

# Molecular phylogeny of oligotrich genera *Omegastrombidium* and *Novistrombidium* (Protozoa, Ciliophora) for the systematical relationships within Family Strombidiidae\*

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**Abstract** The phylogeny of the oligotrich ciliates is currently a hot debate despite the availability of both morphological and molecular data. In the present paper, further small subunit rRNA (SS rRNA) genes were analyzed from the Genera *Omegastrombidium* and *Novistrombidium*, as well as from *Strombidium*, and combined with three new SS rRNA sequences from *Strombidium basimorphum*, *S. sulcatum* population QD-1, and *Novistrombidium testaceum* population GD. The phylogenetic positions of these organisms were inferred using Bayesian inference, Maximum Likelihood, and Maximum Parsimony methods. The main results are: (1) the SS rRNA gene sequence analyses match the recent findings about the molecular evolution of oligotrichs, indicating that the family Strombidiidae is paraphyletic; (2) the Genus *Omegastrombidium* is separated from the Genus *Strombidium*, as shown in recent cladistic analyses; (3) morphospecies in Genus *Novistrombidium*, based on similarity of somatic ciliature, are separated from each other in all topological trees, indicating that this genus could be a paraphyletic group; (4) the molecular data indicate a possibility of parafyly for the genus *Strombidium*; and (5) the similarities of the SS rRNA gene of specimens identified as *S. sulcatum* and *S. inclinatum* are 99.8%–100%. However, present knowledge on the oligotrichs sensu stricto is still insufficient and further studies based on both molecular and other technologies are required.

**Keyword:** *Omegastrombidium*; *Novistrombidium*; *Strombidium*; SS rRNA; phylogeny

## 1 INTRODUCTION

Oligotrichs are an important component of the marine energy flux due to their high abundance and growth rate (Pierce et al., 1992). About 200 oligotrich taxa are known, of which 60% have been described or re-described, using silver impregnation methods (Agatha, 2004a; Liu et al., 2009; Lynn et al., 1993; Modeo et al., 2003; Montagnes et al., 1990; Song et al., 2000;). In a recent classification (Lynn, 2008), the oligotrichs were divided into two subclasses; the Oligotrichia (oligotrichs sensu stricto) and the Choreotrichia, while the halteriids were affiliated with the stichotrichs. Although several gene sequences and cladistic analyses have been

conducted in the past several years (Agatha, 2004a, b; Agatha et al., 2007; Modeo et al., 2003; Snoeyenbos-West et al., 2002; Strüder-Kypke et al., 2003, 2008), the phylogenetic relationships among the families and genera are still uncertain, especially within the subclass Oligotrichia.

The probable main reasons for these uncertainties are the undersampling in the molecular genealogies, methodological constraints, and/or an insufficient knowledge about the taxa in the cladistic analyses.

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Hence, we used new SS rRNA gene sequences to analyse the phylogenetic position of the rarely discussed genera *Omegastrombidium* and *Novistrombidium* and the relationships among the oligotrichs.

## 2 MATERIALS AND METHODS

### 2.1 Sampling, species identification and the terminology

*Novistrombidium testaceum* population GD and *Strombidium basimorphum* were collected from the Daya Bay, Guangdong (22°42'N, 114°32'E; southern China) in 2008. The *S. sulcatum* population QD-1 was collected from the coast of Qingdao (36°08'N, 120°43'E; northern China) in 2003. The specimens were investigated in-vivo and impregnated with protargol following the methods of Wilbert (1975). Species identifications were based on Song et al. (1998) for *N. testaceum*, Granda et al. (2003) for *Strombidium sulcatum*, and Martin et al. (1993) for *S. basimorphum*.

The population identified as *S. sulcatum* in the present paper matches the authoritative redescription by Granda et al. (2003), except for the macronucleus, which lacked an indentation, and the apical protrusion, which was recognizable only in live specimens.

The terminology and systematic classification used are according to Lynn (2008). Additionally, to avoid confusion, the name of one *Strombidium* species, *S. styliferum*, used in the present paper is here re-defined as *S. stylifer*. The name “stylifer” is the correct spelling of the specific epithet, following the original description by Levander (1894), who used the Greek composite *stylifer*. According to Article 31.2.2. of the ICZN (1999), the original spelling is to be retained and the species-group name has to be treated as a noun in apposition without changing the gender.

### 2.2 DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted using standard methods, as suggested by Yi et al., (2009a), for *Strombidium sulcatum* population QD-1 and REExtract-N-Amp Tissue PCR Kit (Sigma, St. Louis, USA) with modifications, suggested by Gong et al. (2009), for *S. basimorphum* and *Novistrombidium testaceum* population GD. PCR reactions and sequencing were according to the methods described by Miao et al. (2009) and Yi et al. (2008, 2009b). The SS rRNA gene sequence analyses

comprised 55 spirotrichs and the karyorelictid, *Loxodes magnus* (Table 1). Sequences from *Omegastrombidium elegans*, *Strombidium apolatum*, *S. stylifer*, *S. sulcatum* were obtained from the NCBI database and were sequenced by the co-author, Yi Zhenzhen. Additionally, these four species were also collected from the coast of Qingdao and were identified by the co-author Xu Dapeng. Sequences of *Novistrombidium sinicum*, *N. orientale*, *Pseudotontonia simplicidens*, *Spirostrombidium* sp., *Spirotontonia turbinata*, and *Strombidium conicum* were provided by Gao et al. (2009). The newly deposited SS rRNA sequences are: *Strombidium basimorphum*, FJ480419; *S. sulcatum* population QD-1, FJ377546; *Novistrombidium testaceum* population GD, FJ377547 (Table 1).

### 2.3 Phylogenetic analyses

The sequences were aligned using the ClustalW program implemented in Bioedit 7.0.0 with default settings (Hall, 1999). The alignment was further manually modified, and 1 834 characters were used in the final analyses. Three different methods were employed to compute the phylogenetic trees; Bayesian inference (BI), Maximum Likelihood (ML), and Maximum Parsimony (MP). The program MrModeltest 3.7 (Nylander, 2004) selected the GTR+I+G as the best model with the Akaike Information criterion (AIC), which was then used for the Bayesian and Maximum Likelihood inferences. The Bayesian inference analysis was performed with MrBayes 3.1.2 (Ronquist et al., 2003). Four simultaneous Markov Chain Monte Carlo algorithms (MCMC) were run for 1 000 000 generations, sampling every 100th generation. The first 2 500 trees were discarded as burn-in. The remaining trees were used to calculate the posterior probabilities, applying the majority rule consensus. A Maximum Likelihood (ML) tree was constructed with the PhyML V2.4.4 program (Guindon et al., 2003). The reliability of the internal branches was assessed using a non-parametric bootstrap method with 1 000 replicates, a variable-site gamma distribution shape parameter (G) of 0.39, and a proportion of invariable sites (I) of 0.28. The Maximum Parsimony analysis was performed with the software package PAUP\* 4.0b10 (Swofford, 2002), and support for the internal branches was estimated using the bootstrap method with 1 000 replicates (Felsenstein, 1985).

The support of the dataset for the monophyly of the family Strombidiidae, genus *Strombidium* and genus *Novistrombidium* was evaluated using A-U (AU) tests (Shimodaira, 2002). The Bayesian inference

**Table 1 Small subunit rRNA gene sequences from GenBank used in this study**

Species name	GB number	Species name	GenBank No.	Species name	GenBank No.
<i>Aspidisca steini</i>	AF305625	<i>Onychodromus quadricornutus</i>	X53485	<i>Strombidinopsis acuminata</i>	AJ877014
<i>Certesias quadrinuclata</i>	DQ059581	<i>Oxytricha granulifera</i>	X53486	<i>Strombidinopsis jeokjo</i>	AJ628250
<i>Codonella</i> sp.	DQ487193	<i>Oxytricha nova</i>	X03948	<i>Strombidium apolatum</i>	DQ662848
<i>Codonellopsis americana</i>	AY143571	<i>Parallelostrombidium</i> sp.	FJ422991	<i>Strombidium inclinatum</i>	AJ488911
<i>Diophrys appendiculata</i>	AY004773	<i>Parastrombidinopsis minima</i>	DQ393786	<i>Strombidium purpureum</i>	U97112
<i>Euplotes elegans</i>	DQ309868	<i>Parastrombidinopsis shimi</i>	AJ786648	<i>Strombidium stylifer</i>	DQ631805
<i>Euplotes rariseta</i>	AF492706	<i>Pelagostrombidium neptuni</i>	AY541683	<i>Strombidium sulcatum</i>	DQ777745
<i>Euplotidium arenarium</i>	Y19166	<i>Pseudotontonia simplicidens</i>	FJ422993	<i>Strombidium sulcatum</i> -QD-1	FJ377546
<i>Eutintinnus pectinis</i>	AY143570	<i>Phacodinium metchnikoffi</i>	AJ277877	<i>Strombidium basimorphum</i>	FJ423447
<i>Favella ehrenbergii</i>	AF399164	<i>Protocruzia adherens</i>	AY217727	<i>Strombidium conicum</i>	FJ422992
<i>Gonostomum strenuum</i>	AJ310493	<i>Protocruzia contrax</i>	DQ190467	<i>Stylonychia lemnae</i>	AM260994
<i>Halteria grandinella</i>	AF194410	<i>Pseudokeronopsis bergeri</i>	DQ777742	<i>Stylonychia mytilus</i>	EF535730
<i>Laboea strobila</i>	AF399154	<i>Pseudokeronopsis rubra</i>	DQ640314	<i>Tintinnidium mucicola</i>	AY143563
<i>Laurentiella strenua</i>	AJ310487	<i>Rhabdonella hebe</i>	AY143566	<i>Tintinnopsis beroidea</i>	EF123709
<i>Loxodes magnus</i>	L31519	<i>Rimostrombidium lacustris</i>	DQ986131	<i>Tintinnopsis tocatinensis</i>	AY143561
<i>Metacylis angulata</i>	AY143568	<i>Novistrombidium sinicum</i>	FJ422989	<i>Urostyla granulifera</i>	AF164129
<i>Novistrombidium testaceum</i>	AJ488910	<i>Novistrombidium orientale</i>	FJ422988	<i>Varistrombidium kielum</i>	DQ811090
<i>Novistrombidium testaceum</i> -GD	FJ377547	<i>Spirotontonia turbinata</i>	FJ422994		
<i>Omegastrombidium elegans</i>	EF486862	<i>Strombidium caudatum</i>	AY143573		

tree was generated with a constraint block, enforcing the constraint of focal group monophyly in MrBayes.

PHYLIP Version 3.67 (Felsenstein, 2007) was applied to calculate the sequence similarity and the evolutionary distances between pairs of nucleotide sequences, using the Kimura (1980) two-parameter model.

### 3 RESULTS

#### 3.1 Comparison of SS rRNA gene sequences

The SS rRNA genetic similarities and distances for the oligotrichs sensu stricto are provided in Table 2. The *Strombidium* species deviate from each other by up to 81.0%. The similarities between *Omegastrombidium elegans* and the *Strombidium* species are 92.1%–95.5%, while the similarities of *O. elegans* to the other strombidiid genera are 94.1%–96.6%. The similarity between the two populations of *Novistrombidium testaceum* is 98.8%, while the intrageneric similarities of *Novistrombidium* are 94.3%–96.7%. The similarities of *Novistrombidium* to the other strombidiid genera are 89.7%–97.8%. Interestingly, the similarities between populations identified as *Strombidium inclinatum* by Modeo et al. (2003) and the two populations determined as *S. sulcatum* in the present paper (see above) are high, i.e., 100% and 99.8%.

#### 3.2 Phylogenetic analyses (Figs.1, 2)

The Bayesian inference and maximum likelihood

trees were nearly identical in their topology, and were thus combined in a single tree (Fig.1). The maximum parsimony tree is also shown, including the support values for the internal nodes (Fig.2).

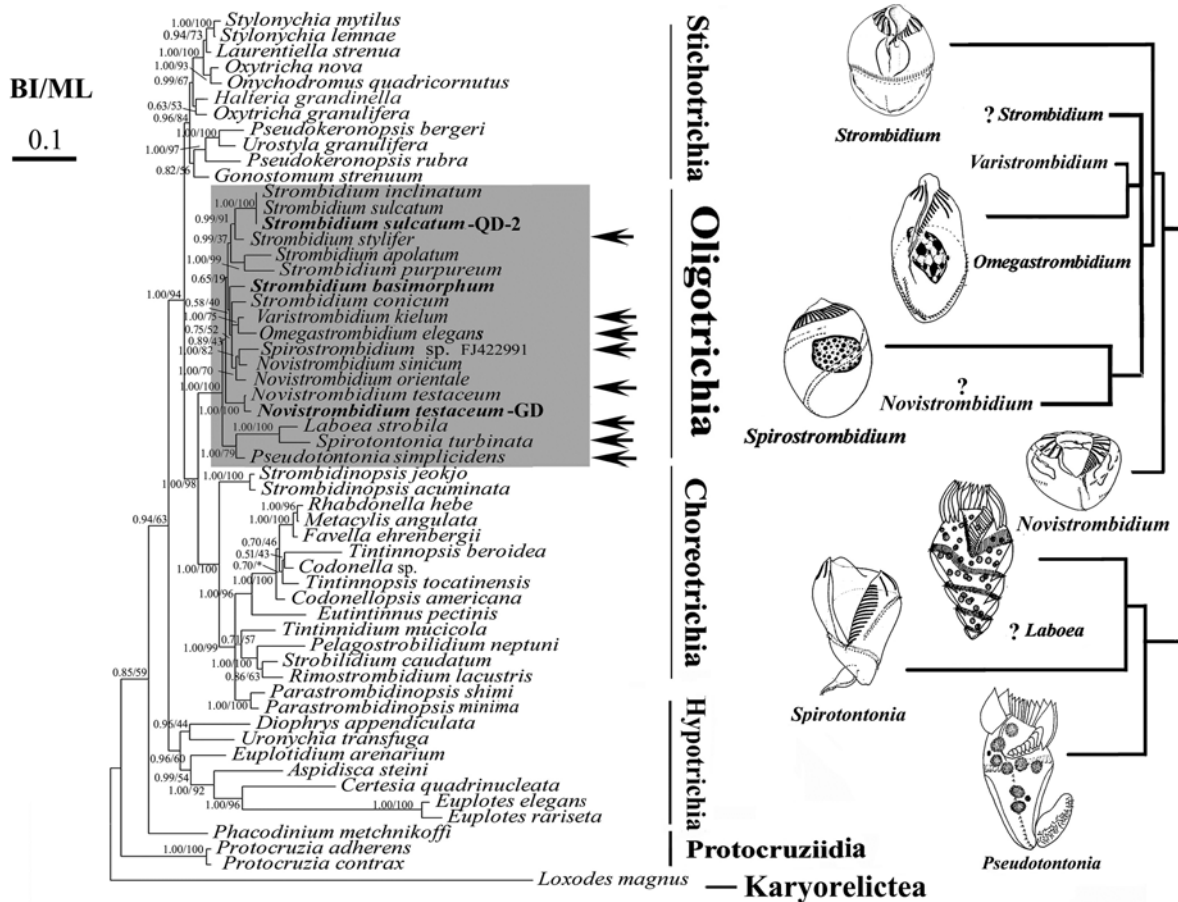
In all the analyses, the monophyly of the choreotrichs is supported by high values (1.00 BI, 100% ML, 99% MP). Likewise, the oligotrichs sensu stricto form a well-supported monophylum (1.00 BI, 100% ML, 99% MP), while the genus *Halteria* groups with the stichotrich ciliate *Oxytricha granulifera*. The family Strombidiidae is paraphyletic, as the monotypic genus *Laboea* is affiliated with the family Tontoniidae (Agatha, 2004); thus, it represents the adelphotaxon of *Spirotontonia*, both clustering with *Pseudotontonia* as a well-supported branch (1.00 BI, 100% ML, 90% MP) basal to the other oligotrichs sensu stricto.

*Omegastrombidium elegans* and *Varistrombidium kielum* cluster with high or moderate supports (1.00 BI, 75% ML, 76% MP) in the phylogenetic analyses. While their relationships with *Strombidium conicum* and a cluster formed by *Spirostrombidium*, *Novistrombidium sinicum*, and *N. orientale* are unresolved in the Bayesian Inference and maximum likelihood trees (Fig. 1), *Omegastrombidium elegans* and *Varistrombidium kielum* form a less-supported cluster with *Novistrombidium testaceum* in the maximum parsimony analysis (Fig.2). However, none of these topologies is well supported (0.75 BI, 52% ML, 28% MP).

**Table 2 Similarity (lower half) and evolutionary distance (upper half) among SS rRNA gene sequences of oligotrichs sensu stricto. For sequence availability, see ‘MATERIALS AND METHODS’**

	<i>L.stro</i>	<i>P.sin</i>	<i>S.tur</i>	<i>O.ele</i>	<i>V.kie</i>	<i>N.tes1</i>	<i>N.tes2</i>	<i>N.sin</i>	<i>N.ori</i>	<i>S.sp</i>	<i>S.inc</i>	<i>S.sul1</i>	<i>S.sul2</i>	<i>S.syl</i>	<i>S.apo</i>	<i>S.pur</i>	<i>S.coi</i>	<i>S.bas</i>
<i>L.stro</i>	–	0.079	0.050	0.103	0.086	0.100	0.103	0.097	0.098	0.098	0.102	0.102	0.105	0.097	0.110	0.113	0.095	0.110
<i>P.sin</i>	0.921	–	0.090	0.054	0.038	0.054	0.062	0.050	0.063	0.049	0.058	0.058	0.061	0.054	0.083	0.078	0.045	0.052
<i>S.tur</i>	0.950	0.910	–	0.102	0.097	0.100	0.103	0.091	0.098	0.100	0.100	0.100	0.103	0.101	0.120	0.113	0.097	0.112
<i>O.ele</i>	0.897	0.946	0.898	–	0.036	0.059	0.063	0.036	0.049	0.034	0.056	0.056	0.056	0.045	0.072	0.080	0.042	0.058
<i>V.kie</i>	0.914	0.962	0.903	0.964	–	0.040	0.046	0.025	0.044	0.023	0.045	0.045	0.047	0.042	0.059	0.065	0.026	0.036
<i>N.tes1</i>	0.900	0.946	0.900	0.941	0.960	–	0.010	0.047	0.054	0.041	0.054	0.054	0.056	0.045	0.067	0.075	0.040	0.057
<i>N.tes2</i>	0.897	0.938	0.897	0.937	0.954	0.990	–	0.049	0.057	0.048	0.058	0.058	0.060	0.049	0.069	0.074	0.045	0.064
<i>N.sin</i>	0.903	0.950	0.909	0.964	0.975	0.953	0.951	–	0.033	0.022	0.048	0.048	0.051	0.046	0.069	0.067	0.030	0.044
<i>N.ori</i>	0.902	0.937	0.902	0.951	0.956	0.946	0.943	0.967	–	0.036	0.043	0.043	0.045	0.045	0.070	0.071	0.028	0.040
<i>S.sp</i>	0.902	0.951	0.900	0.966	0.977	0.959	0.952	0.978	0.964	–	0.061	0.061	0.064	0.056	0.081	0.076	0.043	0.047
<i>S.inc</i>	0.898	0.942	0.900	0.944	0.955	0.946	0.942	0.952	0.957	0.939	–	0.000	0.002	0.040	0.075	0.079	0.035	0.051
<i>S.sul1</i>	0.898	0.942	0.900	0.944	0.955	0.946	0.942	0.952	0.957	0.939	1.000	–	0.002	0.040	0.075	0.079	0.035	0.051
<i>S.sul2</i>	0.895	0.939	0.897	0.944	0.953	0.944	0.940	0.949	0.955	0.936	0.998	0.998	–	0.043	0.075	0.081	0.038	0.053
<i>S.syl</i>	0.903	0.946	0.899	0.955	0.958	0.955	0.951	0.954	0.955	0.944	0.960	0.960	0.957	–	0.066	0.073	0.039	0.053
<i>S.apo</i>	0.890	0.917	0.880	0.928	0.941	0.933	0.931	0.931	0.930	0.919	0.925	0.925	0.925	0.934	–	0.067	0.060	0.078
<i>S.pur</i>	0.887	0.922	0.887	0.920	0.935	0.925	0.926	0.933	0.929	0.924	0.921	0.921	0.919	0.927	0.933	–	0.068	0.070
<i>S.coi</i>	0.905	0.955	0.903	0.958	0.974	0.960	0.955	0.970	0.972	0.957	0.965	0.965	0.962	0.961	0.940	0.932	–	0.040
<i>S.bas</i>	0.890	0.948	0.888	0.942	0.964	0.943	0.936	0.956	0.960	0.953	0.949	0.949	0.947	0.947	0.922	0.930	0.960	–

Abbreviations: *L.stro*: *Laboea strobila*; *P.sin*: *Pseudotonia simplicidens*; *S.tur*: *Spirotonia turbinata*; *O.ele*: *Omegastrombidium elegans*; *V.kie*: *Varistrombidium kielum*; *N.tes1*: *Novistrombidium testaceum*; *N.tes2*: *Novistrombidium testaceum* population GD; *N.sin*: *Novistrombidium sinicum*; *S.sp*: *Spirostrombidium sp.*; *N.ori*: *Novistrombidium orientale*; *S.inc*: *Strombidium inclinatam*; *S.sul1*: *Strombidium sulcatum*; *S.sul2*: *Strombidium sulcatum* population QD-1; *S.syl*: *Strombidium sylifer*; *S.apo*: *Strombidium apotatum*; *S.pur*: *Strombidium purpureum*; *S.coi*: *Strombidium conicum*; and *S.bas*: *Strombidium basimorphum*. Gray shading: similarity between *Omegastrombidium* and *Strombidium* species; similarity between *Novistrombidium* and *Strombidium* species; similarity between *Novistrombidium testaceum* populations; and greatest genetic distance among *Strombidium* species and *Novistrombidium* species



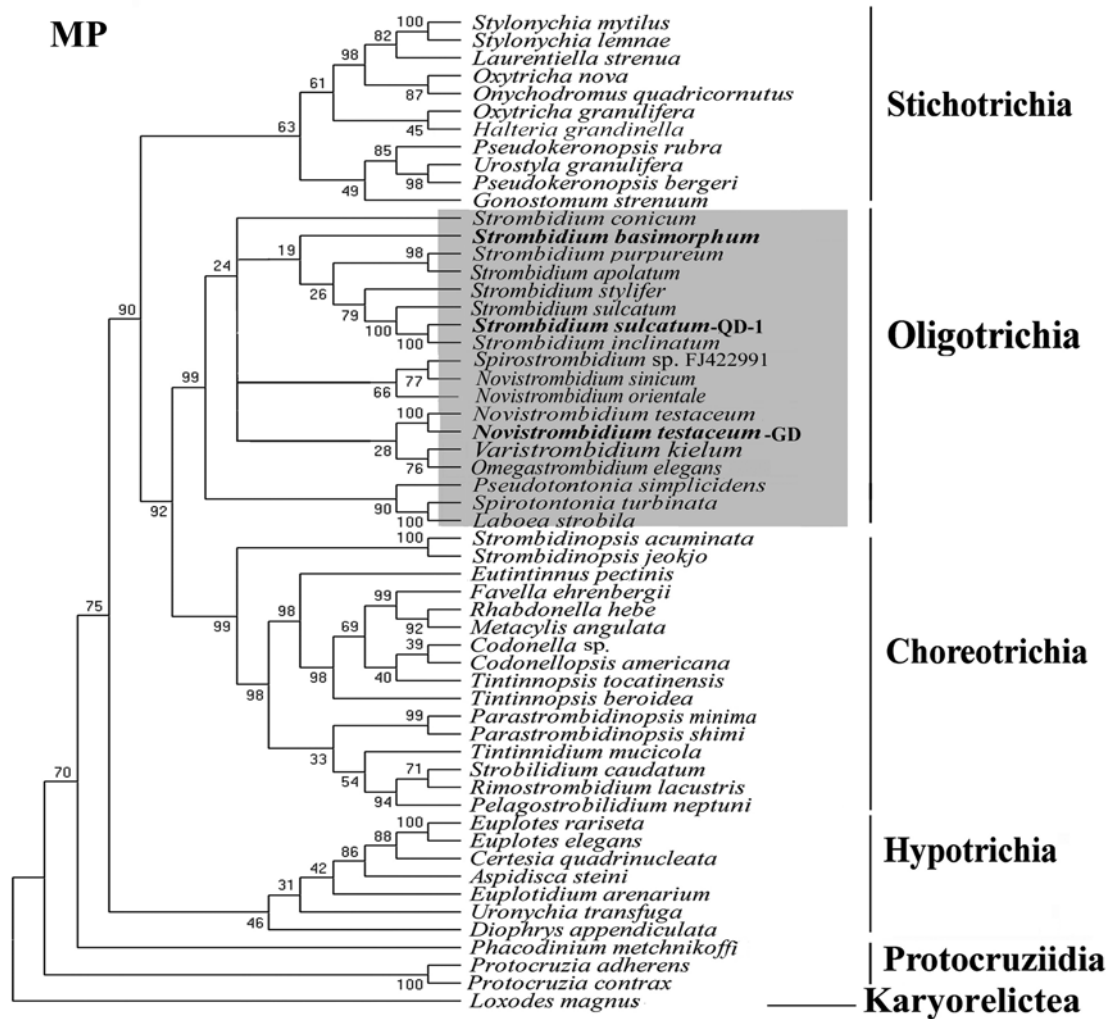
**Fig.1** Phylogenetic tree, based on SS rRNA genes, indicating the relationships among the subclass Oligotrichia by the Bayesian inference (BI) and maximum likelihood (ML) algorithms, applying the GTR + G + I model

The ciliary patterns of the species marked by arrows are shown in the right tree (Lei et al., 1999; Lynn et al., 1993; Montagnes et al., 1988; Petz et al., 1995; Song et al., 1998; Song et al., 2000). Numbers near branches represent the posterior probability value of the BI analysis and the bootstrap value for the ML analysis. ‘\*’ reflects disagreement between the two phylogenies. The scale bar corresponds to 10 substitutions per 100 nucleotides positions. Sequences newly submitted are in bold

*Novistrombidium* is a paraphyletic genus. Two populations of *Novistrombidium testaceum* cluster together with highest support values (1.00 BI, 100% ML, 100% MP). Their divergence of 1.2% corresponds to small morphological differences (Modeo et al., 2003). However, *Novistrombidium sinicum* firstly forms a branch with *Spirostrombidium* sp. with high or moderate supports (1.00 BI, 82% ML, 79% MP), then cluster with *N. orientale* (1.00 BI, 70% ML, 66% MP). *Novistrombidium testaceum* represents the basal branch of the Strombidiidae (excluding *Laboea*; see above) in the Bayesian inference and maximum likelihood trees, while other *Novistrombidium* spp. cluster more closely to the other strombidiid genera. In the maximum parsimony analysis, the relationships of the cluster formed by *Spirostrombidium*, *Novistrombidium sinicum*, and *N. orientale* are unresolved.

Genus *Strombidium* is also paraphyletic, as *S.*

*basimorphum* and *S. conicum* cluster more closely to the other strombidiid genera (Figs.1, 2). However, the positions of *Strombidium conicum* and *S. basimorphum* differ depending on the algorithms applied (Figs.1, 2); they branch basally to the congeners in the Bayesian inference and maximum likelihood analyses (0.75 BI, 52% ML), while they represent a sister group to the other strombidiid genera (excluding *Laboea*, see above) with a low bootstrap value in the maximum parsimony tree (19% MP). The intimate relationship between the specimens identified as *S. sulcatum* in the present paper and as *S. inclinatum* by Modeo et al. (2003) is indicated by all phylogenetic analyses (1.00 BI, 100% ML, 100% MP; Figs.1, 2). This cluster forms, together with *S. stylifer*, a highly or moderately supported sister group (0.99 BI, 91% ML, 79% MP) to the highly corroborated cluster of *S. purpureum* and *S. apolatum* (100 BI, 99% ML, 98% MP). However,



**Fig.2** Maximum parsimony phylogeny of the SS rRNA gene sequences indicating the relationships among the subclass Oligotrichia. Numbers at the nodes are values generated from 1 000 bootstrap replicates. Sequences newly submitted are in bold

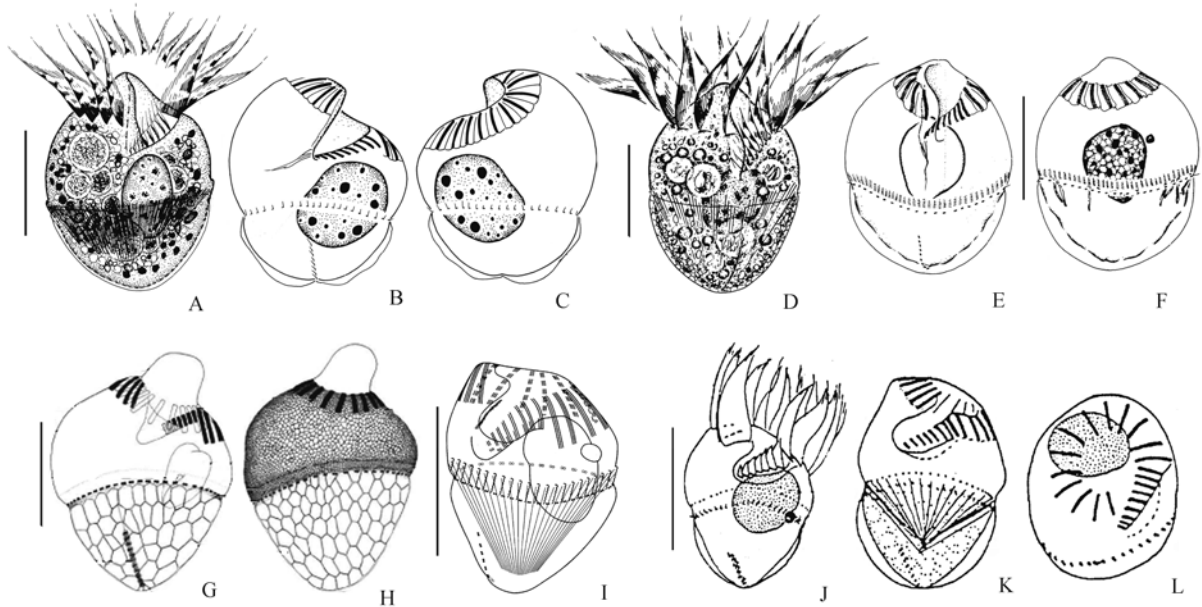
only the Bayesian inference analysis (0.99 BI, 37% ML, 26% MP) strongly supports monophyly of this association.

#### 4 DISCUSSION

Based on the presence of a long and conspicuously contractile tail, Agatha (2004b) separated the family Tontoniidae from the tail-less family Strombidiidae. However, this scheme is only partly corroborated by our phylogenetic trees. The present analyses confirm the monophyly of the order Strombidiida, whereas the family Strombidiidae is paraphyletic in both our study and in a previous investigation (Gao et al., 2009). High values support an affiliation of the tail-less genus *Laboea* with the tontoniid ciliates; it forms an adelphotaxon to *Spirotontonia* and this cluster represents a sister group to *Pseudotontonia*. In addition, the AU test strongly rejects the monophyly of the family Strombidiidae (AU<0.005) by the inclusion of the genus *Laboea*. Finally, the

topologies of the other Strombidiidae are only supported by low bootstrap values (28% MP, 51% ML) and posterior probability value (0.89 BI).

According to the evolution of oligotrichid ciliary patterns suggested by Agatha (2004a), the *Parallelostrombidium* pattern originated first, followed by the *Novistrombidium* pattern. The latter gave rise to the *Omegastrombidium*, *Spirostrombidium*, and *Strombidium* patterns. The *Laboea* pattern again evolved from the *Strombidium* pattern. Indeed, the genus *Pseudotontonia* with its *Strombidium*-like ciliary pattern, branches basally to a cluster formed by taxa with a *Laboea*-like pattern; the genera *Spirotontonia* and *Laboea*. Moreover, without the information for the genus *Parallelostrombidium*, *Novistrombidium testaceum* branches basally to the other Strombidiidae (excluding *Laboea*, see above) in the Bayesian inference and maximum likelihood trees (Fig.1), while *N. orientale* gave rise to *Spirostrombidium* in all the analyses. Furthermore,



**Fig.3** A–E. *Strombidium sulcatum* from the wild (A, D) and after silver impregnation (B, C, E, F, G, H); A–C. Ventral (A, B) and dorsal (C) views of specimens from the newly sequenced Chinese population (original); D–F. Ventral (D, E) and dorsal (F) views of specimens from a further Chinese population (population GD) (from Song et al., 2000); G, H. Ventral and dorsal views of a specimen from the type population collected in the North Atlantic (from Granda et al., 2003); I–L. *Strombidium inclinatum* after protargol impregnation; I. Ventral view of a specimen from a Mediterranean population (from Modeo et al., 2003); J–L. Ventral (J, K) and top (L) views of specimens collected in the brackish waters of the Baltic Sea (from Montagnes et al., 1990). Scale bars: 20  $\mu$ m

the insertion of *Spirostrombidium* to *Novistrombidium* spp. as well as the positions of the remaining taxa, contradict Agatha's (2004a, b) hypotheses. The deviations between the SS rRNA gene trees and the cladistic analyses are possibly due to (i) the distinctly different number of taxa considered (18 vs. 96 oligotrich species *sensu stricto*), (ii) the incongruence between gene and species trees, (iii) the different evolutionary speeds in the various branches, and/or (iv) the dubious identification of the taxa.

*Strombidium sulcatum* is a taxonomically difficult species. Although many data exist, there is only a single study combining live observation and protargol impregnation (Song et al., 2000). Granda et al. (2003) reinvestigated the silver nitrate-impregnated material of Fauré-Fremiet from the North Atlantic and deposited type material. Hence, the two gene sequences provided here are not from the type population, but from a Chinese raw culture, whose specimens differed from the type material in the macronucleus shape (without vs. with an indentation). Likewise, the specimens sequenced by Modeo et al. (2003) are not from the type population of *S. inclinatum* described by Montagnes et al. (1990). The sequences of the specimens identified as *S. sulcatum* in the present paper and as *S. inclinatum* by Modeo et al. (2003) show a similarity of 100% and 99.8%,

respectively. Considering that (i) in other taxa a similar or lower divergence distinguishes morphotypes (Fokin et al., 2004; Schmidt et al., 2006) and (ii) the sequences analyzed are not from the type populations, we refrain from synonymising the species and only conclude a conspecificity of the Chinese populations and the Italian specimens.

The specimens of *Novistrombidium testaceum* sequences in the present paper match the species description by Song et al. (1998), based on a Chinese population (population GD). However, they differ in their SS rRNA gene sequence by 1.2% from Mediterranean specimens. As in *Srombidium sulcatum*, this deviation is accompanied by minute morphological differences, indicating the presence of a cryptic species.

Due to undersampling of SS rRNA sequences, the branching patterns within the genera *Strombidium* and *Novistrombidium* are unstable (this study; Modeo et al., 2003; Snoeyenbos-West et al., 2002; Strüder-Kypke et al., 2003). All the trees presented here indicate a paraphyly of the two genera, and the intrageneric genetic distances are considerable ( $d_{\max} = 0.081$  for *Strombidium*,  $d_{\max} = 0.057$  for *Novistrombidium*). Nevertheless, the AU test shows that the monophyly of *Strombidium* cannot be rejected with this dataset ( $P > 0.05$ ), which agrees

with the rather low support values observed for several of the internal nodes (Fig.1). Furthermore, result of the AU test for *Novistrombidium* spp. cannot reject the monophyly of this genus either ( $P>0.05$ ), though the support values for the internal nodes are middle or high. Accordingly, more information is required to provide features for a meaningful split of these genera.

## 5 CONCLUSIONS

Although SS rRNA gene sequences of several new sequences of oligotrichs ciliates were added, and we now have sequences of eight genera of oligotrichs sensu stricto, the relationships among this subclass remain largely unresolved. The positions of genera *Omegastrombidium*, *Novistrombidium* in subclass Oligotrichia are not definite, as well as the paraphyletic/monophyletic statuses of genera *Novistrombidium* and *Strombidium*. Additionally, the molecular tree topologies are not congruent with morphological classifications. The diversity within oligotrichs sensu stricto is very large, and the comparison and analysis of sequence data of even these many representative genera might not be sufficient to unambiguously infer their phylogeny. Thus, we conclude that the present knowledge about the oligotrichs sensu stricto is insufficient and that further studies on the morphology, ontogenesis, conjugation, ultrastructure, and resting cysts are required to provide evidence to resolve the relationships among these genera.

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