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Unrecognized species-level diversity of terrestrial nemerteans in the UNESCO world heritage Ogasawara Islands revealed by mitogenomics

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Abstract

Background The terrestrial ribbon worm *Geonemertes pelaensis* Semper, 1863 (phylum Nemertea) is widely reported from tropical regions worldwide. In Japan, this species has been recorded from subtropical islands including the Ogasawara Islands, a UNESCO World Heritage Site south of Tokyo recognized for its unique biodiversity, where it has been implicated in the decline of native soil invertebrates. Here, we demonstrate that the nemerteans in the Ogasawara Islands are genetically and morphologically distinct from those found on Yonaguni Island (Okinawa, Japan), indicating the presence of at least two separate species in Japan.

Results We sequenced the complete mitochondrial genomes of both populations (18,755 bp for Ogasawara; 31,745 bp for Yonaguni), revealing substantial differences in genome size and gene arrangement. The mitochondrial genome of the Yonaguni population is unusually large, exceeding typical sizes reported for metazoans. Uncorrected *p*-distances in cytochrome *c* oxidase subunit I (*COX1*) sequences between the two populations ranged from 6.75 to 8.59%, which is above the widely used threshold for intraspecific variation in nemerteans. Morphological comparisons also support species-level distinction: live specimens from Yonaguni have a pale body with a prominent mid-dorsal stripe (body width-to-stripe ratio: 1:0.078–0.110), whereas individuals from Ogasawara are pale to light brown with a narrower and fading stripe (ratio: 1:0.042–0.050). Moreover, accessory-stylet pouches differ between populations: Yonaguni specimens possess four to five pouches, each containing 3–5 stylets, while Ogasawara specimens have two pouches, each with two stylets. Examination of museum specimens collected in the 1980s from Chichijima showed the extremely similar external morphology as our recent Ogasawara specimens, indicating that this form has been the only *Geonemertes* species in the Ogasawara Islands for nearly half a century.

Conclusions Our results indicate the presence of species-level diversity in Japanese terrestrial nemerteans and demonstrate that accurate species identification using molecular barcodes is essential in insular ecosystems.

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Recognizing cryptic or pseudocryptic lineages is critical for effective biodiversity monitoring and for preventing mismanagement in ecologically sensitive regions such as the Ogasawara Islands.

Keywords *Geonemertes pelaensis*, Alien species, Pseudocryptic, Conservation, Endemic

Background

Oceanic islands are globally recognized for their high levels of endemism and evolutionary distinctiveness, yet they are particularly vulnerable to biological invasions [1]. The Ogasawara Islands or the Bonin Islands, a group of more than 30 oceanic islands located approximately 1,000 km south of mainland Japan, were designated a UNESCO World Heritage Site in 2011 due to unique ecosystems and its evolutionary processes including biogeographic isolation [2, 3]. Prolonged spatial isolation has led to high endemism, particularly among land snails, where adaptive radiation has resulted in remarkable species diversification within the islands. However, like other oceanic islands such as the Galápagos, Aldabra, and Lord Howe, the Ogasawara Islands have experienced severe ecological disruptions caused by invasive species [3]. Introduced species—including goats, rats, cats, green anoles, and terrestrial flatworms—have driven the decline or extinction of many native organisms (i.e. plants, birds, bees, and land snails) and have disrupted a delicate ecosystem in the Ogasawara Islands [4–7]. These impacts are exacerbated by the inherent fragility of insular ecosystems and the frequent difficulty in distinguishing native from introduced species [8]. In particular, cryptic or morphologically similar species can obscure biogeographic origins, potentially leading to inappropriate conservation measures, such as the inadvertent protection of invasive species or the failure to safeguard endangered endemics [9].

Nemerteans (phylum Nemertea) are mostly marine predators, with approximately 1,350 described species [10]. They are characterized by an eversible proboscis used to capture prey and occasionally to escape predators [11, 12]. While predominantly benthic marine animals, a few nemertean species inhabit freshwater or terrestrial environments [13]. Although often small, cryptic, and not abundant, nemerteans play important ecological roles as predators [14–16]. At both species and higher taxonomic levels, the necessity of molecular approaches (e.g., DNA barcoding) to reveal cryptic diversity in nemerteans has become increasingly evident over the past decade [17–24]. Many nemertean taxa once thought to be cosmopolitan have turned out to be complexes of cryptic or pseudocryptic species.

The genus *Geonemertes* Semper, 1863 once encompassed all known terrestrial nemerteans [11]. Subsequent taxonomic revisions based on external/internal morphology have narrowed the genus to include *G. pelaensis* Semper, 1863 (the type species), *G. rodericana* (Gulliver, 1879), and *G. philippinensis* Gibson & Moore, 1998. *Geonemertes arboricola* Punnett, 1907, described from the Seychelles, is now regarded as a junior synonym of *G. pelaensis* [25]. These species can be grouped into two general forms based on body coloration: those with a dark background (*G. rodericana*, *G. philippinensis*) and those with a pale background (*G. pelaensis*, *G. arboricola*). While *G. rodericana* is characterized by a white mid-dorsal stripe on dark green, and *G. philippinensis* by a cream stripe on dark brown, *G. pelaensis* and *G. arboricola* exhibit a pale background with a mid-dorsal brown stripe [26]. In *G. pelaensis* occasional faint lateral stripes have been reported [26]. Specimens of *G. pelaensis* from Papua New Guinea deviate from this typical pattern, showing four dark stripes, with lateral stripes joining anteriorly [26, 27]. Despite such morphological variation, no prior study has rigorously examined the color pattern diversity of *G. pelaensis*-like forms using molecular methods. This raises the possibility that what has been referred to as "*G. pelaensis*" may in fact represent a complex of multiple distinct species.

Geographic distributions of *Geonemertes* species are typically limited to small areas on remote oceanic islands [26, 28]. An exception is *G. pelaensis*, which was originally described from Palau (Pelew Islands) [29] and has since been reported—based on morphology—across the Indian, Pacific, and Atlantic Oceans. Recent DNA barcoding using cytochrome *c* oxidase subunit I (*COX1*) sequences has confirmed the wide distribution of *G. pelaensis*-like forms, with identical haplotypes detected in Cuba, Bermuda, and tropical islands in Japan (Minamidaito and Okinawa islands; [30]). One possible explanation for this broad distribution is the species' reproductive strategy: *G. pelaensis* is a cyclic hermaphrodite, a trait associated with high fecundity and dispersal potential [31, 32]. By contrast, its congeners *G. rodericana* and *G. philippinensis* are dioecious, possibly limiting their ability to spread [26, 33].

In Japan, *G. pelaensis* is the only reported land nemertean, with morphological records from subtropical islands such as Okinawa and the Ogasawara archipelago [13, 34–37]. While *G. pelaensis* has been reported to feed on terrestrial snails in the Seychelles Islands [38], recent observations from the Ogasawara Islands show that the local population is a voracious predator of isopods and other soil invertebrates [39, 40]. These differences in feeding ecology may reflect species-level divergence among geographically separated populations. Indeed, some reports have already questioned the identification of the Ogasawara nemerteans as *G. pelaensis*, and have described new ecological characteristics, although these are not based on molecular data [40–42]. In this study, we investigate the taxonomic identity of *Geonemertes* specimens collected from the Ogasawara Islands and compare them with specimens from other localities in Pacific and western Atlantic regions. We also examined *Geonemertes* specimens collected in the 1980s, deposited in museum collections by Prof. Dr. Masanori Kawakatsu, which provide the earliest evidence of the occurrence of *G. pelaensis* in the Ogasawara Islands (e.g. [39]). Using an integrative approach—combining *COX1*-based phylogenetic analysis, complete mitochondrial genome sequencing, and morphological examination for fresh and museum specimens—we assess whether the Ogasawara species represents a distinct lineage.

Materials and methods

Fieldwork, specimen collection, and morphological observation

Geonemertes specimens were collected from the Ogasawara Islands (Chichijima and Hahajima), Tokyo, and Yonaguni Island, Okinawa, Japan, between 2022 and 2025. Living specimens were photographed using digital cameras (Nikon D5600 and Z50II, Japan). For anesthetization, specimens were placed on a piece of paper towel moistened with a few drops of bittern (Tenpi Nigari, Amashio, Japan) and observed under a light microscope by gently squeezing them between a glass slide and a cover slip. Following internal morphological observation, relaxed specimens were cut into two fragments using a razor blade: the proboscis or posterior portion (approximately 3 mm in length) was preserved in 99% ethanol for DNA extraction, while the remaining part was fixed in formalin or preserved whole in 99% ethanol. In addition, we examined archival specimens deposited at the Naturalis Biodiversity Center (formerly Rijksmuseum van Natuurlijke Historie, RMNH), Leiden, the Netherlands, originally collected by Prof. Dr. Masanori Kawakatsu in the 1980s from Chichijima, Ogasawara Island. A specimen of *Malacobdella japonica* Takakura, 1897 was collected from inside the mantle cavity of *Pseudocardium sachalinense* (Schrenck, 1862), which were obtained at the local market in Kashima, Ibaraki, Japan. The sucker tissue was preserved in 99% ethanol for DNA extraction.

DNA extraction, PCR amplification, and electrophoresis

Total DNA was extracted from body wall or proboscis muscle tissues to avoid contamination from gut contents, using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Polymerase chain reaction (PCR) amplification was performed using TaKaRa Ex Taq (Takara Bio, Japan) on a Thermo Fisher Veriti 96-Well Thermal Cycler (Thermo Fisher Scientific, USA) under the following conditions: initial denaturation at 94 °C for 2 min; followed by 37 cycles of 94 °C for 40 s, 52 °C for 60 s, and 72 °C for 60 s; with a final extension at 72 °C for 7 min. The "Folmer region" in the cytochrome *c* oxidase subunit I gene (*COX1*)—the universal DNA barcoding region for metazoan [43]—was amplified using the primer pair LCO1490/HCO2198 [43]. PCR products were checked by electrophoresis on E-Gel EX Double Comb 2% Agarose Gels (Thermo Fisher Scientific, USA). *COX1* amplification was successful for the Yonaguni specimens, but all PCRs failed for specimens from the Ogasawara Islands.

Shotgun sequencing and mitochondrial genome assembly

To obtain the complete mitochondrial genome and design specific *COX1* primers for the Ogasawara specimens, total DNA from one individual from Chichijima (Ogasawara) and one from Yonaguni was subjected to shotgun sequencing using next-generation sequencing (NGS). The protocol largely followed Hiruta et al. (2022) [44]. DNA concentration was quantified using Qubit 4 Fluorometer with a dsDNA HS Assay Kit (Thermo Fisher Scientific, USA). Since the EDTA in AE buffer (Qiagen, Germany) can inhibit enzymatic reactions during library preparation, the buffer was replaced with EB buffer using SeraPure beads [45]. Library preparation was performed using the Collibri ES DNA Library Prep Kit for Illumina Systems with UD Indexes (Thermo Fisher Scientific, USA), using 20 ng of input DNA. DNA fragmentation was conducted at 37 °C for 10 min to obtain fragments ranging from 300 to 800 bp. The library was amplified for 12 cycles. Quantification and quality assessment were conducted using Qubit 4 and a TapeStation 4200 (Agilent Technologies, USA) with a D1000 DNA Assay Kit. Libraries were pooled and sent to Macrogen Japan for sequencing on an Illumina HiSeq X system to generate 150 bp paired-end reads.

In addition, the complete mitochondrial genome of *M. japonica* was determined for a mitogenome-based phylogenetic analysis. The total genomic DNA solution was sent to Macrogen Japan for shotgun library preparation and sequencing. The final libraries were sequenced on an Illumina NovaSeq 6000 system to generate 150 bp paired-end reads. Acquired fastq files were divided into tenths using SeqKit v2.10.0 [46], and only one pair of reads was used for subsequent analyses.

Complete mitochondrial genomes were assembled using GetOrganelle v1.7.5.2 [47]. Annotation was performed with the MITOS2 web server (RefSeq 81 Metazoa; Genetic Code 5) [48], followed by manual curation using Geneious Prime [49]. GenBank accession numbers of the newly obtained mitochondrial genomes are provided in Tables 1 and 2. Genome maps were visualized using Organellar Genome DRAW [50].

Table 1 GenBank accession numbers of *Geonemertes* spp. and the related species used for phylogenetic analyses in this study

Species	Collection localities	Accession No.	References
Geonemertes sp. Ogasawara CMH01	Miyanohamamichi, Chichijima, Ogasawara	PX290021	Present study
Geonemertes sp. Ogasawara CMH02	Miyanohamamichi, Chichijima, Ogasawara	PX290018	Present study
Geonemertes sp. Ogasawara CMK01	Mt. Mikazuki, Chichijima, Ogasawara	PX290022	Present study
Geonemertes sp. Ogasawara CMK02	Mt. Mikazuki, Chichijima, Ogasawara	PX290023	Present study
Geonemertes sp. Ogasawara CMK03	Mt. Mikazuki, Chichijima, Ogasawara	PX290017	Present study
Geonemertes sp. Ogasawara CMK04	Mt. Mikazuki, Chichijima, Ogasawara	PX290019	Present study
Geonemertes sp. Ogasawara MI	Higashimachi, Chichijima, Ogasawara	PX392562	Present study
Geonemertes sp. Ogasawara MNK01	Nakanotaira, Hahajima, Ogasawara	PX290020	Present study
Geonemertes sp. MCZ IZ-132,534	Bocas del Toro, Panama	KF935548	Kvist et al. (2014) [87]
Geonemertes "*pelaensis*" CZACC_18_007	Cuba	MN653922	Morffe et al. (2020) [30]
Geonemertes "*pelaensis*" CZACC_18_008	Cuba	MN653923	Morffe et al. (2020) [30]
Geonemertes "*pelaensis*" CZACC_18_010	Cuba	MN653924	Morffe et al. (2020) [30]
Geonemertes "*pelaensis*" CZACC_18_011	Cuba	MN653925	Morffe et al. (2020) [30]
Geonemertes "*pelaensis*" CZACC_18_012	Cuba	MN653926	Morffe et al. (2020) [30]
Geonemertes "*pelaensis*" CZACC_18_014	Cuba	MN653927	Morffe et al. (2020) [30]
Geonemertes "*pelaensis*" CZACC_18_015	Cuba	MN653928	Morffe et al. (2020) [30]
Geonemertes "*pelaensis*" CZACC_18_016	Cuba	MN653929	Morffe et al. (2020) [30]
Geonemertes "*pelaensis*"	Bermuda	EU255602	Mateos and Giribet (2008) [88]
Geonemertes "*pelaensis*"	Minamidaito Island, Okinawa	LC412491	Unpublished
Geonemertes "*pelaensis*"	Minamidaito Island, Okinawa	LC412492	Unpublished
Geonemertes "*pelaensis*"	Minamidaito Island, Okinawa	LC412493	Unpublished
Geonemertes "*pelaensis*"	Minamidaito Island, Okinawa	LC412494	Unpublished
Geonemertes "*pelaensis*"	Minamidaito Island, Okinawa	LC412495	Unpublished
Geonemertes "*pelaensis*"	Minamidaito Island, Okinawa	LC412496	Unpublished
Geonemertes "*pelaensis*"	Minamidaito Island, Okinawa	LC412497	Unpublished
Geonemertes "*pelaensis*"	Minamidaito Island, Okinawa	LC412496	Unpublished
Geonemertes "*pelaensis*"	Minamidaito Island, Okinawa	LC412497	Unpublished
Geonemertes "*pelaensis*"	Minamidaito Island, Okinawa	LC412498	Unpublished
Geonemertes "*pelaensis*"	Minamidaito Island, Okinawa	LC412499	Unpublished
Geonemertes "*pelaensis*"	Minamidaito Island, Okinawa	LC412500	Unpublished
Geonemertes "*pelaensis*"	Okinawa Main Island, Okinawa	LC412501	Unpublished
Geonemertes "*pelaensis*"	Okinawa Main Island, Okinawa	LC412502	Unpublished
Geonemertes "*pelaensis*"	Yonaguni Island, Okinawa	PX392564	Present study
Prosadenoporus floridensis	Link Port, Florida, USA	EF157596	Maslakova and Norenburg (2008) [12]
Prosadenoporus mooreae	Magnetic Island, Australia	EF157595	Maslakova and Norenburg (2008) [12]
Prosadenoporus winsori	Banks of Ross River, Townsville, Australia	EF157594	Maslakova and Norenburg (2008) [12]

COX1 primer design and sanger sequencing

Based on the complete mitochondrial genome obtained above, specific primers targeting the Folmer region of *COX1* for the Ogasawara specimens were designed using Geneious Prime. PCR products were purified using ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. Sanger sequencing was performed with the same primer pairs used in PCR and outsourced to FASMAC (Kanagawa, Japan). Shotgun sequencing of the mitochondrial genome revealed that the forward primer for the Folmer *COX1* region in the *Geonemertes* specimens collected from Ogasawara Islands (referred to as *Geonemertes* sp. Ogasawara in Fig. 1A, E) showed mismatches at three positions in each primer region: the 1st, 2nd, and 8th positions for the forward primer (LCO1490), and the 3rd, 12th, and 18th positions for the reverse primer (HCO2198). Based on these results, specific primers were designed for PCR amplification: GeoG_98F (TTTGGTCTGGGCTTGTTGGT) / GeoG_1348R (AATAACGACGAGGCATCCCA) for amplification, and GeoG_interval_917F (TGGATGTTGATACTCGGGCT) / GeoG_interval_498R (AGAAGAAACACCAGCAAGATGA) for sequencing. The same PCR conditions as in other PCR reactions were used, allowing for successful *COX1* sequencing of *Geonemertes* sp. Ogasawara.

Phylogenetic analyses based on *COX1*

Sequence alignments were performed in Geneious Prime using Clustal Omega v1.2.3 [51]. Ambiguous regions were trimmed using Gblocks v0.91b [52] under less

Table 2 GenBank accession numbers of the mitochondrial genome of the selected hoplonemerteans used for phylogenetic analyses in this study

Species	Accession No.	References
Amphiporus formidabilis	KC710979	Sun and Sun (2014) [89]
Amphiporus lactifloreus	OR387313	Dodt et al. (unpublished)
Geonemertes "pelaensis" Yonaguni Island	PX392564	Present study
Geonemertes "pelaensis" New Caledonia	PX238424	Gastineau (unpublished)
Geonemertes "pelaensis" Martinique	PQ336987	Gastineau (unpublished)
Geonemertes sp. Ogasawara	PX392562	Present study
Gononemertes parasita	KF572481	Sun et al. (2014) [90]
Emplectonema gracile	JF727825	Unpublished
Malacobdella japonica	PX392563	Present study
Nectonemertes cf. *mirabilis*	HQ997772	Chen et al. (2012) [65]
Nemertopsis tetraclitophila	KF572482	Sun et al. (2014) [90]
Nipponnemertes punctatula	KC710980	Sun and Sun (2014) [89]
Paranemertes cf. *peregrina*	GU564481	Chen et al. (2011) [91]
Prosadenoporus spectaculum	KC710981	Sun and Sun (2014) [89]
Prosorhochmus claparedii	OR397959	Dodt et al. (unpublished)

stringent parameters. From the original 658-bp *COX1* alignment, 573 bp (87%) remained after trimming.

Maximum-likelihood (ML) analyses were conducted using IQ-TREE [53] based on the best-fit substitution model identified by the IQ-TREE web server [54]. Node support was evaluated using 1,000 ultrafast bootstrap (UFboot) replicates. Clades with UFboot support values ≥ 95% were considered strongly supported, following the guidelines provided by IQ-TREE. Three species of *Prosadenoporus* Bürger, 1890 were used as outgroup taxa. *COX1* sequences determined in the present study were deposited in GenBank, with accession numbers listed in Table 1.

Uncorrected pairwise genetic distances were calculated based on the 658-bp *COX1* in Geneious Prime.

Phylogenetic analyses based on protein coding genes (PCGs) in mitochondrial DNA

To infer phylogenetic position of *Geonemertes* in Amphiporiina (Monostilifera), a ML analysis was performed using concatenated sequences of mitochondrial 13 PCGs and two ribosomal RNA genes, ATP synthase subunits (*ATP6* and *ATP8*), cytochrome *c* oxidase subunits (*COX1–3*), NADH dehydrogenase subunits (*ND1–6* and *ND4L*), and cytochrome *b* (*CYTB*) and 16S rRNA (*16S*) and 12S rRNA (*12S*) from 16 operational taxonomic units (OTUs) of hoplonemerteans. A single species of

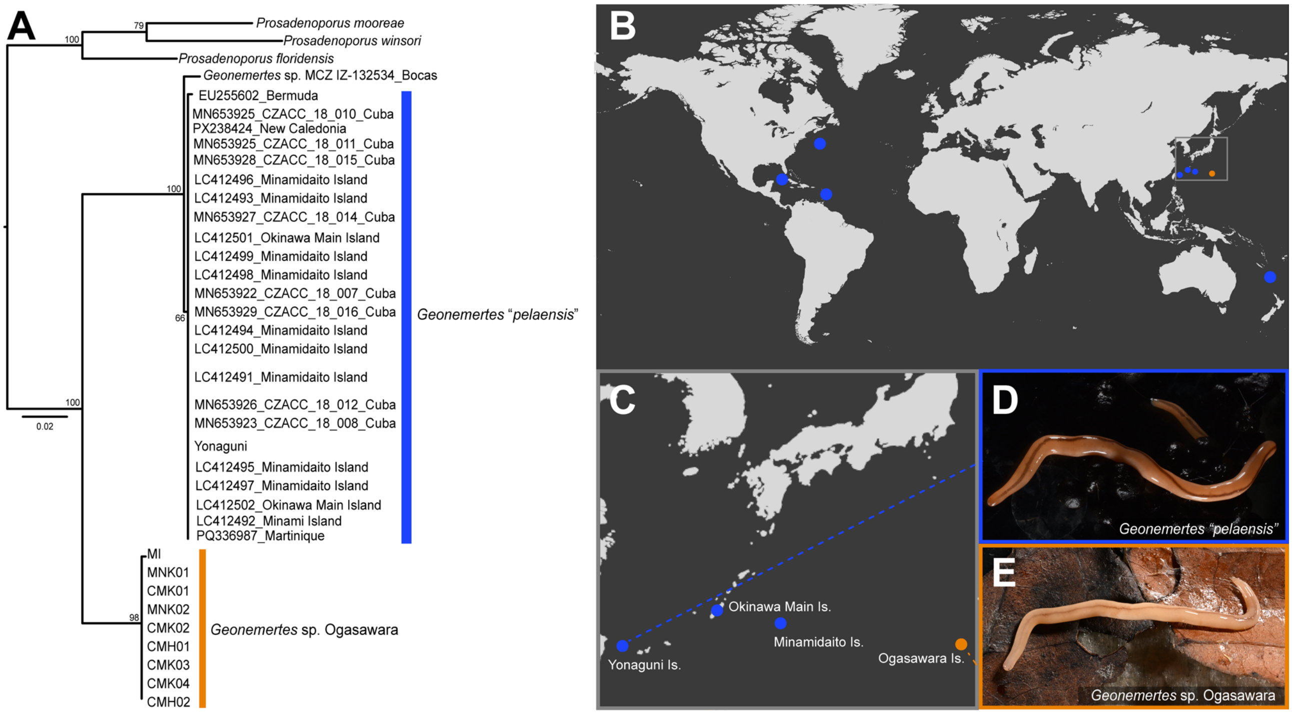

Fig. 1 Genetic and geographic differentiation between *Geonemertes* species in the Pacific and western Atlantic regions. (**A**) Maximum likelihood phylogenetic tree based on *COX1* sequences (573 bp) showing the genetic divergence between *Geonemertes* sp. Ogasawara (orange) and the broadly distributed *Geonemertes "pelaensis"* clade (blue), including specimens from Bermuda, Cuba, Martinique, New Caledonia, and the Okinawa Islands. Bootstrap values ≥ 70 are shown at nodes. (**B**) Global map showing sampling localities. Blue circles indicate records of *G. "pelaensis"*, orange circles indicate *Geonemertes* sp. Ogasawara. (**C**) Regional map of the northwestern Pacific showing localities in Japan where *Geonemertes* specimens were collected. (**D**–**E**) Live specimens: (**D**) *G. "pelaensis"* from Yonaguni Island (blue frame); (**E**) *Geonemertes* sp. Ogasawara from Chichijima Island, Ogasawara (orange frame)

polystiliferan, *Nectonemertes* cf. *mirabilis*, was used as an outgroup taxon. Sequence alignment, ambiguous site trimming, model selection, and phylogenetic analyses follow the methods above.

Results

Sequencing and mitochondrial genome assembly

For *Geonemertes* "*pelaensis*", a total of 94.6 Gbp, corresponding to approximately 626.9 million raw reads, was generated. Assembly of a 10% subset of these reads yielded a complete mitochondrial genome of 31,745 bp with an average coverage of 650×. The assembled mitochondrial genome showed no evidence of nuclear mitochondrial pseudogene contamination, as indicated by continuous read mapping across the genome without gaps or excessive coverage variation. The overall read coverage was uniform across both coding and non-coding regions (Supplementary Fig. 1, Supplementary Table 2).

Sequencing of *Geonemertes* sp. Ogasawara produced 2.5 Gbp of data, comprising approximately 16.6 million raw reads. The subsequent assembly resulted in a mitochondrial sequence of 18,755 bp with an average coverage of 177.7×.

For *Malacobdella japonica*, we obtained 3.8 Gbp of data, consisting of approximately 25.7 million raw reads. The assembly of these reads produced a 14,917 bp mitochondrial genome with an average coverage of 697.39×.

Phylogeny based on *COX1*

Phylogenetic analyses based on *COX1* sequences (573 bp) revealed that *Geonemertes* sp. Ogasawara formed a distinct clade, separate from those identified as *G. pelaensis* based on body coloration (referred to as *Geonemertes* "*pelaensis*" in Fig. 1A, D), including specimens from Okinawa (main island), Minamidaito Island, Yonaguni Island (this study), Bermuda, Cuba, Martinique, and New Caledonia (Fig. 1A, B). All Ogasawara specimens from both Chichijima and Hahajima clustered together with 100% ultrafast bootstrap support (UFboot) and were consistently recovered as sister to a clade comprising *G.* "*pelaensis*" and *Geonemertes* sp. from Bocas del Toro, Panama (Fig. 1A).

Uncorrected pairwise *COX1* sequence divergence between *Geonemertes* sp. Ogasawara and *G.* "*pelaensis*" plus *Geonemertes* sp. Bocas del Toro ranged from 6.75% to 8.59%. These inter-clade divergences far exceed the typical intraspecific variation in nemerteans; for reference, most species show less than 4–5% *COX1* divergence between geographically distant populations, although cryptic species complexes may exhibit higher values (e.g. [55]). In contrast, within-clade divergences were very low: 0.00–0.16% within the Ogasawara clade and 0.00–0.26% within the *G. pelaensis* clade (Supplementary Table 1).

Alignment of the *COX1* sequences also revealed consistent synonymous substitutions distinguishing *Geonemertes* sp. Ogasawara population, further supporting their genetic separation.

Characterization and interspecific comparison of mitochondrial genome

We obtained the complete mitochondrial genomes of *Geonemertes* sp. Ogasawara, *G.* "*pelaensis*" from Yonaguni Island, and the relatively close taxon with *Geonemertes* (e.g. [56]), *Malacobdella japonica* from Kashima-nada, Japan (Fig. 2). The mitogenome of *G. pelaensis* from Yonaguni Island was substantially larger at 31,745 bp in total, with a GC content of 22.3% (Fig. 2A). The mitogenome of *Geonemertes* sp. Ogasawara was reconstructed as 18,755 bp, with a GC content of 24.3% (Fig. 2D). The mitogenome of *M. japonica* was reconstructed as 14,917 bp, with a GC content of 30.4% (Fig. 2G). The mitogenome of *G. "pelaensis"* from Yonaguni Island was comparable in length to the deposited sequence of *G. pelaensis* from Martinique and New Caledonia (Fig. 3C), which shares an identical *COX1* sequence with our Yonaguni specimen.

The 13 PCGs of *Geonemertes* "*pelaensis*", *Geonemertes* sp. Ogasawara, and *M. japonica* encode a total of 3,698 (Fig. 2A), 3,627 (Fig. 2D), and 3,699 amino acids (Fig. 2G), respectively. Among the amino acids, phenylalanine (Phe) is the most frequently used in *M. japonica* and *Geonemertes* sp. Ogasawara, whereas proline (Pro) is the most common in *G.* "*pelaensis*" (Fig. 2C, F, H). Relative synonymous codon usage (RSCU) analysis indicates a strong preference for codons ending with thymine (T) at the third codon position (Fig. 2B, E, H). This pattern is consistent with the general AT-bias commonly observed in mitochondrial genomes of metazoans [57–59]. In particular, the preferred codon for arginine (Arg) differs between lineages: *M. japonica* shows a bias toward GGA (Fig. 2H), whereas both *Geonemertes* species preferentially use GGT (Fig. 2B, E). Moreover, striking differences were observed in leucine (Leu, CUN) codon usage; *G.* "*pelaensis*" predominantly employed CTT for Leu (CUN) codon but *Geonemertes* sp. Ogasawara displayed a more balanced distribution of Leu (CUN) codon, closely resembling the pattern in *M. japonica* (Fig. 2B, E, H).

To evaluate sequencing quality of *G.* "*pelaensis*" from Yonaguni Island, we analyzed coverage depth based on Phred quality scores ≥ 30. The Yonaguni specimen exhibited high-quality sequencing across the genome, with an average per-site coverage of 22,574 reads (median: 23,528; minimum: 881; maximum: 29,187) and a mean base quality score of 37. A small number of sites showed minor alternative bases (Supplementary Fig. 1, Supplementary

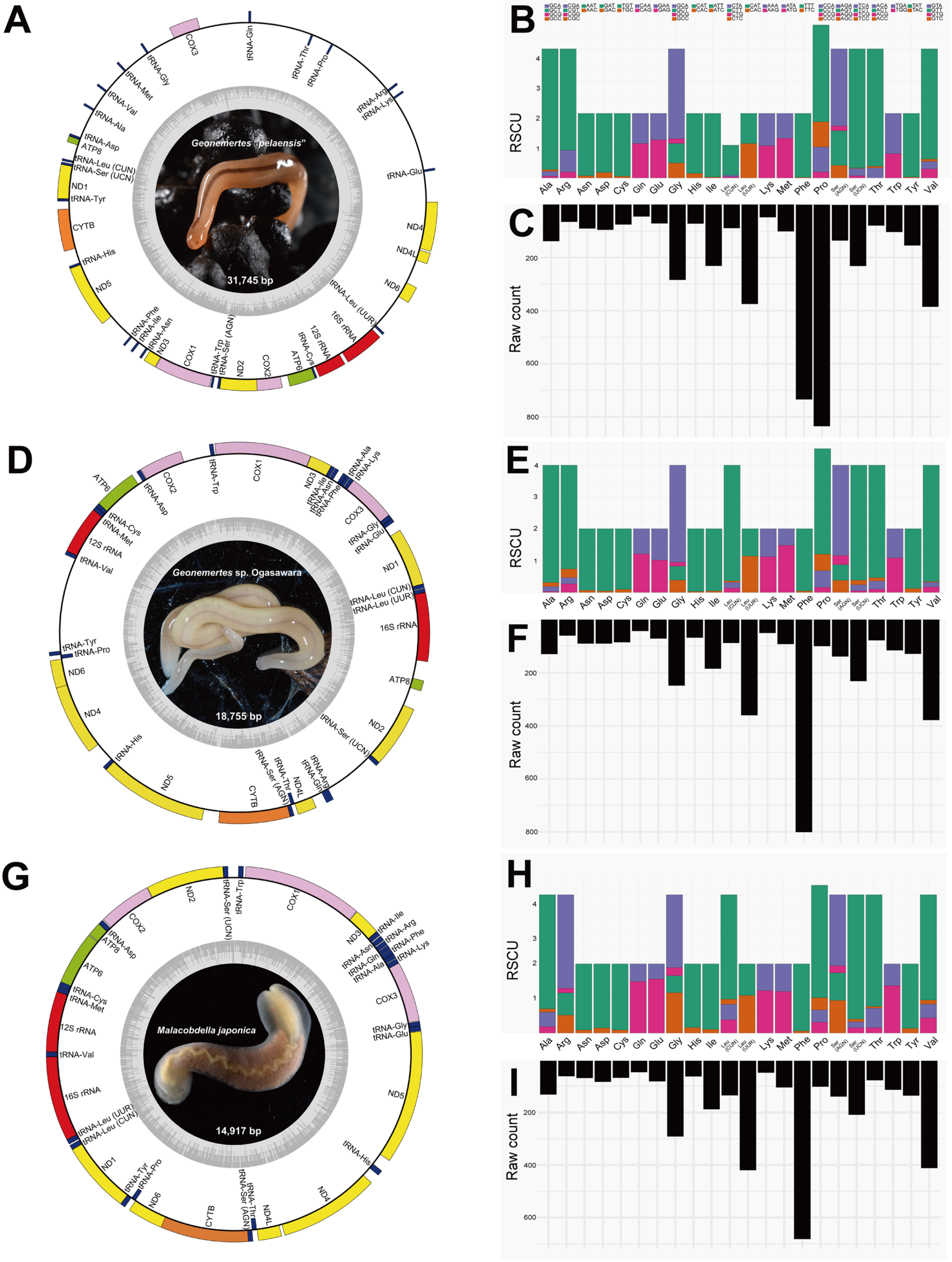

Fig. 2 (See legend on next page.)

(See figure on previous page.)

Fig. 2 Mitochondrial genome organization, codon usage, and relative synonymous codon usage (RSCU) of three nemertean species. (**A**–**C**) *Geonemertes "pelaensis"* from Yonaguni Island, (**D**–**F**) *Geonemertes* sp. Ogasawara, and (**G**–**I**) *Malacobdella japonica*. (**A**, **D**, **G**) Circular maps of the mitochondrial genomes showing gene organization and strand orientation. Protein-coding genes are indicated by standard gene abbreviations (e.g., *COX1*, *ND2*, *ATP6*), ribosomal RNA genes are labeled as *12S rRNA* and *16S rRNA*, and tRNA genes are denoted by their corresponding amino acid codes. The innermost ring plot represents the GC content along the genome, with peaks indicating regions of higher GC composition. Central photographs show the corresponding specimens. (**B**, **E**, **H**) RSCU for each species. Colored bars represent the RSCU values of individual codons grouped by amino acid. RSCU values > 1 indicate preferential usage of that codon relative to others encoding the same amino acid. (**C**, **F**, **I**) Codon usage bias across protein-coding genes, showing the deviation in codon usage frequency

Table 2), likely reflecting sequencing artifacts or low-level heteroplasmy, though their frequencies were low and did not affect the consensus sequence.

All three mitochondrial genomes were circular and contained the full complement of 13 PCGs and two ribosomal RNA genes (*12S* and *16S*). The inferred gene order of *Geonemertes* sp. Ogasawara oriented clockwise, was as follows: *ND6*, *ND4*, *ND5*, *CYTB*, *ND4L*, *ND2*, *ATP8*, *16S*, *ND1*, *COX3*, *ND3*, *COX1*, *COX2*, *ATP6*, *12S* (Figs. 2D, 3B and 4). This arrangement differs markedly from that of *G. "pelaensis"*, which has the following gene order: *ND6*, *ND4L*, *ND4*, *COX3*, *ATP8*, *ND1*, *CYTB*, *ND5*, *ND3*, *COX1*, *ND2*, *COX2*, *ATP6*, *12S*, *16S* (Figs. 2A and 4); the gene arrangement was identical with sequences from the Martinique and New Caledonia specimens (Fig. 3B). Despite the substantially larger genome size (Fig. 3C), we detected no gene duplications in the mitogenome of *G. "pelaensis"*. Expanded non-coding regions were observed between *ND4* and *COX3*, and *COX3* and *ATP8*, which likely contributed to the overall mitochondrial genome size (Fig. 2A).

Interspecific comparisons of PCGs order with marine species of Hoplonemertea, revealed that these taxa share a conserved ancestral gene order typical of Nemertea: *ND6*, *CYTB*, *ND4L*, *ND4*, *ND5*, *COX3*, *ND3*, *COX1*, *ND2*, *COX2*, *ATP8*, *ATP6*, *12S*, *16S*, *ND1* (Fig. 4) (e.g., Chen et al. 2012). Relative to the ancestral hoplonemertean gene order, both *Geonemertes* species exhibit multiple rearrangements; in *G. "pelaensis"*, *CYTB*, *ND1*, *ND5*, and *ATP8* are translocated, whereas in *Geonemertes* sp. Ogasawara, translocations involve *ND4*–*ND5*, *ND2*, *ATP8*, and the *16S*–*ND1* blocks (Figs. 3B and 4).

Phylogeny based on mitochondrial PCGs, *12S*, and *16S*

In the resulting tree (Fig. 3A), the two terrestrial nemerteans, *G. "pelaensis"* and *Geonemertes* sp. Ogasawara, formed a clade; the clade was sister to *M. japonica* with 94% of UFboot. The clade constituted by the two *Geonemertes* species and *M. japonica* was sister to *Prosadenoporus spectaculum* (Yamaoka, 1940) with a full support value (Fig. 3A).

Systematics

Family Prosorhochmidae Bürger, 1895.

Genus *Geonemertes* Semper, 1863.

Type species: *Geonemertes pelaensis* Semper, 1863.

Diagnosis based on Gibson & Moore (1998) [26]: Terrestrial eumonostiliferous nemertean with 2–8 eyes. Rhynchocoel nearly full body length; rhynchocoel wall with two-separated muscular layers, outer circular and inner longitudinal muscle layers. Mid-dorsal vascular vessels with a single vascular plug. Frontal organ massively developed. Cephalic glands extensively developed. Submuscular glands present. Lateral nerve with accessory nerves. Excretory system well developed, consisting of enormous numbers of separate protonephridia distributed throughout body and opening by large numbers of pores; the binucleate flame cells, with both transverse and longitudinal support bars.

Geonemertes pelaensis Semper, 1863.
(Fig. 5A–E)

Material examined: NSMT-Ne 22, a complete body except for a posterior tip used for DNA extraction, preserved in 70% ethanol, collected near Tabaru River, Yonaguni Island, Okinawa, Japan (24°27′38.7′′N, 123°00′15.3′′E), by Naoto Sawada, on April 16 2022.

Habitat and geographic distribution: The specimens collected from Yonaguni were found under stones and among litter layers in the natural forest seemingly remoted from human activities. In other localities, the species was also found from habitats likely affected from human activities such as in botanical gardens and under plant pots or soil sacks, and beneath moist cement blocks and bricks [60]. Currently reported from tropical/subtropical areas in the Atlantic, Caribbean, Indian, and Pacific Oceans.

Description: Body 23.0–120.0 mm in length and 0.7–4.0 mm in maximum width; body ground color uniformly pale to light brown; a single longitudinal dark brown stripe faintly appearing at posterior region of head and

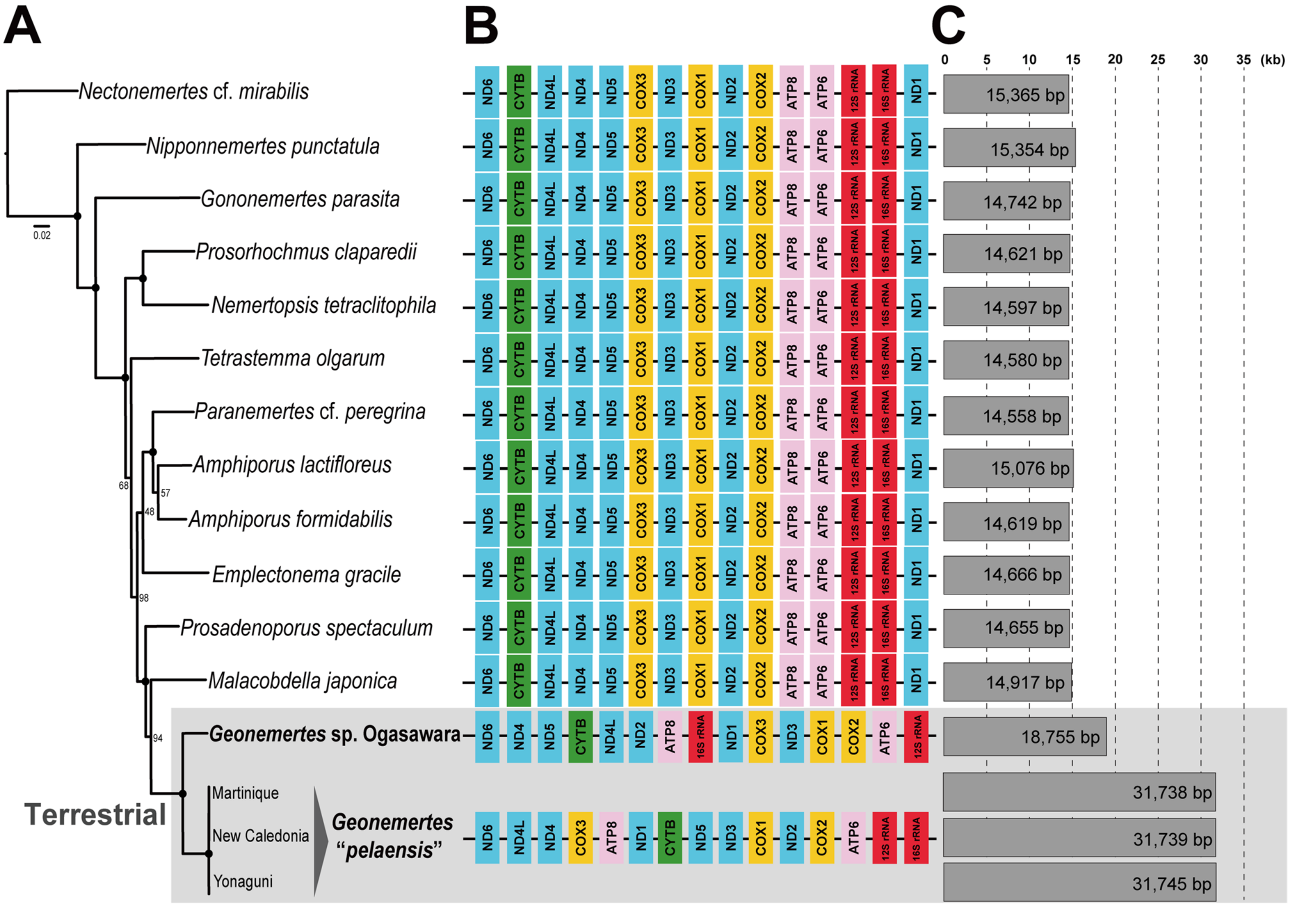

Fig. 3 Mitochondrial phylogeny, gene order, and genome size of *Geonemertes* species and other hoplonemerteans. (**A**) Maximum likelihood phylogeny of selected hoplonemerteans based on concatenated mitochondrial protein-coding genes (PCGs), and two rRNA genes (12S, 16S). (**B**) Mitochondrial gene order of 13 protein-coding genes and 2 rRNA genes for each species. (**C**) Comparison of total mitochondrial genome sizes; *Geonemertes* sp. Ogasawara has a larger genome (18,755 bp) than other hoplonemerteans, while *G.* "*pelaensis*" from Yonaguni Island shows an exceptionally large genome (31,745 bp), more than twice the typical size observed in Nemertea

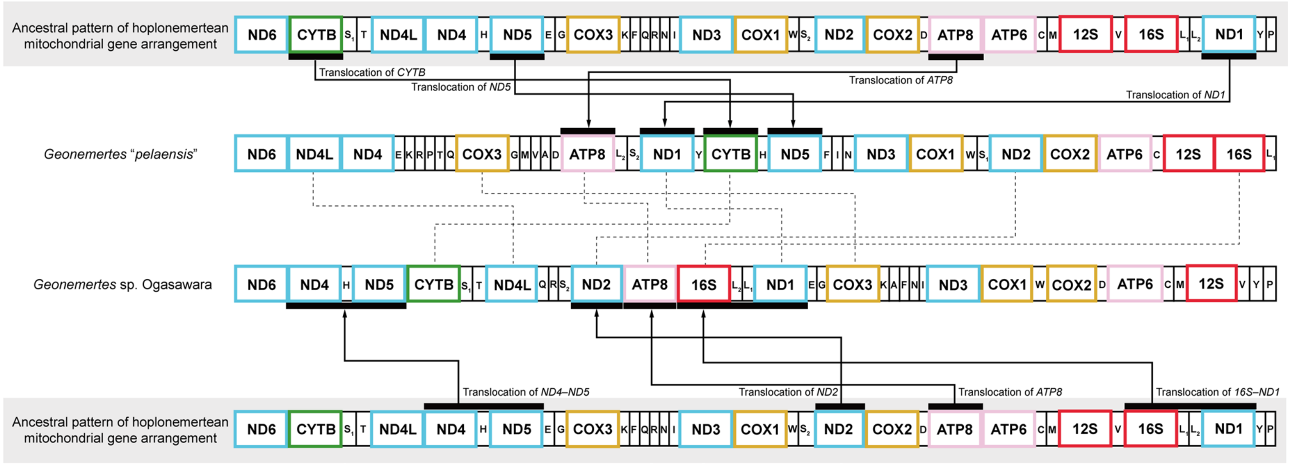

Fig. 4 Comparisons of mitochondrial gene arrangements in *Geonemertes*. Gene translocations are illustrated by comparing the ancestral hoplonemertean mitochondrial gene arrangement with those of *Geonemertes* "*pelaensis*" and *Geonemertes* sp. Ogasawara. Arrows indicate inferred hypothetical translocation events. Single-letter abbreviations denote tRNA genes: A = tRNA-Ala, C = tRNA-Cys, D = tRNA-Asp, E = tRNA-Glu, F = tRNA-Phe, G = tRNA-Gly, H = tRNA-His, I = tRNA-Ile, K = tRNA-Lys, L_1 = tRNA-Leu (UUR), L_2 = tRNA-Leu (CUN), M = tRNA-Met, N = tRNA-Asn, P = tRNA-Pro, Q = tRNA-Gln, R = tRNA-Arg, S_1 = tRNA-Ser (AGN), S_2 = tRNA-Ser (UCN), T = tRNA-Thr, V = tRNA-Val, W = tRNA-Trp, Y = tRNA-Tyr

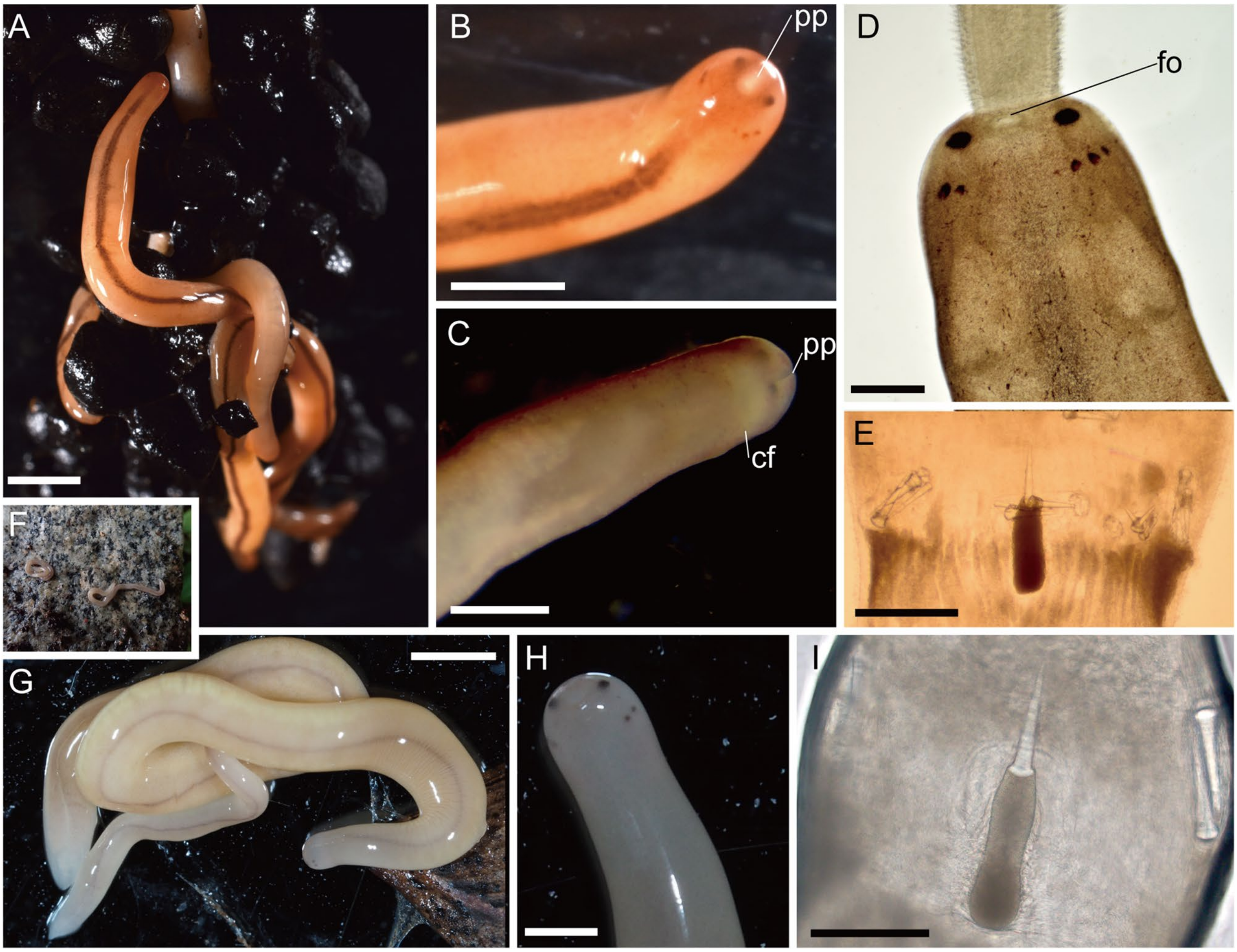

Fig. 5 *Geonemertes* spp. herein reported. (**A**) *Geonemertes* "*pelaensis*", whole body; (**B**) magnification of head, anterodorsal; (**C**) ventral; (**D**) squeezed specimen; (**E**) stylet apparatus; (**F**) *Geonemertes* sp. Ogasawara, specimens photographed in-situ; (**G**) whole body; (**H**) magnification of head, dorsal; (**I**) stylet apparatus. Abbreviations: cf, cephalic furrow; fo, frontal organ; pp, proboscis pore. Scale bars: 1 mm (**A**, **G**); 500 μm (**B**, **C**, **H**); 100 μm (**D**, **E**, **I**)

extending to posterior end of body (Fig. 5A). A single longitudinal lateral stripe very faintly present on both sides of a mid-dorsal stripe (Fig. 5A). Cerebral ganglia invisible through body wall. Cephalic furrows transversely extending on ventral surface and meeting at mid-line (Fig. 5B, C). Eyes arranged in four groups; anterior eyes typically larger than posterior ones; posterior groups containing 2–4 eyes (Fig. 5D). Large frontal organ visible in squeezed specimens (Fig. 5D). Proboscis pore terminal (Fig. 5D). Proboscis white (Fig. 5D). In the Yonaguni specimens, proboscis middle chamber with 4 or 5 accessory-stylet pouches, each containing 3–5 accessory stylets (Fig. 5E). Central stylet smooth; stylet 80–92 μm in length and 16–20 μm in width; basis cylindrical, 100–125 μm in length and 30 μm in width (Fig. 5E).

Remarks: The external features of the Yonaguni specimens including the body coloration and the number of eyes agree with *G. pelaensis* previously reported worldwide [26, 30, 61]. A wide distribution of the present species was genetically confirmed by 0.00–0.16% of *COX1* genetic distances between the specimens from Yonaguni and the specimens from Bermuda, Cuba, Martinique, and New Caledonia, much lower than interspecific divergences (approx. 4–5%) observed among monostiliferans (e.g. [55]).

Geonemertes sp. Ogasawara.
(Fig. 5F–I)

Material examined: NSMT-Ne 23, a complete body except for a posterior tip used for DNA extraction, preserved in 70% ethanol, collected in a private garden in Higashimachi, Chichijima Island, Ogasawara, Tokyo, Japan by Natsumi Hookabe, on December 12, 2020. RMNH.VER.22254 (referred to as "KAWAKATSU's Specimen Lot No. 1899" in Oki et al. (1987) [34]), seven specimens preserved in 70% ethanol, collected under a flowerpot placed in a garden at Nishi-machi, Chichijima Island; altitude, ca. 10 m by Kitagawa Ken'ichi on July 18, 1987; RMNH.VER.22258 (referred to as "KAWAKATSU's Specimen Lot No. 1882" in Oki et al. (1987) [34], five specimens, collected at a roadside bush at Miyanohamamichi, Chichjima Island; altitude, ca. 25 m by Kitagawa Ken'ichi, the specimen label indicating the collection date as August 22, 1986, whereas Oki et al. (1987) [34] reported it as 1981.

Habitat and geographic distribution: This species was found under bricks in backyard gardens and roadsides. It is likely to prefer moist substrates like most land snails and terrestrial flatworms. The similar forms with a lighter brown mid-dorsal single stripe than *G. pelaensis* were reported from southern part of Hahajima Island [39].

Description: Body 21.0–40.0 mm in length and 1.7–2.0 mm in maximum width; body ground color uniformly pale; a single longitudinal narrow pale-brown stripe faintly appearing at posterior region of head; a stripe becoming faded at posterior end of body (Figs. 5F, G and 6). A single longitudinal lateral stripe not well distinguished. Cerebral ganglia invisible through body wall. Eyes arranged in four groups; anterior eyes typically larger than posterior ones; posterior groups containing 2 or 3 eyes (Fig. 5H). Testes visible through body wall as a milk-yellow colored region (Fig. 5G).

Proboscis pore terminal (Fig. 5H). In the specimen examined in this study, proboscis middle chamber with 2 accessory-stylet pouches, each containing 2 accessory stylets. Central stylet smooth; stylet 82 µm in length and 16 µm in width; basis cylindrical with a constricted middle region, 100 µm in length and 27 µm in width (Fig. 5I).

Ecology: Hahajima Island population has been observed preying on a wider range of invertebrates, such as spiders, cicadas (e.g., *Orosanga*), *Drosophila*, *Gryllodes sigillatus*, lepidopteran larvae, amphipods (Talitridae), and isopods (*Burmoniscus kathmandius* and *Venezillo parvus*) [39, 40, 62].

Remarks: The present species most resembles *G. pelaensis* in having a pale body with a dark mid-dorsal longitudinal stripe (Figs. 5G and 6). However, the mid-dorsal stripe is paler and narrower than *G. "pelaensis"* from Yonaguni Island (Fig. 5A, G) and these characters seem consistent with Kawakatsu Collection collected in 1980s from Chichijima Island (Fig. 6). Apart from the external features, there is the other morphological difference between the two—the number of accessory-stylet pouches and accessory stylets (each 2 in 2 pouches in *Geonemertes* sp. Ogasawara vs. each 3–5 in 4 or 5 pouches in *G. "pelaensis"*).

The present study provides clear molecular and morphological evidence that *Geonemertes* sp. Ogasawara and *G. "pelaensis"* represent distinct species (Table 3).

Fig. 6 Museum specimens identified as *Geonemertes pelaensis* in the 1980s from Chichijima Island, Ogasawara. (**A**) RMNH.VER.22,254 collected in 1987 from Chichijima Island; (**B**) RMNH.VER.22,258 collected from Miyanohamamichi (misspelled on label as "Miyano-hama, Nichi"), Chichijima Island; the specimen label indicates the collection date as August 22, 1986, whereas Oki et al. (1987) reported it as 1981. Scale bars: 5 mm

Table 3 Summary of diagnostic morphological and distributional differences between the Yonaguni (*G. "pelaensis"*) and Ogasawara (*Geonemertes* sp. Ogasawara) specimens.

	***Geonemertes "pelaensis"* (based on Yonaguni specimens)**	***Geonemertes* sp. Ogasawara**
Body length (mm)	23–120	21–40
Body width (mm)	0.7–4.0	1.7–2.0
Body color	Pale to light brown	Uniformly pale
Mid-dorsal stripe	Dark brown, distinct and broad; extending to posterior end	Pale brown, narrow and faint; fading posteriorly
Lateral stripes	Faintly visible on both sides of mid-dorsal stripe	Poorly defined or absent
Eyes	Four groups; anterior eyes larger; posterior groups with 2–4 eyes	Four groups; anterior eyes larger; posterior groups with 2–3 eyes
Proboscis pore	Terminal	Terminal
Accessory-stylet pouches (no.)	4–5	2
Accessory stylets per pouch	3–5	2
Stylet basis (μm)	Cylindrical, 100–125 × 30	Cylindrical, constricted medially, 100 × 27
Geographic distribution	Widespread in tropical/subtropical Atlantic, Caribbean, Indian, and Pacific; originally described from Palau	Known only from Chichijima and Hahajima, Ogasawara (Japan)
Remarks	*COX1* identical to specimens from Bermuda, Cuba, Martinique, and New Caledonia; considered a widespread cosmopolitan species	Distinct molecular and morphological lineage

The Ogasawara specimen is potentially undescribed; however, because topotypic DNA sequences for *G. pelaensis* from Palau are currently unavailable, we refrain from describing it formally in this study.

Discussion

Unrecognized species diversity in Japanese terrestrial *Geonemertes*

Our mitochondrial genome and *COX1* analyses revealed the presence of at least two distinct *Geonemertes* lineages in Japan (Fig. 1A). The Ogasawara population—previously identified as *G. pelaensis*—is genetically and morphologically distinct from the widely distributed *G.* "*pelaensis*" found in Yonaguni and other tropical/subtropical regions. Given its unique ecological characteristics and potential impact on native soil fauna, further taxonomic and ecological investigation, particularly regarding its biogeographic origin and pathways of dispersal are required for the Ogasawara population.

Morphological differentiation of the Ogasawara population

Our morphological examination revealed differentiations between *Geonemertes* sp. Ogasawara and *G.* "*pelaensis*" from Yonaguni Island (Fig. 5). Live specimens of *G.* "*pelaensis*" possess a pale body with a solid dark mid-dorsal stripe; (body width): (width of mid-dorsal stripe) = 1: 0.078–0.110 (Figs. 1D and 5A–F). In contrast, *Geonemertes* sp. Ogasawara exhibit a uniformly pale to light brown coloration, with a narrow, posteriorly becoming faint or indistinct (body width): (width of mid-dorsal stripe) = 1: 0.042–0.050, mostly half ratio than *G. pelaensis* (Figs. 1E and 5G–F). In addition, *G.* "*pelaensis*" from Yonaguni Island possess four or five accessory-stylet pouches, each containing 3–5 accessory stylets (Fig. 5E), while Ogasawara specimens consistently had only two pouches, each with two accessory stylets (Fig. 5I). These differences, coupled with 6.75 to 8.59% *COX1* genetic divergence observed in our analyses, support the hypothesis that the Ogasawara population represents a distinct species as suggested in Yoshino (2025) [40].

Examination of specimens collected in the 1980s by Prof. Dr. Kawakatsu, which were later used in Oki et al. (1987) [34] and are now deposited in the museum collections of the Naturalis Biodiversity Center, revealed external morphologies identical to those of our recent Ogasawara specimens (Figs. 5G and H and 6). To the best of our knowledge, *Geonemertes* sp. Ogasawara has been the only *Geonemertes* species present in the Ogasawara Islands for nearly half a century. These findings suggest the crucial role of museum collections in documenting biodiversity through time as mentioned in many previous studies (e.g. [63]). The preservation and accessibility of historical specimens not only allowed us to confirm species identification in Ogasawara Islands over several decades but also highlight the importance of systematic deposition for future taxonomic and conservation studies.

Mitogenomic variation and its evolutionary implications

In addition to *COX1* divergence, our study revealed striking differences in mitochondrial genome size and arrangement among *Geonemertes* sp. Ogasawara, *G.* "*pelaensis*", and other monostiliferans (Figs. 3B, C and 4). The mitogenome of the Ogasawara species, at 18,755 bp, is larger than those reported for other hoplonemerteans (e.g. [64, 65]). Further, the mitogenome of *G.* "*pelaensis*" from Yonaguni Island was 31,745 bp—1.89–2.18 times larger than those of other nemerteans—despite consistently encoding the 13 PCGs and two rRNA genes [64, 66]. The large genome of *G.* "*pelaensis*" seems due to the long intergenic non-coding regions, particularly between *ND4*–*COX3* and *COX3*–*ATP8* (Fig. 2A). These features are also confirmed in *G.* "*pelaensis*" from Martinique and

New Caledonia (Fig. 3A), indicating that this expansion pattern is species-specific and conserved across geographically distant populations.

Although both *Geonemertes* species exhibit extensive rearrangements in gene order, their overall architectures retain partial similarity to the ancestral hoplonemertean pattern (Fig. 4). The two *Geonemertes* species nevertheless differ markedly: in *G.* "*pelaensis*", the translocations of *CYTB*, *ND1*, *ND5*, and *ATP8* have occurred, whereas in *Geonemertes* sp. Ogasawara, rearrangements involve the *ND4–ND5* block, *ND2*, *ATP8*, and the *16S–ND1* block. *ATP8*, *ND1*, and *ND5* are translocated in both the *Geonemertes* species (Fig. 4), suggesting that these loci may represent hotspots of rearrangement in *Geonemertes*. The relative position of *ATP8* is similar in both species, suggesting that its translocation may have occurred once in their common ancestor, while the remaining rearrangements likely arose independently after divergence.

Despite these dynamic mitochondrial gene rearrangements, several elements remain conserved; for example, the *ND3–12S* block is conserved across both *Geonemertes* species as well as in the inferred ancestral pattern (Fig. 4). It suggests that this segment possibly represents relatively conserved gene arrangement in hoplonemertean mitogenomes.

Such mitochondrial plasticity has not been previously reported in Nemertea [64], but well documented in other invertebrate phyla, including Mollusca, Bryozoa, Tunicata, and Acari (e.g. [67–75]). Gene rearrangements and expansions in mitogenomes have been reported in gastropods, bivalves, and isopods (e.g. [76–78]). Several studies hypothesized to be driven by ecological factors such as parasitism, hypoxia, oxidative stress, and short generation times [72, 79, 80]. In addition, striking accelerations of mitochondrial substitution rates have been linked to cytoplasmic male sterility (CMS) in freshwater snails, where genomic conflict between mitochondria and nuclear genes suppresses male function [81]. However, large-scale analyses in annelids found no correlation between gene rearrangements and ecological traits. Instead, substitution rates and nucleotide composition were identified as the main predictors of gene order variability [82].

In annelids and mollusks, mitochondrial gene rearrangements are often associated with molecular mechanisms such as tandem duplication–random loss (TDRL) and slipped-strand mispairing [74, 83]. The relatively simple regulatory system of mitochondrial genomes—typically limited to the recognition of start and stop codons and lacking complex interactions with distant regulatory elements—may facilitate structural changes with minimal functional disruption [84–86]. Flexibility of mitogenome may help explain the frequency of gene translocations, although the evolutionary mechanisms underlying this plasticity appear to vary among taxa and remain incompletely understood.

Although *Geonemertes* lacks gene duplications in mitogenome, the gene rearrangements and size expansions—especially in *G.* "*pelaensis*"—might represent lineage-specific responses to terrestrial stressors such as desiccation or hypoxia. These findings possibly raise important questions about the evolutionary pressures shaping mitochondrial architecture in terrestrial nemerteans. Future studies incorporating transcriptional profiling, regulatory element mapping, and replication dynamics will be essential to determine whether these changes reflect adaptive evolution or neutral processes. Comparative analyses across subtidal, intertidal, and terrestrial nemerteans may further clarify whether mitogenomic reorganization is driven by ecological transitions or phylogenetic constraints.

Conservation implications

The discovery of previously unrecognized biodiversity within the terrestrial nemertean *Geonemertes* provides important insights into biodiversity and ecological risks in island ecosystems. Misidentification of invasive species can lead to underestimation of their ecological impact and delay appropriate management responses [9]. In the case of *Geonemertes*, previous assumptions regarding its limited predatory role may have obscured the true extent of its ecological impact on native soil fauna in Ogasawara [40]. If the Ogasawara population indeed represents a different species with a distinct ecological niche or predatory behavior, its influence on native ecosystems may differ from that of *G. pelaensis* elsewhere.

While our results clearly support the genetic and morphological distinctiveness of the Ogasawara population, its biogeographic origin remains uncertain. Similarly, the population in Okinawa—although genetically distinct from the Ogasawara lineage—also lacks historical records or biogeographic data. As both Ogasawara and Okinawa Islands are partially designated as World Natural Heritage Sites—with the latter inscribed in 2021 and notable for its insect diversity—the potential ecological role and interactions of terrestrial nemerteans within these ecosystems warrant careful investigation. At present, it is premature to definitively conclude the native or non-native status of either population. A comprehensive investigation involving population genetics, ecological field surveys, and historical reconstruction is essential to determine the evolutionary origin, native range, and ecological significance of previously unrecognized *Geonemertes* lineages.

Conclusion

Our study demonstrates that the terrestrial nemertean population in the Ogasawara Islands—long assumed to be *Geonemertes pelaensis*—constitutes a genetically and morphologically distinct lineage from the widely distributed *G.* "*pelaensis*" found in other parts of the Pacific and western Atlantic. This finding suggests the importance of integrating molecular and morphological data to previously overlooked species-level diversity, particularly in ecologically sensitive island systems.

In addition to morphology and *COX1* divergence, mitogenomic traits—including genome size expansion and gene order rearrangement—further distinguish these lineages. Although we refrain from definitive conclusions about the population's origin, the evidence strongly supports the recognition of at least two species-level lineages of *Geonemertes* in Japan.

The species-level divergence between the two Japanese *Geonemertes* species implies the need for accurate species identification in biodiversity monitoring and invasive-species management on oceanic islands such as Ogasawara, a UNESCO World Heritage Site. More broadly, our findings strengthen the importance of accurate species identification not only for taxonomic studies, but also for understanding ecological dynamics, informing conservation strategies, and protecting native biodiversity.

Abbreviations

AT	Adenine–thymine
bp	Base pairs
CMS	Cytoplasmic male sterility
COX1	Cytochrome *c* oxidase subunit I
CYTB	Cytochrome *b*
ML	Maximum likelihood
NGS	Next-generation sequencing
OTU	Operational taxonomic unit
PCG	Protein-coding gene
PCR	Polymerase chain reaction
RSCU	Relative synonymous codon usage
rRNA	Ribosomal RNA
tRNA	Transfer RNA
UFboot	Ultrafast bootstrap

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12862-025-02468-7.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions

NH conceived and designed the study, conducted fieldwork, morphological observations, and molecular analyses, and drafted the manuscript. SFH and AY contributed to mitogenome sequencing and assembly. YH and NS conducted field sampling and ecological observations in Ogasawara. RU contributed to phylogenetic interpretation and manuscript revision. HK supervised the project and contributed to manuscript writing and editing. All authors read and approved the final manuscript.

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Data availability

The mitochondrial genome sequences generated in this study have been deposited in GenBank under accession numbers [PX290017–PX290023, PX392562–PX392564] (see Table 1 and 2).

Declarations

Ethics approval and consent to participate

Not applicable. This study did not involve humans or vertebrate animals.

Consent for publication

Not applicable. This manuscript does not contain data from any individual person.

Competing interests

The authors declare no competing interests.

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