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Original Research

Phylogenetic and Genetic Distance Analysis of the Mangrove Worm (*Namalycastis*) Based on the 18S rRNA Gene Using in Silico Methods

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Abstract

Namalycastis is a polychaete worm that inhabits dynamic aquatic environments and exhibits high genetic and morphological variability. This study investigates the phylogeny of the genus *Namalycastis* through an in silico analysis using the 18S ribosomal RNA (rRNA) gene. This genetic marker is considered ideal as it combines conserved and variable regions, both essential for taxonomic and evolutionary analyses. The objective of this research was to examine the phylogenetic relationships and genetic distances among 12 species of the genus *Namalycastis* based on their 18S rRNA gene sequences. DNA sequences were obtained from the NCBI database and aligned using Clustal-W in MEGA XI software. Phylogenetic reconstruction was performed using the Neighbor-Joining method with 1000 bootstrap replications, applying the Kimura 2-Parameter (K2P) model. The results revealed that *Namalycastis jaya* shares a very close evolutionary relationship with *Namalycastis abiuma*, forming a monophyletic clade that is distinct from *Namalycastis hawaiiensis*. The genetic distances among *N. jaya, N. abiuma*, and *N. abiuma* group sp. indicate a close evolutionary affinity, whereas *N. hawaiiensis* displays greater genetic divergence from the other two species. Overall, this study demonstrates that the 18S rRNA gene is an effective molecular marker for identifying phylogenetic relationships among *Namalycastis* species. The findings also highlight the potential of in silico methods in elucidating evolutionary patterns within the genus *Namalycastis*.

Introduction

Namalycastis belongs to the class Polychaeta and can be found in brackish, marine, and freshwater environments. This worm is known as a deposit feeder, playing an important role in the decomposition of organic matter derived from decaying plant materials. In addition to its function as a decomposer, *Namalycastis* also contributes to the ecological sustainability of coastal ecosystems by serving as an integral component of the food web, supporting the survival of organisms such as fish and shrimp. (Panirman et al., 2023). The high adaptive capability of *Namalycastis* serves as a key factor enabling it to perform these vital ecological roles. This adaptability is evidenced by its ability to survive and reproduce in mangrove forest environments, where food sources are highly diverse, as well as its capacity to tolerate a wide range of salinity levels. Consequently, *Namalycastis* can thrive in mangrove

ecosystems, estuarine habitats, and marine waters, demonstrating remarkable ecological flexibility (Mulyani et al., 2023).

The relatively high adaptive capability of *Namalycastis* is closely associated with the genetic diversity that exists within its populations. This genetic variation enables the species to adapt to dynamic environmental conditions. Understanding the relationship between the level of genetic diversity and a species' adaptive capacity is crucial in evolutionary and conservation studies. Phylogenetic analyses and genetic distance measurements serve as effective tools for examining the evolutionary relationships among populations (Birader, 2023). In line with the importance of understanding evolutionary relationships and genetic variation in phylogenetic studies, the selection of appropriate genetic markers plays a crucial role in determining the accuracy and reliability of the analysis.

One of the most widely used genetic markers for higher taxonomic levels, such as genus and family, is the 18S ribosomal RNA (rRNA) gene. This gene is selected due to its highly conserved nature across all eukaryotic organisms, while still containing several variable regions (V1–V9) that are useful for cross-taxon identification and evolutionary analysis. Additionally, the extensive availability of 18S rRNA sequence data in international databases makes this marker a preferred choice for phylogenetic and taxonomic studies (Abushattal et al., 2024; Romadhona et al., 2024). Previous studies have analyzed phylogenetic relationships by combining morphological data with genetic data from the 18S rRNA gene of several individuals belonging to the same species, such as *Namalycastis hawaiiensis* (Abe et al., 2017)*, Namalycastis jaya* (Magesh et al., 2012)*,* and *Namalycastis abiuma*. Although 18S rRNA gene sequence data from several *Namalycastis* species, such as *N. hawaiiensis, N. jaya,* and *N. abiuma* are already available, an in-depth investigation of the phylogenetic relationships and genetic distances among these species using this marker has not yet been conducted.

In silico methods are widely applied in analyses that utilize digital simulations to predict and examine biological interactions without the need for direct laboratory experimentation. For example, these methods are commonly used for protein and ligand structure modeling (Abdul et al., 2023), as well as for the prediction of physicochemical properties and pharmacokinetic characteristics (Shofi, 2022), and for assessing the potential of DNA barcoding in the analysis of genetic variation (Aulia, 2022). The use of in silico methods enables faster identification, cost efficiency, and the prediction of subsequent research pathways required for further investigation (Ugbaja et al., 2025). In this study, phylogenetic relationships and genetic distances among *Namalycastis* species were analyzed based on the 18S rRNA gene using in silico methods. The results of this research are expected to provide insights into the application of in silico approaches for understanding evolutionary patterns within the genus *Namalycastis*.

Method

2.1 Materials

This study utilized secondary data in the form of 18S rRNA gene sequences from several *Namalycastis* (mangrove worm) species obtained from the National Centre for Biotechnology Information (NCBI) database. A total of 12 samples representing species from the genus *Namalycastis* (Family Nereididae) were analyzed, including *Namalycastis abiuma, N. abiuma* group sp., *N. jaya*, and *N. hawaiiensis*. As an outgroup, another genus from the same family, *Hediste diversicolor*, was selected based on the availability of relevant genetic data and its suitability for providing an optimal evolutionary distance for comparison.

2.2 Methods

This study was conducted by analyzing sequence data from four Namalycastis species, namely *N. abiuma* accession number KF850492.1, KT270940.1 and KT270941.1, as well as KT900287.1 and KT900289.1, *N. abiuma* group sp. accession number HQ157237.1, *N. hawaiiensis* accession number LC213729.1 (Abe et al., 2017), and *N. jaya* accession numbber HQ157238, JX483865.1, JX483866.1 and JX483867.1 (Magesh et al., 2012), as well as KF850493.1. In addition, one species from a different

genus, *Hediste diversicolor* accession number LC381864.1 (Tosuji et al., 2019) (Table 1). All 18S rRNA gene sequences were downloaded from the NCBI GenBank database in FASTA format via the website https://www.ncbi.nlm.nih.gov/. To obtain these sequences, the nucleotide data type was first selected, followed by entering the target gene name (18S rRNA) and the genus (*Namalycastis*) into the NCBI search field. The resulting sequences were subsequently downloaded in FASTA format for further analysis.

Table 1. The list of species was obtained from the National Centre for Biotechnology Information (NCBI) website.

Famili	Spesies	Location	Sequence Length (bp)	Genbank Accession Number
Nereididae	*Namalycastis abiuma*	India	793	KF850492.1
	Namalycastis abiuma	India	1176	KT270940.1
	Namalycastis abiuma	India	1110	KT270941.1
	Namalycastis abiuma	India	1669	KT900287.1
	Namalycastis abiuma	India	1709	KT900289.1
	Namalycastis abiuma group sp.	India	1744	HQ157237.1
	Namalycastis hawaiiensis	Japan	1781	LC213729.1
	Namalycastis jaya	India	1744	JX483865.1
	Namalycastis jaya	India	1744	JX483866.1
	Namalycastis jaya	India	1744	JX483867.1
	Namalycastis jaya	India	1744	HQ157238.1
	Namalycastis jaya	India	839	KF850493.1
	Hediste diversicolor	Japan	1682	LC381864.1

All downloaded DNA sequences were aligned using the Clustal-W algorithm implemented in MEGA XI software to perform sequence editing and alignment construction (Tamura et al., 2021). The 5' and 3' ends of the sequences for all species were trimmed and aligned to ensure uniform sequence length for analysis. Phylogenetic analysis was performed using the Neighbor-Joining (NJ) method (Saitou & Nei, 1987) with 1,000 bootstrap replications. The evolutionary model and genetic distance calculations among species were conducted using the Kimura 2-Parameter (K2P) model (Kimura, 1980). The Neighbor-Joining (NJ) method was selected because it is computationally efficient, suitable for distance-based genetic analyses, evolutionarily assumption-neutral, and produces stable and informative phylogenetic trees for exploratory evolutionary studies. In this research, the phylogenetic data analysis included the construction of phylogenetic trees, calculation of interspecific genetic distances, and identification of single nucleotide polymorphisms (SNPs), all of which were conducted using MEGA XI software.

Results and Discussion

The 18S rRNA nucleotide sequences from 12 *Namalycastis* species and one outgroup species, *Hediste diversicolor*, were reconstructed into a phylogenetic tree using the Kimura 2-Parameter (K2P) model (Figure 1). Based on the constructed phylogenetic tree, a monophyletic relationship was observed among all species of the genus Namalycastis, indicating that the taxonomic grouping converges toward a single common ancestor. The presence of this monophyletic clade serves as a reference point to confirm that previous classification methods applied to this genus were accurate and taxonomically consistent (Gutiérrez & Garbino, 2018; Hayden, 2020). Within the *N. jaya* sequences, the species formed a distinct clade (Clade I) with a bootstrap value of 97, indicating strong support for this grouping. In contrast, *N. abiuma* sequences formed several separate clades with varying bootstrap support: Clade II with a bootstrap value of 27, Clade III with a value of 99, and Clade IV with a value of 53, reflecting differences in sequence divergence and support among the subgroups.

The emergence of cladistic methods revolutionized systematics by proposing that all classifications should be based on the principle of monophyly (Mallet, 2007).

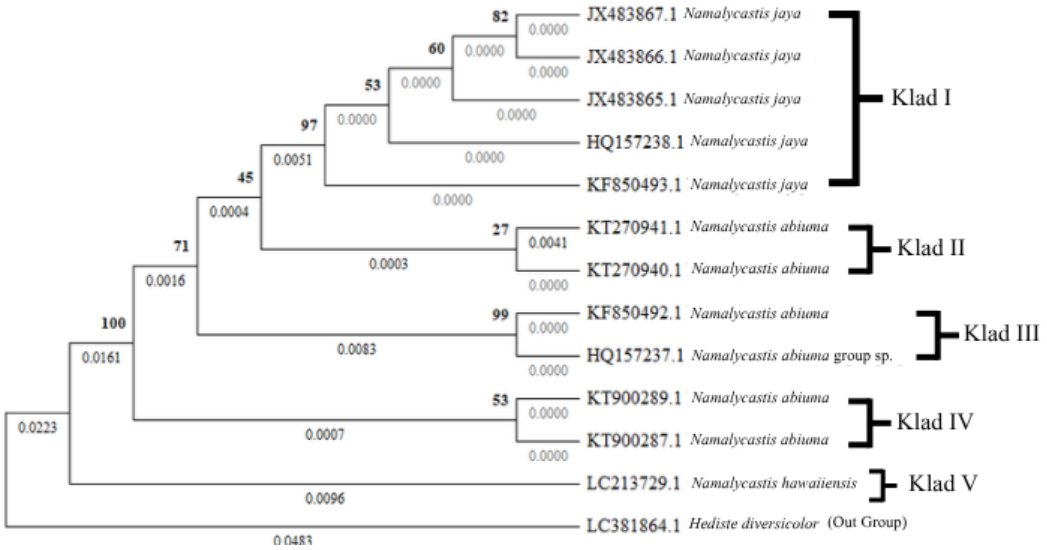

Figure 1. Phylogenetic tree of *Namalycastis* comprising 12 in-group species and 1 out-group species, constructed based on 18S rRNA gene sequences.

The positions of single nucleotide polymorphisms (SNPs) identified among the 12 analyzed species are presented in Table 2. From a total of 770 base pairs compared, four insertions were detected at positions 374, 424, 588, and 758, and eight deletions were observed at positions 118, 680, 709, 719, 728, 737, 761, and 762. Additionally, 32 base substitutions were identified, consisting of 16 transversions and 16 transitions. These insertions, deletions, and base substitutions represent single nucleotide variations that form the basis for calculating genetic distances among species (Siregar & Diputra, 2013). Such variations serve as key data for constructing the phylogenetic tree and determining the evolutionary relationships among *Namalycastis* species.

The analysis revealed that *N. hawaiiensis* exhibited the highest number of deletions and base substitutions compared to *N. abiuma, N. abiuma* group sp., and *N. jaya*. These differences caused *N. hawaiiensis* to separate from the clade containing *N. abiuma, N. abiuma* group sp., and *N. jaya*, with a relatively long branch length (0.0096) in the phylogenetic tree. This finding indicates that *N. hawaiiensis* has undergone greater genetic divergence than the other species within the *Namalycastis* group. From an evolutionary perspective, this may suggest that *N. hawaiiensis* either diverged earlier from the common ancestor or experienced a faster rate of molecular evolution. Furthermore, its genetic relationship with *N. abiuma* and *N. jaya* appears more distant, implying that it may belong to a distinct clade or represent a more genetically differentiated species. Nevertheless, the inclusion of additional genetic markers is required to confirm this hypothesis.

Table 2. Positions of *Single Nucleotide Polymorphisms* (SNPs) identified among the 12 *Namalycastis* species based on sequence *alignment*.

	33	44	47	65	113	118	158	172	177	201	204
KF850492.1 Namalycastis abiuma	G	C	C	C	A	A	T	A	G	G	C
KT900287.1 Namalycastis abiuma	G	C	C	C	A	A	T	A	G	G	C
KT900289.1 Namalycastis abiuma	G	C	C	C	A	A	T	A	G	G	C
KT270941.1 Namalycastis abiuma	G	C	C	C	A	A	T	A	G	G	C
KT270940.1 Namalycastis abiuma	G	C	C	C	A	A	T	A	G	G	C
HQ157237.1 Namalycastis abiuma group sp.	G	C	C	C	A	A	T	A	G	G	C
LC213729.1 Namalycastis hawaiiensis	A	A	T	T	G	-	C	C	A	A	A

KF850493.1 Namalycastis jaya	G	C	C	C	A	A	T	A	G	G	C
HQ157238.1 Namalycastis jaya	G	C	C	C	A	A	T	A	G	G	C
JX483865.1 Namalycastis jaya	G	C	C	C	A	A	T	A	G	G	C
JX483866.1 Namalycastis jaya	G	C	C	C	A	A	T	A	G	G	C
JX483867.1 Namalycastis jaya	G	C	C	C	A	A	T	A	G	G	C
LC381864.1 Hediste diversicolor	A	T	T	C	G	A	T	C	C	A	A

	272	277	278	299	300	305	324	360	374	424	547
KF850492.1 Namalycastis abiuma	G	G	C	G	G	G	C	A	G	A	T
KT900287.1 Namalycastis abiuma	G	T	G	T	G	G	G	G	-	-	T
KT900289.1 Namalycastis abiuma	G	T	G	T	G	G	G	G	-	-	T
KT270941.1 Namalycastis abiuma	T	T	G	T	G	G	G	G	-	-	A
KT270940.1 Namalycastis abiuma	T	T	G	T	G	G	G	G	-	-	T
HQ157237.1 Namalycastis abiuma group sp.	G	G	C	G	G	G	C	A	G	A	T
LC213729.1 Namalycastis hawaiiensis	C	T	G	T	A	A	G	G	-	-	T
KF850493.1 Namalycastis jaya	T	T	G	T	G	G	G	G	-	-	T
HQ157238.1 Namalycastis jaya	T	T	G	T	G	G	G	G	-	-	T
JX483865.1 Namalycastis jaya	T	T	G	T	G	G	G	G	-	-	T
JX483866.1 Namalycastis jaya	T	T	G	T	G	G	G	G	-	-	T
JX483867.1 Namalycastis jaya	T	T	G	T	G	G	G	G	-	-	T
LC381864.1 Hediste diversicolor	T	T	G	T	G	G	G	G	-	-	T

	553	561	582	585	588	618	643	650	680	709	719
KF850492.1 Namalycastis abiuma	T	T	G	G	-	T	A	T	G	A	T
KT900287.1 Namalycastis abiuma	T	T	G	G	-	T	A	T	G	A	T
KT900289.1 Namalycastis abiuma	T	T	G	G	-	T	A	T	G	A	T
KT270941.1 Namalycastis abiuma	T	T	G	G	-	T	A	T	G	-	-
KT270940.1 Namalycastis abiuma	T	T	G	G	-	T	A	T	G	A	T
HQ157237.1 Namalycastis abiuma group sp.	T	T	G	G	-	T	A	T	G	A	T
LC213729.1 Namalycastis hawaiiensis	A	A	C	A	-	C	C	A	-	A	T
KF850493.1 Namalycastis jaya	A	A	G	G	G	T	A	T	G	A	T
HQ157238.1 Namalycastis jaya	A	A	G	G	G	T	A	T	G	A	T
JX483865.1 Namalycastis jaya	A	A	G	G	G	T	A	T	G	A	T
JX483866.1 Namalycastis jaya	A	A	G	G	G	T	A	T	G	A	T
JX483867.1 Namalycastis jaya	A	A	G	G	G	T	A	T	G	A	T
LC381864.1 Hediste diversicolor	T	T	C	A	-	T	C	G	G	A	T

	723	727	728	733	737	757	758	760	761	762	764
KF850492.1 Namalycastis abiuma	C	G	G	A	C	A	-	A	T	G	A
KT900287.1 Namalycastis abiuma	G	G	G	A	C	T	-	A	T	G	A
KT900289.1 Namalycastis abiuma	G	G	G	A	C	T	-	A	T	G	A
KT270941.1 Namalycastis abiuma	G	G	-	A	-	A	-	T	-	-	G
KT270940.1 Namalycastis abiuma	G	G	G	A	C	A	-	A	T	G	A
HQ157237.1 Namalycastis abiuma group sp.	C	G	G	A	C	A	-	A	T	G	A
LC213729.1 Namalycastis hawaiiensis	G	G	G	T	C	T	-	A	T	G	A
KF850493.1 Namalycastis jaya	A	A	G	A	C	A	T	A	T	G	A
HQ157238.1 Namalycastis jaya	A	A	G	A	C	A	T	A	T	G	A
JX483865.1 Namalycastis jaya	A	A	G	A	C	A	T	A	T	G	A

JX483866.1 Namalycastis jaya	A	A	G	A	C	A	T	A	T	G	A
JX483867.1 Namalycastis jaya	A	A	G	A	C	A	T	A	T	G	A
LC381864.1 Hediste diversicolor	G	G	G	T	C	T	-	A	T	G	A

A very close genetic relationship (0.0000) was observed within the *N. jaya* clade, indicating that this group exhibits a monophyletic structure and is genetically homogeneous. Such a minimal genetic distance suggests that the analyzed samples likely originated from the same individual or population. In contrast, *N. abiuma* formed a distinct clade grouping with genetic distances ranging from 0.0003 to 0.0083. The *N. abiuma* sequence data obtained from India indicate a notable level of genetic differentiation among populations, even those originating from the same geographic region. These results corroborate the findings of Magesh et al. (2012) and Alves et al. (2024), which reported the presence of a sensu stricto lineage of *N. abiuma* identified in South America.

Table 3. Matrix of genetic distance values based on 18S rRNA gene sequences for 12 *Namalycastis* species and one *Hediste* species used as the outgroup.

Nama Spesies	1	2	3	4	5	6	7	8	9	10	11	12	13
KF850492.1 Namalycastis abiuma													
KT900287.1 Namalycastis abiuma	0.0095												
KT900289.1 Namalycastis abiuma	0.0095	0.0000											
KT270941.1 Namalycastis abiuma	0.0136	0.0068	0.0068										
KT270940.1 Namalycastis abiuma	0.0095	0.0027	0.0027	0.0041									
HQ157237.1 Namalycastis abiuma group sp.	0.0000	0.0095	0.0095	0.0136	0.0095								
LC213729.1 Namalycastis hawaiiensis	0.0365	0.0265	0.0265	0.0323	0.0280	0.0365							
KF850493.1 Namalycastis jaya	0.0136	0.0082	0.0082	0.0095	0.0054	0.0136	0.0337						
HQ157238.1 Namalycastis jaya	0.0136	0.0082	0.0082	0.0095	0.0054	0.0136	0.0337	0.0000					
JX483865.1 Namalycastis jaya	0.0136	0.0082	0.0082	0.0095	0.0054	0.0136	0.0337	0.0000	0.0000				
JX483866.1 Namalycastis jaya	0.0136	0.0082	0.0082	0.0095	0.0054	0.0136	0.0337	0.0000	0.0000	0.0000			
JX483867.1 Namalycastis jaya	0.0136	0.0082	0.0082	0.0095	0.0054	0.0136	0.0337	0.0000	0.0000	0.0000	0.0000		
LC381864.1 Hediste diversicolor	0.1050	0.0936	0.0936	0.0984	0.0936	0.1050	0.0862	0.1000	0.1000	0.1000	0.1000	0.1000	

The cladogram constructed using the Neighbor-Joining (NJ) method grouped the 12 species into a single ingroup, consisting of *Namalycastis jaya, N. abiuma, N. abiuma* group sp., and *N. hawaiiensis*, with *Hediste diversicolor* serving as the outgroup, showing the greatest genetic distance (0.0223) from the ancestral node. These species are clustered together due to their genetic sequence similarities, allowing them to be classified within the same clade. According to previous morphological studies, these species can be differentiated based on morphological characteristics, particularly parapodial structures and setae patterns. For example, *N. jaya* and *N. abiuma* share the feature of lacking notosetae (Magesh et al., 2012), whereas *N. hawaiiensis* possesses notosetae on the middle region of its body (Abe et al., 2017).

All *N. jaya* and *N. abiuma* sequences exhibited a very close genetic relationship, forming a distinct clade separate from *N. hawaiiensis*. This clade separation is supported by a bootstrap value of 100, indicating strong statistical confidence. As explained by Saleky et al. (2021), the bootstrap value reflects the stability and reliability of the reconstructed phylogenetic tree—low bootstrap values suggest that the resulting tree may be unreliable. In contrast, high bootstrap values indicate robust and well-supported clustering and branching patterns.

Branch length analysis also provides valuable insights into evolutionary distances. The sequence groups of *N. jaya* and *N. abiuma* exhibited branch lengths of 0.0000, indicating no or minimal genetic variation from their most recent common node, thereby reflecting a very high degree of sequence similarity within this cluster. In contrast, *N. abiuma* (Acc. #KT270941.1) displayed a branch length of 0.0041, while *N. hawaiiensis* showed a branch length of 0.0096 from their respective branching points.

The closest phylogenetic relationship was clearly observed between *N. jaya* and *N. abiuma,* which were grouped in a single clade with a bootstrap value of 100. In contrast, *N. hawaiiensis*, although belonging to the same genus *(Namalycastis)*, exhibited a greater genetic distance from the *N. jaya–N. abiuma* pair, as indicated by its branch length of 0.0096 and its distance from the node uniting *N. jaya* and *N. abiuma* (0.0223). As the outgroup, *Hediste diversicolor* displayed the greatest genetic distance (0.0483) from *Namalycastis* spp., thereby confirming its role as a comparative reference rather than a member of the ingroup.

Conclusions

The phylogenetic analysis of three Namalycastis species, based on 18S rRNA sequences using the Neighbor-Joining (NJ) method, produced a monophyletic cladogram. The cladogram shows that *Namalycastis jaya* and *Namalycastis abiuma* have a branch length of 0.0000, indicating an extremely high level of sequence similarity between the two species. In contrast, *N. abiuma* exhibits a branch length of 0.0041, corresponding to approximately 0.41% divergence from its most recent common ancestor. *N. hawaiiensis,* however, displays the highest genetic divergence among the *Namalycastis* species analyzed, with a branch length of 0.0096, or about 0.96% divergence. These results are consistent with the genetic distance values obtained using the Kimura 2-Parameter (K2P) model.

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