

THE TAXONOMIC STATUS OF *HOLOTHURIA (HOLOTHURIA) TUBULOSA* FROM THE ALGERIAN COAST WITH THE DESCRIPTION OF A NEW SPECIES *HOLOTHURIA ALGERIENSIS* NOV. SP. (ECHINODERMATA: HOLOTHURIIDAE)

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ABSTRACT

In the present study we redescribe the systematics of some *holothurian* species collected from different localities of the Algerian coastal waters. Morphological (anatomical and endoskeletal) and previous molecular studies showed the presence of two distinct morphotypes of *Holothuria (Holothuria) tubulosa* (A & B). Morpho type A corresponds to the classical *Holothuria (Holothuria) tubulosa* (Gmelin 1791), described by Koehler (1921); while morpho type B, we believe, represents a species new to science with characteristics significantly different from those of *Holothuria (Holothuria) tubulosa* A. Statistical methods (hierarchical clustering) confirm significant differences between these two morpho types. To better visualize these differences, measurements made on ossicles of *Holothuria (H.) tubulosa* A & B (*Holothuria algeriensis* nov. sp.) were compared to other species that were present in our collection including the classical *Holothuria (H.) tubulosa* from Banyuls sur Mer (France) and the north-eastern Atlantic species, *Holothuria (Roweothuria) arguinensis* (Koehler & Vaney, 1906) recently recorded from Algerian waters and the Mediterranean *Holothuria (Roweothuria) poli* (Delle Chiaje, 1824). We conclude that the two morpho types of *H. (H.) tubulosa* are significantly different to warrant the recognition of a new species for the B form herein called *H. (H.) algeriensis* nov. sp.

Keywords: Sea cucumbers, New species, Systematics, Algerian basin, North-East Atlantic, Mediterranean.

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 of a lot of discussion and dispute 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*. Panning (1939) re-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 ossicles (tables). Cherbonnier (1950) noted that it would have been better to use *H. tubulosa*, established in 1790 by Gmelin, rather than *H. stellati* established by Delle Chiaje (1823) as super-species. However, he recognized *H. tubulosa*, *H. mammata* and *H. dakarensis* as separate 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* regarding the form of the ossicles, the size of the dorsal papillae and the arrangement and number of ventral podia (tube feet). For this reason, specimens of *H. tubulosa* were distinguished from *H. mammata* only by their size and the absence of Cuvierian tubules. Furthermore, 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 coloration 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; *H. tubulosa* and *H. mammata* with buttons of medium size, while the larger buttons are present in *H. dakarensis*. He proposed as a specific character for *H. mammata* the presence of Cuvierian tubules and the large dorsal mammillated 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*. Gustato & Villari (1979) distinguished *H. tubulosa* from *H. stellati* on detailed comparison of their morphology in water (live specimens) and outside water (after physical stress) (Gustato, personal communication, 1997), as well as the morphology and the percentage of specific ossicles. He divided the ossicles into 3 types ("a", "b" and "c") based on the form of the buttons. According to him *H. tubulosa* is devoid of type "b" ossicles (elongated smoothed oval buttons, with symmetrical holes and longitudinal streak) while in *H. stellati* such ossicles are the most common. Both button types "a" and "c" are present in both species (a: oval buttons with scalloped edges, their holes are placed symmetrically, their surface may be smooth or knobbed; c: round buttons without holes and fully bumpy). 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* for the existence of morpho type B (for *H. poli*). According to Mezali's (2008; 2011) DNA sequences results, the *H. (H.) poli* lineage is sister to three well supported lineages composed of *H. (H.) tubulosa*, *H. (H.) tubulosa* B (that we propose to be *H. (H.) algeriensis nov. sp.* and *H. (R.) stellati*. Within this lineage all *H. (R.) stellati* form 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* and *H. (H.) algeriensis nov. sp.* (i.e. *H. (H.) tubulosa* and *H. (H.) algeriensis nov.sp* are sister lineages).

In this study, the taxonomic status of the two morphotypes considered by Mezali (2008; 2011) of *H. (H.) tubulosa* A & B

(*H. (H.) algeriensis nov. sp.*) has been re-assessed based on a more detailed study of the morphology, anatomy and geometric morphology of the ossicles with advanced statistical analysis. In addition, three other holothuriid *holothurians* from the Algerian coast (*Holothuria (R.) arguinensis*, *Holothuria (R.) poli* and *Holothuria (H.) stellate*) are considered for a morphological, an anatomical and an endoskeleton statistical comparison. It would have been interesting if such a study is also undertaken for *H. (Roweothuria) vema* (Thandar, 1988), once fresh material comes to light.

MATERIALS AND METHODS

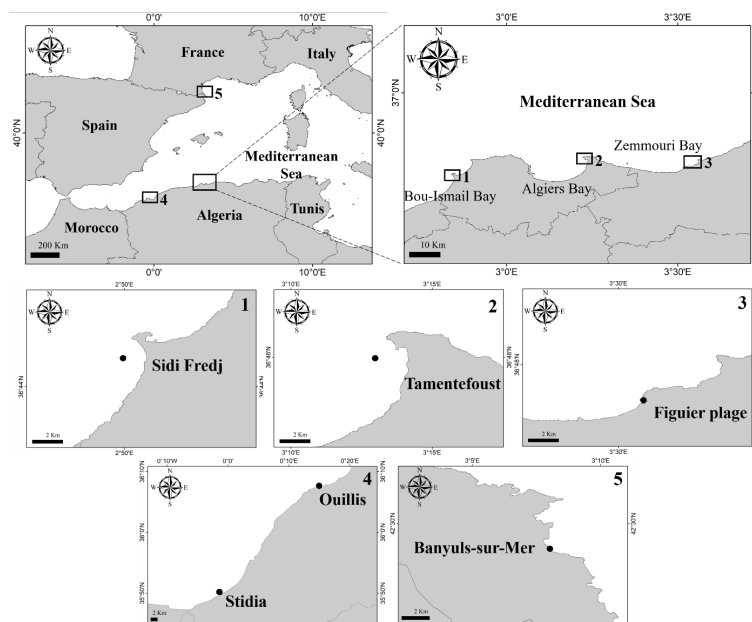
Most individuals of the genus *Holothuria* used in this study were collected from three stations located in the center of the:

- Algerian coast (Sidi Fredj (36°45.000'N, 2°50.000'E),
- Tamentefoust (36°48.000'N, 3°13.000'E) and
- Figuiet-plage (36°46.750'N, 3°30.821'E).

Sampling was also conducted at two other stations, located on the western part of the

- Algerian coast (Stidia-Mostaganem (35° 49.922'N, 0° 1.174'O) 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 (MgCl₂.6H₂O). 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), Tortonesi (1965), Rowe (1969) and Gustato & Villari (1979) (Figure 1).



Figures 1. Geographical localization of the sampling areas (actual area indicated by the black square).

Endoskeleton study

Samples of tissues for ossicle study from the bivium, trivium, podia and tentacles were prepared following the protocol of Samyn *et al.* (2006). We considered only adult individuals. The ossicles were photographed under a polarized light microscope 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 Hitachi S-4000 scanning electron microscopy in Florida and Philips XL 30 FEG scanning electron microscopy in Canada. For morphometric study the ossicles were measured using a micrometer slide (1 graduation=4.9 μm). Fourteen (14) variables (measurements) (Table 1) were used as data in different statistical methods. 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, we treated and reduced data (measures) to eliminate any effect of measurement units, thus our variables are all in the same order to perform hierarchical clustering using the method of Ward (1963). This method was performed using Rstudio software.

Table 1: The different types of measurements carried on ossicles. For the dorsal surface the suffix "fd" is added and for the ventral surface the suffix "fv" is added.

Abbreviation	Variable description
Buttons	
LBfd	Length of buttons of dorsum
LaBfd	Width of buttons of dorsum
Ltrfd	Width of the button's holes of dorsum
Nbtrfd	Number of holes of buttons of dorsum
LBfv	Length of buttons of ventrum
LaBfv	Width of buttons of ventrum
LtrBfv	Width of the button's holes of ventrum
NbtrBfv	Number of holes of buttons of ventrum
Tables	
HTfd	Height of spire of table of dorsum
DTfd	Disk diameter of table of dorsum
Rafd	Ratio between disk diameter and height of the spire of the dorsum tables
HTfv	Height of spire of table of ventrum
DTfv	Disc diameter of table of ventrum
Rafv	Ratio between disk diameter and height of the spire of the ventrum tables

RESULTS

Systematic study

Order: Holothuriida (Miller et al., 2017)

Family: Holothuriidae (Burmeister, 1837)

Genus: *Holothuria* (Linnaeus, 1767)

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

Holothuria (*H.*) *tubulosa* (Gmelin, 1791) (Figure 2) (Table 2)



Figures 2. External morphology and ossicles of *H. tubulosa*. A. Dorsal face. B. Ventral face. C. a: Tables of the dorsal and ventral sides, b: Buttons of the dorsal and ventral sides. D. a: Enlarged plates of the ventral side, b: Elongated plates of the ventral side. E. a: straight rods of the tentacles; b: slightly arched rods

Remarks: The described forms match with the form of *H. tubulosa* described by Kohler (1921) which description is recommended by Rowe (1969) for this species in his revision of the Holothuriidae.

Synonymy:

Holothuria tubulosa (Gmelin, 1791, Grube, 1840; Selenka, 1867; Heller, 1868; Marenzeller, 1874; Ludwig, 1879; Lampert, 1885; Koehler, 1921; Koehler, 1927; Hérouard, 1929; Nobre, 1931; Panning, 1934; Tortonese, 1934; Tortonese, 1935; Mayer, 1937; Tortonese, 1952; Cherbonnier, 1956; Gustato & Villari, 1979;

Tortonese, 1965; Rowe, 1969; Mezali, 1998).

Holothuria tremula (Linnaeus, 1767) *Holothuria columnae* (de Blainville, 1821) *Holothuria pentagnae* (Delle Chiaje, 1824) *Holothuria maxima* (Delle Chiaje, 1823) *Holothuria columnae* (Delle Chiaje, 1824)

Material examined:

- M182, M195, M181, M197, Stidia, Algeria, 35° 49.922'N, 0° 1.174'O, 3 m, Summer 2006, 5 spec.

- M147, Sidi Fredj, Algeria, 36°45.000'N, 2°50.000'E, 0.5-9 m, Summer 2006, 1 spec.

- M111, Banyuls sur Mer, France, 42° 29.040'N, 3° 7.970'E, 3 m, Summer 2006, 1 spec.

- M224, Figuier plage, Algeria, 36°46.750'N, 3°30.821'E, 0.5-9 m, Summer 2006, 1 spec. Ouillis, Algeria, 36° 7.436'N, 0° 15.219' E, 5 m, June 2020, 2 spec.

Description: Body form (Figure 2, Table 2) almost cylindrical, length up to 200 mm, width 30-60 mm when contracted. Body wall thick (until 7 mm) and leathery. Dorsal

surface (bivium) (Figure 2A) arched, bearing small conical tubercles/verruca cities scattered, each terminating in a small, elongated papilla. Ventral surface (trivium) (Figure 2B) flattened 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 podia each). Live colouration varying from light brown, red mahogany, to brown or black, ventral surface generally lighter (light brown). Longitudinal muscles of about 8 to 9 mm. Cuvierian tubules absent. Stone canals several 1-4 (two on left side of dorsal mesentery and two on right side). Polian vesicles between one and four of an average length <10 mm. Tentacular ampullae of about 8 mm.

Ossicles: Ossicle from the dorsal surface and ventral surface appear identical, they include the tables and the buttons. The tables (Figure 2C-a) are small in size and in the form of a “cups”. The average height of the arrow is less than the average diameter of the disc. The later most often has a slightly arched shape. Some tables have a basilar disc with a thorny outline which generally presents 4 large orifices and a variable number of peripheral perforations. Buttons (Figure 2C-b) oval in shape of about 36-50 μm long and 19-22 μm wide in the dorsal and ventral face respectively, not very thick, with a regular, rough surface and have small, conical, pointed and close roughness. Outline irregular, nodular, may be smooth and occasionally thorny. An average of 7 holes with a rough periphery arranged in two rows relative to the central axis. Holes may be partitioned, round, irregular in outline and may become very small or even disappear completely to give the buttons a full and rough appearance. Average width of

the holes 3-5 μm in the dorsal and ventral face respectively. Pseudo-buttons abundant and fenestrated ellipsoids scarce. Papillae and pedicels present perforated plaques. They are of two type; the first are enlarged (Figure 2D-a) which have large central perforations symmetrical about the central axis of the plate surrounded by other smaller one, the second are thick, rough and elongated plates (Figure 2D-b) with an irregular outline which have a few large perforations at their lateral and apical ends. Enlarged plates smooth in surface with an irregular and prickly outline. Two rows of large perforations that are symmetrical about the central axis of the plate and surrounded by several smaller perforations. Tentacles present two forms of thick rods (19 μm); straight (Figure 2E-a) and slightly arched rods (Figure 2E-b).

Holothuria (H.) Algeriensis nov. sp. (Figure 3) (Table 3)

Partial synonymy

Holothuria tubulosa B (Mezali, 2008).

Material examined

- M182, M195, M181, M197, Stidia, Algeria, 35° 49.922'N, 0° 1.174'O, 3 m, Summer 2006, 5 spec.
- M147, Sidi Fredj, Algeria, 36°45.000'N, 2°50.000'E, 0.5-9 m, Summer 2006, 1 spec.
- M111, Banyuls sur Mer, France, 42° 29.040'N, 3° 7.970'E, 3 m, Summer 2006, 1 spec.
- M224, Figuiersplage, Algeria, 36°46.750'N, 3°30.821'E, 0.5-9 m, Summer 2006, 1 spec. Ouillis, Algeria, 36° 7.436'N, 0° 15.219' E, 5 m, June 2020, 2 spec.

Table 2: Mean values of measurements 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.

	LBfd	LaBfd	Ltrfd	Nbtrfd	HTfd	DTfd	Rafd
H. poli	36,25	28,60	6,25	57,00	49,20	56,20	11,47
H. arguinensis	64,85	32,45	9,75	91,00	43,60	43,20	10,05
H. tubulosa	101,25	44,25	6,75	86,00	48,10	51,00	10,60
H. Algeriensis nov. sp.	69,10	35,60	7,80	75,00	37,10	38,10	10,22
H. stellati	61,00	29,85	6,75	74,00	48,50	49,00	10,12

Table 3: Mean values of measurements 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.

	LBfv	LaBfv	Ltrfv	Nbtrfv	HTfv	DTfv	Rafv
H. poli	36,75	26,25	6,00	54,00	31,90	36,60	11,58
H. arguinensis	69,00	33,00	7,75	66,00	31,90	36,60	11,58
H. tubulosa	73,75	38,70	5,75	59,00	42,90	42,85	10,02
H. Algeriensis nov. sp.	58,00	31,00	4,90	61,00	33,85	35,75	10,71
H. stellati	45,25	27,25	2,50	53,00	31,90	40,50	12,74

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 (until 4 mm) and soft in a relaxed state. Pedicels/tube feet irregularly distributed on ventral side. Conical verrucosities of dorsal surface (bivium) minute or lacking. Mouth ventral, tentacles usually 20, anus terminal, anal papillae developed (5 series of 4 podia each). Live colouration of dorsal surface brown mahogany to brown bitumen, ventral surface dark grey/ brown. Longitudinal muscles of about 10 to 14 mm. Cuvierian tubules absent. Stone canals several, 5- 6 on right and 2-3 on left side of dorsal mesentery of an average length <1 cm). Polian vesicle single, until 30 mm long. Tentacular ampullae longer than those of *H. tubulosa*.

Ossicles: Dorsal and ventral surface present the same type of ossicles; tables (Figure 3C-a) and buttons (Figure 3C-b). Tables of the ventral surface with a basilar disc in the shape of a “rudder” which shows a variable number of points. Pillars of the arrow parallel to each other and do not merge in the apical part. Several pointed and thin thorns at the end of the pillars, generally arranged in number of 3 to 4 on the end of each pillar. Buttons rectangular of about 28-34 μm long and 15-17 μm wide in the dorsal and ventral face respectively, relatively thick, oval or “8” shaped. Outline irregular, wavy

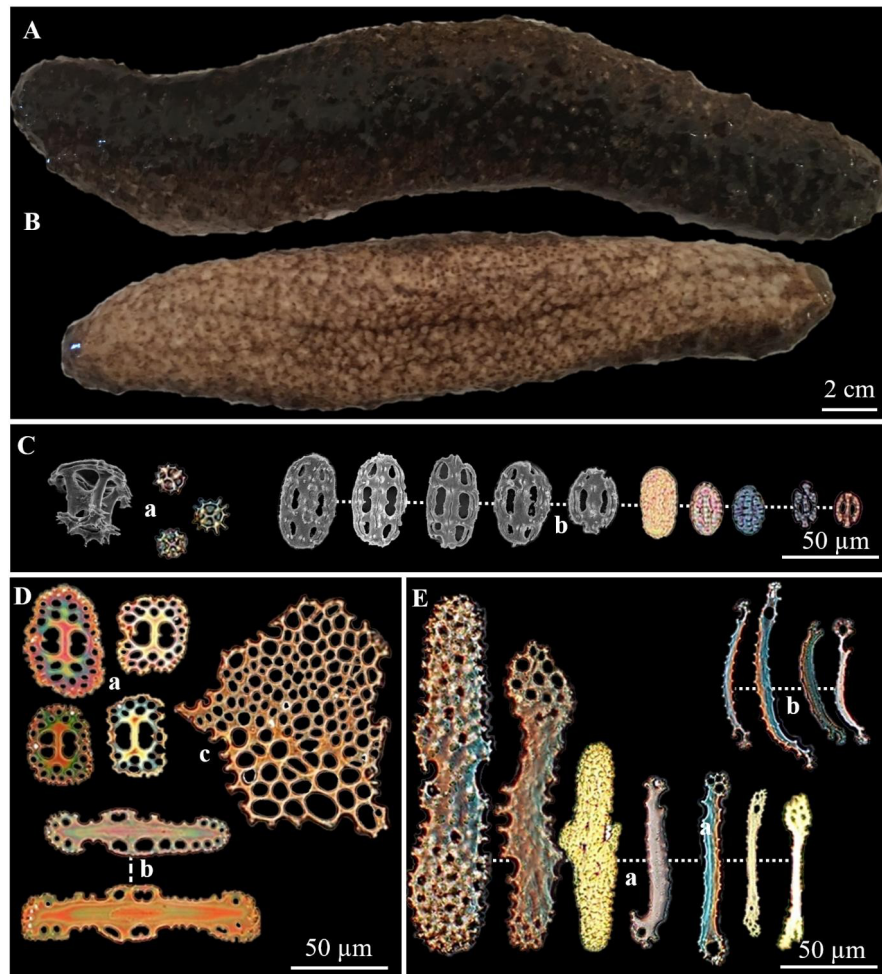
and rough. Holes of an average number of 7 are quite open, usually rounded and may disappear to give the buttons a rough and full appearance. Average width of the holes 2-4 μm in the dorsal and ventral side respectively. Generally, the symmetrical holes in the center of the buttons are wider than the apical ones and have both a rather oval shape. Pseudo-buttons abundant, fenestrated ellipsoids scarce.

Papillae and pedicels present the same type of perforated plaques as *H. tubulosa*. Enlarged plates (Figure 3D-a) rectangular in shape, smoother than those of *H. tubulosa*. The central perforations are surrounded by smaller and circular ones. They can also be oval in shape with an irregular outline. Elongated plates (Figure 3D-b) simple than those of *H. tubulosa* and are irregular in outline. Ventral surface present also terminal plates (Figure 3D-c). Tentacles present same forms of rods as *H. tubulosa* (Figure 3E-a and Figure 3E-b) but they are thinner (12 μm).

Other species of the Genus *Holothuria* present in the Mediterranean Sea Subgenus:

Holothuria (*Roweothuria*) (Thandar, 1988)

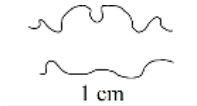
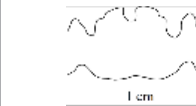


Holothuria (*Roweothuria*) *poli* (Delle Chiaje, 1824) (Figure 4) (Table 4)



Figures 3. External morphology and ossicles of *H. algeriensis* nov. sp. A. Dorsal face. B. Ventral face. C. a: Tables of the dorsal and ventral sides, b: Buttons of the dorsal and ventral sides. D. a: Enlarged plates of the ventral side, b: Elongated plates of the ventral side, c: terminal plate of the ventral side. E. a: straight rods of the tentacles; b: slightly arched rods.

Table 4: Taxonomical (external and internal morphological) characters of Holothuria species

	<i>Holothuria tubulosa</i> A		<i>Holothuria algeriensis</i> nov. sp.		<i>Holothuria poli</i>		<i>Holothuria arguinensis</i>	
Specimen number	spec. 14	spec. 13	spec. 6	spec. 5	spec. 1	spec.15	spec. 3	spec . 5 of arguinensis may be stellati?
External morphological characters								
Body shape	cylindrical	cylindrical	semi cylindrical	semi cylindrical	cylindrical	cylindrical	semi cylindrical	semi cylindrical
Contracted length (cm)	16.5	11	18.5	15.5	13	12	13.5	16.5
Contracted width(cm)	3.2	4.2	4.5	4	3	3.1	5, very contracted	6
Consistency of integument after preservation	Smooth	Smooth	Smooth	Smooth	Rough	Rough	smooth	smooth
Body wall thickness (cm)	0.6	0.2	0.3	0.4	0.4	0.4	0.2	0.7
Shape of trivium	rounded	rounded	somewhat flattened	somewhat flattened	rounded	rounded	somewhat flattened	somewhat flattened
Coloration of trivium	Brown	Brown	Light gray	Light gray	Dark brown with light podia	Dark brown with light podia	Light brown	Brown going to yellow green
Coloration of podia (trivium)	Dark along margin, whitish in middle	Dark along margin, whitish in middle	Dark grey	Dark grey	Dark outline, light brown end	Dark outline, light brown end	Dark brown outline, light end	Dark brown outline, light end
Shape of bivium	Rounded	Rounded	Arched	Arched	Rounded	Rounded	Arched	Arched
Coloration of bivium	Dark brown	Dark brown	Dark grey/ brown	Dark grey/ brown	Dark brown with light areas	Dark brown	Dark brown	Brown (darker than H. arguinensis)
Disposition of podia	Scattered	Scattered	Scattered	Scattered	In rows	In rows	In rows	In rows
Size of conical verrucosities of bivium	0.6	Small	0.3	0.3	0.2	0.3	0.3	0.7
Form of verrucosities of bivium	Large protuberances	Small protuberances	Small protuberances	Small protuberances	Large protuberances	Large protuberances	Large protuberances in rows	Large protuberances in rows
Position of the anus	Ventral	Ventral	Terminal	Terminal	Terminal	Terminal	Terminal	Terminal
Anal papillae	5 series of 3 podia each	5 series of 3 podia each	5 series of 4 podia each	5 series of 4 podia each	5 series of 4 podia each	5 series of 4 podia each	5 series of 4 podia each	5 series of 4 podia each
Number of tentacles	20	20	20	20	20	20	20	20

Internal morphological characters								
Tentacular ampullae	-	0.8 cm	Longer than	Longer than	Very small	Very small	1.2 cm	1.5 cm
Polian vesicles	4 (1 in the middle of 1,8cm and 3 on the left of 0,7/0,4/0,2 cm)	1cm	2 cm	3 cm	0.8 cm	1.5 cm	1 cm	3 cm
Stone canals	2 (one of 1cm on each side)	1 right (1.4 cm)	5 right (0.6/0.5 and 3 of 0.3 cm)	3 right (0.8/0.5/0.2 cm) and 4 left	1 right (1.3 cm)	1 right (1.1 cm)	3 right (0,7/0,6/0,3 cm) and 1 left (0,9)	5 right and 1 left (0,7 cm)
Longitudinal muscles / thickness	Attached /0.9 cm	Attached /0,8 cm	Attached /1,4 cm	Attached /1 cm	Attached /0,6 cm	Attached /0,7 cm	Attached /0,7cm	Attached /1,4 cm
Endoskeleton								
Calcareous ring								
Size range of the discs of the tables	04-6		2.1-5.75		3-7.5		3-5.5	04-Jun
Size range of the spire	4-5.5		2.5-5.1		03-06		03-05	3-5.5
Size range of the buttons	6-12.75		3-10.1		2.25-5.75		4.75-9	3-8.5
Number of holes of the buttons	05-10		02-12		02-09		04-11	02-12
Texture of the buttons	Oval, not very thick with a regular, rough surface and have small, conical, pointed and fairly close		Rectangular, relatively thick, oval or « 8 » shaped		Oval, sometimes with a large perforations. They may show an enlargement on their surface. Often, two pairs of large, symmetrical and successive holes and others smaller toward each end of the buttons		Smooth surface with unequal perforations of large circumferences. Buttons can be solid and « 8 » shaped. The holes are an average of 8	Small, oval buttons with irregular, rough, smooth and thick surface / large buttons with a slightly rectangular surface / enlarged, round and full buttons / « 8 » shaped buttons

Description: Soft cylindrical body, light brown to dark brown in color. Sometimes exceeds 20 cm in length and 4 cm to 5 cm in width. Trivium (Figure 4A-b) clearly separated from bivium (Figure 4A-a) by a groove. Pedicels whitish, very tight and numerous on the ventral surface. Thinner and reduced dorsal papillae. Conical tubercles of reduced size on the dorsal surface with a whitish end. Ventral mouth and terminal anus. Tentacles of yellowish color in number of 20. Cuvierian tubules absent. Collar around the mouth thin.

Ossicles: Tables (Figure 4A-c) with a basilar disc with irregular contour, serrated and pierced with 4 central holes and a variable number of peripheral holes. Buttons (Figure 4A-d) of 3 types:

- (i) Smooth and regular outline;
- (ii) Buttons with a smooth and irregular outline and
- (iii) Buttons with large perforations. Perforated plates of the central surface of two types: enlarged (Figure 4A-f)

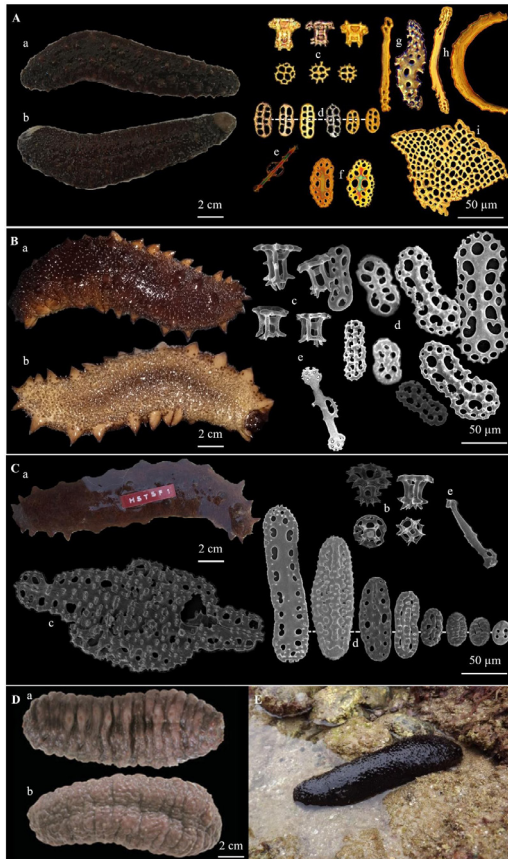
and elongated (Figure 4A-e). Terminal plates on the ventral side (Figure 4A-i). Straight (Figure 4A-g) and arched tentacle rods (Figure 4A-h).

Holothuria (Roweothuria) arguinensis (Koehler & Vaney, 1906) (Figure 4) (Table 4)

Description: Semi cylindrical body. Dorsal face (Figure 4B-a) arched of a dark brown color and ventral face (Figure 4B-b) sole-like of a light brown color. Several large protuberances in two double rows on the dorsal side. Trivium and bivium separated by several arched protuberances. Terminal anus. Mouth with 20 tentacles. Cuvierian tubules absent. Collar around the mouth thick.

Ossicles: Tables (Figure 4B-c) sometimes have a rectangular shape. Straight to slightly arched pillars, parallel to each other and have a point at their ends. Full or 8 shaped buttons (Figure 4B-d) with rough, irregular and prickly edges. Smooth surface with uneven perforations. Numerous

elongated perforated plates (Figure 4B-e) and rare enlarged plates. Rods larger and more complex with an enlarged, branched and perforated middle region.



Figures 4. *Holothuria (Roweothuria) poli*: Aa. Dorsal face. Ab. Ventral face. Ac. Tables. Ad. Buttons. Ae. Elongated plates. Af. Enlarged plates. Ag. Straight rods. Ah. Arched rods. Ai. Terminal plates. B. *Holothuria (Roweothuria) arguinensis*: Ba. Dorsal face. Bb. Ventral face. Bc. Tables. Bd. Buttons. Be. Perforated plates. C. *Holothuria (Roweothuria) stellati*: Ca. Dorsal face Cb. Tables. Cc. Elongated plates. Cd. Buttons. Ce. Straight rods. D. *Holothuria (Roweothuria) dakarensis*: Da. Dorsal face. Db. Ventral face (Pictures of Prataet al. 2014). E. *Holothuria (Roweothuria) mammata* (Picture of Marquet 2017).

Subgenus

Holothuria (Holothuria) (Linnaeus, 1767)

Holothuria (Holothuria) stellate (Delle Chiaje, 1824) (Figure 4) (Table 4)

Description: Body dark brown on the dorsal face (Figure 4C-a) and light brown on the ventral side. Can reach 30 cm in length and 5 cm to 6 cm in width. Rounded bivium and flat trivium with fairly tight pedicels. Integument thicker and rough. A row of 5-6 very large protuberances stretched out, pointed and separated by equal intervals at the limit of the two sides of the body. Cuvierian tubules absent. Collar around the mouth thick.

Ossicles: Circular basilar disc of the tables (Figure 4C-b) with wavy outline, 4 large central and 4 small peripheral perforations. Buttons (Figure 4C-d) of several types:

- (i) Oval with irregular, rough, smooth and thick surfaces;
- (ii) Large sizes with a slightly rectangular surface, smooth and sometimes wavy outline;
- (iii) Enlarged, round and full and 8 shaped.

Perforated plates of the ventral surface of two types: enlarged and elongated plates (Figure 4C-c). Straight (Figure 4C-e) and slightly arched rods. Collar around the mouth thick.

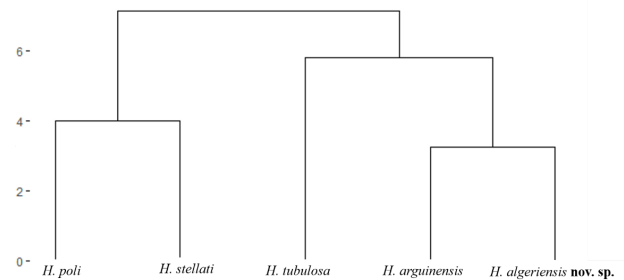
Morphometries of the ossicles:

Holothuria (Roweothuria) (Thandar, 1988)

Why did you not use all the variables from Table 1.

Statistical analysis of ossicle measurements Ascending Hierarchical Classification (AHC)

The obtained dendrogram (Figure 5) helped to separate the two populations of *Holothuria (H.) tubulosa* (A & B). Two groups of species are here observed; the first group includes *H. (R.) poli* and *H. (H.) stellati* and from the second separates *H. (H.) tubulosa* first and then the two species *H. (R.) arguinensis* and *H. (H.) algeriensis nov. sp.* forms another cluster.



Figures 5. Dendrogram representing the distance between the groups of studied species by cluster analysis of ossicles (square of the Euclidean distance, Ward's method).

The results of this classification confirm that *H. (H.) algeriensis nov. sp.* is separated from *H. (H.) tubulosa* and closer to *H. (R.) arguinensis*. We can also conclude that even if *H. (R.) poli* et *H. (R.) arguinensis* belong to the same subgenus *Holothuria (Roweothuria)*, they are in two different cluster.

DISCUSSION

Throughout history of the classification of sea cucumbers, all taxonomists agree that *Holothuria (H.) tubulosa*, the best-known species and most common in the Mediterranean Sea, is a single species well separated from the others. Our results indicate that *Holothuria (H.) tubulosa* encompasses two pseudocryptic species recovered here as *Holothuria (H.) tubulosa* and *Holothuria (H.) algeriensis nov. sp.* According to our results these two varieties/forms are distinct after detailed consideration of morphological, anatomical and statistical analysis of morphometric data of ossicles. The latter indicates that the individuals of *Holothuria (H.) algeriensis nov. sp.* are much closer to *Holothuria (R.) arguinensis* [previously considered a hybrid of *H. stellati*

and *H. poli* (Mezali, 2008)] than to those of *Holothuria (H.) tubulosa*. The latter indicates that the individuals of *Holothuria (H.) algeriensis* nov. sp. are much closer to *Holothuria (R.) arguinensis* [previously considered a hybrid of *H. stellati* and *H. poli* (Mezali, 2008)] than to those of *Holothuria (H.) tubulosa*. According to this result, *Holothuria (R.) arguinensis* is sister to *H. (H.) algeriensis* and *Holothuria (H.) tubulosa* nov. sp. than to *Holothuria (R.) poli*. Borerro et al. (2010) also reported a closer relationship between the *H. (H.) tubulosa* and *H. (R.) arguinensis*. In these cases, the subgenera *Holothuria* and *Roweothuria* do not appear to be monophyletic groups as reported by Thandar (1988) but paraphyletic. We provide a table based on morphological characters to distinguish the very much confused species of the subgenera *Holothuria* and *Roweothuria* to compare species (see Table 4).

H. algeriensis nov. sp. is a Mediterranean species but it was recently also found in the Atlantic Ocean (Morocco) by Haddi I. (ongoing work).

Morphologically and compared to the other species of the genus *Holothuria* studied (Table 4),

the species *H. (H.) algeriensis* is distinguished by the shape of the body which is semi-cylindrical, the absence of large protuberances on the dorsal surface and the difference in color between the dark dorsal face and the very light ventral face thus leaving a clear demarcation between the two without the presence of protuberances at their limit. It can reach 185 mm unlike the two species *H. (H.) mammata* (Figure 4E) *H. (H.) dakarensis* (Figure 4D-a, b) that do not reach a high length (Koehler 1921; Prata et al. 2014).

Anatomically, the tentacular ampullae and the Polian vesicle are larger in size, stone canals are more numerous and Cuvierian tubules are absent. Endoskeletally, the calcareous ring has a square shape with a central notch and a posterior margin with a triangular indentation, the inter radial pieces of triangular shape with an anterior spiral. The difference with that of *H. (H.) tubulosa* is in the shape of the right and left anterior extremities of the radial part, in *H. (H.) tubulosa*, the latter are rather smooth rounded while in *H. (H.) algeriensis* are marked forming a more important angle almost square. At the level of the ossicles, the buttons differ in the shape of their perforations which, when present, are rather oval compared to that of other species which are rounded.

We emphasise that the differences in morphology, form of the ossicles (Table 2) and molecular analysis strongly support that the two populations of *Holothuria (H.) tubulosa* A & B (Mezali 2008; 2011), are different and here regard them as separate species with *H. (H.) tubulosa* Bas a new species which we here in name *Holothuria (Holothuria) algeriensis* nov. 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 the morphology and the morphometrics of the ossicles all

suggest that *H. (H.) tubulosa* and *H. (H.) algeriensis* nov. sp. are two distinct species. Considering the endoskeleton criterion, *Holothuria (H.) algeriensis* nov. sp. has buttons with oval perforations compared to those of *Holothuria (H.) tubulosa*. Morphologically, individuals of *Holothuria (H.) algeriensis* nov. sp. have a different morphological and anatomical characters compared to that of *Holothuria (H.) tubulosa*.

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