Three new species of ciliated Protozoa from the hindgut of both white and black wild African rhinoceroses

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ABSTRACT

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This report deals with the effect of the mode of feeding of the hindgut-fermenting herbivorous rhinoceros on the species of Protozoa fermenting the ingesta, as demonstrated by the proposed three new species of ciliated Protozoa: *Didesmis synciliata* differing from *D. ovalis* in having syncilia in place of simple cilia, *Blepharoconus dicerotos* being twice the size of *B. cervicalis*, and *Blepharosphaera ceratotherii* being one third the size of *B. intestinalis*. The findings are in line with the biological tenet that in herbivores the composition of the diet is the major factor determining the composition of the digestive organisms.

Keywords: *Blepharosphaera ceratotherii* n.sp., *Blepharoconus dicerotos* n.sp., *Didesmis synciliata* n.sp., hindgut-fermenting Protozoa, rhinoceros

INTRODUCTION

A study on the intestinal ciliated protozoa of both black and white wild African rhinoceroses (Skinner & Smithers 1990) has revealed 48 species of which 26 are new. The hook-lipped black rhinoceros (*Diceros bicornis* Linnaeus 1758) is a concentrate browser (Hofmann 1973), while the square-lipped white rhinoceros (*Ceratotherium simum* Burchell 1817) is a roughage grazer (Owen-Smith 1988). The effect of the different modes of feeding on the Protozoa was examined.

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MATERIALS AND METHODS

Gastrointestinal tracts were excised while the carcasses were still warm after the rhinoceroses had been shot. Sets of six samples from the stomach, small intestine, caecum, right ventral ascending colon, right dorsal ascending colon, and descending colon were collected. A slit was made in the wall of the gastrointestinal tract at the sampling point, and the digesta mixed by hand. Using a beaker, digesta was bailed out and strained through a 4-mm-mesh wire sieve. The strained fluid containing the Protozoa was collected. For both light and scanning electron microscopy, 25 mℓ of fluid were added immediately to 25 mℓ of formalin (14% aq.). Clumping of the Protozoa was prevented by shaking the sample vigorously for 30 s on addition of the preservative.

For light microscopy a portion of the formalinized sample was diluted with mineral solution (Bryant & Burkey 1953) and finally with equal parts of glycerol as stabilizing agent (Van Hoven 1983). Total counts were made at x90 magnification with a 0,5-mm-Nageotte counting chamber (W. Schreck, Hofheim, Germany).

The various ciliate species were counted at x400 magnification and converted to a percentage of the total which was in excess of 200 individuals. Detailed anatomy was studied at x1 000 magnification using oil immersion. Drawings were made on the camera lucida principle and all measurements made with a calibrated eyepiece micrometer. Light microphotography was carried out with a Zeiss photomicroscope provided with Nomarski equipment for differential interference contrast. A Kodak Technical Pan Film (Estar-AH Base) TP (35–36) was employed to obtain clear image enlargements.

For scanning electron microscopy (SEM) a portion of the formalinized sample was washed three times with 0,075 M sodium phosphate buffer, postfixed with OSO_4 (1 % aq.) for 2 h, rinsed with distilled water and dehydrated with serial ethanol solutions. It was critical point dried and mounted on stubs, then sputter coated with gold and examined with a JEOL 840 scanning electron microscope, followed by normal photographic procedures.

Size was measured in micrometers (µm). Lubinsky's (1957, 1958) terminology was used for the description and orientation of the species.

RESULTS

No protozoa were found in the stomach or small intestine of either black or white rhinoceroses, and few, if any, of the Protozoa occurring in the caecum and ascending colon occurred in the descending colon.

Bacteria, fungi and flagellates were also encountered in the hindgut. Forty eight different species of Protozoa were found in the caecum and/or ascending colon where active fermentation of the ingesta takes place, and of these, three are considered to be new species and are described below.

Didesmis synciliata n.sp. (Fig. 1, 4–9)

DESCRIPTION (*n* = 35)

The body is oval, rigid, and dorso-ventrally flattened with a blunt anterior end and a tapering posterior end (Fig. 4); length 66 ± 12,3; width 45 ± 6,8; thickness 27 ± 5,8.

The large anterior funnel-shaped oral opening and small posterior round cytopyge are both surrounded by approximately equal numbers of syncilia (Fig. 5, 6), which have the appearance of tentacles under the light microscope (Fig. 7).

The rest of the body is naked. The oval macronucleus is situated in the middle of the body with a small refractive ellipsoidal micronucleus in a depression on its surface (Fig. 4). A concrement vacuole containing refractive granules is present in the anterior part of the body (Fig. 7). A contractile vacuole lies near the cytopyge in the posterior part of the body.

TYPE HOST

Ceratotherium simum. Other hosts unknown.

TYPE LOCALITY

Ellisras district, Northern Province, South Africa (23–24°S; 27–28°E).

OTHER LOCALITIES

Pilanesberg Game Reserve, North-West Province, South Africa (25–26°S; 27–28°E); Hluhluwe Game Reserve, KwaZulu-Natal Province, South Africa (28–29°S; 31–32°E).

SITE OF INFECTION

Dorsal ascending colon.

PREVALENCE

1 x 10^4/mℓ digesta fluid.

ETYMOLOGY

Specific name refers to the syncilia of the organism.

TYPE MATERIAL

Accession no. 3010585 (Ellisras); 4220585 (Pilanesberg); 1130476 (Hluhluwe), deposited in Intestinal Protozoa Collection of Centre for Wildlife Management, University of Pretoria, Pretoria, South Africa.

KEY TO SPECIES OF *DIDESMIS*

1. Body ovate 2
   Body spiral .... *D. spiralis* Hsiung 1930
2. Dorsal groove absent 3
   Dorsal groove present .... *D. quadrata* Fiorentini 1890
3. Cilia simple (Fig. 8, 9) .... *D. ovalis* Fiorentini 1890
   Cilia compound (Fig. 5, 6) .... *D. synciliata* n.sp.

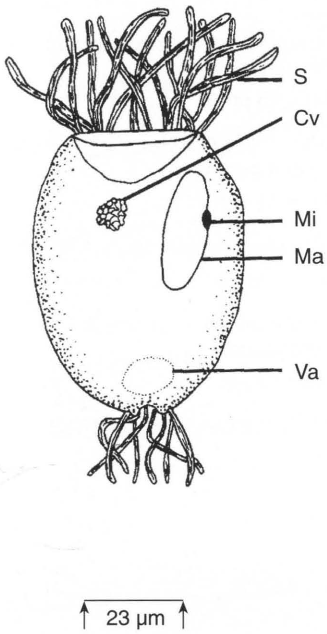
REMARKS

Fig. 8, 9 show the SEM of the simple cilia of *D. ovalis* at the same magnification as the SEM of the syncilia of proposed *D. synciliata* in Fig. 5, 6.

Blepharoconus dicerotos n. sp. (Fig. 2a, 2b, 10–14)

DESCRIPTION (*n* = 31)

The oval-shaped body is dorso-ventrally compressed with rounded posterior end; the anterior terminates in a short neck leading to the oral opening; length 157 ± 34,8; width 99 ± 19,8; thickness 35 ± 5,5 (Fig. 2a). Cuticular folds (Fig. 10) allowing for expansion, radiate out from the cytopharyngeal canal encompassing the oral opening.



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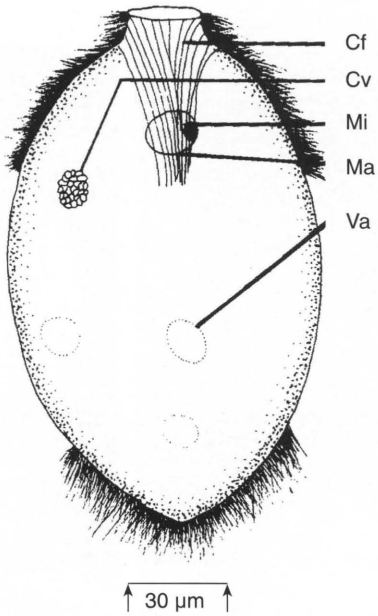

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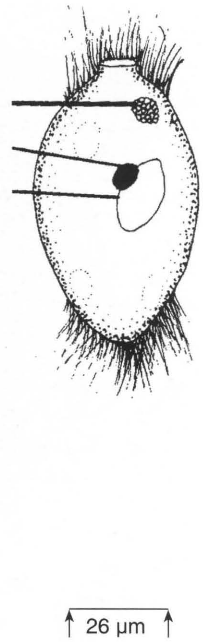

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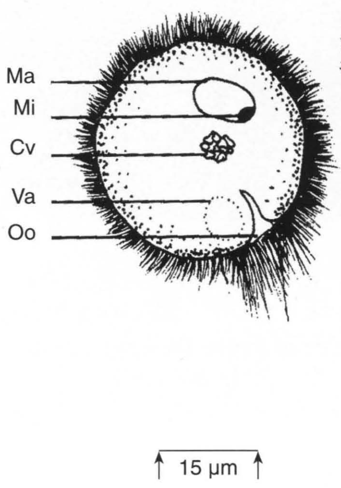

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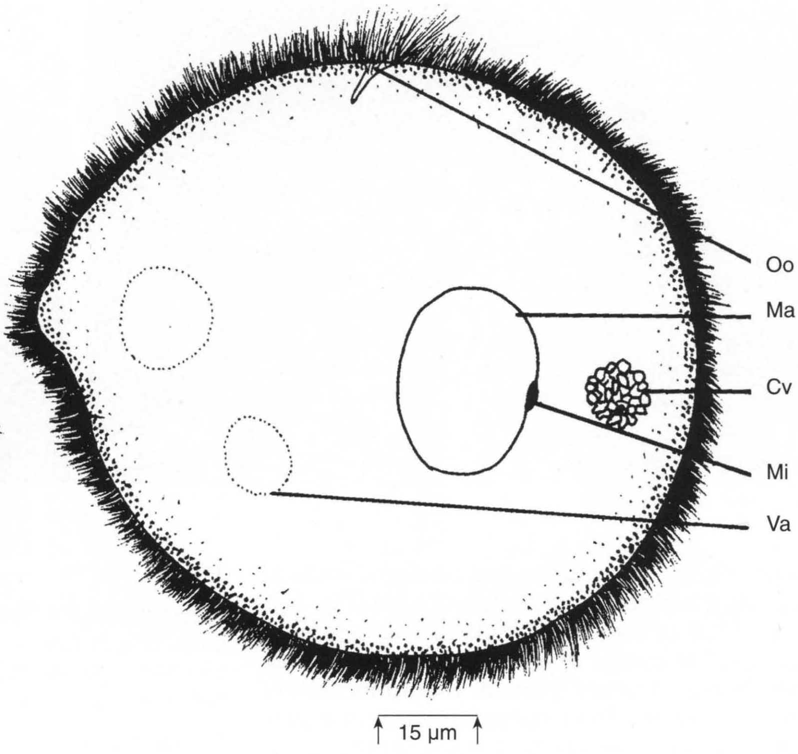


3b

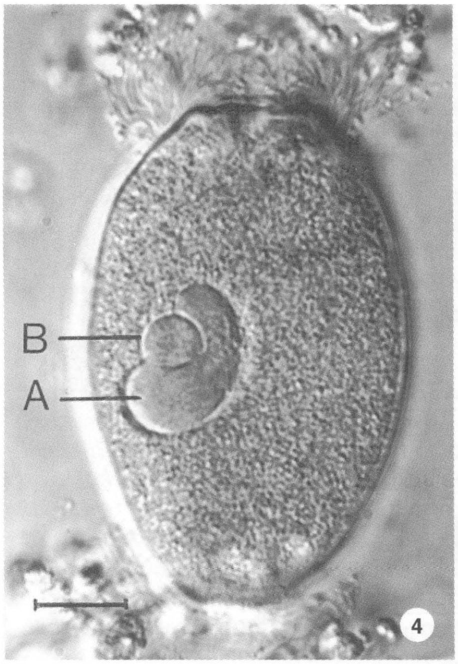
FIG. 1 *Didesmis synciliata* n.sp., S = syncilia, Cv = concrement vacuole, Mi = micronucleus, Ma = macronucleus, Va = contractile vacuole

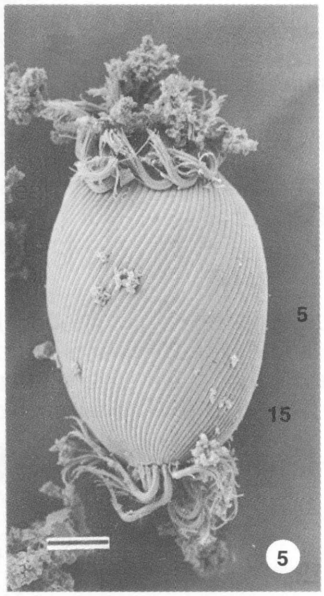
FIG. 2a *Blepharoconus dicerotos*, Cf = cuticular folds

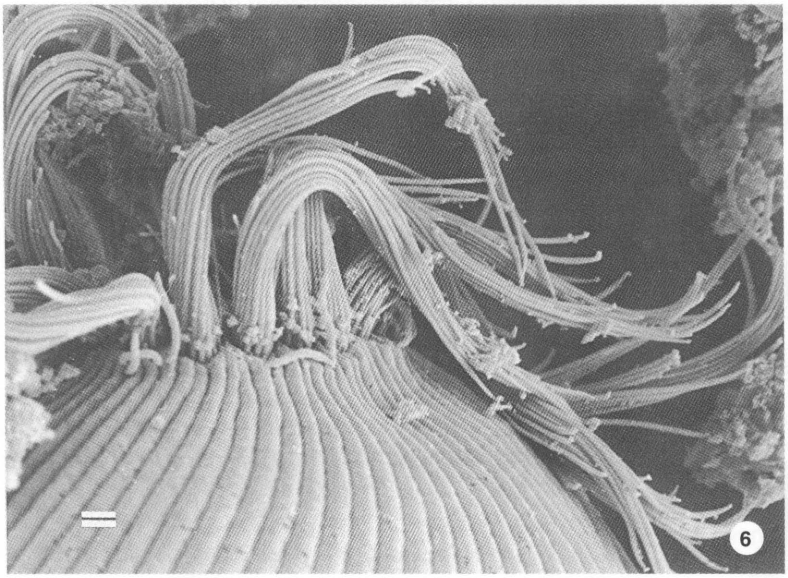
FIG. 2b *Blepharoconus cervicalis*

FIG. 3a *Blepharosphaera ceratotherii*, Oo = oral opening

FIG. 3b *Blepharosphaera intestinalis*







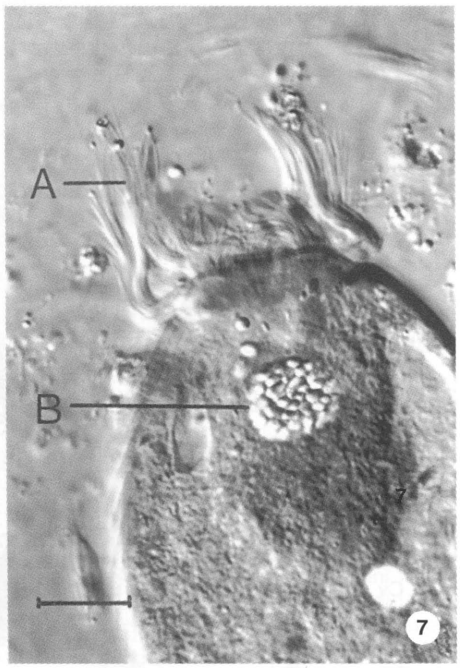


FIG. 4–7 *Didesmis synciliata*

FIG. 4 Ventral view, A = macronucleus, B = micronucleus
Bar = 15 µm

FIG. 5 SEM of syncilia surrounding oral opening and cytopyge
Bar = 10 µm

FIG. 6 SEM showing multiplicity of syncilia
Bar = 1 µm

FIG. 7 Ventral view, A = syncilia, B = concrement vacuole
Bar = 15 µm

A quarter of both the anterior and posterior ends of the body are covered with fine somatic cilia (Fig. 11). The middle half of the body is naked. The cytopyge is located in the centre of the posterior terminal of the body. A disc-shaped granular macronucleus is indefinite in position. The ellipsoidal micronucleus is situated in a depression on the surface of the macronucleus. A concrement vacuole (Fig. 11) containing refractive granules is located in the anterior part of the body. Two or three contractile vacuoles are present in the posterior part of the body.

TYPE HOST

Diceros bicornis. Other hosts unknown.

TYPE LOCALITY

Addo Elephant National Park, Eastern Cape Province, South Africa (33–34°S; 25–26°E).

SITE OF INFECTION

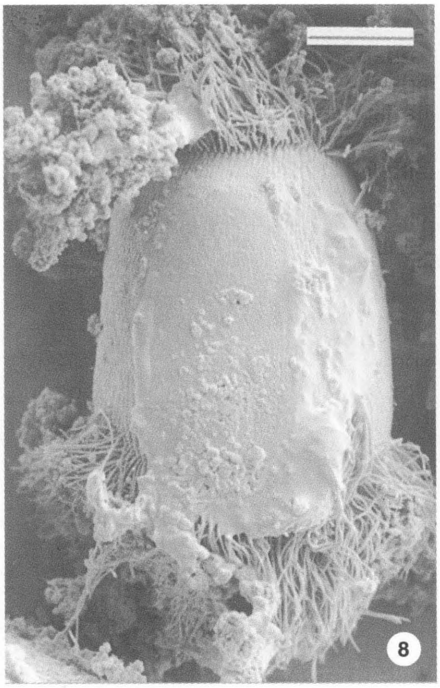
Dorsal ascending colon.

PREVALENCE

1 x 10^4/mℓ digesta fluid.

ETYMOLOGY

Specific name *dicerotos* refers to the organism found in the black rhinoceros *Diceros bicornis*.



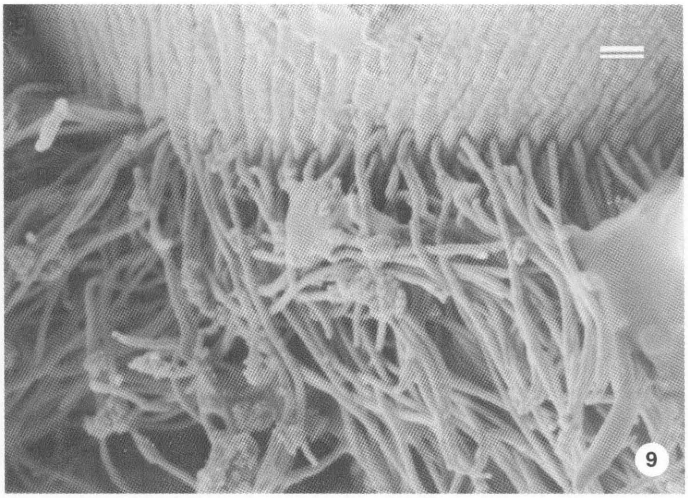


FIG. 8–9 ***Didesmis ovalis***

FIG. 8 SEM of simple cilia surrounding oral opening and cytopyge
Bar = 16 µm

FIG 9 SEM showing simplicity of the cilia
Bar = 1 µm

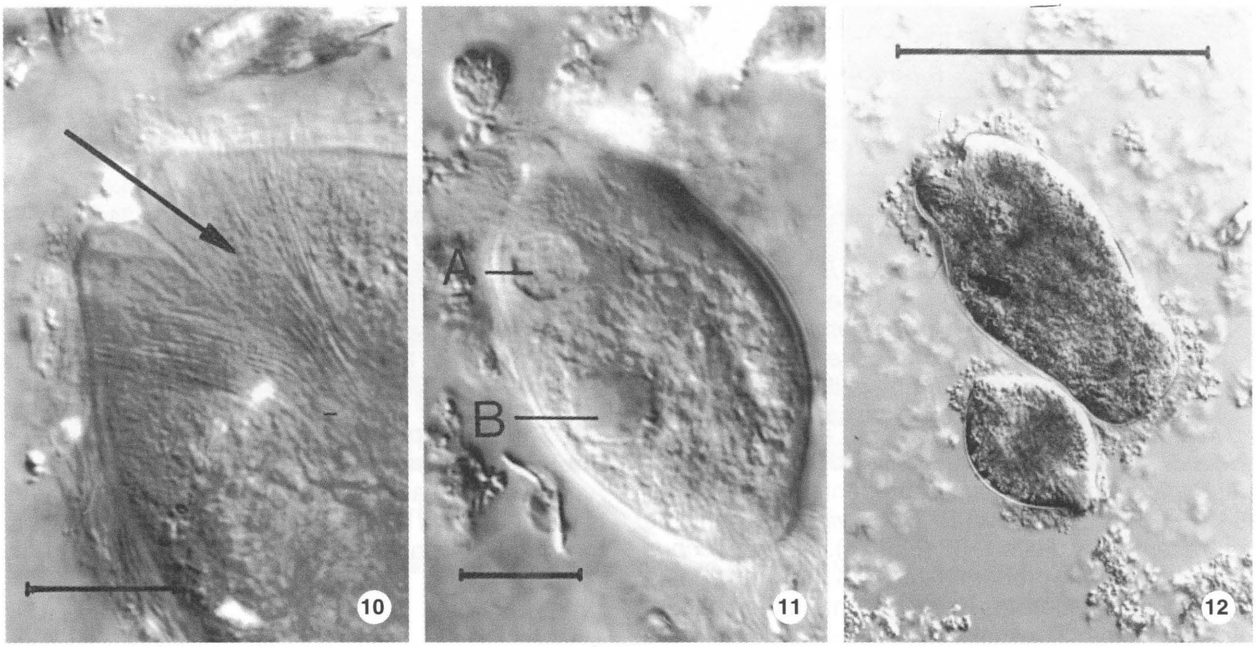


FIG. 10–12 ***Blepharoconus dicerotos***

FIG. 10 Ventral view, arrow on oral opening and cuticular folds radiating out from cytopharyngeal canal
Bar = 20 µm

FIG. 11 Ventral view, fine somatic cilia covering anterior and posterior ends of body, A = concrement vacuole, B = macronucleus
Bar = 16 µm

FIG. 12 A = *B. dicerotos* and B = *B. cervicalis* lying side-by-side and head-to-tail showing differences in size
Bar = 42 µm

Body lengths of *Blepharoconus*

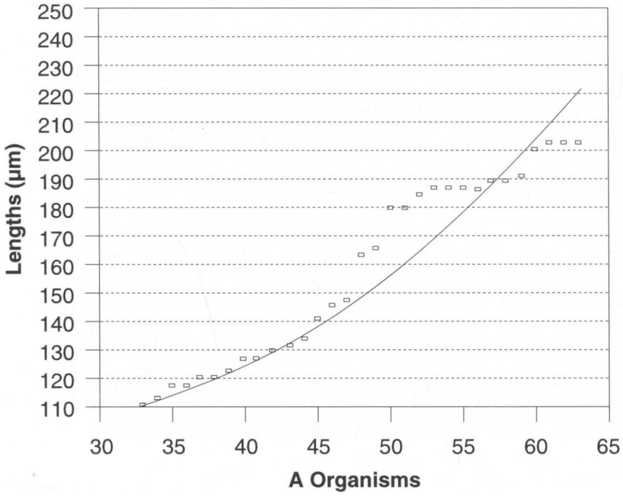


FIG. 13 *B. dicerotos*, group A organisms represented by digits 33–63

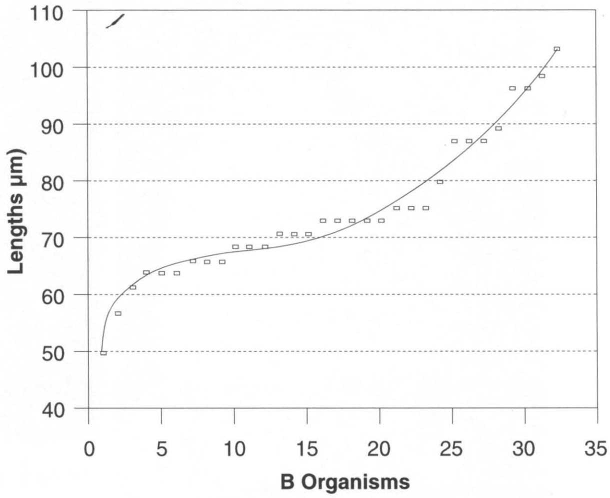


FIG. 14 *B. cervicalis*, group B organisms represented by digits 1–32

TYPE MATERIAL

Accession No. 2240485 (Addo) deposited in Intestinal Protozoa collection of Centre for Wildlife Management, University of Pretoria, Pretoria, South Africa.

KEY TO SPECIES OF *BLEPHAROCONUS*

1. Body conical .... *B. hemiciliatus* Gassovsky 1919
   Body ovate 2
2. Body length 28 ± 0,62 .... *B. benbrooki* Hsiung 1930
   Body length 70 ± 0,80 .... *B. cervicalis* Hsiung 1930
   Body length 157 ± 34,8 .... *B. dicerotos* n. sp.

REMARKS

The superficial characteristics of *B. dicerotos* are similar to those of *B. cervicalis* except for size. That of *B. dicerotos* is twice that of *B. cervicalis* (Fig. 12): when occurring in the rhinoceros length 75 ± 12,7, width 49 ± 10,6; when occurring in the horse: length 70,33 ± 0,80, width 57 ± 0,65 (Hsiung 1930).

No overlapping of lengths occurred between group A, *B. dicerotos* organisms represented by digits 33-64 (Fig. 13), and group B, *B. cervicalis* organisms represented by digits 1–32 (Fig. 14). *Blepharoconus cervicalis* clearly predominated (2,6 x 10^4/mℓ digesta fluid) over *B. dicerotos* (0,5 x 10^4/mℓ) in the colon of the rhinoceros. This makes it unlikely that *B. cervicalis* represents daughter cells of *B. dicerotos* which constituted only one sixth of the total number of cells (3,1 x 10^4/mℓ) of the two species together. Moreover *B. cervicalis* occurred in horses fed fibrous or semi-fibrous diets (Hsiung 1930; Strelkow 1939), while *B. dicerotos* was found together with *B. cervicalis* only in the black rhinoceros consuming a diet of succulent leaves (Hofmann 1973). Rod-like structures, interpreted as reinforcements of the cytopharyngeal canal walls by Hsiung (1930) and Strelkow (1939), have been interpreted here as cuticular folds allowing for expansion in order to accommodate large food particles. Such folds were observed in the carapace of space-restricted *Rhinozeta* species (Van Hoven *et al.* 1988).

Blepharosphaera ceratotherii n. sp. (Fig. 3a, 3b, 15–18)

DESCRIPTION ($n = 38$)

The body is spherical and uniformly covered with somatic cilia (Fig. 3a); length 33 ± 6,5; width 31 ± 6,3; thickness 32 ± 7,0. The oral opening marks the anterior of the body. It is circular and surrounded with long dense cilia (Fig. 15), and leads into a short funnel-shaped cytopharynx. The cytopyge is indistinct. The position of the oval macronucleus (Fig. 16) is variable.

The small subspherical micronucleus is situated in a surface depression at one end of the macronucleus. A concrement vacuole containing refractive granules is located in the middle of the body. The position of the single contractile vacuole is variable.

TYPE HOST

Ceratotherium simum. Other hosts unknown.

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,

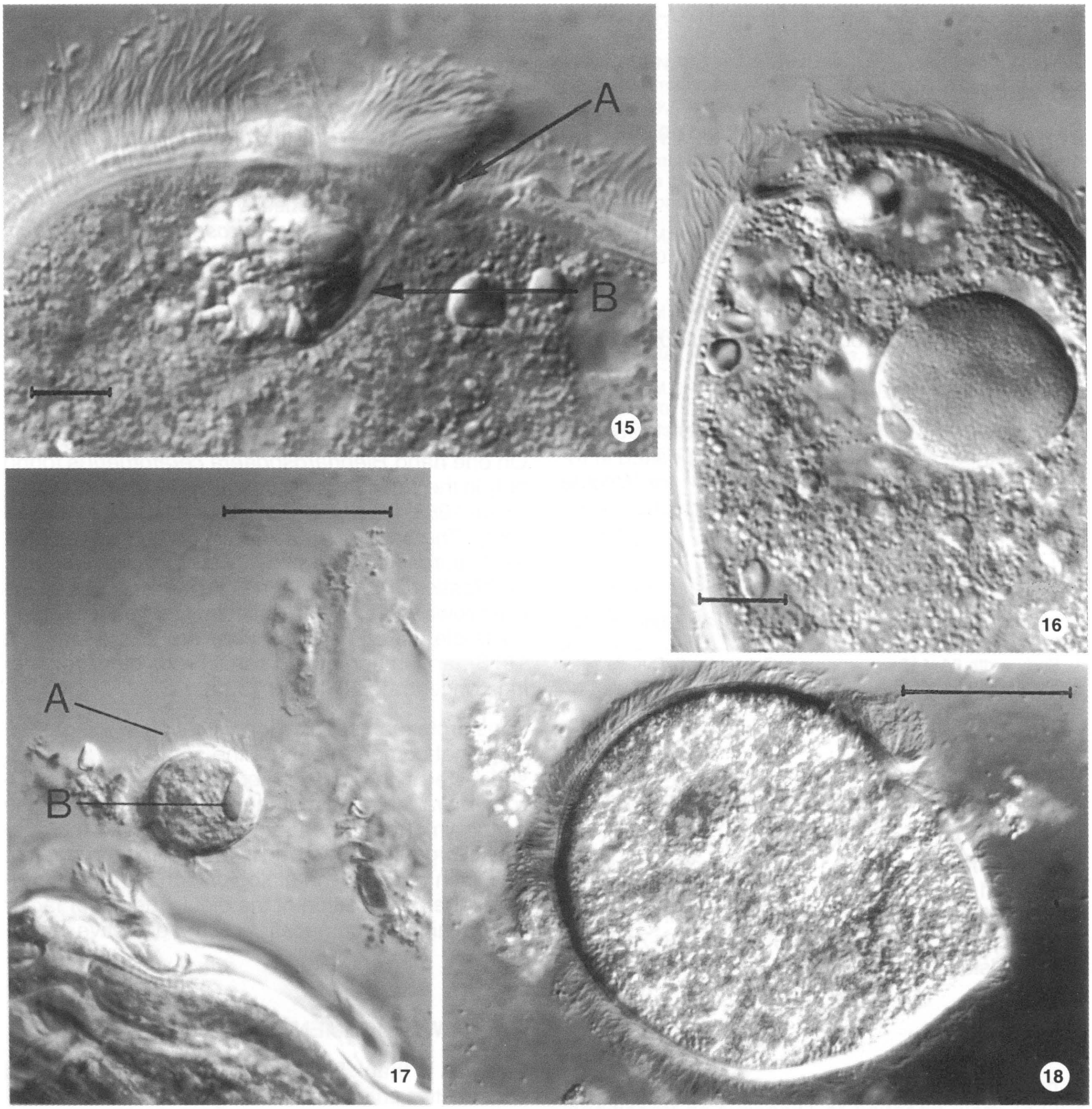


FIG. 15–18 *Blepharosphaera ceratotherii*

FIG. 15 Dorsal view of anterior terminal, A = oral-opening surrounded with dense long cilia, B = cytopharynx
Bar = 13 µm

FIG. 16 Anterior dorsal view, macronucleus with micronucleus
Bar = 15 µm

FIG. 17 Showing A = somatic cilia, B = macronucleus, magnification x696
Bar = 28 µm

FIG. 18 *B. intestinalis* at same magnification x696
Bar = 28 µm

KwaZulu- Natal Province, South Africa (28–29°S; 31–32°E).

SITE OF INFECTION

Caecum, dorsal and ventral ascending colon.

PREVALENCE

1 x 10^4/mℓ caecal and colon fluid.

ETYMOLOGY

Specific name ceratotherii refers to the organism found in the white rhinoceros *Ceratotherium simum.*

TYPE MATERIAL

Accession No. 4220585 (Pilanesberg); 3010585 (Ellisras district); 1130476 (Hluhluwe), deposited in Intestinal Protozoa Collection of Centre for Wildlife Management, University of Pretoria, Pretoria, South Africa.

KEY TO SPECIES OF *BLEPHAROSPHAERA*

1. Body ellipsoidal .... *B. ellipsoidalis* Hsiung 1930
   Body spherical 2
2. Body width 84–115 .... *B. intestinalis* Bundle 1895
   Body width 31 ± 6,3 .... *B. ceratotherii* n.sp.

REMARKS

The superficial characteristics of *B. ceratotherii* are similar to those of *B. intestinalis,* except for size.

The size of *B. ceratotherii* (Fig. 17) is one third the size of *B. intestinalis* (Fig. 18) of the black rhinoceros: length 101 ± 20,8, width 93 ± 19,6, thickness 96 ± 18,7; of the horse: length 82–110, width 84–115, thickness 84–116 (Bundle 1895); length 38–74, width 38–74 (Hsiung 1930).

DISCUSSION

In her phylogenetic investigations (Wolska 1966a; 1966b) drew attention to *Didesmis* as transitional model in the lines of evolution of Holotricha → Gymnostomata → Buetschliidae → *Didesmis* → Entodiniomorphida. On the one hand, *Didesmis* has the typical characteristics of the Buetschliidae including a concrement vacuole. On the other hand, it has the ability to form syncilia as seen in *D. synciliata*, common in the Entodiniomorphida, which still retain the short kineties associated with the concrement vacuole in *Didesmis*, although the concrement vacuole itself is absent (Wolska 1971). These findings are implicated in the Corlissian Classification of 1979 (Corliss 1979).

Of the available characteristics of the formalin-preserved organisms, the proposed new species *Blepharoconus dicerotos* differs from the original species *B. cervicalis* only in size. In this case it is considered that size alone suffices to separate the two species, since both occur in the dorsal ascending colon of the same animal without overlap in the size range of each species: *B. dicerotos* length 110,5–223,3, *B. cervicalis* length 56,4–77,6 and the limits between the two ranges are large (32,9).

The available characteristics of the formalinized proposed new species, *Blepharosphaera ceratotherii,* differ from the original species, *B. intestinalis,* only in size.

In this particular instance, it is considered that size alone is adequate for the purpose of separating the two species, since the numbers of organisms of each species are similar and great, 1 x 10^4/mℓ digesta fluid in the dorsal colon of the black rhinoceros.

On one hand *Blepharosphaera ceratotherii* is found only in the white rhinoceros which is a grazer (Owen-Smith 1988) feeding on fibrous grasses, of which red grass *Themeda trianda* is an important dry season food source (Owen-Smith 1973). On the other hand, *B. intestinalis* is found in the black rhinoceros which is a browser feeding on succulent leaves and plant-parts classified as a concentrate diet (Hofmann 1973), and in horses fed semifibrous diets containing concentrates (Hsiung 1930).

Thus protozoan species identification in the gastrointestinal compartments of the same host species, suggested in the case of *Blepharoconus* and *Blepharosphaera*, at least, that the mode of feeding of the host animal was important in determining the species of organisms involved in fermenting the ingesta. This is in line with the biological tenet that in herbivores the composition of the diet is the major factor determining the composition of the digestive organisms.

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