Molecular Phylogenetics and Evolution 57 (2010) 899–906

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Short Communication

Molecular systematics of the genus *Holothuria* in the Mediterranean and Northeastern Atlantic and a molecular clock for the diversification of the Holothuriidae (Echinodermata: Holothuroidea)

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ARTICLE INFO

Article history:
Received 6 August 2009
Revised 6 August 2010
Accepted 17 August 2010
Available online 4 September 2010

Keywords:
Phylogeny
rrnL
Cox1
mtDNA
Holothuria
Roweothuria
Holothuriidae
Echinodermata

ABSTRACT

This work investigates the systematics of the genus *Holothuria* in the Mediterranean Sea and Northeastern Atlantic in the light of a wider molecular phylogenetic hypothesis of Holothuriidae, and it also provides a time-scale for the family diversification using mitochondrial markers and the molecular clock hypothesis. The subgenera *Holothuria* and *Roweothuria* are retrieved as paraphyletic. At least four separate lineages, with quite different time frameworks were identified. There are at least three species with an apparent long evolutionary history, *H. forskali, H. sanctori* and *H. impatiens* and six species belonging to *Holothuria, Roweothuria* and *Vaneyothuria*, which have diverged relatively recently.

1. Introduction

The sea cucumber family Holothuriidae is the most diverse in the Holothuroidea class, and includes predominantly tropical, shallow water animals; only a few species inhabit warm temperate environments. The family includes five extant genera representing 187 described species: *Actinopyga* Bronn, 1860 (17 species), *Bohadschia* Jaeger, 1833 (11), *Holothuria* Linnaeus, 1767 (150), *Labidodemas* Selenka, 1867 (8), and *Pearsonothuria* Levin et al., 1984 (1) (Samyn et al., 2005, 2006; O'Loughlin et al., 2007). The genus *Holothuria* is currently recognized to include 18 subgenera and is the only holothuriid present in the Mediterranean Sea (MS) and Northeastern Atlantic (NEA), where thirteen species from seven of these subgenera occur. Two of these subgenera, *Holothuria* and *Roweothuria* Thandar, 1988, including six out of the thirteen species, are restricted to the eastern Atlantic, MS and Red Sea, with exception of *H.* (*H.*) *dakarensis* Panning, 1939, which was also recorded from the Gulf of Mexico (Pawson and Shirley, 1977). The subgenus *Panningothuria* Rowe, 1969 was considered a monotypic subgenus exclusive of this area, however, a second species from Australia has been recently described (O'Loughlin et al., 2007). The other four subgenera, *Platyperona* Rowe, 1969, *Vaneyothuria* Deichmann, 1958, *Thymiosycia* Pearson, 1914, and *Semperothuria* Deichmann, 1958, are widely distributed, including one or two species in the area of interest. Some of these species are restricted to the eastern Atlantic; however other ones are common in the western Atlantic (recorded in the Eastern Atlantic only in the Cape Verde Islands (Pérez-Ruzafa et al., 1999)) or reported as circum-tropical.

Sea cucumber fishing is increasing globally mainly for export, and there is overexploitation due to the demand from Asian markets (Toral-Granda et al., 2008). New economically important species include the Mediterranean *H. mammata, H. tubulosa*, *H. polii* and *H. arenicola* (Aydin, 2008; Abdel Razek et al., 2007). Ecological and commercial importance of sea cucumbers has recently spurred systematics work in this group, mainly carried out by the Aspidochirote Working Group, engaged in the taxonomic revision of the Aspidochirotida worldwide (http://guammarinelab.com/peetcukes/index.html). Nevertheless, modern treatises about the higher level systematics of Holothuriidae are scarce. The relationships of the five genera were documented from morphological (Samyn et al., 2005) and molecular (Kerr et al., 2005; Kamarul Rahim

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doi:10.1016/j.ympev.2010.08.019

et al., 2006) studies. However, the relationships among subgenera of *Holothuria* were only hypothesized by Rowe (1969), although some new information was presented by Samyn et al. (2005) using morphological data, with molecular information still being scarce.

Sea cucumbers are soft animals which leave a poor fossil record and often fail to provide useful taxonomic information at low hierarchical levels. These circunstances preclude a solid reconstruction of the time-scale for the evolution of these animals, so that the timing of the diversification of holothuriids using DNA sequence data and the molecular clock hypothesis constitutes an appealing, almost unavoidable strategy. Despite the few fossils recorded for the family, their age (the earliest one is from the middle Triassic (approximately 237 and 245 mya; Reich, 2004) and the seemingly ancestral Tethyan distribution of the group suggest the antiquity of several groups within the Holothuriidae (Kerr et al., 2005).

In this study, we examine the systematics of MS and NEA species of *Holothuria* in the light of a wider phylogenetic hypothesis of the Holothuriidae and provide a time-scale for the family diversification presenting a molecular clock calibration.

2. Material and methods

We sampled nine species out of thirteen distributed in the MS and NEA, including all currently recognized subgenera ocurring in this area, except *Semperothuria*. We also used all sequence data of Holothuriidae available in GenBank, analyzing a total of 25

Table 1
Species of Holothuriidae included in this study, with references, GenBank accession number and sample location. Voucher numbers are also given for individuals sequenced in this study and deposited at the University of Murcia (Ho).

Species	Reference	GenBank Accession number		Voucher/location
		rrnL	cox1	
Holothuria (*Holothuria*) *mammata*	1/This study	EU191949	GQ214743	Ho1293/Canary Islands, Spain (9)
Holothuria mammata	1/This study	EU191957	GQ214744	Ho1804/Azores Islands, Portugal
Holothuria mammata	This study	GQ21 4729	GQ214745	Ho1824/Cabo de Palos, Spain
Holothuria mammata	1/This study	FJ231190	GQ214746	Ho1855/Algarve, Portugal
Holothuria mammata	This study	GQ21 4730	GQ214747	Ho1873/Gerona, Spain
Holothuria (*Holothuria*) *tubulosa*	1/This study	FJ231192	GQ214748	Ho1828/Cabo de Palos, Spain
Holothuria tubulosa	This study	GQ21 4731	GQ214749	Ho1870/Aguilas, Spain
Holothuria tubulosa	1/This study	EU191974	GQ214750	Ho1869/Aguilas, Spain
Holothuria tubulosa	This study	GQ214732	GQ214751	Ho1884/Gerona, Spain
Holothuria (*Holothuria*) *dakarensis*	1/This study	EU191979	GQ214752	Ho321/Cape Verde Islands (9)
Holothuria dakarensis	1	EU191980	no	Ho312/Cape Verde Islands (9)
Holothuria (*Roweothuria*) *arguinensis*	This study	GQ214734	GQ214754	Ho1191/Canary Islands, Spain (9)
Holothuria arguinensis	This study	GQ214735	GQ214755	Ho1841/Algarve, Portugal
Holothuria arguinensis	This study	GQ214736	GQ214756	Ho1741/Canary Islands, Spain
Holothuria arguinensis	This study	GQ214737	GQ214757	Ho1223/Canary Islands, Spain (9)
Holothuria (*Roweothuria*) *polii*	This study	EU191981	GQ214759	Ho1835/Cabo de Palos, Spain
Holothuria polii	This study	GQ214738	GQ214758	Ho1838/Mar Menor, Spain
Holothuria (*Vaneyothuria*) *lentiginosa lentiginosa*	This study	GQ214733	GQ214753	Ho1021/Alboran Island, Spain
Holothuria (*Thymiosycia*) *impatiens*	This study	GQ214739	GQ214760	Ho273/Cabo de Palos, Spain
Holothuria (*Panningothuria*) *forskali*	This study	GQ214740	GQ214761	Ho1856/Algarve, Portugal
Holothuria forskali	1 /This study	EU191983	GQ214762	Ho1857/Algarve, Portugal
Holothuria (*Panningothuria*) *austrinabassa*	2	EU220797	EU220818	West Australia
Holothuria (*Platyperona*) *sanctori*	This study	GQ214741	GQ214763	Ho1451/Canary Islands, Spain (9)
Holothuria sanctori	This study	GQ214742	GQ214764	Ho1833/Cabo de Palos, Spain
Holothuria (*Platyperona*) *excellens*	2	EU220796	EU220817	Palau Island
Holothuria (*Halodeima*) *atra*	2	EU220799	EU220820	Hawaii island
Holothuria (*Halodeima*) *edulis*	2	EU220811	EU220830	Okinawa, Japan
Holothuria (*Halodeima*) *floridana*	2	EU220803	EU220822	Florida
Holothuria (*Halodeima*) *grisea*	2	EU220800	No	Florida
Holothuria (*Halodeima*) *kefersteini*	2	EU220801	No	Panama
Holothuria (*Halodeima*) *mexicana*	2	EU220802	EU220821	Belize
Holothuria (*Halodeima*) *nigralutea*	2	EU220805	EU220824	West Australia
Holothuria (*Halodeima*) *signata*	2	EU220812	EU220831	Rangiroa, French Polynesia
Holothuria (*Microthele*) *fuscogilva*	3	No	AY700769	Fiji Islands
Holothuria (*Microthele*) *nobilis*	3	No	AY1 76775	La Reunion
Holothuria (*Microthele*) *whitmaei*	3	No	AY1 76777	Fiji Islands
Holothuria (*Microthele*) *whitmaei*	4	AY509147	No	Ilot Maitre, New Caledonia.
Holothuria (*Metriatyla*) *scabra*	4	AY509130	No	Ilot Maitre, New Caledonia.
Holothuria (*Metriatyla*) *scabra var. versicolor*	4	AY509145	No	Ilot Maitre, New Caledonia.
Holothuria (*Mertensiothuria*) *leucospilota*	5	AY338419	No	Guam, Mariana Islands
Holothuria (*Mertensiothuria*) *leucospilota*	6	FJ223871	No	Tiom an Island, Malaysia
Actinopyga mauritania	5	AY33841 4	No	Guam, Mariana Islands
Actinopyga miliaris	3	No	AY700773	Linnet Reef, Great Barrier Reef
Actino pygaobesa	2	EU220794	EU220815	Hawaii island
Bohadschia marmorata	7	AY574877	AY574883	Pohnpei Island, Micronesia
Bohadschia argus	7	AY574870	AY574878	Pohnpei Island, Micronesia
Labidodemas semperianum	5	AY338420	No	Guam, Mariana Islands
Pearsonothuria graeffei	5	AY338421	No	Guam, Mariana Islands
Pearsonothuria graeffei	7	AY574868	No	Chuuk Islands, Micronesia
Isostichopus macroparentheses	5	AY33841 5	No	Guana Islands, British Virgin Islands
Isostichopus fuscus	8	No	AF486429	Coast of Jalisco, Mexico
Stichopus ocellatus	2	EU220793	EU220814	Papua New Guinea

Refs. (1) Borrero-Pérez et al. (2009); (2) O'Loughlin et al. (2007); (3) Uthicke et al. (2004); (4) Uthicke et al. (2005); (5) Kerr et al. (2005); (6) Kamarul Rahim et al. (2006); (7) Clouse et al. (2005); (8) Solís-Marín et al. (2004); (9) Individuals collected during Macaronesia 2000 project (Museo de la Naturaleza y el Hombre de Santa Cruz de Tenerife, Canary Islands, Spain).