



First record of *Conidophrys pilisuctor* (Ciliophora: Pilisuctorida) as ectosymbiont of *Hyaella azteca* from Mexico

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Abstract

The pilisuctorid ciliate *Conidophrys pilisuctor* is recorded for the first time as ectosymbiont of the freshwater amphipod *Hyaella azteca* collected in Pátzcuaro Lake, Michoacán, Mexico. The morphometrical data of several phases of its life cycle (except free tomites) and of its prevalence on the host's body regions are presented. The pilisuctorid was observed attached exclusively to the setae of various appendages from which those of the pereopods and antennae had the greatest prevalences. However, the results of χ^2 tests show that there was not a homogeneous ciliate preference for the body regions of *H. azteca*. From the cluster analysis, several regions presented a greater degree of similarity according to prevalence and presence-absence of the ciliate. Some aspects are considered for the establishment of symbiosis.

Introduction

The ciliated protozoa show a wealth of special adaptations and interesting life strategies. The group includes free-living species that also may take part in a symbiosis either as hosts of smaller symbionts (mostly microorganisms) or as symbionts of larger hosts. The symbiotic associations between ciliates and several taxa of Metazoa, in particular the crustaceans, have been documented (Fenchel, 1965; Couch, 1983; Morado & Small, 1995; Fernández-Leborans & Tato-Porto, 2000a, b; Fernández-Leborans, 2001). Epibiotic communities on small crustacea are fantastic examples of the specific colonization of small and often closely adjacent habitat niches by extremely specialized organisms (Görtz, 1996). In Mexico, the study of symbiotic ciliates has been focused on three species of decapod crustaceans, *Penaeus setiferus*, *Cambarus patzcuarensis* and *C. montezumae*

zempoalensis (López-Ochoterena & Ochoa-Gasca, 1971; Mayén-Estrada & Aladro-Lubel, 1994, 1998, 2000, 2001, 2002).

The pilisuctorids have been regarded as aberrant ciliates with a feeding specialization: sucking up the contents of crustacean setae. They spend most of their lives perched on cuticular hairs of Crustacea, growing at the expense of their hosts. Their life cycle is dimorphic and interwoven with the molting cycle of the host. The trophont is long, slender, always completely motionless, without cilia, and surrounded by a substantial cyst wall (Chatton & Lwoff, 1936; Bradbury, 1975; Bradbury & Tyson, 1982). The described species of *Conidophrys* differ from one another in minor details of their structure and in their hosts (Bradbury, 1975).

Hyaella azteca Saussure is a small, epibenthic, detritus-feeding amphipod that lives in close contact with the sediments of ponds and freshwater

lakes throughout North America, Central America, and northern South America (Othman & Pascoe, 2001; González & Watling, 2002). Its common occurrence, together with a short life cycle and ease of culturing, make this amphipod an ideal laboratory organism for ecological studies, assessments of toxicity, and studies of host-parasite interactions (Othman & Pascoe, 2001; Kokkotis & McLaughlin, 2002).

According to Fernández-Leborans & Tato-Porto (2000a) and Morado & Small (1995), the only epibiont species of ciliated protozoa that have been recorded in association with *H. azteca* are *Lagenophrys patina*, *L. lenticula*, *Acineta fluviatus* and *Dendrocometes peragrinus*.

In order to contribute to the record of the pilisuctorid ciliate of the genus *Conidophrys*, the present study provides the morphometrical data of the ciliate and shows its distribution over the setae of the different body regions in a freshwater amphipod host.

Materials and methods

Amphipods (*Hyaella azteca*) inhabiting the bottom of Lake Pátzcuaro, Michoacán, Mexico (19° 32' to 19° 41' N, 101° 32' to 101° 43' W), were collected with a 5 mm mesh net during several months through the years 2000–2002 (Table 1). The amphipods were maintained in laboratory aquaria containing unfiltered water from the lake and submerged plants. Examinations of 584 individuals were subdivided into 12 body parts: head (H), antennae (A), mouth parts (MP), gnathopods (G), coxae (C), pereionites (PE), pleonites (PN), uronites (UN), pereopods (P), pleopods (PL), uropods (U) and telson (T). Living pilisuctorids were observed using light microscopy. After fixa-

tion, permanent preparations of whole body parts stained with Harris hematoxylin and protargol (Lee et al., 1985) were made to reveal major characteristics of the ciliates. For study under the scanning electron microscope (SEM), the material was fixed in 1% glutaraldehyde, 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 morphometrical data of living and stained specimens were taken, and the minimum, maximum, mean, and standard deviation were calculated. The prevalence of pilisuctorid ciliate for the host population and for each body region was calculated; the χ^2 test was performed to estimate the degree of dependence of the ciliate for the body part of the host and for the month of the year. The cluster analysis (Statistica Program V. 60) from the raw data was used to estimate the degree of similarity between body parts of the host in relation to the prevalence and presence-absence of the pilisuctorid in the samples where it was recorded.

Results

Conidophrys pilisuctor Chatton & Lwoff was identified according to the characters that distinguish the species of the genus, which are the shape of its macronucleus, the morphometry of the several phases of their life cycle, and the disposition of the infraciliature in the trophont. These characters are similar to those described by Chatton & Lwoff (1934, 1936).

The pilisuctorid was observed attached only to the setae in several body regions (Table 1, Figs 1–3) of 115 individuals (19.7%) of *Hyaella azteca* collected in the months of June 2001 and February 2002. The greatest prevalences were for the setae of

Table 1. Prevalence (% of inhabited individuals) of *Conidophrys pilisuctor* in the body regions of *Hyaella azteca*

Date	n	%	Body regions											
			H	A	MP	G	C	PE	PN	UN	P	PL	U	T
Jun-01	134	(74) 55.2	0	32.8	2.2	0	6.7	13.4	0	2.9	52.9	1.4	7.4	4.4
Feb-02	175	(41) 23.4	1.7	8.0	2.8	2.8	2.8	1.1	0	1.1	12.0	1.7	1.7	3.4

n = number of hosts; % = prevalence of *C. pilisuctor*, the number in parentheses corresponds to number of hosts. Individuals of January (n = 69) and November (n = 42) 2000, and February (n = 164) 2001, had no ciliates attached. Jun-01 = June 2001; Feb-02 = February 2002. Refer to text for abbreviations of body regions.

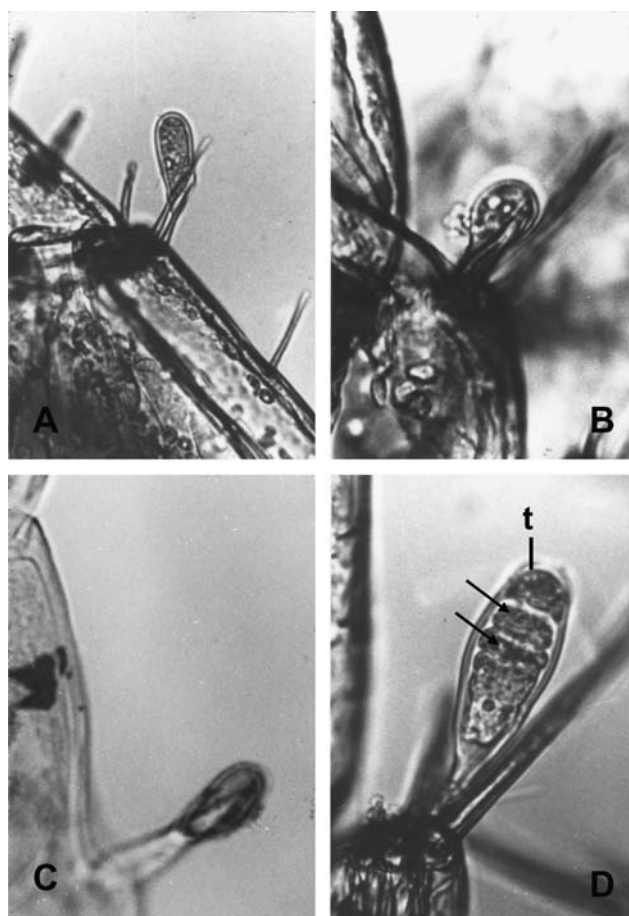


Figure 1. Life cycle phases of *Conidophrys pilisuctor*. (A) Young trophont *in vivo*, 590 \times . (B) Newly settled lacrymoid *in vivo*, 550 \times . (C) Spheroid phase stained with Harris hematoxylin, 690 \times . (D) Trophotomont *in vivo*. Two tomites (arrows) and a tomite before its releasing (t) are observed, 980 \times .

pereiopods (52.9%) and the antennae (32.8%) during June 2001. No pilisuctorids were attached to the pleonites (Table 1). All of the life cycle phases of the ciliate, except the free tomites phase, were observed. However, tomites were observed in the process of formation inside the trophotomont and just before release (Figs 1 and 3). The morphometrical data of the several life cycle phases of *C. pilisuctor* are shown in Table 2. The results of χ^2 test for June 2001 are, 375.29, $p < 0.05$, and for February 2002, $\chi^2 = 62.97$ ($p < 0.05$). The χ^2 test used to assess differences between the body parts with pilisuctorids attached exclusively was $\chi^2 = 239.72$, $p < 0.05$ (June 2001), $\chi^2 = 55.82$, $p < 0.05$ (February 2002), and $\chi^2 = 126.58$, $p < 0.05$ (June 2001 and February 2002). These results suggests that there is no homogeneous cil-

iate preference for the body regions of the hosts, which is consistent with the data of Table 1, where the highest prevalences were found on pereiopods (52.9%) and antennae (32.8%). Nevertheless, the pilisuctorid–amphipod relationship was maintained over all (except pleonites) the surface of exoskeleton on individuals collected during June 2001 and February 2002. However, there were qualitative differences as shown by the results of cluster analysis which could be related to the prevalence, presence–absence and the month of the year. These results are shown in Figure 4. The data resulting by comparing the prevalence of the ciliates on the different body regions of the amphipods show that the head–gnathopods, mouth parts–pleopods, and coxae–uropods (nested in cluster 1) clustered at the highest similarity and

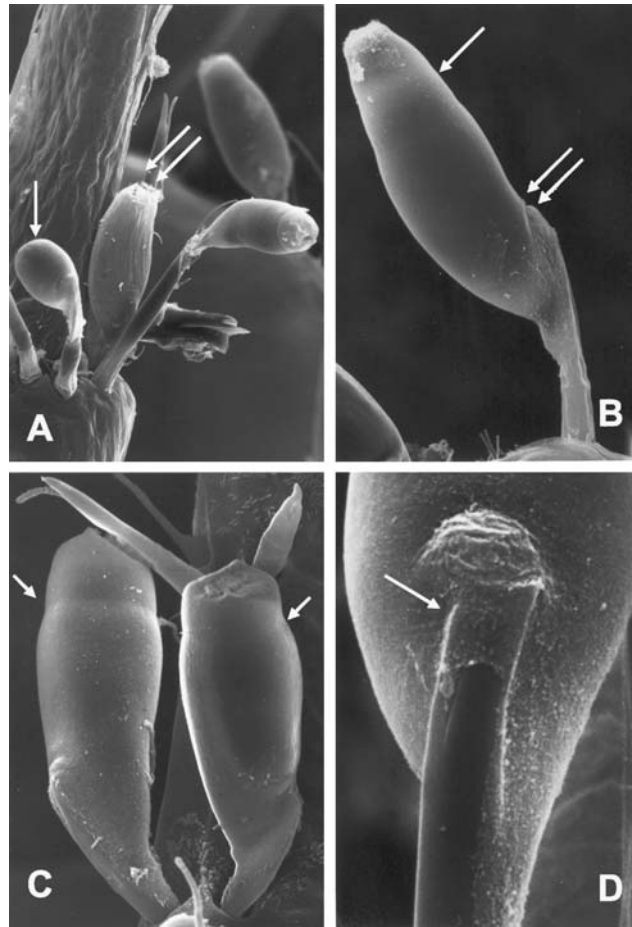


Figure 2. SEM micrographs of stages in the life cycle of *C. pilisuctor*. (A) Lacrymoid phase (arrow) and trophont (double arrow), 550 \times . (B, C) Trophotomonts. Arrows points to external ridges which corresponds to maturing tomites. Double arrow shows the site of the attachment to the seta, B = 1123 \times , C = 1300 \times . (D) Detail of the site of attachment (arrow) of the trophotomont to the seta of the host, 3100 \times .

that antennae–pereopods (cluster 2) clustered at a great dissimilarity (Fig. 4A). Taking into account the ciliate presence–absence on the host body parts, two clusters were obtained, from which antennae–mouth parts, coxae–pereionites, uro–nites–pereopods, and pleopods–uropods show the maximum degree of similarity (Fig. 4B).

Discussion

The species of the genus *Conidophrys* have been documented as ciliates with a high degree of specificity in relation to the host taxa (amphipods, isopods and decapods) and the body parts to which they attach (setae of crustaceans), (Brad-

bury, 1975). *Conidophrys pilisuctor* has been described previously only from marine amphipods and isopods (Chatton & Lwoff, 1934, 1936; Mohr & Leveque, 1948; Fenchel, 1965; Jones & Khan, 1970; Boshko & Dovgal, 2000; Ólafsdóttir & Svavarsson, 2002). The present record is the first for a freshwater host, thus enlarging the number of hosts and expanding the habitat of this species. Jones & Khan (1970) reported *C. pilisuctor* only in *Jaera ischiosetosa* individuals found under stones on the wet parts of the upper shore of an estuary, from which they concluded the preference of this ciliate for freshwater to brackish conditions, usually where water movement is minimal. Mohr & Leveque (1948) reported *C. pilisuctor* on the wood-boring isopod *Limnoria lignorum* and they con-

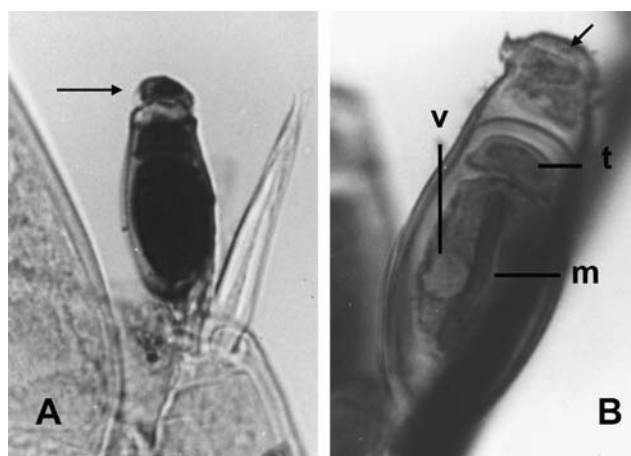


Figure 3. Trophotomonts of *C. pilisuctor*. (A) Harris hematoxilin technique. Tomite before its releasing (arrow), 620x. (B) Protargol technique. Serpentine macronucleus (m), contractile vacuole (v), tomite in formation (t) and a tomite formed but not yet released (arrow), 1180x.

Table 2. Morphometrical data of the life cycle phases of *Conidophrys pilisuctor**

Phase		N		Min		Max		Mean		SD	
		S	A	S	A	S	A	S	A	S	A
Lacrymoid	L	25	18	19.6	16.8	36.4	47.6	26.0	29.8	5.2	8.5
	W	25	18	8.4	11.2	16.8	25.2	12.7	16.2	3.0	4.3
Spheroid	L	10	–	11.2	–	22.4	–	16.8	–	2.9	–
	W	10	–	8.4	–	22.4	–	12.3	–	3.7	–
Trophont	L	52	16	22.4	40.6	75.6	64.4	47.7	54.6	13.3	7.6
	W	52	16	5.6	11.6	22.4	23.2	16.5	20.2	4.4	3.3
Trophotomont	L	25	10	50.4	61.6	78.4	75.4	60.0	69.3	5.7	4.6
	W	25	10	16.8	11.6	25.2	28	21.3	22.9	2.1	4.9
Tomite	L	61	12	5.5	5.6	42	50.4	16.7	22.3	11.8	18.3
	W	61	12	8.4	14	28	25.2	13.1	18.1	2.9	3.8

*Measurements in μm . N = number of individuals, Min = minimum, Max = maximum, SD = standard deviation, L = length, W = width, S = stained, A = alive.

cluded that because the host is cosmopolitan, *C. pilisuctor* may be presumed to have a wider distribution than the localities from which it has been reported. Moreover, for the crustacean isopods, this ciliate species has been recorded on three species of deep-water asellotes of the genus *Ilyarachna* (Ólafsdóttir & Svavarsson, 2002). The present findings support these ideas and demonstrate that the amphipod *H. azteca* serves as host for this ciliate species.

If the symbiotic ciliate gets its nourishment (haemolymph) through any seta, there would seem to be no great advantage to their being located in particular setae of body regions on their hosts, thus, its differential presence on the amphipod host may be caused by physico-chemical variations through the year or behaviour of the hosts, which also could explain the absence of the ciliate in some samples. This is consistent with the results of the study of Mayén-Estrada & Aladro-Lubel

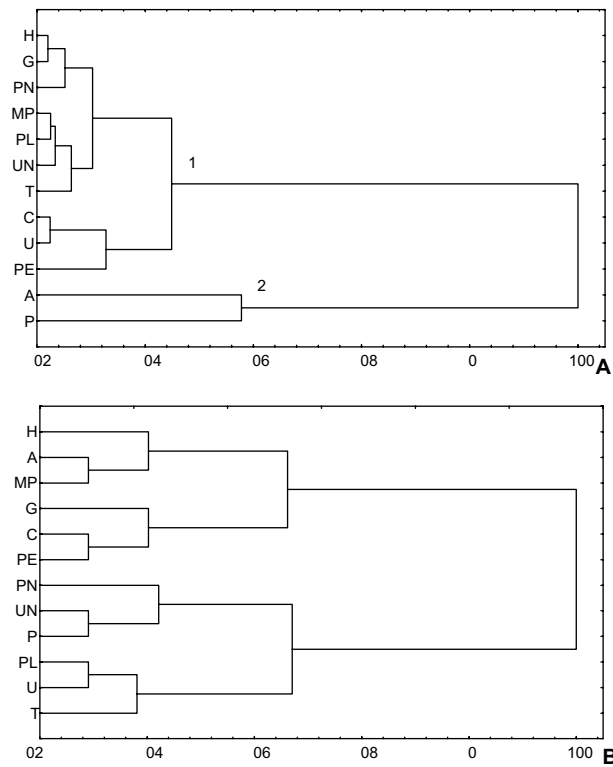


Figure 4. Phenogram of the cluster analysis showing the degree of similarity between body parts of *H. azteca*, according to prevalence (A) and presence-absence (B) of *C. pilisuctor*.

(1994), in which the highest prevalences of *C. pitelkae* were found on the pereopods of individuals of *Penaeus setiferus* of only one sample.

The pilisuctorid–amphipod symbiosis would be influenced by several factors, including reproduction and life cycle of the amphipod host (the mean time spent by a male and female in precopula is 1–7 days; the females are able to resume precopula after moulting in 4–12 days, and up to nine neonates are produced by a single female); the architectural diversity of the setae of the different appendages of the body regions, which creates many microhabitats providing a broad available surface where the ciliates could attach; and other particular characteristics of the pilisuctorid ciliates.

The morphological and physiological adaptations of pilisuctorian ciliates are closely related to their hosts: their life cycle is interwoven with the molting cycle of the host. Some of the most important adaptations are: liberation of the tom-

ites a few hours before the ecdysis of the amphipod; attaching rapidly to new hosts (which explains the high prevalence on the setae of pereopods in a host population); the cyst wall, which completely encloses the trophont, grows along with it, and attaches the ciliate to a seta on its host; and the direct connection to its host through the cyto-stome.

It is possible that greater symbiont infestation causes some damage to the crustacean population. According to Morado & Small (1995) only the scuticociliate *Mesanophrys pugettensis* and a few apostomes such as *Collinia* are capable of producing significant mortalities in the populations of wild crabs and amphipods, respectively. Indeed, limited data on the prevalence and distribution on these hosts suggest that ciliate infections may seriously impact crustacean populations.

At present, there are no references explaining the repercussion of *C. pilisuctor* on its hosts relating to damage caused. Moreover, for *C. pili-*

suctor, the available data are still scarce and, with the exception of the works of Boshko & Dovgal (2000), Ólafsdóttir & Svavarsson (2002), and Dovgal (2003a,b), are not updated.

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