Marine parasite Protaphelidium rhizoclonii gen. et sp. nov. designates the basal environmental cluster at the aphelid phylogenetic tree

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Research Article

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Abstract

Aphelids are a poorly known group of algal parasites that have raised considerable interest because of their pivotal phylogenetic position as a sister lineage to the kingdom Fungi. Recent breakthroughs in sampling, sequencing and bioinformatical analyses of environmental nucleic acids have revealed magnificent biodiversity of aphelids. Nevertheless, only 4 genera have been described (*Aphelidium*, *Paraphelidium*, *Amoeboaphelidium* and *Pseudaphelidium*); 18S rRNA gene sequences are published for all except for the marine genus *Pseudaphelidium.* Most of the environmental nucleic acid data is from analysis of freshwater samples. We isolated two new marine aphelid strains (X-138 and X-139), and herein describe the life cycle of *Protaphelidium rhizoclonii* gen. et sp. nov., a parasite of the green alga *Rhizoclonium* sp., and provide the first 18S rRNA gene sequences for cultivated marine aphelids. The new marine aphelid life cycle is mostly typical for aphelids, but also includes previously undescribed stages. Molecular phylogenetic analysis indicated that *Protaphelidium rhizoclonii* is a member of an environmental clade at the base of the aphelid tree.

INTRODUCTION

Aphelids are parasitic protists with opisthokont zoospores. Knowledge of this group began about 150 years ago with the description of *Aphelidium deformans*, an unusual intracellular parasite of the alga *Coleochaete solute* (Zopf 1885). Aphelids evoked interest of naturalists due to their lifestyle, which superficially resembles that of chytrids. Based on their morphology, Scherffel (1925) considered their phylogenetic importance to be because of their intracellular development. Unlike members of the Chytridiomycota, aphelids have phagotrophic ameboid stages in their life cycle (Gromov 2000) and molecular analysis placed them in the Opisthosporidia sister to Fungi (Karpov et al. 2013, 2014; Letcher et al. 2013).

Electron-microscopy observations during the late 1960s and the 1970s contributed to our understanding of the biology of aphelids (Gromov and Mamkaeva 1968, 1970a,b, 1975, Schnepf et al. 1972). More recent studies have clarified the phylogenetic position of Aphelida as sister to Fungi, which makes them a pivotal group of protists (Torruella et al. 2018; Mikhailov et al. 2022; Wijayawardene et al. 2024).

Although just a few aphelid species are officially described, the group is highly diverse, including many environmental sequences from different ecosystems (Seto et al. 2023; Yang et al. 2023). Currently, 20 species in four genera (*Aphelidium*, *Paraphelidium*, *Amoeboaphelidium* and *Pseudaphelidium*) have been described (Letcher and Powell 2019; Tcvetkova et al. 2019; Karpov et al. 2020; Seto et al. 2020, 2022). All but one of these are freshwater species. The marine species, *Pseudaphelidium drebesii*, a parasite of the diatom *Thallasiosira punctigera*, was described without molecular information(Schweikert and Schnepf 1996, 1997), hence its phylogenetic position is unknown.

Here, we describe the morphology of the main life cycle stages of the marine aphelid strain X-138, which along with strain X-139, represents the new genus and species *Protaphelidium rhizoclonii.* Its novelty and

position is supported by 18S rDNA based molecular phylogeny.

MATERIAL AND METHODS

Isolation and cultivation

Strains were isolated from samples collected from the littoral zone of White Sea Biological Station named after A.N. Pertsov of Moscow State University in August 2023. Sample K-1 was collected using plankton net with mesh 23 μm and transferred into 50 ml falcon, sample Cl-4 was collected by hand squeezing out mass of algae from the littoral zone into 50 ml falcon. Sample K-1 was poured on Petri dishes with the addition of f/2 medium (Guillard and Ryther 1962; Guillard 1975) in 1:1 proportion; dishes were kept at 22°C under permanent light for 2 weeks. Sample Cl-4 was diluted with f/2 medium in proportion 1:1 in the 50 ml falcon centrifuge tubes, transferred to a laboratory and placed at 17°C under permanent light. For cultivation of the alga *Rhizoclonium* sp. we cut off single filaments and put them in Petri dishes with fresh f/2 medium at 17°C. Significant growth of algae occurred after 2–3 weeks, when the filaments of algae occupied the entire Petri dish area. An optimal temperature for parasite growth was 22°C. Each 2–3 weeks infected filaments of *Rhizoclonium* sp. were transferred into new medium and supplied with new host filaments. The strains X-138 and X-139 were established from isolates K-1 and Cl-4 correspondingly.

Light microscopy

Microscopic observations of living cultures were carried out on a Leica DM2500 upright microscope equipped with DIC and Phase contrast optics and a DS-Fi-3 camera (Nikon, USA) powered by NisElements AR software (Nikon). Several filaments of algae were carefully cut off from growth on a Petri dish and transferred to a droplet on a slide.

DNA amplification and sequencing

One or several infected *Rhizoclonium* sp. cells were cut off, manually isolated with a glass micropipette, transferred to 0.2-μl PCR tubes with 1–2 μl of medium and frozen at -21°C. The 18S rRNA gene of strain X-138 and X-139 was amplified with primer combination Q5antiDika (5’ - GCC ATG CAT GTC TAA GTA TAA A – 3’) and Q3Opisth (5’ - GGA AAC CTT GTT ACG ACT TTT A – 3’) using the Encyclo Plus PCR kit with polymerase mix (Evrogen, Russia). The PCR amplification program consisted of 2 min denaturation at 94°C; 35 cycles of a denaturation step at 94°C for 15 s, a 30 s annealing step at 62°C and an extension step at 72°C for 2 min; and a final elongation step of 7 min at 72°C. Sequencing was performed on the ABI Prism 3500 xl sequencer (Applied Biosystems, USA) with the primers, used for amplification, and a number of internal primers. Newly acquired 18S rRNA gene sequences of X-138 and X-139 have been deposited in GenBank with accession numbers: XXX

Molecular phylogenetic analyses

For molecular phylogenetic analyses of aphelids and rozellids we used a data set of 18S rDNA sequences from Seto et al. (2022). Sequences were automatically aligned with MAFFT 7.409 (Kuraku et al. 2013; Katoh et al. 2018). Ambiguously aligned regions were excluded manually. 1875 sites were retained in the final alignment. The maximum likelihood (ML) tree was inferred using RAxML 9.2.7 (Stamatakis 2014). A Bayesian analysis was run using MrBayes 3.2.7a (Ronquist et al. 2012). All parameters were retained as described in Seto et al. (2022). Execution of methods was performed using CIPRES (Miller et al. 2010). Resulting trees were visualized using iTOL (Letunic and Bork 2021).

RESULTS

Light microscopic observations

The life cycle of the strain X-138 is similar to that of the marine aphelid *Pseudaphelidium drebesii*, however, with some differences.

Invasive cysts

Zoospores of X-138 attach to the host alga, encyst and penetrate the host cell wall with a penetration tube (Fig. 1A). The contents of the cyst migrate through the penetration tube into the host cell (Fig. 1B,C). Although most algal cells were infected with a single cyst, we found cells infected by numerous individual cysts or clusters containing up to 7 cysts (not shown) at the same spot on the algal surface (Fig. 1B–E). Generally, it is challenging to determine individual penetration tubes growing from a clump of cysts, but most cysts have their own penetration tube (Fig. 1C). However, we did find a pair of fused cysts with a common penetration tube (Fig. 1D,E). Occasionally, cysts group at the surface of previously infected and already empty algal cells. The contents of these cysts in the form of an amoeba are still capable of invading the algal cell (Fig. 1B,C). We also found simultaneous invasion of two amoebae from two cysts (Fig. 1C).

Trophonts (amoeba - plasmodium)

The intracellular amoeba is a trophic stage of the aphelids - it engulfs the host cytoplasm forming a central vacuole containing a residual body, which consists of red, undigested lipids (Fig. 1F,G). The parasite continues to grow and forms a multinuclear plasmodium, often with a loose and large residual body, while it totally consumes the cytoplasm of the host cell (Fig. 1H). The mature plasmodium then divides into a number of uninucleate cells (Fig. 1I), each of which produces a flagellum and thus, can be interpreted as zoospores (Fig. 1J, K).

Intracellular zoospores

Most zoospores inside host cells are spherical (~ 2.5 µm diam.) and can swim using a flagellum (~ 11 µm in length) (Fig. 1J, K). Flagellated zoospores, which move inside empty algal cells like amoeba using anterior lamellipodium then encyst. We often observed massive zoospore encystment inside the host cell (Fig. 1L–N). Newly formed internal cysts are able to form exit tubes through the host cell wall to release

from the host (Fig. 1M-O). This may be the only way for the zoospores to exit because *Rhizoclonium* cell walls are rather thick and have no any holes. Meanwhile, tubes may connect two or even three adjacent, internal cysts and the contents of one cyst have been observed to move into the other, suggesting cell fusion (Fig. 1L, O). We base our opinion that flagellated cells encyst inside the host on the occurrence of zoospores together with cysts inside the algal cell (Fig. 1L). We also found algal cells containing numerous empty cysts (Fig. 1N).

Extracellular zoospores

We did not observe how aphelid X-138 releases zoospores from inside the host; therefore, we preliminarily propose that it might be the same as invasion. This can result in a number of rounded cells outside the host, which then produce a flagellum and become crawling amoeboflagellates with relatively short and stiff flagella (~ 11 μm) and cell bodies ~ 4 μm in length. These spores form anterior lamellipodia with subfilopodia, and filopodia (Fig. 1P–R). Rarely, we found swimming zoospores with a spherical body 3.5 μm in diameter and long flagellum ~ 17 μm (Fig. 1S). They often attach to the host surface for a very short time, or can reduce their movement, gradually settle down on the slide, and form filopodia convolving flagellum around the cell (Fig. 1T,U).

Resting spores

Resting spore formation is common in the X-138 strain (Fig. 1V–X). Spore walls are composed of thick inner and thin outer layer (Fig. 1X). The body contains lipid globules of different sizes located at the poles and separated with homogenous or granulated cytoplasm. Two residual bodies are always present between the two wall layers at the opposite poles. The shape of resting spores varies from elongated to spherical what can be interpreted as different stages of their maturation from elongated to spherical shape, because the cell wall is thickening and the contents constrain (Fig. 1V-X).

Molecular phylogeny

We amplified and sequenced full 18S rDNA from strains X-138 and X-139 maintained on *Rhizoclonium* sp. The sequences are nearly identical and the isolates certainly belong to the same species. We reconstructed a maximum likelihood (ML) phylogenetic tree including their 18S rDNA sequences with a set of published aphelid sequences using sequences of rozellids and nucleariids as an outgroup (Fig. 2). In our tree, all main aphelid clades are stable and our strains turned out to be in the basal clade (called here Clade I) containing environmental sequences only. X-138 and X-139 form a sister lineage with TAGIRI-24, which is an environmental sequence obtained from the East-China Sea (Takishita et al. 2005) and with Jp13Nb17E and Jp13Nb11E, which are from Japanese freshwater lakes (Ishida et al. 2015).

DISCUSSION

Morphology and life cycle

Our newly described representative of the Aphelida has morphological peculiarities that are unknown for other aphelids. Its life cycle comprises two types of zoospores: swimming flagellates and crawling amoeboflagellates, with different lengths of flagella. One type of zoospore may transform to the other; we are not certain. We saw the beginning of the swimming form settling (Fig. 1T,U), but additional observations are needed to determine whether the swimming form settles and becomes an amoeboflagellate. Although the cell body of the swimming zoospore is similar in size as the amoeboflagellates, the length of the flagellum differs and is an important character distinguishing the two types of flagellates.

Generally, change of locomotion from actively swimming to crawling amoeba occurs in *Aphelidium* and *Paraphelidium* before encystment, when zoospores settle down on algae filament, becoming amoeboflagellate with an immotile flagellum (Karpov et al. 2014; 2017). Transformation of a swimming zoospore to the amoeboid form, when not as a part of infection process, has been documented for *Aph. insulamus* (Karpov et al. 2020, Fig. 2E,F). If no direct transformation between amoeboflagellate zoospores with short (11 μm) flagellum and spherical zoospores with long (17 μm) flagellum takes place, then we conclude that the two types of zoospores have different origins.

Members of the Chytridiomycota and the Blastocladiomycota produce zoospores after sporangial cleavage and gametes after germination of a resting spore, where meiosis is proposed to occur (Sparrow 1960). *Physoderma gerhardti* zoospores from germinating resting spores bear flagella 5–10 μm longer than those on asexual zoospores (Sparrow 1977, Table 1). Zoospores from asexual sporangia and zoospores from the resting spore of *Synchytrium endobioticum* differ in their outline and the length of flagellum, which can be seen on the author's plates (Curtis 1921, Figs. 72 and 171), but was not noted by the author.

Table 1
Comparison of *Pseudaphelidium drebesii* and *Protaphelidium rhizoclonii* gen. et sp. nov.

	***Pseudaphelidium drebesii* (Schweikert and Schnepf, 1996)**	***Protaphelidium rhizoclonii* gen. et sp. nov. (present paper)**
Zoospore body length (μm)	5	4
Zoospore body width (μm)	3	2.6
Zoospore flagellum (μm)	15	11
Zoospore filopodia length (μm)	-	Up to 11
Infective cyst diam. (μm)	3	2.8
Multiple infection	+	+
Cells after plasmodium cleavage (μm)	Amoebae, 4–10 in length	Amoeboflagellates, 4 in length
Non-infective cysts (μm)	6.5	2.8
Penetration tube in non-infective cysts	-	+
stack cysts	-	+
non-infective encystment out of the host	+	-
Host	*Thallasiosira punctigera*	*Rhizoclonium sp*
Salinity (g/l), locality	~ 28, Wadden Sea	~ 25, White Sea

Based on this comparison, we suggest that aphelid X-138 zoospores with long flagella may be produced by germination of resting spores whereas amoeboflagellates with shorter flagella could represent asexual zoospores formed after plasmodium cleavage. Some authors also suggest that resting spore can be a place where sexuality occurs (Seto et al. 2022). Unfortunately, germination of aphelid resting spores has not been described so far and we know little about its nature. The resting spore derives directly from the plasmodium, which contains several nuclei as proposed earlier for *Amoeboaphelidium chlorellavorum* by Gromov and Mamkaeva (1970). We interpreted several images of resting spores (Fig. 1U–W) as consecutive stages of their maturation, as was suggested earlier for other aphelids (Karpov et al. 2017). During this process, the cell changes shape from oval to spherical and produces thick inner and thin outer wall with a residual body between them. Strain X-138 has a residual body at each pole of the spore. A few

TEM images of *Aph. insulamus* resting spores revealed one nucleus and one or several prominent lipid globules (Karpov et al. 2020).

Based on our observations we propose a general scheme of the life cycle as follow (Fig. 3).

Two generations of zoospores have been described in *Pseudaphelidium drebesii*, another marine aphelid (Schweikert and Schnepf 1996, 1997): amoebae emanating from plasmodial division represent first generation; the second generation (the flagellates) appears after the first generation amoebae encyst; each cyst produces from 1 to 4 flagellates, depending on the amoeba's size. The smallest amoebae (5 μm) yield one zoospore and the biggest amoebae (10 μm) yield up to four zoospores. We can explain this by incomplete plasmodium division resulting in amoeboid zoospores probably having 1–4 nuclei.

That is not the case for the strain X-138, which produces a first generation of amoeboflagellates of equal dimensions and the second one (after excystment) of similar spreading amoeboflagellate zoospores.

Cysts

Stacked cysts on a surface of algae were also found in *Amoeboaphelidium protococcorum* (Gromov and Mamkaeva 1970b) and *Aph. parallelum* (Seto et al. 2022). By analogy with some chytrids, this may be related to sexual reproduction that leads to formation of resting spores (Seto et al. 2022): one gamete encysts on the other encysted gamete followed by fusion of their contents (Canter 1969; Couch 1935; Koch 1951).

One more explanation of stacked cysts on the algal surface suggests zoospore encystment on the empty cyst surface, forms penetration tube and injects its contents through empty cyst into the alga. Therefore, if the cysts appear one by one there should up to ten or more, like Gromov and Mamkaeva (1970b) observed. Probably, the cysts just use the previous penetration tube of the first cyst. Moreover, zoospores can encyst on the algal surface nearby an empty cyst and form penetration tube straight in the tube of the neighbor as we observed (Fig. 1E).

This phenomenon might be caused by thick cell wall of the host alga, i.e., it is easier for parasite to use common cyst tube or empty cysts for penetration. In all mentioned cases *Amoeboaphelidium protococcorum* (Gromov and Mamkaeva 1970b), *Aph. parallelum* (Seto et al. 2022) and present paper the green algae with thick cell wall were infected, and we have never seen stacked cysts in the aphelids living on *Tribonema gayanum* having a gap in the cell wall.

If the newly formed cyst just uses an empty tube of previous one, or the empty cyst, it makes nothing with resting spore formation. We really need to observe the cyst development and formation of resting spores to clarify the biological nature of the stacked cysts.

The thick cell wall of *Rhizoclonium* can also cause difficulties for zoospores to release. Possibly, a capability of internal zoospores to encyst and penetrate through the cell wall is an adaptation of parasites to marine littoral algae. The intracellular encystment and formation of a tube through the host

cell wall has been reported for *Aph. insulamus* (Karpov et al. 2020), what means that it is common for aphelids. Numerous intracellular encysted cells of X-138 strain produce tubes through host cell obviously to release from the host. Although we did not observe the moment of zoospore release, the presence of many emptied intracellular cysts support this idea.

We were lucky to capture the moment of simultaneous invasion of two amoebae from two cysts independently (Fig. 1B,C). This leads to formation of two trophonts, what we could often observe (Fig. 1F). This way of penetration was described for all known aphelids and studied with TEM (Schnepf et al. 1971; Karpov and Paskerova 2020). Perhaps, further fusion of two trophonts occurs to produce a single plasmodium, as it was discussed recently for aphelids (Tcvetkova et al. 2023). That suggests a common phenomenon for aphelids.

Identification, molecular phylogeny and taxonomy

Isolates X-138 and X-139 have nearly identical full 18S rDNA sequences, suggesting that they belong to one species even though these strains were isolated from different samples collected ~ 700m from each other along the marine shore.

Our phylogenetic reconstruction saved all main aphelid clades together with paraphyletic genus *Aphelidium* and shows, that *Protaphelidium rhizoclonii* gen. et sp. nov. is a single described species in the basal clade composed of environmental sequences only. This clade turns off the next after highly divergent *Aph. collabens* lineage (Seto et al. 2020, 2022) and called here Clade I. The SSU rDNA of strain X-138 differs from that of sister marine clone TAGIRI-24 by 10.57% and of the closest described species *Aphelidium parallelum* (11.83%). These differences indicate at least genus level, though it is well known that genetic divergence for species level for aphelids is also rather high (Karpov et al. 2020). Despite a sister position to the marine clone, our strains have higher percentage of identity (90.22%) to an isolate under accession number OQ702871 from Seto et al. (2023) (not present on Fig. 2), obtained from fresh water samples in Michigan. Based on the position of X-138 in a clade of environmental sequences, and its unique life cycle features, we declare it as a new genus and new species *Protaphelidium rhizoclonii* Seliuk et Karpov.

Pr. rhizoclonii gen. et sp. nov. resembles in some respects *Pseudaphelidium drebesii*, which parasitizes the marine diatom *Thalassiosira punctigera*, but essentially differs in others (Table 1).

Taxonomy

Among the studied aphelids, strain X-138 is morphologically closest to *Pseudaphelidium drebesii*; both are marine and have two zoospore generations with encystment in between. Theoretically, X-138 could be a *Pseudaphelidium* species, but we choose not to place the new species in this genus because the phylogenetic position of *Pseudaphelidium* is unknown. Even if the genus *Pseudaphelidium* is represented by one of the published environmental sequences in Aphelida Clade I, the rDNA of *Protaphelidium rhizoclonii* differs from the nearest environmental sequence in Clade I by more than 10%, which

corresponds a genus level difference. Thus, we establish a new genus and species for strains X-138 and X-139 of this marine parasite.

Protaphelidium Seliuk et Karpov, present paper.

Amoeboflagellate zoospores with a single opisthokont flagellum. Zoospore attaches to a host cell, encysts, penetrates into the cell interior, and develops into a phagocytotic plasmodium with central big digestion vacuole containing a prominent residual body composed of several lipid globules. Plasmodium cleaves to form uninucleate ameboflagellated cells, which encyst within the algal host. Cells release from algae through exit tubes and give rise to amoeboflagellate zoospores. Resting spore normally contains two opposite resting bodies between the inner and outer walls. Parasites of marine green algae predominantly.

Etymology: πρώτα (greek) – the first, meaning the aphelid Clade I.

Type species: *Protaphelidium rhizoclonii* Seliuk et Karpov, present paper.

Protaphelidium rhizoclonii Seliuk et Karpov. Type: Fig. 1, present paper.

Zoospores 4 μm long and 2.6 μm wide, flagellum 11 μm long. Plasmodium cleaves to form globular cells from which the amoeboflates arise. They form cysts measuring 2.8 μm in

diameter, which penetrate host cell wall to release zoospores.

Host: The marine green alga *Rhizoclonium* sp.

Distribution: sea coastal waters.

Type locality: White Sea, near village Poyakonda, N66.5549°, E33.09907°.

Holotype: Fig. 1.

Type strain: X-138

Etymology: after generic name of the host alga.

Notes. Flagellated cells of *Pr. rhizoclonii* differ from *Ps. drebesii* by shorter flagellum (11 vs 15 μm), much smaller non-infective cysts (2.8 vs 6.5 μm), smaller cells after plasmodium cleavage (3 vs 5–10 μm). Plasmodium of *Ps. drebesii* cleaves to form non-flagellated amoebae, which are slowly (for some hours) released from diatom frustule via the girdle region, and then encyst. Meanwhile the plasmodium of *Pr. rhizoclonii* produces amoeboflagellates that encyst intracellularly and are released from the host through exit tubes without division as in *Ps. drebesii*.

In *Pr. rhizoclonii* both zoospore generations have flagella, while in *Ps. drebesii* the first generation has non-flagellated, amoeboid zoospores. Penetration tube in non-infective cysts and stack cysts were not

shown for *Ps. drebesii.* And lastly, they have different hosts.

Declarations

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Availability of data and material

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests.

The authors have no relevant financial or non-financial interests to disclose.

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Author Contributions

Sample collection, isolation and cultivation, light microscopic observations and sequencing were performed by Alexei Seliuk. Sergey Karpov contributed to the study conception and design. Both authors commented on previous versions of the manuscript, read and approved the final manuscript.

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Figures

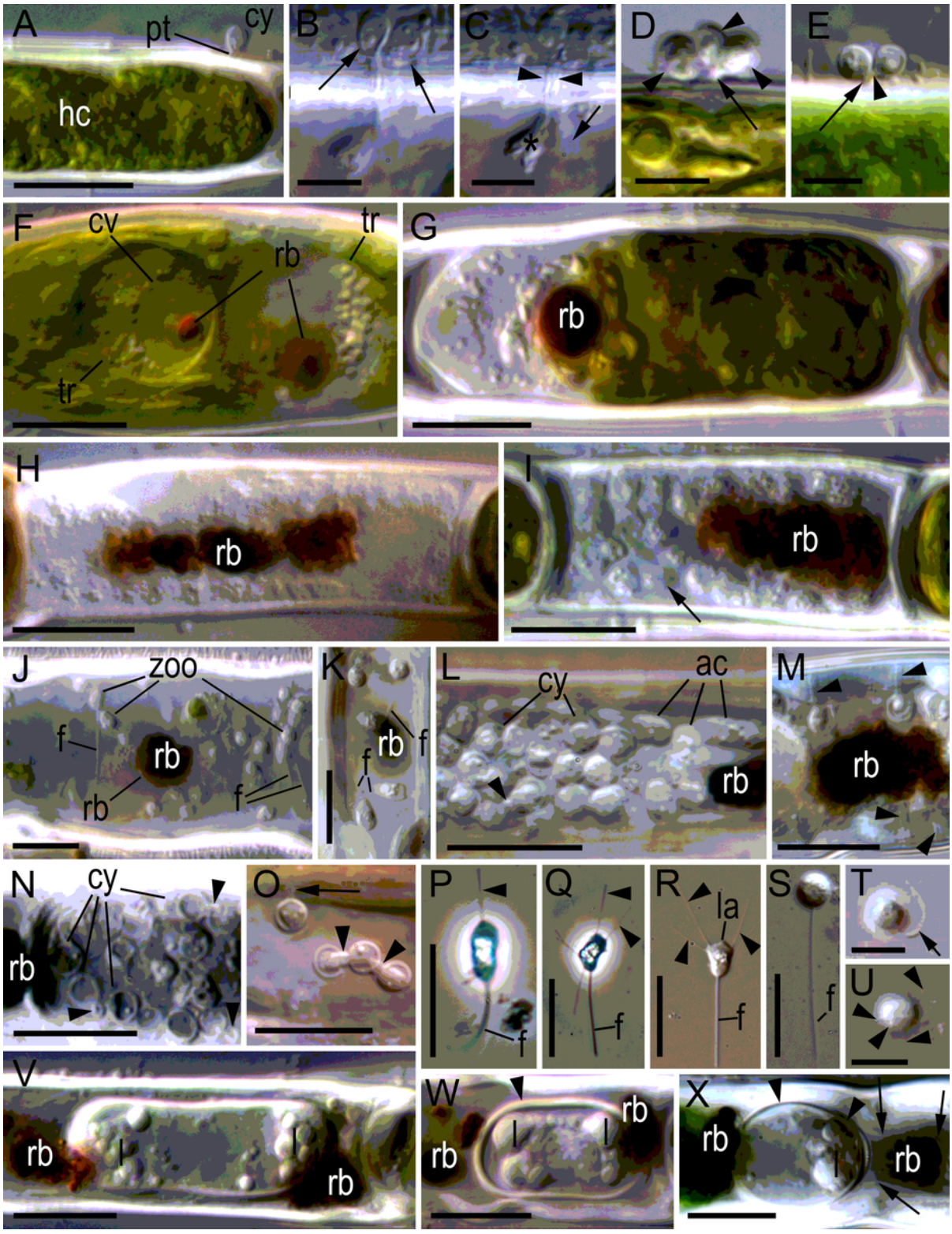

Figure 1

Main stages of the life cycle of *Protaphelidium rhizoclonii* strain X-138 observed in living material with DIC and phase contrast (**O, P**). **A**: cyst (cy) with penetration tube (pt) on the surface of the host cell (hc); **B, C**: two optical sections of two empty cysts (arrows) with contents injected into the host cell; penetration tubes lie extremely close to each other (arrowheads) with penetrating amoeba from the left cyst (asterisk) and second amoeba not in focus (arrow) from the right cyst; **D**: three infective cysts (arrowheads), two

with a common penetrating tube (arrow); **E**: two cysts with connection between them (arrowhead) and common penetration tube (arrow); **F**: two young trophonts (tr) in the host cell with corresponding residual bodies (rb) in the central vacuoles (cv); **G**: maturing trophont, which has engulfed almost half of the host cell; **H**: plasmodium with loose residual body; **I**: mature plasmodium with distinguishable spherical cells (arrow); **J**, **K**: amoeboflagellate zoospores (zoo) with flagellum (f) in the algal cell; **L**: numerous cysts inside a host cell (cy), some of them are stack with connections (arrowheads), with remnant crawling amoebae (ac) which have not encysted yet and residual bodies lying on the opposite sides (rb); **M:** cysts with exit tubes through the cell wall of algae (arrowheads); **N**: numerous empty cysts (cy) with exit tubes (arrowheads); **O**: connections between three cysts (arrowheads) with cytoplasm migration into the middle cyst; exit tube of the cyst directed outside (arrow); **P–Q**: amoeboflagellate zoospores with flagellum (f), lamellipodia (la) and subfilopodia (arrowheads); **S**: zoospore with long flagellum (f) and **T,U:**its settlement down with convolving flagellum (arrow) and small filopodia (arrowheads); **V–X** resting spores with lipid globules (l), ejected residual bodies from opposite sides. Residual bodies lie in between of the spore wall (arrowhead) and outer thin wall (arrow).

Scale bars: **A,F–S,V–X** – 10 μm; **B–E,T,U** – 5 μm.

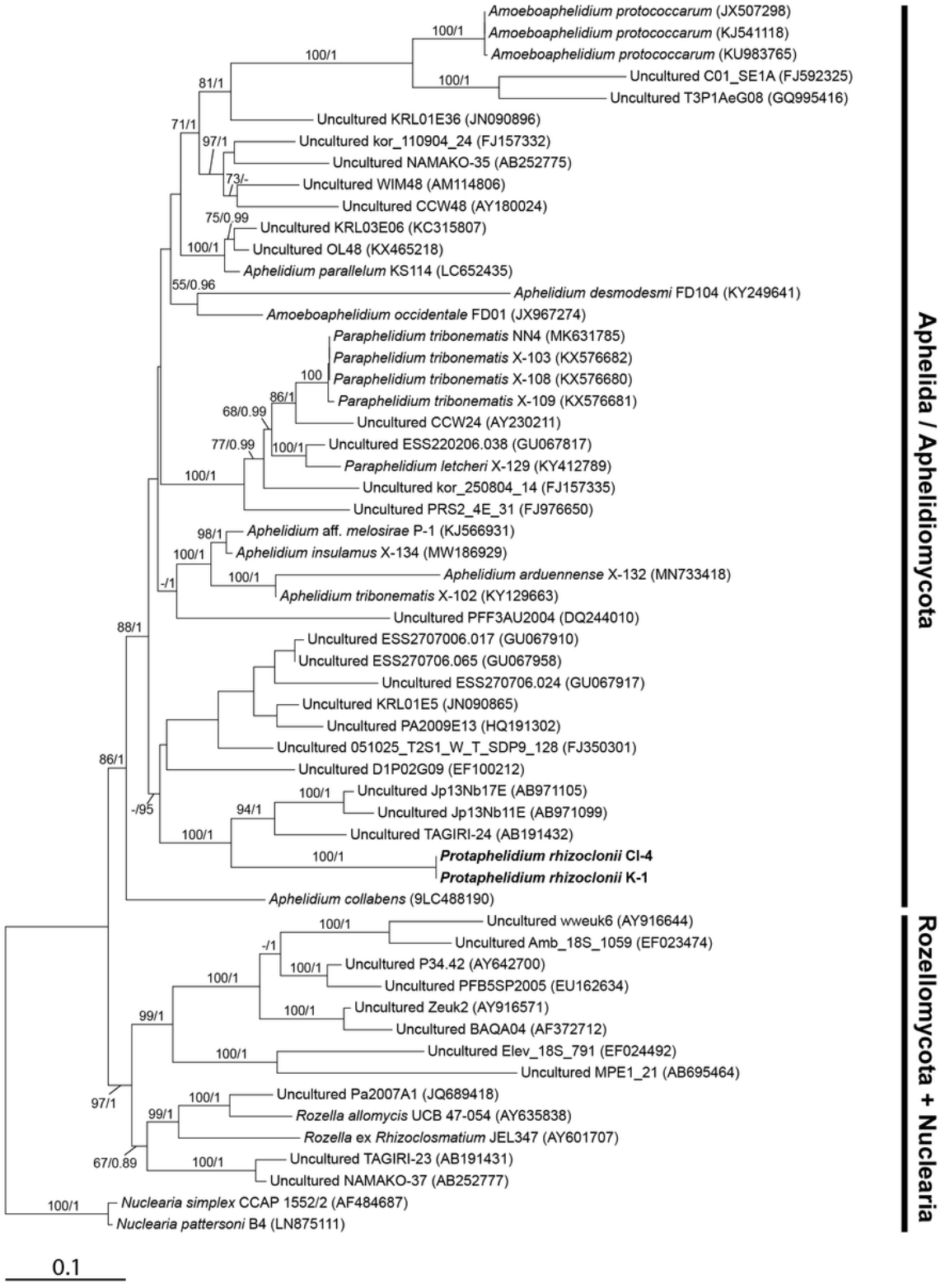

Figure 2

Maximum likelihood consensus tree of aphelids and rozellids with some of nucleariids based on 18S rDNA sequences, showing position of strains X-138 and X-139 (in bold). The tree was constructed using 1875 nucleotide characters. Node support values are as follows: bootstrap values (RAxML; above 50% shown) followed by Bayesian posterior probabilities (MrBayes; above 95% shown).

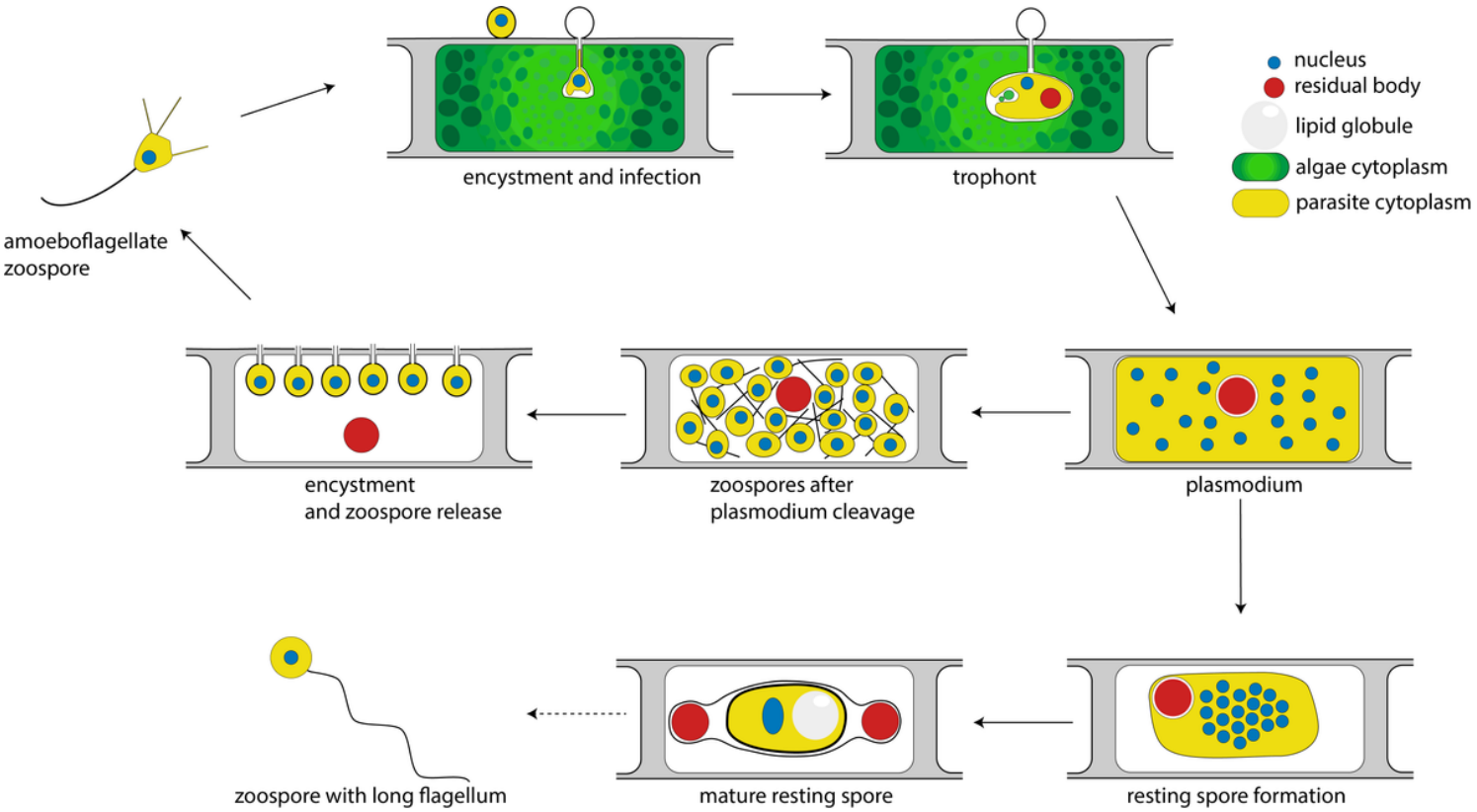

Figure 3

General scheme of the *Protaphelidium rhizoclonii* gen. et sp. nov. life cycle.

Dash arrow shows a proposed way of the origin of zoospores with long flagellum.