Journal of
Marine Science and Engineering

MDPI

Article

Phylogenetic Affinities of Acanthopleurinae Dall, 1889 Chitons (Mollusca: Polyplacophora: Chitonidae) from Jazan Coast in the Red Sea and Western Indo-Pacific

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Abstract: Chitons (Polyplacophora) are marine molluscs that mostly inhabit rocky intertidal shores. Their biological and phylogenetic studies are comparatively sparse in the western Indo-Pacific regions. In addition, chitons belonging to the subfamily Acanthopleurinae Dall, 1889, collected from the Andaman Sea of the northeastern Indian Ocean and the Jizan coast of Saudi Arabia were sequenced and analyzed to study the phylogenetic affinities. The analysis was carried out using a single locus dataset (cytochrome oxidase 1) generated during the present study and integrated with sequences retrieved from GenBank. *Acanthopleura gemmata* (Blainville, 1825) from India was linked to *Acanthopleura vaillantii* Rochebrune, 1882, from the Saudi Arabia coast. *Squamopleura miles* (Carpenter in Pilsbry, 1893) from the Indian coast forms a separate clade representing the genus. Furthermore, the results illustrate several significant instances of misplacement of several species under the wrong genus and the existence of cryptic species within the genera *Acanthopleura* and *Squamopleura*. An integrated approach is required to better understand these important intertidal groups' taxonomy, systematics, and biogeography.

Keywords: Acanthopleurinae Dall, 1889; *Acanthopleura vaillantii* Rochebrune, 1882; chitons; Polyplacophora; marine molluscs; Red Sea; Western Indo-Pacific

Citation: Alnashiri, H.; Thomas, L.; Thaikkottathil, M.; Philip, S.; Kutty, R.; Sivanpillai, S.; Abo Rasain, A.M. Phylogenetic Affinities of Acanthopleurinae Dall, 1889 Chitons (Mollusca: Polyplacophora: Chitonidae) from Jazan Coast in the Red Sea and Western Indo-Pacific. *J. Mar. Sci. Eng.* **2023**, *11*, 158. https://doi.org/10.3390/jmse11010158

Academic Editors: John A. Theodorou and Theofania Tsironi

Received: 31 October 2022
Revised: 21 December 2022
Accepted: 4 January 2023
Published: 9 January 2023

1. Introduction

The class Polyplacophora, with more than a thousand nominal species, is an ancient clade of extant molluscs, common in the intertidal and subtidal zones of tropical and temperate regions, with several representatives from the deep sea [1]. The chitons are considered taxonomically difficult [2] owing to their superficial resemblance in morphology within and between groups. In addition, using novel morphological and anatomical characters in conjunction with molecular phylogeny and population genetics data has revealed the cryptic diversity among chitons and led to the discovery of various new species [3–5]. In the past two decades, more than 200 species of chitons have been discovered and described globally. Monophyly of the two major lineages of Polyplacophora (Order Lepidopleurida and order Chitonida) has been resolved by morphological and molecular characteristics [2,6–8].

The subfamily Acanthopleurinae Dall, 1889 (family Chitonidae) is a group of medium- to large-sized chitons and is a common inhabitant along the rocky intertidal regions [9]. Acanthopleurinae are represented by 20 species (Table 1) grouped under four genera, viz. *Acanthopleura* Guilding, 1830, *Enoplochiton* Gray, 1847, *Liolophura* Pilsbry, 1893; and *Squamopleura* Nierstrasz, 1905 (MolluscaBase, 2022). The ecology, feeding behavior, reproduction, homing behavior, radular biomineralization, shell ocelli, and bioerosion [10–17] of

several Acanthopleurinae species have already been characterized. However, the identification of species based on non-reliable morphological characters over detailed examination (characters on the tegmentum and perinotum), along with the lack of specimens from wide-ranging locations, add to the taxonomic complexity within several species groups of Acanthopleurinae [18–20].

Table 1. Species belonging to the subfamily Acanthopleurinae have been identified in different studies.

Accepted Name (MolluscaBase)	Ferreira, 1986	Brooker, 2003	Kaas et al. 2006
Acanthopleura brevispinosa (G. B. Sowerby II, 1840)	*Acanthopleura brevispinosa*	*Acanthopleura brevispinosa*	*Acanthopleura brevispinosa*
Acanthopleura echinata (Barnes, 1824)	*Acanthopleura echinata*	*Enoplochiton echinatus*	*Acanthopleura echinata*
Acanthopleura gemmata (Blainville, 1825)	*Acanthopleura gemmata*	*Acanthopleura (Gemmata) gemmata*	*Acanthopleura gemmata*
Acanthopleura granulata (Gmelin, 1791)	*Acanthopleura granulata*	*Acanthopleura (Gemmata) granulata*	*Acanthopleura granulata*
Acanthopleura loochooana (Broderip & G. B. Sowerby I, 1829)	*Acanthopleura loochooana*	*Acanthopleura (Squamopleura) loochooana*	*Acanthopleura loochooana*
Acanthopleura planispina Bergenhayn, 1933	*Acanthopleura gemmata*		
Acanthopleura spinosa (Bruguière, 1792)	*Acanthopleura spinosa*	*Acanthopleura spinosa*	*Acanthopleura spinosa*
Acanthopleura vaillantii Rochebrune, 1882	*Acanthopleura gemmata*	*Acanthopleura vaillantii*	*Acanthopleura vaillantii*
Enoplochiton niger (Barnes, 1824)	*Acanthopleura nigra*	*Enoplochiton niger*	*Enoplochiton niger*
Liolophura arenosa (Ferreira, 1986)	*Acanthopleura arenosa*	*Acanthopleura arenosa*	*Liolophura (Liolophura) arenosa*
Liolophura gaimardi (Blainville, 1825)	*Acanthopleura gaimardi*	*Acanthopleura (Liolophura) gaimardi*	*Liolophura (Liolophura) gaimardi*
Liolophura hirtosa (Péron MS, Blainville, 1825)	*Acanthopleura hirtosa*	*Acanthopleura (Squamopleura) hirtosa*	*Liolophura (Clavarizona) hirtosa*
Liolophura japonica (Lischke, 1873)	*Acanthopleura japonica*	*Acanthopleura (Liolophura) japonica*	*Liolophura (Liolophura) japonica*
Liolophura koreana Yeo & U. W. Hwang, 2021			
Liolophura rehderi (Ferreira, 1986)	*Acanthopleura rehderi*	*Onithochiton rehderi*	*Liolophura (Liolophura) rehderi*
Liolophura sinensis Choi, B. Park & U. W. Hwang, 2021			
Liolophura tenuispinosa Leloup, 1939	*Liolophura tenuispinosa*	*Acanthopleura (Liolophura) tenuispinosa*	
Squamopleura araucariana (Hedley, 1898)	*Acanthopleura araucariana*	*Acanthopleura (Squamopleura) araucariana*	*Squamopleura araucariana*
Squamopleura curtisiana (E. A. Smith, 1884)	*Acanthopleura curtisiana*	*Acanthopleura (Squamopleura) curtisiana*	*Squamopleura curtisiana*
Squamopleura miles (Carpenter in Pilsbry, 1893)	*Acanthopleura miles*	*Acanthopleura (Squamopleura) miles*	*Squamopleura miles*
		Acanthopleura (Gemmata) sp nov	

A revision of the genus *Acanthopleura* by Ferreira (1986) identified *Acanthopleura* as the senior synonym of 13 genera [21]. Brooker studied the anatomy of *Acanthopleura* using a scanning electron microscope and included 16 species in the genus [22]. The revision resulted in 15 species, where he coalesced species from three other genera (*Liolophura*, *Squamopleura*, and *Enoplochiton*) into the genus *Acanthopleura*.

In the Western Indo-Pacific Polyplacophora has only four representatives: (*Acanthopleura gemmata* (Blainville, 1825), *Acanthopleura brevispinosa* (G. B. Sowerby II, 1840), *Acanthopleura vaillantii* (Rochebrune, 1882), and *Squamopleura miles* (Carpenter in Pilsbry, 1893)) [9]. *A. gemmata* has a wide distribution in the Indo-Pacific, from the Andaman Islands in the west to Tonga in the east, along the coasts of Australia and the northern Philippines [9], with records also from the East African coast [9]. *A. brevispinosa* is known to be distributed along the east African coast from Somalia to Mozambique and along the coasts of the Seychelles, Comoro Islands, Madagascar, Reunion, and Mauritius [9]. *A. vaillantii* shows distribution along the shores of the Red Sea, Yemen, and Socotra Island to the opening of the Arabian Gulf, and the distributional records of *S. miles* in the western Indo-Pacific are from Sri Lanka in the west to New Guinea in the east and from Taiwan in the north to Australia in the south [9].

The studies on chitons from the western Indo-Pacific are limited compared to other regions. Several researchers have recently studied chitons from the region, primarily focusing on the taxonomy, diversity, and distribution [23–37]. However, no works on taxonomy and systematics based on molecular techniques are available.

In this study, a molecular taxonomic study is undertaken to elucidate the phylogenetic affinities of Acanthopleurinae chitons from the Western Indo-Pacific using the universal barcoding locus and the partial mitochondrial cytochrome oxidase 1 (*COI*) gene sequence data.

2. Materials and Methods

2.1. Sampling

The specimens were collected from the intertidal zone along the coast of the Andaman and Nicobar Islands, India (*A. gemmata* and *S. miles*), and Saudi Arabia's coast of the Red Sea (*A. vaillantii*) during 2020–2021 (Table 2). The specimens were collected by hand from crevices in the exposed rocks during low tide.

Table 2. Details of sampling locations.

Species	Locality	Latitude, Longitude	No. of Samples
Acanthopleura gemmata	Andaman Islands, India	11°34′44.8″ N 92°44′30.0″ E	3
Acanthopleura vaillantii	Jazan, Saudi Arabia	16°56′39.3″ N 42°32′33.0″ E	2
Squamopleura miles	Andaman, Islands, India	11°34′44.8″ N 92°44′30.0″ E	2

2.2. DNA Extraction, PCR Amplification, and Sequencing

The genomic DNA was extracted from ethanol-preserved specimens using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) using the Qiagen QIAcube HT DNA extraction automation system, following the manufacturer's instructions. The partial sequence of the mitochondrial cytochrome oxidase 1 (*COI*) marker was amplified using the LCO1490 and HCO2198 primers [38]. The PCR amplification was carried out in a 25 µL master mix consisting of 15.4 µL dd H_2O, 5 µL 10× buffer (Invitrogen, Waltham, MA, USA), 0.35 µL $MgCl_2$ (Applied Biosystems, Waltham, MA, USA), 0.5 µL of 10 mM dNTPs (Promega, Madison, WI, USA), 1.25 µL of 0.1 µM of each primer, 0.25 µL of 5 U/µL Taq Polymerase (Invitrogen, Waltham, MA, USA) and 1 µL of DNA template. The thermal cycler was programmed for 35 cycles of 94 °C for 60 s, 49 °C for 90 s and 72 °C for 90 s, after an initial denaturation of 95 °C for 5 min. The reaction mixture was subjected to a final elongation of 72 °C for 6 min and held at 4 °C until further steps were taken. The amplified PCR products are purified using Wizard® SV Gel and PCR Clean-Up System (Promega), following the manufacturer's protocol, and outsourced for deep sequencing.

2.3. Sequence Selection and Analysis

In this study, 67 sequences of chitons belonging to the subfamily Acanthopleurinae were assembled. A total of eight sequences were generated for three species from the western Indo-Pacific. The rest of the sequences were retrieved from the GenBank and BOLD databases (Table 3). The sequences generated from the present study were also submitted to GenBank (OM758201-OM758209). In addition, the sequences were aligned using MUSCLE [39] as implemented in SeaView v5.0.5 [40]. The data were partitioned to three codon positions of the *COI* gene to create a full partition, and a partition analysis [41] and ModelFinder [42] were used to find the right partitioning scheme and the best-fit substitution model. The maximum likelihood (ML) analysis was performed using IQ-TREE [43] with the best partition scheme and ultrafast bootstrap support for 1000 iterations [44]. The phylogenetic tree was edited and visualized in FigTree v1.4.4 [45].

Table 3. Accession numbers and sources of sequences of *COI* used in the present study were retrieved from NCBI GenBank. Sequences retrieved from the BOLD database are mentioned as (BIN).

Species	Location	Accession Number	Source
Acanthochitona mahensis Winckworth, 1927	Mahe, India	OM758209	Present study
Acanthopleura brevispinosa (G. B. Sowerby II, 1840)	Suez, Egypt	KX537628	[46]
Acanthopleura echinata (Barnes, 1824)	Quintero, Chile	MN864062	[47]
Acanthopleura echinata (Barnes, 1824)	Paracas, Peru	MK016459	[47]
Acanthopleura echinata (Barnes, 1824)	Paracas, Peru	MK016460	[47]
Acanthopleura echinata (Barnes, 1824)	Paracas, Peru	MK016461	[47]
Acanthopleura gaimardi (Blainville, 1825)	Queensland, Australia	AB066221	Unpublished
Acanthopleura gaimardi (Blainville, 1825)	Unknown	LIMX064 (BIN)	Unpublished
Acanthopleura gaimardi (Blainville, 1825)	Unknown	LIMX065 (BIN)	Unpublished
Acanthopleura gaimardi (Blainville, 1825)	Unknown	LIMX097 (BIN)	Unpublished
Acanthopleura gemmata (Blainville, 1825)	Okinawa, Japan	AB066222	Unpublished
Acanthopleura gemmata (Blainville, 1825)	Penang, Malaysia	MK016466	[47]
Acanthopleura gemmata (Blainville, 1825)	Penang, Malaysia	MK016467	[47]
Acanthopleura gemmata (Blainville, 1825)	Penang, Malaysia	MK016468	[47]
Acanthopleura gemmata (Blainville, 1825)	South Andaman, India	OM758201	Present study
Acanthopleura gemmata (Blainville, 1825)	South Andaman, India	OM758202	Present study
Acanthopleura gemmata (Blainville, 1825)	South Andaman, India	OM758203	Present study
Acanthopleura granulata (Gmelin, 1791)	Unknown	AY377719	[7]
Acanthopleura granulata (Gmelin, 1791)	Unknown	MZ467321	Unpublished
Acanthopleura loochooana (Broderip & G. B. Sowerby I, 1829)	Unknown	MT585716	Unpublished
Acanthopleura loochooana (Broderip & G. B. Sowerby I, 1829)	Unknown	MT585719	Unpublished
Acanthopleura loochooana (Broderip & G. B. Sowerby I, 1829)	Okinawa, Japan	AB066223	Unpublished
Acanthopleura loochooana (Broderip & G. B. Sowerby I, 1829)	Unknown	MH587607	Unpublished
Acanthopleura miles (Carpenter in Pilsbry, 1893)	Okinawa, Japan	AB064988	Unpublished
Acanthopleura planispina Bergenhayn, 1933	Chichijima Island, Japan	AB706351	Unpublished
Acanthopleura spinosa (Bruguière, 1792)	Miyako Island, Japan	AB099505	Unpublished
Acanthopleura tenuispinosa Leloup, 1939	Okinawa, Japan	AB065288	Unpublished
Acanthopleura vaillantii Rochebrune, 1882	Jazan, Saudi Arabia	OM758207	Present study
Acanthopleura vaillantii Rochebrune, 1882	Jazan, Saudi Arabia	OM758208	Present study
Americoliva sayana (Ravenel, 1834)	Unknown	MZ091415	Unpublished
Enoplochiton niger (Barnes, 1824)	Iquique, Chile	MK016414	[47]
Enoplochiton niger (Barnes, 1824)	Iquique, Chile	MK016415	[47]
Enoplochiton niger (Barnes, 1824)	Iquique, Chile	MK016419	[47]
Lepidozona coreanica (Reeve, 1847)	Unknown	MT070411	Unpublished
Ischnochiton hakodadensis P. P. Carpenter, 1893	Hakkaido, Japan	LC214409	Owada, 2018
Liolophura japonica (Lischke, 1873)	Unknown	MT585720	Unpublished
Liolophura japonica (Lischke, 1873)	Unknown	MT585718	Unpublished
Liolophura japonica (Lischke, 1873)	Ehime, Japan	AB064986	Unpublished
Liolophura japonica (Lischke, 1873)	Tsushima, Japan	KT932897	[5]
Liolophura japonica (Lischke, 1873)	Tsushima, Japan	KT932890	[5]
Liolophura japonica (Lischke, 1873)	South Korea	JX503058	Unpublished
Liolophura japonica (Lischke, 1873)	Unknown	AY377717	[7]
Liolophura japonica (Lischke, 1873)	Unknown	HM180654	[48]
Liolophura japonica (Lischke, 1873)	Wakayama, Japan	AB066269	Unpublished

Table 3. *Cont.*

Species	Location	Accession Number	Source
Liolophura japonica (Lischke, 1873)	Zhejiang, China	MF774404	[49]
Liolophura japonica (Lischke, 1873)	Zhejiang, China	MG203946	[49]
Liolophura japonica (Lischke, 1873)	Zhejiang, China	MF774400	[49]
Liolophura japonica (Lischke, 1873)	Geojedo, South Korea	KT932872	[5]
Liolophura japonica (Lischke, 1873)	Tongyeong, South Korea	KT932867	[5]
Liolophura japonica (Lischke, 1873)	Tongyeong, South Korea	KT932866	[5]
Liolophura japonica (Lischke, 1873)	Geojedo, South Korea	KT932875	[5]
Squamopleura miles (Carpenter in Pilsbry, 1893)	South Andaman, India	OM758204	Present study
Squamopleura miles (Carpenter in Pilsbry, 1893)	South Andaman, India	OM758205	Present study
Squamopleura miles (Carpenter in Pilsbry, 1893)	South Andaman, India	OM758206	Present study

3. Results

The present study incorporates 14 of the 20 valid species belonging to four genera under the subfamily Acanthopleurinae. The partition analysis suggested separate nucleotide substitution models for three codon positions of the *COI* gene, with TIM3 + F + I + G4 for the first codon position (BIC = 2105.7466, lnL = −1028.4610), F81 + F + I (BIC = 761.5852, lnL = −367.2302) for the second codon position, and HKY + F + G4 for the third codon position (BIC = 8249.4081, lnL = −4108.4292).

A total of four distinct clades ('Enoplochiton', 'Acanthopleura', 'Squamopleura', and 'Liolophura') representing four genera under the subfamily Acanthopleurinae were resolved in ML analysis (Figure 1). Several species presently included in *Acanthopleura* and *Liolophura* were found to be placed across different genera. Based on the analysis, our study demonstrates the occurrence of cryptic species within the genera *Acanthopleura* and *Squamopleura,* respectively (see Figure 1).

The results of the present analysis also provide insights into the biogeography of Acanthopleurinae chitons. The majority of species in our study are from the Indo-Pacific region. All the species in the clade 'Acanthopleura' are recorded from the Indo-Pacific except *Acanthopleura granulata* (Gmelin, 1791), which is recorded from the Caribbean. The clades 'Liolophura' and 'Squamopleura' include species from the Indo-Pacific, while the species of the clade 'Enoplochiton' are restricted along the west coast of South America (Figure 2).

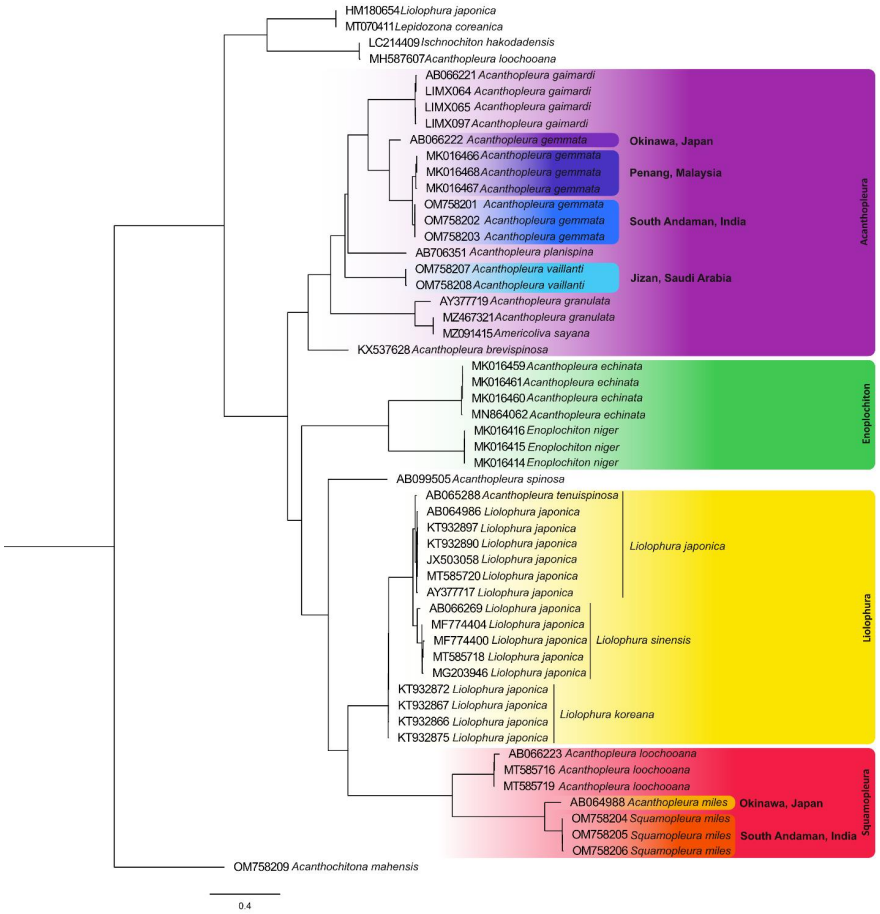

Figure 1. Phylogenetic tree of Acanthopleurinae chitons constructed using cytochrome oxidase c subunit I.

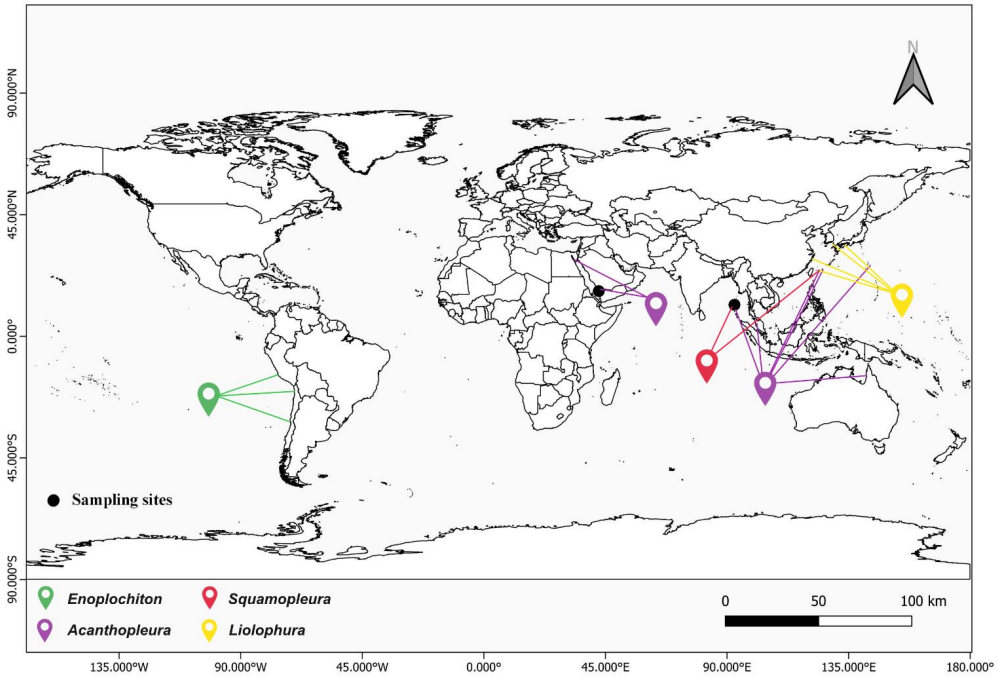

Figure 2. Map showing the sampling locations and the geographical distribution of species from four genera used in the present analysis.

4. Discussion

The phylogenetic affinities of Acanthopleurinae chitons from the western Indo-Pacific were addressed in the present study. The study focused on the phylogenetic relationships of Acanthopleurinae chitons from the western Indo-Pacific. Based on the phylogenetic analysis and morphological characters, the three species collected in the present study belonged to the subfamily Acanthopleurinae. *A. gemmata* from the Andaman coast and *A. vaillantii* from the Red Sea were related and thus belonged to the genus *Acanthopleura,* while *S. miles* from the Andaman coast was grouped under the genus *Squamopleura.* The results also illustrate the ambiguity in the misplacement of several species under the wrong genus and the existence of cryptic species within the genera *Acanthopleura* and *Squamopleura*. The incorrect placement of species could be due to wrong identification prior to sequencing and deposition of the sequences in the GenBank (HM180654, MH587607), and those within the subfamily could be due to a lack of detailed analysis using more advanced taxonomic tools [5].

4.1. Phylogenetic Affinities of Acanthopleurinae

As per the current understanding, the subfamily Acanthopleurinae accommodates 20 validated species belonging to four genera. The present analysis resolves the species of Acanthopleurinae across four clades representing the four genera (Figure 1).

4.1.1. *Acanthopleura* Guilding, 1830

According to our present knowledge, *Acanthopleura* is globally represented by eight species, with distributional records from the western part of the Indian Ocean to the central part of the Pacific and the western coast of South America and the Caribbean Sea [9]. Based on our analysis, species currently validated under the genus *Acanthopleura* are resolved across three clades. The majority of the species currently validated under the nominal genus *Acanthopleura* are included in the clade 'Acanthopleura'. The placement of the type species *Acanthopleura spinosa* (Bruguière, 1792) separately from the rest of the species in the sequence is controversial and requires verification using more data. *Acanthopleura loochooana* (Broderip & G. B. Sowerby I, 1829) was conventionally placed under the genus *Acanthopleura*. The three sequences from the database (AB066223, MT585716, and MT585719) are included in the clade 'Squamopleura' in the present study (Figure 1). Another sequence (MH587607) recorded as *A. loochooana* is a misidentification and falls outside the subfamily Acanthopleurinae. More data is therefore required to ascertain the right placement of this species. The sequences of *Acanthopleura echinata* (Barnes, 1824) from the western coast of South America have shown affinity for the clade 'Enoplochiton' rather than 'Acanthopleura' (Figure 1). Brooker [22], who suggested the placement of *A. echinata* under the genus *Enoplochiton,* supports the results of the present study. Likewise, two sequences (AB065288 and AB066223) that have been reported as *A. tenuispinosa* and *A. miles*, are misidentified specimens, under the genus Acanthopleura. However, in our study, we found their affinity towards the genera *Liolophura* and *Squamopleura,* respectively. The results also demonstrate the probable occurrence of cryptic species within the species currently identified as *A. gemmata* from the coasts of India (Andaman Islands), Malaysia (Penang), and Japan. The specimen of *A. gemmata* collected from the Indian coast has a genetic distance of 10.6% and 3.0% with specimens recorded from Japan and Malaysia, respectively. The type locality of *A. gemmata* is Australia (New Holland), and the unavailability of molecular data from the type locality prevents us from identifying the nominal species from the Andaman Islands. A recent study on Acanthopleurinae chitons from Indonesia [19] also highlighted the probably hidden diversity of *Acanthopleura*. The phylogeny of the rest of the *Acanthopleura* species is resolved within the clade.

4.1.2. *Liolophura* Pilsbry, 1893

Recent studies on genetic polymorphisms [4] and species discrimination with geographical distribution patterns and divergence [5] of *Liolophura japonica* (Lischke, 1873) from

the northwestern Pacific revealed the existence of cryptic species and resulted in the description of two new species (*Liolophura koreana* Yeo & U. W. Hwang, 2021, and *Liolophura sinensis* Choi, B. Park & U. W. Hwang, 2021) [5]. Five species of *Liolophura* were not represented in our analysis due to the unavailability of data: *Liolophura arenosa* (Ferreira, 1986), *Liolophura hirtosa* (Péron MS, Blainville, 1825), *Liolophura rehderi* (Ferreira, 1986), *Liolophura gaimardi* (Blainville, 1825), and *Liolophura tenuispinosa* (Leloup, 1939) [20]. However, sequences of *Acanthopleura gaimardi* (AB066221, LIMX064, LIMX065, and LIMX097) in our study fall in the clade 'Acanthopleura' indicating that the taxonomically accepted *L. gaimardi* could be a species of the genus *Acanthopleura*. More specimens of the species need to be verified with morphological and molecular tools before placing them in the clade 'Acanthopleura'. The sequence (HM180654) seems misidentified as it falls out of the subfamily Acanthopleurinae.

4.1.3. *Squamopleura* Nierstrasz, 1905

Globally, the genus *Squamopleura* is represented by three valid species (see Table 1). The type species, *Squamopleura miles* (Carpenter in Pilsbry, 1893) is known to have a wide distribution from Sri Lanka in the west to New Guinea in the east, and from Taiwan in the north to north-western Australia in the south [9]. The species recorded as *S. miles* from the Andaman Islands in the present study, however, aligned with *A. miles* from Japan (AB064988), indicating that the latter could be a misidentification as they fall entirely outside of the clade 'Acanthopleura'. This indicates that there could be an occurrence of a species complex for *S. miles* since the sequence from the Indian coast has a genetic distance of above 9% from those recorded from Japanese waters. Starger [19] identified the existence of cryptic species of *Squamopleura* in Indonesian waters. The two species of the genus that are not represented in our analysis are *Squamopleura araucariana* (Hedley, 1898) and *Squamopleura curtisiana* (E. A. Smith, 1884). Another noteworthy observation relates to the placement of *Acanthopleura loochooana* under the clade 'Squamopleura.' This needs further study to ascertain the systematics of this species.

4.1.4. *Enoplochiton* Gray, 1847

The genus *Enoplochiton*, restricted to the central part of the western coast of South America, is presently represented by a single species, *Enoplochiton niger* (Barnes, 1824). The present analysis suggests the placement of *A. echinata* and *E. niger* in the clade 'Enoplochiton'. Both *E. niger* and *A. echinata* belong to the same geographical region, along the west coast of South America, suggesting that this phylogenetic replacement is reasonable. A detailed examination of morphological characters is required to justify the placement of *A. echinata* under the genus *Enoplochiton*.

4.2. Acanthopleurinae in the Western Indo-Pacific

Research suggests that three species of *Acanthopleura* (*A. brevispinosa, A. gemmata,* and *A. vaillantii*) and a single species of *Squamopleura* (*S. miles*) represent the diversity of Acanthopleurinae from the Western Indo-Pacific. Our analysis suggests the probability of having more species of *Acanthopleura* and *Squamopleura* from the western Indo-Pacific, reflecting the geographical distribution patterns as inferred by previous studies [5,18]. Population genetic analysis using *COI* and 16S genes on the genus *Liolophura* from the northwestern Pacific coast revealed the occurrence of three species lineages [5]. Along with genetic studies, morphological analysis using field emission scanning electron microscopy (FE-SEM) identified differences in the black spots on the tegmentum and spicules on the perinotum, resulting in the discovery of two new species (*L. koreana* and *L. sinensis*), along with the previously known *L. japonica* [5]. An integrated approach using morphological characters and molecular data from different locations along the Western Indo-Pacific could provide interesting insights into the cryptic diversity of Acanthopleurinae.

5. Conclusions

The rich diversity of chitons also poses a significant challenge for the taxonomists to classify them accordingly. As discussed above, there is a need for an integrated taxonomic approach for identifying many species that show morphological similarities. In our present study, we have made an attempt to understand the complexity of the taxonomic status of species in the subfamily Acanthopleurinae based on molecular analysis using a single locus dataset. Though we understand this is not a comprehensive approach, based on the available data, we have made an attempt to shed light on certain uncertainties in the taxonomy and systematics of certain species from the Western Indo-Pacific region. The analysis also confirms the existence of cryptic diversity within the genus *Acanthopleura*. As a result of their preference for intertidal habitats, chitons form an interesting model for understanding the fluxes in the intertidal zone caused by destructive environmental and climatic events. An integrated approach using morphological and molecular tools with improved sampling and data generation will provide a better understanding of these important intertidal groups' taxonomy, systematics, and biogeography.

Author Contributions: M.T. and L.T. set up and performed the lab experiments, interpreted the data results, and wrote the manuscript. S.P. and R.K. contributed to and supported the lab's experiments. S.S., A.M.A.R. and H.A. contributed to analyzing and inspecting the data and editing the manuscript. H.A., L.T. and M.T. gave the idea for a research project and planned the experiments; they also analyzed and inspected the data and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia, Project Number No. 11613.

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of Department of Biology, College of Science, Jazan University.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Acknowledgments: The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia, for funding this research work through project number (No. 11613) under the Research Groups program of Jazan University for the Ministry of Education's Institutional Support Program ISP20-22. Our appreciation also goes to the Deanship of Scientific Research at Jazan University for their efforts in organizing and supporting this project. Liju Thomas is funded by the Council for Scientific and Industrial Research (CSIR), Senior Research Fellowship (SRF direct). We acknowledge Ramvilas Ghosh (Kerala University of Fisheries and Ocean Studies) for the assistance in sampling from the Indian coast and Shalu Kannan (Kerala University of Fisheries and Ocean Studies) for preparing the study area map.

Conflicts of Interest: The authors declare that there is no conflict of interest regarding the publication of this article.

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