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Paul Alain Nana, Zéphyrin Fokam, Guy Martial Ntoungwa Ebague, Pierre Ngassam, Geneviève Bricheux, et al.. Description of five new species of hysterocinetian ciliates (Ciliophora, Hysterocinetidae), endocommensal in a terrestrial oligochaete of the genus Alma from Cameroon. Protistology, 2012, 7 (2), pp.97-107. hal-00917775

HAL Id: hal-00917775 https://hal.science/hal-00917775

Submitted on 12 Dec 2013

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Summary

Studies on five new species of hysterocinetian ciliates, endocommensal within a terrestrial oligochaete from the genus *Alma* (Cameroon), reveal the existence of three distinct types of cytoskeletal topography within their adhesive apparatus. In the first topographical type, four superimposed antagonistic strata of skeletal fibres that correspond to the arrangement observed in the genus *Ptychostomum*, Stein 1860 are found. The second topographical type is characterized by the existence of an unorganized field of fibrillar structures forming a single dense mass in the central part of the sucker. For this, a new genus is created: *Ptychostomoides*, Nana, Fokam, Ntoungwa et al. n. g. differs from the latter by the existence of a bare patch within the central region of the adhesive apparatus. This new evidence of morphological diversification within the Hysterocinetidae supports a new approach to the systematics of this group.

Key words: *Alma*, ciliates, Hysterocinetidae, phylogeny, *Proptychostomoides* n. g., *Ptychostomoides* n. g., *Ptychostomum*

Introduction

Alma nilotica is a soil dwelling oligochaete widely distributed in a damp mud along the river Sanaga (at

Ebebda and Batchenga) in the region of Yaoundé. The worm harbours within its digestive tract both a rich fauna of Astome ciliates and a variety of ciliates from the family Hysterocinetidae. In these latter organisms, the morphogenetic process associated with cell replication and the ultrastructure of the cortical and buccal architecture has been described by Ngassam (1992), Njiné and Ngassam (1993), Ngassam and Grain (1997, 1998, 2000, 2002), and Ngassam et al. (1993, 1994).

Furthermore, during the course of studying the patterns of morphological variation in those species of hysterocinetian cilates, commensal in Glossoscolecidae, we have noted a certain structural conservatism at the level of the buccal apparatus, which comprises one paroral and three adoral organelles (Ngassam and Grain, 2002). Such morphological variation as occurs in these species is confined essentially to the topography of the anteriorly located adhesion apparatus.

Since that earlier report, further examination of a great number of Glossoscolecidae has resulted in identification of two additional kinds of anterior adhesion apparatus that, to our knowledge, have hitherto remained undescribed. In this paper, we propose a revision of the morphological classification of these hysterocinetians, commensal in the digestive tract of Glossoscolecidae earthworms.

Material and methods

The host of hysterocinetian ciliates is the Glossoscolecidae oligochaete Alma nilotica found in the central region of Cameroon. Portions of this worm's digestive tract were opened either in physiological Ringer's solution or in commercial mineral water (Volvic™ in France or Supermont™ in Cameroon). Ciliates observed under a stereomicroscope to be actively swimming in the electrolyte were collected by micropipette and examined in the living state. After fixation, staining was carried out either by pyridinated ammoniacal silver carbonate method of Fernandez-Galiano (1966, 1994), or by silver proteinate according to Bodian's method modified by Dragesco and Njiné (1971), employed to control and complete our observations. Morphometric data were gathered by the examination of groups of 30 separate cells of each species. Drawings of these cells were made with the aid of a camera lucida attached to a Wild M20 microscope.

Results

1. MORPHOLOGICAL STUDIES

Ptychostomum bacteriophilus Nana, Fokam, Ntoungwa et al. n. sp. (Figs 1-3, Plates 1, 2).

P. bacteriophilus is commonly found, along with Metaptychostomum ebepdae Ngassam and Grain, 1997, *M. piriformus* Ngassam and Grain, 1997, *M. graini* Ngassam and Grain, 1997 and *Proptychostomum commune* Ngassam and Grain, 1997, in the mid- and hind-gut of *Alma* collected along the river Sanaga at Ebebda where they attain a frequency of 60% and an average population density of 43 ciliates per worm.

Rather ovoid in shape, broad in the middle, with a rounded posterior and narrowing at the anterior where the sucker is situated, *P. bacteriophilus* is somewhat flattened laterally. This ciliate is 97-102 μ m in length and 71-76 μ m at its broadest part.

The cylindrical macronucleus (length 40 μ m, width 14 μ m), situated anteriorly, is aligned orthogonally to the anterior-posterior axis of the cell (Fig. 1, Plate 1). The rounded micronucleus (3-4 μ m in diameter) is situated above the macronucleus with respect to which it occupies an anterior-dorsal position.

From 48 to 52 kineties are equally distributed over the two faces of the ciliate. On the lower face, the ends of some of these kineties define a rather discrete secant line, located dorsally above the infundibular part of the buccal cavity.

The components of the buccal apparatus of the hysterocinetian ciliates have been described from several species. These components form a paroral organelle consisted of two rows of kinetosomes of which only the external, at the furthest right of the buccal apparatus, bears cilia. Together they form an undulating membrane parallel to the posterior edge of the cell in the peristome region before descending into the infundibulum in a spiral manner. This paroral organelle, according to de Puytorac (1968), would correspond to the undulating membrane of Hymenostomes. On the left-hand side of the peristome, two longitudinal adoral organelles are found: Ad₁, the more external, is formed by 2 parallel rows of ciliated kinetosomes confined to the peristome itself; Ad₂, the more internal, similarly is composed of 2 ciliated rows that extend into the infundibulum in a spiral path that is named Ad_2 '. The spiral path is called Ad_2 ' because sometimes there is a slight disjunction between the peristomal and infundibular parts of this organelle (Figs 1, 3). A third organelle (Ad₂), absent from the peristome, extends within the infundibulum. It consists of a single row of ciliated kinetosomes accompanied by a row of argentophilic granules and intercalates between the paroral and Ad₂'. Thus, the buccal infraciliature is composed of a paroral and 3 adoral organelles, and this justifies



Figs 1–3. *Ptychostomum bacteriophilus* n. sp. 1 – Left (lower) face showing the sucker (S), the somatic infraciliature and the buccal apparatus (BA); A – anterior part of the cell, D – dorsal side of the cell, V – ventral side of the cell, Mn – macronucleus, Mi – micronucleus. 2 – Detail of the sucker with its bundles of skeletal fibres (F_1 , F_2 , F_3 , F_4). 3 – Detail of the buccal infraciliature; AD₁, AD₂, AD₂', AD₃ – adoral organelles, IN – infundibulum, Pa – paroral, PE – peristome.

the position of *Ptychostomum* within the class Oligohymenophora.

In the descriptions of ciliates that follow below, mention will not always be made of the structural organization of the buccal apparatus. The primary reason for this is that the pattern of buccal ciliature in the representatives of all genera of the family Hysterocinetidae stands in accordance with the description given above.

The left lateral face (also called the "lower face") of the ciliate bears a clearly defined anterior thigmotactic area or sucker, with its cord-like rim, which represents the zone of the cell responsible for securing adhesion to the wall of the host intestine. The ceiling of the sucker, almost at the surface in front, sinks down progressively towards the back, extending below the posterior edge of the opening for a distance of 5 to 8 μ m. Thus, there is a major anterior part (25-31 μ m in diameter) opening directly to the exterior, and a posterior part of the sucker that is invaginated beneath the somatic cortex. The central part is occupied by 5-8 short oblique kineties.

The wall of the sucker ceiling contains skeletal fibres that reinforce the adhesive apparatus. These fibres are disposed in 4 superimposed layers which proceed from the lower to the upper face of the cell as follows (Fig. 2, Plate 2):

- a layer of external fibres F1 is situated close to the lower face of the ciliate and is composed of a dozen curved skeletal fibres; it is lying at the lateral edges of the sucker and arranged orthogonally to the antero-posterior axis of the cell;

- a layer F2 is consisted of 6-8 fibres showing a brush-shaped arrangement; these fibres emanate from a common point at the edge of the right posterior of the cell and spread out in a fan-shape towards its anterior part;

- a most substantial layer F3 is composed of 8-12 fibres that expand anteriorly in a fan-shape from the lower left side of the cell and in crossing beneath the F2 bundle form with the latter an inverted V-shaped structure;

- a layer F4 includes 13-15 short fibres that go from the lower left edge of the cell in a fan-shape



Plate 1. Ammoniacal silver carbonate impregnated specimen of *Ptychostomum bacteriophilus* n. sp.: general view of the cell, left (lower) face showing the buccal apparatus (posterior part of the cell); Bact. – bacteria, Mi – micronucleus, Mn – macronucleus, S – sucker; ×1000.

and terminate at the neighboring F3 layer with which they fuse in the centre to form an obvious thickened zone.

Like many other Hysterocinetidae ciliates found as endocommensals in the oligochaetes of Cameroon, *P. bacteriophilus* harbours many bacteria throughout its cytoplasm. However, unlike phagocytosed prokaryotes, these particular bacteria are not enclosed in host membranes and are in intimate contact with the ciliate cytoplasm.

The cytoskeleton is composed of several groups of fibres arranged in different planes and directions in a manner characteristic of the genus *Ptychostomum*. However, we have found one small departure from this standard pattern concerning the size of the groups F3 and F4 (Fig. 2) that consists respectively of 12 and 15 fibres rather than of 5 and 7 as in Ptychostomum elongatum Njiné and Ngassam, 1993. This is presumably a random change in the transmission of epigenetic information, for example the result of a chance doubling of these fibres, which would be transmitted during successive cell divisions of the ciliate.

However, the superficial F1 bundle is in all respects similar to that of other *Ptychostomum*



Plate 2. Partial view of the anterior part of *Ptychostomum bacteriophilus* n. sp. showing the sucker (S); ×1400.

species, and layers F3 and F4, in spite of their greater size, do crossover deeper in the cytoplasm in the form of the typical inverted "V".

On balance, the above consideration of the morphology of the cytoskeletal fibre system reinforcing the adhesive apparatus of this ciliate does not support the creation of a new genus distinct from *Ptychostomum*. On the other hand, the ubiquitous presence of bacteria naked within its cytoplasm justifies the creation of a new species for it within the existing genus as *P. bacteriophilus*.

Ptychostomoides nilotica Nana, Fokam, Ntoungwa et al. n. g., n. sp. (Figs 4-6, Plate 3).

Although not the most abundant, *P. nilotica* is one of the most commonly found ciliate in the midand hind-gut of *Alma nilotica*. We have collected the worms from different localities along the river Sanaga, and in all cases *P. nilotica* ciliates were found in 60-80% of worms studied.

Ovoid and rounded at the poles, this ciliate is 74-82 μ m long and 54-62 μ m wide at the level of the macronucleus where it is at its broadest (Fig. 4, Plate 3).

The macronucleus is aligned with the anteroposterior axis and is curved back at the anterior pole of the cell where an outgrowth connects it



Figs 4–6. *Ptychostomoides nilotica* n. g., n. sp. 1 – Left (lower) face showing the sucker and the somatic infraciliature; A – anterior part of the cell, D – dorsal side of the cell, F – fibre, FDM – field of dense material, Mn – macronucleus, Mi – micronucleus, S – sucker, V – ventral side of the cell. 5 – Left (lower) face showing the buccal infraciliature apparatus (posterior part of the cell). 6 – detail of the buccal apparatus; IN – infundibulum, PE – peristome.

to the thigmotactic zone. Cylindrical in form, the macronucleus is 29-32 μ m long and 18-21 μ m at its widest. At its antero-dorsal portion, it has a depression in which the ovoid micronucleus about 4 μ m in diameter sits.

The somatic ciliature consists of 22-26 parallel, meridional kineties that are equally distributed on the 2 faces and bipolar in arrangement. On the lower face, the kineties terminate anteriorly at the edge of the rounded sucker.

The peristome region of the buccal apparatus measuring 48-52 μ m long and 16-19 μ m width is very well developed. Aligned obliquely with respect to the antero-posterior axis of the cell, it occupies the entire posterior pole hugging the cell contour (Figs 5, 6).

A prominent feature of the ciliate is the cytoplasmic structure underlying the anterior adhesive apparatus. It is composed of a field of dense material occupying the entire sucker from which fine fibres radiate out.

According to the diagnostic features of the Hysterocinetidae ciliates defined by de Puytorac (1968), the nature of the anterior thigmotactic zone of this organism (reduced to a dense undivided

fibrillar mass in composition but remaining undifferentiated into skeletal fibres) is most likely to be distinct at the generic level. For this reason, we create a new genus for it - *Ptychostomoides* and a new species, *P. nilotica*.

Ptychostomoides gigas Nana, Fokam, Ntoungwa et al. n. g., n. sp. (Fig. 7, Plate 4).

Without a doubt *P. gigas* is the largest member of the genus *Ptychostomoides* found in the alimentary canal of *Alma nilotica* where it occurs at low frequency and in low numbers although being very widespread. Found in the mid-gut along with *Ptychostomum macrostomum* Njiné and Ngassam, 1993 and in the hindgut with Kozloffia de Puytorac, 1968, the number of individuals encountered varies between 10 and 18 per worm.

The cell is elongate, 135-146 μ m long and 72-78 μ m at its widest part, with the anterior being much narrower than the posterior and terminating in a sucker. The macronucleus, median and rounded, measuring 28-32 μ m in diameter, lies along the antero-posterior axis of the cell. It is flanked dorsally by a micronucleus 2.5 μ m in diameter (Plate 4).



Plate 3. Ammoniacal silver carbonate impregnated specimen of *Ptychostomoides nilotica* n. g. n., sp.; FDM – field of dense material, Mi – micronucleus, Mn – macronucleus; ×1400.

The entire cell is covered by 39-44 kineties that are unequally distributed over its two faces; on the lower face there are 18-20 kineties. These kineties have a spacing of 4.5 μ m in the mid-region and 2.5 μ m laterally. On the upper face they are distributed uniformly.

The buccal apparatus is reasonably developed with the peristome measuring 45-48 μ m long and 18-20 μ m wide. Situated obliquely to the anteroposterior axis of the cell, it occupies the entire posterior pole forming its contour. Similarly, the infundibulum is considerably developed enabling the cell to ingest bacteria; 36-41 μ m long, it forms 2 turns of a spiral making an angle of 60° to the peristome and extending to the mid-third of the cell (Fig. 7).

The sucker, appearing like an adhesive button with a network of radially disposed fine skeletal fibres, is in all respects similar to that of the preceding species.

By its size and general appearance, the great development of the buccal apparatus, and the shape of the macronucleus this ciliate can be distinguished from the preceding species which it resembles only in the constitution of the buccal apparatus. It can be regarded as a new species that we name *Ptychostomoides gigas*.

Ptychostomoides minus Nana, Fokam, Ntoungwa et al. n. g., n. sp. (Fig. 8, Plate 5).

P. minus lives in the mid- and hind-gut of large specimens of Alma collected both at Ebebda and Batchenga at low frequency and population density. The ovoid cell is 56-62 μ m long by 34-36 μ m wide. The prominent macronucleus is narrower in its anterior half if compared with its posterior half which is much stouter. The macronucleus is situated along the antero-posterior axis of the cell, closer to the lower face of the cell. On its dorsal side, it is always flanked by an ovoid micronucleus 4 μ m in diameter (Fig. 8, Plate 5).

There are 24-28 kineties equally distributed over both faces of the ciliate.

The buccal apparatus is relatively small; the peristome (18-21 μ m long) leads into a very short (7-9 μ m) infundibulum.

The thigmotactic zone, that forms a dark area in the lower anterior of the cell, has the same appearance as the two preceding species. However, this ciliate differs from those latter ones in its relatively small size and the shape of the macronucleus. Because of this we create a new species for it – *Ptychostomoides minus*.

Proptychostomoides camerounensis Nana, Fokam, Ntoungwa et al. n. g., n. sp. (Fig. 9, Plate 6).

P. camerounensis is a small ciliate, elongate in form and circular in cross-section (Fig. 9); its mean length is 52 μ m (50-55 μ m) and width is 27 μ m (25-30 μ m). We have found it accompanied by *Metaptychostomum graini*, *M. ebepdae*, and *M. piriformis* in the mid- and hind-gut of large specimens of *Alma*, collected both at Batchenga and Ebebda, where it appears at low frequency and population density.

The median nuclear apparatus is always situated along the antero-posterior axis of the cell, closer to its



Plate 4. Ammoniacal silver carbonate impregnated specimen of *Ptychostomoides gigas* n. g., n. sp.; BA – buccal apparatus, Mi – micronucleus, Mn – macronucleus; ×1400.



Fig. 7. *Ptychostomoides gigas* n. g., n. sp. Left (lower) face showing the buccal apparatus (posterior part of the cell); BA - buccal apparatus, Mn - macronucleus, Mi - micronucleus.



Plate 5. Ammoniacal silver carbonate impregnated specimen of *Ptychostomoides minus* n. g. n. sp.; BA – buccal apparatus, FDM – field of dense material, Mi – micronucleus, Mn – macronucleus; $\times 1000$.



Fig. 8. *Ptychostomoides minus* n. g., n. sp.; A – anterior part of the cell, BA – buccal apparatus, FDM – field of dense material, Mn – macronucleus, Mi – micronucleus.



Fig. 9. *Proptychostomoides camerounensis* n. g., n. sp.; A – anterior part of the cell, BA – buccal apparatus, FDM – field of dense material, Mn – macronucleus, Mi – micronucleus.

lower face. It consists of a cylindrical macronucleus, $(17-20 \mu m \log and 10-12 \mu m at its widest, situated posteriorly) and a large micronucleus (4.5 <math>\mu m$ in diameter) always located on the antero-dorsal face of the macronucleus.

On average, there are 22 kineties that are distributed equally over the two faces of the ciliate.

The buccal apparatus is short, with a peristome region some 9-11 μ m long and a similarly reduced infundibulum that makes an angle of 120° with the latter.

One of the striking features of the ciliate rests in the structure of the anterior adhesion apparatus. As in the preceding genus, this apparatus is composed of a fibrillar field forming a single dense mass; however, a major difference is that a discrete bare patch occurs in the centre of this field for which there is no equivalent in the preceding genus (Fig. 9, Plate 6). Emerging from this field are skeletal fibres which layout is modeled on the arrangement of the



Plate 6. Ammoniacal silver carbonate impregnated specimen of *Proptychostomoides camerounensis* n. g. n. sp.; BA – buccal apparatus, FDM – field of dense material, Mi – micronucleus, Mn – macronucleus; ×1000.

kineties of the cell lower face. Morphological and ultrastructual studies mentioned above allow us to set up a new genus – *Proptychostomoides*.

2. DIVERSITY WITHIN THE HYSTEROCINETIDAE

The establishment of the new genera *Ptychostomoides* and *Proptychostomoides* leads us to look a new at diversity within the Hysterocinetia. The buccal infraciliature being the most remarkably homogeneous feature of all those genera so far described, variation in morphology principally involves the structure of the anterior adhesive apparatus (Ngassam and Grain, 2002).

One could suppose the most primitive type of sucker to consist of a thigmotactic zone lacking a ciliature differentiated from the rest of the somatic ciliature and devoid of a cytoskeleton (*Hysterocinetoides* Ngassam and Grain, 2002; *Thurstonia* Ngassam and Grain, 1998; *Amietia* Ngassam and Grain, 1998). From this starting point evolution could have led, on the one hand, to the isolation of parts of the anterior kineties and their self-organisation into an autonomous ciliated zone, and on the other hand, to the acquisition and development of fibres and/or skeletal material there into arrangements of increasing complexity.

Taking into account only species of ciliates commensal in Glossoscolecidae, we can imagine from a lineage consisting of Hysterocinetoides -Thurstonia – Amietia, which is divergent from the genus Kozloffia (a framework of thin fibres spread over the entire surface of the cup arranged for the most part antero-posteriorly). We assume the emergence of a branch based on the genus Preptychostomum de Puytorac, 1968 (some segments of kineties devoid of an associated cytoskeleton in the thigmotactic depression) which itself diverges, on the one hand, into one lineage including Ptychotomoides (whose thigmotactic area consists of a dense fibrillar field), Metaptychostomum, and Ptychostomum (skeletal fibres in superimposed and antagonistic layers), and on the other hand, a separate lineage with Proptychostomoides (glabrous area with a dense fibrillar centre) and Proptychostomum Ngassam and Grain, 1997 (skeletal fibres in a radiating arrangement) (Fig. 10).

Discussion and conclusion

Ptychostomum bacteriophilus sp. n. displays the following features characteristic for the genus *Ptychostomum*: (1) an ovoid shape of the cell, (2) cytoskeletal architecture of the sucker built on a common model and composed always of 4 main layers of interlocking fibres, (3) short cilia-bearing strips in the central depression of the sucker divorced from the kineties wherein they were generated, (4) an ellipsoidal macronucleus aligned with its major axis orthogonal to that of the cell, and (5) the micronucleus situated anterior to the macronucleus. These are indeed the ptychostomian features. However, in its more impressive cytoskeleton (with 4, not 3 layers, of fibres superimposed and antagonistically arranged in a very diverse manner), by the anteriorly situated macronucleus with its major axis orthogonal to that of the cell, this ciliate bears no resemblance to any other *Ptychostomum* so far described.

The skeletal architecture of the adhesive apparatus is considered to be a fundamental character in the definition of Hysterocinetidae genera. From this



Fig. 10. Scheme of diversification in Hysterocinetidae.

viewpoint, the skeletal framework of the adhesive apparatus of the ciliates *Ptychostomoides nilotica* n. g., n. sp., *Ptychostomoides gigas* n. g. n. sp., and *Ptychostomoides minus* n. g., n. sp. composed of a dense field of fibrillar components, probably originating from the infraciliature. This type of sucker, with no equivalent in other genera, defines a new genus called *Ptychostomoides*.

Given that the skeletal fibres have a kinetodesmal origin, the genus *Ptychostomoides* would derive from *Pretychostomum* by the resolution of the short segments of kineties. These kineties are isolated in the central depression of the sucker into a fibrillar field from which those skeletal fibres differentiate that would organize themselves later into superimposed and antagonistic layers (*Metaptychostomum, Ptychostomum*). Also, from *Proptychostomoides* n. g. the edifice of skeletal fibres arranged radially from a central glabrous area (*Proptychostomum*) would derive.

Diagnoses of new genera and species

Ptychostomum bacteriophilus n. sp.

Commensal in the digestive tube of *Alma nilotica*; cell ovoid, with a broad mid-region nar-rowing anteriorly; size $97-102 \times 71-76 \ \mu\text{m}$; 48-52 kineties equally spaced over the two faces; infundibulum in a spiral turn.

Type material. Photoholotype deposited in the 2011 year collections of the Unit of Protistology, Laboratory of General Biology, Faculty of Science, University of Yaoundé I, as well as in the Laboratory of Zoology, Department of Biology, Higher Teacher Training College, University of Bamenda, Cameroon.

Ptychostomoides n.g.

The anterior thigmotactic zone is reduced to a dense and undivided fibrous mass without differentiation of skeletal fibres.

Type species: *P. nilotica* n. sp.

Ptychostomoides nilotica n. sp.

Commensal in the digestive tube of *Alma nilotica*; ovoid in shape and rounded at the extremities; 74- $82 \times 54-62 \mu m$; 22-26 bipolar kineties, distributed equally on the two faces; infundibulum in a spiral turn.

Type material. Photoholotype deposited in the 2011 year collections of the Unit of Protistology, Laboratory of General Biology, Faculty of Science, University of Yaoundé I, as well as in the Laboratory of Zoology, Department of Biology, Higher Teacher Training College, University of Bamenda, Cameroon.

Ptychostomoides gigas n. sp.

Commensal in the digestive tube of *Alma nilotica*; cell elongated, with the anterior part much narrower than the posterior; $135-146 \times 72-78 \ \mu m$; 39-44 kineties unequally distributed on the two faces; infundibulum in a spiral turn.

Type material. Photoholotype deposited in the 2011 year collections of the Unit of Protistology, Laboratory of General Biology, Faculty of Science, University of Yaoundé I, as well as in the Laboratory of Zoology, Department of Biology, Higher Teacher Training College, University of Bamenda, Cameroon.

Ptychostomoides minus n. sp.

Commensal in the digestive tube of *Alma nilotica*; cell elongated, with the anterior part much narrower than the posterior; $56-62 \times 34-36 \mu m$; 24-28 kineties distributed equally on the two faces; infundibulum in a very short spiral turn.

Type material. Photoholotype deposited in the 2011 year collections of the Unit of Protistology, Laboratory of General Biology, Faculty of Science, University of Yaoundé I, as well as in the Laboratory of Zoology, Department of Biology, Higher Teacher Training College, University of Bamenda, Cameroon.

Proptychostomoides n.g.

Possesses a glabrous zone in the centre of a dense anterior fibrillar field.

Type species: P. camerounensis n. sp.

Proptychostomoides camerounensis n. sp.

Commensal in the digestive tube of *Alma nilotica*; elongated cell, circular in cross-section; $50-55 \times 25-30 \mu m$; on average 22 kineties, distributed equally on the two faces of the ciliate; infundibulum in a spiral turn.

Type material. Photoholotype deposited in the 2011 year collections of the Unit of Protistology, Laboratory of General Biology, Faculty of Science, University of Yaoundé I, as well as in the Laboratory of Zoology, Department of Biology, Higher Teacher Training College, University of Bamenda, Cameroon.

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