Acta Protozool. (2004) 43: 163 - 172

ACTA
PROTOZOOLOGICA

A New Species of *Ellobiophrya* Chatton *et* Lwoff, 1923 (Ciliophora: Peritrichia) Attached to *Mantoscyphidia* Jankowski, 1980 (Ciliophora: Peritrichia) Species

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Summary. Surveys carried out along the coast of South Africa revealed the presence of a secondary symbiont of the genus *Ellobiophrya* Chatton *et* Lwoff, 1923 found attached to the narrow basal part adoral to the scopula of *Mantoscyphidia spadiceae* Botes, Basson *et* Van As, 2001 and *M. midae* Botes, Basson *et* Van As, 2001 occurring on the gills of *Haliotis spadicea* Donovan, 1808 and *H. midae* Linnaeus, 1758, respectively. *Mantoscyphidia branchi* Van As, Basson *et* Van As, 1998 found on the gills of *Cymbula* H. *et* A. Adams, 1854 and *Scutellastra* H. *et* A. Adams, 1854 species respectively, had the same ellobiophryid species attached to the narrow part adoral 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.

Key words: *Ellobiophrya maliculiformis* sp. n., *Mantoscyphidia,* marine mollusc, scyphidiid peritrich, secondary symbiont.

INTRODUCTION

Representatives of the family Ellobiophryidae Chatton *et* Lwoff, 1929 attach to the host by means of a scopula that has been adapted to form a ring-like cinctum or caudal process (Clamp 1982). Only parts of the scopula are included in the bouton of the cinctum, as the remainder of the scopula (the principal part) is found in the usual location and secretes the embryonic stalk linking the two daughters that result from binary fission. Currently the family comprises two genera, i.e. *Ellobiophrya* Chatton *et* Lwoff, 1923 and *Caliperia* Laird, 1953. All of the known species of the genus *Ellobiophrya* were found associated with fish, bivalves or bryozoan hosts from marine habitats. *Ellobiophrya donacis* Chatton *et* Lwoff, 1923 was described from the gill filaments of the bivalve *Donax vittatus* (Chatton and Lwoff 1923, 1928, 1929). Nearly sixty years later Clamp (1982) described *E. conviva* from the tentacles of the ectoprocts *Bugula neritina* and *B. turrita*. Another species, *E. oblida* (Naidenova *et* Zaika, 1969) occurs on the marine fish *Proterorhinus marmoratus*. It was originally described as *Clausophrya oblida* by Naidenova and Zaika (1969), but was placed within the genus *Ellobiophrya* by Clamp (1982).

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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 *C. brevipes* in the genus *Ellobiophrya*. This species was renamed as *E. brevipes* (Laird, 1959) with *C. longipes* the sole remaining species in the genus (Clamp and 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 comm.) [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. The same *Ellobiophrya* species was attached around the body of various scyphidiid peritrich hosts adoral to the scopula. The hosts were populations of *Mantoscyphidia spadiceae* Botes, Basson *et* Van As, 2001, *M. midae* Botes, Basson *et* Van As, 2001 and *M. branchi* Van As, Basson *et* Van As, 1998, which occur on the gills of *Haliotis spadicea* Donovan, 1808, *H. midae* Linnaeus, 1758 and different limpet species, respectively (Van As *et al.* 1998, Botes *et al.* 2001). 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.

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, *Mantoscyphidia spadiceae* and *M. midae*. *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. 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 fluid, 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 *et al.* (1985) and Lom and Dykova (1992). This method proved rather unsuccessful, as the ellobiophryids had 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), gave the best results. A brief summary of the method is: Bouins-fixed smears were transferred to 70% ethanol, then 50%, 30% and distilled water: followed by bleaching in 0.5% potassium permanganate for 5 min and washed in distilled water; transferred to 5% oxalic acid for 5 min and washed for 10 min; some slides were placed in 1% protargol solution for 10-15 min at 67-70°C, with copper sheets and others for a period of 12-24 h at room temperature; transferred to 1% hydroquione (in 5% sodium sulfite) for 7-8 min, washed briefly; transferred to 0.5% gold chloride for 15 s, washed briefly; transferred to 2% oxalic acid for up to 3 min; remove and washed for 5 min; transferred to 5% sodium thiosulfate for 5 min and washed in distilled water for 5 min; slides were dehydrated in 30, 50, 70, 95, 100% ethanol; transferred to xylene and mounted using Canada Balsam.

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 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 *et* Van As, 2001, attaches to narrow basal part adoral to the scopula; De Hoop Nature reserve, south coast (34°28'S, 20°30'E) of South Africa.

Other hosts and localities: *M. midae* Botes, Basson *et* Van As, 2001 and *M. branchi* Van As, Basson *et* Van

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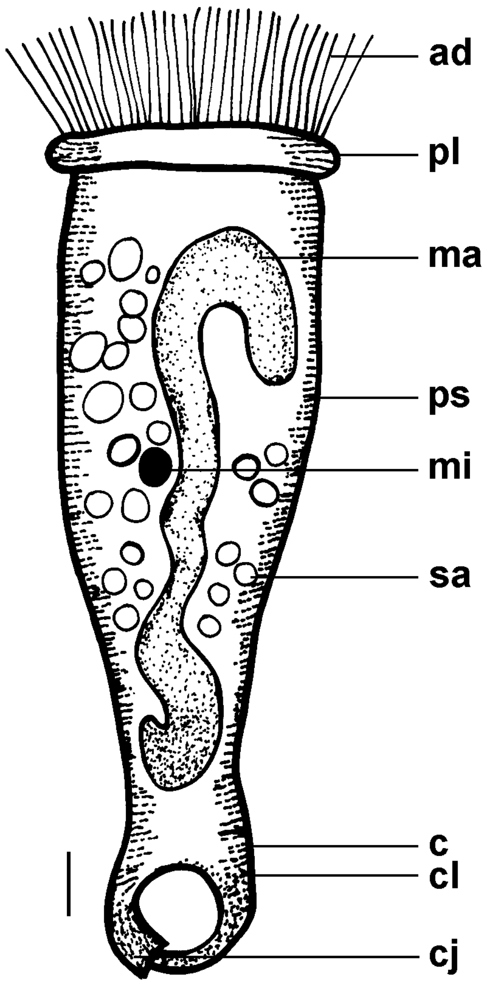


Fig. 1. Microscope projection drawing of *Ellobiophrya maliculiformis* sp. n. found as a secondary symbiont on *Mantoscyphidia spadiceae* Botes, Basson *et* Van As, 2001; *M. midae* Botes, Basson *et* Van As, 2001 and *M. branchi* Van As, Basson *et* Van As, 1998 occurring on the gills of *Haliotis spadicea* 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 - peristomial lip, ps - pellicle striations, sa - symbiotic algae. Scale bar 10 µm.

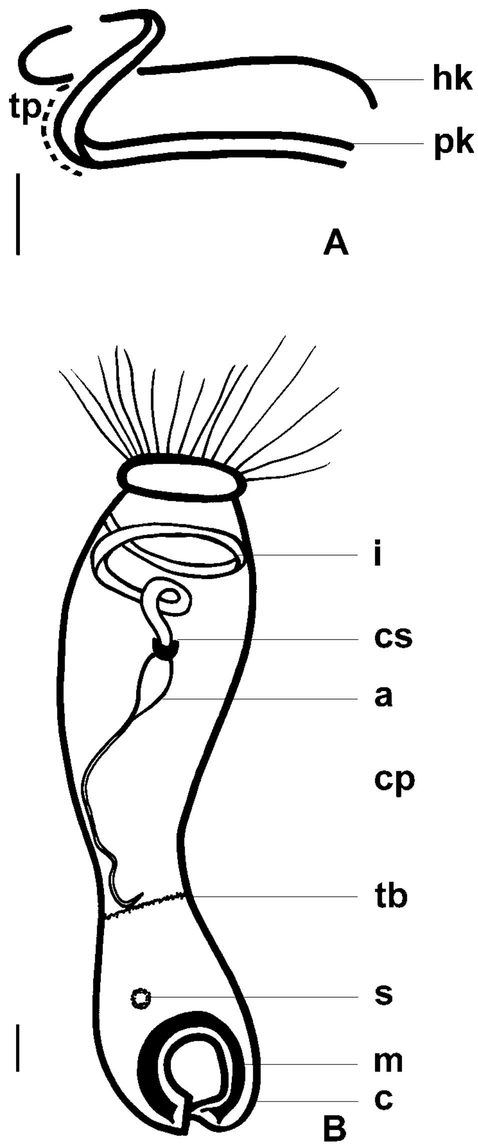


Fig. 2. Diagrams illustrating the infraciliature of *Ellobiophrya maliculiformis* sp. n. found as a secondary symbiont on *Mantoscyphidia spadiceae* Botes, Basson *et* Van As, 2001, *M. midae* Botes, Basson *et* Van As, 2001 and *M. branchi* Van As, Basson *et* Van As, 1998 occurring on the gills of *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, c - cinctum, cp - cytopharynx, cs - cytostomal sphincter, hk - haplokinety, i - infundibulum; m - myoneme, pk - polykinety, s - scopula, tb - telotroch band, tp - third polykinetid. Scale bars 10 µm.

As, 1998, De Hoop Nature reserve, south coast (34°28'S, 20°30'E) 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.

Etymology: Named after the mode of attachment adoral to 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)],

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 *et* Van As, 2001 and *M. branchi* Van As, Basson *et* Van As, 1998 occurring on the gills of haliotid (A,B) and different limpet (C-F) species from the south coast of South Africa.

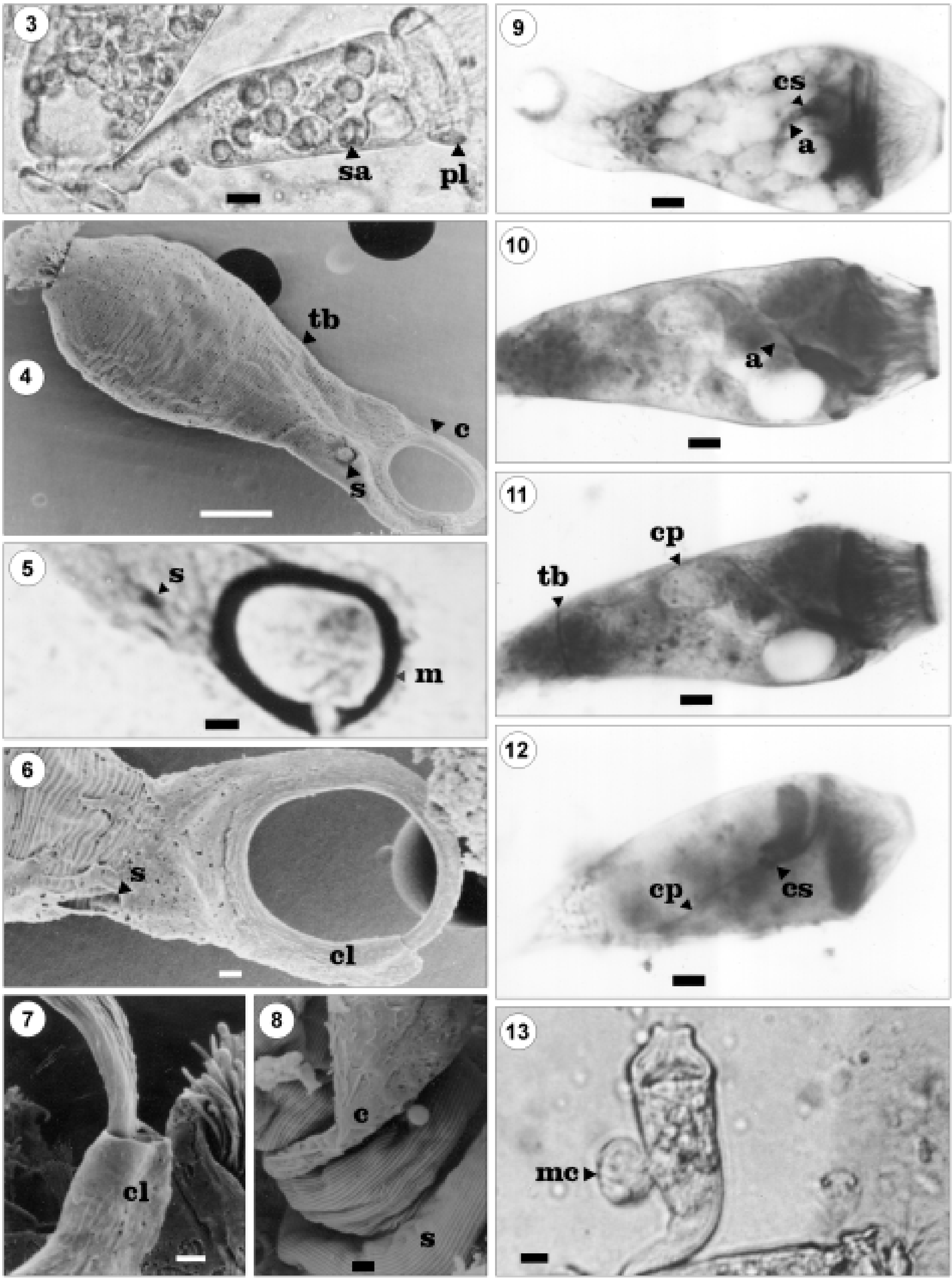
Mollusc host	A (*Haliotis midae*)	B (*H. midae*)	C (*Scutellastra barbara*)	D (*S. argenvilli*)	E (*S. cochlear*)	F (*Cymbula compressa*)
Ciliate host	*M. midae*	*M. midae*	*M. branchi*	*M. branchi*	*M. branchi*	*M. branchi*
Body length	60.0-85.0 (72.9 ± 8.4, 20)	43.0-93.0 (61.9 ± 13, 35)	45.0-65.0 (56.5 ± 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 *et* Van As, 2001 and *M. branchi* Van As, Basson *et* 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	8-22 (14.2 ± 4.6, 10)	7-9 (8.0, 3)
Peristome to cinctum	54-118 (86.7 ± 17.3, 10)	66-96 (78.6 ± 8.8, 13)
Total number of striations	62-140 (100.9 ± 20.5, 10)	66-116 (82.4 ± 12.8, 13)

* A new phylogenetic classification for the patellid limpets was suggested by Ridgway *et al.* (1998), grouping the patellid limpets in four monophyletic genera, namely *Helcion* Montfort, 1810; *Cymbula* H. *et* A. Adams, 1854; *Scutellastra* H. *et* 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*.

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 a secondary symbiont on *Mantoscyphidia spadiceae* Botes, Basson *et* Van As, 2001, *M. midae* Botes, Basson *et* Van As, 2001 and *M. branchi* Van As, Basson *et* 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, m - myoneme, mc - microconjugant, pl - peristomial lip, s - scopula, sa - symbiotic algae, tb - telotroch band. Scale bars 10 µm (3, 4, 9-13), 1 µm (5-8).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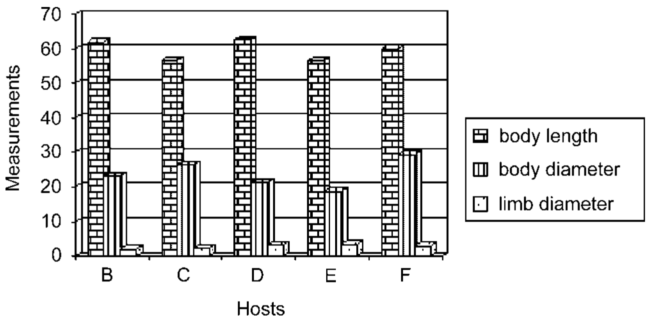

Fig. 14. Comparison in the variation of body length, body diameter and cinctal diameter of different populations of *Ellobiophrya maliculiformis* sp. n. found attached to *Mantoscyphidia midae* Botes, Basson *et* Van As, 2001 (B) and *M. branchi* Van As, Basson *et* Van As, 1998 (C-F) associated with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

diameter of body 15-30 µm (20.5 ± 3.7, 40) [16-37 µm (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; 101 striations on average, spaced 0.5 µm apart. Striations evenly spaced and uniform (Fig. 4, Table 2).

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). Limb that tapers forms bifurcated structure at tip of its myoneme (Fig. 5). Scopula is typical, but nonfunctional, except when it participates in secretion of larval stalk that links two daughters after fission (Figs 2B, 4-6).

Oral infraciliature of *E. maliculiformis* divisible into peristomial part and infundibular part as in other sessiline peritrichs. Adoral zone completes spiral of 360° counter-clockwise 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 parallel one another for entire length before plunging into infundibulum (Fig. 2A).

Haplokinety and polykinety run together around peristome and separate before plunging into infundibulum. Polykinety joined by additional polykinetids after entering infundibulum. P1 and P2 were positively observed in most of the specimens. The third polykinetid, which is normally very short, was observed only in few specimens, running parallel and closely associated with polykinety from the lip of opening up to first turn within infundibulum. Inside infundibulum, polykinetids and haplokinety make one turn (360°- 400°) each 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 and binary fission followed by 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 *et al*. 2001). Live observations of conjugation were made in two instances in populations on *M. midae*, four times in populations on *M. spadiceae* (Fig. 13), and twice in populations on *M. branchi*.

Binary fission and the subsequent formation of telotrochs were observed in ellobiophryid populations associated with all three hosts. After binary fission one daughter individual becomes a telotroch and the other remains a trophont attached by the parental cinctum to the 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 and Clamp 1991). The telotroch is slightly asymmetric, as is the case in other *Ellobiophrya* species.

A larval stalk was identified during an observation of telotroch formation in a live specimen of *Ellobiophrya maliculiformis* attached to *M. midae*. 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 was not beating yet. The parent's peristome was open, with cilia creating a feeding current. This telotroch was found on a gastropod host that had been collected 8-10 h beforehand and was observed for a period of 55 min 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-shaped structure which attached it to the trophont, but it was not attached to the scopula, as would have been the case in a developing telotroch. The apparent 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 of live observations and hematoxylin-stained material of *E. maliculiformis* are summarised in Table 1. The effect of contraction on the body length of live specimens versus hematoxylin-stained specimens is as follows: in the *M. spadiceae* populations there was a 27% body contraction between live observations and those stained with hematoxylin. The length and diameter of the body varied among different populations of *E. maliculiformis*. The average body length of ellobiophryid populations on *M. midae* was 61.9 µm. The average body length of 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. The ratio of body length to diameter in hematoxylin-stained specimens of *E. maliculiformis* found on *M. midae* and *M. branchi* is as follows: 2.68 (*M. midae*), 2.15 (*M. branchi*), 2.94 (*M. branchi*), 3.04 (*M. branchi*) and 2.04 (*M. branchi*). Also no significant differences could be found in the diameter of the cinctal limbs of different populations (see Table 1 and Fig. 14).

Live specimens of *E. maliculiformis* 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 of *M. branchi* could achieve a reduction in body length with the peristome remaining open. In these specimens, groups of elevated striations can be seen aboral to the telotroch band. When the peristome of *M. branchi* is fully closed, the degree of contraction can also vary. Specimens of *M. branchi* can be found in a whole range of body contractions on a single smear. Live ellobiophryids were able to contract to half of their fully extended body length.

Although the nuclear apparatus of all the populations were mostly obscured by algal inclusions, there were no great differences in the shape of the macronucleus. It is coiled and stretches throughout the body, much the same as those of *E. conviva, E. oblida* and *E. brevipes* (Clamp and Bradbury 1997).

The only difference between the ellobiophryid populations of *M. spadiceae* and *M. branchi* was that the latter had slightly fewer body striations (Table 2). This could be due to the fact that *E. maliculiformis* specimens found associated with *M. spadiceae* has a greater body length. The *M. 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. Other records of peritrichs found in a symbiotic association with peritrichs, are that of *Epistylis lwoffi* Fauré-Fremiet, 1943 which attached to the epistylidid *Apiosoma piscicola* (Blanchard), which in turn is found on the skin of freshwater fish (Fauré-Fremiet 1943, Clamp 1982) and *E. colisarum* (Foissner *et* Schubert, 1977) attaching to an epistylidid which lives symphoriontly on a freshwater fish, *Colisa fasciata* (Anabantoidei: Belontiidae) (Foissner and Schubert 1977).

In comparing *E. maliculiformis* with other species of *Ellobiophrya*, it 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 *E. maliculiformis* differs from the other species of *Ellobiophrya* in that it is located much nearer to the cinctum. The cinctum of *E. maliculiformis* is also asymmetrical, with uneven limbs. The longer thinner limb fits into the junction of the shorter much broader limb. The limb diameter of the populations varies be-

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

Species	*E. donacis*	*E. conviva*	*E. oblida*	*E. brevipes*	*E. maliculiformis*	*C. longipes*
Host	*Donax vittatus* Marine bivalve mollusc	*Bugula neritina*, *B. turrita* Marine ectoprocts (Bryozoa)	*Proterorhinus marmoratus* Marine fish	*Raja erinacea* Marine fish	*Mantoscyphidia spadiceae*, *M. midae*, *M branchi*, Scyphidiid peritrichs	*Oliverichtus melobesi*, *Ericentrus rubrus*, Marine fishes
Position on host	Gill filaments	Ciliated tentacles around mouth	Skin	Gills	Narrow basal part adoral to scopula of host	Gills
Collection locality	Morgat, France	North Carolina, USA	Black Sea	New Brunswick, Canada	South coast of South Africa	Wellington, New Zealand
Body length (µm)	50 (100)	46.2	180	60.2 (54.5)	50-125 (78.5)	31.2-68.4 (51.5)
Body diameter (µm)	40 (30)	26.8	36.5	34.6 (35.7)	15-30 (20.5)	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 conical, elongate, tapers towards aboral pole, Ma - coiled, sausage- shaped, Mi - fusiform	Body cylindrical, tapers towards aboral pole, Ma – cylindrical, Mi - fusiform/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 of uneven thickness, fitting into junction, 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	Tubular when empty, bulbous when filled, merges with cytopharynx	Resembles pipette bulb, cytostomal sphincter between infundibulum and ampulla

Table 3 (contd.)

Cytopharynx	Not described	Elongate, ends near aboral end of macronucleus	Not described	Long sineous tube discharging near posterior part of macronucleus	Small, recurving just adoral to trochal band	Large and funnel-shaped, ends blindly posterior at macronucleus
Expanded peristome	Prominent argentophilic cytostomial sphincter, uneverted peristomial 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 peristomial lip	Conspicous cytostomal sphincter at end of infundibulum	Argentophilic cytostomal sphincter present peristomial disc invaginated
Extent of infundibulum	Does not extend far beyond peristome	Approximately a third of distance from peristome to sphincter	Extends approximately a third of distance from peristome to cinctum	Short, ends at ampulla, quarter of distance from peristome to aboral end of body	Approximately a third of distance from peristome to cinctum	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 clearly visible due to algal inclusions	Not described
Pellicular striations	Closely spaced, faint striae	Prominent transverse striae	Closely spaced, faint striae	Closely spaced, faint striae	Evenly spaced and uniform	Smooth pellicle (no annuli)
Inclusions	Cytoplasmic inclusions (type 1 and 2)	Greenish areas in body (diatoms/algal cells)	Not described	Not described	Symbiotic algae, throughout cytoplasm, obscuring body features	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*	Short, rigid stalk, embryophore present	Not described

tween 2.2 and 3.4 µm (see Table 1). Table 3 represents a summary of the taxonomic characteristics of all species of the family Ellobiophryidae. This summary was compiled from Chatton and Lwoff (1923, 1928, 1929), Clamp (1982), Bradbury and Clamp (1991), Clamp and Bradbury (1997), and also includes the summarized characteristics of *E. maliculiformis*.

Up to four specimens of *E. maliculiformis* were observed attached adoral to 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 host's body, gripping it where the nuclear apparatus is situated (Fig. 8). Two ellobiophryids were observed attached between the macro- and micronuclei of a single *Mantoscyphidia spadiceae*. In cases where the ellobiophryids were attached to the peristomial region of the host its buccal cavity was probably obstructed. The ellobiophryids might interfere with the host's feeding. Ellobiophryids attached to the nuclear region of the host's body might have an influence on reproductive processes, possibly interfering with division.

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

Acknowledgement. The authors would like to express their gratitude to Dr. John C. Clamp of the Department of Biology, North Carolina Central University for proofreading the manuscript.

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Received on 21st May, 2003, revised version 28th October, 2003; accepted on 30th October, 2003