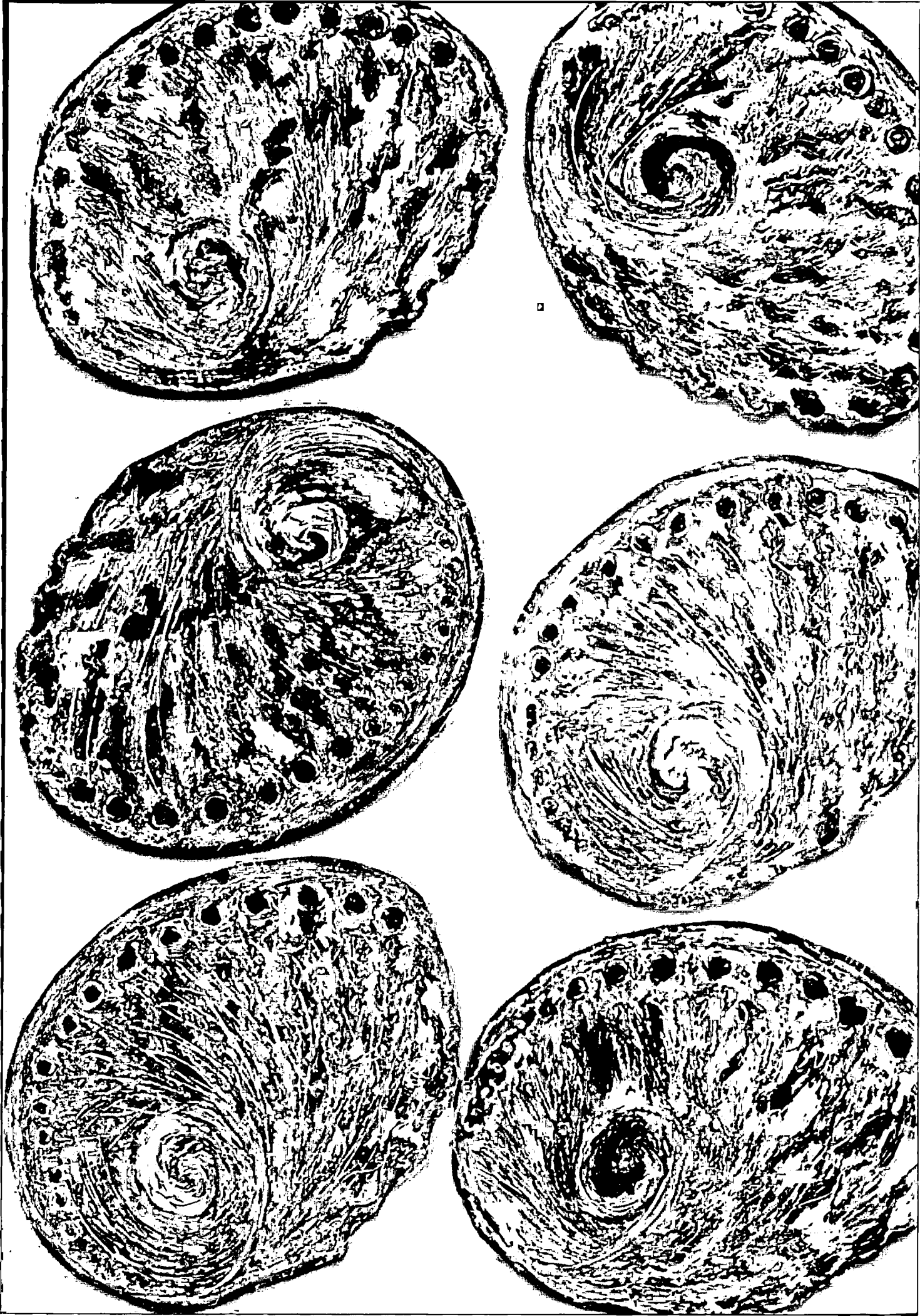

SESSILINE CILIOPHORANS ASSOCIATED WITH *HALIOTIS* SPECIES (MOLLUSCA: ARCHAEOGASTROPODA) FROM THE SOUTH COAST OF SOUTH AFRICA

By

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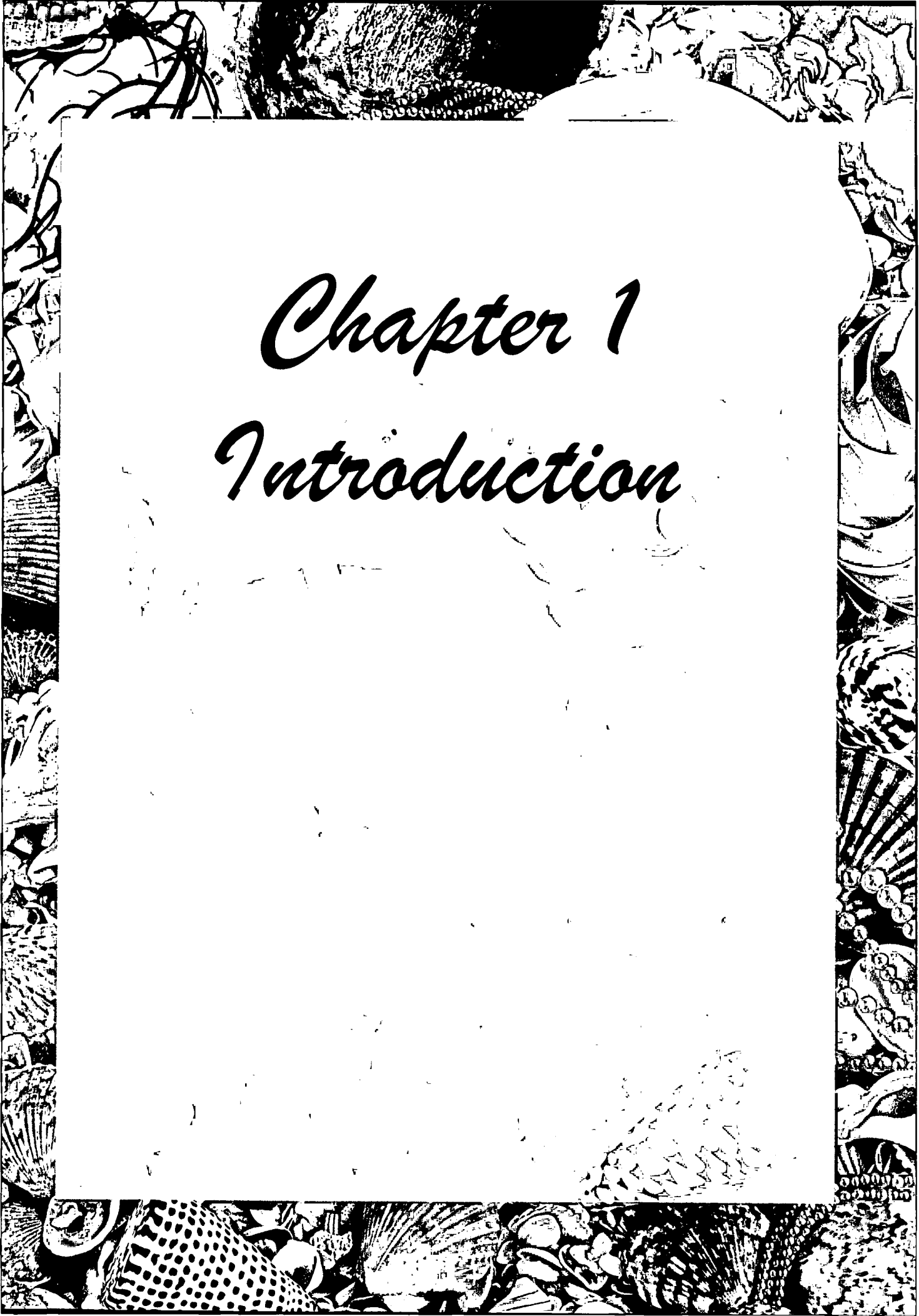


Chapter 1
Introduction

Abalone have been utilised world-wide by humans for thousands of years and recently commercial exploitation has escalated. The demand for the flesh of its foot has led to the development of abalone fisheries in numerous countries. During the present study, the opportunity to study South African perlemoen in their natural habitat as well as from an aquaculture facility, arose. The focus of abalone research has primarily been placed upon culture techniques and potential pathogens of abalone in aquaculture or holding facilities. Very few studies have been done on the parasites and symbionts of natural populations of abalone.

According to Lindberg (1992), there are about 70 species of abalone world-wide, there is, however, considerable discrepancy in the literature regarding the extant *Haliotis* Linnaeus, 1758 species, ranging from 50 to 130 species and subspecies (Knauer 1994). Important abalone fisheries exist in Australia, China, Japan, Mexico, New Zealand, South Africa and the United States of America (California) (Shepherd, Tegner & Guzman Del Proo 1992). The world-wide demand for abalone is centred in the Far East, especially Japan and China (Tarr 1993, 1995).

The Aquatic Parasitology Research Group in the Department of Zoology and Entomology at the University of the Orange Free State has been involved in studying parasites and symbionts of aquatic organisms since 1980. Most of their research has been devoted to freshwater organisms, but also included studies on intertidal species. Currently, most freshwater research in the Group forms part of the Okavango Fish Parasite Project. Since 1994 the Foundation for Research Development (FRD), now referred to as the National Research Foundation (NRF), has been supporting their research project entitled: Intertidal Symbionts of the South African coast. This project falls within the realm of the Coastal Resources Program of the NRF. Within the context of this research program, one Ph.D. and four M.Sc. students have already completed their research on aspects of intertidal parasites and symbionts. Van As (1997) studied

ciliophoran parasites of limpets (Patellogastropoda). The M.Sc. works are that of Botha (1994) who studied ciliophoran symbionts of *Oxystele* Philippi, 1847 species; Loubser (1994) studied the ciliophorans of intertidal fishes; Molatoli (1996) investigated the symbionts of red bait, *Puyra stolonifera* (Heller, 1878) and Smit (1997) who studied gnathiid isopods of intertidal fishes. Currently other projects within this program are also being carried out, i.e. on the myxosporideans, ciliophorans, isopods and caligid copepods of intertidal fishes, turban gastropods, polychaetes and echinoderms.

So far this program has led to the publication of our results in the form of **full length publications** (Basson & Van As 1992; Loubser, Van As & Basson 1995; Van As & Basson 1996; Van As, Basson & Van As 1998; Basson, Botha & Van As in press); **congress proceedings** (Molatoli, Van As & Basson 1995; Van As, Van As & Basson 1995; Molatoli, Van As & Basson 1996; Smit, Van As & Basson 1996; Van As, Van As & Basson 1996a; Botes, Basson, & Van As 1997; Christison, Van As & Basson 1997; Grobler, Van As & Basson 1997; Van As, Basson & Van As 1997; Botes, Basson & Van As 1998; De Villiers, Van As & Van As 1998; Grobler, Van As & Basson 1998; Van As, Van As & Basson 1998; Smit, Van As & Basson 1998 and Reed, Van As & Basson 1998), as well as **extended abstracts** (Botha & Basson 1994; Loubser, Van As & Basson 1994; Van As & Basson 1994; Christison & Van As 1996; Molatoli & Basson 1996; Smit & Van As 1996; Van As, Van As & Basson 1996b).

The genus *Haliotis* comprises six species i.e. *H. midae* Linnaeus, 1758; *H. spadicea* Donovan, 1808; *H. parva* Linnaeus, 1758; *H. speciosa* Reeve, 1846; *H. queketti* Smith, 1910, and *H. pustulata* Reeve, 1846, all endemic to and distributed along the west, east and south coast of South Africa (Jacks 1983; Muller 1984, 1986 & Branch, Griffiths, Branch & Beckley 1994).

Surveys carried out by the Aquatic Parasitology Group, on a small number of perlemoen during 1995 and 1996 from the De Hoop Nature Reserve along the south coast of South Africa, revealed the presence of scyphidiid peritrichs, of the genus *Mantoscyphidia* Jankowski, 1980, occurring in abundance on the gills of *Haliotis spadicea* and *H. midae*. The mantoscyphidians in turn hosted ellobiophryids of the genus *Caliperia* Laird, 1953.

Digenetic trematodes were also found on the gills of *H. spadicea,* as well as in the digestive glands.

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

- to elucidate the morphology, ultrastructure and systematics of the different species of scyphidiid peritrichs as well as the associated caliperid species,
- to determine the infestation pattern of the scyphidiid peritrichs and caliperid fauna of all the South African haliotid species occurring in the De Hoop Nature Reserve,
- to determine whether any other symbionts are regularly associated with perlemoen, and
- to obtain an understanding of the different host/symbiont associations.

In order to collect data to achieve these objectives, field work was carried out in the same season (March/April) of 1997, 1998 and 1999 at the De Hoop Nature Reserve. Back at the laboratory in Bloemfontein light and scanning electron microscopy studies of material collected in the field were carried out. During the study a perlemoen aquaculture facility, Danger Point Abalone Farm, was also visited, where specimens of *H. midae* were examined and found to harbour the same species of scyphidiid peritrich and caliperid than *H. midae* collected from the De Hoop Nature Reserve.

The layout of this dissertation is as follows: **Chapter 2** explains the material and methods used during field and laboratory work. Due to the nature of this project it was necessary to devote attention to aspects of the morphology, life-cycle and biology of the hosts, which forms the contents of **Chapter 3**. In **Chapter 4** the focus is placed on the basic principles of perlemoen/abalone aquaculture, before discussing the known parasites of abalone in **Chapter 5**. The scyphidiid peritrichs and caliperids found in this study are all members of the phylum Ciliophora Doflein, 1901. The higher systematics of this phylum is discussed in **Chapter 6**. In **Chapter 7** the systematics of the scyphidiid peritrichs is dealt with, which includes the description of two new species of the genus *Mantoscyphidia*. In **Chapter 8** the associated caliperid is described as a new species. **Chapter 9** sheds some light on the possible life-cycle of the digenean trematodes that

were found in *Haliotis spadicea*, with some information on their morphology. Although this is not included in the title of the thesis, and the trematodes have not yet been positively identified, the information is included because it may form part of further studies and have an impact on the aquaculture industry. Results of statistical data collected throughout the study are discussed in **Chapter 10**. In **Chapter 11** the concluding remarks are given, which include a hypothesis as to the associations of the scyphidiid peritrichs as well as caliperids and their hosts. **Chapter 12** contains the literature referred to in this manuscript, followed by the Abstract and Acknowledgements.

Chapter 2
Materials and Methods

Study Area

The South African coastline and intertidal life are influenced by two major currents, i.e. the warm **Agulhas Current** along the east coast and the cold **Benguela** along the west coast. The Indian Ocean has a huge gyre of water circulating anticlockwise, driven by the winds. This equatorial water mass splits when it reaches Madagascar, part moving around the island and down the coast of Mozambique, where it is known as the Mozambique current, while a second stream passes around the eastern side of Madagascar. The two currents unite again as they flow along the coast of Natal, forming an input into the Agulhas Current. The edge of the continental shelf swings away from the shore from Transkei southwards, deflecting the Agulhas current away from the coast. As a result, the **warm temperate south coast province** (Fig. 2.1), from about Port St. Johns to Cape Point, has cooler coastal waters and a different set of animals and plants from the Natal and Mozambique coasts. Towards the south the Agulhas swings eastwards as the Return Agulhas Current, and unites with three smaller circuits known as the semi-basin, regional and Return Agulhas circulations (Branch & Branch, 1981).

Collection of haliotids

The distribution of the six South African haliotids is depicted in Fig. 2.2A-F. Hosts were collected during March and April of 1995 to 1999 on the south coast of South Africa at the De Hoop Nature Reserve (Fig. 2.1). No seasonal infestation patterns were studied as the haliotids were always collected during the same season (autumn) of the particular year. Perlemoen from the Danger Point Abalone farm near Gansbaai (Fig. 2.1 & 2.6D) were also examined. Two of the six South African species, i.e. *H. spadicea* (Fig. 2.3A&B) and

H. midae (Fig. 2.3C&D) were collected from infratidal pools on the rocky shore. *Haliotis spadicea* are found in shallow infratidal pools, occupying small crevices. *Haliotis midae* is commonly found in the infratidal zone amongst the red bait, the adults are mostly non-cryptic and readily visible, and most are to be found in depths shallower than 10 m (Newman 1969), in beds of the kelp *Ecklonia maxima*. According to literature, *Haliotis parva* also occurs in the De Hoop Nature Reserve (Fig. 2.2E), but was never collected during the five-year study period. A total of 225 haliotids were collected and examined over the five-year period. The haliotids were collected live (Fig. 2.6A) by inserting a stainless steel spatula, also called an ab-iron by Fallu (1991), between the muscular foot and the substratum, so that the haliotids could be prised from the substratum.

Collections were made during spring low tides or low tides, which allowed maximum access to the intertidal area. The infratidal, or subtidal zone, is only completely exposed during spring low tide, every second week (Fig. 2.6B). The haliotids were taken to a field laboratory (Fig. 2.6C) that was set up as close as possible to the collection site, because the symbionts have to be examined live. Abalone can survive some time out of water, but dry air damages delicate tissues, such as the gills (Fallu 1991). After dissection, the shells were labelled and returned to the laboratory in Bloemfontein for later references. The viscera were either discarded in the ocean, or fixed in 10% buffered neutral formalin, for later examination for the presence of trematodes.

Collection of symbionts

The length, width and mass of the haliotids were determined (e.g. Table 10.2c), after which they were shucked (by inserting a spatula blade between the shell and muscular foot), dissected, and the gills removed. In order to collect symbionts a whole gill was placed on a microscope slide, smeared and examined using a compound microscope. Live symbiont specimens were observed with light microscopy to determine their contractility, and the position of contractile vacuoles and nuclei in their bodies were noted.

Photomicrographs were taken of live specimens in various stages of contraction, for the purpose of determining body measurements. Gills infested with scyphidiid peritrichs were graded according to a scale of infestation (Table 1). In the case of the caliperids and trematodes, only the presence or absence of these symbionts was noted. Wet smears were left to air dry for later processing in the laboratory in Bloemfontein, and supplied with a collection number as follows: **Year/Month/Day - collection number**.

Table 1. Index of the grade of infestation of scyphidiid peritrichs on the gills of haliotids.

Index	Number of scyphidiid peritrichs present
X	< 10
XX	> 10 < 100
XXX	> 100 < 200
>XXX	> 200

A specimen collection number (SCN) was assigned to each haliotid collected during the five-year study period. Specimen collection numbers from 1995 and 1996 are collective Aquatic Parasitology data numbers (thus not collected by me), e.g. Table 10.1c refers to SCN 248. This refers to the 248th marine invertebrate that was collected for examination in a specific survey, and not the 248th specimen of haliotid that were collected. In the present study the SCN starts at number one for each survey, and the numbers follow chronologically. In the tables presented in Chapter 10, however, the numbers do not necessarily appear in chronological form. Data from 1997 to 1999 were collected by the author and these specimen collection numbers only represent haliotid collections, and was thus not part of the other marine invertebrate collection data of the Aquatic Parasitology Group.

The digestive glands and gonads of *H. spadicea* and *H. midae* were also examined for the presence of trematodes. This was done by making a wet smear of the digestive gland contents or examining digestive gland and gonad tissue with the aid of a dissecting microscope. Gills prepared for SEM and histopathology examination (see Preparation of

material) of the scyphidiid peritrichs and caliperids, were also studied for the presence of trematodes.

Preparation of material

Light microscopy

Hematoxylin [scyphidiid peritrichs and caliperids]

Some of the wet smears were fixed in Bouin's, whereafter they were transferred to 70% ethanol. In some cases they were returned to the laboratory in Bloemfontein for further processing and in other cases hematoxylin staining was done in the field laboratory. Mayer's and Harris' Hematoxylin was used to stain the nuclear apparatus and for obtaining body measurements, following the standard procedures as described by Humason (1979).

Protargol [scyphidiid peritrichs and caliperids]

The details of the infundibulum was studied by impregnating Bouin's fixed smears with protargol using a combined method as described by Lee, Hunter and Bovee (1985) and Lom and Dykova (1992). In some cases protargol impregnation was done in the field laboratory, and in other cases impregnation was done after returning to the laboratory in Bloemfontein. Depending on the procedure used, protargol can reveal many cortical and internal structures, such as basal bodies, cilia, various fibrillar systems and nuclear apparatus.

Histopathology [scyphidiid peritrichs, caliperids and trematodes]

Formalin fixed gill filaments were processed at the Anatomical Pathology Department of the Medical School, of the University of the Orange Free State, in order to determine whether the symbionts had any pathological effect on their hosts. The gill tissue was embedded in paraffin wax and sectioned (2 μm), using microtome techniques, stained with hematoxylin and counter stained using eosin.

Scanning electron microscopy (SEM) [scyphidiid peritrichs, caliperids and trematodes]
In the field laboratory the gills were fixed in concentrations of 4-10 % buffered neutral formalin. In some cases gills were fixed in Parducz' solution: first in osmium for 30 minutes at 4 °C and then placed in a sodium cacodylate buffer at 4 °C. In other cases gills were fixed in 2.5 % glutar aldehyde. Thereafter the gills were dehydrated to 70 % ethanol at 4 °C. In the laboratory in Bloemfontein the specimens that were fixed in formalin were cleaned by washing the gills in tapwater for 20 minutes, whereafter these were dehydrated in ethanol concentrations:

30 % ethanol - 10 minutes
50 % ethanol - 10 minutes
70 % ethanol - 10 minutes
80 % ethanol - 10 minutes
90 % ethanol - 10 minutes
96 % ethanol - 10 minutes,
and 100 % ethanol - 20 minutes, renewing each concentration every five minutes.

The gills that were fixed in Parducz's solution were dehydrated in ethanol concentrations, similar to the method used in the case of the formalin fixed gills. The gills that were fixed in 2.5 % glutar aldehyde were dehydrated in ethanol concentrations of 80 % to 100 % approximately 24 hours after fixation.

Thereafter the gills were critical point dried, mounted on SEM stubs using instant Pratley Quickset, and coated with gold using an Emscope sputter coater. Detached specimens were prepared on a nucleopore filter with a pore size of 5 µm. The gills were examined at 5 and 10 kV in a JOEL WINSEM JSM 6400 scanning electron microscope. Silver impregnation is used in freshwater specimens to elucidate the body striations (Lowe, McQueen, Ranganathan & Finley 1967; Carey & Warren 1983), but is unsuccessful for marine specimens due to the incompatibility of silver nitrate and seawater. The body striations of the scyphidiid peritrichs and caliperids can clearly be distinguished using

electron microscopy, and SEM was thus used to count body striations of these ciliophorans.

Morphological measurements

Body and nuclear apparatus measurements of the ciliophorans (Fig. 2.4 & 2.5) were obtained from microscope projection drawings done with the aid of a drawing tube. The statistical analysis of the measurements (in μm) was calculated using the computer program CSS Statistica. Minimum and maximum values are given, followed in parentheses by the arithmetic mean and standard deviation, followed by the number of specimens measured. In the cases where less than ten specimens were measured, the standard deviation has not been provided (e.g. Table 7.3).

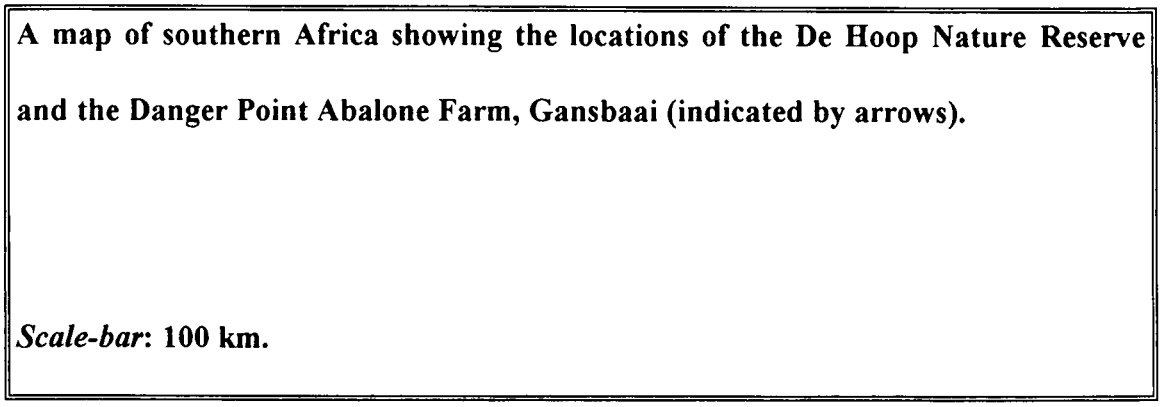
Authors of taxa

Due to the wide spectrum of different taxa mentioned, it was not always possible to find the original authors. In some cases the author could be located but without the date of description. These are indicated by # in the text.

Terminology

More than 25 different common names exist for representatives of the Haliotidae consumed in different parts of the world (Table 3.1). In the text these local names will be used when referring to a specific region's haliotids, and the term "abalone" will be used when referring to haliotids in general. *Haliotis spadicea* specimens are referred to as Venus ears or siffies, whilst the local name for *H. midae* is perlemoen.

Figure 2.1



A map of southern Africa showing the locations of the De Hoop Nature Reserve and the Danger Point Abalone Farm, Gansbaai (indicated by arrows).

***Scale-bar*: 100 km.**

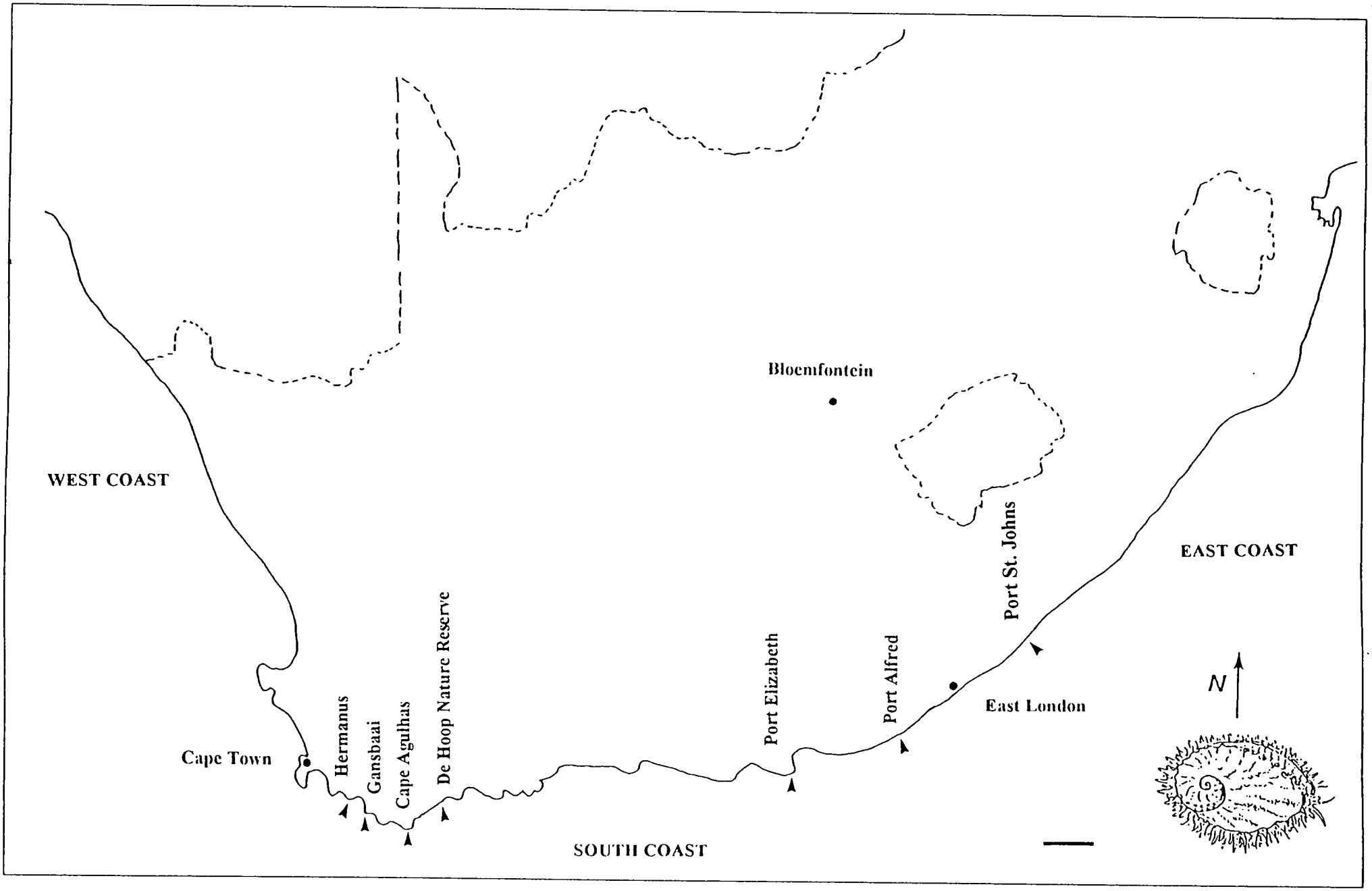

Bloemfontein
WEST COAST
EAST COAST
Port St. Johns
Port Alfred
East London
Port Elizabeth
De Hoop Nature Reserve
Cape Agulhas
Gansbaai
Hermanus
Cape Town
SOUTH COAST
N

Figure 2.2

Geographical distribution of *Haliotis* Linnaeus, 1758 species along the South African coast line (Redrawn from Jacks 1983 & Branch, Griffiths, Branch & Beckley 1994).

A. *H. midae* Linnaeus, 1758.

B. *H. spadicea* Donovan, 1808.

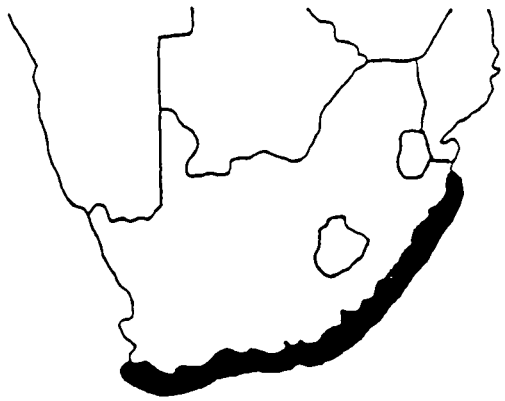
C. *H. parva* Linnaeus, 1758.

D. *H. speciosa* Reeve, 1846.

E. *H. queketti* Smith, 1910.

F. *H. pustulata* Reeve, 1846.

A



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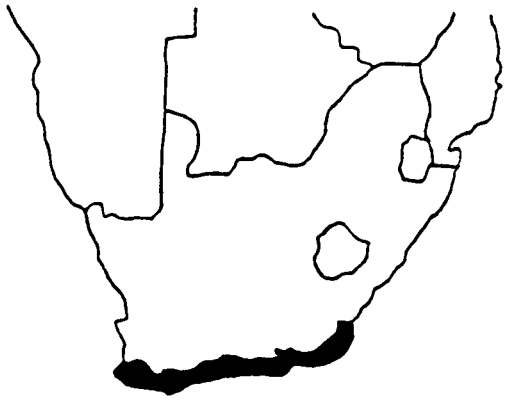

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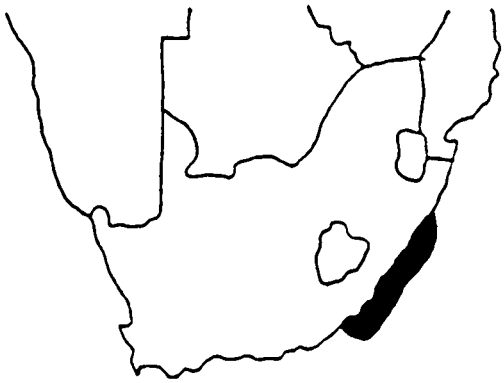

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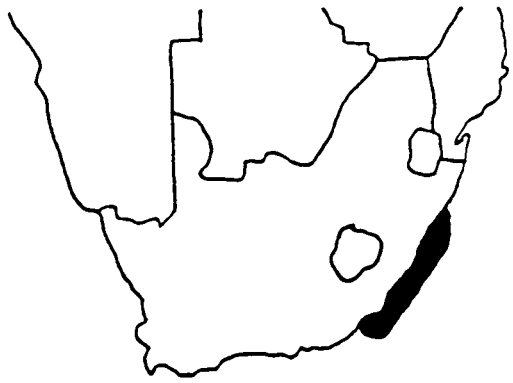

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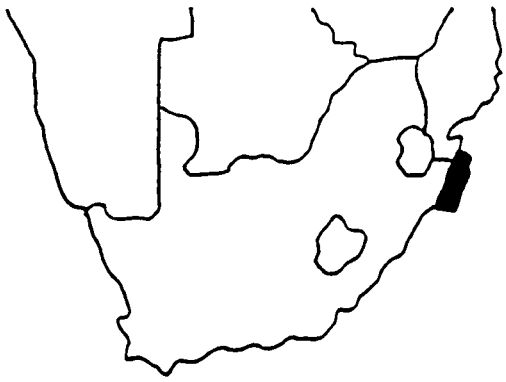

F

Figure 2.3

Photographs of live specimens and shells of the perlemoen (C&D) and Venus ears/siffies (A&B) collected from the De Hoop Nature Reserve, South Africa.

A. Live *Haliotis spadicea* Donovan, 1808 specimens.

B. *H. spadicea* shells.

C. Live *H. midae* Linnaeus, 1758 specimens.

D. *H. midae* shells.

A
B
C
D

Figure 2.4

Diagram of a typical scyphidiid peritrich illustrating morphological features used to determine biometrical measurements.

bl= body length; bd= body diameter; mad= macronucleus diameter; mal= macronucleus length; mid= micronucleus diameter; mil= micronucleus length; sd= scopula diameter; sl= scopula length.

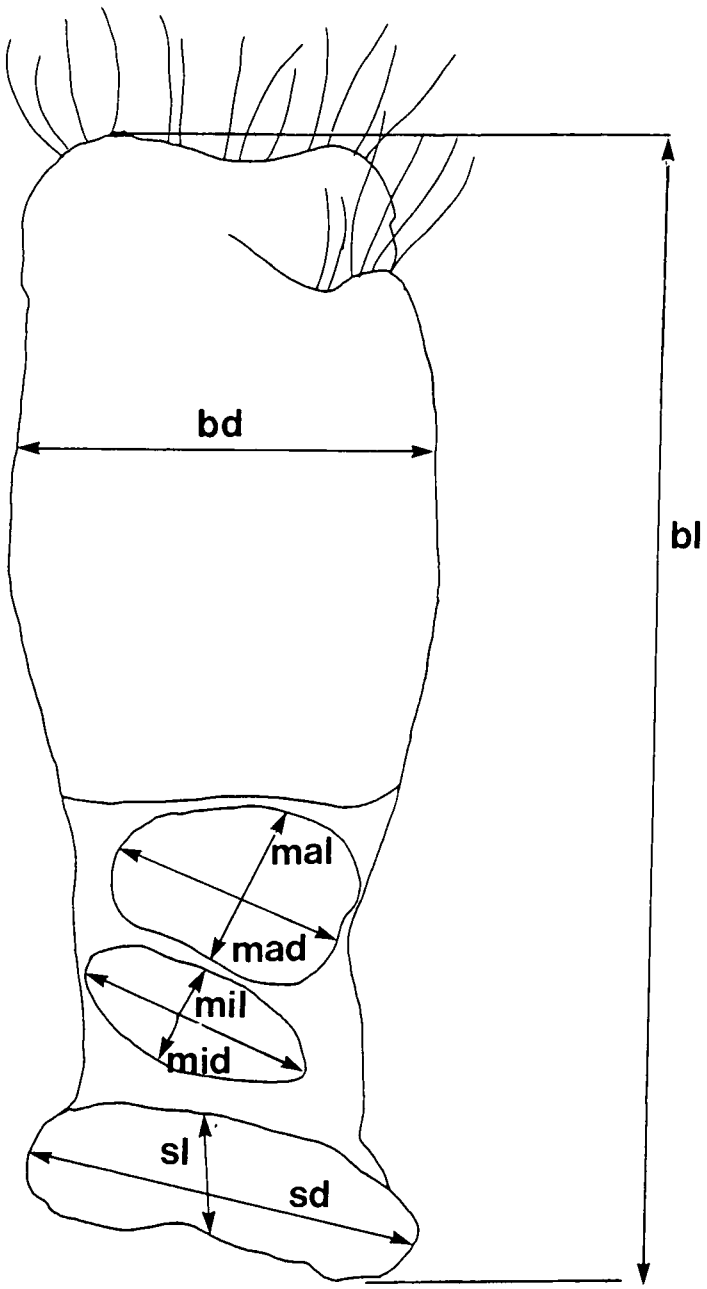
bd
bl
mal
mad
mil
mid
sl
sd

Figure 2.5

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

bl= body length; bd= body diameter; c= cinctum; cld= cinctum limb diameter; icd= inner cinctum diameter; ocd= outer cinctum diameter.

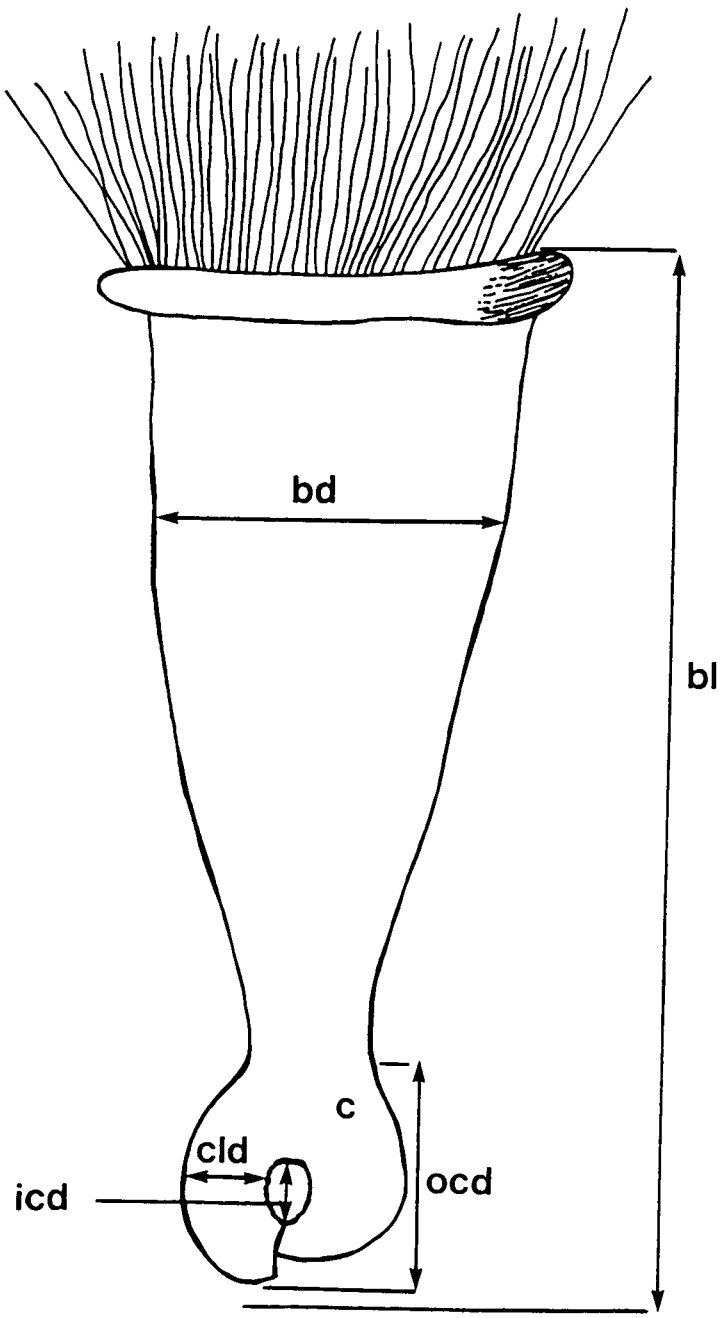
bd
bl
c
cld
icd
ocd

Figure 2.6

Photographs of the collection and study sites along the south coast of South Africa.

A. Author busy collecting perlemoen using an ab-iron.

B. Rocky shore of the De Hoop Nature Reserve, south coast of South Africa.

C. Field laboratory.

D. Danger Point Abalone Farm, Gansbaai, South Africa.

A
B
C
D

Chapter 3

Systematics, biology and life-cycle of the genus Haliotis

The number of described species of molluscs is estimated to be in the order of 100 000, placing them second only to the arthropods as the phylum with the most species (Boyle 1981). There are probably more living species of gastropods than the total in all of the other classes. Widely distributed in all the major marine habitats, they have successfully invaded freshwaters and are the only molluscan group to establish themselves convincingly on land. The use of molluscs' shells as jewellery, such as abalone pearls (Fankboner 1993, 1994) dates back to prehistoric times.

Abalone, locally known as perlemoen, are large, herbivorous marine gastropods with all species in one genus, *Haliotis*. Older scientific papers also refer to *Haliotis* as *Notohaliotis, Euhaliotis* or *Sanhaliotis*. In the 4th century BC Aristotle documented the first natural history of a haliotid (Crofts 1929). According to Olley and Thrower (1977), Aristotle (ca. 347) in Historia Animalium, called abalone "*Agria lepas*" (wild limpet) and '*Thalattion us*" (marine ear). Linnaeus in Systema Naturae, Ed 2 (1740) named the genus "*Haliotis*", which means sea ear (Crofts 1929). The first published figure of a haliotid is believed to be given by Belon in 1553, who calls attention to Aristotle's reference to "the other *Patella major*" under the name "*Aporrhias*" (Cox 1962). More than twenty five different local names exist for Haliotidae eaten in different parts of the world. Olley and Thrower (1977), as well as Hahn (1989a) provide some common names for the species of abalone (Table 3.1).

Table 3. 1 Common names of some of the abalone species consumed in different parts of the world.

Locality	Common name
Adriatic, Dalmatia (Yugosalvia)	Orechio de San Pietro
Australia	Mutton fish, Abalone
Amboina (Molluccas), Ceram	Holley
Canada	Abalone
Channel Islands	Ormer, Sie-ieu, Sea ear
China	Abalone
England	Ormer, Ormier, Omar, Venus ear, Normal shell
France	Ormer, Sie-ieu, Orielle de Mer
Greece	Venus ear
Germany	Ohrsnecke, Meerohren
Italy	Orecchiale
Japan	Kuro awabi, Oni, Onigai, Tokobushi, Madaka, Megai, Mimigai
Malaysia	Telinga Maloli, Ria Scatsjo
Mediterranean	Orecchiale, Orechio de San Pietro
Mexico	Aulone
New Zealand	Paua, Karariwha
Portugal	Lapa Burra
Sicily	Patella Reale
South Africa	Perlemoen, Venus ear or siffie
Spain	Senorinas
Sultunate of Oman	Al sufailah
Thailand	Cholburi
United States	Abalone

Abalone have been commercially exploited since ancient times. The oldest abalone fishery was probably conducted by the Japanese, for it is recorded that "a diver named Osahi, in north Shikoku, collected 'awabi' on September 12, 425 AD" (Cox 1962). Ama abalone divers were exclusively female, because men were taken to serve on war ships (Hahn 1989b). Olley and Thrower (1977) remark that Asian people believe this shellfish has aphrodisiac properties. The shell has been used in Chinese traditional medicine and is called "Shi-Jue-Ming", which is thought to be beneficial for eyesight and the liver (Zong Qing Nie 1992).

According to Lindberg (1992), there are 66 species of abalone world-wide (Table 3.4). Numerous regional and global checklists of *Haliotis* species exist, but knowledge of the evolution and phylogeny of the genus *Haliotis* remains sketchy and poor. The classification system is wholly phenetic and little use in recognizing relationships between the species. Knauer (1994) states that there is considerable discrepancy in the literature regarding the extant *Haliotis* species, ranging from 50 to 130 species and subspecies. All haliotids belong to the family Haliotidae Rafinesque, 1815. *Haliotis* is the only genus in the family, with *Haliotis midae* the genotype (Cox 1962). The genus has been divided into over 15 subgenera, but until new characters have been studied, the division of the genus *Haliotis* into lower taxa is fallacious (Lindberg 1992).

The ancestors of the Haliotidae are unknown. Members of the Haliotidae are monophyletic (all share a common ancestor), but relationships below this taxonomic level are unknown. In most modern systematic treatments the haliotids are grouped with the Pleurotomaridae and Scissurellidae in the taxon Pleurotomariacea (Table 3.2). Members of this group are characterized by the presence of an excurrent opening along the margin of their shells, paired bipectinate ctenidia and the presence of a well-developed epipodium (Lindberg 1992). The gill and internal organs on the right side of the body are typically reduced in size.

Table 3.2 Classification of the genus *Haliotis* Linnaeus, 1758.

Kingdom	Animalia
Phylum	Mollusca
Class	Gastropoda
Subclass	Prosobranchia
Order	Archeogastropoda
Suborder	Zygobranchia
Superfamily	Pleurotomariacea
Family	Haliotidae Rafinesque, 1815
Genus	*Haliotis* Linnaeus, 1758

The genus *Haliotis* has been divided into three morphological groups by Tissot (1992). The characters on which these are based include the ratio of shell to body size, shell sculpture, epipodial structures and the morphology of the tremata (Table 3.3). These characters could be of adaptive significance, as shell sculpture contributes to armour against shell crushing predators, elaborate sensory epipodial extensions of the muscular foot could facilitate the escape response, and large tremata may enhance respiratory exchange passively in areas of low water movement. The haliotid radula has not been studied sufficiently for characters that may prove useful in diagnosing taxa within the family. The structure of the epipodium has been used to diagnose species. These diverse characters co-vary and form three distinct morphological groups within the family.

Abalone are found from the subarctic to antarctic. They are most abundant in temperate and tropical waters, as common inhabitants of rocky intertidal and subtidal zones (Muller 1984). They occur along the rocky shores of all the major continents, with the exception of South America and eastern North America, and among many of the islands in the Pacific, Atlantic and Indian Oceans (Cox 1962; Hahn 1989a). The most abundant populations are found along the coasts of Australia, Japan and western North America. Abalone live in turbulent habitats, with high levels of dissolved oxygen (Fallu 1991).

Table 3.3 Three morphological groupings within the family Haliotidae Rafinesque, 1815 (Tissot 1992).

	I	II	III
Shell shape	Oval, arched	Elongate, flat	Oval, variable
Shell sculpture	Obscure spiral ribs, smooth shell	Obscure strong spiral ribs, imbricate growth lines	Elevated, prominent spiral and axial ribs, conspicuous shell sculpture
Tremata	5-16, flush with dorsal surface, small	4-8, slightly elevated	2-7, highly elevated on tubular projections
Epipodium	Thin, simple plates	Thick, simple and papillate	Medium thick, branched, plated and tubercles
Habitat	Open, shallow (to 10m) intertidal and subtidal habitats	Semi-protected, and open shallow to moderate (to 20m) and deep habitats	Protected, shallow to deep (to 600m) subtidal habitats
Species	*H. asinina* Linnaeus, 1758 *H. australis* Gmelin, 1791 *H. cracherodii cracherodii* Leach, 1814 *H. cyclobates* Péron & Lesueur, 1816 *H. glabra* Gmelin, 1791 *H. iris* Gmelin, 1791 *H. laevigata* Donovan, 1808 *H. midae* Linnaeus, 1758 *H. planata* Sowerby, 1833 *H. virginea virginea* Gmelin, 1791	*H. coccinea* Reeve, 1846 *H. diversicolor* Reeve, 1846 *H. elegans* Philippi, 1848 *H. mariae* Wood, 1928 *H. pustulata* Reeve, 1846 *H. squamata* Reeve, 1846 *H. tuberculata* Linnaeus, 1758 *H. walallensis* Stearns, 1899	*H. brazieri* Angas, 1869 *H. corrugata* Wood, 1828 *H. dalli* Henderson, 1915 *H. discus discus* Reeve, 1846 *H. fulgens fulgens* Philippi, 1845 *H. gigantea* Gmelin, 1791 *H. kamtschatkana kamtschatkana* Jonas, 1845 *H. ovina* Gmelin, 1791 *H. parva* Linnaeus, 1758 *H. pourtalesii* Dall, 1881 *H. scalaris* Leach, 1814 *H. semiplicata* Menke, 1843 *H. sieboldii* Reeve, 1846 *H. sorenseni* Bartsch, 1940 *H. varia varia* Linnaeus, 1758

Table 3.4 Extant abalone species (in bold), with synonyms listed after each species where applicable (Muller 1984; Hahn 1989a & Lindberg 1992). Localities indicated by * could not be found in the literature.

Species	Locality
***H. asinina* Linnaeus, 1758**	Japan
***H. assimilis* #**	Australia
***H. australis* Gmelin, 1791** *H. aleata* Röding, 1798	New Zealand
***H. barbouri* Foster, 1946**	*
***H. brazieri* Angas, 1869**	Australia
H. coccinea Reeve, 1846 *H. janus* Reeve, 1846	*
***H. coccoradiata* Reeve, 1846**	Australia
***H. corrugata* Wood, 1828** *H. nodosa* Philippi, 1845	North America, Mexico
***H. cracherodii cracherodii* Leach, 1814** *H. interrupta* Valenciennes, 1832	North America, Mexico
***H. cracherodii californiensis* Swainson, 1821**	North America
***H. crebisculpta* Sowerby, 1914**	Japan
***H. cyclobates* Péron & Lesueur, 1816** *H. excavata* Lamarck, 1822	Australia
***H. dalli* Henderson, 1915**	*
***H. discus discus* Reeve, 1846**	Japan, Korea
***H. discus hannai* Ino, 1953**	Japan, China, Korea
***H. dissona* Iredale, 1929**	*
***H. diversicolor* Reeve, 1846** *H. tayloriana* Reeve, 1846 *H. gruneri* Philippi, 1848 *H. supertexta* Lischke, 1870	Japan, China
***H. dohrniana* Dunker, 1882**	*
***H. elegans* Philippi, 1848**	Australia
***H. exigua* Dunker, 1877**	Japan
***H. fulgens fulgens* Philippi, 1845** *H. splendens* Reeve, 1846 *H. planilirata* Reeve, 1846	North America, Mexico
***H. fulgens turvei* Bartsch, 1942**	North America
***H. fulgens guadalupensis* Talmadge, 1964**	North America
***H. gigantea* Gmelin, 1791** *H. tubifera* Lamarck, 1822 *H. gigas* Röding, 1798	Japan, Korea

Table 3.4 (continue) Extant abalone species (in bold), with synonyms listed after each species where applicable.

Species	Locality
***H. glabra* Gmelin, 1791** *H. ziczac* Reeve, 1846 *H. picta* Röding, 1798	*
***H. guineensis* Gmelin, 1791** *H. rosacea* Reeve, 1846 *H. decussata* Philippi, 1850 *H. virginea* Reeve, 1846	*
***H. hanlevi* Ancey, 1881**	*
***H. hargravesi* Cox, 1869**	Australia
***H. howensis* Iredale, 1929**	*
***H. iris* Gmelin, 1791**	New Zealand
***H. jacnensis* Reeve, 1846** *H. echinata* Sowerby, 1883	Japan
***H. japonica* Reeve, 1846** *H. aquatilis* Reeve, 1846 *H. incisa* Reeve, 1846	Japan
***H. kamtschatkana kamtschatkana* Jonas, 1845**	Japan, North America, Canada
***H. kamtschatkana assimilis* Dall, 1878** *H. aulaea* Bartsch, 1940 *H. smithsoni* Bartsch, 1940	Australia
***H. laevigata* Donovan, 1808** *H. albicans* Quoy & Gaimard, 1834 *H. excisa* Gray, 1856	Australia
***H. mariae* Wood, 1928**	Sultunate of Oman
***H. melculus* Iredale, 1927**	*

Table 3.4 (continue) Extant abalone species (in bold), with synonyms listed after each species where applicable.

Species	Locality
***H. midae* Linnaeus, 1758** *H. midae* Linnaeus/ Krauss, 1848 / Turton, 1932 *H. capensis* Dunker, 1844 *H. elatior* Pilsbry, 1890 *H. midae elatior* Turton, 1932 *H. midae capensis* Turton, 1932	South Africa
***H. ovina* Gmelin, 1791** *H. caelata* Röding, 1798 *H. latilalabris* Philippi, 1851	*
***H. parva* Linnaeus, 1758** *H. canaliculata* Lamarck, 1822 *H. carinata* Swainson, 1822 *H. cingulata* Röding, 1798 *H. kraussi*, Turton, 1932 *H. parvum* Krauss, 1848 / Smith, 1910 / Turton, 1932 *H. rubicunda* Röding, 1798	South Africa
***H. planata* Sowerby, 1833**	Japan
***H. pourtalesii* Dall, 1881**	*
***H. pustulata* Reeve, 1846** *H. alternata* Sowerby, 1882 *H. ancile* Reeve, 1846 *H. nebulata* Reeve, 1846 *H. pertusa*, Reeve, 1846 *H. scutulum* Reeve, 1846 *H. relevata* Reeve, 1846 *H. zealandica* Reeve, 1846 *H. strigata* Weinkauff, 1883	South Africa
***H. queketti* Smith, 1910** *H. queketti* Turton, 1932 / Macnae & Kalk, 1958	South Africa
***H. roberti* McLean, 1970**	*
***H. roei* Gray, 1827**	Australia
***H. rubra rubra* Leach, 1814** *H. conicopora* Péron, 1816 *H. cunninghami* Gray, 1826 *H. granti* Pritchard & Gatliff, 1903 *H. improbulum* Iredale, 1924 *H. naevosa* Martyn, 1786 *H. vixlirata* Cotton, 1943	Australia
***H. rubra clathrata* Reeve, 1815**	Australia

Table 3.4 (continue) Extant abalone species (in bold), with synonyms listed after each species where applicable.

Species	Locality
***H. rufescens* Swainson, 1822** *H. californiana* Valenciennes, 1832 *H. ponderosa* Adams, 1848	North America, Mexico
***H. scalaris* Leach, 1814** *H. emmae* Reeve, 1846 *H. rubicunda* Gray, 1846 *H. tricostalis* Lamarck, 1822 *H. tricostata* Wood, 1828	Australia
***H. semiplicata* Menke, 1843** *H. lauta* Reeve, 1846	Australia
***H. sieboldii* Reeve, 1846**	Japan, Korea
***H. sorenseni* Bartsch, 1940**	North America, Mexico
***H. spadicea* Donovan, 1808** *H. ficiformis* Menke, 1844 *H. nebulata* Turton, 1932 *H. pertusa* Bartsch, 1915 / Turton, 1932 *H. sanguinea* Hanley, 1840 / Krauss, 1848 / Bartsch, 1915 / Turton, 1932 / Macpherson, 1953	South Africa
***H. speciosa* Reeve, 1846** *H. pertusa* Sowerby, 1900 / Smith, 1903 *H. speciosum* Reeve, 1846 / Talmadge, 1958 *H. alfredensis* Bartsch, 1915 / Tomlin, 1927 / Turton, 1932	South Africa
***H. squamata* Reeve, 1846** *H. elevata* Sowerby, 1883	*
***H. tuberculata* Linnaeus, 1758** *H. incisa* Reeve, 1846 *H. bistriata* Gmelin, 1791 *H. lamellosa* Lamarck, 1822 *H. lucida* Requien, 1848 *H. reticulata* Reeve, 1846 *H. rugosa* Lamarck, 1822 *H. vulgaris* da Costa, 1778	France, Channel Islands
***H. unilateralis* Lamarck, 1822**	*

Table 3.4 (continue) Extant abalone species (in bold), with synonyms listed after each species where applicable.

Species	Locality
***H. varia varia* Linnaeus, 1758** *H. concinna* Reeve, 1846 *H. semistriata* Reeve, 1846 *H. viridis* Reeve, 1846 *H. varia pustulifera* Pilsbry, 1890 *H. astricta* Reeve, 1846 *H. granulata* Röding, 1798 *H. papulata* Reeve, 1846 *H. rubiginosa* Reeve, 1846	Japan
***H. varia stomatiaeformis* Reeve, 1846**	Japan
***H. varia aliena* Iredale, 1928**	Japan
***H. virginea virginea* Gmelin, 1791** *H. subvirginea* Weinkauff, 1833	New Zealand
***H. virginea crispata* Gould, 1847**	New Zealand
***H. virginea huttoni* Filhol, 1880**	New Zealand
***H. virginea morioria* Powell, 1938**	New Zealand
***H. walallensis* Stearns, 1899**	North America

Abalone/perlemoen belong to the order Archaeogastropoda, which are among the oldest and least specialised members of the gastropod subclass Prosobranchia (Kilburn & Rippey 1982). Abalone have a pair of bipectinate gills consisting of rows of filaments on either side of a central axis. The left ctenidium is decidedly larger than the right, because organs on the right are typically reduced as in higher gastropods (Fig. 3.1; 3.2). The right gill can be seen through the transparent mantle. A pair of extensive osphridia is present along the anterior border of each ctenidial support, to test the water passing to the ctenidia (Crofts 1929).

Abalone have developed perforations or tremata to accommodate a central outlet from the mantle cavity, through which stale water containing excreta can be discharged. This prevents contamination of the inhalant current, which is drawn into the mantle cavity from above the head (Kensley 1973; Kilburn & Rippey 1982). These perforations close posteriorly as growth proceeds (Muller 1986), in other words, as the shell grows the hole nearest to the spire closes as another forms at the growing edge (Jacks 1983). *Haliotis midae* and *H. spadicea* have only slightly elevated tremata. Tissot (1992) stated that abalone with larger, elevated tremata are more efficient at promoting induced flow at low external velocities, and therefore are capable of maintaining a constant mantle cavity flow rate in a wide range of habitats, than species with small, unelevated tremata.

The hypobranchial glands are attached to the right and left of the rectum. The quantity of mucus discharged from them into the respiratory chamber increases if the animal is irritated. It is produced for protection as well as cleaning away debris from the anus and renal organs, in order to keep the ctenidia clean (Crofts 1929). The gut, kidneys and reproductive glands empty into the mantle cavity, and their excretory products are passed out in the exhalant currents through the tremata (Fallu 1991).

Haliotis species are characterised by their single oval shell and their large muscular feet whose flesh makes such good eating. The part of the abalone sought after as food is the muscle in the foot and the short stalk joining the foot to the shell. Usually the rest of the

viscera, gut, reproductive glands and outer skin of the foot are discarded (Fallu 1991). The ear-shaped shell covers the entire dorsal side of the animal's body, is depressed and has an enlarged body whorl and a reduced spiral apex (Kilburn & Rippey 1982; Muller 1986). The shell shape has probably evolved from a taller spire, because of the abalone's habit of squeezing into confined spaces between rocks. This flattening results in the inability to retract completely into the shell. An operculum would therefore be useless and is missing except in the larval stage (Crofts 1929). The outside of the shell is usually rough to a greater or lesser degree, often with other molluscs, sponges, algae or hard red coralline encrusting algae growing on it (Fallu 1991). The shell grows by the addition of new material at the anterior right-hand side.

All members of the family are herbivorous. Larvae feed on plankton, spat feed on coralline algae and slime (micro-algae and bacteria) and adults feed on seaweed. Some of the larger species such as *H. midae* devour drifting seaweed which are trapped under the front of the foot, while pieces are rasped off with the powerful rhipidoglossan radula, located inside the mouth. Adult abalone graze on seaweed attached to the seabed and loose seaweed drifting in the currents. Haliotids feed on red, brown or green algae, and are stimulated to feed when the surrounding water is moving vigorously. Feeding in these conditions makes these molluscs less susceptible to predation and increases the chance of seaweed being washed past (Fallu 1991; Knauer, Britz & Hecht 1993; Day & Cook 1995; Knauer, Hecht & Britz 1995 Matthews & Cook 1995; Wood & Buxton 1996a).

The epipodium is more elaborate in *Haliotis* species than in any other mollusc, it is a development of the foot and is elaborately supplied with nerves (Cox 1962). The epipodium consists of very tough skin that forms a shield against predators trying to eat the succulent parts of the foot (Fallu 1991). Most of the body of an abalone is a large muscle mass consisting of the foot, including its epipodium and the large right shell columellar muscle (Fig. 3.1; 3.2).

The cephalic region can be withdrawn under the shell for protection. It carries the snout, cephalic tentacles and the stumpy eye protruberances. Sexes are separate in *Haliotis* species. A single reproductive gland or gonad is extensively developed over the brown digestive gland and extends posteriorly as a conical, horn-shaped structure along the left side of the columellar shell muscle (Fig. 3.1) and up into the coiled apex of the shell. In mature animals, the gonad is cream to white coloured in the male and grey-green to yellow-grey in the female. The gonad of a mature abalone is clearly defined and swollen and is termed fat, conditioned or ripe. The genital products in both sexes escape to the cavity of the right renal organ and are freed into the sea through the tremata, where the ova sink and the spermatozoa swim.

Abalone are dioecious, broadcast spawners and external fertilisation takes place (Fallu 1991; Wood & Buxton 1996b). When these gastropods are ready to spawn, they migrate towards the higher parts of the reef and group together. This minimises losses due to unfertilised eggs. Fertilisation is followed by the development of a lecithotrophic larval stage. The larvae undergoes metamorphosis, through stages which are initially called the trochophore and later, the veliger stage (Fig. 3.3). The pelagic nature of the trochophore stage is thought to facilitate dispersal, and the upward swimming larvae (or risers) avoid predation by benthic filter feeders by staying at the water's surface (Fallu 1991; McShane 1992).

After a week the pre-torsion veliger larvae sink to settle on the seabed. At this stage abalone are termed spat (Fig. 3.3). Spat use their radulae to scrape coralline algae and slime off the surface of rocks. The larvae's body undergoes an internal twist (torsion) and the relative positions of its organs are changed. Just prior to settlement it develops a clearly visible eyespot. This takes place over the next few weeks. Once settled, the creeping larvae eats and grows into juveniles.

Haliotis spadicea is a summer breeder with a protracted breeding season and peak spawning period in December. According to Muller (1984) and Fallu (1991) the Venus

ear or siffie reaches sexual maturity at 40 mm shell length, while being estimated to be three years old at this length. *Haliotis midae* spawns twice a year in certain areas namely during spring and autumn. There are some variations, however, due to locality (Newman 1967). Perlemoen reaches sexual maturity at 80-85 mm shell length and is 7.5 years old (Muller 1984). The reproductive cycles of these molluscs are to a large extent governed by environmental factors and temperature is especially important. Spawning is usually associated with a well-defined increase in water temperature.

The genus *Haliotis* comprises six species (Table 3.5) endemic to and distributed along the southern African coast (Jacks 1983; Muller 1984, 1986 & Branch, *et al.* 1994). The two species *H. spadicea* and *H. midae* show considerable overlap in geographical range and *H. spadicea* may be found in habitats utilised by both adult and juvenile *H. midae*. Muller gave an elaborate discussion on the taxonomic status of the genus *Haliotis* in South Africa.

Table 3.5 Taxonomy of the six haliotid species occurring along the coast of South Africa (Kennelly 1969; Jacks 1983 & Muller 1986).

Haliotis midae Linnaeus, 1758 (Fig. 2.5C&D)	
Synonyms	*H. capensis* Dunker, 1884; *H. elatior* Pilsbry, 1890; *H. midae elatior* Turton, 1932; *H. midae capensis* Turton, 1932; *H. midae* Linnaeus / Krauss, 1848 / Turton, 1932
Common name	Perlemoen
Size range	This is the largest of the South African abalone growing up to 23cm in 30 years
Distribution Fig. 2.2A	From Saldanha Bay to Gonubie. Also reported from Coffee Bay, Transkei (Eastern Cape)
Description	The dorsal surface is reddish, but is often obscured by thick marine growth. Numerous characteristic corrugations run obliquely to the lines of growth and 7-11 deep tremata are slightly raised along the shoulder of the shell. The interior of juveniles is a clear irridescent pink, which becomes more turquoise or green in older specimens. Most shells have a large rough muscular scar in the centre of the interior surface although this is not apparent in juveniles. The foot is pale cream to mottled light brown, and the tentacles and gills are yellow.

Table 3.5 (continue) Taxonomy of the six haliotid species occuring along the coast of South Africa.

Haliotis spadicea Donovan, 1808 (Fig. 2.5A&B)	
Synonyms	*H. sanguinea* Hanley, 1840 / Krauss, 1848 / Bartsch, 1915 / Turton, 1932 / Macpherson, 1953; *H. ficiformis* Menke, 1844; *H. pertusa* Bartsch, 1915 / Turton, 1932; *H. nebulata* Turton, 1932
Common name	Venus ear, siffie
Size range	Grows up to 9.5 cm
Distibution Fig. 2.2B	From Partridge Point, Cape Peninsula to Tongaat, Kwa-Zulu Natal. Also recorded from Mauritius and Western Australia, although this record needs to be investigated.
Description	The dorsal surface has minor ridges radiating from the spire oblique to the growth lines. Specimens average between 6 and 8 tremata, situated almost flush with the shell surface. The predominant colour is a reddish brown with intermittant and random white/green mottling. The spire is bronze and most specimens are free of marine growth. The interior cavity has a characteristic copper stain on the inside of the spire and juveniles tend to be a more irridescent turquoise than larger shells. The foot is a bluish-green colour and the tentacles and outer edges of the mantle a luminescent green.

Table 3.5 (continue) Taxonomy of the six haliotid species occuring along the coast of South Africa.

Haliotis parva Linnaeus, 1758	
Synonyms	*H. parvum* Krauss, 1848 / Smith, 1910 / Turton, 1932; *H. kraussi* Turton, 1932; *H. canaliculata* Lamarck, 1822; *H. carinata* Swainson, 1822; *H. cingulata* Röding, 1798; *H. rubicunda* Röding, 1798
Common name	-
Size range	Grows up to 4.8 cm
Distribution Fig. 2.2C	False Bay, Table Bay, Still Bay through Port Elizabeth, Port Alfred to Gonubie, Kwa-Zulu Natal. Scarce throughout its range.
Description	The dorsal surface is sculptured with numerous fine lirae running parallel to the line of growth. The most distinguishing characteristic is the prominent fold lying parallel to the tremata. This ridge extends beyond the growing edge of the shell. In beach worn specimens or large shells, the ridge can become indistinct. The spire is high and is located approximately one third along the length of the shell. The dorsal surface varies from a beige or green and maroon mottling to a uniform brick red or pumpkin orange. There are 5-7 tremata, they are usually ovate or slightly irregular and are marginally raised along the shoulder. The interior varies from a nacreous pink in juveniles to a more turquoise pink in larger specimens. The parallel fold on the dorsal surface corresponds to a deeply incised groove on the inside of the shell continuing into the fairly deep concave ear. The foot has a greyish colour.

Table 3.5 (continue) Taxonomy of the six haliotid species occuring along the coast of South Africa.

Haliotis speciosa Reeve, 1846	
Synonyms	*H. alfredensis* Bartsch, 1915 / Tomlin, 1927 / Turton, 1932; *H. speciosum* Reeve, 1846 / Talmadge, 1958 / Tomlin, 1927 / Turton, 1932; *H. pertusa* Sowerby, 1900 / Smith, 1903
Common name	-
Size range	4 - 6.3 cm
Distribution Fig. 2.2D	Gonubie, East London, Kowie, Port Alfred, Western Transkei (Eastern Cape) to Kwa-Zulu Natal. Rare throughout its range.
Description	A fairly smooth and flattened shell with 3-6 oval perforations. Dorsally, numerous fine striations run parallel to the growth line. Maroon, dark brown and beige mottling is predominant while the interior is irridescent green/pink. The midwhorl ridge is virtually absent.

Table 3.5 (continue) Taxonomy of the six haliotid species occuring along the coast of South Africa.

Haliotis queketti Smith, 1910	
Synonyms	*H. queketti* Turton, 1932 / Macnae & Kalk, 1958
Common name	-
Size range	Grows up to 7.6 cm
Distribution Fig. 2.2E	Port Alfred, Transkei (Eastern Cape) through Natal-Isezela, Kelso and off O'Niel Peak (Zululand). Rare throughout its range
Description	The dorsal surface shows a raised spire that is far more prominent than in *H. parva*. Five ovate tremata are situated on elevated tubules about 1 mm high. There is a slight groove running parallel to the line of growth between the tremata and the rim of the shell. Also parallel to the line of growth is a rib running from the spire to the growth line. The colour varies from a tan with scarlet mottling to a burnt orange. The shell has a wrinkled appearance with a large spire in relation to the size of the shell. The interior is a nacreous pink to turquoise with a deeply incised ear. The trough corresponding to the dorsal ridge is not as evident in the interior as it is in *H. parva* and it does not extend beyond the growth line.

Table 3.5 (continue) Taxonomy of the six haliotid species occuring along the coast of South Africa.

Haliotis pustulata Reeve, 1846	
Synonyms	*Haliotis pertusa* Reeve, 1846; *H. alternata* Sowerby, 1882; *H. ancile* Reeve, 1846; *H. nebulata* Reeve, 1846; *H. scultulum* Reeve, 1846; *H. relevata* Reeve, 1846; *H. zealandica* Reeve, 1846; *H. strigata* Weinkauff, 1883
Common name	-
Size range	5.3 cm
Distribution Fig. 2.2F	Known from a single specimen from Kosi Bay, northern KwaZulu-Natal
Description	A small, thick elongated oval shell with 5-6 nearly circular tremata. The dorsal surface has strong spiral grooves and ridges crossed by five axial growth lines. Brown mottled with red, brown and green colouring.

Figure 3.1

Illustration of the general morphology of a haliotid, dorsal view with the shell removed (Redrawn from Cox 1962).

cl= left ctenidia; cr= right ctenidia; ct= cephalic tentacle; e= eye; es= eye stalk; ep= epipodium; f= foot; g= gonad; m= mantle; sm= shell muscle; t= tentacle.

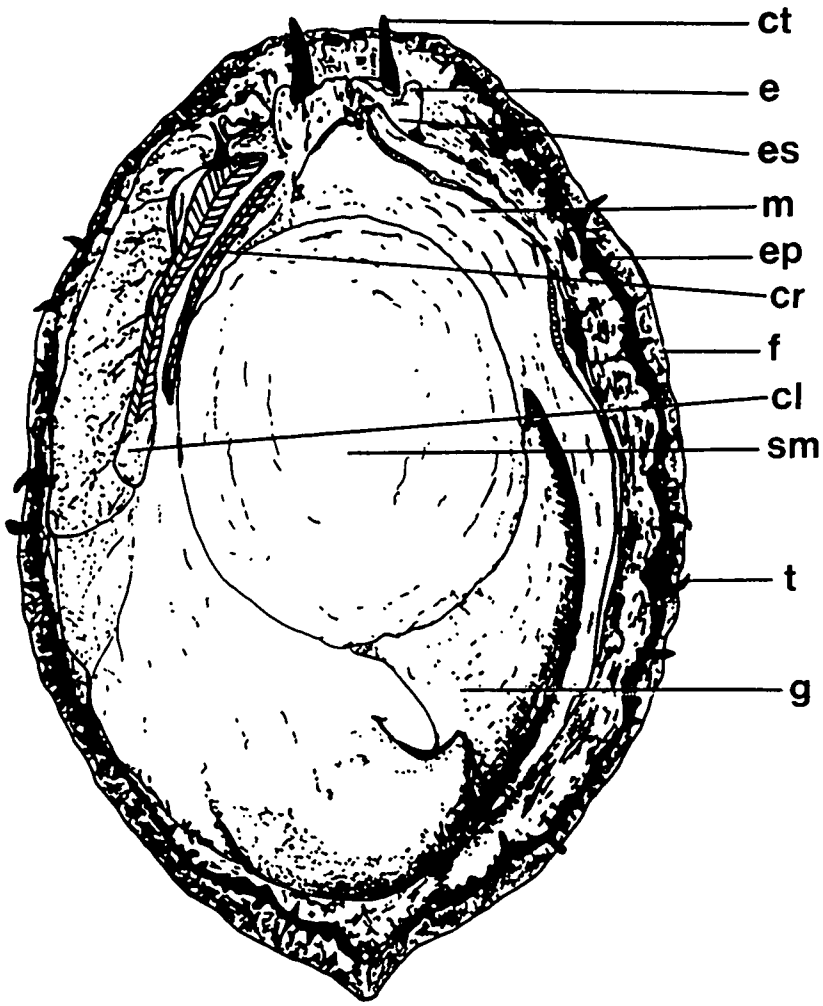
ct
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Figure 3.2

Illustration of the general morphology of a haliotid, alimentary tract with ctenidia and viscera removed (Redrawn from Cox 1962).

a= anus; dg= digestive gland; i= intestine; l= liver; s= stomach; sg= salivary gland.

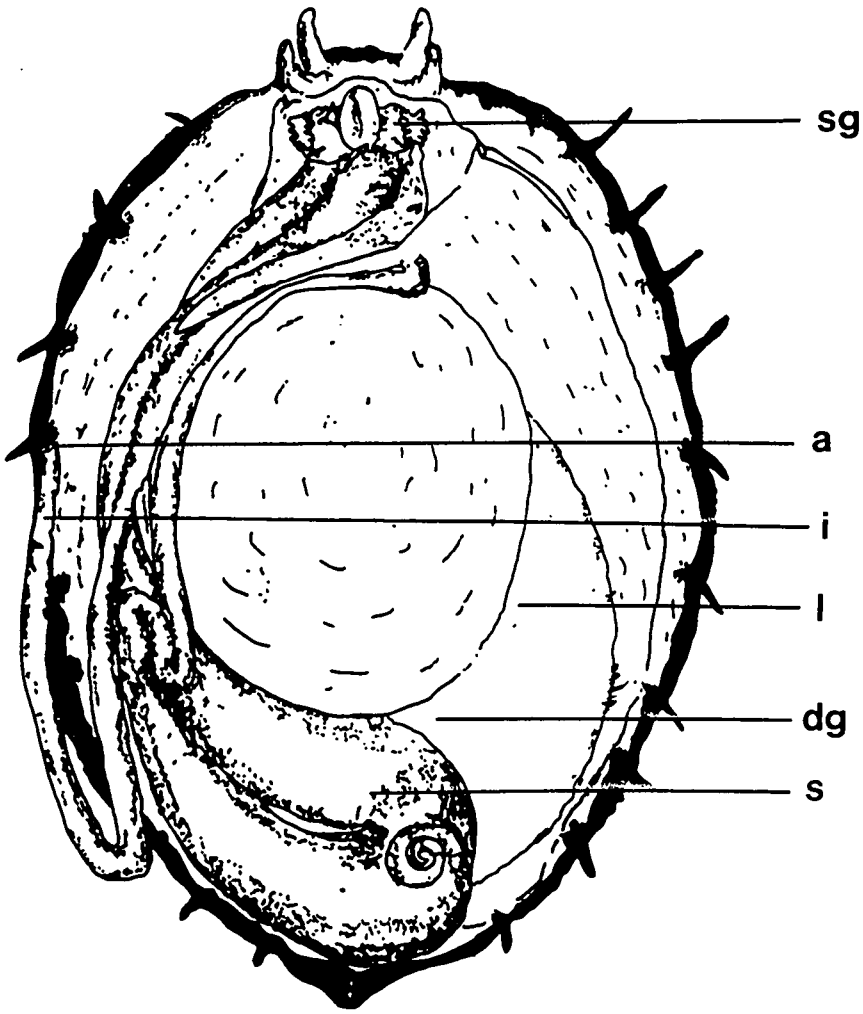
sg
a
i
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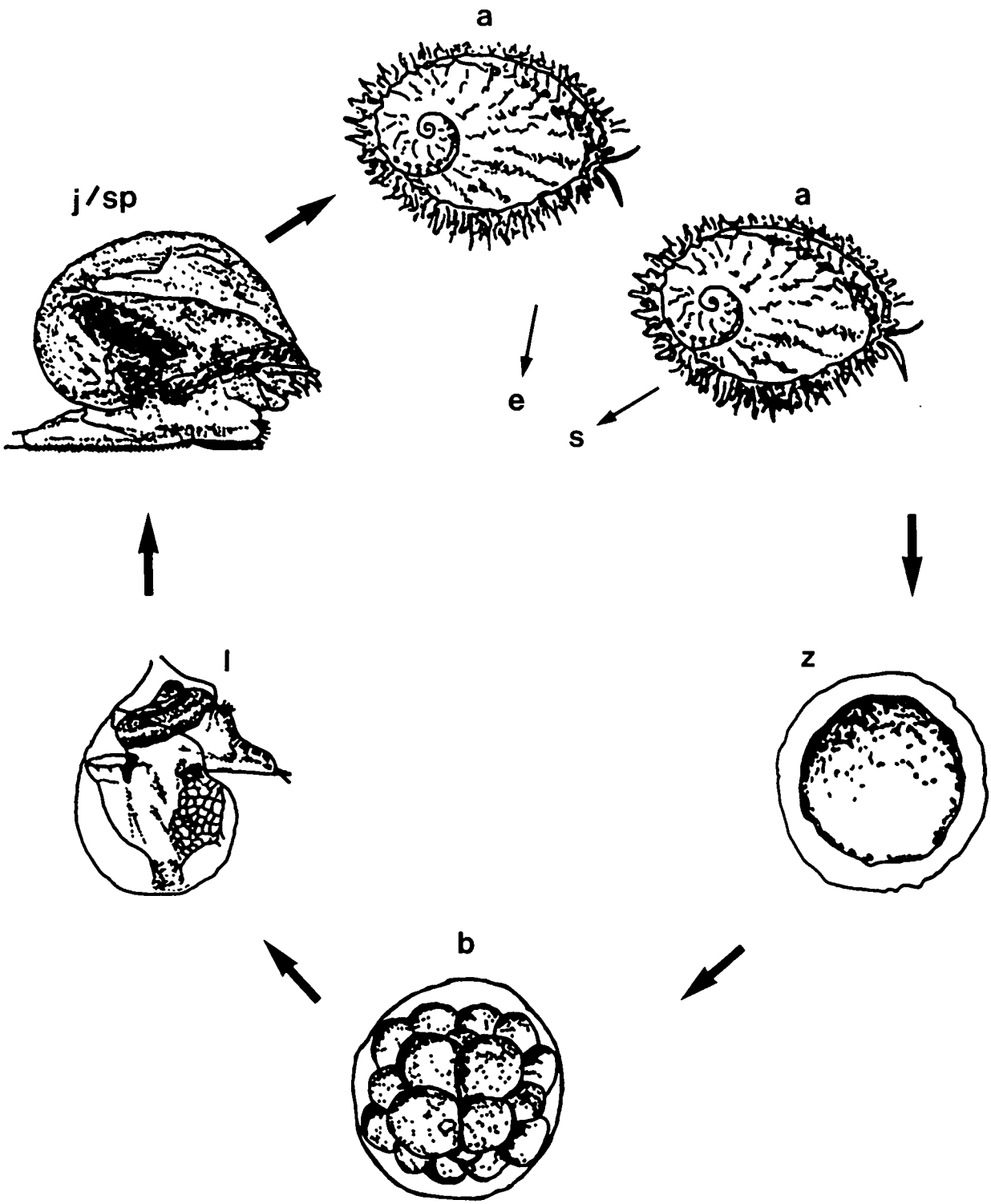
Figure 3.3

Illustration of the general life-cycle of a haliotid (Redrawn from Fallu 1991 and Hahn 1989c).

a= adult; b= blastula; e= eggs; j= juvenile; l= larvae; s= sperm; sp= spat; z= zygote.


a
j/sp
a
e
s
l
z
b

Chapter 4
Abalone/Perlemoen Aquaculture in South Africa and abroad

Abalone have been exploited by humans for thousands of years. The demand for the flesh of its foot has led to the development of abalone fisheries in numerous countries. In recent decades and in some places, exploitation has risen above the level at which abalone can maintain stocks by natural reproduction and fisheries have collapsed. For example: all fishing for abalone in British Columbia (Canada) has been prohibited since 1990 to allow abalone stocks to rehabilitate (Campbell pers. comm.)*[1]. The continued demand for abalone and the existence of farming technology has led to the development of abalone farming and it is possible that this form of aquaculture could evolve into a significant industry in the future. These efforts are not always successful; for example, an abalone culture project was attempted in the early eighties on Vancouver Island, but failed due to parasite infections. The west coast fishery closed in 1990, but abalone populations continue to decline in Canada (Winther pers. comm.)*[2].

Important abalone fisheries exist in Australia, China, Japan, Mexico, New Zealand, South Africa and the United States of America (California) (Shepherd, *et al.* 1992). The world-wide demand for abalone is centered in the Far East, especially Japan and China (Tarr 1993; 1995). In South Africa, the Total Allowable Catch (TAC) is set annually at about 640 tons whole weight for discrete fishing grounds, of which more than 90 % is exported to the Far East. Globally, abalone fishing has declined due to the biology and life history of abalone that lead to overfishing, causing an escalation in price of product. In the early 1970's the Japanese laid the basis for abalone aquaculture, and since then various countries have devoted research to abalone cultivation. During the 1980's sound management practices such as season and size limitations, harvest quotas, and area and fishing method restrictions, have resulted in a stabilisation of annual harvest (Grant 1981). With the high prices obtainable on the export market, abalone fisheries are

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developing rapidly. According to Hahn (1989b) the total annual harvest of abalone in Japan is approximately $5,7 \times 10^6$ kg or 15 % of the total abalone population.

In South Africa, *H. midae* is the only species of commercial importance, due to the small size of the other five South African haliotids (Newman 1968; Barkai & Griffiths 1986; Hecht 1994; Fielding 1995; Tarr 1995). *Haliotis midae* was first harvested from the lower intertidal zone on small scale by natives for at least 6000 years (Tarr 1993). Commercial exploitation of perlemoen, *H. midae*, has taken place along the south and southwest coasts of South Africa since 1950 (Newman 1966). Overfishing led to a decline in the availability of perlemoen during the 1960's, prompting Sea Fisheries to initiate a perlemoen research program covering aspects of the biology of *H. midae*, which may indicate more effective ways of managing available stocks. Strict conservation measures were implemented from 1965 to curb overfishing (Genade, Hirst & Smit 1988). During this time the quota was reduced to 227 tonnes, in order to limit the rapidly declining catch. Between 1980 and 1990 surveys were undertaken by R.J.Q. Tarr to provide management advice on the status and future of the fishery. Perlemoen is currently commercially exploited between Cape Point and Cape Agulhas (Fig. 2.1).

During the early 1990's four perlemoen processing factories were in existence in South Africa (Tarr 1992), three located in or near Hermanus, the center of the perlemoen fishery, and one near Cape Town. Perlemoen farmers in South Africa are still busy completing planned infrastructure, and their aquaculture facilities have not yet become profitable (Loubser, pers. comm.)*[1]. Farming may be the only way to ensure the future of perlemoen, because, world-wide, poachers are busy destroying vast abalone colony structures (Cremer 1998). Their very shortsighted and indiscriminate harvesting disturbs the natural spawning activities by leaving males and females too far apart for breeding. Juveniles that should be left as future breeding stock, are also being removed. The legal status of perlemoen ranching has been cleared up and has resulted in the establishment of a ranching operation at Port Nolloth. There has also been substantial community interaction and involvement in perlemoen reseeding at Hawston.

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Perlemoen are exported to Taiwan where, presently, the price fluctuates around $80/kg of meat, but the export price is generally kept secret amongst companies. *Haliotis midae* doesn't have a very good quality shell for craft use, due to the high incidence of boring polychaetes (*Polydora*) and molluscs.

In South Africa, perlemoen farmers induce the haliotids to spawn monthly. After eggs hatch, the larvae settle on special plates in seawater that has been sterilized, filtered and kept at constant temperatures. After four months the spat, now about 4-5 mm in diameter are transferred to baskets in weaning tanks, where they will stay for three more months. Here, their diet also changes from micro-algae to solid algae, but their growth progress remains a slow 2-3 mm per month. When the juveniles reach about 10 mm in diameter, the colonies are thinned out (Cremer 1998). After the spat have passed the weaning stage, they are ready to feed on seaweed in outdoor enclosures or in the sea. This phase of perlemoen farming is called grow-out. Usually, the rate of growth is unique to the individual farm factors, such as species of abalone, climate, diet and the possible onset of sexual maturity. Knauer, Hecht and Duncan (1994) concluded that the primary constraint in successful cultivation is an adequate supply of a suitable and cost-effective feed.

Cremer (1998) states that if perlemoen farming is successful in South Africa, then perhaps one day the ideal situation will develop where the South African seas are privately farmed as fully guarded marine ranches, like those presently operated in Japan. This approach to aquaculture is to release seed animals directly into the sea. Direct control of the animals is lost, but nature takes its course and the animals feed on natural foods and grow. After the appropriate time, the crop is harvested. In Japan, the government produces abalone seed (shell length 15-20 mm) at a highly subsidized rate. Fisherman's cooperatives liberate the seed and have total rights to any abalone that can be taken from the sea. Officially, the Japanese claim that, after liberation, annual survival of ranched abalone is 0-80 %. At harvest, 2-4 years after seeding, Japanese fisherman recover approximately 10 % of the abalone seeded (Fallu 1991). In June 1978, 28 hatcheries throughout Japan were producing seed abalone for sale and release into the sea. According to Grant (1981) about 20-30 % of the released seed survive until

harvest. In the United States of America and New Zealand experiments have been undertaken to determine possible recovery rates of stocked seed size abalone. These experiments have indicated much lower recovery rates in the order of 1 % (Fallu 1991).

There are several important species of abalone, called "awabi" in the coastal fisheries economy of Japan. These are *Haliotis discus discus* Reeve, 1846, *H. gigantea* Gmelin, 1791 and *H. sieboldii* Reeve, 1846 in warmer waters; *H. discus hannai* Ino, 1953 in colder waters and *H. diversicolor* Reeve, 1846 as well as *H. asinina* Linnaeus, 1758 in the subtropical areas of Taiwan (Du & Guo 1981; Grant 1981; Uki & Kikuchi 1984). *Haliotis discus hannai* is favoured, because it forms about 60 % of the total catch and is the most saught after awabi on the Japanese market (Chen 1984; Fallu 1991).

Seven species of abalone are found in China. They are all small in size, but the two largest, *H. discus hannai* in the north and *H. diversicolor* in the south, have the highest production. According to Zong Qing Nie (1992), abalone production in mainland China is not great. The highest annual yield was about 100 tonnes in the 1950's, but declined to 60 tonnes due to overfishing.

Five species of abalone are found in Korea, four of which are commercially valuable - *H. discus hannai*, *H. discus*, *H. sieboldii* and *H. gigantea*. Since 1974 seed abalone production has increased, reaching a total of 1 155 300 individuals in 1983 (Sung Kyoo Yoo 1989).

In Australia, abalone fisheries are based on blacklip abalone *H. rubra rubra* Leach, 1814 and to a much lesser extent on another two species, namely *H. laevigata* Donovan, 1808 and *H. roei* Gray, 1827. Since 1965 the Australian abalone fishery has grown from small beginnings to a major fishery worth over 100 million annually (Prince & Shepherd 1992), whilst Tasmania has the largest abalone industry in Australia (Shepherd 1973).

In New Zealand there are three species of abalone, locally called the Maori name "paua", two of which are of commercial significance. The favoured species is the common paua *H. iris* Gmelin, 1791. New Zealand paua has a very attractive shell colour and the value

of the shell is worth about twice that of the meat (Fallu 1991). *H. australis* Gmelin, 1791 and *H. virginea virginea* Gmelin, 1791 are also fished occassionally (Hahn 1989d). Schiel (1992) states that the annual value of the fishery, including exported and domestic meat products as well as shell sales and value-added products such as jewellery, were estimated to be about 21 million US $.

In the cold Pacific waters off California red abalone *H. rufescens* Swainson, 1822 are found. Red abalone has traditionally been the most popular and commercially important species in California (Hahn 1989a). Six other slightly less desirable species also occur in this area, namely *H. kamtschatkana kamtschatkana* Jonas, 1845; *H. cracherodii cracherodii* Leach, 1814; *H. corrugata* Wood, 1828; *H. fulgens fulgens* Philippi, 1845; *H. sorenseni* Bartsch, 1940 and *H. walallensis* Stearns, 1899. In Mexico the main species are *H. fulgens fulgens*, *H. corrugata* and *H. cracherodii cracherodii*. The earliest records for the Mexican aulone fishery in Baja California date from 1923, when the catch was 1721 tonnes (Guzman Del Proo 1992).

In Europe, the French are culturing the indigenous ormer *H. tuberculata* Linnaeus, 1758. In 1982 researchers developed a hatchery in Ireland with an approximate annual production of 80 000 juvenile *H. tuberculata* (Hahn 1989d). Some Europeans have also expressed interest in farming *H. kamtschatkana kamtschatkana*, a northern Pacific species.

Al sufailah, the Omani abalone (*H. mariae* Wood, 1928) is harvested in the Sultunate of Oman, About 1.75 million abalone are exported annually with a value of nearly US $4 million (Johnson, Al-Harassy & Al-Harthy 1992). According to Fallu (1991) projects have also been undertaken to grow *H. rufescens* in geothermal water in Iceland where seaweed is abundant.

Chapter 5
Abalone species parasitised world-wide

Molluscs are increasingly being raised for human consumption in both the developed and developing world. Several mass mortalities of shellfish, in particular oysters, throughout the world during the last five decades have aroused the concern of the industry and of the fishery biologists and shell-fishermen. Many natural oysterbeds were wiped out and have yet to recover. In addition to these major mortalities, numerous other mortalities have been reported and several zooparasites have been found in oysters and other commercially important marine molluscs (Barber & Mann 1994). Although the cause and effect relationships in most cases have not been established, there is legitimate concern over such parasites as possible lethal agents. There are thus clear economical needs and a medical importance to obtain full understanding of molluscan pathogens. A wide range of symbiotic associations exists between molluscs and micro-organisms.

Infectious disease occurs when a micro-organism comes to live in, or on, a host and the life processes of the organism damage the host's health. The infecting micro-organism is termed a pathogen. A pathogen may be a virus, bacterium, fungus, protist or even a metazoan. For the purpose of aquaculture, disease may reduce the production of the farmed species and reduce profits; therefore its prevention is of real concern to the abalone farmer. Abalone are sold as a high quality product and the price they obtain depends on their good reputation. Any slurs on the purity of the product may be disastrous for the market.

Unwanted micro-organisms and spores can be introduced with water and become potential pathogens. Filters are usually used to remove pathogens from the water. If pathogens can not be prevented from gaining access to abalone containers, the next best approach is to attempt to eradicate them later. Prophylactic substances may kill a fair percentage of bacteria, but they do not remove the bacterial habitat and nutrients, so that, once the antiseptic effect has abated, bacteria are able to return. Ultra-violet

light and antibiotics are also used to kill bacteria, but some of the substances may have an adverse effect on the abalone.

The types of infectious disease problems that affect abalone vary with the phase of the life-cycle that the abalone are in: each phase has its own set of conditions and problems. For example, brood stock may carry contamination on their shells. These need to be cleaned before the abalone can be introduced to the spawning containers. Abalone may also carry contamination in their viscera and their faeces could contaminate water. After spawning, eggs stick and congregate on the bottom of the container in any sludge left over from spawning. The result is localised poor water quality and conditions ideal for the proliferation of infectious disease (Fallu 1991).

Disease and the occurrence of potential pathogens in abalone have mostly been studied in culture conditions. Only a few studies on natural populations of abalone and their parasites/symbionts have been carried out. In breeding conditions, and more particularly aquaculture, the environment in which a parasite and host are kept are often quite different from the natural milieu. The most evident change concerns the probability of encounter between infecting stages of parasites and their targets, since the dispersion of the parasites is limited while the density of hosts is increased.

One of the most dangerous groups of bacteria belongs to the genus *Vibrio*. In the wild, *Vibrio* commonly lives in the mud of saline waters. On an abalone farm, it thrives on the surfaces of containers holding seawater and can move into mid-water. A *Vibrio* infection can result in extensive larval mortality. Vibriosis seems to occur commonly in abalone with shell lengths of 2-15 mm and the motile rod-shaped *Vibrio* bacteria can be seen on the inside of the shell. Spat are lethargic, and are unable to right themselves if turned over. They may go pale in colour, and seem to shrink in their shells and refuse to eat. The bacterium also invades the inside of the abalone. Lumps, filled with bacteria, appear on the bottom of the abalone's foot. The infection proliferates and moves into the rest of the foot or other tissues via the nerves and blood channels. *Vibrio parahaemolyticus* has been described from Japan, occurring in *Haliotis discus* Reeve, 1846 (Shepherd *et al.* 1992), and *Vibrio fluvialis* causes pustule disease in *H. discus hannai* (Li, Ding, Zhang, Xiang & Liu 1998). Bacterial infections commonly

occur in intensive cultures of *H. rufescens* from California (Elston & Lockwood 1982; 1983).

A protist pathogen, *Labyrinthuloides haliotidis* Bower, 1987 belonging to the phylum Labyrinthomorpha Levine, Corliss, Cox, Deroux, Grain, Honigberg, Leedale, Loeblich, Lom, Lynn, Merinfeld, Page, Poljansky, Sprague, Vavra & Wallace 1980, occurs in the foot muscle and nervous tissue of the head of *H. kamtschatkana* from Japan and in red abalone, *H. rufescens* from California (Bower 1987). An abalone mariculture facility on Vancouver Island, British Columbia (Canada) suffered high mortalities among juvenile abalone of these two species (Hahn 1989e). According to Hatai (1982) and Fallu (1991) a fungus, *Haliphthoros milfordensis*, grows on the outside of the body of *H. sieboldii* and forms lumps on the soft body parts.

Perkinsus parasites are widespread in molluscs of commercial importance around the world (Calvo & Burreson 1994; Goggin & Lester 1995). In Australia, wild blacklip abalone, *Haliotis rubra rubra* have been found infected with the protist parasite *Perkinsus olseni* Lester & Davis, 1981. *Perkinsus* becomes established in the abalone's blood supply system. Initially the abalone fight the infection internally and may develop lumps in their flesh. A chronically infected abalone can develop abscesses and these are of concern to commercial abalone divers, because affected animals are not acceptable to processing companies (Lester & Davis 1981). If the disease runs its full coarse the abalone will die. At normal temperature (15 °C), the abalone can encapsulate the parasite with flesh and kill it. Once *Perkinsus* has invaded an abalone, it circulates in the blood system in much the same way the malaria pathogen does in humans. For a while, the pathogen multiplies and grows, the abalone's immune system fights it, but eventually the abalone may succumb. When the dead abalone decomposes, the pathogen has changed its form and is released into the sea where many zoospores are produced, which in turn invades a new abalone host. *Perkinsus olseni* is also known to infect *Haliotis laevigata* Donovan, 1808 (Shepherd *et al.* 1992; Friedman, Roberts, Kismohandaka & Hedrick 1993).

A coccidian-like protozoan found in the kidneys and nefridia of *H. cracherodii cracherodii* and *H. corrugata*, causes withering syndrome. The infected abalone

becomes weakened, the tissues discolour and become atrophied and are also non-responsive to stimuli (Haaker, Parker, Togstad, Richards, Davis & Friedman 1992).

From time to time a form of discolouration in abalone, referred to as Blue spot, occurs (Thrower 1977). Blue spots appear on the muscular foot, often extending through the muscle. Such abalone are hard to sell and are often discarded by industry. The blue discolouration is due to an excess of oxygenated hemocyanin in the flesh (Olley & Thrower 1977). This is thus a physiological blood condition, and a non-parasitic disorder.

The most common animals inhabiting abalone shells are species of the boring sponge genus *Cliona*. These species bore into and weaken the shell, so that the abalone more easily falls prey to other predators (Shepherd & Breen 1992). *Cliona celata calioforniana* is a yellow encrusting sponge that spreads over the abalone shell and dissolves holes into it to increase attached surface. According to Kojima and Imajima (1982) as well as Shepherd and Breen (1992) boring annelids and boring molluscs often bore into the shell of some abalone species. It was found that the body weight of an abalone, whose shell was entered by ten or more *Polydora* specimens, was significantly lower than that of an abalone with fewer or no *Polydora* in the shell. Along the South African coast *Polydora hoplura* infests the shells of both *H. spadicea* and *H. midae*. Muller (1984) found that all *H. spadicea* specimens with shell lengths greater than 30 mm are susceptible to infestation. The weakening of the shell by *P. hoplura* facilitates predation on them.

Shepherd and Breen (1992) states that Kelsey found 25 species of molluscs on abalone, and Oldroyd listed 59 species of gastropods inhabiting abalone shells. Ectoparasitic gastropods can drill through the shell to feed on the abalone foot.

Abalone are occasionally hosts of trematodes that complete their life-cycle in a vertebrate host (Fretter & Graham 1962). Few infections occur in the muscular foot or gonad, but the gonad can be destroyed in serious cases. Overall, it doesn't appear to affect the abalone's health, the only effect being that the abalone is unable to reproduce. Harrison and Grant (1971) and Keesing (1984) describe digenean

trematodes of the families Opeocoilidae and Allocreadiidae from the gonads of Californian abalone. Digenean trematodes have also been described from Australian abalone: occurring in the gonads of *Haliotis rubra rubra* and *H. roei* (Shepherd & Breen 1992). According to Hahn (1989e) about 3 % of *H. rubra rubra* populations have various stages of infection with trematode cercariae, probably of the family Allocreadiidae. The gonads of infected animals are dull to bright orange in colour, with no other visible abnormalities. The infection usually causes sterilisation, and determining the sex of infected individuals is impossible due to the destruction of the gametes by the parasites. The cercariae develop within sac-like sporocysts in the gonad, which even penetrate into the digestive gland tissue.

Echinocephalus pseudouncinatus has been described from the foot, liver, digestive tract and gonads of *H. corrugata* and *H. laevigata*. The whole surface of the visceral mass, mantle, mucous glands and ctenidia of a female *Haliotis tuberculata* were found to be orange in colour, owing to infection by trematode sporocysts, rediae and cercariae. A male was found with cysts between the gonad and digestive gland. This trematode was identified as *Cercaria* cf. *brachyura* (Crofts 1929; Cox 1962 & Hahn, 1989e).

According to Fallu (1991) copepods, larval nematodes and ciliophorans, all potentially harmful, are often found associated with spat. Parasitic copepods occur in the mantle cavity of *Haliotis gigantea*, whilst the commensal shrimp, *Betaeus harfordi* lives in the mantle cavity of all Californian abalones (Cox 1962; Fallu 1991). In the United States of America, farmers of red abalone, *H. rufescens* had problems with high abalone mortality in the early part of grow-out. It was found that a copepod (*Tigriopus californicus*) was being introduced with the kelp *Macrocystis* sp. offered as food and that the copepod attacked the abalone (Fallu 1991).

The association between representatives of the Peritrichia and marine gastropods is not uncommon. In Europe and America different species of sessiline and mobiline peritrichs were found associated with *Patella* Linnaeus, 1758, *Cellana* Adams, 1869 and *Acmaea* Eschoscholtz, 1833 as well as other molluscan species (Cuénot 1891; Kahl 1933; Hirshfield 1949 & Raabe 1952).

Although various parasitic and symbiotic associations have been recorded and some studies have been done on ciliophorans from molluscs (Penn 1958; Fenchel 1965), there are many species of molluscs in all parts of the world whose ciliophoran faunas have not been examined, especially in Africa. Very little work has been done on the geographical distribution and host-specificity of molluscan ciliophorans. Emphasis has been placed rather upon the taxonomic and morphological features (Penn 1958). Jamadar and Choudhury (1988) emphasise that very little is known about ciliophoran parasites of commercially important marine and estuarine molluscs.

The occurrence of sessiline ciliophorans on perlemoen collected at the De Hoop Nature Reserve in South Africa (Botes *et al.* 1997), is not only the first record in South Africa, but also the first record of sessiline ciliophorans from abalone in the world. Associated ecto-hypersymbionts, representative of the family Ellobiophryiidae (Chatton & Lwoff, 1929) and belonging to the genus *Caliperia*, which attach to the scopula of the ciliophorans, were also found (Botes *et al.* 1998).

Susan Bower found an unusual ciliophoran, resembling a columnar trichodinid in the esophageal folds of starving abalone from an abalone closing facility in British Columbia, Canada, some years ago (pers. comm.)*[1]. This ciliate might probably be a scyphidiid peritrich belonging to the genus *Mantoscyphidia*, as in the case of the present study. Protozoans have caused major damage to abalone gills in the commercial company Irvin & Johnston's live holding facility of wild perlemoen in Hermanus (Loubser, pers. comm.)*[2]. *Haliotis midae* specimens from the Danger Point Abalone farm were examined and the same species of sessiline ciliophoran that was found on the gills of natural populations of *H. midae* from the pristine environment of the De Hoop Nature Reserve, occurred on their gills.

In the present study severe infections of cercarial and metacercarial stages of trematodes were found in the digestive gland of *H. spadicea* as well as on the gill

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filaments. According to Sindermann (1990), trematode larvae are undoubtedly the most important helminth parasites of marine molluscs. Of the various worm parasites, larval trematodes are clearly the most destructive to molluscs, in some instances changes in host population abundance have been attributed to high levels of parasitation. When encystment occurs in the gonads of the molluscs, sterilisation and tissue destruction of the host takes place. Helminth parasites could thus be potential pathogens, as they can cause parasitic castration, directly affecting mollusc reproduction and population dynamics. Larval trematodes may use molluscs as the first intermediate hosts (with sporocyst, redial and cercarial stages) or as second intermediate hosts (metacercarial stage), recognising that in some instances the mollusc may act as the host for both stages (Sindermann 1990). Interest in molluscan pathogens and parasites arises primarily from the role of gastropods in the transmission of trematodes of medical and veterinary importance.

According to Fallu (1991), the pathology of abalone is very poorly understood. Only the most common problems have been looked at and, even with these, the exact causes and fine details of the disease processes are imperfectly known. There may be many diseases of abalone that will ultimately become important to abalone farmers, but are as yet, unknown.

Chapter 6

Phylum Ciliophora

Currently 14 phyla are distinguished within the kingdom Protozoa, including the phylum Ciliophora. Over the years a number of hierarchical classification systems for the Ciliophora were proposed, these include the works of Kahl (1933); Bhatia (1936); Corliss (1959), De Puytorac, Batisse, Bohatier, Corliss, Deroux, Didier, Gragesco, Fryd-Versavel, Grain, Groliére, Hovasse, Iftode, Laval, Roque, Savoie & Tuffrau (1974); Corliss (1977; 1979); Lynn & Small (1988); De Puytorac, Batisse, Deroux, Fleury, Grain, Laval-Peuto & Tuffrau (1993); Corliss (1994) and De Puytorac (1994).

In 1994 two major works, concerning the higher systematics of the Ciliophora, appeared. Batisse, Bonhomme-Florentin, Deroux, Fleury, Foissner, Grain, Laval-Peuto, Lom, Lynn, De Puytorac & Tuffrau (1994) published a book on the anatomy, systematics and biology of the phylum Ciliophora, with different authors responsible for specific chapters. The higher classification of this book, however, differs slightly from the works of Corliss (1994), who proposed a "user-friendly" classification for all protists. Prof. Pierre De Puytorac begins the book with the hierarchical classification of the phylum Ciliophora, with three subphyla and eleven classes in total, whereas in his classification Dr. John Corliss only mentioned eight classes, with no subphylum division (Van As 1997). Listed below is an abridged version of the relevant subphyla, superclasses and classes of De Puytorac's classification, followed by the classification of Corliss (1994). In each classification the relevant taxa for this study are provided in bold format.

Classification of the Phylum Ciliophora Doflein, 1901 (De Puytorac 1994)

Subphylum	Epiplasmata De Puytorac, *et al.*, 1993
Superclass	Ciliostomatophora De Puytorac, *et al.*, 1993
Class	Phyllopharyngea De Puytorac, *et al.*, 1974
Superclass	Membranellophora Jankowski, 1975
Class	Nassophorea Small & Lynn, 1981
	Oligohymenophorea De Puytorac, *et al.*, 1974

Classification of the Phylum Ciliophora Doflein, 1901 (Corliss 1994)

Class:	Karyorelictea Corliss, 1974
	Polyhymnophorea Jankowski, 1967
	Colpodea Small & Lynn, 1981
	Phyllopharyngea De Puytorac, *et al.*, 1974
	Nassophorea Small & Lynn, 1981
	Oligohymenophorea De Puytorac, *et al.*, 1974
	Prostomatea Schewiakoff, 1896
	Litostomatea Small & Lynn, 1981

Without getting involved in a debate on the merits of either system, or favouring the one above the other, the system proposed by De Puytorac (1994), will be followed. This is the same classification system that Van As (1997) used. The main reason being that his work is more comprehensive, including the systematics of taxa below class level, whereas the work of Corliss (1994) does not provide any information below class level. The relevant class for this study is Oligohymenophorea De Puytorac, *et al.*, 1974. Presented below are the systematic characteristics of the relevant taxa, according to Corliss (1979, 1994) and Lom and Dykova (1992).

Kingdom Protozoa Goldfuss, 1818

Phylum Ciliophora Doflein, 1901

Heterotrophic, free-swimming or sessile with cilia (simple and compound) in at least one stage of the life-cycle; possesses a complex somatic and oral infraciliature; pellicular alveoli, microtubular or microfibrillar structures often kinetosome associated; homothetogenic fission and often perkinetal, one or more diploid micro- and one to several polyploid macronuclei; conjugation temporary or total, widespread occurrence; monostomic, some groups are mouthless or polystomic; contractile vacuole present; osmotrophic to phagotrophic feeding methods; broad distribution, diverse aquatic and edaphic habitats, a number of species are ecto- or endosymbionts.

Class Oligohymenophorea De Puytorac, *et al.*, 1974

Oral apparatus distinct from somatic ciliature, comprised of well-defined paroral membrane plus several membranelles of peniculli located in buccal cavity or infundibulum situated on ventral side of body, cytostome situated at base of the cavity; inconspicuous cytopharynx; stomatogenesis parakinetal or buccokinetal; some variation exists in mode of fission; conjugation temporary and only in one group; species are free-living or symbiotic.

Subclass Peritrichia Stein, 1859

Body characteristically inverted bell- or goblet-shaped or conical-cylindrical; morphology dominated by adoral ciliary wreath of buccal ciliature, winding counterclockwise at apical pole and a scopula (holdfast derivatives: contractile stalk or complex adhesive disc) at anti-apical pole; somatic ciliature reduced to subequatorial locomotor fringe (trocheal band); ciliated infundibulum, into which contractile vacuole empties, leading to cytostome; stomatogenesis buccokinetal, plane of fission parallel to major axis of body; dimorphism (migratory telotroch), colonies, loricae or thecae and cysts common to many species; conjugation (total) involves fusion of micro- with macroconjugant; very widespread aquatic distribution, species are free-living or symbionts on diverse host range, some commensals and even ecto- or endoparasites.

Order Sessilida Kahl, 1933

Adults are sedentary or sessile, commonly stalked (or with inconspicuous adhesive disc, scopula), with a few species secondarily mobile; many produce arboroid colonies; some entire groups loricate; mucocysts and pellicular pores universal; adults filter feed on bacteria (larval stage mouthless); habitats ranging from freshwater, brackish and marine, few species live as endozoic forms.

Family Scyphidiidae Kahl, 1935

Solitary stalkless (yet sessile) forms, adherence to substrate with a flattened aboral scopula often prominently distinct from the rest of the body, adoral ciliary spiral, one turn around epistomial disc; species are epibionts on marine and freshwater fishes, molluscs and other invertebrates.

Genera *Scyphidia* Dujardin, 1841; *Ophrydiopsis* Pénard, 1922; *Paravorticella* Kahl, 1933; *Pachystoma* Rudzinska, 1952; *Ambiphrya* Raabe, 1952; *Gonzeela* Kufferath, 1953; ***Mantoscyphidia* Jankowski, 1980**; *Riboscyphidia* Jankowski, 1980; *Speleoscyphidia* Jankowski, 1980; *Myoscyphidia* Jankowski, 1985

Family Ellobiophryiidae (Chatton & Lwoff, 1929)

Solitary, stalkless forms, fission anisotomic; members of type genus are particularly distinguished by the remarkable production, on either side of the scopula, of elongate cylindrical projections (or cinctal limbs) which encircles a gill filament of their host, gluing them together to form a closed circle in firm attachment.

Genera *Ellobiophrya* Chatton & Lwoff, 1923; ***Caliperia* Laird, 1953**

Relevant genera for present study are indicated in bold.

Chapter 7
Scyphidiid peritrichs

FAMILY SCYPHIDIIDAE KAHL, 1935

The family Scyphidiidae comprises stalkless (yet sessile) forms, that adhere to the substrate with a flattened aboral scopula. They mostly occur as epibionts on marine and freshwater fishes, molluscs and other invertebrates, with one genus associated with water plants.

According to Lom and De Puytorac (1994) the following represents the valid genera within the family Scyphidiidae:

***Ambiphrya* Raabe, 1952.** Body cylindrical or conical (40-50 µm) with broad flat or convex epistomial disc, elongated macronucleus, situated in oral half of body, permanent telotroch band. Epizoites on the skin and gills of fish. Species: *A. tholoformis* (Surber, 1943); *A. macropodia* (Davis, 1947); *A. ameiuri* (Thompson, Kikegaard & Jahn, 1947); *A. miri* Raabe, 1952; *A. neobolae* Viljoen & Van As, 1985.

***Gonzeela* Kufferath, 1953.** Conical body, aboral area very reduced, stalkless, juxtaposed and glued with a layer of gel onto large spherical pseudocolonies of *Volvox*. Single species: *G. colonialis* Kufferath, 1953.

***Ophrydiopsis* Pénard, 1922.** Aboral area of cylindrical body (70-80 µm) clearly reduced, diameter of scopula measures about half the width of body. Single species: *O. concava* Pénard, 1922.

***Pachystoma* Rudzinska, 1952.** Single species *P. oslithus* Rudzinska, 1952. According to Corliss (1979) and Lom and De Puytorac (1994) this genus must be re-evaluated and is probably a synonym of *Scyphidia*.

***Paravorticella* Kahl, 1935.** Conical body (100-120 µm), very elongated, small scopula, attaches directly to substrate or with a pad of fixative substance, with a reduced aboral area, containing myoid bundle that quickly retracts it into a spiral. Epizoic on aquatic invertebrates. Species: *P. terebellae* (Fauré-Fremiet, 1920); *P. clymenellae* (Shumway, 1926); *P. lycastic* Chakravorty, 1937.

***Scyphidia* Dujardin, 1841.** Scopula with large surface, often with very large diameter sometimes exceeding peristome width, with or without immobile scopular cilia. Cylindrical or conical body (60-100 μm), adoral flat or slightly convex epistomial disc. Macronucleus more or less elongated, situated in oral half of body.

Until the eighties the genus *Scyphidia* comprised a large number of species from a variety of hosts and habitats, including plants, gastropods, insects, tadpoles, freshwater and marine fishes and even some free living species. Jankowski (1980) proposed a new system of systematics for the phylum Ciliophora and created three new genera to accommodate some of the *Scyphidia* species. One more genus was later created by Jankowski (1985). All these genera are summarised in Table 7.1. They are separated solely on the basis of the host or substrate on which the peritrich attaches.

Table 7.1 Summary of the genera created to accommodate species formally included under the genus *Scyphidia* Dujardin, 1841 (Jankowski 1980, 1985).

Genus	Type species	Substrate or host
Scyphidiella* Guhl, 1979	*S. dentata* (Matthes & Guhl, 1972)	Freshwater Coleoptera
Mantoscyphidia Jankowski, 1980	*M. littorinae* (Issel, 1918)	Marine and freshwater Gastropoda
Myoscyphidia Jankowski, 1985	*M. hyalina* (Biegel, 1959)	Water plants in ponds
Speleoscyphidia Jankowski, 1980	*S. husmanni* (Lüpkes, 1974)	Free living, in clean ground water between detritus
Riboscyphidia Jankowski, 1980	*R. scorpaenae* (Fabré-Domerque, 1888)	Freshwater and marine fishes also tadpoles
Scyphidia Dujardin, 1841		Various freshwater and marine hosts, such as Polychaeta, Hirudinea, Crustacea and other classes of the Mollusca

* Lom and De Puytorac (1994) suggests that the genus *Scyphidiella* Guhl, 1979, move to the family Operculariidae, as previously proposed by Guhl (1979).

Thus, the genera included in the family Scphidiidae are: *Ambiphrya, Gonzeela, Ophryodiopsis, Pachystoma, Paravorticella, Mantoscyphidia, Myoscyphidia, Speleoscyphidia, Riboscyphidia* and *Scyphidia.*

General morphology of scyphidiid peritrichs

The general morphology of a scyphidiid peritrich is illustrated in Fig. 7.1. The following description is based on information taken from Van As (1997). Representatives of this family are all sedentary with **cylindrical bodies**, reaching a length between 40 and 120 µm in expanded specimens. Body diameter ranges from 25 to 50 µm. The exterior body of scyphidiid peritrichs is sculptured with ridges also known as striations. These can be seen with the aid of silver nitrate impregnation as well as scanning electron microscopy. Different patterns of these striations are used as generic differentiation between genera within the Scyphidiidae. The anterior **peristome** has a broad and striated lip. The peristomial disc is characteristically arched or flattened. This feature is also used as a taxonomic characteristic. The margin of the peristomial disc is lined with long oral cilia.

Representatives of the Scyphidiidae have no stalk and attach firmly to the host or substrate with the striated **scopula**, a specialised flattened thigmotactic area of the pellicle equipped with short, imperfect and immobile cilia. The scopula adheres directly to the surface of the host or substrate, being cemented to it with a thin layer of sticky substance secreted by the scopula (Lom & Corliss 1968; Lom & Dykova 1992).

In the case of the peritrichs, the **buccal ciliature** is 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, formally known as the undulating membrane. The second part is made up of membranelles. These cilia arise from three to four closely spaced sets of kinetosomes that is known as the adoral zone of membranelles.

The buccal cavity is lined with a haplokinety (outer row or paroral membrane) and polykinety (inner rows or adoral polikinetids). Outside the buccal cavity the haplo- and polikineties continue to spiral in a clockwise direction making at least one and a half to four turns around the peristome. The haplokinety remains as 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.

Normally between the original haplo- and polykineties, the new cilia resemble peniculi (Lom & Dykova 1992). The erect polykinety causes food particles to be carried to the horizontal haplokinety, where the particles are swept into the **infundibulum**, also known as the gullet or vestibulum. 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 (Davis 1947; Lom & Dykova 1992).

Ciliophorans occupy a variety of niches. Some are particle feeders, feeding on organic particles. Others are herbivores feeding on unicellular algae, whilst some feed on filamentous algae. Many ciliophorans, both small and large, are carnivores. Small prey is simply swept into the cytopharynx and enclosed in food vacuoles. Ciliophorans that feed on large prey have elaborate capture and ingestion mechanisms. In the case of representatives of the Scyphidiidae food particles are swept into the infundibulum through ciliary action. The cytostome is situated at the base where the particles are encapsulated into **food vacuoles**. Two main stimuli induce this formation: One mechanical (presence of particles) and the other chemical (dissolved or particulate nutrients). Once formed, food vacuoles move around in the cytoplasm, until digestion is completed.

Some representatives of the family Scyphidiidae have symbiotic green algae living in their cytoplasm. These algae are also known as zoochlorellae or zooxanthellae depending on the type of water habitat.

A **contractile vacuole** is present in some species of ciliophorans and functions in the control of internal osmotic pressure and excretion of waste matter. In freshwater species the contractile vacuole is mostly present, whilst it may be absent in some marine species. Most contractile vacuoles occur close to the plasma-membrane and swell slowly (diastole) before a sudden contraction (systole), expelling the fluid contents. A constant cycle of diastole-systole occurs which varies in pulse rate. This rate is normally much higher in freshwater than in marine ciliophorans. This observation, together with experimental evidence, suggests the primary function to be osmoregulation. No special

organs for respiration exist, because these ciliophorans are small and respiration occurs through the pellicle by diffusion.

The **pellicle** of scyphidiid peritrichs consists of a single plasma membrane, which overlies a membrane-bound alveolar sac. The latter lies between well developed pellicular ridges, that are supported by single dense microtubular fibres. Silver impregnation reveals two types of striations on the peritrich pellicle, an annular form and a reticulate or lattice type. The majority of ciliophorans have pellicles with the annular form. In the family Scyphidiidae striations circumvent the body. These striations are present all over the scyphidiid peritrich's body, from the peristomial lip to the scopula.

The presence of the **myoneme system** in ciliophorans has already been known for more than a hundred years and consists of contractile organelles. Myonemes are composed of thick packs of bundles of 3-5 nm microfibrils. The microfibrils are responsible for the contractility in ciliophorans.

Various stages of contraction 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 the stages, variation in the peristome and adoral spiral shape can be seen. During this process the peristome changes from flattened to arched. In contracted specimens the adoral cilia are either drawn inwards or, in some cases, a bundle of cilia still protrude from the peristome.

Representatives of the family Scyphidiidae have both a single **macro-** and **micronucleus**. 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, ranging from sphere to oval and elongated. In scyphidiid peritrichs the macronucleus is situated mostly adoral to the telotroch band. The micronucleus is very small, round to oval and always either adorally or aborally to the macronucleus. Since the micronucleus does not vary much in both shape and size, this alone is normally of minor taxonomic importance.

Representatives of the Scyphidiidae reproduce solely asexually by **fission** (including budding and binary fission) (Davis 1947). Budding in peritrichs can be considered as a specialised type of fission according to Corliss (1979).

GENUS *MANTOSCYPHIDIA* JANKOWSKI, 1980

The scyphidiid peritrichs of the present study conforms in morphological features to those of the family Scyphidiidae and due to its marine gastropod host must be placed within the genus *Mantoscyphidia*, (see classification Table 7.2).

Table 7.2. Classification of the genus *Mantoscyphidia* Jankowski, 1980.

Kingdom	Protozoa Goldfuss, 1818
Phylum	Ciliophora Doflein, 1901
Subphylum	Epiplasmata De Puytorac, *et al.*, 1974
Superclass	Membranellophora Jankowski, 1985
Class	Oligohymenophorea De Puytorac, *et al.*, 1974
Subclass	Peritrichia Stein, 1859
Order	Sessilida Kahl, 1933
Family	Scyphidiidae Kahl, 1935
Genus	*Mantoscyphidia* Jankowski, 1980

Fifteen *Mantoscyphidia* species have so far been found associated with freshwater and marine gastropod hosts. Four species are from freshwater: The first two species, i.e. *M. physarum* (Lachman, 1856) and *M. limacina* (Lachman, 1856) were described from freshwater gastropods of the genera *Physa* and *Planorbis* respectively. The other two species, *M. inclinata* (Lom & Corliss, 1968) and *M. capitis* (Boitsova, 1976), are also found associated with freshwater gastropods.

The 11 known marine species of *Mantoscyphidia*, their hosts, locations, habitats and morphological features are summarised below.

Compendium of *Mantoscyphidia* Jankowski, 1980 species known from marine gastropod hosts, based on information from different authors.
[BL= body length; BD= body diameter; SD= scopula diameter; MaL= macronucleus length; MaD= macronucleus width; MiL= micronucleus length; MiD= micronucleus width. Superscript numbers refer to specific authors in the compendium]

Mantoscyphidia patellae (Hutton, 1878)	
TYPE HOST: *Patella argentea* #	
TYPE LOCALITY: New Zealand	
Remarks	
This species was originally described as *Cothurina patellae*. Jankowski (1985) rejects the genus and placed this species within the genus *Mantoscyphidia*. Unfortunately the work of Jankowski is in Russian which causes a slight problem with translation and interpretation of his work. Until now the original description of Hutton could not be traced, thus the South African material can not be compared with *M. patellae* recorded from a limpet in New Zealand.	

Mantoscyphidia lusitana (Cuénot, 1891) *emend* Jankowski, 1985 (Fig. 7.2A&B)	
TYPE HOST: *Patella vulgata* Linnaeus, 1758	
TYPE LOCALITY: Roscoff le Portel, (Calais) (Cuénot 1891)[1]	
OTHER HOSTS & LOCALITIES: *Polymesoda caroliniana* (Bosc #), *Rangia cuneata* (Gray #), Laguna Pom, Campeche, Mexico (*[1]Madrazo-Garibay & López-Ochoterena 1988)[2] (Fig. 7.2C)	
Body features	Body cylindrical when expanded, oval when contracted, no striations, peristome arched, contractile vacuole at base of infundibulum [1, 2]
Measurements	BL= 30-45 BD= 20[1]; BL= 41 BD= 22[2]
Nucleus	Macronucleus beaded[1], forming a band[2]
Position on host	Abundant on gills
Remarks	
This species was described as *Scyphidia patellae* by Cuénot (1891), thus a homonym of the species Hutton (1878) described from *P. argentea*. Jankowski (1985) proposed the name *lusitana* be used in stead of *patellae* to accommodate the material described by Cuénot (1891). It appears that scientists working in this field are unaware of the work of Hutton, as **no references to Hutton (1878) could be found, and it is thus not included in the reference list (Chapter 12)**. Even recent papers only refer to Cuénot (1891) and thus incorrectly to his material as *M. patellae*.	

Compendium (continue) of *Mantoscyphidia* Jankowski, 1980 species known from marine gastropod hosts, based on information from different authors.

Mantoscyphidia fischeri (Vayssiére, 1885)	
TYPE HOST: *Truncatella truncatula* # (Vayssiére, 1885) **TYPE LOCALITY:** Unknown **OTHER REFERENCES, HOSTS & LOCALITIES:** Hirshfield (1949)[1] *Rangia (Rangianella) flexuosa* (Conrad), *R. cuneata* (Gray) (*[2] Madrazo-Garibay & López-Ochoterena 1988)[2] (Fig. 7.3A)	
Body features	Body cylindrical when expanded, peristome flat, no striations[1]
Measurements	BL= 60[1]; BL= 63 BD= 22[2]
Nucleus	Macronucleus ribbon[1], forming a band[2]
Remarks *[1+2] Madrazo-Garibay & López-Ochoterena (1988) also recorded *M. lusitana* and *M. fischeri* from the intestine and gonads of clams. This is unusual as *M. lusitana* and *M. fischeri* are valid species occurring on the gills of limpets. This record needs to be considered cautiously, as it is unlikely that a limpet gill ciliophoran will also be found in the intestine and gonads and clams.	

Mantoscyphidia littorinae (Issel, 1918)	
TYPE HOST: Unknown **TYPE LOCALITY:** Unknown **OTHER HOSTS & LOCALITIES:** *Littorina meritoides* Linnaeus, *L. punctata* Gmelin Yugoslavia (Raabe 1952)[1] (Fig. 7.3B&C)	
Body features	Cylindrical
Measurements	BL= 45 BD= 20[1]
Nucleus	Macronucleus sausage-shaped
Position on host	Unknown

Mantoscyphidia hydrobiae (Kahl, 1933) (Fig. 7.4A)	
TYPE HOST: *Hydrobia* # (Kahl, 1933) **TYPE LOCALITY:** Unknown **OTHER REFERENCES, HOSTS & LOCALITIES:** *Hydrobia ventrosa* #, *Littorina rudis* # (Precht 1935); Hirshfield (1949)[1]	
Body features	Body from goblet, peristome flat, no striations[1]
Measurements	BL= 70[1]
Nucleus	Macronucleus kidney-shaped[1]
Position on host	Unknown

Compendium (continue) of *Mantoscyphidia* Jankowski, 1980 species known from marine gastropod hosts, based on information from different authors.

Mantoscyphidia ubiquita (Hirshfield, 1949) (Fig. 7.5A-D)	
TYPE HOST: *Acmaea pelta* Eschscholtz, 1833 **TYPE LOCALITY:** Southern California (Hirshfield 1949)[1] **OTHER REFERENCES, HOSTS & LOCALITIES:** *A. digitalis* Eschscholtz, 1833; *A. limatula* Carpenter #; *A. scabra* Gould, 1846; *A. insessa* Hinds #; *A. fenestrata fenestrata* Reeve #; *Lottia gigantea* Sowerby, 1834; *Tegula funebralis* Adams #; *T. ligulata* Menke #; *Fissurella volcano* Reeve (Hirshfield 1949)[1] *Acmaea testudinis* Scutum, 1833, San Juan Island, Washington (Lom & Corliss 1968)[2]. *Littorina obtusata* (Linnaeus, 1758), *L. mariae* Sacchi & Rastelli #, West coast of Wales (Fish & Goodwin 1976)[3] *Helcion* Montfort, 1810; *Patella* Linnaeus, 1758, *Siphonaria* Sowerby, 1824, South Africa (Hodgson, Hawkins & Cross 1985)[4]. *L. melanostoma* Gray #; *L. (Littorinopsis) scabra scabra* (Linnaeus, 1758), West Bengal. India (Jamadar & Choudhury 1988)[5] (Fig. 7.6A-C)	
Body features	Body cylindrical when expanded, various stages of contraction, no striations, basal disc broad and flat, peristome when expanded arched and pointed, single row of peristomial cilia, no body cilia, gullet long, extend ½ of the BL, contractile vacuole with long canal, emptying on peristomial disc, myoneme present
Measurements	BL= 30-100 BD= 25-40[1]; BL= 102.3 BD= 43.3[3]; BL= 70-90 BD= 40[4]; BL= 40.8-96.9 BD= 23.8-64.6 SD= 40[2]; SD= 42.8[3]
Nucleus	Macronucleus sausage-shaped[1]; cylindrical, sausage-shaped centre and lower part of body[3]; elongated and centrally placed[4,5] MaL= 33.4-41.5 MaD= 6.9-12.4[3] Micronucleus large lens-shaped located below peristome[1]; small round close to macronucleus[3]; small spherical to oval, anterior to macronucleus[5] MiD= 3-4[3]
Position on host	Very large numbers on gills and mantle cavity
Remarks Hirshfield (1949) in his original description, distinctly noted that in his material there were no striations. Other authors such as Lom and Corliss (1968); Fish and Goodwin (1976) as well as Jamadar and Choudhury (1988) noted the pellicular ridges. Lom and Corliss (1968) provide a detailed description of the ultrastructure and confirm the occurrence of ridges. Later Fish and Goodwin (1976) also described the ultrastructure of *M. ubiquita* from other localities, confirming the findings of Lom and Corliss (1968).	

Compendium (continue) of *Mantoscyphidia* Jankowski, 1980 species known from marine gastropod hosts, based on information from different authors.

Mantoscyphidia acanthophora (Fish & Goodwin, 1976) (Fig. 7.4B)	
TYPE HOST: *Gibbula umbilicalis* da Costa # & *Monodonta lineata* da Costa # **TYPE LOCALITY:** Aberystwyth, West coast of Wales (Fish & Goodwin 1976)	
Body features	Body cylindrical, pellicle ridged, scopula narrow with short stout cilia
Measurements	BL= 88.7-127.4 BD= 32.3-48 SD= 19.6-38.7
Nucleus	Macronucleus C-shaped, located middle to upper part of body Micronucleus small round, lying close to end of macronucleus MiL= 3
Position on host	In the mantle cavity

Mantoscyphidia bengalensis (Jamadar & Choudhury, 1988) (Fig. 7.4C&D)	
TYPE HOST: *Cerithidea cingulata* (Gmelin #) **TYPE LOCALITY:** Sagar Island, West Bengal, India (Jamadar & Choudhury 1988)	
Body features	Body cylindrical vase-shaped, sculptured by concentric pellicular ridges, from base to peristomial disc, very small scopula, highly contractile body, long gullet, twisted and curved ending in middle of body, prominent contractile vacuoles, other small vacuoles (9-12) in central part of body
Measurements	BL= 40.8-71.4 BD= 25.5-61.2 SD= 5.1-11.9
Nucleus	Macronucleus cylindrical, sometimes coiled, great variation in shape and length MaL= 81.6 Micronucleus situated near oral end MiD= 1.7-3.4
Position on host	Abundant in mantle cavity and buccal mass of host

Compendium (continue) of *Mantoscyphidia* Jankowski, 1980 species known from marine gastropod hosts, based on information from different authors.

Mantoscyphidia branchi Van As, Basson & Van As, 1998 (Fig. 7.7A)	
TYPE HOST: *Patella barbara* Linnaeus, 1758 **TYPE LOCALITY:** De Hoop Nature Reserve, south coast of South Africa **OTHER REFERENCES, HOSTS & LOCALITIES:** *P. aphanes* Robson, 1986; *P. argenvillei* Krauss, 1848; *P. cochlear* Born, 1778; *P. compressa* Linnaeus, 1758; *P. concolor* Krauss, 1848; *P. granatina* Linnaeus, 1758; *P. granularis* Linnaeus, 1758; *P. longicosta* Lamarck, 1819; *P. miniata miniata* Born, 1778; *P. m. sanguians* Reeve, 1856; *P. obtecta* Krauss, 1848; *P. oculus* Born, 1778; *P. pica* Reeve, 1854; *P. tabularis* Krauss, 1848; *Cellana capensis* (Gmelin, 1791); *Helcion dunkeri* (Krauss, 1848); *H. pectunculus* (Gmelin, 1791) and *H. pruinosus* (Krauss, 1848). McDougall's Bay and the Olifants River Mouth on the west coast; Goukamma Nature Reserve and Keurboom Beach on the south coast; Bazley and at the rocky shores of Lake St. Lucia on the east coast of South Africa.	
Body features	Body cylindrical when expanded, peristomial area flattened to arched, telotroch band broad, elevated, situated one third of body length from scopula, prominent pellicle folds adoral to telotroch band, scopula very prominent and broad, scopula cilia short, densely grouped together, adoral zone completes spiral of 540°, cytoplasm granular, single contractile vacuole, number of food vacuoles, symbiotic algae in cytoplasm, nuclear apparatus occupies area below telotroch band
Measurements	BL= 44-78 BD= 17-32 SD= 15-25
Nucleus	Macronucleus large, adorally broad, tapering somewhat aborally MaL= 12-19 MaD= 11-20 Macronucleus forms indentation, occupied by round to oval-shaped micronucleus MiL= 7-12 MiD= 6-10
Position on host	Abundant on gills

Mantoscyphidia marioni Van As, Basson & Van As, 1998 (Fig. 7.7B)	
TYPE HOST: *Nacella delesserti* (Phillips, 1849) **TYPE LOCALITY:** Boulder Beach, Marion Island	
Body features	Body cylindrical, somewhat barrel-shaped in expanded forms, distinctly barrel-shaped in contracted forms, epistomial disc flattened to arched with broad lip, elevated pellicle ridges indicate telotroch band position, broad prominent scopula, round, symbiotic algae distributed throughout body
Measurements	BL= 63-128 BD= 26-69 SD= 20-57
Nucleus	Macronucleus large, oval to sausage-shaped MaL= 8-48 MaD= 5-42 Micronucleus very small, round. positioned aborally to macronucleus MiL= 2-6 MiD= 2-6 Both occur in middle or aboral region of body
Position on host	On gills

Compendium (continue) of *Mantoscyphidia* Jankowski, 1980 species known from marine gastropod hosts, based on information from different authors.

Mantoscyphidia fanthami Basson, Botha & Van As, 1998 (Fig. 7.7C)	
TYPE HOST: *Oxystele variegata* (Anton, 1838) **TYPE LOCALITY:** Jeffrey's Bay , south coast of South Africa. **OTHER REFERENCES, HOSTS & LOCALITIES:** *O. sinensis* (Gmelin, 1791); *O. tabularis* (Krauss, 1848); *O. tigrina* (Anton, 1838) and *O. impervia* (Menke, 1843). Margate and Ballito on the east coast; Nature's Valley and Buffel's Bay on the south coast and McDougall's Bay and the Olifants River Mouth on the west coast of South Africa.	
Body features	Body slender, elongated when expanded, with peristome broader than rest of body, in contracted specimens, epistomial disc and adoral cilia completely enclosed in body, adoral zone completes spiral of 540°, epistomial disc smooth, convex with prominently pointed apex, contracted adoral area with prominent elevated tapering point, large oral opening, telotroch band broad, elevated, situated one third of body length from scopula, prominent pellicular folds in area aboral to telotroch band, scopula broader than body, peristome, body and scopula with encircling pellicular striations, sometimes bifurcated, single contractile vacuole, some specimens with large symbiotic zooxanthellae occurring throughout body
Measurements	BL= 80-130 BD= 25-35 SD= 35-50
Nucleus	Macronucleus compact, somewhat rectangular, situated in base of body MaL= 7-16 MaD= 13-28 Micronucleus oval-shaped, situated on aboral side of macronucleus, sometimes in indentation of macronucleus MiL= 3-6 MiD= 5-10
Position on host	On gills

Figure 7.1

Diagram of a typical scyphidiid peritrich to illustrate general morphology.

ad= adoral ciliary spiral; c= cystostome; co= contractile vacuole; f= food vacuole; ma= macronucleus; mi= micronucleus; p= peristome; pd= peristomial disc; pl= peristomial lip; ps= pellicle striations; s= scopula; tb= telotroch band.

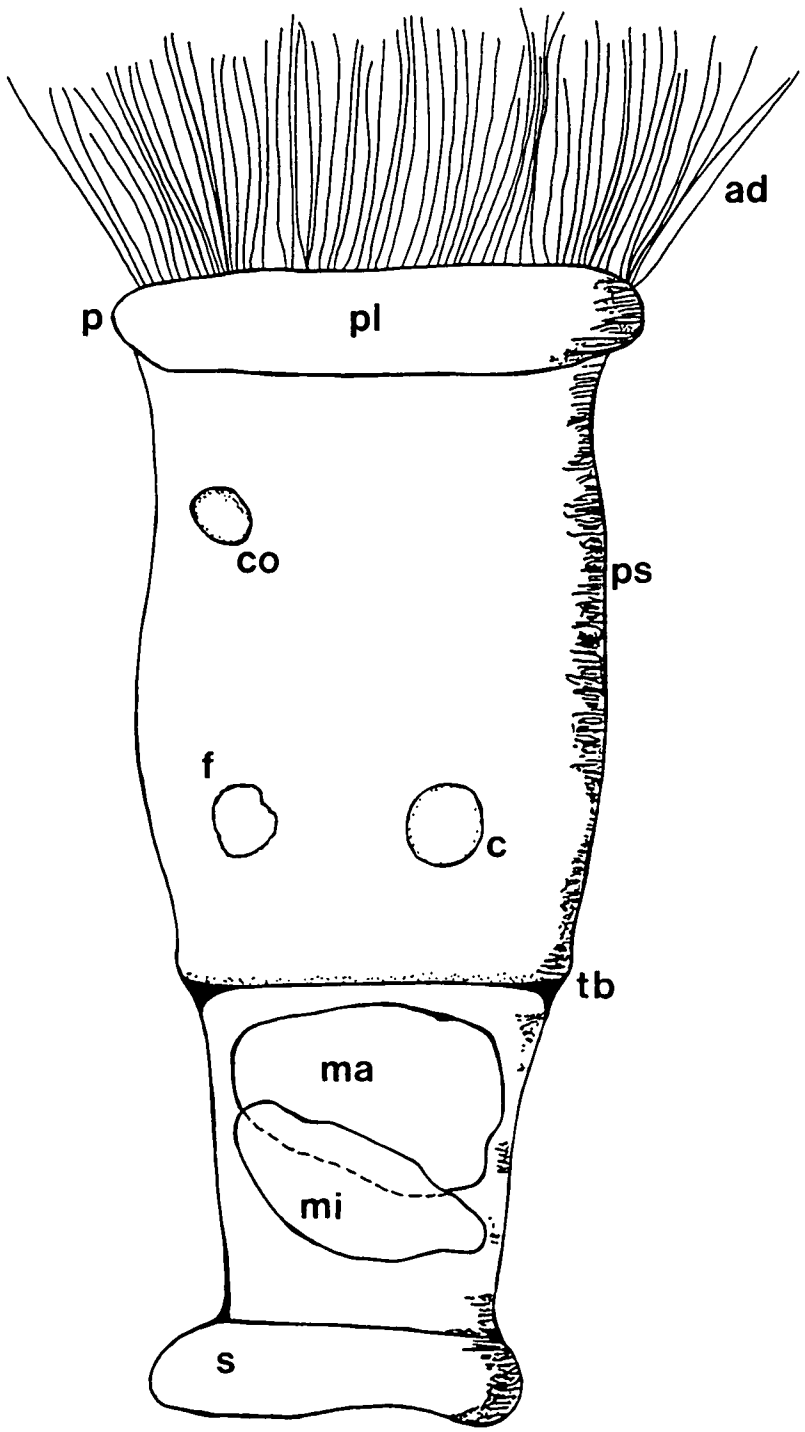
ad
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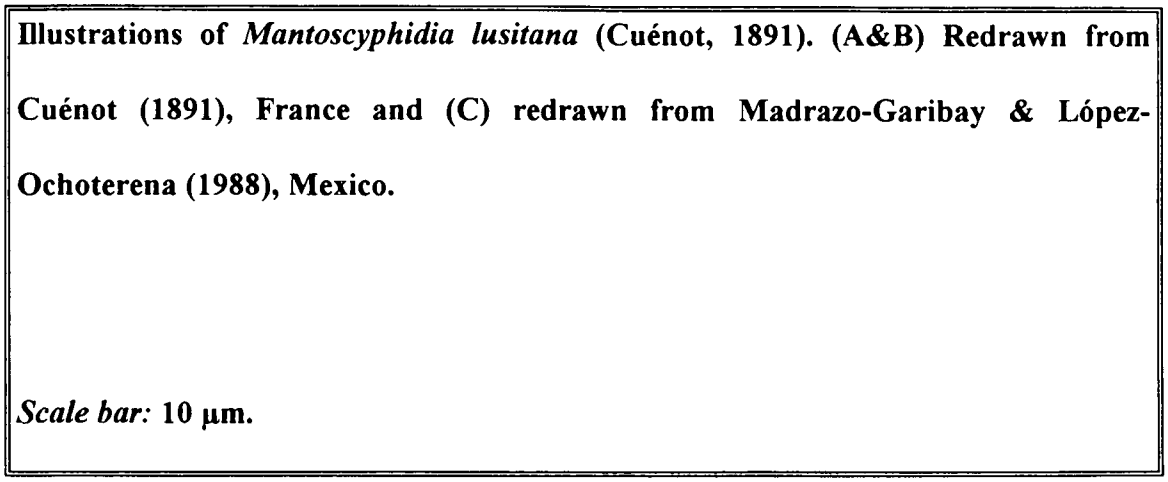
Figure 7.2



Illustrations of *Mantoscyphidia lusitana* (Cuénot, 1891). (A&B) Redrawn from Cuénot (1891), France and (C) redrawn from Madrazo-Garibay & López-Ochoterena (1988), Mexico.

Scale bar: **10 µm.**

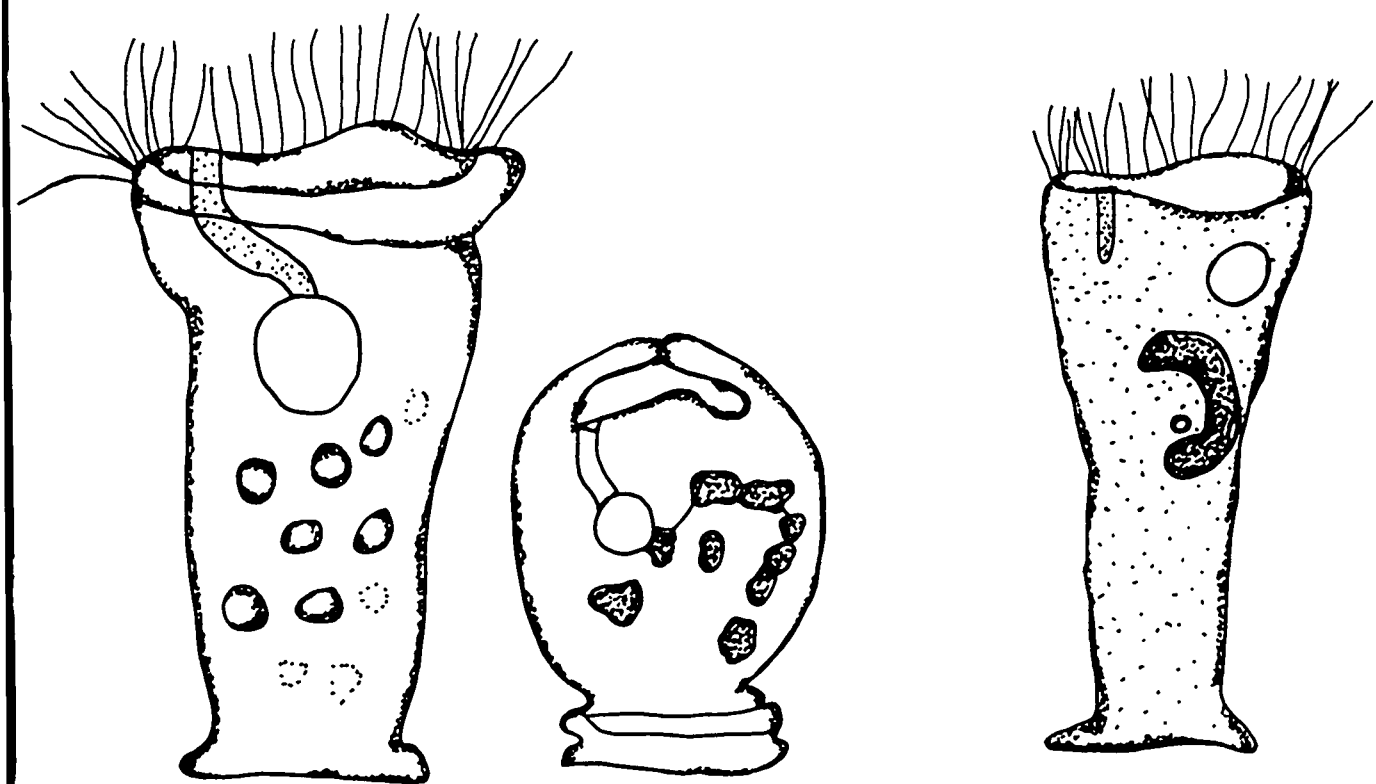
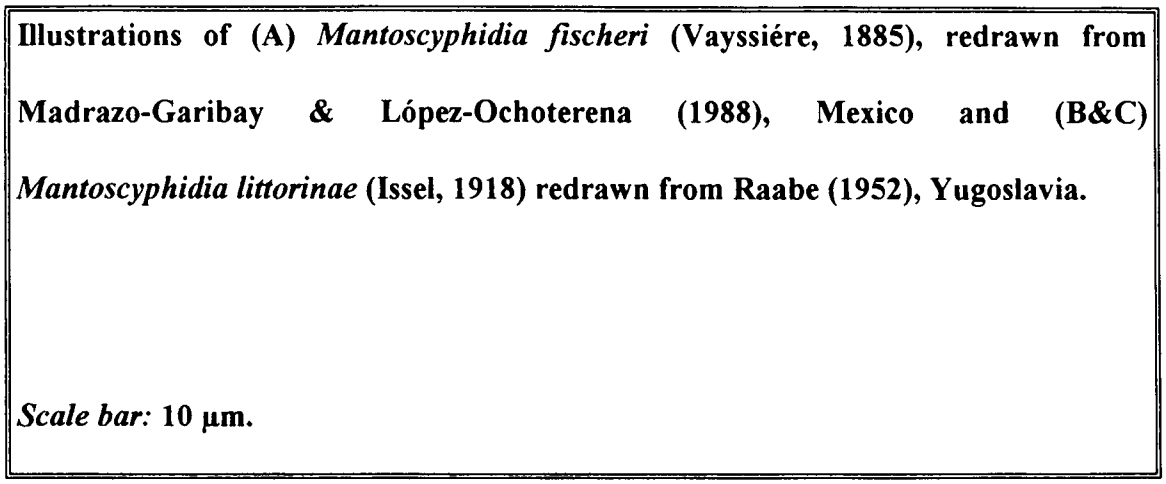

A
B
C

Figure 7.3



Illustrations of (A) *Mantoscyphidia fischeri* (Vayssiére, 1885), redrawn from Madrazo-Garibay & López-Ochoterena (1988), Mexico and (B&C) *Mantoscyphidia littorinae* (Issel, 1918) redrawn from Raabe (1952), Yugoslavia.

Scale bar: 10 µm.

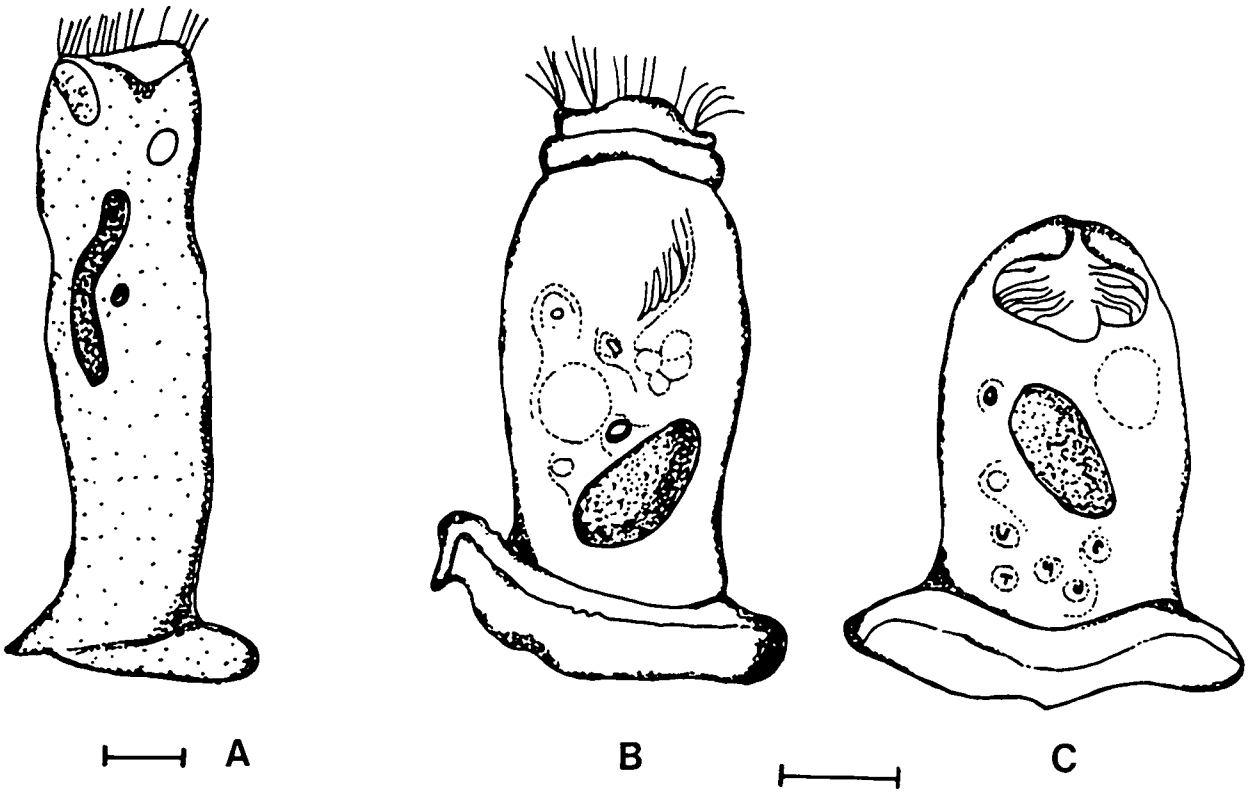

A
B
C

Figure 7.4

Illustrations of (A) *Mantoscyphidia hydrobiae* (Kahl, 1933), redrawn from Kahl (1933), Germany, (B) *Mantoscyphidia acanthophora* (Fish & Goodwin, 1976), redrawn from Fish and Goodwin (1976), Wales and (C&D) *Mantoscyphidia bengalensis* (Jamadar & Choudhury, 1988) redrawn from Jamadar and Choudhury (1988), India.

Scale bar: 10 µm.

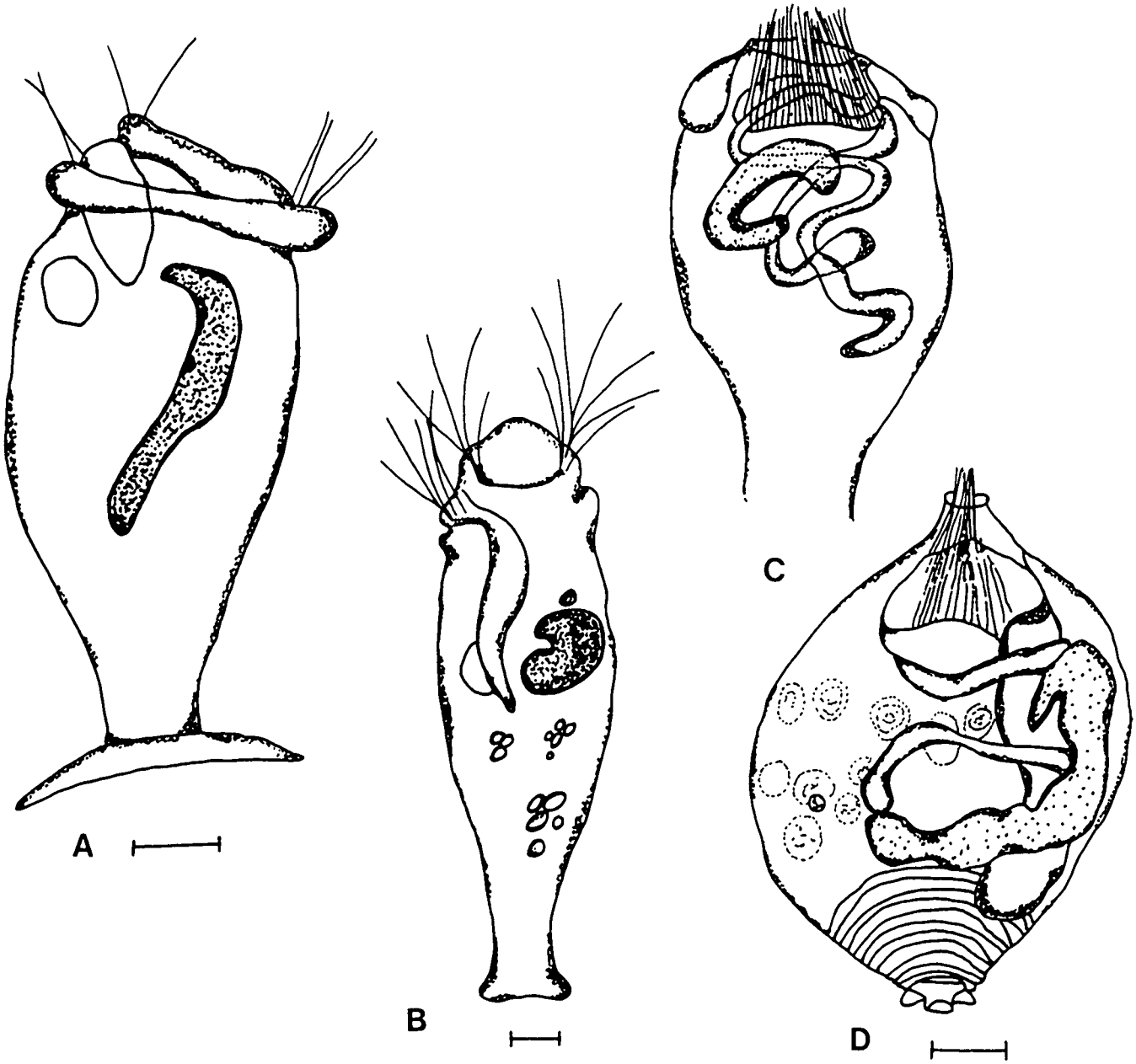
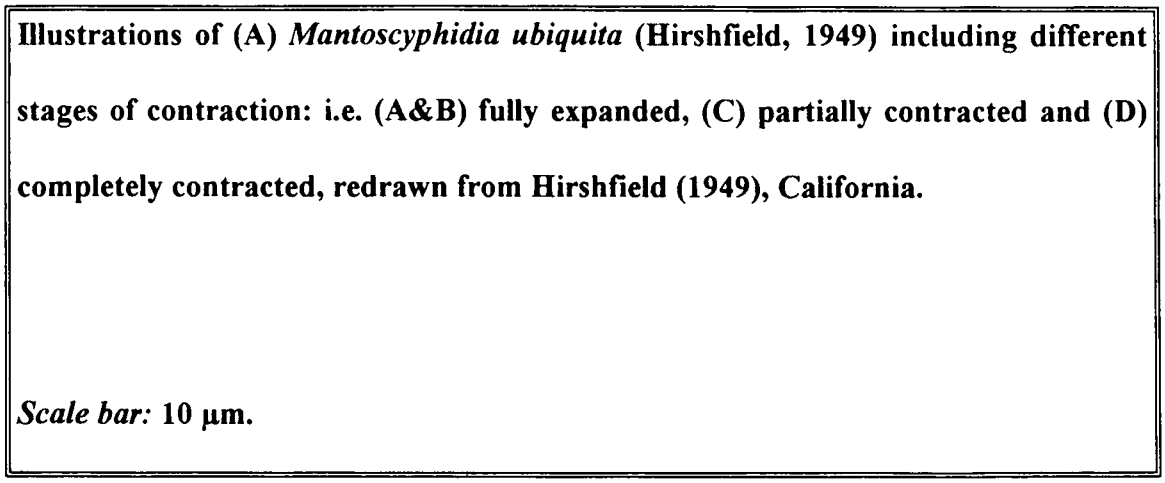

A
B
C
D

Figure 7.5



Illustrations of (A) *Mantoscyphidia ubiquita* (Hirshfield, 1949) including different stages of contraction: i.e. (A&B) fully expanded, (C) partially contracted and (D) completely contracted, redrawn from Hirshfield (1949), California.

Scale bar: **10 µm.**

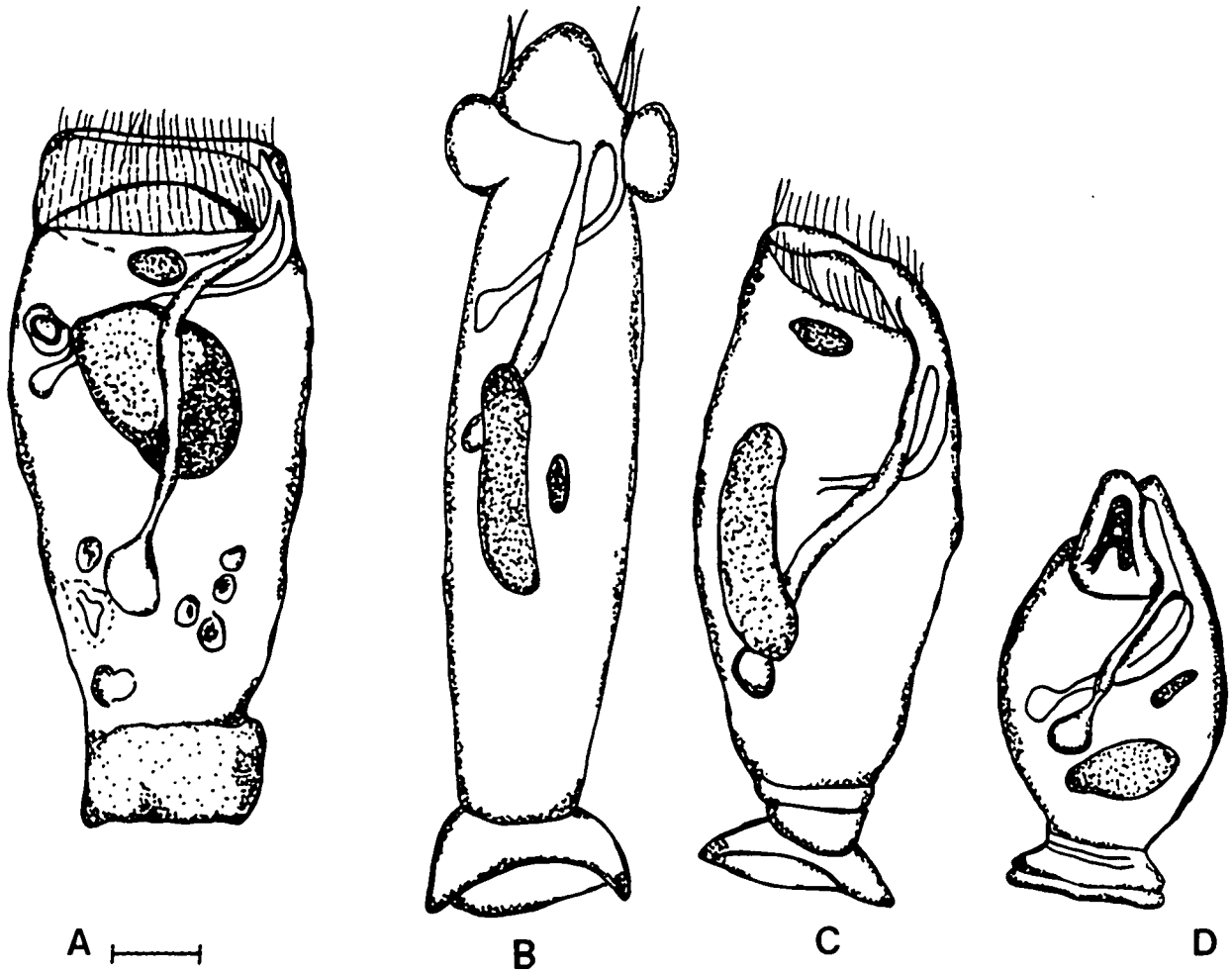
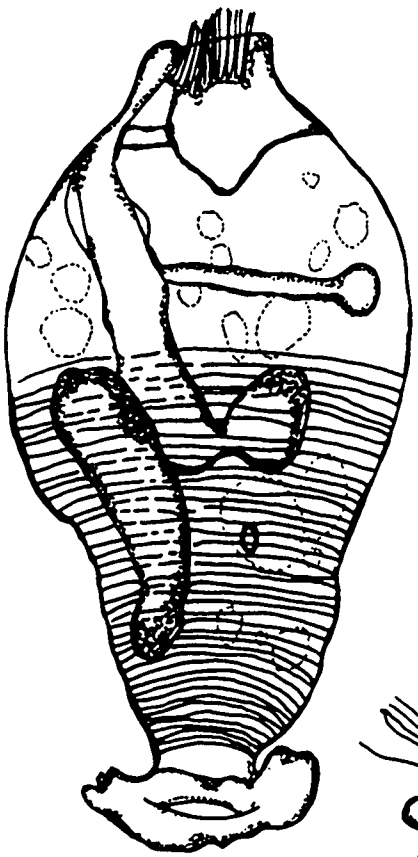

A
B
C
D

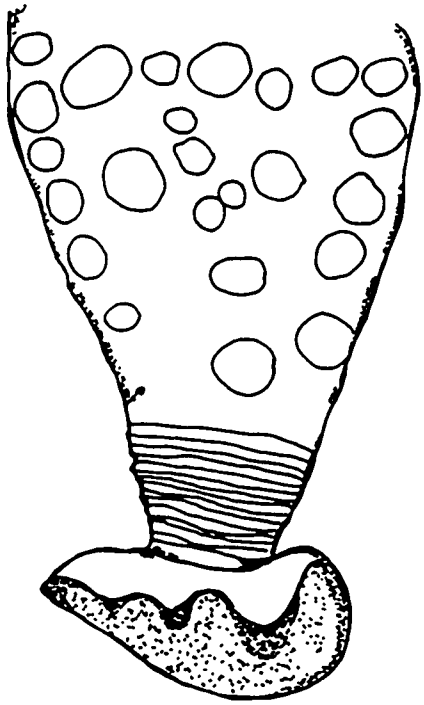
Figure 7.6

Illustrations of (A-C) *Mantoscyphidia ubiquita* (Hirshfield, 1949), redrawn from Jamadar and Choudhury (1988), India.

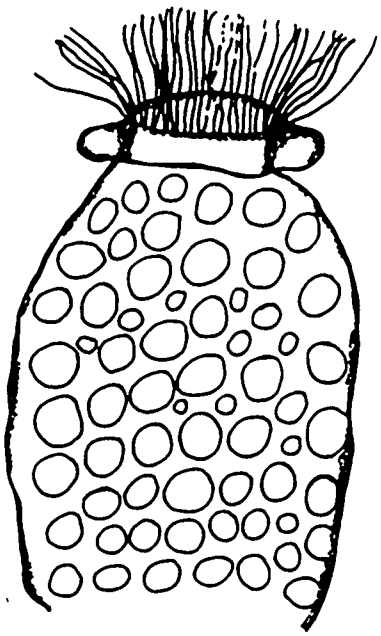
***Scale bar:* 10 µm.**



A

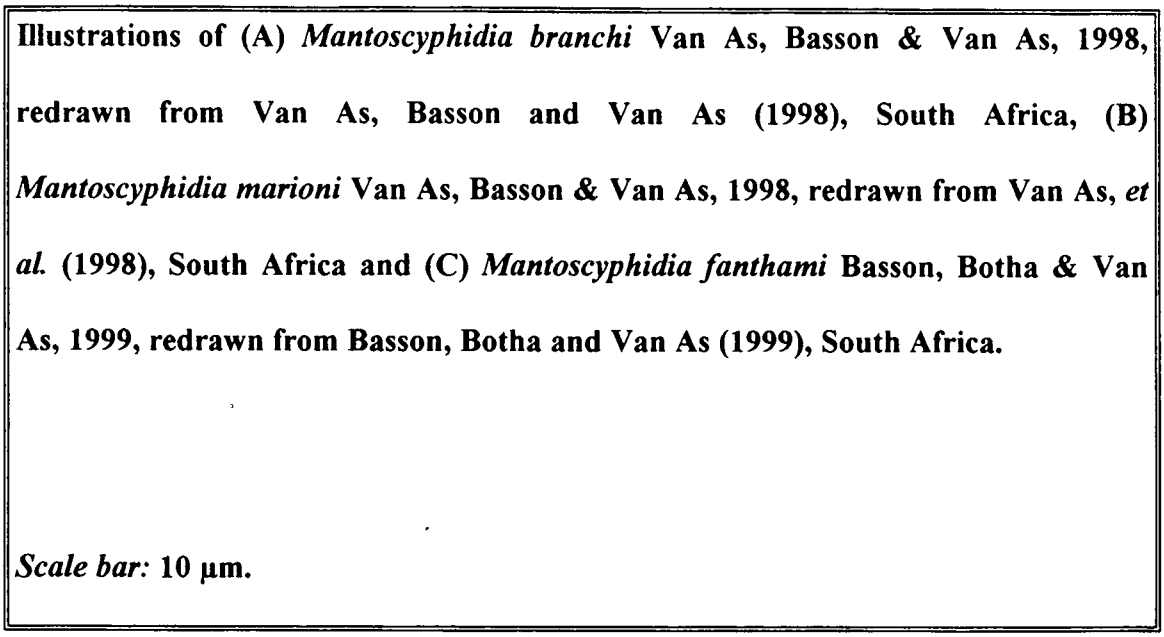


B



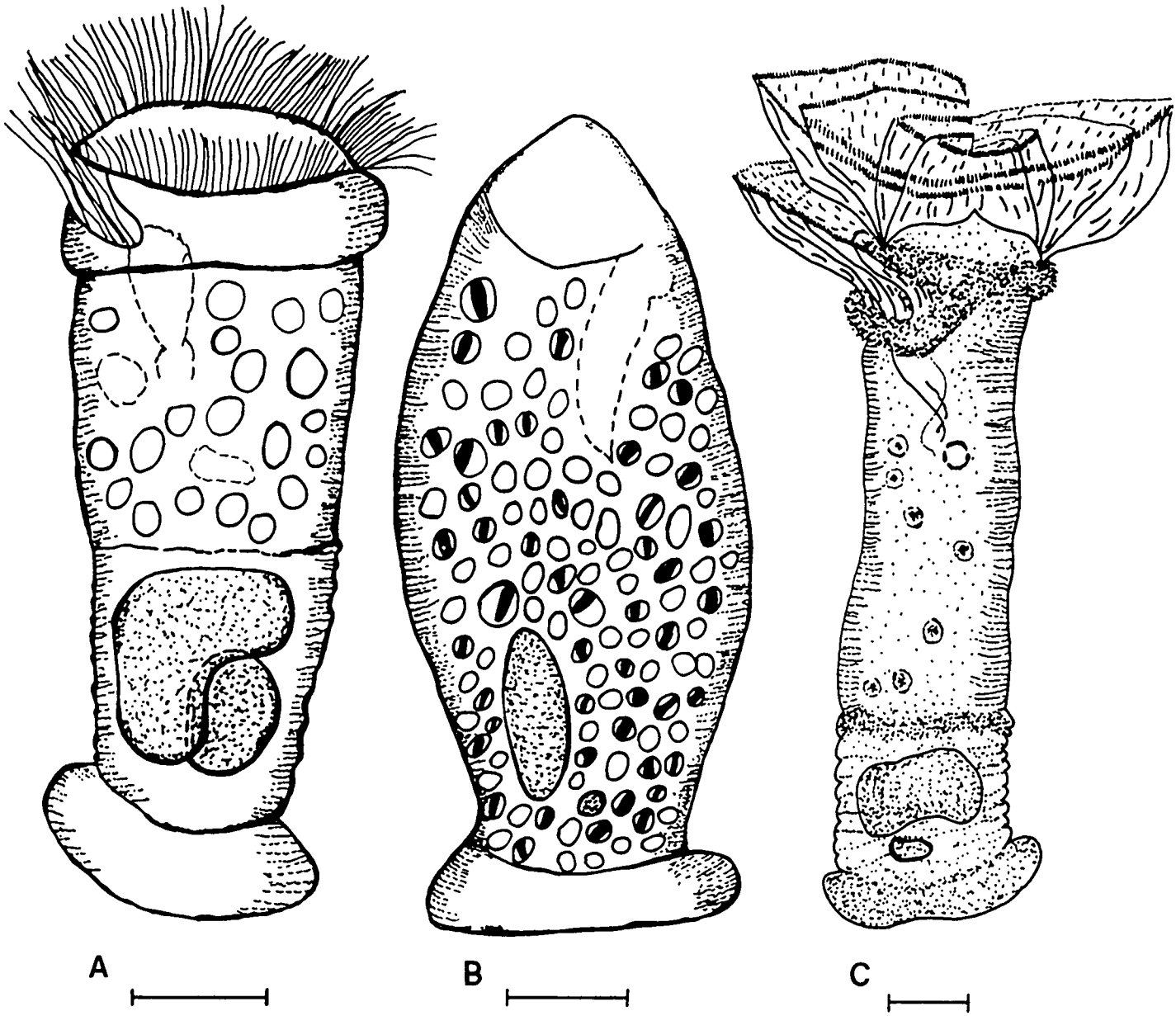
C

Figure 7.7



Illustrations of (A) *Mantoscyphidia branchi* Van As, Basson & Van As, 1998, redrawn from Van As, Basson and Van As (1998), South Africa, (B) *Mantoscyphidia marioni* Van As, Basson & Van As, 1998, redrawn from Van As, *et al.* (1998), South Africa and (C) *Mantoscyphidia fanthami* Basson, Botha & Van As, 1999, redrawn from Basson, Botha and Van As (1999), South Africa.

Scale bar: **10 µm.**


A
B
C

TWO NEW SPECIES OF *MANTOSCYPHIDIA* FROM SOUTH AFRICAN *HALIOTIS* SPECIES

Two of the six South African species of *Haliotis*, i.e. *H. spadicea* and *H. midae* were examined and found to be infested with two different species of *Mantoscyphidia*. These differ from all the known species, previously recorded from marine gastropod hosts, based on body dimensions, morphology and nuclear apparatus. These scyphidiid peritrichs are described as new species. The *Mantoscyphidia* specimens were found in abundance on the gills of the host. Infestation statistics will be presented in Chapter 10.

MANTOSCYPHIDIA SPADICEAE SP. NOV. FROM THE SOUTH AFRICAN VENUS EAR OR SIFFIE

***Mantoscyphidia spadiceae* sp. nov.** (Fig. 7.8-7.11; 7.16-7.18)

Type host: *Haliotis spadicea* Donovan, 1808.

Type locality: De Hoop Nature Reserve (34°28'S;20°30'E), south coast of South Africa.

Type material: Holotype slide, 97/04/05-04c (NMBP 235) and paratype slides, 97/04/07-17 (NMBP 236) and 98/04/05-06b (NMBP 237) in the collection of the National Museum, Bloemfontein, South Africa.

Position on host: Gills

Etymology: Named after the South African Venus ear or siffie, *Haliotis spadicea* on which the scyphidiid peritrich occurs.

Body measurements of live observations as well as hematoxylin stained specimens and data on the number of striations are summarised in Table 7.3.

Table 7.3 Body measurements of live observations as well as hematoxylin stained specimens (µm) and number of striations of specimens of *Mantoscyphidia spadiceae* sp. nov. occurring on the gills of *Haliotis spadicea* Donovan, 1808 from the De Hoop Nature Reserve, South Africa.

Live observations

Body length	50 - 140 (100.3 ± 24.1, 47)
Body diameter	20 - 45 (31.2 ± 7.0, 47)
Scopula length	5 - 15 (9.8 ± 1.8, 47)
Scopula diameter	15 -40 (31.3 ± 7.5, 47)
Macronucleus length	10 - 25 (19.3 ± 3.4, 30)
Macronucleus diameter	20 - 40 (28.3 ± 5.8, 30)
Micronucleus length	10 - 20 (10.8 ± 2.2, 37)
Micronucleus diameter	15 - 40 (22.2 ± 5.6, 37)

Hematoxylin stained specimens

	Completely contracted	Partially contracted	Fully expanded
Body length	49 - 60 (53 ± 4.7, 11)	63 - 80 (72.9 ± 5.5, 31)	82 - 134 (100 ± 12.7, 47)
Body diameter	25 - 43 (34.3 ± 4.8, 11)	23 - 56 (34.5 ± 7.5, 31)	26 - 60 (42 ± 6.5, 47)
Scopula length	3 - 12 (6.8 ± 2.8, 11)	2 - 21 (8.1 ± 4.6, 31)	1 - 24 (10.3 ± 6.1, 47)
Scopula diameter	15 - 38 (27.5 ± 7.7, 11)	19 - 41 (28.2 ± 5.1, 31)	19 - 45 (30.2 ± 5.1, 47)
Macronucleus length	8 - 18 (13.1 ± 3.6, 11)	5 - 22 (14.8 ± 3.5, 31)	11 - 27 (16.8 ± 3.9, 47)
Macronucleus diameter	14 - 33 (20.6 ± 5.1, 11)	16 - 31 (21.1 ± 3.5, 31)	17 - 38 (25.8 ± 4.4, 47)
Micronucleus length	4 - 10 (6.7 ± 1.7, 11)	4 - 12 (8.4 ± 2.1, 31)	3 - 15 (10.2 ± 2.2, 47)
Micronucleus diameter	11 - 17 (12.9 ± 2.3, 11)	10 - 24 (16.2 ± 3.7, 31)	12 - 30 (21.1 ± 3.5, 47)

Number of striations

Telotroch band	5 - 12 (7.7 ± 2.6, 10)
Peristome	19 - 30 (24.6 ± 3.6, 10)
Peristome to Telotroch band	92 - 136 (114.3 ±13.6, 10)
Telotroch band to Scopula	40 - 103 (58.9 ± 19.5, 10)
Scopula	9 - 22 (15.8 ± 4.7, 10)
Total number of striations	188 - 249 (222.1 ± 21.7, 10)

Body cylindrical, widens towards peristome (Fig. 7.8; 7.10A&B; 7.11A&F), extremely contractile. Peristomial disc ranging from flattened to arched, with broad lip (Fig. 7.11B&F) depending on body contraction. Very prominent peristomial apex (Fig. 7.10B; 7.11B). Scopula small (Fig. 7.10A; 7.11A). Cytoplasm smooth to granular (Fig. 7.10A&B). Contractile vacuole present. A number of food vacuoles in cytoplasm. Symbiotic algae occasionally found in cytoplasm, always adoral to telotroch band, varying in number and size. Adoral spiral describes one and a half turns, about 540° (Fig. 7.11D), before plunging into infundibulum (Fig. 7.11C). Row of pores between ciliary spiral and peristomial lip (Fig. 7.11D). Buccal apparatus, haplo- and polykinety

starts almost equal (Fig. 7.11E), first three kinetosomes of polykinety barren. Haplo- and polykinety divided by pellicle ridge or comb, approximately width of a kinetosome (Fig. 7.11D). Polykinety three kinetosomes wide, with proximal row on outside and barren row of kinetosomes, not always visible. Polykinety plunges first into infundibulum divides into peniculi. Haplokinety makes another half turn (180°) before plunging into infundibulum. Impregnable structure associated with haplokinety. Inside infundibulum (Fig. 7.9), polykinety makes one half turn (180°), haplokinety makes less than one half turn (<180°) before reaching cytostome. Cytostome not always clearly visible.

Pellicle striations in expanded individuals (Fig. 7.11F) approximately 0.3 μm apart, adorn whole body, including peristome and scopula. Striations evenly spaced and uniform. Bifurcated pattern of striations found in some individuals on body and scopula. Bottom half of peristomial lip with uniform striation pattern in expanded specimens, upper half showing irregular pattern (Fig. 7.11C). In contracted scyphidiid peritrichs edge of peristome indistinct with striations forming a zig-zag pattern (Fig. 7.11D). Telotroch band consists of seven or eight closely associated striations, forming slight elevations (Fig. 7.11A&F). Pellicle area between telotroch band and scopula, forms five to eight elevations depending on degree of contraction (Fig. 7.11A).

Nuclear apparatus occupies most of area below telotroch band (Fig. 7.10A-F). Definite membrane situated above nuclear apparatus, separating nuclear apparatus from rest of cytoplasm (Fig. 7.8; 7.10A-F). Nuclear apparatus consists of single large round to oval-shaped macronucleus and single smaller oval-shaped micronucleus. Macronucleus adorally broad, tapering somewhat aborally, forming indentation occupied by micronucleus. The latter always situated aboral to macronucleus. Nuclear apparatus strikingly consistent in position and shape (Fig. 7.10C-F).

Remarks

Mantoscyphidia spadiceae is the twelfth species of sessiline peritrich found associated with marine gastropods and is the first record from the genus *Haliotis*. It can be

distinguished from the other species based on the morphology of the nuclear apparatus, scopula and body shape.

Mantoscyphidia fischeri (Vayssiére, 1885) (Fig. 7.3A) and *M. hydrobiae* (Kahl, 1933) (Fig. 7.4A) have ribbon- and kidney-shaped macronuclei, respectively (Kahl 1933; Hirshfield 1949). In the drawings presented of Raabe (1952) of *M. littorinae* (Issel, 1918) (Fig. 7.3B&C), it appears that this species has an oval to sausage-shaped macronucleus, situated in the aboral part of the body. The fourth species, *M. acanthophora* (Fish & Goodwin, 1976) (Fig. 7.4B), has a C-shaped macronucleus (Fish & Goodwin 1976) which is situated in the middle to adoral part of the body. Another character of this species is the small, narrow scopula with cilia. *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 has a broad scopula with much shorter cilia that are densely grouped together. The scopula of *M. spadiceae* also possesses short cilia grouped together. The scopula isn't as broad and prominent as the scopulas of *M. branchi* and *M. marioni* Van As, Basson & Van As, 1998 (Fig. 7.7A&B) (Van As, *et al.* 1998). *M. spadiceae* also has a narrow, small scopula in relation to body diameter, similar to *M. acanthophora*. The fifth species, *M. bengalensis* (Jamadar & Choudhury, 1988) (Fig. 7.4C&D), described by Jamadar and Choudhury (1988) also has a small scopula and a very conspicuous macronucleus, which appears cylindrical and sometimes coiled. In the coiled forms the nucleus fills the whole of the aboral region. A small micronucleus occurs close to the peristome.

In *M. lusitana* (Cuénot, 1891) *emend* Jankowski, 1985, the macronucleus forms a band or is beaded (Cuénot 1891; Hirshfield 1949). Another population of *M. lusitana*, described by Madrazo-Garibay and López-Ochoterena (1988) from clams, has a horseshoe-shaped macronucleus above the telotroch band. *Mantoscyphidia ubiquita* (Hirshfield, 1949) (Fig. 7.5A; 7.6A-C) also has a sausage-shaped macronucleus, which is situated in the middle part of the body in expanded and partially contracted forms. When the individuals are completely contracted the macronucleus occurs closer to the scopula. According to Hirshfield (1949) *M. ubiquita* has two types of micronuclei, depending on the host species. Limpet ciliophorans' micronuclei are larger and lie close to the peristome, whilst the ciliophorans occurring on turbans have small round micronuclei,

lying close to the macronuclei. According to Van As (1997) this could in fact be two distinct species.

The scopula of *Mantoscyphidia fanthami* Basson, Botha & Van As, 1999 is broader than its body (Fig. 7.7C) (Basson, Botha & Van As 1999), while in the case of *M. spadiceae*, it is not as broad as the widest part of the body. The position and shape of the nuclear apparatus of *M. fanthami* and *M. spadiceae* are similar, but *M. spadiceae* has a much larger macro- and micronucleus than that of *M. fanthami*. As in the case of most of the species of *Mantoscyphidia*, except *M. littorinae*, constriction or narrowing is visible adoral to the scopula.

Interspecific variation

The shrinkage effect of live specimens vs. hematoxylin stained specimens on the nuclear apparatus (see Table 7.3) is as follows: The arithmetic mean of the macronucleus length and diameter of fully expanded hematoxylin stained specimens was 16.8 µm and 25.8 µm respectively, compared to 19.3 µm and 28.3 µm in live specimens. This amounts to shrinkage of approximately 13 % and 9 %, respectively. The micronucleus length and diameter showed very little shrinkage between expanded hematoxylin stained and live specimens. Thus, macronucleus length is more affected by shrinkage. This was also the case in *M. branchi* (Van As 1997).

The arithmetic means of the body dimensions of expanded live specimens (Fig. 7.10A&B; Table 7.3) are slightly higher than that of expanded specimens fixed in Bouin's and stained with hematoxylin. This information confirms the necessity of including live observations in species descriptions. The body of *M. spadiceae* is extremely contractile, varying from 49 to 140 µm. These scyphidiid peritrich's body lengths are much greater than any of the other species in the genus, the closest in resembling such extreme contractility is *M. ubiquita*, whose body length varies between 30 and 100 µm, and *M. fanthami* (80-130 µm).

The number of striations, from different parts of the body, counted with the aid of a scanning electron microscope, can be used to describe new species (Van As, Van As &

Basson 1995). Unfortunately this can only be compared to data of *M. branchi*, *M. marioni* and *M. fanthami* as no information on the number of body striations of the other species of *Mantoscyphidia* from marine gastropods is available. *M. spadiceae* and *M. fanthami* have an average total number of 222.1 and 262 striations, respectively, whilst *M. branchi* and *M. marioni* have 106.5 and 115.6 striations respectively. This can be ascribed to the greater body length of *M. spadiceae* and *M. fanthami*, and the fact that both of these species' striations are 0.3 μm apart, whilst in the case of *M. branchi* and *M. marioni* the striations are further apart (0.5 μm).

As in the case of *M. branchi*, the form and position of the nuclear apparatus showed the most consistency. The macronucleus is also shaped in such a way that it leaves a perfect hollow into which the micronucleus snugly fits.

Figure 7.8

Microscope projection drawing of *Mantoscyphidia spadiceae* sp. nov. occurring on the gills of *Haliotis spadicea* Donovan, 1808, collected from the De Hoop Nature Reserve, South Africa.

***Scale bar:* 10 µm.**

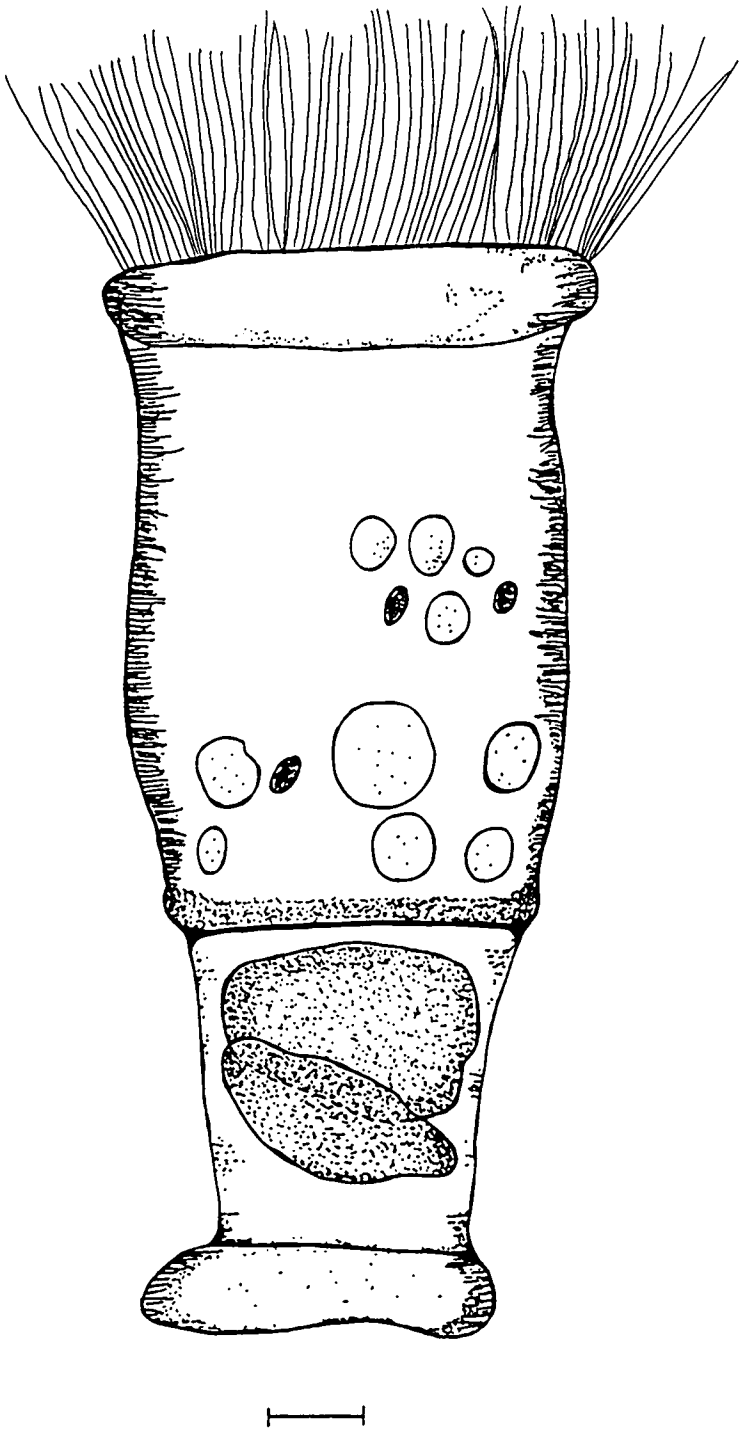


Figure 7.9

Diagram illustrating the infundibulum of *Mantoscyphidia spadiceae* sp. nov. occurring on the gills of *Haliotis spadicea* Donovan, 1808, collected from the De Hoop Nature Reserve, South Africa.

c= cytostome; hk= haplokinety; im= impregnable band; pe= peniculus; pk= polykinety.

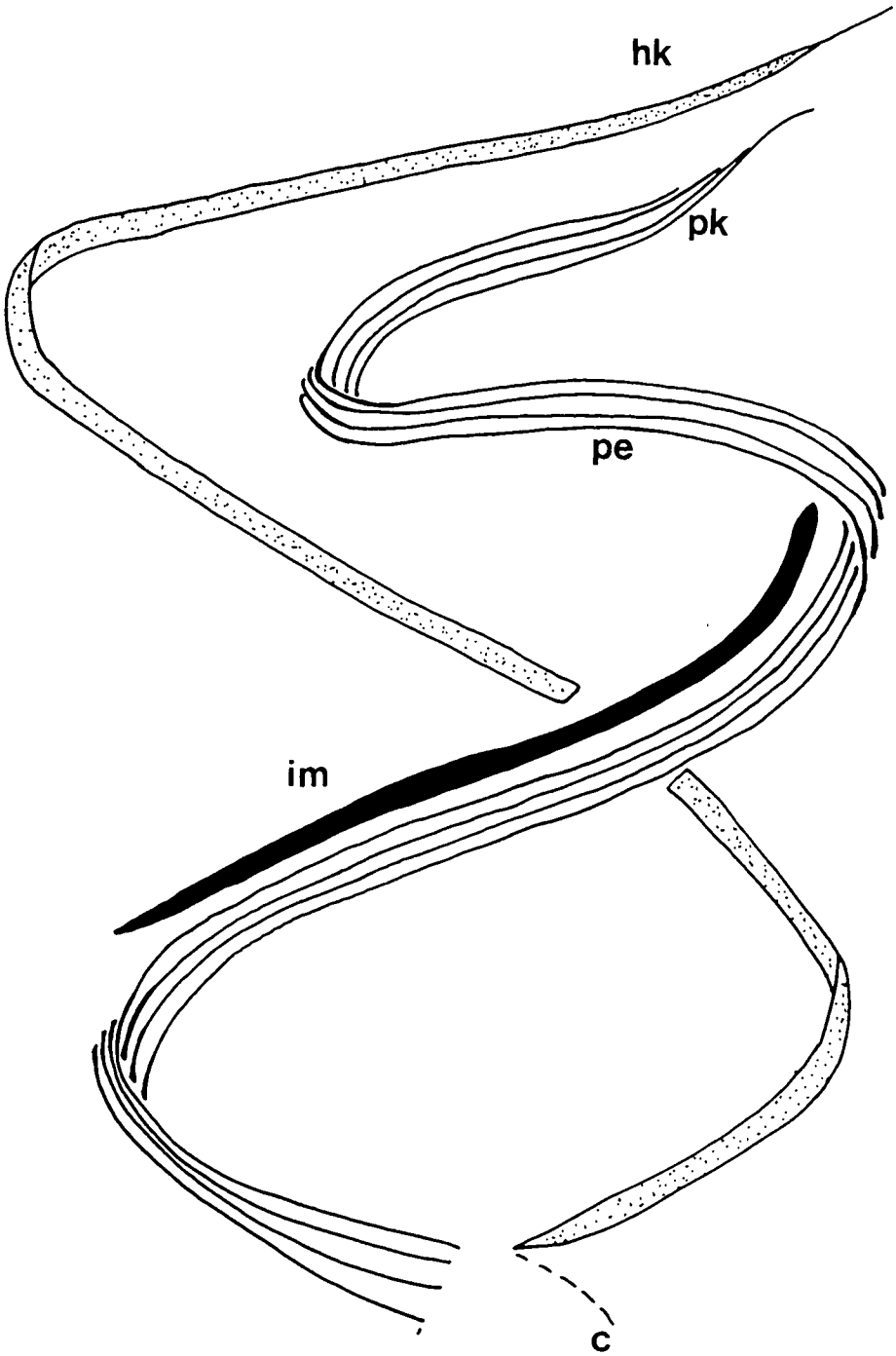

hk
pk
pe
im
c

Figure 7.10

Micrographs of live specimens (A&B) as well as hematoxylin stained specimens (C-F) of *Mantoscyphidia spadiceae* sp. nov. occurring on the gills of *Haliotis spadicea* Donovan, 1808, collected from the De Hoop Nature Reserve, South Africa.

A. Body expanded. Peristome arched. ***Scale bar:* 10 µm.**

B. One expanded and one contracted specimen. ***Scale bar:* 10 µm.**

C-F. Hematoxylin stained specimens. Macro- and micronuclei visible.

***Scale bars:* 20 µm.**

ma= macronucleus; mi= micronucleus.

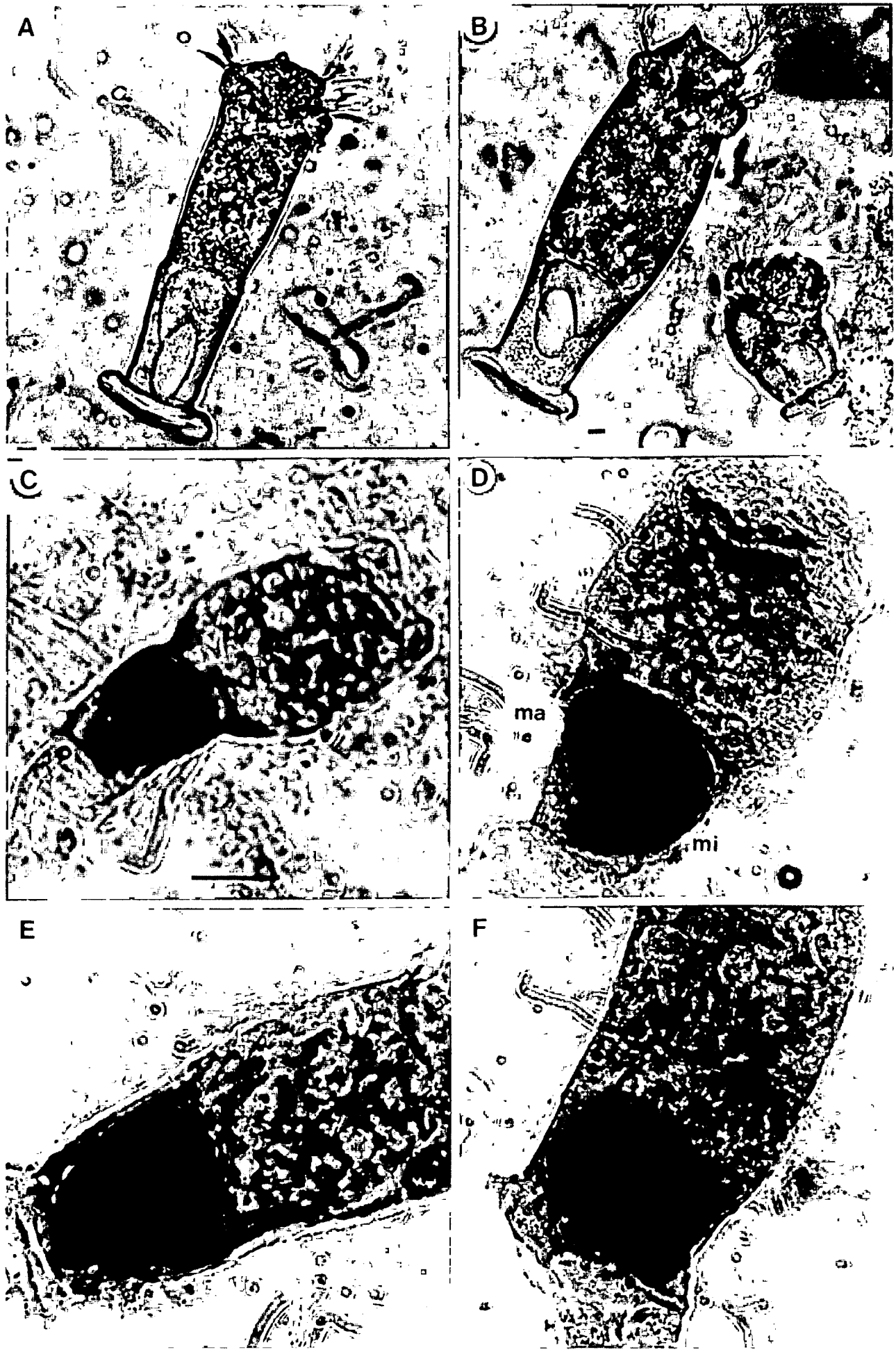

A
B
C
D
ma
mi
E
F

Figure 7.11

Scanning electron micrographs of specimens of *Mantoscyphidia spadiceae* sp. nov. occurring on the gills of *Haliotis spadicea* Donovan, 1808, collected from the De Hoop Nature Reserve, South Africa.

A. Expanded specimens attached to the gill arch.

B. Adoral ciliary spiral with peristomial apex indicated by arrow.

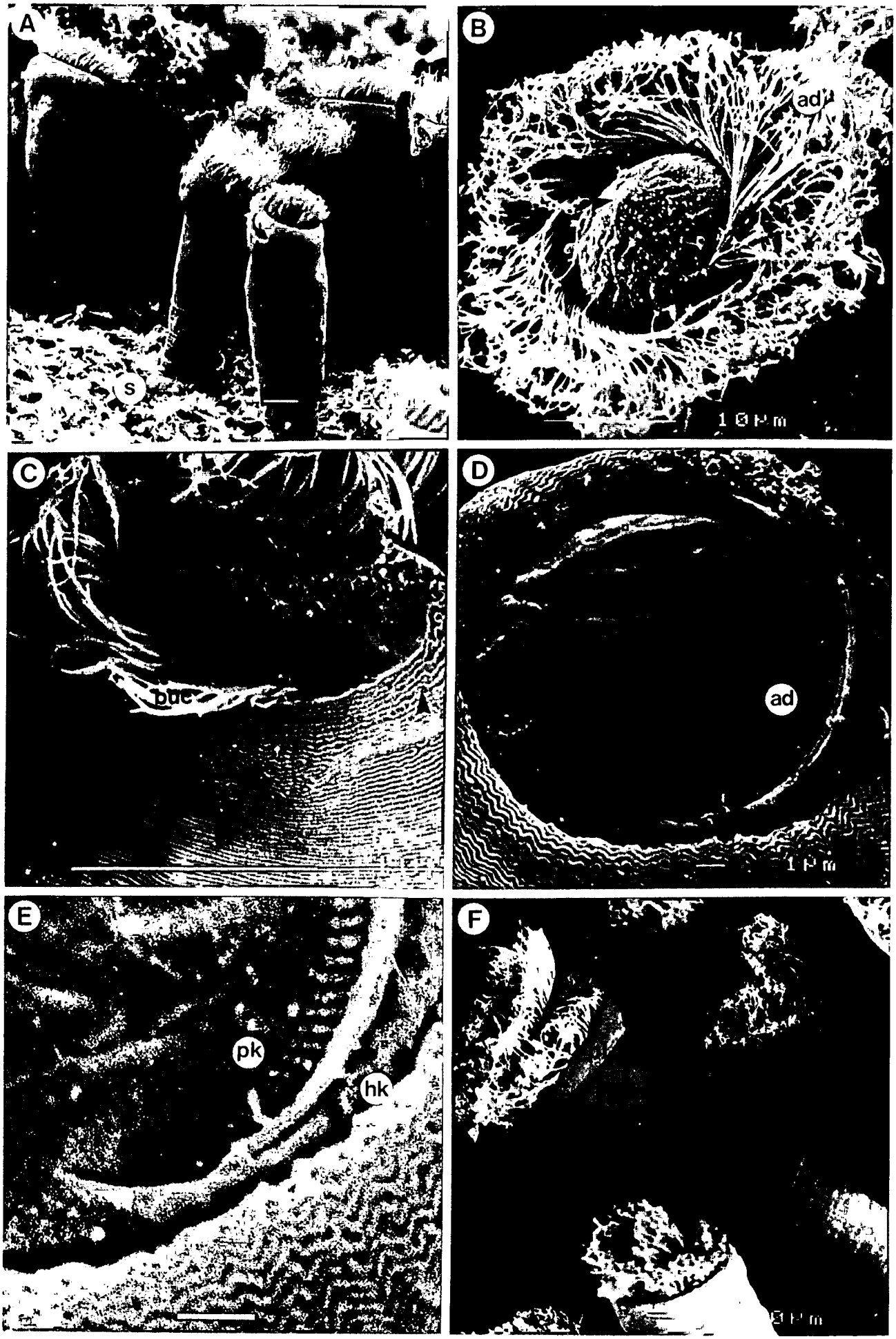
C. Buccal cavity. Uneven zig-zag striations indicated by arrow.

D. Adoral ciliary spiral with kinetosomes where cilia were removed.

E. Beginning of haplo- and polykineties. ***Scale bar*: 1 µm.**

F. Expanded specimen with telotroch band indicated by arrow.

ad= adoral ciliary spiral; buc= buccal cavity; hk= haplokinety; pk= polykinety; pl= peristomial lip; s= scopula.


A
s
B
ad
10µm
C
buc
D
ad
1µm
E
pk
hk
F

MANTOSCYPHIDIA MIDAE SP. NOV. FROM THE SOUTH AFRICAN PERLEMOEN

***Mantoscyphidia midae* sp. nov.** (Fig. 7.12-7.18)

Type host: *Haliotis midae* Linnaeus, 1758.

Type locality: De Hoop Nature Reserve (34°28'S;20°30'E), south coast of South Africa.

Type material: Holotype slide, 97/04/07-14 (NMBP 238) and paratype slides, 97/03/29-02 (NMBP 239) and 97/03/29-09 (NMBP 240) in the collection of the National Museum, Bloemfontein, South Africa.

Position on host: Gills

Etymology: Named after the South African perlemoen *Haliotis midae* on which the scyphidiid peritrichs occur.

Body measurements of live observations as well as hematoxylin stained specimens and data on the number of striations are summarised in Table 7.4.

Table 7.4 Body measurements of live observations as well as hematoxylin stained specimens (µm) and number of striations of specimens of *Mantoscyphidia midae* sp. nov. occurring on the gills of *Haliotis midae* Linnaeus, 1758 from the De Hoop Nature Reserve, South Africa.

Live observations

Body length	50 - 150 (84.3 ± 17.3, 89)
Body diameter	15 - 45 (27.9 ± 6.7, 89)
Scopula length	3 - 20 (9 ± 2.5, 89)
Scopula diameter	15 - 50 (33.8 ± 9.1, 89)
Macronucleus length	10 - 25 (15.5 ± 4.5, 19)
Macronucleus diameter	10 - 30 (20.8 ± 5.8, 19)
Micronucleus length	5 - 15 (7.9 ± 2.7, 27)
Micronucleus diameter	8 - 25 (13.3 ± 4.9, 27)

Hematoxylin stained specimens

	Completely contracted	Partially contracted	Fully expanded
Body length	45 - 60 (55.6 ± 4.3, 36)	61 - 83 (69.5 ± 5.9, 67)	81 - 110 (89.8 ± 8.8, 19)
Body diameter	17 - 43 (23.6 ± 4.8, 36)	17 - 38 (24.9 ± 4.4, 67)	22 - 40 (29.8 ± 5.4, 19)
Scopula length	1 - 18 (5.1 ± 3.7, 36)	1 - 14 (5.9 ± 3.4, 67)	3 - 28 (10.3 ± 6.3, 19)
Scopula diameter	13 - 34 (24.3 ± 5.3, 36)	16 - 43 (27.5 ± 5.4, 67)	6 - 50 (32.6 ± 10, 19)
Macronucleus length	5 - 19 (11 ± 3.1, 36)	7 - 18 (11.1 ± 2.2, 67)	9 - 31 (14.5 ± 5.7, 19)
Macronucleus diameter	11 - 27 (15.5 ± 3.6, 36)	10 - 31 (16.2 ± 4.1, 67)	13 - 41 (20.7 ± 7.3, 19)
Micronucleus length	2 - 12 (5.2 ± 1.8, 36)	3 – 10 (6 ± 1.2, 67)	5 - 14 (7.5 ± 2.7, 19)
Micronucleus diameter	7 - 15 (10.5 ± 2.1, 36)	7 - 16 (10.1 ± 2, 67)	8 - 23 (12.8 ± 4.2, 19)

Number of striations

Telotroch band	4 - 8 (6.2 ± 1.5, 10)
Peristome	20 - 42 (26.8 ± 6.8, 10)
Peristome to Telotroch band	127 - 167 (141.8 ± 11.6, 10)
Telotroch band to Scopula	50 - 83 (61.3 ± 12.4, 10)
Scopula	14 - 31 (22.5 ± 4.9, 10)
Total number of striations	238 - 312 (255.8 ± 23.7, 10)

Body cylindrical (Fig. 7.12; 7.14A-G; 7.15A). Peristomial disc ranging from flattened to arched, with broad lip (Fig. 7.15A&B) depending on body contraction. Very broad, flattened and prominent scopula (Fig. 7.14B; 7.15E). Peristomial apex prominent in expanded specimens (Fig. 7.14A-C; 7.15B). Cytoplasm granular (Fig. 7.14A&B). Contractile vacuole present. A number of food particles in cytoplasm. Symbiotic algae (Fig. 7.14A-G) found throughout cytoplasm, varying in number and size. Adoral spiral describes 450° (Fig. 7.15D), before plunging into infundibulum (Fig. 7.13). Row of pores between ciliary spiral and peristomial lip. Small buccal cavity (Fig.7.15C). Buccal apparatus, haplo- and polykinety starts almost equal, first three kinetosomes of

polykinety barren. Haplo- and polykineties divided by pellicle ridge or comb, approximately width of a kinetosome. Polykinety three kinetosomes wide, with proximal row on outside and barren row of kinetosomes, not always visible. Haplokinety plunges first into infundibulum (Fig. 7.15C). Polykinety divides into peniculus after plunging into infundibulum. Impregnable structure associated with polykinety. Inside infundibulum (Fig. 7.13), both haplo- and polykinety make one turn (360°) before reaching cytostome. Cytostome not always clearly visible.

Pellicle striations in expanded individuals (Fig. 7.15A,F&G) approximately 0.25 μm apart, adorn whole body, including peristome and scopula. Striations evenly spaced and uniform. Bifurcated pattern of striations found in some individuals on body and scopula. Bottom half of peristomial lip with uniform striation pattern in expanded specimens, upper half showing irregular pattern (Fig. 7.15C). In contracted scyphidiid peritrichs edge of peristome indistinct with striations forming a zig-zag pattern (Fig. 7.15B). Telotroch band consists of four to eight closely associated striations, forming slight elevations (Fig. 7.15F).

Nuclear apparatus situated below telotroch band, situated very close to scopula (Fig. 7.14D-G). Nuclear apparatus consists of single, large round to oval-shaped macronucleus and single smaller oval-shaped micronucleus. Micronucleus always found aborally and closely associated with macronucleus. Macronucleus forming indentation, occupied by micronucleus. Nuclear apparatus appear to be surrounded with granular cytoplasm and symbiotic algae (Fig. 7.14A-G). Nuclear apparatus strikingly consistent in position and shape.

Remarks

Mantoscyphidia midae is the thirteenth species of sessiline peritrich found associated with marine gastropods and is the second record from the genus *Haliotis*. It can be distinguished from the other species based on the morphology of the nuclear apparatus, scopula and body shape.

Mantoscphidia midae, *M. marioni* and *M. branchi* all have broad, prominent scopulas (Fig. 7.7A&B; 7.12). *M. midae* (Fig. 7.12) and *M. fisheri* (Fig. 7.3A) have similar cylindrical body forms, but their nuclear apparatus differ significantly. *M. fisheri* has an sausage-shaped macronucleus and the nuclear apparatus is situated in the adoral region of the body, compared to the nuclear apparatus of *M. midae* that is always found below the telotroch band. According to the figure redrawn from Raabe (1952), *M. littorinae* (Fig. 7.3B&C) also has a prominent scopula, much like the scopula of *M. midae*, but its nuclear apparatus is situated further from the scopula. The micronucleus, however, is also much smaller than the macronucleus, as is also the case in *M. midae*. There is a big difference in body length between these two species (Fig. 7.19A&B). *M. fanthami* (Fig. 7.7C) also has the same cylindrical body from as *M. midae*, but is much longer.

The body of *M. midae* does not widen towards its peristome, most specimens rather narrow towards the peristome. *M. midae* has a smaller body diameter (27.9 μm), compared to *M. spadiceae* (31.2 μm), (Fig.7.20A&B) and a much broader and flattened scopula in relation to body diameter. *M. midae* has a much more granular cytoplasm than *M. spadiceae*, and has a much higher occurrence of symbiotic algae occurring throughout the body. The symbiotic algae were not restricted to the area adoral of the telotroch band, as was the case in *M. spadiceae*.

There are significant differences between the infundibula of these two species, which can be used as distinguishing taxonomic characters (See descriptions of two species). *M. midae* has striations that are spaced a bit closer together than those of *M. spadiceae*, therefore accounting for the greater total number of striations adorning the body, even though *M. spadiceae* has a greater average body length. Both live observations and measurements of hematoxylin stained specimens revealed *M. spadiceae* to have a greater body length than *M. midae*. It appears as if the telotroch band of *M. midae* also consists of more striations and elevations.

Mantoscyphidia spadiceae has a definite membrane situated adoral to the nuclear apparatus, thus separating the nuclear apparatus from the rest of the cytoplasm. This membrane is absent in *M. midae* and the nuclear apparatus appears to be surrounded by

symbiotic algae and part of the granular cytoplasm, and is thus not separated from the cytoplasm as in the case of *M. spadiceae*. By comparison, *M. midae* specimens have smaller micronuclei than those of *M. spadiceae*. The size of their macronuclei do not differ significantly.

Mantoscyphidia spadiceae and *M. midae* do have some similarities, however. The nuclear apparatus of both species are consistent in position and shape. Both species show extreme contractility. In some populations, both of these scyphidiid peritrichs had the same caliperid species attached around their scopulas (Chapter 8). Both *M. spadiceae* and *M. midae* attach to the arch of the gill, rather than between the gill filaments. In the case of extremely high infestations, however, the scyphidiid peritrichs were also observed attached to the gill filaments.

In a comprehensive survey of 19 limpet species representing three genera along the South African coast, the same scyphidiid peritrich, *M. branchi*, was found attached to the gills of specimens of all limpet species examined (Van As, *et al.* 1998). Similarly, seven of the limpet species were infested with the same *Licnophora* Claparède, 1867 species, namely *L. limpetae* (Van As, Van As & Basson in press). In a similar investigation of the limpet fauna from a Sub-antarctic Island in the Southern Ocean, one scyphidiid peritrich, *M. marioni* was found associated with the endemic limpet, *Nacella delesserti* (Phillips, 1849).

Parasitological surveys of the marine gastropod *Oxystele* revealed the occurrence of one scyphidiid peritrich, i.e. *M. fanthami* on the gill filaments. This topshell genus comprises five species endemic to and distributed along the South African coast. *M. fanthami* was found associated with all five species and occurred along the southern, western and eastern coastline (Basson, *et al.* 1999). All the topshells were also infested with the same mobiline species, *Trichodina oxystelis* Sandon, 1965 (Botha 1994).

The two species in the genus *Haliotis*, that were examined, namely *H. spadicea* and *H. midae*, were infested with two distinct species of scyphidiid peritrichs. This is unique, when compared to the other South African gastropods, and can perhaps be attributed to

the morphological and ecological differences that exist between the hosts. This will be discussed in Chapter 10 and 11. It would be interesting to know whether the other four *Haliotis* species, occurring in small numbers along the South African coast, also have distinct species of scyphidiid peritrichs occurring on their gills. This will be investigated as the Intertidal Symbiont program develops further.

Figure 7.12

Microscope projection drawing of *Mantoscyphidia midae* sp. nov. occurring on the gills of *Haliotis midae* Linnaeus, 1758, collected from the De Hoop Nature Reserve, South Africa.

Scale bar: **10 µm.**

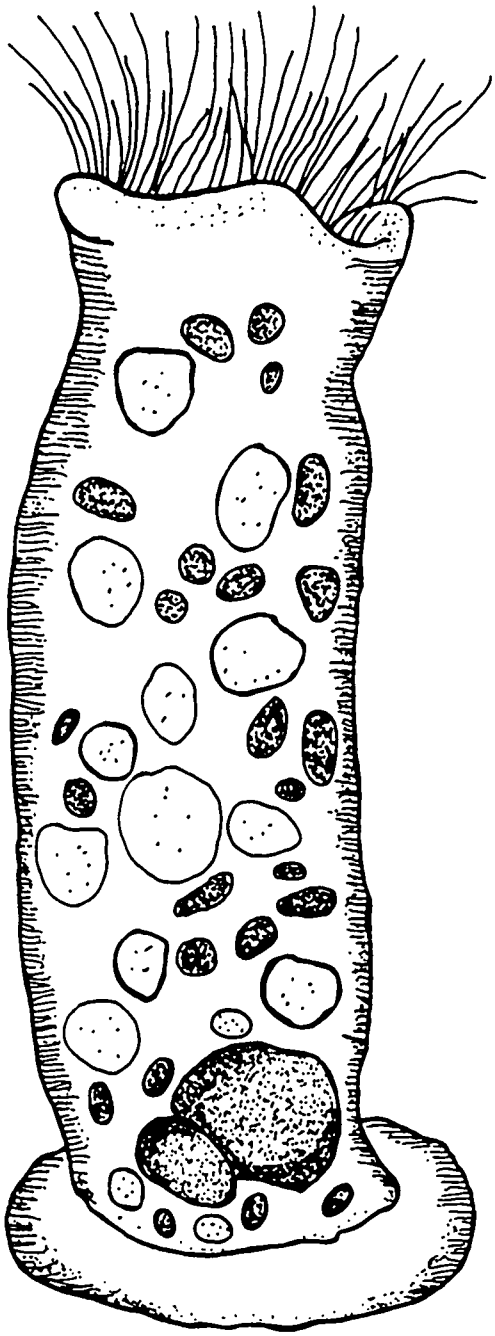


Figure 7.13

Diagram illustrating the infundibulum of *Mantoscyphidia midae* sp. nov. occurring on the gills of *Haliotis midae* Linnaeus, 1758, collected from the De Hoop Nature Reserve, South Africa.

c= cytostome; hk= haplokinety; im= impregnable band; pe= peniculus; pk= polykinety.

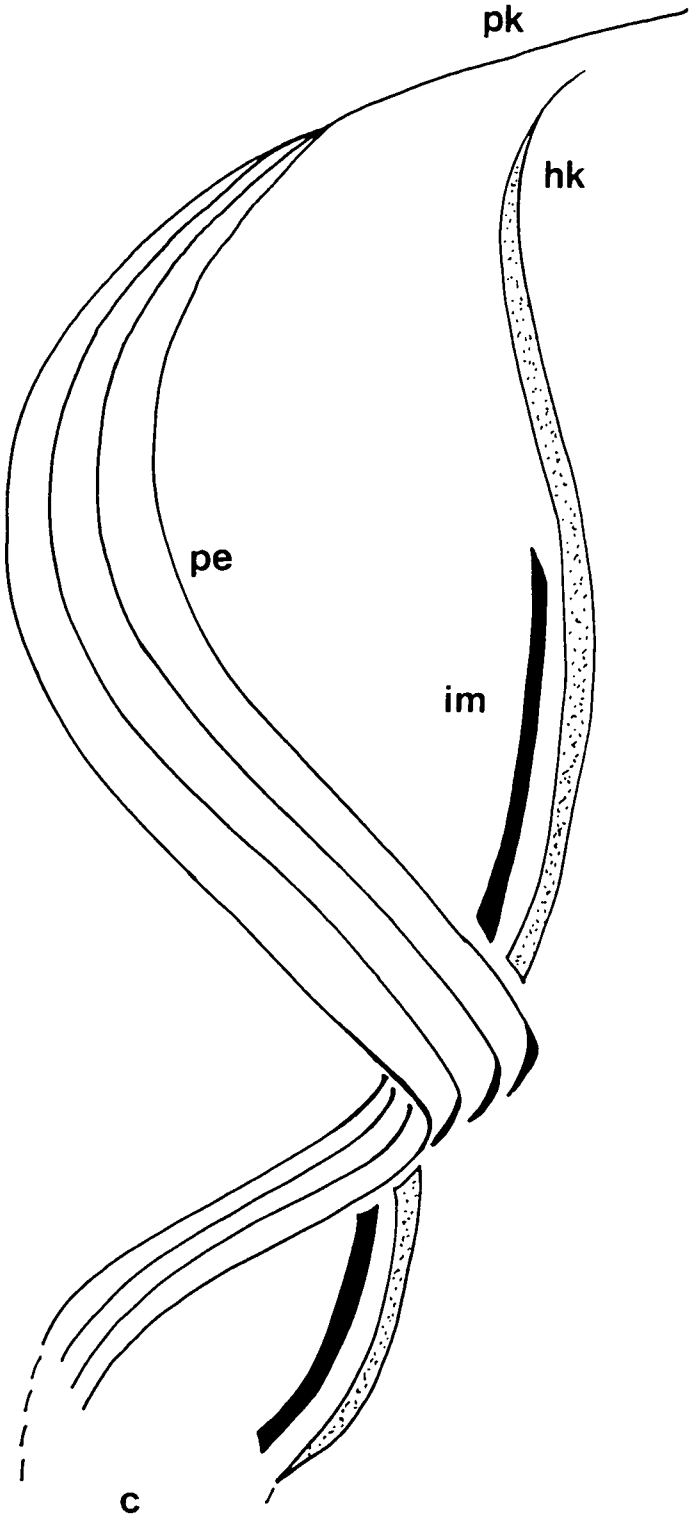

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Figure 7.14

Micrographs of live specimens (A-C) as well as hematoxylin stained specimens (D-G) of *Mantoscyphidia midae* sp. nov. occurring on the gills of *Haliotis midae* Linnaeus, 1758, collected from the De Hoop Nature Reserve, South Africa.

A-C. Bodies expanded. Peristomial discs arched. Symbiotic algae distributed throughout body. ***Scale bars:*** **10 µm.**

D-G. Hematoxylin stained specimens. Macro- and micronuclei visible.

Scale bars: **20 µm.**

ma= macronucleus; mi= micronucleus.

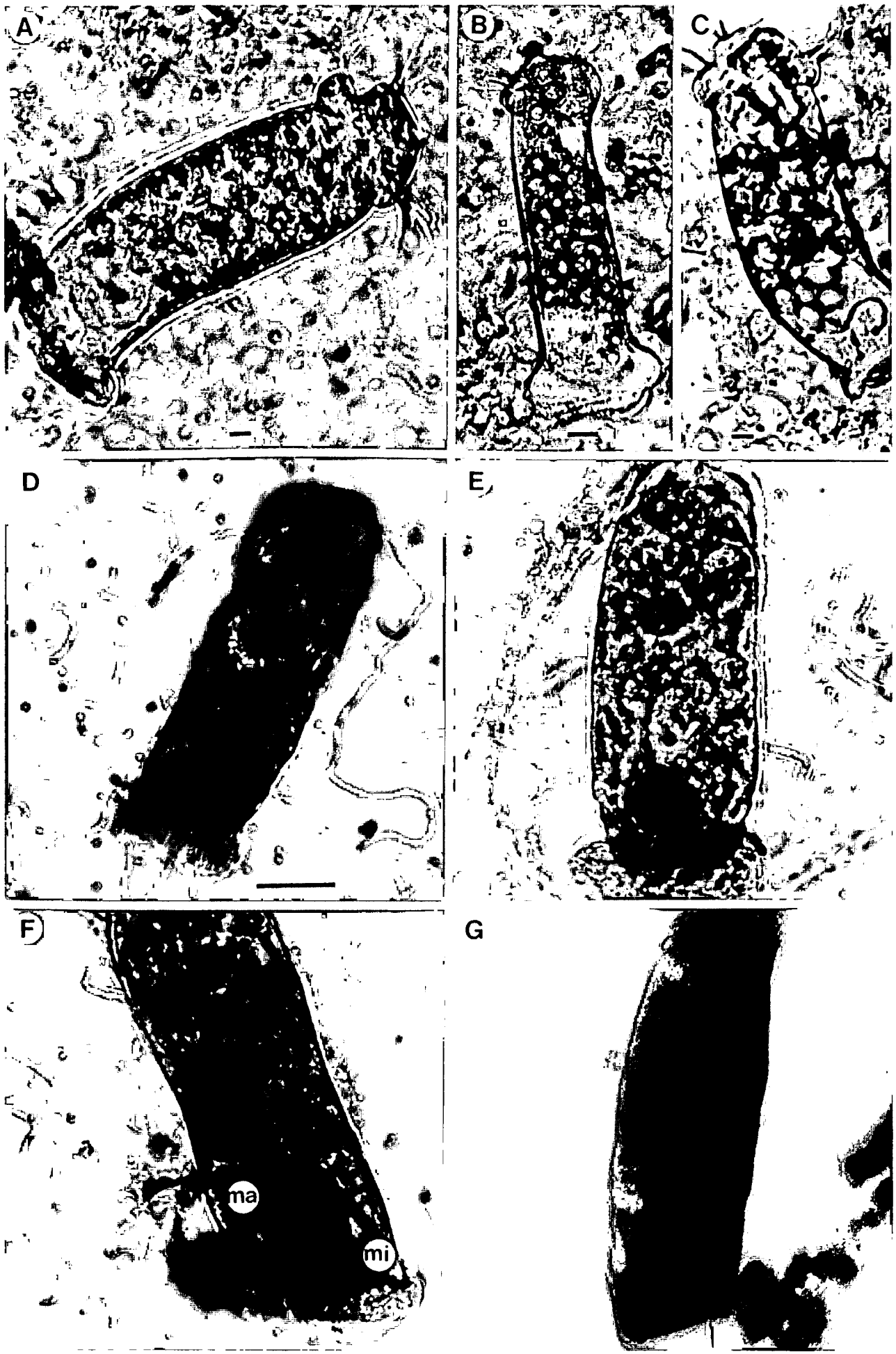
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Figure 7.15

Scanning electron micrographs of specimens of *Mantoscyphidia midae* sp. nov. occurring on the gills of *Haliotis midae* Linnaeus, 1758, collected from the De Hoop Nature Reserve, South Africa.

A. Expanded specimens attached to the gill arch.

B. Adoral ciliary spiral.

C. Buccal cavity. Uneven zig-zag striations indicated by arrow.

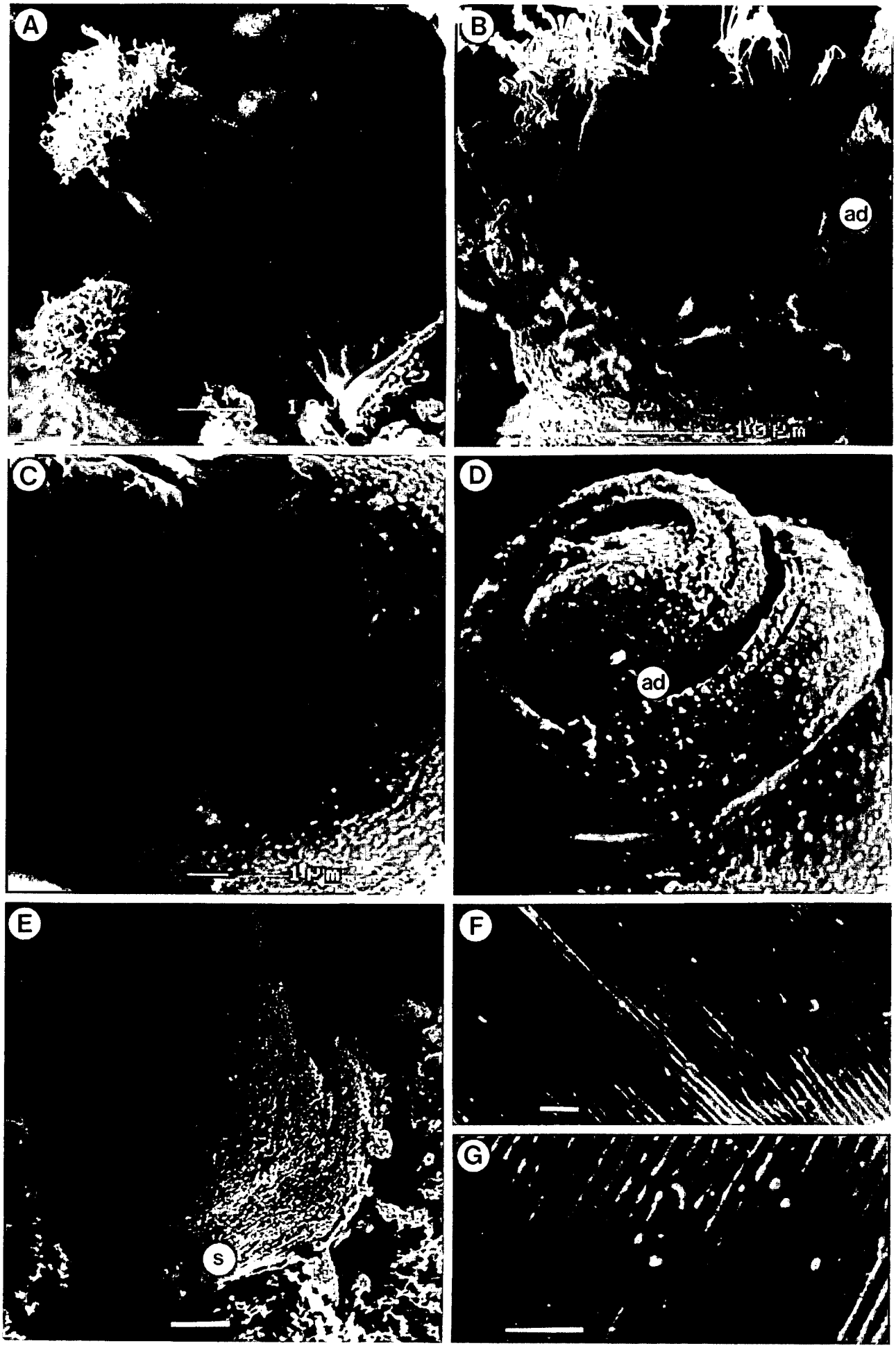
D. Adoral ciliary spiral with kinetsomes where cilia were removed.

E. Broad, flattenend scopula. ***Scale bar*: 10 µm.**

F. Telotroch band and body striations. ***Scale bar*: 0.75 µm.**

G. Body striations. ***Scale bar*: 1 µm.**

ad= adoral ciliary spiral; buc= buccal cavity; pl= peristomial lip; s= scopula.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OBSERVATIONS ON BINARY FISSION AND THE OCCURRENCE OF TELOTROCHS

In the course of examining numerous slides, as well as SEM preparations, different stages of binary fission (Fig. 7.16A-H), telotroch formation (Fig. 7.17A-H) as well as stages of budding or possibly conjugation, were observed. Concerning the latter two processes, budding and or conjugation, no information will be provided, as this requires further in-depth investigation. The aspects of reproduction that will be discussed, concern binary fission and some observations on telotroch formation. The observations presented below are based on information relating to different populations of *M. spadiceae* as well as *M. midae*. The process of binary fission is similar in both species.

During binary fission the cell division is initiated by the micronucleus and is concluded when the two products of fission have acquired approximately equal portions of the macronucleus and cytostome (Finley 1952). The plane of fission is parallel along the oral-aboral axis, thus representing a longitudinal fission process. The scyphidiid peritrich will divide into two parts, resembling small adults that will grow and in time divide again. Before and after the division process the organelles in the body must be duplicated. The nuclei must divide, the number of somatic cilia must duplicate and an oral apparatus must be provided for each of the two cells. The micronucleus divides mitotically, followed by an amitotic division of the macronucleus (Davis 1947).

Scyphidiid peritrichs on the verge of binary fission appear more contracted or plump than those not yet ready for fission (Fig. 7.16A). At the same time both nuclei move from beneath the telotroch band towards the adoral region (Fig. 7.18A&B), with the broad, horseshoe-shaped macronucleus lying adorally or to the side of the micronucleus, which divides first. The scopula divides to form two scopulas (Fig. 7.16B; 7.18D&E), whilst the micronucleus is busy dividing (Fig. 7.18C). During binary fission the primordial peristome region will differentiate in the daughter cell, whilst the parent cell retains its peristome. At this stage the peristome is closed with the cilia drawn inwards (Fig. 7.16C). The plane of fission will become prominent (Fig. 7.16D&E), and the macronucleus separates into two equal parts (Fig. 7.18E).

After separation both nuclei move aborally towards the scopula, to the original place below the telotroch band. Food vacuoles circulate between the parent and daughter cells, whilst the latter will develop a new contractile vacuole. The daughter cell opens its peristome and feeding begins. The ciliary spiral will differentiate and the contractile vacuole will function properly. A combined effort of the scyphidiid peritrich results in the separation of the two cells. The final stage is when only a small string of pellicle is still adorally fused (Fig. 7.16F). The parent separates (Fig. 7.16G&H; 7.18F-I) through a forceful contraction of the body and the daughter cell through rotating along its own longitudinal axis (Finley 1952).

The process of telotroch formation is well documented by Davis (1947); Raabe (1952); Hobbs and Lang (1954); Dobrzañska (1961); Vavra (1961) and Walker, Roberts and Usher (1986) for a number of colonial and solitary ciliophoran species. Telotroch formation is brought about by a gradual deterioration in condition of either the host or the substrate to which the peritrichs attach. The peristome will close and a swelling in the middle region of the body occurs (Fig. 7.17A), where three or four rows of basal kinetosomes will appear (Fig. 7.17B). The emerging ciliary girdle is associated with a tearing of the scopula. Contracting and extending movements of the ciliophoran (Dobrzañska 1961) will draw in the remaining portion of the aboral girdle (Fig. 7.17C). The upward movement of the cytoplasm above the scopula results in an increase in ciliophoran diameter and the macronucleus moves towards the peristome. The fully formed telotroch (Fig. 7.17D&E) moves in much the same way as the mobiline peritrichs. Gradually the scopula increases in size, with the central cytoplasm flowing into the scopula (Fig. 7.17F). The scyphidiid peritrich elongates (Fig. 7.17G&H) and the macronucleus returns to its original place. Finally, the ciliary girdle activity is lost during this process.

As in the case of *M. branchi* and *M. marioni*, numerous telotrochs from *M. spadiceae* and *M. midae* were observed, but no correlation could be made to the binary fission process. It is unclear whether binary fission has to occur before a telotroch can develop, or whether binary fission follows telotroch formation. Wet smears made of the gills of

Haliotis species that had been out of water for approximately ten hours, revealed numerous stages of dividing scyphidiid peritrichs and telotroch formation. This serves as evidence that these scyphidiid peritrichs start to divide when stressed. The exact process of the telotroch formation is unclear, but the following was observed. The locomotory cilia developed on the elevated telotroch band. The scopula decreased in size. In the free moving telotroch, the aboral cilia were very long (10 μm). When the telotroch finds a new suitable substrate it will settle and the scopula will increase in size again.

Figure 7.16

Scanning electron micrographs of specimens of *Mantoscyphidia spadiceae* sp. nov. and *M. midae* sp. nov. occurring on the gills of *Haliotis spadicea* Donovan, 1808 and *H. midae* Linnaeus, 1758 respectively, in different stages of binary fission, collected from the De Hoop Nature Reserve, South Africa.

A. Beginning of binary fission.

B. Bodies still fused, scopula has divided in two.

C-E. Longitudinal division progressing.

F&G. Binary fission almost complete, daughter cells only attached adorally.

H. Binary fission complete. Two daughter cells formed.

***Scale bar*: 10 µm.**

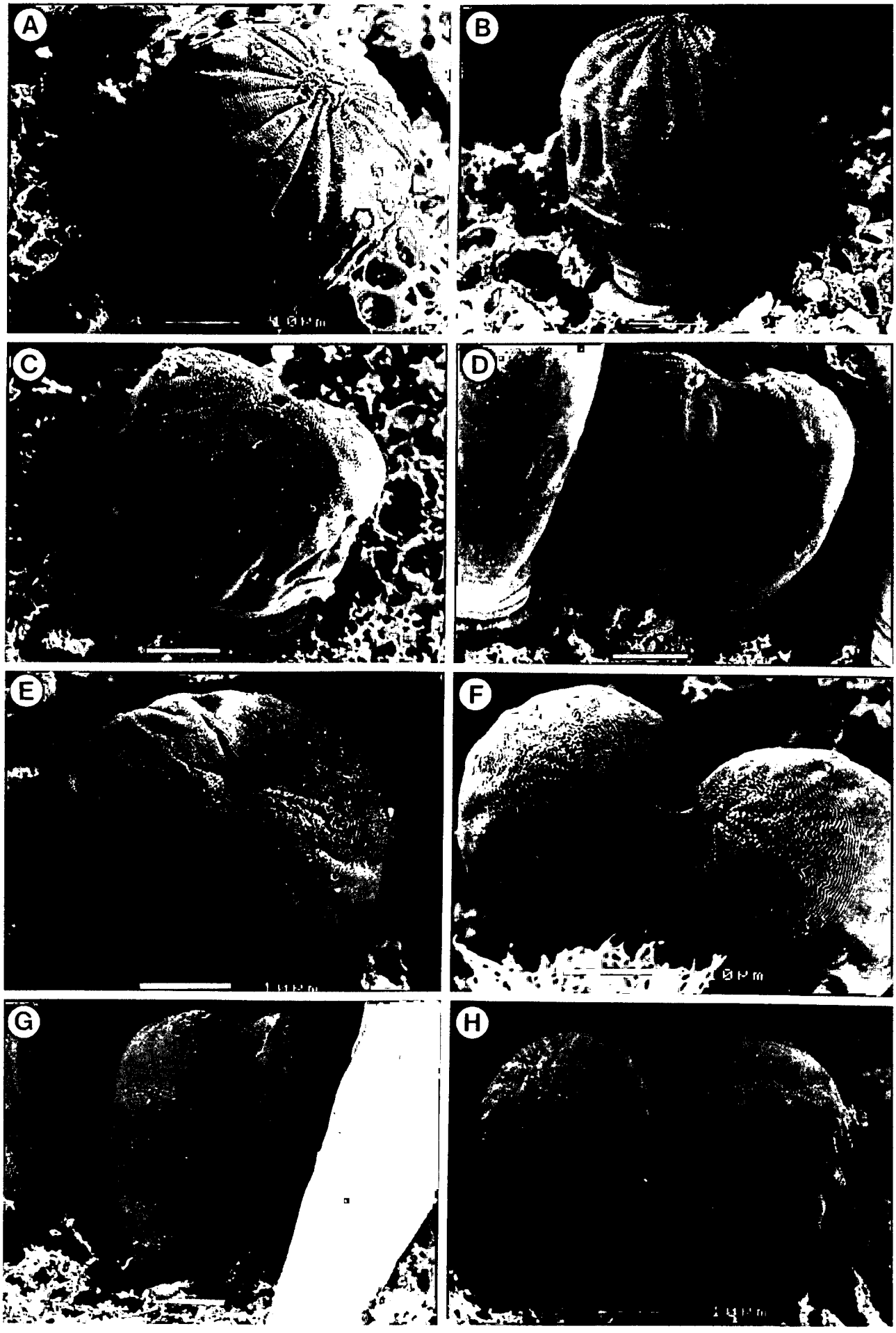
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Figure 7.17

Scanning electron micrographs of different telotroch stages of *Mantoscyphidia spadiceae* sp. nov. and *M. midae* sp. nov. occurring on the gills of *Haliotis spadicea* Donovan, 1808 and *H. midae* Linnaeus, 1758 respectively, collected from the De Hoop Nature Reserve, South Africa.

A&B. Body swells and basal kinetosomes appear.

C. Ciliary girdle emerges (arrow indicates closed peristome).

D&E. Fully developed telotroch.

F-H. Scopula enlarges, scyphidiid peritrich elongates.

***Scale bar*: 10 µm.**

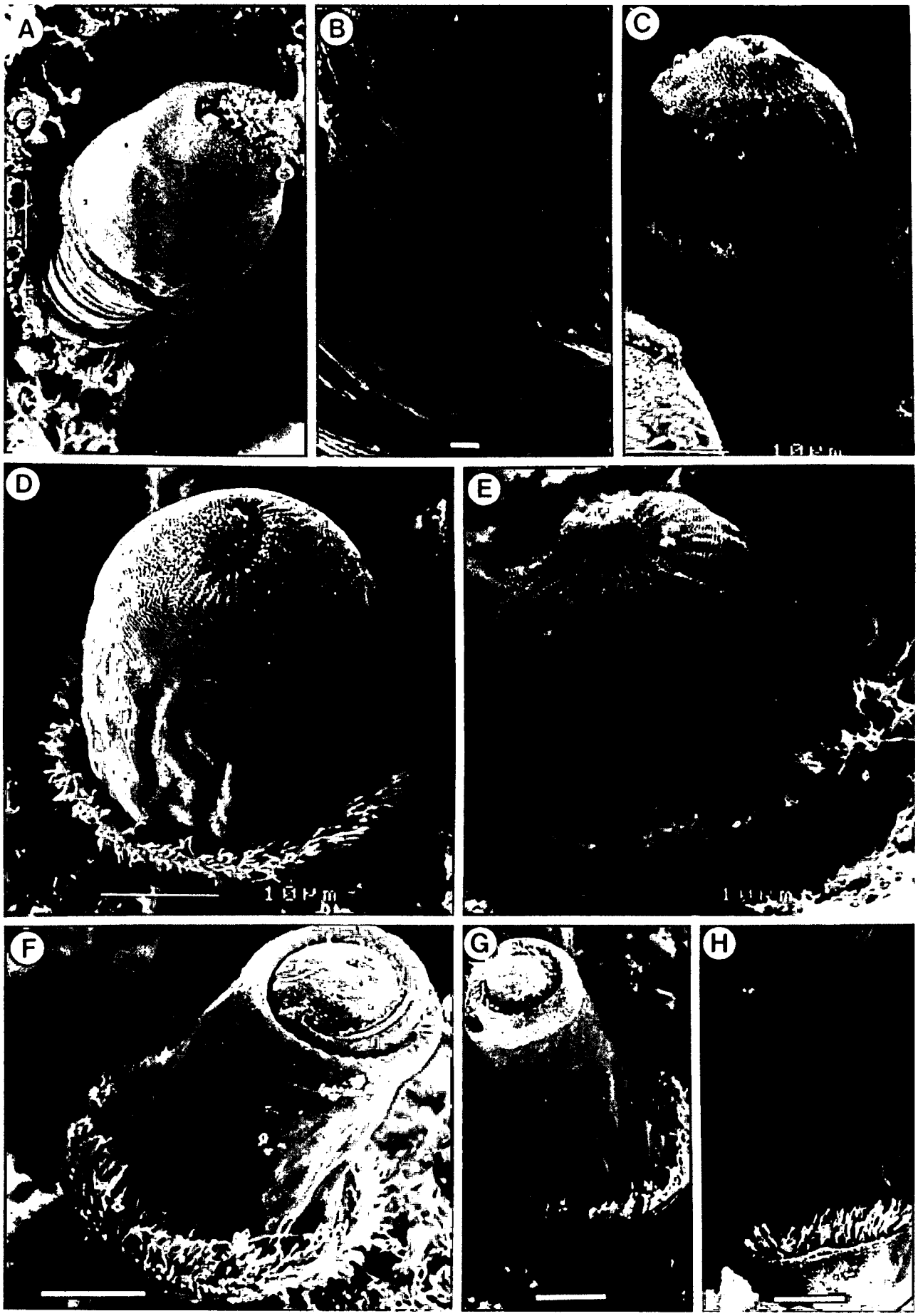
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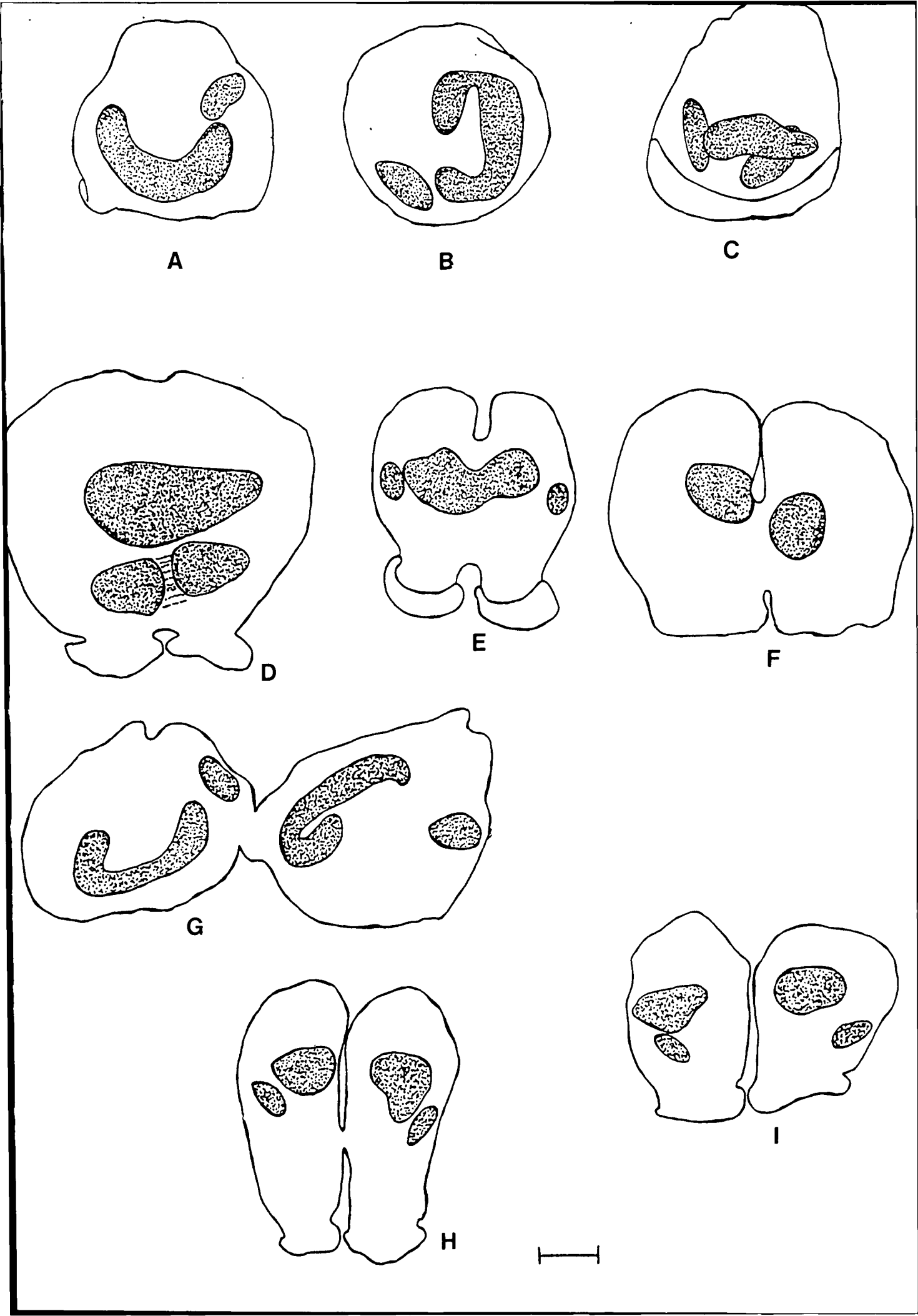
Figure 7.18

Microscope projection drawings of specimens (A-I) of *Mantoscyphidia spadiceae* sp. nov. and *M. midae* sp. nov. occurring on the gills of *Haliotis spadicea* Donovan, 1808 and *H. midae* Linnaeus, 1758 respectively, collected from the De Hoop Nature Reserve, South Africa.

***Scale bar*: 10 µm.**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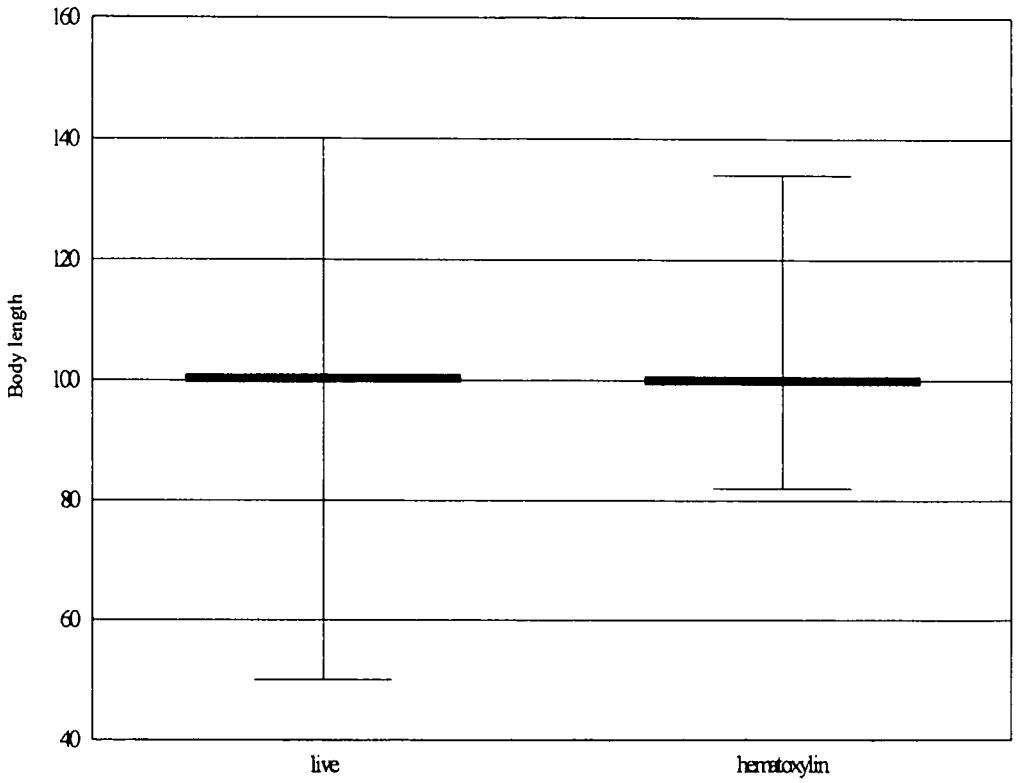


Figure 7.19A Comparison of the variation in expanded body length of *Mantoscyphidia spadiceae* sp. nov. of live observations and hematoxylin stained specimens, from the De Hoop Nature Reserve, South Africa.

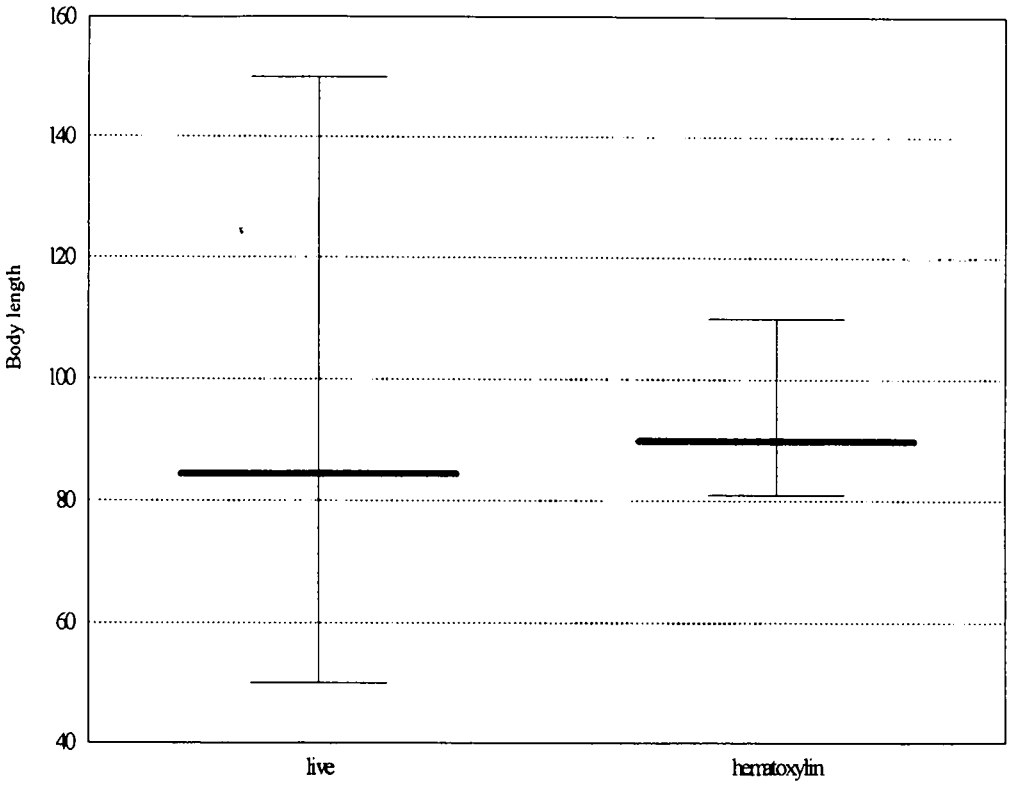


Figure 7.19B Comparison of the variation in expanded body length of *Mantoscyphidia midae* sp. nov. of live observations and hematoxylin stained specimens, from the De Hoop Nature Reserve, South Africa.

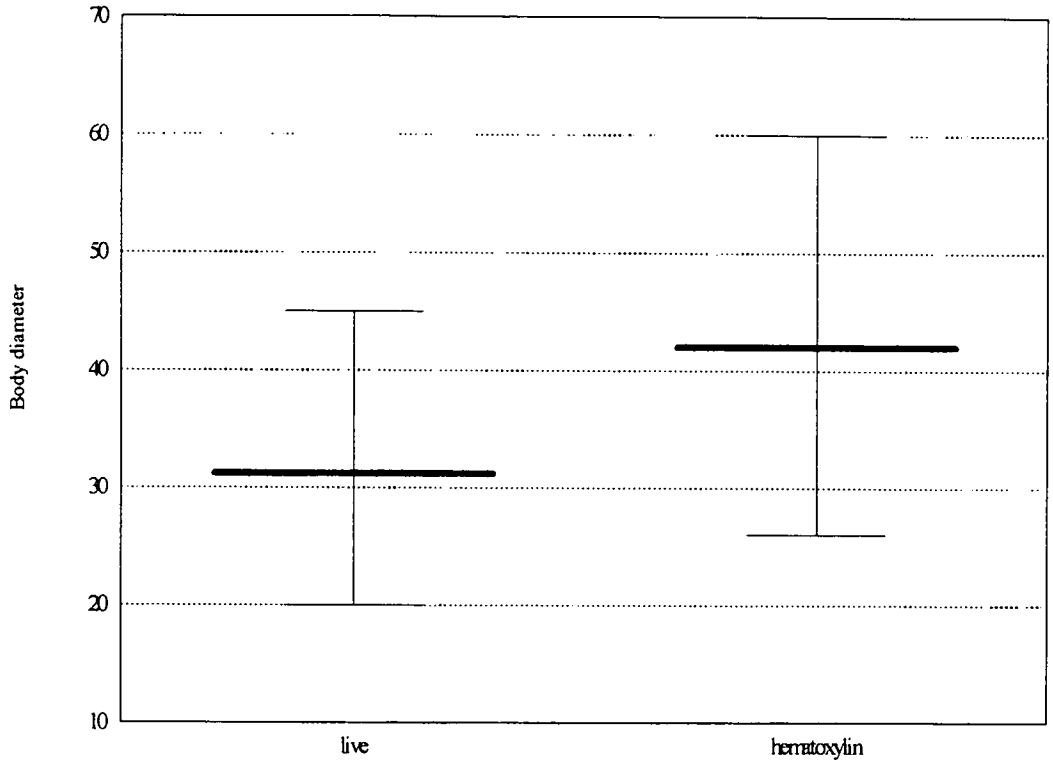


Figure 7.20A Comparison of the variation in body diameter of *Mantoscyphidia spadiceae* sp. nov. of live observations and hematoxylin stained specimens, from the De Hoop Nature Reserve, South Africa.

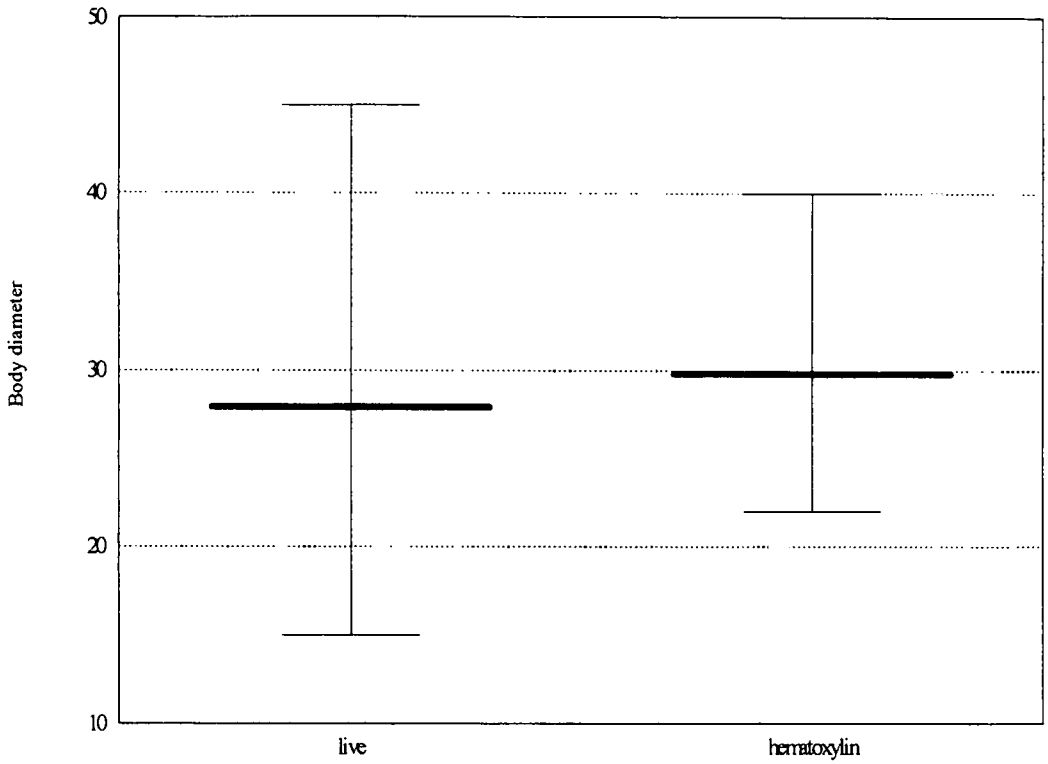
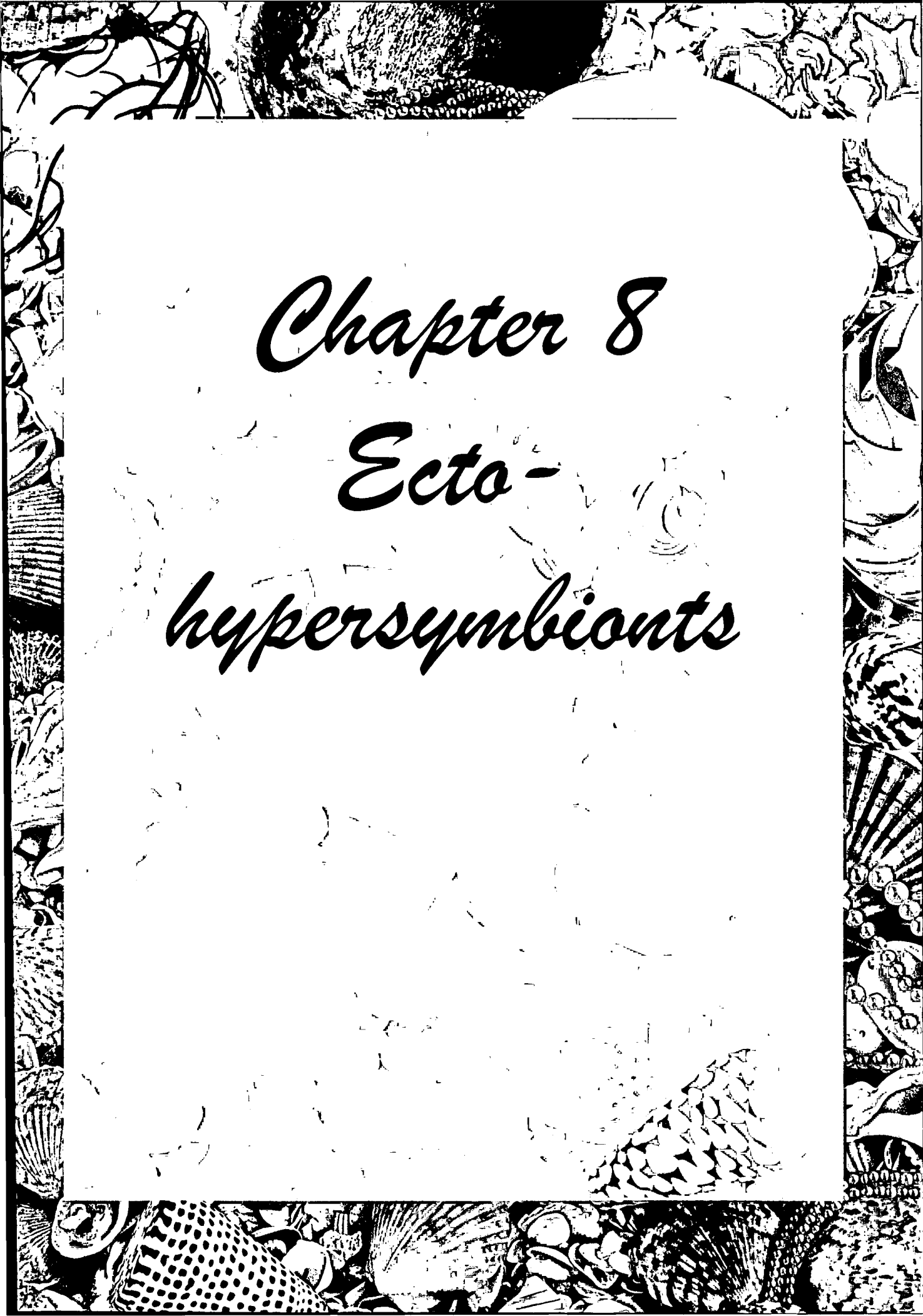


Figure 7.20B Comparison of the variation in body diameter of *Mantoscyphidia midae* sp. nov. of live observations and hematoxylin stained specimens, from the De Hoop Nature Reserve, South Africa.



Chapter 8
Ecto-hypersymbionts

FAMILY ELLOBIOPHRYIIDAE (CHATTON & LWOFF, 1929)

The family Ellobiophryiidae is unique among ectosymbiotic peritrichs in attaching to the host with a scopula that has been adapted to form a ring-like cinctum or caudal process. Representatives of the Ellobiophryiidae (Fig. 8.1) are characterised by solitary, elongated bodies which taper aborally. Some species taper more than others. The cinctal limbs may be joined and may have an internal rod-like structure, also known as an endoskeletal rod. Reproduction is by means of budding and binary fission. General details of the rest of the morphology is the same as for other peritrichs. Ellobiophryids are filter feeders that obtain their food from the surrounding water and do not rely on the host for nourishment.

The family comprises two genera, i.e. *Ellobiophrya* and *Caliperia*. All the species in the genus *Ellobiophrya* are found associated with non-ciliophoran hosts, occurring in the marine environment. Chatton and Lwoff (1923) described *E. donacis* Chatton & Lwoff, 1923 (Fig. 8.2A) from the gill filaments of the bivalve *Donax* Linnaeus, 1758. In 1982, Clamp described the second species, *E. conviva* Clamp, 1982 (Fig. 8.2C), from the tentacles of a bryozoan lophophore. The third species (Fig. 8.2B) from a marine fish, *E. oblida* (Naidenova & Zaika, 1969), was originally described by Naidenova and Zaika (1969) as a species in the genus *Clausophrya* Naidenova & Zaika, 1969, but was later placed within the genus *Ellobiophrya* by Clamp (1982).

Caliperia is separated from *Ellobiophrya* by having a noncontractile skeletal rod in the cinctum and lacking a bouton. *Caliperia longipes* Laird, 1953 and *C. brevipes* Laird, 1953 (Fig. 8.2D&E), redescribed by Clamp and Bradbury (1997) as *Ellobiophrya brevipes*, both occur on the gill filaments of fish. Laird (1959) described *E. brevipes* as having a noncontractile skeletal rod within the arms of its cinctum and by not having the cinctal arms bonded to one another at their tips. Clamp and Bradbury's (1997)

observations revealed that their cinctal arms are linked by a bouton and that the cytoskeletal structure within them has the fine structure of a myoneme. The second species in the genus *Caliperia*, i.e. *C. maliculiformis* Van As, 1997, is found associated with *Mantoscyphidia branchi*, occurring on the gill filaments of *Patella* species occurring around the South African coast (Fig. 8.2F) (Van As 1997). This is the first record of an ellobiophryid found associated with a ciliophoran host.

Figure 8.1

Diagram of a typical ellobiophryid illustrating general morphology.

ad= adoral ciliary spiral; c= cinctum, cj= cinctal junction; cl= cinctal limb; i= inclusions; ir= internal rod like stucture; ma= macronucleus; mi= micronucleus; pl= peristomial lip; ps= pellicle striations.

***Scale-bar*: 10µm.**

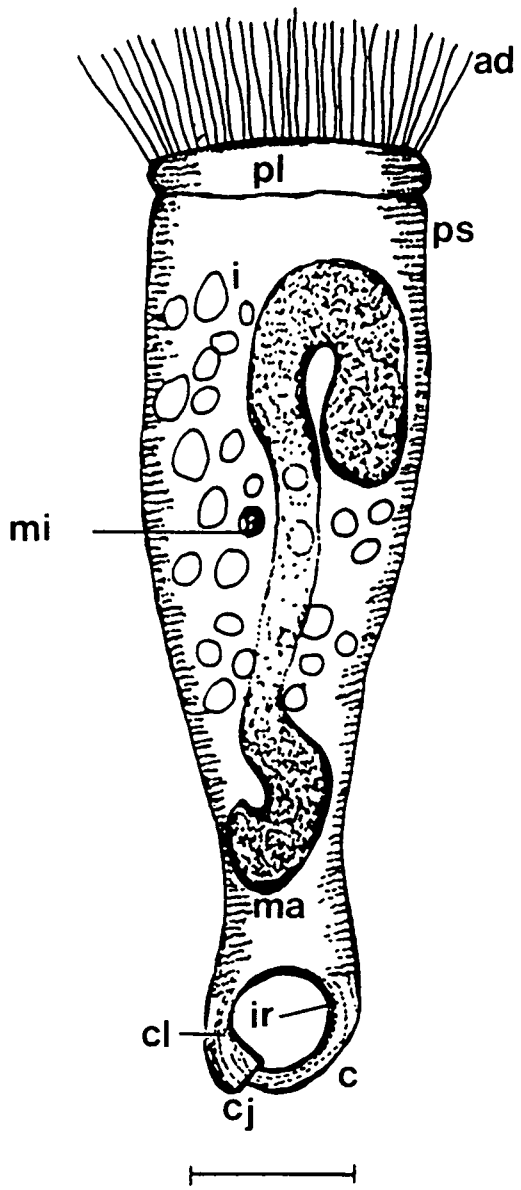

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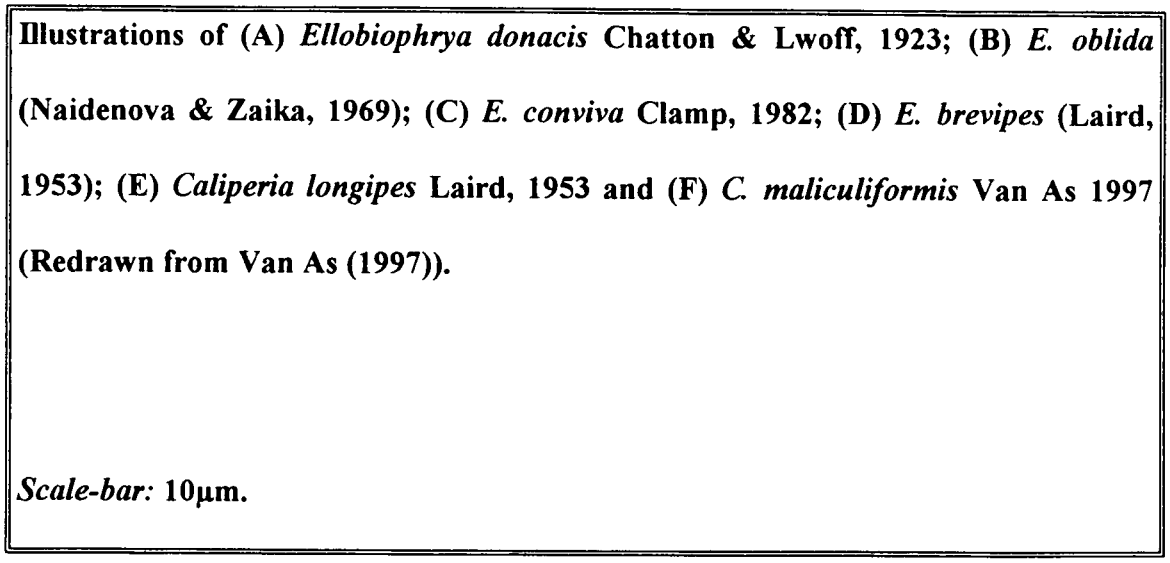
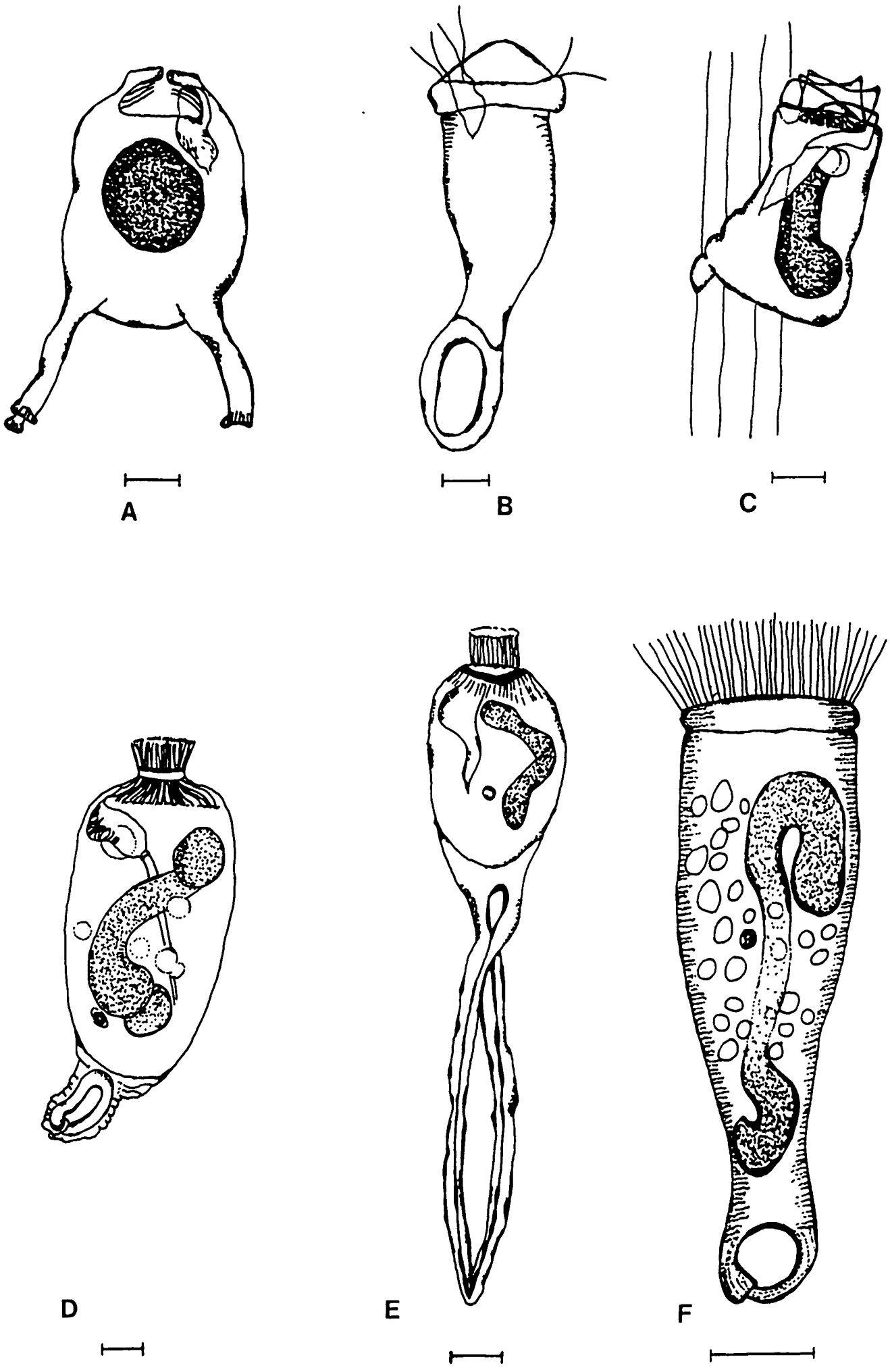
Figure 8.2



Illustrations of (A) *Ellobiophrya donacis* Chatton & Lwoff, 1923; (B) *E. oblida* (Naidenova & Zaika, 1969); (C) *E. conviva* Clamp, 1982; (D) *E. brevipes* (Laird, 1953); (E) *Caliperia longipes* Laird, 1953 and (F) *C. maliculiformis* Van As 1997 (Redrawn from Van As (1997)).

Scale-bar: 10μm.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CALIPERIA PERLEMOENAE SP. NOV. FROM SOUTH AFRICA

The ellobiophryid found in this study, belongs to the genus *Caliperia* Laird, 1953 based on the morphology of its cinctum. The classification is summarised in Table 8.1. Populations of *Mantoscyphidia spadiceae* and *M. midae*, found on *Haliotis spadicea* and *Haliotis midae* were found to be infested with the same *Caliperia* species. It differs from the two known species in the genus based on the morphological features of the macronucleus and cinctum.

Table 8.1 Classification of the genus *Caliperia* Laird, 1953.

Phylum	Ciliophora Doflein, 1901
Subphylum	Epiplasmata De Puytorac, *et al.*, 1993
Superclass	Membranellophora Jankowski, 1975
Class	Oligohymenophorea De Puytorac, *et al.*, 1974
Subclass	Peritrichia Stein, 1859
Order	Sesselida Kahl, 1933
Family	Ellobiophryiidae (Chatton & Lwoff, 1929)
Genus	*Caliperia* Laird, 1953

***Caliperia perlemoenae* sp. nov.** (Fig. 8.3-8.7)

Type host: *Mantoscyphidia spadiceae* found on the gills of *Haliotis spadicea*.

Type locality: De Hoop Nature Reserve (34°28'S;20°30'E), south coast.

Type material: Holotype slide, 98/04/11-04 (NMBP 241) and paratype slides, 98/04/04-05 (NMBP 242) and 97/04/05-04c (NMBP 243) in the collection of the National Museum, Bloemfontein, South Africa.

Position on host: Attached around scopula of host.

Etymology: Named after the South African name for the primary host (perlemoen) on which the scyphidiid peritrichs were found.

Body measurements and number of pellicle striations are presented in Table 8.2.

Table 8.2 Body measurements of live observations (µm) as well as hematoxylin stained specimens as well as number of striations of specimens of *Caliperia perlemoenae* sp. nov. found attached to *Mantoscyphidia spadiceae* sp. nov. and *M. midae* sp. nov. occurring on the gill filaments of *Haliotis spadicea* Donovan, 1808 and *H. midae* Linnaeus, 1758 from the De Hoop Nature Reserve, South Africa.

	Live observations	Hematoxylin stained specimens
Body length	5 - 125 (78.9 ± 15.1, 41)	43 - 98 (70.1 ± 15.3, 52)
Body diameter	15 - 30 (20.5 ± 3.7, 41)	16 - 37 (23.9 ± 4.7, 52)
Outer cinctum diameter	10 - 20 (15, 6)	7 - 17 (12.7, 3)
Inner cinctum diameter	5 - 10 (7.2, 6)	4 - 7 (5.3, 3)
Limb diameter	5 (5, 6)	1 - 5 (2.5 ± 0.9, 33)

Number of striations

Peristome	8 - 22 (14.2 ± 4.6, 10)
Peristome to Cinctum	54 - 118 (86.7 ± 17.3, 10)
Total number of striations	62 - 140 (100.9 ± 20.5, 10)

Conical, elongated body, tapering aborally towards scopula (Fig. 8.3; 8.5A&B; 8.6A&B). Body striated, 100.9 striations on average, with striations about 0.5 µm apart. Peristome with broad striated peristomial lip (Fig. 8.6C), zig-zag striations present on peristome in contracted specimens. Striations on peristome not always visible. telotroch band narrow, approximately a quarter of body length from cinctum, though not clearly visible. Scopula transformed into attachment organelle defined as a caudal process or cinctum. Cinctum flattened (Fig. 8.6F&G) with two cytoplasmic cinctal limbs of equal thickness, forming closed circle (Fig. 8.3; 8.5A). One limb tapers, fitting into cinctal junction. Other limb shorter, terminating somewhat broader at junction (Fig. 8.6E). Cinctum contains a non-contractile skeletal rod and lacks a bouton.

The oral infraciliature of *C. perlemoenae* is divisible into a peristomial part and an infundibular part as in other sessiline peritrichs. Due to the fact that very few specimens were fully expanded, the peristomial part with haplo- and polykineties can not be described. The infundibulum is situated almost horizontally beneath the peristome. It appears as if the infundibulum is shortened and restricted to the upper part of the caliperid's body due to the presence of inclusions (Fig. 8.4). Numerous striations occur, which could possibly have a supportive function. No impregnable structure could be seen associated with the haplo- or polykineties. *Caliperia perlemoenae* does not have a sphincter-like siderophilous rim.

Whole body filled with inclusions, probably symbiotic algae (Fig. 8.5B). Internal organelles and infundibulum not visible due to presence of inclusions. Micronucleus fusiform, but not always visible. Macronucleus coiled and sausage-shaped, extending throughout body. Inclusions obscure position and shape of macronucleus and micronucleus. Prominent sections of nucleus in adoral and aboral sides visible (Fig. 8.5C&D).

Remarks

Caliperia perlemoenae is the second species of the family Ellobiophryiidae found associated with another ciliophoran. *Caliperia longipes* was described from the gill filaments of marine fish and *C. maliculiformis* is associated with *M. branchi*. Both of these species have elongated macronuclei extending throughout the body. As in the case of *C. maliculiformis*, the present species' macronucleus appears to have prominent ad- and aboral areas (Fig. 8.3; 8.5C&D). The area in between is not clearly defined due to the presence of inclusions.

Great morphological differences exist between ellobiophryids, which are related to structural features of the host, the characteristics of the food particles that must be captured and the characteristics of the current that carries the food particles. Ellobiophryids must cope with these features in order to maintain physical attachment. There are significant differences in cinctum morphology. *Caliperia longipes* has a long, flattened cinctum, with limbs of equal size and length. As in the case of *C. maliculiformis*, the limbs of *C. perlemoenae* is locked in a cinctal junction, but it appears as if the inner limb may be able to withdraw from the broader limb at the junction. The two limbs differ in length as well as size at the junction (Fig. 8.6E).

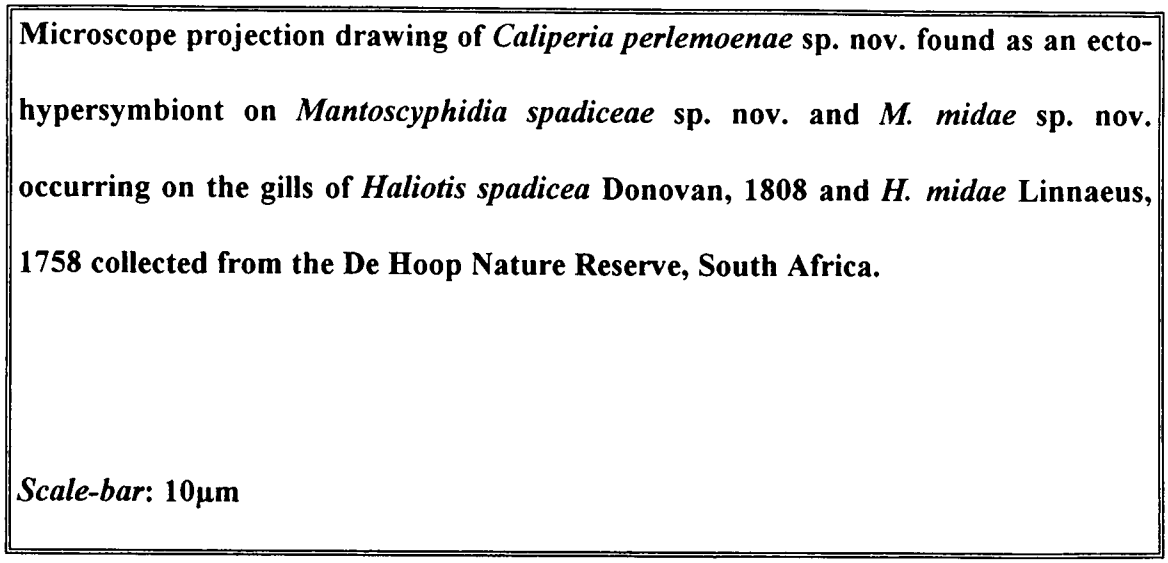
Only a few specimens were detached so that measurements of the cinctum could be made. Mean body length varies from 70.1 µm in hematoxylin stained specimens to 78.9 µm in live specimens (Fig. 8.8), with a mean body diameter of up to 23.9 µm (Table 8.2). Hematoxylin stained specimens have a greater average body diameter than live

specimens (Fig. 8.9). *Caliperia perlemoenae* has a greater mean body length than *C. maliculiformis*, which varies between 56 and 62 μm, but there isn't a significant difference in inner cinctum diameter, outer cinctum diameter and limb diameter measurements. It was observed, however, that the body of *C. perlemoenae* appears to become narrower towards the cinctum, as compared to *C. maliculiformis*. The majority that were attached to scyphidiid peritrichs had their oral cilia only partially protruding, a couple of specimens, however, were fully extended with oral cilia protruding (Fig. 8.6A&C). In *C. perlemoenae*, the peristomial lip is more everted and forms a broad rim in fully extended specimens.

Up to four caliperids were observed attached to one scyphidiid peritrich. Some caliperids were attached to the peristomial region (Fig. 8.5F&G) or even in the region of the telotroch band of the scyphidiid peritrich's bodies, gripping the individual where its nuclear apparatus is situated. In Fig. 8.5E two caliperids are attached between the macro- and micronuclei of the scyphidiid peritrich.

Reproduction is by means of budding and binary fission. Caliperids in various stages of division (Fig. 8.7 A-D), as well as scyphidiid peritrichs in the process of division with attached caliperids, were observed. The one region of the parent that does not participate in binary fission, therefore remaining unaffected by cytokinesis, is the cinctum. The entire cinctum is inherited by one daughter, which stays attached in the parents' place. The other daughter cells become telotrochs (Fig. 8.6D) that do not acquire cinctums until, presumably, they locate other scyphidiid peritrichs and develop further. As in other ellobiophryids, the telotroch is attached during development to the daughter retaining the cinctum by a short, straight, rigid stalk that passes between the scopulas of the two individuals (Clamp & Bradbury 1997). During transformation of the telotroch of *C. perlemoenae* to the trophont, it remains a mystery how the cinctal limbs appear and then grasp around the scopula of the scyphidiid peritrich.

Figure 8.3



Microscope projection drawing of *Caliperia perlemoenae* sp. nov. found as an ecto-hypersymbiont on *Mantoscyphidia spadiceae* sp. nov. and *M. midae* sp. nov. occurring on the gills of *Haliotis spadicea* Donovan, 1808 and *H. midae* Linnaeus, 1758 collected from the De Hoop Nature Reserve, South Africa.

***Scale-bar*: 10µm**

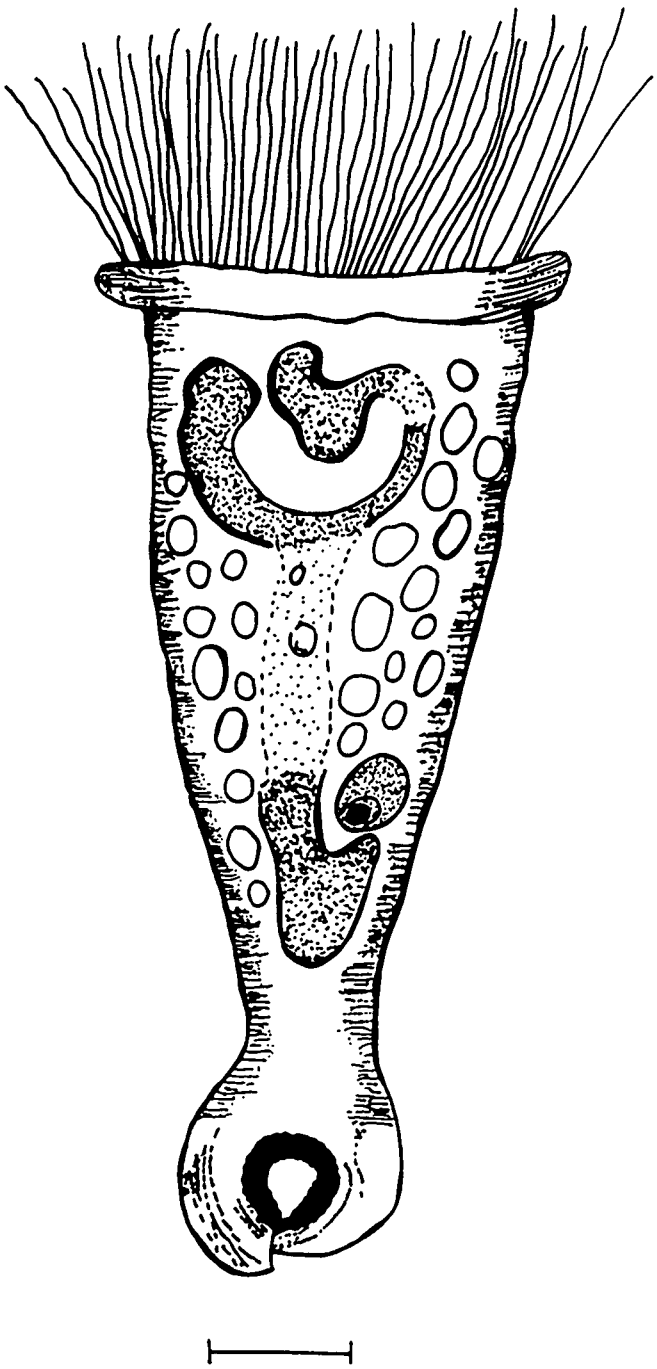


Figure 8.4

Diagram illustrating a part of the infundibulum of *Caliperia perlemoenae* sp.nov. found as an ecto-hypersymbiont on *Mantoscyphidia spadiceae* sp. nov. and *M. midae* sp. nov. occurring on the gills of *Haliotis spadicea* Donovan, 1808 and *H. midae* Linnaeus, 1758 collected from the De Hoop Nature Reserve, South Africa.

ad= adoral ciliary spiral; buc= buccal cavity; i= inclusions; pl= peristomial lip; s= striations.

***Scale-bar*: 10µm.**

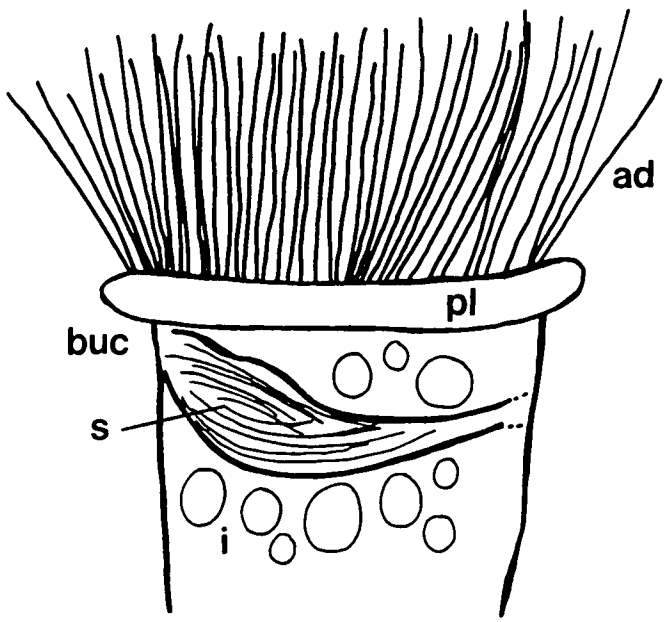

ad
pl
buc
s
i

Figure 8. 5

Light micrographs of live specimens (A&B) and hematoxylin stained specimens (C-G) of *Caliperia perlemoenae* sp.nov. found as an ecto-hypersymbiont on *Mantoscyphidia spadiceae* sp. nov. and *M. midae* sp. nov. occurring on the gills of *Haliotis spadicea* Donovan, 1808 and *H. midae* Linnaeus, 1758 collected from the De Hoop Nature Reserve, South Africa.

A. Detached specimen.

B. Attached specimen (arrow indicates symbiotic algae).

C. Two caliperids attached to a scyphidiid peritrich.

D. Prominent sections of the macronucleus visible in the adoral and aboral areas of *C. perlemoenae*.

E. Two caliperids attached between the macro- and micronucleus of a single *M. spadiceae*.

F. A caliperid attached to the peristomial region of a single *M. spadiceae*.

G. Two caliperids attached to a single mantoscyphidian, one attached to the peristomial region and the other between the macro- and micronucleus.

sa= symbiotic algae.

***Scale-bar*: 10μm.**

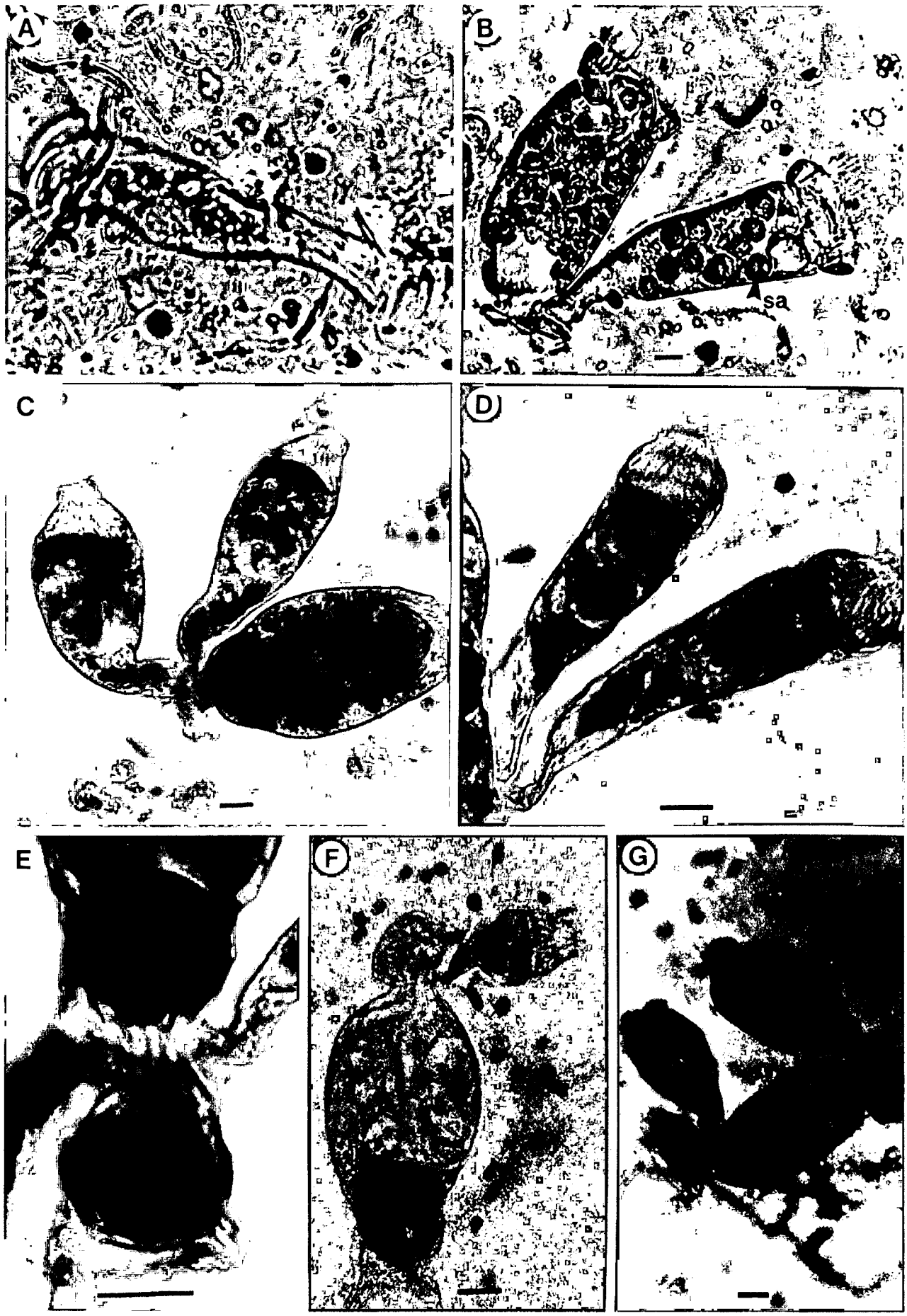

A
B
sa
C
D
E
F
G

Figure 8.6

Scanning electron micrographs of *Caliperia perlemoenae* sp.nov. found as an ecto-hypersymbiont on *Mantoscyphidia spadiceae* sp. nov. and *M. midae* sp. nov. occurring on the gills of *Haliotis spadicea* Donovan, 1808 and *H. midae* Linnaeus, 1758 collected from the De Hoop Nature Reserve, South Africa.

A. *C. perlemoenae* with protruding adoral cilia attached to *M. spadiceae*.

B. Contracted *C. perlemoenae* attached to *M. spadiceae*.

C. Peristome and adoral ciliary spiral.

D. *C. perlemoenae* and a telotroch attached to *M. spadiceae*.

E. Two caliperids attached around the scopula of a single *M. spadiceae* with the cinctum junction visible.

F & G. Flattened cinctum. ***Scale-bar*: 10µm in F.**

ad= adoral ciliary spiral; c= cinctum; pl= peristomial lip; t= telotroch.

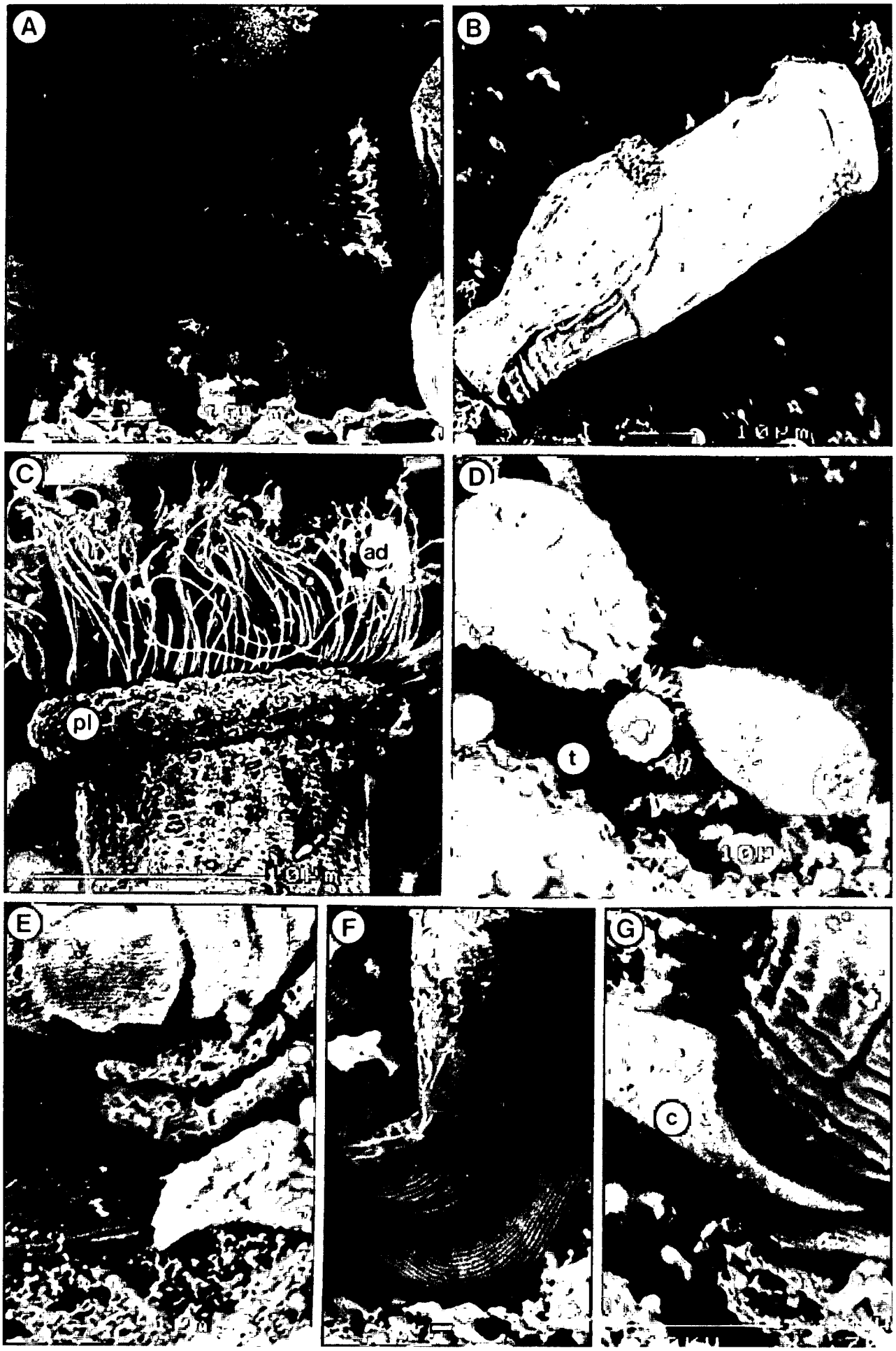
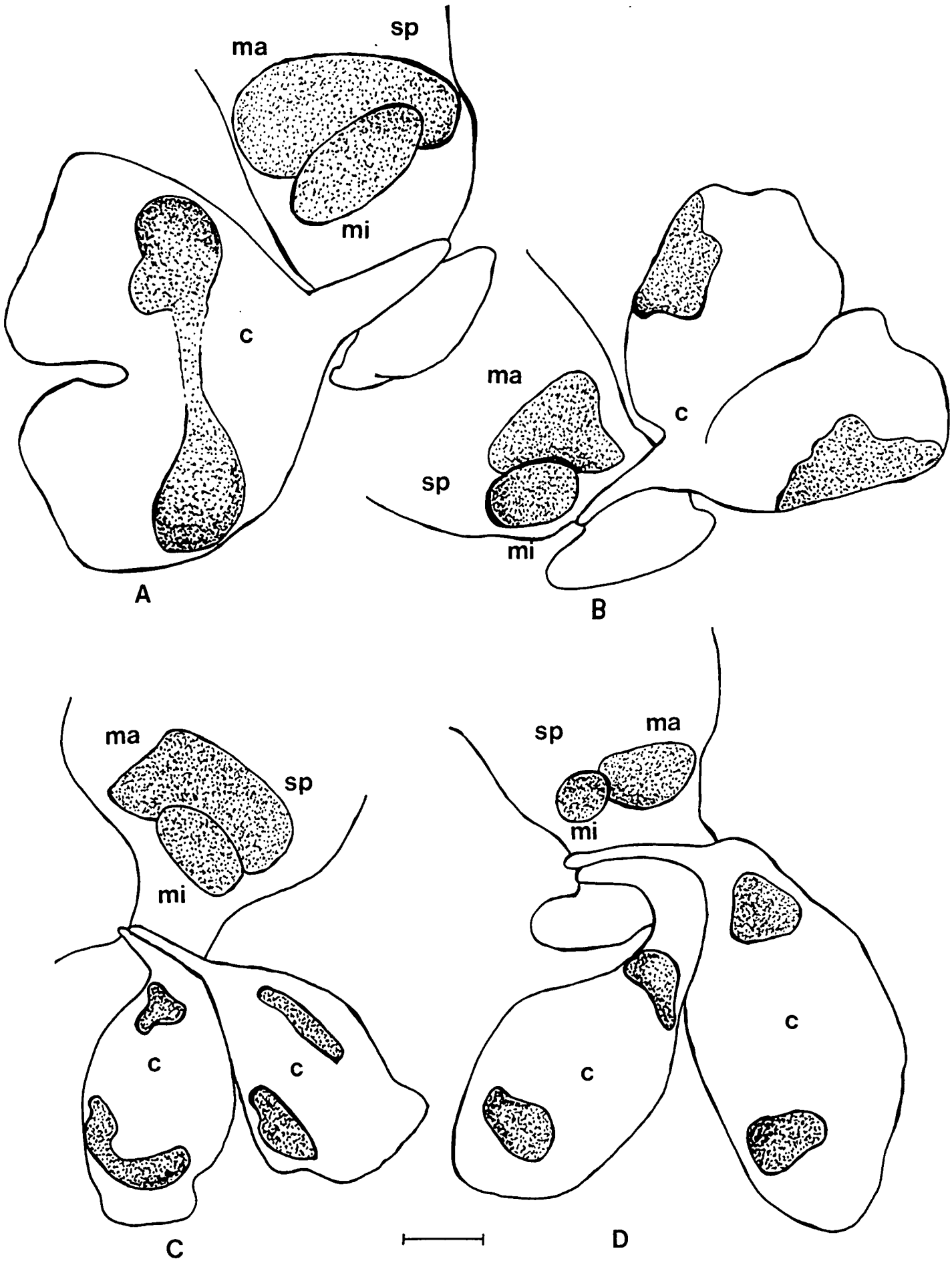

A
B
C
ad
pl
D
t
E
F
G
c

Figure 8.7

A-D: Illustrations of various stages of binary fission observed in *Caliperia perlemoenae* sp. nov. specimens, found as ecto-hypersymbionts on *Mantoscyphidia spadiceae* sp. nov. and *M. midae* sp. nov. occurring on the gills of *Haliotis spadicea* Donovan, 1808 and *H. midae* Linnaeus, 1758 collected from the De Hoop Nature Reserve, South Africa.

c= caliperid; sp= scyphidiid peritrich; ma=macronucleus; mi=micronucleus.

Scale-bar: **10µm.**


sp
ma
mi
c
A
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sp
mi
c
B
ma
sp
mi
c
c
C
sp
ma
mi
c
c
D

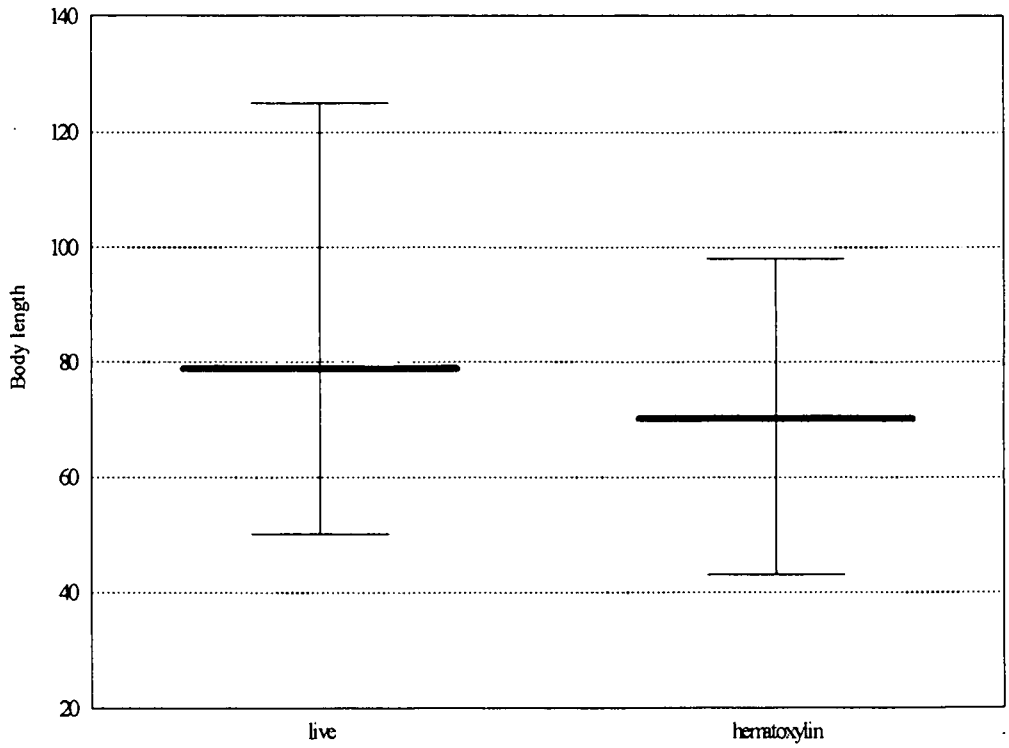


Figure 8.8 .Comparison of the variation in expanded body length of *Caliperia perlemoenae* sp. nov. of live observations and hematoxylin stained specimens, an ecto-hypersymbiont on *Mantoscyphidia spadiceae* sp. nov. and *M. midae* sp. nov., from the De Hoop Nature Reserve, South Africa.

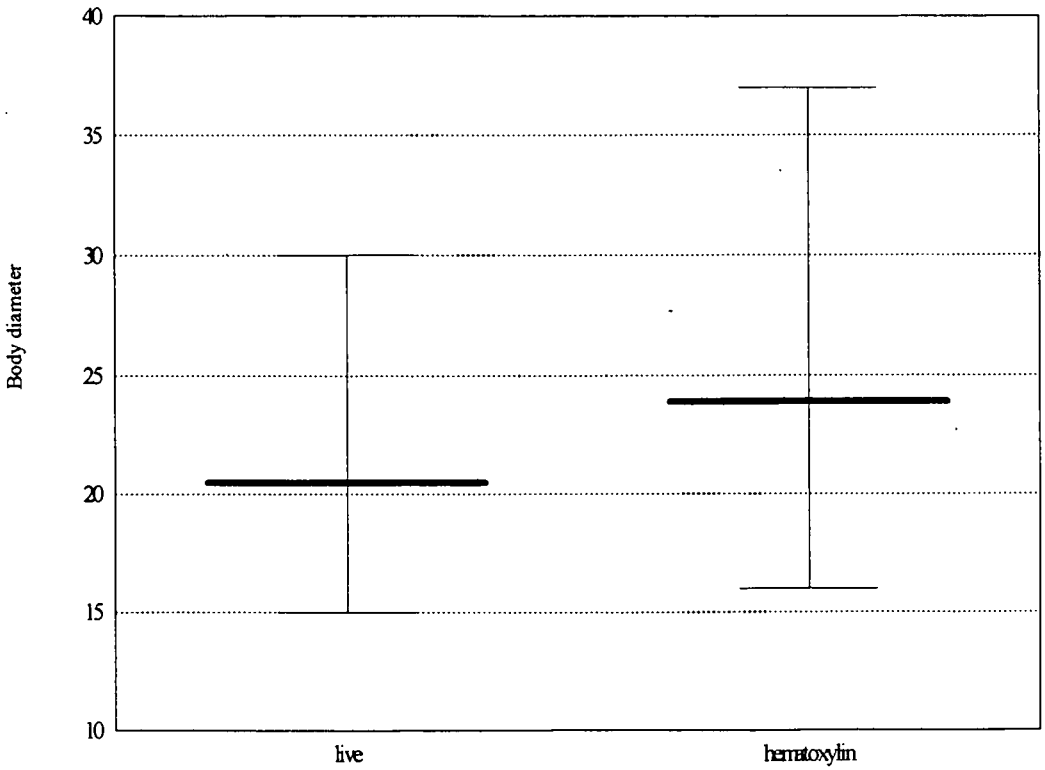


Figure 8.9 Comparison of the variation in body diameter of *Caliperia perlemoenae* sp. nov. of live observations and hematoxylin stained specimens, an ecto-hypersymbiont on *Mantoscyphidia spadiceae* sp. nov. and *M. midae* sp. nov., from the De Hoop Nature Reserve, South Africa.

Chapter 9

Digenean Trematodes found associated with Haliotis spadicea

Trematodes are parasitic flukes in the phylum Platyhelminthes. All trematodes live on the skin, fins, gills, buccal or cloacal surfaces of aquatic hosts, in the alimentary tract and its accessory structures, or in the reproductive, excretory, respiratory, blood and nervous systems (Erasmus 1972). The majority of trematodes are parasitic in vertebrate hosts, although the wide distribution of larval stages in the invertebrate fauna results in the association of most animal taxa with the biology of the trematodes.

Molluscs are involved in the digenean life-cycle as first or second intermediate hosts. The typical digenetic life-cycle consists of a molluscan primary host in which multiplication occurs, an intermediate transport host, and a vertebrate final host. Cross or self-fertilisation occurs in digeneans. Infection of the molluscan primary host results from the penetration of the foot or mantle of the mollusc by a free-swimming miracidium, or by the ingestion and subsequent hatching of eggs containing a fully developed miracidium. Within the snail, a mother and daughter sporocyst generation develops. In other life-cycles, the mother sporocysts give rise to rediae which themselves are able to produce a generation of daughter rediae under certain circumstances. Daughter sporocysts and rediae are mutually exclusive and both do not occur within the same cycle.

These generations usually inhabit the digestive gland or gonad of the mollusc and exhibit polyembryony. Rediae are more active than sporocysts and possesses a mouth, pharynx as well as a gut and thus feed. The next stage, the cercariae, may arise from daughter sporocysts or rediae, depending on the cycle. The cercariae phase is a distributive phase, they possess gland cells for penetration or encystment, eyespots and tactile sense organs as well as a muscular tail, used for swimming. Cercariae give rise to metacercariae. The metacercariae are usually encysted and may live free on vegetation or may be parasitic within a second intermediate host.

The life-cycle is completed by the development of the metacercaria into the adult, after the ingestion of the infested vegetation, or the second intermediate host by the final host. The adult trematode has a digestive tract and may feed on host tissue (Fried & Graczyk 1997).

According to Erasmus (1972) the development of the sporocyst and redial generations within the tissues of the molluscan host must have a tremendous strain on the well-being of the host. The multiplication associated with these stages require ample supplies of nutrients for its continuation, as well as space for daughter sporocysts and rediae. These stages generally occur in the molluscan digestive gland, a structure associated with the digestion of food, but not vital in the sense that a reduction in its size or efficiency would result in the immediate death of the host. The sporocyst and redial stages lie in the connective tissues between the tubules of the digestive gland, and this, in heavy infections, become almost entirely replaced by the parasitic stages.

Changes in the histology of the epithelium lining the digestive gland tubules, have been described by many authors. The epithelium changes from a columnar type to a squamous, more flattened epithelium (Erasmus 1972; Mohandas 1974) and in some cases complete loss of epithelium takes place. The close contact between the digestive gland and the ovotestis and associated ducts, in many molluscs does suggest that the presence of heavy infection might affect the functioning of the reproductive system in some way. Varied effects have been reported, ranging from inhibition of gametogenesis to castration and sex reversal (Miller & Northup 1926; Matta & Rai 1971; Ricker 1977; Lemly & Esch 1984; Sousa 1992; Bretos & Chichuailaf 1993; Taskinen, Valtonen & Mäkelä 1994; Curtis 1995; Sokolova 1995; Tharme, Webb & Brown 1996; Mouritsen, Jensen & Jensen 1997).

Digenetic trematode larval stages are often found associated with limpets. According to Rees (1934) the rediae of *Cercariae patellae* Lebour, 1911 in *Patella vulgata* Linnaeus, 1758 invaded the ovotestis and either consumed the gonad or produced a reduction of the germinal epithelium. Recently, Kollien (1996) concluded that *C. patellae* is a developmental stage of a digenean found in the intestine of the oystercatcher

Haematopus ostralegus and should be referred to as *Echinostephilla patellae* (Lebour, 1911). Immature cercariae and rediae were found in up to 34 % of a natural *P. vulgata* population, in Scotland. Branch (1981) recorded *Cercariae patellae* from *P. granatina* Linnaeus, 1758 in South Africa. Branch found adult stages of *C. patellae* in the gut of the turnstone *Arenaria interpres*, so the possibility exist that this bird may be the final host. Adult stages were also found in the gut content of the European turnstone. These birds migrate between South Africa, Europe and America.

In 1998 Martorelli and Morriconi described a new species of gymnophallid metacercariae from the mantle surface of the limpets *Nacella magellanica* (Gmelin, 1791) and *N. dearutata* (Gmelin, 1791). These metacercariae occur on the mantle surface and no mention of pathology to host tissues was made.

The effect that metacercariae have on their hosts, is largely dependant on whether or not they encyst. In those species where relatively short migratory distances are covered by cercariae and encystment occurs fairly rapidly, relatively little damage to host tissue takes place. The metacercariae don't feed on the hosts' tissues and only stimulate inflammatory response. Some cercariae do not emerge from their molluscan hosts, and encyst within the tissues of the digestive diverticulum. A very different situation exists in the case of metacercariae which do not encyst and continue in an active state, feeding on the tissues of the host. Host responses include phagocytosis, encapsulation, humoral immunity and leucocytosis. The effect which adult trematodes might have upon their hosts is often difficult to predict in specific terms, as considerable variation exists between genera and species occupying similar habitats in the host (Erasmus 1972).

The hooked mussel, *Ischadium recurvum* and platform mussel, *Mytilopsis leucopheuta* are hosts to the metacercarial form (*Cercaria brachidontis*) of the digenean parasite *Proctoeces maculatus*, that reaches sexual maturity in the sheepshead *Archosargus probatocephalus*. This digenean, however, does not need the vertebrate host to complete its life-cycle, in other words, the adult digenean infects the mussels as well (Wardle 1980). Infected mussels had an orange hue to their gonads, and not the bright yellow or brown colour.

In South Africa the indigenous brown mussel, *Perna perna* is commonly infected by two species of digenetic trematodes: metacercariae of the genus *Proctoeces* and bucephalid sporocysts (Calvo-Ugarteburu & McQuaid 1998a). According to Calvo-Ugarteburu and McQuaid (1998b) mussels infected with bucephalid sporocysts are easier to open and lose significantly more water than non-infected individuals. These sporocysts also affect reproduction by castrating the host. *Proctoeces* primarily affects the growth of *P. perna.*

In the present study, as previously mentioned in Chapter 5, severe infections of cercarial and metacercarial stages of trematodes were found in the digestive gland of *Haliotis spadicea* as well as on the gill filaments (Table 9.1). The occurrence of rediae was also noted. What appears to be the adult stages of these trematodes were also identified. Very few of these were, however, observed. *Haliotis spadicea* may be the intermediate host for the trematodes, as Erasmus (1972) suggest molluscs to be, but the possibility exists that *H. spadicea* may infect itself, because all the stages of the digenean life-cycle have been found in the haliotid. Thus, *H. spadicea* may be the intermediate as well as the final host for these trematodes. No trematodes were found on the gills or in the digestive gland of *H. midae* from the De Hoop Nature Reserve or the Danger Point Abalone Farm.

Table 9.1 Prevalence of *Haliotis spadicea* Donovan, 1808 specimens infected with trematodes, collected at the De Hoop Nature Reserve, South Africa from 1995 to 1999.

Haliotis spadicea	1995	1996	1997	1998	1999
Number collected	5	4	53	47	24
Number infected	3	2	32	40	23
Prevalence	60 %	50 %	60.4 %	85.1 %	95.83 %

A normal parasitic distribution in nature has a much lower prevalence than shown in Table 9.1. Parasites are usually overdispersed, a few hosts with high infections, a couple with low infections and most with no infections. In the case of *H. spadicea*, more than half of the specimens, examined during the last five years, were infected with trematodes. Data of 1995 and 1996 should be interpreted with caution though, because very few specimens were examined. An extremely high infection of 95.83 % were recorded during 1999.

As in the case of *H. tuberculata* (Crofts 1929; Cox 1962 & Hahn 1989e) the surface of some of the ctenidia was orange in colour owing to infection by trematode sporocysts, rediae, cercariae and metacercariae. Massive infections were noted during field observations, but SEM examination (Fig. 9.1A-I) of the gills only revealed low infection of trematodes. Only after gills were histologically prepared, it became evident where the massive infections of trematodes occurred. Most of the trematodes had encysted as metacercariae in the gill tissue. Hundreds of the digeneans were either encysted in the gill filaments (Fig. 10.2E & 10.3D) or in the gill arch (Fig. 10.3E-G).

The rediae are between 100 and 200 μm long and contained many cercariae in different stages of development. It appeared narrow anteriorly, widening to accommodate the cercarial stages, and again narrowed before forming a posterior rounded structure.

The cercariae (Fig. 9.1A&B) are approximately 200 μm long and extremely active. Posteriorly four knoblike protrusions (Fig. 9.1C) that resemble suckers are visible. These protrusions can be inverted. The body narrows just after this posterior structure, forming approximately eight segments (Fig. 9.1D). The rest of the body also appears to be segmented. The anterior end possesses an oral opening (Fig. 9.1E), approximately 5 μm wide.

The metacercariae (Fig. 9.1F) encysts within the gill tissue, with their anterior ends protruding. Most of the metacercariae still possess the four knoblike protrusions. Upon encystation the body becomes much shorter and widens (Fig. 9.1F).

The adult trematode (Fig. 9.1G) has an anteriorly located oral sucker (Fig. 9.1H) and an acetabulum (ventral sucker) in the midventral region of the body (Fig. 9.1I). Above the oral sucker, two openings can be seen (Fig. 9.1H). The adults protruded from between gill filaments, where the metacercariae had encysted (Fig. 9.1G), they also invaded the gill filaments themselves (Fig. 10.2E & 10.3D) as well as the gill arch (Fig. 10.3E-G). The adults were never observed attached by means of their suckers, but only protruding from the metacercarial cyst. During 1999 a cushion-like structure was observed attached to the posterior end of the adult trematode (Fig. 9.2). The structure possessed four to

six filaments, and its cytoplasm appeared granular. Segmentation occurs at the posterior end.

In one case where an adult trematode was protruding between two gill filaments, proliferation of gill tissue could be observed. The gill filament's epithelial tissue was damaged and the gill filament was swollen to about three times its normal thickness. Histological sections of the gills of *H. spadicea* show that gill epithelial cells are distorted and that tissue destruction take place where the trematodes had encysted (Fig. 10.3E-G). Further studies are required in order to determine whether these trematodes also cause tissue destruction in the digestive gland and gonads of *H. spadicea*.

Attempts at identifying these trematodes have been unsuccessful. Dr. C.D. McQuaid studied SEM photographs that I had taken of the specimens, but were unable to identify the trematodes (pers. comm.)*[1]. Dr. Gurutze Calvo-Ugarteburu were consequently contacted, hoping that she could shed some light on these trematodes, but was also unable to identify them (pers. comm.)*[2]. Crofts (1929), Cox (1962), Harrison and Grant (1971), Keesing (1984) and Hahn (1989e) all refer to digenean trematodes associated with abalone, possibly of the families Opeocoilidae and Allocreadiidae, but they never provided a description of the trematodes, thus making a positive identification very difficult.

Various mammals, birds, fish, crustaceans, echinoderms and molluscs are predators of *Haliotis spadicea* (Muller 1984). The Cape clawless otter *Aonyx capensis capensis* is known to feed on perlemoen, crabs, fish and octopus. Tegner and Butler (1989) consider sea otters in California to be major predators of midsized and adult abalone. According to Muller (1984), the southern black-backed or kelp gull *Larus dominicanus* and the black oystercatcher *Haematopus moquini* feed on *H. spadicea*. According to Prof. Phil Hockey (pers. comm.)*[3], it is probably very unlikely that the oystercatcher is

*[1] Dr. C.D. McQuaid, Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa.
*[2] Dr. G. Calvo-Ugarteburu, University of Transkei, South Africa.
*[3] Prof. Phil Hockey, Oystercatcher Conservation Programme, Percy Fitzpatrick Institute of African Ornithology, University of Cape Town, Rondebosch, South Africa.

the final host, if they have to eat the perlemoen in order to become infected. He has examined well over 100 000 oystercatcher prey remains, and have recorded *H. spadicea* as being eaten only once by a pair on St. Croix island. Five years of diet studies done by Anne Scott as part of her Ph.D., at the De Hoop Nature Reserve, show that oystercatchers very occasionally eat perlemoen at this site. Among the thousands.of prey items examined, there were two *H. midae*, two *H. parva* and five *H. spadicea* specimens.

Stingray *Pteromylacus bovinus*, black tail *Diplodus sargus capensis*, zebra *D. cervinus hottentotus* and galjoen *Coracinus capensis* have been recorded as predators of *H. spadicea* (Muller 1984). These fish are often infected by digeneans, but adult trematodes found in these fish do not resemble the trematodes from *H. spadicea*.

Crustaceans that feed on *H. spadicea* include the Cape rock crab *Plagusia chabrus*, the common hermit crab *Diogenes brevirostris* and the Cape crayfish *Jasus lalandi* (Tarr, Williams & MacKenzie 1996). Crayfish feed on sea urchins and could possibly ingest juvenile perlemoen associated with the urchins. It is believed that starfish (for example *Marthasterias glacialis*) feeds on larger abalone because smaller abalone have good escape responses (Montgomery 1967; Tegner & Butler 1989). My personal observation at the De Hoop Nature Reserve was that *M. glacialis* do indeed feed on juvenile abalone. In one instance a juvenile *H. midae* was found in the process of being digested by the starfish. There is thus a possibility that *H. spadicea* may fall prey to *M. glacialis* as well. This particular starfish's gut was examined, but no trematodes were found.

Amongst the other molluscans feeding on *H. spadicea*, the common octopus *Octopus* sp. cf *vulgaris* is most likely the final host for the trematodes. Octopuses are scavengers and feed by wrenching abalone off the substrate or by drilling holes through the shell. According to Jacks (1983) a strong predator-prey link exist between octopus and perlemoen. Examination of a single *O. vulgaris* from the De Hoop Nature Reserve revealed the presence of nematodes in its gut, but no trematodes. Along the east coast of South Africa *Octopus vulgaris* is infected with two different trematodes namely *Lobatostoma* (Aspidogastridae) and *Proctoeces maculatus* (Fellodistomidae) (Bray

1984; 1986). Since octopuses and starfish are major predators of perlemoen, the possibility that they are in fact the final hosts for these trematodes, still exist. Further morphological studies on this fascinating trematode need to be done, before the life-cycle can be described in full. It would be interesting to know whether development into an adult trematode in *H. spadicea* is an evolutionary dead-end or not.

Figure 9.1

Scanning electron micrographs of specimens of the digenean trematodes found on the gills of *Haliotis spadicea* Donovan, 1808, collected from the De Hoop Nature Reserve, South Africa.

A. Cercariae and embedded metacercariae.

B. Cercaria. ***Scale-bar*: 10µm.**

C. Four knoblike protrusions located on the posterior end of cercaria.

D. Narrowing of six to eight segments (cercaria).

E. Anterior oral opening (cercaria).

F. Encysted metacercaria.

G. Adult digenean trematode (arrows indicate anterior and posterior suckers).

***Scale-bar*: 10µm.**

H. Anterior or oral sucker with two openings located above it.

I. Ventral sucker.

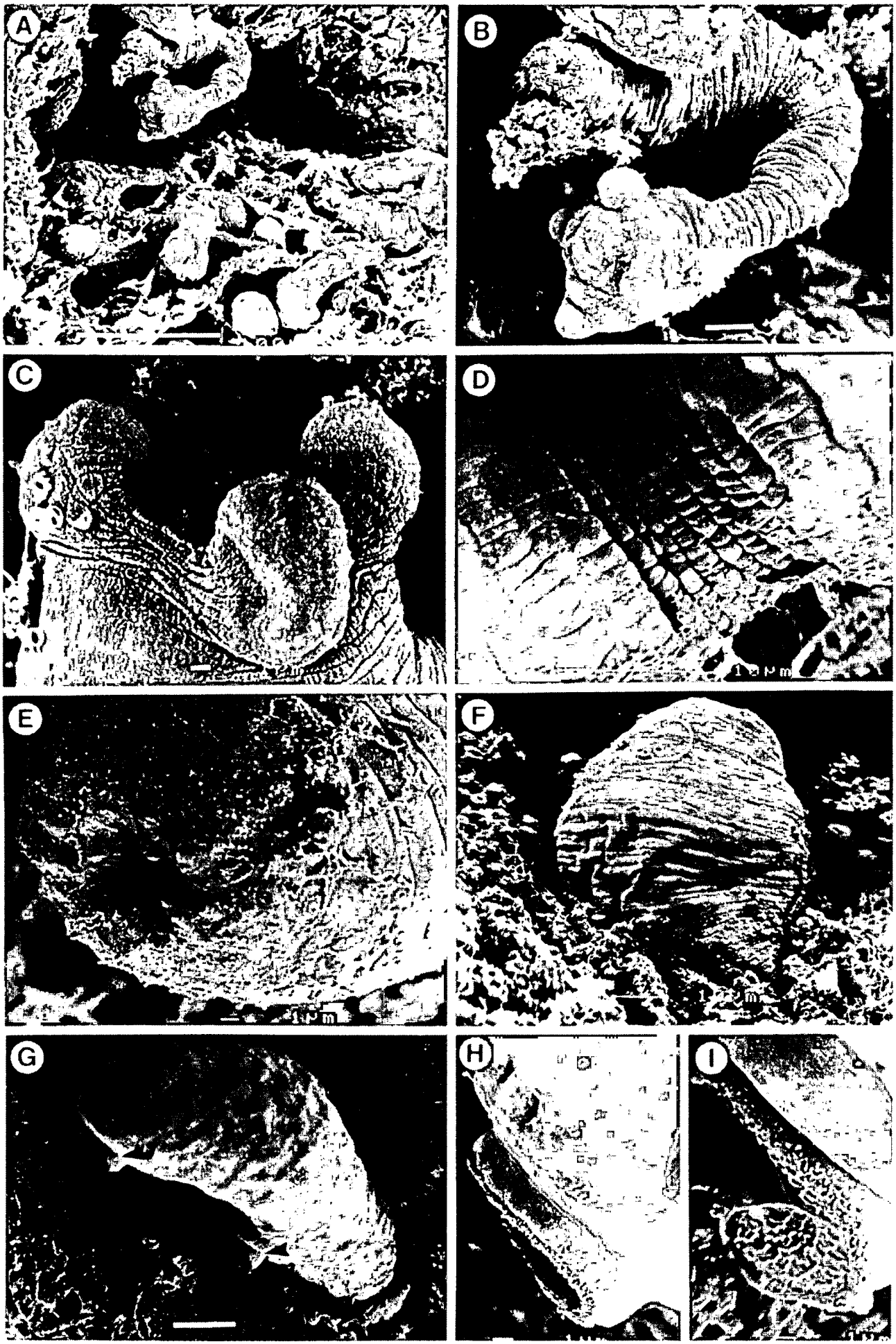
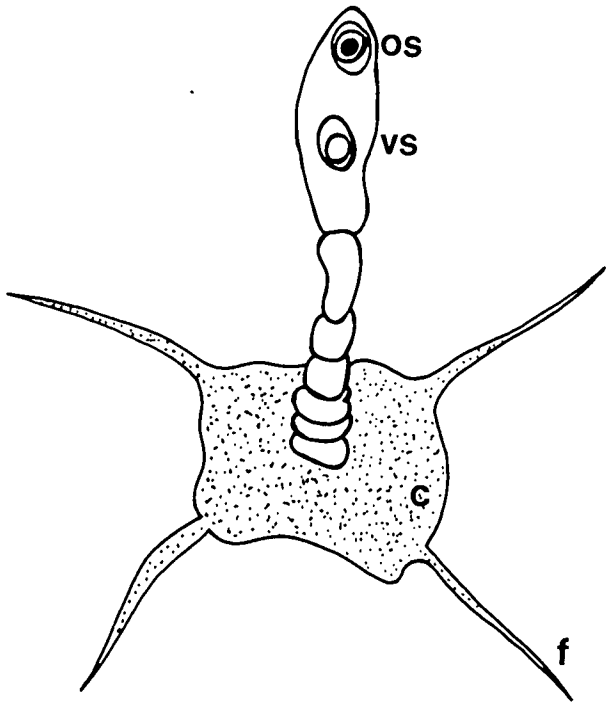

A
B
C
D
E
F
G
H
I

Figure 9.2

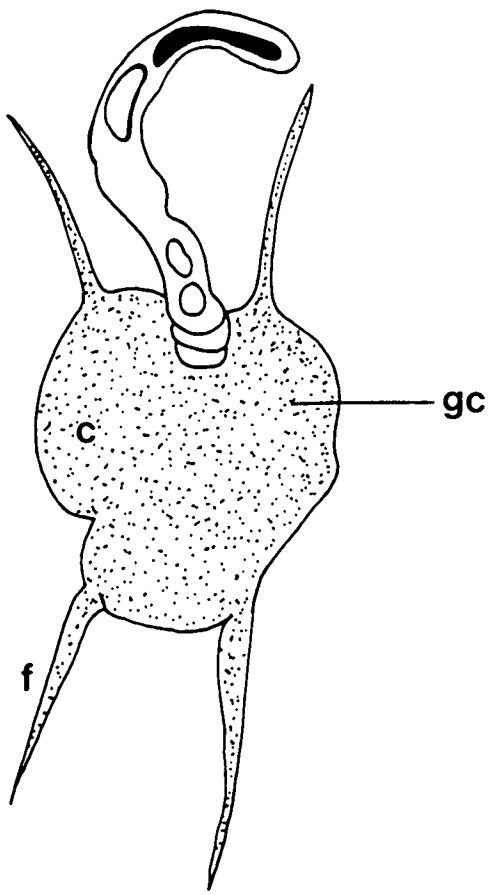
Diagrams illustrating the adult digenean trematodes (A&B) found during 1999 on the gills of *Haliotis spadicea* Donovan, 1808, collected from the De Hoop Nature Reserve, South Africa.

c= cushion-like structure; f= filaments; gc= granular cytoplasm; os= oral sucker; vs= ventral sucker.

***Scale-bar*: 10µm.**



A



B

Chapter 10
Host/Symbiont Associations

In this Chapter I will be answering the following key questions:

- Where do the scyphidiid peritrichs occur on the host?
- How are the scyphidiid peritrichs distributed on the host?
- How many scyphidiid peritrichs occur on a host?
- Which haliotid hosts are infested?
- Are there any differences in the infestation patterns between different haliotid species occurring in the same habitat?
- Does the size/age of the host influence the infestation level of the scyphidiid peritrichs?
- Do the scyphidiid peritrichs cause any damage to the host?
- How many caliperids occur on a primary host?
- What is the influence of the caliperids on the host?
- What is the influence of the trematodes on the host?

Where do the scyphidiid peritrichs occur on the host?

After removing the muscular foot of a perlemoen specimen (called "shucking"), the mantle has to be cut along the shell to expose the gills. It is not possible to distinguish the scyphidiid peritrichs using a dissecting microscope, not because of their small size, but due to the ineffective reflection of light from the white wet gill tissue.

It was necessary to examine the gills with the aid of a compound microscope in order to establish the existence of the scyphidiid peritrichs. Gill filaments were removed as intact as possible, and consequently fixed in buffered neutral formalin. This material was later studied with the aid of SEM, to determine the position of the scyphidiid peritrichs.

Figure 10.1A-F illustrates that a large number of the scyphidiid peritrichs are firmly attached to the gill filaments and gill arch. The highest concentrations of *Mantoscyphidia spadiceae* and *M. midae* were always found on the gill arch (Fig. 10.1B-F). In exceptionally high cases of infestation, the scyphidiid peritrichs also attached on and between the gill filaments (Fig. 10.1A). At no time during field observations were any scyphidiid peritrichs found attached to the muscle of the foot or on the internal shell surface.

From extensive field observations it can be concluded that *M. spadiceae* and *M. midae* are associated primarily with the gill arches of their haliotid hosts.

How are the scyphidiid peritrichs distributed on the host?

Having established that *M. spadiceae* and *M. midae* occur primarily on the gill arches, the question that arises is whether any particular part of the gill arch has higher prevalence of infestation than other parts. With the aid of SEM, it could be concluded that the scyphidiid peritrichs are distributed evenly on the full extent of the gill arch, but seem to favour areas on the gill arch where depressions are formed which provides some form of shelter (Fig. 10.1D&F).

Because *Haliotis spadicea* consistently had higher infestations of scyphidiid peritrichs than *H. midae*, infestations were not restricted to the gill arches, but also occurred on the gill filaments (Fig. 10.1A). The scyphidiid peritrichs do not prefer attachment to or between the gill filaments, except in very high infestations, probably due to their elongated bodies. *Mantoscyphidia branchi*, for example, has a much shorter body length compared to *M. spadiceae* and attachment between gill filaments would therefore be easier. No difference could be observed in the infestation intensities of the left and right gills

It is concluded that *M. spadiceae* and *M. midae* are distributed more or less evenly throughout the whole gill arch of the two different host species, favouring areas of depression or shelter.

How many scyphidiid peritrichs occur on a host?

A four point scale was selected (See Chapter 2) to quantify levels of infestation. The scale does not represent a linear increase. One (X) infestation indicates the presence of scyphidiid peritrichs with not more than 10 specimens per slide, whereas XXX indicates a presence of between 100 and 200 specimens on a wet smear, prepared from one whole or intact gill filament. Exceptionally high levels of infestation were indicated by >XXX. In Fig. 10.1A-F the extent of the infestation referred to, can be seen. Infestation levels as seen on the SEM micrographs, probably represents only about a third to half of the actual level of infestation, due to the fact that many scyphidiid peritrichs are lost during dissection of a live gill, fixation and subsequent SEM preparation.

Which haliotid hosts are infested?

Data on the number of perlemoen examined and their infestation (graded on the four point scale) with scyphidiid peritrichs, caliperids and digenean trematodes are summarised in Tables 10.1-10.5. In some cases the length and mass of the haliotid hosts were determined. No measurements of length were made during 1995. Data of 1995 and 1996 were collected by other members of the Aquatic Parasitology studygroup, whilst data from 1997 to 1999 were collected by the author (See Chapter 2).

Table 10.1a Scyphidiid peritrich infestation data of haliotids collected at the De Hoop Nature Reserve, South Africa during **April 1995**.

	Number collected	Number infested	Prevalence
Total	7	6	99.85 %
H. spadicea	5	5	100 %
H. midae	2	1	50 %

Table 10.1b Infestation intensity of scyphidiid peritrichs found on the gills of both *Haliotis spadicea* Donovan, 1808 and *Haliotis midae* Linnaeus, 1758 collected at the De Hoop Nature Reserve, South Africa during **April 1995**.

Infestation intensity	
<X	0 %
X	33.3 %
X-XX	0 %
XX	0 %
XX-XXX	0 %
XXX	33.3 %
>XXX	33.3 %

Table 10.1c Infestation data of *Haliotis spadicea* Donovan, 1808 collected at the De Hoop Nature Reserve, South Africa during **April 1995**. SCN = Specimen collection number.

SCN	*M. spadiceae* Infestation	Caliperids	Trematodes
4	>XXX	Yes	Yes
5	>XXX	Yes	Yes
9	XXX	No	Yes
48	XXX	Yes	No
248	X	No	No

Table 10.1d Infestation data of *Haliotis midae* Linnaeus, 1758 collected at the De Hoop Nature Reserve, South Africa during **April 1995**. SCN = Specimen collection number.

SCN	*M. midae* Infestation	Caliperids	Trematodes
3	X	No	No
235	-	No	No

Table 10.2a Scyphidiid peritrich infestation data of haliotids collected at the De Hoop Nature Reserve, South Africa during **April 1996**.

	Number collected	Number infested	Prevalence
Total	6	5	99.8 %
H. spadicea	4	3	75 %
H. midae	2	2	100 %

Table 10.2b Infestation intensity of scyphidiid peritrichs found on the gills of both *Haliotis spadicea* Donovan, 1808 and *Haliotis midae* Linnaeus, 1758 collected at the De Hoop Nature Reserve, South Africa during **April 1996**.

Infestation intensity	
<X	0 %
X	40 %
X-XX	40 %
XX	20 %
XX-XXX	0 %
XXX	0 %
>XXX	0 %

Table 10.2c Infestation data of *Haliotis spadicea* Donovan, 1808 collected at the De Hoop Nature Reserve, South Africa during **April 1996**. SCN = Specimen collection number.

SCN	Length of Venus ears (cm)	*M. spadiceae* Infestation	Caliperids	Trematodes
2	6.5	X	Yes	Yes
3	6.5	-	-	Yes
45	5.4	XX	No	No
46	1.8	X-XX	No	No

Table 10.2d Infestation data of *Haliotis midae* Linnaeus, 1758 collected at the De Hoop Nature Reserve, South Africa during **April 1996**. SCN = Specimen collection number.

SCN	Length of perlemoen (cm)	*M. midae* Infestation	Caliperids	Trematodes
1	15	X-XX	No	No
4	6	X	Yes	No

Table 10.3a Scyphidiid peritrich infestation data of haliotids collected at the De Hoop Nature Reserve, South Africa during **April 1997**.

	Number collected	Number infested	Prevalence
Total	68	59	86.76 %
H. spadicea	53	50	93.34 %
H. midae	15	9	60 %

Table 10.3b Infestation intensity of scyphidiid peritrichs found on the gills of *Haliotis spadicea* Donovan, 1808 collected at the De Hoop Nature Reserve, South Africa during **April 1997**.

Infestation intensity	
<X	0 %
X	6 %
X-XX	8 %
XX	30 %
XX-XXX	28 %
XXX	26 %
>XXX	2 %

Table 10.3c Infestation data of *Haliotis spadicea* Donovan, 1808 collected at the De Hoop Nature Reserve, South Africa during **April 1997**. SCN = Specimen collection number.

SCN	Length of Venus ears (cm)	*M. spadiceae* Infestation	Caliperids	Trematodes
2	5.4	XX-XXX	Yes	No
7	5.5	-	-	Yes
9	5.3	XX-XXX	No	Yes
10	5.5	XXX	No	Yes
11	6.5	XX-XXX	No	No
12	8	X	No	No
13	6.7	X	No	Yes
16	6	XX-XXX	Yes	Yes
17	6.2	XXX	No	Yes
19	6	XXX	Yes	No
20	5.3	XXX	Yes	No
21	5	-	-	No
22	5.9	XXX	Yes	Yes
24	6.3	XX-XXX	Yes	Yes
25	5.8	XX	Yes	No
26	7	X-XX	Yes	No
28	6	XXX	Yes	Yes
29	6	XX	Yes	Yes
30	6.3	XXX	Yes	Yes
31	4.7	XX-XXX	Yes	Yes
32	6	XXX	No	Yes
33	5	XX	No	No
34	5.8	XXX	No	Yes
35	5	XXX	No	Yes
36	7	XX-XXX	No	Yes
37	4.8	XX-XXX	No	No
38	6.5	XX	No	No
39	5.6	>XXX	No	Yes
40	6.7	XX-XXX	No	Yes
41	6.3	XX	No	Yes
43	6.1	XX	No	Yes
44	5.8	XX	No	No

Table 10.3c (continue) Infestation data of *Haliotis spadicea* Donovan, 1808.

SCN	Length of Venus ears (cm)	*M. spadiceae* Infestation	Caliperids	Trematodes
45	6.2	XX	Yes	No
46	6.1	X-XX	Yes	No
47	7	X-XX	No	Yes
48	6.4	XX-XXX	No	Yes
49	4.7	XX-XXX	No	No
50	5.3	XX-XXX	No	Yes
51	6.5	XX	No	No
53	6.1	XX-XXX	No	Yes
54	6.2	X	No	Yes
55	6.2	XX	No	Yes
57	1.3	-	-	-
59	6.2	XXX	Yes	Yes
60	6.6	XX-XXX	Yes	Yes
61	3.5	XX	Yes	No
62	5.3	XX	Yes	Yes
63	5.7	XX	No	No
64	5.2	XX	Yes	No
65	5.7	X-XX	Yes	Yes
66	4.2	XXX	Yes	Yes
67	3.3	XXX	Yes	Yes
68	4.7	XX	No	No

Table 10.3d Infestation intensity of scyphidiid peritrichs found on the gills of *Haliotis midae* Linnaeus, 1758 collected at the De Hoop Nature Reserve, South Africa during **April 1997**.

Infestation intensity	
<X	**0 %**
X	**44 %**
X-XX	**11 %**
XX	**11 %**
XX-XXX	**11 %**
XXX	**22 %**
>XXX	**0 %**

Table 10.3e Infestation data of *Haliotis midae* Linnaeus, 1758 collected at the De Hoop Nature Reserve, South Africa during **April 1997**.

SCN	Length of perlemoen (cm)	*M. midae* Infestation	Caliperids	Trematodes
1	7.8	XXX	Yes	No
3	7	X-XX	Yes	No
4	7	X	No	No
5	5.3	XXX	No	No
6	6.3	XX-XXX	Yes	No

Table 10.3e (continue) Infestation data of *Haliotis midae* Linnaeus, 1758.

SCN	Length of perlemoen (cm)	*M. midae* Infestation	Caliperids	Trematodes
8	7	-	-	-
14	10	-	-	-
15	13	-	-	-
18	8	-	-	-
23	8.5	X	No	No
27	11.3	X	Yes	No
42	7.1	XX	No	No
52	9.8	-	-	-
56	2.8	-	-	-
58	7.5	X	No	No

Table 10.4a Scyphidiid peritrich infestation data of haliotids collected at the De Hoop Nature Reserve, South Africa during **April 1998.**

	Number collected	Number infested	Prevalence
Total	97	92	94.85%
H. spadicea	47	45	93.62%
H. midae	50	48	96%

Table 10.4b Infestation intensity of scyphidiid peritrichs found on the gills of *Haliotis spadicea* Donovan, 1808, at the De Hoop Nature Reserve, South Africa during **April 1998.**

Infestation intensity	
X	**4.55%**
X-XX	**11.36%**
XX	**29.55%**
XX-XXX	**34.09%**
XXX	**20.45%**
>XXX	**0%**

Table 10.4c Infestation data of *Haliotis spadicea* Donovan, 1808 collected at the De Hoop Nature Reserve, South Africa during **April 1998.** SCN = Specimen collection number.

SCN	Length of Venus ears (cm)	Weight of abalone(g)	*M. spadiceae* Infestation	Caliperids	Trematodes
1	7.5	60	XX	No	Yes
2	8	96	XXX	Yes	Yes
3	5.5	46	XX-XXX	No	Yes
4	7.5	86	XXX	No	Yes
5	6.5	60	XX-XXX	No	No
6	8	48	X-XX	No	Yes

Table 10.4c (continue) Infestation data of *Haliotis spadicea* Donovan, 1808.

7	7	64	XX-XXX	No	Yes
8	7.5	56	XX-XXX	No	No
9	6.5	38	XXX	No	Yes
11	3	6	-	No	No
12	3	6	-	No	No
17	7.5	68	XX	No	Yes
18	8	86	XX	No	Yes
19	6	76	XX	No	Yes
20	7	50	XX-XXX	No	Yes
21	5.5	30	X-XX	Yes	Yes
22	4.5	14	X	No	Yes
23	7	100	XX-XXX	Yes	Yes
24	6.5	68	XX	No	Yes
25	6	52	XX	No	Yes
26	6	64	XX	No	No
27	6.5	40	XX	No	Yes
28	6	50	X-XX	No	Yes
29	6.5	40	XXX	No	Yes
30	5.5	32	XX	No	Yes
31	4.5	26	XX	No	No
32	4.5	22	X	No	Yes
35	5	18	-	No	Yes
36	6.5	44	X-XX	No	Yes
37	7	62	X-XX	No	Yes
38	7.5	78	XX-XXX	No	Yes
39	9	125	XX-XXX	Yes	Yes
55	5.6	46	XX	Yes	Yes
56	5.5	38	XXX	No	Yes
69	5.4	62	XX-XXX	Yes	Yes
70	6.5	90	XX-XXX	Yes	Yes
74	2.7	8	XX	Yes	Yes
75	4.3	26	XXX	No	No
76	6.3	66	XXX	Yes	Yes
77	5.4	62	XXX	Yes	Yes
78	4.3	26	XX-XXX	Yes	Yes
79	6.1	68	XX-XXX	Yes	Yes
80	6.7	98	XX-XXX	Yes	Yes
81	6.2	74	XX-XXX	Yes	Yes
82	6	46	XX-XXX	Yes	Yes
83	5.6	56	XXX	Yes	Yes
84	5	54	XX	Yes	Yes

Table 10.4d Infestation intensity of scyphidiid peritrichs found on the gills of *Haliotis midae* Linnaeus, 1758 at the De Hoop Nature Reserve, South Africa during **April 1998.**

Infestation intensity	
X	29 %
X-XX	35 %
XX	20 %
XX-XXX	12 %
XXX	4 %
>XXX	0 %

Table 10.4e Infestation data of *Haliotis midae* Linnaeus, 1758 collected at the De Hoop Nature Reserve, South Africa during **April 1998.** *1-12 - *Haliotis midae* Linnaeus, 1758 from the Irvin & Johnston Abalone farm, Danger Point, Gansbaai. SCN = Specimen collection number.

SCN	Length of perlemoen (cm)	Weight of perlemoen (g)	*M. midae* Infestation	Caliperids	Trematodes
1*	6.6	78	XX-XXX	Yes	No
2*	7	78	XXX	No	No
3*	4.4	26	XX	Yes	No
4*	3.7	14	XX	Yes	No
5*	5.7	54	XX	Yes	No
6*	6	62	X-XX	Yes	No
7*	5.1	40	X-XX	No	No
8*	5.2	46	X-XX	Yes	No
9*	2.5	4	X	No	No
10*	2.7	8	X	No	No
11*	1.8	<1	-	No	No
12*	1.6	<1	X	No	No
10	6	56	X	No	No
13	13	348	X	No	No
14	10	190	X-XX	No	No
15	9	160	X	No	No
16	11.5	298	X	No	No
33	10	156	X	No	No
34	8.5	138	X	No	No
40	13	520	XX	Yes	No
41	12	328	XX-XXX	Yes	No
42	11	270	XX	No	No
43	9	156	XX	No	No
44	8.5	138	XX-XXX	No	No
45	8	114	-	No	No
46	7.6	134	X-XX	No	No
47	7.9	156	X-XX	No	No
48	5.6	54	X-XX	No	No
49	10	262	X-XX	Yes	No
50	10	188	XX	No	No
51	9.3	176	X-XX	Yes	No
52	8.3	150	X-XX	No	No

Table 10.4e (continue) Infestation data of *Haliotis midae* Linnaeus.

53	7.2	102	X	No	No
54	7	112	X-XX	No	No
57	7.7	110	X	No	No
58	8.8	130	X-XX	No	No
59	11.5	376	X	No	No
60	2.6	6	X-XX	No	No
61	3.6	14	XX-XXX	No	No
62	7.9	130	XX-XXX	No	No
63	7	88	X-XX	Yes	No
64	8.1	178	X-XX	Yes	No
65	4.3	32	X	Yes	No
66	9	210	XX-XXX	Yes	No
67	14.5	710	X	No	No
68	7.9	134	XX	Yes	No
71	5.7	68	X-XX	No	No
72	8.5	206	XX	Yes	No
73	8.4	172	XX	No	No
85	14.5	894	X-XX	Yes	No

Table 10.5a Scyphidiid peritrich infestation data of haliotids collected at the De Hoop Nature Reserve, South Africa during **April 1999**.

	Number collected	Number infested	Prevalence
Total	44	43	97.73 %
H. spadicea	24	24	100 %
H. midae	20	19	95 %

Table 10.5b Infestation intensity of scyphidiid peritrichs found on the gills of *Haliotis spadicea* Donovan, 1808 collected at the De Hoop Nature Reserve, South Africa during **April 1999**.

Infestation intensity	
<X	**0 %**
X	**25 %**
X-XX	**0 %**
XX	**33.3 %**
XX-XXX	**37.5 %**
XXX	**4.2 %**
>XXX	**0 %**

Table 10.5c Infestation data of *Haliotis spadicea* Donovan, 1808 collected at the De Hoop Nature Reserve, South Africa during **April 1999**. SCN = Specimen collection number.

SCN	Length of Venus ears (cm)	*M. spadiceae* Infestation	Caliperids	Trematodes
1	7	X	Yes	Yes
2	6	X	No	Yes
5	6.5	XX	No	Yes
6	6	XX-XXX	No	Yes
7	6.25	XX-XXX	No	Yes
9	7	XX	No	Yes
10	6	XX	No	Yes
13	5.5	XX-XXX	No	No
14	7	XX	No	Yes
15	6	XX-XXX	Yes	Yes
16	7	X	Yes	Yes
21	7.5	XX-XXX	Yes	Yes
22	6	XX-XXX	No	Yes
23	6.5	XX-XXX	No	Yes
24	4.5	X	No	Yes
25	5.5	XX	Yes	Yes
26	5	XX	No	Yes
27	6	XX	No	Yes
28	5.5	XX	No	Yes
40	7	XX-XXX	No	Yes
41	6	X	No	Yes
42	5.5	XXX	No	Yes
43	5.5	X	No	Yes
44	5.5	XX-XXX	No	Yes

Table 10.5d Infestation intensity of scyphidiid peritrichs found on the gills of *Haliotis midae* Linnaeus, 1758 collected at the De Hoop Nature Reserve, South Africa during **April 1999**.

Infestation intensity	
<X	**15.8 %**
X	**42.1 %**
X-XX	**21 %**
XX	**15.8 %**
XX-XXX	**5.3 %**
XXX	**0 %**
>XXX	**0 %**

Table 10.5e Infestation data of *Haliotis midae* Linnaeus, 1758 collected at the De Hoop Nature Reserve, South Africa during **April 1999**. SCN = Specimen collection number.

SCN	Length of perlemoen (cm)	*M. midae* Infestation	Caliperids	Trematodes
3	7.5	X	No	No
4	10	X	No	No
8	5.5	X	No	No
11	11.5	XX	No	No
12	10	X-XX	Yes	No
17	10	X-XX	Yes	No
18	3.2	-	No	No
19	9.5	X-XX	No	No
20	10	XX	Yes	No
29	3.5	<X	No	No
30	4.5	X	Yes	No
31	5.5	X	Yes	No
32	6.5	<X	No	No
33	8	<X	No	No
34	9	X	No	No
35	10	XX-XXX	No	No
36	10	XX	No	No
37	9	X-XX	No	No
38	8.5	X	No	No
39	13	X	No	No

Of the 133 specimens of *H. spadicea* that were examined from 1995 to 1999, only seven specimens were found to be uninfested. When all the infestation data regarding *H. spadicea* is combined, this results in an infestation of 94.74 % over the five years. Similarly, of the 89 specimens of *H. midae* that were collected, only 10 specimens were uninfested. This results in an infestation of 88.76 % in *H. midae* over five years.

Are there any differences in the infestation patterns between different haliotid species occurring in the same habitat?

When the data of 1995 to 1999 is compared, *Haliotis spadicea* consistently had a higher infestation intensity than *H. midae* (Fig.10.4 & Fig.10.5). Infestation intensities of XX

or XX-XXX were common in *H. spadicea*, while *H. midae* mostly had scyphidiid peritrich infestations of X or X-XX. In Tables 10.6a&b no data of 1995 are used for comparison, as too few specimens were examined in order to give significant data. The highest percentage infestation intensity is given for both species in the case of 1996 to 1999 (See Tables 10.6a&b).

Table 10.6a Infestation intensity and percentage infestation of *Haliotis spadicea* Donovan, 1808 specimens collected from the De Hoop Nature Reserve, South Africa.

Haliotis spadicea	Infestation intensity	Percentage
1996	X	44 %
1997	XX	30 %
1998	XX-XXX	34.09 %
1999	XX-XXX	37.5 %

Table 10.6b Infestation intensity and percentage infestation of *Haliotis midae* Linnaeus, 1758 specimens collected from the De Hoop Nature Reserve, South Africa.

Haliotis midae	Infestation intensity	Percentage
1996	X-XX	40 %
1997	X	44 %
1998	X-XX	35 %
1999	X	42.1 %

Throughout the study, results showed that *Haliotis spadicea* had higher infestations of scyphidiid peritrichs than *H. midae*. No major difference in the total perlemoen infestation patterns between the five years can be observed (Table 10.3a). The lower infestation of 86.76 % during 1997 (pooled data, Table 10.3a) can be ascribed to the fact that only 60 % of the *H. midae* specimens examined, were infested (Fig. 10.6).

Does the size/age of the host influence the infestation level of the scyphidiid peritrichs?

Of the uninfested *Haliotis spadicea*, that were examined over the five year period, three were juveniles (1.3 cm; 3 cm and 3 cm), with a further four (5 cm; 5 cm; 5.5 cm and 6.5 cm) larger/older specimens. The infestation intensities of the other 14 *H. spadicea* specimens (out of a total of 133 specimens) that had body lengths of less than 5 cm is summarised in Fig. 10.7. Figures 10.13-10.15 illustrate the distribution of infestation intensities in the different sizes of *H. spadicea* specimens.

Almost all of the *H. spadicea* specimens with an infestation intensity of XXX, had body lengths in excess of 5 cm, with the exception of three specimens that measured 3.3 cm; 4.2 cm and 4.3 cm respectively.

There is thus an increase in infestation levels with an increase in the size/age of the host, i.e. *H. spadicea*. This is likely to be a result of an accumulative effect. The majority of the haliotids had infestations of XX or more.

Of the uninfested *H. midae* specimens, three were juvenile (1.8 cm; 2.8 cm and 3.2 cm), with a further six larger/older specimens (7 cm; 8 cm; 8 cm; 9.8 cm; 10 cm and 13 cm). One uninfested specimen collected during 1995 was not measured. The infestation intensities of the other 25 *H. midae* specimens that have body lengths of less than 7 cm are summarised in Fig. 10.8. Figures 10.16-10.18 illustrate the distribution of infestation intensities in the different sizes of *H. midae* specimens.

Almost all of the *H. midae* specimens with an infestation intensity of XX-XXX or XXX had body lengths in excess of 7 cm, with the exception of four specimens that measured 3.6 cm; 5.3 cm; 6.3 cm and 6.6 cm respectively.

Thus it can be concluded that there is an increase in infestation levels with an increase in the size/age of the host, *H. midae*. This is also likely to be a result of an accumulative effect. The majority of haliotids had infestations of X or X-XX.

Based on data concerning the five year study period, it can be concluded that *H. spadicea* and *H. midae* juveniles harbour less scyphidiid peritrichs than adult or larger sized haliotids.

Upon comparison between the same size order (7-8 cm) of *H. spadicea* and *H. midae* specimens, the following results are evident from Fig. 10.9 and Fig. 10.10, i.e. that *H. spadicea* specimens of the same size/length as *H. midae* had higher infestation intensities of scyphidiid peritrichs.

The majority of *H. spadicea* specimens had XX-XXX infestations, while *H. midae* mostly had X or X-XX infestations.

The infestation statistics of the largest specimens of the two relevant perlemoen species were also compared: (Fig. 10.11 & Fig. 10.12). The four largest *H. spadicea* specimens (8 cm to 9 cm) had infestation intensities that ranged from X to XXX. Of the five largest *H. midae* specimens (13 cm to 14.5 cm), three specimens had an infestation of X, one an infestation of X-XX and one an infestation of XX. No clear pattern could be observed concerning these statistics.

Do the scyphidiid peritrichs cause any damage to the host?

In Figures 10.2A-D and 10.3A-D micrographs of histopathological sections are presented. These were made from *Mantoscyphidia spadiceae* occurring on *Haliotis*

spadicea and *M. midae* occurring on *H. midae*. In Fig. 10.3C&D the gill filaments can be seen with high infestations of *M. spadiceae*. In Fig. 10.3A&B *M. midae* are attached to the gill arch. Fig. 10.2A&B shows micrographs of *M. midae*'s attachment to the gill epithelium, and Fig. 10.2C&D shows *M. spadiceae*'s attachment. In none of these sections examined, could any pathology be observed. The gill epithelial tissue were not distorted and no proliferation of tissue occurred, as is normally the case when tissue is irritated by the presence of parasites.

Although there may not be clear signs of pathology on the gill filaments and arches, the bulk of the scyphidiid peritrichs present on the gills can result in coverage of gill surface which could interfere with respiration. The fact that such high infestations occurred on the gills of the assumingly healthy perlemoen, and that no sign of pathology could be found, confirms that this association is not a typical parasitic infestation where parasites are normally overdispersed, i.e. that a few hosts harbour high infestations, some with mild infestations and most with none. There can be no doubt that the association between *M. spadiceae* and *H. spadicea* as well as *M. midae* and *H. midae* is not a typical parasitic association.

How many caliperids occur on a primary host?

Caliperids do not seem to prefer one haliotid as primary host (Fig. 10.19 & Fig. 10.20) or mantoscyphidian host above the other. Observations made on the presence of caliperids were based on the examination of wet smears. Caliperids were reported as present or not (e.g. Table 10.4c). No infestation levels, as in the case of the mantoscyphidians, were noted.

Over a three year period it was observed that between 20.83 % and 41.51 % *H. spadicea* specimens, and between 25 % and 34 % *H. midae* specimens were infested with caliperids, indicating that there were no real difference in the infestation intensity between the two *Mantoscyphidia* species. Data concerning 1995 and 1996 are not included in these percentages, because too few specimens were examined.

What is the influence of the caliperids on the host?

The presence of *Caliperia perlemoenae* was observed in different populations of both *Mantoscyphidia spadiceae* and *M. midae*. In some populations of *M. spadiceae*, almost every scyphidiid peritrich had an attached caliperid, in some cases more than one. Up to four caliperids were found attached to the scopula of a single *M. spadiceae*. As previously mentioned, some caliperids were attached to the peristomial region (Fig. 8.5F&G) or even the region of the telotroch band of the scyphidiid peritrichs' bodies (Fig. 10.1G&H), gripping the scyphidiid peritrich where its nuclear apparatus is situated. In Fig. 8.5E two caliperids are attached between the macro- and micronuclei of a scyphidiid peritrich. In the cases where the caliperids are attached to the peristomial region, the buccal cavity and infundibulum are obstructed. The caliperids most likely interfere with the scyphidiid peritrichs' feeding and their attachment might thus be detrimental as they could cause the scyphidiid peritrichs to die. Caliperids attaching to the area where the scyphidiid peritrichs' nuclear apparatus is situated might have an influence on their reproductive process, interfering with nuclear division.

As in the case of *C. maliculiformis* attaching to *M. branchi* (Van As 1997), different specimens of *C. perlemoenae* were also observed attached to specimens of *M. spadiceae* and *M. midae* in the process of budding and binary fission. Some cases were also observed where a single individual of *C. perlemoenae* was found attached to two

specimens of *M. spadiceae* simultaneously - most likely the result of completion of binary fission and suggesting that the association is continuous for at least an extended period.

What is the influence of the trematodes on the host?

In one case where an adult trematode was protruding between two gill filaments, proliferation of gill tissue could be observed. The gill filament's epithelial tissue was damaged and the gill filament was swollen to about three times its normal thickness. Trematodes also invaded gill filaments (Fig. 10.2E). Histological sections of the gills of *H. spadicea* show that gill epithelial cells are distorted and that tissue destruction take place where the trematodes had encysted (Fig. 10.3D-G). Further studies are required in order to determine whether these trematodes also cause tissue destruction in the digestive gland and gonads of *H. spadicea*.

Figure 10.1

Scanning electron micrographs of the gills of *Haliotis spadicea* Donovan, 1808 (B-E,G&H) and *H. midae* Linnaeus, 1758 (A&F) infested with *Mantoscyphidia spadiceae* sp. nov., *M. midae* sp. nov. and *Caliperia perlemoenae* sp. nov., collected from the De Hoop Nature Reserve, South Africa.

A. Scyphidiid peritrichs between gill filaments.

B-F. Scyphidiid peritrichs on gill arches.

G & H. *C. perlemoenae* attached to the telotroch band region of *M. spadiceae* where its nuclear apparatus is situated.

***Scale-bar*: 10µm in A, B, D - G and H..**

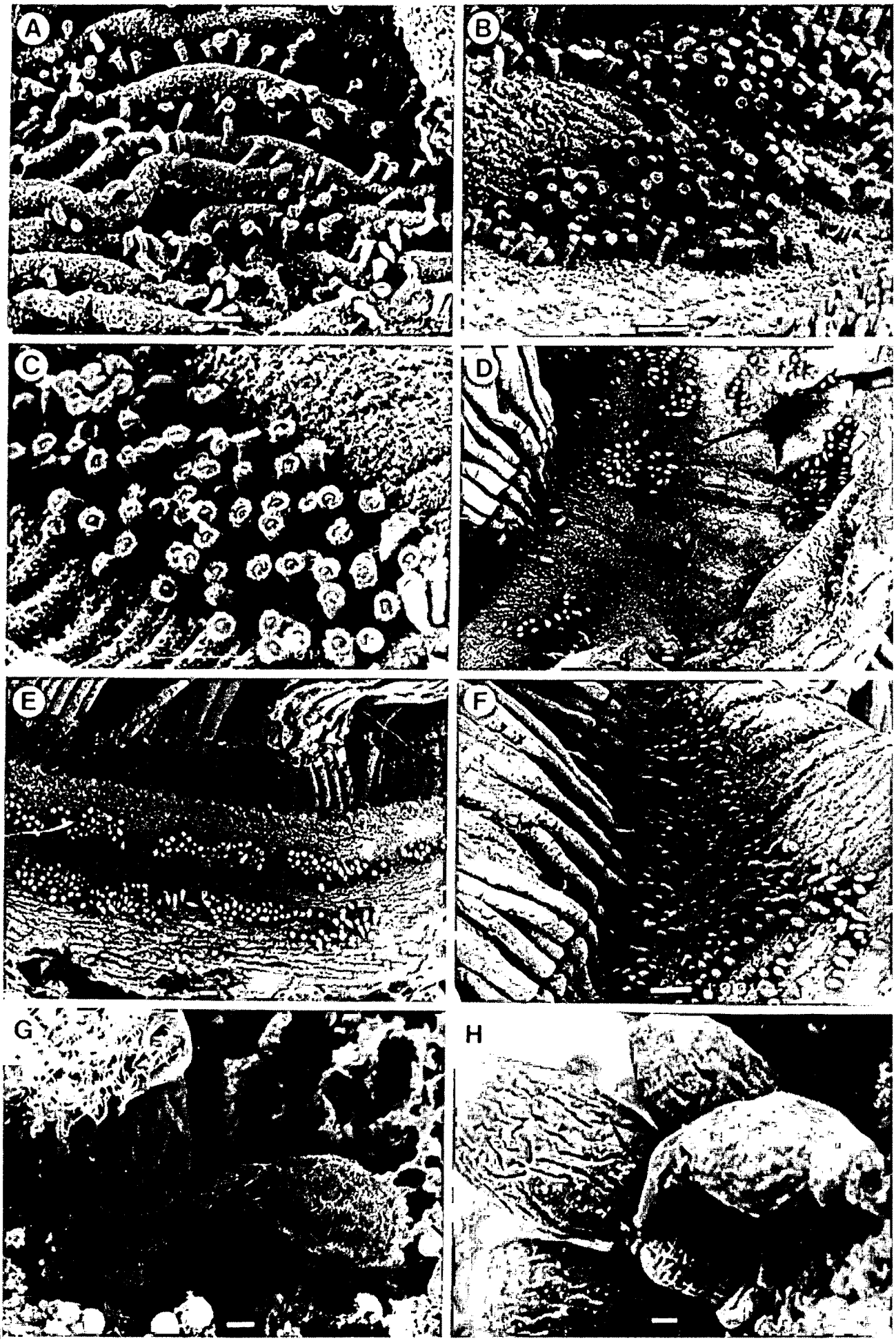

A
B
C
D
E
F
G
H

Figure 10.2

Light micrographs of histological sections of the gills of *Haliotis spadicea* Donovan, 1808 and *H. midae* Linnaeus, 1758 infested with *Mantoscyphidia spadiceae* sp. nov. (C&D), *M. midae* sp. nov. (A&B) as well as digenean trematodes (E), collected from the De Hoop Nature Reserve, South Africa.

A&B. *M. midae*. Enlargement of gill surface, showing no sign of pathology.

C&D. *M. spadiceae*. Enlargement of gill surface, showing no sign of pathology.

E. Trematode (indicated by arrow) encysted within a gill filament of *H. spadicea*.

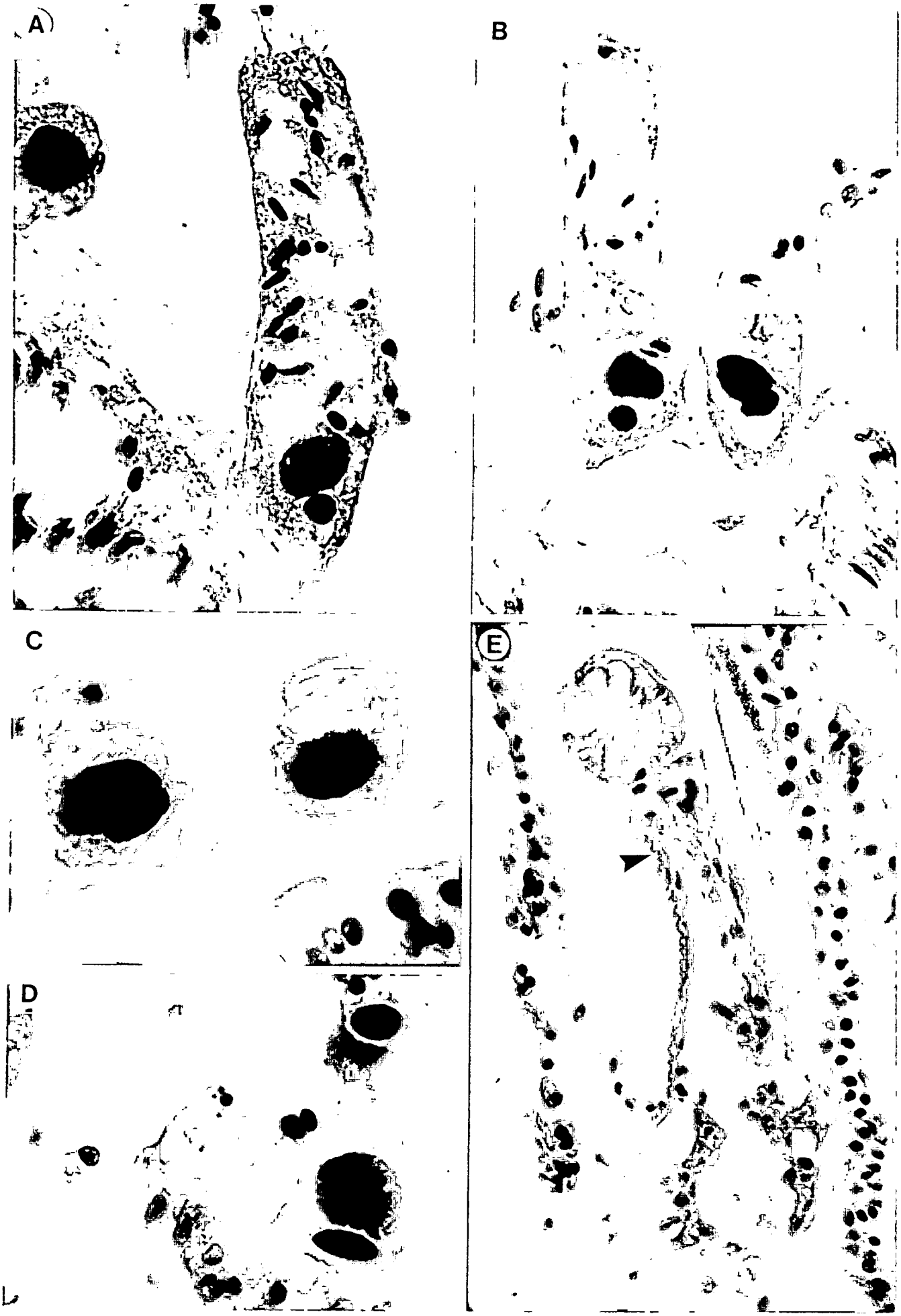
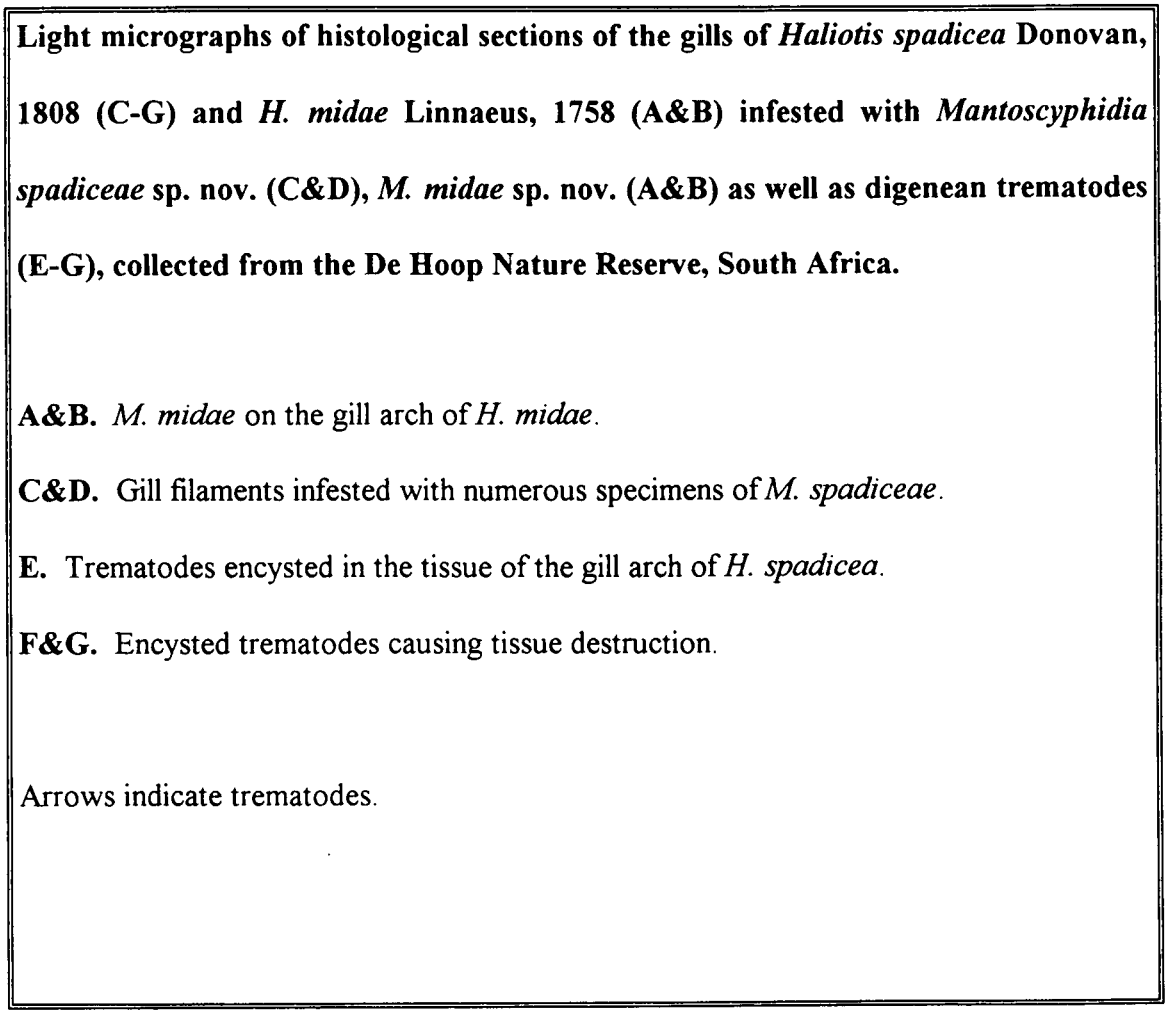

A
B
C
D
E

Figure 10.3



Light micrographs of histological sections of the gills of *Haliotis spadicea* Donovan, 1808 (C-G) and *H. midae* Linnaeus, 1758 (A&B) infested with *Mantoscyphidia spadiceae* sp. nov. (C&D), *M. midae* sp. nov. (A&B) as well as digenean trematodes (E-G), collected from the De Hoop Nature Reserve, South Africa.

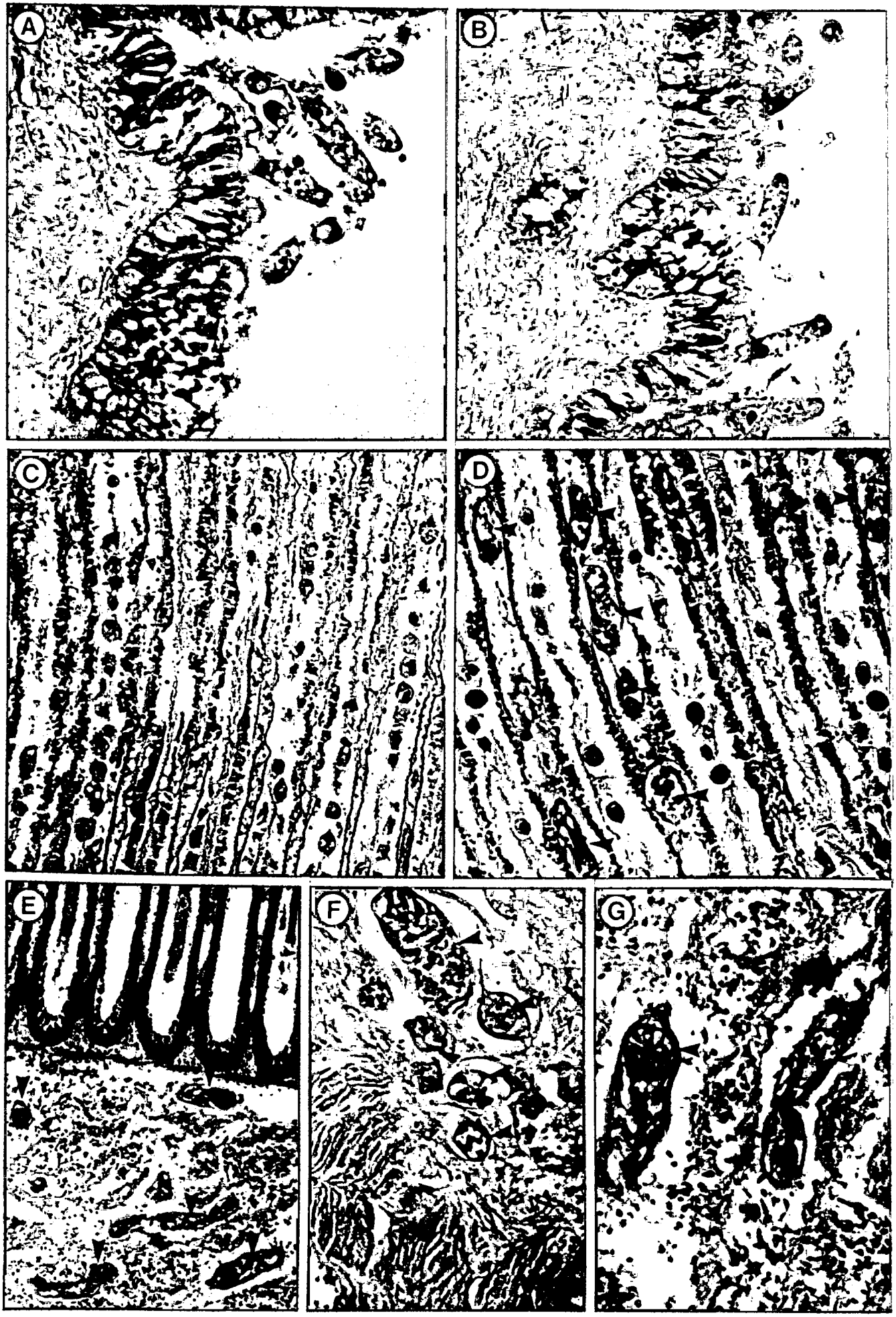
A&B. *M. midae* on the gill arch of *H. midae*.

C&D. Gill filaments infested with numerous specimens of *M. spadiceae*.

E. Trematodes encysted in the tissue of the gill arch of *H. spadicea*.

F&G. Encysted trematodes causing tissue destruction.

Arrows indicate trematodes.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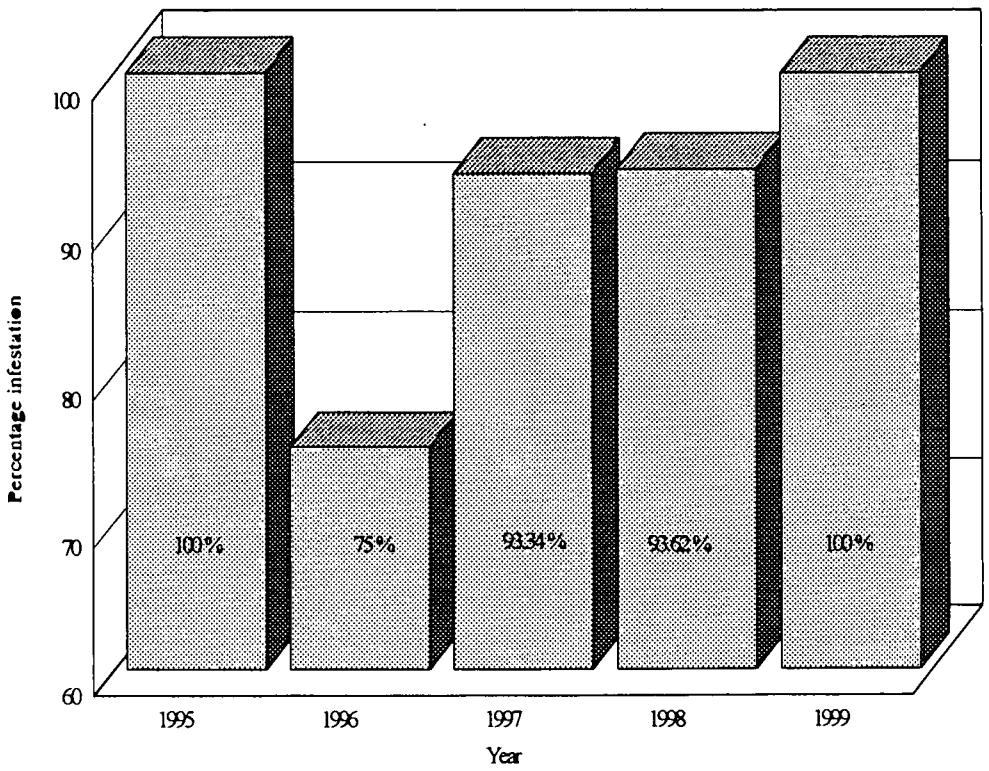

Figure 10.4 Percentage infestation of *Haliotis spadicea* Donovan, 1808 specimens collected over a five year period from the De Hoop Nature Reserve, South Africa.

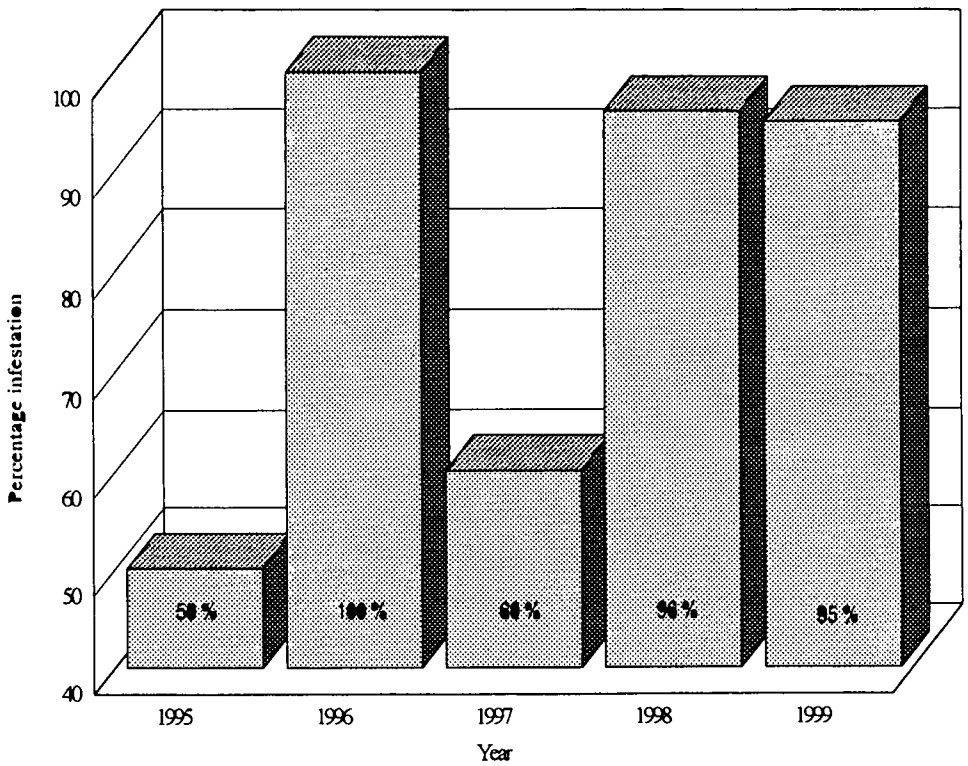

Figure 10.5 Percentage infestation of *Haliotis midae* Linnaeus, 1758 specimens collected over a five year period from the De Hoop Nature Reserve, South Africa.

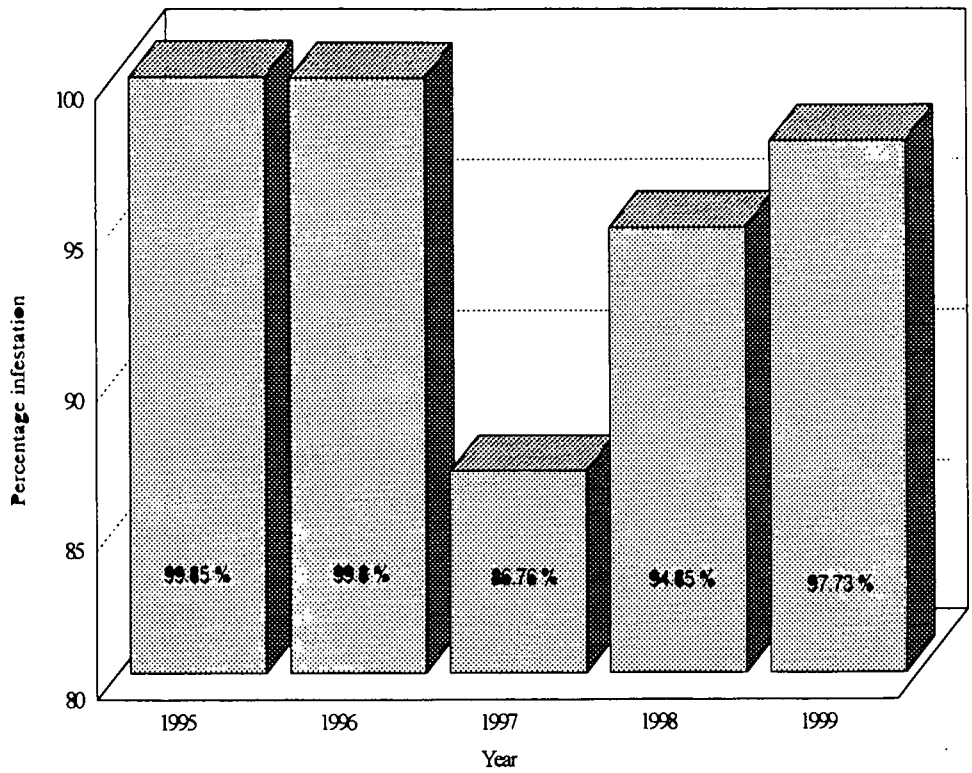

Figure 10.6 Total percentage infestation of both *Haliotis spadicea* Donovan, 1808 and *Haliotis midae* Linnaeus, 1758 specimens collected over a five year period from the De Hoop Nature Reserve, South Africa.

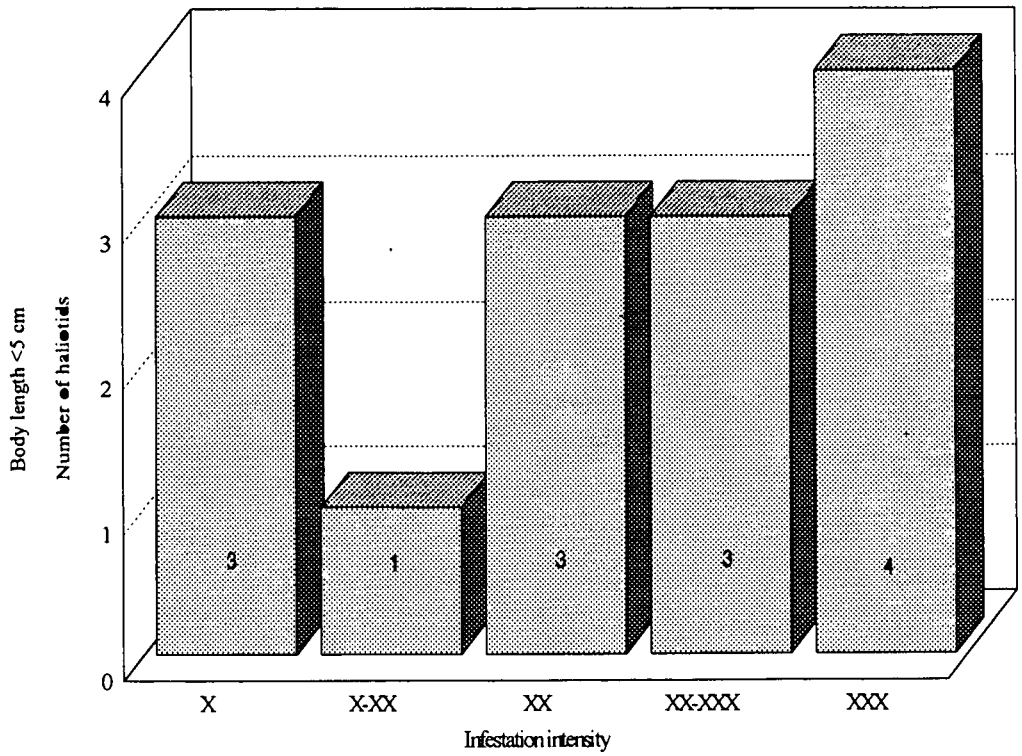

Figure 10.7 Frequency histogram of infestation intensity and number of *Haliotis spadicea* Donovan, 1808 specimens smaller than 5 cm in length, infested with scyphidiid peritrichs collected over a period of five years from the De Hoop Nature Reserve, South Africa.

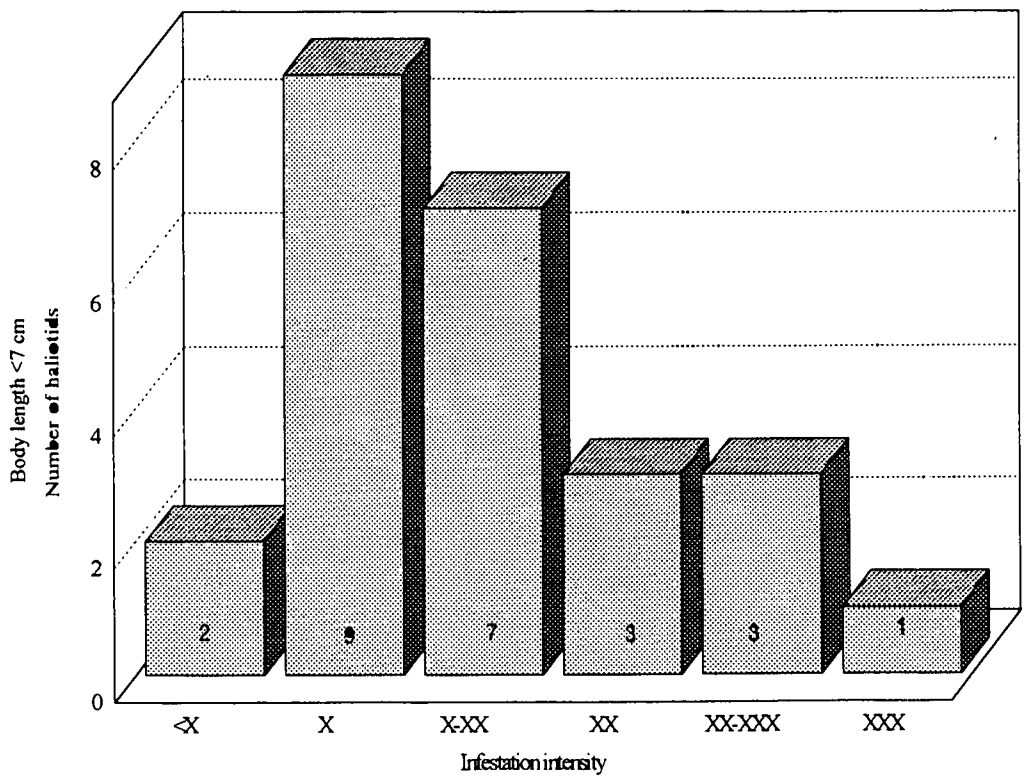

Figure 10.8 Frequency histogram of infestation intensity and number of *Haliotis midae* Linnaeus, 1758 specimens smaller than 7 cm in length, infested with scyphidiid peritrichs collected over a period of five years from the De Hoop Nature Reserve, South Africa.

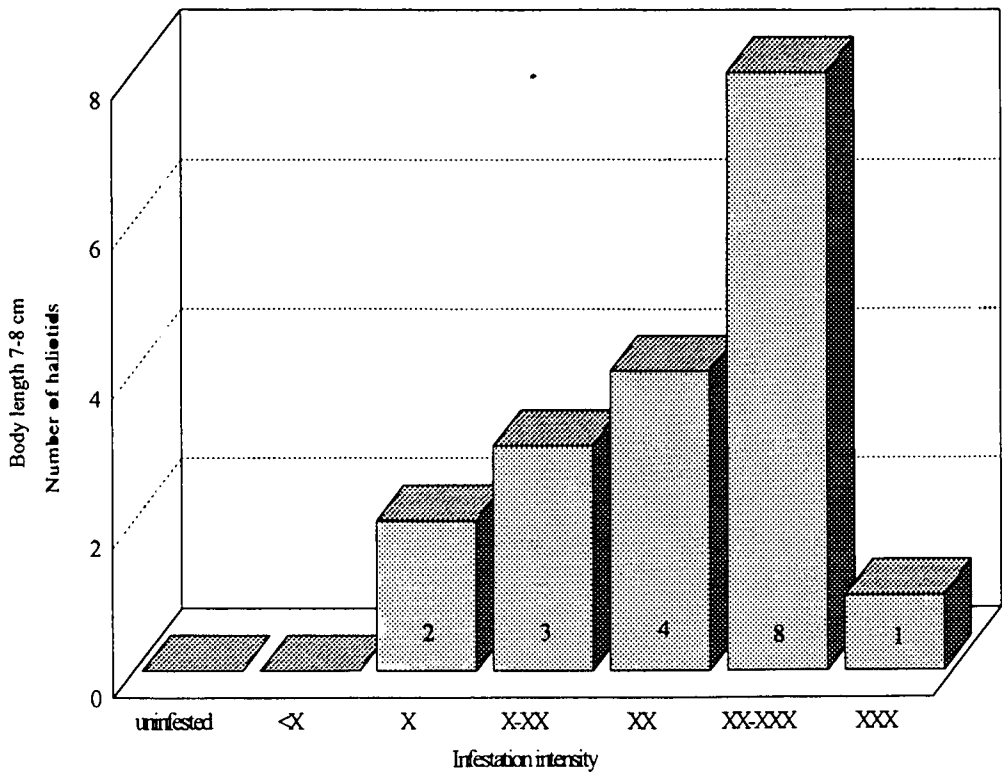

Figure 10.9 Frequency histogram of infestation intensity and number of *Haliotis spadicea* Donovan, 1808 specimens between 7 cm and 8 cm in length, infested with scyphidiid peritrichs collected over a period of five years from the De Hoop Nature Reserve, South Africa.

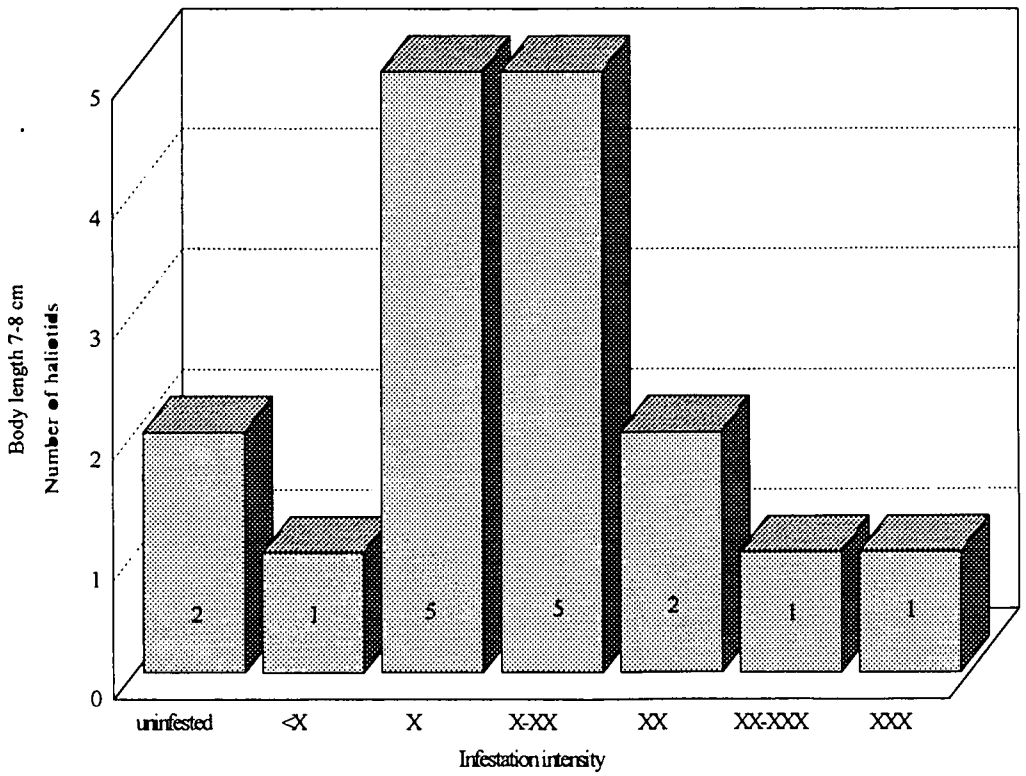

Figure 10.10 Frequency histogram of infestation intensity and number of *Haliotis midae* Linnaeus, 1758 specimens between 7 cm and 8 cm in length, infested with scyphidiid peritrichs collected over a period of five years from the De Hoop Nature Reserve, South Africa.

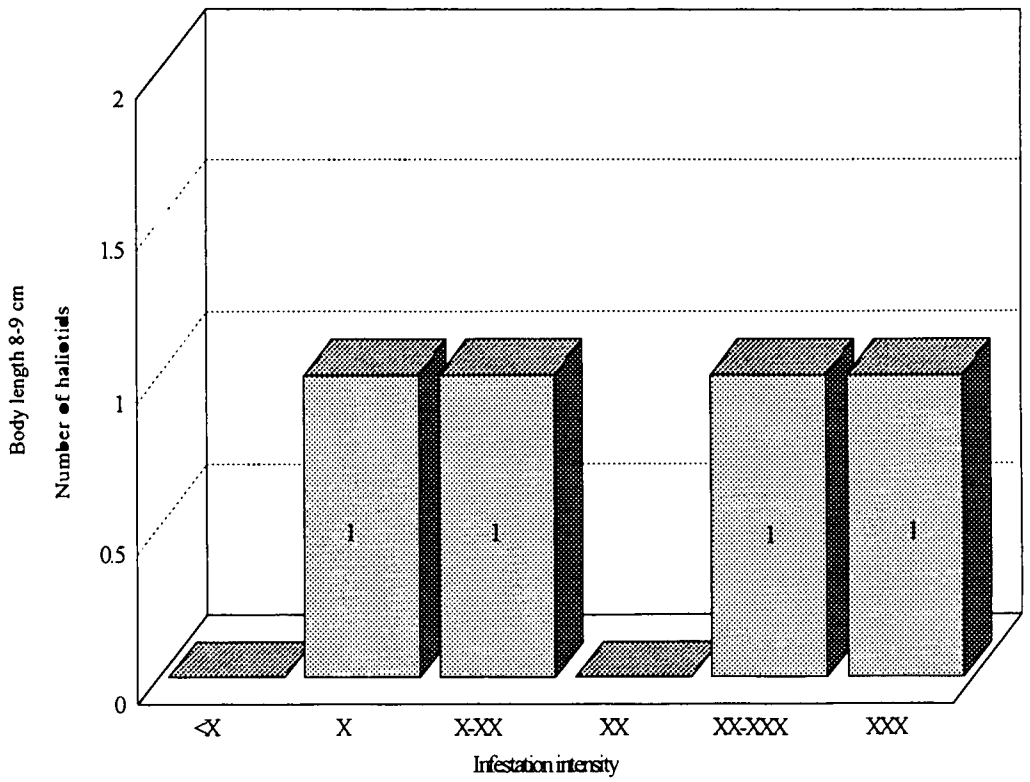

Figure 10.11 Frequency histogram of infestation intensity and number of the four largest *Haliotis spadicea* Donovan, 1808 specimens (between 8 cm and 9 cm in length), infested with scyphidiid peritrichs collected over a period of five years from the De Hoop Nature Reserve, South Africa.

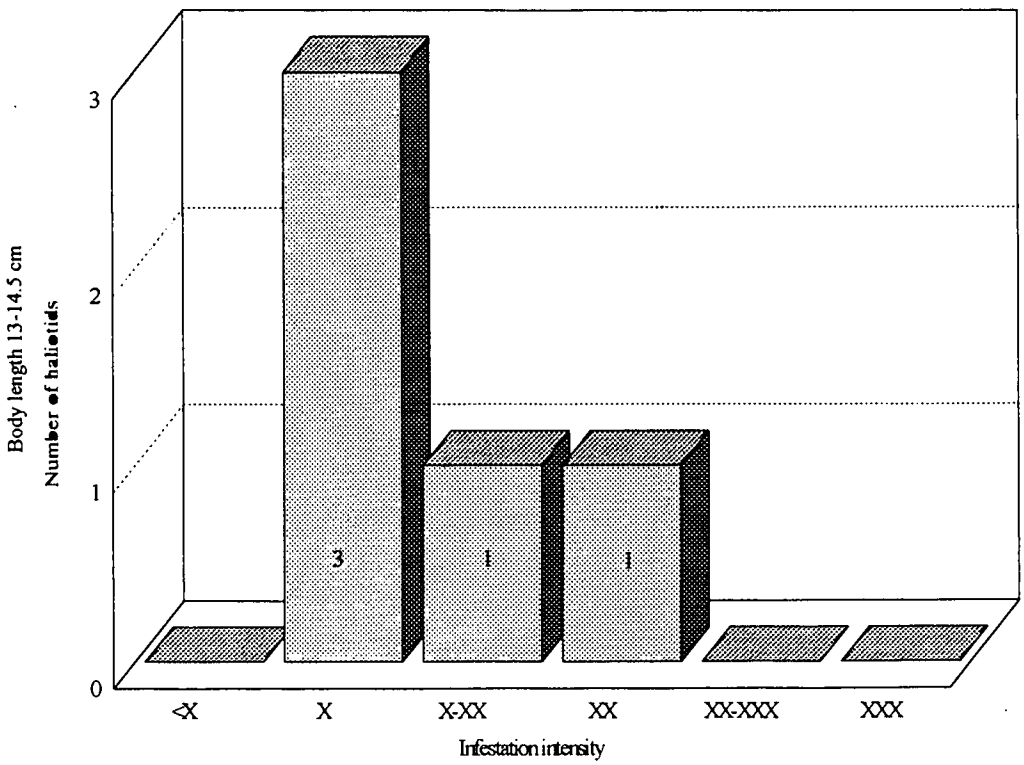

Figure 10.12 Frequency histogram of infestation intensity and number of the five largest *Haliotis midae* Linnaeus, 1758 specimens (between 13 cm and 14.5 cm in length), infested with scyphidiid peritrichs collected over a period of five years from the De Hoop Nature Reserve, South Africa.

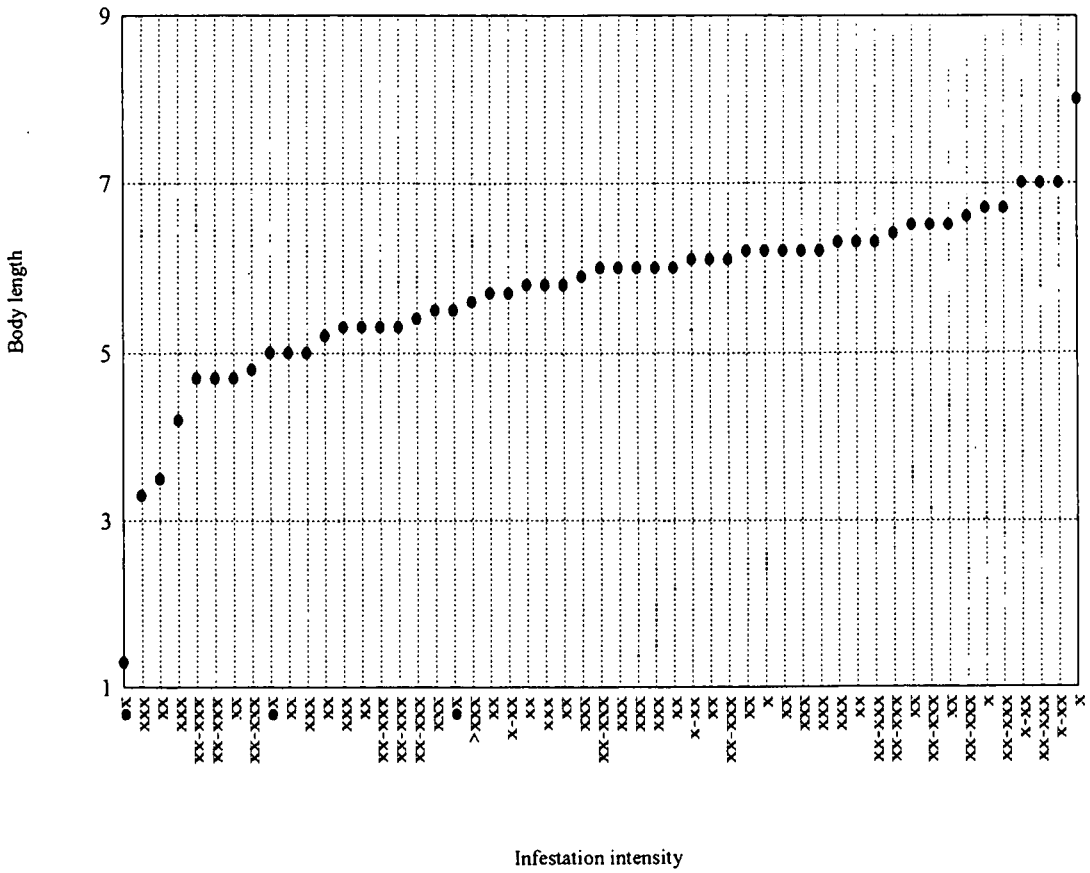

Figure 10.13 Distribution of the infestation intensities in *Haliotis spadicea* Donovan, 1808 specimens collected during April 1997 from the De Hoop Nature Reserve, South Africa.

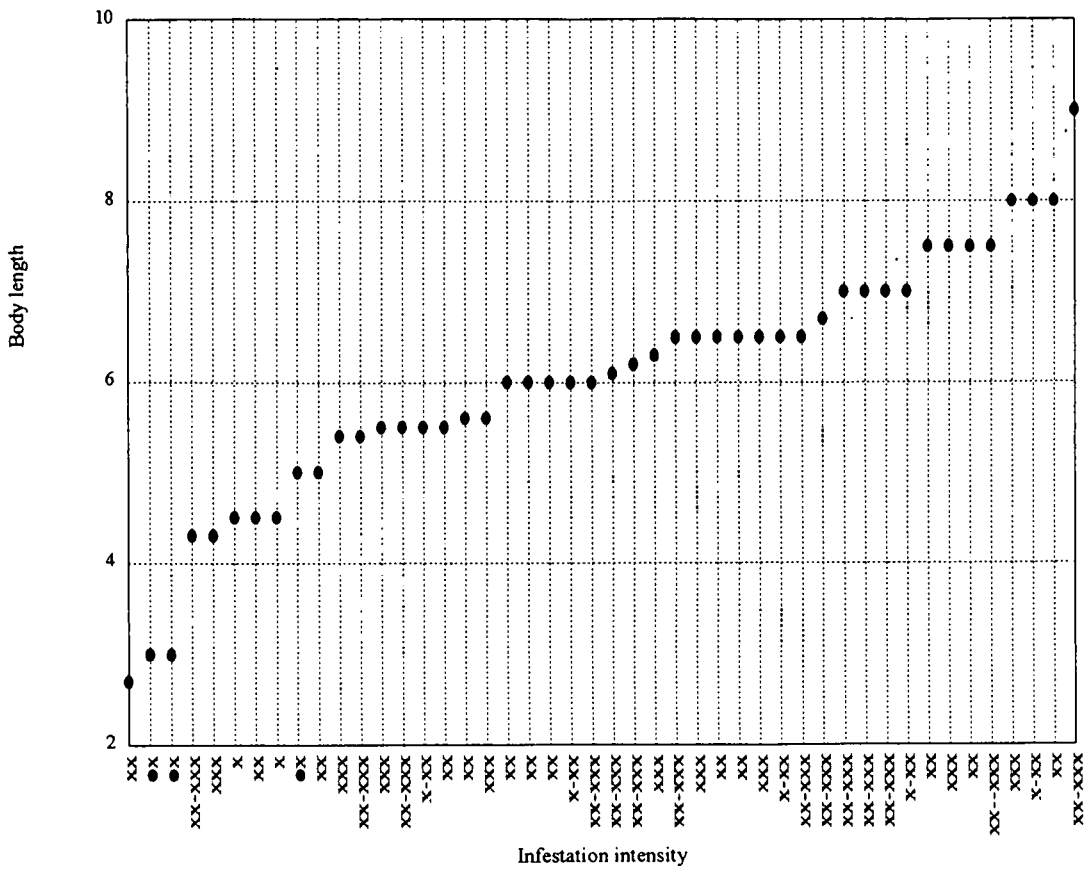

Figure 10.14 Distribution of the infestation intensities in *Haliotis spadicea* Donovan, 1808 specimens collected during April 1998 from the De Hoop Nature Reserve, South Africa.

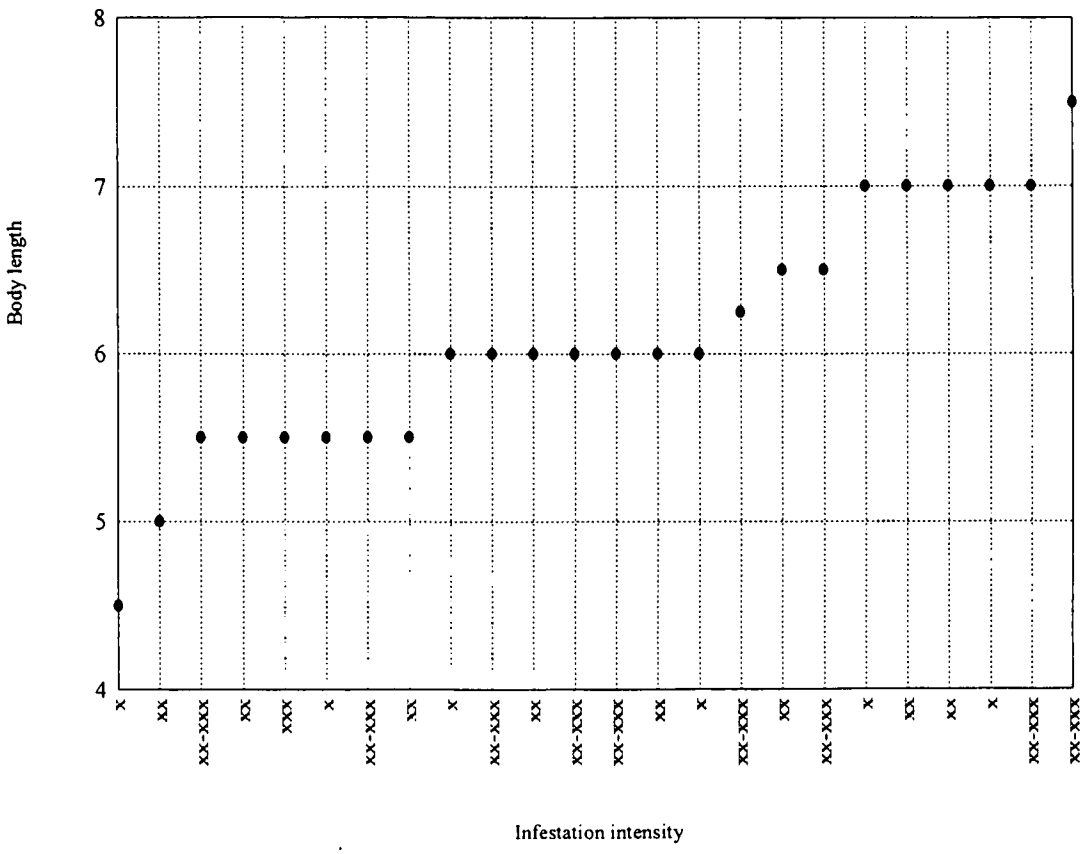

Figure 10.15 Distribution of the infestation intensities in *Haliotis spadicea* Donovan, 1808 specimens collected during April 1999 from the De Hoop Nature Reserve, South Africa.

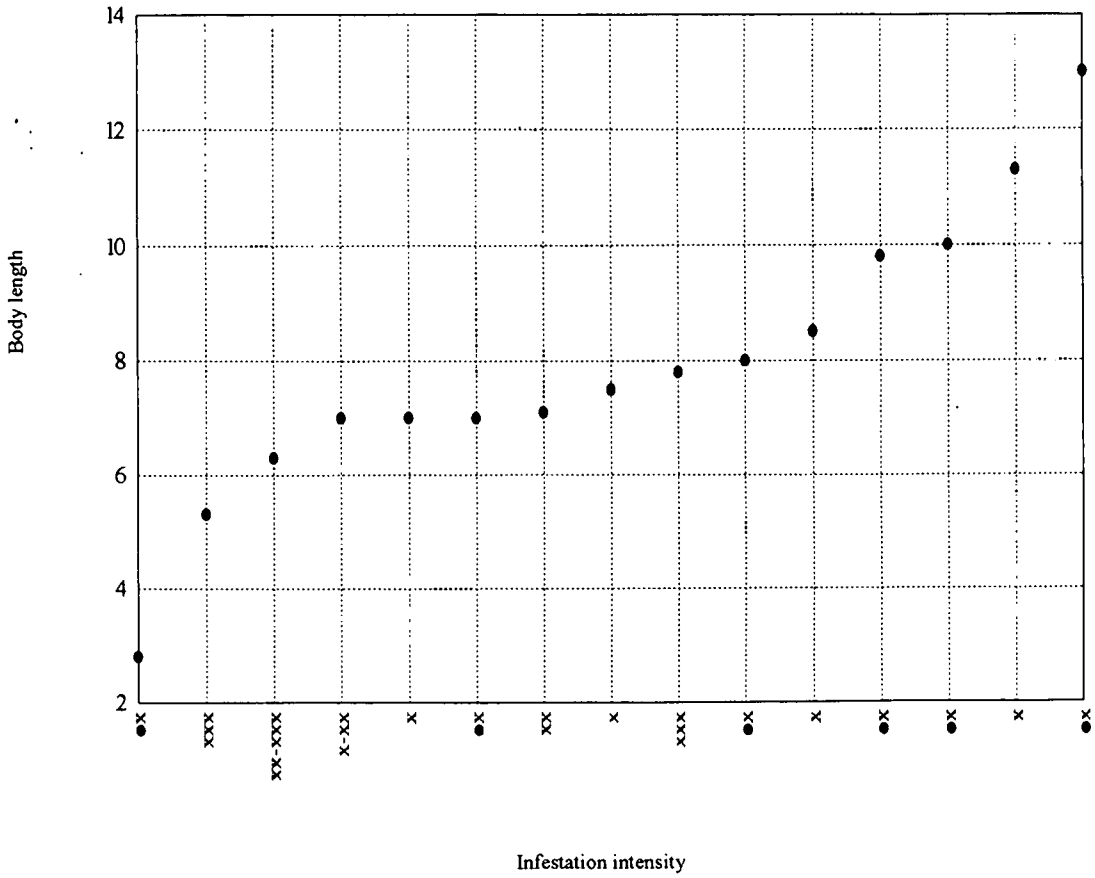

Figure 10.16 Distribution of the infestation intensities in *Haliotis midae* Linnaeus, 1758 specimens collected during April 1997 from the De Hoop Nature Reserve, South Africa.

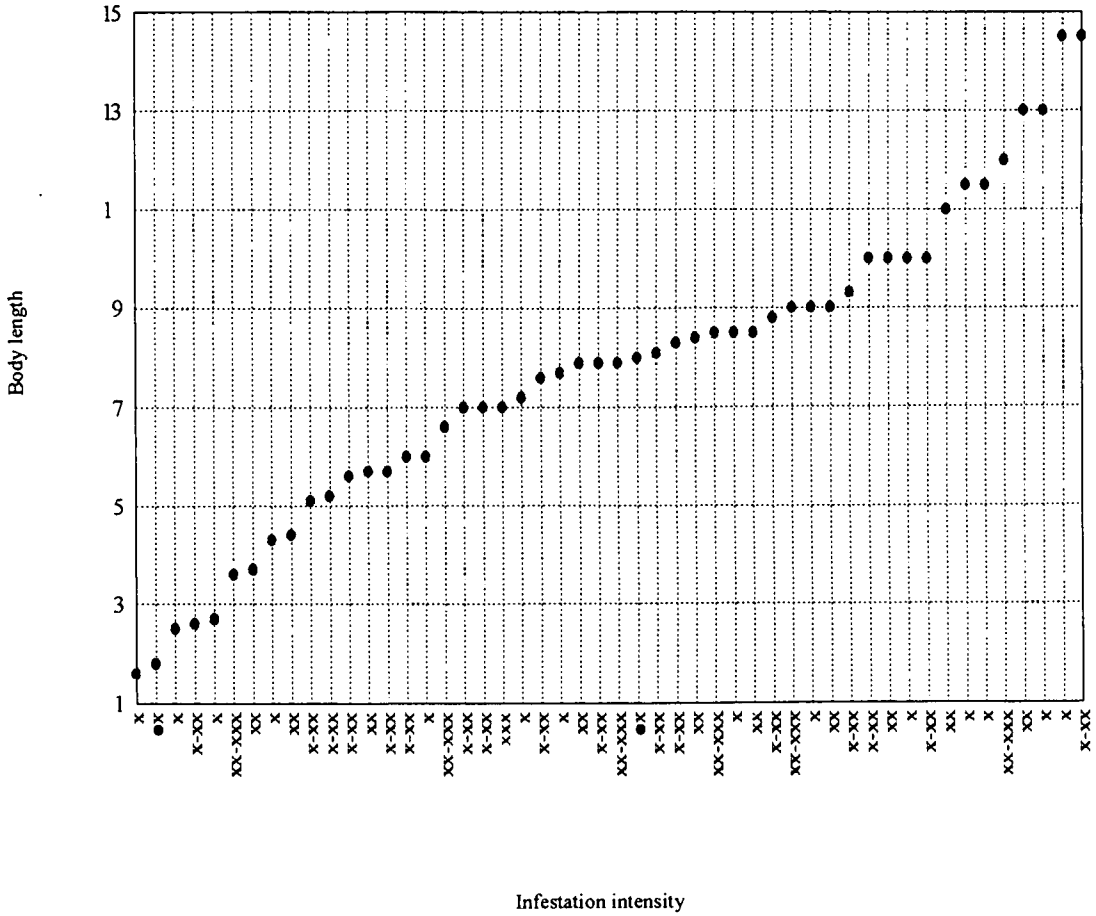

Figure 10.17 Distribution of the infestation intensities in *Haliotis midae* Linnaeus, 1758 specimens collected during April 1998 from the De Hoop Nature Reserve, South Africa.

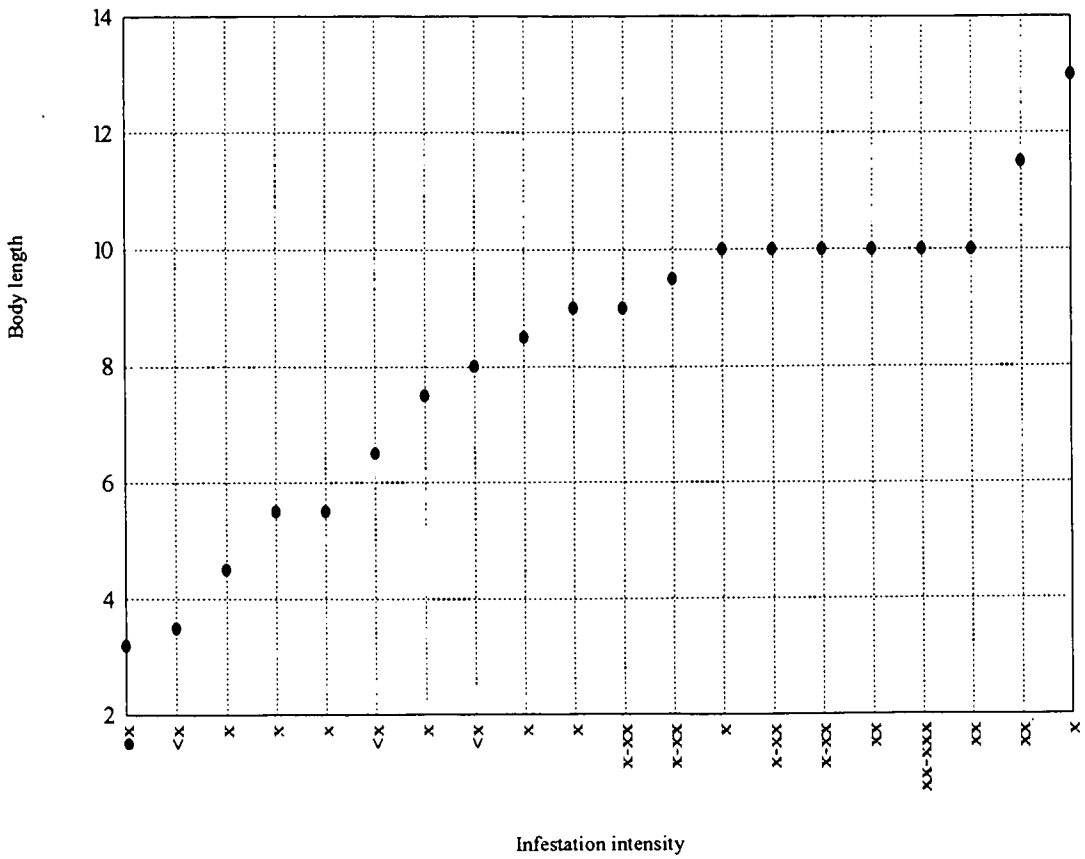

Figure 10.18 Distribution of the infestation intensities in *Haliotis midae* Linnaeus, 1758 specimens collected during April 1999 from the De Hoop Nature Reserve, South Africa.

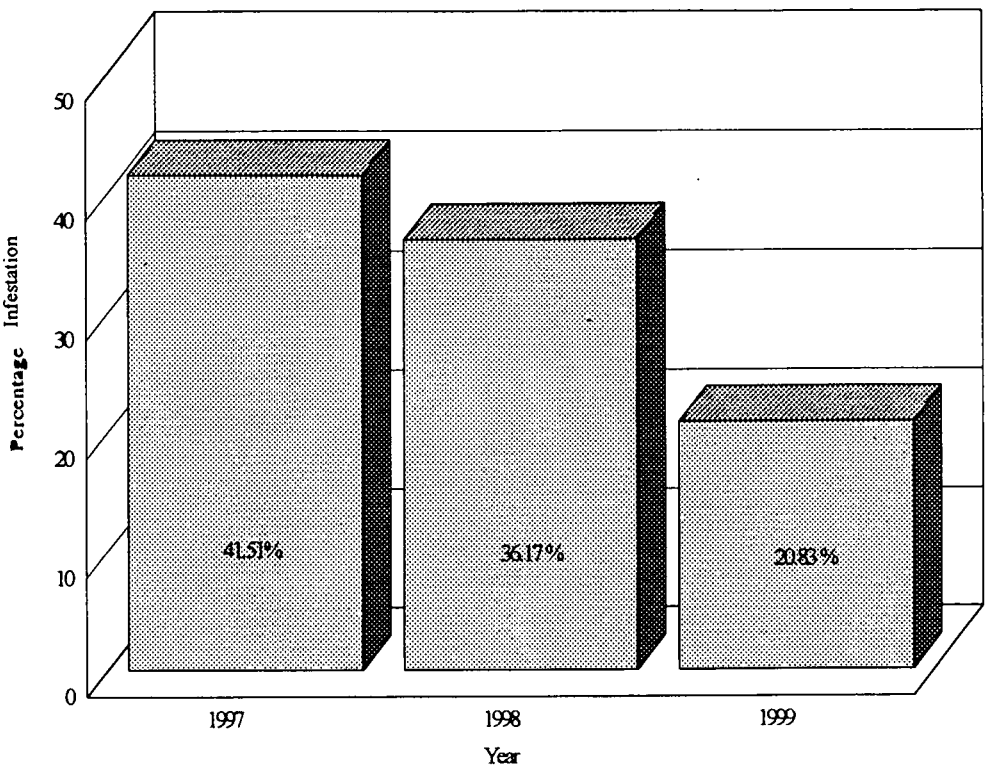

Figure 10.19 Percentage of *Caliperia perlemoenae* sp. nov. infestation occurring on *Mantoscyphidia spadiceae* sp. nov. from *Haliotis spadicea* Donovan, 1808 specimens, collected over a period of three years from the De Hoop Nature Reserve, South Africa.

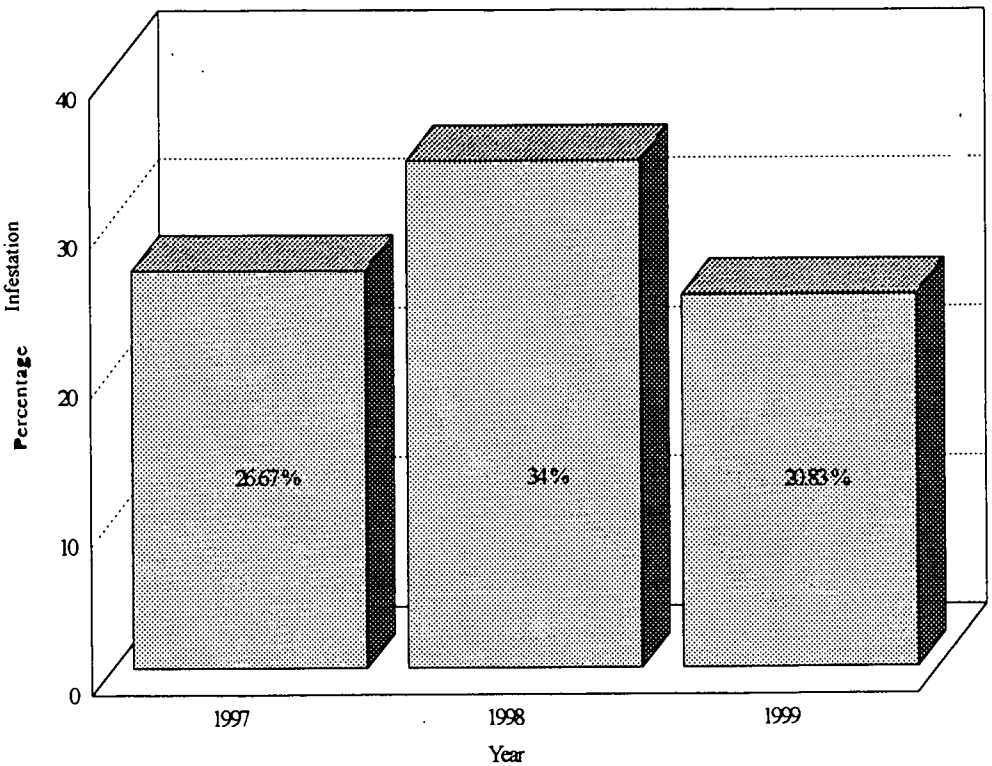

Figure 10.20 Percentage of *Caliperia perlemoenae* sp. nov. infestation occurring on *Mantoscyphidia midae* sp. nov. from *Haliotis midae* Linnaeus, 1758 specimens, collected over a period of three years from the De Hoop Nature Reserve, South Africa.

Chapter 11
Concluding Remarks

Host/Symbiont Associations

Research that has been carried out by the Aquatic Parasitology Group has shown that almost every marine invertebrate collected along the South African coast, harbour ciliophoran infestations. Results have shown that these associations are mostly not typical parasitic associations.

Van As, *et al.* (1998) recorded *Mantoscyphidia branchi* in association with all 19 species of limpets examined (from the South African zoogeographical province). Of these, 13 species are endemic to the South African coast line. *Licnophora limpetae* (the first record of a licnophorid from a true limpet host) is specific to the limpets in the same way that *M. branchi* is (Van As, Van As & Basson in press). Infestation statistics of Van As, *et al.* (1998) show that almost every limpet were infested and that these infestations were enormous. This is unlike any typical parasitic infestation, where parasites are usually overdispersed. No sign of pathology could be found either (Van As, Van As & Basson 1998).

Botha (1994) carried out a comprehensive study on the symbionts of the gastropod genus *Oxystele*. Five species occur along the South African coastline and all five are endemic. The same species of mobile ciliophoran, namely *Trichodina oxystelis* (Basson & Van As 1992) and the same species of scyphidiid peritrich, namely *Mantoscyphidia fanthami*, (Basson, *et al.* 1999) was found associated with all five *Oxystele* species. Botha concluded that the severe infestations recorded from the hosts, most likely do not have a detrimental effect, but rather play a significant role in respiration and cleaning symbiosis.

The fact that *Haliotis spadicea* was found to be infested with a different species of scyphidiid peritrich than *H. midae*, makes the haliotids' host/symbiont associations

unique. This does not conform to the symbiont association patterns that have so far been described from the other South African gastropods, namely where the same scyphidiid peritrich species were found in association with all the host species (*Patella* and *Oxystele*). A question that arises, is whether the other four South African haliotids have scyphidiid peritrichs occurring on their gills and whether each of these are different species to that of *Mantoscyphidia spadiceae* and *M. midae*.

I have had the opportunity to examine *Haliotis rubra rubra* specimens, which were collected by Prof. Linda Basson during her sabbatical study in 1998 in Tasmania, Australia, for the presence of scyphidiid peritrichs on their gills. Light microscopy and SEM studies revealed a different species to that of *M. spadiceae* and *M. midae*. Amongst haliotids it thus appears as if mantoscyphidians are host specific. Further studies on the other four South African haliotids from the east and west coast, as well as abalone from around the world are thus necessary, before it would be possible to confirm this hypothesis.

Host/Scyphidiid peritrich Association

According to the Newton-Harvey equation (Schmidt-Nielsen 1990) diffusion alone is sufficient to supply the centre of a spherical organism, with a body diameter of less than 1 mm, with oxygen. If the organism's oxygen consumption is lower than the oxygen concentration in the surrounding waters, diffusion will take place from the outside, where there is a higher oxygen concentration, to the inside of the organism. The body diameter of the two mantoscyphidian species found in the present study varies between 15 and 45 µm, and can thus not hinder oxygen diffusion from the surrounding water and haliotid gills.

Furthermore, the cylindrical bodies of the scyphidiid peritrichs may in fact enlarge the respiration area of the gills. *Haliotis spadicea* occur higher up on the rocky shore than *H. midae* and is sometimes exposed, especially during low tide. During these stressful times of exposure, the scyphidiid peritrichs may in fact play a more significant role in

enlarging the respiration area of *H. spadicea*'s gills. *Haliotis midae* occur in shallow pools and is usually not exposed during low tides. Statistical analysis has shown that perlemoen's scyphidiid peritrich infestation levels were constantly lower than that of the Venus ears, and the assumption is thus that the perlemoen probably do not need as high a scpyhidiid peritrich infestation as the Venus ears would in order to cope with respiration..

As in the case of *M. branchi*, feeding in the scyphidiid peritrichs is accomplished by the active movement of the adoral ciliary spiral, which consists of a number of long cilia. The mantoscyphidians not only enlarge the respiration area of the host, but because of their abundance and own ciliary activity they also play an important role in the countercurrent flow of circulation of oxygenated water over the haliotid gills.

In unpolluted, pristine environments, such as the De Hoop Nature Reserve, our Research Group has found high ciliophoran infestations on many marine invertebrates. More polluted collection sites, such as the east coast of Senegal (West Africa) and the east coast of South Africa (Basson pers. comm.)*[1] have lower infestations of ciliophoran fauna on their marine invertebrates. It is thus clear that these symbionts are absent in polluted habitats, but thrive in pristine environments, and this also serves as evidence that the associations are most likely not parasitic. A characteristic of parasitic infestations in fish, for example, is a drastic increase or proliferation in parasite numbers in a polluted habitat which results in massive fish mortalities. Khan (1990) found that fish which were experimentally exposed to crude oil, had higher infestations of trichodinids than in natural fish populations, which eventually led to their death. He also found that fish from the Gulf of Alaska, where the Exxon Valdez oil disaster took place, died from secondary effects of the pollution, namely massive parasite infections. On the other hand, the symbionts found on the marine invertebrates by the Aquatic Parasitology Research Group tend to disappear when pollution increases.

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The Aquatic Parasitology Research Group doesn't completely understand the specific type of association between the scyphidiid peritrichs and haliotids, yet we prefer to refer to these scyphidiid peritrichs as symbionts. Whether the association is a mutualistic or commensialistic one, or somewhere in between, will only be clarified as our studies in the marine environment progresses, and to incorporate the data on all the different marine invertebrates and their associated symbionts.

Host/Caliperid Association

As previously mentioned, *Caliperia longipes* occur on the gill filaments of fish. *Caliperia maliculiformis* was the first caliperid recorded from a marine invertebrate as primary host. This ecto-hypersymbiont was found associated with *Mantoscyphidia branchi* which occurred on South African limpets, but was not found in association with *M. marioni*. *Caliperia perlemoenae* was found associated with both *M. spadiceae* and *M. midae*, and is thus less host specific than *C. maliculiformis*.

No ecto-hypersymbionts have so far been found in association with scyphidiid peritrichs from *Oxystele* (*M. fanthami*) and *Turbo* species, around the South African coastline (Basson, pers. comm. *[2]). It seems as if caliperids favour the open and depressed (flat) shell shape of patellid and haliotid gastropods, and not the higher spire and confined shell shape of the top shells and turbans. Although these caliperids could interfere with feeding and reproduction in scyphidiid peritrichs (Fig.8.5E-G & 10.1G&H), no evidence was found that they are pathogenic. In fact, their ciliary activity could also play an important role in the circulation of oxygenated water over the haliotid gills, as in the case of the mantoscyphidians.

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Host/Trematode Association

A tremendous amount of research on freshwater and marine digenean trematodes has been done world-wide, but limited research has been carried out on marine digenean trematodes around the South African coastline. Of all the symbionts found associated with the haliotids in the present study, only the trematodes have the potential to become pathogenic. The alarmingly high infections found in *Haliotis spadicea* could be of concern, as the trematodes can cause tissue destruction and affect the reproductive organs. However, *H. spadicea* specimens collected appeared healthy and in good reproductive condition.

I am not unduly concerned about these infections in the natural milieu, but realise that it could have a devastating effect in aquaculture facilities. In breeding conditions, and more particularly in aquaculture, the environments in which a parasite and host are kept are often quite different from the natural milieu. The most evident change concerns the probability of encounter between infecting stages of parasites and their targets, since dispersion of parasites is limited while the density of the hosts is increased.

During the 1999 survey a high number of adult trematodes were observed on the gills of *H. spadicea*. SEM examination of the gills collected during 1995 to 1998 did not reveal as many adult trematodes as was found during April of 1999 alone. If it is indeed the case that all the stages of the trematode's life-cycle occur in the Venus ear, in other words if self-infection occurs, the importance of fully describing the life-cycle and morphology becomes evident. Trematode infections are usually controlled by breaking the life-cycle at some point, targeting one of the hosts. If all the stages of the life-cycle occur within *Haliotis spadicea*, the possible means of eradicating the trematodes, without affecting the host become limited. This, however, is a study all on its own.

During my study several questions arose that still need to be answered:

- Whether the other four South African haliotids have scyphidiid peritrichs and associated ecto-hypersymbionts occurring on their gills and whether each of these are different species to that of *Mantoscyphidia spadiceae* and *M. midae*,
- Whether other abalone species of the world harbour scyphidiid peritrich and associated ecto-hypersymbiont infestations, and what their host/symbiont associations are, and
- The identification of the digenean trematode found in *Haliotis spadicea*, as well as the unravelling of its life-cycle.

During 1992 the first survey on the Intertidal Symbiont program was carried out. As previously mentioned my study forms part of this program, but it is only a piece put in place in this jigsaw puzzle of trying to understand the host/symbiont associations in the marine environment. This is an ongoing project, which I am sure, will answer some of the questions that arose during my study.

Chapter 12
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Abstract/ Opsomming

Abalone, locally known as perlemoen, are herbivorous marine gastropods with all species in one genus, *Haliotis* Linneaus, 1758. Abalone have been commercially exploited since ancient times. Along the southern African coast, the genus *Haliotis* comprises six endemic species. Surveys carried out from 1995 to 1999 at the De Hoop Nature Reserve along the south coast of South Africa, revealed the presence of scyphidiid peritrichs, of the genus *Mantoscyphidia* Jankowski, 1980, occurring in abundance on the gills of *Haliotis spadicea* Donovan, 1808 and *H. midae* Linnaeus, 1758. These were described as two new species, i.e. *M. spadiceae* sp. nov. and *M. midae* sp. nov. The mantoscyphidians in turn hosted ellobiophryids of the genus *Caliperia* Laird, 1953, which was also described as a new species, *Caliperia perlemoenae* sp. nov., occurring on both mantoscyphidian species. During the study a perlemoen aquaculture facility, Danger Point Abalone Farm, was also visited, specimens of *H. midae* were examined and found to harbour the same species of scyphidiid peritrich and caliperid than *H. midae* collected from the De Hoop Nature Reserve. In the present study, severe infections of redial, cercarial and metacercarial stages and a few adult specimens of a digenean trematode were also found in the digestive gland of *Haliotis spadicea* as well as on the gill filaments. In order to elucidate the symbiont/host associations field experiments and histopathological examinations were carried out. These led to the conclusions that the host/scyphidiid peritrich associations are most likely not parasitic. In the case of the mantoscyphidian/caliperid association, no clear evidence was found that the caliperids are pathogenic to their mantoscyphidian hosts. On the other hand, the trematodes could be potential pathogens since they can cause tissue destruction and affect the reproductive organs in the host.

Verteenwoordigers van die genus *Haliotis* Linnaeus, 1758 is mariene herbivore met ses endemiese spesies versprei rondom die Suid-Afrikaanse kus. Die kommersiële verbruik van spesies in die genus *Haliotis* is al vir baie eeue bekend. Opnames wat tydens 1995-1999 by De Hoop Natuur Reservaat langs die suidkus van Suid-Afrika uitgevoer is, het die voorkoms van groot getalle sessiele siliofore wat aan die genus *Mantoscyphidia* Jankowski, 1980, behoort, op die kieue van *Haliotis spadicea* Donovan, 1808 en *H. midae* Linnaeus, 1758 aangetoon. Twee nuwe spesies, naamlik *M. spadiceae* sp. nov. en *M. midae* sp. nov. is beskryf. Verteenwoordigers van die genus *Mantoscyphidia* speel op hul beurt gasheer vir siliofore van die genus *Caliperia* Laird, 1953, wat ook as 'n nuwe spesie beskryf is, naamlik *Caliperia perlemoenae* sp. nov. Tydens die studie is 'n perlemoen-akwakultuur fasiliteit, "Danger Point Abalone Farm", besoek. *Haliotis midae* individue is ondersoek en daar is gevind dat hulle dieselfde sessiele siliofoor (*M. midae*) en *Caliperia* spesie, as *H. midae* van die De Hoop Natuur Reservaat op hul kieue het. Tydens die huidige studie is massiewe infeksies van redia, serkarië en metaserkarie-stadiums, sowel as 'n paar volwasse digenetiese trematode in die verteringsklier asook op die kieue van *H. spadicea* gevind. Ten einde lig te werp op die simbiont/gasheer verwantskappe, is veldeksperimente sowel as histopatologiese ondersoeke uitgevoer. Hieruit is daar tot die gevolgtrekking gekom dat die gasheer/sessiele siliofoor assosiasie heelwaarskynlik nie parasities van aard is nie. Geen duidelike bewyse is gevind wat daarop kon dui dat die verteenwoordigers van die genus *Caliperia* patogenies vir hul sessiele siliofoor gashere is nie. Daarenteen kan die trematood potensieel patogenies wees omdat hulle weefselvernietiging kan veroorsaak en die voortplantingsorgane van die gasheer nadelig kan affekteer.

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