

## Mini Review

# The Hidden Biodiversity of Ciliate-Endosymbionts Systems

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Submitted: 24 November 2014

Accepted: 24 December 2014

Published: 26 December 2014

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## Abstract

Many bacterial endosymbionts have been recorded in natural populations of different ciliates. However, it is possible to keep only some of them as infected stockcultures under laboratory conditions and then investigate these bacteria more precisely. However, large part of morphological descriptions for ciliate endosymbionts was made in the last quarter of XX c. Thus, these data cannot be compared with more modern discoveries as reliable molecular methods have been introduced in order to study uncultivable bacteria quite recently. The novelty of new bacterial endosymbiont descriptions in ciliates is that among of the recently found species several bacteria closely related to the *Rickettsia* were reported. Mean time, many territories are still either insufficiently sampled or have never been investigated by professional ciliate taxonomists, and we can expect to find many more still hidden ciliate bacterial endosymbionts

## Keywords

- Bacteria
- Ciliates
- Exotic habitats
- Endosymbionts
- *Rickettsia*

## Diversity of Ciliates and Their Bacterial Endocymbionts

Ciliated protozoa (Phylum Ciliophora) are found in all moist habitats [1-3]. They are generally cosmopolitan in their distribution, but some endemics as well as climate depended species are present [4]. About 9 000 species of ciliates, defined as unicellular dikaryotic organisms carrying cilia at some stages in their life cycle, have been described by present [3,5]. Rich, but still poorly studied fauna of soil ciliates and numerous descriptions of new ciliate taxa from exotic habitats and scantily investigated regions [6] provide grounds to believe that a total species number about 20 000 – 25 000 for all Ciliophora is not far from reality. They may be ecto- and endocommensals and parasites of both unicellular and multicellular animals and, at the same time, serve as hosts for bacterial ecto- and endosymbionts, fungi, algae and other protists. Ecological and trophic preferences of ciliates seem to predetermine the possibility of their endosymbiosis with bacteria and the diversity of symbiotic relationships. There is a broad literature about ciliate-endosymbionts systems and relations between ciliates and its bacterial endosymbionts [7-15]. Apparently, the ability to maintain symbionts is different in various ciliate classes as well as in different groups of protists in general. Among the detected prokaryotic endosymbionts, the main part belongs to Alphaproteobacteria. Hundreds of prokaryotic endosymbionts can occupy almost all cellular compartments of ciliated protists [11,12]. In most cases, these intracellular bacteria show some kind of dependence upon their host, and don't grow on standard culture media outside of their natural habitat in the eukaryotic cell [9]. This is one of big difficulties to study the symbiosis under laboratory condition.

Surveys of bacterial endosymbiosis show this phenomenon to be widespread in majority of ciliated protist groups. However, for the host cell this relationship is usually facultative [15,16-19]. Thus, the variety of ecological niches occupied by ciliates as well as their trophic strategies and preferences suggest that endosymbiosis with bacteria may originate in different systematic (especially in different ecological) groups but with unequal probability. At present, bacterial endosymbionts are recorded in the cells of about 300 ciliate species [11,15,20-22], which is definitely an infinitesimal part of their total number. Apparently, heterotrichs and oligohymenophoreans are the most promising Ciliophora groups for such investigations as they manifest quite high level of bacterial endosymbionts biodiversity. For instance, only in the *Paramecium* genus representatives (class Oligohymenophorea) were already found more than 60 different bacterial symbionts [11,12,22].

It should be stressed that up to now there have been almost no special diversity-oriented investigations of ciliate-endosymbionts systems, especially in nature [11,22]. So far, there have been practically no comparative studies of the diversity of bacterial endosymbionts in several isolated populations of the same host species, within a species or within the same genus of ciliates. Only *Paramecium*, *Euplotes* and nowadays *Spirostomum* make an exception [11,15,19,21,23-25]. However, even for these genera the endosymbiotic studies are far from being accomplished [11,15,22,26,27]. One of the most striking features of these ciliate-endosymbionts relationships is their variability (also from evolutionary point of view). Recently, it was shown, for example, that the obligate *Euplotes* endosymbiont *Polynucleobacter necessarius* has closely related

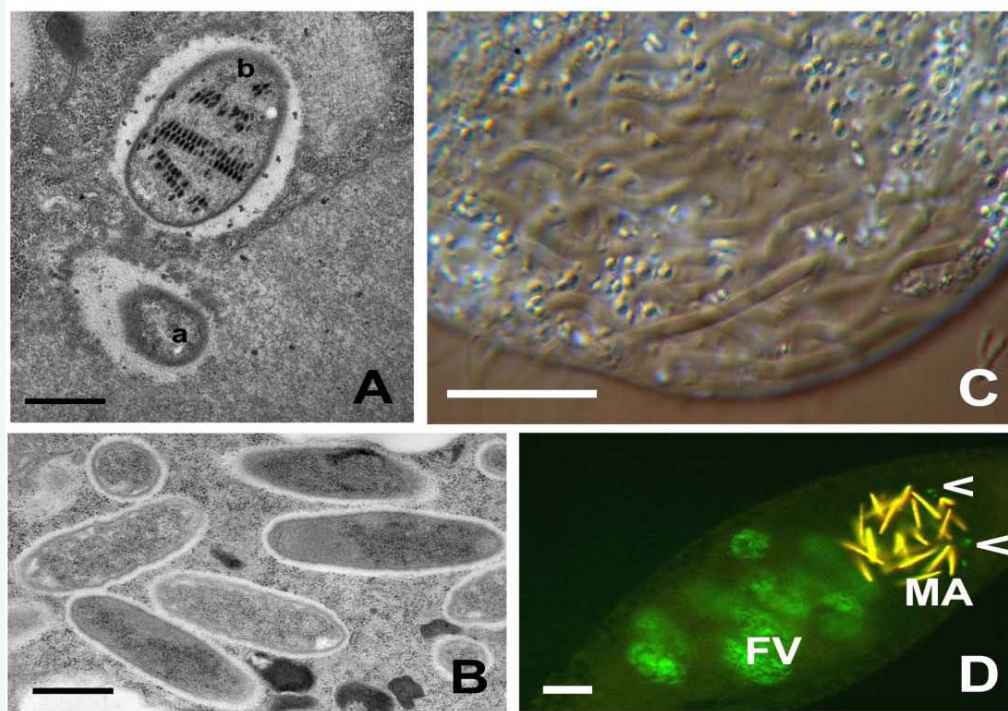
free-living relatives. This association, previously thought as going back to the very ancestor of freshwater *Euplotes* species [23], is nowadays regarded as the evolutionary youngest obligate endosymbiosis so far discovered [27]. This recent result suggests that endosymbiosis in ciliates should be regarded as open and dynamic system, in which different kind of associations can be established in relatively short time. Therefore, it can support a view that some bacterial endosymbionts in ciliates and protists in generally are not necessarily derived from long co-evolutions with certain host taxa.

### Potentially Pathogenic Endosymbionts and Geographical Areas promised for Investigations

A new, intriguing topic emerged, in the last ten years, is about the possible role of ciliates as reservoir for potentially pathogenic endosymbionts. Some of the bacteria are assigned to the group of *Rickettsia*-like organisms, closely related to the family *Rickettsiaceae* (*Alphaproteobacteria*) [10,28-34] (Figures 1A, 1B, 1C), which includes the etiologic agents of diseases produced by *R. prowazekii*, *R. rickettsii* and *Orientia tsutsugamushi*; some – to eukaryotic parasites: kinetoplastida and microsporidia [35,36]. The novelty of some recent reports arises from the fact that, despite the belonging of new bacterial endobiont species to the genus *Rickettsia*, they seem to present unusual features with respect to the other rickettsiae. In detail, the symbiont of *P. multimicronucleatum* is localised in the macronucleus of the host

cell, and the stages with phage capsids were detected both into the macronucleus and the cytoplasm (Figure 1A) the symbiont of *S. minus* presents an elongate cell shape and an unusual big size (up to 20 µm). Surprisingly enough, both the novel species have the ability to move quickly within the nucleoplasm and/or the cytoplasm of the host which is well visible in the living cell [34] (Figure 1C).

Mean time, many territories are still either insufficiently sampled or have never been investigated by professional ciliate taxonomists. Generally, it is more likely to detect a number of new ciliate species in remote and therefore rarely investigated areas such as South America, Africa, and tropical Asia or in the contrary – Arctic and Antarctic regions. But as our current knowledge suggest even in Europe the abundance of ciliate species seems to be higher than it was believed previously, and can be further increased [37]. In this context, exotic habitats and tropical countries, are yet poorly studied under the profile of ciliate biodiversity and thus of their bacterial endosymbionts as well. For instance, such studies of India ciliates and their bacterial endosymbionts, which (for the last point of investigations) just started, already revealed three novel species of endosymbiotic bacteria: “*Candidatus* *Nebulobacter yamunensis*” [32], “*Candidatus* *Cyrtobacter zanobii*” [33], and “*Candidatus* *Trichorickettsia mobilis*” [34] found in an *E. aediculatus* strain, sampled in the region of Yamuna river, in the surrounding of



**Figure 1** A) Transmission electron micrograph of “*Candidatus* *Trichorickettsia mobilis*” inside the macronucleus (flagellated form - a) and in the cytoplasm (form with viral capsids - b) of *Paramaecium multimicronucleatum*. Bar = 0.5 µm. B) Transmission electron micrograph of “*Candidatus* *Cryptoprodotis polytropus*” inside the cytoplasm of *Pseudomicrothorax dubius*. Bar = 0.5 µm. C) Part of the cytoplasm of *Spirostomum minus* (living cell, DIC contrast) with a number of “*Candidatus* *Gigarickettsia flagellata*” bacteria. Bar = 10 µm. D) Double FISH reaction (eubacterial + *Holospora*-specific probes) performed after experimental infection of the macronucleum of *Paramaecium caudatum* with *Holospora obtusa* (long bright particles). *H. obtusa* inside the macronucleus (MA) stained yellow; food bacteria inside food vacuoles (FV) in the cytoplasm stained green as well as some small introduced bacteria inside the MA (arrowheads). Fluorescence microscopy. Bar = 15 µm.

Delhi. It means that we have great perspectives in looking for hidden ciliate-symbionts systems.

### Stability of Bacterial Infection and Its Hidden Biodiversity

Assessing the number of various endosymbionts, one should also take into account the difference between the rate and stability of bacterial infection in natural populations and in laboratory cultures. In the former ones (when samples from natural habitats are examined), intracellular bacteria are much more frequent than in the latter ones. The life of ciliates and their ability to become hosts for intracellular bacteria appear to be determined in nature by some essential abiotic and biotic factors [38], which cannot be reproduced, either separately or in combination, under laboratory conditions [12,21,23]. That is why we can postulate kind of hidden endosymbiont' biodiversity which always can escape from our attention during long time investigations in laboratory. Over 15 different *Holospira* and *Holospira*-like bacterial endosymbionts can also be found inside the nuclei [22]. These infectious bacteria probably play a very special vector role in increasing the diversity of nuclear endosymbionts. Sometimes these infectious bacteria can introduce into the nuclei nonspecific microorganisms which have no own infectivity. Such co-infection could be considered as a way for elevation the hidden endosymbiont biodiversity also [39] (Figure 1D).

Majority of bacterial endosymbionts have relatively poor morphology, and for precise discrimination it is necessary to use molecular approaches which became routine in the last decade only. Since reliable molecular methods have been introduced in order to study uncultivable bacteria, a vast number of less-known and much more diverse organisms were described. However, it means that big volume of morphological descriptions produced in 1960–1990<sup>th</sup> cannot be compared with more modern set of information about ciliate' endosymbionts because all these cultures have never ever been investigated from molecular point of view. These infected populations and stocks do not exist anymore (or even have never been established). Unfortunately, very few of those infected strains were deposited during last quarter of XX century into official culture collections and even smaller number of the cultures survived with its endosymbionts until now. Some of those bacteria apparently were recorded repeatedly, what gives us a hope about rediscovery some part of such hidden bacterial biodiversity «from the past» using molecular and modern microscopy tools.

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#### Cite this article

Fokin SI, Sera V (2014) The Hidden Biodiversity of Ciliate-Endosymbionts Systems. *JSM Microbiology* 2(2): 1015.