Haidadinium ichthyophilum gen.nov. et sp.nov. (Phytodiniales, Dinophyceae), a freshwater ectoparasite on stickleback (*Gasterosteus aculeatus*) from the Queen Charlotte Islands, Canada

J. Buckland-Nicks, T.E. Reimchen, and D.J. Garbary

Abstract: The dinoflagellate *Haidadinium ichthyophilum* gen.nov. et sp.nov. is associated with the threespine stickleback, *Gasterosteus aculeatus* L., in freshwater. This new genus differs from all previously described dinoflagellates in the morphology and ultrastructure of its complex life history stages and in the ecology of its interaction with *G. aculeatus*. Distinguishing characters included (*i*) the absence of thecal plates and the occurrence of chloroplastes in the short-lived swarmer (=dinospore) stage; (*ii*) the development of four distinct amoeboid stages including a spheroidal, rolling amoeba unknown in any other species; and (*iii*) the fact that this dinoflagellate causes epithelial hyperplasia in the stickleback and does not result in massive fish kills. *Haidadinium ichthyophilum* is known only from two acidic lakes in the Queen Charlotte Islands, British Columbia, Canada. *Haidadinium* is tentatively assigned to the family Phytodiniaceae of the order Phytodiniales.

Key words: dinoflagellates, Dinophyceae, *Gasterosteus, Haidadinium ichthyophilum* gen.nov. et sp.nov., Queen Charlotte Islands, Phytodiniales, symbiosis, taxonomy, ultrastructure.

Résumé : La dinoflagellée *Haidadinium ichthyophilum* gen. et sp.nov. est associée à l'épinoche à trois épines, *Gasterosteus aculeatus*, en eau douce. Ce nouveau genre diffère de toutes les dinoflagellées précédemment décrites par la morphologie et l'ultrastructure des stades de son cycle vital complexe, ainsi que par l'écologie de ses interactions avec le *G. aculeatus*. Les caractères distinctifs incluent: (*i*) l'absence de plaques de la thèque et présence de chloroplastes au stade zoé (dinospore); (*ii*) le développement de quatre stades amoeboïdes distincts incluant une amibe sphaeroïdale roulante, inconnue chez toutes les autres espèces; et (*iii*) le fait que cette dinoflagellée causent une hyperplasie épithéliale chez l'épinoche sans conduire à une mortalité massive des poissons. L'*Haidadinium ichthyophilum* n'est connu que dans deux lacs acides des îles de la Reine Charlotte, en Colombie Britannique. Le genre *Haidadinium* est tentativement attribué à la famille des Phytodiniaceae de l'ordre des Phytodinales.

Mots clés : dinoflagellés, Dinophyceae, *Gasterosteus*, *Haidadinium ichthyophilum* gen.nov. et sp.nov., îles de la Reine Charlotte, Phytodinales, symbiose, taxonomie, ultrastructure.
[Traduit par la rédaction]

Introduction

In the early 1980s a population of the three-spine stickleback, *Gasterosteus aculeatus* L. was found from the Queen Charlotte Islands with a heavy infection of cysts (Reimchen 1984), which were later identified as one stage in the life cycle of a parasitic dinoflagellate (Reimchen and Buckland-Nicks 1990). Subsequently these dinoflagellates were extensively studied in terms of their life history, ultrastructure, and interaction with the host fish (Buckland-Nicks et al. 1990; Buckland-Nicks and Reimchen 1995). These studies demonstrated that the dinoflagellate has a series of unique characters that warrant its description as a new genus and species.

In this paper we summarize previous observations (Reimchen and Buckland-Nicks 1990; Buckland-Nicks et al. 1990; Buckland-Nicks and Reimchen 1995) and provide some additional details, as well as formally describe the new genus and species. The new species is compared with the recently described fish parasite, *Pfiesteria piscicida* Steidinger et Burkholder, an estuarine dinoflagellate that is the basis for a new family, Pfiesteriaceae (Burkholder et al. 1992; Burkholder and Glasgow 1995; Steidinger et al. 1995, 1996). We have tentatively assigned the new species to the family Phytodiniaceae of the order Phytodiniales.

Materials and methods

The dinoflagellate described has been collected at two sites in two adjacent watersheds in the Queen Charlotte Islands, British Columbia: Rouge Lake (53°55′46.2″N, 132°1′25.1″W) and Laurel Lake (54°1′57.0″N, 131°52′26.5″W). The sites are small acidic lakes (pH 3.9–4.5) in *Sphagnum* bogs with extremely low cation levels.

Received May 4, 1997.

J. Buckland-Nicks[1] **and D.J. Garbary.** Department of Biology, St. Francis Xavier University, P.O. Box 5000, Antigonish, NS B2G 2W5, Canada.
T.E. Reimchen. Department of Biology, University of Victoria, Victoria, BC V8W 2Y2, Canada.

[1] Author to whom all correspondence should be addressed. e-mail: jbucklan@juliet.stfx.ca

The limnology and biota for Rouge Lake have been described elsewhere (Reimchen 1984; Reimchen and Buckland-Nicks 1990) and appear similar to Laurel Lake (T.E. Reimchen, unpublished data). Methods for collection, cultivation, and light and electron microscopy are described elsewhere (Buckland-Nicks and Reimchen 1995; Buckland-Nicks et al. 1990). Cultures of the dinoflagellate are maintained in distilled water for 6–12 months, with new cultures established each year from vegetative cysts collected at Rouge Lake. Cultures are unialgal but not axenic, and natural populations of bacteria that develop in the cultures provide a food source for the heterotrophic stages.

Results

Phylum Dinophyta Möhn 1984
Class Dinophyceae Pasche 1914
Order Phytodiniales Christensen 1962 ex Loeblich 1970
Family Phytodiniaceae Krebs 1912

Haidadinium Buckland-Nicks, Reimchen et Garbary, gen.nov.

ETYMOLOGY: The new genus is named in honour of the Haida people, the native inhabitants of the Queen Charlotte Islands.

Dinoflagellatum cysta primaria vegetative supra epithelium hospitis crescenti. Nucleus dinokaryoticus in cysta vegetativa ac facies gregaria praesens, nucleus eukaryoticus in faciebus amoeboideis cystique quiescentibus. Facies variae amoeboideae praesentes: rhizopodiales, lobatae, sphaeroideae; omnes hae formae ex cystis vegetativis evolventes. Chloroplastus aliquando pyrenoide manifestus prominensque in cysta vegetativa et in faciebus trophontialibus ac gregariis, sed non in faciebus amoeboideis praesens.

TYPE: As *Haidadinium ichthyophilum* Buckland-Nicks, Reimchen et Garbary (Fig. 6).

Dinoflagellate with primary vegetative cyst developing on host epithelium (Figs. 2 and 6). Dinokaryotic nucleus present in vegetative cyst (Fig. 4) and swarmers, with a more conventional eukaryotic nucleus in amoeboid phases and resting cysts. Various amoeboid stages present including lobose (Fig. 3), rhizopodial (Fig. 5), and spheroidal forms, these developing from vegetative cysts. Chloroplast sometimes with a prominent, projecting pyrenoid, present in vegetative cyst, trophont, and swarmer stages but not in amoeboid stages.

Haidadinium ichthyophilum Buckland-Nicks, Reimchen et Garbary sp.nov.

ETYMOLOGY: The specific epithet is derived from *ichthys*, the Greek for fish, and from *philos*, the Greek for love. It also recognizes the historical symbiosis between the Haida people and the aquatic resources of the Queen Charlotte Islands.

Cystae immobiles, parietibus laevae, 20–150 μm in diametro, cum cellulis externis epithelialibus ac branchiis hospitis piscis consociatae. Cysta vegetativa matrice translucida fragili fenestrali instructa. Cystae in diversas facies amoeboideas vel trophontes varie evolventes, aut intra parietem cystae dividentes ut duas cellulas flagellatas vel plures aplanosporas producunt. Nucleus in cystis vegetativis et faciebus gregariis ac trophontialibus dinokaryoticus; nuclei facierum amoeboidearum eukaryotici. Chloroplastus solummodo in faciebus gregiis et cystis praesens. Reproductio asexualis formatione facierum variarum amoeboidearum: lobatotarum, rhizopodialium, sphaeroidearum. Reproductio sexualis ignota. Facies omnes vitae cycli bacteria endosymbiotica habentes.

TYPE LOCALITY: Rouge Lake, Queen Charlotte Islands, British Columbia.

HOLOTYPE: Figure 6 and formalin-preserved material from nature (paratypes) collected July 1994 and deposited in the National Herbarium of Canada, Ottawa (CANA).

ICONOGRAPHY: Reimchen and Buckland-Nicks (1990, Figs. 1–7); Buckland-Nicks et al. (1990, Figs. 1–18); Buckland-Nicks and Reimchen, (1995, Figs. 1–23).

Smooth-walled, vegetative cysts, 20–150 μm, associated with external epithelial cells and gills of fish host (Figs. 1 and 6). Cyst with translucent, brittle, fenestrated matrix. Cysts variously developing into different amoeboid (Figs. 3 and 5), or trophont (Fig. 2) stages or dividing within cyst wall to produce two flagellated cells or many aplanospores (Fig. 6). Nucleus in vegetative cyst and swarmer stages dinokaryotic (Fig. 4); amoeboid stages with more typical eukaryotic nucleus. Chloroplast present only in swarmer and cyst stages. Asexual reproduction by formation of various lobose, rhizopodial, and spheroidal amoeboid stages and by aplanospore and swarmer production (Fig. 6). Sexual reproduction unknown. All life-history stages with endosymbiotic bacteria.

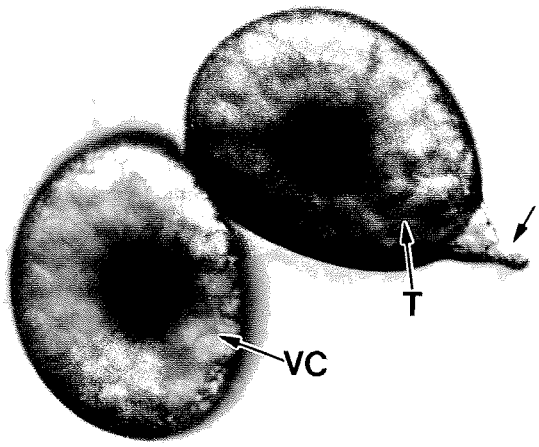
ECOLOGY: The cyst stage occurs in densities of up to 9.3 cells/mm^2 on *Gasterosteus aculeatus*, and the occurrence of the infection varies from nil in January to 99% in July (Reimchen and Buckland-Nicks 1990). *Haidadinium ichthyophilum* was initially observed in 1969, and has been recorded in all five collections of fish made during July from Rouge Lake in the 1980s and 1990s by T.E.R. Current knowledge of the life history of *H. ichthyophilum* is summarized in Fig. 6.

Discussion

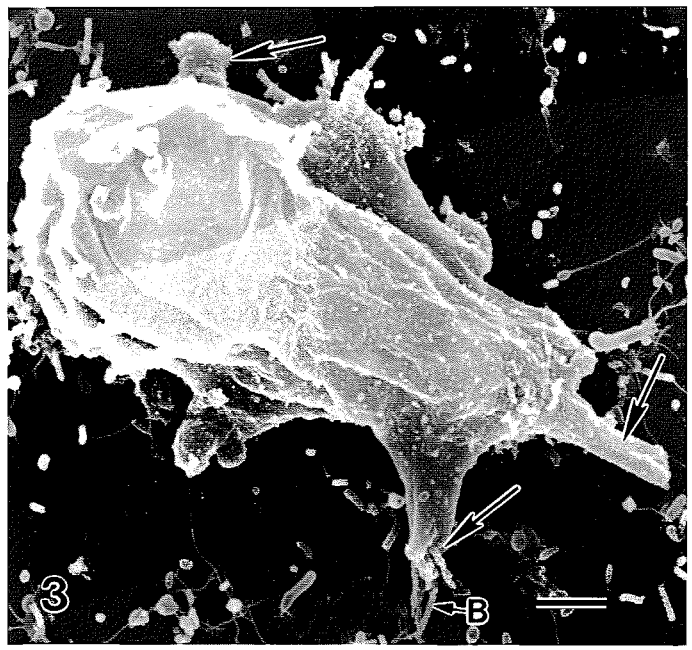
The placing of *Haidadinium* in the order Phytodiniales is somewhat problematic. This is because the order itself is poorly defined and considered to be largely a taxon of "convenience" (Fensome et al. 1993). In addition, the gymnodinioid swarmers have been seen only occasionally over a 5-year period of study, and only one of these cells was successfully prepared for electron microscopy. This cell was apparently nonthecate (Buckland-Nicks and Reimchen 1995); however, it is conceivable that a rapid loss of the theca might have occurred in the 2 min between observation of the flagellated cell and cell fixation. If a theca can be demonstrated in these swarmers, then *Haidadinium* may have to be reassigned according to the tabulation. This problem will likely only be resolved with sequence studies such as the rRNA analyses of Lenaers et al. (1991). Regardless of the eventual higher level classification of *Haidadinium*, it has sufficiently unique characters to warrant generic recognition.

As characterized by Fensome et al. (1993) the Phytodiniales may comprise organisms in which an amoeboid stage is present in a life history that also includes a coccoid stage. The order is generally characterized by having a permanent dinokaryon; however, as found previously by Popovsky and Pfiester (1982), amoeboid stages of Phyto-

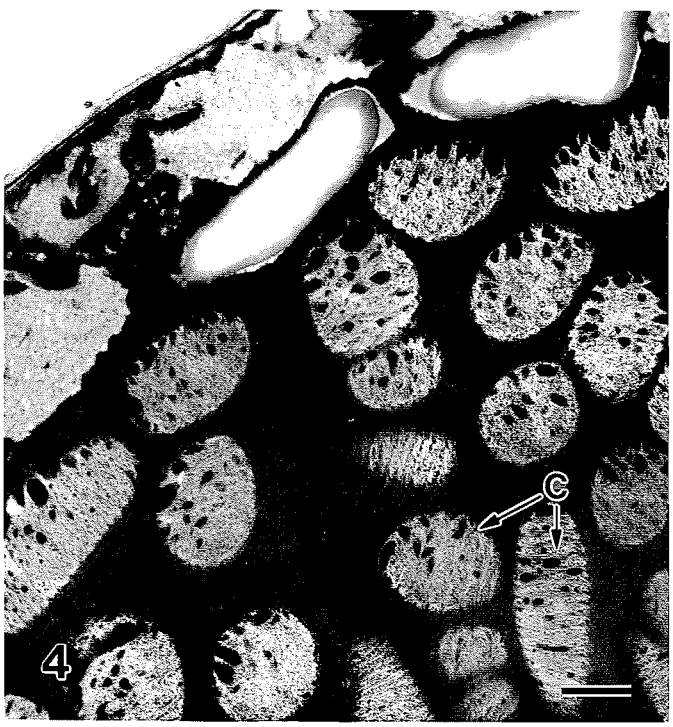
1



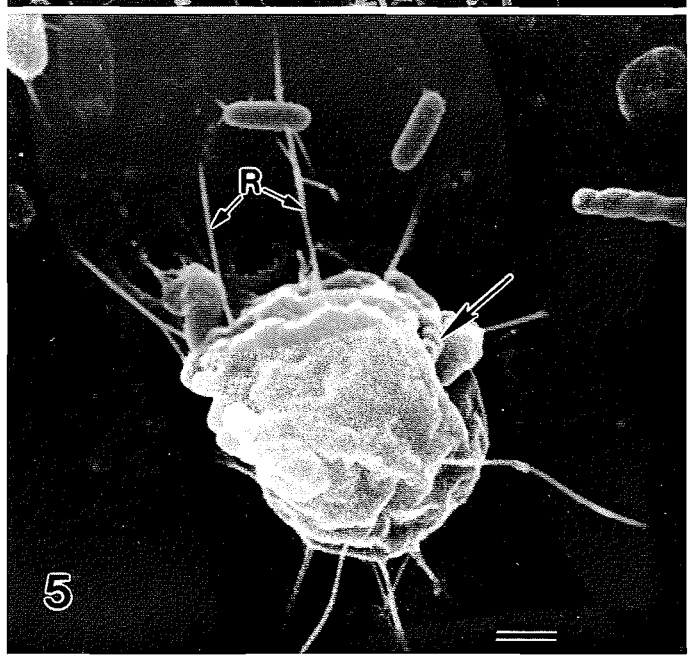
2



3



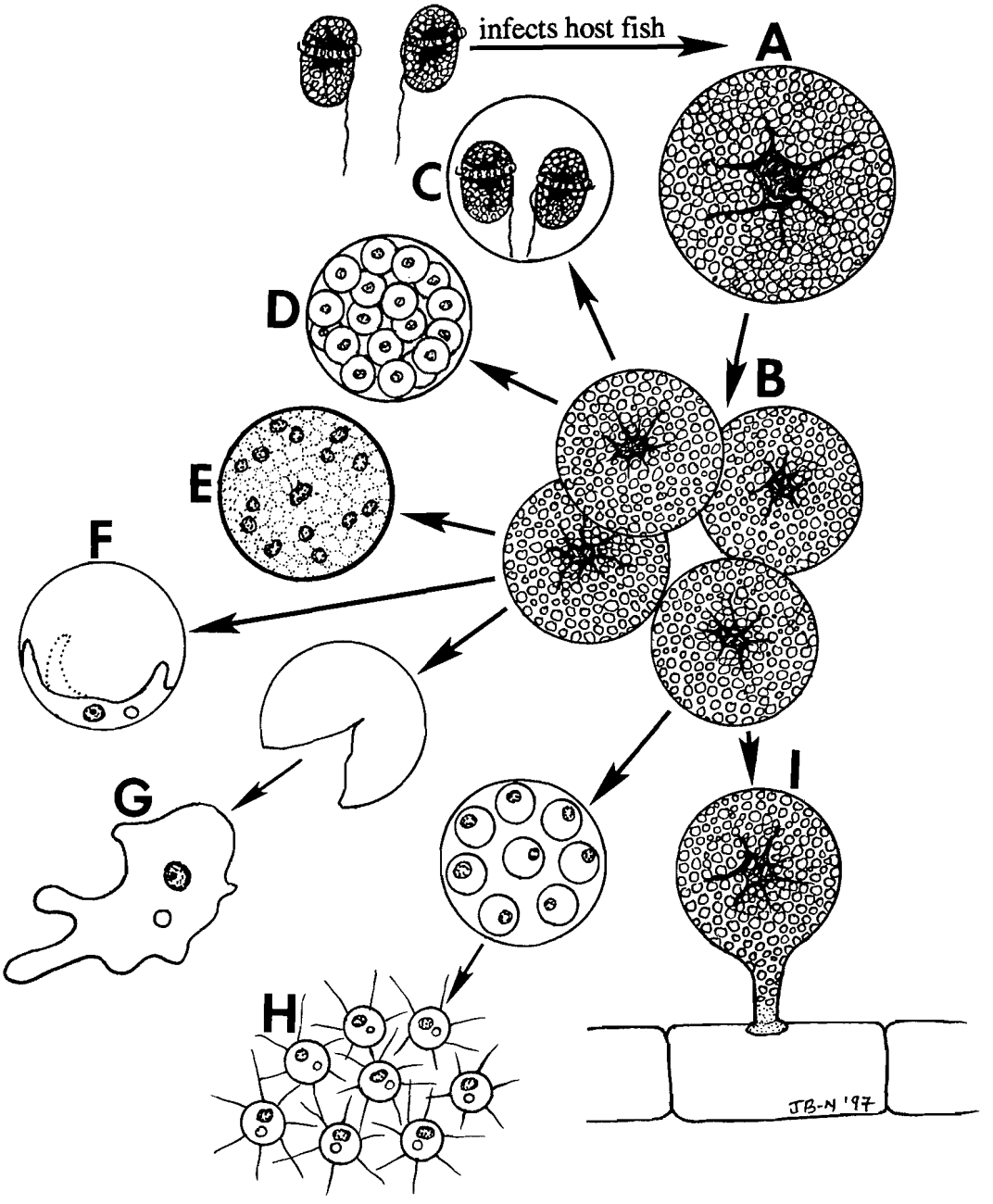
4



5

Fig. 1. Spine-reduced three spine stickleback, *Gasterosteus aculeatus* showing heavy infection with vegetative cysts of *Haidadinium ichthyophilum*. Arrows indicate concentrations of cysts on dorsal surface, operculum, and eye. Scale bar = 1 cm. **Fig. 2.** Vegetative cyst (VC) of *H. ichthyophilum* forms trophont (T) by extension of stalk-like organelle (arrow), which attaches to algal filament. Scale bar = 10 μm. **Fig. 3.** Lobose amoeba of *H. ichthyophilum*, with several extended lobopodia (arrows) phagocytoses bacteria (B). Scale bar = 10 μm. **Fig. 4.** Transmission electron micrograph of dinokaryotic nucleus of newly formed vegetative cyst, showing condensed chromosomes (C). Scale bar = 0.5 μm. **Fig. 5.** Rhizopodial amoeba with fine rhizopods (R). Note engulfed spiral bacterium (arrow). Scale bar = 1 μm. Reprinted with permission from Buckland-Nicks and Reimchen (1995, Fig. 6).

Fig. 6. Diagrammatic representation of established life history of *Haidadinium ichthyophilum*. (A) Primary vegetative cyst on fish host. (B) Vegetative cyst undergoes repeated asexual fission producing cysts as small as 20 μm. At each division the theca is shed (eleutheroschisis). Vegetative cysts give rise to a variety of other life-history stages, including: (C) At 55 μm or less, paired daughter cysts may form swarmers, which emerge by shedding the theca. Swarmers can infect a host fish. (D) Palintomic reproduction within a vegetative cyst produces aplanospores. (E) Multinuclear resting cyst. (F) Spheroidal amoeba. (G) Lobose amoeba. (H) Rhizopodial amoebae. (I) Trophont stage develops by extension of stalk-like structure that attaches to filamentous alga. The mechanism of re-entry into the life cycle is not known for stages D–I. Diagram modified from Buckland-Nicks and Reimchen (1995, Fig. 23).



diniales may not have a dinokaryon. Phytodiniales are also typically associated with algae or macrophytes (Bourrelly 1985; Fensome et al. 1993). Although the primary vegetative cysts of *Haidadinium* are associated with fish, a trophont stage also has been observed associated with a filamentous alga (Buckland-Nicks and Reimchen 1995). This might represent a vestige of an original algal association.

The recently described *Pfiesteria piscicida* was placed in the new family Pfiesteriaceae in a redefined Dinamoebales (Steidinger et al. 1996). *Pfiesteria* and *Haidadinium* are fish parasites, and the presence of both swarmer and elaborate and various amoeboid stages might be suggestive of a relationship between these genera. *Pfiesteria*, however, is basically a heterotroph in which flagellated stages can have cleptochloroplasts in food vacuoles. In addition, the occurrence of triflagellated swarmers, a scaled cyst stage, a cyst with a reticulate surface, and flagellated amoeboid cells in *Pfiesteria* (Burkholder and Glasgow 1995; Steidinger et al. 1996) have not been observed in *Haidadinium*. The rolling, spheroidal, amoeboid stage (Buckland-Nicks and Reimchen 1995) and the fenestrated matrix of the cysts of *Haidadinium* are unknown in *Pfiesteria* or in other parasitic, amoeboid dinoflagellates (Pfeister and Popovsky 1979).

Haidadinium is the only genus of Phytodiniales that parasitizes fish (Cachon and Cachon 1987; Fensome et al. 1993). Like most Phytodiniaceae, *Haidadinium* is found in freshwater where several other genera (e.g., *Hemidinium, Stylodinium*) are also acidophilic (Bourrelly 1985). *Spiniferodinium* is apparently marine (Horiguchi and Chihara 1987), but Chrétiennot-Dinet et al. (1993) suggest that this genus might be a marine species of the otherwise freshwater genus *Cystodinedria*. Previously, members of the Phytodiniaceae were characterized as being primarily photosynthetic; in *Haidadinium*, chloroplasts appear to be confined to the cyst and swarmer stages with the amoeboid stages being devoid of chloroplasts. Proplastids appear to be retained in at least some resting cyst and amoeboid stages (Buckland-Nicks and Reimchen 1995), and these structures may form the basis for regenerating the chloroplasts in the vegetative cysts.

Biogeographic associations of *Haidadinium ichthyophilum* remain unresolved. The dinoflagellate is currently known only from two adjacent watersheds in the *Sphagnum*-dominated lowlands on the northeastern region of the Queen Charlotte Islands. Analyses of the stickleback host, *G. aculeatus*, show that populations with the dinoflagellate have extensive reduction in armor relative to the putative marine ancestor (Reimchen 1984), and the fish have probably had a prolonged history in the calcium-poor and acidic freshwater habitats. Rouge Lake sticklebacks are monomorphic for a divergent and uncommon mitochondrial lineage (O'Reilly et al. 1993), and this lineage has close affinities with sticklebacks from Japan (Orti et al. 1994).

Acknowledgements

We thank K. Steidinger for helpful discussion, Marilyn O'Brien for technical assistance, Dr. R.B. MacDonald for translations into Latin, and Dr. Steve Baldner for timely corrections. All three authors thank the Natural Sciences and Engineering Research Council of Canada for supporting their research programs. This work was also supported in part by the James Chair Professorship to T.E.R.

References

Bourrelly, P. 1985. Les algues d'eau douce. Initiation à la systématique. Tome III. Les algues bleues et rouges. Les Eugléniens, Peridiniens et Cryptomonadines. Boubée, Paris, France.

Buckland-Nicks, J., and Reimchen, T.E. 1995. A novel association between an endemic stickleback and a parasitic dinoflagellate. 3. Details of the life cycle. Arch. Protistenkd. **145**: 165–175.

Buckland-Nicks, J., Reimchen, T.E., and Taylor, M.F.J.R. 1990. A novel association between an endemic stickleback and a parasitic dinoflagellate. 2. Morphology and life cycle. J. Phycol. **26**: 539–548.

Burkholder, J.M., and Glasgow, H.B., Jr. 1995. Interactions of a toxic estuarine dinoflagellate with microbial predators and prey. Arch. Protistenkd. **145**: 177–188.

Burkholder, J.M., Noga, E.J., Hobbs, C.H., Glasgow, H.B., Jr. 1992. New "phantom" dinoflagellate is the causative agent of major estuarine fish kills. Nature (London), **358**: 407–410.

Cachon, J., and Cachon, M. 1987. Parasitic dinoflagellates. *In* The biology of dinoflagellates. *Edited by* M.F.J.R. Taylor. Blackwell, Oxford, U.K. pp. 571–610.

Chrétiennot-Dinet, M.-J., Sournia, A., Ricard, M., and Billard, C. 1993. A classification of the marine phytoplankton of the world from class to genus. Phycologia, **32**: 159–179.

Fensome, R.A., Taylor, F.J.R., Norris, G., Sarjeant, W.A.S., Wharton, D.I., and Williams, G.L. 1993. A classification of living and fossil dinoflagellates. Micropaleontology Press, New York.

Horiguchi, T., and Chihara, M. 1987. *Spiniferodinium galeiforme*, a new genus and species of benthic dinoflagellates (Phytodiniales, Pyrrhophyta) from Japan. Phycologia, **26**: 478–487.

Lenaers, G., Scholin, C., Bhaud, Y., Saint-Hilaire, D., and Herzog, M. 1991. A molecular phylogeny of dinoflagellate protists (Pyrrhophyta) inferred from the sequence of 24S rRNA divergent domains D1 and D8. J. Mol. Evol. **32**: 53–63.

O'Reilly, P., Reimchen, T.E., Beech, R., and Strobeck, C. 1993. Mitochondrial DNA in *Gasterosteus* and Pleistocene glacial refugium on the Queen Charlotte Islands, British Columbia. Evolution, **47**: 678–684.

Orti, G., Bell, M.A., Reimchen, T.E., Meyer, A. 1994. Global survey of mitochondrial DNA sequences in the three-spined stickleback: evidence for recent migrations. Evolution, **48**: 608–622.

Pfiester, L., and Popovsky, J. 1979. Parasitic, amoeboid dinoflagellates. Nature (London), **279**: 421–424.

Popovsky, J., and Pfiester, L.A. 1982. The life-histories of *Stylodinium sphaera* Pascher and *Cystodinedria inermis* (Geitler) Pascher (Dinophyceae), two freshwater facultative predator-autotrophs. Arch. Protistenkd. **125**: 115–127.

Reimchen, T.E. 1984. Status of unarmoured and spine-deficient populations (Charlotte unarmoured stickleback) of three-spine stickleback, *Gasterosteus* sp., on the Queen Charlotte Islands, British Columbia. Can. Field-Nat. **98**: 120–126.

Reimchen, T.E., and Buckland-Nicks, J. 1990. A novel association between an endemic stickleback and a parasitic dinoflagellate. 1. Seasonal cycle and host response. Can. J. Zool. **68**: 667–671.

Steidinger, K.A., Truby, E.W., Garrett, J.K., and Burkholder, J.M. 1995. The morphology and cytology of a newly discovered toxic dinoflagellate. *In* Harmful marine algal blooms. *Edited by* P. Lassus, G. Arzul, E. Erard-Le Denn, P. Gentien, and C. Marcaillou-Le Baut. Lavoisier Publishing, Paris, France. pp. 83–88.

Steidinger, K.A., Burkholder, J.M., Glasgow, H.B. Jr., Hobbs, C.W., Garrett, J.K., Truby, E.W., Noga, E.J., and Smith, S.A. 1996. *Pfiesteria piscicida* gen. et sp. nov. (Pfiesteriaceae fam. nov.), a new toxic dinoflagellate with a complex life cycle and behavior. J. Phycol. **32**: 157–164.