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# Molecular and morphological evidence for a sister group relationship of the classes Armophorea and Litostomatea (Ciliophora, Intramacronucleata, Lamellicorticata infraphyl. nov.), with an account on basal litostomateans

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# Abstract

Based solely on the localization of the cytostome, Cavalier-Smith (2004) divided the ciliate subphylum Intramacronucleata into three infraphyla: the Spirotrichia, including Armophorea and Spirotrichea; the Rhabdophora, containing exclusively Litostomatea; and the Ventrata, comprising the remaining six intramacronucleate classes. This scheme is supported largely by 18S rRNA phylogenetic analyses presented here, except for the placement of the Armophorea. We argue that this group does not belong to the Spirotrichia but forms a lineage together with the Litostomatea because the molecular sister relationship of the Armophorea and Litostomatea is supported by two morphological and morphogenetic synapomorphies: (i) plate-like arranged postciliary microtubule ribbons, forming a layer right of and between the ciliary rows and (ii) a telokinetal stomatogenesis. Thus, we unite them into a new infraphylum, Lamellicorticata, which replaces Cavalier-Smith's Rhabdophora. Further, our phylogenetic analyses consistently classify the most complex haptorian genus *Dileptus* basal to all other litostomateans, though morphological investigations suggest dileptids to be highly derived and possibly originating from a spathidiid ancestor. These discrepancies between molecular and morphological classifications have not as yet been investigated in detail. Thus, we propose an evolutionary scenario, explaining both the sister relationship of the Armophorea and Litostomatea, as well as the basal position of the morphologically complex dileptids.

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# Introduction

The phylum Ciliophora contains two subphyla, the Postciliodesmatophora and the Intramacronucleata. The relationships among the nine intramacronucleate classes are

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largely unresolved and only two, Prostomatea and Oligohymenophorea, are consistently placed together (for review, see Lynn 2008). Based solely on the localization of the cytostome, Cavalier-Smith (2004) proposed the grouping of the nine intramacronucleate classes into three infraphyla (Fig. 3): the Spirotrichia, including Armophorea and Spirotrichea; the Rhabdophora, containing exclusively Litostomatea; and the Ventrata, comprising the remaining six classes (Phyllopharyngea, Nassophorea, Colpodea, Plagiopylea, Oligohymenophorea, and Prostomatea). However,

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**Figs 1–5.** Classification of Armophorea and Litostomatea based on light microscopical features (Bütschli 1889), on ultrastructural data (Small and Lynn 1985), on localization of the cytostome (Cavalier-Smith 2004), and on a combination of morphological and molecular data (Lynn 2008; present study).

this classification was widely ignored by most ciliatologists, for instance, it was not mentioned in the recent monograph of Lynn (2008). Based on 18S rRNA gene phylogeny and comparative analyses of ontogenesis and arrangement of the somatic fibrillar system, we slightly modify Cavalier-Smith's framework and argue that the classes Armophorea and Litostomatea should be united as a novel infraphylum.

Morphologists never hypothesized a close relationship of the Armophorea (e.g. *Metopus*) and Litostomatea (e.g. *Dileptus* or *Balantidium*) because they appear very different. The former are anaerobic bacterivores with somatic dikinetids and a complex oral ciliature (paroral membrane and adoral membranelles), while the latter are aerobic predators (haptorid litostomeans) or anaerobic endosymbionts (trichostomatid litostomateans) with somatic monokinetids and a comparatively simple oral ciliature (oral dikinetids and/or oralized somatic monokinetids). Thus, it was a great surprise when early molecular phylogenetic studies suggested a sister relationship of the armophoreans and litostomateans, though with low bootstrap support ranging from 53% to 72% (Embley and Finlay 1994; Hammerschmidt et al. 1996; Hirt et al. 1995). The monophyly of these two groups was not rejected when further species from all main ciliate lineages were added in the phylogenetic analyses, but the support usually remained poor (e.g. Gong et al. 2009; Strüder-Kypke et al. 2006). Nobody commented in detail on these remarkable results. Only Foissner and Agatha (1999) found some common ontogenetical features for these two groups, but they did not argue for a sister relationship due to the prevailing morphological and ecological dissimilarities.

In the past, the Armophorea were classified as a subgroup of the Heterotrichea, which were assigned to the Spirotrichea due to the prominent adoral zone of membranelles (Fig. 1; Bütschli 1889; Small and Lynn 1985). However, based on morphological characters (somatic kinetids with postciliodesmata and macronucleus divided by extramacronuclear microtubules) and gene sequences, the heterotricheans were separated from the Spirotrichea and were found as a sister group of the Karyorelictea (Figs 3–5; Hammerschmidt et al. 1996; Hirt et al. 1995; Lynn 2008). Further, these analyses showed that the armophoreans do not belong to the heterotricheans, but could form a monophyletic group with the litostomateans.

The Haptoria were assigned, together with the Prostomatea, to the Holotricha because of the completely ciliated body and simple oral apparatus (Fig. 1). Later, both were integrated into the Rhabdophora due to the transverse microtubule ribbons longitudinally lining the wall of the oral basket (Fig. 2; Small and Lynn 1985). However, the inclusion of the Prostomatea was caused by a misinterpretation of the fibrillar associates of the oral dikinetids (Lynn 2008). Further, neither molecular phylogenies nor ontogenetical data support a close relationship of the Haptoria and Prostomatea (Figs 3–5; Bardele 1999; Foissner 1996; Lynn 2008; Strüder-Kypke et al. 2006).

Small and Lynn (1981) recognized that the Haptoria and the Trichostomatia share a unique ultrastructural pattern of the somatic kinetids which are single basal bodies bearing a convergent postciliary microtubule ribbon, a short kinetodesmal fibre, and two transverse microtubule ribbons. Consequently, Small and Lynn (1981) united the Haptoria and Trichostomatia into the class Litostomatea whose monophyly is strongly supported by the molecular phylogenies of the 18S rRNA gene (e.g. Lynn 2008; Strüder-Kypke et al. 2006) and by three additional synapomorphies: (i) the cytopharynx is of a rhabdos type, i.e., it is lined by transverse ribbons (see Foissner and Foissner 1985, 1988 for haptorians and Grain 1966a, b for trichostomatians), whereas by postciliary ribbons in all other ciliate classes, including Prostomatea; (ii) the ciliature of at least one somatic kinety is differentiated to clavate cilia, forming a dorsal brush in the haptorians and a "clavate field" in the trichostomatians (Foissner 1996); (iii) the micronucleus conspicuously increases in size during the first maturation division and the conjugation mode is heteropolar, except for the pleurostomatid haptorians in which it is homopolar (Raikov 1972; Vďačný and Foissner 2008; Xu and Foissner 2004).

The molecular phylogenies of the haptorian ciliates (Gao et al. 2008; Strüder-Kypke et al. 2006) are conflicting with morphology-based evolutionary scenarios and classifications (Foissner 1984; Foissner and Foissner 1988; Lipscomb and Riordan 1990; Vďačný and Foissner 2008, 2009; Xu and

Foissner 2005). Specifically, the morphologically most complex genus, *Dileptus*, branches off at the base of the molecular trees (Gao et al. 2008; Strüder-Kypke et al. 2006), while morphological traits (complex oral ciliature, hybrid circumoral kinety, transiently formed spathidiid and polar ciliary patterns during ontogenesis and conjugation) indicate dileptids as highly derived and possibly originating from a spathidiid ancestor (Foissner 1984; Vďačný and Foissner 2008, 2009; Xu and Foissner 2005). All these discrepancies between molecular and morphological classifications have as yet not been investigated in detail. Thus, we propose an evolutionary scenario, explaining both the sister relationship of the Armophorea and Litostomatea, as well as the basal position of the morphologically complex dileptids.

## Material and Methods

*Metopus es* (Müller, 1776) Lauterborn, 1916 was found in a puddle from the surroundings of the city of Salzburg, Austria. *Enchelys polynucleata* (Foissner, 1984) Foissner et al., 2002 was collected from the upper soil layer (0–2 cm) of a meadow near Salzburg (Schaming near Eugendorf). Species were identified using live observation and protargol impregnation technique (Foissner 1991). *Enchelys polynucleata* was processed for transmission electron microscopy following the procedure of Foissner (1991). For explanation of morphological and ontogenetical terms, see Foissner (1996) and Lynn (2008).

To test whether the Armophorea is sister to the Litostomatea, we performed several preliminary phylogenetic analyses, using 18S rRNA gene sequences of about 180 species from all major ciliate lineages. All 18S rRNA gene sequences were retrieved from GenBank and their alignments were based on primary and secondary structure of the 18S rRNA molecule. The preliminary analyses generally resulted in similar topologies and 59 representative taxa were selected for the final phylogenetic analyses (Table 1). Alignments were constructed using ClustalX (Thompson et al. 1997), and regions that could not be aligned unambiguously were removed from the initial alignment manually in BioEdit (Hall 1999), resulting in a matrix of 1211 characters. The General-Time-Reversible model for nucleotide substitution, considering invariable sites and a gamma distributed substitution rate among sites  $(GTR + I + \Gamma)$ , was chosen using Modeltest (Posada and Crandall 1998). This model (n = 6,rates = invgamma) was implemented in MrBayes (Ronquist and Huelsenbeck 2003). Two parallel runs were performed and the maximum posterior probability of a phylogeny out of 5,000,000 generations was approximated with the Markov chain Monte Carlo (MCMC). Trees were sampled every thousandth generation. The first 25% of sampled trees were considered 'burn-in' trees and were discarded. A 50% majority rule consensus of the remaining trees was used to calculate posterior probability (PP) values for Bayesian inference (BI). The maximum likelihood (ML) analysis was conducted

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Species name	GB number	Species name	GB number	Species name	GB number
Amphileptus aeschtae	EU242510	Dysteria procera	DQ057347	Nyctotheroides parvus	AF145352
Anoplophrya marylandensis	AY547546	Entodinium caudatum	U57765	Nyctotherus ovalis	AY007454
Arcuospathidium muscorum	DQ411859	Ephelota gemmipara	DQ834370	Obertrumia georgiana	X65149
Balantidium coli	AF029763	Epispathidium papilliferum	DQ411857	Ophryoglena catenula	U17355
Blepharisma americanum	M97909	Eudiplodinium maggii	U57766	Ophryoscolex purkynjei	U57768
Bryometopus pseudochilodon	EU039887	Eufolliculina uhligi	U47620	Orthodonella apohamatus	DQ232761
Bursaria truncatella	U82204	Frontonia vernalis	U97110	Oxytricha granulifera	AF164122
Chilodonella uncinata	AF300281	Furgasonia blochmanni	X65150	Paramecium calkinsi	AF100301
Chlamydodon excocellatus	AY331790	Heliophrya erhardi	AY007445	Phialina salinarum	EU242508
Chlamydodon triquetrus	AY331794	Homalozoon vermiculare	L26447	Plagiopyla nasuta	Z29442
Coleps hirtus	AM292311	Isochona sp.	AY242119	Prorodon teres	X71140
Colpoda magna	EU039896	Isotricha intestinalis	U57770	Pseudomicrothorax dubius	X65151
Colpodidium caudatum	EU264560	Lacrymaria marina	DQ777746	Spathidium stammeri	DQ411862
Cyrtolophosis mucicola	EU039899	Leptopharynx costatus	EU286811	Strombidium purpureum	U97112
Dasytricha ruminantium	AM158463	Litonotus paracygnus	EU242509	Tetrahymena corlissi	U17356
Didinium nasutum	U57771	Loxodes striatus	U24248	Tracheloraphis sp.	L31520
Dileptus sp.	AF029764	Loxophyllum rostratum	DQ190465	Trimyema compressum	Z29438
Diophryopsis hystrix	EF486861	Metopus palaeformis	AY007450	Trithigmostoma steini	X71134
Diplodinium dentatum	U57764	<i>Nassula</i> sp.	EU286810	Zosterodasys transversus	EU286812
Discophrya collini	L26446	Notoxoma parabryophryides	EU039903		

 Table 1. List of ciliate species with GenBank accession numbers of corresponding 18S rRNA gene sequences included in the phylogenetic analyses.

online on the CIPRES Portal V 1.15 (http://www.phylo.org), using RAxML with the setting as described in Stamatakis et al. (2008). The ML bootstrapping analyses were carried out with 1000 replicates. The maximum-parsimony (MP) analyses were performed in PAUP\* v4.0b8 with randomly added species and tree bisection-reconnection (TBR) branchswapping algorithm in effect (Swofford 2001). The reliability of internal branches was assessed using the non-parametric bootstrap method with 1000 replicates.

# **Results and Discussion**

# **Phylogenetic analyses**

All phylogenetic analyses (BI, ML, MP) consistently placed the class Armophorea as sister to the class Litostomatea (Fig. 6). This clade was strongly supported by Bayesian interference with a posterior probability (PP) of 0.94, and moderately supported by the 75% ML and 67% MP bootstraps. Thus, based on two morphologic synapomorphies (unique arrangement of the somatic fibrillar system and ontogenetic mode), we unite the armophoreans and litostomateans into a new infraphylum, Lamellicorticata. In all trees, the Lamellicorticata and the Spirotrichea formed a super-clade that was, however, only weakly supported (0.65 PP, 56% ML, 52% MP). Within the large subphylum Intramacronucleata, another super-clade, comprising six classes (Colpodea, Oligohymenophorea, Prostomatea, Plagiopylea, Phyllopharyngea, and Nassophorea), was depicted with very strong (1.00 PP) to moderate (75% ML, 82% MP) support.

The monophyla Armophorea and Litostomatea were fully supported in all analyses (1.00 PP, 100% ML, 100% MP). However, the internal relationships of the class Litostomatea were rather poorly resolved (Fig. 6). The subclass Haptoria was paraphyletic in all analyses, consistent with Gao et al. (2008) and Strüder-Kypke et al. (2006). The genus Dileptus branched basal to all other litostomateans, justifying at least the ordinal rank suggested by Jankowski (1980). This node was strongly supported only in the BI analysis (0.94). The subclass Trichostomatia was classified as a monophyletic group with full posterior probability (1.00 PP) and very strong bootstrap support (99% ML, 99% MP). The trichostomatians branched rather deep within the subclass Haptoria where they clustered together with the free-living, aerobic haptorian Epispathidium papilliferum in the BI and ML trees, while they formed a polytomy pattern along with Arcuospathidium muscorum, Epispathidium papilliferum, and Spathidium stammeri in the MP analysis. The trichostomatian Balantidium coli was placed basal to the cluster formed by other vestibuliferids and entodiniomorphids. The support values for this node were very high in the BI (posterior probability 1.00) and ML (bootstrap 99%) analyses, while moderate in the MP tree (bootstrap 84%).

# Morphological evidence and evolutionary scenario for a sister relationship of Armophorea and Litostomatea

### Arrangement of somatic fibrillar system

The classic set of fibrillar associates of ciliate somatic kinetids includes a kinetodesmal fibre, a postciliary



**Fig. 6.** Phylogenetic tree of 59 18S rRNA gene sequences from the phylum Ciliophora, showing the sister relationship of the classes Armophorea and Litostomatea. Three methods (Bayesian inference, maximum likelihood, and maximum parsimony) were used to construct trees, all resulting in the same topology. Posterior probabilities (PP) and bootstrap values for the maximum-likelihood (ML) and maximum-parsimony (MP) analyses are shown at nodes.



**Figs 7–11.** Comparison of light microscopic (Fig. 7, protargol impregnation) and electron microscopic (Figs 8–11) appearance of the somatic fibrillar system in armophoreans (Fig. 7, *Metopus es*, original; Fig. 9, *M. contortus*, micrograph kindly supplied by Finlay and Esteban) and haptorid litostomateans (Figs 8, 10, 11, *Enchelys polynucleata*, originals). The well-developed postciliary microtubule ribbons are arranged in a unique pattern: they form a single, plate-like layer right of and between the somatic kineties (Figs 7–11, arrows). The cortical granules, which impregnate strongly, follow the slightly oblique course of the postciliary microtubule ribbons (Fig. 7). ER – rough endoplasmic reticulum, G – cortical granules, KD – kinetodesmal fibre, M – mitochondria, PMT – postciliary microtubule ribbons, SK – somatic kineties (kinetosomes), TM – transverse microtubule ribbons.

microtubule ribbon, and a transverse microtubule ribbon. Well-developed postciliary microtubule ribbons occur in only four ciliate groups: karvorelicteans, heterotricheans, armophoreans, and litostomateans (Lynn 2008). In the karyorelicteans and heterotricheans, the postciliary microtubule ribbons are stacked, forming a distinct fibre (postciliodesma) close to the right of the kineties. In the armophoreans and litostomateans, the postciliary microtubules are not stacked but form a single, plate-like layer right of and between the ciliary rows (Figs 7–11). Molecular phylogenies suggest this state as an apomorphy because karyorelicteans and heterotricheans are consistently placed basal to litostomateans and armophoreans. In accordance with the structural conservatism hypothesis (Lynn 2008), we consider this unique pattern as a highly important phylogenetic marker.

#### Stomatogenic mode

The Armophorea and Litostomatea have a telokinetal and purely somatic stomatogenesis. This is pleurotelokinetal in the former and typically holotelokinetal in the latter, except for the pleurostomatid haptorians in which it is monotelokinetal and the trichostomatians in which it is cryptotelokinetal (Cameron and O'Donoghue 2001; Foissner 1996). Therefore, Foissner and Agatha (1999) did not particularly argue for a close relationship between armophoreans and litostomateans. However, our detailed comparison revealed further ontogenetic similarities, all considered here as apomorphies. Firstly, the proliferation of basal bodies commences in the dorsal or dorsolateral kineties in the armophoreans and litostomateans. This mode is unique to these two groups, as basal body proliferation begins in the ventral kineties in all other ciliates. Secondly, the paroral membrane and the circumoral kinety originate from kinetofragments that detach from the somatic kineties and unite into a single kinety. The paroral membrane of all other ciliates develops from an oral primordium or anarchic field. Thirdly, the adoral membranelles and the preoral kineties of the dileptids are migrating kinetofragments, indicating the latter as highly reduced membranelles (Foissner 1996; Foissner and Agatha 1999; Vďačný and Foissner 2009; Xu and Foissner 2005). In all other ciliates with a prominent adoral zone of membranelles, the new membranelles differentiate from a long oral primordium, and thus they are not migrating kinetofragments.

#### **Body shape**

Most armophoreans and some trichostomatians are twisted, and thus spiralization seems to be plesiomorphic for the Lamellicorticata. However, there are three indications that the ancestral body shape was oblong and the twisted body evolved convergently in the armophoreans and trichostomatians: (i) the body is oblong in the Spirotrichia, the closest relative of the Lamellicorticata; (ii) during binary fission of the armophoreans (*Metopus* and *Caenomorpha*), the complicated cell shape becomes oblong and all ciliary rows arrange meridionally (Foissner and Agatha 1999; Martín-González et al. 1987), indicating the ancestral body organization; and (iii) in the molecular phylogenies, the oblong trichostomatians (e.g. *Balantidium*) are placed basally, while twisted ones (e.g. *Ophryoscolex*) appear to be derived (Strüder-Kypke et al. 2006, 2007).

### Structure of somatic ciliature

The complex ciliate cortex undoubtedly evolved from a flagellate dikinetid (Lynn 2008; Orias 1976). Thus, somatic dikinetids are considered as plesiomorphic feature for ciliates. The somatic ciliature of armophoreans is still composed of dikinetids whose anterior basal body is, however, often not ciliated. In litostomateans, the somatic ciliature is monokinetidal except for the dorsal brush which is dikinetidal. This is certainly an apomorphic state for which the most parsimonious explanation is that the somatic dikinetids, except for those of the dorsal brush, lost the anterior basal body and became monokinetids. The somatic ciliature of the last common ancestor of armophoreans and litostomateans was very likely condensed in the anterior body portion. Our rationale is based on the assumption that the armophorean perizonal stripe is homologous with the litostomatean dorsal brush. Both are a specialized field in the anterior portion of the ciliary rows and are composed from narrowly spaced dikinetids with ciliated basal bodies.

#### Localization and structure of oral apparatus

The oral apparatus in most ciliates, including the armophoreans and the basal litostomateans (dileptids), occurs on the ventral side. Thus, the more or less apically or even dorsally located oral opening of all other haptorians is considered as an apomorphy. The paroral membrane is very likely homologous with the circumoral kinety and the adoral membranelles are very likely homologous with the preoral kineties (see "Stomatogenic mode"). Based on the homology of the armophorean and dileptid oral ciliature, we conclude that the oral apparatus of their last common ancestor was complex and included a paroral membrane and an adoral zone of membranelles.

#### Lifestyle

The armophoreans are typically free-living anaerobes, the haptorid litostomateans are free-living aerobes, and the trichostomatid litostomateans are anaerobic endosymbionts in a variety of vertebrates. Thus, more parsimonious would be to infer that anaerobiasis is ancestral within the Lamellicorticata and a transition to an aerobic way of life is a derived feature of the Haptoria. However, the aerobic lifestyle is an old plesiomorphy in ciliates, occurring also in the outgroup, i.e., Spirotrichia. Further, the 18S rRNA gene phylogenies indicate that anaerobic way of life evolved convergently in the armophoreans and trichostomatid litostomateans because trichostomateans branch rather deep within the Haptoria and are grouped with the free-living, aerobic haptorian *Epis*-



**Fig. 12.** An evolutionary scenario for a sister relationship of Armophorea and Litostomatea. The Armophorea maintained the ancestral oral apparatus and the somatic dikinetids, but their body became spiralized and the anterior cilia condensation was transformed into a perizonal stripe. In the Litostomatea, the anterior condensation developed into a dorsal brush, the paroral membrane became a circumoral kinety, and the multi-rowed membranelles were reduced to single-rowed preoral kineties. Asterisks mark convergently evolved features of armophoreans and trichostomatians. AC – anterior condensation of dikinetids, AM – adoral membranelles (polykineties), B – dorsal brush, CK – circumoral kinety, CV – contractile vacuole, KD – kinetodesmal fibre, MA – macronucleus, MI – micronucleus, OO – oral opening, PE – perioral kinety, PM – paroral membrane, PMT – postciliary microtubule ribbons, PR – preoral kineties, PS – perizonal stripe, SK – somatic kineties, TM – transverse microtubule ribbons.

*pathidium papilliferum* (Fig. 6; Strüder-Kypke et al. 2006, 2007).

#### Last common ancestor of Lamellicorticata

Based on the morphology and ontogeny of the extant armophoreans and litostomateans, we hypothesize that their last common ancestor was a bacterivorous ciliate living in aerobic aquatic environments and having the following apomorphies: (i) plate-like arranged postciliary microtubule ribbons, forming a layer right of and between the ciliary rows; (ii) telokinetal stomatogenesis commencing in the dorsal or dorsolateral kineties, and with migrating oral kinetofragments; (iii) oblong and not twisted body; (iv) ventrally located oral apparatus composed of a dikinetidal paroral membrane and several multi-rowed adoral membranelles; and (v) dikinetidal somatic ciliature condensed in the anterior body portion (Fig. 12).

#### Last common ancestor of Armophorea

The armophoreans maintained the complex ancestral oral apparatus and the somatic dikinetids, but their body became more or less spiralized and the anterior cilia condensation was transformed into a perizonal stripe. Finally, the armophoreans exploited oxygen-depleted habitats, developed hydrogenosomes from mitochondria, and gained endosymbiotic methanogenic bacteria (Fig. 12).

#### Last common ancestor of Litostomatea

In the haptorid litostomateans, (i) the anterior dikinetidal cilia condensation was partially reduced and developed into a dorsal brush, while all other somatic dikinetids became monokinetids, loosing the anterior basal body which is often barren in the armophoreans; (ii) the paroral membrane elongated to a U-shaped pattern, forming the circumoral kinety which is still open anteriorly in the dileptids; and (iii) the multi-rowed adoral membranelles were transformed into single-rowed preoral kineties as indicated by the localization (left body margin) and the origin (migrating kinetofragments) of these structures in metopids and dileptids (Foissner and Agatha 1999; Vďačný and Foissner 2009). Finally, the haptorians evolved toxicysts and became predators (Fig. 12). Based on the molecular phylogenies, we propose that the trichostomatid litostomateans evolved from a microaerophilic haptorian having oralized somatic monokinetids. Finally, the trichostomatians became anaerobic endosymbionts of vertebrates, simplifying the oral structures, loosing toxicysts, and transforming the mitochondria to hydrogenosomes (convergence to the Armophorea). For the review of trichostomatian morphological evolution, see Cameron and O'Donoghue (2004).

#### Are dileptids basal litostomateans?

Data on ontogenesis and conjugation (e.g. transiently formed spathidiid and polar ciliary patterns) as well as some morphological traits (complex oral ciliature, hybrid circumoral kinety) indicate that dileptids are highly derived, possibly originating from a spathidiid ancestor by developing a proboscis with a complex ciliature (Vďačný and Foissner 2008, 2009; Xu and Foissner 2005). In contrast, the molecular phylogenies of the small subunit rRNA gene (Fig. 6; Gao et al. 2008; Strüder-Kypke et al. 2006) and the present scenario (Fig. 12) suggest the dileptids as ancestral litostomateans because their oral apparatus still displays important plesiomorphic features, such as a ventral oral opening and preoral kineties possibly homologous to adoral membranelles. There is a further morphological trait sustaining the basal position of the dileptids, viz., the occurrence of small kinetofragments (adesmokineties) in the myriokaryonid and some spathidiid haptorians (Xu and Foissner 2005). These kinetofragments resemble dileptid preoral kineties in location (left of the oral bulge) and structure (short, oblique rows), and thus may be their vestiges (Fig. 12). Accordingly, all other haptorians originated from a Dileptus-like ancestor by reduction of a proboscis-like anterior body portion, causing apicalization (polarization) of the body and loss of the preoral kineties. As concerns trichostomatians, their oral ciliature is considered to be secondarily simplified, comprising only oralized somatic kinetids (Lipscomb and Riordan 1990). Further, there are obvious trends towards simplification of trichostomatid oral structures: the vestibuliferids have a vestibulum with extension of densely packed somatic kineties lining it, while the more derived entodiniomorphids have only short oral kinetofragments, the so-called polybrachykineties (Cameron and O'Donoghue 2004).

A similar kind of evolution occurred in the bacterivorous Oligohymenophorea, from which the rapacious Prostomatea evolved. In the prostomateans, the paroral membrane became an apical circumoral kinety and the subapical adoral membranelles (polykineties) were strongly reduced becoming the brosse, as first recognized by Wilbert and Schmall (1976) and Foissner (1984), and later confirmed by the detailed studies of Bardele (1999). Based on the oral apparatus (paroral membrane and three to several adoral organelles), Puytorac et al. (1993) united prostomateans with oligohymenophoreans and nassophoreans in the superclass Membranellophora. However, molecular trees (e.g. Gao et al. 2008; Kim et al. 2007; Strüder-Kypke et al. 2006) and somatic kinetid ultrastructure (slightly divergent postciliary ribbon, anteriorly directed kinetodesmal fibre, and radially oriented transverse ribbon) recover only a close relationship of the prostomateans and oligohymenophoreans (see Lynn 2008 for a review). Accordingly, we confine the Membranellophora to these two taxa (Fig. 5). On the other hand, Lynn (2008) synonymized pro parte the Membranellophora with the Heterotrichea, Spirotrichea, and Oligohymenophorea.

# A new infraphylum Lamellicorticata and macrosystem of the phylum Ciliophora

In the mid-1990s, the phylum Ciliophora was divided into two subphyla – Postciliodesmatophora and Intramacronu-

cleata (for review see Lynn 2008). The former subphylum comprises two classes (Karyorelictea and Heterotrichea), whereas the latter includes nine classes which Cavalier-Smith (2004) grouped according the localization of the cytostome into three infraphyla: the Spirotrichia, including the class Spirotrichea and Armophorea; the Rhabdophora, containing the single class Litostomatea; and the Ventrata, comprising all other intramacronucleate classes (Phyllopharyngea, Nassophorea, Colpodea, Oligohymenophorea, Plagiopylea, and Prostomatea). We were surprised to find that our 18S rRNA gene phylogeny matches Cavalier-Smith's (2004) higher classification of intramacronucleate ciliates rather well. Both differ only in the placement of the Armophorea. According to our molecular and morphological data, the class Armophorea does not belong to the infraphylum Spirotrichia, but forms a separate lineage together with the class Litostomatea, i.e., Rhabdophora sensu Cavalier-Smith (2004). Unfortunately, Rhabdophora is not eligible as a name for an infraphylum containing only Litostomatea, as it was originally proposed to unite Litostomatea and Prostomatea (Small and Lynn 1985). Therefore, we replace Cavalier-Smith's Rhabdophora with a new infraphylum Lamellicorticata which includes two classes Litostomatea and Armophorea. Thus, the classification of the subphylum Intramacronucleata has been slightly changed as follows: Spirotrichia (excluding Armophorea), Lamellicorticata infraphyl. nov. (including Armophorea and Litostomatea), and Ventrata (as proposed by Cavalier-Smith 2004).

# Why did some of the previous phylogenetic analyses fail to reveal the Lamellicorticata?

We analyzed several datasets with different taxon selections and alignments based on primary as well as secondary structure of the 18S rRNA molecule. When a high number of heterotrichean or spirotrichean sequences was included in our analyses, we observed that some armophoreans (i.e., Metopus spp.) cluster within the Spirotrichea, while others (i.e., Caenomorpha uniserialis) cluster within the Litostomatea (data not shown), an observation reported also by Miao et al. (2009a,b). Thus, the stability of the armophorean clade is somewhat dependent on the number of sequences from other groups included in the alignment. This explains why the sister relationship between Armophorea and Litostomatea may have been missed in some studies (e.g. Miao et al. 2009a,b), while indicated in others (Embley and Finlay 1994; Gong et al. 2009; Hammerschmidt et al. 1996; Hirt et al. 1995; Strüder-Kypke et al. 2006). The consistency of the three phylogenetic analyses used here with our comparative analyses of the ontogenesis and somatic fibrillar system of these two groups strongly suggests that they form a monophylum.

# **Taxonomic Summary**

Lamellicorticata infraphyl. nov.

*Diagnosis*: Intramacronucleate ciliates with postciliary microtubules arranged in a single layer right of and between the ciliary rows. Somatic dikinetids typically very narrowly spaced in anterior body portion, forming a perizonal stripe (in armophoreans), dorsal brush (in haptorid litostomateans) or clavate field (in trichostomatid litostomateans). Stomatogenesis telokinetal, commencing in dorsal or dorsolateral somatic kineties, and with migrating oral kinetofragments. *Etymology*: Composite of the Latin noun *lamella*, the thematic vowel *i*, and the Latin noun *cortex*, referring to the lamellar arrangement of the postciliary microtubules in the cortex.

*Remarks*: The infraphylum Lamellicorticata comprises two classes, viz., the Armophorea Lynn, 2004 and the Litostomatea Small and Lynn, 1981, both basically as diagnosed by Lynn (2008).

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