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Comparative morphology of *Tonicia* (Polyplacophora) geographical ecotypes from Southeastern Pacific

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ABSTRACT. Here we present detailed morphological and molecular comparison of the *Tonicia* species and ecotypes along Southeastern Pacific coast. Several specimens were examined by scanning electron microscopy and based on plates morphology we propose a new taxonomic key for identification of species and ecotypes of *Tonicia*. A total of 566 specimens were measured to explore morphological differences between ecotypes and ecoregions by means of multivariate analyses. Genetic distances and a maximum likelihood (ML) phylogeny were estimated using Cytochrome Oxidase I (*cox1*) to compare species and ecotypes. Morphometry and multivariate analyses revealed morphological differences between ecotypes in each species. However, *cox1* genetic distances were low between ecotypes in both species (*T. calbucensis* and *T. chilensis*) and the ML phylogeny revealed an absence of monophyletic relationships between ecotypes in each species clades. The new key will be useful to identify *Tonicia* species by morphology without molecular analysis.

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Сравнительная морфология географических экотипов *Tonicia* (Polyplacophora) юго-восточной Пацифики

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РЕЗЮМЕ. Представлены подробное морфологическое сравнение и новый таксономический ключ для идентификации по морфологии видов и экотипов *Tonicia* вдоль юго-восточного побережья Тихого океана. Несколько образцов были подготовлены для сканирующей электронной микроскопии, и на основе морфологии пластин мы предложили новый таксономический ключ. В общей сложности было измерено 566 образцов для изучения морфологических различий между экотипами и экорегионами с помощью многомерного анализа. Морфометрия и многомерный анализ выявили морфологические различия между экотипами у каждого вида. Новый ключ будет полезен для идентификации видов *Tonicia* по морфологии без молекулярного анализа.

Introduction

Chitons of the genus *Tonicia* are difficult to identify by morphology, and several articles have failed to give a certain identification [e.g., Leloup, 1956; Aldea, Valdovinos, 2005; Araya, Araya, 2015; Guillén, Urteaga, 2019]. The taxonomic status of two common species in Southeastern Pacific (SEP) *Tonicia chilensis* (Frembly, 1827) and *Tonicia 'elegans'* (Frembly, 1827) has also remained poorly documented and controversial. For instance, various subspecies and forms have been associated with both species [Leloup, 1956; Kaas *et al.*, 2006; Schwabe *et al.*, 2006] based on variations in shell and perinotum colour patterns and the distribution of their aesthetes (shell eyes) [Ibáñez *et al.* 2019]. A recent article identified by morphological and molecular methods the actual diversity of *Tonicia* in South America and proposed a key for species identification [Ibáñez *et al.*, 2019]. However, this taxonomic key is useful to identify species, but two of them (*T. chilensis* and *T. calbucensis* Plate, 1898) found in Chile or Peru have morphologically different geographical ecotypes (northern and southern). As a result, it is not possible to identify the northern ecotype of *Tonicia calbucensis* and southern ecotype of *T. chilensis,* us-

Table 1. Sample size of each ecotype of *Tonicia* species by ecoregions of Southeastern Pacific.

Табл. 1. Размер выборок каждого экотипа видов *Tonicia* по экотипам юго-восточной Пацифики.

Species/Ecotypes	Central Peru	Humboldtian	Central Chile	Araucanian	Chiloense	Channels and Fjords	Total
Tonicia calbucensis northern ecotype	1	28	39	133			201
Tonicia calbucensis southern ecotype					13	17	30
Tonicia chilensis northern ecotype	13	7	105	114			239
Tonicia chilensis southern ecotype					41	55	96
Total	14	35	144	247	54	72	566

ing the key [Ibáñez *et al.*, 2019]. The *T. calbucensis* southern ecotype dwells from Puerto Montt to the Magellan Strait (40–53°S) and is also found in the Falkland Islands, while the *T. calbucensis* northern ecotype has a wide distribution from Arica (Chile, ~18°S) to the Golfo de Ancud (~41°S) (Fig. 1). The *T. chilensis* northern ecotype inhabits the coast of central Chile (~33 to 39°S) while *T. chilensis* southern ecotype is found from Puerto Montt (~41°S) to Tierra del Fuego (~53°S) [Ibáñez *et al.*, 2019] (Fig. 11). These forms have several notable differences of tegmentum sculpture and girdle features; as such we describe each form separately.

In this study we made a detailed morphological comparison and proposed an extension of the previous taxonomic key and several images to help in identification of ecotypes of *Tonicia* species from Southeastern Pacific. In addition, genetic distances were estimated and a maximum likelihood (ML) phylogeny was used to compare species and ecotypes.

Materials and methods

Field sampling and collecting specimens were performed by hand and Hookah or SCUBA diving at intertidal and subtidal zones respectively in Peru and Chile [for details see Ibáñez *et al.*, 2019]. Each sampling session was performed around peak hour of low tide. Some specimens collected in this study were deposited in the Zoological Institute, Russian Academy of Sciences. A tissue sample was cut from each specimen and later was preserved in 96% ethanol for molecular analysis, moreover 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, gridle scales and radula. The valves, perinotum and radula of some small specimens were dissected, mounted on tape, critical-point dried, coated under vacuum with gold and examined with a scanning electron microscope (SEM).

To analyze the morphological variation of each species and ecotypes of *Tonicia* at different ecoregions sampled (Table 1) the following 15 distance variables were measured on 566 ethanol preserved specimens through a calliper: total length (TL), total width (TW), length of plate I and plate VIII (the terminal shell plates), widths of each shell plate (I to VIII) and anterior, lateral and posterior length of perinotum (PL, PA, PP). We made a correction of body length by standardized width of each plate dividing each plate width by total length to compare shell plates across species and ecotypes to avoid allometric effects [Ibáñez *et al.*, 2018]. In addition, the ratio of length/width of plates I and VIII were included, compiling 16 standardized measurements.

A multivariate approach was employed to explore potential shifts in shape-space over ontogeny within each species, using Principal Component Analysis (PCA). As the dataset comprising 16 standardized measurements, the two first components (PC1 and PC2) reflect variation in shape trajectories [Shea, 1985]. Similarity between ecotypes and ecoregions [following Spalding *et al.*, 2007] morphology was evaluated with two-way PERMANOVA using Euclidean distances and 10,000 permutations [Anderson, 2001]. All statistical analyses were performed in PAST ver. 4.11 software [Hammer *et al.*, 2001].

Finally, to demonstrate the genetic differentiation among both species (*T. chilensis* and *T. calbucensis*) and between ecotypes, we calculated the genetic p-distance using Cytochrome Oxidase I (*cox1*) sequences from Ibáñez *et al.* [2019] in MEGA XI software [Tamura *et al.*, 2021]. A Maximum likelihood (ML) phylogeny was included to represent the evolutionary relationships between species and ecotypes at the same time. In short, the phylogenetic relationships of the *Tonicia* species and ecotypes were examined using a Maximum Likelihood reconstruction via the IQ-TREE online server [Trifinopoulos *et al.*, 2016] with hill-climbing NNI tree search strategy [Nguyen *et al.*, 2015]. Statistical support was estimated using 5,000 ultrafast bootstrap

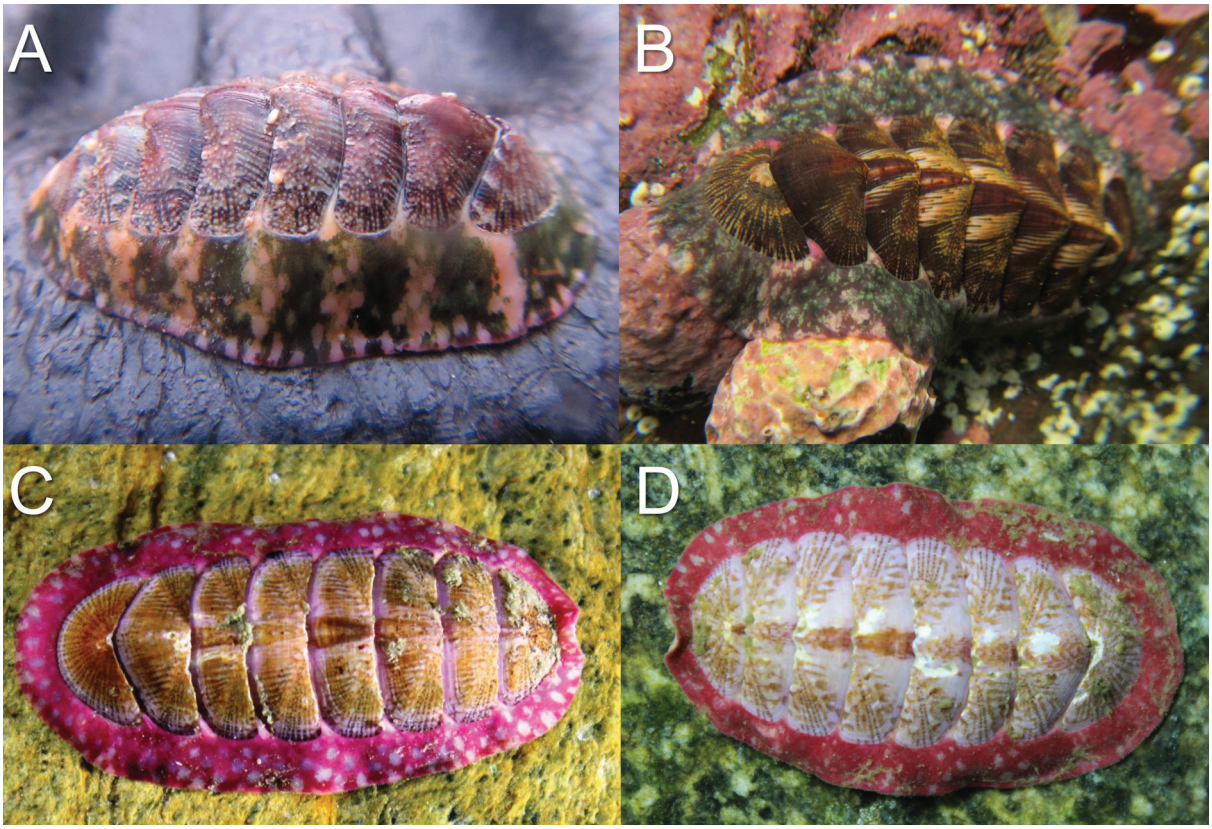

FIG. 1. Photos of live specimens of *Tonicia calbucensis.* **A.** Northern ecotype, Coquimbo (~29°S). **B.** Northern ecotype, Talcahuano (~36°S). **C.** Southern ecotype, Huinay (~41°S). **D.** Southern ecotype Valdivia (~39°S).

РИС. 1. Фотографии живых экземпляров *Tonicia calbucensis*. **A.** Северный экотип, Коквимбо (~29°S). **B.** Северный экотип, Талькахуано, (~36°S). **C.** Южный экотип, Уйнай (~41°S). **D.** Южный экотип, Вальдивия (~39°S).

replicates [Minh *et al.*, 2013]. The trees were rooted using the sister species *Tonicia fremblyana* (Frembly, 1827) as outgroup.

Abbreviations: BL, Body length. SEP, Southeastern Pacific. ZIN, Zoological Institute of Russian Academy of Sciences, St. Petersburg, Russia

Results

Below is a new key and photos of the four ecotypes: the southern and northern ecotypes of *Tonicia calbucensis* and *T. chilensis*. The figures show that, despite the close similarity of features of the girdle and radula in the same species of chiton from different areas, they have significant differences in the sculpture of a shell surface.

Tonicia calbucensis Plate, 1898

Complete synonymy in Ibáñez *et al.*, 2019.

Description of *T. calbucensis* southern ecotype (Figs 1C, D; 2–4)

The animals are of medium size, up to 40 mm long (Table 2). Valves subcarinated, side slopes minimally convex with low elevation (dorsal elevation 0.28). Tegmentum orange brown to brownish red with darker radial streaks on the terminal valves and lateral areas; central areas with streaks oriented longitudinally. Jugal area chestnut brown in general with two wider white streaks on both sides [after Schwabe *et al.*, 2006]. Specimens collected near Punta Huinay (42.36°S, 72.44°W) and Isla Lilihuapi (42.16°, 72.59°W) have typical coloration or have yellow orange tegmentums and girdles. Head valve semicircular, and the posterior margin widely V-shaped. Intermediate valves broadly rectangular, and anterior margin almost straight in the central part and somewhat slanting towards the rounded side margins. Posterior margin straight, not beaked, and lateral areas not raised. Tail valve small and slightly narrower than head valve, mucro antemedian, antemucronal, and post mucronal slopes straight. All areas of tegmentums smooth with exception of 1–4 weak, short, narrow grooves on both sides of the jugum and several growth lines and ocelli. Ocelli on radial streaks of lateral areas and terminal valves only, not numerous. Articulamentum well developed with wide and rather long apophyses connected by a slightly dentate jugal plate. Slit formula 8/1/8,

Table 2. Mean of morphometric measurements (mm) of 16 morphological traits of each ecotype of *Tonicia* species. Total length (TL), total width (TW), length of plate I and plate VIII (LI and LVIII), widths of each shell plate (WI to WVIII) and anterior, lateral and posterior length of perinotum (PL, PA, PP).

Табл. 2. Средние значения морфометрических измерений (мм) 16 морфологических признаков каждого экотипа видов *Tonicia*. Общая длина (TL), общая ширина (TW), длина щитка I и щитка VIII (LI и LVIII), ширина каждого щитка раковины (WI до WVIII) и передняя, латеральная и задняя длина перинотума (PL, PA, PP).

Traits	*Tonicia calbucensis* Northern Ecotype	*Tonicia calbucensis* Southern Ecotype	*Tonicia chilensis* Northern Ecotype	*Tonicia chilensis* Southern Ecotype
TL	32.78	28.40	39.62	40.96
LI	4.29	3.27	4.97	5.26
LVIII	3.95	3.20	4.53	5.16
TW	18.55	12.93	21.19	21.83
WI	9.76	8.23	10.82	12.05
WII	11.22	9.37	12.62	13.55
WIII	12.56	10.47	14.31	15.06
WIV	13.40	10.80	15.34	16.08
WV	13.42	10.90	15.50	16.25
WVI	12.86	10.30	14.84	15.64
WVII	11.50	9.30	13.27	13.83
WVIII	8.82	7.33	9.92	11.23
PL	5.15	3.20	6.86	6.49
PA	2.99	1.93	3.90	3.73
PP	2.21	1.40	2.98	2.71

and teeth strongly pectinated on the outside. Girdle rather wide and leathery, usually rose with spots or rare orange in specimens with orange tegmentum, dorsally covered with rare small elongate sharp-pointed spicules and rare short bristles. Ventral side with radial rows of squarish scales. Specimens with a body length ~20 mm have a ~7.5 mm long radula, with 43 transverse rows of mature teeth. Central tooth narrows with a long curved blade. Major lateral tooth with unicuspid rounded head. From valve II to valve VIII, there are 34 gills on each side of specimens with body lengths of ~20 mm.

Description of *Tonicia calbucensis* northern ecotype (Figs 1A, B; 5–10)

Body length medium sized, up to 50 mm, elongated and oval with low elevation (dorsal elevation 0.24–0.26). Valves minimally beaked (Table 2). Tegmentum very variable in color and streaked with various combinations of dark brown, buff, yellow, pale, reddish brown, and wine red. Tegmentum and girdle are both rare uniform orange in alive specimens. Head valve semicircular, widely V-shaped posterior margin. Intermediate valves broadly rectangular. Ratio of the width of valve V to its length is 2.7–3.0. Anterior margin slightly concave in the jugal area and slanted at the pleural. Posterior margin weakly concave at both sides of the prominent pointed apex. Tail valve slightly less wide than the head valve. Antemedian portion of mucro not raised, antemucronal slope straight or slightly convex and postmucronal slope concave. Tegmentum sculptured with numerous granules or pustules and longitudinal grooves. Head valve, lateral area, pleural area, and postmucronal area sculptured with small granules or pustules. Pleural area with low granules arranged in longitudinal rows, become obsolete in some specimens. Both sides of the jugum have 1–3 longitudinal grooves that reach the anterior margin. Articulamentum white with all valves having a reddish spot in the apical area and in the center of the valve. Apophyses wide, rounded, and connected to the dentate jugal plate. Slit formula is 8/1, rarely 2/9–10. Slit rays are hardly noticeable. Girdle moderately wide and spotted in live specimens, uniformly yellow in fixed specimens. Girdle dorsally beset with few calcareous spicules up to 34 x 16 µm. Ventral side of the girdle covered with radiating rows of subquadrangular scales 35 x 17 µm. Radula of specimens with body lengths of 17.5 mm are 6.7 mm long, with 40 transverse rows of mature teeth. Central tooth narrows to a long curved blade, major lateral tooth has a unicuspid rounded head. There are 43 gills on each side of specimens with body lengths of 17.5 mm; spanning from valve II to valve VIII.

There are intermediate forms with a coloration similar to that of the northern ecotype, but with rare

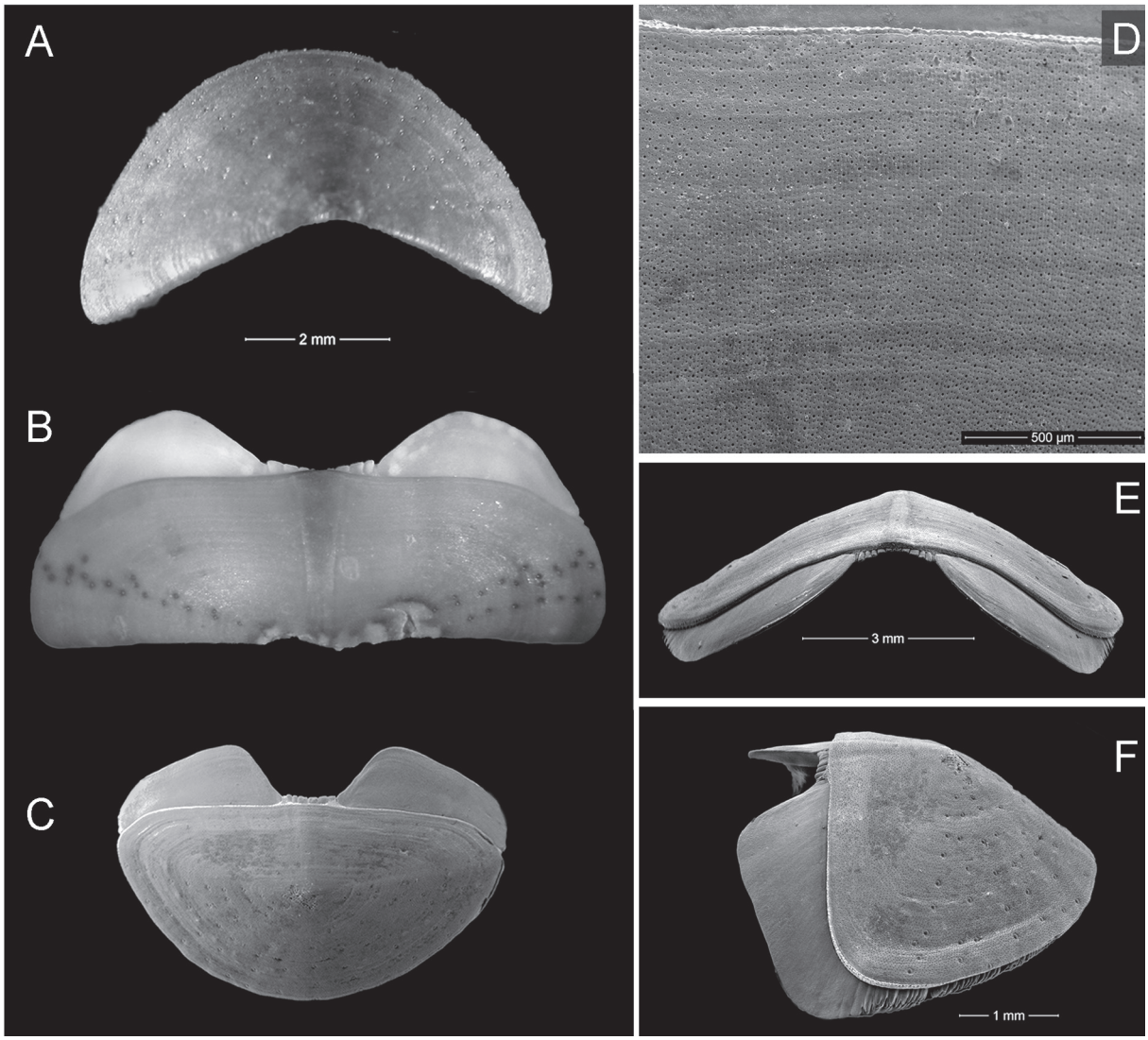

FIG. 2. *Tonicia calbucensis*, southern ecotype, South Chile, 42°22'S, 72°25'W, 5–20 m, BL 20.5 mm, 04.01.2005, leg. B. Sirenko. **A.** Valve I, dorsal view. **B.** Valve V, dorsal view. **C.** Valve VIII, dorsal view. **D.** Valve VII, detail of tegmentum in central area. **E.** Valve VII, rostral view. **F.** Valve VIII, lateral view.

РИС. 2. *Tonicia calbucensis*, южный экотип, южное Чили, 42°22'S, 72°25'W, 5–20 м, BL 20,5 мм, 04.01.2005, собрал B. Sirenko. **A.** Головной щиток вид сверху. **B.** Щиток V, вид сверху. **C.** Щиток VIII, вид сверху. **D.** Щиток VII, деталь тегментума в центральном поле. **E.** Щиток VII, вид спереди. **F.** Щиток VIII, вид сбоку.

grains on tegmentum, as well as with an almost completely smooth shell in Metri Bay and Isla Lilihuapi, Golfo de Ancud.

The northern ecotype differs from the southern ecotype of *Tonicia calbucensis* by having smooth tegmentum without granules except several narrow, short, weak longitudinal grooves on both sides of the jugum and does not have bristles.

Tonicia chilensis (Frembly, 1827).

Complete synonymy in Ibáñez *et al.*, 2019.

Description of *Tonicia chilensis* northern ecotype (Figs 11A, B; 12–14)

Body large sized, up to 81 mm in length (Table 2), oval, minimally elevated (dorsal elevation 0.25), rounded or slightly subcarinated. Side slopes straight, valves are slightly beaked in young specimens. Tegmentum streaked with various combinations of dark brown, yellow, reddish brown, black and red in small specimens with body lengths up to 20–25 mm. Large individuals uniformly black. Head valve semicircular, hind margin slightly concave. Tegmentum sculptured with low pustules arranged without a definite pattern. Growth lines noticeable. Intermediate valves broadly rectangular, front margin slightly concave in jugum and convex in pleural parts. Hind margin weakly concave on both sides of the prominent pointed apex. Lateral area slightly raised and sculptured like the head valve. Central area with 10 or more longitudinal ribs in the central part of the

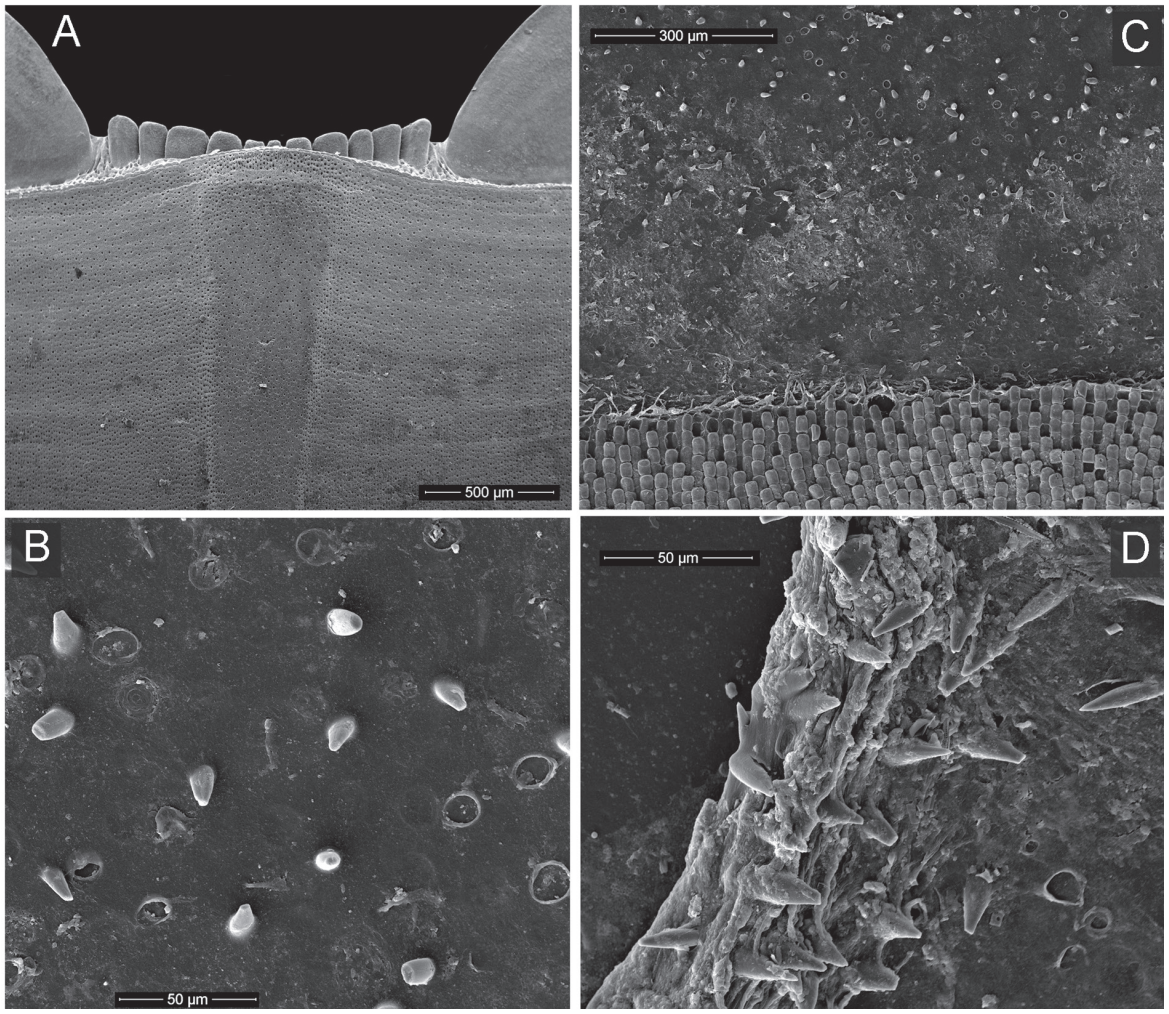

FIG. 3. *Tonicia calbucensis*, southern ecotype, South Chile, 42°22’S, 72°25’W, 5–20 m, BL 20.5 mm, 04.01.2005, leg. B. Sirenko. **A.** Valve VII, jugal area. **B, D**. Dorsal spicules. **C.** Dorsal, marginal and ventral spicules.

РИС. 3. *Tonicia calbucensis*, южный экотип, южное Чили, 42°22’S, 72°25’W, 5–20 м, BL 20,5 мм, 04.01.2005, собрал B. Sirenko. **A.** Щиток VII, югальное поле. **B, D.** Дорсальные спикулы. **C.** Дорсальные, маргинальные и вентральные спикулы.

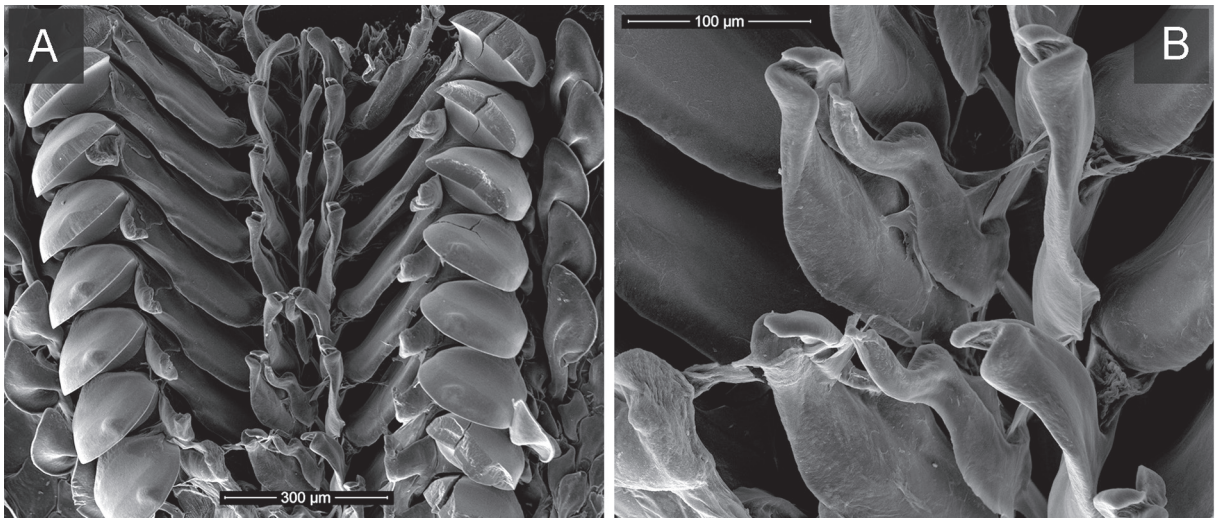

FIG. 4. *Tonicia calbucensis*, southern ecotype, South Chile, 42°22’S, 72°25’W, 5–20 m, BL 20.5 mm, 04.01.2005, leg. B. Sirenko. **A.** Radula. **B.** Central and first lateral teeth of radula.

РИС. 4. *Tonicia calbucensis*, южный экотип, южное Чили, 42°22’S, 72°25’W, 5–20 м, BL 20,5 мм, 04.01.2005, собрал B. Sirenko. **A.** Радула. **B.** Центральные, первые латеральные зубы радулы.

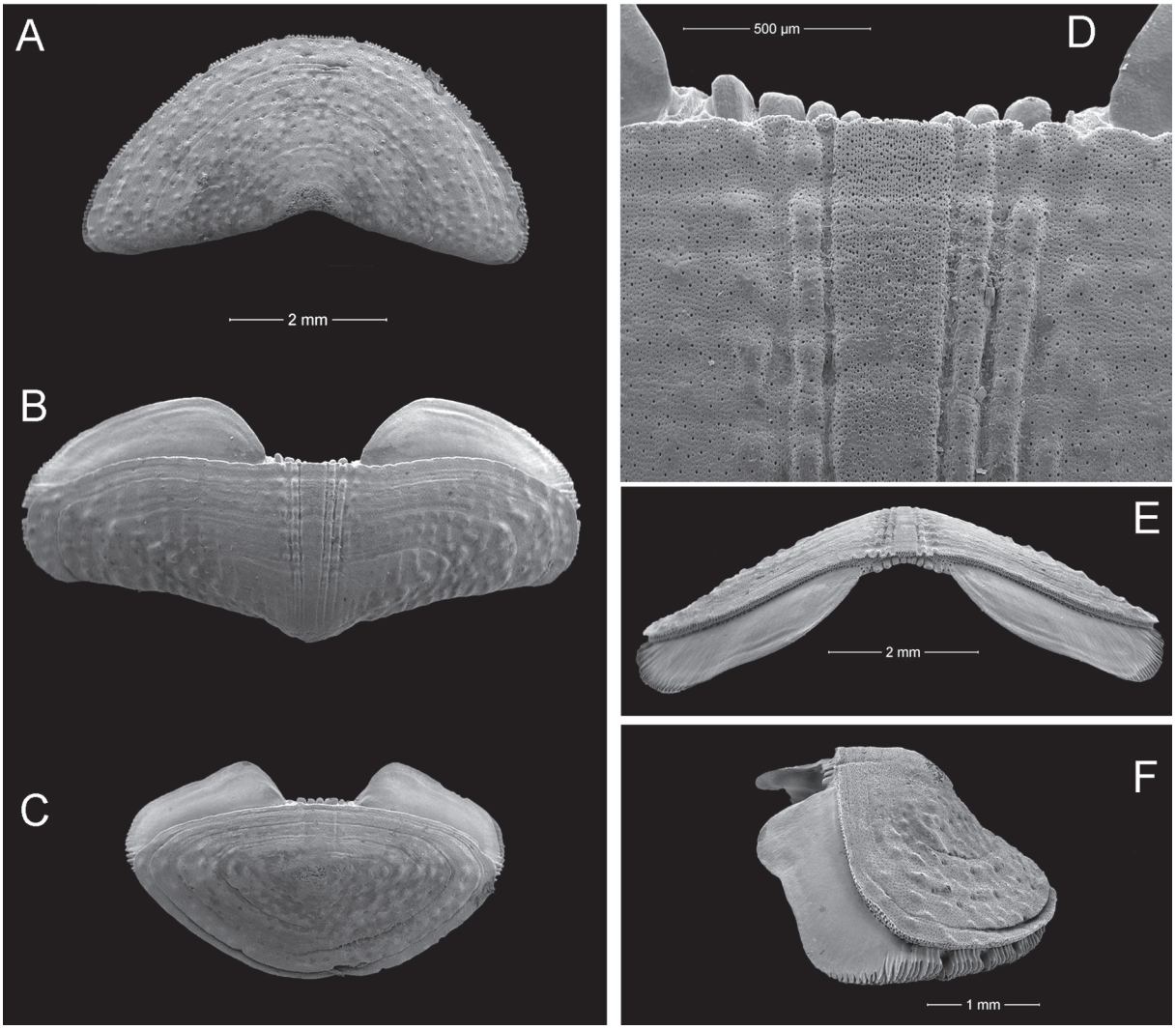

FIG. 5. *Tonicia calbucensis*, northern ecotype, Chile, La Mission, intertidal, BL 17.5 mm, 30.12.2004, leg B. Sirenko. **A.** Valve I, dorsal view. **B.** Valve VI, dorsal view. **C.** Valve VIII, dorsal view. **D.** Valve VI, jugal area. **E.** Valve VI, rostral view. **F.** Valve VIII, lateral view.

РИС. 5. *Tonicia calbucensis*, северный экотип, Чили, Ла Мисьон, литораль, BL 17,5 мм, 30.12.2004, собрал B. Sirenko. **A.** Головной щиток вид сверху. **B.** Щиток VI, вид сверху. **C.** Щиток VIII, вид сверху. **D.** Щиток VI, .югальное поле. **E.** Щиток VI, вид спереди. **F.** Щиток VIII, вид сбоку.

valve. Several low pustules run diagonally between the central and lateral area of small specimens. Tail valve is smaller than the head valve. The front margin is concave. Antemedian portion of mucro not raised, antemucronal area sculptured like the central area and postmucronal area sculptured like the head valve. Articulamentum white with lighter or dark reddish-brown parts in the apical area. Apophyses are wide and rather long. Outer half of the apophyses and teeth sharply pectinated. Sinus has a short, dentate (less 15 denticles) jugal plate. Slit formula is 8/1/10. Girdle rather wide and spotted in young live specimens, black in adult specimens. After fixation, girdle becomes dirty yellow in color and is dorsally covered with small, flattened spicules up to 23 x 18 µm. Marginal spicules twice as large as the dorsal spicules. Ventral side covered with radiating rows of subquadragular scales. The radula of a chiton with body length of 20.6 mm is 7.1 mm long and has 41 transverse rows of mature teeth. The central tooth is long with a curved blade. The major lateral tooth is rounded with a unidentate cusp. Fifty-three gills per side, extending from valve II to valve VIII in the same specimen.

Description of *Tonicia chilensis* southern ecotype (Figs 11 C, D; 15–16)

Body large sized, up to 77 mm in length, elongated, oval (Table 2) with solid valves moderately elevated (dorsal elevation 0.30–0.35), subcarinated and not beaked. Tegmentum color variable, from dark

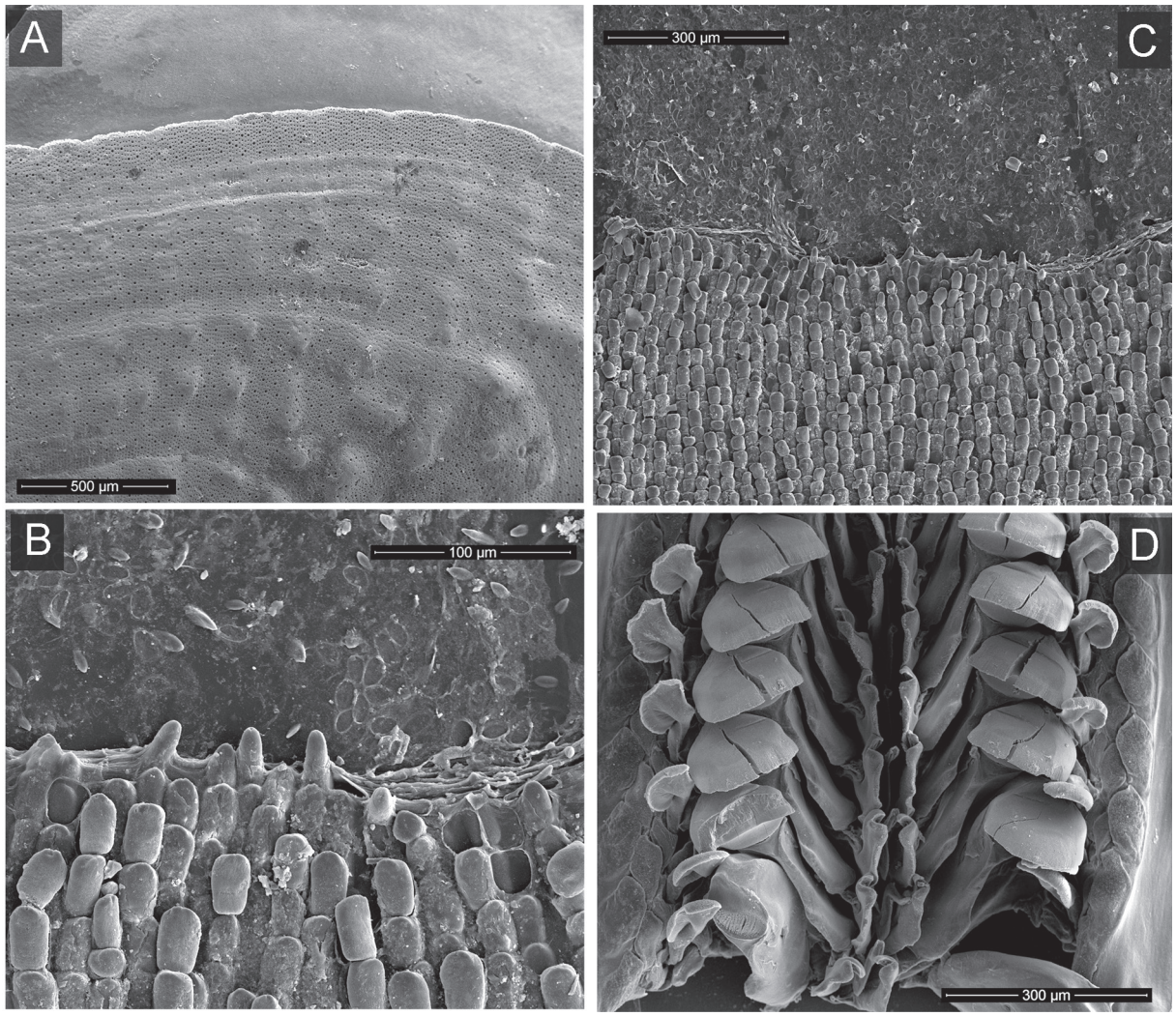

FIG. 6. *Tonicia calbucensis*, northern ecotype, Chile, La Mission, intertidal, BL 17.5 mm, 30.12.2004, leg. B. Sirenko. **A.** Valve VI, pleural area. **B, C.** Dorsal, marginal and ventral spicules. **D.** Radula.

РИС. 6. *Tonicia calbucensis*, северный экотип, Чили, Ла Мисьон, литораль, BL 17,5 мм, 30.12.2004, собрал B. Sirenko. **A.** Щиток VI, плевральное поле. **B, C.** Дорсальные, маргинальные и вентральные спикулы. **D.** Радула.

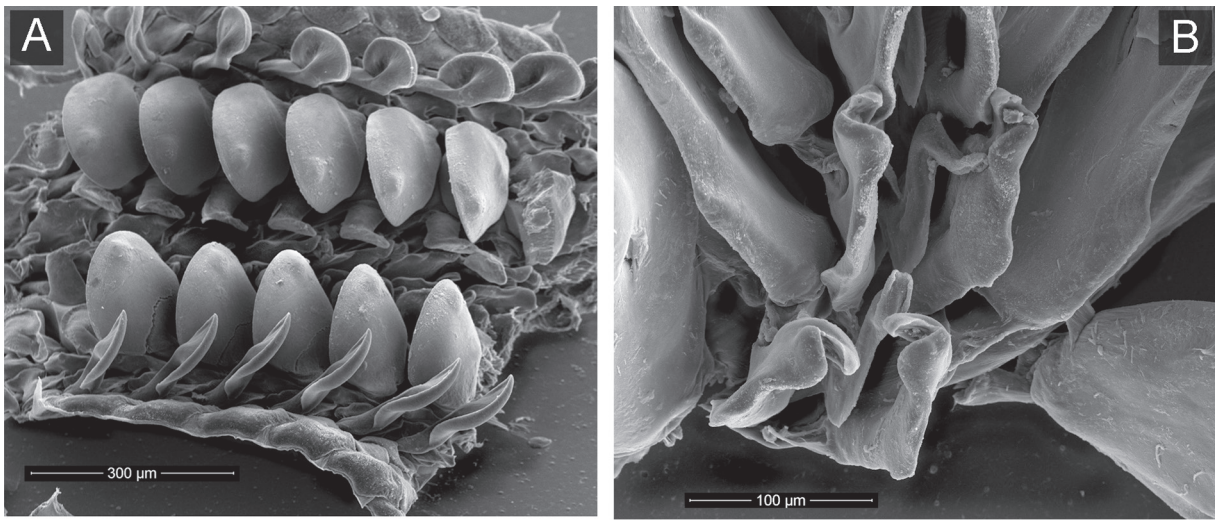

FIG. 7. *Tonicia calbucensis*, northern ecotype, Chile, La Mission, intertidal, BL 17.5 mm, 30.12.2004, leg. B. Sirenko. **A.** Radula. **B.** Central and first lateral teeth of radula.

РИС. 7. *Tonicia calbucensis*, северный экотип, Чили, Ла Мисьон, литораль, BL 17,5 мм, 30.12.2004, собрал B. Sirenko. **A.** Радула. **B.** Центральные и первые латеральные зубы радулы.

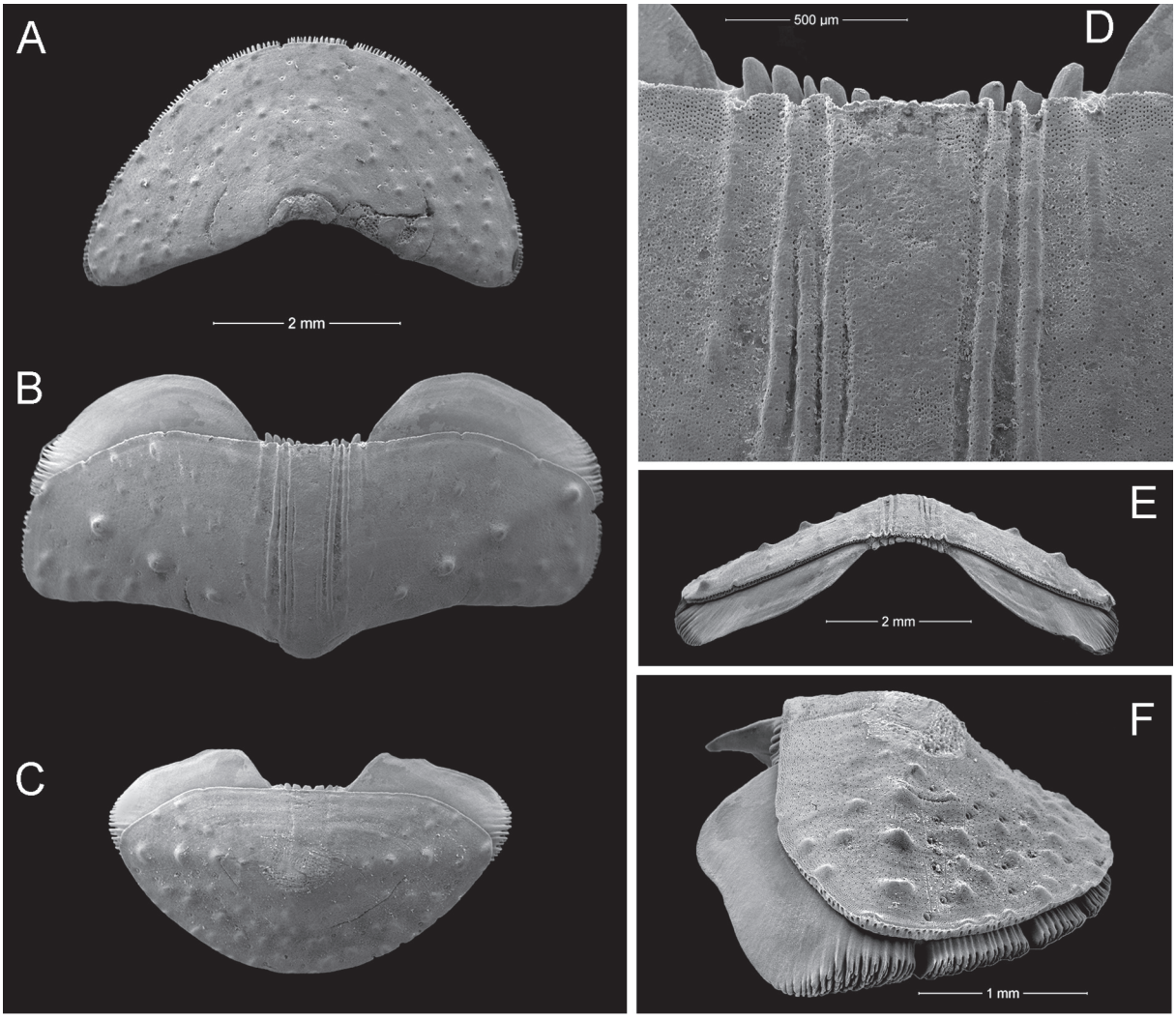

FIG. 8. *Tonicia calbucensis*, northern ecotype, Chile, Las Cruces, intertidal, BL 16.9 mm, 18.11.2008, leg. B. Sirenko. **A.** Valve I, dorsal view. **B.** Valve VI, dorsal view. **C.** Valve VIII, dorsal view. **D.** Valve VI, jugal area. **E.** Valve VI, rostral view. **F.** Valve VIII, lateral view.

РИС. 8. *Tonicia calbucensis*, северный экотип, Чили, Лас Крусес, литораль, BL 16,9 мм, 18.11.2008, собрал B. Sirenko. **A.** Головной щиток вид сверху. **B.** Щиток VI, вид сверху. **C.** Щиток VIII, вид сверху. **D.** Щиток VI, .югальное поле. **E.** Щиток VI, вид спереди. **F.** Щиток VIII, вид сбоку.

green to blackish, often with pale greyish blotches or streaks; occasionally with some valves orange, and black in others. Head valve semicircular, hind margin widely V-shaped. Tegmentum has rare, very small granules, and most of these are arranged near the hind margin. Growth lines evident. Intermediate valves solid and broadly rectangular. Anterior margin almost straight in the central part, distinctly slants towards the rounded side margins. Hind margin concave at both sides of the slightly protruding apex. Lateral area not raised and sculptured like the head valve. Ocelli numerous and arranged in a random manner. Central area almost smooth or vaguely granulose. When present, small granules sometimes coalesce in short wrinkles in front of diagonal lines. Dorsal ridge very low and narrow, with a smooth keel in the middle and 1–5 longitudinal grooves on both sides. Tail valve as wide as the head valve or slightly narrower, front margin convex, mucro elevated subcentral or antemedian, elevated, antemucronal area sculptured like the central areas, postmucronal area sculptured like the head valve. Articulamentum white and blotched with brown in the central part of the valves. Apophyses large, rather wide, rounded, and connected across the narrow jugal sinus by a long dentate plate (13–24 denticles). Slit formula 7–9/1/9–13. Teeth short and deeply pectinated on the upper side and outer edge. Very wide girdle (ratio of the width of valve V to the width of the girdle is 2.1–2.7), nude to the naked eye, and velveteen. Dorsal side of the girdle black or dark green in live specimens and dirty yellow after fixation. Girdle covered with numerous short bristles and small light, striated, flattened spicules up to 42 µm in length.

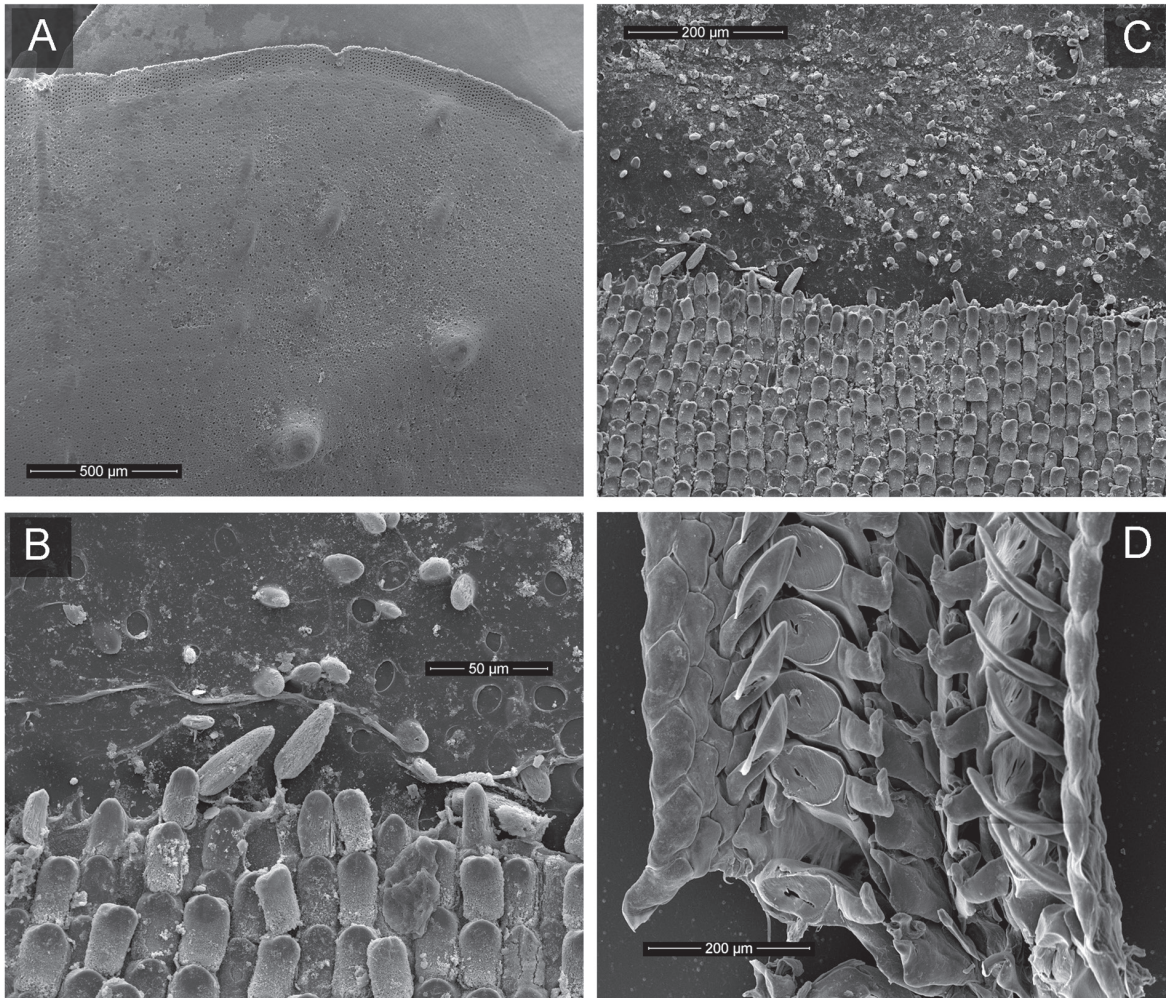

FIG. 9. *Tonicia calbucensis*, northern ecotype, Chile, Las Cruces, intertidal, BL 16.9 mm, 18.11.2008, leg. B. Sirenko. **A.** Valve VI, pleural area. **B, C.** Dorsal, marginal and ventral spicules. **D.** Radula.

РИС. 9. *Tonicia calbucensis*, северный экотип, Чили, Лас Крусес, литораль, BL 16,9 мм, 18.11.2008, собрал B. Sirenko. **A.** Щиток VI, плевральное поле. **B, C.** Дорсальные, маргинальные и вентральные спикулы. **D.** Радула.

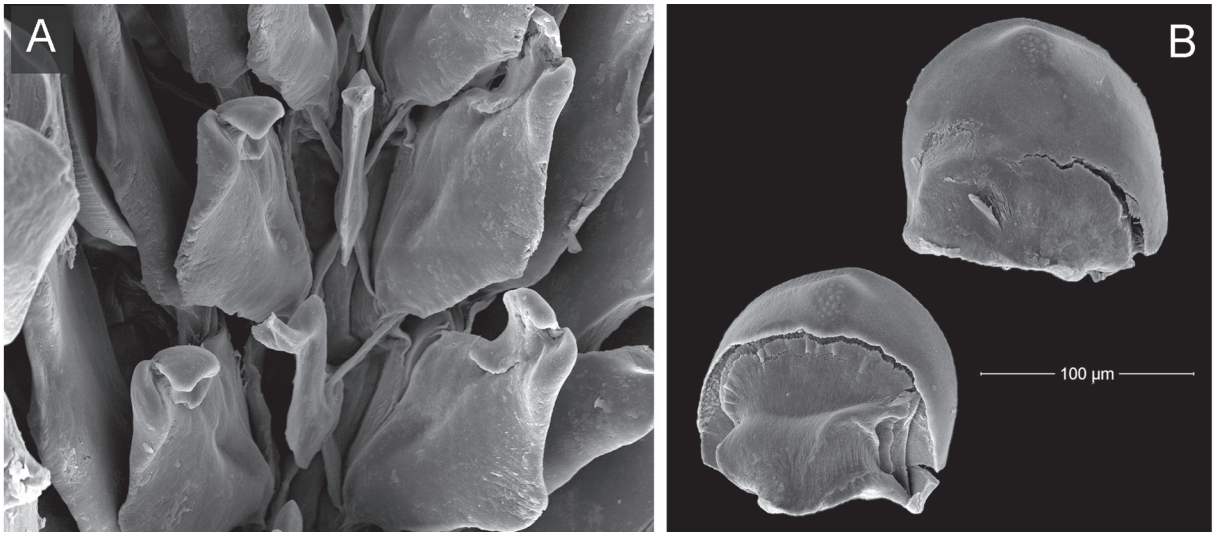

FIG. 10. *Tonicia calbucensis*, northern ecotype, Chile, Las Cruces, intertidal, BL 16.9 mm, 18.11.2008, leg. B. Sirenko. **A.** Central and first lateral teeth of radula. **B.** Head of major lateral tooth of radula.

РИС. 10. *Tonicia calbucensis*, северный экотип, Чили, Лас Крусес, литораль BL 16,9 мм, 18.11.2008, собрал B. Sirenko. **A.** Центральные и первые латеральные зубы радулы. **B.** Наконечник крючковой пластинки радулы.

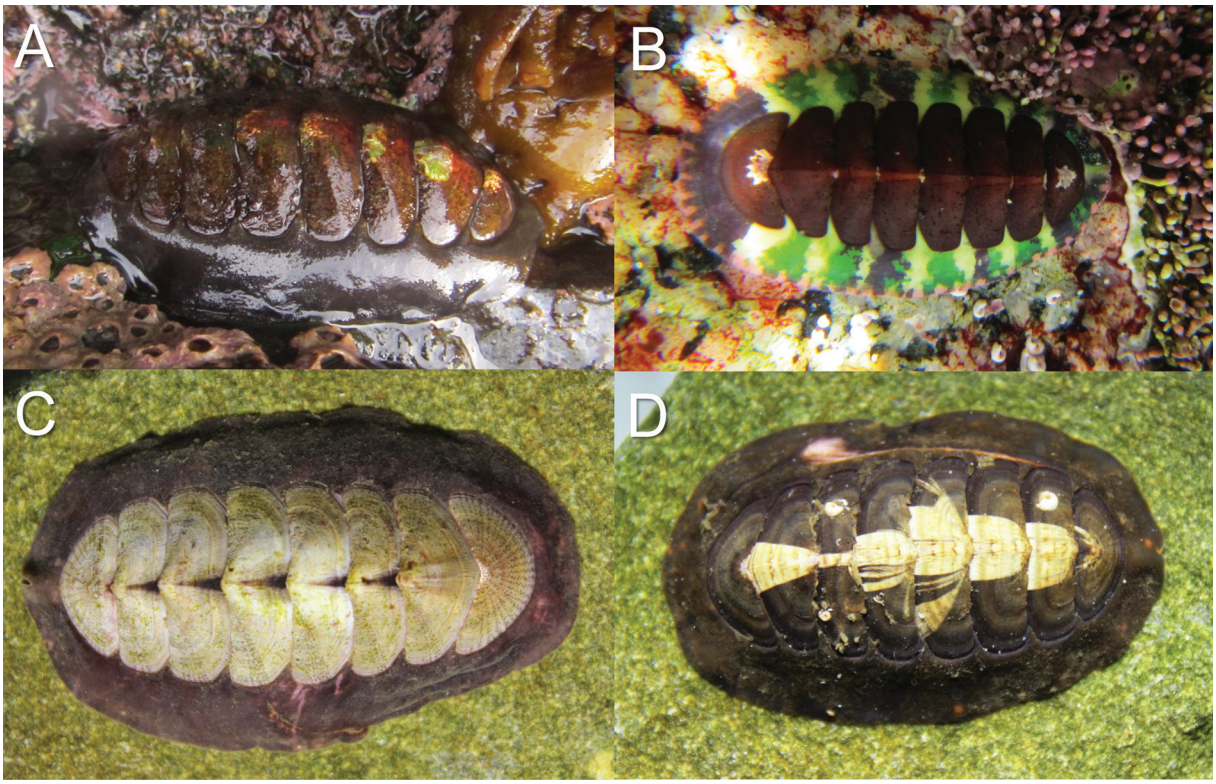

FIG. 11. Photos of live specimens of *Tonicia chilensis.* **A.** Northern ecotype, Ventanas (~33°S). **B.** Northern ecotype, Los Vilos (~32°S). **C.** Southern ecotype, Huinay (~41°S). **D.** Southern ecotype Valdivia (~39°S).

РИС. 11. Фотографии живых экземпляров *Tonicia chilensis*. **A.** Северный экотип, Вентанас (~33°S); **B.** Северный экотип, Лос Вилос (~36°S); **C.** Южный экотип, Уйнай (~41°S); **D.** Южный экотип, Вальдивия (~39°S).

Marginal edge with short bristles and rare spicules that are longer than the dorsal spicules. Ventral side of the girdle with radiating rows of rectangular scales up to 40 μm in length. Major lateral tooth of the radula has a unicuspid roundish head. Gills spanning from valve II to valve VIII, 38 on each side of a specimens with body length of 44 mm.

Adults of the northern ecotype of *Tonicia chilensis* inhabit the intertidal zone in areas with a fairly strong surf, while its juveniles live in the sublittoral, among red calcareous algae and the color of the tegmentum of the juveniles is light with redish-brown spots that hides them against the background of red algae. Apparently, as the chitons of this ecotype grow older, they move to the intertidal zone, where the tegmentum and the strongly expanded girdle become black. The low shell and the expanded belt of large chitons help to retain them during strong surf. Unlike the northern ecotype, all individuals of the southern ecotype of *T. chilensis* live at depths of 0–36 m [Schwabe, 2009] in bays and fjords with a calmer wave action. There is no age segregation in their settlements.

The southern ecotype of *Tonicia chilensis* differs from the northern ecotype of this species by having 1–5 narrow, longitudinal grooves in both sides of the jugum (vs. 10 or more grooves in both sides of the jugum in the northern ecotypes, ratio of the width of valve V to its length is 1.8 or more (vs. usually less 1.8 in the northern ecotypes). Moreover the girdle of the southern ecotype of *Tonicia chilensis* have longer and more numerous bristles.

Taxonomic key for identification of species and ecotypes of genus *Tonicia* from Southeastern Pacific

1(2) Girdle connects between valves. Cusp of major lateral teeth of radula is bidentate. Color of tegmentum is chestnut with blue spots.....*Tonicia disjuncta* (Frembly, 1827)

2(1) Girdle does not connect between valves. Cusp of major lateral teeth of radula is unidentate. Tegmentum with no blue spots.

3(4) Color of sutural laminae is brown. Tegmentum is smooth without longitudinal grooves on both sides of the jugum.…....…… *Tonicia lebruni* Rochebrune, 1884

4(3) Color of sutural laminae is white. Tegmentum is granulated or rare smooth with grooves near the jugal area.

5(6) The head valve, lateral, and postmucronal areas have radiating ribs. The ratio of the width of valve V to its length is 3.4–3.9. The ratio of the length of the postmucronal area to the length of the antemucronal area of the tail valve is 2.5–3.0...*Tonicia swainsoni* (Sowerby, 1832)

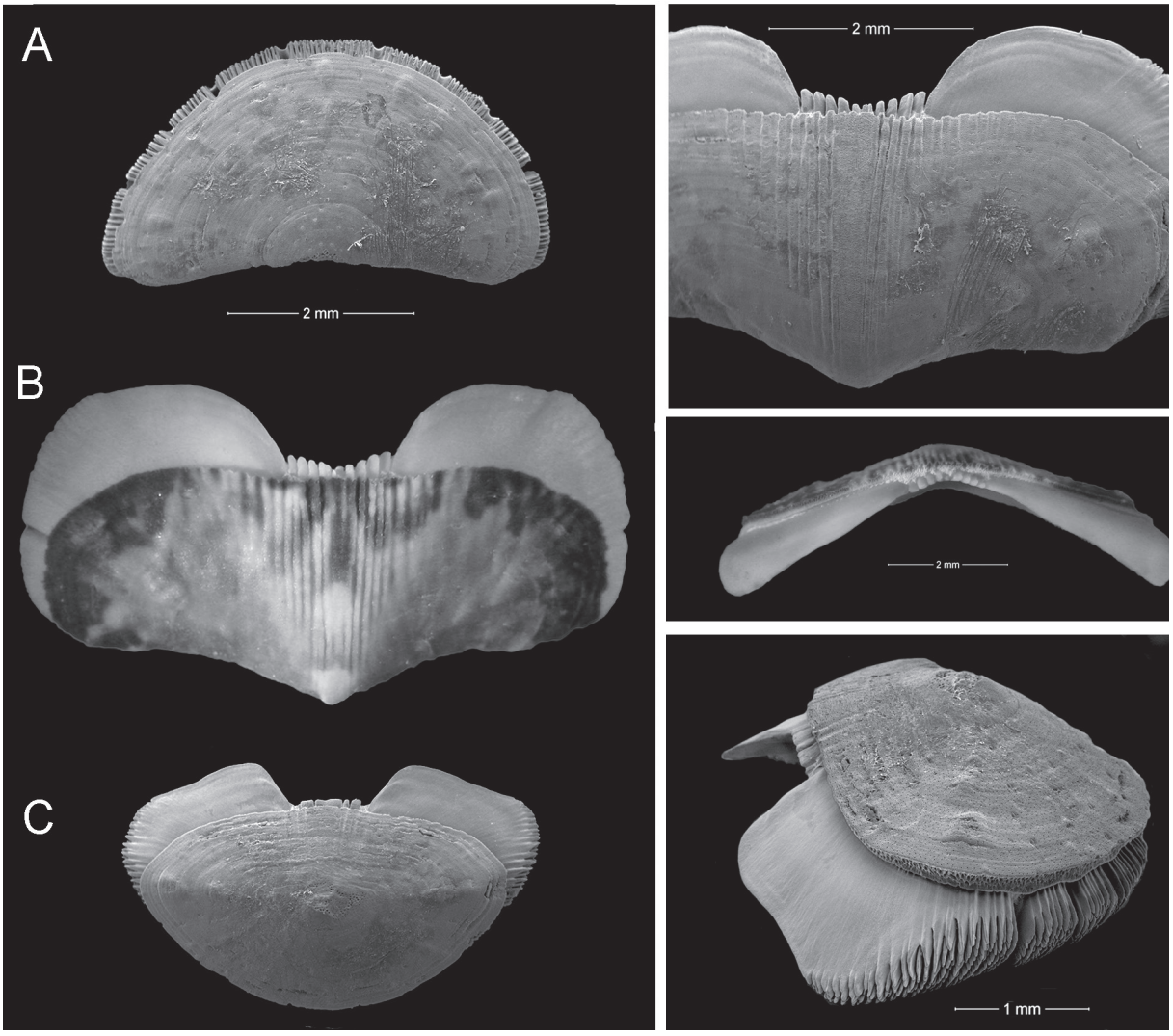

FIG. 12. *Tonicia chilensis*, northern ecotype, Chile, Calfuco, intertidal, BL 20.6 mm, 16.01.2005, leg. B. Sirenko. **A.** Valve I, dorsal view. **B.** Valve V, dorsal view. **C.** Valve VIII, dorsal view. **D.** Valve VII, dorsal view. **E.** Valve V, rostral view. **F.** Valve VIII, lateral view.

РИС. 12. *Tonicia chilensis*, северный экотип, Чили, Калфуко, литораль BL 20,6 мм, 16.01.2005, собрал B. Sirenko. **A.** Головной щиток вид сверху. **B.** Щиток V, вид сверху. **C.** Щиток VIII, вид сверху. **D.** Щиток VII, вид сверху. **E.** Щиток V, вид спереди. **F.** Щиток VIII, вид сбоку.

6(5) Radiating ribs on the head valve, lateral, and postmucronal areas are absent. The ratio of the width of valve V to its length is less than 3.0. The ratio of the length of the postmucronal area to the length of the antemucronal area of the tail valve is 1.9 or less.

7(8) The tegmentum is smooth without granules or pustules except several narrow, short, weak longitudinal grooves on both sides of the jugum ….....…….....
.....*Tonicia calbucensis* southern ecotype

8(7) The tegmentum has grooves, granules or pustules.

9(10) All valves or part of them are colored with numerous undulated concentric lines. The ratio of the length of the postmucronal area to the length of the antemucronal area of the tail valve is 1.9. Both sides of the jugum have few short narrow, bent grooves ….....
.....*Tonicia fremblyana* Kaas, 1957

10(9) The color of the valves is variable. The ratio of the length of the postmucronal area to the length of the antemucronal area of the tail valve is 1.8 or less. Both sides of the jugum have few or numerous long grooves.

11(12) Both sides of the jugum have 10 or more narrow longitudinal grooves……*Tonicia chilensis* northern ecotype

12(11) Both sides of the jugum have 1-5 narrow, longitudinal grooves.

13(14) The ratio of the width of valve V to its length is 1.8–2.1. Tegmentum is very minutely granulose. Perinotum has numerous short bristles .....……..…
.....*Tonicia chilensis* southern ecotype

14(13) The ratio of the width of valve V to its length is 2.3–2.8. Tegmentum has granules of medium size. Perinotum does not have bristles.....
.....*Tonicia calbucensis* northern ecotype

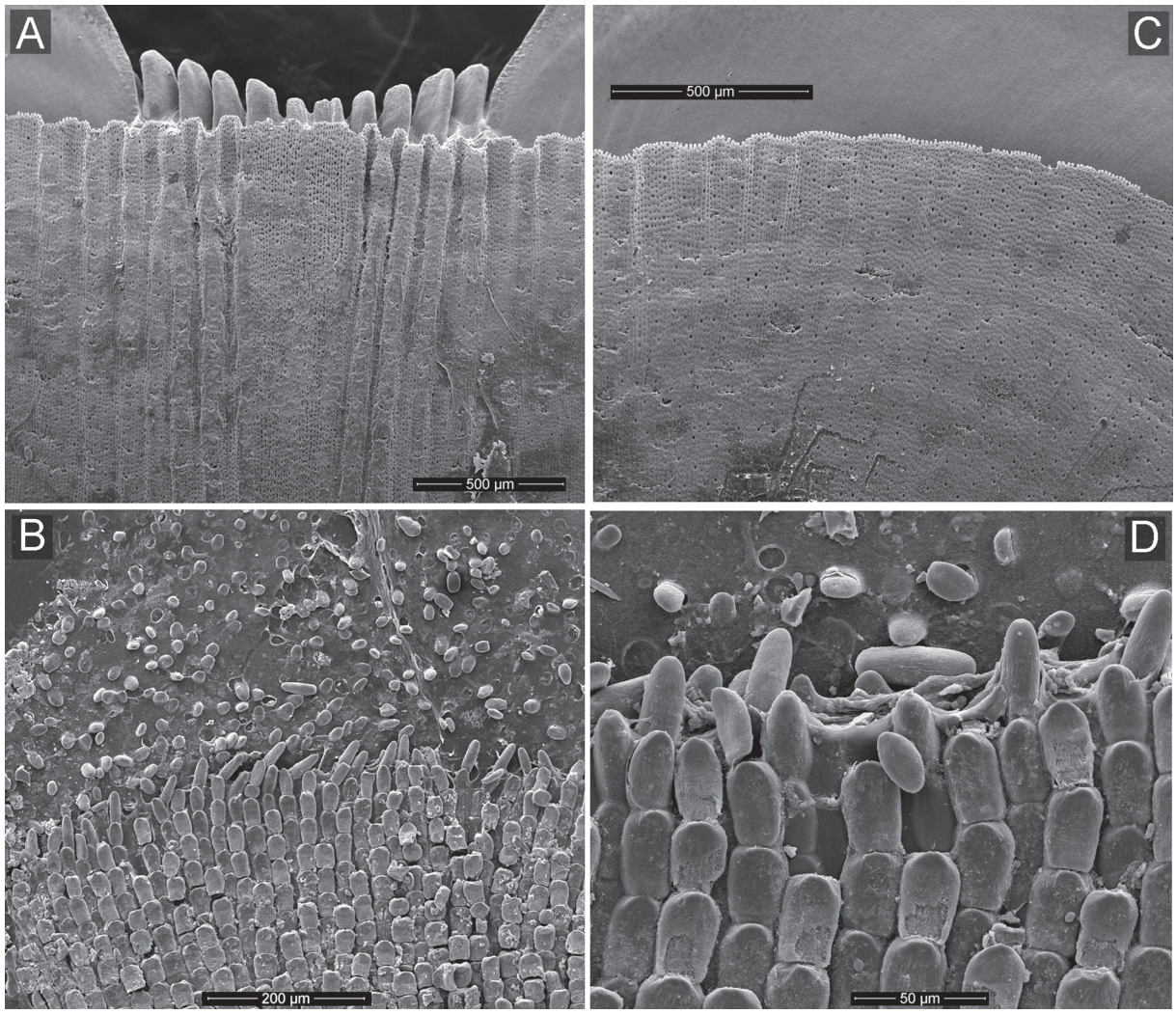

FIG. 13. *Tonicia chilensis*, northern ecotype, Chile, Calfuco, intertidal, BL 20.6 mm, 16.01.2005, leg. B. Sirenko. **A.** Valve VII, jugal area. **B, D.** Dorsal, marginal and ventral spicules. **C.** Valve VII, pleural area.

РИС. 13. *Tonicia chilensis*, северный экотип, Чили, Калфуко, литораль BL 20,6 мм, 16.01.2005, собрал B. Sirenko. **A.** Щиток VII, югальное поле. **B, D.**Дорсальные, маргинальные и вентральные спикулы. **C.** Щиток VII, плевральное поле.

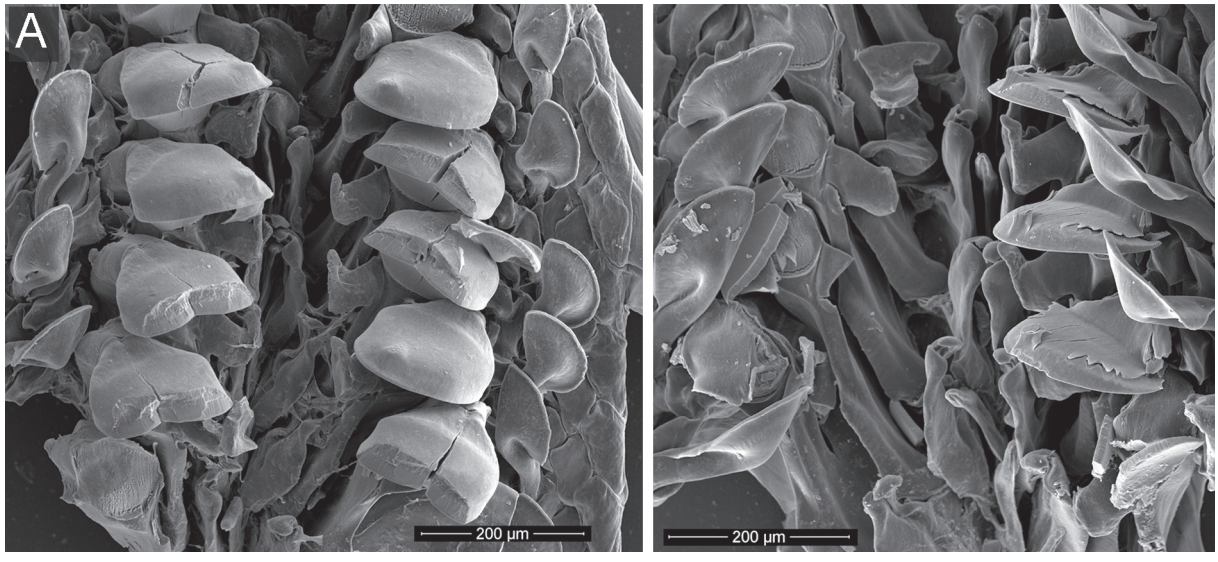

FIG. 14. *Tonicia chilensis*, northern ecotype, Chile, Calfuco, intertidal, BL 20.6 mm, 16.01.2005, leg. B. Sirenko. **A, B.** Radula.

РИС. 14. *Tonicia chilensis*, северный экотип, Чили, Калфуко, литораль BL 20,6 мм, 16.01.2005, собрал B. Sirenko. **A, B.** Радула.

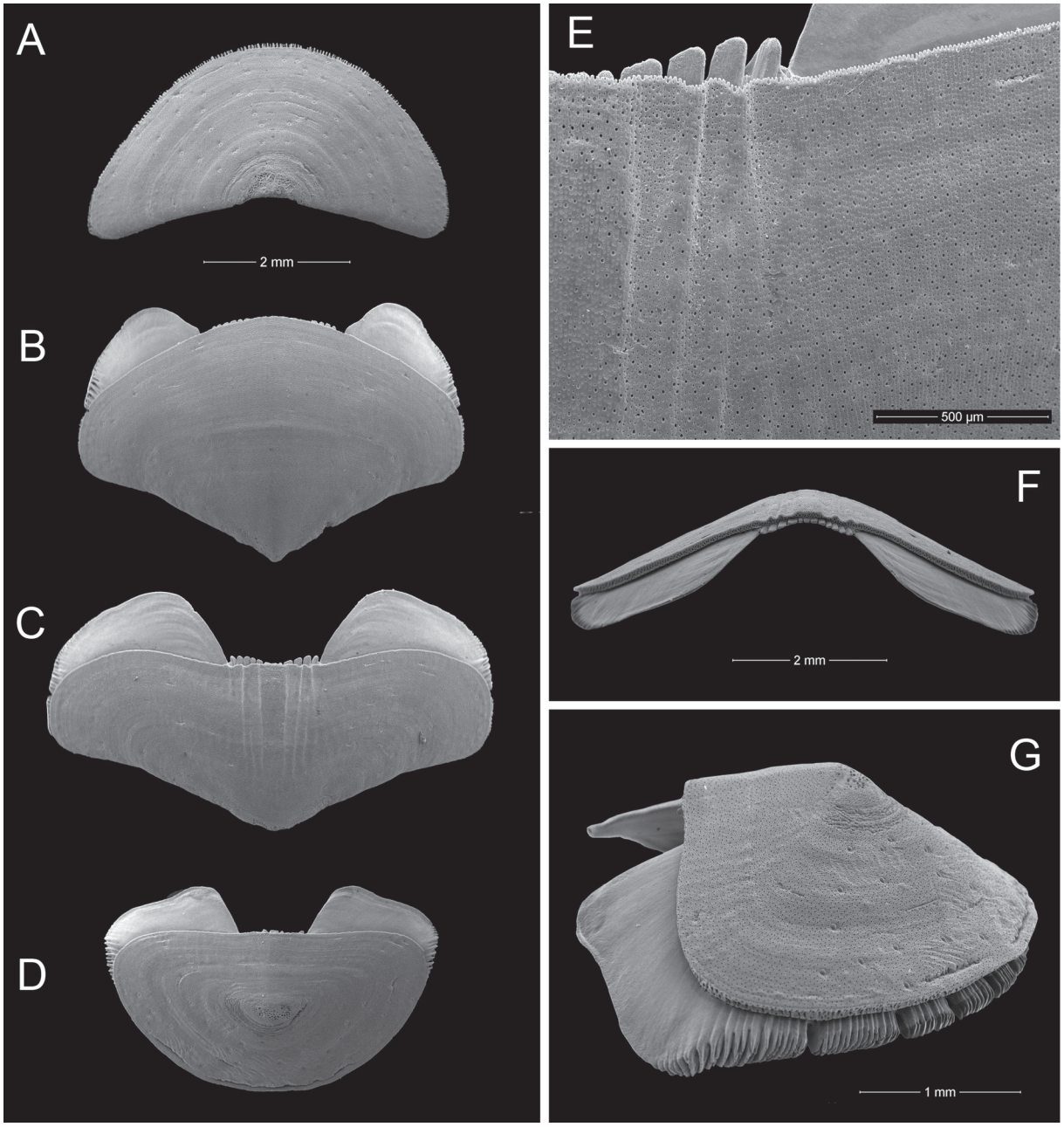

FIG. 15. *Tonicia chilensis*, southern ecotype, Chile, Magellan Strait, 53°37'S, 70°56'W, 0.5–3.0 m, BL 18.5 mm, 18.05.2000, leg. B. Sirenko. **A.** Valve I, dorsal view. **B.** Valve II, dorsal view. **C.** Valve V, dorsal view. **D.** Valve VIII, dorsal view. **E.** Valve V, jugal and pleural areas. **F.** Valve V, rostral view. **G.** Valve VIII, lateral view.

РИС. 15. *Tonicia chilensis*, южный экотип, Чили, пролив Магеллана, 53°37'S, 70°56'W, 0.5–3.0 m, BL 18.5 mm, 18.05.2000, собрал. B. Sirenko. **A.** Головной щиток вид сверху. **B.** Щиток II, вид сверху. **C.** Щиток V, вид сверху. **D.** Щиток VIII, вид сверху. **E.** Щиток V, югальное и плевральное поля. **F.** Щиток V, вид спереди. **G.** Щиток VIII, вид сбоку.

Morphometry

The total length of ecotypes in both species was similar, but total width, width of plates and perinotum were lower in *T. calbucensis* southern ecotype (Table 2). Conversely, widths of the plates of *T. chilensis* northern ecotype were a little lower than in southern ecotype (Table 2).

In multivariate analyses, first principal component (PC1) in species-specific analyses accounted for more than 62% of variation (Fig. 17). The second principal component (PC2) reflects changes in shape; this accounted for more than 27% in both species (Fig. 17). The other components (PC3-PC16) have little variation (<5%). The morphospace of *T. calbucensis* southern ecotype is smaller than of northern one (Fig. 17A). For *T. chilensis* the northern ecotype occupied a little smaller morphospace than the southern one (Fig. 17B). The factors with relatively

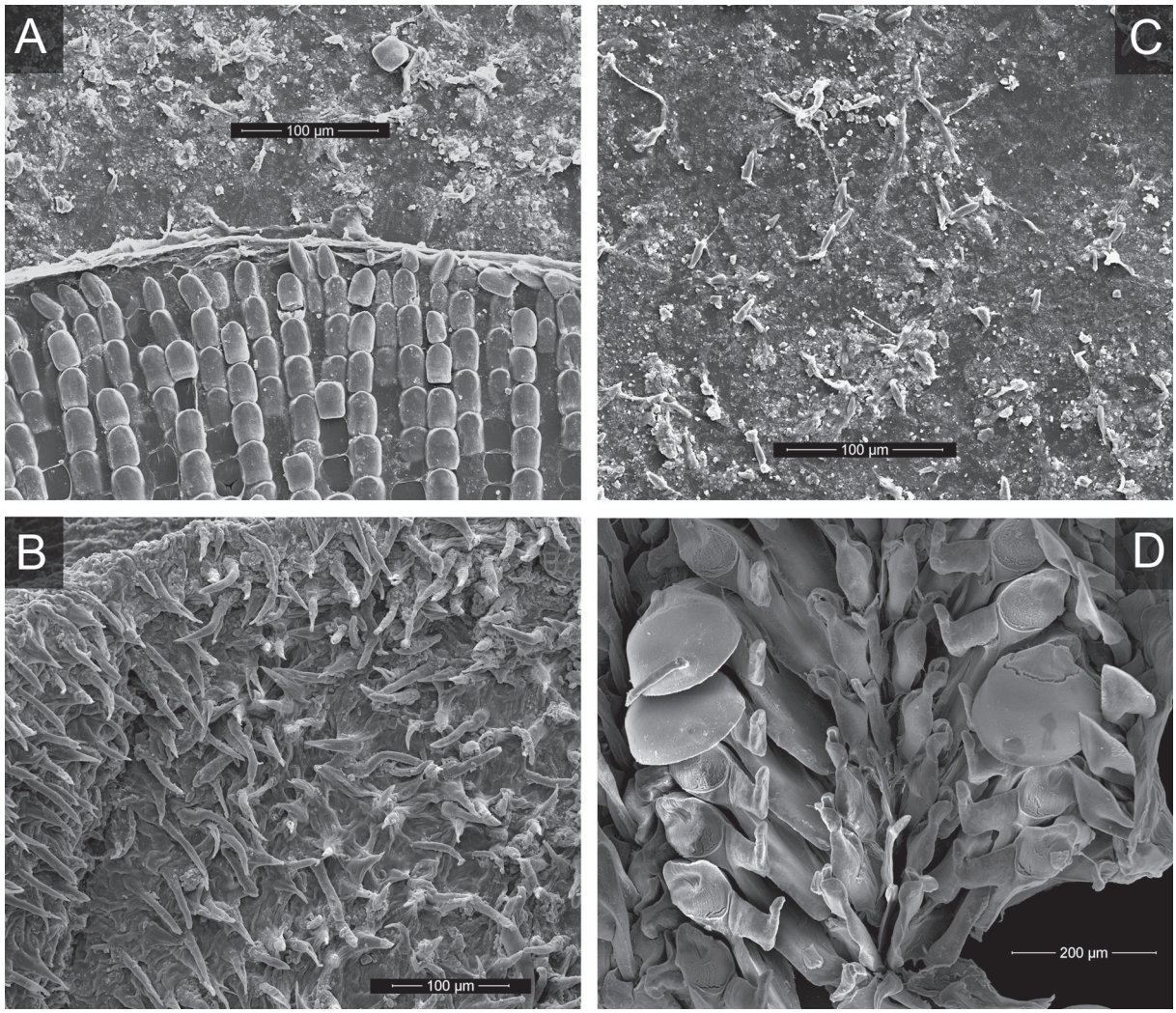

FIG. 16. *Tonicia chilensis*, southern ecotype, Chile, Magellan Strait, 53°37'S, 70°56'W, 0.5–3.0 m, BL 18.5 mm, 18.05.2000, leg. B. Sirenko. **A.** Dorsal, marginal and ventral spicules. **B.** Dorsal bristles and spicules in girdle not treated with KOH. **C.** Dorsal spicules in girdle treated with KOH. **D.** Radula.

РИС. 16. *Tonicia chilensis,* южный экотип, Чили, пролив Магеллана, 53°37'S, 70°56'W, 0.5–3.0 m, BL 18.5 mm, 18.05.2000, собрал. B. Sirenko. **A.** Дорсальные, маргинальные и вентральные спикулы. **B.** Дорсальные щетинки и спикулы на перинотуме не обработанном KOH. **C.** Дорсальные спикулы на перинотуме обработанном KOH. **D.** Радула.

larger loadings for PC1 and PC2 are potentially more relevant to shape variation: these features are length/width ratio of valve I (0.29-0.65 PC1, 0.75-0.95 PC2), and length/width ratio of valve VIII (0.75 to 0.95 PC1, -0.30 to -0.65 PC2). Significant differences in shape between ecotypes and ecoregions were found in both species (Table 3, $P < 0.001$), but the interaction between both variables was not significant (Table 3, $P > 0.05$).

Phylogeny

The genetic distances among species were higher (0.0989-0.1103) than among ecotypes within species (0.0030 – 0.0041). Within ecotype of each species the genetic distances were the lowest (0.0000 – 0.0015). These genetic comparisons evidenced that ecotypes inside each species are geographical variations in shape and colorations. Phylogenetic analysis corroborated this argument based on the absence of monophyly in each ecotype inside species (Fig. 18).

Discussion

This study corroborates the existence of geographical ecotypes in *Tonicia calbucensis* and *T. chilensis* along Southeastern Pacific. These ecotypes were previously treated as subspecies [i.e., Leloup, 1956], but have very low genetic differentiation, absence of monophyly and high latitudinal morphometric variation. The new taxonomic key will allow the identification of *Tonicia* species and ecotypes in Peru and Chile. Some of these species also inhabit the South Atlantic coast and the Falkland Islands so

Table 3. Summary of PERMANOVA analyses for the morphological relationships between ecotypes and ecoregions of each *Tonicia* species.

Табл. 3. Резюме анализов PERMANOVA для морфологических связей между экотипами и экорегионами каждого из видов *Tonicia*.

Source	SS	df	MS	F	P-value
Tonicia calbucensis					
Ecotype	11.81	3	3.937	2.168	0.000
Ecoregion	8.33	5	1.666	0.917	0.003
Interaction	-714.40	15	-47.627	-26.228	0.257
Residual	984.18	542	1.816		
Total	289.92	565			
Tonicia chilensis					
Ecotype	1.06	1	1.058	1.113	0.026
Ecoregion	4.95	5	0.989	1.042	0.001
Interaction	-207.39	5	-41.479	-43.671	0.123
Residual	306.78	323	0.950		
Total	105.39	334			

they will also be of interest to the scientific community of South America. The correct identification of these chitons will allow better understanding of the biodiversity of intertidal invertebrates and the shallow waters.

In the taxonomic key published by Ibáñez *et al.* [2019], it is written that the tegmentum is smooth, without granules or pustules in *T. calbucensis*, which corresponds to the southern ecotype of this species only; the northern ecotype of this species has tegmentum sculptured with numerous granules or pustules. A similar problem will occur if one tries to use their key to identify a southern ecotype specimen of *T. chilensis*. According to the key both sides of the jugum have ten or more narrow, longitudinal grooves. This corresponds to the northern ecotype of *T. chilensis* only, whereas in the southern ecotype of this species both sides of the jugum have one to five longitudinal grooves.

To summarize, *Tonicia calbucensis* northern ecotype differs from southern ecotype by having granules, pustules, and long longitudinal grooves on both sides of the jugum (southern ecotype has a smooth tegmentum with short weak grooves). Additionally, the northern ecotype has no bristles on the dorsal side of the girdle (southern ecotype has short bristles).

The southern ecotype of *T. chilensis* is easily distinguishable from the northern ecotype by having longer intermediate valves. The ratio of the width of valve V to the length is 1.8-2.1 (vs. 2.4–2.5 in *T. chilensis* northern ecotype). It has 2-6 longitudinal ribs near both sides of the flattened keel in the jugal area (vs. more than 10 ribs in *T. chilensis* northern ecotype). The spicules are light (vs. dark in *T. chilensis* northern ecotype), and it has small granules in the pleural and lateral areas. Specimens from the fjords in the Gulf of Ancud in southern Chile (about 42.5°S) at depths of 6–19 m have tegmentum structures that are more similar to the southern ecotype, but the length of the intermediate valves are shorter, and the dorsal spicules of the girdle are dark, like those found in the northern ecotype. The northern ecotype of *T. chilensis* differs from other congeneric species by having 10 or more longitudinal ribs on both sides of the jugum. The tegmentum and girdle are black, and the outer half of the apophyses and teeth are sharply pectinated.

Morphometric analysis helps to discriminate between ecotypes in both species and the morphological differences are associated to width of valves (Table 2). The morphological differences between ecoregions support the existence of the morphological ecotypes in both species. Morphological ecotypes are common in mollusks and are promoted by environmental variation and ecological interactions between species [Rolán *et al.*, 2004; Guerra-Valera *et al.*, 2009; Sepúlveda, Ibáñez, 2012; Sepúlveda *et al.*, 2012]. Chitons exhibit color polymorphism varying in different habitat and probably is promoted by visual predators [Mendonça *et al.*, 2015, 2016].

Divergence times estimated for *Tonicia* species show a recent divergence between all species from SEP (~ 35 My) being the southernmost species (*T. calbucensis*, *T. chilensis*, *T. disjuncta* and *T. lebruni*) the youngest (< 20 My) [Ibáñez *et al.*, 2019]. The divergence among the ecotypes in *T. calbucensis* and *T. chilensis* dated from the Pliocene (< 5 My) [Ibáñez

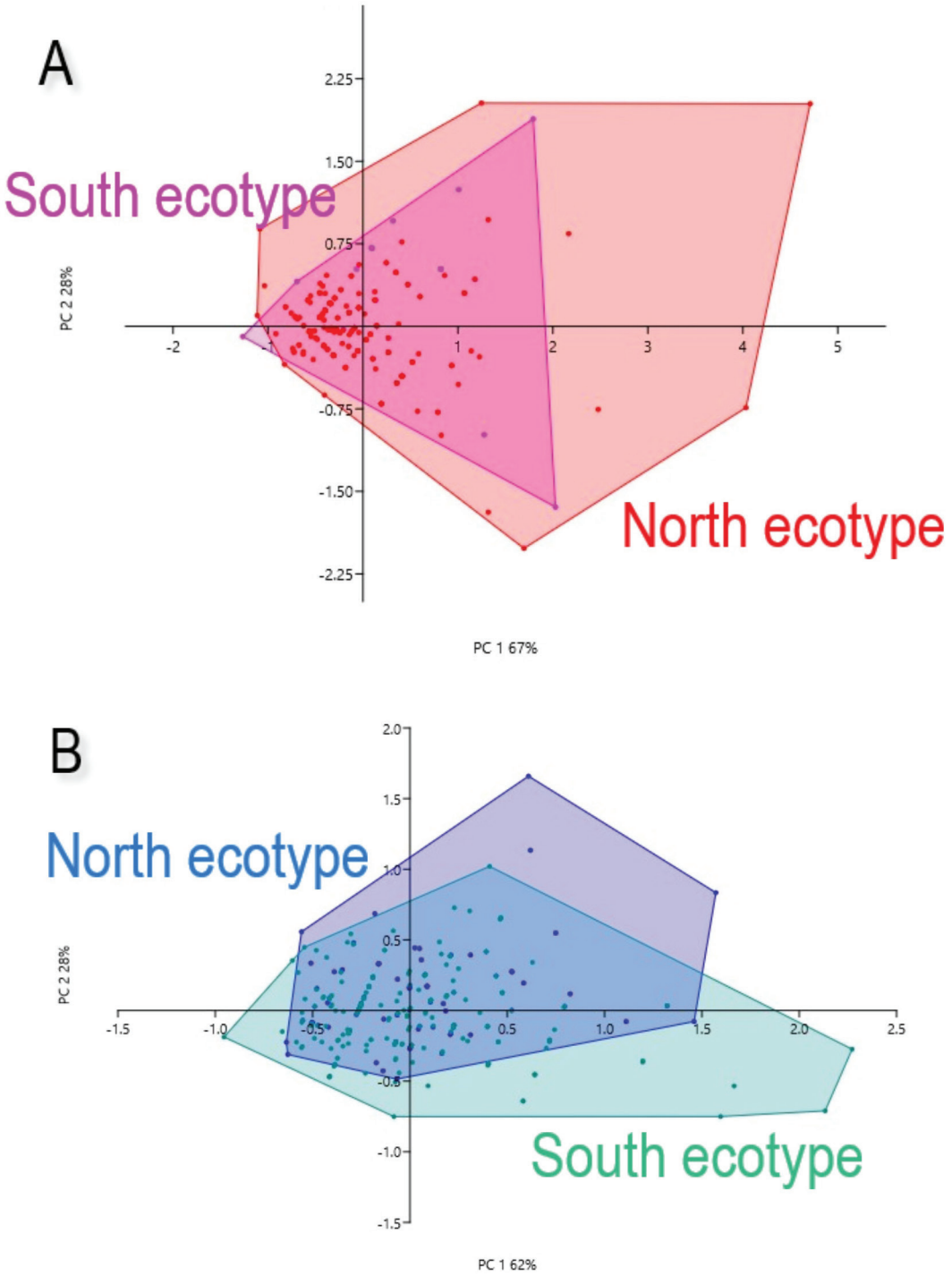

FIG. 17. Shape differences in *Tonicia* ecotypes, resulting from combined principal components analysis. The two species studied are shown in different colors. **A.** *Tonicia calbucensis.* **B.** *Tonicia chilensis*.

РИС. 17. Различия в форме экотипов *Tonicia*, полученные в результате комбинированного анализа основных компонентов. Два изученных вида показаны разными цветами. **A.** *Tonicia calbucensis*. **B.** *Tonicia chilensis*.

et al., 2019] evidencing a little genetic divergence between the ecotypes within each species. The habitat of southern ecotypes of *T. calbucensis* and *T. chilensis* have been impacted by glaciations and constitute a set of islands, fjords and channels formed by deglaciation [Camus, 2001] and contain a set of species adapted to cold environments forming a different biogeographic province [Camus, 2001; Ibáñez *et al.*, 2009]. These southern ecotypes probably have been formed during last glaciation cycle and the current oceanographic conditions (low salinity and temperature) reinforce these phenotypes. Several

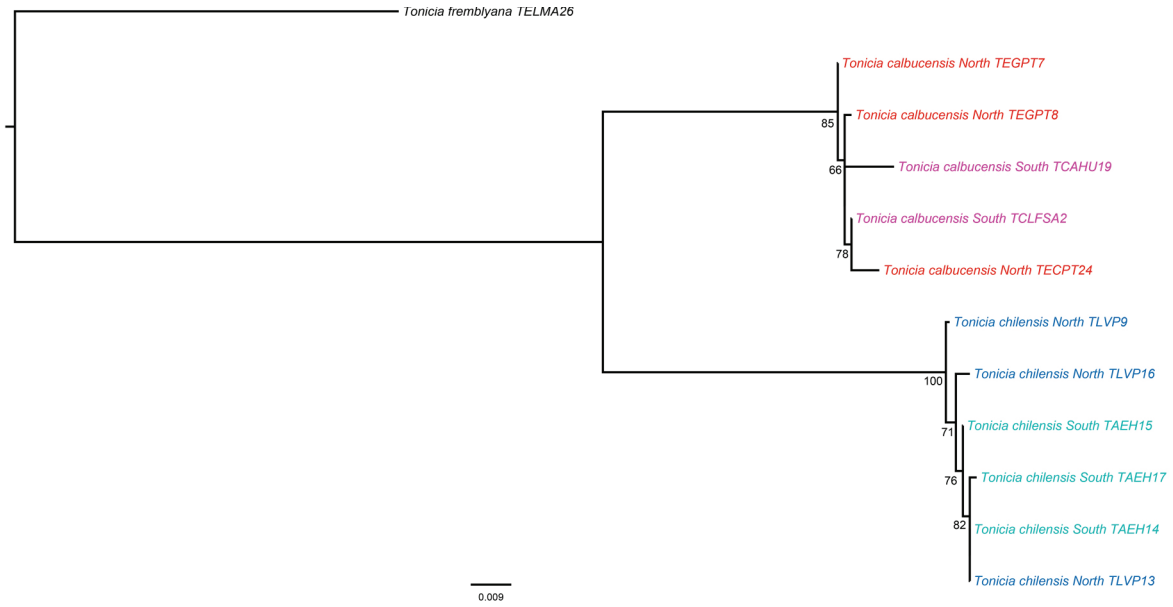

FIG 18. Maximum likelihood phylogram of *Tonicia* species and ecotypes (north and south) from the southeastern Pacific Ocean. Node values represent bootstrap support.

РИС. 18. Филограмма максимального правдоподобия видов и экотипов *Tonicia* (север и юг) из юго-восточной части Тихого океана. Значения узлов представляют поддержку начальной загрузки.

chiton species, including *Tonicia* species, show an increase of body size with higher latitude associated with the gradient of decreasing temperature and different growth rates along SEP [Ibáñez *et al.*, 2021]. Future studies should explore the environmental and genetic factors shaping the ecotypes and their geographic variation in relation to different habitats.

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