



On the taxonomic status of *Holothuria (Holothuria) tubulosa* (s.s.) from the Algerian coast with the description of a new Mediterranean species, *Holothuria (Holothuria) algeriensis* n. sp. (Echinodermata: Holothuroidea: Holothuriidae)

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Abstract

In this study we redescribe the taxonomy of some holothuriid species collected from different localities of the Algerian coastal waters. Morphological (anatomical and endoskeletal) and previous molecular studies show the presence of two distinct morphotypes of *Holothuria (Holothuria) tubulosa* “A” & “B”. Morphotype “A” corresponds to the classical *Holothuria (Holothuria) tubulosa* Gmelin 1791, described by Koehler (1921) and hereafter referred to as *H. (H.) tubulosa* (s.s.), while morphotype “B”, we believe, represents a new species, herein named *H. (Holothuria) algeriensis* n. sp. with characteristics significantly different from those of *Holothuria (H.) tubulosa* (s.s.). Both morphology and statistical analysis (Linear Discriminant Analysis) confirm significant differences between these two morphotypes. To visualize these differences, measurements made on ossicles of *Holothuria (H.) tubulosa* (s.s.) and *Holothuria (H.) algeriensis* n. sp. were compared with other species present in our collections, including the north-eastern Atlantic and Mediterranean *Holothuria (Roweothuria) arguinensis* Koehler & Vaney, 1906, recently recorded from Algerian waters, the Mediterranean *Holothuria (Roweothuria) poli* Delle Chiaje, 1824 and *Holothuria (Holothuria) stellati* Delle Chiaje, 1824. We conclude that the two morphotypes of *H. (H.) tubulosa* are significantly different to warrant the recognition of a new species, *H. (H.) algeriensis* n. sp. and the linear discriminant analysis (LDA) of the five species demonstrate this.

Key words: sea cucumbers, new species, systematics, Mediterranean Sea, Algerian basin

Introduction

The taxonomic status of *Holothuria (Holothuria) tubulosa* Gmelin, 1791, *Holothuria (Holothuria) stellati* Delle Chiaje, 1824, *Holothuria (Holothuria) dakarensis* Panning, 1939 and *Holothuria (Holothuria) mammata* Grube, 1840, has been subject to much discussion and controversy in the literature. According to Koehler (1921, 1927), *H. tubulosa*, *H. stellati* and *H. mammata* are distinct and well separated species. Panning (1934) noted some similarities amongst these species and maintained the specific rank of *H. mammata* while considering *H. stellati* as a variety of *H. tubulosa*. However, in 1939, he considered *H. stellati* as a super-species composed of four subspecies: *H. stellati stellati*, *H. stellati tubulosa*, *H. stellati mammata* and *H. stellati dakarensis*. This was based on similarities in the external morphology and the form of the ossicles (tables). Cherbonnier (1950) noted that it would have been better to use *H. tubulosa*, established in 1791 by Gmelin, rather than *H. stellati* established by Delle Chiaje (1823), as a super-species but, despite this, recognized *H. tubulosa*, *H. mammata* and *H. dakarensis* as distinct species and confirmed their specific taxonomic status while listing the characteristics that differentiate them (size of the dorsal papillae, shape of the tables and presence or absence of Cuvierian tubules). Later, Cherbonnier (1956) described large polymorphism in *H. tubulosa* with regard to the form of the ossicles, the size of the dorsal papillae and the arrangement and number of ventral podia (tube feet). On this basis specimens of *H. tubulosa* were distinguished

from *H. mammata* only by their size, the absence of Cuvierian tubules and the buttons of integument which have a thorny surface and variable dimensions (Koehler 1921). Further, Cherbonnier (1960) commented on the impossibility of confusing juveniles of *H. mammata* with those of *H. stellati* and *H. tubulosa* since in the latter two, the juveniles have many ventral podia and the tables of the ventral surface are never as thick as those of *H. mammata*. In addition, *H. tubulosa* and *H. stellati* do not have Cuvierian tubules. Tortonese (1965) also entered this debate by studying some samples from the Italian coast which he identified as *H. mammata* and *H. tubulosa*. Differences cited by Tortonese (1965) included comparing the morphology and ossicles of the integument, the length of the papillae and colouration of the animals. Rowe (1969) concluded that it is possible to distinguish these species specifically by the size of their ossicles, *H. stellati* having smaller buttons (31–42 μm); *H. tubulosa* and *H. mammata* have buttons of medium size (40–116 μm), while the larger buttons (84–160 μm) are present in *H. dakarensis*. He proposed as a specific character for *H. mammata* the presence of Cuvierian tubules and the large dorsal mammilated papillae, whereas for *H. tubulosa*, he noted the dominance of solid elongated ventral buttons. Gustato & Villari (1979) studied the systematics and frequency of occurrence of some holothurian species in the Gulf of Naples (Italy) with special emphasis on defining the taxonomic status of *H. stellati*. They distinguished *H. tubulosa* from *H. stellati* on detailed comparison of their morphology in water (live specimens) and outside water (after physical stress), as well as the morphology and the percentage of specific ossicles (Gustato, pers. comm. 1997). They divided the ossicles into 3 types (“a”, “b” and “c”) based on the form of the buttons: [(a: oval buttons with scalloped edges, their holes placed symmetrically, their surface may be smooth or knobbed); (b: elongated, smooth-edged, oval buttons, with symmetrical holes and longitudinal streak) and (c: round, knobbed buttons without holes)]. According to Gustato & Villari (1979), *H. tubulosa* is devoid of type “b” ossicles whereas in *H. stellati*, such ossicles are the most common. Button types “a” and “c” are present in both species. Zavodnik (2003) reported that the taxonomic position of *H. stellati* is still very dubious and controversial, Mezali (2008) and Mezali & Paulay (2009) proposed confluent results and concluded that *H. stellati* exists in the Mediterranean according to the results obtained from morphological (including endoskeletal) analysis of this species. The same authors hypothesized the existence of two morphotypes of *H. tubulosa* “A” & “B” and a possible case of hybridism between *H. stellati* and *H. poli*.

According to Mezali’s (2008; 2011) DNA sequence results, the *H. (R.) poli* lineage is sister to three well supported lineages composed of the classical *H. (H.) tubulosa* [i.e. *H. (H.) tubulosa* (s.s.)], *H. (H.) tubulosa* “B” [herein referred to a new species *H. (H.) algeriensis* n. sp.], and *H. (H.) stellati*. Within this lineage, *H. (H.) stellati* forms a separate well supported lineage (100% BS support) and is sister to a fairly well supported lineage (81% BS support) composed of *H. (H.) tubulosa* (s.s.) and *H. (H.) algeriensis* n. sp. [meaning that *H. (H.) tubulosa* (s.s.) and *H. (H.) algeriensis* n. sp. are sister lineages].

In the current study, the taxonomic status of the two morphotypes [*H. (H.) tubulosa* “A” & “B”] considered by Mezali (2008, 2011) and Mezali & Francour (2012) has been re-assessed based on a more intensive study of morphological characters (including biometrical endoskeletal data). In addition, three other holothuriid species from the Algerian coast [*Holothuria (R.) poli*, *Holothuria (R.) arguinensis* and *Holothuria (H.) stellati*] are also considered using similar morphological and endoskeletal characteristics.

Materials and methods

Most individuals of the genus *Holothuria* used in this study were collected from three stations located in the center of the Algerian coast [Sidi-Fredj (36°45.000’N, 2°50.000’E), Tamentefoust (36°48.000’N, 3°13.000’E) and Figuiers-plage (36°46.750’N, 3°30.821’E)]. Sampling was also conducted at three other stations, located on the western part of the Algerian coast [Stidia-Mostaganem (35°49.922’N, 0°1.174’O), Salamandre-Mostaganem (35°55.221’N, 0°03.470’E) and Ouillis-Mostaganem (36°7.436’N, 0°15.219’E)]. A single sample from the French coast [Banyuls-sur-Mer (42°29.040’N, 3°7.970’E)] is also considered (Figure 1). The samples were collected by scuba-diving between 0.5 and 10 m depth. In the laboratory the sea cucumbers were placed separately in containers with sea water and anesthetized with magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$). All anesthetized individuals were photographed with a Canon EOS 1100 camera. For morphological descriptions we adopted the descriptions and/or style of Koehler (1921, 1927), Tortonese (1965), Rowe (1969) and Gustato & Villari (1979). Morphological comparison between the species of the genus *Holothuria* present in our collection were made by considering 2 individuals of *Holothuria tubulosa* (s.s.) and *H. algeriensis* n. sp. each, and one each of *H. poli*, *H. arguinensis* and *H. stellati*.

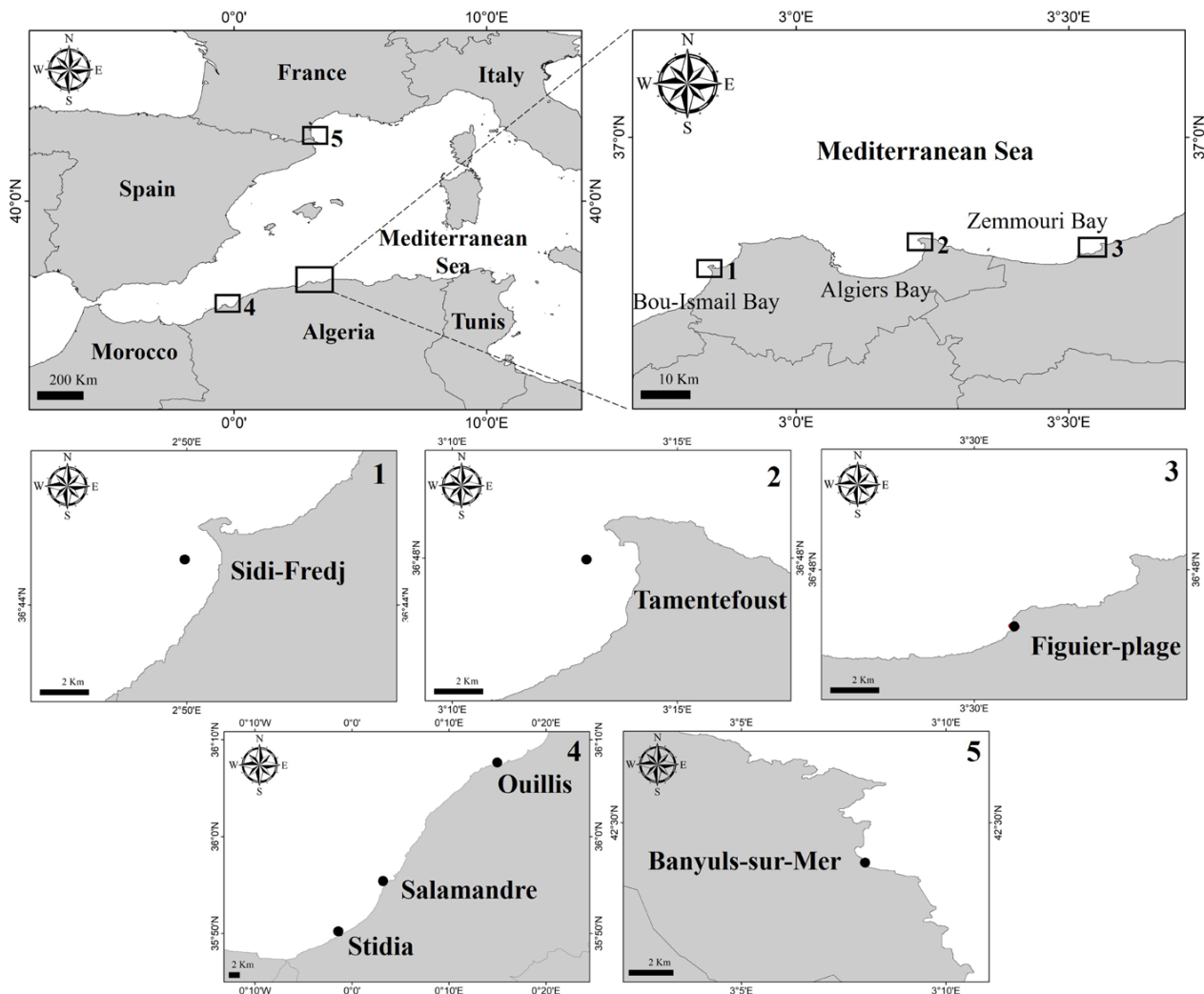


FIGURE 1. Geographical localization of the sampling areas (1, 2, 3, 4 and 5) indicated by the white rectangles in the upper two maps.

Acronyms

Abbreviations used: CMNI—Canadian Museum of Nature Invertebrate collection, Canada; LPVCMRMS—Laboratory of Protection, Valorization of Coastal Marine Resources and Molecular Systematics, Algeria; UF—University of Florida, USA.

Endoskeleton study

Samples of tissues for ossicles study from the bivium, trivium, podia and tentacles were prepared following the protocol of Samyn *et al.* (2006). We considered only adult individuals (see Neghli & Mezali 2019). The ossicles were photographed under a polarized light microscope (LM) LEICA DMLP equipped with a Nikon ME600 camera with DIC (Differential Interference Contrast). The most representative buttons and tables of each individual were selected for illustration and other study, including SEM, using Philips XL 30 FEG scanning electron microscope. For morphometric study the ossicles were measured using a micrometer slide (1 graduation = 4.9 μm). Fourteen (14) variables (measurements) (Table 1) of one representative individual per species were considered and each measure was repeated on 10 ossicles. These measurements were performed using the “ImageJ 1.48” software. The morphology of the most representative ossicles was studied and compared with that in the literature. For statistical analysis, linear discriminant analysis (LDA) was used to distinguish the different species and was performed using the *lda* function (package MASS) in the R environment. Visualization was carried out using the package *ggplot2*. All variables were used since they were not correlated (correlation less than 0.9). A permutational multivariate analysis of variance (PERMANOVA) was conducted on the Software PAST v 1.93 (Hammer *et al.* 2001).

TABLE 1. Different types of measurements carried out on ossicles (for the dorsal surface the prefix “D” is used and for the ventral surface the prefix “V” is used).

Abbreviation	Variable description
Buttons	
LBD	Length of buttons of dorsum
WBD	Width of buttons of dorsum
WhBD	Width of holes of buttons of dorsum
NhBD	Number of holes of buttons of dorsum
LBV	Length of buttons of ventrum
WBV	Width of buttons of ventrum
WhBV	Width of holes of buttons of ventrum
NhBV	Number of holes of buttons of ventrum
Tables	
HSD	Height of spire of tables of dorsum
DDD	Disk diameter of tables of dorsum
RDHD	Ratio between disk diameter and height of spire of the dorsum tables
HSV	Height of spire of ventrum tables
DDV	Disk diameter of ventrum tables
RDHV	Ratio between disk diameter and height of spire of ventrum tables

Systematic results

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

Family Holothuriidae Burmeister, 1837

Genus *Holothuria* Linnaeus, 1767

Subgenus *Holothuria* (*Holothuria*) Linnaeus, 1767

Holothuria (*Holothuria*) *tubulosa* (s.s.) Gmelin, 1791

Figure 2, Table 4

Synonymy.

Holothuria tubulosa Gmelin, 1791, p. 3138; Grube, 1840, p. 35; Selenka, 1867, p. 323, pl. 18, figs. 42–43; Heller, 1868, p. 72; Marenzeller, 1874, p. 314; Ludwig, 1879, p. 570; Lampert, 1885, p. 76, fig. 34; Koehler, 1921, p. 174, fig. 130; Koehler, 1927, p. 215, pl. 16, fig. 25; Hérouard, 1929, p. 48; Nobre, 1931, p. 141, fig. 66, pl. 13; Panning, 1934, p. 77, fig. 60; Tortonese, 1934, p. 226; Tortonese, 1935, p. 261; Mayer, 1937, p. 15, figs. 5–6; Tortonese, 1952, p. 225; Cherbonnier, 1956, p. 22; Gustato & Villari, 1979, p. 107–110, figs. 1–2; Tortonese, 1965, p. 53, figs. 16–17; Rowe, 1969, p. 152–154, fig. 17; Mezali, 1998, p. 59–63, fig. 11.

Holothuria tremula Linnaeus, 1767, p. 1090.

Holothuria columnae de Blainville, 1821, p. 316.

Holothuria petagnae Delle Chiaje, 1824, p. 82.

Holothuria maxima Delle Chiaje, 1823, p. 82.

Holothuria columnae Delle Chiaje, 1824, p. 79.

Remarks. The forms described below comply with the description of *H. tubulosa* by Koehler (1921) which description was recommended by Rowe (1969) for this species, in his excellent revision of the Holothuriidae. Thus, this form is here regarded as *Holothuria* (*H.*) *tubulosa* (s.s.).

Material examined. UF3847; UF5471; UF5476; UF3844, Stidia, Algeria, 35°49.922’N, 0°1.174’O, 3 m, Summer 2006, 4 specs. UF5470, Sidi-Fredj, Algeria, 36°45.000’N, 2°50.000’E, 0.5–10 m, Summer 2006, 1 spec.

UF4471, Banyuls-sur-Mer, France, 42°29.040'N, 3°7.970'E, 3 m, Summer 2006, 1 spec. UF5462, Figuiers-plage, Algeria, 36°46.750'N, 3°30.821'E, 0.5–10 m, Summer 2006, 1 spec. LPVCMRMS2020.201; LPVCMRMS2020.202, Ouillis, Algeria, 36°7.436'N, 0°15.219'E, 5 m, June 2020, 2 specs.

Description. Body form (Figure 2, Table 4) almost cylindrical, length up to 200 mm, width 30–60 mm when contracted. Body wall thick (about 7 mm) and leathery. Dorsal surface (bivium) (Figure 2A) rounded, bearing small conical tubercles/verrucosities, scattered, each terminating in a small, elongated papilla. Ventral surface (trivium) (Figure 2B) rounded in contracted state. Pedicels/tube feet numerous, crowded, no regular arrangement. Mouth ventral, surrounded by 20 tentacles; anus ventral, anal papillae well developed (5 series of 3 each, in each ambulacrum). Live colouration varying from light brown, red mahogany, to brown or black; ventral surface generally lighter (light brown). Longitudinal muscles about 8–9 mm in thickness. Cuvierian tubules absent. Stone canals 1–2. Polian vesicles several, 1–4 (if 4, one on middle and three on right side of dorsal mesentery), average length <10 mm. Tentacular ampullae about 8 mm long. Collar around mouth thin (about 1.30 mm). Calcareous ring not stout, radial plates (Table 4) with broad anterior notch and only slightly concave posterior margin.

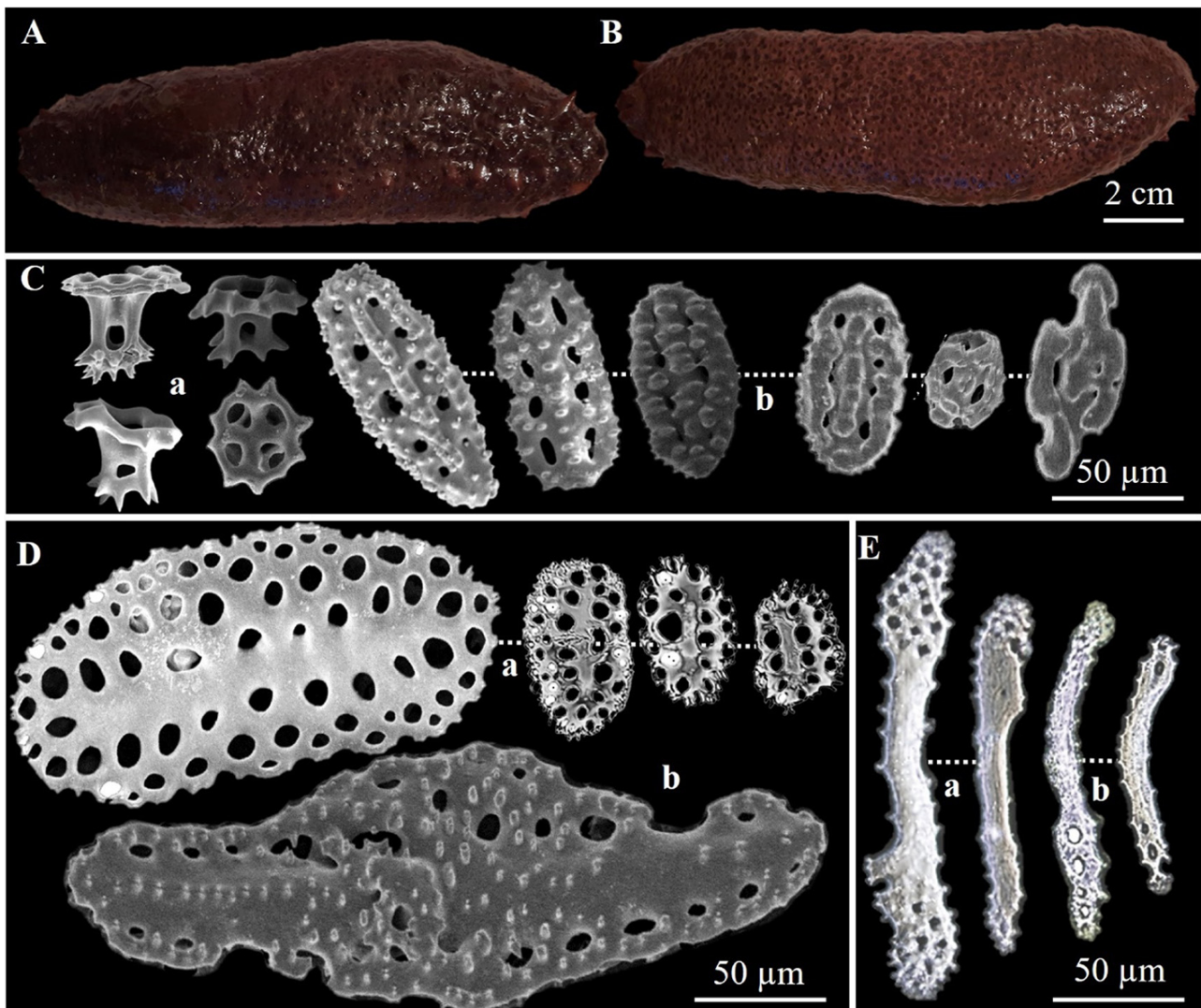


FIGURE 2. External morphology and ossicles of *Holothuria (Holothuria) tubulosa* (s.s.) (CMNI 2021–0195). **A.** Dorsal surface. **B.** Ventral surface. **Ca.** Tables of dorsal and ventral surfaces (SEM). **Cb.** Buttons of dorsal and ventral surfaces (SEM). **Da.** Enlarged plates of pedicels (one enlarged plate left with SEM and the 3 other in right with LM). **Db.** Elongated plate of pedicels (SEM). **Ea.** Straight rods of tentacles (LM). **Eb.** Slightly arched rods of tentacles (LM).

Ossicles. Ossicle types from the dorsal and ventral surfaces appear identical, they comprise tables and buttons. Tables (Figure 2Ca) small and four-pillared, average height of spire less than average diameter of disc. Disc slightly arched. Some tables with a thorny disc generally pierced with 4 large holes and a variable number of peripheral ones. Buttons (Figure 2Cb) appear oval in shape, about 101.25 and 73.75 µm long dorsally and ventrally respec-

tively and 44.25 and 38.70 μm wide dorsally and ventrally respectively, not very thick but with a regular, rough surface with small, conical or pointed spines; holes average about 7, with a roughened periphery, arranged in two rows relative to the central axis of the button; holes may be partitioned, round, irregular in outline, but often become minute or even completely obliterated, then giving the buttons a solid but rough appearance. Average width of the holes of buttons 6.75 and 5.75 μm dorsally and ventrally respectively. Pseudo-buttons abundant but fenestrated ellipsoids scarce. Pedicels contain perforated plates, which are of two types; the first are enlarged (Figure 2Da), with large central perforations symmetrically arranged along the central axis, surrounded by other smaller perforations, the second type of plates are thick, rough and elongated (Figure 2Db) with an irregular outline and with only few large perforations at their lateral and terminal ends. The enlarged plates of the ventral surface have a smooth surface but an irregular and prickly outline and with two rows of large perforations symmetrically arranged along the central axis of the plate and surrounded by several smaller perforations. Tentacles present two forms of thick rods (19 μm); straight rods (Figure 2Ea) and those that are slightly arched (Figure 2Eb).

***Holothuria (Holothuria) algeriensis* n. sp.**

Figure 3, Table 4

Synonymy.

Holothuria (Holothuria) tubulosa “B” Mezali, 2008, p. 116, fig. 23.

Material examined. Holotype. UF5187, Figuier-plage, Algeria, 36°46.750'N, 3°30.821'E, 0.5–10 m, Summer 2006. **Paratype.** UF5475, Figuier-plage, Algeria, 36°46.750'N, 3°30.821'E, 0.5–10 m, Summer 2006, 1 spec. LPVCMRMS2020.203; LPVCMRMS2020.204, Ouillis, Algeria, 36°7.436'N, 0°15.219'E, 5 m, June 2020, 2 specs.

Diagnosis. Length up to 185 mm, body arched, very net demarcation between the dorsal surface brown bitumen and the ventral surface light gray. Mouth ventral, tentacles about 20; anus terminal. Cuvierian tubules absent, stone canals several, Polian vesicle single. Ossicles types are buttons with a large perforations and tables in dorsal and ventral surface; terminal plates in tube feet; enlarged and elongated plates in tube feet and pedicels and rods in tentacles.

Description. Body form generally arched dorsally (Figure 3A), little flattened ventrally (Figure 3B). Length up to 185 mm, width up to 45 mm when contracted. Body wall thin (up to 4 mm) in relaxed state. Pedicels/tube feet irregularly distributed on ventral side. Conical verrucosities of dorsal surface minute or lacking. Mouth ventral, tentacles usually 20, anus terminal, anal papillae well developed (5 series of 4 each, in each radius). Live colouration of dorsal surface brown bitumen, ventral surface light gray. Longitudinal muscles about 10–14 mm in thickness. Cuvierian tubules absent. Stone canals several, 5–6 on right and 2–4 on left side of dorsal mesentery, average length <1 cm. Polian vesicle single, up to 30 mm long. Tentacular ampullae >8 mm. Collar around mouth thin (about 0.69 mm). Calcareous ring (Table 4) large/stout with expanded radial plates, each with a broad anterior notch, slightly bifurcate anterior parts and a flat or slightly indented posterior margin.

Ossicles. Dorsal and ventral surfaces comprise the same type of ossicles; tables (Figure 3Ca) and buttons (Figure 3Cb). Table disc of ventral surface wheel-shaped with a variable number of spokes. The four pillars of spire appear parallel to each other and do not merge at the apical end, each ending in several pointed, thorns/teeth, generally arranged in groups of 3–4 at the end of each pillar. Buttons thick, oval, about 69.10 and 58 μm long dorsally and ventrally respectively and 35.60 and 31 μm wide dorsally and ventrally respectively. Margins of buttons irregular, wavy and rough; holes average about 7, quite large, usually oval but can become obliterated to disappear completely, thus giving the button a solid, rough appearance. Average width of the holes 7.80 and 4.90 μm dorsally and ventrally respectively. Generally, the symmetrical holes in the center of the buttons are wider than the terminal ones which are rather oval in shape. Pseudo-buttons abundant, fenestrated ellipsoids scarce.

Pedicels present perforated plates. Enlarged plates (Figure 3Da) rectangular in shape and smooth. The central perforations are surrounded by smaller, circular ones; other plates generally oval in shape with an irregular outline; elongated plates (Figure 3Db) simple and irregular in outline. Ventral surface also present terminal plates (Figure 3Dc). Tentacles possess thin rods (12 μm) (Figure 3Ea and Figure 3Eb).

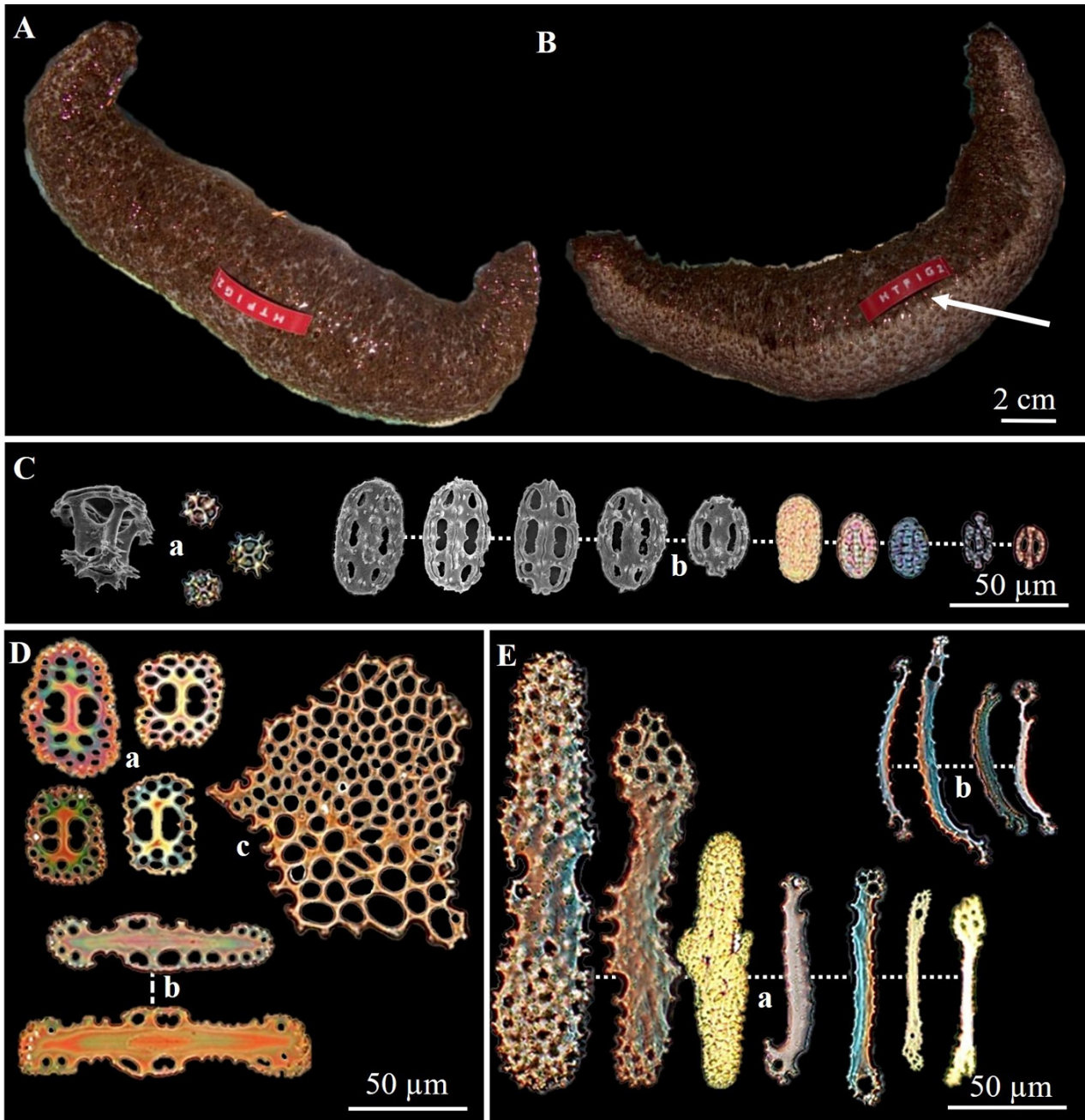


FIGURE 3. External morphology and ossicles of *Holothuria (Holothuria) algeriensis* n. sp. (Holotype UF5187). **A.** Dorsal surface. **B.** Limit between dorsal and ventral surface (arrow). **Ca.** Tables of dorsal (far left with SEM) and ventral (right with LM) surfaces. **Cb.** Buttons of dorsal and ventral surfaces (the 5 buttons left with SEM). **Da.** Enlarged plates of pedicels (LM). **Db.** Elongated plates of pedicels (LM). **Dc.** Terminal plate of pedicels (LM). **Ea.** Straight rods of tentacles (LM). **Eb.** Slightly arched rods of tentacles (LM).

Other Mediterranean species of *Holothuria*

Subgenus *Holothuria (Roweothuria)* Thandar, 1988

Holothuria (Roweothuria) poli Delle Chiaje, 1824

Figure 4A, Table 4

Material examined. UF3857, Tamentefoust, Algeria, 36°48.000'N, 3°13.000'E, 0.5–10 m, Summer 2006, 1 spec. LPVCMRMS2020.205, Ouillis, Algeria, 36°7.436'N, 0°15.219'E, 5 m, June 2020, 1 spec.

Description. Soft cylindrical body, light brown to dark brown in color, sometimes exceeding 20 cm in length and 3–5 cm in width. Trivium (Figure 4Ab) clearly separated from bivium (Figure 4Aa) by a groove. Pedicels whitish (giving the body a white-spotted appearance), spots numerous, dense ventrally, often also dorsally but not obvious. Dorsal papillae thin, whitish, reduced, situated on reduced conical warts/tubercles. Mouth ventral; anus terminal. Tentacles 20, yellowish. Cuvierian tubules absent. Collar around mouth thin (about 0.50 mm). Calcareous ring with broad radial plates with deep, slit-like anterior notch with slightly bifurcate anterior portions and a convex posterior margin (Table 4).

Ossicles. Ossicles of body wall tables and buttons. Tables (Figure 4Ac) with an irregular, marginally serrated disc pierced by 4 central holes and a variable number of peripheral holes. Buttons (Figure 4Ad) of 3 types: (i) smooth with regular outline; (ii) smooth with irregular outline and (iii) with large perforations. Perforated plates of the pedicels of two types: (i) enlarged (Figure 4Af) and (ii) elongated (Figure 4Ae). Terminal plates on the pedicels (Figure 4Ai). Tentacle rods straight (Figure 4Ag) or arched (Figure 4Ah).

***Holothuria (Roweothuria) arguinensis* Koehler & Vaney, 1906**

Figure 4B, Table 4

Material examined. CMNI 2021–0194, Salamandre, Algeria, 35°55.221'N, 0°03.470'E, 2 m, November 2014, 1 spec. LPVCMRMS2020.207, Ouillis, Algeria, 36°7.436'N, 0°15.219'E, 5 m, June 2020, 2 specs.

Description. Semi-cylindrical body with dorsal surface (Figure 4Ba) arched, dark brown in color; ventral surface (Figure 4Bb) sole-like, light brown in color. Several large protuberances in two double rows on the dorsal surface; in addition, dorsal and ventral surfaces separated by several arched protuberances. Anus terminal. Mouth surrounded by 20 tentacles. Cuvierian tubules absent. Collar around mouth thick (about 4.60 mm). Calcareous ring with broad, squarish, radial plates with a shallow anterior notch, almost flat anterior portions and slightly concave posterior margin (Table 4).

Ossicles. Body wall tables (Figure 4Bc) sometimes with a rectangular disc, spire of straight to slightly arched pillars, parallel to each other. Buttons often shaped in the figure of “8” (Figure 4Bd) with rough, irregular and prickly margins but with a smooth surface with unequally-sized perforations. Pedicels also with numerous elongated perforated plates (Figure 4Be) and other rare enlarged plates. Rods of tentacles larger and more complex with an enlarged, branched and perforated middle region, arched (Figure 4Bf) or straight (Figure 4Bg).

Subgenus *Holothuria (Holothuria)* Linnaeus, 1767

***Holothuria (Holothuria) stellati* Delle Chiaje, 1824**

Figure 4C, Table 4

Material examined. UF5468, Sidi-Fredj, Algeria, 36°45.000'N, 2°50.000'E, 0.5–10 m, Summer 2006, 1 spec. UF5495, Stidia, Algeria, 35°49.922'N, 0°1.174'O, 3 m, Summer 2006, 1 spec. LPVCMRMS2020.208, Ouillis, Algeria, 36°7.436'N, 0°15.219'E, 5 m, June 2020, 1 spec.

Description. Body dark brown dorsally (Figure 4Ca), light brown ventrally (Figure 4Cb); sometimes reaching a length of 30 cm and width of 5–6 cm. Dorsal surface arched, ventral surface flattened, the latter with fairly dense, scattered pedicels. Integument thick (0.70 µm) and smooth. A row of 5–6 very large, straight pointed protuberances separated at equal intervals on both sides of body. Cuvierian tubules absent. Collar around mouth thick (about 3.50 mm).

Ossicles. Body wall tables with circular disc (Figure 4Cc) with wavy outline and 4 large central and 4 small peripheral perforations. Buttons (Figure 4Ce) of several types: (i) oval with irregular, rough, smooth and thick surfaces; (ii) larger ones slightly rectangular in shape, with smooth but sometimes with wavy outline; (iii) enlarged, rounded ones and (iv) those that are slightly warped in the middle. Perforated plates of the pedicels of two types: enlarged and elongated (Figure 4Cd). Tentacles with straight (Figure 4Cf) or slightly arched rods.

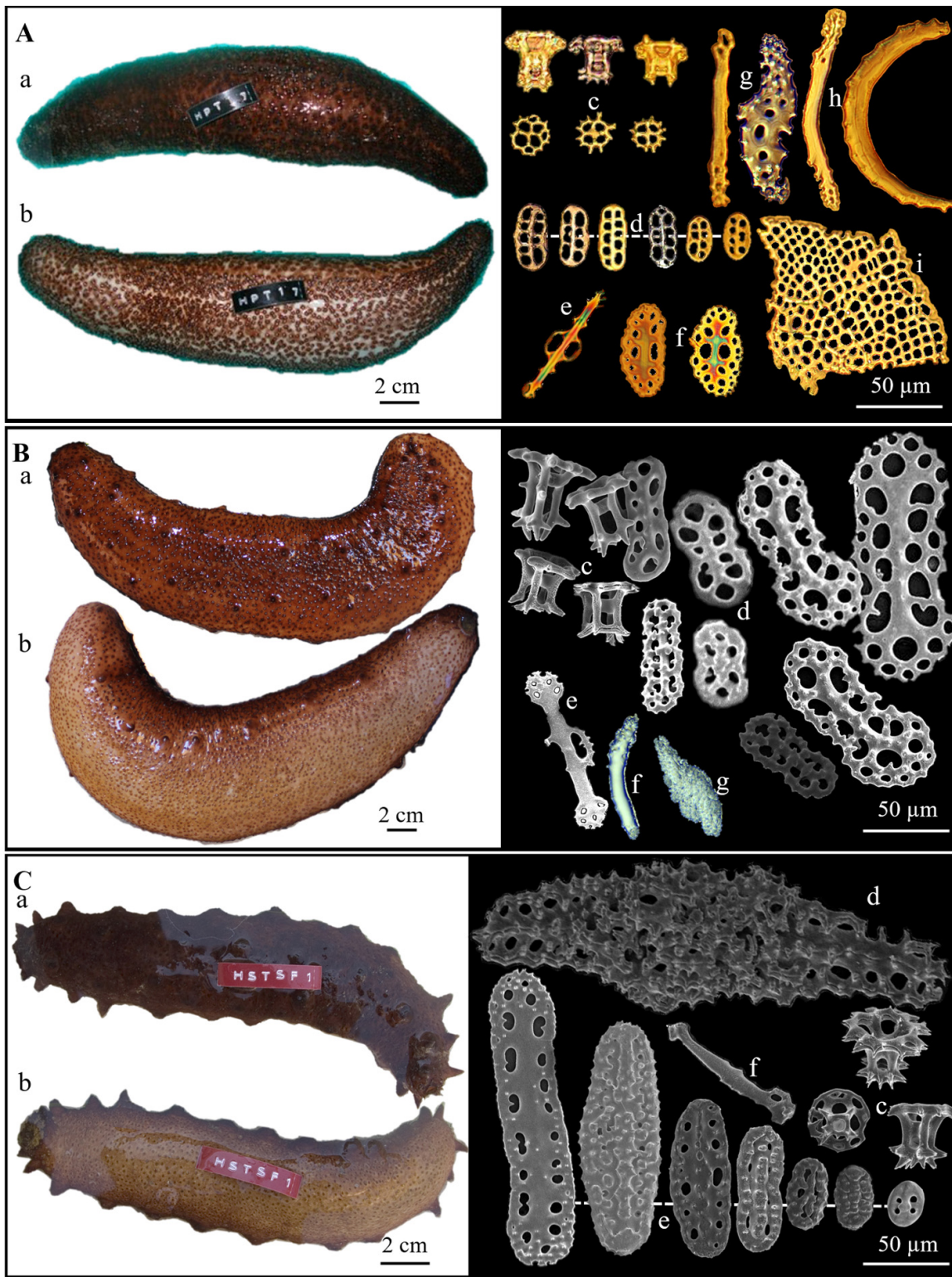


FIGURE 4. **A.** *Holothuria (Roweothuria) poli* UF5491: **Aa.** Dorsal surface. **Ab.** Ventral surface. **Ac.** Tables of dorsal and ventral surfaces. **Ad.** Buttons of dorsal and ventral surfaces. **Ae.** Elongated plates of pedicels. **Af.** Enlarged plates of pedicels. **Ag.** Straight rods of tentacles. **Ah.** Arched rods of tentacles. **Ai.** Terminal plates of pedicels. **B.** *Holothuria (Roweothuria) arguensis* CMNI 2021–0194: **Ba.** Dorsal surface. **Bb.** Ventral surface. **Bc.** Tables of dorsal and ventral surfaces. **Bd.** Buttons of dorsal and ventral surfaces. **Be.** Elongated plates of pedicels. **Bf.** Arched rods of tentacles. **Bg.** Straight rods of tentacles. **C.** *Holothuria (Holothuria) stellati* UF5468: **Ca.** Dorsal surface. **Cb.** Ventral surface. **Cc.** Tables of dorsal and ventral surfaces. **Cd.** Elongated plates of pedicels. **Ce.** Buttons of dorsal and ventral surfaces. **Cf.** Straight rods of tentacles. Ossicles of B and C except Bf and Bg with SEM. Ossicles of A, Bf and Bg with LM.

Morphometrics of the ossicles

The mean values of measurements of the ossicles of both dorsal and ventral surfaces of *H. (H.) tubulosa* (s.s.), *H. (H.) algeriensis* n. sp. and the three other species found in the Algerian waters [*Holothuria* (*R.*) *poli*, *Holothuria* (*R.*) *arguinensis* and *Holothuria* (*H.*) *stellati*], used for comparison, are shown in Tables 2 and 3.

TABLE 2. Mean \pm sd (standard deviations) values of measurements (in μm) of ossicles of the dorsal surface. The average values obtained from ten measurements for each type (tables and buttons). Refer to Table 1 for abbreviations of variables.

	LBD	WBD	WhBD	NhBD	HSD	DDD	RDHD
<i>H. tubulosa</i> (s.s.)	101.25 \pm	44.25 \pm	6.75 \pm	9 \pm	48.10 \pm	51 \pm	10.60 \pm
CMNI 2021–0195	14.11	7.46	2.37	1	4.89	7.38	1.17
<i>H. algeriensis</i> n. sp.	69.10 \pm	35.60 \pm	7.80 \pm	8 \pm	37.10 \pm	38.10 \pm	10.22 \pm
UF5187	21.64	5.14	2.19	3	6.97	8.90	1.08
<i>H. poli</i>	36.25 \pm	28.60 \pm	6.25 \pm	6 \pm	49.20 \pm	56.20 \pm	11.47 \pm
UF5491	12.49	8.24	2.95	2	6.29	8.16	1.27
<i>H. arguinensis</i>	64.85 \pm	32.45 \pm	9.75 \pm	9 \pm	43.60 \pm	43.20 \pm	10.05 \pm
CMNI 2021–0194	9.03	19.73	0.79	2	6.88	7.37	1.88
<i>H. stellati</i>	61 \pm	29.85 \pm	6.75 \pm	7 \pm	48.50 \pm	49 \pm	10.12 \pm
UF5468	15.42	7.16	1.21	2	4.12	5.16	0.76

TABLE 3. Mean \pm sd (standard deviations) values of measurements (in μm) of ossicles of the ventral surface. The average values obtained from ten measurements for each type (tables and buttons). Refer to Table 1 for abbreviations of variables.

	LBV	WBV	WhBV	NhBV	HSV	DDV	RDHV
<i>H. tubulosa</i> (s.s.)	73.75 \pm	38.70 \pm	5.75 \pm	6 \pm	42.90 \pm	36.60 \pm	11.58 \pm
CMNI 2021–0195	8.01	4.83	1.70	0	3.11	5.30	2.20
<i>H. algeriensis</i> n. sp.	58 \pm	31 \pm	4.90 \pm	6 \pm	33.85 \pm	40.50 \pm	12.74 \pm
UF5187	13.06	4.89	2.29	2	4.20	0.71	0.79
<i>H. poli</i>	36.75 \pm	26.25 \pm	6 \pm	5 \pm	31.90 \pm	35.75 \pm	10.71 \pm
UF5491	5.90	3.58	2.11	2	2.23	4.57	1.95
<i>H. arguinensis</i>	69 \pm	33 \pm	7.75 \pm	7 \pm	31.90 \pm	36.60 \pm	11.58 \pm
CMNI 2021–0194	15.01	4.83	1.84	1	2.23	5.30	2.20
<i>H. stellati</i>	45.25 \pm	27.25 \pm	2.50 \pm	5 \pm	31.90 \pm	42.85 \pm	10.01 \pm
UF5468	16.93	6.50	0	3	2.23	3.74	0.90

Statistical analysis of ossicles

The Linear Discriminant Analysis (LDA) (Figure 5), using all 14 endoskeletal variables, was effective in discriminating between the five species of the genus *Holothuria*, supported by the results of PERMANOVA analysis ($F = 10.4$, $p < 0.001$) confirming multivariate differences.

The first and second linear discriminant axes described 62.83% and 23.26% of the among species variation in endoskeletal characters respectively. The classification success of LDA among the five species was 62.50%.

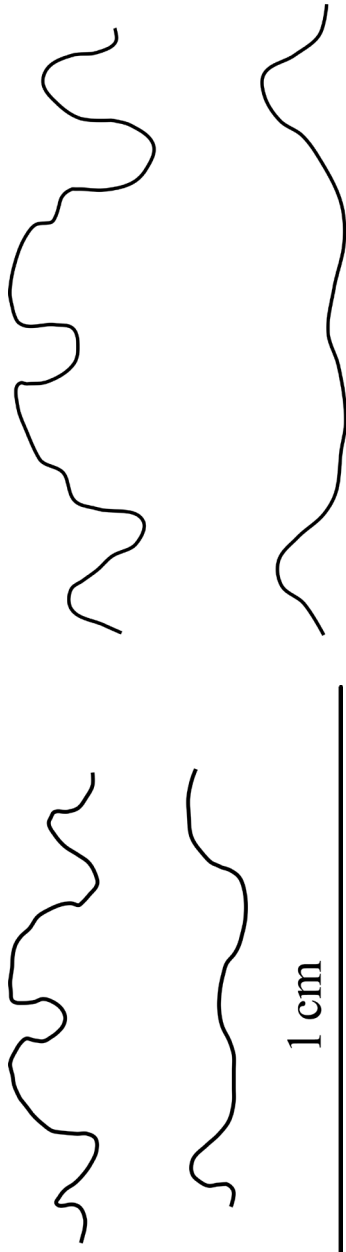
The ratio between the disk diameter and the height of the spire of the dorsum tables and the height of the spire of the tables of dorsum (RDHD and HSD) were the characters with highest positive coefficients in the first discriminant vector (LD1), separating *H. poli* and a group formed of the three species; *H. algeriensis* n. sp., *H. arguinensis* and *H. stellati*. The highest negative coefficients were the disk diameter of the tables of dorsum and length of buttons of ventrum (DDD and LBV), separating mainly *H. tubulosa* (s.s.) from the other species.

TABLE 4. Morphological characters of the species of the genus *Holothuria* of the Algerian coast. mL = mean length.

Specimen code	<i>Holothuria tubulosa</i> (s.s.)			<i>Holothuria algeriensis</i> n. sp.		
	LPVCMRMS 2020.201	LPVCMRMS 2020.202	LPVCMRMS 2020.203	LPVCMRMS 2020.203	LPVCMRMS 2020.204	
External morphological characters						
Body shape	Cylindrical	Cylindrical	Sub-cylindrical	Sub-cylindrical	Sub-cylindrical	
Contracted length (cm)	16.50	11	18.50	18.50	15.50	
Contracted width (cm)	3.20	4.20	4.50	4.50	4	
Consistency of integument after preservation	Smooth	Smooth	Smooth	Smooth	Smooth	
Body wall thickness (cm)	0.60	0.20	0.30	0.30	0.40	
Shape of trivium	Rounded	Rounded	± flattened	± flattened	± flattened	
Colouration of trivium	Brown	Brown	Light gray	Light gray	Light gray	
Colouration of pedicels (trivium)	Margin dark, whitish in middle	Margin dark, whitish in middle	Dark gray	Dark gray	Dark gray	
Shape of bivium	Rounded	Rounded	Arched	Arched	Arched	
Colouration of bivium	Dark brown	Dark brown	Dark gray/brown	Dark gray/brown	Dark gray/brown	
Disposition of papillae	Scattered	Scattered	Scattered	Scattered	Scattered	
Size of conical verrucosities of bivium (cm)	0.60	0.50	0.30	0.30	0.30	
Form of verrucosities of bivium	Large protuberances	Small protuberances	Small protuberances	Small protuberances	Small protuberances	
Position of anus	Ventral	Ventral	Terminal	Terminal	Terminal	
Anal papillae	5 in series of 3 podia each	5 in series of 3 podia each	5 in series of 4 podia each	5 in series of 4 podia each	5 in series of 4 podia each	
Number of tentacles	20	20	20	20	20	
Internal morphological characters						
Tentacular ampullae length	-	0.80 cm	Longer than those of <i>H. tubulosa</i> (s.s.)	Longer than those of <i>H. tubulosa</i> (s.s.)	Longer than those of <i>H. tubulosa</i> (s.s.)	
Polian vesicles	4 (1 in the middle, 1.80 cm and 3 on the left mL: 0.40 cm)	1 (1 cm)	1 (2 cm)	1 (2 cm)	1 (3 cm)	
Stone canals	2 (1cm long on each side)	1 right (1.40 cm)	5 right (mL: 0.55 cm) and 3 left (mL: 0.30 cm)	5 right (mL: 0.55 cm) and 3 left (mL: 0.30 cm)	3 right (mL: 0.50 cm) and 4 left (mL: 0.60 cm)	
Longitudinal muscles / thickness	Attached / 0.90 cm	Attached / 0.80 cm	Attached / 1.40 cm	Attached / 1.40 cm	Attached / 1 cm	

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TABLE 4. (Continued)

	<i>Holothuria tubulosa</i> (s.s.)		<i>Holothuria algeriensis</i> n. sp.	
Specimen code	LPVCMRMS 2020.201	LPVCMRMS 2020.202	LPVCMRMS 2020.203	LPVCMRMS 2020.204
Endoskeleton				
Calcareous ring				
Size range of the discs of the tables (µm)	40–60		21–57.50	
Size range of the spire of the tables (µm)	40–55		25–51	
Size range of the buttons (µm)	60–127.50		30–101	
Number of holes of the buttons	5–10		2–12	
Shape and texture of buttons	Oval, not very thick, with regular, rough surface comprising small, conical, pointed, fairly close asperities		Oval, relatively thick, holes oval and can become obliterated	
Tentacle ossicles	Two forms of rods—straight and slightly arched, thicker than those of the <i>H. algeriensis</i> n. sp. (19 µm)		Same as in <i>H. tubulosa</i> (s.s.), but thinner (12 µm)	

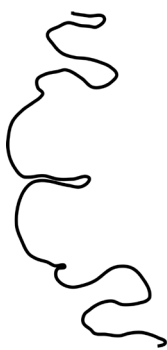

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TABLE 4. (Continued)

Specimen code	<i>Holothuria poli</i>		<i>Holothuria arguinensis</i>		<i>Holothuria stellati</i>	
	LPVCMRMS 2020.205		LPVCMRMS 2020.207		LPVCMRMS 2020.208	
External morphological characters						
Body shape	Cylindrical		Sub-cylindrical		Sub-cylindrical	
Contracted length (cm)	13		13.50		16.50	
Contracted width (cm)	3		5		6	
Consistency of integument after preservation	Rough		Smooth		Smooth	
Body wall thickness (cm)	0.40		0.20		0.70	
Shape of trivium	Rounded		± flattened		± flattened	
Colouration of trivium	Dark brown with light podia		Light brown		Brown to yellowish green	
Colouration of pedicels (trivium)	Margin dark, light brown in middle		Margin dark brown, lighter in middle		Margin dark brown, lighter in middle	
Shape of bivium	Rounded		Arched		Arched	
Colouration of bivium	Dark brown with light areas		Dark brown		Brown (darker than <i>H. arguinensis</i>)	
Disposition of papillae	In rows		In rows		In rows	
Size of conical verrucosities of bivium (cm)	0.20		0.30		0.70	
Form of verrucosities of bivium	Large protuberances		Large protuberances in rows		Large protuberances in rows	
Position of anus	Terminal		Terminal		Terminal	
Anal papillae	5 in series of 4 podia each		5 in series of 4 podia each		5 in series of 4 podia each	
Number of tentacles	20		20		20	
Internal morphological characters						
Tentacular ampullae length	Very small		1.20 cm		1.50 cm	
Polian vesicles	1 (0.80 cm)		1 (1 cm)		1 (3 cm)	
Stone canals	1 right (1.30 cm)		3 right (mL: 0.53 cm) and 1 left (0.90 cm)		5 right (mL: 0.60 cm) and 1 left (0.70 cm)	
Longitudinal muscles / thickness	Attached / 0.60 cm		Attached / 0.70 cm		Attached / 1.40 cm	

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TABLE 4. (Continued)

	<i>Holothuria poli</i>	<i>Holothuria arguinensis</i>	<i>Holothuria stellati</i>
Specimen code	LPVCMRMS 2020.205	LPVCMRMS 2020.207	LPVCMRMS 2020.208
Endoskeleton			
Calcareous ring			
	1 cm	1 cm	
Size range of the discs of the tables (µm)	30–75	30–55	40–60
Size range of the spire of the tables (µm)	30–60	30–50	30–55
Size range of the buttons (µm)	22.5–57.50	47.50–90	30–85
Number of holes of the buttons	2–9	4–11	2–12
Shape and texture of buttons	Oval, smooth, holes large, about 8, never obliterated	Solid or “8” shaped, spinose surface, many, unequal, large perforations, never obliterated but sometimes small	Oval to irregular, of various sizes, surface somewhat spinulated, small-knobbed/holes small, often obliterated, then buttons appearing rough or slightly knobbed
Tentacle ossicles	Two forms of rods: straight and slightly arched, straight ones thick	Large and complex, medial region often enlarged, branched and perforated	Straight and slightly arched rods; straight rods thick, with smooth, sometimes prickly surface

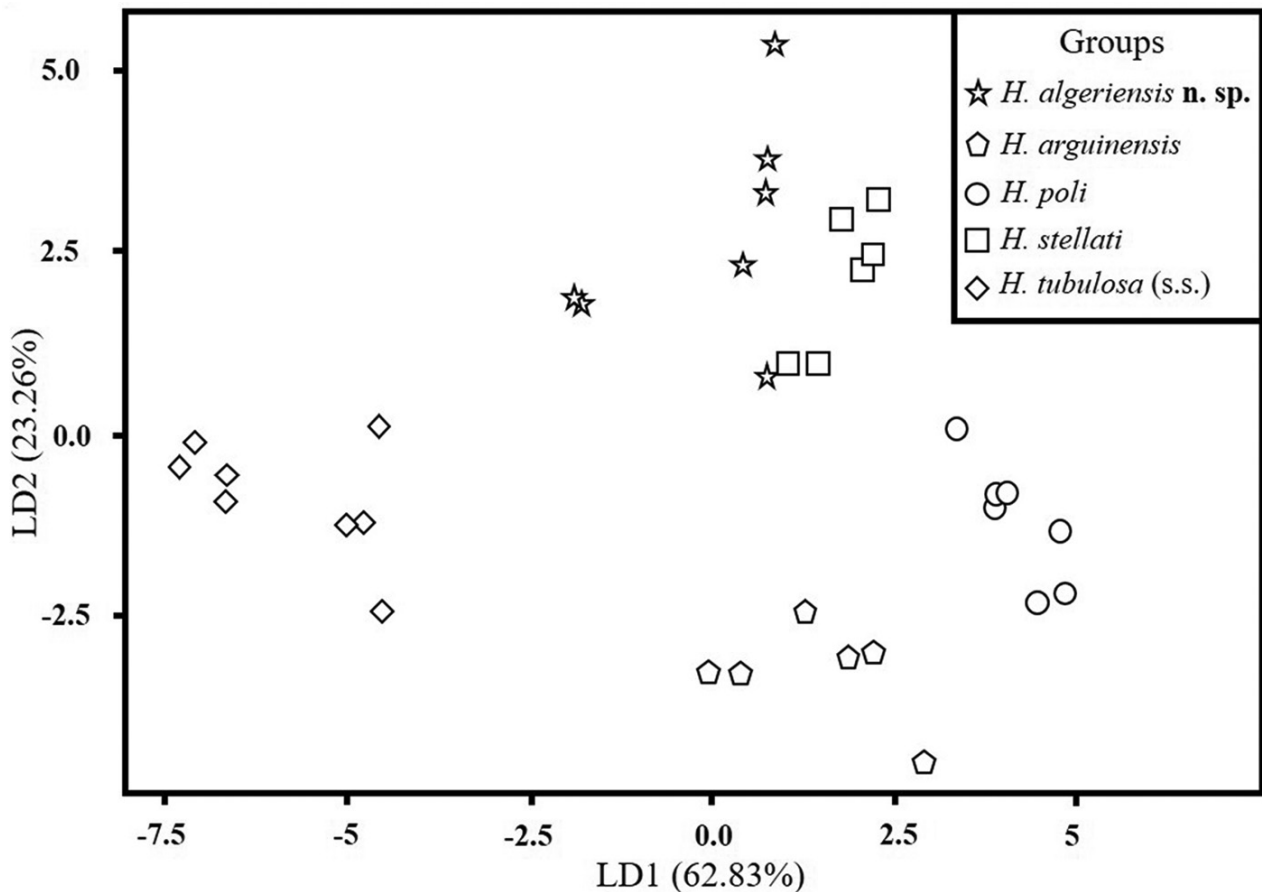


FIGURE 5. Axes 1 and 2 from linear discriminant analysis (LDA) based on 14 endoskeletal variables.

The second discriminant vector (LD2) has Ratio between disk diameter and height of spire of the ventrum tables (RDHV) and disk diameter of tables of dorsum (DDD) as a positive coefficient and height of spire of tables of dorsum (HSD) and Ratio between disk diameter and height of spire of the dorsum tables (RDHD) as a negative coefficient separating *H. arguinensis* from the two species, *H. algeriensis n. sp.* and *H. stellati*. The results of this classification confirm that *H. (H.) algeriensis n. sp.* is well separated from *H. (H.) tubulosa (s.s.)*.

Discussion

Throughout the history of classification of Mediterranean and related sea cucumbers, it is well known that *Holothuria (H.) tubulosa*, the most common Mediterranean species, was the most confused, with some very well-established workers expressing different opinions, some regarding it as a well-established species and others as a species complex (see Introduction). We herein followed the descriptions of this species and several other related Mediterranean forms given by Koehler (1921), which descriptions were recommended by Rowe (1969) after an extensive and excellent revision of the Holothuriidae. Our results indicate that while *H. (R.) poli*, *H. (R.) arguinensis* and *H. (H.) stellati* represent distinct species, *Holothuria (H.) tubulosa* encompasses two pseudocryptic forms herein recovered as *Holothuria (H.) tubulosa (s.s.)* and the new species, *Holothuria (H.) algeriensis n. sp.* According to our results these two species are distinct, considering their anatomy and morphometric analysis of their ossicles.

Statistical analysis demonstrates that *Holothuria (H.) algeriensis n. sp.* is close to *H. (H.) stellati* but distinct from *H. (H.) tubulosa (s.s.)*. In fact, *Holothuria (H.) algeriensis n. sp.* is not strictly Mediterranean as it was recently uncovered in the Moroccan Atlantic waters by Haddi I. (ongoing work).

Based on gross morphological study of the Mediterranean *Holothuria* species here studied (Table 4), *Holothuria (H.) algeriensis n. sp.* is clearly distinguishable by its sub-cylindrical form, the absence of large protuberances on the dorsal surface, and the differences in color between its dark dorsal surface and the very light ventral surface.

It can reach 18.5 cm in length, unlike *H. (H.) tubulosa* (s.s.) which reaches a maximum size of 16.5 cm. *Holothuria (H.) mammata* and *H. (H.) dakarensis* are much shorter [15 cm (Gustato & Villari 1977) and 14 cm (Prata *et al.* 2014) respectively].

In the new species the tentacular ampullae and Polian vesicles are larger in size, stone canals are more numerous and Cuvierian tubules are absent. The calcareous ring (see Table 4) of the new species is larger, with expanded radial plates, each with a broad anterior notch slightly bifurcate anterior parts, and a flat or slightly indented posterior margin. In *H. (H.) tubulosa* (s.s.) the calcareous ring is much smaller and, although the anterior notch of the radial plates is well-formed, the anterior portions are not notched. In *H. (R.) arguinensis*, although the anterior notch is well formed, the anterior radial portions are squarish, while in *H. (R.) poli* the anterior notch is deep and slit-like, the anterior radial parts clearly notched and the posterior end of the radial plates convex. At the level of the ossicles, the buttons of the new species differ in the shape of their perforations, which when not obliterated, are rather oval compared to those of other species which are rounded.

We here emphasise that the differences in morphology, form of the ossicles (see Table 2–4 above) and molecular analysis (see Mezali 2008, 2011; Mezali & Francour 2012) strongly support our proposition that *Holothuria (H.) tubulosa* “A” & “B” as described by Mezali (2008, 2011), represent two distinct species: *Holothuria (Holothuria) tubulosa* (s.s.) and *Holothuria (Holothuria) algeriensis* n. sp.

Conclusion

This taxonomic revision presented in this paper allowed us to re-evaluate the taxonomic status of some sea cucumbers species of the Algerian coast. The DNA sequences results (Mezali 2008, 2011), the morphology and the morphometrics of the ossicles all suggest that *H. (H.) tubulosa* (s.s.) and *H. (H.) algeriensis* n. sp. are two distinct species. Considering the endoskeleton criterion. *Holothuria (H.) algeriensis* n. sp. has buttons with oval perforations compared to those of *Holothuria (H.) tubulosa* (s.s.). Morphologically, individuals of *Holothuria (H.) algeriensis* n. sp. have a different morphological and anatomical characters compared to that of *Holothuria (H.) tubulosa* (s.s.).

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