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Evolutionary relationship of edible mollusks based on phylogenetic analysis using 16s RRNA and COX1 gene sequences

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ABSTRACT: All of the edible mollusk organisms selected for this study exhibited evolutionary relationships which were identified by phylogenetic analysis of DNA sequences (20 16S ribosomal RNA gene (rRNA) sequences and 50 mitochondrial cytochrome c oxidase 1 gene (COX1) sequences). Two separate phylogenetic trees based from the COX1 and 16S rRNA; and phylogenetic tree from the concatenation of the sequences of the taxa with both COX1 and 16S rRNA sequences revealed the distant and shared common ancestors of the selected mollusks. These two genes are widely used markers in molecular biology, particularly for phylogenetic and species identification. Phylogenetic analysis elucidates the evolutionary relationships among a diverse assemblage of molluscan taxa, spanning key families from cephalopods, gastropods, and bivalves. Using robust statistical support—reflected by high bootstrap values—the tree distinctly separates major clades including cephalopod groups.The inclusion of selected outgroup species anchors the tree, thereby establishing a clear evolutionary trajectory and enabling inferences about ancestral states and morphologies. This phylogenetic framework not only advances our understanding of molluscan evolution and taxonomy but also provides a crucial foundation for future studies in comparative morphology, ecology, and conservation biology across these economically and ecologically significant organisms.

KEYWORDS: Edible mollusk, Phylogenetic Analysis, COX1 gene, 16S rRNA, Evolutionary relationship

INTRODUCTION

With about 120,000 species, Mollusca are the second most animal phylum occurring in major environments except aerospace [1]. They are identified as soft bodied organisms that typically secrete a protective outer shell. Besides fishes, edible mollusks are also a major source of protein [2] that can be found in various places including marine and terrestrial regions [3]. Aside from their importance in food, mollusks also provide a significant part both culturally and economically for the production of jewelry and dye [4].

Despite their contributions, still understanding of the early history of mollusks is limited and several phylogenetic explanations are competing [5]. This common unmatched diversity of molluscan morphological phenotypes includes the gastropods (snails, slugs), bivalves (clams, mussels), and cephalopods (nautiluses, squids, and octopuses). However, there are also added groups like spicule-bearing, simple worms (aplacophorans), flattened, ovoid, shell plate-bearing polyplacophorans (chitons), circular monoplacophorans with a single, cap-like shell, and the scaphopods (tusk shells), which get their name from the bent, elephant tooth-like shell in which they live [6]. Due to their remarkable differences in overall body plan shape, mollusks are a perfect group for comparing how evolution has produced from a common ancestor that lived the seafloors of the oceans at least 550 million years ago [7].

The existence and advancement of technology that is progressively transforming to aid in the fulfillment of every research gives also an impact on how organisms are taxonomically identified and offer a more precise approach. Scientists utilize molecular tools to analyze nucleic acids, specifically DNA and RNA, in order to identify every organism according to its specific genetic makeup that can produce their genetic markers. Therefore, to understand the evolutionary relationship between organisms, unique molecular markers of COX1 and 16S rRNA were used for a wide range present in animal genes.

The most commonly used barcode region to animals found in mitochondrial DNA is a portion of cytochrome oxidase I (Mt-COX1) gene and this sequence is more suitable for the DNA barcoding due to its faster rate of mutation and distinguishing between closely related species [8]. Additionally, the barcoding of DNA is an effective approach to address the taxonomic barrier producing problems in species identification [9] and COX1 sequencing data is a strong tool for estimating large-scale species diversity with a great potential for use in ecology and β-diversity studies and for setting conservation priorities. However, error rates can be high in individual lineages [10]. Similar with the genes 16S rRNA sequences which were initially used for phylogenetic analysis in 1985 [11]. It became the most popular marker gene because it includes both highly conserved regions for primer design and hypervariable regions to identify phylogenetic characteristics of microorganisms [12] and the majority of research employs partial sequences of the 16S rRNA gene [13].

The highlights of this present study is to exhibit the evolutionary relationships of the identified edible Molluscs which comprise of 50 species with unique mitochondrial cytochrome c oxidase 1 gene (COX1) sequences and 20 16S ribosomal RNA gene (rRNA).

MATERIALS AND METHODS

Data mining

Fifty COX1 nucleotide sequences and 20 16S rRNA nucleotide sequences were obtained from NCBI (National Center for Biotechnology Information.) These species of mollusks were selected based on their ecological importance and biodiversity status in the Philippines. In addition, they are among the native, endemic and introduced species of mollusks in the country. The class gastropoda, bivalvia andcephalopoda were represented in the study. Moreover, five outgroup species under class clitellate, polychaeta and insecta were also selected. Concatenation of sequences between COX1 nucleotide sequences and 16S rRNA sequences was carried out using Fasta Alignment Joiner by FaBox.

Multiple Sequence Alignment

Multiple sequence alignment was performed using the ClustalW in MEGA. he collected sequences were aligned using ClustalW with 15.00 gap opening penalty and 6.66 gap extension penalty.

Phylogenetic analysis

The phylogenetic tree was directly constructed using the ETE3 (Environment for Tree Exploration) phylogenetic analysis pipeline. Separate phylogenetic trees for COX1, 16S rRNA genes and the combination of COX1 and 16S rRNA sequences were constructed. In addition alignment was done using Clustal Omega v1.2.4 with the default options. Phylogenetic reconstructions were performed using the function "build" of ETE3 3.1.3 [14] as implemented on the GenomeNet(https://www.genome.jp/tools/ete/).

Alignment was performed with Clustal Omega v1.2.4 with the default options [15]. The tree was constructed using FastTree v2.1.8 with default parameters [16]. Values at nodes are SH-like local support. For verification, phylogenetic trees were reconstructed using MEGA X. The aligned sequences were trimmed to eliminate biases due to the variability in the length of the sequences. Then, phylogenetic trees were constructed using maximum-likelihood methods evaluated by a bootstrap analysis with 1000 replicates. Evolutionary relationships of the sampled species were inferred using the bootstrap consensus trees.The best maximum-likelihood model was determined and applied for the construction of each phylogenetic tree. Phylogenetic analysis was carried out through interpretation of the phylogenetic tree based on the relationship of the sample.

Presented in Tables 1 and 2 are the selected mollusks with their correspoding accession number, species, class and family.

Table 1. Species used for phylogenetic analyses of the 16S rRNA gene

Accession code	Species	Class	Family
NC_056385.1:c13539-12160	*Enteroctopus dofleini*	Cephalopoda	Octopodidae
NC_022194.1:16329-17546	*Fulvia mutica*	Bivalvia	Cardiidae
MN449488.1:7879-8989	*Geukensia demissa*	Bivalvia	Mytilidae
NC_031361.1:c14866-13403	*Haliotis iris*	Gastropoda	Haliotidae
NC_036928.1:c11561-10074	*Haliotis rufescens*	Gastropoda	Haliotidae
NC_041247.1:1590-2566	*Helix pomatia*	Gastropoda	Helicidae
NC_011763.1:3419-4715	*Hyriopsis cumingii*	Bivalvia	Unionidae
NC_026908.1:c19932-18527	*Illex Argentines*	Cephalopoda	Ommastrephidae
NC_048487.1:7121-8339	*Mercenaria mercenaria*	Bivalvia	Veneridae
KJ755996.1:5695-7090	*Mya arenaria*	Bivalvia	Myidae
NC_030633.1:15522-16765	*Mytilus chilensis*	Bivalvia	Mytilidae
DQ399833.1:17866-19109	*Mytilus galloprovincialis*	Bivalvia	Mytilidae
KU295559.1:c13476-12125	*Octopus bimaculoides*	Cephalopoda	Octopodidae
PQ163737.1:c8463-7121	*Octopus vulgaris*	Cephalopoda	Octopodidae
NC_050683.1:17176-18217	*Tridacna gigas*	Bivalvia	Cardiidae
MW690579.1:12015-13260	*Tubifex tubifex*	Clitellata	Naididae
NC_051993.1:11426-12617	*Alitta succinea*	Polychaeta	Nereididae
NC_024855.1:c13962-12637	*Musca domestica*	Insecta	Fanniidae
NC_016956.1:c13944-12653	*Periplaneta americana*	Insecta	Blattidae
NC_056279.1:c14580-13577	*Sabella spallanzanii*	Polychaeta	Sabellidae

Table 2. Species used for phylogenetic analyses of the COX1 gene

Accession code	Species	Class	Family
AY569552.1:59-1603	*Aplysia californica*	Gastropoda	Aplysiidae
NC_009687.1:8847-10448	*Argopecten irradians*	Bivalvia	Pectinidae
NC_023080.1:1-1533	*Babylonia areolate*	Gastropoda	Babyloniidae
EU827194.1:1-1533	*Bolinus brandaris*	Gastropoda	Muricidae
NC_040940.1:1-1536	*Buccinum undatum*	Gastropoda	Buccinidae
NC_035728.1:1-1561	*Cerastoderma edule*	Bivalvia	Cardiidae
NC_035734.1:17009-17009,1-1541	*Cipangopaludina chinensis*	Gastropoda	Viviparidae
NC_017886.1:1-1533	*Concholepas concholepas*	Gastropoda	Muricidae
MK392334.1:1-1614	*Corbicula fluminea*	Bivalvia	Cyrenidae
MH016739.1:2916-4682	*Crassadoma gigantea*	Bivalvia	Pectinidae
NC_027653.1:1-1629	*Crassostrea gasar*	Bivalvia	Ostreidae
NC_012649.1:1-1617	*Crassostrea sikamea*	Bivalvia	Ostreidae
AY905542.2:1-1623	*Crassostrea virginica*	Bivalvia	Ostreidae
NC_035985.1:7509-9218	*Donax trunculus*	Bivalvia	Donacidae
NC_009734.1:1425-2957	*Dosidicus gigas*	Cephalopoda	Ommastrephidae
ON367818.1:1-1533	*Eledone cirrhosa*	Cephalopoda	Octopodidae
NC_056385.1:2569-4101	*Enteroctopus dofleini*	Cephalopoda	Octopodidae
NC_022194.1:1-1572	*Fulvia mutica*	Bivalvia	Cardiidae
MN449488.1:4908-6494	*Geukensia demissa*	Bivalvia	Mytilidae
ON920309.1:2795-4336	*Haliotis fulgens*	Gastropoda	Haliotidae
NC_031361.1:3355-4896	*Haliotis iris*	Gastropoda	Haliotidae
NC_005940.1:3352-4893	*Haliotis rubra*	Gastropoda	Haliotidae
NC_036928.1:1-1542	*Haliotis rufescens*	Gastropoda	Haliotidae
NC_013708.1:3267-4808	*Haliotis tuberculata*	Gastropoda	Haliotidae
NC_041247.1:1-1530	*Helix pomatia*	Gastropoda	Helicidae
NC_011763.1:c14881-13346	*Hyriopsis cumingii*	Bivalvia	Unionidae
NC_026908.1:1987-3519	*Illex argentines*	Cephalopoda	Ommastrephidae
NC_007781.1:1-1536	*Ilyanassa obsoleta*	Gastropoda	Nassariidae
NC_048487.1:2109-3677	*Mercenaria mercenaria*	Bivalvia	Veneridae
NC_056622.1:4522-6174	*Mizuhopecten yessoensis*	Bivalvia	Pectinidae
NC_033537.1:1-1551	*Modiolus modiolus*	Bivalvia	Modiolidae
KJ755996.1:1-1755	*Mya arenaria*	Bivalvia	Myidae
NC_030633.1:9089-10753	*Mytilus chilensis*	Bivalvia	Mytilidae
NC_006886.2:9068-10732	*Mytilus galloprovincialis*	Bivalvia	Mytilidae
NC_007687.1:12229-13884	*Mytilus trossulus*	Bivalvia	Mytilidae
KU295559.1:2557-4089	*Octopus bimaculoides*	Cephalopoda	Octopodidae
NC_006353.1:2564-4096	*Octopus vulgaris*	Cephalopoda	Octopodidae
JF274008.1:1-1566	*Ostrea edulis*	Bivalvia	Ostreidae
KM580067.1:1-1563	*Panopea generosa*	Bivalvia	Hiatellidae
NC_007895.1:2058-3590	*Sepia officinalis*	Cephalopoda	Sepiidae
EU880278.1:1-1692	*Sinonovacula constricta*	Bivalvia	Pharidae
NC_017616.1:1-1566	*Solen strictus*	Bivalvia	Solenidae
NC_026081.1:1-1578	*Tegillarca granosa*	Bivalvia	Arcidae
NC_006354.1:1400-2938	*Todarodes pacificus*	Cephalopoda	Ommastrephidae
NC_050683.1:249-1865	*Tridacna gigas*	Bivalvia	Cardiidae
MW690579.1:1-1543	*Tubifex tubifex*	Clitellata	Naididae
NC_016956.1:1474-3012	*Periplaneta americana*	Insecta	Blattidae
NC_056279.1:16-1551	*Sabella spallanzanii*	Polychaeta	Sabellidae
NC_024855.1:1410-2943	*Musca domestica*	Insecta	Fanniidae
NC_051993.1:1-1536	*Alitta succinea*	Polychaeta	Nereididae

*outgroup

RESULTS AND DISCUSSION

The phylogenetic trees of 16S rRNA and mitochondrial COX1 genes were used to assess the evolutionary relationships of the chosen edible mollusk and the combinations of these sequences. Phylogenetic trees containing mitochondrial COX1 and 16S rRNA genes as can be seen in figures 1 and 2, while the concatenated COX1 and 16S rRNA genes are presented in figure 3.

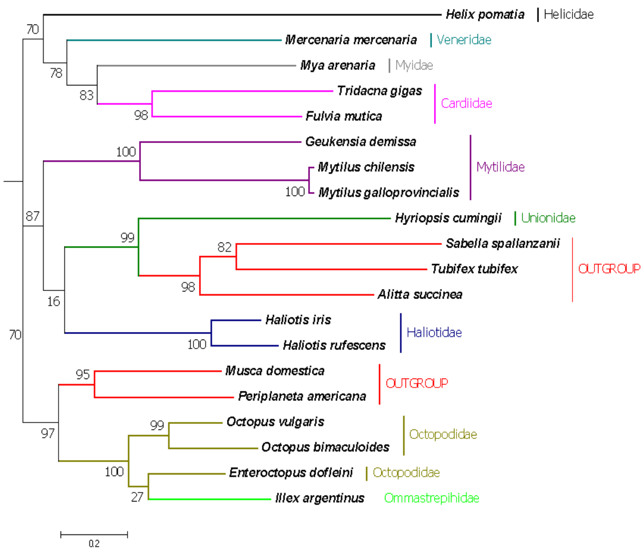

Figure 1. Phylogenetic Tree of Mollusks Based from 16S rRNA Gene Sequences.

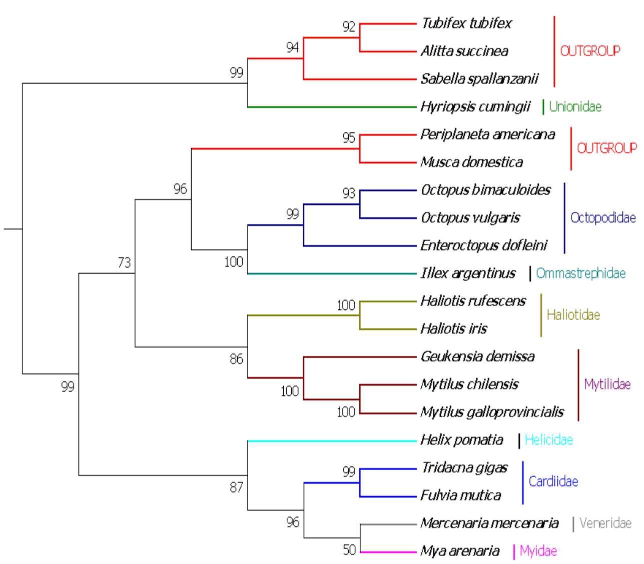

Figure 3. Phylogenetic Tree of the Selected Mollusks based from Concatenated COX1 and 16s rRNA Sequences.

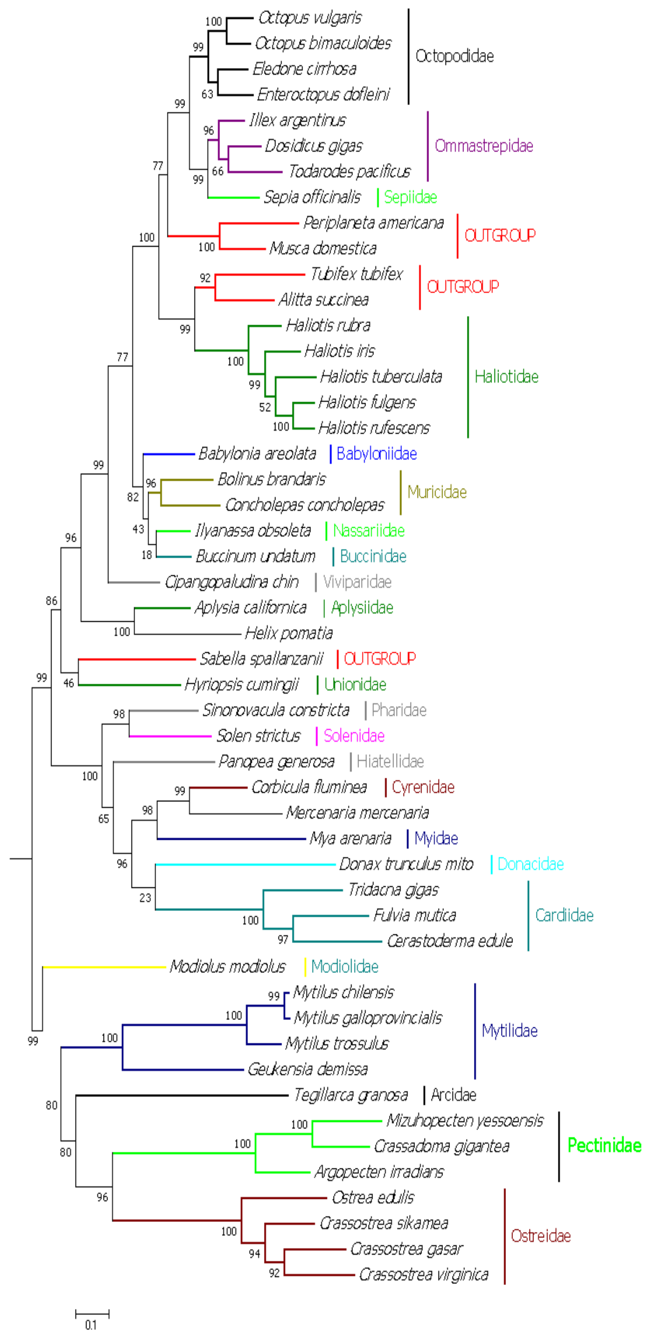

Figure 2. Phylogenetic Tree of the Selected Mollusks based from COX1 Sequences.

Phylogenetic analysis using 16S rRNA

Using the 16S rRNA genes, the generated phylogenetic tree shows the relationships between several edible mollusks and the outgroups belonging to phylum chordata. Figure 1 presented a midpoint- rooted phylogenetic tree using 16S ribosomal RNA genes illustrating the evolutionary relationship between different species. Among all the mollusk species assessed, cephalopods branching at a 99 to 100 bootstrap valuea, such as *Illex argentines*, *Enteroctopus dofleini*, *Octopus bimaculoides*, and *O. vulgaris*, provide clearer grouping patterns in their evolutionary relationships. These species share a common branch, indicating they are closely related cephalopods and likely diverged recently from a shared lineage.

Haliotis rufescens and *H. iris* hold a high value of 100%, indicating their similarity taxonomic level, order and family, as also they are both classified as marine species. Both belong to the *Haliotis* genus (abalones), sharing a branch due to their genetic similarities and a recent common ancestor. Misalignment of the *Helix pomatia*, which is recognized as a land snail, triggered completely its difference due to its taxonomic ranks, despite the fact that they all belong to the same class gastropoda.

In the division of class Bivalvia, *Mytilus galloprovincialis* and *M.chilensis* exhibit a 100 bootstrap value due to their close taxonomic similarity, species *Geukensia demissa* also clusters with them with a 100 bootstrap value indicating near-identical taxonomic traits. Also, as members of the Mytilidae family (mussels), their shared branch highlights evolutionary connections within this family. Meanwhile, *Fulvia mutica* and *Tridacna gigas* show a high bootstrap value of 98. However, *Mercenaria mercenaria* and *Mya arenaria* have a lesser connection, with a bootstrap value of 83, indicating a different standing in order and family.

In terms of the selected outgroups, separate branches were formed from the ingroup which helps clarify the evolutionary relationships within the ingroup. *A. succinea* represents a basal lineage or less closely related species compared to the rest of the tree, whereas the *M. domestica* and *P. americana* showed a very distant evolutionary relationship or divergence with mollusks or worms Sabella (likely *Sabella spallanzanii*, a marine polychaete): This species of tube-dwelling worm is a closer outgroup than insects but still distinct from mollusks and crustaceans. Also, *H. cumingii* and *T. tubifex* represent different lineages connected through distant nodes, emphasizing their divergence from mollusks.

Mercenaria mercenaria and *Mya arenaria* form their own branch as well of the species *Fulvia mutica* and *Tridacna gigas.* A lower bootstrap value of 83%% connecting these two groups may rather due to their evolutionary relationship, which is more reflective in their ecological diversity classifying *M. mercenaria* and *Mya arenaria* are temperate species [17, 18] while *F. mutica* and *T. gigas* are more specialized into tropical environments [19, 20], thus 16S rRNA gene provide limitation resolving specially among species [21]. Furthermore, marine mollusks such as *M. mercenaria*, *Mya arenaria*, *Fulvia mutica*, *G. demissa*, *M. chilensis*, *T. gigas*, and *M. galloprovincialis* are grouped together in a branch [22]. *H. cumingii*, on the other hand, is a freshwater bivalve from the *Unionidae* family within the order Unionida. Unlike the marine species included in the group of bivalves above the tree, *H.cumingii* is native to freshwater environments and is most known for its use in pearl production [23], rather than as a food source. This distinguishing feature sets it apart from the other groups' primarily marine, food-oriented bivalves and stresses its ecological and economic importance in freshwater settings.

The constructed phylogenetic tree also demonstrates that *Helix pomatia*, a gastropod, had a 70% connection to the species class Bivalvia. This weak connection might be what would enable comparisons of the related ecological factors. Since both gastropod and bivalve families are each mainly dioecious. Mollusks' sexual systems have undergone several evolutionary transformations [24]. The 99.4% branching in the tree that ties *Hyriopsis cumingii* to the Phylum Chordata is a result of the two species' similar bilateria, which can lead to the identification of certain conserved molecular traits in the 16S rRNA gene and a highly significant grouping. Additional proposed research would offer a more thorough understanding of the relationship [25]. Clearly in the result of the phylogenetic tree 16S rRNA genes focus on determining wider higher-level taxonomic relationships [26].

Phylogenetic Analysis using COX1

The COX1 phylogenetic tree shows evolutionary relations for most species, majority has strong evolutionary relations for most species having statistical values from ≥70% to 100%, Also, 65.5 support is still a moderated but not a robust or reliable, while low values <50% indicating insufficient or unreliable support for the inferred relationship [27]. This phylogenetic tree reveals the evolutionary connections among mollusk species, including snails, mussels, clams, octopuses, and related invertebrates which serve as outgroups. Based from the inferred tree, the outgroup species (*S. spallanzanii*, *T. tubifex*, *A. succinea*, *M.domestica*, and *P. americana* diverged earlier from mollusks and provide a reference point for comparison and helps determine how mollusks evolved from more distant common ancestors. *Haliotis iris* and *H.rufescens* form a closely related group within the abalone lineage with a bootstrap value of 100. Whereas, *M. chilensis*, *M. galloprovincialis,* and *G. demissa* cluster together within the Mytilidae, showing strong evolutionary ties (100 boostrap value). In addition, *O. vulgaris*, *O. bimaculoides*, and *E. dofleini* share a common ancestor and are closely related within the cephalopod lineage. This highlights the genetic divergence between mollusks and their ancestral relatives.

Bolinus brandaris and *Concholepas concholepas* share a branch with a boostrap value of 96, highlighting a close evolutionary relationship within the Muricidae family. *Babylonia areolata* stands out as part of its own branch but aligns more closely with other carnivorous gastropods like Muricidae species. *Ilyanassa obsoleta* (Nassariidae) and *Buccinum undatum* (Buccinidae) form related branches, showing their shared adaptation to scavenging and detritivorous feeding habits. *Aplysia californica* (Aplysiidae) is part of a more basal gastropod lineage, distinct but related through ancestral traits. Meanwhile, *Helix pomatia* (land snail) diverged significantly from marine gastropods like *Babylonia* and *Concholepas*. Its adaptations to terrestrial life mark it as a more distantly related lineage. This tree emphasizes the incredible diversity and specialization within mollusks, with clear evolutionary pathways connecting these varied species.

This demonstrates that, while they are still closely related, the COX1 gene is more variable and better at resolving differences at the species level than at higher taxonomic levels [28]. The long branch might suggest increased genetic divergence between the species when employing COX1. Since these genes imply mutations [29] that lead to differences at the species level, even if they are still genetically related. The class cephalopoda demonstrates consistency by providing a clearer illustration of their evolutionary history, as evidenced using 16S rRNA gene sequences. Cephalopods such as octopuses, squids, and cuttlefish tend to form their own groups. This is explained by their shared genetic and physical properties such as the development of their distinct head, eyes, specialized locomotion and tentacles and even their complex nervous system as this class seen to meet. It was also suggested in some articles. This implies that cephalopod are mostly independent of other molluscs' ancestral benthic lifestyles [30, 31]. Though two species like *Eledone cirrhosa* and *Enteroctopus dofleini* shows 63 bootstrap value indicates a moderate level of support.

Aplysia californica and *Helix pomatia* belong to order Stylommatophora, despite their ecological variations marine versus terrestrial and even in their taxonomic level specifically from family to genus, but both species are gastropods. The high bootstrap values in COX1 of 100 reflects how gastropods, with their diverse ecological adaptations, showcase a fascinating example of evolutionary innovation. Evolutionary context during the Cenozoic era resulted to evolution of marine to land species, As a result, in comparison to previous history, relatively few new land species evolved. Only a few of these species were able to effectively adapt to life on land during this period, and they did not colonize the land on a significant scale. In which Stylommatophora made their first (or only) transition to land during or before the Cretaceous [32,33].

The clear result of this phylogenetic tree using COX1 genes, causes significant diversity between species, particularly among closely related species. Its variation, caused by mutations, makes it particularly useful for species identification and differentiation. It can identify cryptic species, which are visually similar but genetically distinct, resulting in a more accurate estimation of species diversity [34,36]. However, COX1 is less effective for higher taxonomic levels, as its ability to distinguish between broader taxonomic groups is limited due to lower variability at those levels [32, 34].

Phylogetic Analysis using Concatenated COX1 and 16s rRNA

The result of the phylogenetic analysis of the selected mollusks based on their concatenated COX1 and 16s rRNA sequences is shown in Figure 3. The same major clades like in all the other trees are also observed. The tree is organized into distinct clades based on families, such as Octopodidae (octopuses), Mytilidae (mussels), and Haliotidae (abalones) with bootstrap values of 99 -100 suggesting strong support for the relationships shown. Within the e class Bivalvia, families like Mytilidae (*G. demissa*, *M.chilensis*, *M.galloprovincialis*) and Cardiidae (*T.gigas*, *F. mutica*) form monophyletic groups. Meanwhile *Illex argentines* (from Ommastrephidae) and *O.vulgaris* (from Octopodidae) are part of separate clades, showing their evolutionary divergence within cephalopods. Gastropods such as *Haliotis rufescens* and *H.iris* (family Haliotidae) form a distinct clade, showing their shared evolutionary history.

Species within the same family (*O. vulgaris* and *O. bimaculoides* from Octopodidae, or *M. chilensis* and *M. galloprovincialis* from Mytilidae) are closely related, sharing recent common ancestors. Whereas the placement of bivalves, gastropods, and cephalopods in separate branches reflects their divergence from a shared ancestor, with traits that adapted to different ecological niches.

Thus, the tree inferred from concatenated sequences effectively highlights the evolutionary paths of mollusk families, supported by high bootstrap values. It also illustrates the evolutionary distance between mollusks and the insect outgroups, emphasizing the diversity of life forms and their shared ancestry.

Phylogenetic studies within Mollusca progresses through the years which support the separation of two lineages Aculifera (Solenogastres and Caudofoveata) as a monophyletic Aplacophora, and Conchifera which include Bivalvia, Gastropoda, Cephalopoda, Monoplacophora, and Scaphopoda [37, 38, 39]. These three diverse groups of mollusks (cephalopods, gastropods, and bivalves) display morphological, ontogenetic, and even genomic features [40]. The results inferred in the phylogenetic trees were supported by these claims.

CONCLUSION

COX1 gene and the 16S ribosomal RNA gene are essential tools for taxonomic investigations and each of them provide a specific purpose basing on their distinguishing traits, where COX1 gene is effective particularly in identifying the level of species in animals and 16S rRNA genes consist of excellent conservation that is essential for giving a broader taxonomic survey permitting for a higher-level classification of organisms. The phylogenetic trees based from the selected COX1 and 16s rRNA sequences provides a comprehensive view of the evolutionary relationships among various mollusk families, clearly delineating how these groups have diverged from a common ancestral lineage.

DECLARATION

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Authorship Contributions

Concept and Writing: Ms. Jenelyn E. Tuliuan, Dr. Mary Jhane G. Valentino; Critic and final revision: Ms. Jenelyn Tuliuan, Dr. Mary Jhane G. Valentino, Mr.Wilhelm M. Javier; Dr. Eleonor S. Austria

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Consent for publication

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Competing interests

The authors declared that there is no conflict of interest.

REFERENCES

[1] Haszprunar, G. (2020): Mollusca (Molluscs). 1(3): 565–571. https://doi.org/10.1002/9780470015902.a0029219

[2] Abbott, R.T. (1976): Seashells. The Ridge Press, New York. 156 pp

[3] Fortunato, H. (2015): Mollusks: Tools in Environmental and Climate Research. American Malacological Bulletin 33(2): 310–324.. https://doi.org/10.4003/006.033.0208

[4] Ponder, W., Lindberg, D. (2008): Molluscan evolution and phylogeny: an introduction. Phylogeny and Evolution of the Mollusca, University of California Press, 1–17. https://doi.org/10.1525/california/9780520250925.001.0001

[5] Haszprunar, G., Schander, C., Halanych, K. M. (2008): Relationships of higher molluscan taxa. In phylogeny and evolution of the Mollusca, 19–32. https://doi.org/10.1525/9780520933705-004

[6] Haszprunar, G., Wanninger, A. (2012): Molluscs. Current Biology 22: 510–514.

[7] Parkhaev, P. Y. (2008): The early cambrian radiation of Mollusca. In phylogeny and evolution of the Mollusca (Ponder, W. F., Lindberg, D. R., Eds.), 33–70. Ponder, W.F. & Lindberg, D.R., eds., University of California Press, Berkeley, 33–69.

[8] Jalajakshi, S., Usha, R. N. (2019): Phylogenetic analysis of COXI gene in identification of spiders. Journal of Entomology and Zoology Studies 7(2): 895-897.

[9] Sudhikumar, A.V., Kashmeera, N.A. (2015): Preliminary study on identification of spiders using mitochondrial DNA. Journal of Entomology and Zoology Studies 6(8): 5741-5743.

[10] Hendrich, L., Pons, J., Ribera, I., Balke, M. (2010): Mitochondrial Cox1 sequence data reliably uncover patterns of insect diversity but suffer from high lineage-idiosyncratic error rates. PLoS One 5(12). https://doi.org/10.1371/journal.pone.0014448

[11] Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L., & Pace, N. R. (1985): Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proceedings of the National Academy of Sciences of the United States of America, 82(20), 6955–6959. https://doi.org/10.1073/pnas.82.20.6955

[12] Tringe, S. G., & Hugenholtz, P. (2008): A renaissance for the pioneering 16S rRNA gene. Current Opinion in Microbiology, 11(5), 442–446. https://doi.org/10.1016/j.mib.2008.09.011

[13] Yang, B., Wang, Y., & Qian, P.-Y. (2016): Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. *BMC Bioinformatics*, 17:135. https://doi.org/10.1186/s12859-016-0992-y

[14] 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

[15] 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

[16] Price, M. E., Handley, K., Millar, J., & O'Donovan, B. (2010): Feedback: All that effort, but what is the effect? Assessment &

Evaluation in Higher Education, 35(3), 277-289. https://doi.org/10.1080/02602930903541007

[17] In-Young, H., Malouf, R., & Lopez, G. (1993): Enhanced larval settlement of the hard clam *Mercenaria mercenaria* by the gem clam *Gemma gemma*. Marine Ecology Progress Series, 9(1), 51–59

[18] Emerson, C. W., Minchinton, T. E., & Grant, J. (1988): Population structure, biomass, and respiration of *Mya arenaria* L. on a temperate sandflat. Journal of Experimental Marine Biology and Ecology, 115(2), 99–111. https://doi.org/10.1016/0022-0981(88)90096-2

[19] Yamaoka, Y., Otsuka, Y., & Kondo, Y. (2018): Shell morphology and habitat of *Fulvia* sp., a candidate for ancestral species of extant *F. mutica* (Bivalvia: Cardiidae) from the Pliocene Ananai Formation. *Fossils, 98*, 5-15. https://doi.org/10.14825/kaseki.98.0_5

[20] Neo, M. L., Eckman, W., Vicentuan, K., Teo, S. L.-M., & Todd, P. A. (2015): The ecological significance of giant clams in coral reef ecosystems. Biological Conservation, 181, 111-123. https://doi.org/10.1016/j.biocon.2014.11.004

[21] Bailén, M., Bressa, C., Larrosa, M., & González-Soltero, R**.** (2020): Bioinformatic strategies to address limitations of 16S rRNA short-read amplicons from different sequencing platforms. *Journal of Microbiological Methods* 169, 105811. Journal of microbiological methods, 169, 105811. https://doi.org/10.1016/j.mimet.2019.105811

[22] MolluscaBase eds. (2024): MolluscaBase. Accessed at https://www.molluscabase.org on 2024-12-08. https://doi:10.14284/448

[23] Li, Q., Bai, Z., Han, X., & Li, J. (2017): Freshwater nucleated pearl quality is influenced by host mussel growth traits in *Hyriopsis cumingii*. Aquaculture Research, 48, 4656–4665. https://doi.org/10.1111/are.13288

[24] Collin, R. (2013): Phylogenetic patterns and phenotypic plasticity of molluscan sexual systems. Integrative and Comparative Biology, 53(4), 723–735. https://doi.org/10.1093/icb/ict076

[25] Toxvaerd, S. (2021): The emergence of the bilateral symmetry in animals: A review and a new hypothesis. Symmetry, *13*(2), 261: https://doi.org/10.3390/sym13020261

[26] Ramli, F. F., Munian, K., Othman, N., Sariyati, N. H., Abdullah-Fauzi, N. A. F., Ilham-Norhakim, M. L., & Abdul-Latiff, M. A. B. (2024): A comparative assessment of 16S ribosomal RNA and Cytochrome C Oxidase Subunit I (COI) Primers for Amphibian DNA Barcoding. BIO Web of Conferences, 94, 01003.

[27] Hillis, D. M., & Bull, J. J. (1993): An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology, 42, 182–192. https://doi.org/10.1093/sysbio/42.2.182

[28] Strüder-Kypke, M. C., & Lynn, D. H**.** (2010): Comparative analysis of the mitochondrial cytochrome c oxidase subunit I (COI) gene in ciliates (*Alveolata*, *Ciliophora*) and evaluation of its suitability as a biodiversity marker. Systematics and Biodiversity, 8(1), 131–148. https://doi.org/10.1080/14772000903507744

[29] Dela Cruz, R., Jeong, M.-Y., & Winge, D. R. (2016): Cox1 mutation abrogates need for Cox23 in cytochrome c oxidase biogenesis. Microbial cell (Graz, Austria), *3*(7), 275–284. https://doi.org/10.15698/mic2016.07.511

[30] Lindgren, A. R., Giribet, G., & Nishiguchi, M. K. (2004): A combined approach to the phylogeny of Cephalopoda (Mollusca). Cladistics : The International Journal of the Willi Hennig Society, 20(5), 454–486. https://doi.org/10.1111/j.1096-0031.2004.00032.x

[31] Vermeij, G. J., & Watson-Zink, V. M. (2022): Terrestrialization in gastropods: lineages, ecological constraints and comparisons with other animals. Biological Journal of the Linnean Society, 136(3), 393–404. https://doi.org/10.1093/biolinnean/blac053

[32] Lastuti, N. D. R., Rohman, A., Handiyatno, D., Chrismanto, D., & Desiandura, K**.** (2019): Sequence analysis of the cytochrome c oxidase subunit 1 gene of *Sarcoptes scabiei* isolated from goats and rabbits in East Java, Indonesia. Veterinary World, 12(7): 959–964. https://doi.org/10.14202/vetworld.2019.959-964

[33] Filipe, D., Parreira, R., Pereira, A. (2021): Preliminary comparative analysis of the resolving power of COX1 and 16S-rDNA as molecular markers for the identification of ticks from Portugal. Veterinary Parasitology, Regional Studies and Reports, 24, 100551. https://doi.org/10.1016/j.vprsr.2021.100551

[34] Trobajo, R., Mann, D. G., Clavero, E., Evans, K. M., Vanormelingen, P., & McGregor, R. C. (2010): The use of partial COX1, rbcL, and LSU rDNA sequences for phylogenetics and species identification within the *Nitzschia palea* species complex (Bacillariophyceae). European Journal of Phycology, 45(4), 413–425. https://doi.org/10.1111/j.1529-8817.2006.00291.x

[35] Fuks, G., Elgart, M., Amir, A., Zeisel, A., Turnbaugh, P. J., Soen, Y., & Shental, N. (2018): Combining 16S rRNA gene variable regions enables high-resolution microbial community profiling. Microbiome, **6**(1) 17. https://doi.org/10.1186/s40168-017-0396-x

[36] Paternina, L.E., Verbel-Vergara, D., & Bejarano, E.E. (2016): Comparison of 16S and COX1 genes mitochondrial regions and their usefulness for genetic analysis of ticks (Acari: Ixodidae). Biomedica. 3,36(2): 295–302. https://doi.org/10.7705/biomedica.v36i2.3116

[37] Kocot, K. M. (2013): Recent advances and unanswered questions in deep molluscan phylogenetics. Am. Malacol. Bull. 31, 195–208. American Malacological Bulletin 31, 195–208. https://doi.org/10.4003/006.031.0112

[38] Wanninger, A., & Wollesen, T. (2019): The evolution of molluscs. Biological reviews of the Cambridge Philosophical Society, 94(1), 102–115. https://doi.org/10.1111/brv.12439

[39] Kocot, K. M., Poustka, A. J. , Stöger, I. , Halanych, K. M., & Schrödl, M. (2020): New data from Monoplacophora and a carefully-curated dataset resolve molluscan relationships. Scientific reports, 10(1), 101. https://doi.org/10.1038/s41598-019-56728-w

[40] Wollesen, T., Rodríguez Monje, S.V., Luiz de Oliveira, A., & Wanninger, A. (2018): Staggered Hox expression is more widespread among molluscs than previously appreciated. Proceedings of the Royal Society B 285, 20181513. https://doi.org/10.1098/rspb.2018.1513