FROM THE TETHYS OCEAN TO THE MEDITERRANEAN SEA, HOW TECTONICS SHAPED *ECHINOCARDIUM CORDATUM* EVOLUTION.

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Abstract

Irregular echinoids constitute one of the most important groups of the Mediterranean benthic fauna. The genus *Echinocardium* is well represented but its diversity seems to have been underestimated. Genetic analyses of *E. cordatum* highlighted a complex of at least five cryptic specieswhich appearance seems to have been greatly driven by the paleontological history of the Mediterranean Sea.
Keywords: Biodiversity, Biogeography, Echinodermata, Ocean History

Introduction

Echinocardium [Loveniidae, Spatangoida] is a widespread genus of irregular sea urchins, presently composed of six nominal species. Three of them can be observed in sympatry in the Mediterranean Sea: *E. cordatum* (Pennant, 1777), *E. mediterraneum* (Forbes, 1844) and *E. capense* (Mortensen, 1907). Genetic studies [1] revealed that *E. cordatum* is a complex of five different cryptic species encountered in the Atlantic (A, B1), Pacific (NP, SP) and Mediterranean (B1, B2) basins (Fig 1). Its antitropical distribution raises the question of the formation of these cryptic species complex with respect to paleogeography.
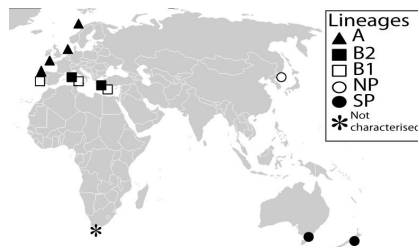

Fig. 1. Distribution of the cryptic species in *E. cordatum*

Material and Methods

A 430 bp portion of the 16S ribosomal gene and a 560 portion of the cytochrome oxidase subunit I (CO1) of the mitochondrial genome were amplified for several Spatangoida species (Species in bold Fig1). Sequences of 16S and CO1 for 12 additional Spatangoid species and one Echinolampadidae were retrieved from genebank (Fig 1). The phylogenetic tree was constructed using Bayesian inferences as performed in MrBAYES software [2], under the GTR+I+G evolutionary model as determined by MODELTEST [3] Topology within *E. cordatum* was confirmed by the intronic region of the Elongation Factor α (EF1) of the nuclear genome. Molecular estimates of divergence times were calculated using the Bayesian method of Thorne et al. (1998) [4] as implemented in the Multidivtime package of PAML [5]. Paleontological data from the litterature were used to calibrate the topology. Hence, the divergence of *E. cordatum* and the other species of the genus of the tree are estimated to be 12 to 40 MYR old [6], and the appearance of the Schizasteridae family, 95MYR old [7]. *Conolampas sigsbei* (Cassiduloida, Echinolampadidae) was used as outgroup for the study establishing the root age at about 200MYR [7].

Results

Bayesian inferences allowed generating a satisfying phylogeny of the studied species, and in particular, of *E. cordatum* nominal species as well as aging its cryptic lineages. The split between cryptic specie A and the remaining lineages is estimated to have occurred some 13MYR ago, and the split between B1 and B2 is dated to 5 MYR.

Discussion

These data are consistent with geological information about the evolution of the Tethys Ocean and Mediterranean Sea under the following scenario. Geographic isolation in the two basins resulting from the closure of the Tethys around 10 MYR ago leaded to allopatric differentiation of the species A in the Atlantic-Mediterranean basin and the common ancestor of lineages P (NP & SP) and B (B1 & B2) in the Pacific basin. Between 13 and 5 MYR ago, this common ancestor evolved to create two modern species in the Pacific Ocean, NP and SP and migrated back to the Mediterranean Sea [via South Africa (where the nominal species *E. cordatum* is found)] to diverge in two

others species, B1 and B2, around 5 MYR ago. Despite the fact that these species are now found in sympatry, they might have been formed by allopatric speciation, as their divergence time concords with the Messinian crisis (6MYR ago) during which the Mediterranean Sea experimented extensive desiccation and sea level changes[8]. The salinity increase that results from the Messinian crisis might have caused the extinction of lineage A into the modern Mediterranean Sea. This hypothesis could be tested via salinity tolerance experiments on lineages A and B.
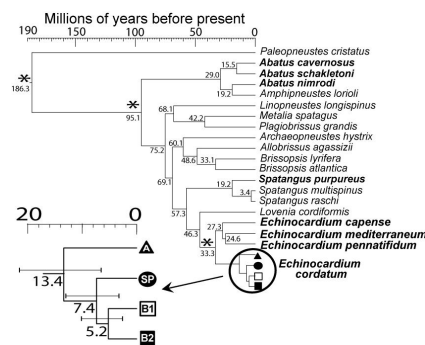

Fig. 2. ML tree obtain from 16S and CO1 concatenated genes using Bayesian inferences with datation of nodes and 95% confidence interval for *Echinocardium cordatum* . * Nodes with fossil records. Cryptic species NP not shown

Long thought to be a single cosmopolitan species, *E. cordatum* revealed to be a complex of cryptic species which evolution has been extensively intertwined with the evolution and history of the Mediterranean Sea.

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