EPIBIONT PERITRICHIDS (CILIOPHORA: PERITRICHIDA: EPISTYLIDIDAE) ON THE CRAYFISH *CAMBARELLUS PATZCUARENSIS* IN LAKE PÁTZCUARO, MICHOACÁN, MEXICO

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ABSTRACT

In this study, eight species of *Epistylis* were observed attached to the cuticular surface of 13 body parts of crayfish *Cambarellus patzcuarensis*. The species found were *E. bimarginata, E. branchiophila, E. carinogammari, E. gammari, E. lacustris, E. niagarae, E. stammeri,* and *E. variabilis*. Some morphological structures of the species observed with optical and scanning electron microscopy are presented. The distribution of each species over the exoskeleton, as well as the occurrence, was recorded for each decapod studied. Specific richness and *G* tests were calculated for all body parts. *Epistylis gammari* was the most widespread species on the surface of *C. patzcuarensis*, being attached to the 13 body parts studied. Seven body parts harboured eight species while the gills supported only one species. Epistylid species did not show any preference for a particular body region but rather utilized the substrate available, and every body part plays an important role as a substrate; this is consistent with the value obtained from the *G* test. Epistylid species are already considered as epibionts without host or body region specificity.

Sessile peritrichs are ciliate protozoans found attached to the exoskeleton of different crustacean hosts. According to Görtz (1996), such epibiotic ciliates are remarkably host specific and use specific sites on their hosts as their habitat niche. Although several members of the genus *Epistylis* Ehrenberg have been described as epibionts on various hosts, the descriptions were made several years ago without the aid of electron microscopy (Kahl, 1935; Nenninger, 1948; Stiller, 1971). However, taxonomic studies using electron microscopy have been made by Walker and Roberts (1982), and Foissner *et al.* (1992).

Epistylids belong to the peritrich ciliates, whose major characteristic is their colonial organization. The colony attaches to the substrate (exoskeleton) by a first-order non-contractile stalk, with zooids attached to the second- and third-order stalks. The features used for specific identification include the type of colonial ramification, the height of colony, and the number of zooids. Each zooid possesses a number of characteristics such as shape and dimensions, the shape of peristomial lip and epistomial disc, and also features of the pellicle such as striations and disposition of the buccal ciliature around the peristomial area and the infundibular region.

Very few papers deal with the distribution and occurrence of ciliates associated with freshwater crayfish in wild conditions (López-Ochoterena and Ochoa-Gasca, 1971; Matthes and Guhl, 1973; Lahser, 1976). The crayfish distributed in Mexico belong to the subfamilies Cambarinae and Cambarellinae, and attention in regard to their epibionts has only been given to those having commercial importance. Subfamily Cambarinae includes genera such as Cambarus Erichson, Orconectes Cope, and Procambarus Ortmann from which only Procambarus is present in Mexico. The subfamily Cambarellinae is confined to the United States and Mexico. Seventeen species are assigned to genus Cambarellus (Ortmann) Hobbs (Hobbs, 1991). The crayfish Cambarellus patzcuarensis Villalobos constitutes an important link in ecological webs, as it represents a considerable biomass for fish nutrition. No studies have been conducted concerning the occurrence and distribution of Epistylis epibiont species on C. patzcuarensis. The aim of the present paper is to provide morphological data of colonial organization of eight *Epistylis* species and their occurrence and distribution on C. patzcuarensis exoskeleton.

MATERIALS AND METHODS

Crayfish Cambarellus patzcuarensis were collected with 5-mm mesh net at two different sites of Lake Pátzcuaro, Michoacán, Mexico: Espíritu (with moderate organic pollution and better water circulation) and Jarácuaro (with higher organic pollution and low water circulation), (from 19°32'-19°41'N and from 101°32'-101°43'W) during 1990-1993 (Mayén-Estrada, 1997; Mayén-Estrada and Aladro-Lubel, 1998). The hosts were maintained in the laboratory for two weeks in aquaria containing unfiltered water of the study sites, with submerged plants, gently aerated at a temperature of 18-25°C. The hosts were dissected into 13 body parts (rostrum, antennules, antennae, scale, chela, carapace, mouthparts, pereiopods, pleopods, abdominal segments, telson, uropods, and gills). Living symbionts were observed under a light microscope using both bright field and phase contrast techniques. Permanent preparations of small pieces of exoskeleton bearing epistylids were made by fixing samples in 5% Formalin, which were then stained with Harris hematoxylin or protargol (Lee *et al.*, 1985; Foissner, 1991). In order to learn if the Epistylis species can colonize artificial substrates, glass slides were also submerged in the aquarium and stained with Harris hematoxylin. Morphometric characters were recorded from living and stained specimens. For scanning electron microscopy (SEM), material was fixed in 1% glutaraldehyde and transferred to 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, critical point dried, and coated with carbon and gold. The distribution of each species of Epistylis on the exoskeleton was recorded for each crayfish, and its occurrence was calculated, and the G test for independence was performed. Species richness was calculated for all body parts.

RESULTS

Eight species of *Epistylis* were found as epibionts on the exoskeleton of the freshwater crayfish *Cambarellus patzcuarensis*. The species were: *Epistylis bimarginata* Nenninger (Figs. 1–3); *E. branchiophila* Perty-Stein (Fig. 4); *E. carinogammari* Stiller (Figs. 5–7); *E. gammari* Precht (Figs. 8–10); *E. lacustris* Imhoff (Figs. 11–14); *E. niagarae* Kellicott (Fig. 15); *E. stammeri* Nenninger (Figs. 16–17); and *E. variabilis* Stiller (Figs. 18–20).

Morphometry of Species

The morphometric features for each species are shown in Table 1. The new data on zooid morphology include the pellicular striations viewed with SEM, and details of buccal ciliature revealed with protargol staining. Details of colonial organization are also included. For *E. bimarginata*, observations show a buccal ciliature consisting of a haplokinety and a three-component polykinety, both extending to the middle of the zooid, where P3 is shorter than P1 and P2 (Fig. 3). *Epistylis carinogammari* shows, in lateral view, the haplokinety and polykinety both forming a figure "8" before reaching the infundibulum, and P1 and P2 being longer than P3, with an evident fusiform space between them (Fig. 6). For E. gammari, 40–50 striations in the pellicle were observed (Fig. 10). The main colony stalk is dichotomously branched into old colonies, is short and broad, is in the form of a pedestal, with evident striations, and has lobulations on the basal disk (Figs. 8, 9). In young colonies of *E. lacustris*, the central stalk is short, and first-order branches are candlestick-shaped (Fig. 13). In older colonies, the central stalk is short; firstorder branches are longer, and second-order branches are short and broad; and the stalk is cup-shaped at the proximal region where the zooids attach (Fig. 12). Pellicular striae were also observed (Fig. 14). In *E. niagarae* the stalk is dichotomously branched, with longitudinal striations and a transversal septum, with the zooids implanted on the short second-order branches (Fig. 15). In E. stammeri, a dichotomously branched broad stalk was observed (Fig. 16). The P1 and P2 showed a conspicuous separation between the two. These run side by side, with a much shorter P3 joining them below. The haplokinety is accompanied by a germinal row of kinetosomes which is almost the same length as the infundibulum (Fig. 17). For *E. variabilis*, the haplokinety and polykinety divide at about a third of the ciliate, reaching opposite walls of the infundibulum. A germinal row appears where both kineties separate, and this runs alongside the haplokinety. The P1 and P2 run parallel, but with a space between them, and are much longer than P3. When seen in lateral view, they appear hook-shaped (Fig. 20).

Distribution and Occurrence of *Epistylis* Species on the Exoskeleton of the Host

The number of hosts examined at each site was 109 (Espíritu) and 65 (Jarácuaro). At Espíritu, 35.77% of the hosts did not have any attached epibiont species, 19.26% had only one species, and 44.9% had more than one species attached. At Jarácuaro, 30.76% of the decapods had no epibiont associated, 24.61% had one epibiont species, and 44.6% had more than one epibiont species on the exoskeleton.

Epistylis species were attached to between two and thirteen body parts (Table 2).

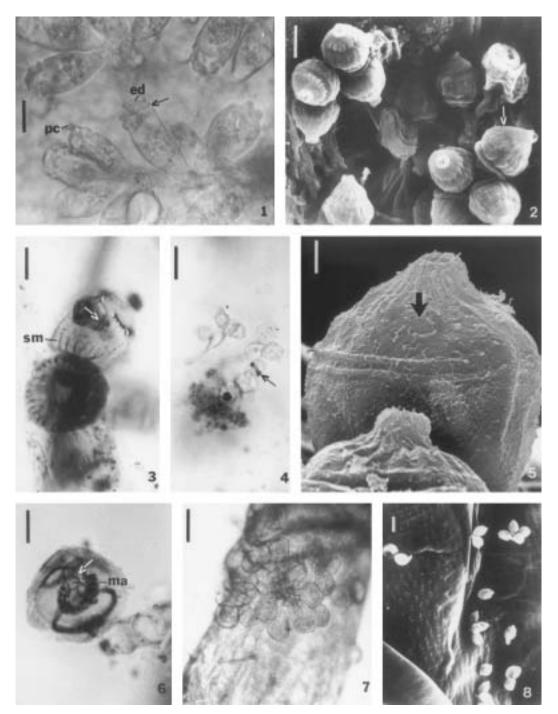


Fig. 1. Living individuals of *Epistylis bimarginata* attached to the uropod surface of the host. Zooids expanded with peristomial cilia (pc), epistomial disc (ed), and peristomial lip divided in two (arrow). Scale bar, 20 μ m. Fig. 2. *Epistylis bimarginata* colonies attached to the decapod uropod. Contracted zooids with pellicular striae (arrow). Scale bar, 20 μ m. Fig. 3. Contracted zooids of *Epistylis bimarginata* on uropods, stained with protargol, showing buccal ciliature (arrow) and somatic myoneme (sm). Scale bar, 20 μ m. Fig. 4. Colony of *Epistylis branchiophila* stained with Harris hematoxylin attached to the uropod showing macronucleus (arrow). Scale bar, 40 μ m. Fig. 5. Contracted zooids of *Epistylis carinogammari* attached to the rostrum of the host. Arrow points to the parallel pellicular striae. Scale bar, 7 μ m. Fig. 6. Zooid of *Epistylis carinogammari* impregnated with protargol. The oral ciliature is shown (arrow); macronucleus (ma). Scale bar, 20 μ m. Fig. 7. *Epistylis carinogammari in vivo*. Colony with contracted zooids on the pereiopod of decapod host. Scale bar, 30 μ m. Fig. 8. Various colonies of *Epistylis gammari* attached to the carapace. Scale bar, 20 μ m.

Species	Length of colony	Number of zooids	Length of zooid*	Width of zooid*	Length of macronucleus*	Width of peristome*
E. bimarginata	73–185	2-6	36.5-74	14.6–37	10.9–25.8	14.8-29.6
)	\bar{x} 99, SD 43.1		\bar{x} 54.3, SD 9.8	$ar{x}$ 24.3, SD 4.6	x 17.8, SD 3.5	\bar{x} 22.1, SD 5.8
	n = 24		n = 54	n = 54	n = 54	n = 24
E. branchiophila	66.6–251	20 - 30	18.5 - 51.4	14.8 - 47.7	14.6 - 22.2	I
	$ar{x}$ 133.2, SD 40		\bar{x} 29.5, SD 6.7	\bar{x} 23.3, SD 6.4	\bar{x} 17.5, SD 3.5	I
	n = 20		n = 55	n = 55	n = 55	I
E. carinogammari	55-182	4–22	44.4–88	29.6–66	22.2–37	37
0	\bar{x} 127.5, SD 32.3		\bar{x} 61.4, SD 10.4	\bar{x} 42.7, SD 6.8	\bar{x} 28.1, SD 3.7	\bar{x} 37, SD 0
	n = 22		n = 37	n = 37	n = 37	n = 22
E. gammari	40.4 - 148	2-4	25.8-74	10.9 - 51.8	14.6–37	18.5-22.2
0	\bar{x} 76.8, SD 27		\bar{x} 38.8, SD 8	\bar{x} 28.6, SD 8.1	\bar{x} 21.8, SD 5	\bar{x} 20.3, SD 1.8
	n = 40		n = 73	n = 73	n = 73	n = 23
E. lacustris	117-590	4-42	44.4–96.2	29.6–62.3	22.2–37	22.2–37
	x 297.6, SD 143.8		\bar{x} 63.6, SD 14.8	\bar{x} 45.4, SD 7	\bar{x} 32, SD 4.9	x 30.7, SD 6.2
	n = 18		n = 39	n = 39	n = 18	n = 10
E. niagarae	182.5 - 740	56	47.7–195.8	29.6–131.4	22.2–74	25-44.4
0	\bar{x} 411.9, SD 160		<i>x</i> 88.2, SD 37.1	\bar{x} 59.7, SD 28.1	x 37.4, SD 12.9	x 34, SD 7.3
	n = 10		n = 31	n = 31	n = 31	n = 0
E. stammeri	73-83.9	2-4	44.1 - 74	22.2-47.4	25.8–29.5	29.6
	\bar{x} 76.6, SD 5.1		<u>x</u> 59.4, SD 11.5	\bar{x} 36, SD 5.6	\bar{x} 28.2, SD 1.7	\bar{x} 29.6, SD 0
	n = 10		n = 14	n = 14	n = 14	n = 10
E. variabilis	127-450	2^{-20}	40.4–96.2	22.2-47	22.2–36.5	18.5–37
	\bar{x} 268.4, SD 108.8		\bar{x} 65.1, SD 17.4	\bar{x} 37.2, SD 5.7	\bar{x} 32.9, SD 4.6	\bar{x} 30.5, SD 7.5
	n = 10		n = 26	n = 26	n = 26	n = 10

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Table 2.Distribution and occurrence of *Epistylis* specieson decapod hosts.

	parts s	of body howing presence	To	
Species	Ε	J	Ε	J
			<i>n</i> = 109	<i>n</i> = 65
E. bimarginata	12	12	35.7	36.9
E. branchiophila	12	2	16.5	3.0
E. carinogammari	12	12	20.1	23.0
E. gammari	13	13	50.4	53.8
E. lacustris	12	3	11.0	4.6
E. niagarae	10	3	4.5	4.6
E. stammeri	10	11	10.0	6.1
E. variabilis	9	6	9.1	13.8

Table 3. Specific richness at host body parts.

	Number of species present		
Body region	Espíritu	Jarácuaro	
Rostrum	7	4	
Antennules	8	5	
Antennae	6	4	
Scale	8	7	
Carapace	8	5	
Chela	7	7	
Mouthparts	6	5	
Pereiopods	8	6	
Pleopods	7	4	
Abdominal segments	8	4	
Telson	8	4	
Uropods	8	6	
Gills	1	1	

E = Espíritu; J = Jarácuaro.

Epistylis branchiophila was associated with two crayfish body parts at the Jarácuaro site, and E. gammari was associated with 13 body parts at both sites, these representing the lowest and higher values respectively. At Espíritu, seven body parts (antennules, scale, carapace, pereiopods, abdominal segments, telson, and uropods) harboured the eight species of *Epistylis*, representing the highest species richness (Table 3). At the Jarácuaro site, the maximum number of epibiont species on the exoskeleton was seven on the scale and chela. In both cases, the body part to which only one species (E. gammari) attached was the gill, representing the lowest species richness (Table 3).

Concerning the occurrence of *Epistylis* species on the total of hosts examined, the greatest value was for *E. gammari*, associated with 50.4% of hosts at Espíritu and 53.8% of the hosts at Jarácuaro. The lowest total occurrence value recorded at Espíritu was that for *E. niagarae* (4.5%), while at Jarácuaro the lowest total occurrence recorded was for *E. branchiophila* (3%) (Table 2).

In relation to the preference for a particular body region the values obtained with the *G* test (3.2–13) fell below χ^2 (18.5 and 21; α 0.1 and 0.05) and therefore was not significant.

Only *E. variabilis* was observed attached to the glass slides submerged in the aquarium.

DISCUSSION

In this study, eight species of *Epistylis* were found attached to any of the 13 body parts of the decapod *C. patzcuarensis. Epistylis bimarginata* was previously recorded on leeches and submerged plants (Nenninger, 1948; Martínez-Murillo and Aladro-Lubel, 1996; Martínez-Murillo, 1997); *E. variabilis* was reported associated to *Epeorus* larva and trichoptera larva (Stiller, 1971; Foissner, 1979). Four species of *Epistylis (E. carinogammari, E. gammari, E. lacustris,* and *E. stammeri*) have been recorded as being associated with crustacean hosts (Nenninger, 1948; Stiller, 1949, 1971; Fenchel, 1965; Piezik, 1975; Foissner, 1979; Santhakumari and Nair, 1985), and only *E. branchiophila* and *E. niagarae* have been recorded on decapods (López-Ochoterena and Ochoa-Gasca, 1971; Matthes and Guhl 1973, 1974).

Although several studies using scanning electron microscopy (SEM) on sessile Epistylis have been carried out (Walker and Roberts, 1982; Foissner et al., 1992), the present paper is the first SEM documentation of five species of Epistylis (E. bimarginata, E. carinogammari, E. gammari, E. lacustris, and E. stammeri) attached to decapods. SEM studies on peritrich ciliates have not been generally undertaken, due to the fact that peritrich ciliates are highly contractile, and normal processing for SEM resulted in gross morphological distortion of the zooid and loss of surface features (Carey and Warren, 1983). In the case of *Epistylis*, we have used SEM to study colony organization and morphology of the zooids of the species. Our examination revealed features, such as the number of pellicular striations, that are important as diagnostic markers for the different species. Likewise, features revealed with standard staining techniques (hematoxylin and protargol) complement the knowledge of cytomorphology of the different species reported earlier (Kahl, 1935; Nenninger, 1948; Stiller, 1949, 1971).

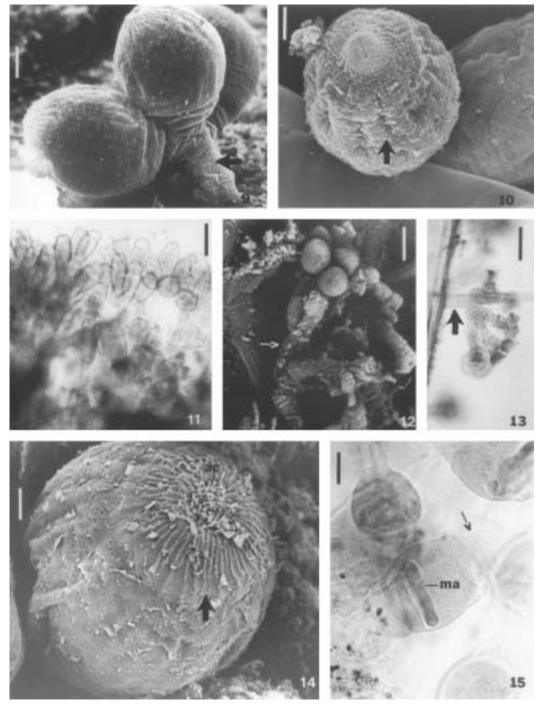


Fig. 9. *Epistylis gammari* colony with contracted zooids, attached to rostrum surface; broad stalk (arrow). Scale bar, 12 μ m. Fig. 10. The oral region of a contracted zooid of *Epistylis gammari* in which the peristomial cilia are completely withdrawn; arrow denotes conspicuous pellicular striae. Scale bar, 4 μ m. Fig. 11. Colonies of *Epistylis lacustris in vivo*, attached to the pereiopods of the host. Scale bar, 60 μ m. Fig. 12. Contracted zooids of *Epistylis lacustris* on carapace surface; arrow points to the stalk. Scale bar, 40 μ m. Fig. 13. Young colony of *Epistylis lacustris* associated to the surface of the pereiopod, arrow indicates the central stalk; the branches of first-order are candlestick shaped. Scale bar, 40 μ m. Fig. 14. The zooid surface of *Epistylis lacustris* attached to the carapace of the host; pellicular striae (arrow) and adoral cilia (ac) are shown. Scale bar, 4 μ m. Fig. 15. Partially expanded zooids of *Epistylis niagarae* stained with Harris hematoxylin; macronucleus (ma), flared scopular region (arrow). Scale bar, 20 μ m.

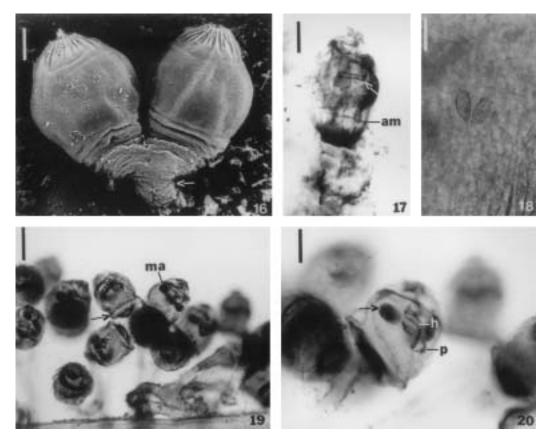


Fig. 16. Two zooids of *Epistylis stammeri* on the surface of the uropod. Arrow points to the broad stalk. Scale bar, 10 μ m. Fig. 17. Zooid of *Epistylis stammeri* attached to the uropod of the host, impregnated with protargol; aboral myoneme (am), arrow indicates oral ciliature. Scale bar, 15 μ m. Fig. 18. Living individuals of one colony of *Epistylis variabilis* attached to the telson of the decapod. Scale bar, 60 μ m. Fig. 19. Colonies of *Epistylis variabilis* impregnated with protargol. Macronucleus (ma); arrow points to myonemes. Scale bar, 30 μ m. Fig. 20. Detail of the oral ciliature of the zooid of *Epistylis variabilis* impregnated with protargol. Haplokinety (h), polykineties (p); arrow points to the macronucleus. Scale bar, 20 μ m.

All surfaces of the host body, except the gills, were covered with more than one epibiont species. In the majority of cases, the ciliates were evenly distributed over the cuticular surface of the different body parts that can be considered suitable for the attachment of the epibionts, but the shape and function of the different body parts to which ciliates attach can be taken into account when explaining specific richness.

On the scales, uropods, and telson, additional settlement was found on the setae, which may favour establishment of peritrichs, that function as areas of shelter from predators and may also act as traps where organic debris and bacteria accumulate. In the cases of pereiopods, antennules, and antennae, ciliates were observed even at the junction between segments. Settlement on these appendages also favoured a constant supply of food and oxygen. With respect to the gills, only one species was observed attached, possibly because the gill provides a feeble substrate without projections.

Careful examination of the communities of epistylids revealed that *E. gammari, E. bimarginata,* and *E. carinogammari* were the three most widespread species on the cuticular surface of crayfish (Table 2). There were very few cases in which colonies of the same or different species overlapped (*E. carinogammari* and *E. gammari*).

Although each species had the same opportunity to utilize the substrate at the same time, if the exoskeleton was crowded due to the presence of other species already attached, the larvae would settle on any other appendage found to be free of epibionts. This is consistent with the values obtained from the G test, which were not significant, demonstrating that species of *Epistylis* did not show any preference for a particular body region, but rather utilized the substrate available. Nevertheless, interactions such as competition are usual, so the suctorians Acineta tuberosa Ehrenberg, Podophrya sandi Collin, and Tokophrya quadripartita (Claparède and Lachmann) Bütschli, and the lagenophryid Lagenophrys dennisi Clamp were also observed attached to the crayfish body parts, seemingly contending for the substrate with epistylids (Mayén-Estrada and Aladro-Lubel, 1998, 2000). Furthermore, each species occupied different surface areas of the exoskeleton, due to their particular colonial organization and growth, according to the disposition of the zooids upon the stalk (large colonies will occupy a larger surface area, thus preventing the attachment of other colonies). Therefore, we observed small colonies with few zooids (E. bimarginata, E. gammari), others with a stick stalk (E. stam*meri*), and tall ones with numerous zooids (E. lacustris, E. niagarae, and E. variabilis), showing that the attachment potential was similar and that the determinant feature for epibiosis to occur was the availability of the resource and the time at which the epibiosis takes place.

According to Fernández-Leborans et al. (1997) some ciliate epibionts such as the chonotrich Chilodochona quennerstedti Wallengren found on *Liocarcinus depurator* Linnaeus are structurally and physiologically adapted to live as ectocommensals and confined to specific areas of the host due to host feeding habits. Species of Lagenophrys Stein have been documented both as site specific and host specific (Corliss and Brough, 1965; Clamp, 1973,1987,1990). This type of dependence cannot be applied to the species assemblage described here. Notwithstanding, E. gammari was observed attached only to antennae of Gammarus oceanicus Segerstrale, G. salinus Spooner, and G. zaddachi Sexton by Fenchel (1965) but had a generalized distribution (13 body parts) upon the exoskeleton in C. patzcuarensis.

Previous records and the present findings of the species of *Epistylis* suggest that they are epibionts and not confined to specific hosts or taxa and that they have a low degree of specificity. According to Nenninger's scheme (Nenninger, 1948) only *E. carinogammari, E. gammari*, and *E. stammeri* would be considered as species with a certain level of specificity (found only on animals of different orders in one class of crustacean hosts).

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