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A new species of *Callochiton* (Mollusca: Polyplacophora) from the Strait of Messina (central Mediterranean)

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Abstract. A new species of *Callochiton*, *C. stefaniae* n. sp., is identified in the framework of investigations aimed to better characterise the Strait of Messina benthic ecosystem. The new species, found in a peculiar facies of hard bottoms densely colonised by the hydrocoral *Errina aspera* (Linnaeus, 1767), is morphologically distinct from the co-generic *Callochiton septemvalvis* (Montagu, 1803), living in the Mediterranean Sea and along other European coasts, and *C. doriae* (Capellini, 1859), living only in the Mediterranean Sea.

Key words. Callochitonidae, Recent, Strait of Messina, *Errina aspera*, new species.

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Introduction

Three species of *Callochiton* Gray, 1847 are known from the Atlantic coasts of Europe and the Mediterranean Sea. *Callochiton calcatus* Dell'Angelo & Palazzi, 1994 is a rare species restricted to the Mediterranean. The other 2 species, *C. septemvalvis* (Montagu, 1803), living in the Mediterranean Sea and along other European coasts, and *C. doriae* (Capellini, 1859), living only in the Mediterranean Sea, are very similar to each other, differing mainly in the absence or presence of longitudinal grooves on the pleural areas of the intermediate valves and antemucronal area of the tail valve. There was previously some room for doubt about whether these 2 morphotypes represented distinct species, or rather the morphological plasticity of *C. septemvalvis* (e.g. Thiele 1902, Bergenhayn 1931, Leloup 1934, Kaas 1978, Carmona Zalvide et al. 2002). Individuals attributed to both these species of *Callochiton* were included in a molecular phylogeny published by by Sigwart et al. (2013) with 1 specimen of *C. septemvalvis* from France (Roscoff, Atlantic Ocean) and 1 of *C.* "*euplaeae*" (O.G. Costa, 1830) (not 1829 as usually indicated; see Fasulo 2013) from Croatia (Adriatic Sea). However, the taxon *Callochiton euplaeae* is a nomen dubium, as reported by Dell'Angelo et al. (2016). To clarify, considering the more appropriate available name *C. doriae*, the Mediterranean members of the species of *Callochiton* without longitudinal grooves on the pleural areas should be attributed to *C. septemvalvis*, whilst the species with longitudinal grooves is *C. doriae*.

In this paper, we describe some small specimens of *Callochiton* sampled in the framework of research cruises carried out in a peculiar habitat in the Strait of Messina. These specimens lack longitudinal grooves (the key character for separating *C. septemvalvis* and *C. doriae*), yet differ from other congeners, and they are therefore described as a new species, *C. stefaniae* n. sp.

The Strait of Messina (Fig. 1) is characterised by strong tidal currents which create a constant upwelling regime. Such special hydrology makes the strait distinct from the major Mediterranean sectors, and it is noted as hosting "a wealth of biogeographic peculiarities, including Pliocene Atlantic remnants and local endemisms" (Bianchi et al. 2012), and recent studies of the region have frequently described new species (Bogi & Campani 2006, 2007, Buzzurro & Russo 2007, Bogi & Bartolini 2008, Crocetta et al. 2009, Tisselli et al. 2009, Bogi et al. 2016, Renda & Micali 2016, Smriglio et al. 2016), all of which combine to make the strait an apparent biodiversity hotspot. One of the most peculiar environments of the Messina Strait is probably the "Sill", a rough seafloor that connects the Calabrian and Sicilian continental slope, where it extends southernmost throughout Rada Paradiso (Giacobbe et al. 2007). Such steep hard bottoms are densely colonised by the hydrocoral *Errina aspera* (Linnaeus, 1767), representing an

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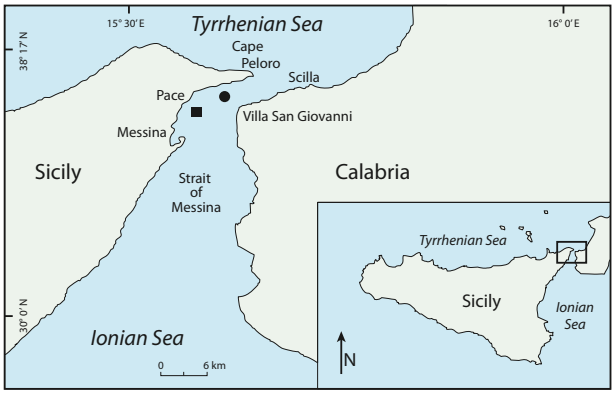

Figure 1. Sampling stations. Circle: dredging stations at "Sill" sites DG001, DG04, and DG05. Square: dredging station at Paradiso roadstead site DG11.

exclusive facies from the subtidal–bathyal transition (Di Geronimo & Fredj 1987).

Materials and Methods

From the "Sill" in the Strait of Messina, 20 small-sized specimens of *Callochiton* sp. were sampled in the framework of 2 distinct cruises (Errina'87 and POP'95) sponsored by the University of Messina (Table 1), which aimed to better define the distribution of the hydrocoral-dominated biotope (Giacobbe 2001, Giacobbe et al. 2007) and to investigate the major associated species (Giacobbe & Rinelli 1991, Giacobbe & Spanò 2001). The *Callochiton* specimens were also compared with other material collected by fishing boats at various times from the same area.

Morphological features and diagnostic characters of the chiton specimens were studied and imaged by scanning electron microscope (SEM) at the Messina University. The digital images were obtained using a Motic SMZ-140 Microscope with the software Motic Images Plus.

The following abbreviations are used:

BD B. Dell'Angelo Collection, Genova, Italy (will be deposited in MZB)

BEL Benthic Ecology Laboratory, Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy

MS M. Sosso Collection, Genova, Italy

MZB Museo di Zoologia dell'Università di Bologna, Bologna, Italy

POP Programma Operativo Plurifondo, Sicilia 1994-99

SMF Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt a. Main, Germany

spm. specimen

WR W. Renda Collection, Amantea (Cosenza), Italy

Systematics

Class Polyplacophora Gray, 1821

Order Chitonida Thiele, 1909

Family Callochitonidae Plate, 1901

Genus *Callochiton* Gray, 1847

Type species. *Chiton laevis* Montagu, 1803 (non Pennant, 1777) [= *Callochiton septemvalvis* (Montagu, 1803), fide Kaas 1978] by subsequent designation (Gray 1847).

Distribution. Tropical and subtropical regions of the Indo-Pacific (including Japan; absent from the northeastern Pacific). In the Atlantic Ocean restricted to the eastern part and to the sub-Antarctic and Antarctic parts (Kaas & Van Belle 1985). Neogene to Recent.

Callochiton stefaniae n. sp.

Figures 2–4

Type material. Holotype: MZB 32137, spm. disarticulated and coated for SEM analysis, length 4.3 mm (Fig. 3A–N). Paratype: BEL 122 POP'95 DG11, length 2 mm.

Material examined. The type material and 18 specimens were collected from the sites reported in Table 1. An additional 6 specimens are in the BD collection from separate collection events, all from fishing boats working in the strait in environments colonised by *Errina aspera*, and probably coming from the Sill in the Strait of Messina. All the material examined is reported in Table 2.

Type locality. Strait of Messina, Rada Paradiso, dredging station DG11 (Table 1, Fig. 1).

Etymology. The species is named after Stefania Minerva, the last author's wife and herself a marine biologist.

Table 1. Sampling data: numbered dredging stations (see Fig. 1) and samples with locality details, number of polyplacophoran specimens collected and the range of their body lengths.

Dredging station	Number of spm.	Length (mm)	Date	Site	Depth (m)	Latitude (N)	Longitude (E)
DG001	4	2.0–3.4	7 April 1987	Sill	90–115	38°14'45"	15°37'28"
DG04	9	1.2–2.5	13 July 1995	Sill	100	38°14'45"	15°37'36"
DG05	5	2.3–3.4	13 July 1995	Sill	80–110	38°14'40"	15°37'30"
DG11	2	2.0–4.3	14 July 1995	Rada Paradiso	75–100	38°13'20"	15°34'48"

Diagnosis. Animal of small size, ovate, moderately elevated, tegmentum and girdle uniformly coloured from very pale brownish to rosaceous. Tail valve semicircular, with a small, hardly raised mucro in anterior position, antemucronal and postmucronal slopes straight. Tegmentum surface appears rough, sculptured with fine, tabulate elongate granules, well separated on the lateral areas and fusing into continuous lines in the central areas. Slit formula 17/2/14. Girdle wide, densely covered with fine, smooth, dorsal imbricating spicules, a marginal fringe of lanceolate spicules, and ventral small, flat spicules. Radula with 30 transverse rows of mature teeth, major lateral tooth with a tridentate cusp, the denticles of almost equal length.

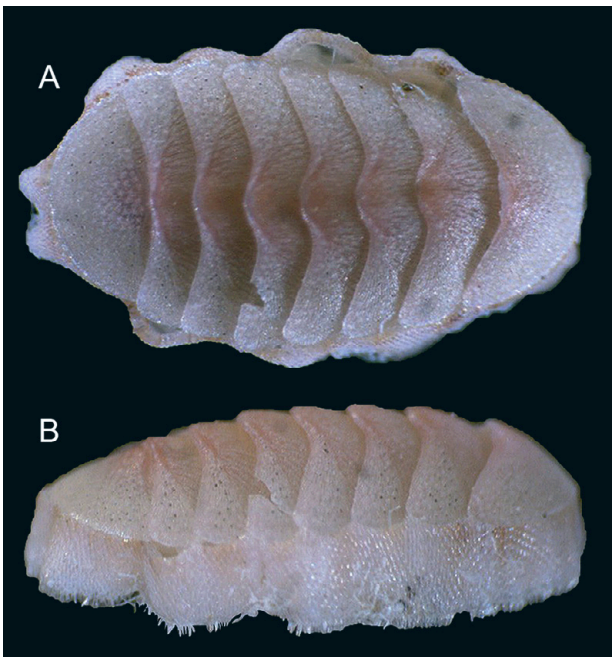

Figure 2. *Callochiton stefaniae* n. sp., MZB 32138, Strait of Messina, "Sill" dredging station DG001, length 3.4 mm. **A.** Dorsal view. **B.** Lateral view.

Description. Animal of small size, 4.3 mm long, ovate, moderately elevated, girdle rather wide, tegmentum and girdle uniformly coloured from very pale brownish to rosaceous.

Head valve semicircular with almost straight anterior slope, posterior margin widely V-shaped, without central posterior notch. Intermediate valves rectangular, subcarinate with the top rounded, dorsal elevation 0.36 (valve IV; Fig. 3I), anterior margin almost straight, side margins rounded, posterior margin slightly concave at both sides of the well-pronounced apex, lateral areas moderately raised. Tail valve semicircular, anterior margin slightly convex, with a small, hardly raised mucro in anterior position, antemucronal and postmucronal slopes straight.

Tegmentum surface appears rough, sculptured with fine, tabulate elongate granules, well separated on the lateral areas and fusing into continuous lines in the central areas (Figs 3H, 4D). Overall appearance of valves is finely striated when examined under magnification, striae longitudinally oriented in central and antemucronal areas, radially oriented in head valve, lateral areas and postmucronal area, marked with a few concentric growth lines toward the outer margins.

Articulamentum rose coloured in the centre, whitish towards the sides, apophyses wide, short, regularly rounded, connected at the jugum by a lamina, insertion plates short, slit formula 17/2/14, teeth irregular, slit rays scarcely visible, eaves very porous, apical area of head and intermediate valves marked by longitudinal fine furrows (Fig. 3L, M).

Girdle wide, densely covered with fine, straight, smooth, imbricating spicules, closely tightened to the surface, directed inwards, c. 75–95 × 21–25 µm (in situ), oval in shape. Some slender, smooth, curved spines, 230–260 µm long (measured in spm. MZB 32138 in situ) are found close to outer margin (Fig. 4J, K). There is a marginal fringe of lanceolate, feather-like striated, somewhat flattened spicules, c. 87–97 µm long and 22–24 µm

Table 2. Material examined. See Materials and Methods for explanations of repository abbreviations.

Dredging station	Number of spm.	Repository	Length (mm)	Figure	Type material
DG11 (Rada Paradiso)	2	MZB 32137	4.3	Fig. 3A–N	Holotype
		BEL 122 POP'95 DG11	2.0		Paratype
DG001 (Sill)	4	MZB 32138	3.4	Figs 2A, B, 4J–L	
		BEL 122 Errina'87 DG001	2.0–2.7 (3 spm.)		
DG04 (Sill)	9	SMF 349105	2.5		
		WR	2.2		
		Sigwart coll.	1.2		
		BEL 122 POP'95 DG04	1.6–2.5 (6 spm.)		
DG05 (Sill)	5	BD 128	2.9		
		MS 102	2.8		
		BEL 122 POP'95 DG05	2.3–3.4 (3 spm.)		
Precise locality unknown, not listed in Table 1	6	BD coll.	Max. 4.6 mm (6 spm.)		

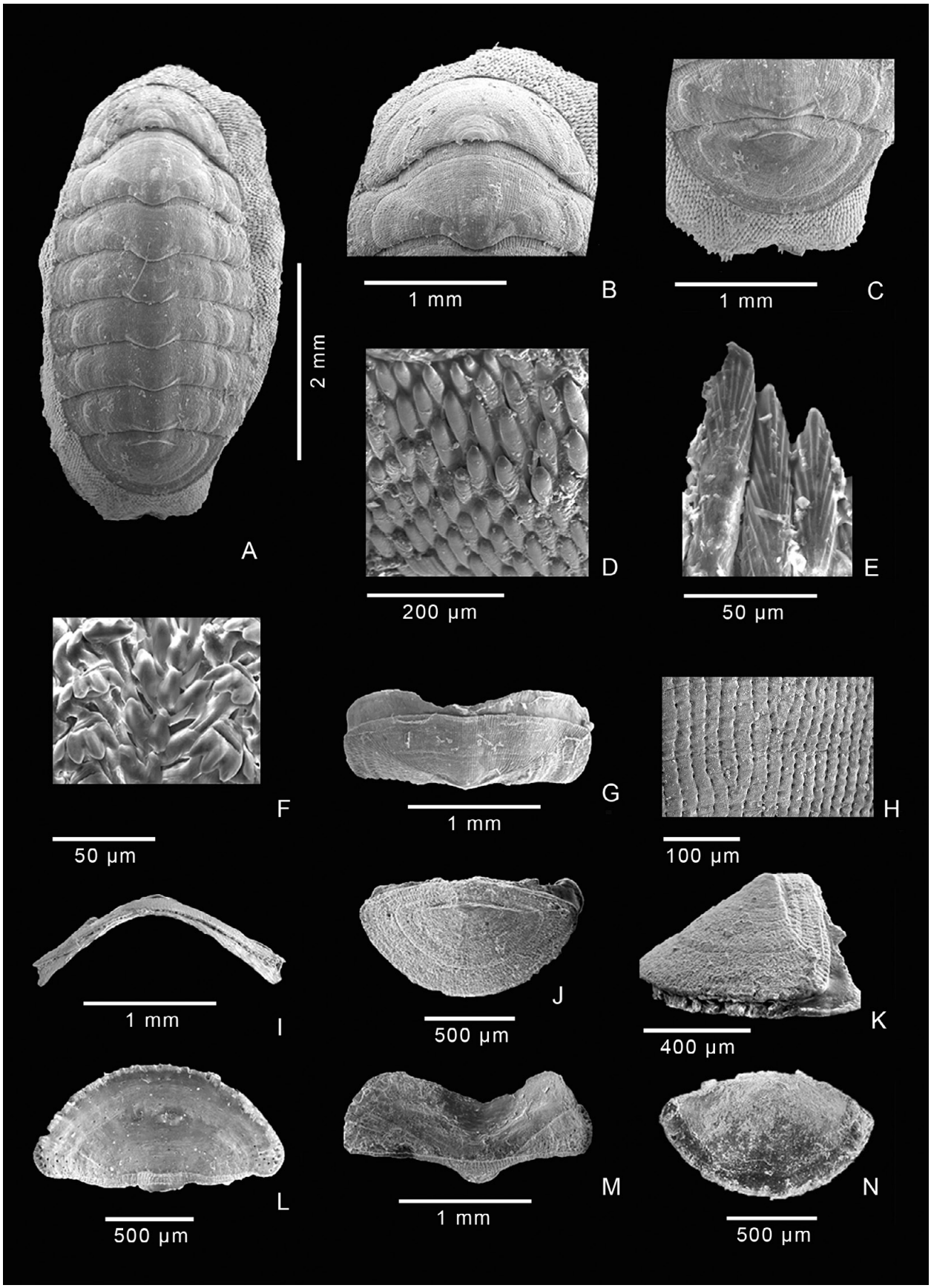

Figure 3. *Callochiton stefaniae* n. sp., MZB 32137, Holotype, Strait of Messina, dredging station DG11, Paradiso roadstead. **A–F.** Whole specimen, length 4.3 mm, coated and disarticulated for SEM analysis: (**A–C**) dorsal view and detail of anterior and posterior parts; (**D**) dorsal girdle spicules in situ; (**E**) marginal girdle spicules in situ; (**F**) central portion of radula. **G–N.** Separate valves, disarticulated from the specimen shown in A: (**G–I**) intermediate valve IV, dorsal view, detail of the sculpture, and frontal view, respectively; (**J, K**) tail valve, dorsal, and lateral views, respectively; (**L**) head valve, ventral view; (**M**) intermediate valve, ventral view; (**N**) tail valve, ventral view.

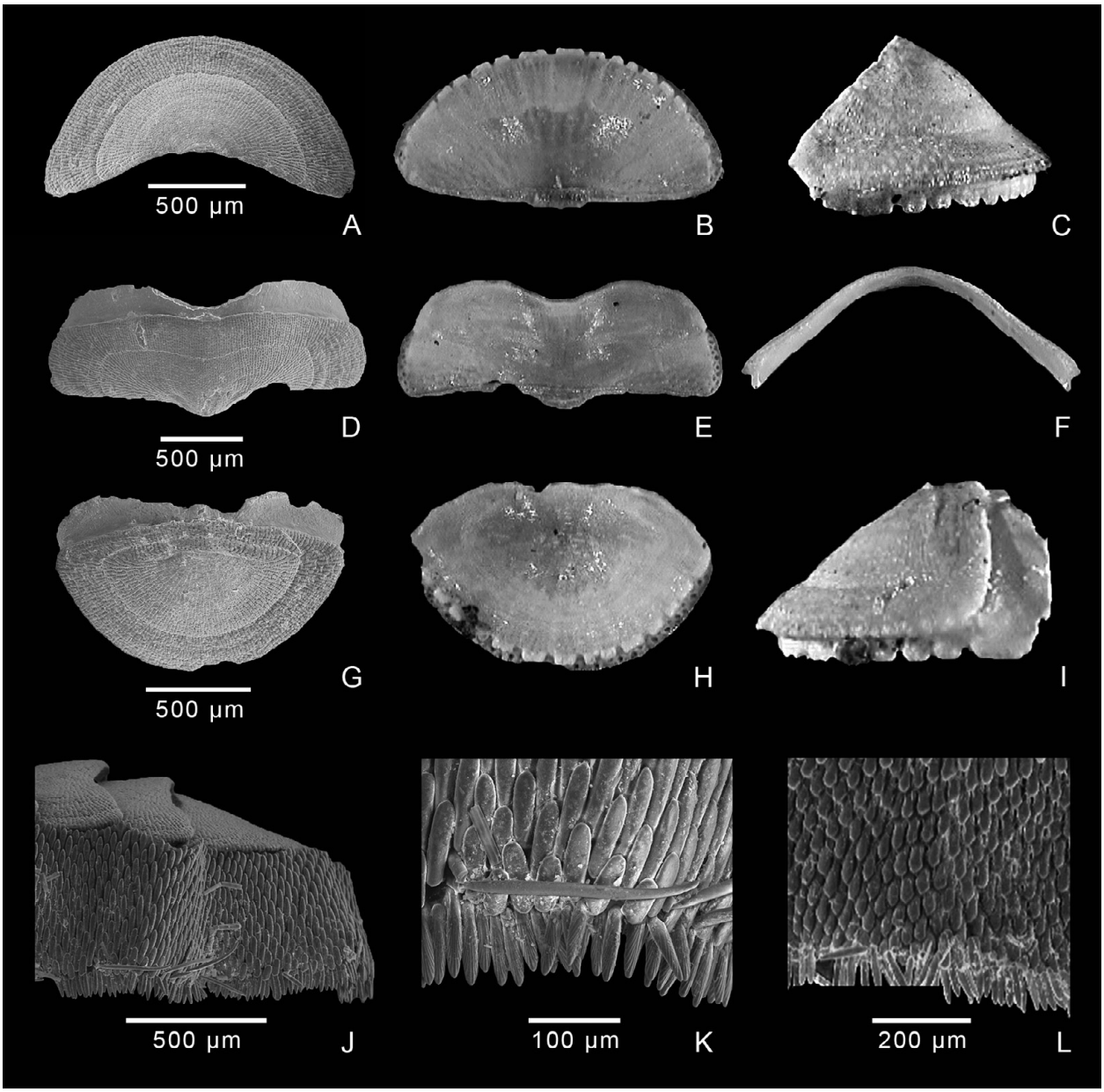

Figure 4. *Callochiton stefaniae* n. sp., Strait of Messina, Sill. **A–I.** Dredging station DG04, BEL: (**A–C**) head valve, width 1.6 mm, dorsal, ventral, and lateral views. (**D–F**) intermediate valve, width 1.9 mm, dorsal, ventral, and frontal views. (**G–I**) tail valve, width 1.4 mm, dorsal, ventral and lateral views. **J–L.** Dredging station DG001, MZB 32138: (**J–K**) girdle formations (dorsal spicules, dorsal spines, and marginal spicules) in situ; (**L**) ventral girdle spicules and marginal spicules in situ.

wide. Ventral side covered with transverse rows of small, bluntly pointed, flat spicules, c. 50 µm long (measured in spm. MZB 32138 in situ).

Radula with 30 transverse rows of mature teeth, asymmetric, central tooth with a narrow blade, first lateral teeth distally widening, major lateral tooth with a tridentate cusp, denticles of almost equal length.

Remarks. No trace of grooves was found in the central areas of any of the listed specimens examined from the Strait of Messina, contrary to what was observed on other specimens of *Callochiton* collected along the Calabrian and Sicilian coasts of the strait and attributed to *Callochiton doriae*, which always has conspicuous longitudinal grooves, even when juvenile (Monterosato 1879, Giovine & Dell'Angelo 1993).

The valves of the specimen disarticulated for SEM analysis (Fig. 3G–N) are quite eroded and some features (especially in the articulamentum) are not clear, so we present additional images of valves (Fig. 4A–I) from another specimen from DG04 (broken in half and therefore not counted in the material examined) with the valves better preserved. The girdle of this specimen also includes some irregularly spaced much longer needles dorsal to the marginal fringe in the form of ringshaft-needles (c. 260 µm long; Fig. 4J, K).

Callochiton stefaniae n. sp. differs from *C. doriae* and *C. septemvalvis* in the following features:

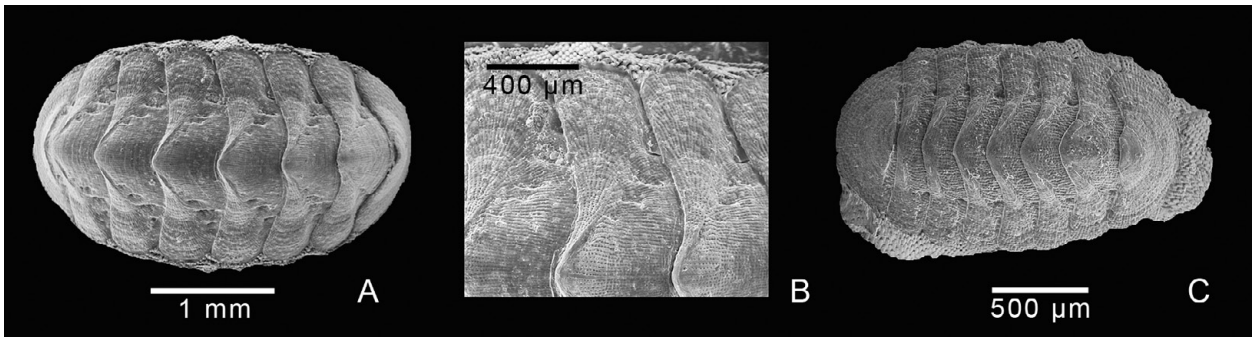

Figure 5. *Callochiton doriae* (Capellini, 1859), juvenile specimens, BD. **A, B.** Lampedusa, length 3.1 mm, whole specimen and detail of ornamentation. **C.** Antignano (Livorno), length 2 mm, whole specimen.

(a) the smaller size, up to 24 mm in length for *C. doriae* (Dell'Angelo & Palazzi 1994) and 22 mm for *C. septemvalvis* (Carmona Zalvide et al. 2002), vs up to 4.6 mm for *C. stefaniae*;

(b) the uniform very pale brownish to rosaceous colour, both for the tegmentum and for the girdle, vs a variable colour in *C. doriae*/*C. septemvalvis*, which are more or less variegated with spots of various colours, and often with lighter coloured bands on the girdle (Kaas & Van Belle 1985, Dell'Angelo & Smriglio 1999);

(c) the shape of tail valve, with the mucro central in *C. doriae*/*C. septemvalvis* (e.g. Kaas & Van Belle 1985: fig. 2(8, 9); Dell'Angelo & Smriglio 1999: pl. 40, fig. G, pl. 41 fig. J), vs anterior in position in *C. stefaniae* (Fig. 3J, K);

(d) the different size of dorsal girdle spicules, 150–280 × 25–32 µm in *C. septemvalvis*/*C. doriae* (Kaas & Van Belle 1985) vs 75–90 × 21–25 µm in *C. stefaniae*. Carmona Zalvide et al. (2002) give 130–190 × 50–60 µm for *C. doriae*, 100–200 × 50–100 µm for *C. septemvalvis*;

(e) the lack of longitudinal grooves on the central areas, always evident in *C. doriae*, including in juvenile specimens. We include SEM photos of 2 juvenile specimens of *C. doriae*, for comparison (Fig. 5A–C).

Callochiton stefaniae n. sp. differs from *C. calcatus* in the shape of the valves, strongly angulated and elevated in *C. calcatus* (dorsal elevation 0.65–0.72 vs 0.36 in *C. stefaniae* n. sp.), the postmucronal slope of tail valve, convex and very characteristic in *C. calcatus* (see Dell'Angelo & Smriglio 1999: 133, fig. 7B), the intermediate valves trapezoidal in *C. calcatus*, and moreover the lack of longitudinal grooves on the central areas in *C. stefaniae* n. sp., clearly apparent in *C. calcatus*.

Species in the genus *Callochiton* possess pigmented aesthetes (Baxter & Jones 1984, Schwabe 2010). Only a subset of the shell pores associated with the aesthete system found in *Callochiton* spp. contain pigments. The pigmented aesthetes in *Callochiton* spp. are regularly arranged over the lateral areas of intermediate valeves and in radial rows of the head valve and postmucronal area of the tail valve (Baxter & Jones 1984); they are surprisingly difficult to see, the visibility depending on the direction of view too. We were not able to confirm the presence of pigmented aesthetes in *C. stefaniae* but we also cannot confirm their absence. The lack of visible pigments in aesthetes has been already reported in literature (e.g. Dell'Angelo et al. 2012 for *Callochiton cupreus* Dell'Angelo, Prelle, Sosso & Bonfitto, 2012 from Madagascar).

The fine longitudinal furrows present in the apical area of head and intermediate valves (Fig. 3L, M) seem of particular interest. The apical areas of *C. septemvalvis*/*C. doriae* are smooth, which has not been previously noted but is apparent from published SEM images of those species (Baxter & Jones 1984: pl. 4, figs (a), (c); Dell'Angelo & Smriglio 1999: pl. 40, figs D, F). Likewise, these fine furrows are not visible in the other Mediterranean *Callochiton* species, *C. calcatus*. Any functional significance of the peculiar furrows is unclear and awaits further studies.

With this material, as for many chitons so far, there are very few data available for molecular genetic comparisons. With regard to *C. septemvalvis*/*C. doriae*, to our knowledge only 2 specimens have been sequenced for *C. septemvalvis* (Okusu et al. 2003, Sigwart et al. 2013), and 1 specimen for *C. doriae* (Sigwart et al. 2013). We have established here the distinctive morphologies of *Callochiton septemvalvis*/*C. doriae*, but we note that there may be more hidden diversity within the Atlantic and Mediterranean members of *Callochiton*.

The habitat of *C. stefaniae* is different from those known for *C. doriae*/*C. septemvalvis*, which are known from shallow water (20–30 cm) to a maximum of 580 m (Kaas & Van Belle 1985, Kaas 1991), preferentially living on coralline algae. The habitat of *C. stefaniae* is inferred from our limited data to be very restricted; to date, the species has been collected only in the "rough bottoms" of the Strait of Messina. The other records, occasionally collected from fishing boats, cannot be precisely localised but plausibly came from the same area and environment. In the "rough bottoms", dense colonies of the hydrocoral *Errina aspera* cover all the hard substrates from almost 90 m to 200 m depth, forming an exclusive facies in association with the giant barnacle *Pachylasma giganteum* (Philippi, 1836) (Di Geronimo & Fredj 1987). Such facies are characterised by highly diversified communities that

include a mixture of Atlantic subtropical (Giacobbe & Spanò 2001) and temperate (Giacobbe & Rinelli 1991) taxa; nevertheless, the *E. aspera* colonies are relatively sparsely covered in epibiota, except for the snail predators specifically associated with *Pedicularia sicula*, preying upon the hydrocoral polyps, and occasional epibionts such as the barnacle *Megatrema anglicum* (Sowerby, 1823) (Di Natale & Mangano 1985). The specimens of *C. stefaniae*, although exclusively recorded in *E. aspera* samples, were always found detached. Nevertheless, their small size together with the peculiar shell roughness and pale rosaceous colour, similar with the *E. aspera* skeleton, suggest a possible association with the hydrocoral bioconstruction.

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