

SESSILINE PERITRICH SYMBIONTS OF FRESHWATER CRUSTACEAN HOSTS

by

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Most planktonic organisms feed on phytoplankton, making them the principal link between phytoplankton and higher trophic levels in almost every aquatic food chain. A major part of the diet of many aquatic animals is comprised of copepods and other planktonic organisms, as it is such a rich source of protein (Rupert & Barnes 1994). If symbionts associated with planktonic organisms cause any pathogenic harm to the hosts, it could cause the total collapse of aquatic food webs, due to the significant role of planktonic organisms in the feeding habits of aquatic communities.

Freshwater copepods in southern Africa occupy a wide variety of habitats, including open waters of large impoundments, a wide range of temporary water bodies, backwaters of rivers, marshy areas and coastal lakes. Copepods do not occur in the main flow of rivers and have not been recorded from subterranean waters, in spite of the association with river systems (Rayner 2001). Within the group Cyclopoida there are several parasitic copepods, while the Calanoida and Harpacticoida do not contain any parasitic forms. The majority of copepods form part of the animal component of plankton.

Ciliophorans are found in all moist habitats, are generally cosmopolitan in their distribution and may be free-living or symbiotic. Ciliophorans are extremely common and frequently numerous. It is rare to find a sample of natural water without some ciliophorans being present. Phenomenal adaptations to a wide variety of ecological niches have evolved, a great many of which resulted in parasitic or other symbiotic associations (Schmidt & Roberts 1977).

Epibiosis is a facultative association of two organisms: the epibiont, an organism that lives attached to the body surface of another organism, whereafter it disperses to other organisms or habitats and the basibiont, the host organism (Wahl 1989). The epibiotic life style is apparent to the casual observer in the form of barnacles attached to whales. Equally conspicuous, on a smaller size scale, are epibionts such as bacteria, algae and protozoans (e.g. ciliophorans), that use crustacean zooplankton as a substrate organism (Green 1974).

Epistylids belong to the peritrich ciliophorans, whose major characteristic is their colonial organisation. The colony attaches to the substrate 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 the colony and the number of zooids.

In the present study, emphasis was placed on *Epistylis* Ehrenberg, 1830 species found associated with freshwater planktonic cyclopoid copepods, collected mainly from the Loch Logan Waterfront in Bloemfontein. During the course of this study, introduced freshwater crayfish of the genus *Cherax* Erichson, 1846 was brought to the laboratory from a commercial crayfish farm, outside Bloemfontein. Epistylids were noted on the antennae and carapace of the crayfish and the question immediately arose whether the epistylids are indigenous to South Africa, whether it has been reported from Africa before or if it was introduced along with the crayfish.

Against this background the present study was undertaken with the following specific objectives:

1. to examine the copepod composition of plankton samples and to identify relevant species using available keys.
2. to study any sessiline ciliophorans associated with crustaceans, in various natural and man made water systems and if found, taxonomically describe such species.
3. to study the effect of epistylid symbionts on an introduced freshwater crayfish.
4. to obtain an understanding of the different host/symbiont associations in both cases.

The layout of this dissertation is as follows: **Chapter 2** explains the materials and methods used during field and laboratory work, in order to collect and fix material. **Chapter 3** is the literature overview on the genus *Epistylis*, including the taxonomic history and position of this cosmopolitan epibiont, as well as a summary of *Epistylis* species associated with crustacean hosts and the general biology of *Epistylis*. In **Chapter 4** the focus is placed on the hosts (both planktonic copepods and crayfish species) including the identification of the hosts, composition of the plankton samples, as well as the influence of introduced crayfish on our natural water systems. Statistical data, morphological descriptions of the *Epistylis* species associated with *Eucyclops* Claus, 1893 sp. and *Cherax destructor* Clark, 1936 and a compendium of known euplanktonic *Epistylis* species follows in **Chapter 5**. **Chapter 6** provides a general discussion on the present study and **Chapter 7** contains the literature referred to in this manuscript, followed by the abstract and acknowledgements.

2.1 Collection Localities

Plankton samples were collected at different localities including the Loch Logan Waterfront, Botanical Gardens and Sewerage Farm, in the vicinity of Bloemfontein from February 2002 until September 2003. A freshwater crayfish farm just outside Bloemfontein was also visited to collect external parasites from two introduced crayfish species; *Cherax destructor* and *Cherax quadricarinatus* Von Martens, 1868.

Although other collection localities included once off collections at the Krugersdrif Dam, Pony Club, Soetdoring Nature Reserve, Botanical Gardens and Sewerage Farm, no ciliophoran infestations were found in these localities. Emphasis will therefore be placed on Loch Logan and the crayfish farm.

2.1.1 Loch Logan Waterfront

Loch Logan (Figs 2.1; 2.2 A - F) was built in one of the channels of Bloemspruit, in the Westdene area near the city centre Bloemfontein. This channel feeds Loch Logan with runoff water collected from the urban areas it runs through. The canal enters Loch Logan at its north-western side, opposite the impoundment wall. Eventually Bloemspruit flows into the Renosterspruit (about 12 km downstream from Loch Logan), which ends up in the Modder River, just outside of Bloemfontein (Vos 2002).

Loch Logan's grid reference is 29°06'50.7"S and 26°12'30.3"E and has a volume of about 95 000 m³, an area of approximately 4.2 ha, excluding the island and a mean depth of 2.26 m. There is an island in the middle of Loch Logan that divides the water mass in almost two equally sized arms. It is located in a summer rainfall area, which receives between 500-700 mm per annum, half of which is due to thunderstorms. This rain is runoff/storm water that is canalised to Loch Logan. Waste flushes into Loch Logan with rainstorms and contribute to algal blooms (Figs 2.2 D, E & F) when organic decomposed materials and inorganic nutrients are released into the water (Vos 2002).

In 1997 a shopping complex was developed on the banks of Loch Logan consisting of several shops, restaurants, pubs, a movie theatre as well as a gymnasium on the eastern bank. On the island there are Barbecue areas (Fig 2.2 A) and a performance stage. Human and commercial activities at the waterfront also contribute to the pollution of Loch Logan. When comparing digital images of Loch Logan Waterfront taken in November 2002 (Figs 2.2 A & B) to those taken in November 2003 (Figs 2.2 D, E & F), the environmental deterioration can clearly be seen in the increased amount of litter in the water. Algal blooms present in 2003 (Figs 2.2 D, E & F) are also an indication of a disrupted ecological system.

2.1.2 Freshwater Crayfish Farm

Freshwater crayfish farming is not a new tendency in South Africa; there are several freshwater crayfish farms in the country. The only farm in the Free State is the small holding, La Menezze, of Dr. Herman Reinach, a well known orthodontic surgeon and business man from Bloemfontein (Fig 2.3 A).

Farming consists mainly of two Australian freshwater crayfish species, *Cherax destructor* and *Cherax quadricarinatus* (Figs 2.3 D, E & F). This farm has existed for thirteen years and crayfish are exported and sold as pets and delicatessens to several countries, including Canada. According to the regulation of the Department of Environmental Affairs the crayfish must be kept indoors (Figs 2.3 A & B). They are kept in heated breeding tanks, which ensure optimum conditions for breeding (Fig 2.3 B). Plastic piping in the tanks, provide shelter for the crayfish (Fig 2.3 C). Quarantine tanks also exist where newly bought or sick crayfish are kept. The necessary permits for keeping, breeding and transporting crayfish are renewed annually. This is essential to prevent the spreading of the crayfish into natural river systems of South Africa, specifically in this case the Orange-Vaal River system.

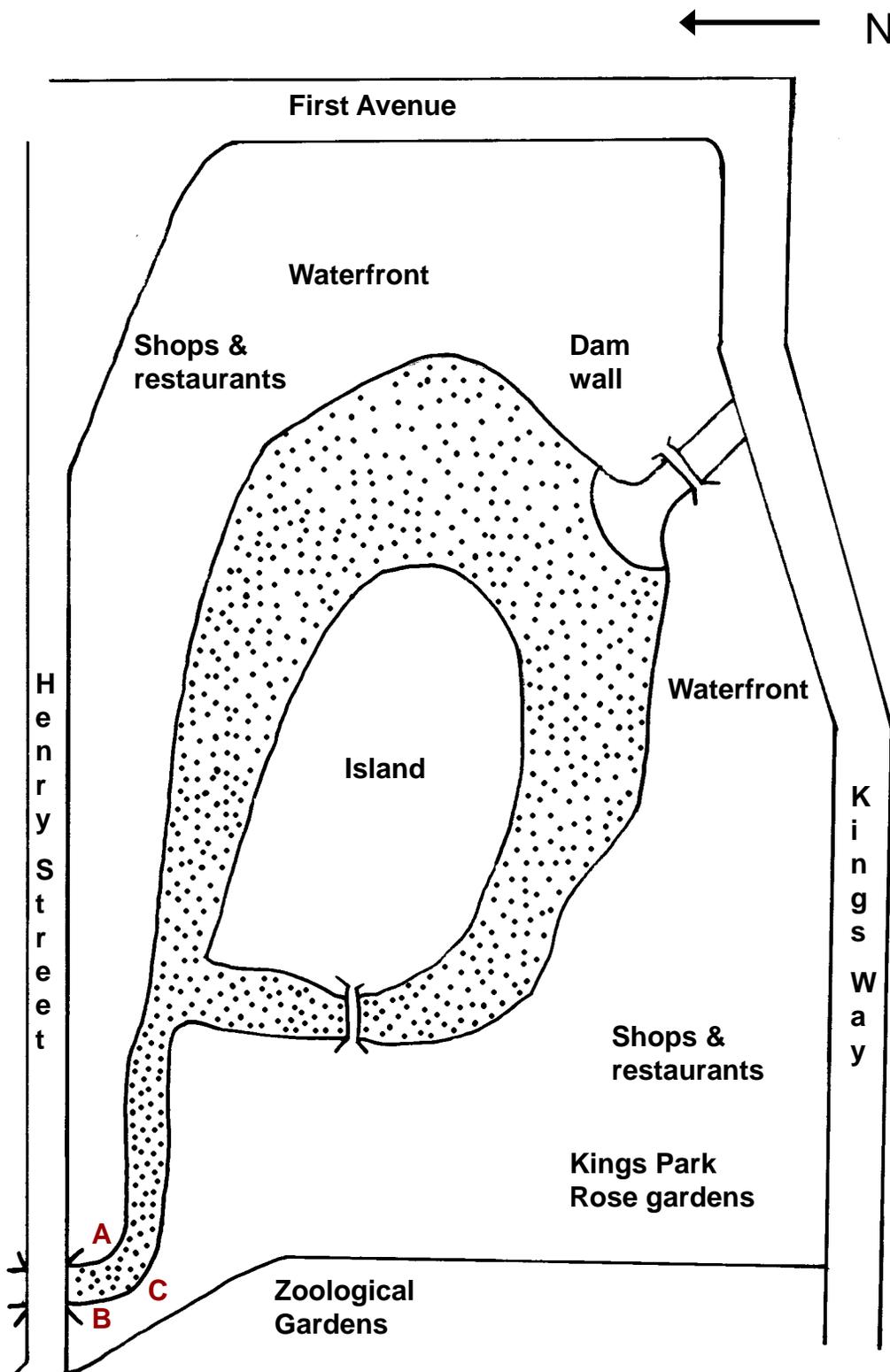


Fig 2.1 Map of Loch Logan, showing the three main sampling points (A – C). Redrawn from Vos (2002).



Fig 2.2 A – F: Collecting plankton samples at the Loch Logan Waterfront in Bloemfontein. Barbecue areas can be seen in A, whilst plankton net & plankton sample can be seen in B & C. Algal blooms and litter can be seen in D, E & F.



Fig 2.3 Collection at the freshwater crayfish farm. A: Outside buildings where crayfish tanks are situated, B: Double row of breeding tanks, C: Pieces of plastic pipe used for shelter for *Cherax destructor* Clark, 1936 and *C. quadricarinatus* Von Maartens, 1868, D: Female *C. quadricarinatus* in bucket, ready for examination. E: Lateral view of female *C. destructor* and F: Ventral view of female *C. destructor*.

2.2 Collection of Hosts

Plankton samples were collected with the aid of a plankton net in various areas of the Loch Logan Waterfront (Figs 2.2 B & C). Directly after the collection of hosts, the samples were transported to the laboratory in containers with water from the same water body. Due to the extremely fast movement of planktonic crustaceans, they were divided into glass Petri-dishes and left in the refrigerator for 5 – 10 minutes to immobilise the copepods, or a few drops of 70% Ethanol was added to the Petri-dish with the same effect. In order to examine the morphology and taxonomy of epibiont sessile ciliophorans attached to copepods, they were observed live under a dissecting microscope, after which they were prepared for light and scanning electron microscopy.

During the present study *Cherax destructor* and *Cherax quadricarinatus* were collected at the crayfish farm. Both were examined for *Epistylis* colonies on the carapace or appendages. Only *C. destructor* was found to be heavily infested with *Epistylis* colonies on the carapace and especially the antennae.

2.3 Live Observations

While examining collected material from both the planktonic copepods and the freshwater crayfish, under the dissecting microscope, the following observations were made:

- size and shape of colonies
- size and shape of single zooids
- and presence of any reproductive stages.

Temporary slides were prepared for live observations where after light microscope photographs and video prints were taken for morphological measurements.

In order to obtain statistical data of prevalence and incidence of *Epistylis* infestations specifically on the copepods, copepods were constantly examined in a Petri-dish with a volume of 50 mm³. The total number of copepods was

counted, as well as the number of infested specimens, providing a prevalence percentage. Although the exact amount of colonies and number of zooids per colony were not established, incidence was determined by allocating a symbol to a certain density of ciliophoran colonies.

Infestation ranged from a single colony (Fig 5.3 E) to the carapace being almost completely covered in colonies (Fig 5.3 B). This data is presented as a table and graphic representation in Chapter 5 as follows:

Symbol	% of copepod community infested with <i>Epistylis</i>
-	No ciliophorans present
+	0 – 25 % of sample
++	25 – 50 % of sample
+++	50 – 75 % of sample

2.4 Processing of Material

2.4.1 Scanning Electron Microscopy

Scanning electron microscopy (SEM) was done to examine the ultra structure of the ciliophorans attached to crustacean hosts. Copepods and ciliophorans were fixed together, while the appendages of the crayfish infested with cilioporans were dissected and handled separately. As far as possible, organisms should be fixed in a relaxed state and therefore fixation for SEM should be done immediately after collecting. Different fixative techniques were adapted to ensure that ciliophorans were fixed before they could contract.

- **Formalin**

Ciliophorans attached to the hosts were fixed in 10% GNF overnight. Specimens were washed with distilled water for 20 minutes, dehydrated through a series of ethanol concentrations (30 – 100%) for 10 minutes in each and critical point dried. Specimens were mounted on stubs with double sided tape, sputter coated with gold and studied with the aid of a JEOL WINSEM JSM 6400 scanning electron microscope at 5 KV. This process was successful for the crayfish

sessiline ciliophorans, but in the case of the copepod's sessiline ciliophorans the zooids were wrinkled and contracted.

To try and eliminate contraction of ciliophorans on the copepods, modification on this technique included transferring live ciliophorans to varying temperatures of Formalin.

Heated Formalin

Live ciliophorans were placed in heated formalin and observations were made to see if the zooids would still contract. This method was unsuccessful, as zooids were still contracted.

Cold Formalin

Live ciliophorans placed in cold (4° C) were still contracted.

Another modification was to kill live copepods and ciliophorans with very hot water, after which it was observed under the dissection microscope to determine if contraction of zooids had taken place. Individual zooids started swimming away from the ciliophoran colony, but contracted as soon as the zooids were killed. Specimens were transferred to a nucleopore filter and prepared for SEM, using standard techniques.

The techniques discussed in the following sections were only for ciliophorans from copepods, since it was not necessary for crayfish material.

- **Glutaraldehyde**

Freshly collected material was fixed overnight in 2.5% glutaraldehyde at 4°C and washed with a phosphate buffer (4°C) for 10 minutes. Specimens were dehydrated through a series of ethanol concentrations, 30 – 70% at 4° C and 80 – 100% at room temperature, critical point dried, mounted and sputter coated with gold.

- **Glutaraldehyde and post fixed in Osmium Tetra Oxide**

Instead of washing material with phosphate buffer after fixation in glutaraldehyde, some specimens were post fixed in a 1:1 osmium tetra oxide and phosphate buffer solution (4° C) for 10 minutes. Standard dehydration, critical point drying and mounting procedures were then followed.

- **Osmium Tetra Oxide**

Some live specimens were fixed in a fume cupboard in osmium tetra oxide (4° C) for 20 minutes directly after collection. Standard dehydration, critical point drying and mounting procedures were then followed.

2.4.2 Light Microscopy

Light microscopy is used to study the stained and impregnated internal structures of ciliophorans, including the nuclear and oral apparatus. Various chemicals and staining techniques including Protargol and Haematoxylin were used to stain internal body structures, which were studied and photographed using an automatic camera system mounted on a light microscope.

- **Protargol**

Protargol methods are indispensable for describing ciliophoran species. Kirby (1945), Moskowitz (1950), Dragesco (1962) and Tuffrau (1964, 1967) were the first scientists promoting and describing the Protargol methods to be used. After them many modifications were proposed by Zagon (1970), McCoy (1974), Wilbert (1975, 1976), Ng & Nelsen (1977), Aufderheide (1982) and Montagnes & Lynn (1987). Protargol reveals many cortical and internal structures, such as basal bodies, cilia, various fibrillar systems and sometimes even information of the nuclear apparatus.

In the present study the combined method of Lee, Hunter & Bovee (1985) and Lom & Dyková (1992) was used for ciliophorans collected from the antennae of *Cherax destructor*, whilst some difficulties were experienced with Protargol

staining of ciliophorans on infested copepods. Several modifications on this technique were experimented with, in search for a suitable one.

The method of Foissner (1991) was unsuccessful, since ciliophorans were stained too darkly to distinguish the oral apparatus. Time variations in various concentrations of this method were also unsuccessful. Wilbert's (1975) method was also unsuccessful because ciliophorans were too darkly stained as well. A modification of Wilbert's (1975) method proved to be successful in staining the oral apparatus and the procedure was as follows:

- Dried smears placed in 1% Protargol 7 min
- Washed with distilled water 5 min
- Developed smears in 1% Hydroquinone (in 5% Sodium Sulphite), 80° C 8 min
- Washed thoroughly with distilled water 5 min
- Placed slides in 0.5% gold chloride solution 20 min
- Bleached smears in 2% Oxalic acid 10 min
- Fixed smears in 5% Sodium Thiosulfate 2 min
- Washed thoroughly with distilled water 5 min
- Dehydrated through a series of ethanol concentrations (30 – 100%) 3 min
- Xylene 3 min
- Mounted with Eukitt and left to dry (48 hours) in a cool, dark place.

- **Harris' Haematoxylin**

In the present study standard Harris' Haematoxylin staining procedures (Humason 1979) worked for ciliophorans on the antennae of *Cherax destructor*, although it was unsuccessful for ciliophorans on copepods, since nuclear apparatus stained too dark to examine. To compensate for this, copepods infested with ciliophorans were placed in Harris' Haematoxylin for a much shorter period of time (3 min) which proved to be successful. The procedure was as follows:

- Fixed in Bouins 30 min
- Transferred to 70% Ethanol 30 min
- Transferred to 50 % Ethanol 3 min
- Harris' Haematoxylin 3 min
- Washed once in tap water 3 min
- Washed twice in distilled water 3 min
- Dehydrated through a series of ethanol concentrations (30 – 100%) 3 min
- Xylene 3 min
- Mounted with Eukitt and left to dry (48 hours) in a cool, dark place.

- **Silver nitrate impregnation**

Silver nitrate impregnation techniques were used to study and count the number of transverse striations on the pellicle. Lom's (1958) method for silver nitrate impregnation proved to be successful for examining the striations of sessiline ciliophorans in the present study.

In the present study both SEM techniques and silver nitrate impregnated specimens were used to count pellicular striations of *Epistylis* species. This is done in accordance to the proposal of Van As, Van As & Basson (1995), who suggested that both of these techniques should be used for counting striations since silver impregnation is unsuccessful for marine specimens due to the incompatibility of AgNO₃ and seawater.

Silver nitrate impregnation was not done on the ciliophorans found on *Cherax destructor* in the present study, but copepods infested with ciliophorans were placed on slides and smears were made. The procedure was as follows:

- Fixed in Bouins solution for 30 minutes
- Transferred to 70 % Ethanol 30 minutes
- Placed in 2 % Silver nitrate solution 10 minutes
- Washed in distilled water 5 min

- Slides were divided, some left under a black light for 10 – 12 minutes, some under a UV light for 45 minutes.
- Washed thoroughly in distilled water 5 min
- Dehydrated through a series of ethanol concentrations (30 – 100%) 3 min
- Xylene 3 min
- Mounted with Eukitt and left to dry (48 hours) in a cool, dark place.

2.5 Morphological Measurements

Body and nuclear measurements (length and diameter) of all the sessiline ciliophorans were made from videoprints, drawings of haematoxylin stained material as well as live material (Fig 2.4). Microscope drawings were made of live specimens using a drawing tube fitted to a light microscope. Measurements, in μm , are presented in the following way: minimum and maximum values are given, followed in parenthesis by the arithmetic mean, standard deviation and total number of specimens measured. Measurements based on Bouin's fixed specimens stained with haematoxylin are presented in square brackets.

Record was kept of all the different dates of collection, as well as the method of processing done for each collection. Spot checks were made, where more than one specimen from each method of processing at different collection dates, were measured. This data was compared and since the morphological measurement variation was not significant, the assumption can be made that I dealt with a constant community of copepods and ciliophorans for the duration of this study.

Figure 2.4

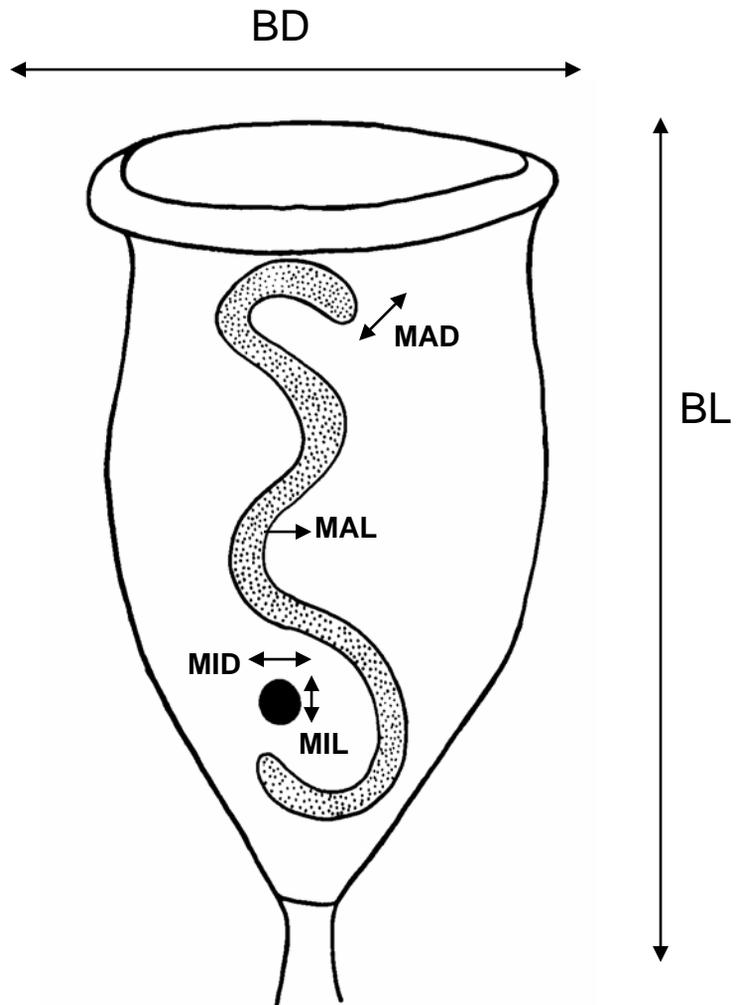


Diagram of a typical epistylid, illustrating morphological features used to determine biometrical measurements.

BL = body length

BD = body diameter

MAD = macronucleus diameter

MAL = macronucleus length-measured from top to bottom with piece of string to obtain total length

MID = micronucleus diameter

MIL = micronucleus length

Peritrichs are a very large group of distinctive-appearing ciliophorans known to scientists since the time of Van Leeuwenhoek, nearly 300 years ago. They are free-living or epizoic. The sessiline peritrichs found associated with fish are essentially ectocommensals or symphorionts that use the hosts as a living, moving substrate to settle on, where they may gain access to a convenient source of food particles – organic debris and waterborne bacteria. Symbiotic ciliophorans are specifically adapted – unlike free-living sessilines – to life on the surface of certain fish species and a variety of other hosts ranging from other peritrichs to molluscs, crustaceans including barnacles and the body or gills of crabs and crayfish, aquatic insects as well as lower vertebrate species in both freshwater and marine habitats (Lom & Dyková 1992).

The subclass Peritrichia is divided into two main groups, the sessiline and mobiline ciliophorans. Sessiline peritrichs are usually stalked and lack somatic ciliature, except as a temporary aboral band of locomotor cilia in the migratory larval stage (telotroch). However, the mobiline peritrichs have a permanent girdle of cilia around the flattened aboral pole of the body, which assists movement across the surface of host animals, vertebrate or invertebrate, marine or freshwater; the aboral pole often also has a characteristic ring of denticles and radial myonemes, which may aid adhesion to the host (Sleigh 1989).

3.1 Taxonomic Position and History

Pelagic ciliophorans and protozoans in general have been ignored for a long time by plankton ecologists, although studies from the sixties and eighties show that they form an integral part of the planktonic food web and contribute significantly to the total zooplankton standing crop (Foissner, Berger & Schaumburg 1999).

There are several obstacles in the scientist's way when handling ciliophorans. They are often difficult to handle, because of small size. It was the concept of the microbial loop, developed by Azam, Fenchel, Field, Gray, Meyer-Reil & Thingstad (1983), which stimulated more detailed and intensive research.

Now, ecology of planktonic protists has attained a high standard in terms of methods and interpretations.

If, however, one looks at the identification of the organisms involved, the standard is often poorer than it was 50 years ago, which may strongly limit the usefulness of data (Foissner *et al.* 1999).

Euplanktonic ciliophorans as such, have been known since the turn of the century (Lauterborn 1894, Zacharias 1897), while some species were already discovered by Linnaeus (1758, 1767), Müller (1773) and Ehrenberg (1838). Later, Fauré-Fremiet (1924), Gajewskaja (1933) and Kahl (1930-1935) studied ciliophoran plankton in more detail; although most species were described by Fauré-Fremiet (1924) from marine habitats.

Recently mainly Foissner & Wilbert (1979), Wilbert (1986), Krainer (1995) and the Russian scientists Alekperov (1984) and Mirabdullaev (1985) contributed significantly to the taxonomy of freshwater plankton ciliophorans. Contemporary morphological studies on marine plankton ciliophorans were performed mainly by Lynn, Montagnes, Dale, Gilron & Strom (1991), Marten & Montagnes (1993) and Petz, Song & Wilbert (1995).

There is as yet no firm conclusion on the phylogenetic position among ciliophorans (Corliss 1979). Some scholars considered that the peritrichs were closest to spirotrichous ciliophorans and allied them based on their reduced somatic ciliature and spiralled oral ciliature. From 1950 – 1970, life history studies on peritrichs emphasized the nature of the infraciliature and suggested that the peritrichs were most closely related to holotrichs (Fauré-Fremiet 1965).

Corliss (1968) placed the peritrichs within the class Oligohymenophorea De Puytorac, Batisse, Bohatier, Corliss, Deroux, Didier, Gragesco, Fryd-Versavel, Grain, Grolière, Hovasse, Iftode, Laval, Roque, Savoie & Tuffrau, 1974 and

elevated them to subclass rank, recognizing the basic similarities in their oral structures to other oligohymenophoreans and their distinct differences in body structure (Miao, Yu & Shen 2001).

During the last twenty years, an increasing number of scientists became involved in studying the systematics and evolution of the protists. As knowledge on the cytoarchitecture and phylogenetic relationships of a large number of species expanded, so has our understanding of a natural scheme of classification to use for these ubiquitous, unicellular organisms.

The development of molecular chronometric techniques (e.g. ribosomal RNA sequencing), combined with ultra structural investigations and the application of sophisticated cladistic analyses, makes the outlook for learning enough about the evolution of protistan groups even more auspicious. This will enable scientists to propose a robust classification system that will withstand the test of phylogenetic principles as monophyly and can thus be expected to endure for a reasonable number of years (Miao *et al.* 2001).

In spite of their abundance, ciliated protozoans are recognized to be a difficult group to identify; partly due to the lack of suitable keys. At present, taxonomy is in a state of flux, being frustratingly trapped between faulty existing classifications of protists and a lack of any better scheme to use.

The present study focuses on the systematics of the genus *Epistylis*. Emphasis is placed on this group's systematics instead of other groups involved, although all the families of the class Oligohymenophorea are listed.

3.2 Classification

There are currently 14 phyla distinguished within the kingdom Protozoa Goldfuss, 1818, including the phylum Ciliophora Doflein, 1901. Within this phylum, there are eight classes, eight subclasses and several families, genera and species.

For the purpose of this study the classification system of De Puytorac (1994) will be used, since it is comprehensive and includes the systematics of taxa below class level.

Class: Oligohymenophorea De Puytorac *et al.* 1974

The oral apparatus is distinct from somatic ciliature, comprised of a well defined paroral membrane plus several membranelles of penicilli located in the buccal cavity or infundibulum, situated on the ventral side of the body, with the cytostome at the base of the cavity. Cytopharynx is inconspicuous.

Subclass: Peritrichia Stein, 1859

Body is characteristically inverted bell- or goblet-shaped or conical-cylindrical. The morphology is dominated by an adoral ciliary wreath of buccal ciliature, whilst somatic ciliature is reduced to a subequatorial locomotor fringe or trocheal band. Very widespread aquatic distribution, free-living or symbionts on diverse host range (Corliss, 1979).

Order: Sessilida Kahl, 1933

Adults are sedentary or sessiline, commonly stalked (or with inconspicuous adhesive disc: scopula), while a few species are secondarily mobile. Many produce arboroid colonies, while some entire groups are loricate. Mucocysts and pellicular pores are universal. Adults are filter feeding bacterivores, whilst larval stages are mouthless. Habitats range from freshwater, brackish and marine environments and a few species live on endozoic forms (Corliss 1979).

Family: Scyphidiidae Kahl, 1935

Genera: *Ophrydiopsis* Pénard, 1922; *Paravorticella* Kahl, 1933; *Pachystoma* Rudzinska, 1952; *Ambiphrya* Raabe, 1952; *Gonzeela* Kufferath, 1953; *Mantoscaphidia* Jankowski, 1980; *Riboscaphidia* Jankowski, 1980; *Spoleoscaphidia* Jankowski, 1980; *Myoscaphidia* Jankowski, 1985

Family: Ophrydiidae Ehrenberg, 1838

Genera: *Ophrydium* Bory de St Vincent, 1826; *Gerda* Claparède & Lachmann, 1858

Family: Epistylididae Kahl, 1935

The **scopula** produces a **non-contractile stalk**, either simple, bearing a solitary zooid, or branched, bearing many **zooids**. The retractile **peristomial lip**, more or less divergent, encircles a wide, slightly elevated **epistomial disc**. The adoral spiral forms about one to five turns, whilst the infundibular fringe forms an incomplete turn to two turns (De Puytorac 1994). A great number of species exist, having a great range in size; some species of the two genera *Campanella* Goldfuss, 1820 (De Puytorac, 1994) and *Epistylis* may have zooids up to 600 µm in length (Lom & Dyková 1992).

The family comprises six genera, ***Apiosoma*** Blanchard, 1885 (syn. *Glossatella*) ***Campanella***, ***Epistylis***, ***Heteropolaria*** Foissner & Schubert, 1977, ***Opisthostyla*** Stokes, 1886 and ***Rhabdostyla*** Kent, 1880.

Apiosoma Blanchard, 1885 (syn. *Glossatella*)

Scopula is typical, but narrow or with a wide scopular disc, sometimes lobular or even with long lateral projections. Stalks rudimentary, generally not detectable with light microscope. Individuals occur solitary. Body is cylindrical or cylindro-conical (60 – 100 µm). Macronucleus is compact, conical and situated in the aboral half of the body. Adoral spiral about one turn; more than half turn of

infundibular fringes. Occurs epizoic on freshwater fish, often in close association with *Epistylis*, without indication of pathogenic action on the epithelium of the host. This genus represents a transition between solitary and colonial epistylids. In some species, the scopula gives rise to a primitive stalk which is wide and very short. Only *Apiosoma gasterostei* has a distinct stalk, branched and carrying colonies of two zooids.

Campanella Goldfuss, 1820 (De Puytorac, 1994)

Giant, colonial epistylids. The hyperthelic adoral spiral makes five turns around the wide, flat epistomial disc, whilst the infundibular fringes make two turns. Zooids are bell-shaped and up to 350 µm in length. The stalks are tubular, long and flexible when the colony is well developed and measures up to 5 - 6 mm long. Zooids are often tinted pale yellow.

Epistylis Ehrenberg, 1830

Colonial ciliophorans with **bell-shaped** or **elongated, cylindrical** or **conical** body. The non-contractile, ramified stalk bears multiple zooids. The vaulted epistomial disc is slightly elevated above the peristomial lips and slanted. Adoral spiral with one to two turns, infundibular fringes about one turn. Telotrochal migrants are often flat, circular and disc-shaped. Macronucleus is either horseshoe-shaped, situated in the anterior region of the body, or very long, ovoid and longitudinally orientated. In adverse conditions cysts are frequently formed. Numerous species, epizoic or free, marine or freshwater.

The first species of the genus *Epistylis* was originally placed under the genus *Vorticella* Linnaeus, 1767 by Linnaeus in 1767 as *Vorticella anastatica*. The genus *Epistylis* was created in 1830 by Ehrenberg and *V. anastatica* was included (Lom & Vavra 1961). Kent (1881 – 1882) was the first person who comprehensively described this species which is regarded as the type species (Vavra 1963).

According to Fernandez-Leborans & Tato-Porto (2000) the genus *Epistylis* contains 45 species associated with crustacean hosts (Table 3.1), whilst there are five species associated with fish (Lom & Dyková 1992) and several species associated with molluscs, insect larvae and freshwater plants.

Epistylids have also been found as **hypersymbionts** associated with the parasitic copepods, *Lernaea barnimiana* (Hartman, 1870), *Lernaea cyprinacea* Linnaeus, 1758 and *Dolops ranarum* (Stuhlmann, 1891) by Van As & Viljoen (1984) as well as *Caligus acanthopagri* Lin, Ho & Chen 1994 and *Caligus engraulidis* Barnard, 1984 by Grobler (2000).

Molatoli (1996) found a new species, *Epistylis* sp from endosymbiotic copepods of the genera *Doropygus* Thorell, 1859 and *Gunenotophorus* Buchholz, 1869 associated with red bait - *Pyura stolonifera* (Heller, 1878).

Heteropolaria Foissner & Schubert, 1977

Foissner & Schubert (1977) established the genus *Heteropolaria* for *Epistylis* species from fish, solely because the scopula in the migratory stage, the telotroch, is shifted excentrically on the aboral surface. According to Lom & Dyková (1992), this is, however, not adequate for describing a new genus and therefore the genus *Epistylis* is retained for fish epistylids.

Opisthostyla Stokes, 1886

Individuals are solitary, supported by a very slender, rather long stalk (one and a half times the length of the body), elastically bouncing back when the ciliophoran retracts itself and loses its traction due to the activity of the peristomial cilia. Body is bell-shaped with an approximate size of 25 µm. An epizoic species, occurring as epiphytes. It is possible that the *Opisthostyla* derives from the small species of the genus *Vorticella* by involution of the stalk myoneme system.

Rhabdostyla Kent, 1880

Individuals are solitary (60 µm), fixed by a stalk which is generally much shorter than the body. Adoral spiral makes about one turn, whilst the infundibular fringes make almost half a turn. Macronucleus is elongated, rarely ovoid, or horseshoe-shaped. Generally occurs as epizoic, marine, or freshwater. If growth is not stopped by certain environmental factors, the solitary state may be replaced by small colonial groups. More than 30 species have been described with rather uncertain characters and many are either representatives of the vorticellids or have ramified stalks, which dissociate them from the main criteria of *Rhabdostyla*, being the solitary state.

Family: Operculariidae Faure-Fremiet in Corliss, 1979

Genera: *Opercularia* Stein, 1854; *Telotrochidium* Kent, 1881; *Opisthnecta* Fauré-Fremiet, 1924; *Operculariella* Stammer, 1948; *Orbopercularia* Lust, 1950; *Ballodora* Dogiel & Fursenko, 1920; *Rovinjella* Matthes, 1972; *Scyphidiella* Guhl, 1979; *Tauriella* Nadanova, 1985

Family: Ellobiophryiidae

Genera: *Ellobiophrya* Chatton & Lwoff, 1923; *Calipera* Laird, 1953

Family: Termitophryidae

Genus: *Termitophrya* Noitot-Timotheé, 1969

Family: Vorticellidae

Genera: *Vorticella* Linnaeus, 1767; *Carchesium* Ehrenberg, 1838; *Zoothamnium* Ehrenberg, 1838; *Intranstylum* Fauré-Fremiet, 1905; *Haplocaulus* Precht, 1935; *Myoschiston* Precht, 1935; *Entziella* Stiller, 1950; *Pseudocarchesium* Sommer, 1950; *Parazoothamnium* Piesik, 1975; *Tucolescoa* Lom in Corliss, 1979; *Rugaecaulis* Lom & De Puytorac, 1994

Family: Astylozoonidae Kahl, 1935

Genera: *Astylozoon* Engelmann, 1862; *Hastatella* Erlanger, 1890

Family: Vaginicolidae De Fromentel, 1874

Genera: *Vaginicola* Lamarck, 1816; *Cothurnia* (Ehrenberg, 1831); *Thuricola* Kent, 1881; *Pyxicola* Kent, 1881; *Platycola* Kent, 1881; *Pachytrocha* Kent, 1881; *Caulicola* Stokes, 1894; *Pseudothuricola* Kahl, 1935

Family: Lagenophryidae

Genera: *Lagenophrys* Stein, 1851; *Operculigera* Kane, 1969; *Stenophrys* Jankowski, 1986; *Clistolagenophrys* Clamp, 1991

Family: Usconophryidae

Genus: *Usconophrys* Jankowski, 1985

Order: Mobilida Kahl, 1933

Conical body form, cylindrical or goblet-shaped. The dominant feature is the aboral disc, which serves as a holdfast organ of considerable complexity. The trocheal band is permanently ciliated. Individuals are stalkless with a vestigial scopula. All species are associated with a host which can either be the gills, the integument, and the digestive and urogenital tracts of freshwater and marine vertebrates including several fish and amphibian species as well as several aquatic invertebrates.

Family: Urceolariidae Dujardin, 1841

Genera: *Urceolaria* Stein, 1867; *Leiotrocha* Fabre-Domerque, 1888; *Polycycla* Poljansky, 1951

Family: Trichodinopsidae Kent, 1881

Genus: *Trichodinopsis* Claparède & Lachmann, 1862

Family: Trichodinidae Raabe, 1963

Genera: *Trichodina* Ehrenberg, 1838; *Vauchomia* Mueller, 1938; *Trichodinella* Šramek-Hušek, 1953; *Semitrichodina* Kazubski, 1958; *Tripartiella* Lom, 1959; *Dipartiella* Stein, 1961; *Paratrichodina* Lom, 1963; *Trichodoxa* Sirgel, 1983; *Hemitrichodina* Basson & Van As, 1989; *Pallitrichodina* Van As & Basson, 1993

3.3 General Morphological Features of Sessiline Peritrichs (Fig 3.1).

Presented below are the systematic characteristics of the relevant sessiline ciliophorans, according to Corliss (1979) & Van As (1997).

The body or **zooid** is carried on a **non-contractile, branched stalk** in the form of **colonies** and the size of the colonies and number of zooids per colony are constant for the same, but may vary for different species (Fig 3.1). The stalks are hyaline and have **transverse striations** (Fig 3.1). The form of the zooids varies greatly from oval and inverted bell-shape to cylindrical and funnel-shaped. A single contractile vacuole can be found in the upper part of the zooid. The **peristomial disc** (Fig 3.1) is not pushed out onto a stalk during bulging and a clear **peristome edge**, which can either be single or double can be distinguished.

In comparison with other ciliophorans, like mobiline trichodinids, the buccal ciliature of peritrichs are more diverse than the somatic ciliature. The cilia on the outer row of the **kinetosomes** form the outermost cilia; this is the first part of the oral ciliature. The second part is made up of membranelles. These cilia arise from three to four closely set kinetosomes that is known as the adoral zone of membranelles (AZM) (Fig 3.2).

The buccal cavity is lined with a **haplokinety** - outer row of paroral membrane and **polykinety** - inner rows or adoral polykinetids (Fig 3.2). Outside the buccal cavity the haplo- and polykineties continue to spiral in a clockwise direction

making at least one and a half to four turns around the peristome. The haplokinety remains a double row of kinetosomes, consisting of a ciliated row and an inner row of barren kinetosomes. The polykinety consists of a band of three rows of cilia, running along-side the haplokinety. In the buccal cavity, two further bands of cilia, each three kinetosomes wide, appear. The erect polykinety causes food particles to be carried to the horizontal haplokinety, where the particles are swept into the **infundibulum** (Fig 3.2). Within the infundibulum the rows of cilia spiral a half to two and a half turns downwards to a single **cytostome**, where food vacuoles are formed. The infundibulum of epistylids is considerably more complex than that of other peritrichs such as scyphidids and trichodinids.

No somatic cilia are found on the normal trophozooids. Epistylids are sessiline organisms thus relying on motile hosts for locomotion. Therefore it is important that the position of attachment enables them to be in contact with flowing water in order to feed on bacteria or debris particles. A **pectinel** occurring as a ridge or groove around the zooid, more or less in the middle thereof, gives rise to a ciliary girdle when the **free-swimming telotroch** is formed.

The pellicle has a number of parallel horizontal lines (grooves) encircling the zooid. A **myoneme** (Fig 3.1) network is found directly under the pellicle. This myoneme network consists of three parts – the systems in the peristomial edge, the peristome disc and those in the rest of the zooid. Myonemes are composed of thick packs of bundles made up of 3 – 5 nm **microfibrils**. The microfibrils are responsible for the contractility in ciliophorans. Although the majority of epistylids have acontractile stalks, some species and especially the closely related vorticellids have highly contractile stalks. This requires a well developed myoneme system in the stalk as well as in the zooid.

Many ciliophorans are capable of actively changing body shape by **contraction**. Various stages of contractility can be identified ranging from fully expanded,

through partially contracted to completely contracted. Contraction is followed by expansion. This involves relaxation of the myonemes and restoration of the original body shape. Between these stages, variation in the peristome and adoral spiral shape can be seen. During this process the peristome changes from flattened to arched. When contracted the adoral cilia are either drawn inwards or, in some cases, a bundle of cilia will still protrude from the peristome. Every zooid contains a single **macro-** and **micronucleus** (Fig 3.3). The macronucleus is polyploid and has a somatic function. During reproduction the macronucleus disintegrates and is replaced, whilst the micronucleus is diploid and carries the genetic information. The macronucleus is variable in shape and size and can either be: horseshoe-shaped or semi circular in the transverse axis of the cell (Fig 3.3 A); ribbon or sausage-shaped in the longitudinal axis of the cell (Fig 3.3 B); J-shaped in the longitudinal axis of the cell (Fig 3.3 C) or C-shaped in the longitudinal axis of the cell (Fig 3.3 D). The micronucleus varies from round or oval-shaped and is usually not as clearly seen as the macronucleus. The nuclear apparatus is an important taxonomic character for ciliophorans. The position of the macronucleus in the zooid and the position of the micronucleus towards the macronucleus are also very important taxonomic characteristics.

Asexual reproduction occurs by means of **binary fission** or the formation of a **free-swimming telotroch** (Figs 3.4 A-E), which leaves the colony in search for another suitable substrate. Telotroch formation is brought about by a gradual deterioration in condition of either the host or the substrate. The peristome will close and a swelling in the middle part of the body occurs, where three or four rows of basal kinetosomes will appear. The telotroch is usually asymmetrical during the developmental process, but symmetrical in the free-swimming condition. It moves by means of a telotroch band, consisting of the pectinel.

Sexual reproduction occurs through **conjugation**, when a sessile macro-conjugant, which does not differ from the trophozooids, fuses with a smaller free-

*Chapter 3: The Genus *Epistylis* Ehrenberg, 1830*

swimming micro-conjugant forming a zooid with a synkarion. This zooid gives rise to a new colony through binary fission.

Figure 3.1

Diagram illustrating general morphology of a typical epistylid. Redrawn from Foissner, Berger & Schaumburg (1999).

AZM = polykinety – adoral membranelles, number of turns on peristomial disc is important for identification and **haplokinety**, the distal portion runs into the adoral ciliary spiral.

CS = cytostome (mouth, where oral ciliature terminates).

CV = contractile vacuole (location depending on the species, either on the ventral, **CV 1** or dorsal **CV 2** wall of the infundibulum).

CY = cytopharynx (non-ciliated tubular passway, leading from cytostome to inner cytoplasm, forming food vacuoles)

FV = food vacuole

I = infundibulum (oral apparatus)

MA = macronucleus

MI = micronucleus

MY = myonemes (contractile fibres)

PC = peristomial collar

PD = peristomial disc (retracted into cell in contracted specimens)

PS = pellicular striations

SC = scopula

ST = stalk, with stalk myoneme in contracting species like *Vorticella* Linnaeus, 1767 sp.

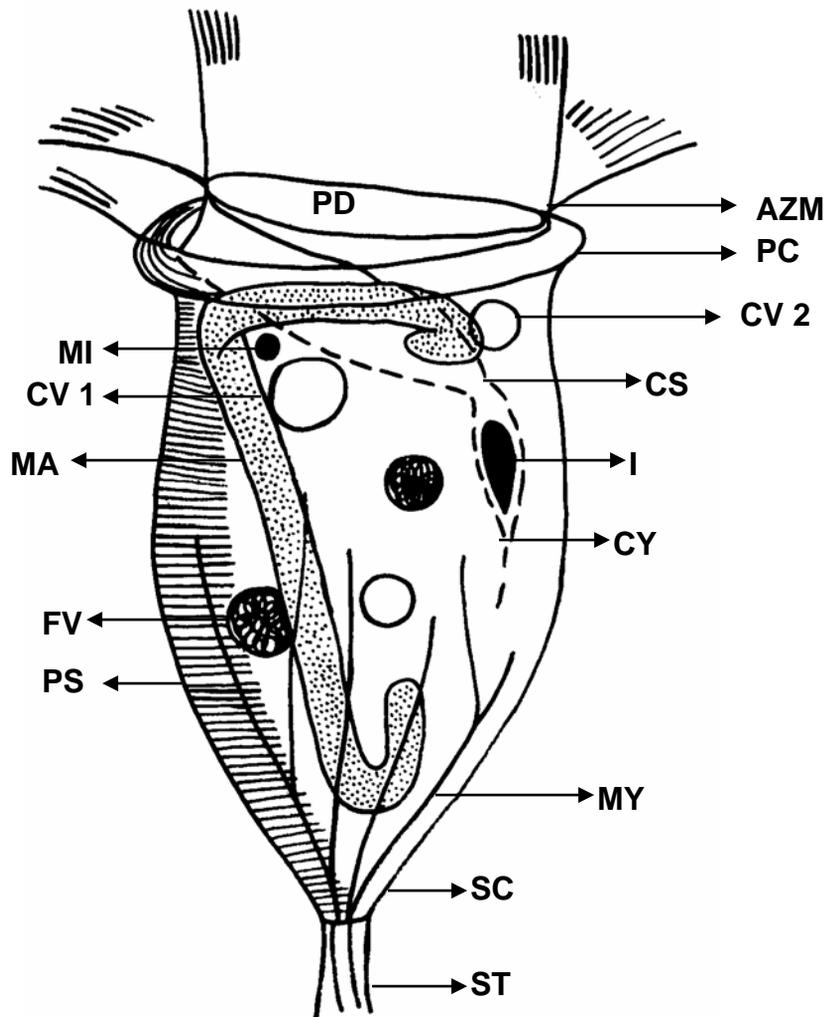


Figure 3.2

Diagram of an oral ciliature, after Protargol impregnation. Redrawn from Foissner, Berger & Schaumburg (1999).

AZM = polykinety – adoral membranelles, number of turns on peristomial disc is important for identification and **haplokinety**, the distal portion runs into the adoral ciliary spiral.

CS = cytostome (mouth, where oral ciliature terminates).

CY = cytopharynx (non-ciliated tubular passway, leading from cytostome to inner cytoplasm, forming food vacuoles).

G = germinal kinety (the new oral ciliature originates unciliated basal body row).

I = infundibulum (oral apparatus).

P = peniculi (adoral membranelles).

PD = peristomial disc (retracted into cell in contracted specimens).

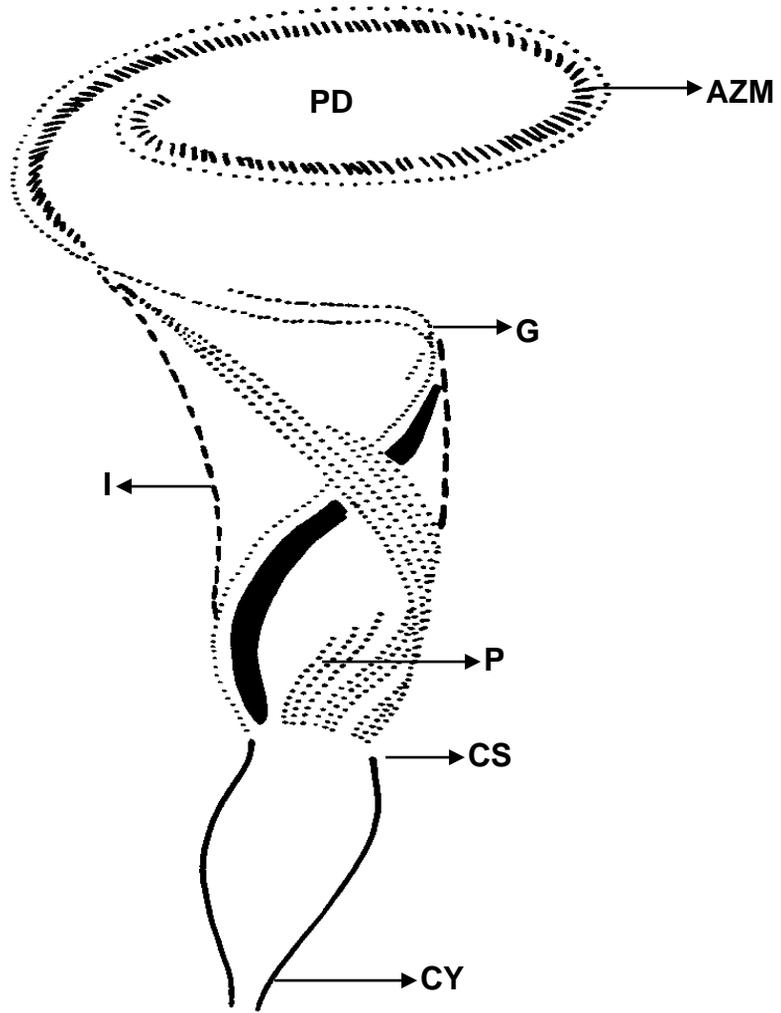


Figure 3.3

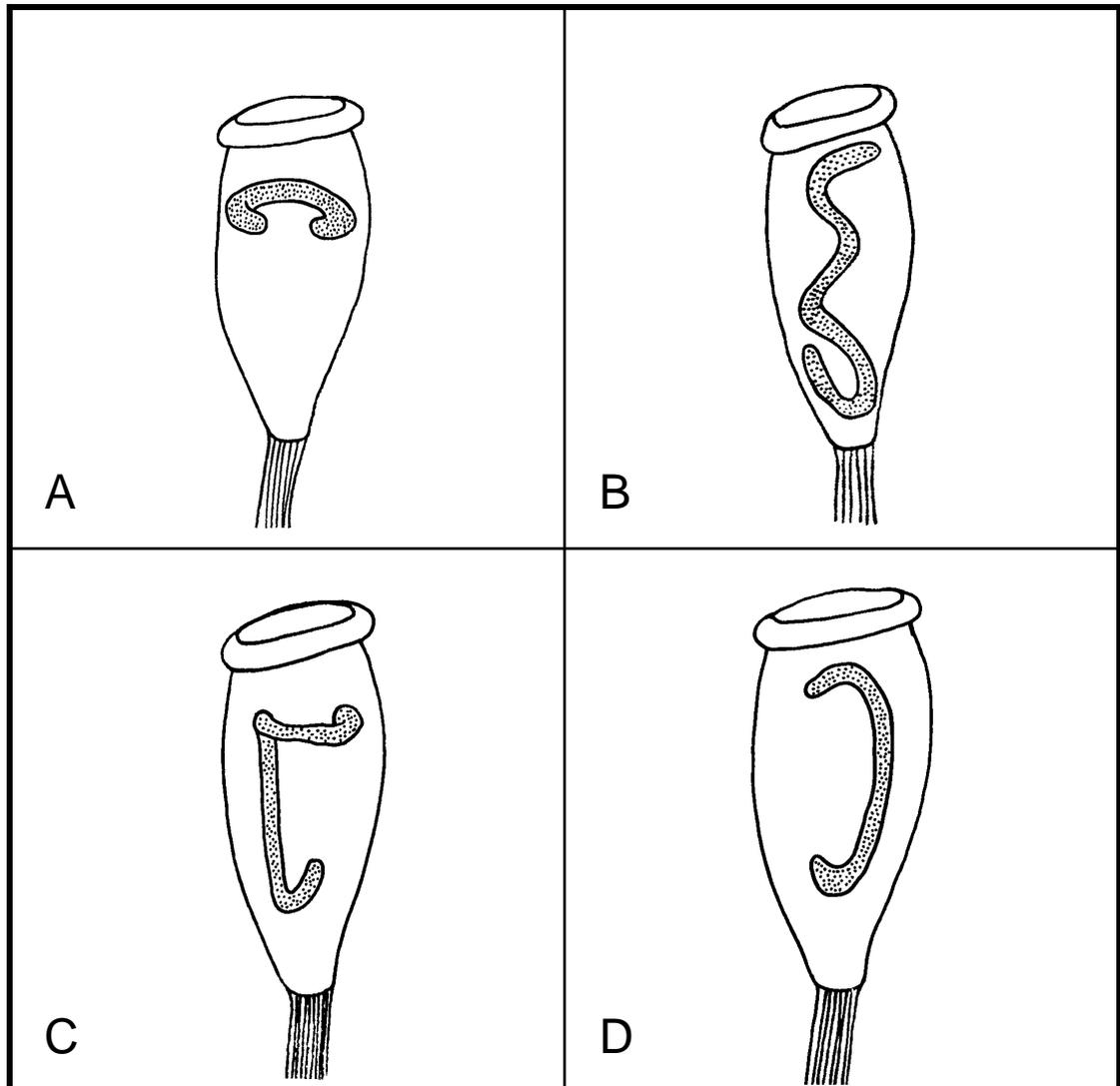


Diagram of a typical epistylid, illustrating different macronucleus shapes. Redrawn from Foissner, Berger & Schaumburg (1999).

- A** = Horseshoe-shaped or semi circular, in transverse axis of cell.
- B** = Sausage- or ribbon-shaped, in the longitudinal axis of cell.
- C** = J-shaped, in longitudinal axis of cell.
- D** = C-shaped, in longitudinal axis of cell.

Figure 3.4

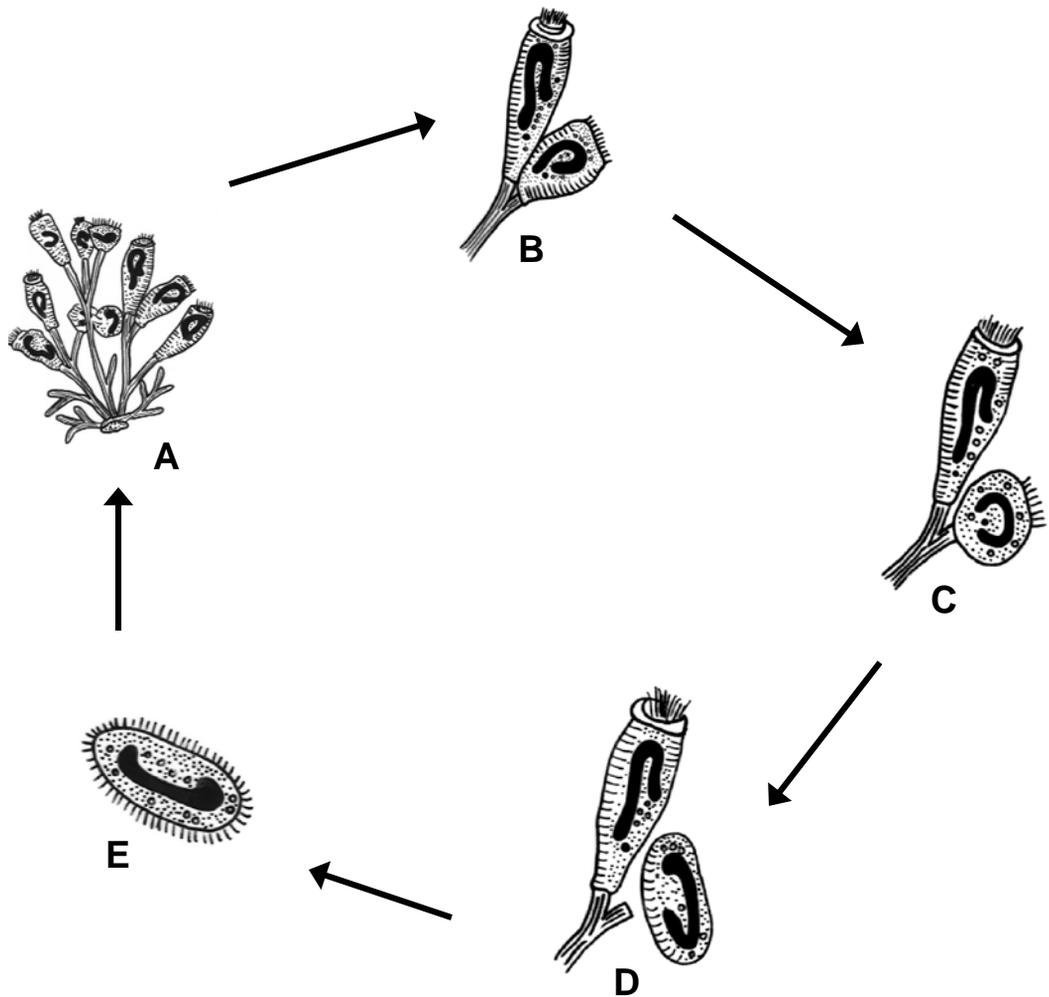


Diagram of a typical epistylid colony, illustrating telotroch formation during asexual reproduction. Redrawn from Rogers (1971).

A = Colony of *Epistylis* sp.

B = Zooid contracts and rounds up, ring of cilia develops near proximal end of zooid.

C = Adoral ring of cilia re-absorbed into body, body changes from round to dorsoventrally flattened, disc-like shaped with a ring of cilia around the margin.

D = Detaches from colony and becomes a free-swimming telotroch.

E = Telotroch seeks a new host or attachment substrate and divides by binary fission to produce a new colony.

Chapter 3: The Genus Epistylis Ehrenberg, 1830

Table 3.1: Summary of Species of the Genus *Epistylis* Ehrenberg, 1830 found associated with Crustacean Hosts worldwide.

Genus and species	Ha	Host	L	Reference
<i>Epistylis anastatica</i> (Linnaeus, 1767) (cf. Kent, 1881) Syn.: <i>Vorticella anastatica</i> Linnaeus, 1767	F	Entomostraca, freshwater plants, cyclopoid copepods & cladocerans <i>Daphnia pulex</i>	C	Fernandez-Leborans & Tato-Porto (2000)
<i>E. astaci</i> Nenninger, 1948	F	Gills of decapods <i>Astacus astacus</i> & <i>A. leptodactylus</i> , gills of <i>Austropotamobius torrentium</i>	G	Fernandez-Leborans & Tato-Porto (2000)
<i>E. bimarginata</i> Nenninger, 1948	F	Appendages of <i>Astacus astacus</i> (Decapoda)	G	Fernandez-Leborans & Tato-Porto (2000)
		Exoskeleton of crayfish <i>Cambarellus patzcuarensis</i>	M	Mayén-Estrada & Aladro-Lubel (2001)
<i>E. branchiophila</i> Perty, 1852 Syn.: <i>E. formosa</i> Nenninger, 1948	F	Parasitic copepod <i>Lernaea cyprinacea</i>	SA	Fernandez-Leborans & Tato-Porto (2000)
		Exoskeleton of crayfish <i>Cambarellus patzcuarensis</i>	M	Mayén-Estrada & Aladro-Lubel (2001)
<i>E. breviramosa</i> Stiller, 1931	F	Antennal filament of the cladoceran <i>Daphnia</i> sp.	H	Fernandez-Leborans & Tato-Porto (2000)
		Cyclopoid copepod <i>Cyclops</i> sp.	CR	Fernandez-Leborans & Tato-Porto (2000)
		Cladocerans <i>Bosmina longirostris</i> & <i>Alona affinis</i>	U	Fernandez-Leborans & Tato-Porto (2000)
<i>E. cambari</i> Kellicott, 1885	F	Gills & maxillae of decapod <i>Cambarus</i> sp.	USA	Fernandez-Leborans & Tato-Porto (2000)
<i>E. carinogammari</i> Stiller, 1949	F	Exoskeleton of crayfish <i>Cambarellus patzcuarensis</i>	M	Mayén-Estrada & Aladro-Lubel (2001)
<i>E. cyprinaceae</i> Van As & Viljoen, 1984	F	Parasitic copepod <i>Lernaea cyprinacea</i>	SA	Van As & Viljoen, (1984)
<i>E. daphniae</i> Fauré-Fremiet, 1905	F	Cladoceran <i>Daphnia</i> sp. & <i>Daphnia magna</i> , copepod <i>Boeckella triarticulata</i> & cladoceran <i>Moina macrocopa</i>	NZ	Fernandez-Leborans & Tato-Porto (2000)
<i>E. diaptomi</i> Fauré-Fremiet, 1905	F	Copepod <i>Diaptomus</i> sp.	U	Fernandez-Leborans & Tato-Porto (2000)
<i>E. digitalis</i> Ehrenberg, 1838	F	Copepod <i>Cyclops</i> sp.	U	Fernandez-Leborans & Tato-Porto (2000)
<i>E. epibarnimiana</i> Van As & Viljoen, 1984	F	Parasitic copepod <i>Lernaea barnimiana</i>	SA	Van As & Viljoen, (1984)
<i>E. fugitans</i> Kellicott, 1887	F	Cladoceran <i>Sida crystallina</i>	NA	Fernandez-Leborans & Tato-Porto (2000)
<i>E. gammari</i> Precht, 1935	Ma	Proximal parts of 1st & 2nd antenna of <i>Gammarus oceanicus</i> & <i>G. salinus</i>	BS	Fernandez-Leborans & Tato-Porto (2000)
	Ma	Antennae of gammarid <i>Gammarus</i> sp.	KC	Fernandez-Leborans & Tato-Porto (2000)
	F	Exoskeleton of crayfish <i>Cambarellus patzcuarensis</i>	M	Mayén-Estrada & Aladro-Lubel (2001)

Chapter 3: The Genus *Epistylis* Ehrenberg, 1830

Table 3.1 continue: Summary of species of the genus *Epistylis* Ehrenberg, 1830

Genus and species	Ha	Host	L	Reference
<i>E. halophila</i> Stiller, 1942	F	Cladocerans <i>Daphnia pulex</i> & <i>D. longispina</i>	H	Fernandez-Leborans & Tato-Porto (2000)
<i>E. harpacticola</i> Kahl, 1933	Ma	Harpacticoid copepods	KC	Fernandez-Leborans & Tato-Porto (2000)
<i>E. helenae</i> Green, 1957	F	Cladocerans <i>Daphnia pulex</i> , <i>D. magna</i> , <i>D. obtuse</i> , <i>D. longispina</i> , <i>D. curvirostris</i> , <i>Ceriodaphnia pulchells</i> , <i>C. reticulate</i> , <i>C. laticaudata</i> , <i>Moina macropa</i> , <i>M. micrura</i> , <i>Chydorus sphaericus</i> , <i>Simocephalus serrulatus</i> & <i>S. vetulus</i>	U	Fernandez-Leborans & Tato-Porto (2000)
<i>E. helicostylum</i> Vavra, 1962	F	Extremities of the ostracod <i>Eucypris virens</i>	U	Vavra (1962)
<i>E. humulis</i> Kellicott, 1887	F	Gammarid <i>Gammarus</i> sp. & other Entomostraca	U	Fernandez-Leborans & Tato-Porto (2000)
<i>E. lacustris</i> Imhoff, 1884	F	Copepod <i>Cyclops</i> sp, buccal appendages of branchiopod <i>Lepidurus apus</i>	AU	Fernandez-Leborans & Tato-Porto (2000);
		Exoskeleton of crayfish <i>Cambarellus patzcuarensis</i>	M	Mayén-Estrada & Aladro-Lubel (2001)
<i>E. magna</i> Van As & Viljoen, 1984	F	Parasitic copepod <i>Lernaea cyprinacea</i>	SA	Van As & Viljoen, (1984)
<i>E. niagarae</i> Kellicott, 1883	F	Carapace, antennae of crayfish <i>Astacus leptodactylus</i> , <i>Austropotamobius torrentium</i> & <i>Orconectes limosus</i> . Exoskeleton of copepod <i>Eucyclops serrulatus</i> & Cladocerans <i>Daphnia pulex</i> , <i>D. rosea</i> , <i>Ceriodaphnia reticulate</i> & <i>Scapholeberis mucronata</i>	USA	Fernandez-Leborans & Tato-Porto (2000)
		Exoskeleton of crayfish <i>Cambarellus patzcuarensis</i>	M	Mayén-Estrada & Aladro-Lubel (2001)
<i>E. nitocrae</i> Precht, 1935	F	Third pereopod of <i>Gammarus tigrinus</i>	U	Fernandez-Leborans & Tato-Porto (2000)
<i>E. nympharum</i> Engelmann, 1862	F	Cladocerans, <i>Cyclops</i> sp. & Branchiuran <i>Dolops ranarum</i>	SA	Fernandez-Leborans & Tato-Porto (2000)
<i>E. ovalis</i> Biegel, 1954	F	Gnathopods of <i>Gammarus tigrinus</i> , third pereopod of <i>G. pulex</i> & spines at end of third uropod of <i>G. tigrinus</i>	U	Fernandez-Leborans & Tato-Porto (2000)
<i>E. plicatilis</i> Ehrenberg, 1831	F	Copepods <i>Eucyclops agilis</i> , <i>Cyclops vernalis</i> & <i>C. bicuspidatus</i>	USA	Fernandez-Leborans & Tato-Porto (2000)
<i>E. pseudovum</i> Lüpkes, 1975	F	Front gnathopods of <i>Gammarus</i> sp.	E	Lüpkes, 1975

Chapter 3: The Genus *Epistylis* Ehrenberg, 1830

Table 3.1 continue: Summary of species of the genus *Epistylis* Ehrenberg, 1830

Genus and species	Ha	Host	L	Reference
<i>E. salina</i> Stiller, 1941	F	First & second antennae, coxae & gills of gammarid <i>Gammarus pulex</i>	U	Fernandez-Leborans & Tato-Porto (2000)
<i>E. stammeri</i> Nenninger, 1948	F	Exoskeleton of crayfish <i>Cambarellus patzcuarensis</i>	M	Mayén-Estrada & Aladro-Lubel (2001)
<i>E. thienemanni</i> Sommer, 1951	F	Gills of <i>Gammarus tigrinus</i>	U	Fernandez-Leborans & Tato-Porto (2000)
<i>E. variabilis</i> Stiller, 1953	F	Exoskeleton of crayfish <i>Cambarellus patzcuarensis</i>	M	Mayén-Estrada & Aladro-Lubel (2001)
<i>E. zscokkei</i> (Keiser, 1921) Syn.: <i>Opercularis zscokkei</i> Keiser, 1921	F	Gnathopods of gammarid <i>Gammarus tigrinus</i> , other Entomostraca & cladoceran <i>Acantolebris curvirostris</i>	U	Nenninger (1948)
<i>Epistylis</i> sp.	Ma	Decapod <i>Penaeus duorarum</i> , Decapod <i>Ploeticus robustus</i>	USA	Fernandez-Leborans & Tato-Porto (2000)
	F	Setae of first antenna of <i>Gammarus tigrinus</i>	USA	Fernandez-Leborans & Tato-Porto (2000)
<i>Epistylis</i> sp.	F	Two species on thoracic appendages of a brachyuran	SA	Viljoen & Van As (1983)
<i>Epistylis</i> sp.	F	Gills of decapod <i>Coenobita clypeatus</i> , <i>Gergrapsus lividus</i> & <i>Pachygrapsus transverses</i>	USA	Fernandez-Leborans & Tato-Porto (2000)
<i>Epistylis</i> sp.	B	Gills of decapod <i>Scylla serrata</i>	A	Hudson & Lester (1994)
<i>Epistylis</i> sp.	B	Estuarine copepods <i>Acartia tonsa</i> & <i>A. clause</i>	USA	Turner, Postek & Collard (1979)
<i>Epistylis</i> sp.	Ma	Endosymbiotic copepods of red bait (<i>Pyura stolonifera</i>)	SA	Molatoli (1996)
<i>Epistylis</i> sp.	Ma	Parasitic copepods <i>Caligus acanthopagri</i> & <i>Caligus engraulidis</i>	SA	Grobler (2000)
<i>Epistylis</i> sp.	Ma	Carapace fringe and ventral surface of adult sea lice <i>Lepeophtheirus salmonis</i>	J, S	Gresty & Warren (1990)
<i>Epistylis</i> sp.	F	Laterally on carapace of <i>Cherax quadricarinatus</i> & <i>C. tenuimanus</i>	A	Herbert (1987)
<i>Epistylis</i> sp.	F	Gills of the blue crab (<i>Callinectes sapidus</i>)	USA	Couch (1966)
<i>Epistylis</i> sp.	F	Eggs of berried Red Claw Crayfish (<i>Cherax quadricarinatus</i>)	S Am	Romero & Jiménez (1997)
<i>Epistylis</i> sp.	F	Exoskeleton of orconectid crayfish (<i>Orconectis rusticus</i>)	NA	Brown, White, Swann & Fuller (1993)
<i>Epistylis</i> sp.	F	Exoskeleton of freshwater crayfish (<i>Cherax tenuimanus</i>)	A	Villarreal & Hutchings (1986)

List of abbreviations used in Table 3.1:

A - Australia
 AU - Austria
 B - Brackish
 BS - Baltic Sea
 C - Cosmopolitan
 CR - Czech Republic

E - Europe
 F - Freshwater
 G - Germany
 H - Hungary
 Ha - Habitat
 J - Japan

KC - Kiel Channel
 L - Locality
 M - Mexico
 Ma - Marine
 NA - North America
 NZ - New Zealand

S - Scotland
 SA - South Africa
 S Am - South America
 U - Unknown
 USA - United States of America

3.4 Notes on the Biology of Epistylids

3.4.1 Adaptations

The two basic problems of planktonic organisms are having to swim continuously to prevent sinking and having no place to hide. According to Foissner *et al.* (1999) all the common morphological adaptations to the pelagic life (Sommer 1994), which reduce sinking rate, can be found in planktonic ciliophorans:

- **Small size** (for example, *Balanion planctonicum*).
- **Shape** (inverted conical – *Paradileptus elephantinus*; parachute like – *Liliimorpha viridis*; body elongation(s) – *Teuthophrys trisulca*; distinct excavations – *Histiobalantium bodamicum*).
- **Spines** (*Hastatella radians*), **tentacles** (*Actinobolina* sp.) and **bristles** (*Halteria* sp).
- **Foamy cytoplasm** (*Bursellopsis* sp).
- **Mucous covers** (*Mucophrya pelagica*).
- **Large locomotor organelles combined with feeding apparatus at top of body** (oligotrichs and peritrichs).
- **Special movement** (like strong metachronal waves – *Urotricha* sp; fast and/or jumping – *Halteria* sp, oligotrichs).
- **Gas production in algae-bearing species** (not yet proved, but likely influences buoyancy).
- **Transport by other plankton organisms** (*Epistylis* sp. on rotifers and crustaceans).

The more than 38 000 known species of the subphylum Crustacea include some of the most familiar arthropods, such as crabs, shrimps, lobsters, crayfish and wood lice. In addition a myriad tiny crustaceans living in the seas, lakes and ponds of the world occupy an important position in the aquatic food chains. The Crustacea is the only large subphylum of arthropods whose members are primarily aquatic. Most crustaceans are marine, but there are some semi-terrestrial groups, but in general, the terrestrial crustaceans have never undergone extensive adaptive evolution for life on land (Rupert & Barnes 1994).

Ancestral crustaceans were probably small, swimming, filter feeding shrimps with a large number of similar appendages; all the appendages behind the antennae took part in feeding, locomotion and respiratory exchange. Somewhere along the evolution line larger bottom-living forms were produced that seek out and grasp their food in larger quantities. They have relatively shorter bodies and fewer, more specialised appendages.

4.1 The Subphylum Crustacea

According to Rupert & Barnes (1994) the subphylum Crustacea comprises 50 orders, the larger groups being:

Branchiopoda - a very diverse group of small filter-feeding shrimps, many in freshwater, including the very well known water flea *Daphnia* Müller, 1785.

Ostracoda - a very separate and ancient group of crustaceans. Often less than a millimetre long, enclosed in a bivalve calcareous shell, even as a nauplius. Sometimes called mussel or seed shrimps, widely distributed in marine and freshwater environments. The head region constitutes much of the ostracod, as the trunk is much reduced in size.

Copepoda – the largest class of small crustaceans, with more than 8500 species described worldwide. Most copepods are marine, but there are many freshwater

species and a few that live in moss, soil-water films and leaf litter. Freshwater copepods exist in enormous numbers and are usually the most abundant and conspicuous component of a plankton sample. Although most copepods are rather pale and transparent, some species may be brilliant red, orange, purple, blue or black. Many luminescent species have been reported.

The copepods include over 1000 species of **parasitic crustaceans**. Some copepods are ectoparasitic on fish, attaching to the skin, fins or gill filaments. These include well known genera like *Caligus* Müller, 1785, *Lernaea* Linnaeus, 1758 and *Ergasilus* Van Nordman, 1932. Among ectoparasitic copepods certain appendages have become specialized as holdfast organs, while mouthparts are adapted for piercing and sucking. Other copepods are commensal or endoparasitic within a variety of hosts including polychaete worms, the intestine of echinoderms, tunicates and bivalves (Rupert & Barnes 1994).

In most parasitic copepods, the adults are adapted for a parasitic way of life, whilst the larval stages are free-swimming, ensuring the distribution and survival of this highly successful group. Parasitic copepods are of great economic importance, because they can cause a serious increase in mortality amongst fish in aquaculture and fisheries where conditions are artificial and therefore very beneficial for the parasites.

Branchiura - although several structurally diverse groups compose the class, all are characterised by trunk appendages that have a flattened, leaf-like structure, compound eyes and reduced mouthparts. All branchiurans are fish parasites, known collectively as fish lice, although there are some species known to parasitise anuran tadpoles. These ectoparasites are found on the skin and fins, branchial chambers, gill filaments and mouth cavities of the hosts in freshwater, marine and brackish habitats. Branchiurans are usually small, but visible to the naked eye. Some have body coloration for camouflage, either by possessing or lacking pigmentation.

Malacostraca - this class contains over half of all the known species of crustaceans, as well as all the larger forms, such as crabs, lobsters and shrimps (Decapoda). The trunk of a malacostracan is typically composed of 14 segments, plus the telson, of which the first eight segments form the thorax and the last six the abdomen. The thorax may or may not be covered by a carapace. All of the segments bear appendages. The first antennae are often biramous and the exopod of the second antenna is frequently in the form of a flattened scale.

Cirripedia - are unique and very much modified crustaceans: includes the familiar marine animals known as barnacles. Cirripedes are known as the only sessile group of crustaceans, aside from the parasitic forms and as a result they are one of the most aberrant groups within the Crustacea. Barnacles are exclusively marine and approximately two thirds of the nearly 900 described species are free-living, attaching to rocks, shells, coral, floating timber and other objects.

For the purpose of the present study, emphasis is placed on crustacean hosts of epistylids. For a better understanding of the hosts, additional information on the three distinctive copepod groups, i.e. **Harpacticoida**, **Calanoida** and **Cyclopoida** is given in the following section of this chapter.

Harpacticoid, Calanoid and Cyclopoid Copepods

According to Wetzel (1975) the free-living copepods of the subphylum Crustacea can be separated into three distinct groups that are important in freshwater habitats: i.e. **Cyclopoida Burmeister, 1835**, **Calanoida Sars, 1903** and **Harpacticoida Sars, 1903** (Table & Fig 4.1). Although accurate identification is based largely on morphological details of appendages, several general characteristics distinguish the major groups. The body consists of the anterior metasome (cephalotorax), divided into the head region bearing five pairs of appendages of antennae and mouthparts and the thorax, with six pairs of mainly swimming legs. The posterior urosome consists of abdominal segments, the first of which is modified in females as the genital segment and the terminal caudal rami, bearing setae.

Cyclopoid copepods are the most abundant and successful group of freshwater copepods occurring in all types of habitats such as lakes, ponds, temporary pools, wells, streams and rivers. They are small, varying around 1 mm in length, usually pale in colouration and extremely fast swimmers – making them difficult to handle during examination.

Calanoid copepods are almost exclusively planktonic in the pelagial zone. The majority of calanoid copepods are marine. Families which have freshwater, estuarine, or coastal lake species are the Centropagidae Giesbrecht, 1892; Pseudodiaptomidae Sars, 1902; Temoridae Giesbrecht, 1892; Diaptomidae Sars, 1903 and Acartiidae Sars, 1900, but the Diaptomidae is the only family which have been recorded from inland waters of southern Africa (Rayner 2001).

Harpacticoid copepods are almost exclusively littoral, habitating macro-vegetation, mosses in particular and the littoral sediments and particulate organic matter. Certain species have life-histories with a diapause similar to that of cyclopoid copepods. Although harpacticoid copepods are primarily littoral

benthic species, those few members that are predominantly planktonic form major parts of the copepod zooplankton, especially in small shallow lakes.

Table 4.1: Characteristics of the three Suborders of Free-living Freshwater Copepods, compiled from Wetzel (1975).

Cyclopoida	Calanoida	Harpacticoida
Anterior part of body much broader than posterior	Anterior part of body much broader than posterior	Anterior part of body slightly broader than posterior
Marked constriction between somites of 4 th and 5 th legs	Marked constriction between somite of 5 th leg and genital segment	Slight or no constriction between somites of 4 th and 5 th legs
Two egg sacs, laterally	One egg sac, medially	One egg sac, medially
First antennae short, extends from proximal 3 rd of head segment to near end of metasome, 6-17 segments in female	First antennae long, extended from end of metasome to near end of caudal setae, 23-25 segments in female	First antennae very short, extends from proximal 5 th to end of head segment, 5-9 segments in female
5 th leg vestigial	5 th leg similar to other legs	5 th leg vestigial
Littoral, a few species planktonic	Planktonic, rarely littoral	Exclusively littoral, on macro-vegetation and sediments

Figure 4.1

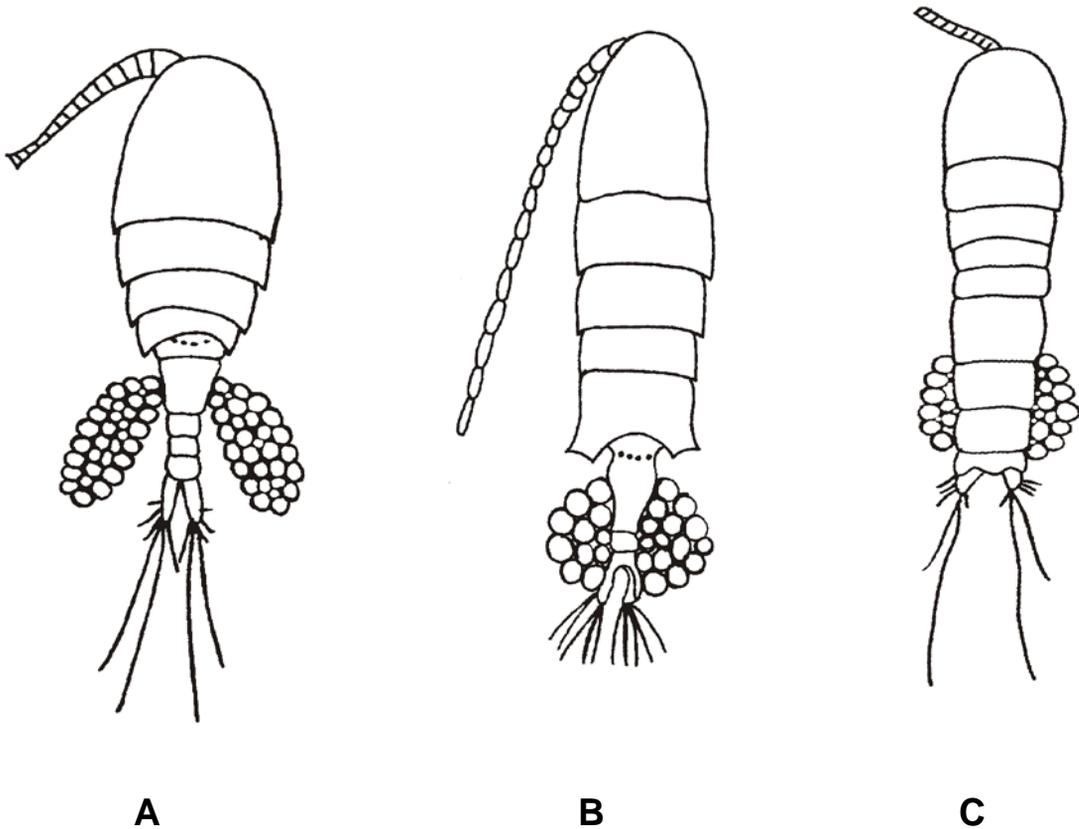


Diagram of typical free-living freshwater copepods, dorsal views of three orders namely Harpacticoida, Calanoida and Cyclopoida, illustrating diagnostic differences. Redrawn from Rayner (2001).

A = Female **cyclopoid** copepod with distinguishing anterior part of body broader than posterior part, and two laterally carried egg sacs.

B = Female **calanoid** copepod with distinguishing antennae longer than body, and single medially carried egg sac.

C = Female **harpacticoid** copepod with distinguishing short antennae, and single medially carried egg sac.

4.2 Crustacean Hosts of Ciliophorans

In the present study, ciliophorans were only found associated with cyclopoid copepods and freshwater crayfish species. For the remainder of this chapter I will discuss these hosts in more detail.

4.2.1 Cyclopoid Copepods

Some of the species of **Cyclopoida** (Table 4.2) which occur in southern Africa have a worldwide distribution. Identification of cyclopoids to species level relies greatly on the characters of mature females, with supplementary information on the males. Copepodites do not have the full complement of segments in the appendages and can therefore not be used in species identification. The main distinguishing character between males and females is the antennules of the males, which are bilaterally geniculated (Figs 4.2 A & B). The female carries paired egg sacs, which is also a good distinguishing characteristic, as calanoids and harpacticoids usually only have one egg sac. The number of segments in the antennules of adult females is consistent for a particular genus. Characters which are important in cyclopoid identification are:

- body length
- number of segments in antennules of female
- length of female antennules relative to prosome length
- structure of female fifth legs
- shape of receptaculum seminis
- the length: breadth ratio of furcal rami
- the relative lengths of furcal terminal setae
- and the position of lateral setae on the furcal rami

The spine formula refers to the number of spines on the last segment of the exopodite of swimming legs 1-4 and is written for example as: *Mesocyclops* sp. 2:3:3:3.

Figure 4.2

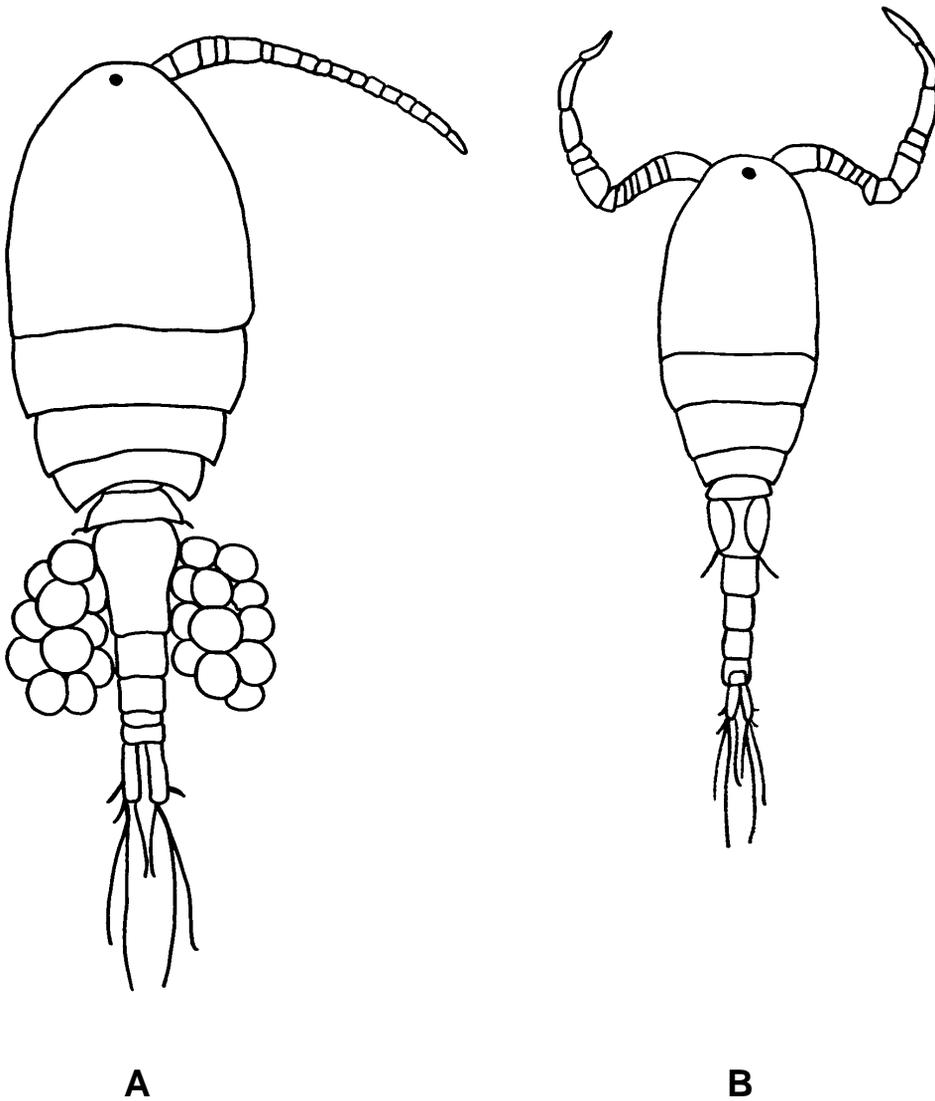


Diagram of typical free-living freshwater cyclopoid copepods, dorsal views of male and female copepods, illustrating sexual differences. Redrawn from Rayner (2001).

A = Female cyclopoid copepod with continuous segmented antennae.

B = Male cyclopoid copepod with bilaterally geniculated antennae.

Table 4.2: Summary of the Genera and Species of the Subclass **Cyclopoida** Burmeister, 1835 occurring in Southern Africa, compiled from Rayner (2001).

Genus & Species	Total Length	Geographic distribution
Genus 1: <i>Acanthocyclops</i> Kiefer, 1927		
<i>A. vernalis</i> Fischer, 1853	Female 1.2 – 1.7 mm	Cosmopolitan
Genus 2: <i>Ectocyclops</i> Brady, 1904		
<i>E. phaleratus</i> (Koch, 1838)	Female 0.86 mm	Cosmopolitan. In southern Africa recorded from South Western Cape & Vaal River Catchments.
Genus 3: <i>Eucyclops</i> Clauss, 1893		
<i>Eucyclops (Afrocyclops) gibsoni</i> (Brady, 1904)	Female 1.06 mm	South Western Cape, Okavango River, Vaal River Catchment & Namibia.
<i>E. sublaevis</i> (Sars, 1927)	Female 1 – 1.3 mm	Botswana, Cape Flats, Vaal River Catchment.
<i>E. serrulatus</i> (Fischer, 1860)	Female 0.9 – 1.4 mm	Cosmopolitan. In southern Africa only from South Western Cape.
Genus 4: <i>Macrocyclus</i> Clauss, 1893		
<i>M. albidus</i> (Jurine, 1820)	Female 1.8 – 2.5 mm	Cosmopolitan. In southern Africa: Linyande River, Okavango River, Vaal River Catchment & Western Cape.
Genus 5: <i>Mesocyclops</i> Sars, 1914		
<i>M. major</i> Sars, 1927	Female 1.1 – 1.5 mm	Widely distributed in southern Africa: Cape Peninsula, KwaZulu Natal, Eastern Cape, Free State, Gauteng, Namibia & Zimbabwe.
Genus 6: <i>Metacyclops</i> Kiefer, 1927		
<i>M. necessaries</i> Kiefer, 1914 [syn. <i>Cryptocyclops assimilis</i> Sars, 1927]	Female 0.9 – 1 mm	Cape Flats
Genus 7: <i>Microcyclops</i> Clauss, 1893		
<i>M. crassipes</i> (Sars, 1927)	Female 0.6 – 0.8 mm	South Western Cape
<i>M. imopinatus</i> (Sars, 1927)	Female 0.7 mm	Namibia

Chapter 4: Freshwater Crustacean hosts

Table 4.2 continue: Summary of the Genera and Species of the Subclass **Cyclopoida** Burmeister, 1835 occurring in Southern Africa, compiled from Rayner (2001).

Genus & Species	Total Length	Geographic distribution
Genus 8: <i>Paracyclops</i> Claus, 1893		
<i>P. poppei</i> (Rehberg, 1880)	Female 0.54 mm	Cosmopolitan. In Southern Africa only from Cape Peninsula.
<i>P. fimbriatus</i> (Fischer, 1853)	Female 0.87 mm	Cosmopolitan. In Southern Africa recorded from South Western Cape & Vaal River catchments.
Genus 9: <i>Thermocyclops</i> Kiefer, 1927		
<i>T. oblongatus</i> Sars, 1927	Female 0.9 mm	Widely distributed in Southern Africa particularly in the southern region of Western Cape & KwaZulu Natal, but is probably also confused with other African species of <i>Thermocyclops</i> namely <i>T. neglectus</i> , <i>T. schuurmanae</i> , <i>T. macracanthus</i> , <i>T. infrequens</i> , <i>T. retroversus</i> and <i>T. emini</i> .
<i>T. macracanthus</i> Kiefer, 1929	Female 0.85 – 1mm	Namibia, Free State
<i>T. schuurmanae</i> Kiefer, 1928	Female 0.88mm	South Western Cape
<i>T. emini</i> (Mrazek, 1895)	Female 0.9mm	Pongola Floodplain, Zululand
Genus 10: <i>Tropocyclops</i> Kiefer, 1927		
<i>T. prasinus</i> (Fischer, 1860)	Female 0.5 – 0.9mm	Cosmopolitan. In Southern Africa only from South Western Cape.

Identification of the copepod host in the present study

The cyclopoid copepods in the present study were identified as a member of the genus *Eucyclops* Claus, 1893 using the key from Pennak (1978).

General Composition of Plankton Samples in the Present Study

The animal component of freshwater plankton includes a wide spectrum of organisms:

- *Volvox* sp.
- Protozoans like *Amoeba* sp. and *Paramecium* sp.
- *Hydra* sp.
- *Moina* sp.
- *Daphnia* sp.
- Insect larvae, including representatives of the Odonata, Coleoptera and Diptera
- Aquatic insects for example representatives of the Notonectidae
- Anuran tadpoles of the genus *Xenopus*

Seasonality, temperature and water quality play a significant role in the composition of a plankton sample, although some species like *Daphnia* are almost always present.

4.2.2 Freshwater Crayfish

Crayfish belong to the largest crustacean order, the Decapoda, which has approximately 1200 genera and 10 000 species. Decapods are distinguished from other malacostracans, in that the first three pairs of thoracic appendages are modified as maxillipeds. The remaining five pairs of thoracic appendages are legs, from which the name decapod is derived (Rupert & Barnes 1994).

Crayfish belong to the same infraorder (Astacoidea) as the marine lobsters (superfamily Nephropoidea), which appear to be their closest relatives. Two superfamilies of crayfish are recognised, the Astacoidea with two families and the monotypic Parastacoidea. Of the three crayfish families, the Astacoidea and Cambaridea occur in the Northern Hemisphere, whilst the Parastacoidea are confined to the Southern Hemisphere (Hobbs 1988).

In some underdeveloped parts of the world native crayfish are a subsistence food. Introduced species are not always accepted by local inhabitants, but in more affluent countries, they and native species, are often popular as luxury food items. Such introduced species may cause local problems with disruption of established wildlife or the introduction of disease and parasites (Holdich & Lowery 1988).

According to Hobbs (1988) the classification of freshwater crayfish is:

Subphylum: Crustacea

Class: Malacostraca

Order: Decapoda

Infraorder: Astacidea Latreille, 1802

Superfamily: Parastacoidea Huxley, 1879

Family: Parastacidea Huxley, 1879

Genus: *Cherax* Erichson, 1846

The group of crayfish which exhibits the widest range of adaptations to adverse habitat conditions are representatives of the Parastacidae of Australia. There are over 100 species of crayfish in 14 genera native to Australia alone and these may be broadly subdivided into the aquatic, semi-aquatic and terrestrial species.

The aquatic species generally inhabit running water and belong to the genera *Eustacus* and *Astacopsis*. These crayfish rarely burrow, although some are capable of doing so if water levels drop. Semi-aquatic species inhabit streams, lakes, dams and quarry holes but when these dry, they burrow to the water table until the next rainfall.

The Genus *Cherax* Erichson, 1846

Of the parastacids, the species group having the most described species and subspecies is the genus *Cherax*, which also has the largest geographic range of any of the parastacid genera. In Australia it is well represented in the temperate region of the country, whilst an isolated eight species occur in the extreme south-western part of Western Australia.

Cherax is also common in South Australia, Victoria, New South Wales and South Eastern Queensland. There, 11 of the 13 species are endemic in the eastern parts of Queensland and New South Wales. Members of this genus have also entered the tropics of the continent.

The most common and well known species are the “yabby” – *C. destructor* and “marron” – *C. tenuimanus* Smith, 1912. Of these the former is a very good digger, whereas the latter is less so and consequently prefers to inhabit permanent water bodies.

All members of the genus are at least part-time inhabitants of streams, lakes, ponds or temporary pools and some are able burrowers. *Cherax destructor* has penetrated far inland, inhabiting mainly flood-plain habitats – swamps, lakes, creeks, farm ponds and irrigation channels. When water temperature drops during the winter, *C. destructor* burrows into the mud and seals the entrance with mud when water levels drop. *Cherax destructor* has been recorded alive in holes beneath dry lake beds which had not been filled for years (Frost 1975).

These species can live for considerable periods without water and may travel overland from one water body to another. Indeed they have been found in artificial ponds miles from the nearest population (Clark 1936). Clearly these crayfish are able to withstand the great physiological stress imposed by inhabiting temporary inland water bodies and are considered to be the hardest of the Australian genera of crayfish (Bishop 1967).

The Situation in Africa

There are no known natural populations of freshwater crayfish in Africa. The only species known from the continent are the introduced *Cherax* species, utilised in crayfish farming. Several freshwater crayfish farms exist in South Africa, including farms in the Western- and Northern Cape regions and a single farm in the Free State. Freshwater crayfish are bred and kept in heated tanks for the purpose of exporting either as pets or delicatessens.

A number of parasites found associated with *C. destructor* (Fig 4.3 A & B) and *C. quadricarinatus* (Fig 4.3 C & D). These are listed below:

- Intracellular bacterium – Ecuador (Jimenez & Romero 1997).
- Two species of opportunistic oomycetous fungi, infecting crayfish, eggs and larvae – Mitchell River, Northern Queensland (Herbert 1987).
- Microsporidian, *Thelohania* sp. restricted to the striated and cardiac muscle - Mitchell River, Northern Queensland (Herbert 1987).
- Peritrich ciliophorans of the genera *Zoothamnium* and *Vorticella* found laterally on the cephalothorax – Mitchell River, Northern Queensland (Herbert 1987).
- Loricated ciliophorans of the genus *Lagenophrys* found on the gills - Mitchell River, Northern Queensland (Herbert 1987).
- Ciliophorans of the *Tetrahymena pyriformis* complex – North Queensland (Edgerton, O'Donoghue, Wingfield & Owens 1996).
- Platyhelminthes including, *Diceratocephala* on the ventral surface of the abdomen and *Notodactylus* attached to the upper surface of the cephalothorax and rostrum - Mitchell River, Northern Queensland (Herbert 1987).
- *Diceratocephala boschmai* (Platyhelminthes; Temnocephalida) – Brisbane, Australia (Jones & Lester 1993).
- Commensal nematodes found on the gills - Mitchell River, Northern Queensland (Herbert 1987).

The turbellarian *Temnocephala chaeropsis* is one of the most common symbionts found associated with freshwater crayfish. Temnocephalans are hermaphroditic and capable of self fertilization, so a viable population can start from a single egg. Heavy temnocephalan infestations affect the marketability of the crayfish and death occurs in some extreme cases when body fluids have been reported to ooze from the thorax and abdomen from infested individuals (Avenant-Oldewage 1993).

In South Africa the temnocephalid *Temnocephala chaeropsis* has been found associated with *Cherax* on several occasions (Mitchell & Kok 1988; Avenant-Oldewage 1993). Temnocephalids were also found on the carapace of *C. destructor* and *C. quadricarinatus* in the present study, but since this parasite falls outside the spectrum of the present study it is therefore not discussed in detail.

If one considers this list of known parasites associated with freshwater crayfish globally as well as the destructive nature of *Cherax* spp., it becomes evident that introducing these crayfish (with their parasites) into our natural water systems poses a significant threat to our ecosystems. This phenomenon should therefore be monitored with extreme caution.



Fig 4.3 Dorsal (A) and ventral (B) view of a female individual of *Cherax destructor* Clark, 1936 with its well developed, robust claw. Dorsal view of female individual (C & D) of *Cherax quadricarinatus* Von Maartens, 1868 with the typical red coloration on the claw.

The association between epistylids and freshwater crustaceans is not uncommon and is found in abundance in almost any water body one can imagine. According to Fernandez-Leborans & Tato-Porto (2000) (Table 3.1) there are 45 *Epistylis* species associated with either freshwater/brackish or marine crustaceans. Although there are several other symbionts also found associated with freshwater crustaceans, for the purpose of the present study, emphasis was placed on freshwater copepods belonging to the genus *Eucyclops* and the introduced freshwater crayfish, *Cherax destructor* and *C. quadricarinatus*.

5.1 Epistylids found on Freshwater Planktonic Copepods

According to Foissner *et al.* (1999) and Fernandez-Leborans & Tato-Porto (2000) there are 13 *Epistylis* species associated with freshwater planktonic copepods i.e.:

- ***Epistylis anastatica*** (Linnaeus, 1767) Ehrenberg, 1830
- ***E. breviramosa*** Stiller, 1931
- ***E. daphniae*** Fauré-Fremiet, 1905
- ***E. diaptomi*** Fauré-Fremiet, 1905
- ***E. digitalis*** (Linnaeus, 1758) Ehrenberg, 1830
- ***E. halophila*** Stiller, 1942
- ***E. harpacticola*** Kahl, 1933
- ***E. lacustris*** Imhoff, 1884
- ***E. niagarae*** Kellicott, 1883
- ***E. nympharum*** Engelmann, 1862
- ***E. plicatilis*** Ehrenberg, 1831
- ***E. procumbens*** Zacharias, 1897
- ***E. pygmaeum*** (Ehrenberg, 1838) Foissner *et al.*, 1999

This list can be narrowed down to nine *Epistylis* species specifically associated with cyclopoid copepods, covering the spectrum of the present study. These known euplanktonic *Epistylis* species, the hosts, location, habitat and morphological features, are summarised below.

Compendium of *Epistylis* species associated with Cyclopoid Copepods.

<i>Epistylis anastatica</i> (Linnaeus, 1767) Ehrenberg, 1830	
Hosts & Locality: Cyclopoid planktonic copepods & aquatic plants in several European countries, Far East and East Africa.	
Body features	Colonies with many zooids, all zooids near the same level. Extended zooids <i>in vivo</i> 60 – 100 µm long, length 2 – 2.5 times width, slightly conical. Contracted specimens ellipsoidal to pyriform.
Site of attachment	All body regions, some preference for rear legs.
Nucleus	Macronucleus in longitudinal axis of cell, slightly curved to C-shaped.
Striations	Pellicle with ± 75 distinct transverse striations, 49 – 58 adoral from telotroch band to peristomial disc, 17 – 26 aboral from telotroch band to stalk.
Infundibulum	Occupies anterior end of cell, peristomial collar narrow, about as wide as body, not distinctly projecting beyond body proper. Peristomial disc slightly convex, asymmetrically raised in feeding specimens. Infundibulum of usual size, extends asymmetrical to middle third of cell.
Stalk	Dichotomously branching, acontractile (myoneme lacking) and smooth, 8 – 12 µm across, last branching up to length of zooids, main stalk often rather long.
Contractile vacuole	Slightly underneath peristomial collar at ventral wall of infundibulum.
Reference	Foissner, Berger & Schaumburg (1999)



Fig 5.1 A: Illustration of *E. anastatica*, redrawn from Foissner *et al.* (1999)

Compendium continued

***Epistylis breviramosa* Stiller, 1931**

Hosts & Locality: Cladoceran *Daphnia* sp. in Hungary and cyclopoid copepod *Cyclops* sp. in the Czech Republic.

Body features	Extended zooids <i>in vivo</i> 40 – 60 µm long. Colonies rarely have more than 4 individuals.
Site of attachment	Antennal filament and carapace.
Nucleus	Unknown.
Striations	Pellicle very weakly striated.
Infundibulum	Unknown.
Stalk	Main colony stalk not much longer than zooids and individual stalks very short.
Contractile vacuole	Unknown.
Reference	Green (1974)

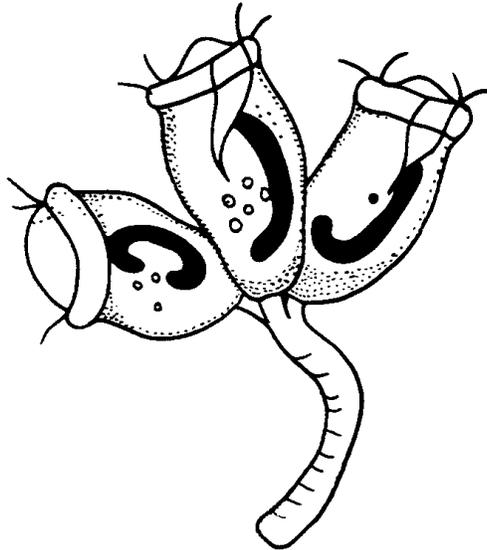


Fig 5.1 B: Illustration of *E. breviramosa*, redrawn from Stiller (1931)

Compendium continued

Epistylis digitalis (Linnaeus, 1768) Ehrenberg, 1830

Hosts & Locality: *Eucyclops serrulatus* in the former USSR. Mites in Germany. *Cyclops* sp. in Bavaria, Macedonia and USA. *Canthocampus* in Germany and the Czech Republic.

Body features	Colonies large, up to 1.6 mm high. Conical, all zooids near the same level. Extended zooids <i>in vivo</i> 60 – 120 µm long, length 3 – 4 times width. Contracted specimens pyriform, anterior end projecting snout-like.
Site of attachment	Thoracal leg, abdomen, furca, rostrum and antennae.
Nucleus	Macronucleus in longitudinal axis of cell slightly curved to C-shaped.
Striations	Pellicle distinct transverse striations, number of striae unknown.
Infundibulum	Occupies anterior end of cell, peristomial collar, about as wide as body, not distinctly projecting. Peristomial disc slightly convex, asymmetrically raised in feeding specimens. Infundibulum inconspicuous, about ¼ of body. Peniculli 1 & 3 distinctly longer than peniculus 2.
Stalk	Dichotomously branching, acontractile (myoneme lacking) and annulated, about 10 µm across. Last branching up to length of zooids, sometimes very short, giving the impression of two zooids attached to single broad stalk.
Contractile vacuole	Slightly underneath peristomial collar at ventral wall of infundibulum.
Reference	Foissner <i>et al.</i> (1999)

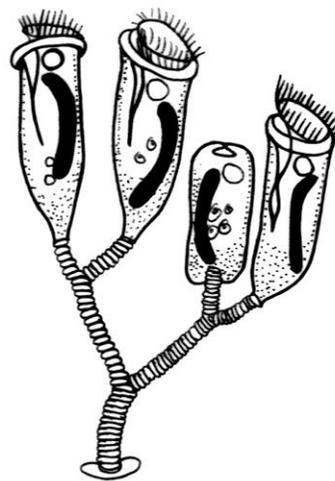


Fig 5.1 C: Illustration of *E. digitalis*, redrawn from Foissner *et al.* (1999)

Compendium continued

Epistylis lacustris Imhoff, 1884

Hosts & Locality: Copepod *Cyclops* sp, branchiopod *Lepidurus apus* in Austria, crayfish *Cambarellus patzcuarensis* in Mexico.

Body features	Extended zooids <i>in vivo</i> 60 – 100 µm, slightly conical to barrel-shaped.
Site of attachment	Exoskeleton and buccal appendages.
Nucleus	Macronucleus C-shaped.
Striations	Unknown.
Infundibulum	Unknown.
Stalk	Knobs and joints at branching.
Contractile vacuole	Ventrally located.
Reference	Foissner <i>et al.</i> (1999)

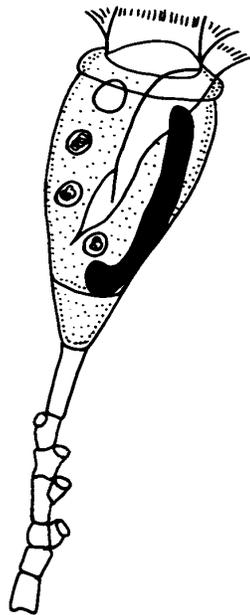


Fig 5.1 D: Illustration of *E. lacustris*, redrawn from Viljoen (1987)

Compendium continued

***Epistylis niagarae* Kellicott, 1883**

Hosts & Locality: Crayfish *Astacus leptodactylus*, *Austropotamobius torrentium* and *Orconectes limosus*. Copepod *Eucyclops serrulatus*, Cladocerans *Daphnia pulex*, *D. rosea*, *Ceriodaphnia reticulata* and *Scapholeberis mucronata* in USA and Mexico.

Body features	Extended zooid urn-shaped with prominent peristomial collar with wavy, crenulated edge. Peristomial disc flattened, forming slight angle with top of peristomial disc. Large colonies with up to 40 – 50 individuals per colony. Contracted specimens pyriform, anterior end projecting snout-like.
Site of attachment	Exoskeleton, carapace and antennae.
Nucleus	Macronucleus band formed in transverse position, enfolding infundibulum. Positioned in anterior third of body with ends directed posteriorly.
Striations	Unknown.
Infundibulum	Spirals clockwise as seen from anterior end. Tapering tube extending almost to the posterior end of body. Food vacuoles spherical, large and not numerous.
Stalk	All branches of stalk longer than body length except terminal branches. Faintly striated longitudinally.
Contractile vacuole	Lies in anterior fourth of body – opening into infundibulum.
Reference	Bishop & Jahn (1941)



Fig 5.1 E: Illustration of *E. niagarae*, redrawn from Viljoen (1987).

Compendium continued

***Epistylis nympharum* Engelmann, 1862**

Hosts & Locality: Cladocerans, *Cyclops* sp. Locality unknown, Branchiuran *Dolops ranarum* in South Africa.

Body features Extended zooid *in vivo* 60 – 150 μm , length 2 – 2.5 times width, slender, bell to barrel shaped. Contacted specimens oval-shaped. Colonies small, 4 – 8 zooids per colony, rarely up to 12 zooids per colony.

Site of attachment Carapace and appendages.

Nucleus Macronucleus sausage-shaped, in the longitudinal axis of the cell, not enfolding the infundibulum.

Striations Pellicle faintly striated, 44 – 61 adoral from telotroch band to peristomial disc, 17 – 26 aboral from telotroch band to stalk.

Infundibulum Unknown.

Stalk Dichotomously branching, acontractile (myoneme lacking) and annulated, about 12 μm across. Bifurcated in colonial forms, short, less than one third of zooid length.

Contractile vacuole Posterior to peristomial collar at the ventral wall of the infundibulum.

Reference Foissner, Berger & Kohmann (1992)

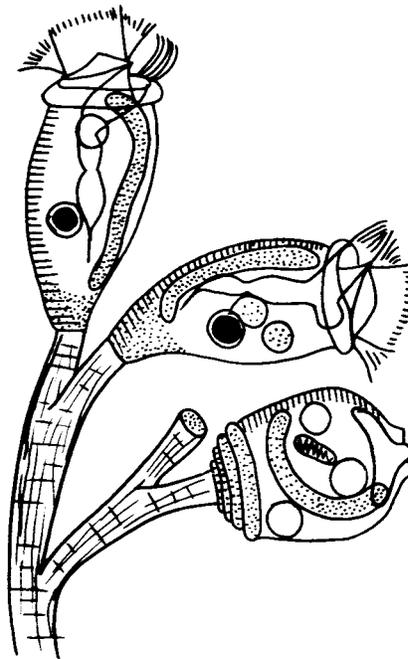


Fig 5.1 F: Illustration of *E. nympharum*, redrawn from Foissner *et al.* (1992)

Compendium continued

***Epistylis plicatilis* Ehrenberg, 1838**

Hosts & Locality: Planktonic copepods: *Eucyclops agilis*, *Cyclops vernalis* and *C. bicuspidatus* in USA, several European countries and the former USSR.

Body features	Extended zooids <i>in vivo</i> 90 – 160 µm x 25 – 50 µm, slender funnel-shaped. Contracted specimens pyriform, anterior end projecting snout-like. Colonies large, up to 4 mm high.
Site of attachment	Carapace and appendages.
Nucleus	Kidney-shaped to semi-circular, enfolding infundibulum.
Striations	Pellicle with distinct transverse striations, 110 - 123 adoral from telotroch band to peristomial disc, 68 - 83 aboral from telotroch band to stalk.
Infundibulum	Peristomial collar 36 – 60 µm in diameter, protruding distinctly over body. Adoral ciliary spiral asymmetrically placed.
Stalk	Dichotomously branching, acontractile (myoneme lacking). Longitudinal striations.
Contractile vacuole	At level of peristomial collar at dorsal wall of infundibulum.
Reference	Foissner <i>et al.</i> (1992)

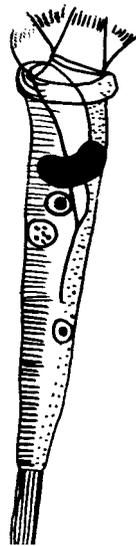


Fig 5.1 G: Illustration of *E. plicatilis*, redrawn from Foissner *et al.* (1992).

Compendium continued

***Epistylis procumbens* Zacharias, 1897**

Host & Locality: Common in summer plankton of lakes and ponds of several European countries, Far East, USA and the former USSR, but also in large running waters, prefers warm season.

Body features	Extended zooids <i>in vivo</i> 60 - 140 μm long, length 2 - 2.5 times width, conspicuously slender, posterior portion narrowed stalk-like. Contracted specimens pyriform and distinctly folded.
Site of attachment	Carapace and appendages.
Nucleus	Macronucleus in transverse axis & anterior half of cell, semicircular.
Striations	Pellicle with \pm 137 indistinct, narrow transverse striations, 62 - 80 adoral from telotroch band to peristomial disc, 52 - 80 aboral from telotroch band to stalk.
Infundibulum	Occupies anterior end of cell, peristomial collar thin, wider than body proper, distinctly projecting. Peristomial disc flat or slightly convex, asymmetrically raised in feeding specimens. Ciliary pattern without peculiarities, except for short basal body row at distal end of adoral ciliary spiral.
Stalk	Dichotomously branching, acontractile (myoneme lacking). Surface smooth with distinct transverse lines, marking separation sites.
Contractile vacuole	At level of peristomial collar at dorsal wall of infundibulum.
Reference	Foissner <i>et al.</i> (1999)

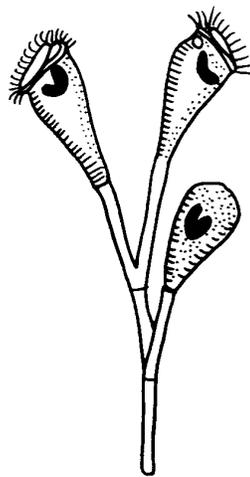


Fig 5.1 H: Illustration of *E. procumbens*, redrawn from Foissner *et al.* (1999)

Compendium continued

Epistylis pygmaeum (Ehrenberg, 1838) Foissner *et al.*, 1999

Hosts & Locality: Planktonic copepods, including cladocerans, copepods and ostracods, rotifers, daphnids, gills of mayfly larvae, aquatic plants in several European countries, Far East and the USA.

Body features	Solitary, colonies if present, composed of 2 – 3 zooids. Extended zooids <i>in vivo</i> 22 - 50 µm long, length 1.3 times width, pyriform, goblet-shaped, almost cylindrical. Contracted specimens ellipsoidal to globular.
Site of attachment	Carapace and appendages.
Nucleus	Macronucleus in transverse axis & anterior half of cell, semicircular.
Striations	Pellicle with ± 30 distinct, transverse striations, 15 - 22 adoral from telotroch band to peristomial disc, 10 - 14 aboral from telotroch band to stalk.
Infundibulum	Occupies anterior end of cell, peristomial collar distinctly narrower to as wide as body, never projecting beyond body proper. Peristomial disc flat or slightly convex, asymmetrically raised in feeding specimens. Infundibulum of usual size.
Stalk	Stalk occasionally dichotomously branched, usually unbranched, acontractile (myoneme lacking) and smooth.
Contractile vacuole	Underneath peristomial collar at dorsal wall of infundibulum.
References	Foissner <i>et al.</i> (1999)

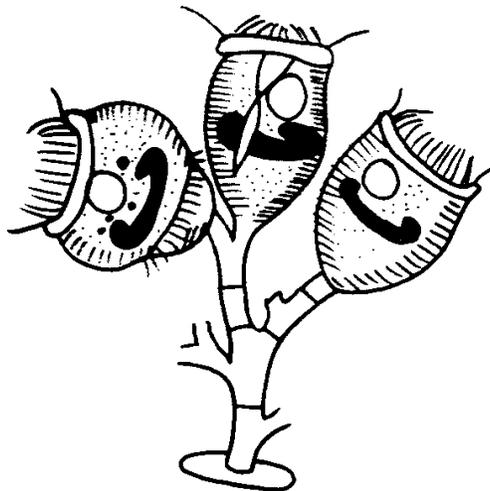


Fig 5.1 I: Illustration of *E. pygmaeum*, redrawn from Foissner *et al.* (1999)

5.2 *Epistylis* sp. associated with *Eucyclops* Claus, 1936 sp.

Taxonomic description and morphology of *Epistylis* sp. A

Hosts: *Eucyclops* Claus, 1936 sp.

Position on host: Carapace and appendages

Locality: Loch Logan Waterfront, Bloemfontein

Reference Material: 2003/10/07-07 LK (protargol)

2003/07/23-03 (haematoxylin)

Biometrical data of *Epistylis* sp. A

Length of fully expanded zooid: 44 – 80 μm (64 ± 9.1 , 28)

Diameter of fully expanded zooid: 21 – 37 μm (28 ± 4.2 , 28)

Macronucleus length: [28 - 68 μm (42 ± 8.7 , 40)]

Macronucleus diameter: [3 – 6 μm (4 ± 0.91 , 40)]

Micronucleus length: [1.5 – 4 μm (2.3 ± 0.5 , 40)]

Micronucleus diameter: [1 – 3 μm (2.1 ± 0.1 , 40)]

Total Pellicular striations (SEM): 62 – 67 (65 ± 1.7 , 11)

Pellicular striations below telotroch band (SEM): 12 – 18 (15 ± 2.01 , 11)

Pellicular striations above telotroch band (SEM): 46 – 53 (50.3 ± 1.8 , 11)

Total Pellicular striations (AgNO₃): 58 – 75 (64 ± 4.9 , 14)

Pellicular striations below telotroch band (AgNO₃): 13 – 24 (16 ± 2.8 , 14)

Pellicular striations above telotroch band (AgNO₃): 43 – 53 (47 ± 3.1 , 14)

Description

Colonies large, many zooids per colony (Figs 5.3 A-H). Zooids attached to bifurcated, dichotomously branched stalks (Figs 5.3 G & H; 5.6 C). Stalks very long, 3 – 4 times zooid length when fully expanded (Figs 5.3 G & H). Colonies found on various body regions, especially antennal, carapace, swimming legs and abdominal segment regions (Figs 5.3 A-E; 5.5 A-D).

Zooids elongated, slightly funnel-shaped (Figs 5.3 F-H; 5.4 A-E). Contracted zooids oval in shape (Figs 5.4 F; 5.6 B), peristomial lip closed and cilia pulled into

body (Figs 5.4 F; 5.6 B). Zooid encircled with pellicle striations approximately 0.75 μm apart. Striations evenly spaced and uniform (Figs 5.6 A & B; 5.7 E & F). A very distinct and well developed myoneme system could be seen, with myonemes extending from the adoral to the aboral region of the zooid (Figs 5.2 B; 5.7 C & D).

Cytoplasm granular, with food vacuoles near infundibulum, contractile vacuoles suspended in cytoplasm (Figs 5.4 A, C & D). **Peristomial disc** slightly convex, not protruding clearly from body proper (Figs 5.4 A-D; 5.6 D), whilst peristomial edge is broad, not clearly indented with a single peristomial lip (Figs 5.2 A; 5.4 C & E).

Nuclear apparatus extends from aboral to adoral region of zooid. Macronucleus large, elongated, sausage-shaped – folding and spiralling in cytoplasm (Fig 5.7 A). Micronucleus small, round and situated near aboral end of macronucleus (Fig 5.7 B).

Infundibulum

Adoral spiral on peristome not clearly visible, due to body contraction of ciliophorans. Buccal apparatus fairly simple, situated anteriorly in body (Fig 5.2 C). Haplo- and polykinety plunges into the infundibulum out of phase, 180 $^{\circ}$ apart, each making a 360 $^{\circ}$ actinomorphic turn. Polykinety comprises two rows of kinetosomes running parallel along one another (Fig 5.2 C). Cytostome not clearly visible.

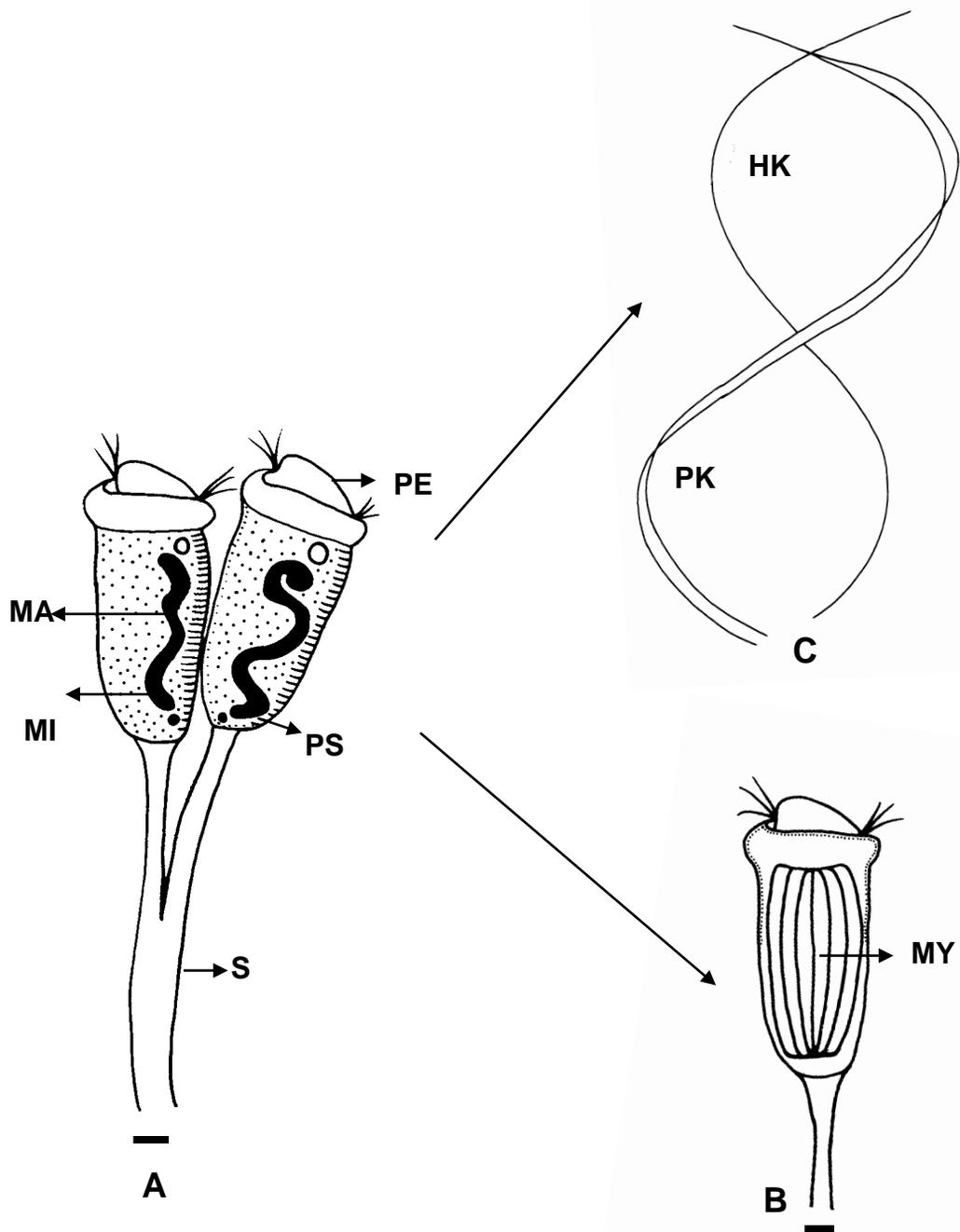


Fig 5.2 Microscope projection drawing of *Epistylis Epistylis* Ehrenberg, 1838 sp. A, associated with *Eucyclops* Clauss, 1893 sp. illustrating different morphological features (A), myoneme system (B) and the infundibulum (C).

HK - Haplokinety

MA – Macronucleus

MI – Micronucleus

MY – Myonemes

Scale bar: 20 μ m

PE – Peristomial edge

PK - Polykinety

PS – Pellicular striations

S – Stalk

Figure 5.3

Light micrographs of live specimens of *Epistylis* Ehrenberg, 1838 sp. A, associated with *Eucyclops* Claus, 1893 sp., collected from Loch Logan Waterfront, Bloemfontein, illustrating:

- A.** Colonies of *Epistylis* attached to the carapace of copepod host.
- B.** Copepod heavily infested with *Epistylis* colonies both on carapace and appendages.
- C.** Close look at copepod in B, colonies with relaxed zooids.
- D.** Copepod with multiple colonies on carapace.
- E.** Copepod with single *Epistylis* colony on carapace.
- F.** Closer look at single colony with relaxed zooids.
- G.** *Epistylis* colony, showing slightly funnel shaped zooids.
- H.** *Epistylis* colony, showing dichotomously branched stalks and relaxed zooids.

Scale bars: A – H:100 μ m

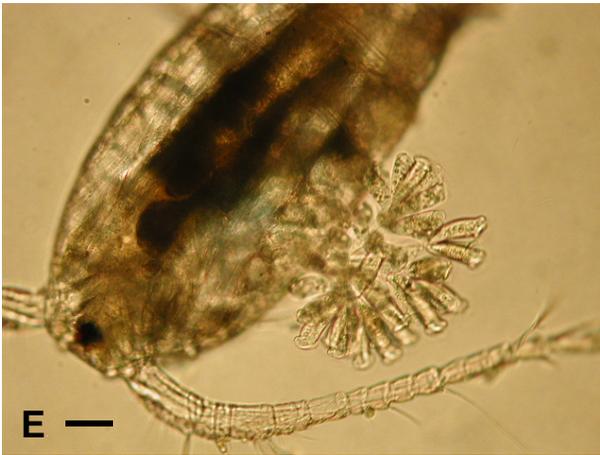
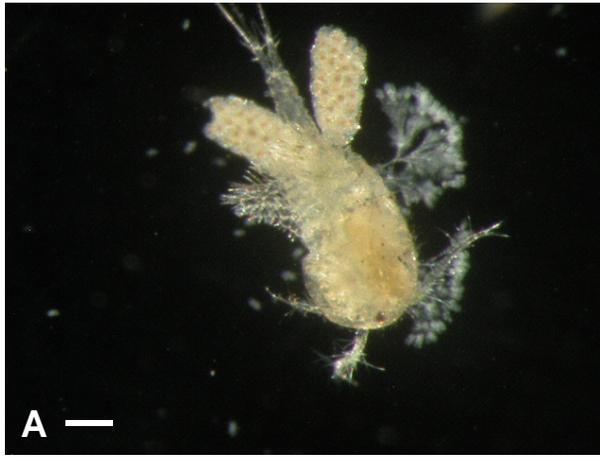


Figure 5.4

Light micrographs of live specimens of *Epistylis* Ehrenberg, 1838 sp. A, associated with *Eucyclops* Clauss, 1893 sp., collected from Loch Logan Waterfront, Bloemfontein, illustrating:

- A.** Colony of *Epistylis* , with several zooids attached to the carapace of copepod host.
- B.** Binary fission of zooids on carapace of copepod.
- C.** Multiple zooids of colony, with peristomial disc and associated cilia.
- D.** Closer look at two zooids, with pellicular striations.
- E.** Free-swimming zooid, with granular cytoplasm.
- F.** Two zooids, one slightly relaxed, one contracted.

Scale bars: A - F: 50 μ m

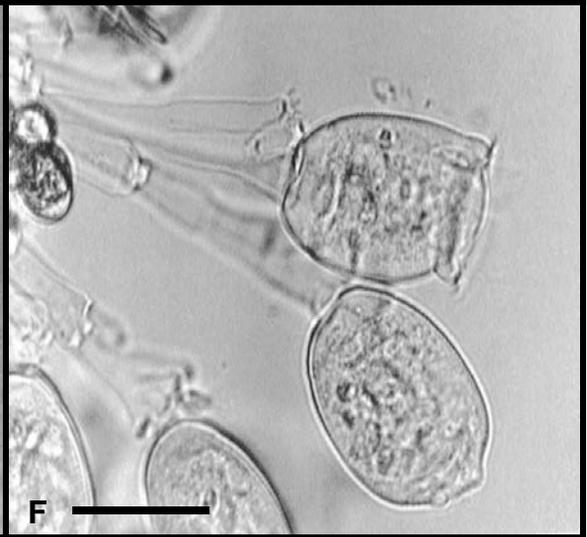
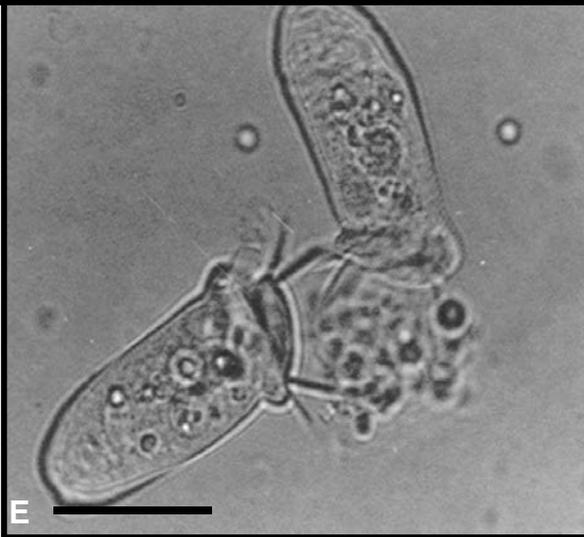
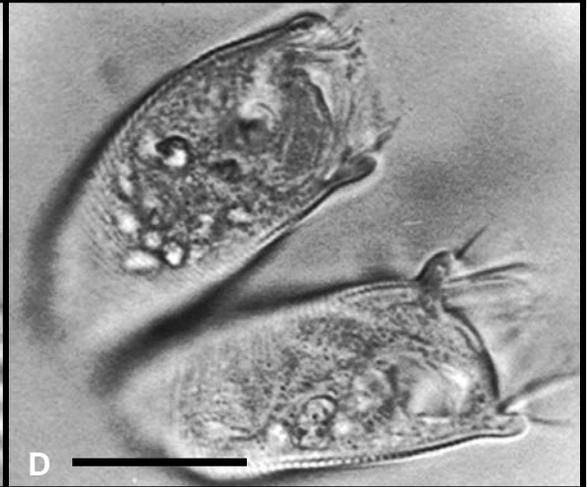
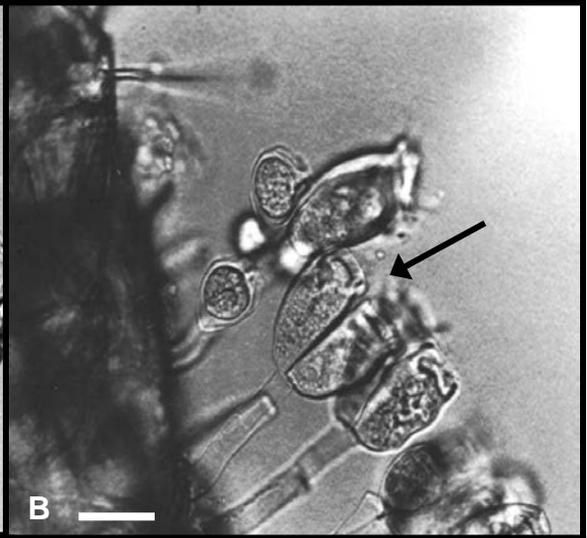
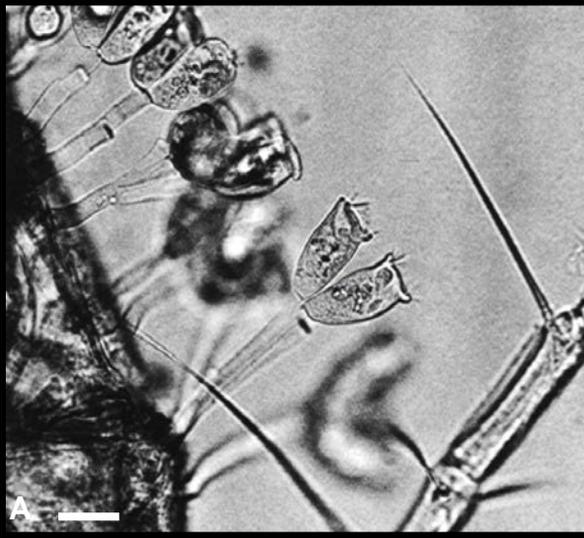


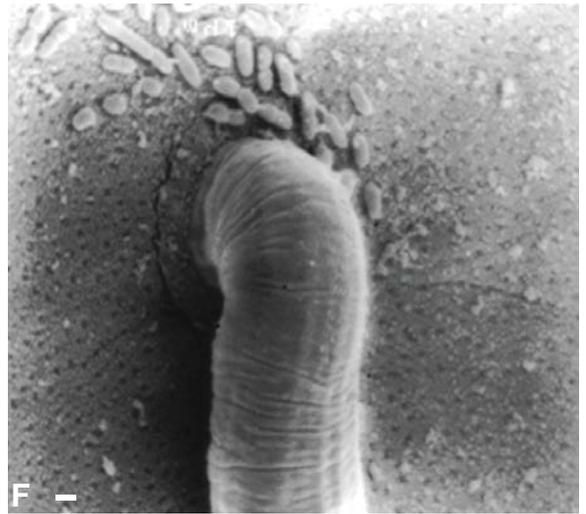
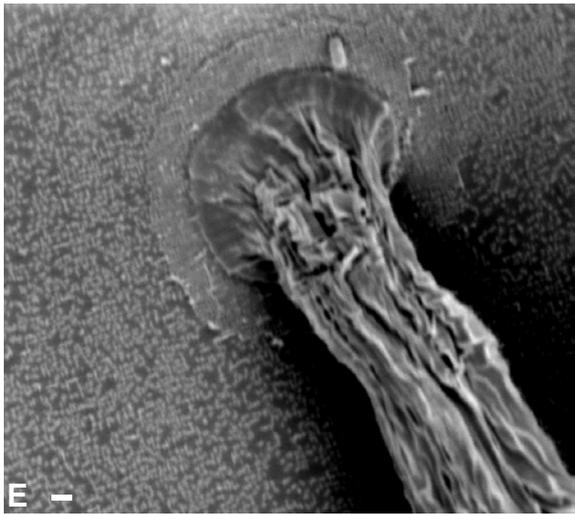
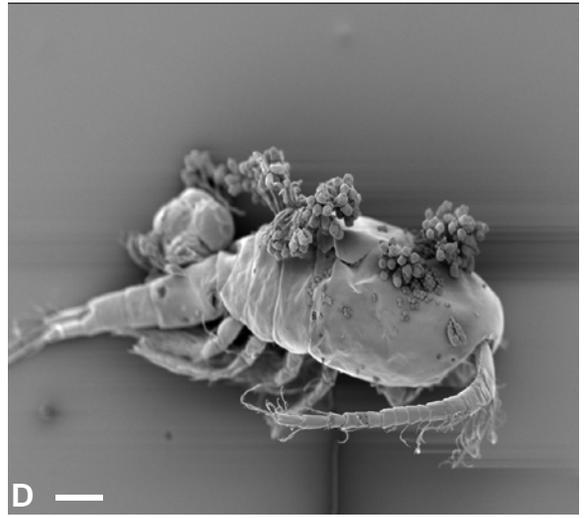
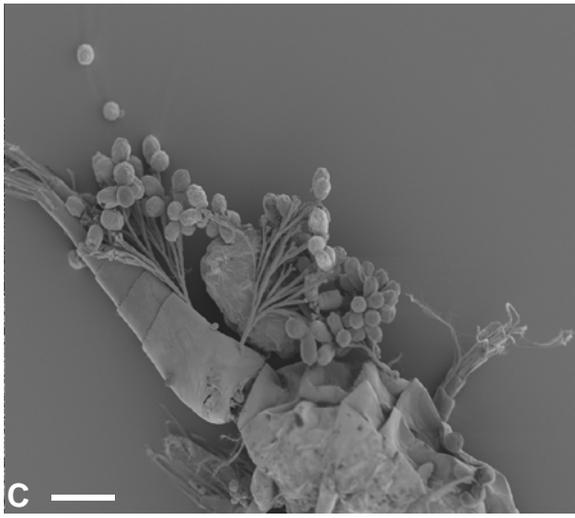
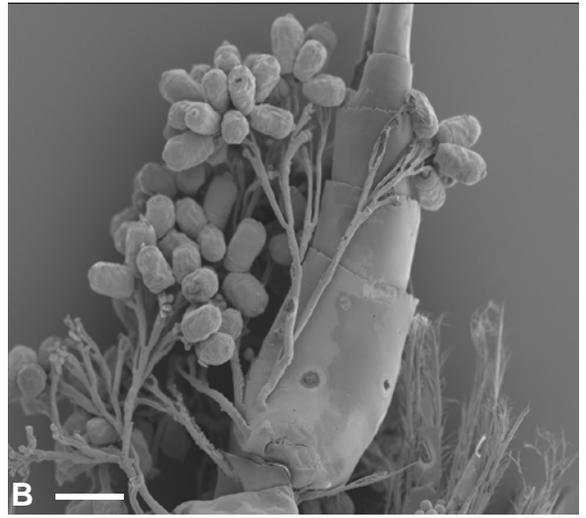
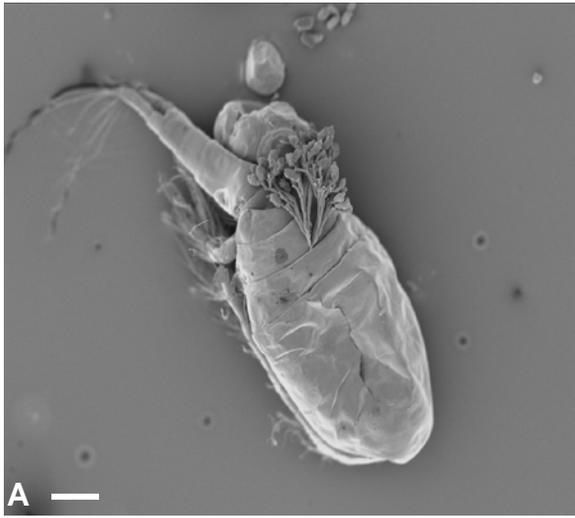
Figure 5.5

Scanning electron micrographs of *Epistylis* Ehrenberg, 1838 sp. A, associated with *Eucyclops* Claus, 1893 sp., collected from Loch Logan Waterfront, Bloemfontein, illustrating:

- A.** Colony of *Epistylis* attached to the carapace of copepod host.
- B.** Closer look at multiple colonies attached to abdominal segment of copepod.
- C.** Colonies of *Epistylis* attached to the carapace & abdomen of copepod host.
- D.** Multiple colonies attached dorsally to carapace of copepod.
- E.** Attachment of ciliophoran colony to body surface of copepod.
- F.** Attachment site with surrounding bacteria.

Scale bars: A - D: 100 μm

E & F: 1 μm



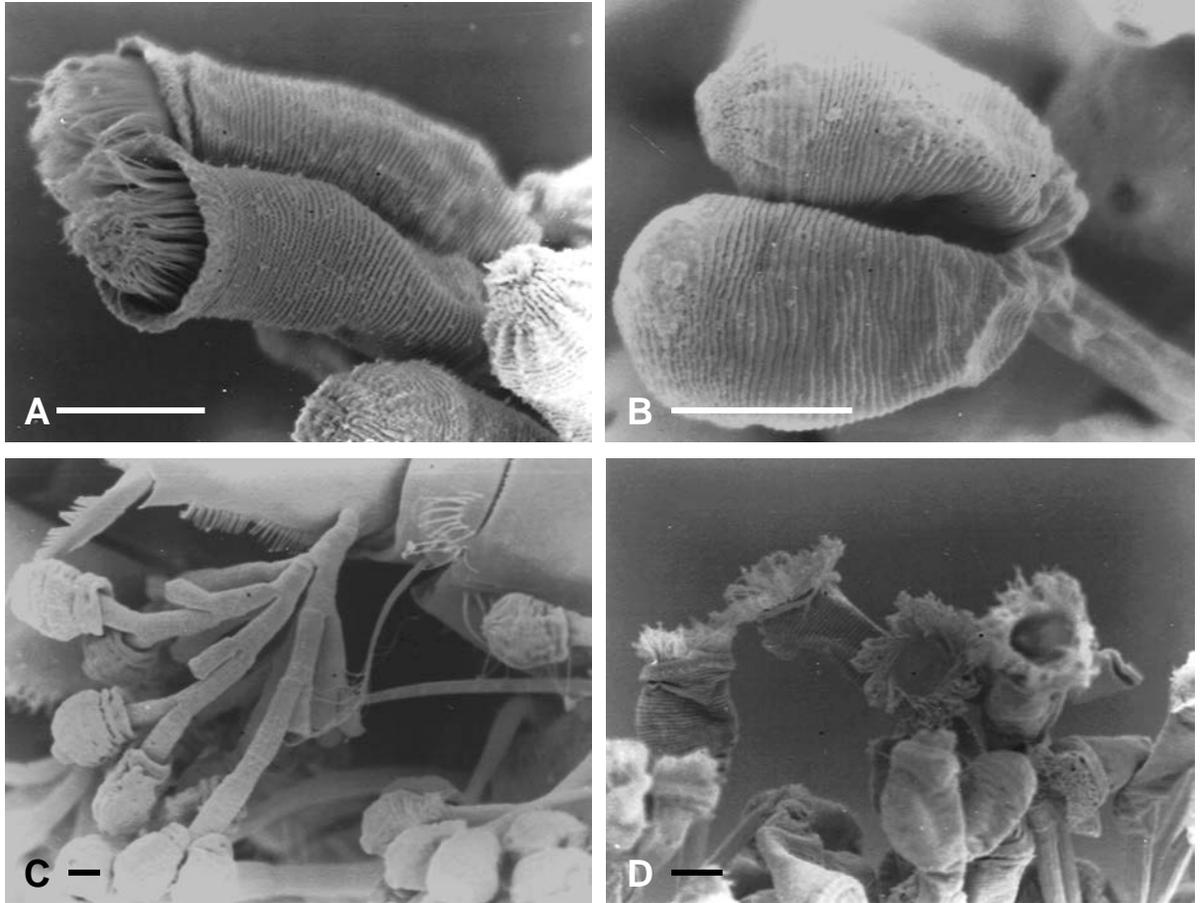


Fig 5.6 Scanning electron micrographs of *Epistylis* Ehrenberg, 1838 sp. A, associated with *Eucyclops* Claus, 1893 sp., collected from Loch Logan Waterfront, Bloemfontein, illustrating:

- A.** Two relaxed zoids with single peristomial lip.
- B.** Two contracted zoids with pellicular striations.
- C.** Colony with dichotomously branched stalk.
- D.** Relaxed zoids, peristomial discs & AZM visible.

Scale bars: A & B: 50 μ m

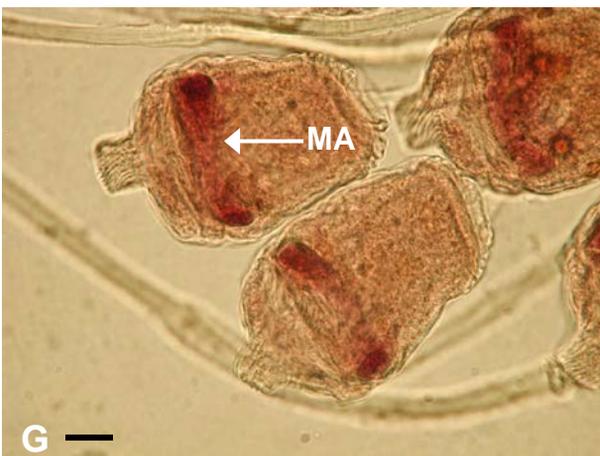
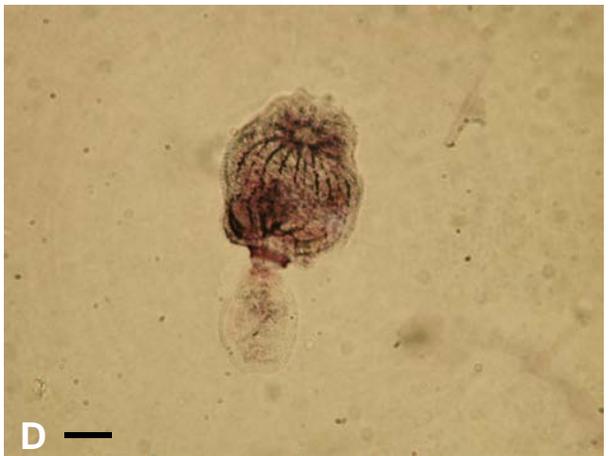
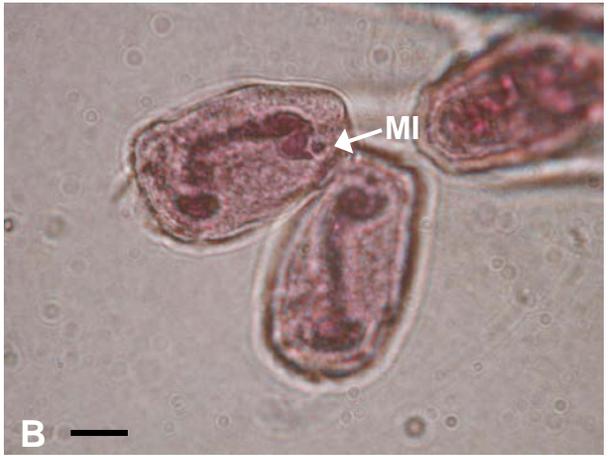
C & D: 10 μ m

Figure 5.7

Light micrographs of haematoxylin stained specimens (A, B, G & H), protargol stained specimens (C & D) and silver nitrate impregnated specimens (E & F) of *Epistylis* Ehrenberg, 1838 sp. A, associated with *Eucyclops* Clauss, 1893 sp., collected from Loch Logan Waterfront, Bloemfontein (A - F) and *Epistylis* sp. B associated with *Cherax destructor* Clark, 1936 (G, H), illustrating:

- A. Elongated sausage-shaped macronucleus (MA).
- B. Small round micronucleus (MI).
- C & D. Well developed myoneme system.
- E & F. Pellicular striations on zooids.
- G & H. Semi circular or C-shaped macronucleus (MA).

Scale bars: A - H: 10 μ m



Remarks

Morphological measurements of different *Epistylis* communities collected at different stages of this study, stayed more or less constant through the study, with very little variation. The conclusion can therefore be made that we dealt with the same community of *Epistylis*, within the planktonic community of the Loch Logan Waterfront.

In comparing *Epistylis* sp. A with known euplanktonic *Epistylis* species from the compendium, several of the species can be eliminated, due to distinct taxonomic differences with respect to:

E. breviramosa – small colonies, rarely having more than four individuals, C-shaped macronucleus, weakly striated pellicle, in contrast with large colonies, elongated sausage-shaped macronucleus and distinctly striated pellicle of *Epistylis* sp. A. Short main and individual stalks also in contrast with long stalks of *Epistylis* sp. A.

E. digitalis – size and conical shape of zooids, slightly curved or C-shaped macronucleus, annulated stalks, in contrast with funnel-shaped zooids, elongated sausage-shaped macronucleus and smooth stalks of *Epistylis* sp. A.

E. lacustris – conical to barrel-shaped zooids, C-shaped macronucleus, knobs and joints present at branching of stalks, in contrast with funnel-shaped zooids and elongated sausage-shaped macronucleus of *Epistylis* sp. A.

E. niagarae – indented, wavy and crenulated peristomial edge, band formed-macronucleus, enfolding infundibulum and faintly striated stalks, in contrast with smooth peristomial edge, elongated macronucleus and smooth stalks of *Epistylis* sp. A.

E. nympharum – size and slender shape of zooids, small colonies, rarely with more than 4 – 8 zooids, number of pellicular striations and distinctly annulated stalks, in contrast with funnel-shaped zooids, large colonies and smooth stalks of *Epistylis* sp. A.

E. plicatilis – size of zooids, kidney-shaped macronucleus, enfolding infundibulum. Number of pellicular striations and longitudinally striated stalks, in contrast with elongated sausage-shaped macronucleus and smooth stalks of *Epistylis* sp. A.

E. procumbens – size of zooids, conspicuously slender-shaped. Distinctly folded contracted zooids and semi-circular macronucleus in transverse axis of cell, in contrast with funnel-shaped zooids, oval-shaped contracted zooids and elongated sausage-shaped macronucleus of *Epistylis* sp. A.

E. pygmaeum – solitary zooids, colonies if present comprises of 4 – 8 zooids. Size and goblet-shape of zooids, semi-circular macronucleus in transverse axis of cell and unbranched stalks, in contrast with large colonies, funnel-shaped zooids, elongated, sausage-shaped macronucleus and dichotomously branched stalks of *Epistylis* sp. A.

***Epistylis* sp. A shows the most taxonomic similarities when compared to *Epistylis anastatica*. These include similar zooid size and shape. In both cases colonies are large with many zooids. Macronuclei and number of pellicular striations also correlates. Therefore *Epistylis* sp. A is identified as *Epistylis anastatica* for the purpose of the present study.**

5.3 Epistylids Found on Freshwater Crayfish

A variety of symbionts and parasites are found associated with the introduced freshwater crayfish of the genus *Cherax*. This includes bacteria and fungi, microsporidians, nematodes, temnocephalids and a number of peritrich ciliophorans including *Epistylis* sp. (see Chapter 4). Epistylids have been recorded from three *Cherax* species, namely *C. quadricarinatus*, *C. destructor* and *C. tenuimanus* in several countries which are listed below:

- **Ecuador** – in early January 1996 a commercial farm producing juvenile red claw crayfish (*C. quadricarinatus*) experienced an outbreak of *Epistylis* in their breeding ponds. The crayfish presented a “mushroom” appearance. The problem was controlled through water exchange and by decreasing phytoplankton biomass in the pond (Romero & Jiménez 1997).
- **Woongoolba, Queensland** – dense colonies of *Epistylis* sp. were found on the exoskeleton of one-year old *C. tenuimanus*, reared at a commercial farm. The ciliophorans were not considered to be directly harmful to *C. tenuimanus*, although large colonies found on the periopods and maxillipeds, extending to the brachiolegite of the abdomen are believed to cause asphyxia of several crayfish (Villarreal & Hutchings 1986).
- **Deception Bay, Queensland** – Heavy infestations of *Epistylis* sp. occurred in mid-summer when water temperatures were 24 – 30 °C. Colonies were found laterally on the carapace of *C. quadricarinatus*. Although the *Epistylis* infestations showed no pathogenic effect on the hosts, it affected the aesthetic appearance of the crayfish and therefore decreased its marketability (Herbert 1987).

Very little is known about the *Epistylis* species associated with *Cherax* and a comprehensive species description could not be found in the literature cited.

5.4 *Epistylis* sp. associated with *Cherax destructor* Clark, 1936.

Taxonomic description and morphology of *Epistylis* sp. B

Hosts: *Cherax destructor* Clark, 1936

Position on host: Carapace and appendages

Locality: La Menereze Freshwater Crayfish Farm, Bloemfontein

Reference Material: 2003/09/09 – 6 (haematoxylin)
2003/09/09 – 7 (protargol)

Biometrical data of *Epistylis* sp. B

Length of fully expanded body: 70 – 120µm (97 ± 12, 22)

Diameter of fully expanded body: 30 - 45 µm (39 ± 4, 22)

Macronucleus length: [40 – 92 µm (63 ± 2, 38)]

Macronucleus diameter: [5 – 13 µm (7.5 ± 2, 38)]

Micronucleus: not clearly visible

Pellicular striations: not enough material was available to do successful AgNO₃ impregnations. Although the pellicular striations could be seen with SEM observations, this was also not enough data to be used in the description.

Description

Colonies large, many zooids per colony (Figs 5.9 B & D). Zooids attached to bifurcated, dichotomously branched stalks (Figs 5.9 C & F). Stalks very long, 3 – 4 times zooid length when fully expanded. Colonies found on various body regions, especially antennae and carapace (Fig 5.10A).

Zooids elongated, distinctly funnel-shaped (Fig 5.9 A). Contracted zooids oval in shape, peristomial lip closed, cilia pulled into the body (Fig 5.9 E). Zooid encircled with pellicle striations, evenly spaced and uniform. Striations not clearly visible.

Cytoplasm granular, food vacuoles near infundibulum and contractile vacuoles suspended in cytoplasm. **Peristomial disc** distinctly convex, protruding snout-

like from body proper, whilst peristomial edge is small, not clearly indented, with a double peristomial lip (Figs 5.8 A; 5.9 A).

Nuclear apparatus situated in adoral region of zooid. Macronucleus small, semi-circular, C-shaped (Figs 5.7 G, H). Micronucleus not always clearly visible.

Infundibulum

Adoral spiral on peristome makes $> 360^\circ$ turn before plunging into infundibulum (Figs 5.10 D & E). First three kinetosomes of polikinity barren (Fig 5.10 F). Buccal apparatus situated in anterior region of body. Polykinety comprises three rows of kinetosomes, two outer rows comprising three rows of penicilli, whilst inner row comprises two rows of peniculi. Haplokinety comprises two rows of kinetosomes, each comprising four rows of peniculi (Fig 5.8 B). Polykinety spirals downwards into infundibulum, making two turns of 180° each. Haplokinety does not spiral inside zooid, but makes a 90° turn, extends further downwards and makes another 90° turn. Haplo- and polykinety ends in more or less the same region of the zooid, approaching from opposite sides of the body (Fig 5.8 B). Although not clearly visible it seems as if the impregnable band runs along the haplokinety. Horse shoe-shaped structure seen in some specimens although cytostome not clearly visible.

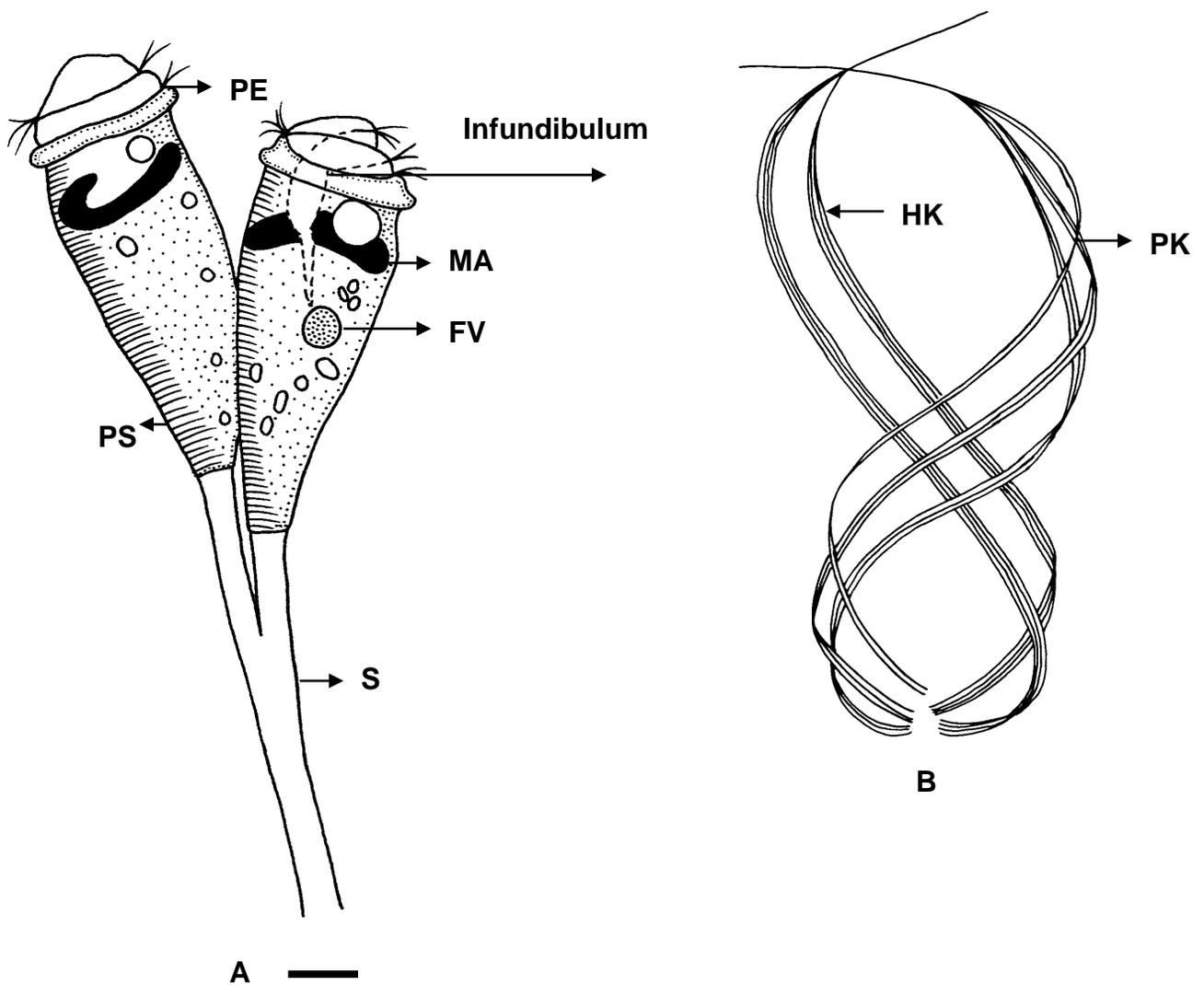


Fig 5.8 Microscope projection drawings of *Epistylis* Ehrenberg, 1838 sp. **B**, associated with *Cherax destructor* Clark, 1936 illustrating different morphological features (A) and the infundibulum (B).

FV – Food vacuole

HK - Haplokinety

PE – Peristomial edge

PK - Polykinety

PS – Pellicular striations

S – Stalk

Scale bar: 20 μ m

Figure 5.9

Light micrographs of live specimens of *Epistylis* Ehrenberg, 1838 sp. B associated with *Cherax destructor* Clark, 1936 illustrating:

- A.** Two zooids of colony, showing distinct funnel-shaped zooids.
- B.** Zooids, showing double peristomial lip, and elevated peristomial disc.
- C.** Telotroch forming – with distinct telotroch band.
- D.** Dichomously branched stalks.
- E.** Contracted specimens with binary fission also visible.
- F.** Relaxed specimens with binary fission visible.

Scale bars: A - F: 50 μ m

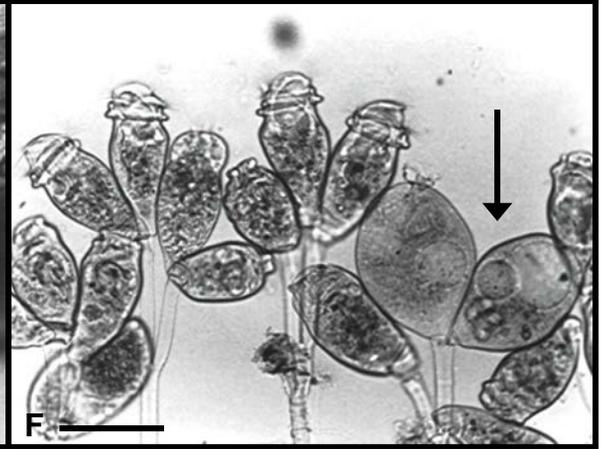
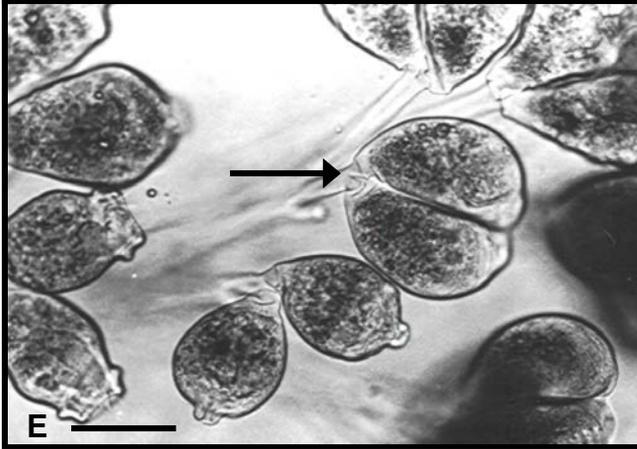
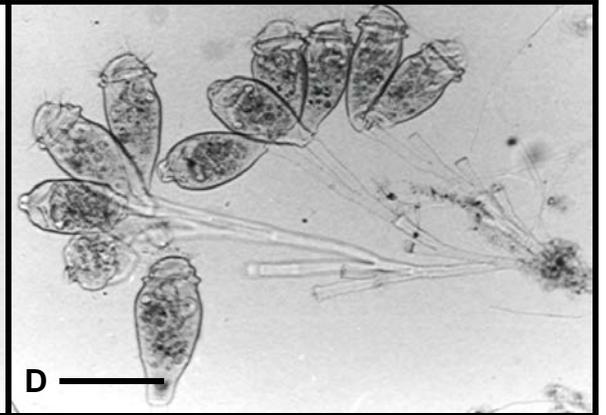
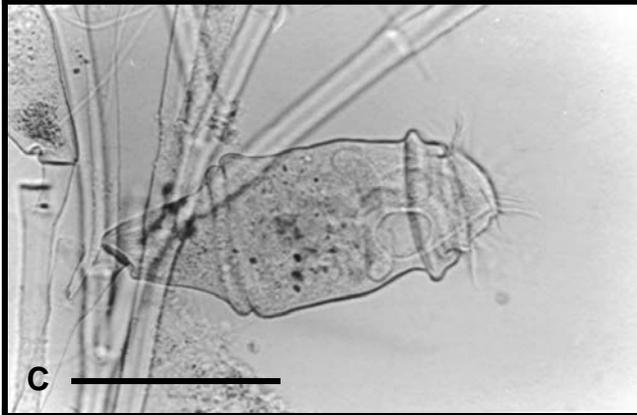
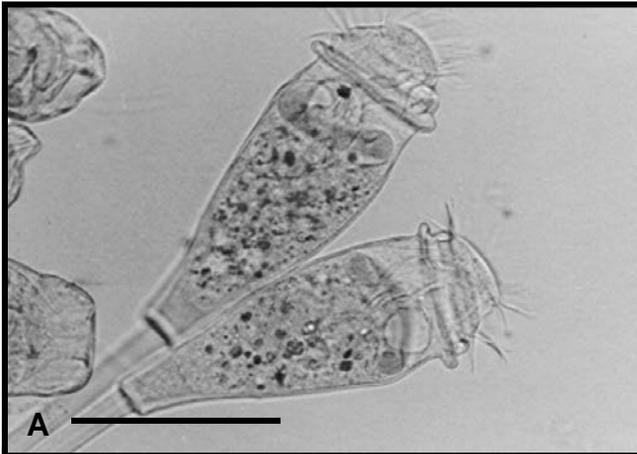
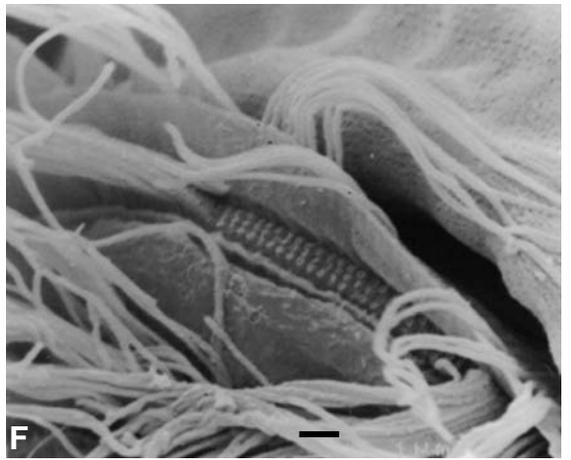
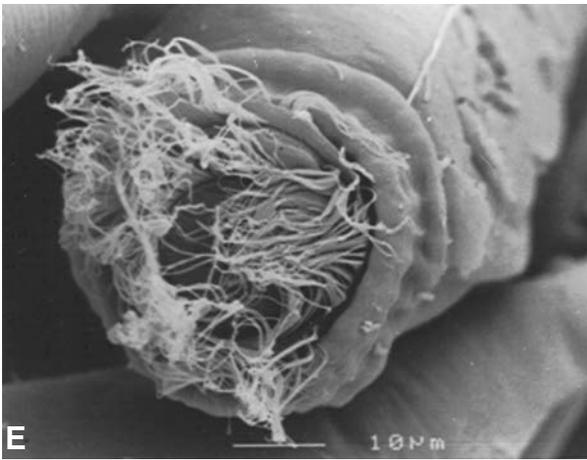
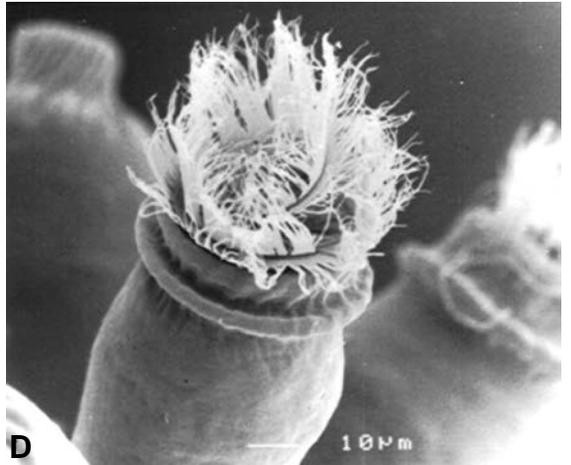
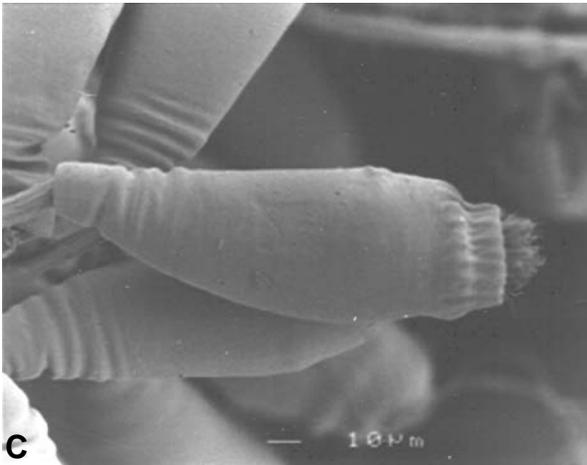
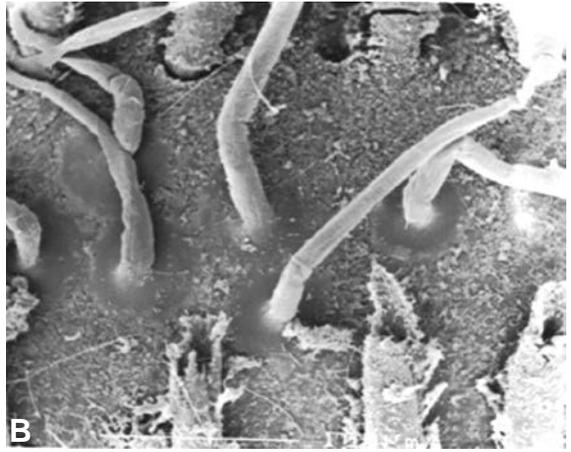
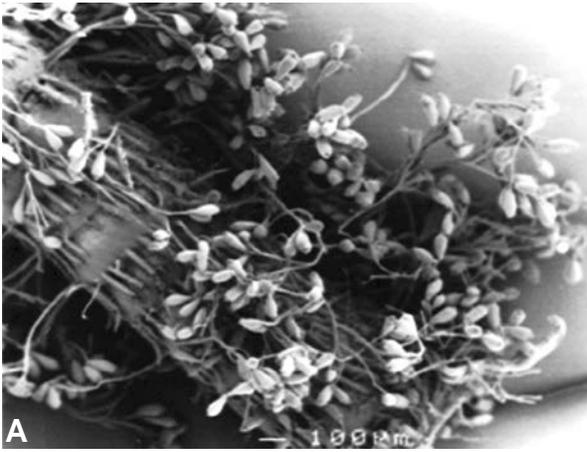


Figure 5.10

Scanning electron micrographs of *Epistylis* Ehrenberg, 1936 sp. B associated with *Cherax destructor* Clark, 1936 illustrating:

- A.** Colonies attached to the antennae.
- B.** Firm attachment of stalks to antennae.
- C.** Slightly contracted zooid, distinctly funnel shaped.
- D.** Double peristomial lip.
- E.** View of peristomial disc, with ciliary spiral.
- F.** Beginning of haplo- and polykinety, with barren kinetosomes.

Scale bars: F: 1 μ m



Remarks

In comparing *Epistylis* sp. B with known *Epistylis* species from the compendium, all the species can be eliminated, due to distinct taxonomic differences with respect to:

E. anastatica – zooid size and shape, number of pellicular striations and macronucleus in longitudinal axis of cell, in contrast with number of pellicular striations and C-shaped macronucleus in transverse axis of cell in *Epistylis* sp. B. Infundibulum of *Epistylis* sp. B significantly more complex than infundibulum of *E. anastatica* found on *Eucyclops* sp. in this study.

E. breviramosa – small colonies, rarely having more than four individuals, in contrast to large colonies of *Epistylis* sp. B. Short main and individual stalks, in contrast with long stalks of *Epistylis* sp. B.

E. digitalis – size and conical shape of zooids, annulated stalks, in contrast with funnel-shaped zooids and smooth stalks of *Epistylis* sp. B.

E. lacustris – conical to barrel-shaped zooids, knobs and joints present at branching of stalks, in contrast with funnel-shaped zooids and absence of knobs and joints at branching of stalks of *Epistylis* sp. B.

E. niagarae – indented, wavy and crenulated peristomial edge, band formed-macronucleus, enfolding infundibulum and faintly striated stalks, in contrast with smooth peristomial edge, C-shaped macronucleus and smooth stalks of *Epistylis* sp. B.

E. nympharum – size and slender to barrel-shape of zooids, small colonies, rarely with more than 4 – 8 zooids, number of pellicular striations and distinctly annulated stalks, in contrast with funnel-shaped zooids, large colonies and smooth stalks of *Epistylis* sp. B.

E. plicatilis – size of zooids, kidney-shaped macronucleus, enfolding infundibulum. Number of pellicular striations and longitudinally striated stalks, in contrast with C-shaped macronucleus and smooth stalks of *Epistylis* sp. B.

E. procumbens – size of zooids, conspicuously slender-shaped. Distinctly folded contracted zooids, in contrast with funnel-shaped zooids, pyriform-shaped contracted zooids of *Epistylis* sp. B.

E. pygmaeum – solitary zooids, colonies if present comprises of 4 – 8 zooids, size and goblet-shape of zooids and unbranched stalks, in contrast with large colonies, funnel-shaped zooids and dichotomously branched stalks of *Epistylis* sp. B.

***Epistylis* sp. B shows no taxonomic similarities when compared to known *Epistylis* sp. found associated with crustacean hosts. Based on the above mentioned description it is suggested that *Epistylis* sp. B is a new species and that the above mentioned serves as a description thereof. In order not to create confusion in literature, a species name is not allocated now. This will be done in the subsequent species description in an accredited journal.**

General remarks

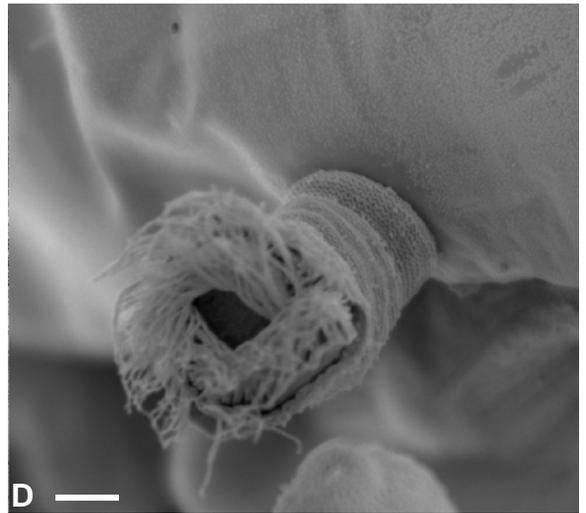
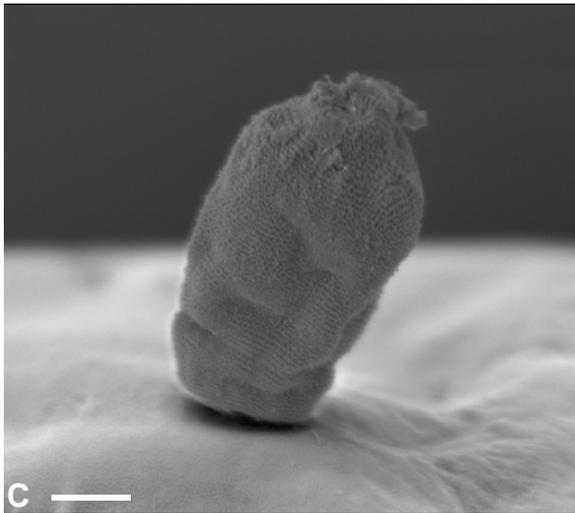
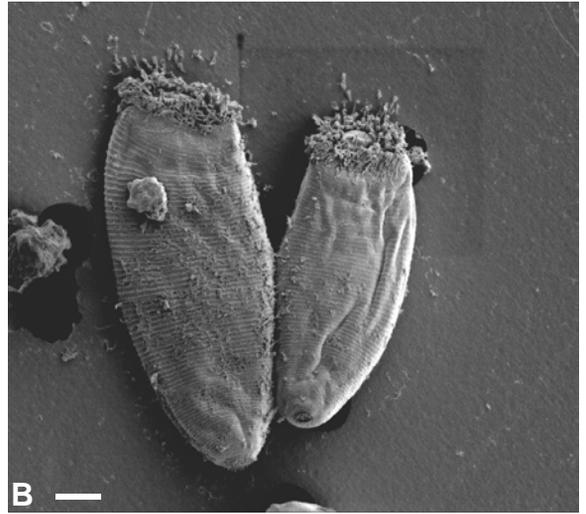
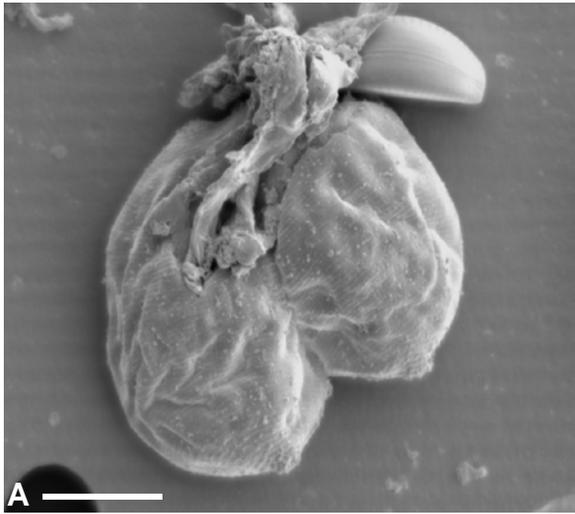
Asexual reproduction in protozoans includes binary fission, multiple fission and budding. Binary fission usually produces two daughter cells through mitotic division. Several examples of the reproductive methods, binary fission (Figs 5.11 A & B) and telotroch formation were observed in the present study (Figs 5.11 C, D & E). Figure 5.11 B clearly shows two individuals almost completely divided and two zooids still in the initial stages of binary fission (Figs 5.9 E & F). Although diffusion was noted, it was, however, not enough data to use in the taxonomic description of the species. Studies conducted on marine peritrichs usually show much more reproductive stages and in the present study very few reproductive stages were observed.

Figure 5.11

Scanning electron micrographs of *Epistylis anastatica* (Linnaeus, 1767) (cf. Kent, 1881), associated with *Eucyclops* Claus, 1893 sp., collected from Loch Logan Waterfront, Bloemfontein, illustrating:

- A.** Binary fission of zooids.
- B.** Zooids that have newly undergone binary fission.
- C.** Contracted telotroch newly attached to new substrate.
- D.** Relaxed telotroch newly attached to the body surface of a copepod.
- E.** Single zooid starting a new colony.

Scale bars: A - E: 10 μ m



5.5 Statistical Data

The highest infestations of ciliophorans on *Eucyclops* sp. were found during May, June and July 2003, while very few and small infestations were found in April and September (Table 5.1 & Fig 5.12). The high infestations found in October can be due to an extreme cold front experienced during this month. No ciliophoran infestations and even significantly lower plankton numbers were found in the warm summer months of November and March (Fig 5.12).

Epistylids from crayfish were only collected on one occasion. Due to the fact that these crayfish are kept in a confined environment of heated breeding tanks, it can be assumed that it was a constant population of *Epistylis* that was dealt with.

Very little intra-specific variation was observed between the striation number obtained from SEM micrographs and silver impregnated specimens. This serves as a motivation for the proposal suggested by Van As, Van As & Basson (1995) that SEM micrographs, as well as silver impregnated specimens should be used to count pellicular striations.

Table 5.1 Summary of total collections of plankton samples at various localities, from January 2002 to October 2003.

Nr	Locality	Date	Infestation [⊙]	Processing of Material
2002				
1	Soetdoring	2002/02/13	-	
2	Botanical Gardens	2002/03/18	-	
3	Botanical Gardens	2002/04/18	-	
4	Botanical Gardens	2002/04/25	-	
5	Krugersdrif dam	2002/05/03	-	
6	Botanical Gardens	2002/06/02	-	
7	Loch Logan	2002/06/06	+	GNF, Bouins
8	Loch Logan	2002/06/11	+++	Protargol & Haematoxylin
9	Loch Logan	2002/09/13	++	Bouins + 70% EtOH
10	Loch Logan	2002/09/19	-	
11	Loch Logan	2002/11/20	-	
12	Loch Logan	2002/12/04	-	
13	Loch Logan	2002/12/20	-	
2003				
14	Botanical Gardens	2003/01/06	-	
15	Loch Logan	2003/01/09	-	
16	Sewerage Farm	2003/01/15	-	
17	Pony Club	2003/01/15	-	
18	Botanical Gardens	2003/02/06	-	
19	Loch Logan	2003/02/21	-	
20	Loch Logan	2003/03/07	-	
21	Loch Logan	2003/04/10	+	SEM – normal GNF
22	Loch Logan	2003/04/11	+	SEM – normal GNF
23	Loch Logan	2003/05/27	++	Live observations
24	Loch Logan	2003/05/30	+++	Haematoxylin
25	Loch Logan	2003/06/12	+++	Live observations
26	Loch Logan	2003/06/13	+++	GNF, Bouins + 70% EtOH
27	Loch Logan	2003/07/14	+++	SEM – glutaraldehyde, osmium tetra oxide
28	Loch Logan	2003/07/23	+++	SEM – osmium tetra oxide, glutaraldehyde & Haematoxylin
29	Loch Logan	2003/07/26	+++	SEM – varying concentrations of glutaraldehyde, post fixed in osmium tetra oxide and phosphate buffer
30	Loch Logan	2003/09/03	+	Bouins + 70% EtOH
31	Loch Logan	2003/09/10	-	
32	Sewerage farm	2003/09/11	-	
33	Loch Logan	2003/10/07	+++	Dried smears – Silver impregnation & Protargol SEM – varying temperatures of Formalin

⊙ prevalence of infestations indicated by:

- no infestations present

+ 0 – 25% infested

++ 25 – 50% infested

+++ 50 – 75% infested

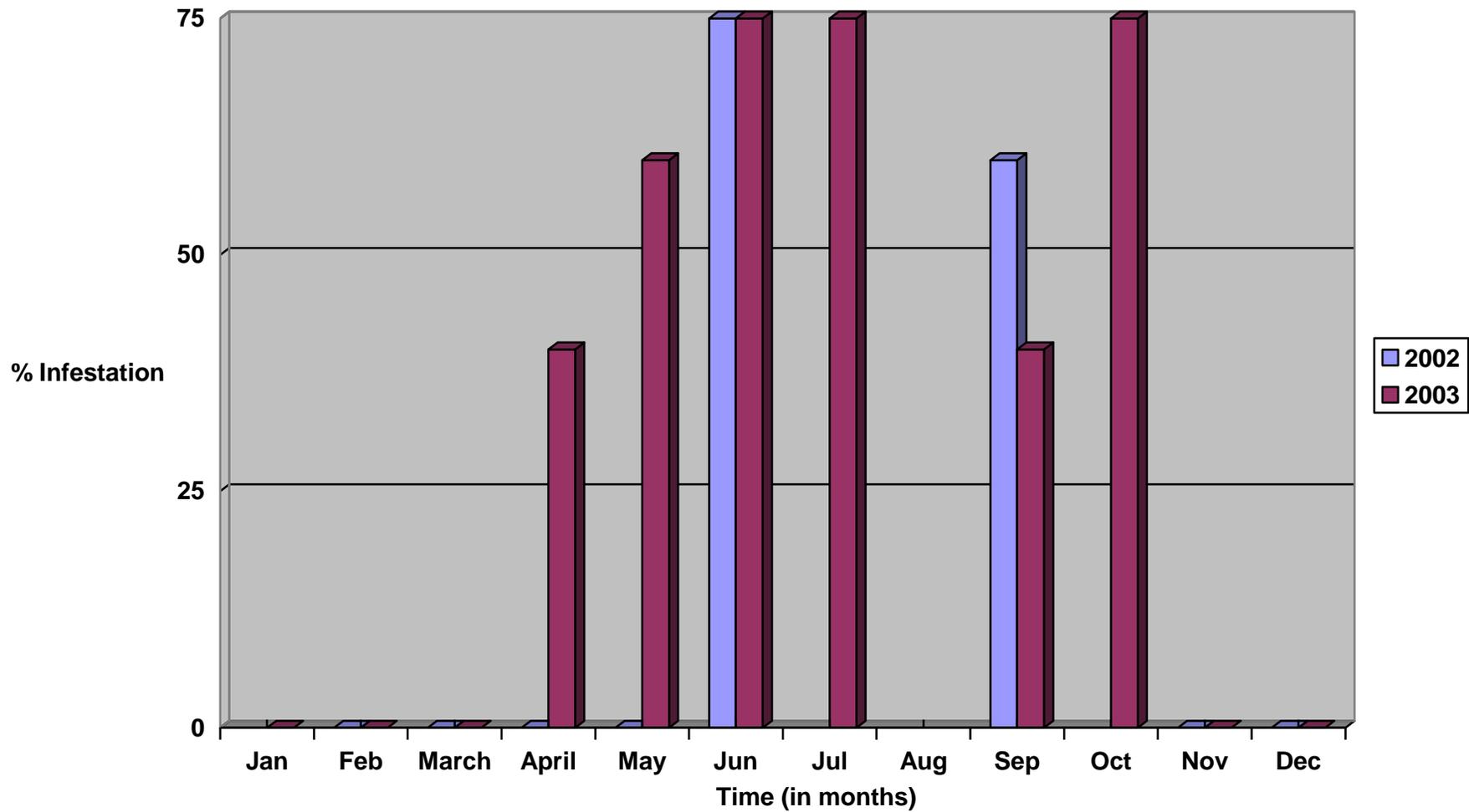


Fig 5.12 Graphic representation of *Epistylis anastatica* (Linnaeus, 1767) (cf. Kent, 1881) infestations on *Eucyclops Clausi*, 1893 sp. collected from the Loch Logan Waterfront for 2002 and 2003.

The significant role that plankton plays in aquatic ecology speaks for itself, if one considers the fact that so many aquatic organisms rely on plankton as an essential food source, e.g. in the marine aquatic environment where most of the great whales feed by gulping water and expelling it through the sieve-like plates in the mouth, capturing plankton in the process. A large blue whale may eat up to four tons of plankton per day.

In freshwater environments, plankton serves as secondary producers and primary consumers, since almost every organism in the freshwater environment is dependant on plankton during some stage of its life cycle, especially in the larval stages, for example predatory fish that feed on plankton during the juvenile stage.

In the present study area, the Loch Logan Waterfront, there are several fish species, i.e. the Sharptooth catfish *Clarias gariepinus* (Burchell, 1822), the introduced carp *Cyprinus carpeo* Linnaeus, 1758 which was introduced in the 1700's and the Mosquito fish *Gambusia affinis* (Baird & Girard, 1853), also introduced, whose primary food source in the larval stage will be plankton. Even in the mature stages plankton continues to be an essential part of these fish species' diets.

Although epistylids found attached to *Eucyclops* sp. in the present study do not feed on the host, they may have a detrimental effect on the host in several respects: **Impaired movement** – copepods heavily infested with epistylids or other epibiont (Fig 5.3 A - F) are not able to swim as fast as those not infested with symbionts. During physical examination of copepods, it could clearly be seen that infested individuals are significantly slower. This also has an energetic implication for the copepod and subsequently these individuals are more exposed and vulnerable to predators.

Although some copepods are colored, the majority of copepods does not possess excessive coloration and is usually very pale or **transparent**. This morphological adaptation is most likely to avoid predators. Epistylids also affect the general appearance of the copepod, making it seem dirty and thus increasing visibility. Subsequently this could increase **predation pressure** on the copepod. This implicates a disadvantage to the epibionts as well, as they will be eaten along with the copepod.

In consideration of the above mentioned, some inevitable **evolutionary questions** arise. If ciliophoran infestations increase predation pressure upon the copepod, it may have a detrimental effect on the existence of the *Epistylis* colony attached to the exoskeleton as well, since the more settled the colony becomes on the host, the more visible the hosts become, making them more vulnerable to predators. If this is true, the association of ciliophorans and planktonic hosts will not last long in evolutionary terms, since it can not overcome natural selective obstacles.

Several forms of pollution occur in natural waters as a result of human activity. Organic pollution is probably most widespread, but toxic chemicals, including heavy metals and also extreme pH and heated effluents all damage ecosystems. In environmental impact studies certain organisms provide valuable information about the chemical, physical, biological and ecological state of the environment through their presence or absence (Sures 2002).

According to MacKenzie, Williams, Williams, McVicar and Siddal (1995) a good bio-indicator must be exceptionally sensitive to environmental changes, so that a significant reduction in the numbers can be used as a warning of deteriorating conditions, before the majority of other less-sensitive organisms are seriously affected.

Parasites as environmental indicators are attracting increasing interest, due to the variety of ways in which they respond to anthropogenic pollution. This is a complex area of research, as it is difficult to link parasite population levels with pollution without considering a number of other abiotic and biotic factors. Much of the work published up to now in this field is inconclusive and largely unconvincing, as there is little evidence to show that the many natural biotic and abiotic factors that regulate the numbers and distribution of parasites have been considered together with the effects of pollutants.

The use of parasites as bioindicators can be subdivided into the two groups, i.e. **effect indicators** and **accumulation indicators**. By using parasites for effect indication, changes of population or community structures reflecting pollution of the environment could be monitored. Parasites also have alternative applications in environmental biomonitoring due to the ability to accumulate heavy metals within body tissues (Sures 2002). Infections by symbionts, either as ecto- or endoparasites, with direct- and indirect cycles, can be better, more sensitive indicators than some lesions – a few of which take long periods to develop and cannot be related to specific conditions (Overstreet 1993).

A large number of **biotic** and **abiotic** factors affect the prevalence and abundance of parasites. **Temperature** is among the most important of these. Esch (1982) divided abiotic factors into two groups: those which are determined and predictable and those which are not. In nature, temperature is a determinate predictable character, according to season, but through human activity like artificial heating of water, is often unpredictable. Most indeterminate factors are artificial and include fire, flood, artificially elevated temperature, radical temperature change on short-term basis and nutrient enrichment of water. Low oxygen levels can also result from the introduction of fertilizers from agriculture, sewage and other organically enriched pollutants (Mackenzie *et al* 1995).

Temperature and salinity are the most studied natural environmental conditions in regard to symbiont biology, because it affects growth, development, reproduction, susceptibility and behaviour of symbionts and hosts, as well as other natural environmental conditions. However, each symbiotic relationship is different and has to be examined separately.

These natural environmental factors, as well as various other ones including water quality, seasonality and human activities, serve to control the **equilibrium** between pathogen (or symbiont) and host. This intricate balance among symbiont, host and environment applies to a population, ecosystem as well as an individual. For example a disease of a dominant fish host species can reduce the number of that species to a density level at which the species is permanently replaced by one or more fish species (Overstreet 1993).

Pollutants have various affects on the parasite/host association:

- The parasite may be directly susceptible to the toxic effects of pollutants, in which case pollution may reduce infection prevalence and intensity.
- On the other hand, if the host is more susceptible than the parasite to the pollutant, its resistance to infection may be lowered, leading to higher prevalence and intensity.
- Being parasitised may itself increase the host's susceptibility to pollution.
- Pollutants may decrease parasitism if infected hosts' numbers decrease because of pollution.
- Habitat alteration – alteration of the hosts or parasites habitat through pollution may increase or decrease parasitism.

Overstreet (1993) suggests that parasitic / symbiotic surveys should become a routine part of any overall base-line or follow up survey of a locality, suspected of being polluted. It should be conducted in conjunction with other disciplines, including pathobiology, biology, ecology and associated fields. Some parasites provide extremely sensitive indicators for specific toxicants, habitats and

geographic areas, because of the complex nature of the multihost life histories. This should be utilised and accumulation of all the parasitic data should provide an important aspect of any such surveys.

Loch Logan is, however, an **artificial**, manmade impoundment and this surely has an effect on the copepod/ciliophoran association. In the study preceding the current one (Kitching 2001), conducted at natural water systems like the Modder River in the Soetdoring Nature reserve and the Okavango River in Botswana, ciliophorans were never found on planktonic copepods. From this the conclusion can be made that ciliophorans are more likely to occur on copepods in artificial environments than in natural ones.

If this statement is to be proven beyond any doubt, this association could be used as a valuable tool in **aquatic biomonitoring** and as **biological indicators** of pollution and environmental disturbance.

Cyclopoid copepods act as intermediate hosts for several internal fish parasites such as tapeworms and acanthocephalans. In this lifecycle copepods are suitable hosts due to the high probability of being eaten by a fish in order for the lifecycle of the parasite to continue. Although this is an advantage for the fish parasite, it is a disadvantage for the sessiline ciliophoran.

Studies by Xu (1993) show that epizoic peritrichs do not affect the growth or development of planktonic crustaceans, although it affects the **reproductive tempo** significantly. The mean number of total offspring per uninfected female was significantly more than for infected females. This could have an economic implication for aquaculture farmers who use plankton as main food source for their fish stock.

Living on a motile substrate has several **advantages** and **disadvantages** for the ciliophoran. Advantages include being in constant contact with its food source

(detritus and bacteria) as many peritrich ciliophorans are located where the feeding currents of filter feeding zooplankton passes over the ciliophoran, creating its own feeding current, reducing the energy costs of creating its own feeding current.

Another advantage lies within the **respiration** of this organism. Circulation of water through the body of ciliophorans is essential for successful respiration. This is easily achieved when living on a motile substrate, as the swimming motion of the host causes sufficient currents surrounding the attached ciliophoran for respiration.

Sessiline organisms have several obstacles to overcome to ensure their existence and distribution of new offspring. Ciliophorans are filter-feeders and are in constant contact with their never-ending food source; obtaining food is therefore not a problem. Finding a **suitable substrate** to attach to is a considerable problem for sessiline ciliophorans. To overcome this obstacle, **attachment** should be very firm, ensuring that once the organism has found a substrate it will not detach easily. *Epistylis* in the present study are clearly very firmly attached to their hosts, with a disc-like structure, almost cemented into the exoskeleton of the host (Figs 5.5 E & F; 5.10 B). There is a significant difference in the attachment of *Epistylis anastatica*, associated with *Eucyclops* sp. and *Epistylis* sp. B associated with *Cherax*. *Epistylis anastatica* has a round, disc- or plate-like structure, with which it is attached to the copepod (Fig 5.5 E & F), whilst *Epistylis* sp. B almost melts into the exoskeleton of the crayfish (Fig 5.10 B).

Reproduction strategies of sessiline organisms can also be problematic. Not only finding a suitable mate if a sexual reproduction strategy is followed, but dispersing of the new offspring to other suitable substrates, outside the territory of the parent organism. Producing a free-swimming migratory stage (in this case a telotroch (Fig 5.11 C) compensates for this problem, avoiding overcrowding on

the host and minimalising intra-specific competition between parent and offspring for suitable attachment space.

The attached stage of the zooplankton-epibiont interaction is limited to the duration of the instars of the zooplankton. When moulting occurs, the epibiont may either remain with the cast skin for a period of time, or disperse. Instar duration varies greatly among zooplankton species and together with seasonal variations in temperature, the expected seasonal prevalence and burden of epibionts should vary considerably among zooplankton species in a planktonic community (Threlkeld, Chiavelli & Whilley 1993).

According to Foissner *et al.* (1999) *Epistylis anastatica*, is a common epibiont of planktonic crustaceans, reported from several European countries including Denmark, Germany, Austria, Hungary and the Czech Republic. It is also reported that *E. anastatica* has been found attached to aquatic plants. Although *E. anastatica* has been reported from eastern Africa (Foissner *et al.* 1999), this is the first record of this *Epistylis* species in southern Africa and the question arises whether *E. anastatica* is a cosmopolitan ciliophoran. If not, one wonders where its origin lies and if it is found in the rest of the African continent. One could speculate that if the host, *Eucyclops* sp. has a cosmopolitan geographic distribution and *E. anastatica* is host specific to *Eucyclops* it may also have a cosmopolitan distribution. This questions could, however, not be answered positively in this study and further research is necessary to verify this statement.

In planning the present study, the main objective was to study planktonic copepods and associated symbionts – it was not part of the planning to examine freshwater crayfish and symbionts associated with it. However, in the duration of the study freshwater crayfish were brought to our laboratory and *Epistylis* infestations were noticed. These crayfish are alien species introduced from Australia and the question immediately arose whether the epistylids are also introduced or if they occurred naturally.

In literature cited epistylids have been recorded from several freshwater crayfish species (Table 3.1), although a comprehensive taxonomic description could not be found. This also emphasises the value of good taxonomic descriptions. The present study is the first taxonomic description of this epistylid, but has some shortcomings due to obstacles with staining techniques. It is also difficult to compare present data to known species of epibionts found on crayfish, by other authors, due to inadequate taxonomic information.

Subsequently we cannot say for sure if the epistylid associated with *Cherax* is indigenous or if it was introduced along with the crayfish. Therefore the question still remains unanswered and can only be answered if further research is done.

In conclusion I would like to take a critical look at the taxonomic chaos of sessiline ciliophorans, especially the genus *Epistylis*. Identifying a ciliophoran to genus level is not as challenging as finding the correct species. Presently several of the available taxonomic descriptions in literature is extremely incomplete and unsuitable for scientific use, lacking several essential components including infundibulum descriptions, micrographs of important taxonomic features such as macro- and micronuclei, line drawings, live observations and morphological measurements. This makes describing a new species or giving a review of known species of *Epistylis* very problematic.

Warren (1981) compiled a complete review of the genus *Vorticella* including, taxonomic characters, a comprehensive key to species as well as species descriptions along with line drawings and morphological measurements of each known species. This makes future reference for scientists in the same field very easy and convenient.

It is my proposal that someone working on the genus *Epistylis* will take the courage of their conviction and do the same with this remarkable group of organisms.

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Forty five *Epistylis* Ehrenberg, 1838 species are found associated with crustaceans. Of these, 13 species are found associated more specifically with planktonic copepods, whilst 19 species are found associated with decapods. The aim of this study was to examine freshwater crustacean hosts for the presence of sessiline ciliophoran symbionts. The crustacean hosts found infested with ciliophorans included a freshwater cyclopoid copepod of the genus *Eucyclops* Clauss, 1893, collected at the Loch Logan Waterfont, Bloemfontein as well as two introduced Australian crayfish species from a commercial crayfish farm, situated in the Vaalbank south vicinity, outside Bloemfontein. These two species were *Cherax destructor* Clark, 1936 and *Cherax quadricarinatus* Von Martens, 1868. Plankton was collected with a plankton net and examined with a dissection microscope. Crayfish were examined externally in search of any symbionts on the carapace and appendages. Ciliophorans found were prepared for Scanning Electron and Light Microscopy using standard techniques. The epistylid found on the copepod *Eucyclops* Clauss, 1893 sp. was identified as *Epistylis anastatica* (Linnaeus, 1767) (cf. Kent, 1881) whilst the one found on the crayfish is a new species and was only named *Epistylis* sp. B for the purpose of this study. Both *Epistylis* species were taxonomically described. Seasonality plays a significant role in the former association and it seems as if low ambient and water temperatures in the winter months are favourable for the occurrence of ciliophorans. The association between ciliophorans and crustaceans in this study seems to be mainly commensal, although the infestation has some detrimental effects on the host, i.e. impaired movement, increased visibility to predators and suffocation of crayfish in extreme cases.

Keywords: copepod, crayfish, crustacean, *Epistylis*, freshwater, planktonic, sessiline ciliophoran, South Africa.

Vyf en veertig *Epistylis* Ehrenberg, 1838 spesies word met verteenwoordigers van Crustacea geassosieer. Hiervan is 13 spesies spesifiek met planktoniese verteenwoordigers van die Copepoda en 19 met verteenwoordigers van die Decapoda (krappe en krewes) geassosieer. Die doel van die huidige studie was om varswater krustasiër gashere vir die teenwoordigheid van sessiele siliofore te ondersoek. Krustasiërgashere, besmet met sessiele siliofore, sluit varswater siklopoïede kopepode in, van die genus *Eucyclops* Clauss, 1893 versamel vanaf die Loch Logan Waterfront in Bloemfontein, asook twee ingevoerde Australiese varswater krewes vanaf 'n kommersiële kreefplaas in die Vaalbank Suid distrik buite Bloemfontein. Hierdie twee kreef spesies is *Cherax destructor* Clark, 1936 en *Cherax quadricarinatus* Von Maartens, 1868. Planktonmonsters is met behulp van 'n planktonnet versamel, waarna dit met 'n disseksiemikroskoop ondersoek is. Varswater krewes is uitwendig vir die teenwoordigheid van simbiote op die karapaks en aanhangsels ondersoek. Sessiele siliofore is volgens standaard tegnieke vir skanderelektroon- en ligmikroskopie voorberei. Die siliofoor gevind op *Eucyclops* Clauss, 1893 sp. is as *Epistylis anastatica* (Linnaeus, 1767) (cf. Kent, 1881) geïdentifiseer, terwyl die siliofoor geassosieer met *Cherax* 'n nuwe spesies is en slegs as *Epistylis* sp. B benoem is vir die doeleindes van die huidige studie. Beide *Epistylis* spesies is taksonomies beskryf. Seisoenale wisseling in temperatuur speel 'n beduidende rol in die voorkoms van sessiele siliofore op krustasiërs en dit wil voorkom asof lae omgewings- en watertemperature gunstig vir die voorkoms van siliofore is. Die assosiasie tussen siliofore en krustasiërs blyk hoofsaaklik kommensalisties te wees, alhoewel belemmerde beweging, verhoogde sigbaarheid vir predatore by kopepode en selfs versmoring van krewes in ekstreme gevalle aangetref word.

Sleutelwoorde: kopepode, kreef, krustasiër, *Epistylis*, varswater, planktonies, sessiele siliofoor, Suid Afrika.

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