Some insights on the phylogeny of Algerian shallow-water sea cucumber species (Holothuroidea: Aspidochirotida)

Karim Mezali[1]

Abstract

Phylogenetic analyses from the fraction of mitochondrial gene (16S mDNA) sequenced for 26 individuals (in general, five species sampled in various shallow-water locations along the Algerian coast) clarified taxonomic uncertainties. This study shows that: 1) the two color morphs of *Holothuria* (*P.*) *sanctori* that have been debated in some of the literature form a well defined clade; 2) *Holothuria* (*H.*) *stellati* whose confusion has always acknowledged, is genetically distinct from the other species; 3) *Holothuria* (*H.*) *tubulosa*, the most common species, and the "best known" species in the Mediterranean Sea, forms a clade with two well separated populations. Few specimens of holothurians analyzed in our collection have given unusual DNA sequences. However, it is clear that one specimen will probably represent either another species previously unknown, or a hybrid between two known species — *H.* (*R.*) *polii* and *H.* (*H.*) *stellati*.

Introduction

Sea cucumbers (Holothuroidea) in the order Aspidochirotida are a conspicuous and diverse group in marine ecosystems. They inhabit soft sediment and Mediterranean *Posidonia oceanica* meadows (Francour 1990; Coulon and Jangoux 1993; Mezali 2008). They provide important ecosystem services by enhancing nutrient cycling and local productivity in oligotrophic carbonate sediments through their bioturbation and deposit feeding activities (Uthicke 1999). They are also fished for beche-de-mer production (Conand and Byrne 1993; Toral-Granda 2008). Despite being large and often the most dominant mobile invertebrates in Mediterranean shallow-water areas, the taxonomy of many specimens collected from Algerian shallow-water areas is uncertain. This is due to the difficulty in applying traditional taxonomic characters (e.g. body profile, skeleton morphology). We undertook a phylogenetic analysis of sampled holothuroids using sequence data for a mitochondrial gene (16S rDNA). The aims of this study were to provide a systematic revision of the Algerian aspidochirotid holothurians, using molecular phylogenetic systematic methods.

Materials and methods

Sampling

Most holothurians species were collected by scuba diving and skin diving in 2006 across the Bay of Algiers (Tamentefoust) and the Bay of Bou-Ismail (Sidi-Fredj) at depths ranging between 1 m and 20 m (Fig. 1). Three additional stations (not shown in Fig. 1) were also explored during 2006 (Stidia and Sidi-Medjdoub in the Bay of Mostaganem and

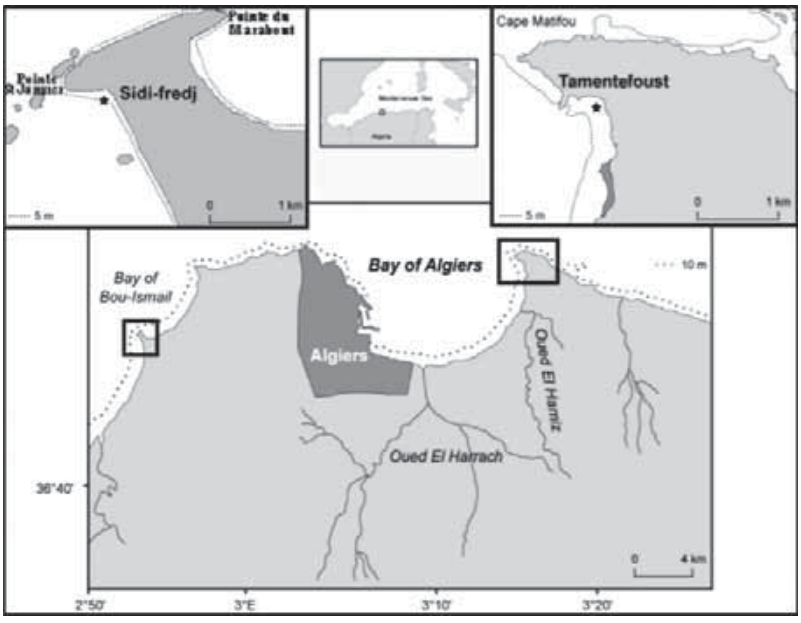

Figure 1. Stations where most of the holothurians were sampled.

1. Département des Ressources Halieutiques, Faculté des Sciences Exactes, de la Nature et de la Vie. Université Abelhamid Ibn Badis-Mostaganem, BP 300 rue Hocine Hamadou, 27000, Mostaganem, Algérie. Email: mezalikarim@yahoo.fr

Figuier-plage in the Bay of Zemmouri-Boumerdes). The fresh sampled holothuroids were grouped into 10 morphotypes: 1) The classical *Holothuria* (*Holothuria*) *tubulosa A* and 2) *Holothuria* (*Holothuria*) *stellati*, characterised by a distinct, round bivium and flat trivium. *Holothuria* (*H.*) *stellati* differs from the classical *H.* (*H.*) *tubulosa A* by its big protuberances; 3) *Holothuria* (*Holothuria*) *tubulosa B* has a flask-shaped integument that is not very thick in a released state; 4) *Holothuria* (*Holothuria*) *tubulosa C* has a soft consistency with an arched bivium that has a pointed conical verrucosities; 5) *Holothuria* (*Holothuria*) *tubulosa D* has a slightly cylindrical form with a flattened trivium and thick integument. This species presents apparent conical verrucosities laid out in several lines on the bivium; 6) The classical *Holothuria* (*Roweothuria*) *poli A* has abundant white pedicels on the trivium; 7) *Holothuria* (*Roweothuria*) *poli B* has a similar aspect as *H.* (*H.*) *stellati* (large protuberances) and with *H.* (*R.*) *poli A* (white pedicels) regularly laid out on the trivium; 8) *Holothuria (Panningothuria) forskali* has a cylindrical soft black body (when it is alive), and a numerous white pedicels on the trivium; 9) *H.* (*Platyperona*) *sanctori A* is brown whereas 10) *H.* (*Platyperona*) *sanctori B* is easily recognized in water by its white spots. The first seven species described above do not have Cuvierian bodies. Species 8, 9 and 10 have Cuvierian bodies.

DNA extraction, PCR protocols and sequencing

Tissue samples preserved in ethanol (90%) were obtained from the tentacle of each individual. In total, sequence data were generated for 26 individuals (all confused species). Additional sequence data (from the French Mediterranean areas) were obtained from the Florida Museum of Natural History (USA). DNA was extracted from a small piece of macerated tissue (10–20 mg) placed in a 1.5 ml microcentrifuge tube and extracted with 750 µL of DNAzol and 5 µL of proteinase K. Sections of the large subunit 16S ribosomal DNA (16S rDNA) genes were amplified using primers 16SA-R (5′-CGCCTGTTTATCAAAAACAT-3′) and 16SB-R (5′GCCGGTCTGAACTCAGATCACGT-3′) (Palumbi et al. 1991).

PCR amplification was performed in 49 mL containing: ddH2O (30.8 mL); 10X (5 mL); dNTPs (5 mL); AR (2 mL); BR (2 mL); Taq polymerase (0.2 mL); MgCl2 (4 mL) and 1 mL template DNA solution. PCR reactions involved denaturation for 60s at 95°C followed by 40 cycles of 30s denaturation at 95°C, 30s annealing at 50°C, and 80s extension at 72°C, and final extension of 10 min. PCR amplicons were purified using PCR DNA Gel Band Purification Kit. All sequencings were done by Interdisciplinary Centre for Biotechnology Research of Florida University (www.biotech.ufl.edu/staff.html). Sequences were aligned using Se-Al 2.011 under default parameters and checked by eye.

Sequence analyses and phylogeny

After trimming some base pairs at the beginning and end of the sequences, sequence size for 16S rDNA was 600 bp. These sequence data used for phylogenetic analyses were treated by Sequencher 4.8 software to make several DNA assemblage sequences relatively short in order to create longer sequences called contigues. Bayesian analyses were performed using MrBayes (v.3.1, Ronquist et al. 2005). Prior to analyses we tested for the most appropriate nucleotide substitution model using Modeltest 3.06 (Posada and Crandall 1998). For the evaluation of the reliability of the reconstituted trees, statistical test was applied using the posterior Bayesian probabilities with GTR (General Time Reversible model). The used out-group sequence was from a species *Cucumaria frondosa*, obtained from Genbank.

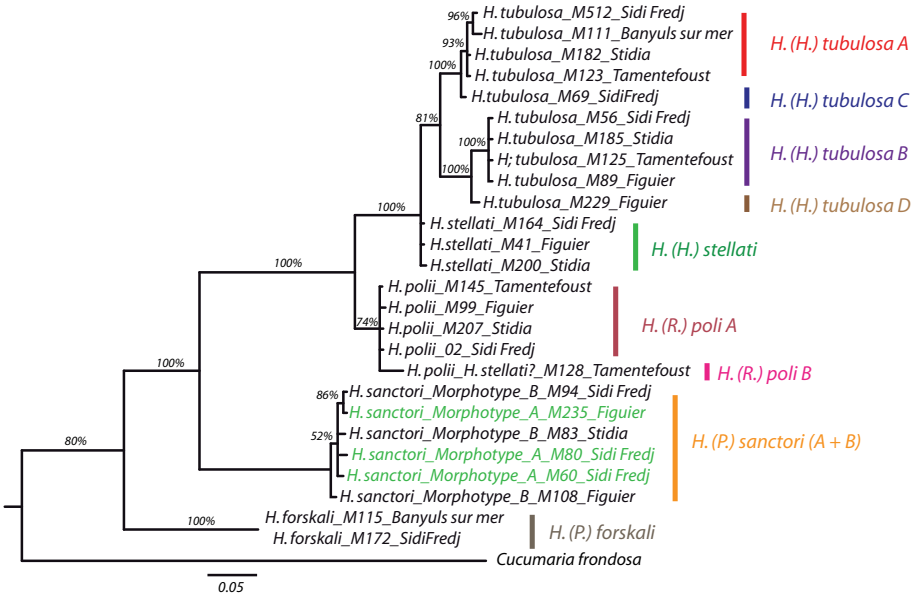

Figure 2. Bayesian consensus tree based on 16S rDNA. The values indicated above the branches represent the posterior probabilities (PP in %).

Results

The Bayesian consensus trees present, in general, five monophyletic clades with high posterior probabilities corresponding to the five examined species (Fig. 2). *Holothuria* (*H.*) *tubulosa* is represented by two well supported clades (AC and BD). *Holothuria (H.) stellati* is monophyletic and is well separated from the two groups (A and B) of *H.* (*H.*) *tubulosa* (high posterior probabilities 100%). The Bayesian consensus tree shows no differences between the two morphotypes of *Holothuria* (*P.*) *sanctori* (A and B*)*.The classical *Holothuria* (*R.*) *poli A* is monophyletic and is well separated from the two groups of *H.* (*H.*) *tubulosa* (A and B) with high posterior probabilities value (100%). *Holothuria* (*R.*) *poli B* included in the clade of the classical *H.* (*R.*) *poli A* is slightly different (high posterior probabilities value 74%) and different from the *H.* (*H.*) *stellati* (high posterior probabilities value 100%).

Discussion

Phylogenetic analyses using the16S rDNA provided insights into the relationships within the aspidochirotid holothurians species of Algeria's coastal areas. The most currently recognised species formed separate clades supported by high posterior probabilities values and the species formed clades that agree with taxonomic revisions based on morphology and anatomy (Mezali 2008). The phylogram shows that *H.* (*H.*) *tubulosa* have two well separated populations (A and B). *Holothuria* (*H.*) *stellati* is well separated from both *H.* (*H.*) *tubulosa* (A and B). This molecular results obtained on fresh *H.* (*H.*) *stellati* samples contradicts the one obtained by Borrero-Pérez et al. (2009) considering *H.* (*H.*) *stellati* to be a junior subjective synonym of *H. tubulosa*. *Holothuria* (*R.*) *poli B* occurs in the same clade than the classical *H.* (*R.*) *poli A*. *Holothuria* (*R.*) *poli B* could be considered as an intermediate form between *H.* (*H.*) *stellati* and *H.* (*R.*) *poli A* because its morphological characters are common to both species. This led us to suspect that *H.* (*R.*) *poli B* is a hybrid species. The taxonomic status of some holothurians specimens analyzed in our collection remains to be confirmed. The two individuals of *H.* (*H.*) *tubulosa* (C and D) gave unusual DNA sequences. The use of other molecular markers (i.e. ITS and COI) could be useful to determine in the future the precise taxonomic status of these two specimens.

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