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Licnophora bassoni sp. n. (Ciliophora: Heterotrichea) from South African Turban Shells (Gastropoda: Prosobranchia)

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Summary. During surveys on the symbionts of intertidal invertebrates, turban shells, *Turbo sarmaticus* Linnaeus, 1758 and *Turbo cidaris cidaris* Gmelin, 1791, were found to host heterotrichous ciliophorans on their gills. The ciliophoran conform to the morphology of the genus *Licnophora* Claparéde, 1867, but differ from all the known species based on body morphology, details of the nuclear apparatus and host preference. Based on light and scanning electron microscopy this species is described as a new species, *Licnophora bassoni* sp. n.

Key words: heterotrichous ciliophoran, *Licnophora bassoni* sp. n., turban shells.

INTRODUCTION

The results presented below was obtained during surveys carried out as part of a comprehensive study on the biodiversity of intertidal symbionts, along the coast of South Africa by the Aquatic Parasitology group of the University of the Free State. This study already resulted in the descriptions of scyphidiid peritrichs (Van As *et al.* 1998, Basson et *al.* 1999), a heterotrichous ciliophoran (Van As *et al.* 1999), blood parasites (Smit and Davies 1999) and isopod symbionts (Smit *et al.* 1999, 2000) found associated with a variety of invertebrate hosts and tidal pool fishes. During these surveys another heterotrichous ciliophoran was found on the gills of two *Turbo* Linnaeus, 1758 species. This licnophorid differs from the known *Licnophora* species based on general body morphology, characteristics of the nuclear apparatus and host preference and is described as a new species. The description is based on Bouin's fixed specimens stained with hematoxylin, specimens impregnated with protargol as well as scanning electron microscopy.

MATERIALS AND METHODS

Specimens of *Turbo sarmaticus* Linnaeus, 1758 and *T. cidaris cidaris* Gmelin, 1791 (Mollusca: Archaeogastropoda: Trochacea: Turbinidae) were collected from the De Hoop Nature Reserve and Jeffreys Bay, South Africa and taken to a field laboratory where wet

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smears were prepared and examined. Positive smears were fixed in Bouin's and transferred to 70 % ethanol. Some smears were stained with Mayer's hematoxylin (Humason 1979) for studying the nuclear apparatus and for obtaining body measurements. Other smears were impregnated with protargol, using the method described by Lom and Dyková (1992).

For scanning electron microscopy, licnophorids were fixed in 2.5 % glutaraldehyde, transferred to 5 µm nuclearpore filters, washed with phosphate buffer, dehydrated through a series of ethanol concentrations and critical point dried. Filters were mounted on stubs, sputter coated with gold and studied at 5 kV, using a JEOL WINSEM JSM 6400 scanning electron microscope (SEM).

Body and micronucleus measurements and the number of macronuclear segments were obtained from microscope projection drawings, using fixed material (Table 1). Measurements of specimens are presented in the following way: minimum and maximum values are given, followed in parentheses by the arithmetic mean (mode in the case of the number of macronuclei and micronucleus diameter), standard deviation (only in n>9) and number of specimens measured. The type material is deposited in the collection of the National Museum, Bloemfontein, South Africa.

RESULTS AND DISCUSSION

Licnophora bassoni sp. n. (Figs. 1-7)

Hosts: *Turbo sarmaticus* Linnaeus, 1758, *Turbo cidaris cidaris* Gmelin, 1791.

Position on host: gills.

Localities: De Hoop Nature Reserves and Jeffreys Bay on the south coast of South Africa.

Type-specimens: holotype slide S98/3/30-1 (NMBP 252), paratype slides, S96/4/8-10 (NMBP 253), S98/3/30-2 (NMBP 254) in the collection of the National Museum, Bloemfontein, South Africa, other material in the collection of the authors.

Type host and locality: *T. sarmaticus* De Hoop Nature Reserve, South Africa (34° 28'S; 20° 30'E).

Etymology: named after Professor Linda Basson in recognition of her contribution to the knowledge of ciliophorans.

Description

Body squat, total length 40-70 µm (56.5 ± 6.7, 71), consists of two distinct regions; oral region and basal region (Figs. 1, 2, 7). Oral region diameter at broadest part 20-41 µm (28.4 ± 5.1, 71). Adoral side of oral region fringed by broad band of adoral zone of membranelles (AZM) describing spiral of 270°, before plunging into infundibulum. AZM comprising 71-129 (96.8 ± 16.5, 14) rows of membranelles (Figs. 1, 2), between 16 and 20 kinetids wide. Rows of membranelles separated by sharply pointed endoplasmic ribs (Fig. 3). First two thirds of AZM follows periphery of body, last third of AZM deviates from body periphery spiralling inward towards centre of oral region (Fig. 1). Spiral diameter at widest point in oral region 10-29 µm (17.94 ± 3.4, 71). Centre of aboral surface smooth without cilia, fringed by AZM. Neck short, not clearly distinguishable from adjacent oral and basal regions. Basal region (Fig. 4) round, surface slightly concave, diameter 22-41 µm (30.3 ± 3.9, 71). Basal disc diameter 15-30 µm (19.9 ± 3.2, 44), disc surrounded by a single circular ring of short, densely packed cilia of uniform length. Three additional rings of cilia extend around two thirds of anterior basal disc, proximal row shortest, distal row longest. Posterior third of disc with six rows of cilia, inner periphery shortest, outer longer (Fig. 4). Anterior part of basal region separated from membranelles by velum (Fig. 5). Single row of 20-25 kinetids and cilia on dorsal side of basal

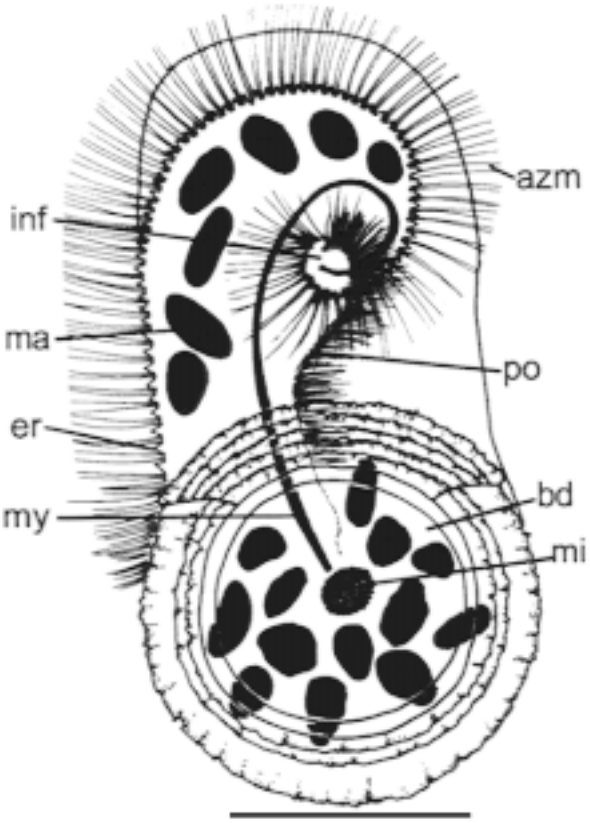
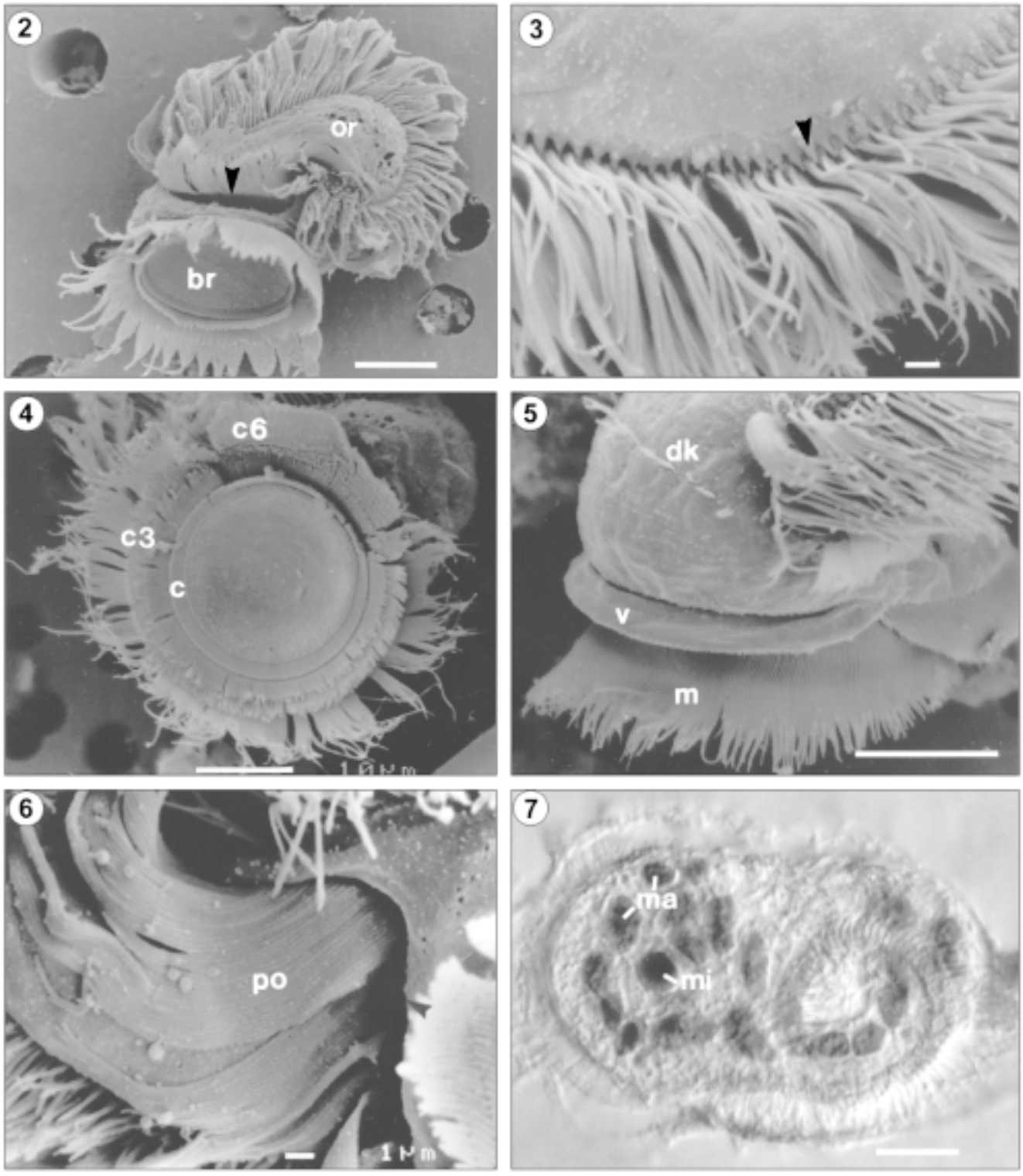


Fig. 1. Microscope projection drawing, based on hematoxylin stained and protargol impregnated specimens, of *Licnophora bassoni* sp. n. occurring on the gill filaments of *Turbo sarmaticus* Linnaeus, 1758 collected from the De Hoop Nature Reserve, South Africa. azm - adoral zone of membranelles, bd - basal disc, er - endoplasmic ribs, inf - infundibulum, ma - macronucleus, mi - micronucleus, my - myoneme, po - paroral organelle. Scale bar - 20 µm



Figs. 2-7. Scanning electron micrographs (**2-6**) and photomicrograph of hematoxylin stained specimen (**7**) of *Licnophora bassoni* sp. n. collected from turban shells from De Hoop Nature Reserve. **2** - adoral view: oral region (or), basal (br) region and ectoplasmic furrow (arrow); **3** - endoplasmic ribs (arrow) and part of azm; **4** - basal region with membranelles: single, circular ring of short cilia (c), three additional cilia rings (c3) and six posterior cilia rows (c6); **5** - basal region with dorsal kinetids (dk), velum (v) and membranelles (m); **6** - paroral organelle cilia (po), plunging into infundibulum (arrow); **7** - aboral view: macronuclear segments (ma) and micronucleus (mi). Scale bars - 10 µm (**2**, **4**, **5**, **7**); 1 µm (**3**, **6**)

Table 1. Morphometric measurements (µm) and numbers of macronuclear segments and membranelles of specimens of *Licnophora bassoni* sp. n. collected from the gills of *Turbo* spp. Linnaeus, 1758, from the south coast of South Africa

Measurements	*Turbo sarmaticus* De Hoop Nature Reserve	*Turbo sarmaticus* Jeffreys Bay	*Turbo cidaris cidaris* De Hoop Nature Reserve
TBL	40-70 (56.5 ± 6.7, 71)	45-70 (53.5 ± 6.1, 19)	44-76 (55.2 ± 7.5, 33)
ORD	20-41 (28.4 ± 5.1, 71)	21-34 (27.5 ± 3.7, 19)	10-34 (23.4 ± 4.5, 33)
SD	10-29 (17.9 ± 3.4, 71)	20-25 (21.9 ± 2.2, 19)	12-21 (17.5 ± 2.3, 27)
BRD	22-41 (30.3 ± 3.9, 71)	20-31 (27.6 ± 2.5, 19)	20-31 (26.3 ± 3.4, 33)
BDD	15-30 (19.9 ± 3.2, 44)	16-24 (20.3 ± 1.9, 18)	15-26 (19.4 ± 3.0, 18)
MaOR	3-13 (7, 71)	5-9 (5, 19)	3-9 (4, 33)
MaBR	9-21 (13, 71)	8-17 (14, 19)	6-14 (9, 33)
TMA	14-32 (21, 71)	13-24 (19, 19)	9-18 (18, 33)
MiD	3-7 (5, 30)	4-6 (4, 6)	4-9 (5, 31)
AZM	71-129 (96.8 ± 16.5, 14)	80-123 (104.5 ± 12.7, 14)	70-102 (87.3 ± 8.7, 25)

TBL - total body length, ORD - oral region diameter, SD - spiral diameter, BRD - basal region diameter, BDD - basal disc diameter, MaOR - number of macronuclei in oral region, MaBR - number of macronuclei in basal region, TMA - total number of macronuclei, MiD - micronuclei diameter, AZM - adoral zone of membranelles

Table 2. Body length (BL), number of macronuclear segments (No. MaS) and hosts of all the species of the genus *Licnophora* Claparéde, 1867, including *L. bassoni* sp. n.

Species	BL	No. MaS	Hosts	Reference
L. auerbachii (Cohn, 1866)	80-120	10-25	Echinoderm Bivalve Nudibranch	Cohn (1866)
L. bassoni sp. n.	40-70	14-32	Gastropod	Present study
L. biecheleri Villeneuve-Brachon, 1940	90-100	40-50	Cnidarian	Villeneuve-Brachon (1940)
L. bullae Dustin, 1915	123-130	15-20	Gastropod	Dustin (1915)
L. chattoni Villeneuve-Brachon, 1939	70-90	10-20	Ascidian	Villeneuve-Brachon (1939)
L. cohnii Claparéde, 1867	55-60	unknown	Polychaete	Claparéde (1867)
L. conklini Stevens, 1904	100-135	5-6	Gastropod	Stevens (1904)
L. hippocampi Meng and Yu, 1985	50-87	70-79	Seahorse	Meng and Yu (1985)
L. limpetae Van As, Van As and Basson, 1999	50-95	13-25	Gastropod	Van As *et al.* (1999)
L. lyngbycola Fauré-Fremiet, 1937	100	13	Algae	Fauré-Fremiet (1937)
L. macfarlandi Stevens, 1901	67-96	25-30	Echinoderm	Stevens (1901)

disc (Fig. 5). Myoneme extends from centre of basal disc, stretching directly upwards before following curve of AZM, plunging into infundibulum (Fig.1). Myoneme in basal region, broader than rest. Paroral organelle extends from inner periphery of basal disc close to micronucleus (Fig. 1), following myoneme curving upwards before extending down to plunge into infundibulum (Fig. 6). Paroral organelle consisting of single row of densely packed kinetosomes from which a single row of long cilia originates, aborally visible in an ectoplasmic furrow (Fig. 2).

Macronucleus consists of round, sometimes elongated, separate nuclei, varying in number between 14 and 32 (21, 71), distributed throughout body (Figs. 1, 7). Number of macronuclear segments in oral region 3-13 (7, 71), in basal region 9-21 (13, 71). Micronucleus round, diameter 3-7 (5, 30) situated in the centre of basal disc (Figs. 1, 7). No food vacuoles observed, endoplasm with granular appearance. No contractile vacuole found.

Remarks

Both *Turbo sarmaticus* and *T. cidaris cidaris* from De Hoop Nature Reserve and Jeffreys Bay were infested with *Licnophora bassoni*. No significant difference between the body measurements of these heterotrich populations could be found (Table 1). A very

consistent feature of *L. bassoni* is the micronucleus, which is distinct in its round shape situated in the center of the basal region.

Licnophora auerbachii (Cohn, 1866); *L. biecheleri* Villeneuve-Brachon, 1940; *L. bullae* Dustin, 1915; *L. chattoni* Villeneuve-Brachon, 1939; *L. conklini* Stevens, 1904; *L. lyncbycola* Fauré-Fremiet, 1937 and *L. macfarlandi* Stevens, 1901 are larger species than *L. bassoni*. All of these species except, *L. bullae* and *L. conklini*, are found on non-gastropod hosts (see Table 2). The number of macronuclear segments of *L. bullae* ranges between 15 and 20, which falls within the range of *L. bassoni*. Nevertheless, *L. bullae*, is a very large licnophorid with an elongated body and long neck found in the pallial cavity of the bubble snail *Bulla* Linnaeus, 1758 (Dustin 1915). *L. conklini* has 5-6 macronuclear segments and was described from the egg capsules of the slipper snail *Crepidula* Lamark, 1799 found in Woods Hole, Massachusetts (Stevens 1904). *L. bassoni* can be distinguished from the remaining three species (*L. cohnii* Claparède, 1867; *L. hippocampi* Meng and Yu, 1985 and *L. limpetae*) in the following ways: *L. cohnii* was found on the gills of an Italian polychaete and according to the drawings provided, this species has a more circular oral region and a very thin neck (Kahl 1932). *L. hippocampi* is a small species with 70-79 macronuclear segments and is so far the only licnophorid known from a vertebrate host (Meng and Yu 1985). *L. bassoni* differs from *L. limpetae*, the other South African species, not only in body length and number of nuclei (Table 2), but also in the arrangement of the nuclei. In the case of *L. limpetae* the segments are in the shape of an eight (Van As *et al.* 1999), whereas those of *L. bassoni* are scattered throughout the body. Another prominent difference between these two species is that *L. limpetae* has a distinct neck region, which is indistinguishable in *L. bassoni.*

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