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Life cycle of *Schizochytriodinium calani* nov. gen. nov. spec., a dinoflagellate parasitizing copepod eggs*

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ABSTRACT: During the Polarstern-cruise ARK IV/2, June 1987, in the Fram Strait, dinophytes parasitizing copepod eggs were observed. In the laboratory on board, vegetative reproduction was documented and re-infection of *Calanus glacialis* and *C. hyperboreus* eggs was experimentally established. During food uptake, a primary cyst produces successively several secondary cysts, all separating immediately after formation from the primary cyst. In every one of these free floating secondary cysts up to 256 dinospores are formed by palintomy. Re-infection only occurred after a "maturation time" of at least 2 days after formation of the dinospores. The life cycle is compared to that of other similar parasitic dinophyte genera: *Apodinium* Chatton, *Chytriodinium* Chatton, *Dissodinium* Klebs in Pascher and *Myxodinium* Cachon, Cachon & Bouquaheux. As the taxon under discussion does not fit in with any species or genus known so far, it is described as *Schizochytriodinium calani* nov. gen. nov. spec.

INTRODUCTION

During the Polarstern-cruise ARK IV/2 in June 1987 to the Greenland Sea, parasitized copepod eggs were observed. The infected eggs were isolated, and at first glance the parasite was classified as a dinoflagellate of the genus *Chytriodinium* Chatton or *Dissodinium* Klebs in Pascher. Subsequently, vegetative reproduction was documented and followed from dinospore infestation of the copepod egg to dinospore release. Vegetative reproduction of this organism shows a new type of life cycle not described so far for parasitic dinoflagellates; in consequence, it is described as a new species belonging to a new genus.

MATERIAL AND METHODS

Samples were taken by vertical tows (20 m to surface) with a plankton net of 20 µm mesh size. Immediately after sampling, one subsample was stored at −1 °C in an incubator, the other placed in a petri dish, and species composition observed with a dissecting microscope (Wild M 400). For observations with higher magnification and long-term documentation , seawater immersion objectives (Leitz SW 25, SW 50) were used in connection with a Leitz Orthoplan. The life cycle of the parasitic dinophyte was

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documented by video with a Sony DXC-101 P camera and a Panasonic NV 180 recorder. Copies of this tape are available from the author.

Infected copepod eggs were isolated by micropipetting into petri dishes with sterile, filtered sea water and placed in an incubator at −1 °C. For re-infection experiments, eggs of *Calanus glacialis* and *C. hyperboreus* were taken from fecundity experiments with these copepods. Eggs were added not later than 20 hours after oviposition. Every day fresh eggs were added.

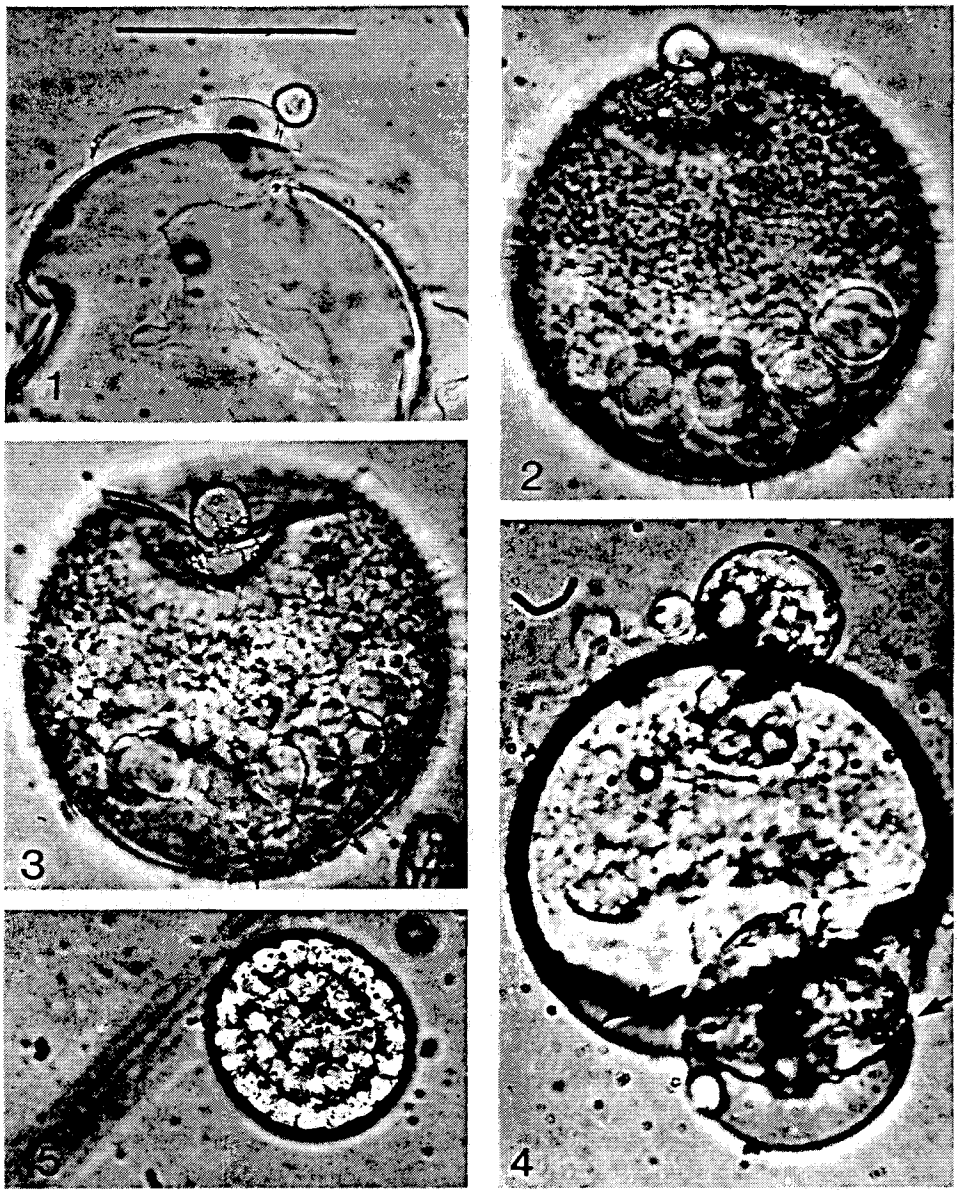
RESULTS

The parasitic dinophyte was found at three stations: St. 207 at 80° 00.0′ N, 01° 40.8′ E on 15. June; St. 213 at 80° 00.0′ N, 06° 31.6′ E on 16. June, and St. 216 at 78° 53.4′ N, 06° 42.6′ E on 17. June 1987. Water temperature was between −1 °C and +1.5 °C, salinity was about 34.5 ‰.

A small dinospore infests a copepod egg and protrudes a peduncle-like organelle into the egg. If the egg has an additional wall this peduncle-like organelle can reach the length of the dinospore itself (Fig. 1) but if the copepod egg has no additional external wall the peduncle-like organelle is shorter (Fig. 2). The dinospore gradually loses its shape and becomes spherical. At about 10 to 15 min after infestation the peduncle-like organelle increases in size, forming an elongated refractive body with a proboscis-like elongation at its end (Fig. 2).

Around the part lying inside the copepod egg, an enlarging hyaline area appears (Fig. 3). This is interpreted as extracellular digestion taking place. Gradually, the exterior part increases in shape. The „sucking forces" may be so large that in some cases the copepod egg invaginates (Fig. 3), but not always (Fig. 4). It seems that no particulate food but only liquid material is taken up by the parasite. Nevertheless, in the external part of the parasite small refractive spheres occur which apparently are formed by the parasite itself (Fig. 4). These spheres gradually change to brownish „food masses" of irregular shape, which later, during cell division of the secondary cysts, are divided during each division step. Inside the copepod egg the parasite also enlarges the zone of apparent extracellular digestion. The egg content gradually retracts from its wall and shrinks whereas the external part of the parasite grows (Fig. 4). The dinoflaggellate does not develope a conspicuous permanent holdfast and sucking organelle as described by Cachon & Cachon (1968) for *Chytriodinium*.

When the size of the external part of the parasite is about 50 to 80 µm a primary cyst wall can be discerned and, shortly after, the cytoplasm starts cytoplasmic division. Live observations did not allow the determination of the exact time of nuclear division and the axis of nuclear division plane. The nucleus of the secondary cyst is spherical to obovate and chromosomes are clearly visible. It is suggested that the nucleus of the primary cyst has the same characteristics. The cytoplasmatic division plane is perpendicular to the long axis of the peduncle-like infestation organelle (Fig. 4). During cytoplasmic division the parasite continues to feed and therefore continues to grow. Thus, wall rupture occurs in the primary cyst shortly before termination of cytoplasmic division. After cell division the so formed secondary cyst detaches itself from the primary cyst and floats free in the water. Due to this process the primary cyst is reduced in size but continues to feed. Therefore it may be designated as a „trophocyte", a term created by Chatton describing the life cycle of the dinophyte *Apodinium*, an ectoparasite of Appendicularia. In this



Figs 1–5. *Calanus hyperboreus*. 1 A dinospore shortly after infection of *Calanus hyperboreus* egg with additional wall; but the nauplius hatched before *Schizochytriodinium calani* had penetrated. Note the long peduncle-like organelle. 2 A dinospore has infected a young egg of *Calanus hyperboreus* without additional wall; the peduncle-like organelle is short. 3 Early stage of infection. Note the indentation of the egg, in contrast to Figure 2, and the hyaline zone of presumably extracellular digestion. 4 Egg of *Calanus hyperboreus* with experimental multiinfection. On the top left an early stage, comparable to that of Figure 2; on the right hand a later stage with clearly visible internal part of holdfast and sucking organelle; on the lower part right a primary cyst forming a secondary cyst inside the primary cyst wall; arrow shows the cytoplasmic fission line. 5 Detached planktonic secondary cyst during palintomic dinospore formation. Scale for Figs 1–5: 100 µm

genus, the primary cyst produces, by repeated cytoplasmatic divisions, several "gonocytes" inside the primary cyst wall which start with dinospore formation, whereas the trophocyte continues to feed and to produce new gonocytes (Chatton, 1920; Cachon & Cachon, 1973).

The primary cyst of the species under discussion forms a new cyst wall and produces in the course of several hours 3 or 4 more secondary cysts, all of which detach themselves after rupture of the newly formed primary cyst wall. Thus, the number of secondary cysts released can be checked by the number of cyst walls still attached to the copepod egg. If the copepod egg is sucked out more or less completely by the primary cyst, the latter stops producing secondary cysts but after termination of food uptake, multiple cell divisions take place inside the cyst wall, resulting in dinospore formation. Additionally, each secondary cyst – after a time lag of at least 6 h up to one day – starts with cytoplasmatic division resulting in the formation of 132 to 264 dinospores in each secondary cyst. Thus, one parasite may produce between about 500 to 2000 dinospores in about 2 days after infestation of a copepod egg. After termination of the last nuclear division inside the secondary cyst the furrows and flagella are formed. In the three cases observed, wall rupture in the secondary cyst occurred prior to final cytoplasmic division of the cells. Thus, long chains of more than 20 dinospores emerge from the cyst wall and swarm together for a short period before they disintegrate into small chains and finally into single cells. Only in one case were 2 single dinospores observed to occur inside the secondary cyst; the others, however, were released as chains. Dinospore morphology could not be determined exactly, as dinospores swim very fast, and they were preferably used for re-infection experiments. They are about 10 to 12 µm long, about 8 µm broad and dorsoventrally slightly compressed. The longitudinal furrow extends onto the epicone, probably to the apex. The cingulum is slightly displaced, resembling that of free living *Gymnodinium* Stein and *Gyrodinium* Kof. & Sw. species. The nucleus is spherical with clearly discernible chromosomes. No chloroplasts are present, as tested by fluorescence microscopy.

The further fate of the dinospores is not exactly known. If copepod eggs are added directly after dinospore liberation, no infestation took place. Only after a „maturation time" of at least 2 days could the next infestation be observed, although fresh eggs were added to the culture vessel every day. Binary fission of the dinospores in the motile stage after disintegration of the chains has not been observed but cannot be excluded due to sporadic observations. In contrast, few encysted dinospores were observed. Whether there is an obligate vegetative resting stage prior to the next infestation, or even a casual or obligate sexual reproduction remains pure speculation at the present state of knowledge. But re-infection can be induced experimentally as shown above. In these experiments several multiinfections resulted (Fig. 4), and up to 6 dinospores infested one copepod egg. Although eggs of both copepod species were infested in the experiments it seems that *C. hyperboreus* eggs were preferred.

DISCUSSION

Dogiel (1906) described three dinoflagellate species parasitizing marine crustacean eggs. As the dinospores were of the *Gymnodinium* type, he placed them into the genus *Gymnodinium* Stein. However, he did not observe dinospores infesting the eggs but he

concluded that the dinospore as a whole penetrates into the egg and later forms the primary cyst. Chatton (1912, 1920) re-interpretated the observations of Dogiel, and created for these parasitic species the genus *Chytriodinium*. He was right in his view that the dinoflagellates were ectoparasites, but he was wrong in postulating – in analogy to the life cycle of *Apodinium* – that sporogenesis is of the palinsporogenetic type. Palinsporogenesis is defined as repeated division with a trophic differentiation into unequal daughter individuals: the trophocyte, which remains associated with the host, continuing to feed and grow, and the gonocyte which continues to divide by linear palintomy while the trophocyte continues to produce new gonocytes. In contrast, palintomy is defined as binary fission repeated over and over again, without the intermediate stage of nutrition and growth, and leading to the formation of completely identical products of reproduction.

Cachon & Cachon (1968) showed that the interpretation of Chatton was in contradiction to the description of Dogiel (1906) but that *Chytriodinium* reproduces clearly by palintomy. The dinospore infests a crustacean egg, forms a conspicuous holdfast and sucking organelle inside the prey; shortly before termination of the food uptake strictly palintomic dinospore formation starts inside the primary cyst wall. This mode of reproduction was also documented on material from the Northwest African upwelling region (Elbr. unpubl.). The life cycle of *Chytriodinium* is depicted schematically by Cachon & Cachon (1987, Fig. 13.15). In *Myxodinium* Cachon, Cachon & Bouquaheux, a parasite of the marine prasinophyte *Halosphaera*, dinospore formation is quite similar, clearly differentiating *Chytriodinium* and *Myxodinium* from the parasite just described. In *Schizochytriodinium*, a primary cyst – equivalent to a trophocyte – subsequently produces several secondary cysts which detach immediatly after formation from the primary cyst. In these secondary cysts palintomic dinospore formation starts after a time lag. The life cycle is depicted schematically in Figure 6.

Dissodinium also parasitizes copepod eggs but its life cycle differs in several cardinal points (Elbrächter & Drebes 1978; Drebes 1978, 1984). In *Dissodinium*, after termination of particulate food uptake, the primary cyst detaches itself from the host and only then do several nuclear divisions take place before the first cytoplasmic division starts. Secondary cysts are formed by multiple cytoplasmic division inside the primary cyst wall which ruptures only after all secondary cysts are formed.

In conclusion, the life cycle of the parasite described above does not fit into any of the genera described so far. Therefore it is designated as *Schizochytriodinium calani* gen. nov. spec. nov. It is included into the family Chytriodiniaceae Cachon & Cachon. It differs from the Apodiniaceae Chatton with regard to the feeding organelles, food uptake and host: feeding organelles are fine rhizoids inside the host epithelium in *Apodinium* but a large holdfast and sucking organelle in *Schizochytriodinium*; slow food uptake by osmotrophy in *Apodinium* but extracellular digestion followed by fast food uptake in *Schizochytriodinium*; hosts are Appendicularia for *Apodinium* but crustacean eggs for *Schizochytriodinium*.

Diagnosis: *Schizochytriodinium* Elbrächter gen. nov.

Ectoparasitic dinophytes of crustacean eggs. Sporogenesis: a dinospore sucks out an egg and grows to a primary cyst (trophont) which subsequently produces 3 to 4 secondary cysts. Inside the primary cyst wall secondary cysts are liberated by rupture of this primary cyst wall and shed into the water. Inside these secondary cysts many dinospores are

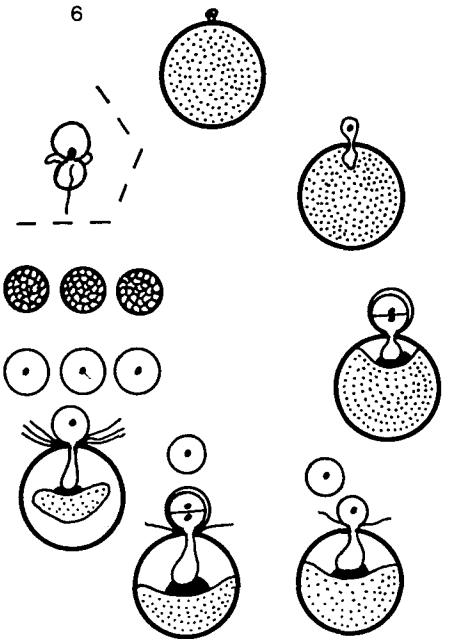


Fig. 6. A schema of the life cycle of *Schizochytriodinium calani*

formed by palintomy. Dinospores hatch from the secondary cyst wall. Dinospores of the gymnodinium- or gyrodinium-type infect new crustacean eggs. Nucleus spherical to obovate. Differs from *Chytriodinium* Chatton by its life cycle.

Type species: *Schizochytriodinium calani* Elbrächter spec. nov.

Dinophyta ectoparasitica de ovo crustaceo. Sporogenesis: una dinospora exsugit ovum et crescit in cystam primam (trophontus), quae postero tempore format 3 ad 4 cystas secundas. Omnis cysta secunda est formata e cysta prima intra involucrum cystae primae et rumpendo huius cystae primae liberata in aquam. Formatio dinosporum divisione cytoplasmae palintomiformi intra cystas secundas planctonicas. Dinosporae efflorescunt ex involucro cystae secundae. Dinosporae gymnodinioides vel gyrodinioides inficiunt ova crustacea nova. Nucleus sphericus vel obovatus. Differt a *Chytriodinium* Chatton cyclo vivendi.

Typus generis: *Schizochytriodinium calani* spec. nov.

Diagnosis: *Schizochytriodinium calani* Elbrächter spec. nov. Life cycle as described for the genus. Primary cyst about 50 to 80 µm in diameter, secondary cyst of similar size. Dinospores 10 to 12 µm long, about 8 µm broad, dorsoventrally slightly compressed. Without chloroplasts. Host: eggs of *Calanus glacialis* and *C. hyperboreus* (Crustacea, Copepoda). Distribution: marine plankton. Type locality: Arctic Atlantic, near Svalbard.

Type: Figs 1–5

Cyclus vivendi idem ac, qui generis. Cysta prima 50–80 µm dimensione, cystae secundae æqualiter dimensionibus. Longitudines latitudinesque dinosporarum 10–12 µm, 8 µm, dorsoventraliter vix applanata. Chloroplasti absentes. Parasiticus

ovarum *Calanus glacialis* et *C. hyperboreus* (Crustacea, Copepoda). Distributio: in aquis marinis maris atlantici arctici prope Svalbardiam insulam. Typus: Figs 1–5.

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