Intestinal protozoa of the African elephant *Loxodonta africana* (Blumenbach)

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The digestive tract of 15 elephants from South Africa and two from Zaïre were sampled in order to determine the identity, density and population composition of the intestinal protozoa. The following orders were represented: Gymnostomatida, Trichostomatida and Entodiniomorphida. Altogether 17 species were identified, of which three are new. The new species as well as a new genus are described.
S. Afr. J. Zool. 1980, 15: 83–90

Die spysverteringskanaal van 15 olifante uit Suid-Afrika en twee uit Zaïre is gemonster om die intestinale protosoë se identiteite, digthede en bevolkingsamestelling te bepaal. Verteenwoordigers van die volgende ordes is gevind: Gymnostomatida, Trichostomatida en Entodiniomorphida. Altesaam 17 spesies is geïdentifiseer. Drie nuwe spesies en 'n nuwe genus word beskryf.
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Gwynne and Bell (1969) have discussed differences in cellulose-content of the diet of ruminants and non-ruminants. The elephant, like the equidae, exists on a diet with a high cellulose content, and the length of the intestinal canal from the stomach to the mesocolon is about 18 m in the adult. As such it forms an anaerobic environment for a unique microfaunal community which ferments foodstuffs and bacteria at a pH ranging from 6,0 to 6,8.

The duodenum of the elephant forms a distinct U-shaped loop into which the bile and pancreatic ducts open about 10 – 15 cm from the pylorus. A coiled jujeno-ileum follows the duodenum and the junction of the ileum and caecum is marked by an ileo-caecal sphincter valve. The colon extends from the sacculated caecum. Both the colon and rectum are suspended on a mesocolon. The length of the rectum is 35 cm long and contains the formed faeces which is released by relaxation of the powerful anal sphincter (Fig. 1).

The first report on protozoa in the caecum of a monogastric herbivorous animal was a study by Gruby and Delafond (1843) on seven species in the horse. Colin (1871) and Fiorentini (1890) described 10 and 14 species, respectively, from the horse. Other authors (Bundle 1895; Da Cunha 1919; Fantham 1921; Buisson 1923; Hsiung 1930; Hoare 1937) published reports on protozoa in non-ruminants.

Buisson (1923) published the first description of a protozoon found in the African elephant. Kofoid (1935) discovered two ciliates in the Indian elephant *Elephas maxima*. The latter two species were redescribed and another species added by Latteur (1958), and he later described another two species (Latteur 1967; Latteur & Bousez 1969). Latteur, Tuffrau, and Wespes (1970) and Latteur and Dartevelle (1971) described two species from the African elephant.

The present paper reports on a project, the purpose of which was to identify, describe, and count the protozoa in the intestine of the African elephant. The orders in the class Ciliata relevant in the investigation are:

i. Gymnostomatida Bütschli 1889
ii. Trichostomatida Bütschli 1889
iii. Entodiniomorphida Reichenow 1929

Methods

During a culling programme of elephants in the Kruger National Park samples of the intestinal fluid of 15 elephants were collected. Another two elephants, from Zaïre,

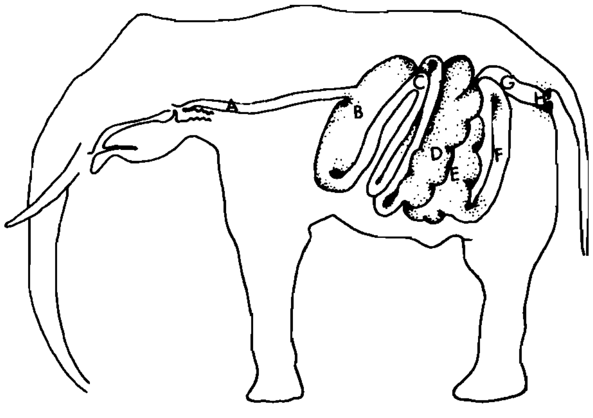

Fig. 1 The alimentary canal of the African elephant.

A: Oesophagus.	E: Stomach.
B: Duodenum.	F: Caecum.
C: Colon.	G: Mesocolon.
D: Rectum.	H: Anus.

were also studied. The different compartments of the alimentary canal were opened, and the contents of each were thoroughly mixed and strained through cheesecloth. The 50 ml samples were preserved by the addition of 10% formalin in a 1:1 ratio and stored in sealed containers.

Counting was performed by mixing one ml of the intestinal fluid with 22 ml of a 9% glycerine solution to prevent rapid sedimentation of the larger ciliates so that a representative sample could be taken. The method of counting protozoa developed by Adam (1951), Boyne, Eadie and Raitt (1957) as modified by Van Hoven (1974) was used, two counts per sample being made using a MacMaster counting chamber.

A drop of the protozoal suspension was placed on a slide with a drop of glycerine and mounted in Canada Balsam for systematic microscopical analysis. This method does not affect the structure of the protozoa. All organelles were clearly visible, even in unstained mounts. Staining with chlorzinciodide was used for study of the skeletal plates.

The movement and way of locomotion of certain species were investigated microscopically by placing a drop of the intestinal fluid on a slide and covering it with a cover-slip. immediately after extracting the fluid in the field.

Permanent slides were used in identification. Twenty-five individuals of each species were measured using a calibrated ocular micrometer. Drawings were made with the aid of a camera lucida. Ciliates were classified according to the classification system proposed by Honigberg (1964).

The 17 species found in the intestine of the elephant belong to the class Ciliata. They possess simple or compound cilia-bundles, contractile and food vacuoles and a micro- and macronucleus (Corliss 1959; Honigberg 1964).

This specialized group of protozoa takes full advantage of the elephant's digestive canal. Their anaerobic existence, normally considered primitive, is in an environment rich in fragmented particles such as cellulose, chromatophores, other cellular components, and partially degraded substances. Bacteria and other protozoa are also ingested.

Species that have previously been described and new species are shown diagrammatically. Descriptive terminology follows the system of Lubinsky (1957–1958). The oral cilia were observed and drawn in the retracted position. The retraction is caused by preservation in formalin.

Systematic account

Subclass: Holotricha
Order: Gymnostomatida
Family: Bütschlidae

Blepharosphaera intestinals Bundle 1895

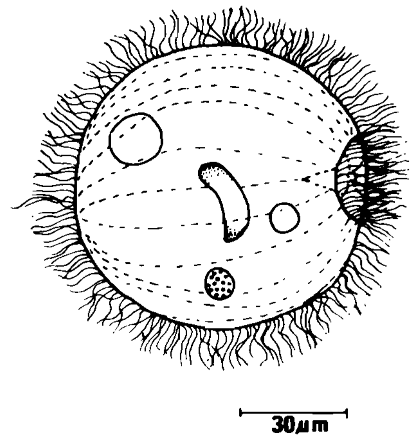

Fig. 2 *Blepharosphaera intestinalis.*

Blepharoconus krugerensis sp. nov.

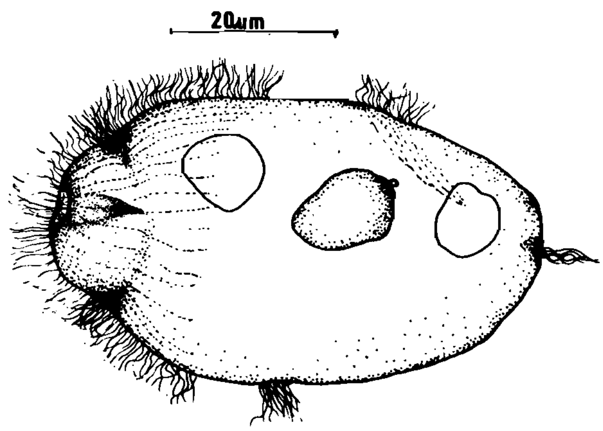

Fig. 3 *Blepharoconus krugerensis.*

Diagnosis

Body ovoid (somewhat flattened dorsoventrally), regularly shaped with a blunt anterior knob and a rounded posterior end separated by a slight groove which extends around the body; anterior half of body covered with regularly spaced, longitudinal rows of long thin cilia; two short bands of cilia in the middle region of body and situated on opposite sides of the body, one twisted towards the left and one towards the right hand side of the body; a bundle of cilia present around the margin of the cytopyge at posterior end of the body; the rest of body covered by a naked cuticle.

Description

Ectoplasm homogeneous, endoplasm coarsely granular and contains particles of food. Cytostome funnel-shaped, located on the knob at anterior end of the body.

Macronucleus disc-shaped, distinctly granular, varies in position. Elliptic micronucleus, situated in a depression in the macronucleus.

Concretion vacuole containing granules, situated near to 'neck'. Two or three contractile vacuoles, vary in position. Transverse fission occurs in reproduction.

Measurements:

	Length	Width
Range	27,4 — 68,3 µm	18,3 — 39,1 µm
Mean	47,8 µm	28,7 µm

Habitat: Duodenum, caecum and colon.

Remarks

The macronucleus of *Blepharoconus krugerensis* closely resembled that of *Blepharoconus cervicalis* (Hsiung 1930) and *Blepharoconus benbrooki* (Hsiung 1930) found in the horse. *B. krugerensis* has two or three contractile vacuoles, while *B. cervicalis* has three and *B. benbrooki* has only one. The vacuoles of *B. cervicalis* were constant in their position, but those of *B. krugerensis* varied in position.

There are two short lateral cilia bundles situated in the middle of the body and deflected toward the anterior end in an anti-clockwise direction in *B. krugerensis*, which are lacking in *B. benbrooki* and *B. cervicalis*. *B. krugerensis* also has a bundle of anal cilia, as had *B. benbrooki*, but this is not present in *B. cervicalis*.

The mean length and width of *B. krugerensis* was found to be intermediate between *B. benbrooki* and *B. cervicalis*.

	Length	Width
B. cercicalis	70,33 µm	57,00 µm
B. krugerensis	47,80 µm	28,70 µm
B. benbrooki	28,40 µm	20,90 µm

Order: Trichostomatida
Family: Paraisotrichidae

Paraisotricha colpoidea Fiorentini 1890

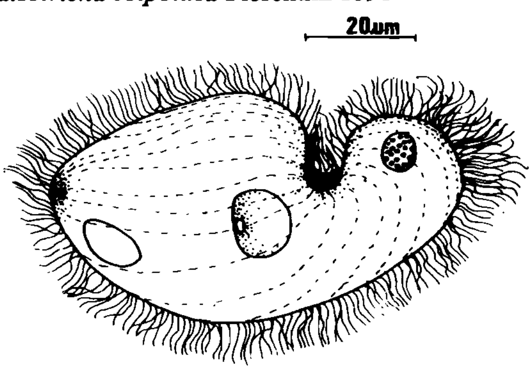

Fig. 4 *Paraisotricha colpoidea.*

Paraisotricha beckeri Hsiung 1930

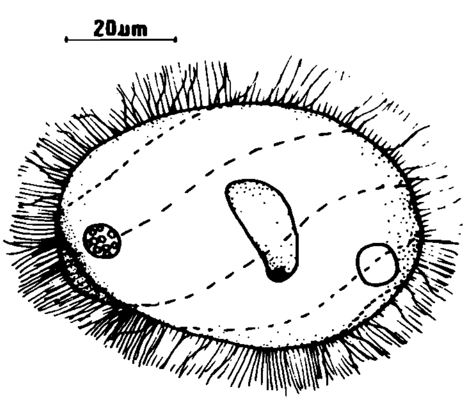

Fig. 5 *Paraisotricha beckeri.*

Paraisotricha minuta Hsiung 1930

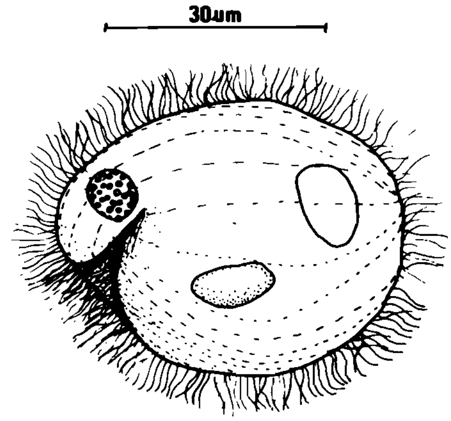

Fig. 6 *Paraisotricha minuta.*

Helicozoster proboscidicus sp. *nov*.

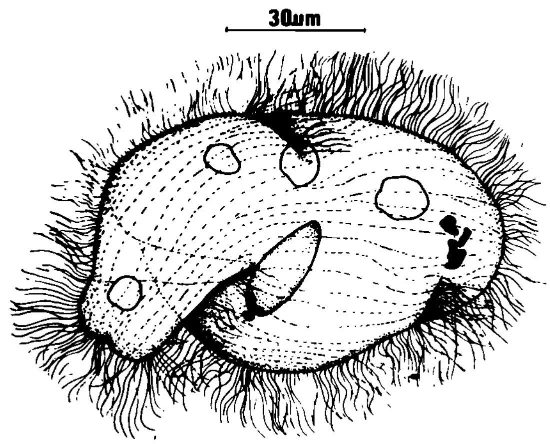

Fig. 7 *Helicozoster proboscidicus* sp. *nov.*

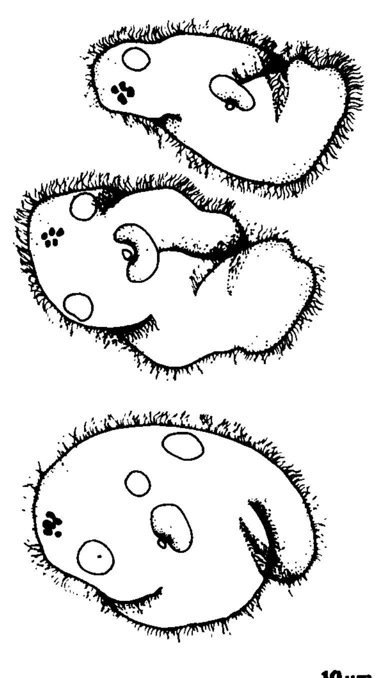

Fig. 8 *Helicozoster proboscidicus* sp. *nov.*

Diagnoses

Body flexible, elliptic and twisted in shape; with a narrow, proboscis-like shaped anterior part folded to the left, followed by the widest part of the body which terminates posteriorly into a granule-filled rounded end. The body's spiral rotating movements was found to give it a range of shapes.

Description

Cuticle with parallel lengthwise arranged cilia rows can be seen. Cilia found on the peristome margin extend from underneath the proboscis – like anterior part, as a funnel-shaped opening.

Macronucleus ellipsoidal, varies in position; round micronucleus embedded in hollow of anterior end of macronucleus. Three to four contractile vacuoles, indefinite in position, except for one situated near posterior end.

	Length	Width
Range	80 — 100 μm	47 — 70 μm
Mean	90 μm	58,5 μm

Remarks

No cytopyge could be found, only an aggregation of granules at posterior end of body.

Macronucleus of *Helicozoster proboscidicus* resembles *H. indicus* (Latteur 1967). The following differences were noted:

- Peristome of *H. proboscidicus* not characterized by anticlockwise spiral, but funnel-shaped.
- Ten contractile vacuoles present in *H. indicus,* arranged irregularly form anterior to posterior in the body, *H. prodoscidicus* only four vacuoles, one regularly arranged.
- Body of *H. indicus* much longer than body of *H. proboscidicus.*

Subclass: Spirotricha
Order: Entodiniomorphida
Family: Ophryscolecidae

Endoralium loxodontae gen et sp. *nov.*

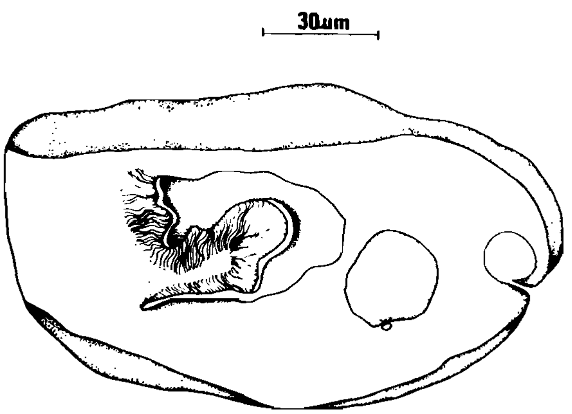

Fig. 9 *Endoralim loxodontae gen. et.* sp. *nov.*

Diagnoses

Body rectangular elliptic in shape; lateral cuticle folds prominent especially on right side of body; cilia found in centre upper side of body; s-shaped as a protruding moving buccal cytosome embedded in buccal floor; funnel-shaped cytopharynx extends from buccal cavity into body; oral cilia move in rotating circle, function to ensure food selection and locomotion.

Description

Endoralium loxodontae has one contractile vacuole posteriorly linked with cytopyge at end of right lateral groove.

A disc-shaped macronucleus with an embedded micronucleus is situated between the anus and the oral zone.

	Length	Width
Range	100 — 177 μm	50 — 90 μm
Mean	138,5 μm	70 μm

Remarks

Like primitive Ophryoscolecidae, *E. loxodontae* has only one zone of membranelles (centrally) and one contractile vacuole, therefore it is classified under the subfamily Entodiniiae subf. nova. *E. loxodontae* is asymmetrical; body not spirally shaped like that of *Entodinium* species, survival value therefore much lower. No ventrad displacement of contractile vacuole. Size of body very large for a primitive Ophryoscolecidae; no skeleton is present.

Lavierella africana Buisson 1923

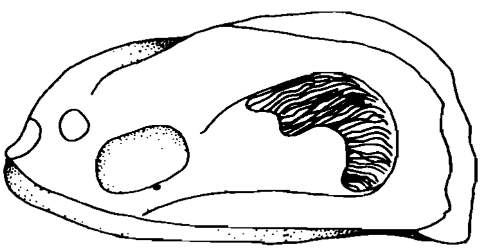

Fig. 10 *Lavierella africana.*

Family Cycloposthiidae
Cycloposthium bipalmatum Fiorentini 1890

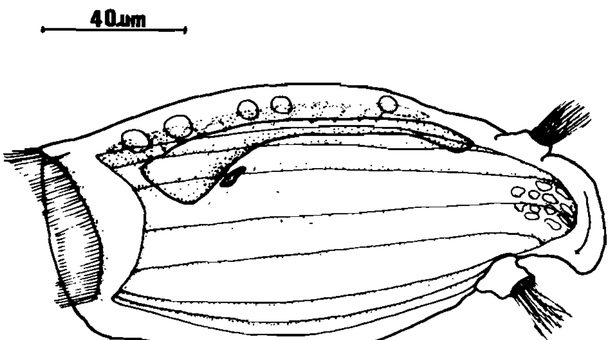

Fig. 11 *Cycloposthium bipalmatum.*

Triplumaria hamertonii Hoare 1937

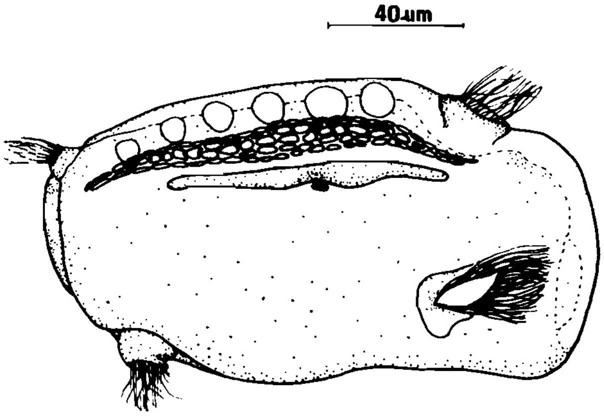

Fig. 12 *Triplumaria hamertonii.*

Triplumaria selenica Latteur 1970

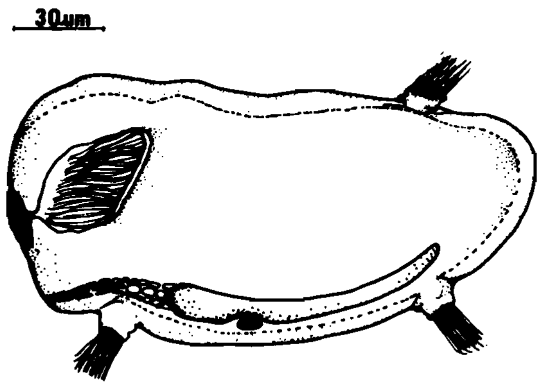

Fig. 13 *Triplumaria selenica.*

Prototapirella intestinalis Da Cunha 1918

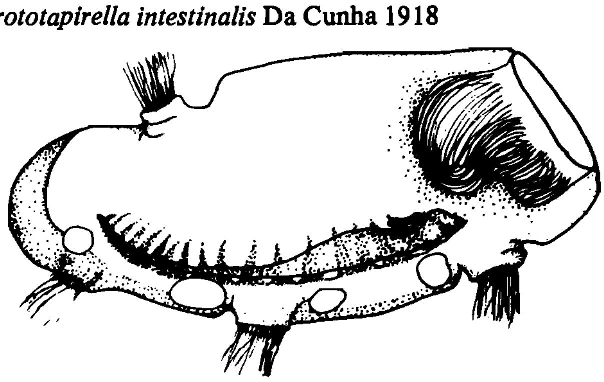

Fig. 14 *Prototapirella intestinalis.*

Prototapirella elephantis Buisson 1923

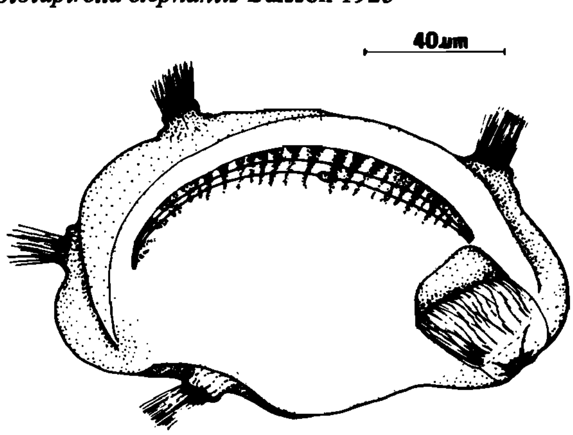

Fig. 15 *Prototapierella elephantis.*

Polydinium mysoreum Kofoid 1935

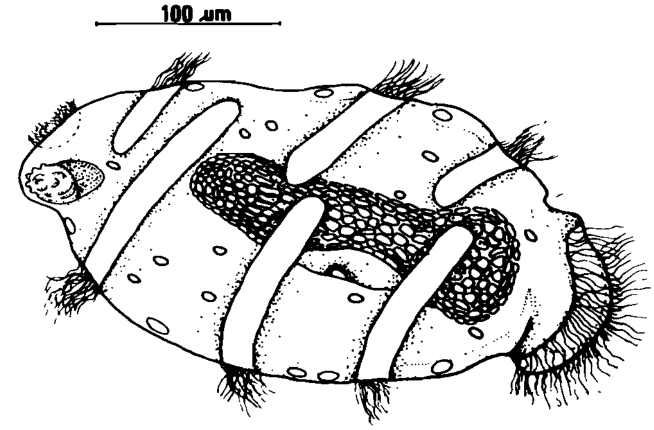

Fig. 16 *Polydinium mysoreum.*

Elepantophilus zeta Kofoid 1935

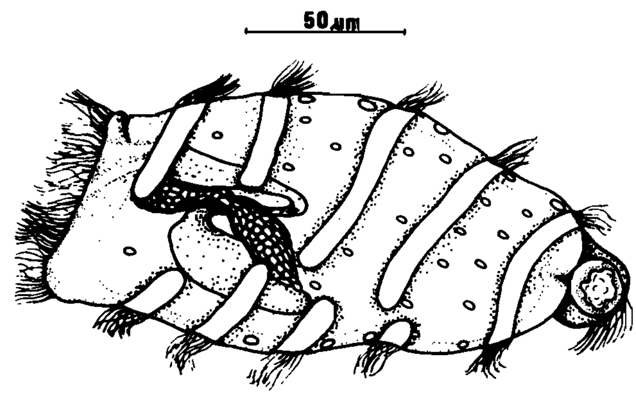

Fig. 17 *Elephantophilus zeta.*

Thoracodinium vorax Latteur 1958

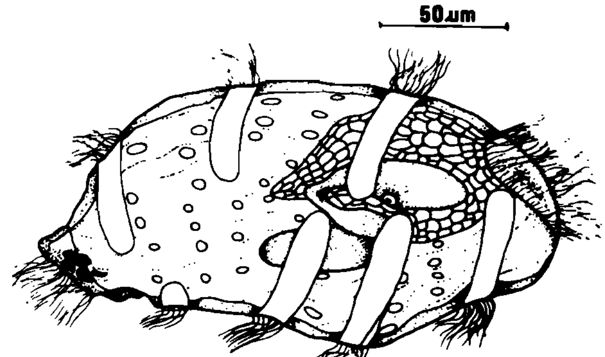

Fig. 18 *Thoracodinium vorax.*

Family Spirodinidae

Pterodinium microlithovorax Latteur 1971

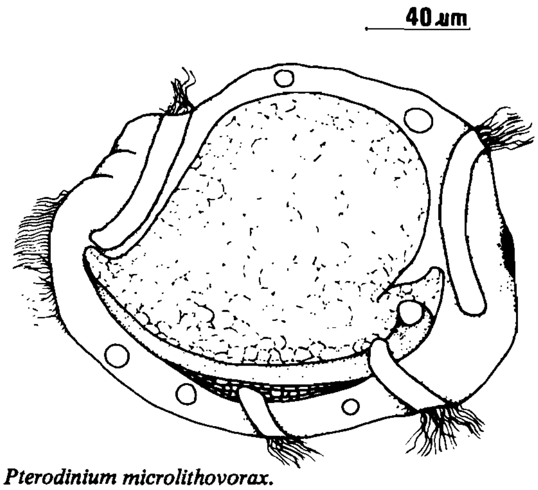

Fig. 19 *Pterodinium microlithovorax.*

Numbers and distribution of the ciliates

No significant variation was found in the numbers of protozoa found in the seventeen elephants investigated. The mean density in the four major parts of the fermentive part of the digestive tract yielded the following figures: duodenum $3,2 \times 10^4$/ml, caecum $4,1 \times 10^4$/ml, colon $4,6 \times 10^4$/ml and rectum $1,1 \times 10^4$/ml.

Most of the protozoa found in the rectum were dead and partly decomposed and no cysts were found. On average there was 57% Spirotricha and 43% Holotricha in the intestine of the elephant, which varies in the different parts of the system as is indicated in Fig. 20.

Seasonal variation in population densities could not be established because all the elephants sampled were shot during July. Field (1971) found that although elephants do not select their food, their diet varies according to availability. In the wet season grass is mainly consumed and the diet gradually changes to browse in the dry season. McCullagh (1969) found that they not only eat the bark of trees as a result of a lack of calcium in the blood, but also to prepare the digestive system for a more ligneous grass diet in the late dry season. One can reason that protozoa numbers may also be influenced by this change in diet; Spirotricha, for instance, increase in numbers to cope with the higher fibre content of the diet.

Only two elephants from Zaire were examined and they contained two species not present in the South African elephants *viz. Polydinium mysoreum* and *Elephantophilus zeta*. Except for species of the genus *Paraisotricha* in the Zaire elephants, all other species were similar in size, appearance and numbers.

Discussion

In ruminants the survival of the protozoa population is ensured by transfaunation from the mother and other members of the herd. The transfer is by licking, grooming and by eating plants on which saliva or undigested food has been dropped. The protozoa population is established in the young ruminant a few weeks after birth (Sleigh 1973).

In monogastric herbivores such as the elephant, transfaunation isn't clearly understood. Gordon (1972) described a process of coprophagy in rabbits, whereby the parents produce a soft gelatine covered light coloured form of faeces which is eaten by the young. The faeces can pass through the low pH in the stomach without harming the

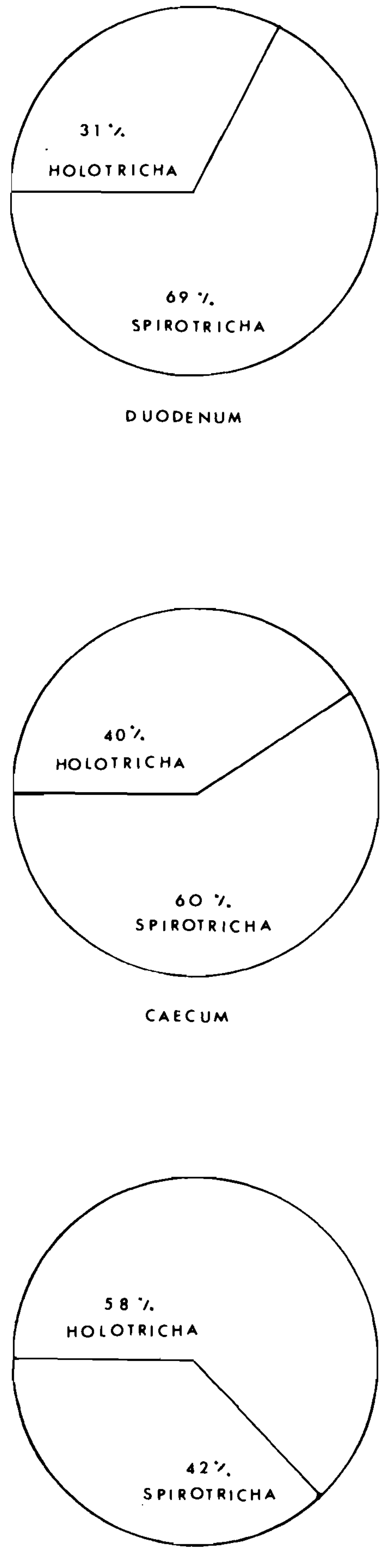

Fig. 20 Diagrammatic representation of the proportions of different ciliates found in the duodenum, caecum and colon of the African elephant.

protozoa it carries and eventually establish a functional protozoa population in the intestine. The normal bolus-shaped faeces have been observed in a different form, light coloured and very fluid, by Sikes (1971) in elephants in captivity and similarly by Guy (1977) in wild elephants in the Sengwa area of Rhodesia. Guy (1977) describes how juvenile elephants picked up faeces immediately after defaecation by cows and placed the faeces in their mouths. The concentration of ciliates in the faeces is very low and it is known that their survival outside the gut is of short duration. (Hungate 1966).

Six species appear to be species specific:

— *Helicozoster proboscidicus*
— *Endoralium loxodontae*
— *Blepharoconus krugerensis*
— *Triplumaria selenica*
—*Prototapirella elephantis*
—*Pterodinium microlithovorax*

The first three have just been described as new species while *Thoracodinium vorax, Polydinium mysoreum* and *Elephantophilus zeta* are also known to occur in the Indian elephant. No ruminant Ciliata have yet been found in elephants.

Hsiung (1930) and Adam (1951) found that differences in the ciliate concentration in different parts of the horse gut are due to the density of the food. In die duodenum and caecum of elephants the protozoa numbers are per unit volume lower and the plant material more diluted. In the colon, due to absorption of water, the plant material and protozoa are more concentrated. Per unit volume the colon may thus also be a site of higher fermentation rate than the caecum.

The order Gymnostomatida can be regarded as the most primitive of the Ciliata (Corliss 1961). This order is represented by two species in elephants. The order Entodiniomorphida has a more complex structure, the family Ophryoscolecidae is almost exclusively found in ruminants and the Cycloposthiidae in a variety of hosts such as the elephants, chimpanzees, gorillas, rhinoceroses, Equidae and Perissodactyla (Thurston & Noirot-Timotheè 1973).

Two Entodinium-like species occur in the elephant namely *Endoralium loxodontae* and *Lavierella africana;* both belong to the family Ophryoscolecidae. Thurston *et al.* (1973) also regarded *Lavierella* as the ancestor of the Cycloposthiidae.

Presently the Cycloposthiidae consists out of 25 genera, six of which occur in elephants. The number of cilia bundles is regarded as a criterion for evolutionary development (Thurston *et al.* 1973)

Two cilia bundles:	*Cycloposthium*
Three cilia bundles:	*Tripumaria*
Four cilia bundles:	*Prototapirella*
Five cilia bundles:	*Polydinium*
	Elephantophilus
	Thoracodinium

Da Cunha (1918, 1919) and Hoare (1937) described all the cilia bundles, including those near the oral cavity, as caudalia. According to Corliss (1959) the term 'caudalium' refers only to a cilia-bundle situated at the posterior end of the body. An alternative term for 'caudalium' is needed; cilia-bundle or motilium is proposed.

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