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Morphological diversity and genetic homogeneity in *Spondylus squamosus* from Lianga Bay, Surigao del Sur, Philippines

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Abstract. *Ruaza FC. 2025. Morphological diversity and genetic homogeneity in* Spondylus squamosus *(Scheiber, 1703) from Lianga Bay, Surigao del Sur, Philippines. Biodiversitas 26: 1768-1776.* This study investigates the two morphotypes of *Spondylus squamosus* found in the northern and southern parts of Lianga Bay, Surigao del Sur, using landmark-based geomorphometry, Field Emission Scanning Electron Microscopy (FESEM) for shell microstructures, and molecular analysis. The research aims to determine the morphological and genetic differences between the two morphotypes and assess their plasticity and potential evolutionary divergence. Geomorphometric analysis revealed distinct clusters among the morphotypes when projected onto a two-dimensional plane based on their relative warp coordinates, suggesting significant morphological plasticity in shell structure. Despite these differences in external morphology, shell microstructure analysis showed that both morphotypes share the same shell structure, characterized by a regular complex crossed lamellar outer layer composed of long rod-shaped mineral crystals stacked parallel to each other. Molecular analysis using mitochondrial 12S and 16S genes revealed a high percentage of identity, confirming that both northern and southern individuals are *S. squamosus*. This genetic consistency supports the conclusion that the two morphotypes belong to the same species, despite their morphological variability. The significance of these findings is underscored by their implications for biodiversity preservation and conservation strategies in Lianga Bay. Understanding the ecological dynamics and the role of *S. squamosus* within the ecosystem provides insights into sustainable management strategies. This study recommends further research into the environmental factors contributing to this morphological diversity, considering the adaptive significance of these differences to provide insights into the evolutionary processes shaping *S. squamosus* populations.

Keywords: 12S and 16S genes, landmark-based geomorphometry, shell microstructures

Abbreviations: FESEM: Field Emission Scanning Electron Microscopy; PCA: Principal Component Analysis, LGU: Local Government Unit

INTRODUCTION

The family Spondylidae, commonly known as thorny or spiny oysters, consists of only one genus, *Spondylus*. These species inhabit rocky reef areas from the intertidal zone down to depths of 1 to 30 meters. The Lianga Bay in Surigao del Sur has an abundant supply of spiny oysters, which are sold in local markets, restaurants, and resorts. Economically, these oysters have become an important source of income for fishers living near the coastal areas of the bay. Additionally, due to their superior taste compared to other oysters, they attract tourists to the province, further enhancing their economic value. Their vibrant and unique shell patterns make them popular among collectors and artisans, adding to their economic importance. The sustainable management of these oyster populations is crucial to preserving their role in the local economy and ecosystem.

However, intricate observations of the unregulated, uncontrolled, and constant harvesting of spiny oysters have led to a drastic decline in their populations. From 2016 to 2019, it was reported that the *Spondylus* fishery in Lianga Bay was already overexploited with the exploitation rates (E) of 0.7 (Ruaza and Ilano 2021). One of the exploited species in Lianga Bay is *Spondylus squamosus* Schreibers 1793, which exhibits distinct morphotypes in the northern and southern regions of the bay. These morphotypes differ in shell morphology, suggesting potential genetic divergence and adaptation to varying environmental conditions (van Bocxlaer et al. 2020). The unregulated harvesting of *S. squamosus* has led to concerns over its declining population, with anecdotal evidence indicating a significant reduction in catch rates in recent years (Ruaza and Ilano 2021). This highlights the need for comprehensive studies to understand the species' morphological and genetic diversity, which are crucial for informing sustainable management practices and ensuring the long-term conservation of this economically and ecologically important species (Bernatchez et al. 2017; Houston et al. 2020; Wood et al. 2020; Ruaza and Ilano 2023).

In response to these taxonomic concerns, this study employs a combination of landmark-based geomorphometry (Boretto et al. 2014; Ruaza et al. 2024), Field Emission Scanning Electron Microscopy (FESEM) for shell microstructure analysis (Kumar et al. 2021) and molecular techniques (Arivalagan et al. 2017; Velázquez-Urrieta et al. 2021). These methodologies are used to explore the morphological and genetic differences between the two morphotypes, aiming to understand how these variations may be linked to environmental factors and the potential for evolutionary divergence within the species (Collado et al. 2014; Wong and Sigwart 2019). By integrating these approaches, the research seeks to uncover the extent of phenotypic plasticity in *S. squamosus* and how it may contribute to the species' adaptability and resilience in varying habitats. This multi-faceted approach allows for a comprehensive understanding of both external and internal shell characteristics. Additionally, it helps in identifying the evolutionary pressures that may have shaped the current diversity within the species.

Given the ecological and economic importance of *S. squamosus* in Lianga Bay, it contributes significantly to the marine food web and holds economic value due to its market demand in local trade. The species is harvested for its meat, which is a source of nutrition for coastal communities, and its shells, which are prized in the decorative arts market. The significance of this study lies in its potential to clarify the taxonomic status and evolutionary relationships of the species' morphotypes, which is crucial for effective conservation and management (Ruaza et al. 2024; Shamily et al. 2024). Understanding the genetic and morphological diversity within the species can inform sustainable harvesting practices and help mitigate the impact of overexploitation (Barrientos-Luján et al. 2021). Furthermore, this research provides valuable baseline data that can be used to monitor changes in the species over time, ensuring that conservation strategies are based on a thorough understanding of the species' biology and ecology (Gerdol 2017).

This study signifies the first comprehensive investigation on its shell microstructural, and molecular characteristics of *S. squamosus*. The research is of paramount importance due to the species' declining population from overexploitation, which threatens both its ecological role and economic value. By integrating morphological and genetic analyses, this research aims to support sustainable management measures, ensuring that the populations of *S. squamosus* remain viable for future generations. Moreover, we cannot effectively conserve organisms that we do not fully understand, highlighting the importance of this study in generating critical baseline data (von der Heyden et al., 2014; van Oppen and Coleman 2022). Conservation strategies informed by these findings will help maintain the species' biodiversity and its continued role in the livelihood of coastal communities in Surigao del Sur, Philippines.

MATERIALS AND METHODS

Study area

The study was conducted at the North (Hornasan, San Agustin) and South (Sua, Barobo) of the Lianga Bay, Surigao del Sur, Philippines. The Bay consists of 60 Barangays with 36 coastal Barangays. The Lianga Bay municipalities occupy a total land area of 1,007.50 km^2. It is located in the central part of the province of Surigao del Sur and lies within a geographical coordinate of 8°34'00" and 8°25'06" latitude and 125°59'00" and 126°22'00" longitude. It is bounded on the north by the Pacific Ocean (Figure 1).

Sample collection and processing

The samples were collected from the intertidal area of the Bay. The collected specimens were brought to North Eastern Mindanao State University-Lianga Campus for processing. The samples were cleaned to detach the epibionts on the surface of the shells. The samples were opened and a tissue sample of 1 cm^3 was removed from the adductor muscle. It was then fixed in 100% absolute ethanol and stored in -20°C.

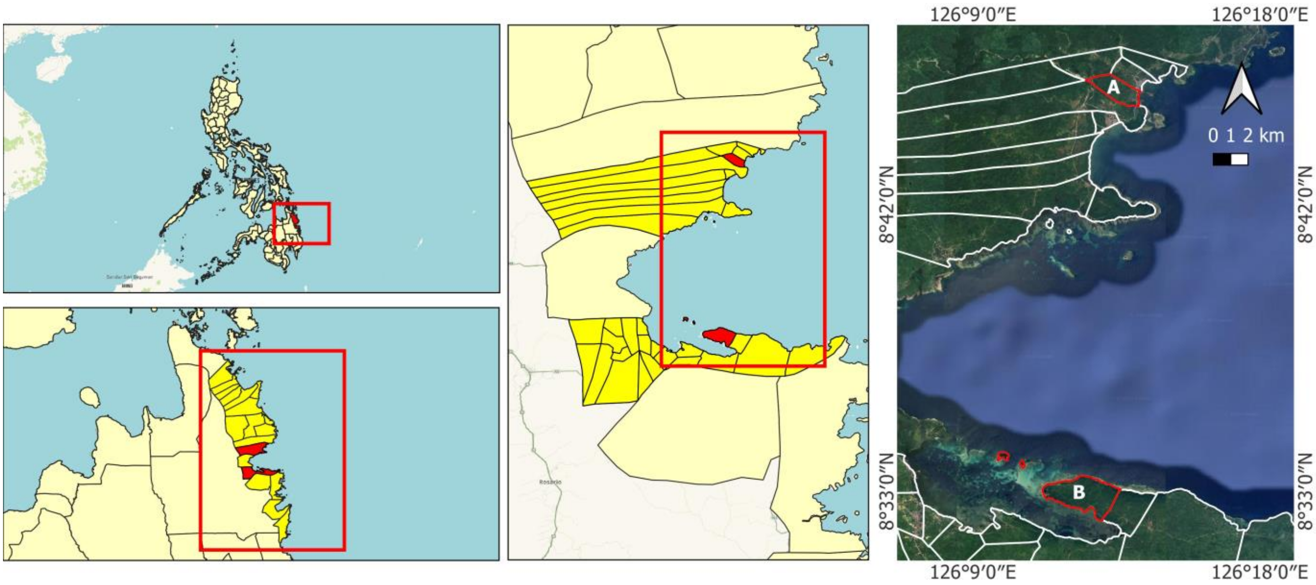

Figure 1. The study site. A. Hornasan, San Agustin; B. Sua, Barobo, Surigao del Sur, Philippines

Shell geo-morphometry

A total of 100 specimen were subjected to landmark-based geometric morphometric analysis. Shell specimen were mounted on modeling clay to ensure standard orientation before photography or measurement, with a scale bar attached for accurate meristic computation. Using a DSLR Canon EOS750D, upper valves were photographed in a constant dorsoventral position to capture clear internal shell structures, with umbo oriented vertically. These images were then transferred to a computer and enhanced using Adobe Photoshop for better visualization and interpretation. A total 15 landmarks configuration the umbo, dorsal tip of anterior cardinal tooth, dorsal tip of anterior cardinal tooth, posterior socket, anterior socket, ventral tip of posterior cardinal tooth, ventral tip of anterior cardinal tooth, postero-dorsal shell curvature, antero-dorsal shell curvature, dorsal tip of adductor/retractor muscle scar, junction of ventral tip of adductor-retractor muscle scar, pallial line, anterior end of the shell, ventral end of the shell and posterior end of the shell (Boretto et al. 2014 and Ruaza et al. 2024). The landmarks were digitized using TPSDig software and analyzed with PaSt v3.01 for geometric and multivariate data, including relative warp, thin plate splines, and 2D warps.

Shell microstructure

The shells were radially sectioned using a precision saw to examine the distribution of the outer and inner shell layers. Afterward, the samples were polished and cleaned in an ultrasonic bath. As elaborated by Sato and Sasaki (2015), the polished planes were filled with epoxy resin before cutting into small fragments. The polished planes were treated by etching with 0.2% HCl for 1-30 minutes. Afterwards, the sodium hypochlorite was added for 10-120 minutes to remove the organic materials. Later, the etching with 0.2% HCl for 1-5 minutes after immersion in sodium hypochlorite. After cleaning with an ultrasonic cleaner, the samples were coated with gold for observations in Field Emission Scanning Electron Microscope (FESEM).

Molecular analysis

Genomic DNA was extracted from the samples of *S. squamosus* collected from the north and south portion of the Bay. The adductor muscle tissue was extracted using the NucleoSpin® Gel and PCR Clean-up Tissue Kit. The 12S and 16S genes were then amplified using specific primers of Barucca et al. (2003). The 12S rRNA gene was amplified with the primers 12S F (5'-AGA CAT GGA TTA GAT ACC C-3') and 12S R (5'-ACC CCT ACC TTG TTA CGA CTT-3'), while the 16S rRNA gene was amplified using the primers 16S arL (5'-CGC CTG TTT AAC AAA AAC AT-3') and 16S rbH (5'-CCG GTT TGA ACT CAG ATC ACG T-3').

The sequences were then aligned and compared using bioinformatics tools to assess genetic similarity of the two morphotypes. This approach allowed for the identification of any potential genetic differences that could indicate evolutionary divergence, while also confirming species identity. The sequences were done by submitting the sequences to Basic Local Alignment Search Tool (Zhang et al. 2000; Morgulis et al. 2008) which compares the sequences to all the database sequences. A phylogenetic tree was constructed using the Neighbor-Joining method with MEGA 11 software, incorporating 1,000 bootstrap replicates (Tamura et al. 2021).

RESULTS AND DISCUSSION

Landmark-based geometric morphometry

Distinct clusters among the morphotypes of *S. squamosus* of the Bay were noticed when projected multivariate analysis (Figure 2) as defined by their relative warp coordinates. The PCA scatter diagram provided a clear differentiation between two groups, indicated by green (North) and purple (South) points, along the first two principal components (PC1 and PC2).

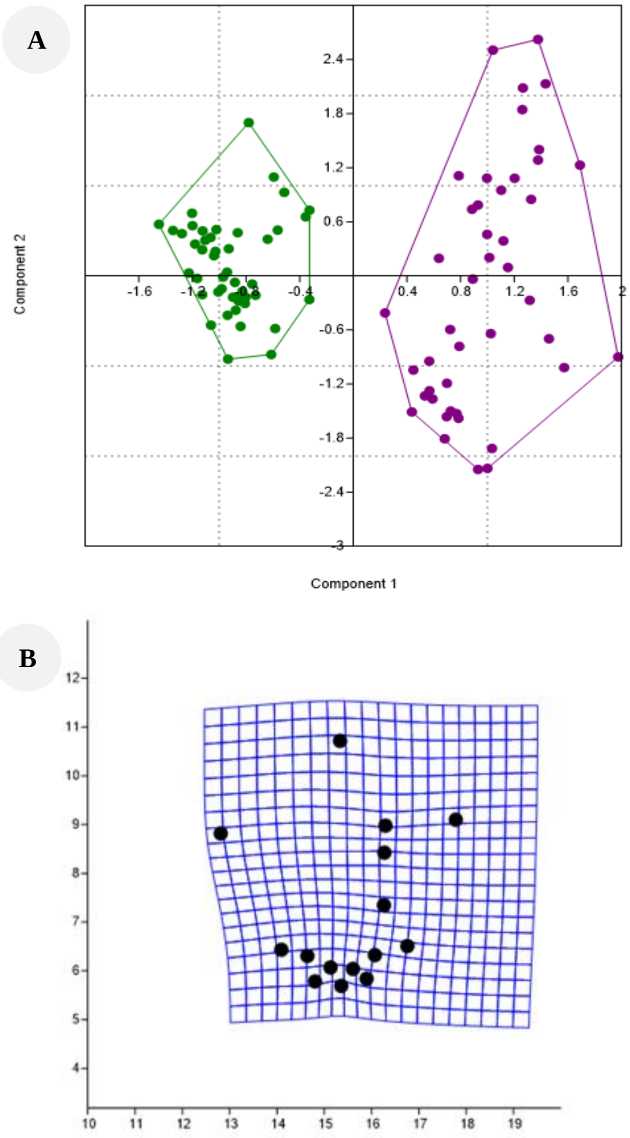

Figure 2. Multivariate Analyses: A. Principal Component Analysis (green dots represent *S. squamosus* North and purple in South); B. Thin-plate Spline Transformation Grid

Spondylus squamosus in North or green group, situated on the left side of the plot, is tightly clustered with relatively little spread, as shown by its convex hull. This tight clustering suggests that the *S. squamosus* in North exhibits low variability, that the individuals within this group are quite similar in the traits analyzed. In contrast, *S. squamosus* in North, located on the right side of the plot, shows a broader distribution with a larger convex hull. This indicates greater variability within the purple group, suggesting that the individuals in this group are more diverse in the traits contributing to PC1 and PC2. The separation between the green and purple groups along PC1 is particularly striking, indicating that the primary source of variation captured by PC1 strongly distinguishes these two groups. PC2 also contributes to the separation but to a lesser extent. In addition, MANOVA revealed that there is significant ($P>0.01$) difference on the two groups of *S. squamosus*.

The thin- plate spline transformation grid observed that areas of the grid that appear stretched the regions exhibit the most significant differences. There is a prominent deformation in the lower middle portion of the grid. This deformation signifies that this specific area has undergone substantial changes between the two shapes being compared, indicating a major variation in that shell shape.

Shell microstructure

The morphotypes of *S. squamosus* from North and South of the Bay were subjected to shell microstructure observation under the FESEM (Table 1). Two shell microstructures were observed and noted through its categories and types across the 10 samples (Figures 3 and 4). It was observed that the two morphotypes had the same shell microstructures. The shell microstructures were distinctly observed in each shell layers. In the outer layer of the shell, all of the species has a category of crossed lamellar structure and according to the type of crossed, it was regular complex crossed lamellar. It was constituted of long rod-shaped mineral crystals stacked parallel to each other.

Table 1. The shell microstructures of the morphotypes of *Spondylus squamosus*

Species	Outer layer	Middle layer	Inner Layer
S. squamosus in North	(crossed) regular complex crossed lamellar	Absent	(prismatic) irregular nondenticular composite prismatic
S. squamosus in South	(crossed) regular complex crossed lamellar	Absent	(prismatic) irregular nondenticular composite prismatic

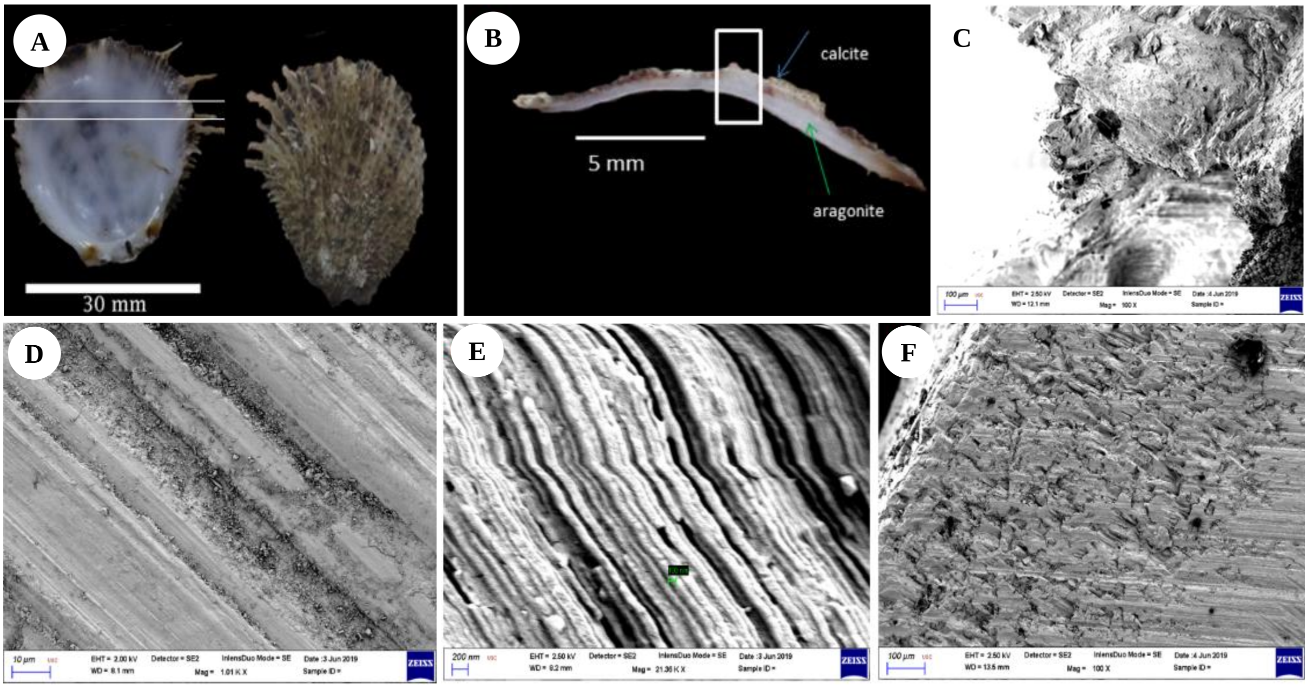

Figure 3. FESEM images of the shell microstructures of *Spondylus squamosus* in North. A. Exterior and interior valve; B. Transverse section of the shell; C. The cemented epibionts of the outer shells; D. The crossed lamellar microstructure of the outer shells; E. The preserved growth lines in the crossed lamellar microstructure; F. The transitional structure of the crossed lamellar structure to the inner layer the irregular nondenticular composite prismatic structure

Molecular analysis

The 12S and 16S rRNA sequences of *S. squamosus* have been submitted to the NCBI GenBank database and are available under accession numbers PV216829, PV216830, PV216831, and PV216832. The molecularly identified morphotypes of *S. squamosus* based on 12S and 16S rRNA sequences (Table 2), have been analyzed using BLAST. The combined results from both genes reveal a high genetic identity, with the northern sample exhibiting 99.76% identity for the 12S gene and 99.79% for the 16S gene, while the southern sample shows comparable levels of genetic similarity.

The phylogenetic analyses based on both mitochondrial 12S and 16S rRNA genes revealed consistent results in confirming the species identity and genetic relationships of the query samples, *S. squamosus* from the North and South of Liang Bay (Figures 5 and 6). In both gene trees, the two isolates clustered together in a well-supported clade with high bootstrap values, indicating strong genetic similarity and suggesting they belong to the same species. In the 12S gene tree, the samples grouped closely with other *S. squamosus* sequences and formed a distinct lineage, while in the 16S gene tree, they again clustered with previously reported *S. squamosus* sequences, reinforcing the species identification. Both trees also showed clear genetic divergence among other *Spondylus* species such as *S. gaederopus*, *Spondylus spinosus* Schreibers 1793, *Spondylus ictericus* Reeve 1856, and *Spondylus victoriae* G.B.Sowerby II 1860, supporting species-level distinction.

Table 2. The molecularly identified morphotypes of *Spondylus squamosus* in 12S and 16S with their BLAST result

Morpho species ID	Strain from GenBank	Author with accession number	Query cover (%)	Percent identity (%)
12s				
S. squamosus in North and South	*S. squamosus*	Puslednik and Serb (2008) EU379425.1	100	99.76
16s				
S. squamosus in North and South	*S. squamosus*	Puslednik and Serb (2008) EU379479.1	95	99.79

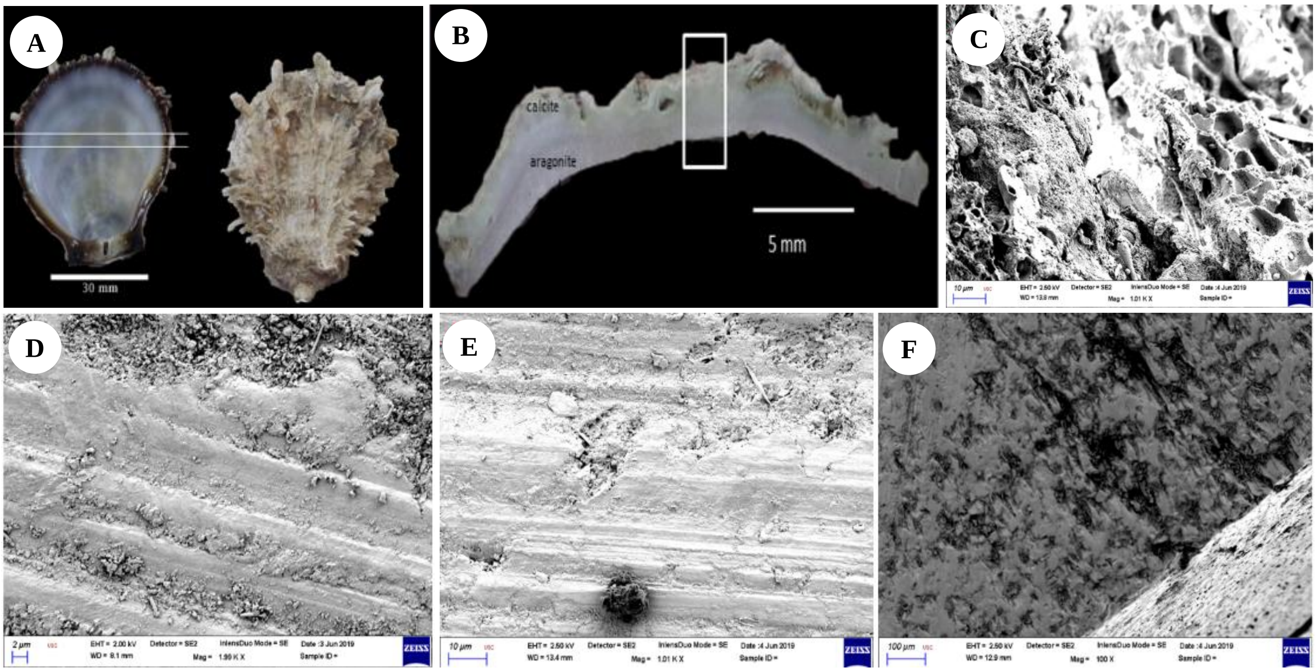

Figure 4. FESEM images of the shell microstructures of *Spondylus squamosus* Schreibers, 1793 in South. A. Exterior and interior valve; B. Transverse section of the shell; C. Thick cemented epibionts of the outer shells; D. The crossed lamellar microstructure of the outer shells with fine aragonitic grains; E. Crossed lamellar microstructure; F. The inner layer of the shell composed of the irregular nondenticular composite prismatic structure

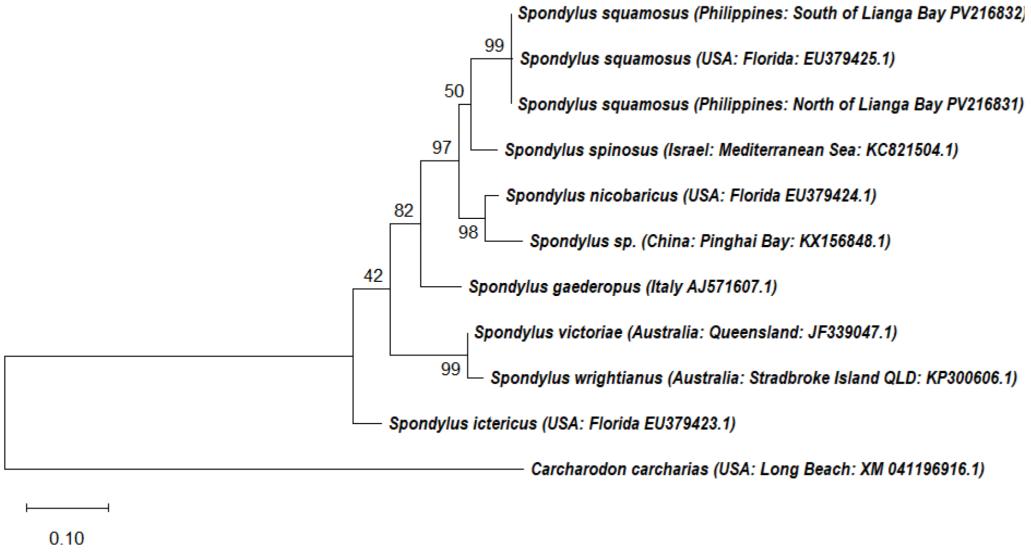

Figure 5. Phylogenetic relationships among *Spondylus* species based on mitochondrial 12S

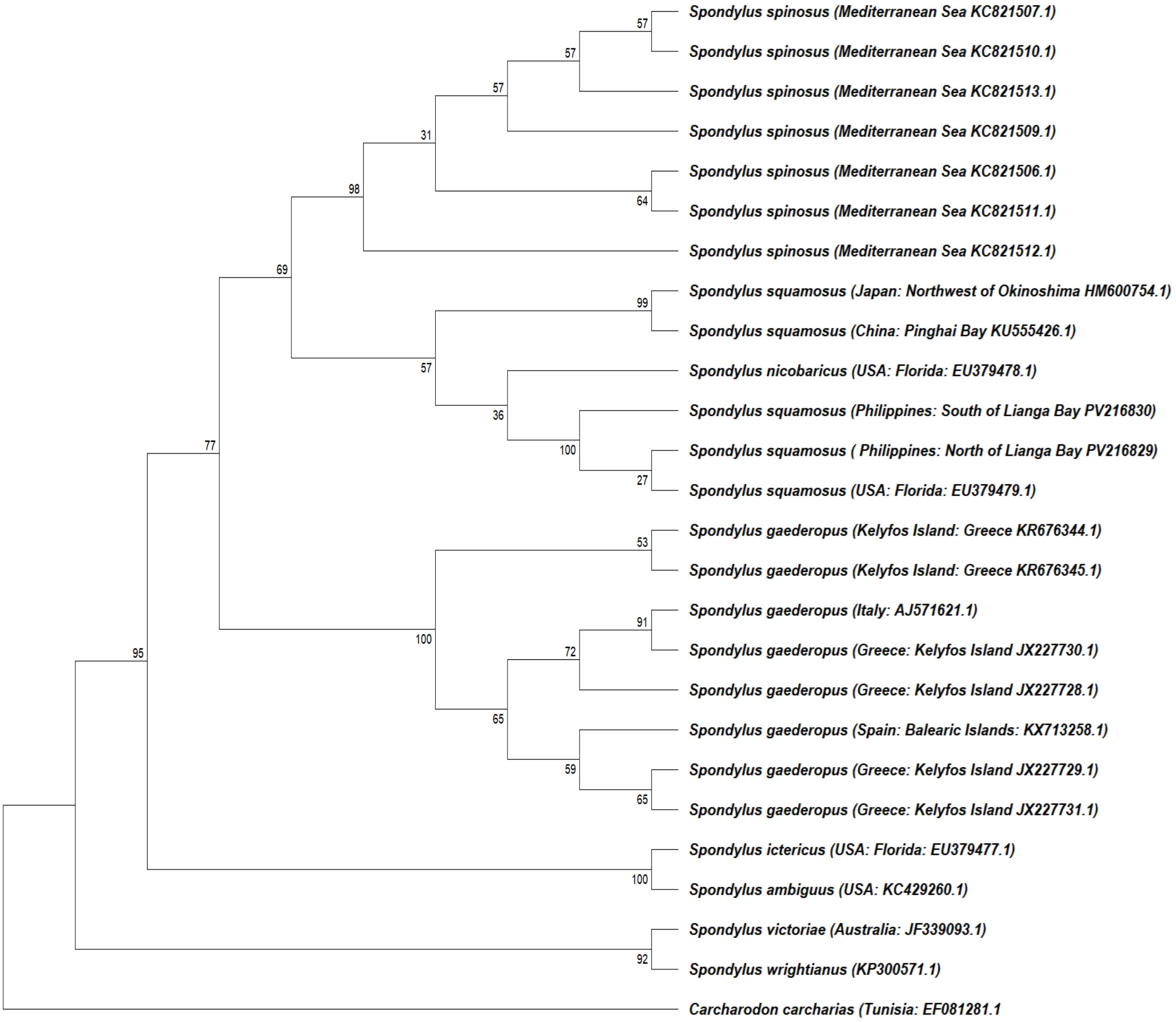

Figure 6. Phylogenetic relationships among *Spondylus* species based on mitochondrial 16S

Discussion

The landmark-based geomorphometric analysis reveals a clear separation between the *S. squamosus* populations in the North and South, strongly suggesting that these groups exhibit plastic characters or different species or populations with significant differences in the traits or measurements analyzed. Hence, detailed morphological and genetic analyses were conducted. The distinct separation aligns with previous studies that emphasize the role of geographic isolation in driving morphological divergence within species (Layton et al. 2016; Barrientos-Luján et al. 2021). The compact nature of the *S. squamosus* population in the North, compared to the more dispersed South group, could reflect adaptations to different ecological niches, varying evolutionary histories, or distinct genetic backgrounds, further supporting the idea that they are distinct entities. This pattern of divergence is consistent with the findings of other studies on species distributed across different environments, where morphological variation often correlates with ecological and genetic differences (Laland et al. 2014; Ord and Summers 2015; Levis and Pfennig 2017).

Apart from the landmark-based geomorphometric analysis, the study employed Field Emission Scanning Electron Microscopy (FESEM) observation to examined its shell microstructure. This approach aimed to determine whether the observed morphotypes represent a distinct species or exhibit plasticity. The Spondylid species composed of 2 types of microstructures. The bivalve groups consist of combinations of two or more microstructures often their external layer is composed of prismatic or crossed lamellar (Böhm et al. 2016). Of all the morphological types, the crossed lamellar and prismatic configurations are the most prevalent microstructures among the Spondylid species observed in the study. The arrangements of the crossed lamellar structure in various molluscan shells have been further explored in recent studies (Bonarski et al. 2015; Ji et al. 2017).

In addition, the *S. squamosus* from the North and South of the Bay has similar microstructure configurations of crossed and prismatic structures. There are several studies that discuss how different species of bivalves within the same genus can have distinct shell microstructures. The study of Popov (2014) has shown that bivalve shell microstructures can vary significantly even within the same family, such as the Veneridae. Also, Sato et al. (2017) shows that the group of bivalves show taxon-specific differences despite morphological similarities. They emphasized that these variations in microstructure are used for systematic classification and understanding evolutionary trends. This study strongly suggests the shell microstructure of the *Spondylus* species provide implications and useful bases in the field of Taxonomy. Thus, for taxonomic revisions of *Spondylu*s species or in other mollusc groups, it will be useful to consider microstructural data. The identification of the microstructural layers and their distribution within the shell of particular taxa has been found to have both phylogenetic and adaptive significance (Popov 2014; Sato and Sasaki 2015).

The molecular analysis reveals an impressive level of genetic similarity between the northern and southern *S. squamosus* samples. Specifically, the 12S gene demonstrates a 99.76% identity in the northern samples and a 99.79% identity in the southern samples, while the 16S gene exhibits comparable levels of genetic identity. These findings suggest that the morphotypes in both regions belong to the same species, despite their observable morphological differences. This genetic homogeneity has several significant implications for our understanding of the species' adaptability and evolution. It highlights the plasticity of *S. squamosus*, indicating that environmental factors might play a more crucial role in shaping the phenotypic variations observed in this species. The similarity in shell microstructure, as revealed by the Field Emission Scanning Electron Microscopy (FESEM) analysis, further supports the idea that these morphotypes are not divergent on a structural level.

The phylogenetic analyses (Figures 4 and 5) using both mitochondrial 12S and 16S rRNA gene sequences provided consistent and reliable insights into the evolutionary relationships among *Spondylus gaederopus* Linnaeus 1758 species. The close clustering of the query samples, *S. squamosus* from North and South of Liang Bay, indicates a high degree of genetic similarity, supporting their classification under the same species. Their association with previously reported *S. squamosus* sequences further validates their species identity. The separation of other *Spondylus* species into distinct clades with strong bootstrap support highlights clear genetic divergence and species-level distinctions. The congruence between the two molecular markers (12S and 16S) underscores their effectiveness in phylogenetic resolution and species delimitation. The inclusion of appropriate outgroups successfully rooted the trees, reinforcing the monophyletic nature of the *Spondylus* genus.

This result was supported by the study of Cen et al. (2022) on the analysis of genetic diversity in two different shell colors of the giant triton snail (*Charonia tritonis* Linnaeus 1758) based on mitochondrial COI sequences, which found that the genetic and size differentiation between the two different shell colors was small, and individuals of *C. tritonis* with different shell colors belong to the same genetic clade. Comparing 12S sequences of oysters from the Mediterranean and Eilat to the same locus sequences of congeners, including *S. gaederopus* which is indigenous to the Mediterranean, revealed that *S. spinosus* is indeed the same species in both seas. In succeeding years he used 16S (Shabtay et al. 2014) sequences of oysters from several sites were used in order to acquire a preliminary insight into their genetic variability and haplotype structure in the newly invaded Mediterranean environment. He further added that the 16S gene of the mtDNA was found to be the more variable of the two sequences, and was chosen for the haplotype analysis within the Mediterranean Sea sites. Studies on bivalve molluscs have demonstrated considerable variability in mtDNA, which has been used to understand phylogenetic relationships and population structures (Lavrov and Pett 2016; Milani and Ghiselli 2020; Ghiselli et al. 2021).

This insight is crucial for biodiversity conservation efforts, as it emphasizes the need to consider both genetic and morphological data when assessing species diversity and developing conservation strategies. In conclusion, the phylogenetic analysis provides robust evidence supporting the genetic consistency and phenotypic plasticity of *S. squamosus* in Lianga Bay. These findings contribute to our understanding of the evolutionary dynamics of this species and inform future research and conservation efforts aimed at preserving the ecological balance in the bay.

In conclusion, this study demonstrates that despite significant morphological plasticity and distinct clusters in the external shell structure of the two morphotypes, both are confirmed to be the same species, *S. squamosus*, based on shell microstructures and genetic analysis. The geomorphometric and multivariate analyses revealed substantial variation in external shell characteristics, yet the microstructural examination showed uniformity in shell structure across both morphotypes. This suggests that the observed morphological differences are not due to genetic divergence but may result from environmental influences. The study underscores the importance of further exploring the environmental factors driving this variability and suggests that a deeper understanding of these dynamics could enhance conservation strategies and provide valuable insights into the evolutionary processes affecting *S. squamosus* populations.

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REFERENCES

Arivalagan J, Yarra T, Marie B, Sleight VA, Duvernois-Berthet E, Clark MS, Marie A, Berland S. 2017. Insights from the shell proteome: Biomineralization to adaptation. Mol Biol Evol 34 (1): 66-77. DOI: 10.1093/molbev/msw219.

Barrientos-Luján NA, Rodríguez-Zaragoza FA, López-Pérez A. 2021. Richness, abundance and spatial heterogeneity of gastropods and bivalves in coral ecosystems across the Mexican Tropical Pacific. J Molluscan Stud 87 (2): eyab004. DOI: 10.1093/mollus/eyab004.

Barucca M, Olmo E, Schiaparelli S, Canapa A. 2004. Molecular phylogeny of the family Pectinidae (Mollusca: Bivalvia) based on mitochondrial 16S and 12S rRNA genes. Mol Phylogenet Evol 31 (1): 89-95. DOI: 10.1016/j.ympev.2003.07.003.

Bernatchez L, Wellenreuther M, Araneda C, Ashton DT, Barth JMI, Beacham TD, Maes GE, Martinsohn JT, Miller KM, Naish KA, Ovenden JR, Primmer CR, Young Suk H, Therkildsen NO, Withler RE. 2017. Harnessing the power of genomics to secure the future of seafood. Trends Ecol Evol 32 (9): 665-680. DOI: 10.1016/j.tree.2017.06.010.

Böhm CF, Demmert B, Harris J, Fey T, Marin F, Wolf SE. 2016. Structural commonalities and deviations in the hierarchical organization of crossed-lamellar shells: A case study on the shell of the bivalve *Glycymeris glycymeris*. J Mater Res 31: 536-546. DOI: 10.1557/jmr.2016.46.

Bonarski JT, Checa AG, Rodriguez-Navarro A, Tarkowski L, Wajda W. 2015. Irregularities of crystallographicorientation and residual stresses in the crossed-lamellar shell as a natural functionally graded material. J R Soc Interface 12: 20150738. DOI: 10.1098/rsif.2015.0738.

Boretto GM, Baranzelli MC, Gordillo S, Consoloni I, Zanchetta G, Morán G. 2014. Shell morphometric variations in a Patagonian Argentina clam (*Ameghinomya antiqua*) from the Mid-Pleistocene (MIS 7) to the present. Quaternary Intl 352: 48-58. DOI: 10.1016/j.quaint.2014.09.03.

Cen X, Zhang G, Liu H, Yao G, Xiong P, He M, Liu W. 2023. Analysis of genetic diversity in two different shell colors of the giant triton snail (*Charonia tritonis*) based on mitochondrial COI sequences. Front Mar Sci 9: 1066750. DOI: 10.3389/fmars.2022.1066750.

Collado GA, Salinas HF, Méndez MA. 2014. Genetic, morphological, and life history traits variation in freshwater snails from extremely high environments of the Andean Altiplano. Zool Stud 53 (1): 14. DOI: 10.1186/1810-522X-53-14.

Gerdol M. 2017. Immune-related genes in gastropods and bivalves: A comparative overview. Intl J Invert Reprod Dev 14 (1): 95-111. DOI: 10.1080/1824-307X.2017.1234567.

Ghiselli F, Gomes-dos-Santos A, Adema CM, Lopes-Lima M, Sharbrough J, Boore JL. 2021. Molluscan mitochondrial genomes break the rules. Philos Trans R Soc B 376: 20200159. DOI: 10.1098/rstb.2020.0159.

Houston RD, Bean TP, Macqueen DJ, Gundappa MK, Jin YH, Jenkins TL, Selly SLC, Martin SAM, Stevens JR, Santos EM, Davie A, Robledo D. 2020. Harnessing genomics to fast-track genetic improvement in aquaculture. Nat Rev Genet 21 (7): 389-409. DOI: 10.1038/s41576-020-0227-y.

Ji HM, Li XW, Chen DL. 2017. Crystallographic texture of crossed-lamellar structure in *Cymbiola nobilis* shell. J Ceram Soc Jpn 125 (5): 16333. DOI: 10.2109/jcersj2.125.16333.

Kumar S, Singh M, Krishna R. 2021. Field emission scanning electron microscopy (FESEM) for shell microstructure analysis. J Mater Sci 56 (12): 345-359.

Laland KN, Matthews B, Feldman MW. 2014. Organisms and their environment: An evolving relationship. Evolution 71 (2): 503-504. DOI: 10.1111/evo.13140.

Layton K, Martel A, Hebert P. 2016. Geographic patterns of genetic diversity in two species complexes of Canadian marine bivalves. J Molluscan Stud 82 (2): 282-291. DOI: 10.1093/mollus/eyv056.

Lavrov DV, Pett W. 2016. Evolutionary genetics of the mitochondrial genome: Insights from molluscs. Genetics 224 (3): iyad036. DOI: 10.1093/genetics/iyad036.

Levis NA, Pfennig DW. 2017. Organisms and their environment: An evolving relationship. Evolution 71: 503-504. DOI: 10.1111/evo.13140.

Morgulis A, Coulouris G, Raytselis Y, Madden T, Agarwala R, Schäffer A. 2008. Database indexing for production MegaBLAST searches. Bioinformatics 24: 1757-1764. DOI: 10.1093/bioinformatics/btn322.

Milani L, Ghiselli F. 2020. Molluscan mitochondrial genomes break the rules. Philos Trans R Soc B 376: 20200159. DOI: 10.1098/rstb.2020.0159.

Ord TJ, Summers TC. 2015. Repeated evolution and the impact of evolutionary history on adaptation. BMC Evol Biol 15: 137. DOI: 10.1186/s12862-015-0424-z.

Popov S. V. 2014. Formation of bivalve shells and their microstructure. Paleontol J 48 (14): 1519-1531. DOI: 10.1134/S003103011414010X.

Puslednik L, Serb JM. 2008. Molecular phylogenetics of the Pectinidae (Mollusca: Bivalvia) and effect of increased taxon sampling and outgroup selection on tree topology. Mol Phylogenet Evol 48 (3): 1178-1188. DOI: 10.1016/j.ympev.2008.05.006.

Ruaza F, Ilano A. 2021. Stock assessment of *Spondylus varius* (Sowerby, 1827) in Lianga Bay, Surigao del Sur, Eastern Mindanao, Philippines. J Bio Env Sci 18 (1): 44-50. DOI: 10.5281/zenodo.638237.

Ruaza F, Ilano A. 2023. Reproductive cycle of *Spondylus varius* (Sowerby, 1827) in Lianga Bay, Surigao Del Sur, Eastern Mindanao. Intl J Aquat Biol 5: 374-382. DOI: 10.22034/ijab.v11i5.170.

Ruaza FC Jr., Maturan DC, Bustillo E. 2024. Interior shell patterns among the Spondylid species (Bivalvia: Spondylidae) in Mindanao, Philippines. Biodiversitas 25 (7): 2901-2906. DOI: 10.13057/biodiv/d250711.

Sato K, Checa AG, Rodríguez-Navarro AB, Sasaki T. 2017. Crystallographic texture analysis of *Protobranchia* (Mollusca: Bivalvia): Interspecific variations, homology and shell microstructural evolution. J Mollusc Stud 83 (3): 304-315. DOI: 10.1093/mollus/eyx021.

Sato K, Sasaki T. 2015. Crystallographic texture analysis of *Protobranchia* (Mollusca: Bivalvia): interspecific variations, homology and shell microstructural evolution. J Mollusc Stud 83 (3): 304-315. DOI: 10.1093/mollus/eyx021.

Shabtay A, Tikochinski Y, Benayahu Y, Rilov G. 2014. Preliminary data on the genetic structure of a highly successful invading population of oyster suggesting its establishment dynamics in the Levant. Mar Biol Res 10: 4. DOI: 10.1080/17451000.2013.814790.

Shamily PC, Nandan SB, Hershey NR. 2024. Diversity of bivalve mollusks, their ecosystem services, and potential impacts of climate change. In: Joseph S, Pradeepkumar A (eds). Ecosystem Services Valuation for Sustainable Development. Springer, Singapore. DOI: 10.1007/978-981-97-4688-0_7.

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol 38 (7): 3022-3027. DOI: 10.1093/molbev/msab120.

van Oppen MJH, Coleman MA. 2021. Advancing the protection of marine life through genomics. PLoS Biol 20 (10): e3001801. DOI: 10.1371/journal.pbio.3001801.

Velázquez-Urrieta Y, Pérez-Ponce de León G. 2021. Morphological and molecular assessment of the diversity of trematode communities in freshwater gastropods and bivalves in Los Tuxtlas tropical rainforest. J Helminthol 95 (1): e44. DOI: 10.1017/S0022149X21000407.

van Bocxlaer B, Ortiz-Sepulveda C, Gurdebeke P, Vekemans X. 2020. Adaptive divergence in shell morphology in an ongoing gastropod radiation from Lake Malawi. BMC Evol Biol 20: 5. DOI: 10.1186/s12862-019-1570-5.

von der Heyden S, Beger M, Toonen RJ, van Herwerden L, Juinio-Meñez MA, Ravago-Gotanco R, Fauvelot C, Bernardi G. 2014. The application of genetics to marine management and conservation: Examples from the Indo-Pacific. Bull Mar Sci 90 (1): 123-158. DOI: 10.5343/bms.2012.1079123.

Wong NL, Sigwart JD. 2019. Natural history clues to the evolution of bivalved gastropods (Mollusca: Gastropoda: Sacoglossa: Juliidae). Mar Biodivers 49 (6):1997-2007. DOI: 10.1007/s12526-019-00960-0.

Wood G, Marzinelli EM, Vergés A, Campbell AH, Steinberg PD, Coleman MA. 2020. Using genomics to optimise and evaluate the performance of underwater forest restoration. J Appl Ecol 57 (10):1988-1998. DOI: 10.1111/1365-2664.13707.

Zhang Z, Schwartz S, Wagner L, Miller W. 2000. A greedy algorithm for aligning DNA sequences. J Comput Biol 7 (1-2): 203-214.