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BIOLOGY, MORPHOLOGY, AND SYSTEMATICS OF HYDROBIONTS

Structure of the Cell of the Amoeboid Flagellate *Thaumatomonas coloniensis* Wylezich et al., 2007 (Thaumatomonadida (Shirkina) Karpov, 1990)

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Abstract—The ultrathin structure of the amoeboid flagellate *Thaumatomonas coloniensis* Wylezich et al. has been studied. The cell is surrounded by somatic scales forming on the surface of the mitochondria. The heterodynamic flagella emerge from the small flagellar pocket. Both flagella are covered by pineal scales and thin twisted mastigonemes. The kinetosomes lie parallel to each other. The transitional zone of the flagella carries the thin-walled cylinder. The transversal plate of the flagella is above the cell surface. The flagellar root system consists of three microtubular bands and a fibrillar rhizoplast. The vesicular nucleus and Golgi apparatus are of the usual structure. The mitochondria contain tubular cristae. The extrusive organelles (kinetocysts) contain amorphous material and a capsule; they are located in cytoplasm. The capsule consists of a muff and cylinder. Osmiophilic bodies of various shapes contain crystalloid inclusions. The pseudopodia capturing the bacteria emerge from the ventral groove. The groove is armored by the two longitudinal groups of the close situated microtubules. Microbodies and symbiotic bacteria have not been discovered. The resemblance of *Th. coloniensis* with other thaumatomonads is discussed.

Keywords: Thaumatomonas coloniensis, ultrastructure, scales, kinetocysts, vesicular bodies, Thaumatomonadida

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INTRODUCTION

Thaumatomonadida (Shirkina) Karpov, 1990 includes bi-flagellar gliding, rarely, swimming flagellates covered by scales that are forming on the mitochondria surface [1–3, 8]. The branched filose pseudopodia usually emerge from a ventral groove that is armored by two groups of microtubules. The scale shape varies from oval to triangle; the cells of some species carry spicules as well [12–14]. Alongside that, there are small scales on the flagellar surface in some species [1, 7].

The studied group of the heterotrophic flagellates traditionally combines several genera: *Allas* Sandon 1927, *Gyromitus* Skuja 1939, *Hyaloselene* Skuja 1956, *Reckertia* Conrad 1920, *Thaumatomastix* Lauterborn 1899, and *Thaumatomonas* de Saedeleer 1931 [3]. The representatives of these genera differ from each other by the size and shape of the cell, the length of flagella, and the structure of the scales. The species of *Gyromitus* and *Thaumatomastix* genera are swimming organisms; the other species are gliding. New genera of Thaumatomonadida have been defined under the analyses of the scale and spicule morphology and under the results of the gene sequencing performed during the taxonomic revision of the order [10, 13, 14].

Thaumatomonadida are important bacteriotrophic components of the benthic and soil biocenoses [9, 10, 14]. The species of *Thaumatomonas* genus are the common representatives of Thaumatomonadida; they are usually found in freshwater and are the most studied representatives of this group [1–6, 8, 11]. The ultrathin structure of the cell has been described for three species: *Th. lauterborni* De Saedeleer, *Th. seravini* Mylnikov et Karpov, and *Th. zhukovi* Mylnikov et Mylnikov [1–5, 7, 11].

This study aims to describe the ultrathin structure of the cell of the recently described flagellate species *Th. coloniensis* Wylezich et al. [18].

MATERIALS AND METHODS

The culture of *Th. coloniensis* (clone T-4) [18] was provided from the collection of the protist cultures (Laboratory of Microbiology, Institute for Biology of Inland Waters). The flagellates were fed by a culture of bacteria *Pseudomonas fluorescens* Migula. The flagellates were reared in a Pratt medium [6].

In order to perform electron microscopy, the flagellates in the medium were centrifuged, then the liquid phase has been removed and the wet residue was fixed

by a 2% solution of OsO_4 and 0.6% solution of glutaraldehyde (final concentration) in a cacodylate buffer for 15–30 min at 1°C. After dehydrating in the ethanol series and 100% acetone, the material was placed into an Epon-Araldite and studied under a JEM-1011 electron microscope (Japan). The slides were prepared at the ultramicrotome LKB (Sweden).

RESULTS

The morphology of the major components of the calls carrying one nucleus has been studied in the slides. The flagellar pocket transforming into the longitudinal ventral groove, two flagella, mitochondria, and the accumulation of the derivates of Golgi apparatus (group of vesicles) are visible at the cross section of the cell (Fig. 1a). Somatic scales of a similar type cover the whole cell surface, except for the ventral groove. The scale of 0.50–0.65 μm × 0.35–0.45 μm comprises the basement and the upper plate that is mounted onto it by the two groups of three to five stalks (Fig. 1b); the upper plate is characterized by the compact edge and four to six perforations on the sides.

Two flagella (the locomotor anterior and trailing posterior) emerge from the flagellar pocket (Figs. 1a, 1c). The posterior flagellum is covered by numerous and very thin primitive curved filaments (mastigonemes) and pineal flagellar scales (Fig. 1c). These scales have been found also on the surface of the anterior flagellum in low numbers (Figs. 1i, 1j). The flagellar transverse plate is elevated above the cell surface by 0.2 μm (Figs. 1d, 1f–1h). The thin-walled hollow cylinder is located above and under the transverse plate (Fig. 1d). A diaphragm with a relatively large aperture is visible at the distal end of kinetosome (Fig. 1d). The flagellar axonemes has a standard microtubular set 9 + 2 (Figs. 1g–1j). The kinetosomes are linked with each other by two fibrillar bridges (Fig. 1e).

When interpreting the root structure of the flagella, we used the terminology provided in [7, 11].

The root system of the flagella consists of three microtubular bands, secondary microtubules, and the fibrillar structure (rhizoplast). Because the serial slides of the flagellate are absent, identifying the microtubular roots is hard. The dorsal root emerges from the kinetosome of the anterior flagellum (Fig. 1d). Two ventral roots differ by the number of their microtubules; these roots lie along the wall of the flagellar pocket (Fig. 1g). Here, ventral root no. 1 consists of 4 + 1 microtubules; ventral root no. 2 consists of three microtubules. As the roots transit to the anterior end of the cell, the number of the microtubules increases in both. Secondary microtubules presented as the bands of 6, 8, and 9 microtubules appear along these structures (Figs. 1f–1k). The microtubules are surrounded by the layer of the osmiophilic material (Fig. 1h). In the ventral groove, the microtubular bands, which probably consist of the extension of the microtubular roots, and the secondary microtubules form bunches (groups), where 8 to 11 microtubules form several closely located rows (Figs. 1k, 1l). Single microtubules are found also in the cytoplasm; no relation was found with kinetosomes (not shown). The fibrillar rhizoplast is characterized by a slight transverse striation; it widens from the proximal end of kinetosome of the posterior flagellum to the nucleus (Fig. 1m).

The nucleus diameter is 2.5–3.0 μm; the nucleus is spherical (Figs. 1n, 1o). No invagination has been found at the anterior end of the nuclei, as opposed to *Thaumatomonas seravini* [2]. Clumps of chromatin and the central nucleolus are visible in the nucleus. The Golgi apparatus is of the usual structure: the pile of the cisterns lies close to the nucleus (Figs. 1n, 1o). The derivates of the Golgi apparatus form the groups of vesicles with a length of 0.5–1.0 μm (Figs. 1a, 1o). The oval-shaped mitochondria 0.8–1.2 μm in length with tubular crists dissipate in the cytoplasm (Figs. 1a, 2a). The mitochondria surface is a place where somatic scales form. Their assembly takes part in the vesicle, which, in turn, is located in the shallow invagination of mitochondria (Fig. 1a).

Kinetocysts that look like vesicles 0.3–0.4 μm in diameter are located under the plasmalemma (Figs. 1b–1d). In the vesicle there is a capsule that looks like a cylinder with the core inside (Figs. 2b, 2c). The capsule length is 0.35–0.40 μm; the diameter is 0.20–0.25 μm. No place of kinetocyst forming was found. Discharged kinetocysts were not found.

Osmiophilic bodies of different stages of formation have been found in cytoplasm (Figs. 2e–2h). The early forms are spheres 0.6–0.7 μm in diameter located in the vesicle. In the bodies, electron-transparent crystalloid inclusions are found. The inclusion in the maturing bodies is increasing, so the vesicle elongates. At the terminal stage of maturation, most of the vesicle is filled by this structure reaching 1.5 μm in length.

The diameter of the contractile vacuole (diastole stage) is 2.3 μm (Fig. 2i). Filose pseudopodia are usually covered by somatic scales; they do not carry mictochondria and form on the ventral side of the cell (Fig. 2j). The bacteria from the external environment are captured by the pseudopodia (Fig. 2k). The reserve material is presented by spherical structures filled with a homogenous content (Fig. 2l). Microbodies and the symbiotic bacteria have not been found.

DISCUSSION

Our studies showed that cells of *Th. coloniensis* carry two flagella emerging from the flagellar pocket, thin fibrils and pineal scales on the flagella surface, thin-walled cylinder in the transitional flagellar zone,

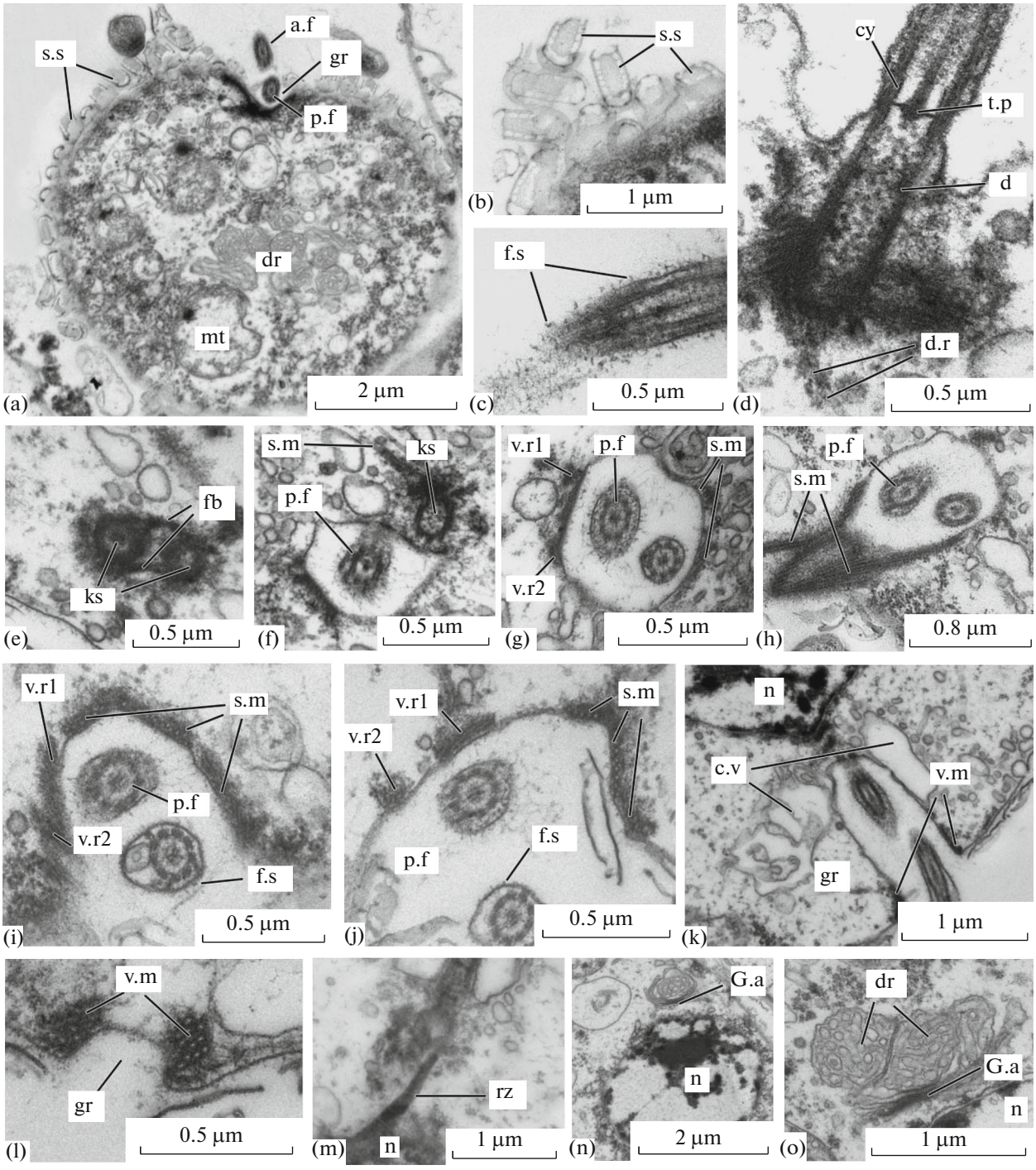

Fig. 1. General morphology of the cell of *Thaumatomonas coloniensis* and its flagella: (a) cross section of the cell, (b) somatic scales, (c, d) posterior flagellum, (e–g) location of kinetosomes, (h–k) transiting of the flagella in the flagellar pocket, (l) the part of the transversal groove in the posterior end of the cell, (m) rhizoplast, (n) nucleus and Golgi apparatus, and (o) Golgi apparatus; (G.a) Golgi apparatus, (gr) groove, (v.r1) ventral root no. 1, (v.r2) ventral root no. 2, (s.m) secondary microtubules, (v.m) ventral bunch of microtubules, (dr) derivates of Golgi apparatus, (d.r) dorsal root, (d) diaphragm in the kinetosome, (f.s) flagellar scales, (p.f) posterior flagellum, (ks) kinetosome, (mt) mitochondria, (a.f) anterior flagella, (t.p) transverse plate, (s.s) somatic scales, (rz) rhizoplast, (c.v) contractile vacuole, (cy) cylinder in the transitional zone of flagella, (fb) fibril, and (n) nucleus.

two root microtubular bands and secondary bands armoring the walls of the ventral groove, somatic scales forming on the mitochondria surface, osmiophilic bodies, and kinetocysts.

The cell architecture of *Th. coloniensis* resembles that of *Th. lauterborni, Th. seravini,* and *Th. zhukovi* [1–4, 7, 8, 11]. In particular, the somatic scales are oval and resemble that of *Th. seravini* and *Th. zhukovi*.

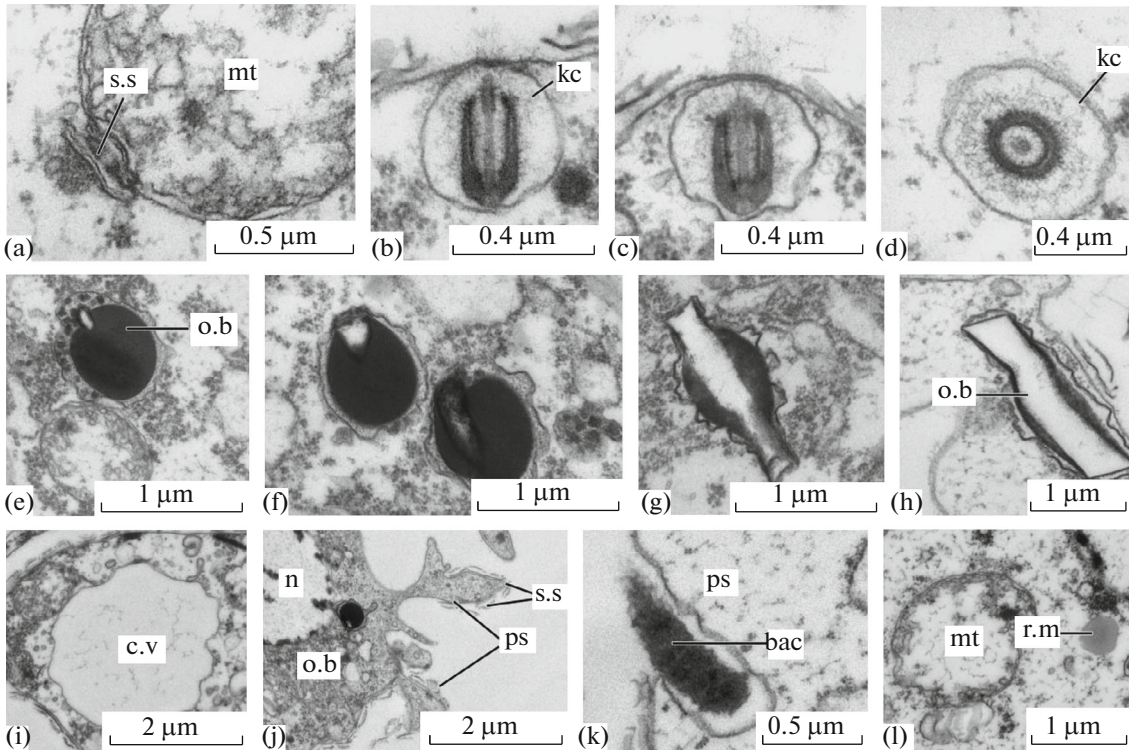

Fig. 2. Kinetocysts and the other components of the cell of *Thaumatomonas coloniensis*: (a) forming of the cover scale in the contact with mitochondria, (b–d) kinetocysts, (e–h) osmiophilic bodies, (i) contractile vacuole, (j) pseudopodia, (k) capture of bacteria, and (l) mitochondria and reserve material; (bac) bacteria, (r.m) reserve material, (kc) kinetocyst, (o.b) osmiophilic body, and (ps) pseudopodia. For the other abbreviations, please refer to Fig. 1.

In all four species, the somatic scales formed on the mitochondria surface. Two kinetosomes of *Th. coloniensis* are oriented parallel to each other and are connected by two fibrillar bridges, as is observed in other species of *Thaumatomonas* genus [3, 7]. The thin-walled cylinder in the transitional flagellar zone is probably a species-specific feature, since it has been found in all studied species. A similarity has been found also for the structure of the flagellar roots. Two roots emerge from the kinetosome of the anterior flagellum and two from the kinetosome of the posterior flagellum [11]. *Th. lauterborni* and *Th. seravini* are characterized by the full number of the root structures, but one root is absent in *Th. coloniensis* and *Th. zhukovi*. The absence of the series of the slides of the proximal end of the cell probably made it impossible to find this root. Two ventral roots and the secondary microtubules are identified doubtless. As they move to the proximal end of the cell, the microtubules of the ventral roots reorganize into two bunches (groups) that lie along the margins of the ventral groove. The number of microtubules in the ventral bunches varies from 9–14 up to 10–19 in *Th. zhukovi* and from 5–6 up to 8–9 in *Th. lauterborni* and *Th. seravini* [3]. In *Th. coloniensis*, there are 8 and 11 microtubules, respectively.

The posterior flagellum in *Th. lauterborni*, *Th. seravini*, and *Th. zhukovi* carries thin curved mastigonemes and pineal scales [1, 2, 4, 7]. The forming of the somatic scales in the invaginations of the mitochondria surface is similar in *Th. coloniensis* and the other species of *Thaumatomonas* genus studied earlier [1, 3, 4, 8].

A significant similarity of the kinetocysts of *Th. coloniensis* and the other *Thaumatomonas* species has been found. These extrusive organelles has the same architecture: they are vesicles with the capsule inside; in the capsule there is a collar with the inner cylinder. *Th. coloniensis* differs from the other species by the presence of the unusual structures—osmiophilic bodies. The electron-transparent structure inside these bodies at different developmental stages resembles the crystalloid formation. The absence of the microbodies found in *Th. lauterborni*, *Th. seravini*, and *Th. zhukovi* is another difference of the studied species.

The cell architecture of *Th. coloniensis* and the other representatives of *Thaumatomonas* genus are similar to that observed in marine *Thaumatomastix* and *Reckertia* [12, 15, 17]. There are only fragmentary data on the ultrathin morphology of the representatives of *Reckertia* genus. In *R. sagittifera* Conrad, 1920,

the somatic and flagellar scales are forming on the mitochondria surface [12]. According to the same work, kinetocysts were not found, the mitochondria contained tubular crists, and the Golgi apparatus was presented by a single dictyosome. In this species the somatic scales covered the cell surface by one layer, small and flat scales covered the short flagellum, and the long (posterior) flagellum was covered by mastigonemes. Similar structures have been found in *Th. coloniensis.* The ultrathin structure of marine *Thaumatomastix salina* Beech et Moestrup, 1986 has been described [13]. In this species, scales are also forming on the surface of mitochondria, as is observed for *Thaumatomonas*. Mainly, *Thaumatomastix salina* differs from *Thaumatomonas coloniensis* and other *Thaumatomonas* species by the length of flagella and the structure of the somatic scales. The micronucleus (absent in *Th. coloniensis*) is close to the nucleus of *Thaumatomastix salina*; in the last species, the kinetocysts are absent and are replaced by fibrillar structures (probably extrusomes). The architecture of the cell of *Thaumatomastix* sp. [19] is similar to that in *Thaumatomonas*. The differences are the shape of the somatic scales and relatively large flagellar scales, the extrusomes in *Thaumatomastix* and *Thaumatomonas* are nearly identical, and thin fibrils are present on the posterior flagellum.

The cells of the flagellate *Th. coloniensis* resemble the cells of planktonic Thaumatomonadida of *Gyromitus* genus by some features [15, 16]. Similar structures are the tubular crists of mitochondria, the location of scale formation (on the mitochondria surface), kinetocysts, and the location of kinetosomes. *Gyromitus* differs from *Thaumatomonas coloniensis* by a heart-shaped cell, its scales are cylinders or plates with arms, the groove is located on the distal end of the cell, and the flagella are of equal length.

CONCLUSIONS

The architecture of the cell of the *Th. coloniensis* clone has been studied. The cell is characterized by the presence of somatic scales and tubular crists of mitochondria, primitive mastigonemes and the pineal scales are located on heterodynamic flagella, the thin-walled cylinder is present in the transitional flagellar zone, and there are kinetocysts and the ventral groove. *Th. coloniensis* differs from the known species of *Thaumatomonas* genus by the presence of osmiophilic bodies and the absence of microbodies. This flagellate and other species of *Thaumatomonas* genus share a number of features with the representatives of *Thaumatomastix*, *Reckertia*, and *Gyromitus* genera according to the data on the ultrathin cell structure and by forming somatic scales on the surface of mitochondria.

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