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THE RECLASSIFICATION OF *PFIESTERIA SHUMWAYAE* (DINOPHYCEAE): *PSEUDOPFIESTERIA*, GEN. NOV.[1]

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***Pfiesteria shumwayae* Glasgow et Burkholder is assigned to a new genus *Pseudopfiesteria* gen. nov. Plate tabulation differences between *Pfiesteria* and *Pseudopfiesteria* gen. nov. as well as a maximum likelihood phylogenetic analysis based on rDNA sequence data warrant creation of this new genus. The Kofoidian thecal plate formula for the new genus is Po, cp, X, 4′, 1a, 6″, 6c, PC, 5 + s, 5‴, 0p, 2⁗. In addition to having six precingular plates, *P. shumwayae* comb. nov. also has a distinctive diamond or rectangular-shaped anterior intercalary plate. Both *Pfiesteria* and *Pseudopfiesteria* gen. nov. are reassigned to the order Peridiniales based on an apical pore complex (APC) with a canal (X) plate that contacts a symmetrical 1′, four to five sulcal plates, and the conservative hypothecal tabulation of 5‴, 0p, and 2⁗. These morphological characters and the life histories of *Pfiesteria* and *Pseudopfiesteria* are consistent with placement of both genera in the Peridiniales. Based on the plate tabulations for *P. shumwayae, P. piscicida*, and the closely related "cryptoperidiniopsoid" and "lucy" groups, the family Pfiesteriaceae is amended to include species with the following tabulation: 4-5′, 0-2a, 5-6″, 6c, PC, 5 + s, 5‴, 0p, and 2⁗ as well as an APC containing a pore plate (Po), a closing plate (cp), and an X plate; the tabulation is expanded to increase the number of sulcal plates and to include a new plate, the peduncle cover (PC) plate. Members of the family have typical dinoflagellate life cycles characterized by a biflagellated free-living motile stage, a varying number of cyst stages, and the absence of multiple amoeboid stages.**

Key index words: **evolution; *Pfiesteria*-like dinoflagellates; *Pfiesteria shumwayae*; *Pseudopfiesteria*; ribosomal genes; taxonomy**

Abbreviations: **APC, apical pore complex; ITS, internal transcribed spacer; ML, maximum likelihood; LSU, large subunit; PC, peduncle cover plate; PLDs, *Pfiesteria*-like dinoflagellates; s.d.p., right (dexter) posterior sulcal plate; s.m., median sulcal plate; s.s, left (sinister) sulcal plate; SSU, small subunit**

Pfiesteria and *Pfiesteria*-like species are morphologically similar thinly armored dinoflagellates with flagellated stages characterized by their distinct Kofoidian thecal plate formulae or plate tabulations. Currently, the plate formula of the genus *Pfiesteria* is characterized

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by an apical pore complex (APC) that contains a pore plate (Po), a closing plate (cp), and a canal plate (X); an apical series comprised of four apical plates (′), one anterior intercalary plate (a), five precingular plates (″); six cingular plates (c); four sulcal plates (s); and an antapical series comprised of five postcingular plates (‴) and two antapical plates (⁗) (Steidinger et al. 1996). The type species, *Pfiesteria piscicida* Steidinger et Burkholder, and a second species, *Pfiesteria shumwayae* Glasgow et Burkholder, are currently assigned to the genus (Steidinger et al. 1996, Glasgow et al. 2001). In contrast to the type plate formulation for the genus *Pfiesteria*, Glasgow et al. (2001) described *P. shumwayae* with a plate formula of Po, cp, X, 4′, 1a, 6″, 6c, 4 s, 5‴, and 2⁗. For this species to be assigned to *Pfiesteria*, either the plate formula for the genus erected by Steidinger et al. (1996) should be amended to include a range of precingular plates from 5″ to 6″ or the species should be placed in a distinct genus based on this unique plate formula.

Historically, one plate difference in the epithecal or hypothecal series of armored dinoflagellates is sufficient to warrant placement of species into separate genera provided there is no confusion over the designation of specific plates, for example, an apical plate counted as a precingular plate (Fensome et al. 1993, Steidinger and Tangen 1997). The genera *Glochidinium*, *Protoperidinium*, *Peridinium*, and *Scrippsiella*, for instance, have nearly identical Kofoidian plate tabulations with species being placed into one genus or another based solely on whether they have three, four, five, or six cingular plates, respectively (Steidinger and Tangen 1997, Boltovskoy 1999). In a parallel situation, *P. shumwayae* has a six-plate precingular series (6″) versus *P. piscicida*, which has a five-plate precingular series (5″). Furthermore, the intercalary plate (1a) of *P. shumwayae* is rectangular and adjoins the second apical (2′) plate, in contrast to the intercalary plate of *P. piscicida*, which is triangular and does not adjoin the 2′ plate.

To determine whether molecular evidence would support placement of *P. piscicida* and *P. shumwayae* in the same or different genera, a separate phylogenetic analysis of rDNA sequences was conducted. Both the morphological and molecular data were consistent and support placement of *P. shumwayae* into a related, but distinct, genus from *P. piscicida* that we designate *Pseudopfiesteria*.

MATERIALS AND METHODS

Collection and Isolation. Isolates of *Pseudopfiesteria shumwayae* were originally made in 1998 from a fish bioassay aquarium at the North Carolina School of Veterinary Medicine, Raleigh, North Carolina, United States (Litaker et al. 1999). The original inoculum for the aquarium was a water sample from the Pamlico River, North Carolina collected in 1991. Isolation and culture procedures were described in Litaker and Tester (2002).

Additional isolates of *P. shumwayae* were obtained from water samples collected from the area of South Point, Pamlico River, North Carolina, 17 November 1999, as in Vogelbein et al. (2001). Briefly, water samples were collected, taking care to include some surficial sediment material that might contain benthic cyst stages. Ambient salinities of these samples ranged from 6 to 12 psu depending on the location of each sample site. In order to assure that heterotrophic cells remained active groups of 10 juvenile tilapia (*Oreochromis niloticus*, 3.0–5.0 cm, Southern States, New Kent Co., VA, USA) were released into 38 L of each water sample, immediately placed into coolers, and transported to the laboratory within 4–6 h. Upon arrival, the water samples containing fish were placed in 40-L aquaria and monitored until fish mortality was observed. Aliquots from the fish-killing aquaria were used to inoculate additional 40-L aquaria containing tilapia maintained in artificial seawater (Crystal Sea [formerly Forty Fathoms] Marine Mix, Marine Enterprises International, Baltimore, MD, USA) at 12 psu.

Clonal cultures of *P. shumwayae* were established from dinoflagellate assemblages present in those tanks where fish mortality occurred as described in Vogelbein et al. (2001). Individual cells were isolated using sterile micropipettes to transfer them into 0.22-μm filter-sterilized York River water adjusted to 12 psu. These cells were subsequently reisolated two to three more times. The resulting clonal cultures were maintained on a diet of *Rhodomonas salina* (CCMP1319), grown at 12 psu in f/2 medium (Guillard and Ryther 1962) and incubated at 24° C at 130 μmol photons $\cdot m^{-2} \cdot s^{-1}$ and a 12:12-h light:dark cycle. SEM and *P. shumwayae*-specific molecular probes were then used to confirm the taxonomic identity of each culture (Litaker et al. 2003). One of these *P. shumwayae* cultures (CCMP2089, Pamlico River, NC, USA), was deposited in the Provasoli–Guillard National Center for Culture of Marine Phytoplankton (West Boothbay Harbor, ME, USA).

Cultures of *Pfiesteria piscicida* (CCMP2091, Neuse River, NC, USA, Litaker et al. 1999), a "cryptoperidiniopsoid species" (CCMP1827, King's Creek, MD, USA, Steidinger et al. 2001), "lucy" Florida isolate (St. Lucie River estuary, FL, USA, Steidinger et al. 2001, died out before it could be deposited at CCMP), and "Shepherd's crook" (CCMP1829, Rhode River, MD, USA) were all grown as described in Litaker and Tester (2002). These cultures were fed either *Rhodomonas salina* (CCMP1319) or *Rhodomonas* sp. (CCMP767). The "cryptoperidiniopsoid," "lucy," and "shepherd's crook" species represent provisionally described genera that are related to *P. piscicida* and *Pseudopfiesteria shumwayae* (Steidinger et al. 2001).

DNA isolation, cloning, and sequencing procedures. Cultures of *P. shumwayae* (CCMP 2089), *Pfiesteria piscicida* (CCMP2091), the "cryptoperidiniopsoid sp." (CCMP1827), "lucy," and "shepherd's crook" (CCMP1829) were concentrated by filtration onto 3-μm Nucleopore (Costar, Cambridge, MA, USA) filters. Genomic DNA was isolated from the concentrated cells using the DNeasy Tissue Kit™ Qiagen, Chatsworth, CA, USA) following the manufacturer's protocol. An approximately 3600-bp region of the rDNA complex, including the small subunit (SSU), internal transcribed spacer (ITS) 1, 5.8S, ITS2, and first approximately 850 bp of the large subunit (LSU), was subsequently PCR amplified using a combination of the primer pairs Dino5′UF and ITSR2, 5.8SF2, and LSUB, or 5.8S_FWR_ PLCR and ITS-B (Table 1). PCR conditions are described in Litaker et al. (2002).

The PCR products were directly sequenced using an ABI 373 system (Applied Biosystems–ABI, Foster City, CA, USA) (Litaker et al. 2003). In instances where direct sequencing gave ambiguous results, the amplification products were cloned into plasmid vector pCR2.1® (Invitrogen, Carlsbad, CA, USA) using the Topo TA Cloning Kit® following the manufacturer's protocol. Plasmids were then sequenced using either 1) the Thermo Sequenase Sequencing Kit® (Amersham Life Science, Piscataway, NJ, USA) and infrared labeled (IRD700 or IRD 800) M13 primers (LI-COR, Lincoln, NE, USA) or 2) the

TABLE 1. Amplification and sequencing primers used to obtain the SSU–5′LSU rDNA sequences for each species.

Primer name	Sequence (5′–3′)	Location
Forward primers		
Dino5′UF	CAACCTGGTGATCCTGCCAGT	bp 1 of SSU
G19F	CATCTAAGGAAGGCAGCAGG	bp 464 of SSU
SSU918	GTTRAAGACGGACTAC	bp 918 of SSU
G17F	ATACCGTCCTAGTCTTAACC	bp 1009 of SSU
G22F	TGGTGGAGTGATTTGTCTGG	bp 1300 of SSU
seq1400	AGGTCTGTGATGCCCTTAGATG	bp 1436 of SSU
ITSF2	TACGTCCCTGCCCTTTGTAC	bp 1675 of SSU
ITSF1	GAAGGAGAAGTCGTAACAAGG	bp 1758 of SSU
5.8S_FRW_PLCR	GATGAAGGGCACAGCGAACT	bp 41 of 5.8S
5.8SF	CATTGTGAATTGCAGAATTCC	bp 70 of 5.8S
LSU500F	GCAAACAAGTACCATGAGGG	bp 351 of LSU
M13F	GTAAAACGACGGCCAGT	In the vector
Reverse primers		
G10R	CCGCGGCTGCTGGCACCAGAC	bp 559 of SSU
G14R	CTGCGAAAGCATTTGCCAAGG	bp 940 of SSU
G18R	GCATCACAGACCTGTTATTG	bp 1470 of SSU
G23R	TTCAGCCTTGCGACCATAC	bp 1121 of SSU
G21R	CCAGACAAATCACTCCACC	bp 1297 of SSU
GCG18SR	AGGTTCACCTACGGAAACCTTG	bp 1784 of SSU
5.8SR	CATCGTTGTTCGAGCCGAGAC	bp 24 of 5.8S
ITSR2	TCCCTGTTCATTCGCCATTAC	bp 66 of LSU
LSU500R	CCCTCATGGTACTTGTTTGC	bp 351 of LSU
ITS-B	TATGCTTAAATTCAGCGGGT	bp 883 of LSU
M13R	GGAAACAGCTATGACCATG	In the vector

ABI377 DNA sequencer using the Deoxy Terminator Cycle Sequencing Kit™ (Applied Biosystems–ABI). Sequencing primers are given in Table 1. The ribosomal sequences for *Pfiesteria piscicida*, *Pseudopfiesteria shumwayae*, "Lucy," "Shepherd's crook," and the "cryptoperidiniopsoid sp." were compiled using Vector NTI® (InforMax™, North Bethesda, MD, USA). The consensus sequences for *Pfiesteria piscicida* (AY245693), the "cryptoperidiniopsoid sp." (AY245690), "lucy" Florida isolate (AY245689), and "shepherd's crook" (AY590479) were deposited in GenBank. The resulting consensus sequence for *P. shumwayae* (AY245694) was the same as that for other single cell isolates previously deposited in GenBank.

Phylogenetic analysis. The consensus sequences for all five species were aligned using the CLUSTAL-W program (Thompson et al. 1994) in MacVector (Accelrys, San Diego, CA, USA) with an open gap penalty of 8 and an extended gap penalty of 3. Minor manual edits were made to these final alignments in cases where misalignments of 1 or 2 bp occurred. The aligned sequences were then analyzed using the MODELTEST program to evaluate which of 56 possible maximum likelihood (ML) models best fit the data using a log likelihood test as the criteria for goodness of fit (Posada and Crandall 1998). The best ML model selected by the MODELTEST program for the combined SSU/5.8S/5′LSU sequence data corresponded to a general-time-reversible model with four substitution types (Lanave et al. 1984, Hasegawa et al. 1985, Yang 1994, 1996).

An ML phylogenetic analysis was then conducted using PAUP* (Swofford 2002) and the general-time-reversible model selected by MODELTEST. The specific model parameters were as follows: substitution rate matrix = (1.000000, 0.846000, 1.000000, 1.000000, 0.162500, 1.00000), assumed nucleotide frequencies A = 0.38080, C = 0.17840, G = 0.15390, T = 0.28690, shape parameter (alpha) = 1.6368, molecular clock not enforced, assumed proportion of invariable sites = none, and the number of distinct data patterns under this model = 147. Starting trees were obtained via stepwise addition with steepest descent option not in effect, topological constraints not enforced, branch-swapping algorithm = tree-bisection reconnection, molecular clock not enforced, starting branch lengths obtained using Rogers-Swofford approximation method, trees with approximate likelihoods of 5% or further from the target score were rejected, and a single tree was held at each step during stepwise addition. Groups retained in 50% or more of the trees were indicated on the final ML tree. Support for each branch in the trees was estimated by doing 1000 bootstrap replicates with 100 random additions per replicate (Felsenstein 1985).

Because the relative evolutionary distance between these species was of interest, and not which species represent ancestral and derived states, the resulting phylogenetic tree was plotted as an unrooted phylogram where branch lengths indicate the relative evolutionary distance between species. This approach made it possible to evaluate whether *Pfiesteria piscicida* was more closely related to *Pseudopfiesteria shumwayae* or to species representing the other related genera.

SEM. Dinoflagellate cells grown in 12 psu seawater were combined 1:1 with ice-cold 3% glutaraldehyde and 1.4% osmium tetroxide buffered with 0.1 M sodium cacodylate (Truby 1997). Fixative solutions were adjusted to iso-osmotic and slightly hyperosmotic (up to + 20 mOsm) conditions, relative to the osmolarity of the sample, by adjusting the concentration of the sodium cacodylate buffer. Sample, fixative, and resulting sample/fixative combination osmolarities were measured using a vapor pressure osmometer (Wescor Inc., Logan, UT, USA). Cells were fixed on ice for 30 min, collected on 3-μm polycarbonate filters, washed in buffer, dehydrated through a graded ethanol series, critical-point dried, and sputter coated with gold-palladium. Analyses were performed on a LEO 435VP scanning electron microscope. Plate tabulations were established using the swollen sutures of intact cells as well as stripped swollen cells. Swollen cells revealed the critical sulcal plate sutures. Using established methods (Truby 1997) proved ineffective in exposing the underlying plates of *P. shumwayae*. Therefore a new procedure was developed using Triton X-100 to strip the outer membranes of cells. This was coupled with a poststripping to swell the cells enabling identification of plates. Briefly, motile

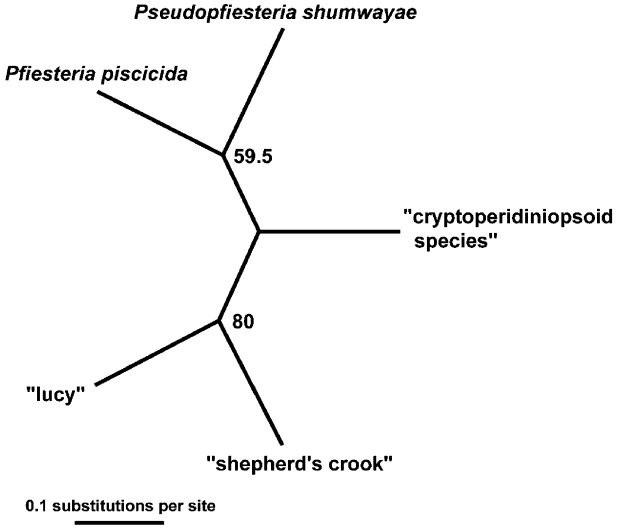

FIG. 1. ML phylogenetic analysis using the combined SSU, 5.8S, and 5′LSU data set for *Pfiesteria piscicida*, *Pseudopfiesteria shumwayae*, "cryptoperidiniopsoid sp.," "Lucy," and "Shepherd's crook." The latter three species belong to separate genera that are related to the *Pfiesteria piscicida* and *Pseudopfiesteria shumwayae* (Steidinger et al. 2001). The results are plotted as an unrooted phylogram with branch lengths indicating the relative genetic distance between species.

cells were combined with an aqueous solution of 20% Triton X-100 and sonicated for 1 h. The stripped cells were collected on 3-μm polycarbonate filters, swelled with diluted seawater for 30 min, and then fixed with buffered paraformaldehyde. After dehydration and critical-point drying, the filters were mounted on specimen stubs, sputter coated with gold-palladium, and analyzed (Mason et al. 2003).

RESULTS

Phylogenetic analysis. There was low bootstrap support (>60%) (Hershkovitz and Leipe 1998) for grouping *P. shumwayae* together with either *Pfiesteria piscicida* or the "cryptoperidiniopsoid species" based on the SSU/5.8S/5′LSU phylogeny (Fig. 1). The genetic distance observed between *P. piscicida* and *Pseudofiesteria shumwayae* was similar to that observed between "lucy" and "shepherd's crook," indicating similar species or genus level divergence between each respective species pair.

Pseudopfiesteria Litaker, Steidinger, Mason, Shields et Tester gen. nov.

Division: Pyrrhophyta Pascher, 1914
Class: Dinophyceae Pascher 1914
Order: Peridiniales Haeckel 1894
Family: Pfiesteriaceae Steidinger and Burkholder 1996

Dinoflagellatum parvum mobile, biflagellatum, unicellulare, parum armatum; circumscriptio ovoidea vel conica. Formula patellae Po, cp, X, 4′, 1a, 6″, 6c, PC, 5+s, 5‴, 0p, 2⁗. Cingulum aequatorium, parum depulsum. Sulcus, non

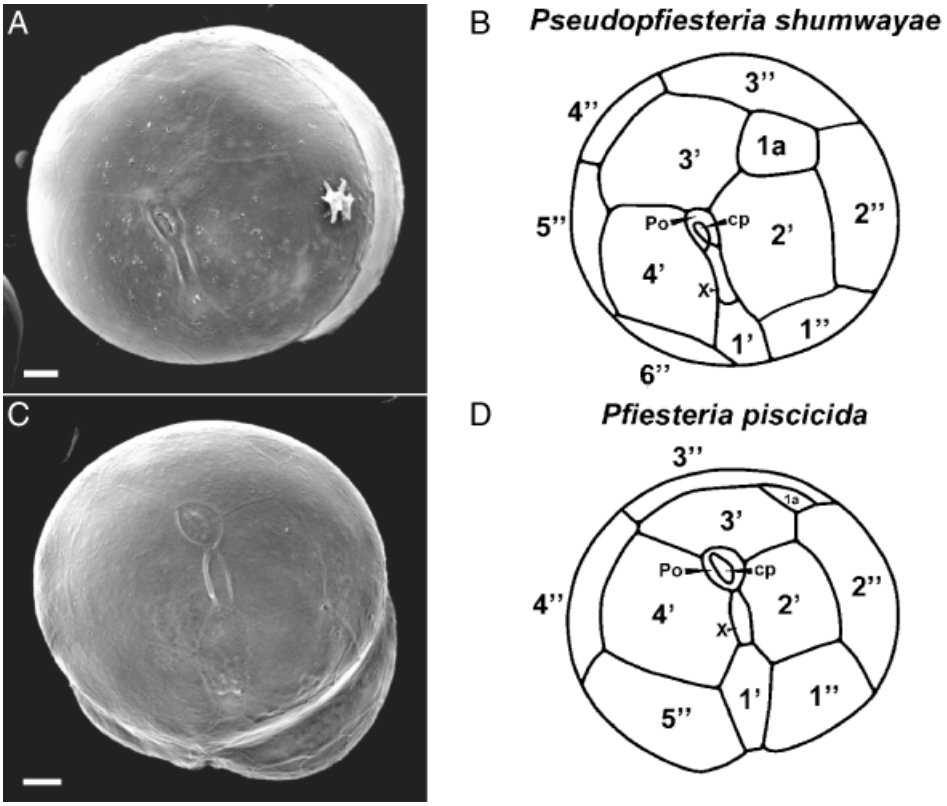

FIG. 2. Apical views of *Pseudopfiesteria shumwayae* (A, B) and *Pfiesteria piscicida* (C, D). Shown are the 6″ and rectangular 1a plates characteristic of *Pseudopfiesteria* species (A, B) and the 5″ and triangular 1a plates characteristic of *Pfiesteria* species (C, D). Cells were stripped and swollen before SEM using the method of Mason et al. (2003). Scale bar, 1μm.

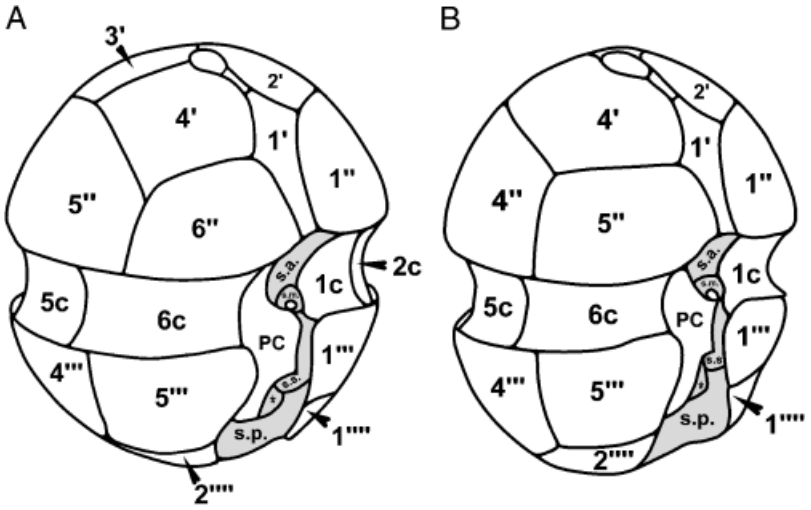

FIG. 3. Line drawing showing the differences in the overall morphology between *Pfiesteria piscicida* and *Pseudopfiesteria shumwayae*. (A) *Pseudopfiesteria shumwayae* showing the 6′′ plate. (B) *Pfiesteria piscicida* showing the 5′′ plate. The individual sulcal plates are shaded in gray. Starting at the apical end of the sulcus, the sulcal plates are arranged in the following order: s.a., anterior sulcal; s.m., median sulcal; s.s., left (sinister) sulcal; s.d.p., right (dexter) posterior sulcal; and s.p., posterior sulcal. The flagellar pore is indicated by the unshaded region in the middle of the s.m. plate. The s.d.p. plates in A and B are indicated by an asterisk. The sulcal plates indicate their relative position in a normal motile vegetative cell. The stripped swollen vegetative cells presented in Figures 4 and 5 show these sulcal plates in greater detail but with their location slightly altered because of the swelling process.

prolongatus ad antapicem sed prolongatus ad epithecam et patellam 1′ et parum amotus dextrosum. Nucleus sphaericus, dinocaryon typicus, locatus in hypotheca. Cellulae mobiles cum nutrimento heterotrophico per pedunculum tubularem. Vacuolae digestivae adsunt.

Small motile, biflagellated single-celled dinoflagellate, lightly armored, ovoid to biconical. Plate formula Po, cp, X, 4′, 1a, 6′′, 6c, PC, 5 + s, 5′′′, 0p, 2′′′′ (Figs. 2–4). Cingulum equatorial, slightly displaced. Sulcus, not extending to antapex but extending to epitheca and 1′, and slightly offset to the right. Nucleus spherical, typical dinokaryon, present in the hypotheca. Motile cells heterotrophic, feed via a tubular peduncle. Food vacuoles present.

Etymology: The genus is named *Pseudopfiesteria* to denote the difficulty in distinguishing this species from *Pfiesteria* using LM.

Type species: Pseudopfiesteria shumwayae (Glasgow et Burkholder) Litaker, Steidinger, Mason, Shields et Tester comb. nov.

Type locality: Pseudopfiesteria shumwayae was isolated from the Neuse and Pamlico Rivers, North Carolina (USA) and cultured using both fish and algae (Litaker et al. 1999, Oldach et al. 2000). Molecular analyses indicate a distribution in the continental United States from at least New York to Texas (Coyne et al. 2001, Rublee et al. 2001). This species has also been reported in northern European waters (Jakobsen et al. 2002), Australia (CSIRO Marine Research, Information Sheet 2001), and New Zealand (Rhodes et al. 2002). *Pseudopfiesteria shumwayae* is likely present in mesohaline estuaries worldwide.

Basionym: Pfiesteria shumwayae Glasgow & Burkholder, 2001 (Glasgow et al. 2001).

Synonyms: Pfiesteria-like species (Litaker et al. 1999).

Holotype: Pseudopfiesteria shumwayae. In Glasgow et al. (2001, Figs. 1–5).

Isotypes: Figures 2, A and B, 3A, and 4.

Habitat: Mesohaline estuarine waters where tidal flushing is relatively low.

Description: Dinoflagellate, small, oval, cryptic peridinioid-like stages with a plate formula of Po, cp, X, 4′, 1a, 6′′, 6c, PC, 5 + s, 5′′′, 0p, 2′′′′. The epitheca convex and biconical. The APC small with an oval pore plate (Po), closing plate (cp), and a canal plate (X). The APC situated between 2′, 3′, and 4′ and connected to 1′ by the X (canal) plate. The Po plate approximately $1.7 \pm 0.3\,\mu m$ long ($n = 29$), touching 2′, 3′, and 4′ plates (Fig. 2, C and D). The 1a plate rectangular or diamond shaped, touching the 2′, 3′, 2′′, and 3′′ plates. The 1′ plate ortho, shifted slightly to the left due to sulcal torsion and positioned over the anterior sulcal plate. The hypotheca symmetrical and composed of 5′′′ and 2′′′′, and subequal in size to the epitheca. Posterior intercalary plates absent. The cingulum equatorial, displaced 0.5× , and composed of six plates not rimmed by lists. The sulcus extends to the 1′ and offset to the right, partially concealed by a peduncle cover (PC) plate, and composed of at least five plates: small and difficult to visualize (Fig. 4). The anterior sulcal plate slightly higher than 1c, under the 1′ and 6′′, and adjacent to the 1′′. Transverse flagellar pore located in the median sulcal (s.m.) plate. Left (sinister) sulcal (s.s.) and right (dexter) posterior sulcal (s.d.p.) plates. PC plate originates at the right side of the sulcus at the left side of the 6c and 5′′′ plates. The PC plate is flexible to allow the extension and retraction of the labiate peduncle underneath. It is not known whether the PC plate is compositionally and structurally different than other sulcal plates. The possibility exists that one or more additional sulcal plates are located anterior to the s.d.p. plate (e.g. an anterior right sulcal plate) but cannot be visualized because of the overlying PC plate (Fig. 4). Posterior sulcal plate extends between 1′′′ and 5′′′.

Diagnosis. Differences between Pfiesteria piscicida *and* Pseudopfiesteria shumwayae *plate structures:* The 2′ plate of *Pfiesteria piscicida* is bounded by six plates compared with seven plates in *Pseudopfiesteria shumwayae* (Fig. 2). Additionally, in *Pfiesteria piscicida* the 2′ does not touch the intercalary, whereas in *Pseudo-*

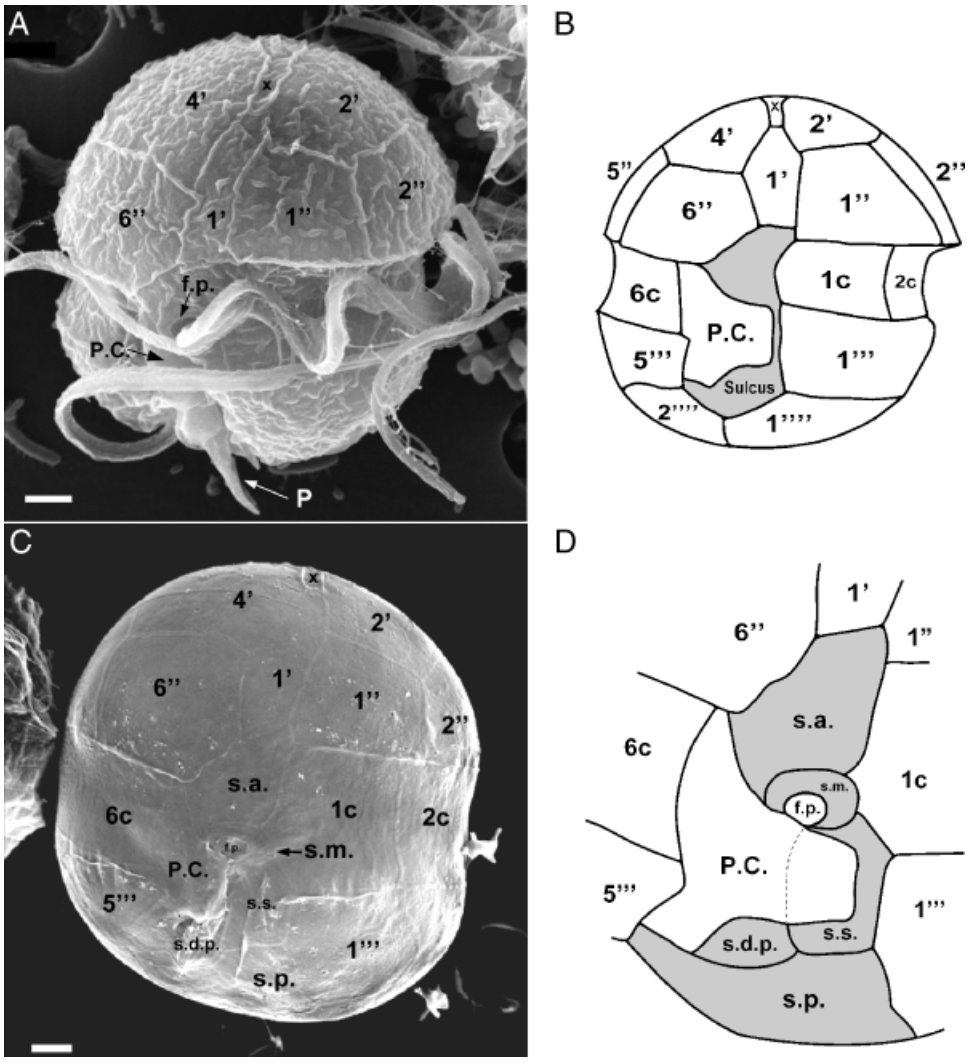

FIG. 4. Ventral view of *Pseudopfiesteria shumwayae*; comparison of intact and stripped motile cells. (A) Intact motile cell showing epithecal plates, the transverse flagellar pore (f.p.), and the peduncle cover (PC) plate extending over the labiate peduncle (P). (B) Composite line drawing of the same ventral view. (C) Motile cell stripped of the plasmalemma and outer amphiesmal membrane and swollen to reveal details of the sulcal region. The PC plate normally extends over the sulcus but often retracts during the swelling process, as shown in C. (D) Composite line drawing of the sulcal region based on the SEM shown in C plus more than 20 additional sulcal region SEMs. Sulcal annotation abbreviations: s.a., anterior sulcal plate; s.m., median sulcal plate; s.s, left (sinister) sulcal plate; s.d.p., right (dexter) posterior sulcal plate; and s.p., posterior sulcal plate. Scale bar, 1μm

pfiesteria shumwayae it shares a common suture with the intercalary plate. As a result of the placement of the 2′/3′ suture, the 2′ plate is smaller and often more square in shape in *Pfiesteria piscicida* than in *Pseudopfiesteria shumwayae*. Overall, the apical series is also more asymmetrical in *Pfiesteria piscicida* than in *Pseudopfiesteria shumwayae* (Fig. 2).

The precingular series (′′) is comprised of five plates in *Pfiesteria piscicida* and six plates in *Pseudopfiesteria shumwayae* (Fig. 2). The s.m. plate of *Pfiesteria piscicida* is smaller than that of *Pseudopfiesteria shumwayae* relative to the size of the transverse flagellar pore (Figs. 4 and 5). The PC plate originates at the right side of the sulcus adjacent to the cingulum (6c) and 5′′′ plate and covers the s.d.p. plate as well as a significant portion of the s.s. plate (Figs. 4 and 5). It is possible that the PC plate also overlays one or more additional sulcal plates located to the right of the s.s and s.d.p plates. In *Pseudopfiesteria shumwayae*, the PC plate is larger and appears to have a prominent elongation that extends further toward the posterior end of the sulcus than in *Pfiesteria piscicida* (Figs. 4 and 5).

The Po plate of *Pseudopfiesteria shumwayae* is narrower relative to the X plate than in *Pfiesteria piscicida*. The suture between the 2′ and 3′ plates of *Pfiesteria piscicida* intersects the Po plate laterally, whereas the same suture is shifted dorsally and intersects the APC in *Pseudopfiesteria shumwayae* from the rear.

Remarks. The same life cycle stages were observed for *Pfiesteria shumwayae* as reported in Parrow and Burkholder (2003a). Amoeboid or chrysophyte-like forms were not evident.

Pfiesteriaceae (Steidinger et Burkholder 1996) Emend Litaker, Steidinger, Mason, Shields et Tester
Division: Pyrrhophyta Pascher, 1914
Class: Dinophyceae Pascher 1914
Order: Peridiniales Haeckel 1894

Type genus: Pfiesteria Steidinger et Burkholder 1996.

Type species: Pfiesteria piscicida Steidinger et Burkholder 1996.

Distribution: Worldwide in mesohaline estuaries.

Motile unicellular stages including gametes and triflagellated planozygotes, and mitotic, meiotic and temporary cysts of a peridiniopsoid type. All stages

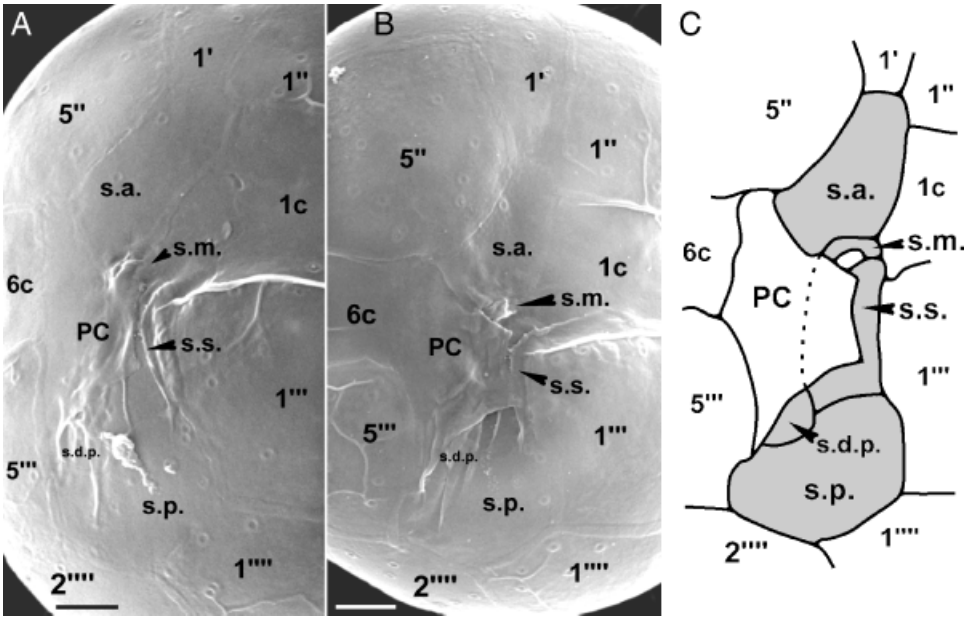

FIG. 5. (A, B) Motile *Pfiesteria piscicida* cells stripped of the plasmalemma and outer amphiesmal membrane and swollen to reveal details of the sulcal region. Sulcal annotation abbreviations: s.a., anterior sulcal plate; s.m., median sulcal plate; s.s, left (sinister) sulcal plate; s.d.p., right (dexter) posterior sulcal plate; and s.p., posterior sulcal plate. (A) The peduncle cover (PC) plate is shown in a retracted position. This is a preservation artifact that is often observed in stripped cells. (B) The PC plate is shown in its normal position extended over the peduncle and sulcal region. (C) Line drawing of sulcal region of *P. piscicida* based on the cell shown in B. The dotted line on the left sulcal plate (s.s.) indicates another suture that delineates the s.s. plate from one or more additional sulcal plates that are obscured by the PC plate. Scale bar, 1μm.

lack chloroplasts. Motile forms capable of phagocytosis.

Remarks: The family Pfiesteriaceae conservatively includes *Pfesteria piscicida* and *Pseudopfiesteria shumwayae* as well as the ''lucy'' and ''cryptoperidiniopsoid species'' (Steidinger et al. 2001). The Kofoidian plate tabulations for species belonging to the ''lucy'' and ''cryptoperidiniopsoid'' groups are Po, cp, X, 4′, 2a, 6′′, 6c, PC, 5 + s, 5′′′, 0p, 2′′′′ and Po, cp, X, 5′, 0a, 6′′, 6c, PC, 5 + s, 5′′′, 0p, 2′′′′, respectively (Steidinger et al. 2001, Kokocinski and Marshall 2003, unpublished data).

Studies indicate that these species lack multiple amoeboid stages (Litaker et al. 2002, Parrow and Burkholder 2003a) that dominated the life cycle as originally described (Burkholder and Glasgow 1995, 1997). The family is therefore amended to include species with the following Kofoidian plate structures: APC containing a pore plate (Po), a closing plate (cp), and a canal plate (X), 4–5′, 0–2a, 5–6′′, 6c, a peduncle cover plate (PC), 5 + s, 5′′′, 0p and 2′′′′, and which have variable life cycles with motile dinoflagellate and sessile cyst stages but lacking multiple amoeboid stages (Litaker et al. 2002). Heterotrophic.

Pfiesteria (Steidinger and Burkholder 1996) Emend Litaker, Steidinger, Mason, Shields et Tester

Type species: Pfiesteria piscicida Steidinger et Burkholder 1996.

Distribution: Worldwide in mesohaline estuaries.

Emendation: The genus *Pfiesteria* was expanded by Glasgow et al. (2001) to include *Pfiesteria shumwayae*, a species with 6′′ precingular plates. Based on the plate structure and molecular data placing *Pseudopfiesteria shumwayae* in a separate genus, the genus *Pfiesteria* is returned to that described in Steidinger et Burkholder 1996 with the following modifications: ''PC, 5 + s'' included in the plate tabulation series and ''Life cycles variable with typical dinoflagellate and cyst stages, but lacking multiple amoeboid stages that dominate the life cycle.''

DISCUSSION

Pfiesteria shumwayae (Glasgow et al. 2001) is reassigned to the new genus *Pseudopfiesteria* based on an ML phylogenetic analysis of rDNA sequences as well as conserved morphological data. The ML analysis used rDNA sequence data to specifically test the null hypothesis that *Pfiesteria piscicida* and *Pseudopfiesteria shumwayae* were in the same genus. The phylogenetic analysis included *Pfiesteria piscicida*, *Pseudopfiesteria shumwayae*, and three other dinoflagellate species, which have been provisionally assigned to separate but related groups based on Kofoidian plate tabulations (Steidinger et al. 2001). Each species exhibited at least one unique Kofoidian plate difference that would distinguish it from the other four species. The molecular analysis showed that *Pfiesteria piscicida* and *Pseudopfiesteria shumwayae* were no more closely related to

TABLE 2. Comparison of morphological differences between *Pseudopfiesteria shumwayae* and *Pfiesteria piscicida*.

	Pseudopfiesteria shumwayae	*Pfiesteria piscicida*
Epitheca	Po, cp, X, 4, 1a, 6″	Po, cp, X, 4′, 1a, 5″
Typical size range	10–25μm	7–14 μm
Mean plate length ± SD	1.7 ± 0.3 μm, $n = 29$	1.5 ± 0.2 μm, $n = 22$
1a plate	4-sided (rectangular)	3-sided (triangular)
1a placement	Touches 2″, 3″, 2′, 3′	Touches 2″, 3″, 3′

each other than to the "cryptoperidiniopsoid species" (Fig. 1). A similar level of divergence was observed between the "Lucy" and "Shepherd's crook" species as between the other three species. These results are consistent with two possible alternatives. The first is that the observed divergences between species represent generic level divergences and that all five species belong to separate genera.

The second alternative is that both the "lucy"; "shepherd's crook" clade and the *Pfiesteria piscicida*; *Pseudopfiesteria shumwayae*; "cryptoperidiniopsoid" clade should be combined into two separate genera. This second alternative would require grouping species with either two or three significant Kofoidian plate tabulation differences into the same genus. Combining species with such divergent plate tabulations into the same genus, however, would compromise currently accepted taxonomic rules governing dinoflagellate classification. Presently, a single Kofoidian plate difference is considered sufficient to place dinoflagellate species into different genera (Fensome et al. 1993, Steidinger and Tangen 1997). The phylogenetic analysis is therefore most consistent with placement of *Pfiesteria piscicida* and *Pseudopfiesteria shumwayae*, as well as the other three species, into separate genera.

Such a reassignment is further supported by the following two major and one minor plate differences between the two species. *Pseudopfiesteria shumwayae* has a rectangular or diamond-shaped intercalary plate (Fig. 1) and six precingular plates, whereas *Pfiesteria piscicida* has a triangular intercalary and five precingular plates (Figs. 2 and 3, Table 2) (Steidinger et al. 2001). Accordingly, we place *Pfiesteria shumwayae* into the genus *Pseudopfiesteria* (Po, cp, X, 4′, 1a, 6″, 6c, PC, 5 + s, 5‴, 0p, 2⁗) and emend the genus *Pfiesteria* to include only species with a Kofoidian plate tabulation of Po, cp, X, 4′, 1a, 5″, 6c, PC, 5 + s, 5‴, 0p, 2⁗ (Figs. 2, 3, and 5).

The sulcal structure revealed by the stripping and swelling techniques used in this study is more complex than originally described. A PC plate was found to cover the peduncle opening and most of the sulcal region in all the species examined (Figs. 4, C and D, and 5). This plate is distinct from the cingular and sulcal plates and may represent a common structural feature in many pedunculate feeding dinoflagellates. The swollen and stripped cells also revealed the presence of five sulcal plates, which varied in shape between species. It is likely that additional sulcal plates occur but cannot be visualized because a portion of the sulcus is consistently covered by the PC plate regardless of what type of cell preparation method is used. This inability to observe the sulcal plates obscured by the PC plate accounts for the 5 + s designation in the Kofoidian tabulation series.

Assignment of the Pfiesteriaceae to the Peridiniales. The taxonomic assignment of *Pfiesteria* and other similar *Pfiesteria*-like dinoflagellates (PLDs) to subclass has proven difficult. Originally, the family Pfiesteriaceae was assigned to the Dinamoebales based on the multiphasic life cycle containing flagellated stages, amoeboid forms, and cysts of multiple forms. The life cycle observations made in this study were consistent with those presented in Litaker et al. (2002) and Parrow and Burkholder (2003a,b). We find no evidence of amoeboid stages in the life cycles of *Pseudopfiesteria shumwayae*, *Pfiesteria piscicida*, or related PLDs. Our results support the conclusion that *Pfiesteria piscicida* and *Pseudopfiesteria shumwayae* have life cycles typical of many marine dinoflagellates. Simple life cycles lacking amoebae are not consistent with placement in the Dinamoebales (Fensome et al. 1993).

The plate structure of *Pfiesteria* and *Pseudopfiesteria* most closely corresponds to species in the Peridiniales that have a 5‴ and 2⁗ plate series, a symmetrical 1′ that touches the X plate in the APC, and relatively few sulcal plates. These similarities in tabulation support placement of the Pfiesteriaceae in the Peridiniales. Accordingly, we reassign the family Pfiesteriaceae from the Dinamoebales to the Peridiniales to reflect similarities in the Kofoidian plate tabulations as well as the results of recent life history studies. A similar reassignment of *Pfiesteria* species to the Peridiniales has been previously suggested by Fensome et al. (1999) and Parrow and Burkholder (2003a).

Additional support for placing Pfiesteriaceae in the Peridiniales comes from a previous ML phylogenetic analysis of the SSU gene from 54 diverse dinoflagellate species that showed that the PLDs were most closely related to a subgroup within the Peridiniphycidae and to *Amyloodinium ocellatum*, which belongs to the Blastodinophycidae (Litaker et al. 1999). The branch support for the association between *Amyloodinium*, the PLDs, and the Peridiniphycidae, however, was not significant, making an unambiguous assignment impossible. The morphological similarities between the Kofoidian plate tabulation series of *A. ocellatum* zoospores and species in the Peridiniales favors inclusion of *Amyloodinium* in the Peridiniphycidae as well (Landsberg et al. 1994). These data are consistent with the Blastodinophyceae and Pfiesteriaceae representing distantly related branches within the ancestral Peridiniphycidae lineage.

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