Two New Species of *Epistylis* (Ciliophora: Peritrichida) on the Blue Crab (*Callinectes sapidus*) in the Gulf of Mexico

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ABSTRACT. Two epibiotic peritrichs infested the blue crab, *Callinectes sapidus*, from the Gulf of Mexico, Mississippi, USA. *Epistylis callinectes* n. sp. was isolated from the epipods of maxillipeds, bases of gill-cleaning setae, and gills, and *Epistylis clampi* n. sp. was isolated from the exterior surfaces of the exoskeleton. *Epistylis callinectes* has short, symmetrically and dichotomously branched stalks; its zooid is elongate ovoid and conspicuously longer than the individual stalk branches, measuring 40-57 (49) × 18-33 (26) µm in vivo and containing a thick, undivided peristomial lip (PL). It has a single contractile vacuole and a transverse horseshoe-shaped macronucleus. Its haplokinety (H) and polykinety (Po) complete one and one-half circuits on the peristome before entering the infundibulum. There is a distal kinetal fragment present at the distal end of both the H and Po. *Epistylis clampi* has long, asymmetrically, and dichotomously branched stalks. Its zooid is elongate vase-shaped, measuring 35-64 (48) × 21-30 (27) µm in vivo and with a thick, transversely folded PL. The stalks supporting zooids are unequal in length. Its zooid has a single contractile vacuole and a transverse horseshoe-shaped macronucleus occurs in the upper half of the body. Its H and Po complete approximately one circuit around the peristome before entering the infundibulum. There is a distal kinetal fragment present at the distal regenent present at the distal end of both the H and Po. This species has 71-112 transverse silverlines from the peristome to the scopula.

Key Words. Ciliate, epibiont, Epistylididae, Epistylis callinectes n. sp., Epistylis clampi n. sp., morphology, taxonomy.

PERITRICHS, such as species of *Epistylis* Ehrenberg, 1830, *Zoothamnium* Bory de St. Vincent, 1826, and *Vorticella* Linnaeus, 1767, are conspicuously diverse, sessile ciliates that are found on a large variety of substrata in marine and freshwater environments, including the surfaces of crustaceans (Foissner, Berger, and Kohmann 1992; Kahl 1935; Morado and Small 1995; Nenninger 1948; Precht 1935; Rustige 1991; Schödel 1987; Stiller 1942). Epibiotic ciliates are often host-specific and restricted in their choice of attachment sites on their hosts as well (Gilbert and Schröder 2003; Görtz 1996; Mayén-Estrada and Aladro-Lubel 2001).

The blue crab, *Callinectes sapidus* Rathbun, 1896, occurs commonly along the Gulf of Mexico; its natural distribution is the western Atlantic Ocean from Nova Scotia to Argentina. It comprises one of the most valuable commercial fisheries in the U.S. (Guillory, Perry, and VanderKooy 2001; Messick 1998); it hosts several ciliates: *Acineta* sp., *Epistylis* sp., *Ephelota* sp., *Mesanophrys chesapeakensis* Messick and Small, 1996, *Mesanophrys* sp., *Hyalophysa chattoni* Bradbury, 1966, *Lagenophrys callinectes* Couch, 1967, and *Lagenophrys* sp. (e.g. Couch 1966, 1973; Landers, Confusione, and Defee 1996; Landers, Zimlich, and Coate 1999; Messick 1998; Messick and Small 1996; Overstreet and Whatley 1975; Sawyer, MacLean, and Ziskowski 1976; Shields and Overstreet 2006).

More than 33 members of *Epistylis* plus seven incertae sedis and several unnamed species have been reported as infesting crustacean hosts (e.g. Fernández-Galiano and Tato-Porto 2000; Morado and Small 1995; Song 1986, 1992, 2003). Aside from some exceptionally detailed descriptions by Foissner et al. (1992) and others, most of the species within this genus are based on observations of live organisms or on ultrastructure (e.g. Guinea, Gil, and Fernández-Galiano 1986; Mayén-Estrada and Aladro-Lubel 2001; Walker and Roberts 1982). Consequently, many descriptions lack important comparative features.

Historically, epistylidid specimens from the blue crab and mostly confined to the gills have been considered one species (e.g. Couch 1966; Overstreet 1978), but at least two species infest the blue crab, even though a single species is confined primarily to the gills (Shields and Overstreet 2006). We detected two undescribed species of *Epistylis* on *C. sapidus* along the Gulf Coast of Mississippi and Florida. One species was discovered on the epipods of maxillipeds, bases of gill-cleaning setae, and gills. The other species was seen on the exterior surfaces of the exoskeleton. The main objective of the present work was to describe the two species of *Epistylis* found on *C. sapidus* using three different methods of silver impregnation in addition to observations of the living ciliates.

MATERIALS AND METHODS

Specimens of *Callinectes sapidus*, including juveniles and adults, were collected from the Gulf of Mexico near Ocean Springs $(30^{\circ}24'N, 88^{\circ}49'W)$ and Biloxi $(30^{\circ}25'N, 88^{\circ}59'W)$, Mississippi, and Pensacola $(30^{\circ}25'N, 87^{\circ}39'W)$, Florida, USA. They were examined either immediately or after being maintained in a 200-L container in filtered, aerated sea water with the same salinity as the capture locality, exchanged weekly, and fed a commercial diet (Rangen Inc., Buhl, ID).

Ciliates were detached or scratched off with a pipette or knife and kept temporarily in filtered sea water at room temperature $(\sim 25 \,^{\circ}\text{C})$. Observations of living specimens were conducted with both bright-field and differential interference contrast microscopy. Three kinds of silver impregnation, protargol staining according to Wilbert (1975) with some modification by adding anhydrous sodium carbonate to give a concentration of 4% (w/v) to the developer solution (HM. & RMO., unpubl. data), Chatton-Lwoff silver nitrate method as described by Corliss (1953), and silver carbonate impregnation according to Ma, Choi, and Song (2003), were used to reveal the infraciliature, silverline system, and nuclear apparatus. Measurements and digital images of live and stained specimens were performed with Olympus optics and an Evolution MP 5.0 RTV digital camera system. Morphological terminology used in the descriptions follows that of Corliss (1979) and Lynn and Small (2002).

RESULTS

Class Oligohymenophora de Puytorac et al., 1974 Subclass Peritrichia Stein, 1859 Order Sessilida Kahl, 1933 Family Epistylididae Kahl, 1933 Genus *Epistylis* Ehrenberg, 1830

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Epistylis callinectes **n. sp.** (Table 1 and Fig. 1–33) Synonyms: *Epistylis* sp. sensu Couch (1966), *Epistylis* sp. sensu

Shields and Overstreet (2006: Fig. 34)

Diagnosis. Epibiotic species with short, symmetrically and dichotomously branched stalk; zooids elongate ovoid in shape, measuring 40–57 (49) × 18–33 (26) μ m in vivo, with length conspicuously longer than individual stalk branches, with thick undivided peristomial lip (PL). Contractile vacuole single, apically located slightly below peristomial disc; macronucleus horseshoe shaped, transversely oriented in upper one-third of body. Infundibulum reaching mid-body; haplokinety (H) and polykinety (Po) completing approximately one and one-half circuits on peristome before entering infundibulum; distal kinetal fragment present at distal end of H and Po. Transverse silverlines numbering 48–70 from oral area to trochal band (TB) and 19–26 from TB to scopula.

Deposited specimens. One protargol-stained slide (USNM 1079447, which we consider the most diagnostic slide) and one wet silver-impregnated slide (USNM 1079448) of syntype specimens were deposited in the Ciliate Type Slide Collection, United States Museum of Natural History, Smithsonian Institution, Washington, DC, USA; additional syntype slides were deposited in the Gulf Coast Research Laboratory Museum, The University of Southern Mississippi (GCRL 2307, protargol slide; and GCRL 2308, wet silver-impregnated preparations).

Etymology. The specific epithet refers to the genus of the host. **Type host.** *Callinectes sapidus* Rathbun, 1896, blue crab, Portunidae.

Type locality. Ocean Springs, Mississippi, USA (30°24'N, 88°49'W); other localities, Biloxi, Mississippi (30°25'N, 88°59'W); Pensacola, Florida (30°25'N, 87°39'W).

Location on host. Most individuals occurred on the epipods of maxillipeds and bases of gill-cleaning setae, but some occasionally occurred on the gill lamella. **Ecological data for collections.** Water temperature 10–25 °C, pH 6.5–7.0, and salinity 15–31‰.

Description. Individual zooid measuring $40-57(49) \times 18-33$ (26) µm in vivo, elongate ovoid in shape, with maximum width at anterior third of body, usually contracted below PL. Length-towidth ratio of fully extended zooid 1:0.3-0.7. Epistomial disc (ED) strongly elevated in expanded individual (Fig. 1, 16). PL thick, 19-24 (22) µm in width, 3-6 (5) µm in height, not divided by circumferential fold (Fig. 1, 10, 13-20). Pellicle appearing smooth at low magnification, with fine striae detectable at magnifications of $400 \times$ and higher. Cytoplasm nearly colorless and transparent in the anterior and posterior third of the body, often with several large refringent food vacuoles (usually 3-5 µm in diam.) present in the mid-body region (Fig. 1, 16, 19-20). Macronucleus horseshoe-shaped, transversely oriented. Contractile vacuole single, $5-12 \,\mu\text{m}$ in diam., apically located slightly below peristomial disc (Fig. 1-3, 10, 14), with intervals of 30-65 s between discharges.

Stalk short, with longitudinally striated surface (Fig. 1, 7, 10, 22), stout, $6-11 \,\mu\text{m}$ in diam., often with base wider than stalk when attached to epipods of maxillipeds or gill-cleaning setae (Fig. 23, arrow). Colony branching symmetrically and dichotomously, usually with four levels of branches, with zooids situated in pairs at regular intervals; zooids conspicuously longer than the terminal stalk, numbering 2–64 per colony but usually eight (Fig. 3, 7, 13–20, 22–23); stalk base 17–28 μ m to the first branching point; scopula 5–11 μ m to the terminal branching point.

Haplokinety and Po both completing approximately one and one-half circuits around ED before entering infundibulum at which point completing one additional circuit around wall (Fig. 4–6); distal end of both H and Po usually with one kinetal fragment (Fig. 4, 6, 29, arrowhead); H on wall of infundibulum opposite infundibular polykinetids. Epistomial membrane (EM)

Table 1. Morphometric data for Epistylis callinectes n. sp.

Character	n	Min	Max	Mean	SD	SE	CV
Length of zooid, extended, in vivo	21	39.8	56.6	48.6	4.84	1.06	10.0
Length of zooid, contracted, in vivo	15	23.3	43.3	30.2	4.90	1.26	16.2
Length of cyst, in vivo	7	31.9	39.7	35.9	2.47	0.93	6.9
Length of zooid within cyst, in vivo	8	25.6	35.0	30.7	3.80	1.34	12.4
Length of zooid, fixed, protargol	33	15.5	38.8	23.8	5.76	1.00	24.2
Length of zooid, fixed, silver carbonate	28	34.9	52.8	41.7	3.79	0.72	9.
Width of zooid, extended in vivo	21	17.8	33.4	26.2	4.13	0.90	15.8
Ratio of width to length of zooid, extended in vivo	21	1: 0.3	1: 0.7	1:0.5	0.02	0.10	20.0
Width of zooid, contracted in vivo	16	15.6	27.6	19.9	2.84	0.71	14.
Width of cyst, in vivo	8	25.6	35.0	30.7	3.80	1.34	12.4
Width of peristomial lip, in vivo	32	18.3	24.2	21.6	1.35	0.24	6.2
Height of peristomial lip, in vivo	30	3.2	6.2	4.9	0.72	0.13	14.
Diameter of contractile vacuole, in vivo	17	4.8	12.1	7.4	1.85	0.45	25.0
Distance from TB to peristome, fixed, protargol	29	17.1	30.9	24.5	3.59	0.67	14.
Distance from TB to scopula, fixed, protargol	28	6.1	16.1	10.9	2.39	0.45	22.0
Width of scopula, fixed, protargol	28	5.0	11.2	8.0	1.64	0.31	20.0
Width of oral apparatus, fixed, protargol	27	8.6	19.8	14.1	3.31	0.64	23.
Width of TB, fixed, protargol	30	12.7	34.5	21.5	5.19	0.95	24.
Perimeter of Ma, fixed, silver carbonate	16	26	56	41.9	6.97	1.74	16.0
Length of Mi, fixed, silver carbonate	24	2.8	8.1	4.4	1.43	0.29	32.7
Distance from stalk base to first branching point, in vivo	11	13.6	28.2	20.3	3.92	1.18	19.3
Distance from scopula to terminal branching point, in vivo	11	5.4	10.7	7.9	1.61	0.49	20.5
Width of stalk, in vivo	22	6.5	10.8	8.7	1.16	0.25	13.4
Number of silverlines from peristome to TB, fixed, silver nitrate	11	48	70	56.8	8.15	2.46	14.
Number of silverlines from TB to scopula, fixed, silver nitrate	10	19	26	23.5	2.12	0.67	9.0
Distance between two adjacent pellicular pores in same silverline, fixed, silver nitrate	28	1.3	4.1	2.7	0.81	0.15	30.0
Number of zooids per colony, in vivo	72	2	64	8.1	10.1	1.19	123.
Time to discharge of contractile vacuole(s)	32	30	65	41.5	8.76	1.55	21.

All measurements in µm; CV, coefficient of variation; Ma, macronucleus; Mi, micronucleus; SD, standard deviation; SE, standard error; TB, trochal band.



Fig. 1–12. *Epistylis callinectes* n. sp. in vivo (1–3, 10), stained with silver carbonate (7, 9), protargol (4–6), silver nitrate impregnations (8), and compared with *E. stammeri* (11–12, after Nenninger 1948). 1, 10. Typical zooids; arrows indicate pellicular striae. 2. Contracted zooid. 3. Colony with six zooids. 4–6. Infraciliature of oral apparatus; arrowhead in 4 and 6 marks the distal kinetal fragment. 7. Colony of eight zooids showing macronucleus and micronucleus as well as structure of stalk. 8. Silverline system; arrows indicate pellicular pores. 9. Zooid stained to show pellicular striae; arrows indicate the trochal band. Abbreviations: CV, contractile vacuole; EM, epistomial membrane; FV, food vacuole; G, germinal kinety; H, haplokinety; Ma, macronucleus; Mi, micronucleus; P1–P3, infundibular polykinetids 1–3; Po, polykinety; Sc, scopula; TB, trochal band. Scale bars = 30 µm.



Fig. 13–21. *Epistylis callinectes* n. sp. in vivo. 13, 15, 20. Colonies of zooids filled with food vacuoles. 19. Typical zooid with fine pellicular striae. Arrows in 14 indicate apical contractile vacuoles; arrows in 16 indicate the epistomial disc; arrowhead in 17 and 18 indicates the base of stalk; arrows in 18 and 21 show the filamentous bacteria on the outer surface of the precyst. Scale bars = $40 \,\mu\text{m}$.

short, located near upper level of infundibulum (Fig. 4–6, 26–27, 29–31, arrow). Germinal kinety (G) consisting of densely packed kinetosomes within upper half of infundibulum, located above and parallel to infundibular part of H (Fig. 4–6, 26–27, 29–32). Poly-kinetids three, located at lower half of infundibulum, with each polykinetid consisting of three rows of kinetosomes; Polykinetid 1 (P1) longer than polykinetid 2 (P2); P2 longer than polykinetid 3 (P3); ratio of P1: P2: P3 approximately 1:0.5:0.3 (Fig. 33); P1 and P2 closely parallel or slightly separated at anterior ends. Aboral TB consisting of two staggered rows of kinetosomes,

encircling posterior region of cell (Fig. 26, 29–30). Silverline system consisting of 48–70 (56.8) pellicular striae between ED and TB, and of 19–26 between TB and scopula, with an average distance between adjacent silverlines of 0.5 μ m. Pellicular pores (= argentophilic dots) numerous, associated with pellicular striae (Fig. 8, 25, arrows). Distance between two adjacent pellicular pores 1–4 μ m. Scopula most argentophilic part of cell (Fig. 8, 25). Myoneme system sparse, staining most intensely on periphery of epistomial area in silver carbonate-impregnated specimens (Fig. 9, 24, arrows).



Fig. 22–33. *Epistylis callinectes* n. sp. stained with silver carbonate (22–24), silver nitrate (25), and protargol (26–33). 22. Colony showing macronucleus, micronucleus, and stalk. 23. Colony showing stalk and stalk base (arrow). 24. Zooid showing the pellicular striae and myoneme (arrows) around epistomial area. 25. Zooids showing silverline system and pellicular pores (arrows). 28. Intermediate stage of dividing zooid showing peristomial apparatus (arrows), macronucleus, and micronuclei (arrowheads). 26–27, 29–32. Non-dividing zooids, with arrows showing the epistomial membrane, and arrowheads indicating the distal kinetal fragment. 33. Early stage of dividing zooid; arrow indicating the gap between infundibular polykinetid 1 and polykinety. Abbreviations: P1–P3, infundibular polykinetids 1–3; TB, trochal band. Scale bars = 40 μm.

Table 2. Morphometric data for *Epistylis clampi* n. sp.

Character	n	Min	Max	Mean	SD	SE	CV
Length of zooid, extended, in vivo	15	35.4	63.8	48.3	7.42	1.91	15.3
Length of zooid, fixed, protargol	56	22.3	38.0	28.1	3.74	0.50	13.3
Width of zooid, extended, in vivo	13	21.4	30.4	27.0	2.61	0.72	9.7
Width of zooid, fixed, protargol	56	16.3	25.8	21.0	2.56	0.34	12.2
Width of peristomial lip, in vivo	11	27.1	34.7	31.9	2.04	0.61	6.4
Distance from scopula to terminal branching point (long), in vivo	24	7.6	73.0	31.4	18.95	3.87	60.4
Distance from scopula to terminal branching point (short), in vivo	24	4.3	30.3	11.6	7.47	1.52	64.6
Ratio of width to length of zooid, in vivo	13	1: 0.4	1: 0.8	1: 0.6	0.12	0.03	20.0
Ratio of short stalk to long one, in vivo	24	1: 0.2	1: 0.6	1: 0.4	0.12	0.02	29.6
Length of colony, in vivo	5	300.9	740.4	594.7	175.21	78.35	29.5
Width of oral apparatus, fixed, protargol	50	13.0	22.3	16.9	2.13	0.30	12.6
Width of trochal band, fixed, protargol	50	11.8	21.9	16.9	2.45	0.35	14.5
Width of scopula, fixed, protargol	45	4.1	7.5	5.5	0.78	0.12	14.1
Distance from trochal band to oral apparatus, fixed, protargol	44	9.2	20.3	13.0	2.28	0.34	17.5
Distance from trochal band to scopula, fixed, protargol	44	4.1	9.8	6.6	1.20	0.18	18.0
Thickness of stalk near scopula, fixed, protargol	9	3.1	4.9	4.0	0.62	0.21	15.4
Perimeter of Ma, fixed, protargol	23	26.1	42.6	34.9	4.18	0.87	12.0
Number of silverlines from peristome to trochal band, fixed, silver nitrate	3	62	77	68.0	7.94	4.58	11.7
Number of silverlines from trochal band to scopula, fixed, silver nitrate	4	17	35	29.3	8.50	4.25	29.1
Number of silverlines from peristome to scopula, fixed, silver nitrate	11	71	112	87.6	12.53	3.78	14.3
Distance between two adjacent silverlines, fixed, silver nitrate	8	0.38	0.62	0.49	0.07	0.03	15.2

All measurements in µm. CV, coefficient of variation; Ma, macronucleus; SD, standard deviation; SE, standard error.

Binary fission. Early stage with G proliferating, nuclear apparatus changing after proliferation of G (Fig. 33). Middle stage with macronucleus beginning to divide, with two oval daughter micronuclei aboral to macronucleus (Fig. 28, arrowheads), with oral apparatus replicated into two sets (Fig. 28, arrows).

Encystment. Precystic stage occurring after environmental deterioration or death of host. Zooid forming thin transparent external wall, with cell becoming oval and leaving large space anteriorly (Fig. 18, 21). Filamentous bacterium (e.g. *Leukothrix* sp.) often attaching to the cyst wall and stalk (Fig. 18, 21, arrows).

Epistylis clampi n. sp. (Table 2 and Fig. 34-53)

Diagnosis. Epibiotic species with long asymmetrically and dichotomously branched stalk; zooid elongate, vase-shaped, measuring 35–64 (48) \times 21–30 (27) µm in vivo, with thick transversely folded PL when extended. Stalks supporting zooids unequal in length. Contractile vacuole single, apically located below peristomial disc; macronucleus horseshoe-shaped, transversely oriented in upper half of body. Infundibulum reaching mid-body; H and Po completing approximately one circuit around ED before entering infundibulum; one distal kinetal fragment present at the distal end of H and Po. Transverse silverlines numbering 71–112 from peristome to scopula.

Type specimens. One protargol-stained slide (USNM 1079449, which we consider the most diagnostic slide) and one wet silver-impregnated slide (USNM 1079450) of syntype specimens were deposited in the Ciliate Type Slide Collection, United States Museum of Natural History, Smithsonian Institution, Washington, DC, USA; additional syntype slides were deposited in the Gulf Coast Research Laboratory Museum, The University of Southern Mississippi (GCRL 2309, protargol slide; and GCRL 2310, wet silver-impregnated preparations).

Etymology. We dedicate this species to the American protozoologist Dr. John C. Clamp, Department of Biology, North Carolina Central University, for his contributions to the taxonomy of epibiotic ciliates.

Type host. *Callinectes sapidus* Rathbun, 1896, blue crab, Portunidae.

Type locality. Ocean Springs, Mississippi, USA (30°24'N, 88°49'W); other localities, Biloxi, Mississippi (30°25'N, 88°59'W); Pensacola, Florida (30°25'N, 87°39'W).

Location on host. Individuals occurred in abundance on the exterior surfaces of the exoskeleton and rarely on the gills, epipods of maxillipeds, and gill-cleaning setae.

Ecological data for collections. Water temperature 10–25 °C, pH 6.5–7.0, and salinity 15–31‰.

Description. Individual zooid measuring 35–64 (48) × 21–30 (27) μ m in vivo, shape like elongate vase; length to width ratio of fully extended zooid 1:0.4–0.8; cell widest at PL; PL with transverse fold, conspicuously thick, 27–35 (32) μ m in width. ED strongly elevated in expanded individual (Fig. 34, 43). Pellicle smooth at low magnification, with fine striae detectable only at magnification of 400× and higher. Cytoplasm nearly colorless and transparent in anterior third of body, with several large refringent food vacuoles (3–5 μ m in diam.) present in mid-body of recently collected specimens (Fig. 34–36, 43). Macronucleus horseshoe-shaped, horizontally oriented; contractile vacuole single, 5–13 μ m in diam., apically located below peristomial disc, with 30–125 s intervals between discharges.

Dichotomously branched colony reaching 0.3–0.7 mm in length, with up to 30–60 zooids (Fig. 46). Terminal branches of stalks unequal in length, bearing individual pairs of zooids, with longer stalk measuring 8–73 (31) μ m in length and shorter stalk, 4–30 (12) μ m; ratio of longer to shorter stalk 1:0.2–0.6 (Fig 37, 46–47, arrows and arrowheads). Primary stalk approximately 10 μ m in diam., narrowing to 4 μ m in distal branches. Stalk smooth, with very fine longitudinal striations visible under high magnification only (Fig. 47). Contracting zooid folding over anterior end of stalk branch (Fig. 35, arrows; 36, arrowheads); zooids flattening in shape before telotroch formation (Fig. 38).

Haplokinety and Po both completing approximately one circuit around ED before entering infundibulum, at which point completing one additional circuit around the wall (Fig. 40, 50); distal end of both H and Po often with one kinetal fragment (Fig. 40, 50). Infundibular polykinetids in lower half of infundibulum three, each consisting of three rows of kinetosomes; P1 longer than P2; P2 longer than P3 (Fig. 40, 51–52); H on wall of infundibulum opposite infundibular polykinetids; G parallel to H, with anterior kinetosomes sparsely distributed (Fig. 40, arrowhead); EM short, near opening of infundibulum (Fig. 40). TB consisting of two staggered rows of kinetosomes, expanded to three or four rows in



Fig. 34-42. *Epistylis clampi* n. sp. in vivo (34-38), stained with protargol (39-40), and stained with silver nitrate (41-42). 34. Typical zooid; showing the elevated epistomial disc (ED), transversely folded PL. 35. Beginning of contraction; arrows show retraction of ED. 36. Fully contracted zooid; arrowheads indicate the depression around scopula. 37. Portion of colony; showing the unequal terminal stalk branches (arrows and arrowheads). 38. A zooid developing to telotroch. 39. Large individual with trochal band consisting of kinetids that are three to four kinetosomes in width. 40. Infraciliature; arrow marks the distal kinetal fragment; arrowhead shows the widely spaced anterior kinetosomes of the germinal kinety. 41. Pellicular pores and portion of silverlines (arrowheads). 42. Silverlines and portion of pellicular pores of large individual. Abbreviations: CV, contractile vacuole; Cy, cytopharynx; ED, epistomial disc; EM, epistomial membrane; FV, food vacuoles; G, germinal kinety; H, haplokinety; Ma, macronucleus; P1-P3, infundibular polykinetids 1–3; PL, peristomial lip; Po, polykinety; Sc, scopula; TB, trochal band. Scale bars = 40 μm.

zooids nearing binary fission (Fig. 39, 53). Pellicular pores conspicuous, mostly concealing silverlines; fine silverlines visible in large expanded individuals only, numbering 71-112 (88) from peristome to scopula, with average distance 0.5 µm between adjacent silverlines. Scopula always appearing as most argentophilic part of stained cell.

DISCUSSION

We assigned the two undescribed peritrich species from the blue crab to the genus *Epistylis* because they were colonial, with a branched, non-contractile stalk, and their zooids had a definite lip encircling the peristome and peristomial ciliature that consisted of no more than 1.5 circuits. The peristomial infraciliature of both species also resembled those described in the zoothamnids *Zoothamnium plumula* Kahl, 1933, *Zoothamnium maximum* Song, 1986, and *Zoothamnopsis sinica* Ji and Song, 2004 (Ji and Song 2004; Song, AL-Rasheid, and Hu 2002) by having a short kinetal fragment at the distal ends of the Po and H. We also observed them in two undescribed species of *Zoothamnium* and *Vorticella aquadulcis* Stokes, 1887 (HM. and RMO., unpubl. data). The fragment, however, can be observed on well-prepared protargol



Fig. 42–53. *Epistylis clampi* n. sp. in vivo (43–47), stained with silver nitrate (48–49), and stained with protargol (50–53). 43. Typical zooid. 44. Developing telotroch almost ready to leave the stalk. 45. Portion of colony, showing the unequal terminal stalks. 46. Typical colony. 47. Longer (arrow) and shorter (arrowhead) terminal stalks. 48. Large individual showing fine silverlines. 49. Typical individual showing pellicular pores. 50, 53. Oral and aboral views of same individual; arrow indicates the distal kinetal fragment. 51–52. Same individual, showing oral apparatus. Abbreviations: EM, epistomial membrane; G, germinal kinety; H, haplokinety; P1–P2, infundibular polykinetids 1–2; Po, Polykinety; Sc, scopula; TB, trochal band. Scale bars = $40 \,\mu$ m.

Species	Body length	Body width	Number of silverlines ^a	Number of silverlines ^b	Number of CV, position	Number of zooids in one colony	Source of data	
E. callinectes n. sp.	40-57	17-33	48-70	19–26	1. below PD	2-64	Present paper	
E. clampi n. sp.	35-64	21-30	Total number	71-112	1, below PD	1-70	Present paper	
E. acuminata	35-55	21-44			1, below PD		Song (1986)	
E. agrionis	42.6				1, near infundibulum		Nenninger (1948)	
E. aselli	25-40				1, below PD	4-8	Song (1992)	
E. balatonica	90-100				1, below PL		Kahl (1935)	
E. bimarginata	75–93	33–39	—	—	1, below PD	—	Kahl (1935), Schödel (1987)	
E. caliciformis	70-100				1, below PD		Kahl (1935)	
E. chrysemydis	120-250	55-110			1, below PL		Foissner et al. (1992)	
E. daphniae	31-37				1, below PL	2-5	Nenninger (1948)	
E. harpacticola	28-40				1, below PD	4-16	Song (1992)	
E. longifila	39.6				1, below PD	5-6	Nenninger (1948)	
E. nigrellii	35-40	33	_	_	1, below PL	_	Arvy et al. (1969)	
E. plicatilis	90-160	25-50	110-123	66-83	1, below PD		Foissner et al. (1992)	
E. poloneci	98-120	38-45		_	1. irregular	_	Matthes (1955)	

Table 3. Morphological comparison among the two new species of Epistylis and other morphologically similar species of Epistylis.

Measurement in µm. -, no data; CV, contractile vacuole; Ma, macronucleus; PD, peristomial disc; PL, peristomial lip; TB, trochal band. ^aFrom peristome to trochal band.

^bFrom trochal band to scopula.

61-62

E. stammeri

specimens only. We do not consider such distal fragments to constitute generic diagnostic features.

With respect to size, position of contractile vacuole, appearance of the PL, number of silverlines, and branching pattern of the specimens, Epistylis callinectes resembles E. stammeri Nenninger, 1948, E. daphniae Fauré-Fremiet, 1905, E. agrionis Nenninger, 1948, E. longifila Nenninger, 1948, E. harpacticola Kahl, 1933, E. acuminata Song, 1986, and E. aselli Stiller, 1941 (Table 3). It differs from the species described by Nenninger (1948) in the following features. Epistylis stammeri discovered on Asellus aquaticus in a freshwater pond and cold well in Orberndorf, Germany, has two contractile vacuoles rather than one, a relatively longer body (61-62 vs. 39-57 µm), and fewer zooids per colony (1-4 vs. 2-64). Epistylis daphniae found on Daphnia magna in Germany differs from E. callinectes by being shorter (30-37 vs. 39-57 µm) and having a V-shaped body with the macronucleus located in the upper third of the zooid. Epistylis agrionis on Agrion sp. in Germany has the contractile vacuole located below the PL (versus below the peristomial disc). Epistylis longifila from Eudiaptomus zachariasi differs from E. callinectes by having prominent pellicular striae and a relatively longer stalk. Moreover, Epistylis harpacticola has prominent pellicular striae compared with fine ones on E. callinectes (Kahl 1935; Song 1992), E. acuminata has asymmetrical and unilaterally arranged zooids rather than symmetrical and bilaterally distributed ones (Song 1986, 2003), and zooids of E. aselli have a shorter and stouter body (Song 1986, 2003; Stiller 1941).

Epistylis clampi resembles E. nigrellii Arvy, Batisse, and Lacombe 1969 by the shape of the zooid. Epistylis nigrellii, which was found on Balanus eburneus in New Jersey and New York, differs from E. clampi by having an unfolded PL and one and one-half rather than one circuit of ciliary bands on the peristome. Epistylis clampi resembles E. chrysemydis Bishop and Jahn, 1941, E. bimarginata Nenninger, 1948, E. balatonica Stiller, 1931, E. caliciformis Kahl, 1933, and E. poloneci Matthes, 1955 by having two peristomial collar bulges. Epistylis chrysemydis (including several junior synonyms listed by Foissner, Berger, and Kohmann 1992) differs from E. clampi by having larger zooids (120-250 µm vs. 35-64 µm in length), with the PL narrower rather

than wider than the body width, the contractile vacuole located below the PL rather than below the peristomial disc, and a polytropic macronucleus (Foissner et al. 1992; Nenninger 1948). Even though the contractile vacuole of E. bimarginata is also located below the peristomial disc, this species differs from E. clampi by having a larger and cylinder-shaped zooid rather than a relatively small and vase-shaped one, and fewer zooids per colony (Nenninger 1948; Schödel 1987). Epistylis caliciformis is oblong ovate with a longitudinally distributed macronucleus, and E. balatonica is ovate and wider at the mid-body region (Kahl 1935). Epistylis poloneci differs from E. clampi by having an irregular rather than a horseshoe-shaped macronucleus and is widest in the mid-body rather than at the level of the PL (Matthes 1955) (Table 3).

1 - 4

Nenninger (1948)

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Epistylis clampi differs noticeably from E. callinectes by having long, slender, and unequal terminal stalk branches rather than short, stout, and equal ones. Moreover, the two species have differently shaped zooids (elongate vase-shaped vs. ovoid), different types of PLs (transversely folded vs. unfolded), and different number of circuits of peristomial ciliature (one circuit of H and Po vs. one and one-half).

Foissner et al. (1992) divided the pellicular topography of peritrichs into three types: concave, convex, and bubble-shaped. Epistylis callinectes and E. clampi both have convex pellicles. The pellicle exhibited fine striae apparent in living organisms and dense silverlines having numerous pellicular pores apparent in specimens impregnated with silver nitrate. The silverline system of the two species was similar to that described by Foissner et al. (1992) and others in E. plicatilis Ehrenberg, 1830, the vorticellid Carchesium polypinum (Linnaeus, 1758), the operculariids Opercularia nutans (Ehrenberg, 1838), and O. articulata Goldfuss, 1820, and the opisthonectid Opisthonecta matiensis Martín-Cereceda, Serrano, and Guinea, 1999. By contrast, the pellicular pores of both were more prominent than those of E. nympharum Engelmann, 1862, as reported by Foissner et al. (1992).

Encystment or resting cyst formation of ciliates is a general strategy against several environmental stresses that involves a general metabolic inactivation. The presence of a cyst wall and gene silencing comprise the main diagnostic features of restingcyst formation (Gutiérrez et al. 2001). For E. callinectes, precystic stages were uncommon in wild blue crabs collected from most localities. The precystic stage was noted in crabs taken from a small, dead-end, boat harbor with poor circulation and in crabs experimentally stressed by confinement. In the former case, no evidence existed to support the precyst or cyst excysting after the crabs were placed in clean sea water for 10–15 d. In the latter case, 10 individual adult crabs without precystic stages and heavily infested with peritrichs and suctorians, Acineta spp., Epistylis callinectes, E. clampi, Zoothamnium spp., and Lagenophrys callinectes, exhibited precystic stages after being crowded in an aquarium with 95 L of static sea water for 5-7 d. In some cases, the gills of presumed stressed individuals or those with heavy ciliate infestations appeared black. The blue crab can tolerate a variety of harsh conditions not tolerated by many other crustaceans; it readily detoxifies several different toxic chemicals (Brouwer and Lee 2006). The encystment of the ciliate may be the mechanism by which it can survive a temporary decline in environmental conditions without abandoning the host. Encystment has been reported for few peritrichs.

Walker, Edwards, and Suchard (1989) and Calvo et al. (2003) described four morphologically distinct layers in the cyst wall of Opisthonecta henneguyi Fauré-Fremiet, 1906, using electron microscopy and cytochemistry. Livingston and Walker (1992) examined the encystment of Epistylis rotans Svec, 1897, by light microscopy as well as by transmission and scanning electron microscopy, and they showed that the precursors of the cyst wall migrated to the cell surface before being secreted to form a thick, unlayered cyst wall. Martín-Cereceda, Serrano, and Guinea (1999) observed Opisthonecta matiensis forming layered structures, which they interpreted as precystic stages surrounding the zooids. By light microscopy, E. callinectes exhibited at least two layers in the cyst wall surrounding the zooid. The inner layer is thick and nearly transparent, with the zooid moving weakly, shrinking to form an oval mass, and leaving a large space between the oral end of the zooid and the cyst wall; the outer layer is thin and semitransparent, covered with a large amount of a filamentous Leucothrix-like bacterium that is also attached to the stalks. Leucothrix sp. covers and penetrates the stalks of Epistylis sp. on a fish (Overstreet and Howse 1977) (not Heteropolaria colisarum Foissner and Schubert, 1977, also reported from sunfishes [e.g. Foissner, Hoffman, and Mitchell (1985)]) and on a brine shrimp (Solangi, Overstreet, and Gannam 1979).

Gilbert and Schröder (2003) hypothesized four pathways to encystment in peritrichs: from a recently attached zooid, a zooid in a colony, a free-swimming zooid, and a telotroch. Our observations suggest that encystment of attached zooids within a colony of *E. callinectes* serves as a primary means to survive adverse conditions. Even though we did not observe telotroch formation for *E. callinectes*, we found occasional empty stalks on the epipods of maxillipeds and bases of gill-cleaning setae of both crabs taken from the wild and those maintained temporarily under stress. Consequently, those empty stalks suggest that *E. callinectes* releases telotrochs as well as cysts to survive adverse conditions. In contrast, we often observed telotroch formation for *E. clampi* as well as numerous empty stalks, sometimes en mass and presumably in relation to premolt of the crab.

Gilbert and Schröder (2003) discovered that when the zooids of *E. pygmaeum* (Ehrenberg, 1838) Foissner, Berger, and Schaumburg, 1999 settled on a transitory substratum, the eggs of three rotifer species of *Brachionus*, they exhibited only short stalks, in contrast with long stalks and branched colonies that often developed in material located on the host's body. They hypothesized that the zooids attached to eggs had little time for stalk development and subsequent division into colonies before the egg substratum was eliminated by hatching. By contrast, zooids attached

to bodies of rotifers would have a much more stable substratum, allowing the adults more than 1 week to develop very long stalks, which divided into branching colonies. From our observations on peritrichs from the blue crab, we recognized differences in the stalks related to site on the host, long and slender stalks on the exoskeleton and short and stout stalks on the epipods of maxillipeds, bases of gill-cleaning setae, and gills, but we considered the two forms as distinct species. That decision was based also on the structures of the zooids, such as the different types of PLs and different lengths of peristomial ciliature.

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LITERATURE CITED

- Arvy, L., Batisse, A. & Lacombe, D. 1969. Péritriches épizoïques dans la chambre branchiale des Balanidae (Crustacean: Cirripedia), *Epistylis* nigrellii n. sp., *E. horizontalis* (Chatton 1930). Ann. Parasitol. Hum. Comp., 44:351–374.
- Brouwer, M. & Lee, R. F. 2006. Responses to toxic chemicals at the molecular, cellular, tissue, and organismal level. *In*: Kennedy, V. S. & Cronin, L. E. (ed.), The Blue Crab, *Callinectes sapidus*. Maryland Sea Grant, p. 405–432. (in press)
- Calvo, P., Fernandez-Aliseda, C., Garrido, J. & Torres, A. 2003. Ultrastructure, encystment and cyst wall composition of the resting cyst of the peritrich ciliate *Opisthonecta henneguyi*. J. Eukaryot. Microbiol., 50:49–56.
- Corliss, J. O. 1953. Silver impregnation of ciliated protozoa by the Chatton–Lwoff technic. *Stain Technol.*, 28:97–100.
- Corliss, J. O. 1979. The Ciliated Protozoa: Characterization, Classification and Guide to the Literature. 2nd ed. Pergamon Press, New York.
- Couch, J. A. 1966. Two peritrichous ciliates from the gills of the blue crab. *Chesapeake Sci.*, **7**:171–176.
- Couch, J. A. 1973. Ultrastructural and protargol studies of Lagenophrys callinectes (Ciliophora: Peritrichida). J. Protozool., 20:638–647.
- Fernández-Galiano, G. & Tato-Porto, M. L. 2000. A review of the species of protozoan epibionts on crustaceans. I. Peritrich ciliates. *Crustaceana*, 73:643–683.
- Foissner, W., Berger, H. & Kohmann, F. 1992. Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. Band II: Peritrichia, Heterotrichida, Odontostomatida. *Informationsber. Bayer. Landesamt. Wasserwirtsch.*, 5/92:1–502.
- Foissner, W., Berger, H. & Schaumburg, J. 1999. Identification and ecology of limnetic plankton ciliates. *Informationsber. Bayer. Landesamt. Wasserwirtsch.*, 3/99:1–793.
- Foissner, W., Hoffman, G. L. & Mitchell, A. J. 1985. *Heteropolaria co-lisarum* Foissner & Schubert, 1977 (Protozoa: Epistylididae) of North American freshwater fishes. J. Fish Dis., 8:145–160.
- Gilbert, J. J. & Schröder, T. 2003. The ciliate epibiont *Epistylis pyg-maeum*: selection for zooplankton hosts, reproduction and effect on two rotifers. *Freshw. Biol.*, 48:878–893.
- Görtz, H. D. 1996. Symbiosis in ciliates. *In*: Hausmann, K. & Bradbury, P. C. (ed.), Ciliates Cells as Organisms. Gustav Fischer Verlag, New York. p. 441–462.
- Guillory, V., Perry, H. & VanderKooy, S. 2001. The blue crab fishery of the Gulf of Mexico, United States: a regional management plan. Gulf States Marine Fisheries Commission, Ocean Springs, MS.

- Guinea, A., Gil, R. & Fernández-Galiano, D. 1986. Ultrastructure de la frange aborale d'*Opisthonecta henneguyi* Fauré-Fremiet, 1906 (Ciliophora, Peritrichida). Acta Protozool., 25:15–22.
- Gutiérrez, J. C., Callejas, S., Borniquel, S., Benítez, L. & Martín-González, A. 2001. Ciliate cryptobiosis: a microbial strategy against environmental starvation. *Int. Microbiol.*, 4:151–157.
- Ji, D. & Song, W. 2004. Note on a new marine peritrichous ciliate (Ciliophora: Peritrichida), *Zoothamnopsis sinica* n. sp. from North China, with reconsideration of *Zoothamnium maximum* Song, 1986. Acta Protozool., 43:61–71.
- Kahl, A. 1935. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 4. Peritricha und Chonotricha. *In*: Dahl, F. (ed.), Die Tierwelt Deutschlands. Fischer, Jena, Germany. p. 651–886.
- Landers, S. C., Confusione, A. & Defee, D. 1996. Hyalophysa bradburyae sp. n., a new species of apostome ciliate from the grass shrimp Palaemonetes kadiakensis. Eur. J. Protistol., 32:372–379.
- Landers, S. C., Zimlich, M. A. & Coate, T. 1999. Variations in the ventral ciliature of the crustacean symbiont *Hyalophysa* (Ciliophora, Apostomatida) from Mobile Bay and Dauphin Island, Alabama. *Gulf Res. Rep.*, 11:57–63.
- Livingston, J. G. & Walker, G. K. 1992. Encystment in the stalked peritrich ciliate, *Epistylis rotans. Cytobios*, 71:201–209.
- Lynn, D. H. & Small, E. B. 2002. Phylum Ciliophora Doflein, 1901. *In*: Lee, J. J., Leedale, G. F. & Bradbury, P. (ed.), An Illustrated Guide to the Protozoa. Organisms Traditionally Referred to as Protozoa, or Newly Discovered Groups. 2nd ed. Society of Protozoologists, Lawrence, KS. p. 371–656.
- Ma, H., Choi, J. K. & Song, W. 2003. An improved silver carbonate impregnation for marine ciliated protozoa. Acta Protozool., 42:161–164.
- Martín-Cereceda, M., Serrano, S. & Guinea, A. 1999. Description of *Opisthonecta matiensis* n. sp. (Protozoa, Ciliophora), a new peritrich ciliate from wastewater. *J. Eukaryot. Microbiol.*, 46:283–289.
- Matthes, V. D. 1955. Eine neue *Epistylis*-Art aus der Adria. Zool. Anz., **154**:193–194.
- Mayén-Estrada, R. & Aladro-Lubel, M. A. 2001. Epibiont peritrichids (Ciliophora: Peritrichida: Epistylididae) on the crayfish *Cambarellus patzcuarensis* in Lake Pázcuaro, Michoacán, Mexico. J. Crust. Biol., 21:426–434.
- Messick, G. A. 1998. Diseases, parasites, and symbionts of blue crabs (*Callinectes sapidus*) dredged from Chesapeake Bay. J. Crust. Biol., 18:533–548.
- Messick, G. A. & Small, E. B. 1996. Mesanophrys chesapeakensis n. sp., a histophagous ciliate in the blue crab Callinectes sapidus, and associated histopathology. Invetebr. Biol., 115:1–12.
- Morado, J. F. & Small, E. B. 1995. Ciliate parasites and related diseases of Crustacea: a review. *Rev. Fish. Sci.*, 3:275–354.
- Nenninger, U. 1948. Die Peritrichen der Umgebung von Erlangen mit besonderer Berücksichtigung ihrer Wirtsspezifität. Zool. Jb. Syst., 77:69–266.
- Overstreet, R. M. 1978. Marine maladies? Worms, Germs, and other Symbionts from the Northern Gulf of Mexico. Blossman Printing Inc., Ocean Springs, MS.

- Overstreet, R. M. & Howse, H. D. 1977. Some parasites and diseases of estuarine fishes in polluted habitats of Mississippi. *Ann. N.Y. Acad. Sci.*, 298:427–462.
- Overstreet, R. M. & Whatley, E. C. Jr. 1975. Prevention of microsporidiosis in the blue crab, with notes on natural infections. *Proceedings of World Mariculture Society* (6th Annual Workshop). p. 335–345.
- Precht, H. 1935. Epizoen der Kieler Bucht. Nova Acta Leopold., 3: 405–474.
- Rustige, K. H. 1991. Eine Bestimmungshilfe f
 ür die epizoischen Ciliaten der einheimischen Gammariden. Ber. Naturwiss. Verein Bielefeld Umgegend, 32:263–290.
- Sawyer, T. K., MacLean, S. A. & Ziskowski, J. 1976. A report on *Ephelota* sp. (Ciliata, Suctorida) as an epibiont on the gills of decapod crustaceans. *Trans. Am. Microsc. Soc.*, **95**:712–717.
- Schödel, H. 1987. Seßhafte Wimpertiere (Peritricha, Chonotricha, Suctoria) auf Asellus aquaticus und Gammariden. Limnologica (Berlin), 18:83–166.
- Shields, J. D. & Overstreet, R. M. 2006. Chapter 8. Diseases, parasites, and other symbionts. *In*: Kennedy, V. S. & Cronin, L. E. (ed.), The Blue Crab, *Callinectes sapidus*. Maryland Sea Grant, p. 223–339. (in press).
- Solangi, M. A., Overstreet, R. M. & Gannam, A. L. 1979. A filamentous bacterium on the brine shrimp and its control. *Gulf Res. Rep.*, 6: 275–281.
- Song, W. 1986. Description of seven new species of peritrichs (Peritricha: Zoothamniidae, Epistylididae) on *Penaeus orientalis. Acta Zootaxon. Sin.*, 11:225–235. (in Chinese with English summary)
- Song, W. 1992. Contribution to the commensal ciliates on *Penaeus orientalis*. III. (Ciliophora, Peritrichida). J. Ocean Univ. Qingdao, 22: 107–117.
- Song, W. 2003. Ectocommensal peritrichs on the cultured shrimps. *In*: Song, W., Zhao, Y., Xu, K., Hu, X. & Gong, J. (ed.), Pathogenic Protozoa in Mariculture. Science Press, Beijing. p. 13–48.
- Song, W., AL Rasheid, K. A. S. & Hu, X. 2002. Notes on the poorlyknown marine peritrichous ciliate, *Zoothamnium plumula* Kahl, 1933 (Protozoa: Ciliophora), an ectocommensal organism from cultured scallops in Qingdao, China. *Acta Protozool.*, **41**:163–168.
- Stiller, J. 1941. Epizoische Peritrichen aus dem Balaton. Arb. Ungar. Biol. Szeged, **2**:211–223.
- Stiller, J. 1942. Einige Gewässer der Umgedung von Szeged und ihre Peritrichenfauna. Arch. Hydrobiol., 38:315–435.
- Walker, G. K., Edwards, C. A. & Suchard, S. J. 1989. Encystment in the peritrich ciliate *Telotrochidium henneguyi*. *Cytobios*, **59**:7–18.
- Walker, M. H. & Roberts, E. 1982. The protozoan epizooites found on the gills of *Gammarus pulex*. *Hydrobiologia*, 88:171–176.
- Wilbert, N. 1975. Eine verbesserte Technik der Protargolimprägnation für Ciliaten. *Mikrokosmos*, 64:171–179.

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