

Article

The Complete Mitochondrial Genome of the Korean Endemic Polychaete *Phyllodoce koreana* (Lee & Jae, 1985) from Jindong Bay, Korea, with Additional Morphological and Ecological Features

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Abstract: *Phyllodoce koreana* was first described in 1985 in Gwangyang Bay, a semi-enclosed bay in Korea affected by significant organic input from the Seomjin River and dredging activities near the Gwangyang Port. Since then, this Korean endemic species has received limited attention in taxonomic and ecological studies. *Phyllodoce koreana* is known for its resilience to mild disturbances but is vulnerable to severe environmental changes. In this study, *P. koreana* specimens were collected from organically polluted Asian stalked tunicate aquaculture farms at eight sites in Jindong Bay, a location with environmental conditions similar to those of Gwangyang Bay, over the course of five sampling events from March to November. Both bays experience benthic hypoxia in summer due to elevated water temperatures and organic matter accumulation. Phyllodocid specimens were primarily collected in March and November 2023, non-hypoxic periods, suggesting potential seasonal adaptations to environmental fluctuations. The morphological features of the collected specimens were consistent with the original description of *P. koreana*, confirming their identification. Additionally, we reported previously overlooked morphological details, contributing to a more comprehensive taxonomic understanding of the species. We also present, for the first time, the complete mitochondrial genome of this species, comprising 15,559 bp, which provides essential genetic data for future taxonomic and phylogenetic studies. The phylogenetic analysis of protein-coding genes shows that, among 17 related polychaete species, *P. koreana* (family Phyllodocidae) is closely related to the family Goniadidae. Future research should expand our knowledge of polychaete taxonomy by integrating additional mitochondrial genomes and investigating the role of conserved gene synteny within Polychaeta.

Keywords: Phyllodocidae; indicator species; morphology; phylogeny; mitochondrial genome

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1. Introduction

The family Phyllodocidae is a taxonomically diverse and ecologically significant group of polychaetes found in a wide range of marine environments worldwide, from deep-sea hydrothermal vents to shallow coastal bays [1]. With three subfamilies, 30 genera,

and approximately 500 species, the family exhibits remarkable adaptability, thriving in both benthic and pelagic zones [2,3]. Some species retain ancestral traits, reflecting their evolutionary continuity within annelids [1,4,5].

Although Phyllodocidae species lack specialized feeding structures, such as jaws or teeth, they have developed effective strategies for predation. They secrete mucus and toxins to immobilize prey and use an eversible pharynx to pierce and consume tissues or bodily fluids from both live prey and carrion, including mollusks, lugworms, and crustaceans [1,6]. These characteristics allow them to act as both predators and scavengers, contributing significantly to nutrient cycling in benthic ecosystems [1]. The distribution of Phyllodocidae across diverse marine habitats makes them valuable bioindicators [1,6]. Their sensitivity to environmental changes—such as organic pollution, hypoxia, and habitat disturbance—renders them essential for ecological monitoring and environmental assessments [6–9].

Despite their ecological importance and high biodiversity, the members of Phyllodocidae in Korean waters have been largely understudied. Of the approximately 400 polychaete species recorded in Korean marine habitats, only 11 belong to this family [3]. Notably, only three species from the genus *Phyllodoce* (which comprises 134 species globally) have been recorded in Korea. Among these, *Phyllodoce koreana* (Lee & Jae, 1985), first described in 1985 in Gwangyang Bay, has remained almost entirely unstudied both ecologically and taxonomically for the past 40 years [10]. Moreover, the original description of this Korean endemic species includes only a brief species description, which falls short of modern taxonomic standards. The AZTI Marine Biotic Index (AMBI) assesses the response of soft-bottom benthic communities to both natural and human-induced disturbances in coastal and estuarine environments and has been successfully applied across various geographical areas and under diverse environmental impact sources [11]. Although the basis for *P. koreana*'s adaptability to environmental disturbances is unclear, this Korean endemic species is classified in Group II of the AMBI, a system originally designed to evaluate the ecological quality of European coastal waters [3,11]. This AMBI classification suggests that *P. koreana* is tolerant of mild environmental disturbances but is vulnerable to significant environmental changes, such as organic pollution and hypoxia [3,6,12,13]. Similarly, the presence of *P. koreana*, especially in aquaculture areas or semi-enclosed bays of Korea, holds high ecological value as it indicates that the benthic environment is relatively healthy [12,13]. However, since its initial morphological description in 1985, this species has been only briefly mentioned in few studies analyzing the spatial and temporal distribution of macrobenthic communities, and its detailed morphological features and environmental adaptability at the species level remain largely unexplored [12,13]. Mitochondrial gene sequences, especially protein-coding genes (PCGs), are widely recognized as reliable tools for species identification, phylogenetic studies, and the investigation of adaptive evolution associated with habitat environments [14–20]. Yet, no mitochondrial genome has been reported for *P. koreana*, leaving a significant gap regarding its phylogenetic relationships with closely related taxa occurring in various marine habitats.

To address this gap, this study aimed to comprehensively analyze *P. koreana*, a Korean endemic species that has remained little studied for 40 years. Specifically, we identified and reported additional morphological details overlooked in its original description, briefly checked its potential as an indicator species for organic pollution associated with hypoxia, and presented the first complete mitochondrial genome of *P. koreana* to clarify its phylogenetic relationships with related taxa.

2. Materials and Methods

2.1. Sampling Effort

Sediment samples were collected in March, July, September, October, and November 2023 from a total of eight sites at Asian stalked tunicate (*Styela clava*) aquaculture farms in Jindong Bay, Korea, using a 0.1 m² Van Veen grab sampler (Figure 1). The collected samples were elutriated with filtered seawater and passed through a 1 mm sieve to retain biological material. Specimens were initially fixed in 5% buffered formalin for two hours to maintain morphological integrity, and then transferred to 80% ethanol for long-term preservation and morphological analysis.

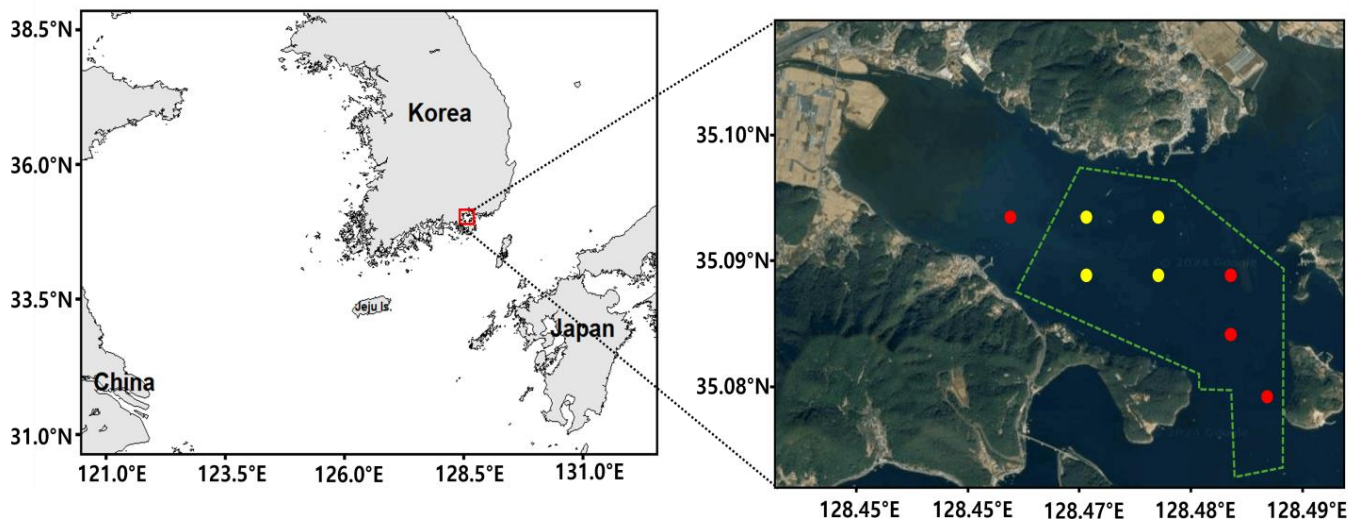


Figure 1. A map of the study area in Jindong Bay on the southern coast of Korea. The circles represent collection sites, with red circles indicating where phyllodocid specimens were collected. The green dotted line indicates the boundary of Asian stalked tunicate aquaculture.

The preserved specimens were sorted and identified under a zoom stereomicroscope (SMZ745T; Nikon, Tokyo, Japan). Key morphological features were documented and photographed using an attached DS-Fi3 camera (Nikon, Tokyo, Japan), ensuring precise observations of diagnostic characteristics for species-level identification.

2.2. DNA Sequencing, Mitochondrial Genome Assembly, and Annotation

Genomic DNA was isolated following the protocol provided with the QIAGEN Blood and Cell Culture DNA Mini Kit (QIAGEN, Hilden, Germany). A 151 bp paired-end library was prepared using the TruSeq DNA Nano 550 Kit (Illumina, Inc., San Diego, CA, USA), and DNA was sequenced on the NovaSeq 6000 platform (Illumina) by DNALink Inc. (Seoul, Republic of Korea) (Table 1).

Prior to mitochondrial genome assembly, raw sequence data were filtered using Trim_Galore (ver. 0.6.10) [21] to remove remaining adapter sequences and bases with Phred quality scores below 20. The trimmed reads were de novo assembled and annotated using MitoZ (ver. 3.6) [22] to construct the mitochondrial genome of *P. koreana*. The assembled genome was visualized using Circos (ver. 0.69-8) [23–25].

Table 1. General information on next-generation sequencing and mitochondrial genome assembly process.

<i>Phyllodoce koreana</i>		
Sequencing	Platform	Illumina NovaSeq 6000
	Library kit	TruSeq DNA Nano
	Read length (bp)	151 × 2
	Insert size (bp)	550
	Number of reads	73,237,515
	Reads of over Q20 (%)	99.1
	Number of bases (bp)	11,058,864,765
After data filtering	Number of reads	73,237,515
	Number of bases (bp)	10,962,924,602
	Total length (bp)	15,559
Mitochondrial genome assembly	GC content (%)	29.14
	Number of protein-coding genes	13

2.3. Substitution Saturation and Phylogenetic Analysis

To elucidate phylogenetic relationships within the class Polychaeta, we conducted a phylogenetic analysis utilizing 13 mitochondrial PCGs extracted from 17 species, including *P. koreana*, with two species from the family Syllidae serving as outgroup representatives. All species included in this study have complete mitochondrial genome sequences available in the National Center for Biotechnology Information (NCBI) database.

To evaluate substitution saturation in the DNA sequences prior to alignment, we used DAMBE (ver. 7.3.32) [26]. Substitution saturation was assessed following the method proposed by Xia et al. [26], which compares the observed substitution saturation index (Iss) to the critical substitution saturation index (Iss.c) under symmetrical and asymmetrical tree assumptions. The DNA sequence data were input into DAMBE, and the program calculated Iss values (0.6462) for both symmetrical and extreme asymmetrical tree topologies. A two-tailed statistical test was used to determine significance. When the observed Iss value is significantly lower than the Iss.c value (0.6628), the data are considered to retain sufficient phylogenetic signal. In this analysis, substitution saturation was not significant (p -value > 0.05) under either tree assumption, indicating that the DNA sequences contained sufficient phylogenetic information for further analysis. Therefore, these sequences were used for phylogenetic reconstruction.

Multiple sequence alignments (MSA) of the 13 PCGs were performed using MAFFT (ver. 7.525) [27] and PAL2NAL (ver. 14.1) [28] with the default parameters. The best-fitting evolutionary model for the dataset was identified based on Bayesian Information Criterion (BIC) analyses using the PartitionFinder2 (ver. 2.1.1) [29]. Based on a partitioning analysis, the 13 PCGs were divided into two subsets, and the GTR+I+G model, a general time-reversible model with Invariant sites (+I) and among-site rate variation modeled using a gamma distribution (+G), was determined to be the most suitable for both subsets.

Phylogenetic trees were reconstructed using both maximum likelihood (ML) and Bayesian Inference (BI) methods. The ML tree was constructed with RAxML-NG (ver. 1.2.2) [30], employing 1000 bootstrap resamples to assess node support. For the BI analysis, we utilized MrBayes (ver. 3.2.7) [31] for DNA sequences. Two independent Markov Chain Monte Carlo (MCMC) runs were performed for 1×10^6 generations with four chains each, sampling every 1000 generations. Chain temperature was set to 0.02 to improve chain mixing. Convergence was assessed using the average standard deviation of split frequencies with a stop value of 0.01. The first 25% of trees were discarded as burn-in, and

a majority-rule consensus tree was constructed from the remaining trees. The resulting phylogenetic trees were visualized using FigTree (ver. 1.4.4) [32].

3. Results and Discussion

3.1. Additional Morphological Characteristics and Ecological Potential

The morphological characteristics of the phyllodocid polychaetes collected from Jindong Bay closely aligned with the original description by Lee and Jae [10] of *Anaitides koreana* from Gwangyang Bay, confirming the species-level identification. However, their 1985 description lacked detailed morphological features, including the precise length and shape of the tentacular cirri on segments 1–3, the shape of the rostrum and denticles on the middle part of the compound spinigerous chaeta, the shape of pre- and post-chaetal lobes, and the exact segment where ventral cirri are first observed—features now considered essential in recent descriptions of new *Phyllodoce* species. Additionally, unclear terminology from the original description (e.g., “upper lip” and “lower lip,” corresponding to supracircular and subacircular lobes, respectively) was revised to align with current taxonomic standards. Species-specific features observed in the Korean specimens from this study are detailed in the following paragraph.

Specimen incomplete, 22 mm long, 2.7 mm wide at median part of body, including parapodia and excluding chaetae for 109 segments. Body long, dorso-ventrally flattened, tapered posteriorly. Prostomium slightly longer than wide, heart-shaped, with anterior protuberance and deep posterior incision with nuchal papilla (Figure 2A,B). Two paired frontal antennae and palps conical, short, robust and of similar lengths (Figure 2B). Antennae and palps approximately one-fourth of prostomial length (Figure 2B). A pair of subepidermal black eyes on the posterior third of prostomium. Proboscis not everted. Peristomium not visible. First two achaetous segments fused. Segment 1 not visible dorsally. Segment 3 with distinct ventral cirri, lacking neuropodial lobes and chaetae (Figure 2C). Four pairs of tentacular cirri on first three segments, cylindrical, biarticulated, tapering towards the tip, with short cirrophores and long cirrostyles. Tentacular cirri arrangement on segments 1–3 expressed as $(O_0^1 + O_1^1) + O_N^1$ (in this formula, symbols indicate the presence or absence of specific features: 1 or 0 = presence or absence of tentacular cirri; S or O = presence or absence of chaetae; N = presence of normal cirri; () = fused segments). Tentacular cirri of segment 1 reaching segment 6. Dorsal and ventral tentacular cirri of segment 2 reaching segments 12 and 5, respectively. Dorsal tentacular cirri of segment 3 extending to segment 10. Parapodia first appearing from segment 4, uniramous, divided into two lobes by an aciculum.

Prechaetal lobes bilobate, asymmetrical, rounded, with supracircular lobes slightly longer than subacircular lobes. Postchaetal lobes rounded, smaller than prechaetal lobes. Dorsal cirri first appearing from segment 4, symmetrical, with well-developed cirrophores, without dorsal expansions. Dorsal cirri of anterior segments symmetrical, heart-shaped, with rounded tip; dorsal cirri of posterior segments slender, asymmetrical, kidney-shaped. Ventral cirri first appearing from segment 3. Ventral cirri horizontally oriented in relation to lobes, rounded, distally pointed, asymmetrical, dorso-ventrally flattened; ventral cirri of posterior segments approximately 1.5 times longer than parapodial lobes, tapering towards posterior end. Compound spinigerous chaetae first appearing from segment 4. All chaetae compound spiniger with one serrated edge; 12–13 chaetae per parapodium (Figure 2D). Rostrum of chaetal shaft surrounded by irregularly distributed conical denticles. Pygidium missing.

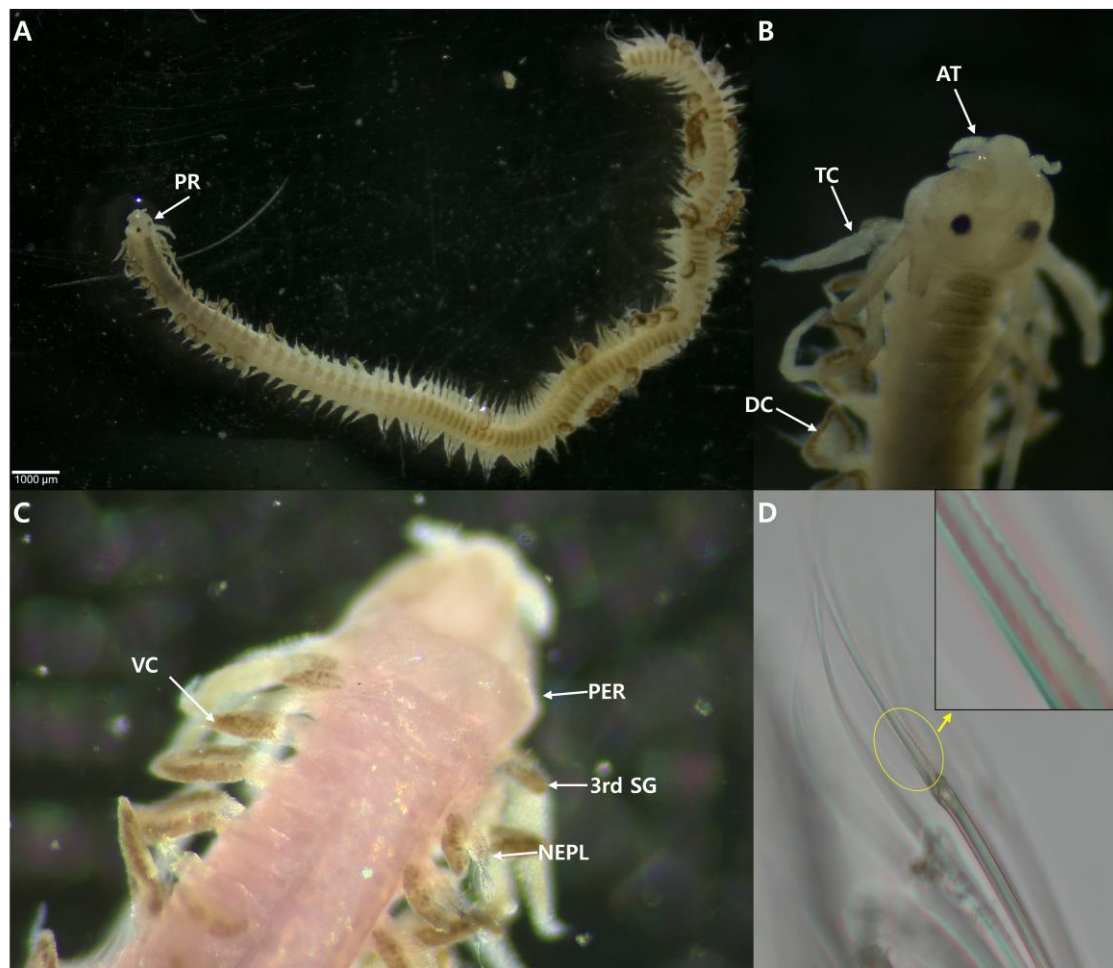


Figure 2. *P. koreana*: (A) dorsal view; (B) prostomium, dorsal view; (C) anterior segment, ventral view; (D) composite chaetae (PR: prostomium; PER: peristomium; AT: antennae; TC: tentacular cirrus; DC: dorsal cirri; VC: ventral cirri; NEPL: neuropodial lobe of 4th segment; SG: segment).

All examined specimens were incomplete; due to damage, the posterior end of the body (i.e., segments near pygidium), including anal cirri, could not be observed in this study. However, the other available key features of our Korean specimens, including the tentacular cirri arrangement (although it was about two segments longer in the original description), parapodial structure, and type of chaetae, align closely with the original description, confirming the accurate identification of *P. koreana* [10]. Notably, *P. koreana* has distinct ventral cirri but lacks neuropodial lobes and chaetae on the third segment. These morphological characteristics distinguish *P. koreana* from the other two Korean *Phyllodoce* species, *Phyllodoce chinensis* Uschakov & Wu, 1959 [33] and *Phyllodoce maculata* (Linnaeus, 1767) [34], which exhibit different combinations of ventral cirri, neuropodial lobes, and chaetae on the third segment. Specifically, ventral cirri are present in *P. koreana* and *P. maculata* but absent in *P. chinensis*; neuropodial lobes and chaetae are present in *P. maculata* but absent in *P. koreana* and *P. chinensis*.

Gwangyang Bay, Korea, the type locality of *P. koreana*, is frequently disturbed by the influx of significant amounts of organic matter from the Seomjin River and periodic dredging around the Port of Gwangyang. The inner part of this semi-enclosed bay experiences poor ocean current circulation, resulting in frequent occurrences of elevated organic pollution and hypoxic water masses during the summer. Since the 1990s, *P. koreana* has mainly been observed in winter and spring, with minimal occurrences during the summer in Gwangyang Bay [35–37]. Although detailed ecological studies at the species level

for *P. koreana* are limited, its spatiotemporal distribution patterns resemble those in other semi-enclosed bays in the western and southern regions of Korea that share similar environmental conditions with Gwangyang Bay [38]. Notably, in Deukryang Bay, *P. koreana* exhibits a distribution pattern that contrasts with the mass occurrences of *Lumbrineris longifolia* and *Heteromastus filiformis*, species known as indicators of organic pollution, suggesting that *P. koreana* may be particularly sensitive to high levels of organic pollution and environmental disturbance [39].

Jindong Bay, the study area for the present research, is a semi-enclosed bay characterized by limited ocean current circulation [40]. Until the 1990s, the bay was a major site for the large-scale aquaculture of clams and oysters; however, production declined due to the degradation of fishing grounds and frequent occurrences of red tides. In 2001, the Asian stalked tunicate (*Styela clava*), locally known as “Mideodeok” in Korea, was officially designated as an aquaculture species. Since then, Jindong Bay has served as a primary aquaculture site for Mideodeok, contributing to approximately 70% of Korea’s total production [40–42]. In the summer, large-scale farming of the Asian stalked tunicate leads to a significant buildup of organic matter on the seafloor, often resulting in low-oxygen conditions in the bottom layer [40,42,43]. Consistent with previously observed distribution patterns, *P. koreana* of the present study was absent in July, September, and October, when organic pollution and low-oxygen conditions were the most prevalent, and was only encountered in March and November, when the benthic environment was relatively stable. Furthermore, phyllodocid specimens were completely absent from sites located in the inner areas of the aquaculture farms (indicated by the yellow circles in Figure 1), where organic pollution was presumed to be more severe, even during months when phyllodocids were observed.

The spatial and temporal distribution patterns of *P. koreana* have been briefly mentioned in fragmented studies on macrobenthic communities in Korea since the species was first described morphologically 40 years ago. Although the species-level environmental adaptability of *P. koreana* remains largely unexplored, the findings of this study, combined with previous reports, suggest that *P. koreana* may serve as a potential bioindicator sensitive to severe organic pollution and hypoxic conditions in benthic environments, particularly during summer. Future research should focus on the long-term monitoring of *P. koreana* populations in various semi-enclosed bays and aquaculture zones to confirm its bioindicator potential. Additionally, investigations into its physiological responses to organic pollution and hypoxia, coupled with molecular approaches, could provide deeper insights into its ecological role and adaptive strategies.

3.2. Mitochondrial Genome Characterization

In this study, we generated a total of 73,237,515 raw paired-end reads from the genome of the endemic polychaete *P. koreana* using Illumina sequencing. Following the quality control process, 10,962,924,602 clean reads with a Q-score of 20 or higher were retained, representing 99.10% of the original reads (Table 1).

Utilizing these high-quality reads, we successfully completed the first mitochondrial genome of *P. koreana* from aquaculture farms in Jindong Bay. This mitochondrial genome covered 15,559 bp (Figure 3; GenBank: PQ510072) and contained a total of 37 genes: 13 PCGs (COX1, COX2, COX3, COB, ND1, ND2, ND3, ND4, ND4L, ND5, ND6, ATP6, and ATP8), 2 rRNA genes (12S rRNA and 16S rRNA), and 22 tRNA genes (Table 2 and Figure 3). All 37 genes were located on the heavy strand, comprising 94.97% of the mitochondrial genome, while the 13 PCGs accounted for 65.06%. All PCGs were initiated with the ATG start codon, and the stop codons were either TAA (for eight genes) or TAG (for five genes) (Table 2). The nucleotide composition of the mitochondrial genome is as follows: A: 28.68%;

T: 42.18%; G: 18.02%; and C: 11.12%. An A + T content of 70.86% and a G + C content of 29.14% were yielded.

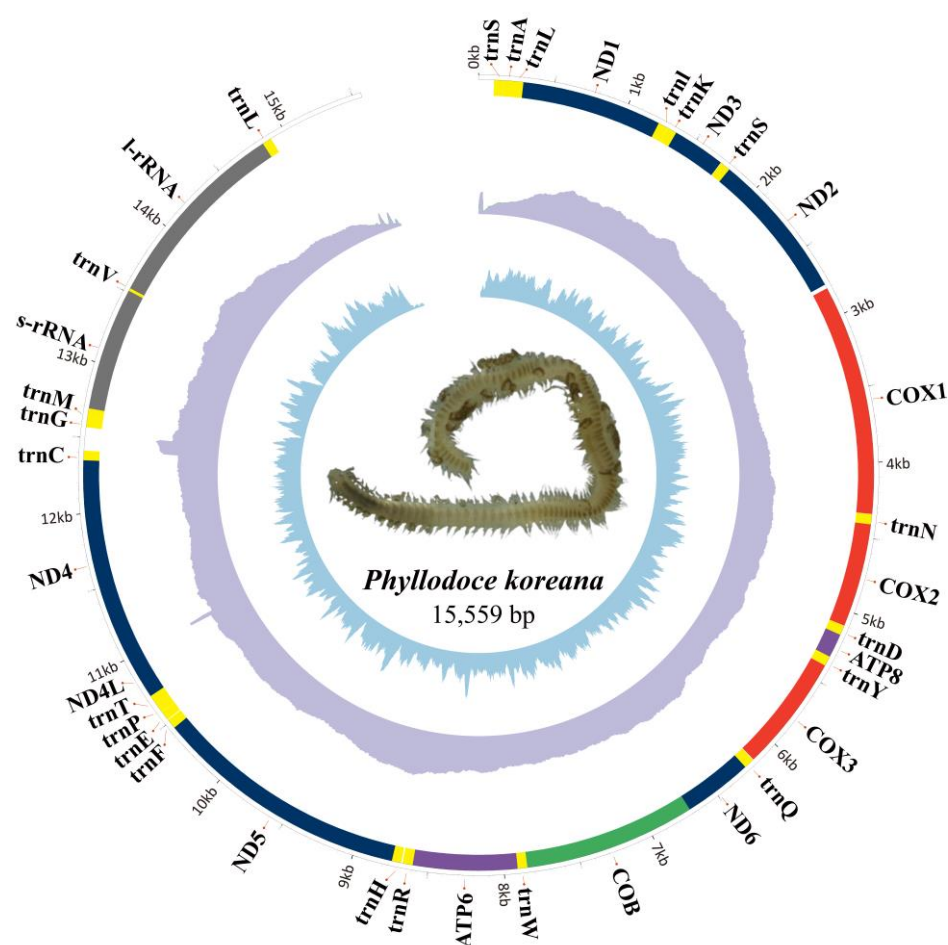


Figure 3. The mitochondrial genome map of *P. koreana* inhabiting Asian stalked tunicate aquaculture farms in Jindong Bay. The innermost ring is a blue bar plot representing the GC content. The middle purple ring shows the sequencing depth. The outermost ring depicts the genes in order according to color: red for COX genes, violet for ATP synthase, green for COB, and navy for ND genes. The two rRNA genes are shown in gray, and the 22 tRNA genes are shown in yellow.

Table 2. Annotation of *P. koreana* mitochondrial genome.

Gene	Position	Length (bp)	Initiation Codon	Stop Codon	Anticodon	Strand
tRNA-Ser (trnS)	103–168	67			TGA	+
tRNA-Ala (trnA)	169–232	65			TGC	+
tRNA-Leu (trnL)	233–296	65			TAA	+
NADH dehydrogenase subunit 1 (ND1)	297–1233	938	ATG	TAA		+
tRNA-Ile (trnI)	1231–1295	66			GAT	+
tRNA-Lys (trnK)	1297–1361	66			TTT	+
NADH dehydrogenase subunit 3 (ND3)	1362–1715	355	ATG	TAG		+
tRNA-Ser (trnS)	1719–1786	69			TCT	+

Table 2. Cont.

Gene	Position	Length (bp)	Initiation Codon	Stop Codon	Anticodon	Strand
NADH dehydrogenase subunit 2 (ND2)	1787–2777	992	ATG	TAA		+
cytochrome c oxidase subunit I (COX1)	2805–4340	1537	ATG	TAA		+
tRNA-Asn (trnN)	4343–4409	68			GTA	+
cytochrome c oxidase subunit II (COX2)	4410–5099	691	ATG	TAG		+
tRNA-Asp (trnD)	5101–5164	65			GTC	+
ATP synthase F0 subunit 8 (ATP8)	5165–5326	163	ATG	TAG		+
tRNA-Tyr (trnY)	5325–5389	66			GTA	+
cytochrome c oxidase subunit III (COX3)	5391–6170	781	ATG	TAA		+
tRNA-Gln (trnQ)	6174–6242	70			TTG	+
NADH dehydrogenase subunit 6 (ND6)	6243–6710	469	ATG	TAG		+
cytochrome b (COB)	6703–7848	1147	ATG	TAA		+
tRNA-Trp (trnW)	7847–7910	65			TCA	+
ATP synthase F0 subunit 6 (ATP6)	7911–8606	697	ATG	TAG		+
tRNA-Arg (trnR)	8605–8671	68			TCG	+
tRNA-His (trnH)	8682–8743	32			GTG	+
NADH dehydrogenase subunit 5 (ND5)	8744–10,451	1709	ATG	TAA		+
tRNA-Phe (trnF)	10,452–10,516	66			GAA	+
tRNA-Glu (trnE)	10,522–10,583	63			TTC	+
tRNA-Pro (trnP)	10,585–10,648	65			TGG	+
tRNA-Thr (trnT)	10,649–10,711	64			TGT	+
NADH dehydrogenase subunit 4L (ND4L)	10,712–10,999	289	ATG	TAA		+
NADH dehydrogenase subunit 4 (ND4)	10,993–12,351	1360	ATG	TAA		+
tRNA-Cys (trnC)	12,351–12,414	65			GCA	+
tRNA-Gly (trnG)	12,569–12,631	64			TCC	+
tRNA-Met (trnM)	12,632–12,695	65			CAT	+
12S ribosomal RNA (s-rRNA)	12,694–13,521	829				+
tRNA-Val (trnV)	13,514–13,577	65			TAC	+
16S ribosomal RNA (l-rRNA)	13,533–14,879	1348				+
tRNA-Leu (trnL)	14,837–14,898	63			TAG	+

3.3. Phylogeny and Synteny

Practical constraints often necessitate the use of genetic data over morphological characteristics in modern phylogenetic studies. Time and resource limitations in examining type specimens, data quality issues from relying on older studies in the literature without direct observation, and the occasional unavailability of original descriptions or specimens make comprehensive morphological comparisons challenging. Therefore, in our study, we conducted a phylogenetic analysis using publicly available genetic data.

To clarify the phylogenetic relationships within the order Phyllodocida, with a particular focus on *P. koreana*, we constructed an ML phylogenetic tree utilizing the nucleotide sequences of 13 mitochondrial PCGs from 13 representative species (Table 3). This multi-

gene approach provides a more comprehensive and informative basis for phylogenetic inference than single-gene analyses [15,16]. Our results delineate clear familial clades, with the Syllidae family being a distinct outgroup (Figure 4, left). Each family formed monophyletic clusters with strong statistical validation (bootstrap value [BS] = 100%), reinforcing topological patterns observed in previous phylogenetic analyses employing diverse genetic markers across various polychaete taxa [14,16].

Table 3. The 13 mitochondrial genomes used in the analysis with their taxonomic information and GenBank accession numbers.

Order	Family	Genus	Species	Accession Number
Phyllodocida	Syllidae	<i>Ramisyllis</i>	<i>Ramaryllis multicaudata</i>	KR534502.1
		<i>Typosyllis</i>	<i>Typosyllis Antoni</i>	KX752426.1
	Aphroditidae	<i>Aphrodita</i>	<i>Aphrodita australis</i>	MN334532.1
	Polynoidae	<i>Eunoe</i>	<i>Eunoe nodosa</i>	MW557378.1
		<i>Harmothoe</i>	<i>Harmothoe imbricata</i>	MK858187.1
	Nereididae	<i>Hediste</i>	<i>Hediste diversicolor</i>	MW377219.1
		<i>Namalycastis</i>	<i>Namalycastis abiuma</i>	KU351089.1
		<i>Nectoneanthes</i>	<i>Nectoneanthes oxypoda</i>	OL782599.1
		<i>Nereis</i>	<i>Nereis pelagica</i>	OL782598.1
	Hesionidae	<i>Leocrates</i>	<i>Leocrates chinensis</i>	OP104125.1
		<i>Sirsoe</i>	<i>Sirsoe methanicola</i>	OM914591.1
	Nephtyidae	<i>Micronephthys</i>	<i>Micronephthys minuta</i>	OR123448.1
	Phyllodocidae	<i>Phyllodoce</i>	<i>Phyllodoce medipapillata</i>	PP035857.1
			<i>Phyllodoce koreana</i>	PQ510072
	Goniadidae	<i>Goniada</i>	<i>Goniada japonica</i>	KP867019.1
	Pilargidae	<i>Pilargis</i>	<i>Pilargis verrucosa</i>	OR123439.1
		<i>Glyphohesione</i>	<i>Glyphohesione klatti</i>	OR123443.1

Our phylogenetic analysis revealed well-supported monophyletic clades within Phyllodocida, with each family showing high bootstrap support (BS = 100%). Notably, Phyllodocidae formed a strongly supported sister-group relationship with Goniadidae (BS = 100%), suggesting their divergence from a common ancestor. This relationship is further corroborated by the mitochondrial gene arrangement patterns observed in both families.

To complement the phylogenetic analysis, we conducted a comparative assessment of mitochondrial gene synteny across the examined polychaete species. The gene arrangement patterns of the 13 PCGs exhibited considerable variability (Figure 4, right), underscoring the dynamic nature of mitochondrial genome evolution in Polychaeta. Notably, we observed that gene order remains relatively consistent within Phyllodocidae and Nereididae, with gene synteny remaining consistent within the genus *Phyllodoce*. This syntenic conservation may serve as an additional phylogenetic signal, complementing sequence-based analyses. Our phylogenetic analysis, based on 17 species across nine families, provides robust support for the placement of *P. koreana* within Phyllodocida. The conserved gene order patterns observed between *P. koreana* and *P. medipapillata* suggest that this genomic feature could serve as a phylogenetically informative marker. However, the limited availability of

comparative data from other *Phyllodoce* species constrains our ability to determine whether this gene order conservation is characteristic of the entire genus.

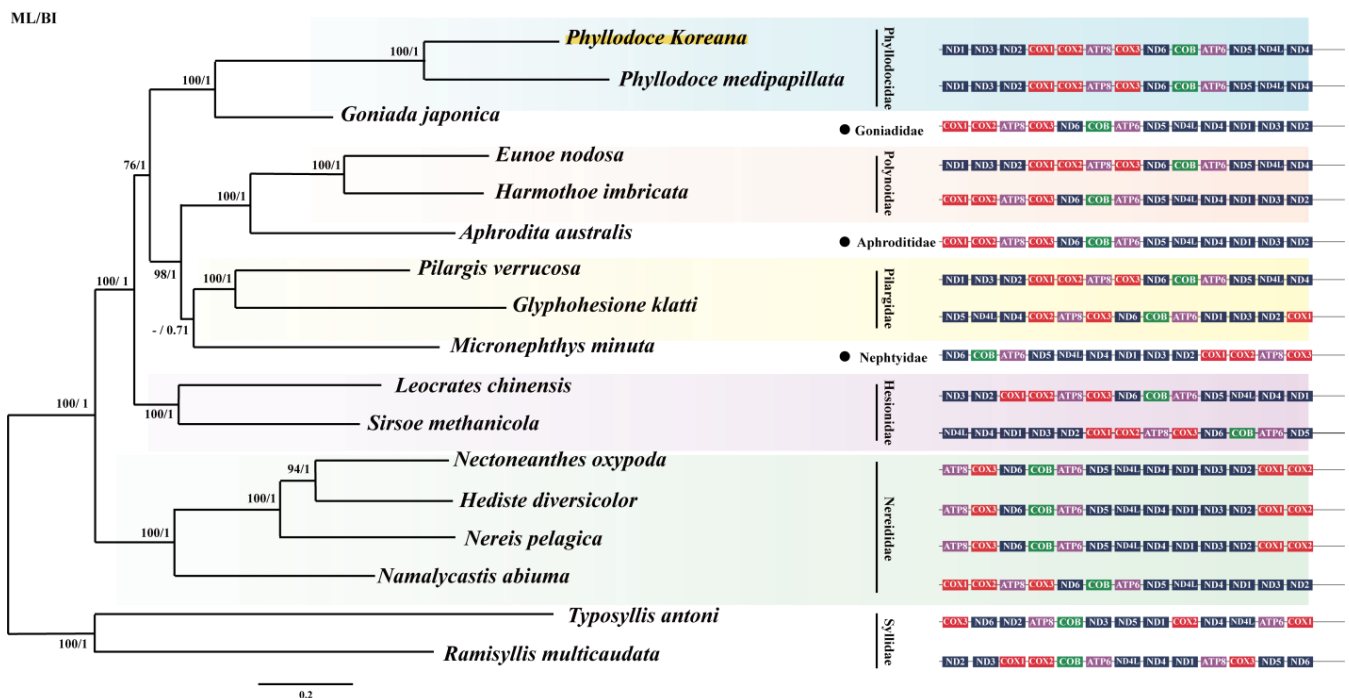


Figure 4. Two species in the family Syllidae were used as the outgroup. Bootstrap support values > 60% based on the maximum likelihood (ML) and Bayesian Inference (BI) methods (ML/BI) are included at each node. The background colors at the right delineate different families. The scale bar represents the relative substitution rates per site. The synteny of the 13 mitochondrial protein-coding genes is shown to the right of the tree.

The results highlight the need for broader taxonomic sampling within Phyllodocidae, particularly including more representatives from the genus *Phyllodoce*, to refine genus-level relationships. Expanding the dataset would not only enhance our understanding of the phylogenetic framework but also contribute to deeper insights into the evolutionary history of polychaetes. Future research should aim to include additional representatives from underexplored lineages, which will not only refine the phylogenetic framework with facilitating a deeper understanding of the evolutionary history of polychaetes as a whole. Enhanced genomic data could provide context for interpreting gene synteny patterns and their potential functional implications, contributing to a more complex view of polychaete evolution.

4. Conclusions

We analyzed comprehensive biological data for the Korean endemic polychaete *P. koreana*, which were collected from Asian stalked tunicate aquaculture farms in Jindong Bay. The morphological and ecological features of the collected specimens align with those in the original description of *P. koreana*. Phylogenetic analyses utilizing all 13 PCGs from the mitochondrial genomes of Phyllodocida species reported to date, including the *P. koreana* genome generated in this study, support phylogenetic relationships found in previous studies. Notably, it provides implications for the monophyly of Phyllodocidae and its sister group relationship with Goniadidae. Furthermore, a synteny analysis of the 13 PCGs revealed within-genus and within-family similarity, but diverse gene arrangement patterns were observed across the order. Our findings provide insights into the evolutionary relationships of mitochondrial genomes in Phyllodocidae. Future studies should aim

to expand the scope of our knowledge of polychaete taxonomy, integrating additional mitochondrial genomes, and investigate the functional significance of conserved gene synteny in Polychaeta.

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