

From *Transactions of the American Microscopical Society*

## Evidence for a Sexual Cycle in the Florida Red Tide Dinoflagellate *Ptychodiscus brevis* (= *Gymnodinium breve*)

Linda M. Walker

A condensation of "Evidence for a Sexual Cycle in the Florida Red Tide Dinoflagellate, *Ptychodiscus brevis* (= *Gymnodinium breve*)," published recently in *Transactions of the American Microscopical Society* (Vol. 101, No. 3). Walker is with the Florida Department of Natural Resources, Marine Research Laboratory, 100 Eighth Ave. SE, St. Petersburg, FL 33701.

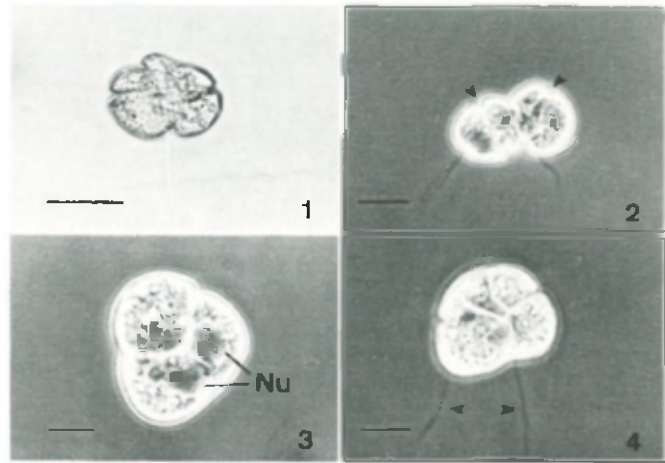
Steidinger (1975a,b) proposed that blooms of the Florida toxic dinoflagellate, *Ptychodiscus brevis* (Davis) Steidinger, began 18–74 km offshore with a "seed" population and that the entire bloom cycle of initiation, support, and maintenance was mediated by hydrologic and meteorologic factors. Finucane<sup>1</sup>, Wilson (1967), and Steidinger and Ingle (1972) had postulated a benthic resting cyst stage for *P. brevis*, and Steidinger suggested that cysts accumulated, forming cyst beds. She further suggested that numerous cysts in these beds, when triggered by proper conditions, could excyst en masse to provide the "seed" population for a bloom.

This report describes gametes, planozygotes, and possible hypnozygotes (cysts) for *P. brevis*. Seven strains of *P. brevis*, isolated from waters off the west coast of Florida, were used in this study: a diploid (Loper et al. 1980) 1953 strain, haploid 1971, 1974, 1976, and 1977 strains, and haploid clonal 1978 P<sub>5</sub> and P<sub>6</sub> strains.

Sexual stages (gametes, planozygotes) were observed in stock cultures of nonclonal strains of *P. brevis* (1971, 1974, 1976, and 1977) in low numbers. Sexual stages could be induced in greater numbers within 7–10 days by subjecting the cultures to nitrogen deficiency or by crossing the six haploid strains. Sexual stages were also observed in 1971 and 1974 isolates subjected to cold temperatures and blue light and in crosses subjected to cold temperatures and blue and green light. Gamete pairing occurred for 2–3 weeks after induction and involved only 5–10% of the total number of cells at a time. Sexuality never was observed in the diploid 1953 isolate or in any of the crosses involving this isolate. Likewise, sexual stages were never observed in the 1978 clonal strains, although they were observed regularly in crosses of these strains. Therefore, *P. brevis* is heterothallic.

Asexual cells (Figure 1) of *P. brevis* are 30–40  $\mu\text{m}$  wide and 10–15  $\mu\text{m}$  deep. The nucleus measures 6–9  $\mu\text{m}$  (Steidinger et al. 1978). The isogamous gametes (Figure 2) are rounder and slightly smaller (about 18–24  $\times$  24  $\mu\text{m}$ ) than asexual cells.

The cingula of fusing pairs are at oblique angles to each other (Figure 2). Cytoplasmic fusion precedes nuclear fusion (Figure 3). The resulting planozygote (Figure 4) is rounded,



**Figures 1–4.** *Ptychodiscus brevis*. 1. Motile, asexual cell. 2. Fusing gametes, note oblique angle of the cingula (arrows). 3. Planozygote. Nuclei (Nu) not yet fused. 4. Planozygote with two longitudinal flagella (arrows). Cingulum of each gamete still distinguishable. Scale bars = 20  $\mu\text{m}$ .

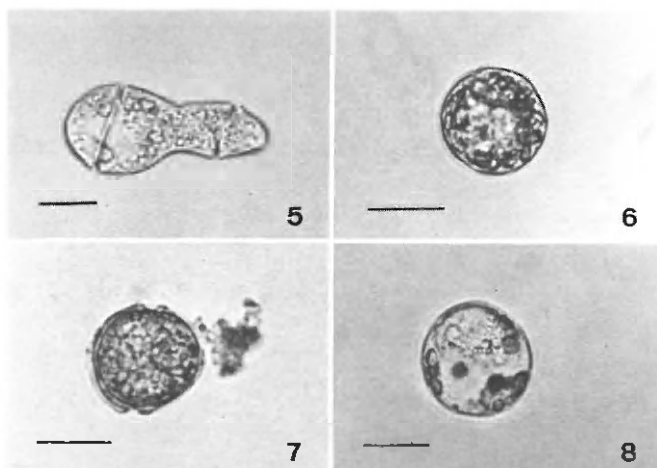
with two longitudinal flagella and a single large nucleus (15  $\mu\text{m}$ ) and ranges in size from 33  $\times$  37  $\mu\text{m}$  to 43  $\times$  43  $\mu\text{m}$ . The planozygote is motile for several days, but eventually settles to rotate on the bottom of the culture dish for 1–3 weeks before dying.

Unusual hypotheca-hypotheca forms (Figure 5) were observed in 1974 and 1976 isolates subjected to lowered salinity and several crosses subjected to cold temperatures and blue light. Whether these forms are the result of aberrant asexual divisions or are sexual stages is unknown.

Possible cysts (Figure 6) were observed during preliminary experiments with multiple crosses and cold temperatures. A possible cyst (Figure 7) has also been observed from a field sample from a monospecific bloom collected during continuous 24-h sampling on a red-tide cruise in January 1980. The field cyst was observed about 10 h after sunset. In both cases, the cysts were about 30  $\mu\text{m}$  in diameter with a thickened wall, dense gold-brown pigmented cytoplasm, and a single large dinokaryotic nucleus (15  $\mu\text{m}$ ). These cells stand in marked contrast to the thin-walled rounded forms (Figure 8) commonly formed in stressed or senescing cultures. The cytoplasmic contents in these forms are degraded with little or no pigment.

Zygotic cysts of other dinoflagellates have been produced in laboratory cultures following induction of sexuality by cold temperatures, nitrogen deficiency, and/or short day lengths (Pfiester 1975, 1976, 1977, Pfiester and Skvarla 1979, von Stosch 1973, Walker and Steidinger 1979). Although manipulation of these environmental parameters produces gametes and planozygotes in *P. brevis*, cyst induction may require different conditions. Experiments with *P. brevis* point to the importance of lowered temperatures. Endogenous rhythms may also play a role (Yentsch et al. 1980). Physical factors may not be effective unless correlated with the appropriate stage of an endogenous rhythm. The occurrence of gametes and planozygotes is seasonal in cultures and field populations of *P. brevis*. In both populations, sexual stages occur only in fall to late winter. Induction in laboratory cultures at other times of the year are rarely successful. Such periodicity may also control encystment.

<sup>1</sup>unpublished data



**Figures 5–8.** *Ptychodiscus brevis*. **5.** Hypothea-hypothea form. **6.** Possible induced cyst from laboratory culture. **7.** Possible cyst from field population. **8.** Rounded form common in stressed or senescing cultures. Scale bars = 20  $\mu$ m.

## REFERENCES CITED

Loper, C. L., K. A. Steidinger, and L. M. Walker. 1980. A simple chromosome spread technique for unarmored dinoflagellates and implications of polyploidy in algal cultures. *Trans. Am. Microsc. Soc.* 99: 343–346.

Pfiester, L. A. 1975. Sexual reproduction of *Peridinium cinctum* f. *ovaplanum* (Dinophyceae). *J. Phycol.* 11: 259–265.

———. 1976. Sexual reproduction of *Peridinium willet* (Dinophyceae). *J. Phycol.* 12: 234–238.

———. 1977. Sexual reproduction of *Peridinium gatunense* (Dinophyceae). *J. Phycol.* 13: 92–95.

Pfiester, L. A. and J. J. Skvarla. 1979. Heterothallism and thecal development in the sexual life history of *Peridinium volzii* (Dinophyceae). *Phycologia* 18: 13–18.

Steidinger, K. A. 1975a. Basic factors influencing red tides. Pages 153–162 in V. R. LoCicero, ed. *Toxic Dinoflagellate Blooms*, Proceedings of the First International Conference, Massachusetts Scientific and Technology Foundation, Wakefield, MA.

———. 1975b. Implications of dinoflagellate life cycles on initiation of *Gymnodinium breve* red tides. *Environ. Lett.* 9: 129–139.

Steidinger, K. A. and R. M. Ingle. 1972. Observations on the 1971 summer red tide in Tampa Bay. *Environ. Lett.* 3: 271–278.

Steidinger, K. A., E. W. Truby, and C. J. Dawes. 1978. Ultrastructure of the red tide dinoflagellate *Gymnodinium breve*. I. General description. *J. Phycol.* 14: 72–79.

von Stosch, H. A. 1973. Observations on vegetative reproduction and sexual life cycles of two freshwater dinoflagellates, *Gymnodinium pseudopalustre* Schiller and *Woloszynkia apiculata* sp. nov. *Br. Phycol. J.* 8: 105–134.

Walker, L. M. and K. A. Steidinger. 1979. Sexual reproduction in the toxic dinoflagellate *Gonyaulax monilata*. *J. Phycol.* 15: 312–315.

Wilson, W. B. 1967. Forms of the dinoflagellate *Gymnodinium breve* cultures. *Contrib. Mar. Sci.* 12: 120–134.

Yentsch, C. M., C. M. Lewis, and C. S. Yentsch. 1980. Biological resting in the dinoflagellate *Gonyaulax excavata*. *BioScience* 30: 251–254.