



Molecular identification of the edible rock scallop, *Spondylus limbatus* (Bivalvia: Spondylidae) from the southwestern Gulf of California

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ABSTRACT

The genus *Spondylus* is of great economic and ecological importance in the Eastern Pacific; however, its taxonomy has historically been complex due to the phenotypic plasticity in shells and cryptic species. This study addresses this problem through an integrative approach, combining morphological and molecular tools to analyse specimens from the southwestern Gulf of California. The markers (28S rRNA and 16S rRNA) were analysed in individuals of *S. limbatus*. Phylogenetic results, obtained using maximum likelihood and Bayesian Inference, confirmed the monophyly of the genus and the identity of *S. limbatus* as a robust and distinct clade. Interspecific genetic distances ranged from 2.5–3.4% (28S rRNA) to 7.6–15.7% (16S rRNA), while intraspecific variability was non-existent (0.0%) for the evaluated markers, reinforcing the stability of these genes for diagnosis. Unlike external ornamentation, which can disappear in adults or be masked by epibionts, constant internal characteristics were identified: an internal purple band and a finely crenulated margin. The study highlights that, given morphological ambiguity induced by environmental factors, the provided DNA sequences – the first 28S rRNA and 16S rRNA sequences for this species – are critical for population monitoring, fisheries management and conservation, raising the total number of species in the genus with global molecular data to 15%.

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Introduction

Scallops of the genus *Spondylus* (Bivalvia: Spondylidae) are conspicuous marine molluscs, widely distributed in tropical and subtropical environments (Lamprell 1987). These species are highly valued for their ornamental shells in jewellery and as collector's items, while some are harvested for human consumption (Lodeiros *et al.* 2016). Ecologically, *Spondylus* species are key components of rocky and coral reefs, acting as filter feeders and providing microhabitats for diverse marine biota (Skoglund and Mulliner 1996; Villalejo-Fuerte *et al.* 2015). In the eastern Pacific Ocean, four spondylid species are recognised, two of which are commercially exploited, *S. crassisquama* Lamarck, 1819, and *S. limbatus* Sowerby, 1847 (Lodeiros *et al.* 2016). The latter is the largest and most morphologically variable species within the Panamic province (Keen 1971; Skoglund and Mulliner 1996; Villalejo-Fuerte *et al.* 2015). *S. limbatus* is of particular interest due to its extensive commercial harvest for its adductor muscle and ornamented shells across most of its range (Villalejo-Fuerte *et al.* 2023).

Although the genus exhibits low diversity in the eastern Pacific Ocean, its taxonomy has been

problematic since the mid-1990s. The available literature has been inconsistent regarding species nomenclature. While Skoglund and Mulliner (1996) and Coan and Valentich-Scott (2012) provided significant documentation, accurate identification remains challenging due to high intraspecific variability in shell ornamentation (Ruaza *et al.* 2024) and the existence of cryptic species (Ruaza 2025). Such morphological ambiguity hinders ecological, biogeographical, and conservation studies, as well as effective fisheries management. Furthermore, the exclusive use of morphological data often provides limited phylogenetic resolution (Barucca *et al.* 2004).

Molecular biology has become an invaluable tool for resolving taxonomic ambiguities, ensuring unequivocal species identification and clarifying phylogenetic relationships (Feng *et al.* 2011). This approach has proven effective for species discrimination in various molluscs (Herbert *et al.* 2003; Mikkelsen *et al.* 2007; Ran *et al.* 2020). Recent phylogenomic studies based on ultraconserved elements (UCEs) have substantially improved the resolution of deep evolutionary relationships within Bivalvia (Li *et al.* 2025), while comparative mitogenomic analyses have provided new insights

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into mitochondrial genome evolution, gene rearrangements and the phylogeny of Pteriomorpha (Zhang *et al.* 2025). At the same time, integrative taxonomy, combining molecular and morphological evidence, has become a cornerstone for species delimitation and the recognition of cryptic diversity in marine bivalves (Lin *et al.* 2025).

For pectinids, molecular markers such as 12S, 18S, 28S, 16S, H3, and COI have been used to reconstruct evolutionary lineages (Matsumoto 2003; Barucca *et al.* 2004; Saavedra and Peña 2006; Puslednik and Serb 2008; Alejandrino *et al.* 2011; Liu *et al.* 2019; Sato *et al.* 2020). However, molecular data for the genus *Spondylus* is scarce, covering just over 10 species worldwide. Existing studies often focus on specific taxa such as *S. gaederopus* Linnaeus, 1758, *S. spinosus* Schreibers, 1793, *S. squamosus* Schreibers, 1793, *S. versicolor* Schreibers, 1793 and *S. virgineus* Reeve, 1856 (Shabtay *et al.* 2014; Galinou-Mitsoudi *et al.* 2019; Liu *et al.* 2019; Li *et al.* 2023; Ruaza 2025). For *S. limbatus*, the only available molecular study in the Gulf of California utilised microsatellites to evaluate population structure (Munguia-Vega *et al.* 2010), later coupling these genetic data with bio-oceanographic models to assess regional larval dispersal (Soria *et al.* 2012). The mitochondrial genome of *S. limbatus* has recently been published (Cotom-Nimatuj *et al.* 2026).

The phenotypic plasticity and presence of cryptic taxa in marine molluscs necessitate the integration of molecular data to complement traditional taxonomy (Sun *et al.* 2016). Establishing reliable molecular reference databases is essential for applications ranging from population monitoring to the control of fishery products (Teletchea 2009; Darling and Mahon 2011). Currently, molecular information is available for less than 13% of the 75 extant *Spondylus* species recorded in WoRMS (2026). The main objective of this study is to provide the first 28S and 16S sequences for morphologically identified specimens of *S. limbatus* from the southern Gulf of California. This research seeks to provide data for the molecular understanding of this species that can serve as a baseline for the development of subsequent taxonomic and phylogenetic studies.

Materials and methods

Material collection and morphological treatment

Samples of spiny rock scallops *Spondylus limbatus* were donated by Sociedad Cooperativa Piripichi SC de RL. Scallops were collected in Punta El Mechudo, La Paz Bay in the southwest of the Gulf of California, at 24° 48'29.98"N and 110°39'30.35"W in April 2024 (Figure 1). The specimens were collected using semi-autonomous diving, also known as hookah diving, at a depth of 33 m.

Specimens were collected (left valve) from the rocky subtidal zone and preserved in a 70% ethanol-sea-water solution. Morphological characterisation was conducted on all individuals ($n = 33$). Shell length was recorded for each specimen (only left valve) as the maximum antero-posterior distance parallel to the hinge line, measured using a digital calliper (TRUPER, CALDI-6MP, Mexico). While tissue samples from the foot from five specimens were sequenced for molecular analysis. Morphological features (e. g. dorsal and ventral views, and the left valve) were documented using a Canon EOS Rebel T6 digital camera (Canon Inc., Tokyo, Japan). Laboratory observations were performed using an Axioscope A1 microscope and a Stemi 2000-C stereomicroscope (Carl Zeiss, Germany). Detailed analysis of the shell sculpture was carried out with an Olympus CX23 optical microscope (Olympus America Inc., PA, USA). Finally, the examined material was deposited in the 'Dr. Antonio García-Cubas' Malacological Collection at the Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México (ICML-UNAM).

The five specimens used in the molecular analyses were selected because they had the typical morphological characteristics of *S. limbatus* and the appropriate size for capture according to local fishing standards; few specimens had muscle mass because they were donated by fishermen, and tissue was obtained from these specimens to carry out the molecular study. Total genomic DNA was extracted using the QIAGEN DNeasy Blood & Tissue kit (Qiagen, USA) from a small portion of the foot and mantle (1 cm²) of the five specimens. PCR reactions were set up with the Thermo Scientific Phire Plant Kit using the following reagent volumes: 10 µl of 2X buffer, 1.0 µl of each forward and reverse primer at 10 pmol, 0.4 µl of Taq Polymerase, 1.0 µl of DNA, and 11.6 µl of sterile water, for a final volume of 25 µl. The primers used for amplifying and sequencing 28S rRNA (forward: 5'-CAGCACCGAATCCCTCAGCCTTG-3' and reverse: 5'-TCTGGCTTCGTCCTACTCAAGCATAG-3') and 16S rRNA (forward: 5'-CGCCTGTTTAACAAAAACAT-3' and reverse: 5'-CCGGTTTGAATCAGATCACGT-3') were those presented in Alejandrino *et al.* (2011) and Palumbi *et al.* (1991). The reactions were run in a Techne Flexi-gene thermal cycler (Bibby Scientific, Maryland, USA) following the cycling protocol for the primers. The PCR products were sequenced by Macrogen (South Korea).

Sequences were edited with the BioEdit (Hall 1999) or Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA) programs and aligned with Clustal W (Thompson *et al.* 1994). The 28S rRNA and 16S rRNA alignments consisted of five new for marker (this study) and 10 GenBank sequences for the other species of *Spondylus* and the outgroups (*Acar domingensis* (Lamarck, 1819) and *Limopsis* sp. Sasso, 1827 for 28S

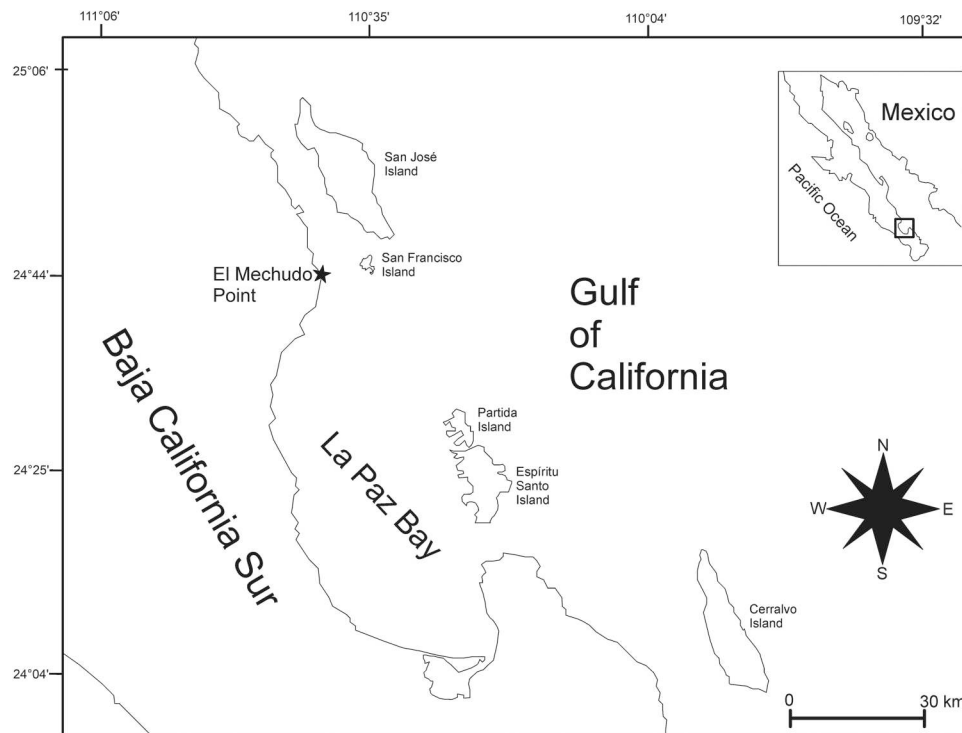


Figure 1. Map of site of study in the Gulf of California.

rRNA and *B. barbata* (Linnaeus, 1758) and *Limopsis* sp. for 16S rRNA (Table 1).

The software MEGA 7.0 (Kumar et al. 2016) was used to calculate genetic distances (Tables S1–S2). The evolutionary model for phylogenetic analyses was GTR + G + I for both data sets, selected as the best fit model with the Bayesian Information Criterion in JModelTest v2.1.10 program (Darriba et al. 2012). Phylogenies of 28S and 16S datasets were inferred using maximum likelihood (ML) and Bayesian inference (BI). The ML analyses were reconstructed using RAxML v8.0.X (Stamatakis 2014) set as follows: a rapid bootstrap analysis and search for the best scoring ML tree in one single program run with 1000 bootstrap replicates under the GTR + G + I substitution model. BI was performed for individual datasets with MrBayes v3.2.1 (Ronquist et al. 2012) using the Metropolis coupled Markov chain Monte Carlo under the GTR + G + I model. For each matrix, two million generations of two independent runs were performed with four chains and sampling trees every 100 generations. The burn-in period was identified graphically by tracking the likelihood at each generation to determine whether they reached a plateau. Twenty-five per cent of the saved trees were removed, and the remainder were used to calculate Bayesian posterior probabilities.

Results

Molecular phylogeny based on 28S

The 28S rRNA alignment consisted of 15 sequences (five obtained in the present work) with 877 bp. ML

(Figure 2) and BI analyses produced trees with similar topologies for this marker. The *Spondylus limbatus* specimens formed a clade supported by high posterior probability and bootstrap values (1 and 100, respectively). The clade branch was well supported (100/1) from the clades formed by the other species of *Spondylus*; the sister species of *S. limbatus* was the clade formed for *S. tenuis* Schreibers, 1793, *S. varius* G. B. Sowerby I, 1827, *S. sinensis* Schreibers, 1793, *S. crassisquama* Lamarck, 1819 and *S. virgineus* Reeve, 1856, the branch presented low values of support (38/0.59). For 28S rRNA, genetic distances among the specimens were 0.0% at the intraspecific level. When comparing these specimens with the other *Spondylus* species, the difference was 2.5–3.4%.

Molecular phylogeny based on 16 S

The 16S rRNA alignment consisted of 15 sequences (five obtained in the present work) with 510 bp. ML (Figure 3) and BI analyses produced trees with similar topologies for this marker. The *Spondylus limbatus* specimens formed a clade supported by high posterior probability and bootstrap values (1 and 100, respectively). The clade was separate from the clades formed by the other species of *Spondylus*, and the branch presented high values of support (100/1). For 16S rRNA, genetic distances among the specimens were 0.0% at the intraspecific level. When comparing these specimens with the other species of *Spondylus*, the distances were 7.6–15.7%.

Table 1. Species, collection sites, GenBank accession numbers and references with which the present work was carried out.

Species	Location	Accession GenBank		Reference
		28S	16S	
<i>Spondylus crassisquama</i> Lamarck, 1819	Egypt:Safaga Bay, Red Sea	AJ307542	–	Hammer (2001)
<i>Spondylus gaederopus</i> Linnaeus, 1758	Greece: Kelifos	–	KR676345	Galinoui-Mitsoudi <i>et al.</i> (2019)
<i>Spondylus limbatus</i> G. B. Sowerby II, 1847	Mexico: Baja California Sur, El Mechudo	PX606553 PX606554 PX606555 PX606556 PX606557	PX606558 PX606559 PX606560 PX606561 PX606562	This study
<i>Spondylus nicobaricus</i> Schreibers, 1793	Papua New Guinea: Kimbe Bay, Vanessa's Reef / West of New Briton	HM600743	EU379478	Alejandrino <i>et al.</i> (2011)/Puslednik and Serb (2008)
<i>Spondylus sinensis</i> Schreibers, 1793	Japan: Okinawa (Seragaki)	AB102750	–	Matsumoto (2003)
<i>Spondylus squamosus</i> Schreibers, 1793	Japan: Northwest of Okinoshima, Tateyama	HM600747	HM600754	Alejandrino <i>et al.</i> (2011)
<i>Spondylus tenuis</i> Schreibers, 1793	USA: Wreck of Thunderbolt, Florida	HM600742	EU379477	Alejandrino <i>et al.</i> (2011)/Puslednik and Serb (2008)
<i>Spondylus varius</i> G. B. Sowerby I, 1827	Japan: Okinawa (Shimoji island)	AB102749	–	Matsumoto (2003)
<i>Spondylus victoriae</i> G. B. Sowerby II, 1860	Australia	–	JF339093	Masters <i>et al.</i> (2011)
<i>Spondylus virgineus</i> Reeve, 1856	Egypt:Safaga Bay, Red Sea	AJ307561	–	Hammer (2001)
<i>Spondylus sp.</i>	China: Pinghai Bay	KU555424	KU555426	Wang (2016)
<i>Acar domingensis</i> (Lamarck, 1819)	Bahamas	KT757814	–	Combosch and Giribert (2016)
<i>Barbatia barbata</i> (Linnaeus, 1758)	ND	–	KC429244	Sharma <i>et al.</i> (2013)
<i>Limopsis sp.</i>	ND	KC429422	KC429247	Sharma <i>et al.</i> (2013)

Morphology

Specimens were identified as *Spondylus limbatus* based on shell sculpture features, which align with morphological descriptions provided by previous authors (Table 2).

Family Spondylidae
Subfamily Spondyliinae

Genus *Spondylus* Linnaeus, 1758

Spondylus limbatus G. B. Sowerby II, 1847 (Figure 4)

Description. The shell is massive and heavy, oval-shaped, 140–170 mm long (left valve), purplish-red with short, spatulate spines arranged irregularly, which are absent in adults. The right valve is attached to rocks by its lower portion. The hinge has large brown teeth and a flat to deep callus. The distinctive



Figure 2. Maximum-likelihood tree inferred from 28S sequences of *Spondylus*. ML bootstrap and Bayesian posterior probabilities values are shown at branches. Bold letters indicate newly generated sequences in this study.

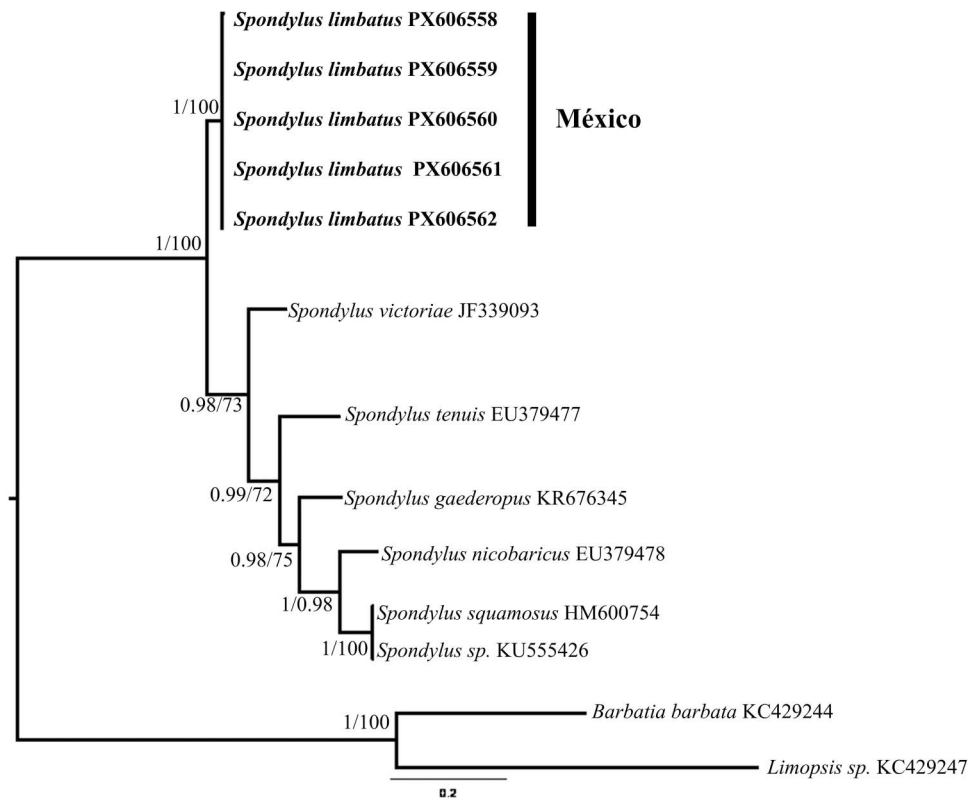


Figure 3. Maximum-likelihood tree inferred from 16S sequences of *Spondylus*. ML bootstrap and Bayesian posterior probabilities values are shown at branches. Bold letters indicate newly generated sequences in this study.

Table 2. Morphological comparison between *Spondylus limbatus* of this work against previous studies.

<i>S. limbatus</i>	Sowerby II (1847)	Carpenter (1857)	Keen (1971)	Skoglund and Mulliner (1996)	Coan and Valentich-Scott (2012)	This study
Exterior features						
Shape	Oval	Suborbicular masive	–	Variable	–	Oval massive
Maximum shell length (mm)	–	152.4	150	249	249	170.4
Colour	Dark purple/yellow	Deep red-purple	–	Purple/orange, orange/yellow, all orange, all purple	Red-purple, yellow orange	Red-purple
Spines	Depressed, smooth, palmate, short, irregular,	Short finely radiately striated	Numerous and evenly distributed	Short, spatulate to absent on adults	short and wide, irregular spatulates, which are reduced to thick cords in some specimens around 20	Short and wide, spatulate to absent on adults
Radial ribs	9–11 finely striated	Vaulted, very irregular intervals, ill-developed	coarsely ribbed	Coarse threads	around 20	Irregular, intervals coarse threads
Attachment area right valve	–	Attached by a portion of the lower valve to the rocks	–	Most of the bottom valve attached to rock	broadly attached, fitting to attachment rocks	Attached by a portion of the lower valve to the rocks
Interior features						
Hinge teeth, left valve	Large	–	–	Large, brown	Heavy, Brown	Large, brown
Hinge teeth left valve	–	Very large, irregularly suborbicular	–	Large, white	–	–
Adductor muscle scar right valve	–	–	–	Flat to deep with callus	From shallow to deep, with ventral callus	Flat to deep with callus
Colour band	Purple	Purple in adults, reddish-orange young shells	A wide band of purplish red marks the inside margin	Broad, red-purple or yellow-orange around entire margin	Red-purple yellow orange	Purple
Margin	–	Finely crenated	–	In adults specimens matching the external colours	Fine crenulations	Finely crenated

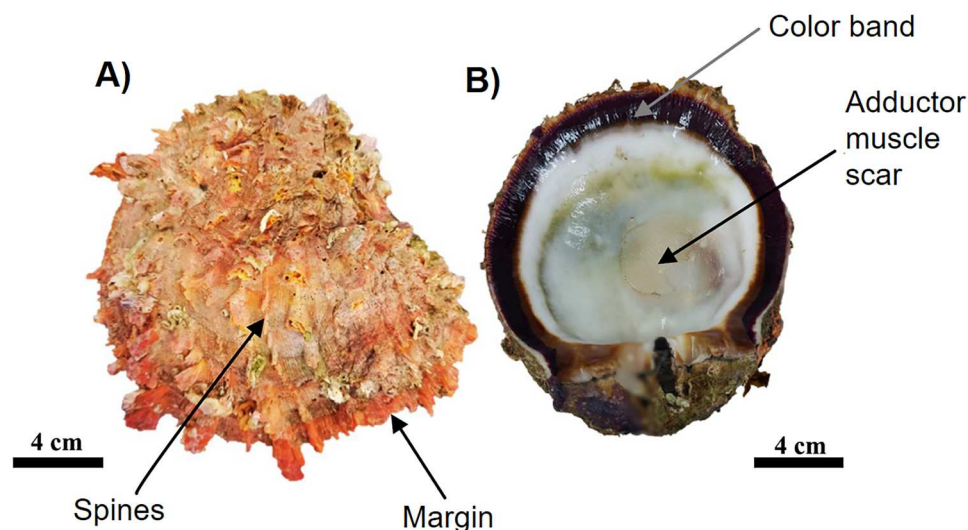


Figure 4. Morphological characteristics of the valve of *Spondylus limbatus*. **A**, External view showing the arrangement of the spines, colouration, and margin. **B**, Internal view highlighting the peripheral coloured band and the central adductor muscle scar.

feature of this species is the presence of a thick purple band on the inner side of the valves, with a finely crenulated margin. The shell surface is commonly covered by various groups of algae and invertebrates as epibionts.

Distribution. From Rocas Alijos, Baja California Sur, and the northern Gulf of California (Bahía La Choya, Sonora) to Caleta Mero, Tumbes, Peru (Coan and Valentich-Scott 2012).

Type specimens. According to White *et al.* (2026), there exist an neotype in the British Museum of Natural History.

Type locality. Persian Gulf.

Material examined. Material examined. 33 specimens; shell length, 105–170 mm; shell width, 110–182 mm. (COMA-701423).

Habitat. It lives from the intertidal zone down to 40–90 m deep on rocky bottoms. It is common to find specimens with epibionts forming assemblages of macroalgae and invertebrates.

Discussion

In this study, nuclear (28S rRNA) and mitochondrial (16S rRNA) sequences were obtained for *Spondylus limbatus*, contributing to the genetic characterisation of the Spondylidae family, aligning with previous efforts (Barucca *et al.* 2004; Ruaza *et al.* 2025). Despite these advances, there is a persistent need to sequence additional species within the genus to accurately infer phylogenetic relationships. Including the data from this study, molecular information is now available for 11 species, representing only 15% of the currently recognised species (WoRMS 2026).

The five individuals characterised here showed no variation across either marker. This lack of divergence necessitates future evaluations using different genes

such as Cox 1 (Matsumoto 2003), which failed to amplify in the present work. To elucidate the genetic landscape of *S. limbatus* further, it is essential to sample a larger number of individuals across different populations. This would allow for the detection of intraspecific variability, the identification of haplotypes, and the assessment of gene flow (Aguilar-Estrada *et al.* 2025).

The segregation of other *Spondylus* species into distinct clades with robust bootstrap support indicates clear specific distinctions. This is consistent with findings by Ruaza (2025), who utilised 16S rRNA and 12S rRNA markers for *S. squamosus* Schreibers, 1793. Furthermore, the present work confirms the monophyly of the genus *Spondylus* using the 28S marker, reinforcing its taxonomic stability at the generic level.

The specimens examined in this study fully coincide with the morphological descriptions of *Spondylus limbatus* provided by various authors (Sowerby 1847; Carpenter 1857; Keen 1971; Skoglund and Mulliner 1996; Coan and Valentich-Scott 2012; Cotom-Nimatuj *et al.* 2026). Morphological variability, driven by both genetic and phenotypic factors, typically increases with environmental heterogeneity (Schlichting and Pigliucci 1998). In marine organisms, substrate and life-style significantly influence biological architecture, a relationship most evident within the class Bivalvia (Sharma *et al.* 2023).

Historically, physical differentiation has allowed for the realisation of taxonomic recognition (Stanley 1970) under the premise that morphology reflects genetic divergence and reproductive isolation (Dobzhansky 1982; Mayr 1999). However, while shell construction is primarily dictated by the species' genetic information (McDougall and Degnan 2018), its final expression is highly plastic.

This phenotypic plasticity is strongly modulated by biotic and abiotic factors, such as hydrodynamics, food

availability, parasitism, predation, and substrate characteristics (Telesca *et al.* 2018; Barbers *et al.* 2019). In bivalves, this variability represents a critical taxonomic challenge, as individuals of the same species can exhibit divergent morphologies depending on their habitat (Seed 1968; Cerdeira-Arias *et al.* 2024). These adaptive processes, which mediate the fate of species along environmental gradients, define key biological traits such as shell shape and thermal tolerance limits (McLachlan *et al.* 1995). Consequently, relying exclusively on external characters can lead to an underestimation of true biodiversity, as intraspecific variability often overlaps between different taxa, thereby invalidating traditional diagnostic criteria (Bonello *et al.* 2016; Deichmann *et al.* 2017).

The implications of such morphological ambiguity extend to the management and conservation of fishery resources. The presence of cryptic species or taxa with few external diagnostic characteristics hinders accurate identification via field guides (Knowlton 1993; Appeltans *et al.* 2012), increasing the risk of erroneous reports and compromising the protection of specific evolutionary lineages (Ko *et al.* 2013; Omer 2017). A clear example is observed in *S. limbatus*, which undergoes significant ontogenetic shifts and morphological variation in its shell ornamentation. Although juvenile specimens are characterised by distinct spatulate spines, the persistence of these structures in adults is highly inconsistent; while they may erode or become obscured, they can also persist into adulthood despite age or epibiont colonisation (Coan and Valentich-Scott 2012; Cotom-Nimatuj *et al.* 2026). This unpredictable retention of juvenile traits can lead to diagnostic confusion during fisheries inspections if this structural variability is not accounted for.

Due to the instability of external ornamentation, this study emphasises the importance of more consistent internal diagnostic characters for *S. limbatus*. Specifically, the continuous purple or violet band on the inner valve surface, as detected by other authors (Sowerby 1932; Carpenter 1857; Keen 1971; Skoglund and Mulliner 1996; Cotom-Nimatuj *et al.* 2026) and the finely crenulated margin (Carpenter 1857; Coan and Valentich-Scott 2012) remain reliable markers. In contrast, *S. crassisquama*, exhibits a white interior with deeply folded red edges turning brown at hinge in old specimens (Villalejo-Fuerte *et al.* 2015). However, it is crucial to note that the internal colouration of *S. limbatus* can also exhibit phenotypic plasticity; some specimens display orange or reddish marginal bands instead of the typical purple, as previously reported by Skoglund and Mulliner (1996), Coan and Valentich-Scott (2012), and Cotom-Nimatuj *et al.* (2026). This chromatic variation introduces a potential risk of misidentification, as these atypical colour patterns can easily be confused with the internal margins of *S. crassisquama* (Cotom-Nimatuj *et al.* 2026).

Such morphological variations in *S. limbatus* may be driven by environmental parameters during development, such as temperature, food availability, and microalgae nutritional value, which alter shell sculpture and pigmentation patterns even under controlled cultivation conditions (Loor *et al.* 2016; Lodeiros *et al.* 2016). In wild populations, this eco-phenotypic variation is further compounded by the presence of various epibionts on the shells (Keen 1971; Logan 1974), which often serve as camouflage (Feifarek 1987), obscuring diagnostic traits in the field and making laboratory processing necessary for accurate identification.

In conclusion, the contrast between high external variability – driven by inconsistent ontogeny, environmental plasticity, and epibiosis – and the stability of the 28S rRNA and 16S rRNA molecular data confirms that genetic analysis is essential for reliable species identification. While shell morphology remains highly susceptible to these confounding modifications, the molecular markers provided in this study represent an indispensable tool to overcome the limitations of classical taxonomy and ensure unambiguous identification in future ecological and fisheries management studies (Lewis *et al.* 2019).

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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