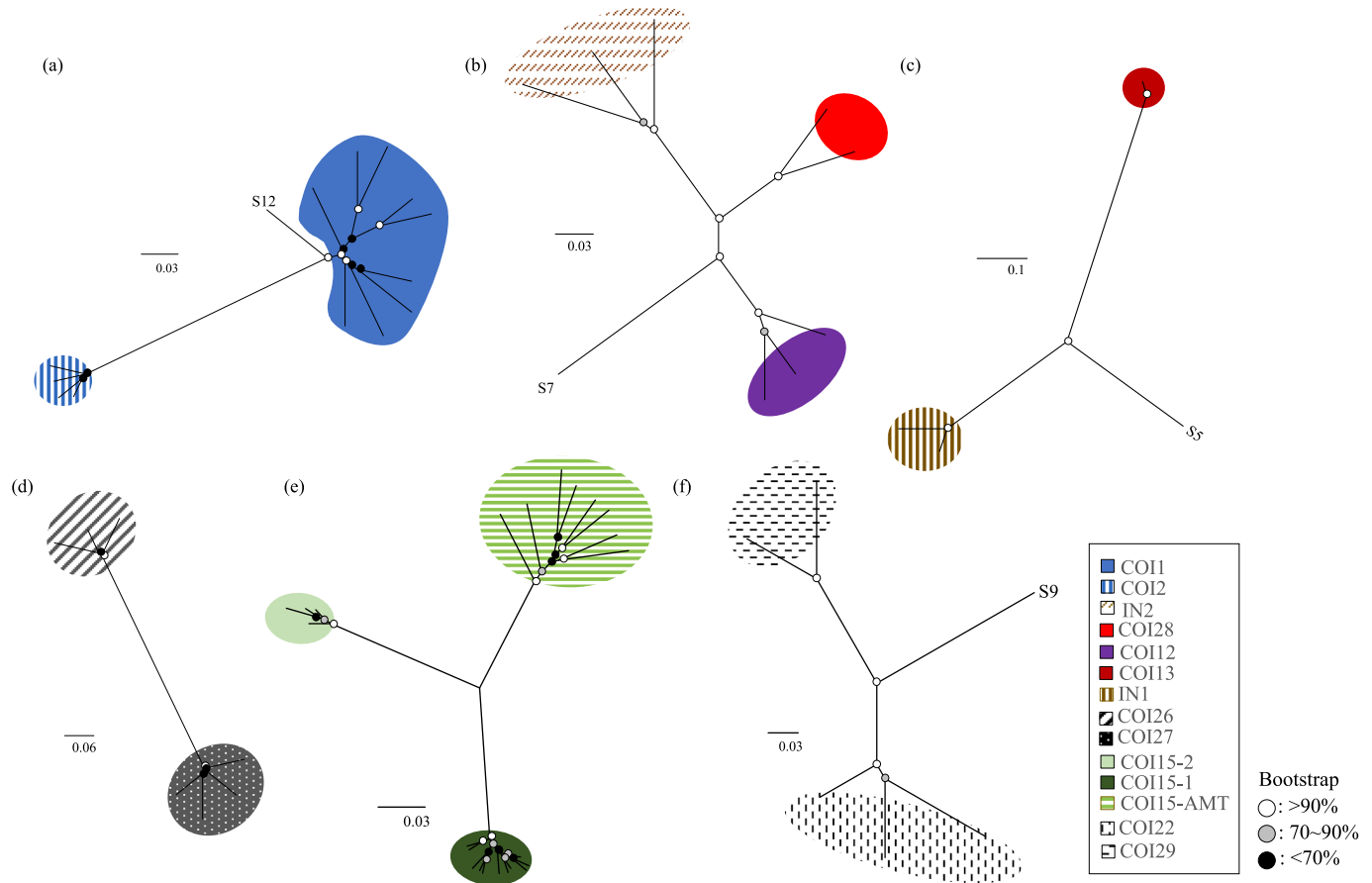


Fig. 2. Unrooted ML trees of genome-wide single-nucleotide polymorphisms (SNPs) for six closely-related groups decided based on the 28S clades, using TVM (a and c), TVMef (b and f), GTR (d and e) models, respectively, with 1000 bootstrap replicates. The scale bar indicates genetic distance.

such as 1.6% in the copepod *Haloptilus longicornis* for mtCOII (Andrews et al., 2014), approximately 8.0% in the copepod *Pleuromamma abdominalis* for mtCOI (Hirai, 2020); and 3.6% in the ctenophore *Bolinopsis microptera* for mtCOI (Johnson et al., 2022). Intra/inter-specific variation (0–2.8% and 2.2–26%, respectively) was observed, with apparent barcoding gaps for each OTU (Table 1). This minimum intra-species distances was smaller than those typically seen with conventional DNA barcoding of mtCOI for described species within a genus of zooplankton, such as 13.5–16.6% and 20.0–24.3% in the copepods *Eucalanus* and *Rhincalanus*, respectively (Goetze, 2003); 3.2–14.2% minimum of intra-OTU p-distances in copepod genus *Paracalanus* (Cornils and Held, 2014), 9–24% inter pairwise variation among 34 congeneric calanoid species in ten genera (Bucklin et al., 2003); 16–18% (nearest neighbor p-distance) among eight species of pterotracheoids (Collin et al., 2023), 5.6–38.1% K2P-mean mtCOI variation between species for 35 genus of Medusozoa (Ortman et al., 2010), and 7–24% inter-species distance for 10 genera of euphausiids (Bucklin et al., 2007). In addition, Waugh (Waugh, 2007) described 0.01–5% intra- and 4–21% inter-species genetic distances (K2P) for mtCOI barcodes from Annelida, Arthropoda, Mollusca, Cnidaria, and Chordata. Collectively, these findings support the high resolution of OTU delimitation in the current study. Pham et al. (Pham et al., 2020) summarized the inter-species, -genera, and -family genetic distances for seven ostracod families in Japan and Thailand, using 18S, 28S, ITS, 16S, and COI, reporting mtCOI variances (p-distance) of 0.2–12.4% depending on genera. The maximum intra-species and minimum inter-species genetic distance vary depending on base pair length, zooplankton taxa, and sampling range. For example, mtCOI barcoding for zooplankton in the Sargasso Sea at low latitudes, the intra-species divergence of less than 1% (Bucklin et al., 2010). The small overlap in genetic distance between

intra- and inter-species distances observed in the current study may be attributed to extensive sampling across ocean basins and genetic variance within a large plankton population size. Collectively, our operational criteria for species boundaries effectively detected tomopterid potential species diversity, including cryptic taxa, crucial for understanding oceanic speciation processes (e.g., Hirai, 2020; Choo et al., 2023).

4.2. Unknown species diversity of Tomopteridae

A total of 35 OTUs and 17 genetically-divergent singletons were identified, corresponding to 52 potential biological species of Tomopteridae. Phylogenetic analyses of mtCOI and genome-wide SNPs confirmed that singletons were distinct from OTUs. Despite our incomplete sampling of all oceanological regions, the number of species identified in this study ($n = 52$) matches with the number of previously reported morphological species ($n = 52$) recorded in the World Register of Marine Species (WoRMS; accessed on Oct. 9, 2024). In addition, considering the taxonomic confusion within Tomopteridae, and the typically 3–4 times higher species richness detected using mtCOI metabarcoding compared to morphological analyses (Ershova et al., 2021), the previous reports on the diversity of Tomopteridae warrant careful consideration. For instance, we detected nine OTUs and three singletons in the western Pacific Ocean, while only seven species had been previously reported in this region (Tebble, 1962; Uschakov, 1974; Dales, 1957). Similarly, our dataset included eighteen OTUs and five singletons in the eastern Pacific Ocean (with two OTUs present only in Monterey Bay), where eleven species were reported earlier (Treadwell, 1943; Fernández-Alamo et al., 2003; Dales, 1957). These findings highlight the insufficient investigation of the diversity based on morphological

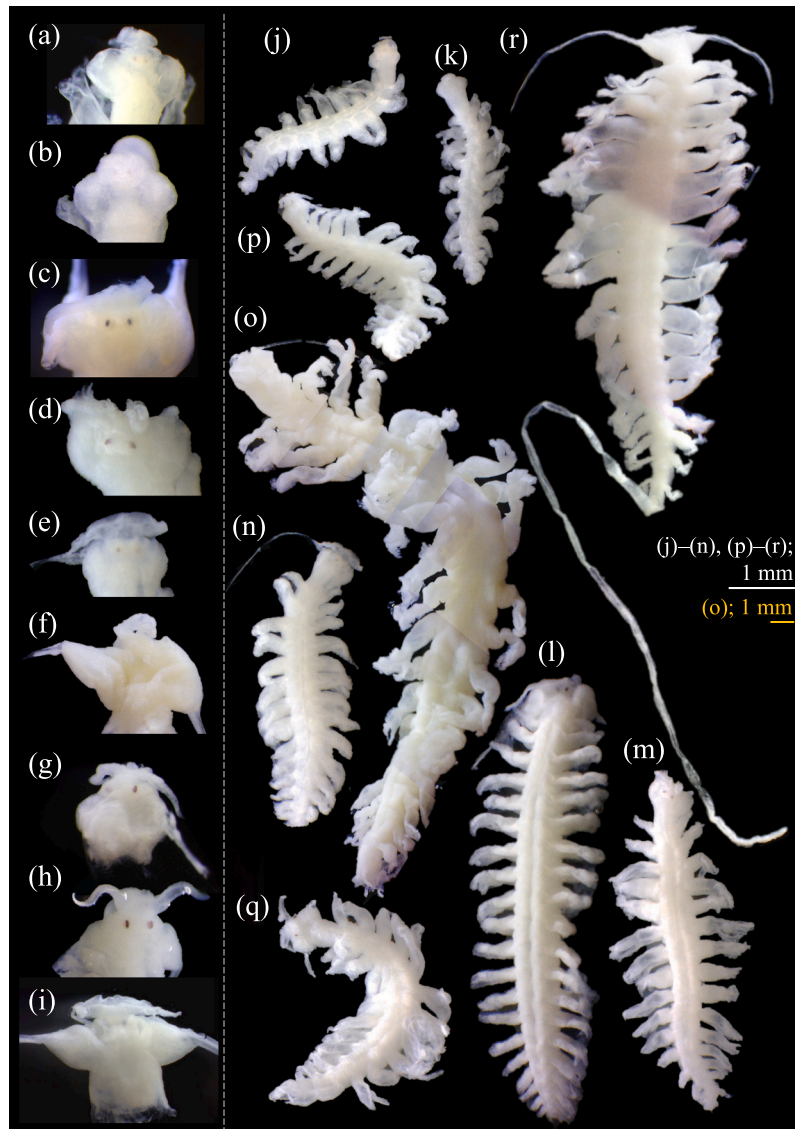


Fig. 3. Images of the head and whole body of selected operational taxonomic units (OTUs). Parapodial numbers and relative body sizes are presented; possible shrinkage may have occurred due to ethanol preservation. COI1 (a, j); COI2 (b, k); COI15-1 (c, l); COI15-AMT (d, m); COI18 (e, n); COI19 (f, o); COI26 (g, p); IN4-COI10 (h, q); COI29 (i, r). Scale bars are indicated for the whole body: orange scale bar for (o) and white scale bar for the other images.

classifications in earlier studies.

To identify factors contributing to the underestimation (e.g., presence of cryptic or pseudo-cryptic species or insufficient research effort) or overestimation (e.g., synonymous species) of tomopterid species diversity, integrated molecular and morphological analyses are necessary. Unfortunately, in the current study, species were not identified based on morphological observations, and basic character information was not collected (number of segments, possession of a tail) for most specimens. Furthermore, species names were assigned to M2 (*T. helgolandica*) and COI15-AMT (*T. septentrionalis*) based on sequences registered in the BOLD database. Although the biogeographical pattern reported in this study for M2 was limited to the Baltic Sea, the type locality was the Canary Islands, requiring further investigation. COI15-AMT was distributed in the North and Central Atlantic, including their type locality. *Tomopteris septentrionalis* is typically cosmopolitan, inhabiting the Pacific, Atlantic, and Southern Oceans (Stop-Bowitz, 1948; Treadwell, 1943; Kolbasova et al., 2023); however, COI15-AMT was restricted to the subarctic North Atlantic Ocean. Species genetically closely related to COI15-AMT (i.e., COI15-1, COI15-2, and COI15-KC) exhibited unique distribution patterns, suggesting that *T. septentrionalis* may represent

multiple species. Hence, detailed taxonomic analyses are warranted for each OTU in our global dataset.

4.3. Global distribution pattern

Our global-scale investigation represents an important step in understanding species-level biogeography using molecular data, complementing previous taxonomic efforts. The review of species recognition by various researchers in the previous works within Tomopteridae has proven challenging, with the current study being the first to employ global-scale molecular data for species-level biogeography. The observed biogeography of Tomopteridae aligns with general marine zooplankton distribution patterns (McGowan, 1971; Miller and Wheeler, 2012). Miller and Wheeler (Miller and Wheeler, 2012) summarized these patterns, citing Frost's global investigation of small *Clausocalanus* copepods (Frost and Fleminger, 1968), identifying five distribution patterns: circumglobal, temperate-tropical; circumglobal, bi-antitropical; Indo-Pacific, temperate-tropical; circumglobal, Southern Ocean; broadly neritic. These latitudinal-belt distribution patterns are general for other zooplankton taxa but vary by species'

Table 2
Morphological characteristics measured for each OTU. Information on body length, segment numbers, and the morphological traits (tentacular cirri,) is listed. Note that the number of specimens used for size measurements was limited. +: present; -: absent; nd: no data.

OTU name	Analyzed specimens	Measured specimens	BL (mm)	Segments (#)	Mean BL/Segmental numbers (±SD)	Morphological traits tentacular cirri	tail
COI1	236	129	1.8–8.4	7–16	0.29 (±0.055)	+	–
COI2	36	19	2.1–4.4	9–13	0.29 (±0.055)	nd	nd
COI5	4	1	16.5	21	0.79	nd	+
COI12	6	1	3.3	13	0.25	nd	nd
COI13	2	1	5.2	14	0.37	nd	nd
COI15-1	62	15	3.6–13	10–21	0.51 (±0.12)	–	–
COI15-2	9	1	2.9	10	0.29	nd	nd
COI15-AMT	12	4	9.1–20	14–20	0.65 (±0.0020)	–	–
COI18	11	5	2.7–8.2	10–17	0.34 (±0.11)	–	–
COI19	65	2	35–41	nd	nd	–	–
COI21	3	3	6.6–9.5	13–19	0.50 (±0.0041)	nd	nd
COI22	9	1	12	17	0.71	+	+
COI26	3	–	nd	nd	nd	nd	–
COI27	4	3	2.1–15	11–15	0.46 (±0.45)	nd	–
COI29	2	1	14	19	0.73	+	+
IN3	2	1	1.9	11	0.17	nd	nd
IN4-COI10	18	11	3.3–9.6	11–18	0.40 (±0.11)	–	–
M1	2	1	7.3	18	0.41	nd	nd
M3	2	1	12	23	0.52	nd	nd

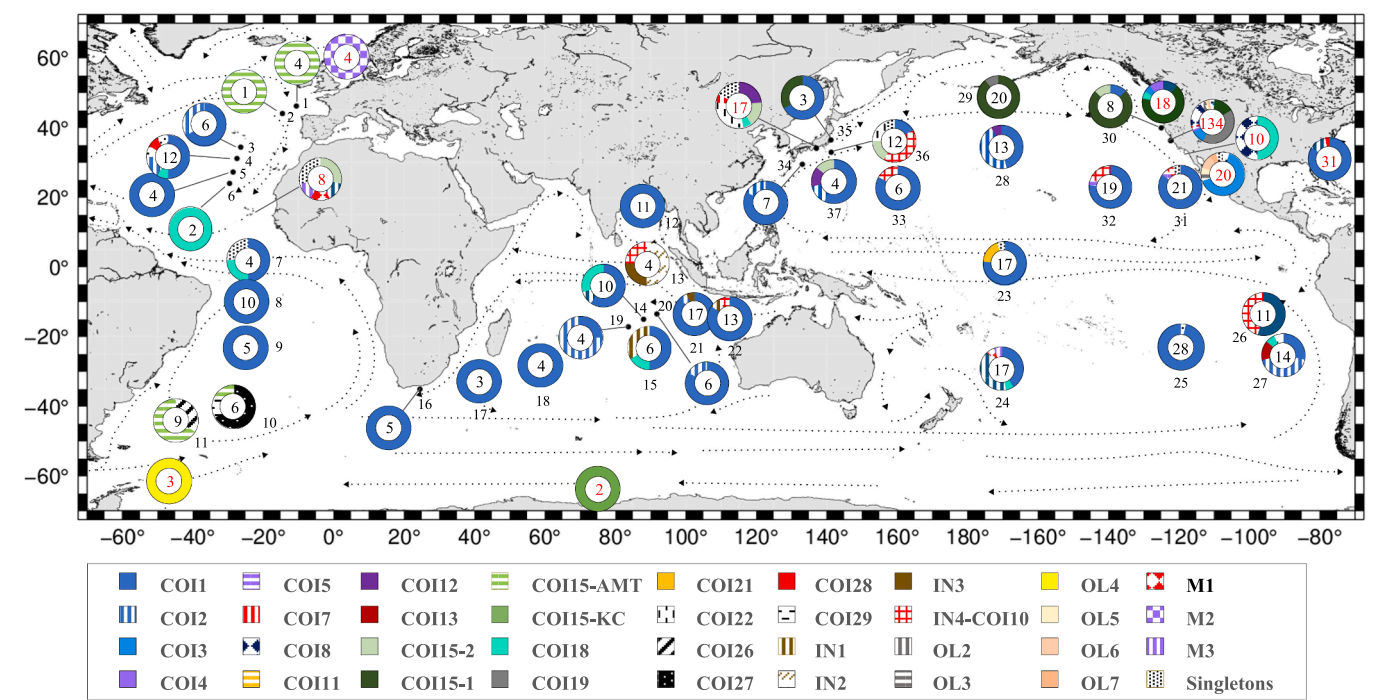


Fig. 4. Composition of operational taxonomic units (OTUs) at each site. Number in the center of each circle is the number of analyzed specimens at site; black colored is newly obtained mtCOI data, and red colored from databases Dotted arrows indicate the major ocean current systems.

environmental tolerances. On the other hand, in higher latitudes, the distribution pattern of cold-water species is simpler than in low latitudes, such as the boreal distribution pattern in the Arctic Ocean of *Pseudocalanus* (genus) in Clausocalanidae (family) (Frost, 1989). Nevertheless, fine-scale differences among species and endemic distributions were also observed.

A significant boundary was observed between the temperate/subtropical and subarctic regions. Subarctic species exhibited differences between ocean basins, with COI15-1 and COI19 confined to areas north of 37°N in the Pacific Ocean, while COI15-AMT (*T. septentrionalis*) was found above 44°N in the Atlantic Ocean, associated with cold waters within the subarctic gyres. Zooplankton species in the subarctic Pacific, such as *Parasagitta elegans* (Bieri, 1959) and *Limacina helicina* (McGowan, 1963), typically do not extend into the California and

Kuroshio Currents, except during shifts caused by large-scale oceanographic conditions such as negative Pacific Decadal Oscillation (PDO) affecting certain cold water zooplanktons (euphausiids *Euphausia pacifica* and *Thysanoessa spinifera* (Lilly and Ohman, 2021); tunicates *Cyclosalpa bakeri* and *C. affinis* (Lavaniegas and Ohman, 2003). Additionally, certain subarctic species exhibit a bipolar distribution pattern, including the Tintinidae *Acanthostomella norvegica* (Pierce and Turner, 1993) and the copepod *Oithona similis* in the Pacific Ocean (Nishida, 1985). Our study identified a bipolar distribution for COI15-AMT (*T. septentrionalis*), suggesting the need for further investigation into vertical distributions, including deeper layers, to elucidate the connectivity of bipolar species.

A broad distribution spanning three ocean basins was observed for temperate/subtropical species (COI1 [n = 238], COI2 [n = 36], and

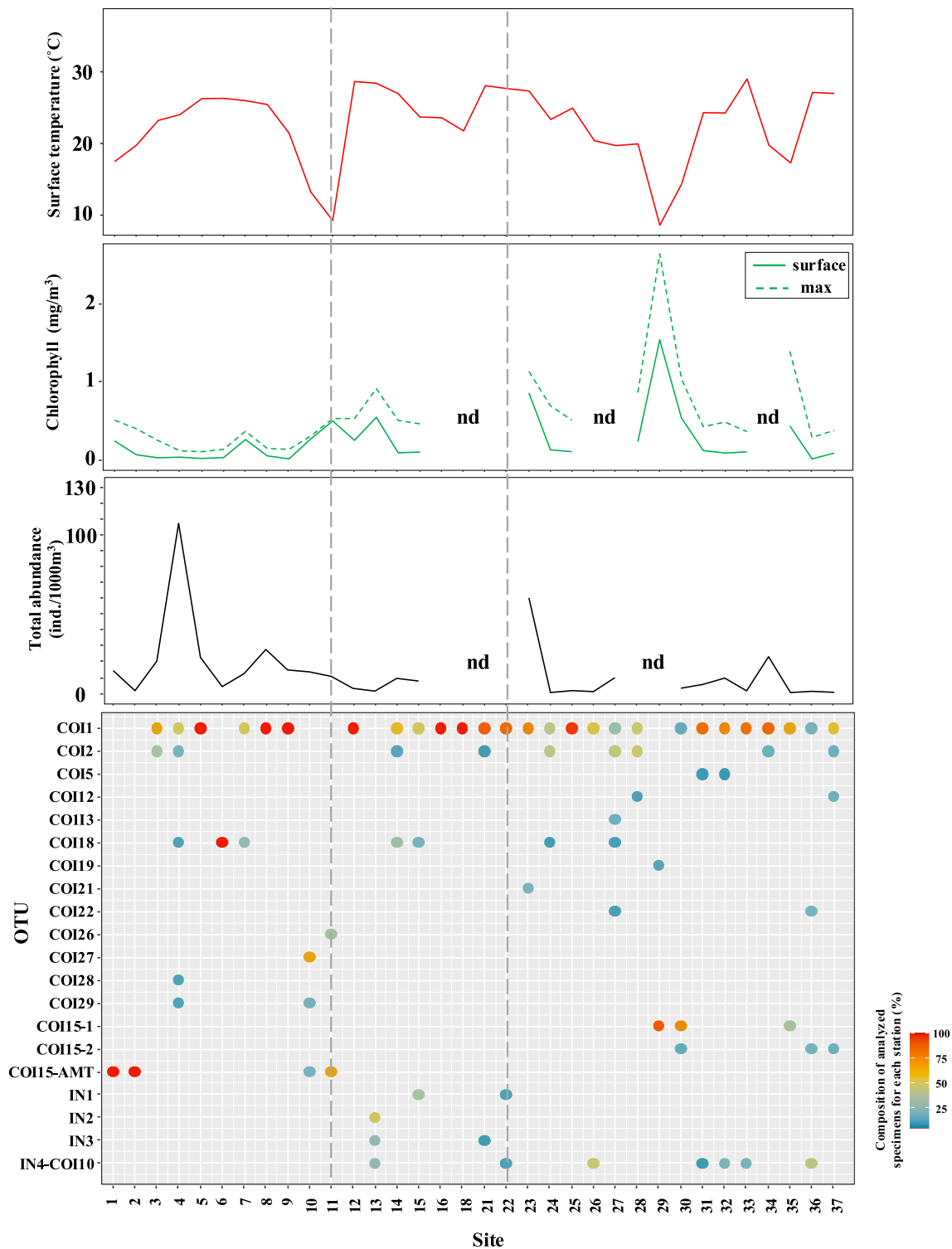


Fig. 5. Distributions of major operational taxonomic units (OTUs) and environmental variables. Surface water temperature, surface chlorophyll a (Chl a), maximum Chl a, total abundance of tomopterids, and relative composition of tomopterid OTUs at each site. Abundance data are also summarized in Table 1. nd: no data.

COI18 [$n = 10$]), as well as the Indo-Pacific (COI10-IN4 [$n = 18$]) and Atlantic-Pacific (COI15-2 [$n = 8$], M1 [$n = 2$], and M3 [$n = 2$]). Distribution extent and peaks varied among OTUs and species. Significant water exchange in the epipelagic layer through the Indo-Pacific Warm Pool (De Deckker, 2016) allows numerous subtropical/temperate species to inhabit the equatorial Pacific Ocean, extending into the Indian and Atlantic Oceans, and the Kuroshio Current (e.g., *Subeucalanus subcrassus* (Goetze and Ohman, 2010; Lang, 1965; Fleminger, 1973) and

Euphausia diomedea (Brinton, 1962). However, only COI10-IN4 was classified as an Indo-Pacific species in our dataset. This may be due to insufficient sampling or detection of endemic species. Thus, the term *endemic* may be subject to interpretation given the sample sizes, requiring confirming true endemism by broader sampling.

Some temperate/subtropical species exhibited restricted distributions in specific areas. For instance, IN1 [$n = 3$], IN2 [$n = 2$], IN3 [$n = 2$], and COI21 [$n = 3$] displayed endemic equatorial distributions in

either the Indian or Pacific Oceans, potentially due to adaptations to equatorial upwelling, as suggested by large-scale zooplankton investigations using both molecular and morphological methods (Hirai et al., 2015; Choo et al., 2021). However, larger distributions may be observed with increased sampling. Other endemic species were found in the coastal regions along the California Current: eastern North Pacific (COI3 and COI8), Monterey Bay and Gulf of California (OL3 [$n = 3$], OL5 [$n = 2$], OL6 [$n = 3$], and OL7 [$n = 2$]), and only Monterey Bay (COI11 [$n = 2$] and OL2 [$n = 4$]). Fernández-Alamo et al. (Fernández-Alamo et al., 2003) documented seven pelagic tomopterids along the California Current transect, including two common northern ($> 37^\circ\text{N}$) and three common southern ($< 22^\circ\text{N}$) species, based on 200 m depth tows using conical nets. Meanwhile, we identified 18 OTUs in this area, indicating a more tomopterid species in the California Current region compared to Fernández's morphological work (Fernández-Alamo et al., 2003).

4.4. Diversification in pelagic environments

Oceanic zooplankton species exhibit ubiquitous distributions due to their high dispersal via ocean currents, with few geophysical barriers (Van der Spoel and Heyman, 1983). However, large-scale molecular studies have revealed significant genetic diversity within certain cosmopolitan species, corresponding to cryptic species adapted to distinct oceanic regions (Hirai et al., 2015; Miyamoto et al., 2012; Andrews et al., 2014; Bucklin, 1986; Peijnenburg et al., 2006). In this study, tomopterids displayed complex genetic structures in pelagic environments. The highest genetic diversity was observed in the cosmopolitan species COI1, suggesting further population-level subdivisions for this circumglobal species. The short mtCOI region assessed in this study provides low bootstrap values ($< 70\%$) in deeper phylogenetic relationships, and long branches were observed in the phylogenetic tree. Thus, additional phylogenetic analyses using multiple molecular markers would be expected to reveal phylogenetic relationships among tomopterid OTUs in more detail.

Distribution pattern differences among closely related species in this study likely resulted from adaptive divergence influenced by dispersal barriers in the open ocean. For instance, the closely related lineages of the Atlantic species COI15-AMT and Pacific species COI15-1 illustrate divergence accelerated by the geographical barrier between ocean basins. COI15-2, a subtropical species in the Atlantic and Pacific Oceans, may have diverged by adapting to different environments from its sibling species COI15-1 and COI15-AMT, which inhabit cold-water regions. Based on a most mtCOI mutation rate of 2% per Ma (Knowlton, 1993; Knowlton and Weigt, 1998), these species are expected to have diverged relatively recently, approximately 1.2 million years ago. Consequently, the speciation of COI15-1, COI15-AMT, and COI15-2 may have occurred following isolation between the Pacific and Atlantic Oceans, exacerbated by ocean circulation changes due to the closure of the Panama Isthmus (O'Dea et al., 2016).

On the other hand, sibling species COI1 and COI2 apparently exhibit sympatric distributions. COI1 is notably dominant in subtropical gyres and demonstrates a greater capacity for adaptation to various oceanic environments, including oligotrophic conditions. In contrast, the distribution of COI2 is limited to the fringes of subtropical gyres. A similar pattern has been observed in sibling species of *Eucalanus hyalinus*, which display distribution peaks at central water mass boundaries, frontal zones, and upwelling systems, whereas *E. spinifer* is found within the oligotrophic subtropical gyre (Goetze, 2005). These findings suggest ecological differentiation among closely related species, likely shaped by speciation in distinct pelagic environments. The process of niche differentiation in tomopterids should be revealed by further ecological investigation, particularly regarding feeding preferences, vertical

distributions, and the environmental conditions they experience.

5. Conclusion

This study represents the first molecular investigation of global Tomopteridae species biodiversity and biogeography. The genomic data reported here are key to understanding zooplankton diversification processes in oceanic regions, especially genome-wide SNPs are informative to specify mitochondrial lineages as OTUs, which are potential biological species. Our global data suggest that physical isolation and environmental differences influence diversity and distribution patterns of Tomopteridae OTUs in the open ocean. Given that zooplankton samples were collected from depths between the sea surface and 400–1000 m, additional species may be found below the mesopelagic layer. In this study, patterns of diversity and biogeography of the Tomopteridae were revealed based on sequence data. For further understanding of species-level diversity of the Tomopteridae beyond molecular data, re-descriptions of morphological traits are essential for accurate taxonomy and ecology of each OTU and species. Since both molecular and morphological data on pelagic polychaetes remain largely unexplored, further molecular and morphological studies are needed to improve our understanding of zooplankton diversity and biogeography in marine ecosystems.

CRedit authorship contribution statement

Kanako Amei: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Junya Hirai:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Erica Goetze:** Writing – review & editing, Resources. **Yuichiro Nishibe:** Writing – review & editing, Supervision, Resources, Conceptualization.

Funding

This work was funded by JSPS KAKENHI, Grant Number JP22KJ1091, and a research project grant from the grants provided by the Environmental Research and Technology Development Fund (JPMEERF20224R03) of the Environmental Restoration and Conservation Agency, provided by the Ministry of the Environment of Japan.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank the researchers and crew who joined the research vessels James Clark Ross, Melville, Hakuho-Marui, and Shioji-Marui during the cruises (AMT24, KH18-6, VANC10MV, KH13-7, KH-11-10, KH-19-6, KH-14-3, KH-17-4, KH-22-5, and SJ-22-29).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.pcean.2026.103689>.

Data availability

Data will be made available on request.

References

- Amei, K., Dobashi, R., Jimi, N., Kitamura, M., Yamaguchi, A., 2021. Vertical changes in abundance, biomass and community structure of pelagic polychaetes down to 1000 m depths at Station K2 in the western subarctic Pacific Ocean covering the four seasons and day-night. *J. Plankton Res.* 43 (3), 442–457. <https://doi.org/10.1093/plankt/fbab031>.
- Amei, K., Dobashi, R., Kitamura, M., Yamaguchi, A., 2023. Diel, seasonal and vertical changes in the abundance, biomass and community structure of pelagic polychaetes at the subtropical station S1 in the western North Pacific: Comparison with the results from the subarctic station K2. *J. Plankton Res.* 45 (4), 661–676. <https://doi.org/10.1093/plankt/fbad023>.
- Andrews, K.R., Norton, E.L., 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. *Mol. Ecol.* 23 (22), 5462–5479.
- Avise, J.C., 2000. *Phylogeography: the history and formation of species*. Harvard University Press, Cambridge, MA.
- Avise, J.C., 2004. *Molecular Markers, Natural history, and Evolution*, Second Edition. Sinauer, Sunderland, MA.
- Benson, D.A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Sayers, E.W., 2013. GenBank. *Nucleic Acids Res.* 41 (Database issue), D36–D42. <https://doi.org/10.1093/nar/gks1195>.
- Bieri, R., 1959. The distribution of the Planktonic Chaetognatha in the Pacific and their Relationship to the Water Masses. *Limnol. Oceanogr.* 4 (1), 1–28. <https://doi.org/10.4319/l.o.1959.4.1.0001>.
- Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30 (15), 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Brinton, E., 1962. The distribution of Pacific euphausiids. *Bull. Scripps Inst. Oceanogr. Univ. Calif.* 8, 51–270.
- Bucklin, A., 1986. The genetic structure of zooplankton populations. *Unesco Techn Papers in Marine Science* 49, 35–41.
- Bucklin, A., Frost, B., Bradford-Grieve, J., Allen, L., Copley, N., 2003. Molecular systematic and phylogenetic assessment of 34 calanoid copepod species of the Calanidae and Clausocalanidae. *Mar. Biol.* 142, 333–343. <https://doi.org/10.1007/s00227-002-0943-1>.
- 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). *J. Plankton Res.* 29 (6), 483–493. <https://doi.org/10.1093/plankt/fbm031>.
- Bucklin, A., Hopcroft, R.R., Kosobokova, K.N., Nigro, L.M., Ortman, B.D., Jennings, R.M., Sweetman, C.J., 2010. DNA barcoding of Arctic Ocean holozooplankton for species identification and recognition. *Deep Sea Res. II* 57 (1–2), 40–48. <https://doi.org/10.1016/j.dsr.2.2009.08.005>.
- Bucklin, A., Steinke, D., Blanco-Bercial, L., 2011. DNA barcoding of marine metazoa. *Annu. Rev. Mar. Sci.* 3, 471–508. <https://doi.org/10.1146/annurev-marine-120308-080950>.
- Bucklin, A., Peijnenburg, K.T.C.A., Kosobokova, K., Machida, R.J., 2021. New insights into biodiversity, biogeography, ecology, and evolution of marine zooplankton based on molecular approaches. *ICES J. Mar. Sci.* 78 (9), 3281–3287. <https://doi.org/10.1093/icesjms/fsab198>.
- Burridge, A.K., Goetze, E., Raes, N., Huisman, J., Peijnenburg, K.T.C.A., 2015. Global biogeography and evolution of *Cuvierina* pteropods: Phylogenetics and phylogeography. *BMC Evol. Biol.* 15 (1), 1. <https://doi.org/10.1186/s12862-015-0310-8>.
- Carr, C.M., Hardy, S.M., Brown, T.M., Macdonald, T.A., Hebert, P.D.N., 2011. A tri-occeanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PLoS One* 6 (7), e22232. <https://doi.org/10.1371/journal.pone.0022232>.
- Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A., Cresko, W.A., 2013. Stacks: an analysis tool set for population genomics. *Mol. Ecol.* 22 (11), 3124–3140. <https://doi.org/10.1111/mec.12354>.
- Choo, L.Q., Bal, T.M.P., Goetze, E., Peijnenburg, K.T.C.A., 2021. Oceanic dispersal barriers in a holoplanktonic gastropod. *J. Evol. Biol.* 34 (1), 224–240. <https://doi.org/10.1111/jeb.13735>.
- Choo, L.Q., Spaggiardi, G., Malinsky, M., Choquet, M., Goetze, E., Hoarau, G., Peijnenburg, K.T.C.A., 2023. Genome-wide phylogeography reveals cryptic speciation in the circumglobal planktonic calcifier *Limacina bulimoides*. *Mol. Ecol.* 32 (12), 3200–3219. <https://doi.org/10.1111/mec.16931>.
- Choquet, M., Lizano, A.M., Le Moan, A., Ravinet, M., Dhanasiri, A.K.S., Hoarau, G., 2023. Unmasking microsatellite deceptiveness and debunking hybridization with SNPs in four marine copepod species of Calanus. *Mol. Ecol.* 32 (24), 6854–6873. <https://doi.org/10.1111/mec.17183>.
- Collin, R., Madrid, R., Venera-Pontón, D.E., Macdonald, K.S., de León, A., Vrdoljak, D., Boyle, M.J., Bryant, P., Arehart, T., Driskell, A.C., 2023. Diversity and genetic connectivity of heteropod (Pterotracheoidea) gastropods in the Tropical Eastern Pacific. *Invertebr. Biol.* 142 (1), e12395. <https://doi.org/10.1111/ivb.12395>.
- Cornils, A., Held, C., 2014. Evidence of cryptic and pseudocryptic speciation in the *Paracalanus parvus* species complex (Crustacea, Copepoda, Calanoida). *Front. Zool.* 11, 19. <https://doi.org/10.1186/1742-9994-11-19>.
- Dales, R.P., 1957. Pelagic polychaetes of the Pacific Ocean. *Bull. Scripps Inst. Oceanogr. Univ. Calif.* 7, 99–168.
- Daniels, J., Aoki, N., Havassy, J., Katija, K., Osborn, K.J., 2021. Metachronal Swimming with Flexible Legs: a Kinematics Analysis of the Midwater Polychaete *Tomopteris*. *Integr. Comp. Biol.* 61 (5), 1658–1673. <https://doi.org/10.1093/icb/ibab059>.
- Darriba, D.I., Posada, D., Kozlov, A.M., Stamatakis, A., Morel, B., Flouri, T., 2019. ModelTest-NG: a New and Scalable Tool for the selection of DNA and Protein Evolutionary Models. *Mol. Biol. Evol.* 37 (1), 291–294. <https://doi.org/10.1093/molbev/msz189>.
- De Deckker, P., 2016. The Indo-Pacific Warm Pool: critical to world oceanography and world climate. *Geosci. Lett.* 3, 20.
- Edgar, R.C., 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32 (5), 1792–1797. <https://doi.org/10.1093/nar/gkh340>.
- Ershova, E.A., Wangenstein, O.S., Descoteaux, R., Barth-Jensen, C., Præbel, K., 2021. Metabarcoding as a quantitative tool for estimating biodiversity and relative biomass of marine zooplankton. *ICES J. Mar. Sci.* 78 (9), 3342–3355. <https://doi.org/10.1093/icesjms/fsab171>.
- Fernández-Alamo, M.A., Sanvicente-Añorve, L., Angel Alatorre-Mendieta, M., 2003. Changes in pelagic polychaete assemblages along the California current system. *Hydrobiologia* 496, 329–336.
- Fleminger, A., 1973. Pattern, number, variability, and taxonomic significance of integumental organs (sensilla and glandular pores) in the genus *Eucalanus* (Copepoda, Calanoida). *Fish. Bull.* 71, 965–1010.
- Francis, W.R., Powers, M.L., Haddock, S.H.D., 2016. Bioluminescence spectra from three deep-sea polychaete worms. *Mar. Biol.* 163, 255. <https://doi.org/10.1007/s00227-016-3028-2>.
- Frost, B.W., 1989. A taxonomy of marine calanoid copepod genus *Pseudocalanus*. *Can. J. Zool.* 67, 525–551. <https://doi.org/10.1139/z89-077>.
- Frost, B., Fleminger, A., 1968. A revision of the genus *Clausocalanus* (Copepoda: Calanoida) with remarks on distributional patterns in diagnostic characters. *Bull. Scripps Inst. Oceanogr. Univ. Calif.* 12, 1–235. [https://doi.org/10.1016/0011-7471\(69\)90080-1](https://doi.org/10.1016/0011-7471(69)90080-1).
- Goetze, E., 2003. Cryptic speciation on the high seas; global phylogenetics of the copepod family Eucalanidae. *Proc. R. Soc. Lond. B* 270 (1531), 2321–2331. <https://doi.org/10.1098/rspb.2003.2505>.
- Goetze, E., 2005. Global population genetic structure and biogeography of the oceanic copepods *Eucalanus hyalinus* and *E. spinifer*. *Evolution* 59 (11), 2378–2398. <https://doi.org/10.1111/j.0014-3820.2005.tb00948.x>.
- Goetze, E., Ohman, M.D., 2010. Integrated molecular and morphological biogeography of the calanoid copepod family Eucalanidae. *Deep-Sea Res. II* 57 (24–26), 2110–2129. <https://doi.org/10.1016/j.dsr.2.2010.09.014>.
- Gouveaux, A., 2016. Bioluminescence of Tomopteridae species (Annelida): multidisciplinary approach. Centre Natl. la Recherche Scientifique, Ottignies-Louvain-la-Neuve Belgium. PhD thesis.
- Gouveaux, A., Mallefet, J., 2013. Physiological control of bioluminescence in a deep-sea planktonic worm. *Tomopteris Helgolandica*. *J. Exp. Biol.* 216 (22), 4285–4289. <https://doi.org/10.1242/jeb.090852>.
- Gouveaux, A., Flood, P.R., Erichsen, E.S., Olsson, C., Lindström, J., Mallefet, J., 2017. Morphology and fluorescence of the parapodial light glands in *Tomopteris helgolandica* and allies (Phyllodocida: Tomopteridae). *Zool. Anz.* 268, 112–125. <https://doi.org/10.1016/j.jcz.2016.08.002>.
- Guglielmo, R., Gambi, M.C., Granata, A., Guglielmo, L., Minutoli, R., 2014. Composition, abundance and distribution of holoplanktonic polychaetes within the Strait of Magellan (southern America) in austral summer. *Polar Biol.* 37, 999–1015.
- Guglielmo, R., Bergamasco, A., Minutoli, R., Patti, F.P., Belmonte, G., Spanò, N., Zagami, G., Bonanzinga, V., Granata, A., 2019. The Otranto Channel (South Adriatic Sea), a hot-spot area of plankton biodiversity: Pelagic polychaetes. *Sci. Rep.* 9 (1), 19490.
- Hays, G.C., Richardson, A.J., Robinson, C., 2005. Climate change and marine plankton. *Trends Ecol. Evol.* 20 (6), 337–344.
- Hirai, J., 2020. Insights into reproductive isolation within the pelagic copepod *Pleuromamma abdominalis* with high genetic diversity using genome-wide SNP data. *Mar. Biol.* 167 (1). <https://doi.org/10.1007/s00227-019-3618-x>.
- Hirai, J., Tsuda, A., Goetze, E., 2015. Extensive genetic diversity and endemism across the global range of the oceanic copepod *Pleuromamma abdominalis*. *Prog. Oceanogr.* 138, 77–90. <https://doi.org/10.1016/j.pocean.2015.09.002>.
- Jennings, R.M., Bucklin, A., Pierrot-Bults, A., 2010. Barcoding of arrow worms (Phylum Chaetognatha) from three oceans: Genetic diversity and evolution within an enigmatic phylum. *PLoS One* 5 (4), e9949.
- Johnson, S.B., Winnikoff, J.R., Schultz, D.T., Christianson, L.M., Patry, W.L., Mills, C.E., Haddock, S.H.D., 2022. Speciation of pelagic zooplankton: Invisible boundaries can drive isolation of oceanic ctenophores. *Front. Gen.* 13. <https://doi.org/10.3389/fgene.2022.970314>.
- Kin, I., Jimi, N., Ohtsuka, S., Mizuno, G., Nakamura, T., Maekawa, Y., Oba, Y., 2022. The COI haplotype diversity of the pelagic polychaete *Tomopteris* (Annelida: Tomopteridae) collected from the Pacific coast off Kii Peninsula, central Japan. *Plankton and Benthos Res.* 17 (2), 214–220. <https://doi.org/10.3800/pbr.17.214>.
- Knowlton, N., 1993. Sibling species in the sea. *Annu. Rev. Ecol. Syst.* 24, 189–216.
- Knowlton, N., 2000. Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia* 420, 73–90. <https://doi.org/10.1023/A:1003933603879>.
- Knowlton, N., Weigt, L.A., 1998. New dates and new rates for divergence across the Isthmus of Panama. *Proc. R. Soc. Lond. B* 265 (1412), 2257–2263.
- Kolbasova, G., Syomin, V., Mishin, A., Molodtsova, T., Neretin, N., Neretina, T., 2023. Holopelagic Annelida from the Atlantic sector of the Southern Ocean and adjacent

- South Atlantic with descriptions of three new species. *Mar. Biodivers.* 53 (6). <https://doi.org/10.1007/s12526-023-01380-x>.
- Kozlov, A.M., Darriba, D., Flouri, T., Morel, B., Stamatakis, A., 2019. RAXML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* 35 (21), 4453–4455. <https://doi.org/10.1093/bioinformatics/btz305>.
- Lang, B.T., 1965. Taxonomic review of the copepod genera *Eucalanus* and *Rhincalanus* in the Pacific Ocean. Scripps Institute of Oceanography, University of California, San Diego. PhD Thesis.
- Lavaniegos, B.E., Ohman, M.D., 2003. Long-term changes in pelagic tunicates of the California current. *Deep-Sea Res. II* 50 (14–16), 2473–2498. [https://doi.org/10.1016/S0967-0645\(03\)00132-2](https://doi.org/10.1016/S0967-0645(03)00132-2).
- Lilly, L.E., Ohman, M.D., 2021. Euphausiid spatial displacements and habitat shifts in the southern California Current System in response to El Niño variability. *Prog. Oceanogr.* 193, 102544. <https://doi.org/10.1016/j.pocean.2021.102544>.
- 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 (4), e10278. <https://doi.org/10.1371/journal.pone.0010278>.
- Marlétaz, F., le Parco, Y., Liu, S., Peijnenburg, K.T.C.A., 2017. Extreme Mitogenomic Variation in Natural Populations of Chaetognaths. *Genome Biol. Evol.* 9 (6), 1374–1384. <https://doi.org/10.1093/gbe/evx090>.
- McGowan, J.A., 1963. Geographical variation in *Limacina helicina* in the North Pacific. In: Speciation in the sea. Harding JP & Tebbel N (eds). Syst. Ass. London 5, 109–128.
- 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.
- Miller, C.B., Wheeler, P.A., 2012. *Biological Oceanography*. John Wiley & Sons.
- Miyamoto, H., Machida, R.J., Nishida, S., 2012. Global phylogeography of the deep-sea pelagic chaetognath *Eukrohnia hamata*. *Prog. Oceanogr.* 104, 99–109. <https://doi.org/10.1016/j.pocean.2012.06.003>.
- Nishida, S., 1985. Taxonomy and distribution of the family Oithonidae (Copepoda-Cyclopoida), in the Pacific and Indian oceans. *Bull. Ocean Res. Inst. Univ. Tokyo*.
- Norris, R.D., 2000. Pelagic species diversity, biogeography, and evolution. *Paleobiology* 26 (S4), 236–258.
- O'Dea, A., Lessios, H.A., Coates, A.G., Eytan, R.I., Restrepo-Moreno, S.A., Cione, A.L., Jackson, J.B., 2016. Formation of the Isthmus of Panama. *Sci. Adv.* 2 (8), e1600883. <https://doi.org/10.1126/sciadv.1600883>.
- Ortman, B.D., Bucklin, A., Pages, F., Youngbluth, M., 2010. DNA barcoding the Medusozoa using mtCOI. *Deep-Sea Res. II* 57 (24–26), 2148–2156.
- Osborn, K.J., Rouse, G.W., 2008. Multiple origins of pelagicism within Flabelligeridae (Annelida). *Mol. Phylogenet. Evol.* 49 (1), 386–392.
- Paris, J.R., Stevens, J.R., Catchen, J.M., 2017. Lost in parameter space: a road map for stacks. *Methods Ecol. Evol.* 8 (10), 1360–1373. <https://doi.org/10.1111/2041-210X.12775>.
- Passamanek, Y.J., Schander, C., Halanych, K.M., 2004. Investigation of molluscan phylogeny using large-subunit and small-subunit nuclear rRNA sequences. *Mol. Phylogenet. Evol.* 32 (1), 25–38. <https://doi.org/10.1016/j.ympev.2003.12.016>.
- 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. *Mol. Ecol.* 15, 3319–3338. <https://doi.org/10.1111/j.1365-294X.2006.03002.x>.
- Pham, H.T.M., Tanaka, H., Karanovic, I., 2020. Molecular and Morphological Diversity of *Heterodesmus* Brady and its Phylogenetic Position within Cypridinidae (Ostracoda). *Zool. Sci.* 37 (3), 240–254. <https://doi.org/10.2108/zs190118>.
- Pierce, R.W., Turner, J.T., 1993. Global biogeography of marine tintinnids. *Mar. Ecol. Prog. Ser.* 94, 11–26.
- Purschke, G., Helm, C., 2023. Development and structure of the anterior nervous system and sense organs in the holopelagic annelid *Tomopteris* spp. (Phyllodocida, Errantia). *Org. Divers. Evol.* 23 (3), 505–527. <https://doi.org/10.1007/s13127-023-00603-0>.
- R Core Team, 2023. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna <https://www.R-project.org/>.
- Ratnasingham, S., Hebert, P.D.N., 2007. BOLD: the Barcode of Life Data System (www.barcodinglife.org). *Mol. Ecol. Notes* 7, 355–364. <https://doi.org/10.1111/j.1471-8286.2006.01678.x>.
- Richardson, A.J., 2008. In hot water: zooplankton and climate change. *ICES J. Mar. Sci.* 65 (3), 279–295.
- Rosa, D., 1908. Raccoglie planktoniche fatte della R. Nave Liguria nel vaggio di circonnavigazione del 1903–1905 sotto il comando di S. A. R. Luigi di Savoia. *Duea Degli Abruzzi. Inst. Firenze* 1, 245–327.
- Rozas, J., Ferrer-Mata, A., Sanchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., et al., 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol. Biol. Evol.* 34 (12), 3299–3302. <https://doi.org/10.1093/molbev/msx248>.
- Schroth, W., Jarms, G., Streit, B., Schierwater, B., 2002. Speciation and phylogeography in the cosmopolitan marine moon jelly, *Aurelia* Sp. *BMC Evol. Biol.* 2, 1. <https://doi.org/10.1186/1471-2148-2-1>.
- Siciński, J., 1998. Pelagic Polychaeta in the Scotia Front west of Elephant Island (BIOMASS III, October–November 1986). *Pol. Polar Res.* 9 (2–3), 277–282.
- Støp-Bowitz, C., 1948. Polychaeta from the “Michael Sars” North Atlantic deep-sea expedition 1910. *Rep. Sci. Res. m. Sars North Atlantic Deep-Sea Exp.* 5, 1–91.
- Struck, T.H., Schult, N., Kusen, T., Hickman, E., Bleidorn, C., McHugh, D., Halanych, K. M., 2007. Annelid phylogeny and the status of Sipuncula and Echiura. *BMC Evol. Biol.* 7, 1–11.
- Suyama, Y., Matsuki, Y., 2015. MIG-seq: an effective PCR-based method for genome-wide single-nucleotide polymorphism genotyping using the next-generation sequencing platform. *Sci. Rep.* 5 (1), 16963.
- Suyama, Y., Hirota, S.K., Matsuo, A., Tsunamoto, Y., Mitsuyuki, C., Shimura, A., Okano, K., 2022. Complementary combination of multiplex high-throughput DNA sequencing for molecular phylogeny. *Ecol. Res.* 37 (1), 171–181. <https://doi.org/10.1111/1440-1703.12270>.
- Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* 38 (7), 3022–3027.
- Tebbel, N., 1962. The distribution of pelagic polychaetes across the North Pacific. *Bull. British Mus. Nat. History Zool.* 7, 371–492.
- Tilic, E., Stiller, J., Campos, E., Pleijel, F., Rouse, G.W., 2022. Phylogenomics resolves ambiguous relationships within Aciculata (Errantia, Annelida). *Mol. Phylogenet. Evol.* 166. <https://doi.org/10.1016/j.ympev.2021.107339>.
- Treadwell, A.L., 1943. Scientific results of Cruise VII of the Carnegie during 1928–1929 under Command of Captain J.P. Ault. *Biology - IV. Biological results of the last cruise of the Carnegie. III. Polychaetous annelids*. Carnegie Inst. Wash. Publ. 555, 29–59.
- Uschakov, P.V., 1974. Fauna of the U.S.S.R. Polychaetes. Russian. translated by the Israel Program for Scientific Translations. Jerusalem.
- Van der Spoel, S., Heyman, R.P., 1983. *A Comparative Atlas of Zooplankton*. Springer-Verlag, Berlin.
- Waugh, J., 2007. DNA barcoding in animal species: progress, potential and pitfalls. *Bioessays* 29 (2), 188–197.
- Weigert, A., Helm, C., Meyer, M., Nickel, B., Arendt, D., Hausdorf, B., Santos, S., Halanych, K., Purschke, G., Bleidorn, C., Struck, T.H., 2014. Illuminating the base of the annelid tree using transcriptomics. *Mol. Biol. Evol.* 31 (6), 1391–1401.