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A new record and redescription of *Acanthochitona crinita* (Pennant, 1777) from the Bulgarian Black Sea coast

(Mollusca, Polyplacophora)

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The chiton species *Acanthochitona crinita* (Pennant, 1777) is newly recorded from the Bulgarian sector of the Black Sea and is redescribed. New morphological, ecological, and chorological data about the *Acanthochitona crinita* populations from the Black Sea are provided. The differences between *Acanthochitona crinita* and *A. fascicularis* (Linnaeus, 1767) are clarified.

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Introduction

Between 1989 and 2014 zoological material was extensively collected in the rocky sublittoral biocoenosis of the Bulgarian Black Sea coast. While paying attention to diverse invertebrate groups and especially to epibionts on diverse hard substrates (such as concrete tetrapods, stones, shells, plastic debris) a few specimens of *Acanthochitona crinita* (Pennant, 1777), a species previously unknown to the Bulgarian malacofauna, were collected.

Until now, this species has been only reported by Van Belle (1983–1986: fig. 105) for the Ukrainian Black Sea coast and for the Bosporus region (Fig. 1). Nevertheless, the literature dealing with the Black Sea fauna contains information solely about another representative of this genus – the great bristly chiton *Acanthochitona fascicularis* (Linnaeus, 1767). This species has been mentioned by many authors for the Black Sea shores of Romania, Ukraine, Georgia, and Turkey (Fig. 2) (Ulyanin 1872 (after Anistratenko & Anistratenko 2001), Milashevich 1909 (after Anistratenko & Anistratenko 2001), Zernov 1913, Milashevich 1916, Bekman 1940, Yakovleva 1952, Băcescu et al. 1971, Starobogatov 1972, Müller 1973, Vlastov & Matekin 1988, Anistratenko & Anistratenko 2001, Demmir 2003, Revkov 2003, Mazlumyan et al. 2003, 2004, Gönlügür-Demirci & Katagan 2004, Revkov & Sergeeva 2004, Demirci 2005, Çulha et al. 2007, 2010, and Gozler et al. 2010).

The apparent lack of detailed information on *Acanthochitona crinita* from the Black Sea stimulated the inclusion of extensive information on the morphology and the ecology of the species. Moreover, while identifying the *Acanthochitona crinita* material and comparing it to specimens of a similar species (*Acanthochitona fascicularis*) some discrepancies in the Russian and Ukrainian literature were observed, where the characters of *A. fascicularis* are systematically confused with these of *A. crinita*. Because these literature sources are still used for determination of malacological samples from the Black Sea, the most important taxonomical/morphological traits for both species are summarised and the data on their distribution throughout the Black Sea basin is reviewed in the hope of providing an update to the information on the morphology and chorology of these related species and to facilitate their reliable identification in the future.

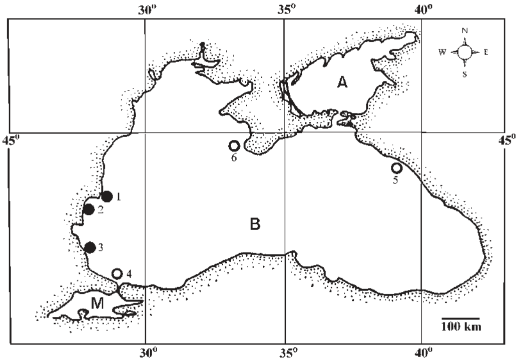


Fig. 1. Distribution map of *Acanthochitona crinita* (Pennant, 1777) in the Black Sea. Localities: **1**, region of Kamen Bryag village (Bulgaria); **2**, resort Sts Constantine and Helena (Bulgaria); **3**, city of Akhtopol (Bulgaria); **4**, Bosporus region (Turkey); **5**, Tuapse: on Cliff Slyoz (= cliff Kiselyova) (Russia); **6**, west-southwest Crimean region (? Sevastopol). **A**, Sea of Azov; **B**, Black Sea; **M**, Sea of Marmara. **○**, literature records; **●**, present data. For literature records see sources in "Chorological data", Discussion part.

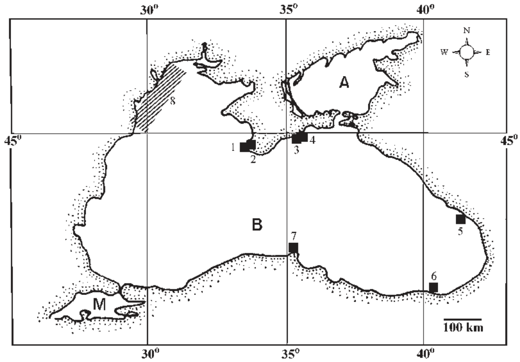


Fig. 2. Distribution map of *Acanthochitona fascicularis* (Linnaeus, 1767) in the Black Sea based on literature records. Localities: Crimea: **1**, Sevastopol Bay; **2**, Juzhnaya buhta (= Juzhnaya Bay); **3**, Lisya Bay; **4**, in the region of Karadag biological station, on Ivan Razboynik-rock. Georgia: **5**, Sukhumi shore. Turkey: **6**, Rize shore; **7**, Sinop coastline. **8**, near Romanian-Ukrainian Black Sea coast (the exact locality position is unknown). **A**, Sea of Azov; **B**, Black Sea; **M**, Sea of Marmara (from the literature; for references see "Introduction").

Material and methods

Collecting sites and effort

It is known that the chitons occurring in the Black Sea are petrophilous species, common in shallow water (Marinov 1990, Hubenov 2005, 2007a,b).Therefore collecting efforts were focussed mainly on the rocky sublittoral, with a total of 29 sites sampled (25 sites along the northern Black Sea coast and 4 along the southern Black Sea coast).

At each locality a minimum of 15 stones were gathered from depths between 0.4 and 2.0 m, and examined on land for the presence of epibionts; the stones had dimensions suitable for collecting and subsequent handling – i.e. with sizes between 0.09 × 0.20 × 0.08 and 0.5 × 0.2 × 0.2 m, and weight below 15 kg. It was found that except on stones, chitons may often be collected on the shells of *Rapana venosa* Valenciennes, 1846, so the collecting efforts were also focused at recovering some veined whelks as well. The latter were lighter and less voluminous than the stones where chitons occur, with shell and body wet weight ranging from 46.0 g to 553.9 g (see Gollasch 2006) which were much more easily and convenient to collect and handle. Additionally, other hard substrates, i.e. concrete tetrapods, empty bivalve shells, plastic pieces, were also examined for chitons by snorkelling (free-diving). Only the *Rapana venosa* shells from the shore of Bulgarevo village [locality of Dulboka (the mussel farm)] and Akhtopol city were taken by scuba-diving from 10–24 m depth. Altogether 420 stones and 3185 *Rapana venosa* shells from the northern Black Sea coast were examined for chitons, while on the southern Black Sea coast 45 stones and 200 kg *Rapana venosa* shells (approximately 1000–1250 specimens of 160-200 g each; T. Stoyanov, pers. comm.) were sampled.

Below is the list of sites (in order from north to south) where the newly recorded chiton was sampled; for each site, the number of collected and examined *Rapana venosa* shells is provided:

Northern Bulgarian Black Sea coast

Site No 1: SW of Kamen Bryag village, Archaeological Reserve Yailata. 43°25'31.63"N, 28°31'52.52"E (Fig. 26), rocky bottom, 02.VIII.2010, depth 1–3 m, leg. Plamen Mitov (PM) & Zahari Petkov: 51 specimens *Rapana venosa* (h/w: 5.5/4.5 – 9.0/7.0 cm); 15.VIII.2010, depth 0.5–1.5 m, leg. PM: stones checking.

Site No 2: Sts Constantine and Helena resort. International Home of Scientists "Frederic Joliot-Curie" beach (Joliot-Curie beach) (Fig. 27), 43°13'47.54"N, 28°00'55.00"E, sandy bottom, on rocks and on breakwater rocks, leg. PM, 01.VII.2002, depth 1–2.5 m: 205 specimens *Rapana venosa*; 25.–29.VI.2004, depth 1–1.5 m: stones checking; 26.VI.–14.VII.2005, depth 0.4–1 m: stones checking; depth 2–4 m: 348 specimens *Rapana venosa*; 03.VIII.2008, depth 1–2 m: 128 specimens *Rapana venosa* (h/w: 2.1/1.5 – 9.0/6.5 cm); 25.VII.–10.VIII.2009, depth 1–2.5 m: 130 specimens *Rapana venosa* plus one empty *R. venosa* shell; 14.VIII.2010, depth 1–4 m: 158 specimens *Rapana venosa* (h/w: 4.0/2.8 – 8.3/6.0 cm) and 5 empty *R. venosa* shells; 11.–16.VII.2011, depth 1–4 m: 122 specimens *Rapana venosa* (h/w: 5.0/3.2 – 9.0/6.5 cm); 20.VII. 2011, depth 2–4 m: 73 specimens *Rapana venosa* (h/w: 4.3/3.2 – 8.5/6.5 cm) and stones checking; 10.IX.2011, depth 1–3 m: 76 specimens *Rapana venosa*; 04.X.2011, depth 2–4 m: 46 specimens *Rapana venosa* (h/w: 5.2/4.0 – 8.0/5.5 cm); 03.VIII.2012, depth 2–4 m: 110 specimens *Rapana venosa* (h/w: 4.5/3.0 – 8.5/6.6 cm); 19.VII.–07.VIII.2013, depth 1.5–2 m: 63 specimens *Rapana venosa* (h/w: 4.0/2.9 – 8.3/6.8 cm) and stones checking.

Southern Bulgarian Black Sea coast

Site No 3: Akhtopol. 42°06'23.82" N, 27°56'43.84" E, 300 m opposite the old Greek school (Fig. 28), approx. 200 kg *Rapana venosa* (estimated approximately 1000–1250 specimens) have been hand-collected by scuba-diver Mr. Todor Stoyanov (city of Ahtopol) from rocky bottom, covered with *Mytilus galloprovincialis* at depths ranging from 18 to 24 m; these shells have been kindly checked for epibionts by Dr. Zdravko Hubenov (NMNHS).

Acanthochitona crinita material

Northern Bulgarian Black Sea coast

Site No 1: (Figs 1, 26), depth 1 m, under stones (among 15 stones), 15.VIII.2010, leg. PM (CPM) – 1 adult specimen (L × W: 14 × 7.5 mm) (Figs 3, 29) from underneath a stone covered with red algae *Phymatolithon lenormandii* (Areschoug) W. H. Adey, 1966 and the bay barnacle *Amphibalanus improvisus* (Darwin, 1854), the chiton was partly covered by the beadlet anemone *Actinia equina* (Linnaeus, 1758) at the moment of capture (Fig. 29).

Site No 2: (Figs 1, 27), depth 1-4 m, on *Rapana venosa* shells (among 1465 *Rapana*-shells), leg. PM (CPM), 01.VII.2002 – 1 mature female specimen (L × W: 9.9 × 5.8 mm) (with eggs) (Fig. 4); 14.VII.2005 – 1 juvenile specimen (L × W: 2.55 × 1.65 mm); 14.VIII.2010 – 1 adult specimen (L × W: 14 × 7.5 mm) (Figs 5, 30).

Southern Bulgarian Black Sea coast

Site No 3: (Figs 1, 28), depth 18-24 m, on *Rapana venosa*-shell (among 1000-1250 *Rapana*-shells), 30.VII.2006, leg. Z. Hubenov, (CPM) – 1 juvenile specimen (L × W: 4.7 × 3.8 mm).

Other chitons

The following *Acanthochitona fascicularis* were available for comparative study: 5 specimens (one used for the SEM study) (L$\bar{x}$: 25.9 mm (22.9–28.9 mm, n = 5); W$\bar{x}$: 15.5 mm (13.6–17.0 mm, n = 5)), unknown locality, label: “automne 1919”, det. P. Kaas, 1981, MHNG, No 981/717 (Fig. 6); 5 specimens (L$\bar{x}$: 20.7 mm (15.3–29.7 mm, n = 3; the other 2 specimens were rolled and couldn’t be measured accurately); W$\bar{x}$: 14.6 mm (13.3–16.1 mm, n = 3)), unknown locality, det. P. Kaas, 1981, MHNG, No 981/719.

A few more *Acanthochitona fascicularis* specimens were available for comparative examination, i. e. 2 specimens (labelled as *Acanthochitona communis* Risso, 1826): (L × W: 22.1 × 16.7 mm; and L × W: 16.2 × 12.4 mm)), north-western France, “Roscoff”, 1955, leg. & det. (?), MHNG, No 955/102.

The collected chiton material was preserved in 75 % ethanol and is deposited in the personal malacological collection of the author (CPM, see “Symbols and abbreviations used” below). All the measurements were made under a MBS-9 stereoscopic binocular microscope (JSC “Lytkarino Optical Glass Factory”, Russia) and a Zeiss microscope equipped with an ocular micrometer. The rhachidian teeth were measured according to the scheme in Fig. 24h. Chitons were photographed under an Olympus BX41 SZ61 stereo microscope with a mounted Olympus Colour View 1 digital camera. Digital images captured at different focal planes were assembled using the Combine ZM application. Light microscope-photos of the radula were taken with a Canon PowerShot A520 digital camera through the oculars of the Zeiss microscope.

To investigate the spicule morphology, parts of the perinotum in fixated chitons were carefully cut off. Some of these, after rinsing with distilled water and dehydrated for ½ hour in 95 % and 100 % alcohol, were used for the SEM investigation, while others after cleaning for about 5–6 hours in 10 % KOH and following the same pre-treatment as the material for SEM, were mounted on microscopic slides in glycerol. To establish the tegmentum granule density in the adults of *Acanthochitona* Gray, 1821, the granules per 1 mm^2, from 10 areas on the tegmentum of IV valve were counted.

The radulae were studied by SEM and from temporary glycerol mounts, after cleaning for about 5–6 hours in 10 % KOH, washing in distilled water, and dehydrated in a graded ethanol series to 100 % ethanol. Only typically shaped and normally developed radular teeth from the medial parts of the radula were measured and photographed.

To investigate the egg hull surface in *A. crinita*, the eggs were dissected from the ovary. Thereafter the eggs were rinsed with distilled water and dehydrated for ½ hour in 95 and 100 % alcohol, before being used for the SEM investigation.

All the investigated structures – valve pieces, perinotum parts, girdle elements, radulae, eggs – were air-dried, mounted onto aluminium stubs coated with double-sided sticky tabs and sputter-coated with a 300–400 Å gold layer (Jeol JFC-1200 sputter), then examined in a Jeol JSM-5510 scanning electron microscope at 10–20 kV in high vacuum mode.

The *Acanthochitona* material was identified by the features listed in Kaas (1985), Van Belle (1983 1986), Jones & Baxter (1987), and Dell’Angelo & Smriglio (1999). The taxonomy has been updated to reflect the modern classification status, mostly relying on Schwabe & Gofas (2009) from the World Register of Marine Species website (http://www.marinespecies.org/aphia.php?p=taxdetails&id=55). The synonymy follows Kaas (1985), Kaas & Van Belle (1981, 1998), Dell’Angelo & Smriglio (1999, 2001), Anistratenko & Anistratenko (2001), and Dell’Angelo & Zavodnik (2004). Chiton terminology follows Schwabe (2010). The terminology in egg morphology follows Eernisse & Reynolds (1994).

Symbols and abbreviations used

AWR	width of apical part of rhachidian tooth
BWR	width of basal part of rhachidian tooth
CPM	in malacological collection of the author
h	shell height
L	body length
LR	length of rhachidian tooth
L$\bar{x}$	average body length
max	maximal
MHNG	Muséum d'Histoire Naturelle de Genève, Switzerland
MWR	width of middle part of rhachidian tooth
NMNHS	National Museum of Natural History, Sofia, Bulgaria
n	sample size
R	rhachidian tooth
SC	first lateral teeth
SD	standard deviation
SEM	scanning electronic microscopy/scanning electron micrographs
UAWR	width under apical part of rhachidian tooth
W	body width
w	shell width
W$\bar{x}$	average body width
$\bar{x}$	mean
±	standard error of the mean

Results

According to the summarised literature data and direct observations, the total number of chiton species found in the Bulgarian Black Sea shores increases to three. These include the hitherto known *Lepidochitona cinerea* (Linnaeus, 1767), *Lepidochitona caprearum* (Scacchi, 1836) (cf. Chichkoff 1912, Paspaleff 1933, Valkanov 1957, Hubenov 2007b), and the newly recorded *Acanthochitona crinita*. The latter seems to be relatively rare, as until now only five specimens have been collected (three adults and two juveniles) from the coast of Kamen Bryag village, Sts Constantine and Helena resort, and the city of Akhtopol (Figs 1, 3–5, 26–30).

In the literature there is apparently no data concerning *Acanthochitona crinita* from the Black Sea; therefore, a detailed morphological study based on the materials collected from the Bulgarian seashore, with ecological notes, as well as maps which summarise the chorological data currently available for both representatives of genus *Acanthochitona* in the Black Sea basin (Figs 1–2) is presented here.

Characteristics of the Bulgarian Black Sea *Acanthochitona crinita* sample

Morphological notes

Length. The Bulgarian adult specimens of *Acanthochitona crinita* measure L = 9.9–14.0 mm and W = 5.8–7.5 mm (female: L = 9.9 mm, W = 5.8 mm).

Tegmentum colour. The valve granules in the Bulgarian specimens of *A. crinita* are brown, yellowish-white and bluish-green with a light centre, and form patches of the same colour. The jugal area (excl. that on VIII valve) each with 8–11 dark (brown, blackish-brown) longitudinal dotted lines (Figs 3–5).

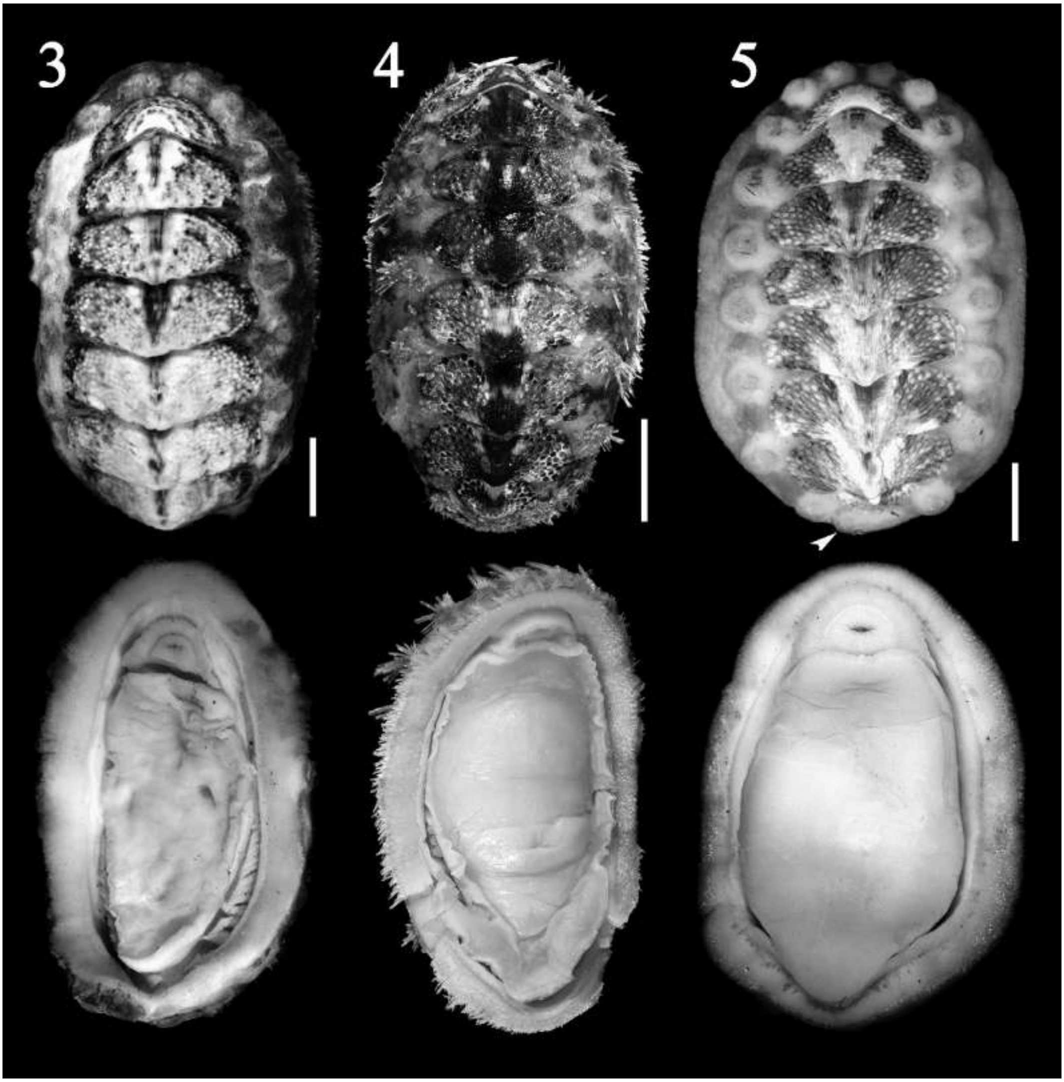
Tegmentum sculpture/ornamentation (Figs 7, 9). The valve tegmentum is covered with ellipsoidal, teardrop-, and pear-shaped, distinctly separate granules (see also information in Table 1: "Morphology of the tegmentum granules"). The jugal area is hardly raised, not sharply separated from the latero-pleural areas (see also Kaas 1985, Dell'Angelo & Smriglio 2001).

Perinotum is yellowish-white, with brown reticulate patches formed by short spicules aligned in circles between the bristle tufts (Figs 3–5, 14–15). In some specimens, these brown spicular patches coalesce in broader spicular fields at the periphery of the perinotum (see Fig. 3).

Perinotum elements
Dorsal girdle spicules and bristly tufts
In *A. crinita* (the female specimen from Sts Constantine and Helena resort, Fig. 4) the girdle tufts consist of three types of transparent elements (Fig. 12):

1) bristles – length: $\bar{x}$ = 676.2 ± 32.28 µm, SD = 125,03 (415.86–811.44 µm, n = 15), with diameter: $\bar{x}$ = 35.62 ± 1.03 µm (27.5–40.6 µm, n = 19);
2) shorter and thinner spicules – length: $\bar{x}$ = 216.8 ± 29.18 µm, SD = 116.72 (120–456.4 µm, n = 16), with diameter: $\bar{x}$ = 15.23 ± 2.51 µm, SD = 8.32 (7.5–30 µm), n = 11);
3) very short spicules – length: $\bar{x}$ = 181.86 ± 11.44 µm, SD = 32.35 (142–225 µm, n = 8), with diameter: $\bar{x}$ = 7.75 ± 0.25 µm, SD = 0.56 (7.5–8.75 µm, n = 5).

The bristly tufts are surrounded by very tiny, translucent and sharply-pointed spicules with an ellipsoidal cross-section (length: $\bar{x}$ = 36.43 ± 2.17 µm, SD = 5.75 (30–42.5 µm, n = 7); with width: $\bar{x}$ = 5.59 ± 0.39 µm, SD = 1.04 (5.0–7.5 µm, n = 7)) (Fig. 16). Among the dorsal girdle spicules there are also small, translucent obtusely-pointed spicules, which are laterally compressed and have an ellipsoidal cross-section (Figs 14–15), which may be brown or colourless (length: $\bar{x}$ = 39.48 ± 1.44 µm, SD = 7.74 (27.5–55 µm, n = 29); with width: $\bar{x}$ = 6.85 ± 0.2 µm, SD = 1.09 (5.0–7.5 µm,



Figs 3–5. *Acanthochitona crinita* from the Bulgarian Black Sea coast; adults in dorsal (top) and ventral (bottom) views: **3.** specimen from Kamen Bryag village; **4.** female, from Sts Constantine and Helena; **5.** specimen from the same locality as in "**4.**" with an extra tuft behind the tail valve (arrowed). Scale bars: 2 mm.

n = 29). These spicules form round patches between the bristly tufts (Fig. 14, arrowed).

Large curved spicules/spines also occur randomly (Fig. 17) (length (measured from top to top): $\bar{x} = 174.5 \pm 13.95$ µm, SD = 31.19 (125–200 µm, n = 5), with max diameter: $\bar{x} = 21.5 \pm 1.0$ µm, SD = 2.24 (20–25 µm, n = 5)) and are found on the perinotum (most commonly in the area between the bristly tufts, the zone beneath these and margin).

The girdle margin is fringed with spicules bearing longitudinal grooves (Fig. 18), most being translucent and without colour, but some are yellowish-brown and longer than the dorsal ones (length: $\bar{x} = 387.9 \pm 27.8$ µm, SD = 96.53 (172.4–517.3 µm, n = 12); with diameter: $\bar{x} = 28.5 \pm 0.78$ µm, SD = 2.71 (25–32.5 µm, n = 12)).

Hyponotum elements (Fig. 20)**.** These are represented only by transparent and colourless, finely striate scales, with length: $\bar{x} = 96.6 \pm 9.86$ µm, SD = 36.9 (57.5.0–180.0 µm, n = 14) and max width: $\bar{x} = 16.34 \pm 1.24$ µm, SD = 4.64 (12.5–27.5 µm, n = 14).

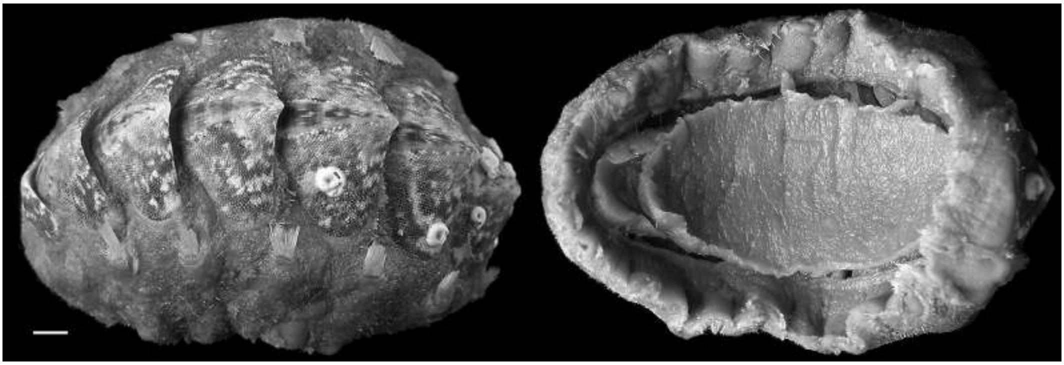


Fig. 6. *Acanthochitona fascicularis*: complete specimen in dorsal (left side), and ventral (right side) view (MHNG: No 981/717; L: 26.24 mm). Scale bar: 2 mm.

Radula (Figs 22, 24a–c, Table 1). Rhachidian teeth in *A. crinita* are bottle-shaped, apically wide, subapically constricted, centrally widened again, and basally strongly constricted (Figs 22, 24a–c). The meristic data for the examined *A. crinita* are presented in Table 1 and Figure 24h. In *A. crinita* the distal angle of the first lateral tooth is rounded (Fig. 24a–c, arrowed), its frontal part is strongly bent medially, and encompasses the broadened median part of the rhachidian tooth.

Arrangement of the gills. Abanal, merobranchial type, investigated adult specimens have 13/13 ctenidia (female, L: 9.9 mm), 14/14 (L: 14 mm), and 16/16 (L: 14 mm) (see also Matthews 1953, Sirenko 1993).

Egg hull surface structure. In the gonads of a female specimen from Sts Constantine and Helena resort (site No 2; 01.VII.2002) eggs with a diameter of 203–284 µm ($\bar{x}$ = 236 µm, n = 4) were found. The egg hull surface structure in *A. crinita* was hitherto unknown (cf. Sirenko 1993, John Buckland-Nicks, pers. comm.) and is documented in Figure 25 for the first time. The collapsed hull cupules are hexagonal in shape, with wide bases, similar to those in the other members of the genus (see Eernisse 1984: plate 23a, Sirenko 1993: fig. 11D). In *A. crinita* series of micropores are visible between cupules where their hexagonal bases meet (Fig. 25b, arrowheads). The micropores present in these specific regions of the egg surface is typical for species that have egg hulls with closed cupules (i. e. *Acanthochitona* species, see Buckland-Nicks, 1993, 1995, 2006, 2008; Buckland-Nicks & Hodgson 2000); the area covered by the cupules is unavailable to the sperm thus directing them to the intercupule area, where the micropores provide direct access to the vitelline layer (Buckland-Nicks 1993).

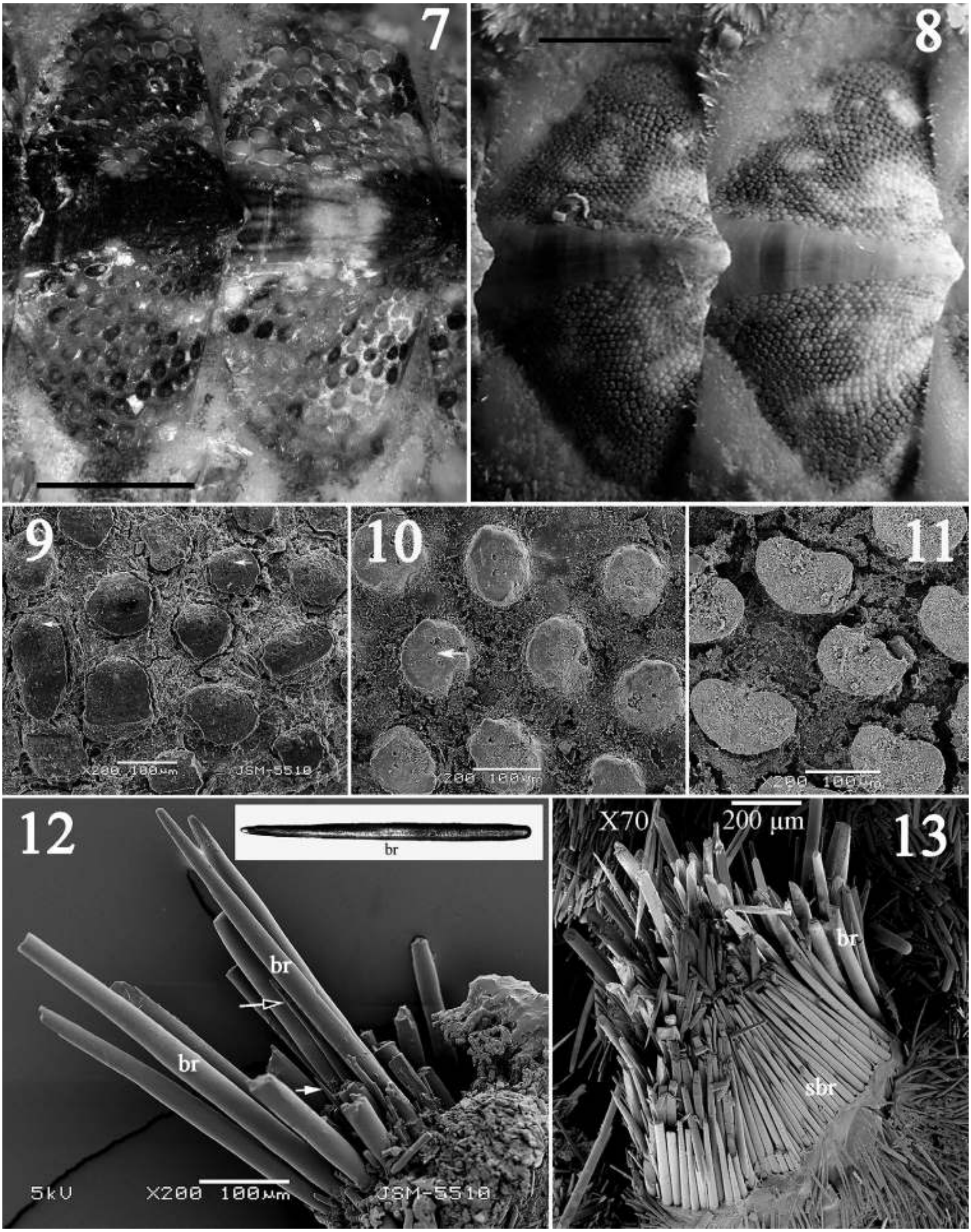
Discussion

Dorsal girdle elements

The number of girdle tufts in four of the collected specimens of *A. crinita* is typically 18, only in one of the specimens it reaches 19 – there is an extra tuft behind the tail valve (Fig. 5, arrowed). A similar case in *A. crinita* was reported by Kaas (1985: p. 601): "[…] I possess a fine and in all respects normal specimen of *crinita* from Pointe de Barfleur, Manche, Normandy, with one extra tuft (K 4935)". These states are for

Table 1. *Acanthochitona crinita* rhachidian tooth morphology (µm).

Locality	Akhtopol juvenile (L: 4.7 mm)			Sts Constantine and Helena female (L: 9.9 mm)			Kamen Bryag adult (L: 14 mm)		
Rhachidian tooth	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n
LR	43.44	2.97 (37.5–45)	8	53.75	2.62 (50–57.5)	6	63.75	1.77 (60–65)	10
AWR	21.07	1.97 (17.5–22.5)	7	30.00	0.00	6	42.75	1.29 (41.25–45)	10
UAWR	20.00	0.00	7	27.50	0.00	6	40.25	1.29 (38.75–42.5)	10
MWR	23.04	0.98 (22.5–25)	7	31.88	1.04 (30–32.5)	6	47.25	0.79 (45.0–47.5)	10
BWR	12.86	1.72 (10–15)	7	13.33	1.29 (12.5–15)	6	12.12	0.84 (10–12.5)	10



Figs 7–13. 7–8. Intermediate valves IV and V, dorsal view: **7.** female *Acanthochitona crinita*, Sts Constantine and Helena, CPM; **8.** *Acanthochitona fascicularis*, MHNG: No 955/102. Scale bars: 1 mm. **9–11.** Sculpture of pleurolateral areas of intermediate valves IV, SEM: **9.** juvenile *Acanthochitona crinita*, detail showing the ovoid/elongate granules, on the left side of valve, close to jugum (Akhtopol, CPM); **10.** *Acanthochitona fascicularis*, roundish granules, central part of valve area, left side (MHNG: No 981/717); **11.** *Acanthochitona fascicularis*, heart-shaped/reniform granules, anterior valve periphery, left side (MHNG: No 981/717); megalaesthete are arrowed. **12–13.** *Acanthochitona* dorsal girdle elements: bristle tufts, SEM: **12.** *Acanthochitona crinita*, bristles (br), shorter and thin spicules (a long arrow with black arrowhead), and very short, thin spicules (a short white arrow); **13.** *Acanthochitona fascicularis*, long bristles (br), surrounded by belt of shorter bristles (sbr).

sure a rare variation in this character or an anomaly in the number of girdle tufts occurring in species of the genus *Acanthochitona*.

Concerning the radula, *A. crinita* differs from the other representative of genus *Acanthochitona* in the Black Sea basin – *A. fascicularis* mainly in the shape of rhachidian and first lateral teeth – for details see Figs 22–24 and Table 2: "Radula" (see also Kaas 1985, Jones & Baxter 1987: plate 3B, figs 22–23; Dell'Angelo & Smriglio 1999, 2001: tavola/plate 67N; Bonfitto et al. 2011: fig. 4).

Ecological notes

According to the literature sources, *A. crinita* lives in very shallow waters (typically at depths of 0.3–0.5 m, occasionally up to 1 meter) (Kattoulas et al. 1973, Van Belle 1983–1986, Jones & Baxter 1987, Strack 1988, 1990, Bode 1989, Slieker et al. 1994, Öztürk et al. 2000, De Bruyne 2003, Dell'Angelo & Smriglio 2001, Dell'Angelo & Zavodnik 2004, Koukouras & Karachle 2005). This chiton has also been collected in shallow waters (at depths between 1 and 24 m) along the Bulgarian Black Sea coast.

Acanthochitona crinita is an herbivorous grazer that occurs on the hard substrates (e.g. underside of stones (which are often lightly embedded in coarse sand or gravel), on and under (solid) rocks in the intertidal zone, on calcareous algae; found in association with acorn barnacles (Balanomorpha) and in coralligenous formations (Kattoulas et al. 1973, Van Belle 1983–1986, Jones & Baxter 1987, Strack 1988, Bode 1989, Slieker et al. 1994, Öztürk et al. 2000, Dell'Angelo & Smriglio 2001, De Bruyne 2003, Dell'Angelo & Zavodnik 2004, Koukouras & Karachle 2005). According to Slieker et al. (1994) overhanging rocks are the preferred habitat of *A. crinita* (but not of *A. fascicularis*). During investigations along the Bulgarian Black Sea coast it was observed that this chiton prefers hard substrates – four of the five *A. crinita* specimens were found on shells of living *Rapana venosa*, and one was collected on a stone. Juvenile *A. crinita* (L: 2.55–4.7 mm) were found in July, adults in July-August, and a female bearing mature ova in early July.

Chorological data

Currently, *Acanthochitona crinita* is known from only a few localities in the Black Sea (Fig. 1), where it is rather infrequent. The three new findings from the Bulgarian sector of the Black Sea provide further information to previous Black Sea records (Van Belle 1983–1986). It may be assumed that the distribution of *A. crinita* reaches the eastern Black Sea coast. Confirmation for that is the original photo of *Acanthochitona crinita* (collected in the Krasnodar region (Russia), Fig. 1, No 5) published in the work of Vershinin (2003, pp. 111, 173), wrongly labelled by the author as "*Lepidochitona cinerea*".

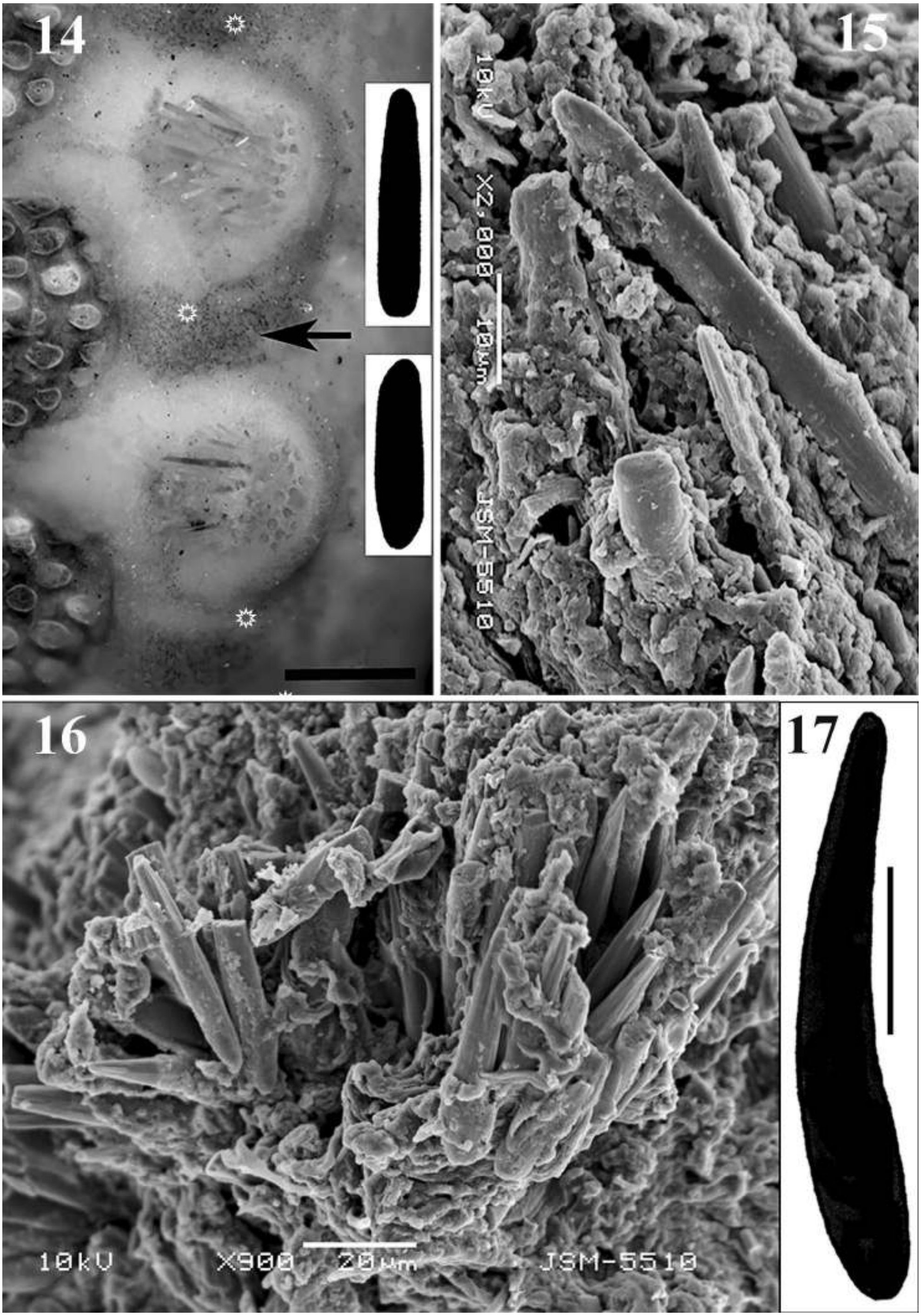
It appears that there are no published data documenting the occurrence of *Acanthochitona crinita* in the southern Black Sea region (pers. obs. and Öztürk et al. 2014). According to the literature, the other *Acanthochitona* species in the Black Sea, *A. fascicularis*, occurs at eight localities; some of these sites are shared between both *Acanthochitona* species (Fig. 2).

Discrepancies in diagnostic characters of *Acanthochitona* species found in the literature concerning the Black Sea region

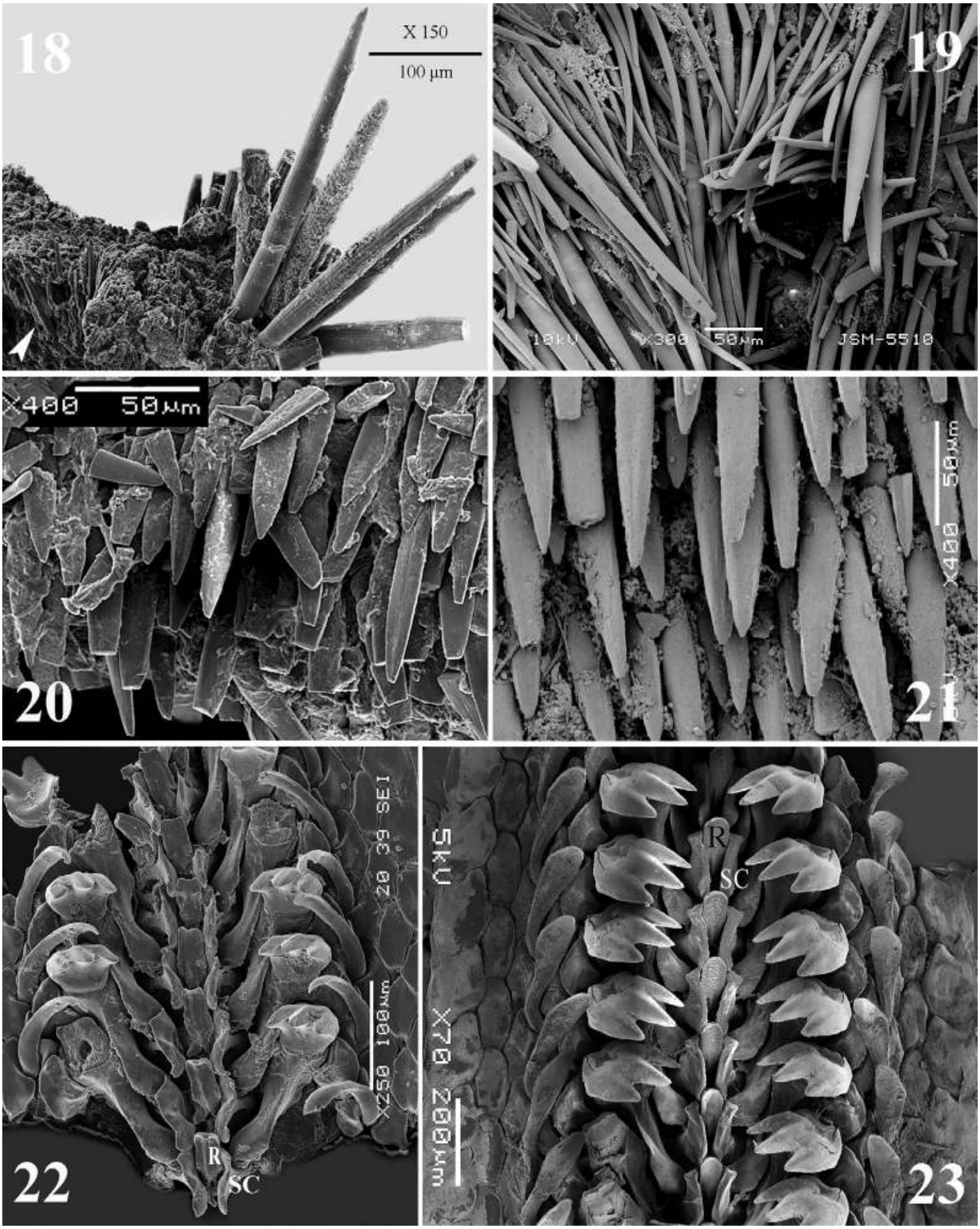
Milashevich (1916, as *Anisochiton* (*Acanthochites*) *fascicularis*) and Yakovleva (1952, as *Acanthochiton fascicularis*) list only *Acanthochitona fascicularis* as being part of the Black Sea fauna. While identifying the *A. crinita* specimens collected in the Black Sea and comparing these with material of *A. fascicularis,* it was noted that there were some discrepancies regarding the details for *A. fascicularis* by Milashevich (1916) and Yakovleva (1952). These concern some important taxonomical characters that differ from those typical for *A. fascicularis* as mentioned in more recent chiton works (i.e. Kaas 1985, Van Belle 1983-1986, Jones & Baxter 1987, Dell'Angelo & Smriglio 1999, and Bonfitto et al. 2011). In particular, Milashevich (1916) and Yakovleva (1952) mentioned characters that do not occur in *A. fascicularis* but are typical for *A. crinita* (cf. Kaas 1985, Van Belle 1983–1986, Jones & Baxter 1987, Dell'Angelo & Smriglio 1999, and Bonfitto et al. 2011). For instance, Milashevich (1916: p. 146) writes that the sculpture of the valves consist of scattered oval-shaped granules/grains ("nechastujmi ovalnujmi zernami" [="rare oval grains"]), but this sculpture type is in fact characteristic for *A. crinita*. Yakovleva (1952, p. 91) also mentions in her redescription of *A. fascicularis* that the granules on the tegmentum are large and oval-shaped, a feature typical for individuals of *A. crinita*. The same holds also for the drawings of spicules that Yakovleva presents in her work (Yakovleva 1952): the figures labelled as showing *A. fascicularis* (fig. 44: 3, 4) in fact depict the features corresponding to *A. crinita* (cf. Kaas 1985, Jones & Baxter 1987, Dell'Angelo & Smriglio 2001).

Discrepancies appear also in the description of the radular teeth of *A. fascicularis* in Milashevich (1916) and Yakovleva (1952). The description of the rhachidian tooth and the drawing of the radular teeth in Milashevich (1916, p. 147) are rather similar to those of *A. crinita* (see Figs 22, 24a–c,f).

Similar is the confusion in Yakovleva (1952, p. 91: fig. 44, 1), where the redescription of the



Figs 14–17. *Acanthochitona crinita,* dorsal girdle spicules: **14.** the dark patches between the bristle tufts = aggregation of small, obtusely-pointed spicules (*), the inlets on the right = the image of these spicules, X700. Scale bar: 0.5 mm; **15.** SEM image of the obtusely-pointed spicules; **16.** tiny, pointed spicules, SEM; **17.** Image of bigger, curved spicules. Scale bar: 50 μm.



Figs 18–23. 18–19. Girdle margin-elements, SEM: **18.** *Acanthochitona crinita* (from Sts Constantine and Helena, CPM), the ribbed marginal spicules, (the arrow marked the position of the tiny, pointed spicules, shown on Fig. 16); **19.** *Acanthochitona fascicularis* (MHNG: No 981/717), the smooth marginal spicules; **20–21.** Ventral girdle elements, SEM: **20.** *Acanthochitona crinita*, (Sts Constantine and Helena, CPM); **21.** *Acanthochitona fascicularis*, (MHNG: No 981/717); **22–23.** Anterior portions of radulae, SEM: **22.** *Acanthochitona crinita* (female, Sts Constantine and Helena, CPM); **23.** *Acanthochitona fascicularis* (MHNG: No 981/717). **R**, rhachidian tooth; **SC**, first lateral tooth.

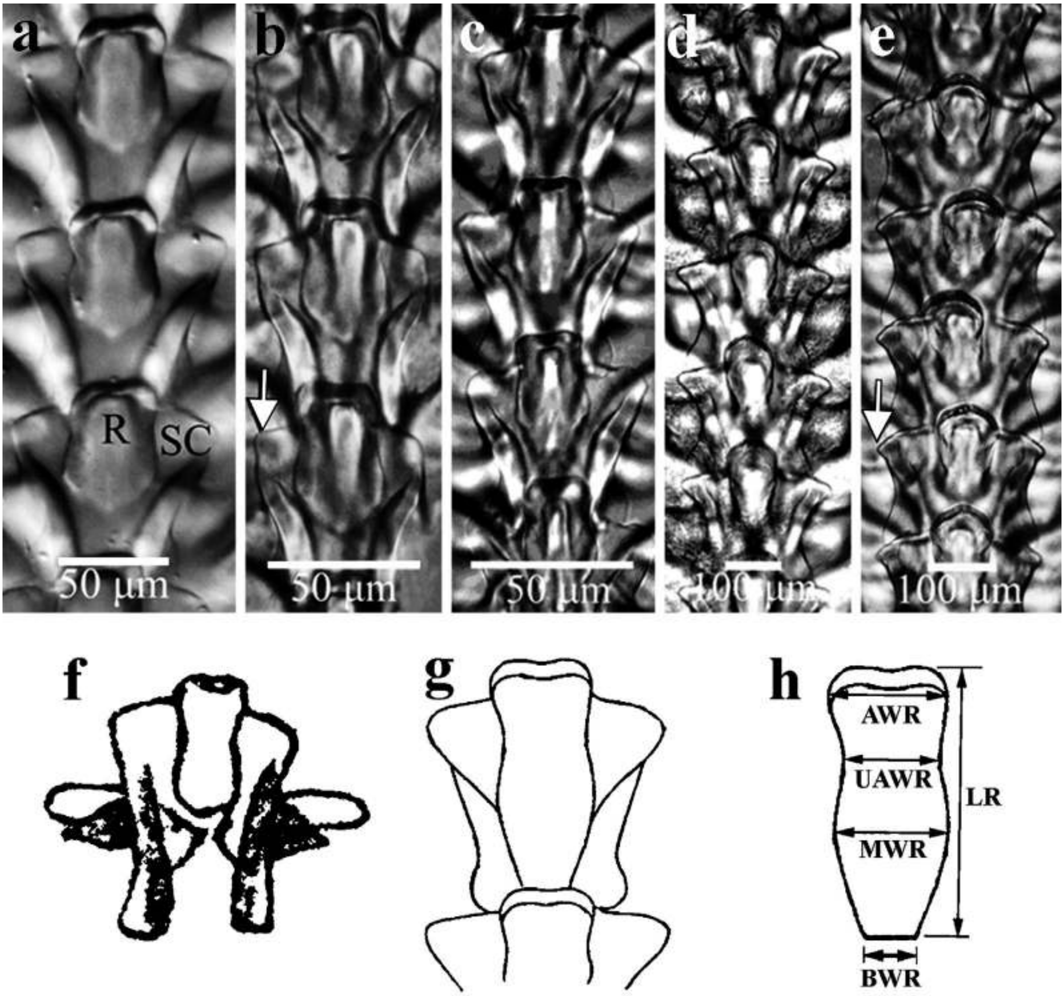


Fig. 24. Light optical microscope images of *Acanthochitona* radulae: rhachidian and first lateral teeth in: **a–c.** *Acanthochitona crinita*: **a.** adult specimen from Kamen Bryag, **b.** female specimen from Sts Constantine and Helena, **c.** juvenile specimen from Akhtopol; **d–e.** *Acanthochitona fascicularis*: **d.** adult specimen, MHNG: No 981/717, **e.** adult specimen, MHNG: No 981/719; **f.** *Anisochiton* (*Acanthochites*) *fascicularis*, after Milashevich (1916); **g.** *Acanthochiton fascicularis*, after Yakovleva (1952); **h.** rhachidian tooth measurements. The arrows (**b, e**) show the distal angle of SC. **R**, rhachidian tooth; **SC**, first lateral teeth.

rhachidian and first lateral teeth are shown to be those of *A. crinita* (see Figs 22, 24a–c,g); the same author mentions also that the head (cusp) points (denticles) of the second lateral tooth are almost equal, while in the published figure the denticles (fig. 44, 2) are clearly more similar to these in *A. crinita* rather than for *A. fascicularis* (Table 2 and cf. Kaas 1985: fig. 38, Bonfitto et al. 2011: fig. 4B). Thus it appears that Milashevich (1916) and Yakovleva (1952) have misidentified Black Sea specimens of *A. crinita* as *A. fascicularis*. Moreover, it is worth noting that Starobogatov (1972) and Anistratenko & Anistratenko (2001) appear to repeat this error in their redescriptions of *A. fascicularis*; therefore using these sources as an identification aid will lead to misidentification of *Acanthochitona* specimens from the Black Sea. In this connection it appears also strange that Anistratenko & Anistratenko (2001) have included in their list only one representative of genus *Acanthochitona*, i. e. *A. fascicularis*, but appear to have missed *A. crinita* previously mentioned by Van Belle (1983–1986: fig. 105) for the Ukrainian Black Sea coast and the Bosporus region.

The records by Çulha et al. (2007, 2010), Demirci

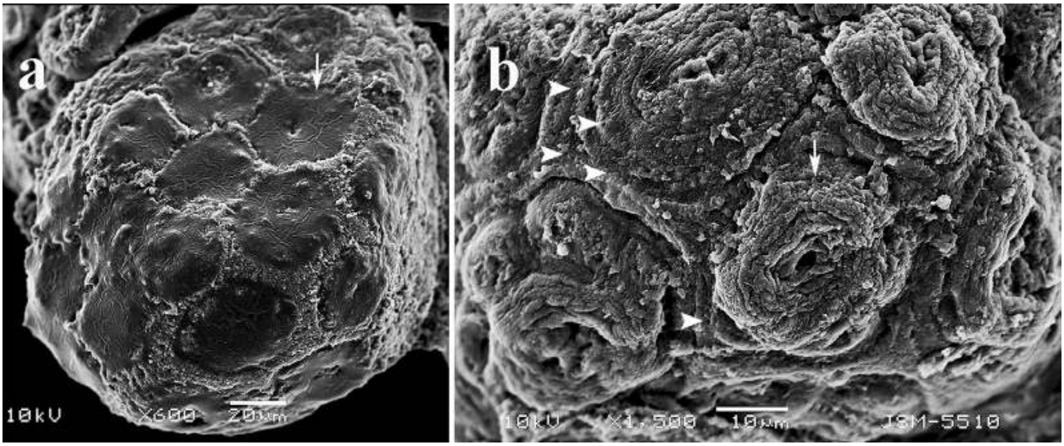


Fig. 25. *Acanthochitona crinita* unfertilized eggs, dissected from the ovary, SEM: **a.** Micrograph of egg with layer of follicle cells (one is marked with arrow); **b.** close up of the egg hull surface showing the collapsed hull cupules (one of them is marked with arrow), and series of micropores in the intercupule region (arrowheads).

(2005), Gozler et al. (2010) concerning *A. fascicularis* from the southern and southeastern Black Sea coast merit validation as well because according to the details found in their Materials and methods and References, the materials have been identified using Milashevich (1916), Starobogatov (1972), and Anistratenko & Anistratenko (2001); these sources have been shown to be unreliable for the identification of *Acanthochitona* species. Gozler et al. (2010) mention that molluscs and platyhelminthes were classified following Riedl (1963); this work, however, provides information on *A. communis* and *A. fascicularis*. While studying the newer literature on chitons, Riedl's (1963) comment on the sculptural elements in *A. communis*, as well as the drawing of the body and the valve, should in fact refer to *A. fascicularis* (see Riedl 1963: p. 347, table 119); the comment concerning the sculpture elements in *A. fascicularis* and the presented scheme of its valve should refer to *A. crinita* (see Riedl 1963: p. 347, table 119). Presumably, Riedl (1963) has used the drawings of *A. communis* and *A. fascicularis* from Leloup & Volz (1938: p. 27, fig. 40; p. 30, fig. 44a), as he cites that work in his publication, but it has been known for a long time (cf. Dell'Angelo & Smriglio 1999, 2001, Dell'Angelo & Zavodnik 2004) that these taxa described in Leloup & Volz (1938) are synonyms of *A. fascicularis* and *A. crinita*, respectively. Hence it follows that the information regarding *A. fascicularis* and *A. crinita* cited in Riedl (1963) have most probably been confused, and so the Turkish *Acanthochitona* specimens from the southeastern Black Sea coast have been misidentified.

All these observations suggest that the Black Sea material of *Acanthochitona fascicularis* has been largely misidentified and so the question of whether *A. fascicularis* is indeed a part of the Black Sea fauna still remains open; it was not found in this study.

Comparative remarks

Acanthochitona crinita and *A. fascicularis* are very similar and variable and consequently have been often confused (see Kaas 1985, Jones & Baxter 1987, Dell'Angelo & Smriglio 1999, 2001, see above/below). To facilitate the correct determination of *Acanthochitona* materials from the Black Sea, Table 2 summarises the morphological differences between both chiton species.

Both *Acanthochitona* species can be easily distinguished by 1) the outline of the intermediate valves, 2) the morphology and arrangement of the granules, 3) the length of the girdle spicules, 4) the structure of the marginal fringe spicules, and 5) the morphology of some of the radular teeth (Kaas 1985, Kaas & Van Belle 1985, Jones & Baxter 1987, Dell'Angelo & Smriglio 1999, 2001, Dell'Angelo & Zavodnik 2004, Bonfitto et al. 2011, the present study) (Table 2).

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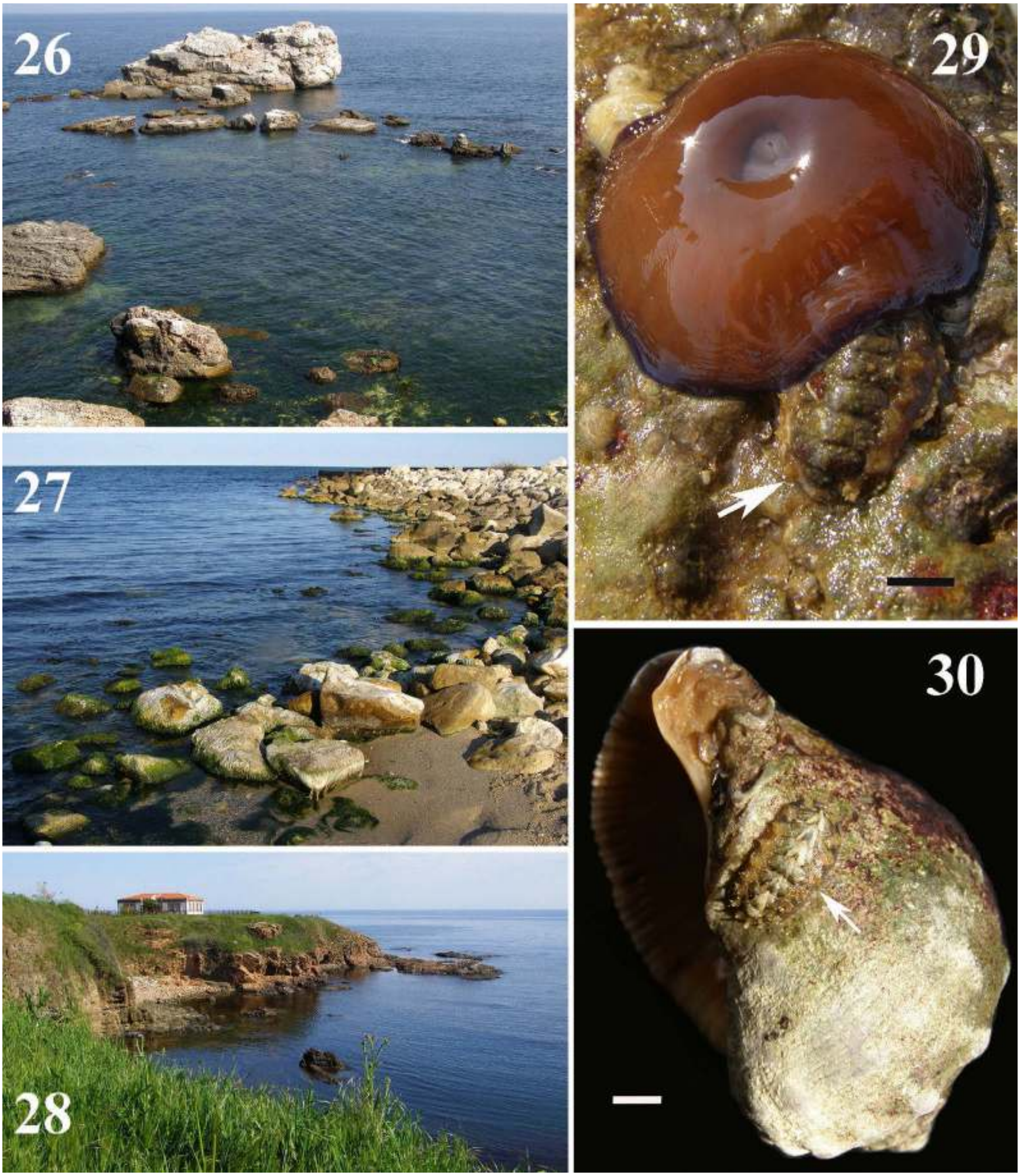
Table 2. Basic differences between *Acanthochitona fascicularis* (Linnaeus, 1767) and *A. crinita* (Pennant, 1777).

Characters	*A. fascicularis* (Fig. 6)	*A. crinita* (Figs 3–5)
Body length (L), Body width (W)	L: 15.0–60.0 mm, W: 8.0–26.0 mm [1], [*]	L: 9.9–34.0 mm, W: 5.8–12.0 mm [2], [*]
Intermediate valve outline	from triangular to pentagonal [3] (Fig. 8)	more ellipsoidal [3] (Fig. 7)
Jugal area	somewhat raised, sharply separated from the latero-pleural areas [4], with fine longitudinal striae [5]	hardly raised, not sharply separated from the latero-pleural areas [4]
Sculpture of intermediate valves: Morphology of the tegmentum granules:	granules, rather elevated, concave; roundish, in some cases heart/reniform, with incision on the anterior margin [6] (Figs 8, 10, 11) granules rather densely arranged (from 37 to 47 granules per 1 mm^2, average 42, n = 10 samples of 1 mm^2) [*]; granules relatively small, with diameter from 0.07 to 0.126 mm (n = 15) [*]	granules lower, flat or slightly concave; ovoid to a more or less elongate drop; only adjacent to the jugum the granules are much more elongated (Figs 7, 9) (but not very narrow and lanceolate which is typical for *A. oblonga* (Leloup, 1981)) [6] granules less densely arranged, more spaced (from 26 to 34 granules per 1 mm^2, average 33, n=10 samples of 1 mm^2) [*]; granules visibly larger: their length ranges between 0.14 and 0.238 mm, and their breadth between 0.07 and 0.098 mm (n = 15) [*]
Morphology of the granules on tegmentum: megalaesthete (macroaesthete) and micraesthete (microaesthete) number and position	megalaesthete: single, in central position, surrounded by 1 to 5 micraesthete (sometimes 0) [7]; see also Fig. 10	megalaesthete: single, posteriorly located, surrounded by 12–16 micraesthete [7]; see also Fig. 9
Length of the bristles forming the girdle tufts (Figs 12–13)	$\bar{x}$ = 846 ± 153.98 µm, SD = 671.2 (507.15–2059 µm, n = 14) [*]	$\bar{x}$ = 676.2 ± 32.28 µm, SD = 125.03 (415.86–811.44 µm, n = 15) [*]
Marginal fringe spicules	smooth (without longitudinal grooves) [8], (Fig. 19)	ribbed (with longitudinal grooves) [8], (Fig. 18)
Radula: form and size (in µm) of rhachidian tooth [*]	almost trapezoidal (see Figs 23, 24d–e) LR: $\bar{x}$ = 163 ± 3.54, SD = 12.78 (142.5–182.57, n = 13); AWR: $\bar{x}$ = 85.4 ± 1.74, SD = 7.59 (77.5–100.0, n = 19); MWR: $\bar{x}$ = 51.4 ± 0.55, SD = 2.33 (50.0–57.5, n = 18); BWR: $\bar{x}$ = 54.4 ± 0.99, SD = 4.08 (50.0–60.0, n = 17)	bottle-shaped (see Figs 22, 24a–c) LR: $\bar{x}$ = 53.65 ± 2.11, SD = 10.32 (37.5–65.0, n = 24); AWR: $\bar{x}$ = 31.88 ± 2.09, SD = 10.24 (17.5–45.0, n = 24); MWR: $\bar{x}$ = 34.53 ± 2.42, SD = 11.87 (17.5–47.5, n = 24); BWR: $\bar{x}$ = 12.14 ± 0.31, SD = 1.54 (10.0–15.0, n = 24)
Radula: form of first lateral tooth	strongly arched [7]; its tip is distinctly concave [7]; the distal angle of first lateral tooth forms a beak-shaped spine (Figs 23, 24d–e, arrowed); its frontal part is weakly impressed medially and encompasses the distal, widened part of the central tooth [*]	more or less straight [7]; its tip is slightly concave, giving the impression of a truncated end [7]; the distal angle of first lateral tooth is rounded (Figs 22, 24a–c, arrowed); its frontal part is strongly bent medially and encompasses the broadened median part of the central tooth [*]
Radula: second lateral tooth: head (cusp) points (cone, denticles) size	the mesocone larger than the endo- and ectocone [7], Fig. 23	the endo- and mesocone are of similar size and the ectocone is smaller [7], Fig. 22

[*] – original data; **[1–8]** – literature data: **1.** after Malatesta 1962, Kattoulas et al. 1973, Barash & Danin 1977, Van Belle 1983-1986, Kaas 1985, Jones & Baxter 1987, Dell'Angelo & Smriglio 1999, 2001, Koukouras & Karachle 2005; **2.** after Malatesta 1962, Kattoulas et al. 1973, Van Belle 1983-1986, Kaas 1985, Jones & Baxter 1987, Bode 1989, Dell'Angelo & Smriglio 2001, De Bruyne 2003, Rowley 2005, Koukouras & Karachle 2005; **3.** see Dell'Angelo & Smriglio 1999, 2001; **4.** cf. Kaas 1985; **5.** cf. Dell'Angelo & Smriglio 2001; **6.** see Kaas 1985, Kaas & Van Belle 1985, Jones & Baxter 1987, Dell'Angelo & Smriglio 1999, 2001, Bonfitto et al. 2011; **7.** see Bonfitto et al. 2011; **8.** Jones & Baxter 1987; Dell'Angelo & Smriglio 2001.



Figs 26−30. 26−28. The Bulgarian Black Sea coast, rocky shore habitats of *Acanthochitona crinita*: **26.** SW of Kamen Bryag village, Archaeological Reserve Yailata (02.VIII.2010); **27.** Sts Constantine and Helena resort, International Home of Scientists "Frederic Joliot-Curie" beach, the breakwater (10.IX.2011); **28.** Akhtopol city, the building of the old Greek school (09.V.2009); **29−30.** *Acanthochitona crinita* (arrowed): **29.** on submerged stones (partially covered by *Actinia equina* (Linnaeus, 1758)), Kamen Bryag village, Archaeological Reserve Yailata, 15.VIII.2010; **30.** on *Rapana venosa* shell, Sts Constantine and Helena resort, 14.VIII.2010. Scale bars: 5 mm.

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