J. Appl. Biol. Sci., 19 (3): 203-209, September 2025
https://dx.doi.org/10.71336/jabs.1454
RESEARCH ARTICLE

Journal of Applied Biological Sciences
EISSN: 2146-0108; License: CC BY 4.0
Anatolia Academy of Sciences Open Access

A phylogenetic analysis of the polychaeta based on mitochondrial CO1 and 16S rRNA gene sequences

Jared M. Corpuz[1], Wilhelm M. Javier[1], Eleanor S. Austria[1], Mary Jhane G. Valentino[2]

[1]*Biology Department, Adamson University, 900 San Marcelino St., Ermita Manila, Philippines*

[2]*Department of Biological Sciences, College of Science, Science City of Numon, Nueva Ecija, Philippines*

*Correspondence:
Mary Jhane G. Valentino
Email: maryjhane.valentino@clsu2.edu.ph

Received: February 3, 2025
Revised: September 08, 2025
Accepted: September 10, 2025

ABSTRACT: Annelids, a phylum of segmented worms, are a diverse group inhabiting terrestrial, freshwater, and marine environments. Recent molecular studies challenge traditional classifications, revealing the inclusion of taxa like *Echiura*, *Sipuncula*, and non-monophyletic relationships within the group. This study utilized mitochondrial cytochrome c oxidase I (CO1) and 16S ribosomal RNA (16S rRNA) gene sequences to construct phylogenetic trees for 50 Polychaeta species using maximum likelihood analysis. CO1 gene exhibited limitations in phylogenetic relationship, while 16S rRNA excelled in elucidating broader taxonomic relationships with strong support for evolutionary relationships. Results reaffirmed the non-monophyly of Polychaeta and highlighted their distinct evolutionary patterns, such as independent lineage development within the Polynoidae family. These findings contribute to understanding polychaete diversity and evolutionary history, emphasizing the need for integrating multiple molecular markers for comprehensive phylogenetic analysis.

KEYWORDS: CO1, non-monophyletic, polychaeta, 16S rRNA

INTRODUCTION

Annelids also known as segmented or ringed worms, is a diverse phylum within the Lophotrochozoa that inhabits various environments, including terrestrial, freshwater, and marine ecosystems. Many species are highly adapted to specific ecological niches like intertidal and pelagic zones, as well as hydrothermal vents [1].

Traditionally, Annelida is divided into two main groups: Clitellata that includes earthworms and leeches; and Polychaeta which are called marine worms. But recent molecular studies indicate that Annelida may encompass other taxa previously classified as separate phyla, such as Echiura and Sipuncula. Additionally, these studies suggest that Clitellata are derived from annelids, which challenges the traditional classification of Polychaeta [2].

The present study focused on polychaetes. They are important in benthic community dynamics and contribute significantly to processes like recycling, sediment reworking, bioturbation, and the burial of organic matter in marine sediments. Polychaetes often dominate microbenthic taxa in terms of both species diversity and abundance, sometimes comprising over half of the organisms in soft-bottom habitats [3]. The diversity of annelids is clearly reflected in the significant morphological variations observed among different polychaete representatives. Historically, this led taxonomists to establish as many as 24 distinct 'orders' within this group [4].

Phylogenetic analysis is a tool in understanding the evolutionary relationships among organisms. The molecular markers such as the mitochondrial cytochrome c oxidase I (CO1) gene and the nuclear 16S ribosomal RNA (16S rRNA) gene, are commonly used in constructing phylogenetic trees. The CO1 gene, widely recognized for its role in DNA barcoding, is particularly effective in distinguishing closely related species due to its high variability. In contrast, the 16S rRNA gene, known for its conserved regions, is often used to resolve relationships at broader taxonomic levels [5].

Understanding the deeper relationships within the group has been difficult thus, the main working hypothesis for polychaeta phylogeny remained to be based on morphological cladistic analysis [6]. This study aims to compare phylogenetic trees derived from mitochrondrial genes of CO1 and 16S rRNA gene sequences for polychaete and explore their evolutionary relationships.

METHODOLOGY

Data Collection

Sequences of the mitochondrial cytochrome oxidase subunit 1 (CO1) gene and 16S rRNA were obtained from National Center for Biotechnology Information Genbank. The study is focused on selecting representative species from Polychaeta. Presented in table 1 is the list of the species and their corresponding GenBank accession numbers that is used for this study.

Phylogenetic Analysis

The Phylogenetic reconstruction was performed in the function "build" of Environment for Tree Exploration (ETE3 3.1.3) [7] as implemented on the GenomeNet (https://www.genome.jp/tools/ete/). The selected sequences of CO1 and 16s rRNA gene were aligned with Clustal Omega v1.2.4 with the default options [8] and the tree was constructed using FastTree v2.1.8 with default parameters [9].

Table 1. list of the species and their corresponding GenBank accession numbers used in this study.

Order	Subclass	Family	Species	Accession number
Errantia	Eunicida	*Dorvilleidae*	*Veneriserva pygoclava*	OR449961
Errantia	Eunicida	*Onuphidae*	*Hyalinoecia robusta*	PP790749
Errantia	Eunicida	*Onuphidae*	*Diopatra cuprea*	NC_058588
Errantia	Eunicida	*Amphinomidae*	*Eurythoe complanata*	KT726962
Errantia	Eunicida	*Eunicidae*	*Marphysa victori*	NC_060759
Errantia	Phyllodocida	*Pilargidae*	*Pilargis verrucosa*	NC_087805
Errantia	Phyllodocida	*Pilargidae*	*Synelmis amoureuxi*	NC_087806
Errantia	Phyllodocida	*Nereididae*	*Perinereis wilsoni*	NC_085286
Errantia	Phyllodocida	*Nereididae*	*Perinereis vancaurica*	NC_065095
Errantia	Phyllodocida	*Nereididae*	*Perinereis linea*	NC_063944
Errantia	Phyllodocida	*Nereididae*	*Perinereis camiguinoides*	NC_065094
Errantia	Phyllodocida	*Nereididae*	*Nereis pelagica*	OL782598
Errantia	Phyllodocida	*Nereididae*	*Nectoneanthes uchiwa*	ON960182
Errantia	Phyllodocida	*Nereididae*	*Laeonereis culveri*	KU992689
Errantia	Phyllodocida	*Nereididae*	*Nectoneanthes oxypoda*	NC_086458
Errantia	Phyllodocida	*Polynoidae*	*Eunoe nodosa*	NC_060302
Errantia	Phyllodocida	*Polynoidae*	*Branchinotogluma segonzaci*	NC_062818
Errantia	Phyllodocida	*Polynoidae*	*Hyperhalosydna striata*	NC_063122
Errantia	Phyllodocida	*Hesionidae*	*Sirsoe methanicola*	NC_064058
Errantia	Phyllodocida	*Hesionidae*	*Micropodarke fujianensis*	PP003976
Errantia	Phyllodocida	*Hesionidae*	*Leocrates chinensis*	NC_066969
Errantia	Phyllodocida	*Nephtyidae*	*Micronephthys minuta*	NC_087810
Errantia	Phyllodocida	*Phyllodocidae*	*Phyllodoce medipapillata*	NC_087881
Errantia	Phyllodocida	*Syllidae*	*Clavisyllis tenjini*	NC_077651
Errantia	Phyllodocida	*Chrysopetalidae*	*Chrysopetalum debile*	NC_060816
Errantia	Phyllodocida	*Polynoidae*	*Harmothoe imbricata*	NC_081955
Errantia	Phyllodocida	*Polynoidae*	*Branchipolynoe onnuriensis*	NC_064376
Errantia	Phyllodocida	*Microphthalmidae*	*Struwela camposi*	PP035858
Errantia	Phyllodocida	*Syllidae*	*Ramisyllis kingghidorahi*	NC_065765
Errantia	Phyllodocida	*Glyceridae*	*Glycera capitata*	KT989320
Errantia	Phyllodocida	*Antonbruunidae*	*Antonbruunia milenae*	NC_087808
Errantia	Phyllodocida	*Chrysopetalidae*	*Craseoschema thyasiricola*	NC_060815
Sedentaria	Sabellida	*Siboglinidae*	*Riftia pachyptila*	PQ468431
Sedentaria	Sabellida	*Siboglinidae*	*Lamellibrachia columna*	NC_082190
Sedentaria	Sabellida	*Siboglinidae*	*Lamellibrachia barhami*	NC_082191
Sedentaria	Sabellida	*Siboglinidae*	*Escarpia spicata*	ON929996
Sedentaria	Sabellida	*Siboglinidae*	*Oligobrachia dogieli*	OR804078
Sedentaria	Sabellida	*Siboglinidae*	*Siboglinum plumosum*	NC_084115
Sedentaria	Sabellida	*Siboglinidae*	*Arcovestia ivanovi*	NC_082193
Sedentaria	Sabellida	*Siboglinidae*	*Seepiophila jonesi*	NC_026861
Sedentaria	Sabellida	*Siboglinidae*	*Alaysia spiralis*	ON929998
Sedentaria	Spionida	*Spionidae*	*Polydora hoplura*	NC_061377
Sedentaria	Spionida	*Spionidae*	*Prionospio cirrifera*	OR935937
Sedentaria	Spionida	*Spionidae*	*Prionospio fallax*	OR935929
Sedentaria	Spionida	*Spionidae*	*Aurospio banyulensis*	OR935933
Sedentaria	Sabellida	*Serpulidae*	*Ficopomatus enigmaticus*	LC757642
Sedentaria	Scolecida	*Maldanidae*	*Lumbriclymenella robusta*	OP537514
Sedentaria	Scolecida	*Maldanidae*	*Asychis amphiglyptus*	NC_069297
Sedentaria	Terebellida	*Terebelliformia*	*Paralvinella palmiformis*	NC_064503
*Cestoda	*Eucestoda	**Diphyllobothriidae*	**Spirometra erinaceieuropaei*	KJ599680

*Outgroup

RESULTS AND DISCUSSION

The reconstruction of the phylogenetic tree of polychaete worms based on the nucleotide sequences of CO1 (Figure 1) and 16s rRNA genes (Figure 2.) using Maximum likelihood approach shows the topology tree having different bootsrap values. The CO1 tree provided finer resolution within some species-level clades, whereas the 16S rRNA tree excelled in identifying broader taxonomic groupings.

As shown in Figure 1, the phylogenetic tree of the mitochondrial genes (CO1 gene) shows that the 50 selected species are within the polychaeta class. Under the order Errantia order, Nereididae family shows a strong support with 100 bootstrap values. The Polynoidae family also indicates a strong support within the Errantia order with 99.8 bootstrap value. In addition, Sabellida, Eunicida and Spionida share common ancestor, and it is grouped in order Sedentaria. Meanwhile, Eunicida diverges within the Sedentaria with a bootstrap value of 36, that indicates a weak support. This means that Polychaeta were reaffirmed to be non-monophyletic because of the exclusion *of Echiura*, *Pogonophora*, and *Clitellata* [10]. Accordingly, a non-monophyletic group exclude some descendants of the common ancestor or include members from different evolutionary lineages which indicates that a group is not a true natural grouping in terms of evolutionary relationships [11]. This is also shown in the phylogenetic tree in Figure 1, wherein *Clavisylis tenjini*, *Antonbruunia milenae*, and *Phyllodoce medipapillata* diverged earlier which means they have undergone more recent evolutionary changes.

The outgroup which is a Platyhelminthes species *Spirometra erinaceieuropaei* and the *Ficopomatus enigmaticus* a polychaete shows in the CO1 phylogenetic tree that they are related with strong support of 99.5 bootsrap value. In the study of Kobayashi et al. [12], a phylogenetic analysis using all annelid mitogenomes was not conducted because *Serpulidae* mitogenomes which is the family of the *Ficopomatus enigmaticus* are not suitable for inferring phylogenetic relationships within Annelida, due to the long branches of serpulids.

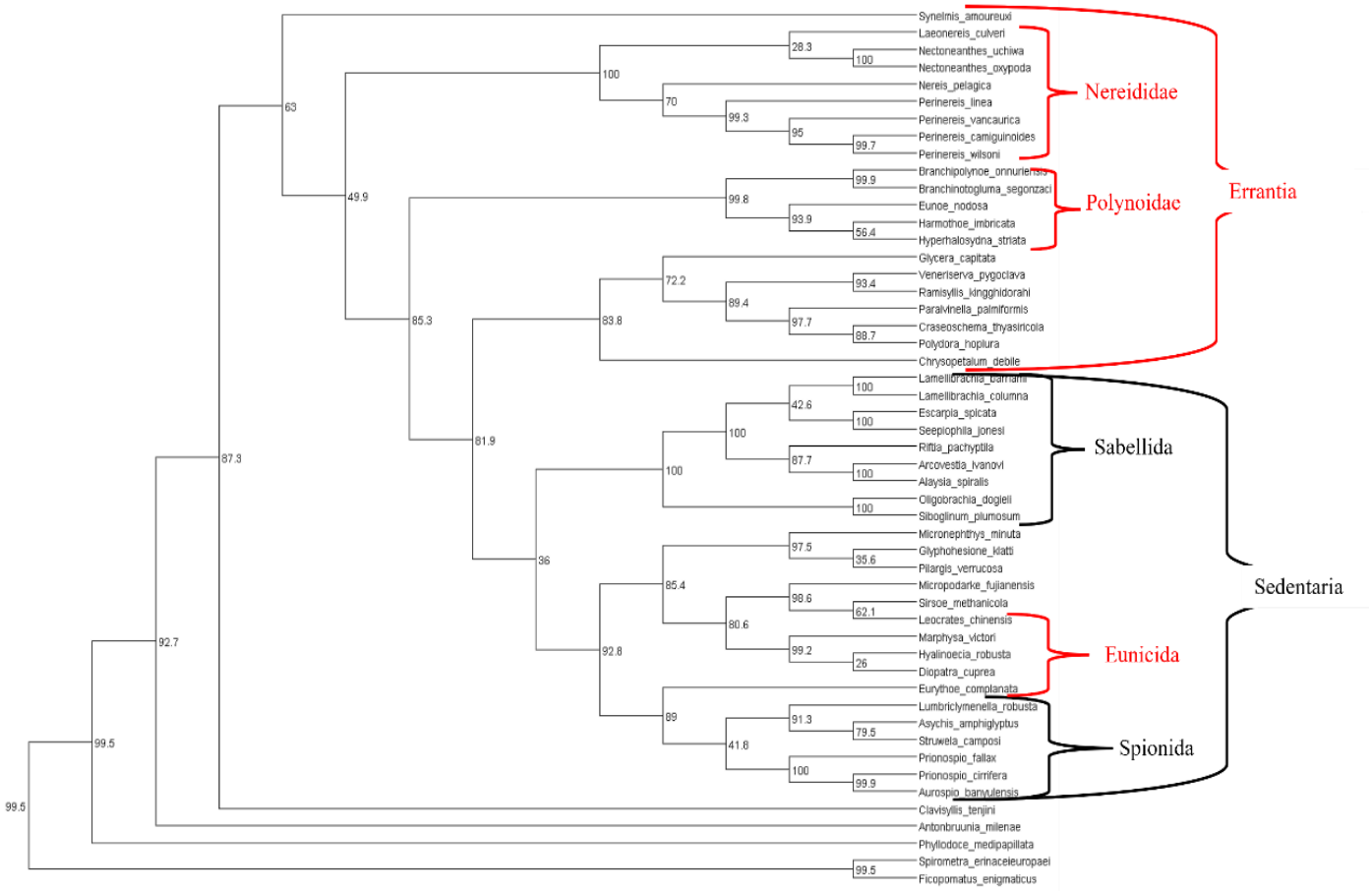

Figure 1. Phylogenetic Tree Based on CO1 Sequences. Bootstrap values are shown at branch points, indicating the reliability of the inferred relationships.

The COI gene may not be ideal for phylogenetic analysis when used as the sole molecular marker. Another study of Halanych et al. [6] using the CO1 gene might not produce reliable or well-supported phylogenetic trees in some annelids, primarily because the standard primers do not amplify the gene effectively in certain annelid groups. This primer inefficiency could lead to incomplete or poor-quality data, affecting the accuracy of the phylogenetic tree. But Canales-Aguirre et al. [13], explained that their phylogenetic tree of CO1 gene in benthic polychaetes in Figure 2. In Terebellida and Eunicida do not form a monophyletic group. When analyzing the molecular taxonomic position of the species sequenced it is clustered within a specific clade, but not necessarily within the same broader morphological clade. So they conclude that the using CO1 gene at taxonomic higher classification it tends to be unreliable.

Using the of 16s rRNA of 20 selected species of polychaeta (Figure 3), *Polynoidae* family shows a strong support of 100 bootsrap value. The branching pattern in this clade shows that Polynoidae is distinct from other Errantia . The distinct placement of *Polynoidae* suggests that while they are part of Errantia, they have been evolving independently for a long time. This could explain why they form a separate branch in the tree, showing a higher degree of divergence from other Errantia spp , which may have undergone more recent evolutionary changes.

The Errantia including *Craseoschema thyasinicola, Micronephthys minuta, Nectoanthes oxypoda, Glycera capitata, Antonbruunia milenae* and *Sedentaria* spp which includes *Lamlibrachia columna, Lamelibracha barhami, Arcovestia ivanovi, Escarpia spicatam Lumbriclymenella robusta, Asychis amphiglypus* were derived from a common ancestor together with the Sedentaria (*Lamelibrachia columna, lamilbrachia barhami, Arcovesta ivanovi, Escarpia spicata, Lumbriclymenella robusta, Ficopomatus enigmaticus* and *Asychis amphiglyptus)*with 100- bootsrap value which means that they closely related to each other. The 16s rRNA genes shows a strong support for inferring evolutionary relationships.

The 16s rRNA in most studies so far have focused on a short 450–500 nucleotide fragment of this gene, using primers designed by Palumbi's group [14]. This segment is generally effective for analyzing relationships at the intraspecific and intrageneric levels [6]. In the clade Sedentaria species including the *Aurospio banyulensis* and the other species of *Errantia* that includes *Diopatra cuprea, Marphysa victori and Chrysopetalm debile* is poorly supported relationship indicating weak support of 17.2 bootstrap values because Eunicida (*Diopatra cuprea*) and Phylodocida (*Marphysa victori and Chrysopetalm debile*) is unexpectedly have a relationship within the Sedentaria clade. In the study of Hall et al. [15] that they need to increase taxon sampling in underrepresented groups like Phyllodocida, Spionida, Eunicida, and Sabellida could improve the congruence between morphological and molecular inferences, potentially leading to a reevaluation of some higher taxa within Polychaeta [15].

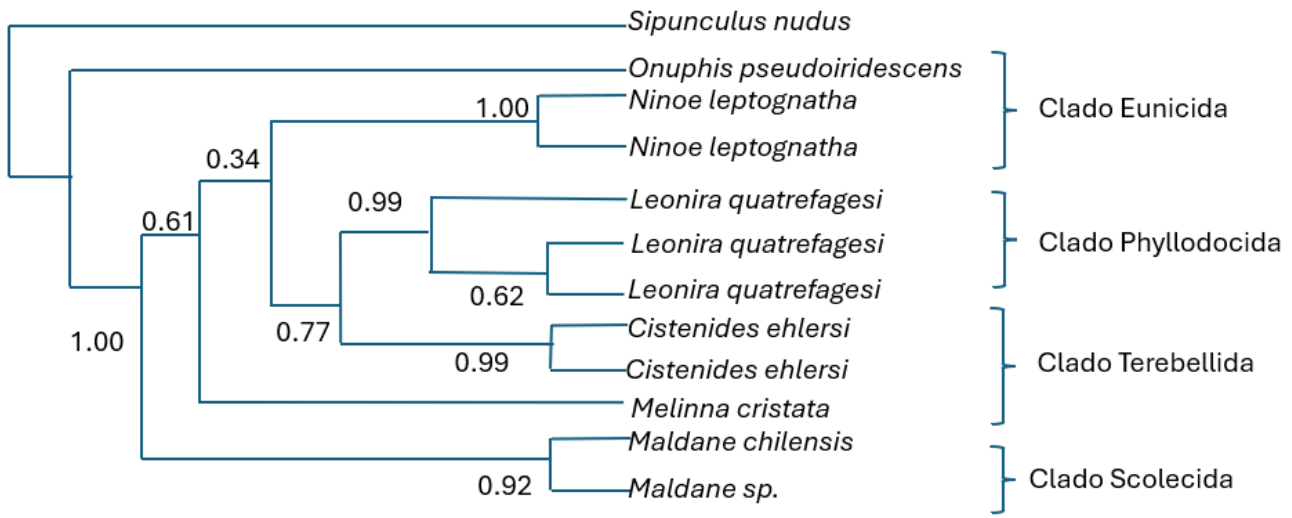

Figure 2. The consensus tree of the 4977 phylogenetic trees obtained in the convergence zone of the Markov chain by means of the Bayesian approach. The values above the nodes correspond to the posterior probability of the nodes. Lifted from Canales-Aguirre et al. [13].

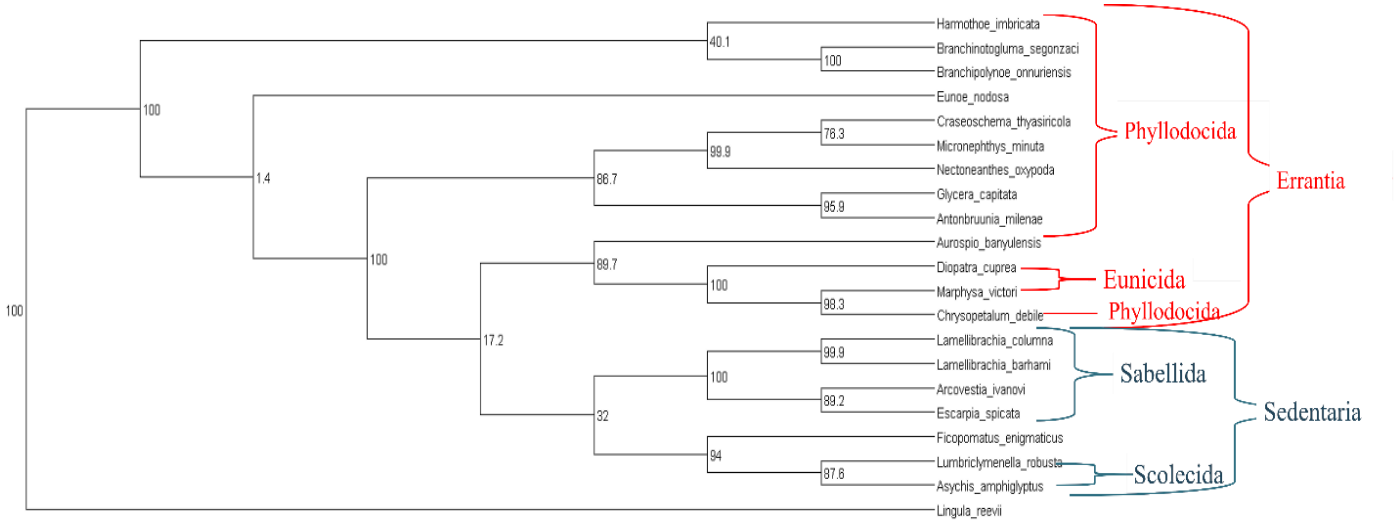

Figure 3. Phylogenetic Tree Based on 16S rRNA Sequences Species are clustered into well-supported clades.

The comparison between the two phylogenetic trees reveals distinct approaches to resolving evolutionary relationships within Annelida, particularly among polychaete families. In the phylogenetic tree with CO1 sequences focuses more narrowly on selected polychaete families (Nereididae, Polynoidae, and Eunicidae within the Errantia and Sedentaria clades). While it retains statistical support at branching nodes, its scope is more targeted, likely designed for ecological or functional comparisons among these families [16]. The phylogenetic tree with 16sRNA sequences presents a broad, high-resolution view of annelid diversification, incorporating multiple clades such as Errantia, Sedentaria, Eunicida, Phyllodocida, Sabellida, and Scolecida). Both trees reflect the modern understanding that Errantia and Sedentaria are monophyletic sister clades within Annelida, diverging from a common lophotrochozoan ancestor. The inclusion of basal groups like Scolecida and Oligochaeta suggests a broader taxonomic sampling on morphologically and ecologically distinct families allowing finer resolution of traits such as jaw morphology, parapodial structure, and reproductive strategies. The traits that may have contributed to the phylogenetic relationship of the selected species are presented in Table 2.

Table 2. Distinctive characters of the selected species of Polychaetes.

Taxon	Evolutionary Importance	Properties	Diversity	Biological Importance	Other Characteristics
Dorvilleidae	Early diverging Errantia; jawed polychaetes with cryptic speciation	Small, interstitial; jaw apparatus; rapid reproduction	Moderate; often overlooked due to size and habitat	Important in sediment turnover and microbial interactions	Found in extreme habitats (e.g., hydrothermal vents); high tolerance to pollutants
Onuphidae	Late-diverging Eunicida; tube-builders with complex jaws	Construct tubes; strong jaws; benthic	High; cosmopolitan distribution	Key bioturbators; prey for fish and invertebrates	Used in environmental monitoring; some species bioindicator-sensitive
Amphinomidae	Basal annelid lineage; sister to most Sedentaria and Errantia	Fireworms; chaetae with toxins; slow-moving	Moderate; mostly tropical	Coral reef inhabitants; some species cause dermatitis	Unique chaetal structure; ancient lineage with Cambrian origins
Eunicidae	Highly diverse jawed polychaetes; key in Eunicida clade	Large-bodied; strong jaws; active predators	Very high; over 400 species	Important predators and scavengers; regulate benthic food webs	Used in bait fisheries; jaw morphology aids taxonomy
Pilargidae	Errantia; poorly studied but ecologically relevant	Flattened body; burrowing; reduced parapodia	Low to moderate	Sediment dwellers; contribute to nutrient cycling	Often misidentified; cryptic diversity suspected

Nereididae	Model organisms in annelid research; key Errantia family	Well-developed parapodia; epitoky; jawed	High; widespread in marine and estuarine systems	Crucial in trophic dynamics; used in ecotoxicology and regeneration studies	*Platynereis dumerilii* used in evo-devo and neurobiology
Polynoidae	Scaled polychaetes; part of Errantia; commensalism common	Elytra (scales); often commensal with echinoderms	High; over 800 species	Symbiotic relationships; benthic diversity indicators	Morphologically diverse; used in phylogenetic studies
Hesionidae	Small Errantia; often interstitial or epibenthic	Slender body; reduced parapodia	Moderate	Important in meiofaunal communities	Taxonomically challenging; molecular data improving resolution
Nephtyidae	Sedentaria; muscular burrowers	Strong musculature; pharyngeal proboscis	Moderate	Sediment mixing; prey for demersal fish	Used in benthic impact assessments
Phyllodocidae	Errantia; fast-moving predators	Long-bodied; large parapodia; sensory appendages	High	Active predators; influence benthic prey populations	Colorful; used in behavioral studies
Syllidae	Highly diverse; reproductive plasticity	Small; branching reproduction; epitoky	Very high; >700 species	Key in microhabitats; biofouling and reef systems	Complex life cycles; model for reproductive evolution
Chrysopetalidae	Errantia; ornate chaetae and scales	Fan-shaped chaetae; epibenthic	Low to moderate	Minor role in benthic systems	Taxonomically distinct; limited ecological data
Microphthalmidae	Deep-sea and interstitial; poorly known	Small eyes; reduced body structures	Low	Likely important in deep-sea sediment ecology	Understudied; molecular data needed
Glyceridae	Errantia; venomous jawed predators	Proboscis with jaws; venom glands	Moderate	Regulate prey populations; used in neurotoxin studies	Jaw morphology used in phylogenetics; venom studied for biomedical use
Antonbruunidae	Deep-sea; rare and enigmatic	Long-bodied; reduced parapodia	Very low	Unknown ecological role	Named after deep-sea explorer; few specimens known
Siboglinidae	Sedentaria; chemosynthetic symbiosis	No digestive tract; symbiosis with bacteria	Moderate; includes vestimentiferans	Key in hydrothermal vent ecosystems	Model for symbiosis and deep-sea adaptation
Spionidae	Sedentaria; tube-dwellers with palps	Long palps; selective deposit feeders	High	Important in sediment sorting and bioturbation	Used in pollution studies; larval dispersal well studied
Serpulidae	Sedentaria; calcareous tube builders	Tube-dwelling; filter feeders	High	Reef builders; biofouling organisms	Used in biomineralization and larval ecology studies
Maldanidae	Sedentaria; bamboo worms	Cylindrical body; head and tail specialization	Moderate	Sediment processors; influence benthic structure	Tube-building; used in sediment toxicity studies
Terebelliformia	Sedentaria clade; includes spaghetti worms	Long feeding tentacles; tube-dwelling	High	Major bioturbators; enhance sediment oxygenation	Includes *Terebellidae*, *Ampharetidae*; key in benthic restoration

CONCLUSION

The CO1 gene provided high resolution within species-level clades but faced limitations in broader taxonomic inference. In contrast, 16S rRNA gene sequences showed strong support for evolutionary relationships across broader taxonomic groups, reaffirming its reliability as a molecular marker for phylogenetic studies. The results also underscored the non-monophyly of Polychaeta, reflecting complex evolutionary

histories and lineage divergence. These findings emphasize in employing complementary molecular markers to resolve evolutionary relationships accurately, contributing to the broader understanding of annelid phylogeny and diversity.

DECLARATION

Acknowledgement

The authors would like to express their sincerest gratitude to Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines and Adamson University, Ermita Manila, Philippines

Authorship Contributions

Concept and Writing: Mr. Jared Corpuz, Dr. Mary Jhane G. Valentino; Critic and final revision ; Mr. Jared Corpuz, Dr. Mary Jhane G. Valentino, Wilhelm Javier; Eleonor Austria

Funding

The present research received no grant from any funding agency.

Consent for publication

Not applicable.

Competing interests

The authors declared that there is no conflict of interest.

REFERENCES

[1] Weigert, A. & Bleidorn, C. (2016): Current status of annelid phylogeny. Organisms Diversity and Evolution 16:345–362. https://doi.org/10.1007/s13127-016-0265-7.

[2] Struck, T.H., Schult, N., Kusen, T., Hickman, E., Bleidorn,C., McHugh, D.,& Halanych, K.M. (2017): Annelid phylogeny and the status of Sipuncula and Echiura. BioMed Central Ecology and Evolution 7(57). https://doi.org/10.1186/1471-2148-7-5.7.

[3] Bleidorn, C., Vogt, L. & Bartolomaeus, T. (2003): A contribution to sedentary polychaete phylogeny using 18S rRNA sequence data. Journal of Zoological Systematics and Evolutionary Research 41: 186-195. https://doi.org/10.1046/j.1439-0469.2003.00212.x.

[4] Olsgard, F., Brattegard, T. & Holthe, T. (2003): Polychaetes as surrogates for marine biodiversity: lower taxonomic resolution and indicator groups. Biodiversity and Conservation 12, 1033–1049. https://doi.org/10.1023/A:1022800405253

[5] Chan, A.H.E., Saralamba, N., Saralamba, S., Ruangsittichai, J., & Thaenkham, U. (2022): The potential use of mitochondrial ribosomal genes (12S and 16S) in DNA barcoding and phylogenetic analysis of trematodes. BioMed Central Genomics 23, 104. https://doi.org/10.1186/s12864-022-08302-4.

[6] Halanych, K. & Janosik, A. (2006): A review of molecular markers used for annelid phylogenetics. Integrative and Comparative Biology, 46 (4): 533–543. https://doi.org/10.1093/icb/icj052

[7] Huerta-Cepas, J., Serra, F., & Bork, P. (2016): ETE 3: Reconstruction, analysis, and visualization of phylogenomic data. Molecular Biology and Evolution 33(6): 1635–1638. https://doi.org/10.1093/molbev/msw046

[8] [8]Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J. D., & Higgins, D. G. (2011): Fast, scalable generation of high-quality protein multiple sequence alignments using clustal omega. Molecular systems biology 7, 539. https://doi.org/10.1038/msb.2011.75 .

[9] Price, M. N., Dehal, P. S., & Arkin, A. P. (2010): Fast Tree 2--approximately maximum-likelihood trees for large alignments. Public Library of Science ONE *5*(3): e9490. https://doi.org/10.1371/journal.pone.0009490.

[10] Almeida, W. O., Christoffersen, M. L., Amorim, D. S., Garraffoni, A. R. S., & Silva, G. S. (2003): Polychaeta, annelida, and articulata are not monophyletic: articulating the metameria (Metazoa, Coelomata). Revista Brasileira de Zoologia 20(1) 1-7. https://doi.org/10.1590/S0101-81752003000100006.

[11] Alström, P., Höhna, S., Gelang, M. Ericson, P.G.P., & Olsson, U. *(*(2011): Non-monophyly and intricate morphological evolution within the avian family cettiidae revealed by multilocus analysis of a taxonomically densely sampled dataset. BMC Ecology and Evolution 11, 352 https://doi.org/10.1186/1471-2148-11-352.

[12] Kobayashi, G., Itoh, H., & Nakajima, N. (2023): First report of the mitogenome of the invasive reef-building polychaete *Ficopomatus enigmaticus* (Annelida: Serpulidae) and a cryptic lineage from the Japanese Archipelago. Molecular Biology Reports 50, 7183–7196. https://doi.org/10.1007/s11033-023-08647-3.

[13] Canales-Aguirre, C.B., Rozbaczylo, N., & Hernández, C.E. (2011): Genetic identification of benthic polychaetes in a biodiversity hotspot in the southeast pacific. Revista De Biologia Marina Y Oceanografia 46, 89-94. http://dx.doi.org/10.4067/S0718-19572011000100014

[14] Palumbi, S., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G. (1991): The simple fool's guide to PCR. Version 2. Distributed by author, 1991(pg. 1-46)

[15] Hall, K. A., Hutchings, P. A., & Colgan, D. J. (2004): Further phylogenetic studies of the polychaeta using 18S rDNA sequence data. Journal of the Marine Biological Association of the United Kingdom 84(5): 949–960. https://doi:10.1017/S0025315404010240h.

[16] Zanol, J., Carrera-Parra, L. F., & Steiner, T. M. (2021): The current state of Eunicida systematics and biodiversity. Diversity 13(2), 74. https://doi.org/10.3390/d13020074