

Interesting Images

Hiding in Fouling Communities: A Native Spider Crab Decorating with a Cryptogenic Bryozoan in a Mediterranean Marina

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Camouflage is the method by which animals conceal by blending in with the environment, and may be achieved by fixed or changing color, shape, texture, chemical secretions, and/or behavior [1–4]. Among various forms of camouflage, the visual one mostly consists of hiding into the background, and can be attained through various methods, including the decoration through the attachment of environmental materials and objects on the exterior part of the organism [1–4].

Decoration is an active process that involves costs in terms of time and energy, and has been studied so far across a range of different taxa [3,5]. The most widely studied decorators are the crabs of the superfamily Majoidea Samouelle, 1819, a group composed of over 900 species, of which about 75% show decorations over some or all of their body. Although some examples are known of decapod species that put edible materials on their bodies and later remove these materials to eat them, the majority of majid crabs decorate themselves to camouflage against predators either entangling (in parallel) or impaling (in perpendicular) biological material through the hooked setae of the exoskeleton [1,3,6–11].

During a recent BioBlitz (an event that focuses on finding and identifying as many species as possible in a specific area over a short period of time) carried out on the 3 May 2020 at Port Saplaya (Valencia, Spain, 39.512049° N, 0.321490° W), a small marina in the western Mediterranean Sea (Figure 1A), the local biota was investigated by one of the authors, and in particular, the fouling communities of the mooring lines were observed with the help of scalpels, scissors, and a magnifying glass. Among fishes, specimens of two native predators were noticed, namely *Sparus aurata* Linnaeus, 1758 (Figure 1B) and *Balistes caprisicus* Gmelin, 1789 (Figure 1C). With regards to the fouling organisms, the area resulted to be dominated by the cirriped *Amphibalanus amphitrite* (Darwin, 1854), the bryozoans *Schizoporella errata* (Waters, 1878) and *Bugula neritina* (Linnaeus, 1758) [complex], and the ascidians *Styela plicata* (Lesueur, 1823) and *Microcosmus squamiger* Michaelsen, 1927 (Figure 1D,E), all alien or potential cryptogenic species already known for the Spanish coast of the Mediterranean Sea (e.g., [12–16]). However, a crab specimen decorating with a number of colonies of an arborescent bryozoan was also found living among mooring lines (Figure 1F). As such decoration was never encountered by us during fieldwork in various localities of the Mediterranean Sea, it attracted our attention, and thus the crab was sampled, photographed, and fixed in 99.9% ethanol for subsequent integrative taxonomic analyses. The crab sample and some bryozoan colonies detached during manipulation were deposited in the collection of the Laboratory of Benthos-Napoli, Stazione Zoologica Anton Dohrn (Naples, Italy), with the following codes: SZN-B-1358CR106C (Arthropoda) and SZN-B-936BR3A (Bryozoa).

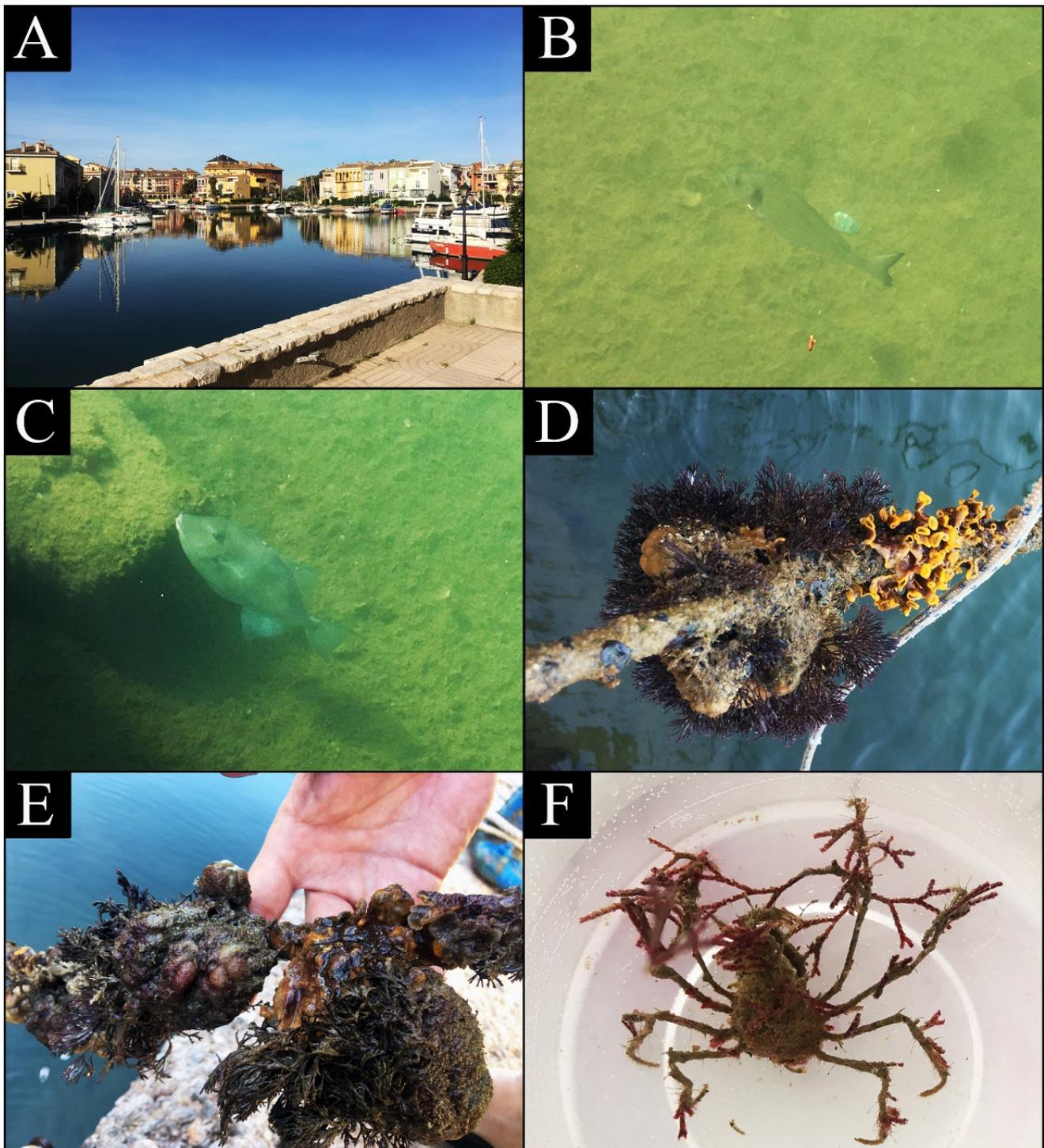


Figure 1. Port Saplaya (Valencia, Spain, western Mediterranean Sea) and its biota. (A) The local marina. (B) The gilt-head seabream *Sparus aurata*. (C) The gray triggerfish *Balistes capriscus*. (D,E) Fouling communities of mooring lines. (F) *Macropodia czernjawszkii* decorating with colonies of the *Bugula neritina* complex.

The crab specimen was identified to genus level following guides on the decapod biota of the Atlanto-Mediterranean area [17,18], and subsequently to species level according to guides and specialist literature [17–22]. In particular, among other diagnostic features, the specimen showed basal antennal segments with spines visible in ventral and/or lateral view (Figure 2A), a downcurved rostrum whose length did not exceed the antennal peduncles (Figure 2B,C), and a pyriform cephalothorax with a single median protogastric bilobed

spine (Figure 2B,C). In addition, the carapace surface, sternal area, pleon, and chelipeds were unevenly and densely covered with pile, whereas the gastric region presented a large protogastric protuberance on each lateral side. All these features suggested the morphological identification of the crab as *Macropodia czernjawszkii* (A.T. Brandt, 1880). It was a female, measured 13.74 mm × 9.40 mm in total length (from the anterior part of the rostrum to the posterior tip of the telson) × width, and hosted 43 colonies (or colony fragments) of a single arborescent bryozoan species, distributed in the following way: Cephalothorax (8 colonies), L(ef) P(ereiopod) 1 (carpus: 1), LP2 (carpus: 3, propus: 3), LP3 (merus: 2, carpus: 2, propus: 2), LP4 (merus: 2, carpus: 1, propus: 1), LP5 (merus: 1, propus: 1), R(ight) P2 (carpus: 2, propus: 1), RP3 (merus: 2, carpus: 1, propus: 2), RP4 (merus: 1, carpus: 1, propus: 3), RP5 (merus: 1, carpus: 1, propus: 1) (Figure 2D). The bryozoan colonies were identified to genus level following guides on the bryozoan biota of the Atlanto-Mediterranean area [23–26], and subsequently to species level according to guides and specialist literature [24,26–30]. In particular, the colonies were brown-purple in color, the autozooids lacked spines and avicularia but had a distal margin well marked, and the ovicells were white, big, and globular, jointed by a peduncle to the maternal zooid and with an oblique orientation (Figure 2D,E). All these features suggested the morphological identification of the colonies as *Bugula neritina* (Linnaeus, 1758), an apparently worldwide-distributed species discovered to be a species complex in the last decades [31–36].

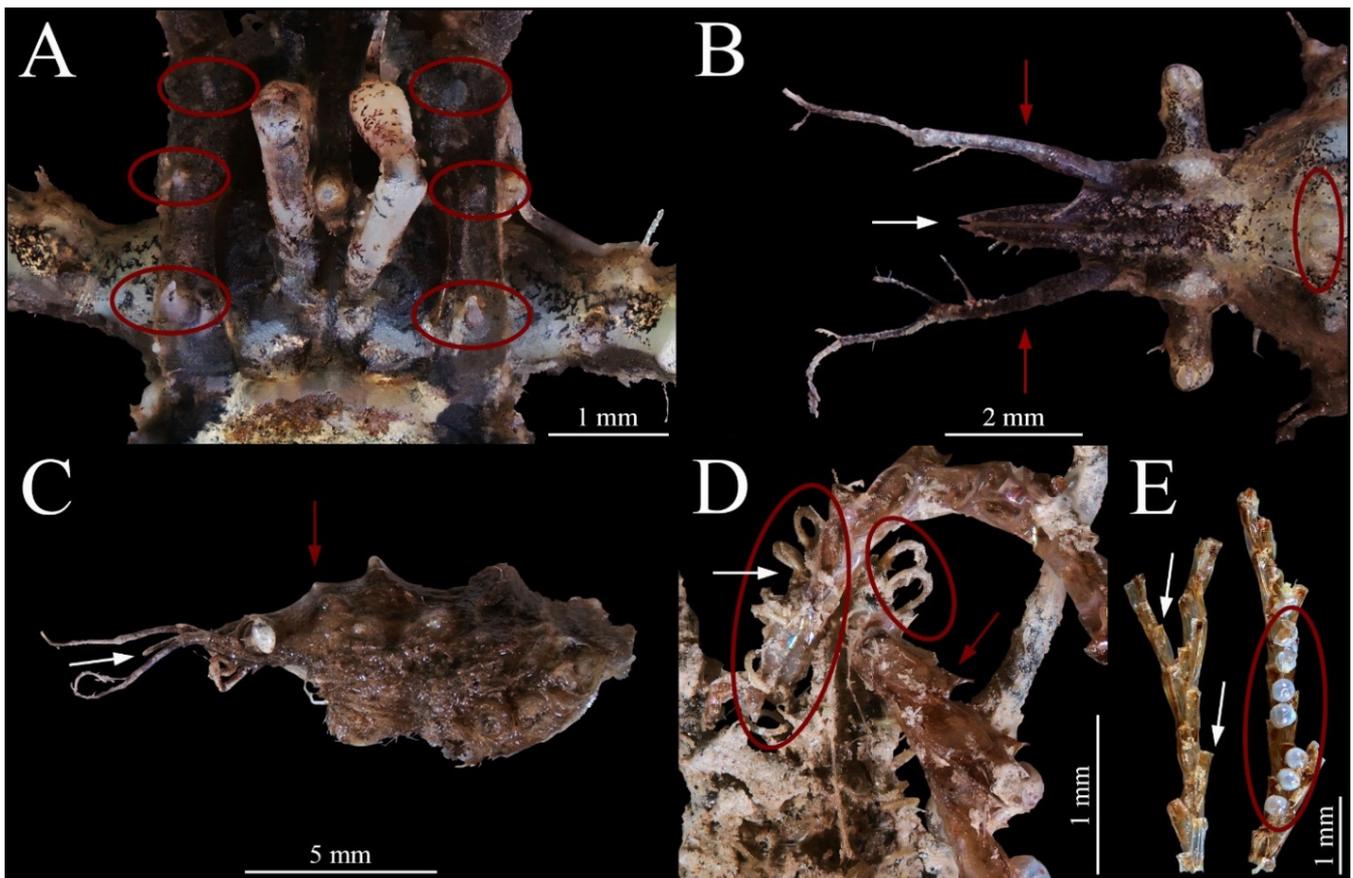


Figure 2. *Macropodia czernjawszkii* from Port Saplaya (Valencia, Spain, Mediterranean Sea). (A) Basal antennal segments with spines highlighted (red circles). (B) Detail of dorsal view of cephalothorax (cleaned), highlighting rostrum (white arrow), antennal peduncles (red arrows), and the median protogastric bilobed spine (red circle). (C) Lateral view of cephalothorax (cleaned), highlighting rostrum (white arrow) and median protogastric spine (red arrow). (D) Hooked setae of the rostrum (red circles) with colonies of the *Bugula neritina* complex entangled (white arrow) and impaled (red arrow). (E) Bryozoan colonies detached from the carapace highlighting distal margins of autozooids (white arrows), and ovicells (red circle).

To further verify the morphological identifications, and considering that both species were never sequenced from the Mediterranean Sea, total genomic DNA was extracted from the crab host species and five bryozoan colonies as described by Crocetta et al. [37]. Partial sequence of the *Cytochrome c Oxidase subunit I* (COI) gene was amplified for *Macropodia* using the primers COL6b (5'-ACAAATCATAAAGATATYGG-3') and COH6 (5'-TADACTTCDGGRTGDCCAAARAAYCA-3') [38], whereas for *Bugula* using the primers Neri-11f (5'-GGTTCATATCCACTAACCATAAAG-3') and Neri-725r (5'-TATACTTCGGGGTGACCAAAAAACCA-3') [34]. Polymerase chain reactions (PCRs) were conducted in 25 µL volume reaction as in Tanduo et al. [39]. Amplification in *Macropodia* was performed with an initial denaturation at 95 °C (5 min), followed by 39 cycles of denaturation at 95 °C (1 min), annealing at 48 °C (1 min), extension at 72 °C (1 min), with a final extension at 72 °C (5 min), whereas in *Bugula* with an initial denaturation at 95 °C (3 min), followed by 35 cycles of denaturation at 94 °C (1 min), annealing at 55 °C (1 min), extension at 72 °C (1 min), with a final extension at 72 °C (5 min). The PCR products were purified and Sanger sequenced at the Molecular Biology and Sequencing Service of SZN through an Automated Capillary Electrophoresis Sequencer 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA), using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies, Renfrew, UK). The chromatograms for each sequence obtained were checked, assembled, and edited using Sequencher v.5.0.1 (GeneCodes, Ann Arbor, MI, USA) and compared with reference sequences from the NCBI nucleotide (NT) database using BLASTn [40]. The *Macropodia* sequence (662 base pairs; GenBank MZ044965) showed a very high (98.81–99.39%) similarity with six sequences (MT311173–75, MT311177–79) from the Black Sea ascribed to *M. czernjawszkii* by Spiridonov et al. [22], whereas all the other taxa deposited in GenBank showed lower similarities ($\leq 93.46\%$). Interestingly, the bryozoan sequences showed differences among them. In particular, one sequence type (662 base pairs; GenBank MZ045377) showed a 100% similarity with several sequences (e.g., KC129725, KC129730–33, KC129735, KC129822), among which those ascribed to the clade S of the *B. neritina* complex *sensu* Fehlaue-Ale et al. [34], whereas the other sequence type (662 base pairs; GenBank MZ045393) showed a very high (99.55–100%) similarity with six sequences, among which three (KC129721, KC129734, KC129748) ascribed to the clade N of the same species complex. Thus, molecular results confirm the morphological identification for both species. At the same time, they first suggest here the conspecificity between Mediterranean and Black Sea samples of *M. czernjawszkii* through molecular data and that two different clades of the *B. neritina* complex, which presumably account for different species [34], live in the Mediterranean basin. While the clade S is already widespread worldwide, the clade N was only known from few localities so far (eastern and western U.S.A. and eastern Australia) [34]. The contemporary finding of both clades on *M. czernjawszkii* suggests that they also live in syntopy in Port Saplaya.

Decoration in European majid species has been studied since the 19th century, although early authors did not provide accurate descriptions, mixed up decoration with epibiosis, or mostly reported the usage of algae. Graeffe [41] and Aurivillius [42] provided the earliest description of the biota associated with the carapace in several majid species, and also recognized the role of the hooked setae. Both authors also mentioned a preference for macroalgae, although, mostly based on aquarium observations, they noticed the potential usage of other taxonomic groups. More recently, other authors focused on decoration in European species, among which Cruz-Rivera [43] tested the decoration preferences of *Macropodia rostrata* (Linnaeus, 1761), *Acanthonyx lunulatus* (Risso, 1816), and *Pisa tetraodon* (Pennant, 1777) in plastic containers filled with seawater, highlighting a high degree of specificity in decorating preferences based on algal morphology. Fürböck and Patzner [44] studied the decoration preferences of *Maja crispata* Risso, 1827, revealing that it is an opportunist species that uses several algae based on the abundance and the morphological structure of the available algae. Martinelli et al. [45,46] highlighted the usage of sponges by *Inachus phalangiium* (J.C. Fabricius, 1775) and of filamentous green algae by juveniles of *Inachus communissimus* Rizza, 1839. Rorandelli et al. [47] investigated the choice preferences

of *I. phalangium* when using algae for feeding and masking. Finally, Spiridonov et al. [22] mentioned decoration and epibiosis in almost all *Macropodia* species in Europe, although he specifically focused on *M. czernjawska* in the Black Sea, that showed preferences for various autotrophic taxa (Chlorophyta, Phaeophyta, and Rhodophyta). In addition, hydroids and sponges, sedentary polychaetes, and an encrusting bryozoan colony were rarely found as epiphytes on the dorsal part of the cephalothorax of this species. Therefore, to the best of our knowledge, the present record accounts for the first observation of such decoration in a European species, or at least the first confirmed one. A similar situation also holds true for majid taxa worldwide: In fact, although several authors generally stated that crabs may be able to decorate with bryozoans (among other phyla), field observations mostly or even only reported macroalgae (e.g., [7–9,48,49]), and this statement is presumably based on the few observations listed thereafter. Wicksten [50] reported small- and medium-sized specimens of *Loxorhynchus crispatus* Stimpson, 1857 and *Loxorhynchus grandis* Stimpson, 1857 decorating with branched bryozoans in California, among which specimens of the *B. neritina* complex. The same author also highlighted that, in particular, one *Loxorhynchus* specimen found on a piling was decorating with colonies of *Pomocellaria californica* (Trask, 1857), but that this happened because this bryozoan was the only “material” readily available for decorating. A photo of a *L. crispatus* juvenile covered by branched bryozoans was also subsequently published by Guinot and Wicksten [4]. In another article, Wicksten [51] drew a specimen of the California crab *Ericerodes hemphillii* (Lockington, 1877) decorating with a high number of unidentified arborescent bryozoans as the *M. czernjawska* found here, and again highlighted that the species was found in docks and wharf pilings. The same specimen was so peculiar that it was then redrawn by Hultgren and Stachowicz [2]. More examples may have been published as scattered records or oddities in the worldwide literature, but we did not trace them.

Decoration is generally considered a plastic process that occurs on a short timescale (hours to days), with the animal that must find the materials and manipulate and attach them to itself, but also sometimes exchange old materials for the new ones, especially during growth or when changing substrate/environment. Moreover, although crabs have been often considered to be generalists, subsequent studies have demonstrated material or phyla preference or fidelity, and thus this issue is still discussed in the current literature [1,10,49]. *Macropodia czernjawska* is a crab species reported to live until about 100 m depth, although it is mostly found in meadows and algal fringes of the infralittoral zone [21,52,53], and was only known to decorate with photophilic algae so far [22]. However, at least in the present case, it proved to be a generalist and a very opportunistic species, able to camouflage in fouling communities by using what was available at the site at the specific moment. The similar observations reported by Wicksten [50,51] on other majid species found worldwide, but in the same habitat investigated here, would suggest that arborescent bryozoans, whether native, introduced, or cryptogenic, may have an important ecological role acting positively at least on some crab populations or species by facilitating their camouflage in the fouling communities of harbors. Finally, Skelhorn et al. [54] highlighted decoration in terrestrial caterpillars with non-edible items, thus causing repellence in predators, a phenomenon also noticed in majid crabs and that may apply here to bryozoans too [2,8,55]. In fact, marinas and harbors often host variegated fish assemblages [56–58], including top-level predators as also found in Port Saplaya. Specific studies to be carried out in harbors and marinas, and subsequent laboratory experiments on potential repellence of bryozoans (including native vs. alien and cryptogenic species), would confirm all these statements based so far on a few opportunistic findings worldwide.

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