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Life Cycle of Heterotrophic Dinoflagellate *Cryptoperidiniopsis brodyi* (Dinophyceae)

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Abstract

Pfiesteria and *Pfiesteria*-like organisms were reported to be linked to major fish kills (involving well over a billion fish) in North Carolina and Maryland estuaries on the U.S. east coast during the 1990s. Occurrences of these species have been recently reported from Korean waters including Chinhae Bay and the coast of Yeosu. In this study, the life cycle of *Cryptoperidiniopsis brodyi* and *Pfiesteria piscicida* were examined using DAPI staining. Their excystment and growth were stimulated directly by the addition of prey cells such as *Rhodiminas salina*. Amoeboid stages in *C. brodyi* and *P. piscicida* were never observed in culture, even after addition of filter-sterile fish mucus and tissue. The dominant life cycle stages consisted of motile flagellated zoospores and cysts. A typical dinoflagellate life cycle was demonstrated by direct observation and DAPI staining.

Key Words : *Cryptoperidiniopsis brodyi*, DAPI staining, Harmful algal blooms, Life cycle, *Pfiesteria piscicida*, Red tide

1. Introduction

Dinoflagellates can be classified by characteristics such as life history stages as well as morphological and genetic features[1~3]. *Pfiesteria piscicida* Steidinger et Burkholder and *Cryptoperidiniopsis brodyi* Steidinger et Litaker were initially proposed to have a complex life cycle containing more than 20 stages, including amoeboid forms[4~6]. It has been claimed that the amoeboid stages could only be found in the presence of fish or fish excreta[4,6]. However, other studies suggested that the complex life stages may be induced by environmental contaminants and that *Pfiesteria* species instead have a typical homothallic dinoflagellate life cycle. The simple life cycle of *Pfiesteria* species includes an asexual phase and a sexual phase. The asexual phase has vegetative cells and three cyst types including division cysts, resting cysts, and temporary cysts. The sexual phase involves gametes, a planozygote resulting from fusion of two daughter cells, and a hypnozygote that eventually germinates. *Pfiesteria* species were originally placed in the order Dinamoebales because it was thought that the dominant stage was amoeboid[7,8]. However, more recently these dinoflagellates have been transferred to the order Peridiniales, considering morphological, phylogenetic and haplontic life history data[1,9]. In the present study, the life cycle of *C. brodyi* was observed by using DAPI stain and compared to closely related dinoflagellate *P. piscicida*. Sterile-filtered fish mucus/tissue was added in *C. brodyi* and *P. piscicida* cultures for testing the absence or presence of amoeboid stages.

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2. Materials and Methods

2.1. Cultures

Three strains of *C. brodyi* (CBWA12, CBSA4, and CBDE10 from Australia) and two strains of *P. piscicida* (CCMP1975 and CCMP1974 from USA) were prepared for life cycle observations. They were provided from University of Tasmania culture collection and CCMP (Provasoli-Guillard National Center for Culture of Marine Phytoplankton). Cultures were maintained in 15 psu f/2 medium at 20℃ in the dark by adding *Rhodomonas salina* prey every 2-3 days.

2.2. DNA staining and light microscopy (LM)

Nuclear staining was conducted as described by Litaker et al.[10]. Zoospores and cysts were fixed in a final concentration of 1% glutaraldehyde. The fixed cells were settled on slides coated with poly-L- lysine, and dried. The slides containing cells were alternately dipped in 95% ethanol and double distilled water. This procedure allowed greater dye penetration into hypnocysts. DAPI (300-500 ng ml^{-1}) was added to the cells and allowed to stain for 5 min in the dark. The cells were examined with an Axioskop 2 Plus microscope with epifluorescence (λex=350 nm, λex =480 nm) (Zeiss, Gottingen, Germany) connected to AxioCam HR digital camera (Zeiss, Gottingen, Germany).

2.3. Additions of sterile-filtered fish mucus/tissue to dinoflagellate cultures

To confirm the absence of amoeboid stages in *C. brodyi* and *P. piscicida* cultures, they were observed under light microscopy with and without additions of sterile-filtered fish mucus/tissue. To obtain contaminant-free fish water, fresh damselfish tissue (*Polycanthus* sp.) was placed in 100 ml of 15psu f/2 medium and homogenized using a wooden stick. The medium was sterile-filtered on glass fiber filters (0.45 μm) and 0.2 μm membrane syringe filter (Pall corporation, Ann Arbor, MI, USA), and then stored at 4℃ up to 1 week. The filtered medium (2 μl) was added daily to *P. piscicida* and *C. brodyi* for 3 weeks. Following the 3 weeks, seawater (35 psu) in the fish tank with damselfish was sterile-filtered on the membrane filters, and added daily into the same cultures for 1 week. The experiments were conducted in triplicated containers. For controls, cultures duplicated from the dinoflagellate were prepared with and without the addition of a non-sterile fish mucus/tissue. For the non-sterile fish medium control, fresh fish mucus/tissue was directly added into dinoflagellate cultures. Small amounts of *R. salina* were added to all cultures at 1-2 day intervals. The cultures containing the fish medium were maintained in a vacuum chamber at room temperature for the experimental period because *Pfiesteria* species were claimed to become toxigenic upon the addition of fish mucus and tissue[6]. Changes in the cultures were monitored using light microscopy connected to digital camera and examined by nuclear staining using DAPI.

3. Results

3.1. Additions of fish tissue/excreta to *C. brodyi* and *P. piscicida*

Cryptoperidiniopsis brodyi and *P. piscicida* cultures were monitored with the additions of fish tissue/excreta for 4 weeks. After additions of fish tissue/excreta, an attractant response to the materials was observed in both *C. brodyi* and *P. pisicicda* cultures. They actively swarmed for the first 2-3 hours, and gradually slowed in response to the fish materials. But no amoebae stages or unusual transformations of life cycle stages were observed in these cultures. *Cryptoperidiniopsis brodyi* and *P. piscicida* in the control with non-sterile fish medium also showed an attractant response to the fish medium for the first 1-2 hours. Few zoospores remained in the cultures after day 1, and no zoospores were observed after day 2 because of contamination by bacteria. Since the isolations of *C. brodyi* and *P. piscicida* strains from environmental samples, all of the clonal cultures were monitored regularly by light microscopy for 2-3 years, but unusual life stages were never found.

3.2. Observation of life cycle stages of *C. brodyi* stained with a nuclear-specific dye

Haplontic dinoflagellate life stages including zoo-

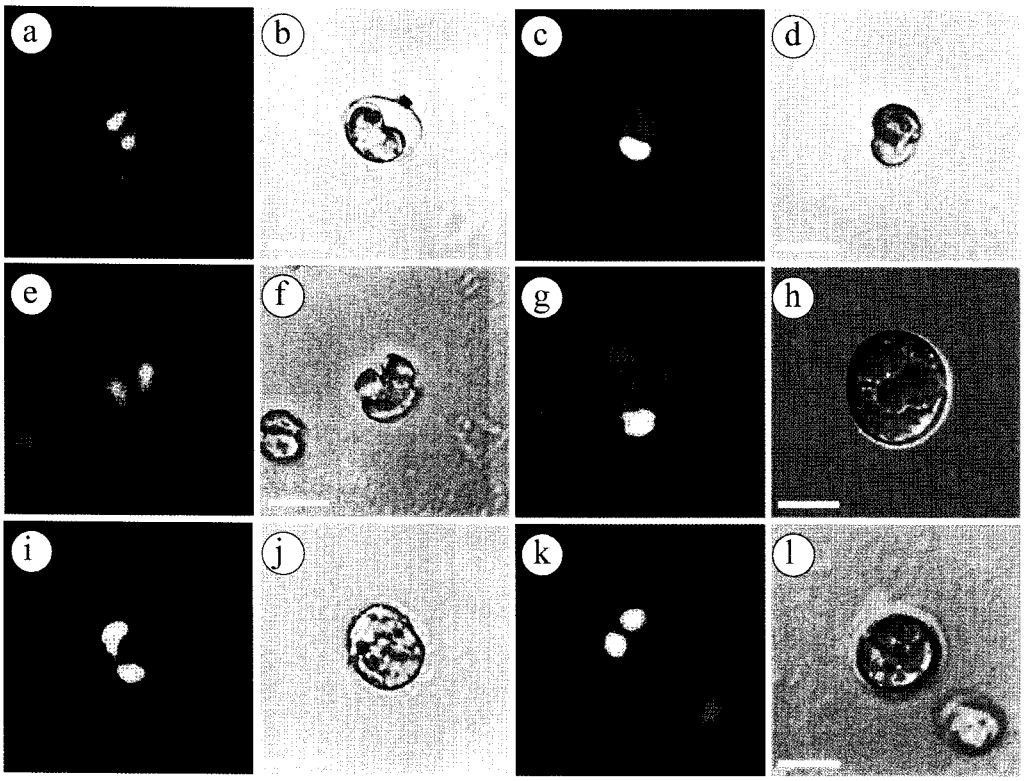

Fig. 1. Light micrographs of *C. brodyi* and cells stained with DAPI. Light micrographs of *C. brodyi* (b,d,f,h,j,i) and DAPI-stained nucleus (a,c,e,g,i,k). (a,b) Cyst from a food limited culture. (c,d) Enlarged epitheca of zoospore with prey. (e,f) Gamete pair in mid-fusion. (g,h) Zygotic cyst with a single nucleus. (i,j) Division cyst with nuclei migrating to opposite poles of the cell. (k,l) Division cyst with two distinct nuclei. Scale bars=10 mm.

spores, gametes, planozygote, and division cyst (Fig. 1) were observed in the *C. brodyi* cultures as described in previous studies[11,12]. Two general phases observed under light microscopy were motile zoospores and nonmotile cysts. The zoospore stage dominated when prey was sufficient, while few flagellated cells and many colorless cysts were observed in prey-depleted cultures (Fig. 1a, b; Fig. 2a). The colorless cysts were generally smaller than pigmented cysts in cultures with abundant prey. The small and colorless cysts could survive without addition of prey for approximately 10 months. However, after addition of prey, these cysts quickly germinated and became flagellated cells in 2 to 24 h (Fig. 2f). Size and shape of flagellated cells varied considerably and depended on the amount of ingested prey (Fig. 1c, d; Fig. 2b). Zoospores fed by attaching to a prey cell with a peduncle (Fig. 2d).

Fusion of flagellated gametes occurred and paired gametes rotated rapidly (Fig. 1e, f). The paired gametes formed a planozygote of typical dinoflagellate shape and with two longitudinal flagella (Fig. 2e). Encystment occurred by loss of the flagella and rounding off of surface features (Fig. 1g, h; Fig. 2c). Cysts also varied in size and shape as did the flagellated cells. When cell division occurred, daughter nuclei began moving toward opposite ends of the cyst (Fig. 1i, j) and formed a 2N nucleus (Fig. 1k, l). Excystment of hypnozygote occurred and released biflagellate cells. Figure 3 shows a schematic diagram of the life history of *C. brodyi*.

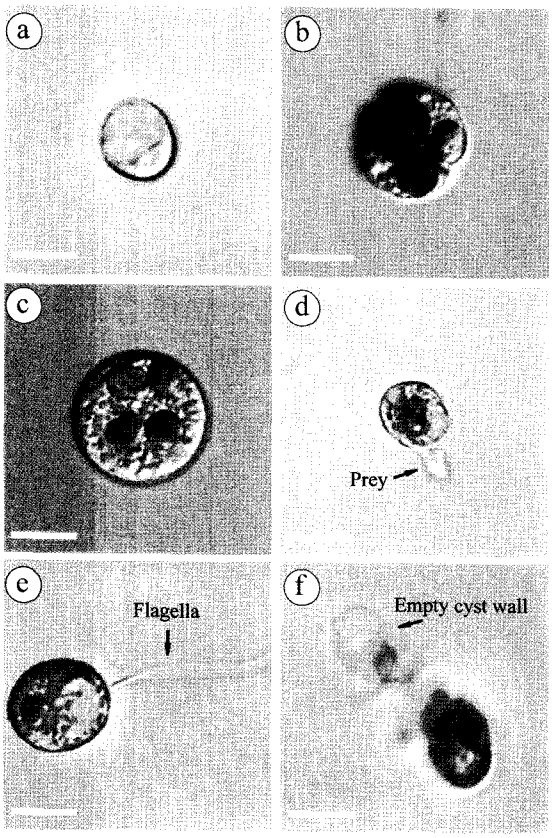

Fig. 2. Light micrographs of *C. brodyi*. (a) Colorless cyst from a prey starved culture. (b) Zoospore with pigmented food vacuole. (c) Cyst with pigmented food vacuole. (d) Zoospore attached to and feeding prey. (e) Planozygote with two longitudinal flagella. (f) Germination oftemporary cyst. Scale bars=10 mm.

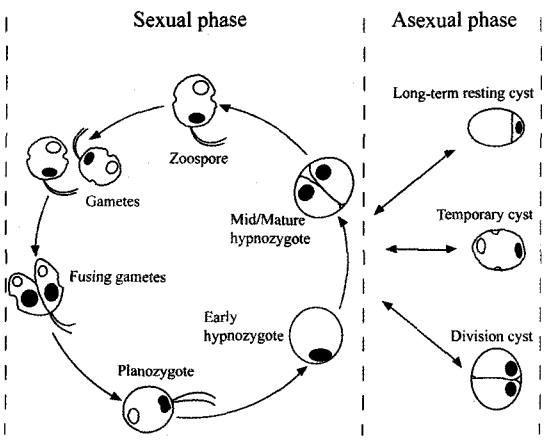

Fig. 3. Schematic diagram showing the life history of *C. brodyi*.

4. Discussion

Most dinoflagellates have a life cycle of two phases which represent the asexual and the sexual phases[13]. The dominant phase can be either zoospores or cysts. *Pfiesteria* species have been claimed to have a complex life cycle (at least 24 life cycle stages including amoebae), not previously known in dinoflagellates[6,14,15]. The complex life cycle including amoeboid stages was also suggested to exist in *Pfiesteria*-like dinoflagellates including *Cryptoperidiniopsis* species[5]. It was reported that the amoeboid stages could be induced only in the presence of live fish or fresh fish excreta[15,16]. However, the present study could not demonstrate any amoeboid stages in *Cryptoperidiniopsis* and *Pfiesteria* species when cultured in filter-sterilized fish excreta. Attractant responses to fish tissue/excreta were shown in *Cryptoperidiniopsis* and *Pfiesteria* cultures, but stimulation of those cultures did not induce any unusual transformations of life cycle stages. It was suggested that the chemosensory attraction to fish excreta is not simple because of a nutritional response to organic molecules like amino acid or bovine serum albumin (BSA) solution but a more specific stimulation by a substance in fish excreta[17]. Absence of amoeboid forms in *Pfiesteria* was also suggested by Litaker et al.[10] who examined the life cycle of *Pfiesteria* with fluorescent in situ hybridization using peptide nucleic acid probes (PNA). Amoebae were found in cultures of *Pfiesteria* grown in the presence of live fish, but the amoebae were true amoebae closely related to *Korotnevella*[18]. Fish cultures contain a number of micro-organisms, including bacteria, fungi, rotifers, and amoebae[19,20,21]. The contaminants carried with fish make it difficult to track the life cycle of dinoflagellates and can lead to misidentification of life cycle stages[10,22]. Considering the results from the previous and the present studies, no amoeboid forms exist

in *C. brodyi* and *P. piscicida* even when cultures were grown in the presence of fish tissue/excreta, and biflagellated cells and cysts are dominant stages when cultured with prey cells.

5. Conclusions

These findings suggest that *C. brodyi* and *P. piscicida* have a typical marine dinoflagellate life cycle and no amoeboid stages were observed.

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