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RESEARCH

Relationship of *Hediste* (Annelida, Polychaeta) and the description of the complete mitochondrial genome of *Hediste sinesimplex* Costa et al., 2025, including a status reassessment of some Nereididae species

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Abstract

In this study, we update the phylomitogenomic relationships of *Hediste* (Annelida, Polychaeta, Nereididae), with the inclusion of *Hediste sinesimplex* as a recently described species from the Mediterranean and Northeast Atlantic. Phylogenetic inference using maximum likelihood, based on 13 Protein-Coding Genes (PCGs) from nereidid species, confirms the position of the recently described *H. sinesimplex* within the genus *Hediste* showing a closed relationship with the sympatric *Hediste diversicolor*. We also describe the complete mitochondrial genome of *H. sinesimplex* which is composed by of 15,876 bp genome size, with a GC content of 34.4%, and encodes 13 Protein-Coding Genes (PCGs), 22 transfer RNA (tRNA) genes, and two ribosomal RNA (rRNA) genes. Most PCGs start with the ATG codon and terminate mainly with TAA or TAG. However, three genes present incomplete stop codons: ND5 and ND1 end with T-, whereas *cox1* ends with TA-. These results provide new genomic resources for nereidid systematics and contribute to a better understanding of phylomitogenomic relationships. Finally, our findings to provide a status reassessment of some species of Nereididae based on mitochondrial genes.

Keywords Mitogenome · Nereidinae · Ragworm · Mediterranean Sea · Baltic and North Seas

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Introduction

The species *Hediste sinesimplex* Costa at al. 2025 was recently described, with distribution in the Western Mediterranean Sea (Spain and Italy), and in the Baltic and Eastern North Seas. This polychaete is distinguished by the absence of fused simple chaetae in the posterior supra-acicular neurochaetae, which are replaced by heterogomph falciger chaetae, a condition that possibly represents a microevolutionary phenomenon within the genus *Hediste* (Costa et al. 2025).

The mitochondrial genome of polychaetes are generally a circular molecule ranging in size from ~15–17 kb, and, like other metazoans, usually encode the set of 37 genes, comprising 2 rRNAs, 22 tRNAs, and 13 proteins, e.g. (Chen et al. 2019; Kim et al. 2024; Putignano and Tilic 2025). Mitochondrial phylogenomic (phylomitogenomics), the use of entire or partial mitochondrial genomes (mitogenomes) within a phylogenetic framework, has proven to be more powerful in taxonomic, phylogenetic, population, and evolutionary studies than individual gene sequence comparison (Wang et al. 2017; Winn et al. 2024).

In this way, the main goal of our study is updating the phylomitogenomic relationships of Nereididae (Annelida), focusing on the recently described species of *Hediste*, *H. sinesimplex* and characterize its complete mitogenome. In addition, we evaluated the status reassessment of some Nereididae species.

Materials and Methods

Genomic DNA was extracted from the holotype of *Hediste sinesimplex*, deposited at the Natural History Museum of the Iberian Peninsula, Aquamuseu do Rio Minho (NatMIP-APPh-0019 – BioSample: SAMN52019640, collected in Pisa – Italy, from Costa et al. 2025) and whole genome sequencing with 150 bp Illumina paired-end (PE) reads were performed by Novogene (Cambridge, United Kingdom), (SRA data: PRJNA1335767) (Fig. 1A, B). The mitogenome was assembled *de novo* using NOVOPlasty v.4.3.5 (Dierckxsens et al. 2017), with a k-mer size of 33, genomic range of 12,000 bp to 22,000 bp, read length of 150 bp, and an insert size of 305 bp. A *cox1* sequence available in GenBank (Accession Number PV449855) was used as seed. Mitogenome annotation was performed with MITOS v.2.1.7 (Donath et al. 2019) and subsequently curated with Artemis v.18.2.0 (Carver et al. 2012). The mitogenome was visualised using the online platform OGDRAW v.1.3.1 C (Greiner et al. 2019).

Coverage statistics, including mean depth and per-base coverage, were calculated using samtools depth and plotting through software R (The R Foundation 2026). Codon usage and the Relative Synonymous Codon Usage (RSCU) values for each protein-coding gene (PCG) were estimated based on the invertebrate mitochondrial genetic code using PhyloSuite v1.2.2 (Xiang et al. 2023). The tRNA genes

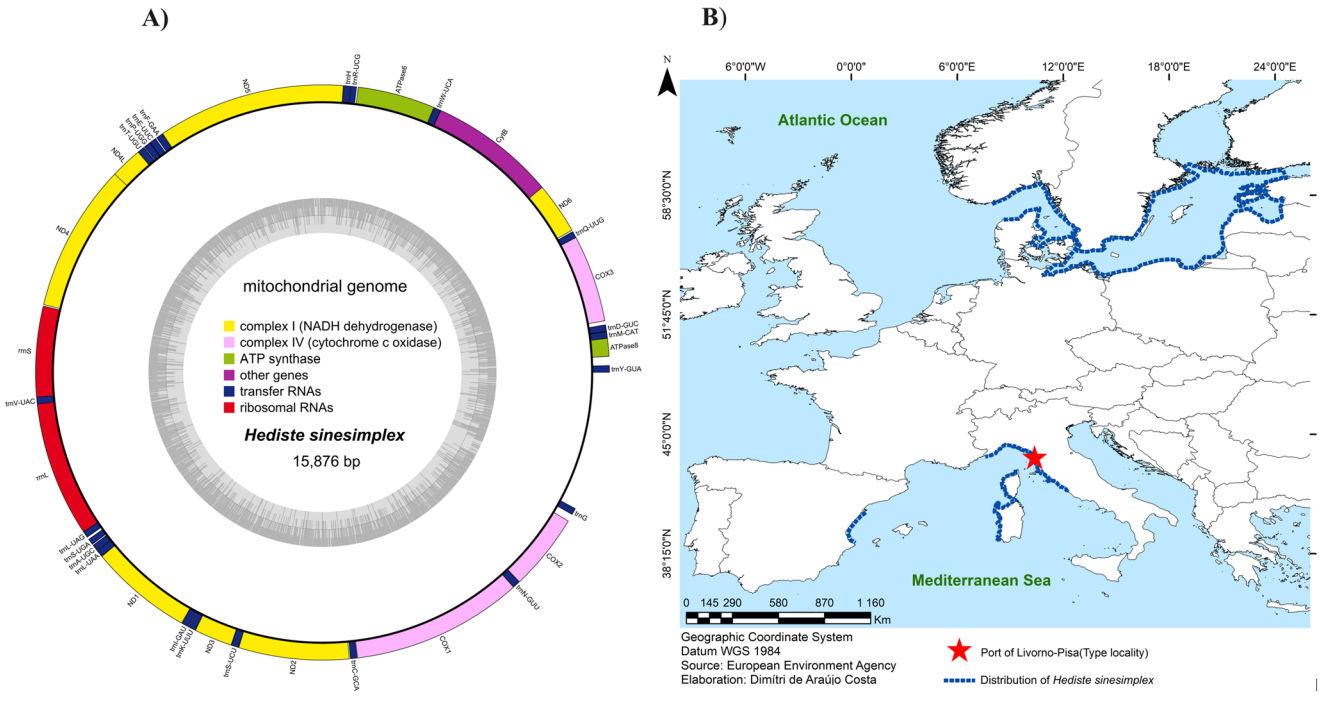

Fig. 1 (**A**) Circular map of the assembled mitogenome of *Hediste sinesimplex* Costa at al. 2025 (GenBank Accession Number PX427683) composed of 13 protein-coding genes (light green, lilac, and yellow), 22 tRNA genes (blue), two rRNA genes (red), and the non-coding control region, which has not been annotated. Read coverage is represented in the inner circle in grey. (**B**) Updated distribution of *H. sinesimplex*, including type locality

(transfer RNA) were identified using ARAGORN v1.2.41 (Laslett and Canback 2004) with default settings for the invertebrate mitochondrial genetic code. Additionally, the secondary structures of the tRNAs were predicted using the ViennaRNA Package 2.0 (Lorenz et al. 2011) and the Forna web server (Kerpedjiev et al. 2015) was used to visualize these structures.

Phylogenetic analyses were conducted using a dataset comprising 15 mitochondrial genes from representatives of the family Nereididae. The selection of taxa followed recent phylomitogenomic studies, which constitute the most comprehensive samples available for the group (Villalobos-Guerrero et al. 2024; Pamungkas et al. 2025). The mitochondrial genome attributed to *Neanthes glandicincta* (Southern, 1921) (GenBank Accession Number KY094478) was excluded from the data matrix as it corresponds to a previously recognised case of misidentification (Villalobos-Guerrero et al. 2024).

In addition to the taxa used in these studies, seven further mitochondrial genomes not included in previous analyses were incorporated, as well as the newly sequenced mitochondrial genome of *Hediste sinesimplex* (Table S1). In total, 35 mitochondrial genome sequences belonging to species of the family Nereididae Blainville, 1818 were compiled. Sequence alignment was performed using the SPLACE software (Oliveira et al., 2022) and subsequently checked manually in BioEdit v. 7.7.1.0 (Hall, 2021) to ensure the quality of the alignment. The species *Goniada japonica* Izuka, 1912 (GenBank Accession Number KP867019) was used as the outgroup.

To increase the accuracy of phylogenetic inference, we excluded start and stop codons from all protein-coding genes (PCGs) in strand H, due to their highly conserved structure, as well as the third position of the codon, due to the high variability that can generate synonymous mutations with no functional impact (Näsvall et al. 2023; Wesp et al. 2023). The alignment of the PCGs of strand H was then separated into first and second codon positions. For ribosomal subunits, sites containing gaps or ambiguous alignments were removed using trimAl v.1.2 (Capella-Gutiérrez et al. 2009), with the command "-automated1". Segments of the rRNA units and PCGs of strand H were concatenated in PhyloSuite v.1.2.3 (Xiang et al. 2023). The rRNA subunits and the first and second codon positions were defined as independent partitions. The best partition scheme and the most appropriate evolutionary models were estimated with ModelFinder 2 (Kalyaanamoorthy et al. 2017), using the Bayesian Information Criterion (BIC) (Minin et al. 2003). As a result, the following models were selected: rRNAs (GTR + F+I+G4), first codon position (GTR + F+I+G4), and second codon position (TVM + F+R5).

Phylogenetic inference by Maximum Likelihood (ML) was performed in IQ-TREE v.3 (Wong et al. 2025). Branch support was assessed using 1,000 ultrafast bootstrap replicates with 1,000,000 interactions (Minh et al. 2013; Hoang et al. 2018) and the Bayesian approximation test (aBayes) (Anisimova et al. 2011). Bayesian inference (BI) was conducted in BEAST v.1.8 (Drummond and Rambaut 2007). For this analysis, the GTR + F+I+G4 evolutionary model was applied to the three partitions, as estimated by ModelFinder 2 (Kalyaanamoorthy et al. 2017). The molecular clock was set as lognormal, with the substitution rate estimated from the data itself (clock model: estimate), and the Yule process was used as the speciation prior. Two independent chains were run, each with 60 million generations, with sampling every 60,000 generations and initial burn-in of 25% of the samples. Parameter convergence and estimate quality were assessed in Tracer v.1.7.1 (Rambaut et al. 2018), considering ESS values greater than 200. The resulting trees were summarized in TreeAnnotator v.2.4.8 (Bouckaert et al. 2019). Phylogenetic trees were visualised and edited in iTOL web server (Letunic and Bork 2024). Genetic similarity between mitogenomes was assessed using BLASTN (Nucleotide BLAST) tool within the Basic Local Alignment Search Tool (BLAST) package (Altschul et al. 1990), comparing the sequences against the NCBI (National Center for Biotechnology Information) nucleotide database.

Results

The complete mitochondrial genome of *Hediste sinesimplex* (GenBank Accession Number PX427683) has a total length of 15,876 bp and a GC content of 34.4% bp, comprising 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes, and an unannotated control region (D-Loop), all encoded on the same strand (Fig. 1A).

The length, gene composition, and single-stranded orientation are as expected for polychaetes of the family Nereididae. All 13 protein-coding genes (PCGs) were initiated with the standard start codon ATG. Eigth genes terminated with the stop codon TAA (*ATPase8*, *cox3*, *CytB*, *ATPase6*, *ND4L*, *ND3*, *ND2*, *cox1* and *cox2*), two genes used the alternative stop codon TAG (*ND6* and *ND4*), and three genes terminated with an incomplete stop codon T- (*ND5*, *ND1*) and TA- (*cox1*) (Table 1). The average sequencing coverage was 34 times. The mitochondrial genome of *H. sinesimplex* has a high A + T content (65%), comprising 33.5% T and 32.0% A, whilst C and G occur in lower proportions, at 20.0% and 14.4%, respectively. The mitochondrial tRNA genes had a total length of 1396 bp, varying individually between 59

Table 1 Gene composition of the complete mitochondrial genome of *Hediste sinesimplex* Costa et al. 2025, detailing gene position, length, anticodon, start and stop codons, and transcriptional direction

Gene name	Start	Stop	Length (bp)	AntiCodon	Start codon	Stop codon	Strand
tRNA-Tyr	1	65	65	GAT	-	-	*Forward*
ATPase8	138	302	165		ATG	TAA	*Forward*
tRNA-Met	298	360	63	CAT	-	-	*Forward*
tRNA-Asp	360	422	63	GTC	-	-	*Forward*
cox3	454	1236	783		ATG	TAA	*Forward*
tRNA-Gln	1232	1295	64	TTG	-	-	*Forward*
ND6	1305	1769	465		ATG	TAG	*Forward*
CytB	1762	2898	1137		ATG	TAA	*Forward*
tRNA-Trp	2897	2959	63	TCA	-	-	*Forward*
ATPase6	2960	3655	696		ATG	TAA	*Forward*
tRNA-Arg	3666	3719	54	TCG	-	-	*Forward*
tRNA-His	3720	3783	64	GTG	-	-	*Forward*
ND5	3784	5464	1681		ATG	TAT	*Forward*
tRNA-Phe	5465	5528	64	GAA	-	-	*Forward*
tRNA-Glu	5536	5599	64	TTC	-	-	*Forward*
tRNA-Pro	5598	5661	64	TTG	-	-	*Forward*
tRNA-Thr	5664	5729	66	TGT	-	-	*Forward*
ND4L	5730	6017	288		ATG	TAA	*Forward*
ND4	6011	7339	1329		ATG	TAG	*Forward*
rrnS	7348	8158	811		-	-	*Forward*
tRNA-Val	8152	8216	65	TAC	-	-	*Forward*
rrnL	8201	9457	1257		-	-	*Forward*
tRNA-Leu	9418	9480	63	TAG	-	-	*Forward*
tRNA-Ser	9499	9557	59	TGA	-	-	*Forward*
tRNA-Ala	9564	9626	63	TGC	-	-	*Forward*
tRNA-Leu	9627	9688	62	TAA	-	-	*Forward*
ND1	9689	10,613	925		ATG	TAT	*Forward*
tRNA-Ile	10,614	10,678	65	GAT	-	-	*Forward*
tRNA-Lys	10,680	10,745	66	TTT	-	-	*Forward*
ND3	10,746	11,090	345		ATG	TAA	*Forward*
tRNA-Ser	11,089	11,156	68	TCT	-	-	*Forward*
ND2	11,157	12,146	990		ATG	TAA	*Forward*
tRNA-Cys	12,152	12,213	62	GCA	-	-	*Forward*
cox1	12,215	13,749	1535		ATG	ATA	*Forward*
tRNA-Asn	13,750	13,814	65	GTT	-	-	*Forward*
cox2	13,815	14,507	693		ATG	TAA	*Forward*
tRNA-Gly	14,570	14,633	64	TCC	-	-	*Forward*

and 68 bp. The ribosomal (rRNA) genes totalled 2068 bp, comprising 811 bp for rrnS and 1257 bp for rrnL. (Table 1).

The coverage depth across the *H. sinesimplex* mitochondrial genome was uniform, with an average of approximately 37× and moderate variations between genes (Fig. 2A). No regions without coverage or abrupt drops were observed, indicating high reliability in the assembly of the mitochondrial genome. Thus, the RSCU analysis comparing the species with available mitochondrial genomes for *Hediste* revealed that the 13 protein-coding genes (PCGs) exhibit similar patterns across the four analysed species (*H. sinesimplex*, *Hediste diversicolor* (Müller, 1776), *Hediste diadroma* Sato & Nakashima, 2003 and *Hediste japonica* (Izuka, 1908), with comparable relative frequencies across all amino acids (Fig. 2B). Isoleucine exhibited the highest percentage values in all species, ranging approximately between 9.5% and 10.0% of the total. High values were also observed for leucine (Leu1), threonine, alanine, serine (Ser2), valine and glycine, with average frequencies of approximately 5% to 8%. Proline also showed relatively high values, close to 4.5%–4.7% across the four species.

However, the lowest percentages were observed for cysteine, aspartic acid, arginine and tryptophan, all with frequencies close to or below 3%, with cysteine having the lowest value of all amino acids, at around 1%. Aspartic acid and arginine had values of around 1.7%–1.9%. Glutamine, glutamate and lysine also showed low relative frequencies, close to 2%. The codons UUA, UUG, CUU, CUA and

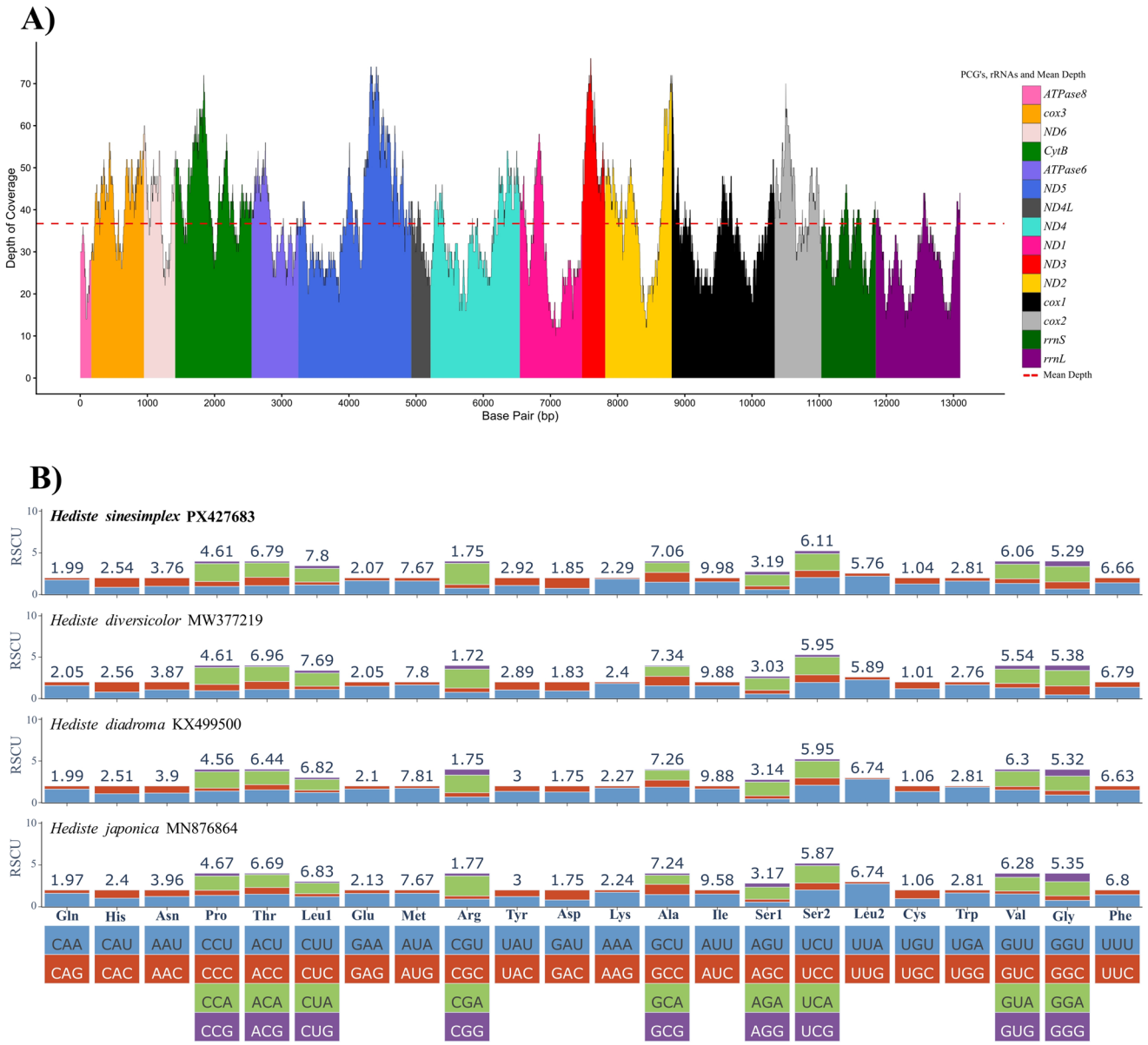

Fig. 2 (**A**) Sequencing coverage of the genes used in the analyses of this study, including phylogenetic inferences and Relative Synonymic Codon Usage (RSCU) analysis. (**B**) Relative Synonymic Codon Usage (RSCU) plots for representatives of the genus *Hediste*

CUG were widely used, reflecting the strong bias towards A- and U-rich codons (Fig. 2B).Twenty-two tRNA genes were identified in the mitochondrial genome analysed, with lengths ranging from 54 to 68 bp. Most tRNAs exhibited the typical cloverleaf secondary structure, comprising the acceptor arm, DHU stem (D arm), anticodon and TΨC, with well-defined stems and clearly delineated terminal loops. Nevertheless, structural variations were observed in some tRNAs, particularly in the serine (TGA and TCT anticodons) and arginine (TCG) genes, which lacked a DHU stem, forming an elongated D-loop (Fig. 3).

Base pairings in the stems were predominantly canonical (A–U and G–C), although non-canonical wobble-type base pairings (G–U) were also observed in different regions, contributing to structural stability even in reduced conformations. The anticodon loop was clearly identified in all tRNAs, highlighting the functional conservation of these molecules. Among the identified genes, the two leucine isoacceptors (TAA and TAG) and two serine isoacceptors (TGA and TCT) were observed (Fig. 3).

Our phylomitogenomic analyses based on 15 mitochondrial genes (Fig. 4), performed using BI and ML identified two major, well-supported clades in the mitochondrial phylogeny, although with some minor differences in topology (Fig. 4). Clade I showed high support in the Bayesian analysis (PP = 1) and moderate support in the ML analysis

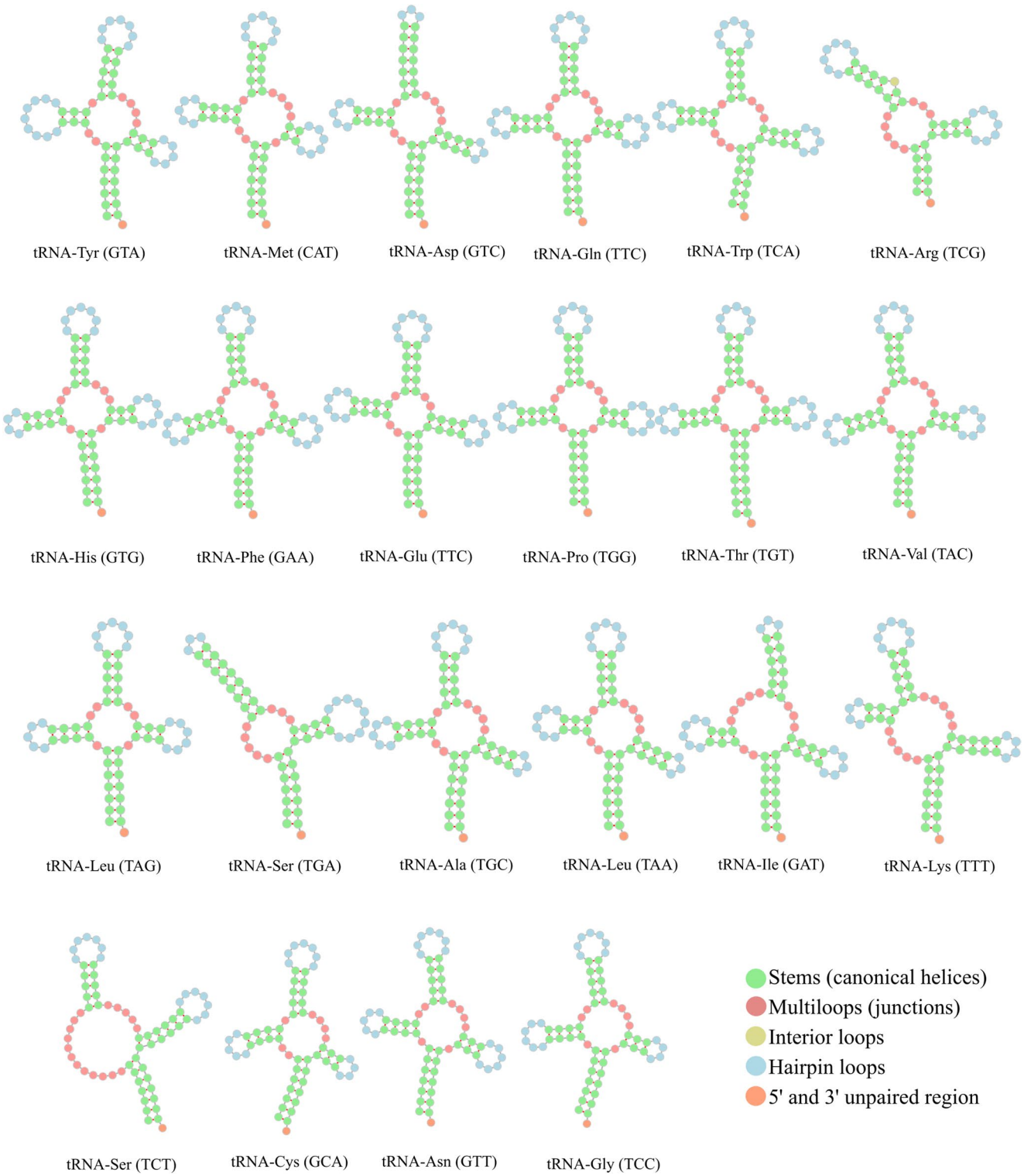

Fig. 3 Presumed secondary structure of 22 tRNAs identified in the *Hediste sinesimplex* mitochondrial genome. The tRNAs are listed in the order in which they appear in the mitochondrial genome, starting with tRNA-Thr. The anticodons are shown in brackets after the name of each tRNA

Fig. 4 Bayesian inference (**A**) and Maximum likelihood (**B**) phylogeny of *Hediste sinesimplex* Costa et al. 2025 and all other species of *Nereididae* with available mitogenomes, reconstructed from 13 mitochondrial PCGs and two ribosomal RNA (rRNA) genes. Only support values ≥ 0.95 for posterior probabilities (BI) and ≥ 80% for Maximum Likelihood (aBayes/UFBoot2) are shown

(aBayes/UFBoot2 = 1/91), comprising *Paraleonnates uschakovi* Chlebovitsch & Wu, 1962 as the sister group to Dendronereidinae, Namanereidinae and Tylorrhynchinae (Fig. 4A). Nonetheless, this species was not recovered within this clade in the ML analysis (Fig. 4B), indicating instability in the phylogenetic position of this taxon, highlighted with a blue line (Fig. 4).

In Clade II, the Bayesian analysis (PP = 1; Fig. 4A) placed Nereidinae as a paraphyletic group, as *P. uschakovi* was not included in this clade. In contrast, the ML analysis supported the monophyly of Nereidinae, placing *P. uschakovi* as the sister group to the other members of the subfamily (aBayes/UFBoot2 = 1/98; Fig. 4B). In both topologies (IB and ML), the other subfamilies, Dendronereidinae, the recently proposed Tylorrhynchinae, and Namanereidinae [represented solely by *Namalycastis abiuma* (Grube, 1872)] were recovered as monophyletic, with high support (PP = 1; aBayes/UFBoot2 = 1/≥ 95% on all branches) (Fig. 4). The subfamily Gymnonereidinae was not included in the present analysis due to the lack of available mitogenomes. We also observed differences in the phylogenetic position of *Laeonereis* cf. *pandoensis* (Monro, 1937) across the topologies. In the Bayesian analysis, this species was recovered with high support (PP = 1) as the sister group to the other Nereidinae species in Clade II, except for *P. uschakovi* (Fig. 4A). Conversely, in the ML analysis, *L.* cf. *pandoensis* was positioned as the sister group to the *Hediste* spp. Although the node showed high support in the aBayes test (0.99), the UFBoot2 value of less than 80% indicates low support, suggesting uncertainty in this phylogenetic relationship (Fig. 4B).

In addition, the mitochondrial genome attributed to *Cheilonereis cyclurus* (Harrington, 1897), as described by Park et al., (2017), likely corresponds to a *Nereis* species; in our analyses, for example, this species was recovered within a well-supported monophyletic subclade together with of *Nereis* spp. (PP = 1; aBayes/UFBoot2 = 1/91). Likewise, we have detected that the mitochondrial genome attributed to *Perinereis aibuhitensis* (Grube, 1878) by Kim et al. (2015) likely corresponds to *Perinereis linea* (Treadwell, 1936). The position of *H. sinesimplex* was consistent in both phylomitogenomic analyses, being highly supported (PP = 1; aBayes/UFBoot2 = 1/100) and always recovered as the sister species of *H. diversicolor*, within a monophyletic

subclade of the genus *Hediste* (Fig. 4A–B). Sequence similarity between the mitogenome of *H. sinesimplex* and other *Hediste* spp. ranged from 85 to 93%, whereas similarity with other analysed nereidid species was <80%.

Discussion

The mitochondrial genome of the recently described species *H. sinesimplex* (Costa et al. 2025), presented in this study, is the fourth mitochondrial genome sequenced for the genus *Hediste*, which currently comprises nine valid species (Read and Fauchald 2026). Overall, the mitochondrial genome exhibited characteristics consistent with those previously described for Nereididae, including a total length of 15,876 bp, a GC content of 34.4% and a high A+T content (65%), values that fall within the known range for polychaete mitochondrial genomes. Among the family's representatives, *Laeonereis culveri* (Webster, 1879), treated here as *L.* cf. *pandoensis* based on Villalobos-Guerrero et al. (2024), has the smallest mitogenome (14,918 bp) and the lowest GC content (26.2%). In contrast, the largest available mitogenome belongs to *H. diadroma* – 17,765 bp (Kim et al. 2016), whilst the highest GC content (38.6%) was recorded in *N. abiuma* (Lin et al. 2016) (see Table S1).

The start and stop codons identified in the protein-coding genes of *H. sinesimplex*, including incomplete stop codons (TA- and T-), follow the pattern commonly reported for Nereididae mitogenomes (Kim et al. 2016; Chen et al. 2016; Park et al. 2020; Gomes-dos-Santos et al. 2021; Villalobos-Guerrero et al. 2024; Pamungkas et al. 2025). These incomplete codons are subsequently completed by post-transcriptional polyadenylation, resulting in functional stop signals (Ojala et al. 1981). Furthermore, the gene order observed in *H. sinesimplex* falls within group 1 proposed for Nereididae (Park et al. 2016), indicating that the mitochondrial architecture of this species is conserved and consistent with the pattern previously described for the family (Fig. 1A).Analysis of the relative usage of synonymous codons (RSCU) (Fig. 2B) showed that the 13 protein-coding genes exhibit similar patterns across *Hediste* species, indicating compositional stability and reflecting the strong A+T bias typical of the Nereididae. This pattern, also observed in other Nereididae (Kim et al. 2015; Seixas et al. 2016; Park et al. 2020; Gomes-dos-Santos et al. 2021), is associated with a preference for codons ending in A or U, such as UUA, UUG, CUU, CUA and CUG, and with the predominance of amino acids such as isoleucine, leucine, threonine and alanine, consistent with this bias; this preference for codons ending in A or U is described for mitochondrial genomes of nemertines, suggesting that mutational and selective forces likely act on codon usage bias (Chen et al. 2014).

Most tRNA genes exhibited the typical cloverleaf secondary structure, indicating that the expected functional pattern for these molecules is maintained. The main exceptions occurred in the serine (Ser1 and Ser2) and arginine tRNAs, which lacked a DHU stem (D-arm), forming more simplified structures (Fig. 3). This type of modification is documented in the mitochondrial genomes of annelids (Boore 2001; Jennings and Halanych 2005; Bleidorn et al. 2006; Zhong et al. 2008) and does not appear to compromise the functionality of these molecules, particularly as the canonical and wobble-type base pairings observed in the stems likely contribute to structural stability. Although it is believed that this DHU stem acts as a recognition site for aminoacyl-tRNA synthetase (Clark 2006). However, both the pattern of codon usage and the organisation of the tRNAs reinforce the idea that the mitogenome of *H. sinesimplex* is structurally conserved and evolutionarily compatible with the other known representatives of *Hediste*.

From a phylogenetic perspective, our phylomitogenomic analyses identified two major clades within the Nereididae (Fig. 4A–B), a pattern also observed in recent studies (Alves et al. 2020, 2023; Villalobos-Guerrero et al. 2024; Pamungkas et al. 2025). This result reinforces the usefulness of complete mitogenomes for reconstructing evolutionary relationships within the family, whilst confirming a broad phylogenetic structure. Nevertheless, Bayesian and Maximum Likelihood analyses were not entirely consistent regarding some internal relationships, as also reported in recent studies on Nereididae (Villalobos-Guerrero et al. 2024; Pamungkas et al. 2025), indicating that certain nodes remain sensitive to the inference method, the set of sampled taxa, and possibly the nature of the mitochondrial signal itself (Yang et al. 2024).

The main inconsistencies observed in our phylogenetic analyses concerned the position of *P. uschakovi* and *L.* cf. *pandoensis*. Depending on the method, *P. uschakovi* was sometimes placed close to Clade II, and sometimes as a sister group to Nereidinae, a pattern already observed in previous studies using different datasets and analytical methods (Villalobos-Guerrero et al. 2024; Yang et al. 2024; Pamungkas et al. 2025). This inconsistency suggests that the position of *P. uschakovi* remains unresolved, possibly due to the limitations of the data currently available for this genus. Morphologically, the genus *Paraleonnates* is characterised by the presence of spines on the maxillary proboscis ring, dorsal and ventral transverse rows of paragnaths, and papillae in the dorsal and ventral positions of the oral ring (Hong et al. 2012).

The species *L. culveri* represents a case of misidentification, as the available mitochondrial genome likely belongs to *L.* cf. *pandoensis* (Villalobos-Guerrero et al. 2024), whose correspondence with MOTU 7 and wide distribution across

the western South Atlantic is suggested by DNA barcoding data (Sampieri et al. 2021). Its position within the Nereidinae is uncertain based on our analyses (Fig. 4A–B). We partly agree with Yang et al. (2024) that, based on the characteristics of the pharyngeal armour (Wu et al. 1981), the hypothesis is suggested that *Paraleonnates* and *Laeonereis* occupy early positions in the diversification of Nereidinae, this is reflected in our ML phylogeny (Fig. 4B), but it is also possible that *Paraleonnates* lies well outside the boundaries of Nereidinae (Villalobos-Guerrero et al. 2024; Pamungkas et al. 2025), and may be associated with a subfamily not yet described; therefore, considering this body of evidence, we suggest that the status of these two genera within Nereididae should be re-evaluated on the basis of a comprehensive review, integrating morphological and molecular data from all taxa currently assigned to the genera.

In contrast, the monophyly of Dendronereidinae, Tylorrhynchinae and Namanereidinae was consistently recovered with high support in our analyses. This result reinforces the phylogenetic stability of these subfamilies and supports, in particular, the delimitation of the recently proposed Tylorrhynchinae (Pamungkas et al. 2025). Our results also corroborate hypotheses of misidentification in some mitogenomes available in public databases. The genome attributed to *C. cyclurus* by Park et al. (2017) was recovered within a monophyletic clade of *Nereis*; this species was initially described as *Nereis cyclurus* and redescribed as *C. cyclurus*, based solely on morphological data (Bakken and Wilson 2005) (see Fig. 4A-B), reinforcing the interpretation that this sequence likely corresponds to a misidentified species of *Nereis*. Similarly, the mitochondrial genome attributed to *P. aibuhitensis* by Kim et al. (2015) likely corresponds to *P. linea*, as suggested by Yang et al. (2024) (see Fig. 4A-B). These cases illustrate how comparative phylomitogenomic analyses can be useful not only for inferring evolutionary relationships, but also for detecting taxonomic inconsistencies in molecular databases, reinforcing the need for careful curation of sequences and the availability of raw data for independent validation (Yang et al. 2024).

With specific regard to the genus *Hediste*, the position of *H. sinesimplex* was stable in both analyses and received strong support, consistently being recovered as the sister species of *H. diversicolor* (Fig. 4A–B), corroborating with Costa et al. (2025). The mitogenomic similarity of 85–93% between *H. sinesimplex* and its congeners reinforces this proximity, whilst maintaining its specific distinction. Furthermore, the sympatric occurrence of *H. sinesimplex* (Fig. 1B), *H. diversicolor* and *H. astae* in the Baltic Sea, previously treated as part of the same species complex, provides further evidence that this group harbours previously underestimated cryptic diversity (Costa et al. 2025).

Morphologically, *H. sinesimplex* is distinguished primarily by the replacement of fused simple chaetae with heterogomph falcigers on the supra-acicular neurochaetae of the posterior parapodia (Costa et al. 2025). Nevertheless, this species shares with the other *Hediste* spp. the diagnostic synapomorphies, such as the presence of supra-acicular homogomph falcigers on the posterior parapodia and the absence of paragnaths in position V of the proboscis (Costa et al. 2025). In this way, the congruence between mitogenomic and morphological evidence reinforces the taxonomic validity of *H. sinesimplex* and confirms its placement within the genus *Hediste*.

Conclusion

The mitogenome of *H. sinesimplex* expands the genomic knowledge available for the Nereididae, exhibiting structural, compositional and organisational characteristics consistent with the conserved pattern of the family. Phylomitogenomic analyses confirm the species' position within *Hediste* and its close relationship with *H. diversicolor*, whilst highlighting the utility of complete mitochondrial genomes for reconstructing evolutionary relationships within the Nereididae. Although the family still lacks mitogenomes representative of the subfamily Gymnonereidinae, limiting a more comprehensive phylomitogenomic assessment, the results obtained here provide a framework for future investigations. We highlight that the phylogenetic instabilities involving *Paraleonnates* and *Laeonereis*, associated with possible misidentifications in public databases, underscore the need for an integrative taxonomic revision. In addition, our results reinforce the importance of careful sequence curation and the availability of raw data to ensure the reliability of phylogenetic inferences. Taken together, this study contributes to the refinement of evolutionary relationships within Nereididae and highlights the need for integrative approaches that combine mitogenomic, nuclear and morphological data to achieve a more complete understanding of the group's diversity and evolution.

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Data Availability The genome sequence data of *Hediste sinesimplex* Costa, Rocha, Martins, Venâncio, Ureña, Almeida, Ferreira, Christoffersen & Antunes, 2025 that support the findings of this study are openly available in GenBankNCBI (https://www.ncbi.nlm.nih.gov/) under the accession no. PX427683. The associated BioProject, BioSample and SRA numbers were PRJNA1335767, SAMN52019640 and SRR35653029, respectively.

Declarations

Competing interests The authors declare no competing interests.

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