

Editorial

Biodiversity of Ciliates and Their Symbionts: A Special Issue

Martina Schrallhammer 

Microbiology, Institute of Biology II, Albert Ludwig University of Freiburg, 79104 Freiburg, Germany;
martina.schrallhammer@biologie.uni-freiburg.de

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Interests to estimate and assess the diversity of ciliates have a centuries-long history [1]. In recent decades, these efforts have been furthered by technological advances in molecular biology, sequencing techniques and barcoding strategies. Similar huge impacts were caused by advances in systematics and in phylogenetic reconstructions of the phylum Ciliophora [2,3].

With our increasing understanding of ciliates' diversity, our insights into their ecologies have also grown. Ciliates' distributions and frequencies are influenced, next to abiotic factors, by their interactions with predators, prey organisms, and their symbionts. Many ciliates have the capacity to harbor bacterial [4–6], archaeal or eukaryotic [7–10] endosymbionts. Their contributions to or impairments of the survival of their hosts are, in many (if not in most) cases, not yet understood. We know even less about how symbionts affect the diversification and evolution of ciliates.

This Special Issue (SI) aims to highlight new research and significant advances in the description of ciliates and their symbionts. The seven studies discuss heterotrophic and mixotrophic ciliates and their prokaryotic and eukaryotic endosymbionts originating from five continents (Europe, Asia, Africa, North and South America; [4–10]). These organisms have been isolated from diverse habitats, such as a lake in the Austrian Central Alps [8], brackish water pools in the littoral zone of the White Sea, Russia [6], a pond in a Japanese temple garden [9], or the bladder traps of the carnivorous aquatic plant *Utricularia reflexa* [10]. The manuscripts of this SI comprise the microscopic and molecular characterization of ciliates and of new or rediscovered endosymbionts. They provide insights into the biology of the intracellular symbionts such as their infection cycle [5], their host range [5,6,9], and the potential to protect their hosts against detrimental ultraviolet irradiation [8]. Several studies provide tools for species identification [5–7,9] and highlight the different species concepts applying to Ciliophora and their respective prokaryotic and eukaryotic symbionts as challenges for future studies [4,5,7,9].

A sampling campaign collected ca. 130 samples from different regions of Mexico and addressed the diversity of the ciliate genus *Paramecium* by microscopical and molecular analyses using mitochondrial cytochrome C oxidase subunit I and subunit II genes [4]. Representatives of six *Paramecium* morphological species were detected. In approximately one third of the isolated *Paramecium* strains, cytoplasmic or nuclear endosymbionts were observed [4]. Furthermore, the authors present the description of a novel species, *Paramecium quindecimcaurelia* [4]. Overall, the collected strains belonged to different clades within the respective *Paramecium* species. This finding points to the presence of hidden sibling species complexes comparable to the *Paramecium aurelia* complex [11] and others [12–14]. Members of those species might serve as model organisms to address questions of speciation, such as genetic isolation and gene flow between species.

Two manuscripts of this SI were concerned with the diversity of *Paramecium bursaria* and its green algal endosymbionts [7,9] analyzing nine [9] respectively 19 strains [7]. This *Paramecium* species is often called the “green *Paramecium*” as nearly all strains live in symbiosis with intracellular green algae, usually one of the closely related species *Chlorella variabilis* and *Micractinium conductrix*. The symbiosis with *P. bursaria* is facultative and the algae can be cultivated outside their host. Spanner and colleagues

describe a three-step isolation procedure for the establishment of axenic algal cultures from *P. bursaria* cells [7], prerequisites for subsequent detailed analyses. Additionally, the authors present a simple diagnostic PCR approach facilitating the rapid discrimination between *Chl. variabilis* and *M. conductrix* [7].

Flemming and co-authors also study the symbiosis between *P. bursaria* and its algal symbionts [9]. They report a double algal infection in a *Paramecium* strain harboring *M. conductrix* in its cytoplasm and additionally the nearly bacteria-sized picoalgae *Choricystis parasitica*. In their work they use aposymbiotic *P. bursaria* cells generated by cycloheximide treatment as receiver for either *Chl. variabilis*, *M. conductrix*, or *Cho. parasitica*. The authors use re- and cross-infections to demonstrate that, in all tested combinations, the algae can establish a long-term stable association. They conclude that the various *P. bursaria* syngens have no divergent preference for a specific algal partner [9].

Another case of a facultative endosymbiosis with a green algae has been reported from the recently described species of *Tetrahymena utriculariae* [15], which lives in the bladder traps of the carnivorous aquatic plant *Utricularia reflexa*. The description of its symbiont *Micractinium tetrahymenae* is included in this SI [10]. While *T. utriculariae* is the first mixotrophic member of this genus it remains to be verified if *M. tetrahymenae* occurs only in this host species or if it can also live in association with other ciliates [10].

The descriptions of two further novel endosymbiont species have been included in this SI—i.e., “*Candidatus* *Mystax nordicus*” [6] and “*Candidatus* *Gortzia yakutia*” [5]. Both are alphaproteobacterial endosymbionts of *Paramecium* with “*Ca. M. nordicus*” found in the cytoplasm of *Paramecium nephridiatum* [6] and “*Ca. G. yakutia*” in the macronucleus of *Paramecium putrinum* [5]. The latter belongs to a rather well-studied group of intranuclear bacteria with a complex life and infection cycle. Characteristic for these so-called HLB (*Holospora*-like bacteria) is the presence of two morphologically distinct forms specialized in either reproduction or infection. In the article, included in this SI, the authors demonstrate the ability of “*Ca. G. yakutia*” to infect naïve *P. putrinum* strains by exposing them to isolated infectious forms and following the completion of the bacterial life cycle in the macronucleus of its new host [5].

“*Ca. M. nordicus*” represents the rediscovery and detailed morphological and molecular characterization of a symbiont which was first reported 30 years ago [16]. It exhibits two unusual features: it occurs, at least sometimes, in very close association to the host’s mitochondria and in one of the examined strains it shares its host’s cytoplasm with a second cytoplasmic symbiont, “*Candidatus* *Megaira venefica*” [6]. Consequently, it represents the second example of a double infection within this SI. This article reports the case of two co-occurring bacteria [6] whereas two different algae sharing their host’s cytoplasm were characterized in the above mentioned study [9].

Coming back to algal symbionts for a last time. Usually, symbioses including algae are considered mutualistic due to photosynthesis products shared with the host, such as sugars or oxygen. Sonntag and Sommaruga [8] tested and discuss another advantage supplied by algal partners: photoprotection against UV exposure provided by, for example, self-shading or the production of sunscreen compounds like mycosporine-like amino acids. Despite this potential, they observed unexpected high levels of lethality in the three tested ciliate species *Pelagodileptus trachelioides*, *Stokesia vernalis*, and *Vorticella chlorellata* under UV irradiation similar to natural doses found at lake surfaces [8]. Thus, the authors conclude that at least the tested ciliates need to shift their position in the water column to escape highest exposure levels around noon.

All studies of this SI share a strong interdisciplinary aspect. Ciliates have been traditionally associated with the fields of zoology or protistology, the characterized symbionts with microbiology, botany, or phycology. The here performed experiments ask for experience typically found in ecology or evolutionary biology departments, cell biology, or bioinformatics. Thus, endosymbiosis research is concerned with diversity not only in regard to the studied organisms and the applied methods but also at the level of the involved researchers.

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