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Dawn of astome ciliates in light of morphology and time-calibrated phylogeny of *Haptophrya planariarum*, an obligate endosymbiont of freshwater turbellarians

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Abstract

Morphology, systematic position and time-calibrated phylogeny of *Haptophrya planariarum* were investigated. This endosymbiont of freshwater turbellarians is characterized by: (i) a length of about 200–900 μm; (ii) a campanulate to truncate claviform body carrying an anterior adhesive sucker; (iii) an ellipsoidal macronucleus localized in the rear body end; (iv) a contractile canal extending along the dorsal margin; and (v) usually more than 150 meridional ciliary rows, a horseshoe-shaped suture line along the sucker, and two inconspicuous secant systems at lateral ends of the suture line. In 18S rRNA gene phylogenies, astomes were depicted as a non-monophyletic group within the scuticociliate clade, whereby *H. planariarum* clustered with the loxocephalid genus *Dexiotricha*. After considering morphological evidence, statistical tree topology tests and evolutionary distances, we find astomes as a distinct group that evolved from a free-living scuticociliate ancestor in the early Paleozoic. Molecular clock analyses indicated that astomes living in annelids diverged from those inhabiting turbellarians within about 50 Ma during the Late Cambrian and the Upper Ordovician. This comparatively short time span might have not sufficed for fixation of molecular synapomorphies in the 18S rRNA gene and/or they might have been erased by substitutions during the almost 500 Ma-long evolutionary history of astomes.

Keywords: Adhesive sucker; Contractile canal; Molecular clock; Morphology; Taxonomy; 18S rRNA gene

Introduction

Astomatia Schewiakoff, 1896 represent one of the six subclasses of the highly diverse class Oligohymenophorea de Puytorac et al., 1974. As the very name Astomatia indicates, taxa belonging to this subclass completely lost the cytostome and oral ciliature. These mouthless ciliates are obligate endosymbionts of a variety of animals, a fact causing that their distribution is determined by the geographic range of their hosts (Lynn, 2008). Astomes are comparatively commonly found in the digestive tract of turbellarians and oligochaetes, but some live in polychaetes, leeches, mollusks and one group infects newts and frogs (e.g., Cépède, 1910; Corliss et al., 1965; de Puytorac, 1957, 1963; de Puytorac and Schrével, 1965; Dixon, 1975; Fokam et al., 2008, 2011, 2012; Georgévitch, 1941, 1950; Kay, 1942; Kijenskij, 1926a,b; Lom, 1959; McAllister and Trauth, 1996; McAllister et al., 1993; Meyer, 1939; Muzzall, 1990; Powders, 1967; Sauvadet et al., 2017; Sikora, 1963; Woodhead, 1928 etc.). These mouthless ciliates attach to host's tissues by an elaborate holdfast organelle located on the anterior pole of the cell.

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This organelle can take the form of hooks, spines, spicules or of an adhesive sucker. Concerning the body size, some astome ciliates are small (<80 μm), while others are quite large (>200 μm). Their body shape ranges from flattened ovoid to worm-like. The macronucleus is globular to elongate ellipsoidal, sometimes even almost as long as the whole cell. The contractile vacuole apparatus is comparatively diverse and vacuoles can be arranged in one or two rows or may form a long canal emptying via several excretory pores (Cépède, 1910; Kijenskij, 1926a; MacLennan, 1944). The somatic ciliation is usually very dense, holotrichous and formed by monokinetids (for a review, see Lynn, 2008).

According to Lynn (2008), the subclass Astomatia contains only a single order, the Astomatida Schewiakoff, 1896, which is traditionally divided into nine families typically associated with a particular host spectrum. Endocommensals occupying the digestive tract of fresh- and saltwater turbellarians are gathered in the family Haptophryidae Cépède, 1923, namely, in its subfamilies Haptophryinae Cépède, 1923 and Lachmannellinae Cépède, 1923. On the other hand, the third haptophryid subfamily Cepediettinae Corliss et al., 1965 includes exclusive endocommensals of amphibians (Corliss et al., 1965).

The subfamily Haptophryinae contains only a single genus, *Haptophrya* Stein, 1867, living in freshwater planarians, with five nominal species described so far: *H. planariarum* (von Siebold, 1839) Stein, 1867; *H. acetabulifera* (Georgévitch, 1941) Corliss et al., 1965; *H. ohridensis* (Georgévitch, 1941) Corliss et al., 1965; *H. oweni* (Gillespie in de Puytorac, 1963) Corliss et al., 1965; and *H. stankovici* (de Puytorac, 1957) Corliss et al., 1965. Characteristic common features of these species are (i) the presence of a conspicuous adhesive sucker covered by a thigmotactic ciliary zone and (ii) binary fission without chain formation. The subfamily Lachmannellinae comprises three genera whose species inhabit marine as well as freshwater turbellarians. Lachmannellids attach to their hosts with the aid of hooks: *Lachmannella recurva* (Claparède and Lachmann, 1859) Cépède, 1910 with a single hook; *Steinella uncinata* (Schultze, 1851) Cépède, 1910 with two hooks of an unequal size; and *Annelophrya sphaeronucleata* (Georgévitch, 1950) Lom, 1959 with numerous, small spines surrounding the thigmotactic area. No chain formation was reported during binary fission of lachmannellids, similarly as in *Haptophrya* species.

For more than 80 years, there was a nomenclatorical and taxonomic problem, concerning the usage of the generic names *Haptophrya* and *Cepedietta* Kay, 1942. Maupas (1879) described a mouthless ciliate from the rectum of frogs, which he named *Haptophrya gigantea* Maupas, 1879. Interestingly, chain formation was recorded during reproduction of this species. In 1942, Kay discovered another peculiar chain-forming ciliate in the intestinal tract of a salamander. Considering morphological differences from ciliates living in planarians, he erected a new genus for his species that he named *Cepedietta fibrillata* Kay, 1942. However, most authors followed Maupas' generic classification and associated their chain-forming ciliates from amphibians with the genus *Haptophrya* (Bush, 1933, 1934; MacLennan, 1944; Meyer, 1939; Woodhead, 1928 etc.). Although de Puytorac (1963) recognized that Kay's species is congeneric with Maupas' endocommensal isolated from frogs, he still classified astomes with a prominent adhesive sucker from freshwater planarians in the genus *Sieboldiellina* Collin, 1911 which was superfluously erected for *H. planariarum* by Collin (1911). Finally, Corliss et al. (1965) ended this problem by transferring chain-forming ciliates from amphibians into the genus *Cepedietta* and ciliates with simple binary fission from turbellarians into the genus *Haptophrya*.

Just few studies have attempted to clarify phylogenetic relationships among astome ciliates. The pioneer study of Fokam et al. (2011), based on SSU rRNA gene sequences from 10 species isolated from terrestrial oligochaete hosts, revealed the subclass Astomatia to be monophyletic, with a possible kinship to the subclass Scuticociliatia though with poor statistical support. Sauvadet et al. (2017) extended datasets by adding sequences from three morphotypes of the genus *Durchoniella* de Puytorac, 1954 from marine polychaete hosts. *Durchoniella* was classified in a sister position to the clade containing astomes from terrestrial oligochaete hosts. Similarly as in the study of Fokam et al. (2011), astomes were depicted as closely related to scuticociliates, but now with much higher statistical support.

During examination of freshwater turbellarians, we were successful in isolating two populations of an outstanding astome ciliate with a huge adhesive sucker, *Haptophrya planariarum*. The newly obtained SSU rRNA gene sequences from *H. planariarum* enabled us to address some outstanding questions about (i) monophyly of the subclass Astomatia; (ii) association of astomes with their hosts during the course of evolution; and (iii) phylogenetic position of astome ciliates within the class Oligohymenophorea.

Material and Methods

Material collection and taxonomic methods

Planarians were collected from the bottom side of at least partially immersed stones in two slowly running streams, the Malá Vydrica stream in the Kačínska dolina valley (48°12′05.9″N 17°04′34.7″E) and the Drieňová dráha stream near the Drieňovská lúka meadow at the locality Železná studnička (48°11′39.3″N 17°05′50.8″E) between October and November 2016. Železná studnička is a municipal forest park in the town of Bratislava, located in the foothills of the Malé Karpaty Mts. Planarians were placed together with stones, some detritus and 0.5 liters of stream water into glass vessels and were transported to the laboratory, where they were kept in dark at a temperature of 8 °C for several weeks without nutrient addition. On the basis of morphological and molecular criteria, they were identified as *Dugesia gonocephala* (Dugès, 1830) Girard, 1850.