REPRODUCTIVE PROCESSES OF SCYPHIDIID PERITRICHS ASSOCIATED WITH LIMPET AND HALIOTID HOSTS ALONG THE COAST OF SOUTH AFRICA

by

Helene Peters

Thesis submitted in fulfilment of the requirement for the degree Philosophiae Doctor in the Faculty of Natural and Agricultural Sciences, Department of Zoology and Entomology, University of the Free State

Promotor	Prof. Linda Basson
Co-promotor	Dr. Liesl L. van As

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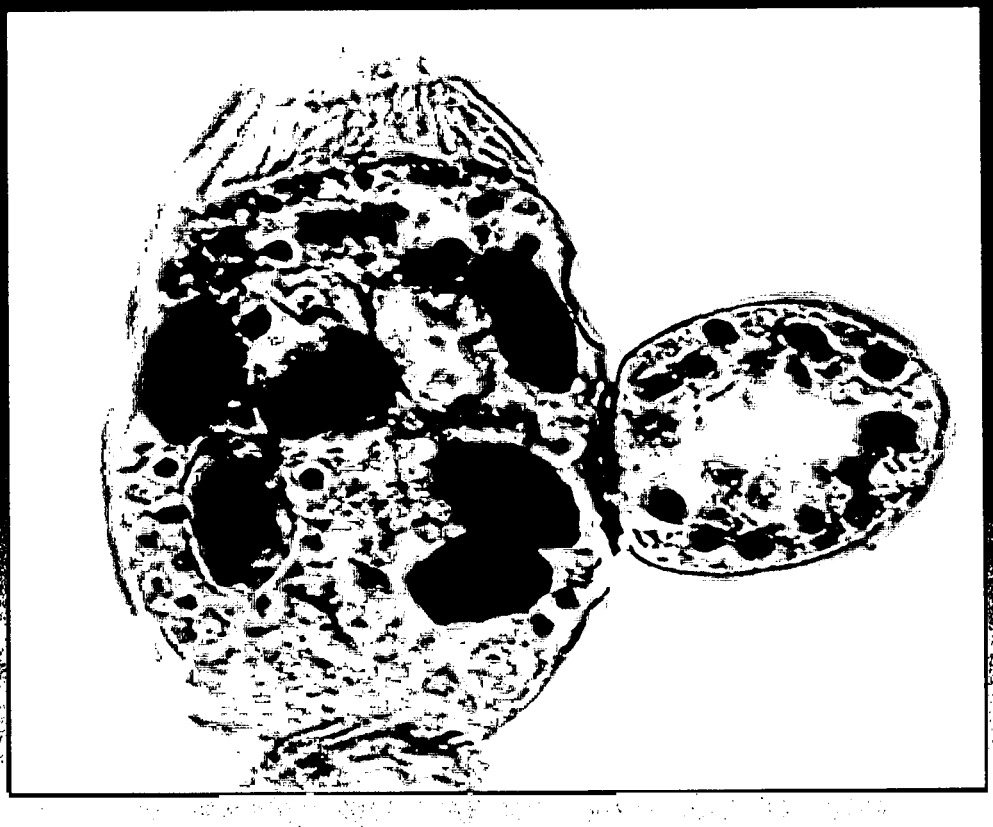

"Lord, you have made so many things!
There is the ocean, large and wide,
Where countless creatures live,
Large and small alike."

Psalm 104:24 - 25

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Chapter 1

Introduction

During the early 19th century a lot of progress was made in clarifying reproduction patterns and correlating cytological events with reproductive cycles in the kingdom Protozoa Goldfuss, 1818. During the first half of the 20th century various scientists studied the reproductive processes of peritrichs and made valuable contributions (Enriques 1907; Popoff 1908; Awerinzew 1912; Caullery & Mesnil 1915; Diller 1928; Finley 1936,1939; Rosenberg 1940; Padnos & Nigrelli 1942; Finley 1943; Colwin 1944; Davis 1947; Finley 1952; Dobrzañska 1961; Vávra 1961). Although thousands of recent papers exist on reproduction of ciliates, not much work has been done on epibiontic peritrichs (Lom personal communication)[1].

Some of the most interesting reproductive phenomena are to be found in the subclass Peritrichia Stein, 1859. Binary fission, the formation of telotrochs, pre-conjugation fission and conjugation are well-established phenomena in this subclass (Finley 1952; Walker, Roberts & Usher 1986). Binary fission, budding and the formation of telotrochs are asexual methods of reproduction while pre-conjugation fission is the conjugant or sex differentiating fission, and conjugation is the sexual method of reproduction.

The Aquatic Parasitology Research Group in the Department of Zoology and Entomology at the University of the 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 Research Group forms part of the Okavango Fish Parasite Project. This project was initiated in 1997 and has already led to a number of scientific publications.

[1] Prof. Jiri Lom, Institute of Parasitology, Academy of Sciences of the Czech Republic, Branisovska 31, 370 05 Ceské Budejovice, Czech Republic.

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, two Ph.D. and six M.Sc. students have already completed their research on aspects of intertidal parasites and symbionts.

Van As (1997) studied ciliophoran parasites of limpets (Patellogastropoda) and Smit (2000) studied the biology of gnathiid isopods and their role as vectors of fish blood parasites, both of these were Ph.D. studies. The M.Sc. works are that of Botha (1994) who studied ciliophoran symbionts of *Oxystele* Philippi, 1847 species; while Loubser (1994) studied the ciliophorans of intertidal fishes; Molatoli (1996) investigated the symbionts of red bait, *Puyra stolonifera* (Heller, 1878); Smit (1997) studied gnathiid isopods of intertidal fishes and Grobler (2000) investigated caligid fish parasites. **The current author, Peters (néé Botes) studied scyphidiid peritrichs associated with *Haliotis* species (Botes 1999)**. These studies have led to the publication of various articles, congress proceedings and extended abstracts. Currently other M.Sc. projects are also being carried out and various Honours and Final year Zoology projects have also been completed within this program.

Since the current study only deals with peritrichs only research concerning this will be listed. **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 1999; Van As, Van As & Basson 1999a; Van As & Van As 2000; Botes, Basson & Van As 2001a, Van As & Van As 2001; Peters, Van As, Basson & Van As in prep [see Appendix C]); **congress proceedings** (Van As, Van As & Basson 1995; Van As, Van As & Basson 1996a; Botes, Basson & Van As 1997; Van As, Basson & Van As 1997; Botes, Basson & Van As 1998; Van As, Van As & Basson 1998; Van As, Van As & Basson 1999b; Botes, Van As, Basson & Van As 2001, as well as **published abstracts** (Botha & Basson 1994; Loubser, Van As & Basson 1994; Van As & Basson 1994; Van As, Van As & Basson 1996b and Botes, Basson & Van As 2001b).

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

During fieldwork carried out from 1993 to 1999 occasional observations were made of binary fission, telotroch formation and conjugation in populations of *Mantoscyphidia* Jankowski, 1980 a species associated with haliotids and limpets. These observations led to the present study in which the reproductive processes of *Mantoscyphidia* species are described.

Objectives:

- to determine if binary fission, telotroch formation and conjugation occur in *Mantoscyphidia branchi* Van As, Basson & Van As, 1998, *M. spadiceae* Botes, Basson & Van As, 2001, *M. marioni* Van As, Basson & Van As, 1998 and *M. midae* Botes, Basson & Van As, 2001. These scyphidiid peritrich species have all been described by the Aquatic Parasitology Research Group as new species from marine mollusc hosts.

  - to describe the process of **binary fission** in these *Mantoscyphidia* species.
  - to describe the process of **telotroch formation** in these *Mantoscyphidia* species.
  - to describe the process of **conjugation** in these *Mantoscyphidia* species.

The following objectives were not the main focus of the study, but were included as part of the study:

- to determine if binary fission, telotroch formation and conjugation occur in *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep, a new species that has recently been submitted for publication (see Appendix C).

  - the reproductive processes in *E. maliculiformis* were described when observed, but these processes were not studied in detail. In some cases the occurrence of the reproductive processes was just noted.

In order to collect data to achieve these objectives, fieldwork was carried out by The Aquatic Parasitology Research Group from 1993 to 1999, and this data was used for comparative studies. Field work was also carried out during 2000 to 2002 (March, April and November) at the De Hoop Nature Reserve along the south coast of South Africa. Light and scanning electron microscopy studies of material collected in the field were carried out in the laboratory in Bloemfontein.

The layout of this thesis 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 the most important and relevant contributions of other scientists who described the reproductive processes of some peritrichs. This will be presented in **Chapter 3**. Chapter 4, 5 and 6 present the results of this study. In **Chapter 4** the process of binary fission in the genus *Mantoscyphidia* is discussed. The formation of the free swimming migratory stage, the telotroch, in *Mantoscyphidia* is discussed in **Chapter 5**. **Chapter 6** presents a description of the process of conjugation in *Mantoscypidia* species. In this chapter the first record of conjugation in the genus *Ellobiophrya* (Chatton & Lwoff, 1923) is also provided.

The results are interpreted and discussed in **Chapter 7**. Valuable contributions and suggestions for future studies are also provided in this chapter. **Chapter 8** contains the literature referred to in this thesis, followed by Appendix A to C. **Appendix A** contains additional data from field trips from 1996 to 1999. **Appendix B** contains a glossary of terms used throughout this thesis. In **Appendix C** the article on *Ellobiophrya maliculiformis* that will be submitted for publication in Acta Protozoologica is presented. The Abstract and Acknowledgements follow after Appendix C.

Chapter 2
Materials and Methods

Study Area

Haliotids were collected from the De Hoop Nature Reserve (34°28'S, 20°30'E) on the south coast of South Africa (Figs. 2.1 & 2.2A - E). Haliotids were also obtained from the Danger Point Abalone Farm near Gansbaai, and the Abagold Farm in Hermanus.

Limpets were collected from the Goukamma Nature Reserve (34°20'S, 22°55'E), De Hoop Nature Reserve (34°28'S, 20°30'E) and Keurboom Beach (23°28'S, 34°0'E) on the south coast; Mc Dougall's Bay (29°45'S, 16°45'E) (Figs.2.3B & C), Alexanderbaai (Fig. 2.3D) and the Olifants River Mouth (31°22'S, 18°18'E) on the west coast; Bazley (30°22'S, 30°40'E) and at the rocky shores of Lake St. Lucia (28°10'S, 32°30'E) (Fig. 2.3A) on the east coast of South Africa; and on the east coast of Marion Island at Boulder Beach (46°54'S, 37°45'E) (Fig. 2.3E & F) which is situated in the southern Indian Ocean, 2300 km south-east of Cape Town, South Africa.

Collection of molluscs

Two of the six South African abalone species, i.e. *H. midae* Linnaeus, 1758 (perlemoen) (Fig. 2.2F) and *Haliotis spadicea* Donovan, 1808 (venus ears) (Fig. 2.2G) were collected from infratidal pools on the rocky shore. *Haliotis spadicea* is found in shallow infratidal pools, occupying small crevices. *Haliotis midae* is commonly found in the infratidal zone amongst 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 Branch, Griffiths, Branch and Beckley (1994) *Haliotis parva* Linnaeus, 1758 also occurs in the De Hoop Nature Reserve but was never collected during the study period. A

total of 284 haliotids were collected and examined over a six-year period, these included 24 haliotids from the Danger Point Abalone Farm near Gansbaai, and eight haliotids from the Abagold Farm in Hermanus.

Seventeen endemic limpet species occur between Cape Point and Cape Agulhas in the South African zoogeographic marine province (Branch and Branch 1995; Ridgeway, Reid, Taylor, Branch & Hodgson 1998). A total of 130 limpets represented by three genera, namely *Cellana* H. Adams, 1869, *Cymbula* H. & A. Adams, 1854 and *Scutellastra* H. & A. Adams, 1854 were collected from 2000 to 2002 at the De Hoop Nature Reserve. These included *Cellana capensis* (Gmelin, 1791) (Fig.2.4E), *Cymbula compressa* (Linnaeus, 1758) (Fig. 2.4F); *C. miniata* (Born, 1778) (Fig. 2.4G); and *C. oculus* (Born, 1778) (Fig. 2.4H); *Scutellastra argenvillei* (Krauss, 1848) (Fig.2.4D); *S. barbara* (Linnaeus, 1758) (Fig. 2.4A); *S. cochlear* (Born, 1778) (Fig. 2.4B) and *S. longicosta* (Lamarck, 1819) (Fig. 2.4C) (Table 2.1). Additional material was examined that had been collected during previous field trips (1993 - 1999) by the Aquatic Parasitology Research Group, namely *Cymbula granatina* (Linnaeus, 1758) (Fig. 2.5F); *Helcion concolor* (Krauss, 1848) (Fig.2.6A); *H. dunkeri* (Krauss, 1848) (Fig. 2.6B); *H. pectunculus* (Gmelin, 1791) (Fig. 2.6B) and *H. pruinosus* (Krauss, 1848) (Fig. 2.6B); *Scutellastra aphanes* (Robson, 1986) (Fig. 2.5A); *S. exusta* (Reeve, 1854) (Fig. 2.5B); *S. granularis* (Linnaeus, 1758) (Fig. 2.5C); *S. obtecta* (Krauss, 1849) (Fig. 2.5D) and *S. tabularis* (Krauss, 1848) (Fig. 2.5E). The Sub-Antarctic limpet *Nacella delesserti* (Philippi, 1849) was collected on the east coast of Marion Island (Fig. 2.6C).

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. The molluscs were collected alive by inserting a stainless steel spatula between the muscular foot and the substratum, so that they could be dislodged from the substratum. The molluscs were taken to a field laboratory (Figs. 2.2C & D, 2.3B) that was set up as close as possible to the collection site since the symbionts have to be examined live. After dissection the viscera were either discarded in the ocean or fixed in 10% buffered, neutral

formalin for later examination for other parasites that do not form part of the present study.

Table 2.1 Molluscs collected during the study period. In cases where species names have changed the previous name is also provided. Common names of all the molluscs are provided, as well as a map of the distribution along southern Africa.

Molluscs collected	Previous name	Common name	Distribution
	HALIOTIDAE	**Rafinesque, 1815**	
Haliotis midae Linnaeus, 1758	–	Perlemoen/abalone	
Haliotis spadicea Donovan, 1808	–	Siffie/Venus ear	
	NACELLIDAE	**Thiele, 1891**	
Cellana capensis (Gmelin, 1791)	*Cellana radiata capensis* (Gmelin, 1791) Stephenson, 1948	Resembles the variable limpet	
Nacella delesserti (Philippi, 1849)	*Nacella (Patinigera) delesserti Patinigera* Dall, 1905	–	Marion and Prince Edward Islands
	PATELLIDAE	**Rafinesque, 1815**	
Cymbula compressa (Linnaeus, 1758)	*Patella compressa* Linnaeus, 1758	Kelp limpet	
Cymbula granatina (Linnaeus, 1758)	*Patella granatina* Linnaeus, 1758	Granite limpet	
Cymbula miniata (Born, 1778)	*Patella miniata miniata* Born, 1778	Pink-rayed or cinnamon limpet	
Cymbula oculus (Born, 1778)	*Patella oculus* Born, 1778	Goat's eye or eye limpet	
Helcion concolor (Krauss, 1848)	*Patella concolor* Krauss, 1848	Variable limpet	
Helcion dunkeri (Krauss, 1848)	*Patella dunkeri* Krauss, 1848. *Helcion (Patinastra) dunkeri* (Krauss, 1848). *Patinastra* Thiele in Troschel & Thiele (1891)	Rayed limpet	

Table 2.1 continued Molluscs collected during the study period. In cases where species names have changed the previous name is also provided. Common names of all the molluscs are provided, as well as a map of the distribution along southern Africa.

Molluscs collected	Previous name	Common name	Distribution
Helcion pectunculus (Gmelin, 1791)	*Patella pectunculus* Gmelin, 1791	Prickly or spiny ribbed limpet	
Helcion pruinosus (Krauss, 1848)	*Helcion (Patinastra) pruinosus* (Krauss, 1848). *Patinastra* Thiele in Troschel & Thiele (1891)	Rayed or shimmering limpet	
Scutellastra aphanes (Robson, 1986)	*Patella aphanes* Robson, 1986	Resembles a small Argenville's limpet	
Scutellastra argenvillei (Krauss, 1848)	*Patella argenvillei* Krauss, 1848	Argenville's limpet	
Scutellastra barbara (Linnaeus, 1758)	*Patella barbara* Linnaeus, 1758	Bearded limpet	
Scutellastra cochlear (Born, 1778)	*Patella cochlear* Born, 1778	Pear limpet	
Scutellastra exusta (Reeve, 1854)	*Patella pica* Reeve, 1854	–	
Scutellastra granularis (Linnaeus, 1758)	*Patella granularis* Linnaeus, 1758	Granular or beaded limpet	
Scutellastra longicosta (Lamarck, 1819)	*Patella longicosta* Lamarck, 1819	Duck's foot, long-spined, spiked or spider limpet	
Scutellastra obtecta (Krauss, 1849)	*Patella obtecta* Krauss, 1849	Resembles a small duck's foot limpet	
Scutellastra tabularis (Krauss, 1848)	*Patella tabularis* Krauss, 1848	Giant limpet	

Collection of symbionts

The molluscs 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 undergoing reproduction were observed with light microscopy. Photomicrographs were taken of live specimens in various stages of binary fission, conjugation and telotroch formation, as well as for the purpose of determining body dimensions. Positive wet smears were left to air dry in some cases and in other cases fixed in Bouin's fluid and transferred to 70% ethanol for later processing in the laboratory in Bloemfontein. Samples were supplied with a collection number as follows: **Year/Month/Day - collection number, e.g. 2000/10/24-01**. Mantoscyphidians occurring on the gills of haliotids and limpets are referred to as primary symbionts thoughout this thesis, while ellobiophryids attached to the mantsoscyphidians are referred to as secondary symbionts.

Preparation of material

Light microscopy

Hematoxylin

Wet smears were fixed in Bouin's fluid, after which 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, Harris' and Heidenhain's Iron Hematoxylin were used to stain the nuclear apparatus, following the standard procedures as described by Humason (1979). Striations of muscle and some protozoan structures, especially nuclei, are better differentiated by Heidenhain's Iron Hematoxylin (Humason 1979).

Mayer's Hematoxylin method

1. Specimens fixed in Bouin's (minimum 30 minutes)

2. Transfered to 70 % ethanol for storage
3. 70 % ethanol (3 minutes)
4. 50 % ethanol (3 minutes)
5. Mayer's hematoxylin (11 minutes)
6. Rinsed in tapwater (3 minutes)
7. Scotts solution (3 minutes)
8. Rinsed in tapwater (3 minutes)
9. 50 % ethanol (3 minutes)
10. 70 % ethanol (3 minutes)
11. 80 % ethanol (3 minutes)
12. 90 % ethanol (3 minutes)
13. 96 % ethanol (3 minutes)
14. 100 % ethanol (6 minutes)
15. Xylene (6 minutes)
16. Mounted cover slips with Eukitt

Harris' Hematoxylin method

1. Specimens fixed in Bouin's (minimum 30 minutes)
2. Transfered to 70 % ethanol for storage
3. 70 % ethanol (3 minutes)
4. 50 % ethanol (3 minutes)
5. 30 % ethanol (3 minutes)
6. Rinsed in tapwater (3 minutes)
7. Harris Hematoxylin (15-18 minutes)
8. Rinsed in tapwater (3 minutes)
9. 50 % ethanol (3 minutes)
10. 70 % ethanol (3 minutes)
11. 80 % ethanol (3 minutes)
12. 90 % ethanol (3 minutes)
13. 96 % ethanol (3 minutes)
14. 100 % ethanol (6 minutes)
15. Xylene (6 minutes)

16. Mounted cover slips with Eukitt

Heidenhain's Hematoxylin method

1. Specimens fixed in Bouin's (minimum 30 minutes)
2. Transfered to 70 % ethanol for storage
3. 70 % ethanol (10 minutes)
4. 50 % ethanol (10 minutes)
5. 30 % ethanol (10 minutes)
6. Rinsed in tapwater (10 minutes)
7. 4 % iron alum (15 minutes)
8. Rinsed in tapwater (5 minutes)
9. Saturated aqueous picric acid (time varied from 23 minutes to 1 hour and 50 minutes) Observed development under light microscope
10. Rinsed in tapwater (15-30 minutes)
11. 30 % ethanol (5 minutes)
12. 50 % ethanol (5 minutes)
13. 70 % ethanol (5 minutes)
14. 80 % ethanol (10 minutes)
15. 90 % ethanol (10 minutes)
16. 96 % ethanol (10 minutes)
17. 100 % ethanol (20 minutes)
18. Xylene (10 minutes)
19. Mounted cover slips with Eukitt

Protargol

Details of the infundibulum were initially studied by staining smears fixed Bouin's fluid with protargol using a combined method as described by Lee, Hunter and Bovee (1985) and Lom and Dykova (1992). This method proved rather unsatisfactory in some cases, as the peritrichs have many symbiotic algae and inclusions, which obscures the position of the infraciliature. Dr. Clamp's "quick method" (personal

communication)[11], which was slightly amended, proved to give the best results. Square pieces of copper sheets were placed vertically between slides in a staining jar with a 70°C protargol solution. The copper suppresses the staining of cytoplasmic inclusions or vacuoles. In some cases protargol staining was done in the field laboratory, and in other cases staining was done after returning to the laboratory in Bloemfontein.

Method

1. Specimens fixed in Bouin's (minimum 30 minutes)
2. Transfered to 70 % ethanol for storage
3. 50 % ethanol (5 minutes)
4. 30 % ethanol (5 minutes)
5. Washed in distilled water (5 minutes)
6. Bleached in 0.5 % potassium permanganate (5 minutes)
7. Washed in distilled water, until no more purplish colour washed out
8. 5 % oxalic acid (5 minutes)
9. Washed in distilled water (10 minutes)
10. 1 % protargol solution at 70°C (12-14 minutes)

- with copper wire placed between slides (Field laboratory)
- with copper sheet, 66mm X 26mm; 1mm thickness between slides (Laboratory in Bloemfontein)

11. 1 % hydroquinone in 5 % sodium sulphite (6.5-8 minutes)

- observed development under light microscope

12. Washed in distilled water (5 minutes)
13. 0.5 % gold chloride solution (15-25 seconds)
14. Washed briefly in distilled water
15. 2 % oxalic acid (2.5 minutes)

- observe development under light microscope

16. Washed in distilled water (5 minutes)
17. 5 % sodium thiosulphate (5 minutes)

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[1] Dr. John C. Clamp, Department of Biology, North Carolina Central University, Durham, North Carolina, 27704, USA.

18. Washed in distilled water (5 minutes)
19. Dehydrated through a series of alcohols (30-100 %), 3 minutes in each except two changes of 5 minutes each in 100 %
20. Xylene
21. Mounted cover slips with Eukitt

Scanning electron microscopy (SEM)

In the field laboratory the gills were fixed in concentrations of 4 or 10 % buffered, neutral formalin. The formalin was diluted with fresh seawater. In some cases gills were fixed in Parducz' solution, post-fixed in osmium tetroxide 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 % glutaraldehyde. Thereafter, the gills were dehydrated to 70 % ethanol at 4 °C, and then transported to the laboratory in Bloemfontein.

In the laboratory in Bloemfontein the specimens that were fixed in formalin were cleaned by washing the gills in tapwater for 20 minutes, after which 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 % glutaraldehyde (that were dehydrated up to 70 % ethanol at 4°C in the field) were dehydrated in ethanol concentrations of 80 % to 100 % at room temperature, approximately 24 hours after fixation.

Thereafter, the gills bearing peritrichs were critical point dried, mounted on stubs using instant Pratley Quickset, and sputter coated with gold using an Emscope sputter coater. The gills were examined at 5 and 10 kV in a JOEL WINSEM JSM 6400 scanning electron microscope.

Morphological measurements

Body dimensions and nuclear apparatus measurements of the peritrichs undergoing binary fission, conjugation and telotroch formation were obtained from microscope projection drawings done with the aid of a drawing tube. Photomicrographs and videoprints were also taken for additional measurements. Scale bars will be indicated in all figures presenting the author's own work. In the work of other authors scale bars are not given because only microscope magnifications were given in the orginal literature.

Nuclear material of the telotroch stages was measured using the same parameters as used in measuring the nuclear apparatus of species of *Trichodina* Ehrenberg, 1830 (Lom 1958), namely Ma = diameter of the macronucleus; $-y^1$, +y, -y = different positions of the micronucleus in relation to the macronucleus (Fig. 2.7A). Body width and diameter were also noted.

The body dimensions of peritrichs undergoing binary fission were determined, as well as the position and shape of the nuclei and the development of the infraciliature (Fig. 2.7B).

The macro- and microconjugants of peritrichs undergoing conjugation were measured in width and in length. The shape and position of nuclei were also noted (Fig. 2.7C).

The statistical analysis of the measurements (in µm) was done using the computer program Microsoft Excel. For measurements of live specimens, minimum and

maximum values are given, followed in parentheses by the arithmetic mean and standard deviation (only in n>9), followed by the number of specimens measured. Measurements based on Bouin's-fixed specimens stained with hematoxylin are presented in square brackets.

Authors of taxa

Due to the wide range of different taxa mentioned, it was not always possible to find the original authors. 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. 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 for using De Puytorac's system is 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.

Jankowski (1980, 1985) proposed six new genera to accommodate species formerly included under the genus *Scyphidia* Dujardin, 1841. Lom and Dykova (1992) described the systematic characteristics of the relevant taxa.

The present study focuses on the reproductive processes of scyphidiid peritrichs, and it is thus not a taxonomic study. Although many species have undergone name changes in literature that have been referred to in this study, the author will give the original species names, with taxonomic changes indicated in footnotes.

Terminology

Throughout this thesis the term "daughter" is used for presumptive telotroch and the term "parent" refers to the presumptive trophont. The terminology that have been used in this study is provided in Appendix B.

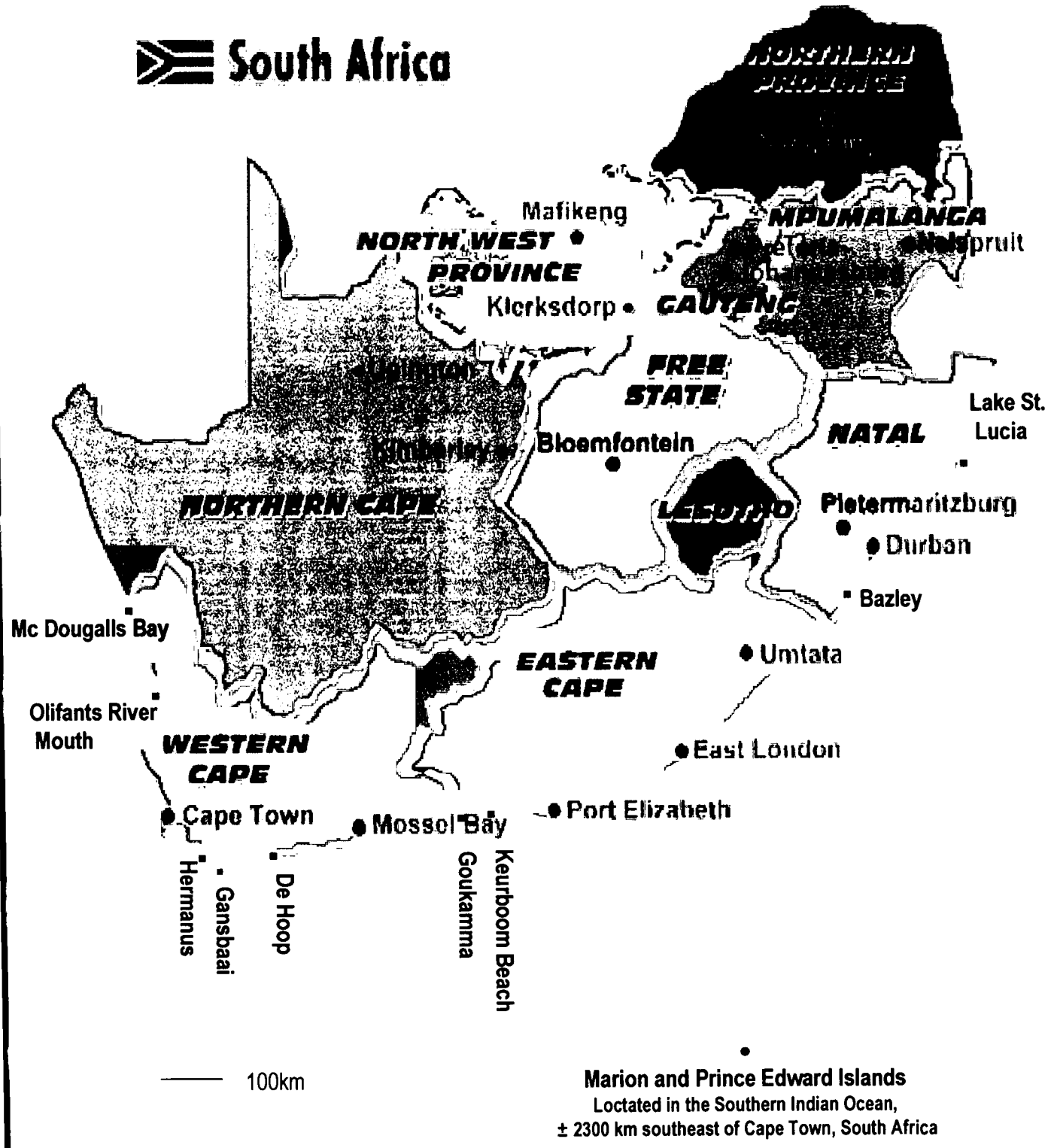


Fig. 2.1 Map of South Africa showing the major centres various collection localities (▪) along the coast, namely Mc Dougalls Bay, Olifant's River Mouth, Hermanus, Gansbaai, De Hoop Nature Reserve, Goukamma Nature Reserve, Keurboom Beach, Bazley and Lake St. Lucia. Scale bar: 100km. Figure taken from http://www.places.co.za/html/visualfind.html.

Figure 2.2 **A** - De Hoop Nature Reserve, south coast of South Africa. **B** - Tidal pools at De Hoop Nature Reserve, south coast of South Africa. **C** - Field laboratory at Koppie Alleen, De Hoop Nature Reserve. **D** - Author in field laboratory at Potberg, De Hoop Nature Reserve. **E** - Author busy collecting abalone at the De Hoop Nature reserve. **F** - *Haliotis midae* Linnaeus, 1758 shells. **G** - *Haliotis spadicea* Donovan, 1808 shells.

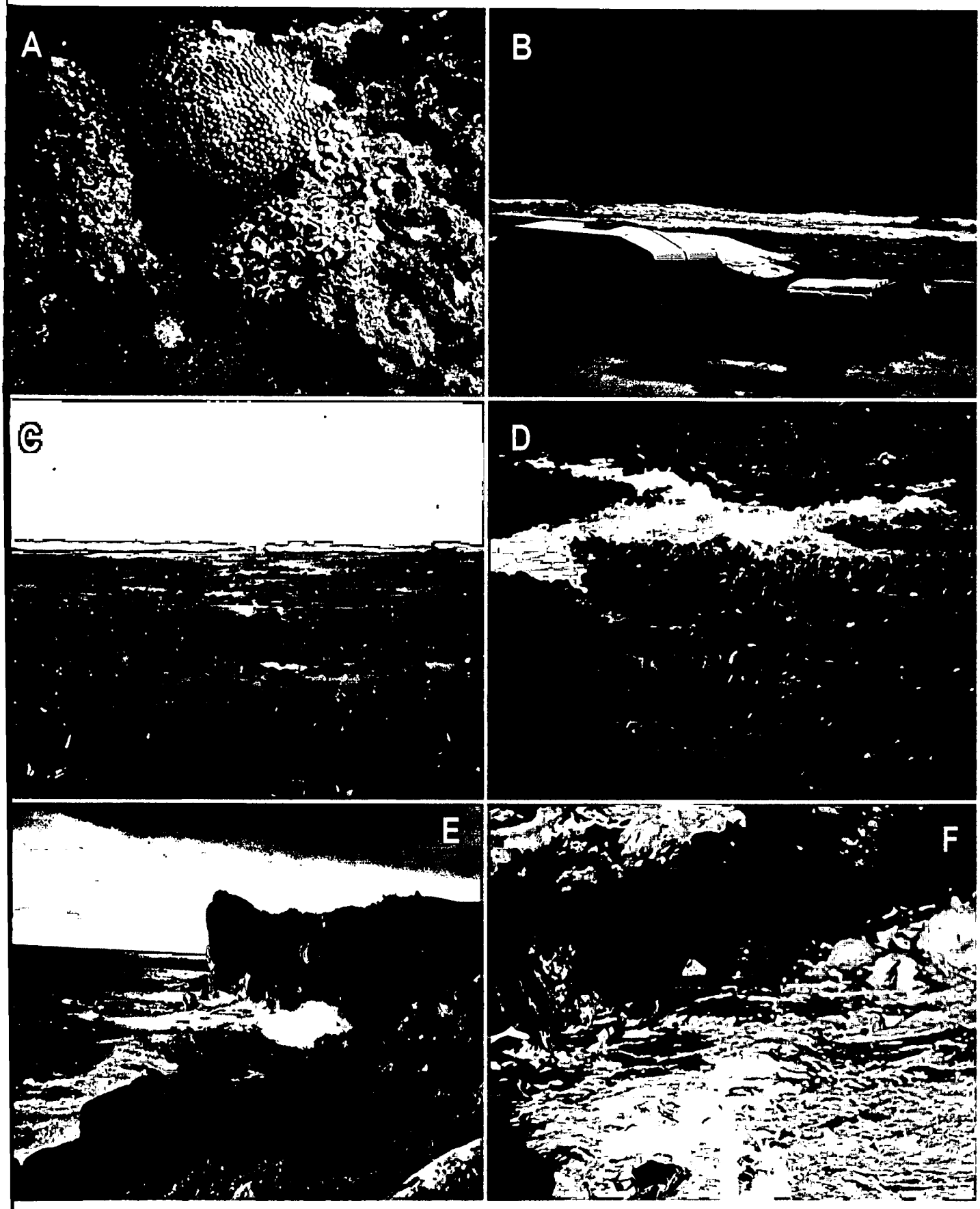


Fig. 2.3 Collection localities. **A** - Intertidal zone of Bazley on the east coast of South Africa. **B** - Field laboratory set up at Mc Dougalls Bay, west coast of South Africa. **C** - Kelp beds along the west coast, Mc Dougalls Bay of South Africa. **D** - Rocky shore of Alexanderbaai, west coast of South Africa. **E** - Boulder Beach, MarionIsland. **F** - Intertidal zone at Boulder Beach, Marion Island.

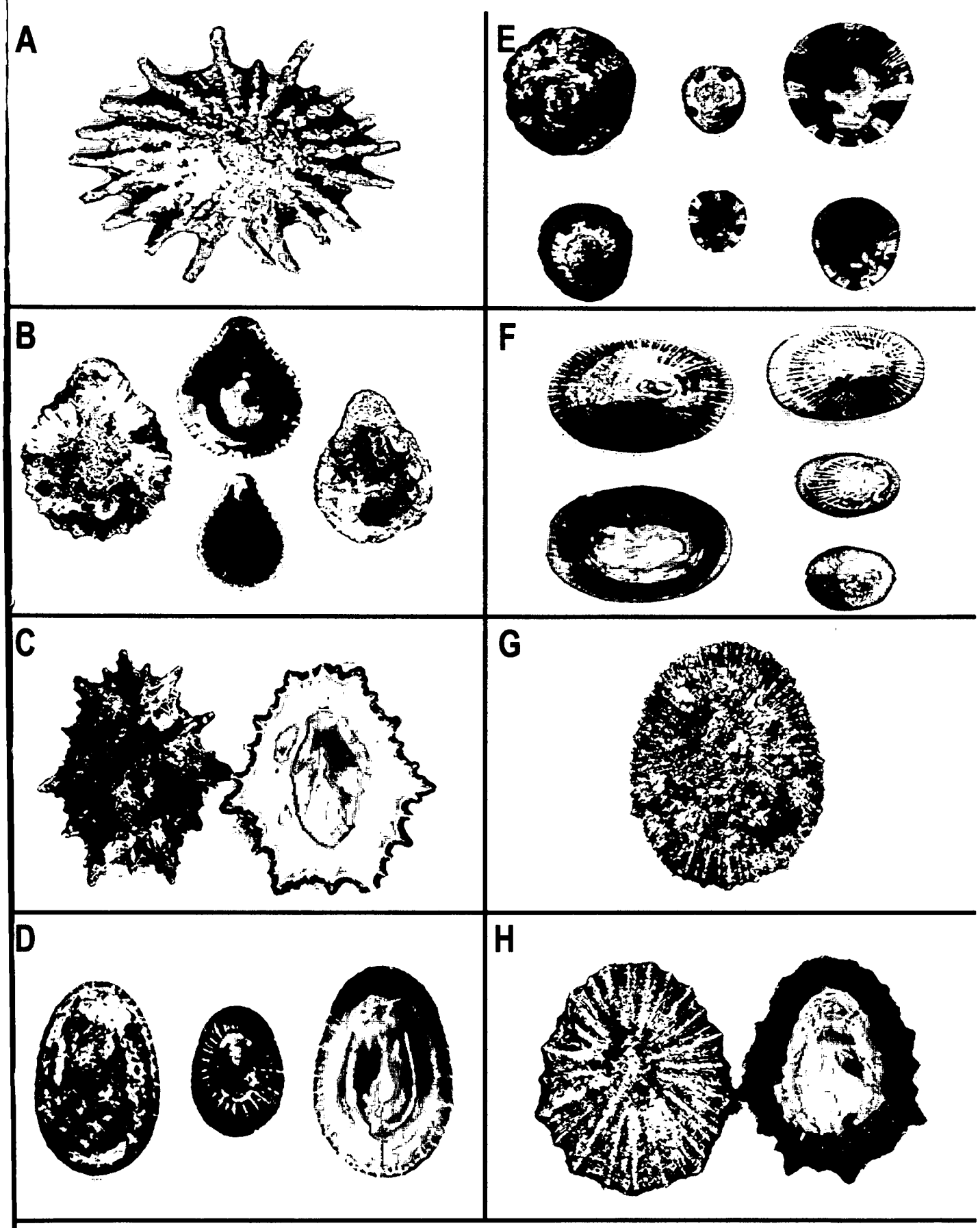


Fig. 2.4 Limpets collected: **A** - *Scutellastra barbara* (Linnaeus, 1758). **B** - *S. cochlear* (Born, 1778). **C** - *S. longicosta* (Lamarck, 1819). **D** - *S. argenvillei* (Krauss, 1848). **E** - *Cellana capensis* (Gmelin, 1791). **F** - *Cymbula compressa* (Linnaeus, 1758). **G** - *C. miniata* (Born, 1778). **H** - *C. oculus* (Born, 1778). Figures taken from Van As (1997).

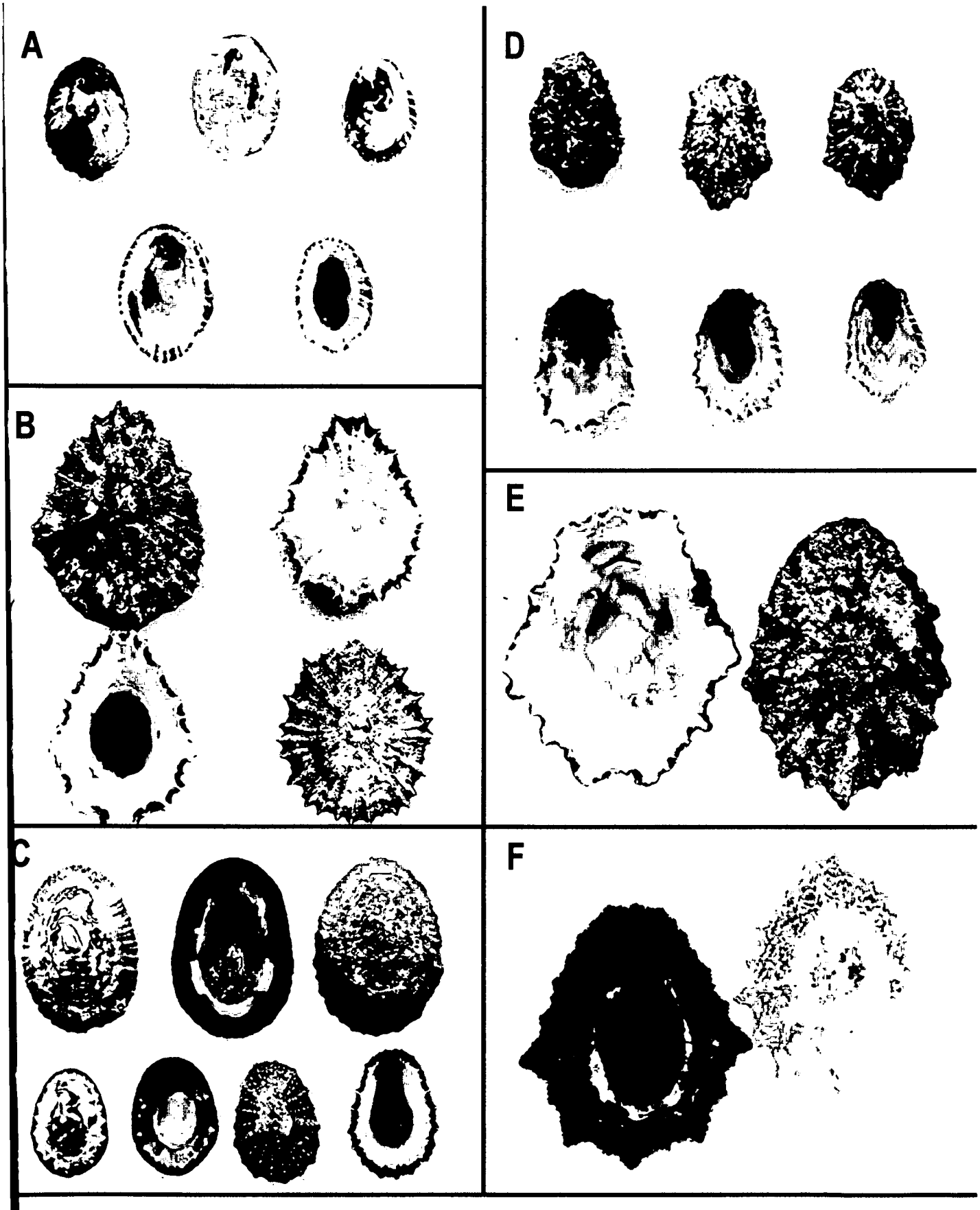


Fig. 2.5 Limpets collected: **A** - *Scutellastra aphanes* (Robson, 1986). **B** - *S. exusta* (Reeve, 1854). **C** - *S. granularis* (Linnaeus, 1758). **D** - *S. obtecta* (Krauss, 1849). **E** - *S. tabularis* (Krauss, 1848). **F** - *Cymbula granatina* (Linnaeus, 1758). Figures taken from Van As (1997).

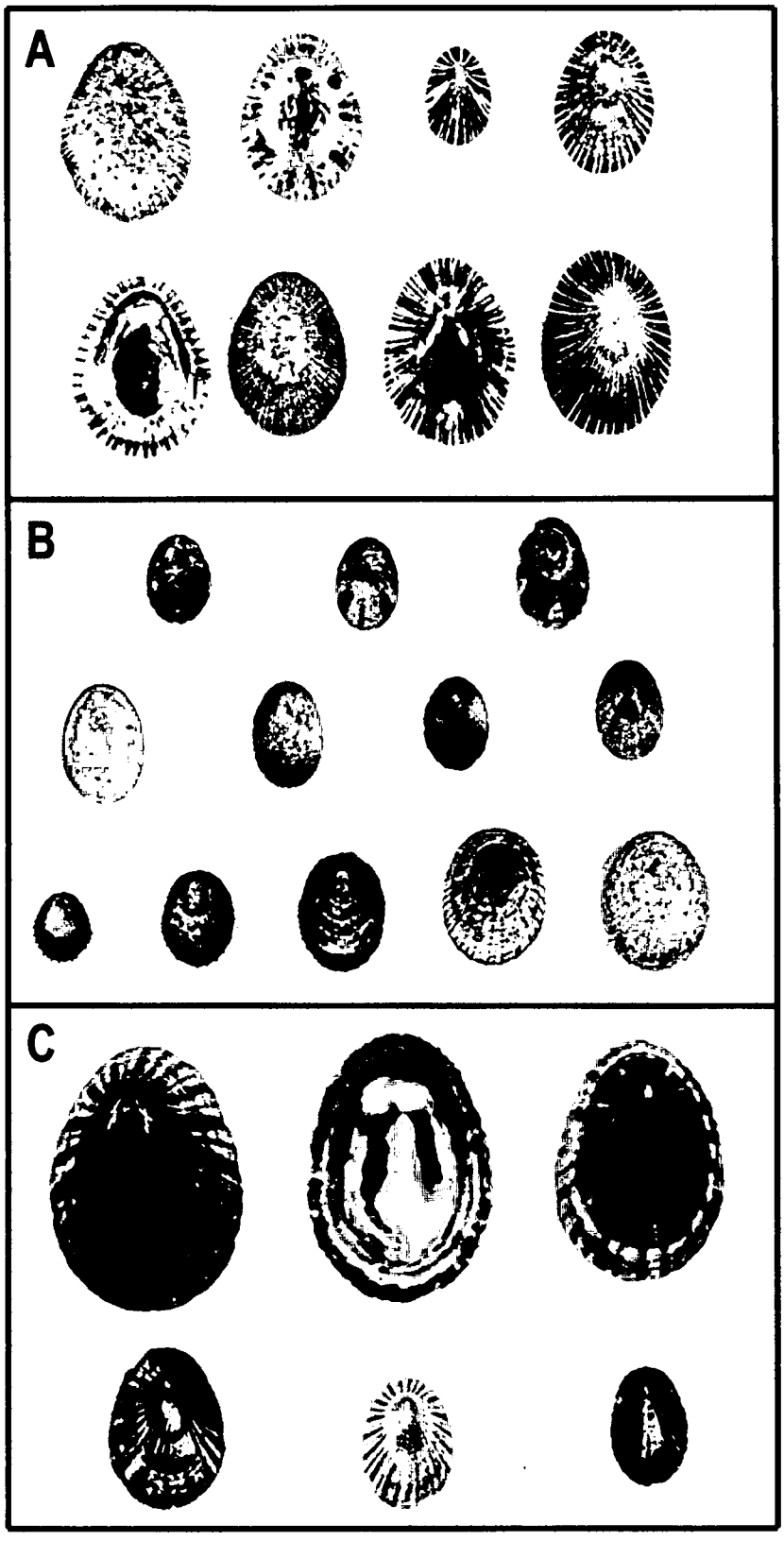


Fig. 2.6 Limpets collected: **A** - *Helcion concolor* (Krauss, 1848). **B** - *Helcion dunkeri* (Krauss, 1848), top row, *Helcion pectunculus* (Gmelin, 1791), middle row and *Helcion pruinosus* (Krauss, 1848) bottom row. **C** - *Nacella delesserti* (Philippi, 1849). Figures taken from Van As (1997).

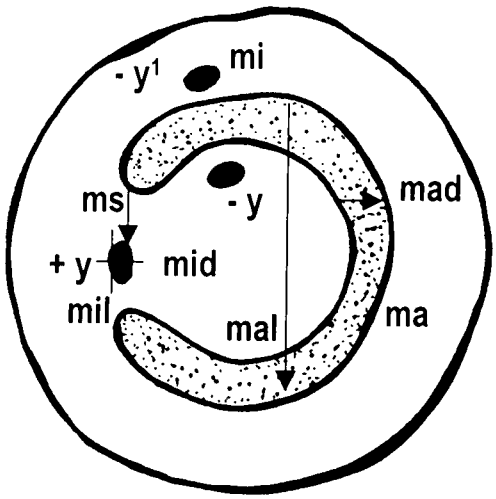
Figure 2.7

Diagrammatical illlustrations of morphological features used to determine body dimensions during reproduction.

A. Telotroch stage – with dimensions indicated by Lom (1958) for trichodinid species.
B. Binary fission.
C. Conjugation – macroconjugant and attached microconjugant.

bd = body diameter; **bl** = body length; **ma** = macronucleus; **mad** = macronuclues diameter; **mal** = macronucleus length; **mi** = micronucleus; **mid** = micronucleus diameter; **mil** = micronucleus length; **ms** = length of the sector between the terminations of the macronucleus; **-y^1**, **+y**, **-y** = different positions of the micronucleus in relation to the macronucleus.

A


- y1
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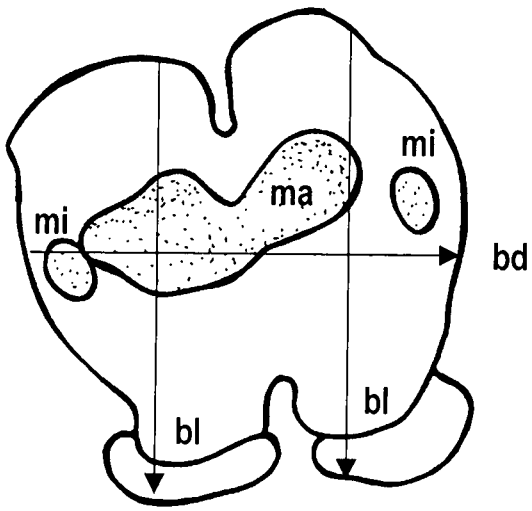
mi
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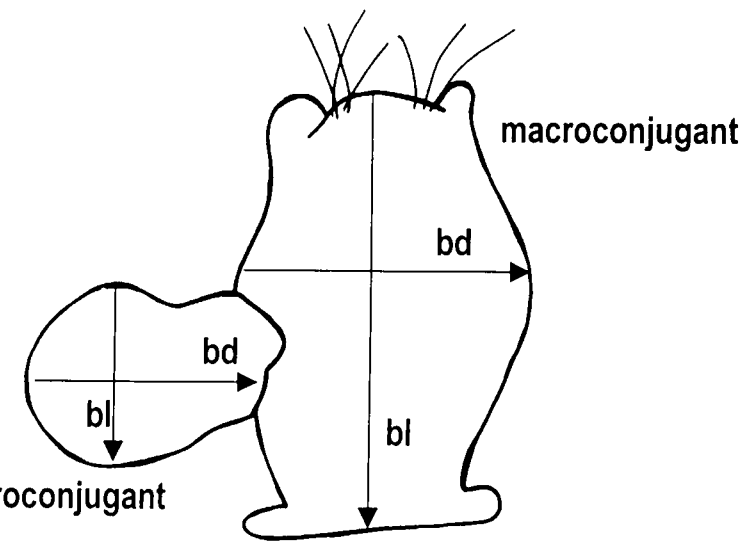
C


macroconjugant
bd
bd
bl
bl
microconjugant

Chapter 3

Literature Review: Reproduction in some Peritrichs

During the early 19th century much progress was made in clarifying reproductive patterns and correlating cytological events with reproductive cycles in the kingdom Protozoa. The various reproductive processes illustrate the diversity of protozoan species. Some of the most interesting reproductive phenomena are to be found in the subclass Peritrichia. Binary fission, telotroch formation, pre-conjugation fission and conjugation are well-established phenomena in this subclass (Finley 1952; Walker, *et al.* 1986).

Binary fission, budding and the formation of telotrochs are asexual methods of reproduction. Pre-conjugation fission is the conjugant or sex-differentiating fission, and conjugation is the sexual method of reproduction. The asexual method of reproduction in peritrichs conforms to the pattern which characterizes ciliophorans in general, but the sexual method does not. Sexual reproduction in representatives of the Peritrichia not only differs strikingly from that of other ciliophorans in having a different number of progamic divisions but also in the microconjugant being incorporated into the macroconjugant. Sexual reproduction is also characterized by a sex-differentiating preconjugation fission that seems to occur in all the families.

ASEXUAL REPRODUCTION

Asexual reproduction in protozoans includes **binary fission**, **multiple fission** and **budding**. Binary fission usually produces two identical daughter cells through mitotic division. According to Anderson (1988) two kinds of fission namely symmetrogenic and homothetogenic fission can be distinguished based on the orientation of the fission plane relative to the long axis of the cell (when one exists) and the geometric relations between major morphogenetic characteristics of the two daughter cells.

Symmetrogenic Fission (mirror image fission)

This type of fission is typical of many flagellates, opalinids and many other non-

ciliophoran protozoans. The fission plane is coincidental with the long axis of the cell. The separation proceeds along the fission plane and two daughter cells with a mirror image relationship are produced (Anderson 1988).

Homothetogenic Fission (transverse or perikinetal fission)
This type of fission typically occurs in ciliophorans. The parent cell gives rise to two daughter cells by a fission plane that is transverse to the long axis or to the anterior-posterior axis. The twin-like development occurs in tandem to one another and not parallel to each other as in the symmetrogenic form of fission.

Multiple fission
Multiple fission occurs in a wide range of protozoans. According to Anderson (1988) this mode of reproduction involves repeated division of the nucleus to produce several to many daughter nuclei that eventually give rise to multiple progeny by repeated cellular fission.

Anderson (1988) states that **budding** can be considered to be a specialized type of fission. Nuclear division produces daughter nuclei. Each nucleus migrates into a cytoplasmic bud that is released by cytoplasmic fission. In some cases, the released cell is a motile dispersal stage that migrates from the mother cell and develops into a mature reproductive individual. There are many variations among species, and it is therefore not possible to give a generalized model of reproduction by budding. Budding can either be internal (endogenous) or external (exogenous) (Lom & Dykova 1992).

In the phylum Ciliophora Doflein, 1901 division takes place by transverse (homothetogenic) binary fission, rarely by budding or multiple fission (Lom & Dykova 1992).

In peritrichs, **binary fission** is essentially cell division of a neuter (vegetative)

individual initiated by mitotic activity of the micronucleus and concluded when the two products of fission have acquired approximately equal portions of the macronucleus and cytostome (Finley 1952). The plane of fission parallels the oral-aboral axis instead of passing at a right angle to it: therefore, it is generally interpreted as a longitudinal fission. Thus, in the subclass Peritrichia binary fission separates two daughter individuals along the longitudinal, i.e., apical-antapical axis and seems to be an exception to the transverse cell division in ciliophorans. The division plane in peritrichs is probably homologous to the transverse plane of other ciliates. The body of a peritrich is radically distorted from the probable shape or symmetry of the ancestor. Thus, it is impossible to find the true longitudinal axis. According to Lom and Dykova (1992) the functional apical-antapical polarity is a secondary adaptation to the sessile way of life, i.e. the distortion has obliterated the original symmetry.

Telotroch formation

The formation of telotrochs in the scyphidiid peritrich life cycle is a well established feature and is well documented by Davis (1947); Raabe (1952); Dobrzañska (1961); Vávra (1961); Hobbs and Lang (1964); and Walker, *et al.* (1986). In the sessiline (scyphidiid) peritrichs one of the newly formed individuals resulting from binary fission may become a swarmer (telotroch) that has a disc-shaped form (Lom & Dykova 1992). This larval stage develops an locomotory wreath of cilia in the posterior third of the body. Telotroch stages form in response to unfavorable living conditions, following binary fission or in preparation for conjugation by migratory conjugants (Hobbs & Lang 1964).

Hobbs and Lang (1964) studied the ultrastructure of the telotroch stages of the peritrichs *Zoothamnium arbuscula* Ehrenberg, 1838, *Carchesium polypinum* Linnaeus, 1758, *Epistylis* Ehrenberg, 1830 sp., *Epistylis vittata* Stokes, 1889 and *Vorticella microstoma* Ehrenberg, 1830. The location of the ciliary wreath is marked by a single row of basal bodies. As the individual transforms into a telotroch, the ciliary belt or girdle becomes wider due to the multiplication of the basal bodies. They are arranged in short, compact, oblique rows (polykinetids) similar to those of the peristomial region. According to Rouiller and Fauré-Fremiet (1957) the cilia can attain normal size and

activity very quickly, within ten or 15 minutes. Hobbs and Lang (1964) further mention that telotrochs must be formed regularly if there are to be new locations for individuals or colonies.

Telotrochs will often form during prolonged observation of the species, detach at the scopula from the stalk and swim away, with the aboral end leading, until they again attach with the scopulas to an appropriate substratum.

The **preconjugation fission** process is a modification of binary fission that yields macro- and microconjugants. It is initiated by mitotic activity of the micronucleus, which divides equally, and terminates when products of the fission have acquired unequal portions of the macronucleus and cytostome. Various authors point out that, morphologically, all peritrichous macro-and microconjugants are different, the former possessing a larger volume of cytoplasm and macronuclear protoplasm (Padnos & Nigrelli 1942; Willis 1942; Finley 1943; Colwin 1944; Davis 1947). The morphological differences are derived from preconjugation fission, therefore, the very existence of peritrichous conjugants that differ in size, is evidence that preconjugation fission has occurred. Preconjugation fissions impose cytological and macronuclear differentiation upon microconjugants which, in turn, is additional evidence of sexual differentiation (Finley 1952).

Finley (1952) successfully activated reproduction in populations of the peritrich *Rhabdostyla vernalis* Stokes, 1887 (Family Epistylididae Kahl, 1933)[1], which attaches to aquatic animals by means of a non-contractile stalk, or it may lead a non-epizoic existence. A series of recurring binary fissions continued for approximately 36 hours and culminated in the unique preconjugation fissions. Preconjugation fissions yielded macro-and microconjugants which, in turn, conjugated.

[1] This species was originally described as a *Rhabdostyla* Kent, 1881 species. The status of this genus is doubtful (Stiller 1971, Lom & Dykova 1992) since these may be solitary or freshly attached zooids of *Epistylis* in which colony formation has been suppressed by environmental factors or had not yet begun. Pending further study, these organisms are better considered as *Epistylis*. The species is now known as *Epistylis vernalis* (Stokes, 1887).

SEXUAL REPRODUCTION

According to Anderson (1988) there are three fertilization modes:

1. Gametogamy – gametes are released in the water, individual gametes fuse at random and form a zygote, followed by reduction division (meiosis) at some point in the preparation for the next gamete release.
2. Autogamy – occurs when gametes from the same parent (gamont) fuse to form a zygote. Autogamy is a special case of obligate monoecy (both gametes come from a single gamont). Formerly known as endomixis.
3. Gamontogamy – occurs when gametes from two gamonts unite during fertilization. Gametes from one gamont fuse with gametes from only one other gamont (more restrictive than gametogamy, less restrictive than autogamy). The gamonts, gametes or gamete nuclei can express sexual differentiation.

GAMONTOGAMY

Conjugation in ciliophorans, though fundamentally similar among a wide variety of species, varies in details of gamete nuclei production, development and fate of the nuclei after syngamy, and form of the gamonts, in other words, whether both gamonts are morphologically identical (isogamonty) or of different morphology (anisogamonty) (Anderson 1988).

Anisogamonty occurs when conjugants are morphologically different in size and sometimes in general form, as is the case in ciliophorans. The stationary nucleus in the macroconjugant is fertilized by the migratory nucleus of the microconjugant. The microconjugant is resorbed by the macroconjugant, the latter being the only conjugant to complete fertilization. Subsequent division of the macroconjugant, either by binary fission or a variety of multiple fission patterns, gives rise to daughter cells of unequal size, thus reconstituting the macro-and microconjugant forms. There is considerable variation in the form and fate of the macronuclei during ciliophoran reproduction.

Conjugation begins when a microconjugant attaches to a macroconjugant. As a consequence of this union the macronuclei of both conjugants disintegrate and disappear. Ultimately, macronuclear anlagen are derived from a synkaryon that owes its origin to the fusion of pronuclei. The macronuclear anlagen are distributed to neuter (vegetative) individuals by means of reorganization (binary) fissions, that denote that the sexual process has ended. Gamete exchange occurs when environmental conditions are less than optimal and individuals that are produced have the potential to be better adapted (Anderson 1988). Conjugation restores vitality to a population. The advantage of sexual reproduction is that it permits gene recombinations, thus increasing genetic variation in the population.

In the phylum Ciliophora, the two conjugants are sexually, but not morphologically, differentiated (Lom & Dykova 1992), but the peritrichs, chonotrichs and suctorians seem to be an exception. The preconjugants come into close contact with one another, fuse (often in the oral regions) and then establish a cytoplasmic bridge between one another. The macronuclei start to disintegrate, while the micronucleus undergoes meiotic divisions resulting in four haploid nuclei. Three nuclei are resorbed, the fourth divides once more to produce two nuclei, a stationary and a migratory pronucleus. The migratory pronucleus finds its way to the macroconjugants' stationary nucleus to fuse with it, forming a diploid synkaryon.

The macro- and microconjugant then separate and a series of divisions follow that restores the original state. The macronucleus arises from one of the division products of the synkaryon, called the macronuclear anlage, which becomes polyploid by amplification of certain parts of the genome. Both exconjugants are genetically identical.

Autogamy does occur in some ciliophorans (Lom & Dykova 1992). The nuclear phenomena takes place in one partner, and the fusing pronuclei are the products of division of only one micronucleus. In other words, no exchange of genetic material takes place.

REPRODUCTIVE PROCESSES OF SOME REPRESENTATIVE CILIOPHORAN GENERA

Some major features of the processes of binary fission, preconjugation fission, conjugation and telotroch formation in representative ciliophoran genera that have been studied thoroughly are described below.

BINARY FISSION

Binary fission in *Rhabdostyla vernalis* Stokes, 1887 according to Finley (1952)
Family: Epistylididae Kahl, 1933 (Fig. 3.1)
This asexual process involves four steps:

- Swelling or growth of the micronucleus. Neuter individuals on the verge of binary fission are plumper than those not ready for fission; the endoplasm undergoes vigorous cyclosis, as indicated by the forceful movement of food vacuoles. Frequently the organism ceases its feeding and the contractile rim of the peristome border encompasses the peristome.
- The micronucleus rapidly passes through the stages of mitosis (Fig. 3.1 no. 1), two nuclei are formed, one is transmitted into the "daughter" individual and the other remains in the "parent".
- The macronucleus grows, elongates and takes a position that will allow each fission product to receive an equal portion (Fig. 3.1 no. 2).
- Amitotic separation of the macronucleus takes place. The macronuclear cleavage seems to be a clean one without the loss or extrusion of chromatin. Approximately six minutes later the plane of fission is distinguishable (Fig. 3.1 no. 3). The primordial peristome region differentiates *de novo* in the daughter; the parent retains its peristome. Shortly after the plane of fission appears the parent resumes its feeding and the pulsation of its contractile vacuole. Meanwhile, food vacuoles circulate between parent and daughter (Figs. 3.1. no. 4 & 5).

The next cytostomal change is characterized by the development of the contractile vacuole in the daughter. Shortly after this, the new vacuole begins pulsating, the daughter opens its peristome and takes in food. The posterior ciliary wreath differentiates soon after the new peristome and contractile vacuole are properly functioning and plasmotomy has become well advanced (Fig. 3.1. no. 5).

The combined efforts of the daughter and parent result in separation, the parent contributing to the process by making forceful contractions of its body, the daughter assisting by rotating along its own longitudinal axis. The entire process of binary fission requires 40 to 50 minutes for completion (Figs. 3.1 no. 6 - 10).

Binary fission in *Urceolaria synaptae* Cuénot, 1891[2] according to Colwin (1944)
Family: Urceolariidae Dujardin, 1840 (Fig. 3.2)
Binary fission was observed during the summer months and occasional examinations during the winter sometimes showed a few dividing ciliophorans. The activity of the ciliophorans don't seem to diminish, as specimens were seen in rapid locomotion while undergoing the various phases of fission.

As division begins the peritrich broadens and thickens orally. Many vacuoles appear in the plane of the long axis of the cell, where the cleavage furrow will cut through. Separation of the daughters begins in the oral region, then in the aboral region with the last point of union part way between the two ends. The oral ciliary spiral, denticulate ring and the aboral (discal) ciliary apparatus are divided between the two daughters. The new denticulate rings do not appear in the parent cell and develop only after complete separation of the daughters.

[2] Now known as *Polycycla synaptae* (Cuénot, 1891).

Colwin (1944) observed that fission is occasionally unequal, producing daughters of slightly different, or rarely very different, sizes. Fission seems to be initiated by the nuclei. The macronucleus shortens and condenses, then appears bilobed with a wavy outline, then compact and elliptically, and then increasingly long until it finally divides. Its tendency to curve appears in all, but its most compact stages (Figs. 3.2 no. 1 - 9).

Meanwhile the micronucleus undergoes mitosis (Fig. 3.2 no. 4). It begins to swell and elongate at approximately the time of the first macronuclear changes, then it migrates from its vegetative position to an oral position.

The separated daughters develop into adults (Figs. 3.2. no. 10 & 11). At first the macronucleus remains almost rectangular, then its ends bend orally and lengthens, especially the end the furthrest away from the cytopharynx. The arms of the macronucleus bend aborally, toward the rim of the adhesive disc, restoring the typical vegetative state. The micronucleus leaves its position near the cleavage line and moves to a location slightly adoral (oral) to the center of the macronucleus. Both nuclei are now ready for the vegetative activities.

Binary fission in *Trichodina spheroidesi* Padnos & Nigrelli, 1942[3] according to Padnos and Nigrelli (1942)

Family: Trichodinidae Raabe, 1959 (Fig. 3.3)

- The trophomacronucleus undergoes vacuolization and clefts appear in the ground substance. The macronucleus condenses and contracts (Figs. 3.3. no. 1 - 3).
- The micronucleus swells and becomes spheroidal, then divides mitotically (Figs. 3.3 no. 2 & 3).
- The mitotic division continues as the macronucleus pulls apart, and two daughter micronuclei are formed before the macronucleus is completely divided (Figs. 3.3. no. 4 - 6).

[3] *Trichodina spheroidesi* Padnos & Nigrelli, 1942 is a nomen nudum (Lom & Laird 1969).

- Plasmotomy occurs about the time of the late telophase and the adhesive disc and denticle ring separate into approximately equal halves. Final cleavage of the macronucleus takes place and two daughter cells are formed (Figs. 3.3. no. 7 - 10).
- The adoral and aboral zones of cilia as well as the contractile vacuole are retained throughout division.

Binary fission in *Scyphidia ameiuri* Thompson, Kirkegaard & Jahn, 1947[4] according to Thompson, Kirkegaard and Jahn (1947)

Family: Scyphidiidae Kahl, 1933 (Fig 3.4)

Scyphidia ameiuri Thompson, Kirkegaard & Jahn, 1947 is a peritrichous ciliophoran that occurs on the gills of young bullheads, *Ameiurus melas melas*. Thompson, *et al.* (1947) noted that division begins at the adoral (basal) and aboral (distal) ends (Figs. 3.4 no. 1 & 2) and proceeds from both ends until cleavage is complete (Figs. 3.4 no. 3 - 5). The peritrich was never observed to be attached while undergoing fission.

Binary fission in *Scyphidia micropteri* Surber, 1940[5] according to Surber (1940)

Family: Scyphidiidae Kahl, 1933

Surber (1940) observed longitudinal fission in *Scyphidia micropteri* Surber, 1940, and he only observed the late stages of division of three individuals into six new individuals. In all instances, upon initial observation, the macronuclei are already largely divided and are connected by narrow isthmi of nuclear material. The rounded masses of nuclear material of the macronucleus are near the centers of the already largely separated halves, and at this stage, the cilia are undifferentiated. In two of the three individuals, separation of the halves occurs rather quickly. Death of all the resultant individuals made further observation impossible.

[4] Now known as *Ambiphrya ameiuri* (Thompson, Kirkegaard & Jahn, 1947).

[5] Now known as *Ambiphrya micropteri* (Surber, 1940).

Binary fission in *Scyphidia tholiformis* Surber, 1943[6] according to Surber (1943)

Family: Scyphidiidae Kahl, 1933

Scyphidia tholiformis Surber, 1943 occurs on the external body surface and gills of largemouth and smallmouth black bass. Surber (1943) states that longitudinal fission takes place in this peritrich, but relatively few dividing individuals were observed. The macronucleus becomes rounded, moves to the central part of the body and is in close association with the micronucleus that is situated posterior to the macronucleus. Constriction of the body and macronucleus occurs after the division of the micronucleus.

TELOTROCH FORMATION

Telotroch formation in *Rhabdostyla scyphidiformis* Vávra, 1961[7] according to Vávra (1961)

Family: Epistylididae Kahl, 1933

Vávra (1961) described *Rhabdostyla scyphidiformis* Vávra, 1961 from the branchial sacs of *Rana esculenta* tadpoles. Telotroch stages develop when the peritrich detaches from the host or when the host dies. The telotroch stages come spontaneously into existence and serve to propagate the peritrich. The aboral wreath of cilia appears in the first third of the body, usually above the 18th pellicle annulus counted from below. Vávra (1961) observed contraction of the peristome while the ciliary girdle forms. The body becomes bell-shaped, flattens progressively and is detached from the host after reaching a disc shape. The telotrochs were observed to whirl around for up to 20 hours.

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[6] Now known as *Ambiphrya tholiformis* (Suber, 1943).

[7] Now known as *Epistylis scyphidiformis* (Vávra, 1961).

Telotroch formation in *Circolagenophrys ampulla* (Stein, 1851)[8] according to Walker, Roberts and Usher (1986)

Family: Lagenophryidae Bütschli, 1889

In *Circolagenophrys ampulla* (Stein, 1851) both first and second type divisions have been reported (Willis 1942). In the first a single telotroch is formed and the parent remains within the lorica and resumes feeding. In the second type, the parent rapidly undergoes further division that results in the formation of a second telotroch and a very small residual organism that remains attached around the aperture of the lorica. According to Walker *et al.* (1986), the aboral surface is completely surrounded by the ciliary girdle that is composed of eight or nine kinetosomes per row.

In fully developed telotrochs there are very few adoral cilia that protrude from the peristome. A developing telotroch was observed and appeared to show cilia protruding from the peristome. In this particular telotroch, the aboral cilia do not appear to have grown to the full length, and it is possible that the disappearance of the adoral cilia coincides with the growth of the aboral cilia (Walker *et al.* 1986).

Telotroch formation in *Orbopercularia raabei* Dobrzañska,1961 according to Dobrzañska (1961)

Family: Operculariidae Fauré-Fremiet in Corliss (1979) (Fig. 3.5)

Orbopercularia raabei Dobrzañska, 1961 is an epizoic ciliophoran that occurs on the amphipod *Talitrus saltator*. Dobrzañska (1961) observed telotroch formation *in vivo* on several occasions. No division or conjugation was observed.

The telotrochs appeared either as fully developed forms in colonies (Fig. 3.5 no. 1) or the formation was due to the deteriorating conditions of the host's surroundings while being cultivated. During telotroch formation the peristome closes and the middle part of the zooid swells. Three rows (perhaps more) of basal corpuscles appear around it and

[8] Jankowski (1980) removed all *Lagenophrys* species whose loricae are circular in outline to the new genus *Circolagenophrys*. According to Clamp (1987) lorica shape is not a valid character in the Lagenophryidae, and the name *Lagenophrys* is preferred to that of *Circolagenophrys*.

the ciliary girdle emerges while the zooid tears off its stalk. The basal portion of the zooid, posterior to the aboral girdle is drawn in by the contraction and extension of the whole specimen. This contraction increases the specimen's diameter, the macronucleus moves towards the peristomial region and the peristome is plugged more tightly (Figs. 3.5 no. 2 - 4). According to Dobrzańska (1961) the fully developed telotroch moves around in a manner similar to mobiline peritrichs just after the drawing-in process is completed. This drawing-in process lasts only a few minutes. The telotroch dimensions are 16 - 25 µm in diameter at the aboral girdle and 15 - 22 µm in height.

Dobrzańska (1961) further describes the transformation of the telotroch into a settled form as the reverse of telotroch formation (Figs. 3.5 no. 5 - 8). The scopular part gradually protrudes with the cytoplasm from the central parts flowing into it. Granular structures that relate to the process of peduncle (stalk) formation could be seen in this protruding cytoplasm. The whole protozoan becomes leaner, the macronucleus returns to its original position and the peristome is unblocked. The ciliary girdle disappears at about the same time and the peduncle forms.

Telotroch formation in *Scyphidia ameiuri* Thompson, Kirkegaard & Jahn, 1947 according to Thompson, Kirkegaard and Jahn (1947)

Family: Scyphidiidae Kahl, 1933 (Fig. 3.6)

According to Thompson, *et al.* (1947) telotrochs form directly from the sessile form by contraction of the body and folding of the basal disc. Contraction causes a change in proportions resulting in the width being almost twice the length of the peritrich (Fig. 3.6). The macronucleus becomes more folded, the median row of cilia is used for locomotion and the body striations become very inconspicuous. The bell is usually closed.

Telotroch formation in *Scyphidia macropodia* Davis, 1947[9] according to Davis (1947)

[9] Now known as *Ambiphrya macropodia* (Davis, 1947).

Family: Scyphidiidae Kahl, 1933

According to Davis (1947) the transformation into the free-swimming or telotroch stage takes place very rapidly when the parasites are removed to a slide, but occurs only in a small percentage of organisms. The body undergoes extreme shortening and assumes a disc shape that resembles a trichodinid. Davis (1947) states that the cilia in the central membranelle become free, increase in length and forms the locomotive organ in the same way as in the ciliary girdle of trichodinids.

Telotroch formation in *Scyphidia tholiformis* Surber, 1943 according to Surber (1943)

Family: Scyphidiidae Kahl, 1933

Surber (1943) also described telotroch stages for *Scyphidia tholiformis* Surber, 1943. The peritrich becomes disc- or dome-shaped when it detaches from its host and resembles *Trichodina* species. The peristome contracts, the cilia around the peristome are drawn inwards and the scopula is drawn into the body. Surber (1943) states that the band of central cilia becomes posteriorly located after contraction and it is used for locomotion. He measured one of these individuals: 51.4 µm in diameter and 28.6 µm in depth.

PRECONJUGATION FISSION

Preconjugation fission in *Rhabdostyla vernalis* Stokes, 1887 according to Finley (1952)

Family: Epistylididae Kahl, 1933 (Fig. 3.7)

These observations were done during various experiments that Finley (1952) carried out on *Rhabdostyla vernalis*.

Nuclear phenomena

The process of preconjugation fission is a modification of binary fission that differentiates a neuter individual into conjugants of peritrichous ciliophorans. The micronucleus initiates this reproductive process (Fig. 3.7 no. 1), it is mitotic in character,

and it reaches a climax when approximately equal portions are distributed to the preconjugation-fission products. One of the striking features of preconjugation fission is the unequal, amitotic division of the macronucleus. The smaller individual receives a relatively small amount of macronuclear material; therefore, conjugant differentiation has occurred (Figs. 3.7 no. 2 & 3).

The second very noticeable feature is that four microconjugants are produced, this being accomplished by two fissions of the microconjugant (Figs. 3.7 no. 4 - 8) which occur consecutively. The micronuclei divide mitotically, while the macronuclei divide amitotically. These macronuclear divisions are equal rather than unequal.

Cytostomal phenomena

The third striking feature of preconjugation fission is the unequal division of the cytostome accompanying the macronuclear division mentioned above. Living peritrichs on the verge of preconjugation fission and those about to undergo binary fission all look alike, until the plane of fission becomes evident. The first microconjugant is called the undifferentiated microconjugant; its peristome and contractile vacuole differentiate *de novo*. The unequal fission takes 40 minutes to complete. The undifferentiated microconjugant does not develop a posterior ciliary wreath. Instead it begins fission after 5-10 minutes, which produces two equal microconjugants after 30 minutes. Each of these microconjugants then divide.

Thus, four microconjugants are derived by means of two consecutive equal fissions (Figs. 3.7 no. 9 - 13). The posterior ciliary wreath does not differentiate until the "four cell stage" is attained, so that the microconjugants remain in association with the macroconjugant practically the same length of time. No sign of a stalk between differentiating micro- and macroconjugants was ever seen. As soon as a microconjugant acquires the posterior ciliary wreath, it detaches itself. A macroconjugant may accept a microconjugant for conjugation, while undifferentiated microconjugant-fissions are in progress.

This unequal fission contributes to the differentiation of the macro- and microconjugant lineage. The undifferentiated microconjugant's fate has been determined by this fission – it must follow a path of development, which ultimately leads either to sexual reproduction, or to death. The larger individual's fate has also been determined, although it can undergo asexual reproduction as well as sexual reproduction. The larger individual is a macroconjugant for approximately two hours, possessing the attributes of sex. The macroconjugant's potency for conjugation must be utilized within this time. Otherwise its physiological differentiation disappears, and it reverts to a neuter status in which survival depends on asexual reproduction.

Preconjugation fission in *Vorticella microstoma* Ehrenberg, 1830 according to Finley (1939, 1943)

Family: Vorticellidae Ehrenberg, 1838 (Fig. 3.8)

Vorticella microstoma Ehrenberg, 1830 is a symphoriont that occurs in soil. Finley (1939, 1943) described the process of preconjugation fission and interpreted this process as the conjugant or sex differentiating fission. The micro- and macroconjugants originate from a vegetative individual as the result of equal division of the micronucleus accompanied by an unequal division of the macronucleus and endoplasm (Figs. 3.8 no. 1 & 2). The morphological differences between the preconjugation division and the ordinary division are found in the amount of macronuclear chromatin and endoplasm distributed to the microconjugant by the sister cell (Fig. 3.8 no. 3).

Preconjugation fission in *Lagenophrys tattersalli* Willis, 1942 according to Willis (1942, 1948) and Finley (1952)

Family: Lagenophryidae Bütschli, 1889 (Fig. 3.9)

Lagenophrys tattersali Willis, 1942 is an epizoic (ectocommensal) organism restricted to the gill-plates of the amphipod crustacean *Gammarus marinus*. The author described a "first-type division" and a "second-type division". The first type division is

ordinary binary fission which does not occur during ecdysis in the host. The second type division occurs at ecdysis and has three variations: Mode a; Mode b and Mode c (Figs. 3.9 no. 1 - 3).

"Mode a" involves the rapid succession of two binary fissions, the latter being unequal with respect to cytostome only and producing a small enucleated residual organism which degenerates after two telotrochs escape from the lorica. The telotrochs establish new colonies on the host.

In "Mode b" the micronucleus divides equally, but the macronucleus and cytostome divide unequally, producing a smaller "protoconjugant" and larger "parent" organism. The protoconjugant separates from the parent and undergoes fission before developing a posterior ciliary wreath. The products of this protoconjugant fission transform directly into functional microconjugants. Each microconjugant develops a posterior ciliary wreath, but the peristome disappears.

"Mode c" seems to be identical to "Mode a", except that only one unequal cytostomal fission yields a telotroch and an enucleated residual organism. The functional microconjugants may escape from the mouth of the lorica, or die before escaping, or one microconjugant may conjugate with the parental organism while the other microconjugant dies.

Preconjugation fission in *Scyphidia tholiformis* Surber, 1943 according to Surber (1943)

Family: Scyphidiidae Kahl, 1933

Surber (1943) states that conjugation of anisogametes or heterogametes takes place in this peritrich. Microconjugants are formed by exogenous budding or gemmation which apparently takes place very rapidly. This is evidence that the process of preconjugation fission precedes conjugation in *S. tholiformis*.

CONJUGATION

Conjugation in *Rhabdostyla vernalis* Stokes, 1887 according to Finley (1952)
Family: Epistylididae Kahl, 1933 (Fig. 3.10)
Nuclear- and cytoplasmic phenomena of *Rhabdostyla vernalis* are similar to those reported for *Vorticella microstoma* (Finley & Nicholas 1950). Conjugation requires about 24 to 36 hours for completion and can be divided into five intervals:

- Firm attachment and entering, 50-60 minutes (Fig. 3.10 no. 1)

The microconjugant firmly fuses with the upper third of the macroconjugant's body. The endoplasmic bridge is formed slower in individuals of *Rhabdostyla vernalis* than in individuals of *Vorticella microstoma* (Finley 1943). The macroconjugant normally continues feeding for more than an hour after the firm union has been established.

- Progamic nuclear divisions and shedding of the microconjugant's pellicle (Figs. 3.10 no. 2 - 7)

After the microconjugant's endoplasm enters the macroconjugant and its pellicle has become dislodged, it is impossible to distinguish the living, conjugating individual from the neuter individuals (no significant morphological differences). Both continue feeding until shortly before they are ready to divide; each can differentiate a posterior wreath of cilia and detach itself from its stalk; each can secrete a new stalk. Each microconjugant produces one functional pronucleus; the pronuclei fuse to form the synkaryon which gives rise to eight metagamic nuclei from which the nuclear complex is derived. The microconjugant's micronucleus undergoes three consecutive progamic divisions. The micronuclear membrane remains intact during karyokinesis. Only one of the progamic micronuclei functions in fertilization (synkaryon formation), all others become chromophilic and disappear slowly.

The macroconjugant's micronucleus undergoes only two progamic divisions. Only one of the macroconjugant's progamic nuclei functions in fertilization, all others disappear slowly.

- Synkaryon formation, probably about 15 minutes or less (Figs. 3.10 no. 8 & 9)

The synkaryon undergoes three successive metagamic divisions. Each division is presumed to be mitotic.

- Metagamic nuclear divisions and first reorganization fission, 12-18 hours (Figs. 3.10 no. 10 & 11)

One of the eight metagamic nuclei shrinks in size and becomes restored to the shape and size of the vegetative (neuter) micronucleus; the remaining seven metagamic nuclei differentiate into macronuclear anlagen which are distributed between the products of the reorganization fissions.

A mitotic division of the micronucleus initiates all reorganization fissions. As a result of the first reorganization fission, one of the fission products receives four macronuclear anlagen and one the other three; each receives one micronuclear anlage. Seven *Rhabdostyla* individuals are produced by the reorganization fissions, each containing one macro- and one micronuclear anlage and some old macronuclear fragments. The fragments of the macronucleus seem to be resorbed into the endoplasm, and they do not fuse with the macronuclear anlagen. The macronuclear anlagen of each individual *Rhabdostyla* elongate, grow and ultimately assume the characteristic shape and position of the neuter's macronucleus.

- Completion of reorganization, 10-16 hours (Figs. 3.10 no. 12 & 13)

Segments of the old macronuclei persist for a long time, but they are finally resorbed into the endoplasm. The first visible change in these organelles occurs about the time when progamic micronuclear divisions are in progress. The macronuclear segments seem to become inflated (called the balloon stage) and give the impression of being less viscous than they were before conjugation got under way. Following the balloon stage the macronuclear segments spin out into a tangled mass (skein stage). The macronuclear skein subsequently breaks into shorter and shorter segments. Eventually these short segments become round bodies that slowly disappear.

Conjugation in *Vorticella microstoma* Ehrenberg, 1830 according to Finley (1939, 1943)

Family: Vorticellidae Ehrenberg, 1838 (Fig. 3.11)

Observations made by Finley (1943) on free-living *Vorticella microstoma* have shown that the microconjugant is physiologically different from the vegetative individual in that it is capable of positive searching reactions and is able to carry out the conjugation reaction on contact with a macroconjugant. The macroconjugant does not differ physiologically from a vegetative individual (Fig. 3.11 no. 1), but is able to attract microconjugants and undergo conjugation immediately upon arrival for a limited time.

The microconjugant does not immediately attach itself to the macroconjugant, instead it usually touches about the body and stalk of the macroconjugant as if seeking for the right spot for fusion. According to Finley (1943) microconjugants of *Vorticella* Linnaeus, 1767, *Opisthonecta* Fauré-Fremiet, 1906 and *Cothurnia* Ehrenberg, 1831 always attach themselves to the lower third of the macroconjugant's body. Attachment to the adoral third of the macroconjugant's body has been reported for *Urceolaria* Stein, 1884, *Trichodina*, *Glossatella* Buetschli, 1889[10], *Pyxidium* Kent, 1882[11], *Epistylis*, *Campanella* Goldfuss, 1820, *Opercularia* Goldfuss, 1820, *Carchesium* Ehrenberg, 1830, *Zoothamnium* Bory de St. Vincent, 1826, *Ophrydium* Bory de St. Vincent, 1826 and *Lagenophrys* Stein, 1851.

The microconjugant assumes a position approximately perpendicular to the long axis of the macroconjugant's body and by turning on its own long axis it appears to bore its way into the pellicle of the macroconjugant. More than one microconjugant frequently fuses with the same macroconjugant (Fig. 3.11 no. 8). Microconjugants usually attach close to each other and only rarely attach on opposite sides of the macroconjugant. Many fusions of microconjugants with macroconjugants are made before the preconjugation division is completed (Figs. 3.11 no. 2 - 4), thus microconjugants do not search for a point from which a differentiating microconjugant detached itself.

[10] Now known as *Apiosoma* Blanchard, 1885 (syn. *Glossatella*).

[11] *Propyxidium* Corliss, 1979 nomen nudum (for *Pyxidiella* Corliss, 1960, for *Pyxidium*).

In *Vorticella microstoma* conjugation requires 18 to 24 hours to be completed. The process can be divided into six intervals:

- Firm attachment and entering, 30 minutes (Fig. 3.11 no. 5)

The microconjugant explores the macroconjugant and fuses firmly with it. About 10 minutes later the endoplasm surges inward for a slight distance, moving slowly, but steadily into the macroconjugant.

- Progamic nuclear divisions and shedding of the microconjugant's pellicle, 30 minutes

About 30 minutes after the time of attachment the only external evidence of the microconjugant is the shriveled pellicle that gradually slips toward the macroconjugant's stalk. The shrivelled pellicle is mostly dislodged from the body of the macroconjugant. After the microconjugant disappears into the macroconjugant's body, it is impossible to distinguish the living, conjugating individual from the ordinary vegetative individual. Both continue feeding until the conjugants are ready to divide; each can secrete a posterior ciliary wreath and detach itself from its stalk; each can secrete a new stalk, and some conjugants can encyst.

The first visible change of the macronucleus occurs at about 40 to 50 minutes after fusion. The macronuclei seem to be inflated (the balloon stage) (Figs. 3.11 no. 13 & 14). The balloon stage is followed by the macronuclear skein stage, during which the macronucleus elongates and spins out into a tangled mass that fills the whole body of the conjugant. The macronucleus subsequently divides into several segments that in turn forms shorter and shorter fragments. These short segments become dumb-bell shaped and the rounded ends pull away from each other until the rounded ends are only joined by a thread. These macronuclear fragments are slowly resorbed into the cytoplasm. The microconjugant's macronucleus also passes through the balloon stage, the skein stage, and then fragments in the same manner. These processes occur in the macroconjugant. The macroconjugant and the daughter cells resorb the microconjugant's old macronucleus.

The microconjugant's micronucleus undergoes three progamic divisions. The first is a preliminary division that begins shortly after firm attachment and ends at the time that the microconjugant's endoplasm passes into the body of the macroconjugant. The second progamic division follows without an interphase. The first and second progamic divisions are equational divisions, while the third is a reductional division (Figs. 3.11 no. 5 - 7 & 9 - 13).

The macroconjugant's micronucleus undergoes two progamic divisions. The second progamic division is the reductional division. Three of the four nuclei degenerate, while one of them assumes the spindle shape that characterizes the functional pronucleus (Fig. 3.11 no. 15).

- Synkaryon formation, five to 15 minutes (Figs. 3.11 no. 16 - 18)

Each conjugant produces one spindle-shaped functional pronucleus. The pronuclei fuse very quickly in the anterior third of the macroconjugant's body. The fused pronuclei form the synkaryon that gives rise to the metagamic nuclei from which the new nuclear complex is formed. The remaining seven pronuclei degenerate and are resorbed by the macroconjugant.

- Metagamic nuclear divisions and first reorganizational fission, 12 to 18 hours (Figs. 3.11 no. 19 - 24)

Three metagamic nuclear divisions (divisions of the synkaryon) result in the formation of eight nuclei of which one becomes a micronucleus and seven form macronuclear anlagen. The first metagamic division forms the first two anlagen of the new nuclear complex, and they are known as the metagamic nuclei.

One of the eight metagamic nuclei shrinks in size and is restored to the shape and size of the vegetative micronucleus. Each of the remaining seven metagamic nuclei are retained to form the seven macronuclei, which are distributed during the first reorganization fission.

As a result of the first reorganization fission, one of the daughters receives four macronuclear anlagen and one daughter receives three. Each daughter receives one micronuclear anlage.

- Second and third reorganizational fissions, about two hours (Figs. 3.11 no. 25 - 31)

Three reorganization fissions distribute the seven macronuclear anlagen to seven individuals and the micronucleus divides at each fission. Each individual also has some old macronuclear fragments.

- Growth of the new macronucleus and resorption of the remaining macronuclear fragments, about two hours.

When all of the fragments of the old macronucleus have been resorbed and nucleoli appear in the functional macronucleus, the reorganization process may be considered completed.

Conjugation in *Urceolaria synaptae* [12] **Cuénot, 1891** according to Colwin (1944)
Family: Urceolariidae Dujardin, 1840 (Fig. 3.12)

Colwin (1944) states that conjugation was noted often during the summer months but found no diurnal or lunar correlations. Regular binary fission occurred together with conjugation in any given population. According to Finley (1952), this observation is strong presumptive evidence of preconjugation fission in *Urceolaria synaptae* Cuénot, 1891, especially in view of the fact that Colwin found that conjugation was "anisogamous" (conjugants of unequal size). What is of more significance is cytoplasmic and macronuclear differentiation preceding the actual union of conjugants in *Urceolaria*.

[12] Now known as *Polycycla synaptae* Poljansky, 1951.

Conjugation begins when two ciliophorans of very unequal size join together. The smaller individual uses the adhesive disc to attach to the lateral, aboral or fairly often to the oral side of the larger individual, ordinarily at a point slightly below the apex of the larger cell (Colwin 1944) (Fig. 3.12 no. 1). The size variations in *U. synaptae* are so wide, that a micro-conjugant of one pair may be almost as large as the macroconjugant of some other pair. Preconjugants are not morphologically distinct from vegetative cells and therefore a cell about to conjugate can only be identified after it has taken up the typical position with another cell.

In living material various pseudo-conjugation associations may also be seen, as these peritrichs are apt to use the adhesive discs for attaching to any object, whether it is host gut wall, detached host cells or even other ciliophorans. However, only those cells that are truly beginning to conjugate are likely to remain attached throughout the processes of fixation.

As conjugation continues, a cytoplasmic bridge forms between the two cells, after which the conjugants are apparently unable to separate. The bridge generally links the side of the macroconjugant with an area of the microconjugant near the aboral end of the disc, sometimes at the adoral surface, sometimes at the area between the aboral and adoral surfaces (oral).

The concave portion of the disc of the microconjugant becomes everted, making the former innermost part of the cup the aboral extremity of the cell. The disc is no longer the means of attachment. When the bridge is established the cells appear to exchange material and the microconjugant, being the greater donor, diminishes in size, until finally only a small quantity of cytoplasm within a shriveled pellicle remains attached to the macroconjugant. This depleted cell presumably eventually drops off.

The cilia can be seen beating during the shrinkage process. The cytopharynx with the cilia disappears during the latter stages. Conjugation effects the incorporation by the macro-conjugant of most of the cytoplasm and nuclear material of the micro-conjugant.

DETAILED EVENTS: **Conjugation in *Urceolaria synaptae***

- The nuclear activity is usually synchronous and seems to begin after members of a pair have come together.
- The only nuclear difference is that the macronucleus of the microconjugant is smaller than that of the macroconjugant.
- The micronucleus shows the first activity. It enlarges, becomes spherical then oval. It's chromatic material increases in volume and appears to consist of a faintly staining mass of many granules (Fig. 3.12 no. 2).
- Enlargement and elongation continues and the micronucleus leaves the vegetative position.
- The macronucleus has begun to disintegrate; it starts with a thinning and a spreading. The fragments are usually larger in the microconjugant and smaller and more numerous in the macroconjugant.
- The micronucleus undergoes the first pregamic division. The daughter nuclei begin the next division without undergoing a resting period. The second pregamic division proceeds like a regular mitosis. Four micronuclei are produced in each conjugant, and enter a resting stage (Figs. 3.12 no. 3 - 17).
- There is some evidence that a third pregamic division also takes place in the microconjugant.
- After the resting stage, there are two micronuclei in each conjugant that play an active part in conjugation. The micronuclei take up positions near the point of attachment of the conjugants.
- One nucleus in each conjugant migrates towards the junction point of the conjugant. A true break in the cell membranes never appears before this stage (Figs. 3.12 no. 18 - 23).
- The migratory and stationary pronuclei unite to form a synkaryon, mostly in the macroconjugant, sometimes in the microconjugant. The first synkaryon division then follows (Figs. 3.12 no. 24 - 27).
- From this point the histories of the two conjugants diverge. Cytoplasmic and macronuclear fragments from the microconjugant pour into the macroconjugant.

The movement starts during synkaryon formation. Little is left in the microconjugant except the contractile vacuole and the micronuclei. Occasionally a synkaryon forms that, in turn, may go on to divide once or even twice. Sometimes even the micronuclei join the movement into the macroconjugant (Figs. 3.12 no. 28 & 29).

- Finally the shriveled and depleted microconjugant becomes detached and disappears, leaving only the macroconjugant, now known as the exconjugant to undergo the re-organization process that follows conjugation (Figs. 3.12 no. 30 & 31).
- Exconjugants have a swollen shape attributable to the extra cytoplasm not yet evenly distributed.
- The first two post-zygotic divisions (typical mitosis separated by a brief resting period) of the synkaryon usually occur before the microconjugant disappears.
- A rest period of longer duration follows the second division and specimens containing four resting nuclei are found fairly often. The third post-zygotic division occurs rapidly, since few cells in these phases are found (Figs. 3.12 no. 32 & 33).
- Eight synkaryon products are formed – four of the nuclei are macronuclear - and four are micronuclear anlagen, only one of the latter will become a functional micronucleus. Old macronuclear fragments disappear (Figs. 3.12 no. 34 - 37).
- The metamorphosis of the micronuclei goes on while the macronuclear anlagen are enlarging. Three of the micronuclei fade from view, but the fourth and only functional one condenses still further and moves orally (towards the buccal opening) and prepares to undergo mitosis.
- The first post-zygotic cell division gives rise to daughters that contain two macronuclear anlagen. The micronucleus undergoes mitosis, producing one daughter nucleus for each daughter cell. It is a rapid fission period followed by a resting period before the next division. New denticulate rings form in the daughters, as is the case after binary fission (Figs. 3.12 no. 38 - 46).
- The second post-zygotic division also takes places rapidly and produces daughter cells with one micro- and one macronucleus. As the small daughters grow the macronuclei enlarge and assume the typical vegetative position (Figs. 3.12 no. 47 - 50).

- The macronucleus changes gradually from a heterogeneous body to a vegetative macronucleus before, during and after the second post-zygotic division. The macronuclear membrane disappears, presumably because the increasing nuclear material fills the membrane completely.

Anomalous conditions:

- In one pair almost the entire nuclear content of the large member entered the body of the smaller one.
- Exconjugants bearing unusual numbers of macronuclear anlagen, from three up to as high as twelve occur occasionally.
- Triple conjugation has been seen a number of times.

Conjugation has been observed in several other urceolariids, Caullery and Mesnil (1915) and Brouardel (1936) reported stages of conjugation in *Leiotrocha patellae* (Cuénot, 1891). Peschkowsky (1923) stated that conjugation in *Trichodina steinii* Claparéde & Lachmann, 1858[13] is apparently like that of the vorticellids in all essential features. Anisogamous conjugation was described by Zick (1928) for *Urceolaria korschelti* Zick, 1928 and by Hunter (1936) for two types of mobiline peritrichs found in the intestines of sea cucumbers.

Padnos and Nigrelli (1942) present the most complete account of trichodinid conjugation in their study of *T. spheroidesi* Padnos & Nigrelli, 1942 (Colwin 1944). Conjugation has also been studied in *Trichodina reticulata* Hirschmann & Partsch, 1955; there are micro- and macroconjugants, not differing markedly in size (Ahmed 1977). Davis (1947) has also reported the occurrence of conjugation in *Trichodina bulbosa* Davis, 1947[14]; *T. californica* Davis, 1947[15]; *T. discoidea* Davis, 1947[16] and *T. symmetrica* Davis, 1947[17]. The process is essentially the same for all these trichodinid

[13] *Trichodina steinii* Claparéde & Lachmann, 1858 is a nomen nudum.
[14] Now known as *Tripartiella bulbosa* (Davis, 1947). [15] Now known as *Tripartiella californica* (Davis, 1947).
[16] *Trichodina discoidea* Davis, 1947 is a nomen nudum (Lom & Laird 1969).
[17] Now known as *Trichodinella symmetrica* (Davis, 1947).

species. Colwin (1944) has also described the process of binary fission for *U. synaptae* and it is essentially the same as the process in *T. spheroidesi.*

Conjugation in *Trichodina spheroidesi* Padnos & Nigrelli, 1942[3] according to Padnos and Nigrelli (1942)

Family: Trichodinidae Raabe, 1959 (Fig. 3.13)

The process in *Trichodina spheroidesi* is very similar to that reported for *Vorticella nebulifera* Müller, 1773 var. *similis* Stokes, 1887 (Maupas 1888; Doflein 1927).

- Padnos and Nigrelli (1942) emphasize the fact that the conjugants are always unequal in size (Fig. 3.13 no. 1).
- The aboral surface of the microconjugant is fitted over the adoral surface of the macroconjugant. They may or may not be oriented in the same direction. After the conjugants have assumed these positions, the micronucleus of each begins to swell, eventually becomes vesicular and passes from the original posterior position to a more central location in the cell.
- The macronucleus undergoes vacuolization, and at the time of spindle formation, the macronucleus twists and pulls apart into large coarse fragments which continue to break up into smaller parts until minute spherical bodies with deeply staining granules are formed.
- During the final fragmentation of the macronucleus, two micronuclear divisions take place in each conjugant. Conjugation in *T. spheroidesi* differs from conjugation in *V. nebulifera* var. *similis* in that there are only two micronuclear divisions in each conjugant instead of three (Figs. 3.13 no. 2 - 10).
- Protoplasmic continuity is established between the conjugating individuals and the content of the smaller individual passes into the larger one.
- It is assumed that the gametic nuclei then combine to form the synkaryon, and the remaining nuclei are resorbed. The remains of the microconjugant collapse and the ensuing processes of conjugation are confined to the single large exconjugant (Figs. 3.13 no. 11 & 12).

- It is assumed that three mitotic divisions subsequently occur, resulting in eight micronuclei. Seven of these become the macronuclear anlagen and one becomes the functional micronucleus (Figs. 3.13 no. 13 & 14).
- The functional micronucleus divides and in the cell division that takes place, the macronuclei are distributed between the daughter individuals (Fig. 3.13 no. 15). The most frequent distribution is three and four (sometimes it may be two and five, or one and six) (Figs. 3.13 no. 16 - 19).
- Cell division continues until each of the daughter cells formed contains one macronuclear anlage. The macronucleus then increases in size and develops the characteristic horseshoe shape (Fig. 3.13 no. 20).
- Reorganization of the denticle ring occurs in the macroconjugant shortly after the fusion of the protoplasmic contents of the conjugants has occurred.

Conjugation in *Scyphidia ameiuri* Thompson, Kirkegaard & Jahn, 1947 according to Thompson, Kirkegaard and Jahn (1947)

Family: Scyphidiidae Kahl, 1933 (Fig. 3.14)

The microconjugant resembles the sessile form, but it is smaller (half the length and diameter of the macroconjugant) and has a rounded base (Fig. 3.14). Numerous small individuals were found that varied in size from that of the sessile (vegetative) form to that of the microconjugant. At least four microconjugants (probably eight) are formed by repeated binary fission. They become free swimming and then attach to the distal one-third of the macroconjugant. In a few cases two microconjugants were observed to be attached to one macroconjugant. The macroconjugant has always been observed to be attached to the gill during conjugation.

Conjugation in *Scyphidia macropodia* Davis, 1947 according to Davis (1947)

Family: Scyphidiidae Kahl, 1933

Scyphidia macropodia Davis, 1947 is a ciliophoran that occurs on the gills and body of the bullhead *Ameiurus nebulosus* and the channel catfish *Ictalurus punctatus*. Although individuals in various stages of conjugation were not uncommon, it has been

impossible to follow the entire process in detail. The process of conjugation appears to be similar to that of *Vorticella microstoma* as described by Finley (1943). Finley (1952) and Davis (1947) reported that when conjugating forms are present, vegetative forms undergoing binary fission were also common. Davis also indicated that microconjugants of *Scyphidia* are produced in the same way as microconjugants in the genus *Rhabdostyla*, namely as a result of a preconjugation fission followed by two successive fissions of the undifferentiated microconjugant. Thus, in *Trichodina* and in *Scyphidia* Dujardin, 1841, cytoplasmic and macronuclear differentiation are encountered (Davis 1947).

- According to Davis (1947), the small free-swimming microconjugants are apparently formed from an ordinary individual through budding [Finley (1952) refers to this as preconjugation fission].
- The microconjugant becomes attached to the macroconjugant near the peristome, but does not unite with it immediately.
- The micronucleus of the microconjugant forms eight nuclei of equal size by three rapidly occurring divisions.
- At the same time, the micronucleus in the macroconjugant divides twice to form four nuclei of equal size.
- During the divisions of the micronuclei the macronuclei of both conjugants remain unchanged.

- Davis (1947) has not followed the next stage in the process, but from analogy with other vorticellids, it is assumed that the eight micronuclei pass from the microconjugant into the macroconjugant.
- One of the micronuclei becomes a pronucleus and unites with the pronucleus of the macrogamete to form the synkaryon.
- The remaining seven micronuclei of the microconjugant and three micronuclei of the macroconjugant then degenerate and take no further part in the process.
- During formation of the synkaryon, the macronuclei of both conjugants break down in rounded structures similar to those formed in *Trichodina* species (Davis 1947).

- The synkaryon then divides twice to form four nuclei, three of which increase in size to form macronuclear anlagen, while the fourth becomes the functional micronucleus.

This does not agree with the findings of Padnos and Nigrelli (1942) and Finley (1943) who have found that the postconjugants in peritrich ciliophorans contain eight nuclei, seven of which develop into a macronuclear anlagen as in *Trichodina*. The only exception is Enriques (1907) who found that in *Opercularia* only four nuclei were present in the postconjugant, three of which became macronuclear anlagen.

Postconjugants in *Scyphidia macropodia*, containing three macronuclear anlagen and one micronucleus, were quite common, but although a special search was made, no individuals containing more than four nuclei could be found (Davis 1947).

- The macronuclear anlagen increase in size and become elongate and eventually U-shaped.
- The macronuclear anlagen are spherical at first, finely granular and stain less deeply than the rounded remnants of the macronuclei.
- The postconjugant then divides by two rapidly occurring divisions (binary fission) into four individuals that are much smaller than usual.

Davis (1947) also studied conjugation in *Scyphidia tholiformis*, which appears to be essentially the same as in *S. macropodia*. The macronuclei, however, break down before the conjugants unite and consequently it is very difficult to follow the divisions of the micronuclei. Only a few postconjugants were found, but no more than three macronuclear anlagen could be distinguished in any individual.

Conjugation in *Scyphidia tholiformis* Surber, 1943 according to Surber (1943)
Family: Scyphidiidae Kahl, 1933
Surber (1943) states that individuals in the process of gamete formation can easily be distinguished from those that are already undergoing conjugation. During gamete

formation, the macronuclear material is divided into one to eight large, rounded bodies, the endoplasm is unvacuolated and the microconjugant in the process of being cut off contains four large masses and four smaller ones. In the macroconjugant the macronucleus divides into about thirty-two, large, irregularly shaped bodies. Macronuclear division begins at the posterior end and is preceded by micronuclear division.

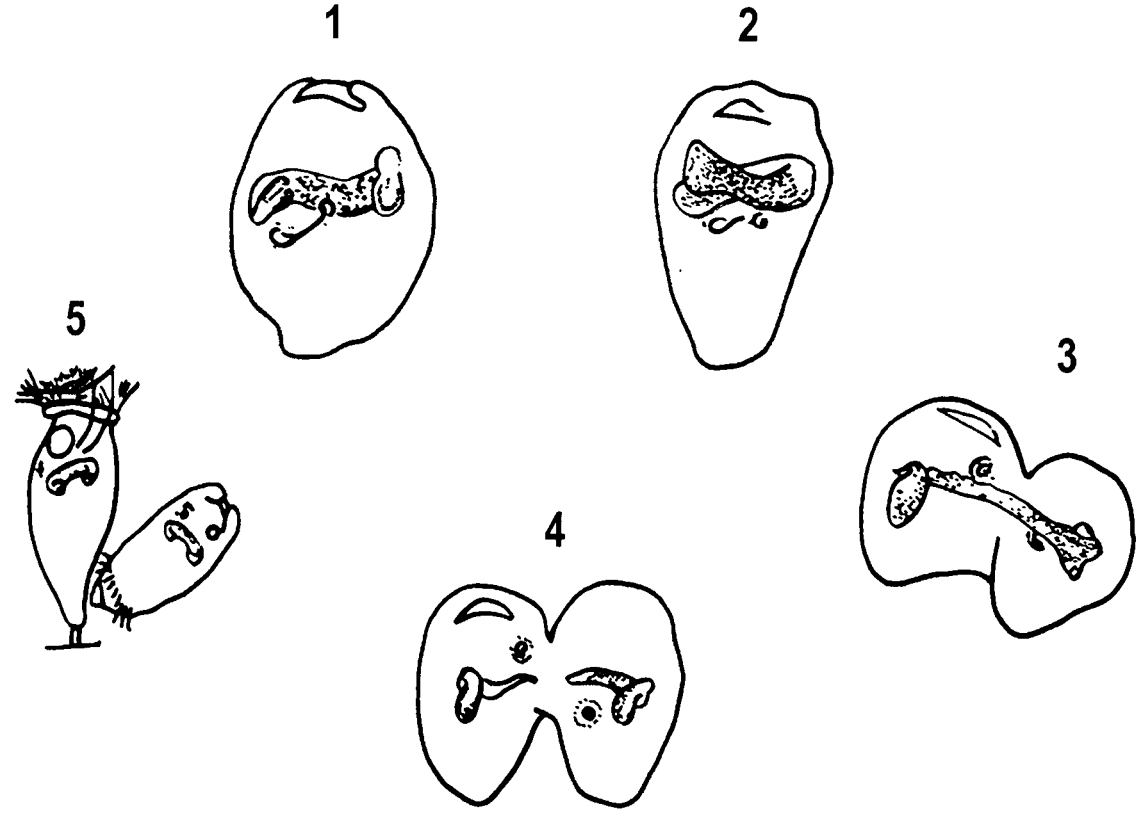
Macroconjugants in the process of conjugation usually have one microconjugant attached near the anterior end of the peristomial border. The microconjugants are about 11.0 μm by 8.0 μm in size. At the time of conjugation the microconjugants have eight large nuclear bodies within them. Conjugation occurs at a slower rate than the formation of conjugants because large numbers of conjugating individuals are found. Surber (1943) did not describe the series of micro- and macronucleur divisions.

Figure 3.1

Diagrammatic illustration of binary fission in *Rhabdostyla vernalis* Stokes, 1887 [Redrawn from Finley (1952)].

1. The micronucleus has almost completed the telophase stage. The macronucleus has not begun division.
2. Separation of the micronuclei. The macronucleus is enlarged in preparation for amitotic division.
3. The cytoplasmic plane of fission has become clearly defined. The peristome is retained by the parent, but not yet developed in the daughter. The macronucleus has become elongated in preparation for separation into two approximately equal portions. Only one of the two micronuclei is in focus.
4. Showing one micronucleus and approximately equal portions of the macronucleus in each product of binary fission. The cytoplasmic plane of fission is clearly defined.
5. The end result of binary fission. The parent is feeding and the daughter's peristome and posterior ciliary wreath have become differentiated.

6-10. Binary fission summarized.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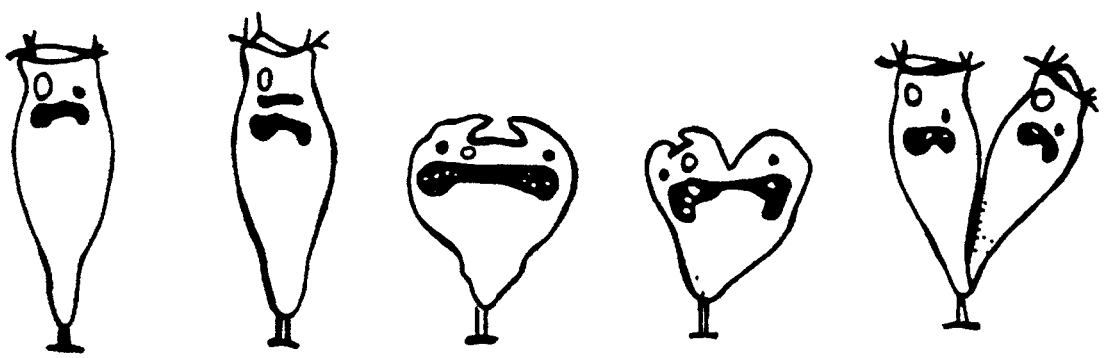
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Figure 3.2

Diagrammatic illustration of binary fission in *Urceolaria synaptae* Cuénot, 1891 [Redrawn from Colwin (1944)].

1. Vegetative individual. Type II. Anti-discal aspect. Macronucleus appears "seamy".
2. Fission. Slight consolidation of macronucleus. Micronucleus slightly enlarged, has begun migration orally.
3. Macronucleus shows wavy outline. Micronucleus migration continued. Threads of cytoplasm still link micronucleus with former position. Beaded threads of chromatin in micronucleus appear double.
4. Further consolidation of macronucleus; longitudinal streaking. Condensation of beaded threads in micronucleus. Micronucleus now in typical location for mitosis.
5. Micronucleus in optical section. Slightly earlier than illustrated in no.4.
6. Two cytopharynges. Macronucleus beginning elongation. Micronucleus in anaphase; separation spindle does not show any heavily staining fibers.
7. Micronucleus in optical section, later anaphase. Separation spindle has three fibers staining more heavily than others.
8. Partial constriction; a vacuole in plane of cleavage furrow; one denticle out of alignment. Macronuclear elongation continued and constriction begun. Micronucleus in late telophase; membranes of daughters seem completed; separation spindle. Polar view of micronucleus on left; lateral view of right.
9. Constriction nearly completed. Micronucleus on right has reached the typical vegetative location near the center of the aboral side of macronucleus.
10. Post-fission: new denticle ring developing outside old one. Arms of macronucleus lengthening; pitting still clear. Micronucleus seen in polar view.
11. Late post-fission. Macronucleus still enlarging; cross striations apparent.

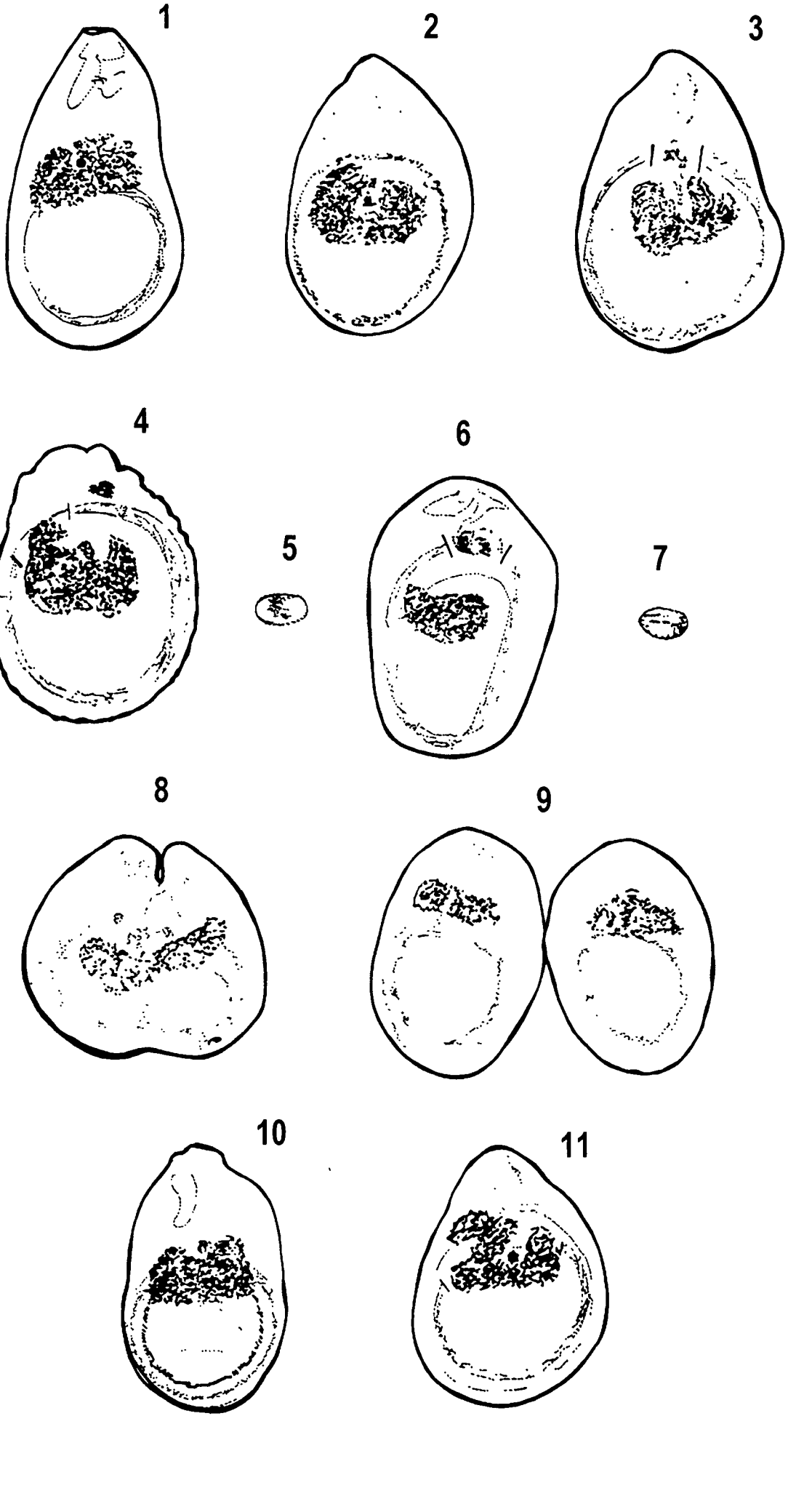
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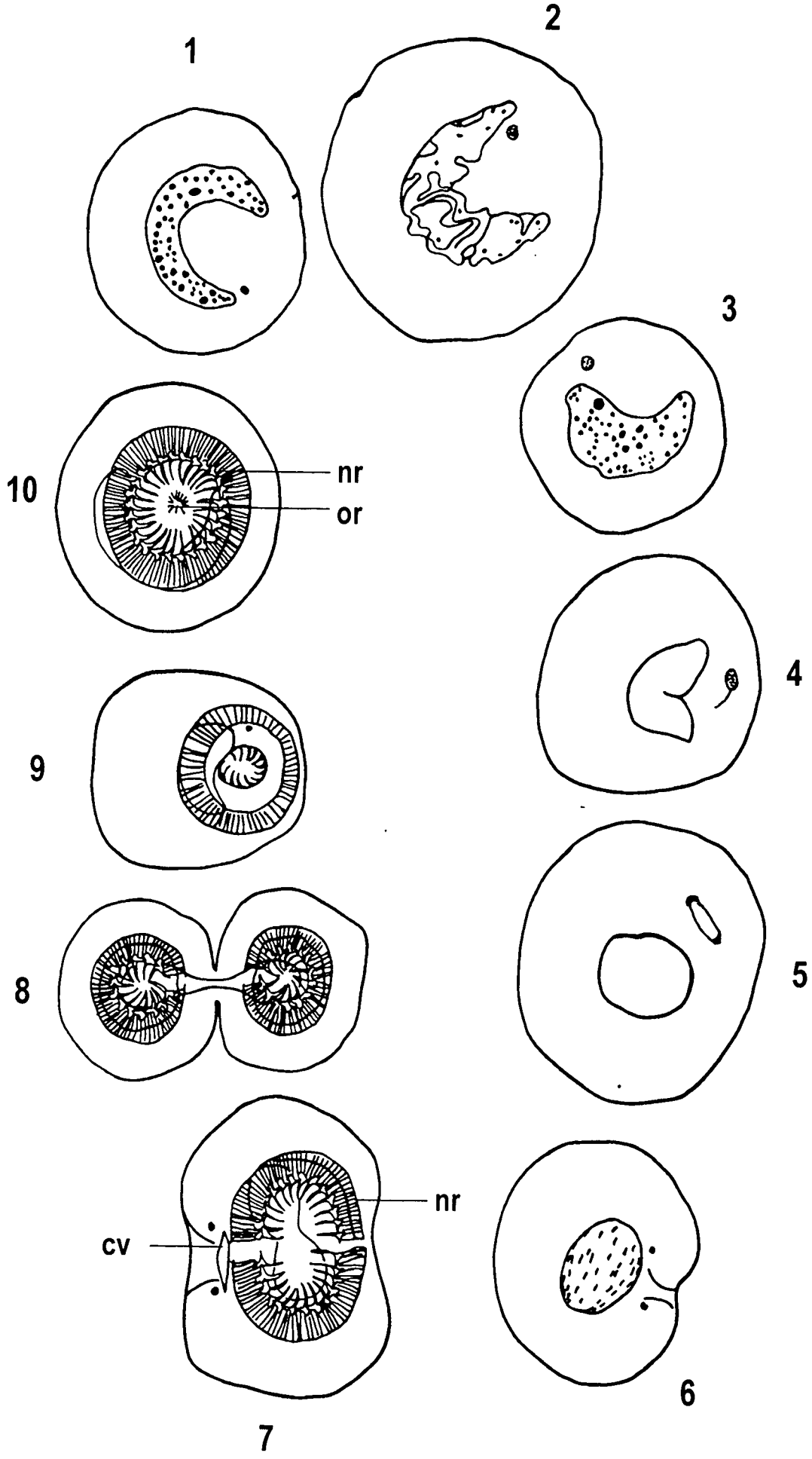
Figure 3.3

Diagrammatic illustration of binary fission in *Trichodina spheroidesi* Padnos & Nigrelli, 1942 [Redrawn from Padnos and Nigrelli (1942)].

1. Trophic stage.
2. Macronucleus in stage of contraction.
2,3. Note swelling of micronucleus.
3-5. Further contraction of the macronucleus.
4. Micronucleus in metaphase.
5. Telophase.
6. Completed micronuclear division.
7-10. Binary fission stages showing the final division of the macronucleus and the reorganization of the denticle ring.

cv = contractile vacuole, **nr** = new ring, **or** = old ring.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or
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cv

Figure 3.4

Diagrammatic illustration of binary fission in *Scyphidia ameiuri* Thompson, Kirkegaard & Jahn, 1947 [Redrawn from Thompson, Kirkegaard and Jahn (1947)].

1,2. Early stages in division.
3-5. Later stages in division.

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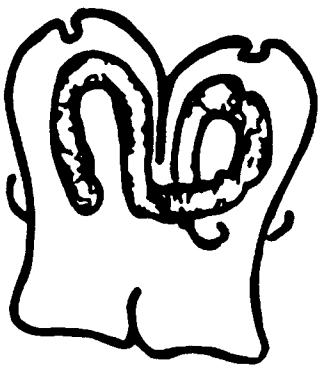

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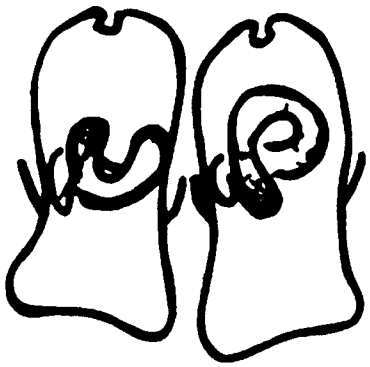

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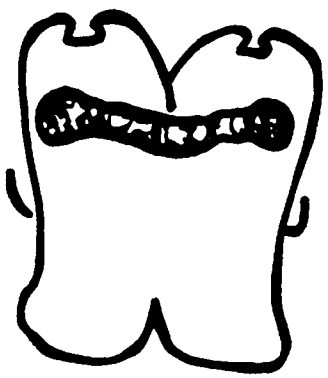

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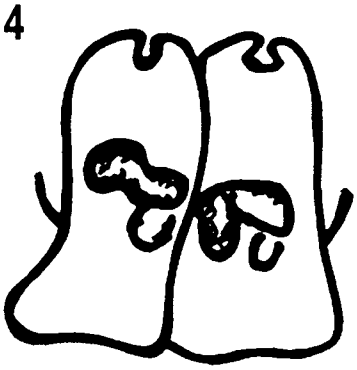

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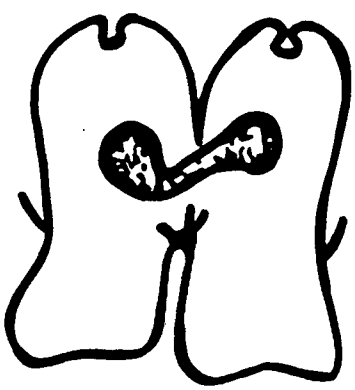
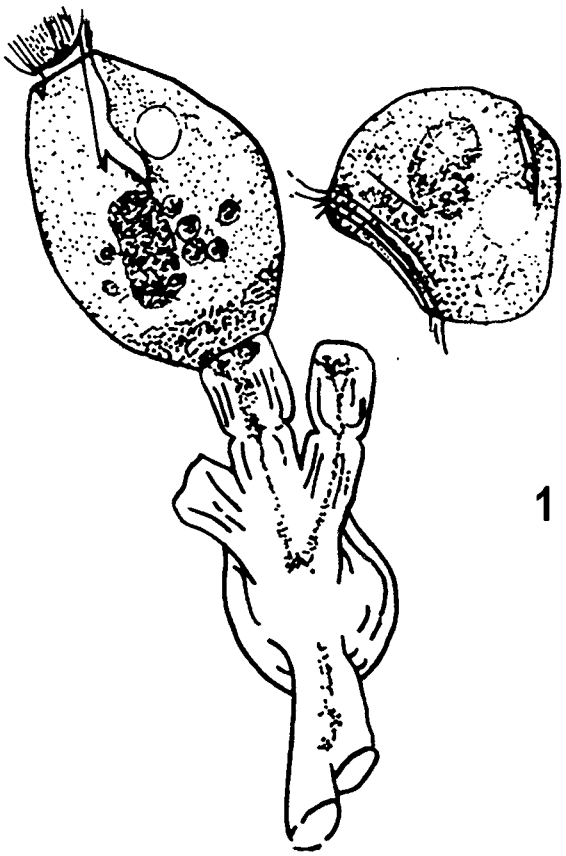


Figure 3.5

Diagrammatic illustration of telotroch formation in *Orbopercularia raabei* Dobrzañska, 1961 [Redrawn from Dobrzañska (1961)].

1. A colony with telotrochs forming after Heidenhain's Iron hematoxylin preparation.
2-4. Stages of telotroch formation. Infravital drawings.
5-8. Stages of telotroch transforming to become a settled form; after infravital photographs.



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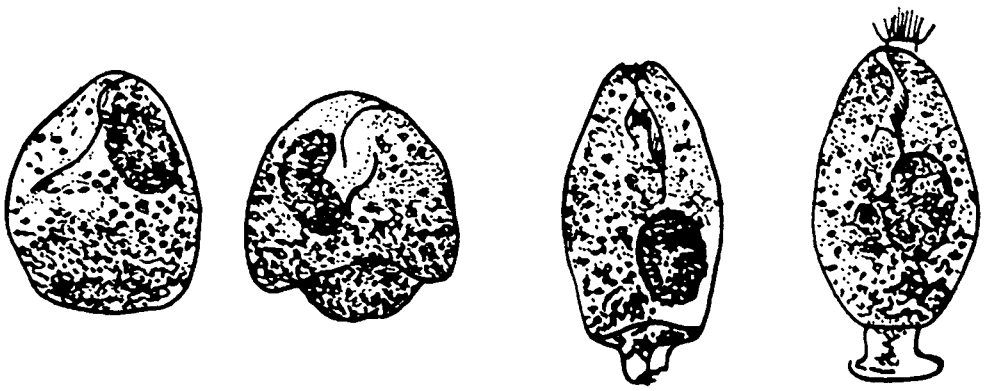

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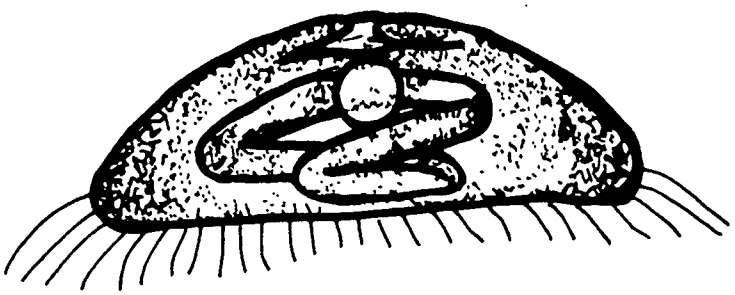


Figure 3.6

Diagrammatic illustration of telotroch formation in *Scyphidia ameiuri* Thompson, Kirkegaard & Jahn, 1947 [Redrawn from Thompson, Kirkegaard and Jahn (1947].

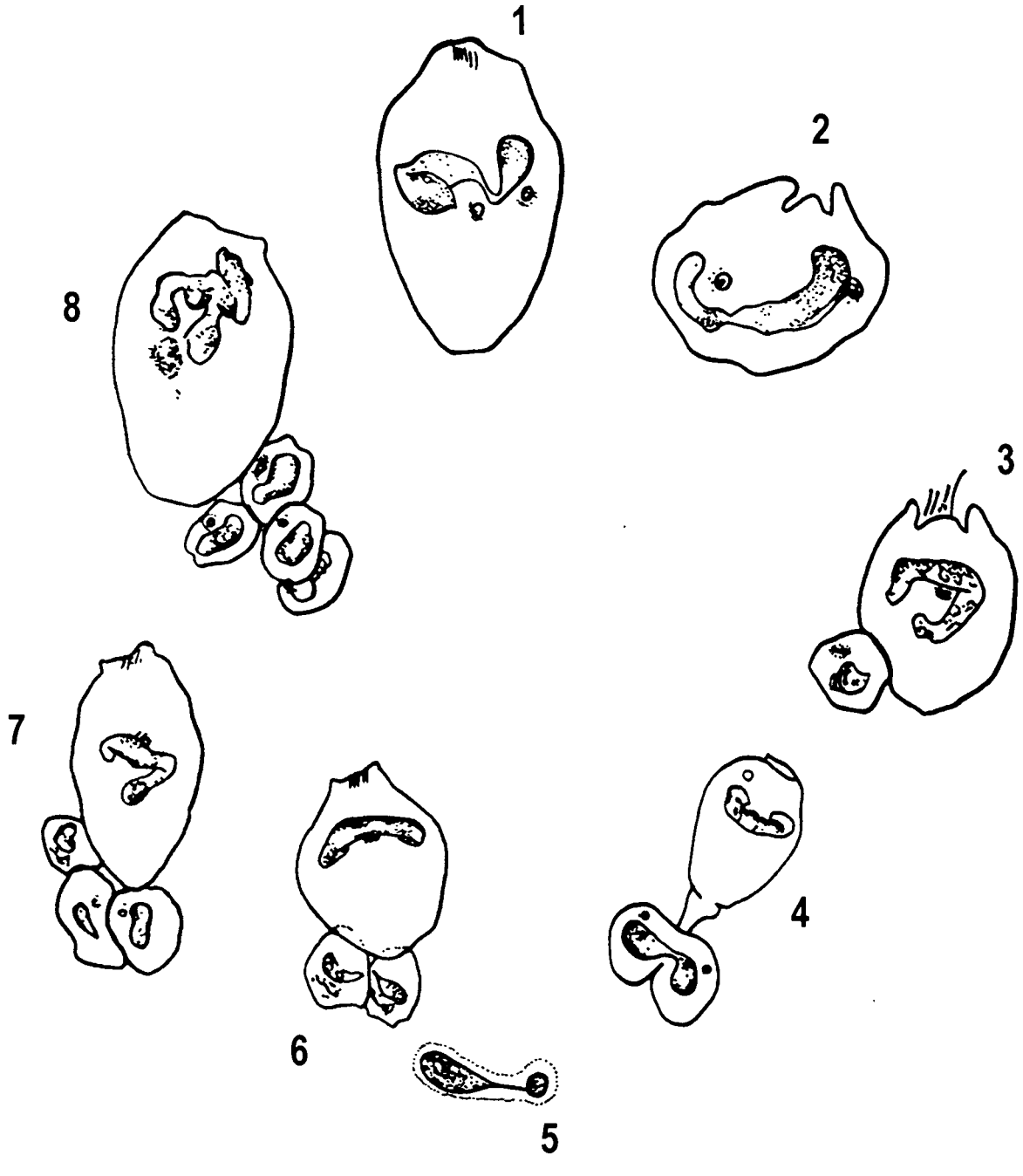
Telotroch, side view. Circular in oral view.

Figure 3.7

Diagrammatic illustration of preconjugation fission in *Rhabdostyla vernalis* Stokes, 1887 [Redrawn from Finley (1952)].

1. Preconjugating (sex differentiating) fission. Showing the two mitosis-derived micronuclei approximately equal in size; macronucleus preparing to separate amitotically, into equal portions.
2. Preconjugation fission; cytostome "transversely" elongated in preparation for the unequal division. Macro- and micronuclei similar to the stages shown in Figure 3.7 no. 1.
3. Preconjugation fission, showing the protoconjugant (undifferentiated microconjugant) and macroconjugant. Protoconjugant's micronucleus undergoing mitosis in preparation for fission. Macroconjugant's micronucleus, protoconjugant's peristome not in focus.
4. Protoconjugant undergoing equal fission; its micronuclei having completed the mitosis before the macronucleus divides by amitosis. The macroconjugant's nuclei are in focus.
5. Enlarged view showing details in a micronucleus.
6. Preconjugation fissions, showing two protoconjugants, the macronuclei have divided very recently and the micronuclei are in telophase. Macroconjugant's micronucleus not in focus.
7. Two microconjugants, one protoconjugant and the macroconjugant. The protoconjugant's micronucleus is undergoing mitosis.
8. Preconjugation fissions completed, conjugation in progress. Six specimens are illustrated here. Four microconjugants clearly shown in association with the macroconjugant's aboral region. Macroconjugant's macronucleus in the balloon stage because it is in the act of conjugation with the microconjugant which is illustrated in dotted outline (dotted outline because the microconjugant is barely in focus). The conjugant's micronuclei are not in focus.

9-13. Sketches from live specimens summarizing preconjugation fission. A "foreign" microconjugant approaches the macroconjugant in Figure 3.7 no. 12 and 13.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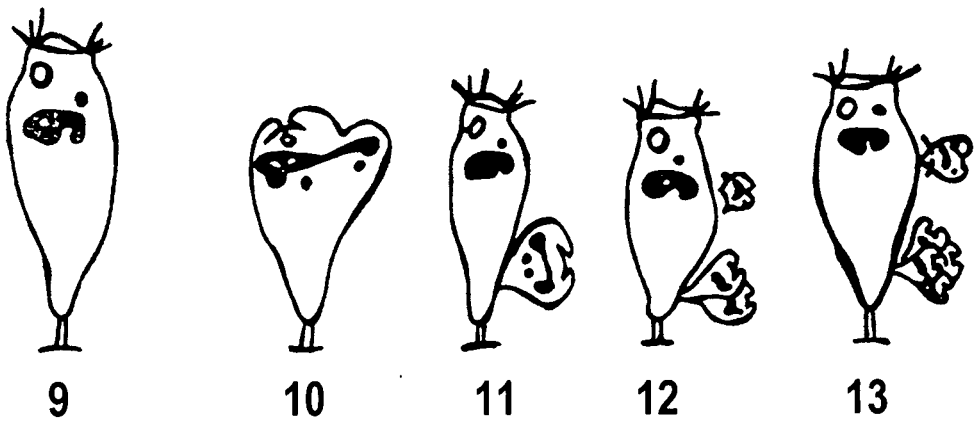
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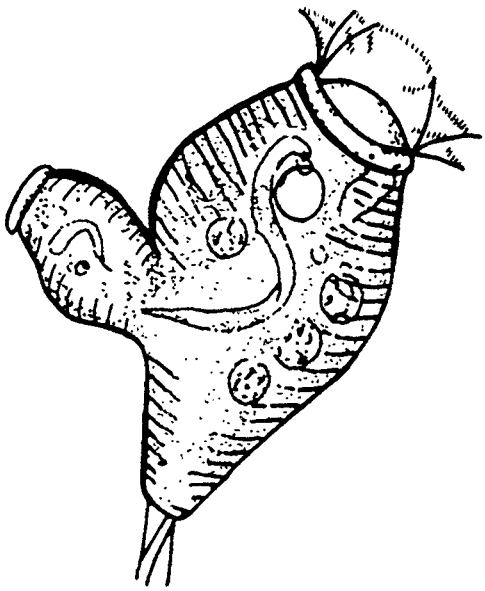
Figure 3.8

Diagrammatic illustration of preconjugation fission in *Vorticella microstoma* Ehrenberg, 1830 [Redrawn from Finley (1943)].

1. A late phase of the sex-differentiating or preconjugation fission, showing the equal micronuclei and the unequal amounts of the endoplasmic components distributed between the macroconjugant and the microconjugant.
2. Preconjugation division showing daughter micronuclei. One micronucleus is entering the developing macroconjugant. The macronuclear division has not yet begun (Bouin's fixation, Feulgen stain).
3. Three microconjugants associated with the same macroconjugant. The microconjugant on the left is a daughter product of the macroconjugant shown in this figure. The other two microconjugants are in the act of fusing with the macroconjugant; the micronuclei are elongated in preparation for the first progamic (preliminary) nuclear division (Bouin's fixation, Feulgen stain).



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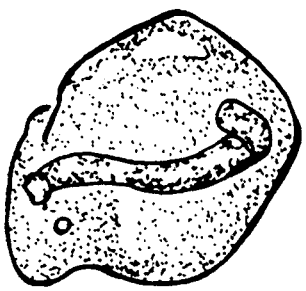

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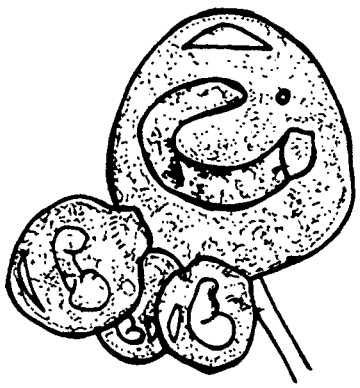

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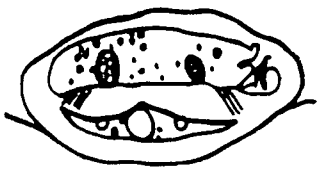
Figure 3.9

Diagrammatic illustration of preconjugation fission in *Lagenophrys tattersalli* Willis, 1942 [Redrawn from Willis (1942)].

1. Section of a division stage after treatment by the Weigl process. Note swarmer cut in longitudinal section and a portion of the residual organism.
2. Swarmer and residual organism produced by second type division. In this case cleavage was not preceded by nuclear division. Feulgen light-green, after mercuric-acid. Dorsal view.
3. Two swarmers and a residual organism produced by the rapid succession of first and second type divisions. Note the rotation of the first (left) swarmer. The cleavage of the residual organism from the second swarmer is here preceded by micronuclear and unequal macronuclear division. Feulgen light-green, after mercuric-acid. Dorsal view.



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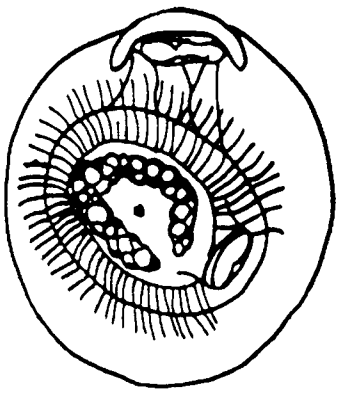

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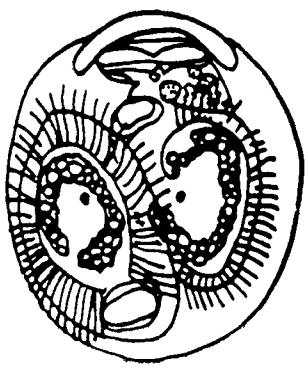

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Figure 3.10

Diagrammatic illustration of conjugation in *Rhabdostyla vernalis* Stokes, 1887 [Redrawn from Finley (1952)].

1. Conjugation. Showing the microconjugant fused to the upper adoral portion of the macroconjugant. Nuclear complements visible in both conjugants.
2. The micronucleus enlarged.
3. Conjugation showing two pronuclei in the microconjugant, both of them derived from the preliminary mitotic division and one of them preparing to undergo the second progamic division. The macroconjugant's micronucleus has elongated in preparation for its first progamic division.
4. The microconjugant's peristome is in focus, its endoplasm and nuclear complement entering the macroconjugant. One of the macroconjugant's progamic nuclei is in focus.
5. Showing six active progamic nuclei and four small degenerating pronuclei. The spindle-shaped progamic nucleus was probably derived from the microconjugant. Undoubtedly, other micronuclei are obscured by the macronuclei.
6. Macronuclei ballooning in preparation for skein formation. Only three progamic nuclei are in focus.
7. Early stage in the formation of macronuclear skeins. Three micronuclei are in focus, those two in the upper adoral zone are functional pronuclei on the verge of fusing to form the synkaryon.
8. Showing the spindle-shaped synkaryon undergoing the first metagamic mitotic division. Macronuclear segments and degenerating progamic nuclei are also visible.
9. The synkaryon enlarged.
10. Seven macronuclear anlagen are visible although they lie at different levels; they may be located by means of the definite halos. Nine degenerating macronuclear segments are visible, the borders are irregular in shape. The micronucleus lies directly below and to the left of the peristome; it is undergoing mitosis.
11. Product of the first reorganization fission. Four large macronuclear anlagen, nine degenerating macronuclear segments, micronucleus in mitosis.
12. Product of the second reorganization fission. The micronucleus lies near the peristome; it has become enlarged in preparation for division. Two macronuclear anlagen and seven degenerating macronuclear segments are visible.
13. Showing an exconjugant's macronucleus differentiating in the direction of the normal morphology. This stage represents the conclusion of the reorganization process.

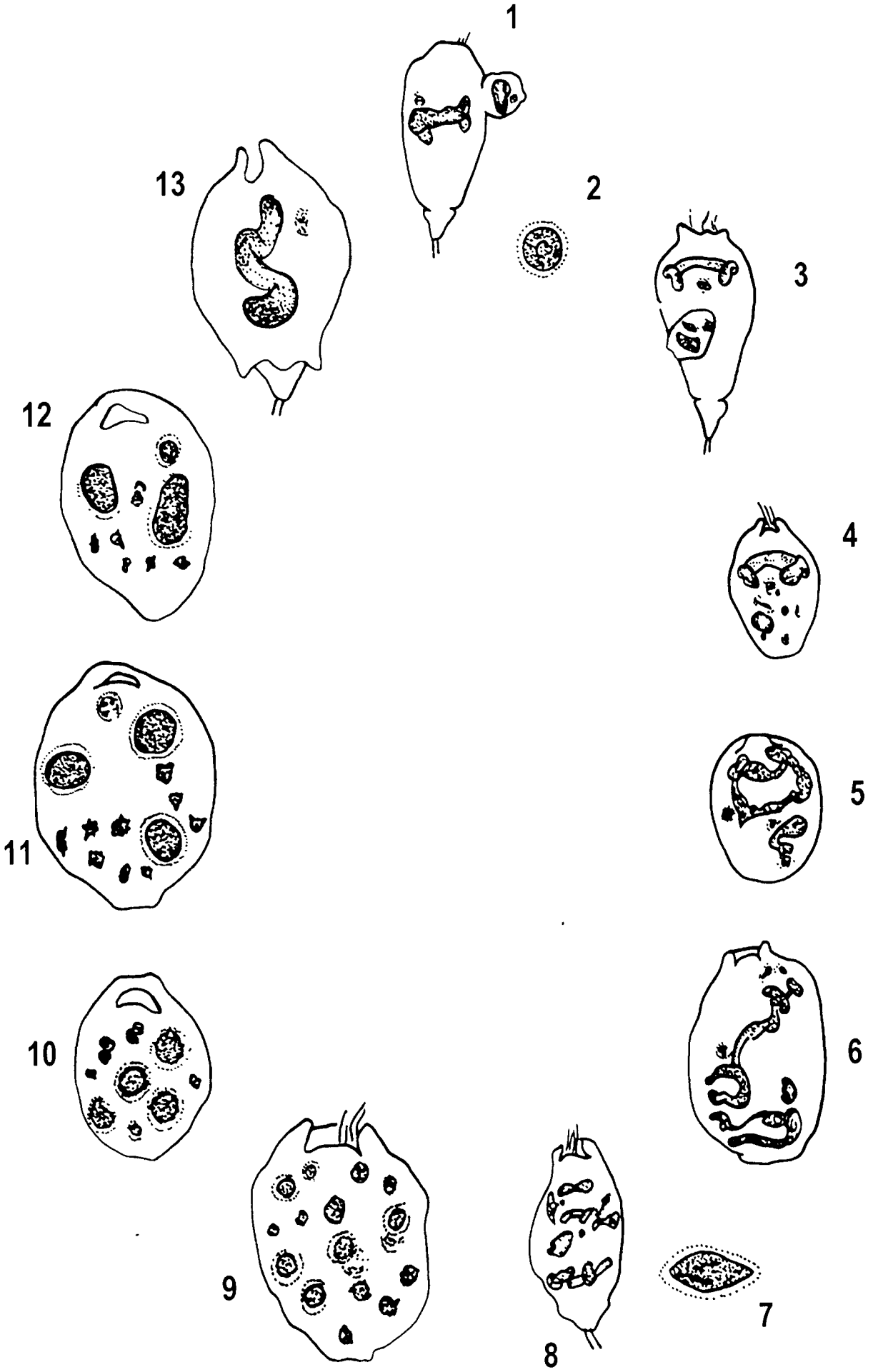
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Figure 3.11

Diagrammatic illustration of conjugation in *Vorticella microstoma* Ehrenberg, 1830 [Redrawn from Finley (1943)].

1. A normal expanded individual, showing the elongated macronucleus, the contractile vacuole, four food vacuoles and a part of one spasmoneme spiral in the stalk.

2,3,4 Preconjugation fission.

5 Interval 1 in the conjugation period. Microconjugant's endoplasmic components entering the macroconjugant. The microconjugant's micronucleus is in a late prophase stage of the first progamic division and the four chromosomes have assumed the definite form. Macroconjugant's micronucleus in early (growth) stage of the first progamic division (Bouin's fixation, Feulgen stain).

6 Microconjugant's micronucleus (Bouin's fixed, Feulgen stained).

7 Nucleoli in the macronucleus of each conjugant. In the macroconjugant's macronucleus one nucleolus opens to the outside. Microconjugant's micronucleus in early prophase stage of the first progamic division. Macroconjugant's micronucleus enlarged. Five food vacuoles are shown in the macroconjugant (Bouin's, Feulgen).

8 Two microconjugants fused with the same macroconjugant. Chromatids visible in the micronucleus of the microconjugant at the lower right of the figure (Bouin's, Feulgen).

9 Double strands (chromatids) in the micronucleus (Bouin's, Feulgen).

10 Interval 2, 45 minutes after fusion (Bouin's, Heidenhain's hematoxylin).

11 Micronucleus showing collections of granules connected by a delicate strand (Bouin's, Heidenhain's hematoxylin).

12 Chromosomes assuming definite form (Bouin's, Heidenhain's hematoxylin).

13 Interval 2, showing products of the microconjugant's second progamic nuclear division. The macroconjugant's micronucleus is in a prophase stage of the first progamic nuclear division. Both macronuclei are entering the balloon stage (Bouin's, Feulgen).

14 Macronuclei in the balloon stage. Products of the macroconjugant's first progamic nuclear division are shown. Only three of the microconjugant's micronuclei are visible. A food vacuole is shown just above and to the right of the macroconjugant's macronucleus (Bouin's, Feulgen).

15 Showing twelve progamic nuclei, two of which have elongated preparatory to forming the functional pronuclei (Bouin's, Feulgen).

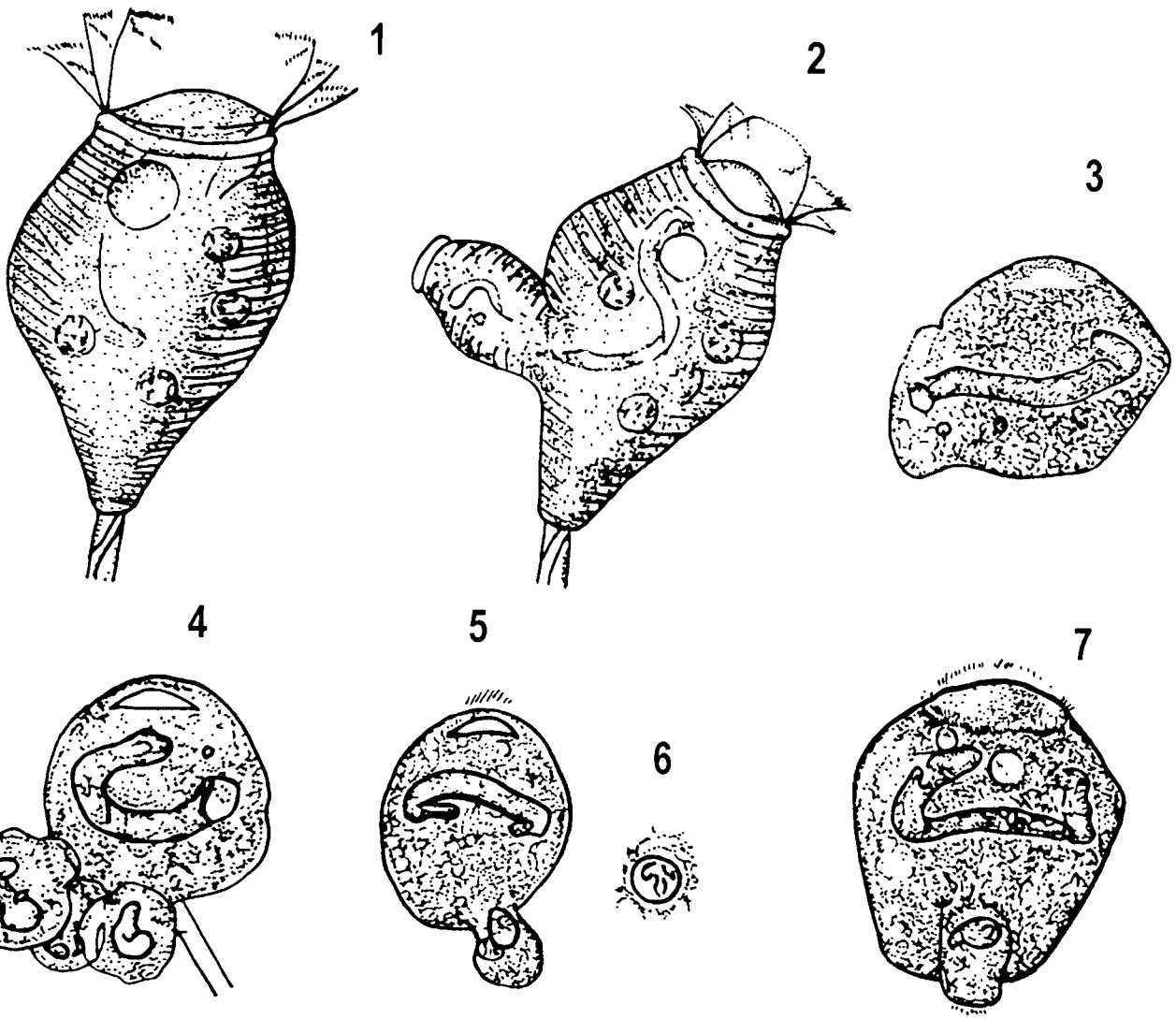
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Figure 3.11 continued

Diagrammatic illustration of conjugation in *Vorticella microstoma* Ehrenberg, 1830 [Redrawn from Finley (1943)].

16 An early stage in the formation of the macronuclear skeins. Showing the macroconjugant's four progamic nuclei; one has assumed the spindle shape which characterizes a functional pronucleus. Only one of the microconjugant's micronuclei is shown (Bouin's, Feulgen).
17 Showing the functional pronuclei just before they fuse to form the synkaryon. Six degenerating progamic nuclei clearly visible (Bouin's, Feulgen).
18 Interval 3. Synkaryon after assuming the spherical shape of a metagamic nucleus. One small degenerating progamic nucleus slightly posterior to the synkaryon (Bouin's, Feulgen).
19 Interval 4, showing three metagamic nuclei and three degenerating progamic nuclei (Schaudinn's, Heidenhain's).
20 An exconjugant after the third metagamic nuclear division. The micronucleus lies between the peristome and the contractile vacuole. Seven small degenerating progamic nuclei are shown in the posterior part of the body. Seven larger functional metagamic nuclei are also beginning the enlargement to form the macronuclear anlagen (Bouin's, Feulgen).
21 Seven enlarged (growing) metagamic nuclei and a micronuclear division figure. The old macronuclear segments are in the process of dissolution; they are characterized by the chromophobic centers and chromophilic peripheral portions (Bouin's, Feulgen).
22 The first reorganization fission. Showing three macronuclear anlagen in one daughter cell and four macronuclear anlagen in the other. Incomplete plasmotomy. The micronuclei may be located by means of the definite halos. One is in the upper right of the specimen on the right; the other is in the lower right of the specimen on the left (Bouin's, Feulgen).
23 A product of the first reorganization fission. Macronuclear anlagen somewhat elongated (Bouin's, Feulgen).
24 Four macronuclear anlagen in an individual resulting from the first reorganization fission (Bouin's, Feulgen).
25 A division figure in a product of the second reorganization fission (Bouin's, Feulgen).
26 Showing two microconjugants within the body of one macroconjugant (Bouin's, Feulgen).
27 A conjugant possessing a posterior ciliary wreath. Progamic nuclear divisions in progress (Goldsmith's, Heidenhain's).
28 A synkaryon showing four chromosomes (Bouin's, Feulgen).
29 Prophase stage of a microconjugant's nucleus entering the second progamic division. In the macroconjugant's nucleus this type of configuration has been found only during the first progamic division (Bouin's, Feulgen).
30 Anaphase stage of an exconjugant's micronucleus (Bouin's, Feulgen).
31 Two functional pronuclei, lying in the same halo, just before fusing to form the synkaryon (Bouin's, Feulgen).

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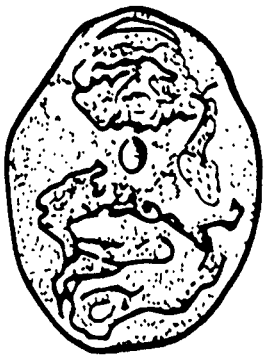

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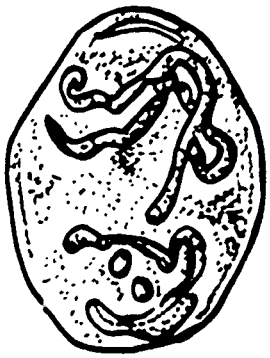

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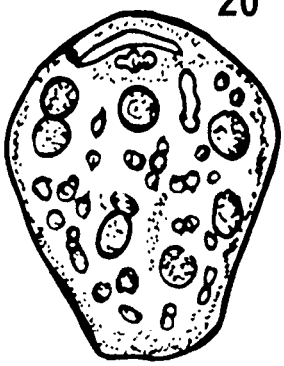

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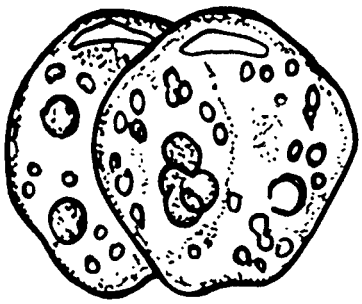

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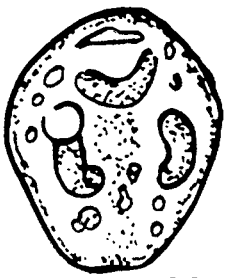

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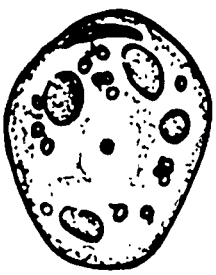

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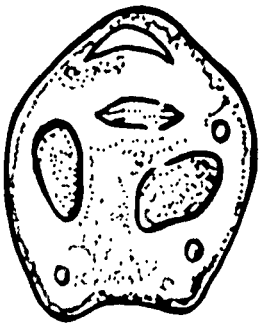

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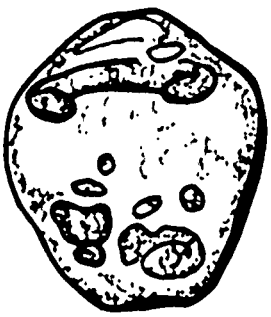

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Figure 3.12

Diagrammatic illustration of conjugation in *Urceolaria synaptae* Cuénot, 1891 [Redrawn from Colwin (1944)].

1. Pair in typical conjugation position, but with all nuclei still in vegetative conditions.
2. Conjugating pair. Consolidation of macronuclei, with disappearance of cross-striations. In macroconjugant part of macronucleus has been drawn lightly as to reveal micronucleus beneath it at lower level of focus. Micronuclei enlarging; endosome a granular mass with faint central line of cleavage.
3. Optical sections of the micronuclei from a pair of conjugants in a slightly more advanced stage than illustrated in no. 2.

3a. From macroconjugant, upper level of focus.
3b. Same, lower level of focus.
3c. From microconjugant, upper level of focus.
3d. Same, lower level of focus.

4. Conjugating pair, nuclei of microconjugant in more advanced stage than in macroconjugant. In microconjugant, macronucleus shows consolidation and more prominence of granules in pits; micronucleus shows loss of alignment of granules and greater prominence of chromatic mass.
5. Micronucleus in early parachute stage; finger-like projection of endosome.
6. Micronucleus in slightly later parachute stage; end masses separated by long thin neck containing corkscrew-shaped core.
7. Conjugating pair. Macronuclei in early ribbon stage. Part of macronucleus in macroconjugant omitted in order to show micronucleus. Micronuclei in parachute stage; later than in previous figure.
8. Conjugating pair. The macronucleus of the macroconjugant is in a slightly earlier stage than illustrated in the previous figure. Macronucleus of microconjugant shows fragmenting ribbon, part of which has been omitted in order to show the micronucleus. Micronuclei in parachute stage.
9. Micronucleus in late parachute stage; projection now resolved into long filament, the tip of which is toward observer in this specimen; rest of endosomal mass spherical with more deeply staining material near center.
10. Conjugating pair. Macronucleus of macroconjugant in late ribbon stage; that of microconjugant fragmented. Micronuclei in later parachute stage, showing increase of granular material and thickening of membrane at one side. Part of macronucleus omitted in macroconjugant in order to show micronucleus.
11. Micronucleus of very late parachute stage; darker mass in center of endosome, and thickened membrane still present.
12. Micronucleus. Membrane still shows trace of thickening. Granules of endosome gathered into elongate groups. Center of each group darker, giving impression of bar across nucleus.
13. Conjugating pair. Later than in following figure. Macronuclei fragmenting, note larger pieces in microconjugant. Micronuclei probably in early anaphase of meiotic division; groups of chromatic material separated into three parts; delicate fibers between and among groups.
14. Conjugating pair. Earlier than in previous figure. Macronuclei fragmenting; some parts omitted in microconjugant in order to show micronucleus. Micronuclei in metaphase of first pregamic division; coarse granules form chains near center of spindle; spindle in microconjugant happens to have one heavier fiber.
15. Macronuclei in fragments. Micronuclei in telophase of first pregamic division.

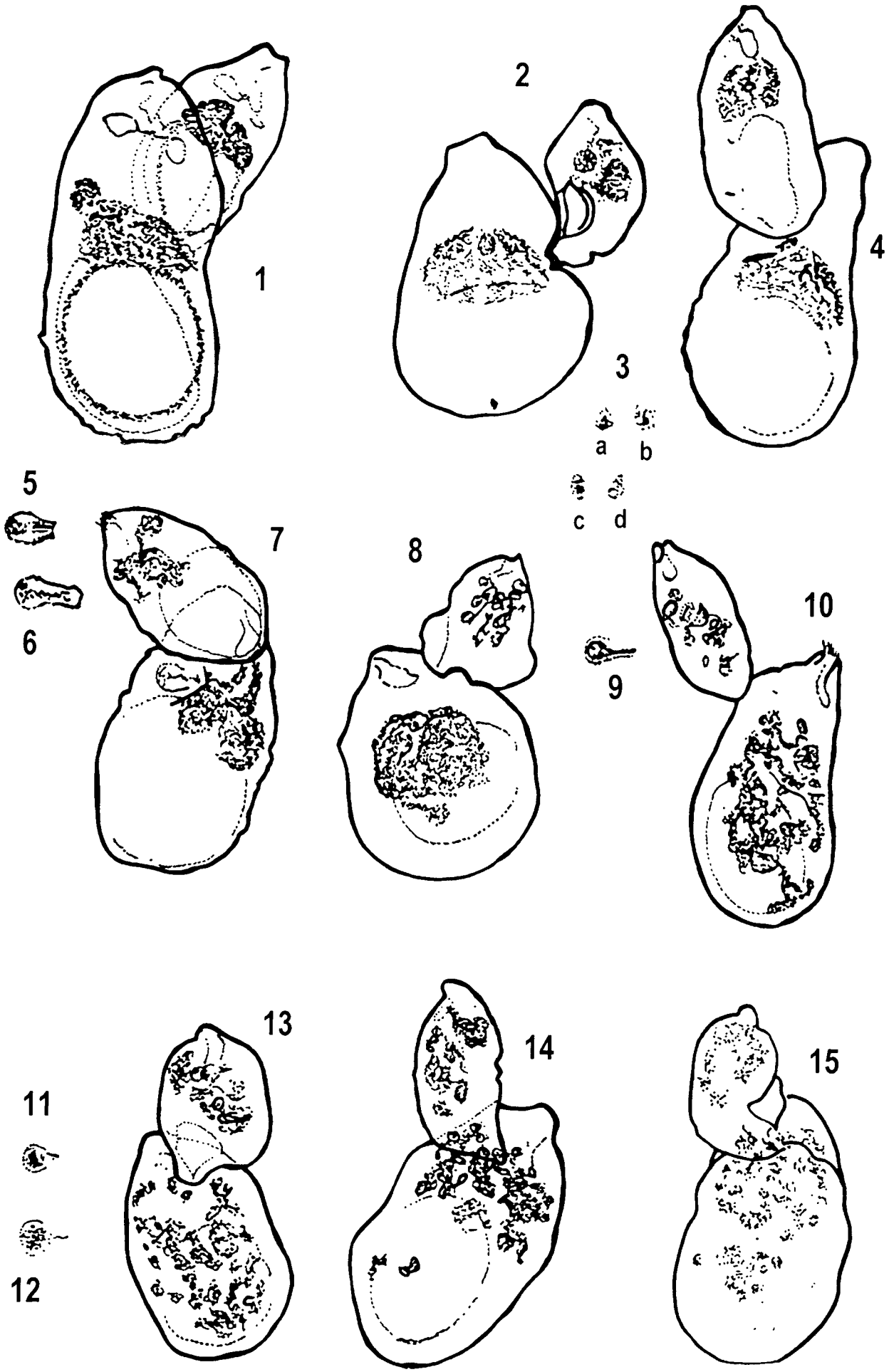
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Figure 3.12 continued

Diagrammatic illustration of conjugation in *Urceolaria synaptae* Cuénot, 1891 [Redrawn from Colwin (1944)].

16. Macronuclei in fragments. In the macroconjugant one micronucleus is in anaphase, other in telophase of second pregamic division. In the microconjugant the micronuclei are in prophase of second pregamic division; one in polar view, the other in oblique view.
17. Macronuclei in fragments. All micronuclei products of second pregamic division. In microconjugant, micronucleus at left of group possibly in early prophase of third pregamic division; other three in "resting" condition.
18. Macronuclei in fragments, one large fragment omitted in order to show micronuclei in microconjugant. Two incipient pronuclei in each conjugant.
19. Macronuclei in fragments. Incipient pronuclei in "resting" condition, the two in the macroconjugant seemingly slightly larger than those in microconjugant.
20. Note larger size of macronuclear fragments in microconjugant. Micronuclei of macroconjugant probably future pronuclei. Micronuclei of microconjugant undergoing synchronous division, interpreted as follows: one small nucleus is "resting", presumably after second pregamic division: one nucleus is in telophase, presumably of belated second pregamic division; one nucleus is in prophase-metaphase, presumably of third pregamic division.

21-22.Conjugating pair, drawn at two levels of focus. Stationary and migratory pronuclei are shown.

21. Lower level. Migratory pronucleus of microconjugant appears to be pushing against cell-membrane.
22. Upper level. Migratory pronucleus of macroconjugant appears to be breaking through cell-wall in narrow prong, but thick membrane of nucleus may represent part of cell-wall in narrow prong. Free-hand 2 X enlargement of cells shown in previous figure.
23. Apparent continuity of cells on either side of migratory nucleus shown at upper level of focus. (Continuity not apparent at lower focus, suggesting one pronucleus crosses before the other). Note "flowing" appearance of migratory nucleus at lower level.
24. Conjugating pair, showing outline of protoplasmic bridge, and apparent continuity of cells. Some macronuclear fragments of microconjugant seem to have entered macroconjugant. Synkaryon in macroconjugant: probably in microconjugant. Small granule seemingly attached to synkaryon in microconjugant actually lies above it.
25. Later synkaryon in each cell. Cell membranes appear to be intact. Nuclear membranes actually present around all fragments, drawn only in a few.

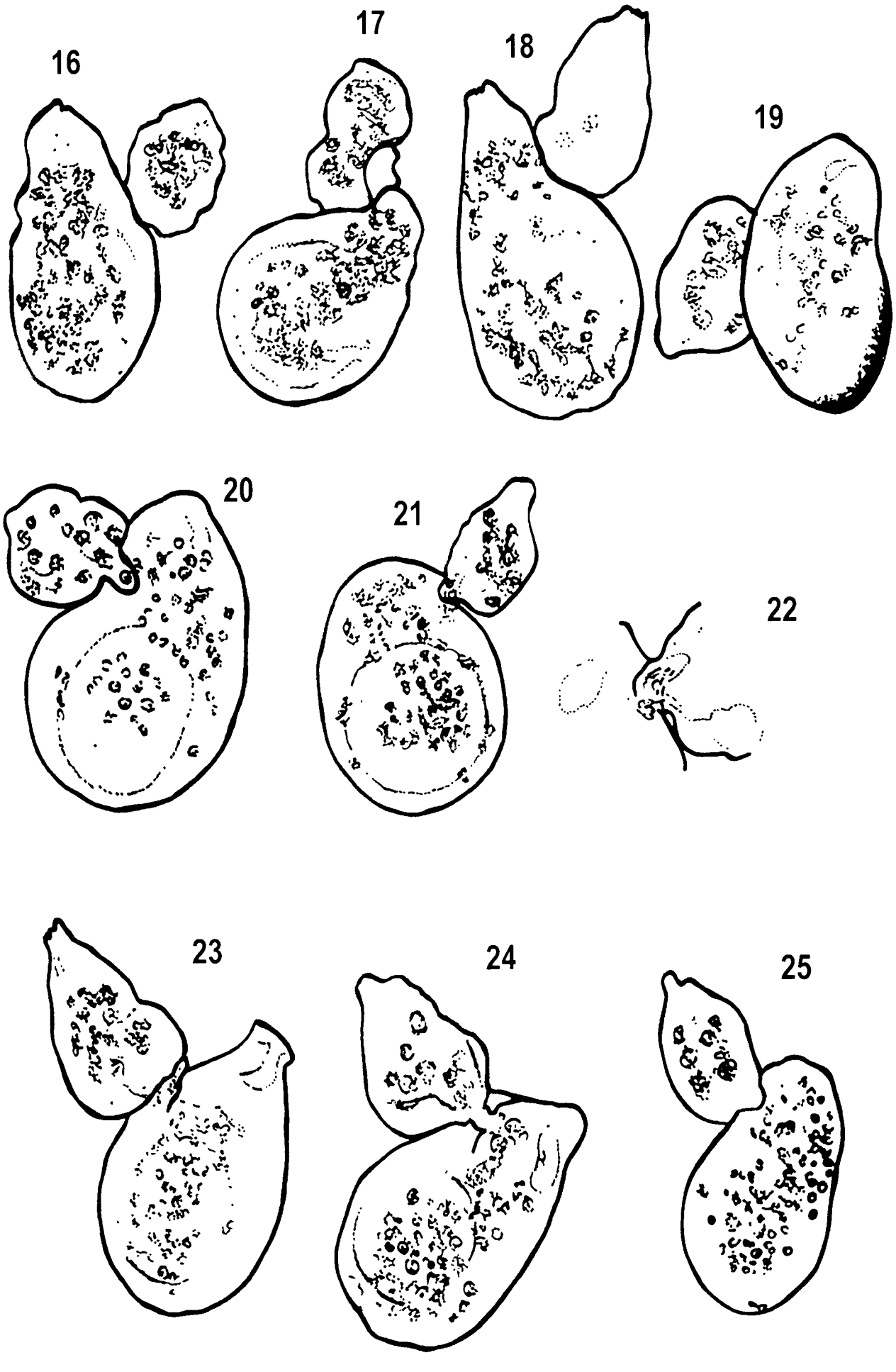
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Figure 3.12 continued

Diagrammatic illustration of conjugation in *Urceolaria synaptae* Cuénot, 1891 [Redrawn from Colwin (1944)].

26. Conjugating pair, showing migration of macronuclear fragments from microconjugant into macroconjugant. Synkaryon in metaphase of first post-zygotic division, in each conjugant.
27. Microconjugant a shriveled pellicle containing four nuclei interpreted as products of second post-zygotic division of synkaryon. Macroconjugant shows synkaryon in telophase of first post-zygotic division; separation spindle.
28. Exconjugants. Shriveled microconjugant was separated from macroconjugant during injury to slide. Two nuclei in microconjugant may be result of division of synkaryon. Nuclei in macroconjugant in metaphase of second post-zygotic division.
29. Exconjugants. Microconjugant still contains some macronuclear fragments and degenerating (author questioned this) nuclei. Macroconjugant shows two nuclei in late anaphase of second post-zygotic division.
30. Exconjugants. Shriveled microconjugant contains two degenerating nuclei. Four synkaryon products in macroconjugant are in "resting" stage after second post-zygotic division. One of macronuclear fragments shows chromatin extrusion.
31. Exconjugant. Four nuclei in third post-zygotic division.
32. Exconjugant. Four nuclei in third post-zygotic division; three in polar view, one in lateral view.
33. Exconjugant. Six nuclei "resting"; two being formed, in late telophase of third post-zygotic division.

34-37. Exconjugants showing metamorphosis of macro- and micronuclei from eight products of third post-zygotic division.

34. Very early differentiation in two types, there is a difference in size.
35. Nuclei in two groups, differing only in size.
36. Three or four central masses of more heavily staining granules apparent in macronuclear anlagen.
37. Condensation of micronuclear anlagen. Macronuclear anlagen show additional granules in outer part of endosome, separated from inner granules by lightly staining area. First appearance of chromatin granules to be extruded from macronuclear anlagen. Most of old macronuclear fragments have disappeared; membranes shown about two of the three which remain.

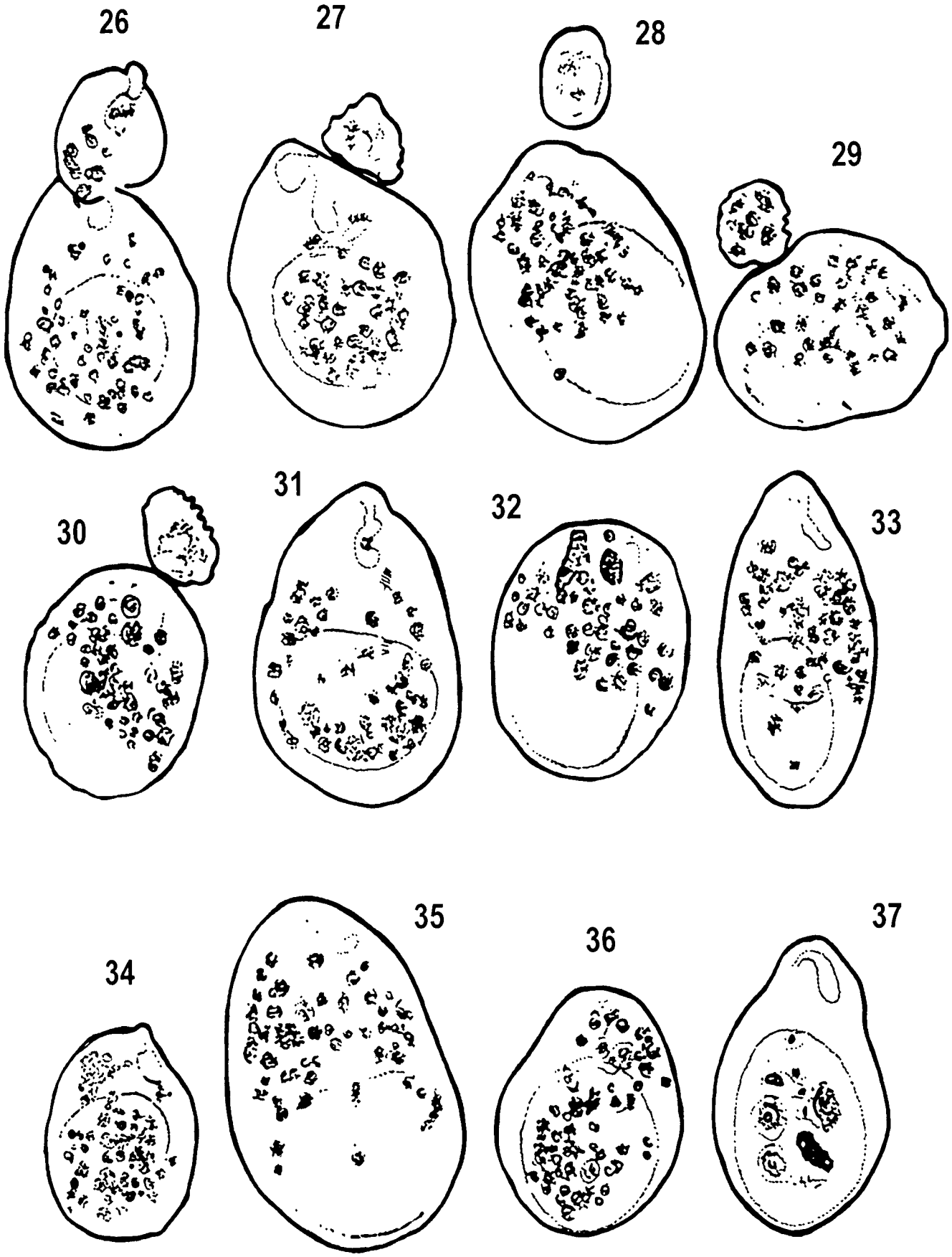
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Figure 3.12 continued

Diagrammatic illustration of conjugation in *Urceolaria synaptae* Cuénot, 1891 [Redrawn from Colwin (1944)].

38. First post-conjugant cell division. Micronucleus shows further condensation; has migrated orally. Macronuclear anlagen show large chromatin bodies to be extruded. All fragments of old macronuclei have disappeared.
39. First post-conjugant cell division. Micronucleus enlarging for mitosis. Three of the macronuclear anlagen contain two granules for chromatin extrusion, one contains one larger one, not all shown.
40. First post-conjugant cell division. Micronucleus shows further enlargement. Macronuclear anlagen migrating to typical positions for this division. Lateral views of two anlagen illustrate flattening of these bodies, characteristic shape. One of degenerating micronuclei still present.
41. First post-conjugant cell division. Later stages and positions of both types of nucleus.
42. First post-conjugant cell division; beginning of constriction, two cytopharynges. Micronucleus in telophase (absence of spindle fibers attributed to faint stain.) Macronuclear anlagen have moved apart. Note chromatin extrusion.
43. First post-conjugant cell division; constriction nearly completed. Daughter micronucleus on left side has assumed vegetative position between macronuclear anlagen. Anlagen still extruding chromatin but central granules no longer notable and general appearance is approaching that of a vegetative macronucleus. Part of one anlage omitted to show micronucleus.
44. Daughter of first post-conjugant division. Nuclei have not yet moved into interkinetic positions.
45. Daughter of anomalous first post-conjugant division. Contains additional piece of macronuclear material, though to be derived by "pinching off" from one of anlagen in sister cell.
46. Daughter of first post-conjugant division, this stage sometimes contains new denticulate ring. Slight cytoplasmic condensation at discal end of micronucleus suggests possible movement orally, to prepare for next division. Macronuclear anlagen still in interkinetic position.
47. Second post-conjugant cell division. Micronucleus in early prophase. Migration of both types of nucleus.
48. Second post-conjugant cell division; two cytopharynges. Micronuclei in late telophase; absence of spindle fibers attributed to faint staining.
49. Anomalous second post-conjugant cell division; constriction almost complete. One cell receiving extra piece of macronucleus of uncertain origin (i.e. might be either a fragment of an old macronucleus, lasting longer than usual, or a piece separated from an anlage). Macronuclei have nearly attained appearance of vegetative macronucleus; membrane no longer visible around one on right. Micronuclei show endosomes as in typical vegetative cells.
50. Daughters of second post-zygotic cell division. Macronuclei less far advanced than previous figure.

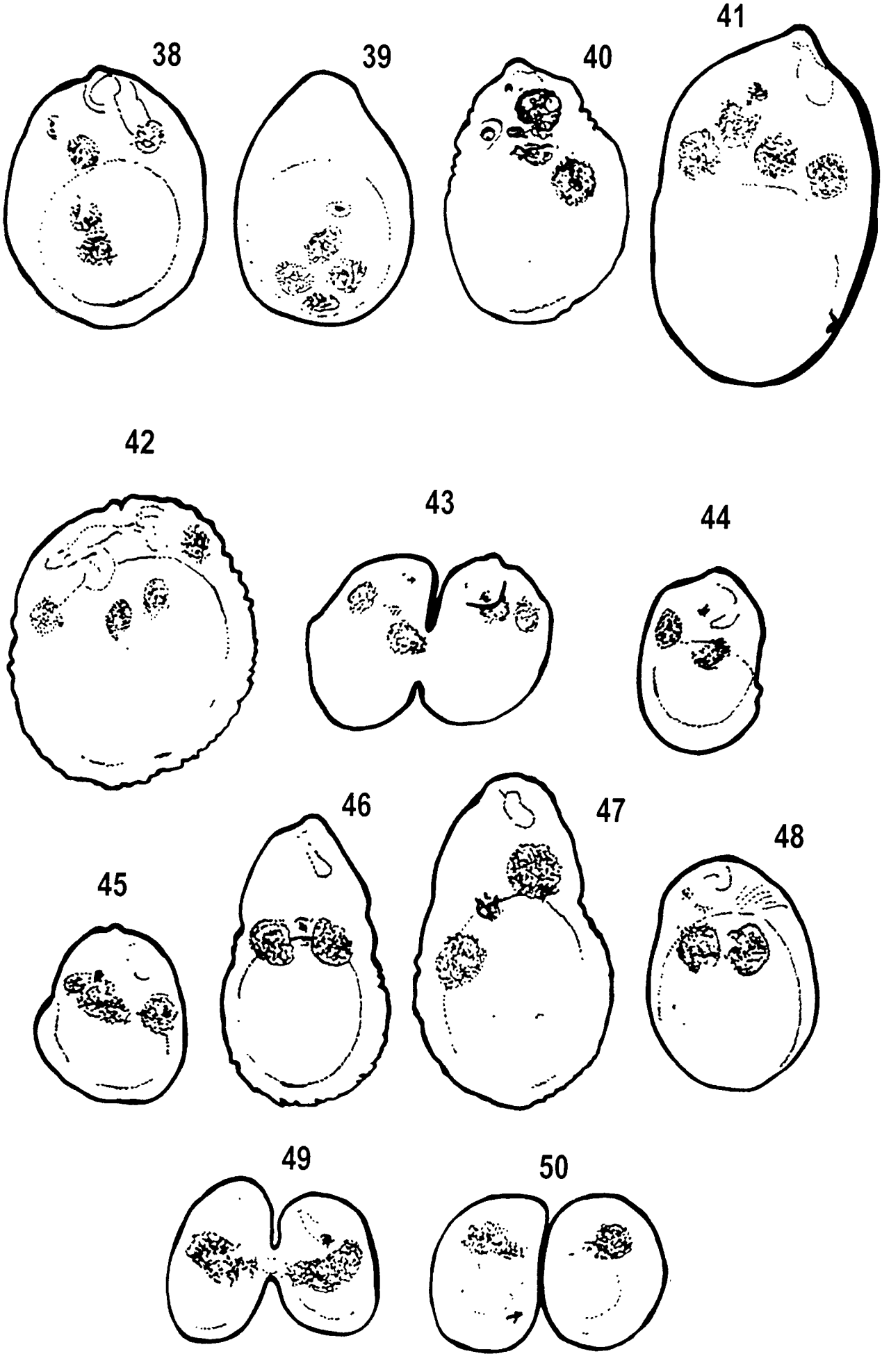
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Figure 3.13

Diagrammatic illustration of anisogamous conjugation in *Trichodina spheroidesi* Padnos & Nigrelli, 1942 [Redrawn from Padnos and Nigrelli (1942)]. All figures drawn from material stained with iron-hematoxylin.

1. Start of conjugation.
2. Macronucleus in the process of fragmentation. Note lumping of nuclear material. Micronucleus in pre-metaphase stages of meiosis.
3. First macronuclear fragmentation. Micronucleus still in pre-metaphase stage.
4. Further macronuclear fragmentation.
5. Metaphase of meiotic nucleus clearly evident. Fragmentation of the macronucleus continues.

6-8. Continued meiotic division and completion of fragmentation of the macronucleus into many spherical and oval shaped bodies of various size.

9. Continuance of the meiotic process seen in no. 8.

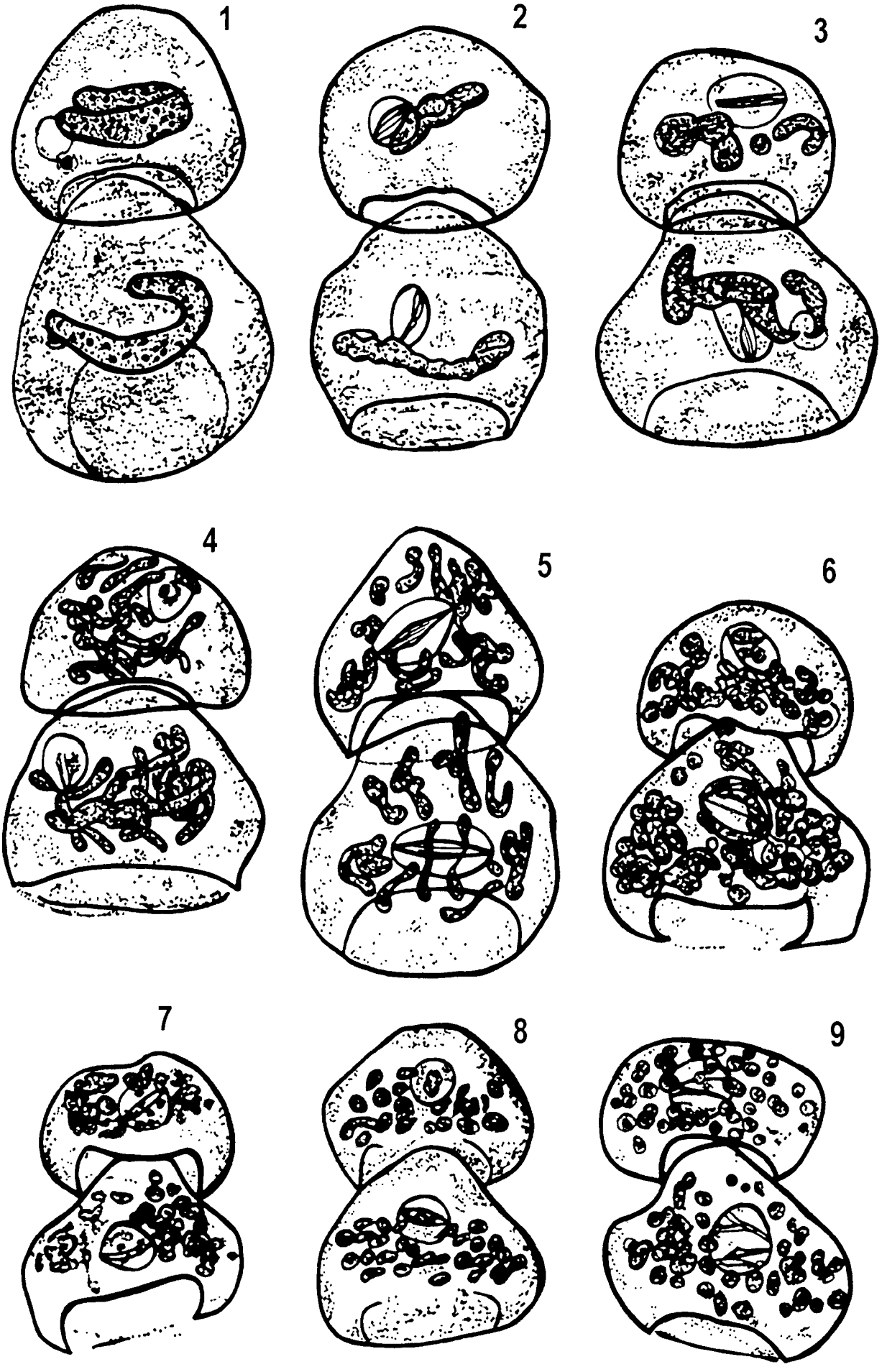
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Figure 3.13 continued

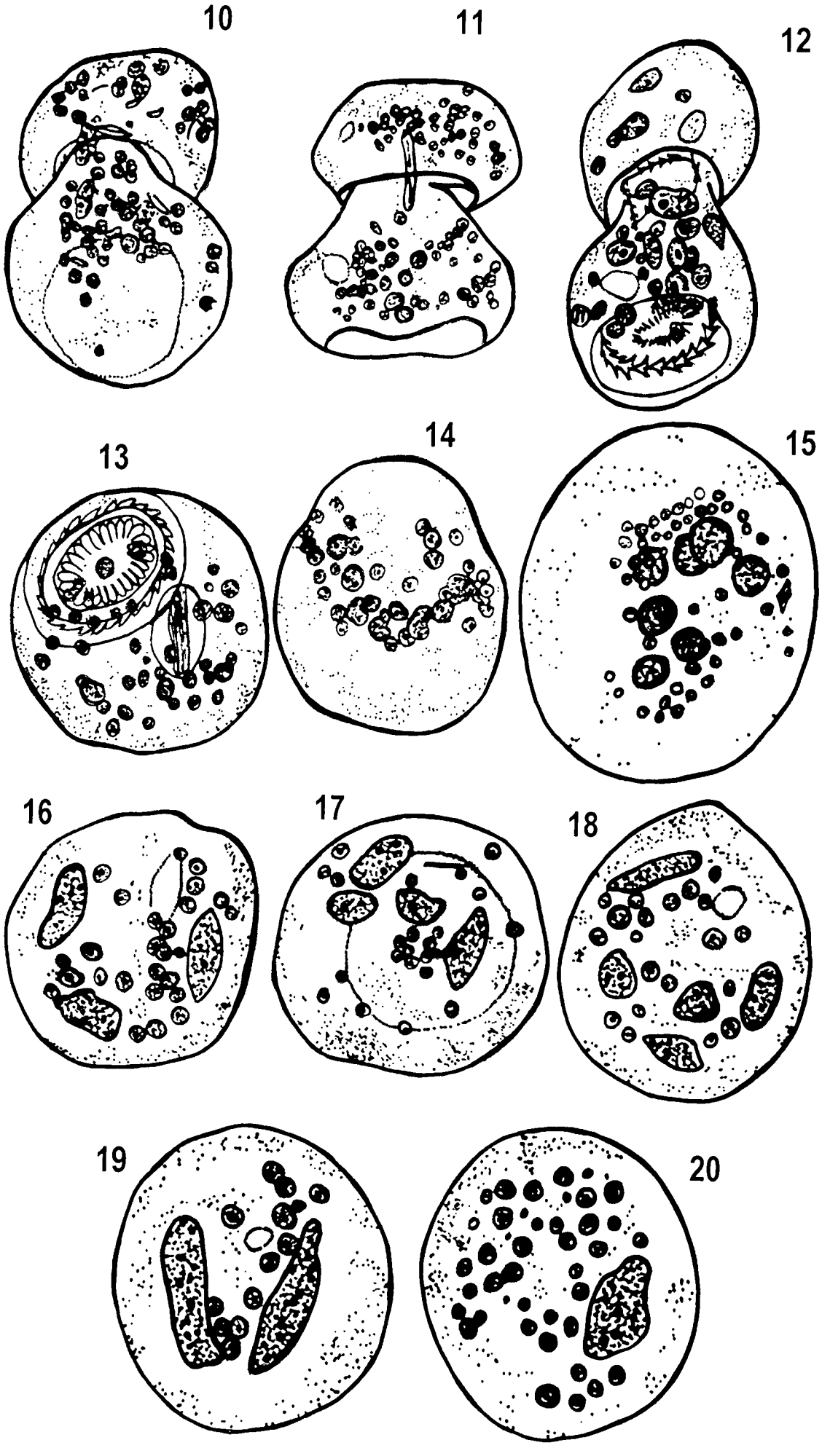
Diagrammatic illustration of anisogamous conjugation in *Trichodina spheroidesi* Padnos & Nigrelli, 1942 [Redrawn from Padnos and Nigrelli (1942)]. All figures drawn from material stained with iron-hematoxylin.

10. Second micronuclear division. Note persistent gametic micronucleus and the degeneration of other three micronuclei in each conjugant.
11. Gametic nuclei in an early stage of fusion to give rise to the synkaryon. The other micronuclei completely disappeared. Note the completion of cytoplasmic continuity between the conjugants.
12. The cytoplasmic contents of the microconjugant pass into the macroconjugant. The new denticulate ring develops while the old ring is resorbed. In this individual the macronuclear fragments are coarser than in the preceding stage.
13. Post-conjugant stage. Macroconjugant with large zygotic nucleus in metaphase stage. New denticulate ring and a remnant of the old ring still present.
14. Initial stage in development of the macronuclear anlage. There are seven of these larger bodies present, indicating that three divisions of the zygotic nucleus had taken place.
15. Further development of the macronuclear anlage. The start of the first binary fission. Micronucleus in metaphase.

16,17. Daughter cells have the three and four macronuclear anlage distribution.

18,19. A two and five distribution of macronuclear material.

20. Each cell will continue to divide until only a single macronuclear anlage is present in each individual. The last step in this process is shown in this figure.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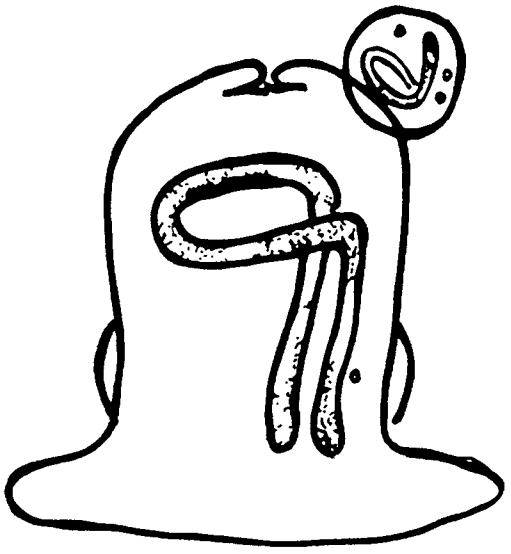

Figure 3.14

Diagrammatic illustration of conjugation in *Scyphidia ameiuri* Thompson, Kirkegaard & Jahn, 1947 [Redrawn from Thompson, Kirkegaard and Jahn (1947)].

Conjugation in *Scyphidia ameiuri*.

Chapter 4
Results:
Binary Fission
in mantoscyphidians and ellobiophryids

In the present study binary fission was observed in populations of the scyphidiid peritrichs *Mantoscyphidia midae*, *M. spadiceae*, *M. branchi* and *M. marioni*, (Van As, Basson & Van As 1998; Botes, *et al.* 2001a) as well as in the secondary symbiont *Ellobiophrya maliculiformis* that is associated with *M. midae*, *M. spadiceae* and *M. branchi* (Peters, *et al.* in prep).

Binary fission was mostly observed in *M. spadiceae* and the description of the process of binary fission will therefore be based upon observations made on populations of *M. spadiceae*. Binary fission was observed in *M. branchi* populations occurring on most of the limpet hosts (Table 2.1), except the following species: *Scutellastra aphanes*, *S. obtecta* and *Helcion concolor*. Observations of binary fission in populations of *M. branchi*, *M. marioni* and *M. midae* will be compared with that of the process in *M. spadiceae*. The process of binary fission is also described for *E. maliculiformis*.

Table 4.1 - 4.3 summarizes the occurrence of binary fission, conjugation and telotrochs in populations of scyphidiid peritrichs from 2000 to 2002. Data of the occurrence of the secondary symbiont *E. maliculiformis* is also presented in these tables. From 1995 to 1999 the reproductive processes were not specifically studied, but if it was observed it was noted. This data is presented in the Appendix A.

Table 4.1: Data from March 2000. Limpets and haliotids collected at the De Hoop Nature Reserve on the south coast of South Africa: *Cellana capensis* (Gmelin, 1791), *Cymbula compressa* (Linnaeus, 1758), *Cymbula oculus* (Born, 1778), *Scutellastra argenvillei* (Krauss, 1848), *Scutellastra barbara* (Linnaeus, 1758), *Scutellastra cochlear* (Born, 1778), *Scutellastra longicosta* (Lamarck, 1819), *Haliotis midae* Linnaeus, 1758 and *Haliotis spadicea* Donovan, 1808, indicating the presence of *Mantoscyphidia midae* Botes, Basson & Van As, 2001; *M. spadiceae* Botes, Basson & Van As, 2001 and the occurrence of the secondary symbiont *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep; binary fission; conjugation, telotrochs and the time the process was studied after collection. M = *Mantoscyphidia*, E = *Ellobiophrya*.

Specimen number	Molluscan Host	*Mantoscyphidia*	*Ellobiophrya*	Binary fission	Conjugation	Telotrochs	Time after collection (hours)
2000/03/22-01	*H. midae*	*M. midae*	-	-	-	-	8-10
2000/03/22-02	*H. midae*	*M. midae*	✔	-	-	-	8-10
2000/03/22-03	*H. midae*	*M. midae*	✔	-	-	-	8-10
2000/03/22-04	*H. midae*	*M. midae*	✔	-	✔ M	-	8-10
2000/03/22-05	*H. midae*	*M. midae*	✔	-	-	-	8-10
2000/03/22-06	*H. midae*	*M. midae*	✔	✔ M	-	✔ M	8-10
2000/03/22-07	*H. midae*	*M. midae*	-	-	-	-	8-10
2000/03/23-08	*H. midae*	*M. midae*	-	-	-	-	24
2000/03/23-09	*H. midae*	*M. midae*	-	-	-	-	24
2000/03/23-10	*H. midae*	*M. midae*	✔	-	✔ E	✔ E	24
2000/03/23-11	*H. midae*	*M. midae*	-	-	-	-	24-27
2000/03/23-12	*H. midae*	*M. midae*	✔	-	-	-	24-27
2000/03/23-13a	*H. midae*	*M. midae*	-	-	-	-	24-27
2000/03/23-13b	*H. midae*	uninfected	-	-	-	-	24-27
2000/03/23-13c	*H. midae*	uninfected	-	-	-	-	24-27
2000/03/23-13d	*H. midae*	uninfected	-	-	-	-	24-27
2000/03/23-13e	*H. midae*	uninfected	-	-	-	-	24-27
2000/03/23-13f	*H. midae*	uninfected	-	-	-	-	24-27
2000/03/23-13g	*H. midae*	uninfected	-	-	-	-	24-27
2000/03/23-13h	*H. midae*	uninfected	-	-	-	-	24-27
2000/03/19-02b	*H. spadicea*	*M. spadiceae*	-	-	-	-	6.75
2000/03/20-06	*H. spadicea*	*M. spadiceae*	-	✔ M	-	-	8
2000/03/20-09	*H. spadicea*	*M. spadiceae*	-	✔ M	-	✔ M	11
2000/03/20-10	*H. spadicea*	*M. spadiceae*	✔	-	-	✔ E	12
2000/03/20-11	*H. spadicea*	*M. spadiceae*	-	✔ M	-	-	12.5
2000/03/26-04	*H. spadicea*	uninfected	-	-	-	-	6
2000/03/26-05	*H. spadicea*	*M. spadicea*	-	-	-	-	7
2000/03/26-06	*H. spadicea*	*M. spadicea*	-	-	-	-	7.5
2000/03/25-01c	*C. capensis*	*M. branchi*	-	-	-	-	4.5
2000/03/23-13i	*C. compressa*	*M. branchi*	-	-	-	-	24-27
2000/03/23-14	*C. oculus*	*M. branchi*	-	-	-	-	6.5
2000/03/23-15	*C. oculus*	*M. branchi*	-	-	-	-	7
2000/03/25-02	*C. oculus*	*M. branchi*	-	-	✔ M	-	5
2000/03/25-03	*C. oculus*	*M. branchi*	-	-	✔ M	-	5.25
2000/03/20-02a	*C. oculus*	*M. branchi*	-	-	-	-	29
2000/03/19-07	*S. argenvillei*	*M. branchi*	-	-	-	-	22
2000/03/19-01b	*S. barbara*	*M. branchi*	-	-	✔ M	-	5.5
2000/03/19-03	*S. barbara*	*M. branchi*	-	-	✔ M	-	10

Table 4.1 continued: Data from March 2000. Limpets and haliotids collected at the De Hoop Nature Reserve on the south coast of South Africa: *Cellana capensis* (Gmelin, 1791), *Cymbula compressa* (Linnaeus, 1758), *Cymbula oculus* (Born, 1778), *Scutellastra argenvillei* (Krauss, 1848), *Scutellastra barbara* (Linnaeus, 1758), *Scutellastra cochlear* (Born, 1778), *Scutellastra longicosta* (Lamarck, 1819), *Haliotis midae* Linnaeus, 1758 and *Haliotis spadicea* Donovan, 1808, indicating the presence of *Mantoscyphidia midae* Botes, Basson & Van As, 2001; *M. spadiceae* Botes, Basson & Van As, 2001 and the occurrence of the secondary symbiont *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep; binary fission; conjugation, telotrochs and the time the process was studied after collection.

Specimen number	Molluscan Host	*Mantoscyphidia*	*Ellobiophrya*	Binary fission	Conjugation	Telotrochs	Time after collection (hours)
2000/03/19-04	*S. barbara*	*M. branchi*	-	✔ M	✔ M	✔ M	11.75
2000/03/20-01	*S. barbara*	*M. branchi*	-	✔ M	-	✔ M	22.5
2000/03/20-07	*S. barbara*	*M. branchi*	-	-	-	-	8.5
2000/03/20-08	*S. barbara*	*M. branchi*	-	-	✔ M	-	9
2000/03/21-01	*S. barbara*	*M. branchi*	-	-	✔ M	-	29
2000/03/21-03	*S. barbara*	*M. branchi*	-	-	✔ M	-	5.5
2000/03/21-04	*S. barbara*	*M. branchi*	-	-	-	✔ M	8
2000/03/21-05	*S. barbara*	*M. branchi*	-	-	✔ M	-	9
2000/03/21-06	*S. barbara*	*M. branchi*	-	-	✔ M	-	12
2000/03/21-07	*S. barbara*	*M. branchi*	-	-	✔ M	✔ M	12.5
2000/03/23-13j	*S. barbara*	*M. branchi*	-	-	-	-	48
2000/03/23-13k	*S. barbara*	*M. branchi*	-	-	-	-	48
2000/03/26-01a	*S. barbara*	*M. branchi*	-	-	-	-	3
2000/03/26-02	*S. barbara*	*M. branchi*	-	-	✔ M	-	4
2000/03/26-03	*S. barbara*	*M. branchi*	-	-	-	-	5.5
2000/03/26-07	*S. barbara*	*M. branchi*	-	-	✔ M	-	9
2000/03/26-08	*S. barbara*	*M. branchi*	-	-	✔ M	-	9.25
2000/03/26-09	*S. barbara*	*M. branchi*	-	✔ M	-	-	9.5
2000/03/26-10	*S. barbara*	*M. branchi*	-	-	✔ M	-	9.75
2000/03/26-11	*S. barbara*	*M. branchi*	-	-	-	-	10
2000/03/19-01a	*S. cochlear*	*M. branchi*	-	-	✔ M	-	4.5
2000/03/19-02a	*S. cochlear*	*M. branchi*	-	-	-	-	6.5
2000/03/19-05	*S. cochlear*	*M. branchi*	-	-	✔ M	-	12.5
2000/03/20-02b	*S. cochlear*	*M. branchi*	-	✔ M	✔ M	-	29.5
2000/03/20-03	*S. cochlear*	*M. branchi*	-	-	✔ M	-	30
2000/03/26-01b	*S. cochlear*	*M. branchi*	-	-	-	-	3.5
2000/03/19-06	*S. longicosta*	*M. branchi*	-	✔ M	✔ M	✔ M	13
2000/03/21-02	*S. longicosta*	*M. branchi*	-	-	-	-	29
2000/03/25-01a	*S. longicosta*	*M. branchi*	-	-	-	-	4
2000/03/25-01b	*S. longicosta*	*M. branchi*	-	-	-	-	4.25
2000/03/25-05	*S. longicosta*	*M. branchi*	-	-	-	-	5.75
2000/03/25-06	*S. longicosta*	*M. branchi*	-	-	-	-	6

Table 4.2: Data from November 2000. Limpets and haliotids collected at the De Hoop Nature Reserve on the south coast of South Africa: *Cymbula miniata* (Born, 1778), *Cymbula oculus* (Born, 1778), *Scutellastra barbara* (Linnaeus, 1758), *Scutellastra cochlear* (Born, 1778) and *Haliotis spadicea* Donovan, 1808, indicating the presence of *Mantoscyphidia midae* Botes, Basson & Van As, 2001; *M. spadiceae* Botes, Basson & Van As, 2001 and the occurrence of the secondary symbiont *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep; binary fission; conjugation, telotrochs and the time the process was studied after collection. M = *Mantoscyphidia*, E = *Ellobiophrya*.

Specimen number	Molluscan Host	*Mantoscyphidia*	*Ellobiophrya*	Binary fission	Conjugation	Telotrochs	Time after collection (hours)
2000/11/08-01	*H. spadicea*	*M. spadiceae*	-	-	-	-	28
2000/11/08-02	*H. spadicea*	*M. spadiceae*	-	-	✔ M	-	29
2000/11/08-03	*H. spadicea*	*M. spadiceae*	-	✔ M	✔ M	-	8
2000/11/08-11	*H. spadicea*	*M. spadiceae*	-	✔ M	-	✔ M	12.75
2000/11/08-12	*H. spadicea*	*M. spadiceae*	-	-	-	-	13
2000/11/09-01	*H. spadicea*	*M. spadiceae*	-	✔ M	-	-	29.5
2000/11/09-09	*H. spadicea*	*M. spadiceae*	-	-	✔ M	-	13.5
2000/11/09-10	*H. spadicea*	*M. spadiceae*	-	-	-	-	27
2000/11/10-07	*H. spadicea*	*M. spadiceae*	✔	-	-	-	9
2000/11/10-10	*H. spadicea*	*M. spadiceae*	-	✔ M	-	✔ M	13.25
2000/11/07-04	*C. miniata*	*M. branchi*	-	-	-	✔ M	13.5
2000/11/08-04	*C. miniata*	*M. branchi*	-	✔ M	✔ M	-	10
2000/11/08-05	*C. miniata*	*M. branchi*	-	-	✔ M	-	10.25
2000/11/11-10	*C. miniata*	*M. branchi*	-	-	-	-	12.5
2000/11/03-01	*C. oculus*	*M. branchi*	-	✔ M	-	-	6
2000/11/03-02	*C. oculus*	*M. branchi*	-	-	✔ M	✔ M	6.25
2000/11/03-03	*C. oculus*	*M. branchi*	-	-	-	-	6.5
2000/11/03-04	*C. oculus*	*M. branchi*	-	-	-	-	6.5
2000/11/04-01	*S. barbara*	*M. branchi*	-	✔ M	✔ M	-	21
2000/11/07-01	*S. barbara*	*M. branchi*	-	-	✔ M	-	7
2000/11/07-02	*S. barbara*	*M. branchi*	-	-	✔ M	-	9
2000/11/07-03	*S. barbara*	*M. branchi*	-	-	✔ M	-	9.5
2000/11/08-06	*S. barbara*	*M. branchi*	-	-	✔ M	✔ M	10.5
2000/11/08-07	*S. barbara*	*M. branchi*	-	-	✔ M	-	11.5
2000/11/08-08	*S. barbara*	*M. branchi*	-	-	✔ M	-	12
2000/11/08-09	*S. barbara*	*M. branchi*	-	✔ M	✔ M	-	12.25
2000/11/08-10	*S. barbara*	*M. branchi*	-	-	✔ M	-	12.5
2000/11/09-02	*S. barbara*	*M. branchi*	-	-	✔ M	✔ M	7
2000/11/09-03	*S. barbara*	*M. branchi*	-	-	✔ M	✔ M	9
2000/11/09-04	*S. barbara*	*M. branchi*	-	-	✔ M	-	10
2000/11/09-05	*S. barbara*	*M. branchi*	-	-	✔ M	-	10.25
2000/11/09-06	*S. barbara*	*M. branchi*	-	-	✔ M	✔ M	12
2000/11/09-07	*S. barbara*	*M. branchi*	-	-	✔ M	-	13
2000/11/09-08	*S. barbara*	*M. branchi*	-	-	✔ M	-	13.25
2000/11/10-01	*S. barbara*	*M. branchi*	-	-	✔ M	-	6
2000/11/10-03	*S. barbara*	*M. branchi*	-	-	✔ M	-	7.25
2000/11/10-04	*S. barbara*	*M. branchi*	-	✔ M	✔ M	-	8
2000/11/10-05	*S. barbara*	*M. branchi*	-	-	✔ M	-	8.5
2000/11/10-06	*S. barbara*	*M. branchi*	-	-	✔ M	-	9

Table 4.2 continued: Data from November 2000. Limpets and haliotids collected at the De Hoop Nature Reserve on the south coast of South Africa: *Cymbula miniata* (Born, 1778), *Cymbula oculus* (Born, 1778), *Scutellastra barbara* (Linnaeus, 1758), *Scutellastra cochlear* (Born, 1778) and *Haliotis spadicea* Donovan, 1808, indicating the presence of *Mantoscyphidia midae* Botes, Basson & Van As, 2001; *M. spadiceae* Botes, Basson & Van As, 2001 and the occurrence of the secondary symbiont *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep; binary fission; conjugation, telotrochs and the time the process was studied after collection.

Specimen number	Molluscan Host	*Mantoscyphidia*	*Ellobiophrya*	Binary fission	Conjugation	Telotrochs	Time after collection (hours)
2000/11/10-08	*S. barbara*	*M. branchi*	-	-	✔ M	-	12.5
2000/11/10-09	*S. barbara*	*M. branchi*	-	-	✔ M	-	12.75
2000/11/11-01	*S. barbara*	*M. branchi*	-	-	✔ M	-	7
2000/11/11-02	*S. barbara*	*M. branchi*	-	-	✔ M	-	7.5
2000/11/11-03	*S. barbara*	*M. branchi*	-	-	-	-	7.5
2000/11/11-04	*S. barbara*	*M. branchi*	-	-	✔ M	-	7.75
2000/11/11-05	*S. barbara*	*M. branchi*	-	-	✔ M	-	7.75
2000/11/11-06	*S. barbara*	*M. branchi*	-	-	✔ M	-	8
2000/11/11-07	*S. barbara*	*M. branchi*	-	-	✔ M	-	8.5
2000/11/11-08	*S. barbara*	*M. branchi*	-	-	✔ M	-	8.75
2000/11/11-09	*S. barbara*	*M. branchi*	-	-	✔ M	-	12
2000/11/11-11	*S. barbara*	*M. branchi*	-	-	✔ M	✔ M	12.75
2000/11/11-12	*S. barbara*	*M. branchi*	-	-	✔ M	-	28
2000/11/12-01	*S. barbara*	*M. branchi*	-	-	✔ M	-	7.5
2000/11/12-02	*S. barbara*	*M. branchi*	-	-	✔ M	-	8
2000/11/12-03	*S. barbara*	*M. branchi*	-	✔ M	✔ M	✔ M	8.5
2000/11/12-04	*S. barbara*	*M. branchi*	-	-	✔ M	-	8.75
2000/11/12-05	*S. barbara*	*M. branchi*	-	-	✔ M	-	8.75
2000/11/12-06	*S. barbara*	*M. branchi*	-	-	✔ M	-	9
2000/11/12-07	*S. barbara*	*M. branchi*	-	-	✔ M	✔ M	9.5
2000/11/10-02	*S. cochlear*	*M. branchi*	-	-	✔ M	-	7

Table 4.3: Data from March 2002. Limpets and haliotids collected at the De Hoop Nature Reserve on the south coast of South Africa: *Cymbula miniata* (Born, 1778), *Scutellastra barbara* (Linnaeus, 1758), *Scutellastra cochlear* (Born, 1778), and *Haliotis spadicea* Donovan, 1808, indicating the presence of *Mantoscyphidia midae* Botes, Basson & Van As, 2001; *M. spadiceae* Botes, Basson & Van As, 2001 and the occurrence of the secondary symbiont *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep; binary fission; conjugation, telotrochs and the time the process was studied after collection. M = *Mantoscyphidia*, E = *Ellobiophrya*.

Specimen number	Molluscan Host	*Mantoscyphidia*	*Ellobiophrya*	Binary fission	Conjugation	Telotrochs	Time after collection (hours)
2002/03/26-01	*H. spadicea*	*M. spadiceae*	-	-	-	-	3.5
2002/03/26-02	*H. spadicea*	*M. spadiceae*	-	-	-	-	3.75
2002/03/26-03	*H. spadicea*	*M. spadiceae*	-	-	-	-	4
2002/03/28-01	*H. spadicea*	*M. spadiceae*	-	-	-	-	3.5
2002/03/28-02	*H. spadicea*	*M. spadiceae*	-	-	-	-	3.75
2002/03/28-03	*H. spadicea*	*M. spadiceae*	-	✔ M	-	✔ M	4
2002/03/28-04	*H. spadicea*	*M. spadiceae*	✔	✔ M	-	-	5
2002/03/28-05	*H. spadicea*	*M. spadiceae*	✔	-	-	-	6
2002/03/28-06	*H. spadicea*	*M. spadiceae*	-	-	-	-	6.25
2002/03/28-07	*H. spadicea*	*M. spadiceae*	✔	✔ M	-	-	6.5
2002/03/28-08	*H. spadicea*	*M. spadiceae*	-	✔ M	-	-	6.75
2002/04/01-01	*H. spadicea*	*M. spadiceae*	✔	✔ M	-	-	3
2002/04/01-02	*H. spadicea*	*M. spadiceae*	✔	✔ M	-	-	3.25
2002/04/01-03	*H. spadicea*	*M. spadiceae*	-	-	-	-	3.5
2002/04/01-04	*H. spadicea*	*M. spadiceae*	-	✔ M	-	-	3.75
2002/04/01-05	*H. spadicea*	*M. spadiceae*	-	✔ M	-	-	4
2002/04/01-06	*H. spadicea*	*M. spadiceae*	-	-	-	-	4.25
2002/04/01-07	*H. spadicea*	*M. spadiceae*	-	-	-	-	4.5
2002/04/01-08	*H. spadicea*	*M. spadiceae*	-	✔ M	-	-	4.75
2002/04/01-09	*H. spadicea*	*M. spadiceae*	-	✔ M	-	-	5
2002/04/01-10	*H. spadicea*	*M. spadiceae*	-	✔ M	-	-	5.25
2002/03/25-03	*C. miniata*	*M. branchi*	-	-	-	-	13
2002/03/25-01	*S. barbara*	*M. branchi*	-	-	-	-	53
2002/03/25-02	*S. barbara*	*M. branchi*	-	-	-	-	7
2002/03/25-04	*S. barbara*	*M. branchi*	-	-	✔ M	-	13.5
2002/03/25-05	*S. barbara*	*M. branchi*	-	-	-	-	13.75
2002/03/25-06	*S. barbara*	*M. branchi*	-	-	-	-	14
2002/03/25-07	*S. barbara*	*M. branchi*	-	-	-	-	14.5
2002/03/26-04	*S. barbara*	*M. branchi*	-	-	-	-	28.5
2002/03/26-05	*S. barbara*	*M. branchi*	-	-	-	-	29
2002/03/26-06	*S. barbara*	*M. branchi*	-	-	-	-	9
2002/03/26-07	*S. barbara*	*M. branchi*	-	-	-	-	9.5
2002/03/26-08	*S. barbara*	*M. branchi*	-	-	-	-	10.5
2002/03/28-09	*S. barbara*	*M. branchi*	-	-	✔ M	-	7
2002/03/30-01	*S. barbara*	*M. branchi*	-	✔ M	✔ M	✔ M	8.5
2002/03/30-02	*S. barbara*	*M. branchi*	-	✔ M	✔ M	-	9
2002/03/30-03	*S. barbara*	*M. branchi*	-	✔ M	✔ M	-	9.75
2002/03/30-04	*S. barbara*	*M. branchi*	-	-	✔ M	-	10
2002/03/30-05	*S. barbara*	*M. branchi*	-	-	✔ M	✔ M	11
2002/03/30-06	*S. barbara*	*M. branchi*	-	-	✔ M	-	11.25

Table 4.3 continued: Data from March 2002. Limpets and haliotids collected at the De Hoop Nature Reserve on the south coast of South Africa: *Cymbula miniata* (Born, 1778), *Scutellastra barbara* (Linnaeus, 1758), *Scutellastra cochlear* (Born, 1778), and *Haliotis spadicea* Donovan, 1808, indicating the presence of *Mantoscyphidia midae* Botes, Basson & Van As, 2001; *M. spadiceae* Botes, Basson & Van As, 2001 and the occurrence of the secondary symbiont *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As,(in prep; binary fission; conjugation, telotrochs and the time the process was studied after collection.

Specimen number	Molluscan Host	*Mantoscyphidia*	*Ellobiophrya*	Binary fission	Conjugation	Telotrochs	Time after collection (hours)
2002/03/30-07	*S. barbara*	*M. branchi*	-	-	-	✔ M	11.5
2002/03/30-08	*S. barbara*	*M. branchi*	-	✔ M	-	-	11.75
2002/03/30-09	*S. barbara*	*M. branchi*	-	-	-	-	12
2002/03/30-10	*S. barbara*	*M. branchi*	-	✔ M	✔ M	-	12.25
2002/04/02-01	*S. barbara*	*M. branchi*	-	-	-	-	7
2002/04/02-02	*S. barbara*	*M. branchi*	-	-	✔ M	-	8
2002/04/02-03	*S. barbara*	*M. branchi*	-	-	✔ M	-	8.5
2002/04/02-04	*S. barbara*	*M. branchi*	-	-	-	-	9
2002/04/02-05	*S. barbara*	*M. branchi*	-	-	✔ M	-	9.25
2002/04/02-06	*S. barbara*	*M. branchi*	-	-	✔ M	-	9.5
2002/04/02-07	*S. barbara*	*M. branchi*	-	-	✔ M	-	9.75
2002/04/02-08	*S. barbara*	*M. branchi*	-	-	-	-	10
2002/04/02-09	*S. barbara*	*M. branchi*	-	-	✔ M	-	10.25
2002/04/02-10	*S. barbara*	*M. branchi*	-	-	-	-	10.5
2002/04/02-11	*S. barbara*	*M. branchi*	-	-	✔ M	-	10.5
2002/04/02-12	*S. barbara*	*M. branchi*	-	-	-	✔ M	11
2002/03/26-09	*S. longicosta*	*M. branchi*	-	-	✔ M	-	10

Binary fission in *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001

Family: Scyphidiidae Kahl, 1933

The plane of fission in *M. spadiceae* was parallel to the oral-aboral axis and could therefore be interpreted as a longitudinal fission. Division is probably not truly symmetrogenic. It looks this way because of the distorted and rearranged body of peritrichs. It is likely that the division plane of peritrichs is homologous to the true homothetogenic plane of other ciliates. Similar reorientations of the division plane are evident in suctorians and chonotrichs but are less obvious in them because of unequal division (=budding). In general, all sessile ciliates have atypical division planes. The lack of somatic cilia in peritrichs makes their division plane harder to reconcile with that of other ciliates. However, if one considers the orientation of the trochal band to correspond to the original orientation of somatic kineties in the ancestor of peritrichs, it can be seen that their division plane is homothetogenic.

The first occurrence of binary fission in *M. spadiceae* was observed from three hours after the host was collected and continued until approximately 30 hours after collection. After 30 hours specimens rarely survived and very few live specimens could be seen on the smears. In *M. branchi* binary fission occurred from six to 29.5 hours after collection and in *M. midae* the first specimens undergoing binary fission were observed after eight hours (Table 4.1 - 4.3)

Individuals on the verge of binary fission were more contracted or plump, compared to those that were not yet ready for fission (Figs. 4.1A; 4.2A; 4.3A). The peristome was tightly closed with no adoral cilia protruding, and the peritrich ceased all feeding activities. The peristomial region's striations formed a zig-zag pattern and the closed peristomial region was elevated in the middle to form a knob-like protrusion (Figs. 4.2B; 4.3A).

Plump individuals of *M. spadiceae* measured 18.3 - 37.0 µm (27.2;7) in length and 20.0 - 36.7 µm (30.1;7) in diameter. Plump individuals of *M. branchi* measured 21.7 - 32.2 µm (25.9±4.1;11) in length and 23.3 - 44.9 µm (33.6±7.1;11) in diameter. Compared to measurements of live and hematoxylin stained specimens that were not

in a stage of reproduction (Table 4.4), it was clear that individuals on the verge of binary fission were more contracted or plump, with a smaller body length.

Table 4.4: The body dimensions of live as well as hematoxylin-stained specimens of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 and *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 that are not in a stage of reproduction, according to Van As, Basson & Van As (1998) and Botes, Basson & Van As (2001a). Raw data are given in Botes (1999)* and Van As (1997).

Species	Length	Diameter
Mantoscyphidia branchi live specimens	44-78 µm (65.0±8.8;14)	17-32 µm (24.2±4.4;14)
Mantoscyphidia branchi hematoxylin stained specimens	45-76 µm (57.1±7.6;39)	20-35 µm (25.33±3.3;39)
Mantoscyphidia spadiceae* live specimens	70-140 µm (104.3±21.1;43)	20-40 µm (31.2±6.7;43)
Mantoscyphidia spadiceae* hematoxylin stained specimens	82-134 µm (100±12.7;47)	26-60 µm (42±6.5;47)

The micronucleus enlarged, left its vegetative position and divided through mitosis to form two micronuclei (Fig. 4.3B). One of the micronuclei moved into the daughter, whilst the other stayed in the parent (Figs. 4.1B; 4.3B). During and immediately after the division of the micronucleus, the plane of fission at the adoral and aboral ends was already evident (Figs. 4.3B & C). The two newly formed micronuclei mostly moved to a position closer to the adoral or middle region of the dividing peritrich's body (Fig. 4.1D).

Simultaneously, the macronucleus grew, elongated and assumed a position in the middle of the peritrich's body that ensured equal distribution of the macronucleus between the daughter and parent. The broad, elongated macronucleus very often stretched to occupy the whole width of the peritrich's body and was sometimes horseshoe-shaped (Figs. 4.1C & D; 4.3C). Development and duplication of the infraciliature and differentiation of adoral cilia could be distinguished while the macronuclear growth was in progress (Figs. 4.1D & E; 4.3C). The peristomial region differentiated in both daughter cells (the "presumptive" trophont and the "presumptive" telotroch). At this stage the peristomial region was still closed with cilia drawn inwards. Both individuals are daugthters but are not synchronized with respect to the timing of their stomatogenesis. While the micronucleus was busy

dividing, the scopula divided into two scopular regions (Figs. 4.2C; 4.3B). The scopula often divided before the plane of fission was apparent (Fig. 4.1B - F).

While the macronucleus prepared for cleavage through amitotic division, spindle-shaped structures could be seen (Figs. 4.1B; 4.3D). Similar threadlike spindle-shaped structures were also observed in the micronuclei. Macronuclear cleavage into two equal parts ended just before final fission took place (Figs. 4.1F; 4.3E). Specimens of *M. spadiceae* and *M. branchi* were observed in the final stages of fission with peristomial regions open and adoral cilia protruding. These peritrichs resumed feeding before the process of binary fission was completed. It was also observed that both individuals were able to contract independently of one another, but if one individual contracted, the other was influenced to also contract to some degree.

Before final separation took place, each individual's infundibulum could be observed in protargol- (Fig. 4.1G) and hematoxylin stained as well as live specimens. The plane of fission progressed until only a small string of pellicle joined the individuals adorally, and finally, complete separation of the two individuals took place through forceful contractions and rotations of both individuals (Figs. 4.2D - I). At this stage, the macronuclear material had a more granular appearance (Figs. 4.1H & I). The macro- and micronucleus of each individual moved back to the vegetative positions below the telotroch band (Figs. 4.3F – H).

The individuals formed after binary fission were two slightly unequal daughters. One grew into the "presumptive" trophont and the other into the "presumptive" telotroch (Figs. 4.1I; 4.2J). Specimens attached to the gill epithelium of the host (viewed by SEM) and detached specimens on smears (observed under a light microscope) were both seen undergoing binary fission.

The molluscs that were collected from 1996 to 1999 were dissected immediately upon returning to the field laboratory, and were not left for a couple of hours before dissection as in the 2000 to 2002 collections. During the first three years the

occurrence of various reproductive processes was noted when it was observed, but the 2000 to 2002 study focused on inducing reproduction in the scyphidiid peritrichs. The hosts were collected and purposely dissected later so that the scyphidiid peritrichs would become stressed and undergo reproduction.

The observations of binary fission in *Mantoscyphidia* populations from 1996 to 1999 illustrate that this reproductive process regularly occurs in normal populations of these scyphidiid peritrichs.

Binary fission in *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep

Family: Ellobiophryiidae (Chatton & Lwoff, 1929)

Longitudinal fission takes place in *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep. Binary fission was only observed three times in live populations of *E. maliculiformis*; twice during March - April 1997 and once in March - April 1998. Observations of binary fission are presented in Fig. 4.4.

The presence of symbiotic algae obscured most of the internal organelles, and the exact process of binary fission could not be followed in detail. The macro-and micronuclear divisions could be observed, however, as well as the progression of the cleavage furrow along the plane of fission.

Individuals on the verge of binary fission contracted and the peristome was tightly closed with no adoral cilia protruding. The micronucleus left its vegetative position and divided through mitosis to form two micronuclei. The macronucleus grew and assumed a position in the middle of the ellobiophryid's body, this ensured that the macronucleus was distributed evenly between the daughter and parent. The macronucleus very often stretched to occupy the whole width of the ellobiophryid's body. The largest part of the macronucleus was situated close to the sides (laterally) in each ellobiophryid's body during this stage of binary fission (Figs. 4.4A & E – G).

The plane of fission was evident at this stage, and it progressed until the daughters were completely separated (Fig. 4.4 B). Figs. 4.4C & D most probably represent two daughters of binary fission. One daughter inherited the entire cinctum, and it stayed attached in the parent's place. The other daughter cells became telotrochs that do not acquire cinctums until, presumably, the daughter cells locate other scyphidiid peritrichs to which they can attach and develop further. Clamp (1982) observed later stages of binary fission in *Ellobiophrya conviva* Clamp, 1982. He noted that the scopula appeared to be the last point of attachment between offspring and parent in the terminal stage of division, when a thin, twisted, dark strand could be seen connecting the scopular areas of each in protargol preparations.

The presence of a telotroch attached to the embryophore of the daughter retaining the cinctum was observed quite often during scanning electron microscopy of fixed specimens of *E. maliculiformis* (see Chapter 7).

Transformation of the telotroch stage in *Mantoscyphidia* and *Ellobiophrya* will be dealt with in Chapter 5, where this process is discussed.

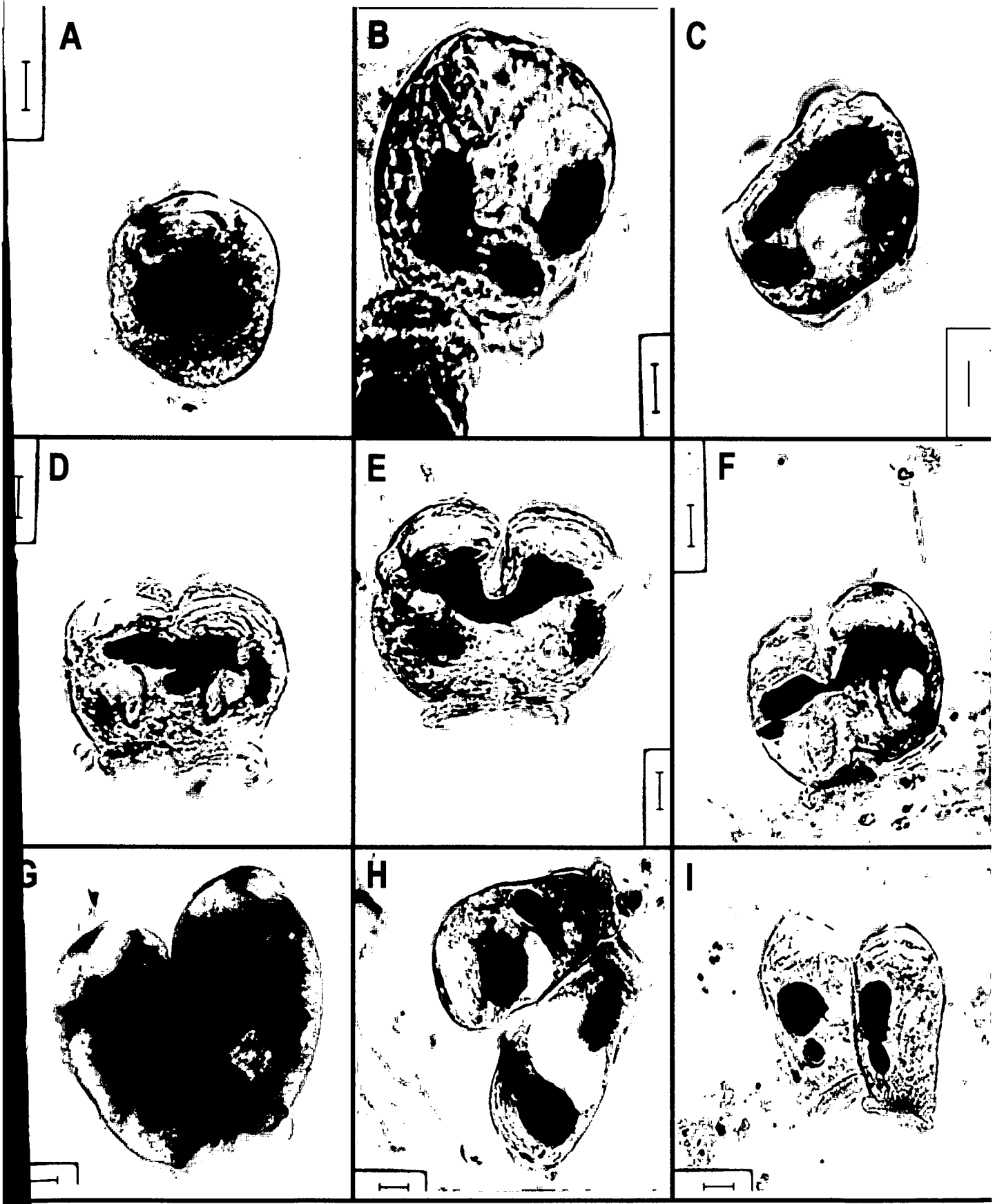

Figure 4.1 Light micrographs of hematoxylin stained (A-F,H&I) and protargol stained (G) specimens of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 (A) associated with *Scutellastra barbara* (Linnaeus, 1758) and *S. oculus* (Born, 1778), and *M. spadiceae* Botes, Basson & Van As, 2001 (B-I) associated with *Haliotis spadicea* Donovan, 1808 undergoing binary fission. **A** - Plump individual. **B** - Macronuclei formed, Macronucleus busy dividing. **C** - Scopulas divided, plane of fission apparent. **D** - Infraciliature differentiated. **E & F** - Final separation of macronuclei and onset of cleavage. **G** - Protargol stain of infraciliatures. **H** - Micronuclei move to vegetative positions. **I** - Daughter individuals separate. Scale bars: 20 µm.

Figure 4.2

Scanning electron micrographs of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 (A-D) associated with *Scutellastra barbara* (Linnaeus, 1758) and *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 (E-J) associated with *Haliotis spadicea* Donovan, 1808 undergoing binary fission.

A. Plump individual.
B. Peristomial region's striations form a zig-zag pattern and is elevated in the middle to form a knob-like protrusion.
C. The scopula divides into two scopular regions.
D. Scopular division completed.
E. Peristomial regions become evident.
F. Plane of fission becomes evident.
G. The plane of fission progresses.
H. Separation almost complete.
I. A small string of pellicle joins the individuals adorally.
J. Final separation. Two daughter individuals are formed.

Scale bars: 10 µm.

A
F
B
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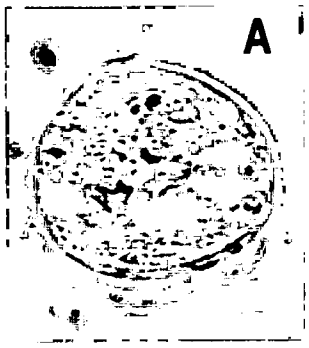
Figure 4.3

Diagrammatic illustrations and photomicrographs of live specimens of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 (A-C,F) associated with *Scutellastra barbara* (Linnaeus, 1758) and *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 (D,E,G,H) associated with *Haliotis spadicea* Donovan, 1808 summarizing the process of binary fission.

A. Plump individual on the verge of binary fission.
B. The scopula divides into two scopular regions. Micronucleus has undergone mitosis.
C. Scopular division almost completed. Peristomial regions become evident (indicated by arrows). Macronucleus stretches along the whole width of the scyphidiid peritrich's body. Micronuclei assume positions inside each daughter individual. Infindubular structures differentiate in each daughter individual.
D. Plane of fission becomes evident. Spindle formation evident in macronucleus as it undergoes mitosis.
E. The plane of fission progresses. Only a small strand of macronuclear material is situated in the region of the plane of fission.
F. Separation complete. Micronuclei move back to vegetative positions.
G. Micronuclei assume vegetative positions below telotroch band.
H. Two daughter individuals. Macronuclei move to vegetative positions.

i = infraciliature, **ma** = macronucleus, **mi** = micronucleus.

Scale bar: 20 µm.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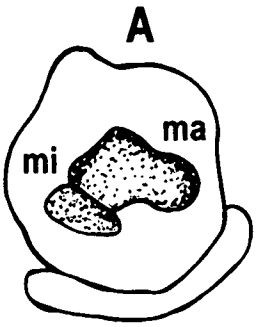
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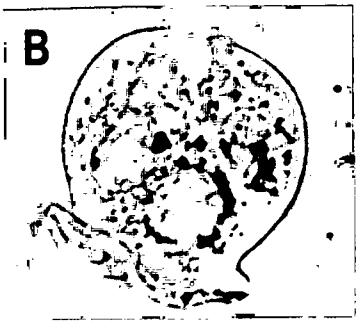
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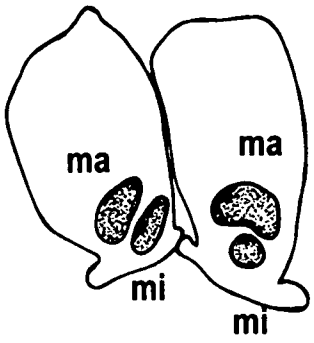
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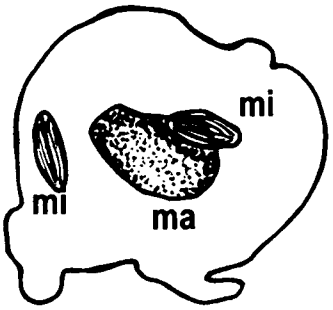
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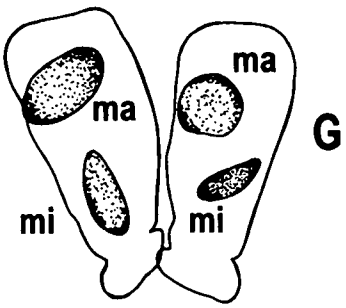
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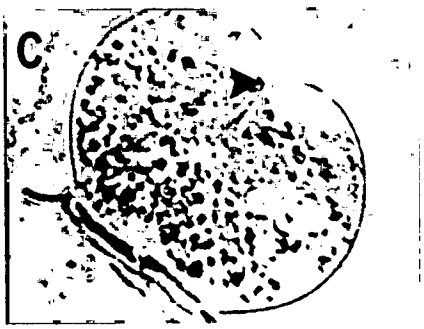
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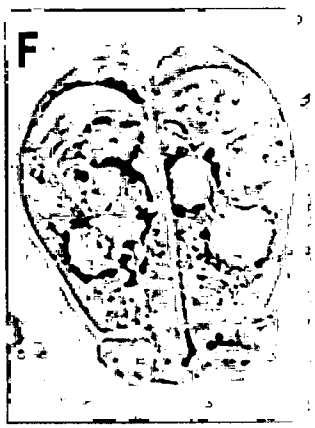
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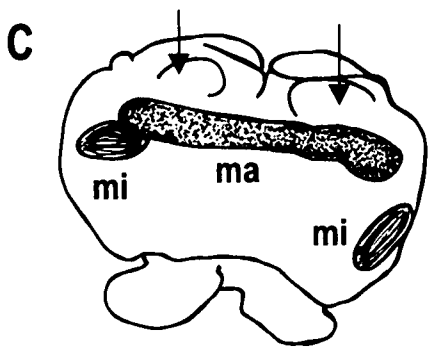
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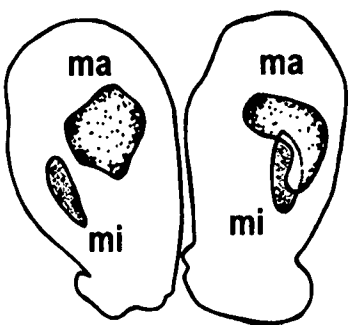
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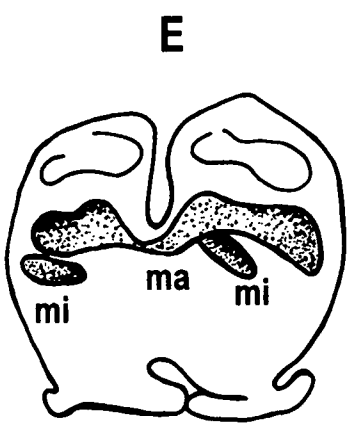
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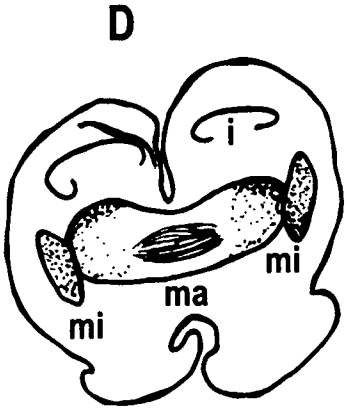
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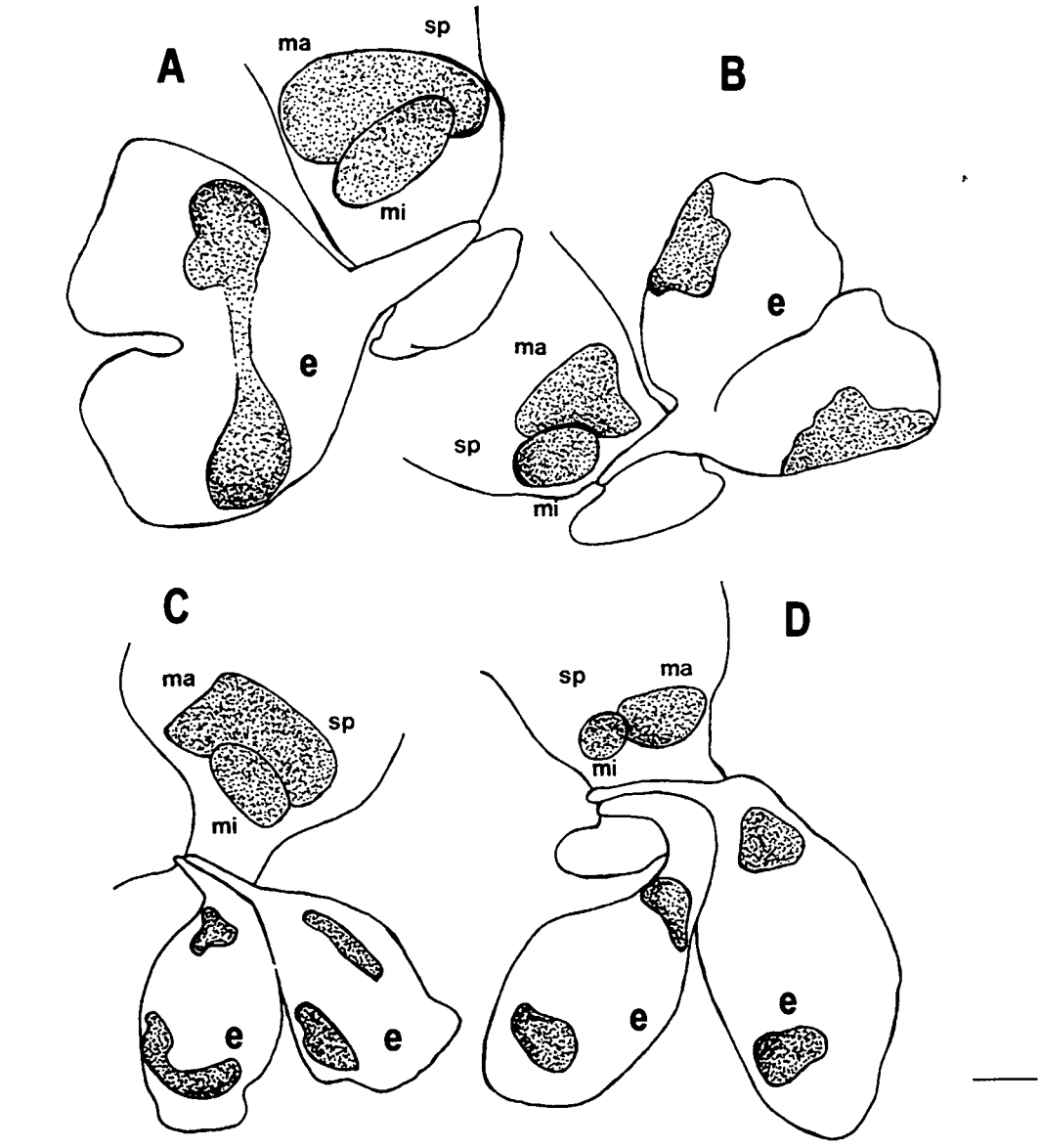
Figure 4.4

Diagrammatic illustrations (A-D) and photomicrographs (E-G) of hematoxylin stained specimens of *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in press associated with *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 undergoing binary fission.

A. Macronuclear division. Plane of fission is evident.
B. Separation of daughter indiduals. Nuclear material situated laterally.
C. Most probably two daughter individuals.
D. Most probably two daughter individuals.
E. Hematoxylin stained specimens. Nuclear material visible in lateral regions of daughter individuals.
F. Hematoxylin stained specimens. Macronuclear division.
G. Hematoxylin stained specimens.

e = ellobiophryid, **ma** = macronucleus, **mi** = micronucleus, **sp** = scyphidiid peritrich.

Scale bars: 10 µm.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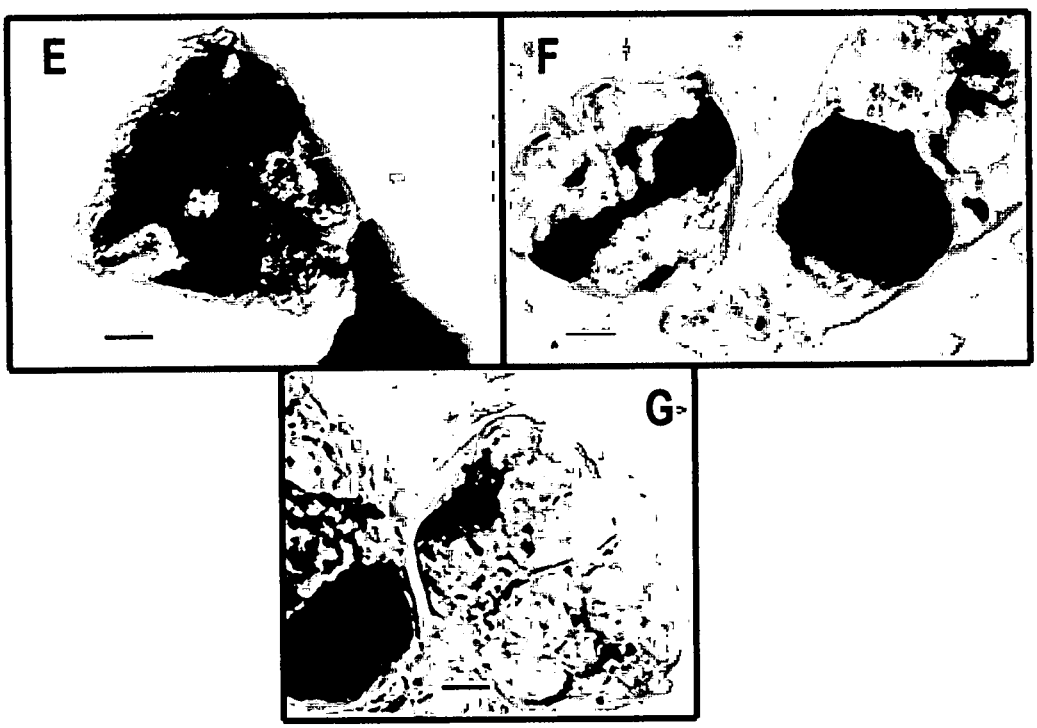
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Chapter 5

Telotroch formation

in mantoscyphidians and ellobiophryids

Telotroch formation has been observed in populations of the scyphidiid peritrichs *Mantoscyphidia midae*, *M. spadiceae*, *M. branchi* and *M. marioni* (Van As 1997, Botes 1999) as well as in the secondary symbiont *Ellobiophrya maliculiformis* that is associated with *M. midae*, *M. spadiceae* and *M. branchi* (Peters, *et al.* in prep). The formation of telotrochs was mostly observed in populations of *M. branchi* and *M. spadiceae* and the description of the process will therefore be based upon observations made on these populations. Observations of telotroch formation in populations of *M. midae* and *M. marioni* will be compared with that of the process in *M. branchi* and *M. spadiceae*. The process of telotroch formation is also described for *Ellobiophrya maliculiformis*.

According to Foissner and Schubert (1977) the following morphological characteristics are important in describing the telotroch stages of peritrichs: size, shape, surface structures, behaviour, the way in which the telotroch is formed, swimming action and the method of attachment to the substrate. The description of the telotroch stages of *M. branchi*, *M. spadiceae* and *E. maliculiformis* will be based upon these taxonomic characteristics.

Telotroch formation in *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 and *M. spadiceae* Botes, Basson & Van As, 2001

Family: Scyphidiidae Kahl, 1933

The first observation of telotrochs in *Mantoscyphidia branchi* occurred approximately six to 22.5 hours after collection. In *M. spadiceae*, telotrochs were observed from four to approximately 13 hours after collection, and in populations of *M. midae* from eight to 10 hours after collection (Tables 4.1 - 4.3). Telotrochs were also occasionally observed in populations of *M. spadiceae*, *M. marioni* and *M. midae* during collections made from 1996 to 1999 (Tables 1 – 5, Appendix A).

Telotroch formation was also observed in populations of *M. branchi* collected during 1994 to 1997 (Van As 1997). These observations illustrate that telotroch formation occurs at any given time in a population. The length and width of *M. branchi* and *M. spadiceae* telotrochs measured from scanning electron micrographs is presented in Tables 5 and 6 (Appendix A), respectively.

SHAPE AND SURFACE STRUCTURES

Telotrochs had a spherical shape and the whole pellicle was adorned with striations. The striations were 0.2 - 0.25 µm apart.

SIZE

The average lengths of *Mantoscyphidia branchi* and *M. spadiceae* telotrochs (Table 5.1) differed significantly, with *M. spadiceae* telotrochs having a much greater average length (38.3 µm) than *M. branchi* telotrochs [26.3 µm (*Cymbula miniata* population) and 24.8 µm (*Scutellastra barbara* population)]. The average width of the two species' telotrochs did not differ significantly (22.7 µm for *M. spadiceae*; *M. branchi*: 20.6 µm (*C. miniata* population) and 22.2 µm (*S. barbara* population). In both species the average width was less than the average length. Thompson, *et al.* (1947) found that the width of *Scyphidia ameiuri* was twice the length. In some individuals of *M. branchi* and *M. spadiceae*, the width of the telotrochs did exceed that of the length of the telotrochs (Table 5 & 6, Appendix A), but it was not twice the length.

Table 5.1: The average body length and width (µm) of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 telotrochs from *Cymbula miniata* (Born, 1778) and *Scutellastra barbara* (Linnaeus, 1758) and *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 telotrochs from *Haliotis spadicea* Donovan, 1808 measured from scanning electron micrographs.

Telotrochs	Mollusc Host	Average body length	Average body width
M. branchi	*C. miniata*	14.9-35.3 (26.3±5.7;14)	16.0-25.5 (20.6±2.4;14)
M. branchi	*S. barbara*	14.5-37.5 (24.8±5.1;50)	13.0-30.0 (22.2±3.4;50)
M. spadiceae	*H. spadicea*	21.4-56.2 (38.3±8.4;34)	16.2-30.0 (22.7±3.7;34)

Lom (1958) contributed to the systematics and the morphology of trichodinids by proposing that diagnostic characteristics be used in the determination of a new species. Amongst these characteristics he included the position of the micronucleus with regard to the macronucleus (+y, -y or -y[1]); the length and diameter of the macronucleus; the length of the sector between the terminations of the macronucleus and the length and width of the micronucleus (Fig 2.7A). For instance, if the position of the micronucleus relative to the macronucleus is constant, it can serve as a differentiating structure (Lom & Vávra 1961).

Various authors have observed that the telotroch stages of scyphidiid peritrichs resemble the mobiline trichodinids. The characteristics that Lom (1958) proposed for mobiline peritrichs were used in this study to determine body dimensions of the telotroch stages of *Mantoscyphidia branchi*, *M. marioni* and *M. spadiceae* (Table 5.2 - 5.4).

Table 5.2: Body dimensions (µm) of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 telotrochs from different limpet hosts, indicating macronucleus length, macronucleus diameter, the length of the sector between the terminations of the macronucleus, y-value, micronucleus length and micronucleus diameter.

Number	Macronucleus length	Macronucleus diameter	Macronucleus (Length of the sectors of termination)	Y value	Micronucleus length	Micronucleus diameter
1	19.5	8.5	15	5 +y	9	6
2	16	7	12	4.5 -y	8	5
3	16	6	12	2.5 -y	9	4
4	17	9	12	3 -y	11	5
5	13.5	7	10	2 +y	6	4
6	13.5	8	11	5 +y	8	3.5
7	14	10	9.5	2.5 +y	9.5	4
8	15	13	12	5 +y	9.5	3
9	14.5	7.5	12	9.5 +y	8	4.5
10	19	9	14.5	1.5 +y	13	6
11	19.5	10	12.5	7.5 +y	11	5.5
12	17	8	12	5 +y	7	6
13	22	7.5	19	5 -y	12	6
14	13.5	9	9	2 -y	9.5	4
15	14.5	8	7.5	7.5 +y	10	4.5
16	15	13.5	11.5	7 +y	13	7
17	14.5	7.5	9	6 +y	11	5
18	14.5	12	7.5	4 +y	8	4.5
19	15	11	13	7.5 +y	7	5
20	12	7	8	6.5 +y	7.5	3
21	15.5	7	12	6 +y	6.5	4
22	12	7.5	6.5	5 +y	5.5	3.5
23	12	10	10	4 +y	6	5
24	15	6.5	10	4.5 +y	8	6
25	14	13	11	2.5 +y	10	7
26	16.5	4.5	16	4 +y	12	9
27	19	8	15	4 -y	7.5	6
28	15	7.5	9	13 +y	8	4
29	13.5	8	6	7 +y	14	5
30	18.5	9.5	14.5	11 +y	11	4.5
31	18.5	6	13	5 -y	9	4
32	21	6.5	17.5	13 +y	9.5	6.5
33	24	6	21	3 +y	13	3
34	22	5	20	6 +y	5.5	4.5
35	22	5	20	4 +y	12	5
36	20	8	8	8 +y	11	5
37	12	7.5	5.5	5 +y	7	4.5
38	13	8.5	11	6 +y	7.5	4
39	14	13	7.5	5 +y	7	5

Table 5.3: Body dimensions (µm) of *Mantoscyphidia marioni* Van As, Basson & Van As, 1998 telotrochs from *Nacella delesserti* Philippi, 1849, indicating macronucleus length, macronucleus diameter, the length of the sector between the terminations of the macronucleus, y-value, micronucleus length and micronucleus diameter.

Number	Macronucleus length	Macronucleus diameter	Macronucleus (Length of the sectors of termination)	Y value	Micronucleus length	Micronucleus diameter
1	18	11	13.5	5 +y	5	3
2	15.5	12	11.5	4 -y	4	3
3	17.5	12	17	24 +y	4	2
4	19	12	12	11.5 +y	5	4
5	19.5	13	12.2	8.5 +y	6	4
6	26	7.5	20	9 +y	4.5	4

Table 5.4: Body dimensions (µm) of *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 telotrochs from *Haliotis spadicea* Donovan, 1808 indicating macronucleus length, macronucleus diameter, the length of the sector between the of terminations of the macronucleus, y-value, micronucleus length and micronucleus diameter.

Number	Macronucleus length	Macronucleus diameter	Macronucleus (Length of the sectors of termination)	Y value	Micronucleus length	Micronucleus diameter
1	24	6	9	4.5 -y	11	5
2	19	4	18	9 -y	7.5	6
3	19	5	11	2.5 +y	11.5	5
4	21.5	6	6	20 +y	11	6
5	22	5	9	5 -y	10	5
6	24.5	5	20	8 -y	10	5
7	21	5	15.5	3.5 -y	11	6
8	20	5.5	5	2.5 +y	6	5
9	20.5	5	17	6 +y	6	5
10	19	5	17	10 -y	12	6
11	19.5	3	17	2 +y	8	6.5
12	22.5	6.5	18.5	10 -y	13	4.5
13	24.5	5	13	5 -y	11	6.5
14	22	4	18.5	2 +y	6	4.5
15	20	5	19	6.5 -y	8.5	6
16	29.5	5	19	5 -y'	10	6
17	16	5	7	13.5 +y	12.5	6
18	19	6	16	7.5 -y	9	4.5
19	26.5	4	21	14.5 -y'	8	5.5
20	21	4.5	4.5	4 -y	7	5.5
21	23.5	5	18	6.5 +y	9	5
22	20	6	4.5	9 +y	11	5
23	21	7	7	16 +y	14.5	5
24	18	4.5	11	11.5 +y	7.5	6
25	18	5	0	19 +y	11	5
26	18	4.5	0	5 -y'	10	5
27	20	5	8	8.5 +y	12.5	6
28	19.5	5	16	6.5 +y	9	4.5
29	22	5	1	5.5 +y	10	5

Table 5.5: Average values of the body dimensions (µm) of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998, *M. marioni* Van As, Basson & Van As, 1998 and *M. spadiceae* Botes, Basson & Van As, 2001 telotrochs indicating macronucleus length, macronucleus diameter, the length of the sector between the terminations of the macronucleus, micronucleus length and micronucleus diameter.

Species	*M. branchi*	*M. marioni*	*M. spadiceae*
Macronucleus length	12.0-24.0 (16.1±3.2;39)	15.5-26.0 (19.5;6)	16.0-29.5 (21.1±2.8;29)
Macronucleus diameter	4.5-13.5 (8.3±2.3;39)	7.5-12.0(11.1;6)	3.0-7.0 (5.1±0.8;29)
Macronucleus (Length of the sectors of termination)	5.5-21.0 (11.8±3.9;39)	11.5-20.0 (14.5;6)	0-21.0 (11.9±6.6;29)
Micronucleus length	5.5-14.0 (9.2±2.3;39)	4.0-6.0 (4.8;6)	6.0-14.5 (9.8±2.2;29)
Micronucleus diameter	3.0-9.0 (4.9±1.2;39)	2.0-4.0 (3.3;6)	4.5-6.5 (5.4±0.6;29)

Position of the micronucleus with regard to the macronucleus:

In *Mantoscyphidia branchi* and *M. marioni* the majority of the specimens measured were situated in the +y position, externally in front of the right termination of the macronucleus. In *M. spadiceae* 15 of the measured specimens were situated in the +y position, 11 specimens were situated in the –y position, a distance from the right termination of the macronucleus towards the left and three specimens were situated in the $-y^1$ position, in front of the right termination of the macronucleus internally. Thus the micronuclei in all three species were mostly situated in front of the right termination of the horseshoe-shaped macronucleus (Table 5.2 - 5.4).

In *M. spadiceae* telotrochs, macronucleus length was greater than *in M. branchi* or *M. marioni* telotrochs, although *M. branchi* telotrochs have the greatest macronuclear diameter as well as the greatest length of the sectors of termination in the macronucleus. *Mantoscyphidia spadiceae* telotrochs, however, also have the greatest micronucleus length and diameter (Table 5.5).

The macronucleus of a *M. spadiceae* telotroch occupied on average 19.5 % more of the body diameter, than the macronucleus of a *M. branchi* telotroch (Table 5.6).

Table 5.6: Average values of the body diameter and macronucleus length (µm) of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 and *M. spadiceae* Botes, Basson & Van As, 2001 telotrochs, and the percentage of the body diameter that the macronucleus occupies.

Species	Body diameter of telotroch	Macronucleus length	Percentage of body diameter occupied by macronucleus
Mantoscyphidia branchi	21.9	16.1	73.5 %
Mantoscyphidia spadiceae	22.7	21.1	93 %

BEHAVIOUR AND SWIMMING ACTION

Telotrochs were active and swam rapidly. The fully formed telotrochs (Figs. 5.2A & B) moved in much the same way as do mobiline peritrichs, with ciliary wreaths beating, in search of a suitable host or substrate to settle on.

TELOTROCH FORMATION

At the onset of telotroch formation the peristome closed and a swelling occurred in the middle region of the body (Fig. 5.1A), where three or four rows of basal kinetosomes appeared (Fig. 5.1B). This emerging ciliary girdle is associated with a tearing of the peritrich's scopula from the gill epithelium. The locomotory cilia developed on the elevated telotroch band. Contracting and extending movements of the peritrich drew in the remaining portion of the aboral girdle (Figs. 5.1C & D). This was similar to what Dobrzañska (1961) described for *Orbopercularia raabei*.

The upward movement of the cytoplasm above the scopula resulted in an increase in scyphidiid peritrich diameter and the macronucleus moved towards the peristome. The peristome was closed with no adoral cilia protruding. In the fully formed mobile telotrochs, the aboral cilia were very long (10 µm) (Fig. 5.1E).

Shortening during telotroch formation

Live, extended specimens of *M. spadiceae* had an average body length of 104.3 µm and the average body length of this species' telotroch was 38.3 µm. There was thus an average shortening of 36.7 % that took place during transformation into the telotroch stage (Table 5.7). In *M. branchi* this shortening was 38.6 %, with live, extended specimens attaining an average length of 65.0 µm and the average length

of the telotrochs was 25.1 µm (Table 5.7). Both species exhibited a similar percentage of shortening. Body width of live, extended specimens and telotrochs are compared in Table 5.8.

Table 5.7: Body lengths (µm) of live, extended specimens and telotrochs of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 from different limpet hosts and *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 from *Haliotis spadiceae* Donovan, 1808.

	Body length of living, extended specimens	Body length of telotrochs
M. branchi	44-78 (65.0±8.8;14)	14.6-37.5 (25.1±5.2;64)
M. spadiceae	70-140 (104.3±21.1;43)	21.4-56.2 (38.3±8.4;34)

Table 5.8: Body widths (µm) of live, extended specimens and telotrochs of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 from different limpet hosts and *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 from *Haliotis spadiceae* Donovan, 1808.

	Body width of living, extended specimens	Body width of telotrochs
M. branchi	17-32 (24.2±4.4;14)	13.0-30.0 (21.9±3.2;64)
M. spadiceae	20-40 (31.2±6.7;43)	16.2-30.0 (22.7±3.7;34)

METHOD OF ATTACHMENT TO SUBSTRATE

During the transformation of the telotroch into a settled form, the scopula gradually increased in size, with the central cytoplasm flowing into the scopula (Fig. 5.1F). The scyphidiid peritrich elongated (Fig. 5.1G) and the macronucleus returned to its original place, unblocking the peristome. When the telotroch had settled onto a suitable substrate, the peristome opened and adoral cilia appeared as the scyphidiid peritrich prepared to resume its feeding activities (Fig. 5.1H). Finally, the ciliary girdle lost its activity.

LIVE OBSERVATIONS AND COMPARISON WITH OTHER SPECIES

Living daughter cells of *M. branchi* and *M. spadiceae* that had undergone binary fission were not seen to develop into telotrochs, but it is assumed that these daughter cells could develop into telotrochs after completion of binary fission, in order to locate new attachment sites. Telotroch stages on the verge of either detaching from the substrate or settling were observed on numerous

occasions. The peristomial regions of these telotrochs were open with adoral cilia protruding and it can be assumed that these telotrochs could be able to feed. Infraciliature movement was also noted. In most cases mature telotroch stages' peristomes were closed, no infraciliature activity was observed and the telotrochs were detached from the gill epithelium.

Table 5.9 Comparison between the body length and body width (µm) of the telotrochs *of Mantoscyphidia branchi* Van As, Basson & Van As, 1998; *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 (pooled data from different limpet hosts); *Orbopercularia raabei* Dobrzañska, 1961; and *Scyphidia tholiformis* Surber, 1943 (Surber 1943, Dobrzañska 1961). *Only a single specimen of *S. tholiformis* was measured.

Telotrochs	Length	Width
Mantoscyphidia branchi	14.6-37.5 (25.1±5.2; 64))	13.0-30.0 (21.9±3.2; 64)
Mantoscyphidia spadiceae	21.4-56.2 (38.3±8.4;34)	16.2-30.0 (22.7±3.7;34)
Orbopercularia raabei	15-22	16-25
Scyphidia tholiformis *	28.6	51.4

A comparison of the measurements of the telotrochs of *M. spadiceae* and *M. branchi* with measurements of *Orbopercularia raabei* and *Scyphidia tholiformis* indicated that the size ranges for telotrochs were almost the same for these four species (Table 5.9).

Telotroch formation in *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep

Family: Ellobiophryiidae (Chatton & Lwoff, 1929)

As is the case generally in sessiline ciliophorans, it was presumed that after binary fission was completed, the daughter individuals became telotrochs that detach and swim away to settle on a new host. These migratory stages did not acquire cinctums until, it is thought, they located other scyphidiid peritrichs to which they could attach, and develop further.

Telotroch formation was observed three times in live populations of *E. maliculiformis* (Tables 4.1-4.3; Tables 2 – 4 in Appendix A). Two of these observations were of *E. maliculiformis* attached to *Mantoscyphidia spadiceae* and the third observation was

of *E. maliculiformis* attached to *M. midae*. Telotroch stages were also seen in specimens that were prepared for SEM and subsequently photographed (Fig. 5.3).

SHAPE AND SURFACE STRUCTURES

Fully formed *Ellobiophrya maliculiformis* telotrochs were round (Figs. 5.3H – J) with striations adorning the whole pellicle. The striations were 0.2 µm apart. Telotrochs without wreaths of cilia and still in the process of formation were observed attached to ellobiophryids (Fig. 5.3D & E). Telotrochs with developing wreaths of cilia were also observed attached to the embryophore of *E. maliculiformis* (Fig. 5.3F & G).

SIZE

Ellobiophrya maliculiformis telotrochs had a greater body length (16.54 µm) than body diameter (14.57 µm) (Table 5.10). Telotrochs were often observed to have narrow scopular regions (Figs. 5.3A – C).

Table 5.10: Average body length and diameter (µm) of *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep telotrochs associated with *Mantoscyphidia midae* Botes, Basson & Van As, 2001 and *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 measured from scanning electron micrographs.

Body length	Body diameter
10.32–24.83 (16.54; 5)	10.29–20.0 (14.57; 5)

BEHAVIOUR AND SWIMMING ACTION

It was observed that the fully formed telotrochs of *E. maliculiformis* moved in the same way as the mobiline peritrichs, with the ciliary wreaths beating, in search of a suitable host or substrate to settle on.

TELOTROCH FORMATION

After binary fission one daughter individual became a telotroch and the other stayed attached with the cinctum to the scyphidiid peritrich host and is known as the parent individual. As in other ellobiophryids, the telotroch was attached during development

to the daughter retaining the cinctum (parent) by a short, rigid stalk that passed between the scopulas of the two individuals (Chatton & Lwoff 1923, 1928, 1929; Bradbury & Clamp 1991).

During an observation of telotroch formation in a living specimen of *Ellobiophrya maliculiformis* attached to *M. midae* (Table 4.1; Fig. 5.2C), a larval stalk was identified. The telotroch was attached to the parent by this short stalk and the trochal band of cilia was in the process of differentiating, but not beating yet. The parent's peristome was open, with cilia creating a feeding current. This telotroch was observed eight to 10 hours after collection of the gastropod host, and for a time period of 55 minutes before it separated from the parent and swam away. The aboral end (larval stalk) that was attached to the embryophore (Figs. 5.4A - C) of the parent ellobiophryid became broader after separation. Figure 5.3D – G illustrates the attachment of the telotroch to the embryophore, which is located just above and to the side of the parent's cinctum.

COMPARISON WITH OTHER ELLOBIOPHRYIDS

According to Bradbury and Clamp (1991) the telotroch is briefly linked to the parent. Telotrochs of *E. conviva* Clamp, 1982 secrete a temporary stalk, like the larval stalk described in *E. donacis* Chatton & Lwoff, 1923 that attaches them to the parent during the period between completion of division and their liberation. The embryonic stalk was larger in diameter and more conspicuous in *E. brevipes* (Laird, 1959) than in *E. conviva*, but less conspicuous than in *E. donacis* (Chatton & Lwoff 1923, 1928, 1929; Clamp & Bradbury 1997). The embryophore, a raised area on the aboral surface of the body *of E. donacis* in which the embryonic stalk is anchored, is likewise present in *E. conviva*. This larval stalk of ellobiophryids superficially resembles the noncontractile stalks of many sessiline peritrichs. According to Chatton & Lwoff (1923, 1928, 1929) and Clamp (1982) the stalk is secreted precociously, anchoring the telotroch to its parent for a short time instead of attaching the telotroch to a suitable substrate after metamorphosis. It is not clear what the

purpose of this stalk is, although it is thought to keep the telotroch from being swept away from the parent before its trochal band of cilia is fully developed. The presence of a cinctum and a larval stalk in ellobiophryids is unique among peritrichs.

METHOD OF ATTACHMENT TO SUBSTRATE

A question that arises is how the newly settled ellobiophryid maintains its hold on the host while the cinctum forms?

Clamp (1982) stated that the obvious need to make an initial attachment to the host and to continue this attachment until its permanent holdfast organ, the cinctum, has developed, suggests that ellobiophryid telotrochs, like those of other sessiline peritrichs, fasten themselves to the host's body with the scopula while metamorphosis takes place. The juvenile ellobiophryid will maintain the connection only until the cinctum is fully developed, whereupon the scopula would become nonfunctional, but remain in its proper position, as is seen in *Ellobiophrya conviva*.

Secondly, how does the cinctum develop during metamorphosis from telotroch to adult?

According to Clamp (1982) the cinctal limbs are gross extensions of body mass and must originate by rapid outgrowth of cytoplasm from specific points on the body during metamorphosis. Superficial similarity between each of the swollen structures at the tips of the cinctal limbs in *E. donacis* and a peritrich scopula suggested to Chatton and Lwoff (1929) that division of the scopula occurs during its metamorphosis, enabling the two products to cement the cinctal limbs to one another. Clamp (1982) did not observe any such structures in other ellobiophryid species, but stated that it is still possible that two small fragments can detach from the scopula early in the transformation of an *Ellobiophrya* telotroch and could be carried along at the tips of the cinctal arms as they lengthen, cementing them together when they meet. Bradbury and Clamp (1991) confirmed the presence of scopular structures at the tips of the arms.

Telotrochs of *E. conviva* are similar to those of *E. donacis*. They are cylindrical to pyriform or almost globular in shape, with the widest portion of the body adoral to the

trochal band of cilia. In both *E. donacis* and *E. conviva* the scopula of the telotrochs appear to be displaced slightly away from the aboral body pole. Chatton and Lwoff (1923, 1928 & 1929) observed numerous short cilia on the aboral pole of the telotroch of *E. donacis*, but Clamp (1982) did not observe any on *E. conviva* telotrochs. No cilia were observed during the present study in *E. maliculiformis*.

During transformation of the telotroch of *E. maliculiformis* to the mature adult, it was unknown how the cinctal limbs (Figs. 5.4A - E) appeared and then grasped around the scopula of a scyphidiid peritrich. It is presumed that the telotrochs fastened themselves to the host's body with the scopula while metamorphosis of the cinctum took place. This needs to be an effective attachment and cinctum formation was presumed to be a quick process otherwise the ellobiophryid will lose its grip around the new scyphidiid peritrich host.

The site of attachment of *E. maliculiformis* was usually just above the scopula of the scyphidiid peritrich host. In some cases they were observed to be attached around the middle region of the scyphidiid peritrich (Figs. 5.4F & G) or even around the peristomial region, thus obstructing the peristome and buccal cavity of the scyphidiid peritrich. This could possibly be detrimental to the mantoscyphidians, either interfering with nuclear division during reproductive processes or preventing the mantoscyphidians from feeding.

Ellobiophryid telotrochs differed from mantoscyphidian telotrochs in that the mantoscyphidian telotrochs were larger and more elongate (Table 5.11; Figs. 5.1; 5.3). *Ellobiophrya maliculiformis* telotrochs were smaller in both length and diameter than *Mantoscyphidia branchi*, *M. midae* and *M. spadiceae* telotrochs.

Table 5.11: Comparison of the body length and diameter (µm) of *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep telotrochs associated with *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 [from *Haliotis spadicea* Donovan, 1808], *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 [*Cymbula miniata* (Born, 1778) population], *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 [*Scutellastra barbara* (Linnaeus, 1758) population].

Telotrochs	Body length	Body diameter
E. maliculiformis	10.32–24.83 (16.54; 5)	10.29-20.0 (14.57; 5)
M. branchi (*C. miniata* population)	14.9-35.3 (26.3±5.7; 14)	16.0-25.5 (20.6±2.4; 14)
M. branchi (*S. barbara* population)	14.5-37.5 (24.8±5.1; 50)	13.0-30.0 (22.2±3.4; 50)
M. spadiceae	21.4-56.2 (38.3±8.4; 34)	16.2-30.0 (22.7±3.7; 34)

Figure 5.1

Scanning electron micrographs of the telotroch stages of *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 (A - F) associated with *Haliotis spadicea* Donovan, 1808 *and Mantoscyphidia midae* Botes, Basson & Van As, 2001 (G & H) associated with *Haliotis midae* Linnaeus, 1758.

A. The scyphidiid peritrich's body swells.
B. Basal kinetosomes appear (arrow).
C. Ciliary girdle emerges (arrow indicates closed peristome).
D,E. Fully developed telotroch.
F. Transformation into a settled form begins.
G. Scopula enlarges and scyphidiid peritrich elongates.
H. Adoral ciliary spiral open, transformation completed.

Scale bars: 10 µm.

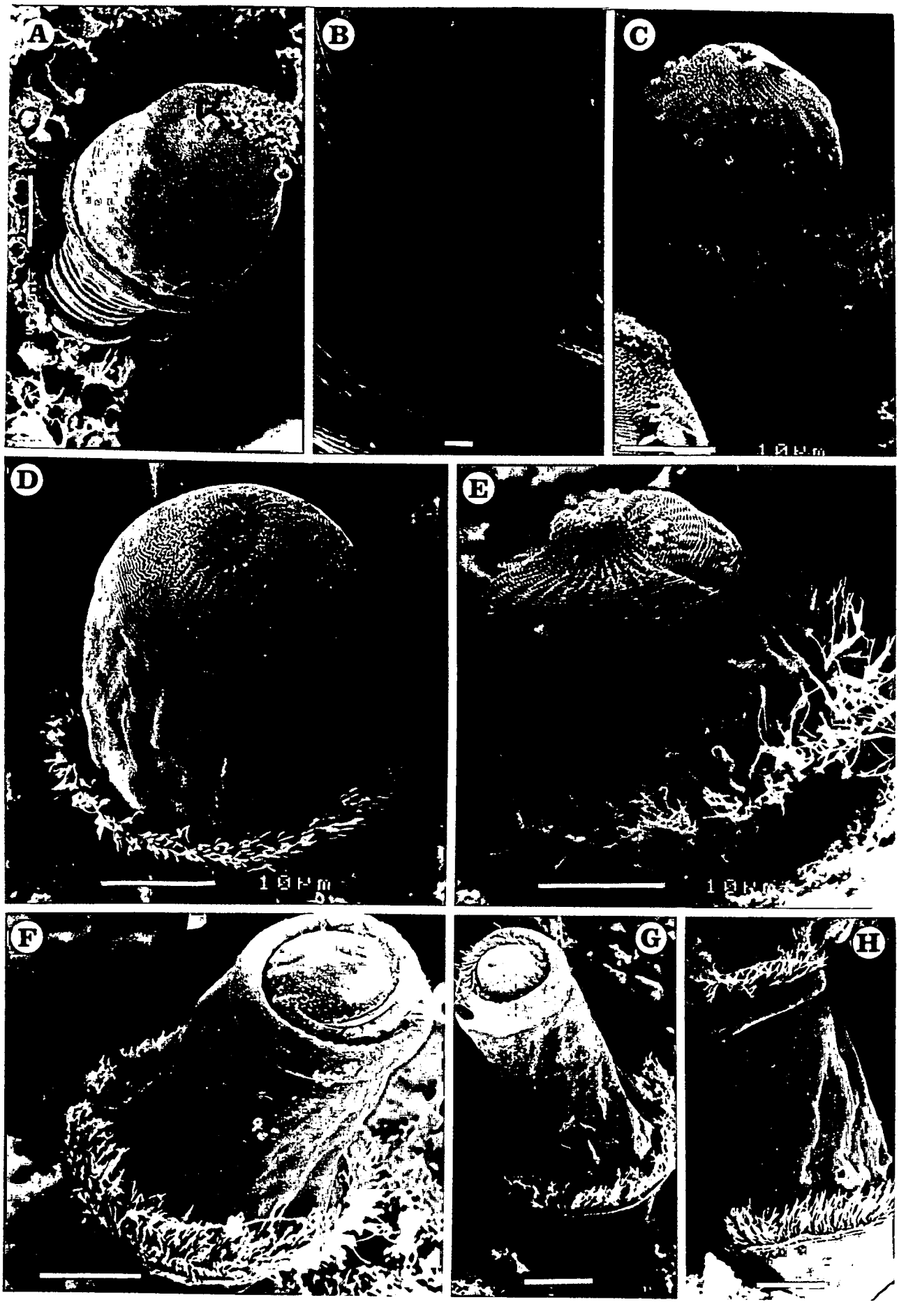
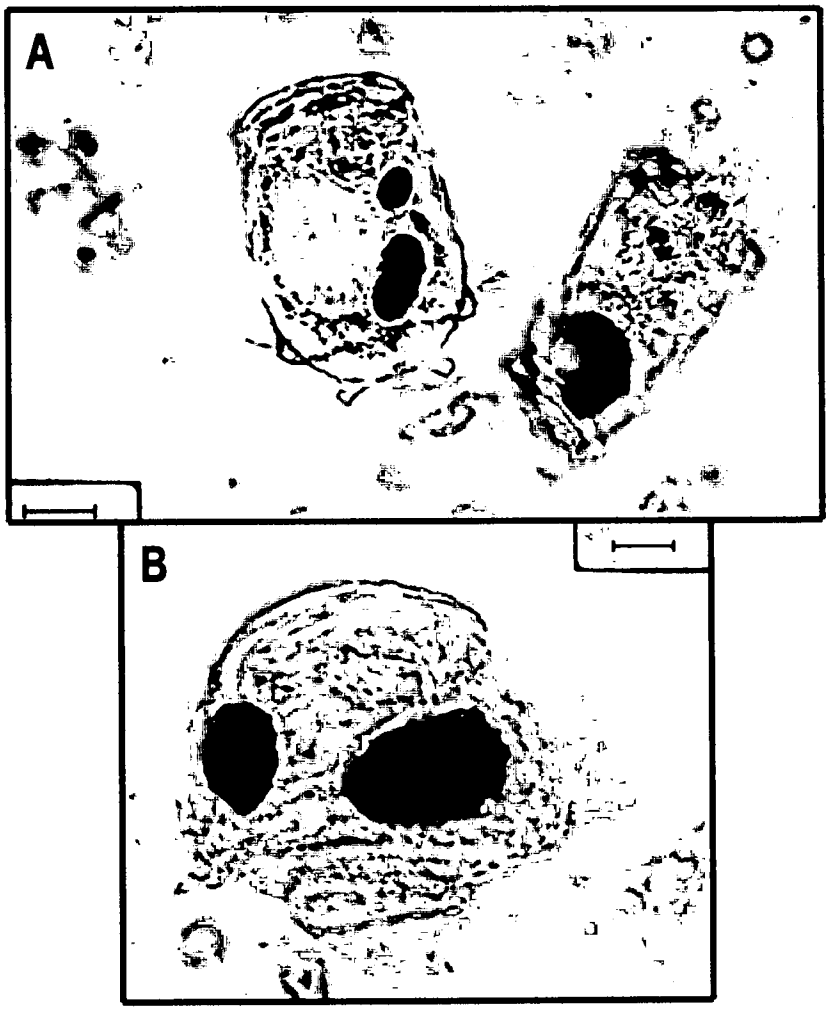

A
B
C
D
E
F
G
H

Figure 5.2

Photomicrographs (A & B) and diagrammatic illustrations (C) of the telotroch stages of live specimens and hematoxylin stained specimens of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 (A-B) associated with *Scutellastra barbara* (Linnaeus, 1758) and *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep, associated with *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 (C).

A. *Mantoscyphidia branchi* telotroch and vegetative individual to the right.
B. *Mantoscyphidia branchi* telotroch.
C. *Ellobiophrya maliculiformis* telotroch attached by a larval stalk to the parent ellobiophryid.

Scale bars: 10 µm.


A
B

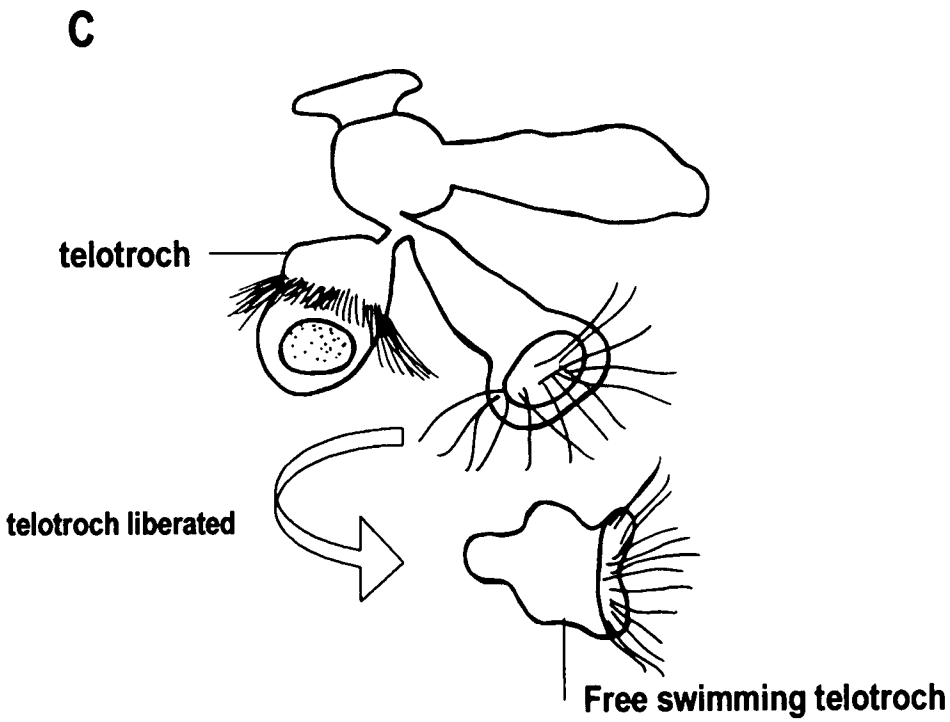
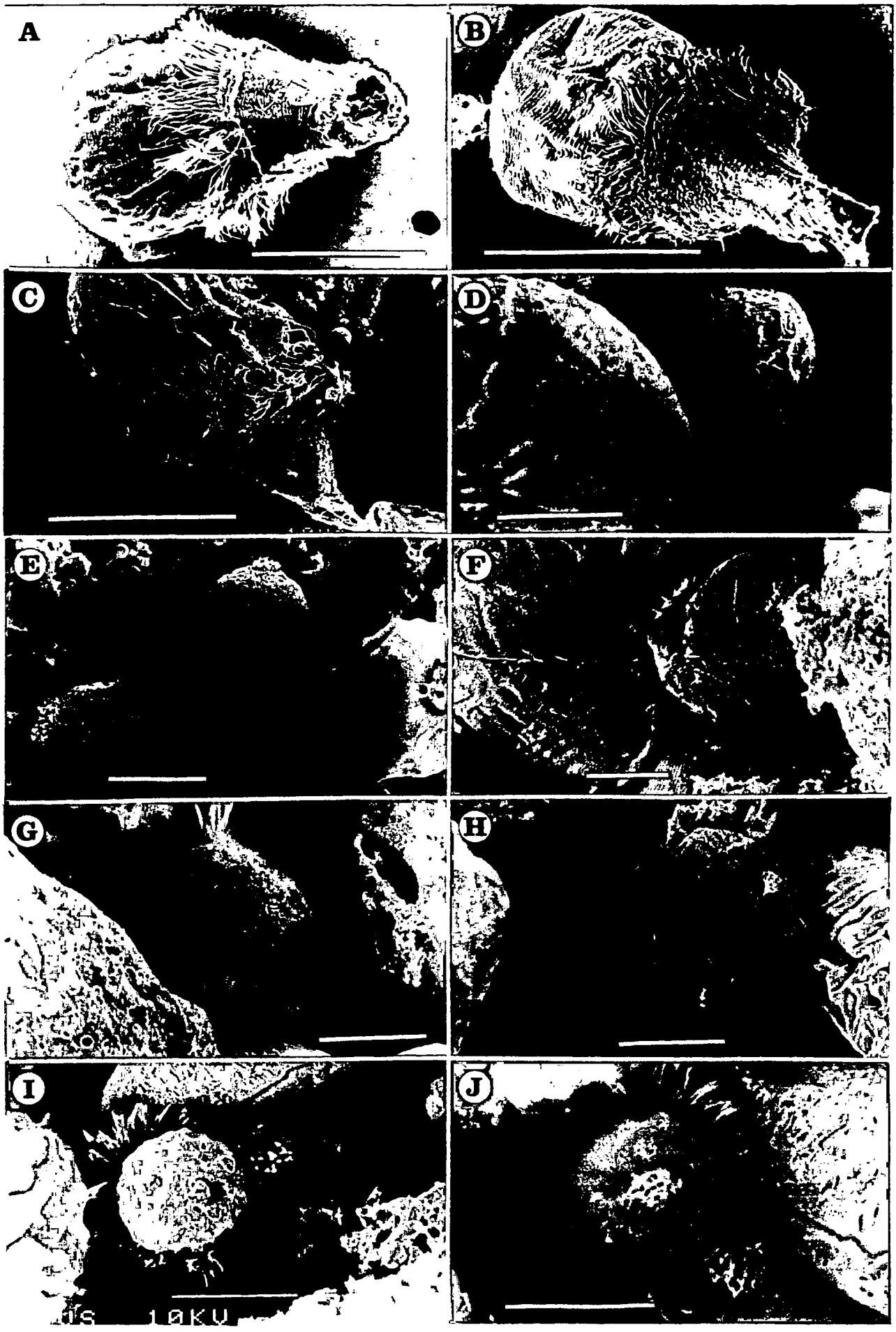

C
telotroch
telotroch liberated
Free swimming telotroch

Figure 5.3

Scanning electron micrographs of the telotroch stages of *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep associated with *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 (A-C), *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 (D-H) and *Mantoscyphidia midae* Botes, Basson & Van As, 2001 (I&J).

A – C Telotroch with narrow scopula.
D – E Telotroch attached to an ellobiophryid.
F Telotroch attached to an ellobiophryid. Trochal band of cilia visible.
G Telotroch attached to an ellobiophryid. Trochal band of cilia visible and peristomial cilia protruding.
H – J Telotroch with long locomotory cilia.

Scale bars: 1µm in A – E and 10 µm in F & G.


A
B
C
D
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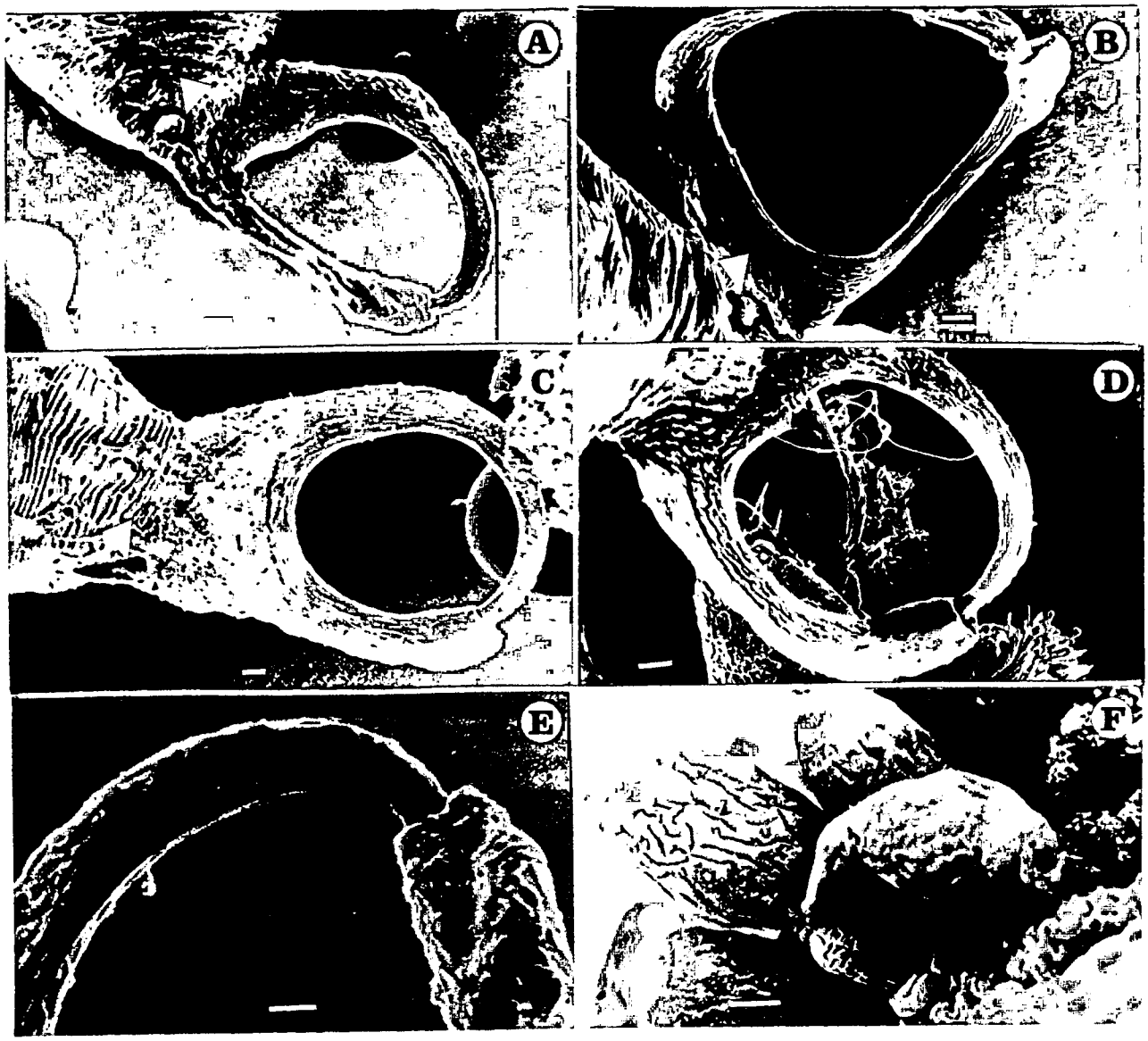
10KV

Figure 5.4

Scanning electron micrographs (A - F) and hematoxylin stained specimen (G) of the cinctum and method of attachment of *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep associated with *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 (A-E) and *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 (F&G).

A – E Cinctum. Scopula indicated by arrow.
F Ellobiophryid attached around the middle part of the scyphidiid peritrichs' body.
G Two ellobiophryids attached around two scyphidiid peritrichs.

Scale bars: 10 µm.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Chapter 6

Conjugation

in mantoscyphidians and ellobiophryids

The sexual reproduction process of conjugation was observed on numerous accounts in populations of the scyphidiid peritrich *Mantoscyphidia branchi* (See Chapter 4, Tables 4.1 - 4.3). Conjugation was also observed in populations of *M. midae* and *M. spadiceae* (Tables 4.1 - 4.3, Tables 1 - 4 in Appendix A). Conjugation was mostly observed in *M. branchi* and *M. midae* and the description is therefore based on observations made of these populations. Conjugation was also observed in populations of *Ellobiophrya maliculiformis* (Table 4.1, Tables 2 & 3 in Appendix A). This is the first record of conjugation in the genus *Ellobiophrya*.

Conjugation in *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 and *M. midae* Botes, Basson & Van As, 2001

Family: Scyphidiidae Kahl, 1933

The first observation of conjugation in *Mantoscyphidia branchi* occurred four hours after collection of hosts and in *M. midae* and *M. spadiceae* from eight to ten hours after collection. Various stages of conjugation were observed in all the populations up until 29 hours after collection (Tables 4.1 - 4.3). The occurrence of conjugation in *M. branchi* was much higher than the occurrence of binary fission and telotroch formation.

Size

Body dimensions of micro- and macroconjugants are presented in Table 7 (Appendix A). There was a large variation in microconjugant length (8.24 µm to 45.91 µm). This variation indicated that a variety of sizes of microconjugants occurred in a population, from the initial stage of attachment to the macroconjugant to the shriveled microconjugant that had entered all of its content into the macroconjugant. The average macroconjugant length was 30.10 µm (Table 6.1) and the average body length of live vegetative individuals of *M. branchi* were 65.0 µm (Table 5.7). Macroconjugants were 46.3 % shorter than live vegetative individuals and were able to attract microconjugants.

Table 6.1: Average length and width (µm) of the microconjugants and macroconjugants of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 measured from scanning electron microscopy photographs occurring on the gills of *Scutellastra barbara* (Linnaeus, 1758).

	Length	Width
Mantoscyphidia branchi microconjugants	8.24–45.91 (19.23 ± 5.01,105)	7.06-24.14 (15.38 ± 3.61,105)
Mantoscyphidia branchi macroconjugants	19.92-49.09 (30.10 ± 6.40,102)	18.64-36.36 (25.71 ±3.66,102)

Appearance of peristomial regions

The peristomial regions of macroconjugants were not always completely closed during the process of conjugation. The peristomes of some macroconjugants were slightly open, with a small tuft of adoral cilia protruding (Figs. 6.6E - H), while others had open peristomes with quite a number of adoral cilia protruding (Figs. 6.4B & G; 6.5E). The peristomes of microconjugants were always closed with no adoral cilia protruding. It is presumed that the microconjugants accumulated enough food during preparation for conjugation, because the microconjugants can not feed during conjugation.

Attachment

The microconjugant always assumed a position perpendicular to the long axis of the macroconjugant's body (Figs. 6.4A – J). More than one microconjugant frequently fused with the same macroconjugant and the microconjugants were always observed attached at opposite sides of the macroconjugant (Figs. 6.6I & J). In *Mantoscyphidia branchi* conjugation required at least 24 hours to be completed.

FIRM ATTACHMENT AND ENTERING

Initial attachment to a macroconjugant took place quickly. Firm attachment and entering of the microconjugant's endoplasm into the macroconjugant required at least one hour and 15 minutes. During this time a protoplasmic bridge was established between the two conjugants and the endoplasm moved slowly into the macroconjugant (Figs. 6.6A – D, 6.8A – C).

PROGAMIC NUCLEAR DIVISIONS AND SHEDDING OF THE MICROCONJUGANT'S PELLICLE

The macro- and micronucleus of the microconjugant enlarged and filled the whole of the microconjugant's body (Figs. 6.1A & B,D,E & F; 6.8A - C). The macronucleus undergoes fragmentation. Spindle formation occurred in the nuclei (Figs. 6.1C & D; Fig. 6.2A) and micronucleus underwent three progamic divisions through meiosis to form seven nuclei in the microconjugant (Figs. 6.2B – F). On occasion up to eleven nuclei or rounded structures were observed in microconjugants. These nuclei moved into the macroconjugant (Figs. 6.3A - C). The macroconjugants illustrated in Figures 6.8D – G, contain different numbers of pronuclei.

The microconjugant became depleted and thinner in diameter as it transferred the endoplasm to the macroconjugant (Figs. 6.3D & E, 6.5A - J). The shriveled and depleted microconjugant eventually fell off (Fig. 6.3F). Figures 6.7A – D illustrate enlarged views of the microconjugants. Figures 6.7E – H illustrates the progressive shrinking of the microconjugant until the microconjugant is just attached to the macroconjugant with a small piece of pellicle. Inside the macroconjugant progamic divisions had taken place in the micronuclei. This division took place very quickly and was not observed very often. The exconjugant had a swollen shape due to the extra cytoplasm that was not yet evenly distributed (Fig. 6.6C).

SYNKARYON FORMATION

One nucleus from the microconjugant and one from the macroconjugant fused to form the synkaryon (Figs. 6.8H & I). All the other pronuclei took no further part in reproduction; they degenerated and were resorbed into the cytoplasm of the macroconjugant or exconjugant. The synkaryon was observed to be located adorally or aborally, and a darker, granular area was frequently observed in the center of the exconjugant's body or close to the protoplasmic bridge. In this area the non-functional pronuclei were resorbed. During this process infraciliature movement was observed in the adoral region and it is assumed that the exconjugants were able to feed during the process of conjugation.

METAGAMIC (POST-ZYGOTIC) NUCLEAR DIVISIONS AND REORGANIZATIONAL FISSIONS

The metagamic or post-zygotic divisions usually occured before the shriveled microconjugant disappeared. The synkaryon divided through two metagamic divisions and formed three macronuclear anlagen and one micronucleus (Fig. 6.8J). The micronucleus shrunk and the macronuclear anlagen fused to form the functional macronucleus (Fig. 6.8K & L). It is presumed that the exconjugant could then undergo two consecutive divisions (binary fission) into four individuals, although this was not observed. These individuals will grow and the nuclei will assume the typical vegetative positions.

In *M. branchi* it was observed that a "telotroch" had attached to a vegetative individual (Fig. 6.9A). It is assumed that this "telotroch" was actually a microconjugant preparing to undergo conjugation. This could not have been an occurrence of preconjugation fission preceding the process of conjugation, given the position at which the "telotroch" was attached. This is a true microconjugant that had attached to the macroconjugant so recently that it had not yet disassembled its trochal band cilia. It is unclear whether binary fission always has to occur before a telotroch can develop, or whether binary fission follows telotroch formation. It is unlikely that this "telotroch" just settled temporarily on the scyphidiid peritrich in search of a new site of attachment, as the specimens remained fused together throughout the whole fixation and staining process. A protoplasmic connection or bridge had to have been established.

Conjugation in *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep

Family: Ellobiophryiidae (Chatton & Lwoff, 1929)

Conjugation of *E. maliculiformis* was observed twice in populations of *Mantoscyphidia midae*, four times in populations of *Mantoscyphidia spadiceae* (Tables 4.1 - 4.3, Tables 1 – 4 in Appendix A), and twice in populations of *Mantoscyphidia branchi* (Aquatic Parasitology Research Group observations from 1993 to 1997). This is the first record of the occurrence of conjugation in the genus *Ellobiophrya* (Figs. 6.7I & J) (Peters, *et al.* in prep).

A telotroch was observed attached to the body of another *E. maliculiformis* (Fig. 6.9B). In this case the telotroch was attached to the middle region of the ellobiophryid's body, and it is assumed that this is a differentiation division that produced a microconjugant. This telotroch also had a short stalk with which it attached to the ellobiophryid, but was not attached to the scopula, as would have been the case in a developing telotroch. There would not have been a larval stalk if this was a microconjugant in the process of fusing with the macroconjugant.

The process of conjugation in the secondary symbiont, *E. maliculiformis* was not studied in detail as it did not form part of the initial objectives of this study. Not all scyphidiid peritrich populations had associated secondary symbionts. The probability of observing ellobiophryids was not very high and observations of the reproductive processes was also less. It is assumed that the process of conjugation in *E. maliculifomis* is essentially the same as for other ellobiophryid peritrichs. Further studies will aim at describing conjugation in *E. maliculiformis*.

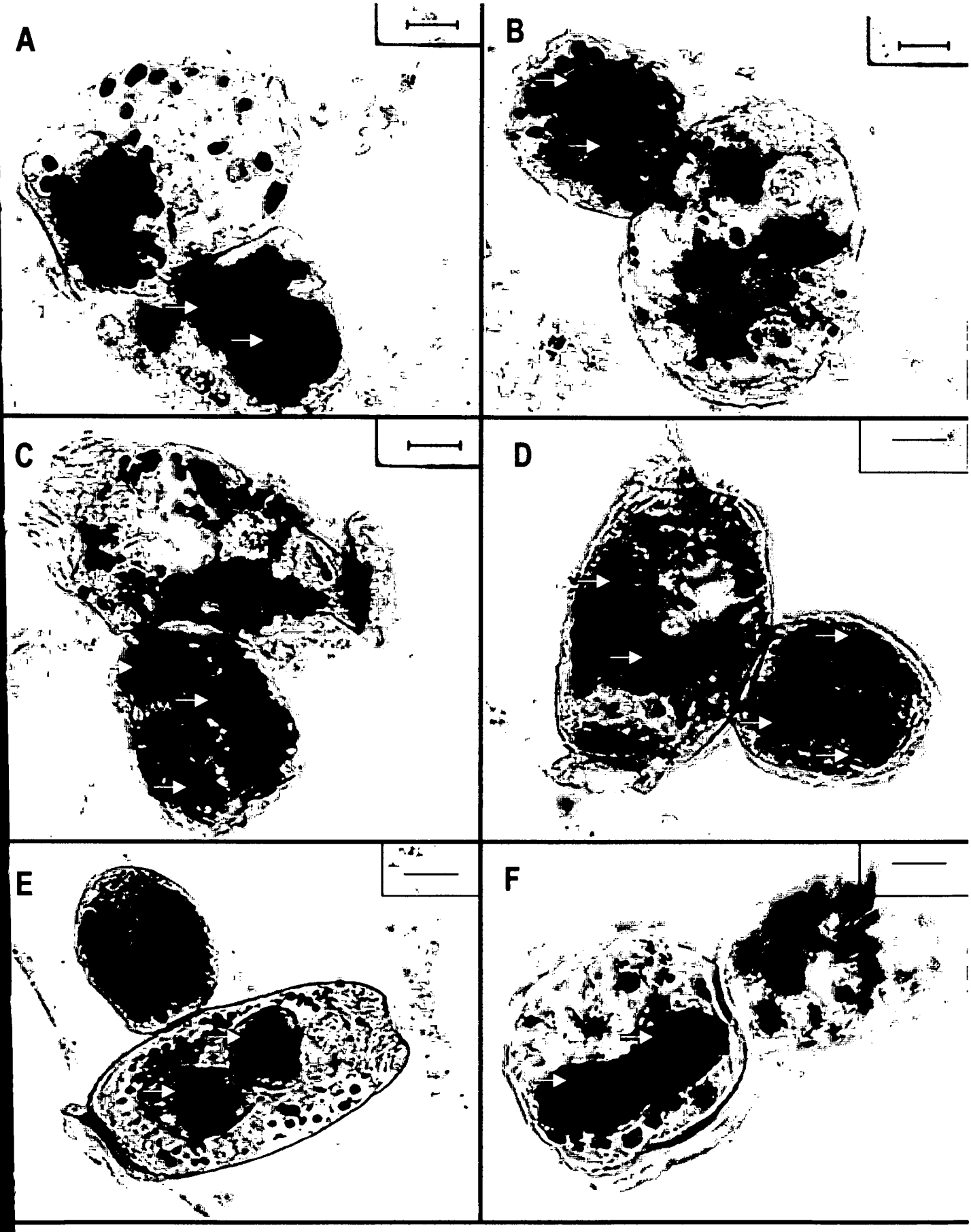

Fig. 6.1 Photomicrographs of hematoxylin stained specimens of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 undergoing conjugation. Small dark stained granules are symbiotic algae. **A&B** - macro- and micronucleus (indicated by arrows) of microconjugant undergoing mitosis. **C** - Spindle formation in the nuclei of both conjugants. **D** - Three nuclei undergoing mitosis in the microconjugant and two in the macroconjugant. **E&F** - Macroconjugant's nuclei undergoing mitosis. Scale bars = 10µm.

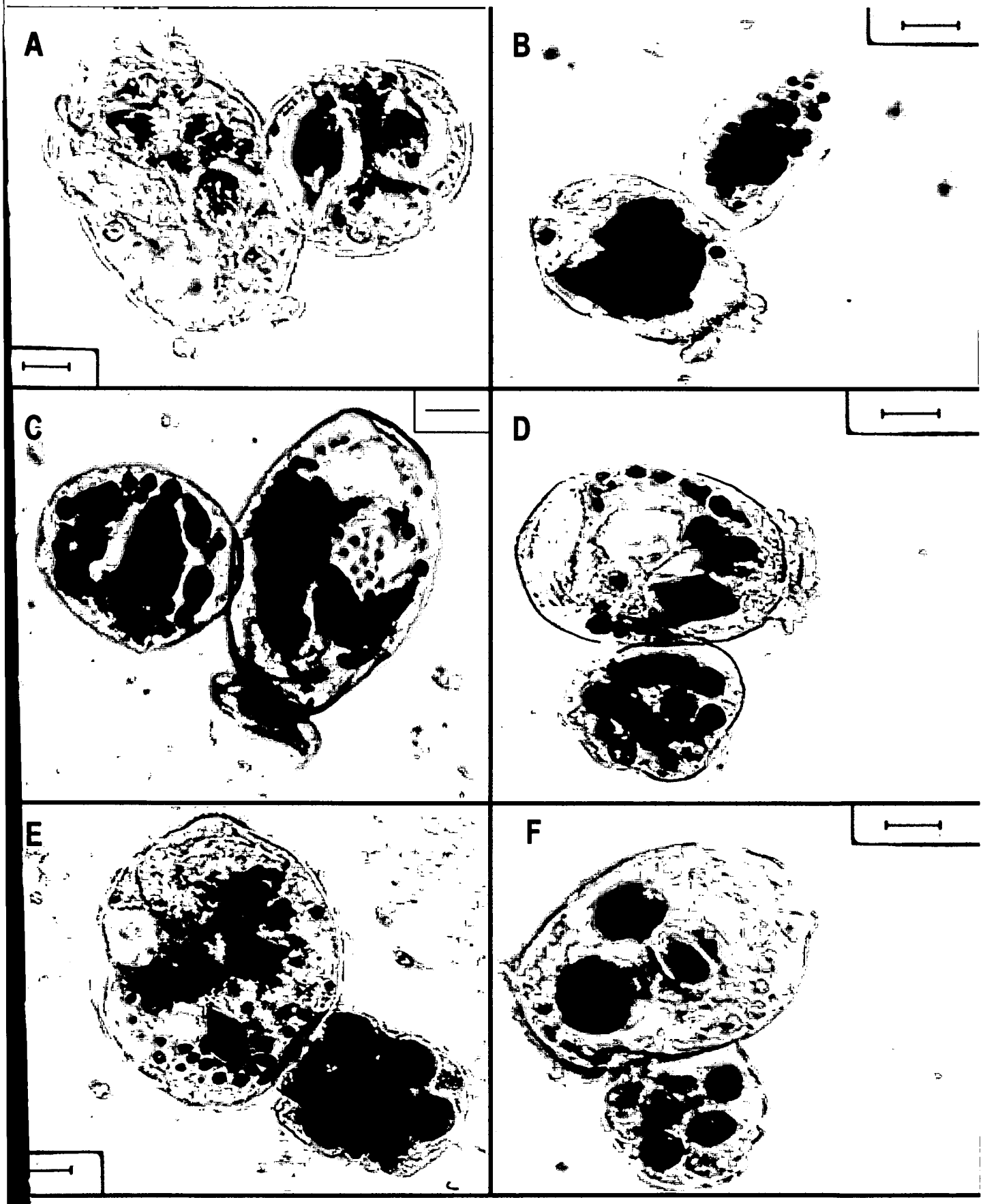

Fig. 6.2 Photomicrographs of hematoxylin stained specimens of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 undergoing conjugation. Small dark stained granules are symbiotic algae. **A** - Three nuclei in microconjugant undergoing mitosis. **B,C&D** - Progamic divisions taking place in both conjugants. **E&F** - Seven nuclei formed in microconjugant through progamic divisions. Scale bars = 10 µm.

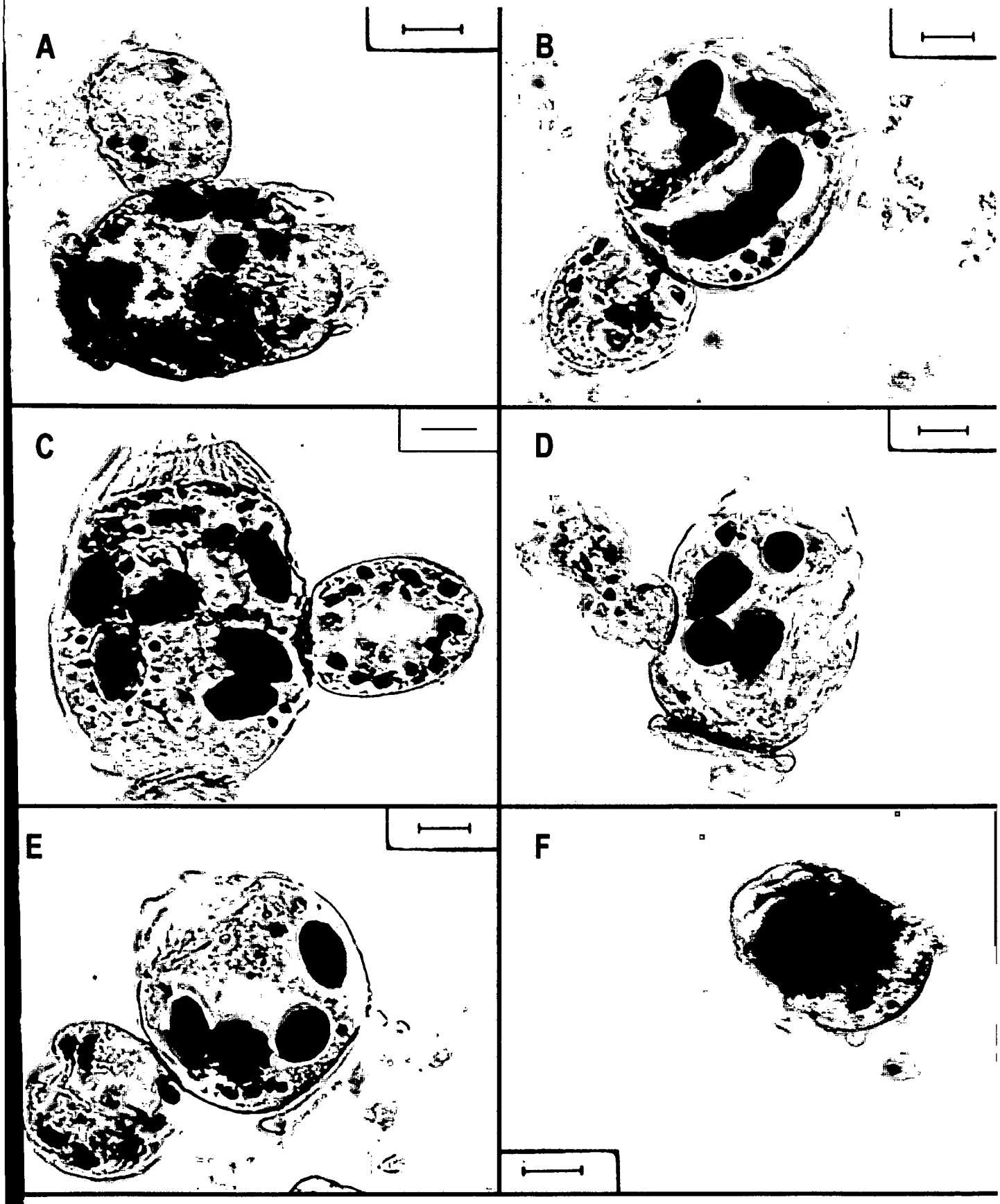

Fig. 6.3 Photomicrographs of hematoxylin stained specimens of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 undergoing conjugation. Small dark stained granules are symbiotic algae. **A** - Nuclei have moved from microconjugant to macroconjugant, seven pronuclei in macroconjugant. **B-D** Six pronuclei in macroconjugant. Microconjugant becoming shriveled. **E** - Four pronuclei in macroconjugant. **F** - Depleted microconjugant about to fall off. Scale bars = 10 µm.

Figure 6.4

Scanning electron micrographs of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 associated with *Cymbula compressa* (Linnaeus, 1758) (E&G), *Scutellastra barbara* (Linnaeus, 1758) (F, H, I&J), *S. cochlear* (Born, 1778) (A&B,D) and *S. longicosta* (Lamarck, 1819) (Fig. 6.4C) undergoing conjugation. Firm attachment of the microconjugant to the macroconjugant. Microconjugants indicated by arrows. *Ellobiophrya maliculformis* Peters, Basson & Van As, in prep is attached to *M. branchi* in B and D.

A. The microconjugant is attached perpendicular to the long axis of the macroconjugant. The peristome of the microconjugant is closed, but there are some adoral cilia protruding from the partially opened peristome of the macroconjugant.
B. *Ellobiophrya maliculiformis* attached around the scopula of *M. branchi.* The peristome of the microconjugant attached to the macroconjugant is slightly open, but no adoral cilia are protruding. The macroconjugant's peristome is open with a number of protruding adoral cilia.
C. The microconjugant appears to be swollen, it has a greater diameter than length.
D. An enlargement of figure B.
E. A protoplasmic bridge between the microconjugant and macroconjugant is established, with the pellicle fused.
F. Microconjugant seen from above. A small tuft of adoral cilia protrudes from the macroconjugant's peristome.
G. The body of the macroconjugant is extended and it's peristome is open with adoral cilia protruding.
H. A small tuft of adoral cilia protrudes from the macroconjugant's peristome.
I. The peristome of the microconjugant is closed tightly and it has a grooved appearance. The macroconjugant's peristome is slightly open and there are some adoral cilia protruding from the partially opened peristome of the macroconjugant.
J. The macroconjugant's peristome is slightly open and there are some adoral cilia protruding from the partially opened peristome of the macroconjugant. The microconjugant's peristome is closed tightly.

Scale bars: 10 µm.

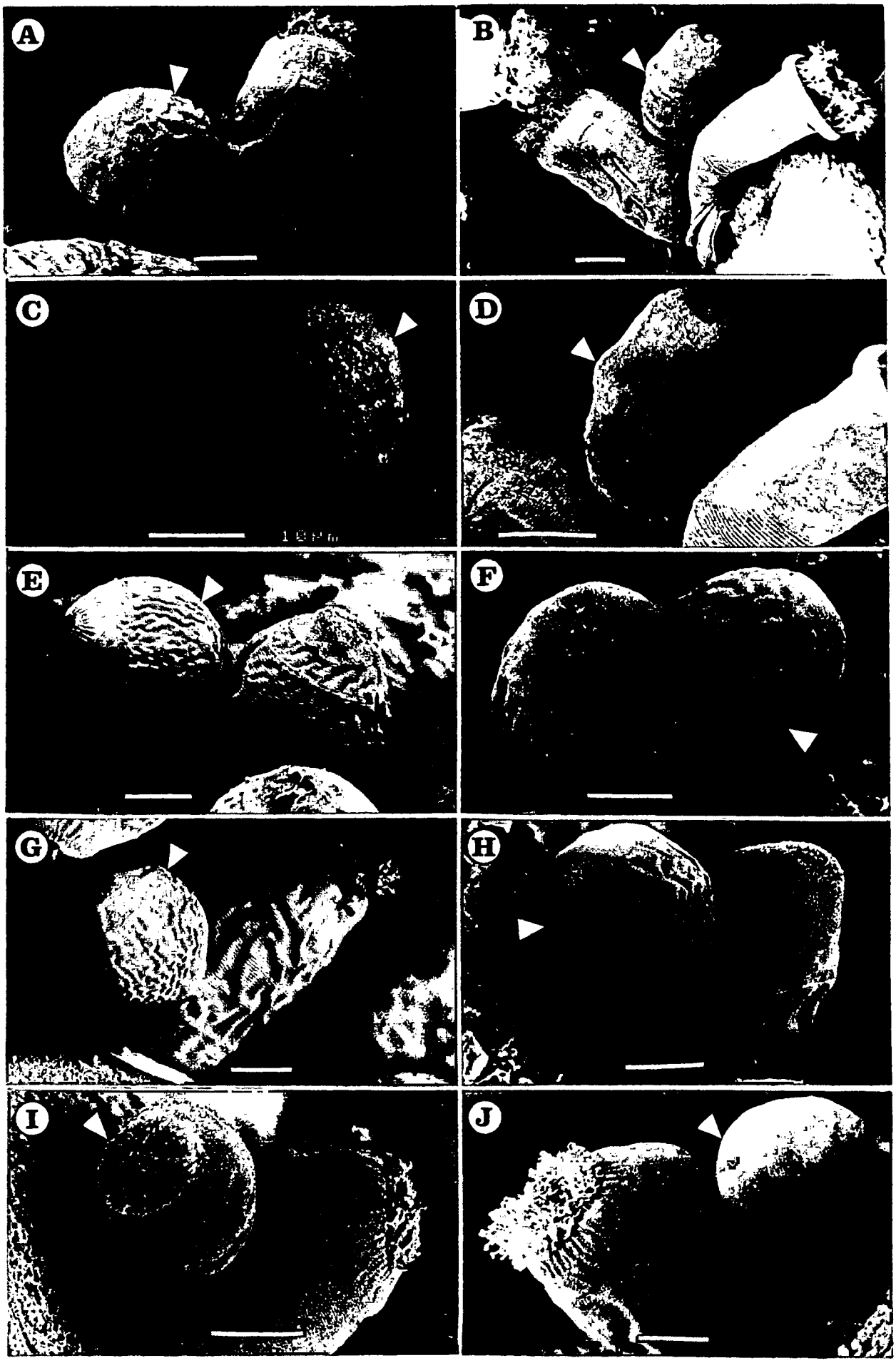
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Figure 6.5

Scanning electron micrographs of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 associated with *Scutellastra barbara* (Linnaeus, 1758) undergoing conjugation. Microconjugants indicated by arrows.

In all figures except G and J, the macroconjugants' peristomes are slightly open with a small number of adoral cilia protruding.

A. Microconjugant becomes progressively more depleted and shriveled as its nuclear material and endoplasm is transferred into the macroconjugant. The peristome of the microconjugant is closed tightly.
B,C. Microconjugant becomes progressively more depleted and shriveled as its nuclear material and endoplasm is transferred into the macroconjugant.
D. The microconjugant's peristome is closed and it forms a knob-like protrusion at the tip of the peristome. The macroconjugant is contracted, with folds forming at the aboral end above the scopula.
E. Microconjugant attached just above the telotroch band of the macroconjugant.
F. The microconjugant's peristome is closed and it forms a knob-like protrusion at the tip of the peristome, similar to figure D.
G. The microconjugant's pellicle is now more shriveled as in the previous figures.
H-J. The appearance of the microconjugants change from a rounded to a more elongated shape.

Scale bars: 10 µm.

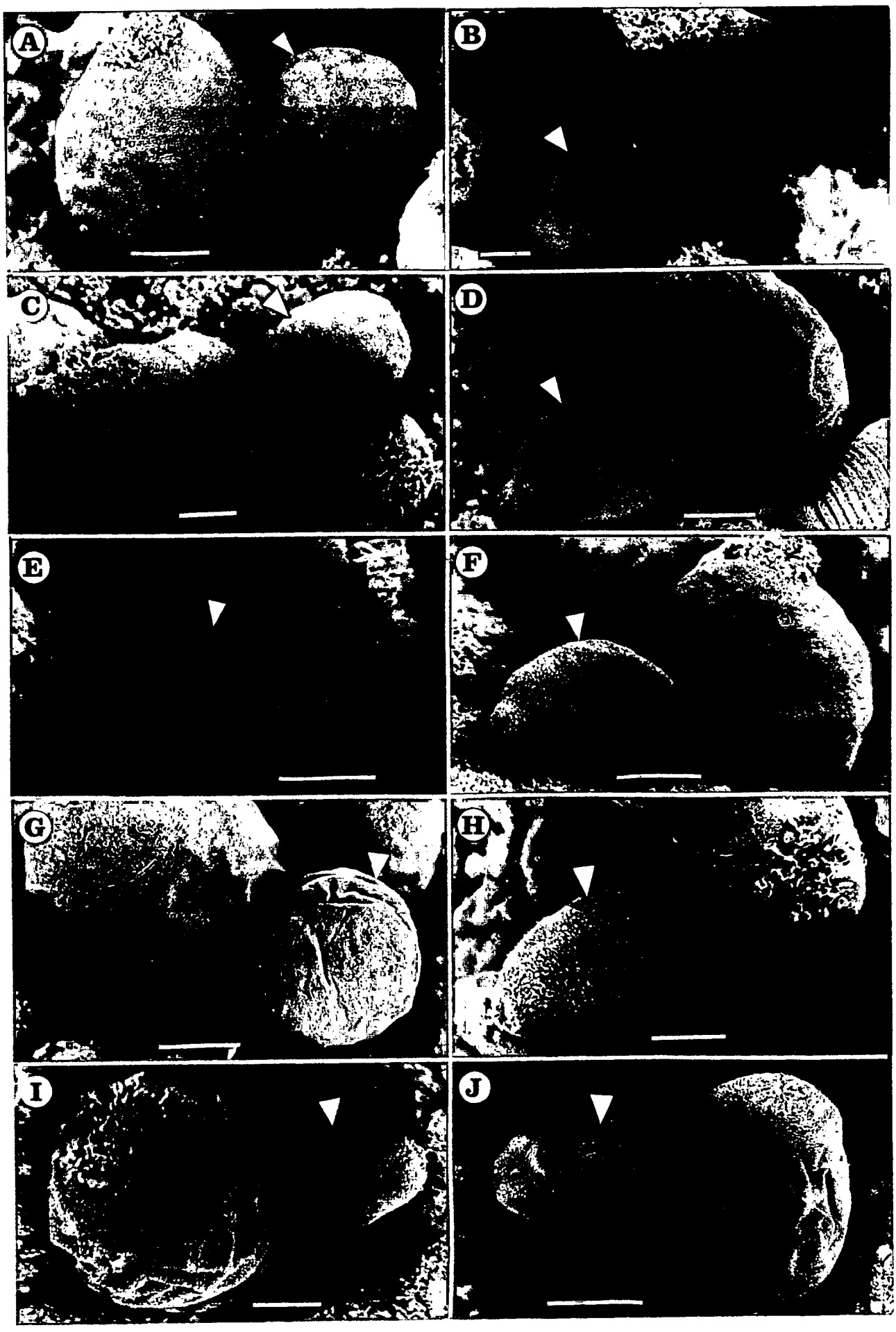
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Figure 6.6

Scanning electron micrographs of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 associated with *Cymbula compressa* (Linnaeus, 1758) (A) and *Scutellastra barbara* (Linnaeus, 1758) (B-J) undergoing conjugation. Microconjugants indicated by arrows.

A. Enlargement of the area where the macro- and microconjugant fuse to form a protoplasmic bridge. The pellicle of the macroconjugant fuses with the microconjugant's pellicle.

B. The attachment site of a microconjugant to a macroconjugant photographed from above. The entire width of the microconjugant's aboral region fuses with the microconjugant.

C. Attachment site of the microconjugant to the macroconjugant (lateral view) with fused pellicles. Microconjugant has a swollen and round shape.

D. Attachment site of the microconjugant to the macroconjugant (lateral view). Microconjugant is more shriveled than in previous figure. Enlargement of figure E.

E. Macroconjugant's peristome is slightly open with a small number of adoral cilia protruding. Microconjugant has emptied about half of its contents into the macroconjugant.

F. The microconjugant's diameter is greatest in the middle and it has a narrower adoral end. Macroconjugant's peristome is slightly open with a small number of adoral cilia protruding

G. The microconjugant folds as its contents are transferred into the macroconjugant. The macroconjugant's peristome is slightly open with a small number of adoral cilia protruding.

H. The microconjugant is now much smaller than in previous figures. The macroconjugant's peristome is slightly open with a small number of adoral cilia protruding. Macroconjugant is swollen due to the extra cytoplasm that has not yet been evenly distributed.

I. Two microconjugants attached to a single macroconjugant. The microconjugant located to the left is long while the other is shorter and smaller.

J. Two microconjugants attached to a single macroconjugant. Both microconjugants are long and thin. The contents of both have been transferred into the macroconjugant.

Scale bars: 10 µm.

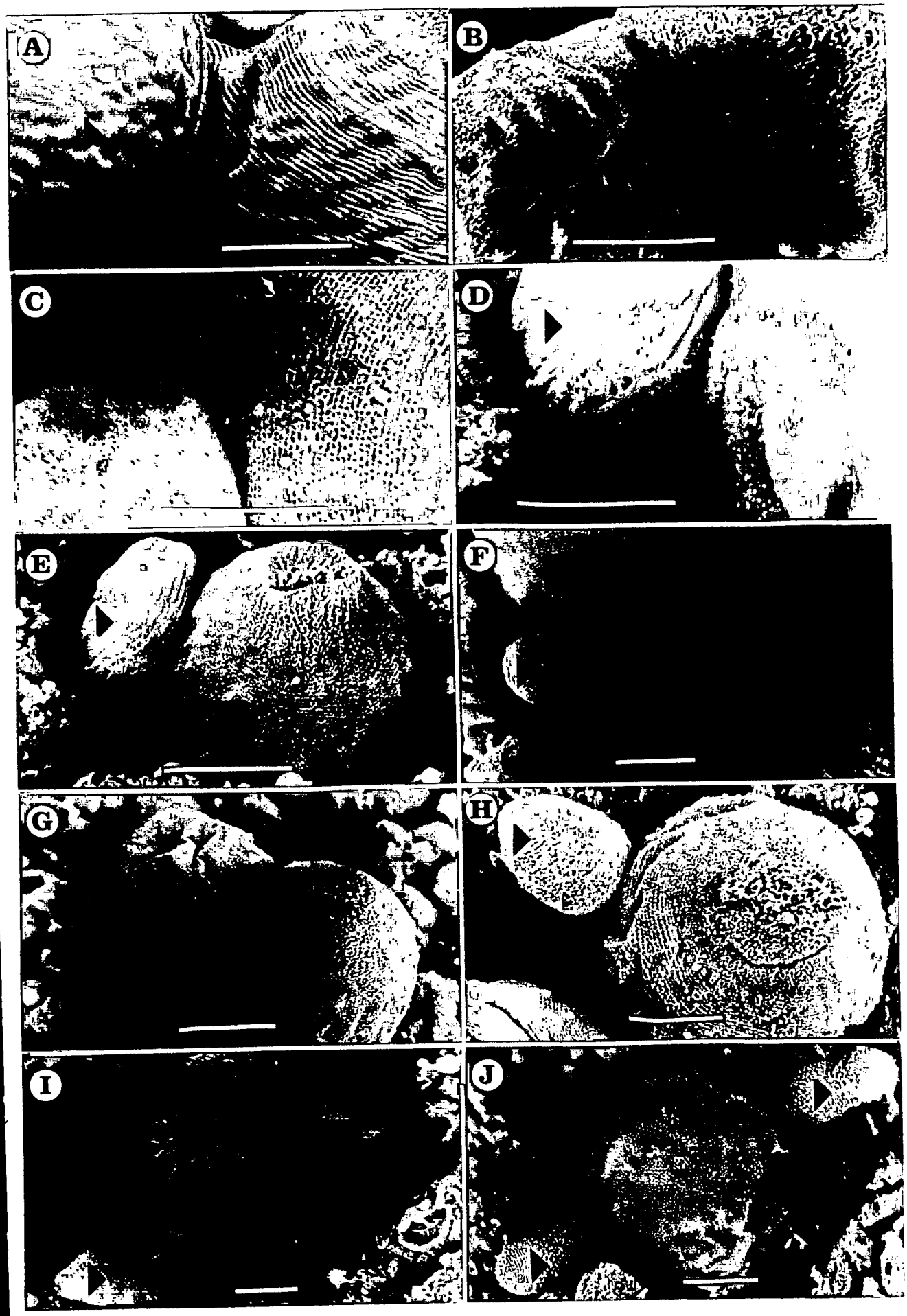
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Figure 6.7

Scanning electron micrographs of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 associated with *Scutellastra barbara* (Linnaeus, 1758) (A–H) and *Ellobiophrya maliculiformis* Peters, Basson & Van As, in prep (I&J) undergoing conjugation. Microconjugants indicated by arrows.

A. Enlargement of the microconjugant attached to a macroconjugant. The microconjugant is long, but not very thin.

B. Enlargement of the microconjugant attached to a macroconjugant. The adoral end of the microconjugant is narrower than the middle region.

C. Enlargement of the microconjugant attached to a macroconjugant. The pellicle of the microconjugant is flattened at the attachment site.

D. The macroconjugant's peristome is slightly open with a small number of adoral cilia protruding. Microconjugant is becoming thinner and more depleted.

E. The macroconjugant's peristome is closed and its body is shriveled. The microconjugant is thin and depleted.

F-G Thin and depleted microconjugants attached to macroconjugants with slightly opened peristomes.

H. Microconjugant almost completely depleted and ready to fall off the macroconjugant. The macroconjugant is shriveled.

I-J. Microconjugants attached to macroconjugants of *Ellobiophrya maliculiformis*. In both cases the microconjugant is attached perpendicular to the long axis of the ellobiophryid's body and just above the telotroch band.

Scale bars: 10 µm.

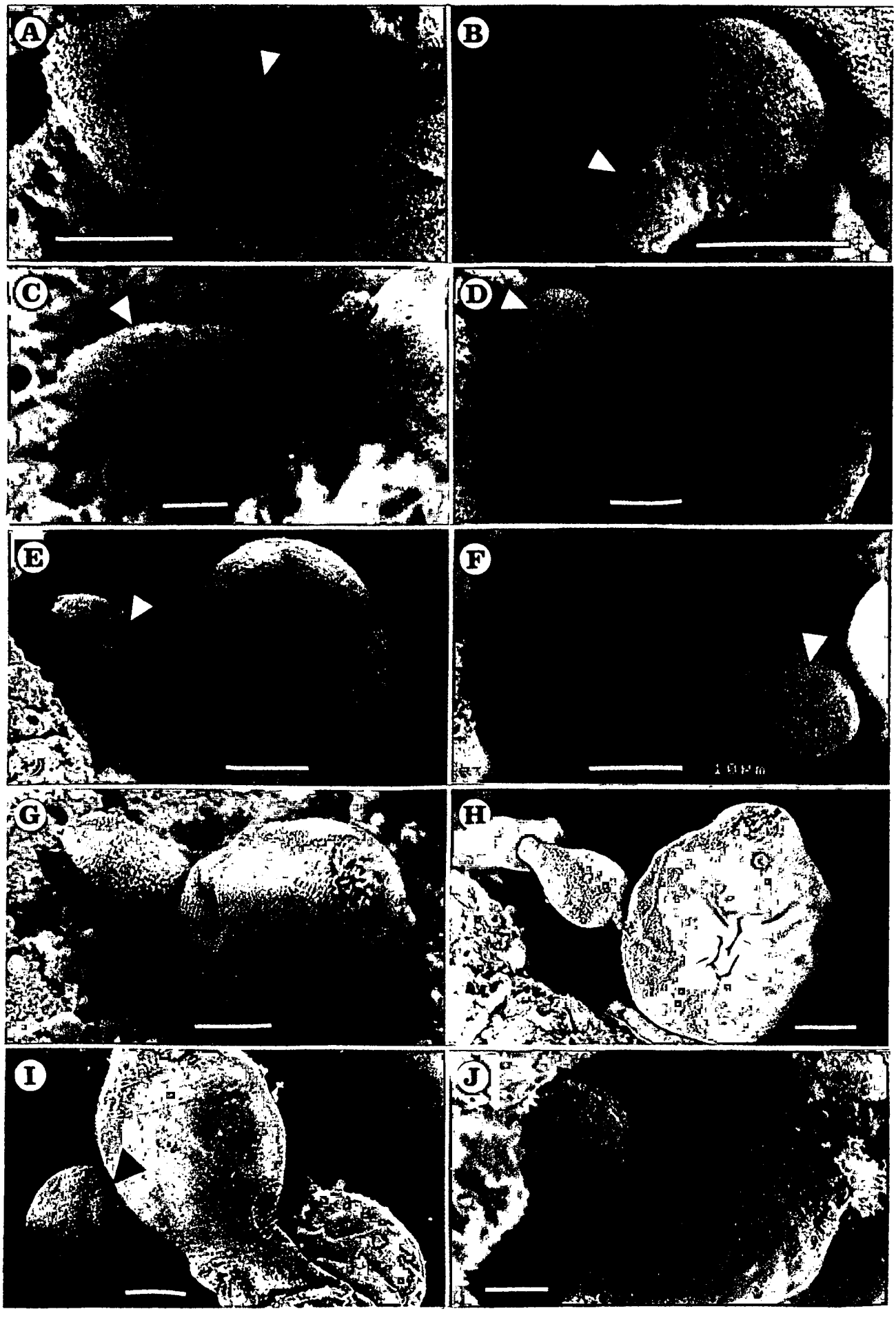
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Figure 6.8

Diagrammatic illustrations of live observations of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 associated with *Scutellastra barbara* (Linnaeus, 1758) undergoing conjugation.

A-C. The macro- and micronucleus of the microconjugant enlarge and fill the whole of the microconjugant's body.

D. Pronuclei are visible in the macroconjugant, which have moved from the microconjugant into the macroconjugant. The microconjugant is shriveled and depleted because its endoplasm is transferred into the macroconjugant.

E. Pronuclei are visible in the macroconjugant, which have moved from the microconjugant into the macroconjugant. Thin, depleted microconjugant.

F. Five pronuclei visible. Degeneration of pronuclei is taking place. Microconjugant thin and depleted.

G. Four pronuclei visible. Degeneration of pronuclei is taking place. Microconjugant thin and depleted.

ma = macronucleus, **mac** = macroconjugant, **mi** = micronucleus, **mic** = microconjugant, **p** = pronucleus.

Scale bar: 10 µm.

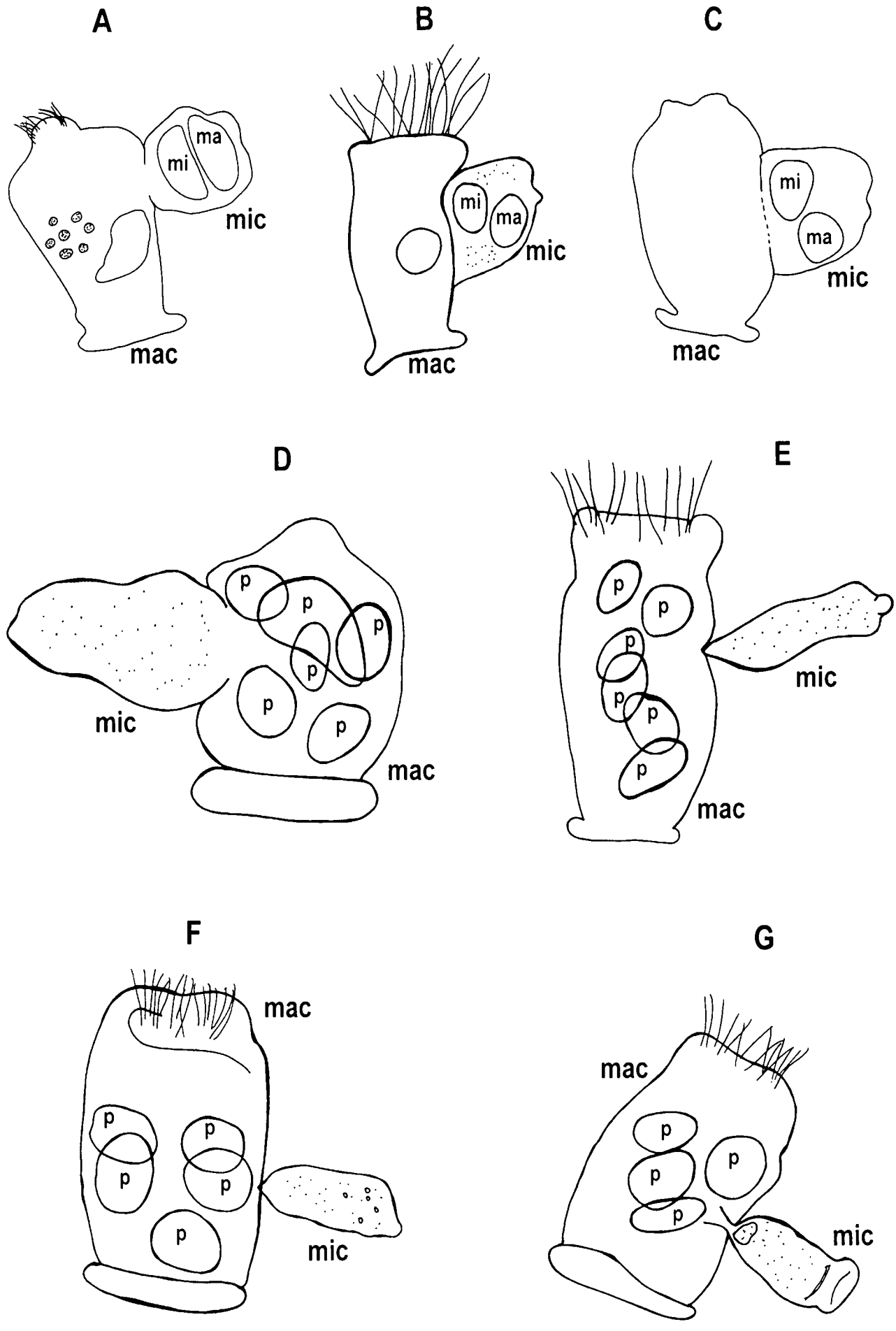
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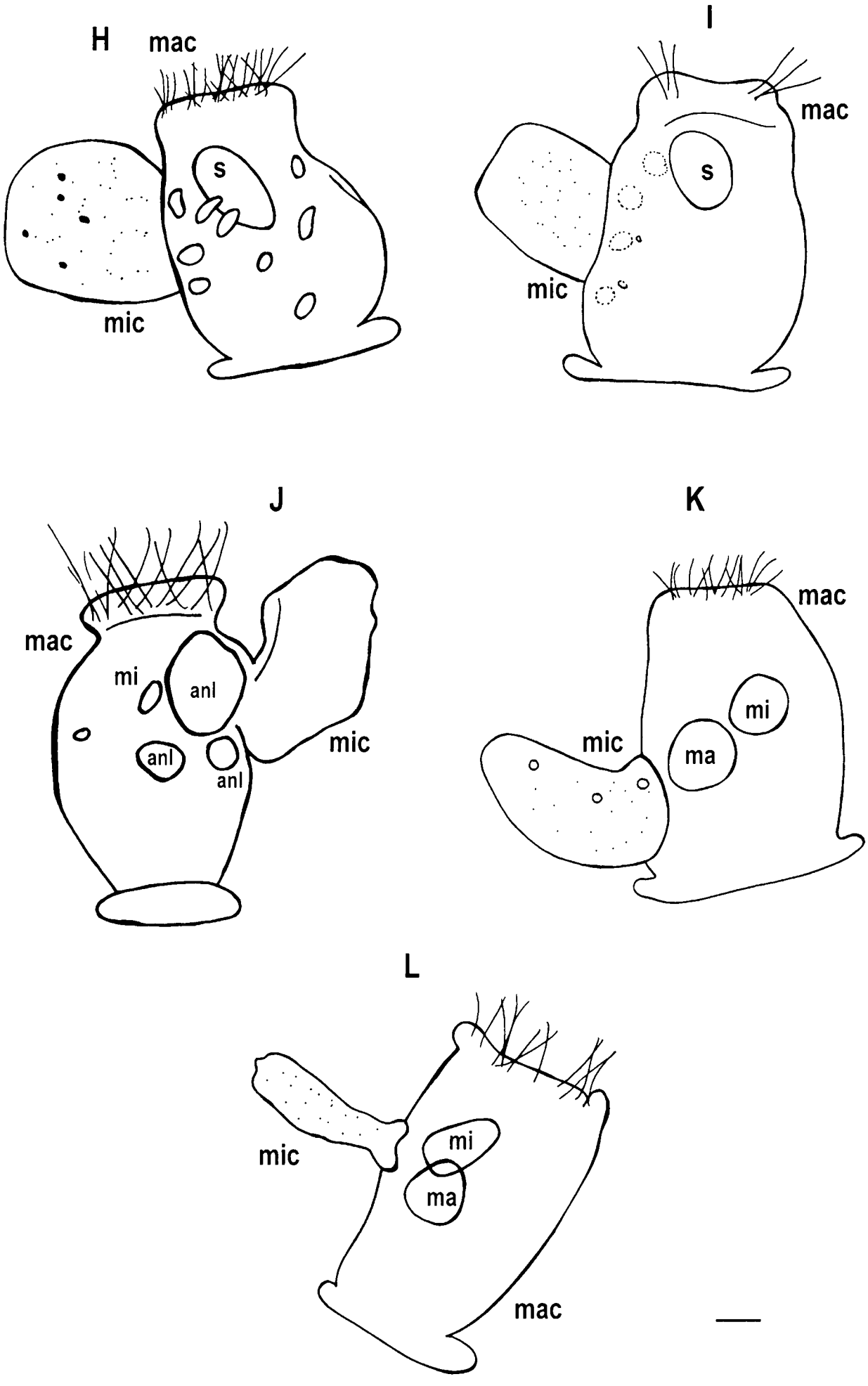
Figure 6.8 continued

Diagrammatic illustrations of live observations of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 associated with *Scutellastra barbara* (Linnaeus, 1758) undergoing conjugation.

H-J. Synkaryon formation. All other pronuclei degenerate.
K&L. The synkaryon divides into three macronuclear anlagen and a micronucleus. The macronuclear anlagen develop into the functional macronucleus.

anl = macronucleur anlagen, **ma** = macronucleus, **mac** = macroconjugant, **mi** = micronucleus, **mic** = microconjugant, **s** = synkaryon.

Scale bar: 10 µm.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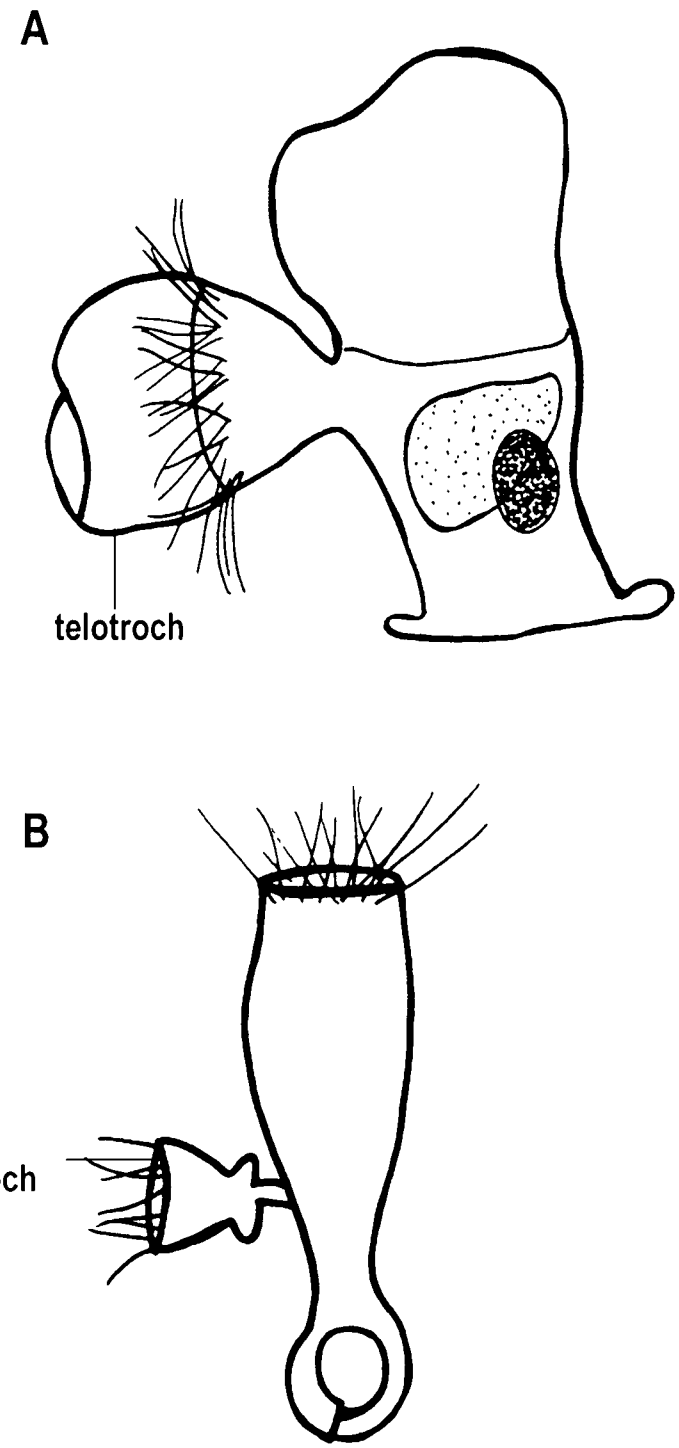

Fig. 6.9 Diagrammatic illustrations of hematoxylin stained (A) and live (B) specimens of *M. branchi* Van As, Basson & Van As, 1998 (A) associated with *Scutellastra barbara* (Linnaeus, 1758) and *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep. associated with *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 in the process of conjugation.

Chapter 7
Discussion

Aquatic organisms may live in habitats in which physical and chemical water quality parameters can remain relatively constant or in habitats where these factors change drastically. Aquatic organisms have adapted to the environment by either developing a narrow range of tolerance or a wide range of tolerance to changes in physical factors. Stenothermic and stenohaline species for instance are intolerant of high temperature fluctuations and high salinities, respectively, while eurythermic or euryhaline species can tolerate a wide range of temperatures or salinities.

The **freshwater habitat** presents several challenges to the aquatic animals that live there but the **intertidal region** is both the harshest and richest of all marine environments. Intertidal organisms are submerged twice a day for six hours when the tide rises, experiencing cold water and wave action, and are exposed to the air during low tides. They are subjected to the blazing sun with temperatures of up to 40°C and lose as much 70% of their body water to the air due to dessication during this time. All the vital functions of life such as respiration, excretion and reproduction must be adapted to function in two totally different environments; marine at high-tide and essentially terrestrial at low tide (Branch & Branch 1995).

There is a gradient in the physical stress that indertidal organisms have to suffer. Low on the shore the period of exposure at low-tide will be brief. Moving upshore conditions become more harsh and the period of exposure increases. Thus, fewer organisms are found to occur higher upshore. Because animals and plants are adapted to certain conditions, zonation occurs on the shore.

Intertidal organisms live in an environment with threats of water loss; extreme temperature fluctuations; differences in light intensities, salinity and pH; fluctuating oxygen levels; competition for food and space; and also the pounding wave action of the tides. As a result of this, these marine organisms are unique and have many adaptations to physical stress in the intertidal zone.

7.1 Molluscs and their scyphidiid peritrichs

A parasite that is attached to another parasite is known as a hyperparasite. The term hypersymbiont (a symbiont associated with another symbiont) however, is not an accepted term amongst parasitologists. The lack of a term that decribes the specific associations between hosts and scyphidiid peritrichs, and between scyphidiid peritrichs and the associated symbionts, led to the formulation of new terminology.

The scyphidiid peritrichs (*Mantoscyphidia branchi*, *M. marioni*, *M. midae* and *M. spadiceae*) that occur on the gills of the mollusc hosts will be referred to as primary symbionts, because the scyphidiid peritrichs attach **directly** to the gills of the primary host. *Ellobiophrya maliculiformis* is a secondary symbiont, because it attaches around the primary symbionts, namely the scyphidiid peritrichs, and thus **indirectly** to the mollusc host.

Haliotis midae (perlemoen)

Adults occupy crevices or exposed positions on shallow reefs and are commonly found in kelp beds (Branch, *et al.* 1994). The scyphidiid peritrich *Mantoscyphidia midae* occurs on the gills of *Haliotis midae* and is referred to as a primary symbiont. The secondary symbiont, *Ellobiophrya maliculiformis*, is associated with *M. midae*.

Haliotis spadicea (Venus ear or siffie)

This is a more common, but cryptic species. It is found in rock crevices or amongst red bait close to low water (Branch, *et al.* 1994). The scyphidiid peritrich *Mantoscyphidia spadiceae* occurs on the gills of *Haliotis spadicea* and is the primary symbiont. The secondary symbiont, *Ellobiophrya maliculiformis*, is found associated with *M. spadiceae*.

Scutellastra barbara (bearded limpet)

This limpet occurs in the lower balanoid zone and subtidally, usually submerged inside tidal pools coated with *Lithothamnion*. During high- and spring tides these

limpets are sometimes exposed. Along the east coast of South Africa it defends its territory against other grazers, but not along the more productive west coast (Branch, *et al.* 1994). The scyphidiid peritrich *Mantoscyphidia branchi* occurs on the gills of *Scutellastra barbara* and is the primary symbiont. The secondary symbiont, i.e. *Ellobiophrya maliculiformis* is found associated with *M. branchi.*

Other limpet hosts

In the present study the various reproductive processes of scyphidiid peritrichs also occurred in populations of *M. branchi* associated with *Scutellastra argenvillei*, *S. cochlear*, *S. longicosta*, *Cellana capensis*, *Cymbula compressa*, *C. oculus* and *C. miniata.* Limpets which are mostly submerged in water (during high and low tide) and which occur in tidal pools are *S. argenvillei*, *S. cochlear, C. compressa* and *C. miniata. Scutellastra longicosta* and *C. oculus* occurs on exposed rock surfaces with *S. longicosta* sometimes occurring in tidal pools and between *S. cochlear.* Limpets that occur on exposed rock surfaces are better adapted to dessication than submerged species. The subantarctic limpet, *Nacella delesserti* occurs on rock surfaces covered with algae in the lower littoral and kelp zone and the scyphidiid peritrich *M. marioni* occurs on the gills and is a primary symbiont.

Host adaptations to physical stress

Both **haliotids** and **limpets** live in areas of strong wave action and the morphology of their shells reflectsthe adaptation to these conditions, for flatness reduces resistance to waves while the wide mouth allows development of a very broad foot to cling securely to the substratum (Branch & Branch 1995). These molluscs cling to the rocks by an adhesion mechanism effected by the release of mucus between the foot and the substratum. The large foot, however, takes up a considerable amount of heat by conduction from the rock and a flat shell absorbs more radiation from the sun. A wide mouth also increases the amount of water that is lost and the absence of an operculum, which blocks off the shell mouth when the snail retreats into its shell, is also an disadvantage.

Limpets are able to return to a fixed position on the rock after feeding, where the shells have grown to fit the contours of the rock precisely, forming an almost watertight seal (Branch & Branch 1995) that reduces the risk of water loss. Like all Prosobranchia, limpets have lost both paired gills. The gills of limpets are not located inside the mantle cavity, but there is a band of secondary gills arranged around the mantle instead. This arrangement of the gills leads to a higher threat of desiccation of the gill surfaces. Haliotids do have bipectinate gills, but the right gill is reduced as organs on the right side are typically reduced in the higher gastropods. Haliotid gills are more protected from desiccation than limpets, as the mantle covers most of the gill surface. Haliotids also have perforations to accommodate a central outlet from the mantle cavity, through which stale water containing excreta can be discharged.

Host/Symbiont Associations

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

Van As, Basson & Van As (1998) recorded *Mantoscyphidia branchi* in association with all 17 species of limpets examined (from the South African zoogeographical province). All of these limpet 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, *et al.* 1999a). Infestation statistics of Van As, Basson & Van As (1998) show that almost every limpet were infested and that these infestations were enormous.

Botes (1999) also found extremely high infestations of *M. spadiceae* and *M. midae*: 94.74 % in *Haliotis spadicea* and 88.76% in *Haliotis midae* over five years. This is unlike any typical parasitic infestation, where parasites are usually overdispersed, i.e. most hosts have no parasites, some have a small number of parasites, and one or two hosts will have a high infestation. In addition no sign of pathology could be

found. These results together with similar observations by the Aquatic Parasitology Research Group on the majority of other intertidal invertebrates have led to the assumption that this is not a typical parasitic association.

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* Sandon, 1965 (Fantham 1930, Sandon 1965, Basson & Van As 1992) and the same species of scyphidiid peritrich, namely *Mantoscyphidia fanthami* Basson, Botha & Van As, 1999 (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 related host species (*Patella* and *Oxystele*).

Haliotid and limpet hosts that are exposed to various stress factors may experience stress, but their symbionts may react directly or indirectly to these stress factors. This is not the case in parasitic associations. Parasites will inhibit the physiological processes of the host and will increase in numbers to ensure their own survival, but this population increase is detrimental to the host. The host and its parasites could eventually die. There is a much closer association between the scyphidiid peritrichs in the present study and their hosts, because the association might be mutualistic in nature and therefor to the advantage of at least one or both parties. The Aquatic Parasitology Research Group assumes that the symbionts might eventually react to the stress of the host if it is detrimental to the host/symbiont's survival. This

assumption is based on research on a variety of marine organisms during the past 10 years (Botha 1994, Loubser 1994 & Van As 1997).

Host/Scyphidiid peritrich Association

The application of the Newton-Harvey equation (Schmidt-Nielsen 1990) in respiration is that diffusion alone is sufficient to supply the centre of a spherical organism with oxygen if the organism has a body diameter of less than 1 mm. Since the organism's consumption of oxygen lowers its internal concentration relative to that of the surrounding waters, diffusion will take place from the higher oxygen concentration (outside), to the inside of the organism. The body diameter of the five mantoscyphidian species described by the Aquatic Parasitology Research Group varies between 15 and 69 µm, and should thus in theory not hinder oxygen diffusion from the surrounding water and haliotid gills (Van As 1997 & Botes 1999). 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 (Botes 1999), and the assumption is that the perlemoen probably do not need as high a scpyhidiid peritrich infestation as the Venus ears would in order to cope with respiration, or perhaps perlemoen are just not as suitable to the symbionts.

Water flow over the gills is achieved in two ways: either by movement of the gills through the water, as in actively moving organsisms or alternatively by circulating water over the gills (Schmidt-Nielsen 1990). In the case of slow moving limpets and haliotids the first method would be impossible, thus water circulation is achieved by

additional ciliary action on the gills (Van As 1997). 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 current created by the movement directs debris and food particles towards the peristomial region and buccal cavity. The particles are then ingested and transported into the infundibulum for absorption and digestion. The Aquatic Parasitology Research Group hypothesises that the abundance and own ciliary activity of the scyphidiid peritrichs most probably play an important role in the countercurrent flow of circulation of oxygenated water over the limpet and haliotid gills. It is also their opinion that the mantoscyphidians enlarge the respiration area of the host, because the scyphidiid peritrichs mostly occur on the margins of the gill filaments and the bodies actually form extensions of the avaliable respiratory surface.

The secondary symbiont *Ellobiophrya maliculiformis* may have a similar association with its mantoscyphidian host, but not being detrimental. It could interfere with the feeding and reproduction if the ellobiophryid is attached around the middle part or the peristomial region of the scyphidiid peritrich's body (Fig. 7.3) (Peters, *et al.* in prep). The Aquatic Parasitology Research Group assumes that the ellobiophryids most probably also play an important role in the circulation of oxygenated water over the haliotid and limpet gills, as in the case of the mantoscyphidians. Up until now low infestations of ellobiophryids were found, but if the infestation levels were to increase, the effect that the ellobiophryids will have on respiration in the mollusc hosts could most probably be more pronounced.

The effect of pollution

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 subtropical east coast of

Senegal (West Africa) and the east coast of South Africa (Basson pers. comm.) [1] have lower infestations of ciliophoran fauna on the marine invertebrates. This might be explained due to the fact 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. Lom and Basson (pers. comm.)[2] have found that the species diversity of the genus *Trichodina* is the highest in temperate marine habitats and that species diversity decrease in arctic, subantarctic, tropical and subtropical regions. Along the east coast of South Africa there is a high diversity of fish hosts, but these hosts had very low trichodinid infestations. This may be attributable to the higher levels of pollution along the subtropical east coast or merely point to the lower trichodinid biodiversity in subtropical waters. This comparison has not been made for the genus *Mantoscyphidia* yet, because not enough species have been studied.

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 Exon 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.

More research is obviously needed to completely understand the specific type of association between the scyphidiid peritrich symbionts and the mollusc hosts. 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,

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*[1] Prof. Linda Basson, Aquatic Parasitology Research Group, Department of Zoology and Entomology, University of the Free State, Bloemfontein, South Africa.

[2] Prof. Jiri Lom, Institute of Parasitology, Academy of Sciences of the Czech Republic, Branisovska 31, 370 05 Ceské Budejovice, Czech Republic.

and to incorporate the data on all the different marine invertebrates and the associated symbionts.

Various points of discussion concerning binary fission, preconjugation fission, telotroch formation and conjugation are presented in this chapter. These subjects follow each other chronologically as the formation of telotrochs in peritrichs for instance, often follows the process of binary fission. The author gives her own conclusions and opinions in this chapter and they are highlighted with a shaded border.

7.2 Binary fission

Mantoscyphidia

1.

Binary fission was mostly encountered in populations of *Mantoscyphidia spadiceae* occurring on *Haliotis spadicea*, that is more often exposed during low tide than *H. midae* or the limpet *Scutellastra barbara*, hosting *M. midae* and *M. branchi* respectively.

Higher infestations of *M. spadiceae* were always found on the host *Haliotis spadicea*, than in the case of *M. midae* associated with *Haliotis midae*. The host, *Haliotis spadicea* occur higher up on the rocky shore than *H. midae* and is sometimes exposed, especially during low tide. During these stressful times the scyphidiid peritrichs may in fact play a more significant role in enlarging the respiration area of *H. spadicea*'s gills. It would thus be an advantage for the host (*H. spadicea*) if the population numbers of *M. spadiceae* increase during this time in order to facilitate more effective respiration. Binary fission will ensure a rapid increase in the numbers of the *M. spadiceae* population. *Haliotis spadicea* occur in rock crevices among red bait and algae and this may possibly contribute more food to their symbionts. Another possibility may be that the high intertidal habitat of *H. spadicea* somehow makes their peritrich populations less vulnerable to whatever physical or biotic factors

cause mortality. A third possibility may be that the high intertidal hosts are simply easier to colonize.

Furthermore, due to the greater number of specimens of *M. spadiceae* present on the gills, there is a greater possibility that these specimens could be observed while they were busy undergoing binary fission. The opposite is true for *M. branchi*, where the process of conjugation was mostly encountered in the populations studied.

2.

Binary fission also occurred earlier in *M. spadiceae* populations (from three hours after collection of hosts) than in *M. branchi* (six hours) and *M. midae* (eight hours) populations.

As was mentioned previously it would be an advantage for the host (*H. spadicea*) if the population numbers of *M. spadiceae* increase quickly during the time of exposure (low tide) in order to facilitate more effective respiration. A probable explanation for the earlier occurrence of binary fission is that *Mantoscypidia spadiceae* is adapted to start dividing quicker than the other species, when the hosts are exposed during low tide or sping tide. The time of exposure between a high- and low tide is usually about 3 hours. Perhaps reproduction at low tide favours colonization of the correct host. At this time, the host's palial cavity is essentially a "closed system". Forming telotrochs during this time might be especially advantageous because it would prevent them from being swept away by strong wave action to areas where ther are no suitable hosts.

3.

Comparison of plump, completely contracted and live specimens:

Plump individuals are in a stage of reproduction and differ from contracted and fully extended vegetative individuals. Plump individuals are contracted with the peristome tightly closed with no adoral cilia protruding, and the peritrich ceases all feeding

activities. The peristomial region is usually elevated to form a knob-like protrusion. **Completely contracted specimens** are vegetative individuals that are contracted into a very short form, but these individuals are not preparing to undergo binary fission. **Fully extended specimens** are also vegetative, they have expanded peristomial regions with protruding adoral cilia and the bodies are extended to the maximum obtainable length.

The body length and body diameter of plump specimens preparing to undergo binary fission, completely contracted vegetative specimens, and live fully extended vegetative specimens were compared (see Table 7.1 & Table 7.2). Some of this information has already been given and comparisons were made in Chapter 4. In this chapter, however, additional information is given and will be compared to the previous information in order to present a more meaningful discussion.

Table 7.1: Body length (µm) of plump specimens preparing to undergo binary fission, completely contracted vegetative specimens and fully extended, live specimens of *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 and *M. branchi* Van As, Basson & Van As, 1998. (Van As 1997; Botes; Basson & Van As 2001a).

	M. spadiceae	***M. branchi***
Plump specimens	18.3-37.0 (27.2; 7)	21.7-32.2 (25.9±4.1; 11)
Completely contracted specimens	49.0-60.0 (53.0±4.7; 11)	20.0-35.0 (28.0±4.1; 17)
Fully extended, live specimens	70.0-140.0 (104.3±; 43)	44.0-78.0 (65.0±8.8; 14)

Table 7.2: Body diameter (µm) of plump specimens preparing to undergo binary fission, completely contracted vegetative specimens and fully extended, live specimens of *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 and *M. branchi* Van As, Basson & Van As, 1998. (Van As 1997; Botes; Basson & Van As 2001).

	M. spadiceae	***M. branchi***
Plump specimens	20.0-36.7 (30.1; 7)	23.3-44.9 (33.6±7.1; 11)
Completely contracted specimens	25.0-43.0(34.3±4.8; 11)	19.0-30.0 (21.1±2.5; 17)
Fully extended, live specimens	20.0-40.0 (31.2±6.7; 43)	17.0-32.0 (24.2±4.4; 14)

Plump individuals on the verge of binary fission were compared with live and hematoxylin stained specimens in Chapter 4 and it was concluded that plump

specimens had a smaller body length than live and hematoxylin stained vegetative specimens. Van As (1997) and Botes, *et al.* (2001a) also measured the body dimensions of completely contracted specimens of populations of *M. branchi* and *M. spadiceae*.

Plump specimens of *M. spadiceae* and *M. branchi* had a smaller average body length (27.2 µm and 25.9 µm) than completely contracted specimens (53.0 µm and 28.0 µm). The difference was greater in the case of *M. spadiceae* (plump individuals are 25.8 µm shorter than completely contracted specimens) because fully extended specimens of *M. spadiceae* had a greater body length. Plump individuals of *M. branchi* were only 2.1 µm shorter than completely contracted specimens (Table 7.1).

In the case of body diameter, plump individuals of *M. spadiceae* had a smaller average body diameter (30.1 µm), compared to 34.3 µm in completely contracted specimens. Plump individuals of *M. branchi*, however, had an average body diameter of 33.6 µm compared to 21.1 µm in completely contracted specimens (Table 7.2).

Specimens of *Mantoscyphidia spadiceae* and *M. branchi* preparing to undergo binary fission contract to a shorter body length (and wider in the case of *M. branchi*) than normal vegetative individuals, suggesting a higher degree of contraction in the entire body. This contraction is longer in duration than that of normally contracted specimens because the process of binary fission needs a period of time to be completed. Vegetative individuals that are not in a stage of reproduction are able to expand and contract within seconds.

The scyphidiid peritrichs most probably need to build up energy reserves before binary fission for the time during fission when they are unable to feed. Binary fission influences normal physiological processes and metabolism and may even slow them down until the two daughter individuals have acquired new infraciliature and start to feed again. This might explain why individuals preparing to undergo binary fission have a different shape than nondividing individuals.

Another explanation for the plump appearance in mantoscyphidians preparing to undergo binary fission might be the influence of the movement of the nuclear material inside the body. The macro and micronuclei move from the vegetative positions to an adoral position, and the macronucleus elongates and stretches across the whole width of the peritrich's body. This could also cause the peritrich's body diameter to increase and to become plumper. Furthermore, the amount of symbiotic algae that occur in the scyphidiid peritrichs may also influence the appearance of the peritrichs. Vegetative individuals as well as individuals preparing to divide may be plumper if they contain more algal cells. Another possible cause may be the movement of new buccal structures after their duplication.

Further study on this aspect needs to be done in order to conclude what exactly causes the increase in body diameter of a scyphidiid peritrich preparing to undergo binary fission.

4.

Does binary fission play an important role in the sexual reproductive process of scyphidiid peritrichs?

Colwin (1944) observed in *Urceolaria synaptae* that fission is occasionally unequal, producing daughters of slightly different, or rarely very different, sizes. The possibility exists that these daughters could just have been unequal sized products of binary fission that will grow into mature adults of more or less the same size, but it may also have been observations of preconjugation fission, which is a modified process of binary fission. Padnos and Nigrelli (1942) also observed that the conjugants of *Trichodina spheroidesi* are always unequal in size, and although they did not state it in their descriptions, it can be interpreted that preconjugation fission is responsible for this conjugant differentiation.

Binary fission is regarded as an asexual reproductive process, but in various peritrich species (e.g. *Rhabdostyla vernalis*, *Vorticella microstoma*, *Lagenophrys tattersali* and *Scyphidia tholiformis*) it precedes conjugation (preconjugation fission – a specialised type of binary fission) and conjugation is also concluded by a series of binary fissions (ordinary fissions) to form daughter individuals. Binary fission produces daughter

individuals with equal amounts of macro- and micronuclear material and endoplasm, whilst preconjugation fission produces daughter individuals with equal amounts of micronuclear material, but unequal amounts of macronuclear material and endoplasm. Preconjugation fission often yields four microconjugants (through two successive binary fissions) and a macroconjugant, whilst ordinary binary fission produces two daughter individuals.

If binary fission is the only reproductive process occurring in a population of scyphidiid peritrichs, no nuclear material would ever be exchanged between individuals, and this would lead to a weakening of the population's genetic material. Conjugation ensures that this genetic exchange does take place.

Binary fission forms an important part of the sexual process of reproduction in scyphidiid peritrichs, although it is regarded as the asexual component thereof. My opinion is that binary fission does not exist as a process on its own, because it mostly precedes and/or follows sexual reproduction. The reproductive process in peritrichs does not begin with binary fission and end with conjugation. It is my opinion that binary fission precedes conjugation and that binary fission follows conjugation again. Asexual reproduction (binary fission) as such does not exist as a process on its own. If asexual reproduction did exist as a process on its own, no exchange of genetic material would ever take place between the individuals in the population and this would lead to a decrease in both the reproductive fitness and vitality of the population.

Ellobiophrya maliculiformis

1.

Binary fission was observed in *E. maliculiformis* and compared to the asexual reproductive processes of *E. brevipes* (Clamp & Bradbury 1997), *E. conviva* (Clamp 1982) and *E. donacis* (Chatton & Lwoff 1923, 1828, 1929).

The presence of symbiotic algae obscured most of the internal organelles, and the exact process of binary fission could not be followed in detail (see Future studies in

Discussion). The macro-and micronuclear divisions could be observed, however, as well as the progression of the cleavage furrow along the plane of fission. Cleavage produces two unequal products of division: the parent retaining the cinctum is larger than the other daughter individual which eventually becomes the free swimming telotroch stage. Chatton and Lwoff (1923, 1929) also observed this for *E. donacis* (Fig. 7.1).

The presence of a telotroch attached to the embryophore of the daughter retaining the cinctum was observed quite often during scanning electron microscopy of fixed specimens of *E. maliculiformis* (Fig. 7.2). The larval stalk, which attaches this telotroch to the embryophore, appears to be smaller than in *E. brevipes*, *E. conviva* and *E. donacis*. As in the case of *E. conviva*, it is often difficult to see the larval stalk of *E. maliculiformis*, because most of its length is buried within the embryophore of the parent.

In order to study details of the infundibulum and other internal organelles Bouin's fixed smears were stained with hematoxylin and Protargol, but these methods proved rather unsuccessful, as the ellobiophryids have many symbiotic algae and inclusions, which obscures the position of the infraciliature and internal organelles. A method needs to be developed for removing the symbiotic algae *from E. maliculiformis* so that the internal organelles and especially the nuclear phenomena of binary fission can be studied in detail. Recently Tanaka, Muraka-Hori, Kadono, Yamada, Kawano, Kosaka and Hosoya (2002) developed a method to eliminate endosymbiotic algae from *Paramecium bursaria* by treatment with a herbicide, paraquat. This method of using a herbicide to remove symbiotic algae could prove useful in future studies of the internal structures of *E. maliculiformis* and *Mantoscyphidia* species.

7.3 Telotroch formation

The telotroch stage is regarded as the temporary, migratory larval stage of scyphidiid peritrichs, but this stage is also the evolutionary precursor of the permanent "larval" stage of the mobiline peritrichs.

Mantoscyphidia

1.

Live observations of telotroch stages:

Live observations of telotroch stages were difficult, because telotrochs lose vitality before settling on a new substrate. Telotrochs could only be observed for a short time before they would die. During live observations wet smears had to be made and the specimens were removed from the gill tissue. Telotrochs swam vigorously once liberated, but no suitable substrate for attachment was available. Cultivation of marine species is difficult, whereas the reproductive processes of freshwater peritrich species are easily studied in cultured mediums (more details follow, see 7.4.1).

In order to make direct observations of telotrochs settling on a new host substrate possible, a method for culturing these species on the host tissue is essential. The reproductive processes of these scyphidiid peritrichs can then be studied in future with the aid of a light microscope.

2.

Studying the transformation of telotrochs:

During transformation of the mature vegetative scyphidiid peritrich into a telotroch stage and vice versa, it is difficult to conclude whether telotrochs are actually being formed or whether transformation of telotrochs into vegetative individuals is taking place. This can be attributed to the fact that the one process is a reversal of the other.

In Chapter 5 (Fig. 5.1) scanning electron micrographs of the formation of telotrochs and the subsequent settling on a new host substrate are presented. These stages were arranged in the order in which I reconstructed telotroch formation and settling based on information from the literature (Stein 1851, Surber 1943, Davis 1947, Thompson, *et al.* 1947, Dobrańska 1961 & Vávra 1961), but the actual living process was not observed. The exact process is difficult to describe from fixed material and the living telotrochs observed on wet smears died quickly.

The formation and settling of telotrochs is a process that happens rather quickly, so it would be possible to study this process in detail if a suitable medium could be cultured in future in which these scyphidiid peritrichs could survive (see 7.4.1).

3.

Does the telotroch develop before or after binary fission has taken place?

In some scyphidiid peritrich populations binary fission and telotrochs were observed simultaneously (Tables 4.1 - 4.3), but binary fission and telotroch formation also occurred independently of each another. During scanning electron microscopy studies telotrochs were frequently seen attached to the substrate right next to each other. These specimens probably had undergone binary fission and then immediately developed into telotrochs, judging by the close proximity of the telotrochs that were on the verge of detaching to locate a new substrate.

Throughout this whole study it was uncertain whether binary fission always has to occur before a telotroch can develop, or whether binary fission follows telotroch formation. It would be possible to study this process in detail if a suitable medium could be cultured in future (see 7.4.1).

Ellobiophrya maliculiformis

1.

Differences between mantoscyphidian and ellobiophryid telotrochs:

Newly liberated ellobiophryid telotrochs had narrower and thinner scopular regions than mantoscyphidian telotrochs. There is a very quick formation of the cinctal limbs during re-attachment of the telotroch stage to the new host.

When the fully formed ellobiophryid telotrochs are liberated they are released from the embryophore as soon as the larval stalk separates from the embryophore. These telotrochs were observed to have narrow, off centre and thin scopular regions (Figs. 5.2 D; Figs. 5.3 A - C). The telotroch will attach again to a new suitable host and quick formation of the cinctal limbs will take place. This was never observed in *E. maliculiformis* and it is assumed that this process happens very quickly, otherwise the telotroch will lose its attachment to the host.

Chatton and Lwoff (1929) observed telotroch stages of *E. donacis* without larval stalks and noted that the larval stalk often remains behind in the embryophore which will reject it after a while by retraction of the embryophore. They did, however, also see telotrochs with larval stalks, but these were abandoned quickly, because they would otherwise prevent the telotrochs from attaching to the new host. Larval stalks were never observed in liberated telotrochs of *E. maliculiformis*.

2.

Cinctum action:

Can the cinctum of *Ellobiophrya maliculiformis* open after initial attachment and can the cinctum open and close during attachment to adjust its grip around its scyphidiid peritrich host?

No evidence was found to indicate that *E. maliculiformis* is able to open and close its cinctum. Detached specimens were frequently observed during scanning electron microscopy, with cinctums open and cinctal limbs unlocked as well as detached specimens with closed cinctums.

In the case of detached specimens with open cinctums, the loosening could possibly have occurred during fixing and processing of specimens. Ellobiophryids could have detached from the scyphidiid peritrich hosts that died during the fixation process. Specimens with closed cinctums could possibly have "lost" the scyphidiid peritrich hosts when the scyphidiid peritrichs died naturally. It is assumed that detached ellobiophryids could possibly have stayed alive for a while after detachment, but the ellobiophryids won't survive for long without a host. The ellobiophryids are not able to attach to the gill epithelium.

According to Chatton and Lwoff (1929) *E. donacis* is also not able to free itself once it is attached, but they state that the aboral zone with the cinctum can be resorbed and formed again. If *E. maliculiformis* can be studied over a longer period of time in a cultured medium, it can be determined whether or not resorption takes place in this species (see 7.4.1).

Ellobiophrya maliculiformis may possibly be able to adjust its grip around the scyphidiid peritrich hosts, because specimens were observed attached around the normal attachment site (around the host's scopula), between the macro- and micronucleus of the host (Figs. 7.3 A & B), around the host's peristomial region (Figs. 7.3 B & C) and around the middle part of the host's bodies (Figs. 5.4 F & G). Ellobiophryids were even attached around scyphidiid peritrichs that were in the process of binary fission.

These attachment sites of ellobiophryids around scyphidiid peritrichs all vary in diameter, thus *E. maliculiformis* must be able to adjust its grip. *Ellobiophrya maliculiformis* has a myoneme in its cinctum and the presence of a myoneme implies contractility. In my opinion *E. maliculiformis*

can not detach itself at will, but it is able to adjust its grip around its scyphidiid peritrich host. When an ellobiophryid is attached around more than one scyphidiid peritrich (Fig. 5.4G) the cinctum has to be able increase its diameter to accommodate the scyphidiid peritrichs in its grip. Circumstantial evidence suggests that *E. maliculiformis* can increase or decrease the diameter of its cinctum at will.

3.

The relationship of multiple ellobiophryids attached around a single scyphidiid peritrich host:

Up to four individuals of *Ellobiophrya maliculiformis* were observed attached around a single scyphidiid peritrich host. Two or three ellobiophryids were very often found associated with a single scyphidiid peritrich (Fig. 5.4G). Are these ellobiophryids related to one another? (in other words, were they produced by the same parent?).

Chatton and Lwoff (1929) observed that a hardened scar forms around the embryophore after the larval stalk of the telotroch is ejected, and that this scar persists even when the embryophore is completely retracted. They observed many specimens of *Ellobiophrya donacis* with two scars. The scars were always next to one another, the one fresh and the other old and fading. They concluded *that E. donacis* might produce at least two telotrochs and probably more, since the scar fades away with time.

In *E. maliculiformis* no more than one embryophore with its surrounding scar tissue was observed in a single individual. These structures could have faded in *E. maliculiformis*, or the embryophore may have the ability to accept more than one larval stalk during development and growth of telotroch stages.

In my opinion there may be two explanations: Firstly, one ellobiophryid could have undergone three consecutive binary fissions, producing three daughter individuals. The possibility even

exists that one or all of these daughter individuals might have undergone binary fission, depending on how fast they reach maturity.

Secondly, four separate, unrelated ellobiophryids could have selected the same scyphidiid peritrich host for initial attachment, although this seems very unlikely. *Mantoscyphidia spadiceae*, *M. midae* and *M. branchi* populations had prevalences of 35.4 %, 34.4 % and 17 % respectively of *E. maliculiformis* associated with the scyphidiid peritrichs (Peters, *et al.* in prep). There is a much higher prevalence of scyphidiid peritrichs on the mollusc hosts and the scyphidiid peritrichs occur in much higher numbers on the hosts than the ellobiophryids. Van As (1997) has calculated this value to be more than 2000 individuals *of M. branchi* per 10 mm of gill tissue. From these statistics the conclusion can be made that the probability of more than one ellobiophryid attaching to the same scyphidiid peritrich is very small.

It is my opinion that in the cases where more than one ellobiophryid are attached to a scyphidiid peritrich, these ellobiophryids are probably related to one another.

7.4 Conjugation

Mantoscyphidia

1.

Conjugation was mostly encountered in populations of *Mantoscyphidia branchi* and it is assumed that conjugation occurred when scyphidiid peritrichs reacted to the host's stress.

Throughout the study period conjugation was most frequently seen in populations of *M. branchi* (Tables 1 – 4, Appendix A). Although conjugation occurs normally in any given population, more conjugating pairs were seen if the host was dissected six hours or more after collection. Conjugation yields more daughter individuals than

binary fission, and could most likely lead to an increase in the population numbers, which in turn will increase the species chance for survival. The process of conjugation, however, requires more time (up to 29 hours) to be completed than binary fission and the process may also be more energy consuming.

Freshwater peritrich species may easily be activated to undergo preconjugation fissions and conjugation. Finley and Nicholas (1950) for instance took old cultures containing sparse animated populations and a few encysted *Rhabdostyla* and activated preconjugation fissions by decanting the old culture medium and replacing it with three changes of distilled water. They then replaced the water with a sterile culture medium and distilled water. Marine organisms are used to a variety of fluctuating physical factors in the intertidal zone as mentioned at the beginning of this discussion and the marine peritrich species associated with them should not react in the same way as freshwater peritrich species. If a culture medium could be developed for marine peritrichs the reproductive processes might be studied easier, but the stimuli for inducing reproduction might not be as simple as it is for freshwater species.

Botha (1994) tried to induce stressful conditions in the topshells of the genus *Oxystele*, which hosts the scyphidiid peritrich *Mantoscyphidia fanthami*, by placing these molluscs in the sun for hours, but no stressful conditions or stimuli to which the hosts were exposed to could successfully activate reproduction in this scyphidiid peritrich species. *Oxystele* species have remarkable adaptations (such as the presence of an operculum) to prevent desiccation and this might imply that *M. fanthami* do not react to the stress of its host.

As mentioned previously, the scyphidiid peritrichs do not stress as soon as there are fluctuations in the external environment. The mollusc host will react to the physical stresses, and then the scyphidiid peritrichs will react to the stress of the host. The peritrichs may then undergo reproduction to ensure survival, depending on the specific association between the host and the symbiotic scyphidiid peritrichs.

The limpet, *S. barbara* is more exposed to external fluctuations than *Oxystele* species, because it has a open shell and does not have an operculum that protects the gills from the environment. The limpet's gills are exposed and *M. branchi* may react more quickly to the stress of the host than *M. fanthami.* Removal of a limpet from its habitat will be sensed immediately, while an *Oxystele* species can just retract into its shell and close its operculum. Conjugation and other reproductive processes may therefore be more easily induced in *M. branchi. Haliotis* species also have an open shell and no operculum, but the gills are partially protected from the external environment in the mantle cavity. Conjugation was observed in scyphidiid peritrichs associated with haliotids, but no to the same extent as in *S. barbara*.

Populations of *M. branchi* found on the gills of the limpet host *Scutellastra barbara* had the highest occurrence of conjugating scyphidiid peritrichs. This limpet mostly occurs submerged in tidal pools, but may be exposed during spring or low tides.

During the limpet's stressful time of exposure the scyphidiid peritrichs may react indirectly or directly to the stress of the host and may reproduce through conjugation, because this might increase the vitality and population numbers. Six hours after collection of the hosts, the incidence of conjugation increased, and this may possibly be because the limpet host is not usually exposed for more than six hours during low tides. I assume that this increase in the number of scyphidiid peritrichs might benefit the limpet host, because higher numbers of scyphidiid peritrichs most probably facilitate respiration in the limpet.

Conjugation was observed more frequently than binary fission in *M. branchi*, perhaps because binary fission as well as the specialized type of binary fission called preconjugation fission, requires less time for completion than conjugation itself. See Chapter 4 and Chapter 6 for time estimates of binary fission and conjugation.

Ellobiophrya maliculiformis

1.

Conjugation has never been observed in any other species of *Ellobiophrya.*

Clamp and Bradbury (1997) did see a few specimens of *E. brevipes* where the macronucleus appeared to be broken apart into vesicles, and to them, these possibly looked like exconjugants (a macroconjugant in the very earliest period after contact with a microconjugant). They concluded that these observations could also have been early stages of autogamy to be followed by binary fission, based on the appearance of the peristomial sphincter and oral infraciliature (appearance of impending division), macronuclear fragmentation and the absence of microconjugants. In autogamy, all the nuclear phenomena takes place in one partner, the fusing pronuclei are the products of division of only one micronucleus, in other words no exchange of genetic material takes place.

In *Ellobiophrya maliculiformis* conjugation was observed. This is the first record of conjugation in the genus *Ellobiophrya*. Ellobiophryids with attached microconjugants were observed during live observations, in hematoxylin stained specimens and were also photographed using scanning electron microscopy. The process of conjugation was not studied in this species, because it was only observed a few times and was not part of the initial objectives of this study.

In the future this process needs to be studied, as it is the only species of *Ellobiophrya* in which conjugation has ever been observed, and it would greatly increase our knowledge on ellobiophryid reproduction. It is strange that telotroch formation and binary fission have been described for other ellobiophryid species, as they occur as primary symbionts on the hosts, yet there has not been any observations of conjugation in the genus *Ellobiophrya*.

Ellobiophrya maliculiformis is a secondary symbiont, and it seemed to be a more delicate or sensitive species than primary symbionts (such as all the other *Ellobiophrya* species). Throughout this study scanning elelctron micrographs revealed that the pellicle of *E. maliculiformis* was always damaged more than the pellicles of the *Mantoscyphidia* species. Very few fully extended specimens of *E. maliculiformis* were also viewed with SEM, because the ellobiopryids contracted more easily during fixation and standard microscopy procedures.

It seems as though *E. maliculiformis* is stressed more easily and that might be why conjugation did occur, but these observations of conjugation could just have been normal occurrences of reproduction in the population. The process of conjugation in *E. maliculiformis* did not form part of the initial objectives of this study and for future purposes it is a whole study on its own.

7.5 FUTURE STUDIES

- During the final stages of the completion of this thesis new information has been published on the use of a herbicide treatment that can remove symbiotic algae from *Paramecium* species. Tanaka *et al.* (2002) developed a method to eliminate endosymbiotic algae from *Paramecium bursaria* by treatment with a herbicide, paraquat. This method of using a herbicide to remove symbiotic algae could prove useful in future studies of the internal structures of *E. maliculiformis* and *Mantoscyphidia* species. This will ensure that detailed studies on the internal organelles and reproductive processes can be performed in future.

- A suitable medium needs to be developed in which scyphidiid peritrichs and ellobiophryids can be cultured in order to study all the reproductive processes in detail.
    - ➢ It would be a great advantage if the peritrichs could be studied alive, because the prevalence of the different reproductive processes could then be determined.
    - ➢ Another important contribution could be to investigate if binary fission does occur alone in a population or together with sexual reproduction,
    - ➢ whether telotroch formation and the transformation of telotrochs into the settled form are exact reversed processes,

- whether preconjugation fission occurs in *Mantoscyphidia* and *Ellobiophrya* species and
- which reproductive processes of the symbionts occur during different types of external stimuli to the host and its symbionts.

- New methods of how to stress intertidal organisms, such as the limpet and haliotid hosts need to be investigated in order to determine if they stimulate certain reproductive processes.

- The possible use of transmission electron microscopy for ultrastructural studies in order to elucidate internal structures such as the nuclei.

- The possible use of new technology such as confocal laser scanning microscopy (CLSM) which allows three-dimensional imaging of fully hydrated, living, thick, procaryotic and eukaryotic organisms. This technique has a large potential to image protozoans stained by fluorescent compounds (Packroff, Lawrence and Neu 2002).

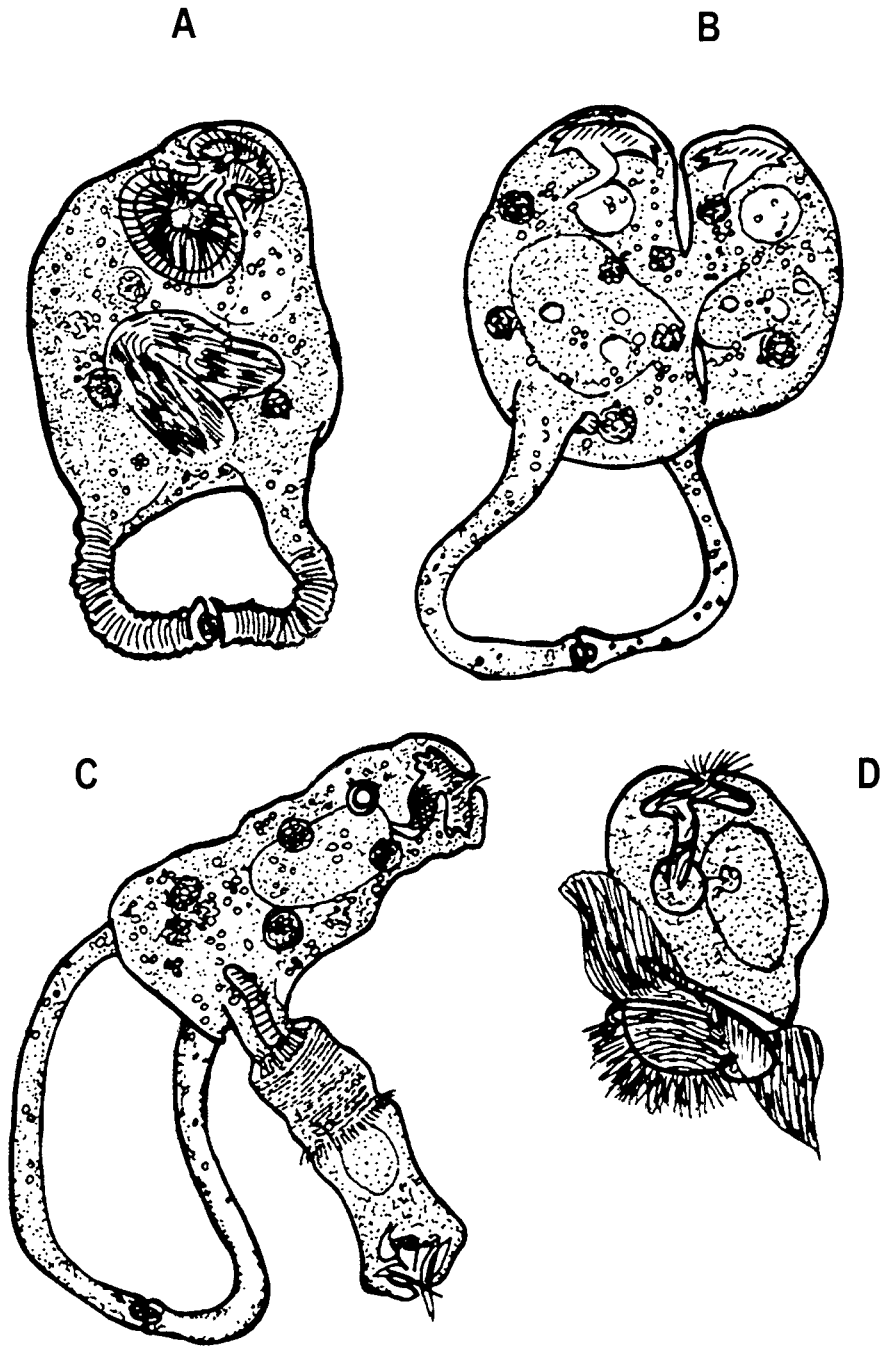

Fig. 7.1 Reproduction in *Ellobiophrya donacis* Chatton & Lwoff, 1923. **A** - Start of division. Constriction of the peristome and lengthening of the macronucleus. **B** - Protoplasmic cleavage. The protoplasm of the pseudo-bud contains digestive vacuoles. **C** - Pseudo-bud having eliminated its digestive vacuoles. Protoplasm finely granulated. Start of development of the aboral zone. The stalk is well developed. **D** - Free embyo. Note the striation and cilliation of the posterior end. Redrawn from Chatton and Lwoff (1928).

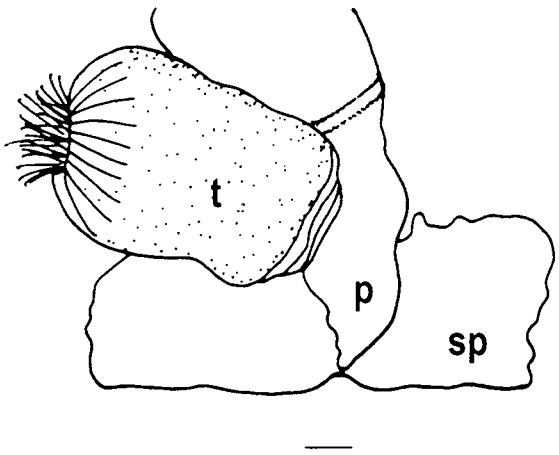

Fig. 7.2 Telotroch attached to the parent individual of *Ellobiophrya maliculifomis* Peters, Basson, Van As & Van As, in press.

t = telotroch, **p** = parent, **sp** = scyphidiid peritrich.

Scale bar: 10 µm.

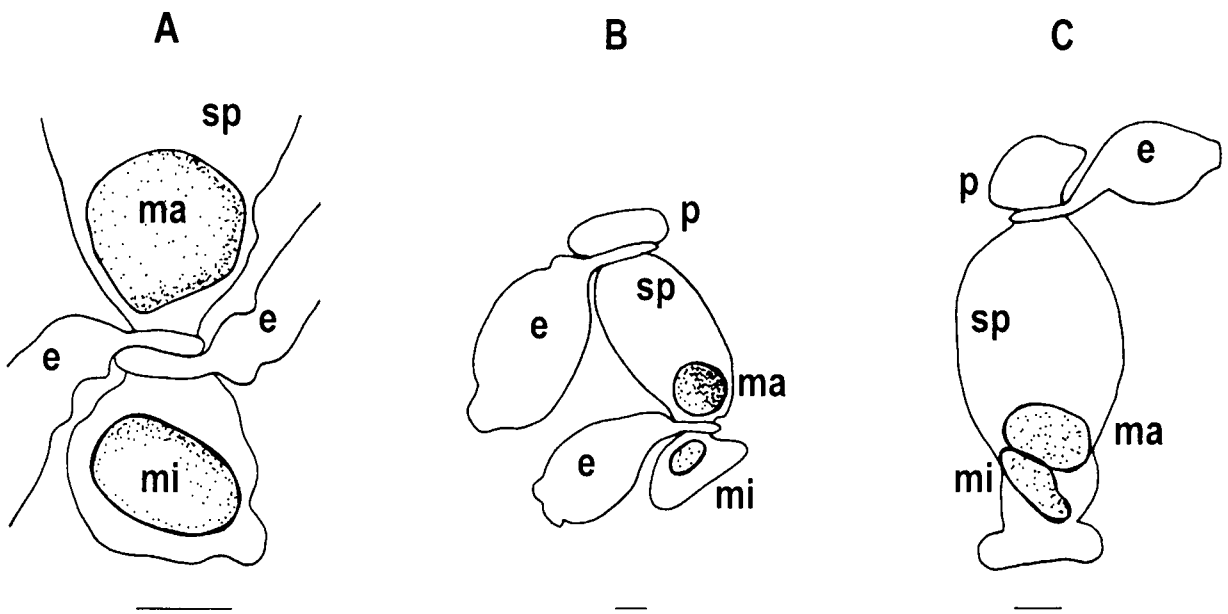

Fig. 7.3 Various attachment sites of *Ellobiophrya maliculiformis* Peters, Basson, Van As & Van As, in press around its scyphidiid peritrich hosts. **A** - Two ellobiophryids attached between the macro- and micronucleus of a scyphidiid peritrich. **B** - One ellobiophryid attached between the macro- and micronucleus and one attached around the peristomial region of a scyphidiid peritrich. **C** - One ellobiophryid attached around the peristomial region of a scyphidiid peritrich.

e = ellobiophryid, **ma** = macronucleus, **mi** = micronucleus, **p** = peristome, **sp** = scyphidiid peritrich.

Scale bars: 10 µm.

Chapter 8
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* Articles not seen in the original form

Appendix A

Tables/Raw data

Table 1: Data from April-May 1996. Limpets collected at Boulder Beach, Marion Island: *Nacella delesserti* Phillips, 1849 indicating the presence of *Mantoscyphidia marioni* Van As, Basson & Van As, 1998 and the occurrence of the secondary symbiont *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep; binary fission; conjugation and telotrochs. M = *Mantoscyphidia*, E = *Ellobiophrya*.

Specimen number	Host	*Mantoscyphidia*	*Ellobiophrya*	Binary fission	Conjugation	Telotrochs
1996/04/31-03	*N. delesserti*	*M. marioni*	-	-	-	-
1996/04/31-04	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/02-01,02	*N. delesserti*	*M. marioni*	-	-	-	✔
1996/05/02-03	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/02-04	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/02-05-10	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/02-11	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/02-12	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/02-13	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/02-14	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/02-15-17	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/02-18-21	*N. delesserti*	*M. marioni*	-	✔	-	-
1996/05/02-24	*N. delesserti*	*M. marioni*	-	-	-	✔
1996/05/02-24b	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/02-25	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/02-26-29	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/05-06	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/05-09,10	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/07-07	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/07-18-25	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/07-26-32	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/07-33-38	*N. delesserti*	*M. marioni*	-	-	-	✔
1996/05/08-04	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/08-05	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/08-06	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/08-07	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/08-08	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/08-09	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/08-10	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/08-11	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/08-12	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/08-13	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/08-14	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/08-16	*N. delesserti*	*M. marioni*	-	✔	-	-
1996/05/09-04-07	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/09-08	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/09-09-11	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/11-01	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/11-02	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/11-03,04	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/12-22	*N. delesserti*	*M. marioni*	-	-	-	-
1996/05/12-23	*N. delesserti*	*M. marioni*	-	-	-	-

Table 2: Data from March-April 1997. Haliotids collected at the De Hoop Nature Reserve on the south coast of South Africa: *Haliotis midae* Linnaeus, 1758 and *Haliotis spadicea* Donovan, 1808, indicating the presence of *Mantoscyphidia midae* Botes, Basson & Van As, 2001; *M. spadiceae* Botes, Basson & Van As, 2001 and the occurrence of the secondary symbiont *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep; binary fission; conjugation and telotrochs. M = *Mantoscyphidia*, E = *Ellobiophrya*.

Specimen number	Host	*Mantoscyphidia*	*Ellobiophrya*	Binary fission	Conjugation	Telotrochs
1997/03/29-01	*H. midae*	*M. midae*	✔	-	-	-
1997/03/29-02	*H. midae*	*M. midae*	-	-	-	-
1997/03/29-05	*H. midae*	*M. midae*	✔	-	-	-
1997/03/29-06	*H. midae*	*M. midae*	-	-	-	-
1997/03/29-07	*H. midae*	*M. midae*	-	-	-	-
1997/03/29-08	*H. midae*	*M. midae*	-	-	-	-
1997/03/29-09	*H. midae*	*M. midae*	✔	-	-	-
1997/03/30-02	*H. midae*	uninfected	-	-	-	-
1997/04/01-07	*H. midae*	uninfected	-	-	-	-
1997/04/01-08	*H. midae*	uninfected	-	-	-	-
1997/04/04-03	*H. midae*	uninfected	-	-	-	-
1997/04/05-05	*H. midae*	*M. midae*	-	-	-	-
1997/04/05-09	*H. midae*	*M. midae*	✔	-	-	-
1997/04/07-14	*H. midae*	*M. midae*	-	-	-	-
1997/04/07-24	*H. midae*	uninfected	-	-	-	-
1997/04/08-01	*H. midae*	uninfected	-	-	-	-
1997/04/10-01	*H. midae*	*M. midae*	-	-	-	-
1997/03/29-03	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/03/29-04	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/03/30-01	*H. spadicea*	uninfected	-	-	-	-
1997/04/01-01	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/01-02	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/01-03	*H. spadicea*	*M. spadiceae*	-	✔ M	-	-
1997/04/01-04	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/01-05	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/01-06	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/04-01	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1997/04/04-02	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/05-01	*H. spadicea*	*M. spadiceae*	✔	✔ E	-	-
1997/04/05-02	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1997/04/05-03	*H. spadicea*	uninfected	-	-	-	-
1997/04/05-04	*H. spadicea*	*M. spadiceae*	✔	-	✔ E	✔ E
1997/04/05-06	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1997/04/05-07	*H. spadicea*	*M. spadiceae*	✔	✔ M	✔ E	-
1997/04/05-08	*H. spadicea*	*M. spadiceae*	✔	✔ E	-	-
1997/04/06-01	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1997/04/07-01	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1997/04/07-02	*H. spadicea*	*M. spadiceae*	✔	✔ M	-	-
1997/04/07-03	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/07-04	*H. spadicea*	*M. spadiceae*	-	✔ M	-	-
1997/04/07-05	*H. spadicea*	*M. spadiceae*	-	✔ M	-	✔ M
1997/04/07-06	*H. spadicea*	*M. spadiceae*	-	-	-	-

Table 2 continued: Data from March-April 1997. Haliotids collected at the De Hoop Nature Reserve on the south coast of South Africa: *Haliotis midae* Linnaeus, 1758 and *Haliotis spadicea* Donovan, 1808, indicating the presence of *Mantoscyphidia midae* Botes, Basson & Van As, 2001; *M. spadiceae* Botes, Basson & Van As, 2001 and the occurrence of the secondary symbiont *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep; binary fission; conjugation and telotrochs.

Specimen number	Host	*Mantoscyphidia*	*Ellobiophrya*	Binary fission	Conjugation	Telotrochs
1997/04/07-07	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/07-08	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/07-09	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/07-10	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/07-11	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/07-12	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/07-13	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/07-15	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/07-16	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1997/04/07-17	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1997/04/07-18	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/07-19	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/07-20	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/07-21	*H. spadicea*	*M. spadiceae*	-	✔ M	-	-
1997/04/07-22	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/07-23	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/07-25	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/07-26	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/07-27	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/08-02	*H. spadicea*	uninfected	-	-	-	-
1997/04/10-02	*H. spadicea*	*M. spadiceae*	✔	-	-	✔ M
1997/04/10-03	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1997/04/10-04	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1997/04/10-05	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1997/04/10-06	*H. spadicea*	*M. spadiceae*	-	-	-	-
1997/04/10-07	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1997/04/10-08	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1997/04/10-09	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1997/04/10-10	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1997/04/10-11	*H. spadicea*	*M. spadiceae*	-	-	-	-

Table 3: Data from March-April 1998. Haliotids collected at the De Hoop Nature Reserve on the south coast of South Africa: *Haliotis midae* Linnaeus, 1758 and *Haliotis spadicea* Donovan, 1808, indicating the presence of *Mantoscyphidia midae* Botes, Basson & Van As, 2001; *M. spadiceae* Botes, Basson & Van As, 2001 and the occurrence of the secondary symbiont *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep; binary fission; conjugation and telotrochs. M = *Mantoscyphidia*, E = *Ellobiophrya*.

Specimen number	Host	*Mantoscyphidia*	*Ellobiophrya*	Binary fission	Conjugation	Telotrochs
1998/03/28-10	*H. midae*	*M. midae*	-	-	-	-
1998/03/29-01	*H. midae*	*M. midae*	-	-	-	-
1998/03/29-02	*H. midae*	*M. midae*	-	-	-	-
1998/03/29-03	*H. midae*	*M. midae*	-	-	-	-
1998/03/28-04	*H. midae*	*M. midae*	-	-	-	-
1998/03/30-11	*H. midae*	*M. midae*	-	-	-	-
1998/03/30-12	*H. midae*	*M. midae*	-	-	-	-
1998/04/01-01	*H. midae*	*M. midae*	✔	-	-	-
1998/04/01-02	*H. midae*	*M. midae*	✔	-	-	-
1998/04/01-03	*H. midae*	*M. midae*	-	-	-	-
1998/04/01-04	*H. midae*	*M. midae*	-	-	-	-
1998/04/01-05	*H. midae*	*M. midae*	-	-	-	-
1998/04/01-06	*H. midae*	uninfected	-	-	-	-
1998/04/04-01	*H. midae*	*M. midae*	✔	-	-	-
1998/04/04-02	*H. midae*	*M. midae*	-	-	-	✔ M
1998/04/04-03	*H. midae*	*M. midae*	✔	-	-	-
1998/04/04-04	*H. midae*	*M. midae*	✔	-	-	-
1998/04/04-05	*H. midae*	*M. midae*	✔	-	-	-
1998/04/04-06	*H. midae*	*M. midae*	✔	-	✔ M	-
1998/04/04-07	*H. midae*	*M. midae*	-	-	-	-
1998/04/04-08	*H. midae*	*M. midae*	✔	-	-	-
1998/04/04-09	*H. midae*	*M. midae*	-	-	-	-
1998/04/04-10	*H. midae*	*M. midae*	-	-	-	-
1998/04/04-11	*H. midae*	uninfected	-	-	-	-
1998/04/04-12	*H. midae*	*M. midae*	-	-	-	-
1998/04/06-01	*H. midae*	*M. midae*	-	-	-	-
1998/04/06-02	*H. midae*	*M. midae*	-	-	-	-
1998/04/06-03	*H. midae*	*M. midae*	-	-	-	-
1998/04/06-04	*H. midae*	*M. midae*	✔	-	-	-
1998/04/06-05	*H. midae*	*M. midae*	-	-	-	-
1998/04/06-06	*H. midae*	*M. midae*	✔	-	-	-
1998/04/06-07	*H. midae*	*M. midae*	-	-	-	-
1998/04/06-08	*H. midae*	*M. midae*	-	-	-	-
1998/04/06-09	*H. midae*	*M. midae*	-	-	-	-
1998/04/08-01	*H. midae*	*M. midae*	-	-	-	-
1998/04/08-02	*H. midae*	*M. midae*	-	-	-	-
1998/04/08-03	*H. midae*	*M. midae*	-	-	-	-
1998/04/08-04	*H. midae*	*M. midae*	-	-	-	-
1998/04/08-05	*H. midae*	*M. midae*	-	-	-	-
1998/04/08-06	*H. midae*	*M. midae*	-	-	-	-
1998/04/08-07	*H. midae*	*M. midae*	✔	-	✔ E	-
1998/04/08-08	*H. midae*	*M. midae*	✔	-	✔ M	-

Table 3 continued: Data from March-April 1998. Haliotids collected at the De Hoop Nature Reserve on the south coast of South Africa: *Haliotis midae* Linnaeus, 1758 and *Haliotis spadicea* Donovan, 1808, indicating the presence of *Mantoscyphidia midae* Botes, Basson & Van As, 2001; *M. spadiceae* Botes, Basson & Van As, 2001 and the occurrence of the secondary symbiont *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep; binary fission; conjugation and telotrochs.

Specimen number	Host	Mantoscyphidia	Ellobiophrya	Binary fission	Conjugation	Telotrochs
1998/04/08-09	*H. midae*	*M. midae*	✔	-	-	-
1998/04/08-10	*H. midae*	*M. midae*	✔	-	-	-
1998/04/09-01	*H. midae*	*M. midae*	-	-	-	-
1998/04/09-02	*H. midae*	*M. midae*	✔	✔ M	-	-
1998/04/11-01	*H. midae*	*M. midae*	-	-	-	-
1998/04/11-02	*H. midae*	*M. midae*	✔	-	-	-
1998/04/11-03	*H. midae*	*M. midae*	-	-	-	-
1998/04/12-01	*H. midae*	*M. midae*	✔	-	-	-
1998/03/28-01	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/28-02	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1998/03/28-03	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/28-04	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/28-05	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/28-06	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/04/28-07	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/04/28-08	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/28-09	*H. spadicea*	*M. spadiceae*	-	✔ M	-	-
1998/03/28-11	*H. spadicea*	uninfected	-	-	-	-
1998/03/28-12	*H. spadicea*	uninfected	-	-	-	-
1998/03/29-05	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/29-06	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/29-07	*H. spadicea*	*M. spadiceae*	-	✔ M	-	-
1998/03/29-08	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/29-09	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1998/03/29-10	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/30-01	*H. spadicea*	*M. spadiceae*	✔	✔ M	-	-
1998/03/30-02	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/30-03	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/30-04	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/30-05	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/30-06	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/30-07	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/30-08	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/30-09	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/30-10	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/31-01	*H. spadicea*	uninfected	-	-	-	-
1998/03/31-02	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/31-03	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/31-04	*H. spadicea*	*M. spadiceae*	-	-	-	-
1998/03/31-05	H. spadicea	M. spadiceae	✔	-	-	-
1998/04/06-10	*H. spadicea*	M. spadiceae	✔		✔ E	-

Table 3 continued: Data from March-April 1998. Haliotids collected at the De Hoop Nature Reserve on the south coast of South Africa: *Haliotis midae* Linnaeus, 1758 and *Haliotis spadicea* Donovan, 1808, indicating the presence of *Mantoscyphidia midae* Botes, Basson & Van As, 2001; *M. spadiceae* Botes, Basson & Van As, 2001 and the occurrence of the secondary symbiont *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep; binary fission; conjugation and telotrochs.

Specimen number	Host	Mantoscyphidia	Ellobiophrya	Binary fission	Conjugation	Telotrochs
1998/04/06-11	*H. spadicea*	*M. spadiceae*	-	-	? (9 hours)	✔
1998/04/09-03	*H. spadicea*	*M. spadiceae*	✔	-	-	✔ M
1998/04/09-04	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1998/04/11-04	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1998/04/11-05	*H. spadicea*	*M. spadiceae*	-	✔ M	-	-
1998/04/11-06	*H. spadicea*	*M. spadiceae*	✔	✔ M	-	-
1998/04/11-07	*H. spadicea*	*M. spadiceae*	✔	✔ M	-	-
1998/04/11-08	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1998/04/11-09	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1998/04/11-10	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1998/04/11-11	*H. spadicea*	*M. spadiceae*	✔	✔ M	-	-
1998/04/11-12	*H. spadicea*	*M. spadiceae*	✔	✔ E	-	✔ M
1998/04/11-13	*H. spadicea*	*M. spadiceae*	✔	✔ M	✔ E	-
1998/04/11-14	*H. spadicea*	*M. spadiceae*	✔	-	-	-

Table 4: Data from March-April 1999. Haliotids collected at the De Hoop Nature Reserve on the south coast of South Africa: *Haliotis midae* Linnaeus, 1758 and *Haliotis spadicea* Donovan, 1808, indicating the presence of *Mantoscyphidia midae* Botes, Basson & Van As, 2001; *M. spadiceae* Botes, Basson & Van As, 2001 and the occurrence of the secondary symbiont *Ellobiophrya maliculiformis* Peters, Van As, Basson & Van As, in prep; binary fission; conjugation and telotrochs. M = *Mantoscyphidia*, E = *Ellobiophrya*.

Specimen number	Host	Mantoscyphidia	Ellobiophrya	Binary fission	Conjugation	Telotrochs
1999/03/22-01	*H. midae*	*M. midae*	-	-	-	-
1999/03/22-02	*H. midae*	*M. midae*	-	-	-	-
1999/03/23-01	*H. midae*	*M. midae*	-	-	-	-
1999/03/27-01	*H. midae*	*M. midae*	-	-	-	-
1999/03/27-02	*H. midae*	*M. midae*	✔	-	-	-
1999/03/30-01a	*H. midae*	*M. midae*	✔	-	-	-
1999/03/30-01b	*H. midae*	*M. midae*	-	-	-	-
1999/03/30-02	*H. midae*	*M. midae*	-	-	-	-
1999/03/30-03	*H. midae*	*M. midae*	✔	-	-	-
1999/03/30-11a	*H. midae*	*M. midae*	-	-	-	-
1999/03/30-11b	*H. midae*	*M. midae*	-	-	-	-
1999/03/30-12	*H. midae*	*M. midae*	✔	-	-	-
1999/03/30-13	*H. midae*	*M. midae*	✔	-	-	-
1999/03/30-14	*H. midae*	*M. midae*	-	-	-	-
1999/03/30-15	*H. midae*	*M. midae*	-	-	-	-
1999/03/30-16	*H. midae*	*M. midae*	-	-	-	-
1999/04/01-01	*H. midae*	*M. midae*	-	-	-	-
1999/04/01-03	*H. midae*	*M. midae*	-	-	-	-
1999/04/01-04	*H. midae*	*M. midae*	-	-	-	-
1999/04/01-05	*H. midae*	*M. midae*	-	-	-	-
1999/04/01-06	*H. midae*	*M. midae*	-	-	-	-
1999/03/21-01	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1999/03/21-02	*H. spadicea*	*M. spadiceae*	-	-	-	-
1999/03/22-03	*H. spadicea*	*M. spadiceae*	-	-	-	-
1999/03/22-04	*H. spadicea*	*M. spadiceae*	-	-	-	-
1999/03/22-05	*H. spadicea*	*M. spadiceae*	-	-	-	-
1999/03/23-02	*H. spadicea*	*M. spadiceae*	-	-	-	-
1999/03/24-01	*H. spadicea*	*M. spadiceae*	-	-	-	-
1999/03/27-03	*H. spadicea*	*M. spadiceae*	-	-	-	-
1999/03/28-01	*H. spadicea*	*M. spadiceae*	-	-	-	-
1999/03/28-02	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1999/03/30-04	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1999/03/30-05	*H. spadicea*	*M. spadiceae*	-	-	-	-
1999/03/30-06	*H. spadicea*	*M. spadiceae*	-	-	-	-
1999/03/30-07	*H. spadicea*	*M. spadiceae*	-	-	-	-
1999/03/30-08	*H. spadicea*	*M. spadiceae*	✔	-	-	-
1999/03/30-09	*H. spadicea*	*M. spadiceae*	-	-	-	-
1999/03/30-10	*H. spadicea*	*M. spadiceae*	-	-	-	-
1999/04/01-07	*H. spadicea*	*M. spadiceae*	-	-	-	✔ M
1999/04/01-08	*H. spadicea*	*M. spadiceae*	-	-	-	-
1999/04/01-09	*H. spadicea*	*M. spadiceae*	-	-	-	✔ M
1999/04/01-10	*H. spadicea*	*M. spadiceae*	-	-	-	-
1999/04/01-11	*H. spadicea*	*M. spadiceae*	-	-	-	-

Table 5: Body length and width (µm) of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 telotrochs measured from scanning electron microscopy photographs, occurring on the gills of *Scutellastra barbara* (Linnaeus, 1758) and *Cymbula miniata* (Born, 1778).

Number	Mollusc Host	Length	Width
1	*Scutellastra barbara*	25.64	21.54
2	*Scutellastra barbara*	30.51	21.54
3	*Scutellastra barbara*	25.56	24.17
4	*Scutellastra barbara*	35.38	23.33
5	*Scutellastra barbara*	25.88	23.82
6	*Scutellastra barbara*	31.28	24.10
7	*Scutellastra barbara*	18.18	20.91
8	*Scutellastra barbara*	16.86	17.84
9	*Scutellastra barbara*	22.27	23.18
10	*Scutellastra barbara*	20.91	20.91
11	*Scutellastra barbara*	18.43	17.65
12	*Scutellastra barbara*	16.67	12.96
13	*Scutellastra barbara*	21.82	18.18
14	*Scutellastra barbara*	17.33	16.33
15	*Scutellastra barbara*	20.56	24.17
16	*Scutellastra barbara*	22.73	19.32
17	*Scutellastra barbara*	19.81	21.54
18	*Scutellastra barbara*	25.13	27.18
19	*Scutellastra barbara*	29.73	24.32
20	*Scutellastra barbara*	25.00	21.25
21	*Scutellastra barbara*	14.55	20.45
22	*Scutellastra barbara*	29.41	21.47
23	*Scutellastra barbara*	25.45	23.18
24	*Scutellastra barbara*	37.50	21.67
25	*Scutellastra barbara*	28.24	25.00
26	*Scutellastra barbara*	26.14	26.82
27	*Scutellastra barbara*	35.14	25.41
28	*Scutellastra barbara*	24.62	22.82
29	*Scutellastra barbara*	25.41	26.49
30	*Scutellastra barbara*	25.13	23.08
31	*Scutellastra barbara*	26.47	27.06
32	*Scutellastra barbara*	28.89	30.00
33	*Scutellastra barbara*	27.06	27.65
34	*Scutellastra barbara*	23.33	21.67
35	*Scutellastra barbara*	30.56	21.67
36	*Scutellastra barbara*	24.17	23.33
37	*Scutellastra barbara*	19.09	20.00
38	*Scutellastra barbara*	22.27	22.27
39	*Scutellastra barbara*	24.09	21.82
40	*Scutellastra barbara*	29.74	21.54
41	*Scutellastra barbara*	28.18	21.36
42	*Scutellastra barbara*	19.67	19.00
43	*Scutellastra barbara*	26.47	24.71
44	*Scutellastra barbara*	22.69	18.85
45	*Scutellastra barbara*	21.67	20.00

Table 5 continued: Body length and width (µm) of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 telotrochs measured from scanning electron microscopy photographs, occurring on the gills of *Scutellastra barbara* (Linnaeus, 1758) and *Cymbula miniata* (Born, 1778).

Number	Mollusc Host	Length	Width
46	*Scutellastra barbara*	16.67	17.33
47	*Scutellastra barbara*	23.18	19.55
48	*Scutellastra barbara*	28.65	27.57
49	*Scutellastra barbara*	27.57	27.57
50	*Scutellastra barbara*	28.64	18.64
51	*Cymbula miniata*	33.75	16.00
52	*Cymbula miniata*	31.50	20.75
53	*Cymbula miniata*	23.33	17.08
54	*Cymbula miniata*	29.32	20.45
55	*Cymbula miniata*	32.78	21.67
56	*Cymbula miniata*	23.64	25.45
57	*Cymbula miniata*	22.27	19.32
58	*Cymbula miniata*	35.29	22.35
59	*Cymbula miniata*	21.03	18.97
60	*Cymbula miniata*	14.87	22.31
61	*Cymbula miniata*	27.88	21.21
62	*Cymbula miniata*	23.78	23.24
63	*Cymbula miniata*	23.18	20.00
64	*Cymbula miniata*	25.00	19.32

Table 6: Body length and width (µm) of *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 telotrochs measured from scanning electron micrographs, occurring on the gills of *Haliotis spadicea* Donovan, 1808.

Number	Mollusc Host	Length	Width
1	*Haliotis spadicea*	40.00	27.59
2	*Haliotis spadicea*	38.46	28.72
3	*Haliotis spadicea*	43.45	18.62
4	*Haliotis spadicea*	39.20	25.60
5	*Haliotis spadicea*	34.38	20.63
6	*Haliotis spadicea*	34.62	21.54
7	*Haliotis spadicea*	49.60	22.40
8	*Haliotis spadicea*	32.41	24.83
9	*Haliotis spadicea*	31.35	20.00
10	*Haliotis spadicea*	25.00	18.89
11	*Haliotis spadicea*	36.30	28.15
12	*Haliotis spadicea*	31.03	27.59
13	*Haliotis spadicea*	39.17	23.33
14	*Haliotis spadicea*	29.09	26.67
15	*Haliotis spadicea*	47.69	17.69
16	*Haliotis spadicea*	36.92	26.92
17	*Haliotis spadicea*	38.82	18.24
18	*Haliotis spadicea*	21.43	25.00
19	*Haliotis spadicea*	21.74	25.22
20	*Haliotis spadicea*	41.00	21.00
21	*Haliotis spadicea*	43.00	24.00
22	*Haliotis spadicea*	35.29	19.41
23	*Haliotis spadicea*	49.66	21.38
24	*Haliotis spadicea*	40.00	21.54
25	*Haliotis spadicea*	56.15	16.15
26	*Haliotis spadicea*	40.00	23.10
27	*Haliotis spadicea*	34.38	26.88
28	*Haliotis spadicea*	25.00	30.00
29	*Haliotis spadicea*	51.25	23.75
30	*Haliotis spadicea*	45.45	21.82
31	*Haliotis spadicea*	35.45	20.91
32	*Haliotis spadicea*	48.28	17.93
33	*Haliotis spadicea*	46.00	18.00
34	*Haliotis spadicea*	40.69	18.62

Table 7: Body length and width (µm) of the microconjugants and macroconjugants of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 measured from scanning electron micrographs, occurring on the gills of *Scutellastra barbara* (Linnaeus, 1758).

Number	Mollusc Host	Micro conjugant Length	Micro conjugant Width	Macro conjugant Length	Macro conjugant Width
1	*Scutellastra barbara*	21.30	21.30	36.67	30.00
2	*Scutellastra barbara*	21.25	16.88	32.14	27.86
3	*Scutellastra barbara*	20.67	17.67	26.25	23.44
4	*Scutellastra barbara*	20.63	19.38	26.00	22.67
5	*Scutellastra barbara*	29.17	20.42	26.88	25.00
6	*Scutellastra barbara*	14.55	17.88	34.17	27.50
7	*Scutellastra barbara*	25.19	20.00	43.00	30.00
8	*Scutellastra barbara*	16.67	12.22	37.78	32.78
9	*Scutellastra barbara*	21.79	11.43	23.03	22.73
10	*Scutellastra barbara*	10.70	12.09	28.15	24.07
11	*Scutellastra barbara*	8.64	15.00	31.85	30.37
12	*Scutellastra barbara*	16.76	16.76	27.86	29.64
13	*Scutellastra barbara*	12.75	7.50	23.72	24.65
14	*Scutellastra barbara*	12.00	15.00	20.45	20.45
15	*Scutellastra barbara*	16.47	17.65	23.78	21.62
16	*Scutellastra barbara*	15.41	15.68	23.50	22.00
17	*Scutellastra barbara*	21.33	9.33	26.00	22.50
18	*Scutellastra barbara*	13.78	15.14	23.53	22.06
19	*Scutellastra barbara*	21.33	16.00	20.54	21.35
20	*Scutellastra barbara*	23.64	13.64	28.00	28.33
21	*Scutellastra barbara*	22.50	20.00	21.62	21.62
22	*Scutellastra barbara*	23.33	18.33	27.33	27.00
23	*Scutellastra barbara*	26.07	18.57	47.27	36.36
24	*Scutellastra barbara*	26.09	20.00	35.00	31.67
25	*Scutellastra barbara*	18.53	18.24	31.67	23.33
26	*Scutellastra barbara*	23.70	17.78	38.57	27.86
27	*Scutellastra barbara*	15.00	18.24	27.83	23.04
28	*Scutellastra barbara*	16.92	16.67	26.47	23.82
29	*Scutellastra barbara*	17.95	16.92	39.63	22.22
30	*Scutellastra barbara*	23.33	19.33	25.29	22.94
31	*Scutellastra barbara*	15.00	15.00	22.31	21.03
32	*Scutellastra barbara*	20.00	15.38	23.33	22.56
33	*Scutellastra barbara*	13.54	16.04	36.00	31.33
34	*Scutellastra barbara*	24.07	18.52	22.73	18.64
35	*Scutellastra barbara*	21.48	18.15	26.67	20.00
36	*Scutellastra barbara*	22.76	17.59	17.92	18.96
37	*Scutellastra barbara*	20.00	16.47	41.48	28.89
38	*Scutellastra barbara*	22.76	18.97	31.11	28.15
39	*Scutellastra barbara*	28.70	19.13	33.79	26.90

Table 7 continued: Body length and width (µm) of the microconjugants and macroconjugants of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 measured from scanning electron micrographs, occurring on the gills of *Scutellastra barbara* (Linnaeus, 1758).

Number	Mollusc Host	Micro conjugant Length	Micro conjugant Width	Macro conjugant Length	Macro conjugant Width
40	*Scutellastra barbara*	17.93	20.00	29.71	24.41
41	*Scutellastra barbara*	28.80	19.20	32.41	25.52
42	*Scutellastra barbara*	20.00	18.62	37.39	28.70
43	*Scutellastra barbara*	21.07	18.93	37.93	30.34
44	*Scutellastra barbara*	13.94	14.24	43.20	28.80
45	*Scutellastra barbara*	21.85	21.48	35.86	31.72
46	*Scutellastra barbara*	22.86	18.57	34.29	27.14
47	*Scutellastra barbara*	24.14	20.00	35.76	26.06
48	*Scutellastra barbara*	22.07	24.14	37.04	34.07
49	*Scutellastra barbara*	16.11	9.72	37.14	29.64
50	*Scutellastra barbara*	22.12	18.18	25.52	25.52
51	*Scutellastra barbara*	16.67	8.33	31.03	25.86
52	*Scutellastra barbara*	27.50	17.86	32.22	25.56
53	*Scutellastra barbara*	25.00	15.21	32.12	27.88
54	*Scutellastra barbara*	18.24	17.94	-	-
55	*Scutellastra barbara*	23.03	12.12	36.43	24.64
56	*Scutellastra barbara*	19.41	17.06	-	-
57	*Scutellastra barbara*	16.67	19.33	42.94	26.47
58	*Scutellastra barbara*	20.00	15.56	49.09	22.42
59	*Scutellastra barbara*	8.24	9.41	35.29	29.41
60	*Scutellastra barbara*	20.00	8.82	26.67	26.00
61	*Scutellastra barbara*	26.67	15.33	29.17	21.67
62	*Scutellastra barbara*	16.92	18.97	28.48	27.88
63	*Scutellastra barbara*	17.04	8.52	26.47	30.59
64	*Scutellastra barbara*	19.63	8.89	34.00	28.00
65	*Scutellastra barbara*	20.63	16.25	26.67	24.62
66	*Scutellastra barbara*	17.65	17.06	32.22	28.89
67	*Scutellastra barbara*	24.00	16.00	36.88	28.75
68	*Scutellastra barbara*	13.54	12.92	34.12	31.18
69	*Scutellastra barbara*	45.91	12.73	38.40	35.20
70	*Scutellastra barbara*	21.39	13.89	27.92	18.75
71	*Scutellastra barbara*	17.22	11.94	25.00	23.64
72	*Scutellastra barbara*	16.92	10.00	30.00	24.44
73	*Scutellastra barbara*	24.12	10.00	27.78	24.44
74	*Scutellastra barbara*	13.85	10.26	26.67	24.10
75	*Scutellastra barbara*	13.70	15.37	34.12	25.29
76	*Scutellastra barbara*	18.79	15.76	26.67	27.18
77	*Scutellastra barbara*	17.04	11.85	22.22	19.26
78	*Scutellastra barbara*	23.13	16.25	30.30	27.27

Table 7 continued: Body length and width (µm) of the microconjugants and macroconjugants of *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 measured from scanning electron micrographs, occurring on the gills of *Scutellastra barbara* (Linnaeus, 1758).

Number	Mollusc Host	Micro conjugant Length	Micro conjugant Width	Macro conjugant Length	Macro conjugant Width
79	*Scutellastra barbara*	19.40	13.33	48.15	29.63
80	*Scutellastra barbara*	20.00	13.33	30.63	26.56
81	*Scutellastra barbara*	12.22	9.44	27.58	23.64
82	*Scutellastra barbara*	13.78	15.14	28.33	25.33
83	*Scutellastra barbara*	17.50	9.83	27.22	25.56
84	*Scutellastra barbara*	17.65	18.82	25.95	22.43
85	*Scutellastra barbara*	17.78	16.11	-	-
86	*Scutellastra barbara*	13.53	13.53	24.12	24.71
87	*Scutellastra barbara*	14.09	13.64	26.11	21.67
88	*Scutellastra barbara*	17.65	18.82	27.06	22.35
89	*Scutellastra barbara*	16.76	15.14	22.73	20.45
90	*Scutellastra barbara*	16.22	15.14	24.71	24.71
91	*Scutellastra barbara*	17.30	11.89	22.70	23.24
92	*Scutellastra barbara*	19.41	15.29	29.19	23.78
93	*Scutellastra barbara*	15.71	12.86	23.78	22.70
94	*Scutellastra barbara*	17.86	10.36	25.29	22.94
95	*Scutellastra barbara*	21.25	10.00	32.86	27.14
96	*Scutellastra barbara*	16.75	16.50	28.13	25.63
97	*Scutellastra barbara*	15.56	16.67	24.50	24.00
98	*Scutellastra barbara*	15.64	13.08	27.22	25.28
99	*Scutellastra barbara*	17.57	15.14	30.26	26.67
100	*Scutellastra barbara*	14.12	7.06	24.86	23.24
101	*Scutellastra barbara*	17.30	17.57	21.18	23.53
102	*Scutellastra barbara*	16.22	11.35	23.78	24.86
103	*Scutellastra barbara*	23.20	12.40	30.27	28.11
104	*Scutellastra barbara*	16.67	16.92	35.20	32.00
105	*Scutellastra barbara*	18.33	18.33	30.26	25.13

Appendix B
Terminology

The terminology list has been compiled from Finley (1939, 1943), Corliss (1979) and Lom and Dykova (1992).

Aboral away from or opposite the mouth or oral area in those groups of animals that have no clear-cut dorsal or ventral surfaces. In ciliatology almost always used in the most extreme sense, meaning at the opposite (usually antapical) pole from the (other) en (usually apical) of the body bearing the mouth; but – as in the case of peritrichs) – the aboral pole is not necessarily the posterior pole of the organism, functionally and/or morphologically and/or evolutionary.

Adhesive disc thigmotactic, cup-shaped organelle at the aboral pole of mobiline peritrichs and some other ciliophorans used for attachment to the substratum (usually the surface of another organism serving as host).

Adoral (of an organism) the side on which the mouth is situated.

Anisogamete (anisogamy) the state in which the gametes are different from each other in size and activity, i.e. male and female, usually the former is smaller and more active than the latter. One or two conjugating motile gametes differing in form and size.

Anlage (plural anlagen) the first structure or cell group indicating development of a part or organ. Primordium, a developing, differentiating, or even presumptive structure or organelle; usable with many a preceding modifier: nuclear, cytoplasmic, cortical, oral, somatic, ciliary, etc.; in ciliophoran morphogenesis, it is often a group of kinetosomes.

Annulus (striation) ring-like striations on the external morphology of peritrichs. Various ring-like structures or markings in general, including the pellicular striations on the zooid of certain peritrichs.

Aperture any type of opening.

Apex tip or summit.

Balloon stage not a general biological term. Term used by Finley (1939, 1943) to describe the inflation of macronuclei about 40 to 50 minutes after fusion of conjugants during the process of conjugation.

Basal bodies (basal corpuscles) kinetosome. A structure similar to (and homologous with) a centriole connected to the axial filament of the flagellum and cilium, e.g. in sperms. A structure undistinguishable from centriole, acting as an organizing centre (nucleation) for eukaryotic cilia and flagella, unlike which its axoneme is a ring of nine triplet microtubules, each comprising one complete microtubule fused into two incomplete ones. It is a permanent feature at the base of each such flagellum or cilium.

Bell body proper (minus the stalk) of many sessiline peritrichs.

Buccal opening (buccal cavity) related to the mouth cavity. Part of the alimentary tract between the mouth and pharynx. Pouch or depression, typically quite deep though sometimes secondarily flattened out or everted, often at or near the apical end of the body and/or on the ventral surface; contains (the bases of) the compound ciliary organelles and inwardly leads ultimately to the organism's cytostome-cytopharyngeal complex, sometimes via a specialized portion of itself known as the infundibulum; especially characteristic, under the name "buccal cavity", of many oligohymenophorans, but it is considered to be the structural equivalent of the peristome of the polyhymenophorans.

Chromophilic chromaffin. Staining readily.

Ciliary belt, girdle or wreath (locomotor fringe) in a general way, the term is

restricted to peritrichs, yet it is also used for any encircling band of somatic ciliature. A ring of specialized "compound" ciliature (sometimes called pectinelles) around the posterior part of the body of the telotrochs of sessiline peritrichs and around the basal disc of mature mobilines; used in swimming by the migratory larval forms, generally resorbed in the adult forms; also popularly known as a trochal band; locomotor fringe *sensu strictu* is unique to peritrichs.

Cyclosis (cytoplasmic streaming) the movement of cytoplasm from one region of a cell to another, often in definite currents, thought to be controlled by microfilaments composed of protein similar to actin.

Cytostome It is an area of cell membrane without underlying pellicle. In ciliates it is usually associated with microtubular ribs.

Denticle ring (denticulate ring) skeletal organelle, made up of denticles, found in mobiline peritrichs.

Diurnal (rhythm) a pattern of activity based upon a 24-hour cycle, in which there are regular light and dark periods. Occurring every day, with a cycle of 24 hours. Active in the daytime.

Ecdysis moulting, the periodic shedding of cuticular exoskeleton in insects and some other arthropods to allow for growth.

Endoplasm (plasmasol) any cytoplasm present within the plasma membrane and ectoplasm of a cell. Cytoplasm that is granular and streams actively and lies in the interior of the cell, surrounded by a thin peripheral layer of rigid, non-granular ectoplasm.

Epizoic (ectocommensal) living on or attached to the outside of the body surface of an animal.

Heterogamete Male and female gametes that are morphologically different.

Isthmi [pl]. (isthmus) narrow structures connecting two larger parts.

Karyokinesis nuclear division.

Kinetosome the basal body of a cilium. Organelle found in cells of those eukaryotic organisms which have cilia or flagella at some stage in their life cycle. Subpellicularly located tubular cylinder of nine longitudinally orientated (at right angles to a cell's surface) equally spaced, skewed, peripheral structures each composed of three microtubules; typical size of a kinetosome in ciliophorans is 1.2 x 0.3 μm; when ciliferous, this embedded basal body (or, by accepted homology, centriole) produces a cilium at its distal end; when viewed from deeper in the cytoplasm of the organism looking outward, the nine triplets of microtubules are skewed inwardly, clockwise.

Lorica Envelope surrounding some flagellated and ciliophoran protists, fitting loosely enough to allow the cell to move fairly freely. A protective external case. Envelope, case or shell (or, occasionally, theca) secreted and/or assembled (using various materials) by a number of ciliophorans from several different taxa (most commonly peritrichs, folliculinids, and tintinnines), with the important properties of fitting (the body) loosely, opening at one (anterior) end (or occasionally both ends), and being either attached to the substratum (common) or carried about by the freely swimming organism (true of the tintinnines); may occur in a multiple (arboroid-tree) state; such a "house" or "tube" may be occupied only temporarily (e.g., as is true in the case of some hypotrichs); may be calcareous, composed of some proteinaceous or mucopolysaccaride secretion, including chitin, pseudochitin, or tectin, or made up of foreign matter (sand grains, diatom frustules, coccoliths, debris) ingeniously cemented together in a species-specific pattern.

Lunar regarding influences from the moon.

Neuter (vegetative) individual an individual that is not in a state of sexual reproduction. Stage of growth where sexual reproduction does not occur.

Orad towards the mouth or mouth region.

Oral of or relating to the mouth. Pertaining to or belonging to the mouth.

Peduncle a stalk. Term generally used as a synonym of stalk when referring to ciliophorans; often reserved for long, highly visible stalks, such as those, not necessarily homologous organelles found in many peritrichs and suctorians; the adjectival form, "peduncular", is also often used with reference to stalk structures.

Pellicle a thin membrane. Any delicate surface or skin-like growth. Outer "living" covering of a ciliophoran, composed of the typical cell or plasma membrane plus the membrane-lined alveoli and, often, the closely apposed underlying fibrous layer known as the epiplasm; sometimes loosely used as synonymous with cortex, but the majority of the infraciliary cortical structures and organelles are mostly subpellicular in location.

Peristome (peristomial region) any structure that occurs around the mouth or opening of an organ. In a broad sense, a synonym of buccal cavity; but the term is well entrenched in the literature to mean the entire expansive oral area (peristomial) field) of the so-called higher ciliophorans (peritrichs and various of the spirotrichs) in which the buccal infraciliature has often emerged from a true cavity to encircle (though usually only partially) much of the anterior (or ventro-anterior or oral) end or pole of the organism's body.

Plasmotomy division of a plasmodium by cleavage into multinucleate parts.

Primordial original, existing from the beginning. Primitive. First formed.

Pronuclei haploid nuclei after fertilization but before nuclear fusion and the first mitotic division.

Scopula attachment region at the aboral pole of the cell body. Compound organelle or structure (or area, in effect), often cup-shaped with a thickened peripheral border or lip, at the aboral pole of (especially sessiline) peritrichs which is comprised of a plaque or field of numerous kinetosomes typically equipped with very short immobile cilia; pellicular (?) pores (=parasomal sacs) are also present; the scopula may function directly as a holdfast or, more commonly, may be involved (presumably mostly via its kinetosomes and/or its pores) in secretion or elaboration of a peduncle or stalk of considerable complexity (contractile or noncontractile, etc.).

Skein stage not a general biological term. According to Finley (1939, 1943), the macronuclear skein stage follows the balloon stage, during which the macronucleus elongates and spins out into a tangled mass that fills the whole body of the conjugant during the process of conjugation.

Spindle formation a network of fibres or microtubules formed during late prophase and early metaphase of mitosis and meiosis, and which serves as an attachment point for chromosomes before they are pulled to the spindle poles during anaphase.

Swarmer (telotroch) free-swimming migratory stage in the life-cycle of, especially, sessiline peritrichs.

Syngamy (fertilization) the fusion of gametes.

Synkaryon a zygote nucleus resulting from fusion of gamete nuclei.

Vacuolization the formation of vacuoles.

Vesicular possessing a sac-like or membranous spherical structure. Composed of or marked by the presence of small cavities.

Zooid term generally restricted in ciliatology to mean only the body proper of an attached sessile form (e.g. the bell of many peritrichs), minus the stalk; the term also refers to the individual members of a colony (free or attached), but usually (again) only of the arboroid colony so typical of peritrichs; macro- and microzooids are distinguishable by size and exhibition of certain functional differences (e.g. in *Zoothamnium* only macrozooids are capable of starting new colonies.

Appendix C

To be submitted for publication in Acta Protozoologica:

Peters, H., Van As, L..L., Basson, L. & Van As, J.G. In press. A new species of *Ellobiophrya* (Chatton & Lwoff, 1923) (Ciliophora: Peritrichia attached to *Mantoscyphidia* Jankowski, 1980 (Ciliophora: Peritrichia) species.

A new species of *Ellobiophrya* (Chatton & Lwoff, 1923) (Ciliophora: Peritrichia attached to *Mantoscyphidia* Jankowski, 1980 (Ciliophora: Peritrichia) species.

Helene PETERS[1], Liesl L. VAN AS[2], Linda BASSON[2] and Jo G. VAN AS[2]

[1]Department of Zoology and Entomology, Qwa-Qwa Campus, University of the Free State, Private Bag X13, Phuthaditjhaba, 9866, South Africa

[2]Department of Zoology and Entomology, University of the Free State, PO Box 339, Bloemfontein, 9300, South Africa

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Page heading. *Ellobiophrya* attached to scyphidiid peritrichs

Address for correspondence: Liesl L. van As, Department of Zoology and Entomology, University of the Free State, PO Box 339, Bloemfontein, 9300, South Africa.

Fax: (+2751) 448 8711. E-mail: VANASLL@SCI.UOVS.AC.ZA

Summary. Surveys carried out along the coast of South Africa revealed the presence of a secondary symbiont of the genus *Ellobiophrya* (Chatton & Lwoff, 1923) found attached to the scopula of *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 and *M. midae* Botes, Basson & Van As, 2001 occurring on the gills of *Haliotis spadicea* Donovan, 1808 and *H. midae* Linnaeus, 1758, respectively. *Mantoscyphidia branchi* Van As, Basson & Van As, 1998 found on the gills of *Cymbula* H. & A. Adams, 1854 and *Scutellastra* H. & A. Adams, 1854 species respectively, had the same ellobiophryid species attached to the scopula. This ellobiophryid differs from all the known *Ellobiophrya* species with respect to morphology of the body, features of the nuclear apparatus, and host preference and is therefore described as a new species, *Ellobiophrya maliculiformis* sp. n.

INTRODUCTION

Representatives of the family Ellobiophryiidae (Chatton & Lwoff, 1929) attach to the host by means of a scopula that has been adapted to form a ring-like cinctum or caudal process. Currently the family comprises two genera, i.e. *Ellobiophrya* (Chatton & Lwoff, 1923) and *Caliperia* Laird, 1953. All the known species of the genus *Ellobiophrya* were found associated with fish, bivalves or bryozoan hosts from marine habitats. *Ellobiophrya donacis* Chatton & Lwoff, 1923 was described from the gill filaments of the bivalve *Donax vittatus* (Chatton & Lwoff 1923, 1928, 1929). Nearly sixty years later *E. conviva* Clamp, 1982 was described from the tentacles of bryozoan ectoprocts, i.e. *Bugula neritina* and *B. turrita* (Clamp 1982). Another species, *E. oblida* (Naidenova & Zaika, 1969) from the marine fish *Proterorhinus marmoratus*, was originally described as *Clausophrya oblida* by Naidenova and Zaika (1969) but was placed within the genus *Ellobiophrya* by Clamp (1982).

Caliperia longipes Laird, 1953 and *C. brevipes* Laird, 1959 were both described from the gill filaments of marine fishes (Laird 1953, 1959). This genus is characterised by a non-contractile skeletal rod within the arms of the cinctum and by not having the cinctal arms bonded to one another at the tips. Clamp and Bradbury's (1997) observations, however, revealed that the cinctal arms of *C. brevipes* are linked by a bouton and that the cytoskeletal structure within them has the fine structure of a myoneme. These characteristics place the previously known *C. brevipes* in the genus *Ellobiophrya*. This species is renamed as *E. brevipes* (Laird, 1959) with *C. longipes* the sole remaining species in the genus (Clamp & Bradbury 1997). According to Clamp, the genus *Caliperia* may not exist at all, and if *C. longipes* could be recollected someday, it may also turn out to be an *Ellobiophrya* (Clamp, personal communication)[1].

The ellobiophryid found in this study belongs to the genus *Ellobiophrya*, based on the morphology of the cinctum and the presence of a bouton. Populations of *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001, *M. midae* Botes, Basson & Van As, 2001 and *M. branchi* Van As, Basson & Van As, 1998 which occur on the gills of *Haliotis spadicea* Donovan, 1808, *H. midae* Linnaeus, 1758 and different limpet species respectively, had the same *Ellobiophrya* species attached, around the body of the various scyphidiid peritrich hosts above the scopula.

This ellobiophryid differs from the known species with respect to morphological features of the body, characteristics of the nuclear apparatus, and host preference and is described as a new species.

[1] Dr. John C. Clamp, Department of Biology, North Carolina Central University, Durham, North Carolina, 27704, USA.

MATERIALS AND METHODS

South African haliotids, i.e. *Haliotis spadicea* (venus ears) and *H. midae* (perlemoen) were collected from infratidal pools on the rocky shores along the south coast of South Africa. The haliotids hosted two scyphidiid peritrich species, i.e. *M. spadiceae* and *M. midae* (Botes, Basson & Van As 2001). *Mantoscyphidia branchi* was found on the gills of all the limpet species collected from the rocky shore along the south, west and east coast of South Africa (Van As, Basson & Van As 1998). An ellobiophryid species was found associated with all three *Mantoscyphidia* Jankowski, 1980 species. Gills were dissected, placed on a microscope slide, smeared, and examined using a compound microscope. Live specimens of ellobiophryids were observed and photomicrographs were taken of ellobiophryids found associated with *Mantoscyphidia spadiceae* and *M. midae* for the purpose of measuring body dimensions.

The species is described from the type population, found attached to the host *Mantoscyphidia spadicea*. Additional data and measurements from the other host populations, namely *M. midae* and *M. branchi*, are given in Table 1.

Additionally, wet smears were fixed in Bouin's, transferred to 70% ethanol and stained with Heidenhain's Iron, Mayer's and Harris' Hematoxylin for studying the nuclear apparatus and for measuring body dimensions. In order to study details of the infundibulum, Bouin's-fixed smears were stained with protargol, initially using a combined method as described by Lee, Hunter and Bovee (1985) and Lom and Dykova (1992). This method proved rather unsuccessful, as the ellobiophryids have many symbiotic algae and inclusions, which obscures the position of the infraciliature. Clamp's "quick method" (Clamp, personal communication) which is an adaptation of the method of Wicklow and Hill (1992), proved to give the best results.

For scanning electron microscopy (SEM), gills were fixed in 4% and 10% buffered neutral formalin. In some cases, gills were fixed in Parducz and 2.5% glutaraldehyde. In the laboratory in Bloemfontein, the specimens were cleaned by washing gills in tapwater, dehydrated in a series of ethanol concentrations and critical point dried. Gills bearing ellobiophryids attached to the mantoscyphidians were mounted on stubs, sputter coated with gold and studied at 5kV and 10kV, using a JOEL WINSEM JSM 6400 scanning electron microscope.

For measurements of live specimens, minimum and maximum values are given, followed in parentheses by the arithmetic mean, standard deviation and number of specimens measured. Measurements based on Bouin's-fixed specimens stained with hematoxylin are presented in square brackets. Body length is measured from the epistomial disc to the cinctum and body diameter at the widest part of the body. Description of pellicular striations was done from specimens viewed by SEM. The type material is in the collection of the National Museum, Bloemfontein, South Africa.

RESULTS AND DISCUSSION

***Ellobiophrya maliculiformis* sp. n.** (Figs. 1-13)

Type host and locality: *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001, attaches around the scopula; De Hoop Nature reserve, south coast (34°28'S, 20°30') of South Africa.

Other hosts and localities: *M. midae* Botes, Basson & Van As, 2001 and *M. branchi* Van As, Basson & Van As, 1998, De Hoop Nature reserve, south coast (34°28'S, 20°30') and Papendorp, Olifants River mouth, west coast of South Africa (31°40'S, 18°15'E).

Type specimens: holotype, slide 98/04/11-04 (NMBP 282), Paratype slides 98/04/04-05 (NMBP 283), 97/04/05-04c (NMBP 284), in the collection of the National Museum, Bloemfontein, South Africa.

Reference material: S94/01/17-10.

Etymology: named after the mode of attachment around the scopula of the hosts which resembles handcuffs.

Description

Trophont conical, elongate, tapering aborally towards scopular region (Figs. 1,3,4,9-12). Length of body 50-125 μm (78.5 ± 15.1,40) [60-98 μm (70.2 ± 17.5,43)], diameter of body 15-30 μm (20.5 ± 3.7,40) [16-37μm (23.9 ± 5,43)]. Peristome with broad striated peristomial lip (Fig. 3); zig-zag striations present on peristome in contracted specimens. Prominent peristomial sphincter (Figs. 10 & 11). Striations on peristome not always visible. Body striated; 100.9 striations on average, spaced 0.5μm apart. Striations evenly spaced and uniform (Fig. 4).

Trochal band narrow, slightly elevated, one quarter length of body from cinctum, not always clearly visible (Figs. 4 & 11). Cinctum flattened with two cytoplasmic cinctal limbs of uneven thickness forming closed circle (Figs. 4-8). One limb tapers, fitting into cinctal junction of shorter somewhat broader limb, both limbs terminate at bouton (Fig. 7). The limb that tapers forms a bifurcated structure at the tip of its myoneme (Fig. 5). The scopula is typical but nonfunctional, except when it participates in secretion of a larval stalk that links two daughters after fission (Figs. 2B, 4,5,6).

Oral infraciliature of *E. maliculiformis* divisible into peristomial part and infundibular part as in other sessiline peritrichs. Adoral zone completes spiral of 360°

counterclockwise around epistomial disc, with haplo- and polykinety starting almost at same point. Peristomial part consists of outer band of kinetosomes, polykinety and inner band of kinetosomes, haplokinety which parallels one another for whole of length before plunging into infundibulum (Fig. 2A).

Haplokinety and polykinety run together around the peristome and separate before plunging into infundibulum. Polykinety separates into two polykinetids after entering infundibulum. Inside infundibulum, polykinety and haplokinety make one turn (360° - 400°) on opposite walls, before reaching cytostome.

Conspicuous cytostomial sphincter visible at end of infundibulum which constricts area between infundibulum and cytostome (=ampulla) (Figs. 9-11). Ampulla tubular when empty and slighty bulbous when filled with food (Figs. 9-12). Ampulla merges with cytopharynx that is very small in diameter throughout its length, recurving slightly just adoral to trochal band (Figs. 2B,11,12).

Symbiotic algae present throughout cytoplasm, varying in number and size, obscuring position and shape of nuclear apparatus (Fig. 3). Micronucleus fusiform, but not always visible. Macronucleus coiled and sausage-shaped, extending throughout body. Prominent sections of nucleus visible in adoral and aboral sides (Fig. 1).

Reproduction is by means of conjugation, binary fission and telotroch formation. Ellobiophryids in various stages of binary fission were observed as well as individuals with attached microconjugants (Fig. 13), which confirms the first record of conjugation (Fig. 13) in the genus *Ellobiophrya* (Botes, Van As, Basson & Van As 2001). Live observations of conjugation were made in two instances in populations for *M. midae* and was observed four times in populations for *M. spadiceae* (Fig. 13), and twice in populations for *M. branchi*.

Binary fission and the subsequent formation of telotrochs were observed in ellobiophryid populations associated with *M. spadiceae*, *M. midae* and *M. branchi*. After binary fission one daughter individual becomes a telotroch and the other remains a trophont attached by the parental cinctum to the scyphidiid peritrich host. As in other ellobiophryids, the telotroch is attached during development to the trophont daughter by a short, rigid stalk that passes between the scopulas of the two individuals (Bradbury & Clamp 1991). The telotroch is slightly asymmetric, as is the case in other *Ellobiophrya* species.

During an observation of telotroch formation in a live specimen of *Ellobiophrya maliculiformis* attached to *M. midae* a larval stalk was identified. The telotroch was attached to the trophont daughter by this short stalk and the trochal band of cilia was in the process of differentiating, but not beating yet. The parent's peristome was open, with cilia creating a feeding current. This telotroch was observed eight to ten hours after collection of the gastropod host, and for a time period of 55 minutes before it separated from the parent and swam away. The aboral end (scopula) that was attached to the embryophore of the parent ellobiophryid became broader after separation.

A telotroch-like individual was also observed attached to the body of a trophont of *E. maliculiformis*. It was attached to the middle region of the trophont, and it may have been a microconjugant that had just attached in preparation for conjugation, rather than a telotroch that was preparing to separate from the other daughter. This telotroch had a short stalk with which it attached to the trophont, but was not attached to the scopula, as would have been the case in a developing telotroch. The stalk may have been a slender cytoplasmic connection, because a larval stalk is expected to be linked to the scopula (embryophore) of the trophont daughter.

Intraspecific variation

Body measurements for *E. maliculiformis* material are summarised in Table 1. The shrinkage effect on the body length of live specimens versus hematoxylin-stained specimens is as follows: In the *M. spadiceae* population there is a 27% shrinkage between live observations and those stained with hematoxylin. By comparing the different populations of *E. maliculiformis* stained with hematoxylin, no clear pattern concerning body length and diameter could be found (Fig. 14). The average body length of the populations found on *Mantoscyphidia spadiceae* and *M. midae* varied between 70.1 and 61.9 μm. The average body length of the different populations found associated with *M. branchi*, ranged between 56.5 and 62.6 μm (Table 1).

Ellobiophryids from *M. branchi, M. spadiceae* and *M. midae* had the same body form. By comparing body diameter and also the diameter of the cinctal limbs no significant differences could be found.

Live specimens from *M. spadiceae* were extremely contractile, with body length ranging between 50 and 125 μm. The body of *M. branchi* is also extremely contractile, with fully expanded specimens varying from 40 μm to 95 μm. Van As, *et al.* (1998) observed during fieldwork that the same individual showed a reduction in body length, but with the peristome remaining open. In these specimens groups of elevated striations can be seen below the telotroch band. When the peristome is fully closed, the degree of contraction can also vary. Specimens can be found in a whole range of body contractions on a single smear. Live ellobiophryids were observed and it was found that they are able to contract to half of their fully extended body length.

Although the nuclear apparatus of all the populations are mostly obscured by the algae inclusions, there are no great differences, it is coiled and stretches throughout the

body, much the same as those of *E. conviva*, *E. oblida* and *E. brevipes* (Clamp & Bradbury 1997).

The only difference between the ellobiophryid populations of *M. spadiceae* and *M. branchi* is that the latter has slightly fewer body striations (Table 2). This could be due to the fact that *Ellobiophrya maliculiformis* specimens found associated with *M. spadiceae* has a greater body length. The *Mantoscyphidia spadiceae* population had a prevalence of 35.4% of ellobiophryids associated with the scyphidiid peritrichs, and the *M. midae* population had a prevalence of 34.3%, whilst those ellobiophryids found associated with *M. branchi* had a prevalence of 17%.

Remarks

This is the first record of an ellobiophryid from Africa and the first found associated with another ciliophoran host in the marine environment. The only other record of peritrichs found in a symbiotic association with another peritrich is that of *Heteropolaria lwoffi* (Faure-Fremiet, 1943), attached to the epistylid *Apiosoma piscicola* (Blanchard) which in turn is found on the skin of freshwater fish (Fauré-Fremiet 1943; Clamp 1982). *Heteropolaria colisarum* Foissner & Schubert, 1977 also attaches to an epistylid which lives symphoriontly on its freshwater host, *Colisa fasciata* (Anabantoidei, Belontiidae) (Foissner & Schubert 1977). *Heteropolaria horizontalis* (Chatton, 1936) is also found associated with a freshwater epistylid.

In comparing *Ellobiophrya maliculiformis* with the known species (*E. donacis, E. conviva, E. oblida* and *E. brevipes)*, the present species shows the most resemblance to *E. oblida* in respect to body form. In both *E. oblida* and *E. maliculiformis*, the expanded peristome is wider in diameter than the rest of the body, and the peristomial lip is everted. *Ellobiophrya oblida* is, however, a much larger species than *E. maliculiformis* and has a different host and site preference as it occurs on the skin of

marine fish. The position of the scopula of *Ellobiophrya maliculiformis* differs from the other species of *Ellobiophrya* in that it is located far toward the cinctum. Table 3 represents a summary of the taxonomic characteristics of the known specimens of the family Ellobiophryidae. This summary was compiled from Chatton and Lwoff (1923, 1928, 1929), Clamp (1982), Bradbury and Clamp (1991), and Clamp and Bradbury (1997).

Up to four specimens of *E. maliculiformis* were observed attached around the scopula of a single scyphidiid peritrich. Some ellobiophryids were attached to the peristomial region or even in the region of the telotroch band of the scyphidiid peritrich's bodies, gripping the ciliophoran where the nuclear apparatus is situated (Fig. 8). Two ellobiophryids were observed attached between the macro- and micronuclei of a single *Mantoscyphidia spadiceae*. In the cases where the ellobiophryids were attached to the peristomial region, the buccal cavity and infraciliature were probably obstructed. The ellobiophryids might interfere with the scyphidiid peritrichs' feeding and their attachment in these instances might be detrimental, causing the peritrichs' death. Ellobiophryids attached to the nuclear apparatus region of the scyphidiid peritrichs' bodies, might have an influence on the reproductive processes, possibly interfering with division.

The present ellobiophryid has a distinctive host situation, as all four other *Ellobiophrya* species are found attached to the host's gills or skin. It is interesting to note that the two haliotid species, namely *Haliotis spadicea* and *H. midae* each has a different mantoscyphidian species occurring on the gills, namely *M. spadiceae* and *M. midae*, whilst all seventeen limpets species only have one species of mantoscyphidian, i.e. *M. branchi*, but all three *Mantoscyphidia* species had the same species of ellobiophryid attached around the scopulas, i.e. *Ellobiophrya maliculiformis*.

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Table 1. Body measurements (μm) of live observations (A) and hematoxylin stained specimens (B-F) of *Ellobiophrya maliculiformis* sp. n. from *Mantoscyphidia midae* Botes, Basson & Van As, 2001 and *M. branchi* Van As, Basson & Van As, 1998 occurring on the gills of haliotid (A,B) and different limpet (C-F) species from the south coast of South Africa.

Mollusc host	A (*Haliotis midae*)	B (*H. midae*)	C (*Scutellastra barbara*)	D (*S. argenvilli*)	E (*S. cochlear*)	F (*Cymbula compressa*)
Ciliate host	*M. midae*	*M. midae*	*M. branchi*	*M. branchi*	*M. branchi*	*M. branchi*
Body length	60.0-85.0 (72.9±8.4, 20)	43.0-93.0 (61.9±13, 35)	45.0-65.0 (56.5±6.4, 9)	45-83 (62.6±11.9, 18)	40-70 (56.5±9.8, 12)	51-70 (60.0, 5)
Body diameter	15.0-25.0 (20.1±2.4, 20)	13.0-29.0 (23.1±3.9, 35)	20-31 (26.3±3.6, 9)	15-30 (21.3±3.7, 18)	13-26 (18.6±4.1, 12)	18-39 (29.4, 5)
Outer cinctum diameter	12.0-15.0 (13.5±2.1, 2)	-	11	9-17 (12.6, 5)	13-16 (14.5, 4)	-
Inner cinctum diameter	-	-	1	2-10 6, 5)	5-10 (7.2, 4)	-
Limb diameter	-	1.0-6.0 (2.2± 1, 30)	2-4 (2.3, 8)	2-6 (3.1±1.1, 17)	2-5 (3.4±0.9, 11)	2-3 (2.8, 5)

Table 2. Body striations of *Ellobiophrya maliculiformis* sp. n. found attached to *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001 and *M. branchi* Van As, Basson & Van As, 1997 occurring on the gill filaments of *Haliotis spadicea* Donovan, 1808 and *Scutellastra barbara* (Linnaeus, 1758)* respectively from the south coast of South Africa.

	Number of striations Host *M. spadiceae*	Number of striations Host *M. branchi*
Peristome	8.0-22.0 (14.2±4.6,10)	7-9 (8.0, 3)
Peristome to Cinctum	54.0-118.0 (86.7±17.3,10)	66.0-96.0 (78.6±8.8,13)
Total number of striations	62.0-140.0 (100.9±20.5,10)	66-116 (82.4±12.8, 13)

* A new phylogenetic classification for the patellid limpets was suggested by Ridgway, Reid, Taylor, Branch and Hodgson (1998), grouping the patellid limpets in four monophyletic genera, namely *Helcion* Montfort, 1810; *Cymbula* H. & A. Adams, 1854; *Scutellastra* H. & A. Adams, 1854 and *Patella* Linnaeus, 1758, with the latter genus not occurring in South Africa. All limpets were formerly placed in the genus *Patella*.

Table 3. Summary of the taxonomic characteristics of the species of the family Ellobiophryidae: *Ellobiophrya donacis* Chatton & Lwoff, 1923, *E. conviva* Clamp, 1982, *E. oblida* (Naidenova & Zaika, 1969), *E. brevipes* (Laird, 1959) and *Caliperia longipes* Laird, 1953. Ma – macronucleus, Mi – micronucleus.

Ellobiophrya species	***E. donacis***	***E. conviva***	***E. oblida***	***E. brevipes***	***C. longipes***
Host	*Donax vittatus* Marine bivalve mollusc	*Bugula neritina, B. turrita* Marine ectoprocts (Bryozoa)	*Proterorhinus marmoratus* Marine fish	*Raja erinacea* Marine fish	*Oliverichtus melobesi, Ericentrus rubrus*,Marine fish
Position on host	Gill filaments	Ciliated tentacles around mouth	Skin	Gills	Gills
Collection locality	Morgat, France	North Carolina, USA	Black Sea	New Brunswick, USA	Wellington, New Zealand
Body length (μm)	50 (100)	46.2	180	60.2 (54.5)	31.2-68.4 (51.5)
Body diameter (μm)	40 (30)	26.8	36.5	34.6 (35.7)	24.0 – 52.6 (38.8)
Body and nuclei	Body subcylindrical, elongate, tapers towards oral pole Ma= compact and spherical, Mi= fusiform	Body subcylindrical, elongate slightly, tapers towards aboral pole Ma= cylindrical, length of soma, Mi= fusiform/oval	Body cylindrical, subconical, tapers towards aboral pole Ma=cylindrical, Mi= fusiform	Body cylindrical, elongate, subconical, tapers towards aboral pole Ma= cylindrical, long and flat Mi= fusiform	Body cylindrical, tapers towards aboral pole Ma=cylindrical Mi=fusifrom/lenticular
Cinctum	Limbs joined, bouton No internal rod Myoneme, contractile	Limbs joined (cemented at tips), bouton No internal rod, contractile	Limbs joined, bouton No internal rod Myoneme	Limbs joined, bouton No internal rod Myoneme	Limbs not joined No bouton 5-6 μm
Ampulla shape	Narrow and lanceolate	Wide and bulbous	Not described	Long, slender, tapering smoothly into cytopharynx Small in diameter, elongate, almost tubular , narrow, lanceolate when not filled	Resembles pipette bulb Cytostomal sphincter between infundibulum and ampulla
Cytopharynx	Not described	Elongate, ends near aboral end of macronucleus	Not described	Long sineous tube discharging near posterior part of macronucleus	Large and funnel-shaped Ends blindly posterior at macronucleus
Expanded peristome	Prominent argentophilic cytostomal sphincter Uneverted perstomial lip Peristome smaller in diameter than body	Argentophilic cytostomal sphincter not prominent Uneverted perstomial lip Subequal in diameter	Argentophilic cytostomal sphincter not described Widest at peristome with thickended peristomial lip	Argentophilic cytostomal sphincter at entrance to peristome Uneverted peritomial lip	Argentophilic cytostomal sphincter present Peristomial disc invaginated
Extent of infundibulum	Does not extend far beyond peristome	Approximately a third of the distance from peristome to sphincter	Extends approximately a third of the distance from peristome to cinctum	Short, ends at ampulla, quarter of distance from peristome to aboral end of body	Haplo- and polykinety make one and one quarter turns before plunging in
Pattern of infundibular kinetids	Not described	Rows in P2 end abstomally far short of junction of P1 with polykinety	Not described	Rows in P2 extend abstomally almost to junction of P1 with polykinety	Not described
Pellicular striations	Closely spaced, faint striae	Prominent transverse striae	Closely spaced, faint striae	Closely spaced, faint striae	Smooth pellicle (no annuli)
Inclusions	Cytosplasmic inclusions (type 1 and 2)	Greenish areas in body (diatoms/algal cells)	Not described	Not described	Greenish, yellowish spherules (algal origin)
Larval stalk and embryophore	Well developed larval stalk and cylindrical embryophore	Temporary stalk in telotroch Embryophore present (shorter and not as thick as in *E. donacis*)	Not described	Short, straight, rigid stalk, larger in diameter than *E. conviva*, less conspicuous than in *E. donacis*	Not described

Fig. 1. Microscope projection drawing of *Ellobiophrya maliculiformis* sp. n. found as an secondary symbiont on *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001, *M. midae* Botes, Basson & Van As, 2001 and *M. branchi* Van As, Basson & Van As, 1998 occurring on the gills of *Haliotis spadicea* Donovan, 1808, *H. midae* Linnaeus, 1758 and *Scutellastra barbara* (Linnaeus, 1758) collected along the south coast of South Africa. ad – adoral ciliary spiral, c – cinctum, cj – cinctal junction, cl – cinctal limb, ma – macronucleus, mi – micronucleus, pl – peristomal lip, ps – pellicle striations, sa – symbiotic algae. Scale-bar - 10μm.

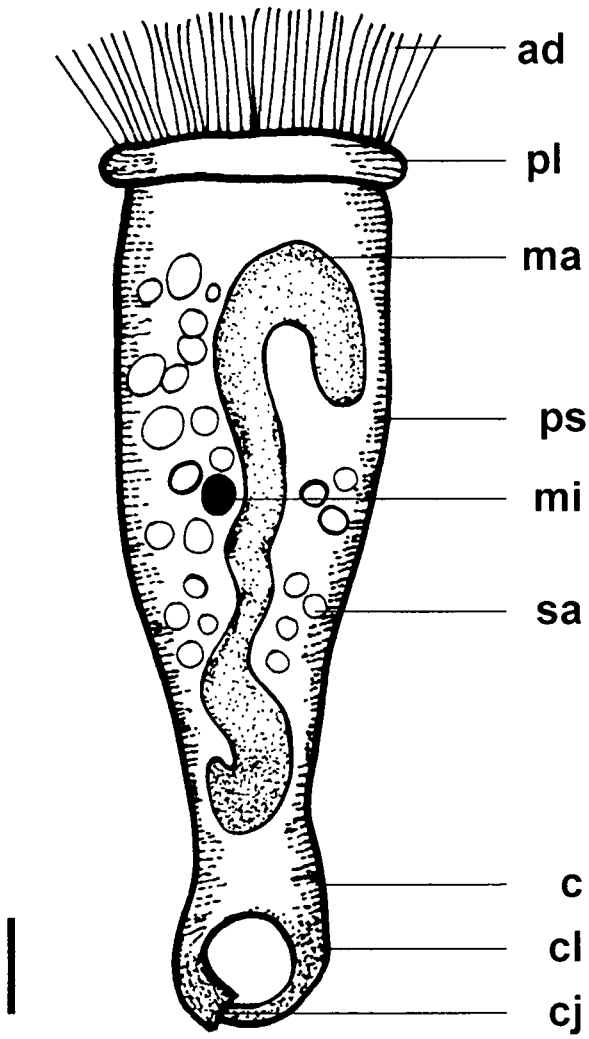
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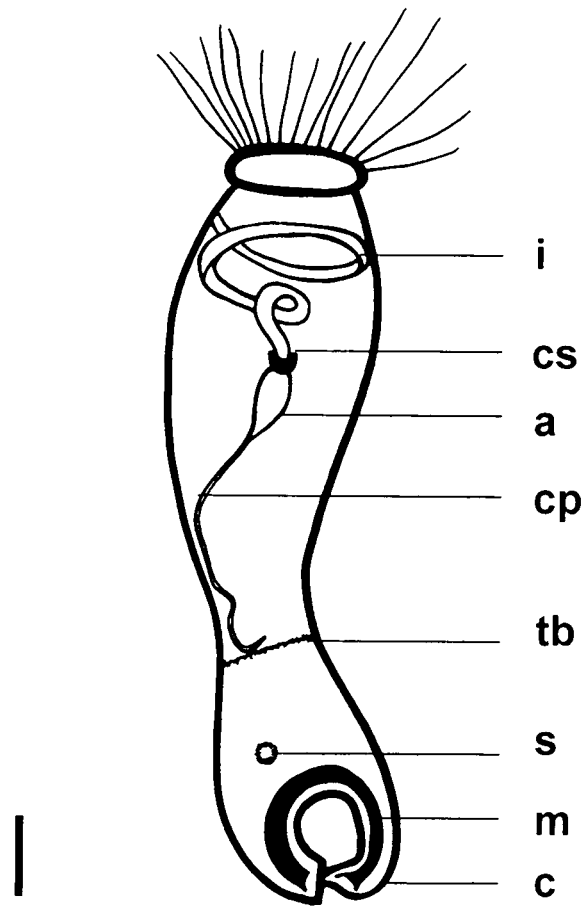
Fig. 2. Diagrams illustrating the infraciliature of *Ellobiophrya maliculiformis* sp.n. found as an secondary symbiont on *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001, *M. midae* Botes, Basson & Van As, 2001 and *M. branchi* Van As, Basson & Van As, 1998 occurring on the gills of *Haliotis spadicea* Donovan, 1808, *H. midae* Linnaeus, 1758 and *Scutellastra barbara* (Linnaeus, 1758) collected along the south coast of South Africa. **A** – Haplo- and polykinetids. **B** – Infundibulum. a – ampulla; cp – cytopharynx; cs – cytostomal sphincter; hk – haplokinety; i – infundibulum; pk – polykinety; s – scopula; tb – telotroch band; m – myoneme; c – cinctum. Scale-bars - 10μm.

A

B



Figs. 3-13. Scanning electron micrographs (**4,6-8**) and photomicrographs of live (**3,13**) and protargol stained specimens (**5,9-12**) of *Ellobiophrya maliculiformis* sp.n. occurring as an secondary symbiont on *Mantoscyphidia spadiceae* Botes, Basson & Van As, 2001, *M. midae* Botes, Basson & Van As, 2001 and *M. branchi* Van As, Basson & Van As, 1998 on the gills of *Haliotis spadicea* Donovan, 1808, *H. midae* Linnaeus, 1758 and *Scutellastra barbara* (Linnaeus, 1758) collected along the south coast of South Africa. **3** – Live specimen of *E. maliculiformis* with protruding adoral cilia attached to *M. spadiceae*, **4** - Detached *E. maliculiformis*, upper part of the body partially contracted, **5** – Bifurcated structure at the tip of the myoneme in the cinctum. Scopula indicated by arrow, **6** – Cinctum. Scopula indicated by arrow, **7** – One limb of cinctum tapers, fitting into the cinctal junction of the shorter, broader limb, **8** – Attachment of cinctum around *Mantoscyphidia spadiceae*, **9-12** – Protargol stained specimens, **13** – Microconjugant attached to ellobiophryid associated with *M. spadiceae*. a – ampulla; c – cinctum; cl – cinctal limb; cp – cytopharynx; cs – cytostomial sphincter; mc – microconjugant; pl - peristomial lip, s – scopula; sa - symbiotic algae; tb – telotroch band. Scale bars - 10μm (**3,4,9-13**) and 1μm (**5-8**).

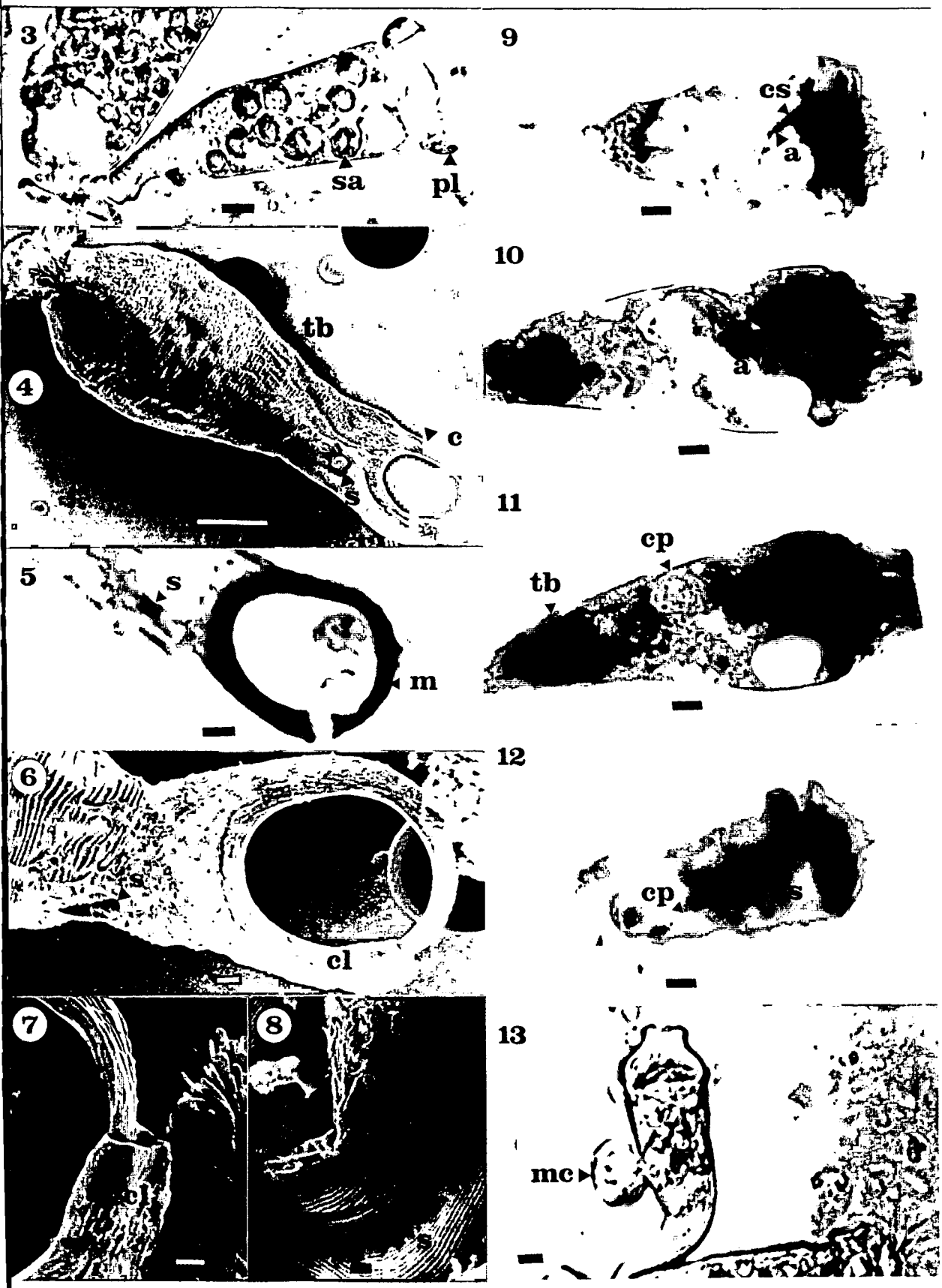

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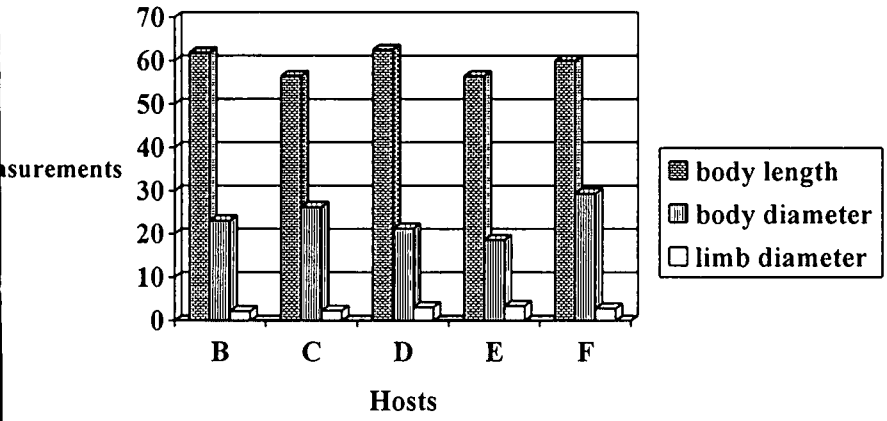
Fig. 14. Comparison in the variation of body length, body diameter and cinctal diameter of different populations of *Ellobiophrya maliculiformis* sp.n. found attached to *Mantoscyphidia midae* Botes, Basson & Van As, 2001 (B) and *M. branchi* Van As, Basson & Van As, 1998 (C-F) associated with haliotid (B) and limpet species (C-F) collected along the south coast of South Africa.



Abstract / Opsomming

ABSTRACT

During the early 19th century a lot of progress was made in clarifying reproduction patterns and cycles in the kingdom Protozoa Goldfuss, 1818. During the first half of the 20th century various scientists studied the reproductive processes of peritrichs and made valuable contributions. While thousands of recent papers exist on reproduction of ciliates, not much work has been done on epibiontic peritrichs. In this study the processes of binary fission, telotroch formation and conjugation are described for *Mantoscyphidia branchi* Van As, Basson & Van As, 1998, *M. marioni* Van As, Basson & Van As, 1998, *M. midae* Botes, Basson & Van As, 2001 and *M. spadiceae* Botes, Basson & Van As, 2001. It was also determined whether binary fission, telotroch formation and conjugation occur in a new species of *Ellbiophrya* Peters, Van As, Basson & Van As, in prep. Haliotids were collected from 1997 to 2002 at the De Hoop Nature Reserve on the south coast of South Africa and were also obtained from the Danger Point Abalone Farm near Gansbaai, and the Abagold Farm in Hermanus. Limpets were collected from 1993 to 2002 from the Goukamma Nature Reserve, De Hoop Nature Reserve and Keurboom Beach on the south coast; Mc Dougall's Bay and the Olifants River Mouth on the west coast; Bazley and at the rocky shores of Lake St. Lucia on the east coast of South Africa; and on the east coast of Marion Island at Boulder Beach which is situated in the southern Indian Ocean. Live symbiont specimens undergoing reproduction were observed with light microscopy and photomicrographs were taken of the various stages of binary fission, conjugation and telotroch formation. Mayer's, Harris' and Heidenhain's Iron Hematoxylin were used to stain the nuclear apparatus. The details of the infundibulum were studied by staining Bouin's fixed smears with protargol. Gills were also examined using scanning electron microscopy. Binary fission was mostly observed in *M. spadiceae* and also occurred earlier in *M. spadiceae* than in *M. branchi* and *M. midae* populations. The formation of telotrochs was mostly observed in populations of *M. branchi* and *M. spadiceae*. Throughout this study it was uncertain whether binary fission always has to occur before a telotroch can develop, or whether binary fission follows telotroch formation. Conjugation was mostly observed in populations of *M. branchi* and *M. midae*. In the *Ellobiophrya* species conjugation was also observed and this is the first record of conjugation in the genus *Ellobiophrya* (Chatton & Lwoff, 1923). It would be possible to study all these processes in detail if a suitable medium could be cultured in future.

Keywords: reproduction, scyphidiid peritrichs, limpets, haliotids, binary fission, telotroch formation, conjugation.

OPSOMMING

Gedurende die vroeë 19de eeu is baie vooruitgang in die verklaring van voortplantings patrone en siklusse in die koninkryk Protozoa Goldfuss, 1818 gemaak. Gedurende die eerste helfte van die 20ste eeu het verskeie wetenskaplikes die voortplantings prosesse van die verteenwoordigers van die Peritrichia Stein, 1859 bestudeer en waardevolle bydraes gelewer. Alhoewel daar duisende onlangse wetenskaplike publikasies bestaan oor voortplanting in siliate, is daar nie veel werk op verteenwoordigers van epibiontiese Peritrichia gedoen nie. In hierdie studie word die prosesse van tweedeling, telotrogvorming en konjugasie vir *Mantoscyphidia branchi* Van As, Basson & Van As, 1998, *M. marioni* Van As, Basson & Van As, 1998, *M. midae* Botes, Basson & Van As, 2001 en *M. spadiceae* Botes, Basson & Van As, 2001 beskryf. Tydens die studie is daar ook bepaal of tweedeling, telotrogvorming en konjugasie in 'n nuwe spesie van *Ellbiophrya* Peters, Van As, Basson & Van As, in voorbereiding voorkom. Perlemoen is vanaf 1997 tot 2002 by die De Hoop Natuurreservaat langs die suidkus van Suid Afrika en ook vanaf die Danger Point perlemoenplaas naby Gansbaai en die Abagold perlemoenplaas in Hermanus versamel. Klipmossels is vanaf 1993 tot 2002 by die Goukamma Natuurreservaat, De Hoop Natuurreservaat en Keurboomstrand langs die suidkus; Mc Dougall's Baai en die Olifantsriviermond langs die weskus; Bazley en die rotsstrande van die St. Lucia Meer langs die ooskus van Suid Afrika; en langs die ooskus van Marion Eiland by Boulder Beach wat in die suidelike Indiese Oseaan geleë is, versamel. Lewende simbionte wat besig was om voort te plant is waargeneem deur ligmikroskopie en fotos is van die verskillende stadiums van tweedeling, telotrogvorming en konjugasie geneem. Mayer, Harris en Heidenhain Yster Hematoksilien is gebruik om die kerne te kleur. Die besonderhede van die infundibulum is bestudeer deur Bouin's gefikseerde smere met protargol te kleur. Kieue is ook met skandeerelektronmikroskopie bestudeer. Tweedeling is meestal in *M. spadiceae* waargeneem en is ook vroeër in *M. spadiceae* as in *M. branchi* en *M. midae* bevolkings waargeneem. Die vorming van telotroë is meestal in bevolkings van *M. branchi* en *M. spadiceae* waargeneem. Tydens hierdie studie was dit egter nie duidelik of tweedeling altyd voor telotrogvorming plaasvind en of tweedeling telotrogvorming volg nie. Konjugasie is meestal in bevolkings van *M. branchi* en *M. midae* waargeneem. Konjugasie is ook waargeneem in die *Ellobiophrya* spesie en hierdie is die eerste rekord van konjugasie in die genus *Ellobiophrya* (Chatton & Lwoff, 1923). Dit sal moontlik wees om hierdie prosesse in die toekoms in meer besonderhede te bestudeer as 'n geskikte kultuurmedium ontwikkel kan word.

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