Rumen ciliate protozoa of the sable antelope *Hippotragus niger*

W. van Hoven, V.L. Hamilton-Attwell and J.H. Grobler

Of the 11 ciliate protozoa present in the rumen of the sable antelope, two are holotrichs and nine entodiniomorphs. One new species *Diplodinium (Eudiplodinium) sablei* is described. The seven antelopes investigated gave an average total number of protozoa per cm^3 rumen fluid of $1,79 \pm 0,39 \times 10^5$, a relatively low figure typical of grazing wild ungulates.

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Van die 11 siliaat-protozoa teenwoordig in die rumen van die swartwitpens was twee holotriche en nege entodiniomorphe vorme. Een nuwe spesies *Diplodinium (Eudiplodinium) sablei* word beskryf. Die sewe swartwitpense wat ondersoek is, het gemiddeld $1,79 \pm 0,39 \times 10^5$ protozoa per cm^3 rumenvloeistof gehad, 'n relatief lae syfer tipies van weiende wildsbokke.

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The sable antelope belongs to the subfamily Hippotraginae and the southern subspecies *Hippotragus niger niger* Harris, 1838 occurs south of the Zambesi River in Rhodesia, northern Botswana and south western Mocambique. The Rhodes Matopos National Park, some 43 200 ha in size, lies 48 km south-west of Bulawayo and consists of large broken granite hills with gorges, surrounded by woodland interspersed with grassland and savanna woodland of variable tree density. It is a habitat ideally suited for a grazer such as the sable antelope.

Several sable antelopes were culled from this park in Rhodesia during February 1977. The study was undertaken because no information existed about the population composition and identities of the rumen protozoa of this antelope.

Material and Methods

Soon after an animal was killed the digestive tract was removed and an incision of approximately 25 cm was made in the rumen wall. A sample of the contents was thoroughly mixed and filtered through cheese cloth. The rumen fluid was preserved in a 1:1 ratio with 10% (v/v) formalin.

Total numbers of protozoa were counted as described by van Hoven (1974). The organelles were found to be readily visible and recognizable in unstained preparations. A microscope with interference contrast colouring was used. When necessary for the study of skeletal plates, staining with chlorzinc iodine was used. Slides were made by mixing a drop of filtered rumen fluid with a drop of glycerine and smearing it over the slide. After placing a cover-slip over the mixture the edges were sealed with Depex mounting medium.

Glycerine-mounted preparations were used in determining the species composition of the total protozoan community in the rumen. Measurements were made with a calibrated ocular micrometer and drawings were done with the aid of a drawing tube. Terminology used in descriptions follows the system of Lubinsky (1958).

The formalin-fixed samples were also prepared for scanning electron microscopy. When it became apparent that the protozoans were covered by excess debris the samples were cleaned by hand. It was observed that some of the protozoans sedimented faster than the debris, thus the sample was pipetted into a petri dish and after a few moments the debris was drawn off with a fine Pasteur pipette. The protozoans were now clean and concentrated. During the SEM study it became apparent that the entodinia possibly sedimented slower and were thus removed with the debris.

The concentrated protozoans were post-fixed in Bouin's fluid for a week. From this stage the procedure was completed in a small funnel on a Nuclepore filter (Hamilton-Attwell 1976). After fixing the picrate sections in 70% alcohol the protozoans were dehydrated through a series of alcohols to 100%. The 100% alcohol was substituted by amyl acetate and the samples were then critical-point dried by the liquid CO_2 method of Anderson (1951). To prevent the protozoans on the filter from being washed away during the drying phase, the filter was sandwiched between layers of cellulose acetate membranes (CAM).

After completion of the drying phase, the filter and the protozoans that were stuck to it were mounted with colloidal silver on a stub. The rest of the protozoans, caught between the CAM, were spread on double-sided masking tape, stuck to stubs. The mounted samples were twice coated with silver and viewed with a Cambridge Stereoscan at 10 or 20 kV.

The protozoa

In classifying the ophryoscolecid species from the rumen the system of Dogiel (1927) as amended by Noirot-Timothee (1960) was used. The isotrichid species were classified according to the system used by Becker & Talbott (1927).

Dogiel (1927) used the term forma to indicate a certain type of infraspecific variation. This term was originally used by Sharp (1914) to indicate the number of caudal spines present in *Epidinium ecaudatum*. Dogiel (1927) extended its use to indicate differences in size (*Diplodinium costatum* forma *major* and *minor*), the occurrence of species which vary slightly in different hosts (*Diplodinium neglectum* forma *bovis* and *impalae*), and also major deviations from the basic form (*Diplodinium neglectum* forma *neglectum* and *giganteum*). These variations have no ecological or geographical basis since different formae of a certain species can be present in different individuals of a host species from the same locality, and several formae of a species can occur in a single host.

The formae as used by Dogiel (1927) were all elevated to the rank of species by Kofoid & MacLennan (1932 and 1933). Since caudal spination varies under changing environmental conditions, and it has been shown specifically with *Entodinium caudatum* by Poljanskij & Strelkow (1938) that caudal spination varies according to the quality of nutrition, Kofoid & MacLennan's system becomes invalid. The original system of classification as used by Dogiel (1927) is therefore used.

Dogiel (1927) distinguished four subgenera within the genus *Diplodinium, viz Anoplodinium, Eudiplodinium, Polyplastron* and *Ostracodinium*. Kofoid & MacLennan (1932) assigned generic rank to all of them and established six additional genera. According to Noirot-Timothée (1960) not all the genera described by Kofoid & MacLennan (1932) are recognizable and he therefore recommended that the subdivision of the genus *Diplodinium* into subgenera as proposed by Dogiel (1927) should be retained but modified. For this purpose *Diplodinium* was subdivided into six subgenera. Two of these subgenera were adopted from two genera established by Kofoid & MacLennan (1932), *viz Elytroplastron* and *Enoploplastron,* while the subgenus *Anoplodinium* of Dogiel (1927) was replaced by the subgenus *Diplodinium.* This was done to comply with the rules of nomenclature (Article 44a) which require that one subgenus of a subdivided genus must be the nominate subgenus. The genus *Diplodinium* therefore contains the following subgenera: *Diplodinium, Eudiplodinium, Polyplastron, Ostracodinium, Elytroplastron* and *Enoploplastron.*

The following rumen ciliates were present in the sable antelopes investigated:

Class: Ciliata, Perty, 1852
Subclass: Spirotricha, Bütschli, 1889
Order: Entodiniomorphida, Reichenow, 1929
Family: Ophryoscolecidae, Stein, 1859
Genus: *Diplodinium* Schuberg, 1888
- *D.* (*Eudiplodinium*) *maggii* Fiorentini, 1889
- *D.* (*Eudiplodinium*) *medium* forma *medium* Awerinzew and Mutafowa, 1914
- *D.* (*Eudiplodinium*) *neglectum* forma *bovis* Dogiel, 1925
- *D.* (*Eudiplodinium*) *sablei* sp. n.
- *D.* (*Diplodinium*) *rangiferi* forma *minor* Dogiel, 1925
- *D.* (*Diplodinium*) *denticulatum* Fiorentini, 1889
- *D.* (*Diplodinium*) *costatum* forma *minor* Dogiel, 1925
- *D.* (*Enoploplastron*) *garstangi* van Hoven, 1975

Genus: *Entodinium* Stein, 1858
- *E. rostratum* Fiorentini, 1889

Subclass: Holotricha, Stein, 1859
Order: Trichostomatida, Bütschli, 1889
Family: Isotrichidae, Bütschli, 1887
Genus: *Isotricha* Stein, 1859
- *I. prostoma* Stein, 1859

Genus: *Dasytricha* Schuberg, 1888
- *D. ruminantium* Schuberg, 1888

Diplodinium (*Eudiplodinium*) *neglectum* forma *impalae* was found to be 55 μm long and 40 μm wide which gives it a somewhat shorter and rounder appearance than the forma described by Dogiel (1927). The macronucleus varied in shape as is shown in Fig. 1.

All the specimens of *D.* (*Enoploplastron*) *garstangi* present in the sable antelope were found to have two caudal spines, whereas those described from the tsessebe had only a single caudal spine (van Hoven 1975). Both caudal spines in this case have short, irregular spikes on the left surface (Fig. 2).

The genus *Entodinium* was represented by the single species *rostratum.* Of this species the forma *bifidum* as well as specimens with three spines were present (Fig. 3).

The percentage composition of the protozoan species and the total numbers of the population per cm^3 rumen fluid in the seven sable antelopes investigated are listed in Table 1. Table 2 contains some information on the antelopes used.

Table 1 Percentage composition of species of the protozoan population and their total numbers × 10^5 per cm^3 in seven sable antelopes

	Antelope Number							
Species	1	2	3	4	5	6	7	X̄ ± S.D.
Diplodinium (Eudiplodinium) maggi	4	9	8	18	2	19	6	9,43 ± 6,63
D. (E.) medium f. *medium*	3	1	0	3	8	1	3	2,71 ± 2,63
D. (E.) neglectum f. *bovis*	22	7	17	22	25	19	16	18,29 ± 5,88
D. (E.) sablei sp. n.	6	4	7	8	3	12	18	8,29 ± 5,19
D. (Diplodinium) rangiferi f. *minor*	4	21	4	6	5	15	6	8,71 ± 6,63
D. (D.) denticulatum	18	15	8	7	13	7	13	11,57 ± 4,31
D. (D.) costatum f. *minor*	4	4	2	8	3	8	3	4,57 ± 2,44
D. (Enoploplastron) garstangi	1	0	6	6	0	8	0	3,00 ± 3,51
Entodinium rostratum	38	36	45	21	39	10	35	32,00 ± 12,14
Isotricha prostoma	0	0	0	2	0	0	0	0,29 ± 0,76
Dasytricha ruminantium	0	0	3	1	3	0	1	1,14 ± 1,35
TOTAL PROTOZOA	2,16	1,38	2,31	2,13	1,57	1,60	1,37	1,79 ± 0,39

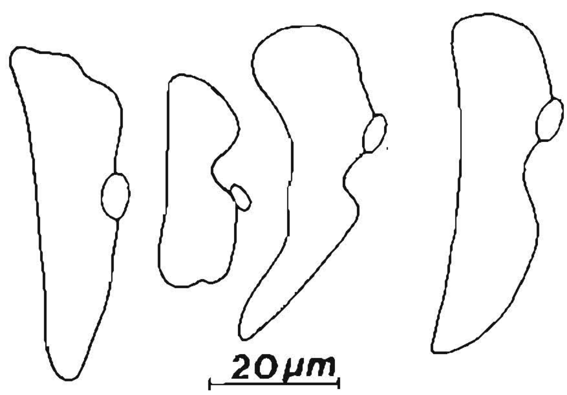

Fig. 1 Variations in macronuclear shape of *Diplodinium (Eudiplodinium) neglectum* forma *impalae*.

Table 2 Details of the seven sable antelope studied

Date	No.	Sex	Age (months)	Body mass (kg)	Rm[b] (kg)
7.2.77	1	Male	48	189	28
9.2.77	2	Female	60	169	34
9.2.77	3	Female	60	157	26
8.2.77	4	Male	60	171	24
7.2.77	5	Female	72	166	24
7.2.77	6	Male	72	196	26
9.2.77	7	Female	120	139	26

[a] No. 7 was destroyed because of a large wound in perineal area and its very poor condition. All other animals were in average to good condition.

[b] Wet mass of rumen contents.

Diplodinium (Eudiplodinium) sablei sp. n.

Diagnosis Body oval in shape. Prominent circular shield encloses the anus; anteriorly two skeletal plates which fuse in the middle of upper side of body; right plate wider than left; macronucleus has typical indentations and depressions partly containing vacuoles and micronucleus. (Figs. 8 and 9).

Description Body viewed from the side has an oval shape with the upper and lower surfaces more compressed than the lateral ones. The left and right sides are convex, the left side more convex than the right side which has a wider rim due to the ectoplasmic extension beyond the anus enclosing the latter in a hollow cap (Figs. 4 and 5). Both oral and adoral membranelle zones are slightly inclined to the lower surface.

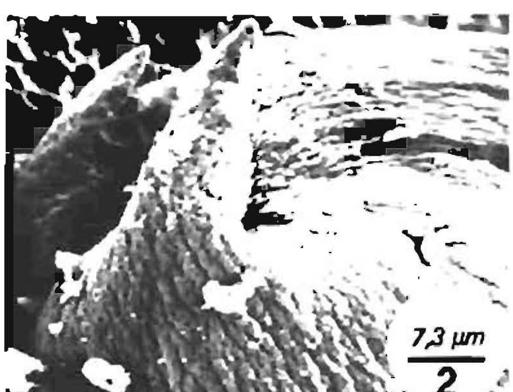

Fig. 2 Caudal spines of *Diplodinium (Enoploplastron) garstangi*.

Fig. 3 *Entodinium rostratum* forma with three caudal spines.

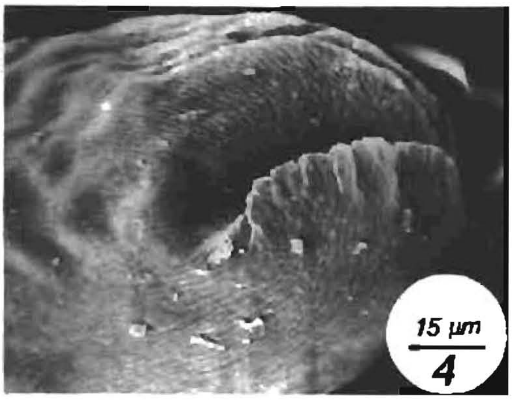

Fig. 4 Caudal view of anal flap of *D. sablei*.

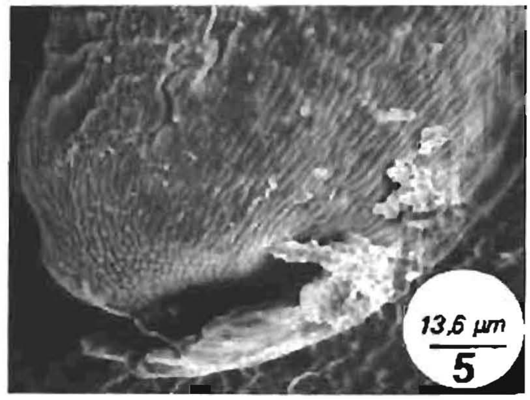

Fig. 5 Lateral view of anal flap of *D. sablei*.

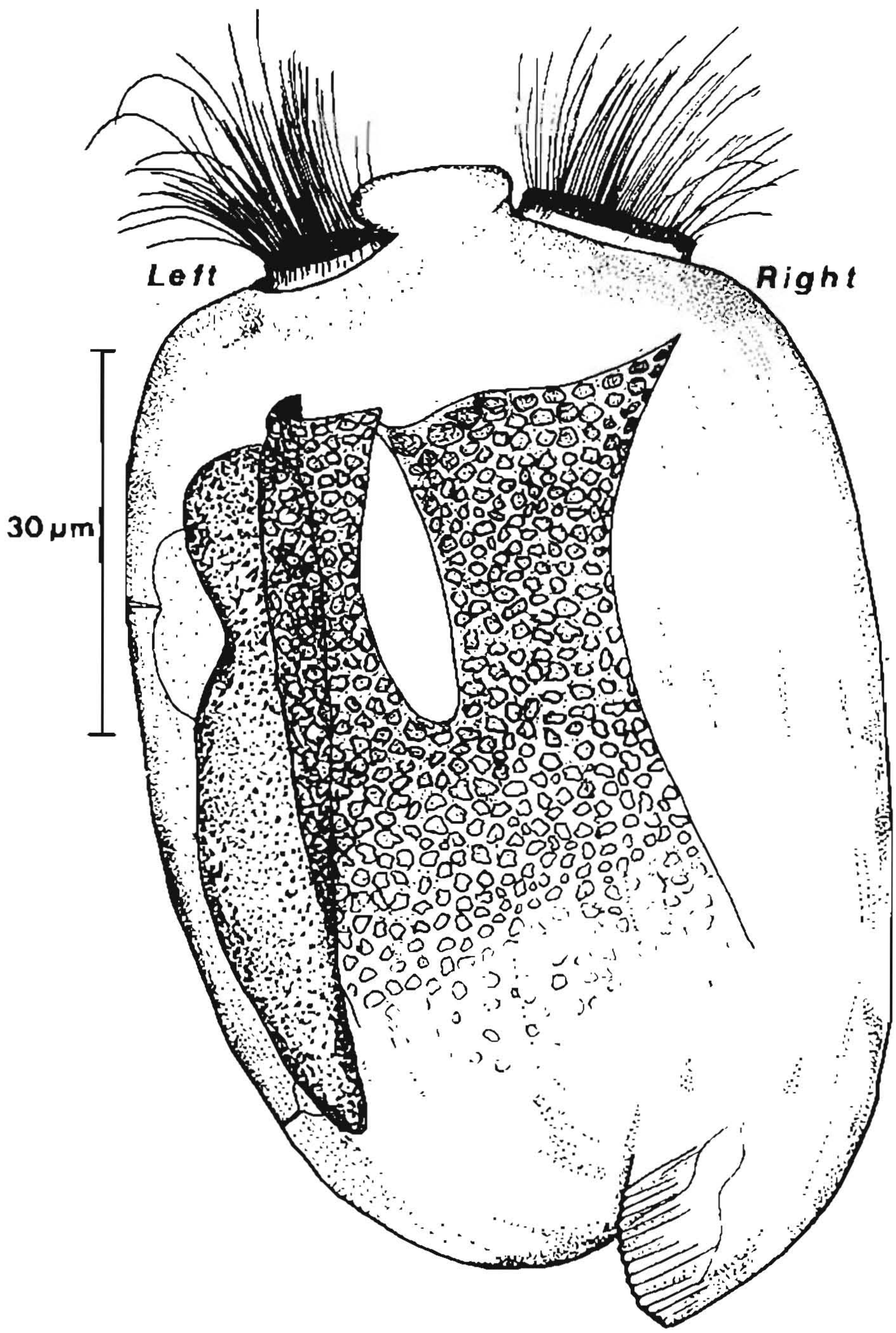

Fig. 8 *Diplodinium* (*Eudiplodinium*) *sablei* sp. n. viewed from upper side.

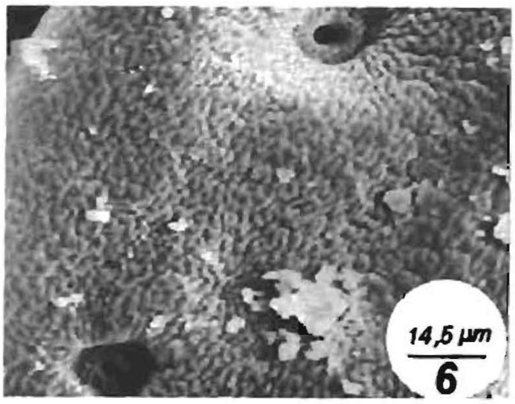

Fig. 6 Contractile vacuoles' excretory channel openings on the left cellular surface of *D. sablei*.

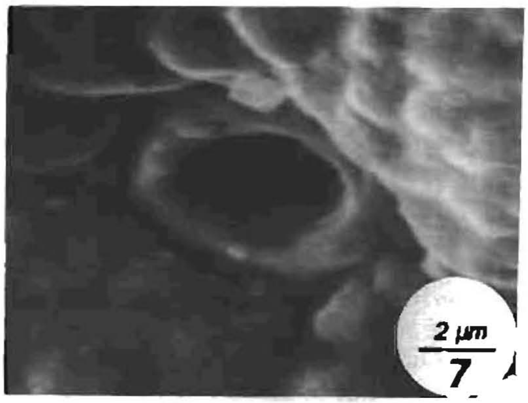

Fig. 7 Opening of excretory channel of contractile vacuole of *D. sablei*.

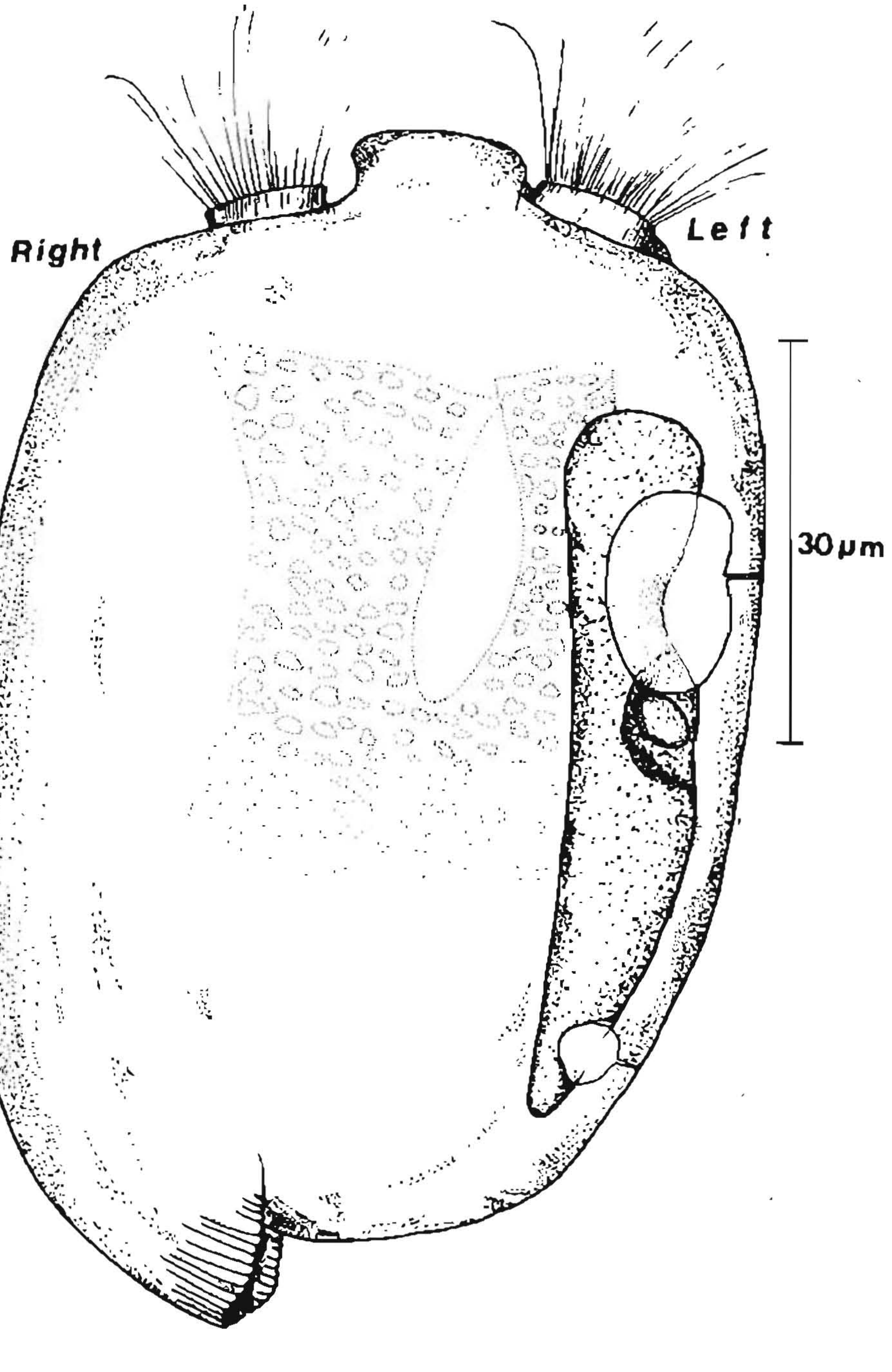

Fig. 9 *Diplodinium* (*Eudiplodinium*) *sablei* sp. n. viewed from lower side.

The skeletal plate lies on the upper surface of the body. Anteriorly the skeletal plate is split into two parts, forming a narrow oval slit. The left side of the skeletal plate folds above the macronucleus inwards along its entire length. Anteriorly the right side of the skeletal plate is triangular in shape with the anterior right extreme curving partly around the right body side. The two anterior parts of the plate join again in the middle of the body at the level of the micronucleus. In the posterior third of the body the skeletal plate becomes increasingly thinner, diminishing towards the posterior of the body.

The macronucleus is basically long and straight. It is in most cases just as long as the skeletal plate and tapers to a fairly sharp point posteriorly. The posterior quarter of the macronucleus exhibits a shallow indentation, which partly houses the posterior contractile vacuole on its lower side. The right side of the macronucleus is concave and partly overlain on the upper side by the skeletal plate. Anteriorly the macronucleus is wide and rounded. The left side of the macronucleus, about a third of the distance from the anterior end, has an indentation which partly contains the anterior vacuole. Just posterior to the anterior contractile vacuole another hollow depression on the lower left side of the macronucleus is found which contains the oval-shaped micronucleus.

As a rule the anterior contractile vacuole was found to be about double the size of the posterior one. The excretory canals open from the middle of the vacuoles to the cell surface (Figs. 6 and 7).

The mouth opens into a narrow tubular gullet. Prominent oesophageal fibrils arise in the gullet. These fibrils form a parallel network close to the upper side immediately below the skeletal plate and extend over the same width as the skeletal plate across the posterior two-thirds of the body.

The rectum is a narrow cylinder lying in the ectoplasm at the upper right side of the body and widens on the inside of the endoplasm. The anus opens inside the rounded hollow capsule which totally encloses it.

Dimensions

Length	89,2 (76-102) μm
Width	57,4 (52-69) μm
Max. length	57,4 (52-71) μm
Min. length	7,6 (7-9,5) μm
L/W	1,55

Relationships This species relates to *Diplodinium* (*Eudiplodinium*) *ypsilon* f. *ypsilon*, particularly the skeletal plate which is also divided into two anteriorly. In *D. ypsilon*, however, the two parts of the skeletal plate are flat and equal in size. In size *D. ypsilon* is about a third larger than *D. sablei*.

The anal cover of *D. sablei* relates to the situation found in *D.* (*Eudiplodinium*) *neglectum* f. *rugosum*. In *D. sablei*, however, the lateral sides of the cover are more firmly attached to the sides of the body.

Type material is available for study at the Zoology Department, University of Pretoria.

Discussion

The only other existing information on the rumen protozoa of the sable antelope is that mentioned by van der Wath & Myburgh (1941). They sampled two animals and identified *Diplodinium* (*Eudiplodinium*) *maggi*, *Entodinium caudatum* and *E. nanellum*. The animals were shot in the Transvaal lowveld.

A count of the total numbers of protozoa by van der Wath & Myburgh (1941) yielded $1{,}84 \times 10^5$ and $3{,}1 \times 10^5$ respectively which compares favourably with the average of $1{,}79 \pm 0{,}39 \times 10^5$ found for seven animals in this study. These relatively low numbers are typical of a grazer with a lower intake of protein and soluble carbohydrates than a browser.

Comparing Tables 1 and 2 proves no difference between age, sex, body mass, rumen size and protozoan population of the rumen. Even antelope no. 7, which was in a very poor condition because of large wounds in the perineal area, had a normal rumen population in comparison with the other healthy animals.

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