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Insights into the Phylogeny of the Genus *Stentor* (Heterotrichea, Ciliophora) with Special Emphasis on the Evolution of the Macronucleus Based on SSU rDNA Data

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Summary. Representatives of the genus *Stentor* (Stentoridae, Heterotrichea) are striking ciliates in environmental water samples because of their size (up to 4 mm) and their trumpet-like shape. Important for species identification are the following main characteristics: (1) the presence or absence of endosymbiotic algae (zoochlorellae); (2) the colour of the pigmented cortical granules, and (3) the shape of the macronucleus. The complete small subunit rDNA (SSU rDNA) of 19 further representatives of the genus *Stentor* was sequenced to examine the phylogenetic relationships within this genus and to determine the taxonomic value of these main characteristics. The detailed phylogenetic analyses yielded a separation of all species possessing a single compact macronucleus from those species with an "elongated" macronucleus (moniliform or vermiform). The data also indicate that the uptake of algae as well as the loss of pigmentation happened independently in different lineages. Furthermore, a high level of intraspecific variation within several species was found. Thus, *S. muelleri* and *S.* (sp.) cf. *katashimai* appear to represent distinct species and *S. multiformis* is composed of a species complex.

Key words: Heterotrichea, *Stentor*, macronuclear evolution, phylogeny.

INTRODUCTION

During recent years, the phylogenetic relationships within the Heterotrichea have been the subject of several studies, all based on SSU rDNA sequence analyses (e.g. Rosati *et al.* 2004; Miao *et al.* 2005, 2009; Gong *et al.* 2007; Schmidt *et al.* 2007a). These analyses yielded a monophyletic group for the representatives of the genus *Stentor* and detected a close relationship of the Stentoridae and the Blepharismidae. In addition, the species *Maristentor dinoferus* and *Condylostentor auriculatus* were shown unambiguously separated from the Stentoridae in the SSU rDNA sequence analyses. Instead, *M. dinoferus* seems to be a close relative to the Folliculinidae (Miao *et al.* 2005), which resulted in the establishment of the new family Maristentoridae (Lobban *et al.* 2002, Miao *et al.* 2005). The species *Condylostentor auriculatus* was originally described as *Stentor auriculatus* by Kahl (1932) and later transferred

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to the genus *Condylostoma* by Fauré-Fremiet (1936). This assignment was also resolved by Foissner and Wölfl (1994) and resulted in the description of the new species *Condylostoma wangi*. Based on SSU rDNA sequence data a close relationship of *C. wangi* (originally published as *S. auriculatus* in Genbank database) and all other members of the family Condylostomatidae was shown (Schmidt *et al.* 2007a). Recently, this isolate was then assigned to *Condylostentor auriculatus* (Miao *et al.* 2009) following the redescription of this species by Chen *et al.* (2007) and based on Jankowski (1978).

Although an extensive amount of taxonomical, morphological and ecological data for the genus *Stentor* exists, a comprehensive molecular analysis of the phylogenetic relationships between species of this genus is still lacking. Previous phylogenetic studies (Gong *et al.* 2007, Schmidt *et al.* 2007a) included only three or four representatives of this genus, which contains about 20 valid species characterised by their medium to very large sized (up to 4 mm) and trumpet-like shape (Foissner and Wölfl 1994). Important for the species identification are the following characters: (1) the presence or absence of endosymbiotic algae (zoochlorellae), (2) the colour of the pigmented cortical granules just below the cell surface, and (3) the shape of the macronucleus. For the species of the genus *Stentor* four different macronuclei types were described: single compact, moniliform, nodular, and vermiform (e.g. Foissner and Wölfl 1994). Analogous, such macronucleus types were described for the heterotrichous genera *Blepharisma* and *Spirostomum* (cf. Aescht and Foissner 1998).

Recently, Schmidt *et al.* (2007a) showed in their SSU rDNA sequence analyses that all species possessing a single compact macronucleus (*Blepharisma steinii*, *B. hyalinum*, *B. elongatum*, *Spirostomum teres*, *Stentor amethystinus*) branched off first within their genera. Therefore, they concluded that the single compact macronucleus type represents the ancestral state, whereas the other macronucleus types (e.g. moniliform, vermiform) might be derived. In this study we tested this hypothesis and analysed the SSU rDNA sequences of eight species of the genus *Stentor* possessing three of the four types of macronuclei: (1) single compact (*S. amethystinus*, *S. elegans*, *S. multiformis*), (2) moniliform (*S. coeruleus*, *S.* (sp.) cf. *katashimai*, *S. muelleri*, *S. polymorphus*), and (3) vermiform (*S. roeselii*). Furthermore and with reference to Gong *et al.* (2007), we tested the phylogenetic significance of the characteristics presence or absence of zoochlorellae and pigmentation using the enlarged dataset.

MATERIALS AND METHODS

Collection of organisms

Specimens of *Stentor* were isolated from environmental water samples. The samples from Embalse de Alarcón (Spain), Plymouth (England), Rio de Janeiro (Brazil) and Averstra (Sweden) were kindly provided by T. U. Berendonk (Universität Dresden), cultures of *S. coeruleus* from Federsee (Germany) were kindly provided by K. Eisler (Universität Tübingen). *S. coeruleus* from Chiemsee (Germany) was purchased at www.lebendkulturen.de. The sampling locations of all specimens are listed in Table 1. Species identification was based on morphological characters through *in vivo* observation, different staining methods, and in comparison with the current literature (Kumazawa 1974, 2002; Foissner *et al.* 1992; Foissner and Wölfl 1994).

DNA isolation, amplification, and sequencing

DNA was isolated from fixed cells (in 70% EtOH) using a modified Kavenoff and Zimm procedure (e.g. Steinbrück and Schlegel 1983). Applying the Chelex 100 extraction method published by Regensbogenova *et al.* (2004), DNA was isolated from single cells.

Universal Eukarya-specific primers (e.g. Korte *et al.* 2004) were used for amplification of nuclear SSU rDNA. The PCR conditions were as follows: (1) initial denaturation 5 min. at 95°C; (2) 35 cycles of 1 min. denaturation at 92°C, 2 min. primer annealing at 40°C, and 4 min. primer elongation at 72°C, and (3) final primer elongation for 10 min. at 72°C. PCR-products were purified using the NucleoSpin® Extract Kit II of Macherey-Nagel (Düren, Germany) and sequenced directly.

Sequencing reactions were performed for both DNA strands using the same standard Eukarya-specific primers and additional internal primers (Wylezich *et al.* 2002) on an ABI PRISM® 3100 Genetic Analyzer (Darmstadt, Germany).

Phylogenetic analyses and sequence availability

All analyses contained a total of 58 SSU rDNA sequences: whereof 28 sequences were from representatives of the heterotrichous genus *Stentor*, 27 sequences of further heterotrichous ciliates, and three karyorelictids served as outgroup. Based on the study of Schmidt *et al.* (2007a) and Miao *et al.* (2009), the data set was expanded by the inclusion of the newly sequenced stentorids (Table 1). Alignment was carried out using CLUSTAL X 1.83 (Thompson *et al.* 1997) with default parameters. Primer sequences were removed from the resulting alignment using BioEdit (Hall 1999).

Maximum-likelihood (ML) analysis was performed with PHYML v.2.4.4 (Guindon and Gascuel 2003) with 100 replications, using the evolutionary model of Tamura and Nei (1993) with $I = 0.5064$ and $\Gamma = 0.4921$, which was selected by Modeltest 3.6 (Posada and Crandall 1998). Bayesian analysis was carried out with MrBayes v3.1 (Huelsenbeck and Ronquist 2001), using the same model and parameters, 1,000,000 generations, and an initial burn in of 2500. Neighbour-joining (NJ) analysis and Maximum parsimony (MP) analysis were performed with the program package MEGA 3.1 (Kumar *et al.* 2004). NJ-analysis was carried out using the same model as described above with 10,000 replication steps. MP-analysis was performed with 2000 resamplings and characters not weighted.

Table 1. Classification of the analysed species in accordance with Lynn (2003b, 2008).

Family	Species	Location	Accession No	Reference
Loxodidae	*Loxodes magnus*		L31519	Hirt *et al.* (1995)
	Loxodes striatus		U24248	Hammerschmidt *et al.* (1996)
Trachelocercidae	*Tracheloraphis* sp.		L31520	Hirt *et al.* (1995)
Blepharismidae	*Blepharisma americanum*		AM713182	Schmidt *et al.* (2007a)
	Blepharisma elongatum		AM713186	Schmidt *et al.* (2007a)
	Blepharisma hyalinum		AM713184	Schmidt *et al.* (2007a)
	Blepharisma japonicum		AM713185	Schmidt *et al.* (2007a)
	Blepharisma steinii		AM713187	Schmidt *et al.* (2007a)
	Blepharisma undulans		AM713183	Schmidt *et al.* (2007a)
Chattonidiidae	*Chattonidium setense*		AM295495	Modeo *et al.* (2006)
Climacostomidae	*Climacostomum virens* [1]		X65152	Hammerschmidt *et al.* (1996)
	Climacostomum virens [2]		EU583990	Miao *et al.* (2009)
	Fabrea salina LesF		DQ168805	Angeli *et al.* (unpubl. observ.)
	Fabrea salina TorF		DQ168806	Angeli *et al.* (unpubl. observ.)
	Fabrea salina		EU583991	Miao *et al.* (2009)
Condylostomatidae	*Condylostentor auriculatus*		DQ445605	Miao *et al.* (2009)
	Condylostoma minutum		DQ822482	Miao *et al.* (2009)
	Condylostoma spatiosum		DQ822483	Miao *et al.* (2009)
	Condylostoma sp.		AM295496	Modeo *et al.* (2006)
	Condylostomides n. sp.		AM713188	Schmidt *et al.* (2007a)
Folliculinidae	*Eufolliculina uhligi*		U47620	Hammerschmidt *et al.* (1996)
	Folliculina simplex		EU583992	Miao *et al.* (2009)
Maristentoridae	*Maristentor dinoferus*		AY630405	Miao *et al.* (2005)
Peritromidae	*Peritromus kahli*		AJ537427	Rosati *et al.* (2004)
	Peritromus faurei		EU583993	Miao *et al.* (2009)
Spirostomidae	*Gruberia* sp.		L31517	Hirt *et al.* (1995)
	Spirostomum ambiguum [1]		L31518	Hirt *et al.* (1995)
	Spirostomum ambiguum [2]		AM398201	Schmidt *et al.* (2007a)
	Spirostomum minus		AM398200	Schmidt *et al.* (2007a)
	Spirostomum teres		AM398199	Schmidt *et al.* (2007a)
Stentoridae	*Stentor amethystinus*[1]		AY775566	Angeli *et al.* (unpubl. observ.)
	Stentor amethystinus [2]		AM713191	Schmidt *et al.* (2007a)
	Stentor amethystinus [3]	Dübener Heide, Germany	FN659808	**This study**
	Stentor amethystinus [4]	Averstra, Sweden	FN659807	**This study**
	Stentor coeruleus [1]		AF357145	Gong *et al.* (2007)
	Stentor coeruleus [2]		AM713189	Schmidt *et al.* (2007a)
	Stentor coeruleus [3]		DQ132978	Angeli *et al.* (unpubl. observ.)
	Stentor coeruleus [4]		DQ136037	Angeli *et al.* (unpubl. observ.)
	Stentor coeruleus [5]	Federsee, Germany	FN659809	**This study**
	Stentor coeruleus [6]	Markleeberg, Germany	FN659810	**This study**
	Stentor coeruleus [7]	Machern, Germany	FN659811	**This study**
	Stentor coeruleus [8]	Chiemsee, Germany	FN659812	**This study**
	Stentor coeruleus [9]	Droyßig, Germany	FN659813	**This study**

Family	Species	Location	Accession No	Reference
	Stentor coeruleus [10]	Machern, Germany	FN659814	**This study**
	Stentor coeruleus [11]	Markleeberg, Germany	FN659815	**This study**
	Stentor coeruleus [12]	Embalse de Alarcón, Spain	FN659816	**This study**
	Stentor elegans	Plymouth, England	FN659817	**This study**
	Stentor* (sp.) cf. *katashimai	Groitzsch, Germany	FN659818	**This study**
	Stentor muelleri [1]	Cranzahl, Germany	FN659819	**This study**
	Stentor muelleri [2]	Rio de Janeiro, Brazil	FN659820	**This study**
	Stentor multiformis [1]	Pegau, Germany	FN659821	**This study**
	Stentor multiformis [2]	Liebenwalde, Germany	FN659822	**This study**
	Stentor polymorphus [1]		AF357144	Gong *et al.* (2007)
	Stentor polymorphus [2]		AM713190	Schmidt *et al.* (2007a)
	Stentor polymorphus [3]	Cranzahl, Germany	FN659823	**This study**
	Stentor roeselii [1]		AF357913	Gong *et al.* (2007)
	Stentor roeselii [2]	Groitzsch, Germany	FN659824	**This study**
	Stentor roeselii [3]	Embalse de Alarcón, Spain	FN659825	**This study**

Numbering of the species refers to Fig. 1.

Nucleotide sequences

All new SSU rDNA sequences are deposited at GenBank database. The accession numbers are listed in Table 1.

RESULTS

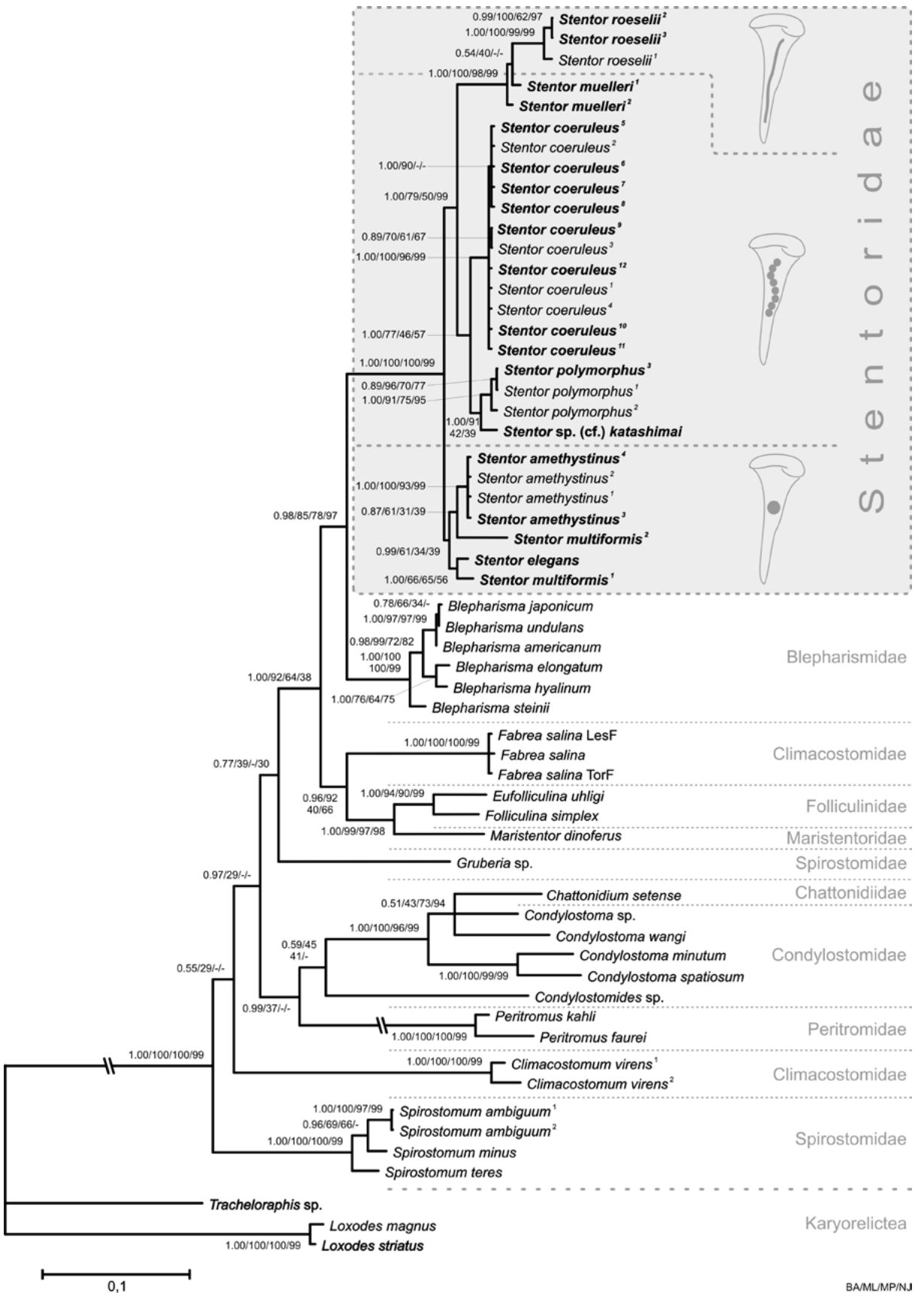
In this study a data set comprising a total of 58 SSU rDNA sequences was analysed, 55 sequences are from heterotrichous species, 28 sequences are from members of the genus *Stentor*. The complete alignment contains 1,713 positions, 593 positions are found to be variable and 512 sites are parsimony informative.

The results of the present phylogenetic analyses (Fig. 1) are largely in accordance to the findings of Schmidt *et al.* (2007a) and Miao *et al.* (2009). Differences occur in the basal branching pattern concerning the Spirostomidae, Climacostomidae, Peritromidae, Condylostomidae (always including Chattonidiidae), and *Gruberia*. However, most of these basal nodes are weakly supported and differ between analyses methods used. Furthermore, the analyses published by Schmidt *et al.* (2007a) and Miao *et al.* (2009) did also not resolve the basal relationships unambiguously. All other branches are in agreement with the results of Schmidt *et al.* (2007a) and Miao *et al.* (2009), particularly the sistergroup relationship between the genera *Stentor* and *Blepharisma* was found again with high support by all analyses.

All representatives of the genus *Stentor* clustered together constantly supported by high bootstrap values or posterior probabilities. Furthermore, all methods revealed a subdivision into two groups within this genus. The first group comprised all species possessing a single compact macronucleus (*S. amethystinus*, *S. elegans*, and *S. multiformis*), whereas the second group contained all species with an "elongated" macronucleus (moniliform or vermiform). Within the first group, all isolates of *S. amethystinus* clustered together, whereas both isolates of *S. multiformis* grouped separately: *S. multiformis*[1] with *S. elegans and S. multiformis*[2] with *S. amethystinus*. The division of the *S. multiformis* isolates was caused by 41 nucleotide differences within the whole SSU rDNA.

▸▸

Fig. 1. Phylogenetic analyses of the Heterotrichea inferred from SSU rDNA sequences based on Bayesian analysis. The karyorelictean species *Loxodes magnus*, *L. striatus*, and *Tracheloraphis* sp. were chosen as outgroup. The numbers at the nodes represent the posterior probabilities of the Bayesian analysis (first number; 1,000,000 generations), the values of the ML analysis after 100 replication steps (second number), the bootstrap values of the MP analysis (third number; 2,000 replications), and bootstrap values of the NJ analysis (fourth number; 10,000 replications). All newly investigated species are in bold. Numbering of the species refers to Table 1.


Stentoridae
Stentor roeselii 2
Stentor roeselii 3
Stentor roeselii 1
Stentor muelleri 1
Stentor muelleri 2
Stentor coeruleus 5
Stentor coeruleus 2
Stentor coeruleus 6
Stentor coeruleus 7
Stentor coeruleus 8
Stentor coeruleus 9
Stentor coeruleus 3
Stentor coeruleus 12
Stentor coeruleus 1
Stentor coeruleus 4
Stentor coeruleus 10
Stentor coeruleus 11
Stentor polymorphus 3
Stentor polymorphus 1
Stentor polymorphus 2
Stentor sp. (cf.) katashimai
Stentor amethystinus 4
Stentor amethystinus 2
Stentor amethystinus 1
Stentor amethystinus 3
Stentor multiformis 2
Stentor elegans
Stentor multiformis 1
Blepharisma japonicum
Blepharisma undulans
Blepharisma americanum
Blepharisma elongatum
Blepharisma hyalinum
Blepharisma steinii
Blepharismidae
Fabrea salina LesF
Fabrea salina
Fabrea salina TorF
Climacostomidae
Eufolliculina uhligi
Folliculina simplex
Folliculinidae
Maristentor dinoferus
Maristentoridae
Gruberia sp.
Spirostomidae
Chattonidium setense
Chattonidiidae
Condylostoma sp.
Condylostoma wangi
Condylostoma minutum
Condylostoma spatiosum
Condylostomidae
Condylostomides sp.
Peritromus kahli
Peritromus faurei
Peritromidae
Climacostomum virens 1
Climacostomum virens 2
Climacostomidae
Spirostomum ambiguum 1
Spirostomum ambiguum 2
Spirostomum minus
Spirostomum teres
Spirostomidae
Tracheloraphis sp.
Loxodes magnus
Loxodes striatus
Karyorelictea
0,1
BA/ML/MP/NJ

The second cluster, characterised by an "elongated" macronucleus, was split into two distinct groups: (1) *S.* (sp.) cf. *katashimai* together with *S. polymorphus* and *S. coeruleus*, and (2) *S. muelleri* together with *S. roeselii*.

All analyses revealed a basal position of *S.* (sp.) cf. *katashimai* to the isolates of *S. polymorphus* (Fig. 1). Within *S. coeruleus* a cluster of five isolates occurred in Bayesian and ML analyses, which resulted from two derived nucleotide substitutions in their SSU rDNA sequences. A further substitution resulted in a second cluster, consisting of *S. coeruleus*[3] and *S. coeruleus*[9].

S. muelleri and *S. roeselii* always clustered together with highest support values in all analyses. The two species differ from each other in the shape of their macronucleus. *S. muelleri* is characterised by a moniliform macronucleus like all other species of the second cluster, while *S. roeselii* possesses a vermiform macronucleus.

DISCUSSION

Phylogenetic analyses

The phylogenetic analyses within the Heterotrichea are largely congruent with previous studies (Rosati *et al.* 2004; Miao *et al.* 2005, 2009; Gong *et al.* 2007; Schmidt *et al.* 2007a). The basal branching pattern within this class still remains unresolved due to insufficient phylogenetic information which may be based on fast radiation events. Such limitations of the SSU rDNA to resolve basal nodes are also known from investigations of other ciliate classes or subclasses (e.g. Dunthorn *et al.* 2008, Schmidt *et al.* 2007b) and within the Intramacronucleata in general (Lynn 2003a).

The present study focussed on the relationships within the genus *Stentor*, the only genus of the Stentoridae, using a total of 28 sequences belonging to eight different species. The Stentoridae was revealed as a monophyletic group, forming the sister taxon to the Blepharismidae.

Within the Stentoridae all species possessing a single compact macronucleus (*S. amethystinus*, *S. multiformis*, and *S. elegans*) clustered together and formed the sistergroup to all remaining species with a moniliform (*S. coeruleus*, *S. polymorphus*, *S.* (sp.) cf. *katashimai*, and *S. muelleri*) or vermiform (*S. roeselii*) macronucleus (Fig. 1). Based on this enlarged dataset the view that a single compact macronucleus might be the ancestral state whereas the "elongated" forms are derived seems not as clear as stated in Schmidt *et al.* (2007a). However, the cluster comprising all species with a single compact macronucleus was only weakly supported by all analyses methods (bootstrap values 34–61%) with the exception of Bayesian analyses (posterior probabilities 0.99) compared to the group containing species with an "elongated" macronucleus (bootstrap values 50–99% and posterior probabilities 1.0). Therefore, a multiple basal branching for those *Stentor* species, characterised by a single compact macronucleus, might also possible from the current data.

All analyses also revealed basal branches for species possessing a single compact macronucleus in the genera *Blepharisma* and *Spirostomum* (*Blepharisma steinii*, *B. hyalinum*, *B. elongatum*, and *Spirostomum teres*). Therefore, we would like to renew our conclusion (Schmidt *et al.* 2007a) that a single compact macronucleus might represent the ancestral state.

Based on the possession of endosymbiotic algae and/or pigments Gong *et al.* (2007) divided the *Stentor* species into four types: a) species with neither symbiotic algae nor pigments (*S. roeselii*, *S. muelleri*, *S. elegans*, *S.* (sp.) cf. *katashimai*); b) species with no algae but with pigments (*S. coeruleus*, *S. multiformis*); c) species with algae but without pigments (*S. polymorphus*); and d) species with both, algae and pigments (*S. amethystinus*). Furthermore, Gong *et al.* (2007) speculated that "species with no pigments or algae represented the primitive species of *Stentor* (...) and with the evolution, some species obtained pigment granules, and some others obtained symbiotic green algae to adapt special niche." However, the authors also stated that this speculation needs to be validated by analysing more taxa. Now our data provide the opportunity to reconsider these speculations because clearly more representatives, particularly belonging to all four groups were analysed (Table 2). The analyses revealed a different picture of the evolution within the genus *Stentor*. Species without algae and pigments (*S. elegans*, *S. muelleri*, and *S. roeselii*, see Table 2), which should present the ancestral state, occur in both major clusters of the genus (Fig. 1). Species possessing pigments (*S. amethystinus*, *S. multiformis*, and *S. coeruleus*) group with non-pigmented species, and both species with symbiotic algae (*S. polymorphus* and *S. amethystinus*) are found on different branches. Therefore, it seems to be more likely that the uptake of algae happened two times independently. Pigments are found in all taxa of the first group with the exception of *S. elegans,* which probably reflects a loss of the pigment inventory for this species. *S. coeruleus* is the only member of the second

Table 2. Summary of some important characters of the species of the genus *Stentor* analysed in the present study.

Species	Characters		
	macronucleus	symbiotic green algae	pigmentation
S. amethystinus	single compact	yes	purple
S. multiformis	single compact	no	blue-green
S. elegans	single compact	no	no
S. coeruleus	moniliform	no	blue-green
S. (sp.) cf. *katashimai*	moniliform	no	no
S. polymorphus	moniliform	yes	no
S. muelleri	moniliform	no	no
S. roeselii	vermiform	no	no

group possessing pigments. Therefore, we can hypothesise that pigments either evolved independently in this species, or they were lost in all other lineages independently. Based on the latter presumption, pigmentation could represent the ancestral state of the genus *Stentor* followed by multiple losses in several lineages.

Intraspecific variation

With the exception of *S. elegans* and *S.* (sp.) cf. *katashimai*, the SSU rDNA was sequenced from several isolates originated from different locations (see Table 1). Several species showed a high level of intraspecific variation within their SSU rDNA sequences. Only in the sequences of *S. amethystinus* no difference could be found, whereas in *S. coeruleus* three, in *S. polymorphus* four, in *S. roeselii* seven, and in *S. muelleri* 10 nucleotide differences occurred between the isolates. Despite these molecular differences, four of the five species each formed a monophyletic cluster. In contrast, both isolates of *S. multiformis* branched off separately in all analyses, caused by an extreme variation of 41 nucleotides in their SSU rDNA sequences corresponding to an evolutionary distance of 2.5%. Such a conspicuous sequence diversification of a highly conserved gene like the SSU rDNA indicates that the two isolates belong to different species. However, the isolates could not be distinguished based on morphological characters, which suggest the existence of a species complex. Such species complexes are defined by closely related species, the so-called sibling species, which are nearly or fully indistinguishable by morphological or morphogenetic characters. Within ciliates many species complexes or sibling species are known, e.g. within the genera *Paramecium*, *Tetrahymena*, *Vorticella*, and *Stylonychia*. For example, the species *Stylonychia lemnae* and *S. mytilus*, members of the *S. mytilus*-complex, differ in only one single nucleotide within their whole SSU rDNA sequences (Schmidt *et al.* 2006). Wright and Lynn (1995) and Strüder-Kypke *et al.* (2001) investigated the phylogenetic relationships within the genus *Tetrahymena* based on SSU rDNA analyses and showed that the sequences of all studied *Tetrahymena* species differ only in 69 positions. Furthermore, closely related species, for example within the *T. australis* group, are separated by evolutionary distances of no more than 0.5% or nine nucleotide differences (Wright and Lynn 1995). Our analyses revealed evolutionary distances of 0.0% (*S. amethystinus*) up to 0.6% (*S. muelleri*). For *S. coeruleus*, the existence of a species complex was already suggested based on RAPD data (Kusch 1998). Our analyses yielded an evolutionary distance of up to 0.2% between the 12 isolates belonging to this species. In addition, two distinct clusters of five and two isolates occurred. These results may also point to the existence of a *S. coeruleus* species complex comprising different species. Based on these data, the occurrence of species complexes for *S. polymorphus*, *S. roeselii*, and *S. muelleri* seem to be likely because the isolates of these species possess even more nucleotide differences (see also Fig. 1). Further studies should focus on other genes because the SSU rDNA and even the non-coding and thus more variable ribosomal internal transcribed spacer (ITS) regions might be too conserved for detailed intraspecific analyses. A good candidate gene could be the mitochondrial cytochrome c oxidase I (COI); Barth *et al*. (2006) showed that this gene is better suited for the separation of *Paramecium* species than ITS sequences. Gentekaki and Lynn (2009) compared the suitability of ITS, LSU rDNA and COI sequences to study the intraspecific genetic variation within *Carchesium polypinum* clades and also obtained the best resolution with the COI gene. However, at the moment no mitochondrial genomes are available from *Stentor* or other members of the Heterotrichea which hampers the primer design for COI or other mitochondrial genes to analyse the intraspecific genetic variation within this genus.

Stentor* (sp.) cf. *katashimai

The species *S. katashimai* Kumazawa, 1973 represents the background of a controversial dispute. The features, which caused Kumazawa to describe a new species, *S. katashimai*, were not accepted by Foissner and Wölfl (1994) and Foissner *et al.* (1992), who sug-

gested that *S. katashimai* is a synonym for *S. muelleri*. We found a specimen displaying the features outlined by Kumazawa (1974) for *S. katashimai*. The genetic analyses resulted in 53 or 55 nucleotide differences between the SSU rDNA sequence of the isolate of *S.* (sp.) cf. *katashimai* and the two sequences of *S. muelleri*. These differences lead to a clear separation of *S.* (sp.) cf. *katashimai* and *S. muelleri* (Fig. 1). Based on these data *S. katashimai* appears to be a separate species. This result is additionally supported by the close relationship of *S. katashimai* and *S. polymorphus* in each of our analyses (Fig. 1) based on 17 nucleotide differences, because *S. katashimai* was depicted as "a miniature of *S. polymorphus*" which "does not have zoochlorellae" (Kumazawa 2002). Although we paid special attention to this species we could not find another specimen displaying the characteristics of *S. katashimai*. Sequence analyses of further isolates as well as other genes (see above) will be necessary to clarify the status of this species in the future.

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