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Six New Ciliated Protozoan Species of Trichostomatida, Entodiniomorphida and Suctorida from the Intestine of Wild African Rhinoceroses

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Summary. A survey of ciliated protozoan endocommensals of both black and white wild African rhinoceroses revealed six new species from the caecum and colon in concentrations of $2x10^3$ - $5x10^4$/ml digesta fluid. Two uniformly somatically ciliated species of Trichostomatida, *Helicozoster africanus* with an external ciliated peristomal trough spiralling the entire body-length, was found in both black and white rhinoceroses; while *H. apicalis* with an external ciliated peristomal through spiralling half body - lenght, was found in white rhinoceroses only. Three species of Entodiniomorphida were found in white rhinoceroses only. These were *Rhinozeta pedale* with a foot-like posterior end of the body; *Triplumaria corrugare* with a corrugated triangular posterior terminal tail, and *Lavierella klipdrifi* with a distinctive club-shaped body. One adult species of Suctorida was present in both black and white rhinoceroses, *Allantosoma multisuctores* distinguishable by its numerous ingestory suctorial tentacles scattered uniformly over its entire body. Counts of the total endocommensals stressed the possible role of the colon in the fermentation of the hosts's diet by these Protozoa. In all, nine protozoan families have been demonstrated to be associated with fermentation of the digesta by the nine species of hindgut-fermenting mammals thus far examined by various workers in morphological studies.

Key words: hindgut Protozoa, new species, rhinoceros.

INTRODUCTION

In a survey of ciliated protozoan endocommensals of both black and white wild African rhinoceroses (Mammalia: Rhinocerotida), 48 species of ciliated Protozoa have been recovered from the hindgut and faeces to date. Twenty three of these were new species which have been identified systematically by Buisson (1923), Hoare (1937), van Hoven *et al.* (1987, 1988), and Gilchrist *et al.* (1994). In addition there are the six new species as reported here, large populations of fungi (Teunissen *et al.* 1991), flagellates and anaerobic bacteria (Dehority 1986).

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The six new species belong to three of the four orders of ciliated Protozoa of herbivorous mammals. The holotrich Protozoa are represented by two uniformly somatically ciliated species of Trichostomatida. *Helicozoster* Latteur 1967, *africanus* sp. n. was found in both black rhinoceros (*Diceros bicornis* Linnaeus 1758) and white rhinoceros (*Ceratotherium simum* Burchell 1817); while *H. apicalis* sp. n. was found in white rhinoceros only. Three species belonging to Entodiniomorphida were found in white rhinoceros only. *Rhinozeta pedale* sp. n. with typical upper and lower skeletal plates and short lateral somatic ciliary bands in between, is distinguishable by the foot-like posterior end of the body. *Triplumaria corrugare* sp. n. encased in a cuticular carapace with 3 typical stalked ciliary tufts is distinguishable by its corrugated triangular tail. *Lavierella klipdrifi* sp. n. is covered with a rigid naked striated cuticle and has a tuft of cilia on a retractable peristomal cone similar to that of the type species, but is distinguishable by its distinctive club-shaped body. *Allantosoma multisuctores* sp. n. of the Suctorida is present in the large intestine of both black and white rhinoceroses. The adult form is small solitary with numerous ingestory suctorial tentacles scattered uniformly over the elongate-cycloid body, which distinguishes it from other species of the genus.

MATERIALS AND METHODS

Gastro-intestinal tracts were excised while the carcasses were still warm, between 1 and 2 h after each of the 4 rhinoceroses were shot. Sets of 6 samples from stomach, small intestine, caecum, right ventral ascending colon, right dorsal ascending colon, and descending colon were collected from a slit made in the wall of the gastro-intestinal tract at the sampling point. Of one white rhiniceros, only microscope slides were available for analysis.The pH of the unmixed stomach digesta of one white rhinoceros were determined at different levels using a freshly calibrated Crison pH-meter (Labotec; Industria, South Africa). Otherwise, the digesta were mixed by hand, bailed out with a beaker and strained through a 4 mm-wire sieve. The strained fluid containing the Protozoa was collected. For light microscopy, 25 ml of fluid was added immediately to 25 ml of formalin (14% aq.) (van Hoven *et al.* 1987). For electron microscopy, 2 ml of fluid was added to 10 ml of instantaneous killing prefixative, which contained osmic acid (2% aq.) and $HgCl_2$ (sat. aq. soln.) mixed in the ratio of 5:1 (Parducz 1966) and which prevented retraction of the cilia (Small and Marszalek 1969). Clumping of the Protozoa was obviated by shaking vigorously for 30 s on addition of the preservative.

On reaching the laboratory, formalinized samples were diluted with a mineral solution (Bryant and Burkey 1953) and finally with equal parts of glycerine as stabilising agent (van Hoven 1983). Total counts were made at x 90 magnification with a 0.50 mm-Nageotte counting chamber (W. Schreck, Hofheim, Germany). The different ciliate species, in wet unstained, permanently sealed, slide preparations, were counted at x 400 magnification and converted to a percentage of the total, which was in excess of 200 individuals per host (van Hoven *et al.* 1987). Detailed anatomy was studied at x1000 magnification using an oil immersion objective. Drawings were made with the aid of camera lucida equipment and all measurements of length, width and thickness were made with a calibrated eyepiece micrometer. Thickness was measured by the distance travelled by the vertical stage micrometer between the values observed when the organism comes into and disappears from view under the oil immersion (x1000) objective. Size was measured in micrometers (μm). Lubinsky's terminology (1957, 1958) was used for description of the species, except in the use of motilia in place of caudalia for stalked ciliary tufts occurring anteriorly and posteriorly on the body (Eloff and van Hoven 1980). With the oral end of the organism directed to 12 o'clock, the orientation used was that of Kofoid and MacLennan (1933), Dehority (1985). Light microphotography was carried out with either an Olympus BH2 microscope with a PM-10 AD photomicrographic system or a Reichert Polyvar photomicroscope, each provided with Nomarski-optics for differential interference contrast. An AGFA PAN 25 ASA film was employed in order to obtain well resolved image enlargements.

For scanning electron microscopy (SEM), the prefixed field sample was washed with tap water as soon as possible through stacked geological sieves (125-20). The subsamples from each sieve were fixed for 24 h with Karnovsky fixative (Hayat 1972) and 1% picric acid (v/v aq.) (Bullock 1984) or, where necessary, stored in that fixative. From this stage, standard dehydration protocols were carried out in a holder containing a Nucleopore filter (10-12) under slight negative pressure. During the last change of ethanol, the funnel was removed, and a similar filter was inserted to close the microchamber. After critical-point drying, both filters were removed and mounted on stubs for carbon and gold/palladium coating (2-3 nm). All preparations were viewed with a Cambridge Stereoscan microscope at 5-10 kV.

Since most intestinal ciliated Protozoa are strictly anaerobic and active only at about 39°C, specimens had to be preserved at the site of the kill with instantaneous-acting fixatives incompatible with techniques for study of subpellicular systems. Thus the descriptions presented here exclude subpellicular ciliary morphology.

RESULTS

The stomach and small intestine were void of Protozoa. The stomach was packed tight with ingesta. The pH at the internal surface of the stomach wall was expectedly low, and showed a gradient toward neutrality approaching the centre of the stomach. This gradient in the Pilanesberg white rhinoceros from stomach-wall → halfway to stomach centre → stomach centre was 2.13 → >3 → 6.6. On mixing the stomach contents the pH became 5.5. In the young rhinoceros this would facilitate the passage of ingested inocula of mother's faeces to the hindgut habitat of the acid sensitive intestinal Protozoa.

The distribution of protozoan families and apparent genera and species in the caecum and colon of both black and white rhinoceroses is described in Table 1. The

Table 1. Distribution of families apparent genera and species in the hindgut of both one black and three white rhinoceroses

Family	Black rhinoceros				White rhinoceros			
Genus species	caecum	colon			caecum	colon		
		ventral	dorsal	descending		ventral	dorsal	descending
Buetschliidae								
Blepharosphaera intestinalis	+	+	+	+	+	0	0	0
B. ceratotherii	+	0	+	+	+	+	+	+
B. epsoidalis	0	0	+	+	0	+	0	0
Didesmis ovalis	+	+	+	+	+	0	+	+
D. quadrata	0	+	+	0	0	0	+	0
D. synciliata	0	0	0	0	0	+	+	0
Holophryoides ovalis	0	0	+	0	0	+	+	0
Alloiozona trizona	0	0	0	0	0	+	0	0
Polymorphella ampulla	0	0	+	+	+	+	+	+
Blepharoconus cervicalis	0	0	+	+	0	0	0	0
Paraisotrichidae								
Helicozoster africanus sp. n.	+	+	0	0	+	0	0	0
H. apicalis sp. n.	0	0	0	0	+	0	0	0
Paraisotricha colpoidea	+	+	+	+	+	+	+	0
P. minuta	0	0	+	0	+	+	+	+
Blepharocorythidae								
Charonina odotophora	0	0	0	0	0	+	+	+
C. tenuis	0	0	0	0	0	+	+	+
C. dicerotis	0	0	+	+	0	0	0	0
C. tortuosa	0	0	+	+	0	0	0	0
C. tetragona	0	+	+	+	0	0	0	0
Cycloposthiidae								
Lavierella klipdrifi sp. n.	0	0	0	0	+	+	0	0
L. africana	+	+	0	0	0	0	0	0
Triplumaria hamertoni	+	+	+	+	+	+	+	+
T. selenica	0	0	0	0	0	0	+	0
T. corrugata sp. n.	0	0	0	0	0	0	+	0
Monoposthium vulgaris	0	0	0	0	0	+	+	+
M. brachium	0	0	0	0	0	+	+	+
M. latus	0	0	0	0	0	+	+	+
Cycloposthium bipalmatum	0	0	0	0	0	+	+	0
Diplolophus hydrochoeri	0	0	0	0	0	+	+	0
Arachnodinium noveni	0	0	0	0	0	+	+	+
Phalodinium digitalis	0	0	0	0	0	+	+	+
Prototapirella intestinalis	0	0	0	0	+	0	+	+
Rhinozetidae								
Rhinozeta addoensis	+	0	0	0	+	0	0	0
R. unilaminatus	+	0	0	0	+	0	0	0
R. caecalis	+	+	0	0	0	0	0	0
R. rhinozeta	+	+	+	0	+	0	0	0
R. triciliata	+	+	0	0	+	+	0	+
R. cristata	0	0	0	0	+	+	+	0
R. multiplatus	0	0	0	0	+	0	0	+
R. pedale sp. n.	0	0	0	0	0	0	+	0
Ditoxidae								
Triodinium galea	0	0	0	0	+	+	+	0
Tr. minimum	0	0	0	0	+	+	+	+
Ophryoscolecidae								
Endoralium loxodontae	0	0	+	+	0	+	+	+
Acinetidae								
Allantosoma intestinale	0	0	0	0	+	0	0	0
A. biseriale	0	0	0	0	+	0	+	+
A. multisuctores sp. n.	+	+	+	0	+	+	+	0
Telamodiniidae								
Telamodinium onyx	+	+	0	0	0	0	0	0

Table 2: Protozoan counts, total number of species, new species number of ciliates in the caecum and colon of black (B) and white (W) rhinoceroses

Protozoa	Gut compartments							
	caecum		colon					
			ventral[a]		dorsal[b]		descending	
	B	W	B	W	B	W	B	W
Total counts x 10^3/ml/digesta	362	178(150-233)	266	100(80-120)	259	85(40-130)	31	25(15-35)
Total No. (av.) of species per animal	29	16(11-20)	20	19(13-25)	20	24(23-24)	15	15(9-20)
New species No (av.) of ciliates x 10^3/ml/digesta								
Trichostomatida								
Paraisotrichidae								
Helicozoster africanus sp. n.	50	13 (10-20)	0	0	0	0	0	0
H. apicalis sp. n.	0	30	0	0	0	0	0	0
Entodiniomorhida								
Rhinozetidae								
Rhinozeta pedale sp. n.	0	0	0	0	0	1	0	0
Cycloposthiidae								
Triplumaria corrugata sp. n.	0	0	0	0	0	2(1-2)	0	0
Lavierella klipdrifi sp. n.	0	60(40-80)	0	21(1-40)	0	0	0	1
Suctorida								
Acinetidae								
*Allantosoma multisuctores*n. sp. n.	8	4(2-9)	7	1(0-2)	2	2(0-3)	0	0

Dorsal[b] - small colon; ventral[a] - large colon

numbers and variety of species in the colon were greater than those in the caecum. This was to be expected since food fermentation was initiated and subsequently maintained in the colon as the main locus of fermentation in large herbivorous mammals like rhinoceroses (Hume and Warner 1980).

Protozoan counts x10^3/ml digesta fluid of the proposed new species separately and together with the total number of species in the caecum and colon of 1 black and 2 white rhinoceroses is given in Table 2. The counts of the browsing black rhinoceros outnumbered, by a factor of 2 or 3, those of the grazing white rhinoceroses in every fermentative hindgut compartment. High counts of *Helicozoster* and *Lavierella* were encountered in caecal fluid. Counts x10^3/ml of 50 *H. africanus* for the black rhinoceros, 30 *H. apicalis* for the white rhinoceroses, and 60 *L. klipdrifi* for the white rhinoceroses only. A similar high count of *L. africana* was found for the black rhinoceros only in our study and that of Buisson (1923).

Helicozoster africanus sp. n. (Figs. 1, 8A, 9, 10)

Description (n=33): body ovoid covered with uniformed somatic ciliation (Figs. 8A,9); length 157 ±13.1 µm, width 114±11.0 µm optically measured dorso-ventral thickness top 27 ±7.8 µm, middle 48 ± 9.3 µm, bottom 36 ±11.0 µm. Apical oral-opening (Fig. 10) at termination of elongate peristomal channel. External ciliated peristomal trough (Latteur 1967) (Figs. 8A,9) spiralling the entire body-length and connected anteriorly (Latteur 1967) with peristomal channel leading into cytopharynx. Inner sides of trough bear parallel striations. Outer edges of trough bear dense long flexible cilia (Fig. 10). Under the oil immersion objective (x1000) the entire body covered with 72 close parallel longitudinal ridges (Figs. 8A,9) bearing dense short cilia. Agglomerate of inclusions in posterior third of body (Fig. 8A). Position of round/oval granular macronucleus variable (Fig. 8A); small oval micronucleus recessed in anterior end of macronucleus (Fig.1). Variable number of contractile vacuoles scattered throughout body. Position of cytoproct as a small indention on posterior pole of body.

Type host: *Diceros bicornis* Linnaeus 1758, black rhinoceros.

Other host: *Ceratotherium simum* Burchell 1817, white rhinoceros.

Type locality: black rhinoceros Addo Elephant National Park, Eastern-Cape Province, South Africa (33°-34°S; 25°-26°E).

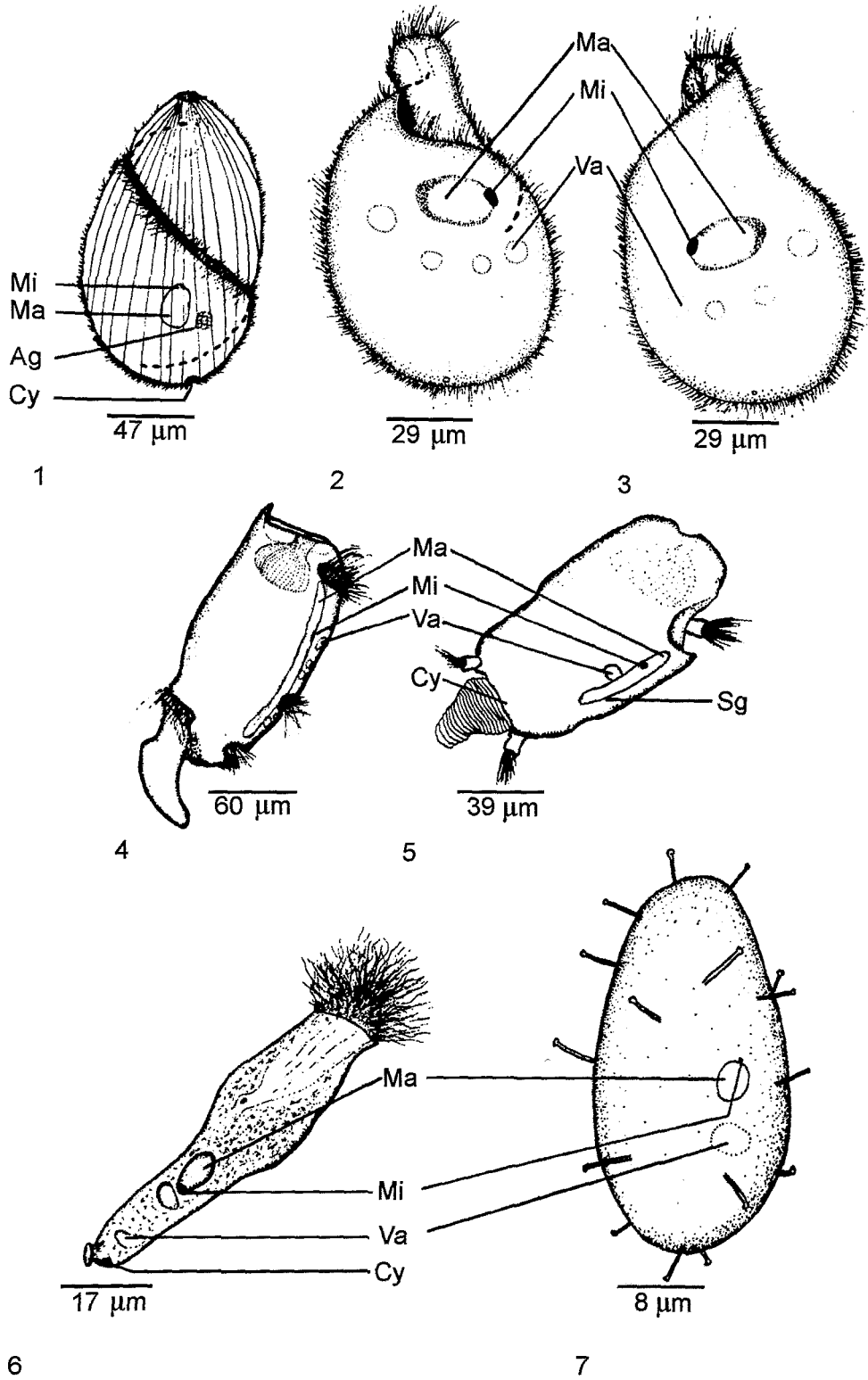


Fig. 1. *Helicozoster africanus* sp. n., ventral view, dotted line indicating length of external trough. Figs. 2,3. *H. apicalis* sp. n. 2 - ventral view, dotted line indicating length of external trough. 3 - dorsal view. Fig. 4. *Rhinozeta pedale* sp. n., ventral view. Fig. 5. *Triplumaria corrugata* sp. n. , ventral view. Fig. 6. *Lavierella klipdrifi* sp. n., ventral view. Fig. 7. *Allantosoma multisuctores* sp. n., ventral view. Ag - agglomerate of inclusions, Cy - cytoproct, Ma - macronucleus, Mi - micronucleus, Sg - skeletal groove, Va - vacuole

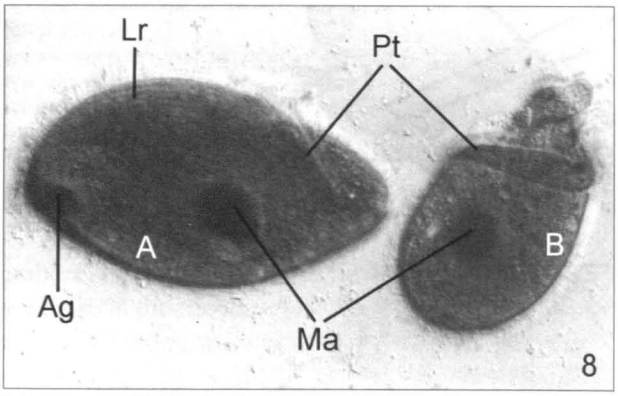
Other localities: white rhinoceros Pilanesberg Game Reserve, North-West Province, South Africa (25°-26°S; 27°-28°E); Ellisras district, Northern Province, South Africa (23°-24°S; 27°-28°E); Hluhluwe Game Reserve, Kwazulu-Natal Province, South Africa (28°-29°S; 31°-32°E).

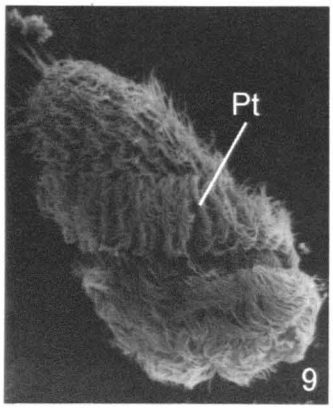
Site of infection: caecum and ventral colon.

Prevalence: Addo black rhinoceros $5x10^4$/ml; Hluhluwe white rhinoceros $2x10^4$/ml; Pilanesberg white rhinoceros $1x10^4$/ml; Ellisras white rhinoceros $1x10^4$/ml.

Etymology: specific name refers to the continent on which hosts were found.

Type materials: black rhinoceros, Addo Elephant National Park, Accession No. 2240485, deposited in the Intestinal Protozoa Collection of the Centre of Wildlife Management, University of Pretoria, Pretoria 0002, South Africa.





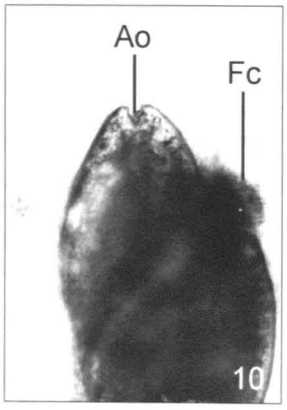


Fig. 8. *Helicozoster* spp. venral view, light Nomarski microphotograph (LNM), x 1250. A - *H. africanus*.; B - *H. apicalis*. Ag - agglomerate of inclusions, Lr - parallel longitudinal ridges, Ma - macronucleus, Pt - external peristomal troughs. Fig. 9. *H. africanus*, scanning electron micrograph (SEM), x 1333. Pt - external peristomal troughs. Fig. 10. *H. africanus* (LMN), x 1250. Ao - apical oral-opening, Fc - long flexible cilia edging external peristomal trough

***Helicozoster apicalis* sp. n. (Figs. 2, 3, 8B, 11, 12)**

Description (n=32): body-shape ovate, with uniform somatic ciliation, narrowing anteriorly to a short column terminating in a rounded apex (diam. 13.3µm); length 118 ±17.1 µm, width 73 ±14.7 µm, dorso-ventral thickness 33 ±7.4 µm. Oral-opening is a slit in tip of apex opening into elongate peristomal channel. External ciliated peristomal trough spiralling anterior half of body-length and connected anteriorly with peristomal channel leading into cytopharynx. Edges of oral-opening slit and of peristomal trough bear dense long flexible cilia (Figs. 11,12). Entire body covered with 72 close parallel ridges bearing dense short cilia. Agglomerate of inclusions in anterior third of body-length (Fig. 12). Oval macronucleus on central longitudinal axis at half body-length (Figs.2,3,8B). Oval micronucleus recessed in one end of macronucleus. Variable number of contractile vacuoles scattered throughout body at half body-length (Figs. 2,3). Cytoproct in small indention in posterior pole of body.

Type host: *Ceratotherium simum* Burchell 1817, white rhinoceros.

Other host: no other hosts known.

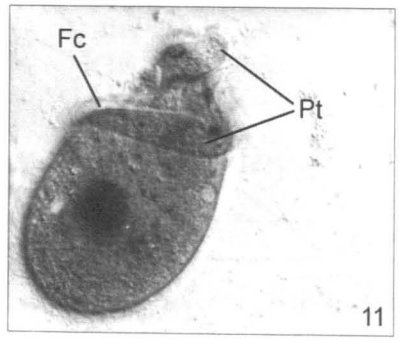
Type locality: Hluhluwe Game Reserve, Kwazulu-Natal Province, South Africa (28°-29°S; 31°-32°E).

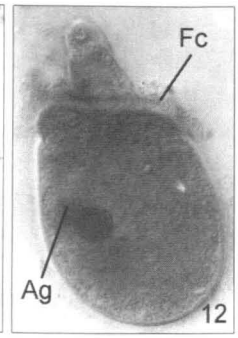
Other localities: Ellisras district, Northern Province, South Africa (23°-24°S; 27°-28°E); Pilanesberg Game Reserve, North West Province, South Africa (25°-26°S; 27°-28°E).

Site of infection: caecum.

Prevalence: $3x10^4$/ml.

Etymology: specific name refers to distinctive anterior peak of organism.





Figs. 11,12. *H. apicalis*, (LNM), x 1250. 11 - ventral view. Fc - long flexible cilia edging external peristomal trough, Pt - external peristomal trough. 12 - dorsal view. Ag - agglomerate of particles, Fc - long flexible cilia edging external peristomal trough

Type materials: white rhinoceros, Accession No. 1130476 (Hluhluwe), deposited in the Intestinal Protozoa Collection of the Centre of Wildlife Management, University of Pretoria, Pretoria 0002, South Africa.

Key to species of *Helicozoster*

The following key is based on body-shape, anterior oral-opening, external peristomal trough.

1 Body-shape ovate terminating in anterior column, external peristomal trough spiralling anterior half of body-length.....*H. apicalis* sp. n.
Body-shape simple ovoid, external peristomal trough spiralling entire body length.....2

2 Anterior oral-opening subterminal .....*H.indicus* Latteur, 1967
Anterior oral-opening terminal.....*H. africanus* sp. n.

***Rhinozeta pedale* sp. n. (Figs. 4, 13)**

Description (n=30): body ovate-longate, dorso-ventrally compressed like the other members of the genus (van Hoven *et al.* 1988), with foot-like posterior end and 2 skeletal plates (Fig. 13); length 228±62.2 µm, width 112 ±39.7 µm, dorso-ventral thickness 23 ±3.0 µm. Oral-opening stretching across entire anterior end of body with characteristic hook-like protrusion on left side (Fig. 13). Adoral zone of cilia borne on retractable cone within peristome. Four semicircular slits forming 4 somatic ciliary bands wrapped partially around the lateral surfaces of body: 3 on the ventral side and 1 on the dorsal side according to Kofoid and MacLennan (1933). First ventral band below oral-opening, second ventral band one half body-length from anterior end of body, third ventral and dorsal bands immediately above foot-like posterior end of body (Fig. 13). All ciliary bands lie across longitudinal axis of body and are housed in the space between 2 thin skeletal plates covering ventral and dorsal surfaces of the body. Cytoproct below posterior end of upper plate. Elongate (173-188 µm) macronucleus beneath ventral edge of upper skeletal plate. Small oval micronucleus recessed in ventral side of macronucleus one-quarter of length of macronucleus from its anterior end. Three or 5 contractile vacuoles on edge of right lateral surface (Fig.4).

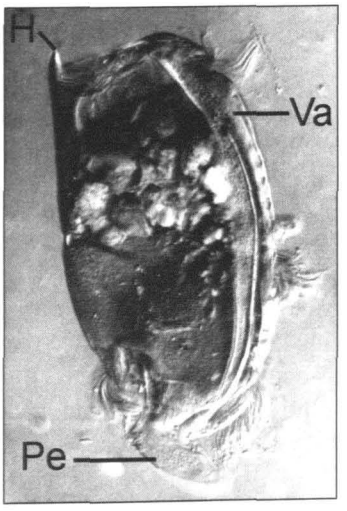


Fig. 13. *Rhinozeta pedale*, ventral view, (LNM), x 1250. H - hook-like protrusion on oral-opening, Pe - foot-like posterior end, Va - contractile vacuoles

Type host: *Ceratotherium simum* Burchell 1817, white rhinoceros.

Other host: no other hosts known.

Type locality: Pilanesberg Game Reserve, North-West Province, South Africa (25°-26°S; 27°-28°E).

Site of infection: dorsal ascending colon.

Prevalence: $1x10^3$/ml.

Etymology: specific name refers to foot-like posterior end of the ciliate.

Type materials: white rhinoceros, Accession No. 4220585 (Pilanesberg) deposited in the Intestinal Protozoa Collection of the Centre of Wildlife Management, University of Pretoria, Pretoria 0002, South Africa.

Key to species of *Rhinozeta*

The following key is based on body-shape, body-size, number of ciliary bands, type of skeletal plate material, cuticular folds (van Hoven *et al.* 1988).

1 With 3 ciliary bands present.....*R. triciliatum* van Hoven *et al.* 1988
With 4 or 5 ciliary bands present.....2

2 With 4 ciliary bands present.....3
With 5 ciliary bands present.....5

3 Small, 94-150 µm long no clear skeletal plate cut-off on left side of body.....*R. rhinozeta* van Hoven *et al.* 1988
Large, 120-331 µm long.....4

4 Posterior end rounded, with clear cuticular folds on the right side and rear of body.....*R. caecale* van Hoven *et al.* 1988
Posterior end foot-like without cuticular folds.....*R. pedale* sp. n.

5 Body largely covered in skeletal plate material.....6
Body partly covered in skeletal plate material.....7
6 Clear lengthwise stripes of skeletal plates.....
.....*R. multiplatum* van Hoven *et al.* 1988
Body mostly uniformly covered by a thin layer of skeletal material.....*R. unilaminatus*
7 Prominent skeletal plate with undulating edge protruding from left body-surface.....
.....*R. cristatum* van Hoven *et al.* 1988
Narrow single plate in the middle of the ventral and dorsal surfaces covering about 50% of each suface...
.....*R. addoense* van Hoven *et al.* 1988

Triplumaria corrugata sp. n. (Figs. 5, 14, 15)

Description (n=34): body-shape ovate-elongate, posterior and terminating in corrugated triangular tail, dorso-ventrally compressed with skeletal rod, and encased in cuticular carapace (Fig. 14); length 153 µm ±23.5, width 79 µm ±12.5, dorso-ventral thickness 22 µm ±3.8. Oral-opening apical (Figs. 14,15). Adoral zone of cilia borne on retractable cone within peristome. Three motilia; one a quarter body-length from anterior end on outside ventral side of body, 2 immediately anterior to corrugated tail on either side of posterior end of body (Fig. 14). Skeletal rod in groove on ventral side of body beneath macronucleus extending full length of body. Cytoproct beneath small raised portion of upper carapace between posterior motilia. Elongate macronucleus posterior to anterior motilium on ventral side of body. Oval micronucleus recessed in dorsal anterior side of macronucleus. Three or more contractile vacuoles in groove on ventral side of body (Fig. 5).

Type host: *Ceratotherium simum* Burchell 1817, white rhinoceros.

Other host: no other hosts known.

Type locality: Pilanesberg Game Reserve, North-West Province, South Africa (25°-26°S; 27°-28°E).

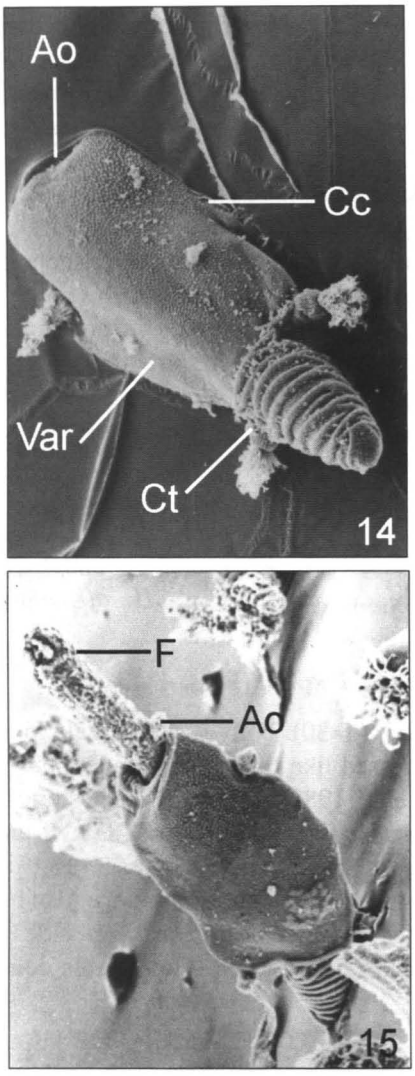
Other locality: Ellisras district, Northern Province, South Africa (23°-24°S; 27°-28°E).

Site of infection: dorsal ascending colon.

Prevalence: Pilanesberg white rhinoceros $0.3x10^3$/ml, Ellisras white rhinoceros $1x10^3$/ml

Etymology: specific name refers to corrugated tail.

Type material: white rhinoceros, Accession No. 4220585 (Pilanesberg) deposited in the Intestinal Protozoa Collection of the Centre for Wildlife Management, University of Pretoria, Pretoria 0002, South Africa.



Figs. 14,15. *Triplumaria corrugata,* ventral view. 14 - SEM, x 500. Ao - apical oral-opening, Cc - cuticular carapace, Ct - corrugated triangular tail, Var - region of vacuoles. 15 - SEM, x 357. Ao - apical oral-opening, F - piece of vegetation being ingested

Key to species of *Triplumaria*

The following key is based on body-shape, body-size, position of oral-opening.

1 Body long: posterior end rounded and smooth.....2
Body short: posterior end somewhat triangular and corrugated.....*T. corrugata* sp. n.
2 Oral-opening subapical on ventral side of body.....
.....*T. selenica* Latteur *et al.* 1970
Oral-opening apical .....*T. hamertoni* Hoare, 1937

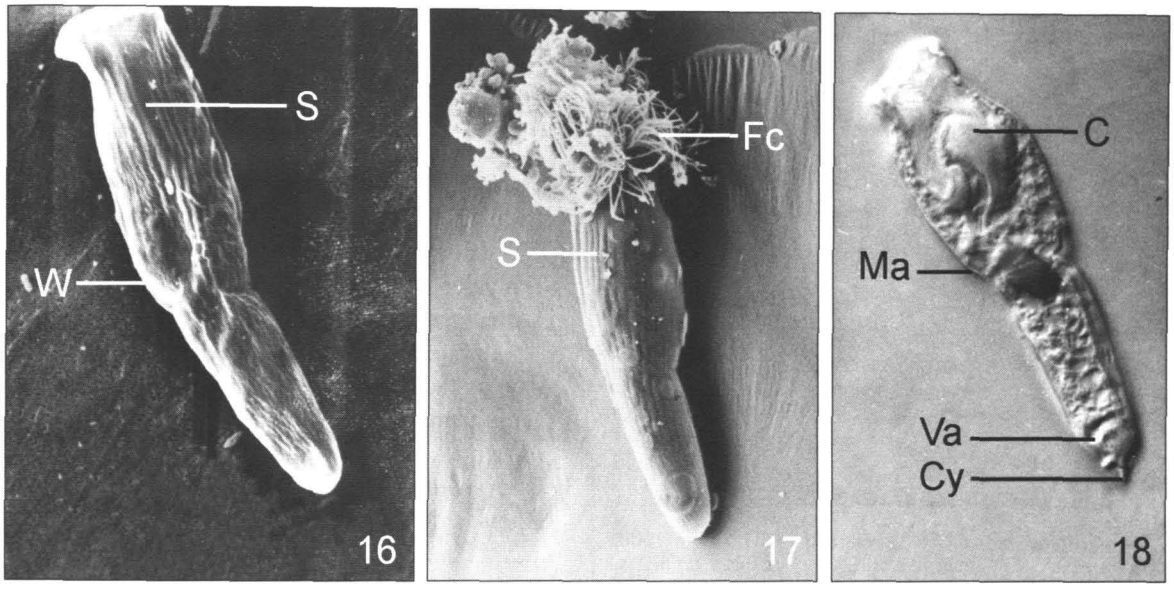


Fig. 16 - 18. *Lavierella klipdrifi*, ventral view. 16 - LNM, x 1250. S - oral cilia of retracted ciliate showing striations of naked cuticle, W - widening at half body-length giving club-shape to body. 17 - SEM, x 867. Fc - tuft of long flexible cilia borne on a cone within peristome, S - cuticular striations. 18 - LNM, x 1250. C - retracted cone within peristome, Cy - covering projecting cytoproct, Ma - macronucleus, Va - contractile vacuoles

***Lavierella klipdrifi* sp. n. (Figs. 6, 16-18)**

Description (n=33): body club-shaped, anterior widening at half body-length to 1.5 times posterior body-width; body covered with rigid naked striated cuticle (Fig.16,17); length 74 µm±8.5, anterior body-width 22 µm ±4.2, anterior body-dorso-ventral-thickness 20 µm ±2.7. Oral-opening apical. Tuft of slender long flexible cilia borne on retractable cone within peristome of oral-opening (Fig. 17). Oval macronucleus in posterior half of body. Oval micronucleus recessed in posterior end of macronucleus (Fig. 6). Position of one or more contractile vacuoles variable. Distinct cytoproct at posterior pole of body (Fig. 6,18).

Type host: *Ceratotherium simum* Burchell 1817, white rhinoceros

Other host: no other hosts known.

Type locality: Pilanesberg Game Reserve, North-West Province, South Africa (25°-26°S; 27°-28°E).

Other localities: Ellisras district, Northern Province, South Africa (23°-24°S; 27°-28°E). Hluhluwe Game Reserve, KwaZulu-Natal Province, South Africa (28°-29°S; 31°-32°E).

Site of infection: caecum, ventral ascending colon.

Prevalence: caecal fluid: Hluhluwe rhinoceros $6x10^4$/ml; Ellisras rhinoceros $4x10^4$/ml; Pilanesberg rhinoceros $8x10^4$/ml. Ventral ascending colon fluid: Pilanesberg rhinoceros $4x10^4$/ml; Ellisras rhinoceros $1x10^3$/ml; Hluhluwe rhinoceros no sample.

Etymology: species name refers to the bottle shape of the ciliate.

Type material: white rhinoceros, Accession No. 4220585 (Pilanesberg), deposited in the Intestinal Protozoa Collection of the Centre for Wildlife Management, University of Pretoria, Pretoria 0002, South Africa.

Key to species of *Lavierella*

The following key is based on body-shape.

1 Body-shape ovate-elongate.....
.....*L. africana* Buisson, 1923
Body-shape club-shaped.....*L. klipdrifi* sp. n.

***Allantosoma multisuctores* sp. n. (Figs. 7, 19)**

Description (n=33 adults): body-shape elongate-cycloid with 20 to 24 ingestory suctorial tentacles scattered uniformly over entire body (Fig. 19); length 35 µm ± 5.3; width 17 µm ±2.6, dorso-ventral thickness 10 µm ±3.0. Distinct sucker borne at end of each tentacle.

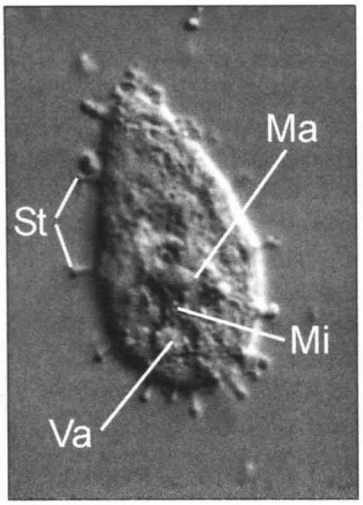


Fig. 19. *Allantosoma multisuctores,* ventral view (LNM) x 1000. Ma - macronucleus, Mi - micronucleus, St - suctorial tentacles, Va - contractile vacuole

Centrally placed spherical macronucleus with spherical micronucleus lying closely along side. Single contractile vacuole. No visible cytoproct (Fig. 7).

Type host: *Disceros bicornis* Linnaeus 1758, black rhinoceros.

Other host: *Ceratotherium simum* Burchell 1817, white rhinoceros.

Type locality: black rhinoceros, Addo Elephant National Park, Eastern-Cape Province, South Africa (33°-34°S; 25°-26°E).

Other localities: white rhinoceros Hluhluwe Game Reserve, KwaZulu-Natal Province, South Africa (28°-29°S; 31°-32°E); Pilanesberg Game Reserve, North-West Province, South Africa (25°-26°S; 27°-28°E); Ellisras district, Northern Province, South Africa (23°-24°S); 27°-28°E).

Site of infection: caecum, ventral and dorsal ascending colon.

Prevalence: caecal fluid: Addo black rhinoceros $8x10^3$/ml, Hluhluwe white rhinoceros $9x10^3$/ml, Pilanesberg white rhinoceros $2x10^3$/ml; fluid of ventral ascending colon: Addo black rhinoceros $7x10^3$/ml, Pilanesberg white rhinoceros $2x10^3$; fluid of dorsal ascending colon: Addo black rhinoceros $2x10^3$/ml, Ellisras white rhinoceros $3x10^3$/ml.

Etymology: species name refers to numerous scattered tentacles with suckers.

Type material: black rhinoceros, Accession No. 2240485 (Addo) deposited in the Intestinal Protozoa Collection of the Centre for Wildlife Management, University of Pretoria, Pretoria 0002, South Africa.

Key to species of *Allantosoma*

The following key is based on the number of tentacles, position of tentacles, body-width, body-length (Imai, 1979).

1 Tentacles confined to each end of body.....2
Tentacles scattered uniformly over entire body surface .....*A. multisuctores* sp. n.

2 One tentacle confined to each end of body.....3
More than one tentacle confined to each end of body 4

3 Body-width 3-7 µm.....*A. lineare* Strelkow, 1939
Body-width 7-11 µm.....
.....*A. brevicornigerum* Hsiung, 1928
Body-width 10-20 µm.....
.....*A. dicornigerum* Hsiung, 1928

4 Body-length 16-35 µm.....5
Body-length 33-67 µm.....6

5 Body-width 7-10 µm.....*A. japonense* Imai, 1979
Body-width 6-17 µm.....
.....*A. biseriale* Strelkow, 1939

6 Body-width 10-20 µm.....
.....*A. cucumis* Strelkow, 1939
Body-width 18-37 µm.....
.....*A. intestinale* Hsiung, 1930

DISCUSSION

In comparing counts of ciliated protozoan endocommensals occurring in the hindgut of rhinoceroses with those of horses and elephants, only those for the 3 grazing white rhinoceroses were used. The higher counts for the black rhinoceros browsing on highly nutritious "spekboom" *Portulacaria afra* were considered exceptional. In fed animals the average total ciliate counts x 10^3/ml digesta in white rhinoceroses, horses (Ozeki *et al.* 1973) and African elephants (Eloff and van Hoven 1980) were respectively, 178, 220, 41 for caecum, 100, 770, 46 for ventral/large colon, 85, 240, no count, for dorsal/small colon. This indicated that the colon probably played an important role in the fermentation of the diet of these 3 groups of large herbivorous mammals as predicted by Hume and Warner (1980).

Of the 48 species recovered to date from the digesta of the rhinoceroses, 15 species (*Blepharosphaera episoidalis*,

Didesmis ovalus, *D. quadrata*, *Holophryoides ovalis*, *Alloiozona trizona*, *Polymorphella ampulla*, *Blepharoconus cervicalis*, *Paraisotricha colpoidea*, *P. minuta*, *Cycloposthium bipalmatum*, *Triadinium galea*, *T. minimum*, *Allantosoma intestinale*, *A. biseriale*, *Telamodinium onyx* are in common with those of horses (Hsiung 1930, Strelkow 1939, Adam 1951, Latteur and Dufrey 1967, Ozeki *et al.* 1973), 8 species (*Blepharosphaera intestinalis*, *Paraisotricha colpoidea*, *P. minuta*, *Lavierella africana*, *Triplumaria hamertoni*, *T. selenica*, *Cycloposthium bipalmatum*, *Prototapirella intestinalis*) are in common with those of african elephants (Latteur *et al.* 1970, Eloff and van Hoven 1980), and 6 species (*Holophryoides ovalis*, *Blepharosphaera intestinalis*, *B. episoidalis*, *Didesmis ovalis*, *D. quadrata*, *Paraisotricha colpoidea*) are in common with those of bushpigs (van Hoven and Gilchrist 1991).

These 48 species from rhinoceroses belong to 22 apparent genera belonging to 9 families [Buetschliidae, Paraisotrichidae, Blepharocorythidae, Cycloposthiidae, Ditoxidae, Ophryoscolecidae, Acinetidae, Telamodiniidae (Corliss 1979), and a recently new family Rhinozetidae (van Hoven *et al.* 1988)], 7 more than the 2 Dehority (1986) reported. These families also contain ciliated protozoan species recovered from the intestines of other herbivorous hindgut-fermenting mammals such as zebras, asses, Indian elephants, tapirs, warthogs, guinea pigs, capybaras (Dehority 1986), and bushpigs (van Hoven and Gilchrist 1991).

Only the family Rhinozetidae appeared to be specific for the rhinoceroses as in the case of the Polydiniidae for the elephants and the Troglodytellidae for the anthropoid apes (Dehority 1986). The 25 systematically identified, proposed, and apparent new species are rhinoceros-specific (*Blepharosphaera intestinalis minor*, *Didesmis synciliata*, *Helicozoster africanus*, *H. apicalis*, *Charonina odontophora*, *C. tenuis*, *C. dicerotis*, *C. tortuosa*, *C. tetragona*, *Lavierella klipdrifi*, *Triplumaria corrugata*, *Monoposthium vulgaris*, *M. bracchium*, *M. latus*, *Arachnodinium noveni*, *Phalodinium digitalis*, *Rhinozeta addoense*, *R. unilaminatum*, *R. caecale*, *R. rhinozeta*, *R. triciliatum*, *R. cristatum*, *R. multiplatum*, *R. pedale*, *Allantosoma multisuctores*). Much more detailed research is required in respect of host specificity of the ciliated Protozoa recovered from the intestines of herbivorous mammals, as well as measured counts of these organisms in the gastro-intestinal tract.

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