

Biol. Mar. Mediterr. (2015), 22 (1): 109-110

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DNA-BARCODING TO SOLVE THE TRICKY CASE OF CO-OCCURRING *SABELLARIA* (ANNELIDA) SPECIES IN THE MEDITERRANEAN SEA

DNA-BARCODING PER LA CORRETTA IDENTIFICAZIONE DELLE SPECIE DEL GENERE SABELLARIA (ANNELIDA) IN MEDITERRANEO

Abstract - *Intraspecific morphological variation of key taxonomic features is probably responsible for frequent misidentification of Sabellaria (Lamarck, 1818) species. This is the case of Sabellaria alcocki Gravier, 1906 whose Mediterranean records probably refer to juveniles of S. spinulosa (Leuckart, 1849). We hereby provide molecular tools (DNA barcoding) to improve correct delimitation of the Mediterranean species of this genus.*

Key-words: *Polychaeta, Sabellaria alveolata, Sabellaria spinulosa, Sabellaria alcocki, COI.*

Introduction - *Sabellaria* (Lamarck, 1818) species are gregarious tube-builder polychaetes which produce complex reefs representing valuable and diverse habitats in coastal areas (La Porta and Nicoletti, 2009). Today three *Sabellaria* species are recorded for the Mediterranean Sea: *S. alveolata* (L., 1767), *S. spinulosa* (Leuckart, 1849) and *S. alcocki* Gravier, 1906 (Castelli *et al.*, 2008). This last is a valid species but according to several authors it is exclusive of the Indian Ocean and its Mediterranean records should be carefully revised (Lezzi *et al.*, 2015). The operculum is of paramount taxonomic importance for *Sabellaria* species identification, but its intraspecific variation, especially during development, may lead to confusion. This is the case of *S. spinulosa* showing remarkable variation in operculum morphology during development to a level that juveniles of this species resemble *S. alcocki*. These facts lead to hypothesize that Mediterranean records of *S. alcocki* could refer to juveniles of *S. spinulosa* (Lezzi *et al.*, 2015). During faunal characterization of *Sabellaria* reefs in Sicily, *S. alveolata*, *S. spinulosa* and some small specimens resembling *S. alcocki* in operculum features (*sensu* Kirtley, 1994) were collected. As the discriminatory power of DNA analysis is especially valuable in case of morphological identification of species leading to confusion (Landi *et al.*, 2014), we used DNA barcoding to correctly delimiting the studied species, thus providing a tool to avoid future misidentification of *Sabellaria* species in the Mediterranean area.

Materials and methods - Twenty-four core samples of *Sabellaria* reefs were collected in June 2013 at 3 Sicilian locations (Donnalucata, Eraclea Minoa, Triscina) at 1.5-3 m depth. The specimens of *Sabellaria* were counted and identified to species level. A 610 base pairs (bp) fragment of the COI gene and a 308 bp of the nuclear 28S rDNA region were sequenced for *S. alveolata*, *S. spinulosa* and *S. cf. alcocki*. The sequences were deposited in GenBank and compared with a public COI sequence of *S. spinulosa* (A.N. KF808174) collected in the eastern Atlantic. Measuring of COI genetic distances was based on K2P model. A Neighbor Joining tree was built, its nodes are supported by a high proportion (>90%) of replicates in the bootstrap analysis.

Results - As a whole 17361 *Sabellaria* specimens were collected; *S. alveolata* was dominant at each station, followed by *S. spinulosa* (Donnalucata 3724 vs 42; Triscina 10167 vs 65; Eraclea Minoa 3350 vs 13 individuals, respectively). Though rare specimens per station were identified as *S. cf. alcocki*, the COI sequences of *S. spinulosa* and *S. cf. alcocki* matched (Fig. 1). Both haplotypes clustered with the Atlantic *S. spinulosa*, though showing a discrete genetic differentiation which could be caused by a geographic isolation. Within the *spinulosa*-clade the distances were less than 0.9%; while the inter-species distance was estimated more than 2% supporting the species delimitation between the *alveolata* vs *spinulosa* group. In 28S sequences only a single transition mutation occurred between *S. alveolata* and *S. spinulosa*.

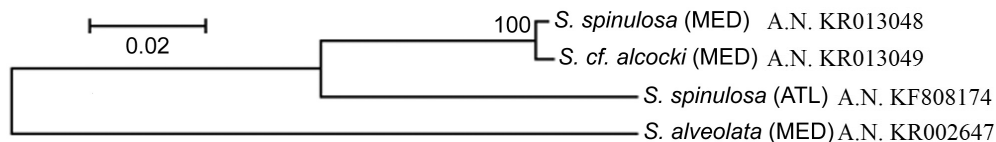


Fig. 1 - NJ tree constructed on the K2P model from COI sequences. MED: collected in the central Mediterranean; ATL: collected in the Atlantic; A.N.: accession number Genbank. *Albero NJ costruito sul modello K2P da sequenze di COI. MED: raccolto in Mediterraneo; ATL: raccolto in Atlantico; A.N.: codice d'accesso Genbank.*

Conclusions - To our knowledge we herein provide the first COI sequences allowing molecular identification of *S. alveolata* and Mediterranean *S. spinulosa* specimens. We confirm that identification of *Sabellaria* spp. only based on morphology may lead to misidentification. Our results strengthen the hypothesis that *S. alcocki* records in the Mediterranean Sea probably derive from misidentification with *S. spinulosa* juveniles, and that the former species is not present in the area (Lezzi *et al.*, 2015). Combination of morphological and molecular taxonomic approaches may further contribute to solve problems due to species misidentification.

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