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Binary Fission, Telotroch Formation and Conjugation in *Mantoscyphidia* Jankowski, 1980 (Ciliophora: Peritrichia)

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Summary. Mantoscyphidians occur in high numbers on the gills of South African *Haliotis* and limpet species. This provided the ideal opportunity to study asexual and sexual reproduction, for the first time in different African *Mantoscyphidia* Jankowski, 1985 populations. Descriptions of the reproductive processes found in scyphidiid peritrichs were until now, mostly based on line drawings. Scanning electron- and light microscopy contributed uniquely, in providing detail information regarding binary fission, telotroch formation and conjugation. *Mantoscyphidia spadiceae* Botes, Basson and Van As, 2001 shed the most light on binary fission. Micronuclear division occurred first with the plane of fission already evident and macronuclear cleavage ended just before final separation took place. Fission is not complete until a small string of pellicle that joins the daughter cells finally separates. Information on telotroch development was gathered from *M. branchi* Van As, Basson and Van As, 1998 and *M. spadiceae* populations. Telotrochs were between 36.7 and 38.6% shorter than live, extended mantoscyphidians and their swimming action resembled mobiline peritrichs. *Mantoscyphidia branchi* populations also proved to be most useful for conjugation studies. Conjugation included three progamic nuclear divisions and preceded synkaryon formation and two metagamic nuclear divisions. The process required 24 hours to be completed.

Key words: Reproduction, Scyphidiidae, *Mantoscyphidia branchi*, *M. spadiceae*, *M. midae*, South Africa.

INTRODUCTION

The mode of reproduction in ciliophorans is through fission, also known as cell division (Corliss 1979). Various authors mention many kinds of fission and according to Foissner (1996) three basic types of cell division occur, i.e. enantiotropic, homothetogenic and parallel

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fission. Division can furthermore also be monotomic or polytomic, the former is typical of most ciliophorans and results in two filial products, the proter (anterior daughter) and the opisthe (posterior daughter). According to Foissner (1996), polytomic fission by palintomy, strobilation or budding is common amongst apostomes, astomes and suctorians. In the phylum Ciliophora Doflein, 1901, division mostly takes place by transverse (homothetogenic/homopolar) binary fission and rarely by budding or multiple fission (Lom and Dykova 1992, Foissner 1996). In homothetogenic cell division, the axes of the proter and opisthe have the same

orientation, i.e. the proter's posterior end is in contact with the opisthe's anterior end. Enantiotropic division, as is found amongst the oligotrichs, results in an inverse homothety and slight shift of the body axes via pronounced morphogenetic movements during stomatogenesis (Corliss 1979, Foissner 1996). This mode of division is not restricted to oligotrichs, but is also found in a modified form in peritrichs and licnophorids.

The process of binary fission was observed in populations of the scyphidiid peritrichs *Mantoscyphidia branchi* Van As, Basson and Van As, 1998, *M. midae* Botes, Basson and Van As, 2001 and *M. spadiceae* Botes, Basson and Van As, 2001 (Van As *et al.* 1998, Botes *et al.* 2001). The description of the process is based upon observations made mostly on populations of *M. spadiceae*.

The process of telotroch formation is well documented by Surber (1943), Davis (1947), Thompson *et al.* (1947), Raabe (1952), Hobbs and Lang (1954), Dobrzańska (1961), Vávra (1961) and Walker *et al.* (1986) for numerous colonial and solitary ciliophorans.

In the present study telotroch formation was frequently observed in populations of *M. branchi* and *M. spadiceae* and the description of the process will therefore be based upon observations made on these two populations.

According to Anderson (1988) there are three fertilisation modes in sexual reproduction, namely gametogamy, autogamy and gamontogamy. Conjugation (which is a type of gamontogamy) in ciliophorans, is fundamentally similar among a wide variety of species, but 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).

The sexual process of conjugation was mostly observed in *M. branchi* and to a lesser extent in *M. midae* and *M. spadiceae*, therefore the description is based on observations made on the first species.

MATERIALS AND METHODS

South African haliotids and various limpets were collected where after the gills were dissected and examined using a compound microscope for the presence of ciliophorans in the process of asexual and sexual reproduction. Photomicrographs were taken of live specimens for the purpose of determining body dimensions. Wet smears were prepared as described in Van As *et al.* (1998),

Botes *et al.* (2001) and Peters *et al.* (2004). For scanning electron microscopy, gills were fixed and prepared as described in Peters *et al.* (2004).

The body dimensions as well as the position and shape of the nuclei and the development of the infraciliature of peritrichs undergoing binary fission and conjugation were determined with microscope projection drawings, light micrographs and scanning electron micrographs. For measurements of live and Bouin's-fixed haematoxylin stained specimens, minimum and maximum values are given, followed in parentheses by the arithmetic mean, standard deviation (in the case of more than ten specimens) and number of specimens measured.

RESULTS

Bodies of *Mantoscyphidia* Jankowski, 1980 species are cylindrical and extremely contractile, the peristomial areas range from flattened to arched, and the scopula is prominent and broad. The telotroch band is elevated and situated about one third of body length from the scopula. The adoral zone completes a spiral of 540°, before plunging into infundibulum. The cytoplasm is homogenous to granular depending on the species. In all the species the macronucleus is large with an ovoid micronucleus. The occurrence of symbiotic algae is a common phenomenon amongst ciliophoran species, but even today there is still a lack of understanding of the systematics and true association of these algae. Symbiotic algae, which varied in size (3–6 μm) and numbers, were found in all the *Mantoscyphidia* species and in some populations details of the internal organelles were severely obscured by the algae. In the case of *M. branchi* and *M. spadiceae* the algae occurred adoral to the trochal band, whilst in *M. midae* it was found throughout the cytoplasm even surrounding the nuclear apparatus (Van As *et al.* 1998, Botes *et al.* 2001).

Binary fission

This process was observed in all the species, but the description is based mostly on populations of *Mantoscyphidia spadiceae*. Division took place in the active condition, individuals on the verge of binary fission were more contracted or plump (Figs 1A, 2A), compared to those that were not yet ready for fission (see Table 1). The peristome was tightly closed with no adoral cilia protruding, and the peritrich ceased all feeding activities. As a result of body contraction the peristomial region's striations formed a zigzag pattern and this closed region was elevated in the middle to form a knob-like protrusion.

The micronucleus enlarged, left the vegetative position and divided through mitosis to form two micronuclei (Fig. 2B). One of the micronuclei stayed in the one daughter cell, whilst the other moved into the second daughter cell (Figs 1B, 2B). During and immediately after division of the micronucleus the plane of fission at the adoral and aboral ends was already evident (Fig. 2C). The two newly formed micronuclei usually moved to a position closer to the adoral or middle region of the dividing peritrich's body (Fig. 1C).

Simultaneously, the macronucleus had enlarged, elongated and assumed a position in the middle of the peritrich's dividing body. The broad, elongated macronucleus (Fig. 2D) very often stretched to occupy the whole width of both peritrich bodies and was sometimes horseshoe-shaped. Development and duplication of the infraciliature and adoral cilia differentiation could be distinguished, while the macronuclear growth was in progress (Figs 1C, 2C). At this stage the peristomial region was still closed with cilia drawn inwards.

While the macronucleus prepared for cleavage through amitotic division, spindle-shaped structures could be seen (Fig. 2D). Similar thread-like spindle-shaped structures were also observed in the micronuclei (Fig. 1B). Macronuclear cleavage into two equal parts ended just before final fission took place (Figs 1D, 2E).

Specimens of both *M. branchi* and *M. spadiceae* 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 fully completed. Each individual's infundibulum could be observed in the stage before final separation took place in Protargol- (Fig. 1E) and haematoxylin-stained as well as live specimens examined. It was also observed that both individuals were able to contract independently of the other, but if one individual contracted, the other was influenced to also contract to some degree.

While the micronucleus was dividing, the scopula divided into two scopular regions (Figs 2B, 4A). The scopula often divided before the plane of fission was apparent (Figs 1B, 4A). The next stage of fission between the two individuals was observed adoral to the scopula as well as in the adoral region of the peristome (Fig. 4B) however; a pellicle bridge is still evident (Figs 4C, D). The plane of fission progressed both adorally and aborally (Fig. 1F), until the two individuals were completely separate, as is illustrated in Fig. 2H. Throughout this fission procedure a strong bridge of pellicle, only visible in scanning electron micrographs, remained until only a small string of pellicle joined the individuals at the adoral region (Fig. 4D). Finally, complete separation of the two daughters took place through forceful contractions and rotations of both individuals (Figs 2F – H), finally severing the string of pellicle.

At this stage, the macronuclear material had a more granular appearance. The macronucleus and micronucleus of each individual moved back to the vegetative positions below the telotroch band (Fig. 2H). The individuals formed after binary fission, were two slightly unequal daughters. It is assumed that one will probably grow into the presumptive trophont and the other into the presumptive telotroch. In most peritrichs the smaller daughter transforms into a telotroch without delay. Immediate transformation was not observed in species of *Mantoscyphidia*. Stages on the verge of either detaching

Table 1. The body dimensions (μm) of fully extended live specimens (Botes *et al.* 2001, Van As *et al.* 1998), plump, completely contracted vegetative specimens preparing to undergo binary fission and the telotroch stages of *Mantoscyphidia branchi* Van As, Basson and Van As, 1998 and *Mantoscyphidia spadiceae* Botes, Basson and Van As, 2001.

Species	Body length	Body diameter
M. branchi fully extended live specimens	44–78 (65.0 ± 8.8, 14)	17–32 (24.2 ± 4.4, 14)
M. branchi completely contracted specimens	20.0–35.0 (28.0 ± 4.1, 17)	19.0–30.0 (21.1 ± 2.5, 17)
M. branchi plump specimens	21.7–32.2 (25.9 ± 4.1, 11)	23.3–44.9 (33.6 ± 7.1, 11)
M. branchi telotroch	14.6–37.5 (25.1 ± 5.2, 64)	13.0–30.0 (21.9 ± 3.2, 64)
M. spadiceae fully extended live specimens	70–140 (104.3 ± 21.1, 43)	20–40 (31.2 ± 6.7, 43)
M. spadiceae completely contracted specimens	49.0–60.0 (53.0 ± 4.7, 11)	25.0–43.0(34.3 ± 4.8, 11)
M. spadiceae plump specimens	18.3–37.0 (27.2, 7)	20.0–36.7 (30.1, 7)
M. spadiceae telotroch	21.4–56.2 (38.3 ± 8.4, 34)	16.2–30.0 (22.7 ± 3.7, 34)
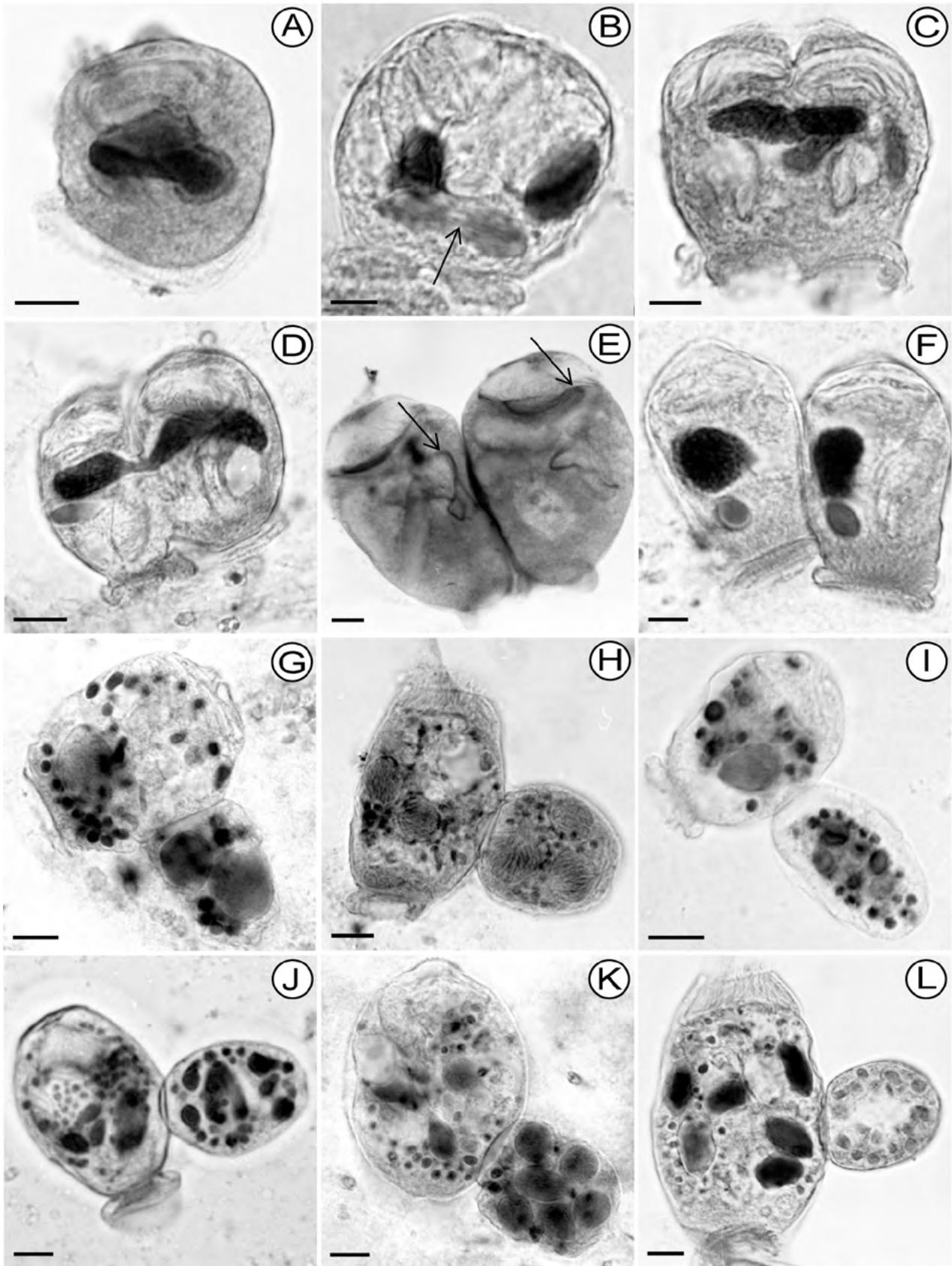
from or settling on the substrate had adoral cilia protruding and infraciliature movement occurring (these were feeding trophonts). Telotrochs partially resorbed their cilia and did not feed after settling and completing metamorphosis.

Telotroch formation

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

At the onset of telotroch formation the peristome closed and a swelling occurred in the middle region of the body (Fig. 3A), where three or four rows of basal kinetosomes appeared. This emerging ciliary girdle is associated with a concurrent 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 cilia (Fig. 3B).

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 (Fig. 3C). In the fully formed mobile telotrochs, the cilia of the ciliary girdle were about 10 μm long.

Telotrochs were round and the whole pellicle was adorned with striations, 0.2–0.25 μm apart, and possessed a ciliary band. Telotrochs were active and swimming fast. It was observed that the fully formed telotrochs moved in much the same way as the mobiline peritrichs, with the ciliary wreaths beating, in search of a suitable host or substrate to settle on.

During the transformation of the telotroch into a settled form, the scopula gradually increased in size, with the central cytoplasm flowing into the scopula. The scyphidiid peritrich elongated (Fig. 3D) 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. 3D). Finally, the ciliary girdle lost its activity.

The macronucleus length of a *M. spadiceae* telotroch was greater than that of *M. branchi* although *M. branchi* telotrochs had the greatest macronuclear diameter as well as the greatest length of the sectors of termination in the macronucleus. *Mantoscyphidia spadiceae* telotrochs, however, had the greatest micronucleus length and diameter (Table 2). The macronucleus of the telotroch of *M. spadiceae* occupied on average 93% of the body diameter, while the macronucleus of *M. branchi* telotrochs occupied 73.5%.

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 therefore an average shortening of 36.7% that took place during transformation into the telotroch stage. In *M. branchi* this shortening was similar, namely 38.6% (see Table 1).

The average body lengths of *M. branchi* and *M. spadiceae* telotrochs (Table 1) differed significantly, with *M. spadiceae* telotrochs having a much greater average body length than those of *M. branchi*. The average body width of the two species' telotrochs did not differ significantly and the average body width was less than the average body length.

Conjugation

The peristomial regions of macroconjugants were not always completely closed during the process of conjugation. Some were slightly open, with a small tuft of adoral cilia protruding, while others had open per-

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Fig. 1. Light micrographs of hematoxylin stained (A–D, F–L) and protargol impregnated (E) specimens of *Mantoscyphidia* spp. before, during, and after binary fission (A–F) and undergoing conjugation (G–L). Small dark stained granules are symbiotic algae. The species are *Mantoscyphidia branchi* Van As, Basson and Van As, 1998 (A, G–L) associated with *Scutellastra barbara* (Linnaeus, 1758) and *M. spadiceae* Botes, Van As and Basson, 2001 (B–F) associated with *Haliotis spadicea* Donovan, 1808. **A** – plump individual; **B** – micronucleus has divided and macronucleus is undergoing division. Spindle-shaped structure is visible in macronucleus (arrow); **C** – scopulas divided, plane of fission apparent, infraciliature differentiated; **D** – final separation of macronuclei and onset of cleavage. Separate infraciliatures; **E** – protargol impregnation of infraciliatures of re-extending cells (arrows); **F** – nuclei move to vegetative positions, daughter individuals nearly separate; **G** – macro- and micronucleus of microconjugant undergoing mitosis; **H** – three nuclei undergoing mitosis in the microconjugant and two in the macroconjugant; **I, J** – progamic divisions taking place in both conjugants; **K** – seven nuclei formed in microconjugant through progamic division; **L** – six pronuclei in macroconjugant. Scale bars: A–F 20 μm, G–L 10 μm.
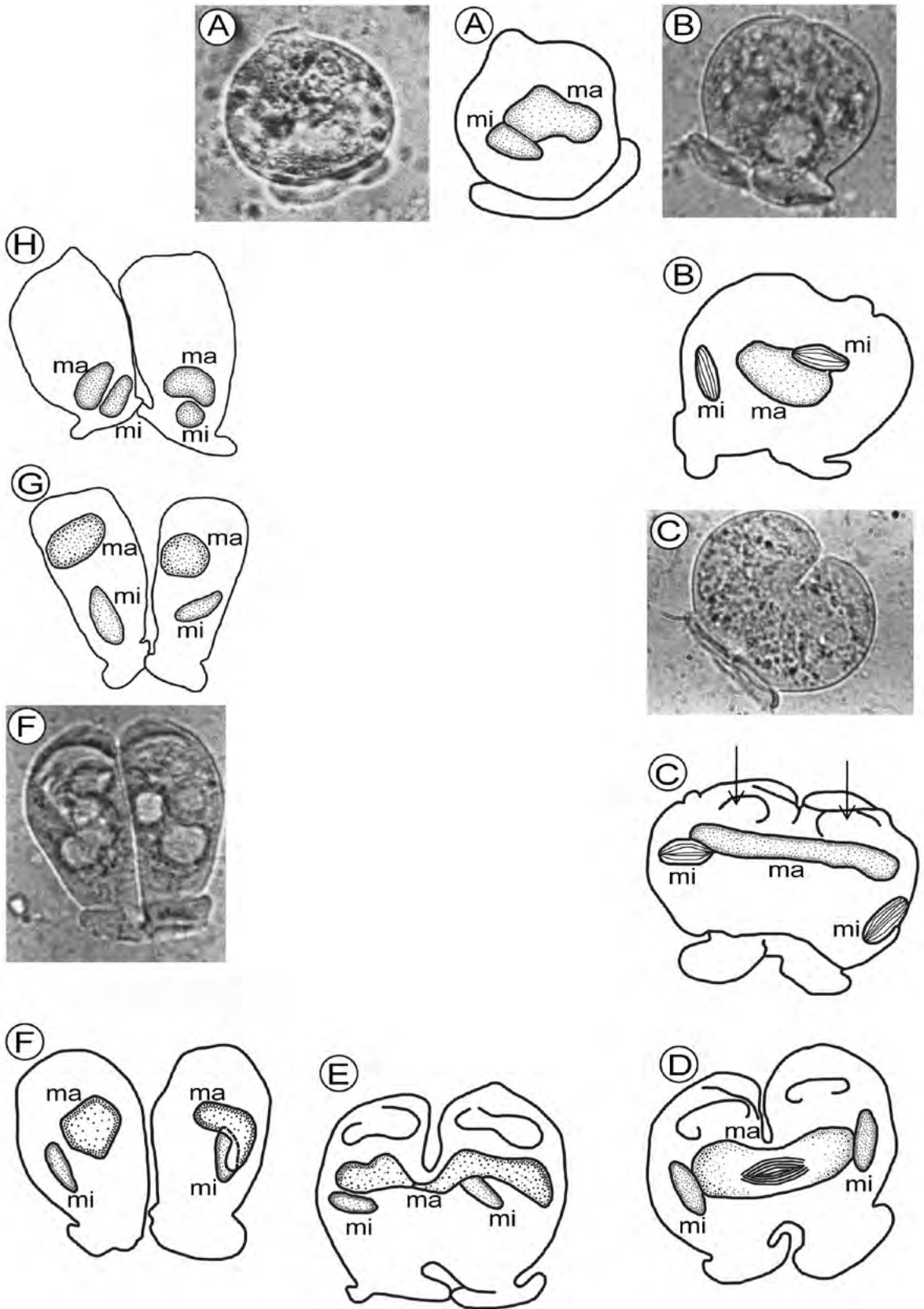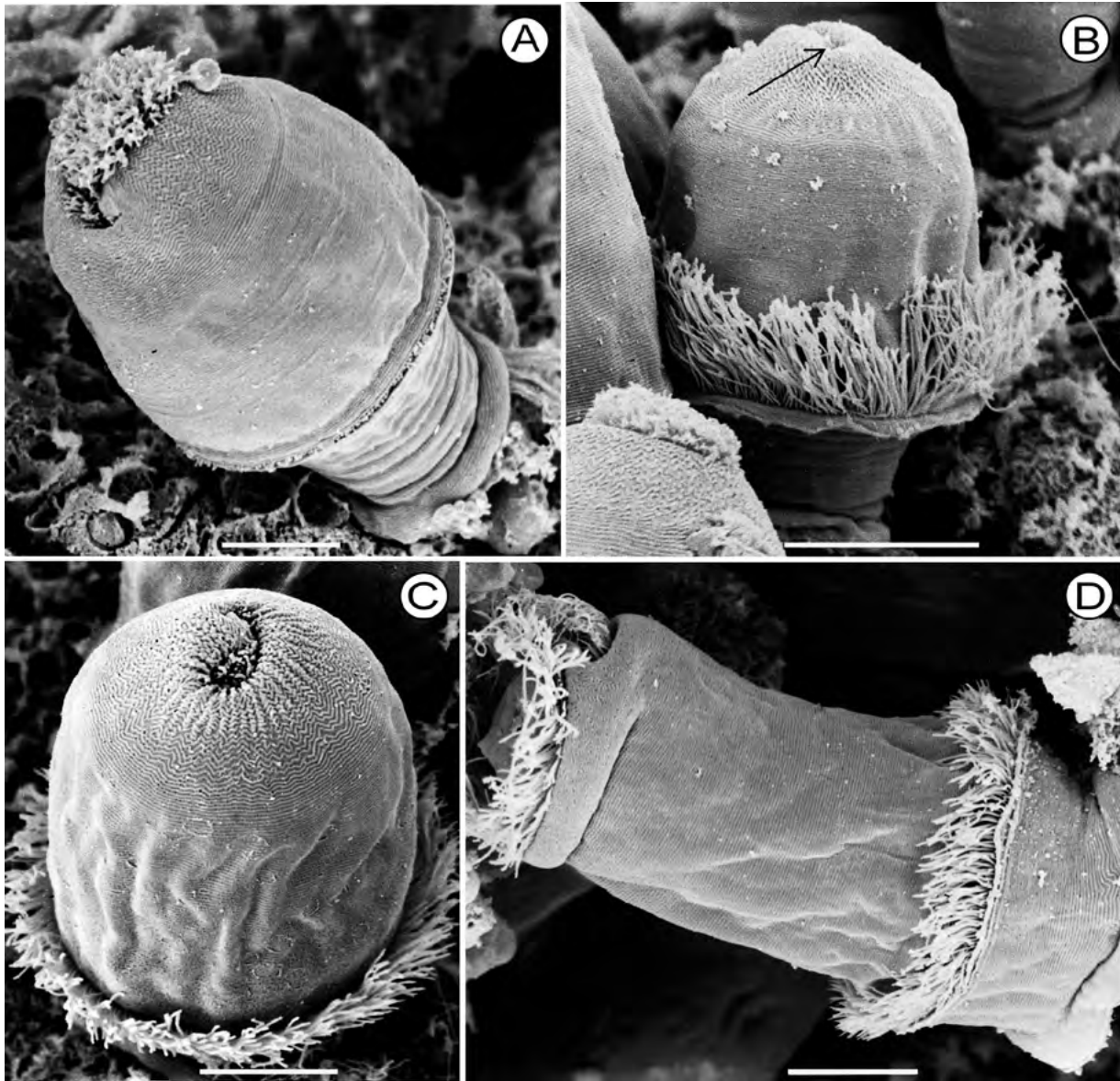
Fig. 3. Scanning electron micrographs of the telotroch stages of *Mantoscyphidia spadiceae* Botes, Basson and Van As, 2001 (A–C) associated with *Haliotis spadicea* Donovan, 1808 and *Mantoscyphidia midae* Botes, Basson and Van As, 2001 (D) associated with *Haliotis midae* Linnaeus, 1758. **A** – the scyphidiid peritrich's body swells; **B** – ciliary girdle emerges (arrow indicates closed peristome); **C** – fully developed telotroch; **D** – transformation into a settled form begins. Scopula enlarges and scyphidiid peritrich elongates. Adoral ciliary spiral open. Scale bars: 10 µm.

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Fig. 2. Diagrammatic illustrations and photomicrographs of live specimens of *Mantoscyphidia branchi* Van As, Van As and Basson, 1998 (A–C, F) from *Scutellastra barbara* (Linnaeus, 1758) and *Mantoscyphidia spadiceae* Botes, Van As and Basson, 2001 (D, E, G, H) from *Haliotus spadicea* Donovan, 1808 summarising the process of binary fission. **A** – plump individual about to commence binary fission; **B** – 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 whole width of scyphidiid peritrich's body. Micronuclei assume positions inside each daughter individual. Infundibular structures differentiate in each daughter individual; **D** – plane of fission becomes evident. Spindle formation evident in macronucleus as it undergoes mitosis; **E** – plane of fission progresses, with only small strand of macronuclear material situated in region of 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. ma – macronucleus, mi – micronucleus. Scale bar: 20 µm.

Table 2. Average nuclear dimensions measurements (μm) of *Mantoscyphidia branchi* Van As, Basson and Van As, 1998 and *M. spadiceae* Botes, Basson and 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. spadiceae
Macronucleus length	12.0–24.0 (16.1 ± 3.2, 39)	16.0–29.5 (21.1 ± 2.8,29)
Macronucleus diameter	4.5–13.5 (8.3 ± 2.3, 39)	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)	0–21.0 (11.9 ± 6.6, 29)
Micronucleus length	5.5–14.0 (9.2 ± 2.3, 39)	6.0–14.5 (9.8 ± 2.2, 29)
Micronucleus diameter	3.0–9.0 (4.9 ± 1.2, 39)	4.5–6.5 (5.4 ± 0.6, 29)

istomes with quite a number of cilia protruding (Fig. 4E). The peristomes of microconjugants were always closed with no cilia protruding. It is presumed that the conjugants accumulated enough food during preparation for conjugation, as these usually cannot feed during conjugation.

The microconjugant always assumed a position perpendicular to the long axis of the macroconjugant's body (Figs 1G, 4F). More than one microconjugant frequently fused with the same macroconjugant and the microconjugants were always observed attached at opposite sides of the macroconjugant (Fig. 4G). 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 (Fig. 4H) between the two conjugants and the microconjugant's endoplasm moved slowly into the macroconjugant (Fig. 5A).

Macro- and micronuclei of the microconjugant enlarged and filled the whole of the microconjugant's body (Fig. 1G). Spindle formation occurred in the nuclei (Fig. 1H) and the micronucleus underwent three progamic nuclear divisions through mitosis (Fig. 1I) to form seven nuclei in the microconjugant (Figs 1J, K). On occasion up to eleven nuclei were observed in

microconjugants. These nuclei moved into the macroconjugant (Fig. 1L). The latter also contained different numbers of pronuclei (Figs 5B, C). The macronuclei degenerated and were resorbed into the micro-and macroconjugants' cytoplasm.

The microconjugant became depleted and thinner in diameter (Fig. 4G) as it transferred its endoplasm to the macroconjugant (Fig. 5C). The metagamic divisions usually occurred before the shrivelled microconjugant disappeared. The synkaryon divided through two metagamic divisions and formed three macronuclear anlagen and one micronucleus (Fig. 5E). The micronucleus shrunk and the macronuclear anlagen fused to form the functional macronucleus (Fig. 5F). A progressive shrinking of the microconjugant occurred until it was just attached to the macroconjugant with a small piece of pellicle (Fig. 4I). The shrivelled and depleted microconjugant eventually fell off. Inside the macroconjugant progamic divisions had taken place in the macro- and 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.

One micronucleus from the microconjugant and one from the macroconjugant fused to form the synkaryon. All the other pronuclei took no further part in repro-

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Fig. 4. Scanning electron micrographs of dividing individuals (A–D) and specimens undergoing conjugation (E–I) of *Mantoscyphidia branchi* Van As, Van As and Basson, 1998 from *Cymbula compressa* (Linnaeus, 1758) (A, E, H), *Scutellastra barbara* (Linnaeus, 1758) (F, G, I) and *Mantoscyphidia spadiceae* Botes, Van As and Basson, 2001 (B–D) from *H. spadicea* Donovan, 1808. **A** – peristomial region's striations form a zig-zag pattern, scopula divides into two scopular regions; **B** – two peristomial regions become evident, plane of fission progresses, scopulas further divided; **C, D** – a small string of pellicle joins the individuals adorally, nearly final separation of two daughter individuals has taken place; **E** – the body of the macroconjugant is extended and it's peristome is open with adoral cilia protruding; **F** – microconjugant indicated by arrow. The appearance of the microconjugant changes from a rounded to a more elongated shape; **G** – two microconjugants attached to a single macroconjugant. Both microconjugants are long and thin. The contents of both have been transferred into the macroconjugant; **H** – enlargement of the area where the macro- and microconjugant fuse to form a protoplasmic bridge; **I** – microconjugant almost completely depleted and ready to fall off the macroconjugant. The macroconjugant is shrivelled. Scale bars: 10 μm.
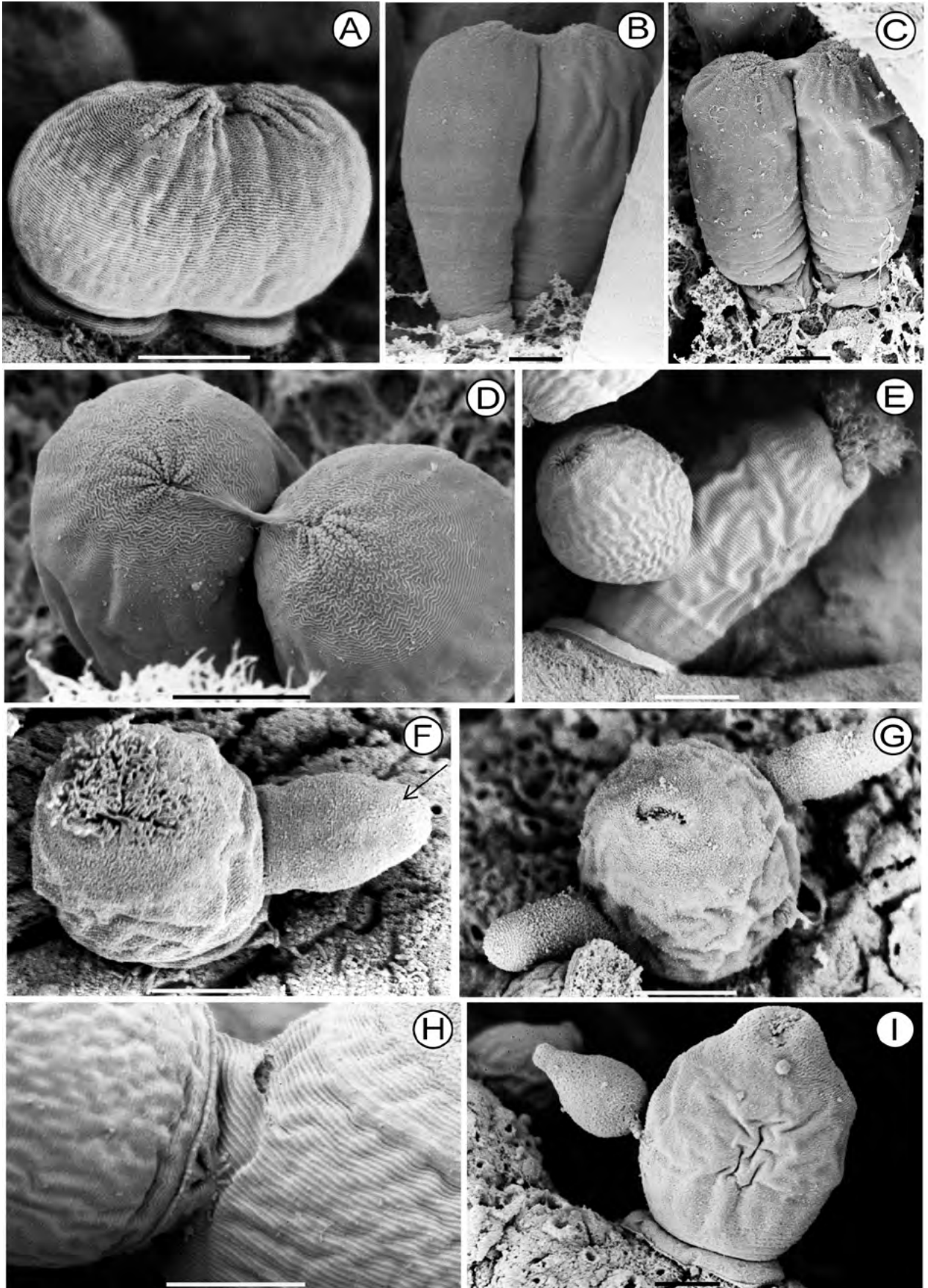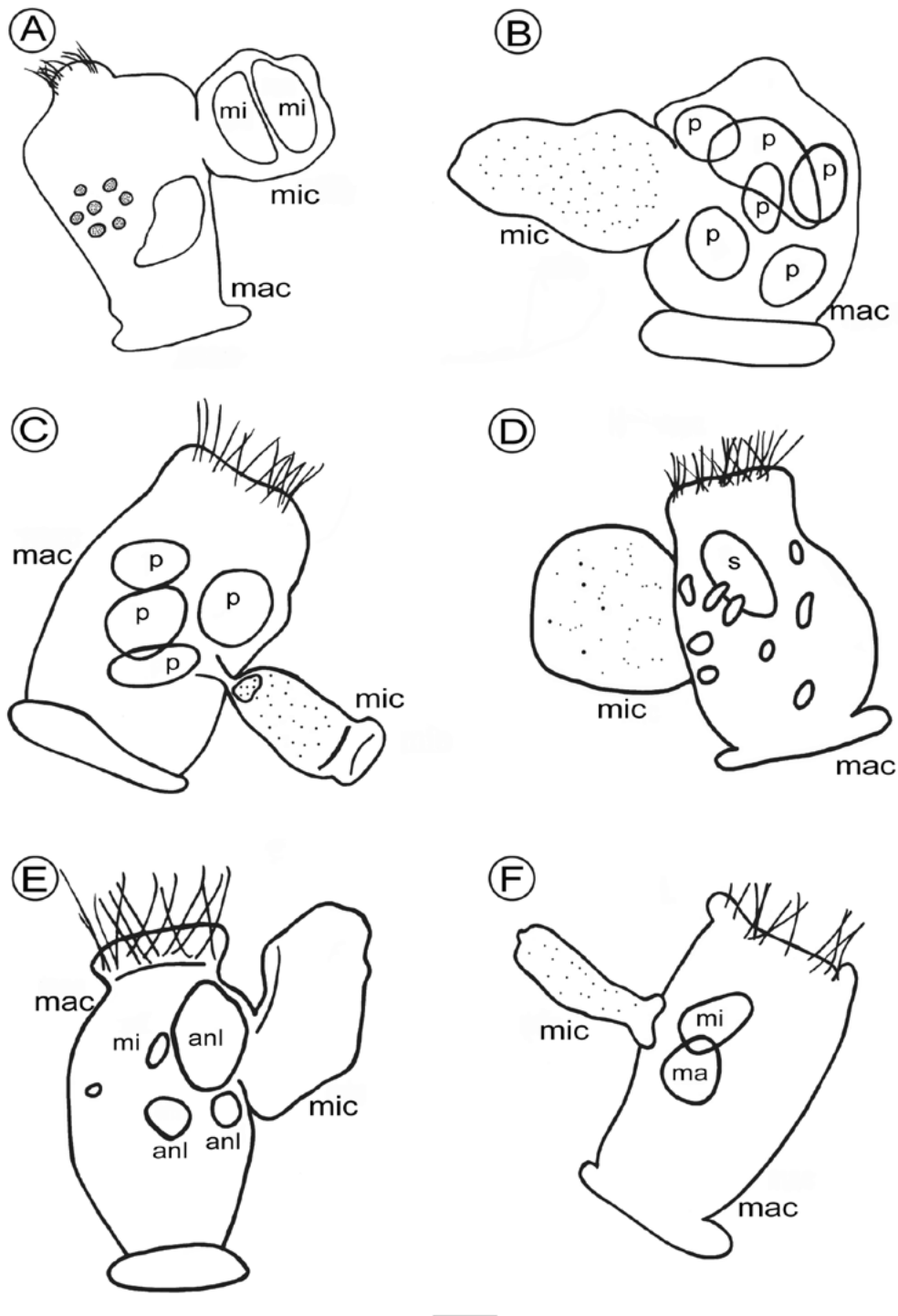
Fig. 5. Diagrammatic illustrations of live observations of *Mantoscyphidia branchi* Van As, Basson and Van As, 1998 associated with *Scutellastra barbara* (Linnaeus, 1758) undergoing conjugation. **A** – macro- and micronucleus of the microconjugant enlarges and fills whole cytoplasm of microconjugant's body; **B** – pronuclei visible in macroconjugant, which have moved from microconjugant into macroconjugant. The microconjugant is shriveled and depleted because the endoplasm is transferred into macroconjugant; **C** – four pronuclei visible. Degeneration of pronuclei is taking place; **D**, **E** – synkaryon formation. All other pronuclei degenerate, synkaryon divides into three macronuclear anlagen and a micronucleus; **F** – macronuclear anlagen develop into the functional macronucleus. Microconjugant thin and depleted. anl – macronuclear anlagen, ma – macronucleus, mac – macroconjugant, mi – micronucleus, mic – microconjugant, p – pronucleus, s – synkaryon. Scale bar: 10 µm.

duction; these degenerated and were resorbed into the cytoplasm of the exconjugant (Fig. 5F). The synkaryon was observed to be located adorally or aborally, and a darker, granular area was frequently observed in the centre of the exconjugant's body or close to the protoplasmic bridge (Fig. 5D). 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 start feeding during this part of the process of conjugation.

It is further presumed that the exconjugant could now undergo two consecutive divisions (binary fission) into four individuals, although these reorganisational fissions were not observed. These individuals will grow with the nuclei assuming the typical vegetative positions.

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 shrivelled microconjugant that emptied all of its content into the macroconjugant. The average macroconjugant length was 30.10 µm (Table 3) and the average body length of live vegetative individuals of *M. branchi* was 65.0 µm (Table 1). Macroconjugants were 46.3 % shorter than live vegetative individuals.

DISCUSSION

Binary fission

The process of binary fission in mantoscyphidians appears to be similar in most cases to that described for various other peritrichs. The plane of division in *Lagenophrys tattersalli* Willis, 1942, is parallel to the axis of the organism and not obliquely transverse to it (Willis 1942). Such longitudinal fission is typical of peritrichs, and means that it is impossible to identify the filial products as proter and opisthe, in the manner applicable to most ciliophorans. In *Ambiphrya ameiuri* Thompson, Kirkegaard and Jahn, 1947 longitudinal fission was observed with binary fission beginning at the basal and

distal ends and proceeded from both ends, until cleavage was completed (Thompson *et al.* 1947). This was also the case in *Mantoscyphidia,* but with the cleavage progressing slightly faster at the basal end resulting in a small string of pellicle remaining connected at the adoral end. The last stage of division in *A. micropteri* (Surber 1940) and longitudinal fission in *A. tholiformis* (Surber 1943) were observed in both cases of only a few individuals (Surber 1940, 1943). The macronucleus became rounded and centrally located and was in close association with the micronucleus lying posterior to it. The constriction of the body and the macronucleus occurred after division of the micronucleus. In the present study the micronucleus also maintained a position posterior to the macronucleus in all the individuals that were in the process of binary fission.

The small string of pellicle that always joined the adoral regions of separating daughter cells at the end of binary fission in the mantoscyphidians has not been reported in any other peritrichs to date. This was not observed in the present study when examining the mantoscyphidians using light microscopy, but was very clear in scanning electron micrographs. Fission is therefore not fully complete, as observed and accepted by most workers, after the plane of division has progressed adorally and aborally, since the two individuals, although seemingly completely separated, are still joined adorally by this small string of pellicle until they finally separate from one another.

In *Mantoscyphidia* the slightly unequal-sized daughter cells will grow into mature adults of more or less the same size. In preparation for binary fission the scyphidiid peritrichs need to build up energy reserves for when they won't be able to feed. The number of symbiotic algae in the peritrich may affect the appearance; it definitely obscures the internal changes that occur during binary fission and the other reproductive stages. These symbiotic algae are beneficial to the mantoscyphidians as an extra source of energy and probably even more important when the peritrichs are in the process of fission and therefore not capable of feeding.

Table 3. Average length and width (µm) of the microconjugants and macroconjugants of *Mantoscyphidia branchi* Van As, Basson and Van As, 1998 measured from scanning electron microscopy photographs.

Conjugants	Length	Width
Microconjugants	8.24–45.91 (19.23 ± 5.01, 105)	7.06–24.14 (15.38 ± 3.61, 105)
Macroconjugants	19.92–49.09 (30.10 ± 6.40, 102)	18.64–36.36 (25.71 ±3.66, 102)

Telotroch formation

Up to the early nineties, telotroch to zooid transformation had not been described for non-colonial peritrichs. Vacchiano *et al.* (1992) described a method to obtain large numbers of telotrochs specifically from mass cultures of *Vorticella convallaria* Linnaeus, 1758. Telotroch formation is brought about by gradual deterioration in conditions of either the host or the environment. Utz and Coats (2008) summarised conditions of telotroch formation obtained from laboratory results such as the observation that telotroch formation of *V. convallaria* occurred when the peritrichs were exposed to harsh environmental conditions and that stalked-type peritrichs (*Carchesium* sp. Ehrenberg, 1830) undergo telotroch formation if damaged as well as that telotrochs form as one product of binary fission during asexual division or by direct transformation of the zooids. The telotroch stage is regarded as the temporary, migratory larval stage of the scyphidiid peritrichs.

The peristomial regions of the mantoschypidian trophonts were open with adoral cilia protruding, enabling them to feed as infraciliature movement was noted. Mature telotrochs' peristomes were closed and no infraciliature activity was observed. It appears as if mature *Mantoscyphidia* telotrochs cannot feed because of the contracted peristome, which is similar to what was found for other peritrichs (Gilbert and Schröder 2003), but the cytoskeletal structures associated with feeding, such as the infundibulum and cytopharynx, are visible in cytoskeletal preparations of non-feeding telotrochs and the structures appeared to persist during telotroch formation (Vacchiano *et al.* 1992).

It is unclear whether binary fission in *Mantoscyphidia* always has to occur before a telotroch can develop, or whether binary fission always follows telotroch formation. Viljoen and Van As (1987) noted that in the case of *Vorticella convallaria* binary fission had to occur before a telotroch could develop. In *Epistylis anastatica* Linnaeus, 1767, however, the telotroch developed directly from a vegetative zooid. Gilbert and Schröder (2003) illustrated that telotrochs of *Epistylis pygmaeum* Ehrenberg, 1838 can give rise to swimming zooids and that swimming zooids can both reproduce themselves and also produce telotrochs.

A comparison of the measurements of the telotrochs of *M. branchi* and *M. spadiceae* with measurements of *Orbopercularia raabei* Dobrzańska, 1961 and *Ambiphrya tholiformis* indicated that the size ranges were very similar (Surber 1943, Dobrzańska 1961). Thomp-

son *et al.* (1947) found that the body width of *Ambiphrya ameiuri* was twice the body length. In some individuals of *M. branchi* and *M. spadiceae*, the body width of the telotrochs did exceed that of the length, but not twice the body length (see Table 1).

During live observations, the telotrochs swam vigorously once liberated, but no suitable substrate for attachment was available on the wet smears. During the transformation into a telotroch stage and vice versa, it was difficult to conclude from scanning electron microscopy photographs whether telotrochs were actually being formed or whether transformation into vegetative individuals was taking place because the one process seems to be a reversal of the other.

Conjugation

Conjugation occurred in dense populations of *Mantoscyphidia*. This might be a means of creating many individuals with recombined genes that are able to disperse to other suitable hosts.

Time: According to Finley and Nicholas (1950) the nuclear- and cytoplasmic phenomena during conjugation are similar for *Vorticella microstoma* Ehrenberg, 1830 and *Rhabdostyla vernalis* Stokes, 1887, requiring 18 to 24 and about 24 to 36 hours respectively for completion. In the case of *Mantoscyphidia branchi* and *M. midae* at least one hour and 15 minutes were required to form an endoplasmic bridge and conjugation also required 24 hours to be completed.

Fusion position: Attachment of the microconjugant to the upper, anterior or adoral third of the macroconjugant's body has been reported for *R. vernalis*, *Apiosoma* Blanchard, 1885, *Propyxidium* Corliss, 1979, *Epistylis* Ehrenberg, 1830, *Campanella* Goldfuss, 1820, *Opercularia* Goldfuss, 1820, *Carchesium, Zoothamnium* Bory de St. Vincent, 1826, *Ophrydium* Bory de St. Vincent, 1826 and *Lagenophrys* Stein, 1851 species. The microconjugants of *Vorticella* Linnaeus, 1767, *Opisthonecta* Fauré-Fremiet, 1906 and *Cothurnia* Ehrenberg, 1831 attached to the lower third of the macroconjugant's body (Finley 1943). In *Ambiphrya macropodia* Davis, 1947 and *A. tholiformis* the microconjugants attached to the macroconjugant near the peristome or to the anterior end of the peristomial border (Surber 1943, Davis 1947), whilst in *Ambiphrya ameiuri* the microconjugants attached to the distal one-third of the macroconjugant (Thompson *et al.* 1947). In *M. branchi* and *M. midae* the microconjugants fused to the middle region and lower third of the macroconjugant's body and

always assumed a position perpendicular to the long axis of the macroconjugant's body.

Number of micro-conjugants: *Ambiphrya tholiformis* macroconjugants usually have one microconjugant attached (Surber 1943). The phenomenon of two or more microconjugants fusing with the same macroconjugant has been reported for numerous ciliophorans (Finley 1939, 1943; Thompson *et al.* 1947). Amongst the mantoscyphidians more than one microconjugant frequently fused with the same macroconjugant and the microconjugants were always observed to be attached at opposite sides of the macroconjugant.

Feeding: *Vorticella microstoma* and *Rhabdostyla vernalis* conjugants continue feeding until they are ready to divide; each can secrete a posterior ciliary wreath and detach itself from its stalk; each can secrete a new stalk, whilst some conjugants can encyst (Finley 1939, 1943; Finley and Nicholas 1950). In *Mantoscyphidia*, some of the macroconjugants' peristomial regions were slightly open, with a small tuft of adoral cilia protruding while others were open with quite a number of cilia protruding. The peristomes of microconjugants, however, were always closed with no cilia protruding.

Progamic nuclear divisions: In *R. vernalis*, *V. microstoma* and *A. macropodia* three consecutive progamic divisions took place in the microconjugant's micronucleus (Finley 1952). The macroconjugant's micronucleus underwent two progamic divisions in *R. vernalis* and *A. macropodia* and resulted in only one functional pronucleus in *V. microstoma* functioning in fertilisation. In *Mantoscyphidia* the microconjugant's micronucleus also underwent three progamic nuclear divisions through mitosis to form seven nuclei. On occasion up to eleven nuclei or rounded structures were observed in microconjugants. Spindle formation was observed in the nuclei. Many ciliophorans' micronuclei separated by pulling apart in such a manner that a long thin thread or ribbon of material extended between the two separating daughter nuclei. This was described in *Vorticella monilata* Tatem, 1870; *V. nebulifera* Müller, 1786; *Opercularia coarctata* Claparede and Lachman, 1858 and *Paramecium aurelia* (Ehrenberg, 1838) (Finley 1943).

Shrivelling of micro-conjugant: The progressive shrinking and shedding of the microconjugant until it was just attached to the macroconjugant by a small piece of pellicle and its eventual detachment, was also observed for *V. microstoma* by Finley (1939, 1943).

Appearance of micro- and macroconjugant: The microconjugant of *V. microstoma* was physiologically different from the vegetative individual in that it was capable of positive searching reactions and was able to carry out the conjugation reaction on contact with a macroconjugant (Finley 1939, 1943). The macroconjugant did not differ from a vegetative individual, but was able to attract microconjugants and undergo conjugation immediately upon arrival for a limited time. In *Ambiphrya ameiuri* the microconjugant resembled the sessile form, but it was smaller (Thompson *et al.* 1947). Surber (1943) stated that *A. tholiformis* individuals in the process of gamete formation could easily be distinguished from those that are already undergoing conjugation. In *Mantoscyphidia* micro- and macroconjugants could only be identified after they had already attached to each other and had formed a protoplasmic bridge.

Appearance of ex- or post-conjugant: Finley (1939, 1943, 1952) noted that after the endoplasm of the *R. vernalis* microconjugant entered the macroconjugant and its pellicle became dislodged, it was impossible to distinguish the living, conjugating individual (exconjugant) from the neuter individuals. *Vorticella microstoma* microconjugants disappeared into the macroconjugant's body. In *Mantoscyphidia* the exconjugant had a swollen shape due to the extra cytoplasm that was acquired and not yet evenly distributed, and could easily be distinguished from a normal vegetative individual.

Synkaryon formation and metagamic nuclear divisions: Synkaryon formation in *R. vernalis* and *V. microstoma* lasted up to 15 minutes and the synkaryon underwent three successive metagamic divisions, presumably mitotic (Finley 1939, 1943; Davis 1947). Three metagamic nuclear divisions that lasted 12 to 18 hours resulted in the formation of eight nuclei of which one became a micronucleus and seven formed macronuclear anlagen. In *Mantoscyphidia* the synkaryon divided through two metagamic divisions and formed three macronuclear anlagen and one micronucleus. These post-zygotic divisions usually occurred before the shrivelled microconjugant disappeared. The time necessary for formation is unknown.

Number of macronuclear anlagen: *Rhabdostyla vernalis* and *V. microstoma* both contained seven macronuclear anlagen, while three anlagen were observed in *A. macropodia* and *A. tholiformis* each (Finley 1939, 1943, 1952; Surber 1943; Davis 1947). Three anlagen were also observed for *Mantoscyphidia* species.

End products of conjugation: Seven *R. vernalis* and *V. microstoma* individuals are normally produced by the reorganization fissions (Finley 1939, 1943, 1952). The postconjugant in *A. macropodia* divided by

two rapidly occurring binary fissions, resulting into four individuals that were much smaller than usual (Davis 1947). In *Mantoscyphidia* it is presumed that the exconjugant can undergo at least two consecutive divisions into four individuals, although these reorganisational fissions were not observed in living specimens. Colwin (1944) noted that regular binary fission seemed to occur together with conjugation in any given population and in *Mantoscyphidia* this was also the case. In conclusion, preconjugation fission was never observed directly in *Mantoscyphidia*.

Descriptions of binary fission, telotroch formation and conjugation in scyphidiid peritrichs published in the first half of the previous century, were mostly illustrated by line drawings. The major contribution of this study is most importantly, the confirmation of the various reproductive processes in *Mantoscyphidia* and secondly, the inclusion of scanning electron- and light-micrographs that aided detailed descriptions and illustrations of binary fission, telotroch formation and conjugation in the genus *Mantoscyphidia* for the first time. This is also the first reproductive study of scyphidiid peritrichs from molluscan hosts occurring in Africa.

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