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Checklist with first records for the Echinoderms of northern Tunisia (central Mediterranean Sea)

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Summary: Tunisia occupies a strategic biogeographic position in the Mediterranean Sea and the Strait of Sicily is considered a biogeographical boundary that separates the eastern and western basins. Despite the importance of marine biodiversity in Tunisia, the few studies of Echinodermata fauna in this region data from long ago. In order to update and produce a validated checklist of the echinoderms that occur in northern Tunisia, a study of this phylum was carried out between 2012 and 2016. Forty-five species were inventoried and distributed into the five living Echinodermata classes (Crinoidea, Asteroidea, Ophiuroidea, Echinoidea and Holothuroidea). New occurrences of four species from Tunisian marine waters [*Asterina pancerii* (Gasco, 1876), *Luidia atlantidea* (Madsen, 1950), *Ophiactis virens* (Sars, 1859) and *Leptopentacta tergestina* (Sars, 1857)], are cited and discussed here for the first time.

Keywords: echinoderms; new occurrences; biodiversity; Tunisia; Mediterranean Sea.

Listado con primeros registros de los equinodermos del norte de Túnez (Mediterráneo central)

Resumen: Túnez ocupa un área biogeográfica estratégica en el Mediterráneo. El estrecho tunecino-siciliano es considerado una frontera biogeográfica que separa las cubetas oriental y occidental. Sin embargo, a pesar de su interés, los estudios sobre la fauna de equinodermos de Túnez son antiguos y escasos. Con el fin de elaborar el inventario de los equinodermos de la región septentrional del mar de Túnez, se realizó un estudio de este filum entre los años 2012 y 2016. Se han inventariado cuarenta y cinco especies pertenecientes a las cinco clases actuales de Echinodermata (Crinoidea, Asteroidea, Ophiuroidea, Echinoidea y Holothuroidea). Cuatro especies [*Asterina pancerii* (Gasco, 1876), *Luidia atlantidea* (Madsen, 1950), *Ophiactis virens* (Sars, 1859) y *Leptopentacta tergestina* (Sars, 1857)] se han recolectado por primera vez en estas aguas.

Palabras clave: equinodermos; nuevas citas; biodiversidad; Túnez; mar Mediterráneo.

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INTRODUCTION

Tunisia occupies a central position between the western and eastern Mediterranean Sea. It is the northernmost point of the African continent (36°347′N, 9°129′E). The Strait of Sicily, from Cape Bon (northeastern Tunisia) to Mazara dell Vallo (Sicily, southern Italy), has been considered a biogeographical barrier that separates the eastern and western Mediterranean basins (Bianchi and Morri 2000, Mejri et al. 2009). This separation is not only important from the point of view of connectivity between two basins with different hydrographical conditions, but also because of its geological history after the Messinian crises that isolated the two basins and a time lag in recolonization by Atlantic species (Zenetos 2010, Coll et al. 2010, Lipej et al. 2017). In fact, several genetic investigations on fish and macro-invertebrates in their different life stages, have demonstrated that the Strait of Sicily acts as a genetic boundary for African Mediterranean Sea species (Pérez-Losada et al. 2007, Zitari-Chatti et al. 2009, Deli et al. 2017). The colonization of new species in the Mediterranean Sea, first by the Lessepsian invasions after the opening of the Suez Canal and, more recently, through the Strait of Gibraltar as climate change becomes more evident (Zenetos 2010), makes Tunisia the point of convergence of the two processes, with a significant stretch of coastline on each side of this "boundary".

The phylum Echinodermata includes marine invertebrate species and is composed of five living classes: Crinoidea, Asteroidea, Ophiuroidea, Echinoidea and Holothuroidea. They cover a wide range of biological strategies, habitats and depths. Echinoderms are found from the shallow intertidal to the abyssal zone, where they play an important role in the ecological processes of marine ecosystems.

Lack of research on Echinodermata is a knowledge gap regarding Tunisian marine biodiversity. Only two studies, by Cherbonnier (1956) and Gautier-Mechaz (1958), have published checklists of Tunisian echinoderms. These checklists are old and need to be updated regarding aspects such as climate change, invasive species, diversity estimation and marine protected areas. This phylum is currently cited associated with the megabenthic invertebrate inventories of Le Danois (1925), Azouz (1973), Ben Othman (1973), El Lakhrach et al. (2012). Other authors have focused on one particular class, generally one of economic interest such as Echinoidea (Sellem et al. 2001) or Holothurioidea (Louiz et al. 2003).

In order to update the inventory of marine diversity of Echinodermata species in the Tunisian Sea, research was performed between 2012 and 2016. The acquired data were used to produce a validated checklist of the Echinodermata of northern Tunisia.

MATERIALS AND METHODS

Study area

Echinoderms were sampled at 93 sites in eight locations along the northern coasts of Tunisia (Supplementary material Table S1). The study area extends over 300 km of the Tunisian coastline, from the Algerian-Tunisian border (37°01′06.0″N, 8°44′04.5″E) to the Cape Bon Peninsula (36°26′53.1″N 10°51′36.5″E). (Fig. 1).

This area of the central Mediterranean Sea is constantly affected by incoming Atlantic marine currents (Lubet and Azouz 1969, Azouz 1973). It is characterized by a continental shelf with a small, irregular plateau and a steep slope (Azouz 1973). The heterogeneity of its bottom type, with hard and soft substrates, enriches the biodiversity of northern Tunisia (Azouz 1973, Ayari and Afli 2003).

Data collection

The Echinodermata inventory was carried out from March 2012 to July 2016. A variety of sampling strategies were adopted depending on the substrate type (rocky or soft bottom, depth) and respecting the benthic bionomics of the Mediterranean Sea (Table 1). Specimens were collected using a dredge for inshore shallow areas at depths of less than 50 m and a professional benthic fishing trawl for offshore waters where the depth exceeds 50 m. Hand collection and diving were used for mid- and infralittoral levels (<5 m) (Supplementary material Table S2).

Taxonomic work

The collected material was measured, photographed and preserved in ethanol. Specimens were identified based on external morphology and internal

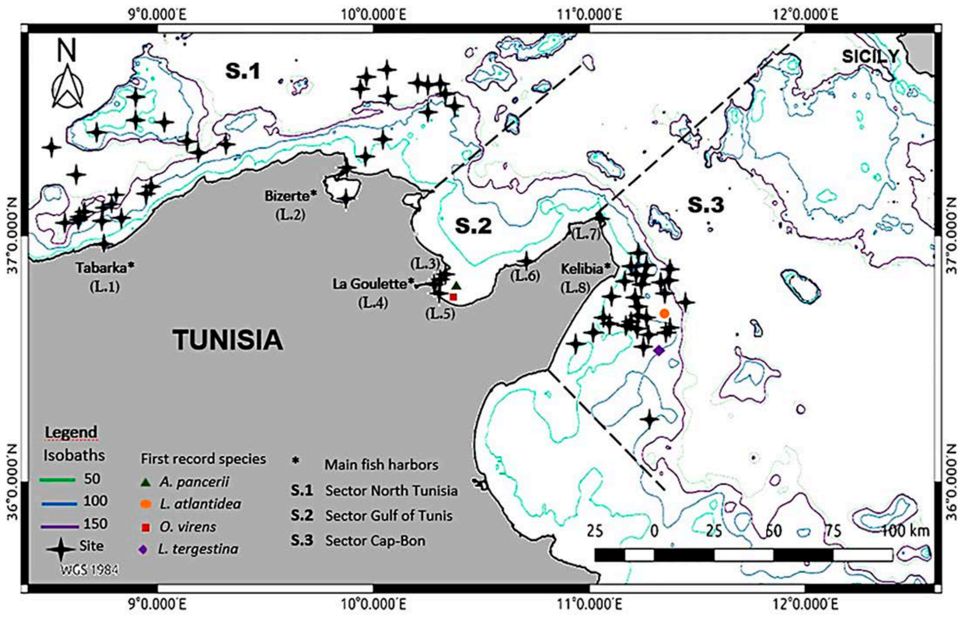

Fig. 1. – Map of the locations (L.) in northern Tunisian waters sampled for echinoderms, also showing the sites of new records: *Asterina pancerii* (green), *Luidea atlantidea* (orange), *Ophiactis virens* (red), *Leptopentacta tergestina* (purple).

Table 1. – The checklist of Echinodermata of northern Tunisia. M, Mediterranean Sea; A, Atlantic Ocean; ME, Mediterranean endemics; C, cosmopolitan; R, Red Sea; A, Algae; Cy, *Cymodocea*; G, gorgonian; S, sandy bottom; M, muddy bottom; R, rocky bottom; *, first records of species; Abundance, total number of individuals; Location (L) from Figure 1.

Taxa	Distribution	Depth range (m)	Habitat	Abundance	Location (L)
Class CRINOIDEA					
Family Antedonidae					
Antedon bifida (Pennant, 1777)	M, A	50-190	S, M, R	50	1, 2, 8
Antedon mediterranea (Lamarck, 1816)	ME	50-190	S, M, R	68	1, 2, 8
Leptometra phalangium (Müller, 1841)	M, A	72-194	S, M	33	1, 8
Class ASTREROIDEA					
Family Asteriidae					
Coscinasterias tenuispina (Lamarck, 1816)	M, A	20-51	S	2	2, 8
Marthasterias glacialis (Linnaeus, 1758)	M, A	75-220	S, M, R	11	2, 8
Family Asterinidae					
Anseropoda placenta (Pennant, 1777)	M, A	185-220	S	1	8
Asterina gibbosa (Pennant, 1777)	M, A	0.45-0.65	R, A	13	3, 5
Asterina pancerii (Gasco, 1876) *	ME	3-5	Cy	3	4
Family Astropectinidae					
Astropecten aranciacus (Linnaeus, 1758)	M, A	51-177	S, M	24	2, 8
Astropecten bispinosus (Otto, 1823)	M, A	1-35	S, M	11	2, 3, 4
Astropecten irregularis (Pennant, 1777)	M, A	50-220	S	33	1, 2, 8
Astropecten jonstoni (Delle Chiaje, 1827)	ME	3-5	S	1	4
Tethyaster subinermis (Philippi, 1837)	M, A	50-220	S, M	39	1, 2, 8
Family Chaetasteridae					
Chaetaster longipes (Retzius, 1805)	M, A	70-170	S, M	21	1, 2, 8
Family Echinasteridae					
Echinaster (*Echinaster*) *sepositus* (Retzius, 1783)	M, A	3-220	S, M, R	96	1, 2, 8
Family Luidiidae					
Luidia atlantidea Madsen, 1950 *	A	65-95	S	1	8
Luidia sarsii sarsii Düben and Koren in Düben, 1844	M, A	175-193	M	1	1
Family Ophidiasteridae					
Hacelia attenuata Gray, 1840	M, A	70-85	R	1	1
Class OPHIUROIDEA					
Family Amphiuridae					
Amphipholis squamata (Delle Chiaje, 1828)	C	0.4-0.6	A	16	5
Family Gorgonocephalidae					
Astrospartus mediterraneus (Risso, 1826)	M, A	98-105	S	2	8
Family Ophiacanthidae					
Ophiacantha setosa (Bruzelius, 1805)	M, A	70-165	G	54	1
Family Ophiactidae					
Ophiactis savignyi (Müller and Troschel, 1842)	C	3-5	S	1	5
Ophiactis virens (M. Sars, 1859) *	M, A	0.4-0.6	A	184	5
Family Ophiocomidae					
Ophiocomina nigra (Abildgaard in O.F. Müller, 1789)	M, A	50-58	M	1	2
Family Ophiodermatidae					
Ophioderma longicauda (Bruzelius, 1805)	M, A	0.65	R, A	1	7
Family Ophiomyxidae					
Ophiomyxa pentagona (Lamarck, 1816)	M, A	50-210	S, M, R	81	2, 8
Family Ophiotrichidae					
Ophiothrix quinquemaculata (Delle Chiaje, 1828)	ME	72-175	S, M, R	21	2
Family Ophiuridae					
Ophiura ophiura (Linnaeus, 1758)	M, A	3-194	S, M, R	124	1, 2, 4, 8
Class ECHINOIDEA					
Family Arbaciidae					
Arbacia lixula (Linnaeus, 1758)	M, A	0.25-5	S, R	12	1, 2, 7
Family Cidaroidae					
Cidaris cidaris (Linnaeus, 1758)	M, A	50-220	S, M, R	114	1, 2, 8
Stylocidaris affinis (Mortensen, 1909)	C	50-220	S, M, R	114	1, 2, 8
Family Diadematidae					
Centrostephanus longispinus (Philippi, 1845)	M, A	50-220	S, M	74	2, 8
Family Echinidae					
Gracilechinus acutus Lamarck, 1816	M, A	50-125	S, R	18	8
Family Parechinidae					
Paracentrotus lividus (Lamarck, 1816)	M, A	0.2-6	S, R	48	1, 2, 7
Family Spatangidae					
Spatangus purpureus (O.F. Müller, 1776)	M, A	3-5	S	1	8
Family Toxopneustidae					
Sphaerechinus granularis (Lamarck, 1816)	M, A	0.6-5	R, A	2	7
Class HOLOTHUROIDEA					
Family Cucumariidae					
Hemiocnus syracusanus (Grube, 1840)	M	3-5	S	1	4
Leptopentacta elongata (Düben and Koren, 1846)	M, A	77-145	S	1	8
Leptopentacta tergestina (M. Sars, 1857) *	ME	77-145	S	3	8
Family Holothuriidae					
Holothuria (*Holothuria*) *mammata* Grube, 1840	ME	3-8	S, R, A		2
Holothuria (*Holothuria*) *tubulosa* Gmelin, 1791	M, A, R	0.2-185	S, M, R, A	40	1, 2, 7
Holothuria (*Platyperona*) *sanctori* Delle Chiage, 1823	M, A, R	0.2-0.4	R, A	8	7
Holothuria (*Roweothuria*) *poli* Delle Chiaje, 1824	M, A, R	0.2-8	S, R, A	16	2, 7
Holothuria (*Thymiosycia*) *impatiens* (Forsskål, 1775)	C	0.45	R	1	6
Family Stichopodidae					
Parastichopus regalis (Cuvier, 1817)	M, A	0.2-194	S, M, R	58	1.8

anatomy following the taxonomic criteria of Mortensen (1927), Tortonese (1965), Koehler (1969) and Clark and Downey (1992). The nomenclature followed the World Register of Marine Species (WoRMS Editorial Board 2019). Sometimes, morphological characters can be ambiguous. For example, spicules of some of our individuals of sea cucumber from the genus *Holothuria* showed confusing anatomical and morphological characteristics. In fact, buttons of our individuals of *Holothuria* [*Holothuria (Holothuria) tubulosa* (Gmelin, 1791) and *Holothuria (Roweothuria) poli* (Delle Chiaje, 1824)] were twisted, which is a typical button characteristics of the eastern Atlantic species *Holothuria (Vaneyothuria) lentiginosa* (Marenzeller von, 1892) (Miller and Pawson 1979). The same species was cited in the Alboran Sea by Pérez-Ruzafa and López-Ibor (1988). In these cases, for the determination and identification of individuals, morphological studies were completed with genetic analyses.

Molecular analysis

Samples. To clarify the taxonomic status of these individuals, a genetic analysis was performed on 28 samples from the genus *Holothuria,* including the 17 doubtful specimens from northern Tunisia, in addition to 10 specimens of *H. poli* and *H. tubulosa* from Spain and a single specimen of *H. lentiginosa* from the Canary Islands from the collection of Dr Angel Pérez-Ruzafa at the Department of Ecology and Hydrology of the Faculty of Biology (University of Murcia). We used as an outgroup taxon 6 individuals of *H. (Panningothuria) forskali* Delle Chiaje, 1823 and *H. (Platyperona) sanctori* Delle Chiaje, 1823. The sequences were taken from Genebank (GenBank accession numbers GQ214761-GQ214762, EU220819, KY774322, GQ214763-GQ214764).

DNA extraction, PCR amplification and sequencing. DNA was extracted from 15-25 mg of muscle tissue of holothurian samples, which was conserved in ethanol following the standard protocol of Sambrook et al. (1989). Only the mitochondrial gene subunit I of cytochrome oxidase (COI) (*ca.* 650 bp) was amplified. The primers used for the amplification were CO1eI 5'ATAATGATA GGAGGRTTTGG 3' and CO1eII 5'GCTCGTGTRTCTACRTCCAT 3' (Palumbi 1996, Borrero-Pérez et al. 2009). Amplifications were carried out in a12 μL final volume of reaction mixture containing 1.2 μL of 10× buffer (Biotools), 0.6 μL MgCl2 (50Mm), 0.24 μL dNTP (10 mM), 0.6 μL of each primer (10 μM), 0.6 μL BSA (20 mg/ml), 0.1 μL of Taq DNA polymerase (5U/ μL) (Biotools) and 1 μL of genomic DNA (10 ng/uL). The complete PCR cycle was 94°C for 3 minutes, then 40 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 20 s, followed by a 20 min final extension time at 72°C (Uthicke et al. 2005). PCR products were visualized on 1% agarose gels. Purified DNA was sequenced at the Molecular Biology section of the Research Support Service at the University of Murcia (Spain) using Big Dye Terminator Cycle Sequencing v. ABI Prism 310 technology (Applied Biosystems).

Phylogenetic reconstruction. Twenty-eight sequences of 500 bp were edited and aligned using ClustalW as a default alignment parameter of the MEGA program, version 7 (Kumar et al. 2016). The DNA sequences were analysed to conduct a neighbour-joining tree using MEGA version 7 (Kumar et al. 2016). Pairwise nucleotide distances were calculated using the Kimura 2-parameter (K2P) model of base substitution (Kimura 1980).

Samples of the collected martial are deposited in the zoology collection of the University of Murcia (UMCZ).

RESULTS

Faunal diversity

Forty-five echinoderm species were collected and identified in northern Tunisia waters (Table 1). They belonged to the five classes of Echinodermata and comprised three sea lilies (Crinoidea), 15 starfishes (Asteroidea), 10 brittle stars (Ophiuroidea), 8 sea urchins (Echinoidea) and 9 sea cucumbers (Holothuroidea). They were divided into 32 genera and 27 families.

All the inventoried species are present in the Mediterranean Sea, except for the starfish-*Luidia atlantidea* (Madsen, 1950), which is an Atlantic species recently recorded in the Alboran Sea (Gallardo-Roldán et al. 2015).

Six of the collected species are endemic in the Mediterranean Sea, namely: *Asterina pancerii* (Gasco, 1876), *Astropecten jonstoni* (Delle Chiaje, 1827), *Holothuria (Holothuria) mammata* (Grube, 1840), *Leptopentacta tergestina* (Sars, 1857), *Ophiothrix quinquemaculata* (Delle Chiaje, 1828) and *Antedon mediterranea* (Lamarck, 1816). Four others have a wide distribution and are cosmopolitan: *Amphipholis squamata* (Delle Chiaje, 1828), *Ophiactis savignyi* (Müller and Troschel, 1842), *Holothuria (Thymiosycia) impatiens* (Forsskål, 1775) and *Stylocidaris affinis* (Mortensen, 1909).

Four collected species were first records for Tunisia: *Asterina pancerii* (Gasco, 1876), *Luidia atlantidea* (Madsen, 1950), *Ophiactis virens* (Sars, 1859) and *Leptopentacta tergestina* (Sars, 1857). Two are exclusively Mediterranean species (*Asterina pancerii* and *Leptopentacta tergestina*), and one is an Atlantic species (*Luidia atlantidea*) (Fig. 2)

New occurrences

The new species recorded for the first time in the present work (Fig. 3, Table 1) are two asteroids (*Asterina pancerii, Luidia atlantidea*), one ophiuroid (*Ophiactis virens*) and one holothurian (*Leptopentacta tergestina*) belonging to three Echinodermata classes.

Class ASTEROIDEA Blainville, 1830
Order VALVATIDA Perrier, 1884
Family ASTERINIDAE Gray, 1840
Genus *Asterina* Nardo, 1834

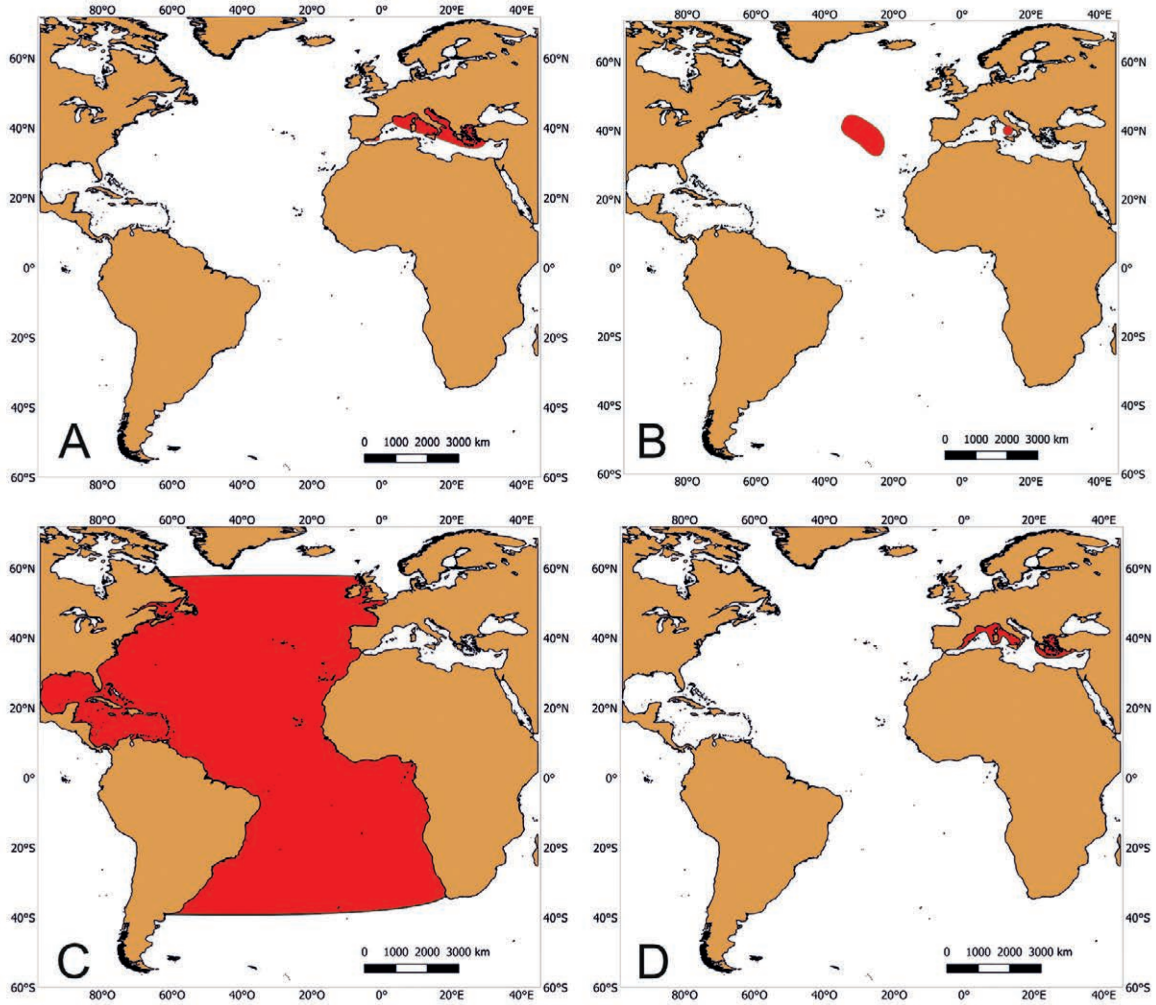

Fig. 2. – Maps of the geographic distribution (in black) of the species newly recorded in Tunisia: *Leptopentacta tergestina* (A), *Ophiactis virens* (B), *Luidea atlantidea* (C) and *Asterina pancerii* (D) (according to WoRMS, and López-Márquez et al. 2018, for *Asterina pancerii*).

Asterina pancerii (Gasco, 1876)
Figs 2D, 3A-B

Asteriscus pancerii Gasco 1870: 86-90. Gasco 1876: 38-40
Asterina gibbosa var. *panceri* Khohler 1924: 133-134
Asterina pancerii Tortonese 1965: 172-173. Oliver et al. 1997: 103-107. Tanti and Schembri 2006: 163-165.

Diagnosis. Flat body with a noticeable pentagonal shape; five rays, short and rounded with two or three papulae; abactinal plates close to each other and covered by spinelets; actinal gonopore are present; subambilacral and supactinal plates are absent; skeletal plates are few and large; actinal plates are distinct with three actinal spines per plate; numerous suboral spines with three usually tending to form a row parallel to the oral furrow spines.

Description. A very small starfish, it is pentagonal in shape and has several colours (brick red or purple, green, olive green or blue) (Tortonese 1965, Oliver et al. 1997). Its diameter does not exceed 15 mm. It has a flat form, with no superambulacral and superactinal plates. It has three suboral spines and gonopores on the ventral side (Clark and Downey 1992).

Examined material. Three specimens. Sector and location: Gulf of Tunis (S.2/L.4). Depth: 3-5 m. Substrates: associated with seagrass *Cymodocea nodosa* (Ascherson, 1870) (Table 1).

Distribution. Mediterranean Sea. It has been reported in several Mediterranean regions: France (Tortonese 1965), Murcia (Galán et al. 1982) and Mallorca (Oliver et al. 1997) in Spain, Athens (Tortonese 1965), Turkey (Özaydın et al. 1995) and Tripoli (Tortonese 1965). The species has been recorded in several localities in Spain, including Ibiza and Mallorca (Ballesteros et al. 1987, Oliver et al. 1997), Almeria, Murcia and Alicante (Luque and Templado 2004, Moreno et al. 2008). Recently López-Márquez et al. (2018) provided molecular evidence that the morphological identification of the specimens of *Asterina pancerii* from Alicante is incorrect and corresponds to *A. phylactica* (Emson and Crump, 1979).

Remarks. Asterina pancerii is a very small asteroid. Its morphology, which is extremely similar to that of juvenile *Asterina gibbosa* (Pennant, 1777), has led some authors (Hattour and Ben Mustapha 2015) to re-

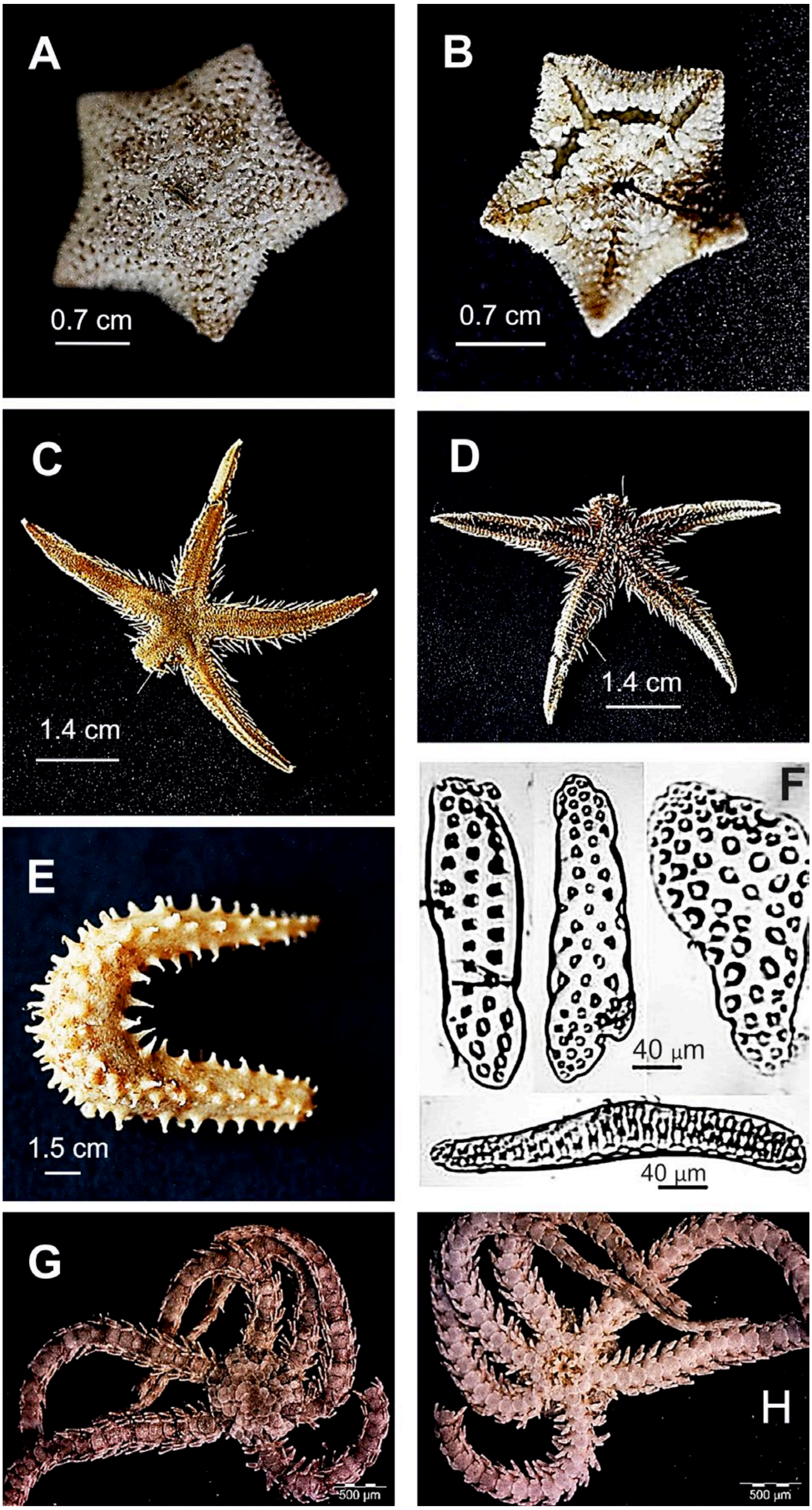

Fig. 3. – *Asterina pancerii* aboral view (A) and oral view (B); scale bar 1 cm. *Luidea atlantidea* aboral view (C) and oral view (D); scale bar 1 cm. *Leptopentacta tergestina* lateral view (E); scale bar 0.5 cm. Body wall ossicules of *Leptopentacta tergestina* (F); scale bar 0.5 cm. *Ophiactis virens* aboral view (J) and oral view (H).

port its presence in Tunisian waters (Gulf of Gabès). However, it was not cited in the final checklist of the same study.

Order PAXILLOSIDA Perrier, 1884
Family LUIDIIDAE Sladen, 1889
Genus *Luidia* Forbes, 1839
Luidia atlantidea Madsen, 1950
Fig. 2C, 3C-D

Luidia africana Doderlein 1920: 288-289 [Non *L. africana* Sladen, 1889]
Luidia atlantidea Madsen 1950: 192-198. Nataf and Cherbonnier 1973: 76-80. A.M. Clark and Downey 1992: 10-11.

Diagnosis. Flat body with five long thin arms; rays not very robust and narrow; abactinal paxillae with two marginal longitudinal series on each side with a white colour; coarser spinelets; the number of supermarginal paxillae is around 15 to 20, with rounded and flattened shape; lateral alignment of inferomarginal plates with two or three large and erect spines; marginal spines with dark base and white tips; presence of large pedicellaria on furrow face of each oral plate.

Description. It has five long, flattish arms with the presence of a marked main line of paxillae, arranged longitudinally (Clark and Downey 1992, Gallardo-Roldán et al. 2015). Central spinelets are distinctly coarser than peripheral ones. Supermarginal paxillae are rounded. Abactinal paxillae with two matching longitudinal lateral series on each side. Adambulacral plates with three large spines in a line at right-angles to the furrow. The central spinelets are distinctly coarser than the peripheral ones. Colour is grey with a white stripe along the supermarginal paxillae, white below, with dark purple marginal spines and white tips (Clark and Downey 1992). The diameter is about 6 cm.

Examined material. One specimen. Sector and location: Cape Bon (S.3/L.8). Substrates: Sand. Depth: 65-95 m (Table 1).

Distribution. Atlantic Ocean. It is present along the Atlantic coast from Morocco to Zaire, including the Cape Verde Islands (Clark and Downey 1992, Entrambasaguas 2008).

Remarks. The genus *Luidia* is represented by two species in the Mediterranean Sea: *L. sarsii sarsii* (Düben and Koren in Düben, 1844) and *L. ciliaris* (Philippi, 1837) (Cherbonier 1956, Tortonese 1965, Koehler 1969). The main difference between these two Mediterranean species is the number of arms: more than five in *L. ciliaris* (Cherbonier 1956, Tortonese 1965, Koehler 1969). In addition, *L. atlantidea* differ from *L. sarsii sarsii* in the number of lateral paxillae (more than 17 for *L. sarsii sarsii*) and the central and peripheral spinelets, which are uniform (Clark and Downey 1992).

Class OPHIUROIDEA Gray, 1840
Order OPHIURIDA Müller and Troschel, 1840
Family OPHIACTIDAE Matsumoto, 1915
Genus *Ophiactis* Lütken, 1856
Ophiactis virens (M. Sars, 1859)
Figs 2B, 3G-H

Amphiura virens Sars 1859: 95.
Ophiactis virens Simroth 1876: 417-485. Koehler, 1924: 294. Tortonese 1965: 238-239.

Diagnosis. Small brittle star with six long, thin arms; small disc, rounded and convex, covered by small irregular plates; peripheral plates have a very short and conical spinelet; six triangular radial shields, very small, more or less sunken and distally joined; two mouth papillae; four radial spines; dorsal plates of arms very broad; no genital slits.

Description. It is a very small brittle star, with a disc diameter of 3-5 mm, characterized by the presence of six arms (Tortonese 1965, Koehler 1969). Disc colour is a yellowish-grey or is greenish with darker spots (Koehler 1924, 1969, Tortonese 1965). Dorsal disc is covered by plates with six triangular radials shields. Two mouth papillae on each side of jaw with four small arm spines. Dorsal plates of arms are very broad and without genital slits (Mortensen 1927, Koehler 1969).

Examined material. 184 individuals. Sector and location: Gulf of Tunis (S.2/L.5). Depth: 0.40-0.60 m. Substrates: Algae (Table 1).

Distribution. Atlantic Ocean and Mediterranean Sea. It has been recorded from the west coast of Africa to the archipelagos of Azores, Madeira, Cape Verde and the Gulf of Gascony (Marques 1980, Entrambasaguas 2008), Italy (Koehler 1924, Tortonese 1965), and Turkey (Özaydın et al. 1995, Öztoprak 2014).

Remarks. *Ophitactis virens* is morphologically close to *Ophiactis savignyi* (Müller and Troschel, 1842), which is a cosmopolitan species characterized by the absence or the presence of one or two papillae, oral shields with rounded edges and five thorny arm spines (Clark 1918).

Class HOLOTHUROIDEA Brin, 1860
Order DENDROCHIROTIDA Grube, 1840
Family CUCUMARIIDAE Ludwig, 1894
Genus *Leptopentacta* Clark, 1938
Leptopentacta tergestina (Sars, 1857)
Figs 2A, 3E-F

Cucumaria incurvata R. Perrier 1886: 497.
Cucumaria tergestina Sars M. 1859: 127. Koehler 1924: 158-160.
Trachythyone tergestina (Sars, 1857) Panning 1949: 426. Tortonese 1965: 83-85.

Diagnosis. Small species with a curved body; ambulacral feet are small, rigid, pointed and conical; they are arranged in two parallel rows; spicules are large and have an irregular shape; perforated plates which are large and irregular (30-50 μm) with numerous perforations; irregular and curved rods.

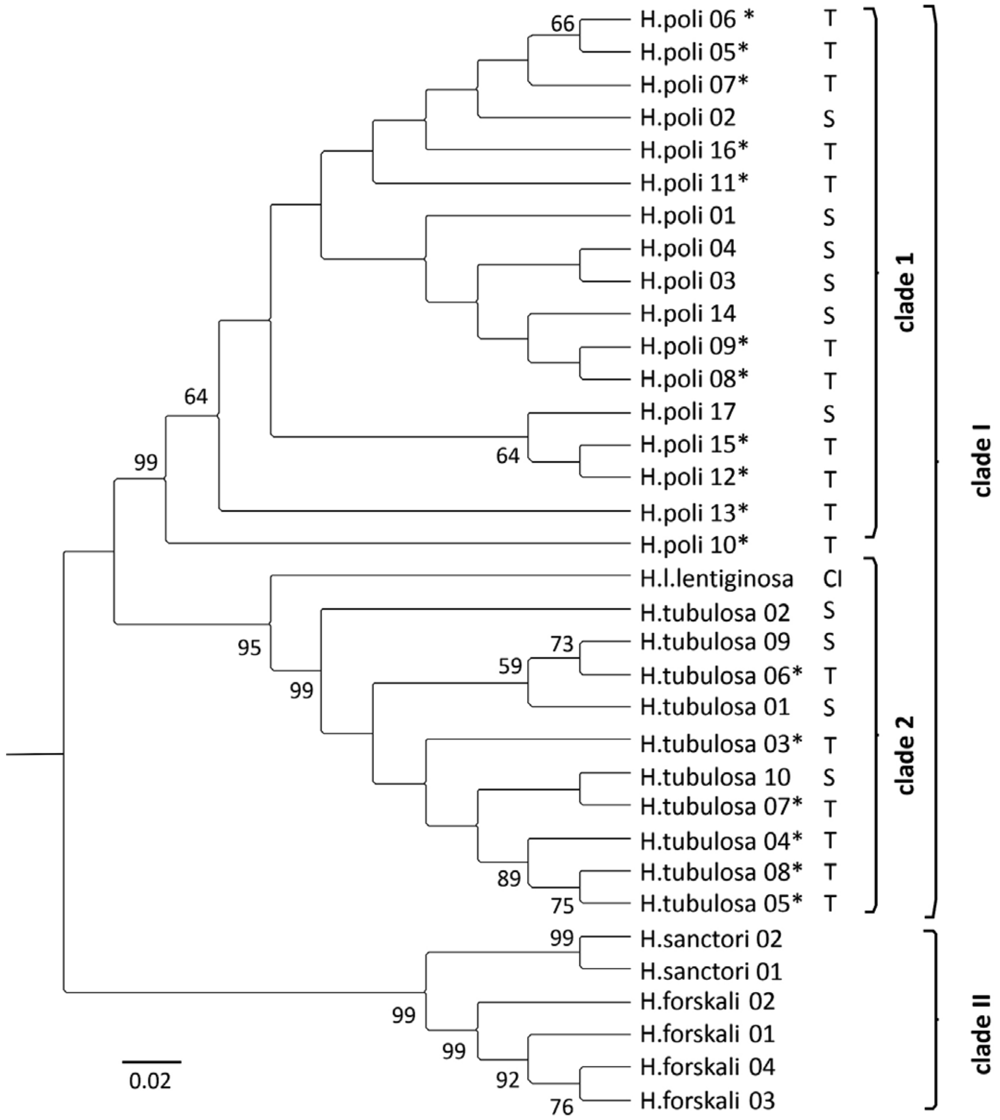

Fig. 4. – DNA sequence analysis of sea cucumbers from the genus *Holothuria.* Neighbour-joining tree analysis of 500 bp COI fragments based on p-distance. The bootstrap consensus tree inferred from 10000 replicates. Only bootstrap value branches exceeding 50% are indicated. The p distances were computed using the Kimura 2-parameter method and they were in the units of the number of base substitutions per site. Analyses were conducted in MEGA7. H, *Holothuria*; *, doubtful species; T, Tunisia; S, Spain; C-I, Canary Islands.

Description. This species has a curved U-shaped body and is between 5 and 7 cm long. It is usually a brownish-yellow colour (Tortonese 1965, Koehler 1969). Spicules of body have the form of large and elongated plates pierced with many holes, accompanied by irregular knobbed buttons and smooth elongated rods.

Examined material. Three specimens. Sector and location: Cape Bon (S.3/L.8). Depth: 77-145 m. Substrates: Sand (Table 1).

Distribution. Mediterranean Sea. It has been reported at many sites along the Italian coast (Koehler 1924, Tortonese 1965) and in France (Koehler 1924, Tortonese 1965), Spain (Tortonese 1965), Turkey (Özaydın et al 1995, Öztoprak 2014) and the Maltese Islands (Tanti and Schembri 2006).

Remarks. L. tergestina may have been confused with *L. elongata* (Düben and Koren, 1846), which very often has the same shape and colour. The main difference between these two species is the form of spicules.

Molecular study

Genetic analysis identified the doubtful specimens of the genus *Holothuria* as the species *H. poli* and *H. tubulosa,* while neighbour-joining analysis showed 28 monophyletic lineages supported by a high bootstrap value (99%). The molecular analyses favour the subdivision of all the group taxa into two major clades: Clade I and Clade II (Fig. 4). Clade I is divided into two subclades: Clade (1) and (2), both highly supported. Clade (1) comprises only members of *H. poli* from Mediterranean locations (Tunisia and Spain), with a high bootstraps value (99%). However, Clade

Table 2. – Genetic distances between the three *Holothuria* species obtained from the phylogenetic reconstruction based on the Kimura two-parameter model (K2P).

	H. tubulosa	*H. sanctori*	*H. poli*	*H. lentiginosa lentiginosa*	*H. forskali*
H. tubulosa	-				
H. sanctori	0.209	-			
H. poli	**0.165**	0.223	-		
H. lentiginosa lentiginosa	**0.092**	0.192	**0.157**	-	
H. forskali	0.260	0.189	0.259	0.249	-

(2) also gains high support (95%) and comprises both specimens of *H. lentiginosa lentiginosa* with an Atlantic origin (Canary Islands) and the Mediterranean specimens *H. tubulosa* (Spain and Tunisia).

Clade II, comprising the outgroup species *H. forskali* and *H. sanctori*, was separated from Clade I with high bootstraps values (99%).

The K2P distances, based on COI sequences, are shown in Table 2. The highest divergence distance was found between *H. poli* and *H. tubulosa* (16.5%) and the lowest between *H. tubulosa* and *H. lentiginosa lentiginosa* (9.2%). The distance between *H. poli* and *H. lentiginosa lentiginosa* (15.7%) was very close to that between *H. poli* and *H. tubulosa* (16.5%).

DISCUSSION

Species first record

The Asteroidea are characterized by two new findings: *Asterina pancerii* and *Luidia atlantidea*. The starfish *Asterina pancerii* is an endemic species of the Mediterranean Sea (Tortonese 1965). According to Annex II of the Bern Convention in the protocol of Specially Protected Areas and Biological Diversity in the Mediterranean Sea from the Barcelona Convention and the Spanish Catalogue of Threatened Species (López-Márquez et al. 2018), it is listed as an endangered and protected species in the Mediterranean Sea. *Asterina pancerii* was found for the first time in Tunisia in northern inshore waters (3-5 m). However, several authors, including Ballesteros et al. (1987), Oliver et al. (1997) and López-Márquez et al. (2018), have reported that this species is typical of *Posidonia oceanica* ((Linnaeus) Delile, 1813) meadows. The only specimens of *A. pancerii* found so far in Tunisia were associated with *Cymodocea nodosa* beds.

A single *Luidia atlantidea* specimen was collected for the first time in the northeastern Tunisian Sea (Cape Bon, East Mediterranean Sea) by trawl-fishing gear at a depth of 65-95 m. *Luidia atlantidea* is an Atlantic species. It was recently found and reported for the first time in the Mediterranean Sea, in the northern Alboran Sea (western Mediterranean Sea) (Gallardo-Roldán et al. 2015), 31 individuals being collected by mechanized dredging performed at depths of between 0.9 and 11.6 m (Gallardo-Roldán et al. 2015). The present report on *Luidia atlantidea* is the first in Tunisia and the second in the Mediterranean Sea.

We report new findings of the ophiuroid *Ophiactis virens*, an eastern and northern Atlantic species. In the Mediterranean Sea, it has been so far reported only in Naples (Koehler 1924, Tortonese 1965) and in the Turkish Levantine Sea (Özaydın et al. 1995, Öztoprak 2014). Over 184 specimens were found for the first time off the northeastern coast of Tunisia (Gulf of Tunis), at a depth of 40-60 cm. The presence of diverse *Ophiactis virens* individuals over several years (from 2012 to 2015) may indicate the persistence of a local population on the shallow circalittoral Tunisian coast, as this species is well known for its asexual reproduction and fission of its body into two equal parts (Wilkie 1984). The presence of *Ophiactis virens* is the first to be reported in Tunisia and the third in the Mediterranean Sea.

Holothuroidea is represented by one new record for the Tunisian Sea: *Leptopentacta tergestina*. This sea cucumber is an endemic Mediterranean species (Koehler 1924, Tortonese 1965). In Tunisia, three individuals were found off Cape Bon (northeastern Tunisia). The *Leptopentacta tergestina* specimens were collected by commercial trawling at depths of between 60 and 150 m.

Two species of the Ophiuroidea class, *Astrospartus mediterraneus* (Risso, 1826) and *Ophiacantha setosa* (Bruzelius, 1805), were found and reported for the second time in this present study after the first finding by Cherbonnier (1956). More than 30 *Ophiacantha setosa* specimens were found associated with the yellow gorgonian, *Eunicella cavolini* (Koch, 1887), close to the Algerian deep sea border. Because of its evasiveness, *Ophiacantha setosa* is recorded for the second time in Tunisia in this study.

Species diversity

The echinoderms recorded from northern Tunisian marine water in this study are quite diverse (45 species). Among the recorded groups, Asteroidea were the most diverse, with 15 species, followed by Ophiuroidea (10 species), Holothuroidea (9), Echinoidea (8) and Crinoidea (3). This can be explained by the techniques and gears used to sample them (hand collection, dredging, trawling and diving). Accordingly, the present research method increased the collection area by covering the marine benthic zones of the Mediterranean Sea, from the infralittoral level to the bathyal level.

Most of the new recorded species (*Asterina pancerii, Luidia atlantidea* and *Ophiactis virens*) were found in the northeastern Tunisian Sea (Cape Bon peninsula), close to the Strait of Sicily, which marks the transition between the two major western and eastern Mediterranean basins (Boudouresque 2004, Coll et al. 2010). This result confirms the importance of the Strait of Sicily as a highly primary production area with a wide range of biodiversity due its moderate depth, hydrography and diversity of habitat types (Bianchi and Morri 2000, Lejeusne et al. 2010). It is one of the biodiversity hotspots in the Mediterranean Sea (Lejeusne et al. 2010, Coll et al. 2010).

A review of the relevant literature of megabenthic Tunisian inventories, including the Echinodermata phylum, by Le Danois (1925), Cherbonnier (1956), Lubet and Azouz (1969), Azouz (1971, 1973), Ben Othman (1973), Boudouresque (1997), Anonymous (1997) and El Lakhrach et al. (2012) shows the presence of 73 valid species in Tunisia. The present work increases the number of echinoderms to 77, with four new occurrences in Tunisian marine waters.

Northern Tunisia alone (from the Algerian-Tunisian border to Ras Kapudia) showed the highest number, with 69 species against 61 in the south (from Ras Kapudia to the Libyan border, including the Gulf of Gabès). However, some species present in the northern part are absent in the south and vice versa (Ben Othman 1973, Boudouresque 1997, El Lakhrach et al. 2012). Some previously recorded species were not found in the present work, since the adopted methodology and fishing gears depend on the depths frequented by fishermen.

Little research has been done on Echinodermata in deep Mediterranean waters (Koukouras et al. 2007, Coll et al. 2010), and the knowledge gap includes especially the north African coast of the Maghreb (Dauvin et al. 2013).

Echinodermata marine biodiversity along the Algerian coast, from the Moroccan border to the Tunisian border, is very low compared with that in northern Tunisia, with 48 species being recorded in Algeria (Dauvin et al. 2013). According to Koukouras et al. (2007), about 144 echinoderms are known from the western Mediterranean Sea, only 53.5 % of which have been found in Tunisia. On the other hand, Tunisia shares over 83.7 % of a total of 91 echinoderms reported from the central Mediterranean Sea.

These findings confirm the importance of northern Tunisia area, which emerging a large number of exotic marine species and a high rate of endemic species (Ayari and Afli 2003, Ounifi Ben Amor et al. 2016). Indeed, there are more endemic species in the western part of the Mediterranean and the number of non-native species entering through the Suez Canal in the eastern basin and the Strait of Gibraltar in the western basin has increased spectacularly since the early 20th century (Boudouresque 2004, Zenetos et al. 2010, Ben Souissi et al. 2011). Most have been introduced by maritime transport.

Overall, the present work enhances the importance of the studied fauna in northern Tunisia. To maintain the diversity of echinoderms in Tunisia's marine waters, it is necessary to promote efforts and acquire knowledge about this macrobenthic group by involving southern and eastern Tunisia.

Systematic and molecular

Systematic studies based on taxonomical and anatomical criteria have often been confusing and doubtful because of the large morphological similarity between species. Many authors have been involved in research on systematic identification and/or revision of the taxonomical status of different classes of Echinodermata and have provided molecular evidence to support their findings (Borrero-Pérez et al. 2009, Laakman et al. 2016, López-Márquez et al. 2018).

For the class Holothuroidea, Borrero-Pérez et al. (2009) evaluated the taxonomic status of some Atlanto-Mediterranean species of the subgenus *Holothuria* using molecular analysis and showed that the combination of the two approaches may solve the taxonomical problems associated with species identification, as was the case with *H.* (*Holothuria*) *stellati* Delle Chiaje, 1824 and *H. tubulosa*. The same authors confirmed the morphological variability in the specimens of *H. stellati* and *H. tubulosa*, as mentioned in the literature, but their molecular results showed *H. stellati* to be a junior subjective synonym of *H. tubulosa.*

As regards our doubtful species, *H. poli* and *H. tubulosa*, the outcome of the phylogenetic neighbour-joining analysis showed a close relation between *H. tubulosa* and *H. lentiginosa lentiginosa*. Although *H. poli* and *H. tubulosa* are different species with different clades, the sequences of the sea cucumber *H. lentiginosa lentiginosa* were between those of the two holothurians, confirming the spicule similarity between the studied taxa.

In addition, the present study points to great morphological and molecular similarities between sea cucumbers from the Atlantic Ocean and the Mediterranean Sea. They were all characterized by elongated and twisted buttons. However, these characteristics are very common in *H. lentiginosa lentiginosa* species (Miller and Pawson 1979) and have recently been observed in *H. poli* and *H. tubulosa* individuals from the northern Tunisian Sea.

Though spicule morphology is an effective taxonomic character, it may show some overlap in some genera, such as the *Holothuria* genus and subgenus (Rowe 1969, Borrero-Pérez et al. 2009). This could be due to phylogenetic relationships between species that are still not well studied, or perhaps to environmental influences such as temperature, which could condition spicule formation and carbonate precipitation—another aspect worthy of study.

At present, the systematic position of the sea cucumbers of the genus *Holothuria* is dubious (Rowe 1969, Zavodnik 1999, Borrero-Pérez et al. 2009), so molecular and morphometric approaches are required if morphological identification is uncertain or impossible. Supported by ecological and biogeographical parameters, these techniques are a strong driving force in taxonomic study.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available through the online version of this article and at the following link:
http://scimar.icm.csic.es/scimar/supplm/sm04899esm.pdf

Table S1. – Collection sites and sampling methods of the echinoderms from different localities of northern Tunisia.
Table S2. – Samples name's and diameters. With the habitat type, depth (maximum and minimum) and date of collection of each sample.