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***Acanthochitona pilosa* spec. nov.,
a new species of *Acanthochitona* Gray, 1821
from the Mediterranean**

(Mollusca, Polyplacophora)

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The Mediterranean chiton fauna was known to comprise three representatives of the genus *Acanthochitona*: *A. fascicularis*, *A. crinita* and *A. oblonga*. Between 1990 and 2014, in Banyuls sur Mer, France a fourth, previously undescribed species was collected inside the so called “trottoir”, a eulittoral area mainly dominated by encrustations of the red alga *Lithophyllum tortuosum* (Esper) Foslie, 1900. The chiton’s presence in this highly oxygenized habitat is in contrast to most other *Acanthochitona* spp., which mainly live under stones. *Acanthochitona pilosa* spec. nov., herein described based on light and scanning electron microscopy (SEM), differs considerably from its congeners mainly by having oval, densely packed granules with very small microaesthetes (2–3 µm), thick dorsal spicules scattered on the perinotum and a consistent dark brown colour usually interrupted by white spots on individual valves.

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Introduction

The genus *Acanthochitona* Gray, 1821, comprises about 80 species (Gofas 2013) of small to medium sized chitons all over the world. The genus is defined by a reduced tegmental area, and a merging of the pleural and lateral areas of the shell plates, which are usually covered by raised granules on the dorsal side; ventrally the plates have five incisions in the head, a single slit on each side in the intermediate plates, and usually two in the tail valve. The most conspicuous characteristic, however, is the presence of usually 18 tufts of aragonitic bristles on the

perinotum, mostly corresponding to the underlying plate incisions. In the Mediterranean, fossil evidence for *A. crinita*, *A. oblonga* and *A. fascicularis* was found dating back to the early Pliocene (Dell’Angelo et al. 2004), just after the Mediterranean had been refilled with seawater from the Atlantic after the Messinian salinity crisis (Sierro et al. 1999). Today there are three recognized species in the Mediterranean. Kaas (1985) made a first attempt to unravel a complex of taxa by considering them to be synonyms of only two accepted species, *Acanthochitona fascicularis* (Linnaeus, 1767) and *A. crinita* (Pennant, 1777). Later on, Bonfitto et al. (2011) accepted *Acanthochitona*

oblonga Leloup, 1981 as a third species, based on molecular and morphological data. The finding of a yet undescribed *Acanthochitona* species at Banyuls sur Mer, France, which is described herein, raises the number of Mediterranean representatives of this genus to four.

Material and methods

Specimens were handpicked in 0–2 m depths during low tide and snorkelling, respectively, along the rocky shore in front of the Laboratoire Arago in Banyuls sur Mer, during biannual field courses of the Ludwig-Maximilians-University of Munich in the years 1996 to 2014. After collection, individuals were transferred alive into petridishes containing seawater. As soon as they had uncurled and sucked to the surface, seawater was exchanged with isotonic MgCl₂-seawater solution in several steps within half an hour, which prevented curling of the specimens. Afterwards, specimens were preserved in 4 % formaldehyde and transferred to 75 % ethanol after two weeks. Preparation of specimens for scanning electron microscopy followed Schwabe (2006). For comparisons with the new species, herein described, we used specimens of *A. crinita*, *A. fascicularis* and *A. oblonga* from the extensive molluscs collection of the Bavarian State collection of Zoology (ZSM). In addition, we studied the neotype of *A. crinita* (Royal Saskatchewan Museum of Natural History; RSMNH 1978.052.02601). Based on the obtained data, an identification key was developed for the four species, entirely based on macroscopic, external morphological features.

Systematic taxonomy

The classification used herein follows Sirenko (2006)

Class Polyplacophora Gray, 1821
Subclass Neoloricata Bergenhayn, 1955
Order Chitonida Thiele, 1909
Suborder Acanthochitonina Bergenhayn, 1930
Superfamily Cryptoplacoidea H. & A. Adams, 1858
Family Acanthochitonidae Pilsbry, 1893

Acanthochitona Gray, 1821

Type species: *Chiton fascicularis* Linnaeus, 1767, by monotypy.

Genus distribution: Worldwide except for polar regions. Oligocene–Recent.

Acanthochitona pilosa spec. nov.

? *Acanthochitona crinita* (non Pennant, 1777); Dell'Angelo & Smriglio 2001: figs 124, 125.

Material examined. Holotype: SNSB-ZSM Mol 20070223, 13 mm, now partly disarticulated, Banyuls sur Mer (42°28'57" N, 03°08'13" E), September 2011. Paratypes: ZSM Mol 19960590, 8.5 mm; ZSM Mol 19960589, 20 mm; ZSM Mol 20070223, 3 specimens, 12 mm, 13 mm, 17 mm, all from type locality. Additional material examined: ZSM Mol 20034395, 3 mm; ZSM Mol 1996589, 5 mm; ZSM Mol 1996589, 7 mm; ZSM Mol 1996589, 9 mm, from type locality.

Diagnosis. Small to medium sized, body elongate oval, tegmentum colouration generally brown, usually with white spots on single valves. Shape of intermediate valves posterior triangular and anterior circular, with hardly raised, distinctly striated jugal area. Granules oval to pear-shaped, densely arranged. Tail valve roundish, mucro at posterior third, postmucronal slope steep and straight. Articulamentum solid, brownish. Perinotum with densely arranged small, sharp-pointed spicules, scattered with larger, slightly striated, medially thickened spicules. Tufts with about 30 straight, solid needles, around 1 mm long, additionally with same amount of much smaller spicules among them. Hyponotum with elongate, flat and smooth spicules. Radula with tulip-shaped central tooth and tricuspidate head of second lateral tooth.

Description

Body outline elongate oval, with a girdle about twice as long as broad. All bodyparts generally of a brown colour with white spots on both sides of the anterior jugal part of valves V and VII, and a white jugal area in tail valve. Dorsum of valve II subcarinate, not keeled, and moderately elevated (elevation ratio 0.36, Fig. 2a). Side slopes slightly concave. Head valve slightly wider than long and more or less semicircular with straight, un-notched posterior margin (Fig. 2c). Second valve more or less elongate with slightly protruding apex (Fig. 2d). Tegmentum of intermediate valves (except valve II) about as long as wide with curved anterior edge and triangle shaped posterior end. Jugal area striated and hardly raised, clearly distinguishable from pleurolateral area, broadly wedge shaped in valve II, to a lesser degree also in valves III, IV, VI, and about parallel in valves V and VII. Striations giving anterior margin a slightly serrated appearance. Tail valve more or less circular, with prominent, posteriorly directed mucro, situated in the posterior third. Antemucronal area straight, postmucronal slope steep and straight. Comarginal growth lines visible in all valves.



Fig. 1. Holotype of *Acanthochitona pilosa* spec. nov.; dorsal view, length 13 mm (SNSB-ZSM Mol 20070223).

Tegmentum, except for jugal areas, densely covered with oval to pear shaped granules. Granules densely packed, well separated, up to 15 μm in length, and usually up to 1.5 times longer than wide. Those close to the jugum more elongated and eventually merge into the jugum (Fig. 3a). Granules raised, flat topped to slightly concave, with a concave anterior slope, and posteriorly slightly raised from tegmental surface. Single macroaesthete (about 8 μm) located at the posterior third of each granule, surrounded by about 14 irregularly arranged microaesthetes (about 2–3 μm). Jugum with macro- and microaesthetes arrangement equivalent to the granules, only with microaesthetes spread evenly between two macroaesthetes (Fig. 3b).

Articulamentum solid, translucent, bright brown. Insertion plates well developed and extending far over the tegmentum. Apophyses large, wing-shaped to triangular in intermediate valves, and trapezoid in the tail valve. Slits sharp, moderately deep and dorsally extend into shallow channels (slit formula: 5/1/2). Eaves not visible.

Radula consisting of 44 teeth rows, 34 rows mineralized. Central tooth 3 μm in width, tulip-shaped and centrally slightly tapered. Blade straight, slightly backwards directed (Fig. 3c). First lateral tooth wing shaped, covering lower half of central tooth. Antero-dorsal corner distinctly knobbed, showing no accessory process. Second lateral tooth elongated, slightly keeled at base, head more or less rectangular, with three large denticles, of which middle one hardly

overlaps the other. First uncinial tooth hammer-head shaped, the second more or less rectangular with anterior margin being notched. Third uncinial tooth slender and spoon-shaped. First marginal tooth diamond shaped, second oval, and third marginal rectangular and larger than first two (Fig. 3d).

Perinotum wide, fleshy, generally brighter than tegmentum, deeply encroaching sutural areas, densely covered with two types of spicules (Fig. 2f). One type solid, rather blunt, medially thickened, and distally slightly striated, measuring up to 320 \times 60–62 μm and second type, straight, pointed and smooth, measuring 80 \times 12–17 μm . Tufts dark brown to greenish, comprising ca. 30 large, straight needles, measuring 1000–1300 \times 70–76 μm , and equal number of smaller, sharp pointed spicules of 400 \times 10–12 μm . Marginal fringe with round, solid spicules up to 40 μm in length with a diameter of 49–53 μm . Hyponotum densely covered with flat, elongated spicules of 120 \times 18–20 μm (Fig. 2g).

Type locality. France, Banyuls sur Mer (42°28'57" N, 03°08'13" E), eulittoral, in the "trottoir" (see habitat notes).

Etymology. The species' name refers to the dense mantle coverage with six spicule types. *Pilosa* is derived from the Latin "pilosus", meaning "covered by hair".

Habitat note. Most specimens, including the holotype, were found in the "trottoir", cavernous rock platforms built by the coralline red alga *Lithophilum tortuosum* (Esper) Foslie, 1900 in the mid-littoral

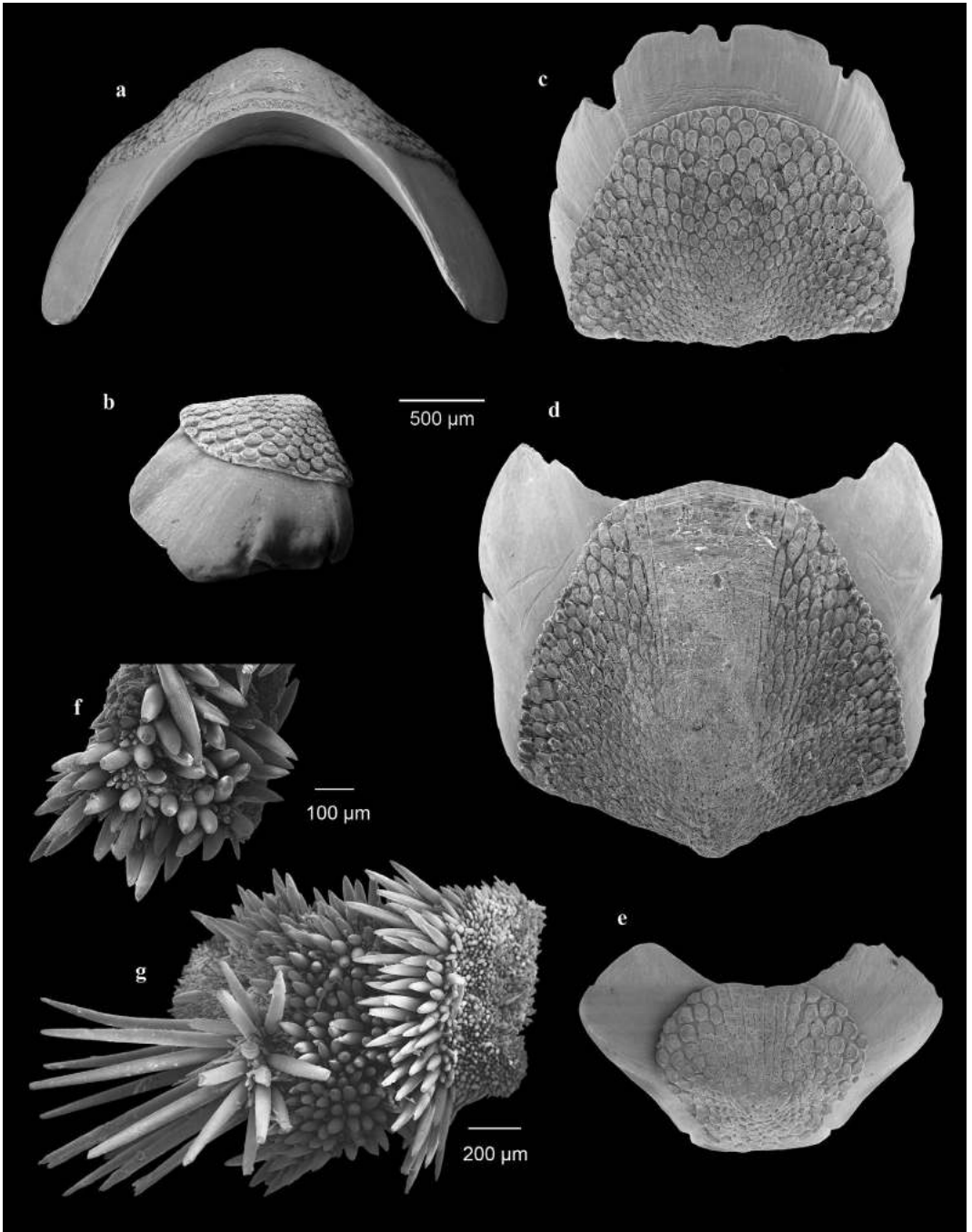


Fig. 2. Valves and girdle elements of the holotype of *Acanthochitona pilosa* spec. nov. (ZSM Mol 20070223) body length 13 mm (SEM). **a.** Frontal view of valve II. **b.** Lateral view of tail valve. **c.** Dorsal view of head valve. **d.** Dorsal view of valve II. **e.** Dorsal view of tail valve. **f.** In situ spicules of the perinotum. **g.** Section through the girdle showing from left to right a tuft, mid-girdle (perinotum) spicules, marginal fringe, and hyponotum spicules. Scale bar: 50 μm for a-e.

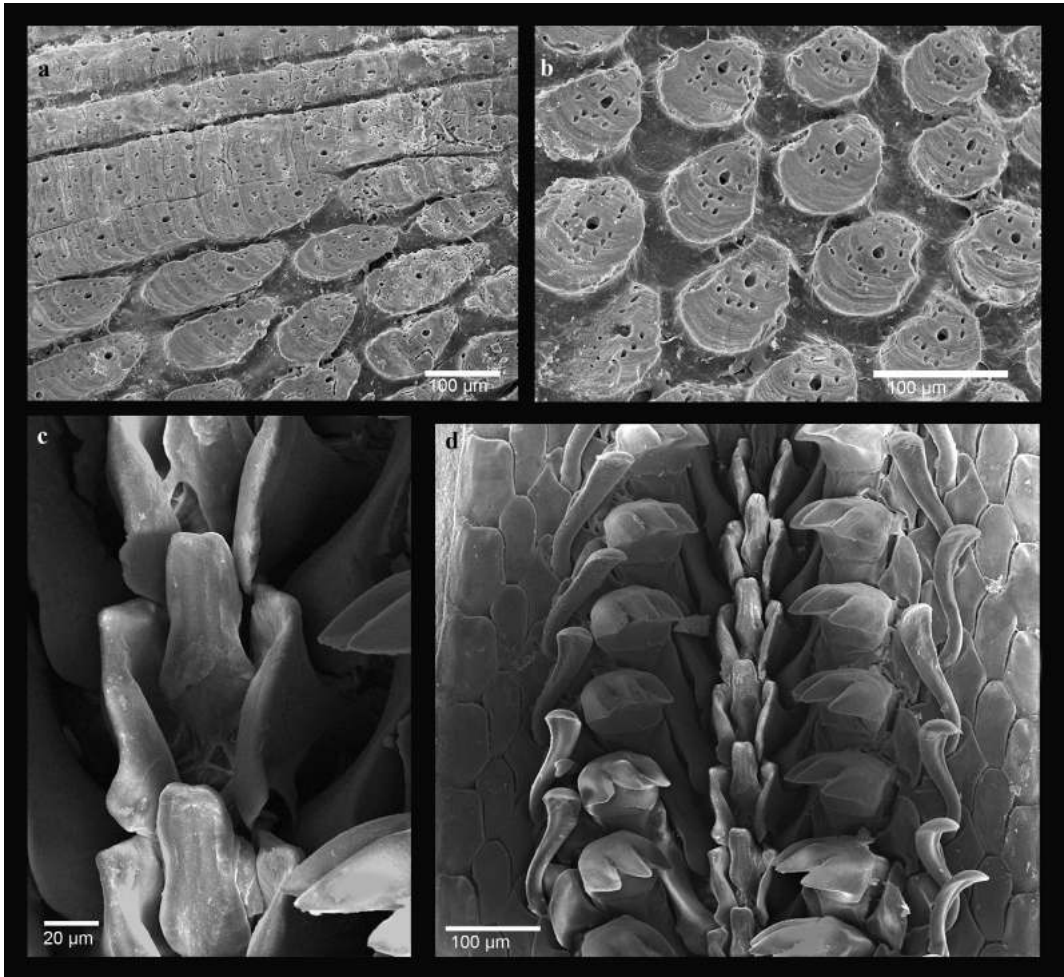


Fig. 3. Radula and tegmentum sculpture of the holotype of *Acanthochitona pilosa* spec. nov. (SEM) (ZSM Mol 20070223). **a.** Dorsal view of valve II junction of jugal area and pleurolateral area. **b.** Detail of pleurolateral area of valve II, showing the aesthete arrangement. **c.** Detail of central tooth and first lateral teeth. **d.** Anterior portion of radula.

zone. These encrustations are commonly found at the Northern coast of the Western Mediterranean (Bressan et al. 1996). Few specimens of *A. pilosa* spec. nov. (10–20 mm) were found below the “trottoir”, attached to and partly within rocky substrate built up again by various coralline red algae, but none were found below 2 m depth.

Distribution. So far recorded only from the type locality within a range of about 2 km, but see discussion.

Observed variability. (Fig. 4) Apart from scattered occurrence of white spots on the tegmentum and a growth-dependent number of teeth rows (up to 58

teeth rows, of which 46 are mineralized, in a 20 mm long specimen), the over-all morphology is quite constant in the observed material (10 specimens; see Table 1).

Remarks. *Acanthochitona pilosa* spec. nov. differs from all other Atlantic and Mediterranean species (see below, and Table 1). All Caribbean congeners, reviewed by Lyons (1988), differ considerably in their shape of valves and granules: *A. hemphilli* (Pilsbry, 1893), *A. rhodea* (Pilsbry, 1893) and *A. ferreirai* Lyons, 1988 have a prominent anterior extension of the jugum that is missing in *A. pilosa* spec. nov. The intermediate valves of *A. balesae* Abbott, 1954 differ

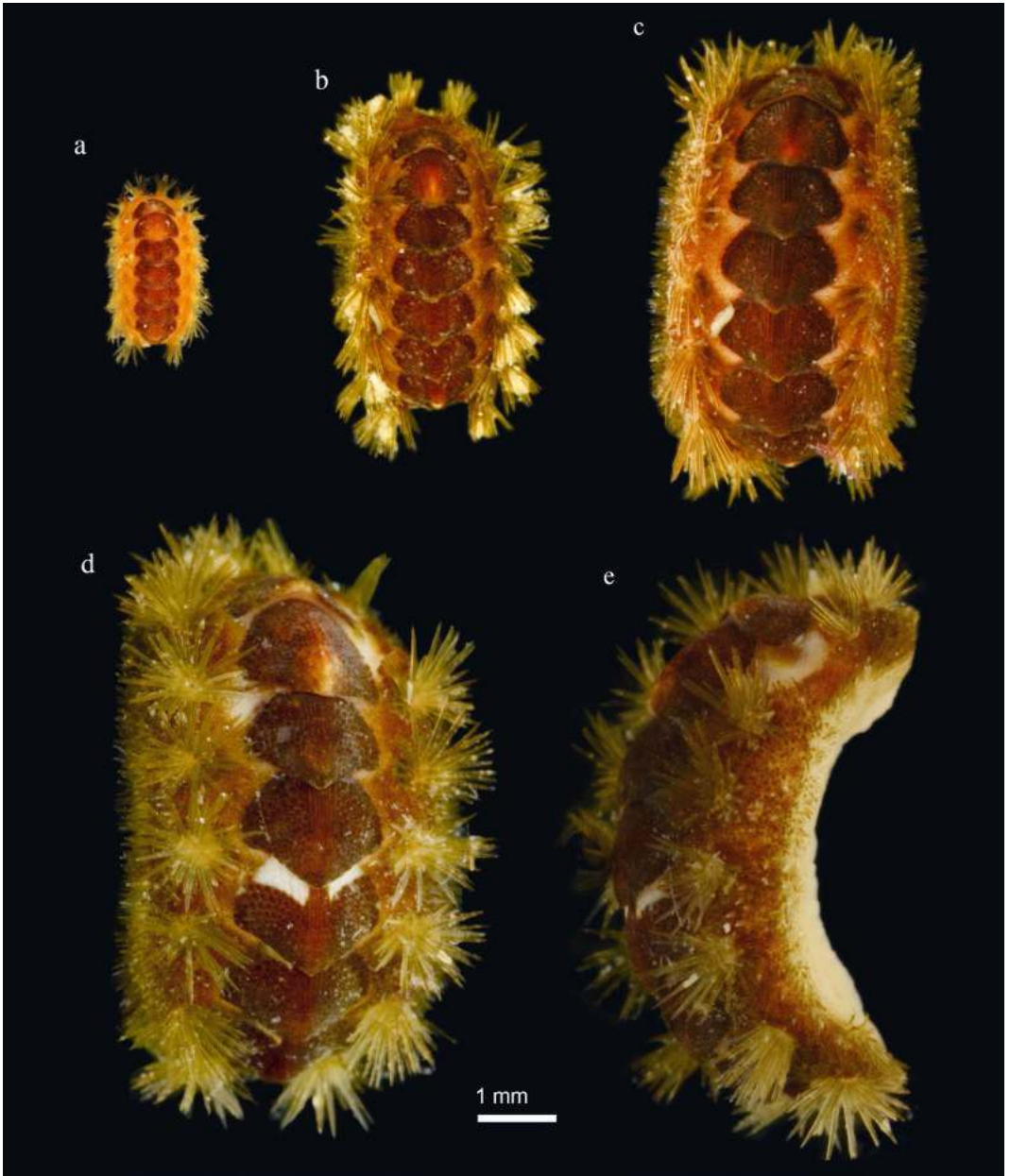


Fig. 4. Different growth stages of *Acanthochitona pilosa* spec. nov. a. Length 3 mm (ZSM Mol 20034395). b. Length 5 mm (ZSM Mol 1996589). c. Length 7 mm (ZSM Mol 1996589). d,e. Length 9 mm (ZSM Mol 1996589).

from the new species in being longer than broad, and of *A. pygmaea* (Pilsbry, 1893), *A. bonairensis* Kaas, 1972, *A. roseojugum* Lyons, 1988 and *A. terezae* Guerra Júnior, 1983 being broader than long, respectively. In contrast to the uniform colour of *A. pilosa* spec. nov., *A. lineata*, Lyons 1988, and *A. zebra* Lyons, 1988, both

show prominent coloured bands on the tegmentum. Specimens of *A. worsfoldi* Lyons, 1988 and *A. spiculosa* (Reeve, 1847) with 21–32 mm body lengths do not show any thick dorsal spicules, which are always present in the herein described species when it exceeds 8 mm. *Acanthochitona astrigera* (Reeve, 1847)

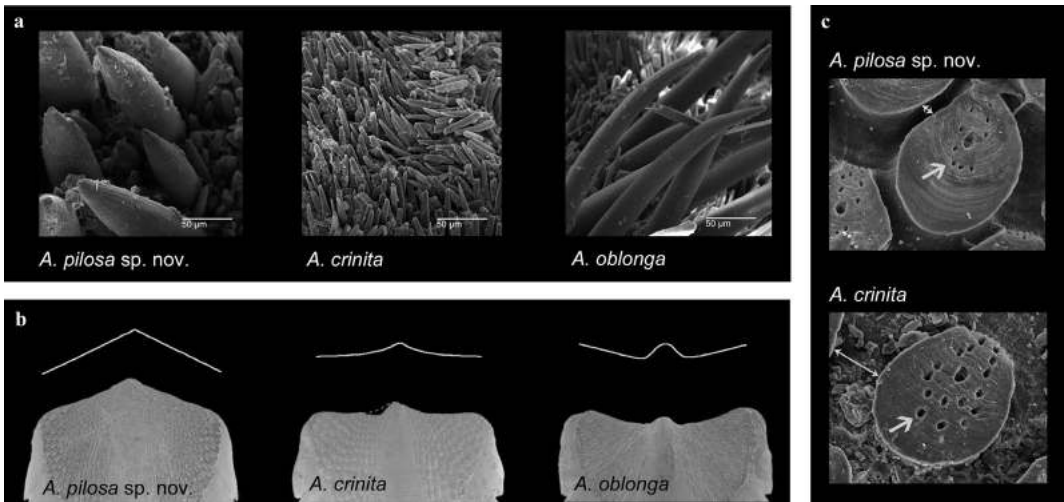


Fig. 5. Morphological differences between *A. pilosa* spec. nov., *A. crinita* and *A. oblonga* (SEM). a. Section of the dorsal part of the girdle (perinotum), scale bar: 50 µm. b. Posterior shape of valve IV. c. Granule with macro- and microaesthetes.

differs by having tufts consisting of considerably more and slender spines. The new species differs from *A. andersoni* Watters, 1981 by the shape of the valves and the distribution of the microaesthetes. It is also distinct from *A. venezuelana* Lyons, 1988 by having microaesthetes that are about $\frac{1}{3}$ of the diameter of the macroaesthetes, whereas they are almost the same size in the latter.

Acanthochitona pilosa spec. nov. can be distinguished from the east Atlantic *A. bowieri* (Rochebrune, 1881) by the shape of the valves, the latter having triangular intermediate valves according to the original description, and also by having a broader jugum. A paralectotype (Kaas 1985: figs 76–83) of *A. joallesi* (Rochebrune, 1881) shows valve IV with a completely different shape than in *A. pilosa* spec. nov., and according to the original description (Rochebrune 1881), the spines are white, in contrast to the dark brown and greenish spines of *A. pilosa* spec. nov. It also differs in colour from *A. subrubicunda* Leloup, 1941, which has rose valves, and further the intermediate valves are as broad as long in the new species, whereas they are much broader than long in *A. subrubicunda*. Brown (1844) describes *A. discrepans* (Brown, 1827) with round granules, acutely carinated valves and an overall colour of an orange-yellow, which all makes *A. pilosa* spec. nov. very distinct from this species with its drop shaped granules, round-backed valves and uniform dark brown colour. Additionally, after Kaas (1985), who designated a lectotype of *A. discrepans*, the dorsal girdle spicules do not exceed 4 µm, which makes them half the size of our smaller spicule type.

Acanthochitona pilosa spec. nov. differs from its three Mediterranean congeners (Kaas 1985), namely *A. fascicularis*, *A. crinita* and *A. oblonga* (Table 1), particular by showing thick, short dorsal spicules (Fig. 5a) on the girdle and by its distinctly different shape of the valves (Fig. 5b). Additionally, the microaesthetes of *A. pilosa* spec. nov. are considerably smaller ($\frac{1}{2}$ of the diameter, Fig. 5c) than those in *A. crinita* (Pennant, 1777). *A. fascicularis* (Linnaeus, 1767) shows a distinct incision in all granules, which is never present in *A. pilosa* spec. nov. Moreover, *A. fascicularis* has a well separated, raised jugal area and roundish granules. The distinctive feature of *Acanthochitona oblonga* Leloup, 1981, are extremely elongated, widely spaced granules, whereas they are small, oval and packed in *A. pilosa* spec. nov. *Acanthochitona pilosa* spec. nov. has nothing in common with the three congeners from the Red Sea, *A. penicillata* (Deshayes, 1863), *A. mahensis* Winckworth, 1927 and *A. mastalleri* Leloup MS, Strack, 1989 (revised in Anseeuw & Terryn 2004). Superficially, the most similar species is the recently described *Acanthochitona britayevi* Sirenko, 2012 from shallow water oyster beds in Viet Nam. This species has a comparable size, a chestnut colouration interrupted with blackish and white blotches, a deeply encroaching girdle and similar shaped valves of equal dimensions. It differs, however, from the present species in having a) flattened valves with elongate flat pustules, b) a perinotum coverage of tiny oval scale (up to 15 µm in length), c) “hundreds” of shorter (up to 80 µm in length) white tuft needles, d) marginal spicules of about 68 µm in length, and e) ventral girdle scales

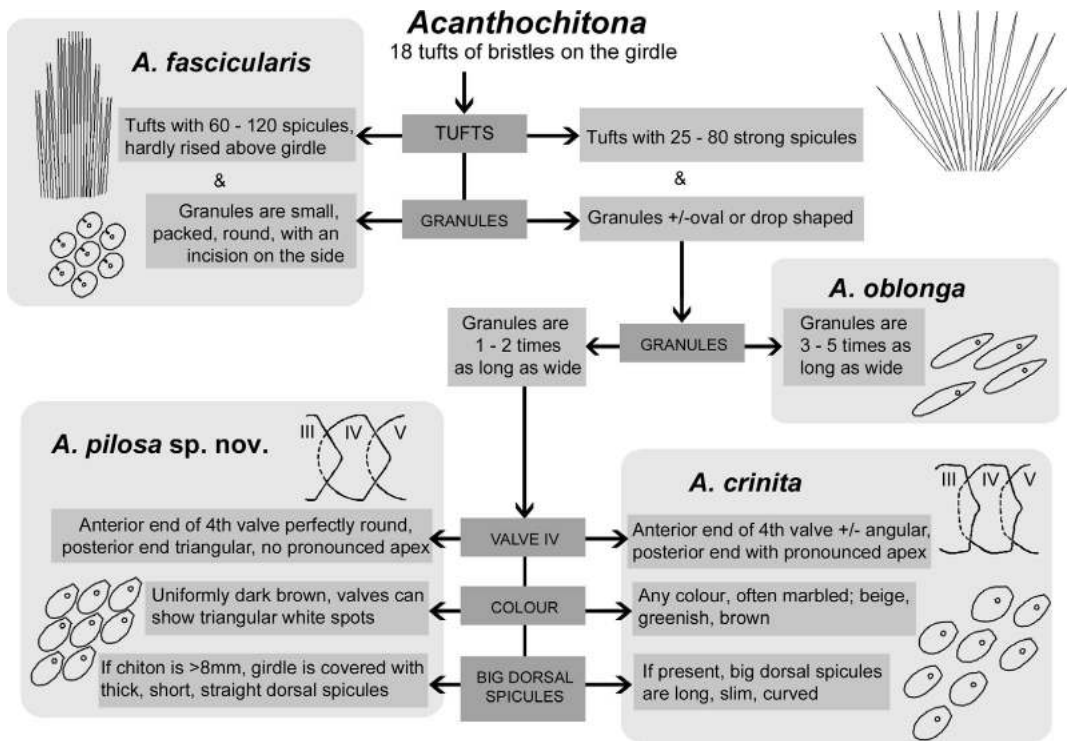


Fig. 6. Illustrated key to the *Acanthochitona* of the Mediterranean Sea. Key is based on external, macroscopic morphological features.

with a maximum dimension of $60 \times 11 \mu\text{m}$. Additionally, despite an overall similarity of the intermediate valves, their shape is more squeezed in *A. britayevi*, due to the lesser pronounced apical region.

Discussion

Acanthochitona pilosa spec. nov. was exclusively found in habitats within the well mixed eulittoral zone at the type locality. Co-occurring *Acanthochitona* species were observed mostly in low-oxygen microhabitats under stones or in clefts and with a substantially larger depth range, conforming to existing literature (Dell'Angelo & Smriglio 2001). Although some small *A. crinita* specimens were found being attached to the surface of the "trottoir", only *A. pilosa* spec. nov. seemed to claim the ecological niche of living inside the encrustations of the red algae *Lithophyllum tortuosum*. To the best of our knowledge, there is no literature mentioning evidence for any invertebrate species restricted to the "trottoir".

So far the only known specimens of *A. pilosa* spec. nov. were collected in close vicinity of the type locality. However, images of two specimens of

"*Acanthochitona crinita*" in Dell'Angelo & Smriglio (2001: figs 124, 125) collected in unknown habitat in Riomaggiore, Italy, in the Ligurian Sea, about 500 km away from Banyuls sur Mer, appear to be *A. pilosa* spec. nov., which suggests a distribution range at least throughout the Ligurian Sea. Those specimens also show dense packed granules, thick spicules, a striated jugum and white spots on the dark valve. Re-examination of this material however failed, as the specimens are missing since the book was published (Bruno Dell'Angelo, pers. comm.). Considering their close association, the distribution of *A. pilosa* spec. nov. may follow the distribution of the "trottoir", formed by *Lithophyllum tortuosum*, which is present only in the Western Basin of the Mediterranean Sea (Péres & Picard 1952). In Tunisia, a similar habitat, build by other coralline algae, is inhabited by several mollusc taxa, but no Polyplacophora are mentioned in the study (Thornton et al. 1978). That's why we suspect the species to be indeed restricted to the Western Basin of the Mediterranean Sea. However, this is speculative and requires further sampling for verification.

Acanthochitona pilosa spec. nov. shows a very consistent morphology at all sizes that could be

collected, ranging from 3 mm to 20 mm specimens. Although in specimens smaller than 8 mm the thick dorsal spicules are absent and the other types of spicules are proportionally thinner and shorter, the specimens can readily be assigned to *A. pilosa* spec. nov., with naked eye by the shape of valve IV, and their consistent uniform colour pattern. There is no single morphological feature with which all four Mediterranean species can be distinguished. The size, shape and arrangement of the granules, combined with the number and especially the size of the microaesthetes is certainly the most reliable way to assign a specimen to the species. Other factors, such as the perinotum have been considered of minor importance by some authors because of their high variability (Bonfitto et al. 2011). However, as the granules are hardly visible with naked eye, and the microaesthetes can only be counted and measured using scanning electron microscopy, we consider the spicules and more general the appearance of the girdle as valuable feature to distinguish the species in the field. In combination with the outline of valve IV and the shape and arrangement of the granules it allows to unambiguously discriminate all four Mediterranean species (see Fig. 6).

Considering the morphological characters, especially the shape and size of the granules, microaesthetes and spicules, *A. pilosa* spec. nov. shows most

similarities with *A. crinita* and *A. oblonga*, but more difference than between the two of them. Therefore we could speculate that *A. pilosa* spec. nov. might be sister taxon of *A. crinita* and *A. oblonga*, being closer related to them than to *A. fascicularis*. Although molecular data, due to the fixation of the specimens in formalin, could not be included in this study, it will be interesting to use these data to correctly place this species in the existing tree of *Acanthochitona* (Bonfitto et al. 2011).

Measurements of the spicules and granules differ slightly between authors (Table 1). This could be due to the fact that especially *A. crinita* is considered as a very variable species (Kaas 1985), but also, these measurements were taken by different authors, with different methods, and some measurements (in parentheses) are only inferred from published figures and pictures. Also, own measurements on the specimens available of *A. pilosa* spec. nov. and *A. crinita* suggest that, even though granules and spicules are proportionally smaller in a large individual, the absolute size is slightly larger in big individuals. But overall, the differences between species are larger than the variability within a species. The high variability of Mediterranean *Acanthochitona* and contradicting descriptions by different authors could also indicate the presence of cryptic species, as supported by this example of a new species, found

Table 1. Overview of morphological differences between the *Acanthochitona* species of the Mediterranean, including *A. pilosa* spec. nov. Information from: ¹ Personal observation, ² Bonfitto et al. 2011, ³ Kaas 1985, ⁴ Leloup 1981, ⁵ Fischer 1979; parenthesized information inferred from figures and pictures.

	<i>A. pilosa</i> spec. nov. ¹	<i>A. crinita</i>	<i>A. oblonga</i>	<i>A. fascicularis</i>
Shape of granules	Oval to pear shaped	Round, oval or pear shaped ³ Oval to drop shaped ¹ Oval to ± elongated drop shaped ²	Oval, very elongated ^{1,2,4}	Round to oval, with deep incision ^{1,2,3}
Granules	Densely packed	Moderately wide spread ^{1,3}	Widely spread ^{1,2,4}	Densely packed ^{1,3}
Microaesthetes (number)	0–18	12–16 ^{1,2}	6–9 ^{1,2}	0–3 ⁵ , 0–5 ²
Microaesthetes size (diameter)	2–3 µm diameter	3.5–5 µm ¹ (4.5–6 µm ²)	4.5–6 µm ¹ (4.5–8 µm ²)	(7 µm diameter ⁵)
Macroaesthetes size (diameter)	9–12 µm	7–8 µm ¹ (11–14 µm ²)	8–11 µm ¹ (8–12 µm ²)	(6.5–8 µm ² ; 10 µm ⁵)
Diameter of small spicules on perinotum	12–17 µm	6–9 µm ¹ (7–11 µm ²)	(11–14 µm ²)	(17–21 µm ²) 20 µm ³
Large spicules on perinotum	Always present (individuals with length <8 mm), dense, straight	Absent or sparse; if present, curved ^{1,2,3,4}	Sparse, long, curved ^{1,2}	Absent ² /present ^{1,2,3}
Diameter of large spicules on perinotum	60–62 µm	27–31 µm ¹	(14 µm ⁴) 23–28 µm ¹	12 µm ³
Long needles in tufts	<30 spicules; 70–76 µm diameter	25–80 spicules ^{1,2,3} 40–45 µm diameter ²	30–50 spicules ¹ 26–35 µm diameter ¹	60–120 spicules ¹ 13–24 µm diameter ²

in a relatively well accessible habitat. It suggests that chitons could be understudied in the Mediterranean, and that a more detailed investigation might reveal even more species of Polyplacophora.

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