



HAL
open science

Four new species of Hysteroecinetida (Protozoa: Ciliophora) from the digestive tract of earthworms collected to the lower Nyong estuary (South Coast, Cameroon)

Paul Alain Nana, Siméon Tchakonté, Anselme Crepin Mama, Fils Mamert Onana, Zéphyrin Fokam, Pierre Ngassam, Estelle Masseret, Moïse Nola, Telesphore Sime-Ngando

► **To cite this version:**

Paul Alain Nana, Siméon Tchakonté, Anselme Crepin Mama, Fils Mamert Onana, Zéphyrin Fokam, et al.. Four new species of Hysteroecinetida (Protozoa: Ciliophora) from the digestive tract of earthworms collected to the lower Nyong estuary (South Coast, Cameroon). *Zootaxa*, 2022, 5194 (2), pp.233-244. 10.11646/zootaxa.5194.2.5 . hal-03865315

HAL Id: hal-03865315

<https://hal.science/hal-03865315v1>

Submitted on 22 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright



Four new species of Hysteroecinetida (Protozoa: Ciliophora) from the digestive tract of earthworms collected to the lower Nyong estuary (South Coast, Cameroon)

PAUL ALAIN NANA^{1*}, SIMEON TCHAKONTE², ANSELME CREPIN MAMA¹,
FILS MAMERT ONANA³, ZEPHYRIN FOKAM⁴, PIERRE NGASSAM⁵, ESTELLE MASSERET⁶,
MOÏSE NOLA⁵ & TELESOPHORE SIME-NGANDO⁷

¹Department of Oceanography, Institute of Fisheries and Aquatic Sciences, University of Douala, P.O. Box 7236, Douala, Cameroon

²Laboratory of Natural Resources and Environmental Management, Faculty of Science, University of Buea, P.O. Box 063, Buea, Cameroon

³Department of Aquatic Ecosystems Management, Institute of Fisheries and Aquatic Sciences, University of Douala, P.O. Box 7236, Douala, Cameroon

⁴Department of Biology, Higher Teacher Training College, University of Bamenda, P.O. Box 39, Bamenda, Cameroon

⁵Laboratory of Hydrobiology and Environment, Faculty of Science, University of Yaounde 1, P.O. Box 812, Yaounde, Cameroon

⁶MARBEQ, University of Montpellier; IRD, Ifremer, CNRS, Montpellier, France

⁷Laboratory Microorganisms: Genome and Environment, UMR CNRS 6023, Clermont Auvergne University, P.O. Box 80026, 63178 Aubière Cedex, France

*Corresponding author: ✉ nanapaul4life@yahoo.fr; 🌐 <https://orcid.org/0000-0002-0121-652X>

Abstract

Hysteroecinetida are a large assemblage of ciliates characterized by sucker and buccal apparatus. They have a wide distribution in the digestive tract of oligochaete annelids of the genus *Alma* that inhabit hydromorphic parts of non-anthropogenic hydrosystems. On the basis of morphological, morphometric and infraciliature characteristics revealed by ammoniacal silver carbonate and 4',6-Diamidino-2-Phenyl Indole (DAPI) staining techniques, four forms were identified as new members belonging to three genera: *Preptychostomum*, *Proptychostomum* and *Thurstonia*. *Preptychostomum donendaensis* n. sp. is ovoid with a slightly narrowed anterior part and is commensal to the digestive tract of *Alma emini* and *A. nilotica*. There are 20–23 kineties on the lower side and 42–45 on the upper side. *Proptychostomum gigas* n. sp. is pear-shaped, with a narrowed anterior part and a wide posterior part (125–138 µm × 80–85 µm). There are 40 to 44 kineties, equally distributed on both sides of the ciliate. Its sucker is circular and measures 31–34 µm in diameter. The peristome follows the contour of the cell and the infundibulum has a turn of spire. *Thurstonia emini* n. sp. is identified in the digestive tract of *A. emini*. The cell is elongate, with the anterior pole narrower than the truncate posterior pole (90–115 µm × 40–55 µm). The kineties are equally distributed on both sides of the ciliate. The sucker is in the shape of an inverted U. *Thurstonia nilotica* n. sp. is commensal of the digestive tract of *A. nilotica*. The cell is pear-shaped, with the anterior part narrowed and the posterior part swollen (110–114 µm × 85–92 µm). 46 to 50 kineties are unevenly distributed on both sides of the ciliate. The sucker is shaped like an inverted lunar crescent.

Key words: *Alma*, Hysteroecinetidae, Nyong estuary, *Preptychostomum*, *Proptychostomum*, *Thurstonia*

Introduction

The phylum Ciliophora of the protists constitutes a branching widely distributed in the most diverse biotopes, as much in the fresh and salt water as in the body of many animals. Free-living ciliates are both pelagic and planktonic (Sanchez Rodriguez *et al.* 2011; Santo Ferrara & McManus, 2017), or littoral (Doherty *et al.* 2010; Wang *et al.* 2009). They are either attached to algae and aquatic metazoans (Šimek *et al.* 2016, Sparvoli *et al.* 2018), or swim on the bottom of dead leaves or in the middle of algae (Šimek *et al.* 2016). Some are catharobes and live in clear water, clean and well oxygenated, others are more or less saprobies, putrid waters affectionate, very charged in organic compounds. In addition, there is a rich and very specialized fauna of ciliates in the sand (Jiang *et al.* 2013). This fauna is called interstitial because it lives in gaps, between grains of sand. Recent research has shown that there is

a huge biomass of ciliates in soil and sediments in edaphic form (Li *et al.* 2018, Dong *et al.* 2016). Ectoparasites forms have been found in marine molluscs (Xu & Song, 2008; Xu *et al.* 2000). The commensal forms were found in the rectal bulb of the Batrachians and in the digestive tract of the Oligochaete Annelids (Fokam *et al.* 2013; 2014; Nana *et al.* 2018). This category, identified in the digestive tract of oligochaetes, is very varied and classified in three groups. The first group is the Heterotrichida who have a buccal apparatus housed in the anterior pole of the cell (Ngassam, 1983; Fokam *et al.* 2014). The second group, that of Hysteroconetidae, is characterized by a suction-cup type of fixation device located at the anterior pole of the cell and a buccal apparatus that has migrated to the posterior pole (Nana *et al.* 2012a). The third group, Astomes, are ciliates with no buccal apparatus (Nana *et al.* 2010; Fokam *et al.* 2012; Obert & Vďačný, 2021). The group that interests us in this study is that of the Hysteroconetidae who colonize the digestive tract of Oligochaetes Glossoscolecidae of genus *Alma*. Knowledge about the rich diversity of this group of ciliated protozoa remains rudimentary. The existing data are those of Ngassam & Grain (2000; 2002), Nana *et al.* (2012a). Based on morphological, morphometric and infraciliature analyzes, our study aims to contribute to the knowledge of the biodiversity of this poorly known and largely underestimated protozoan group in the marine and terrestrial national park reserve of the Nyong estuary in southern Cameroon.

Material and methods

Collection and identification of earthworms

The study was conducted in the Nyong estuary, which constitutes the natural boundary between the Department of Sanaga Maritime in the Littoral Region and the Department of Ocean in the South Cameroon Region (Figure 1). Two sampling stations were surveyed according to their accessibility. Earthworms were collected on the banks of the Nyong River at Donenda (station 1) and Dikobe (station 2). The physical characteristics of each station are listed in Table I. Worms were identified as belonging to the family Glossoscolecidae and to the genus *Alma*, according to the keys described by Sims & Gerard (1999).

TABLE 1. Characteristics of the sampling stations

	Station 1 (Donenda)	Station 2 (Dikobe)
Latitude N	03°20'00.7"	03°19'15.5"
Longitude E	010°02'09.1"	010°00'47.6"
Altitude (m)	10	5
Bed width (m)	284	531
Bed depth (m)	4.40	4.80
Flow speed (m/s)	3	1
Substrate	Rocky & sandy	Muddy & sandy

Collection, staining and identification of ciliates

Fragments of the digestive tract pertaining to each of the three main portions (fore-, mid-, and hindgut) were incised under a binocular dissecting microscope Wild M5 (Heerbrugg, Germany) in a Petri dish containing a physiological Ringer's solution. The ciliates were removed from the gut using micropipettes, counted, washed out, and then observed *in vivo* before fixation for cytological studies. Their shape and mobility were registered. Silver staining was performed using the ammoniacal pyridinated silver carbonate technique described by Fernandez-Galiano (1994). The nuclear apparatus was fluorescently stained using 4',6-Diamidino-2-Phenyl Indole (DAPI) (Johnson *et al.* 1982). Double staining was performed using DAPI and FITC-conjugated anti-tubulin antibody as described previously (Diogon *et al.* 2001). Stained cells were observed under a Leica DMR epifluorescence microscope coupled to a Q-FISH (quantitative fluorescence *in situ* hybridization) image acquisition system. All measurements were made with a calibrated ocular micrometer. The following parameters were calculated: arithmetic mean; standard deviation; minimum and maximum values. Morphometric data were generated after the examination of groups of 40 stained cells of each species. Abundance and frequency of infestation was obtained from the 40 worms analyzed from the study site. The cells were manually drawn under a Wild M20 microscope equipped with a camera Lucida. A digital camera was used for light micrographs. Classification follows Puytorac (1994).

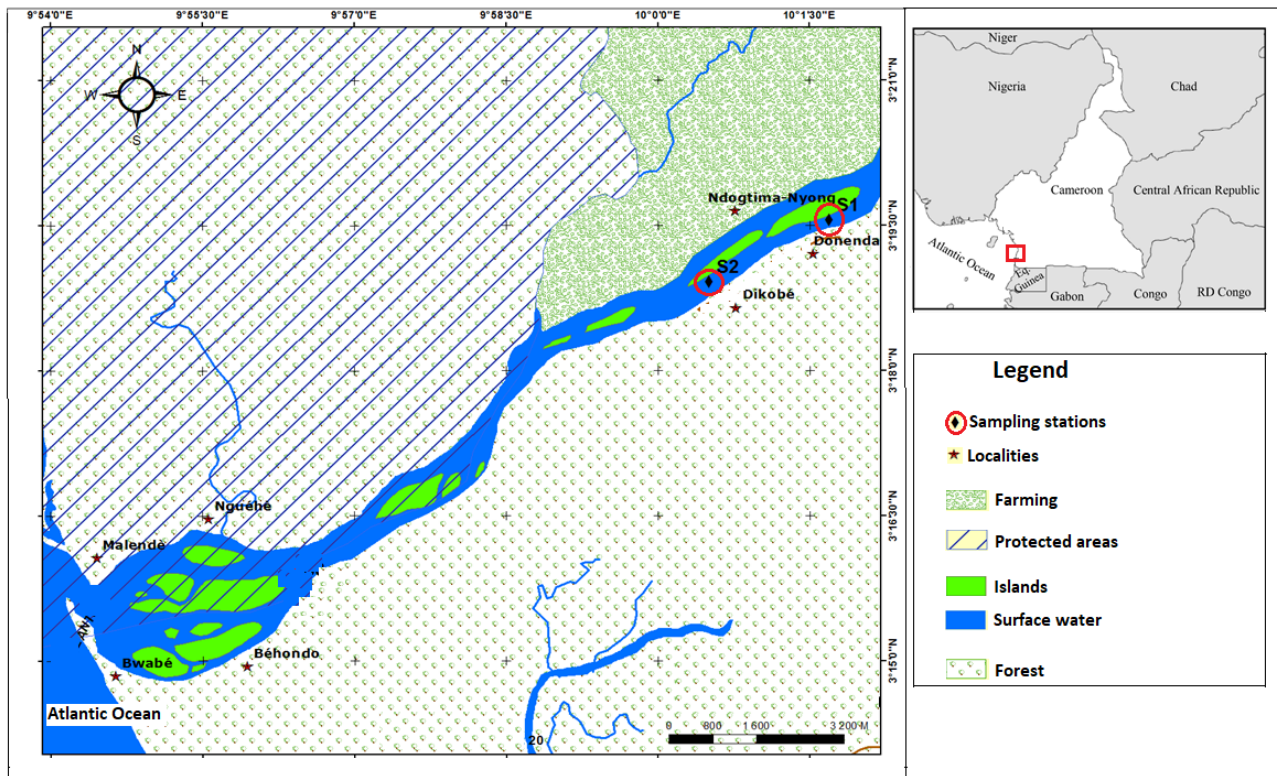


FIGURE 1. Earthworm sampling sites

Results

Taxonomic description

Phylum Ciliophora Doflein, 1901

Sub-phylum Epiplasmata Puytorac *et al.*, 1993

Super class Postciliodesmatophora Gerassimova & Seravin, 1976

Class Oligohymenophora Puytorac, 1994

Subclass Hysterocinetia Diesing, 1866

Order Hysterocinetida Diesing, 1866

Genus *Preptychostomum* Puytorac, 1968a

Preptychostomum ciliates are usually globular in shape. They are characterized by a large oral cavity, an anterior bare area more or less developed without skeletal fibers. The elongated macronucleus perpendicular to the antero-posterior axis of the cell is surmounted by a bulky micronucleus. There is the presence of a posterior pulsatile vacuole. In the genus *Preptychostomum*, we describe a new species: *P. donedaensis* **n. sp.**

Pretychostomum donendaensis n. sp.

This ciliate cohabits with *Pretychostomum magnum* Ngassam *et al.* (1993), *P. microstomum* Ngassam *et al.* (1993) and *P. katashimae* Ngassam *et al.* (1993) in the midgut of the earthworms *A. emini* and *A. nilotica*.

General morphology and morphometry: The cell is ovoid, with a slightly narrowed anterior part (Figures 2A–B). It measures 93–105 μm in length and 65–71 μm in its greatest width. The nuclear apparatus consists of a club-shaped macronucleus measuring 22–27 μm in diameter. In a depression of this macronucleus, is housed a rounded micronucleus of 2.5 μm in diameter on average. The very small buccal apparatus is isolated in a glabrous area of the posterior part of the ciliate. The peristome measures on average 10 μm long and the infundibulum 5 μm . The flattened sucker is ovoid and measures 27–35 μm wide. This sucker is bordered posteriorly by an incomplete bead forming two thin symmetrical lips.

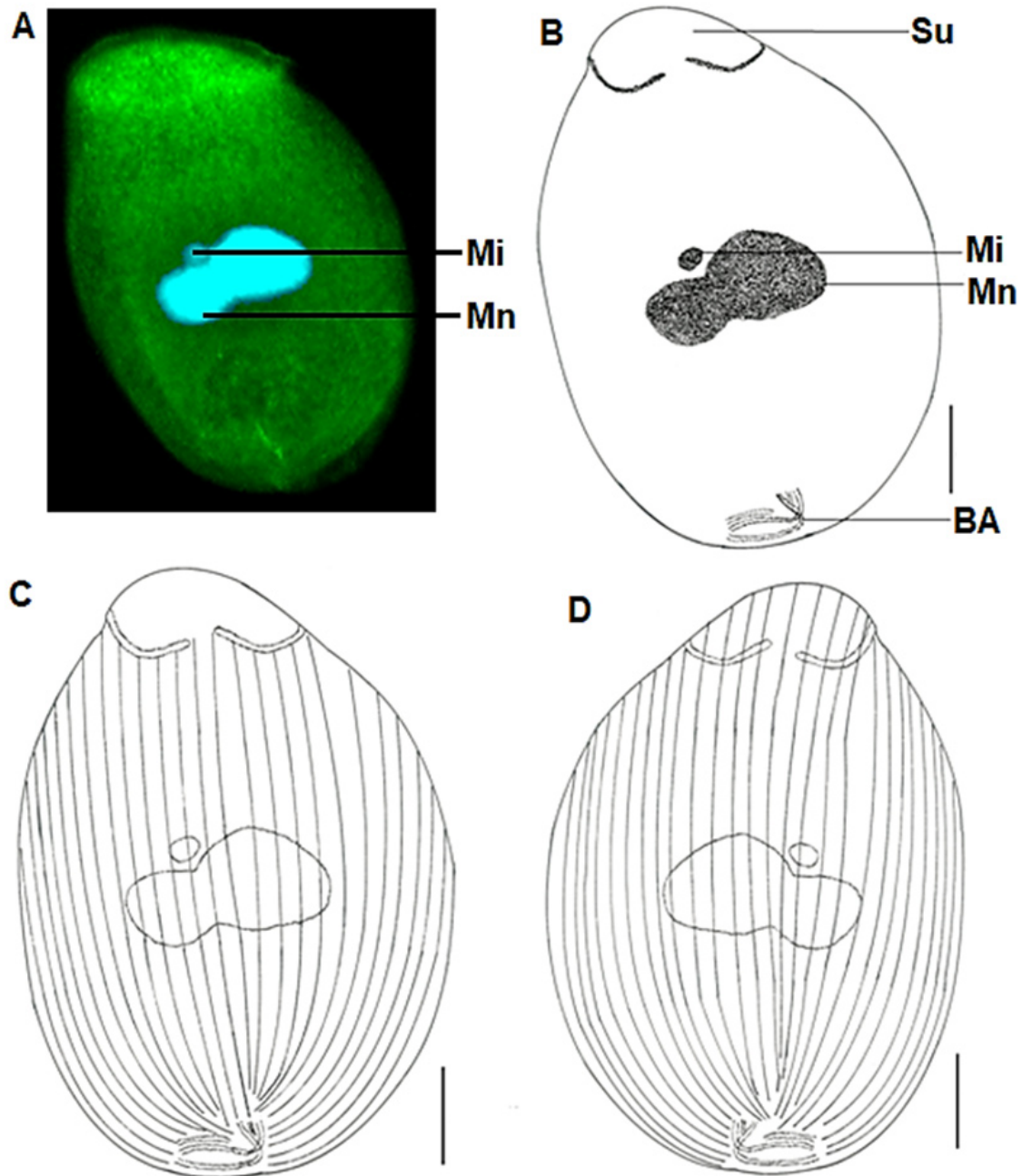


FIGURE 2. *Pretychostomum donendaensis* n. sp. **A.** General morphology after double staining by DAPI (blue) and immunofluorescence microscopy (IF) using FITC-conjugated anti-tubulin antibody (green); **B.** General morphology (Drawings after silver staining); **C.** Ciliature of the upper face; **D.** Ciliature of the lower face; **BA.** Buccal Apparatus; **Ma.** Macronucleus; **Mi.** micronucleus; **Su.** Sucker. Scale bar: 20 μm .

Infraciliature : The ciliature topography consists of 75–80 kineties unevenly distributed on both sides of the ciliate : 20–23 cover the lower face and 42–45, the upper face (Figures 2C–D).

Diagnosis : Commensal of the digestive tract of *A. emini* and *A. nilotica*. Ovoid cell, with a slightly narrowed anterior part, 93–105 71–76 μm . 20–23 kineties cover the lower face and 42–45 the upper face. Sucker diameter : 27–35 μm . Frequency of the ciliate : 94 %. Mean abundance: 25 cells per infested worm.

Type host: Midgut, in earthworms *A. emini* and *A. nilotica*.

Type locality: Donenda (0320'00.7"N–01002'09.1"E), South region, Cameroon.

Etymology: The species is named “donendaensis” in reference to the locality of harvest of the host.

Type material: Slides of the holotype (MNHN-IR-2017-0013) are deposited to the protist collection of the National Museum of Natural History, Paris, France.

Genre *Proptychostomum* Ngassam & Grain, 1997

We have found a new species in this genus.

Proptychostomum gigas n. sp.

This infusoria would be the largest of the ciliates of the genus *Proptychostomum*. It cohabits the middle and posterior portions of the digestive tract of *A. emini* with ciliates of the genus *Ptychostomum* Stein, 1860.

General morphology and morphometry: The cell is pear-shaped with a narrowed anterior part and a bulged posterior part (Figures 3A–B). It measures 125–138 μm in length and 80–85 μm in its greatest width. The buccal apparatus is developed. The peristomal region, arranged perpendicular to the anteroposterior axis, occupies more than half of the posterior pole of the cell whose contour it follows. The infundibular region which makes an angle of about 30 with the peristome, is less developed and forms spirals. The cylinder-shaped macronucleus reaches 47–51 μm long and 15–17 μm wide. It is arranged perpendicularly to the axis of the ciliate. A rounded micronucleus, 4–5 μm in diameter, is in the anterodorsal position with respect to the macronucleus. The circular thigmotactic area is very small and measures only 31–34 μm in diameter. There are 29–32 radiating skeletal fibers, crisscrossed and resting in the deep part of the sucker. These fibers are neither bundled nor layered.

Infraciliature : The somatic ciliature has 40 to 44 kineties, equitably distributed on both sides of the ciliate (Figures 3C–D).

Diagnosis : Commensal of the digestive tract of *A. emini* and *A. nilotica*. pear-shaped cell, with a narrowed anterior part and a wide posterior part, 125–138 80–85 μm . 40 to 44 kineties, evenly distributed on both sides of the ciliate. Circular sucker: 31–34 μm in diameter, peristome marrying the contour of the cell, infundibulum at a spiral turn. Frequency of the ciliate : 35 %. Mean abundance: 23 cells per infested worm.

Type host: Midgut and hindgut, in earthworms *A. emini* and *A. nilotica*.

Type locality: Donenda (03°20'00.7"N–010°02'09.1"E) and Dikobe (03°19'15.5"N–010°00'47.6"E), South region, Cameroon.

Etymology: The species is named “*gigas*” in reference to the large size of this cell.

Type material: Slides of the holotype (MNHN-IR-2017-0011) are deposited to the protist collection of the National Museum of Natural History, Paris, France.

Genre *Thurstonia* de Puytorac, 1968b

The ciliates of the genus *Thurstonia* are typically characterized by an inverted-U shaped sucker and devoid of skeletal fibers and isolated segments of kineties. Two new species of the genus *Thurstonia* have been identified and described: *T. emini* n. sp. and *T. nilotica* n. sp.

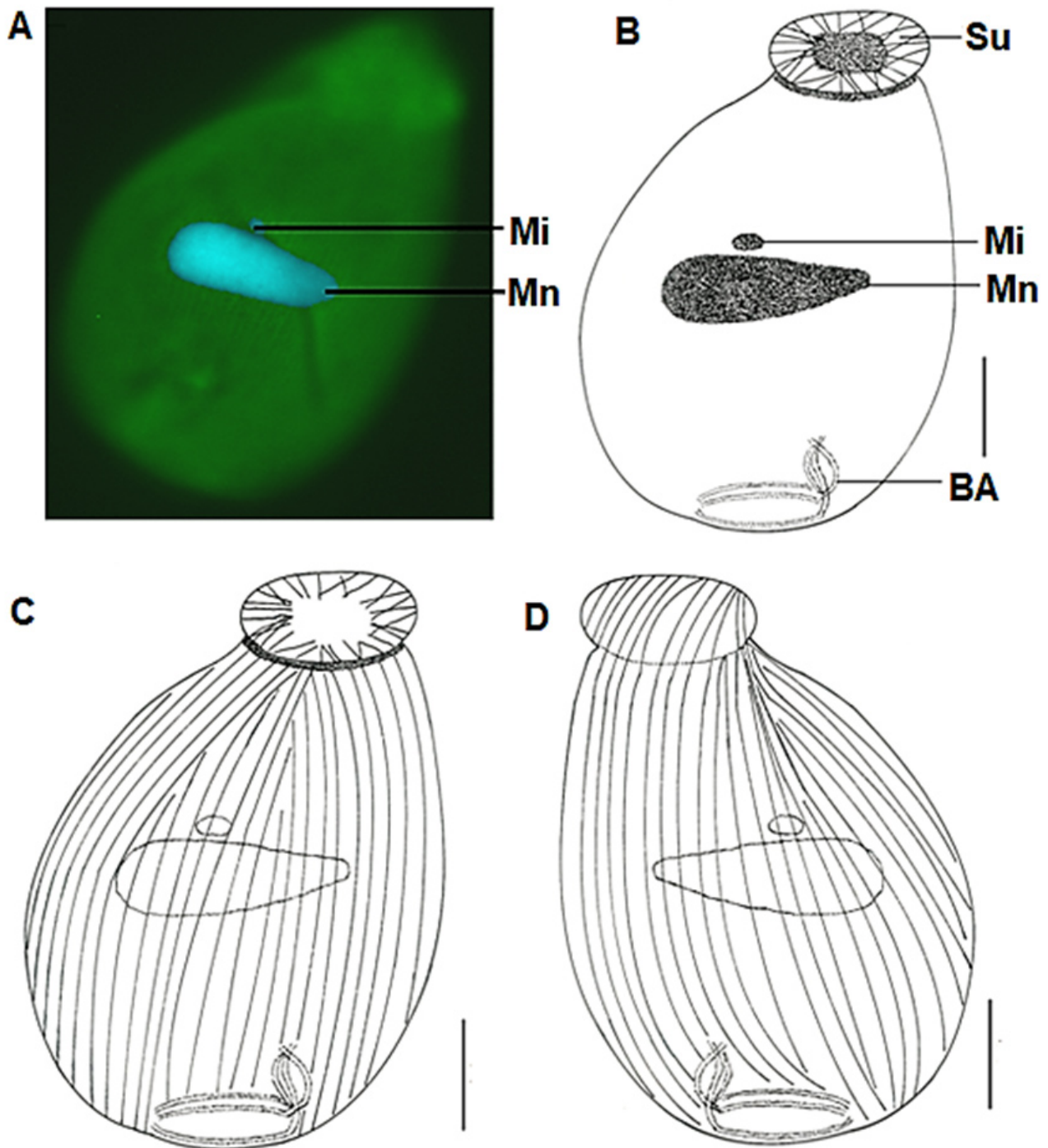


FIGURE 3. *Proptychostomum gigas* n. sp. **A.** General morphology after double staining by DAPI (blue) and immunofluorescence microscopy (IF) using FITC-conjugated anti-tubulin antibody (green); **B.** General morphology (Drawings after silver staining); **C.** Ciliature of the upper face; **D.** Ciliature of the lower face; **BA.** Buccal Apparatus; **Ma.** Macronucleus; **Mi.** micro-nucleus; **Su.** Sucker. Scale bar: 20 μ m.

Thurstonia emini n. sp.

This species lives in the hindgut of *A. emini* collected at Donenda and Dikobe. Its frequency is 45 % and its average abundance 22 ciliates per worm.

General morphology and morphometry: The cell is elongated, with the anterior pole narrower than the pos-

terior pole (Figures 4A–B). It measures an average of 105 μm (90–115 μm) long over 48 μm (40–55 μm) wide. The macronucleus is housed in the anteroposterior axis of the cell and measures an average of 11 μm in diameter. It is flanked by a small rounded micronucleus averaging 2 μm in diameter. The buccal apparatus is very small, with a peristomal part 15–17 μm long and 2–3 μm wide. The infundibular part of the buccal apparatus measures only 5 μm on average. The sucker has the shape of an inverted U, deep of 15 microns on average with its two slightly symmetrical arms devoid of skeletal fibers.

infraciliature : The somatic ciliature is formed of 70 to 74 kineties equitably distributed on both sides of the cell. On the underside, 20–25 kineties extend into the sucker (Figures 4C–D).

Diagnosis : Commensal of the digestive tract of *A. emini*. Elongate cell, with the anterior pole narrower than the truncated posterior pole, 90–115 μm 40–55 μm . 70 to 74 kineties, equally distributed on both sides of ciliate. Sucker in the shape of an inverted U. Frequency of the ciliate : 45 %. Mean abundance: 22 cells per infested worm.

Type host: Foregut and midgut, in earthworms *A. emini*.

Type locality: Donenda (0320'00.7"N–01002'09.1"E) and Dikobe (0319'15.5"N–01000'47.6"E), South region, Cameroon.

Etymology: The species is named “*emini*” in reference to the specificity of the host.

Type material: Slides of the holotype (MNHN-IR-2017-0010) are deposited to the protist collection of the National Museum of Natural History, Paris, France.

Thurstonia nilotica n. sp.

This ciliate lives in the mid and hindgut of the *A. nilotica* harvested in Donenda and Dikobe. He cohabits with *Thurstonia almae* Ngassam & Grain, 1998 and *T. kaczanowskii* of Puytorac, 1968b.

General morphology and morphometry: The cell is pear-shaped and reaches 110 μm long and 85 μm wide. The anterior part is narrowed (20 to 25 μm wide) and the posterior part swollen (55 to 65 μm) (Figures 5A–B). The nuclear apparatus consists of a macronucleus located perpendicularly to the anteroposterior axis of the cell. This macronucleus is oval and is 25 to 28 μm long and 10 to 15 μm wide. It is dorsally attached to a spherical micronucleus 2.5 μm in average diameter. The buccal apparatus is reduced, with a peristomal part long 10 to 15 μm and wide 2 to 4 μm . The infundibular part is very short and measures on average 3 μm long. The sucker, devoid of skeletal fibers, has the shape of an inverted lunar crescent.

Ciliature : The body of the ciliate is covered with eyelashes whose kinetosomes form long rows parallel to each other. There are 46 to 50 kineties, unequally distributed on both sides of the ciliate. On the upper side, there are 20 to 22 sparse kineties and on the lower face 25 to 31 tighter (Figures 5C–D).

Diagnosis : Commensal of the digestive tract of *A. nilotica*. pear-shaped cell, narrowed anterior part and bulging posterior part, 110–114 μm 85–92 μm . 46 to 50 kineties, unequally distributed on both sides of the ciliate. Sucker: inverted lunar crescent shape. Frequency of the ciliate : 11 %. Mean abundance: 10 cells per infested worm.

Type host: Foregut and midgut, in earthworms *A. nilotica*.

Type locality: Donenda (0320'00.7"N–01002'09.1"E) and Dikobe (0319'15.5"N–01000'47.6"E), South region, Cameroon.

Etymology: The species is named “*nilotica*” in reference to the specificity of the host.

Type material: Slides of the holotype (MNHN-IR-2017-0010) are deposited to the protist collection of the National Museum of Natural History, Paris, France.

Discussion and taxonomic affinities

***Preptychostomum donendaensis* n. sp.** The described species is related by its general somatic ciliature to *Preptychostomum katashimae* Puytorac, 1968. However, it differs in its ovoid form, and its very small buccal apparatus, bringing it closer to *Preptychostomum microstomum* Ngassam *et al.* (1993). *Preptychostomum donendaensis* n. sp. is morphologically close to *Preptychostomum magnum* Ngassam *et al.* (1993). The difference lies in the denser somatic ciliature and the reduced thigmotactic zone in *Preptychostomum magnum*.

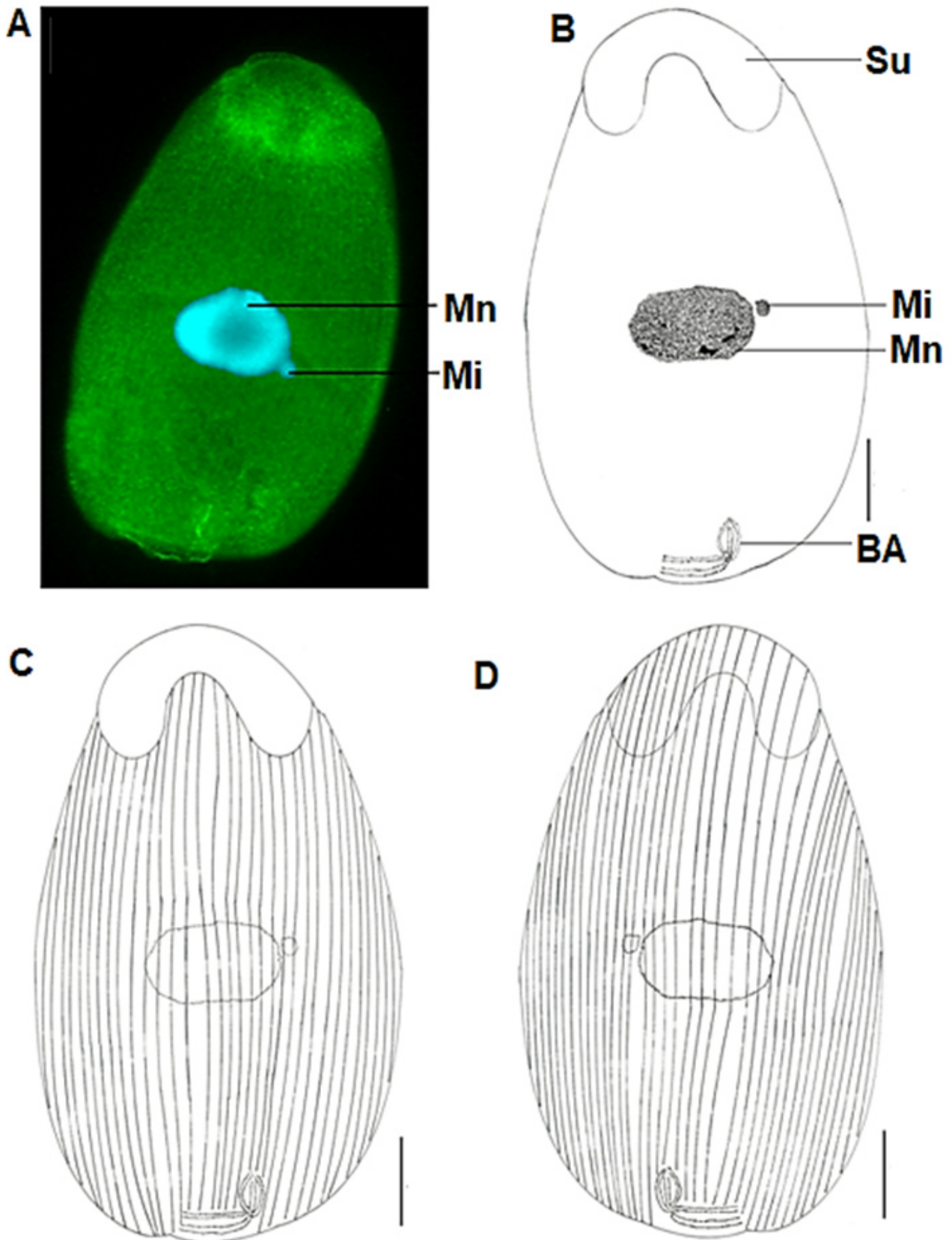


FIGURE 4. *Thurstonia emini* n. sp. **A.** General morphology after double staining by DAPI (blue) and immunofluorescence microscopy (IF) using FITC-conjugated anti-tubulin antibody (green); **B.** General morphology (Drawings after silver staining); **C.** Ciliature of the upper face; **D.** Ciliature of the lower face; **BA.** Buccal Apparatus; **Ma.** Macronucleus; **Mi.** micronucleus; **Su.** Sucker. Scale bar: 20 μ m.

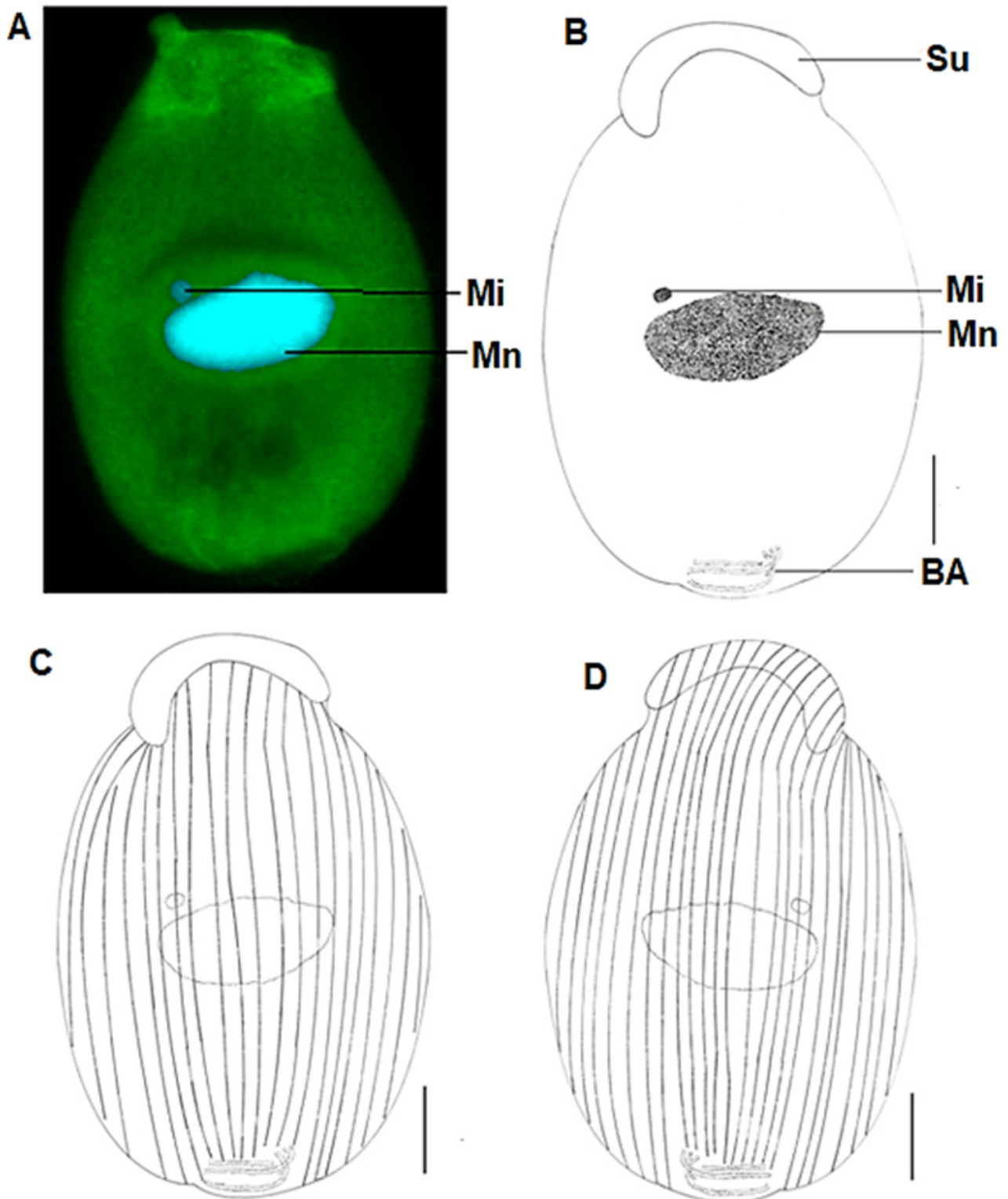


FIGURE 5. *Thurstonia nilotica* n. sp. **A.** General morphology after double staining by DAPI (blue) and immunofluorescence microscopy (IF) using FITC-conjugated anti-tubulin antibody (green); **B.** General morphology (Drawings after silver staining); **C.** Ciliature of the upper face; **D.** Ciliature of the lower face; **BA.** Buccal Apparatus; **Ma.** Macronucleus; **Mi.** micronucleus; **Su.** Sucker. Scale bar: 20 μ m.

***Proptychostomum gigas* n. sp.** By the general appearance of its circular suction cup, the great development of its buccal apparatus, this ciliated protozoan recalls *Ptychostomum macrostomum* Njin & Ngassam, 1993. However, the ciliate described differs from *P. macrostomum* in the distribution of the less tight kinetics on its two faces and especially the arrangement of the skeletal fibers, radial, without arrangement in bundles and superimposed strata. One of the salient features of the new species lies in its Pear-shape morphology, distinct in *Proptychostomum commune* and *Proptychostomum simplex* Ngassam & Grain, 1997, the number and arrangement of intertwined skeletal fibers that support in the deep part of the sucker. Due to its shape and measurements, the described species differs from known forms

***Thurstonia emini* n. sp.** The general morphology of this infusoria (elongated cell, uncoupled thigmotactic area devoid of skeletal fibers) brings it closer to *Thurstonia kaczanowskii* Puytorac, 1968b and *Thurstonia almae* Ngassam & Grain, 1998b. The described species, however, differs from all other known ciliates of the genus *Thurstonia* by the great development of its macronucleus lodged in the antero-posterior axis of the cell.

***Thurstonia nilotica* n. sp.** *Thurstonia nilotica* is close to *Thurstonia emini* due to its uncapillated thigmotactic area without skeletal fibers. Discrepancies are observed on the one hand in the rather elongated form in *T. emini*, pear-shaped in *T. nilotica*; on the other hand, by the shape and dimensions of their sucker.

The newly described species (Table 2) have been localized to the middle and posterior parts of the earthworm digestive tract. This preference is thought to be due to their feeding habits and variations in physicochemical parameters along the digestive tract of *Oligochaetes* (Nana *et al.* 2012b; 2014). In our future work, in addition to morphological and morphometric analyses, we plan to perform molecular analyses of these still poorly understood organisms.

TABLE 2. Main characteristics of the described species

	Described species			
	<i>Proptychostomum donendaensis</i> n. sp.	<i>Proptychostomum gigas</i> n. sp.	<i>Thurstonia emini</i> n. sp.	<i>Thurstonia nilotica</i> n. sp.
Cell form	Ovoid	Pear-shape	Elongated	Pear-shape
Cell length (µm)	93–105	125–138	90–115	110–114
Cell width (µm)	71–76	80–85	40–55	85–92
Kineties lower face	20–23	20–22	35–37	26–28
Kineties upper face	42–45	20–22	35–37	20–22
Sucker diameter (µm)	27–35	31–34	20–25	26–31
Type host	<i>A. emini</i> & <i>A. nilotica</i>	<i>A. emini</i> & <i>A. nilotica</i>	<i>A. emini</i>	<i>A. nilotica</i>
Type locality	Donenda	Donenda & Behonde	Donenda & Behonde	Donenda & Behonde
Frequency (%)	94	35	45	11
Average abundance	24 ± 6	23 ± 4	22 ± 5	10 ± 6

Conclusion

This study, based on morphological, morphometric and infracilature characteristics, has made it possible to highlight the rich, under-explored ciliated fauna, commensal to the digestive tract of oligochaetes of the Nyong estuary. Future work will combine the ammoniacal silver carbonate impregnation technique and 4',6-Diamidino-2-Phenyl Indole (DAPI) staining with sequence analysis of the small subunit of rRNA, to more confidently assess phylogenetic relationships among Hysterocinetida ciliates.

Acknowledgments

P.A. NANA highly thanks the University of Clermont Auvergne (France) for its logistical support and the mobility grant for the year 2022.

References

- Diesing, K.M. (1866) Revision der Prothelminthen. Abteilung: Amasteigen. *Sitzungsberichte der kaiserlichen Akademie der Wissenschaften in Wien*, 52, 505–579.
- Diogon, M., Henou, C., Ravet, V., Bouchard, P. & Viguès, B. (2001) Evidence for regional differentiations in the dynamics of centrin cytoskeletal structures in the polymorphic ciliate *Tetrahymena paravorax*. *European Journal of Protistology*, 37, 223–231.
<https://doi.org/10.1078/0932-4739-00824>
- Doherty, M., Tamura, M., Vriezen, J.A., McManus, G.B. & Katz, L.A. (2010) Diversity of oligotrichia and choreotrichia ciliates in coastal marine sediments and in overlying plankton. *Applied and Environmental Microbiology*, 76 (12), 3924–3935.
<https://doi.org/10.1128/AEM.01604-09>
- Dong, J., Lu, X., Shao, C., Huang, J.A. & Al-Rasheid, K.A.S. (2016) Morphology, morphogenesis and molecular phylogeny of a novel saline soil ciliate, *Lamtostyla salina* n. sp. (Ciliophora, Stichotrichida). *European Journal of Protistology*, 56, 219–231.
<https://doi.org/10.1016/j.ejop.2017.04.005>
- Fernandez-Galiano, D. (1994) The ammoniacal silver carbonate method as a general procedure in the study of protozoa from sewage (and other) waters. *Water Research*, 28, 495–496.
- Fokam, Z., Ngassam, P., Nana, P.A., Bricheux, G., Bouchard, P. & Sime-Ngando, T. (2012) Révision de la Sous-Famille des Metaracoelophryinae de Puytorac, 1972 (Oligohymenophora: Hoplytophryida: Hoplytophryidae), Ciliés Astomes du tube digestif d'Oligochètes terricoles d'Afrique: Description de cinq espèces nouvelles. *Parasite*, 19, 41–56.
<https://doi.org/10.1051/parasite/2012191041>
- Fokam, Z., Nana, P.A., Ngassam, P., Bricheux, G., Bouchard, P., Vigus, B. & Sime-Ngando, T. (2013) Description of two new species of Sicuophoridae and Nyctotheridae (Heterotrichina), endocommensal in the rectal ampulla of *Bufo regularis* (Amphibia: Anura) from the Northwest of Cameroon). *Protistology*, 8 (1), 16–21. [<http://hal-01114647v2>]
- Fokam, Z., Nana, P.A., Ngassam, P., Bricheux, G., Bouchard, P., Vigus, B. & Sime-Ngando, T. (2014) Morphological description of four new species of *Nyctotherus* (Ciliophora: Nyctotheridae: Heterotrichida), commensal ciliates of the digestive tract of a terrestrial Oligochaete (Megascolecidae) from the Northwest Region of Cameroon. *Protistology*, 8 (2), 62–70.
- Gerassimova, Z.P. & Seravin, L.N. (1976) Ectoplasmic fibrillar system of Infusoria and its role for the understanding of their phylogeny. *Zoologicheskij Zhurnal*, 55, 645–656.
- Jiang, J., Huang, J., Li, J., Al-Rasheid, K.A., Al-Farraj, S.A., Lin, X. & Hu, X. (2013) Morphology of two marine euplotids (Ciliophora: Euplotida), *Aspidisca fusca* and *A. hexeris*, with notes on their small subunit rRNA gene sequences. *European Journal of Protistology*, 49 (4), 634–43.
<https://doi.org/10.1016/j.ejop.2013.04.005>
- Johnson, G.D., Davidson, R.S., McNamee, K.C., Russell, G., Goodwin, D. & Hol Borow, E.J. (1982) Fading of immunofluorescence during microscopy: a study of the phenomenon and its remedy. *Journal of Immunological Methods*, 55 (2), 231–242.
- Li, F., Li, Y., Luo, D., Miao, M. & Shao C. (2018) Morphology, Morphogenesis, and Molecular Phylogeny of a New Soil Ciliate, *Sterkiella multicirrata* sp. nov. (Ciliophora, Hypotrichia) from China. *Journal of Eukaryotic Microbiology*, 65 (5), 627–636.
<https://doi.org/10.1111/jeu.12508>
- Nana, P.A., Ngassam, P., Fokam, Z., Bricheux, G., Bouchard, P., Coffe, G., Sime-Ngando, T. & Zébaz Togouet, S.H. (2010) Morphological variations of the nuclear apparatus of astome ciliates *Almophrya bivacuolata* and *A. maediovacuolata* (protozoa: ciliophora) endocommensal of terricolous oligochaetes in Cameroon. *Parasite*, 17 (4), 293–297.
<https://doi.org/10.1051/parasite/2010174293>
- Nana, P.A., Fokam, Z., Ntougwa Ebague, G.M., Ngassam, P., Bricheux, G., Bouchard, P. & Sime-Ngando, T. (2012a) Description of five new species of hysteroecinetian ciliates (Ciliophora, Hysteroecinetidae), endocommensal in a terrestrial oligochaete of the genus *Alma* from Cameroon. *Protistology*, 7 (2), 97–107. [<https://hal.archives-ouvertes.fr/hal-00917775>]
- Nana, P.A., Fokam, Z., Ngassam, P., Zébaz Togouet, S.H., Ajeegah, G.A., Bricheux, G., Bouchard, P. & Sime-Ngando, T. (2012b) Distribution of Astomatia Schewiakoff, 1896 and Hysteroecinetidae Diesing, 1866 (Ciliophora, Oligohymenophora) along the digestive tract of *Alma emini* (Oligochaete, Glossoscolecidae) is correlated with physico-chemical parameters. *Protistology*, 7 (4), 193–202. [<https://hal.archives-ouvertes.fr/hal-00918063>]
- Nana, P.A., Fokam, Z., Ngassam, P., Ajeegah, G.A., Moche, K., Bricheux, G., Bouchard, P., Vigus, B. & Sime-Ngando, T. (2014) Influence of ionic contents on the stratification of Astomatia and Hysteroecinetidae (Ciliophora, Oligohymenophora) along the digestive tract of *Alma emini* (Oligochaete, Glossoscolecidae) from the Center region of Cameroon. *International Journal of Current Research*, 6 (1), 4468–4474.
<https://hal.archives-ouvertes.fr/hal-01114626v2>
- Nana, P.A., Fokam, Z., Viguès, B., Bricheux, G., Ajeegah, G.A., Ngassam, P., Nola, M. & Sime-Ngando, T. (2018) Morphology and infraciliature of two new earthworm ciliates, *Hoplitophrya polymorphus* sp. nov. and *Anoplophrya simplex* sp. nov. (Ciliophora: Oligohymenophorea: Astomatia). *Zootaxa*, 4392 (1), 169–178.
<https://doi.org/10.11646/zootaxa.4392.1.9>
- Ngassam, P. (1983) Description de quatre espèces nouvelles de Ciliés Hétérotriches des genres *Nyctotherus* Leidy et *Paranycto-*

- therus* nov. gen. endocommenseaux d'un oligochète terricole au Cameroun. *Protistologica*, 19 (2), 167–175.
- Ngassam, P. & Grain, J. (1997) Six espèces nouvelles d'Hysterocinetidés appartenant aux deux genres nouveaux *Metaptychostomum* et *Proptychostomum*, Ciliés endocommenseaux d'un Oligochète terricole du Cameroun. *Annales des Sciences naturelles et de Zoologie, Paris*, 13e Série, 18, 41–49.
- Ngassam, P. & Grain, J. (1998) Ciliés Hysterocinetidae des genres *Amietia* et *Thurstonia*: description de 4 espèces, dont 3 nouvelles, endocommensales de l'Oligochète *Alma nilotica*. *Annales des Sciences naturelles et de Zoologie, Paris*, 13e Série, 19 (2), 73–79.
- Ngassam, P. & Grain, J. (2000) Contribution to the study of Hysterocinetidae ciliates of the genus *Ptychostomum*. Description of six new species. *European Journal of Protistology*, 36, 285–292.
[https://doi.org/10.1016/S0932-4739\(00\)80004-8](https://doi.org/10.1016/S0932-4739(00)80004-8)
- Ngassam, P. & Grain, J. (2002) Description of the new genus *Hysterocinetoides* (Ciliophora, Hysterocinetidae) and revision of the systematics of the subclass Hysterocinetia. *European Journal of Protistology*, 38, 11–17.
<https://doi.org/10.1078/0932-4739-00833>
- Ngassam, P., Grain, J. & Njin, T. (1993) Contribution à l'étude des Ciliés Hysterocinetidae: le genre *Preptychostomum* de Puytorac, 1968. *Annales des Sciences Naturelles et de Zoologie, Paris*, 13ème Série, 14, 127–135.
- Njin, T. & Ngassam, P. (1993) Morphogenèse de bipartition de deux espèces de Ciliés Hysterocinetidae du genre *Ptychostomum* Stein, 1890, endocommenseaux d'Oligochètes Glossoscolecidae. *European Journal of Protistology*, 29, 396–406.
[https://doi.org/10.1016/S0932-4739\(11\)80402-5](https://doi.org/10.1016/S0932-4739(11)80402-5)
- Obert, T. & Vďačný, P. (2021) Multi-gene phylogeny of the subclass Astomatia (Protista: Ciliophora) refreshed with two rare astome ciliates from the digestive tube of endogeic earthworms. *Organisms Diversity & Evolution*, 21, 59–77.
<https://doi.org/10.1007/s13127-020-00469-6>
- Puytorac, P. de (1968a) Sur quelques Ciliés Hysterocinetidae endoparasites de Vers Oligochètes au Gabon. Révision de la famille. *Biologia Gabonica*, 4, 241–279.
- Puytorac, P. de (1968b) Structure et ultrastructure de quatre nouvelles espèces de Ciliés Hysterocinetidae Diesing. *Protistologica*, 4, 453–467.
- Puytorac, P. de, Batisse, A., Deroux, G., Fleury, A., Grain, J., Laval-Peuto, M. & Tuffrau, M. (1993) Proposition d'une nouvelle classification du phylum des protozoaires Ciliophora Doflein, 1901. *Comptes Rendus de l'Académie des Sciences, Life Sciences*, 316, 716–720.
- Puytorac, P. de (1994) Proposition d'une classification du phylum Ciliophora. Doflein, 1901. (Réunion de systématique, Clermont-Ferrand). *Centre de Recherche Académique de Paris*, 278, 5–27.
- Sanchez Rodriguez, M. del R., Lugo Vazquez, A., Oliva Martinez, M.G., Verver y Vargas, J.G., Rodriguez Rocha, A. & Peralta Soriano, L. (2011) Planktonic ciliates in a hypertrophic pond: functional role and importance. *Journal of Environmental Biology*, 32 (4), 497–503.
- Santo Ferrara, L.F. & McManus, G.B. (2017) Integrating dimensions of biodiversity in choreotrichs and oligotrichs of marine plankton. *European Journal of Protistology*, 61, 323–330.
<https://doi.org/10.1016/j.ejop.2017.04.004>
- Šimek, K., Pitsch, G., Salcher, M.M., Sirov, D., Shabarova, T., Adamec, L. & Posch, T. (2016) Ecological Traits of the Algae-Bearing *Tetrahymena utriculariae* (Ciliophora) from Traps of the Aquatic Carnivorous Plant *Utricularia reflexa*. *Journal of Eukaryotic Microbiology*, 64 (3), 336–348.
<https://doi.org/10.1111/jeu.12368>
- Sims, R.W. & Gerard, B.M. (1999) *Earthworm*. FSC Publication, London, 167 pp.
- Sparvoli, D., Richardson, E., Osakada, H., Lan, X., Iwamoto, M., Bowman, G.R., Kontur, C., Bourland, W.A., Lynn, D.H., Pritchard, J.K., Haraguchi, T., Dacks, J.B. & Turkewitz, A.P. (2018) Remodeling the Specificity of an Endosomal CORVET Tether Underlies Formation of Regulated Secretory Vesicles in the Ciliate *Tetrahymena thermophila*. *Current Biology*, 28 (5), 697–710.
<https://doi.org/10.1016/j.cub.2018.01.047>
- Stein, F. (1860) *Ueber die Eintheilung der holotrichen Infusionsthieru. einige neuere Gattungen u. Arten dieser Ordnung*. Sitzber. K. böhm. Sitzungsberichte der königlichen böhmischen Gesellschaft der Wissenschaften, Prague, 265 pp.
- Wang, Y., Song, W., Warren, A., Al-Rasheid, K.A., Al-Quraishy, S.A., Al-Farraj, S.A., Hu, X. & Pan, H. (2009) Descriptions of two new marine scuticociliates, *Pleuronema sinica* n. sp. and *P. wilberti* n. sp. (Ciliophora: Scuticociliatida), from the Yellow Sea, China. *European Journal of Protistology*, 45 (1), 29–37.
<https://doi.org/10.1016/j.ejop.2008.06.001>
- Xu, K. & Song, W. (2008) Two trichodinid ectoparasites from marine molluscs in the Yellow Sea, off China, with the description of *Trichodina caecellae* n. sp. (Protozoa: Ciliophora: Peritrichia). *Systematic Parasitology*, 69 (1), 1–11.
<https://doi.org/10.1007/s11230-007-9094-6>
- Xu, K., Song, W. & Warren, A. (2000) Observations on trichodinid ectoparasites (Ciliophora: Peritricha) from the gills of *mari-cultured molluscs* in China, with descriptions of three new species of *Trichodina* Ehrenberg, 1838. *Systematic Parasitology*, 45 (1), 17–24.
<https://doi.org/10.1023/A:1006284629307>