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The Echinoderms of northern Tunisia: Biodiversity, Biology, Biogeography, Phylogeny and Valorisation

Los Equinodermos del norte de Túnez: Biodiversidad, Biología, Biogeografía, Filogenia y Valoración

Dña. Hayfa Chammem
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Dª HAYFA CHAMMEM
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i. RESUMEN

Túnez ocupa una posición biogeográfica estratégica en el mar Mediterráneo, entre las cuencas oriental y occidental y cerca del estrecho de Sicilia que se ha considerado como un límite biogeográfico que separa el mar Mediterráneo en dos ecorregiones diferentes. A pesar de su importancia, los estudios sobre la biodiversidad marina en Túnez y, en particular, en la fauna de equinodermos son antiguos y escasos. Además, los recursos biológicos marinos están expuestos a la sobreexplotación, contaminación y cambio climático, lo que lleva a la degradación de los ecosistemas y la pérdida de biodiversidad. Se llevó a cabo un estudio del filo Echinodermata entre 2012 y 2016 a lo largo de la costa norte de Túnez, en el que se estudia la ecología, la biología y la clasificación de los equinodermos que se dan en esta zona, con el fin de actualizar el inventario de equinodermos en Túnez, prevenir los impactos del cambio climático sobre esta fauna megabentónica y analizar las afinidades biogeográficas de este grupo con las regiones y las provincias vecinas. Además, se estudia la diversidad filogenética de las especies de pepinos de mar del género *Holothuria* de origen Atlanto-Mediterráneo, mediante la introducción de la herramienta molecular “DNA Barcoding” como técnica molecular para el pepino de mar y la identificación de divergencias genéticas de este género. También se analizaron las espículas de pepino de mar para probar su similitud morfométrica y molecular y aclarar su identificación sistemática.

Así que, el capítulo 2 de la presente tesis explora la biodiversidad de los equinodermos en el norte de Túnez y describe el material recolectado en las diferentes campañas de 2012 a 2016. Además, proporciona una lista de verificación validada de los equinodermos con la descripción morfológica y sistemática de todas las especies. También, actualiza el inventario del filo Echinodermata en Túnez. Las campañas se realizaron en las costas del norte de Túnez, desde la frontera con Argelia hasta la península de Cape-Bon. Se utilizaron varios métodos de muestreo, recolección manual con cuadrante para fondos menores a 1 m de profundidad, draga experimental para aguas someras menores a 50 m de profundidad, y arrastres de pesca bentónicos para profundidades superiores a 50 m. Además, el buceo se empleó para lugares restringidos con fondos duros. En total, se recolectaron 1430 individuos de las cinco clases actuales de equinodermos y solo 45 especies fueron inventariadas en el norte de Túnez. Por tanto, comprende 3 de la clase Crinoidea, 15 de la clase Asteroidea, 10 de la clase Ophiuroidea, 8 de la clase Echinoidea y 9 de la clase Holothuroidea, donde cuatro de las especies identificadas fueron los primeros registros para Túnez. Dos de ellas son especies exclusivamente mediterráneas (*Asterina pancerii* (Gasco, 1876) y *Leptopentacta tergestina* (M. Sars, 1857)), una es una especie atlántica (*Luidia atlantidea* (Madsen, 1950)) y la última es una especie cosmopolita (*Ophiactis virens* (M. Sars, 1857)). Otras seis especies aparecen por primera vez en el norte de Túnez. Estas son las tres estrellas del mar *Astropecten auranciacus* (Linnaeus, 1758), *Chaetaster longipe* (Retzius, 1805) y *Hacelea atenuar* (Gray, 1840), un pepino de mar Hemioconus syracusnus (Grube, 1840), las dos ofiuras *Ophiactis savignyi* (Müller y Troschel, 1842) y *Astrospartus mediterraneus* (Risso, 1826). De hecho, la ubicación geográfica de Túnez le confiere un estatus especial para los estudios de fauna marina. El presente trabajo ha aumentado el número de equinodermos registrados en Túnez, desde el primer inventario del filo Echinodermata realizado por Cherbonnier

(1956). Actualmente, es necesario impulsar esfuerzos y adquirir un conocimiento sólido sobre este grupo de invertebrados macrobentónicos, su configuración taxonómica y sus implicaciones ecológicas y socioeconómicas.

A continuación, el capítulo 3 analiza la población de equinodermos del norte de Túnez y define los factores ecológicos que explican su distribución cuantificando y calificando su composición faunística desde las aguas someras (0m) hasta el nivel batial (hasta 100m). Además, explora la relación entre los patrones biológicos y ecológicos de las especies estudiadas. En consecuencia, el análisis de MDS no detectó diferencias entre los tres sectores estudiados sino entre los sectores anidados de las localidades. Los mismos resultados también se confirmaron mediante pruebas por pares. Sin embargo, el análisis SIMPER reveló una diferencia significativa entre las localidades de (S1 = Norte de Túnez) y ninguna diferencia entre las localidades de (S2 = Golfo de Túnez). Al contrario, la distribución de las clases a lo largo de los diferentes niveles batimétricos muestra un aumento de la clase Asteroidea y una disminución de la clase Holothuroidea de los niveles superiores a los inferiores. En contraste, las clases Crinoidea y Ophiuroidea estuvieron ausentes o muy escasas en aguas someras. La distribución de equinodermos en el norte del mar de Túnez varía según la profundidad, que es el principal factor de agrupación de especies. En otra parte, la composición de equinodermos se modifica a través del gradiente batimétrico donde la media fue alrededor de 100 m. Finalmente, los estudios faunísticos y/o botánicos sobre la composición de la biodiversidad marina son fundamentales para comprender los patrones ecológicos de distribución de especies. El filo Echinodermata, que es bien conocido por su adaptabilidad y plasticidad a diversas condiciones ambientales, es uno de los componentes más dominantes y diversos de las comunidades marinas. El conocimiento de la biodiversidad marina y, en concreto, el de un grupo tan importante como los equinodermos, es de gran utilidad para la gestión del ecosistema marino y, por tanto, para identificar las prioridades de conservación desde tres vías distintas: la conservación de especies para proteger y salvar especies en peligro de extinción y sobreexplotación o para mantenerlo vivo y reintegrarse nuevamente en sus hábitats nativos. Además, aprovechando las especies con interés nutricional, ecológico y económico o luchando contra las especies invasoras y exóticas que tienen efectos penetrantes y disruptivos en el ecosistema.

Por otra parte, la clase Holothuroidea, conocida como pepino de mar, es uno de los grupos más diversos del filo Echinodermata. Tiene una amplia distribución mundial y se presenta desde la zona intermareal hasta las profundidades del océano y desde la zona polar hasta la tropical. En muchos casos, el género *Holothuria* fue objeto de discusión y su literatura estaba siempre en confusión, ya que su identificación morfológica sistemática basada en el "estudio de espículas" era dudosa e incierta. El capítulo 4 se centra en los análisis morfométricos y genéticos de especies de holoturia del género *Holothuria*, de origen atlántico y mediterráneo. Así que, evalúa la posición sistemática y la diversidad genética de ocho especies de pepinos de mar del género *Holothuria,* que son: *H. (Roweothuria) arguinensis* Koehler y Vaney, 1906, *H. (Holothuria) dakarensis* Panning, 1939, *H. (Vaneyothuria) lentiginosa lentiginosa* Marenzeller von, 1892, *H. (Holothuria) mammata* Grube, 1840, *H. (Roweothuria) poli* Delle Chiaje, 1824, *H. (Platyperona) sanctori* Delle Chiaje, 1823, *H. (Panningothuria) forskali* Delle Chiaje , 1823 y *H. (Holothuria) tubulosa* Gmelin, 1791. De hecho, investiga la variación morfométrica entre las especies estudiadas en base a la forma de las espículas, en particular sus características de botones y torres y discute su identificación sistemática de acuerdo a la similitud entre la morfometría y el análisis molecular. Por eso, se tomaron muestras de un total de 40 individuos del género *Holothuria* de varias localidades a lo largo del Océano Atlántico y el Mar Mediterráneo. Después, se generaron y compararon un dendrograma de similitud de análisis morfométricos y los árboles filogenéticos de ambos genes de mitocondriales, COI (Citocromo Oxidasa I) y 16S (ARN ribosómico). Los resultados obtenidos revelaron una concordancia en los dendrogramas filogenéticos y morfométricos en las especies del Atlántico y el Mediterráneo. Los análisis morfometrícos de espículas de los botones del pepino de mar mostraron una amplia variación morfológica entre las especies estudiadas. Sin embargo, no existe una diferenciación molecular geográfica entre las especies Atlánticas y Mediterráneas. Actualmente, el estatus de distribución geográfica del pepino de mar está cambiando, con respecto a la disminución tangible de la temperatura y los efectos del calentamiento global. Con respecto a la supervivencia y la adaptación a nuevos hábitats, la plasticidad genotípica y fenotípica de las especies ha aumentado.

Mientras que, el capítulo 5 evalúa la estructura genética de las especies objetivo (clave o diana) *Holothuria (Roweothuria) poli* (Delle Chiaje, 1824), en tres localidades diferentes del Mediterráneo occidental, oriental y central y de dos ecosistemas marinos diferentes: el mar abierto y la laguna, con el fin de investigar la filogeografía de esta especie en el mar Mediterráneo y establecer el potencial de sus poblaciones en Túnez. La misma especie mostró una alta diversidad filogeográfica en el Mar Mediterráneo, en particular una gran conectividad genética entre las cuencas del Mediterráneo central y oriental y una variabilidad genética entre el Mar Mediterráneo oriental y occidental. Además, reveló una gran diferenciación genética entre el ecosistema lagunar y el mar abierto. Los ecosistemas costeros, como las lagunas costeras, son diversos en Túnez y se extienden a lo largo de la costa tunecina estando conectados principalmente con el mar Mediterráneo (Bejaoui et al. 2008, 2010; Guetat et al. 2012). Son muy conocidos por su diversidad de fauna y flora y por importantes actividades pesqueras (Bejaoui et al. 2008; Ben Mustapha y Hattour 2016; Sellem et al. 2019). Además, las poblaciones de *H. poli* tienen una gran abundancia en este ecosistema y la pesca de pepino de mar es común en las lagunas costeras de Túnez. De hecho, las poblaciones de pepino de mar están expuesta a muchas perturbaciones ambientales, como las actividades industriales fuente principal de contaminación de aguas residuales y polución. Además de esto, estos ecosistemas en Túnez están sufriendo la presión de la pesca ilegal. Por ello, los pepinos de mar son vulnerables y requieren protección debido a la sobreexplotación y las actividades pesqueras ilegales (Ben Mustapha y Hattour 2016; Sellem et al. 2019). Comprender el flujo de genes de las poblaciones de holoturias es muy importante para evaluar el manejo de las especies de interés comercial y para aumentar el conocimiento sobre la ecología y la biología de las especies amenazadas. El conocimiento sobre la conectividad de las especies explotadas permite prevenir las consecuencias ecológicas de la sobrepesca y enfatizar la importancia del área marina protegida como medida de protección de las especies (Uthicke y Benzie 2001).

Finalmente, el último capítulo (capítulo 6) analiza la biogeografía del filo Echinodermata a lo largo de las costas tunecinas con las diferentes regiones y las provincias del Mar Mediterráneo y del Océano Atlántico. La diversidad de los

equinodermos se comparó por países y por clases. La lista de los equinodermos actualizada de los conjuntos de datos disponibles, proporcionó 79 especies en Túnez. Estas, fueron divididas en 18 órdenes y 38 familias. Entre los grupos registrados, la clase Asteroidea fue la más diversa con 22 especies, seguida de la clase Ophiuroidea (19 especies), la clase Holothuroidea (18 especies) y la clase Echinoidea (17 especies). La última clase es Crinoidea con solo 3 especies. En comparación con las regiones vecinas de la costa mediterránea del Magreb, el filo Echinodermata es bastante diverso en Túnez. El filo Echinodermata está desigualmente distribuido entre los tres golfos tunecinos: el golfo de Túnez al norte, el golfo de Hammamet al este y el golfo de Gabès al sur. La mayor riqueza de especies con 68 especies, se atribuyó a la zona norte, que representan el 84% del total de especies de Túnez, la mayoría tienen afinidades Atlanto-Mediterráneas con una sola especie atlántica (*Luidea atlantidea*). Sin embargo, solo 36 especies de la parte centro-este de Túnez representan el 51% del total y 63 especies en las costas del sur (82%). De hecho, los patrones biogeográficos mostraron que la riqueza de especies disminuyó del norte al sur del país y del oeste al este del Mediterráneo, con predominio de especies cosmopolitas en todas las regiones. La actualización de los equinodermos registrados en Túnez revela una importante diversidad en abundancia y riqueza de especies en comparación con la región y provincias vecinas. Actualmente, las costas tunecinas están bajo la colonización de especies de origen mixto. La ubicación de Túnez como unión entre las ecorregiones mediterráneas occidental y oriental, permite la transición de especies atlánticas en norte y migrante ''Lessipsian'' en el sur.

El uso de la técnica del ''DNA Barcoding'' en especies del filo Echinodermata parece un método muy confiable, rápido y eficiente para la identificación de especies, y es muy útil para el conocimiento biológico de las especies objetivo. Una de las ventajas de usar el gen COI es que los cebadores universales para este gen son muy robustos (Folmer et al. 1994; Zhang y Hewitt 1997). Muchas veces, y cuando la base de datos genética es coherente, esta técnica tiene ventajas en cuanto a precisión y exactitud para la identificación segura de especies en comparación con las observaciones morfológicas. Este método ha sido probado con éxito en diferentes organismos marinos, también en equinodermos (Jefri et al. 2015; Prehadi et al. 2015; Layton et al. 2016; Madduppa et al. 2016). La falta de información sobre el filo Echinodermata en Túnez es un vacío de conocimiento para la biodiversidad marina. En consecuencia, ampliar el conocimiento de su diversidad y distribución también permitirá una correcta gestión de su extracción y la valorización de sus productos y, en última instancia, su conservación y sostenibilidad.

ii. RÉSUMÉ

La Tunisie occupe une position stratégique dans Méditerranée, près du détroit de Sicile, qui est considéré comme une barrière biogéographique séparant les deux bassins méditerranéens occidental et oriental. Malgré son importance, les études sur la biodiversité marine en Tunisie et en particulier sur la faune Echinodermata sont très anciennes et peu nombreux. De même, les ressources marines sont en face de multiple de menaces tels que, la surexploitation, la pollution et les changements climatiques, qui ont entraîné la dégradation des écosystèmes marins et la perte de leur biodiversité.

Une étude sur cette faune benthique a été menée au nord de la Tunisie septentrionale entre les années 2012 et 2016. Ce travail a pour objectif de caractériser les échinodermes du nord tunisien sur le plan écologique, biologique et de classification taxonomique, afin d'actualiser l'inventaire d'Echinodermata en Tunisie et d'analyser leurs affinités biogéographiques avec les régions et provinces voisines. En outre, elle vise également à déterminer la diversité phylogénétique de différentes espèces de concombre de mer du genre *Holothuria* qui sont d'origine Atlantique et/ou Méditerranéen, en introduisant l'usage de la technique moléculaire, « le code barre ADN » (DNA Barcoding) comme un outil d'identification et de divergence génétique. Cette méthodologie a été également utilisée afin de tester la similarité entre la morphométrie des spicules d'holothuries et leur identité moléculaire, ainsi que de vérifier et d'évaluer leur classification systématique qui était toujours en confusion et discussion.

Au total, environ quarante-cinq espèces d'échinodermes ont été inventoriées. Ceux-ci appartenant aux 5 classes actuelles Echinodermata et comprenant 3 crinoides (Crinoidea), 15 étoiles de mer (Asteroidea), 10 ophiures (Ophiuroidea), 8 oursins de mer (Echinoidea) et 9 concombres de mer (Holothuroidea). Parmi ces espèces quatre taxa sont signalés pour la première fois en Tunisie dans ce présent travail. Deux espèces sont méditerranéennes (*Asterina* pancerii (Gasco, 1876) et *Leptopentacta tergestina* (M. Sars, 1857)), une troisième est exclusivement atlantique (*Luidia atlantidea* (Madsen, 1950)) alors que la dernière est une espèce cosmopolite (*Ophiactis virens* (M. Sars, 1857)). Par conséquence, les résultats génétiques des concombres de mer ont révélé une grande similitude et concordance à ceux de la morphométrie des spécules.

Alors que, pour l'holothurie comestible *Holothuria (Roweothuria) poli* (Delle Chiaje, 1824) on a décelé une grande diversité phylogéographique au sein de la Mer Méditerranée A l'égard d'une connectivité génétique entre les bassins central et oriental plus importante que celle des bassins oriental et occidental où on détecte une grande variabilité génétique. On a révélé également une grande différenciation génétique entre l'écosystème lagunaire et la mer. Finalement, la reactualisation de la liste des échinodermes à partir des données disponibles dans la bibliographie, a fourni 79 espèces pour la Tunisie. En outre, les analyses biogéographiques ont montré que la richesse spécifique des echinodermesdiminue du nord au sud du pays et de l'ouest à l'est de la mer Méditerranée avec une dominance des espèces cosmopolites dans toutes les régions.

Cependant, l'approche moléculaire du code barre ADN prouve une grande fiabilité, rapidité et efficacité pour l'identification des échinodermes, elle a permis une meilleure connaissance biologique des spécimens étudiés. La connaissance de notre richesse en faune Echinodermata est majeur pour la biodiversité marine tunisienne face aux changements climatiques et à la prolifération des espèces indigènes. Ainsi que, le savoir de cette biodiversité permet la bonne gestion et conservation de ces ressources marines ainsi qu'une meilleure valorisation de ses produits.

iii. ABSTRACT

Tunisia occupies a strategic biogeographic position in the Mediterranean Sea, between the eastern and western basins and close to the strait of Sicily which has been considered as a biogeographical boundary that separates the Mediterranean Sea in two differentecoregions. Despite its importance, studies on marine biodiversity in Tunisia and, in particular, in Echinodermata fauna are old and scarce. Besides, marine bioresources are suffering from overexploitation, pollution and climate change, leading to the ecosystem degradation and the biodiversity loss. A study of the phylum Echinodermata was carried out between 2012 and 2016 along the northern coast of Tunisia. It explores the ecology, biology and the classification of the echinoderms that occur in this area, in order to update the inventory of Echinodermata in Tunisia and to analyse their biogeographical affinities with neighbors' regions and provinces. Furthermore, it studies the phylogenetic diversity of sea cucumber species from genus *Holothuria* with Atlanto-Mediterranean origin, by introducing the molecular tool "DNA Barcoding" as a molecular technique for sea cucumber and their genetic divergence identification. Sea cucumber spicules were also analysed in order to test their morphometric and molecular similarity and to clarify their systematic identification since the genus *Holothuria* is, in many cases, under discussion and its literature is currently in confusion.

A total of 45 echinoderm species were inventoried in northern Tunisia. They belong to the five living classes of Echinodermata and comprise 3 Crinoidea, 15 Asteroidea, 10 Ophiuroidea, 8 Echinoidea and 9 Holothuroidea. Amongst all, four species were first records for Tunisia. Two of them are exclusively Mediterranean species (*Asterina pancerii* (Gasco, 1876) and *Leptopentacta tergestina* (M. Sars, 1857)), one is an Atlantic species (*Luidia atlantidea* (Madsen, 1950)) and the last one is a cosmopolitan species (*Ophiactis virens* (M. Sars, 1857)).

Otherwise, the sea cucumber genetic results showed a big similarity between spicule's morphometric and molecular results. While, the target species *Holothuria (Roweothuria) poli* (Delle Chiaje, 1824) showed a high phylogegraphic diversity on the Mediterranean Sea, in particular a large genetic connectivity between the central and the eastern Mediterranean basins and genetic variability between the eastern and the western Mediterranean Sea. Also, it reveald a big genetic differenciation between lagoon ecosystem and open sea. Eventually, the updated Echinoderma list from the availible datasets provided 79 species in Tunisia. The biogeographical patterns showed that species richness decreasing from the north to the south of the country and from the western to the eastern mediterranean sea with the dominance of cosmopolitan species in all the regions.

The use of the DNA barcoding approach on Echinodermata species seems very reliable, fast and efficient method for species identification, and is very useful for biological knowledge of target species. Lack of information of the phylum Echinodermata in Tunisia is a knowledge gap for marine biodiversity. Consequently, amplifying the knowledge of its diversity and distribution will also allow its right fishery management and the valorization of its products, and ultimately its conservation and sustainability.

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"...I salute the echinoderms as a noble group especially designed to puzzle the zoologist..." Libby Hyman, 1955

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CHAPTER I

GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

1.1. Overview of the Phylum Echinodermata

The Phylum Echinodermata is constituted by animals exclusively marine, being despite it one of the most diverse phyla of the animal kingdom (Ocaña and Pérez-Ruzafa 2004). They are present in all the seas and oceans, from tropics to poles and from shallows to deep seas, showing important differences in sizes, colours, and all possible trophic strategies.

Currently, they count over 7000 to 8000 species divided into five living Classes: Asteroidea (starfish), Ophiuroidea (brittle stars), Echinoidea (sea urchins), Holothuroidea (sea cucumbers) and Crinoidea (comatulids or sea lilies) (Pawson 2007).

Echinoderms are classified as Triploblastids, Bilaterians, Coelomates, Deuterostomians, and Epithelioneurians. They possess the deuterostomial character just like the Chordates, because the mouth is neoformed in the second place and their embryonic blastopore evolves into an anus. But they differ from the Chordates by their Epithelioneuric nervous system which have been remained diffuse under the ectoderm, unlike the Epineurian nervous system of the Chordates. Other defining characteristics of the phylum are to present a radial and indeterminate segmentation of the egg, to develop an enterocelic coelom and to possess simple larval forms. They are also animals that are not cephalized and have a pentaradial symmetry, developed from the primary bilateral symmetry that the larva possesses (Ocaña and Pérez-Ruzafa 2004; Chammem 2015).

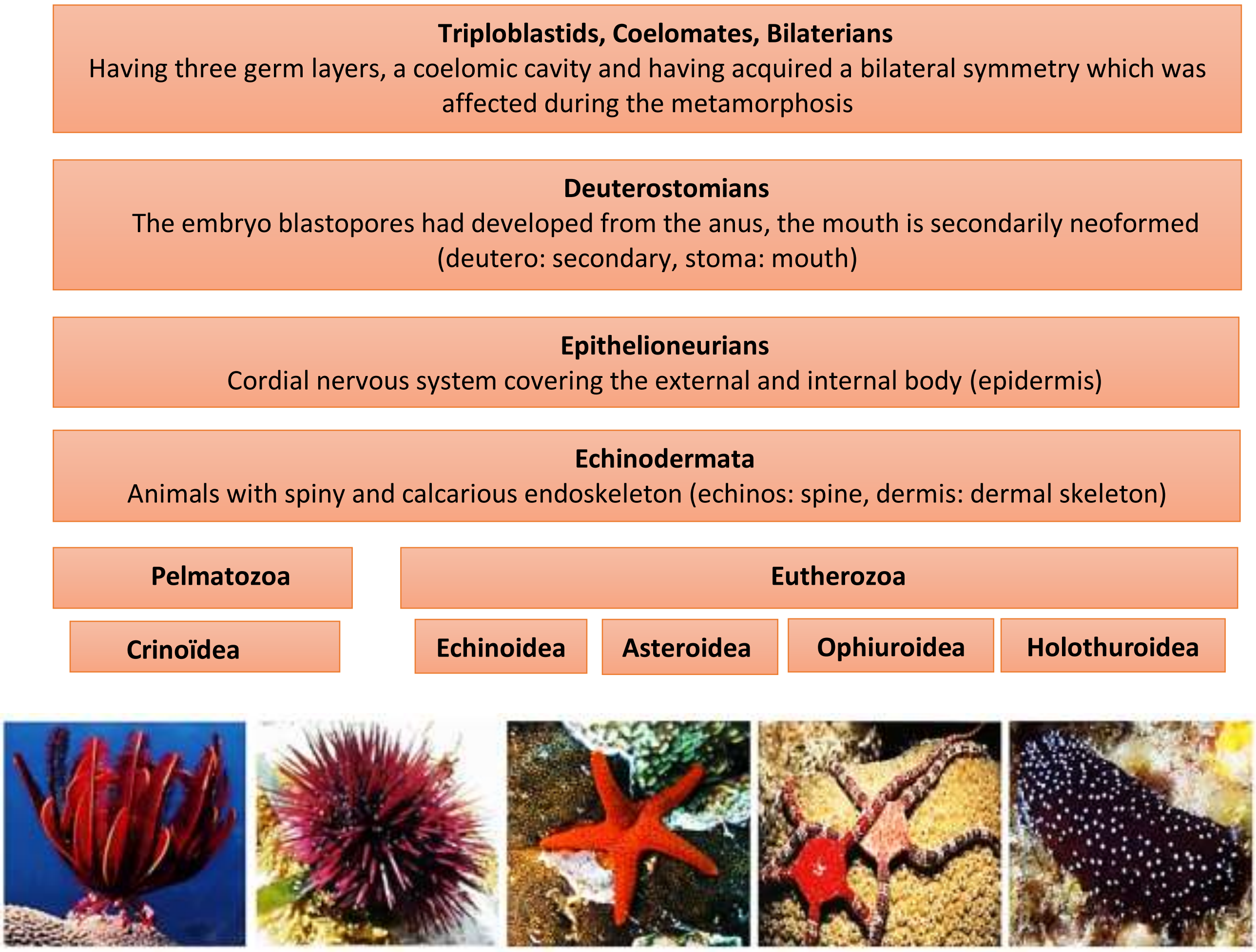
They are typically gonochoristic animals, without sexual dimorphism, which usually present external fecundation of the gametes, although exceptionally there may be hermaphroditism cases and even parthenogenesis and internal fertilization with egg incubation. They usually have asexual reproduction, often by fission or autotomy, and are animals with an extraordinary capacity for self-regeneration (Ocaña and Pérez-Ruzafa 2004; Chammem 2015).

All of them have an endoskeleton that gives rigidity and protection to the body, secreted by cells of the dermis, although it often appears almost superficial given the scarce development of the epidermis that covers it. This skeletal system is exclusive to the group and consists of plates formed by a calcite monocrystal. The shape and function of these plates are very varied, from the microscopic spicules of the holothurians to the large plates or spines of other classes, but they allow a great plasticity based on differential depositions and reabsorptions that facilitate an increase or decrease in the size of the animal according to the energy availability (Ocaña and Pérez-Ruzafa 2004). Finally, among the defining characteristics of the phylum, it should be noted that they have an ambulacral or vascular aquifer system, equipped with ambulatory feet that echinoderms use in functions as varied as respiration, excretion, feeding, movement or sensory perception (Ocaña and Pérez-Ruzafa 2004; Chammem 2015).

The actual Echinodermata living forms are currently subdivided in two Subphyla (fig. 1.1):

Pelmatozoa: fixed to the substrate (sessile), but able to be released in the adult state, with only one class: Crinoidea

Eleutherozoa: vagiles, they include four Classes: Asteroidea, Ophiuroidea, Echinoidea and Holothuroidea.



Triploblastids, Coelomates, Bilaterians
Having three germ layers, a coelomic cavity and having acquired a bilateral symmetry which was affected during the metamorphosis

Deuterostomians
The embryo blastopores had developed from the anus, the mouth is secondarily neoformed (deutero: secondary, stoma: mouth)

Epithelioneurians
Cordial nervous system covering the external and internal body (epidermis)

Echinodermata
Animals with spiny and calcarious endoskeleton (echinos: spine, dermis: dermal skeleton)

Figure 1.1. Taxonomic position, main characteristics and distribution of the five living Echinodermata Classes into the two Subphyla Pelmatozoa and Eleutherozoa (from Chammem 2015).

From an ecological point of view, they play an important number of functional roles as detritivores, filtering feeders, herbivorous, bioturbators, or in general predators or preys, occupying a wide variety of substrates. They could burrow into the sand providing more oxygen at greater depths of the sea floor, eat seaweeds preventing their growth on rocks and coral reefs, participate in the organic matter dynamic and decomposition in the seabed, or provide a habitat for parasites such as worms and snails, among others. Moreover, they are part of the diet of many animals, including the sea otter.

This marine invertebrate group dominate quantitatively and, in many cases, qualitatively the benthos dynamic. The alteration of their populations will thus affect the whole community, threatening the equilibrium of the ecosystems where they live (Coteur *et al.* 2003). Otherwise they are known as bioindicators of marine pollution and in toxicity studies (Portocali *et al.* 1996; Temara *et al.* 1998; Schweitzer *et al.* 2000; Coteur *et al.* 2003).

On the other hand, they have a great interest from the commercial and human consumption point of view (Micael *et al.* 2009), mainly the sea urchin eggs and the body wall or the musculature of some species of holothurians. In this sense, the fishing industry has a greater large impact on the survival of echinoderm species and their population dynamics. By one hand, the fishing industry removes species that constitute their diet, reducing their food availability, but fishing also directly extract species of echinoderms of commercial interest. Also, the pet or ornamental trade has several direct or indirect effects on many of their species. So, modifications or destruction of the natural marine habitats, pollution or global climate change (with a special mention to water acidification), and their overexploitation could be highlighted as the main threats at broader scales that these animals suffer.

1.2. Geographical framework of the Mediterranean Sea

The Mediterranean is a semi-enclosed sea with a total area of approximately 2,536,000 Km^2, a maximum North to South distance of 1600 Km, an East to West width of 3860 Km, and an average depth of 1500 m (Zambianchi *et al.* 2014). It is located at the crossroads of three continents, Africa, Europe and Asia, and it is connected to the Atlantic Ocean by the Gibraltar Strait, to the Red Sea by the Suez Canal and to the Black Sea by the Dardanelles and Bosphorus Straits (Coll *et al.* 2010; Würtz 2010; Paim *et al.* 2015).

It is composed of several seas or sub-basins, being the main ones the Adriatic, Aegean and Tyrrhenian Seas, or the Algerian, Liguro-Provencal, Ionian and Levantine basins.

The Strait of Sicily is considered as a biogeographical barrier that separates the island of Sicily from the Tunisian coast (Cap Bon) and divides the Mediterranean into two more or less similar basins: the western basin, which is most influenced by the Atlantic, and the eastern basin, which is the deepest and the most stretched (Bianchi and Morri 2000; Coll *et al.* 2010; Mejri *et al.* 2011; Zambianchi *et al.* 2014).

The circulation of the water current in the Mediterranean is very active and complex, usually influenced by the winds and the exchanges of water through the different straits and basins, as well as by the thermohaline forces. Moreover, the Mediterranean acts like an ocean system in which several temporal and spatial scales (basin, sub-basin and mesoscale) interact to form a highly complex and variable water circulation (Fernández *et al.* 2005). Generally, oceanographic conditions are characterized by the strong evaporation in the East causing the water level to decrease and the salinity to increase from the West to the East basin (Coll *et al.* 2010; Würtz 2010; Rohling *et al.* 2015). This water deficit is compensated by the entry of water from outside, especially from the Atlantic Ocean through the Strait of Gibraltar, but also to a lesser extent from the Black Sea through the Bosphorus and the Dardanelles straits and from the Red Sea through the Suez Canal.

The assembling of both Atlantic and Mediterranean streams tends to balance, and it generates cyclonic trajectories in the different sub-basin by the changes of the surface water density and the Coriolis force (fig. 1.2). Eventually, Mediterranean Sea currents control the movement and the orientation of many marine vertebrate animals, such as cetaceans, sharks and turtles, that orient themselves and follow the Mediterranean current to swim easier and save energy.

The biodiversity in the Mediterranean Sea is very important, since it represents 7% of marine species in less than 0.8% of the total ocean area. This can be explained by its geomorphological and hydrographical features, its geological history and evolution and its position as interface between temperate and tropical conditions (Bazairi *et al.* 2010).

Following the revision of Coll *et al.* (2010), approximately 17,000 species can be cited in the Mediterranean Sea. Within the Animalia, the greater proportion of them belongs to the subphylum Crustacea (13.2%) and phyla Mollusca (12.4%), followed by Annelida

(6.6%), Platyhelminthes (5.9%), Cnidaria (4.5%), the subphylum Vertebrata (4.1%), Porifera (4.0%), Bryozoa (2.3%), the subphylum Tunicata (1.3%), and Echinodermata (0.9%).

Moreover, it hosts a high percentage of endemisms, being 20.2% the average of the total endemics species, occuring the highest percentages in Porifera (48%), followed by Mysidacea (36%), Ascidiacea (35%), Cumacea (32%), and Echinodermata (24%) (Coll *et al.* 2010).

From the point of view that interests us, 134 echinoderm species have been cited in the Mediterranean, of which 32 are endemic.

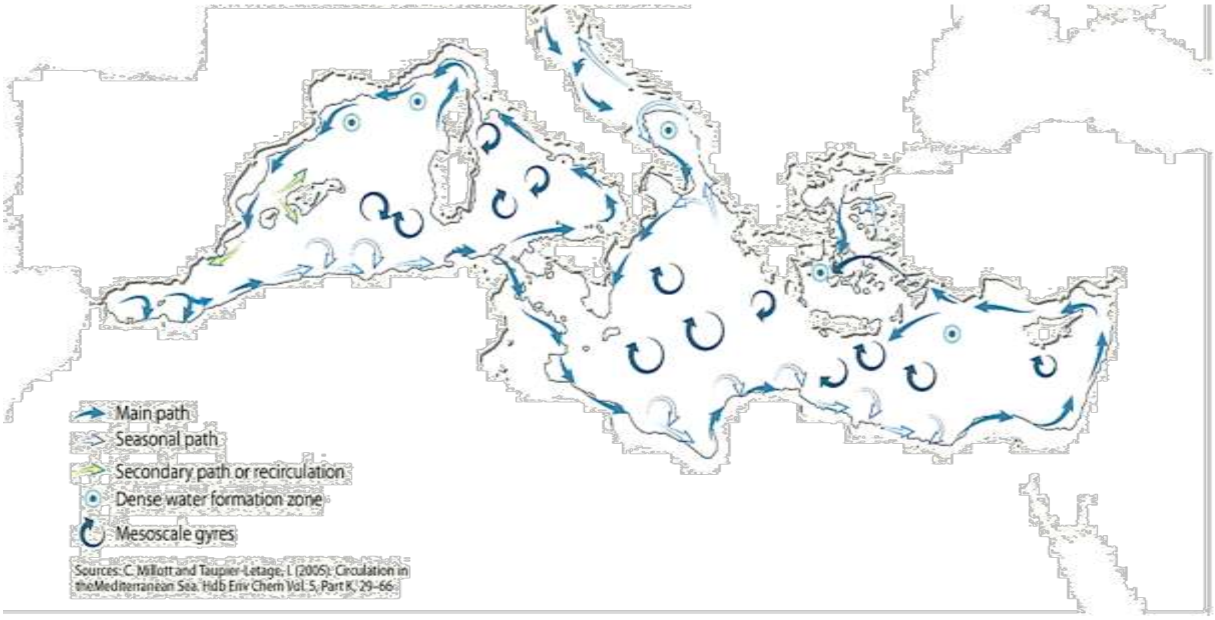


Figure 1.2. Map of the marine circulation in the Mediterranean Sea (from Millot and Taupier-Letage 2005; Zambianchi *et al.* 2014).

1.3. Tunisia framework area

Tunisia is located in the central Mediterranean at the northeastern tip of Africa (fig. 1.3). It is limited by Algeria on the west, Libya on the southeast and the Mediterranean Sea on the north and east. Its coasts extend over 1,300 km and constitute a privileged transition zone between the western and eastern Mediterranean basins with three large gulfs: Tunis, Hammamet and Gabès.

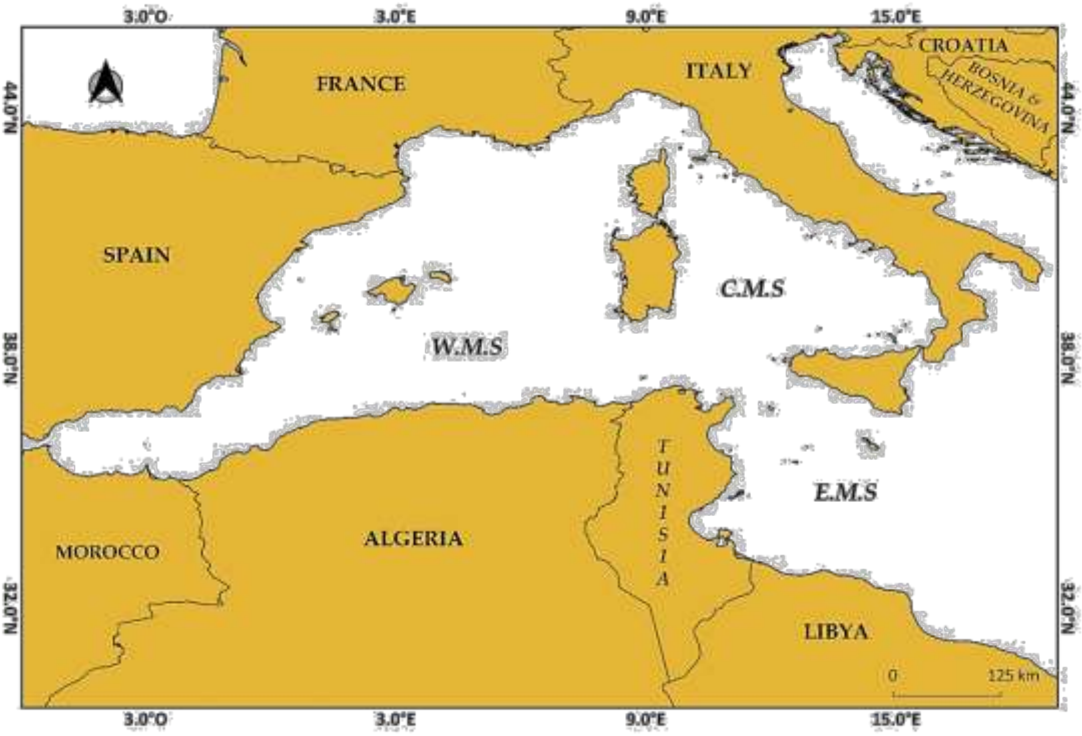


Figure 1.3. The position of Tunisia in the Mediterranean Sea. W.M. S= Western Mediterranean Sea, C.M. S=Central Mediterranean Sea, E.M. S= Eastern Mediterranean Sea.

Tunisia is administratively divided into three mean areas (fig. 1.4):

- North area stretching from the Tunisian-Algerian border to the Cape Bon point, from the region of Tabarka to Kélibia. This area includes the Gulf of Tunis.
- East area, to which belongs the Gulf of Hammamet and that covers the coast from Cape Bon in Kélibia region to Ras Caboudia in the region of Mahdia.
- South area, from Ras Caboudia to the Tunisian-Libyan border, that includes the Gulf of Gabès which is the largest Tunisian gulf.

The northern region of Tunisia extends from the Algerian-Tunisian border (37°01′06 N, 8°44′04 E) to Cape Bon (36°26′53 N, 10°51′36 E) covering 300 km of coastline. It belongs to the western basin of the Mediterranean Sea, although it is in the border between both basins. It is characterized by a distinctive morphology and a heterogeneous bottom type, with a reduced and rugged continental shelf with a steep slope to the North. This region is formed by a complex bathymetry with the extension of a series of marine mountains separated by deep and shallow channels, which make their benthic habitats diverse and patchy with a dominance of coralligenous assemblages (Azouz 1973).

The bottom types vary between rocky, detrital and soft (Lubet and Azouz 1969; Azouz 1971, 1973), offering, mainly in the northern rocky substrata, the best conditions for a rich marine biodiversity (Azouz 1973).

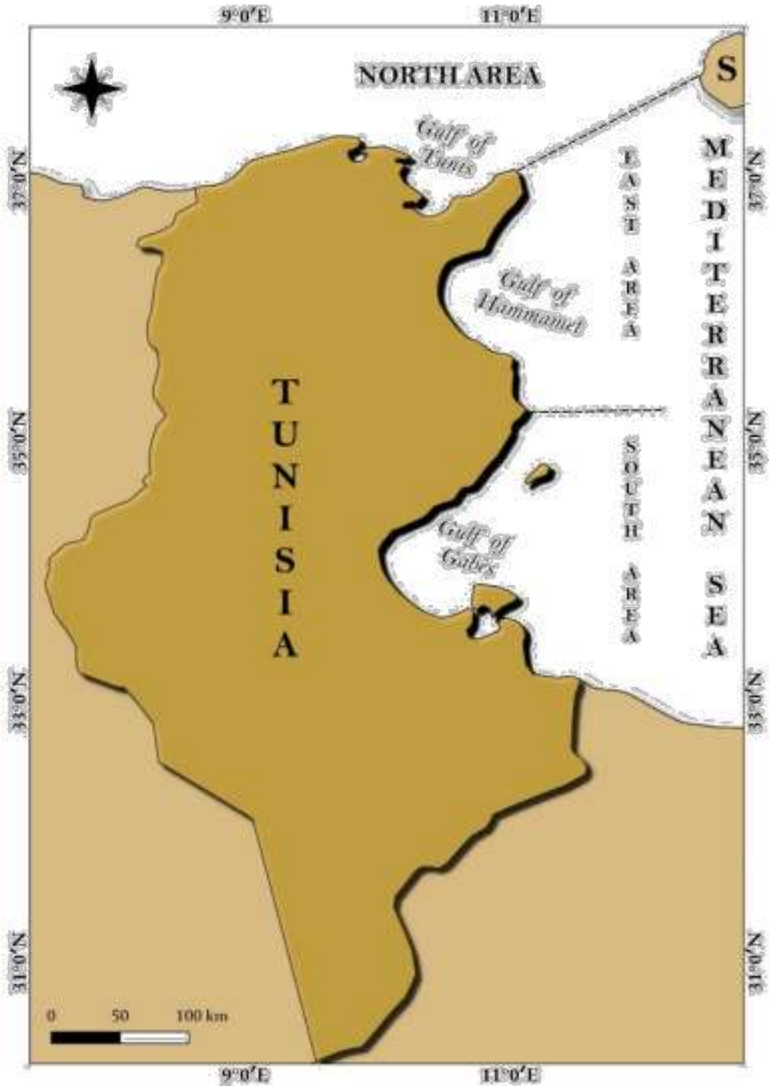


Figure 1.4. Different areas and gulfs of the Tunisian littoral. S = Sicily Island

The marine circulation along the northern Tunisian coasts is linked to the general circulation of the Mediterranean and under the influence of Atlantic currents. In fact, Tunisia is in the junction between the western and the eastern Mediterranean basins, where currents are strongly conditioned by the geomorphology. The water mass flows from the Atlantic through the Algerian basin generating the Algerian current. It enters the Sardinian channel and spreads to the eastern Mediterranean through the Sicily Strait, after skirting the North-Tunisian coasts (Millot 1987; Hattour1995).

Some two thirds of the Atlantic surface water enter the eastern Mediterranean basin through the Strait of Sicily. In this Strait, the circulation is complex due to its large width and the topography with numerous islands and banks.

At the exit of the Strait of Sicily, three kinds of processes occur, so that the eastern gyre at its beginning starts to split into several components, one of those is the regular flow along the Tunisian coast flowing eastward (El-Geziry and Bryden 2010).

So, Tunisia occupies a strategic biogeographic position in the Mediterranean and regarding the Sicily-Strait, considered, as mentioned above, a biogeographical boundary that separates the eastern and western basins. Despite its importance, studies on marine biodiversity in this area are old and scarce. There are some benthic community studies that have investigated the spatio-temporal development of marine ecosystems as well as the distribution of communities and species in the Tunisian coasts (Zaouali and Baeten 1985; Boudouresque 1997a, 1997b; Ben Mustapha *et al.* 2002; Ayari and Afli 2003; Afli *et al.* 2008; Zaâbi *et al.* 2010; Zakhama-Sraieb *et al.* 2011; Sghaier *et al.* 2016; Ghanem *et al.* 2018), but there is an important lack of echinoderms data and publications.

Echinoderms of Tunisia are poorly studied and their inventories are scarce and, actually, no researchers are involved specifically in this group. Except for the two specific checklists of Tunisian marine echinoderms by Cherbonnier (1956) and Gautier-Michaz (1958), this group is usually englobed in other extensive invertebrate megabenthic faunal studies (Le Danois 1925; Azouz 1973; Ben Othman1973; El Lakhrach *et al.*2012), or other authors have focused on studying a single class, generally with economical interest such as Echinoidea (Sellem *et al.* 2000, 2001, 2011; Sellem and Guillou 2007) or Holothurioidea (Louiz *et al.* 2003).

Lack of information on macrobenthic marine groups is a knowledge gap in Tunisian marine biodiversity. In this way, in order to recognize the echinoderms that occur in the northern Tunisia and to update the checklist of the Tunisian echinoderms, a study of this phylum was carried out between 2012 and 2016. Besides, their biogeography and ecology have also been studied.

1.4. Thesis structure and objectives

Therefore, the present doctoral thesis is focused in these aims:

1. Explore the biodiversity of echinoderms in northern Tunisia in order to produce a validated checklist and update the inventory of the echinoderms in Tunisia.
2. Analyze the structure of the phylum Echinodermata in northern Tunisia and define the ecological factors that explain their distribution, according to the locality, habitat and

depth occupied by each species. As well as explore the relationship between the biological and the ecological patterns of the studied species and, in summary, their biogeography in Tunisian waters and in the Mediterranean Sea.

3. Evaluate the systematic identification and the genetic diversity patterns of eight *Holothuria* species with Mediterranean and Atlantic origin, and discuss their systematic position according to the similarity of the morphometric and molecular analyses of the spicules (buttons and tables).
4. Asses the genetic structure of the target species *Holothuria poli* through its phylogeographic study, in three different localities from western, eastern and central Mediterranean Sea and from two different marine ecosystems: open sea and lagoon, in order to establish the potential stocks of this species in Tunisia.
5. Analyze the biogeography of the phylum Echinodermata along the Tunisian coasts as well as, in different regions and provinces from the Mediterranean Sea and from the Atlantic Ocean. Thus by exploring and comparing the diversity of this group by country and by classes.

In order to present and discuss these objectives, this thesis has been organized in seven chapters, including the general introduction and general discussion and conclusions. The different chapters have been structured as independent, with their own objectives, results and discussions which are original and specific to each section.

CHAPTER II

FAUNISTIC STUDY OF THE ECHINODERMS OF NORTERN TUNISIA: CHECKLIST WITH FIRST RECORDS

2. FAUNISTIC STUDY OF THE ECHINODERMS OF NORTERN TUNISIA: CHECKLIST WITH FIRST RECORDS

2.1. Introduction

Tunisia is the northernmost point of the African continent (36°51′44 N, 10°11′44 E) occupying a central position between the western and the eastern Mediterranean Sea. The Strait of Sicily, located between Cape Bon (Tunisia) and Mazara dell Vallo (Sicily), has been considered a biogeographical barrier that separates these two main Mediterranean basins (Bianchi and Morri 2000; Mejri *et al.* 2011; Zambianchi *et al.* 2014). This separation is relevant not only from the point of view of connectivity between the two basins with different hydrographical conditions, but also because the Mediterranean geological history after the Messinian crises that produced an isolation of the basins and a time lag in the processes of recolonization by Atlantic species (Coll *et al.* 2010; Zenetos 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 species (Pérez-Losada *et al.* 2007; Zitari-Chatti *et al.* 2009; Deli *et al.* 2017). Moreover, the colonisation of new species in the Mediterranean Sea, by the Lessepsian invasions after the opening of the Suez Canal, and through the Strait of Gibraltar as climate change becomes more evident (Zenetos 2010), makes Tunisia the convergence point 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, and are found from the shallow intertidal to the abyssal zone, where they play an important role in the ecological processes of marine ecosystems (Souto *et al.* 2014).

Lack of research on Echinodermata represents a knowledge gap concerning Tunisian marine biodiversity. Only two studies, Cherbonnier (1956) and Gautier-Michaz (1958), have published original checklists of Tunisian echinoderms, which, on the other hand, are outdated. This group is also cited in the invertebrate megabenthic faunal inventories of Le Danois (1925), Azouz (1973), Ben Othman (1973), or El Lakhrach *et al.* (2012), and some other authors have focused on one particular class, generally of economic interest, such as Echinoidea (Sellem et *al.* 2001, 2011; Sellem and Guillou 2007) or Holothuroidea (Louiz *et al.* 2003; Sellem *et al.* 2019).

In this context, the present chapter aims to identify the collected material using taxonomical keys and following the World Register of Marine Species nomenclature. Furthermore, to explore the faunal composition of the recorded species and to update the biological diversity of the echinoderms in Tunisia by actualizing the checklist of the phylum Echinodermata.

2.2. Material and methods

2.2.1. Sampling area

Echinoderms were sampled at 93 sites in eight locations along the northern coasts of Tunisia (Table 2.1; fig. 2.1). The study area extends over 300 km of the Tunisian coastline, from the Algerian-Tunisian border (37°01′06 N, 8°44′04 E) to the Cape Bon Peninsula (36°26′53 N, 10°51′36 E).This area of the central Mediterranean Sea is constantly affected by incoming Atlantic marine currents (Lubet and Azouz 1969; Azouz 1973) and is characterized by a continental shelf with a small, irregular platform and a steep slope (Azouz 1973).The heterogeneity of its bottoms, with hard and soft substrates, favors the biodiversity of northern Tunisia (Azouz 1973; Ayari and Afli 2003).

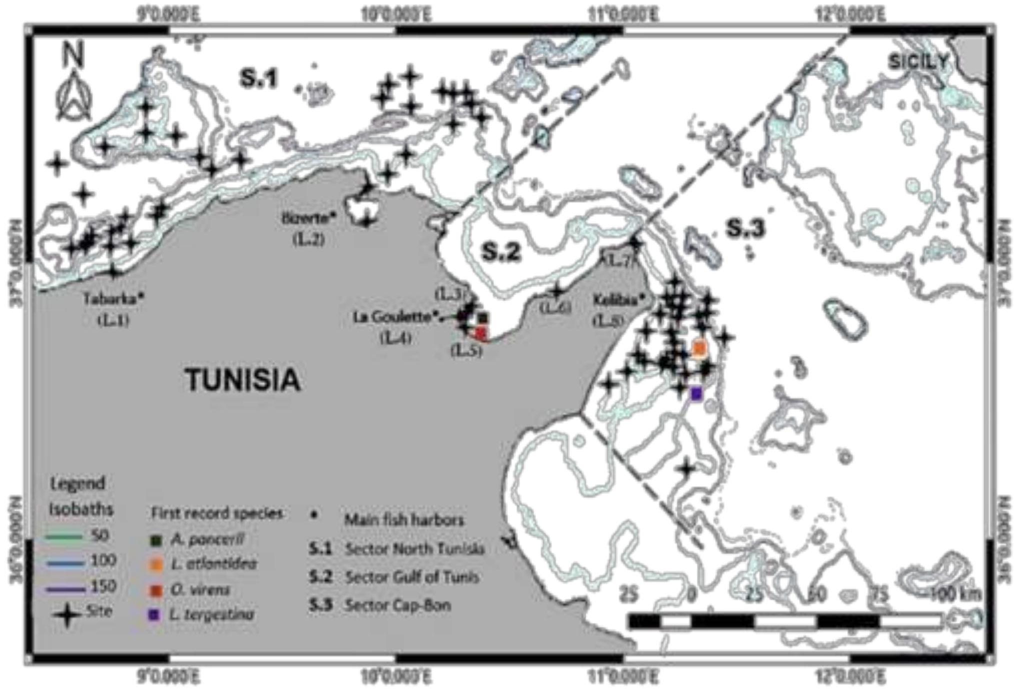


Figure 2.1: Map of the studied locations (L1-L8) in northern Tunisian waters, also showing the sites of newly echinoderms records: *Asteria pancerii* (Green), *Luidea atlantidea* (Orange), *Ophiactis virens* (Red), *Leptopentacta tergestina* (Purple) (Chammem *et al.* 2019).

Table 2.1. Locations and sites studied along the northern coast of Tunisia between March 2012 to July 2016.

Sectors (S)	Locations (L)	Sit Nu.	Geographic coordinates		Sampling methods	Number of specimens	Year
			Latitude	Longitude			
S1	L.1 - Tabarka	S.1	37.3614°S	8.5078°W	Hand collection	6	2016
		S.2	37.2458°S	8.6238°W	Experimental Dredge	4	2016
		S.3	37.0633°S	8.6267°W	Experimental Dredge	6	2016
		S.4	37.0556°S	8.6383°W	Experimental Dredge	4	2016
		S.5	37.0771°S	8.6440°W	Experimental Dredge	5	2016
		S.6	37.0882°S	8.6443°W	Experimental Dredge	6	2016
		S.7	37.0864°S	8.6459°W	Experimental Dredge	5	2016
		S.8	37.0582°S	8.7455°W	Experimental Dredge	6	2016
		S.9	36.9644°S	8.7551°W	Diving	4	2016
		S.10	36.9591°S	8.7554°W	Diving	3	2016
		S.11	37.1157°S	8.7579°W	Benthic Trawling	27	2016
		S.12	37.1226°S	8.7839°W	Benthic Trawling	11	2016
		S.13	37.1219°S	8.7847°W	Benthic Trawling	11	2016
		S.14	37.1312°S	8.7909°W	Benthic Trawling	9	2016
		S.15	37.0655°S	8.8366°W	Benthic Trawling	15	2016
		S.16	37.5643°S	8.8978°W	Benthic Trawling	26	2016
		S.17	37.4715°S	8.8992°W	Benthic Trawling	11	2016
		S.18	37.1731°S	8.9538°W	Benthic Trawling	11	2016
		S.19	37.2006°S	8.9716°W	Benthic Trawling	9	2016
		S.20	37.5015°S	8.8893°W	Benthic Trawling	12	2016
		S.21	37.4462°S	8.6197°W	Benthic Trawling	21	2016
		S.22	37.5664°S	8.9713°W	Benthic Trawling	15	2016
	L.2 - Bizerte	S.1	37,4189°S	8,7187°W	Benthic Trawling	18	2016
		S.2	37,4550°S	9,0303°W	Benthic Trawling	9	2016
		S.3	37,3833°S	9,1417°W	Benthic Trawling	16	2016
		S.4	37.3799°S	9.0167°W	Benthic Trawling	17	2016
		S.5	37,3375°S	9,1923°W	Benthic Trawling	11	2016
		S.6	37,3675°S	9,3199°W	Benthic Trawling	9	2015
		S.7	37,1506°S	9,8706°W	Experimental Dredge	3	2015
		S.8	37,1426°S	9,8726°W	Experimental Dredge	3	2015
		S.9	37,1470°S	9,8740°W	Experimental Dredge	5	2013
		S.10	37,2716°S	9,8793°W	Hand collection	1	2013
		S.11	37,2717°S	9,8795°W	Hand collection	2	2016

		S.12	37,2715°S	9,8843°W	Hand collection	1	2016
		S.13	37,6016°S	9,9434°W	Benthic Trawling	14	2016
		S.14	37,6445°S	9,9682°W	Benthic Trawling	10	2016
		S.15	37,3217°S	9,9670°W	Benthic Trawling	20	2016
		S.16	37.4550°S	9.0303°W	Benthic Trawling	23	2016
		S.17	37.1426°S	9.8726°W	Experimental Dredge	3	2016
		S.18	37.3833°S	9.1417°W	Experimental Dredge	2	2016
		S.19	37,3934°S	10,0499°W	Benthic Trawling	19	2016
		S.20	37,6782°S	10,0633°W	Benthic Trawling	12	2016
		S.21	37.5448°S	10.1377°W	Benthic Trawling	17	2016
		S.22	37.5598°S	10.0670°W	Benthic Trawling	22	2016
		S.23	37.6205°S	10.2040°W	Benthic Trawling	19	2016
		S.24	37.6124°S	10.2516°W	Benthic Trawling	16	2016
		S.25	37.4995°S	10.2526°W	Benthic Trawling	10	2016
		S.26	37.5643°S	10.2075°W	Benthic Trawling	12	2016
		S.27	37.6191°S	10.3120°W	Benthic Trawling	19	2016
		S.28	37.4431°S	10.3229°W	Benthic Trawling	16	2016
		S.29	37.5757°S	10.3278°W	Benthic Trawling	10	2016
		S.30	37.5236°S	10.3729°W	Benthic Trawling	13	2016
S2	L.3 – Carthage	S.1	36.8405°S	10.3323°W	Hand collection	17	2012
		S.2	36.8441°S	10.3292°W	Hand collection	13	2013
	L.4 – La Goulette	S.1	36.8071°S	10.3066°W	Experimental Dredge	6	2012
		S.2	36.8067°S	10.3075°W	Experimental Dredge	4	2013
		S.3	36.8220°S	10.3137°W	Experimental Dredge	4	2013
	L.5 – Radès	S.1	36.7880°S	10.2878°W	Hand collection	200	2012
		S.2	36.8159°S	10.5611°W	Hand collection	2	2013
	L.6 – Korbous	S.1	37.2717°S	9.8795°W	Hand collection	1	2013
S3	L.7 – Haouaria	S.1	37.0762°S	11.0526°W	Diving	10	2014
		S.2	37.0760°S	11.0530°W	Hand collection	20	2015
		S.3	37.0758°S	11.0539°W	Hand collection	22	2016
	L.8 – Kélibia	S.1	36.5593°S	10.9349°W	Benthic Trawling	9	2015
		S.2	36.5507°S	11.0163°W	Benthic Trawling	29	2015
		S.3	36.6083°S	11.0249°W	Benthic Trawling	17	2015
		S.4	36.6683°S	11.0667°W	Benthic Trawling	17	2015
		S.5	36.6433°S	11.0899°W	Benthic Trawling	10	2015
		S.6	36.7500°S	11.1017°W	Benthic Trawling	11	2015

		S.7	36.8138°S	11.1609°W	Benthic Trawling	22	2015
		S.8	36.6267°S	11.1784°W	Benthic Trawling	16	2015
		S.9	36.6507°S	11.1812°W	Benthic Trawling	26	2015
		S.10	36.7463°S	11.1583°W	Benthic Trawling	35	2015
		S.11	36.6132°S	11.3574°W	Benthic Trawling	14	2016
		S.12	36.6322°S	11.1861°W	Benthic Trawling	42	2016
		S.13	36.8645°S	11.1996°W	Benthic Trawling	13	2016
		S.14	36.7517°S	11.2083°W	Benthic Trawling	24	2016
		S.15	36.8445°S	11.2182°W	Benthic Trawling	12	2016
		S.16	36.8812°S	11.2269°W	Benthic Trawling	14	2016
		S.17	36.6183°S	11.2194°W	Benthic Trawling	17	2016
		S.18	36.9323°S	11.2289°W	Benthic Trawling	15	2016
		S.19	36.7099°S	11.2233°W	Benthic Trawling	13	2016
		S.20	36.6767°S	11.2233°W	Benthic Trawling	16	2016
		S.21	36.8087°S	11.2505°W	Benthic Trawling	19	2016
		S.22	36.8367°S	11.2583°W	Benthic Trawling	24	2016
		S.23	36.5467°S	11.2517°W	Benthic Trawling	20	2016
		S.24	36.8752°S	11.2653°W	Benthic Trawling	13	2016
		S.25	36.6667°S	11.2683°W	Benthic Trawling	18	2016
		S.26	36.5957°S	11.2796°W	Benthic Trawling	16	2016
		S.27	36.2534°S	11.2768°W	Benthic Trawling	20	2016
		S.28	36.8078°S	11.3333°W	Benthic Trawling	16	2016
		S.29	36.7713°S	11.3447°W	Benthic Trawling	18	2016
		S.30	36.6132°S	11.3574°W	Benthic Trawling	16	2016

The study area was divided into 3 sectors: North Tunisia (Sector 1), from the Algerian border to Bizerte, including the two principal harbors of Tabarka and Bizerte, Gulf of Tunis (Sector 2) from La-Goulette to Radès with 4 localities, and Cape Bon (Sector 3), from the region of Haouaria to Korba, where is located the Kèlibia harbor.

2.2.2. Data collection

The Echinodermata inventory was carried out from March 2012 to July 2016, along sixteen seasonal sampling campaigns from Tabarka to Cape Bon. Different sampling strategies were adopted depending on the substrate type (rocky or soft bottom), depth, and according to the type of community. 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, using a quadrat, were employed for mid and infralittoral levels (<5 m) (Table 2.1, fig. 2.2).

The dredge had a half cylindrical metal opening, provided with teeth. It was 40 cm long and had a radius of 22 cm in which was fixed a bag with 1 cm of mesh light and 2 m long. It was provided with 3 strong and solid cables whose length were 21 m for each one. This gear was generally used for sandy bottom.

Benthic trawls consisted in a rigid and hard conical net whose opening is held vertically by floats and horizontally by two diverging panels. it meshes varied between 40 to 100 mm, and their length and width depended on the boats size, but always being Italian type.

The used quadrat was a wood rectangular structure of 25 cm surface. It was placed mainly on rocks rich on algae. The sampling was done quickly in order to collect the maximum number of animals. The harvested content was placed in a net of 0.3 mm mesh to filter the water and to keep the animals fresh. Moreover, we used a brush to collect fragile samples such as ophiuroids.

The material was labelled and kept in ethanol 95°, and then taken to the laboratory for their separation and identification. For each site, the geographical coordinates (longitude, latitude), the duration of the trawling (start and end time of trawling) and the depth, were recorded and registered for each trawling hauls.

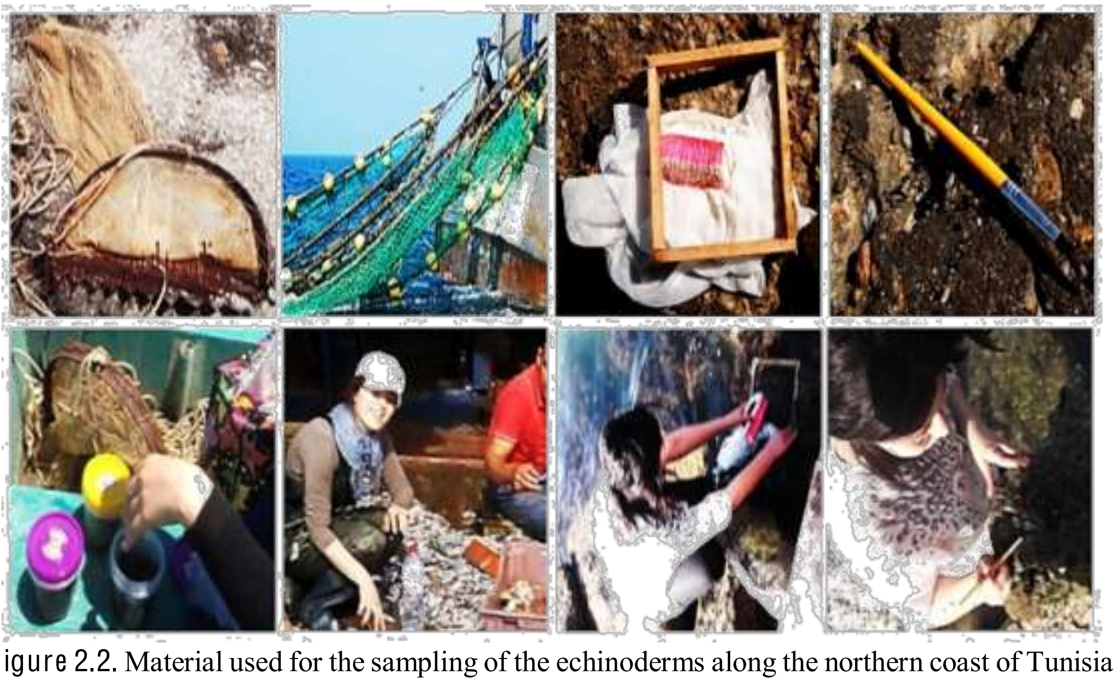


Figure 2.2. Material used for the sampling of the echinoderms along the northern coast of Tunisia: experimental dredge, commercial benthic trawl, quadrat and brush (from left to right).

2.2.3. Taxonomic determinations

The collected material was measured, photographed and preserved in ethanol. Specimens were identified based on their external morphology and internal anatomy following the taxonomic criteria of Koehler (1921, 1969), Mortensen (1927), Caso (1948, 1957), Tortonese (1965), Clark and Downey (1992) and Hendler *et al.* (1995). The nomenclature follows the World Register of Marine Species (WoRMS, http://www.marinespecies.org/).

Moreover, the study relied on the works of Madsen (1950), Blake (1981) and Clark and Downey (1992) for the class Asteroidea; Fell (1960) and Madsen (1970) for the class Ophiuroidea; Koehler (1924) and Tortonese (1965) for Crinoidea; Clark (1925) and Mortensen (1927) for Echinoidea; and Rowe (1969), Pawson (1982) Pérez-Ruzafa (1984) and Pérez-Ruzafa and Marcos (1985) for Holothuroidea. Additionally, many scientific guides and references of taxonomic and faunistic articles, catalogs and museum bulletins, as well as faunistic lists of various scientific expeditions have also been used (Mortensen 1925; Chapman 1951; Cherbonnier 1965; Cherbonnier and Sibuet 1973; Entambasaguas *et al.* 2008).

Echinoderm species previously cited in Tunisia have been found in several works and scientific journals (Koehler 1921; Le Danois 1925; Chambost 1928; Seurat 1929, 1934; Cherbonnier 1956; Pérés and Picard 1956; Maurin 1962; Laban *et al.* 1963; Tortonese 1965; Azouz 1966, 1971, 1974; Lubet and Azouz 1969; De Gaillande 1970; Zaouali 1980; Azouz and Capapé 1971; Ben Othman 1971, 1973; Ktari-Chakroun and Azouz 1971; Prunus and Pantoustier 1974; Azouz and Ben Othman 1975; Darmoul *et al.* 1980; Guelorget *et al.* 1982; Boudouresque *et al.* 1986; Fehri-Bedoui 1986; Sellem 1990; Anonymous 1997; Boudouresque 1997; Ben Souissi and Zaouali 2007; Aloui-Bejaoui and Afli 2012; El Lakhrach et *al.* 2012).

The study of the external characters of the specimens was made by direct observation or with a binocular loupe. The biometric measurements were taken with calipers and expressed in cm. Similarly, the colour and number of the studied specimens were recorded.

For the class of Holothuroidea, other fundamental internal characterstics for the determination of order, family, genera and species were also studied, such as the number, arrangement and colour of the muscular bands, the presence or absence of Cuvier organs and presence (number) or absence of gonads.

The preparation of the spicules was performed according to Pérez-Ruzafa and Marcos (1985), while the subsequent observation of the preparations was made with an optical microscope (magnification 40x-100x).

2.3. Results

2.3.1. Diversity of the echinoderms in northern Tunisia

Forty-five species of echinoderms were collected and identified in northern Tunisia waters, from a total of 1,430 studied specimens (Table 2.2). They belonged to the five classes of Echinodermata and comprised 3 sea lilies (Crinoidea), 15 starfishes (Asteroidea), 10 brittle stars (Ophiuroidea), 8 sea urchins (Echinoidea) and 9 sea cucumbers (Holothuroidea).

They were divided into 27 families and 32 genera. All the inventoried species had previously been cited as present in the Mediterranean Sea, except the starfish *Luidia atlantidea* (Madsen, 1950), which is an Atlantic species that has been referenced only very recently in the Alboran Sea (Gallardo-Roldán *et al.* 2015). Six of the collected species are endemic in the Mediterranean, 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). It is remarkable that four collected species were first records for Tunisia (*Asterina pancerii*, *Luidia atlantidea, Ophiactis virens* (Sars, 1857) and *Leptopentacta tergestina*) (Table 2.2).

Table 2.2. Systematic list of the studied echinoderm species in northern Tunisia (present work). New occurrences are marked by (*).

Class CRINOIDEA Miller JS (1821)
Order Comatulida
Family ANTEDONIDAE Norman, 1865
Genus *Antedon* Fréminville, 1811
Antedon bifida (Pennant, 1777)
Antedon mediterranea (Lamarck, 1816)
Genus *Leptometra* AH Clark, 1908
Leptometra phalangium (Müller, 1841)

Class ASTEROIDEA Blainville, 1830
Order Forcipulatida Perrier, 1884

- Family ASTERIIDAE Gray, 1840
  - Genus *Coscinasterias* Verrill, 1867
    - *Coscinasterias tenuispina* (Lamarck, 1816)
  - Genus *Marthasterias* Jullien, 1878
    - *Marthasterias glacialis* (Linnaeus, 1758)
- Order Valvatida Perrier, 1884
  - Family ASTERINIDAE Gray, 1840
    - Genus *Anseropoda* Nardo, 1834
      - *Anseropoda placenta* (Pennant, 1777)
    - Genus *Asterina* Nardo, 1834
      - *Asterina gibbosa* (Pennant, 1777)
      - *Asterina pancerii* (Gasco, 1876) *
  - Family CHAETASTERIDAE Sladen, 1889
    - Genus *Chaetaster* Müller and Troschel, 1840
      - *Chaetaster longipes* (Retzius, 1805)
  - Family OPHIDIASTERIDAE Verrill, 1870
    - Genus *Hacelia* Gray, 1840
      - *Hacelia attenuata* (Gray, 1840)
- Order Paxillosida Perrier, 1884
  - Family ASTROPECTINIDAE Gray, 1840
    - Genus *Astropecten* Gray, 1840
      - *Astropecten aranciacus* (Linnaeus, 1758)
      - *Astropecten bispinosus* (Otto, 1823)
      - *Astropecten irregularis* (Pennant, 1777)
      - *Astropecten jonstoni* (Delle Chiaje, 1827)
    - Genus *Tethyaster* Sladen, 1889
      - *Tethyaster subinermis* (Philippi, 1837)
  - Family LUIDIIDAE Sladen, 1889
    - Genus *Luidia* Forbes, 1839
      - *Luidia atlantidea* (Madsen, 1950) *
      - *Luidia sarsii sarsii* (Düben and Koren in Düben, 1844)
- Order Spinulosida Perrier, 1884
  - Family ECHINASTERIDAE Verrill, 1867
    - Genus *Echinaster* Müller and Troschel, 1840
      - *Echinaster (Echinaster) sepositus* (Retzius, 1783)

Class OPHIUROIDEA Gray, 1840

- Order Euryalida Lamarck, 1816
  - Family GORGONOCEPHALIDAE Ljungman, 1867
    - Genus *Astrospartus* Döderlein, 1911
      - *Astrospartus mediterraneus* (Risso, 1826
- Order Ophiurida Müller and Troschel, 1840
  - Family AMPHIURIDAE Ljungman, 1867
    - Genus *Amphipholis* Ljungman, 1866
      - *Amphipholis squamata* (Delle Chiaje, 1828)
  - Family OPHIACANTHIDAE Ljungman, 1867
    - Genus *Ophiacantha* Müller and Troschel, 1842
      - *Ophiacantha setosa* (Bruzelius, 1805)
  - Family OPHIACTIDAE Matsumoto, 1915
    - Genus *Ophiactis* Lütken, 1856
      - *Ophiactis savignyi* (Müller and Troschel, 1842)
      - *Ophiactis virens* (M. Sars, 1857) *
  - Family OPHIOCOMIDAE Ljungman, 1867
    - Genus *Ophiocomina* Koehler in Mortensen, 1920
      - *Ophiocomina nigra* (Abildgaard in O.F. Müller, 1789)
  - Family OPHIODERMATIDAE Ljungman, 1867
    - Genus *Ophioderma* Müller and Troschel, 1840

Ophioderma longicauda (Bruzelius, 1805)
Family OPHIOMYXIDAE Ljungman, 1867
Genus *Ophiomyxa* Müller and Troschel, 1840
Ophiomyxa pentagona (Lamarck, 1816)
Family OPHIOTRICHIDAE Ljungman, 1867
Genus *Ophiothrix* Müller and Troschel, 1840
Ophiothrix quinquemaculata (Delle Chiaje, 1828)
Family OPHIURIDAE Müller and Troschel, 1840
Genus *Ophiura* Lamarck, 1801
Ophiura ophiura (Linnaeus, 1758)

Class ECHINOIDEA Bronn, 1860
Order Arbacioida Gregory, 1900
Family ARBACIIDAE Gray, 1855
Genus *Arbacia* Gray, 1835
Arbacia lixula (Linnaeus, 1758)
Order Cidaroida Claus, 1880
Family CIDARIDAE Gray, 1825
Genus *Cidaris* Leske, 1778
Cidaris cidaris (Linnaeus, 1758)
Genus *Stylocidaris* Mortensen, 1909
Stylocidaris affinis (Mortensen, 1909)
Order Diadematoida Duncan, 1889
Family DIADEMATIDAE Gray, 1855
Genus *Centrostephanus* Peters, 1855
Centrostephanus longispinus (Philippi, 1845)
Order Camarodonta Jackson, 1912
Family ECHINIDAE Gray, 1825
Genus *Gracilechinus* Fell and Pawson in Moore, 1966
Gracilechinus acutus (Lamarck, 1816)
Family PARECHINIDAE Mortensen, 1903
Genus *Paracentrotus* Mortensen, 1903
Paracentrotus lividus (Lamarck, 1816)
Family TOXOPNEUSTIDAE Troschel, 1872
Genus *Sphaerechinus* Desor, 1856
Sphaerechinus granularis (Lamarck, 1816)
Order Spatangoida L. Agassiz, 1840
Family SPATANGIDAE Gray, 1825
Genus *Spatangus* Gray, 1825
Spatangus purpureus (O.F. Müller, 1776)

Class HOLOTHUROIDEA Brin, 1860
Order Dendrochirotida Grube, 1840
Family CUCUMARIIDAE Ludwig, 1894
Genus *Hemiocnus* Mjobo and Thandar, 2016
Hemiocnus syracusanus (Grube, 1840)
Genus *Leptopentacta* Clark, 1938
Leptopentacta elongata (Düben and Koren, 1846)
Leptopentacta tergestina (M. Sars, 1857) *
Order Holothuriida Miller, Kerr, Paulay, Reich, Wilson, Carvajal & Rouse, 2017
Family HOLOTHURIIDAE Burmeister, 1837
Genus *Holothuria* Linnaeus, 1767
Holothuria (Holothuria) mammata (Grube, 1840)
Holothuria (Holothuria) tubulosa (Gmelin, 1791)
Holothuria (Platyperona) sanctori (Delle Chiaje, 1823)
Holothuria (Roweothuria) poli (Delle Chiaje, 1824)
Holothuria (Thymiosycia) impatiens (Forsskål, 1775

Order Synallactida Miller, Kerr, Paulay, Reich, Wilson, Carvajal & Rouse, 2017
Family STICHOPODIDAE Haeckel, 1896
Genus *Parastichopus* Clark, 1922
Parastichopus regalis (Cuvier, 1817)

2.3.2. Species description

2.3.2.1. Crinoidea

A total of 151 specimens of Crinoidea were studied and identified. They are grouped in two genera and 3 species: *Antedon bifida* (Pennant, 1777), *Antedon mediterranea* and *Leptometra phalangium* (Müller, 1841).

Class CRINOIDEA Miller, 1821

Order Comatulida Clark, 1908
Family Antedonidae Norman, 1865

Genus *Antedon* Fréminville, 1811

Antedon bifida (Pennant, 1777)
Asterias bifida Pennant, 1777.

Diagnosis: This feather star is formed by a small central disc, the centrodorsal, with a conical shape and doesn’t exceed 10 mm of diameter (Koehler 1921; Hayward and Ryland 2005). Around the central disc there are from 8 to 13 articulated arms well curved at their edges. The feathery appearance of the arms is a common characteristic of sea lilies. The calyx provided with at least 20 tiny and jointed cirri that are shaped on claws to ensure the attachment of the crinoid to the substrate. The number of cirrals is less than 18 and very often between 12 to 16 pieces (Simeonidis 1997; Hayward and Ryland 2005). It has a bright colour like red, pink and red-white stripes or red and pink spots (Tortonese 1965; Simeonidis 1997; Hayward and Ryland 2005).

Description: Small body with conical form; composed of more and less of 10 feathery arms with long and thin pinnules; pinnules are poorly curved; coloured in red or red stained with white; cirri are small and well curved formed by 15 to 16 large cirrals; terminals part of cirri is small with conical shape; very fragile creatures and the majority of the specimens have broken arms.

Habitat and ecology: These macrobenthic animals are free and sedentary, living in sandy bottoms and hard substrates. They are usually found from the first meters up to 450 m depth (Koehler 1921; Tortonese 1965).

Distribution: It occurs in the Atlantic (East and West), from Portugal to Açores, and in the South-West Mediterranean, in Algeria, northern Tunisia and in the South of Sardinia and Sicily (Koehler 1921; Tortonese 1965; Hayward and Ryland 2005).

Examined Material: 50 specimens, size ranging from 1 to 4 cm. They were collected by benthic trawling between 50 to 190 meters deep from three localities: Tabarka (L1, S1), Bizerte (L2, S1), Kélibia (L8, S3).

Antedon mediterranea (Lamarck, 1816)

Antedon adriatica (A.H. Clark, 1911); *Comatula annulata* (Risso, 1826); *Comatula mediterranea* (Lamarck, 1816).

Diagnosis: The colour of this species varies from red to brown, orange, yellow and white. It has a central calyx, surrounded by more than ten fragile feathery arms (Cherbonnier 1956; Hayward and Ryland 2005). The number of its cirri does not exceed 40 and the number of the cirrals ranged from 20 to 23. They are claw-shaped to ensures its attachment to the substrate (Koehler 1921; Tortonese 1965; Beaumont and Cassier 2000).

Description: Calyx with conical form large and rounded in the top; feathery articulated arms composed of long and thin pinnules; pinnules are well curved; colour ranged from bright orange, yellow and red; more than 25 cirri long and poorly curved; cirri have between 18 to 23 long and large cirrals; very fragile animals; the majority of the collected material has broken arms.

Habitat and ecology: This species could be found from 10 to 80 meters deep. This benthic feather star lives free and sedentary, in rocky and sandy bottoms as well as in coral reefs (Beaumont and Cassier 2000; Hayward and Ryland 2005).

Distribution: It is a typical Mediterranean species, relatively abundant throughout this sea (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Examined material: 68 individuals were collected by benthic trawling between 50 and 190 meters deep. Their sizes ranged between 4 and 8 cm. They were collected from Tabarka (L1, S1), Bizerte (L2, S1), Kélibia (L8, S3).

Genus *Leptometra* A.H. Clark, 1908

Leptometra phalangium (Müller, 1841)

Alecto phalangium (Müller, 1841); *Antedon phalangium* (Müller, 1841); *Comatula phalangium* (Müller, 1841).

Diagnosis: This species has a dirty green, whitish or brownish colour. It has a fine feathery form. The arms, very long and thin with the appearance of a feathery plume, that could reach between 10 to 25 cm of diameter (Koehler 1921; Tortonese 1965; Ocaña *et al.* 2000). The number of the arms is usually never exceeding 13 and the most common is between 8 and 10 arms. They are held by a small conical calyx with a rounded apex limited by a centrodorsal plate. Cirri are long and thin they have 25 mm of diameters (Koehler 1921; Ocaña *et al.* 2000). Cirrals are thin and elongated, the distal segments are larger than the proximal one close to the center.

Description: Large and vigorous sea lily of greenish or whitish colour; centrodosrsal plate is large and conical; arms are long and cirris are large and thin; cirrals are elongated and thin; from 8 to 13 large and thin arms; pinnules are small and few in number.

Habitat and ecology: This species lives on sandy and muddy bottoms, from 60 to 1300 meters deep (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Distribution: It is an endemic species of the Mediterranean Sea (Koehler 1921; Tortonese 1965).

Examined material: 33 specimens were sampled in depths ranging from 72 to 194 meters, using the benthic trawl. As they were collected by trawling the majority of the individuals were broken and their diameter doesn't exceed 8 cm of diameter. They were collected from two localities, Tabarka (L1) and Bizerte (L2) from sector (1).

2.3.2.2. Asteroidea

This is the Class with the largest number of species, with 272 individuals belonging to 15 species of starfish, grouped in 10 genera and 7 families. Most of the identified species come from the family Astropectinidae with 5 species, followed by the family Asterinidae with only 3 species.

Class ASTEROIDEA Blainville, 1830

Order Forcipulatida Perrier, 1884
Family Asteriidae Gray, 1840

Genus *Coscinasterias* Verrill, 1867

Coscinasterias tenuispina (Lamarck, 1816)

Asteracanthion tenuispinum (Lamarck, 1816); *Asterias (Stolasterias) tenuispina* (Lamarck, 1816); *Asterias atlantica* (Verrill, 1868); *Asterias savaresi* (Delle Chiaje, 1827); *Asterias tenuispina* (Lamarck, 1816); *Stolasterias tenuispina* (Lamarck, 1816).

Diagnosis: This starfish, whose diameter does not exceed 15 cm, is an atypical star of irregular shape. It has unequal arms whose number range between 6 and 10 (Koehler 1921; Ocaña *et al.* 2000). The dorsal surface of the body is covered with irregularly arranged spines and each one is surrounded by a small pedicellaria (Bergbauer and Humberg 2000; Ocaña *et al.* 2000). Its colour is widely variable, usually white and brown, spotted with red, yellow, and often purple (Koehler 1921; Cherbonnier 1956).

Description: Atypical starfish with 7 to 8 irregular arms; aboral side is covered by thick spines with the presence of two madreporites; colour is white with red, purple and blue spots.

Habitat and ecology: It is a very common sea star which lives on rocky and algal bottoms of the infralittoral zone, from the surface up to more than one hundred meters deep (Koehler 1921; Cherbonnier 1956).

Distribution: It is an exotic species of tropical origin, accidentally imported into the Mediterranean. It is widely distributed across the Atlantic Ocean and the Mediterranean (Koehler 1921; Bergbauer and Humberg 2000; Ocaña *et al.* 2000).

Examined material: Two individuals, with 6 and 12 cm of diameters. They were found in sandy bottoms and collected by dredging and benthic trawling, in the depth between of 20 and 51 meters. They were collected respectively from Bizerte (L2, S1) and Kélibia (L8, S3).

Genus *Marthasterias* Jullien, 1878

Marthasterias glacialis (Linnaeus, 1758)
Asteracanthion glacialis (Linnaeus, 1758); *Asterias madeirensis* (Stimpson, 1862); *Asterias rarispina* (Perrier, 1875); *Asterias spinosa* (Pennant, 1777); *Asterias undulata* (O.F. Mueller, 1784); *Coscinasterias (Stolasterias) glacialis* (Linnaeus, 1758); *Marthasterias foliacea* (Jullien, 1878).

Diagnosis: This starfish is commonly large, its size ranging between 10 to 20 cm, although sometimes it could reach 40 cm (Koehler 1921; Bergbauer and Humberg 2000). It has 5 robust and rigid arms, rounded and tapered with spines. The hall body is covered by thick spines, spiky and surrounded at their bases by large grills (Bergbauer and Humberg 2000). The pedicellaria are so tiny and appear as small clear dots (Koehler 1921). Its colour is extremely variable depending on its location and habitat, it could be green, red, orange and even yellow, purple or pink (Koehler 1921; Bergbauer and Humberg 2000).

Description: A large starfish their size ranged from 12 to 18 cm; five robust, firm and curved arms; aboral side totally covered by large, pointed and thick spines; spines form two distinct rows; spines from oral side are much smaller and thin; body colour ranged from pink reed and purple.

Habitat and ecology: It is present from the first meters up to more than 200 meters deep, living in cavities on hard, rocky substrates, and on sandy or muddy bottoms (Koehler 1921; Cherbonnier 1956).

Distribution: It has a wide geographical distribution over the Mediterranean Sea and the Atlantic Ocean. It is present from the Norwegian coast to South Africa, through Cape Verde, Canary and the Azores Islands (Cherbonnier 1956; Bergbauer and Humberg 2000; Entrambasaguas 2008).

Examined material: 11 specimens from two stations of sandy, rocky and muddy substrates. The depths are ranging from 75 to 220 meters. Their sizes measured between 14 and 23 cm. They were sampled from two localities Kélibia (L8, S3) and Bizerte (L2, S1).

Order Valvatida Perrier, 1884

Family Asterinidae Gray, 1840

Genus *Anseropoda* Nardo, 1834

Anseropoda placenta (Pennant, 1777)

Anseropoda membranacea (Nardo, 1834); *Anseropoda membranacea* (Retzius, 1783); *Asterias cartilaginea* (Fleming, 1828); *Asterias palmipes* (Olivi, 1792); *Asterias placenta* (Pennant, 1777); *Asteriscus membranaceus* (Retzius, 1783); *Asteriscus placenta* (Pennant, 1777).

Diagnosis: It is a particularly flattened and completely pentagonal starfish with an irregular outline and very wide arms. Its body has an ambulacral furrow, fairly thin and straight (Koehler 1921; Bergbauer and Humberg 2000). Very often has orange colour on the median line of the arms and on their edges.

Description: Pentagonal shape; arms are poorly marked; body is thin, flattened and very fragile; median line of orange colour on the arms and the edge.

Habitat and ecology: This starfish is found on sandy and muddy bottoms between the depth of 5 and 200 meters (Koehler 1921; Tortonese 1965; Bergbauer and Humberg 2000).

Distribution: It lives in the Mediterranean and Atlantic, from the Faroe Islands to the coasts of west Africa and North Sea (Koehler 1921; Cherbonnier 1956; Bergbauer and Humberg 2000).

Examined Material: One single individual of 12 cm of diameter. It was collected by benthic trawling on muddy bottoms, between 185 and 220 meters deep in Bizerte (L2, S1).

Genus *Asterina* Nardo, 1834

Asterina gibbosa (Pennant, 1777)
Asterias exiguas (Delle Chiaje, 1827); *Asterias gibbosa* (Pennant, 1777); *Asterias papyracea* (Konrad, 1814); *Asterias pulchella* (de Blainville, 1834); *Asterias verruculata* (Bruzelius, 1805); *Asterina crassispina* (H.L. Clark, 1928); *Asterina minuta* (Nardo, 1834); *Asteriscus ciliatus* (Lorenz, 1860); *Asteriscus gibbosa* (Pennant, 1777).

Diagnosis: It is a small sea star that could reach 7 cm as maximum of diameter (Koehler 1921). It has a wide diversity of colours, ranging from yellow to brown through greenish, grey or beige. It has five very short arms with rounded ends and barely distinct from the body. The body of the animal is slightly bulging (Koehler 1921; Cherbonnier 1956; Tortonese 1965). It is formed of a thin epidermis and nested skeletal plates, with isolated or grouped short spines (Koehler 1921; Tortonese 1965).

Description: Small starfish of pentagonal shape; aboral side is slightly symmetric domed covered by small and thin spines; arms are small and relatively flat; oral view is characterized by the presence of ambulacral groove surrounded by a large number of tube fits.

Habitat and ecology: It is a photophilic benthic species, preferring sandy and rocky bottoms. Also under rocks and stones or on the *Posidonia oceanica* ((Linnaeus) Delile, 1813) meadows. It is present from the surface up to the depths of 100 meters (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Distribution: This sea star is frequently found in the Atlantic Ocean and Mediterranean Sea. It is a ubiquitous and polymorphic species (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Examined material: 13 specimens, ranging from 1 to 5 cm, collected by hand at the infralittoral level. They were found between red and green algae or under the rocks.

Specimens presented different shapes and colours that varies according to the substrate. They were found in three localities Carthage (Punic Harbor) (L3, S2), La Goulette (L4, S2), Radès (L5, S2).

Asterina pancerii (Gasco, 1876) *
Asteriscus pancerii Gasco, 1870; *Asterina gibbosa var. panceri* Koehler, 1924

Diagnosis: 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).

Description: 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.

Habitat and ecology: This species is typical of *Posidonia oceanica* ((Linnaeus) Delile, 1813) meadows (Ballesteros *et al.* 1987; Oliver *et al.* 1997; López-Márquez *et al.* 2018).

Distribution: Its endemic to the 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). This 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).

Examined material: Three specimens collected at a depth between 3-5 m associated with the seagrass *Cymodocea nodosa* (Ascherson, 1870) from Gulf of Tunis (L4, S2).

Family Chaetasteridae Sladen, 1889

Genus *Chaetaster* Müller and Troschel, 1840

Chaetaster longipes (Retzius, 1805)
Asterias longipes (Bruzelius, 1805); *Asterias subulata* (Lamarck, 1816); *Asterias verrucosa* (Risso, 1826); *Chaetaster tessellata* (Gray, 1840); *Nepanthia tesselata* (Gray, 1840).

Diagnosis: Starfish of 30 cm maximum diameter, coloured in yellow or orange, characterized by the presence of 5 long, thin and rounded arms with a very small central disc (Koehler 1921; Cherbonnier 1956). Aboral side devoid of pedicellaria and covered by numerous respiratory papula. Flatty oral side characterized by the presence of two ambulatory grooves sounded by two rages of podia (Clark and Downey 1992; Ocaña *et al.* 2000).

Description: Long-armed sea stars; small central disc; five long and round arms; thick aboral side with many papula; oral side with five ambulatory grooves with two rows of podia; colour ranged from yellow to orange.

Habitat and ecology: This species lives on rocky, sandy and muddy bottoms, beyond 40 meters deep and up to 600 meters (Koehler 1921; Cherbonnier 1956; Tortonese 1965; Entrambasaguas 2008).

Distribution: This starfish can be found throughout the Mediterranean and in the Atlantic, beyond the Strait of Gibraltar to the Canary and Azores Islands (Koehler 1921; Cherbonnier 1956; Tortonese 1965; Entrambasaguas 2008).

Examined material: There were 21 specimens identified. They measured from 6 to 12 cm of diameter. They were found on sandy and deep muddy bottoms between 70 and 170 meters deep. They were collected from Bizerte (L2, S1) and Tabarka (L1, S1) from sector (1) and Kélibia (L8, S3) from sector (3).

Family Ophidiasteridae Verrill, 1870

Genus *Hacelia* Gray, 1840

Hacelia attenuata (Gray, 1840)
*Asterias coriacea (*Grube, 1840); *Ophidiaster (Hacelia) attenuatus* (Gray, 1840); *Ophidiaster lessonae* (Gasco, 1876); *Ophidiaster superba* (H.L. Clark, 1921).

Diagnosis: Starfish with a smooth, satiny surface, its colour ranging from orange to red and marked by darker circular of longitudinal rows. It has a small disc and 5 cylindrical and conical arms. Its body is covered by calcareous plates regularly arranged in rows (Koehler 1921; Tortonese 1965; Bergbauer and Humberg 2000).

Description: Small central disc with five narrow arms; silky skin with orange colour; aboral side characterized by the presence of rows of circular longitudinal depressions.

Habitat and ecology: This species inhabits rocky and coralligenous bottoms, between 3 to 150 meters deep (Bergbauer and Humberg 2000; Entrambasaguas 2008).

Distribution: It is present in the Mediterranean Sea and also in the Atlantic Ocean (Koehler 1921; Tortonese 1965; Bergbauer and Humberg 2000; Entrambasaguas 2008).
Examined material: One single specimen of 8 cm of diameter, was found in Tabarka (L1, S1) on rocks, between 70 and 85 meters deep.

Order Paxillosida Perrier, 1884
Family Astropectinidae Gray, 1840

Genus *Astropecten* Gray, 1840

Astropecten aranciacus (Linnaeus, 1758)
Asterias aranciaca (Linnaeus, 1758); *Asterias aurantiaca* (Tiedemann, 1816); *Astropecten antarcticus* (Studer, 1884); *Astropecten aurantiaca* (Gray, 1840).

Diagnosis: It is the largest species of the comb starfish in the Mediterranean Sea, and could reach up to 60 cm (Koehler 1921; Bergbauer and Humberg 2000; Ocaña *et al.* 2000). Its dorsal surface is curved and equipped with numerous paxillae of orange-red to pale brown colour. It has a lateral row of spines, pointed and in white colour. It could be distinguished from the other species of genus *Astropecten* by its colour and its size (Ocaña *et al.* 2000; Pillon 2009).
Description: Large starfish of reddish colour; arms have large bases and become much thinner at their tips; tips not pointed; aboral side covered by paxillae red-orange to light brown; its devoid of pedicellaria; inferomarginal spines are long, pointed and strong; superomarginal plates have beige and colour equipped with one to three thin and small spines.
Habitat and ecology: This starfish inhabits sandy and muddy bottoms, from the surface up to hundreds of meters deep (Koehler 1921; Ocaña *et al.* 2000; Entrambasaguas 2008; Pillon 2009).
Distribution: This species occurs in the Atlantic and the Mediterranean (Koehler 1921; Bergbauer and Humberg 2000; Ocaña *et al.* 2000; Entrambasaguas. 2008).
Examined material: 24 individuals from 5 to 20 cm of diameter. They were collected by benthic trawling from sandy and muddy bottoms, in depths between 51 and 177 m from two localities Bizerte (L2, S1) and Kélibia (L8, S3).

Astropecten bispinosus (Otto, 1823)
Asteria bispinosa (Otto, 1823); *Astropecten myosurus* (Perrier, 1869).

Diagnosis: Its maximum diameter does not exceed 18 cm (Koehler 1921; Ocaña et al. 2000; Pillon 2009). Its arms are tapered and slandered. Their marked by five light grooves and they are lined with two rows of inferomarginal and superomarginalrigid spines (Koehler 1921; Tortonese 1965; Pillon 2009). The aboral side is covered with tiny brown paxillae. The oral side is lightly brown or orange and the ambulacral feet are devoid of suction cups. The supramarginal plates could raise 77 on each arms (Pillon 2009).

Description: *Astropecten* starfish with small disc and long and flat arms; aboral side covered by paxillae of dark brown colour; oral side is lightly coloured in yellow or orange; inferomarginal spines are long, large and not pointed; superomarginal plates were equipped with one large yellow or white spine; thin and small spines; pair of superomarginal spines between the arms are much longer than the other.

Habitat and ecology: This starfish usually lives on sandy soft bottom, as well as on detrital and shelly bottoms, from the surface to one hundred meters deep (Koehler 1921; Tortonese 1965; Pillon 2009).

Distribution: It is a typical Mediterranean starfish (Koehler 1921; Pillon 2009).

Examined material: 11 specimens were collected at depths between 1 to 35 meters by dredging and benthic trawling. Their diameters varied from 5 to 8 cm. They were found in the localities of Bizerte (L2, S1), Carthage (Punic Harbor) (L3, S2), La Goulette (L4, S2).

Astropecten irregularis (Pennant, 1777)
Asterias pentacantha (Delle Chiaje, 1827); *Astropecten muelleri* (Müller and Troschel, 1844).

Diagnosis: This common starfish is small, with the size between 5 and 15 cm. Its flattened body is covered with tiny yellow or pink paxillae with a slightly convex disc (Koehler 1921; Bergbauer and Humberg 2000; Ocaña *et al.* 2000). The lateral surface of the supramarginal plates is devoid of spines. the inferomarginals spines are short and thin of white colour. They aren't rigid and parallel as they are in the other *Astropecten* species (Bergbauer and Humberg 2000; Ocaña *et al.* 2000; Pillon 2009). The aboral side has very often a bulge on the center of the disc (Koehler 1921; Ocaña *et al.* 2000; Pillon 2009).

Description: Flattened starfish with slightly domed disc; small disc and long and flat arms; aboral side covered by paxillae of dark brown colour; oral side is lightly coloured in yellow or orange; inferomarginal spines are long, large and not pointed; superomarginal plates were equipped with one large yellow or white spine; thin and small spines; pair of superomarginal spines between the arms are much longer than the other.

Habitat and ecology: This species lives almost exclusively on sandy bottoms and *Posidonia* meadows, from the surface up to 400 meters deep (Koehler 1921; Bergbauer and Humberg 2000; Ocaña *et al.* 2000; Entrambasaguas 2008).

Distribution: Its distribution extends in the Atlantic, from Norway to Morocco, and in all the Mediterranean basins (Koehler 1921; Bergbauer and Humberg 2000; Ocaña *et al.* 2000; Entrambasaguas 2008).

Examined material: 33 specimens were sampled by benthic trawling at depths between 50 and 220 meters from Tabarka (L1, S1) and Bizerte (L2, S1) from sector 1 and Kélibia (L8, S3) from sector (3). They were found only on sandy substrates. Their sizes varied between 5 and 7 cm.

Astropecten jonstoni (Delle Chiaje, 1827)
Asterias jonstoni (Delle Chiaje, 1827); *Astropecten squamatus* (Muller and Troschel, 1844).

Diagnosis: It is the smallest species of *Astropecten* in the Mediterranean Sea, its size doesn't exceed 8 cm (Koehler 1921; Pillon 2009). The dorsal surface, flattened and pierced by a single madreporic plate. The paxillae has a colour that varied from gray, green to pale blue (Koehler 1921; Ocaña *et al.* 2000; Pillon 2009). It has a very large disc comparing to the other *Astroprcten* species. The arms are court, triangular and very pointed. The superomarginal plates are devoid or equipped with one single spine. The inferomarginal plates have short and external spines (Koehler 1921; Ocaña *et al.* 2000; Pillon 2009).

Description: Small specimen; aboral side very flattened with gray colour; central disc is very large; arms have a triangular shape; arms are pointed at their ends; superomarginal plates are equipped one short spine; inferomarginal plates are equipped with double row of rigid and pointed spines; absence of spines between the arms.

Habitat and ecology: This species is almost exclusively found on sandy bottoms, *Posidonia* and seagrass meadows, from the surface up to a dozen meters deep (Koehler 1921; Ocaña *et al.* 2000; Pillon 2009).

Distribution: It is endemic to the Mediterranean Sea (Koehler 1921; Ocaña *et al.* 2000; Pillon 2009).

Examined material: Only one single specimen with 4 cm of diameter, collected by dredging from sandy bottoms between 3 and 5 meters of deep in the locality of La Goulette (L4, S2).

Genus *Tethyaster* Sladen, 1889

Tethyaster subinermis (Philippi, 1837)
Archaster subinermis (Philippi, 1837) ; *Asterias subinermis* (Philippi, 1837) ; *Astropecten subinermis* (Philippi, 1837) ; *Goniopecten subinermis* (Philippi, 1837).

Diagnosis: It is a large starfish, whose diameter can exceed 40 cm. Its disc is very wide and its arms are long, large at their bases and pointed at their ends (Koehler 1921; Ocaña *et al.* 2000). Its dorsal surface is covered with several tight paxillae and has a colour that varies between light orange to light pink. The paxillae form on the arms has are transversal and parallel series (Koehler 1921; Ocaña *et al.* 2000).

Description: Flattened starfish; central disc is very large; arms are triangular, long and very pointed at their ends; aboral side covered with small paxillae with light orange to light pink colour; marginal plates are equipped with very small spines; oral surface has whitish colour.

Habitat and ecology: This species inhabits sand and mud bottom from 40 meters to great depths (Koehler 1921; Ocaña *et al.* 2000).

Distribution: It occurs in the Mediterranean Sea and in the eastern Atlantic (Koehler 1921; Ocaña *et al.* 2000).

Examined material: 39 individuals from 4 to 15 cm of diameter, collected by benthic trawling from 50 to 220 deep on sand and mud substrate from the localities of Tabarka (L1, S1), Bizerte (L2, S1) and Kélibia (L8, S3).

Family Luidiidae Sladen, 1889

Genus *Luidia* Forbes, 1839

Luidia atlantidea (Madsen, 1950) *
Luidia africana Doderlein, 1920; *Luidia atlantidea* Madsen, 1950

Diagnosis: 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; Chammem *et al.* 2019). The diameter is about 6 cm.

Description: 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.

Habitat and ecology: It lives in muddy, sandy and rocky substrates between 10 and 80 m deep (Entrambasaguas 2008).

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; Chammem *et al.* 2019).

Examined material: One specimen collected on sand at a depth between 65 and 95 meters in Kélibia (L8, S3).

Luidia sarsii sarsii (Düben and Koren in Düben, 1844)
Luidia paucispina (von Marenzeller, 1893); *Luidia sarsi* (Düben and Koren in Düben, 1844).

Diagnosis: *L. sarsii sarsii* has a flattened body with a very small disc and long and narrow arms. At the sides of the arms there are two distinct longitudinal rows. The dorsal surface is covered by extremely tight paxilles and constituted by fine spines. It has darker brown line in the middle of each arm (Koehler 1921; Tortonese 1965; Clark and Downey 1992).

Description: Flat body with small disc; five long and thin arms; dark line in the middle of each arms; arms bordered on both sides by large whitish spines; aboral side covered by small and very tight paxillae; abactinal paxillae with three marginal longitudinal series on each side.

Habitat and ecology: It inhabits mainly the muddy but also the sandy and rocky bottoms, between 10-1000 meters of deep (Koehler 1921; Cherbonnier 1956; Entrambasaguas 2008).

Distribution: It is a common species in the Mediterranean and Atlantic (Koehler 1921; Entrambasaguas 2008).

Examined material: A single individual that was collected by benthic trawling from muddy bottom between 175 and 193 meters of deep, in the locality of Tabarka (L1, S1).

Order Spinulosida Perrier, 1884

Family Echinasteridae Verrill, 1867

Genus *Echinaster* Müller and Troschel, 1840

Echinaster (Echinaster) sepositus (Retzius, 1783)
Asterias seposita (Retzius, 1783); *Cribrella seposita* (Retzius, 1783); *Echinaster sagenus* (Bruzelius, 1805); *Echinaster sepositus* (Retzius, 1783); *Rhopia seposita* (Retzius, 1783).

Diagnosis: It has a brighter or orange-red colour. It has 5 long arms, cylindrical and are covered with many small papules arranged irregularly on the aboral side. Oral side is much lighter in colour and it has from two to three longitudinal grooves of ambulacral feet. Its diameter is between 10 to 20 cm (Koehler 1921; Tortonese 1965; Clark and Downey 1992; Entrambasaguas 2008).

Description: Small disc with five long, cylindrical and slightly conical arms; coloured in bright orange or red; aboral side is totally covered by papulae; abactinal plate has from 3 to 5 pores; nodal plates are equipped with one small and thin spine; supromarginal plates are armed by two spines; oral side has lighter orange colour; amdambulacral plates are equipped in general with two spines; two lines of margin groove in each arm.

Habitat and ecology: It lives on all types of substrates from the surface to great depths (Koehler 1921; Cherbonnier 1956; Bergbauer and Humberg 2000; Entrabasaguas 2008).

Distribution: This species is mainly found in the Mediterranean and in the eastern Atlantic (Koehler 1921; Cherbonnier 1956; Bergbauer and Humberg 2000; Entrabasaguas 2008).

Examined mmaterial: Over 110 specimens their size is between 4 and 12 cm. They were collected by trawling on sandy, muddy and rocky bottoms between 50 and 220 meters deep in Bizerte (L2, S1) and Kélibia (L8, S3). Also some specimens were collected by diving in Tabarka (L1, S1) between 3 and 5 meters of deep.

2.3.2.3. Ophiuroidea

This class had the largest number of studied specimens, with 483 individuals belonging to 10 species distributed respectively in 9 genera and 9 families.

Class OPHIUROIDEA Gray, 1840

Order Euryalida Lamarck, 1816

Family Gorgonocephalidae Ljungman, 1867

Genus *Astrospartus* Döderlein, 1911

Astrospartus mediterraneus (Risso, 1826
Astrospartus arborescens (L. Agassiz, 1839); *Euryale arborescens* (L. Agassiz, 1839); *Euryale mediterraneus* (Risso, 1826); *Gorgonocephalus verrucosus* (Grube, 1840).

Diagnosis: This basket star has the shape of a gray or beige ball with 10 strong and branched arms. They have a curled end. It has a small and pentagonal disc of 5 to 8 cm of diameter. It sizes ranged between from 6 to 20 cm (Koehler 1921; Cherbonnier 1956; Bergbauer and Humberg 2000).

Description: It Has the form a basket of gray colour; Small disc with the form of star; ten strong and highly branched arms.

Habitat and ecology: This ophiuroid lives from 50 meters to great depths, on muddy and sandy bottoms also hanging on the gorgonians and sponges (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Distribution: It is widely distributed from the western Mediterranean to the eastern Atlantic Ocean (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Examined material: Two individuals were collected by benthic trawling from sandy bottoms, between 93 and 105 meters of deep in the water of Kélibia (L8, S3).

Order Ophiurida Müller and Troschel, 1840

Family Amphiuridae Ljungman, 1867

Genus *Amphipholis* Ljungman, 1866

Amphipholis squamata (Delle Chiaje, 1828)
Amphioplus squamata (Delle Chiaje, 1828); *Amphipholis elegans* (Farguhar, 1897).

Diagnosis: It's a very small ophiuroid. It discs diameter doesn't exceed 5 mm while their arms can reach 20 mm in length. The disc has a scaly appearance with highly variable colour. *A. squamata* is a polymorphic species. It has between 6 to 8 short and conical spines per segment and two radials shields in the beginning of each arm (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Description: A tiny brittle star; small disc of 3 to 5 mm of diameters; five long, thin and fragile arms; each segment is equipped by three or four spines on both side of the arm;

aboral view the disc covered by many small plates giving it a scaly appearance; presence of two small contiguous radial plates at the beginning of each arm.

Habitat and ecology: It is found from the surface up to 250 meters of deep. It lives under rocks, between the algae, and sponges, or in sandy and muddy substrates (Koehler 1921; Cherbonnier 1956; Tortonese 1965; Entambasaguas 2008).

Distribution: It is a cosmopolitan species, worldwide distributed (Koehler 1921; Cherbonnier 1956; Tortonese 1965; Entambasaguas 2008).

Examined material: 16 small specimens where the diameter of the disc didn't exceed 4 mm. They were found among algae and sponge or under rocks in the localities of Carthage (Punic Harbor) (L3) and Radès (L5) from sector 2 (S2).

Family Ophiacanthidae Ljungman, 1867

Genus *Ophiacantha* Müller and Troschel, 1842

Ophiacantha setosa (Bruzelius, 1805)
Asterias setosa (Bruzelius, 1805); *Ophiacantha scabra* (M. Sars, 1859); *Ophiolimna setosa* (Bruzelius, 1805); *Ophiura setosa* (Bruzelius, 1805).

Diagnosis: Ophiuroid with a pentagonal disc that doesn't exceed 1 mm of diameter. The arms are very long and thin (up to 91 mm). The dorsal disc surface is covered with small and thin spines and is provided by 5 radial shields at the extremities. The lateral arm shields are conical and pointed. The aboral arm shields are triangular and the adoral ones have a pentagonal shape. Its colour is usually brown or purplish brown (Koehler 1921; Mortensen 1927; Cherbonnier 1956).

Description: A tiny and fragile ophiuroid; small disc of a pentagonal shape; five long and thin arms; diameter of the arms 8 or 10 times equal to the disc diameter; disc covered by very small and thin spines; a small couple of radial shields at the extremity of the disc; four lateral arm shields are conical and pointed.

Habitat and ecology: It inhabits rocky and muddy bottoms, between 40 to1500 meters deep (Koehler 1921; Cherbonnier 1956).

Distribution: Species are present in the Mediterranean and Atlantic, from the Bay of Biscay to West Africa (Koehler 1921; Cherbonnier 1956).

Examined material: A total of 54 specimens were collected by benthic trawl, attached to gorgonians in depths between 70 and 165 meters from the locality of Tabarka (L1, S1).

Family Ophiactidae Matsumoto, 1915

Genus *Ophiactis* Lütken, 1856

Ophiactis savignyi (Müller and Troschel, 1842)
Ophiactis brocki (de Loriol, 1893); *Ophiactis conferta* (Koehler, 1905); *Ophiactis krebsii* (Lütken, 1856); *Ophiactis maculosa* (von Martens, 1870); *Ophiactis reinhardti* (Lütken, 1859); *Ophiactis sexradia* (Grube, 1857); *Ophiolepis sexradia* (Grube, 1857).

Diagnosis: The dorsal disc face is covered with imbricated scales and small spines. The spines are very numerous at the edges of the disc. It is bordered by 6 large and triangular dark brown radial shields of dark. Their diameter is more than the half of the size of the disc. It has 6 articulated arms that are long, thin and tapered. They are equipped with four or five arms spines. The colour is usually varying between green and brown. The disc does not exceed 9 mm of diameters (Tortonese 1965; Entambasaguas 2008; Gondim e*t al.* 2013)

Description: Small brittle star with six arms; disc never exceed the 6 mm; rounded disc covered by imbricated scales; radial shields are large and triangular; size of radial shields could rich two third of the disc diameter; each couple of radial shields are distally separated; aboral side covered by small and tipped spines; oral shields have a sub-lozenge shape; adoral side equipped by one to two spatulas of oral papillae in both side of the jaw; lateral arm plates with four or five arm spines; coloured in olive green with dark brown; radial shields with white spots.

Habitat and ecology: They are found among algae, on sponge and coral or under rocks, being very common for shallow water, but they could be also found in deep waters (Tortonese 1965; Entambasaguas 2008; Alvarado and Solis-Marin 2013; Prata *et al.* 2017).

Distribution: A cosmopolitan species (Tortonese 1965; Entambasaguas 2008).

Examined material: One single specimen was found on *Cymodocea* meadow between 3 and 5 meters deep in La Goulette (L4, S2).

Ophiactis virens (M. Sars, 1857) *
Amphiura virens M. Sars, 1859

Diagnosis: 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).

Description: 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.

Habitat and ecology: It could be found from shallow water to up to 90 meters deep (Tortonese 1965; Koehler 1969; Entambasaguas 2008)

Distribution: Atlantic and Mediterranean. It has been recorded from the west coast of Africa to the archipelagos of Azores, Madeira, Cape Verde and Gulf of Gascony (Marques 1980; Entrambasaguas 2008), Italy (Koehler 1921; Tortonese 1965) and Turkey (Özaydın *et al.* 1995; Öztoprak *et al.* 2014).

Examined material: Over 184 individuals collected in algae at depths between 0,40-0,60 m, in the locality of Radès (L5, S2).

Family Ophiocomidae Ljungman, 1867

Genus *Ophiocomina* Koehler, 1920 in Mortensen

Ophiocomina nigra (Abildgaard in O.F. Müller, 1789)
Asterias nigra Abildgaard (in O.F. Müller, 1789); *Ophiacantha danae* (Mortensen, 1933); *Ophiocoma granulata* (Forbes, 1839); *Ophiocoma nigra* (Abildgaard in O.F. Müller, 1789).

Diagnosis: This black ophiuroid has a slightly rounded to pentagonal disc, it could reach 4 cm of diameter. Its dorsal surface is finely granulated. It has five long arms, with 3 times the disc diameter. They are thin and segmented. It has between 5 to 7 fine and smooth spines in each side of the arm lateral shield. Its spines have a lightly brown colour. It is dark in colour, most often intense black or brown (Koehler 1921; Mortensen 1927; Cherbonnier 1956; Bergbauer and Humberg 2000).

Description: Big brittle star of black colour; central disc slightly rounded of 2 cm of diameter; five long, flexible and thin arms; oral shields have an oval shape; four oral papillae in the edges of the jaws; lateral arm shields equipped with 6 to 7spines.

Habitat and ecology: It lives on rocky, coralligenous and muddy substrates from the intertidal zone to up 400 meters deep (Koehler 1921; Mortensen 1927; Cherbonnier 1956).

Distribution: It is found from the Scandinavian coasts to the Mediterranean Sea and Azores (Koehler 1921; Mortensen 1927; Cherbonnier 1956).

Examined material: Only one specimen was collected by benthic trawling on muddy substrates between 50 and 58 meters deep in the water of Tabarka (L1, S1).

Family Ophiodermatidae Ljungman, 1867

Genus *Ophioderma* Müller and Troschel, 1840

Ophioderma longicauda (Bruzelius, 1805)
Asterias longicauda (Retzius, 1805); *Ophioderma lacertosum* (Lamarck, 1801).

Diagnosis: Large brittle star with a slightly convex and pentagonal disc. Its disc diameter is between 2 to 5 cm. Five long, thin and cylindrical arms, animated by fast and vigorous movements. Its ventral brachial plates are more or less visible through the integument. The arm spines are short and folded, which gives them smooth and pectinate appearance. Its colour ranged from orange-red to black. Currently, it has a dark disc and its arms surface have the alternation of dark and light green bands (Koehler 1921; Mortensen 1927; Bergbauer and Humberg 2000; Entrambasaguas 2008).

Description: Large ophiuroid; pentagonal and convex disc; aboral side is very smooth; five long cylindrical and flexible arms; dark brown disc with white spots; arm alternating between light green and dark bands; very short and numerous arm spines; adoral side light in colour; oral shields devoid of papillae.

Habitat and ecology: It is a sciaphilic animal that is found on sandy, rocky and coralligenous bottoms from the surface to up 70 m deep (Mortensen 1927; Cherbonnier 1956; Entrambasaguas 2008).

Distribution: This ophiuroid is found in the West African coasts, as well as in the Mediterranean Sea and the tropical and eastern Atlantic Ocean (Koehler 1921; Mortensen 1927; Cherbonnier 1956; Entrambasaguas 2008).

Examined material: One single specimen with 12 cm of diameter, collected by hand from the rocks at midlittoral level from the locality of Haouaria (L7, S3).

Family Ophiomyxidae Ljungman, 1867

Genus *Ophiomyxa* Müller and Troschel, 1840

Ophiomyxa pentagona (Lamarck, 1816)
Ophiomyxa lubrica (Forbes, 1843); *Ophiura pentagona* (Lamarck, 1816).

Diagnosis: Species with a pentagonal disc relatively large, it measures between 2 to 5 cm. The arms are long, cylindrical, thin and flexible. Its ventral brachial plates are more or less visible through the integument. The oral shields papillae are from four to five they are short and flat. They become much larger at the end of the jaw. Its colour varies from very dark brown to red and orange. The arms could have a uniform colour or they have the alternation of light and dark bands (Koehler 1921; Mortensen 1927; Tortonese 1965; Bergbauer and Humberg 2000).

Description: Flat and pentagonal disc; soft disc surface; five long and cylindrical arms with 5times disc diameter; five pentagonal oral shields covered by thin tegument on the adoral side; five to four short and flat oral shields papillae that become gradually much larger at the ends of the jaws; disc coloured in dark brown, orange or red; arms have a uniform colour or the alternation between light and dark bands.

Habitat and ecology: This ophiuroid frequents rocks, sands and muds, usually between 50 and 1000 meters deep (Koehler 1921; Mortensen 1927; Tortonese 1965; Entrambasaguas 2008).

Distribution: It is very common in the Mediterranean Sea and the Atlantic Ocean (Koehler 1921; Mortensen 1927; Tortonese 1965; Entrambasaguas 2008).

Examined material: A total of 81 specimens were collected by benthic trawling from sandy, muddy and rocky bottoms, between 50 and 210 meters deep from the localities of Tabarka (L1, S1), Bizerte (L2, S1) and Kélibia (L8, S3).

Family Ophiotrichidae Ljungman, 1867

Genus *Ophiothrix* Müller and Troschel, 1840

Ophiothrix quinquemaculata (Delle Chiaje, 1828)
Ophiura quinquemaculata (Delle Chiaje, 1828)

Diagnosis: It is a very fragile ophiuroid with small disc, between 10 and 15 mm of diameter with very long arms, equal to 8 or 10 times of disc diameter. The disc is equipped with very thin spines pointed and elongates. It is easily identified by the presence of a very large triangular and long radial shields. Its arms have 6 arms spines colorless, pointed, elongated and more or less variable in length. They are often ringed from pink to gray. The disc is yellowish gray, pink or greenish (Koehler 1921; Weinberg 2007).

Description: Small circular disc, never exceed 15 mm of diameter; couple of five radial shields at the extremity of the disc; radial shields are triangular and long; disc covered by thin, elongated and pointed spines, except the radial shields which are without of spines; 5 long, thin, fragile and spiny arms, eight to ten time diameter disc; 6 thin, transparent, strong and tight arm spines in each lateral arm shield; first and the sexist arm spines are much shorter than the third, fourth and fifth ones; colour of the disc ranged from pink to gray; arms with the alternation of two different colour.

Habitat and ecology: This species prefers muddy and sandy substrates, from 40 meters deep (Koehler 1921; Weinberg 2007).

Distribution: It is very common in the Mediterranean (Koehler 1921; Weinberg 2007).

Examined material: 21 individuals were collected by benthic trawl between 72 and 175 meters deep, from sandy rocky and muddy bottoms. They were found in Tabarka (L1, S1) and Bizerte (L2, S1).

Family Ophiuridae Müller and Troschel, 1840

Genus *Ophiura* Lamarck, 1801

Ophiura ophiura (Linnaeus, 1758)
Ophiura ciliaris (Linnaeus, 1766); *Ophiura lacertosa* (Pennant, 1777); *Ophiura texturata* (Lamarck, 1816); *Ophiura textura* (Linnaeus, 1758).

Diagnosis: It is a big and robust ophiuroid, whose central disc measures between 35 to 40 mm. The radial shields are large and their diameter is about the half of disc radius. The length of the five arms is equal to four times disc diameter. They are strong and very thick at the base and tapered at their ends, equipped by three pairs of short and flat spines. Its colour varies from light pink, orange to dark brown. The aboral is convex while the adoral surface is flat. They are covered by several small plates arranged in mosaic (Koehler 1921; Mortensen 1927; Tortonese 1965; Weinberg 2007).

Description: Large circular disc with a smooth surface; disc plates are small and arranged in mosaic; triangular radial shield, large at their bases; five strong arms, thick at the bases and tapered at their ends; arms comb with thin papillae; lateral arm shield equipped with three short and strong arm spines; adoral side covered by light yellow or white plates; two pores in each side of the ventral plates at the base of the arm; about 4 to 6 oral shield papillae in each side of the jaw; colour varied from light pink to light brown and orange.

Habitat and ecology: This ophiuroid prefers fine sandy-muddy bottoms as well as gravelly bottoms and more or less coarse shell debris from the shallow circalittoral level to great depths (Koehler 1921; Mortensen 1927; Cherbonier 1956; Tortonese 1965).

Distribution: It is a cosmopolitan species, worldwide distributed (Koehler 1921; Mortensen 1927; Cherbonier 1956; Tortonese 1965).
Examined material: 124 specimens sampled by dredging and benthic trawling from 3 to 194 meters of deep. They were found in Tabarka (L1, S1), Bizerte (L2, S1), La Goulette (L4, S2) and Kélibia (L8, S3).

2.3.2.4. Echinoidea

A total of 383 specimens from 8 species (8 genera and 7 families) of Echinoidea were studied. Only one of these species is an irregular sea urchin (*Spatangus purpureus* O.F. Müller, 1776), while the others are regular sea urchins. More than half of the studied specimens belongs to the family Cidaridae represented by the two most abundant species, *Cidaris cidaris* (Linnaeus, 1758) and *Stylocidaris affinis*.

Class ECHINOIDEA Bronn, 1860

Order Arbacioida Gregory, 1900
Family Arbaciidae Gray, 1855

Genus *Arbacia* Gray, 1835

Arbacia lixula (Linnaeus, 1758)
Arbacia pustulosa (Ludwig, 1789); *Echinus lixula* (Linnaeus, 1758); *Cidaris pustulosa* (Leske, 1778); *Echinus pustulosa* (Ludwig, 1789); *Echinus loculatus* (Blainville, 1825).

Diagnosis: It is a regular sea urchin covered with smooth and pointed spines coloured in black purple or dark brown. They have 3 cm of diameters. The test is hemispherical with a gray or light pink colour. Its aboral side characterized by the presence of 4 or 5 large subtriangular genital plates with three gonopores. Its diameter measures between 4 to 6 cm. It has over 5 brown bands surrounded by 4 bands of pore arcs (holes for tube feet) (Koehler 1921; Tortonese 1965; Bergbauer and Humberg 2000; Weinberg 2007).
Description: Test with hemispherical shape; flat oral side; dense, pointed and smooth spines; spines with 3 cm of diameter; mouth is devoided of spines; test without spine has a gray to light pink colour; spines of brown or intense purplish black colour.
Habitat and ecology: This sea urchin inhabits shallow water from the surface to up 15 meters deep, sometimes even up to 50 meters, living mainly on rocks and sands (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Distribution: It is very common and can be found throughout the Mediterranean basin. It has been also reported in the coasts of Ireland and West of Great Britain (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Examined material: 12 specimens were collected by hand and dredging from rocks and sands, from the surface to 5 meters deep, from the localities of Tabarka (L1, S1), Bizerte (L2, S1) and Haouaria (L7, S3).

Order Cidaroida Claus, 1880
Family Cidaridae Gray, 1825

Genus *Cidaris* Leske, 1778

Cidaris cidaris (Linnaeus, 1758)

Cidaris (Dorocidaris) papillata (Leske, 1778); *Cidaris (Stephanocidaris) cidaris* (Linnaeus, 1758); *Cidaris borealis* (Düben, 1844); *Cidaris histrix* (Lamarck, 1816); *Cidaris papillata* (Leske, 1778); *Dorocidaris papillata* (Leske, 1778); *Echinus cidaris* (Linnaeus, 1758).

Diagnosis: It is a regular sea urchin that has long and large spines, quite numerous and robust, rigid and with light brown or gray colour. Their size is 3 times test diameter. The test is formed by ambulacral plates with two pores and a single primary tubercle. The apical disc from the aboral side has five genital plates with five gonopores. The test diameter is between 1 to 7 cm. It has a light gray to light pink colour (Koehler 1921; Tortonese 1965; Bergbauer and Humberg 2000).

Description: Very large and long spines; size of the spine is 3 times test diameter; coloured in gray or light brown; spines are covering all the test; small test; ambulacral plates have double pores for tube-feet and one large primary tubercle; perforated tubercles.

Habitat and ecology: This species is usually found in hard substrates and on sandy and muddy bottoms from 30 to 1000 meters (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Distribution: It is present in the Mediterranean, Atlantic and Caribbean (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Examined material: 114 individuals, of 4 to 6 cm in diameter. They were collected by benthic trawling between 50 and 220 meters deep on sandy, muddy and rocky bottoms, from the localities of Tabarka (L1, S1), Bizerte (L2, S1) and Kélibia (L8, S3).

Genus *Stylocidaris* Mortensen, 1909

Stylocidaris affinis (Mortensen, 1909)
Cidaris affinis (Philippi, 184); *Cidaris stockesii* (Dujardin and Hupé, 1862); *Leiocidaris affinis* (Dujardin and Hupé, 1862); *Leiocidaris stockesii* (Dujardin and Hupé, 1862).

Diagnosis: It is a regular sea urchin that has long and large spines. The length of primary radioles is equal or longer than the test, they are large and conical with light brown colour. They are surrounded at their base very strong secondary spine. The test colour varied from orange to red. The test diameter is between 3 to 5 cm. The ambulacral plate has two pores and the interambulacral ones have one primary tubercle. The apical disc, round or pentagonal, composed of more than five genital plates with five gonopores (Koehler 1921; Tortonese 1965; Bergbauer and Humberg 2000; Weinberg 2007).

Description: Large, conical and thick spines; small test between 3 to 5 cm of diameter; ambulacral plates with two pores; interambulacral plates equipped with one large and long primary tubercle; perforated tubercles; apical disc with more than five genital plates; five gonopores; test colour varied from light orange to red.

Habitat and ecology: It inhabits coralligenous, rocky bottoms and in general hard and soft substrates from 30 to 1000 meters deep (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Distribution: It is only present in the Mediterranean Sea (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Examined material: Over 114 specimens (4-7 cm diameter) were collected by benthic trawling between 50 and 220 meters deep on rocky and sandy substrates, from of Tabarka (L1, S1), Bizerte (L2, S1) and Kélibia (L8, S3).

Order Diadematoida Duncan, 1889

Family Diadematidae Gray, 1855

Genus *Centrostephanus* Peters, 1855

Centrostephanus longispinus (Philippi, 1845)
Diadema longispinna (Philippi, 1845); *Diadema europaeum* (Dujardin and Hupé, 1862).

Diagnosis: It is a regular brown to black sea urchin, with very long, thin and fragile spines of white and purple brown colour. Its test is flat and conical with 7 cm of maximum diameter. The ambulacral plate has three pairs of pores (Koehler 1921; Cherbonnier 1956: Tortonese 1965; Bergbauer and Humberg 2000; Weinberg 2007).

Description: Long, thin and fragile spines; spines with the alteration of two colour, brown and white colour; conical and fragile test; perforated tubercles.

Habitat and ecology: It lives on hard substrates and sandy or muddy bottoms from 50 m to great depths (Koehler 1921; Tortonese 1965).

Distribution: This species is mainly located throughout the Mediterranean (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Examined material: 74 individuals of 4-8 cm diameter were collected by benthic trawl from sandy, muddy and rocky substrates between 50 and 220 meters deep from Kélibia (L8, S3), Bizerte (L2, S1).

Order Camarodonta Jackson, 1912

Family Echinidae Gray, 1825

Genus *Gracilechinus* Fell and Pawson in Moore, 1966

Gracilechinus acutus (Lamarck, 1816)
Echinus acutus (Lamarck, 1816); *Echinus acutus acutus* (Lamarck, 1816); *Echinus acutus mediterraneus* (Mortensen, 1906); *Echinus acutus var. mediterraneus* (Diakonov, 1923).

Diagnosis: Regular sea urchin with large size, it could exceed 15 cm of diameter. It has a conical and pentagonal shape with the alternation of dark red and white longitudinal bands. The spines are numerous, thin and with two different sizes. The secondary spines are much shorter than the primary ones. The tubercles are imperforated (Koehler 1921; Tortonese 1965; Bergbauer and Humberg 2000; Weinberg 2007).

Description: Large sea urchin; white test with five reddish bands; round and subpentagonal test; spine with reddish tips; numerous spines of two different size; spines cover all the test; long and thin primary spines; secondary spines are much shorter with white or red brown colour; each two or three interambulacral plates have one primary tubercle; imperforated tubercles.

Habitat and ecology: This sea urchin lives on muddy, mixed and sandy bottoms, from 20 m to greater depths. It also frequents rocks and coralligenous (Koehler 1921; Cherbonnier; Tortonese 1965; Bergbauer and Humberg 2000).

Distribution: This species is found in all the Mediterranean Sea, and in the Atlantic Ocean too (Koehler 1921; Cherbonnier 1956; Tortonese 1965; Bergbauer and Humberg 2000).

Examined material: A total of 18 individuals were collected by benthic trawl, between 50 and 125 meters deep and on sandy and rocky substrates. Their sizes varied from 7 to 14 cm. They were found in Kélibia (L8, S3).

Family Parechinidae Mortensen, 1903

Genus *Paracentrotus* Mortensen, 1903

Paracentrotus lividus (Lamarck, 1816)
Echinus saxatillis (Tiedeman, 1815); *Echinus lividus* (Lamarck 1816); *Echinus vulgaris* (Blainville, 1825); *Toxopneuste complanatus* (Agassiz and Desor, 1846).

Diagnosis: Common sea urchin with a rounded test slightly depressed, whose size does not exceed 8 cm, its colour varied from green to purple. It has long spines around 3 cm of diameters, smooth and thick, ranging from dark purple to brownish or olive green colour. It also has imperforate tubercles and ambulacral plates composed from 5 to 6 pairs of pores. The suranale plates have variable size and they are surrounded with 5 large subtriangular genital plates (Koehler 1921; Cherbonnier 1956; Tortonese 1965; Bergbauer and Humberg 2000; Weinberg 2007).

Description: Rounded test with flattened oral side; imperforated tubercles; from 5 to 6 pairs of ambulacral plate; aboral side characterized by the presence of 5 large subtriangular genital plates; numerous suranale plates of variable size; long, think and smooth spines.

Habitat and ecology: This common sea urchin lives on rocky bottoms, *Posidonia* meadows or on sandy and coralligenous bottoms up to 30 m (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Distribution: Very common in the Mediterranean Sea, although it is also found in the Atlantic Ocean (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Examined material: 48 individuals with 1 to 7 cm of diameter collected by hand and dredging from algae, and rocks at the mid and infralittoral levels from the localities of Tabarka (L1, S1), Bizerte (L2, S1) and Haouaria (L8, S3).

Family Toxopneustidae Troschel, 1872

Genus *Sphaerechinus* Desor, 1856

Sphaerechinus granularis (Lamarck, 1816)

Echinus albidus (L. Agassiz, 1841); *Echinus granularis* (Lamarck, 1816); *Echinus subglobiformis* (Blainville, 1825); *Strongylocentrotus granularis* (Lamarck, 1816).

Diagnosis: It is a regular sea urchin, whose size is up to 8 cm in diameter, with a very variable colour ranging from dark purple to pink or reddish brown. It has a globular, quite high and hemispherical test, slightly flattened on the oral side. Its spines are quite short and thick, and may have white tips or be totally white. The ambulacral plate is composed of 3 pairs of pores and the tubercles are imperforated (Koehler 1921; Cherbonnier 1956; Tortonese 1965; Bergbauer and Humberg 2000; Weinberg 2007).

Description: Globular sea urchin; short, thick and tight spines of purple colour; spine with white tip; test is rounded, high and hemispherical; imperforated tubercles; ambulacral plate with 3 pairs of pores.

Habitat and ecology: It lives in various habitats, such as soft bottoms, marine meadows or rocks, from the surface to up to 100 meters deep (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Distribution: This sea urchin is found in the Mediterranean, the Atlantic coasts of Europe and the English Channel (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Examined material: Two specimens between 8 and 12 cm of size, collected by diving from Haouaria (L7, S3).

Order Spatangoida L. Agassiz, 1840
Family Spatangidae Gray, 1825

Genus *Spatangus* Gray, 1825

Spatangus purpureus (O.F. Müller, 1776)
Prospatangus purpureus (O.F. Müller, 1776); *Spatagus purpureus* (O.F. Müller, 1776); *Spatangus spinosissimus* (Desor in L. Agassiz and Desor, 1847b).

Diagnosis: This irregular purple sea urchin is large and with big heart shape. It has a flat oral side and rounded adoral side. It is covered by two types of spines, the longest are transparent in colour. The apical side is devoid of anus and teeth. The madreporite is surrounded by 5 gonopores. The ambulacral area has a petaloid shape. Its size varies between 10 to 12 cm (Cherbonnier 1956; Tortonese 1965; Bergbauer and Humberg 2000; Weinberg 2007).

Description: Large irregular sea urchin; two type of spines; longest spines have a beige colour; flat test with heart shape; perforated tubercles; test of purple colour; rounded aboral side; flattened oral side.

Habitat and ecology: It inhabits the sandy and muddy bottoms between 5 to 900 meters deep (Bergbauer and Humberg 2000; Weinberg 2007).

Distribution: This species is found in all Mediterranean and Atlantic coasts (Bergbauer and Humberg 2000; Weinberg 2007).

Examined material: One single broking specimen, was found on sand, between 3 and 5 meters deep in the locality of Tabarka (L1, S1).

2.3.2.5. Holothuroidea

This Class is represented by 9 species belonging to 4 genera, 3 families and 2 orders. The family Holothuriidae has the most abundant in numbers of specimens.

Class HOLOTHUROIDEA Brin, 1860

Order Dendrochirotida Grube, 1840
Family Cucumariidae Ludwig, 1894

Genus *Hemiocnus* Mjobo and Thandar, 2016

Hemiocnus syracusanus (Grube, 1840)
Cladodactyla syracusana (Grube, 1840); *Cucumaria syracusana* (Grube, 1840); *Ocnus syracusanus* (Grube, 1840); *Pseudocnella syracusana* (Grube, 1840).

Diagnosis: This is a cylindrical sea cucumber that can grow up to 50 cm in length. Its colour varies from greenish brown to dark purple through orange-brown red. It has 10 dendritic tentacles of orange colour and 5 longitudinal rows of podia. The body wall is thick and rigid, it is characterized by the presence of round and knobbed fir-cone-shaped plates. Also, it has rods and rosette in the tentacle which varied from small to large (Koehler 1921; Cherbonnier 1956; Tortonese 1965; Mjobo and Thandar 2016).

Description: Sub-cylindrical body; 10 tentacles; rigid and thick body; purplish colour; body wall plates are fir-cone-shaped, round and knobbed; small and large tentacle rods; large rosette in the tentacle.

Habitat and ecology: It is usually found in soft substrates like sandy bottoms, from 1 meter to greater depths (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Distribution: It is present in the Mediterranean and in the coasts of West Africa (Koehler 1921; Cherbonnier 1956; Tortonese 1965; Mjobo and Thandar 2016).

Examined material: One single specimen of 6 cm of diameter, collected by dredging between 3 to 5 m deep in La Goulette (L4, S2).

Genus *Leptopentacta* Clark, 1938

Leptopentacta elongata (Düben and Koren, 1846)
Cucumaria elongata (Düben and Koren, 1846); *Cucumaria pentactes* (Forbes, 1841); *Holothuria fusiformis* (Forbes and Goodsir, 1839); *Trachythyone elongata* (Düben and Koren, 1846).

Diagnosis: It is a small species between 6 to10 cm of diameter with dark brown or dark grey colour. Its body is elongated and cylindrical, and its skin thick and leathery. It has two rows of pedicles and 5 longitudinal rows of podia. The body wall spicules are large, perforated and irregular plates (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Description: Small sea cucumber; cylindrical and elongated body; 10 oral tentacles; 3 anal teeth; big and small irregular plates with many perforations; some plates with regular edge.

Habitat and ecology: This species inhabits mixed and sandy substrates, and sometimes algae, beyond 50 meters deep (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Distribution: It is distributed in the Mediterranean and the Atlantic (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Examined material: One single specimen of 6 cm was collected by benthic trawl in sands at 77 meters deep from Kélibia (L8, S3).

Leptopentacta tergestina (M. Sars, 1857) *
Cucumaria incurvata (Perrier E., 1886); *Cucumaria tergestina* (Sars M, 1857); *Siphothuria incurvata* (Perrier E., 1886); *Trachythyone tergestina* (M. Sars, 1857).

Diagnosis: 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.

Description: 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.

Habitat and ecology: It lives muddy and sandy substrates, and sometimes algae, beyond 50 meters deep (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Distribution: It is found in the Mediterranean Sea. It has been reported at many sites along the Italian coast, France, Spain, Turkish waters and Maltese Islands (Koehler1924; Tortonese 1965; Özaydın *et al.* 1995; Tanti and Schembri 2006; Öztoprak *et al.* 2014).
Examined material: 3 specimens collected on sand at a depth between 77-145 m from the locality of Kélibia (L8, S3).

Order Holothuriida Miller, Kerr, Paulay, Reich, Wilson, Carvajal & Rouse, 2017
Family Holothuriidae Burmeister, 1837

Genus ***Holothuria*** Linnaeus, 1767

Holothuria (Holothuria) mammata (Grube, 1840)
Holothuria mammata (Grube, 1840).

Diagnosis: This is a species whose diameter does not exceed 13 cm, has a dark brown or black color. Its body is covered by numerous long and thin papillae arranged in irregular rows. It is very similar to *Holothuria tubulosa* but it is distinguished by the presence of cuvieran tubules which are few in number and small. Its spicules are large and perforated plates with small tables and perforated rods (Koehler 1921; Cherbonnier 1956; Tortonese 1965; Rowe 1969; Fischer *et al.* 1987).
Description: Elongated and cylindrical body; dark brown or black color; 6 rows of thick mamelon; thin papillae; presence of the cuvierian tubules tubes; perforated rods; small tables with rectangular disc; large and perforated plates; buttons from different size.
Habitat and ecology: This sea cucumber lives on mixed substrates and algae from the surface up to 50 meters deep (Koehler 1921; Cherbonnier 1956; Tortonese 1965).
Distribution: It is found in the Mediterranean and the Eastern Atlantic (Koehler 1921; Cherbonnier 1956; Tortonese 1965).
Examined material: 2 individuals were collected using the dredge on sands between 3 and 8 meters deep. They measured between 6 and 8 cm, from locality Bizerte (L2, S1).

Holothuria (Holothuria) tubulosa (Gmelin, 1791)
Holothuria maxima (Delle Chiaje, 1823).

Diagnosis: Its body is elongated and cylindrical, up to 40 cm long. It has a light brown colour, sometimes with reddish or brownish reflections. It is usually covered by large, highly visible papillae, more or less pointed. Its spicules are perforated tables and buttons of different sizes, irregular plates and perforated rods (Koehler 1921; Cherbonnier 1956; Tortonese 1965; Fischer *et al.* 1987).

Description: Big sea cucumber; elongated and cylindrical body; 20 oral tentacles; conical oral papillae; perforated tables; large perforated plates; perforated rods; small and large knobbed buttons with rugose appearance.

Habitat and ecology: It lives on sandy and hard bottoms from surface up to 7 m deep (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Distribution: It is common in the Mediterranean Sea and eastern Atlantic Ocean (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Examined material: 40 specimens, between 6-15 cm long, were collected by diving, dredging and benthic trawling from algae and sand, mud and rock substrates, from the surface level up to 185 meters deep. They were found in Tabarka (L1, S1), Bizerte (L2, S1) and Haouaria (L7, S3).

Holothuria (Platyperona) sanctori (Delle Chiaje, 1823)
Holothuria farcimen (Selenka, 1867); *Holothuria flavocastanea* (Théel 1886).

Diagnosis: *H. sanctori* is cylindrical and elongated species, measuring up to 30 cm of diameter. It has a dark brown colour. Its dorsal surface is covered by numerous conical and pointed papillae well developed and of identical size. *H. sanctori* may reject Cuvier tubes in case of aggression. Its characteristic spicules are knobbed buttons with regular outline and median line, large table with rectangular bases, big knobbed plates and elongated rods (Koehler 1921; Cherbonnier 1956; Tortonese 1965; Fischer *et al.* 1987).

Description: Cylindrical body; flattened ventral side; thick and rough skin; 20 tentacles; large papillae; three rows of ventral tube feet; darkish brown colour; smooth and knobbed buttons with median longitudinal line; tables with rectangular and knobbed bases; large and knobbed plates; long and thin rods.

Habitat and ecology: It frequents anfractuosities, caves and troughs of rocky bottoms from the surface up to 50 meters deep (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Distribution: Eastern Atlantic, Mediterranean and Red Sea (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Examined material: 8 specimens hand collected from rocks and algae from offshore waters in the locality of Haouaria (L7, S3). Their length is ranging from 10 to 20 cm.

Holothuria (Roweothuria) poli (Delle Chiaje, 1824)
Holothuria polii (Delle Chiaje, 1824).

Diagnosis: It is body is cylindrical in shape, elongated and little flattened. It has a thick and very rough skin. Its dorsal surface is black, against a background of brown or gray. The dorsal surface is covered with conical, irregular tubercles and surmounted by small white papillae. The podia are white. The mean spicule are buttons from different shape with regular outline and with an odd number of holes. Also, it has small tables with rounded and knobbed bases and perforated rods (Koehler 1921; Cherbonnier 1956; Tortonese 1965; Fischer *et al.* 1987).

Description: Cylindrical body; brown colour with white, thin and small and papillae; dense and white ventral tube feet; knobbed buttons with odd hole's number; small tables with rounded disc; elongated and perforated rods.

Habitat and ecology: It lives on mixed bottom, rocks and seagrasses, from the surface up to 20 meters deep (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Distribution: Mediterranean, Atlantic and Red Sea (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Examined material: 40 specimens with the diameter ranging from 5 to 20 cm, collected by hand from the algae and rocks at the infralittoral level and by dredging in the sandy bottoms between 3 and 8 meters deep. They were found in three localities Tabarka (L1, S1), Bizerte (L2, S1) and Haouaria (L7, S3).

Holothuria (Thymiosycia) impatiens (Forsskål, 1775)
Holothuria bottelus (Selenka, 1867); *Holothuria fulve* (Quot and Gaimard 1834).

Diagnosis: It is vermiform, with light and dark bands of variable colour between pink and brown. Its body is firm and flexible, covered by soft and reduced papillae. Spicules are table with rectangular and knobbed disc, few and small buttons and perforated rods (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Description: Vermiform body; thick body wall; pointed papillae are irregularly arranged in the body; species with curvierian tubules; 20 tentacles; light brown colour with transversally dark brown bands; fairly tables with perforated disc and irregular outline; few small and large buttons; large perforated plates.

Habitat and ecology: It inhabits reefs and algae, rocky and sandy bottoms from the surface to greater depths (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Distribution: Cosmopolitan species with a wide geographical distribution (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Examined material: One single specimen, 7 cm long, found among the rocks of the midlittoral level in Korbous (L6, S3).

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

Family Stichopodidae Haeckel, 1896

Genus *Parastichopus* Clark, 1922

Parastichopus regalis (Cuvier, 1817)
Eostichopus regalis (Cuvier, 1817); *Gastrothuria limbata* (R. Perrier, 1899); *Holothuria regalis* (Cuvier, 1817); *Holothuria triquetra* (Delle Chiaje, 1828); *Stichopus regalis* (Cuvier, 1817).

Diagnosis: Elongated and flattened body; large species; thick and conical papillae; dorsal papillae ended with white point; three rows of ventral tube feet; mouth with 20 tentacles; tables with perforated bases; straight and perforated rods.

Description: It has a flattened body, with many large, conical and thick papillae. The dorsal papillae ended with white point. It is colour is brownish yellow and it is very lighter on the ventral side. It is characterized by the presence of very long rods perforated and straight in shape and big tables perforated at their bases (Koehler 1921; Cherbonnier 1956; Tortonese 1965; Fischer *et al.* 1987).

Habitat and ecology: This species inhabits algae and hard and mixed substrates from the surface to greater depths (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Distribution: It is widely distributed in the Mediterranean and Atlantic basins (Koehler 1921; Cherbonnier 1956; Tortonese 1965).

Examined material: 58 individuals, between 8 and 23 cm in length. They were collected by benthic trawling from sandy and muddy bottoms at 53-194 meters of depth. Only one specimen was collected by hand from the midlittoral rocks. The specimens were collected from Tabarka (L1, S1), Bizerte (L2, S1) and Kélibia (L8, S3).

2.3.3. Updating of the inventory of the phylum Echinodermata of Tunisia

An inventory was created from the echinoderms cited in Tunisian literature and updated with the present work. It is based on the echinoderms found and mentioned in several works and scientific articles, such as Koehler (1921), Le Danois (1925), Chambost (1928), Seurat (1929, 1934), Cherbonnier (1956), Pérés and Picard (1956), Maurin (1962), Laban *et al.* (1963), Tortonese (1965), Azouz (1966, 1973), Lubet and Azouz (1969), De Gaillande (1970), Zaouali (1980), Azouz and Capapé (1971), Ben Othman (1971, 1973), Ktari-Chakroun and Azouz (1971), Prunus and Pantoustier (1974), Azouz and Ben

Othman (1975), Darmoul *et al.* (1980), Zaouali (1980), Guelorget *et al.* (1982), Boudouresque *et al.* (1986), Fehri-Bedoui (1986), Sellem (1990), Anounymous (1997), Boudouresque (1997), Sellem *et al.* (2002), Ben Souissi and Zaouali (2007), Aloui-Bejaoui and Afli (2012) and El Lakhrach *et al.* (2012).

The new list count over 75 species belonging to the five living classes Echinodermata. They were reported throughout littoral and unequally distributed on three the Tunisian areas (Figure 1.4). These are grouped into 19 orders, 37 families and 50 genera. The present work has provided four new finding which update the echinoderms for Tunisia to 79 species. They are listed below (Table 2.3).

Table 2.3. Inventory of the 79 species of the Echinoderms cited in Tunisian, updated to the present work.

Class CRINOIDEA
- Order Comatulida
  - Family ANTEDONIDAE Norman, 1865
    - Genus *Antedon* Fréminville, 1811
      - *Antedon bifida* (Pennant, 1777)
      - *Antedon mediterranea* (Lamarck, 1816)
    - Genus *Leptometra* AH Clark, 1908
      - *Leptometra phalangium* (Müller, 1841)

Class ASTEROIDEA Blainville, 1830
- Order Brisingida Fisher, 1928
  - Family BRISINGIDAE G.O. Sars, 1875
    - Genus *Hymenodiscus* Perrier, 1884
      - *Hymenodiscus coronata* (Sars, 1871)
- Order Forcipulatida Perrier, 1884
  - Family ASTERIIDAE Gray, 1840
    - Genus *Coscinasterias* Verrill, 1867
      - *Coscinasterias tenuispina* (Lamarck, 1816)
    - Genus *Marthasterias* Jullien, 1878
      - *Marthasterias glacialis* (Linnaeus, 1758)
- Order Paxillosida Perrier, 1884
  - Family ASTROPECTINIDAE Gray, 1840
    - Genus *Astropecten* Gray, 1840
      - *Astropecten aranciacus* (Linnaeus, 1758)
      - *Astropecten bispinosus* (Otto, 1823)
      - *Astropecten irregularis* (Pennant, 1777)
      - *Astropecten irregularis pentacanthus* (Delle Chiaje, 1827)
      - *Astropecten jonstoni* (Delle Chiaje, 1827)
      - *Astropecten scoparuis* (Müller and Troschel, 1842)
      - *Astropecten spinulosus* (Philippi, 1837)
    - Genus *Tethyaster* Sladen, 1889
      - *Tethyaster subinermis* (Philippi, 1837)
  - Family LUIDIIDAE Sladen, 1889
    - Genus *Luidia* Forbes, 1839
      - *Luidia atlantida* (Madsen, 1950)
      - *Luidia ciliaris* (Philippi, 1837)

- *Luidia sarsii sarsii* Düben and Koren in Düben, 1844

Order Spinulosida Perrier, 1884
- Family ECHINASTERIDAE Verrill, 1867
  - Genus *Echinaster* Müller and Troschel, 1840
    - *Echinaster (Echinaster) sepositus* (Retzius, 1783)

Order Valvatida Perrier, 1884
- Family ASTERINIDAE Gray, 1840
  - Genus *Anseropoda* Nardo, 1834
    - *Anseropoda placenta* (Pennant, 1777)
  - Genus *Asterina* Nardo, 1834
    - *Asterina gibbosa* (Pennant, 1777)
    - *Asterina pancerii* (Gasco, 1876)
- Family CHAETASTERIDAE Sladen, 1889
  - Genus *Chaetaster* Müller and Troschel, 1840
    - *Chaetaster longipes* (Retzius, 1805)
- Family GONIASTERIDAE Forbes, 1841
  - Genus *Peltaster* Verrill, 1899
    - *Peltaster placenta* (Müller and Troschel, 1842)
- Family OPHIDIASTERIDAE Verrill, 1870
  - Genus *Hacelia* Gray, 1840
    - *Hacelia attenuata* (Gray, 1840)
  - Genus *Ophidiaster* L. Agassiz, 1836
    - *Ophidiaster ophidianus* (Lamarck, 1816)

Class OPHIUROIDEA Gray, 1840

Order Euryalida Lamarck, 1816
- Family GORGONOCEPHALIDAE Ljungman, 1867
  - Genus *Astrospartus* Döderlein, 1911
    - *Astrospartus mediterraneus* (Risso, 1826)

Order Ophiurida Müller and Troschel, 1840
- Family AMPHIURIDAE Ljungman, 1867
  - Genus *Acrocnida* Gislén, 1926
    - *Acrocnida brachiata* (Montagu, 1804)
  - Genus *Amphiura* Forbes, 1843
    - *Amphiura chiajei* (Forbes, 1843)
    - *Amphiura filiformis* (O.F. Müller, 1776)
    - *Amphiura mediterranea* (Lyman, 1882)
  - Genus *Amphipholis* Ljungman, 1866
    - *Amphipholis squamata* (Delle Chiaje, 1828)
- Family OPHIACANTHIDAE Ljungman, 1867
  - Genus *Ophiacantha* Müller and Troschel, 1842
    - *Ophiacantha setosa* (Bruzelius, 1805)
- Family OPHIACTIDAE Matsumoto, 1915
  - Genus *Ophiactis* Lütken, 1856
    - *Ophiactis savignyi* (Müller and Troschel, 1842)
    - *Ophiactis virens* (M. Sars, 1857)
- Family OPHIOCOMIDAE Ljungman, 1867
  - Genus *Ophiocomina* Koehler, 1920 in Mortensen
    - *Ophiocomina nigra* (Abildgaard in O.F. Müller, 1789)
- Family OPHIODERMATIDAE Ljungman, 1867
  - Genus *Ophioderma* Müller and Troschel, 1840
    - *Ophioderma longicauda* (Bruzelius, 1805)
- Family OPHIOMYXIDAE Ljungman, 1867
  - Genus *Ophiomyxa* Müller and Troschel, 1840
    - *Ophiomyxa pentagona* (Lamarck, 1816)
- Family OPHIOPSILIDAE Matsumoto, 1915
  - Genus *Ophiopsila* Forbes, 1843

Ophiopsila annulosa (M. Sars, 1859)
Ophiopsila aranea (Forbes, 1843)
Ophiopsila guineensis (Koehler, 1914)
Family OPHIOTRICHIDAE Ljungman, 1867
Genus *Ophiothrix* Müller and Troschel, 1840
Ophiothrix fragilis (Abildgaard in O.F. Müller, 1789)
Ophiothrix quinquemaculata (Delle Chiaje, 1828)
Family OPHIURIDAE Müller and Troschel, 1840
Genus *Ophiura* Lamarck, 1801
Ophiura albida (Forbes, 1839)
Ophiura ophiura (Linnaeus, 1758)

Class ECHINOIDEA Bronn, 1860
Order Arbacioida Gregory, 1900
Family ARBACIIDAE Gray, 1855
Genus *Arbacia* Gray, 1835
Arbacia lixula (Linnaeus, 1758)
Order Cidaroida Claus, 1880
Family CIDARIDAE Gray, 1825
Genus *Cidaris* Leske, 1778
Cidaris cidaris (Linnaeus, 1758)
Genus *Stylocidaris* Mortensen, 1909
Stylocidaris affinis (Mortensen, 1909)
Order Diadematoida Duncan, 1889
Family DIADEMATIDAE Gray, 1855
Genus *Centrostephanus* Peters, 1855
Centrostephanus longispinus (Philippi, 1845)
Order Camarodonta Jackson, 1912
Family ECHINIDAE Gray, 1825
Genus *Gracilechinus* Fell and Pawson in Moore, 1966
Gracilechinus acutus (Lamarck, 1816)
Family PARECHINIDAE Mortensen, 1903
Genus *Paracentrotus* Mortensen, 1903
Paracentrotus lividus (Lamarck, 1816)
Genus *Psammechinus* L. Agassiz and Desor, 1846
Psammechinus microtuberculatus (Blainville, 1825)
Family TOXOPNEUSTIDAE Troschel, 1872
Genus *Sphaerechinus* Desor, 1856
Sphaerechinus granularis (Lamarck, 1816)
Family TRIGONOCIDARIDAE Mortensen, 1903
Genus *Genocidaris* A. Agassiz, 1869
Genocidaris maculata A. Agassiz, 1869Order Clypeasteroida A. Agassiz, 1872
Family ECHINOCYAMIDAE Lambert and Thiéry, 1914
Genus *Echinocyamus* van Phelsum, 1774
Echinocyamus pusillus (O.F. Müller, 1776)
Order Spatangoida L. Agassiz, 1840
Family BRISSIDAE Gray, 1855
Genus Brissopsis L. Agassiz, 1840
Brissopsis lyrifera (Forbes, 1841)
Genus *Brissus* Gray, 1825
Brissus unicolor (Leske, 1778)
Family LOVENIIDAE Lambert, 1905
Genus *Echinocardium* Gray, 1825
Echinocardium cordatum (Pennant, 1777)
Echinocardium flavescens (O.F. Müller, 1776)
Echinocardium mediterraneum (Forbes, 1844)
Family SCHIZASTERIDAE Lambert, 1905

Genus *Ova* Gray, 1825
Ova canalifera (Lamarck, 1816)
Family SPATANGIDAE Gray, 1825
Genus *Spatangus* Gray, 1825
Spatangus purpureus (O.F. Müller, 1776)

Class HOLOTHUROIDEA Brin, 1860
Order Apodida Brandt, 1835
Family SYNAPTIDAE Burmeister, 1837
Genus *Oestergrenia* Heding, 1931
Oestergrenia digitata (Montagu, 1815)
Genus *Leptosynapta* Verrill, 1867
Leptosynapta sp.
Order Holothuriida Miller, Kerr, Paulay, Reich, Wilson, Carvajal & Rouse, 2017
Family HOLOTHURIIDAE Burmeister, 1837
Genus *Holothuria* Linnaeus, 1767
Holothuria (Panningothuria) forskali (Delle Chiaje, 1823)
Holothuria (Holothuria) helleri (Marenzeller von, 1877)
Holothuria (Holothuria) mammata (Grube, 1840)
Holothuria (Holothuria) tubulosa (Gmelin, 1791)
Holothuria (Platyperona) sanctori (Delle Chiaje, 1823)
Holothuria (Roweothuria) poli (Delle Chiaje, 1824)
Holothuria (Thymiosycia) impatiens (Forsskål, 1775)
Family MESOTHURIIDAE Smirnov, 2012
Genus *Mesothuria* Ludwig, 1894
Mesothuria intestinalis (Ascanius, 1805)
Order Dendrochirotida Grube, 1840
Family CUCUMARIIDAE Ludwig, 1894
Genus *Hemiocnus* Mjobo and Thandar, 2016
Hemiocnus syracusanus (Grube, 1840)
Genus *Leptopentacta* Clark, 1938
Leptopentacta elongata (Düben and Koren, 1846)
Leptopentagta tergistina (M. Sars, 1857)
Genus *Ocnus* Forbes and Goodsir in Forbes, 1841
Ocnus petiti (Cherbonnier, 1957)
Ocnus planci (Brandt, 1835)
Genus *Pawsonia* Rowe, 1970
Pawsonia saxicola (Brady & Robertson, 1871)
Family PHYLLOPHORIDAE Östergren, 1907
Genus Phyllophorus Grube, 1840
Phyllophorus (Phyllophorus) granulatus (Grube, 1840)
Phyllophorus (Phyllophorus) urna (Grube, 1840)
Order Synallactida Miller, Kerr, Paulay, Reich, Wilson, Carvajal & Rouse, 2017
Family STICHOPODIDAE Haeckel, 1896
Genus *Parastichopus* Clark, 1922
Parastichopus regalis (Cuvier, 1817)

2.4. Discussion

2.4.1. Species first record

In this chapter it is worth mentioning that four of the collected species were first records in northern Tunisia (*Asterina pancerii*, *Luidia atlantidea, Ophiactis virens* and *Leptopentacta tergestina*).

There are two new records for the Class Asteroidea, *Asterina panceri* and *Luidia atlantidea*. The starfish *Asterina pancerii* is an endemic species of the Mediterranean Sea (Tortonese 1965) and, according to the Bern Convention, the Barcelona Convention and the Spanish Catalogue of Threatened Species, it is listed as an endangered and protected species in the Mediterranean (López-Márquez *et al.* 2018). Only one individual was found for the first time in Tunisia in northern inshore waters (3-5 m), associated to *Cymodocea nodosa* beds. 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.

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 that has been recently reported for first time in the Mediterranean, in the northern Alboran Sea (western Mediterranean Sea) (Gallardo-Roldán *et al.* 2015). In this case, 31 individuals were collected by mechanized dredging performed at depths of between 0.9 and 11.6 m (Gallardo-Roldán *et al.* 2015). So, the present report on *Luidia atlantidea* is the first in Tunisia and the second in the Mediterranean Sea.

We also report the first record 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). In our study, 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, and maybe facilitated by the fact that this species is well known for its asexual reproduction and the fission of its body into two equal parts (Wilkie 1984). The presence of *Ophiactis virens* is, at the same time, the third time to be reported in the Mediterranean.

Leptopentacta tergestina is an endemic Mediterranean holothuria (Koehler 1924; Tortonese 1965), which has also been found for the first time in Tunisia. Three individuals were collected by commercial trawling at depths between 60 and 150 m off Cape Bon (north-eastern Tunisia).

By other hand, two species belonging to the Class Ophiuroidea (*Astrospartus mediterraneus* (Risso, 1826) and *Ophiacantha setosa* (Bruzelius, 1805)) were found and reported for the second time in northern Tunisia in this study after the first finding by Cherbonnier (1956), and its mentioning later in many megabenthic checklist fauna following the same author. Over 54 specimens of *O. setosa* were found associated with the yellow gorgonian *Eunicella cavolini* (Koch, 1887), close to the Algerian deep-sea border. Because of its evasiveness this species is recorded for the second time in the Tunisia in this study.

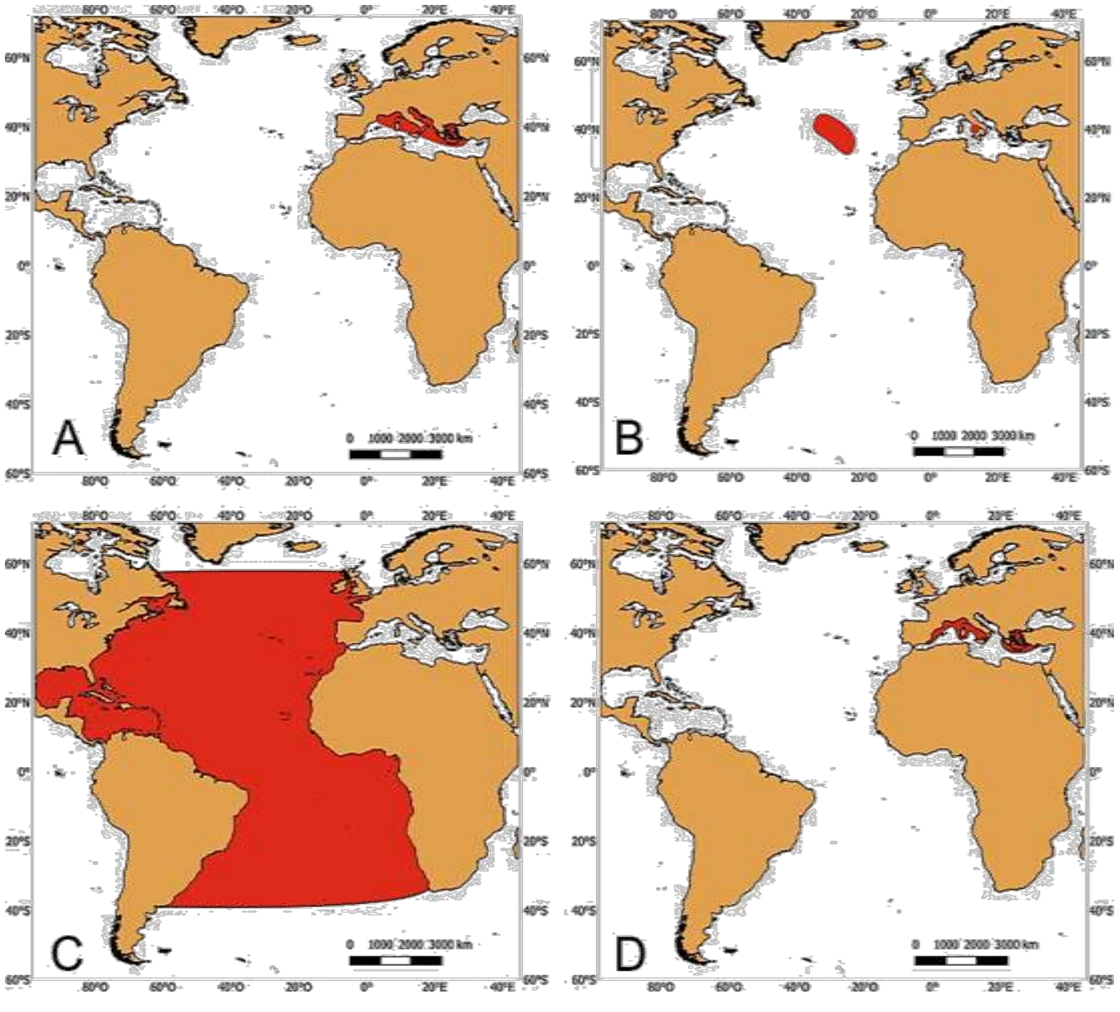


Figure 2.3. Maps of the geographic distribution (in Red) of the species newly recorded in Tunisia: (A) *Leptopentacta tergestina*, (B) *Ophiactis virens*, (C) *Luidia atlantidea* and (D) *Asterina pancerii* (According to WoRMS, marine species distribution: http://www.marinespecies.org/ and López-Márquez *et al.* 2018, for *Asterina pancerii*) (Chammem *et al.* 2019).

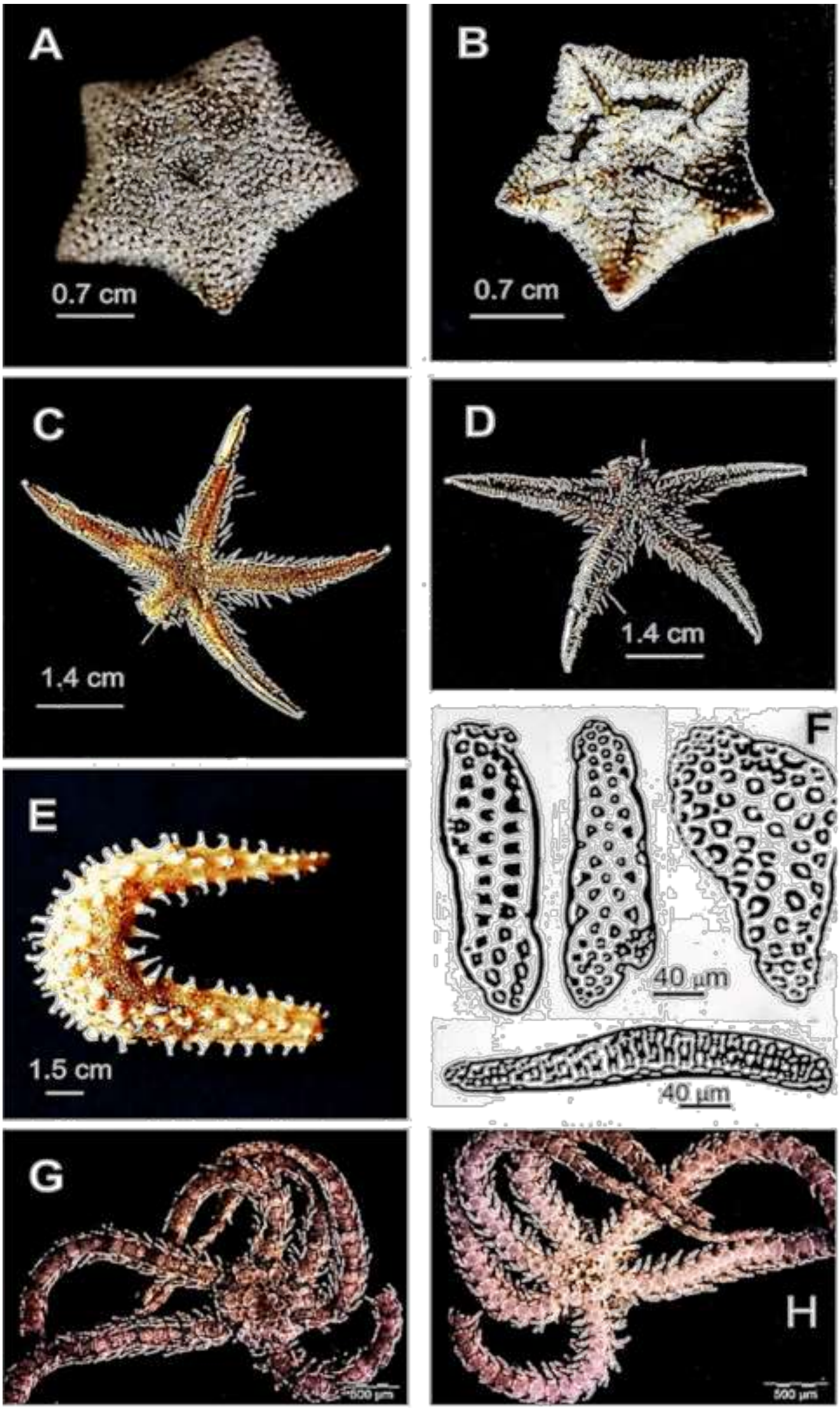


Figure 2.4. A) *Asterina pancerii* aboral view and B) Oral view. Scale bar: 1 cm; c) *Luidia atlantidea* aboral view and D) Oral view. Scale bar: 1 cm; E) *Leptopentacta tergestina* lateral view. Scale bar: 0.5 cm; F) Body wall ossicules of *Leptopentacta tergestina*. Scale bar: 0.5 cm. J) *Ophiactis virens* aboral view and H) Oral view (Chammem *et al.*2019).

2.4.2. Species diversity

Forty-five echinoderm species were collected and identified in this study in northern Tunisia waters. They belonged to the five classes of Echinodermata and weregrouped into 32 genera and 27 Families.

All the inventoried species can be considered as Mediterraneans or Atlanto-Mediterranean, except for the starfish *Luidia atlantidea*, 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, namely: *Asterina pancerii*, *Astropecten jonstoni, Holothuria (Holothuria) mammata*, *Leptopentacta tergestina*, *Ophiothrix quinquemaculata* and *Antedon mediterranea*. Another four have a wide distribution and are cosmopolitan: *Amphipholis squamata, Ophiactis savignyi, Holothuria (Thymiosycia) impatiens* and *Stylocidaris affinis*.

The echinoderms recorded in this study from northern Tunisian marine waters are quite diverse. 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, in part, by the techniques and gears used to sample them (hand collection, dredging, trawling and diving) that increased the collection area by covering a wide variety of substrates and depths, from the infralittoral to the bathyal levels.

As a result, the Tunisian Echinoderm checklist has been updated to 79 species belonging to the five living Classes of Echinodermata. Only the north of Tunisia (from the Algerian-Tunisian border to Ras Kapudia) contributes with the highest number, with 68 species against 63 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). Over six species which were exclusive for south and east Tunisian subregion were found in this study for the second time in the north area. Thus concerned the three fishstars, *Astropecten auranciacus*, *Chaetaster longipe* and *Hacelea attenuata*, one sea cucumber *Hemioconus syracusnus* and the brittle star *Ophiactis savignyi* from south area and the basket star *Astrospartus mediterraneus* from eastern area. Some previously recorded species were not found in the present work, surely because of the adopted methodology and fishing gears which depended on the places and depths frequented by fishermen.

Indeed, only few research has been done on Echinodermata in deep Mediterranean waters (Koukouras *et al.* 2007; Coll *et al.* 2010), a lack of knowledge which especially includes the north African coast of Maghreb (Dauvin *et al.* 2013; Chammem *et al.* 2019). 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, of which only 53.5 % 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.

Most of these new recorded species were found 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 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 habitats (Bianchi and Morri 2000; Lejeusne *et al.* 2010). All this make us consider it as one of the biodiversity hotspots in the Mediterranean Sea (Coll *et al.* 2010; Lejeusne *et al.* 2010). These findings confirm the importance of the marine fauna of northern Tunisia area, in which there are an important 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.

Eventually, Tunisian geographical location confers it a special status for marine fauna studies. Since Cherbonnier work, conducted in 1956, dealing with the first inventory of the Echinodermata of Tunisia, the number of the recorded echinoderms has increased. However, it is necessary to promote efforts and acquire a solid knowledge about this macrobenthic invertebrate group, its taxonomical configuration and its ecological and socioeconomic implications (Chammem *et al.* 2019).

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.

Table 2.4. Checklist of northern Tunisia Echinodermata with their biogeographical origin, distribution and type of substrate on which they are found. 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; Loc(L), Locationfrom Figure 2.1.

Taxa	Distri-bution	Depth range (m)	Habitat	Ab/ 1000m^2	Loc. (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	110	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, 1857) *	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	2
Holothuria (Holothuria) tubulosa Gmelin, 1791	M,A,R	0,2-185	S,M,R,A	40	1,2,7
Holothuria (Platyperona) sanctori Delle Chiaje, 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	40	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

CHAPTER III

ECOLOGICAL DISTRIBUTION OF THE ECHINODERMS OF NORTHERN TUNISIA

3. ECOLOGICAL DISTRIBUTION OF THE ECHINODERMS OF NORTHERN TUNISIA

3.1. Introduction

Faunal and/or floral studies on the composition of marine biodiversity are essential to understand the ecological patterns of species distribution as well as relations between and within communities in a biogeographic and also more local context. Knowledge on the species that constitute marine communities and the environmental conditions that define their distribution are a key issue to the correct management of marine ecosystems and to identify conservation priorities in the same (Iken *et al.* 2010; Souto *et al.* 2014; Arribas *et al.* 2016).

Echinoderms are well known by their adaptability and plasticity to various environmental conditions (Ebert 1996) and are considered as one of the most dominant and diverse components of marine communities (Birkeland 1989). Moreover, they play a relevant role in the functioning of the environments in which they live (Alvarado *et al.* 2012). They present a wide bathymetric distribution and inhabit a large variety of marine habitats, showing a large spectrum of ecological feeding and reproductive strategies. They also occupy different trophic positions in the marine food webs, from top predators to herbivorous and filter feeders or detritivorous, or also being a source of food. Thus, they contribute to nutrient cycle regulation, the control of some other species populations, the aeration and remineralization of sediments, or the secondary production (Uthicke 1999, 2001; Ravest Presa 2001).

Moreover, some sea urchin or starfish species have been recognized as indicator of metal pollution or bioindicators of sea water quality (Portocali *et al.* 1996; Temara *et al.* 1998; Schweitzer *et al.* 2000; Coteur *et al.* 2003). In this way, several echinoderm species have been identified as “keystone species” (Paine 1969; Lawrence 1975; Elner and Vadas 1990; Power *et al.* 1996; Lessios *et al.* 2001; Uthicke *et al.* 2009; Arribas *et al.* 2016).

Some studies can be found on echinoderms of the southern Mediterranean coasts (Soualili *et al.* 1999; Mezali and Thandar 2014; Mezali *et al.* 2014; Amri *et al.* 2017; Belbachir and Mezali 2018; Rouane-Hacene *et al.* 2018; Belbachir *et al.* 2019), most of them paleoecological or applied, however, knowledge and research on the ecology and spatial distribution of echinoderms in this area are still very scarce (Pérez-Ruzafa and López-Ibor

1987), and this is even more evident in the coasts of Tunisia (Sellem and Guillou 2007; Sellem *et al.* 2000, 2011, 2019; Chammem *et al.* 2019).

So, the objectives of the present chapter have consisted in understand the distribution of the echinoderms in the northern coast of Tunisia, quantifying and qualifying the echinoderm faunal composition of the communities from the benthic coastal area and the continental slope, and relating them to the depth and type of substrate in which they are found.

3.2. Material and Methods

3.2.1. Sampling area

To explore the distribution of echinoderm assemblages in our study area, a total of 16 sampling campaigns were performed from 2012 to 2016, including 93 sampling points in 8 localities along northern Tunisia coastline (from 8°30’E to 11°00’E). Samplings were carried out from midlittoral to infralittoral, circalittoral and bathyal levels, obtaining a total of 1,430 individuals. Hand collection and quadrats were used for bottoms less than 1m depth, experimental dredging for shallow waters less than 50 m depth, and benthic fishing trawls for depths exceeding 50 m. Scuba diving was employed for restricted places with hard bottoms where none of the previous gears could be used (Fig. 2.1, Table 2.1).

Benthic fishing trawls were those used in commercial fishing vessels, depending on their length and width of the size of the boats. While the dredger was an experimental tool, specific to our laboratory, made of metal with a cylindrical opening, in which a net bag is fixed. It was generally used in sandy bottoms not accessible with the benthic trawls. The quadrat is square wood frame with 25 by 25 cm of size.

For each sampling place or vessel itinerary, geographical coordinates, maximum and minimum depth and bottom characteristic were registered. The collected species were separated, measured, identified in a first vision and preserved in ethanol for its posterior confirmation and study (Chapter 2).

3.2.2. Data analyses

In order to understand echinoderms assemblages and to analyse the type of substrate and the depth that affect their distribution, total abundance (A) and species richness (S) were calculated for each sampling station.

Frequency of occurrence (FO) was estimated as a percentage to classify the identified taxa as frequent (FO ≥ 75%), common (75% > FO ≥ 50%), occasional (50% > FO ≥ 25%), rare (25% > FO ≥ 10%) or accidental (FO < 10%) (Thai *et al.* 2013; Selfati *et al.* 2019). Density for each species was calculated by the number of individuals per area of collection normalized to an area of 1000 m^2.

To evaluate species and classes distribution according to some habitat factors, the depths ranges and substrate types were established as follow: midlittoral level (from 0 to 0.2 m), infralittoral level (from 0.2 to 50 m), circalittoral (from 50 to 100 m), bathyal (more than 100 m), and Algae, Mud, Mud–Sand, Sand and Rock in the case of types of bottoms or habitats (Pérès 1967) (Table 2.1).

Main echinoderm assemblages were identified using multivariate analyses. To explore the spatial scales of variation we divided the area in three sectors (S1 to S3) corresponding respectively to “North Tunisia”, “the Gulf of Tunis”, and “Cap Bon”. Sector 1 (S1) includes the localities of Bizerte (L1) and Tabarka (L2), Sector 2 (S2) includes Carthage (L3), La Goulette (L4), Radès (L5) and Korbous (L6), and Sector 3 (S3) includes Haouaria (L7) and Kélibia (L8) (Fig. 3.1). The number of samples at each locality was from 2 to 32. The localities of Korbous from sector 2 and Haouaria from sector 3, were sampled only one time and has not been included in the analyses.

Statistical analyses were performed using PRIMER 6.0 software (Clarke and Gorley 2006). Spatial variations in the structure of the echinoderm assemblages were assessed by non-metric multi-dimensional scaling (MDS). Values were transformed to $\log_{10}(x+1)$ before the analyses so that each species contributed more evenly (Clarke and Green 1988). The resemblance matrix was generated using Bray Curtis distance.

Permutational multivariate analysis of variance PERMANOVA was performed to test the differences within groups, considering two factors: Sector with three levels (S1, S2 and S3), and locality nested in Sector (Lo(Se)). The species contributing to discriminate between groups were identified using SIMPER test on the using Bray-Curtis dissimilarities.

The relationships between the different faunistic groups and habitat descriptors were tested using R software (2019). A series of Correspondence Analyses (CA), biplots, and boxplots, were performed using the packages FactoMiner and Factoextra for Correspondence Analyses (Annex 1). For the five Echinodermata Classes in each depth we have used a code (c) reassembling the first letter of the class's name (cC = Class Crinoidea, cA = Class Asteroidea, cO = Class Ophiuroidea, cE = Class Echinodea, cH = Class Holothuroidea) and the depth levels together. The depth varied from 0 (hand collection) to 220 m (benthic trawl). Thereafter, the abundance of each Class in each depth according to the five habitats, Algae, Sand, Sand-Mud, Mud and Rock, was recorded on an Excel table.

The variation of the identified species assemblages through the studied area and their spatial distribution patterns according to latitudinal and longitudinal gradients were analysed using the CANOCO 5 package (ter Braak 1990).

A canonical correspondence analysis (CCA) (ter Braak and Prentice 1988), using also transformed abundance [ln (x + 1)], was performed and the results of the ordination analysis were displayed in a biplot after scaling the axes by adjusting the species scores to the species variances. The obtained scores are the correlations between species and eigenvectors.

Rare species were downweighted following the procedure offered by CANOCO in order to prevent their excessive influence on the ordination. Average values of echinoderms assemblage variables and the log-transformed data of abundance of the most frequent species (FO ≥10%) were compared between different substrates using ANOVA.

3.3. Results

3.3.1. Tunisian species diversity and distribution

The species, their abundances and frequency of occurrence, as well as the depths, habitats and localities in which they were found are listed in the table 3.1.

We have identified 45 species from a total of 1,430 captured specimens, of which none results frequent, two species were common, (*Cidaris cidaris* and *Stylocidaris affinis*), eight were considered occasional (*Antedon bifida*, *Antedon mediterranea*, *Astropecten irregularis*, *Tethyaster subinermis*, *Echinaster (Echinaster) sepositus*, *Ophiomyxa pentagona*, *Ophiura ophiura*, and *Centrostephanus longispinus*), and others five were rare species (*Leptometra phalangium, Marthasterias glacialis, Astropecten aranciacus, Chaetaster longipes* and *Parastichopus regalis*). The majority (30) was considered as accidental species, these last with an abundance under 10 individuals.

Table 3.1. Echinodermata species sampled from northern Tunisia with the abbreviation of species (Abv) and locations (Loc), depth range (Dep), habitats (Hab; A= Algae; Cy= *Cymodocea*; G= gorgonian; S=sandy bottom; M=muddy bottom; R=rocky bottom), percentage of total abundance (%N), and the frequency of occurrence (Fo%; F=frequent, C= common; O= occasional; R=Rare; A= Accidental).

Taxa	Abv	Loc	Dep (m)	Hab	%N	Fo %	Fo
Class: CRINOIDEA							
Family: Antedonidae							
Antedon bifida (Pennant, 1777)	An^{bif}	1,2,8	50-250	S,M,R	3,5	27	O
Antedon mediterranea (Lamarck, 1816)	An^{med}	1,2,8	50-250	S,M,R	4,8	38	O
Leptometra phalangium (Müller, 1841)	Le^{pha}	1,8	72-250	S,M	2,3	15	R
Class: ASTEROIDEA							
Family: Asteriidae							
Coscinasterias tenuispina (Lamarck, 1816)	Co^{ten}	2,8	0-51	S	0,1	2,5	A
Marthasterias glacialis (Linnaeus, 1758)	Ma^{gla}	2,8	1-250	S,M,R	0,8	12	R
Family: Asterinidae							
Anseropoda placenta (Pennant, 1777)	An^{pla}	8	185-220	S	0,07	1,2	A
Asterina gibbosa (Pennant, 1777)	As^{gib}	3,5	0,4-0,6	R,A	0,9	5	A
Asterina pancerii (Gasco, 1876) *	As^{pan}	4	3-5	Ca	0,2	1,2	A
Family: Astropectinidae							
Astropecten aranciacus (Linnaeus, 1758)	As^{ara}	2,8	51-177	S,M	0,02	11	R
Astropecten bispinosus (Otto, 1823)	As^{bis}	2,3,4	1-35	S,M	0,8	1,2	A
Astropecten irregularis (Pennant, 1777)	As^{irr}	1,2,8	50-220	S	2,4	26	O
Astropecten jonstoni (Delle Chiaje, 1827)	As^{jon}	4	3-5	S	0,07	1,2	A
Tethyaster subinermis (Philippi, 1837)	Te^{sub}	1,2,8	50-220	S, M	2,7	30	O
Family: Chaetasteridae							
Chaetaster longipes (Retzius, 1805)	Ch^{lon}	1,2,8	70-170	S,M	1,5	10	R
Family: Echinasteridae							
Echinaster (Echinaster) sepositus (Retzius, 1783)	Ec^{sep}	1,2,8	3-220	S,M,R	7,7	43	O
Family: Luidiidae							
Luidia atlantidea Madsen, 1950 *	Lu^{atl}	8	65-95	S	0,07	1,2	A
Luidia sarsii sarsii Düben and Koren in Düben, 1844	Lu^{sar}	1	175-193	M	0,07	1,2	A
Family: Ophidiasteridae							
Hacelia attenuatea Gray, 1840	Ha^{att}	1	70-85	R	0,07	1,2	A
Class: OPHIUROIDEA							
Family: Amphiuridae							
Amphipholis squamata (Delle Chiaje, 1828)	Am^{squ}	5	0,3-0,6	A	1,1	1,2	A
Family: Gorgonocephalidae							
Astrospartus mediterraneus (Risso, 1826)	As^{med}	8	98-105	S	0,1	1,2	A
Family: ophiacanthidae							
Ophiacantha setosa (Bruzelius, 1805)	Op^{set}	1	70-165	G	3,8	5	A
Family: Ophiactidae							
Ophiactis savignyi (Müller and Troschel, 1842)	Op^{sav}	5	3-5	S	0,07	1,2	A
Ophiactis virens (M. Sars, 1857) *	Op^{vir}	5	0,4-0,6	A	13	1,2	A
Family: Ophiocomidae							
Ophiocomina nigra (Abildgaard in O.F. Müller, 1789)	Op^{nig}	2	50-58	M	0,07	1,2	A
Family: Ophiodermatidae							
Ophioderma longicauda (Bruzelius, 1805)	Op^{lon}	7	0,65	R, A	0,07	1,2	A

Family: Ophiomyxidae							
Ophiomyxa pentagona (Lamarck, 1816)	Op^{pen}	2,8	50-210	S,M,R	5,7	26	O
Family: Ophiotrichidae							
Ophiothrix quinquemaculata (Delle Chiaje, 1828)	Op^{qui}	2	72-175	S,M,R	1,5	6	A
Family: Ophiuridae							
Ophiura ophiura (Linnaeus, 1758)	Op^{oph}	1,2,4, 8	3-194	S,M,R	8,7	35	O
Class: ECHINOIDEA							
Family: Arbaciidae							
Arbacia lixula (Linnaeus, 1758)	Ar^{lix}	1,2,7	0,25-5	S,R	0,8	6	A
Family: Cidaroidae							
Cidaris cidaris (Linnaeus, 1758)	Ci^{cid}	1,2,8	50-220	S,M,R	8,2	57	C
Stylocidaris affinis (Mortensen, 1909)	St^{aff}	1,2,8	50-220	S,M,R	8,0	56	C
Family: Diadematidae	Ce^{lon}						
Centrostephanus longispinus (Philippi, 1845)		2,8	50-220	S,M	5,2	36	O
Family: Echinidae							
Gracilechinus acutus Lamarck, 1816	Gr^{acu}	8	50-125	S,R	1,3	7	A
Family: Parechinidae							
Paracentrotus lividus (Lamarck, 1816)	Pa^{liv}	1,2,7	0,2-6	S,R	3,4	7	A
Family: Spatangidae							
Spatangus purpureus (O.F. Müller, 1776)	Sp^{pur}	8	3-5	S	0,07	1,2	A
Family: Toxopneustidae							
Sphaerechinus granularis (Lamarck, 1816)	Sp^{gra}	7	0,6-5	R,A	0,1	2,5	A
Class: HOLOTHUROIDEA							
Family: Cucumariidae							
Hemiocnus syracusanus (Grube, 1840)	He^{syr}	4	3-5	S	0,07	1,2	A
Leptopentacta elongata (Düben and Koren, 1846)	Le^{elo}	8	77-145	S	0,07	1,2	A
Leptopentacta tergestina (M. Sars, 1857) *	Le^{ter}	8	77-145	S	0,3	6	A
Family: Holothuriidae							
Holothuria (Holothuria) mammata Grube, 1840	Ho^{mam}	2	3-8	S,R,A	0,1	1,2	A
Holothuria (Holothuria) tubulosa Gmelin, 1791	Ho^{tub}	1,2,7	0,2-185	S,M,R, A	2,9	9	A
Holothuria (Platyperona) sanctori Delle Chiaje, 1823	Ho^{san}	7	0,2-0,4	R, A	0,6	1,2	A
Holothuria (Roweothuria) poli Delle Chiaje, 1824	Ho^{pol}	2,7	0,2-8	S,R,A	1,1	2,5	A
Holothuria (Thymiosycia) impatiens (Forsskål, 1775)	Ho^{imp}	6	0,2	R	0,07	1,2	A
Family: Stichopodidae							
Parastichopus regalis (Cuvier, 1817)	Pa^{reg}	1,8	0,2-194	S,M,R	5,9	23,5	R

The presence of founded echinoderms and their abundance varied regarding the horizontal zonation of benthic communities. Class Holothuroidea dominated the midlittoral level (0-0.2 m) with 49% of individuals, followed by Echinoidea with 46%, Asteroidea with 4% and Ophiuroidea with only 1%.

In the infralittoral (0.3-50 m), Ophuroidea individuals dominated with 58% followed by Holothuroidea (18%), Echinoidea (16%) and Asteroidea (8%). *Ophiactis virens* and *Ophiura ophiura* were characteristic of the infralittoral level.

For the circalittoral level (50-100 m) we found the first appearing of the class Crinoidea which represent 13% of the total specimen's frequency. Echinoidea dominated this level with 34%, followed by Ophiuroidea (25%), Asteroidea (23%), the mentioned Crinoidea and Holothuroidea with 5%. Finally, at the bathyal level (100-230 m), 30% of the specimens belongs to the Class Echinoidea, followed by Ophiuroidea (26%), Asteroidea (22%), Crinoidea (15%), and Holothuroidea representing only 7%.

We stand out the increasing of Asteroidea from the infralittoral to the circalittoral and bathyal levels and, on the contrary, the decrease in presence of Holothuroidea. Echinoidea were well distributed and represented in all the benthic zones, while Ophiuroidea and Crinoidea were scarce or do not appear in shallow areas (Fig. 3.1).

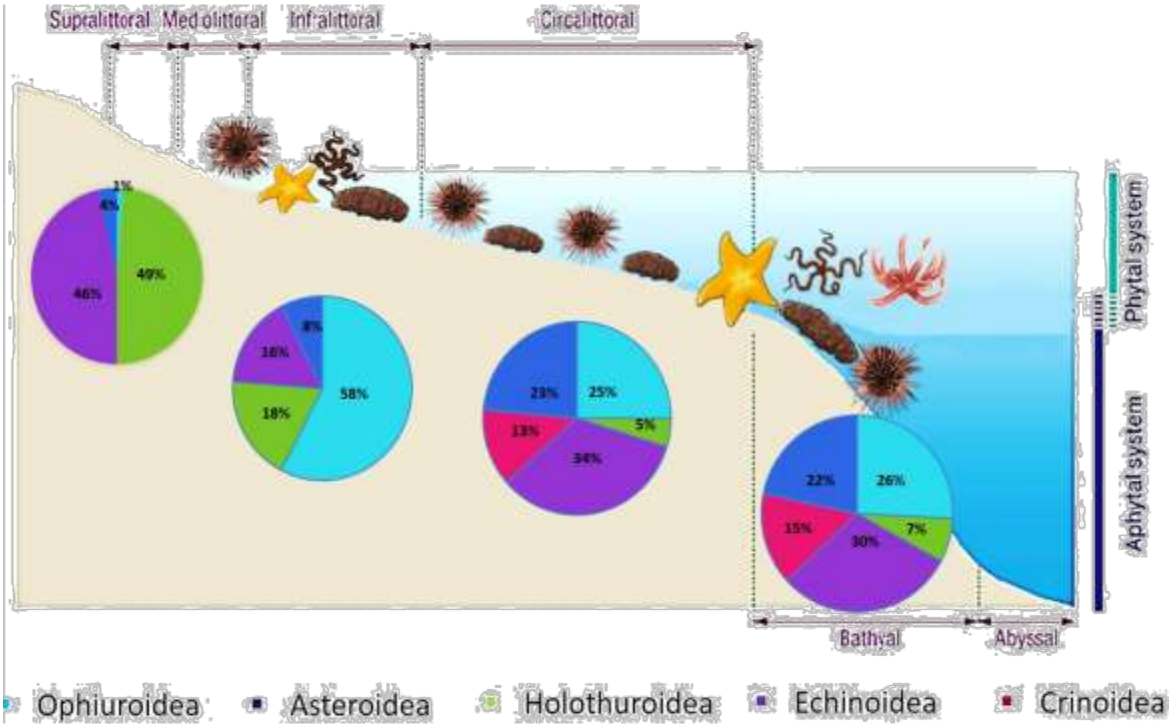


Figure 3.1. Species abundance of the five Classes of Echinodermata registered in the northern coasts of Tunisia according to the marine horizontal zonation.

3.3.2. Echinoderm assemblages and their similarities or differences

The MDS ordination based on Bray-Curtis similarity matrix was used to identify the similarities and the differences of the echinoderms assemblages according to sectors and localities sampled with only hand collection with quadrat, experimental dredging and professional trawling where the sampling was standarised by unity of surface. According to the MDS result, there were no differences between sectors (Fig. 3.2) but between localities nested sectors (Fig.3.3).

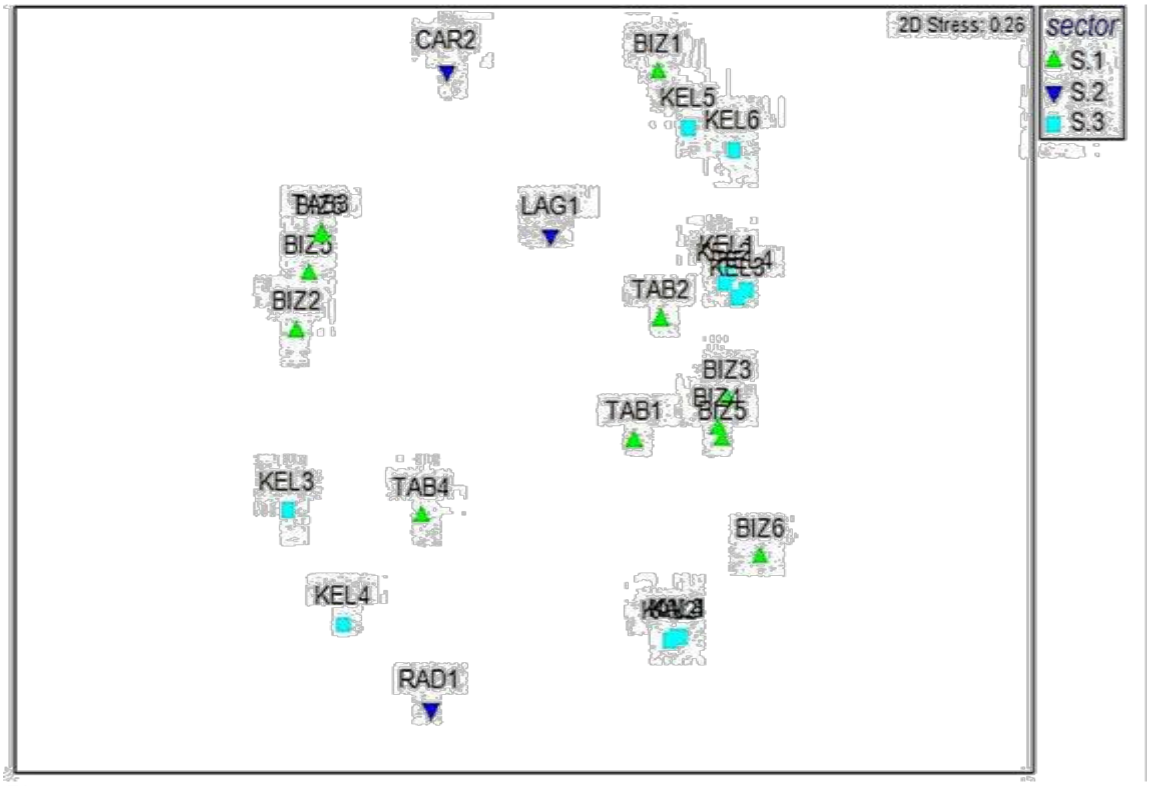


Figure 3.2. Multidimensional scaling analysis (MSD) ordination on the basis of Bray-Curtis similarity, showing the relation between the two studied sector and difference sites nested within localities. KEL= Kelibia, TAB= Tabarka, BIZ= Bizerte, CAR= Carthage, LAG= La Goulette, RAD= Radès S1= north sector with the localities of Tabarka end Bizerte; S2: Sector of Gulf of Tunis with the localities of Cartage, La Goulette, Radès; S3: Sector of Cap Bon with the locality of Kélibia

The average of Bray-Curtis similarity between assemblages from the locality of Tabarka from sector 1 was larger than that between Bizerte and Kelibia respectively from sector 1 and sector 3 (Fig. 3.2). Tabarka shows an extensive ordination on both extreme of the MDS. The difference of species composition between localities nested sector in this case could be attributed mainly to depth, where Tabarka stands out with the record at greater depth (250 m) and the greater heterogeneity of habitats.

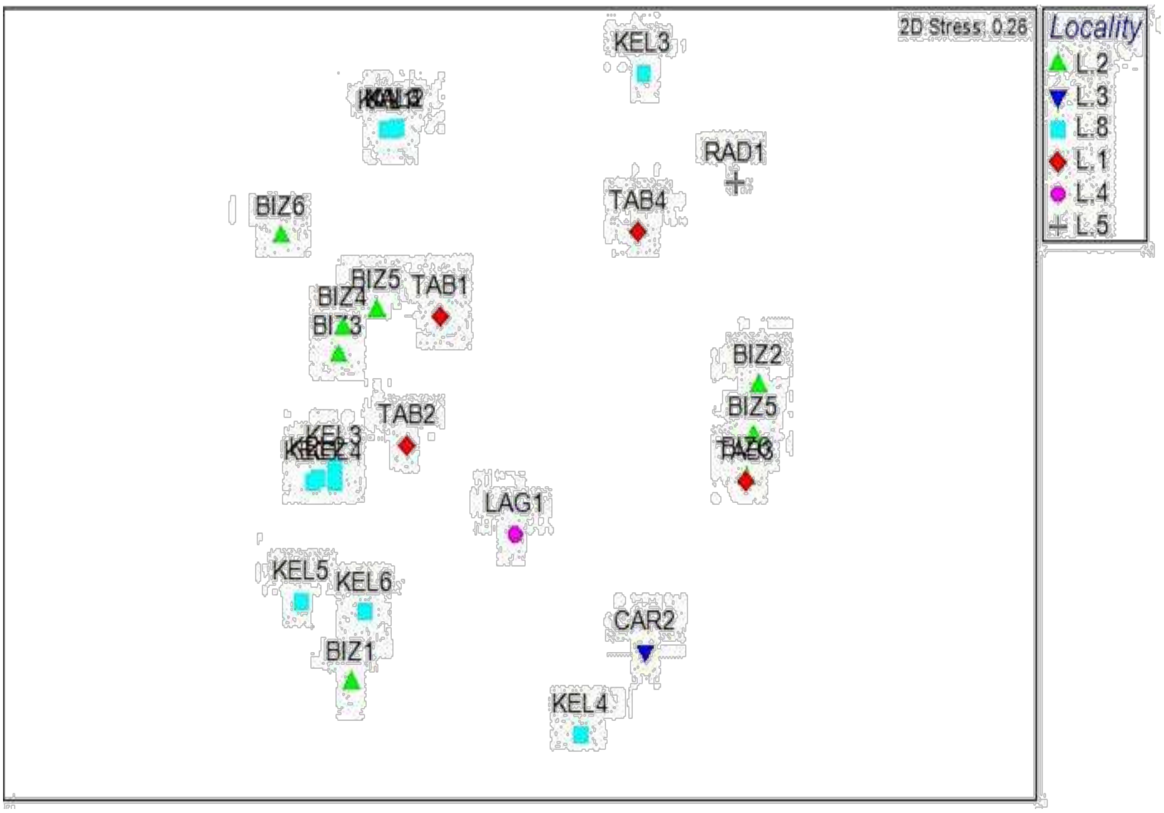


Figure 3.3. Multidimensional scaling analysis (MSD) ordination on the basis of Bray-Curtis similarity, showing the relation between the localities. L.1= Tabarka; L.2= Bizerte; L.3=Carthage =; L.4= La Goulette; L.5= Radès: L.8= Kélibia.

PERMANOVA analyses have investigated the spatial difference between assemblages. The obtained results show a significant difference between sectors and between localities nested sector (Table 3.2). However, the variability between sectors is lower (0.3922) than the one between localities nested in sector (0.0001).

Table 3.2. PERMANOVA analyses performed on the basis of Bray-Curtis similarity for the echinoderm faunal abundance considering the two factors, sector and locality nested in sector. Only localities with significant differences are shown. Se= Sector; Lo(Se)= Locality nested sector; Res= Residual; MS= Mean square.

	df	SS	MS	Pseudo-F	P(MC)
Se	2	26107	13054	1	0.3922
Lo(Se)	2	17182	8590.9	3	0.0001
Res	65	1.75E+05	2685.2		
Total	69	2.19E+05			

Unlike to PERMANOVA results, the Pair wise test shows differences between localities nested sector but not between sectors. At the same time that has shown a large difference between localities. The most differentiated assemblages were found between the localities of Tabarka (S1) and Kelibia (S3) (P = 0.0001) followed by Kelibia (S3) and La Goulette (S2) (P = 0.0002).

Table 3.3. Pair-wise tests analyses performed for the echinoderm faunal abundance matrix considering the factors locality. Only localities with significant differences are shown.

PAIR-WISE TESTS				
Term 'Lo'				
Groups	t	P(perm)	perms	P(MC)
BIZERTE, TABARKA	2.1044	0.0014	9936	0.0014
BIZERTE, CARTHAGE	1.7005	0.0595	18	0.0144
BIZERTE, KELIBIA	1.7664	0.0014	9909	0.0029
BIZERTE, LA GOULETTE	1.8501	0.0131	171	0.0045
TABARKA, CARTHAGE	1.3537	0.1092	18	0.1271
TABARKA, KELIBIA	2.8504	0.0001	9936	0.0001
TABARKA, LA GOULETTE	1.4461	0.0399	172	0.0797
CARTHAGE, KELIBIA	1.823	0.0616	32	0.0024
CARTHAGE, LA GOULETTE	0.8825	1	2	0.5544
KELIBIA, LA GOULETTE	2.0396	0.0058	529	0.0002

As there are no differences between sectors but between localities nested sectors, we have performed a second analyses considering only the factor locality. The SIMPER test result reveals a significant difference between Tabarka and Bizerte (S1) with 81.60% and no differences between la Goulette and Carthage (S2). This could be explaining by the distance which is very important between localities inside the first sector (S1) and the variability of the marine topography inside the same sectors.

Table 3.4. SIMPER analysis performed for the echinoderm faunal abundance matrix considering the factors sector and locality nested in sector.

Groups	BIZERTE and TABARKA
Average dissimilarity	= 81.60 %

Besides, there are over 13 taxa identified by SIMPER as being important to differentiate assemblages located in Tabarka and Bizerte (Table 3.5). Three species were characterizing both localities: the brittle star *Ophiura ophiura*, and the two sea urchin, *Stylocidaris affinis* and *Cidaris cidaris*. The presence of the brittle star *Ophiocanta setosa* exclusively at Tabarka contribute to the strong difference towards the studied localities. The absence of *Ophiomyxa pentagona, Ophiothrix quinquemaculata* and *Centrostephanus longispinus* in Tabarka and *Ophiocantha setosa* and *Parastechopus regalis* in Bizerte also made an important contribution to discriminate between both localities nested sector 1.

Table 3.5: SIMPER analyses performed for the echinoderm faunal abundance matrix considering the factors locality nested in sector 1.

	BIZERTE	TABARKA				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Saffi	0.14	0.16	9.27	1.34	11.35	11.35
Ccida	0.14	0.16	9.15	1.36	11.21	22.57
Esepo	0.1	0.1	7.96	0.93	9.76	32.32
Oseto	0	0.26	7.67	0.52	9.4	41.72
Oophi	0.11	0.1	7.41	1.14	9.08	50.8
Lphal	0.03	0.09	5.52	0.89	6.77	57.57
Prega	0	0.09	5.24	0.79	6.43	63.99
Opent	0.1	0	5.12	0.7	6.28	70.27
Clong	0.1	0	4.64	0.92	5.68	75.96
Clong	0.05	0.05	3.93	0.66	4.82	80.78
Amedi	0.08	0	3.85	0.92	4.72	85.49
Tsubi	0.06	0.02	3.3	0.85	4.05	89.54
Oquin	0.06	0	3.19	0.61	3.9	93.44

3.3.3. Echinoderm assemblage's distribution in relation with depth and bottom types

The CA biplot (Fig. 3.4) show a wide distribution of the species on the four axes of CA. In fact, the two axes didn't show a big difference, the first axe (Dim1) of CA represent 32, 3% of the total CA however, the second one (Dim2) represent only 28,5 %.

The Mud –Sand and Sand habitats were present on both negative sides of (Dim1) and (Dim2). These habitats were emerging the most complex communities of echinoderms and they were the most frequented by fishermen.

Classes characteristic of Algae habitat were Ophiuroidea and Holothuroidea. However, muddy substrate was mainly emerging species from Ophiuroidea and Astroidea. In fact, Rocky and muddy substrates were the lowest frequented by fish men perhaps because of the difficulties of trawling on such habitats and also forfish availability.

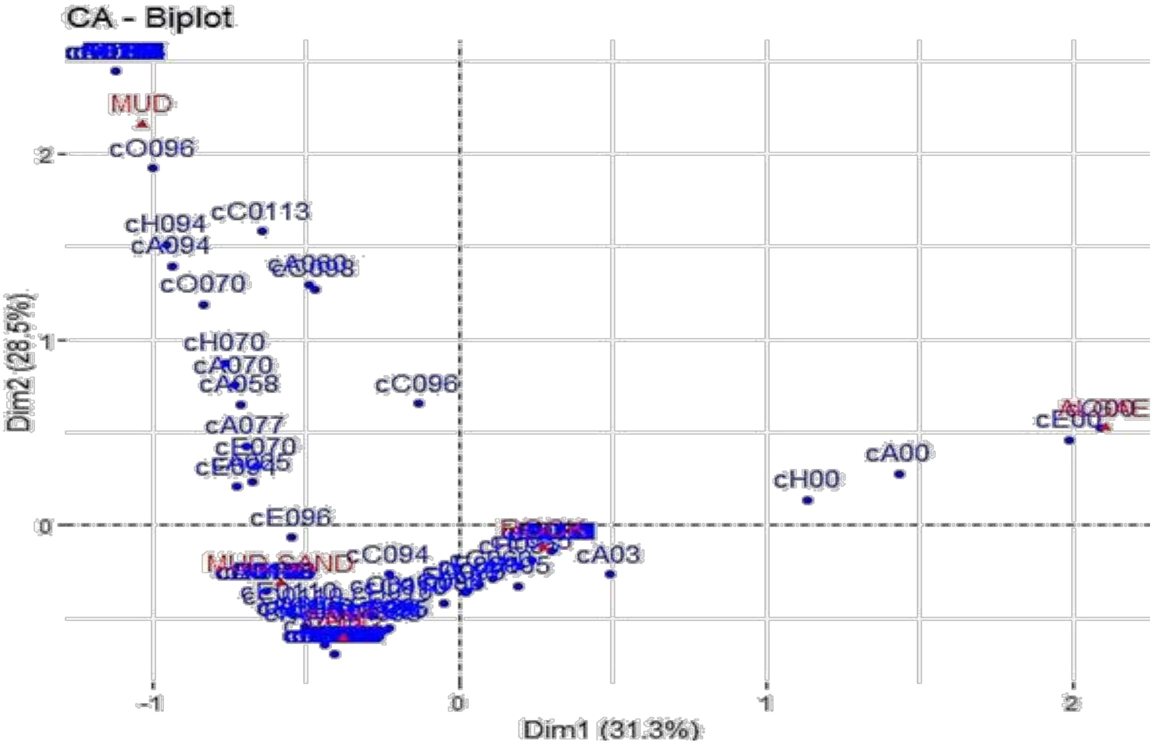


Figure 3.4. Correspondence analayses (CA) biplot of the axes Dim 1 and Dim 2 showing the destribution specimens per classes according to the habitat types and depths.

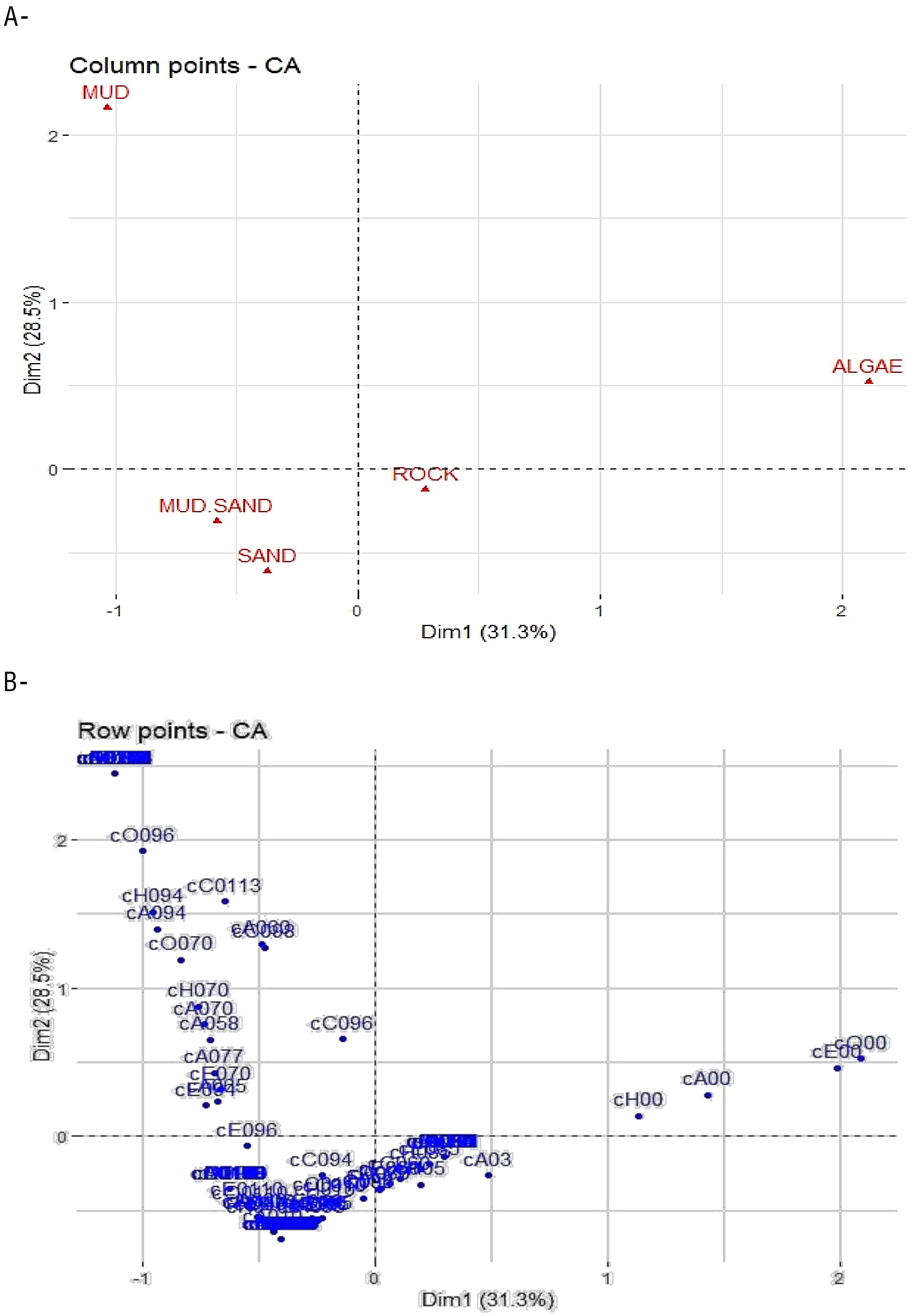


Figure 3.5. Correspondence analyses (CA) biplots of the two axes separately column (A) and row (B) showing the distribution of the habitats types as column (A) and the depths per class as a row (B).

The figure 3.6 represents the distribution of the classes Echinodermata along the axes of the Correspondent Analysis. Firstly, we see the separation of the class Ophiuroidea from the rest of the group and its appearing alone on the positive side of the (Dim2). The rest of the studied were present on the negative sides from both (Dim1) and (Dim2).

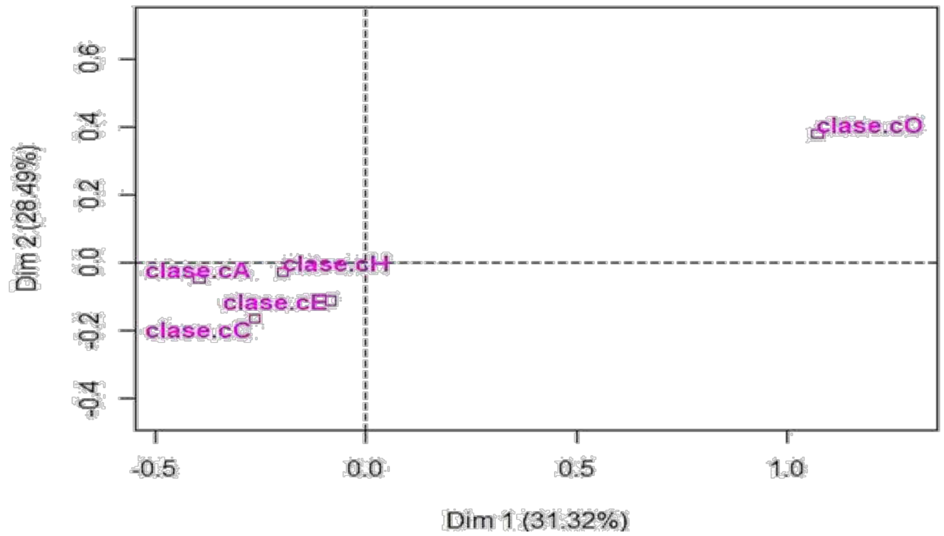


Figure 3.6. Correspondence analyses CA factor map showing classes distribution on the two axes Dim 1 and Dim 2.

For the second CA (Figure 3.7), we set depth as a supplementary colomnas to asses the distribution of species according to habitat type and depth range. The obtained result which is presented in the figure below (Figure 3.76),,shows the correlation between species and habitat according to the depth. In fact, in relation with precited figures (figure 3.4) species from Muddy habitat were positively correlated with depath range however the one from the Mud-Sandy and Sandy were negatively correlated with depth.

This mean that both communities from Mud-Sand and Sand are species ubiquitous with a big ecological tolerance to defferent depth condition.

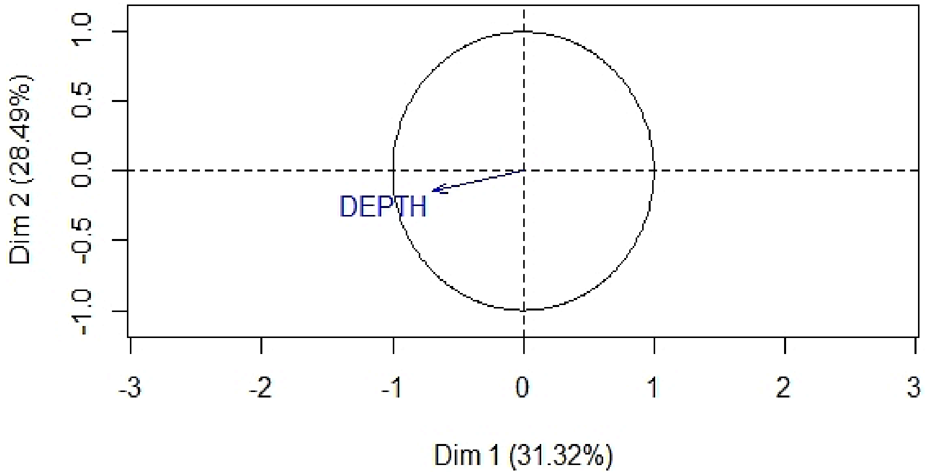


Figure 3.7. Supplementary variable on the Correspondence analyses (CA) factor map.

The generated boxplot (fig. 3.8) shows a significative difference between abundances of the species according to depth ranges. It shows different levels of colonisation of the Echinodermata communities from the infralittoral to the bathyal level. Some species from three classes, Astroidea, Echinoidea and Ophiuroidea, were present from the continental to bathyal level (more than 200m). The rest two classes (Crinoidea and Holothuroidea) were present on depths between 50 to 100 meters. The Crinoidea shows a restricted distribution from 60 to 110 m with the maximum of abundance for more than 100 m. While, the Echinoidea was the most showing a wide bathymetric range (from 0m to 230m). It was present from the continental to depths over 200 meters.

All the groups were highly occurring the on depths between 100 and 110 meters. The presence or rather the absence of the classes is depending on the species preferences, exigence and it capacities to support the different range of temperatures and light. Also, it could be attributed to the predation, thecompetition and/ the availability of food.

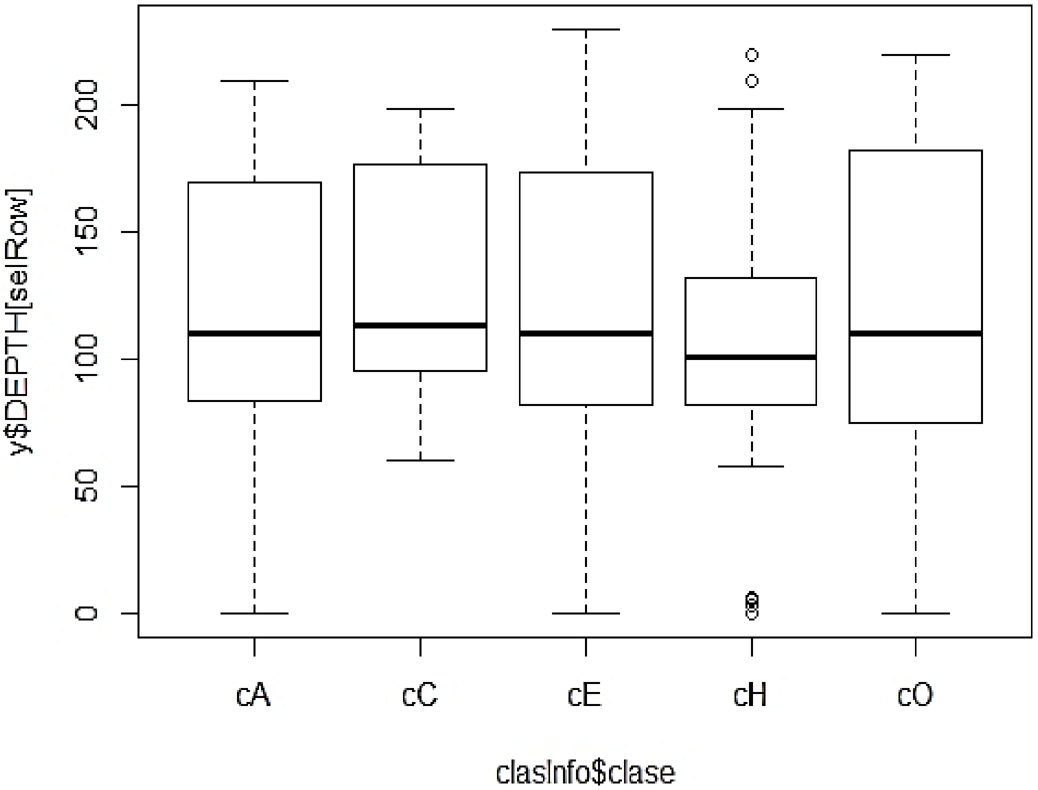


Figure 3.8. Boxplots of the distribution of the five living classes Echinodermata according to the frequented depths. Depth (Y$ DEPTH) is present on meters deep.

3.3.4. Correlation of echinoderm assemblage with other environmental drivers

Original data matrix included 72 cases 38 species and 12 environmental variables. Samples RAD1Q and LA 1E (Annexe) were out layers that differentiates too much from the others due to the abundance of algae and *Cymodocea*, respectively, and the presence of the species *Ophiactis virens* and *Asterina panceri*, precluding to detect differences between the other samples. They have been removed from the analyses.

The total variation is about 6,4 and explanatory variables account only for 27,69% (adjusted explained variation is 18,21%), suggesting that other variables not considered like salinity assemblages.

Table 3.6. Table of the five first axes of Canonical Correspondence Analysis (CCA) with the values of eigenvalue's variations, cumulative variation and their Pseudo-canonical correlation.

Analysis 'Copy of Copy of Copy of CCA-spp-env ext-allvariables removing samples'

Method: CCA

Total variation is 6.44349, explanatory variables account for 27.69%

(adjusted explained variation is 18.21%)

Summary Table:

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.7193	0.3767	0.2980	0.2348
Explained variation (cumulative)	11.16	17.01	21.63	25.28
Pseudo-canonical correlation	0.8940	0.9487	0.6538	0.6296
Explained fitted variation (cumulative)	40.31	61.43	78.13	91.29

The first two axis of the analyses performed including all measured variables explain 61.4% of the fitted variance of species-environment relationship. The first axis, explain 40.31% and in the right side, is mainly related to shallow bottoms dominated by sand with the presence of species like *H. tubulosa* and *H. mammata* or *H. poli*. In deeper areas with some rocks are also present *Paracentrotus lividus* or *Arbacia lixula*, *Ophiactis savighyi*, *Astropecten bispinosus*, *Hemiocnus syracusanus*. At the left side, are deepest areas dominated by mud and include species like *Anseropoda placenta, Marthasterias glacialis, Coscinasterias tenuispina, Ophiomyxa pentagona* and *Gracilechinus acutus*.

The second axis explain an additional 21.1% of the variance and in the positive part is determined by the presence of gorgonians and the species *Ophiacantha setosa* and *Hacilia attenuatea*.

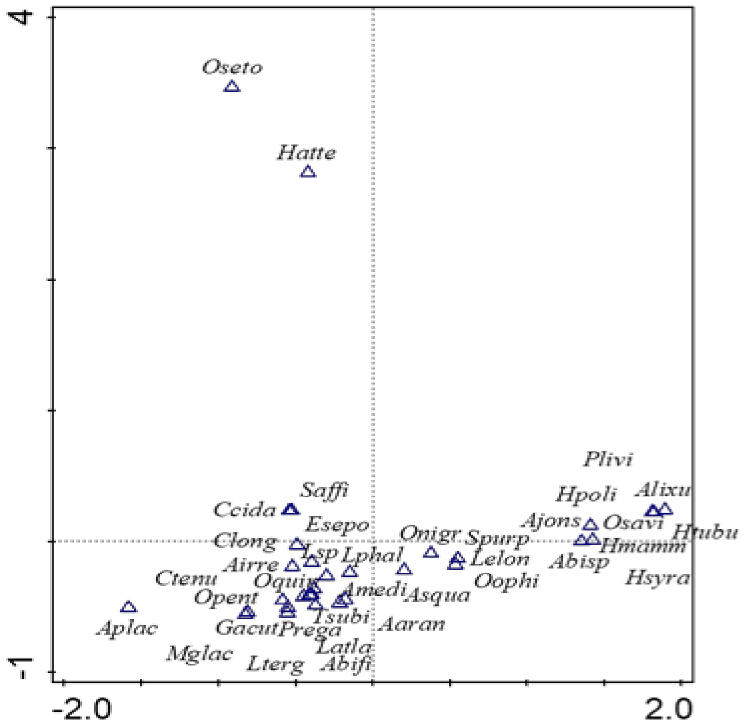


Figure 3.9. Canonical Correspondence Analysis (CCA) diagram showing the ordination of the species along the first two axes of the eigenvalue and their correlation with localities.

However, most of the variables used in the analyses was no significant and a new analysis with forward selection of variables was performed. The results showed that only four variables were significant ($p<0.005$), MaxDepth, longitude, Gorgonia and Rock.

Table 3.7. Table of the four releveling environmental parameters of the Canonical Correspondence Analysis (CCA) promoting species distribution.

Forward Selection Results:				
Name	Explains %	Contribution %	pseudo-F	P
MaxDepth	8.4	30.4	6.2	0.002
Gorgonian	5.8	21.1	4.6	0.038
long	4.6	16.6	3.7	0.002
Rock	4.9	17.5	4.1	0.002

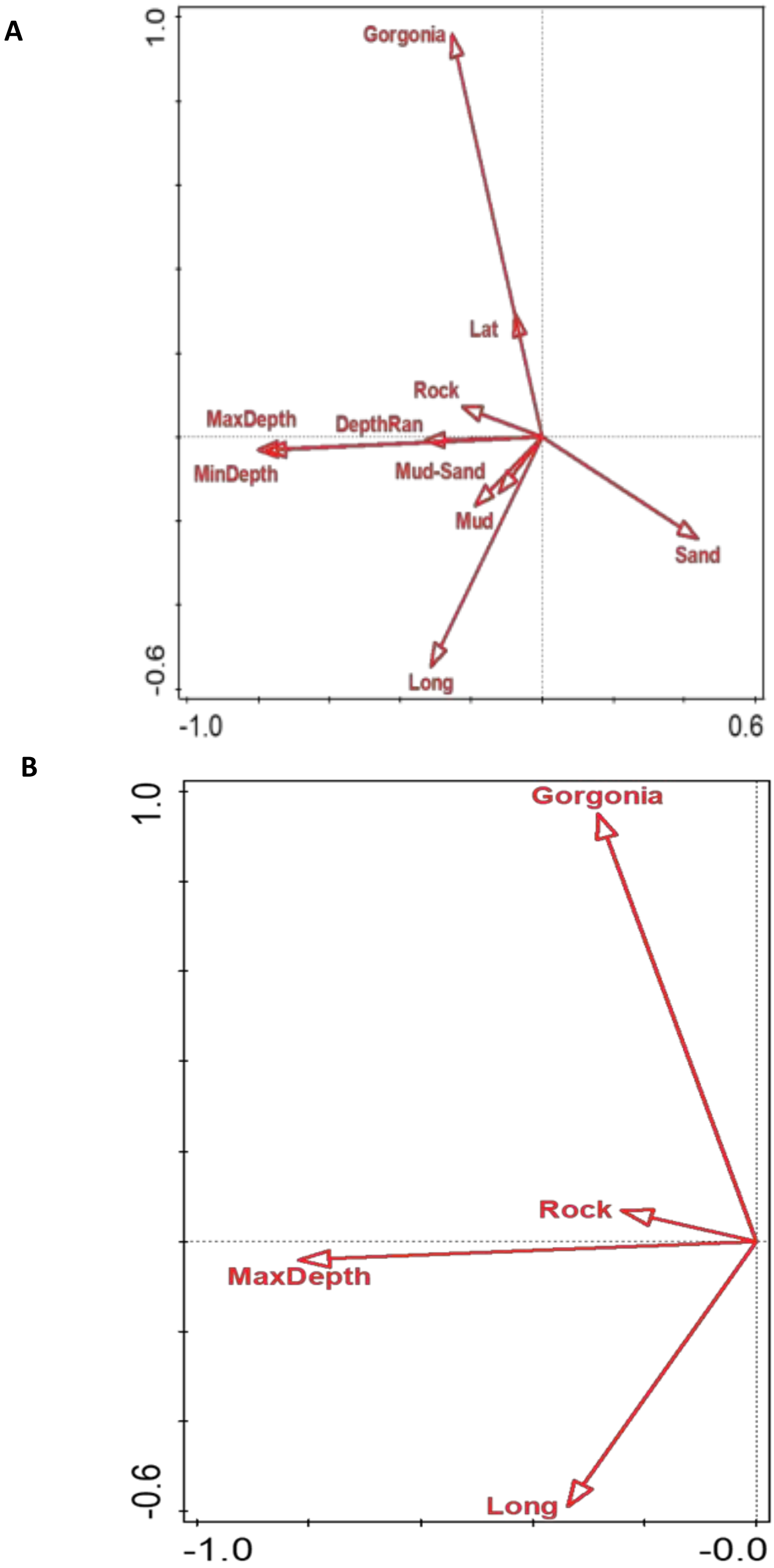


Figure 3.10. Canonical Correspondence Analysis (CCA) diagram showing the distribution of the species according to the environmental parameters depth, latitude and longitude, habitats and maximum and minimum depth (A), and for only maximum depth rocky and gorgonian habitats and longitude (B).

3.4. Discussion

We have analysed the presence and the distribution of 45 species of Echinodermata determined from a total of 1,430 individuals collected in 93 sampling points in the northern coasts of Tunisia. This investigation relies in a first job of the authors where the first checklist of Echinoderms of this area was provided (Chapter 2 of this PhD Thesis, Chammem et *al.,* 2019), and that is the first to study the distribution and some aspects of the ecology of this group in Tunisia.

The Mediterranean Sea is characterized by a diversity of habitats that exhibit a large spatial variability both along its entire surface and at different depths, from the intertidal zone to the continental shelf and the abyssal environments. Each horizontal zone is identified by numerous factors such as topographic structure, light, currents, substrate characteristics or temperature, among the most importants.

The few studies on echinoderms biodiversity in the southern Mediterranean barely explore their assemblages and their species diversity across this horizontal zonation.

These depth parameters condition the biota repartition inside each benthic zone, and also some species and communities could be fixed and identified as key habitats species. Such is the case of the common mollusc *Patella rustica* Linnaeus, 1758, which usually mark the transition between supra and midlittoral or photophilic algae which mark the transition between infra and circalittoral zone (Pérès and Picard 1964; Ros *et al.* 1984; Gili *et al.* 2014).

Regarding the benthic research of marine invertebrates, the evaluation of the biodiversity of each community has not normally been focused or discussed in detail. In general, the least amount of research on the study of echinoderms biodiversity has not explored their composition across the benthic bionomics zoning of the Mediterranean.

In fact, the distribution of fauna is conditioned by an environmental gradient which is very often defined by two major parameters: water temperature and light intensity (Gili *et al.* 2014). Light, in fact determines the physical structure and the trophic organization of the communities as it is the main determinant of the presence of algal cover and primary production and the last will be determined by phytoplankton or macrophytes depending of nutrients availability and in a lesser extent the hydrodinamism. This will affect the dominance or the absence of echinoderm classes and species depending on their food preferences (herbivores, like the sea urchin *P. lividus*, carnivores like most sea stars and some ophiuroids like *Ophiderma longicauda*, detritit feeders as aspidochirote holothurians and some ophiures or filter feeders like crinoids, somo ophiuroids or dendrochirote holothurians).

As the light and the agitation of the water diminish with depth, and the amount of decomposing matter increases, the species that preferentially inhabited these areas, such as Echinoidea and Holothuroidea, supposedly are favored by these types of conditions.

The circalittoral zone has even lower light intensity (less than 1% of surface intensity) (Dauvin *et al.* 2008). It has been defined as the limit of *Posidonia* beds and algae and it is a very diverse area in terms of species and biotopes. It is an offshore zone which mainly consists of detritic and fossil or sub-fossil detrital material (Pérès and Picard 1964; Ros *et al.* 1984). The resuspension of the organic matter generated by the detritus decomposition, make it an ideal habitat for Crinoids and Ophiuroids.

In fact, very few investigations have studied the biodiversity and the composition of the communities of benthic organism in the circalittoral and bathyal zones. The bathyal zone is characterized by the total absence of light and by a temperature that is usually around 13 °C (Pérès and Picard 1964). In this zone we found the presence of all the echinoderm classes but with the dominance of Echinoidea and Ophiuroidea.

The outcome results of the correspondence analysis (CA) show that the echinoderms distribution on the north Tunisian sea is varying according to the depth which is the main factor of species assemblage. The composition of Echinodermata modifying through the bathymetric gradient (figure 3.8) where the mean was around 100 m and the change is mostly observed from 60 m (figure 3.9)

Likewise, a study on the distribution patterns and the species richness of echinoderms from south-western Atlantic Ocean (Souto *et al.* 2014) has shown that the specific distribution of Echinodermata changes through bathymetric gradient and the significant changes were recorded at the depths exceeding the 100 m. Although, we must take on consideration the big difference between the ocean and the sea concerning the size, the depth and water mass and so the oceanographic feature and the abiotic components specific for each ecosystem.

Species patterns distribution was usually associated to the non-living features, the chemical and the physical factors as the salinity, temperature, dissolved oxygen and edaphic parameters. In addition to the living biological factors such as predation and intra- and inter-specific competition (Ventura and Fernandes 1995; Souto *et al.* 2014). However, according to Souto *et al.* (2014) water temperature is the preeminent factor for the echinoderms assembling in south-western Atlantic Ocean which has been so long considered as a limiting factor for species distribution (Stuardo 1964; Okolodkov 2010; Souto *et al.* 2014). Same authors found that shallow water from coastal area is warm and it becomes much colder by moving to the deep.

Indeed, very often it has been proven that coastal areas are a high energetic and productive region (Leigh *et al.* 1987; Arribas *et al.* 2016) with a high diversity of Echinodermata taxa in comparison with the deep waters (Price *et al.* 1999; Rowe and Richmond 2004; Iken *et al.* 2010; Arribas *et al.* 2016). However, it wasn't the case for the present study, where our results from species distribution (figure 3.3) show that both abundance and species richness are highly present in deep waters (from 50 to 250 m). Thus, are characterised by a large heterogeneity of habitat and not in coastal and shore waters which are in our case, dominated by rocky and sandy shores.

Inshore echinoderms were mostly found under the rock or in rocks covered by red algae (Rhodophyta). Similarly, according to the study of Iken *et al.* (2010) on the assemblage of the echinoderms from nearshore rocky habitats where the sampling was done on several localities (76 sampling sites), from northern to southern hemisphere as well as from western to eastern hemisphere. Species richness of the collected material from this area was lower, from one to five species. Nevertheless, they haven't encountered echinoderms in several sampling localities like it was our case.

This result, maybe could be attributed to the oceanographical parameters and the topography of the Mediterranean Sea that limits the species distribution in the Tunisian nearshore waters. Or the adopted sampling methodology and the sampling effort on nearshore and deep sea (Gray 2000; Iken *et al.* 2010).

The relevant results from the CCA analyses have shown that species richness is positively correlated with longitude for regional local scale habitats, maximum depth and muddy substrates. Oppositely, rocky and gorgonian were positively correlated with the latitudinal gradient. There are two species dominating at lower longitudes (the western part of

Tunisian waters), the ophiuroid *Ophiacantha setosa* (Bruzelius, 1805) and the seastar *Hacelia attenuata* Gray, 1840. Further than these species, the longitudinal species gradient is narrower. In the other extreme, at the eastern part, are more abundant *Antedon bifida* (Pennant, 1777), *Marthasterias glacialis* (Linnaeus, 1758) and *Leptopentacta tergestina* (M. Sars, 1857).

We could conclude that Echinodermata richness on northern Tunisia water follow a longitudinal gradient diversity and evenness echinoderms follow a latitudinal diversity gradient. In addition to rocky habitat related to the Tunisian marine topography and to the Mediterranean physical and oceanographical process.

In fact, few studies had documented the longitudinal gradients in the biodiversity of marine invertebrate and fish (O'Hara and Poore 2000; Smith *et al.* 2014) in spite of the one of latitudinal gradient which are much more diverse and common (Roy *et al.* 1998; De Domenico *et al.* 2006; Iken *et al.* 2010; Escolar *et al.* 2013; Souto *et al.* 2014);

Some studies in marine fishes have found longitudinal patterns to be stronger (Mora *et al.* 2003; Smith *et al.* 2014). Smith *et al.* (2014), have found that the species composition of fishes from Australasian temperate reef were clearly following a longitudinal gradient. Same authors have supposed that the historical events such as: the oceanographical connectivity, the microclimate and the niche specialization, were the main reason of the longitudinal gradient. In the case of Tunisian waters, such longitudinal gradient coincides with the transition from the western to the eastern Mediterranean basins through the Silicu-Tunisian strait.

Investigation on Echinodermata assessment from the Mediterranean Sea were few and incomplete. Indeed, the present study is the first to be in Tunisia on Echinodermata assessment from small-scale patterns distribution on northern Tunisian waters.

The study of biodiversity at different scales and in relation to environmental variables is very useful for the management of species from different points of view, the conservation and protection of overexploited and endangered species, identifying and protecting their natural habitats at the same time. New lines of research are also opening up in the identification of invasive species that compete and modify the characteristics of coastal ecosystems and compete with autochthonous species, many of them with great nutritional, ecological and/or economic interest.

CHAPTER IV

MORPHOMETRIC AND GENETIC COMPARISON OF SPECIES OF THE GENUS *HOLOTHURIA* FROM THE ATLANTIC OCEAN AND THE MEDITERRANEAN SEA

4. MORPHOMETRIC AND GENETIC COMPARISON OF SPECIES OF THE GENUS *HOLOTHURIA* FROM THE ATLANTIC OCEAN AND THE MEDITERRANEAN SEA

4.1. Introduction

It has already been commented in the previous chapters that the class Holothuroidea is one of the most diverse groups of Echinodermata, comprising about 1,400 sea cucumbers species from a total of 7,000 living echinoderm species (Lane and Vandenspiegel 2003; Pawson 2007). They are divided in 7 orders (Dendrochirotida, Synallactida, Persiculida, Elasipodida, Apodida, Molpadida and Holothuriida), 25 families and 200 genera (Pawson 2007; Miller *et al.* 2017; WoRMS 2019).

Approximately 150 species of the genus *Holothuria* have a worldwide distribution. They are found in all types of marine habitats, from the intertidal zone to the deep ocean and from polar to tropical, and more than 37 species exist in the Mediterranean Sea (Fischer *et al.* 1987; Moussa and Wirawati 2018). But, even today, they are one of the least studied group of echinoderms (Kerr and Kim 1999; Kerr *et al.* 2009).

They present a wide variety of shape and colours and are characterised by a distinct trivium and bivium and bilateral symmetry. Also, they can be distinguished by the presence or absence of ambulacral feet, oral retractor muscles and Cuvierian tubules, the shape and form of the tentacles of the mouth (digits, dendritic, pinnate or shovel-shaped) and their respiratory trees (Tortonese and Vadon 1987; Hendler *et al.* 1995; Kerr *et al.* 2009), as well as by the presence, absence and morphology of their ossicles.

As mentioned, the derma of sea cucumbers is usually covered by ossicles (or spicules) that constitute a crucial element for species identification (Pawson 2007; Kamarudin and Rehan 2015). These microscopic calcareous components occur in a wide variety of shapes and forms, the most common ones resembling tables, buttons, rods, anchors, perforated plates and rosettes.

However, systematic studies on holothurians based on morphological and anatomical criteria, like spicules, could be confusing and dubious because of the large similarity of these characters between species, and research on these aspects is still scarce or sometimes incomplete, since not many studies have documented the morphological and molecular relationships in them, or the changes that some of them undergo throughout individuals growth or under different environmental conditions (Kerr *et al.* 2005; Samyn *et al.* 2005; Mezali and Francour 2012).

Although the morphology of spicules is an effective taxonomic character, it may show some overlap in some taxa, 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 spicules formation and carbonate precipitation. 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 complementary molecular and morphometric approaches are required if morphological identification is uncertain or impossible. Supported by ecological and biogeographical parameters, these techniques and complementary approaches are a strong driving force in taxonomic study (Trivedi *et al.* 2016).

Many authors (Knowlton 2000; Clouse *et al.* 2005; Uthicke *et al.* 2005; Borrero-Pérez *et al.* 2009, 2010; Mezali and Francour 2012; Kamarudin and Rehan 2015; Laakman *et al.* 2016; López-Márquez *et al.* 2018) have been involved in research on systematic identification and/or revision of the taxonomical status of different classes of Echinodermata in this line of work, combining morphometric and genetic analyses to identify species and/or to clarify their systematic position, and have provided molecular evidence to support their findings.

Identifying sea cucumbers using DNA barcoding techniques seems to overcome the disadvantages of using morphological keys, since for example they can be used for all life-stages from larvae to adults. Therefore, morphological and molecular approaches, complement each other to produce more accurate results, being a very useful and reliable tool for species identification.

For this reason, the present chapter aimed to assess the level of genetic and morphometric variation in different populations of genus *Holothuria* in the Atlantic Ocean and the Mediterranean Sea. We aim also to investigate morphometric differentiation based on the characteristics of their spicules, especially their tables and buttons, in order to clarify the systematic position according to the similarities and dissimilarities between the genetic and morphometric results.

4.2. Material and methods

4.2.1. Biological material

A total of 41 specimens of sea cucumbers of the genus *Holothuria,* from the Atlantic Ocean and Mediterranean Sea, were the subject of the present objective (Table 4.1). Seventeen (17) of the studied specimens are from Tunisia (Bizerte, Haouaria), where they were collected by dredging and diving (Chammem *et al.* 2019) at a depth of 1 to 5 m, while the rest (24 individuals) are Atlantic and Mediterranean species from the collection of Dr. Angel Pérez–Ruzafa located in the Department of Ecology and Hydrology at the University of Murcia. These material was representivemany localities of the Spanish Mediterranean region (Cape of Palos, Mar Menor lagoon, Gerona), and Atlantic Ocean (Cape Verde islands, Portugal (Azores and Algarve), Canary Islands). The studied material blongs to eight species from the genus *Holothuria*: *H. (Roweothuria) arguinensis* Koehler and Vaney, 1906, *H. (Holothuria) dakarensis* Panning, 1939, *H. (Vaneyothuria) lentiginosa lentiginosa* Marenzeller von, 1892, *H. (Holothuria) mammata* Grube, 1840, *H. (Roweothuria) poli* Delle Chiaje, 1824, *H. (Platyperona) sanctori* Delle Chiaje, 1823, *H. (Panningothuria) forskali* Delle Chiaje, 1823 and *H. (Holothuria) tubulosa* Gmelin, 1791.

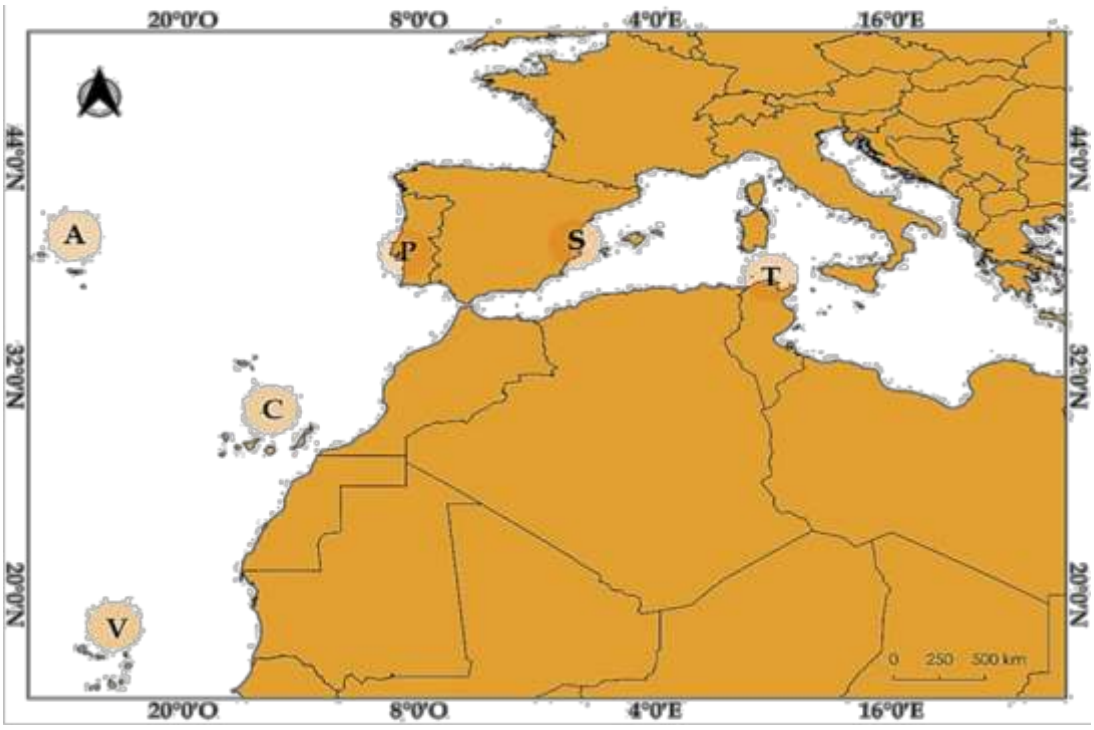


Figure 4.1: Map showing the sampling location in the Atlantic Ocean and the Mediterranean Sea: V= Cape Verde islands, C= Canary Islands, A= Azores, P=Portugal, S= Spain, T= Tunisia.

Table 4.1: Sampling locations and general information of the 41 studied specimens of genus *Holothuria* from the Atlantic Ocean and Mediterranean Sea. (n.) = number of specimens.

Species	n.	Country	Region	Abrevi.	References
Holothuria (Holothuria) tubulosa	01	Spain	Cape Palos	Htub-S	Ho 1828
Holothuria (Holothuria) tubulosa	02	Spain	Aguilas	Htub-S	Ho 1870
Holothuria (Holothuria) tubulosa	09	Spain	Aguilas	Htub-S	Ho 1869
Holothuria (Holothuria) tubulosa	10	Spain	Gerona	Htub-S	Ho1884
Holothuria (Holothuria) tubulosa	03	Tunisia	Haouaria	Htub-T	H1TN
Holothuria (Holothuria) tubulosa	04	Tunisia	Haouaria	Htub-T	H2TN
Holothuria (Holothuria) tubulosa	05	Tunisia	Haouaria	Htub-T	H3TN
Holothuria (Holothuria) tubulosa	06	Tunisia	Haouaria	Htub-T	H4TN
Holothuria (Holothuria) tubulosa	07	Tunisia	Bizerte	Htub-T	H5TN
Holothuria (Holothuria) tubulosa	08	Tunisia	Bizerte	Htub-T	H6TN
Holothuria (Roweothuria) arguinensis	01	Spain	Canary Islands	Harg	Ho 1707
Holothuria (Roweothuria) arguinensis	02	Portugal	Algarve	Harg	Ho 2
Holothuria (Platyperona) sanctori	01	Spain	Cape Palos	Hsan	Ho 7101
Holothuria (Platyperona) sanctori	02	Spain	Cape Palos	Hsan	Ho 15956
Holothuria (Holothuria) mammata	02	Portugal	Azores	Hmam	Ho 7101
Holothuria (Holothuria) mammata	01	Spain	Canary Islands	Hmam	Ho 1705
Holothuria (Holothuria) dakarensis	02	Portugal	Azores	Hdak	Ho 1630
Holothuria (Holothuria) dakarensis	01	Spain	Lanzarote	Hdak	Ho 1612
Holothuria (Roweothuria) poli	01	Spain	Mar Menor	Hpol-S	AL
Holothuria (Roweothuria) poli	02	Spain	Mar Menor	Hpol-S	AL2
Holothuria (Roweothuria) poli	03	Spain	Mar Menor	Hpol-S	AL3
Holothuria (Roweotuhria) poli	04	Spain	Mar Menor	Hpol-S	AL4
Holothuria (Roweothuria) poli	14	Spain	Mar Menor	Hpol-S	AL5
Holothuria (Roweothuria) poli	17	Spain	Mar Menor	Hpol-S	URRU
Holothuria (Roweothuria) poli	05	Tunisia	Bizerte	Hpol-S	Hsp11
Holothuria (Roweothuria) poli	06	Tunisia	Bizerte	Hpol-T	Hsp1
Holothuria (Roweothuria) poli	07	Tunisia	Bizerte	Hpol-T	Hsp2
Holothuria (Roweothuria) poli	08	Tunisia	Bizerte	Hpol-T	Hsp3
Holothuria (Roweothuria) poli	09	Tunisia	Bizerte	Hpol-T	Hsp4
Holothuria (Roweothuria) poli	10	Tunisia	Bizerte	Hpol-T	Hsp5
Holothuria (Roweothuria) poli	11	Tunisia	Bizerte	Hpol-T	Hsp6
Holothuria (Roweothuria) poli	12	Tunisia	Bizerte	Hpol-T	Hsp7
Holothuria (Roweothuria) poli	13	Tunisia	Bizerte	Hpol-T	Hsp8
Holothuria (Roweothuria) poli	15	Tunisia	Bizerte	Hpol-T	Hsp9
Holothuria (Roweothuria) poli	16	Tunisia	Bizerte	Hpol-T	Hsp10
Holothuria (Vaneyothuria) lentiginosa lentiginosa	01	Spain	Canary Islands	Hlen	Ho 1021 GBP
Holothuria (Vaneyothuria) lentiginosa lentiginosa	02	Cape Verde	Cape Verde	Hlen	Ho 5 -1998
Holothuria (Vaneyothuria) lentiginosa lentiginosa	03	Cape Verde	Cape Verde	Hlen	Ho 384 - 1998
Holothuria (Vaneyothuria) lentiginosa lentiginosa	04	Cape Verde	Cape Verde	Hlen	Ho 370 A1: E410-1998
Holothuria (Panningothuria) forskali	01	Portugal	Algarve	Hfor	Ho1856
Holothuria (Panningothuria) forskali	02	Portugal	Algarve	Hfor	Ho1857

4.2.1.1. Study of ossicles

The spicules from eight sea cucumbers species were identified according to the classical method described by Pérez-Ruzafa and Marcos (1985, 1987). A small part was cut from both sides of the trivium and bivium teguments and then put on a watch glass with a few drops of sodium chloride (NaCL), before heating until all the liquid was evaporated. Next, it was washed several times with distilled water and with a few drops of 70° alcohols. The spicules were placed on a slide using a micropipette and a few drops of Xylene and Canada Balsam were added before observation under microscope (Nikon Labophot Binocular Microscope).

Images of the spicules, buttons and tables, of all the studied sea cucumber specimens were taken at the Image Analysis Department of the University of Murcia. A total of 20 buttons and five tables from trivium and bivium sides of five different specimens per species were studied. Analyses were made using the Imp Software 2.0 program.

The maximum and minimum diameter, area, perimeter, convex perimeter and the number of holes per button were measured manually on the computer screen, as well as the maximum and minimum diameter, area and perimeter, height and turn width of tables. Using the resulting data, we calculated the area per hole (π*(length*width)/2), circular shape (4*π*Area/perimeter), elongated shape (Minimum diameter/Maximum diameter), wrinkled shape (convex perimeter/perimeter) and slightness shape (Total area of holes/Area) for buttons and tables. We have referred to Borrero-Pérez *et al.* (2009) for the definition and calculation of the latter parameters.

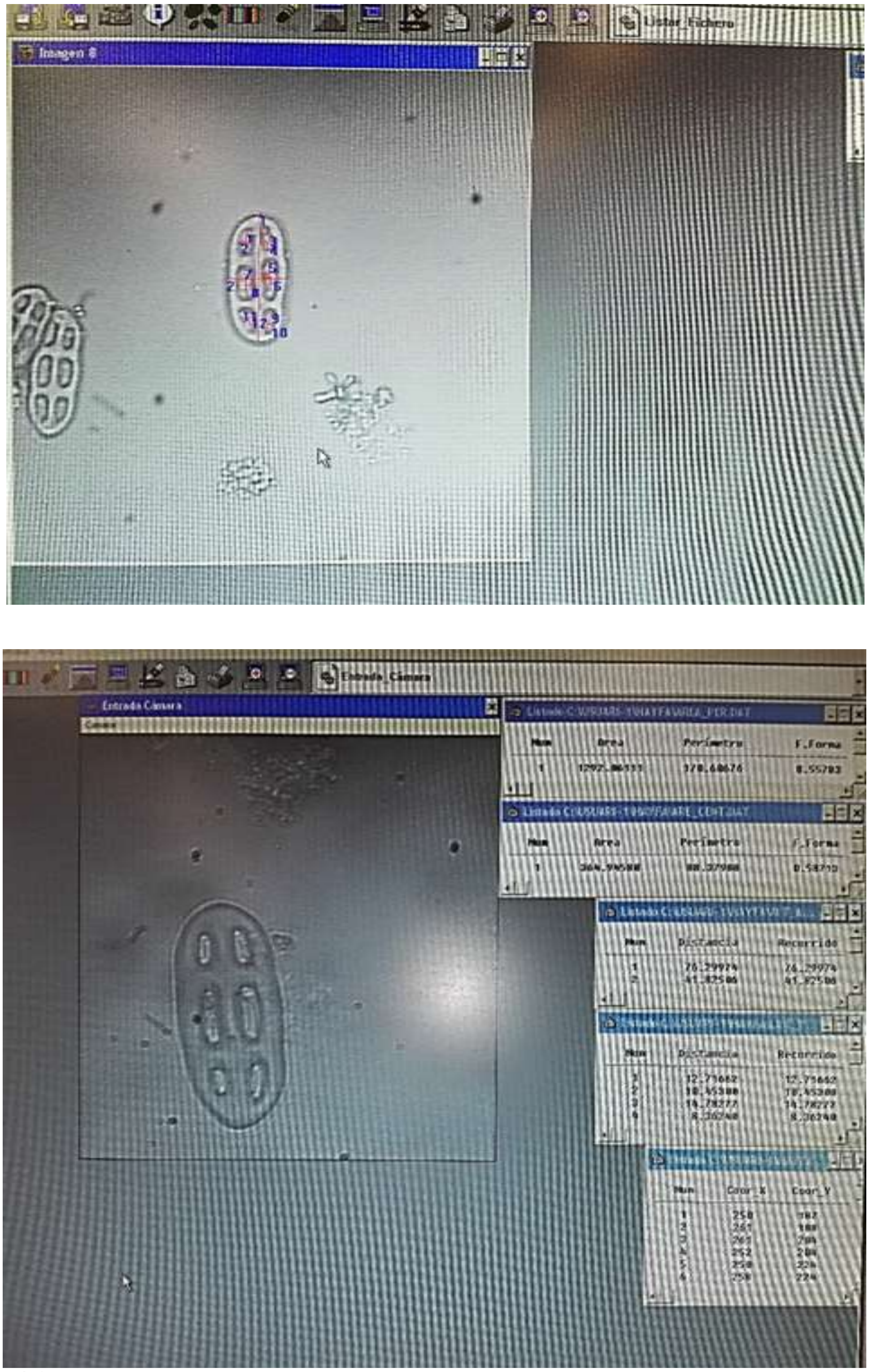


Figure 4.2: Picture showing the processing of analyse with IMP 2.0 software and the measurements done on one of the selected button of the species *H. tubulosa.*

Table 4.2: Morphometric variables and indices used for the measurement of the holothurian specimen's buttons and tables (According to Borrero-Pérez *et al.* 2009).

BUTTONS VARIABLE'S		
Abbreviations	Description	
MaDB	Maximum diameter tables	
MiDB	Minimum diameter tables	
ArB	Area of the button	
PrB	Perimeter of the button	
CoPrB	Convex perimeter of the button	
Holes Buttons Variable's		
Abbreviations	Description	Formula
NbHB	Number of the button hole s	
MaDHB	Maximum diameter of the button hole s	
MiDHB	Minimum diameter of the button hole s	
ArHB	Area of the button hole s	p*MaDHB*MiDHB/2
TArHB	Total area of button hole s	
Indices Calculated		
Abbreviations	Description	Formula
CiSB	Circular shape button	4πArB/PrB
ElSB	Elongation shape button	MiDB/MaDB
WrSB	Wrinkled shape button	CoPrB/PrB
SlHB	Slightness shape button	TArHB /ArB)
TABLES VARIABLE'S		
Abbreviations	Description	
MaDT	Maximum diameter of the table	
MiDT	Minimum diameter of the table	
ArT	Area of the table	
PrT	Perimeter of the table	
CoPrT	Convex perimeter of the table	
NbHT	Number of the tables hole's	
Indices Calculated		
Abbreviations	Description	Formula
CiST	Circular shape table	4πArT/PrT
ElST	Elongation shape table	MiDT/MaDT
WrST	Wrinkled shape table	CoPrT/PrT

4.2.1.2. Statistical analysis of morphometric data

The morphometric variables were statistically analysed using PRIMER–E 6 (Clarke and Gorley 2006) software program. Morphometric data were standardized, transformed to square root and a Principal component analysis (PCA) was conducted using five buttons and tables per species (belonging to five different specimens for each studied species). The Principal component analysis has been used to simplify the problem of data reduction. It is applied to reduce the dimensionality of a data set while retaining as much as possible the variability present and allowing the assessment of associations between variables (Gower 1966).

The same morphometric data were reassembled to perform the principal coordinates analysis (PCO) that is used to obtain a significant representation of a set of items whose relationships are measured by any similarity or distance coefficient (Gower 1966). It is applied to permit the positioning of objects in a space of reduced dimensionality while preserving their distance relationships as much as possible (Gower 1966).

Also, a Bray Curtis Similarity cluster was generatedusing the average of the total data for tables and buttons, to detect the morphometric similarity between the different studied taxa. Itwill be compared later, with the phylogenetic tree from the genetic study.

4.2.2. DNA barcoding and genetic study

DNA barcoding tool has been so long used for identifying animal species. The method is based on DNA sequence analysis of about approximately 650-bp region from the 5' end of the mitochondrial cytochrome oxidase I (COI) gene (Hebert *et al.* 2003). The short DNA barcoding gene sequence contains enough information to distinguish between species. A reference library of barcodes is established based on sequences from verified specimen identifications, backed up wherever possible by voucher specimen retention, and unknown specimens identified by matching their barcodes to the reference library (Ratnasingham and Hebert 2007).

One of the advantages of using the COI gene is that the universal primers for this gene are very robust (Folmer *et al.* 1994; Zhang and Hewitt 1997). Many times, and when the genetic database is consistent, DNA barcoding has advantages in precision and accuracy for the safe identification of species compared with the morphological observations. This method has been successfully proven in different marine organisms, also in echinoderms (Ward *et al.* 2008b; Jefri *et al.* 2015; Prehadi *et al.* 2015; Layton *et al.* 2016; Madduppa *et al.* 2016).

In our study, genetic analyses were performed following the standard protocol of Sambrook *et al.* (1989) for extraction and polymerase chain reaction (PCR) amplification. Both mitochondrial fragments of DNA corresponded to the Cytochrome (C) oxidase subunit (I), (COI) gene and 16S ribosomal RNA gene (rRNA), were amplified using specific primers. They were after followed by DNA sequencing. All the sequences were kept in the Molecular Biology section of the Research Support Service at the University of Murcia (Spain).

4.2.2.1. DNA Extraction, Amplification and sequencing

DNA was extracted from 15-25 mg of longitudinal muscle tissue of holothurian samples, which were conserved in ethanol, following the mentioned standard protocol of Sambrook *et al.* (1989) detailed below:

Reagents:
1. Cell Lysis Buffer (0.5M Tris, 0.1M EDTA, 2% SDS, pH = 8.8)
2. Proteinase Precipitation Solution (5M Ammonium Acetate, pH = 8)
3. Proteinase K (25 mg / ml)
4. Isopropanol (-20 ° C)
5. Ethanol 70% (-20 ° C)
6. Distilled sterile water

Procedure:
1. 20 to 30 mg of the tissue is placed in 1.5 ml Eppendorf tubes.
2. about 600 µl of Cell Lysis buffer and 5 µl of Proteinase K are added and mixed altogether using a Vortex.
3. The mixture is incubated at 55° C overnight, with occasional inversion stirring.
4. The samples are placed in the freezer for 20 minutes until the temperature drops below 20 °C.
5. 400 µl of the solution of Proteinase precipitation solution are added to the previously frozen tubes and they are well mixed with a Vortex for 5 minutes.
6. The tubes are centrifuged for 15 minutes at 13,000 rpm.
7. After centrifugation, the supernatant is carefully removed using a micropipette without removing the precipitation.
8. The supernatant is placed in 1.5 ml Eppendorf tubes, adding 600 µl of Isopropanol at -20 °C.
9. After shaking the tubes, they are placed in the freezer for one hour at 20 °C.
10. Centrifuged again for 30 minutes at 13,000 rpm. The supernatant is carefully discarded without losing the precipitated DNA.
11. 1 ml of 70% ethanol at -20 °C is added to the tubes and they are rigorously mixed by vortex in order to obtain a homogeneous solution.
12. After centrifugation for 15 minutes at 13,000 rpm, the precipitated DNA is keeped in room temperature to dry or in the oven until total evaporation of the alcohol.
13. At list, 50-200 ml of ultra-pure water is added to the tubes and are stored at 20 °C.

Two fragments the mitochondrial genes (mtDNA) of subunit I of cytochrome oxidase (COI) (ca. 650 bp) and 16S genes were amplified by standard PCR techniques. The primers used for the amplification of COI genes were CO1eI 5' ATAATGATAGGAGGRTTTGG 3'; CO1eII 5' GCTCGTGTRTCTACRTCCAT 3'. 16S primers were as follows: 16SI 5' CGCCTGTTTATCAAAAACAT 3'; 16SII 5' CTCCGGTTTGAACTCAGATCA 3' (Palumbi 1996; Borrero-Pérez *et al.* 2009; Chammem *et al.* 2019). Only the specimens from *H. tubulosa* and *H. poli* were identified to 16S. For the rest of the species, werefred to the sequences of Borrero-Pérez *et al.* (2009) from GenBank

Amplifications were performed using standard conditions. They were carried out in reactions (12 µL final volume) involving 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). The temperature wasdepending on the quality of tissue and gene to be amplified. Old tissues were amplified at 39 °C, while recent tissues of COI gene were amplified at 46 °C and the one of 16S gene were amplified at 47 °C. The concentrations of the PCR reagents and the cycle are the same. A Thermocycler PTC100 (MJ Research) and a MJ Mini (Bio-Rad) were used (Borrero-Pérez *et al.* 2009).

To visualize the results of DNA and PCR extractions, an 1% agarose gel electrophoresis was used. It is based on the migration of all molecules with different bands through the gel. The agarose gel was used at a concentration of 1% to verify the results of the extractions (90 ml 1x TBE + 0.9 g Sigma agarose) and of 2% for the results of PCRs (90 ml 1x TBE + 1 8 g of agarose). The gel strips were stained with ethidium bromide and visualized with UV light. To estimate the size and amount of DNA amplified fragment, the HyperLader II (Bioline) tag was used which bands between 50 and 2000 base pairs (Borrero-Pérez *et al.* 2009; Chammem *et al.* 2019).

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) (Borrero-Pérez *et al.* 2009; Chammem *et al.* 2019).

4.2.2.2. Phylogenetic study

The phylogenetic inference from the alignment of homologue molecular sequences is one of the keys for analyzing evolutionary biology in ecology. A phylogenetic tree can be generated from phylogenetic analyses methods using statistical model parameters as Parsimony (P), Bayesian, Maximum and Minimum Likelihood (ML) or Neighbour-Joining (NJ). The appropriate model is chosen usually regarding each genes type (16S or COI).

The NJ parameter is based on the principle of minimum evolution (Saitou and Nei 1987), by calculating the evolutionary distances that separates the homologous sequences using algorithmic agglomeration. The more different the two homologous sequences, the greater the evolutionary distance between them. And, on the contrary, the closer the two homologous sequences approach, the weaker is the evolutionary distance that separates them. This allows to obtain phylogenetic trees in a simple and fast way, what is suitable for groups with big data and gives good results for preliminary approaches.

The phylogenetic analysis was carried out on a total of 64 sequences of 1050bp on both genes 16S and COI. The DNA sequences were respectively, edited and aligned using ClustalW as a default alignment parameter of the MEGA program, version X (Kumar *et al.* 2018). Then they were analysed to conduct a Neighbour Joining tree using MEGA version X (Kumar *et al.* 2018). The two aligned sequences were concatenated in one single matrix using PhyloSuite software v1.2.2 (Zhang *et al.* 2020). The generated fast file was analysed and a Neighbour-Joining phylogenetic tree was generatedwith MEGA X. Finally, pairwise nucleotide distances were calculated using the Kimura 2-parameter (K2P) model of base substitution (Kimura 1980).

4.3. Results

4.3.1. Morphometries of the spicules

The morphometric analyses concern all the studied species of the genus *Holothuria* with the exception of *Holothuria forskali* which is characterized by the absence of buttons and broken or reduced tables. So, this last species appears only on the molecular cluster. They showed close similarity in shape between species, which is why morphological taxonomical identification is uncertain and doubtful in most cases.

The Principal Component Analysis (PCA) (Fig. 4.3) shows the main variables that explain the differences among the studied species of *Holothuria*. Results showed that the first and the second principal axes (PCA1 and PCA2) jointly accounted for 99 % of the total variance of the morphometric data, and the total PCA explained 99,8% of the variance. PCA1 explained 92.1% of the variance and PCA2 explained only a 6.9%. The parameters that justify the variation among species are those with highest eigenvectors such as: the area of the tables (ArT) and their convex perimeter (CoPrT), the area of the buttons (ArB), the area of the button holes (ArHB) and the number of the button holes (NbHB).

The hole's area (ArHB) and the area (ArB) of the buttons are the main parameters which separate *H. poli, H. dakarensis, H. l. lentiginosa* and *H. arguinensis* from the other species. The area of the tables (ArT) and the number of holes of the buttons (NbHB) allow to distinguish *H. poli* and *H. tubulosa* from the rest of the species. The convex perimeter of the tables (CoPrT) was the main parameter that separate H. *mammata, H. tubulosa* and *H. poli* from the rest of the four species. Only *H. arguinensis* and *H. sanctori* are clearly separated from the rest of holothurian species. The individuals of *H. arguinensis*, *H. lentiginosa* and *H. mammata* are present in the positive side of PCA2, while those of *H sanctori* are present on the positive side of PCA1.

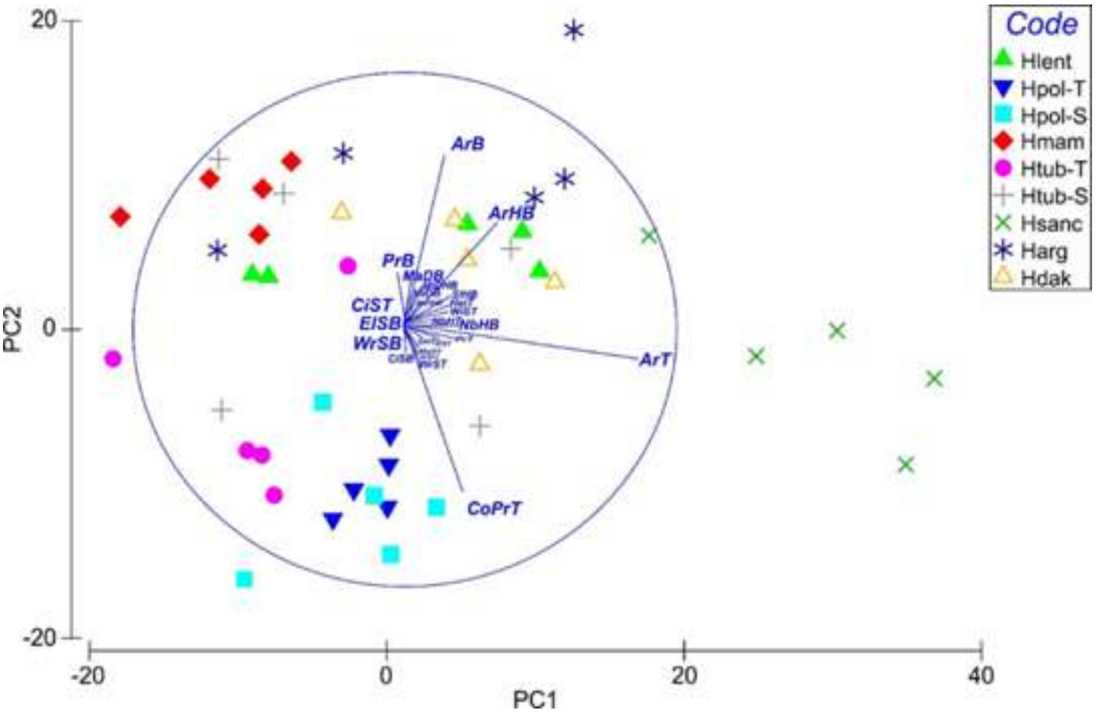


Figure 4.3: Principal Component Analyses (PCA) of the seven holothurian species according to their morphometric tables and buttons parameters. Hlen= *Holothuria l. lentiginosa,* Hpol TN= *Holothuria poli* Tunisia; Hpol SN= *Holothuria poli* Spain; Hmam= *Holothuria mammata*; Htub TN=*Holothuria tubulosa* Tunisia; Htub SN=*Holothuria tubulosa* Spain; Hsan=*Holothuria sanctori;* Harg=*Holothuria arguinensis;* Hdak=*Holothuria dakarensis.*

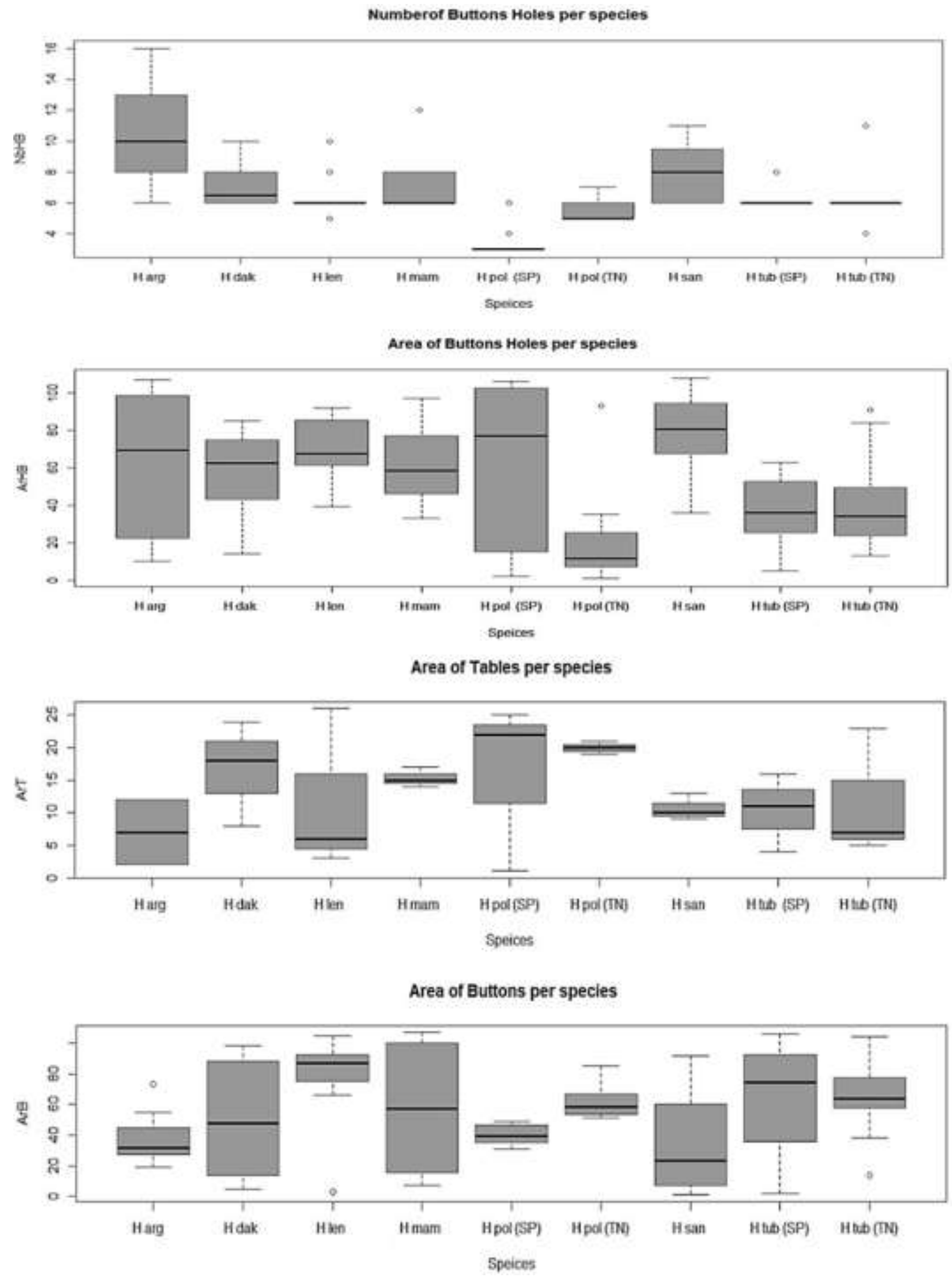


Figure 4.4: Box plots of the four main parameters belonging to the seven species of the genus Holothuria and controlling their distribution according to the PCA Fig. 4.3: the area of the buttons (ArB), the number of the holes (NbHB), the area of the button holes (ArHB), and the area of the table (ArT). For the area the unit is (Um2). The line inside the boxes indicates the medium value and the lines outside the boxes are the maximum and the minimum quartiles. The spots represent the interval between the lowest and highest data of the interval interquartile (minimum and maximum). *Hlen= Holothuria l. lentiginosa, Hpol* TN= *Holothuria poli* Tunisia; *Hpol* SN= *Holothuria poli* Spain; *Hmam= Holothuria mammata; Htub* TN =*Holothuria tubulosa* Tunisia; *Htub* SN =*Holothuria tubulosa* Spain; *Hsan=Holothuria sanctori; Harg=Holothuria arguinensis; Hdak=Holothuria dakarensis* .

On the other hand, the total Principal Coordination Analysis PCO resulted in the first and the second principal axes (PCO1 and PCO2) jointly explained 98.8% of the total variance of the morphometric data, very similar to the PCA results. PCO1 explained 96.8% of the variance and PCO2 explained only 2% of the total variance (Fig. 4.5).

H. poli specimens from Tunisia and Spain (Hpol–T and Hpol–S) had the highest positive eigenvalues in the positive part of the axis 1, while, *H. arguinensis* (Harg) had the highest negative eigenvector. The PCO showed that the spicules of the Atlantic species *H. l. lentiginosa* (Hlen) are much closer to the spicules of both Mediterranean *H. tubulosa* and *H. poli* (from Tunisia and Spain), than the spicules of the Atlantic species *H. dakarensis* (Hdak), *H. mammata* (Hmam) and *H. arguinensis* (Harg). The spicules of *H. l. lentiginosa* (Hlen), *H. tubulosa* (Htub) and *H. poli* (Hpol) species are located on the positive side of PCO1, whereas, the Mediterranean specimens of Hsan (Spain) are present in the negative side of PCO1 together with the Atlantic individuals of *H. dakarensis* (Hdak), *H. mammata* (Hmam) and *H. arguinensis* (Hag). Only *H. arguinensis* (Harg) and *H. poli* (Hpol-T and Hpol-S) are located on the positive side of PCO2, while the rest of the studied species are on the positive negative one. According to the PCA results, H. *arguinensis* (Harg) and *H. poli* seem to be more clearly separated from the other holothurian species by the shape of its buttons and the number of its holes.

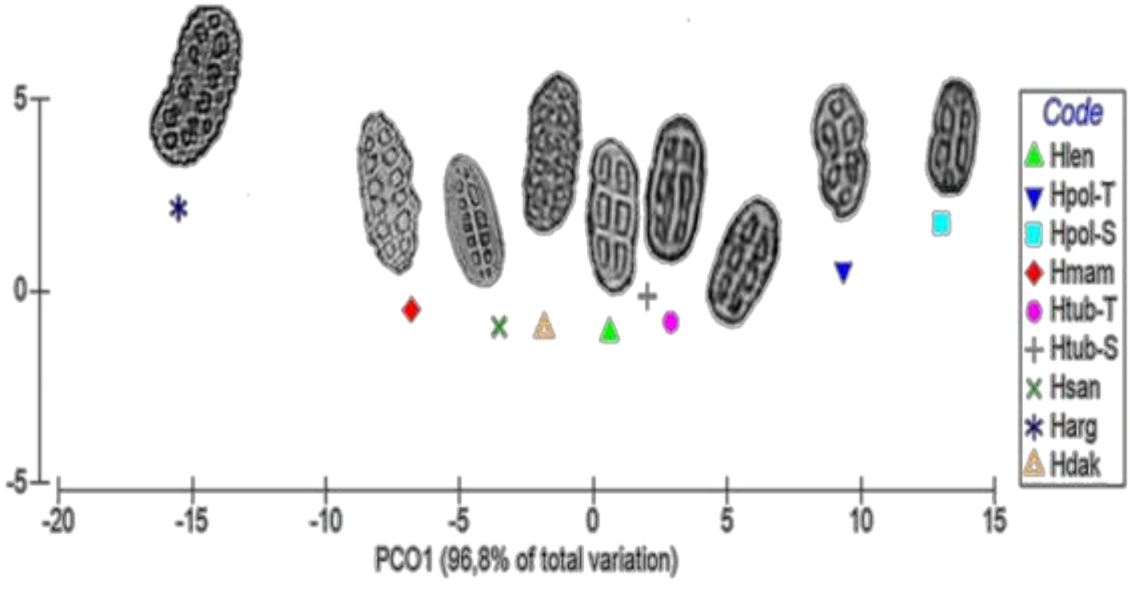


Figure 4.5: Principal Coordinates Analyses (PCO) for the seven species holothurian depending on their morphometric parameters. A, the distribution of the species on the two axes of PCO (PCO1 and PCO2). B, variability of the buttons in the PCO axes according to the species distribution. Hlen= Holothuria l. lentiginosa, Hpol TN= Holothuria poli Tunisia; Hpol SN= Holothuria poli Spain; Hmam= Holothuria mammata; Htub TN=Holothuria tubulosa Tunisia; Htub SN=Holothuria tubulosa Spain; Hsan=Holothuria sanctori; Harg=Holothuria arguinensis; Hdak=Holothuria dakarensis.

A dendrogram was generated (Fig. 4.6) fromthe PCO results, grouping the *Holuthuria* species according to the similarities in button database which were the most confusing because of their big similarity between the studied species. The eight studied species were divided into three clear clades. The first one grouped *H. tubulosa* (Tunisia and Spain) and *H. l. lentiginosa* with 96% similarity, a second clade grouped *H poli* from Tunisia and Spain with about 96% similarity, and the third one grouped *H. mammata* (Hmam), *H. dakarensis* (Hdak) and *H. sanctori* (Hsan) with 95% similarity. *H. arguinensis* (Harg) was separate from the three clades showing the lowest degree of similarity with the rest of the species.

So, these resemblances between species (Table 4.5) indicate that the highest similarity in term of buttons occur between *H. sanctori* (Hsan) and *H. dakarensis* (Hdak) (98.08%) and the lowest between *H. arguinensis* (Harg) and *H. poli* (Hpol) (71.51% for the Spanish specimens and 75.11% for the Tunisian specimens). Although *H. poli* (Hpol) and *H. arguinensis* (Harg) form part from the same subgenus *(Roweothuria*), they shared the lowest degree of morphometric resemblance of all the Atlanto-Mediterranean species.

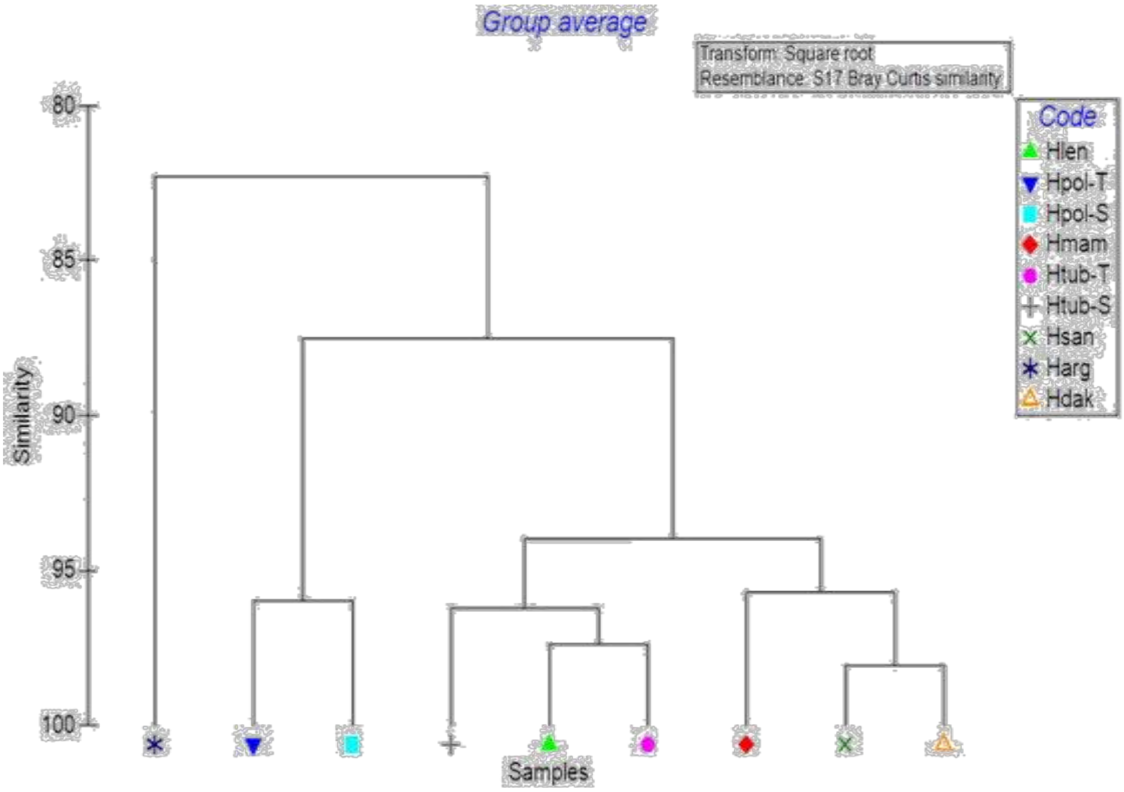


Figure 4.6: Tree diagram of cluster analyses of the species of Genus: Holothuria based on morphometric data (biological parameters) obtained by using the S17 Bray Curtis Similarity and transformed to Square root. *Hlen= Holothuria l. lentiginosa, Hpol* TN= *Holothuria poli* Tunisia; *Hpol* SN= *Holothuria poli* Spain; *Hmam= Holothuria mammata; Htub* TN*=Holothuria tubulosa* Tunisia; *Htub* SN*=Holothuria tubulosa* Spain; *Hsan=Holothuria sanctori; Harg=Holothuria arguinensis; Hdak=Holothuria dakarensis.*

Table 4.3: Principal Component Analysis (PCA) eigenvalues variations and cumulative variation between the 5 major PCA.

Eigenvalues			
PC	Eigenvalues	%Variation	Cumulative % Variation
1	121	92,1	92,1
2	9,02	6,9	99,0
3	0,661	0,5	99,5
4	0,258	0,2	99,7
5	0,154	0,1	99,8

Table 4.4: Principal Coordinates Analysis (PCO) eigenvalue’s variations and cumulative variation between the 9 individuals axes major PCA.

Variation explained by individual axes			
Axis	Eigenvalue	Individual%	Cumulative%
1	571,02	96,78	96,78
2	12,052	1,04	97,82
3	8,8136	1,49	99,32
4	1,4198	0,24	99,55
5	0,83075	0,14	99,69
6	0,13798	0,02	99,71
7	4,7045E-2	0,01	99,72
8	4,1118	0,05	99,77
9	4,3218	0,23	100

Table 4.5: Resemblances of the different species *Holothuria* (H) according to buttons morphometries Hlen= *Holothuria lentiginosa*, Hpol-T= *Holothuria poli*-Tunisia; Hpol-S= *Holothuria poli*-Spain; Hmam= *Holothuria mammata*; Htub-T= *Holothuria tubulosa*-Tunisia; Htub-S= *Holothuria tubulosa*-Spain; Hsan= *Holothuria sanctori*; Harg= *Holothuria arguinensis*; Hdak= *Holothuria dakarensis*; Med= Mediterranean species and Atl= Atlantic species.

	Hlen (Atl)	*Hpol (Tunisia)*	*Hpol (Spain)*	*Hmam (Med)*	*Htub (Tunisia)*	*Htub (Spain)*	*Hsan (Med)*	*Harg (Atl)*	*Hdak (Atl)*
Hlen (Atl)									
Hpol (Tunisia)	91,10								
Hpol (Spain)	87,25	95,98							
Hmam (Med)	92,49	83,80	80,07						
Htub (Tunisia)	97,41	93,30	89,57	90,24					
Htub (Spain)	95,86	92,46	88,68	91,09	96,60				
Hsan (Med)	95,65	87,00	83,25	96,45	93,55	94,36			
Harg (Atl)	83,54	*75,11*	*71,51*	90,89	81,35	82,16	87,64		
Hdak (Atl)	97,27	88,66	84,90	94,98	95,18	96,07	98,08	85,99	

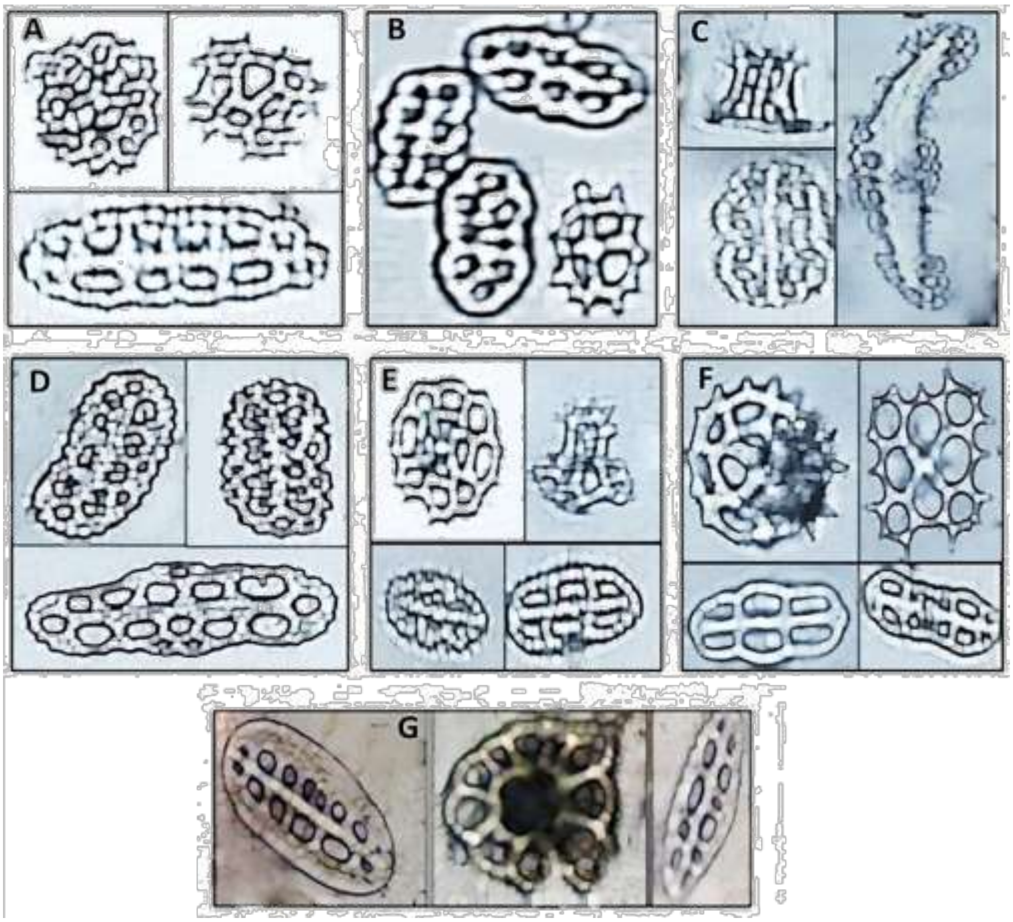


Figure 4.7: Spicules from buttons, tables and rods of sea cucumber including species from chapter 2 and 3. Scale bar: 0.5 um and grossisemnt (x 100); A- *Holothuria tubulosa*; B- *Holothuria poli*; C- *Holothuria mammata*; D- *Holothuria arguinensis*; E- *Holothuria dakarensis*; F- *Holothuria lentiginosa lentiginosa;* G- *Holothuria sanctori.*

4.3.2. Molecular analyses

A total 41 specimens of the genus Holothuria representing eight different species (*H. poli, H. tubulosa, H. sanctori, H. forskali*, *H. mammata*, *H. l. lentiginosa, H. arguinensis* and *H. dakarensis*) from four different localities in the Mediterranean and the Atlantic (Tunisia, Spain (Mediterranean Sea and Canary Islands), Portugal and Cape Verde) were genetically identified and analyzed at genes COI and 16S levels (Fig. 4.8/4.9, Table 4.6/4.7).

All the mtDNA data analyses of both genes placed the sequences of the eight species of Holothuria genus in four separate clades, which were supported by high bootstrap values. The first clade (clade I) from gene COI (Fig. 4.8) is divided into two subclades, both were highly supported (97%).

The first subclade (1) includes the Mediterranean specimens of *H. tubulosa* from the south western Mediterranean Sea (Spain) and central Mediterranean Sea (Northern Tunisia) with *H. l. lentiginosa* and *H. arguinensis*. While the second subclade (2) comprises only Atlantic species of, H. *mammata* and *H. dakarensis* with high bootstraps values (99%).

The second clade (Clade II) has strong support (100%) and it groups only the Mediterranean individuals of *H. poli* (Tunisia and Spain). The last clade (Clade III) is also highly supported (100%) and contains *H. sanctori* and *H. forskali* individuals from the Mediterranean Sea.

The holothurian sequences from the 16S gene cluster were similar to COI gene (Fig. 4.9). The species have the same position. The only difference was shown on the first clade (Clade I) which includes same species as COI with different position. The first subclade (Clade 1) from clade (I) comprises only Mediterranean species from *H. tubulosa* while, the second one (Clade2) includes species from *H arguinensis, H. mammata*, *H. dakarensis* and *H. lentiginosa.* Both were highly supported (97%).

As the COI gene cluster, the second clade (Clade II) from 16S gene, incudesonly individuals from *H. poli* and the third clade (Clade III) groups species of *H. forskali* and *H. sanctori* together. They were respectively highly supported with 98% (Clade III) and 100% (Clade II).

The lastclades (Clade IV) from COI and 16S genes was including outgroup species *H. leucospilota.*

The K2P genetic distances, based on the COI and 16S sequences, shown in Table 4.6 and 4.7, demonstrate that the greatest divergence occurs between *H. poli* and *H. forskali* (27.4%) and the lowest one between *H. mammata* and *H. dakarensis* (0.9%). Indeed, *H. forskali* showed the highest divergence values which varied from 19% to 27.4%. Distances between *H. poli* and *H. l. lentiginosa* was 15,8% and 9,1% between *H. l. lentiginosa* and *H. tubulosa*, while it was 16,9% between *H. poli* and *H. tubulosa*.

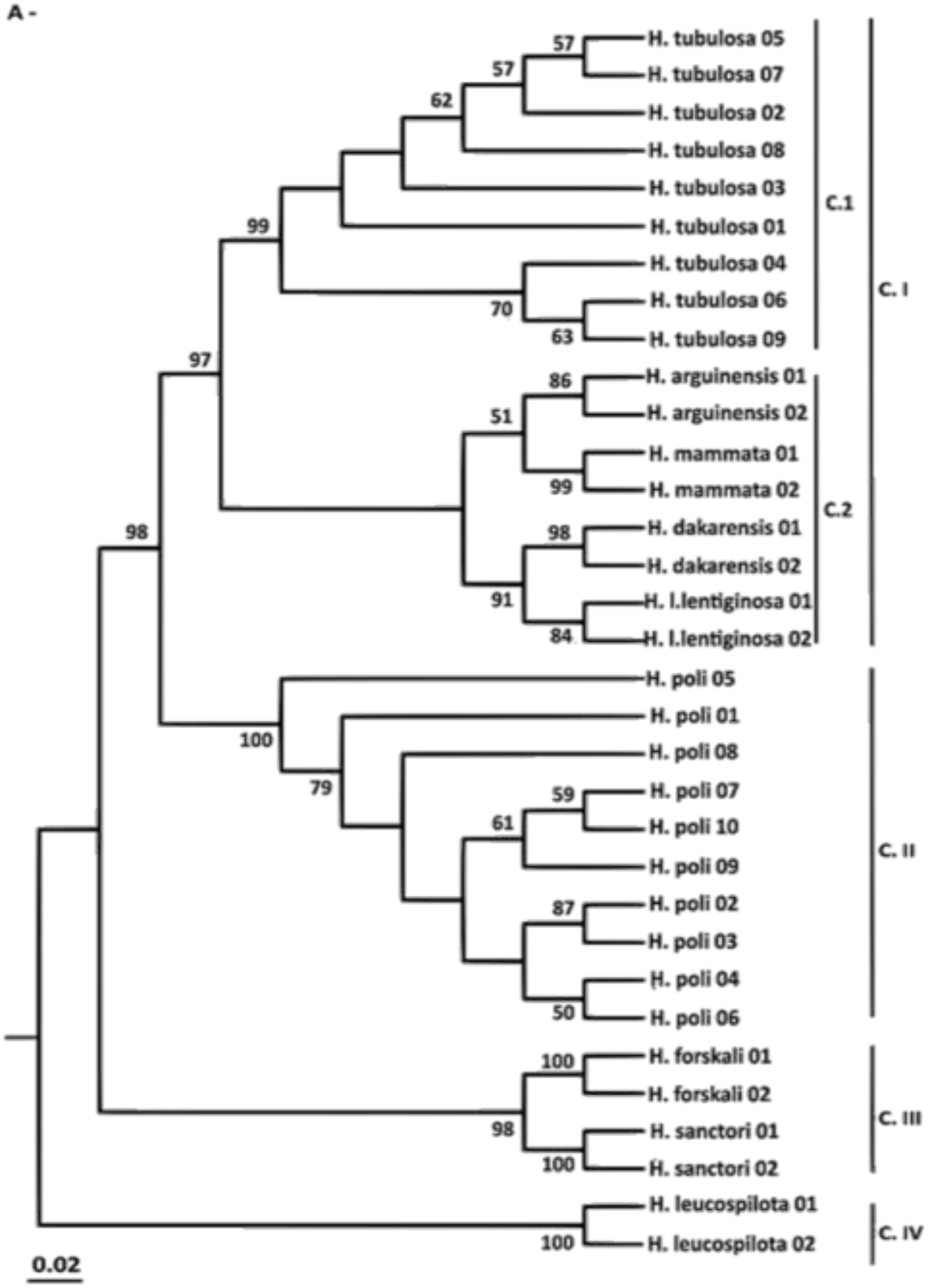


Figure 4.8: DNA sequences of 16S (A) genus of sea cucumbers from the genus *Holothuria*. Neighbour–Joining tree analysis of 500 bp 16S fragment based on p–distance. The bootstrap consensus tree inferred from 10000 replicates. Only bootstrap values of branches exceeding 50% are indicated. The p distances were computed using the Kimura 2–parameter method and are expressed in the units of the number of base substitutions per site. Analyses were conducted in MEGA7. H=Holothuria. C= Clade.

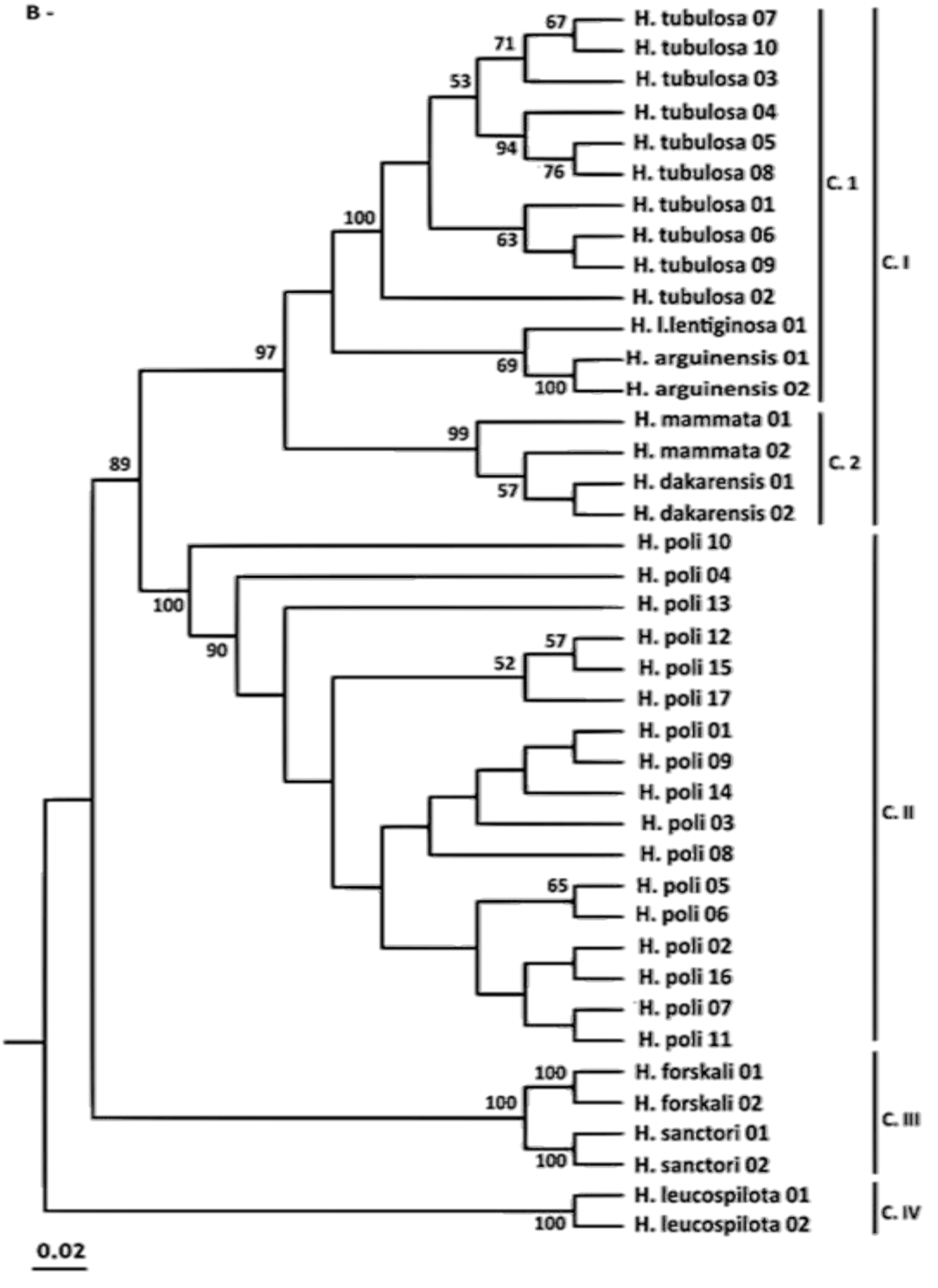


Figure 4.9: DNA sequences COI (B) genus of sea cucumbers from the genus *Holothuria*. Neighbour–Joining tree analysis of 553 bp COI fragment based on p–distance. The bootstrap consensus tree inferred from 10000 replicates. Only bootstrap values of branches exceeding 50% are indicated. The p distances were computed using the Kimura 2–parameter method and are expressed in the units of the number of base substitutions per site. Analyses were conducted in MEGA7. H=Holothuria. C= Clade.

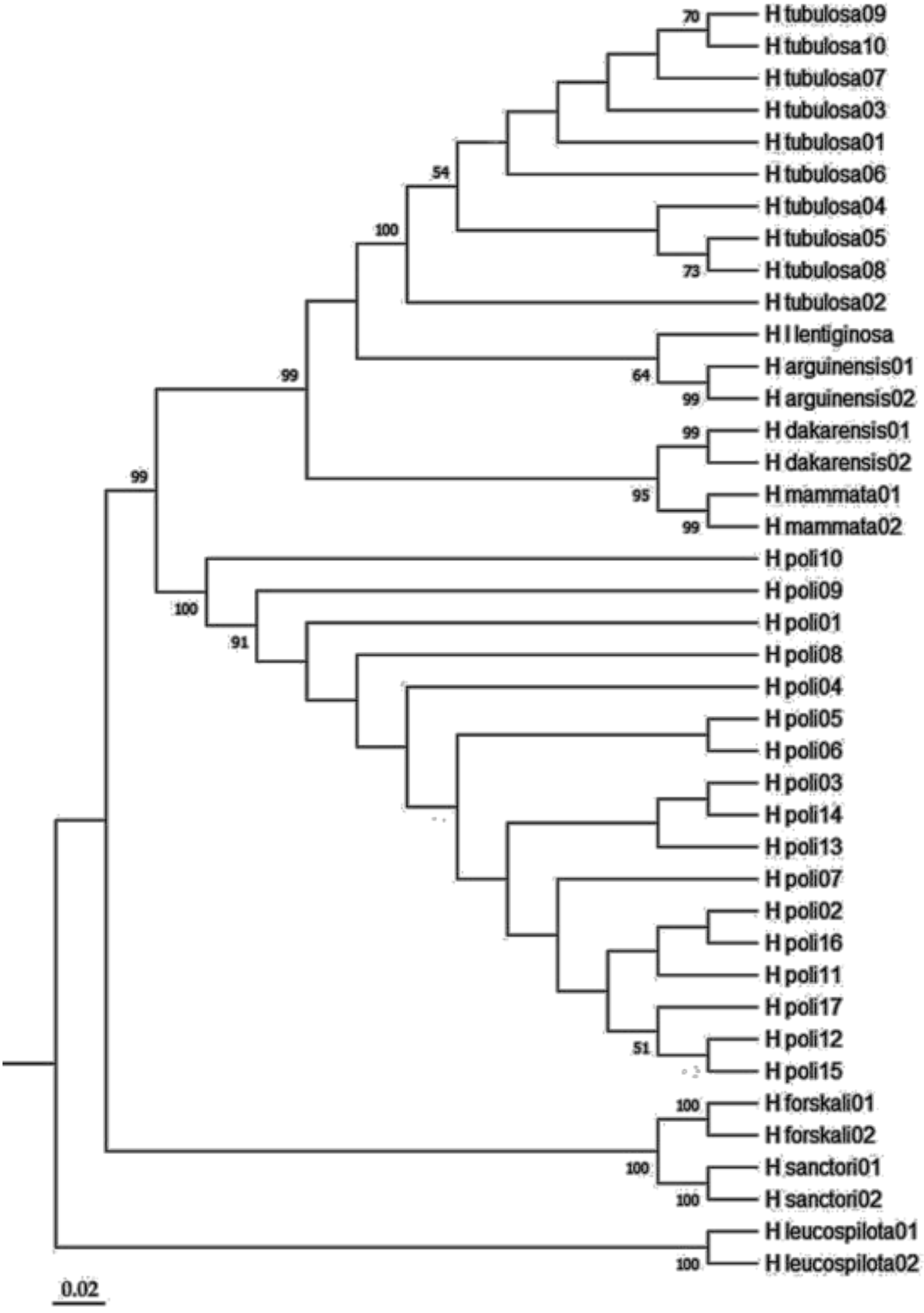


Figure 4.10: A-Neighbour–Joining tree analysis of the DNA sequences of concatenated matrix of mitochondriales genes COI and 16S of sea cucumbers from the genus *Holothuria*. of 553 bp. The optimal tree with the sum of branch length = 0.8 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) are shown next to the branches. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. This analysis involved40 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1051 positions in the final dataset. Evolutionary analyses were conducted in MEGA X. H=Holothuria.

Table 4.6: Genetic distances between *Holothuria* (H.) species obtained from the phylogenetic reconstruction of the genes COI and 16S based on the Kimura 2–parameter model (K2P). Estimates of Evolutionary Divergence over Sequence Pairs between Groups. The number of base substitutions per site from averaging over all sequence pairs between groups are shown. Evolutionary analyses were conducted in MEGA7. *H.arg= H. arguinensis; H.dak= H. dakarensis; H.for= H. forskali; H.l.len= H.l. lentiginosa; H.leu= H. leucospilota; H.mam= H. mammata; H.pol= H. poli; H.san= H. sanctori; H.tub=H. tubulosa*; Med= Mediterranean species and Atl= Atlantic species.

	H.arg (Atl)	H.dak (Atl)	H.for (Med)	H.l.len (Atl)	H.leu (Atl)	H.mam (Med)	H.pol (Med)	H.san (Med)	H.tub (Med)
H.arg (Atl)									
H.dak (Atl)	0,064								
H.for (Med)	0,274	0,293							
H.l.len (Atl)	0,067	0,085	0,292						
H.leu (Atl)	0,216	0,212	0,319	0,240					
H.mam (Med)	0,063	0,031	0,295	0,077	0,218				
H.pol (Med)	0,118	0,121	0,273	0,165	0,229	0,124			
H.san (Med)	0,251	0,271	0,225	0,245	0,306	0,258	0,268		
H.tub (Med)	0,071	0,072	0,289	0,096	0,223	0,076	0,129	0,277	

4.3.3. Comparison between molecular and morphometric analyses

The comparison between molecular and morphometric analyses was made based on dendrograms results (Figs. 4.6, 4.7). Both similarity and gene COI cluster showed a high concordance. In fact, in both cases the Mediterranean species of *H. poli* (Spain and Tunisia) formed a separated clade with more than 90% similarity. Similarly, the Atlantic species *H. l. lentiginosa* was associated with the Mediterranean species *H. tubulosa* (Spain and Tunisia). Also, *H. dakarensis* and *H. mammata* specimens were grouped together, in both genetic and morphometric clusters. However, the *H. arguinensis* individuals, which are genetically grouped with *H. l. lentiginosa* and *H. tubulosa,* were morphometrically grouped in a separate clade with the rest of the species. The *H. sanctori* specimens which are genetically distinct from the other sea cucumbers, were morphometrically grouped with *H. dakarensis* and *H. mammata.* Indeed, geographic separation of the Mediterranean and Atlantic species which was most evident in the genetic cluster, was totally absent from the morphometric cluster.

On the one hand, *H. arguinensis* and *H. poli*, which represent the same subgenus were genetically and morphometrically different. *H. arguinensis* showed a morphometric dissimilarity from all the holothurian species but, genetically, it was in the same clade as the two Mediterranean *H. tubulosa* specimens (Spain and Tunisia) and the Atlantic specimens of *H. l. lentiginosa*. On the other hand, *H. poli* formed a separate clade in both the genetic and morphometric similarity dendrograms.

4.4. Discussion

This chapter focus on the genetic and morphometric resemblance of holothurian species from genus *Holothuria* across the Atlantic Ocean and Mediterranean Sea. Our results revealed a concordance on the clustering partners using both genetic and morphometric criteria.

The spicules morphometry analyses of *Holothuria*'s of the buttons marked an extensive morphological variation among the different species more than the table. The outcome of the Principal Component Analysis (PCA) revealed that the five parameters: area of buttons (ArB), number of button's holes (NbHB) and area of button´s holes (ArHB), also the area (ArT) and the convex perimeter of tables (CoPrT), are the major contributors for the morphometric spicules variability recorded among the different species from genus *Holothuria*.

Based on the Principal Coordinate Analysis (PCO), the above parameters are specific for each species although it should be taken into account that our specimens were all from adult stages. In fact, the spicules of Holothuriidae might change during the different life-history stages and the size of the ossicles, their shape and prevalence, which are specific for each sea cucumber, could differ in the same species as individuals develop from being early juveniles to adult specimens (Rowe 1969; Thandar 1987; Massin *et al.* 2000; Rasolofonirina and Jagoux 2005).

As examples, according to Massin *et al.* (2000), the materials from *H. scabra* sea cucumber collected at regular intervals through every stage of development, demonstrate that increases in body size produce a shorter button with a decreasing number of pairs of holes while the button changes from smooth to knobbed.

At the same time, although the diameter of its tables does not increase, its spire become higher and wider. However, only the tube feet and plates increase in size as body length increases. In studies of juvenile's sea cucumbers, Cutress (1996) and Massin *et al.* (2000), have reported the presence of tables and the absence of buttons, which are considered plesiomorph characters in the evolution of the Holothuriidae.

Moreover, the individuals of *H. forskali,* described by Pérez-Ruzafa *et al.* (1987), showed a reduction in the spicules number during their growth. Adults of this species are characterized by the absence of buttons and the presence of a large number of rods (Tortonese 1965; Pérez-Ruzafa *et al.* 1987). Spicule shape and number were depended on the life stage of the specimen. The juveniles of *H. forskali* had spicules that were much more developed and abundant than those of adults, while the area of table bases decreased compared with body diameter.

Besides, the genetic position of *H. forskali*, a unique species from the subgenus *Panningothuria*, and its presence in the same clade that *H. sanctori* may reflect the morphological resemblance between these two species. Indeed, they are very often confused because of the great similarity in body color and the presence of the Cuvierian tubule.

The results from the morphometric similarity dendrogram revealed the existence of a significant morphological resemblance between Atlantic Ocean and Mediterranean Sea species. The Atlantic species *H. l. lentiginosa* is shared the maximum degree of the similarity with the Mediterranean Tunisian species *H. tubulosa*. The same observation was applicable to the Mediterranean species *H. sanctori,* which was grouped with the two Atlantic species *H. dakarensis* and *H. mammata.* In fact, from so long the status of the last cited species was ambiguous and confused.

Previously, the species of *H. dakarensis* and *H. mammata* were placed in the same rank with *H. tubulosa* and *H. stellati*. Panning (1939) and Rowe (1969) have treated *H. dakarensis, H. mammata* and *H. tubulosa,* as subspecies of *H. stellati,* and described a new species *H. stellati dakarensis*. Cherbonnier (1952), maintained *H. tubulosa* and *H. mammata* in the same rank and put *H. dakarensis* in another. The same author has suggested that *H. tubulosa, H. mammata* and *H. dakarensis* can be distinguished by body form and by spicule size, the smallest spicules belonging to *H. tubulosa* and the largest one to *H. dakarensis.* However, according to Rowe (1969), the size of spicules of *H.*

tubulosa and *H. mammata* is intermediate between those of *H. stellati* and *H. dakarensis.* Accordingly, *H. dakarensis* presents the largest tables and buttons, while *H. stellati* has the smallest tables and *H. tubulosa* comes in the middle of the preceded species, with its very elongated and solid buttons. According to Cherbonnier (1950), the only way to distinguish *H. mammata* from *H. tubulosa* is the form of the body and the presence of a Cuvierian organ. However, the last organ can be easily eviscerated and so is considered an unsatisfactory distinguishing character (Rowe 1969).

Zavodnik (1999, 2003) recognized *H. stellati, H. tubulosa* and *H. mammata,* but concluded that the systematics of these species and also that of *H. dakarensis* to still be ambiguous. Indeed, Borrero-Pérez *et al.* (2009) confirmed the morphological variability of the specimens of *H. stellati* and *H. tubulosa,* as described in the literature but the molecular results obtained by these authors considered *H. stellati* to be a junior subjective synonym of *H. tubulosa.*

Our results obtained from morphometric cluster and PCO analyses concerning spicules variability confirm the close morphological similarity between the different holothurian species, as described on the literature cited above. Moreover, the results clearly show and explain the high degree of ambiguity regarding the classification and taxonomic position of Holothuriidea species.

The ossicles of *H. dakarensis* species comprises large tables with small holes, accompanied by large buttons that are regular and knobbed. In *H. mammata*, the buttons range from small to large, and they are elongated, knobbed and rough with a median ridge, while the tables are large and perforated. While, *H. sanctori* has a large rectangular table perforated with small holes accompanied by regular, slight and knobbed large buttons with a median longitudinal line (Rowe 1969).

According to the outcome of the Principal Component Analysis (Fig. 4.11), the parameters of circular shape buttons (CiSB), elongation shape buttons (ElSB), wrinkled shape buttons (WrSB) and the slightness shape buttons (SlHB) are resembling *H. dakarensis*, *H. mammata* and *H. sanctori* together. The same figure shows that H. *l. lentiginosa* and *H. tubulosa* have in common the area of the holes (ArHB), the area of the buttons (ArB), the perimeter and the convex perimeter of the button (PrB; CoPrB). The ossicles of *H. l. lentiginosa* are characterized by delicate tables of moderate height, the disk is rounded, perforated with a smooth margin, accompanied by buttons which are flat and relatively smooth, irregular and twisted, with two rows of holes. *H. tubulosa* has tables that are performed by four large holes, accompanied by buttons that are irregular, elongated, knobbed and perforated by two rows of holes, very often incomplete, with a rough margin.

The rest of parameters (Fig. 4.11), the number of the button holes (NbHB), maximum and minimum diameter of the button holes (MaDHB; MiDHB), the maximum and minimum diameters of the buttons (MaDB; MiDB), grouped *H. poli* species in an individual clade and *H. arguinensis* species in another clade separate from the rest of holothurian species. The latter two species have the same subgenus, *Roweothuria.* The spicules of *H. poli* are easy to recognize from its regular and smooth buttons with an odd number of holes (Panning 1939; Massin 1996). The last characteristic is confirmed typical for *H. poli* by the results of the Principal Component Analysis.

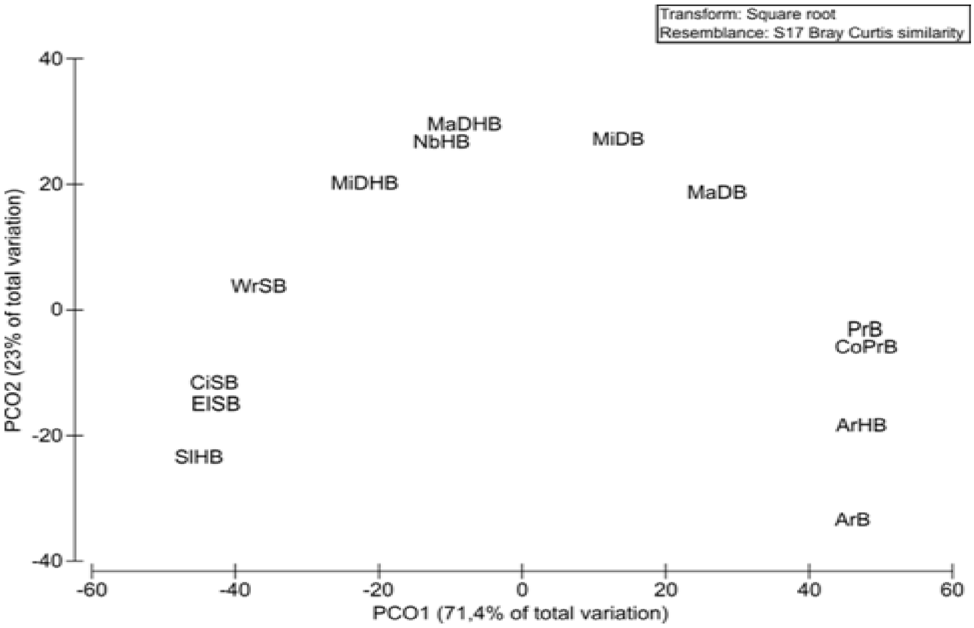


Figure 4.11: Principal Coordinates Analyses (PCO) for the different studied parameters affecting the distribution of the seven holothurian species according to the PCO from the in figure 4.4.

However, *H arguinensis* presents morphological characters similar to *H. poli* and *H. stellati* as described by Koehler (1921) and Gustato and Villari (1980). In many cases, this species was defined as *H. stellati* (Mezali and Francour 2012) or considered as a hybrid species between *H. stellati* and *H. poli* (Mezali 2011). The study made by Mezali and Francour (2012) on systematic and phylogenetic holothurian species from the Algerian border, generated a Bayesian 16S phylogenetic tree that closely coincides with our genes COI and 16S phylogenetic clusters, although *H. stellati* species occupies the position of our *H. arguinensis* specimens. We conclude that the specimens of *H. stellati* from the above studies are almost certainly specimens of *H. arguinensis*, the spicules of which are distinguished by large flat tables accompanied by long large buttons with numerous holes and rough margin, while the number of the holes may be even or odd. This makes the characteristic spicules of *H. arguinensis* match with the parameters defined by the PCO from (Fig. 4.11) and explains its morphometric divergence reflected in the different statistical analyses.

The results of both the genetic (16S and COI) and morphometric dendrograms revealed clear concordance between the spicules of the species from genus *Hotothuria.* This was the case of *H. l. lentiginosa* and *H. tubulosa* species and *H. mammata* which was associated with *H. dakarensis.* Also, the *H. poli* specimens were grouped together forming a clade apart from the others. Besides, the molecular dendrogram, pointed to a geographical convergence between the sea cucumber species. Analysis of the mtDNA of the eight species pointed to a significant genetic similarity between Atlantic and Mediterranean species, except for *H. poli* which were molecularly and morphometrically separated from all the species.

Currently, the geographical distribution of species from genus *Holothuria* has been changing from the Atlantic Ocean to include a new colonization of many species in the Mediterranean Sea. For example, *H. l. lentiginosa,* an amphi-Atlantic species, reported from several localities in the eastern and the western Atlantic Ocean (Miller and Pawson 1979; Pérez-Ruzafa and Lopez Ibor 1986, 1988) has been reported with *H. dakarensis* in the Alboran Sea in the south-western Mediterranean Sea (Pérez-Ruzafa and Lopez Ibor 1986, 1988). In fact, reports and studies about the occurrence of *H. l. lentiginosa* are very few, and Cherbonnier (1965) offered little information about this species.

H. arguinensis, is a north-eastern Atlantic species, distributed from Portugal to Morocco and Mauritania including the Canary Islands (Pérez-Ruzafa *et al.* 1992; González-Wangüemert and Borrero-Pérez 2012). However, it has also been reported in the Alboran Sea and Algeria in the western Mediterranean basin (Ocaña and Pérez-Ruzafa 2004; González-Wangüemert and Borrero-Pérez 2012; Mezali and Thadar 2014).

This change in spatial distribution due to climate change and maritime transport, could engender changes in the holothurian metabolism and body as an adaptation to new habitats. The diving mechanism accompanying this change has already been seen to generate a morphometric variability within this species.

Such phenomena have been observed in cold water (Massin 1994) and tropical species (Cutress 1996; Massin 1996). Indeed, species from *H. l. lentiginosa* show differences in ossicle shape according to their geographic position. In the eastern Atlantic Ocean, *H. l. lentiginosa* has a twisted button, while specimens from the western Atlantic have a smooth regular button (Miller and Pawson 1979). Furthermore, ossicle shape and number of the species *H. (Selenkothuria) carere* (Honey-Escandón, Solís–Marín and Laguarda-Figueras, 2011) differ, depending of its geographical position in the Gulf of Mexico or the Gulf of California (Honey-Escandón *et al.* 2011).

In fact, the morphometric variation among the eight atlanto mediterranean species from genus *Holothuria,* is probably associated to the genetic variation and not to their geographical position or zoogeographical origin. Further studies comprising the identification of the biotic and abiotic factors in the different localities that species inhabit are still required to evaluated their effects on ossicle diversity at intra and inter-population level.

Furthermore, the genus *Holothuria* has long been a subject of discussion and the literature reflects this confusion; in this respect special attention should be paid to the subgenus (*Roweothuria*), which clearly needs to be revised and re-evaluated. The geographical distribution of sea cucumber, including the family of Holothuriidea, is changing because of the declining of the temperature, the global warming and human effects. In response, in an attempt to survive and adapt to new habitats, the plasticity of genotypic and phonotypic characters has increased. However, to achieve more reliable results, further studies into this macrobenthic group need to be carried out, including more species and various localities, and studying the physicochemical factors involved.

CHAPTER V

GENETIC OF *HOLOTHURIA (ROWEOTHURIA) POLI,* ONE OF THE MOST IMPORTANT HARVESTED AND EXPLOITED SPECIES IN THE MEDITERRANEAN SEA, FROM LAGOONS TO OPEN SEA

5. GENETIC OF HOLOTHURIA (ROWEOTHURIA) POLI, ONE OF THE MOST IMPORTANT HARVESTED AND EXPLOITED SPECIES IN THE MEDITERRANEAN SEA, FROM LAGOONS TO OPEN SEA

5.1. Introduction

Sea cucumbers have been exploited for human consumption for centuries and have numerous species of commercial importance. They are consumed in more than 70 countries worldwide, raw or processed and/or dried, usually under the name of “bêche-de-mer” or “trepang” (Conand and Byrne 1993; Sommerville 1993; Ahmed 2006; Purcell *et al.* 2012). They are exported mostly to Asian markets, where are considered a delicacy and therapeutic, being China the main market for sea cucumber products (Conand and Sloan 1988; Toral-Granda and Martínez 2007; Purcell *et al.* 2012).

The oldest record of its use dates from the Ming Dynasty (1368-1644 BC) also in China (Chen 2003; González-Wangüemert *et al,* 2016). Nowadays, fresh holothurians become a premium product on gastronomy because of their flavors and nutritional profiles with high protein content, antioxidant and bioactive compounds, and their bio-extracts components are very common on pharmaceutical, nutraceutical, and cosmetic products (Bordbar *et al.* 2011; Purcell *et al.* 2012, 2013). Their therapeutic properties are widely recognized as holothurian are very rich on chondroitins and glucosamines, which are responsible for building of cartilage blocks and other bioactive substances with anti-inflammatory and anti-tumor properties (González Neira and Vera Figueroa 2006). So, they are used in different ways to cure and to prevent diseases like arthritis and cancer, to regulate blood pressure and cholesterol for human being and as vitamin supplement for pets.

Artisanal fishermen and industrial fleets from over 40 countries exploit a total of more than 60 species of sea cucumbers (Toral-Granda and Martínez 2007). Maximum number of harvested species comes from the Asian continent with a total of 52 species followed by the Pacific with 36 exploited species, (Toral-Granda and Martínez 2007) indeed,

Holothurian stocks have been overfished are over-exploited in many countries as a result of the over-increasing of the market demand. And, so, numerous sea cucumber species became vulnerable and over-exploited because of the uncontrolled exploitation and/or the inadequate fisheries management or due to their illegal marketing, and very often they are sold with unclear taxonomic identification (Kinch 2002; Conand 2004). Also, in many cases, there is a lack of knowledge about their ecology, their biology, or the stock status of the species.

Already, over 16 species of sea cucumbers are classified as “vulnerable” or “endangered” on the IUCN red list (Conand *et al.* 2014; González-Wangüemert *et al.* 2018). There is a clear need to distinguish to species level by fishery managers, customs, trade officers and for the pharmaceutical or other industries (Purcell *et al.* 2012).

In the Mediterranean, sea cucumbers are poorly exploited and their fishery is not currently an active industry. However, Turkey was the first Mediterranean country to harvest and export these species (mainly to Singapore and Hong Kong) (Cakly *et al.* 2004; Aydin 2008; Conand *et al.* 2014). More recently, commercial fishermen from Italy have initiated small-scale harvesting and processing of sea cucumbers for export also to Asiatic markets (Sicuro and Levine 2011).

The most important harvested and exploited species in the Mediterranean Sea in general are *Holothuria poli, H. tubulosa, H. mammata, H. sanctori, H. forskali, H. arguinensis and Parastichopus regalis* (Sicuro and Levine 2011; González-Wangüemert et *al.* 2015, 2016, 2018; Seigenthaler *et al.* 2015). In Turkey stand out the fisheries of *Holothuria tubulosa, H. poli* and *H. mammata* (Aydin 2008; Sicuro *et al.* 2012; González-Wangüemert *et al.* 2018), in Tunisia there are some references about the exploitation of of *Holothuria tubulosa, H. poli*, *H. sanctori* and *Holothuria forskali* (Telahigue *et al.* 2014; Ben Mustapha and Hattour 2016; Sellem et *al.* 2017; Sellem *et al.* 2019), and in Spain about *Paratichopus regalis, H. tubulosa*, *H. forskali, H. mammata* and *H. arguinensis* (some of them as illegal harvesting) (Ramónd *et al.* 2010; González-Wangüemert *et al.* 2018), although references in this regard are very scarce.

Holothuria poli is one of the most frequent and exploited sea cucumber species in the Mediterranean (Tortonese 1965; González-Wangüemert *et al.* 2014, 2018). González-Wangüemert and collaborators (2014, 2018) estimates the collect of around 720,000 to 1,080,000 sea cucumbers per day in Turkish waters, of which *H. poli* account for 80% of the total. However, despite its great use in Turkey, its exploitation in other countries of the Mediterranean is not documented, although some authors point out that it is becoming a novel fishing target (Rakaj *et al.* 2019).

This species mainly inhabits the littoral in soft sediment and seagrass meadows, usually up to 20 m depth, being an important deposit feeder and playing a fundamental role in benthic dynamics, including those of coastal lagoons environments (Koehler 1921, 1969; Tortonese 1965). It is a gonochoric species without sexual dimorphism, and spawning and fertilization are external during the summer, with a distinct annual reproductive cycle (Aydin and Erkan 2015; Sellem et *al.* 2017).

From the nutritional point of view, *H. poli* has a high content in proteins, micronutrients and polyunsaturated acids, but very low in lipids, being a "functional" and nutraceutical food for human consumption (Rakaj *et al.* 2019). It has also been studied as a potential source of bioactive compounds with antifungal, anti-inflammatory and antibacterial activity (Rakaj *et al.* 2019).

Coastal lagoons represent habitats with widely heterogeneous environmental conditions, which fluctuate in both space and time. These characteristics suggest that physical and ecological factors could contribute to the genetic divergence among populations occurring in coastal lagoon and open coast environments (Vergara-Chen *et al.* 2010). In this sense, the objective of this chapter is the study of different specimens of *Holothuria poli* from coastal lagoons and open sea environments in different Mediterranean localities in order to known their genetic characteristics, divergences and relationships. Chapter 4 has provided the information regarding the whole genus *Holothuria* and the purpose of the present chapter is to understand the genetic diversity of *H. poli* and its spatial variability in the Mediterranean within sub-basins and in two different environments, costal lagoon and open sea ecosystems, in the framework of the ecological parameters that could drive this distribution.

5. 2. Material and methods

5.2.1. Biological material

A total of 36 individuals of *Holothuria poli* from three localities and 3 sites of the Mediterranean Sea, Spain (Western Mediterranean), Tunisia (Central Mediterranean) and Turkey (Eastern Mediterranean), were analysed. We have studied 12 specimens from Bizerte lagoon (Biz) in Tunisia, 12 from Mar Menor lagoon (M-Me), Torre de la Horadada (T-Ho), Cabo de Palos (C-Pa) and Mallorca (Mal) in Spain and 12 from Kusadasi in Turkey.

For the Turkish and Spanish (T-Ho, C-Pa, Mal) marine specimens, we referred to the sequences of *H. poli* of González-Wangüemert *et al.* (2014) and Valente *et al.* (2015)

present in the GenBank. The other specimens were collected from two Mediterranean lagoons, Bizerte and Mar Menor, between 3-5 meters deep. After the morphological identification, tissue samples from internal longitudinal muscles bands were removed from each specimen and preserved in 100% ethanol for the molecular analyses.

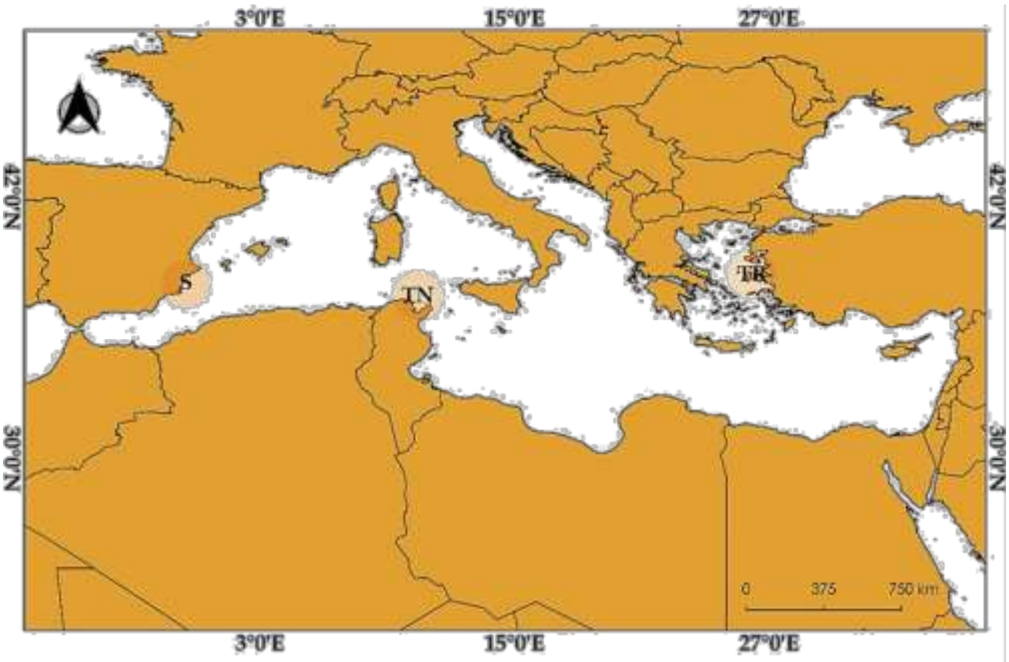


Figure 5.1: Map showing the sampling countries of *Holothuria poli* from the Mediterranean Sea: S= Spain (Mar Menor lagoon), TN= Tunisia (Bizerte lagoon) and TR=Turkey (Kusadasi).

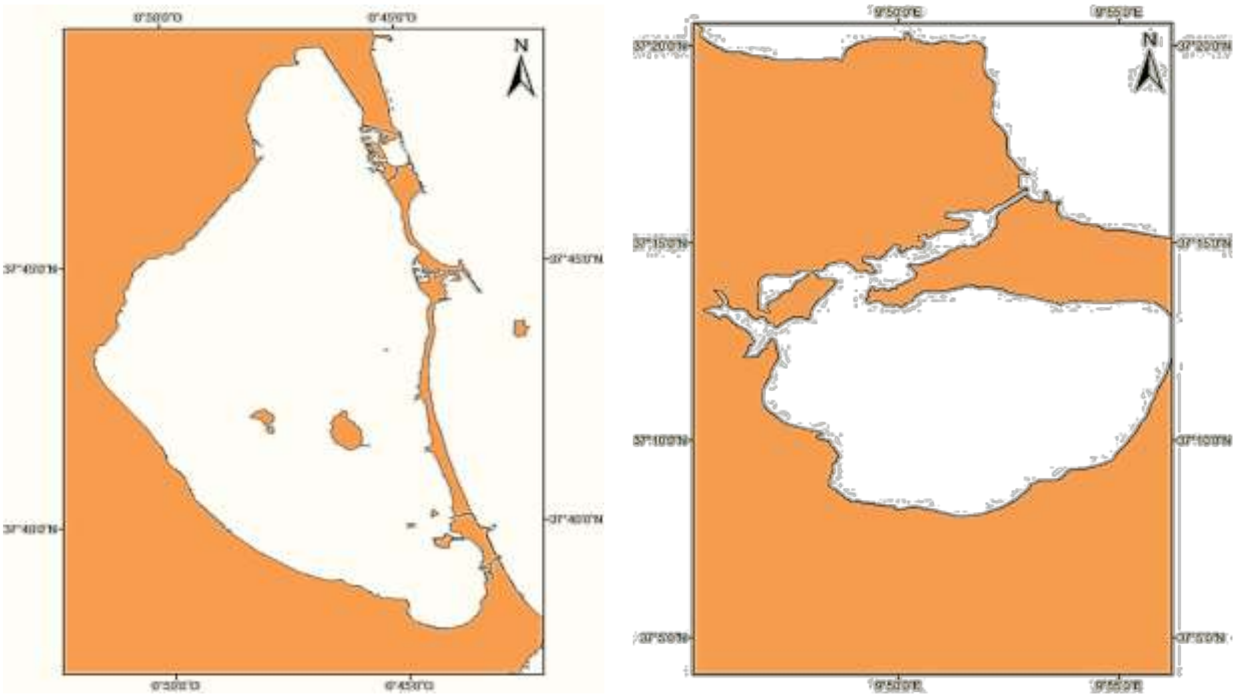


Figure 5.2: Maps of the two Mediterranean costal lagoon sampling localities Mar Menor lagoon (on the left) and Bizerte lagoon (on the right).

5.2.2. DNA Extraction, PCR amplification and sequencing

The extraction, amplification and sequencing of the genes 16S and COI have been done following the protocol of Sambrook *et al.* (1989) as described before in Chapter 4.

Table 5.1: Sampling locations and GenBank references andgenes (COI) and (16S) general information of the studied specimens of *Holothuria poli.* M-Me= Mar Menor; C-Pa=Capo de palos; T-Ho= Torre de la Horadada; Mal=Mallorca; Biz=Bizerte; Kus=Kusadasi.

Code	Species	Country	Habitat	Region	GenBank	GenBank Number	
						COI	16S
H.poli01	*Holothuria (Roweothuria) poli*	Spain	Lagoon	M-Me	No	Ho1835	Ho1835
H.poli02	*Holothuria (Roweothuria) poli*	Spain	Lagoon	M-Me	No	Ho1838	Ho1838
H.poli03	*Holothuria (Roweothuria) poli*	Spain	Lagoon	M-Me	Yes	EU750793	EU750755
H.poli04	*Holothuria (Roweotuhria) poli*	Spain	Lagoon	M-Me	Yes	EU750799	EU750762
H.poli05	*Holothuria (Roweothuria) poli*	Spain	Lagoon	M-Me	Yes	EU750800	EU750754
H.poli06	*Holothuria (Roweothuria) poli*	Spain	Lagoon	M-Me	Yes	EU750813	EU750791
H.poli07	*Holothuria (Roweotuhria) poli*	Spain	Lagoon	M-Me	Yes	EU750814	EU750792
H.poli08	*Holothuria (Roweothuria) poli*	Spain	Sea	C-Pa 5	Yes	EU750819	EU750768
H.poli09	*Holothuria (Roweothuria) poli*	Spain	Sea	C-Pa 14	Yes	EU750820	EU750767
H.poli10	*Holothuria (Roweothuria) poli*	Spain	Sea	C-Pa 28	Yes	EU750821	EU750770
H.poli11	*Holothuria (Roweothuria) poli*	Spain	Sea	T-Ho 24	Yes	EU750824	EU750777
H.poli12	*Holothuria (Roweothuria) poli*	Spain	Sea	T-Ho 25	Yes	KJ493923	KJ408301
H.poli13	*Holothuria (Roweothuria) poli*	Tunisia	Lagoon	Biz	No	H3	H3
H.poli14	*Holothuria (Roweothuria) poli*	Tunisia	Lagoon	Biz	No	H7	H7
H.poli15	*Holothuria (Roweothuria) poli*	Tunisia	Lagoon	Biz	No	H01	H01
H.poli16	*Holothuria (Roweothuria) poli*	Tunisia	Lagoon	Biz	No	H010	H010
H.poli17	*Holothuria (Roweothuria) poli*	Tunisia	Lagoon	Biz	No	H02	H02
H.poli18	*Holothuria (Roweothuria) poli*	Tunisia	Lagoon	Biz	No	H03	H03
H.poli19	*Holothuria (Roweothuria) poli*	Tunisia	Lagoon	Biz	No	H04	H04
H.poli20	*Holothuria (Roweothuria) poli*	Tunisia	Lagoon	Biz	No	H05	H05
H.poli21	*Holothuria (Roweothuria) poli*	Tunisia	Lagoon	Biz	No	H06	H06
H.poli22	*Holothuria (Roweothuria) poli*	Tunisia	Lagoon	Biz	No	H07	H07
H.poli23	*Holothuria (Roweothuria) poli*	Tunisia	Lagoon	Biz	No	H08	H08
H.poli24	*Holothuria (Roweothuria) poli*	Tunisia	Lagoon	Biz	No	H09	H09
H.poli25	*Holothuria (Roweothuria) poli*	Turkey	Sea	Kus	Yes	KJ812194	KJ812160
H.poli26	*Holothuria (Roweothuria) poli*	Turkey	Sea	Kus	Yes	KJ812195	KJ812158
H.poli27	*Holothuria (Roweothuria) poli*	Turkey	Sea	Kus	Yes	KJ812196	KJ812162
H.poli28	*Holothuria (Roweothuria) poli*	Turkey	Sea	Kus	Yes	KJ493928	KJ408280
H.poli29	*Holothuria (Roweothuria) poli*	Turkey	Sea	Kus	Yes	KJ493908	KJ812164
H.poli30	*Holothuria (Roweothuria) poli*	Turkey	Sea	Kus	Yes	KJ812189	KJ812161
H.poli31	*Holothuria (Roweothuria) poli*	Turkey	Sea	Kus	Yes	KJ493888	KJ408306
H.poli32	*Holothuria (Roweothuria) poli*	Turkey	Sea	Kus	Yes	KJ493894	KJ812154
H.poli33	*Holothuria (Roweothuria) poli*	Turkey	Sea	Kus	Yes	KJ493897	KJ812158
H.poli34	*Holothuria (Roweothuria) poli*	Turkey	Sea	Kus	Yes	KJ493898	KJ812155
H.poli35	*Holothuria (Roweothuria) poli*	Turkey	Sea	Kus	Yes	KJ493899	KJ812157
H.poli36	*Holothuria (Roweothuria) poli*	Turkey	Sea	Kus	Yes	KJ493906	KJ408281

5.2.3. Phylogeographic study

We have analyzed both COI and 16S rRNA genes fragments, as independent genetic markers because current evidence suggests that several invertebrate species show mitochondrial DNA recombination (Rokas *et al.* 2003; Tsaousis *et al.* 2005; Vergara-Chen *et al.* 2010). Also, mitochondrial COI gene is a proteincoding region and 16S rRNA gene is non-protein-coding region, with different mutation rates. The different sequences of sea cucumbers studied were treated in order to achieve more concrete results (Vergara-Chen *et al.* 2010). To define the genetic diversity and population structure a set of analyses was made, using an appropriate software, the number of haplotypes (Nh) and the diversity of haplotypes (H). The haplotype is the allelic composition of a several loci on a chromosome. It is referred also to a unique mtDNA sequences for a particular locus. Haplotype diversity is defined as the probability that any two randomly selected haplotypes in a specimen will be different in the sample. It is also known as the gene diversity. Adding to this the number of polymorphic sites (S) or of sites or locus that are variable in a population, the nucleotide diversity (π) which is the probability that two randomly chosen homologous nucleotides sits for an individual will be different. It is known also as the average of gene diversity over loci. This is the equivalent of the measuring of genetic diversity at the nucleotide level (Excoffier *et al.* 2006) and fixation index (FST) defined by the measure of the genetic variation and differentiation between population structure on bases of allele frequencies. Its known as the deviation due to subpopulations differentiation must be conducted.

5.2.3.1. Genetic diversity

Genetic diversity is used to determine intra-population diversity among individuals in the same population based on the level and number of nucleotides since it is most appropriate for mitochondrial sequences.

With the aim of exploring the phylogeography of *Holothuria poli* from lagoon and sea, we have analyzed a fragment of 413 bp of cytochrome oxidase I (COI) and about 355 bp of (16S). The sequences were aligned using the Clustal-W in MEGA 7 software (Kumar *et al.* 2016). Nucleotide diversity (π), number of holotypes (h), and number of polymorphic sites (S) were calculated for each locality using the ARLEQUIN 3.1 program (Excoffier *et al.* 2006).

5.2.3.2. Statistical study

The genetic differentiation between pairs of samples was evaluated by the rate of fixation (F_{ST}), while the significance of F_{ST} values was tested using 10,000 random permutations (Weir and Cockerham 1984). The distribution of variation within and between samples was inferred using an analysis of molecular variance AMOVA (Excoffier *et al.* 1992) considering 4 groups from both costal lagoon and open sea samples: Spain lagoon, Tunisia lagoon, Spain Sea and Turkey Sea. For the F_{ST} and AMOVA analyses, ARLEQUIN 3.1 program (Excoffier *et al.* 2006) was used. Beside, correspondence analyses (CA) were conducting in Past Software (Hammer *et al.* 2001) for both COI and 16S genes, as to detect the difference between the four groups of marine sea and costal lagoon. Haplotype networks were estimated using the PopART software (Leigh and Bryant 2015).

5.3. Results

5.3.1. Genetic diversity

Sequences of *Holothuria poli* were obtained for cytochromeoxidase I (COI) and 16S rRNA (16S) mitochondrial genes among a total of 53 specimens from the three studied locations (Table 5.2). A total of 40 different haplotypes of gene COI sequences with 413-bp were obtained from 44 individuals (Table 1.5; GenBank Accession numbers KJ493908–KJ493899; KJ812189– KJ812196; EU750793–EU750824). There are 15 haplotypes from Bizerte and Mar Menor lagoons against 25 from Turkish and Spanish open coasts. Five haplotype are shared between the two lagoons and ten haplotypes are exclusive, with 6 exclusive haplotypes for Bizerte lagoon. Overall, 355-bp of the 16S sequences with 29 haplotypes were detected among 40 individuals (Table 1.5; GenBank Accession numbers KJ408280–KJ493899; KJ812155–KJ812164; EU750754–EU750792) from the studied localities (Tables: 5.1 and 5.2).

Twenty-one haplotypes are present on the sea against eight for lagoons. The maximum of exclusive haplotypes was found in sea with 9 exclusive haplotypes from Turkey and only 6 from Spanish sea. The sea is owning the maximum number of exclusive haplotypes for both genes with 20 haplotypes for COI and the 16S with 15 haplotypes. However, Bizerte Tunisian lagoon is sharing the maximum of haplotypes with Mar Menor Spanish lagoon, Turkish and Spanish coasts, for gene COI and 16S.

The COI sequences were characterized by a high polymorphism and haplotype diversity (lagoon: S=22; H=0.96; $\pi=0.09$, Sea: S=25; H=0.99; $\pi=0.1$) comparing to the 16S sequences (lagoon: S=10; H=0.7; $\pi=0.07$, Sea: S=24; H=0.94; $\pi=0.1$). Indeed, sea samples showed the highest number of haplotype, haplotype and nucleotides diversity for both genes COI (Nh= 25; S=25; H=0.99; $\pi=0.1$) and 16S (Nh= 21; S=24; H=0.94; $\pi=0.1$).

Table 5.2: Measures of the molecular diversity of the three populations of *Holothuria (Roweothuria) poli* in the Mediterranean Sea (N) Number of individuals, (Nh) number of haplotypes, (Nh) (ex) number of exclusive haplotypes, (S) polymorphic sites, (H) haplotype diversity, (π) nucleotide diversity.

Species	Genes	Localities	Population	N	Nh (ex)	S	H	π
Holothuria poli	COI	*Lagoon COI*		*19*	15(10)	22	0.9591	0.0909
		Tunisia	Bizerte	12	8(6)	14	0.9242	0.0848
		Spain	Mar Menor	7	7(4)	11	1.0000	0.1034
		Sea COI		*25*	25(20)	25	0.9967	0.1053
		Turkey	Kusadasi	12	12(10)	16	1.0000	0.1056
		Spain		13	13(10)	15	1.0000	0.1040
	16S	*Lagoon 16S*		*15*	8(4)	10	0.7238	0.0748
		Tunisia	Bizerte	8	3(1)	6	0.4643	0.0536
		Spain	Mar Menor	7	5(3)	8	0.9048	0.1020
		Sea 16S		*25*	21(15)	24	0.9467	0.1079
		Turkey	Kusadasi	12	11(9)	16	0.9848	0.0952
		Spain		13	10(6)	13	0.9231	0.1081

5.3.2. Population differentiation

The pairwise estimates of Fixation Indices Test (table 5.3) of the mtDNA gene (COI) and rRNA (16S) show a restricted gene flow through costal lagoon and open sea from Mediterranean Sea. The obtained F_{ST} values are in general negative or low. The pairwise test using COI haplotypes show only a significant difference between Tunisian costal lagoons from and Spanish marine samples (P=0.089). F_{ST} values ranged from negative (-0.026) to (0.69). However, the 16S haplotype data revealed a significant difference between only localities from sea only (Turkey and Spain) and no difference between sea and lagoon (F_{ST}= 0 .10063).

The Mar Menor coastal lagoon pointed to no significant difference of both gene value with Sea-Spain, Sea-Turkey and Lagoon–Tunisia. Although, P values for both genes 16S (P= 0.32304+-0.0042) and COI (P=0.34858+-0.0043) are much higher with Spain than Turkey.

Table 5.3: Pairwise estimates of Fixation Indices (F_{ST}) between the three populations of *Holothuria (Roweothuria) poli* based on mtDNA sequences COI and 16 S, F_{ST}, values are below the diagonal and *P value*, above the diagonal. P is the probability that any random value obtained after 1000 permutations is > observed value; Significant F_{ST} values and P <0.05 are in bold and market with *.
(Fst values; P values; * = P value < 0.05; * = significant value).

Gene COI	Lagoon_Spain	Lagoon_Tunisia	Sea_Spain	Sea_Turkey
Lagoon_Spain	-	*0.50586*	*0.69336*	*0.33301*
Lagoon_Tunisia	-0.01132	-	*0.21289*	*0.08984**
Sea_Spain	-0.02607	0.01711	-	*0.29590*
Sea_Turkey	0.00811	0.03614*	0.00980	-

Gene 16S	Lagoon_Spain	Lagoon_Tunisia	Sea_Spain	Sea_Turkey
Lagoon_Spain	-	*0,46973*	*0,34082*	*0,37109*
Lagoon_Tunisia	-0,02823	-	*0,15039*	*0,99902*
Sea_Spain	-0,00074	0,06786	-	*0,03516**
Sea_Turkey	0,01079	-0,05180	0,10063*	-

The analyses of molecular variance AMOVA test using COI don't reveal a significant difference among groups, Lagoon and Sea, or nether populations within groups. Similarly, for the results obtained from 16S gene. The highest value of total variance was attributed to difference for gene COI within populations (57.10 %; P= 0.29619) and the rest percentage of variation was attributed to the difference among groups (lagoon and Sea).

Table 5.4: AMOVA between the three populations of *Holothuria (Roweothuria) poli* based on mtDNA sequences COI and 16S. 1000 permutations; Significance Level=0.0500.
(Groups = Lagoon and Sea; Populations = Lagoon Spain, Lagoon Tunisia, Sea Spain and Sea Turkey).

Genes	Total variance (%)	Fixation indices	P value
COI			
Among groups	-49,02	FCT = -0,49020	1,00000
Among populations within groups	91,92	FSC = 0,61686	0,56598
Within populations	57,10	FST = 0,42904	0,29619
16S			
Among groups	-16,56	FCT = -0,13556	1,00000
Among populations within groups	103,3	FSC = 0,90966	0,94917
Within populations	10,26	FST = 0,89741	0,11046

5.3.3. Populations genetic structure

The correspondence analyses (CA) using the haplotype frequencies of COI explained 36% of the total variance for the two first ordination axes showing three main groups on the positive side of CA: Sea-Spain from the positive side of the axe 1 (CA1), both lagoon, Lagoon-Spain and Lagoon-Tunisia on the positive side of the axis 2 (CA2) and Sea-Turkey on the positive side of both axes CA1 and CA2. However, the CA with the haplotypes frequencies of 16S explained 43 of the total variance for the two first components also with three main groups: Sea-Turkey and Lagoon-Tunisia from both positive side of CA1 and CA2, Lagoon-Spain on the positive side of the axis CA2 and Sea-Spain on the negative side of both axis CA1 and CA2.

Sea-Turkey is occupying the positive side of the two axes 1 and 2 of (CA) in the two cases of genes COI and 16S.

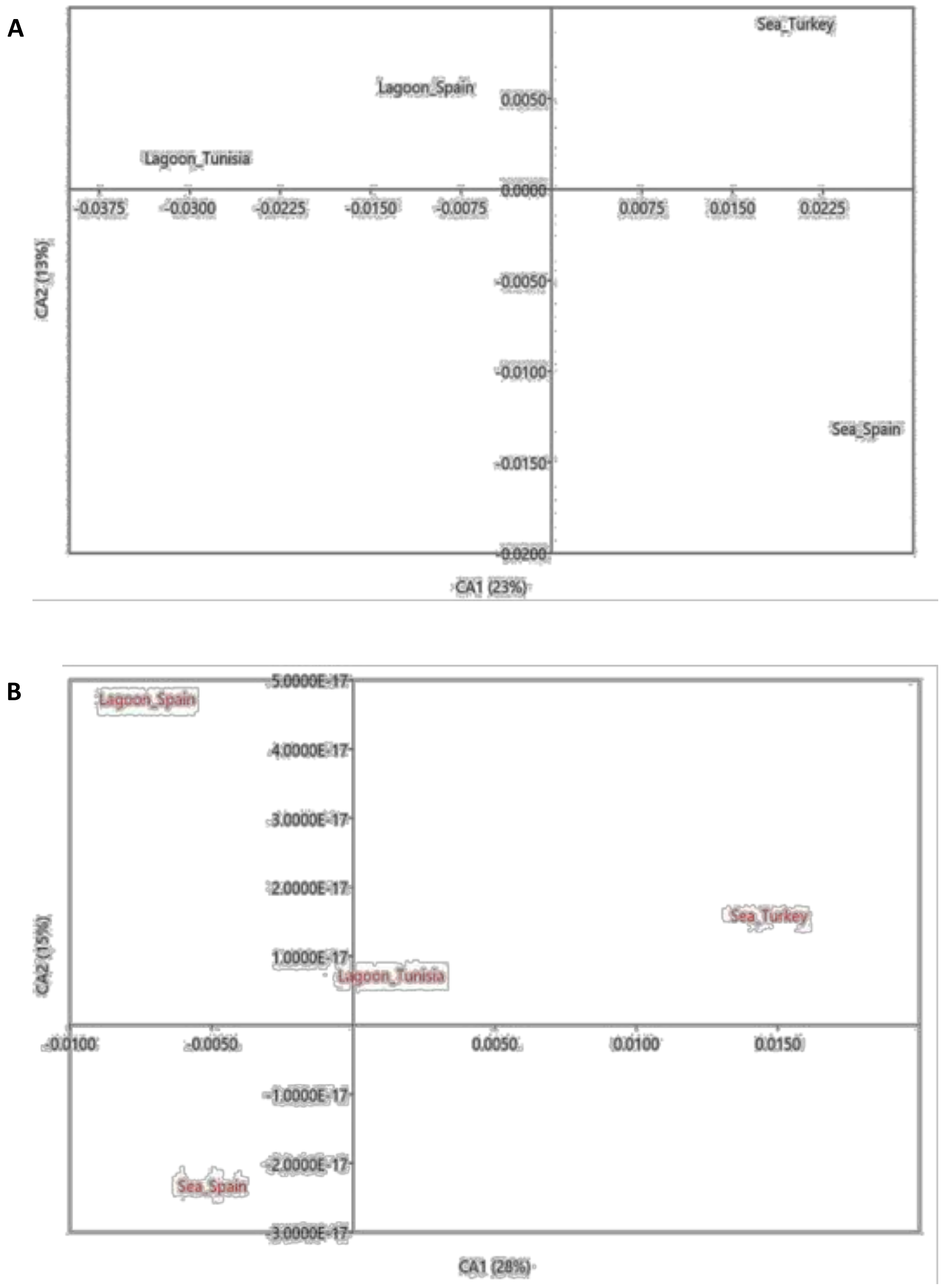


Figure 5.3: Correspondence analyses of COI (A) and 16S (B) haplotypes frequencies of *Holothuria poli* for the four groups: Lagoon-Tunisia, Lagoon-Spain, Sea-Turkey and Sea-Spain.

5.3.4. Analysis of haplotype network

The haplotype parsimony network based on the16S rRNA data (Figure 5.2) showed several ambiguous connections and pointed to three haplotypes shared between all sampling locations (H02, H10 and H16). The most common haplotype is H10, it was present in four areas Spanish lagoon and Sea, Tunisian lagoon and Turkish Sea. This haplotype could be a costal lagoon haplotype with Tunisian (central Mediterranean Sea) origin with a high adaptive character leading it to disperse throw the open marine sea. The second haplotype H16 is shared by Tunisian lagoon and Spanish sea have generated a new haplotype (H15) in Mar Menor lagoon (Spain). However, only one haplotype is shared in open sea area from Spain and Turkey (H02), it could be a marine haplotype that dispersed with larvae and following the marine realm to colonize the western and eastern Mediterranean basins. Fourteen (14) exclusive haplotypes have characterized the open Mediterranean Sea area with nine (9) typical for Kusadasi from Turkish Sea, against four (4) haplotypes exclusive haplotype for both costal lagoons with only one exclusive haplotype for Bizerte lagoon. This may be because of it central position in the Mediterranean Sea so its shared haplotypes with eastern and western Sea more than owning exclusive haplotypes.

For COI data, the statistical parsimony network obtained (Fig. 3) revealed the presence of three major haplotypes (H02, H14 and H24) as well as, for 16S. They were shared among the four Mediterranean locations from coastal lagoon and open sea. One haplotype (H14) was present on all the samples. However, two haplotypes were shared between sea and lagoon respectively (H02) between Spanish lagoon and sea samples and (H24) between Bizerte lagoon and Kusadasi sea. Seven (7) haplotypes are exclusive for sea and seven other are exclusive for lagoon with four (4) exclusive haplotypes for Turkish sea and four (4) Tunisian lagoon.

The COI gene show no significant difference between costal lagoon and marine sea exclusive haplotype samples. However, we notice that Kusadasi Turkey had the highest number of exclusive haplotype for both genes COI and 16S. The ancestral haplotype H01 was with Spain sea origin with low frequency for both gene populations.

The dispersal of gene flow of *H. poli* specimens from coastal lagoon to marine Mediterranean Sea or vice versa, derives a big number of mutations among COI mitochondrial genes more than 16S rRNA sequences.

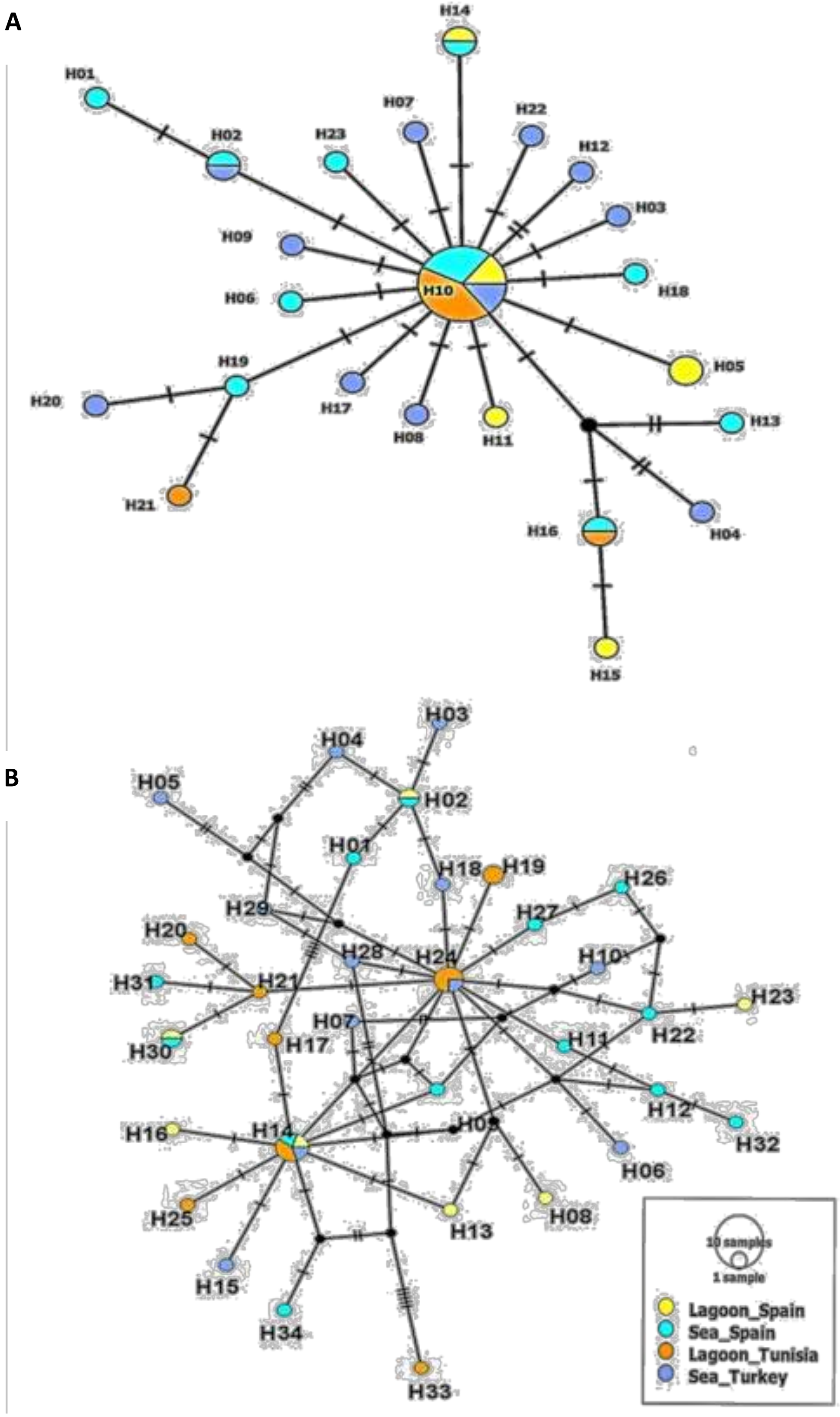


Figure 5.4: Parsimony network based on 16S (A) and COI (B) genes sequence's haplotypes of *Holothuria polii*. Area of each circle is proportional to the number of specimens per locality. Each haplotype is corresponding to a number.

5.4. Discussion

The study of *Holothuria poli* populations from three different localities of the Mediterranean Sea, Spain, Tunisia and Turkey, and two different environments, coastal lagoons and open sea, allows us to discuss the phylogeographical status of this species. Despite of the moderate number of the studied samples (12 specimens per locality) the present work has reviled concrete results on mitochondrial gene flow and its divergence throw coastal lagoon and open sea environments within the eastern, central and western Mediterranean basins.

In fact, the genetic study of *H. poli* using two mtDNA markers 16S and COI genes, shows a high level of haplotype and genetic diversity and a low level of nucleotides diversity (COI: H=0.95; π=0.09; 16S: H=0.94; π=0.07). Previous studies on *H. poli* populations from Vergara-Chen *et al.* (2010), Valente et al. (2014), and more recently González-Wangüemert *et al.* (2018), have showed similar results for genetic diversity on the genes 16S (H=0.65; π=0.003) and COI (H=0.93; π=0.005). Further study focused on *H. arguinensis*, from the same subgenus (*Roweothuria*), revealed a concordance partner with our results, the later species has presented also a high values of genetic diversity on the genes 16S (H=0.88; π=0.005) and COI (H=0.90; π=0.01) (Rodríguez *et al.* 2015). However, the genetic diversity of *H. poli* compared to other Atlanto-Mediterranean cucumbers such as *H. mammata* shows a similar results of gene COI and a high diversity of gene16S. Indeed, Borrero-Pérez *et al.* (2011) and Valente *et al.* (2014), have studied *H. mammata*, using mtDNA markers (COI and 16S genes) and they detected a high genetic diversity (16S gene: H=0.93; π=0.006; COI gene: H=0.92; π=0.007) on most of the populations distributed along the Mediterranean Sea and the Atlantic oceans.

We reveal an important nucleotide and genetic diversity for the genes COI and 16S on sea more than in lagoon ecosystem. However, lagoon results inferred to COI gene are showing a high haplotypes diversity in Mar Menor costal lagoon more than Bizerte lagoon with a high number of exclusive haplotypes. While the nucleotide diversity was almost similar for both areas. Which is on concordance with the investigation of Rodríguez *et al.* (2015) on *H. arguinansis* specimens collected from the Atlantic costal lagoon and marine waters.

Kusadasi from marine sea has the highest haplotype diversity between the four studied groups for both genes however, the ancestral haplotypes for COI and 16S genes were present in Spain sea. Which could confirm the marine sea origin of the studied species.

The pairwise test results show a significant difference between eastern and western Mediterranean Sea from Sea Spain and Sea Turkey for 16S gene only.

Although, there are no difference between central Mediterranean Sea and both Mediterranean basins. The Fst value from gene COI show a significant difference between Sea Turkey and Lagoon Tunisia. The P value of central Mediterranean Sea for both 16S (P= 0.32304+-0.0042) and COI genes (P=0.34858+-0.0043) is higher in the eastern more than the western part.

The present investigation admits a clear difference of *H. poli* gene flow passage through the marine Mediterranean Sea and lagoon ecosystem for large geographic scale, while the investigation of Vergara-Chen *et al.* (2010), showed non-significant genetic differentiation between samples of *H. poli* collected at the micro-geographic scale from Mar Menor coastal lagoon and adjacent marine locations (COI and 16S for AMOVA; COI for FST values and exact test). Correspondingly, Gharbi *et al.* (2011), have studied populations from *H. poli* from several localities of Tunisia from both western and eastern sides of Sicily strait. Their investigations have showed a low genetic variability on *H. poli* samples comparing to other holothurian species with a relatively low and argues alleles frequencies, furthermore, the correlation between F_{ST} test and the geographical distance, provides no evidence for isolation by distance at the studied scales.

The outcome of the haplotype parsimony showing Tunisia as the highly locality that’s sharing haplotypes with other sites from open sea and lagoon ecosystem from both sides of the Mediterranean Sea. The highest number of shared haplotypes is between Spain and north Tunisia.

The sea cucumber *H. poli* has been found and reported in many locations along the Mediterranean Sea from Egypt, Turkey, Aegean Sea, and South-eastern Adriatic Sea to Spain, France, Italy, Algeria and Tunisia (Cherbonnier 1956; Tortonese 1965; Zavodnik 2003; Aydin 2008; Mezali 2010; Mercier 2013; Öztoprak *et al.* 2014). It has a wide distribution from west and east Mediterranean Sea to the Red Sea. According to our result the important genetic diversity of *H. poli* in Turkey could be the evidence of its first presence in eastern Mediterranean basin. Thereafter, its gene flow has spread it out with larva to the western Mediterranean Sea throw the Suez Canal after colonizing the Red Sea.

On the other hand, the material of *H. poli* was collected from two different Mediterranean lagoons: Mar Menor and Bizerte, shows that *H. poli* could support different environmental conditions (Table 5.6). Indeed, Mediterranean lagoon is considered as a highly dynamic environment with great temporal and spatial variability since it is communicating with the open sea. Such coastal area is in general characterized by calm conditions and high richness of nutritive salts. The presence of *H. poli* in this area alike to open sea includes a big support to different condition (biotic and abiotic parameters) and reveals its big plasticity and adaptation to different biotopes and climates.

Which may justify *H. poli* as an euryoecious species with a large ecological valence leading it to support the highest fluctuation within the coastal lagoon and to colonize different types of biotopes from the Red Sea throughout to the Mediterranean Sea.

However, some studies have considered echinoderms species with low adaptation to salinity changes (Lawrence 1987, 1990), H. *poli* shows from the present study (table 5.) a tolerance for high salinities. Confirming that is one of sea cucumber species from genus *Holothuria*, that are able to survive in hypersaline coastal lagoons condition (Vergara-Chen *et al.* 2010).

Beside, a work from Sellem *et al.* 2019, on the diversity of sea cucumber species on two Tunisian lagoons with differente physiological characteristics, Bizerte Lagoon from northern Tunisia and Boughrara Lagoon from southern Tunisia, reveals the presence of *H. poli* in both lagoons with very importante proportion.

Table 5.5: Mean physiological parameters and characteristics from two different Mediterranean costal lagoon Mar Menor and Bizerte. (according to: Dolbeth et *al.*2016; González-Wangüemert 2015; Bejaoui *et al.* 2010; Pérez-Ruzafa et *al.* 2005).

Lagoon	Country	Localities	Area (Km^2)	Average Depth (m)	Length of connection	T (C°)	S (psu)
Mar Menor	Spain	Southeastern Spain	135	3.6	Three canals, the most important with 645 m long;5 m deep	13-30	36–47
Bizerte	Tunisia	North Tunisia	150	7	One canal with 6 km long; 12 m deep	11-29	33,9 -35,8

Mediterranean Sea is almost a closely sea. Its connecting an important network of straits and channels which are playing a crucial role on water exchange inside out basins sub basin and on the dispersal or the isolation of gene flow within larvae and species (Astraldi *et al.*1999; Ramos *et al.* 2017).

Mediterranean basins are occurring different hydrographical conditions and geological history (Coll *et al.* 2010; UNEP RAC/SPA 2010; Zenetos 2010; Lipej *et al.* 2017). The hydrographical changes that took place on Pleistocene led to the isolation of Black Sea, Aegean Sea and east Mediterranean Sea (Svitoch *et al.* 2000). It promoted the genetic convergence inside each ecosystem which justify the high genetic diversity in the eastern basin.

Also, the notable differentiation in temperature and salinity occurred on both eastern and western basins could be one of the reasons of the genetic divergence of *H. poli* in the Mediterranean Sea.

The genetic differentiation among Mediterranean basins has been observed for various species with different dispersal capabilities (Zardoya *et al.* 2004; Mejri *et al.* 2009). Many previous genetic investigations on fish and macro-invertebrates from African Mediterranean Sea and with same life history traits have identified a major genetic break between eastern and western Mediterranean Sea. They identified the strait of Sicily as a boundary for gene flow connectivity for several marine species (Costagliola *et al.* 2004; Domingues *et al.* 2005; Pérez-Losada *et al.* 2007; Zitari-Chatti *et al.* 2009; Zulliger *et al.* 2009; Deli *et al.* 2017).

Borrero-Pérez *et al.* (2009, 2011) have studied the historical processes and oceanographical patterns on the phylogeography study of *H. mammata* populations from different Atlanto-Mediterranean locations. Same authors have reported the colonization or recolonization of into the Mediterranean from the Atlantic was generally related to palaeoecological history and oceanographic processes after the Messinian Salinity Crisis. Moreover, they have proved the genetic break between the population of *H. mammata* from the Aegean Sea and the other from the west Mediterranean. Aegean Sea has been considered as one of the last colonized seas which may explain the low nucleotide diversity and the presence of private haplotypes on it.

Otherwise, there is an imported number of protected marine areas (MPA) in the eastern part of the Mediterranean Sea. The Aegean-Levantine Sea alone comprises over 221 MPA sites against to 274 sites in Ionian Sea and Central Mediterranean Sea (Fraschetti *et al.* 2001, 2002; Gabrié *et al.* 2012; European Environment Agency (EEA) 2015). Kusadasi from Turkey is one of MPA area. This could be one of the reasons of the presence of the important number of exclusive haplotypes in Kusadasi more than in the Mar Menor and Bizerte lagoon and Sea-Spain.

Turkey, where about 4% of its territorial waters is protected, is one of the countries that planning a significant expansion of its protected area system. In fact, the eastern basin has become a Lessepsian province causing serious threat for the native species, the habitats and the fisheries. Turkey emerge the second place of non-native species in the Mediterranean Sea with 182 species (Zenetos and Polychronidis 2010; Queiroz and Pooley 2018). Therefore, marine protected areas are an extremely essential tool for protecting species and habitats, beyond that conserving their genetic identity and marine diversity, in face of human impacts, overfishing and global warming effects. The development of networks of marine protected areas (MPAs) is an essential pre-requisite to understand population connectivity and to implement effective fishery management either in delineation of appropriate scales for management (Fogarty *et al.* 2007; Ross *et al.* 2009; Marandel *et al.* 2017; Pérez-Ruzafa *et al.* 2019).

Besides, coastal wetlands such as ecosystem lagoon are diverse in Tunisia, they extend along Tunisian seashore and are mainly connected with mediterranean sea (Bejaoui *et al.* 2008, 2010; Guetat *et al. 2012*). They are well known by their faunal and floral diversity and by importante fishing activities (Bejaoui *et al.* 2008; Ben Mustapha and Hattour 2016; Sellem *et al.* 2019). However, sea cucumber fishery is prevalent in Tunisian lagoon, it is exposed to many enviremental disturbance such as industrial and harbourated activities main source of wastewater and swage pollution (Ben Garali *et al,* 2009; Guetat et *al.* 2012). Adding to this, ecosystem lagoons in Tunisia are suffering from illegal fishing pressure. Thereafter, sea cucumber stocks become vulnerable and require protection because of the overexploitation and the innacurate fishing activities (Ben Mustapha and Hattour 2016; Sellem *et al.* 2019).

Understanding gene flow of Holothurian populations is very importante to assess the best management of the species whether commercial interest and to increase the knowledge about the ecology and the biology of cached species. According to Uthicke and Benzie 2001, the khowledge on the connectivity of exploited species allows to prevent the ecological consequences of overfishing and to emphasize the importance of marine protected area as a measure of species protection.

Overall, the obtained results confirm that *H. poli* started on the eastern Mediterranean Sea and speared out to the whole Mediterranean Sea as suggested by Valente *et al.* 2014. Furthermore, we detect a phylogeographic differentiation for large scale *H. poli* species from open sea area and lagoon ecosystem.

However, lagoon ecosystems show a restricted gene flow compared to marine open sea which could be explained by the small planktonic larval phase duration of sea cucumber in the water which is around 16 to 21 days (Ivy and Giraspy 2006; Valente *et al.* 2014). Also, by the small potential of larval dispersal between population (Uthicke and Benzie 2001).

For more concrete results we recommend the use of microsatellite analysis, as it the mitochondrial genes studying only the female flow. As well as, including more localities from north coast of Africa and eastern Mediterranean Sea and Red Sea.

CHAPTER VI:

BIODIVERSITY AND BIOGEOGRAPHIC RELATIONSHIPS OF TUNISIAN ECHINODERM FAUNA.

6. BIODIVERSITY AND BIOGEOGRAPHIC RELATIONSHIPS OF TUNISIAN ECHINODERM FAUNA.

6.1. Introduction

Despite the fact that echinoderms constitute a relatively well-known group of fauna, with conspicuous and relatively large species, this group is little prone to the description of new species compared to other more cryptic or diversified groups, and the knowledge of their populations and species geographical distribution is very heterogeneous and discontinuous in time, depending on the areas in which the expert taxonomists of the group have worked.

In the Mediterranean, after Tortonese's monograph for the echinoderms of the Italian coasts, considered a reference for the entire Mediterranean (Tortonese, 1965), works are reduced to local fauna studies, that cover limited depth ranges, and some dispersal references with the first citations of species. It could be said that, at the basin level, the distribution and abundance patterns of echinoderms in the Mediterranean are not well known. Some works have analyzed the biogeographic relationships of specific areas, such as the South-Western Mediterranean (Pérez-Ruzafa and López-Ibor 1987) or the Aegean Sea (Koukouras *et al.* 2007), with the compilation of faunal data from some other areas. If we take into account that genetic studies are showing the existence of cryptic species and that in recent decades there has been a process of colonization of species, both Lesepsian through the Suez Canal, and through the Strait of Gibraltar, we could conclude that the Echinoderm diversity in the Mediterranean Sea is still not well studied (Koukouras *et al.* 2007; Mecho *et al.* 2014).

The Mediterranean Sea is a semi-enclosed relictic ocean composed of an important number of basins and sub-basins, where paleo-geological processes, like plate tectonics or Messinian crises, geographical and physiographic characteristics of the different semi-isolated seas that compose it, like the Black, Aegean, Adriatic, Balearic or Alboran seas, determining a complex circulatory pattern and irregular distribution of water masses and front,s and the physiological adaptations of the different species and populations, lead to an unequal biogeographical distribution of the echinoderm species.

This chapter reviews the distribution of echinoderm species in the Mediterranean, in accordance with the existing bibliography, and analyzes the relationships of the fauna of Tunisia with the different regions and geographic areas. The knowledge of these biogeographic relationships can help to understand the evolutionary processes through which the different species and the respective populations have passed and help to understand the risks of the invasion of species, both Lesepsian and through the Strait of Gibraltar, and the possible trends in said fauna as a consequence of climate change.

6.2. Materials and Methods

According to Briggs (1995) and Briggs and Bowen (2012), Atlanto-Mediterranean area can be divided in two main climatic regions: Cold (including cold-temperate and polar waters) and Warm (tropical and warm-temperate waters). Cold regions include the northern hemisphere with the Western Atlantic, Eastern Atlantic and Arctic Regions. Warm regions include Eastern Atlantic Region with Lusitania, Black Sea, Caspian, Aral, Tropical Eastern Atlantic, Benguela, St Helena, Ascension, Tristan–Gough and Amsterdam–St Paul Provinces, and Western Atlantic Region with the Carolina, Caribbean, Brazilian and Argentinian Provinces.

Spalding *et al.* (2007) consider the Atlantic area divided into five biogeographical realms with 12 provinces (excluding the Artic) and 47 ecoregions inside them. Most of these ecoregions correspond with Clark and Downey (1992) areas considered for their study of the starfishes of the Atlantic (see also Pérez-Ruzafa *et al.*, 2013). In general, there is an agreement in the main biogeographic boundaries considered by the different authors. In the western Atlantic region, these boundaries are located at Bermuda (Southern Florida) and Cape Frio (Brazil). The area between them includes the Caribbean, Brazilian and West Indian provinces. At the eastern Atlantic, La Mancha channel and Cape Blanco and Cape Verde are the corresponding boundaries. with the Cantabric, Atlantic coasts of the Iberian Peninsula, Atlantic Morocco, Canary Islands and Cabo Verde Island belonging to the Lusitania Region (fig. 6.1).

The Mediterranean Sea is considered part of the Lusitania Province (Briggs, 1995), with biogeographic boundaries through the narrow straits with the Red Sea and Black sea provinces, respectively. However, it also maintains restricted connectivity with the Atlantic through the Gibraltar strait. Internally, as commented in previous chapters, the Silicu-Tunisian strait is also usually considered a biogeographic boundary (Moullec *et al.* 2019), based on the isolation between western and eastern basins in the late Miocene, during the Messinian Salinity Crisis (5.96 to 5.33 Ma) (Krijgsaman *et al.* 1999) and the differential colonization of new species through the Gibraltar strait since then and the Suez channel since its opening in the second half of 19th century.

The studied area covers a wide biogeographical and climate range and it is affected from one side by the main currents in the Atlantic Ocean, and the Atlantic and Mediterranean currents from another side (Pérez-Ruzafa *et al.* 2013). Inside the Mediterranean there is also strong climatic differentiation. The annual mean Sea Surface Temperature (SST; 0–50 m depth) and the mean Sea Surface Salinity (SSS; 0–50 m depth) of the Mediterranean Sea are around 17.6º C (± 1.3º C; standard deviation) and 37.9 (± 0.7), respectively (Moullec *et al.* 2019). The coolest areas are the Gulf of Lions and the Northern Adriatic Sea, with a mean SST of 15.3º C and 15.6º C, respectively, and the warmest areas correspond to the Levantine Sea and the Gulf of Gabès, with mean SST of 19.4º C and 18.9º C, respectively (Moullec *et al.* 2019).

According to these considerations and the available faunistic data, to analyze the biogeographic relationships of the echinoderm fauna of Tunisia with the other faunas of the Mediterranean and the Atlantic, the area considered was divided into 64 areas or agregation of areas (Table 6.1, fig. 6.1).

Echinoderms faunistic data for each area were collected from different checklists and studies from the Atlantic, including Portugal, Canary Islands, Morocco and different areas from Latin America, and the Mediterranean, with data from Turkey, Malta Tunisia, and Alboran Islands and the Southern Mediterranean coast of Spain (Koehler 1921; Le Danois 1925; Chambost 1928; Seurat 1929, 1934; Cherbonnier 1956; Pérés and Picard 1956; Maurin 1962; Laban *et al.* 1963; Tortonese 1965; Azouz 1966, 1971, 1974; Lubet and Azouz 1969; De Gaillande 1970; Azouz and Capapé 1971; Ben Othman 1971, 1973; Ktari-Chakroun and Azouz 1971; Prunus and Pantoustier 1974; Azouz and Ben Othman 1975; Darmoul *et al.* 1980; Zaouali 1980; Guelorget *et al.* 1982; Boudouresque *et al.* 1986; Fehri-Bedoui 1986; Pérez-Ruzafa and Lopez-Ibor 1988; Sellem 1990; Özaydın *et al,* 1995; Anonymous 1998; Tanti *et al.* 2006; Ben Souissi and Zaouali 2007; Koukouras *et al,* 2007; Aloui-Bejaoui and Afli 2012; El Lakhrach et *al.* 2012; Pérez-Ruzafa *et al.* 2012; Öztoprak *et al,* 2014).

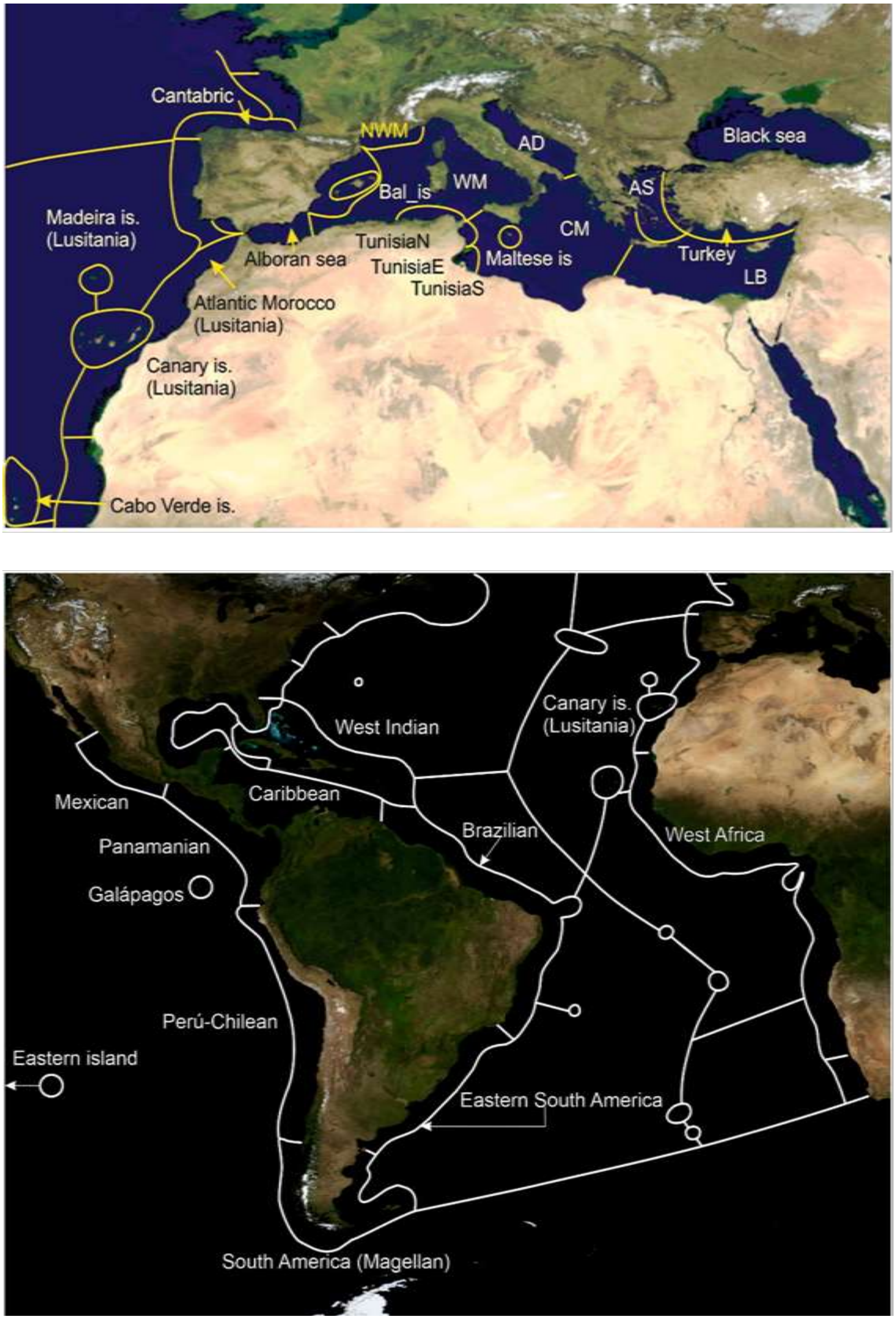


Figure 6.1. Biogeographical regions and areas considered in the Mediterranean Sea (up) and Atlantic Ocean coasts (down) according to Pérez-Ruzafa *et al.* (2013), Briggs (1995) and Clark and Downey (1992).

Table 6.1. Main areas or group of areas used in the biogeographic study and the biogeographic region they belong according to Briggs (1995).

Area	Code	Region	Ocean/sea
Atlantic coast of Mexico	MEXatl	Caribean	Western-Atlantic
Belize	BEL	Caribean	Western-Atlantic
Atlantic coast of Guatemala	GUAatl	Caribean	Western-Atlantic
Atlantic coast of Honduras	HONatl	Caribean	Western-Atlantic
Atlantic coast of Nicaragua	NICatl	Caribean	Western-Atlantic
Atlantic coast of Mexico	CRCatl	Caribean	Western-Atlantic
Atlantic coast of Mexico	PANatl	Caribean	Western-Atlantic
Atlantic coast of Mexico	COLatl	Caribean	Western-Atlantic
Venezuela: Caribean coast	VENcar	Caribean	Western-Atlantic
Atlantic coast of Mexico	VENatla	Brazilian	Western-Atlantic
Brazil	BRA	Brazilian	Western-Atlantic
Uruguay	URG	Eastern_South_America	Western-Atlantic
Argentina	ARG	Eastern_South_America	Western-Atlantic
Malvinas islands	MAV	Eastern_South_America	Western-Atlantic
Cuba	CUB	West_Indian	Western-Atlantic
Haiti	HAI	West_Indian	Western-Atlantic
República Dominicana	RDO	West_Indian	Western-Atlantic
Puerto Rico	PRI	West_Indian	Western-Atlantic
Belle Isle to Cape Cod	Belle	Western_Atl_Boreal	Western-Atlantic
Cape Cod to Cape Hatteras	CCod	Western_Atl_Boreal	Western-Atlantic
Cape Hatteras to Northern Florida	CHatt	North_Florida	Western-Atlantic
Florida to Yucatan, including Gulf of Mexico	Flo	Caribbean_West Indian	Western-Atlantic
Bahamas and Caribbean area, including Greater and Lesser Antilles and Belize to Venezuela on the mainland	Bah	Caribean	Western-Atlantic
Guyanas to Cape Frio, Brazil	Guy	Brazilian	Western-Atlantic
Cape Frio to Uruguay (Rio de la Plata)	CFrio	Eastern_South_America	Western-Atlantic
Uruguay to Eastern Tierra del Fuego and the Falkland Islands	Uru	Eastern_South_America	Western-Atlantic
Trondheim, Norway to the Kattegat	Tron	Eastern-Atlantic Boreal	Eastern-Atlantic Boreal
British Isles, southern North Sea and the Channel (incl. Rockall)	Brit	Eastern-Atlantic Boreal	Eastern-Atlantic Boreal
Ushant to Cape Finisterre (i.e. Bay of Biscay)	Ush	Lusitania	Eastern-Atlantic Boreal
Cape Finisterre to Cap Blanc, Mauritania	CFini	Lusitania	Eastern-Atlantic Boreal
Atlantic coast of Morroco	MARRUECOS_AT	Lusitania	Eastern-Atlantic Boreal
Cap Blanc to S Angola	CBlan	Eastern-Atlantic	Eastern-Atlantic Boreal
West Africa	WESTAFRICA	Eastern-Atlantic	Eastern-Atlantic Boreal
S Angola to False Bay, South Africa	SAng	South-west_Africa	Eastern-Atlantic Boreal
Bermuda	Berm	West_indian	Western-Atlantic
Azores islands	Azor	Lusitania	Eastern-Atlantic
Cabo Verde islands	Cabo Verde	Eastern-Atlantic	Eastern-Atlantic

Madeira islands	Madeira	Lusitania	Eastern-Atlantic
Ascension Island and St Helena	Asce		Eastern-Atlantic
Tristan da Cunha and Gough Island	Trist		Eastern-Atlantic
Newfoundland and North American Basins	NewF		Western-Atlantic
Deep Gulf od Mexico, Caribbean and Guyana Basins	DeepG		Western-Atlantic
Brazilian and Argentine Basins	Braz		Western-Atlantic
West European Basin, including Rockall Trough	Weur		Eastern-Atlantic
Cape Verde to Angola Basins	Cver		Eastern-Atlantic
Cape Basin	CBas		Eastern-Atlantic
Mediterranean: North Africa	MedNAfr	S_Mediterranean	Mediterranean
Mediterranean: Balearic islands	MedIBal	W_Mediterranean	Mediterranean
Mediterranean: Adriatic	MedAdr	Adriatic	Mediterranean
Mediterranean: NortWest	MedMENO	W_Mediterranean	Mediterranean
Atlantic North coast of Spain	D. NOR	Eastern-Atlantic Boreal	Eastern-Atlantic Boreal
Mediterranean: SE Spain	D. SUD	Lusitania	Eastern-Atlantic Boreal
Mediterranean: Alboran sea	D. ESAL	W_Mediterranean	Mediterranean
Mediterranean: Levantin Spanis Coast	D. LEBA	W_Mediterranean	Mediterranean
Canary Islands	D.CAN	Lusitania	Eastern-Atlantic
North Tunisia	North Tunisia-N	C_Mediterranean	Mediterranean
East Tunisia	East Tunisia- E	C_Mediterranean	Mediterranean
South Tunisia	South Tunisia -S	C_Mediterranean	Mediterranean
Malthese Islands	Malthese Islands	C_Mediterranean	Mediterranean
Turkey	Turkey	E_Mediterranean	Mediterranean
Central Mediterranean	CentralMed	C_Mediterranean	Mediterranean
Aegean Sea	AegeanS	E_Mediterranean	Mediterranean
Mediterranean Levantin basin	LB	E_Mediterranean	Mediterranean
Black Sea	BS	Black_S	Mediterranean

The available information suggested the division of the Mediterranean Sea into six main areas: The North-Western Mediterranean, from the Strait of Gibraltar to the southern Mediterranean coast of France including the Alboran sea and the Balearic Islands, the Western Mediterranean Sea with the Northern Tunisian coast and the Tyrrhenian Sea area, the Central Mediterranean Sea that includes the eastern and southern coast of Tunisia, the Maltese Island and the Ionian Sea, the Western Mediterranean Sea, including the Mediterranean coast of Turkey, the Aegean Sea and the Levantine basin, and finally the Adriatic Sea and the Black Sea.

Species names were uniformized for all area’s checklists according to the World Register of Marine Species: WoRMS (http://www.marinespecies.org/). A matrix of Bray-Curtis similarity was built from presence-absence data species matrix and the areas were clustered using complete linkage method of Primer 7 software. Graphical representations of areas relationships were performed using Gephi software.

6.3. Results

The species richness is variable between the different provinces and areas. It deccreases from eastern Atlantic Ocean to the Mediterranean Sea as well as from the western to the eastern Mediterranean Sea basin (fig. 6.3). In the eastern Atlantic and the Mediterranean, the Lusitanic region, with the Canary Islands and the Atlantic coasts of the Iberian Peninsula, show the highest species richness, with more than 150), followed by the western Mediterranean basin. The most impoverished areas correspond to the central Mediterranean, particularly the east coast of Tunisia, and the Black Sea. The Spanish Cantabric coast in the Lusitania province holds the maximum number of echinoderms with over 225 species following by Atlantic South Spain (162 species) and Canary Islands (155 species).

Inside the Mediterranean, the species richness ranges from 110 species in Levantine Spain (western basin) to 77 species in northern Tunisia and Maltese Island (central basin). Eastern basin reached 100 echinoderm species in the Adriatic and 112 species in the Aegean Sea. In Tunisian coasts, north Tunisia reach the highest species richness while, the east Tunisian holds the lowest number with only 40 species. The number of Crinoids is decreasing from the eastern Atlantic Ocean to the eastern Mediterranean Sea. The class Crinoidea and Echinoids are totally absent in the Black sea, while the number of species of Holothuroidea and Ophiuroidea is very abundant.

Our bibliographic review reports the presence of 173 species in the present Mediterranean echinoderm fauna, including the Black sea, that include five Crinoidea, 40 Asteroidea, 38 Ophiuroidea, 31 Echinoidea and 59 Holothuroidea (Pérez-Ruzafa unpublished data; Koukouras *et al.* 2007; Oztoprak *et al.* 2014; Chammem *et al.* 2019). At the same tome, we have compiled a list of 308 species for the Eastern-Atlantic Ocean (Pérez-Ruzafa unpublished data) against 433 species reported just on the Caribbean (Alvarado *et al.* 2010, 2011; Miloslavivich *et al.* 2010; Pérez- Ruzafa *et al.* 2013). The majority of the species have an Atlanto-Mediterranean origin. 138 species are Atlantic, 108 of them exclusives of the eastern Atlantic, 29 are anfiatlantic and only 1 being present only in the western Atlantic. Five of them are also present in the Pacific. Four species are Lessepsian migrants. Finaly, 27 can be considered endemic species.

The western Mediterranean basin presented the highest number of species and thus has the greatest biodiversity of echinoderms. This area extends from Spain to Italy coastlines as well as Moroccan and Tunisian North African coastlines, and in it have been reported 145 species equivalents to 93.5% of the total Mediterranean echinoderm fauna. It is characterised by lower temperature and salinity regarding the eastern Mediterranean basin, but with an intense north-south gradient well represented by the Gulf of Lion front, introducing a large heterogeneity of environmental conditions. Since this basin is connected to the Atlantic Ocean by the strait of Gibraltar, most of the inhabiting species have an Atlanto-Mediterranean origin. However, at the same time, it is emerging a large number of endemic species (Pérez-Ruzafa and Lopez-Ibor 1988; Koukouras *et al.* 2007).

The central Mediterranean, located between western and eastern basin and including the north Tunisian coastline and Sicily and Malta Islands, holds only 91 species meaning 58.7% of the known Mediterranean echinoderm fauna (Fiego and Liao 1996; Koukouras *et al.* 2007). It is considered as a transit area for species from both eastern and western basins.

The eastern district is covering the coast of Cyprus, Greece and Turkey from Europe, Libya and Egypt from north eastern Africa as well as a part from the middle east region. It hosts over 121 echinoderms (78.1% of the total species in the Mediterranean) which are distributed into the Aegean Sea, the Adriatic Sea and the Levantine basin (Pérez-Ruzafa and Lopez-Ibor 1988; Ozaydin *et al.* 1995; Zibrowis and Bitar 2003; Koukouras *et al.* 2007). This area has a high salinity and temperature and is a typical province of lessepsian species that are spreading in the Mediterranean Sea through the Suez channel.

Same as in the Mediterranean Sea, knowledge on Echinodermata taxonomy and biogeography in Tunisia still unaccomplished. The few existing Tunisian reports provide only species lists without studying the ecology or the biogeography (Mecho *et al.* 2014; Koukouras *et al.* 2007).

As we already know, geographically, Tunisian coasts are composed by three large gulfs: The Gulf of Tunis, the Gulf of Hammamet and the Gulf of Gabès, and so it is divided into three mean areas what are north, east and south area (fig. 1.5). Echinoderms diversity and species richness changes from one area to another. In this sense, the present chapter compile and analyse the available historical information on echinoderms in Tunisia in order to evaluate their geographic diversity by area and by class, and, in addition, to investigate the biogeographical patterns of this group.

Only 79 species from the five living classes of Echinodermata were present and distributed in the three Tunisian areas (fig. 1.5). Asteroids comes in first place with a total of 22 species, followed by Ophiuroids with 19 species, Holothuroids (18 species), Echinoids (17 species), and Crinoids with only three species.

The echinoderm species richness shows uneven distribution from North to South Tunisia. The highest species richness (68 species) is accounted in northern area, which represent 84% from total number of species against 36 species from the eastern Tunisian part equally to 51%. and 63 species (82%) in the southern coasts.

From Tunisian echinoderms, 36 (47%) species are ubiquitous, while, only 21 (27%) species are specific of one subregion from which, thirteen species are specific to the north and eight taxa are found only in south area (Peltaster placenta (Müller & Trochel, 1842), Ophiopsila aranea Forbes, 1843, Ophiopsila guineensis Koehler, 1914, Ocnus petiti (Cherbonnier, 1958), Astropecten scoparius Müller & Troschel, 1842, Astropecten spinulosus (Philippi, 1837), Amphiura mediterranea Lyman, 1882, Acrocnida brachiata (Montagu, 1804)).

Despite the Tunisian echinoderm fauna is constituted by a high proportion of world widely distributed species (fig. 6.4) (33 to 42 species, depending on the Tunisian region), it is clearly grouped in the South Eastern Mediterranean region (fig. 6.5) with species with a warm-temperate, wide-temperate or tropical-temperate distribution. Over 53 species are distributed throughout the Atlanto-Mediterranean region and 21 are exclusively Mediterranean species. Only, one species is exclusively Atlantic (*Luidia atlantidea*) and one is from the pacific (*Astropecten scoparuis* (Müller and Troschel, 1842)).

According to the assemblage composition of the Echinodermata fauna of Tunisia (fig. 6.5), over 42% of northern Tunisian echinoderms are cosmopolitan. This component reach 33% in the east and 39% inn south part. The rest are species with warm temperate wide temperate and tropical affinities. The same range of thermal distribution was characterizing the biogeographical regions of the Central Mediterranean and Western Mediterranean with the dominance of cosmopolitan species which present respectively 52% and 78% from the total Echinodermata assemblage. Besides, Black Sea region from eastern Mediterranean basin is dominated by cosmopolitan species with 8% from the total assessment and the rest are warm temperate and temperate tropical species.

The Tunisian echinoderm fauna sows few Mediterranean endemic species: three holothurians (*Phyllophorus (Phyllophorus) urna; Phyllophorus (Phyllophorus) granulatus* and *Holothuria (Holothuria) tubulosa*) and one asteroidea, *Astropecten scoparius* that is present only in South Tunisian coasts. Four species were restricted to the central Mediterranean Sea, (Med MENO) and Maltese Islands (Maltese Is). Over 58 are from Eastern Atlantic, 52 of them in North Tunisia, 30 in East Tunisia and 49 in South Tunisia. Most species were from eastern Mediterranean Sea with Adriatic Sea (MedAdr), Aegean Sea (AegeanS), Turkey and Levantine Basin (LB), and only 6 species are shared with the Black Sea (BS).

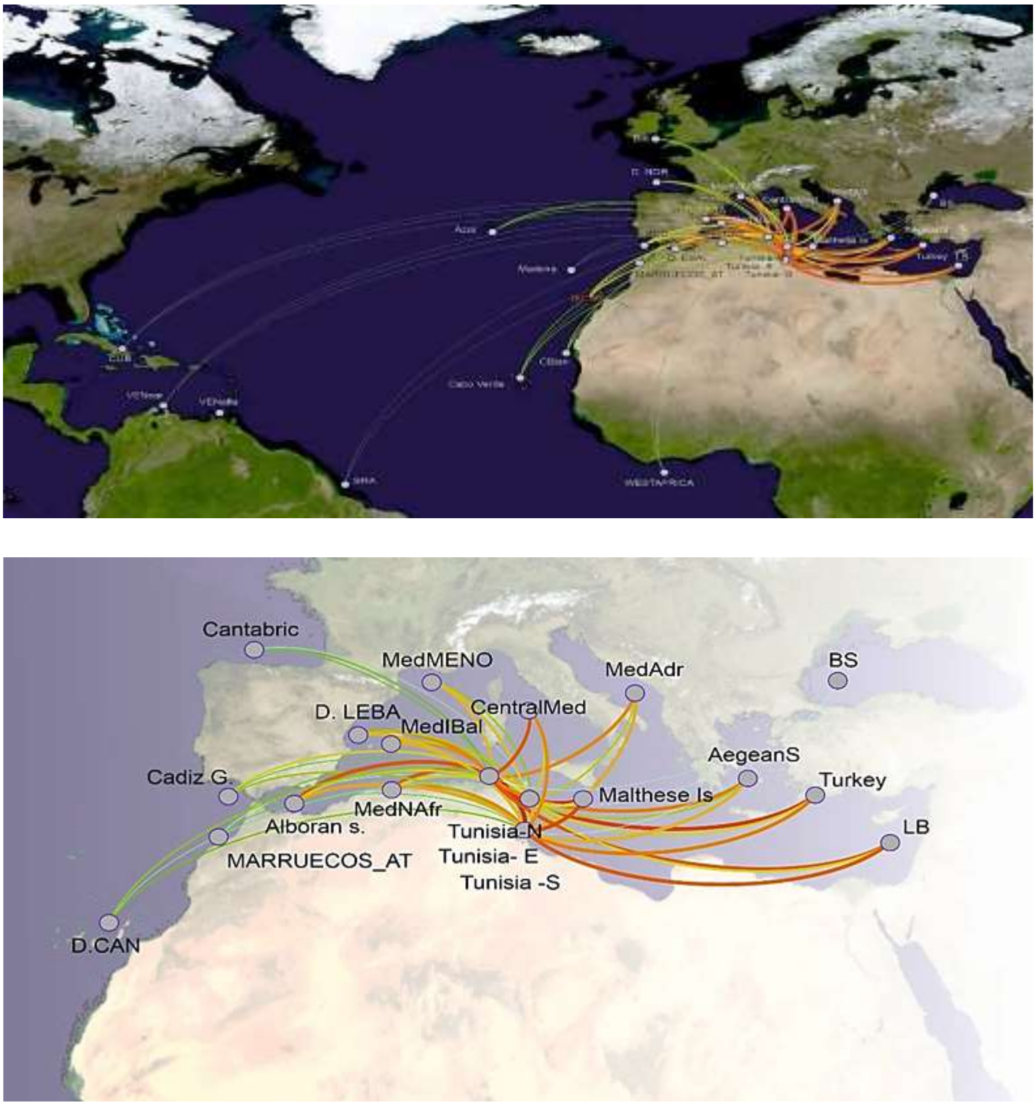


Figure 6.2. Biogeographic relationships estimated as Bray-Curtis similarities, between the echinoderm faunas of Tunisia and other Atlanto-Mediterranean areas. Simmilarity is proportional to wide of the lines. Red color represent highest similarity and blue and green the lowest.

The percentage of the five classes od echinoderms differ in Tunisia from one area to another (fig. 6.3). Holothuroidea was the dominant Echinodermata classes, with highlighting the class Asteroidea, very abundant in the Tunisian areas and Levantin basin. However, the Maltese Island from central Mediterranean Sea was dominated by the class Echinoidea, and the Lusitania Atlantic area was dominated by the Ophiuroidea. The Black Sea emerges the highest percentage of Holothuroidea species (50%). The class Crinoidea is very scarce along the Atlantic Ocean, Mediterranean Sea and Black sea. The percentage of the species from the last group is decreasing from the atlantic to the eastern Mediterranean Sea. It was very abundante in central mediterranean Sea exactly in Tunisia.

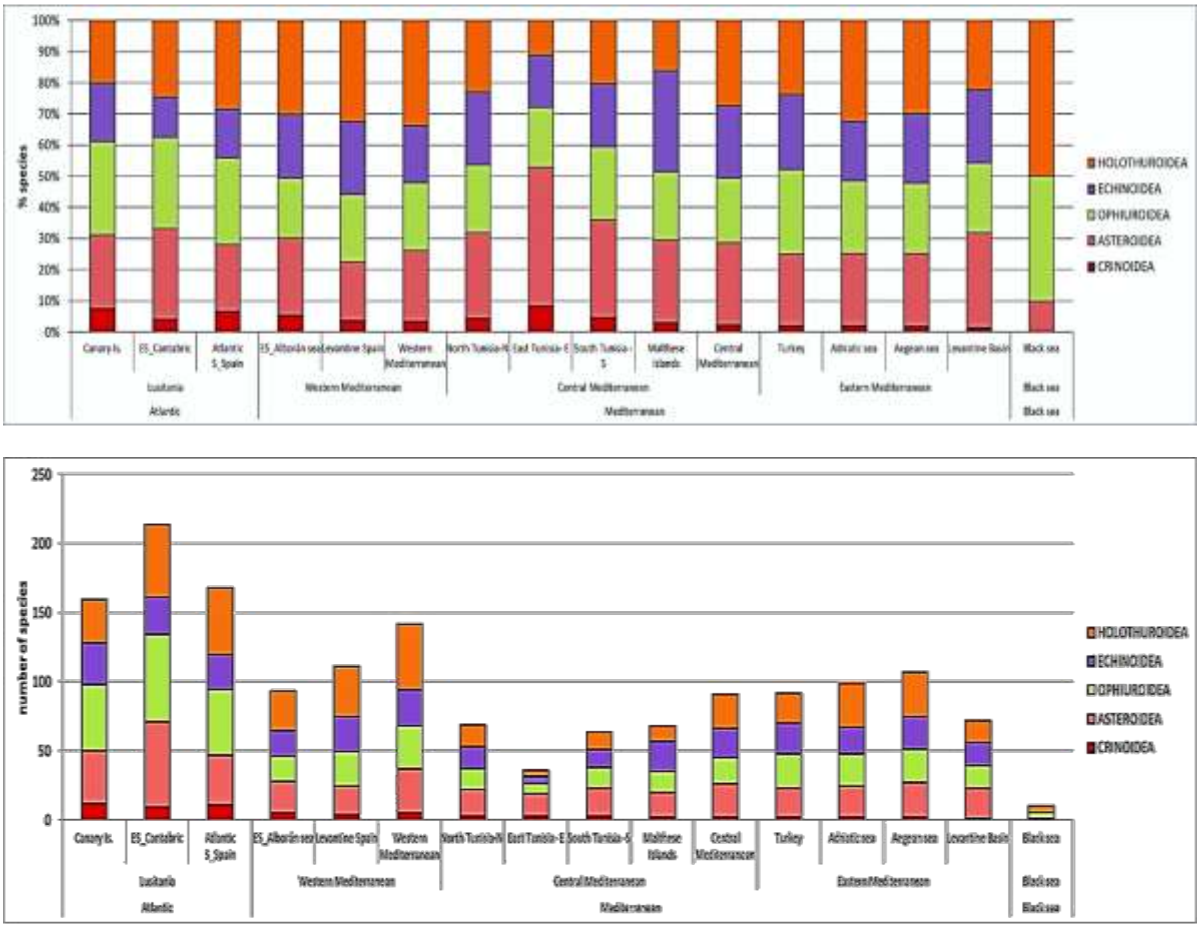


Figure 6.3. Number of species (A) and percentage (B) of the of the five classes Echinodermata along the Atlantic Ocean, Mediterranean Sea and the Black Sea including: Canary Islands (Canary Is), Es_Cantabric and Atlantic South_Spain (Atlantic S_Spain) from Lusitania Atlantic; Alboran Sea (S_Alboran Sea), levanting coast of Spain in the Western Mediterranean, North, South and East Tunisia and Maltese is. in central Mediterranean; and Turkey, Adriatic, Aegean and Levantin basin in Eastern Mediterranean; and the Black sea.

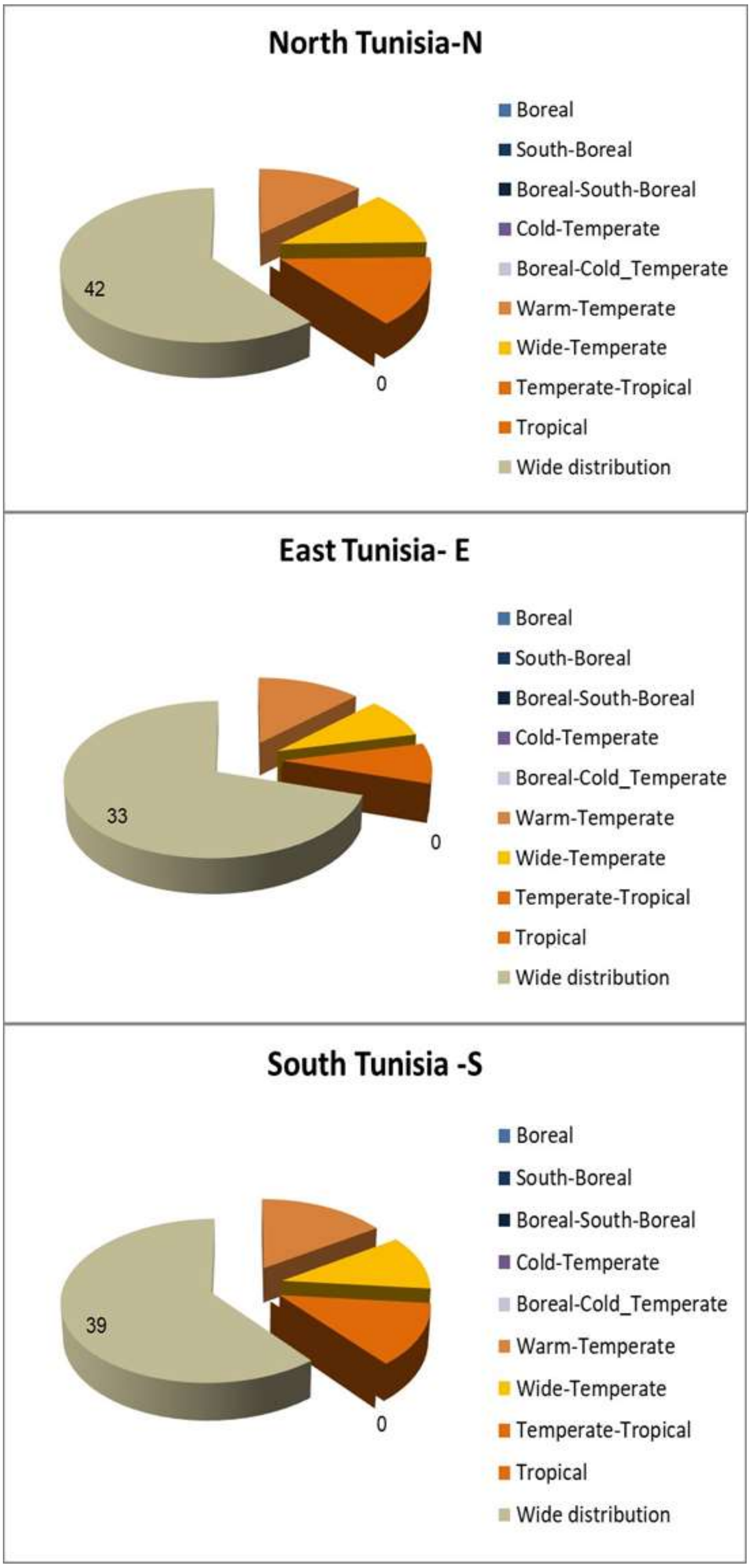


Figure 6.4. Climatic affinities of the echinoderm fauna of the three Tunisian regions.

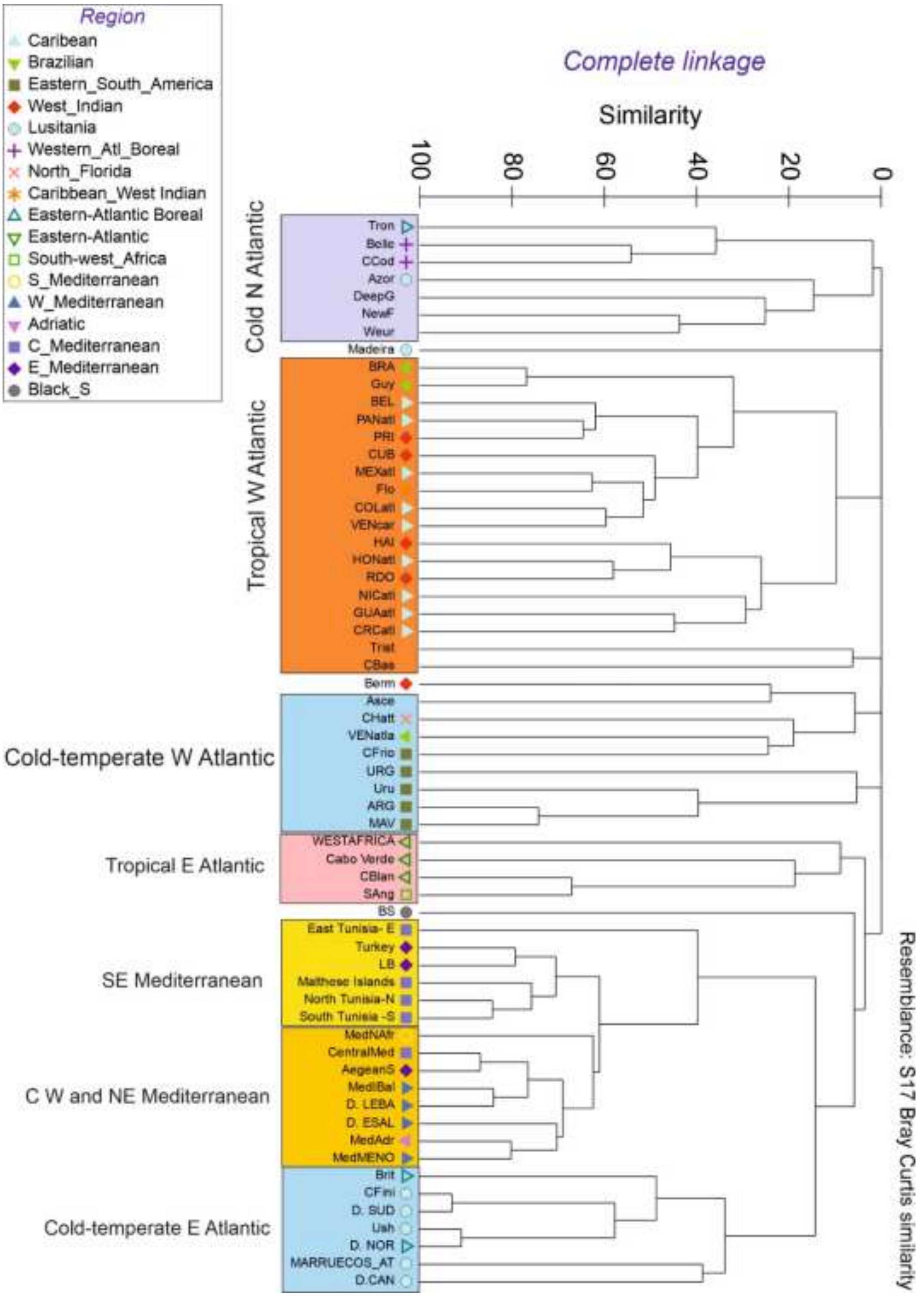


Figure 6.5. Dendrogram built using the Bray-Curtis distances matrix of the echinoderm fauna composition among all the biogeographic areas considered. It is worthy to note than the main clusters aggregate according to the Biogeographic regions and surface water temperature.

According to the composition of assemblage of Echinodermata fauna of Tunisia (Fig. 6.5) over 42% of northern Tunisian echinoderms are cosmopolitan against 33% on the east and 39% on south part. The rest are species with warm temperate wide temperate and tropical affinities. The same range of thermal distribution was characterizing the biogeographical regions of the Central Mediterranean and Western Mediterranean with the dominance of cosmopolitan species which present respectively 52% and 78% from the total Echinodermata assemblage. Besides, Black Sea region from eastern Mediterranean basin is dominated by cosmopolitan species with 8% from the total assessment and the rest are warm temperate and temperate tropical species.

6.4. Discussion

The collected historical data of this macro benthic group, yielded to interesting information about the species richness, their faunistic affinities and their biogeography, within Mediterranean ecoregions and Tunisian subregions.

The available datasets provided the presence of 78 echinoderm species in Tunisia. They are belonging to the five living classes Echinodermata and are divided into 18 orders and 37 families. Among the recorded groups, Asteroidea was the most diverse with 22 species, followed by Ophiuroidea (19 species), Holothuroidea (18 species) and Echinoidea (16 species). The last class is Crinoidea with three species only.

Comparing to neighbor regions from the Maghreb Mediterranean coast, the phylum Echinodermata is quite diverse in Tunisia. The Algerian echinoderms recorded by Dauvin *et al.* (2013) are estimated to 48 species (Chammem *et al.* 2019). The Moroccan coastline on its both Atlantic and Mediterranean facades emerges 167 species of the phylum Echinodermata (Menioui 1998) from which only 25 echinoderms were specific to the Mediterranean coast (Rac/Spa rapport 2003). The researches on the present group from north African coast are old and un-updated, and the knowledge on echinoderms diversity is very limited in space and time (Koukouras *et al.* 2007; Coll *et al.* 2010). Other areas, like the Libyan Mediterranean coast have also very scarces records of echinoderms.

The Tunisian Echinodermata represent 51.3% from the echinoderm’s diversity of the Mediterranean Sea. Over the assortment of the echinoderms known from the western Mediterranean Sea, only 53.5% have been found in Tunisia. Besides, Tunisia is sharing 58.33% of the echinoderms with eastern Mediterranean Sea and 77% with central Mediterranean Sea.

The specific richness shows a decreasing from eastern Atlantic to the Mediterranean Sea, as well as from the western to the eastern Mediterranean basin. This could be in part explained by the sampling effort in this area which is very lower comparing to the Atlantic Ocean, and the lack of information and efforts on Echinodermata assessment on deep Mediterranean Sea, where the eastern ecoregion is the deepest.

The Tunisian echinoderm's fauna is dominated by Atlanto-Mediterranean species with temperate, wide-temperate or tropical-temperate species. Likewise, western, central and eastern districts are mainly constituted from world widely distributed species. However, the Mediterranean Sea is an enclosed basin, with a restricted connectivity with the Atlantic Ocean through the Strait of Gibraltar during a long geological and biogeographical history (Tortonese 1985; Garcia-Castellanos *et al.* 2009; Coll *et al.* 2010). Around 27 echinoderms are endemic or possibly endemic to the Mediterranean Sea (Koukouras *et al.* 2007; Coll *et al.* 2010). Besides,138 are shared with the Atlantic, four are cosmopolitan and four are considered as Lessepsian migrant species (Koukouras *et al.* 2007; Coll *et al.* 2010).

Although the Mediterranean sea is considered as a hotspot of marine biodiversity with high level of endemic species (Danovaro *et al.* 2010; Coll *et al.* 2010; Mecho *et al.* 2014), endimicity of the Echinodermata in the Mediterranean could be the result of the historical events during the evolution of this sea (Tortonese 1985; Garcia-Castellanos *et al.* 2009; Danovaro *et al.* 2010), the global warming and climate changes whith an increasing of temperature much more in the Mediterranean than any other region in the world (Gattaneo-Vietti 2018; Bianchi *et al.* 2019), the maritime traffic and shipping (Bianchi and Morri 2000; Mecho *et al.* 2014) and the human activities impacts (Coll *et al.* 2010; Ounifi-Ben Amor *et al.* 2016).

Likewise, it could be explained as well, by the benthic lifestyle and the pelagic dispersal of the taxa. According to Costello *et al.* 2017, this phylum is characterized by low endimicity with wide spread pelagic lifestyle and slightly influenced by biogeographic barrier realms. The percentage of the endimicity of pelagic Echinodermata species in one realm is equal to 3% in the Mediterranean Sea and from 1% to 2% in northeastern and north western Atlantic Ocean against, 25% in Tasmanian Sea from Pacific Ocean.

In fact, Tunisia is considered as a transitional region between both western and central Mediterranean basins with one main historical sill break, the strait of Sicily that separates the eastern basin from western one (Bianchi and Morri 2000; Boudouresque 2004; Coll *et al.* 2010; Lejeusne *et al.* 2010). The Tunisian coastline, with 1300 km long, endowed three mains gulfs: The Gulf of Tunis (north area), the Gulf of Hammamet (eastern area) and the Gulf of Gabès (southern area). The sea substrates from the north to the south is ranging from rocky to sandy and sandy muddy (Azouz 1971, 1973; Ben Mustapha and Hattour 1992). So, the echinoderms richness in Tunisia is declining from north to south. The highest specific richness was recorded at the northern coast with 68 echinoderms, mostly they have an atlanto-mediterranean affinities with one Atlantic species (*Luidea atlantidea*). Up to 21.5% of the recorded species are specific to this area.

This area is exposed to Atlantic currents and is characterized by rocky sea bottom with wide heterogeneity of habitats (Azouz 1971, 1973; Ben Mustapha and Hattour 1992; Chammmem *et al.* 2019). The assemblages of coralligenous and *Posidonia* meadows from this area, are well developed and very frequented (Azouz 1973; Ben Mustapha and Hattour 1992; Ghanem *et al.* 2018).

The brittle star, *Ophiocanta setosa,* from this area was the unique species found associated to the yellow gorgonian *Eunicella cavolini* (Koch, 1887). Also, the specimens of the starfish *Asterina panceri* were collected from meadows habitat (*Cymodocea nodosa*). Despite, the importance of the macrobenthic diversity inhabited the coralligenous biocenosis and seagrass beds, studies on the assemblages of Echinodermata on coral reef and meadows are rare in Tunisia.

The lowest value of the species richness is observed in eastern area. This coast from central Tunisia, stretches from south Cap Bon peninsula to the north and Ras Caboudia to the south. The gulf of Hammamet which lies within this subregion, is characterized by wide sandy beaches and it hosts one of the largest marina in Tunisia, the Yasmine Hammamet harbor. It is well known by an important industrial and tourism activities, over of 60% of Tunisia’s tourism potential is concentrated in this coast (Elgharsalli *et al.* 2015; Atoui *et al.* 2016), sources of coastline degradation and loss of biodiversity.

Also, the gulf of Hammamet is emerging an important area of seagrass beds of *Posidonia oceanica* and *Cymodocea nodosa*. They are present from 4 meters’ depth and occupies the surface of 167 Km^2 (Ben Mustapha and Hattour 1992; Ben Brahim *et al.* 2014; Atoui *et al.* 2016). They extend out to the gulf of Gabès from southern subregion.

Nowadays, meadows habitats are suffering from destruction and their surface is declining because of human activities the very common are, industrial and domestic effluents pollution and illegal trawling (Ben Mustapha and Hattour 1992; Sghaier *et al.* 2011; Atoui *et al.* 2016). Alike, such disturbance may distress the diversity of the echinoderms in the gulf of Hammamet. According to Walag and Canencia 2016, the anthropogenic activities and the physicochemical factors like temperature, pH and salinity, could affect the distribution and the abundance of the echinoderms.

The Gulf of Gabès from south ecoregion holds the second place of specific richness with 63 species of echinoderms. This area extends from Ras Kapoudia to the Tunisian-Libyan border. It is considered the largest gulf from the space area, with approximatively 90 km wide and 100 km long (Zaouali 1993; Bradai *et al.* 2004). It is characterized by the presence of a big continental shelf and by unique geomorphological, climatic and oceanographic conditions (Ben Mustapha *et al.* 2002; Ben Mustapha and Afli 2005; Bejaoui *et al.* 2019). It is much influenced by Mediterranean currents. Only eight echinoderms were specific to this Gulf.

During the beginning of the 20th century, *Posidonia* meadows was very frequented and widespread along the gulf's coasts. It was considered as one of the most extensive communities of seagrass beds (*Posidonia oceanica)* in the Mediterranean Sea (Zaouali 1993; Hattour and Ben Mustapha 2015), that constitutes an important reproduction and nursery site for several marine species (Francour 1997; Francour *et al.* 1999; Aloulou *et al.* 2012; Bejaoui *et al.* 2019). The discharge of phosphogypsum in sea gulf by phosphate industries lead to drastic loss in the cover of *Posidonia oceanica,* which estimated up to 90% (Bejaoui et *al.* 2004; Ben Brahim *et al.* 2010; El kateb *et al.* 2017; Bejaoui *et al.* 2019). Many investigations on the impacts of the phosphate industry in Gabès documented the decrease of marine biodiversity, heavy metal pollution and the degradation of the seafloor and water quality (Darmoul *et al,* 1980; Zaouali 1993; El Lakhrach *et al.* 2012; Ayadi *et al.* 2014; El kateb *et al.* 2017; Bejaoui *et al.* 2019).

Adding to this, after the opening of the Suez Channel the gulf became a province for several Lessepsian species coming from Red Sea and Indo Pacifc area (Ounifi-Ben Amor *et al.* 2016). Two echinoderms recognized as Lessepsian for the Mediterranean Sea were cited first in the gulf of Gabès thus are the brittle stars *Ophiactis savignyi* (Müller and Troschel, 1842) (Koukouras *et al.* 2007; Ben Souissi *et al.* 2013) and the sea star

Astropecten scoparuis (Müller and Troschel, 1842), which was only limited to this zone (M.E.A.T. 1998; Boudouresque 1997^{b}).

Overall, the phylum Echinodermata is in equally distributed between the three gulfs. The Gulf of Hammamet from east area which represented a moderate echinoderms diversity is facing threats such as the destruction of habitat, marine pollution and illegal fishing. Also, these could be attributed to the lowest sampling effort and to the limited macrobenthic investigations on this subregion, where the interest is much giving to north and south area (Ben Mustapha *et al.* 2002; Ben Mustapha and Afli 2005). The strongest investments are majority destined to the Gulf of Gabès and the Gulf of Tunisia because of their important geographic position and the variability of their habitats which confer a wealth of biological resources.

The update of the recorded echinoderms reveals a diversity in number and species comparing to the neighboring region and provinces. Nowadays, the Tunisian coasts are under the colonization of species from mixed origins. It is a transition between Atlantic species on the north and the Lessepsian one on the south. As to achieve a better understanding on the echinoderm's diversity in Tunisia, different sampling methods and collecting efforts must concern all the coast from north to south. Furthermore, it is recommended that sampling covert all the range depth with a special interest to the east subregion and deep-water area.

Table 6.2. The checklist of the recorded echinoderms along the Tunisian coast with the area of distribution, the habitats and the depth range (Dep. Range. REF.) available on the reference dataset, depth from the present work (Dep. PRE.WORK) and the biogeographical origin, distribution and type of substrate on which they are found. N= Northern Tunisia; E= Estern Tunisia; S=Southern Tunisia; S^a=Sandy bottom; M=Muddy bottom; R=Rocky bottom; M^{ed}=Mediterranean Sea; A=Atlantic Ocean; C= cosmopolitan; L=Lessiepsian; P=Posidonea beds; += first records on Tunisia/second record en northern Tunisia; *=presence; -= no availible data. N^b=Number of the taxa.

Species	N^b	Tunisian Areas			Habitats REFERENCES				Dep. Range REF. (m)	Dep. PRE .WORK (m)	Biogeographical origin				
		N	*E*	*S*	M	R	S^a	P			M^{ed}	AM	A	L	C
Class CRINOIDEA Family ANTEDONIDAE Norman, 1865															
Antedon bifida (Pennant, 1777)	1	*					*		110	50-190		*			
Antedon mediterranea (Lamarck, 1816)	2	*	*	*	*	*	*		20-250	50-190	*				
Leptometra phalangium (Müller, 1841)	3	*	*	*	*		*		50-400	72-194	*				
Class ASTEROIDEA Blainville, 1830 Family BRISINGIDAE G.O. Sars, 1875															
Hymenodiscus coronata (Sars, 1871)	4	*	*	*	*			*	450-600	-		*			
Family ASTERIIDAE Gray, 1840															
Coscinasterias tenuispina (Lamarck, 1816)	5	*	*	*	*			*	25-60	20-51		*			
Marthasterias glacialis (Linnaeus, 1758)	6	*	*	*	*	*	*		30-150	75-220		*			
Family ASTROPECTINIDAE Gray, 1840															
Astropecten aranciacus (Linnaeus, 1758)	7	+	*	*	*	*	*		30-200	51-177		*			
Astropecten bispinosus (Otto, 1823)	8	*	*	*	*		*	*	10-100	1-35		*			
Astropecten irregularis (Pennant, 1777)	9	*	*	*	*		*		40-190	50-220		*			
Astropecten irregularis pentacanthus (Delle Chiaje, 1827)	10	*	*	*	*	*	*	*	15-400	-	*				
Astropecten jonstoni (Delle Chiaje, 1827)	11	*		*	*		*	*	2-100	3-5	*				
Astropecten scoparuis (Müller and Troschel, 1842)	12			*	-	-	-	-	-	-				*	
Astropecten spinulosus (Philippi, 1837)	13			*			*		30-60	-	*				
Tethyaster subinermis (Philippi, 1837)	14	*	*	*	*		*		30-250	50-220		*			
Family LUIDIIDAE Sladen, 1889															
Luidia atlantidea (Madsen, 1950)	15	+								65-95			*		
Luidia ciliaris (Philippi, 1837)	16	*	*	*		*	*		50-400	-		*			
Luidia sarsii sarsii Düben and Koren in Düben, 1844	17	*	*	*		*			30-100	175-193		*			

Family ECHINASTERIDAE Verrill, 1867															
Echinaster (Echinaster) sepositus (Retzius, 1783)	18	*		*	*		*	*	20-250			*			
Family ASTERINIDAE Gray, 1840															
Anseropoda placenta (Pennant, 1777)	19	*	*	*	*		*		30-300	185-220		*			
Asterina gibbosa (Pennant, 1777)	20	*	*	*		*	*	*	2-50	0,45-0,65		*			
Asterina pancerii (Gasco, 1876)	21	+						*	3-5	3-5	*				
Family CHAETASTERIDAE Sladen, 1889															
Chaetaster longipes (Retzius, 1805)	22	+	*	*	*	*	*		35-400	70-170		*			
Family GONIASTERIDAE Forbes, 1841															
Peltaster placenta (Müller and Troschel, 1842)	23			*	*		*		185-300	-		*			
Family OPHIDIASTERIDAE Verrill, 1870															
Hacelia attenuata (Gray, 1840)	24	+		*						70-85		*			
Ophidiaster ophidianus (Lamarck, 1816)	25	*	*	*		*			50-100	-	*				
Class OPHIUROIDEA Gray, 1840															
Family GORGONOCEPHALIDAE Ljungman, 1867															
Astrospartus mediterraneus (Risso, 1826)	26	+	*				*		190	98-105		*			
Family AMPHIURIDAE Ljungman, 1867															
Acrocnida brachiata (Montagu, 1804)	27			*			*		50-100	-		*			
Amphiura chiajei (Forbes, 1843)	28	*		*	*			*	5-110	-		*			
Amphiura filiformis (O.F. Müller, 1776)	29	*	*	*					-	-		*			
Amphiura mediterranea (Lyman, 1882	30			*	*				-	-	*				
Amphipholis squamata (Delle Chiaje, 1828)	31	*		*			*		-	0,4-0,6					*
Family OPHIACANTHIDAE Ljungman, 1867															
Ophiacantha setosa (Bruzelius, 1805)	32	*							300	70-165		*			
Family OPHIACTIDAE Matsumoto, 1915															
Ophiactis savignyi (Müller and Troschel, 1842)	33	+		*		*			3	3-5					*
Ophiactis virens (M. Sars, 1857)	34	+							-	0,4-0,6		*			
Family OPHIOCOMIDAE Ljungman, 1867															
Ophiocomina nigra (Abildgaard in O.F. Müller, 1789)	35	*			*		*		50-250	50-58		*			
Family OPHIODERMATIDAE Ljungman, 1867															
Ophioderma longicauda (Bruzelius, 1805)	36	*	*	*	*		*	*	30-300	0,65		*			
Family OPHIOMYXIDAE Ljungman, 1867															
Ophiomyxa pentagona (Lamarck, 1816)	37	*	*	*	*		*	*	4-250	50-210		*			
Family OPHIOPSILIDAE Matsumoto, 1915															
Ophiopsila annulosa (M. Sars, 1859)	38	*	*	*			*		50-100	-		*			
Ophiopsila aranea (Forbes, 1843)	39			*			*		30-35	-		*			

Ophiopsila guineensis (Koehler, 1914)	40			*			*		30-35	-		*			
Family OPHIOTRICHIDAE Ljungman, 1867															
Ophiothrix fragilis (Abildgaard in O.F. Müller, 1789)	41	*	*	*		*	*	*	20-300	-		*			
Ophiothrix quinquemaculata (Delle Chiaje, 1828)	42	*	*	*	*	*	*		25-200	72-175	*				
Family OPHIURIDAE Müller and Troschel, 1840															
Ophiura albida (Forbes, 1839)	43	*	*				*	*	20-250	-		*			
Ophiura ophiura (Linnaeus, 1758)	44	*	*	*	*	*	*	*	1-450	3-194		*			
Class ECHINOIDEA Bronn, 1860															
Family ARBACIIDAE Gray, 1855															
Arbacia lixula (Linnaeus, 1758)	45	*					*	*	5-15	0,25-5		*			
Family CIDARIDAE Gray, 1825															
Cidaris cidaris (Linnaeus, 1758)	46	*	*	*	*		*		60-400	50-220		*			
Stylocidaris affinis (Mortensen, 1909)	47	*		*	*		*		20-300	50-220		*			
Family DIADEMATIDAE Gray, 1855															
Centrostephanus longispinus (Philippi, 1845)	48	*	*	*	*		*		30-300	50-220		*			
Family ECHINIDAE Gray, 1825															
Gracilechinus acutus (Lamarck, 1816)	49	*	*	*	*		*	*	20-450	50-125		*			
Family PARECHINIDAE Mortensen, 1903															
Paracentrotus lividus (Lamarck, 1816)	50	*		*	*	*	*	*	0-80	0,2-6		*			
Psammechinus microtuberculatus (Blainville, 1825)	51	*		*			*	*	5-100	-	*				
Family TOXOPNEUSTIDAE Troschel, 1872															
Sphaerechinus granularis (Lamarck, 1816)	52	*		*		*	*	*	25-100	0,6-5		*			
Family TRIGONOCIDARIDAE Mortensen, 1903															
Genocidaris maculata A. Agassiz, 1869	53	-	-	-	-	-	-	-	-	-		*			
Family ECHINOCYAMIDAE Lambert and Thiéry, 1914															
Echinocyamus pusillus (O.F. Müller, 1776)	54	*		*	*		*		15-100	-		*			
Family BRISSIDAE Gray, 1855															
Brissopsis lyrifera (Forbes, 1841)	55	*			*				80-110	-		*			
Brissus unicolor (Leske, 1778)	56	*	*	*	*		*	*	7-50	-		*			
Family LOVENIIDAE Lambert, 1905															
Echinocardium cordatum (Pennant, 1777)	57	*			*		*		10-25	-					*
Echinocardium flavescens (O.F. Müller, 1776)	58	*	*	*			*		10	-		*			
Echinocardium mediterraneum (Forbes, 1844)	59	*		*	*				25-30	-		*			
Family SCHIZASTERIDAE Lambert, 1905															
Ova canalifera (Lamarck, 1816)	60	*	*	*				*	15-30	-	*				
Family SPATANGIDAE Gray, 1825															
Spatangus purpureus (O.F. Müller, 1776)	61	*		*	*		*	*	30-300	3-5		*			

Class HOLOTHUROIDEA Brin, 1860 Family SYNAPTIDAE Burmeister, 1837															
Oestergrenia digitata (Montagu, 1815)	62	*			*				50-100	-		*			
Family HOLOTHURIIDAE Burmeister, 1837															
Holothuria (Panningothuria) forskali (Delle Chiaje, 1823)	63	*		*	*				25-45	-		*			
Holothuria (Holothuria) helleri (Marenzeller von, 1877)	64			*					20-260	-	*				
Holothuria (Holothuria) mammata (Grube, 1840)	65	*		*					31-51	3-8		*			
Holothuria (Holothuria) tubulosa (Gmelin, 1791)	66	*	*	*	*		*	*	0-200	0,2-185		*			
Holothuria (Platyperona) sanctori (Delle Chiaje, 1823)	67	*		*	*		*		20-55	0,2-0,4		*			
Holothuria (Roweothuria) poli (Delle Chiaje, 1824)	68	*	*	*	*		*	*	0-250	0,2-8		*			
Holothuria (Thymiosycia) impatiens (Forsskål, 1775)	69	*		*		*	*	*	0-15	0,45					*
Family MESOTHURIIDAE Smirnov, 2012															
Mesothuria intestinalis (Ascanius, 1805)	70	*			*				200-450	-		*			
Family CUCUMARIIDAE Ludwig, 1894															
Hemiocnus syracusanus (Grube, 1840)	71	*		*					22-45	3-5		*			
Leptopentacta elongata (Düben and Koren, 1846)	72	*	*	*			-	-	-	77-145	*				
Leptopentagta tergistina (M. Sars, 1857)	73	+					*		-	77-145		*			
Ocnus petiti (Cherbonnier, 1957)	74			*			*		30-35	-		*			
Ocnus planci (Brandt, 1835)	75	*		*	*		*		50-250	-	*				
Pawsonia saxicola (Brady & Robertson, 1871)	76	-	-	-	-	-	-	-	-			*			*
Family PHYLLOPHORIDAE Östergren, 1907												*			*
Phyllophorus (Phyllophorus) granulatus (Grube, 1840)	77	*	*	*					8-15	-					
Phyllophorus (Phyllophorus) urna (Grube, 1840)	78	*	*	*	*				150	-	*				*
Family STICHOPODIDAE Haeckel, 1896											*				
Parastichopus regalis (Cuvier, 1817)	79	*		*	*		*	*	20-400	0,2-194		*			

CHAPTER VII:

GENERAL DISCUSSION AND CONCLUSIONS

7. GENERAL DISCUSSION AND CONCLUSIONS

The present thesis had explored the faunal diversity and biology of the phylum Echinodermata in northern Tunisia. It had evaluated its distribution along the northern coast according to the marine zonation of the Mediterranean Sea and habitats types. In the other hand, the inventory of echinoderms in Tunisia has been updated and the biodiversity and biogeographic information from north to south of the country, as well as along the Mediterranean ecoregions and provinces, have been studied.

Besides, in order to clarify the systematic position of sea cucumber species from the genus *Holothuria*, this thesis had tested the spicules characteristics of eight species with Atlantic and Mediterranean origins, in the bases of their button and table shapes. It revealed a remarkable similarity between the morphometry of the spicules and the genetic characteristics of mitochondrial genes (16 S and COI), using the barcoding molecular technique at the level of gene COI.

Otherwise, by studying the genetic diversity and the gene flow of the target sea cucumbers species *Holothuria poli,* along the Mediterranean Sea and in two different biotopes, open sea and coastal lagoon ecosystems, this work highlights the importance of protection for holothurian conservation and the maintaining of their genetic identity.

The northern Tunisia shows a hight diversity of echinoderms. In the present work we have identified 45 species that belong to the 5 living classes of Echinodermata and comprise 15 starfishes (Asteroidea), 10 brittle stars (Ophiuroidea), nine sea cucumbers (Holothuroidea), eight sea urchins (Echinoidea) and three sea lilies (Crinoidea). All of them included into 32 genera and 27 Family. All of them were species previously recorded in the Mediterranean Sea, with the exception of the starfish *Luidia atlantidea*, which is an Atlantic species recently recorded in the Alboran sea (Gallardo-Roldán et al., 2015). Four of the collected species are endemic of the Mediterranean Sea and four others have a wide distribution and are cosmopolitan. Four recorded species were the first mention for Tunisia (*Asterina pancerii*, *Luidia atlantidea*, *Ophiactis virens* and *Leptopentacta tergestina*). Two are exclusively Mediterranean species (*Asterina pancerii* and *Leptopentacta tergestina*), *Luidia atlantidea*, as mentioned, is an Atlantic species recently cited in the Mediterranean and the last one is a cosmopolitan species (*Ophiactis virens*).

The faunistic study of the echinoderm communities across the northern coast of Tunisia shows a wide distribution in time and species. The most diverse species are belonging to the class Asteroidea, while the Crinoidea and the Echinoidea were the less diverse groups.

Also, we notice the general scarcity of irregular urchins which are presented by a unique exampler from *Spatangus purpureus* (O.F. Müller, 1776). This could be attributed to the adopted methodology and fishing gears which were depended on the depths and area frequented by fishermen. According to the available historical data in Tunisia the Gulf of Gabès from southern subregion emerges the highest richness of irregular echinoid species (Lakhrach *et al.* 2012).

We have constated the presence of the sea star *Ophidiaster ophidianus* which is an atlanto mediterranean species that was observed by diving in Tabarka at 5 meters deep. Because of its status, as one of the threatened and protected species from the phylum Echinodermata in the Mediterranean Sea, it was not sampled and so, it did not appear in the present checklist of the echinoderms of northern Tunisia.

The present work has elevated the diversity of the echinoderms in Tunisia to 78 species with only one sea cucumber species that werei dentified to the genus level (*Leptosynapta* sp). Moreover, six species, that were found before restricted to the south and east Tunisian areas, were found in this study for the first time in the northern area too.

This phylum showed no difference between localities but a significant difference between sectors and localities nested sectors. Tabarka and Kelibia from sectors one and three were emerging with the greatest number of echinoderms. However, Korbous from sector two showed the lowest number of individuals with only one specimen. Two major species *Stylocidaris affinis* and *Cidaris cidaris* were the most abundant in sector one (Bizerte and Tabarka), *Ophiactis virens* (Radès) from sector two and *Echinaster sepositus* (Kèlibia) from sector three. Furthermore, echinoderm species distribution shows a variability of species richness and abundance along the Mediterranean Sea horizontal marine zonation. We stand out the increasing of Asteroidea from the infralittoral to the circalittoral and bathyal levels and, on the contrary, the decrease in presence of Holothuroidea. Echinoidea were well distributed and represented in all the benthic zones, while Ophiuroidea and Crinoidea were scarce or do not appear in shallow areas. These could be explained by the fact that the distribution of the Echinodermata fauna in the Mediterranean Sea and particularly, in northern Tunisia depends on the biotic and abiotic parameters, living and non-living factors, that affects a marine ecosystem and which are specific for each habitat and to each level.

Otherwise, in many cases, the systematic study based on the taxonomical and anatomical criteria could be confused and doubtful because of the large similarity of morphological characters between the species. Eight species from genus *Holothuria* (*H. (Roweothuria) arguinensis*, *H. (Holothuria) dakarensis*, *H. (Vaneyothuria) lentiginosa lentiginosa*, *H. (Holothuria) mammata*, *H. (Roweothuria) poli*, *H. (Platyperona) sanctori*, *H. (Panningothuria) forskali* and *H. (Holothuria) tubulosa*), that have shown a big spicules resemblance, have been subjected to a molecular study to clarify the differences between species. The obtained results from spicules morphometry shows that buttons have marked an extensive morphological variation among the different species more than tables. Accordingly, three major parameters were the contributors to the variability of these buttons: area of buttons (ArB), number of button's holes (NbHB) and area of button´s holes (ArHB). In the case of tables, the area (ArT) and the convex perimeter of tables (CoPrT) were the main parameters contributing to the significant difference related to the morphometric trait. Thus, data points to a significant morphometric similarity between Atlantic and Mediterranean species, except for the Mediterranean *H. poli* specimens, which were molecularly and morphometrically separated from the rest of the taxa. Indeed, the morphometric variation among the atlanto Mediterranean species is probably associated with the genetic variation and not to their geographical position.

In general, both ,16S and COI mtDNA, go accordingly with morphometric data as it follows by the big concordance between the morphometric cluster and the phylogenetic NJ tree. The specimens from *H. tubulosa, H. l. lentiginosa, H. dakarensis, H. mammata* and *H. poli* were occupying the same position in both dendrograms. However, the phylogenetic results obtained from gene COI of *H. poli, H. tubulosa* and *H.l. lentiginosa* from three different localities: Tunisia, Mediterranean Spain and Canary Islands, shown a clear differentiation between the Atlantic and the Mediterranean specimens. Mediterranean specimens of *H. tubulosa* from Spain and northern Tunisia were separated from the Atlantic species *H. l. lentiginosa* (Canary Islands) and the Mediterranean *H. poli* inviduals (Tunisia and Spain). However, there is no differentiation between the Mediterranean specimens from eastern and central basins, which were clearly connected. Both of *H. poli* and *H. l. lentiginosa,* and *H. poli* and *H. tubulosa* are sharing the same divergence distance. The systematic position of the species of genus *Holothuria* was so far dubious. Therefore, the use of both molecular and morphometric approaches is required when sea cucumbers morphological identification is uncertain or impossible. Concurrently, supported by ecological and biogeographical parameters they represent a strong driving force for taxa.

The phylogeny of the target sea cucumber *H. poli* obtained on the base of the gene 16S and COI had admitted an importante haplotype and genetic diversity of *H. poli* species across the the three Mediterranean basins: west, central and east. In particular, the results of COI gene of *H. poli* are showing a high haplotypes diversity in Bizerte and Mar menor costal lagoons more than, Kusadasi from marine sea. While the nucleotide diversity was almost similar for both ecosystems. There was a significant difference between eastern and western Mediterranean Sea for 16S gene only. However, there was no difference between central Mediterranean Sea and both Mediterranean basins.

H. poli shows a wide distribution from the east to the west of the Mediterranean Sea which is explained by the connectivity and the passage of gene flow leading to the important genetic diversity in eastern basin with the presence of ancestral haplotypes particularly in Kusadasi in Turkey. Kusadasi is a MPA ecosystem which explain the presence of the important number of exclusive and ancestral haplotypes of *H. poli* comparing to the one from Mar Menor and Bizerte lagoon. Nowadays, the eastern Mediterranean Sea has become a Lessepsian province due the colonization of numerous invasive species. Thus, are endangered native species, habitats, and fisheries. Therefore, in face of human impacts, overfishing and global warming effects, as well as to maintain the genetic identity and the marine diversity MPA are the right solution for protecting species identities and habitats.

Besides, lagoon ecosystems are quite diverse the Tunisian coasts and they are holding a high abundance in the population of *H. poli* (Chammem *et al.* 2019; Sellem *et al.* 2019). According to the study done by Sellem *et al.* (2019) on the diversity of sea cucumber from two Tunisian lagoons, Bizerte Lagoon (from northern Tunisia) and Boughrara Lagoon (from southern Tunisia), seven holothurian species are occurring the ecosystem lagoon. They are *H. poli*, *H. tubulosa, H. impatiens*, *H. forskali, H. sanctori*, *H. mammata* and *Hemiocnus syracusanus*, With the first appearing of the latter species in lagoon ecosystems.

Comparing to other harvested species such as *Parastichopus regalis, H. poli* remained one of the most commonly cultured holothurian species in the Mediterranean Sea and one of the popular exploited sea cucumber species. Despite of its smaller and thinner body, its nutritional profile shows a large amount of protein and mineral rate and low-fat rate (Sellem *et al.* 2017; de Walle *et al.* 1993).

Inferred from the data of sea cucumber commercialization in Tunisia collected from the Tunisian INS (national statistical data) of Foreign Trade and the ministry department of fishing and fish farming, *H. poli* is one of the most exploited and exported species in Tunisia, in the second place after *H. tubulosa,* and it occupies the first place on population densities (Karim and Hattour 2016; Sellem *et al.* 2019). Sea cucumber fishery in Tunisia is mainly concentrated in lagoon ecosystem where *H. poli* is common and present with high potential.

Nowadays, the population of *H. poli* is facing threats such as overexploitaon, illegal fishing and habitats destruction. Bearing in account the important ecological role of sea cucumber in maintaining the function of marine ecosystem, it’s necessary to organize the holothurian exploitation in Tunisia for the persistence and the good management of species stocks. Although until now sea cucumber commercialization seems to be limited and disorganized, it is recommended to limit the harvesting in number, in time and in space. Karim and Hattour (2016), have suggested to limit the catches to 50 individuals per a day during three months only one period per year. However, harvesting is strictly prohibited during summer (from June to august) which corresponding to sea cucumber reproduction season. The number of the catches should be controlled and it is important to register all the biological information about the harvested specimen such as the systematic classification, the maturity age, and the biometric data.

Overall, the Tunisian coast with it three gulfs emerging an important diversity of Echinodermata fauna comparing to the neighboring regions and provinces from the Mediterranean Sea. Thus, could be attributed to the diversity of the topography of the Tunisian shoreline and the contrast of the Atlantic and the Mediterranean hydrodynamics. The majority of the echinoderms have an atlanto mediterranean origin with temperate, wide-temperate and tropical-temperate species. Indeed, the location of Tunisia as a junction between the western and the eastern Mediterranean ecoregions, allows the transition of Atlantic species from the north and the Lessipsian migrant from the south. In this sense, we pointed out the presence of Lessepsian species on the gulf of Gabès and the Atlantic species from northern area. The distribution of the species was unequally divided from north to south, which is mainly linked to the restricted scientist interest, the limited investment and the lack of taxonomist.

Indeed, a special attention must be given to deep sea areas which are relatively poorly known comparing to the shallow water levels. For this reason, we suggest the use of developed material such as: the advent of remotely operated vehicules (ROVs), video and photos with very professional material image. Furthermore, multiply samples and fishing gear according to the accurate biotope which allows the covering of the maximum of surface.

The distribution of many taxa from the phylum echnodermata is changing such as for the genus *Echinaster* from the class Asteroidea which show a wide genetic divergence across the atlantic and the Mediterranean. In the present study, this genus as well has shown doubtful morphological characterstic, hence genetic approach and phylobiogeographical analysis are recomunded to verify species idendity. Also, the class Crinoidea presented high morphological variability. Regarding the genus *Antedon*, De Domenico *et al.* (2009) have proved the existence of three different phenotypes of sea lilies species of the family Antenoidea on the Mediterranean Sea which are *Antedon mediterranea, Antedon bifida* and *A. bifida moroccana.* According to same authors, the last species have occurred the Mediterranean basin because of the hydrodynamic marine currents and the upwelling phenomena that favor the proliferation of *A. bifida moroccana* in the Mediterranean Sea and particulary in the Strait of Messina where it tends to replace the endemic species *A. mediterranea.* Recently, this class present changes in the biogeographical area of some species such is the case of the atlantic species *Leptometra celtica* which is colonising the Mediterranean. Same species was recorded on the western basin of the Mediterranean Sea (Koukouras, 2007; Fonseca *et al.* 2014). Hence, the presence of high morphological variabilities between species from same genus, the occurance of new ones and/or the colonisation of new habitat required the usage of molecular tool to identify species taxonomic position and their phylgeographical position within the Mediterranean Sea. Some starfish from the genus *Echinaster* have shown too a doubtful morphological caracteristec with the species from genus *Hacilia*, all of these further aspects worthy for study.

7. DISCUSSION ET CONCLUSIONS GENERALES

La Tunisie occupe une position géographique stratégique en Mer Méditerranée, entre les bassins oriental et occidental et près du détroit de Sicile qui est considérée comme un barrière biogéographique qui sépare la Mer Méditerranée en deux écorégions différentes. Malgré son importance, les études sur la biodiversité marine en Tunisie et, en particulier, sur la faune des échinodermes sont anciennes et rares. De plus, les ressources biologiques marines sont exposées à la surexploitation, à la pollution et au changement climatique, ce qui entraîne la dégradation des écosystèmes et la perte de biodiversité. Une étude du phylum Echinodermata a été réalisée entre 2012 et 2016 le long de la côte nord de la Tunisie. Au cours de laquelle on a étudié l'écologie, la biologie et la classification des échinodermes présents dans cette zone, afin de mettre à jour l'inventaire des échinodermes en Tunisie, prévenir les impacts du changement climatique sur cette faune mégabenthique et analyser les affinités biogéographiques de ce groupe avec les régions et provinces de la Mer Méditerranée et de l'Océan Atlantique. De même, la diversité phylogénétique des espèces d'holothuries du genre *Holothuria* d'origine atlanto-méditerranéenne a été étudiée, à travers l'introduction de l'outil moléculaire le code à barre (DNA Barcoding) comme technique moléculaire d'identification des divergences génétiques des concombres de mer de genre *Holothuria.* Les spicules d'holothuries ont également été analysés pour tester leur similarité morphométrique et moléculaire.

Le chapitre 2 de la présente thèse explore la biodiversité des échinodermes dans le nord de la Tunisie et décrit le matériel collecté durant les différentes campagnes d'échantillonnage entre 2012 et 2016. En outre, il fournit une liste validée des échinodermes collectés avec une description taxonomique et systématique de toutes les espèces. Il a également actualisé l'inventaire du phylum Echinodermata en Tunisie. Les campagnes ont été menées sur les côtes du nord de la Tunisie, depuis la frontière Tuniso-Algérienne jusqu'à la péninsule du Cap-Bon. Des diverses méthodes d'échantillonnage ont été utiliser, la collecte à l'aide d'un quadrat pour les fonds inférieurs à 1 m de profondeur, la drague expérimentale, fournie par le laboratoire, pour les eaux peu profondes de moins de 50 m de profondeur et les chaluts de pêche benthique pour les profondeurs supérieures à 50 m. De plus, les plongées sous-marines ont été utilisées pour les endroits restreints avec des fonds durs. Au total, 1430 individus appartenant aux cinq classes actuelles d'Echinodermata ont été collectés et seulement 45 espèces ont été inventoriées dans le nord de la Tunisie. Ils comprennent 3 espèces de Crinoidea, 15 espèces de étoiles de mer (classe Asteroidea), 10 espèces d'ophiures (classe Ophiuroidea), 8 espèces d'oursin de mer (classe Echinoidea) et 9 espèces appartenant à la classe Holothuroidea. Quatre des espèces identifiées ont été signalées pour la première fois en Tunisie dans le présent travail. Deux d'entre elles sont exclusivement des espèces méditerranéennes (*Asterina pancerii* (Gasco, 1876) et *Leptopentacta tergestina* (M. Sars, 1857)), une est une espèce atlantique (Luidia atlantidea (Madsen, 1950)) et la dernière est une espèce cosmopolite (*Ophiactrice virens* (M. Sars, 1857)). Six autres espèces qui ont été citées dans la partie sud ou la partie est de la Tunisie, ils ont apparu pour la première fois dans la partie nord dans le présent travail. Ces espèces sont les trois étoiles de mer : *Astropecten auranciacus* (Linnaeus, 1758), *Chaetaster longipe* (Retzius, 1805) et *Hacelea atténué* (Gray, 1840), le concombre de mer *Hemioconus syracusnus* (Grube, 1840) et les deux ophiures *Ophiactis savignyi* (Müller et Troschel, 1842) et *Astrospartus mediterraneus* (Risso, 1826). En effet, la situation géographique de la Tunisie lui a confère un statut particulier pour l'étude de la faune marine. Le présent travail a augmenté le nombre d'échinodermes recensés en Tunisie, depuis le premier et le seul inventaire du phylum Echinodermata réalisé par Cherbonnier (1956). En effet, il est nécessaire de promouvoir les efforts et d'acquérir les connaissances sur ce groupe d'invertébrés macrobenthiques.

Le chapitre 3 analyse la population d'échinodermes du nord de la Tunisie et détermine les facteurs écologiques qui expliquent leur distribution en quantifiant et en qualifiant leur composition faunistique depuis la littorale (0m) jusqu'à l'étage bathyal (jusqu'à 100m). De plus, il explore la relation entre les modèles biologiques et écologiques des espèces étudiées. Par conséquent, l'analyse MDS n'a pas détecté de différences entre les trois secteurs étudiés mais entre les localités de chaque secteur. Les mêmes résultats ont également été confirmés par des paires test. Cependant, l'analyse SIMPER a révélé une différence significative entre les localités de (S1 = Nord de la Tunisie) et aucune différence entre les localités de (S2 = Golfe de Tunisie). Alors que, la distribution des classes le long des différents étages benthiques montre une augmentation de la richesse spécifique de la classe Asteroidea et une diminution de la classe Holothuroidea vers les niveaux supérieurs des étages benthiques. En revanche, les classes Crinoidea et Ophiuroidea étaient absentes ou très rares dans les eaux peu profondes. La répartition des échinodermes dans le nord de la mer tunisienne varie en fonction de la profondeur, qui est le principal facteur de regroupement des espèces. Par ailleurs, la composition des échinodermes est modifiée par le gradient bathymétrique où la moyenne était d'environ 100 m. Enfin, les études fauniques et/ou floristique sur la composition de la biodiversité marine sont essentielles pour comprendre l'écologie et la distribution des espèces. Le phylum Echinodermata, qui est bien connu pour son adaptabilité et sa plasticité à diverses conditions environnementales, est l'une des composantes les plus dominantes et les plus diversifiées des communautés marines. La connaissance de la biodiversité marine et, en particulier, celle d'un groupe aussi important que les échinodermes, est très utile pour la gestion de l'écosystème marin et, par conséquent, pour identifier les priorités de conservation de cette embranchement à partir de trois voies différentes : la conservation des espèces pour protéger les espèces menacées et surexploitées ou pour les maintenir en vie et les réintégrer dans leurs habitats naturels. De plus, pour gérer d'espèces à intérêt nutritionnel, écologique et économique ou lutter contre les espèces envahissantes et exotiques qui ont des effets néfastes sur l'écosystème.

D'autre part, la classe Holothuroidea, connue sous le nom de concombre de mer, est l'un des groupes les plus diversifiés du l'embranchement Echinodermata. Il a une large distribution mondiale et il est présent de la zone intertidale aux grands profondeurs et depuis la zone polaire jusqu'à la zone tropicale. Dans de nombreux cas, le genre *Holothuria* a fait l'objet de discussions et sa littérature a toujours été en confusion, car son identification morphologique systématique qui est basée sur « l'étude des spicules » douteuse et incertaine. Le chapitre 4 analyse la morphométrie et la génétique des espèces d'holothuries du genre *Holothuria*, d'origine atlantique et méditerranéenne. Il a évalué la position systématique et la diversité génétique de huit espèces de concombres de mer du genre *Holothuria*, qui sont : *H. (Roweothuria) arguinensis* Koehler and Vaney, 1906, *H. (Holothuria) dakarensis* Panning, 1939, *H. (Vaneyothuria) lentiginosa lentiginosa* Marenzeller von, 1892, *H. (Holothuria) mammata* Grube, 1840, *H. (Roweothuria) poli* Delle Chiaje, 1824, *H. (Platyperona) sanctori* Delle Chiaje, 1823, *H. (Panningothuria) forskali* Delle Chiaje, 1823 and *H. (Holothuria) tubulosa* Gmelin, 1791. En effet, il analyse la variation morphométrique entre les huit espèces en fonction de la forme de leurs spicules, en particulier les boutons et les tours, et discute leur identification systématique en fonction de la similitude entre les analyses morphométriques et moléculaires. Un total de 40 individus du genre *Holothuria* ont été échantillonnés dans des divers endroits de l'océan Atlantique et de la mer Méditerranée. Ensuite, le dendrogramme de similitude des analyses morphométriques a été généré et comparé avec les arbres phylogénétiques des deux gènes mitochondriaux, COI (cytochrome oxydase I) et 16S (ARN ribosomal). Les résultats obtenus ont révélé une concordance entre les deux dendrogrammes phylogénétique et morphométrique chez les espèces atlantiques et méditerranéennes. Les analyses morphométriques des spicules des boutons d'holothuries ont montré une large variability morphologique entre les espèces étudiées. En effet, la répartition géographique de l'holothurie a changé a cause de la baisse tangible de la température et des effets du réchauffement climatique. Pour s'adapter aux nouveaux habitats, la plasticité génotypique et phénotypique des espèces de concombre de mer s'est accrue.

Le chapitre 5 a évalué la structure génétique de l'espèce comestible Holothuria *(Roweothuria) poli* (Delle Chiaje, 1824), dans trois localités différentes de la Méditerranée occidentale, orientale et centrale et dans deux écosystèmes différents : la mer au large et la lagune, afin d'étudier la phylogéographie de cette espèce en Méditerranée et établir le potentiel de ses populations en Tunisie. En effet, cette espèce a montré une grande diversité phylogéographique en mer Méditerranée, en particulier une connectivité génétique élevée entre les bassins méditerranéens central et oriental et une variabilité génétique entre la Méditerranée orientale et occidentale. De plus, elle a révélé une grande différenciation génétique entre l'écosystème lagunaire et la mer. Les écosystèmes côtiers, tels que les lagunes côtières, sont très diversifiés en Tunisie et s'étendent tout au long du littoral tunisien. Ils sont principalement connectés à la mer Méditerranée (Bejaoui *et al.* 2008, 2010 ; Guetat *et al.* 2012) et ils sont bien connus pour leur diversité en faune et en flore avec une importante activité de pêche (Bejaoui *et al.* 2008 ; Ben Mustapha et Hattour 2016 ; Sellem *et al.* 2019). De plus, les populations de *H. poli* sont très abondantes dans cet écosystème et la pêche au concombre de mer est très courante dans les lagunes côtières de Tunisie. En effet, les populations d'holothuries sont exposées à de nombreuses perturbations environnementales, les activités industrielles comme étant la principale source de pollution et de pollution par les eaux usées. En plus de cela, ces écosystèmes en Tunisie sont sous la pression de la pêche illégale. Par conséquence, les concombres de mer sont vulnérables et nécessitent une protection car ils souffrent de la surexploitation et de la pêche illégales (Ben Mustapha et Hattour 2016 ; Sellem *et al.* 2019). Comprendre le flux génétique des populations d'holothuries est très important pour évaluer la gestion des espèces à intérêt commercial et pour améliorer les connaissances sur l'écologie et la biologie des espèces menacées. La connaissance de la connectivité des espèces exploitées permet de prévenir les conséquences écologiques de la surpêche et mis en valeur l'importance de l'aire marine protégée comme mesure de protection de l'espèce (Uthicke et Benzie 2001).

Le dernier chapitre (chapitre 6) analyse la biogéographie du phylum Echinodermata le long des côtes tunisiennes avec les différentes régions et provinces de la mer Méditerranée et de l'océan Atlantique. La diversité des échinodermes a été comparée par pays et par classe. La liste des échinodermes a été actualisée partir de la bibliographie disponible, elle a fourni 79 espèces en Tunisie. Ceux-ci ont été divisés en 18 ordres et 38 familles. Parmi les groupes recensés, la classe Asteroidea était la plus diversifiée avec 22 espèces, suivie par la classe Ophiuroidea (19 espèces), la classe Holothuroidea (18 espèces) et la classe Echinoidea (17 espèces). La dernière classe est Crinoidea avec seulement 3 espèces. Comparé aux régions voisines de la côte méditerranéenne du Maghreb, le phylum Echinodermata est assez diversifié en Tunisie. La richesse spécifique de l'embranchement Echinodermata est inégalement répartie entre les trois golfes tunisiens : le golfe de Tunis au nord, le golfe d'Hammamet à l'est et le golfe de Gabès au sud. La plus grande richesse en espèces avec 68 espèces a été attribuée à la zone nord, qui représente 84% du total des espèces en Tunisie, dont la plupart ont des affinités atlanto-méditerranéennes avec une seule espèce atlantique *(Luidea atlantidea).* Cependant, seules 36 espèces de la partie centre-est de la Tunisie représentent 51% du total et 63 espèces des côtes sud (82%). En fait, les analyses biogéographiques ont montré que la richesse en espèces diminuait du nord au sud du pays et de l'ouest à l'est de la Méditerranée, avec une prédominance d'espèces cosmopolites dans toutes les régions. La mise à jour des échinodermes enregistrés en Tunisie révèle une importante diversité en nombre et en espèces par rapport à la région et aux provinces voisines. En effet, les côtes tunisiennes sont sous la colonisation d'espèces d'origine mixte. La situation de la Tunisie en tant que jonction entre les basins occidentale et orientale de la Méditerranée, permet la transition des espèces atlantiques au nord et les migrantes « lessipsiennes » au sud.

L'utilisation de la technique code a barre ou « ADN Barcoding » sur les espèces des échinodermes semble être une méthode très fiable, rapide et efficace pour l'identification des espèces. L'un des avantages de l'utilisation du gène COI est que les amorces universelles de ce gène sont très robustes (Folmer *et al.* 1994 ; Zhang et Hewitt 1997). Plusieurs fois, et lorsque la base de données génétique est cohérente, cette technique présente des avantages en termes de précision pour l'identification des espèces par rapport aux observations morphologiques. Cette méthode a été testée avec succès sur différents organismes marins, également sur des échinodermes (Jefri *et al.* 2015 ; Prehadi *et al.* 2015 ; Layton *et al.* 2016 ; Madduppa *et al.* 2016). Le manque des informations sur le phylum des échinodermes en Tunisie, est une perte pour la connaissance de la biodiversité marine.

VIII. REFERENCES

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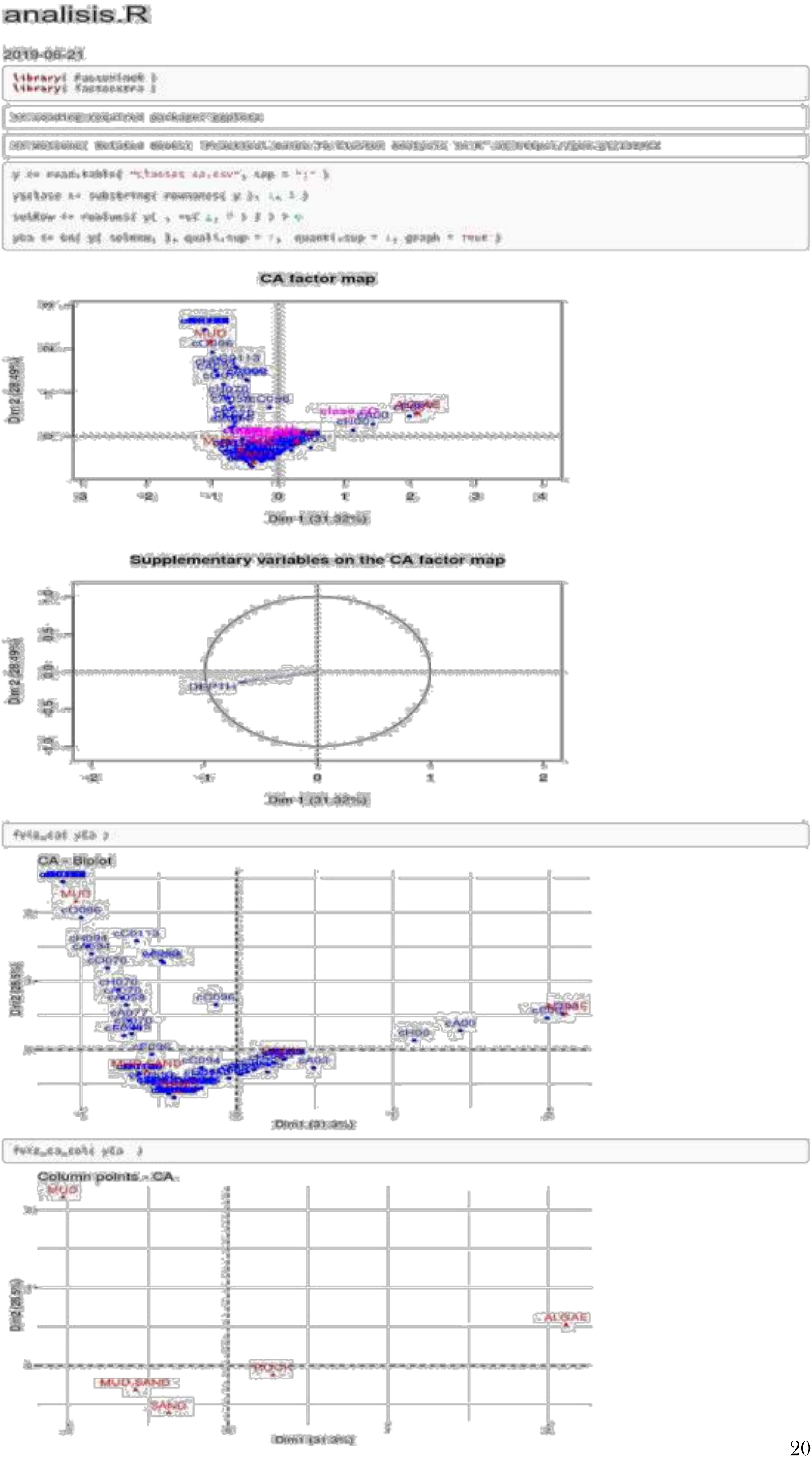
ANNEXES

Annex 1



analisis.R

2019-06-21

```
library( FactoMineR )
library( factoextra )
```

```
## Loading required package: ggplot2
```

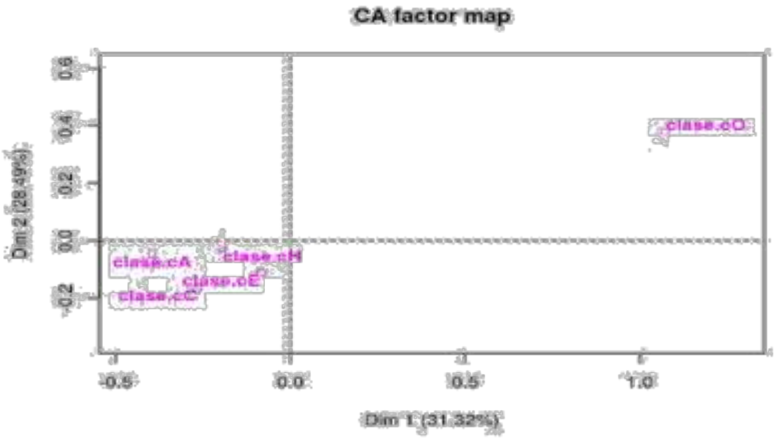
```
## Welcome! Related Books: `Practical Guide To Cluster Analysis in R` at https://goo.gl/13EFCZ
```

```
y <- read.table( "clases ca.csv", sep = ";" )
yclase <- substring( rownames( y ), 1, 1 )
selRow <- rowSums( y[ , -c( 1, 8 ) ] ) > 0
yca <- CA( y[ selRow, ], quali.sup = 7, quanti.sup = 1, graph = TRUE )
```

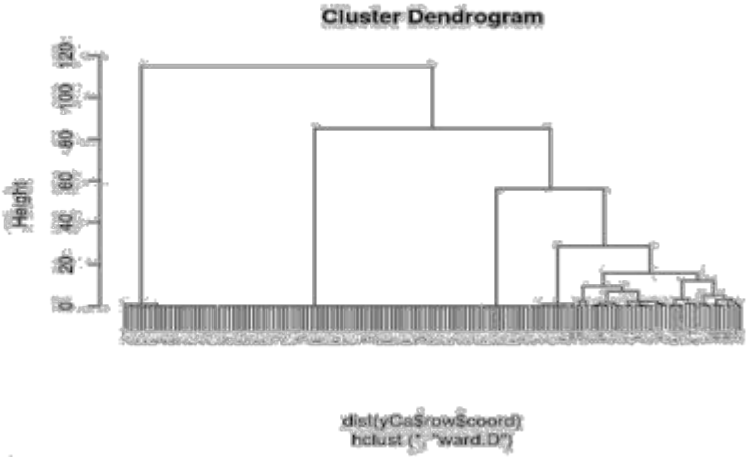
```
fviz_ca( yca )
```

```
fviz_ca_col( yca )
```

```
plot.CA( yCa, invisible = c( "row", "col", "row.sup", "col.sup" ) )
```



```
rowClus <- hclust( dist( yCa$row$coord ), method = "ward.D" )
plot( rowClus, cex = 0.1 )
```



```
clasRow <-  cutree( rowClus, 6 )

clasInfo <- data.frame( clasRow = clasRow, row = rownames( y )[ selRow ], clase = y$clase[ selRow ] )

table( clasInfo$clasRow, clasInfo$clase )
```

```
## 
##     cA cC cE cH cO
##   1  4  9  5  5  1
##   2 15 14 22 24 13
##   3  8  4  3  4  3
##   4  8  3  7  6  6
##   5  3  1  4  5  1
##   6  5  2  3  1  3
```

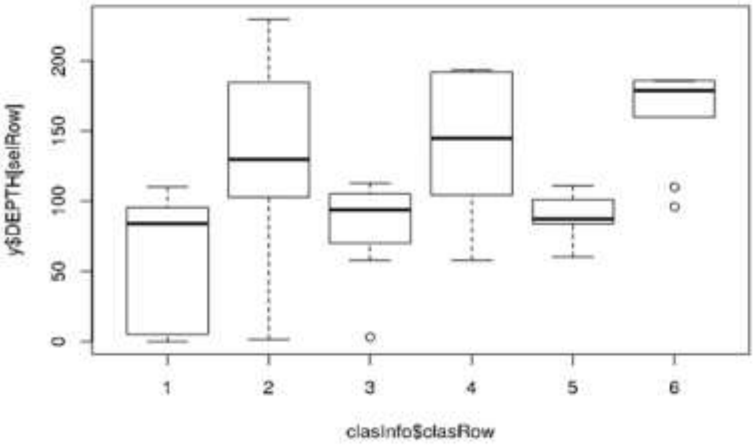
```
clasInfo[ clasInfo$clasRow == 1, ]
```

```
##         clasRow    row clase
## cE00          1   cE00    cE
## cH00          1   cH00    cH
## cA00          1   cA00    cA
## cO00          1   cO00    cO
## cH03          1   cH03    cH
## cA03          1   cA03    cA
## cE05          1   cE05    cE
## cH05          1   cH05    cH
## cE051         1  cE051    cE
## cC060         1  cC060    cC
## cE060         1  cE060    cE
## cC070         1  cC070    cC
## cC077         1  cC077    cC
## cC084         1  cC084    cC
## cC085         1  cC085    cC
## cA085         1  cA085    cA
## cC090         1  cC090    cC
## cC095         1  cC095    cC
## cE095         1  cE095    cE
## cH095         1  cH095    cH
## cE096         1  cE096    cE
## cC010         1  cC010    cC
## cE010         1  cE010    cE
## cH010         1  cH010    cH
## cA010         1  cA010    cA
## cE0105        1 cE0105    cE
## cC0110        1 cC0110    cC
```

```
clasInfo[ clasInfo$clasRow == 1,  ]
```

```
##        clasRow    row clase
## cE00         1   cE00    cE
## cH00         1   cH00    cH
## cA00         1   cA00    cA
## cO00         1   cO00    cO
## cH03         1   cH03    cH
## cA03         1   cA03    cA
## cE05         1   cE05    cE
## cH05         1   cH05    cH
## cE051        1  cE051    cE
## cC060        1  cC060    cC
## cE060        1  cE060    cE
## cC070        1  cC070    cC
## cC077        1  cC077    cC
## cC084        1  cC084    cC
## cC085        1  cC085    cC
## cA085        1  cA085    cA
## cC090        1  cC090    cC
## cC095        1  cC095    cC
## cE095        1  cE095    cE
## cH095        1  cH095    cH
## cE096        1  cE096    cE
## cC010        1  cC010    cC
## cE010        1  cE010    cE
## cH010        1  cH010    cH
## cA010        1  cA010    cA
## cE0105       1 cE0105    cE
## cC0110       1 cC0110    cC
```

```
boxplot( y$DEPTH[  selRow ] ~ clasInfo$clasRow )
```



```
boxplot( y$DEPTH[  selRow ] ~ clasInfo$clase )
```