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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*.

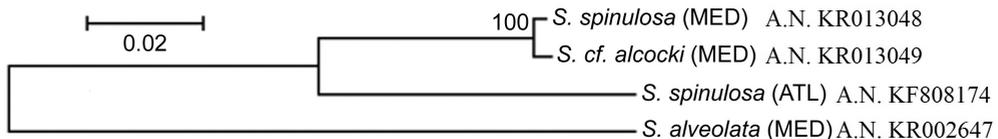


Fig. 1 - NJ tree constructed on the K2P model from COI sequences. MED: collected in the central Mediterranean; ATL: collected in the Atlantic; A.N.: accession number Genbank. *Albero NJ costruito sul modello K2P da sequenze di COI. MED: raccolto in Mediterraneo; ATL: raccolto in Atlantico; A.N.: codice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