Classification and identification of Pfiesteria and Pfiesteria-like species

K Steidinger
et al

Patrice Mason
Virginia Institute of Marine Science

Kimberly S. Reece
Virginia Institute of Marine Science

LW Haas
Virginia Institute of Marine Science

Follow this and additional works at: https://scholarworks.wm.edu/vimsarticles

Part of the Marine Biology Commons

Recommended Citation
Steidinger K, Landsberg J, Vasta G, et al. Classification and Identification of Pfiesteria and Pfiesteria-Like Species. Environmental Health Perspectives Supplements [serial online]. October 2001;109:661. Available from: MasterFILE Premier, Ipswich, MA. Accessed December 12, 2017.

This Article is brought to you for free and open access by W&M ScholarWorks. It has been accepted for inclusion in VIMS Articles by an authorized administrator of W&M ScholarWorks. For more information, please contact scholarworks@wm.edu.
Classification and Identification of *Pfiesteria* and *Pfiesteria*-Like Species

Karen Steidinger,1 Jan Landsberg,1 R. William Richardson,1 Earnest Truby,1 Barbara Blakesley,1 Paula Scott,1 Patricia Tester,2 Torstein Tengs,3 Patrice Mason,4 Steve Morton,5 David Seaborn,6 Wayne Litaker,7 Kimberly Reece,4 David Oldach,3 Leonard Haas,8 and Gerardo Vasta8

1Florida Fish and Wildlife Conservation Commission, Florida Marine Research Institute, St. Petersburg, Florida, USA; 2National Oceanic & Atmospheric Administration/National Ocean Service, Beaufort Laboratory, Beaufort, North Carolina, USA; 3University of Maryland School of Medicine, Baltimore, Maryland, USA; 4Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, Virginia, USA; 5National Oceanic & Atmospheric Administration/National Ocean Service, Charleston, South Carolina, USA; 6Department of Biological Sciences, Old Dominion University, Norfolk, Virginia, USA; 7Program in Molecular Biology and Biotechnology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; 8Center for Biotechnology, University of Maryland, Baltimore, Maryland, USA

Dinoflagellates can be classified both botanically and zoologically; however, they are typically put in the botanical division Pyrrophyta. As a group they appear most related to the protistan ciliates and apicomplexans at the ultrastructure level. Within the Pyrrophyta are both unarmored and armored forms of the dominant, motile flagellated stage. Unarmored dinoflagellates do not have thecal or wall plates arranged in specific series, whereas armored species have plates that vary in thickness but are specific in number and arrangement. In armored dinoflagellates, the plate pattern and tabulation is a diagnostic character at the family, subfamily, and even genus levels. In most cases, the molecular characterization of dinoflagellates confirms the taxonomy on the basis of external morphology; this has been demonstrated for several groups. Together, both genetic and morphological criteria are becoming increasingly important for the characterization, separation, and identification of dinoflagellates species. *Pfiesteria* and *Pfiesteria*-like species are thinly armored forms with motile dinospore stages characterized by their distinct plate formula. *Pfiesteria piscicida* is the best-known member of the genus; however, there is at least one other species. Other genetically and morphologically related genera, for example, include *Pfiesteria* and *Kofoidia* species. These series constitute a plate pattern and formula and are useful in separating genera. There is a good correlation between the phylogenetic relationships among dinoflagellates based on plate structure and those based on small subunit rRNA and tRNA sequences.

Harmful algal bloom (HAB) species can produce a variety of biotoxins that can cause human illness, mass mortalities of aquatic organisms, and disease. Many of the biotoxins can be detected or assayed directly. In known toxin producers it is possible to determine when potentially harmful algal species are present in sufficient high abundance levels to produce toxins, then to employ the more sensitive analytical techniques or bioassays to quantify the amount of toxin(s) present in the environment. Unfortunately, for some toxins there are currently no assays or appropriate analytical techniques because the toxins remain uncharacterized. In this situation the most efficient strategy for identifying a potential public health threat is to rapidly determine if high, potentially toxic concentrations of the organisms are present so that appropriate response plans can be implemented. Quantifying the number of HAB species present in a sample can present difficulties because morphologically similar, but benign, species can be mistaken for the toxin-producing species. This is true for *Pfiesteria piscicida*, a small heterotrophic dinoflagellate that has been associated with fish kills and the production of a putative biotoxin in North Carolina and Maryland (1–3). Originally, it was assumed that small heterotrophic dinoflagellates of a particular shape, and approximately 10 μm in length, were all *P. piscicida*. That morphologically similar co-occurring species could be misidentified with *P. piscicida* has been recognized (4). Because a number of related nontoxic *Pfiesteria*-like species co-occur with *P. piscicida*, assessment of the potential public health threat of this species is complicated. This article is part of the National Conference on "Pfiesteria: From Biology to Public Health" and covers what is currently known about the higher-level placement of the *Pfiesteria*-like organisms and the taxonomic relationship between *P. piscicida* and co-occurring morphologically similar species.

**Higher-Order Taxonomic Placement of *P. piscicida* and Related Dinoflagellates**

*Pfiesteria* and several *Pfiesteria*-like genera are dinoflagellates classified in the botanical division Pyrrophyta. They are morphologically and genetically related and may derive from a common ancestor. The only valid and available named species as of this conference (5) is *P. piscicida* Steidinger and Burkholder (6), which has a zoospore stage characteristic of armored dinoflagellates. A second species, to honor Dr. Sandra Shumway, was named after the conference (7). Armored dinoflagellates have an outer wall of segments, or plates, arranged in specific, mostly horizontal series (Kofoidian series) (Figure 1). These series constitute a plate pattern and formula and are useful in separating genera. There is a good correlation between the phylogenetic relationships among dinoflagellates based on plate structure and those based on small subunit rRNA and tRNA sequences (9,10).

Because dinoflagellates have characteristics that historically have been considered "botanical," e.g., presence of chloroplasts, as well as "zoological," e.g., flagella and heterotrophic mode of feeding, there are several classification schemes. Recently, Femmer et al. (11) proposed a single classification scheme that addressed fossil and extant groups and followed the International Botanical Code of Nomenclature. In the same article, the authors reviewed 39 classification schemes for living dinoflagellates dating back to the 1800s. Some of the classification characteristics included plate tabulation, life cycle, and the unique dinokaryon nucleus. In that work (11) and elsewhere, plate tabulation is considered the most important morphological characteristic to differentiate within the division Pyrrophyta at the family or subfamily rank. It is also used at the generic level in...
most of the orders containing the majority of armored species, e.g., Gonyaulacales and Peridiniales (12).

In general, dinoflagellates are thought to have affinities with ciliates and apicomplexans at the ultrastructure level because of similar organelle structures such as tubular mitochondrial cristae, type of spindle, rod-shaped triechyns, and cortical vesicles, as well as at the genetic level (9,11,13). Dinoflagellates distinctly have a dinokaryon nucleus with continually condensed chromosomes throughout the entire life cycle, or in at least one stage of the life cycle, and characteristic flagella insertion.

A possible phylogenetic scenario or tree was presented by Pienkowski et al. (11), with support from the fossil record. This proposed phylogeny suggests that, structurally, unarmed dinoflagellates with unfilled cortical vesicles (hundreds) preceded armored forms in the evolutionary process; the armored forms then evolved from groups having many plates (vesicles filled with polysaccharide plate material) to those having only a few plates, as in the order Procentrales. In this scheme, the placement of Pfiesteria and related genera depends on several attributes that need further clarification. Originally, Pfiesteria was placed in the order Dinamoebales, which incorporates species with multiple life history stages, including dominant amoeboid forms. However, there are concerns that the dominant life history stage is a motile dinospore or coccoid stage with closer morphological affinities to the order Peridiniales (14). Does Pfiesteria have dominant amoeba and/or coccoid stages? Even if the dominant stage is an amoeba, there have been proposals to transfer Pfiesteria from the order Dinamoebales to the order Phytodiniales (11) or to place Pfiesteria specifically into the order Blastodiniales (9). Landsberg et al. (15) raised similar questions about life-cycle stages in the parasitic dinoflagellate Amyloodinium, and it was suggested that A. ocellatum belonged in the order Peridiniales on the basis of the morphology of the dinospore stage. Recent phylogenetic work on the rRNA ITS and 5.8S regions supports the placement of Pfiesteria in the Peridiniales or in a group between the Peridiniales and the Bolidiniales (16). All these options need to be carefully considered and, for now, most are theoretical schemes based on limited morphological, genetic, and physiological life history data for these orders.

Methods and Materials Used to Isolate and Specifically Identify Pfiesteria and Pfiesteria-Like Organisms

Single cells of flagellated motile stages of Pfiesteria and Pfiesteria-like organisms were isolated from water samples collected in
Identification of *Pfisteria* and *Pfisteria*-like species

Maryland, North Carolina, and Florida estuaries or from estuarine water incubated with microalgal prey. Isolated single cells were washed by transferring them between drops of diluted seawater using a micropipette. The cells were then introduced to individual wells in a 24-well plate containing filtered natural seawater diluted to the salinity of the original sample. These heterotrophs were fed cryptomonad algal prey (Provasoli-Guillard CCMP 1319; Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME, USA) and when growth occurred, contents of the well were transferred to flasks for maintenance (15 psu, 22°C, and 12:12 light:dark cycle) as a research culture in collection facilities at the Florida Marine Research Institute in St. Petersburg, Florida. Other cultures (17) were similarly isolated and maintained at other institutions. Clonal isolates were used to prepare specimens for scanning electron microscopy (SEM) so that the thecal plate patterns and tabulations could be determined reliably from one population. Over 63 clonal cultures were prepared for SEM, and they resulted in more than 1,000 micrographs of individual cells. In addition, more than 3,000 micrographs of individual cells were reviewed from field samples. The most frequently used fixation and preparation protocol was that of Truby (18), in which cells were either stripped or osmotically swollen to visualize sutures. Sutures (Figure 1B) are the visible lines between plates, like grooving between tiles. These same isolates were used for genetic studies (9, 19–22) and for the development of whole cell and other molecular probes.

**Results and Discussion**

All the *Pfisteria* and *Pfisteria*-like dinospores are heterotrophic, typically with a dinokaryon nucleus in the hypotheca and food vacuoles in the epitheca (Figure 1A). The longitudinal flagellum has two parallel parts, and the peduncle, a feeding organelle, emerges from under the flexible right sulcal plate. The sulcus is not straight but offset. Food vacuoles can contain whole phytoplankton cells or whole chloroplasts. At least in *P. piscicida*, Lewitus et al. (23) documented the chloroplasts to be functional for a period of time. The genus *Pfisteria* is characterized as having a plate formula/tabulation of apical pore complex (APC) (pore plate, closing plate, and X plate), 4’, 1a, 5’, 6c, 4s, 5”’, 0p, 2”’ (Figures 2A, 2B, 3). An unnamed marine *Pfisteria* (*Pfisteria* sp. *marina*, Figure 4) (24) has the same plate formula (Table 1). Both have a triangular 1a (first intercalary plate) (Figures 1B, 2B) on the left shoulder, which is one of the characteristics of the genus. Another proposed *Pfisteria* (7), *Pfisteria shumwayae* (Figure 5), has a larger, almost rectangular 1a plate and a plate formula distinct from that of *Pfisteria* (six precingular plates instead of five). This species, because of its difference in plate tabulation, should be reevaluated as to its genetic and morphologic affinity with *Pfisteria*. Both species have been implicated as producing toxins (3,6,7), though a purified toxin has yet to be isolated and characterized for either species.

*Pfisteria*-like organisms, by our definition, include numerous species that are superficially similar when viewed at the light microscopic level but distinct at the ultrastructural level. These are small (10–20 μm) cells lightly armored with defined plate formulas/tabulations and occupying ecological niches similar to *Pfisteria*. Many of these species are not harmful but may be closely related genetically to those that are. The following summarizes the current knowledge regarding the taxonomic relationships of these similar groups and genera.

Cryptoperidinioide (inferring that they are cryptically related to *Peridiniopsis*) are morphologically similar heterotrophs but have a plate formula of APC, 5’, 0a, 6’, 6c, 4s, 5”’, 0p, 2”’ (Figures 2C, 2D, Table 1). The

- Table 1: Kofoidian plate tabulations for *Pfisteria* and *Pfisteria*-like species.

|                | APC | Apicals | Anterior intercalaries | Precingulars | Cingulars | Sulci | Postcirculars | Posterior intercalaries | Antapical |
|----------------|-----|---------|------------------------|--------------|-----------|------|---------------|------------------------|-----------|
| *Pfisteria*    | APC | 4’      | 1a                     | 5’           | 6c        | 4s   | 5”           | 0p                     | 2”’       |
| Cryptoperidinioide | APC | 5’      | 0a                     | 6’           | 6c        | 4s   | 5”’          | 0p                     | 2”’       |
| “Lucy”         | APC | 4’      | 0/2a                   | 5’           | 6c        | 4s   | 5”’          | 0p                     | 2”’       |
| *Peridiniopsis*| APC | 3-5’    | 0/1a                   | 6/7”’        | 6c        | 4s   | 5”’          | 0p                     | 2”’       |

Abbreviations and symbols: a, anterior intercalary; c, cingular; s, sulcal; p, posterior intercalary; ”’, precingular; “’’, postcircular; “”, antapical. Figure 2 illustrates the ventral view and plate designations for *Pfisteria*, “Lucy,” and cryptoperidinioide.
genus *Peridinium* has a plate formula APC, 3-5', 0-1a, 6-7", 6c, 3-5s, 5", 0p, 2'". When a range in epithelial (top half of the cell) in plate tabulation is evident, in the epithelial series (top half of the cell), the presence of more than one genus is indicated (although there are several exceptions to this general rule, most notably the genus *Pyrophacus*). An example of a similar situation occurred with the genus *Gonnyaulax*. In the 1930s, the plate formula was APC, 3-6', 0-3a, 6", 6c, 6", 1p, 1". It is now recognized that there are at least four genera: *Gonnyaulax* sensu stricto, *Alexandrium*, *Amylax*, and *Lingulodinium* (Table 2). Each genus has a specific number of apical and anterior intercalary plates (8). In the order *Peridiniales*, genera are even differentiated on the basis of the number of cingular plates, e.g., 3 versus 4 versus 5 versus 6 (Table 3) (12). Cryptoperidinioids representing several species are one of the most common groups of dinoflagellates found in samples with *Pfisteria*. There is no evidence that the cryptoperidinioid species produce ichthyotoxins, but they do have bioactive compounds (25). Hence, misidentifying the cryptoperidinioids as *P. plicatilis* or *P. shumwayae* may overestimate any potential public health threat.

Another group of new species, with a plate formula of APC, 4', 2a, 6", 6c, 4s, 5", 0p, 2'" (Figure 2D), is being referred to as “Lucy” (Figure 7), after a common name given to a Florida isolate. These species also have a posterior dinokaryon nucleus in the dinospore, are heterotrophic, possess a peduncle that is extendable from under the right sulcal plate, and have a sulcus that is offset to the right. “Lucy” cells have two diamond-shaped anterior intercalaries (1a and 2a) at the left and right side of the 3' plate (Figure 2D). “Lucy” is distinct from *Pfisteria* and the cryptoperidinioids, both morphologically and genetically, yet these groups are closely related. Preliminary evidence indicates that “Lucy” produces bioactive ichthyotoxins (25).

Another new heterotrophic species in the order *Peridiniales*, with a common name, “Shepherd’s crook,” because of its unusual crook-shaped canal or X plate, co-occurs geographically with species in the family *Pfiesteriaceae*, but it does not have the appropriate plate formula or offset sulcus to be included in that family (Figure 8). The species needs further workup to properly assign it to a genus. Its potential to produce toxins is unknown, but the species is thought to be benign.

*Pfisteria* and its relatives are widely distributed, and like other dinoflagellates, they can have benthic stages, which could account for their recurrence in specific areas. Burkholder and Glasgow (26) and colleagues (27) have found there are benign stages of *P. plicatilis* and that when exposed to fish, the organism may become toxic and produce an ichthyotoxins compound. However, neither the morphological (4, 6) nor the biochemical identification of species, using molecular probes (19, 20, 28), differentiates toxic from nontoxic or non-inducible *P. plicatilis* forms. Hence, from a public health standpoint, the presence of *P. plicatilis* alone can potentially overestimate any toxic threat from this species. The same situation applies to *P. shumwayae*, as both species are part of the same toxic *Pfiesteria* complex (26).

In addition to *Pfisteria* and its related species, many other toxic or potentially toxic species of HAB species occur in the coastal regions of the United States. These species include armored and unarmored dinoflagellates, raphidophytes, diatoms, and blue-green algae or cyanophytes such as *Karenia* spp., *Chettomella* spp., *Pseudonitzschia* spp., and *Microcystis* spp. Many of these species produce well-characterized, highly toxic compounds that adversely affect both ecosystems and human health. The economic and ecological impacts from these blooms can be substantial, and the public health risks are still being assessed. It is important that we not lose sight of these harmful algae and their impacts in our pursuit to address harmful algal issues regarding *Pfisteria*.

---

**Table 2.** Kofoidian plate tabulations for *Gonyaulax*.

| Kofoid's *Gonyaulax* | APC | Apicals | Anterior intercalaries | Precingulars | Cingulars | Sulcels | Postcingulars | Posterior intercalaries | Antapicals |
|----------------------|-----|---------|-----------------------|-------------|----------|--------|---------------|------------------------|-----------|
| APC 3-6'             | 2a  | 6"      | 6c                    | 5s          | 3c       | 5'     | 0p            | 1p                     | 1"        |

**Table 3.** Kofoidian plate tabulations for *Peridinium*.

| *Glochadinium*       | APC 4' | 2a | 6' | 5c | 0p | 7c | 1p | 5" |
|----------------------|--------|----|----|----|----|----|----|----|
| *Protoxidinium*      | APC 4' | 2a | 6' | 5c | 0p | 7c | 1p | 5" |
| *Peridinium*         | APC 4' | 2a | 6' | 5c | 0p | 7c | 1p | 5" |
| *Scoposphaera*       | APC 4' | 2a | 6' | 5c | 0p | 7c | 1p | 5" |
| *Amphiesma*          | APC 4' | 2a | 6' | 5c | 0p | 7c | 1p | 5" |

---

Figure 7. "Lucy," SEM micrograph showing diamond-shaped 1a and 2a plates separated by 3'. Cell < 15 μm.

Figure 8. "Shepherd's crook." SEM micrograph of adjoining 1a and 2a plates. Cell > 15 μm.
REFERENCES AND NOTES

1. Burkholder JM. Chronic impacts from toxic microalgae on finfish, shellfish and human health. In: Proceedings of the Symposium on Conservation Medicine (Barkat, C, ed.). New York Academic Press, in press.
2. Gageone HB Jr, Burkholder JM. Water quality trends and management implications from a five-year study of a eutrophic estuary. Esti Apl 10:1024–1046 (2000).
3. Kim K-E, Linfccok W, McEwen PDR, Buxton M, Gardner HB Jr, Burkholder JM, Ramsdell JS. Identification of a PDI receptor in the phytoplankton cells: a potential target for a biocidal substance produced by Pfiesteria piscicida. Environ Health Perspect 108:457–482 (2000).
4. Steidinger KA, Lundeberg JL, Truby EW, Blakeley BA. The use of scanning electron microscopy in identifying small "greenish" dinoflagellates. Nova Hedwigia 112:415–422 (1995).
5. Centers for Disease Control and Prevention. CDC National Conference on Pfiesteria: from biology to public health. October 19–20, Stone Mountain, Georgia, USA, 2000. (Environ Health Perspect 108(suppl 6):631–807 (2001).
6. Steidinger KA, Burkholder JM, Glasgow HB Jr, Hebbes CW, Garrett JK, Truby EW, Noga EJ, Smith SA. Pfiesteria piscicida gen. et. sp. nov. (Pfiesteriaceae fam. nov), a new toxic dinoflagellate with a complex life cycle and behavior. J Phycol 22:157–164 (1986).
7. Glasgow HB Jr, Burkholder JM, Morton SL, Springer J, Turner T, O’Doherty DW. A second species of ichthyotoxic Pfiesteria (Dinophyceae, Pyrrophyta). Physiological Ecology 180(3):234–245 (2001).
8. Steidinger KA, Tangen K. Dinoflagellates, Jr: Identifying Marine Phytoplankton (Tomas C, ed.). San Diego, CA: Academic Press, 1997:367–584.
9. Litaker RW, Tester PA, Colton A, Levy MG, Noga EJ. The phylogenetic relationship of Pfiesteria piscicida, cryptoperidinopid sp., Amynodon aculeatum, and a Pfiesteriidae-like dinoflagellate to other dinoflagellates and apicomplexans. J Phycol 35:1379–1390 (1999).
10. Taylor FJR. Morphological and molecular evidence for dinoflagellate phylogeny: reinvestigating each other. J Phycol 35:1–6 (1999).
11. Fensome RA, Taylor FJR, Norris G, Sarjakoski JAT, Wharton DI, Williams GL. A classification of fossil and living Dinoflagellates. Micropaleontological Species, Publ 7. New York: American Museum of Natural History, 1983.
12. Batch E. On the thecal morphology of dinoflagellates with special emphasis on circular and subcircular plates. In: Cen Cim Mar Limnol Univ Nac Alco Mar 7:57–68 (1988).
13. Cavalier-Smith T. Kingdom protozoa and its 18 phyla. Microbiol Rev 67:553–594 (1993).
14. Fensome RA, Saltmania JF, Taylor FJR. Dinoflagellate phylogeny—reviewed: reconciling morphological and molecular-based phylogenies. Gcano 38:66–80 (1996).
15. Lutzner JL, Steidinger KA, Blakeley BA, Zander RW. Scanning electron microscope study of dinoflagellates of Astaxanthin and Cox. A new pathogenic dinoflagellate parasite of marine fish, and comments on its relationship to the Peridiniace. Di Aquat Org 20:23–32 (1984).
16. Litaker RW. Personal communication.
17. Colonial cultures have been supplied from Florida State Laboratory of Natural History Laboratory of the National Oceanic & Atmospheric Administration in Blakley, NC.
18. Truby EW. Preparation of single-celled dinoflagellates for electron microscopy. Microsc Res Tech 28:327–340 (1994).
19. Bowers HA, Turner T, Glasgow HB Jr, Burkholder JM, Rublee PA, Litaker RW. Development of real-time PCR assays for rapid detection of Pfiesteria piscicida and related dinoflagellates. Appl Environ Microbiol 66:4641–4648 (2000).
20. Oldach DW, Delorme OF, Jacobsen KS, Tengs T, Brown EG, Kempton JW, Schaefer EF, Bowers HA, Glasgow HB Jr, Burkholder JM, et al. Heteroduplex mobility assay-guided sequence discovery: elucidation of the small subunit (18S) rDNA sequences of Pfiesteria piscicida and related dinoflagellates from complex algal culture and environmental sample DNA pools. Proc Natl Acad Sci USA 97:9303–9308 (2000).
21. Woods Hole Oceanographic Institution. Pfiesteria website. http://www.red tide.whoi.edu/pfiesteria/molecular/ molecular.html [cited 30 March 2001].
22. National Institutes of Health. Genbank website. http://www.ncbi.nlm.nih.gov/GeneBank [cited 30 March 2001].
23. Lewis UK, Willis AM, Hayes KS, Burkholder JM, Glasgow HB Jr, Gillett PM, Burie MK. Mitophylogeny and nitrogen uptake by Pfiesteria piscicida (Dinophyceae). J Phycol 35:1430–1437 (1999).
24. Lundeberg JL, Steidinger KA, Blakesly BA. Fish-killing dinoflagellates in a tropical marine aquarium. In: Hamiltom Marine Algal Blooms (Lassus P, Arzel G, Ertul-Le Dern G, Cornion P, Marsall-Le Bost C, edd). Paris: Les Ulis, 1996; 65–70.
25. Morton SL. Personal communication.
26. Burkholder JM, Glasgow HB, Deamer-Melia NJ, Springer J, Parrow MW, Zhang C, Cancelleri PJ. Species of the toxic Pfiesteria complex and the importance of functional type in data interpretation. Environ Health Perspect 109(suppl.S):679–679 (2001).
27. http://www.red tide.whoi.edu/Pfiesteria/glucanid.html [cited 30 March 2001].
28. Rublee PA, Kempton JW, Schaefer EF, Aiken C, Harris J, Oldach DW, Bowers H, Tengs T, Burkholder JM, Glasgow HB. Use of molecular probes to assess geographic distribution of Pfiesteria species. Environ Health Perspect 109(suppl 5):765–767 (2001).