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First Record of *Lagenophrys dennisi* (Ciliophora: Peritrichia) on the Exoskeleton of Crayfish *Cambarellus patzcuarensis*

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ABSTRACT. *Lagenophrys dennisi,* a peritrich ciliate, was observed attached to the exoskeleton of the crayfish *Cambarellus patzcuarensis* in Lake Pátzcuaro, Michoacán, Mexico. *Lagenophrys dennisi* presents a hemispheroidal, suboval or oval lorica in dorsal view, the distinctive lorica aperture consists of a pair of lips highly arched, unthickened, and smooth. Comparison of morphometric characters of the ciliate with previous records is made. Structures such as a "V"-shaped lorica suture, collar ridges, and myoneme are proposed for species identification. An anterior crescentic thickening on the dorsal surface of the lorica was observed under the scanning electron microscope. Lagenophryids were associated with 11 of 13 body parts with antennules and rostrum showing the highest prevalence. *Lagenophrys dennisi* was also found attached to submerged glass slides. This study represents the first record of *L. dennisi* on *C. patzcuarensis* and the first record of its presence in Mexico.

Key Words. Distribution, ectocommensal, epibiont, morphology, occurrence, symbiosis.

PROTOZOAN epibionts may be found on diverse substrates including a variety of invertebrates. The crustacean exoskeleton provides a suitable attaching site; since most crustacea are mobile, a constant flow of water and nutrients across the exoskeleton supplies an optimal habitat for epibionts (Felgenhauer and Schram 1978). Species of the peritrich ciliate, *Lagenophrys,* have been described on a number of crustaceans. These sessile ciliates spend all but a short, dispersive phase of their lives in transparent, secreted loricae that attach only to the cuticle of crustacean hosts (Couch 1983). Lagenophryids are obligate ectocommensals of crustaceans (Clamp 1987, 1990a, 1991), restricted to certain species of hosts (Clamp 1973; Corliss and Brough 1965), are specific in their distribution over the exoskeleton (Clamp 1987, 1990b). Features of *Lagenophrys* that have the greatest utility as taxonomic characters (Clamp 1987) are shape and proportions of the lorica, structure and shape of the lips of the lorica aperture, and shape of the macronucleus and its position in the body. The pattern of kinetosome rows comprising the penicular infraciliature may be useful as a taxonomic character in *Lagenophrys,* though the pattern has been described in relatively few of its species (Clamp 1990a).

Fifty seven species of *Lagenophrys* have been described to date (Clamp 1991, 1992, 1994). *Lagenophrys dennisi* was found associated with decapods, *Orconectes illinoiensis, Cambarus bartonii bartonii,* and *C. chasmodactylus* in the United States (Clamp 1987). The present report constitutes the first record of *L. dennisi* associated with the crayfish, *Cambarellus patzcuarensis.*

MATERIALS AND METHODS

Crayfish, (*C. patzcuarensis*) inhabiting the bottom of Lake Pátzcuaro, Michoacán, Mexico (lat. 19° 32′ to 19° 41′ N, long. 101° 32′ to 101° 43′ W), were collected in the littoral zone at the Jarácuaro site with 5-mm mesh net and maintained in the laboratory in an aquarium with oxygen supply. For an artificial substrate, coated glass slides were submerged in the aquarium. A few decapods were fixed with 5% formaldehyde. Crayfish were dissected into 13 body parts (i.e. rostrum, antennules, antennae, scale, chela, carapace, mouthparts, pereiopods, pleopods, abdominal segments, telson, uropods, and gills) and observed under a light microscope. Permanent preparations of whole body parts or sections were made and stained either with Harris hematoxylin or protargol (Lee et al. 1985). For scanning electron microscopy (SEM) the material fixed in 1% glutaraldehyde was transferred to 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, critical point dried, and coated with carbon and gold. Measurements on stained specimens were taken at magnifications of 200, 400 and 1000×. The ratios between characters were calculated. The minimum, maximum, mean, mode, standard deviation, and coefficient of variation were also calculated. The "Z" test was used to assess differences between the previous data and present results on measurements of the length of lorica and width of lorica. The distribution of the lagenophryid peritrich over the crustacean exoskeleton was recorded for every decapod as well as its occurrence noted.

RESULTS

Morphometric analysis. *Lagenophrys dennisi* presents a hemispheroidal, suboval or oval lorica in dorsal view, both lips being highly arched, unthickened, smooth, without projections or indentations (Fig. 1–4), the lorica being 51.4–66.6 μm in length and 40.4–59.2 μm in width, and the ratio length of lorica/width of lorica being 1.0–1.28 μm. (Table 1). The macronucleus is elongate, extending from the approximate center of the body to the anterior part of the body (Fig. 2).

Lagenophrys dennisi loricae attached to crayfish, showed several features not described in the original description of the species (Clamp 1987). There was a fold in the lorica wall posteriad to the lorica aperture, here designated as a "V"-shaped suture, which extends from the anterior borders to the middle of the anterior half of the lorica (Fig. 5). One or two slightly curved ridges on the wall of the external collar of the lorica aperture were observed under SEM (Fig. 6). There was also a curved thickening or anterior crescentic thickening of the dorsal surface of the lorica, situated either below or slightly above the anterior lip, surrounding the anterior and lateral margins of the aperture, with the posterior edge lying close and under the collar (Fig. 6).

The myoneme at the edge of the peristomial lip serves to open and close the aperture of the lorica, and was found as a compact structure beneath the lower right-hand corner of the buccal cavity; it formed a thick structure which stained heavily with protargol (Fig. 4). The morphometry of this structure and the length and thickness of both lips are reported for the first time here. (Table 1).

The infundibular polykinetids showed the same pattern as the original description (Clamp 1987). Rows of P1 equal in length, ending at cytostome; rows of P2 equal in length, ending at distal curvature of P1. Peniculus 2 separated from P1 by wide gap. Peniculus 3 with two rows; row 1 slightly longer than row 2 (Fig. 7).

Distribution and occurrence of *L. dennisi* on the host. A

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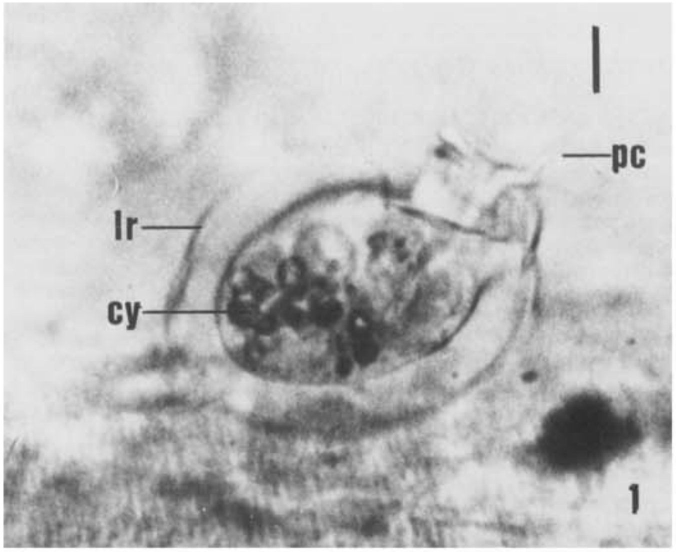
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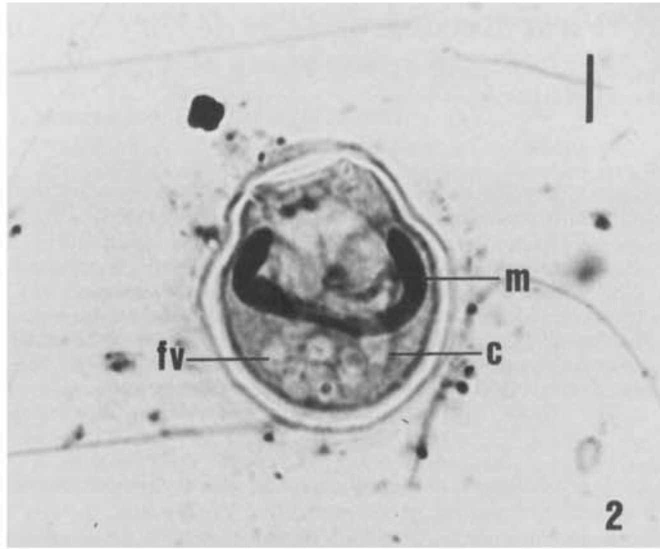
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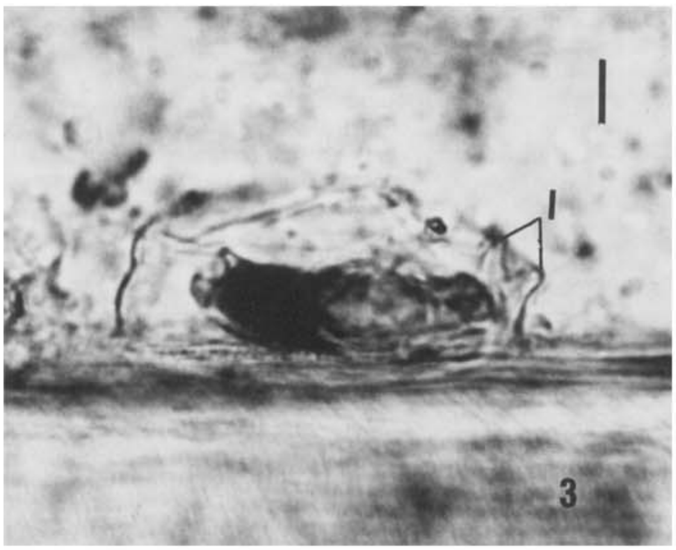
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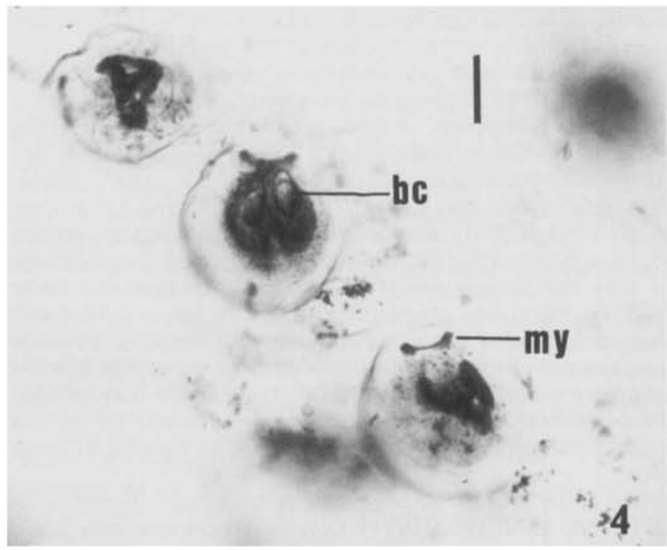
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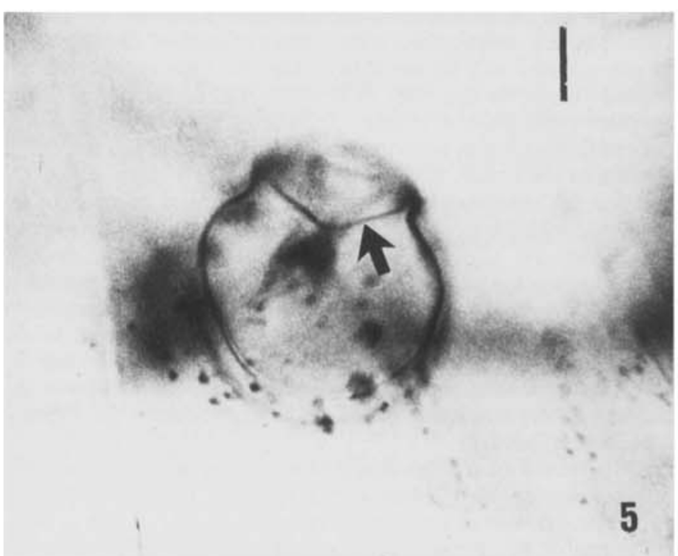
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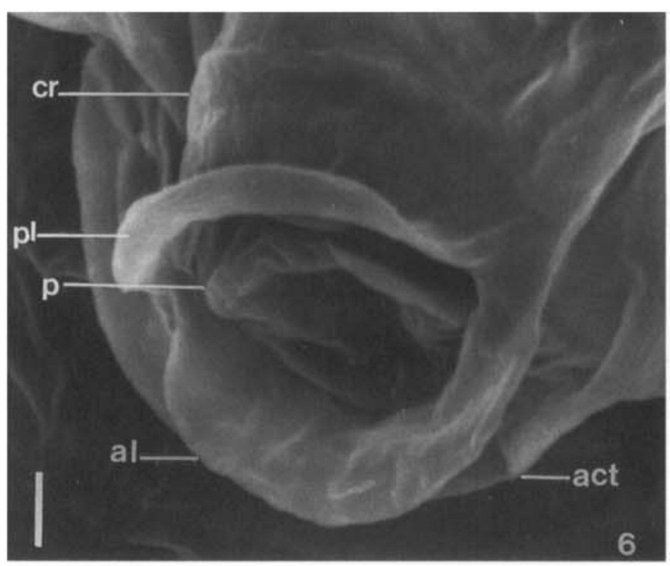
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Table 1. Measurements of *Lagenophrys dennisi*[a] (n = 89).

	Min	Max	M	Mo	SD	CV
Length of lorica[b]	51.4	66.6	60.3	62.3	3.84	6.37
Width of lorica[b]	40.4	59.2	53.4	55.05	3.25	6.07
Length of anterior lip of lorica	14.6	14.6	14.6	14.6	0	0
Thickness of anterior lip of lorica	1.8	1.8	1.8	1.8	0	0
Length of posterior lip of lorica	14.6	14.6	14.6	14.6	0	0
Thickness of posterior lip of lorica	1.8	1.8	1.8	1.8	0	0
Length of myoneme	17.5	18.5	18.4	18.5	0.2	1.1
Width of myoneme	1.7	3.6	3.5	3.65	0.38	10.86
Length of "V" suture	14.6	25.8	20.1	18.5	3.64	18.0
Width of "V" suture	31.5	37.0	33.1	33.1	1.5	4.52
Length of zooid	36.5	51.8	44.0	44.1	4.11	9.33
Width of zooid	25.8	44.4	36.5	36.5	4.14	11.34
Length of macronucleus	21.0	36.5	29.88	29.5	4.41	14.75
Width of macronucleus	6.0	10.9	7.8	7.3	1.65	21.09
Length of lorica/width of lorica	1.0	1.28	1.13	1.13	0.06	5.53
Length of "V" suture/width of "V" suture	0.44	0.81	0.61	0.55	0.12	20.66
Length of myoneme/width of myoneme	5.06	10.0	5.47	5.06	1.42	25.98
Length of macronucleus/width of macronucleus	1.92	5.51	4.03	4.04	0.9	22.4
Length of zooid/width of zooid	0.91	1.7	1.21	1.2	0.18	15.66

[a] Measurements in μm. CV, coefficient of variation (%); M, mean; Max, maximum; Min, minimum; Mo, mode; SD, standard deviation.
[b] By using "Z" test the results were Z = 10.9 ($p < 0.05$) for length of the lorica and Z = 13.3 ($p < 0.05$) for width of the lorica.

total of 65 decapods were sampled; of these, 24.6% had *L. dennisi* attached to host exoskeleton. The peritrich was found on all body parts except the chelae and the gills. Antennules had the prevalence (16.9%) whereas pleopods and abdominal segments had the lowest (3%) (Table 2). *Lagenophrys dennisi* was also observed attached to slides submerged in aquaria (Fig. 2).

DISCUSSION

Morphometry of *L. dennisi*. The major diagnostic feature for species identification of *Lagenophrys* is the morphology of the lips of the oral aperture. Other characteristics that may be utilized are shape and dimensions of the lorica. The individuals observed attached to the exoskeleton of *C. patzcuarensis* showed statistically significant differences in length and width of the lorica (Table 1) compared to *L. dennisi* recorded on decapods of the genera *Orconectes* and *Cambarus,* the lorica being 62.4–71.5 μm in length and 52.8–65.9 μm in width (Clamp 1987). Notwithstanding these differences between the data of the original description (n = 25) (Clamp 1987) and the present results (n = 89), it would be more convenient to perform this test with the ratio of lorica length/lorica width, but this was not possible due to the lack of a standard deviation of the first record (Clamp 1987). The shape and dimensions of the lorica do not show greater variations at the specific level, but differences of this type have been observed on *Setonophrys communis* [syn. *L. communis* and *L. latispina* (Clamp 1991)] in relation to the host genus (Kane 1965). Clamp (1988a), attributed intrapopulational variation in lorica size in *L. anticthos* to the different location on the gills and their differing success in capturing particles of food. In the cases of *L. aselli* and *L. patina,* intraespecific differences in lorica size were correlated with the symbiont's position on the host (Clamp 1988c, 1990a). Despite these differences in dimensions of the lorica, the individuals observed attached to *C. patzcuarensis* were confirmed as *L. dennisi* mainly on the basis of disposition of the lorica lip, its hemispheroidal, suboval or oval lorica, the elongated macronucleus extending from approximate center of the body to the anterior part of the body, and the disposition of infundibular polykinetids (rows of P1 equal in length, ending at cytostome; rows of P2 equal in length, ending at distal curvature of P1; peniculus 2 separated from P1 by wide gap; peniculus 3 with two rows, row 1 slightly longer than row 2), which have also been considered as major features of the species (Clamp 1987, 1990a; Corliss and Brough 1965; Kane 1965) (Fig. 2, 6–7).

The "V"-shaped suture, a ridge located immediately posteriad to the aperture, was observed on *L. dennisi* (Fig. 5), and should be considered as a new diagnostic feature of the lorica. Since Willis (1942) described the lips in *L. tattersalli* as situated in a depression of the lorica, which is bounded posteriorly by a curved rim or fold, ridges like this "V"-shaped suture have also been observed in other species within the genus, such as *L. aegleae* (Clamp 1988a). However, they have a different shape (i.e. a curved fold or ridge, immediately posteriad of the lorica aperture), and have not been used to distinguish the species.

The external collar of the lorica aperture is a tubular extension of the lorica leading to the lorica mouth (Kane 1965); it has not been observed in detail in all species. In *L. labiata* (Felgenhauer 1979) a deep indentation of the lorica wall pos-

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Fig. 1–6. Light (1–5) and scanning electron micrographs (6) of *Lagenophrys dennisi* attached to the crayfish *Cambarellus patzcuarensis*. **1.** Extended individual of *L. dennisi* attached to an antennule. cy = cytoplasm with food vacuoles, lr = lorica, pc = peristomial cilia. Live. Bar = 14 μm. **2.** Contracted individual attached to microscope slide showing cytopharynx (c), food vacuoles (fv), and elongate macronucleus (m). Harris hematoxylin. Bar = 15 μm. **3.** Lateral view of an individual attached to the carapace. Note both lips (l). Harris hematoxylin. Bar = 10.5 μm. **4.** Three specimens attached to a uropod. Note buccal ciliature (bc) of an individual in division. my = myoneme. Protargol. Bar = 22 μm. **5.** Lorica attached to carapace of host. Note "V"- shaped suture (arrow). Harris hematoxylin. Bar = 15 μm. **6.** Detail of the lips of the lorica aperture. Anterior crescentic thickening (act), anterior lip (al), curved thickened ridge (cr) on the dorsal wall of external collar of the lorica. Peristomial lip (p), posterior lip (pl). Bar = 2.8 μm.

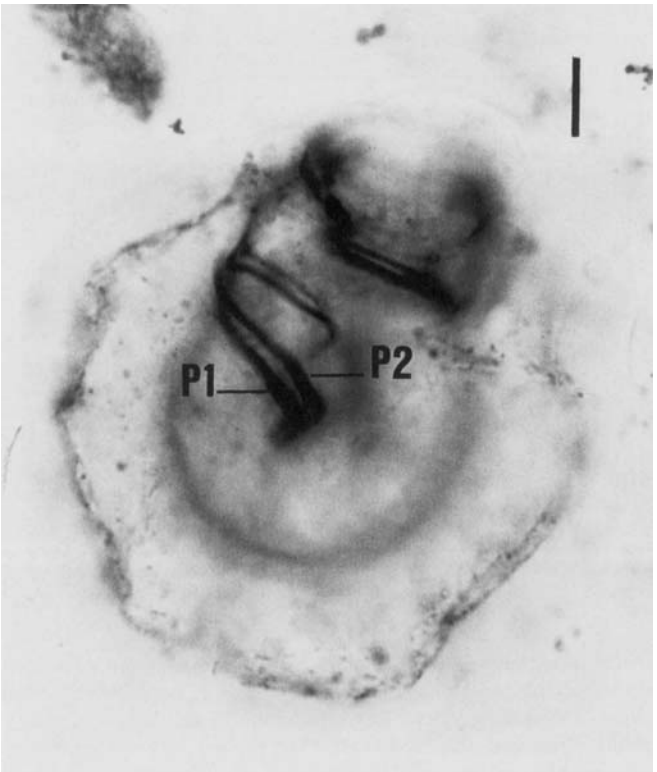

Fig. 7. Light micrograph of *Lagenophrys dennisi* showing the infundibular polykinetids. P1 = peniculus one, P2 = peniculus two. Protargol. Bar = 6.5 μm.

terior to the oral aperture, which is located on the collar, was described. This is the first time that these ridges have been observed on the external collar wall (Fig. 6) in *L. dennisi.*

The curved ridges on the wall of the external collar of the lorica aperture, and the dimensions and form of the myoneme also are features that could be considered as diagnostic characteristics.

The remarkable ridge anteriad to the lorica aperture (Fig. 6), a curved thickened ridge observed in our specimens, probably corresponds to the thickened anterior rim of the lorica of the original description of *L. dennisi* (Clamp 1987), and to the anterior crescentic bulge seen in other species of *Lagenophrys,* such as *L. anticthos* and *L. limnoriae* (Clamp 1988a, b).

Distribution and occurrence of *L. dennisi* on host. Examination of crayfish body parts revealed that this ectocommensal had no preference in establishing itself on certain regions of the host's body except for the chelae and gills: *L. dennisi* was attached to 11 of 13 body parts, and was distributed sparsely or abundantly. Its distribution on the host coincided with those exposed body parts reported previously (Clamp 1987), exclusive of gills or branchial chamber. This distribution has not been explained, but in other species it has been attributed to the form and function of the different appendages. *Lagenophrys lunatus* [according to Clamp (1989), junior subjective synonym of *L. eupagurus*] selects areas on or near the appendages where water movement over the exoskeleton is strongest but attaches to other areas of the exoskeleton when the preferred locations are filled (Clamp 1973). Thus, those appendages with the high rates of movement, which provide the most nourishment for the ciliates, are preferred sites for attachment. The degree of protection against predators offered by the appendage, which varies according to the presence or absence

Table 2. Occurrence of *Lagenophrys dennisi* on body parts of *Cambarellus patzcuarensis* (n = 65).

	Prevalence (%)
Rostrum	10.76
Antennules	16.92
Antennae	4.61
Scale	7.69
Carapace	9.23
Mouthparts	6.15
Pereiopods	6.15
Pleopods	3.07
Abdominal segments	3.07
Telson	4.61
Uropods	4.61

of projections such as setae, could also be a factor. Both factors could explain the distribution of *L. dennisi,* where the highest prevalence was found on antennules, the body parts with the strongest movement and numerous setae.

One well known characteristic of the genus *Lagenophrys* is the host specificity shown by its members (Clamp 1973); the degree of host specificity between species of *Lagenophrys* can be quite variable. Since nearly 300 species of cambarine and cambarelline crayfish are known from North and Central America, the likelihood of finding *L. dennisi* on additional hosts is very great (Clamp 1987). This is confirmed by our new host record: *C. patzcuarensis* belongs to the same superfamily as the hosts of the original records, is found within the limits of the reported biogeographical distribution, and has a freshwater habitat. According to Nenninger's scheme (Nenninger 1948), *L. dennisi* would be considered as a species that associates with members of different families (group II, d) with a high degree of specificity. However, its ability to colonize on slides shows that swarmers are not sensitive to substrate differences. The absence of substrate specificity occurs in other *Lagenophrys*: *L. lunatus* attached to coated slides, submerged in aquaria, where they developed to the trophont stage (Debaisieux 1959), and *L. aselli* settled on glass slides suspended in Mobile Bay, Alabama, USA (Jones 1974).

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LITERATURE CITED

Clamp, J. C. 1973. Observations on the host-symbiont relationships of *Lagenophrys lunatus* Imamura. *J. Protozool.,* **20**:558–561.

Clamp, J. C. 1987. Five new species of *Lagenophrys* (Ciliophora, Peritricha, Lagenophryidae) from the United States with observations on their developmental stages. *J. Protozool.,* **34**:382–392.

Clamp, J. C. 1988a. *Lagenophrys anticthos* n.sp. and *L. aegleae* Mouchet-Bennati, 1932 (Ciliophora, Peritricha, Lagenophryidae), ectocommensals of South American crustaceans. *J. Protozool.,* **35**:164–169.

Clamp, J. C. 1988b. A new species of *Lagenophrys* (Ciliophora: Peritrichia: Lagenophryidae) ectocommensal on the wood-boring isopod *Limnoria* Flabellifera:Limnoridae). *Trans. Amer. Microsc. Soc.,* **107**: 2–16.

Clamp, J. C. 1988c. The occurrence of *Lagenophrys aselli* (Ciliophora: Peritricha: Lagenophryidae) in North America and a description of environmentally-induced morphological variation in the species. *Trans. Amer. Microsc. Soc.*, **107**:17–27.

Clamp, J. C. 1989. Redescription of *Lagenophrys eupagurus* Kellicott (Ciliophora, Peritricha, Lagenophryidae) and a comparison of it with three similar species. *J. Protozool.*, **36**:596–607.

Clamp, J. C. 1990a. Redescription of three species of *Lagenophrys* (Ciliophora: Peritricha: Lagenophryidae) and a new North American species of *Lagenophrys* from hypogean amphipods. *Trans. Amer. Microsc. Soc.*, **109**:1–31.

Clamp, J. C. 1990b. A new species of *Lagenophrys* (Ciliophora: Peritrichia: Lagenophryidae) ectocommensal on North American species of *Gammarus* (Crustacea: Amphipoda). *Trans. Amer. Microsc. Soc.*, **109**:121–128.

Clamp, J. C. 1991. Revision of the family Lagenophryidae Bütschli, 1889 and description of the family Usconophryidae n. fam. (Ciliophora, Peritricha). *J. Protozool.*, **38**:355–377.

Clamp, J. C. 1992. Three new species of lagenophryid peritrichs (Ciliophora) ectocommensal on freshwater decapod crustaceans from Madagascar. *J. Protozool.*, **39**:732–740.

Clamp, J. C. 1994. New species of *Lagenophrys* (Ciliophora, Peritrichia) from New Zealand and Australia. *J. Eukaryot. Microbiol.*, **41**: 343–349.

Corliss, J. O. & Brough, I. M. 1965. A new species of *Lagenophrys* (Ciliata: Peritrichida) from the jamaican crab *Metopaulias depressus*. *Trans. Amer. Micros. Soc.*, **84**:73–80.

Couch, J. C. 1983. Diseases caused by Protozoa. *In*: Provenzano, A. J. (ed.), The biology of Crustacea. Vol. 6. Pathobiology, Academic Press, New York. p. 79–111.

Debaisieux, P. 1959. *Lagenophrys lunatus*, Ima. (Ciliate, Péritriche). *Cellule*, **59**:359–383.

Felgenhauer, B. E. 1979. A note on the scanning electron microscopy and hosts of the widespread peritrich ciliate *Lagenophrys labiata*. *Trans. Amer. Micros. Soc.*, **98**:591–595.

Felgenhauer, B. E. & Schram, F. R. 1978. Differential epibiont fouling in relation to grooming behavior in *Palaemonetes kadiakensis*. *Fieldiana Zoology*, **72**:83–100.

Jones, E. E. 1974. The Protozoa of Mobile Bay, Alabama. University of South Alabama Monographs, Vol. 1. University of South Alabama Press, Mobile.

Kane, J. R. 1965. The genus *Lagenophrys* Stein, 1852 (Ciliata, Peritricha) on australasian Parastacidae. *J. Protozool.*, **12**:109–122.

Lee, J. J., Small, E. B., Lynn, D. H. & Bovee, E. C. 1985. Some techniques for collecting, cultivating and observing Protozoa. *In*: Lee, J. J., Hutner, S. H. & Bovee, E. C. (ed.), An Illustrated Guide to the Protozoa, Society of Protozoologists, Allen Press, Lawrence, Kansas. p. 1–8.

Nenninger, U. 1948. Die Peritrichen der Umgebung von Erlangen mit besonderer Berücksichtigung ihrer Wirtsspezifität. *Zool. Jb.*, **77**:169–266.

Willis, A. G. 1942. Studies on *Lagenophrys tattersalli* sp.n. (Ciliata, Peritricha, Vorticellinae). Part I. Structure, asexual reproduction and metamorphosis. *Quart. J. Microsc. Sci.*, **83**:171–196.

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