

# Taxonomic status and phylogenetic relationship of some Algerian shallow-water sea cucumber species (Holothuroidea: Echinodermata) deduced from mitochondrial DNA sequences

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**Abstract**— The present study focuses on the taxonomic revision and phylogeny of some shallow-water Algerian sea cucumber species belonging to the genus *Holothuria* [the two morphotypes (A & B) of *Holothuria (Holothuria) tubulosa* (Gmelin, 1791); *Holothuria (Roweothuria) poli* (Delle Chiaje, 1824) and *Holothuria (Roweothuria) arguinensis* (Koehler et Vaney, 1906)]. The nucleotide sequences of the 16S rRNA gene obtained by [1] were used to construct the phylogenetic tree of the studied specimens. Our results showed that the two morphotypes (A & B) of *Holothuria (H.) tubulosa* are genetically separated. We also reported a close genetic relationship between the two morphotypes of *Holothuria (H.) tubulosa* (A & B) and *Holothuria (R.) arguinensis*. *Holothuria (R.) poli* is the most divergent species and is in a paraphyletic position with the other studied specimens. Sequence analyses of the 16S rRNA gene revealed the presence of 58 polymorphic sites: 79.25% of these sites are parsimoniously informative and 20.75% are singletons. The means of haplotype diversity (Hd) and nucleotide diversity ( $\pi$ ) were very high.

**Keywords**— *Echinodermata*, *Holothuria*, *Phylogenetic systematics*, *16S rRNA*, *Haplotypes*, *Algerian basin*.

## INTRODUCTION

Aspidochirotid holothurians, commonly known as "sea cucumbers", are marine invertebrates belonging to the echinoderm group. There are over 1400 species occurring from the intertidal to the deepest oceanic trenches [2]. Holothurians are among the main sediment bioturbators in marine ecosystems [3]. They ingest the soft substratum and remove the organic matter which is necessary for their growth. This process prevents the accumulation of organic matter in the marine sediment and could help to control the effect of

pathogenic agents [4]. The ecological role of the holothurians species is largely well known by scientists, but there is a lack of informations concerning the taxonomy of several groups of species belonging to the genus *Holothuria* [5]. In fact, this genus contains individuals with few taxonomically informative morphological characteristics and with a high degree of intraspecific phenotypic diversity [6].

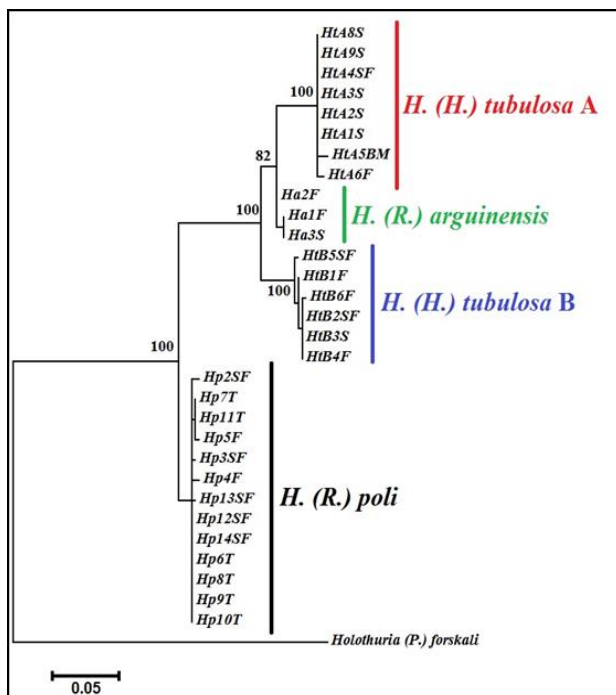
## MATERIAL AND METHODS

The present study is a part of a further systematic review for data already obtained by [1] on the phylogeny of some aspidochirotid holothurians species of the Algerian coast. Two Mediterranean species [*Holothuria (Holothuria) tubulosa*; *Holothuria (Roweothuria) poli* and one exotic species originating from Atlantic Ocean [*Holothuria (Roweothuria) arguinensis* have been considered. Most "sea cucumbers" individuals were collected from three stations located in the centre of the Algerian coast [Sidi-Fredj (2° 50.55'E-36° 45.45'N), Tamentefoust (3° 14.00'E-36° 48.33'N) and Figuier-plage (3° 30.92'E-36° 47.05'N)]. Sampling was also carried out in two other stations, the first located in the western part of the Algerian coast [Stidia (0° 1.11'O-35° 49.85'N)] and the second in the French coast [Banyuls sur mer (3° 7.87'E-42° 28.95'N)]. The samples were collected by scuba diving between 0.5 and 10m depth [1]. The sequences data obtained from the portion of mitochondrial gene 16S rRNA by [1] were classified on the software "BioEdit" [7] and aligned using the extension "CLUSTAL W" integrated in "BioEdit" [8]. After alignment, the sequences were classified on the "Mega 5" software [9]. Relationships between individuals or populations are visualized through phylogenetic tree using the Maximum

Likelihood method [10]. We used the software "jModeltest2.1.3" to find the best model of nucleotide substitutions. The Kimura model with 2 parameters (K2P + gamma distribution) was the most adequate. For evaluating the robustness of the root nodes of the reconstituted tree, we performed 100 Bootstrap replicas. The sequence of *Holothuria (Platyperona) forskali* obtained from "Genbank" was used as an out group. Sequencing of a 411Pb fragment along the mitochondrial rRNA16S gene of the studied species revealed the existence of 58 polymorphic sites; 79.25% of these sites are parsimoniously informative and 20.75% are singletons. For this, the haplotype diversity (Hd), nucleotide diversity ( $\pi$ ) and McDonald's-Kreitman test [11] were estimated by the DNASP 5.10 program [12].

## RESULTS

The obtained phylogenetic tree present four clades with high Bootstrap values (Fig. 1). The two morphotypes of *Holothuria (H.) tubulosa* (A & B) are genetically separated. *Holothuria (R.) arguinensis* is much closer to *H. (H.) tubulosa* (morphotype A) than *H. (R.) poli* (Fig.1). Both varieties of *H. (H.) tubulosa* (A & B) and *H. (R.) arguinensis* are related and monophyletic species. *Holothuria (R.) poli* appears to be the most divergent and distant species (Fig. 1). The means values of haplotype diversity (Hd) and nucleotide diversity ( $\pi$ ) are presented in Table I. The results of McDonald's-Kreitman's test are summarized in Table II.



**Figure 1:** Phylogenetic tree established for the studied aspidochirotid holothurians species by the Maximum likelihood method [HtA: *H. (H.) tubulosa* A; HtB: *H. (H.) tubulosa* B; Ha: *H. (R.) arguinensis*; Hp: *H. (R.) poli*. S: Stidia; F: Figuiet-Plage; T: Tamentefoust; BM: Banyul-sur-Mer] (100 Bootstrap replicas).

**Table I:** Genetic diversity of the studied aspidochirotid holothurians species. NS: number of sequences, h: number of haplotype, Hd: haplotype diversity,  $\pi$ : nucleotide diversity.

Species	NS	h	Hd	$\pi$
<i>H. (R.) poli</i>	14	8	0.82	0.004
<i>H. (H.) tubulosa</i> A	8	4	0.64	0.004
<i>H. (H.) tubulosa</i> B	6	4	0.80	0.005
<i>H. (R.) arguinensis</i>	3	3	1.00	0.005

**Table II:** The McDonald-Kreitman (1991) test between the studied holothurians species.

Species	KN/KS	ON/OS	p
<i>H. (R.) poli</i> and <i>H. (R.) arguinensis</i>	3.16	2.00	0.69
<i>H. (R.) poli</i> and <i>H. (H.) tubulosa</i> A	2.30	2.50	1.00
<i>H. (R.) poli</i> and <i>H. (H.) tubulosa</i> B	2.50	3.33	0.70
<i>H. (R.) arguinensis</i> and <i>H. (H.) tubulosa</i> A	2.33	1.00	0.63
<i>H. (R.) arguinensis</i> and <i>H. (H.) tubulosa</i> B	0.62	1.33	0.64
<i>H. (H.) tubulosa</i> B and <i>H. (H.) tubulosa</i> A	1.37	2.00	0.70

## DISCUSSION

Along the history of the classification of the aspidochirotid holothurians species, taxonomists were sure and all agreed that *Holothuria (H.) tubulosa*, the best-known and the most common species in the Mediterranean Sea, presents only one well separated species. The obtained phylogenetic tree indicates that *Holothuria (H.) tubulosa* is represented by two varieties of species: The first is named in the present work classical *Holothuria (H.) tubulosa* A, because of its morphological characteristics which are very similar to those already described by [13] and the second named in the present work *Holothuria (H.) tubulosa* B, which has different morphological characteristics and could be considered as a new to science. According to the obtained results, these two species varieties are genetically distinct (Fig. 1). This result has already been reported by [1] and [14]. These differences do not concern only the obtained molecular datas, but also the anatomical, morphological and endoskeletal datas [1-15]. *Holothuria (R.) arguinensis* is much closer to both populations of *H. (H.) tubulosa* (A & B) than to *Holothuria (R.) poli* (Fig. 1). Reference [16] reported a very close relationship between *H. (H.) tubulosa* and *H. (R.) arguinensis* and this is the case in this study. *Holothuria (R.) arguinensis* is an Atlantic exotic species not endemic to the Mediterranean Sea. This species is reported for the first time in the Algerian coast by [17]. The phylogram (Fig. 1) also illustrates that *Holothuria (R.) poli* is the most divergent species. Our results are in contradiction with previous works, which are based only on the endoskeletal criterion. More precisely, the *Holothuria* and *Roweothuria* subgenera do not appear as reciprocally monophyletic groups [18-19]. According to our results both subgenus are paraphyletic. The phylogenetic tree gives us an indication of

the subgenus *Platyperona* (Fig. 1). This subgenus is among the oldest and most divergent lineages in the genus *Holothuria* [16], which is in agreement with the work of [20]. This justifies its choice as an external group. Their basal position on the phylogenetic tree (Fig. 1) is in agreement with the plesiomorphic calcareous rings found in individuals of this genus [16]. Calcareous rings are very similar to those observed in the fossils reported by [21]. This supports the idea of treating it as genera and not as subgenera. The low nucleotide diversity ( $\pi$ ) reflects the presence of a dominant haplotype and many related haplotypes, which explains the high value of haplotypic diversity ( $H_d$ ) (Table I). Concerning the McDonald's-Kreitman's test, the p-values were not significant (Table II). We noted a Darwinian selection between taxa, more specifically between *H. (R.) poli* and *H. (R.) arguinensis* and between *H. (H.) tubulosa* A and *H. (R.) arguinensis* (Tab. II). A retention of an ancestral polymorphism between taxa in the case where  $KN/KS < \Theta N/\Theta S$  (Tab. II).

## CONCLUSION

The present study allowed to reevaluate the taxonomic status of some aspidochirotid holothurians species of the Algerian coast. The obtained results indicate that *Holothuria (H.) tubulosa*, the most common species in the Mediterranean Sea, has two distinct species that have been noted in the present work as morphotype A & morphotype B. The morphotype "A" corresponds to the classical *Holothuria (Holothuria) tubulosa* previously described by [13]; while the morphotype "B" should correspond to a new species whose molecular characteristics are different from those of *Holothuria (H.) tubulosa* A. We also reported a close relationship between *Holothuria (H.) tubulosa* A and *Holothuria (R.) arguinensis* which corroborates with other studies carried out on the genus *Holothuria* in the Mediterranean Sea and the Atlantic Ocean. The genetic tests showed very high haplotypic and nucleotide diversities.

## References

- [1] K. Mezali, "Phylogénie, systématique, dynamique des populations et nutrition de quelques espèces d'Holothurie aspidochirotes (*Holothuroidea: Echinodermata*) inféodées aux herbiers de Posidonies de la région Algérienne". PhD thesis, Université des Sciences et de la Technologie Houari Boumediene, Algérie, 208 pp.
- [2] A. M. Kerr and J. Kim, "Phylogeny of Holothuroidea (Echinodermata) inferred from morphology," *Zoological Journal of the Linnean Society*, vol. 133, no. 1, pp. 63-81, 2001/09/01/ 2001.
- [3] C. Massin, "Food and feeding mechanism: Holothuroidea".1982a. Pages 43–55 in M. Jangoux and J.M. Lawrence, eds. Echinoderm nutrition. A. A. Balkema, Rotterdam.. 654 p.
- [4] S. Uthicke, "Sediment bioturbation and impact of feeding activity of *Holothuria (Halodeima) atra* and *Stichopus chloronotus*, two sediment feeding holothurians, at Lizard Island, Great Barrier Reef," *Bulletin of Marine Science*, vol. 64, no. 1, pp. 129-141, 1999.

- [5] K. Mezali, "Some insights on the phylogeny of Algerian shallow-water sea cucumbers species (Holothuroidea: Aspidochirotida)". *SPC Béche-de-mer Information Bulletin* 11, 45–47. 2011.
- [6] R. Clouse, D. Janies, and A. M. Kerr, "Resurrection of *Bohadschia bivittata* from *B. marmorata* (Holothuroidea: Holothuriidae) based on behavioral, morphological, and mitochondrial DNA evidence," *Zoology*, vol. 108, no. 1, pp. 27-39, 2005/03/15/ 2005.
- [7] T. A. Hall, "BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT". *Nucl. Acids. Symp. Ser.* 41: 95-98. 1999
- [8] D. G. Higgins and A. J. Bleasby, R. Fuchs, "CLUSTAL V: improved software for multiple sequence alignment". *CABIOS*, 8 : 189-191. 1992
- [9] K. Tamura, N. Peterson D Fau - Peterson, G. Peterson N Fau - Stecher, M. Stecher G Fau - Nei, S. Nei M Fau - Kumar, and S. Kumar, "MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods," (in eng), no. 1537-1719 (Electronic), 2011.
- [10] L. L. Cavalli-Sforza and A. W. F. Edwards, "Phylogenetic analysis. Models and estimation procedures," *American Journal of Human Genetics*, vol. 19, no. 3 Pt 1, pp. 233-257, 1967.
- [11] J. H. McDonald and M. Kreitman, "Adaptive protein evolution at the Adh locus in *Drosophila*," *Nature*, vol. 351, p. 652, 06/20/online 1991
- [12] J. Rozas, P. Librado, J.C. Sánchez-DelBarrio, X. Messeguer and R. Rozas, "DNA SP (DNA Sequence Polymorphism v5.10.01)". *Departament de Genètica, Universitat de Barcelona*. Spain. 2010
- [13] R. Koehler, "Echinodermes". Faune de France, 1, 1–210. 1921
- [14] T. Mansouri, "Phylogénie et phylogéographie de quelques espèces d'holothuries aspidochirotes de la côte algérienne (Ouest et centre)". Mémoire de Magister. Université d'Abdelhamid Ibn Badis, Mostaganem, Algérie, 119 p. 2015
- [15] K. Mezali and G. Paulay, "Species limits and cryptic species in Mediterranean Sea cucumber (Holothuroidea: Echinodermata)". Mediterranean Sea Grass Workshop 2009 – MSW09, Hvar, Croatie, 06-10 September 2009.
- [16] G. H. Borrero-Pérez, J. Gómez-Zurita, M. González-Wangüemert, C. Marcos, and A. Pérez-Ruzafa, "Molecular systematics of the genus *Holothuria* in the Mediterranean and Northeastern Atlantic and a molecular clock for the diversification of the Holothuriidae (Echinodermata: Holothuroidea)," *Molecular Phylogenetics and Evolution*, vol. 57, no. 2, pp. 899-906, 2010/11/01/ 2010.
- [17] K. Mezali and A. S. Thandar, "First record of *Holothuria (Roweothuria) arguinensis* (Echinodermata: Holothuroidea: Aspidochirotida: Holothuriidae) from the Algerian coastal waters," *Marine Biodiversity Records*, vol. 7, p. e40, 2014, Art. no. e40.
- [18] F.W.E. Rowe, "A review of the family Holothuriidae". Bulletin British Museum Natural History (Zoology) 18(4), 117–170. 1969
- [19] A.S. Thandar, "A new subgenus of *Holothuria* with a description of a new species from the south-east Atlantic Ocean". *Journal of Zoology* 215, 47-54. 1988
- [20] P. M. O'Loughlin, G. Paulay, D. VandenSpiegel, and Y. Samyn, "New *Holothuria* species from Australia (Echinodermata: Holothuroidea: Holothuriidae), with comments on the origin of deep and cool holothuriids," *Memoirs of Museum Victoria*, vol. 64, pp. 35-52, 2007.
- [21] M. Reich, "Aspidochirote holothurians (*Echinodermata*) from the Middle Triassic of southern Germany".2004. In: Heinzeller, T., Nebelsick, J. (Eds.), Proceedings of the 11th International Echinoderm Conference. Balkema, Netherlands, pp.485–486.