

# Phylogenetic position and morphological descriptions of *Chiton* species from the south-eastern Pacific

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In the south-eastern Pacific Ocean (SEP), six species of the genus *Chiton* have been recorded. However, the taxonomic status and geographic distribution of some of these species is controversial. In this study, we compare and describe *Chiton* species in the SEP. More than 3000 specimens of six species from 49 localities were analysed for morphological features. Among these, 27 specimens of the six species from the SEP were sequenced for the mitochondrial barcode region (COI) and ribosomal 16S and 18S to infer phylogenetic relationships. Phylogenetic reconstruction indicates that the genus *Chiton* in the SEP represents a polyphyletic group and confirms the placement of *C. barnesii* in *Chiton* and not in *Radsia*, as previously suggested. Among *Chiton* s.s., *C. cumingsii* is the sister-species of *C. magnificus*, while *C. granosus* is a sister-species of *C. barnesii*. This analysis also indicates that *C. bowenii* is a junior synonym of *C. magnificus*. Our results improved the knowledge of systematics and distribution of *Chiton* species in the SEP, suggesting the presence of five valid species of the genus. In this study, we designate a lectotype for *C. cumingsii* and a neotype for *C. granosus*.

ADDITIONAL KEYWORDS: Chile – Peru – Taxonomy – Mollusca – phylogenetics.

## INTRODUCTION

Chitons are molluscs that belong to the class Polyplacophora, characterized by eight articulated and overlapping dorsal valves forming a shell. They are exclusively marine, are found in all oceans and occur from the intertidal zone to great depths (Eernisse, 2004; Sigwart, 2009; Schwabe, 2010). The genus *Chiton* Linnaeus, 1758 is the first named genus and gave the common name to the group. It has a global distribution and comprises over 80 species, from temperate to tropical habitats (Kaas *et al.*, 2006). Chitons of this genus are common in Peru and Chile, where they have an important ecological role in intertidal ecosystems (Camus *et al.*, 2008). In Chile, four species (*C. barnesii* Gray, 1828, *C. cumingsii* Fremby, 1827, *C. granosus*

Fremby, 1827 and *C. magnificus* Deshayes, 1827) are common on high, medium and low intertidal zones of exposed coasts (Otaíza & Santelices, 1985; Araya & Araya, 2015). *Chiton granosus* is often found in crevices in densities of up to 600 ind./m<sup>2</sup> in central Chile (Otaíza, 1986). Species of *Chiton* are often abundant in the south-eastern Pacific Ocean (SEP), and their ecology and natural history have been extensively studied (Otaíza & Santelices, 1985; Aguilera, 2005; Aguilera & Navarrete, 2007; Camus *et al.*, 2008; Araya & Araya, 2015). However, there are still significant problems with their taxonomy and systematics. In the SEP, six *Chiton* species have been recorded, but the taxonomic status and geographic distribution for several of them remains controversial (Bullock, 1988a, b; Valdovinos, 1999; Araya & Araya, 2015). For example, *C. barnesii* Gray, 1828 has been alternately considered as a member of *Chiton* or *Radsia* Gray, 1847, sometimes as

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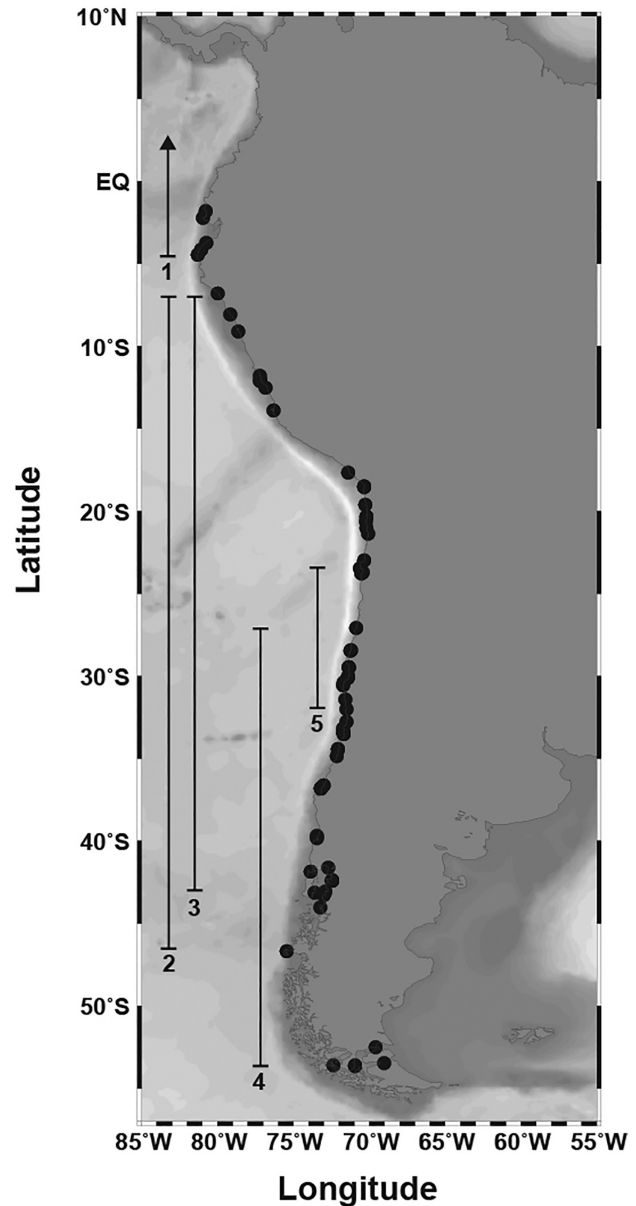
a separate genus or as a subgenus of *Chiton* (Bullock, 1988a, b; Valdovinos, 1999; Kass *et al.*, 2006; Araya & Araya, 2015). Another complex taxon is *Chiton bowenii* King & Broderip, 1831, originally described from Tierra del Fuego, but considered a subspecies of *C. magnificus* (Bullock, 1988b) or, more recently, a morphologically distinct species (Schwabe *et al.*, 2006; Sirenko, 2006). There is still controversy related to polymorphic coloration and patterns in this taxon, but this could be influenced by habitat (Rodrigues & Absalo, 2005; Mendonça *et al.*, 2014; Sigwart, 2018). If shell colour is plastic, or naturally highly variable, it may not be a useful feature for the delimitation of species, at least within the genera *Chiton* and *Tonicia* Gray, 1847 (Ibáñez *et al.*, 2019). Therefore, more information is needed to select the appropriate morphological characters with diagnostic value, to be used in taxonomic identification. Molecular data are a good independent line of evidence to improve the accuracy of the identification in the case of chitons.

In this study, we perform the first integrative morphological and molecular analyses of the genus *Chiton* in order to resolve systematic problems in species from the SEP, including specimens from coastal Ecuador, Peru and Chile.

## MATERIAL AND METHODS

### SAMPLING SITES AND DATA COLLECTION

A geographically distributed dataset of chitons was obtained from sampling field trips along the Pacific coast (Ecuador, Peru and Chile) from 1.8°S to 53.6°S between 2011 and 2016 (Fig. 1). During these field trips, we collected a total of 3175 *Chiton* specimens. Additionally, we sampled a selection of relevant outgroup species, also from the SEP: *Acanthopleura echinata* (Barnes, 1824), *Enoplochiton niger* (Barnes, 1824), *Tonicia chilensis* (Fremby, 1827) and *T. swainsoni* (Sowerby, 1832). We also sampled additional related species from a broader geographical sampling: three specimens of *Acanthopleura gemmata* (De Blainville, 1825) from Malaysia, four *Chiton glaucus* (Gray, 1828) from New Zealand and five *Sypharochiton pelliserpentis* (Quoy & Gaimard, 1835) (Table 1). Specimens were identified at the lowest taxonomic level possible, following relevant published descriptions (Leloup, 1956; Bullock, 1988a, b; Kass *et al.*, 2006; Schwabe *et al.*, 2006; Sirenko, 2006; Schwabe, 2009). Field sampling and specimen collecting were performed by hand and Hookah or SCUBA diving at intertidal and subtidal zones, respectively. Each sampling session was performed during two hours, around peak hour of low tide. Specimens collected in this study were



**Figure 1.** Map showing sampling localities. Lines represent the geographical distribution of each *Chiton* species: 1, *Chiton stokesii*; 2, *Chiton granosus*; 3, *Chiton cumingsii*; 4, *Chiton magnificus*; 5, *Chiton barnesii*.

deposited in the Sala de Colecciones Biológicas de la Universidad Católica del Norte (SCBUCN) and Museo Nacional de Historia Natural de Chile (MNHNCL). A tissue sample was cut from each specimen and preserved in 96% ethanol for molecular analysis. The whole animal was preserved for morphological analysis. Small chitons of each species were selected for scanning electron microscopy (SEM) to study morphology of plates, griddle scales and radula.

Table 1. Species collected, localities and Genbank numbers

Species	Locality	Latitude, Longitude	Code	COI	16S	18S
<b>This study</b>						
<i>Acanthopleura echinata</i>	Paracas, Peru	-13.849, -76.282	AEPPI	MK016459	MK097371	MK480243
<i>Acanthopleura gemmata</i>	Penang, Malaysia	5.481, 100.253	AGPMA3	MK016468	MK097373	MK480245
<i>Acanthopleura gemmata</i>	Penang, Malaysia	5.481, 100.253	AGPMA2	MK016467	MK097374	MK480246
<i>Acanthopleura gemmata</i>	Penang, Malaysia	5.481, 100.253	AGPMA1	MK016466	MK097375	MK480247
<i>Chiton barnesii</i>	El Sauce, Chile	-30.519, -71.694	RBSC9	MK016433	MK097397	MK480268
<i>Chiton barnesii</i>	El Sauce, Chile	-30.519, -71.694	RBSC22	MK016434		
<i>Chiton barnesii</i>	El Sauce, Chile	-30.519, -71.694	RBSC26	MK016435		
<i>Chiton bowenii</i>	Puerto del Hambre, Chile	-53.612, -70.929	CBPA1	MK016395	MK097376	MK480248
<i>Chiton bowenii</i>	Puerto del Hambre, Chile	-53.612, -70.929	CBPA4	MK016396		
<i>Chiton bowenii</i>	Puerto del Hambre, Chile	-53.612, -70.929	CBPA6	MK016397		
<i>Chiton cumingsii</i>	Antofagasta, Chile	-23.774, -70.477	CCCO5	MK016398		
<i>Chiton cumingsii</i>	Antofagasta, Chile	-23.774, -70.477	CCCO6	MK016399	MK097378	MK480250
<i>Chiton cumingsii</i>	Antofagasta, Chile	-23.774, -70.477	CCCO7	MK016400		
<i>Chiton glaucus</i>	Cape Palliser, New Zealand	-41.613, 175.275	CGPNZ1		MK097379	MK480251
<i>Chiton glaucus</i>	Cape Palliser, New Zealand	-41.613, 175.275	CGPNZ2		MK097380	MK480252
<i>Chiton glaucus</i>	Cape Palliser, New Zealand	-41.613, 175.275	CGPNZ3		MK097381	
<i>Chiton glaucus</i>	Cape Palliser, New Zealand	-41.613, 175.275	CGPNZ4			MK480253
<i>Chiton granosus</i>	Rio Seco, Chile	-21.001, -70.165	CGRS1	MK016401		
<i>Chiton granosus</i>	Rio Seco, Chile	-21.001, -70.165	CGRS4	MK016402	MK097382	MK480254
<i>Chiton granosus</i>	Rio Seco, Chile	-21.001, -70.165	CGRS5	MK016403		
<i>Chiton granosus</i>	Rio Seco, Chile	-21.001, -70.165	CGRS2	MK016404		
<i>Chiton magnificus</i>	El Sauce, Chile	-30.519, -71.694	CMSC1			
<i>Chiton magnificus</i>	Valdivia, Chile	-39.796, -73.401	CMV22			
<i>Chiton magnificus</i>	Punta de Tralca, Chile	-33.426, -71.704	CMPT30			
<i>Chiton magnificus</i>	Valdivia, Chile	-39.796, -73.401	CMV21			
<i>Chiton magnificus</i>	Los Vilos, Chile	-31.979, -71.499	CMCV19			
<i>Chiton magnificus</i>	El Sauce, Chile	-30.519, -71.694	CMSC14	MK016405		
<i>Chiton magnificus</i>	Los Vilos, Chile	-31.979, -71.499	CMCV6	MK016406		
<i>Chiton magnificus</i>	Valdivia, Chile	-39.796, -73.401	CMV12	MK016407		
<i>Chiton magnificus</i>	Valdivia, Chile	-39.796, -73.401	CMV20	MK016408	MK097383	MK480255
<i>Chiton magnificus</i>	Valdivia, Chile	-39.796, -73.401	CMV11	MK016409		
<i>Chiton magnificus</i>	El Sauce, Chile	-30.519, -71.694	CMSC19	MK016410		
<i>Chiton stokesii</i>	Santa Rosa, Ecuador	-2.211, -80.946	CSSR1	MK016411	MK097388	MK480260
<i>Chiton stokesii</i>	Santa Rosa, Ecuador	-2.211, -80.946	CSSR3	MK016412		
<i>Chiton stokesii</i>	Santa Rosa, Ecuador	-2.211, -80.946	CSSR11	MK016413		
<i>Enoplochiton niger</i>	Iquique, Chile	-20.309, -70.136	ENT137	MK016416	MK097390	MK480262
<i>Sypharochiton pelliserpentis</i>	Wellington, New Zealand	-41.289, 174.790	SPWNZ1	MK016470		
<i>Sypharochiton pelliserpentis</i>	Wellington, New Zealand	-41.289, 174.790	SPWNZ2	MK016471		

Table 1. Continued

Species	Locality	Latitude, Longitude	Code	COI	16S	18S
<i>Sypharochiton pelliserpentis</i>	Wellington, New Zealand	-41.289, 174.790	SPWNZ3	MK016472		
<i>Sypharochiton pelliserpentis</i>	Wellington, New Zealand	-41.289, 174.790	SPWNZ4	MK016473		
<i>Sypharochiton pelliserpentis</i>	Wellington, New Zealand	-41.289, 174.790	SPWNZ5	MK016474		
<i>Tonicia chilensis</i>	Ventanas, Chile	-32.743, -71.497	TILVP16	MK016458	MK097421	MK480284
<i>Tonicia swainsoni</i>	Isla San Lorenzo, Peru	-12.090, -77.224	TCSL4	MK016444	MK097403	MK480272
<b>Species from Genbank</b>						
<i>Liolophura japonica</i>	Wakayama, Japan			AB066269	KT932934	
<i>Liolophura japonica</i>	Wakayama, Japan				KT932935	
<i>Acanthopleura brevispinosa</i>	Red Sea, Egypt			KX537628	KX5376131	
<i>Acanthopleura granulata</i>	Cozumel Island, Mexico			AY377719	AY377608	
<i>Acanthopleura planispina</i>	Tokyo, Japan			AB706351		
<i>Chiton glaucus</i>	Dunedin, New Zealand			JN376139		
<i>Chiton tuberculatus</i>	Bermuda			KJ909661		
<i>Leptochiton asellus</i>	Sweden				AY377586	
<i>Leptochiton kerguelensis</i>	Antarctica			HQ907864		
<i>Leptochiton medinae</i>	Antarctica			HQ907865		
<i>Onithochiton hirasei</i>	Japan			AB714507		
<i>Onithochiton neglectus</i>	New Zealand			JX123117		
<i>Rhyssoplax olivacea</i>	Girona, Spain			AY377716	KF052802	
<i>Rhyssoplax olivacea</i>	Naples, Italy			KJ500161	KJ500172	
<i>Rhyssoplax olivacea</i>	Sardinia, Italy			KJ500165	KF052793	
<i>Sypharochiton aorangi</i>	Mokohinau, New Zealand			JN376136		
<i>Sypharochiton aorangi</i>	Mokohinau, New Zealand			JN376137		
<i>Sypharochiton pelliserpentis</i>	New Zealand			NC024174	NC024174	
<i>Sypharochiton pelliserpentis</i>	New Zealand				AY377607	
<i>Sypharochiton pelliserpentis</i>	New Zealand				KJ534307	
<i>Sypharochiton sinclairi</i>	Kaka Point, New Zealand			JN376132		
<i>Sypharochiton sinclairi</i>	Kaka Point, New Zealand			JN376134		
<i>Sypharochiton sinclairi</i>	New Zealand			NC024173		
<i>Sypharochiton torri</i>	Stewart Island, New Zealand			JN376135		
<i>Tonicia forbesii</i>	Baja California, Mexico			KJ574101		



## GENETIC AND PHYLOGENETIC ANALYSIS

To estimate phylogenetic relationships and genetic distances, we sequenced the partial mitochondrial gene cytochrome *c* oxidase subunit I (*COI*), and the 16S and 18S ribosomal RNA (16S and 18S). These were combined in an analysis with additional previously published sequences (Table 1).

A small portion of tissue was cut from the foot of each specimen, following the saline extraction protocol proposed by Aljanabi & Martinez (1997). The polymerase chain reaction (PCR) and amplifications were carried out using a total reaction volume of 25  $\mu$ L, with 2.5  $\mu$ L of buffer 10 $\times$  [200 mM Tris-HCL (pH 8.4), 500 mM KCL], 2.0  $\mu$ L of dNTPs (2.5 mM), 1.0  $\mu$ L MgCl<sub>2</sub> (50 mM), 0.3  $\mu$ L of Invitrogen Platinum Taq DNA polymerase, 0.1  $\mu$ L of DNA and 0.25  $\mu$ L of each primer (10 pmol) [see primers in Sigwart *et al.* (2013)]. For amplification, the optimum condition had an initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C (60 s); 50 °C (60 s) for *COI* and 55 °C (60 s) for 16S and 18S and 72 °C (60 s) followed by a final extension at 72 °C (10 min) using a thermal cycler. Double-stranded PCR products were purified and sequenced in both directions using an Automatic Sequencer AB13730  $\times$  1 by Macrogen, Inc. (Seoul, Korea). Sequences were edited and aligned with the MUSCLE software implemented in MEGA 7.0 (Kumar *et al.*, 2016).

Genetic distances (p-distance) between species were calculated for *COI* and 16S in MEGA software to describe and compare the variation between genera and species.

Before phylogenetic analysis, we performed the saturation test of *COI* gene in DAMBE v.6.0 (Xia, 2013). This analysis found little saturation of *COI* gene (Iss = 0.221 < Iss.c = 0.718,  $P < 0.001$ ), suggesting these sequences are useful for phylogenetic analyses. Thus, the phylogenetic reconstructions were inferred from a combined analysis with all three molecular markers (*COI*+16S+18S), using Bayesian inference. To evaluate the monophyly of sampled species in the genus *Chiton* we included additional species of different genera of the family Chitonidae noted above (*Acanthopleura*, *Enoplochiton*, *Rhyssoplax*, *Sypharochiton*, *Onithochiton* and *Tonicia*) and additional published sequences, importantly including the type species of the genus, *Chiton tuberculatus* (Table 1). We rooted the trees using previously published sequences of *L. kerguelensis* Haddon, 1886 and *L. medinae* (Plate, 1899) (Table 1). The dataset was analysed with a general likelihood based mixture model of gene sequence evolution, which considers rate and pattern heterogeneity in the data (Pagel & Meade, 2004, 2008); thus, no prior knowledge for partition of the data is needed. Moreover, a variety of evolution models and parameters can be used to

run the analysis several times, based on general time reversible substitution model (GTR). This mixed model was implemented using Markov chain Monte Carlo methods with Reversible Jump within a Bayesian framework (rjBMCMC; Pagel & Meade, 2008), using BayesPhylogenies 1.1 (Pagel & Meade, 2004). This approach allowed us to select from different models, and their associated parameters, the model that best fits the data in the sample trees. We ran three independent rjBMCMC analyses, using 10 000 000 generations of phylogenetic trees, sampling every 1000 trees to assure that successive samples were independent. To assess the stationary distribution of the Markov chain, we visually inspected the log-likelihood values of the iterations of the Markov chain until it reached convergence using the software TRACER v.1.5 (Rambaut & Drummond, 2009). Moreover, the effective sampling size was checked to get a number higher than 500. Trees that did not reach the convergence zone (10–25%) were discarded and the remaining trees 10 000 were randomly selected to reconstruct the consensus tree by the majority rule and to estimate the posterior probability values using the program BayesTrees v.1.3 (Meade, 2011). Additionally, phylogenetic reconstruction was conducted with maximum likelihood (ML) using IQ-TREE (Nguyen *et al.*, 2015) with the ModelFinder automatic option (Kalyaanamoorthy *et al.*, 2017). Statistical support for the nodes was estimated by ultra-fast bootstrap algorithm with 2000 pseudoreplicates (Minh *et al.*, 2013).

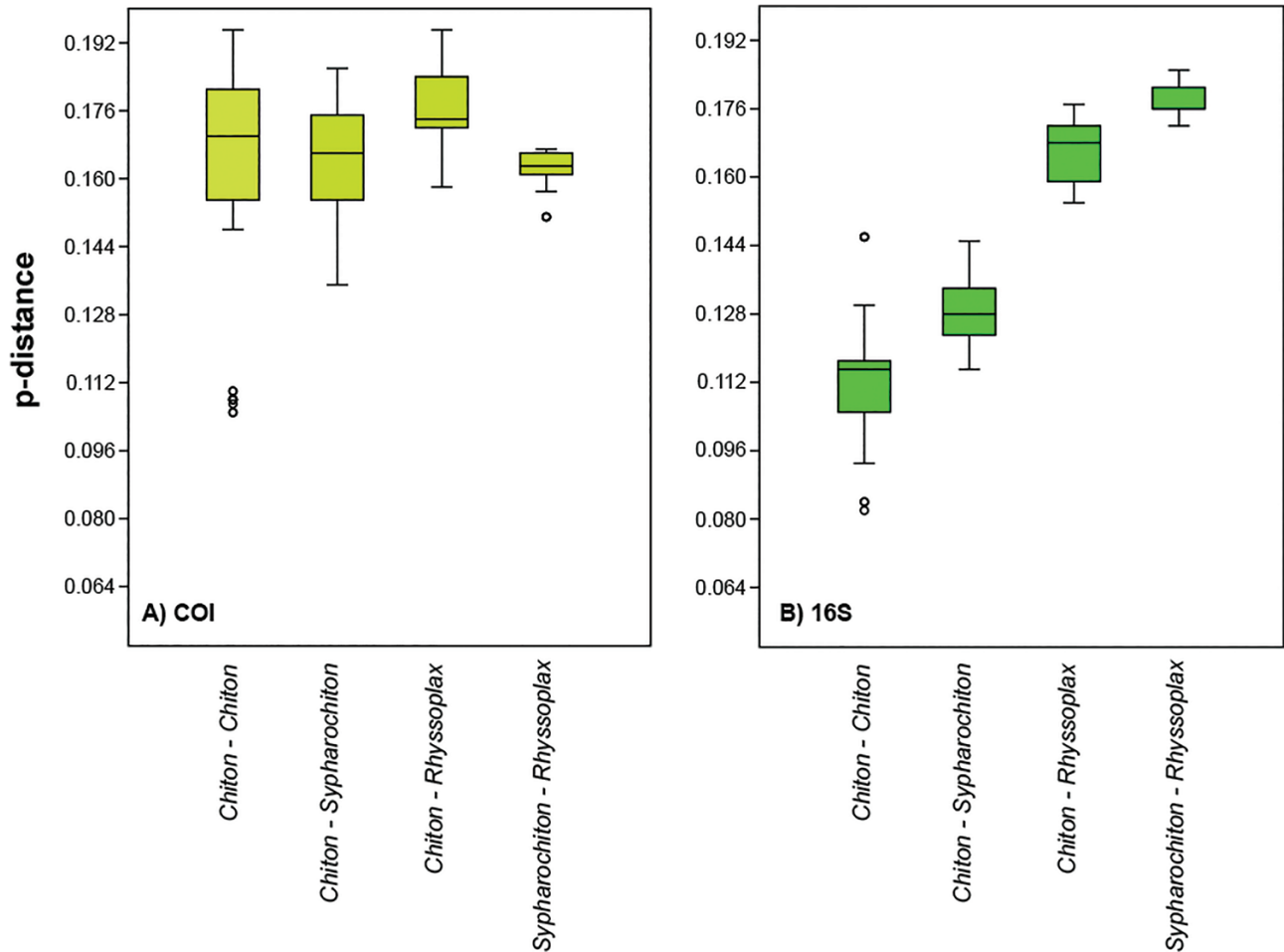
## ABBREVIATIONS

NHMUK, Natural History Museum, London, UK; MNHN, Muséum national d'Histoire naturelle, Paris, France

## RESULTS

The genetic distance among identified chiton species differs between 10.5 and 19.5% (subst./bp) in *COI* and between 8.2 and 18.1% (subst./bp) for 16S (Fig. 2). There is similar pairwise variation with *COI* in comparing species from different genera (Fig. 2A), without a clear distance metric that could indicate genus-level separation. With 16S pairwise distances, comparing *Chiton*–*Sypharochiton* there is a clear overlap in the distances that separate species within *Chiton*, while *Chiton*–*Rhyssoplax* and *Rhyssoplax*–*Sypharochiton* comparisons were clearly different from *Chiton*–*Sypharochiton* (Fig. 2B).

The combined phylogenetic reconstruction (*COI*+16S+18S) shows high posterior probabilities (> 0.95) and high bootstrap support (> 90) in almost all



**Figure 2.** Comparison of genetic distances of *COI* (A) and *16S* (B) of the chiton species using in this study.

nodes of the phylogeny (Fig. 3). The topology shows a clade composed of *Chiton*, *Sypharochiton* and *Rhyssoplax* (Clade 1), but the *Chiton* species from SEP do not resolve as monophyletic. We note three other subgroups of interest: Clade 2 is composed of *C. cumingsii* and *C. magnificus*; Clade 3 of *C. granosus* and *C. barnesii*; and Clade 4 of *C. glaucus* and *S. pelliserpentis* (Fig. 3).

#### SYSTEMATICS

For clarification of the systematics of the genus, we present brief remarks on each species from the SEP in the genus *Chiton*, based on prior descriptions and specimens from material assembled for the present phylogenetic study.

#### *CHITON* LINNAEUS, 1758

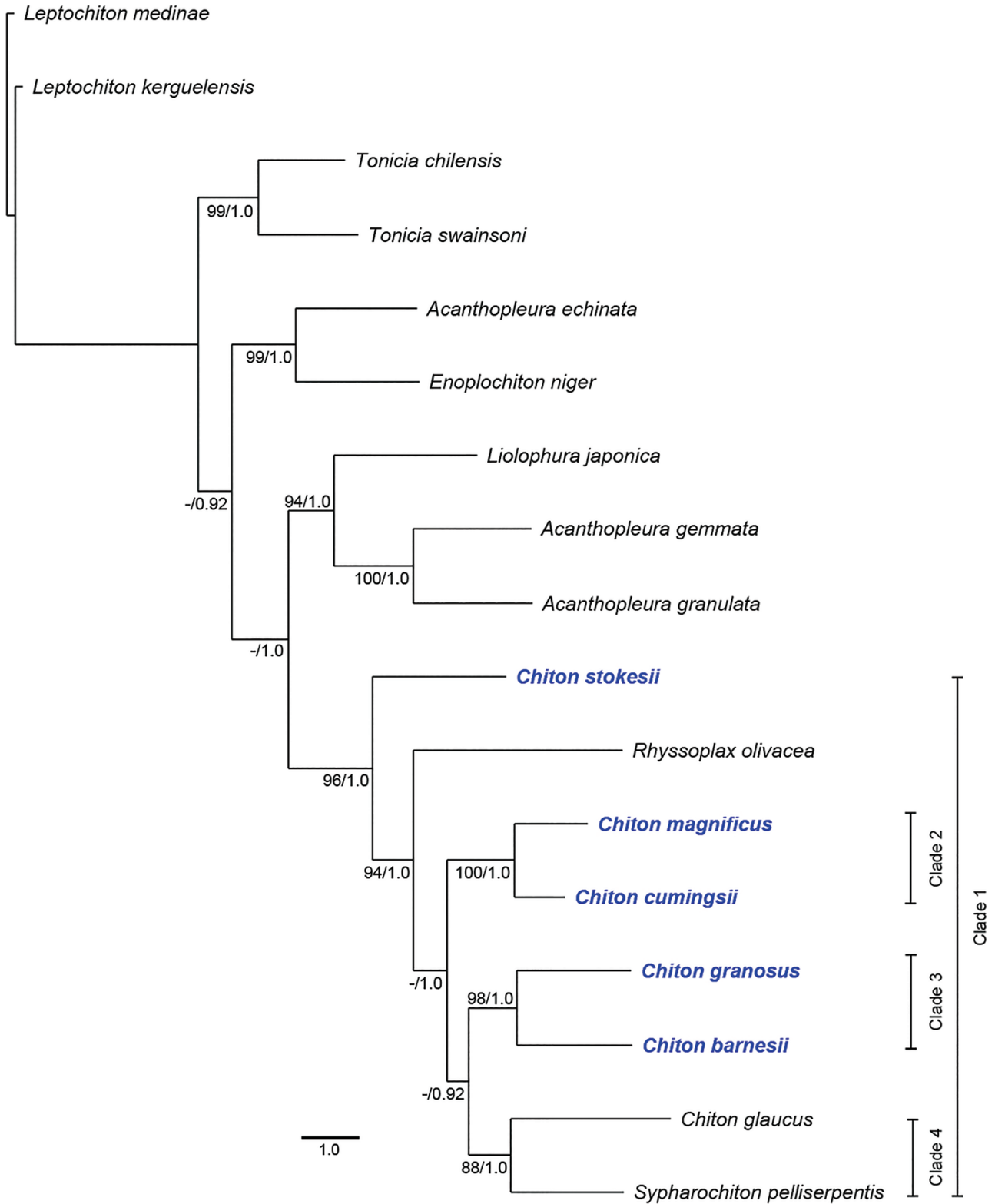
*Type species:* *Chiton tuberculatus* Gray, 1828, by subsequent designation, Dall (1919: 297).

*Diagnosis:* Large, oval organisms (> 30 mm total length). Valves flattened to elevated and carinate, with pectinated insertions. Tegmental structure variable, subtegumentum poorly to highly developed. Girdle scales variable in shape and size, smooth or with striations, pustules or granules. Denticle cap of major lateral tooth of radula variable, blunt or somewhat elongate or round; angle of denticle cap greater than 90° from axis of the tooth itself; distal edge of centrolateral tooth perpendicular to longitudinal axis of animal.

#### *CHITON (CHITON) BARNESII* GRAY, 1828

(FIGS 4A, 5, 6)

*Chiton barnesii* Gray, 1828: 5; Reeve 1847: pl. 1, sp. 1. *Chiton (Radsia) barnesii*: Pilsbry, 1893: 190, pl. 29, figs 10–12; Dall, 1919: 247. *Chiton barnesii*: Plate, 1899: 59, pl. 4, fig. 191; Leloup, 1956: 46. *Chiton barnesi*: Otaiza & Santelices, 1985. *Radsia barnesii*:



**Figure 3.** Bayesian phylogeny of chitons using combined dataset *COI*+16S+18S. Nodes values are posterior probabilities over 0.9 are showed. Blue colours are the *Chiton* species from the south-eastern Pacific.

**Bullock, 1988b**: 282, figs 2, 4, 6, 7, 11–13, 15, 31, 32. *Chiton (Radsia) barnesii*: **Kaas et al., 2006**: 147, fig. 53, map. 27. *Chiton barnesii* **Schwabe, 2009**. *Radsia barnesii* Araya & **Araya 2015**: 52, fig. 2.

*Type material*: NHMUK (holotype).

*Type locality*: Coquimbo, Chile.

*Descriptive notes*: Size: animal reaching a length of 44 mm and width of 27 mm (**Table 2**). Colour: creamy white plates with reddish brown stains in the central areas (**Fig. 4A**), irregular longitudinal bands of reddish brown in lateral triangles. Body shape: wide, oval, low dorsal elevation. Slit formula: 16/1–2/20. Valves fairly flat. Plates: head valve with > 25 ribs with small grains (**Figs 4A, 5A**). Intermediate plates usually with two slits. Intermediate plate with numerous aesthetes (**Fig. 5E, F**). Tail valve back straight to slightly concave (**Fig. 5D**). Mucro somewhat sharp, the front of the posterior valve is acentric. Articulamentum: broad, moderately and uniformly extended processes, short insertion plates (**Fig. 5B–D**). Anterior valve with 16 teeth, posterior valve with 16–20 teeth. Perinotum: wide, with overlapping scales; scales rounded triangular in shape and weakly ridged (**Fig. 6A, C**). Spicules at the girdle margin (**Fig. 6C**). Radula: central tooth rounded and broad. The major lateral rounded (**Fig. 6B, D**).

*Distribution*: We found this species from Antofagasta (~23°S), to Los Vilos (~32°S), Chile (**Fig. 1**). It was previously reported from Coquimbo (~29°S) to the Chonos Archipelago (~45°S) (**Bullock, 1988a**) or from Caldera (~27°S) to the Chonos Archipelago (~45°S) (**Araya & Araya 2015**).

*Remarks*: **Bullock (1988a)** placed *Chiton barnesii* **Gray, 1828** in the genus *Radsia* based on the slitting of the insertion plate. Later, **Kaas et al. (2006)** removed *barnesii* to the subgenus *Radsia*, and recently **Araya and Araya (2015)** also used the name *R. barnesii*. Our molecular results suggest that *barnesii* is nested within the genus *Chiton*. This species is often confused with *C. granosus*, but the principal difference is the slit

formula: *Chiton barnesii* (16–17/2–4/19–20), *Chiton granosus* (12–18/1/14–17).

*CHITON (CHITON) STOKESII* BRODERIP IN BRODERIP & SOWERBY, 1832

(**Figs 4B, 5, 8, 9**)

*Chiton stokesii* Broderip in **Broderip & Sowerby, 1832**: 25. *Chiton (Chiton) granosus*: **Kaas et al., 2006**: 122, fig. 41, maps 14–16 (bibliography and synonymy).

*Type material*: NHMUK (syntypes).

*Type locality*: Port St Elena, Panama.

*Descriptive notes*: Size: animal reaching 88 mm long, 44 mm wide (**Table 2**). Body shape: the angle of the valves is about 110°. Slit formula: 16/1/18. Plates: anterior valve is slightly convex, the postmucronal, posterior valve region is straight (**Figs 4B, 8A**). The intermediate valve central region and tail valve antemucronal region, have numerous longitudinal ribs (**Figs 4B, 8B, F**). Intermediate plate with numerous aesthetes (**Fig. 8E, F**). Tail valve back straight (**Fig. 8D**). Colour: the plates are black, brown, sometimes green (**Figs 4B, 8A–C**), with little white stripes in the central area. Articulamentum white, smooth, with narrow jugal separation with inconspicuous jugal lamina between wide apophyses. Perinotum: wide, with overlapping scales; smooth scales rounded triangular (**Fig. 9A, C**). Spicules at the ventral side of the perinotum (**Fig. 9C**). Radula: central tooth triangular and hooked. The major lateral triangular (**Fig. 9B, D**).

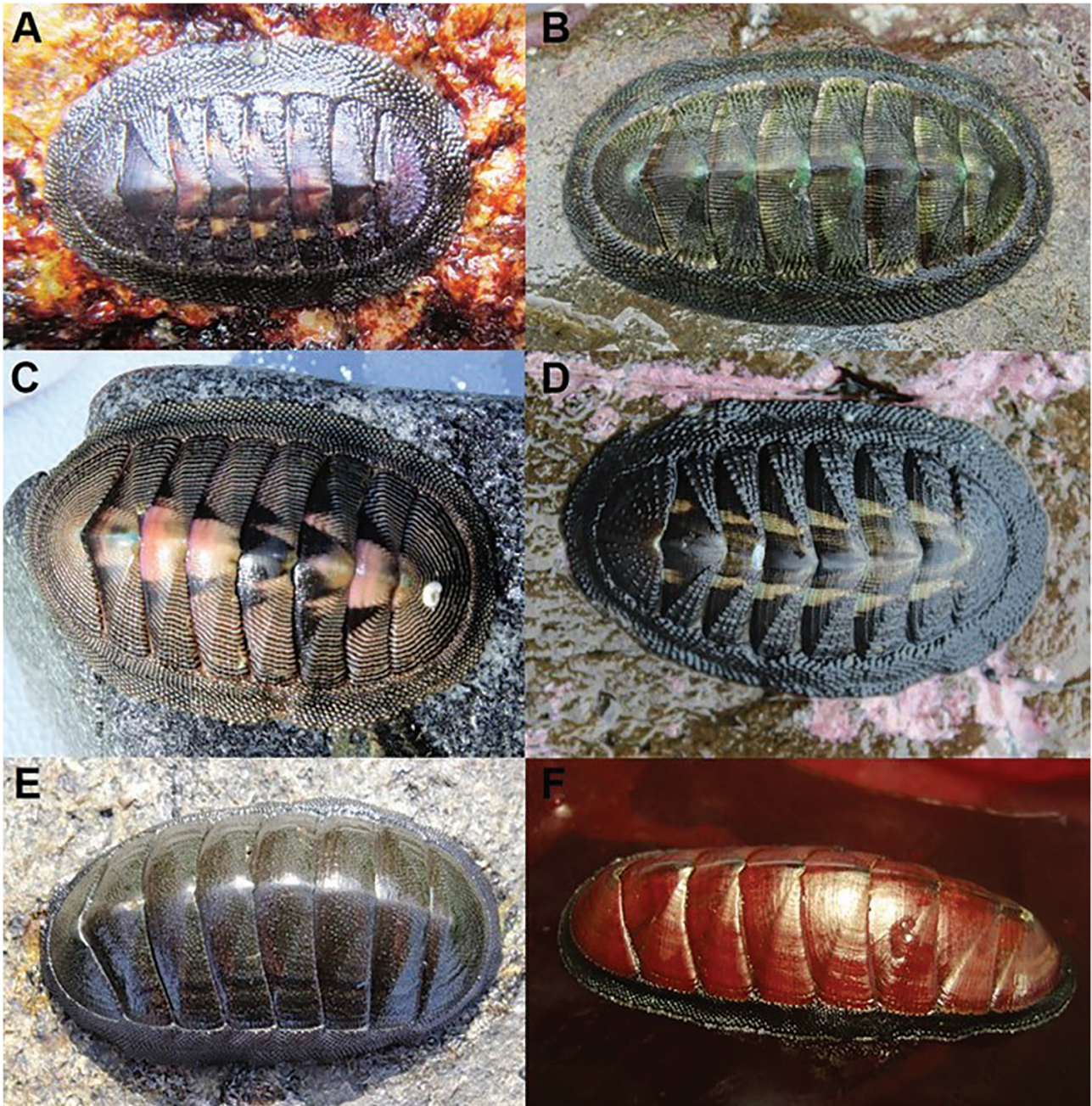
*Distribution*: Guatemala (~12°N) to south Ecuador (~2°S) (**Bullock, 1988a; Schwabe, 2009**). In this study, we found this species from Montañita (~2°S), Ecuador to Bonanza (~4°S), Peru (**Fig. 1**).

*Remarks*: *Chiton stokesii* was erroneously included in the new genus *Chondroplax* by **Thiele (1893)**, but **Bullock (1988b)** placed it in *Chiton s.s.*

**Table 2.** Morphometric measures of each species collected. Range (min–max) in parenthesis

Species	N	Total length (mm)	IV plate width (mm)	Total width (mm)
<i>Chiton barnesii</i>	155	17.4 (3–45)	6.5 (1–19)	9.9 (2–27)
<i>Chiton cumingsii</i>	1383	30.3 (5–84)	12.9 (2–35)	16.7 (3–40)
<i>Chiton granosus</i>	921	42.2 (6–76)	17.4 (2–39)	23.8 (3–51)
<i>Chiton magnificus</i>	661	46.1 (5–160)	21.9 (2–74)	28.3 (3–81)
<i>Chiton stokesii</i>	55	41.5 (13–88)	13.8 (4–27)	19.7 (6–44)





**Figure 4.** Pictures of *Chiton* species from the south-eastern Pacific. A, *Chiton barnesii* live specimen from La Pampilla, Coquimbo, Chile (29°26'11"S, 71°18'20"W). B, *Chiton stokesii* live specimen from Santa Rosa, Ecuador (2°12'39"S, 80°56'45"W). C, *Chiton cumingsii* live specimen from Lagunillas, Coquimbo, Chile (30°06'13"S, 71°22'59"W). D, *Chiton granosus* live specimen from Antofagasta, Chile (23°40'10"S, 70°24'29"W). E, *Chiton magnificus* live specimen from Lagunillas, Coquimbo, Chile (30°06'13"S, 71°22'59"W). F, *Chiton magnificus* live specimen from Puerto del Hambre, Punta Arenas, Chile (53°36'44"S, 70°55'43"W).

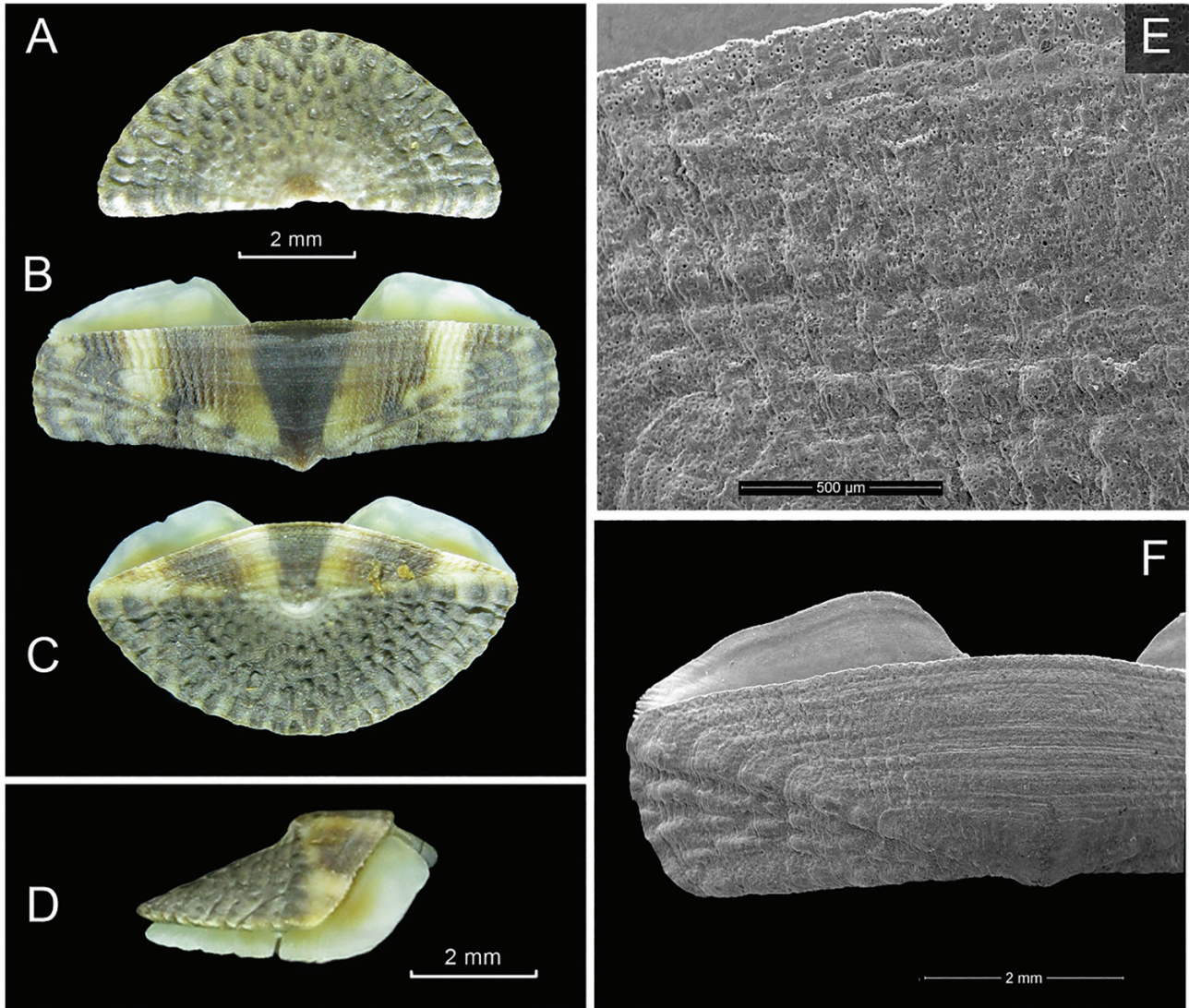
*CHITON (CHITON) CUMINGSII* FREMBLY, 1827

(FIGS 4C, 7A, B, 10, 11)

*Chiton cumingsii* Fremby, 1827: 198, suppl. pl. 16, fig. 3; Dall, 1919: 247. *Chiton cumingsii*: Reeve, 1847, pl. 1,

figs 2a, b; Plate, 1902: 46, pl. 3, figs 179–184, pl. 4. *Chiton cumingi*: Pilsbry, 1893: 164, pl. 30, figs 29–31. *Chiton (Amaurochiton) cumingsii*: Bullock, 1988b: 166, figs 59–63, 69, 74 and 77. *Chiton (Chiton) cumingsii*: Kaas *et al.*, 2006: 113, fig. 37, map 14 (bibliography and synonymy).





**Figure 5.** *Chiton barnesii*, Chile, Coquimbo, BL 20.0 mm, 05.01.2015: A, valve I, dorsal view; B, valve V, dorsal view; C, valve VIII, dorsal view; D, valve VIII, lateral view; E, valve VI, detail of tegmentum in central area; F, valve VI, central and lateral areas.

*Type material:* NHMUK 20190538/1 (lectotype from the J. E. Gray collection, no locality data), NHMUK 20190538/2 (paralectotype, teratological specimen), NHMUK 20190538/3–4 (paralectotypes), all designated herein.

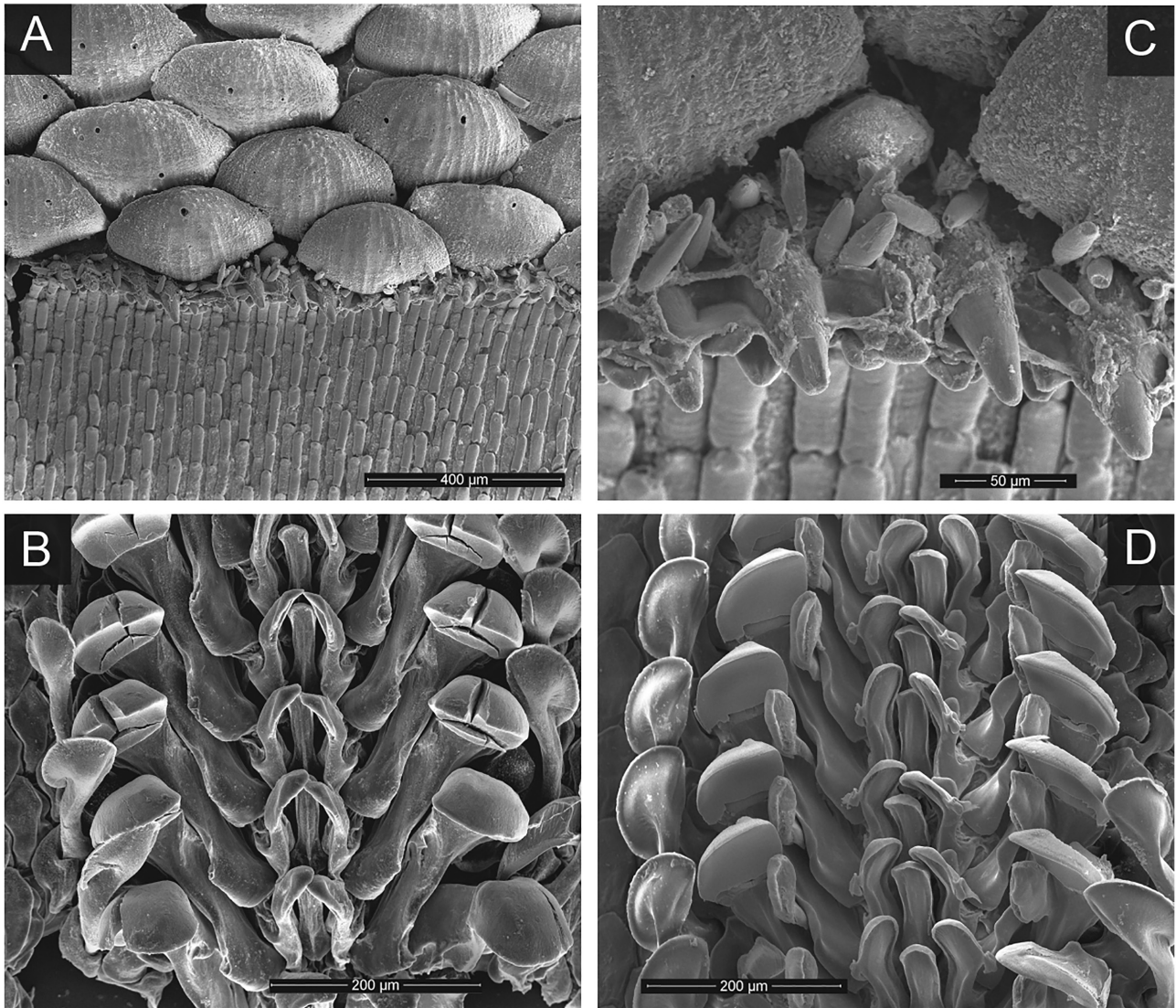
*Type locality:* Valparaíso, Chile, *vide* Frembly, 1927.

*Descriptive notes:* Size: animal up to 84 mm long and 40 mm width (Table 2). Colour: usually pink with black concentric lines compact (Fig. 4C), some individuals can be almost black; jugal area usually more vivid colour. Body shape: oval to elongated-oval, middle dorsal elevation. Slit formula: 12–15/1/10–16. Plates: intermediate plates separated by space not exposed

mantle, slightly overlapping one over the other, lines of growth in intermediate plates apparent to the naked eye (Fig. 10). Intermediate plate with numerous aesthetes (Fig. 11E, F). Tail valve back straight (Fig. 10D). Apophyses: moderately short, connected by short jugal lamina, delicately denticulate, clearly triangular and trapezoidal intermediate valves in valve series (Fig. 10B, C, F). Perinotum: moderately broad, dorsally with imbricated, flat and triangular scales (Fig. 11A, C). Spicules at the ventral side of the perinotum (Fig. 11C). Radula: central tooth rounded and hooked. The major lateral rounded (Fig. 11B, D).

*Distribution:* We found this species from Lobitos (~4°S), Peru to Quellón (~43°S), Chile (Fig. 1). It was





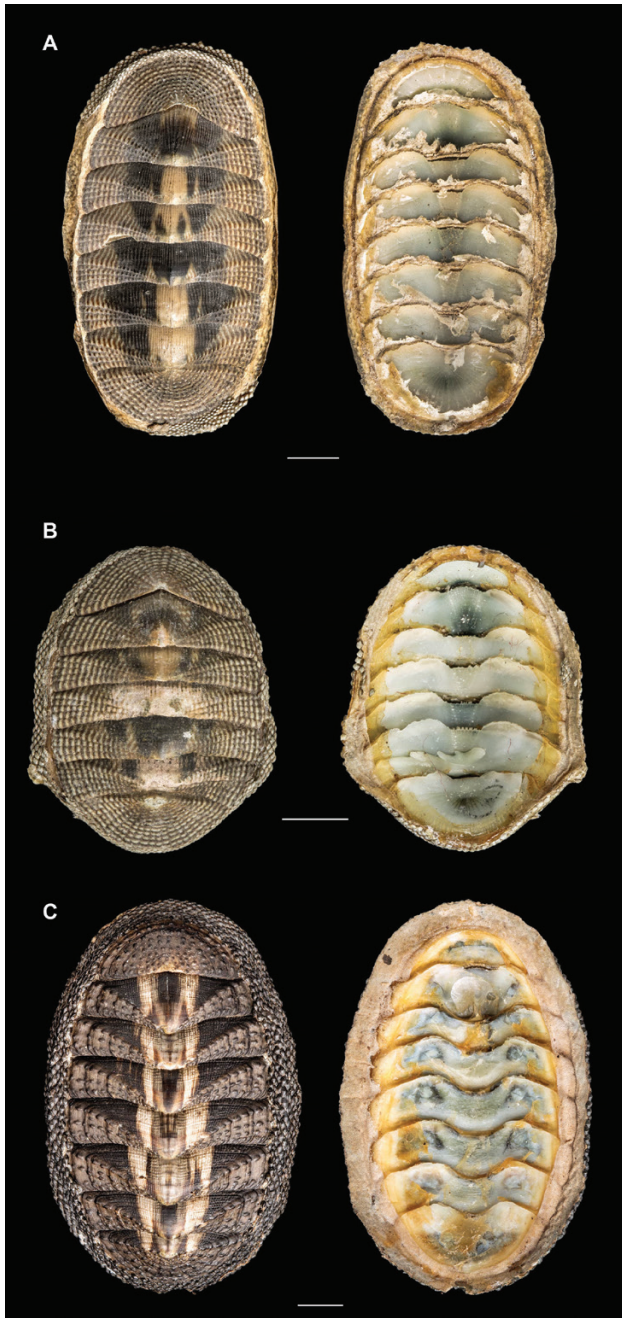
**Figure 6.** *Chiton barnesii*, Chile, Coquimbo, BL 20.0 mm, 05.01.2015: A, C, dorsal and ventral scales and marginal spicules; B, D, radula, head of major lateral tooth has one denticle.

previously reported from Paita (~5°S), Peru to Puerto Montt (~41°S), Chile (Marincovich, 1973).

**Remarks:** This species is easy to differentiate from all other *Chiton* species in the area by their high number of ribs in all plates with small grains. In the head valve, they have more than 40 ribs of grains. This species has been introduced in the Canary Islands (28°06'N, 15°25'W) (Arias & Anadón 2013).

**Notes on designation of type material:** The lectotype and paralectotype material comprise a single lot in the collections of the Natural History Museum, London (NHMUK) (Fig. 6). It is probable that some of Frembly's specimens were deposited in the NHMUK via the

collections of contemporaries, such as Hugh Cuming and J. E. Gray, although there is nothing to link any of this early material to Frembly directly (T. S. White, pers. comm). Type material for other species described by Frembly has also been attributed to the NHMUK collection (Kaas *et al.*, 2006; Bullock, 1988b), although the status of these specimens requires further careful investigation. In the original description of *Chiton cumingsii*, Frembly (1827: 199) noted 'A specimen also was found [showing] ... only seven valves, but inside the articulation of the other valve was plainly seen'. *Chiton* teratologies are rare (Torres *et al.*, 2018). There was only one teratological specimen of this species in the NHMUK collection, amongst a lot of four specimens (NHMUKMUK 20190538/1–4) from the J. E. Gray



**Figure 7.** Type specimens of chitons: A, lectotype of *Chiton cumingsii* NHMUK 20190538/1; B, paralectotype of *Chiton cumingsii* NHMUK 20190538/2; C, neotype of *Chiton granosus* NHMUK 20190537/1. Scale bar 10 mm.

collection; these specimens have been designated as the lectotype (Fig. 7A) and paralectotypes (Fig. 7B) on this basis. The teratological paralectotype (NHMUK 20190538/2) has a coalescence between valve II and valve III; the fragmented valve is visible on the ventral side but the dorsal view appears to be a simple hypomeric seven-valved chiton, as described by

Frembly (1927) (Fig. 7B). The specimen closest to the potentially generalized dimensions given in Frembly's original description ('length two inches, breadth 1 3/10'; 5.0 × 3.3 cm) has been designated the primary lectotype (NHMUK 20190538/1, dimensions, 4.2 × 2.3 cm).

*CHITON (CHITON) GRANOSUS* FREMBLY, 1827

(FIGS 4D, 7C, 12, 13)

*Chiton granosus* Frembly, 1827: 200, suppl. pl. 17, fig. 1; Plate, 1902: 56, pl. 4, fig. 190; Pilsbry, 1893: 167, pl. 30, figs 27, 28; Dall, 1919: 247. *Chiton (Chiton) granosus*: Marinovich, 1973: 43, fig. 98. *Chiton (Chondroplax) granosus*: Bullock, 1988b: 185, figs 96, 97, 103, 140, 141, 143 and 144. *Chiton (Chiton) granosus*: Kaas et al., 2006: 116, fig. 38, map 14 (bibliography and synonymy).

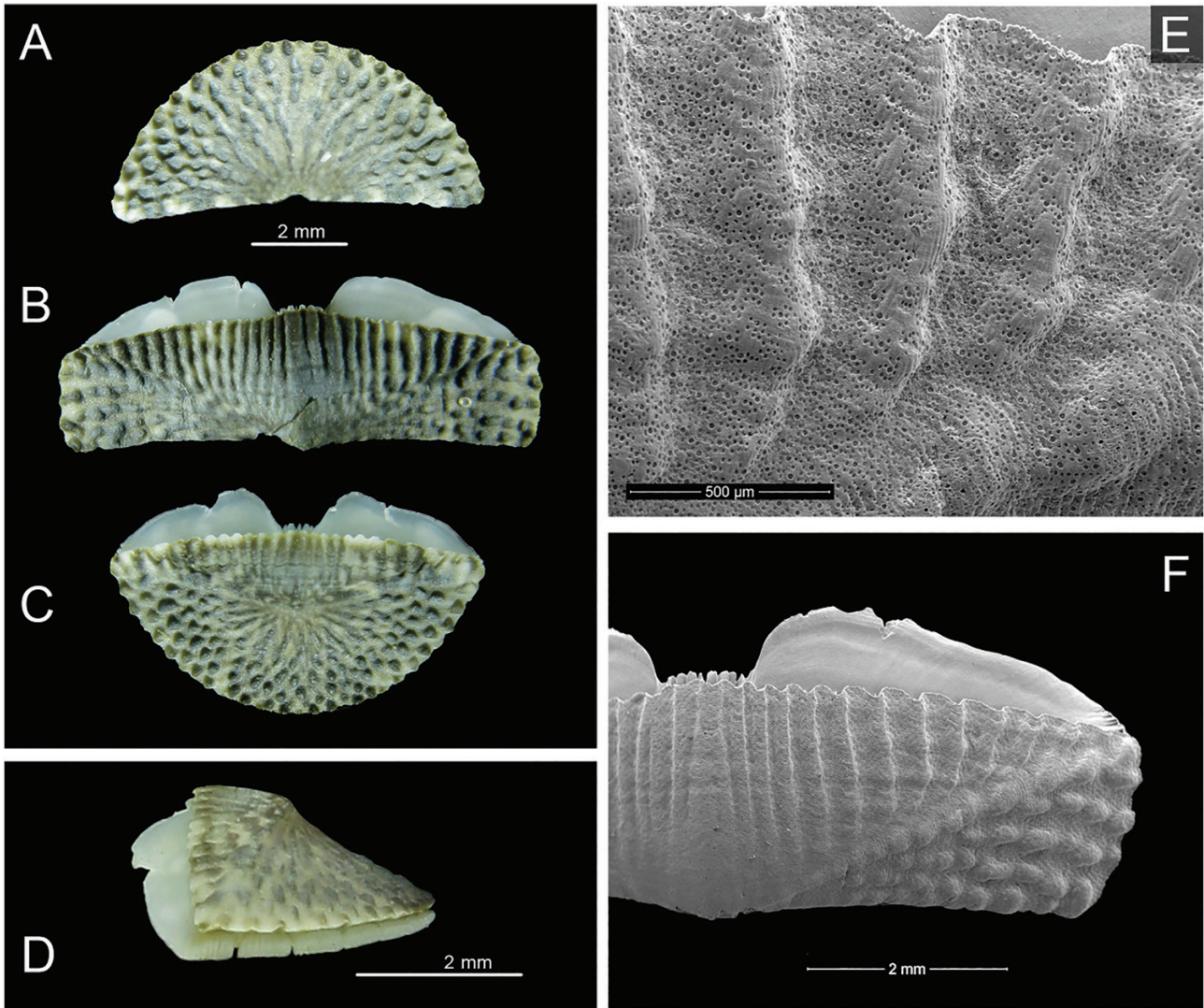
*Type material*: NHMUK 20190537/1, neotype from the Hugh Cuming collection from 'Valparaiso, Chili', designated herein.

*Type locality*: 'Valparaiso, Chili' (Valparaiso, Chile).

*Description of neotype specimen*: Dried specimen 91 mm long by 59 mm wide, overall colour black with pale central stripe divided by a darker midline in intermediate valves (Fig. 6C). Head valve with approximately 13 irregular radiating lines of large, round, raised granules; intermediate valves lateral areas with three to four radiating lines of widely spaced elongate raised granules, solid black; central areas of intermediate valves with anterior-posterior ribs formed in part of fused granules creating a latticed appearance, with symmetrical colour blocks forming the midline stripe, from distal to proximal black, pale cream, dark brown, grey-brown and centrally dark brown to black. Tail valve antemucronal area as central areas of intermediate valves; postmucronal area with approximately 13 irregular radiating lines of elongate granules of similar size but slightly smaller than those on the lateral areas of intermediate valves. Articulamentum blue in middle areas of valves. Girdle perinotum covered in black, imbricating scales; hyponotum with fine elongate flat sclerites.

*Descriptive notes*: Size: animals of medium to large size, typically 76 mm long by 51 mm wide (Table 2). Colour: black with brown or green tone, usually eroded and sometimes with white lines on the central area (Fig. 4D). Body shape: oval with moderate dorsal elevation. Slit formula: 12–18/1/14–17. Plates: head valve with < 20 ribs composed of large granules (Fig. 4D). Juvenile head valve with large granules





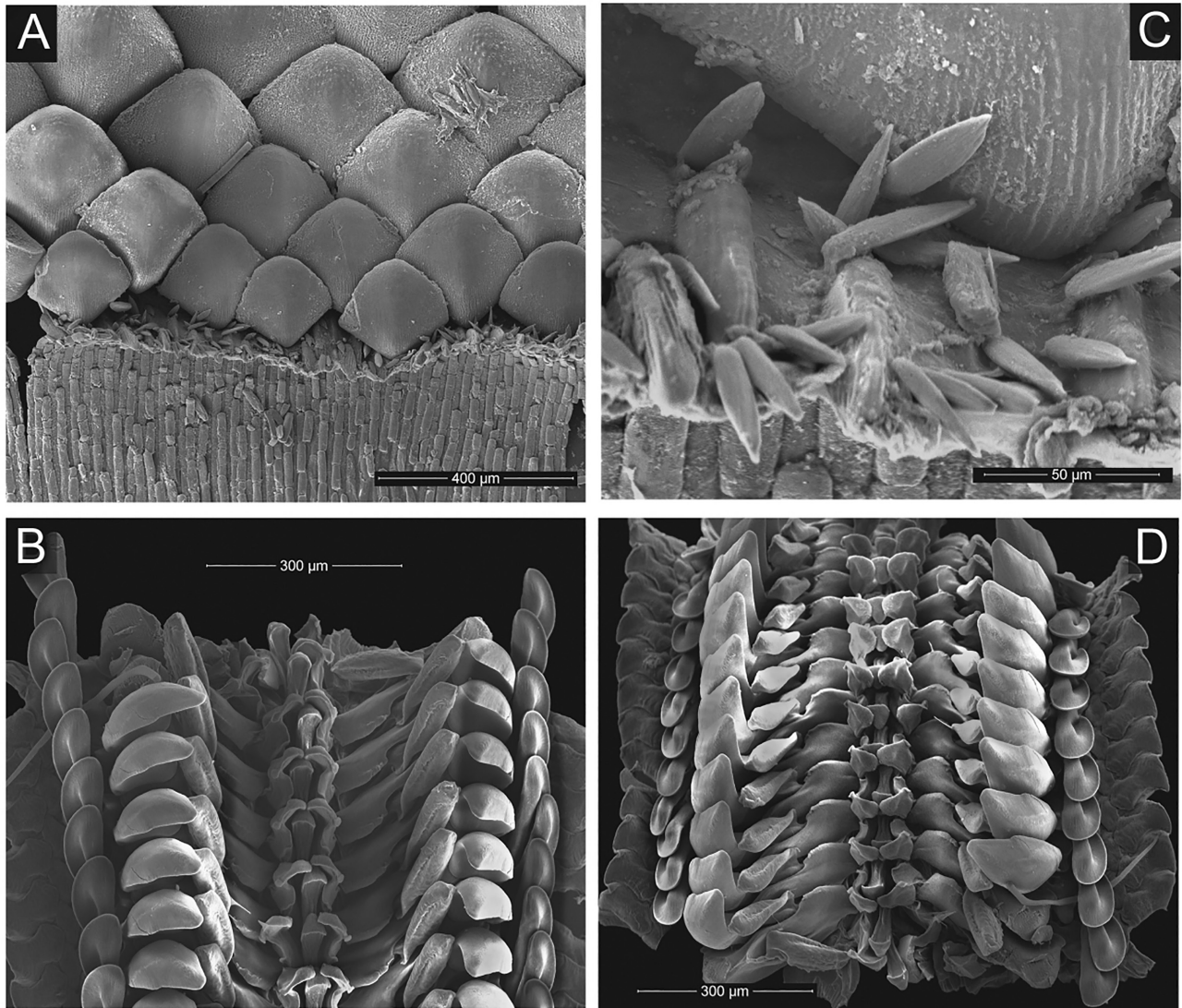
**Figure 8.** *Chiton stokesii*, Ecuador, Santa Rosa, Salinas, CSSR 18, BL 20 mm, 22.09.2013: A, valve I, dorsal view; B, valve V, dorsal view; C, valve VIII, dorsal view; D, valve VIII, lateral view; E, valve VII, detail of tegmentum in central area; F, valve VII, central and lateral areas.

forming < ten ribs (Fig. 12A). Commonly eroded intermediate plates, central area with many fine ribs, valve slightly wider than long, sloping postmucronal tilt (Fig. 13A). Intermediate plates with one slit. Articulamentum: apophyses rounded, short and wide, connected by a pectinate jugal lamina, insertion plates pectinate with short insertion slit (Fig. 12B). Perinotum: wide, solid black with rounded scales arranged irregularly (Fig. 13B, C). Radula: central tooth rounded, major lateral tricuspid (Fig. 12D).

**Distribution:** Paita (~5°S), Peru to Boca del Guafo (~43°S), Chile (Marincovich, 1973; Osorio *et al.*, 2005).

In this study, we found this species from Chiclayo (~8°S), Peru to Peninsula Taitao (~46°S), Chile (Fig. 1).

**Remarks:** This species is easily confounded with *C. barnesii* (see remarks for *C. barnesii*). Sanhueza & Ibáñez (2016) erroneously stated that *C. granosus* was a *nomen nudum*, but the original description is sufficient, complete and appropriately published, and the name is valid. The International Code for Zoological Nomenclature requires seven qualifying conditions (ICZN Art. 75.3). A neotype is designated herein to clarify the taxonomic status of the species and prevent confusion with similar species (ICZN Art. 75.3.1). The

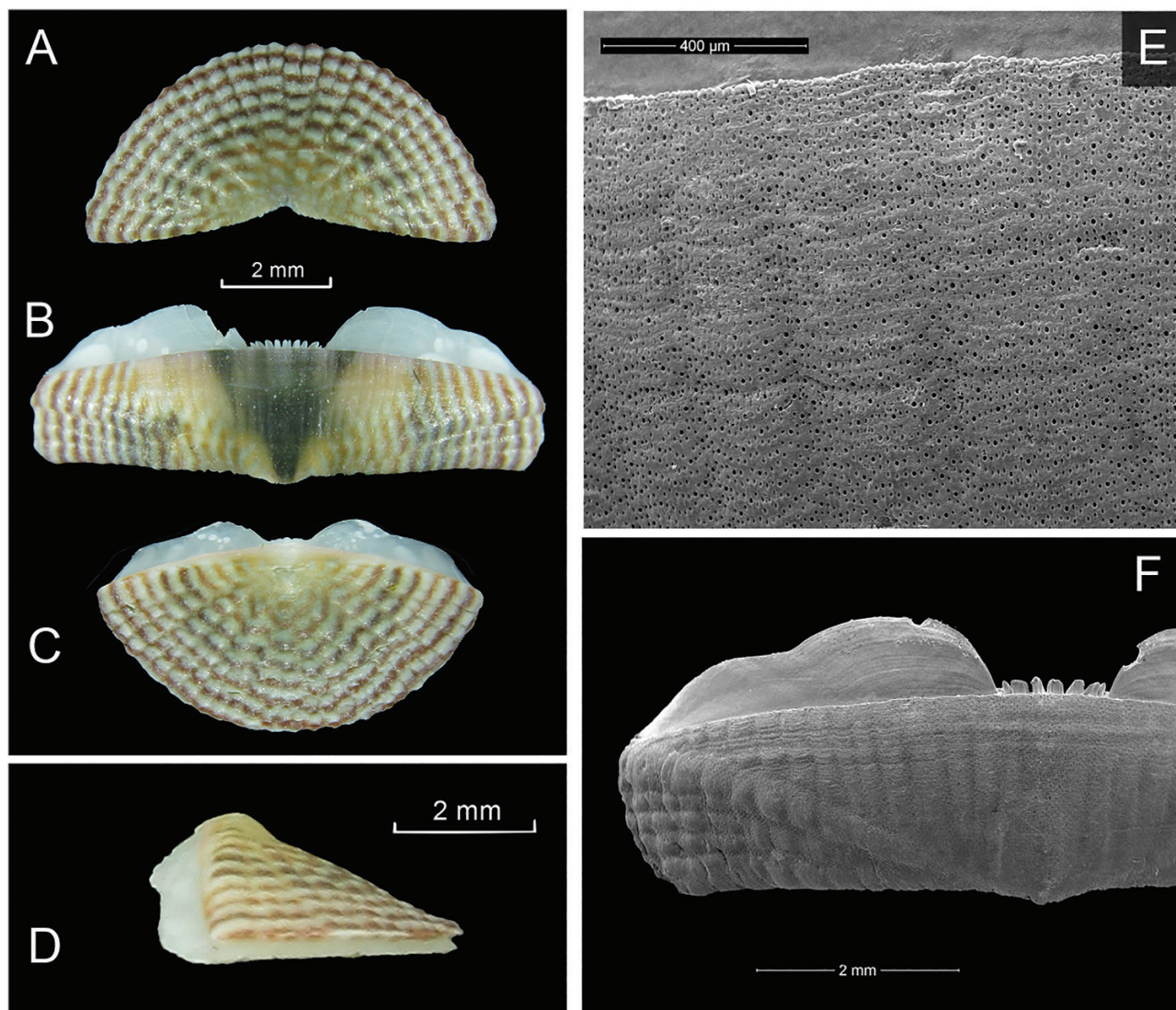


**Figure 9.** *Chiton stokesii*, Ecuador, Santa Rosa, Salinas, CSSR 18, BL 20 mm, 22.09.2013: A, C, dorsal and ventral scales and marginal spicules; B, D, radula.

characters that differentiate the species are included in the descriptive notes above (ICZN Art. 75.3.2). Prior descriptions, and our additional remarks and novel genetic data from additional specimens, are sufficient to ensure the species can be correctly recognized and identified, and this description is consistent with the selected neotype (ICZN Art. 75.3.3). The original description does not include a specific designation of type material (Frembly, 1827). Therefore, the original type material is not lost or destroyed, but a type specimen was never previously designated. From the historical context, we understand that the relevant material was all deposited in NHMUK. One of us (JDS) has examined every known specimen in the relevant part of the polyplacophoran collections

of NHMUK and could not find any specimen that convincingly matches the illustration in the original description (Frembly, 1827: pl. 17, fig. 1). We note that the illustration is somewhat stylized, and may not be sufficiently accurate to match an individual specimen, including the description of specimens in the text of the original description. We have concluded that it is not possible to connect the original description to an extant specimen (ICZN Art. 75.3.4). The designated neotype specimen was selected to ensure consistency with what is known of the material in multiple specimens collected by Frembly and which informed the original description. The neotype is connected to the type locality, and of the available specimens, the individual closest to the generalized dimensions stated in the



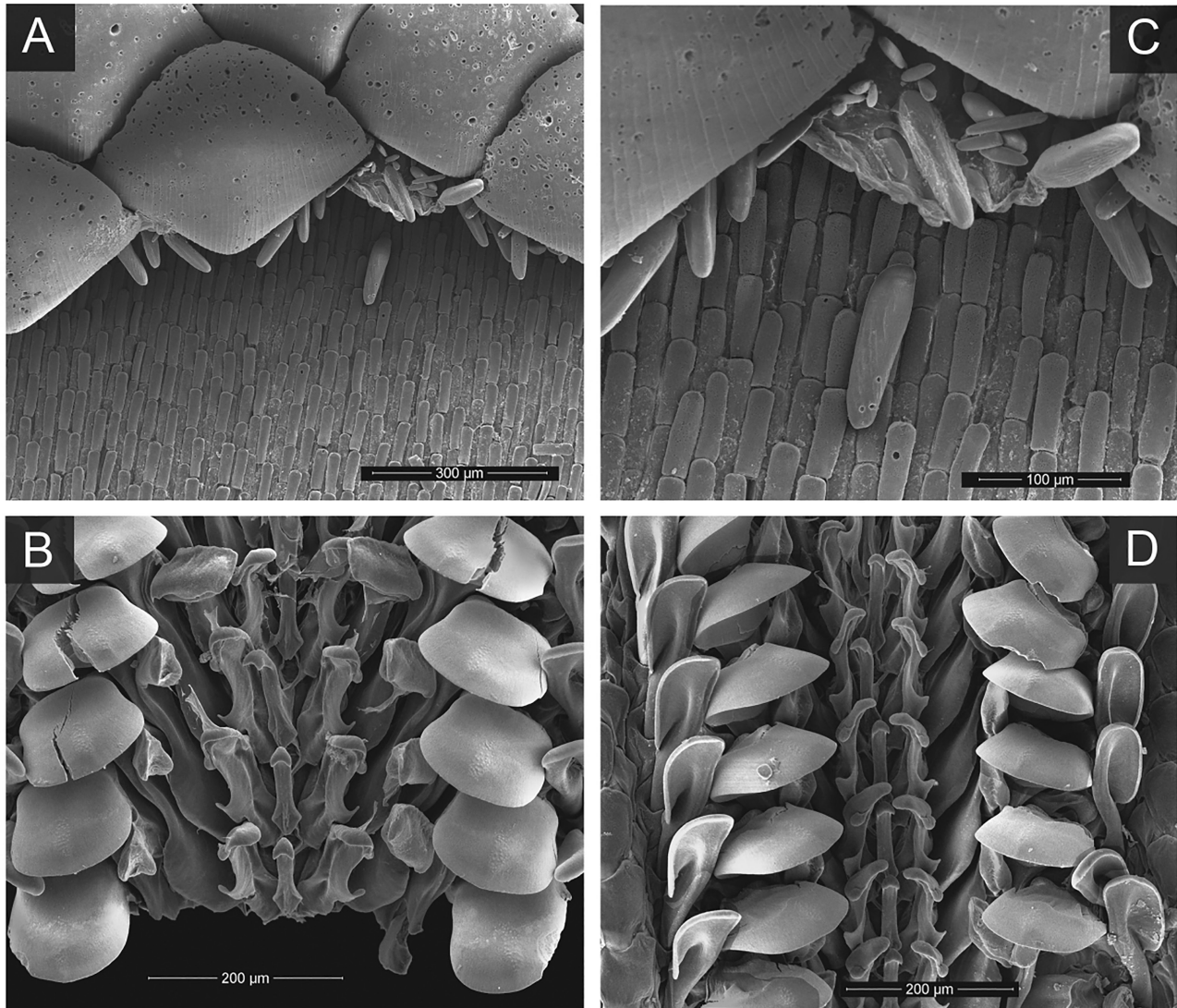


**Figure 10.** *Chiton cumingsii*, Chile, Coquimbo, BL 19.5 mm, 05.01.2015: A, valve I, dorsal view; B, valve V, dorsal view; C, valve VIII, dorsal view; D, valve VIII, lateral view; E, valve VI, detail of tegmentum in central area; F, valve VI, central and lateral areas.

original description (ICZN Art. 75.3.5) is as follows: neotype dimensions,  $4.8 \times 3.2$  cm compared to original description approx.  $4.4 \times 3.3$  cm, 'length  $1 \frac{3}{4}$  inches, breadth  $1 \frac{3}{10}$ ' (Frembly, 1927: 201). The type locality is clearly stated as Valparaiso, Chile (Frembly, 1827) and the selected specimen is also clearly recorded as from Valparaiso (ICZN Art. 75.3.6). As the specimen is part of the permanent collections of NHMUK, it is the property of a recognized institution with appropriate facilities for maintaining this specimen (ICZN Art. 75.3.7).

Circumstantial evidence suggests that some or all of Frembly's material was and is present in the collections of NHMUK (see remarks under *C. cumingsii*, above). The type material for this species

was already noted as 'location of type unknown' in a prior revision (Bullock, 1988b). We were unable to identify any individual specimen or specimen lot that could be connected to the original description or to Frembly. Among the contemporary (early 19<sup>th</sup> century) specimens, two lots from J. E. Gray were considered as potential Frembly-associated material. One lot has two specimens mounted on a card with handwriting that matches the designated lectotype for *C. cumingsii* and has a pencil circle drawn around one specimen. We note that the Gray material is potentially relevant to future research, but these specimens have no locality data, whereas the type locality for *C. granosus* is clearly stated in the original description as Valparaiso. There is also one lot of three specimens of *C. granosus*



**Figure 11.** *Chiton cumingsii*, Chile, Coquimbo, BL 19.5 mm, 05.01.2015: A, C, dorsal and ventral scales and marginal spicules; B, D, radula.

in the Senckenberg Gesellschaft für Naturforschung, Frankfurt am Main, Germany, donated by Cuming. These specimens also have no documented connection to Fremby and the locality is given only as 'Chili'. Therefore, we selected a neotype specimen from the Cuming collection (contemporary to Fremby) in the NHMUK with their collecting locality as Valparaiso.

*CHITON (CHITON) MAGNIFICUS* DESHAYES, 1827

(FIGS 4E, F, 14–18)

*Chiton magnificus* Deshayes, 1827: 454. *Chiton latus*: Fremby, 1827. *Chiton magnificus*: Pilsbry, 1893: 160, pl. 30, figs 23, 24. *Chiton (Amaurochiton) magnificus magnificus*: Bullock, 1988b: 163, figs 52–54, 57, 58, 64,

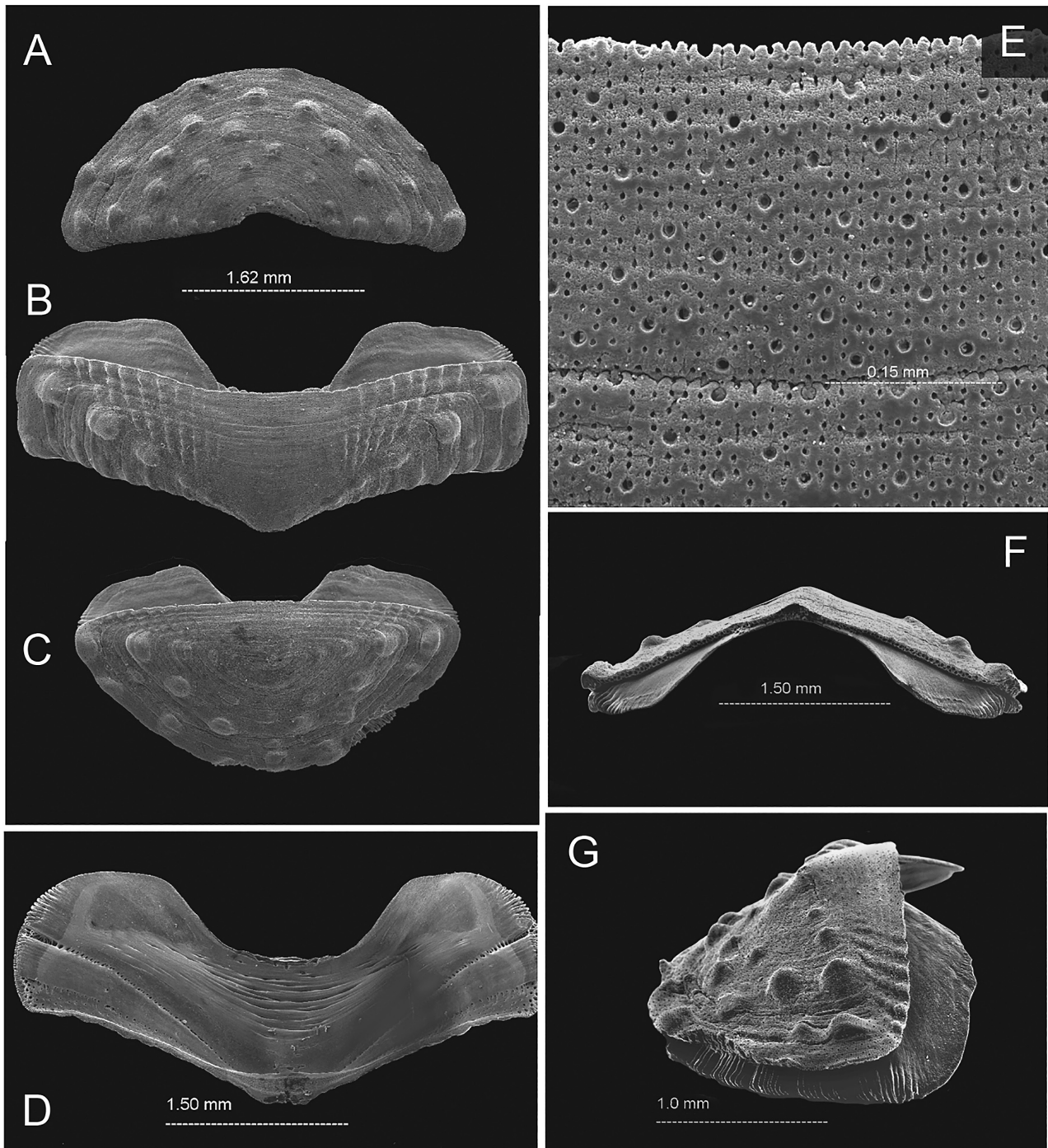
65, 68, 72, 73 and 76. *Chiton magnificus*: Schwabe *et al.*, 2006: 9, figs 7, 8 and 15. *Chiton bowenii*: Schwabe *et al.*, 2006: Sirenko, 2006: 84. *Chiton (Chiton) magnificus*, *Chiton (Chiton) bowenii*: Kaas *et al.*, 2006: 111, 120, fig. 36, 40, map 14 (bibliography and synonymy). *Chiton bowenii*: Schwabe, 2009: 404–405.

*Type material*: MNHN-IM-2000–6039 (syntypes) (not seen). <https://science.mnhn.fr/institution/mnhn/collection/im/item/2000-6039>

*Type locality*: Chile.

*Descriptive notes*: Size: large, 160 mm long and 81 mm wide on average (Table 2), although larger individuals have been reported (> 190 mm; Brito, 2017). Colour



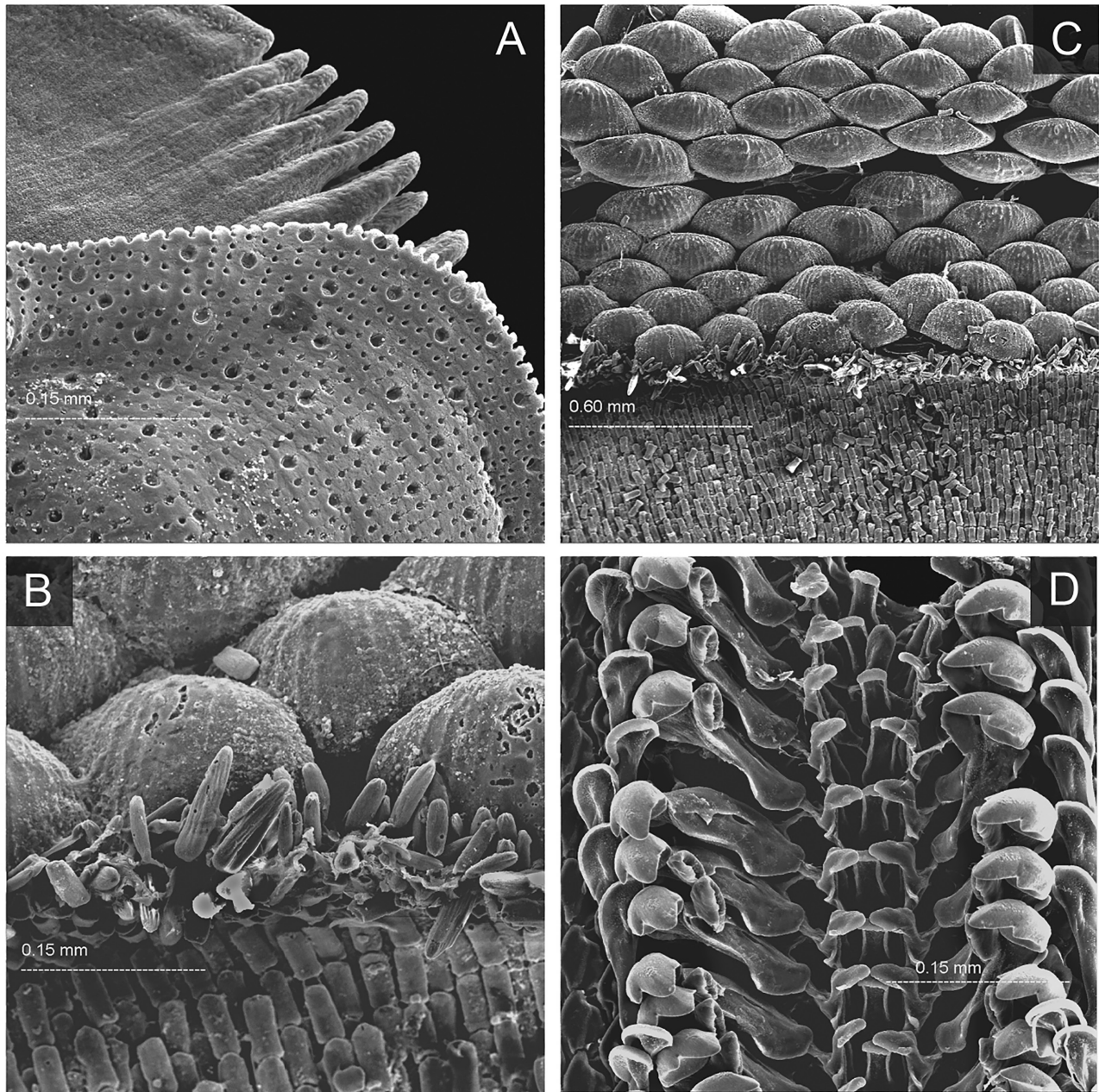


**Figure 12.** *Chiton granosus*, Chile, Las Cruces, BL 9.5 mm, 20.11.2008: A, valve I, dorsal view; B, valve V, dorsal view; C, valve VIII, dorsal view; D, valve V, ventral view; E, valve V, detail of tegmentum in central and lateral areas; F, valve V, rostral view; G, valve VIII, lateral view.

varies geographically: usually black with light blue dots in the northern part of its range (Figs 4E, 14A, 15A–C), blue and blue with orange or red with black in Golfo de Ancud (Figs 14B–D, 16A–C) and reddish orange or black in Estrecho de Magallanes (Figs 4F,

17A–C). Body shape: elongate oval with low dorsal elevation. Slit formula: 14/1/12–14. Plates: slightly convex head plate with numerous weak radial ribs (about 40 longitudinal ribs per side), with lines on the lateral areas (Fig. 4E). Articulamentum: apophyses





**Figure 13.** *Chiton granosus*, Chile, Las Cruces, BL 9.5 mm, 20.11.2008: A, valve V, detail of tegmentum and apophyses; B, C, dorsal and ventral scales and marginal spicules; D, radula, head of major lateral tooth has three denticles.

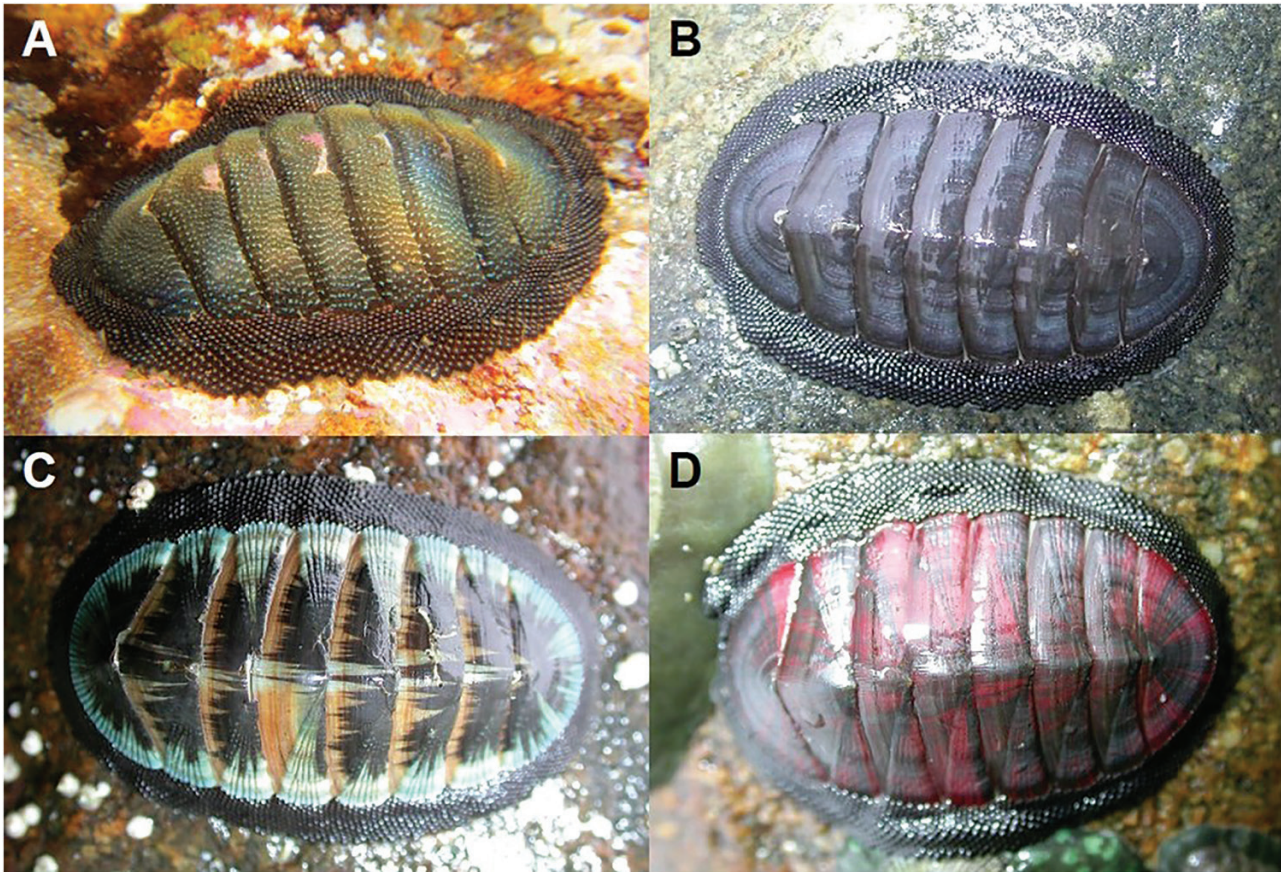
trapezoidal, insertion plate pectinate (Fig. 15A, B). Perinotum: black, moderately broad, dorsally covered with smooth rounded scales of two different sizes (Fig. 18A, C), the largest located near the margin and the small scales near the valves. Radula: central tooth rounded. The major lateral is the biggest tooth rounded in shape (Fig. 18B, D).

**Distribution:** Isla San Lorenzo (~12°S), Peru to Bahía Tictoc (~43°S), Chile (Bullock, 1988a; Sirenko, 2006;

Schwabe, 2009). In this study, we found this species from Huasco (~28°S) to Fuerte Bulnes (~53.6°S), Chile (Fig. 1).

**Remarks:** King (1832) described *Chiton bowenii* from Tierra del Fuego, and Bullock (1988a) provided the new combination as a subspecies *C. magnificus bowenii*. Schwabe et al. (2006) suggested sufficient morphological differences to separate *C. magnificus* and *C. bowenii*. Based on the long-standing doubt





**Figure 14.** Images of *Chiton magnificus*. A, black specimen with light-blue dots from El Soldado, Talcahuano, Chile (36°46'01"S, 73°10'17"W). B, blue specimen from Metri, Chile (41°35'58"S, 72°42'16"W). C, light-blue specimen with black lines from Metri, Chile. D, red specimen from Metri, Chile.

about whether *C. bowenii* represents a taxon at the species-level, and the molecular results indicated no separation based on *COI*, we consider *C. bowenii* to be a junior synonym of *C. magnificus*, representing one particular morphotype among many colour patterns in this variable species (see Discussion).

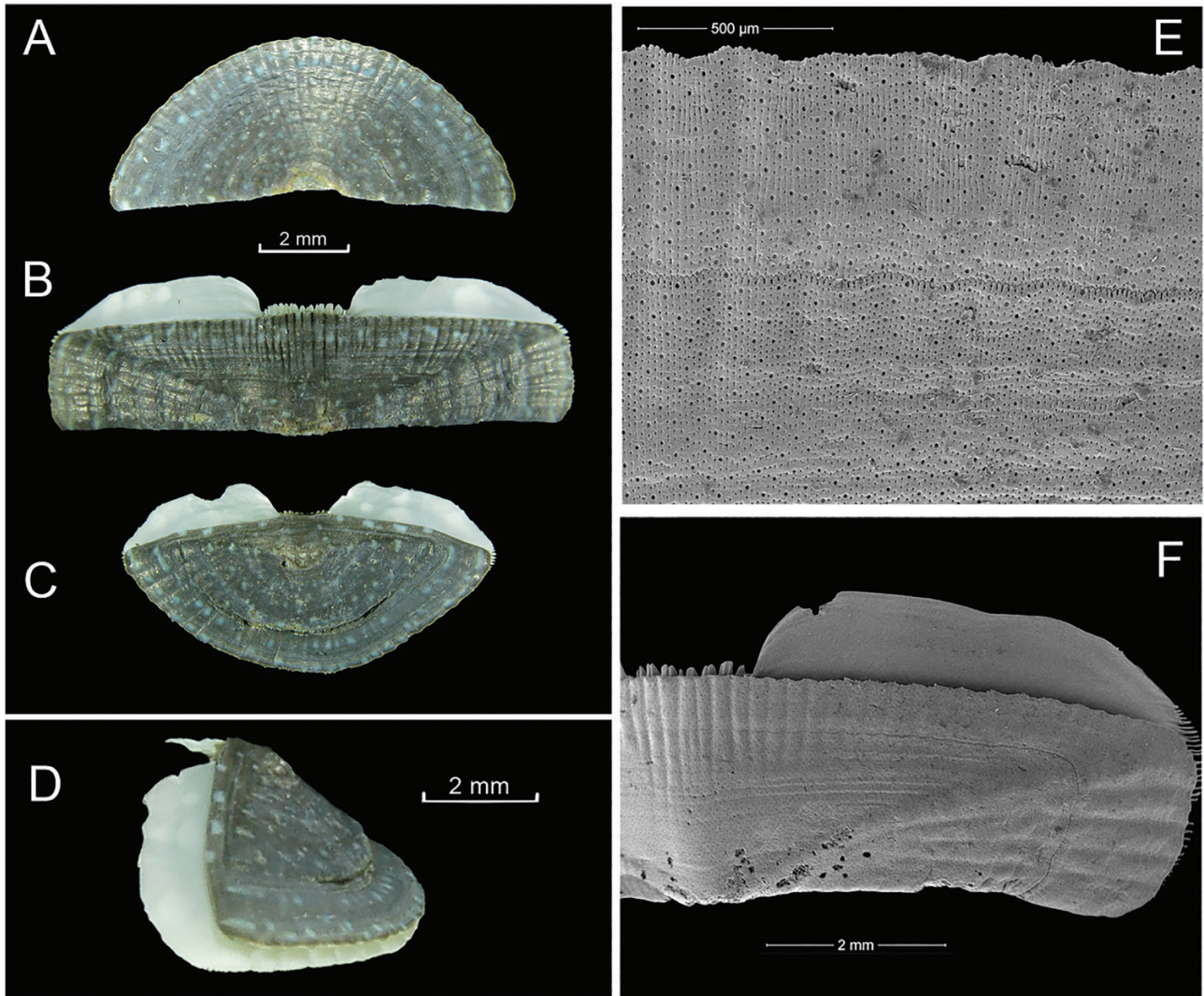
## DISCUSSION

In this study, we reviewed species in the genus *Chiton* from Ecuador to southern Chile (1.8°S–53.6°S), comprising five valid species. This is only a fraction of the global diversity of the genus, which includes around 70 currently accepted species. The monophyly or not of the members in a regional fauna is indicative of broader issues for the global diversity of the group. Furthermore, establishing taxonomic clarity on a regional subset of species is prerequisite to larger scale revisions of the broader group. Two of the SEP species (*C. barnesii* and *C. magnificus*) are endemic to Chile; two species (*C. cumingsii* and *C. granosus*)

have a wider distribution (> 4000 km) from northern Peru to southern Chile. Only one species (*C. stokesii*) is tropical and endemic to the Panamanian province. Our sampling records suggest a different distribution for four of the five *Chiton* species in the SEP, compared to previously reported records. First, we recorded new southernmost distributions of *C. stokesii*, *C. granosus* and *C. magnificus*, as well as northernmost records for *C. barnesii* and *C. magnificus* (Fig. 1). We have found some errors in previous literature, which were related to misidentifications and confusion around some species that consequently became errors in describing the distribution of species. Our new descriptions of shells, which were based primarily on their sculpture, and not coloration, will markedly improve the knowledge and confidence in identifications of the chiton fauna in the SEP.

Morphology of plates (head, intermediate and tail) are different among SEP *Chiton* species (Fig. 4). The head plate is semicircular with a straight posterior margin only in *C. stokesii* and *C. magnificus*. Intermediate plates (III–VII) are relatively straight



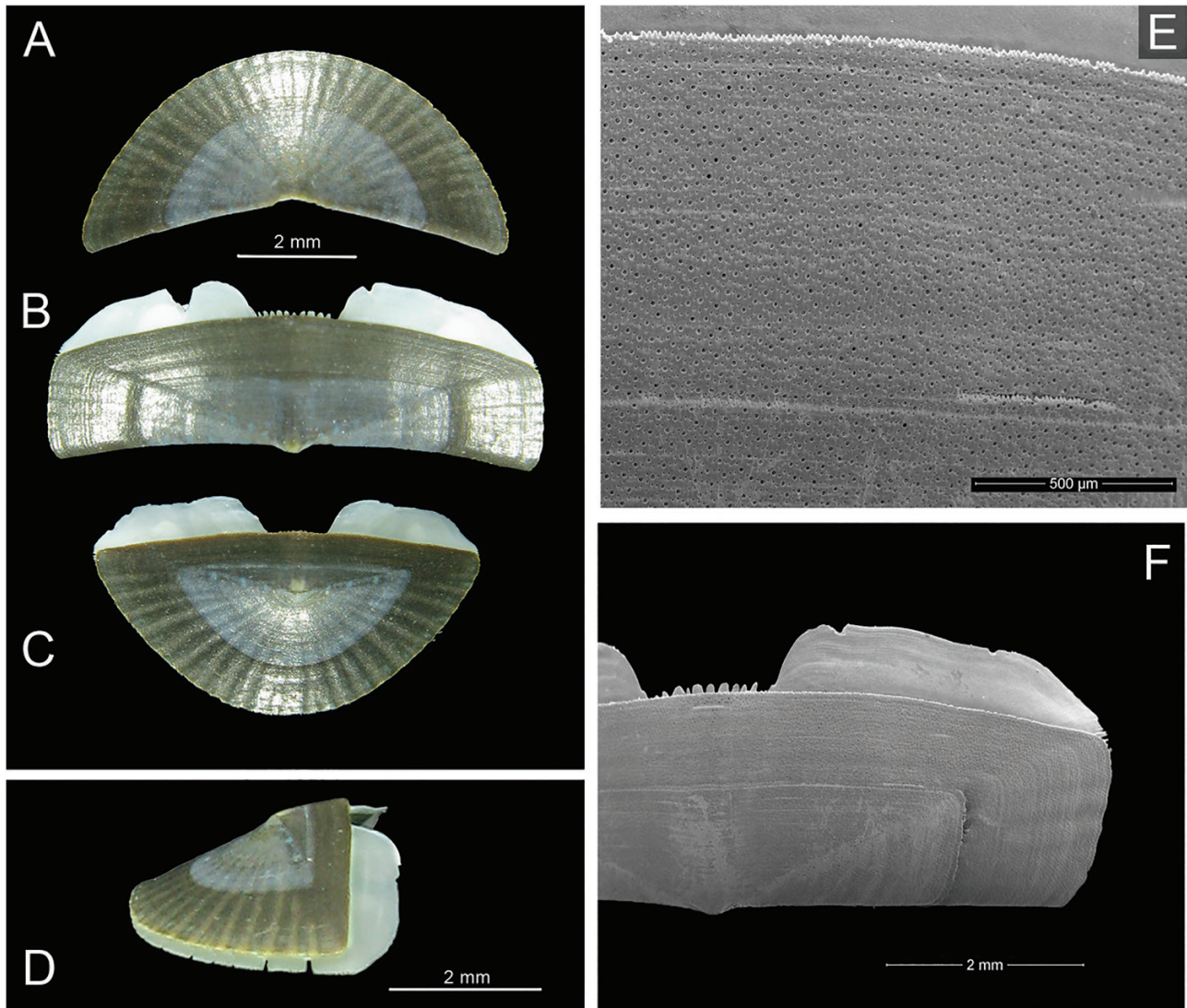


**Figure 15.** *Chiton magnificus*, Chile, La Mission, BL 24.0 mm, 30.12.2004: A, valve I, dorsal view; B, valve V, dorsal view; C, valve VIII, dorsal view; D, valve VIII, lateral view; E, valve VI, detail of tegmentum in central area; F, valve VI, central and lateral areas.

in outline in all species from the SEP, except for *C. granosus*, which shows a distinct boomerang shape (Fig. 4D). The tail plate is semicircular in all species from the SEP but with V-shaped type margins in three species: *C. barnesii*, *C. cumingsii* and *C. magnificus*. The tegmentum sculpture is ornamented to a different degree in each species of *Chiton* from the SEP. Smooth valves are rare and still show microstructure, visible texture under low magnification (Schwabe, 2010). This ‘smooth’ valve surface is a feature only in the *C. magnificus* form *bowenii*, although from the balance of evidence these are apparently not separate species-level lineages. All other species in this genus from the SEP have radial ribs or series of raised granules, in the head,

intermediate and tail plates, radiating from a valve apex or the mucro to the valve margin. The size of the granules and the number of ribs differentiated all species (see species descriptions).

Applying a biogeographical perspective to our molecular phylogenies (*COI* and multigene analysis), we found that species of the genus *Chiton* from the SEP may represent a polyphyletic group under current taxonomy (Fig. 3). The species cluster formed by SEP *Chiton* is part of the genus *Chiton* s.s., because the type species, *C. tuberculatus* resolves within that clade. However, in the combined analysis, the sampled *Chiton* spp. are polyphyletic with respect to sampled taxa from other related genera. New Zealand species of *Sypharochiton* Thiele, 1893 plus *Chiton glaucus* are



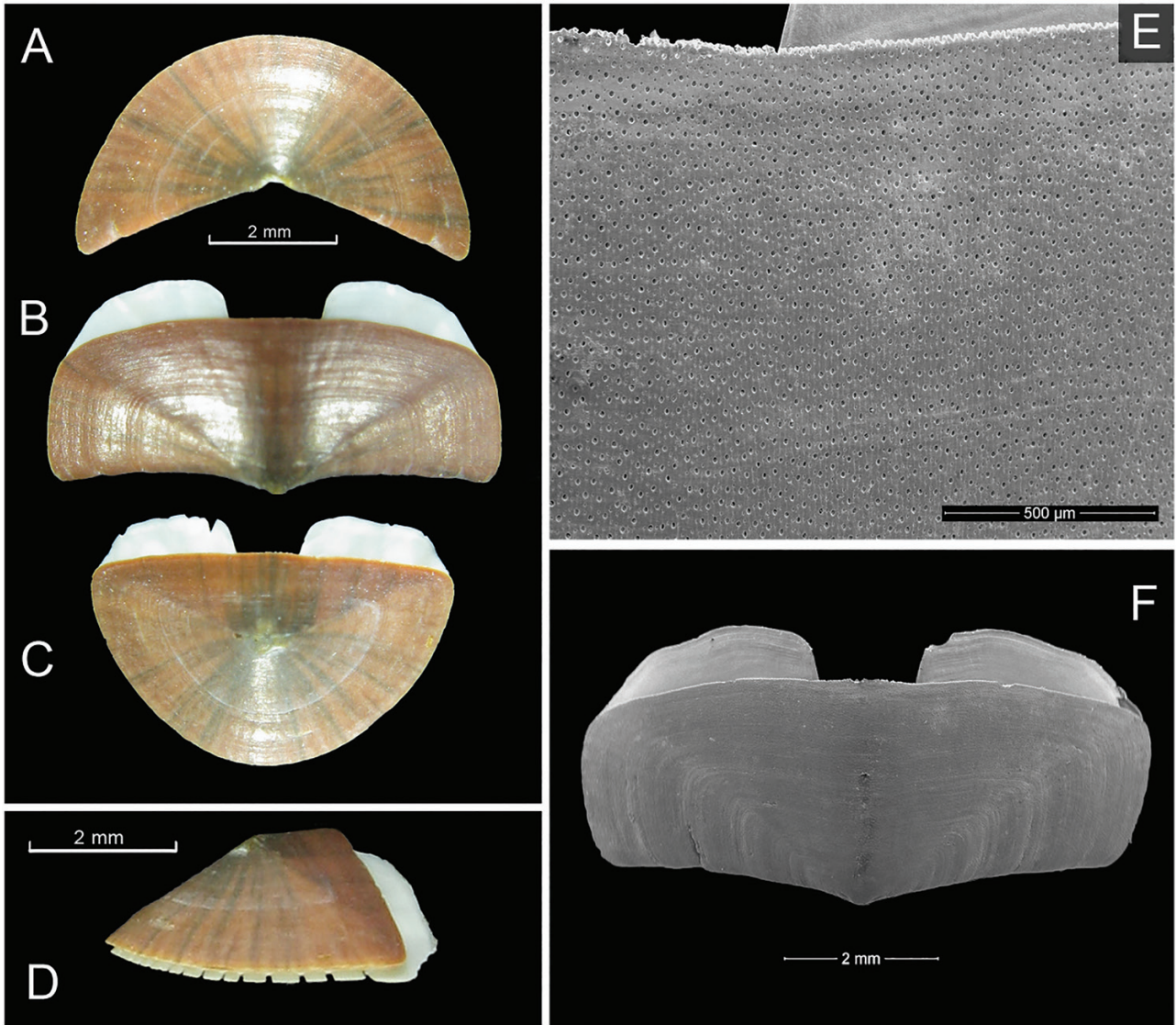
**Figure 16.** *Chiton magnificus*, Chile, Huinay, BL 20.0 mm, 05.01.2005: A, valve I, dorsal view; B, valve V, dorsal view; C, valve VIII, dorsal view; D, valve VIII, lateral view; E, valve VI, detail of tegmentum in central area; F, valve VI, central and lateral areas.

monophyletic with *COI* and in the combined analyses, suggesting that they have a common recent ancestor.

There is a clear phylogenetic substructure of relationships among the sampled *Chiton* species, which can be compared to previous morphological taxonomic proposals. We note that phylogenetic positions are limited to the taxon sampling within this biogeographic region, but nonetheless are informative about certain taxonomic points. *Chiton cumingsii* was consistently resolved as the sister-species of *C. magnificus* in our phylogenetic analysis and also supported by relatively close pairwise genetic distances. Bullock (1988a) proposed that both species belong to the subgenus *Amaurochiton* Thiele, 1893, and our

resultssupport this inference, but under current levels of taxonomic and phylogenetic resolution it seems premature to assign any subgeneric classification. *Chiton granosus* is resolved as the sister-species of *C. barnesii*, indicating that this species should not be assigned to the genus *Radsia*, but could potentially be part of a previously proposed subgenus *Chondroplax*. Bullock (1988b) had proposed assigning the species *C. barnesii* to the genus *Radsia* based on the radula shape. Radula shape is highly variable in the genus *Chiton* and it remains necessary to study the inter- and intraspecific variation of radular structures in more detail. Certainly, a single radular character in this genus does not seem to be sufficient evidence





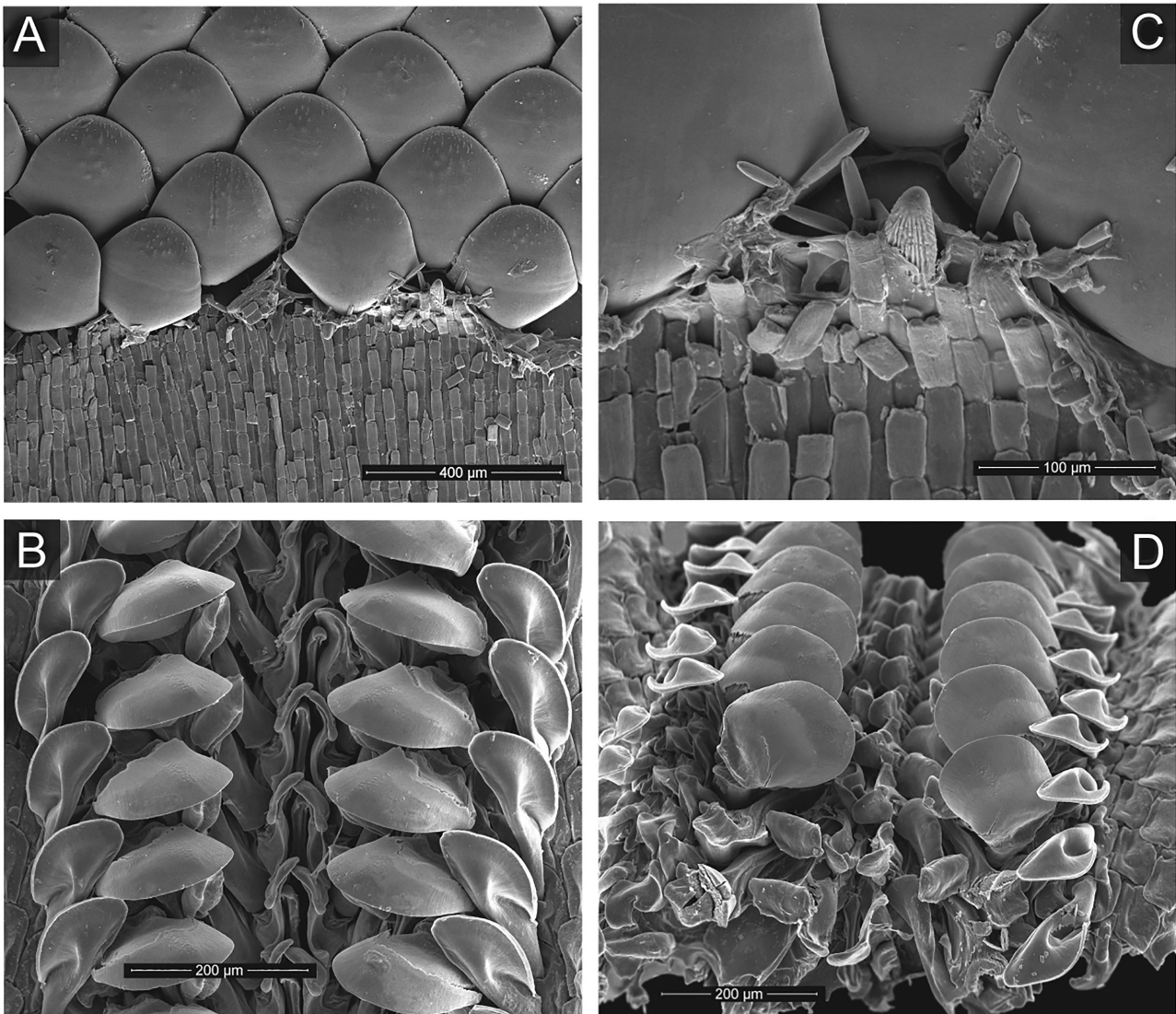
**Figure 17.** *Chiton magnificus*, Chile, Magellan Strait, Fuerte Bulnes, BL 23.0 mm, 17.05.2000: A, valve I, dorsal view; B, valve V, dorsal view; C, valve VIII, dorsal view; D, valve VIII, lateral view; E, valve VII, detail of tegmentum in central area; F, valve VII, dorsal view.

for a genus-level revision. The type species of the genus, *C. tuberculatus*, is from the Caribbean Sea, and we tentatively conclude that, among our study species, only *C. stokesii* belongs to *Chiton* s.s. However, a worldwide sampling of taxa from this large and widespread genus is needed to construct a more robust phylogeny as a basis for further taxonomic resolution.

Among the other taxa we were able to sample, members of the genus *Sypharochiton* represents a clade with a high support (PP = 0.9–1.0), but it falls within the *Chiton* clade. Bullock (1988a) proposed to move the species *S. pelliserpentis* and *S. sinclari* from the genus *Chiton* based on their radula shape.

However, the shape of denticle caps of *S. pelliserpentis* vary from unicuspid to tricuspid in specimens from the same locality (Bullock, 1988a). Our phylogenetic results, genetic distances and the radula morphology of species from the SEP suggest placing *Sypharochiton* in the genus *Chiton*. Kaas et al. (2006) already classified several species of *Sypharochiton* in *Chiton*. The sampled species from the three genera (*Chiton*, *Rhyssoplax* and *Sypharochiton*) conform to a clade with high support, suggesting that they share a common origin.

Along the Chilean coast, *C. magnificus* has three morphotypes: the first one is a black form with



**Figure 18.** *Chiton magnificus*, Chile, Huinay, BL 20.0 mm, 05.01.2005: A, C, dorsal and ventral scales and marginal spicules; B, D, radula.

light-blue spots, observed mainly from northern Chile to Valdivia (6E, 7E, 9A); the second one is a coloured form (red with black, blue, blue with orange and black) with or without lines, mainly found south of Puerto Montt (Figs 15B–D, 16); the third form (i.e. *bowenii*) is slenderer and more coloured (reddish orange or black) with or without the black lines that characterize the southernmost population at Punta Arenas (Figs 4F, 5F). Reid & Osorio (2000) suggested that the second form (coloured, but not slender) could be the subspecies proposed by Bullock (1988a), such as *C. magnificus bowenii*, but the genetic distance (0.000–0.002 subst./bp) and the phylogenetic position do not support even a subspecies level. In addition,

the morphological differences in the width/length ratio, the body is narrower and lower in *C. bowenii* (0.28–0.54) compared to the more regular form of *C. magnificus* (0.40–1.03). Our molecular analysis, including all three morphotypes, does not support the subspecies hypothesis. Differences in body form and coloration patterns could be attributed to the different environmental conditions of habitats from Chiloé southwards. Along the austral Chilean coast, from 41°30'S and southward, there is a geomorphologic discontinuity due to the complete fragmentation of the coast, where the high input of freshwater into coastal water masses promotes estuarine conditions (Ibáñez *et al.*, 2009).



Our phylogeny represents the first robust evaluation of the genus *Chiton*. These are distinctive, shallow-water species that seem to be easily distinguished by multiple morphological characters. Nevertheless, this analysis suggests an urgent review is needed for the subfamily Chitoninae, because the morphological characters used to separate the genera *Radsia*, *Sypharochiton* and *Rhyssoflax* are not consistently supported by molecular phylogeny.

Finally, further molecular phylogenetic analyses, including additional molecular markers (mitochondrial and nuclear), are required to clarify the origin, spatial and temporal diversification of the species of the genus *Chiton* and allies. This study, using integrative taxonomy, has allowed us to clarify the identification of many common and important intertidal chitons, but, as usual, the results propose as many new questions as answers.

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