



Multiple records and polymorphism of *Parastichopus regalis* (Cuvier, 1817) (Echinodermata: Holothuroidea: Stichopodidae) along the Algerian coast

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Abstract

The family Stichopodidae is represented in the Mediterranean Sea by the genus *Parastichopus* which includes two non-endemic species; *Parastichopus tremulus* (Gunnerus, 1767) and *Parastichopus regalis* (Cuvier, 1817). On the Algerian coast (southwestern Mediterranean Sea), two morphotypes of *P. regalis* were observed, one with dark spots on the dorsal surface and the other non-spotted. In total, 65 individuals of *P. regalis* were recorded from 22 stations along the Algerian coast during an oceanographic campaign. Twelve individuals (6 of each morphotype) were used for a comparative study of the morphological (including endoskeletal) characteristics. Table ossicles, the only ossicles of the body wall of the two morphotypes of *P. regalis*, are here compared with regard to the disk diameter of the tables and the total area of the surface of the disc. Statistical analysis did not show any significant differences between the spotted and the non-spotted morphotypes.

Key words: Holothurians, morphotype, ossicles, southwestern Mediterranean Sea, statistical analysis

Introduction

The family Stichopodidae includes a diverse assemblage of sea cucumber species, most of which are found in the Indo-Pacific (Byrne *et al.* 2010). It includes 9 genera and 35 species (Worms 2020). It is represented in the Mediterranean Sea by only two species of the genus *Parastichopus*. This genus was established by Clark (1922) and, according to Byrne *et al.* (2010), it is restricted to the North Atlantic Ocean. It differs from the genus *Stichopus* which is characteristic of the tropical and subtropical regions of the world. The genus is characterized by the absence of “button” type ossicles (Byrne *et al.* 2010). The family was, until recently, classified in the order Aspidochirotida which was found to be paraphyletic and replaced with the monophyletic Holothuriida. The family Stichopodidae, formerly of the Aspidochirotida, has been transferred to the order Synallactida, which also includes the families Deimatidae and Synallactidae (see Miller *et al.* 2017).

Parastichopus regalis occurs between 5–800 m in depth (Clark 1922; Tortonese 1965; Mercier & Hamel 2013; Ramón *et al.* 2019) on sea-bottoms composed mainly of sand, silt and clay (Ramón *et al.* 2019). In the circalittoral zone of the Mediterranean Sea, there is a sticky mud setting formed in association with the cnidarian *Alcyonium palmatum* Pallas, 1766 in the biocenosis of coastal terrigenous muds (Michez *et al.* 2011, 2014). Like most Synallactida, *Parastichopus regalis* is a deposit-feeder, which ingests fine sediment in size ranging from 0.103 µm to 1 mm (Ramón *et al.* 2019). This species is distributed in the East Atlantic [from the Angola (Cherbonnier 1965) to Northern Ireland, including the Canary Islands (Hernández *et al.* 2013), the Azores (Madeira *et al.* 2018), Madeira (Augier 1985) and Portugal (Santos *et al.* 2015)], the West Atlantic [West Indies and the Gulf of Mexico (Ramón *et al.* 2010)] and the Mediterranean Sea where it has been reported from several regions [Alboran Sea, including Spain and West Algeria (Abad *et al.* 2007; Ramón *et al.* 2010; González-Wangüemert *et al.* 2014; Maggi & González-

Wangüemert 2015; Galimany *et al.* 2018; Benzait *et al.* 2020); the Ionian Sea (Tursi *et al.* 2004); Malta Island (Leonard *et al.* 2020); the Sea of Marmara (Aydin 2008) and the Aegean Sea (Aydin 2018)].

Few studies have been devoted to *P. regalis* in the Mediterranean Sea. These include studies on its distribution and fishery (Ramón *et al.* 2010), its endosymbiont *Carapus acus* Brünnich, 1768 (Gonzalez-Wangüemert *et al.* 2014), the genetic differentiation between its populations (Maggi & Gonzalez-Wanguemert 2015), its immune responses at different temperatures for its implication in aquaculture (Galimany *et al.* 2018) and some qualitative aspects of its feeding behaviour (Elakkeri *et al.* 2021).

From an economic point of view, *P. regalis* is of great commercial interest, its longitudinal muscle bands called *espardeña* or *llongo* in Spain, are highly relished by local consumers and Asian tourists. It is considered to be the most expensive sea-product, reaching a price of USD 154.47 / kg in markets (Ramón *et al.* 2010) and USD 51.09 / 165 g in luxury restaurants (Conand, pers. comm.). In Catalonia, the Balearic Islands and Valencia, only longitudinal muscle bands, *espardeña* are sold, unlike in Galicia where the entire animals are sold (Gonzalez-Wanguemert *et al.* 2018). From a pharmaceutical and nutritional point of view, it has been shown that the extract from the body wall of *P. regalis* exhibits antimicrobial, antifungal and antitumor activity (Santos *et al.* 2015) and also a balanced nutrition suitable for human consumption (Roggatz *et al.* 2018) due to its significant percentage of polyunsaturated fatty acid (Santos *et al.* 2015). This is what allows it to be used as food or nutraceutical supplements to improve the risk of certain diseases, such as improving the brain's resistance to damage and preventing atherosclerosis (Massaro *et al.* 2010; Blondeau 2016; Mecheta *et al.* 2020).

Intraspecific morphological variation has been observed in several species of sea cucumbers, ranging from differences in colour pattern, as is the case in *Holothuria (Metriatyla) scabra* Jaeger, 1833 (Al-Rashdi *et al.* 2007), *Bohadschia vitiensis* Semper, 1868 (Kim *et al.* 2013) and *Apostichopus japonicus* Selenka, 1867 (Xing *et al.* 2017), and/or the appearance of spots in some individuals of *Holothuria (Platyperona) sanctori* Delle Chiaje, 1923 (Mezali 2008, 2011, 2013) and *Bohadschia ocellata* Jaeger, 1833 (Kim *et al.* 2013), thus necessitating a study of polymorphism to determine if one is dealing with one or more species/morphotypes. Indeed, phylogenetic analyses based on mitochondrial DNA sequences have revealed that the spotted and non-spotted variety of *H. (P.) sanctori* and the three-colour variants of *A. japonicus* (red, green and black) each represent a single species (Mezali 2011, 2013; Mezali & Francour 2012; Zhang *et al.* 2015).

The main purpose of this study was therefore to determine, based only on their morphological (including endoskeletal) characters, if the two-colour morphs (morphotypes) of *P. regalis*, co-existing on the Algerian coast, are just colour variants of the same species or represent two different species.

Material and methods

Sampling area

The study was carried out along the Algerian coast between 35° 11.392' N and 37° 05.766' N and 02° 05.491' W and 08° 18.627' E (Figure 1). The individuals of *P. regalis* were collected aboard the Oceanographic Research Vessel *Grine Belkacem* between June 13 and July 16, 2019, under the auspice of the MEDITS program (International bottom trawl survey in the Mediterranean). This used the GOC73 fishing gear rigged with Morgère bottom doors, in order to assess the fishery resources of target species, discover new fishing areas and collect benthic invertebrate samples for systematic study. We relied on an indirect method to recover by-catches generally consisting of non-target benthic invertebrates such as echinoderms (sea cucumbers and starfish) and decapod crustaceans (i.e. crabs). For the systematic study of *P. regalis*, we concentrated on four stations in the west [two in Mostaganem (14 and 15), one in Arzew (17) and one in Bouzedjar (18)] (Table 1; Figure 1).

Biological material examined

In total, 65 individuals of *P. regalis* were collected from 22 stations at depths varying between 36 m and 532 m. Each individual was immediately photographed (dorsal and ventral surfaces) using a Canon EOS 1200D camera, measured (contracted length) and weighed (total wet weight), and then preserved in Ethanol (96%). Preservation was done immediately because the specimens tend to degrade very quickly. Among the collected individuals, twelve (12) were selected for morphological (including endoskeletal) study. Of these, six (6) were spotted and six (6) non-spotted. All measurements of relevant organs of each individual (viz. body wall thickness, calcareous ring diameter,

longitudinal muscle thickness and lengths of Polian vesicle, stone canal and tentacle ampullae) were done with a digital caliper to the precision of 0.01 mm.

Tentacles, papillae, pedicels, cloaca and fragments of the body wall from the dorsal, ventral and anal regions, were dissolved in pure commercial bleach and rinsed several times with distilled water to extract ossicles (Samyn *et al.* 2006). A drop of the solution was then placed on a slide, covered with a coverslip and observed under the Optika light microscope at X4 and X10 magnification for a general view, then at X40 magnification for details of the ossicle assemblage. The ossicles were then photographed using the Optika View Lite software. Measurements of the ossicles were done using ImageJ software to the precision of 0.001 μm . For statistical analysis only the diameter and area of a 120 table discs of spotted and non-spotted individuals are here considered (10 for each individual). Differences between samples were tested with the Kruskal-Wallis H test because the data was found to be non-parametric after testing the normality and homogeneity of variance using the Shapiro-Wilk test and the Levene test, respectively. When significant differences were detected, a Dunn's multiple comparison post-hoc test was performed to identify the source of the differences.

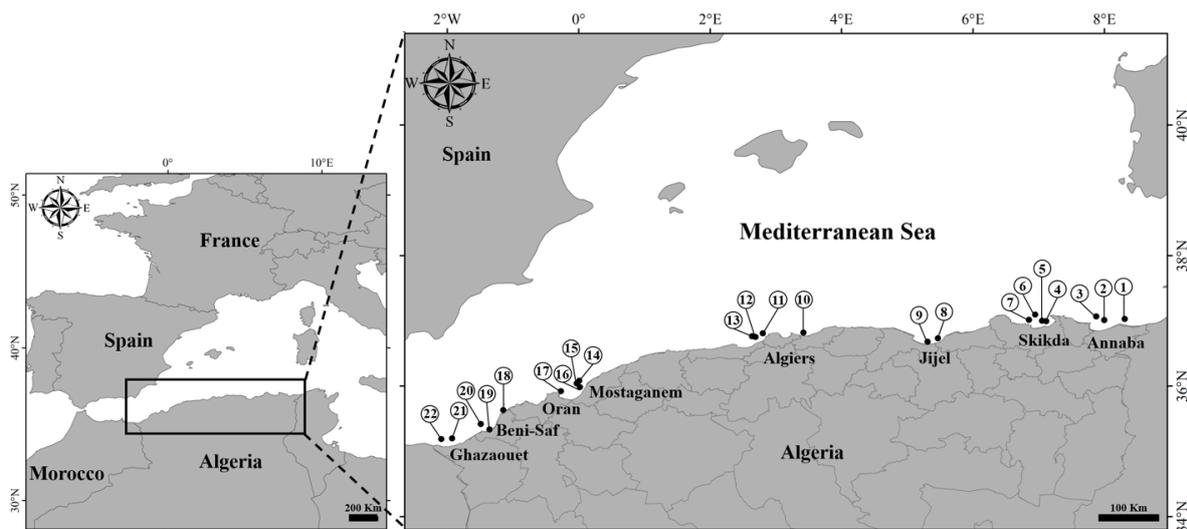


FIGURE 1. Sampling stations of *Parastichopus regalis* along the Algerian coast (the numbered circles represent the sampled stations - see Table 1 for details of the stations).

TABLE 1. *Parastichopus regalis* sampling stations and their geographic coordinates. (D) Depth in meter. Numbers in parentheses indicate station number.

Stations	Coordinates	D	Stations	Coordinates	D
El Kala (1)	37°01.808'N, 8°18.627'E	85	Bou-Ismaïl (12)	36°45.266'N, 2°41.519'E	159
Annaba (2)	37°00.715'N, 7°59.982'E	97	Bou-Ismaïl (13)	36°45.854'N, 2°38.281'E	318
Annaba (3)	37°04.127'N, 7°52.547'E	180	Mostaganem (14)	36°05.077'N, 0°00.490'E	225
Skikda (4)	37°00.272'N, 7°03.249'E	148	Mostaganem (15)	36°02.611'N, 0°01.823'W	117
Skikda (5)	36°59.679'N, 7°06.926'E	110	Mostaganem (16)	35°59.109'N, 0°01.089'E	68
Skikda (6)	37°05.766'N, 6°56.790'E	532	Arzew (17)	35°55.443'N, 0°16.166'W	77
Skikda (7)	37°01.114'N, 6°51.236'E	254	Bouzedjar (18)	35°38.068'N, 1°08.766'W	96
Ziama (8)	36°43.998'N, 5°28.031'E	138	Beni-Saf (19)	35°20.186'N, 1°21.456'W	36
Bejaia (9)	36°40.992'N, 5°18.612'E	79	Beni-Saf (20)	35°25.160'N, 1°29.428'W	125
Boumerdes (10)	36°49.377'N, 3°25.259'E	171	Ghazaouet (21)	35°12.066'N, 1°55.639'W	118
Ain Benian (11)	36°48.756'N, 2°48.164'E	157	Ghazaouet (22)	35°11.392'N, 2°05.491'W	113

Results and discussion

Systematic results

Order Synallactida Miller, Kerr, Paulay, Reich, Wilson, Carvajal & Rouse, 2017

Family Stichopodidae Haeckel, 1896

Genus *Parastichopus* Clark, 1922

Parastichopus regalis (Cuvier, 1817) non-spotted morph

Figures 2–3, Table 2

Pudendum regale Cuvier, 1817: 22.

Holothuria triquetra Delle Chiaje, 1828: 71; Delle Chiaje, 1841: 4.

Holothuria regalis Grube, 1840: 33; Sars, 1859: 96–98, Figures 78–81.

Stichopus regalis Selenka, 1867: 317; Koehler, 1921: 182, Figure 138; 1927: 237, Figure 24; Théel, 1886: 194; Marenzeller, 1893: 15; Mortensen, 1927: 391; Cattaneo, 1981: 15; Peres & Picard, 1964: 91–92, 104, 116; Clark, 1922: 63, plate 1, Figures 17–18; Deichmann, 1940: 193, plate 32, Figures 1–8.

Eostichopus regalis Cutress & Miller, 1982: 720.

Parastichopus regalis Rowe unpublished data (Worms 2020).

Material examined. LPVCMRMS2019.301; LPVCMRMS2019.302; LPVCMRMS2019.303; LPVCMRMS2019.304, Mostaganem, Algeria, 36° 05.077' N, 00° 00.490' E, 225 m, 03 June 2019, 4 specs. LPVCMRMS2019.308, Mostaganem, Algeria, 36° 02.611' N, 00° 01.823' W, 117 m, 03 June 2019, 1 spec. LPVCMRMS2019.309, Mostaganem, Algeria, 35° 59.109' N, 00° 01.089' E, 68 m, 03 June 2019, 1 spec.

Description Body length 10.50–20.50 cm, contracted width 3–5.90 cm, weight 6.34–71.24 g. Body soft, body fairly depressed, trivium flattened, sole like, bivium arched, both separated by a distinct lateral fold on each side bearing large papillae. Dorsal surface (Figure 2A) reddish-brown, characterized by evenly distributed white conical papillae. Ventral surface (Figure 2B) with several rows of podia arranged in three distinct zones, two outer zones whitish brown in colour and mid-ventral one bright red colour forming a distinct groove. Mouth ventral, surrounded by 20 peltate tentacles arranged in two circles; internal and external (Figure 2C). Anus terminal. Mouth and anus each bordered by two papillae (about 1 cm), larger than those of the general body wall (Figure 2D1). Small black spots, each about 1 mm in diameter, cover the both dorsal and ventral surfaces. Thickness of the dorsal integument 1.69–4.74 mm and that of the ventral integument 1–5.01 mm. Calcareous ring delicate, average diameter 10.65 mm. Longitudinal muscles flat, attached to body wall, paired, each about 5.66 mm thick, whitish in life but changing to cream in alcohol, thin at point of insertion to the calcareous ring. Cuvierian tubules absent. Tentacle ampullae about 1.10 to 13 mm (Figure 2E2) in length (Table 2). Polian vesicle (Figure 2E3) single, mean length about 19.23 mm, arising from water vascular ring (Figure 2F) ventrally. Stone canal also single, mean length 9.68 mm, arising from water vascular ring dorsally; madreporite spherical, attached to dorsal body wall (Figure 2G4). Gonadal tubules thin, white, in two tufts, one on each side of dorsal mesentery (Figure 2H). Dissected specimen, based on microscopic observations of the gonad; determined to be a mature female at stage IV of development.

Ossicles. Ossicles of the anal region (Figure 3A), dorsal surface (Figure 3B) and ventral surface (Figure 3C) appear identical and include only tables. Tables (Figures 3Aa; 3Ba; 3Ca) appear more or less symmetrical, disc flat, perforated by 3–6 central holes (4 holed ones most abundant), and 20–60 marginal holes; disc margin smooth; mean diam. of disc, 99.667 µm and mean area 7571.660 µm². Spire 4-pillared (Figure 3Ac), about 67.521 µm high, tapered at end, and with 3–4 cross-bars. Rods of papillae and pedicels (Figures 3Ab; 3Bb; 3Cb) appear straight or curved, smooth or spiny, with an enlarged, perforated, central part and slightly expanded perforated ends; mean length 222.837 µm. Anal region comprises also plates (Figure 3Ad). End plates of pedicels complete or reduced (Figure 3Cc), the former oval to irregular, the latter rounded, slightly concave medially, all plates with small holes, the medial holes the largest; smaller plates average 146.424 µm in length, 86.150 µm in width; average diameter of circular plates 209.472 µm. Tentacles (Figure 3D) present three types of rods; elongated (Figure 3Da), arched (Figure 3Db) and robust, branched type with perforated ends (Figure 3Dc). Cloaca (Figure 3E) possesses dichotomous branched rods.

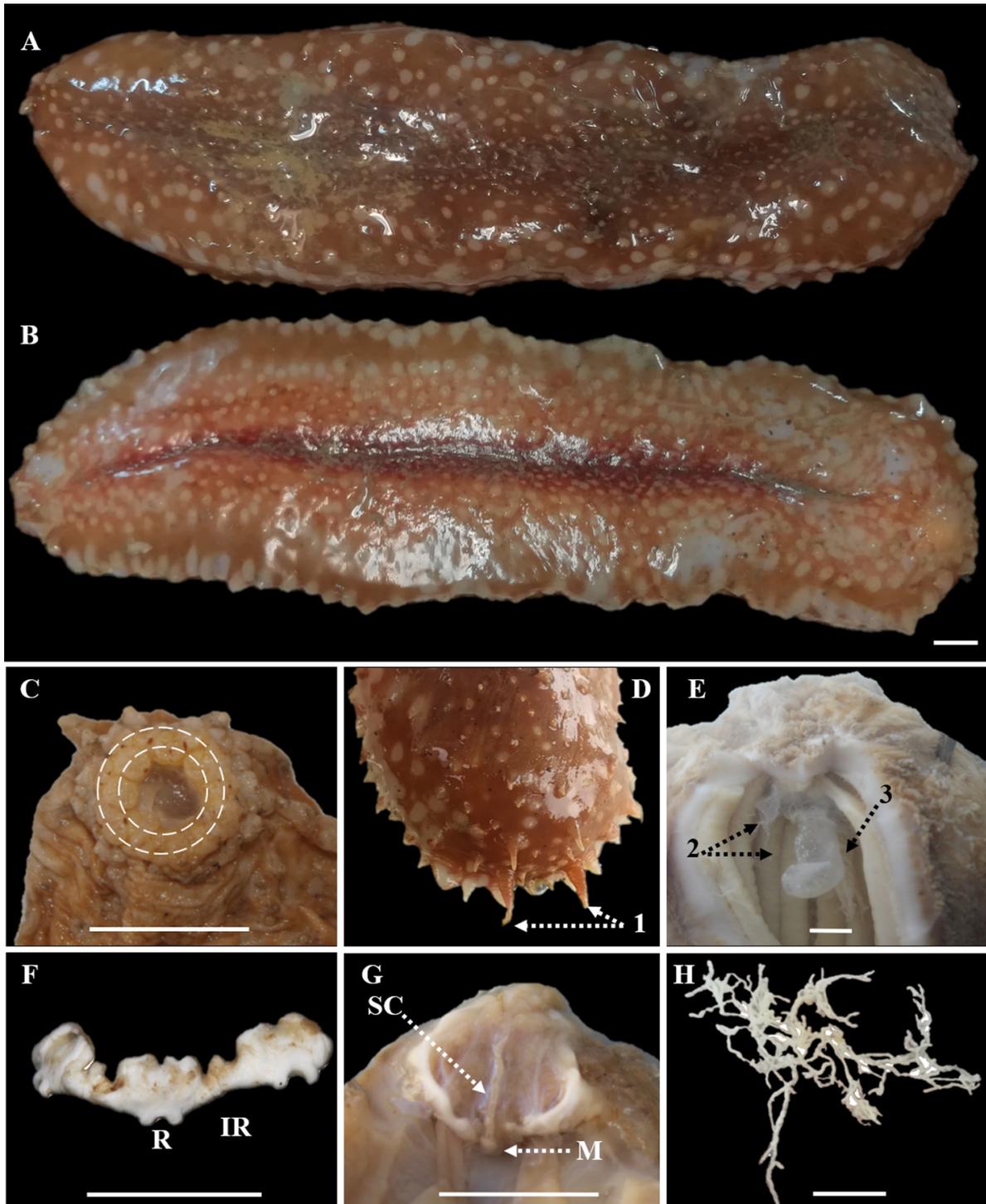


FIGURE 2. Morphological characters of non-spotted *P. regalis*. **A.** Dorsal view. **B.** Ventral view. **C.** Arrangement of tentacles in two circles (indicated by the dotted circles). **D.** **1.** Larger papillae on either side of mouth. **E.** **2.** Tentacle ampullae. **E.** **3.** Polian vesicle. **F.** Calcareous ring (R: Radial and IR: Interradial plates). **G.** **4.** Stone canal (SC) and madreporite (M). **H.** Gonadal tubules. Scale bar = 1 cm.

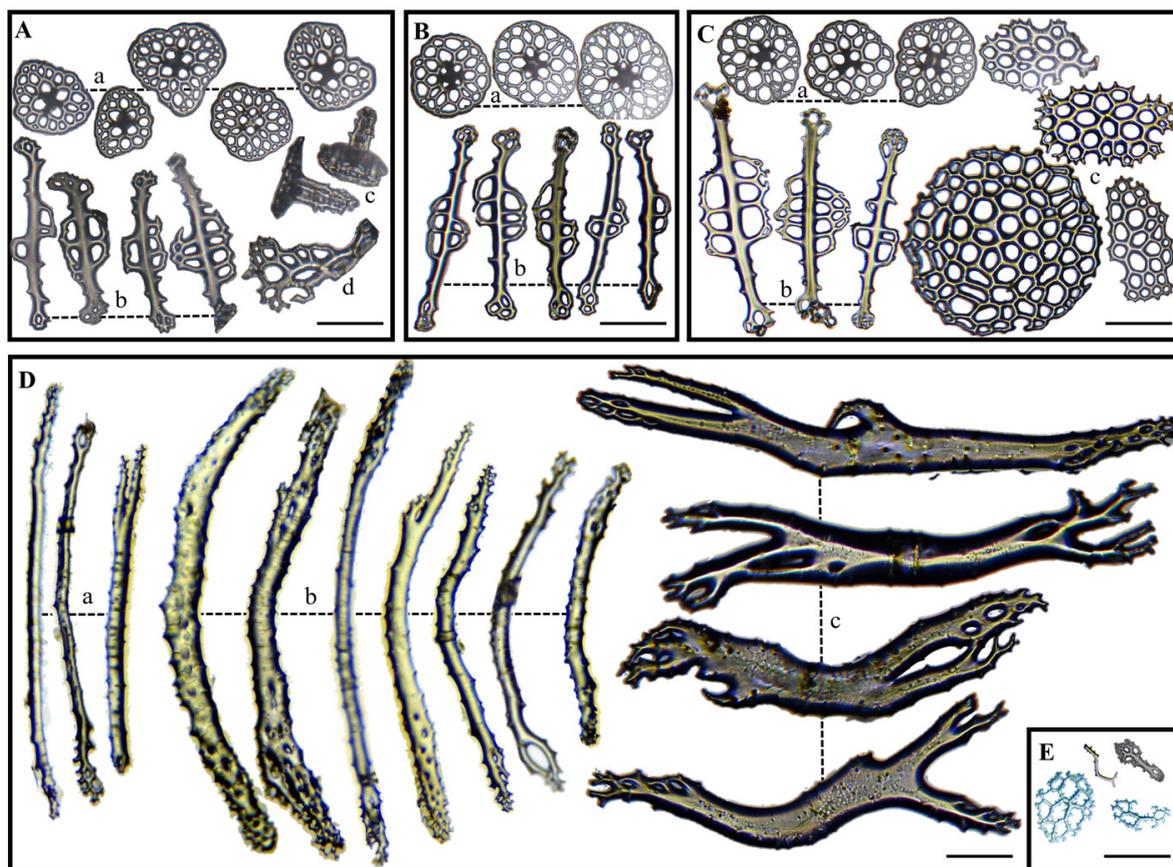


FIGURE 3. Ossicles of *P. regalis* (non-spotted) from different parts of the body. **A.** Anal region. **a.** Tables. **b.** Rods. **c.** Spires of the tables. **d.** Plates. **B.** Dorsal surface and papillae. **a.** Tables. **b.** Rods. **C.** Ventral surface and tube feet. **a.** Tables. **b.** Rods. **c.** End plates. **D.** Tentacles. **a.** Elongated rods. **b.** Arched rods. **c.** Branched rods. **E.** Cloaca. Scale bar = 100 µm.

Parastichopus regalis (Cuvier, 1817) spotted morph

Figures 4–5, Table 2

Material examined. LPVCMRMS2019.305; LPVCMRMS2019.306, Mostaganem, Algeria, 36° 05.077' N, 00° 00.490' E, 225 m, 03 June 2019, 2 specs. LPVCMRMS2019.307, Mostaganem, Algeria, 36° 02.611' N, 00° 01.823' O, 117 m, 03 June 2019, 1 spec. LPVCMRMS2019.311; LPVCMRMS2019.312, Bouzedjar, Algeria, 35° 38.068' N, 01° 08.766' O, 96 m, 04 June 2019, 2 specs. LPVCMRMS2019.310, Arzew, Algeria, 35° 55.444' N, 00° 00.490' E, 77 m, 04 June 2019, 1 spec.

Description. Specimens (Figures 4A and 4B) preserved length 14–24.90 cm, contracted width 4–7.50 cm; total weight 25.07–143.50 g. Each with 5–7 brownish to dark red blotches on the bivium when alive (Figure 4A1) disappearing after storage in ethanol. Morphological characters similar to non-spotted specimens described above. Dorsal body wall 1.23–6.19 mm thick; ventral body wall 1.22–6.77 mm thick. Polian vesicle single, average length 17.57 mm; stone canal also single, average length about 10.71 mm. Calcareous ring about 13 mm in diameter. Longitudinal muscles flat, attached to body wall, about 6.75 mm in thickness (Table 2).

Ossicles. Anal region (Figure 5A), dorsal (Figure 5B) and ventral surfaces (Figure 5C) present the same type of ossicles as non-spotted specimens. Table discs (Figures 5Aa; 5Ba; 5Ca) 97.926 µm in average diameter and 7099.090 µm² in total area. Spire 4-pillared, about 67.521 µm high, 64.691 µm wide, with 3–4 cross-bars. Papillae and pedicels include perforated rods (Figures 5Ab; 5Bb; 5Cb) as in the non-spotted morph, 265.026 µm in average length. Complete pedicel end plates (Figure 5Cc) circular, average diameter 439.218 µm; oval end plates 197.766 µm in mean length and 84.328 µm in mean width. Cloaca (Figure 5D) presents dichotomously branched rods. Ten-

tacle rods (Figure 5E) elongated (Figure 5Ea), or arched (Figure 5Ec), branched (Figure 5Eb) and dichotomously branched rods (Figure 5Ed).

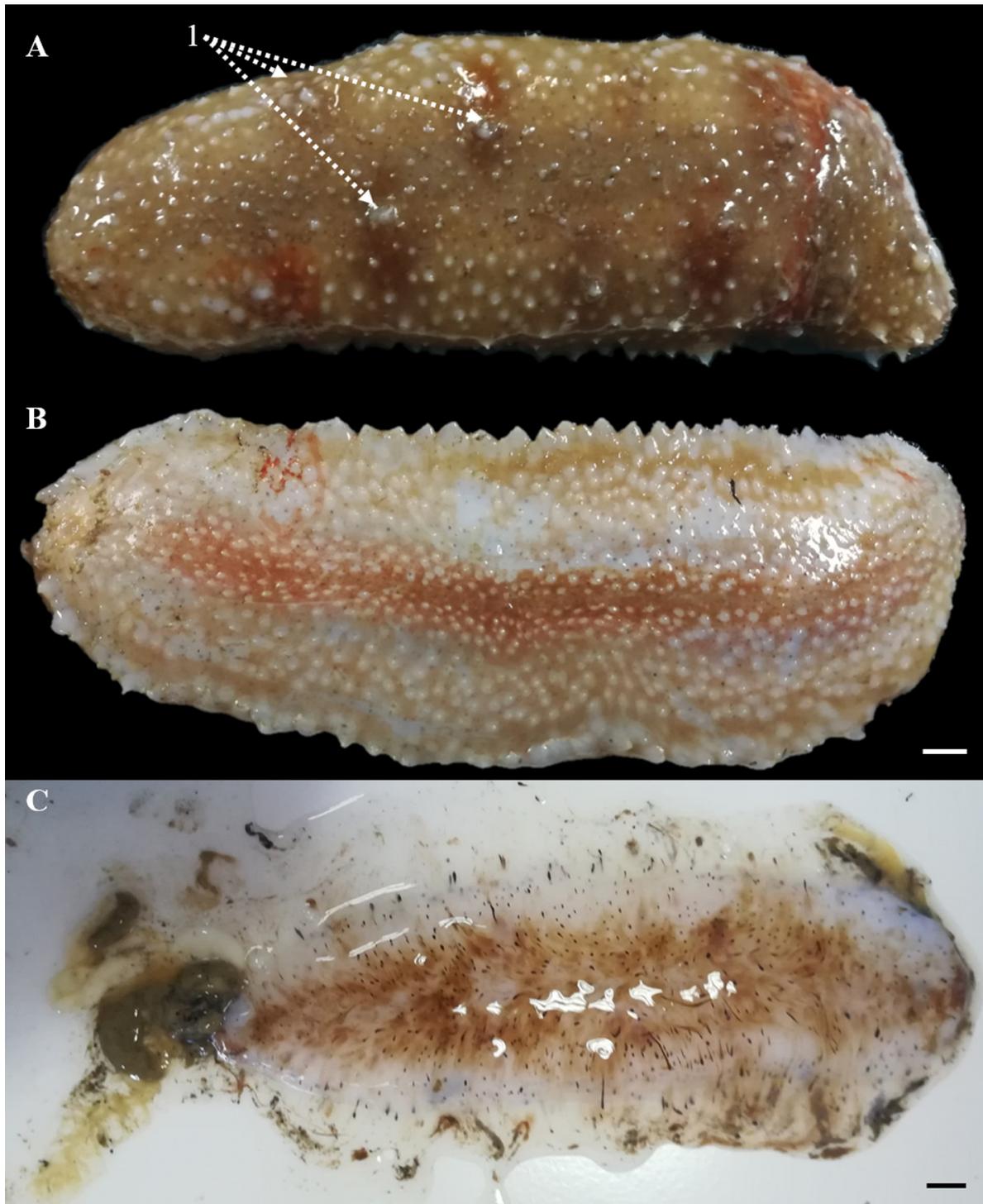


FIGURE 4. Morphological characters of spotted *P. regalis*. **A.** Dorsal view, blotches indicated by arrows (1). **B.** Ventral view spotted individual (note no blotches). **C.** Degradation of the dermis after 2 hours (individual not immediately preserved in ethanol). Scale bar = 1 cm.

TABLE 2. Morphological and anatomical characters of the two morphotypes of *P. regalis* (non-spotted and spotted).

Morphotype	Non-spotted	Spotted
Morphological characters		
Ventral body shape	sole like	
Contracted length (cm)	10.50–20.50	14–24.90
Contracted width (cm)	3–5.90	4–7.50
Consistency of body wall	soft, gelatinous	
Total weight (g)	9.34–71.24	25.07–143.50
Dorsal body wall thickness (mm)	1.69–4.74	1.23–6.19
Ventral body wall thickness (mm)	1–5.01	1.22–6.77
Colour of the trivium	brown and bright red in the center	
Colour of the papillae	White	
Shape of the bivium	Arched	
Colour of the bivium	reddish brown	reddish brown with 5–7 dark blotches
Disposition of the pedicels (bivium)	In more or less regular rows	
Position of the mouth	Ventral	
Position of the anus	Terminal	
Number of tentacles	20	20
Internal characters (mm)		
Tentacle ampullae length	1.10–13	5.94–13
Polian vesicle length	12.54–25.19	12.53–21.69
Stone canal length	6.97–13	8.12–12.37
Shape of the madreporite	Spherical	
Longitudinal muscles edges	Flat, attached	
Average width of longitudinal muscles	3.39–7.30	4.49–13.79
Diameter of calcareous ring	8.75–12.28	9.28–17.20
Endoskeletal characters mean (μm)		
Diameter of table discs	99.668	97.926
Length of perforated rods of papillae and pedicels	222.837	265.026
Length of oval end plates	146.424	197.766
Width of oval end plates	86.150	84.328
Diameter of the complete end plates	209.472	439.218
Length of elongated rods of tentacles	600.410	809.232
Length of arched rods of tentacles	663.903	459.545
Length of branched rods of tentacles	788.369	688.710

Remarks. Both spotted and non-spotted *Parastichopus regalis* individuals degrade very quickly if they are not immediately preserved in ethanol, after collection. The dermis rapidly disintegrates and the texture of the body becomes gelatinous, spreading out on the support on which the specimen is placed (Figure 4C). This reaction is probably due to the stress during fishing operation and/or exposure to light or change in pressure and/or temperature, since fished from waters more than 36 m deep. The ability to lose a piece of dermis from the body wall when in danger has been observed in many holothuroids (Kropp 1982).

The use of formalin, although good for fixation, does not allow for better preservation because the body does not keep its original shape and the ossicles tend to corrode rapidly. Hence, alcohol is recommended although the bright red colour of the ventral surface as well as the dark spots on the dorsal surface (when present) do disappear. The thickness of the body wall can vary from one individual to another since the general texture of the body wall is very soft, thus not rigid enough to maintain the original form.

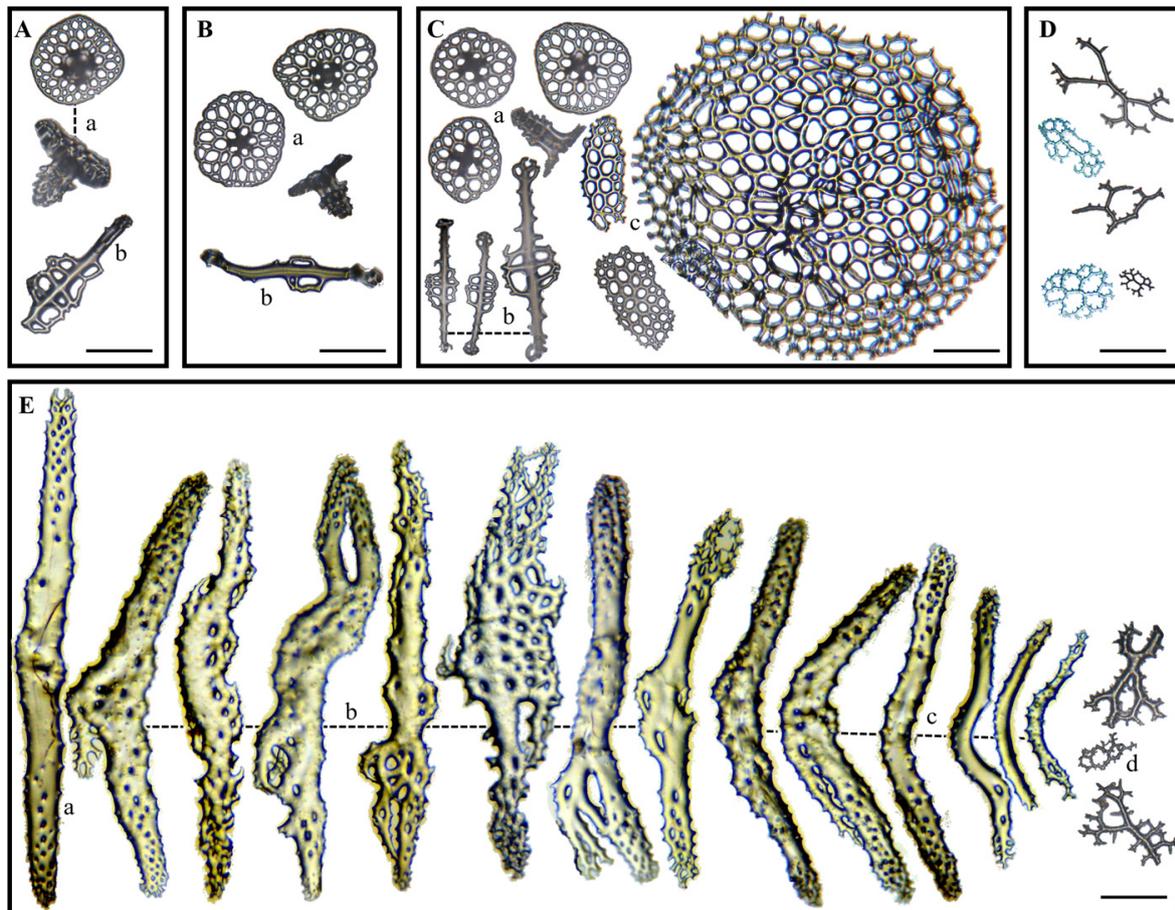


FIGURE 5. Ossicles of *P. regalis* (spotted) from different parts of the body. **A.** Anal region. **a.** Tables. **b.** Rods. **B.** Dorsal surface and papillae. **a.** Tables. **b.** Rods. **C.** Ventral surface and tube feet. **D.** Cloaca. **a.** Tables. **b.** Rods. **c.** End plates. **E.** Tentacles. **a.** Elongated rods. **b.** Branched rods. **c.** Arched rods. **d.** Dichotomously branched rods. Scale bar = 100 μm .

Statistical analysis

Significant differences were found between individuals [Kruskal-Wallis test ($H = 34.724$, $df = 11$, $p\text{-value} = 2.75 * 10^{-4}$) and ($H = 34.006$, $df = 11$, $p\text{-value} = 3.61 * 10^{-4}$)] for the diameter and the area of the table discs.

Multiple comparisons of the diameter and surface means allowed us to highlight these differences (Figure 6). As far as the diameter of the table discs is concerned, there is a significant difference between the individuals 2019.303 NS/ 2019.308NS/ 2019.305S (first group) and the individuals 2019.307S/ 2019.3010S/ 2019.311S (second group), the latter having a significantly smaller table diameter than the first group. As far as the area of the disc is concerned, a significant difference exists between the same groups except individual 2019.311S for the second one. The other specimens showed no significant differences in diameter and area of the table discs.

Discussion

Parastichopus regalis has been recorded from several localities along the Algerian coast from West to East, the regions where the species was not recorded on the map does not necessarily indicate its absence there, as trawling was not conducted in these areas.

External morphology of *P. regalis* showed the presence of two morphotypes, non-spotted and spotted individuals. The latter differ only in the presence of brown to dark red blotches on the dorsal surface. Comparison of the internal anatomy (including the endoskeleton) showed no differences between the two morphotypes. Both present

the same anatomical characters: a single Polian vesicle, a single stone canal with spherical madreporite, longitudinal muscles thin at anterior extremities where they insert on the anterior part of the radial plates of the calcareous ring (Figure 2F). A statistical analysis (Figure 6) with regard to the diameter and area of the table discs showed no significant differences between the spotted and non-spotted individuals, however, within each group, we can observe significant differences between some individuals which of course are not related to the presence or absence of blotches but perhaps to the size of the specimens.

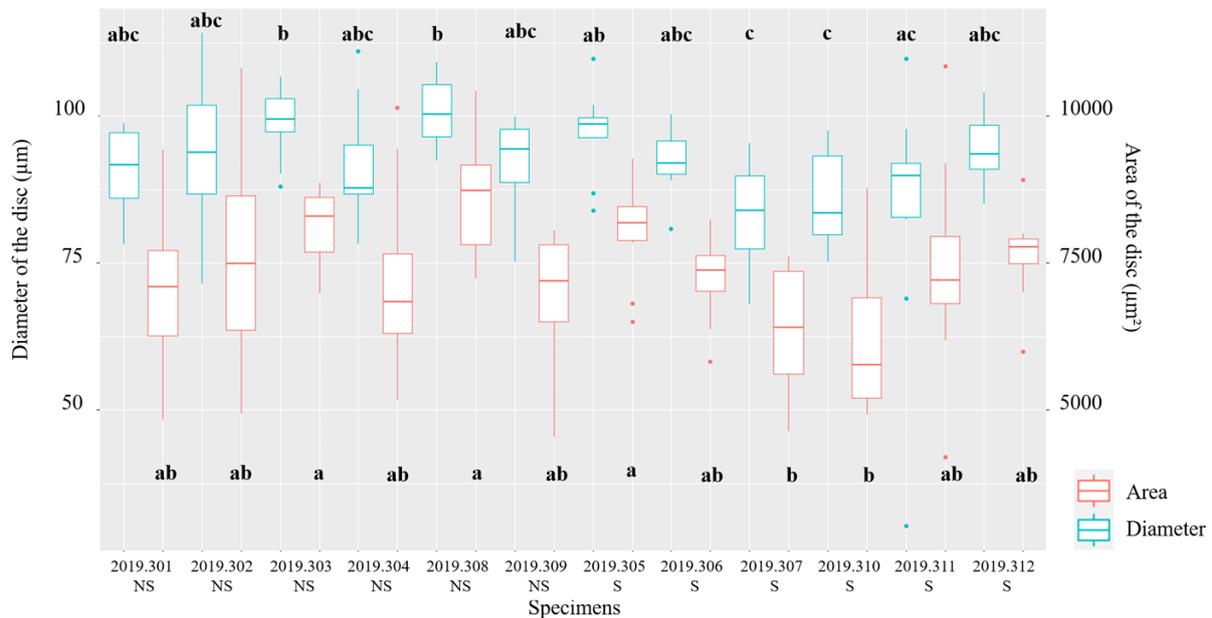


FIGURE 6. Boxplot showing the comparison of the diameter (blue) and the area (red) of the table discs of the two morphotypes of *P. regalis* (S: Spotted and NS: Non-spotted), using the Kruskal-Wallis test with Dunn’s multiple comparison. Different letters represent a significant difference (p -value < 0.05) between individuals, while equal letters mean there is no significant difference.

Examination of the endoskeleton revealed the same types of ossicles in both morphotypes: tables of similar form in body wall; perforated rods of similar form in papillae and pedicels; end plates of various form and size in the pedicels, in cloaca with spiny dichotomous branched deposits; and tentacle rods of different shapes, some with spiny dichotomous branches. Table 2 shows that certain measurements of the various morphological or anatomical (including endoskeletal) characters (e.g. length of tentacle ampullae, width of longitudinal muscles, diameter of calcareous ring, length of body wall plates, diameter of the complete end plates, size of the reduced end plates, length and robustness of some tentacle rods) are greater in spotted individuals. We speculate that these differences, are probably due to their larger size and degree of contraction and perhaps not due to taxonomic differences between the two forms.

According to Zhang *et al.* (2015), body colour may be related to environmental factors. It is probable that the blotches observed in some specimens of *P. regalis* may change or disappear, depending on the light intensity in their habitats or alternatively appear when they move into lower light intensity. Such changes have been reported for *Holothuria (P.) sanctori* (see Mezali 2013). Previous studies on sea cucumbers revealed that they are active nocturnally (Lin *et al.* 2013) and exhibit negative phototaxis (Zhou *et al.* 1999). According to Liu *et al.* (2020), the light-sensitive organs are the tentacles, tube feet and papillae which contract by light stimulation and recover when the stimulation is removed, thus suggesting that these organisms have a photoreceptor system. In the synaptid sea cucumber, *Opheodesoma spectabilis*, pigmented spots on the tentacles base have been shown to be involved in phototactic behavior (Berill 1966) and histologically, the ocellus of this species is composed of sensory cells underlain by numerous bundles of tentacular nerve fibers (Yamamoto & Yoshida 1978). Although these ocelli-like structures were detected in the connective tissue and at the base of tentacles, the mechanism underlying the photoreceptor system has not been elucidated (Zhao *et al.* 2013). Hence, phototactic responses reported for various holothuroids cannot be ruled out for changes in body colouration of the *P. regalis* and would make an interesting future study.

Body colour is a factor affecting the sensitivity of sea cucumbers to light, these animals gradually inhabit the deeper seabed with the development of body colour and thickening of the dermis (Liu *et al.* 2020). In fact, those species which are light-coloured live mainly at shallower depths where the light intensity is high while the dark-coloured forms frequent the deep shadow areas where there is low or no light at all (Lin 2011), thus, the light-coloured individuals are more resistant to high light intensity (Lin 2011). A study carried out on two morphotypes of *Apostichopus japonicus* (albino and normal), showed that the albinos can tolerate longer periods of exposure to intense light (Lin *et al.* 2013). One of the hypotheses that has been made to explain this phenomenon assumes that the body wall of light individuals can reflect more and absorb less light than a dark-coloured body surface (Lin *et al.* 2013). In the case of *P. regalis*, we speculate that the spotted individuals may be found in dark or shaded areas and non-spotted individuals occur at shallower depths and hence exposed to more light, to allow for better reflection of light and hence resistant to high light exposure. However, during our collections we observed that a single fishing haul (same depth and substrate), would bring up individuals of the two morphotypes (spotted and non-spotted). This may debunk the above speculation but can be explained by the fact that this species tends to be covered with sand, thus creating a cover on its dorsal surface, causing the disappearance of the spots in some individuals which are buried deeper, as a single haul covers a long distance varying between 0.926 to 3.704 km.

To confirm our results, we anticipate molecular and histological work to elucidate our conclusions regarding the lack of any significant taxonomic differences between the two morphs here reported on. This will also allow to determine the possible presence of the other non-endemic species *Parastichopus tremulus* which has been reported in the Alboran Sea (Western Mediterranean) (Ordines *et al.* 2019). In addition, we anticipate that, in order to justify our speculation regarding the phototactic responses, to conduct experiment on the two morphs by controlling the intensity and time of light exposure in order to determine if they behave the way we speculate.

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