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Comparative mitogenomics and biodiversity studies of Nemertea

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Abstract

This study presents genome skimming data from 20 nemertean specimens collected globally encompassing diverse habitats. Complete mitochondrial genomes were sequenced and assembled for all species, and phylogenetic analyses of the relationships recovered their evolutionary positions. Due to the limited presence of external morphological traits and a lack of previously published sequence data, it was not possible to make species-level identifications for several of the samples. The large scale analysis of mitochondrial gene order in Nemertea shows that this phylum has a comparatively conserved mitochondrial genome. Heteronemertea and some palaenemertean genera have the protein-coding gene order of the ancestral lophotrochozoans, while Hoplonemertea and *Cephalothrix* Örsted, 1843, (Archinemertea) exhibit gene translocations. In total, three protein-coding gene orders and five tRNA gene orders have been described from Nemertea. In addition, analysis of mt genomes and the nuclear 28S recovered a possible inter-species hybrid with an anomalous genome size. Genome skimming offers a fast and convenient method to analyze nemertean mt genomes on a large scale. Next generation sequencing offers a promising future for nemertean research as it opens new possibilities to study nemertean evolution.

Keywords Nemertea · Phylogeny · Genome skimming · Mitochondrial genomes

Introduction

Despite being the Earth's largest ecosystem, the deep ocean remains one of the least understood and explored areas. Oceans span 71% of the planet's surface, with half lying deeper than 3000 m, and an average depth of 3800 m. Only 5% of the deep sea has been investigated using remote technologies, and less than 0.01% of the deep ocean floor has been thoroughly sampled and analyzed (Ramirez-Llodra et al. 2024). Moreover, oceans are home to a very large number of undescribed and cryptic species, which are important to understand biodiversity. Nemertea is a phylum of worm-like animals consisting of about 1300 described species, that mostly occur in marine environments (Gibson 1972; Kajihara et al. 2008, WORMs 2025) and generally inhabit the littoral and sublittoral zones of the World Oceans. However, most of the almost 100 species of the bathypelagic nemerteans known to date are commonly found within a depth range of a few hundred to a few thousand meters, becoming most abundant at 600–3000 m (Chernyshev and Chaban 2005). *Galathenemertes giribeti* Chernyshev & Polyakova, 2019 is the deepest known symbiotic nemertean, collected at a depth of 7256 m and originally identified as *Galatheanthemum* sp.

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Carlgren, 1956. This is also the second known species to have an association with an *Actina* sea anemone.

Nemertean species are usually soft-bodied with morphological features that do not preserve well when fixed in ethanol or formalin (Gibson 1982, 1995; Sundberg and Strand 2010; Sundberg et al. 2016). In addition, nemerteans have very few distinguishing external characters, and their internal characters are difficult to assess without microscopic analysis (Gibson 1982; Roe et al. 2007). Therefore, both histological sectioning and genetic analyses are required to properly identify the species (Sundberg et al. 2016, Maskalova et al. 2025). In addition, the few external characters are often homoplastic which have led to frequent taxonomic misidentifications (Kajihara et al. 2022). An alternative approach is species identification based on DNA barcoding. However, this is only feasible as long as a well-curated database of correctly identified nemertean species is available (Strand and Sundberg 2010, Sundberg et al. 2016). Although a large number of nemertean COI barcode sequences are found in databases such as GenBank, many have not been allocated a species name, making it difficult to perform taxonomic classifications.

The mitochondrial (mt) genome is a circular molecule present in all eukaryotic cells (Boore 1999). Animal mt genomes are about 16,000 nucleotides (nt) long frequently containing 37 genes (22 tRNAs, 13 protein-coding genes and 2 ribosomal RNAs), but the organization and gene number can differ dramatically in non-bilaterian animals (Lavrov and Pett 2016). Complete mt genomes contain about 25-fold the number of variable sites compared to barcoding genes like COI, providing a significant advantage in phylogenetic reconstruction, particularly for accurately placing highly divergent taxa. In addition, the mt protein-coding gene order can also be used as a marker for phylogenetic relationships, in some cases (Tan et al. 2018). Genome skimming is a fast and affordable approach to generate data to extract complete mt genomes (Dodsworth 2015). In genome skimming experiments, a small tissue sample is sequenced at a very low genomic coverage (0.1X), and the overrepresentation of the mtDNA compared to the nuclear genome in cells allows the assembly of complete mt genomes at a high coverage. The de novo assembly approach allows reconstructing gene order and other mt features without any bias. Nemertea is so far only represented by about 50 complete mt genomes in GenBank (status of 10.2025), but many of them lack proper gene annotations (Sevigny et al. 2021). Using the genome skimming approach, we generated mt genomes for 20 nemerteans from the North Atlantic as well as the Southern Ocean. The nemerteans with the largest body size are found in Heteronemertea, which consists of 500 described species worldwide. We sequenced and analyzed 15 mt genomes from Heteronemertea originating from both the Northern and Southern Hemisphere. Palaeonemertea is the least species-rich nemertean taxonomic group, with only 100 described species. Previously, mt genomes from only one paleonemertean genus, *Cephalothrix* Örsted, 1843, were sequenced (Chen et al. 2009, 2011), and the analysis is now expanded to include *Tubulanus* Renier, 1804, *Carinoma* Oudemans, 1885 and *Carinina* Hubrecht, 1885. In addition, we sequenced two species from the species-rich hoplonemertean clade. The new mt genomes were used for phylogenetic reconstruction, and the gene content and order were investigated. Many of the samples were not classified based on outer morphology at the point of collection, and the resulting molecular data was used for post hoc species identification. Nuclear genome size is an important factor when designing genome sequencing projects. Normally, the genome size differs marginally within a species and genus. Here, we present an unusual case of genome size variation within a species coupled with deviant mt and nuclear phylogenies of one individual. The results highlight the advantages of next generation sequencing and associated molecular methodologies for expanding research possibilities to study this fascinating, morphologically challenging and understudied phylum.

Methods

Samples were collected opportunistically during expeditions (Supplementary Table 1). Several nemerteans were collected during the PS118 expedition on board the RV *Polarstern* in February/March 2019 along the Antarctic Peninsula, in the Weddell Sea (Southern Ocean) (Dorschel 2019). Sampling took place at three different stations at depth ranging from 403 to 442 m, between 63 and 64° South. The nemertean samples from PS118 were collected using an Agassiz Trawl. Once on board, the specimens were sieved using a 300 µm mesh and/or directly transferred into precooled (− 20 °C) 96% ethanol. All ethanol-preserved samples were then stored at − 20 °C for at least 48 h to prevent DNA degradation before further processing. No formal morphological characterization was made of the individual Southern Ocean samples. The COI barcode region was sequenced for eight individuals, and the complete mt genomes were obtained from four of these.

Paleonemertean, hoplonemertean, and heteronemertean species were captured in the French coast intertidal regions of Roscoff, Le Cabellou, and Trégunc (Supplementary Table 1). The species were identified based on external morphological traits during collection as well as COI sequences. Another lineid species commonly encountered in the North Sea around Helgoland was sampled from uncatalogued museum samples stored in ethanol. These lineid nemerteans are relatively large and have a bright yellow to orange coloration. Only few samples have been brought back to the Senckenberg Museum where they remained uncatalogued

due to lack of species identification. Most nemertean samples were preserved in 70% or 96% ethanol, while some were flash frozen and stored at − 80 °C or − 20 °C until further processing, depending on available equipment. DNA was isolated from whole body tissue using the Qiagen DNA mini amp kit according to the manufacturer specifications. For barcoding, the COI region was amplified using conserved primers (Folmer et al. 1994) (LCO1490: GGTCAA CAAATCATAAAGATATTGG and HCO2198: TAAACT TCAGGGTGACCAAAAAATCA). PCR products were sequenced from both strands on an ABI sequencer 3730 DNA analyzer (Applied Biosystems, Foster City, USA) by the laboratory center of the Senckenberg Biodiversity and Climate Research Centre Frankfurt. All sequencing was done in accordance with Nagoya legislation. The COI barcode sequences were analyzed using BLAST to retrieve the best sequence match. Where necessary, alignments were constructed using the COI barcoding region (500–600 nt) and aligned using MUSCLE (Edgar 2004) in AliView v1.28 (https://github.com/AliView, Larsson 2014). Pairwise p-distances were calculated using MEGA v10.2.6 (Kumar et al. 2018; Stecher et al. 2020). Selected individuals were sent for short read paired end sequencing to Novogene or BGI. For each sample between 4 and 8 Gbp of short read data was generated. Mitogenomes were assembled with MitoZ v2.3 (Meng et al. 2019). Annotation of the mt genomes was done with MITOS2 (Donath et al. 2019) using [Refseq 63 Metazoa] and the invertebrate genetic code [code 5] and manually curated in Geneious Prime v2023.1 (https://www.geneious.com/). All annotated mt sequences have been deposited in GenBank (Supplementary Table 2).

The short read sequences from the three *Lineus acutifrons*-like individuals were mapped to the *Lineus acutifrons* Southern, 1913, 28S sequence (2588 nt) (HQ856855). The software BWA-MEM v0.7.17-r1188 mapped the reads to the reference, and Picard (available at http://broadinstitute.github.io/picard/) was used to mark duplicate reads. The nucleotide variants were called from the mapped reads using freebayes (Garrison and Marth 2012) (-report monomorphic, -min-mapping-quality 20, -C 4, -F 0.3). The final consensus sequences were generated using bcftools (Danecek et al. 2021). Additional 28S sequences were selected from GenBank based on the phylogeny in Puerta et al. (2010).

The new mt genomes were included in a final data set of 42 taxa consisting of available annotated nemertean mt genomes from GenBank. The data set was analyzed using PhyloSuite (Zhang et al. 2020; Xiang et al. 2023) which extracted and concatenated the 13 mt protein-coding genes. Two data sets were created, one based on nucleotides and one based on amino acids (aa). The alignments were trimmed using clipKIT v1.1.6 using the kpi-gappy setting (Steenwyk et al. 2020). Phylogenetic analysis was performed with Maximum Likelihood (ML) using IQ-TREE v1.6.12 (Nguyen et al. 2015; Trifinopoulos et al. 2016). The best fitting model was identified using ModelFinder implemented in IQ-TREE (Kalyaanamoorthy et al. 2017). Branch support was calculated by both 1000 bootstrap replicates using SH-aLRT and ultrafast bootstrap support (Hoang et al. 2018). The mt gene order was analyzed using PhyloSuite (Zhang et al. 2020; Xiang et al. 2023). PhyloSuite extracted the gene order for each analyzed species, and it was visualized using iTOL (Letunic and Bork 2024) (available at https://itol.embl.de/). The linear mt genome order, starting at COI, including protein-coding genes, tRNAs, and rRNAs was visualized in OGDRAW (Greiner et al. 2019). Nemertean genome sizes (2C-value; Greilhuber 2005) were estimated using flow cytometry with the Partec CyFlow Space (Partec, Münster, Germany) equipped with a green solid-state laser using the two-step Otto protocol (Otto 1990), with an internal standard *Glycine max* cv. Polanka (2C = 2.50 pg; Doležel et al. 1994) as described by Paule et al. (2021).

Note on recent taxonomical changes

The name of one of the species in our dataset, *Lineus acutifrons* (sensu Puerta et al. 2010), has recently been revised to *Junoya acutifrons* (Southern 1913) (Chernyshev 2025). However, due to the recency of the revision, the accession numbers for the respective nucleotide sequences on NCBI Genbank presently still refer to the former name, *Lineus acutifrons*. As such, we have retained the name *Lineus acutifrons* for the species rediscovered by Puerta et al. (2010) throughout this publication. Another species which is part of the mt genome analysis, *Micrura ignea*, was also revised, and the genus name changed to *Archimicrura* (Chernyshev 2025).

Results

Mt genome organization

We sequenced the complete mt genome from 20 marine nemerteans from the Northern and Southern Hemispheres, collected across various habitats. The sampling includes three paleonemerteans, two hoplonemerteans, and 15 heteronemerteans (Supplementary Table 1). Additionally, the mt genome of the Baltic clam (*Macoma balthica* Linnaeus, 1758) was sequenced (see below). The mt genome of all 20 newly sequenced individuals conforms with previously described "nemertean patterns" and contains 37 genes, similar to most animal mt genomes (Boore 1999). The total length of the mt genomes ranges from 14,558 nt (*Paranemertes peregrina* Coe, 1901) to 16,847 nt (*Micrura bella* Stimpson, 1857) with Heteronemertea having the longest genomes overall and Hoplonemertea the shortest (Fig. 1).

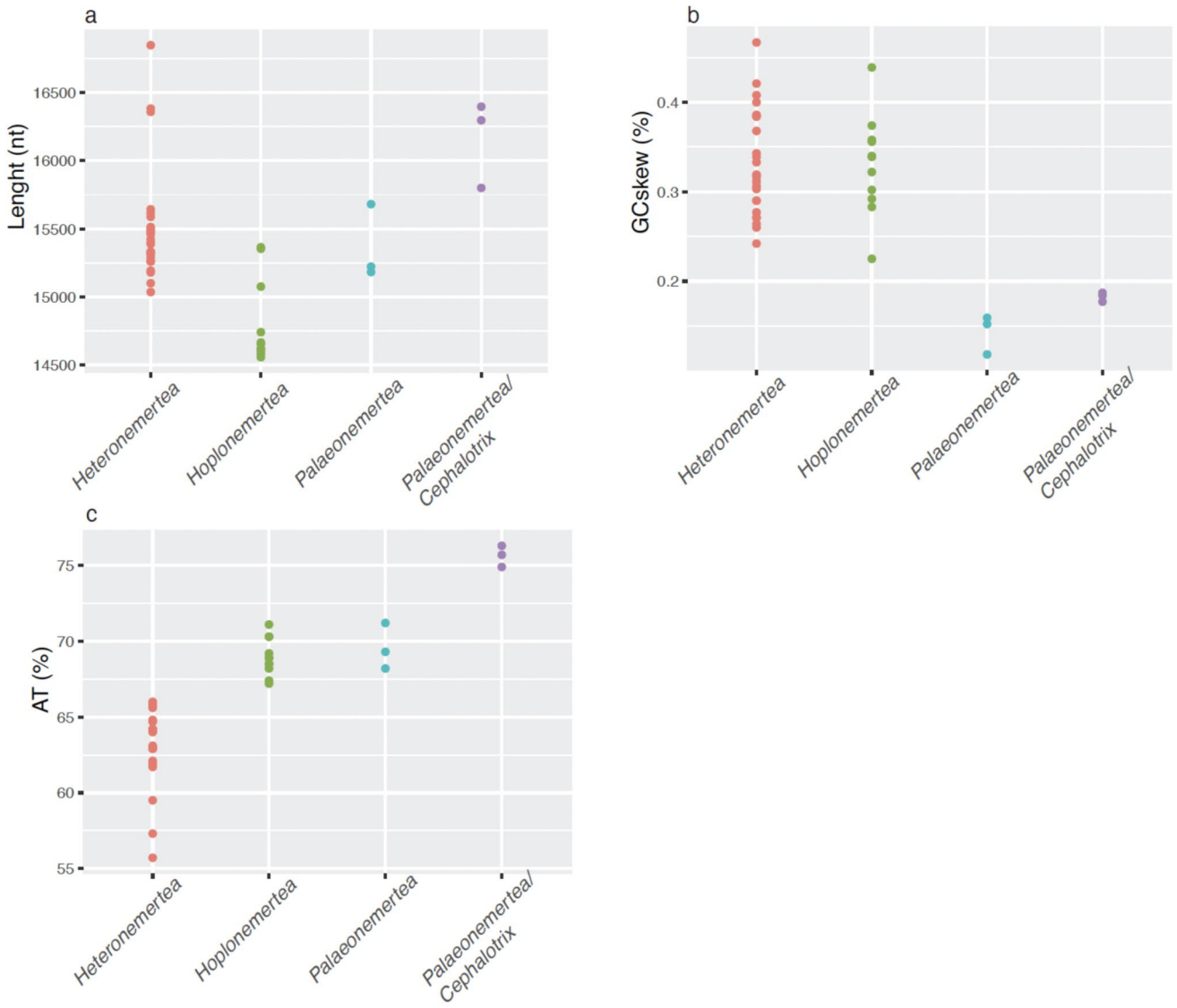

Fig. 1 **a** Length of the nemertean mt genomes in nt; **b** GC-skew (%); and **c** AT-content (%). The species are grouped taxonomically, with Palaeonemertea split into Palaeonemertea and Palaeonemertea/*Cephalotrix*. Palaeonemertea/*Cephalotrix* contain only three *Cephalotrix* species

The AT-content of the nemertean mt genome differs between the major clades, where *Cephalothrix* (Archinemertea) has a significantly higher percentage of AT compared to the other clades. Heteronemertea has the lowest AT-content. A similar pattern is observed in the GC-skew, with both Palaeonemertea clades having very low values.

As previously identified (Podsiadlowski et al. 2009), all 13 protein-coding genes are encoded on the same strand with only 2 tRNAs (tRNA-P (Proline) and tRNA-T (Threonine)) encoded on the opposite strand. The mt gene order does not differ from previously described heteronemerteans and hoplonemerteans (Chen et al. 2012), differing only by a translocation of tRNA-S1 (Serine) and NAD2 between the groups. In contrast, Palaeonemertea displays a more complex gene arrangement. The newly sequenced mt genomes of the three paleonemerteans (*Carinoma*, *Carinina*, *Tubulanus*) share the same protein-coding gene order as Heteronemertea (Fig. 2).

However, the tRNA gene order differs both between groups and within the Palaeonemertea. Compared to Heteronemertea, there is one tRNA translocation in Hoplonemertea, four in the non-cephalothricid Palaeonemertea and eight in *Cephalotrix* species (Supplementary Fig. 1). Within non-cephalothricid Palaeonemertea, an additional tRNA translocation of tRNA-C (Cysteine) is observed in the tRNA cluster located between the genes ATP6 and 12S of *Carinoma armandi* McIntosh, 1875 relative to *Tubulanus polymorphus* Renier, 1804, and *Carinina ochracea* Sundberg, Chernyshev, Kajihara, Kånneby and Strand, 2009.

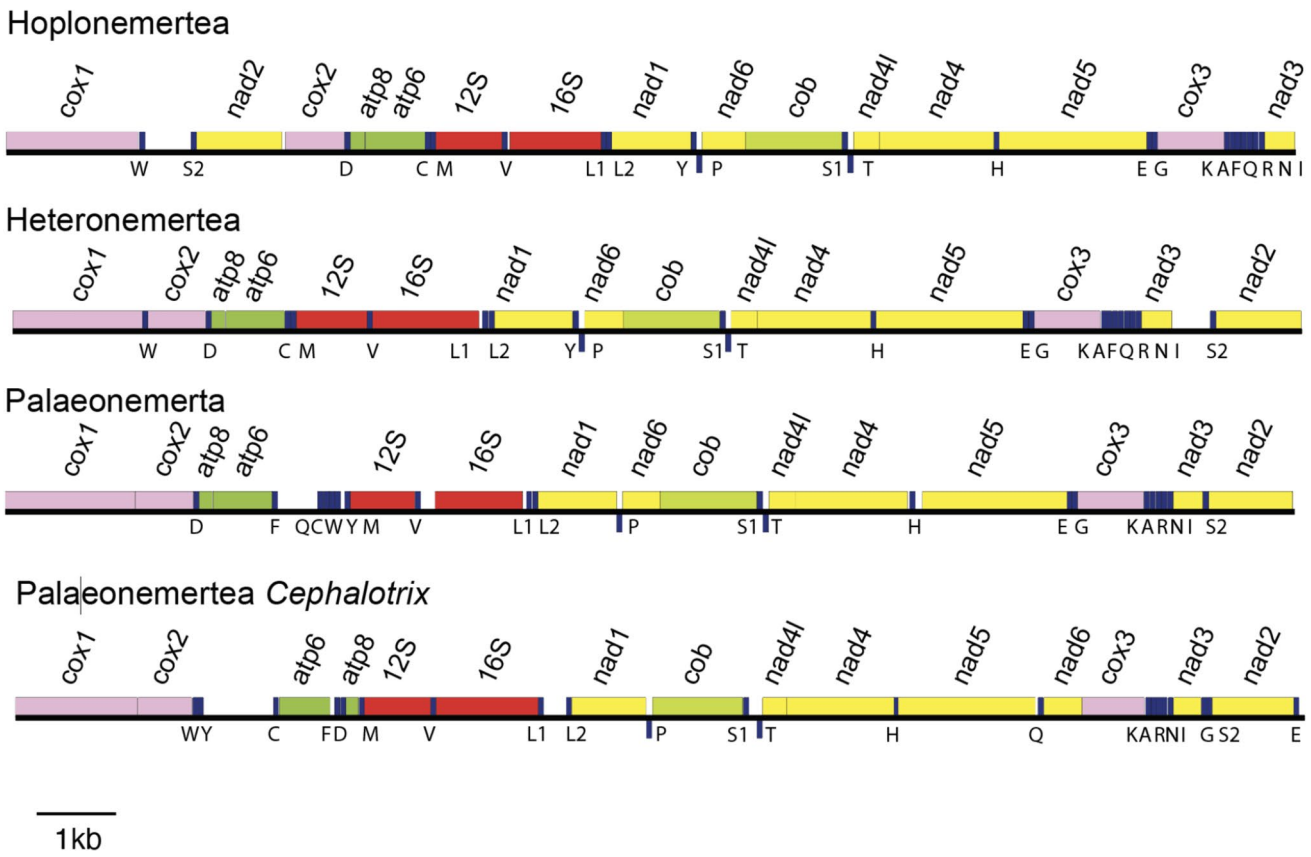

Fig. 2 The mitochondrial protein-coding, rRNA, and tRNA gene order of the major groups of nemerteans. The scale bar indicates 1 Kb. The figure has been generated by OGDRAW (Greiner et al. 2019), and the genes are color coded: pink: COX1-3, green: ATP8, ATP6, and cob, yellow: NAD1-6. The two ribosomal genes are colored in red. The tRNAs are indicated in single case aa letter below the genes. Only Proline (P) and Threonine (T) are encoded on the opposite strand

Phylogenetic reconstruction

The final alignments consisted of 42 taxa and a total length of 2226 aa and 7426 nt. The best fitting model for the ML analyses was selected by ModelFinder (Kalyaanamoorthy et al. 2017), implemented in IQ-TREE (Nguyen et al. 2015; Trifinopoulos et al. 2016), as mtInv + F + G4 according to BIC for the aa data and GTR + F + ASC + G4 for the nt data set. Within Palaeonemertea, the species *Tubulanus* and *Carinoma* are most closely related with *Carinina* as their next closest relative (Fig. 3). *Cephalothrix* species form a lineage separate from species of *Tubulanus*, *Carinoma*, and *Carinina*. Among the heteronemertean species from France, *Lineus ruber* Müller, 1773, named after its bright red coloration, is the sister species to *L. viridis* Müller, 1773 (Fig. 3), as found in previous studies (Zattara et al. 2019, Ament-Velásquez et al. 2016). *L. longissimus* Gunnerus, 1770, the bootlace worm, the presumably longest animal on earth, is the sister species to *L. lacteus* Rathke, 1843 and *L. sanguineus* Rathke, 1799 (Zattara et al., 2019, Ament-Velásquez et al. 2016). The monotypic *R. occultus* Rogers, Junoy, Gibson & Thorpe, 1993 is the sister species to *L. lacteus*, *L. sanguineus*, and *L. longissimus*. However, the internal branches placing both *R. occultus* and *L. longissimus* are short (Fig. 3), with support values of 62% and 32%.

For one species, *Carinina ochracea,* the MitoZ de novo assembly resulted in two contigs, one from *Carinina ochracea* and the other from *Macoma balthica* (OR451556). *Macoma balthica* is likely part of the diet of *Carinina ochracea*, as there were no clams captured with the nemerteans, making gut content the most likely source (*cf.* Maslakova and Hiebert 2014).

New or unclassified species

For the southern ocean samples that previously were not formally characterized, the sequence-based phylogenetic analyses indicate that the eight samples constitute three species from Lineidae. The COI barcode sequences were BLASTed against NCBI (https://www.ncbi.nlm.nih.gov/nucleotide/) and BOLD (https://www.boldsystems.org/) databases, which

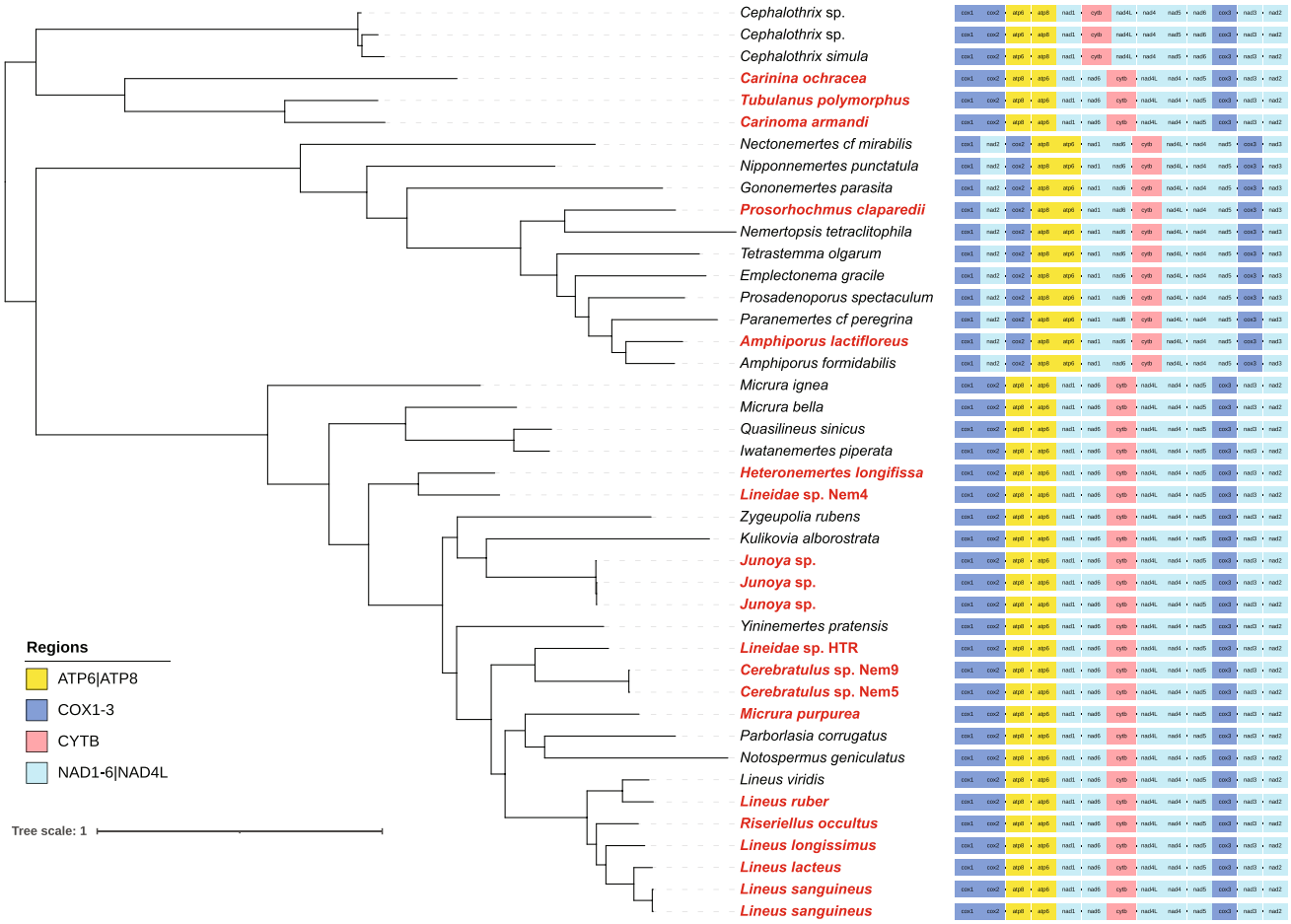

Fig. 3 Maximum likelihood phylogeny based on an aa alignment of 13 protein-coding genes and 42 taxa. The phylogeny was visualized in iTOL. Species names in red indicate new sequences. The gene order for each species is located next to the species name. All mitochondrial genomes are displayed beginning with the COI gene. The root of the phylogeny has been placed on Palaeonemertea

resulted in one full species name and genus level identification for two.

Five of the eight collected Southern Ocean nemerteans belong to the same species. These five individuals had a black coloration when alive. COI sequences were obtained for all five (PV012552-54), and two (Nem5 and Nem9) were selected for complete mt genome sequencing and assembling. The closest BLAST matches for the COI sequences are cf. *Cerebratulus* sp. (GU227125) and *Cerebratulus* sp. (OQ450482) (Chernyshev and Polyakova 2023; Heimeier et al. 2010). The pairwise COI p-distances of 0.2–0.7% between all five sequenced individuals to *Cerebratulus* sp. suggest that these are the same species. This species belongs in the Lineidae clade J + K (Chernyshev and Polyakova 2023) but has not been formally described at the species level and we refer to this as *Cerebratulus* sp. (Fig. 3).

The closest COI sequence similarity for two of the collected individuals (Nem2 (PV012551) and Nem6) is to the recently described species *Heteronemertes longifissa* Hubrecht, 1887 (Chernyshev and Polyakova 2023). The pairwise COI p-distance is 0.2% to the published sequences *Heteronemertes longifissa* (OQ450488, OQ450487, OQ450485). Both of the individuals had a pinkish live coloration which conforms to the description of *Heteronemertes longifissa* (Chernyshev and Polyakova 2023). Phylogenetic analysis of the complete mt genome infers *Heteronemertes longifissa* as the sister species to another Southern Ocean nemertean (Nem4) with high support (100%) (Fig. 3). The closest COI matches, with p-distances 0.5–0.6%, are Lineidae sp. (OM456687) and cf. Heteronemertea sp. (GU227120) both collected in the Southern Ocean (Chernyshev and Polyakova 2022; Heimeier et al. 2010). Given that there is no close COI match with a full species name, we refer to this species as *Lineidae* sp. Both *Lineidae* sp. and *Heteronemertes longifissa* belong to the Lineage D of the lineid heteronemerteans (Chernyshev and Polyakova 2023).

The sample from the Helgoland Trench, North Sea, did not have a close COI p-distance match in the databases. Given the higher number of phylogenetically informative sites in the complete mt genome compared to the COI

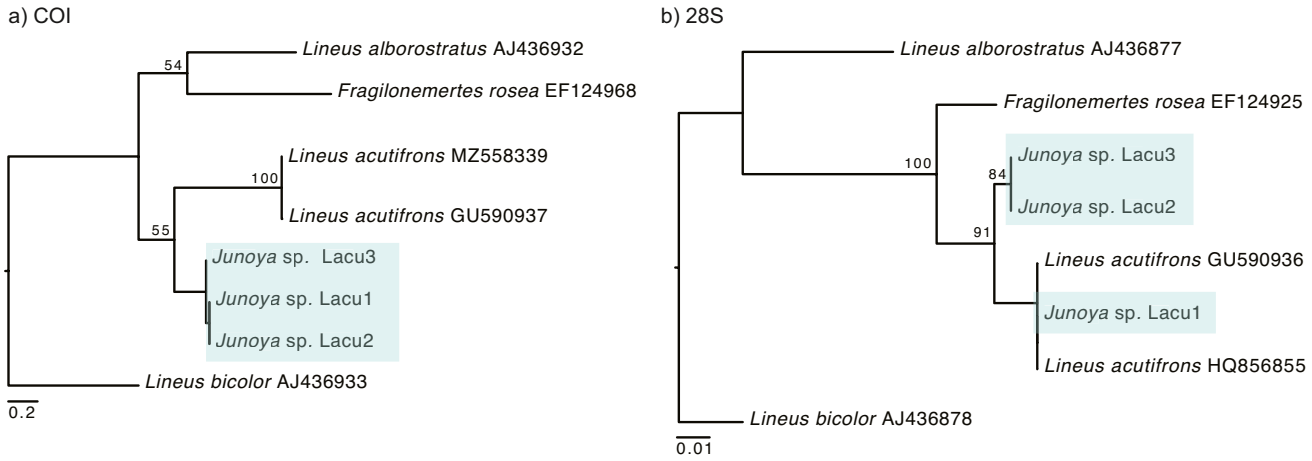

Fig. 4 *Lineus acutifron*s ML phylogeny. **a** COI phylogeny; **b** 28S phylogeny. The three *Junoya* sp. individuals from this study are indicated in light blue shading, when analyzing COI in (a) the three individuals cluster as the sisterspecies to *L. acutifrons*. When 28S is analyzed, one sample of the three *Junoya* sp. individuals, Lacu1, cluster with *L. acutifrons*, while the samples Lacu2 and Lacu3 cluster as the sisterspecies to *L. acutifrons*. Values above branches are SH-aLRT support values. Scale bars indicate substitutions per site

region, it was possible to phylogenetically place the nemertean species. The lineid nemertean is reconstructed as the sister species to the Southern Ocean species *Cerebratulus* sp (Nem5/Nem9) with 100% support (Fig. 3). For this reason, the species is deposited in GenBank as *Lineidae* sp. until a formal species description is made.

Genome size estimation

Three individuals externally similar to the species *Lineus acutifrons* (sensu Puerta et al. 2010) were found along the French Atlantic coast (Trégunc, Anse de Pouldohan) in the lower intertidal region. The COI p-distance to two previously published COI sequences from *L. acutifrons* (Puerta et al. 2010) is 14–18%, suggesting that the collected *L. acutifrons*-like specimens represent a cryptic species, here tentatively referred to as *Junoya* sp. Chernyshev, 2025 (Sagorny et al., submitted). The complete mt genomes reconstruct *Junoya* sp. as the sister species to *Kulikovia alborostrata* Takakura, 1898 (former genus name *Lineus*) (Fig. 3). The COI p-distance is 0–2% between the three individuals (Fig. 4). The nuclear gene 28S was extracted from the Illumina short reads. Phylogenetic reconstruction of 28S shows that one individual (Lacu1) clusters with *L. acutifrons* instead of *Junoya* sp. The genome size was determined for three individuals. Individual 1 (Lacu1) had a genome size of 835 Mb, while individual Lacu2 had a genome size of 1895 Mb, individual Lacu3 had a genome size of 1994 Mb, and the previously published Lacu4 had a genome size of 1904 Mb (Paule et al. 2021). The genome size for *Junoya* sp. ranges between 1895 and 1994 Mb, whereas that of *L. acutifrons* is 1904 Mb.

Discussion

Members of the phylum Nemertea are notoriously difficult to taxonomically classify based on outer morphology alone due to their few distinguishing characters. A fast and efficient alternative approach is to use DNA sequence data to classify the individuals (Emerson 2025). With the advent of next generation sequencing, genome skimming has become a rapid and efficient method to generate large mt genomic datasets for phylogenetic inference. Complete mt genomes contain a much greater number of variable sites than the COI barcode region. This allows for more reliable phylogenetic tree building for confidently placing species in their correct evolutionary context.

We sequenced the mt genome from 20 nemerteans using genome skimming. Analysis of the mt genomes suggests a conserved gene order within Nemertea (Chen et al. 2012). To date, no gene duplications or introns have been identified in nemertean mt genomes, and mt genome sizes do not exceed 16.8 kb. Although recently , a mitochondrial genome of *Geonemertes* (PX238424) became available which is almost 32 kb large (Gastineau et al. 2026). Among the analyzed nemerteans, only three distinct protein-coding gene orders and five tRNA gene orders were identified (Fig. 2). The order of the protein-coding genes in Heteronemertea and the investigated non-cephalothricid Palaeonemertea aligns with the ancestral lophotrochozoan mt protein-coding gene order (Podsiadlowski et al. 2009). However, species in the genus *Cephalothrix* (Archinemertea) deviate from other paleonemerteans due to translocations of ATP6 and NAD6 (Chen et al. 2011). These findings suggest that the basal gene order occurs in some paleonemerteans and Heteronemertea, while Hoplonemertea and at least one paleonemertean

genus exhibit derived rearrangements. In contrast, within Annelida, at least 28 gene orders—or 73 when including tRNAs—have been reported (Struck et al. 2023), and even greater mt genome variation has been found in Mollusca (Ghiselli et al. 2021; Bergmeier et al. 2024). This suggests that protein-coding gene order is stable in nemerteans compared to many other invertebrate phyla (Struck et al. 2023; Shtolz and Mishmar 2023).

The phylogenetic relationships within Palaeonemertea have been difficult to resolve with many competing hypotheses based on different datasets (e.g., Andrade et al. 2012, Kvist et al. 2014, 2015, Thollesson and Norenburg 2003, Sundberg et al. 2001, 2003, 2009; Sundberg and Strand 2007, Chernyshev and Polyakova 2018a, b, 2019). Our analysis of complete mt genome sequences includes only six species and four genera but their relationships have not been recovered in any previous studies. Previous studies have relied on limited data sets of barcoding genes or cladistic analysis (e.g., Andrade et al. 2012); therefore, additional mt genomes, nuclear genes, and more complete taxon sampling will be needed to understand the relationships within Palaeonemertea. In Hoplonemertea, the most species-rich group, *Amphiporus lactifloreus* Johnston, 1825 was reconstructed as sisterspecies to *Amphiporus formidabilis* Griffin, 1898 and *Prosorhochmus claparedii* Keferstein, 1862 as the sister species to *Nemertopsis tetraclitophila* Gibson, 1990. The hoplonemertean relationship conforms with previous phylogenies (Andrade et al. 2012; Kvist et al. 2014, 2015; Chernyshev and Polyakova 2018a). The phylogenetic relationship between the species from France, *L. longissimus*, *L. ruber, L. lacteus*, *R. occultus*, and *L. sanguineus* (Fig. 3), in Lineage H (Chernyshev and Polyakova 2023), generally conforms to previous studies using single genes or transcriptomics (Zattara et al. 2019, Ament-Velásquez et al. 2016, Chernyshev and Polyakova 2023). Considering the close phylogenetic relationship between *R. occultus* and *Lineus*, it may be more appropriate to reclassify *R. occultus* under *Lineus* rather than maintaining it as the sole species of the monotypic genus *Riseriellus* (Kajihara et al. 2022). However, due to the significant challenges in nemertean taxonomy, changes are not easily applied (Kajihara et al. 2022).

Several of the sequenced and analyzed species were not identified to the species level. Genome skimming allowed species determination for only one of these, due to the limited number of correctly classified database entries. Among the three Southern Ocean nemertean species analyzed, only one was assigned to a known species based on sequence data. The two other species had close matches in the database; however, neither of these could be confidently identified to the species level, highlighting significant taxonomic problems. From the Southern Ocean, there have been more than 28 described nemertean species (Gibson 1985; Chernyshev and Polyakova 2023). Many Southern Ocean nemerteans are available only as COI sequences without a proper species identification (e.g., Heimeier et al. 2010). In addition, there is likely a large number of undescribed species inhabiting this region. One nemertean frequently encountered during monitoring expeditions by the Senckenberg Research Institute in the North Sea has remained as uncatalogued and unclassified in museum collections. This species was captured in the Helgoland Deep Trench where the sea is around 50–60 m, which is the deepest point in the North Sea, and considered a biodiversity hotspot (Schwinn et al. 2014). In addition, onboard observations suggest that this colorful species is capable of swimming (Moritz Sonnewald, personal communication). Despite being found in a relatively shallow and highly trafficked marine environment, it was not possible to identify this organism to the species level using DNA sequences. It may be a previously morphologically described species which has not been sequenced or may represent an entirely new species requiring taxonomic description.

The combined analysis of nuclear genes, mt genomes, and genome sizes from three individuals morphologically similar to *L. acutifrons*, discovered along the French coast shows how sequence data and other molecular methodologies can shed light on nemertean evolution. The three individuals are morphologically similar to *L. acutifrons* but likely a new species, *Junoya* sp. , based on sequence data . Analysis of genome size shows that both the new species *Junoya* sp. and *L. acutifrons* have similar genome sizes, around 1.8–1.9 Gb. One individual, however, has a significantly smaller genome size (0.8 Gb), and phylogenetic analysis of 28S and mt genes indicate that it is a distinct maternal lineage.

Based on the genome size, we can hypothesize that this taxon could be one of the parental progenitors of the possibly allopolyploid *L. acutifrons* or *Junoya* sp. . Nemertean hybrids have previously been described. For instance, the hybrid status of the species *Lineus pseudolacteus* Gontcharoff, 1951 was recently confirmed by population genomic and phylogenomic methods (Ament-Velásquez et al. 2016). The extent of hybridization in Nemertea is unexplored, but the results suggest that it may be common even between distantly related species.

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Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval No animal testing was performed during this study.

Sampling and field studies All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements, if applicable. The study is compliant with CBD and Nagoya protocols.

Data availability Sequence data has been submitted to genbank. Mitochondrial genomes: OR387313-21, OR397955-60, OR405543-46, OR419502, OR451556. COI: PV012551-54. 28S: PV012573-75.

Author contribution JD, AB, and DF collected samples; JP, WD, and MN analyzed the data; MN and JD wrote the manuscript, and all authors read and approved the manuscript.

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