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Preliminary study on the systematic relationships of Sabellinae (Polychaeta, Sabellidae), based on the C1 domain of the 28S rDNA, with discussion of reproductive features

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ABSTRACT

Systematic relationships of some taxa within the subfamily Sabellinae (Polychaeta: Sabellidae) based on the sequence of the C1 domain of the 28S rDNA coding gene are assessed, and the cladogram obtained are discussed in the light of some reproductive and morphological features. Sixteen different species belonging to 12 genera, were analysed. The cladograms, obtained with different methods (distance UPGMA, maximum parsimony and maximum likelihood) are consistent, except for the ambiguous position of *Amphiglena*. Three main groups of species, showing similarity in reproductive features and sperm morphology (acrosome structure) were identified, the taxa included in group A (*Myxicola, Chone,* and *Euchone*) show different reproductive strategies and sperm morphology with elongated nucleus, rounded mitochondria and pointed acrosome. Group B contains large sized free-spawning forms, generally with an ect-aquasperm type of spermatozoa (*Bispira, Sabella, Sabellastarte,* and *Branchiomma*). Taxa included in group C (*Eudistylia, Schizobranchia, Pseudopotamilla, Perkinsiana* and *Demonax*) show high variability in sperm morphology, but with a peculiar acrosome structure. The position of *Amphiglena* is variable, being closer to group A in the distance and maximum parsimony trees, and to group C in the maximum likelihood tree. From previous morpho-functional analyses, the genera clustering in group A represent the most plesiomorphic taxa, while those in group C contain the most apomorphic ones. Although the general patterns obtained with this preliminary molecular analysis are highly consistent with those arising from the morpho-functional characters available form the literature, the resolution among genera and species within each of the main groups identified is not consistent, and some of the polytomies and phylogenetic problems among taxa still remain unresolved.

KEY WORDS: Polychaeta - Sabellidae - Phylogeny - C1 domain - 28S - rDNA - Reproductive biology - Sperm morphology.

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INTRODUCTION

During the last decades the polychaete Sabellidae has been the subject of major systematic arrangements (Knight-Jones, 1983; Perkins, 1984; Fitzhugh, 1989; Knight-Jones & Perkins, 1998). Sabellidae represents one of the polychaete families where a phylogenetic hypothesis was constructed based on cladistic analysis (Fitzhugh, 1989). This first analysis, based on morphological characters, revealed a high level of consistency when other genera and features were added, including reproductive characters (Rouse & Fitzhugh, 1994; Fitzhugh & Rouse, 1999).

Sabellidae is also one of the most well-known polychaete families with regard to reproductive characters (Giangrande, 1997; Rouse, 1999a), showing great variability in both sexual and asexual reproductive modes, despite considerable uniformity in design and feeding type (McEuen *et al.*, 1983; Knight-Jones & Bowden, 1984; Gambi *et al.*, 2001). The plesiomorphic conditions for this family were postulated to be brooding associated with small body size and sperm with elongate heads; the presence of lecithotrophic development is considered an autapomorphy for the whole family (Rouse & Fitzhugh, 1994).

To date, about 470 species of Sabellidae have been described and divided over 43 genera. Sabellidae is defined as a monophyletic group give the pattern of dentition, and the presence of the handle in the thoracic hooks, and the hooded nature of the abdominal chaetae. According to Fitzhugh (1989), two subfamilies can be recognised within Sabellidae: Fabriciinae and Sabellinae. Only the genus *Caobangia* remains *incertae sedis* with respect to subfamily placement (Fitzhugh & Rouse, 1999). Monophyly is well assessed for Fabriciinae on the basis of a series of autapomorphies (Fitzhugh, 1989). This group is a more homogeneous clade compared to Sabellinae, both from a morphological and a reproductive point of view. Fabriciinae seem, in fact, to be canalised with regard to their reproductive features: they are all small sized and brooders with modified spermatozoa (Rouse, 1995, 1996b). The Sabellinae subfamily, containing the larger number of sabellid genera, is instead a quite heterogeneous clade, defined only by the presence of a radiolar skeleton with at least two rows of cells (Fitzhugh, 1989). Sabellinae also shows a great variety of reproductive strategies (Giangrande, 1997).

The Sabellinae cladogram, based on cladistic analysis of morpho-functional features, still contains some unsolved nodes. Polytomies are present in three main groups of genera: 1) *Chone, Euchone,* and *Myxicola*; 2) *Sabella, Bispira, Branchiomma,* and *Sabellastarte*; 3) *Perkinsiana, Eudistylia, Schizobranchia,* and *Pseudopotamilla* (Fitzhugh, 1989; Fitzhugh & Rouse, 1999). For most of these genera, autapomorphies are also lacking, as is the case for *Perkinsiana* which also shows some inconsistency in reproductive features (Gambi *et al.*, 2000).

The present study, carried out in the framework of a larger research dealing with the reproductive biology of Sabellinae and its phylogenetic and ecological significance (Giangrande & Petraroli, 1994; Gambi & Patti, 1999; Giangrande *et al.*, 2000; Gambi *et al.*, 2000, 2001; Licciano *et al.*, 2001), represents a preliminary study of systematic relationships within the Sabellinae based on a molecular marker, the C1 domain of the 28S rDNA.

Molecular analysis has already been successfully utilized within the Annelida (McHugh, 1997, 2000), also to solve some taxonomic problems among and within polychaete families and genera (Lenaers & Bhaud, 1992; Feral *et al.*, 1994; von Soosten *et al.*, 1998; Schmidt & Westheide, 1999). The molecular marker utilized here is the C1 domain of the 28S rDNA, which is a coding conservative region suitable for detecting differences at genus and species level, and has already been utilized for this purpose in the polychaete clade Alvinellidae (Feral *et al.*, 1994).

The spermatozoa ultrastructure, as well as other reproductive features, of some of the selected species, were also described and discussed to better evaluate the phylogenetic significance of the molecular cladograms. Molecular data were then compared with previous cladistic analyses based on morpho-functional characters, available in the literature.

MATERIALS AND METHODS

Studied species

Sixteen species of Sabellinae belonging to 12 genera were obtained by collecting or receiving specimens from different geographic locations, and in the framework of different projects. The taxa available include most of the genera involved in the polytomies arising from the previous morpho-functional cladistic analysis, and are also representative of both plesiomorphic and apomorphic genera, according to the existing phylogenetic hypothesis (Fitzhugh, 1989; Rouse & Fitzhugh, 1994). *Sabella spallanzanii* (Gmelin, 1791), *Branchiomma luctuosum* Grube, 1869, and *Amphiglena mediterranea* (Leydig, 1851) were collected from the Gulf of Naples (Tyrrhenian Sea, Italy); *Bispira* sp. and *Chone* sp. come from soft bottoms (fine sand) off the Gulf of Salerno (Tyrrhenian Sea, Italy), and *Bispira mariae* Lo Bianco, 1893 from Otranto (Apulia, Southern Adriatic Sea, Italy). *Eudistylia vancouveri* (Kinberg, 1867), *Schizobranchia insignis* Bush, 1904, and *Pseudopotamilla occelata* Moore, 1905 were collected at Friday Harbor (San Juan Island, Washington, U.S.A.), while *Myxicola infundibulum* (Renier, 1804) came from Puget Sound (Seattle, Washington, U.S.A., kindly provided by Dr B. Pernet). The species *Perkinsiana littoralis* (Hartman, 1967), *Demonax polarsterni* Gambi *et al.*, 2001, *Euchone pallida* Ehlers, 1908, and *Myxicola* cf. *sulcata* Ehlers, 1912 were collected in the shelf off the Eastern Weddell Sea (Antarctica) (Gambi, 1999). The species *Sabellastarte australiensis* Haswell, 1884 was collected in the Sydney area (Australia, kindly provided by Dr G. W. Rouse). Data regarding *Perkinsiana antarctica* Kinberg, 1867 came from Genebank file (reported as *Potamilla antarctica*) (Feral *et al.*, 1994). Finally, the taxon chosen as outgroup was *Protula* sp. (collected in Banyuls-sur-Mer, France), belonging to the Serpulidae, which is considered the sister group of Sabellidae within the Sabellida clade (Rouse & Fauchald, 1997).

Gamete ultrastructural analysis

Data on reproductive features of some of the analysed species were already available from previous papers (Lee, 1977; Dean *et al.*, 1987; Sordino & Gambi, 1992; Giangrande & Petraroli, 1994; Gambi & Patti, 1999; Giangrande *et al.*, 2000; Gambi *et al.*, 2000, 2001; Licciano *et al.*, 2001). Data on other species presented here are original, e.g., *Eudistylia vancouveri, Schizobranchia insignis* and *Myxicola infundibulum*. Both transmission (TEM) and scanning (SEM) electron microscopy were performed on the coelomic content of mature specimens of these species. For TEM analysis samples were fixed for 2 h with 2.5% glutaraldehyde, washed in 0.1 M cacodylate buffer (pH 7.4) containing 0.4 M sucrose for 1 h, and then post-fixed 1% osmium tetroxide in the same buffer for 1 h. All fixation steps were performed at 4 °C. Fixed material was dehydrated through graded concentrations of ethanol (20 min for each alcohol concentration) followed by propylene oxide (10 min), and a mixture of epoxy resin and propylene oxide (1:1, for 5 h in a drier), and embedded in epoxy resin (2 days at 60 °C). Silver-grey sections were stained with alcoholic uranyl acetate followed by lead citrate. Micrographs were taken with a Phillips 400 TEM. For SEM analysis, coelomic fluid was fixed in 2.5% glutaraldehyde for 1 h at 4 °C. Samples were washed in 0.1 M cacodylate buffer, dehydrated with graded alcohol concentrations, and gold coated after critical point drying. Micrographs were taken with a Phillips 505 SEM.

Specimens and DNA extraction

For some species DNA was extracted from fresh or frozen (-80 °C) animals, and from absolute or 80% ethanol preserved samples for others. The DNA was extracted from approximately 1 g tissue which, immediately after dissection was reduced to a fine powder under liquid nitrogen, then dissolved in lysis buffer (75 mM NaCl, 25 mM EDTA, pH 8) and incubated with proteinase K (Merck, Germany) (0.4 mg/ml) and 0.5 % SDS for 2 h at 37 °C. Samples were subsequently extracted with phenol/chloroform and precipitated with 2.5 M NH_4Ac and 75% isopropanol. Following centrifugation, the DNA pellet was washed with 70% ethanol, dried and resuspended in HPLC water to a final concentration of 1 mg/ml (Sambrook *et al.*, 1989). This technique has also been used for samples fixed in ethanol, that were successfully extracted by vacuum drying the samples prior to extraction.

PCR amplification and sequencing

Because of the high variability of the ITS regions (Patti & Gambi, 2001; Famà *et al.*, 2000), to increase the annealing specificity, the forward primer ITS-3F (5'-GCAKCGATGAAGARCGCAGC-3') was designed on the more conservative 5.8S ribosomal gene region, while the D1-R reverse primer (5'-AATCCCAARCAACYCGACTC-3') was designed to anneal in the middle of the 28S region.

PCR amplifications were run on 200 ng of purified genomic DNA using a 9,700 Perkin Elmer thermocycler. The program included, after 95 °C denaturising time for 1 min, two cycles with an annealing temperature of 43 °C, two cycles with 48 °C and finally 30 cycles with 50 °C, followed by 2 min extension time at 72 °C.

Amplification from genomic DNA with ITS-3F and D1-R, produced the nucleotide fragment that consisted of about 600 bp. The C1 domain of the 28S yielded approximately a 200 nucleotide fragment.

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Eudistylia vancouveri          TTCGACCTGA GATCAGACGA GATTACCCGC TGAATTTAAG CATATCACTA AGCGGAGGAA AA [62]
Schizobranchia insignis        -----A---- -T-------- ---------- ---------- ---------- ---------- --
Demonax polarsterni            ---A------ ---------- ---------- ---------- ---------- ---------- --
Perkinsiana littoralis         ---A------ ---------- ---------- ---------- ---------- ---------- --
Pseudopotamilla occelata       ---N------ ---T------ ---------- ---------- ---------- --------G- --
Perkinsiana antarctica         ---N------ ---T------ ---------- ---------- ---------- ---------- --
Sabellastarte australiensis    ---------- ---------- ---------- ---------- ---C---A-- ---------- --
Sabella spallanzanii           ---------- ---------- ---------- ---------- -------A-- ---------- --
Bispira sp.                    ---------- ---------- ---------- ---------- -------A-- ---------- --
Bispira mariae                 ---------- ---------- ---------- ---------- -------A-- ---------- --
Branchiomma luctuosum          ---------- ---------- ---------- ---------- -------A-- ---------- --
Myxicola infundibulum          ---------- ----G----- -GA------- ---------- ---------- ---------- --
Myxicola cf sulcata            ---------- ----G----- -GC------- ---------- ---------- ---------- --
Euchona pallida                ---------- ----G----- -GC------- ---------- ---------- ---------- --
Chone sp.                      ---------- ----G----- -TC------- ---------- ---------- ---------- --
Amphiglena mediterranea        ---A------ ---------- --C------- ---------- ---------- ---------- --
Protula sp. (outgroup)         --------C- ---------T ---------- ---------- ---------- ---------- --

Eudistylia vancouveri          GAAACTAACA AGGATTCCCC TAGTAACGGC GAGTGAAGCG GGATGAGCCC AGCACCGAAT C [123]
Schizobranchia insignis        ---------- ---------- ---------- ---------- ---------- ---------- -
Demonax polarsterni            ---------- ---------- ---------- ---------- ---------- ---------- -
Perkinsiana littoralis         ---------- ---------- ---------- ---------- ---------- ---R------ -
Pseudopotamilla occelata       ---------- ---------- ---------- ---------- ---------- ---------- -
Perkinsiana antarctica         ---------- ---------- ---------- ---------- ---------- ---------- -
Sabellastarte australiensis    ---------T ---------- C--------- ---------- ---A------ ---------- -
Sabella spallanzanii           ---------T ---------- C--------- ---------- ---A------ ---------- -
Bispira sp.                    ---------- ---------- C--------- ---------- ---A------ ---------- -
Bispira mariae                 ---------- ---------- C--------- ---------- ---AC----- ---------- -
Branchiomma luctuosum          ---------- ---------- C--------- --------T- ---A------ ---------- -
Myxicola infundibulum          ---------- ------A--A C--------- ---------- ---A------ ---------- -
Myxicola cf sulcata            ---------- ------A--A C--------- ---------- ---A------ ---------- -
Euchona pallida                ---------- ---------A C--------- --------A- ---A------ ---------- -
Chone sp.                      ---------G ---------A C--------- --------A- ---A------ ---------- -
Amphiglena mediterranea        ---------- ---------- C--------- ---------- ---A------ ---------- -
Protula sp. (outgroup)         ---------- ---------T C--------- ---------- ---A------ ---------- G
```

Fig. 1 - Nucleotide sequences comparison of the C1 domain of the 28S ribosomal gene among the different species. The identities are plotted versus the first sequence (*E. vancouveri*) with a dot.

To avoid interference in the sequence reaction by unincorporated primers and dimers during the PCR reaction, all the PCR products were purified using a GeneClean columns kit according to the manufacturer's instructions (Qiagen S.p.A., Italy).

The sequencing reaction was performed in the Biometra Tgradient thermocycler (25 cycles, with 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min). After the sequencing reaction, all the samples were precipitated in an equal volume of 75% Isopropanol and resuspended in HPLC water. The direct sequences were obtained using the ABI 3100 multicapillary machine, with a specific module designed for the analysis of the ITS-3/D1-R fragment.

Sequence alignments and Phylogenetic analysis

Based on previous alignment of 28S rDNA sequences of other polychaetes (Feral *et al.*, 1994), DNA sequences were aligned using CLUSTALW 1.6b (Thompson *et al.*, 1994, Thompson & Gibson, 1998) and manually edited using MUST2000, software distributed by the Author (Philippe, 2000), and available from: *http://sorex.snv.jussieu.fr/must2000.html.* Using PAUP* version 4.0b8, (Swofford, 2000), trees were constructed with Kimura's (1980) two-parameter distance (UPGMA), by maximum parsimony (MP) with heuristic search command, (gaps were treated as fifth base), and with maximum likelihood (ML) method selected in Modeltest version 3.06 (Posada & Crandall, 1998) (JC69+G model, JukesCantor, gamma distribution, shape parameter set to 0.24), using a Windows 32-bit front end, distributed by the Author (Patti, 2001), and available from: *http://www.oeb.harvard.edu/ /palumbi/people%20pages/Francesco.HTML.* The JukesCantor model returns a model of evolution corresponding to JC69, where the transition (TS), and transversion (TV) rates are set to be equal (by not specifying a TS/TV ratio) (Jukes & Cantor, 1969). The gamma distribution is related to the parameter g, which specifies the range of rate variation among sites (large g means less variation).

To estimate the phylogenetic signal, the maximum likelihood mapping method (Strimmer & von Haeseler, 1997) was used; the analysis was carried out using the TREE-PUZZLE program (Strimmer & von Haeseler, 1996).

One potential problem with bootstrapping is that it assumes all characters are independent (Kitching *et al.*, 1998). The small size of the data set may increase this effect, and bootstrap values can be used in conjunction with Bremer support values to assess clade strength (Bremer, 1994; Zhaxybayeva & Gogarten, 2002). Bremer values consider how strongly each node is supported at the strict consensus of consecutively longer trees, and show how many autapomorphy mutations support the single branches of the tree. Bremer support indices were calculated for all clades in the maximum parsimony analysis using PAUP* and TreeRot version 2 (Sorenson, 1999).

Bootstrap values, to find a majority rule consensus unrooted tree, were calculated for UPGMA, MP, and ML methods, with 1000 replicates.

RESULTS

Molecular analysis

The C1 domain of the 28S in the whole species studied (16) was highly conserved (the number of differences between species was evaluated to be an average of five) and sequence alignment was relatively easy. All the sequences analysed were characterised by single mutations, inversions or transitions (Fig. 1). The TS/TV ratio was 1.3 indicating larger number of transitions. The analysis of the divergence and distance matrix revealed a strong separation between the outgroup *Protula* sp. (Serpulidae) and all the Sabellinae studied (Table I). Within the Sabellinae, the highest values were recorded between *Sabellastarte australiensis* and the two species of *Perkinsiana.*

The topology of maximum parsimony (MP) and distance analysis (UPGMA) trees was very similar, except for the position of *Amphiglena mediterranea* (Fig. 2). The bootstrap values for all of the deepest nodes were high in both analyses, although bootstrap values of some nodes were relatively low. The Bremer values are indicated in the maximum parsimony tree. The two trees showed similar groups of taxa: a first group (boxed as group A in Figure 2), containing *Myxicola, Chone,* and *Euchone* species, originates closer to the outgroup. A second branch separated the remaining taxa which were arranged in two consistent groups, although bootstrap values for these two groups were relatively low (33 in the MP, and 38 in the UPGMA analyses): *Bispira, Sabella, Sabellastarte* and *Branchiomma* species on the one hand (group B), and *Eudistylia, Schizobranchia, Pseudopotamilla, Perkinsiana* and *Demonax* on the other (group C). Within group C the two *Perkinsiana* species appeared separated, with *P. antarctica* being closer to *Pseudopotamilla occelata,* and *P. littoralis* closer to *Demonax polarsterni.* The

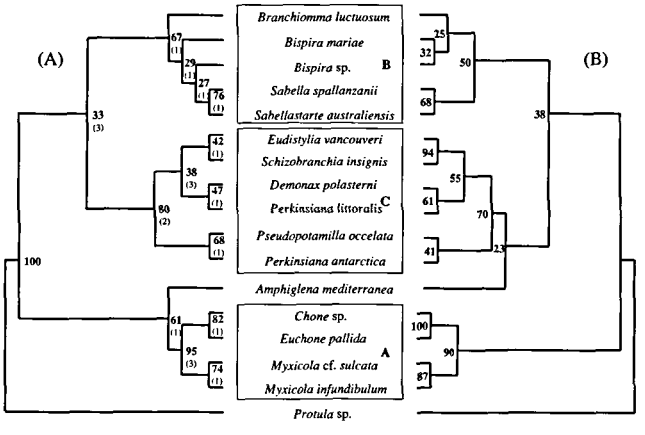

Fig. 2 - Bootstrap consensus trees (1000 replicates) derived from (**A**) maximum parsimony and (**B**) distance (UPGMA) analyses based on the C1 domain of the 28S ribosomal gene. In both cladograms bootstrap values are in bold characters; in the maximum parsimony cladogram the Bremer values (decay index) are indicated in normal characters and in parentheses.

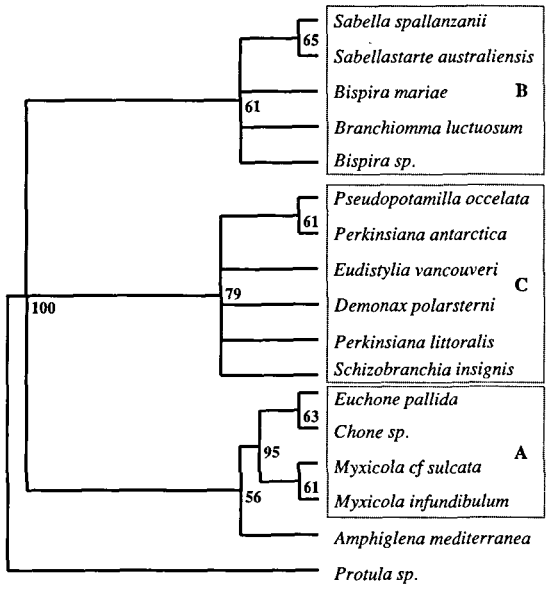

Fig. 3 - Bootstrap consensus trees (1000 replicates) derived from maximum likelihood analysis, showing the three clusters.

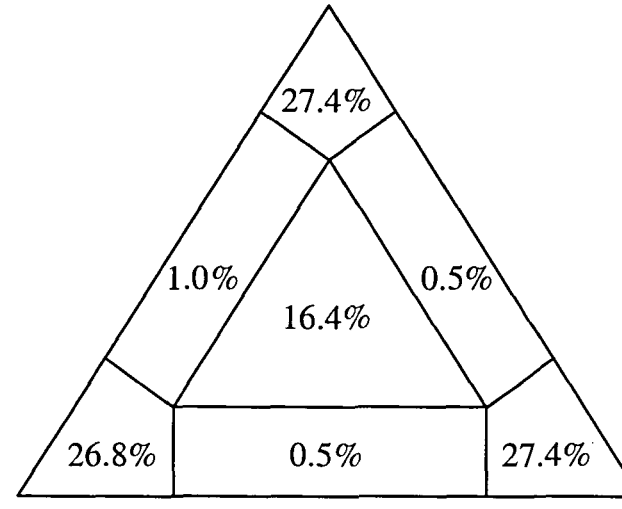

Fig. 4 - Phylogenetic information content by maximum likelihood mapping. Corner regions contain the partition of fully resolved quartet trees, lateral regions contain the partition of partially resolved trees, and the centre region contains the partition of completely unresolved quartet trees.

topology of the maximum likelihood (ML) tree showed a different pattern in which the three groups of taxa A, B, and C were still recognisable, but they originated as a basal trichotomy from the outgroup, and some polytomies within the single branches were unresolved, indicating a lower phylogenetic resolution (Fig. 3). Bootstrap values were high for all of the nodes (Fig. 3). The position of *A. mediterranea* in the latter analysis is close to group A, as in the MP tree (Fig. 2).

Despite these differences, the three cladograms supported similar groups of taxa and a generally consistent pattern. The maximum likelihood mapping analysis (see Materials and Methods) (Fig. 4) showed that about 81%

TABLE I - *Kimura two-parameter distance (above the diagonal) and absolute value of K/SE, estimated by bootstrap method (replications = 1000 and random number seed = 17114), (below the diagonal) among 16 sabellid species and the outgroup: 1,* Euchone pallida*; 2,* Chone *sp.; 3,* Myxicola *cf* sulcata*; 4,* Myxicola infundibulum*; 5,* Amphiglena mediterranea*; 6,* Sabella spallanzanii*; 7,* Sabellastarte australiensis*; 8,* Bispira *sp.; 9,* Bispira mariae*; 10,* Branchiomma luctuosum*; 11,* Eudistylia vancouveri*; 12,* Schizobranchia insignis*; 13,* Perkinsiana littoralis*; 14,* Demonax polarsterni*; 15,* Pseudopotamilla occelata*; 16,* Perkinsiana antartica*; 17, the outgroup sp.; OTUs, Operational Taxonomic Units.*

OTUs	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1		0.0110	0.0115	0.0115	0.0170	0.0231	0.0249	0.0210	0.0231	0.0210	0.0225	0.0225	0.0225	0.0225	0.0265	0.0243	0.0428
2	0.0154		0.0115	0.0115	0.0170	0.0231	0.0249	0.0210	0.0231	0.0210	0.0225	0.0225	0.0225	0.0225	0.0265	0.0243	0.0428
3	0.0167	0.0167		0.0112	0.0171	0.0232	0.0248	0.0212	0.0231	0.0228	0.0229	0.0229	0.0229	0.0229	0.0268	0.0247	0.0432
4	0.0167	0.0167	0.0163		0.0171	0.0232	0.0248	0.0212	0.0231	0.0228	0.0229	0.0229	0.0229	0.0229	0.0268	0.0247	0.0432
5	0.0338	0.0338	0.0338	0.0338		0.0147	0.0167	0.0121	0.0148	0.0145	0.0147	0.0147	0.0147	0.0147	0.0197	0.0172	0.0385
6	0.0602	0.0602	0.0602	0.0602	0.0252		0.0081	0.0079	0.0114	0.0117	0.0170	0.0170	0.0170	0.0170	0.0215	0.0192	0.0393
7	0.0692	0.0692	0.0692	0.0692	0.0338	0.0083		0.0111	0.0138	0.0144	0.0187	0.0187	0.0187	0.0187	0.0230	0.0208	0.0407
8	0.0514	0.0514	0.0514	0.0514	0.0167	0.0083	0.0167		0.0080	0.0083	0.0145	0.0145	0.0145	0.0145	0.0195	0.0171	0.0383
9	0.0602	0.0602	0.0602	0.0602	0.0252	0.0167	0.0253	0.0083		0.0117	0.0172	0.0172	0.0172	0.0172	0.0217	0.0195	0.0404
10	0.0514	0.0514	0.0604	0.0604	0.0253	0.0167	0.0252	0.0083	0.0167		0.0168	0.0168	0.0168	0.0168	0.0216	0.0191	0.0395
11	0.0604	0.0604	0.0604	0.0604	0.0253	0.0338	0.0425	0.0252	0.0338	0.0338		0.0122	0.0154	0.0000	0.0125	0.0084	0.0390
12	0.0604	0.0604	0.0604	0.0604	0.0253	0.0338	0.0425	0.0252	0.0338	0.0338	0.0310		0.0148	0.0000	0.0125	0.0084	0.0390
13	0.0604	0.0604	0.0604	0.0604	0.0253	0.0338	0.0425	0.0252	0.0338	0.0338	0.0123	0.0253		0.0000	0.0125	0.0084	0.0390
14	0.0604	0.0604	0.0604	0.0604	0.0253	0.0338	0.0425	0.0252	0.0338	0.0338	0.0156	0.0312	0.0156		0.0125	0.0084	0.0390
15	0.0789	0.0789	0.0789	0.0789	0.0428	0.0514	0.0602	0.0426	0.0514	0.0515	0.0168	0.0168	0.0168	0.0168		0.0086	0.0427
16	0.0695	0.0695	0.0695	0.0695	0.0340	0.0425	0.0513	0.0338	0.0425	0.0426	0.0083	0.0083	0.0083	0.0083	0.0083		0.0407
17	0.1771	0.1771	0.1771	0.1771	0.1463	0.1573	0.1679	0.1469	0.1573	0.1566	0.1566	0.1566	0.1566	0.1566	0.1766	0.1665	

of all possible quartet trees were fully resolved, indicating well supported phylogenetic information. However, all the fully resolved quartet trees had the same score (Fig. 4), indicating that a single best represented tree became unpredictable.

Reproductive features

Reproductive traits of the studied species (Table II) were superimposed within the groups defined by the molecular analysis. Species in group A were gonochoric and probably free spawners. The only brooder species in this group was *Myxicola* cf. *sulcata* (Gambi *et al.*, 2001). The morphology of spermatozoa was relatively homogeneous among species (Fig. 5a-d) and with an elongated nucleus, rounded mitochondria and pointed acrosome, except *M.* cf. *sulcata* (Fig. 5a), which had a sub-conical acrosome. Ultrastructure analysis of the acrosomes revealed for both *Myxicola* species a simple structure with three rounded sub-acrosomal spaces in *M.* cf. *sulcata* (Fig. 5a-b), and without internal invagination in *M. infundibulum* (Fig. 5c).

Species in group B were all free spawners; some were gonochoric, such as *Sabella spallanzanii* (Giangrande *et al.*, 2000), while others were simultaneous hermaphrodites with male and female gametes in the same segments, such as *B. luctuosum* (Sordino & Gambi, 1992; Licciano *et al.*, 2001). The spermatozoa within this group presented the highest homogeneous morphology with rounded nucleus, spherical mitochondria, and a small sub-spherical acrosome (Fig. 5e-f). The acrosome had a sub-acrosomal space where the inner membranes were invaginated (Fig. 5e-f).

Species in group C were gonochoric and probably all free spawners, except for *P. antarctica* which is a brooder and simultaneous hermaphrodite with both gametes in the same segments (Gambi & Patti, 1999). These species showed the greatest diversity in sperm morphology. *E. vancouveri*, *S. insignis* and *P. littoralis* had a relatively large acrosome with numerous invaginations concentrated in the upper marginal zone (Fig. 6a-d). *Demonax polarsterni* had a spermatozoa with an external structure similar to species of group B, but with a larger acrosome (Fig. 6e), while *P. antarctica* had a pointed acrosome (Fig. 6f). Finally, *A. mediterranea*, a brooding, simultaneous hermaphrodite with male and female gametes developing in different segments, had highly modified spermatozoon related to storage in spermathecae (Rouse & Gambi, 1998a). The main types of spermatozoa related to the identified group of species are shown in Figure 7.

DISCUSSION

The C1 domain within the Sabellinae subfamily was relatively conservative; however, the 12 informative variable sites identified in the analysis were sufficient for differentiating the closely related taxa studied. The cladogram topologies obtained using the three different

TABLE II - *Reproductive features of most of the studied species of Sabellinae. For* Bispira mariae, Bispira *sp.*, Chone *sp., and* Sabellastarte australiensis *data on reproductive biology are lacking. Oogenesis type: EXT, extraovarian solitary; EXT A, extraovarian with amoebocytes. Spermatozoan type: ENT, ent-aquasperm; ECT, ect-aquasperm. Egg fate: BR IN, intratubular brooding; FS, free spawning; BR EX, extratubular brooding. Developmental mode: LEC, lecithotrophic; DIR, direct. Ecology: I, intertidal; S, sublittoral; BW, brackish-waters; Poll, polluted waters. Distribution: A, Atlantic; M, Mediterranean; RS, Red Sea; An, Antarctic; P, Pacific; NS, North Sea.*

Species	Max. size (mm)	Oogenesis type	Spermat. type	Egg diameter	No. eggs per brood	Egg fate	Develop. mode	Reproductive events	Life span (year)	Ecology	Distribution	Reference
Amphiglena mediterranea	4		ENT	173	5	BR	LEC DIR	Continuous	1	I, S	A, M	Knight-Jones & Bowden (1984),
Branchiomma uctuosum	80	EXT	ECT	150		FS	LEC	Seasonal		BW	RS, M	Sordino & Gambi (1982),
Demonax polarsterni	80		ECT	250		FS?	LEC			S	An	Gambi *et al.* (2001)
Euchone pallida	60		ENT	133		FS?	LEC			S	An	Gambi *et al.* (2001)
Eudistylia vancouveri	250	EXT	ECT	182	658000	FS	LEC			S	P	Mc Euen *et al.* (1983), Eckelbarger (1984), Gambi & Giangrande (unpublished data)
Myxicola infundibulum	110	EXT A		130	58000	FS	LEC	Seasonal		S	P, NS, A, M	Dales (1961), Dean *et al.* (1987)
Myxicola cf *sulcata*	45		ENT	500		BR EX	LEC, DIR?			S	An	Gambi *et al.* (2001)
Perkinsiana antarctica	28		ENT	235	300	BR EX	LEC, DIR?			I, S	An	Gambi & Patti (1999)
Perkinsiana littoralis	200		ECT	140		FS	LEC			S	An	Gambi *et al.* (2000)
Pseudopotamilla occelata	100	EXT		103	75000	FS	LEC			I	A	Rouse & Fitzhugh (1994)
Sabella spallanzanii	400	EXT A	ECT	250	50000	FS	LEC	Seasonal	3 - 4	Poll	M, P	Giangrande & Petraroli (1994); Giangrande *et al.* (2000)
Schizobranchia insignis	100	EXT		108	190000	FS	LEC			Poll	P	Dales (1961), Lee (1977)

algorithms (distance, maximum parsimony, and maximum likelihood) were consistent, notwithstanding some variability in the position of the different taxa within the main groups identified.

The trees obtained with the different analyses discriminated the same main groups, even though with relatively low bootstrap values. Due to the ambiguous position of *A. mediterranea* among the three analyses, group A was the only group with the same structure in all the analyses performed.

An overall similarity between the topologies obtained from molecular analysis and that arising from morphological and morpho-functional analyses (Fitzhugh, 1989; Rouse & Fitzhugh, 1994; Fitzhugh & Rouse, 1999) was observed. The taxa clustering in group A contained the more plesiomorphic genera, while those clustering in group C contained the more apomorphic ones. *Amphiglena mediterranea* appeared closely related to the more plesiomorphic group (A) in the MP and ML trees, and to the more apomorphic group (C) in the distance UPGMA tree. The pattern arising from the distance tree was characterised by higher bootstrap values than that of maximum parsimony; moreover, its configuration was also the closest to that obtained from the morpho-functional analysis, where the genus *Amphiglena* was closely related to *Perkinsiana* (Fitzhugh & Rouse, 1999). Therefore it is probable that the position of *A. mediterranea* among apomorphic genera is the most appropriate one.

In the ML tree, the basal trichotomy of the three main groups, does not solve their relationships, while the polytomies within taxa of groups B and C show that the systematic relationships of these taxa are still unresolved, as occurs in the morpho-functional cladogram. The position of the genus *Perkinsiana,* whose examined species seem more closely related to other genera than

to each other, remains ambiguous. In a recent cladistic analysis performed by Fitzhugh & Rouse (1999) utilizing morpho-functional characters, different species of the genus *Perkinsiana* were similarly located far from each other, *Perkinsiana rubra* was within the clade containing *Eudistylia, Schizobranchia,* and *Pseudopotamilla,* while *Perkinsiana riwo* was closely related to *Potamilla* and *Amphiglena* genera.

The maximum likelihood mapping analysis (Fig. 4), although indicating well-supported phylogenetic information (81% of all possible tree quartets, see Materials and Methods), suggests that a single best represented tree became unpredictable. In other words, the molecular marker utilised was able to discriminate three main groups, whose general topology is informative and well supported, as well as consistent among the different analyses performed, but, the marker is not fully appropriate for solving systematic relationships among and within groups.

Reproductive features of the species analysed can be discussed and compared, to support molecular analyses and better discuss its possible phylogenetic significance. Reproductive characters have been used, in fact, within the Sabellidae, coupled with the morphological ones, to infer systematic relationships (Rouse & Fitzhugh, 1994; Fitzhugh & Rouse, 1999; Rouse, 1999b). However, reproductive features may have strong adaptive implications and should be carefully evaluated for their use in phylogenetic inference. The approach here followed was a superimposition of some reproductive features on the tree obtained from molecular characters which could be equally informative. Based on this, the ultrastructure of the acrosome seems more consistent with systematic relationships than with reproductive strategy of species. For example, some species which show the same reproductive features (external fertilization) and possess spherical spermatozoa have a different acrosome shape. In this respect, closely related taxa show similar acrosomes, like the sperm of *Branchiomma* and *Sabella* species (group B), and those of *Schizobranchia* and *Eudistylia* ones (group C). In particular, the acrosome type of species in the clade containing *Sabella* species (B) shows a uniform pattern: it is small, subspherical, and with a subacrosomal space and membrane invaginations. A similar acrosome structures is found in *Sabella pavonina* (Graebner & Kryvi, 1973),

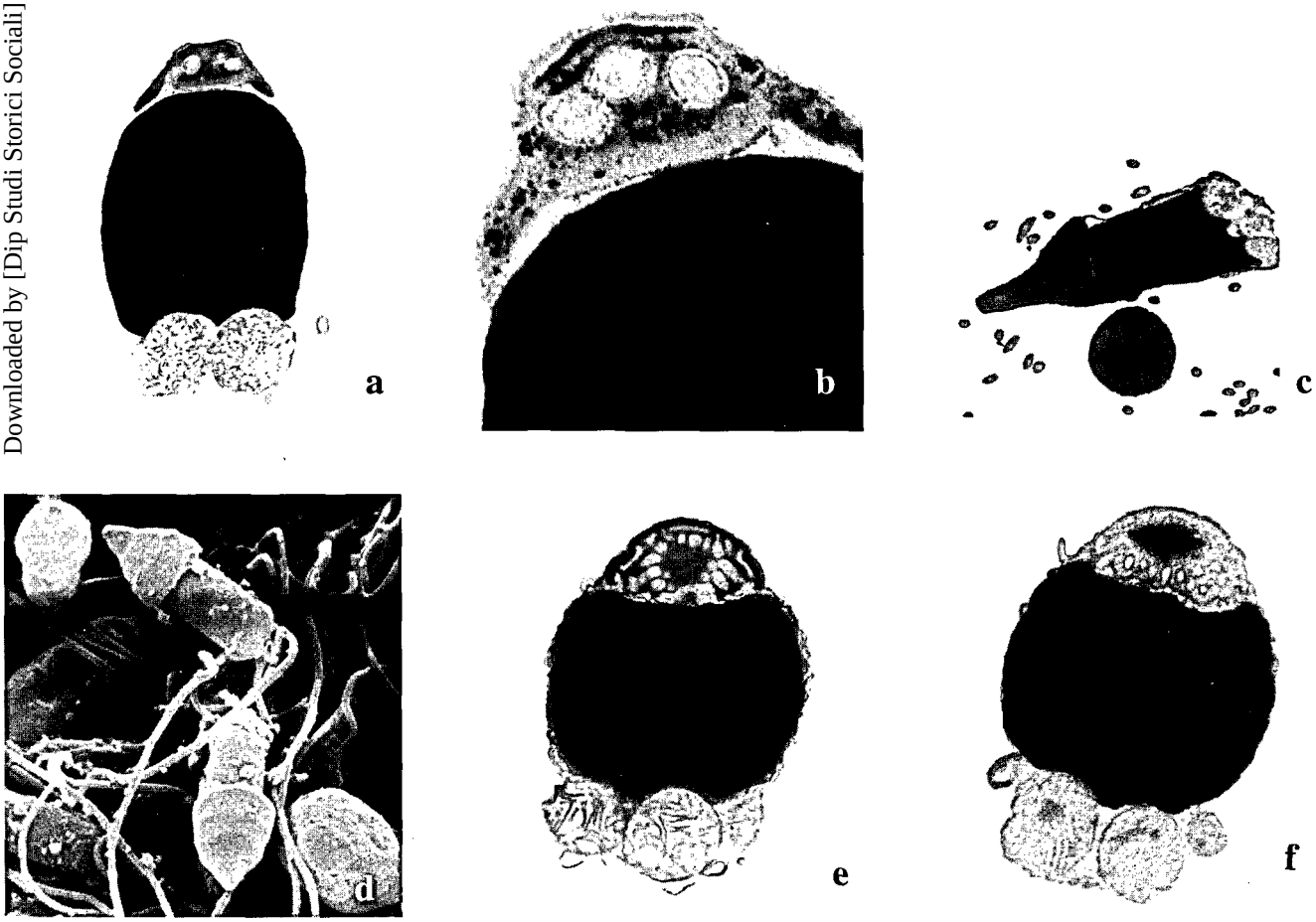

Fig. 5 - Spermatozoa morphology of: **a-b**, *Myxicola* cf *sulcata* TEM (a, entire sperm ×13,000; b, detail of the acrosome, ×36,000); **c**, *Myxicola infundibulum* TEM (×12,000); **d**, *Euchone pallida* SEM (×5,000); **e**, *Sabella spallanzanii* TEM (×28,000); **f**, *Branchiomma luctuosum* TEM (×28,000).

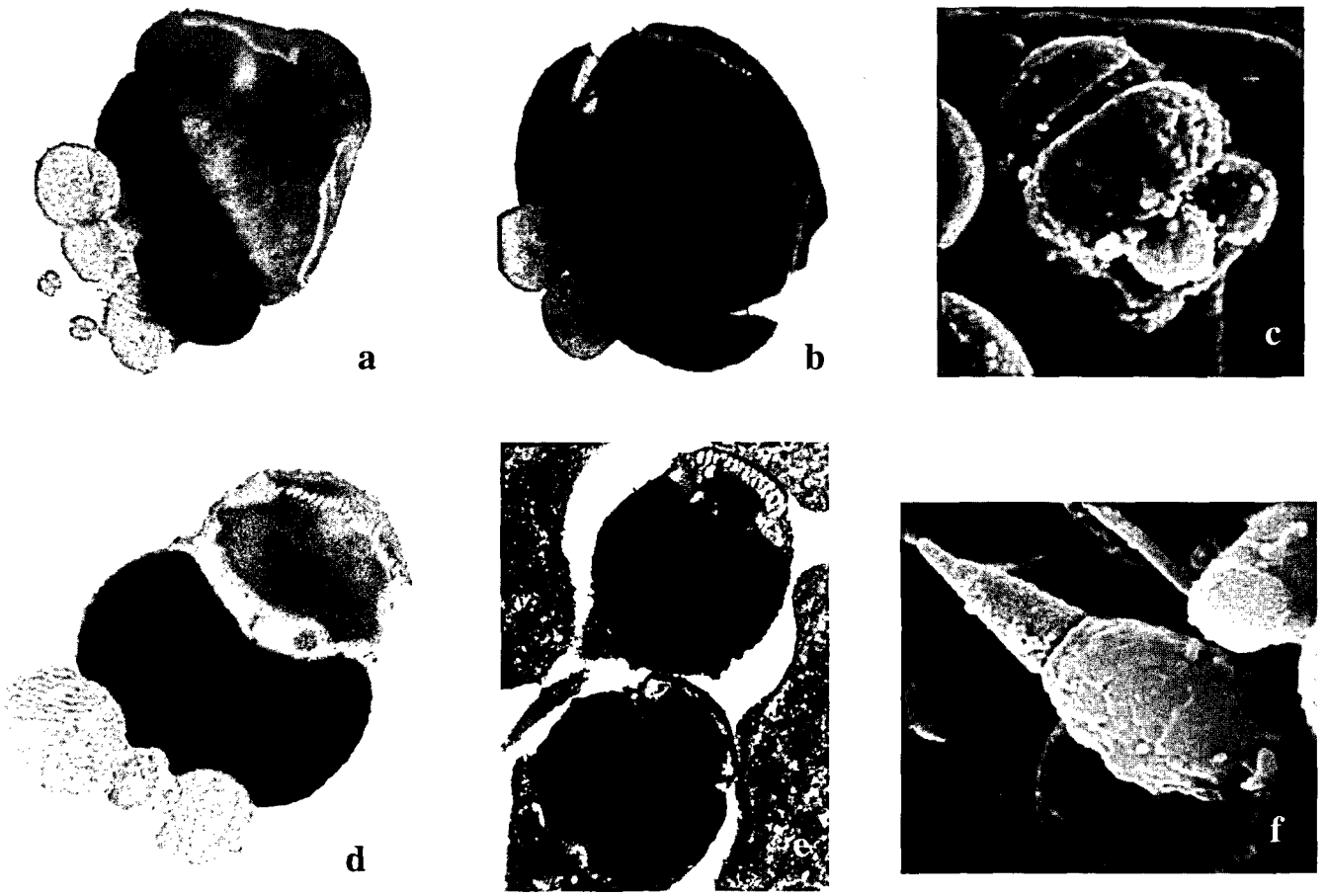

Fig. 6 - Spermatozoa morphology of: **a**, *Eudistylia vancouveri* TEM (×20,000); **b**, *Schizobranchia insignis* TEM (×20,000); **c**, *Demonax polarsterni* SEM (×15,400); **d-e**, *Perkinsiana littoralis* TEM (d, nearly mature sperm, ×22,000; e, mature sperm, ×17,000); **f**, *Perkinsiana antarctica* SEM (×14,800).

Branchiomma nigromaculata, and *Bispira melanostigma* (Rouse, 1999a). The acrosome of the more apomorphic genera *Schizobranchia* and *Eudistylia*, having external fertilisation as well, seems more complex. It is bigger than the nucleus with more regular invaginations concentrated in the upper marginal zone. This kind of structure is also found in *Pseudopotamilla reniformis* (Chugtai, 1986), a genus clustered in the same group C of the molecular tree.

Acrosome morphology could also clarify the relationships within the genus *Perkinsiana*, which is not defined by any synapormophy. Among the species analysed here, *P. littoralis* shows a similarity with *D. polarsterni* (the closest species in molecular trees). In both taxa the acrosome seems to be bigger than that found in species from group B. A completely different acrosome morphology is found in the brooder species *P. antarctica*, whose structure resembles that of *P. rubra*, which is not a brooder form (Chugtai, 1986). Finally, *P. riwo* (Rouse, 1996a) with a reproductive strategy similar to *P. antarctica* (brooding within radioles), has a completely different sperm morphology with a relatively simple subacrosomal canal. The sperm morphology of *P. riwo* seems to be different also from the most closely related genera *Potamilla* and *Amphiglena* (Rouse & Gambi, 1998b). In *Amphiglena*, and probably also in *Potamilla*, spermatozoa are greatly modified and elongated for sperm storage within spermathecae. A similar sperm morphology occurs in *Amphicorina*, the most plesiomorphic genus within Sabellinae. In this case the spermatozoa structure is closely correlated with fertilization biology (Rouse, 1996b).

One of the more plesiomorphic genera considered in the present analysis (*Myxicola*), is characterized by a more simple acrosomal structure, without invaginations, a structure also observed in the closely related genus *Jasmineira* (Rouse, 1999a).

Within broadcasting species a trend of elaboration of the internal structure of the acrosome may be recognised proceeding from the plesiomorphic genera (most of those in group A) to the apomorphic ones (most of those in group C) (see Fig. 7). The trend of increasingly elaborated acrosomes when moving from plesiomorphic to apomorphic genera with rounded spermatozoa was already pointed out by Rouse (1999a). The three groups of taxa, defining the common general pattern in all the molecular analyses performed, are consistent with the trend suggested by previous morpho-functional analyses

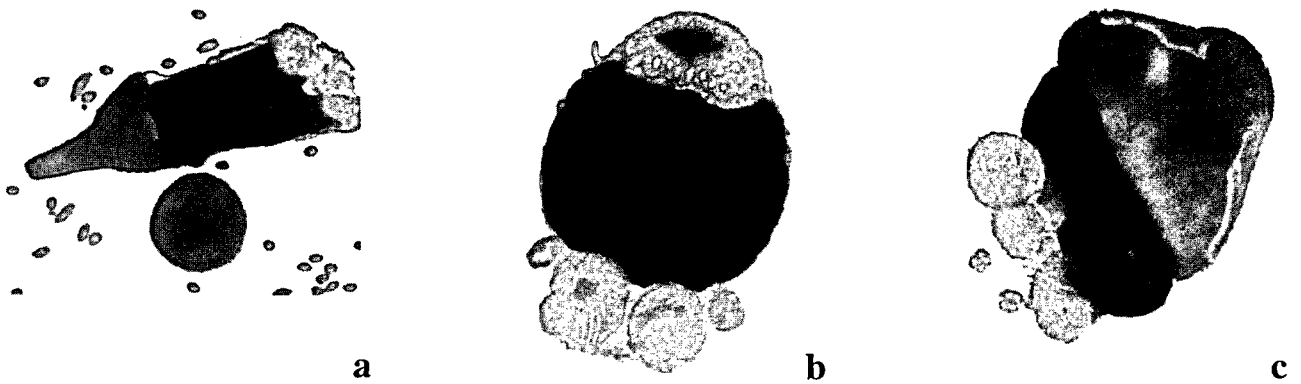

Fig. 7 - Changes of acrosomal internal structure in broadcasting species, proceeding from the more plesiomorphic genera (a) to the more apomorphic one (c): **a**, *Myxicola infundibulum*; **b**, *Branchiomma luctuosum*; **c**, *Schizobranchia insignis*.

which considered reproductive features, including acrosome structure, and morphological characters.

In conclusion, although the present molecular analysis, based on a limited set of species, should be considered only a preliminary screening not fully informative of the fine systematic relationships among the Sabellinae, the pattern here discussed have a heuristic value in increasing the knowledge of polychaete gene sequences, and represents a baseline for future analyses in Sabellinae as well as in other related polychaete clades. On the other hand, the discussion of the molecular general pattern, in the light of morphological and reproductive features of the studied taxa, may increase the reliability of their possible systematic relationships, and the phylogenetic inference of our analyses.

REFERENCES

Bremer K., 1994 - Branch support and tree stability. Cladistics, 10: 295-304.

Chughtai I., 1986 - Fine structure of the spermatozoa in *Perkinsiana rubra* and *Pseudopotamilla reniformis* (Sabellidae: Polychaeta). Acta Zool., 67: 165-171.

Dales R. P., 1961 - The coelomic and peritoneal cell systems of some sabellid polychaetes. Q. J. Microsc. Sci., 102: 327-346.

Dean S. R., Chapman M., Chapman C. S., 1987 - Reproduction and development of the sabellid polychaete *Myxicola infundibulum*. J. Mar. Biol. Assoc. U.K., 67: 431-439.

Famà P., Olsen J. L., Stam W. T., Procaccini G., 2000 - High level of intra- and inter-individual polymorphism in the rDNA ITS1 of *Caulerpa racemosa* (Chlorophyta). Eur. J. Phycol., 35: 349-356.

Feral J-P., Philippe H., Desbruyères D., Laubier L., Derelle E., Chenuil A., 1994 - Phylogénie moléculaire de polychètes Alvinellidae des sources hydrothermales actives de l'océan Pacifique. C. R. Acad. Sci. Paris Sci. Vie, 317: 771-779.

Fitzhugh K., 1989 - A systematic revision of the Sabellidae-Caobangiidae-Sabellongidae complex (Anellida: Polychaeta). Bull. Am. Mus. Nat. Hist., 192: 1-104.

Fitzhugh K., Rouse G. W., 1999 - A remarkable new genus and species of fan worm (Polychaeta: Sabellidae: Sabellinae) associated with marine gastropods. Invertebr. Biol., 118: 357-390.

Gambi M. C., 1999 - Preliminary observations on distribution, biology and feeding ecology of some suspension feeding polychaetes in the Eastern Weddell Sea. Ber. Polarforsch., 301: 83-94.

Gambi M. C., Giangrande A., Patti F. P., 2000 - Comparative observations on reproductive biology of four species of *Perkinsiana* (Polychaeta, Sabellidae). Bull. Mar. Sci., 67: 299-309.

Gambi M. C., Patti F., 1999 - Reproductive biology of *Perkinsiana antarctica* (Kinberg) (Polychaeta, Sabellidae) in the Straits of Magellan (Chile): systematics and ecological implications. Sci. Mar., 63 (suppl.): 253-259.

Gambi M. C., Patti F. P., Micaletto G., Giangrande A., 2001 - Diversity of reproductive features in some Antarctic polynoid and sabellid polychaetes with the description of *Demonax polarsterni* n. sp. (Polychaeta, Sabellidae). Polar Biol., 24: 883-891.

Giangrande A., 1997 - Polychaete reproductive patterns, life cycles and life histories: an overview. Oceanogr. Mar. Biol. Annu. Rev., 35: 323-386.

Giangrande A., Licciano M., Pagliara P., Gambi M. C., 2000 - Gametogenesis and larval development in *Sabella spallanzanii* (Polychaeta, Sabellidae) from the Mediterranean Sea. Mar. Biol., 136: 847-862.

Giangrande A., Petraroli A., 1994 - Observation on reproduction and growth of *Sabella spallanzanii* (Polychaeta, Sabellidae) in the Mediterraenan sea. Mém. Mus. Natl. Hist. Nat. Paris, 162: 51-56.

Graebner I., Kryvi H., 1973 - The spermiogenesis and mature sperm of *Sabella penicillum* (Polychaeta). An electron microscopical investigation. Norw. J. Zool., 21: 211-226.

Jukes T. H., Cantor C. R., 1969 - Evolution of protein molecules. *In*: H. N. Munro (ed.), Mammalian protein metabolism III. Academic Press, New York, pp. 21-132.

Kimura M., 1980 - A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol., 16: 111-120.

Kitching I., Forey P., Humphries C., Williams D., 1998 - Cladistics: the theory and practice of parsimony analysis. The Systematics Association, Oxford.

Knight-Jones P., 1983 - Contributions to the taxonomy of Sabellidae (Polychaeta). Zool. J. Linn. Soc., 79: 245-295.

Knight-Jones P., Bowden N., 1984 - Incubation and scissiparity in Sabellidae (Polychaeta). J. Mar. Biol. Assoc. U.K., 64: 809-818.

Knight-Jones P., Perkins T. H., 1998 - A revision of *Sabella*, *Bispira* and *Stylomma* (Polychaeta: Sabellidae). Zool. J. Linn. Soc., 123: 385-467.

Lee Y. R., 1977 - Oogenesis in tubicolous polychaetes *Schizobranchia insignis* Bush. I Microscopy and biometric studies. Korean J. Zool., 20: 77-92.

Lenaers G., Bhaud M., 1992 - Molecular phylogeny of some annelids: an initial approach to the Atlantic-Mediterranean speciation problem. J. Mol. Evol., 35: 429-435.

Licciano M., Giangrande A., Gambi M. C., 2001 - Reproduction and simultaneous hermaphroditism in *Branchiomma luctuosum* (Polychaeta: Sabellidae) from Mediterranean Sea. Invertebr. Biol., 121: 55-65.

McEuen F. S., Wu B. L., Chia F. S., 1983 - Reproduction and development of *Sabella media*, a sabellid polychaete with extratubular brooding. Mar. Biol., 76: 301-309.

McHugh D., 1997 - Molecular evidence that echiurans and pogonophorans are derived annelids. Proc. Natl. Acad. Sci. U.S.A., 94: 8006-8009.

McHugh D., 2000 - Molecular phylogeny of the Annelida. Can. J. Zool., 78: 1873-1884.

Patti F. P., 2001 - Modeltest WinFrontend version 1b. Distributed by the Author. Organismic and Evolutionary Biology, Harvard University, Cambridge (Mass.).

Patti F. P., Gambi M. C., 2001 - Phylogeography of the invasive polychaete *Sabella spallanzanii* (Gmelin) (Sabellidae) based on the nucleotide sequence of internal transcribed spacer 2 (ITS2) of nucler DNA. Mar. Ecol. Prog. Ser., 215: 169-177.

Perkins T. H., 1984 - Revision of *Demonax* Kinberg, *Hypsicomus* Grube, and *Notaulax* Tauber, with a review of *Megalomma* Johansson from Florida (Polychaeta: Sabellidae). Proc. Biol. Soc. Wash., 97: 285-368.

Philippe H., 2000 - Must version 2000 beta1. Distributed by the Author. UMR 7622, Université Pierre et Marie Curie, Paris.

Posada D., Crandall K. A., 1998 - Modeltest: testing the model of DNA substitution. Bioinformatics, 14: 817-818.

Rouse G. W., 1995 - Is sperm ultrastructure useful in polychaete systematics? An example using 20 species of Fabriciinae (Sabellidae, Polychaeta). Acta Zool., 76: 57-74.

Rouse G. W., 1996a - A new species of *Perkinsiana* (Sabellidae, Polychaeta) from Papua-New Guinea; with a description of larval development. Ophelia, 45: 101-114.

Rouse G. W., 1996b - Variability of sperm storage by females in the Sabellidae and Serpulidae (Polychaeta). Zoomorphology, 116: 179-193.

Rouse G. W., 1999a - Polychaeta, including Pogonophora and Myzostomida. *In*: K. G. Ayoldi, G. Ayolgi & B. G. M. Jamieson (eds), Reproductive biology of invertebrate. Oxford and IBH Publishing CO.PVT.LTD, New Delhi & Calcutta, pp. 82-124.

Rouse G. W., 1999b - Polychaete sperm: phylogeny and functional considerations. Hydrobiologia, 402: 215-224.

Rouse G. W., Fauchald K., 1997 - Cladistics and polychaetes. Zool. Scr., 26: 139-204.

Rouse G. W., Fitzhugh K., 1994 - Broadcasting fables: Is external fertilization really primitive? Sex, size and larvae in sabellid polychaetes. Zool. Scr., 23: 271-312.

Rouse G. W., Gambi M. C., 1998a - Sperm ultrastructure and spermathecal structure in *Amphiglena* spp. (Polychaeta: Sabellidae). Invertebr. Biol., 117: 114-122.

Rouse G. W., Gambi M. C., 1998b - Evolution of reproductive features and larval development in *Amphiglena* Claparède (Polychaeta: Sabellidae). Mar. Biol., 131: 743-754.

Sambrook J., Fritsch E. F., Maniatis T., 1989 - Molecular cloning, 2nd ed. Cold Spring Harbor Lab Press, Cold Spring Harbor (NY).

Schmidt H., Westheide W., 1999 - Genetic relationships (RAPD--PCR) between geographically separated populations of the interstitial polychaete *Hesionides gohari* (Hesionidae) and the evolutionary origin of the freshwater species *Hesionides riegerorum*. Biol. Bull., 196: 216-226.

Sordino P., Gambi M. C., 1992 - Prime osservazioni su biologia riproduttiva e sul ciclo vitale di *Branchiomma luctuosum* (Grube, 1869) (Polychaeta, Sabellidae). Oebalia, 17 (suppl.): 425-427.

Sorenson M. D., 1999 - TreeRot version 2. Boston University, Boston (Mass.).

Strimmer K., von Haeseler A., 1996 - Quartet puzzling. A quartet maximum-likelihood method for reconstructing tree topologies. Mol. Biol. Evol., 13: 964-969.

Strimmer K., von Haeseler A., 1997 - Likelihood-mapping: A simple method to visualize phylogenetic content of a sequence alignment. Proc. Natl. Acad. Sci. U.S.A., 94: 6815-6819.

Swofford D. L., 2000 - PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4.0. Sinauer Associates, Sunderland (Mass.).

Thompson J. D., Gibson T. J., 1998 - ClustalX version 1.64b. Distributed by the Authors. European Molecular Biology Laboratory, Heidelberg.

Thompson J. D., Higgins D. G., Gibson T. J., 1994 - ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res., 22: 4673-4680.

Von Soosten C., Schmidt H., Westheide W., 1998 - Genetic variability and relationships among geographically widely separated populations of *Petitia amphophthalma* (Polychaeta: Syllidae). Results from RAPD-PCR investigations. Mar. Biol., 131: 659-669.

Zhaxybayeva O., Gogarten J. P., 2002 - Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. BMC Genomics, 3: 1-15.