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Systemic infection of freshwater crayfish *Cherax quadricarinatus* by hymenostome ciliates of the *Tetrahymena pyriformis* complex

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ABSTRACT: A survey of cultured freshwater crayfish *Cherax quadricarinatus* in north Queensland revealed systemic infections by hymenostome ciliates in moribund crayfish from one location. The ciliates were identified following protargol impregnation as belonging to the *Tetrahymena pyriformis* species complex on the basis of their somatic and oral ciliature and morphometric characteristics. Live ciliates were observed in the haemal sinuses of the gills browsing on tissue fragments. Histological examination revealed the ciliates to have invaded most organs and tissues, causing extensive necrosis particularly in the hepatopancreas and antennal gland. Lipid reserves were not depleted in the hepatopancreas, suggesting the rapid development of acute disease. This is the first record of systemic ciliate infections in freshwater decapods.

KEY WORDS: Decapoda · *Cherax quadricarinatus* · Ciliophora · *Tetrahymena pyriformis* · Morphology · Histopathology

INTRODUCTION

Systemic infections by ciliated protozoa have only occasionally been recorded in crustacean hosts, most involving small scuticociliates in marine decapods (cf. review by Morado & Small 1995). Several species of *Mesanophrys* (synonyms *Mugardia*, *Paranophrys* and *Anophrys*) have been described from crabs (Bang et al. 1972, Groliére & Leglise 1977, Sparks et al. 1982, Morado & Small 1994), one *Anophryoides* sp. from lobster (Cawthorn et al. 1996) and one *Parauronema* sp. from prawns (Couch 1978). In comparison, systemic infections by related hymenostome ciliates (including *Ichthyophthirius*, *Cryptocaryon*, *Tetrahymena* and *Uronema* spp.) occur more frequently in other aquatic hosts, particularly in fish and insect larvae (Elliott 1973, Lom & Dykova 1992). A variety of other ciliates have been recorded in association with aquatic hosts, predominantly as endozoic or ectocommensal organisms (Corliss 1979).

In the course of a disease survey of freshwater crayfish from commercial farms in north Queensland, systemic infections by hymenostome ciliates were detected in moribund *Cherax quadricarinatus*. This is the first record of a systemic ciliate in a freshwater decapod. This paper describes the morphological characteristics of the ciliate and the histopathological changes associated with infections.

MATERIALS AND METHODS

A survey for pathogens of cultured redclaw crayfish *Cherax quadricarinatus* was conducted in north Queensland in 1993, and the results of the virological and bacteriological investigations have been presented elsewhere (Edgerton et al. 1995). During the survey, systemic infections by ciliates were detected in 3 of 32 (9.4%) moribund crayfish from one location near Townsville. The crayfish exhibited weakened or failed tail-flick responses and were unable to right themselves when placed upside down. The crayfish

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were killed by severing the cephalothorax from the abdomen. The cephalothorax was fixed in Bouin's fluid, and histological sections of the internal organs and tissues were prepared and stained with haematoxylin and eosin (H&E) using routine procedures (Culling et al. 1985). Ciliates were detected in gill filaments of an additional moribund crayfish from the same farm by light microscopic examination of wet mounts counterstained with 0.2% toluidine blue. Infected gill filaments were fixed in Bouin's fluid, thoroughly washed in distilled water and the tissues teased apart to recover intact ciliates, which were then stained by protargol (silver proteinate) impregnation using standard techniques (Foissner 1991). Ciliates were examined by light microscopy, measured using a calibrated eye-piece graticule, drawn with the aid of a camera lucida and photographed in association with tissue lesions.

RESULTS

Live observation

Many ciliates were observed moving around in the haemal sinuses of the gills of the infected crayfish. The ciliates were variable in size, ranging from 30 to 75 µm in length and from 20 to 50 µm in width, but they were consistent in shape, being pyriform and slightly flattened anteriorly. The oral apparatus was located in a small subapical depression and the rest of the body was covered with short isokont cilia. The ciliates were granular in appearance due to the presence of numerous refractile vacuoles particularly in the posterior half of the body. A translucent contractile vacuole was also located in the posterior half of the body. The ciliates were highly motile and continually moved up and down the haemal sinuses while slowly rotating (predominantly clockwise) around their long axes. Individual ciliates were observed to feed on host tissues by circling around clumps of cells and ingesting small fragments as the cells disintegrated. The extent of their histophagous behaviour was evident when examining wet gill mounts over several hours. The ciliates readily consumed all the internal tissues, leaving only the outer cuticle. Cyst formation by the ciliates was not observed even when wet mounts became depleted of tissue or dried out.

Silver impregnation

Details of the oral and somatic ciliature were readily discerned in ciliates impregnated with protargol (Figs. 1 & 2). Their key morphometric characteristics are presented in Table 1. The ciliates contained a microstome oral apparatus consisting of a paroral membrane on the right and a tripartite adoral zone of membranelles on the left (Fig. 1). The somatic ciliature consisted of 20 to 26 longitudinal kineties arranged in meridional rows. All meridians extended to the anterior pole or the suture above the buccal apparatus except for 2 rows which only reached the posterior border of the buccal apparatus. By convention, the right postoral meridian is counted as the first kinety (K1). The ciliates possessed 1 to 2 contractile vacuole pores which were located posteriorly between kineties 5 and 6. All the somatic cilia were uniform in

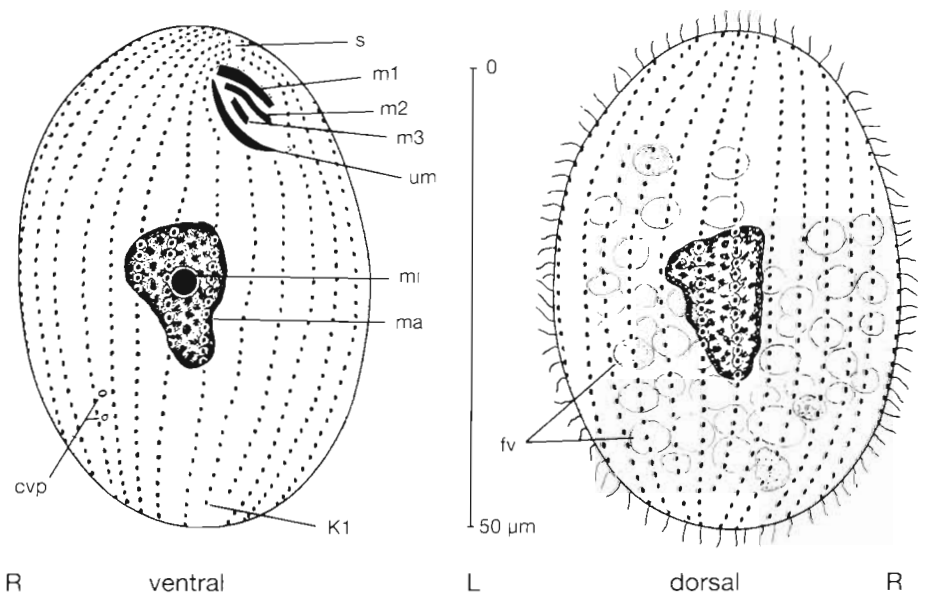


Fig. 1. Diagram of hymenostome ciliate belonging to *Tetrahymena pyriformis* species complex recovered from gills of freshwater crayfish *Cherax quadricarinatus*. Ventral and dorsal views of protargol impregnated specimens. Scale bar = 50 µm cvp: contractile vacuole pores; fv: food vacuoles; K1: first kinety = right postoral meridian; L: left side; ma: macronucleus; mi: micronucleus; m1, m2, m3: first, second and third adoral membranelles; R: right side; um: undulating membrane; s: preoral suture

Table 1 *Tetrahymena pyriformis*. Morphometric characterization of hymenostome ciliate recovered from tissues of freshwater crayfish *Cherax quadricarinatus*. $\bar{x}$: mean; SD: standard deviation; CV: coefficient of variation; n: number of observations

Character	$\bar{x}$	SD	CV	Minimum	Maximum	n
Body dimensions						
Length (µm)	52	7.9	15.2	38	65	10
Width (µm)	36	5.7	15.6	28	45	10
Nuclei						
Macronucleus length (µm)	13	2.4	17.6	10	16	10
Macronucleus width (µm)	10	1.5	15.1	8	12	10
Micronucleus diameter (µm)	2.6	0.45	17.1	2.0	3.2	10
Somatic ciliature						
Total number of kineties	24	1.6	7.0	20	26	10
Number of post-oral kineties	2	–	–	2	2	10
Length of first kinety, K1 (µm)	35	4.2	12.2	30	42	10
Number of basal bodies in K1	25	4.2	16.8	20	32	10
Oral ciliature						
Length of oral ciliary field (µm)	10.7	1.3	12.3	9	13	10
Width of oral ciliary field (µm)	5.5	0.6	11.3	4.8	6.5	10
Length of undulating membrane (µm)	8.2	0.7	8.1	7.0	8.9	10
Length of first membranelle, M1 (µm)	6.8	0.4	6.4	6.0	7.2	10
Length of second membranelle, M2 (µm)	6.0	0.4	6.3	5.2	6.5	10
Length of third membranelle, M3 (µm)	3.2	0.2	8.2	2.9	3.7	10

length and no elongate caudal cilium was detected. The ciliates contained an irregular ovoid to elliptical macronucleus located in the centre of the cell next to a single spherical micronucleus. On the basis of their morphological characteristics (summarized by Elliott 1973, Dragesco & Dragesco-Kernéis 1986), the ciliates were identified as belonging to the species *Tetrahymena pyriformis* (Ehrenberg 1830) Lwoff 1947

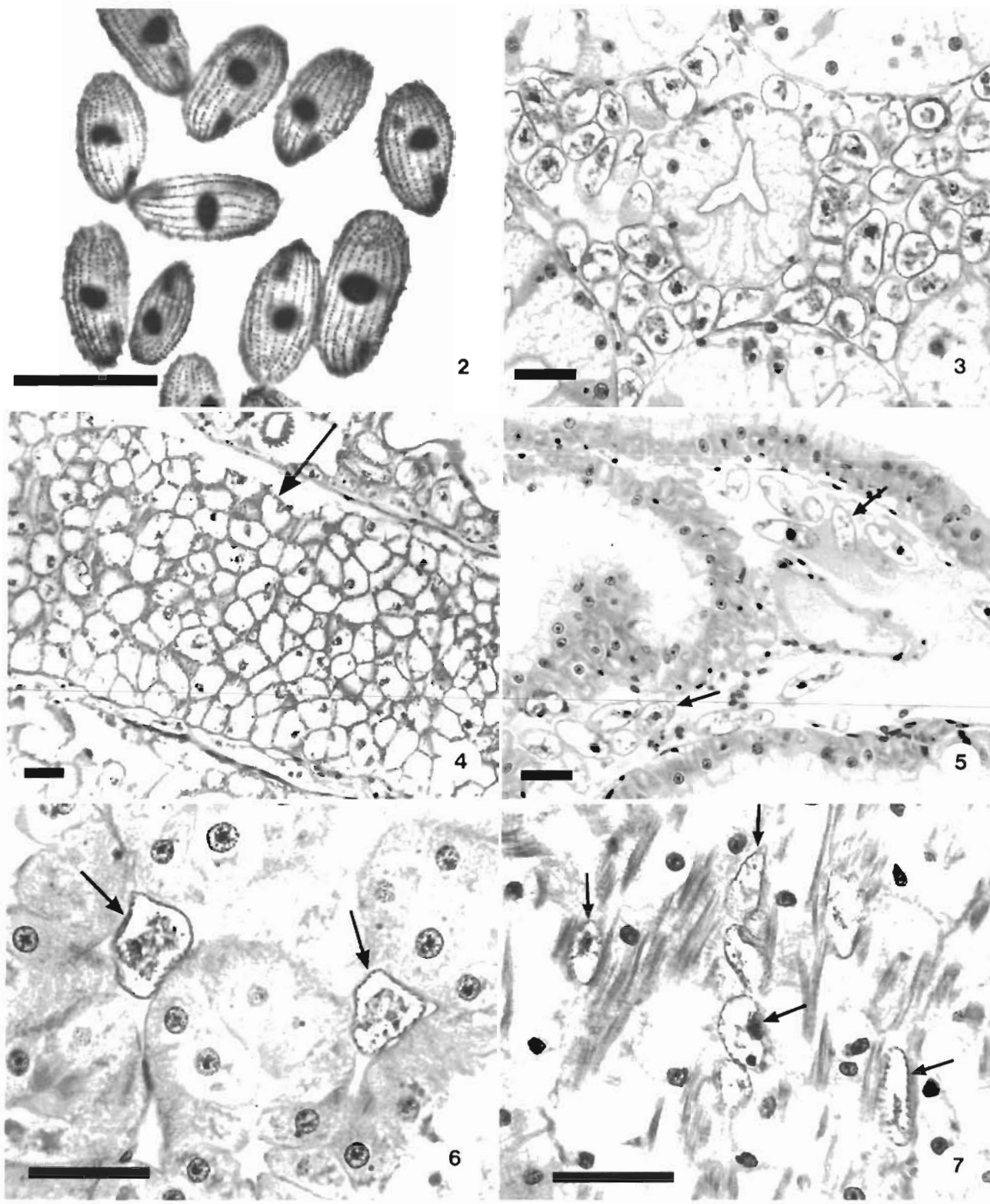
Histopathology

Of the 3 crayfish examined by histology and found to be systemically infected by the ciliate, 1 was intensely co-infected with *Psorospermium* sp. and had a severe bacteremia. The other 2 crayfish had mild co-infections with *Cherax quadricarinatus* bacilliform virus (= *Cherax* baculovirus) and a bacteremia (Edgerton et al. 1995). The ciliates were more numerous in the latter 2 crayfish.

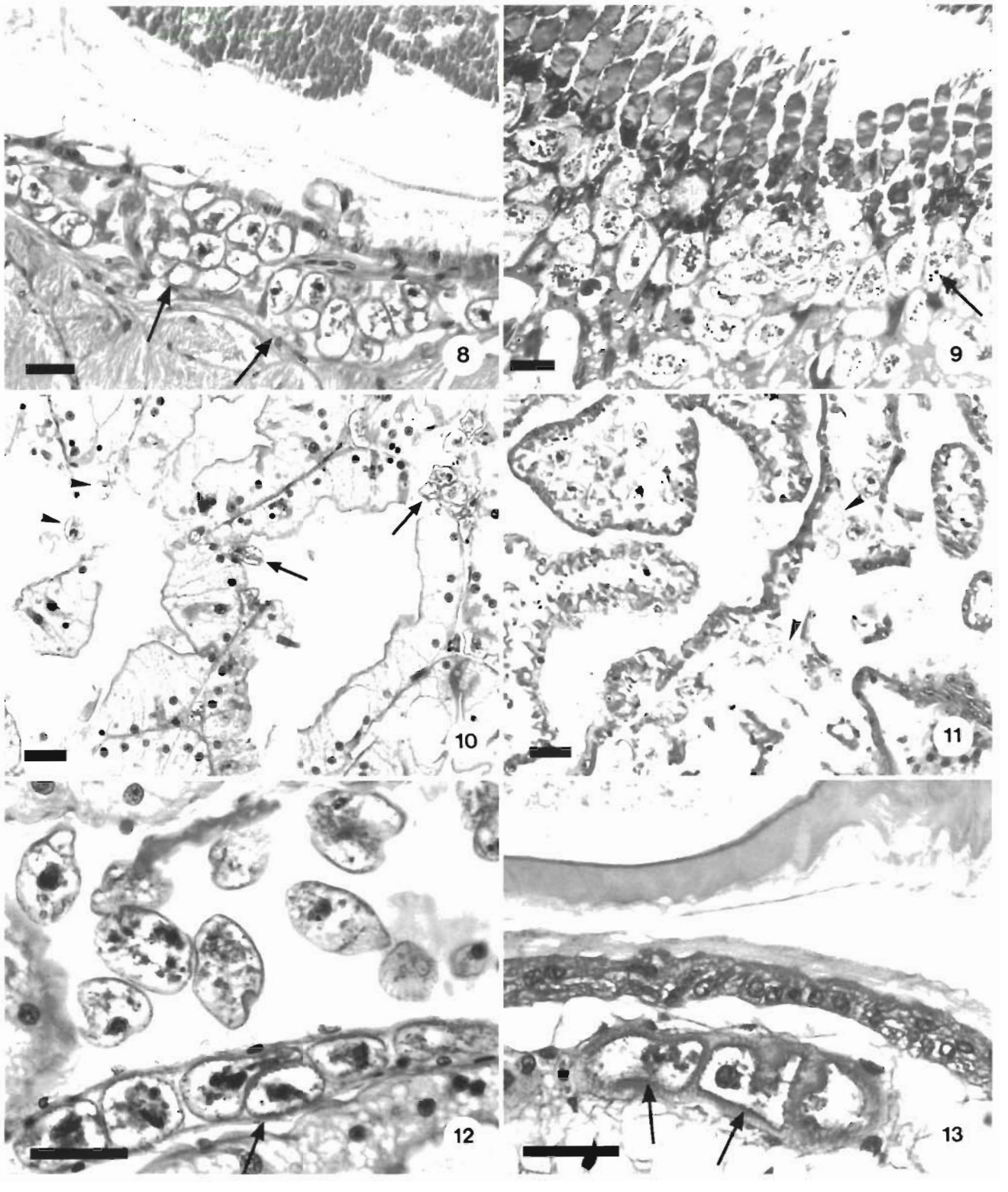
Ciliates were detected in histological sections of most organs and tissues from infected crayfish. They were recognized on the basis of their size, dense basophilic nuclei and prominent cell walls, which occasionally exhibited granular striations due to the presence of the somatic kineties (Figs. 3 to 7). Their cytoplasmic contents, however, were not well preserved and most sections of ciliates revealed extensive shrinkage artefacts and irregular aggregations of amorphous material. The majority of ciliates were detected in the haemocoel and haemal spaces within the tissues. They were frequently detected in the intertubular spaces of the hepatopancreas, often forming dense aggregates around tubules (Fig. 3). They were also commonly found in the main gill arches and were so densely packed in some instances that they filled the entire haemal sinus (Fig. 4). Heavy infections in the secondary lamellae often obscured any distinction between the afferent and efferent channels of the haemal sinus.

Ciliates were detected within the antennal gland, particularly in the large haemal sinuses surrounding the nephridial canal (Fig. 5), and occasionally in the haemal sinuses surrounding the labyrinth (Fig. 6), coelomosac and bladder. Numerous ciliates were found in the interstitial spaces and sometimes in the lumen of the myocardium (Fig. 7), but only rarely in the epicardium. They were frequently detected in the haemal sinuses between skeletal muscle bundles. Ciliates were found in the connective tissues surrounding the epithelium of the vas deferens (Fig. 8) and 1 organism was observed within the lumen of the vas deferens. Numerous ciliates were observed in the eye of 1 crayfish, and were most numerous in the retina at the base of the crystalline cones (Fig. 9), in the primary optic nerve region and in the lamina ganglionaris. Those organisms found in the retina contained dark granules similar to the proximal pigment in retinular cells (Johnson 1980).

Focal necrosis of tissues occurred in all infected crayfish. However, the necrosis was more extensive in those crayfish with less intense concomitant infections, particularly in the hepatopancreas (Fig. 10) and the nephridial canal of the antennal gland (Fig. 11). In



Figs. 2 to 7. *Tetrahymena pyriformis* from tissues of freshwater crayfish *Cherax quadricarinatus*. Scale bars = 50 µm. Fig. 2. Ciliates recovered from gill filaments. Protargol impregnation. Fig. 3. Section through cluster of ciliates in haemal spaces of hepatopancreas. H&E. Fig. 4. Numerous vacuolated ciliates packed within haemal sinus (arrow) of gill filament. H&E. Fig. 5. Ciliates (arrows) within haemal space surrounding the nephridial canal of antennal gland. H&E. Fig. 6. Two ciliates (arrows) in haemal space of labyrinth region of antennal gland. H&E. Fig. 7. Ciliates (arrows) located interstitially in myocardium. H&E



Figs. 8 to 13. *Tetrahymena pyriformis* from tissues of freshwater crayfish *Cherax quadricarinatus*. H&E. Scale bars = 50 µm. Fig. 8. Sections through ciliates (arrows) in connective tissue of vas deferens. Fig. 9. Ciliates in retina of eye, many containing dense black granules (arrow) similar to proximal pigment granules. Fig. 10. Necrotic area in hepatopancreas showing ciliates (arrows) in varying stages of penetration through epithelium. Fig. 11. Ciliates (arrowheads) located in necrotic area in nephridial canal of antennal gland. Fig. 12. Ciliates packed in haemolymph vessel (arrow) and lying free in haemocoel of hepatopancreas. Fig. 13. Ciliates (arrows) located in subcuticular connective tissue

these areas, the intima, endothelium and associated cells and connective tissue of the haemal system were diminished. Moreover, the ciliates had breached the hepatopancreatic tubule and nephridial canal and were in the lumen. Ciliates were more commonly seen in the haemolymph vessels of the crayfish with intense co-infections (Fig. 12). Ciliates invaded the connective tissues of various organs (Fig. 13). Circulating haemocytes were rare in the sections, and recent haemocytic whirling was not observed around the haemocytic nodules formed in response to the bacteraemias.

The lipid reserves in the hepatopancreas of the crayfish with minor concomitant infections were not depleted as numerous nutrient storage (R) cells containing lipid vacuoles were still present; nor was the hepatopancreas atrophied. These changes were, however, evident in the crayfish with acute concomitant infections. The exoskeletons of the infected crayfish were not soft or pale and there was no other evidence of recent ecdysis.

DISCUSSION

While many species of ciliated protozoa have been described as endozoic or ectocommensal organisms of aquatic hosts, few systemic infections have been recorded. Several hymenostome ciliates have been detected in the blood or internal organs of marine and freshwater fishes and various aquatic invertebrates, especially insect larvae (Elliott 1973). Systemic infections by 3 genera of scuticociliates have been described in crustacean hosts, all marine decapods, namely in crabs, lobster and prawns (Morado & Small 1995). The present study represents the first record of systemic infections by ciliated protozoa in a freshwater decapod.

The ciliates were clearly hymenostomes with well-defined oral and somatic ciliature, the former comprising an undulating membrane and 3 membranelles and the latter containing 2 discrete postoral meridians. No evidence was found of postoral thigmotactic areas, scutica or scutico-vestiges, which are characteristic of scuticociliates (Corliss 1979). Instead, their morphological characteristics were consistent with those of the genus *Tetrahymena*, in particular, those species belonging to the *T. pyriformis* complex (Elliott 1973, Corliss 1979, Dragesco & Dragesco-Kernéis 1986, Foissner et al. 1994). This complex comprises *T. pyriformis*, *T. setifera* and *T chironomi*, which are generally less than 60 µm in length, have fewer than 24 somatic meridians, possess spherical micronuclei and do not form cysts (Elliott 1973). They differ from species belonging to the *T. rostrata* complex (*T. rostrata*, *T. limacis*, *T. corlissi* and *T. stegomyiae*), which are typically greater than 60 µm in length, have more than 25 somatic meridians, possess ovoid micronuclei and do form cysts. These species have often been recorded as histophagous parasites but only in fish, amphibians, slugs and snails (Elliott 1973, Corliss 1979). The ciliates were also different from those of the *T. patula* complex (*T. patula*, *T. vorax* and *T. paravorax*), which are all free-living, greater than 100 µm in length and form distinct microstome and macrostome morphotypes, the latter having large cytopharyngeal pouches (Elliott 1973).

The ciliates detected in the crayfish had 20 to 26 somatic meridians, 2 contractile vacuole pores located between kineties 5 and 6 and they lacked a caudal cilium. Within the *Tetrahymena pyriformis* complex, these characters are similar to those of *T. pyriformis* although smaller free-living forms with as few as 15 meridians have been described (Elliott 1973, Dragesco & Dragesco-Kernéis 1986). They were different from those of *T. setifera*, which has a caudal cilium and 2 contractile vacuole pores located between kineties 8 and 9. They were also different from *T. chironomi*, which has 23 to 28 meridians, 2 contractile vacuole pores located between kineties 6 and 9 and has only been found in chironomid larvae (Elliott 1973, Dragesco & Dragesco-Kernéis 1986). *T. pyriformis* has been recorded throughout the world as a free-living organism commonly found in aquatic and terrestrial habitats ranging from freshwater ponds and streams to salt marshes and soils (Elliott 1973). However, it has also been found to be parasitic in the tissues of various vertebrate and invertebrate hosts. Infections have been reported in a variety of freshwater fish from Asia, Europe and North America (Elliott 1973, Hoffman 1978, Shulman 1984). Most infections have been confined to surface tissues and associated with skin lesions, raised scales, epidermal sloughing and extensive necrosis of the underlying musculature sometimes accompanied by neutrophil infiltration (Hoffman 1978). Systemic infections by *T. pyriformis* have only occasionally been detected in fish in association with moderate to extensive necrosis of various internal organs (Shulman 1984). More often, similar clinical and pathological signs have been associated with infections by *T. corlissi* and *T. rostrata*-like organisms in both marine and freshwater fish (Elliott 1973, Ferguson et al. 1987).

Despite the strong histophagous tendencies demonstrated by several *Tetrahymena* spp., they are considered to be facultative parasites with infections being accidental or opportunistic in nature. Ciliates are thought to gain entry to the host tissues through lesions or injuries in the external surfaces of the host (Elliott 1973). The portal of entry for ciliates into the crayfish is not known but most moribund crayfish

were missing some appendages and many had small abrasions and cracks in their exoskeletons. Nevertheless, detailed experimental transmission studies are required to establish the actual route of infection. Previous attempts to infect the American freshwater crayfish *Cambarus* sp. with 5 different *Tetrahymena* spp. by inoculation into the haemocoel, the alimentary tract and into artificial wounds were unsuccessful (Thompson 1958).

Once within host tissues, the ciliates were actively histophagous but the actual mechanisms used to break apart host cells are not known. It has been suggested that ciliary action and extracellular lysosomes provide both mechanical and chemical means for disrupting tissues and cells (Armstrong et al. 1981). All systemic ciliates detected in crustaceans possess small subapical mouthparts and their oral cilia are used to sweep small fragments to the cytostome rather than to actively break apart cells. The role of the somatic ciliature in feeding processes is not known but many ciliates appeared to repeatedly probe clumps of cells with their anterior cilia. Further studies are required to determine the mechanisms by which histophagous ciliates disrupt host tissues and destroy cells. The ciliates were observed in various tissues throughout the crayfish and were often associated with extensive necrosis in several organs, particularly the hepatopancreas. Haemocytopenia has been reported to be characteristic of most systemic infections by scuticociliates in crustaceans (Bang et al. 1972, Sparks et al. 1982, Cawthorn et al. 1996) although lesions in other tissues have been described (Armstrong et al. 1981, Sparks et al. 1982). The ciliate infections in the crayfish were not considered to be long-standing (chronic or latent) as the hepatopancrea of the crayfish with only minor concomitant infections were not depleted of lipid reserves and there were no indications of organ atrophy. These changes in the 1 other crayfish were almost certainly a result of the acute concomitant infections. These findings suggest the recent acquisition of *Tetrahymena pyriformis* infections by the crayfish and the rapid development of acute clinical disease. Even if infections are opportunistic, the ciliates must be regarded as potential pathogens of freshwater crayfish. Their impact on both wild and cultured crayfish populations remains to be determined by future surveys and disease surveillance programs.

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