Taxonomic distinction of Ophelia barquii and O. bicornis (Annelida: Polychaeta) in the Mediterranean as revealed by ISSR markers and the number of nephridiopores

Ferruccio Maltagliati*∫, Marco Casu†, Tiziana Lai†, Daniela Iraci Sareri‡, Daniela Casu*†, Marco Curini Galletti†, Grazia Cantone‡ and Alberto Castelli*

*Dipartimento di Scienze dell'Uomo e dell'Ambiente, Università di Pisa, Via A. Volta 6, 56126 Pisa, Italy.
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[illegible] scaling and assignment [illegible] nephridiopore pairs) and O. barquii (five nephridiopore pairs). This paper represents a further contribution to the estimation of biodiversity within marine invertebrates.

INTRODUCTION

In recent years a number of molecular studies on marine invertebrates have greatly contributed to the assessment of local biodiversity through the resolution of complex taxonomic cases. This led some authors to recommend the deposition of molecular data such as DNA samples of one syntype specimen and a list of diagnostic DNA banding patterns for formal taxonomic nomenclatural purposes (Westheide & Schmidt, 2003). However, a more powerful taxonomic approach requires the integration of molecular data with additional types of information such as morphology, behaviour, physiology, etc. (Lee, 2004). This cross-validation is needed to obtain a sound interpretation of the observed differences in a taxonomic perspective.

In the marine environment there is a growing suspicion that the present perception of biodiversity is based on non-representative data. The problem is experienced even in conspicuous invertebrate macrofaunal groups, in which the number of described species is often underestimated. In particular, polychaetes are characterized by a large number of apparently widely distributed or cosmopolitan species. Detection of diagnostic differences at the molecular level has revealed that many of these formerly alleged [illegible] Savigny, 1818 release their gametes on the sand where they will be swept away by the tidal or wave current into the water-column. Their larvae are potentially capable of long-distance dispersal, adopting a benthic lifestyle after several weeks of planktonic life.

The debate on the taxonomic status of a number of species within the genus Ophelia has a long history owing to the limited reliability of morphological diagnostic traits usually employed for species identification in the group (Maltagliati et al., 2004 and references therein). Ophelia bicornis Savigny 1818 is the type species of the genus as well as the Family Opheliidae Savigny 1818. Fauvel (1927) distinguished in European Ophelia the species O. radiata and O. bicornis and the variety O. bicornis barquii in the

Table 2. Inter simple sequence repeats primers, approximate band sizes (bp) and proﬁles obtained for 30 individuals of *Ophelia barquii* and *O. bicornis* from Italian localities. Morphotype(species)-private bands are in bold.

and higher annealing temperatures of their primers decrease the amount of template primer mismatch artefacts typical of RAPD amplifications.

We used four primers provided by Proligo® Primers and Probes, Proligo France SAS (Table 1). The PCR reaction mixture of 25 µl volume contained 0.5 units of Taq DNA polymerase (Pharmacia®), 1× reaction buffer (Pharmacia®), 2.5 mM $MgCl_2$, 0.2 µM primer, 200 µM of each dNTP (Roche®), and up to 30 ng of genomic DNA. The PCR amplification was performed in a MJ PTC-100 Thermal Cycler (MJ Research®) programmed for 1 cycle of 3 min at 94°C, 45 cycles of 40 s at 94°C, 45 s at 55°C and 1 min and 40 s at 72°C to complete partial amplification. At the end a post-treatment for 5 min at 72

real but samples are not, so that randomizations occurred within morphotypes; and for Φ_{CT} (between morphotypes), it was assumed that the samples were real and the morphotypes were artificial, so that randomizations of samples were made across morphotypes.

The UPGMA cluster analysis on pairwise Nei's (1978) genetic distances between samples was carried out to construct a dendrogram using the program TFPGA. Nodes of the dendrogram were tested using bootstrapping with 10,000 replicates. The ISSR patterns were further analysed by the program RAPDPLOT 3.0 (web site ftp://lamar.colostate.edu/pub/wcb4/) to generate a matrix of 1 − Nei & Li's (1985) similarity index between individuals. Similarity index was defined as $S = 2NAB/(NA+NB)$, where NAB is the number of bands that individuals A and B share in common and NA is the number of bands in individual A and NB is the number of bands in individual B. Non-metric multidimensional scaling (nMDS) was applied to the dissimilarity matrix in order to reveal possible groupings of the individuals using the program STATISTICA 5.1 (web site http://www.stasoft.com). This analysis uses a function minimization algorithm that evaluates different configurations with the goal of maximizing the goodness-of-fit. Stress index measures reliability of the nMDS plot: the smaller the stress index, the better the fit of the reproduced distance matrix to the relative distances on the plot. Further, an assignment test was performed on individuals of the six localities using the calculator available online at http://www2.biology.ualberta.ca/jbrzusto/Doh.php. This calculator takes genotypesc2221.4al

from each population and determines from which population each individual is most likely to have come, by using the highest probability of an individual's genotype in any of the populations. Probabilities were obtained by permuting 10,000 times genotypes within populations.

RESULTS AND DISCUSSION

Integration and cross-validation of di¡erent types of data are desirable tools for good taxonomic practice. This is particularly true with polychaetes, a taxon often comprising widely distributed species which lack reliable morphological characters. Indeed, a number of studies reported that molecular techniques coupled with morphological analyses allowed di¡erentiation of taxonomic di¡erences at a very ¢ne scale (e.g. Schmidt & Westheide, 1999; Scaps et al., 2000; Maltagliati et al., 2001, 2004).

In this study specimens collected at Mugoni, Catania and Calabernardo exhibited ¢ve nephridiopore pairs, while those collected at Platamona, Porto Ferro and Cefalù six pairs. In general, band presences varied in several ways across samples (Table 2). The number of nephridiopore pairs and ISSR patterns yielded perfectly congruent results in distinguishing two groups of individuals, denoting the absence of hybridization and introgression between the two morphotypes. This also suggested complete reproductive isolation between individuals of di¡erent morphotypes, resolving the controversy regarding the taxonomic status of Ophelia bicornis sensu lato in the Mediterranean. Indeed, in this region, Ophelia barquii (Fauvel, 1927), with ¢ve nephridiopore pairs, is a valid species, separated from Ophelia bicornis Savigny, 1818, with six nephridiopore pairs. This result represents a sound con¢rmation based on non-proteic genetic markers of the recent morphological and allozyme survey on western Mediterranean populations (Maltagliati et al., 2004).

The DNA fragment IT3-800 was produced by all 15 individuals of O. bicornis analysed and no bands were exhibited by the 15 individuals of O. barquii conversely,