

1 Habitat, morphology and trophism of *Tritonia callogorgiae* sp. nov., a  
2 large nudibranch inhabiting *Callogorgia verticillata* forests in the  
3 Mediterranean Sea

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6 A B S T R A C T

7 The continuing exploration of the Mediterranean deep sea reveals that a complete census of the biodiversity  
8 of one of the most studied marine areas in the world is yet to be fully accomplished. A tritoniid nudibranch  
9 new to science is described upon material recovered off the Montenegro margin, Adriatic Sea, associated  
10 with the alcyonacean Primnoidae *Callogorgia verticillata* (Pallas, 1766). Here, a coral forest dominated by *C.*  
11 *verticillata*, together with other alcyonaceans, antipatharians and scleractinians, represents the habitat of the  
12 new species. As documented by Remotely Operated Vehicle (ROV) observations, the coral forest covers an  
13 area of 600 m<sup>2</sup> at 420–426 m depth, with dense growth of *C. verticillata* colonies ( $1.34 \pm 0.08$  colonies  
14 m<sup>-2</sup>). As many as 66 nudibranch specimens have been observed on *C. verticillata*. *Tritonia callogorgiae* sp.  
15 nov. (Gastropoda, Nudibranchia, Tritoniidae) is up to 12 cm long, yellow to orange/reddish in colour with  
16 white papillae all over the body. It has a velum with six appendages (three per side), and 4–5 pairs of  
17 dichotomous gills. Internally, the species is distinguished from other tritoniids for the absence of stomach  
18 plates and a distinctive radula with formula  $50 \times 110.1.1.1.108$ . It represents the first case of *Tritonia* species  
19 living in the deep Mediterranean Sea and the only one associated to *C. verticillata*. Stomach content  
20 documents that *Tritonia callogorgiae* sp. nov. feeds on *C. verticillata*. Further visual ROV records from other  
21 sectors of the Mediterranean Sea indicate that the distribution of this new described species is almost basin-  
22 wide. The discovery of another undescribed member of the benthic megafauna related to deep-sea coral  
23 grounds further highlights the need for conservation of such habitats.

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25 1. Introduction

26 The deep Mediterranean Sea (>200 m) is a hotspot of biodiversity of recognized global significance (Coll et  
27 al., 2010). In spite of being probably the most studied marine basin in the world, its fauna is not yet fully  
28 censused (Danovaro et al., 2010; Sabelli and Taviani, 2014). Not surprisingly, the growing effort of the last  
29 decades to better understand the Mediterranean deep-sea realm, with special focus on the charismatic deep-  
30 (or cold-) water coral habitats, has resulted in a number of discoveries of new biotopes (e.g. Freiwald et al.,  
31 2009; Bo et al., 2011; De la Torre et al., 2014; Grynio´ et al., 2018; Knittweis et al., 2019; Taviani et al.,  
32 2019) and updating of taxonomic lists (e.g. Gofas et al., 2007; Taviani et al., 2009; Mastrototaro et al., 2010,  
33 2017; D’Onghia et al., 2015; Rueda et al., 2019). Corals are among the main deep-sea habitat formers, being  
34 able to create large scleractinian frameworks, forests of black corals and alcyonaceans, as well as extensive  
35 sea pen fields (e.g. Roberts et al., 2006, 2009; Chimienti et al., 2019a). Within those habitats, a remarkable  
36 amount of both structuring and associated fauna can be present, enhancing the complexity and the  
37 biodiversity of these communities (Mastrototaro et al., 2010; Rueda et al., 2019). Besides representing  
38 feeding, nursery and spawning areas for many species (e.g. Auster, 2005, 2007; Baillon et al., 2012, 2014;  
39 D’Onghia, 2019), deep-sea coral habitats can act as exclusive habitat for certain organisms. This is the case  
40 of a distinct tritoniid nudibranch exclusively found thus far within the forests of the alcyonacean primnoid  
41 *Callogorgia verticillata* (Pallas, 1766), a gorgonian characterized by fan-like colonies whose feathery  
42 branches resemble palm leaves, distributed in the Atlantic Ocean and Mediterranean Sea. Tentatively  
43 identified as *Marionia blainvillea* (Risso, 1818), one of the first occurrences of this nudibranch was  
44 reported by Bo et al. (2011) within a mixed coral assemblage in the Southern Tyrrhenian Sea, locally  
45 dominated by *C. verticillata*. Morphologically similar nudibranchs have been observed at bathyal depths in  
46 the Gulf of Lions (Fabri et al., 2014), Central Tyrrhenian Sea (Ingrassia et al., 2016), Sicily Channel  
47 (Knittweis et al., 2019) and Southern Adriatic Sea (Angeletti et al., 2014), always on *C. verticillata*, but they  
48 remained undetermined. The lack of actual samples hampered a formal identification and description of this  
49 nudibranch until a few specimens were at last collected by means of a Remotely Operated Vehicle (ROV) in  
50 the south-eastern Adriatic Sea. The specimens were found within a *C. verticillata* forest off Montenegro and  
51 proved to be a new species of the genus *Tritonia* Cuvier, 1798. Here we describe and discuss the  
52 morphology, the anatomy, the stomach contents and the distribution of this new species, as well as the main  
53 features of its habitat.

55 2. Materials and methods

56 The study area is located in South-eastern Adriatic Sea, off the Montenegro margin (41°38.85' N - 18°41.50'  
57 E). The margin here is incised by small canyons, some of which host a deep-sea sessile mega- fauna  
58 dominated by cnidarians and, subordinately, sponges (Angeletti et al., 2014, 2015; 2019; Taviani et al.,  
59 2016). A coral forest dominated by *C. verticillata* with associated nudibranchs was detected at 420–426 m  
60 depth (Angeletti et al., 2014). The survey was carried out during cruise ALTRO (December 2012–January  
61 2013), aboard R/V *Urania*, using the ROV Pollux III of Stella Maris s.r.l., equipped with a low definition  
62 CCD video camera for navigation and a high-resolution (2304 × 1296 pixels) video camera Sony HDR-HC7  
63 for detailed observation. The ROV also hosted an ultra short baseline positioning system (LinkQuest inc.  
64 mod. Tracklink 1500 MA; georeferenced position provided every second), a depth sensor, a compass, a  
65 grabber arm for sampling and three parallel laser beams providing a 20-cm scale to measure objects and  
66 define the width of the transects for subsequent video analysis. A total of 2 h and 30 min of video were  
67 recorded on the coral forest. Four nudibranch specimens and two samples of *C. verticillata* branches (10 cm)  
68 were collected during the dive using the ROV grabber arm and a small basket attached to the front of the  
69 ROV.

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71 2.1. Video analysis

72 Video analysis was performed using Adobe Premiere Pro software to quantify the *C. verticillata* forest and to  
73 collect in situ information about the nudibranch. Sampling units of  $2.5 \pm 0.2$  m<sup>2</sup> were defined along each  
74 transect, according to the minimal area proposed by Weinberg (1978) and recently used for ROV imaging on  
75 octocoral habitats (Ambroso et al., 2014; Chimienti et al., 2018, 2020). Sequences with bad visibility, due to  
76 water turbidity or distance from the seabed, were discarded. A total of 227 sampling units were obtained.  
77 The presence of *C. verticillata* was quantified both by occupancy (frequency of occurrence in the set of  
78 sampling units) and by abundance (number of colonies per sampling unit). Density (colonies m<sup>-2</sup>) was  
79 calculated for each sampling unit, then the density of the coral forest was expressed as mean  $\pm$  standard  
80 error. The same approach was used to quantify other conspicuous spe- cies, including the new nudibranch, as  
81 well as for lost fishing gears, when appropriate.

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83 2.2. Sample analysis

84 Two specimens of nudibranch were immediately preserved in ethanol 99% on board, while other two were  
85 photographed in vivo in aquarium, then anesthetized using menthol crystals and preserved in 4%  
86 formaldehyde solution in seawater. External morphological examina- tion was carried out on all the  
87 specimens, while internal anatomy was investigated on two of them. The other two samples were left intact  
88 to be the holotype and the paratype of the species.

89 A dorsal incision near the anterior end of the body was performed for dissection. The reproductive and  
90 digestive systems were photographed and drawn. The buccal mass was removed to extract the radula and  
91 jaws by dissolving the surrounding tissue overnight in a cold mixture of so- dium hydroxide (KOH; 3 g),  
92 H<sub>2</sub>O 150 ml, Polysorbate 80 (0.5 ml) and Sodium Dodecyl Sulfate (C<sub>12</sub>H<sub>25</sub>O<sub>4</sub>SNa; 0.5 ml): a mixture used  
93 in entomology (Porcelli, 2019) that resulted to be non-invasive on the radula structure. Residual soft tissues  
94 were removed manually, then radula and jaws were rinsed in distilled water and dehydrated in a graded  
95 ethanol-series solution. Dried radula and jaws were first observed and photographed under the  
96 stereomicroscope, then coated with gold-palladium in an Edwards S150A sputter coater and examined with a  
97 Hitachi TM3000 Scanning Electron Microscope (SEM). The stomach content of the dissected specimen was  
98 processed with gentle rinses in diluted sodium hypochlorite (NaClO) solution, then washed with distilled  
99 water and dehydrated in a graded ethanol series samples. The calcareous material (sclerites) found in the  
100 stomach was mounted on a stub and sputter coated for examination under SEM. Same pro- cedure was  
101 repeated for the sub-samples of *C. verticillata* in order to remove tissues from polyp whorls, sclerites and  
102 axis for comparison.

103 Taxonomically informative characteristics, such as the arrangement and shape of the reproductive system,  
104 the radula and jaws morphology, the rhinophore ornamentation, the position of the anal and genital openings,  
105 and the body colour pattern, were used as the primary diag- nostic traits according to extensive literature  
106 (e.g. Odhner, 1934, 1936; 1963; Schmekel and Portmann, 1982; Marcus, 1983; Willan, 1988; Smith and  
107 Gosliner, 2003; Ballesteros and Avila, 2006; Vald'es, 2006; Pola and Gosliner, 2010; Silva et al., 2013,  
108 2014; Furfaro et al., 2017).

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110 3. Results

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### 3.1. Habitat

The benthic community observed in the study area appears dominated by large colonies of the fan-shaped alcyonacean *C. verticillata* (up to more than 1 m in height and width) (Fig. 1a), 762 colonies of which were observed over an area of about 600 m<sup>2</sup>, with 89.43% of occupancy.

Abundance ranged from 0 to 15 colonies per sampling unit, with a mean density of  $1.34 \pm 0.08$  colonies m<sup>-2</sup> (maximum: 6 colonies m<sup>-2</sup>). The coral forest is also characterized by the sparse co-occurrence of *Paramuricea macrospina* (Koch, 1882) (41 colonies; 4.41% of occupancy) and *Swiftia dubia* (Thomson, 1929) (15 colonies; 3.96% of occupancy) (Fig. 1c and d). Although not abundant, the stony corals *Madrepora oculata* Linnaeus, 1758, *Desmophyllum pertusum* (Linnaeus, 1758), *Desmophyllum dianthus* (Esper, 1794) and *Dendrophyllia cornigera* (Lamarck, 1816), as well as the black corals *Antipathes dichotoma* Pallas, 1766 and *Leiopathes glaberrima* (Esper, 1792) were occasionally observed (Fig. 1e–g). Massive and fan-shaped sponges, among which *Pachastrella monilifera* Schmidt, 1868, commonly occur on the silty-muddy substrate, but also in close association with *C. verticillata* (Fig. 1b,h).

Sixty-six specimens of the nudibranch here described as *Tritonia callogorgiae* sp. nov. were identified ( $0.12 \pm 0.05$  specimens m<sup>-2</sup>), all of them resident on *C. verticillata* colonies except for two individuals crawling on muddy bottom in the proximity of the base of the coral colonies (Fig. 2a and b). Two or more specimens could be present on the same colony, and several colonies hosted egg coils, orange in colour and embedded in mucus (Fig. 2c and d). Although spawning was not observed, it is highly likely that the fresh egg coils observed belonged to the nudibranch. Apart from the nudibranch and its eggs, other macrobenthos observed on the colonies of *C. verticillata* included the spiny crab *Anamathia rissoana* (Roux, 1828) and, unusually, one colony of *P. monilifera* (Fig. 2e).

Anthropogenic impacts observed in the consisted of lost longlines and scant litter. Fifteen longlines ( $0.03 \pm 0.03$  items m<sup>-2</sup>) were observed entangling the rocky substrate and the colonies of *C. verticillata*, that were injured by this fishing gear. The remaining litter observed was mainly represented by plastic bags, glass bottles and cans (Fig. 3).

### 3.2. Systematics

Order Nudibranchia Cuvier, 1817

Family Tritoniidae Lamarck, 1809.

Genus *Tritonia* Cuvier, 1798

*Tritonia callogorgiae* Chimienti, Furfaro & Taviani, sp. nov. Figs. 4 and 5.

Zoobank: [urn:lsid:zoobank.org:pub:0C2C3802-66AF-4F0C-A928-55A8DFC7BC58](https://zoobank.org/pub:0C2C3802-66AF-4F0C-A928-55A8DFC7BC58).

Etymology: The name identifies the consistent association of the new species with the Primnoidae sea fan *Callogorgia verticillata*.

Holotype: sample A\_31\_2, 30 mm in length, preserved in ethanol 99%, and deposited in the collection of the Natural History Museum of Paris (France; MNHN-IM-2000-35,803).

Paratypes: paratype A\_31\_1, 49 mm in length, preserved in ethanol 99%, dissected, and deposited in the collection of the Zoological Museum of the University of Bari (Italy; MUZAC-6555); paratype A\_31\_3, 53 mm in length, preserved in 4% formaldehyde solution in seawater, and deposited in the collection of the Zoological Museum of the University of Bari (Italy; MUZAC-6556); paratype A\_31\_4, 38 mm in length, preserved in 4% formaldehyde solution in seawater, dissected, and deposited in the collection of the Zoological Museum of the University of Bari (Italy; MUZAC-6557).

Type locality: Southern Adriatic Sea (Mediterranean Sea), 30 nautical miles offshore of the city of Bar, Montenegro, 420–426 m depth, 41°38.85'N - 18°41.50'E, January 2, 2013.

Description, external anatomy: length in contracted specimens was between 30 and 55 mm, but living specimens were up to ca. 120 mm long. The body is slender with a background colour ranging from yellow to orange and reddish. The body is granular with scattered white papillae (Fig. 4a and b) from the velum to the tail. The oral velum (or frontal veil) is a flattened contractile projection characterized by six finger-like processes, three on each side (Fig. 4a and b). The rhinophoral sheath is a simple chalice-shaped ring colored as the rest of the body (Fig. 4c). The retractable rhinophoral club has unipinnate white plumes. Specimens have 4–5 gills per side, some of them showing an asymmetrical number of gills on the two sides of the body. The gills are long and with the same coloration of the body, except for their apical part that is white transparent. They are dichotomous and with a different degree of subdivision (Fig. 4d), as they divide into two main basal branches but each main branch divides into two or three smaller branches, although some gills do not branch. The foot is linear, narrow and it tapers posteriorly, with the same colour pattern of the

167 notum (Fig. 4a and b).  
168 Description, internal anatomy: the digestive system (Fig. 4e) starts in the cephalic portion with the mouth  
169 located ventrally between the muscular lips. Chitinous jaws are concave, amber-colored with the masticatory  
170 border darker, and with denticles arranged in four rows (Fig. 5a–c). Two well-developed salivary glands are  
171 positioned between pharynx and oesophagus (Fig. 4f). Inside the pharynx is present the buccal bulb, where  
172 the radula is inserted, connecting to the oesophagus in the dorsal region. The radular formula is  $50 \times$   
173  $110.1.1.1.108$ . The analysed radula (Fig. 5d) measures  $5.5 \times 11.5$  mm. The rachidian tooth is  $70 \mu\text{m}$  wide, it  
174 has a short and thin central cusp and two sharp lateral wings on each side of the median cusp (Fig. 5e and f).  
175 The first lateral teeth are thick, differing from the remaining long sharp cuspidate lateral teeth. Their shape is  
176 simple, hooked and smooth, with a wide base (Fig. 5g and h). The oesophagus extends from the buccal mass  
177 until the stomach (Fig. 4i). Stomach and intestine form a loop around the heart that is visible roughly in the  
178 middle of the digestive system (Fig. 4f,i). No stomach plates nor girdles were found in the stomach. The  
179 intestine exits the stomach and narrows toward the anus on the right side of the body, between the 2nd and  
180 the 4th gill. Four cerebralpleural ganglia (Fig. 4e,i) are connected with three cerebral nerves and three pedal  
181 nerves per each side of the body. Reproductive system is dialucic (Fig. 4g and h). The anterior part of the  
182 ovotestis is below the female gland mass and it is connected to the ampulla through a long hermaphroditic  
183 duct (Fig. 4h, j). The ampulla is rounded and convoluted. It bifurcates into the oviduct (and the female  
184 nidamental gland) and the male deferent duct (or vas deferens). The thin deferent duct continues into the  
185 enlarged prostate to become the penial sac, where the penis lies. The receptaculum seminis is ovoid and  
186 connected to the vagina through a narrow duct. The female gland mass is well developed and next to the  
187 female nidamental gland. The genital opening is located in the right side of the body, broadly below the 2nd  
188 gill. The egg masses are orange in colour, and are arranged in a long cylindrical and narrow coil woven  
189 deposited on the branches of *C. verticillata* (Fig. 2d).

190 Distribution: Besides the type locality here reported (Southern Adriatic Sea, offshore Montenegro margin),  
191 the species has been observed also in the Balearic Sea (Gulf of Lions canyon system, Sici'e Canyon, 261 m  
192 depth, reported as *Nudibranchia Tritoniidae*: Fabri et al., 2014), in the Tyrrhenian Sea (Central Tyrrhenian,  
193 Lamezia Gulf, 119–124 m depth, and Southern Tyrrhenian, Pontine Islands, Palmarola Seamount, 194–220  
194 m depth; reported as *Marionia blainvillea*: Bo et al., 2011; Ingrassia et al., 2016), and in the Sicily Channel  
195 (South Malta, unidentified: Knittweis et al., 2019; t. Evans pers. comm.) (Fig. 6). These records are based  
196 on external morphological features observed in vivo using ROVs. The species is thought to be present all  
197 over the Mediterranean Sea, in association with the forests of *C. verticillata*, and it cannot be excluded that  
198 its areal of distribution also includes part of the North Atlantic, where the gorgonian is present.

199 Remarks. The species here described fits to the family Tritoniidae, represented by soft, elongated nudibranch  
200 gastropods with the edge of the mantle usually bearing branched secondary gills, possessing an oral velum  
201 with protuberances (appendages, finger-like processes or oral tentacles), and a broad radula with a median  
202 tooth and numerous lateral teeth (Marcus, 1983). The dissected specimens showed morphological features  
203 congruent with the diagnostic characters associated to the genus *Tritonia*, such as finger-like processes on the  
204 oral velum, rhinophore clubs with simple or feathered extensions, a radula with the first lateral teeth  
205 differentiated from the others (radular formula N1.1.1.N), a digestive gland forming a single complex and the  
206 absence of stomach plates (Schmekel and Portmann, 1982). Identification at a species level is unambiguous  
207 because it is externally different to all the *Tritonia* species described so far in terms of size, colour, anatomy  
208 and ecology. Tritoniidae is a very specialized family with species showing a strong relationship with one or  
209 few species of alcyonacean or sea pen, on which they feed, mate and live (Gomez, 1973; McDonald and  
210 Nybakken, 1999; Smith and Gosliner, 2003; García-Matucheski and Muniain, 2011; Furfaro et al., 2017),  
211 with few exceptions that can feed on zoanthids (Bertsch et al., 2009). The species described here is the first  
212 case of a Mediterranean *Tritonia* species living in deep waters and the only one associated to *C. verticillata*.  
213 Considering such ecological niche, the only comparable species could be *Marionia tedi* Ev. Marcus (1983),  
214 that has been found in the Gulf of Mexico, the Straits of Florida, and the south-eastern Caribbean Sea at 60–  
215 600 m depth, on *Callogorgia americana* Cairns and Bayer (2002) (Valdés, 2006). Differently from our  
216 specimens,

217 *M. tedi* shows a uniformly translucent white body with a pinkish tinge, bilobed velum with about 6 processes  
218 on each lobe, 12–16 short and ramified dorso-lateral cerata, a radular formula N.1.N and numerous stomach  
219 plates. To date, *T. callogorgiae* sp. nov. is a Mediterranean endemic, but its presence in the Atlantic Ocean  
220 cannot be excluded a priori given the areal of distribution of *C. verticillata* (Cairns and Bayer, 2002).

221  
222 3.3. Feeding

223 Specimens of *T. callogorgiae* sp. nov. has been observed exclusively on *C. verticillata*. The stomach contents  
224 of the dissected specimen was characterized by numerous sclerites, fragments of apical axes and partially  
225 digested polyps of *C. verticillata*. In Fig. 7a–n we compare the calcareous structures collected from a colony  
226 with those found in the stomach of the specimen collected on the same colony. Sclerites of both *C.*  
227 *verticillata* and nudibranch's stomach had a glossy, carinated or slightly sculptured outer surface, while  
228 numerous and complex tuber- culate sculptures characterized the inner surface (Fig. 7a–g). Opercular  
229 sclerites were 300–600 µm long and 150–250 µm wide, broadly trian- gular, with a large base and a slender  
230 apex. Body-wall sclerites were irregularly elliptical and ornately ridged, 150–350 µm long and 250–500 µm  
231 wide, oriented in 8–10 rows on the external side of the polyps. Coenenchymal sclerites were thick, elongate  
232 and similar to irregularly polygonal plates, 300–750 µm long and 120–200 µm wide. Due to its large size and  
233 the wide radular surface, the nudibranch is able to bite the branchlets of *C. verticillata*, including part of the  
234 axis with the polyps attached, exposing the axis of the gorgonian. It can remove pieces of up to 6 mm with a  
235 single bite, according to the longest axis portion found in the stomach. The feeding activity of the nudibranch  
236 can dam- age the colonies of *C. verticillata*, eliminating most of the terminal parts of the branches (Fig. 2c).  
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## 238 4. Discussion

### 239 4.1. Habitat

240 is not the first sighting of this nudibranch. Although previously observed, it has neither been collected nor  
241 described before and, considering that the species remained so far unidentified or mis- identified, it is  
242 difficult to track all the documented records in the literature. Some previous observations refer to mixed  
243 coral assemblages dominated by *C. verticillata* South of Malta (Knittweis et al., 2019) and in the Lamezia  
244 Gulf (Bo et al., 2011). The latter showed density of *C. verticillata* comparable to our observations, being  $1.2$   
245  $\pm 0.3$  colonies  $m^{-2}$  (Bo et al., 2011). Further observations occurred in areas where *C. verticillata* was present  
246 but not abundant, such as the Palmarola Seamount, with only two colonies found (Ingrassia et al., 2016), and  
247 the Sicie' Canyon, where the nudibranch was observed within a small pop- ulation of *C. verticillata* but not  
248 in the nearby forests of the Bourcart Canyon (Fabri et al., 2014). However, the distribution of *T. callogorgiae*  
249 sp. nov. might overlap with that of *C. verticillata*, particularly where dense forests occur. The specimens can  
250 spend some time on the mud in search of a new colony to exploit or a partner for mating, but it is assumed  
251 that a certain degree of habitat integrity is needed to guarantee the connectivity among populations. Although  
252 not comprehensively assessed, the distribution of these coral forests seems to be fragmentary and uncommon  
253 in the basin (Chimienti et al., 2019a).  
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### 255 4.2. Taxonomy

256 The taxonomy of the family Tritoniidae has been unclear for a long time and the identification of genera is  
257 still problematic almost 60 years after the last revision (Odhner, 1963). The description of several species is  
258 based mainly on the external anatomy and it often lacks information about important internal anatomical  
259 characters such as the radula, jaws and stomach plates (Odhner, 1936, 1963; Willan, 1988; Smith and  
260 Gosliner, 2003; Pola and Gosliner, 2010). Moreover, there is not a clear consensus among specialists  
261 regarding the diagnostic features within the family Tritoniidae and some specimens do not appropriately fit  
262 into any of the currently recognized genera (Willan, 1988; Schro'dl, 2003). As a consequence,  
263 standardization in the use of taxonomic characters for the understanding of evolutionary relationships among  
264 established species and newly discovered ones is missing (Ballesteros and Avila, 2006).  
265 Tritoniidae currently includes the following accepted genera: Tritonia Cuvier, 1798, Tritoniopsis Eliot,  
266 1905, Tritoniella Eliot, 1907, Marionia Vayssi`ere, 1877, Marioniopsis Odhner, 1934, Marianina Pruvot-Fol,  
267 1930, Paratritonia Baba, 1949, and Tochuina Odhner, 1963. The specimens described here did not have  
268 chitinous stomach plates and the digestive gland was not divided into two distinct masses, thus the genera  
269 Marionia, Marioniopsis, Paratritonia and Tochuina were excluded (Odh- ner, 1934, 1936; 1963; Marcus,  
270 1983; Wa`gele, 1989). Differently from our specimens, the genus Marianina, with the only species *M. rosea*  
271 (Pruvot-Fol, 1930), is characterized by a pink-reddish colour, small size (about 2 mm) and leaf-like dorsal  
272 lamina instead of branched gills (Pruvot-Fol, 1930). The genus Tritoniella has no gills on the two sides of the  
273 body (Wa`gele, 1989), excluding our specimens from this genus as well. The distinguishing between the two  
274 remaining genera, Tritonia and Tritoniopsis, is usually based on the radular traits. In particular, the Tritonia  
275 species have a tricuspid rachidian tooth and a differentiated first lateral tooth, whereas the Tritoniopsis  
276 species have a unicuspid rachidian tooth and the first lateral teeth do not differ from the others (Odhner,  
277 1934; Marcus, 1983). Our species has characteristics of both genera: it has a unicuspid rachidian tooth and  
278 the first lateral teeth are clearly different from the rest. However, the rachidian tooth has two sharp lateral

279 wings on each side of the median cusp that can be considered as a vestigial state of a tricuspid tooth. Another  
280 Tritonia species recently described in the intertidal zone of Cear´a (Brazil), *T. khaleesi* F. V. Silva, Azevedo  
281 and Matthews-Cascon (2014), showed similar intermediate characters of the radula. Moreover, Schmekel  
282 and Portmann (1982) reported that the anus is on the right side, in the middle of the body length in  
283 Tritoniopsis, whereas in Tritonia the anus and genital opening do not have a constant positional relationship  
284 to a specific right bundle of gills, as in our specimens. Finally, based on the number of gills, the fleshy  
285 extension on the outer side of the rhinophoral sheath, and the number of velum processes, the species here  
286 described can be assigned to genus Tritonia.

287 Tritonia includes at least 35 species distributed all around the world, none of which have been previously  
288 reported in the deep Mediterranean Sea. At least seven species are known to be present in the Mediterranean  
289 Sea, but all of them in coastal waters. In particular, Tritonia hombergii Cuvier, 1803 is the only species that  
290 can reach a remarkable size (up to 20 cm) but, differently from our specimens, it has a white to dark purple  
291 colour, velum with numerous finger-like processes, many gill pairs, and numerous soft tubercles on the  
292 mantle used to release an irritant compound. This species has been found down to 80 m depth in the Medi-  
293 terranean and Northeast Atlantic, always associated with the soft coral smaller in size: Tritonia lineata Alder &  
294 Hancock, 1848 is a small species (up to 1.5 cm) living in shallow water down to 15 m depth. It is  
295 distinguishable by its translucent body colored with two white lines along the length of the notum; Tritonia  
296 manicata Deshayes, 1853 lives in the euphotic zone (infralittoral rocks and seagrass meadows) where it feeds  
297 on stoloniferans, such as Cornularia cornucopiae (Pallas, 1766); Tritonia nilsodhneri Marcus (1983) is up to  
298 3 cm long and lives down to 50 m depth where it feeds on coastal gorgonians, such as those of the genera  
299 Eunicella and Leptogorgia; Tritonia coralliumrubri Doneddu, Sacco and Trainito (2014), is a 1-cm  
300 nudibranch, whitish-gray in colour with irregular black spots, 15 pairs of gills on the mantle and a 12-lobed  
301 velum, that has been found associated only with the precious red coral Alcyonium digitatum Linnaeus,  
302 1758 (Gofas et al., 2011). The other Tritonia species known in the Mediterranean Sea are typically coastal  
303 and Corallium rubrum (Linnaeus, 1758), from 35 to 100 m depth; Tritonia striata Haefelfinger, 1963 is  
304 whitish with black stripes and lives down to 30 m depth where it feeds on Paralcyonium spinulosum (Delle  
305 Chiaje, 1822), Maasella edwardsii (de Lacaze-Duthiers, 1888) and other stoloniferans; Tritonia plebeia  
306 tohnston, 1828 is up to 3 cm long, pale yellow colored with extensive greenish-brown mottling, and feeds on  
307 corals of the genus Alcyonium in shallow water (Gofas et al., 2011; Trainito and Doneddu, 2014; Furfaro  
308 and Mariottini, 2016; Furfaro et al., 2017).

309  
310

#### 311 4.3. Conservation issues

312 Tritonia callogorgiae sp. nov. seems to be exclusively associated with *C. verticillata*, a species considered  
313 ‘near threatened’ by the International Union for the Conservation of Nature (IUCN) (Otero et al., 2017),  
314 contemplated in the Annex II of the SPA-BIO Protocol of the Barcelona Convention and able to form coral  
315 forests considered Vulnerable Marine Ecosystems (VMEs) of great importance under a conservation  
316 perspective (FAO, 2009). The occurrence of an exclusively associated nudibranch further strengthens the  
317 need for conservation of *C. verticillata* habitats. Destructive fishing practices, resource exploitation, marine  
318 debris impacts and climate change effects are increasingly threatening deep-sea habitats in the Mediterranean  
319 Sea as everywhere else. Fishing practices using bottom-contact gears represent one of the biggest threats for  
320 deep-sea coral habitats, for which the ongoing conservation measures are not always effective (e.g. FAO,  
321 2009, 2016; Dura´n Mun˜oz et al., 2012; Sampaio et al., 2012; Bo et al., 2014; Fabri et al., 2014; Aguilar et  
322 al., 2017; Chimienti et al., 2019b, 2020; Giusti et al., 2019; Otero and Marin, 2019; Taviani et al., 2019;  
323 Danovaro et al., 2020). This study also provides further evidence of the remarkable presence of lost longlines  
324 entangled and abandoned in sensitive habitats such as the forests of *C. verticillata*. These gears affect  
325 negatively the integrity of the colonies, whose mechanical injuries are evident. Similarly, plastic litter can  
326 have an impact on corals when remaining entangled on the branched structure of the colonies, choking the  
327 polyps. Moreover, current trends in climate change could severely affect deep-sea coral habitats in the North  
328 Atlantic and Mediterranean Sea, with drastic habitat loss that could lead to local extinctions with limited  
329 refugia for both hard and soft corals (Morato et al., 2020). Proper conservation initiatives aiming at reducing  
330 anthropogenic stressors on the deep-sea and protecting vulnerable coral habitats could indirectly contribute  
331 to preserving the associated biodiversity, including taxa not yet described and species strictly related to  
332 these habitats for their survival, like the new tritoniid species described here. Although the deep-sea  
333 nudibranch fauna is still poorly known, the increasing use of visual and sampling technologies will  
334 predictably entail the identification of many more species in the future (Vald´es et al., 2018). Further steps in

335 the study of *T. callogorgiae* sp. nov. include the understanding of the species' life cycle and its role in the  
336 ecology of the *C. verticillata* forests, that could unveil new cryptic dynamics among species on the deep  
337 benthos. For instance, Gomez (1973) hypothesized that, along with other physical and biological agents,  
338 tritoniids can play an important role in preparing the substrate for the settlement of epi- bionts that in turn  
339 can contribute in diversifying the community. On the other hand, population outbreaks of the congeneric  
340 *Tritonia hamnerorum* Gosliner & Ghiselin, 1987 have been documented representing a natural threat for the  
341 purple sea fan *Gorgonia ventalina* Linnaeus, 1758 in Florida and Bermuda (Cronin et al., 1995; Murdoch,  
342 2006). However, healthy and dense coral populations can show higher resilience against nudi- branch  
343 outbreaks.

## 344 345 5. Conclusions

346 The presence of rare or uncommon taxa represents a robust argument for the consideration of their host  
347 habitats as meritorious for proper conservation measures. In this respect, forests of *C. verticillata* represent a  
348 unique habitat for several associated species, including the newly discovered *Tritonia callogorgiae* sp. nov.  
349 This study provides the elements to identify this noticeable species even based solely on images and without  
350 the need of complex sampling operations. It is expected that further records of *T. callogorgiae* sp. nov. will  
351 be added in the future, as part of new deep-sea explorations and monitoring programs all over the  
352 Mediterranean Sea. Colorful and extravagant invertebrates like nudi- branches are often noted for their vibrant  
353 colour palettes and widely appreciated from an aesthetic point of view. They could thus act as 'flag species'  
354 to push general public and decision makers towards the importance to protect the vulnerable habitats where  
355 they live.

## 356 357 Declaration of competing interest

358  
359 The authors declare that they have no known competing financial interests or personal relationships that  
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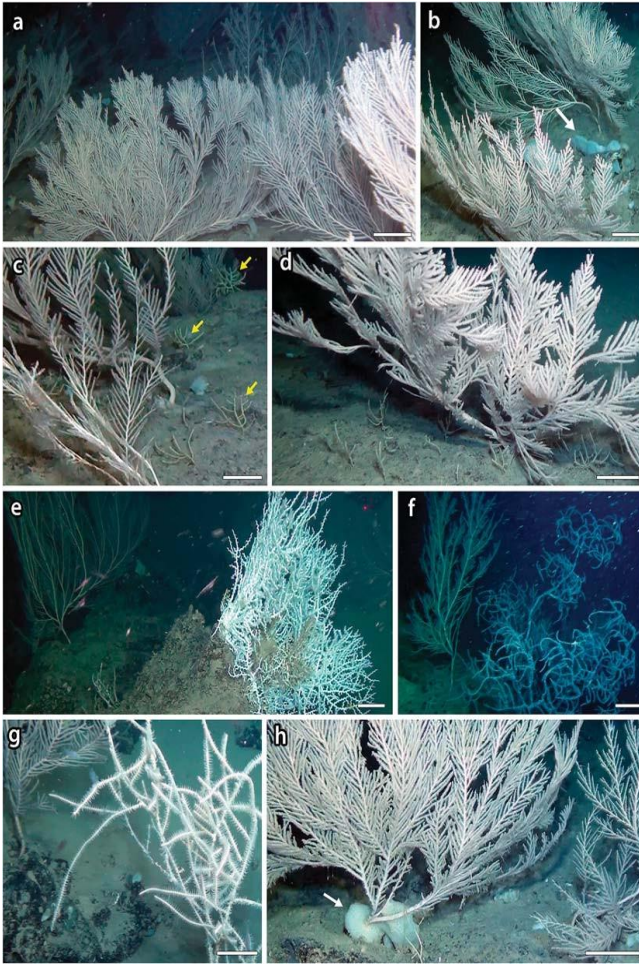
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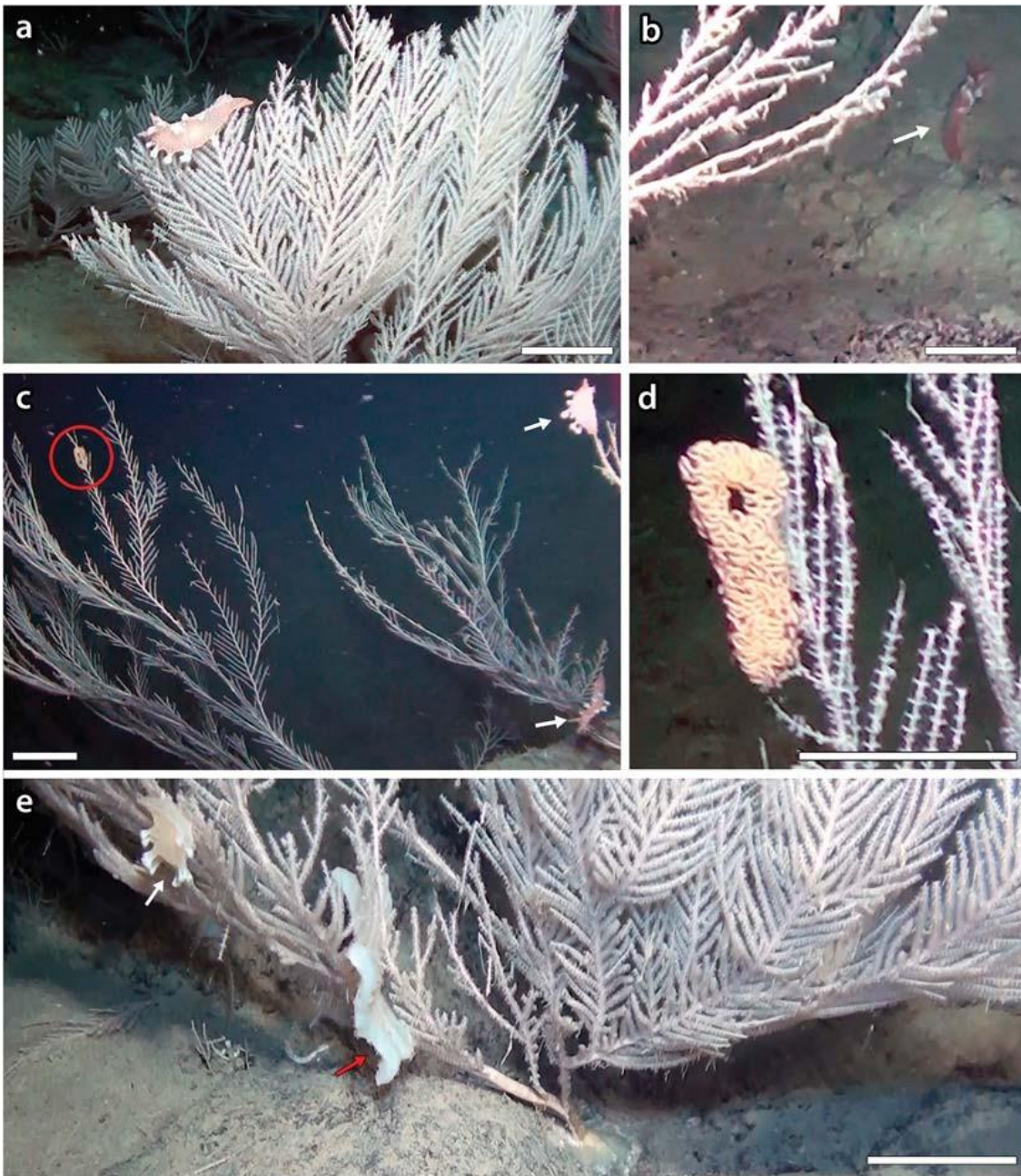
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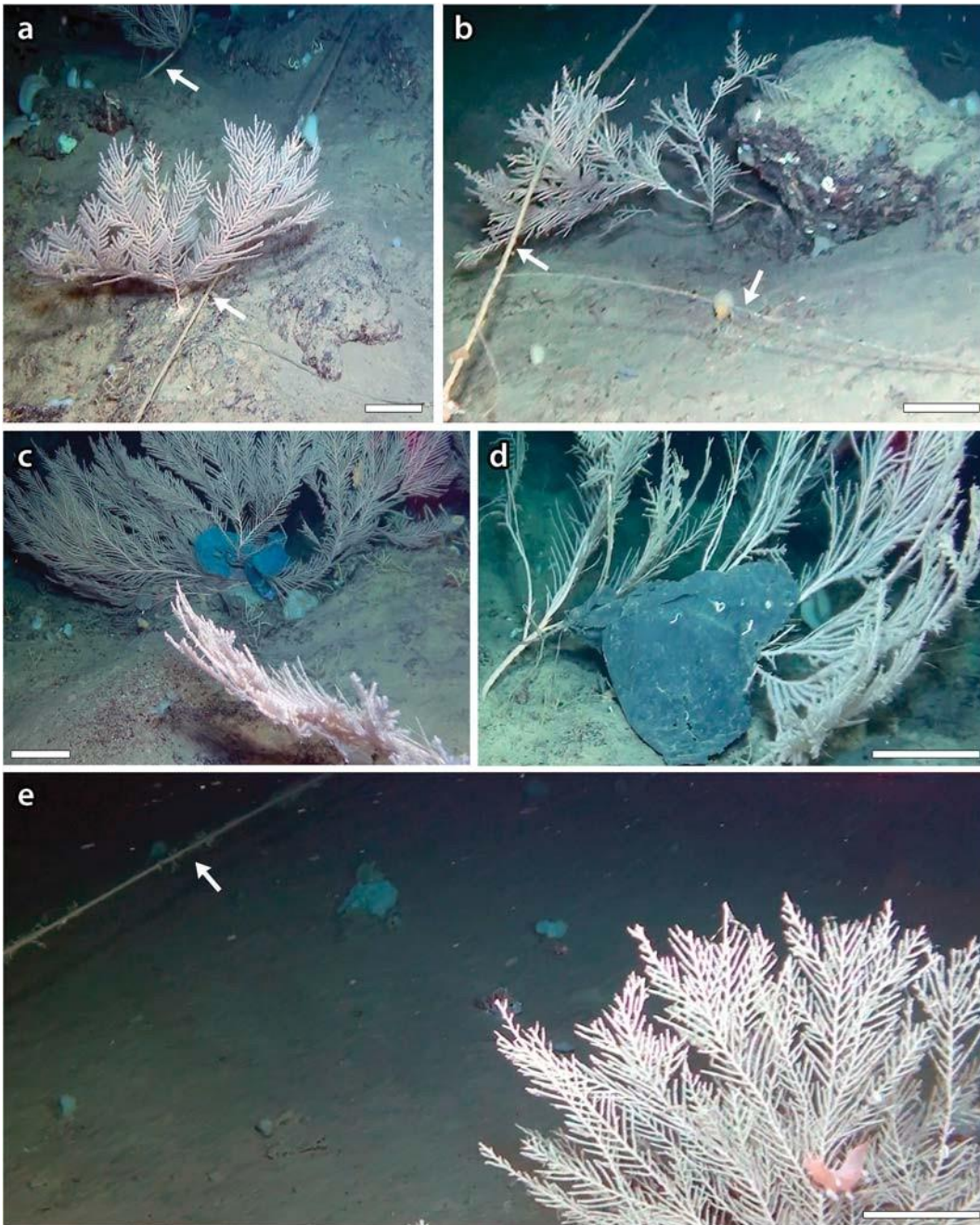
Fig. 1. Forest of *Callogorgia verticillata* off Montenegro, 420–426 m depth. a) dense colonies with associated habitat formers: b) the sponge *Pachastrella monilifera* (white arrow); c) the gorgonians *Paramuricea macrospina* (yellow arrows) and d) *Swiftia dubia*; e) the scleractinian *Madrepora oculata*; f) the black corals *Leiopathes glaberrima* and in the background a specimen of *C. verticillata*; g) *Antipathes dichotoma* in the foreground; h) *P. monilifera* (white arrow) growing around the base of *C. verticillata*. Scale bars: 10 cm.



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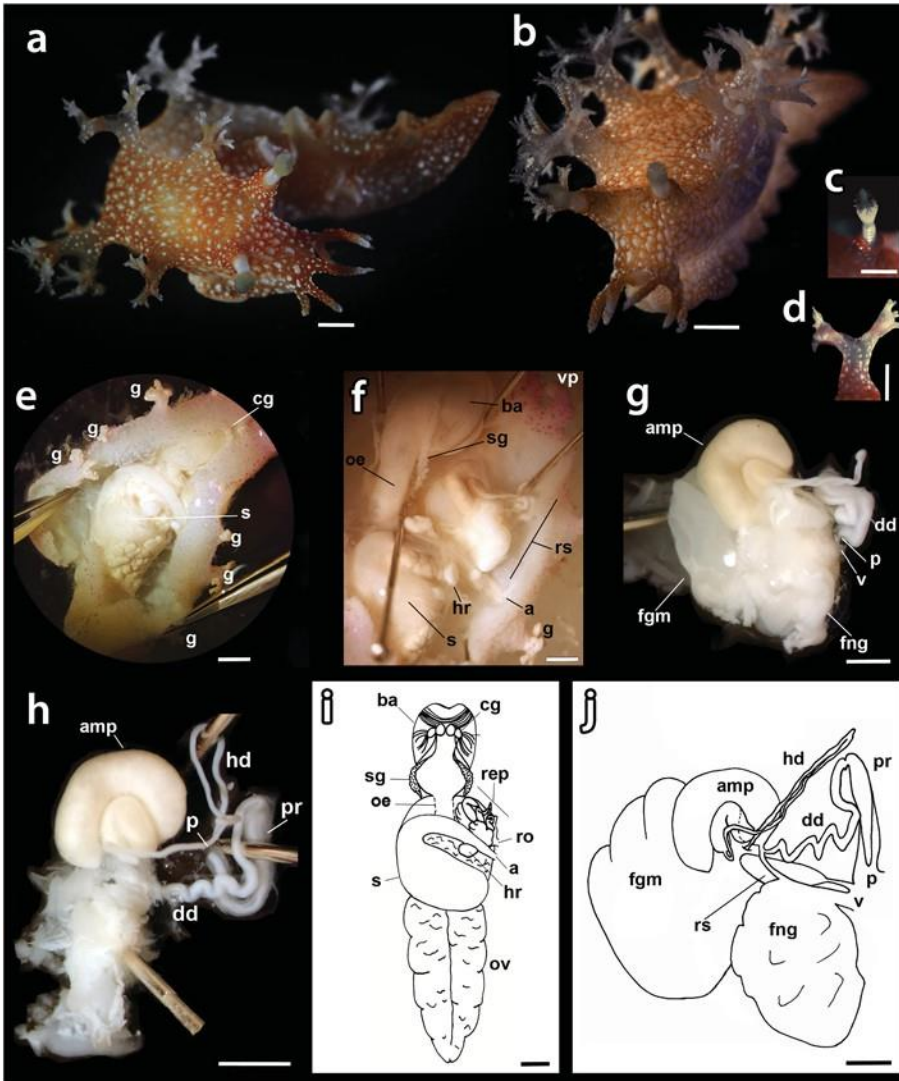
Fig. 2. In situ observations (off Montenegro, 420–426 m depth). Specimens of *Tritonia callogorgiae* sp. nov. a) feeding on *Callogorgia verticillata* and b) crawling on the muddy bottom (white arrow); c) two specimens on the same colony (white arrows) and egg coils (red circle) on another; d) detail of egg coils; e) *Pachastrella monilifera* (red arrow) growing as epibiont on *C. verticillata* in presence of a specimen of *T. callogorgiae* (white arrow). Scale bars: a–c,e) 10 cm; d) 5 cm.

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Fig. 3. Anthropogenic impacts on the forest of *Callogorgia verticillata* off Montenegro. a–b) longlines (white arrows) entangled in colonies of *C. verticillata*; c–d) plastic litter entangled on *C. verticillata*; e) Longline in proximity of a colony where is present a specimen of *Tritonia callogorgiae* sp. nov. Scale bars: 10 cm.



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Fig. 4. *Tritonia callogorgiae* sp. nov. (paratype A\_31\_1; MUZAC-6555). External anatomy: a–b) specimens in vivo, with details of c) rhinophore and d) dichotomous gill. Internal anatomy: e–f) digestive and reproductive systems; g–h) reproductive system; i) schematic drawing of the internal anatomy; j) schematic drawing of the reproductive system. Scale bars: 5 mm. Abbreviations: a: anus; amp: ampulla; ba: buccal apparatus; dd: deferent duct; oe: oesophagus; fgm: female gland mass; fng: female nidamental gland; g: gill; hd: hermaphroditic duct; hr: heart; cg: cerebralpleural ganglia; ov: ovotestis; p: penis; pr: prostate; rep: reproductive system; ro: reproductive openings; rs: receptaculum seminis; s: stomach; sg: salivary gland; v: vagina; vp: velum process.

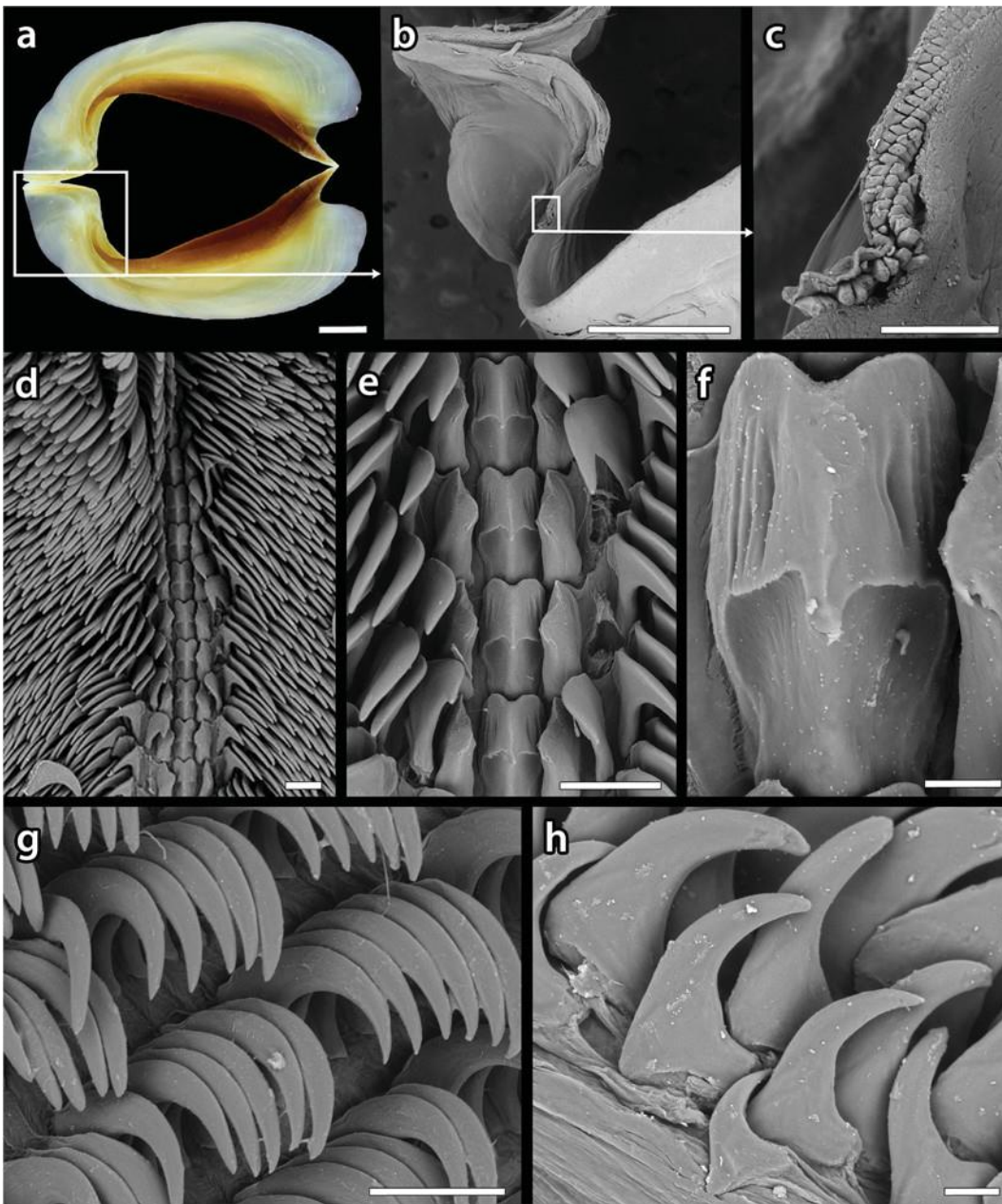
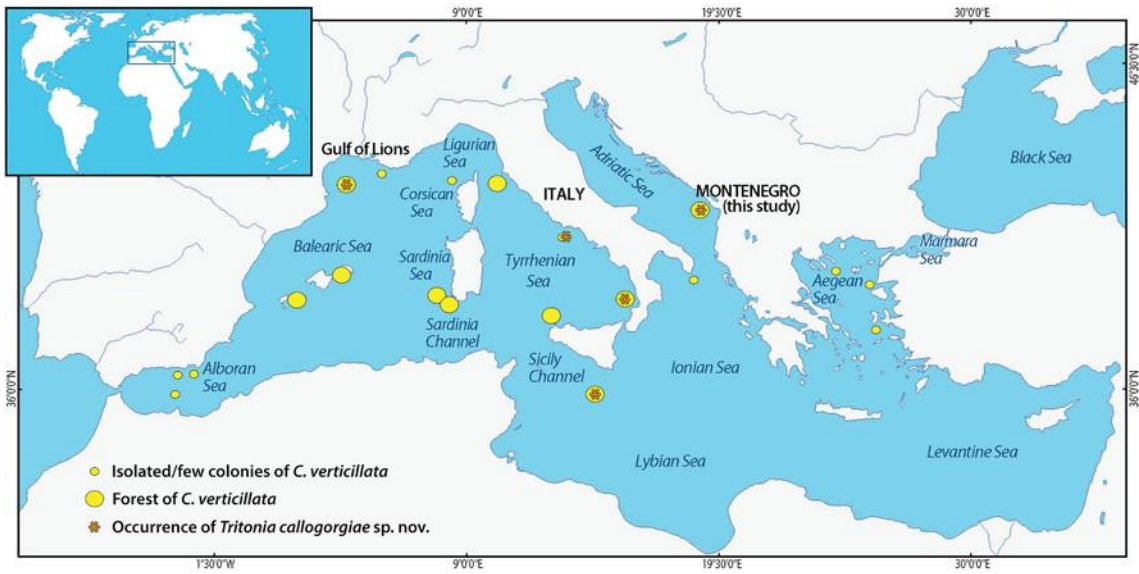


Fig. 5. taws and radula of *Tritonia callogorgiae* sp. nov. (paratype A\_31\_1; MUZAC-6555). taws: a) view at the stereomicroscope; b) detail of the area that hosts denticles; c) detail of denticles arranged in rows. Radula: d) rachidian tooth with numerous lateral teeth; e) rachidian tooth and lateral teeth (first lateral tooth different from the others); f) detail of the rachidian tooth; g–h) lateral teeth. Scale bars: a–b) 1 mm; c–e,g) 100 µm; f,h) 20 µm.

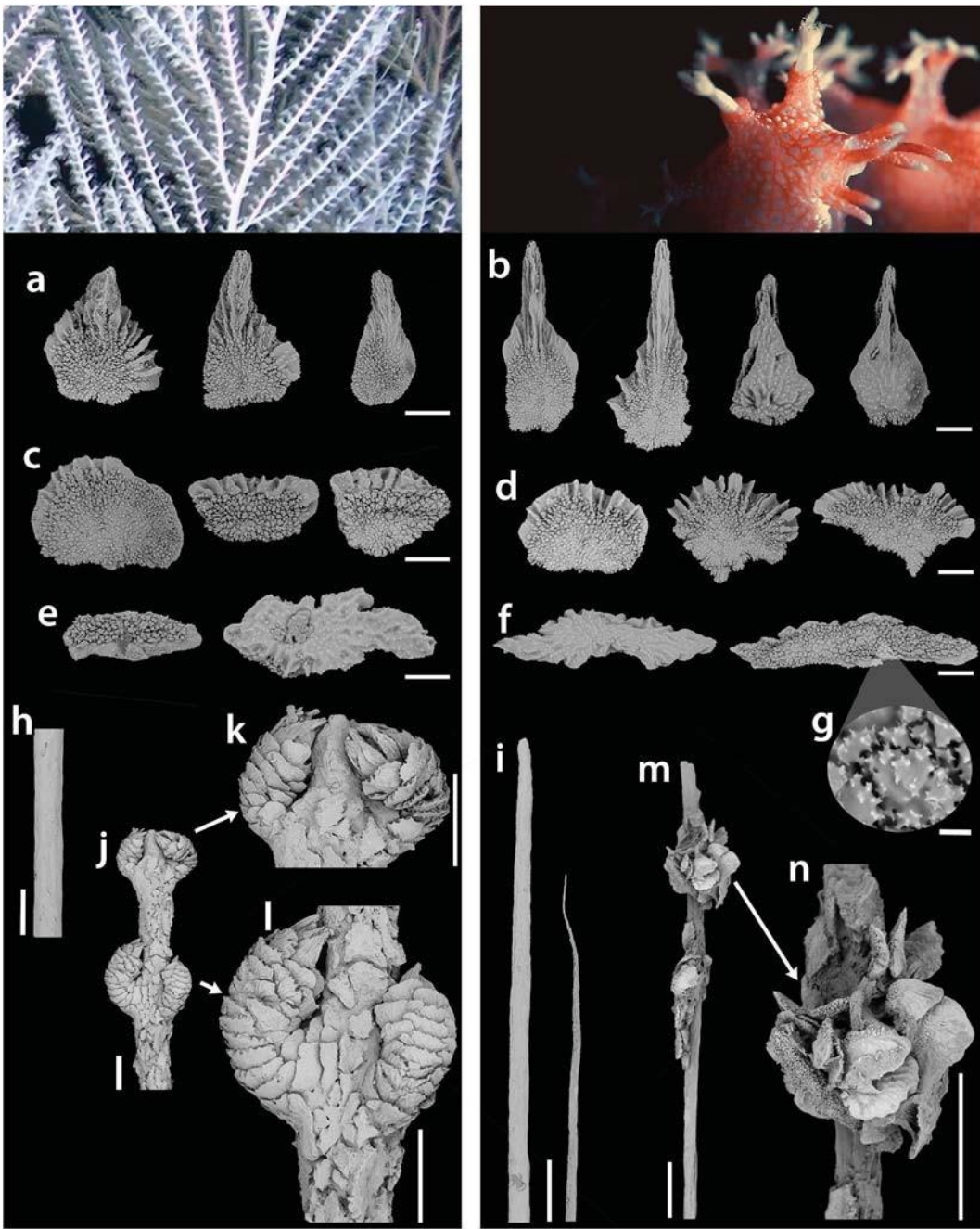


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Fig. 6. Map of the distribution of *Callogorgia verticillata* in the Mediterranean Sea (modified from Chimienti et al., 2019a) and occurrences of *Tritonia callogorgiae* sp. nov. (references in the text). Note that putative records outside the Montenegro type location are only based upon ROV images.



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**Fig. 7.** Comparison between sclerites, axis and polyps of *Callogorgia verticillata* (left) and those found in the stomach of *Tritonia callogorgiae* sp. nov. (right). a–b) opercular sclerites; c–d) body-wall sclerites; e–f) coenenchymal sclerites; g) detail of the tuberculate structures on the inner surface of the sclerites. h–i) internal axis; j) polyps, with details of f) terminal polyp and l) polyp whorls (external lateral side of the calyx); m) partially digested axis with some polyps attached; n) detail of one polyp. Scale bars: a–f) 100  $\mu\text{m}$ ; g) 10  $\mu\text{m}$ ; h–n) 500  $\mu\text{m}$ .