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A New Family, Genus, and Seven New Species of Entodiniomorphida (Protozoa) from the Gut of African Rhinoceros

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ABSTRACT. This report deals with a group of ciliated protozoa with short ciliary bands found mainly in the cecum of black rhinoceros, *Diceros bicornis* (Linnaeus, 1758), and white rhinoceros, *Ceratotherium simum* (Burchell, 1817) from southern Africa. A new genus, *Rhinozeta*, based on the sum total of the characteristics of seven new related species is described. The species described are *R. rhinozeta* n. sp., *R. triciliata* n. sp., *R. caecalis* n. sp., *R. addoensis* n. sp., *R. cristata* n. sp., *R. multiplatus* n. sp., and *R. unilaminatus* n. sp. The specific features of the new genus make it incompatible with any of the known families of the Order Entodiniomorphida containing the ciliates present in the digestive tract of herbivorous mammals. This merits the creation of a new family, the Rhinozetidae.

IN our first report on the intestinal ciliated protozoa in rhinoceros from southern Africa we described two new genera and five new species belonging to the Family Cycloposthiidae of the Order Entodiniomorphida (10). The present report deals with a group with short ciliary bands, which forms 46% of the total ciliated protozoa in the cecum of black rhinoceros *Diceros bicornis* (Linnaeus, 1758) and 32% in that of white rhinoceros *Ceratotherium simum* (Burchell, 1817) from southern Africa. Seven new species are distinguished under a new genus, *Rhinozeta*, for which a new family, Rhinozetidae, has been created.

MATERIALS AND METHODS

Field samples were the same as those employed in our first report (10). Materials and methods for light microscopy and scanning electron microscopy (SEM) were similar to those used in our first report (10). In the description of species the terminology according to Lubinsky (7) was used.

RESULTS

Characteristics of the Family Rhinozetidae n. fam.

Diagnosis. The adoral zone of cilia is borne on a retractable cone. Nonretractable somatic cilia occur in three, four, or five short bands on the left and right lateral body surfaces. From two to six contractile vacuoles lie between the macronucleus and the left body surface. Skeletal plates are present in varying numbers and sizes. These ciliates are found in the intestinal tract of the rhinoceros from southern Africa, a hindgut fermenter.

Discussion. Of the seven existing families included by Corliss (2) in the Order Entodiniomorphida, the Family Ophryoscolecidae is restricted almost exclusively to the foregut fermenters, whereas the other six families occur in the hindgut fermenters (2). The Family Rhinozetidae differs structurally from these six families. The somatic cilia of the Cycloposthiidae occur as tufts and not as bands. Tufts are round bundles of cilia like those found on *Prototapirella elephantis* and *P. intestinalis* by Eloff & van Hoven (3). Long ciliary bands encircle more than half of the body like those seen on *Polydinium mysoreum* and *Elephantophilus zeta* by Kofoid (6). The short bands of the Rhinozetidae encircle less than half of the body. The adoral zone of the Polydiniellidae differs from that of the Rhinozetidae in having nonretractable tufts. Unlike the Rhinozetidae, members of the Spirodiniidae and the Ditoxidae have no skeletal plates. The long somatic ciliary bands partially encircling the body of the Telamodiniidae are more than five times as long as those of the Rhinozetidae. The fusiform, often tailed, body shape with long partially encircling somatic ciliary ribbons of the Troglodytellidae differs from the elongated, oval body shape with short ciliary bands of the Rhinozetidae.

Characteristics of the Genus Rhinozeta *n. g.*

The presence of short nonretractable somatic ciliary bands is typical of the genus. A total of three, four, or five bands occur on the lateral body surfaces only: one or two on the right side, two or three on the left side. The posterior right ciliary band is always larger than the others, except for *R. multiplatus* where the posterior left band is also large. The adoral zone ciliature is borne on a retractable cone. The oval-shaped oral opening is large and occupies virtually the entire anterior end of the body. The elongated macronucleus is three-quarters of the body length and follows the convex curvature of the body surface. An oval-shaped micronucleus is found in the anterior quarter of the macronucleus close to its left surface. From two to six, generally four, contractile vacuoles lie between the macronucleus and the left body surface. Skeletal

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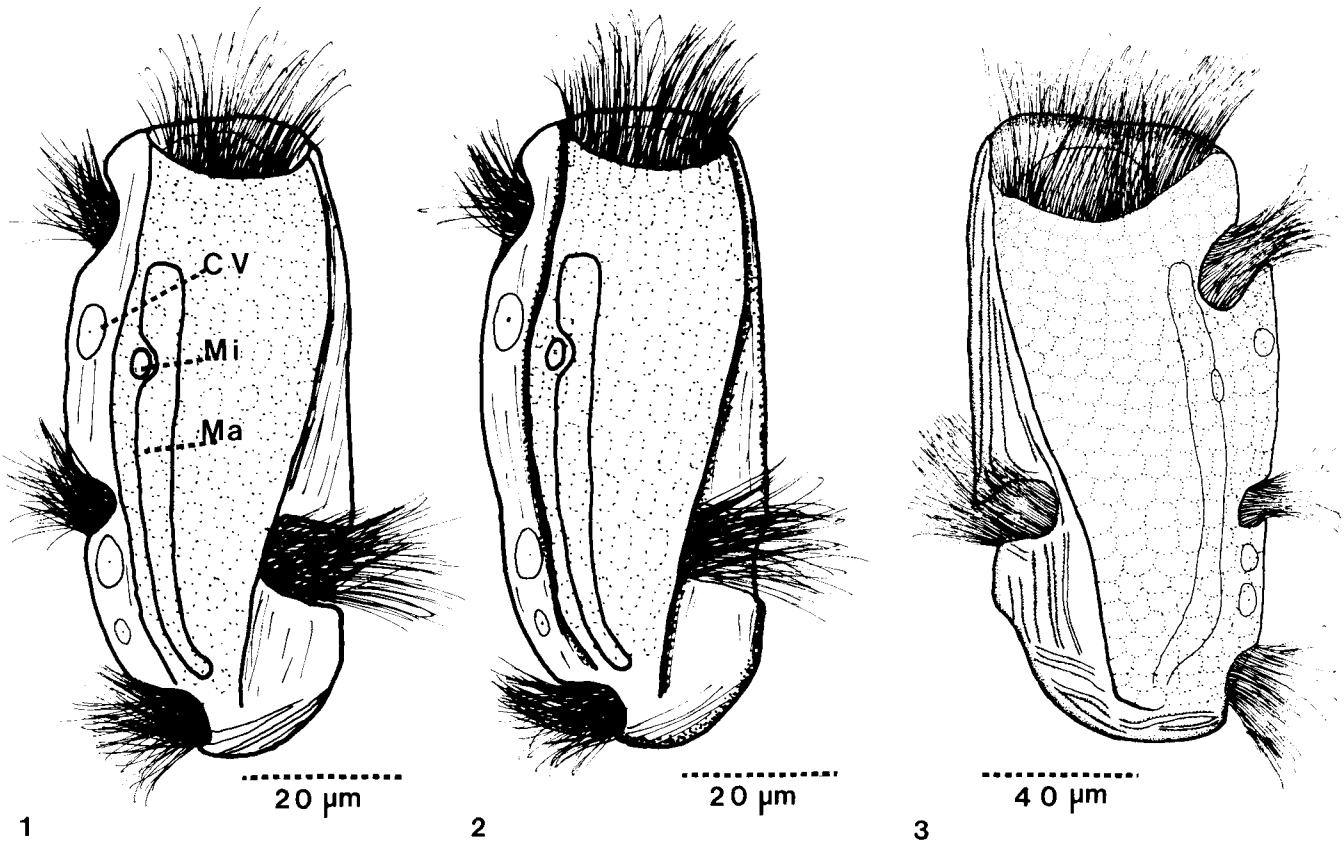


Figs. 1–3. 1. *Rhinozeta rhinozeta* n. g., n. sp., cv = contractile vacuole, mi = micronucleus, ma = macronucleus. 2. *Rhinozeta triciliata* n. sp. 3. *Rhinozeta caecalis* n. sp.

plate material is present in all species. The cytoproct is situated at the base of the upper skeletal plate. The dorso-ventrally flattened body of all species within the genus has an elongated, oval shape, with a length which varies from 65 to 236 µm and a width varying from 24 to 117 µm.

Type-Species *Rhinozeta rhinozeta* n. g., n. sp.

Structure (Figs. 1, 8, 9). The oval-shaped oral opening stretches across the entire anterior end of the body. The adoral zone of cilia is borne on a retractable cone, which can protrude beyond the rim of the mouth.

The elongated, oval-shaped body has an average length of 124 (103–156; n = 33) µm in the black rhinoceros and 86 (71–94; n = 34) µm in the white rhinoceros. The average width is 54 (40–60) µm and 38 (31–43) µm in black and in white rhinoceros, respectively. Thus, except for being about 30% larger in black rhinoceros, no other differences in body structure were seen.

There are four ciliary bands: three on the left and one on the right lateral surface. The bands on the left are evenly spaced: an anterior one near the mouth, one in the middle, and one at the posterior end of the body. The one on the right side is situated in the posterior third of the body. This band is wider and longer than those on the left side, and its housing is thicker and protrudes further from the body. Cuticular folds occur lengthwise anteriorly and posteriorly each side of this band in the form of parallel ridges. These folds, in close proximity to the band, provide the necessary elasticity for efficient motility and allow for expansion (Fig. 8). All ciliary bands lie across the long axis of the body. The large one on the right side is 25 µm long while the three on the left side are not more than 15 µm long. The bands are housed in the space between two thin skeletal plates covering the upper and lower body surfaces. Near the lateral body surfaces, the plates cut off abruptly and taper towards the posterior end (Fig. 9). The cytoproct is situated at the base of the V-shaped tip of the upper skeletal plate.

The macronucleus is about three-quarters of the body length and lies beneath the left edge of the upper skeletal plate. It is widest at the anterior end and follows the posterior curve of the body where it tapers to termination. At about a quarter of its length from its anterior end, the macronucleus forms an indentation which accommodates an oval-shaped micronucleus. On the left side of the body, two contractile vacuoles occur between the middle and posterior ciliary bands and one or two between the middle and anterior ciliary bands.

Habitat. Rhinozeta rhinozeta n. sp. was found in large numbers in the cecum of black (5×10^4/ml liquid digesta) and white (2×10^4/ml liquid digesta) rhinoceros from southern Africa (10) and in the colon (2×10^4/ml liquid digesta) of the black rhinoceros only.

Taxonomic diagnosis. The oval-shaped, elongated body is between 71 and 156 µm long and between 31 and 60 µm wide. Four ciliary bands are present: three on the left side and one larger one situated two-thirds of the way from the anterior end of the body on the right side. Distinct parallel cuticular folds occur lengthwise anteriorly and posteriorly on either side of the right ciliary band.

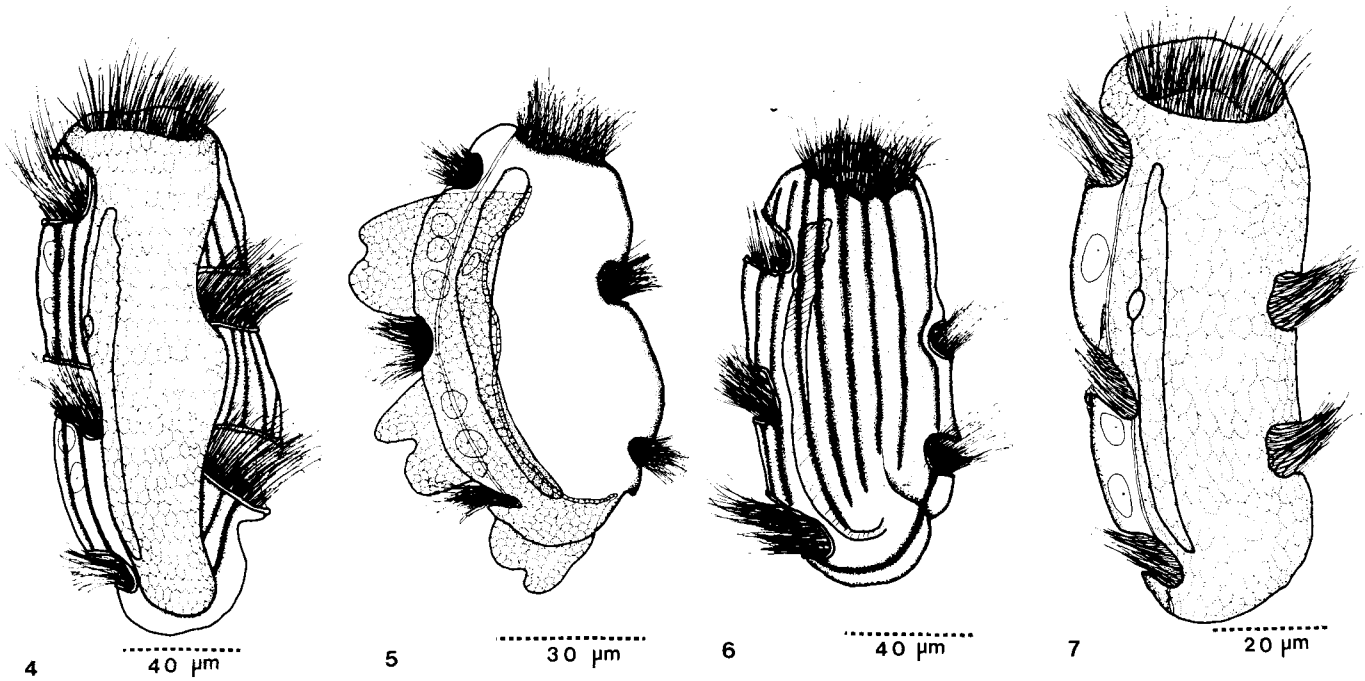
Type material. Type material is deposited in the intestinal protozoa collection of the Department of Zoology, University of Pretoria.

Rhinozeta triciliata n. sp.

Structure (Figs. 2, 10). This species is closely related to the type-species *R. rhinozeta.* The main difference is that it has only three instead of four ciliary bands, the middle left ciliary band as in *R. rhinozeta* being absent. The oval-shaped oral opening stretches across the entire anterior end of the body. The adoral zone of cilia is borne on a retractable cone, which can protrude beyond the rim of the mouth.

The elongated, oval-shaped body has an average length of 98 (84–119; n = 35) µm and an average width of 42 (36–50) µm in the black rhinoceros. In the white rhinoceros of Hluhluwe Game Reserve, this species was found to be 88 (72–97; n = 35) µm long and 36 (30–46) µm wide.

There are three ciliary bands: two on the left and one on the right



Figs. 4–7. 4. *Rhinozeta addoensis* n. sp. 5. *Rhinozeta cristata* n. sp. 6. *Rhinozeta multiplatus* n. sp. 7. *Rhinozeta unilaminatus* n. sp.

lateral surface. On the left, one band is near the mouth and the other is near the posterior end of the body. The single band on the right side is situated in the posterior third of the body. This band is wider and longer than those on the left side, and its housing is thicker and protrudes further from the body. Cuticular folds occur lengthwise anteriorly and posteriorly on each side of this band in the form of parallel ridges. These folds, in close proximity to the band, provide the necessary elasticity for efficient motility and allow for expansion (Fig. 10). The ciliary bands lie across the long axis of the body. The large ciliary band on the right side is 25 μm long while the two on the left side are not more than 15 μm long. The bands are housed in the space between two thin skeletal plates covering the upper and lower body surfaces. Near the lateral body surfaces, the plates cut off abruptly and taper towards the posterior end. The cytoproct is situated at the base of the V-shaped tip of the upper skeletal plate.

The macronucleus is about three-quarters of the body length and lies beneath the left edge of the upper skeletal plate. It is widest at the anterior end and follows the posterior curve of the body where it tapers to termination. At about a quarter of its length from its anterior end, the macronucleus forms an indentation which accommodates an oval-shaped micronucleus. On the left side of the body, two or three contractile vacuoles occur between the two ciliary bands.

Habitat. Rhinozeta triciliata n. sp. occurs in the cecum of both black (7×10^4/ml liquid digesta) and white (3×10^4/ml liquid digesta) rhinoceros from southern Africa (10), and in the colon of the black (3×10^4/ml liquid digesta) and the white (2×10^4/ml liquid digesta) rhinoceros.

Taxonomic diagnosis. The elongated oval-shaped body is between 72 and 119 μm long and between 30 and 50 μm wide. Three ciliary bands are present: two on the left side of the body, one placed anteriorly and the other posteriorly; one on the right side in the posterior third of the body. These bands are in the order of 10 μm long.

Type material. Type material is deposited in the intestinal protozoa collection of the Department of Zoology, University of Pretoria.

Rhinozeta caecalis n. sp.

Structure (Figs. 3, 11, 12). The oral opening is 30–35 μm wide, and typical of the genus in that it virtually occupies the entire anterior end of the body. The adoral zone of cilia is also typically borne on a retractable cone.

The elongated, oval-shaped body is 191 (163–236; n = 32) μm long and 80 (70–117) μm wide. The middle portion of the body is the widest; thereafter the body tapers toward the posterior end (Fig. 11). There are four ciliary bands: three on the left side of the body and one on the right side. These bands are typical of the genus. Those on the left side are evenly spaced, without cuticular folds, and smaller than the single band on the right side, which occurs two-thirds of the distance from the anterior end of the body. The single band follows the lateral curve of the body from the upper to the lower surface and varies between 40 and 50 μm in length. Anterior and posterior to this band, there is a series of lengthwise parallel cuticular folds which give plasticity to the body for expansion and motility (Fig. 11).

Along the entire length of the left side of the body, the skeletal plates of the upper and lower surfaces form a groove, which is interrupted by the three ciliary bands leaving smaller individual lengthwise plates between the bands. On the right side of the body, there is a large, lancet-shaped, skeletal gap, which is widest where it houses the large ciliary band (Fig. 11). This gap often stretches from the rim of the oral opening to the cytoproct at the base of the upper V-shaped skeletal plate. At the posterior end of the body behind the termination of the skeletal plates, there is a series of oblique cuticular folds, which run from the sides to the central line of the upper body surface (Fig. 12).

The elongated macronucleus is widest at its anterior tip, which lies

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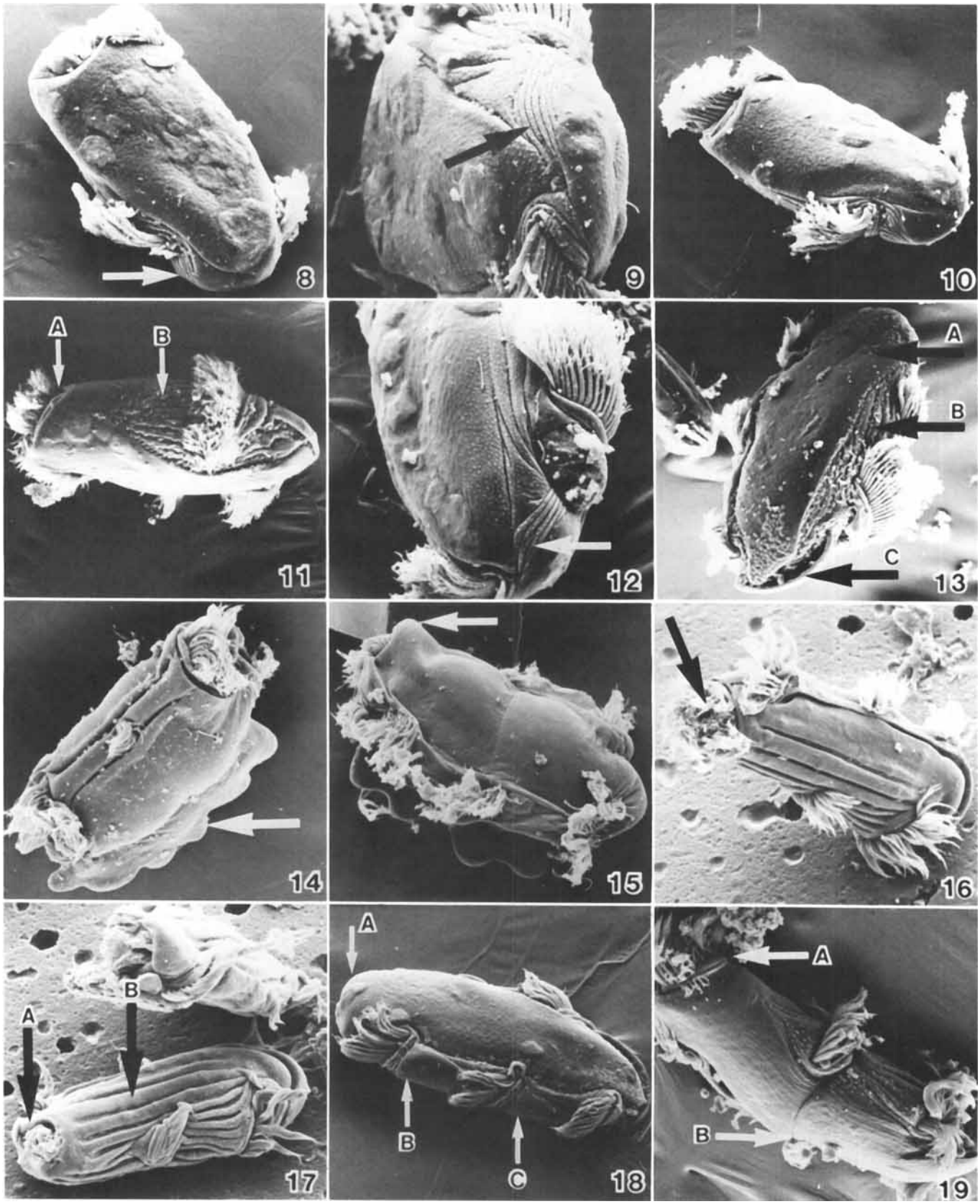
Figs. 8–19. Scanning electron micrographs of new species. 8. *Rhinozeta rhinozeta,* lower view, arrow on parallel ridges of cuticular folds. 9. *Rhinozeta rhinozeta,* posterior view, arrow on parallel ridges of cuticular folds. 10. *Rhinozeta triciliata,* lower view. 11. *Rhinozeta caecalis,* right side, arrow (a) on oral opening, arrow (b) on cuticular folds. 12. *Rhinozeta caecalis,* posterior right side, arrow on oblique cuticular folds. 13. *Rhinozeta addoensis,* posterior upper side, arrow (a) on upper skeletal plate, arrow (b) on cuticular folds in between plates, arrow (c) on lower skeletal plate. 14. *Rhinozeta cristata,* lower right side, arrow on skeletal plate extension. 15. *Rhinozeta cristata,* upper left side, arrow on oral



opening. **16**. *Rhinozeta multiplatus,* lower side, arrow on oral opening. **17**. *Rhinozeta multiplatus,* lower right side, arrow (a) on oral opening, arrow (b) on skeletal plates. **18**. *Rhinozeta unilaminatus,* upper left side, arrow (a) on oral opening, arrows (b) and (c) on smaller longitudinal plates in between cilia bands. **19**. *Rhinozeta unilaminatus,* upper right side, arrow (a) on oral opening, arrow (b) on line of division.

at the level of the anterior ciliary band on the left side of the body. It follows the curve of the body and tapers to termination just behind the left rear ciliary band. At about one-third of the distance from the anterior end of the macronucleus lies an oval-shaped micronucleus. The number of contractile vacuoles varies from two to four. They are found anterior and posterior to the middle ciliary band on the left side.

Habitat. Rhinozeta caecalis n. sp. was found in the cecum of only the black rhinoceros from southern Africa at the rate of 3×10^4/ml liquid digesta and occasionally in the colon.

Taxonomic diagnosis. The elongated, oval-shaped body is 191 (163–236) μm long and 80 (70–117) μm wide. On the right side of the body there is a large, lancet-shaped, skeletal gap stretching virtually over the entire length of the body. Parallel cuticular folds occur in the skeletal gap across the posterior extremity of the body. One large ciliary band (ca. 50 μm long) occurs on the right side of the body and three smaller bands occur on the left side.

Type material. Type material is deposited in the intestinal protozoa collection of the Department of Zoology, University of Pretoria.

Rhinozeta addoensis n. sp.

Structure (Figs. 4, 13). A wide, oval-shaped, oral opening at the anterior end has a retractable cone of cilia inside the oral orifice. It has the typical elongated oval-shaped body of the genus with an average length of 206 (179–230; n = 36) μm and an average width of 89 (71–97) μm.

Five ciliary bands are present: three on the left side and two on the right side of the body. The posterior band on the right side is the longest. Typical of this species are the parallel cuticular folds running lengthwise between the ciliary bands on both left and right sides of the body. Two wide skeletal plates are present: one on the upper and one on the lower body surface. These plates cover about 50% of the entire body surface and cease near the lateral edges of the ciliary bands. The upper skeletal plate has a sharper posterior termination than the lower plate (Fig. 13). The cytoproct is found at the base of the upper plate.

The elongated macronucleus and oval micronucleus are typically situated on the left side of the body. Two to four contractile vacuoles are present between the ciliary bands on the left side of the body.

Habitat. Rhinozeta addoensis n. sp. was found at the rate of 2×10^4/ml liquid digesta in the cecum only of the black rhinoceros from southern Africa.

Taxonomic diagnosis. Body size is large, being 206 (179–230) μm long and 80 (71–97) μm wide. The five ciliary bands are housed in wide gaps between the upper and lower skeletal plates on both sides of the body. Parallel cuticular folds run lengthwise between the ciliary bands on both sides of the body.

Type material. Type material is deposited in the intestinal protozoa collection of the Department of Zoology, University of Pretoria.

Rhinozeta cristata n. sp.

Structure (Figs. 5, 14, 15). The wide, oval-shaped, anterior oral opening with retractable ciliature is typical of the genus. The overall body shape is elongated but not symmetrical. The right side is straight while the left side is convex, thus giving the body a somewhat bean shape. In white rhinoceros of Hluhluwe Game Reserve and Ellisras district, the average length of the body was found to be 111 (95–189; n = 36) μm and the width 58 (46–104) μm. Specimens (n = 36) found in white rhinoceros of Pilanesberg Game Reserve were 30% larger.

Five ciliary bands are present: three on the left and two on the right lateral surface. Of the bands on the left, one occurs anteriorly near the mouth, one in the middle, and one in the posterior quarter of the body. Of the bands on the right, one occurs in the anterior third of the body and a larger one at the posterior end. A striking feature of this species is the unusual skeletal plate structure in the left half of the body. There is only one plate. One end of this is folded into the cytoplasm near the upper surface of the body to form a ridge which runs from the cytostome, alongside the macronucleus, across the posterior end of the body following the body's convex curvature, and terminates near the right rear ciliary band. From the entire length of this ridge, the skeletal plate extends along the lower body surface to the left lateral surface, where it protrudes like a comb. The outer edge of the protruding portion of the skeletal plate undulates in five to seven prominent waves into which the three ciliary bands fit. The normal curvature of the left side of the body continues on the inside of the protruding portion of the skeletal plate.

A large macronucleus, 12 μm wide, arises at the anterior end and tapers to termination at the posterior end of the body. An oval-shaped micronucleus lies a third of the distance from the anterior end of the macronucleus. Four to six contractile vacuoles are distributed equally on either side anteriorly and posteriorly of the middle ciliary band.

Habitat. Rhinozeta cristata n. sp. occurs in the cecum at the rate of 3×10^4/ml fluid digesta from white rhinoceros from the three widely spaced localities investigated in southern Africa and at the rate of 0.6×10^4/ml fluid digesta in the colon of the Pilanesberg animal.

Taxonomic diagnosis. Five ciliary bands are present. The body shape resembles a bean, being straight on the right side and convex on the left side. One large skeletal plate forms a ridge near the upper surface and extends along the lower body surface protruding characteristically beyond the left lateral surface to terminate in conspicuous undulations.

Type material. Type material is deposited in the intestinal protozoa collection of the Department of Zoology, University of Pretoria.

Rhinozeta multiplatus n. sp.

Structure (Figs. 6, 16, 17). The overall body shape of this species is an elongated oval with a wide, anterior, oval-shaped orifice typical of the genus. The average body length is 154 (65–201; n = 35) μm and the width 74 (24–100) μm. The adoral zone of the cilia is borne on a retractable cone. Five ciliary bands are arranged as in other species with five bands: three on the left side and two on the right side of the body. Both left and right rear ciliary bands are longer than the rest.

The feature which clearly separates this species from the rest in the genus is a series of conspicuous lengthwise parallel skeletal plates. These plates surround the entire body surface. They form ridges separated from each other by trenches. The plates are of equal width except between the ciliary bands where they and the trenches are narrower and packed more closely together. The posterior end of the body is smoothly rounded and separated from the rest of the body by a deep trench which makes it look like an operculum.

The elongated macronucleus terminates in a 90° hook at the posterior end of the body. The oval-shaped micronucleus can be seen at the level of the left front ciliary band. Four contractile vacuoles are usually present and are divided equally on either side of the left middle ciliary band.

Habitat. Rhinozeta multiplatus n. sp. occurs at the rate of 2×10^4/ml fluid digesta from the cecum of only white rhinoceros from the three localities (10) investigated in southern Africa.

Taxonomic diagnosis. The oval body shape is typical of the genus. Five ciliary bands are present: three on the left side and two on the right side of the body. Body size is 154 (65–201) μm long and 74 (24–100) μm wide. The main diagnostic feature is the presence of a series of prominent, parallel skeletal plates, which run parallel along the length of the body with trenches in between them.

Type material. Type material is deposited in the intestinal protozoa collection of the Department of Zoology, University of Pretoria.

Rhinozeta unilaminatus n. sp.

Structure (Figs. 7, 18, 19). Structurally this species has the main features of the genus. It has five ciliary bands arranged in the same manner as in the other five-banded species. Its average body length is 110 (101–119; n = 25) μm and width 46 (40–54) μm. The determinative characteristic of this species is a thin skeletal plate covering the entire body, interrupted only by the ciliary bands. Here small, individual, lengthwise plates and trenches form a link between the bands (Fig. 18). The posterior end is smoothly rounded. A straight, elongated macronucleus accommodates an oval micronucleus one-third of the distance from its anterior end. Two to four contractile vacuoles occur on the left side of the body.

Habitat. Rhinozeta unilaminatus n. sp. was found in the cecum (0.7×10^4/ml fluid digesta) and colon (0.1×10^4/ml fluid digesta) of the black rhinoceros, and the cecum (1×10^4/ml fluid digesta) only of the white rhinoceros of southern Africa.

Taxonomic diagnosis. The main characteristics of the genus are present. A large single skeletal plate encompasses the entire body with fissure joints to smaller individual plates between the ciliary bands. It is 110 μm (ave.) long and 46 μm (ave.) wide.

Type material. Type material is deposited in the intestinal protozoa collection of the Department of Zoology, University of Pretoria.

Key to Identification of Species of the Genus Rhinozeta

1. Three ciliary bands present ..... *R. triciliata*
2. Four ciliary bands present 4
3. Five ciliary bands present 5
4. Large, 167–228 μm long, clear cuticular folds on the right side and the rear of body ..... *R. caecalis*
   Small, 94–150 μm long, no clear skeletal plate cut-off on left side of body ..... *R. rhinozeta*
5. Body largely covered in skeletal plate material 6
   Body partly covered in skeletal plate material 7
6. Clear lengthwise stripes of skeletal plate ..... *R. multiplatus*
   Body mostly uniformly covered by a thin layer of skeletal material ..... *R. unilaminatus*
7. Prominent skeletal plate with undulating edge protruding from the left body surface ..... *R. cristata*
   Narrow single plate in the middle of the upper and lower surfaces covering about 50% of each surface ..... *R. addoensis*

DISCUSSION

Corliss (2) arranged seven families under the Order Entodiniomorphida, which contains the ciliated protozoan endocommensals present in the digestive tract of herbivorous mammals. The Family Ophryoscolecidiae includes all the genera found in ruminants, and the other six families occur in hindgut fermenters. This arrangement of the Entodiniomorphida emphasizes the much wider structural diversity found among the ciliated protozoa occupying the large intestine of monogastric herbivorous mammals as opposed to those occupying the reticulorumen of multigastric ruminants.

Structurally, ciliate species of the hindgut fermenters tend to be more characteristic of the different animal species which harbor them than those of the ruminant foregut fermenters. A single hindgut species does not usually occur in a variety of host species in the way that a single species of the Ophryoscolecidae can occur in a variety of wild antelope and domestic species of ruminants. The Family Polydiniellidae is restricted to elephants, the Spirodiniidae to horses, the Telamodiniidae to wart-hog, and the Troglodytellidae to anthropoid apes. The Family Ditoxidae occurs predominantly in horses. This leaves the Cycloposthiidae as the only family of Entodiniomorphida occupying the hindgut that occurs in seven different host species viz., horses, zebras, rhinoceroses, tapirs, elephants, capybaras, and hippopotami (2).

The host specificity exhibited by ciliates of hindgut fermenters is very likely due to the manner in which the young of these animals become inoculated with their particular burden of ciliated protozoa from generation to generation. These young are most probably coprophagic like those of lagomorphs (4, 8, 9) and rodents (1, 9). In this way they become infected with ciliates voided in the feces (5) of their mother and members of the small group of their own species with whom they generally associate. More observations on this aspect of the hindgut fermenters, particularly those in zoos, are needed. On the other hand, ruminant mouth parts become infected with ciliates present in the bolus, which they regurgitate, and from there are spread by licking, grazing, and drinking. Thus young and adults alike constantly receive cross infections from other groups of different ruminant species grazing the same pasture and drinking together at the same water source. Moreover, a group of a single ruminant species generally consists of a greater number of animals than a similar group of hindgut fermenters, and this also facilitates cross infection among ruminants.

Although Entodiniomorphida families tend to be host specific, the division into families is based only on structural differences. The genus *Rhinozeta* is based on the sum total of the characteristics of seven related species found in both black and white rhinoceros. The specific features of the genus, namely, an elongated, oval-shaped body with three to five short ciliary bands at definite positions on the body, the straight, elongated macronucleus, and the presence of skeletal plate material makes this genus incompatible with any of the known families of the order. This merits the creation of an eighth family, the Rhinozetidae.

This family is notable for the structural adaptations which six species of the genus *Rhinozeta* employ to overcome restriction on body size imposed by skeletal material, which encompasses the major portion of the body surface. Cuticular folds running lengthwise between the upper and lower skeletal plates on one or both lateral body surfaces provide a means of expansion in four species viz., *R. rhinozeta, R. triciliata, R. caecalis,* and *R. addoensis.* Small skeletal plates separated by trenches running lengthwise between the ciliary bands perform a similar function in *R. multiplatus* and *R. unilaminatus.* It is noteworthy that these adaptations are lacking in *R. cristata* where a single layer of skeletal material occurs only in the left half of the body, thus the other half of the body is capable of expansion.

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