

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

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Summary

The morphology and ciliature of four *Nyctotherus* species (*Nyctotherus orthostomatus* n. sp., *Nyctotherus ndoumeleleensis* n. sp., *Nyctotherus ngassami* n. sp. and *Nyctotherus atunibaensis* n. sp.), isolated from the mid and hindgut of a Megascolecidae annelid of the genus *Eupolytoreutus* from the northwest region of Cameroon, were investigated using living observation (methylene blue) and silver impregnation methods (Fernandez-Galiano, 1994). *Nyctotherus orthostomatus* n. sp. is recognized by a pear-shaped body (128–152 × 95–104 µm); a conspicuous buccal apparatus with the peristome and the infundibulum perpendicular to each other; about 41–46 somatic kineties covering each side of the cell; one transversally elongated massive macronucleus located in the anterior third of the cell, surmounted by a very small spherical micronucleus. *Nyctotherus ndoumeleleensis* n. sp. is distinguished by the combination of the following characters: ovoid body (151–163 × 87–92 µm) with rounded poles; about 52–68 somatic kineties uniformly covering the cell body; an anterior, transversal and trapezoidal macronucleus carrying laterally a small micronucleus (2–3.5 µm in diameter). *Nyctotherus ngassami* n. sp. can be distinguished from its congeners by the following set of features: size about 153–269 × 102–148 µm; body ovoid, with 64–86 ciliary rows covering its surface; a small and globular micronucleus hidden by a transversal macronucleus located slightly above the equatorial part of the cell. *Nyctotherus atunibaensis* n. sp. is characterized by size about 145–253 × 95–162 µm, ovoid cell body, 108–122 somatic kineties,

a transversal and arc-shaped macronucleus hiding a small micronucleus. In the present study, new locality (northwest region of Cameroon) and new species of the genus *Nyctotherus* are explored.

Key words: Cameroon, megascolecid earthworms, *Nyctotherus*, *N. orthostomatus* n. sp., *N. ndoumeleleensis* n. sp., *N. ngassami* n. sp., *N. atunibaensis* n. sp., systematics

Introduction

Ciliates are ubiquitous eukaryotic unicellular microorganisms which have adapted to a wide range of habitats (terrestrial, marine and fresh waters), using different morphological and physiological strategies; some ciliates are symbiotic (Sauvadet, 2010). The symbiotic ciliates could be parasitic, mutualistic and mostly commensal of the digestive tract of vertebrates and invertebrates, particularly annelids and amphibians (de Puytorac, 1994). Thus, the digestive tract of megascolecid annelids can be considered as a microhabitat suitable for the development of diverse microorganisms, notably many types of ciliates including nyctotherans. These ciliates have been targeted for studies by many researchers (Albaret, 1975; Albaret and Njiné, 1975; Ngassam, 1983; Affa'a, 1991; Affa'a and Amiet, 1994; Finler and Yildiz, 2000). The Nyctotherans that live endocommensally only in the mid and hindgut of Megascolecidae annelids of the genus *Eupolytoreutus* collected in the central Cameroon are presently classified basing on their ciliary pattern. The one characterized by an apical secant system on the right side of the cell, belongs to the genus *Nyctotherus* Leidy, 1849; another, with an apical and a postoral secant system, is typical of the genus *Paranyctotherus* Ngassam, 1983. So far there is no information concerning these ciliates from other regions of Cameroon.

In this paper, we describe four new nyctotheran species obtained from Megascolecidae earthworms of the genus *Eupolytoreutus* which distribution is likely to expand from the central region where they have been studied (Albaret and Njiné, 1975; Ngassam, 1983) up to the Northwest region of Cameroon.

Material and methods

The samples were collected in August–November 2012 in Bambui and Bambili (Tubah Subdivision of the north-west region of Cameroon). This study area is located in the southern part of the meridional Niger basin (5° 59' 00" N and 10° 15' 00" E), at an

altitude of 1350 m (Fig. 1). Worms were identified as belonging to the genus *Eupolytoreutus* according to the key proposed by Sims and Gerard (1999).

Fragments of the digestive tract pertaining to each of the three main portions (fore-, mid-, and hindgut) were opened under a binocular dissecting microscope Wild M5 (Heerbrugg, Germany) at 250× magnification. This was done in a Petri dish containing either physiological Ringer's solution or mineral water (Volvic™ in France or Supermont™ in Cameroon). The ciliates were removed from the ingesta using micropipettes, washed and observed *in vivo* before fixation for cytology. Their shape and mobility were registered. Cell staining was made with the ammoniacal pyridinated silver carbonate technique of Fernandez-Galiano (1976, 1994). All cell measurements were made with a calibrated ocular micrometer. The following parameters were calculated: arithmetic mean; standard deviation; minimum and maximum values. Morphometric data were obtained by examination of 30 cells of each species. Drawings of these cells were performed with the aid of a camera lucida attached to a Wild M20 microscope. A digital camera was used for light micrographs. Identification and classification was done according to the key of Albaret (1975).

Results

NYCTOTHERUS ORTHOSTOMATUS FOKAM, NANA, NGASSAM ET AL. N. SP.

This species occurs in the mid and hindgut of *Eupolytoreutus* sp., where it lives in cohabitation with many other species of nyctotherans and astomes, specifically *Eudrilophrya complanata* de Puytorac and Dragesco, 1970 and *Anoplophrya vulgaris* Ngassam, 1883.

Diagnosis. The body is pear-shaped. Both poles are rounded. The peristome starts far from the apex and joins the infundibulum orthogonally in the equatorial region. The infundibulum ends with a short and slender cytopharynx. The macronucleus is transversal, surmounted by an eccentric micronucleus and located in the anterior half of the cell.

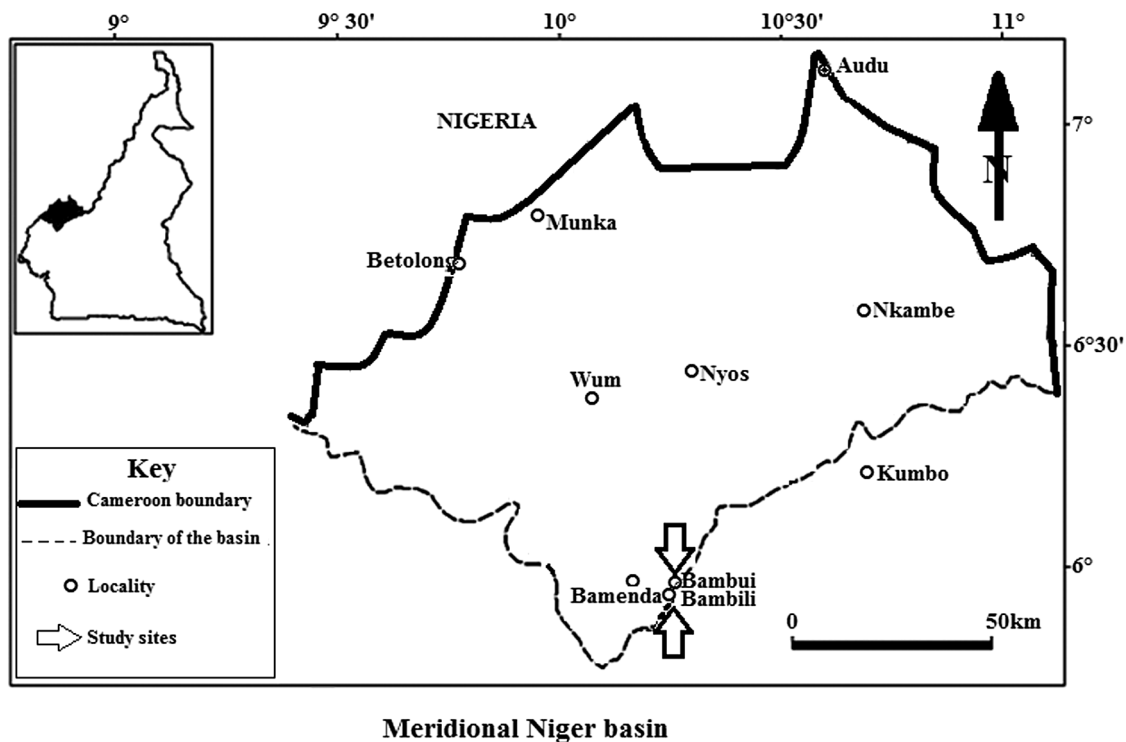


Fig. 1. The study area (modified from Olivry, 1986).

There is one preoral suture line, but no caudal secant system. One to two contractile vacuoles are located posteriorly.

Type host. *Eupolytoreutus* sp.

Type locality. Bambili and Bambui, Tubah Subdivision, Bamenda, Cameroon.

Habitat. Mid and hindgut.

Type specimens. Permanent preparations belonging to this species are kept in the Department of Biology (Higher Teacher Training College, The University of Bamenda, Cameroon).

Description. The cell is elongated and laterally flattened into concave left and convex right sides. The posterior end is wider than the anterior end conferring a pear shape on the specimen. The anterior end is slightly pointed (Fig. 2).

Morphometric characteristics are: 143.5 ± 7.1 μm in length and 94.8 ± 37 μm in width (Table 1). The macronucleus (20.03 ± 1.6 μm long and 47.7 ± 1.4 μm across) is massive and kidney shaped (Fig. 2), lodged in the anterior portion of the ciliate, set horizontally and very close to the peristome. The micronucleus (2.7 ± 0.6 μm in diameter) is spherical, situated to the left side on top of the macronucleus. No caryophore surrounding the nuclear apparatus has been observed. One to two contractile vacuoles are located in the posterior zone of the cell.

The body is covered by 82–91 bipolar somatic kineties that extend longitudinally from a prominent

anterior preoral suture line to the posterior part of the cell without converging into a suture line (Fig. 3). The kineties of both the left and the right sides of the cell are evenly distributed. Below the preoral secant system there is an area devoid of kineties that is occupied by the buccal apparatus. No proliferation of kineties by delineation at the level of secant systems has been observed. The boundaries of the somatic kineties are marked by the position occupied by the buccal aperture. The preoral suture line delimits anteriorly the left and the right sides of the cell (Fig. 3). The buccal apparatus consists of two conspicuous parts: the peristome, situated anteriorly at the left lateral margin of the cell, and the infundibulum. The peristomial part is roughly perpendicular to the infundibular zone.

NYCTOTHERUS NDOUMELELEENSIS FOKAM, NANA, NGASSAM ET AL. N. SP.

This species colonizes the median and posterior intestine of *Eupolytoreutus* sp. collected in Bambili (Ndoumelele quarters) and Bambui in the North West region. It develops there, concomitantly with the previous species.

Diagnosis. Commensal of the digestive tract of *Eupolytoreutus* sp. Ovoid shape. Anterior and posterior poles rounded. Size $151\text{--}163 \times 87\text{--}92$ μm . Transversal and trapezoidal macronucleus.

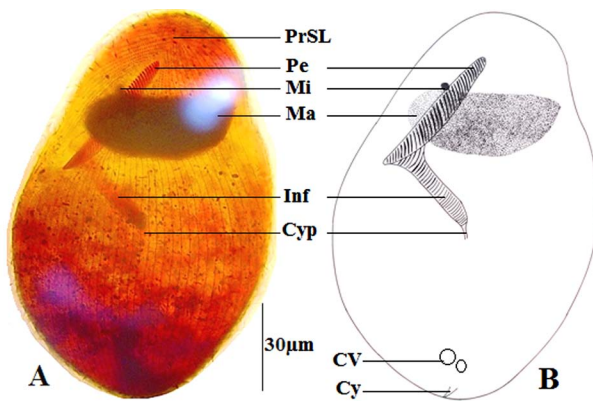


Fig. 2. General morphology of *Nyctotherus orthostomatus* n. sp. A – Light micrograph after silver staining ($\times 400$); B – drawing. *Abbreviations:* Cv – contractile vacuoles, Cy – cytopyege, Cyp – cytopharynx, Inf – infundibulum, Ma – macronucleus, Mi – micronucleus, Pe – peristome, PrSL – preoral suture line.

Micronucleus spherical. Peristome vertical, starting far from the apex. Infundibulum curved, ending with a short cytopharynx. One preoral suture line, but no caudal secant system.

Type host. *Eupolytoreutus* sp.

Type locality. Bambili and Bambui, Tubah Subdivision, Bamenda, Cameroon.

Habitat. Mid and hindgut.

Type specimens. Permanent preparations belonging to this species are kept in the Department of Biology (Higher Teacher Training College, The University of Bamenda, Cameroon).

Description. The ciliate lives in the middle and posterior intestine of a megascolecid earthworms *Eupolytoreutus* sp., collected in the moist soil in Bambili and Bambui. The cell is ovoid (Fig. 4), with the anterior end narrow and the posterior end more or less rounded. It measures 151–163 μm long and 87–92 μm across. The macronucleus (27–35 \times 54–60 μm) is transversally elongated and

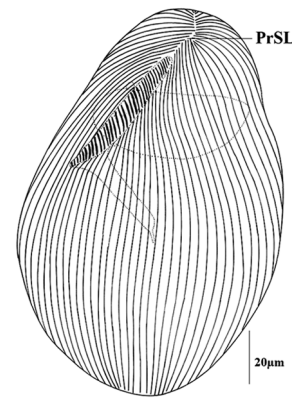


Fig. 3. Ciliary pattern of *Nyctotherus orthostomatus* n. sp. *Abbreviations:* PrSL – preoral suture line.

has a trapeze shape. The micronucleus (2–3.5 μm in diameter) lies on the dorsal posterior side of the macronucleus (Fig. 4, Table 2).

There are 52–68 somatic kineties covering the two sides regularly (Fig. 5). On the right side anteriorly, there is an axial suture line deriving from the convergence of kineties. The left side is devoid of secant system.

The buccal apparatus is composed of a vertical peristome (42–58 μm long) and a curved infundibulum (38–52 μm) linking equatorially at an average angle of 103.3° (Table 2).

NYCTOTHERUS NGASSAMI FOKAM, NANA, NGASSAM ET AL. N. SP.¹

This ciliate co-inhabits the mid and hindgut of *Eupolytoreutus* sp. with the previously described species.

Diagnosis. Commensal of the digestive tract of *Eupolytoreutus* sp. Ovoid shape. Size 153–269 \times 102–148 μm . Longitudinally extended macronucleus with a crescent shape. Curved peristome

¹ Graciously dedicated to Professor Pierre Ngassam.

Table 1. Morphometric characters of *Nyctotherus orthostomatus* n. sp.

	Cell length (μm)	Cell width (μm)	Mn length (μm)	Mn width (μm)	Mi diam. (μm)	Kin.	Pe length (μm)	I length (μm)	AIP (°)
Max	152	104	28	51	3	91	60	51	98
Mean	143.5	94.8	20.3	47.7	2.7	64.4	53.1	43.6	91.3
Min	128	85	21	40	1	82	41	32	87
SD	7.1	3.7	1.6	1.4	0.6	4.2	3.2	5.5	10.4

Notes. AIP – angle infundibulum-peristome in degree, Kin – kineties, Max – maximum, Min – minimum, Mi diam. – micronucleus diameter, Mn – macronucleus, Pe – peristome, SD – standard deviation; N=30.

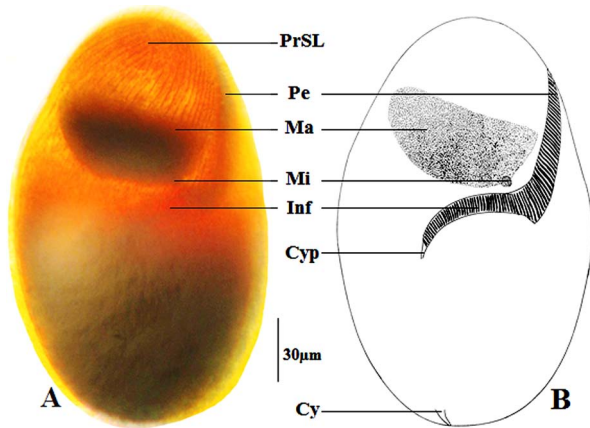


Fig. 4. General morphology of *Nyctotherus ndoumeleleensis* n. sp. A – Light micrograph after silver staining ($\times 400$); B – drawing. *Abbreviations:* Cy – cytopyge, Cyp – cytopharynx, Inf – infundibulum, Ma – macronucleus, Mi – micronucleus, Pe – peristome, PrSL – preoral suture line.

starting far from the apex. Rectilinear infundibulum at an angle of 150° with the peristome; 64–86 somatic kineties with a preoral and acaudal secant systems.

Type host. *Eupolytoreutus* sp.

Type locality. Bambili and Bambui, Tubah Subdivision, Bamenda, Cameroon.

Habitat. Mid and hindgut.

Type specimens. Permanent preparations belonging to this species are kept in the Department of Biology (Higher Teacher Training College, The University of Bamenda, Cameroon).

Description. Cells are ovoid and rounded at both ends; they are flattened on the lower side and slightly convex on the upper side. Cell measurements are: length, 153–269 μm , and width, 102–148 μm . The crescent-shaped macronucleus ($16\text{--}31 \times 36\text{--}53 \mu\text{m}$) is located transversally in the anterior third of the cell (Fig. 6, Table 3). The spherical micronucleus

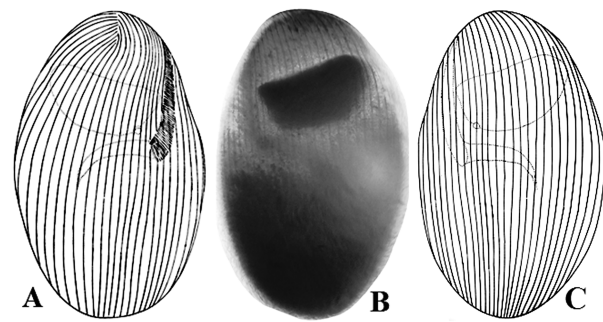


Fig. 5. Ciliary pattern of *Nyctotherus ndoumeleleensis* n. sp. A – Drawing of the right side; B – light micrograph of the left side after silver staining ($\times 400$); C – drawing of the left side.

(3–5 μm) is found in a concavity of the anterior part of the macronucleus. The peristome begins at the anterior end of the body, is vertically oriented and extends slightly backwards and down towards the posterior end leading to the infundibulum at an angle of 150.8° . The infundibulum, shaped like a long oblique funnel, is supported by a slender monitorium and ends with a short cytopharynx (Fig. 6). The cell is covered by 64–86 meridional somatic kineties. The anterior and posterior ends of the right face (upper surface) kineties converge forming two median suture lines (a preoral suture line and a posterior suture line) (Fig. 7).

NYCTOTHERUS ATUNIBAENSIS FOKAM, NANA, NGASSAM ET AL. N. SP.

Diagnosis. Commensal of the digestive tract of *Eupolytoreutus* sp. Ovoid shape. Average size $194.2 (145\text{--}253) \times 125.0 (95\text{--}162) \mu\text{m}$. Arc-shaped laterally extended macronucleus. Vertically oriented peristome starting far from the apex. Curved infundibulum; 108–122 somatic kineties with a preoral but not a caudal secant system.

Table 2. Morphometric characters of *Nyctotherus ndoumeleleensis* n. sp.

	Cell length (μm)	Cell width (μm)	Mn length (μm)	Mn width (μm)	Mi diam. (μm)	Kin.	Pe length (μm)	I length (μm)	A I P ($^\circ$)
Max	163	92	35	60	3.5	68	58	52	111
Mean	158.0	89.7	33.1	58.3	2.7	55.1	53.4	46.9	103.2
Min	151	87	27	54	2	52	42	38	89
SD	2.4	1.8	2.1	1.6	1.1	5.3	1.7	2.0	8.3

Notes. AIP – angle infundibulum-peristome in degree, Kin – kineties, Max – maximum, Min – minimum, Mi diam. – micronucleus diameter, Mn – macronucleus, Pe – peristome, SD – standard deviation; N=30.

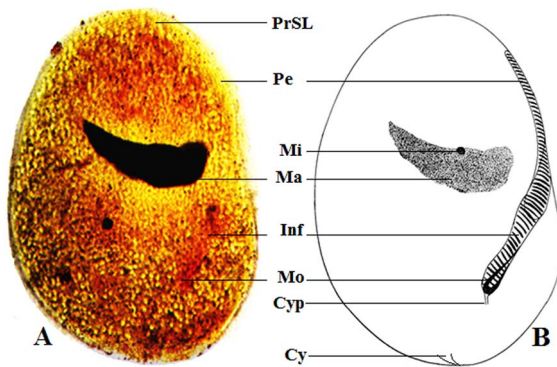


Fig. 6. General morphology of *Nyctotherus ngassami* n. sp. A – Light micrograph after silver staining ($\times 400$); B – drawing. *Abbreviations:* Cy – cytopyge, Cyp – cytopharynx, Inf – infundibulum, Ma – macronucleus, Mi – micronucleus, Mo – monitorium, Pe – peristome, PrSL – preoral suture line.

Type host. *Eupolytoreutus* sp.

Type locality. Bambili and Bambui, Tubah Subdivision, Bamenda, Cameroon.

Habitat. Mid and hindgut.

Type specimens. Permanent preparations belonging to this species are kept in the Department of Biology (Higher Teacher Training College, The University of Bamenda, Cameroon).

Description. The body is kidney-shaped, 145–253 μm long and 95–162 μm wide, somewhat flattened laterally, and rounded at both ends. The right margin is slightly depressed in the equatorial part of body (Fig. 8). The dual nuclear apparatus includes a transversally curved macronucleus (20–32 \times 26–44 μm) located anteriorly; and a tiny micronucleus (3.3 μm in diameter) situated axially in the concave side of the macronucleus (Fig. 8, Table 4). The buccal apparatus comprises a slightly vertical and curved peristome that starts far from the apex of the cell and is in continuity in the equatorial part of the cell with

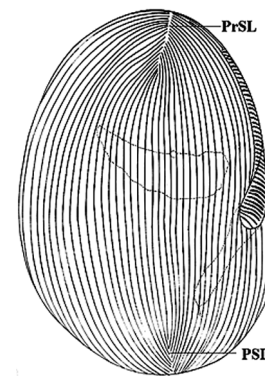


Fig. 7. Ciliary pattern of *Nyctotherus ngassami* n. sp. *Abbreviations:* PrSL – preoral suture line, PSL – posterior suture line.

the internal infundibulum. The junction between these two structures forms an obtuse angle of about 102.2° (mean). The infundibulum ends in a short and thin cytopharynx. The cell is evenly covered by 108–122 somatic meridian kineties. These kineties converge in the anterior part of the cell to form an apical suture line (Fig. 9).

Discussion

With respect to morphology and the ciliature pattern (absence of postoral secant system), the four ciliate species described above (Table 5) belong to the genus *Nyctotherus*, as was originally described by Leidy (1849) and some subsequent authors: Grassé (1928), Puytorac and Öktem (1967), Albaret and Njiné (1975) and Ngassam (1983).

By its general appearance, *Nyctotherus orthostomatus* can be confused at a first glance with *Nyctotherus polymorphus* found by Ngassam (1983) in megascolecid earthworms collected in the central region (Yaounde). However, it differs from the latter by the higher number of kineties and the

Table 3. Morphometric characters of *Nyctotherus ngassami* n. sp.

	Cell length (μm)	Cell width (μm)	Mn length (μm)	Mn width (μm)	Mi diam. (μm)	Kin.	Pe length (μm)	I length (μm)	A I P (°)
Max	269	148	31	53	5	86	48	26	162
Mean	204.3	122.5	23.4	44.2	3.1	76.7	34.6	23.5	150.8
Min	153	102	16	36	3	64	29	18	137
SD	15.3	12.4	1.9	3.4	1.8	8.3	2.0	4.5	8.3

Notes. AIP – angle infundibulum-peristome in degree, Kin – kineties, Max – maximum, Min – minimum, Mi diam. – micronucleus diameter, Mn – macronucleus, Pe – peristome, SD – standard deviation; N=30.

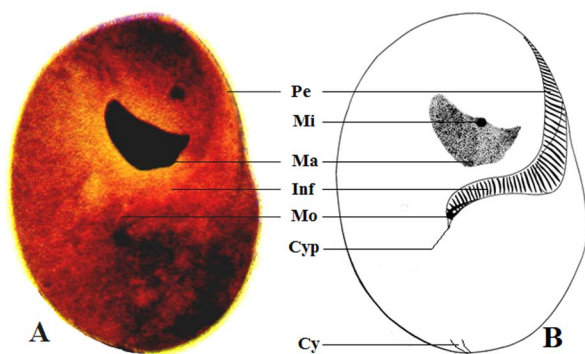


Fig. 8. General morphology of *Nyctotherus atunibaensis* n. sp. A – Light micrograph after silver staining ($\times 400$); B – drawing. Abbreviations: Cy – cytopye, Cyp – cytopharynx, Inf – infundibulum, Ma – macronucleus, Mi – micronucleus, Mo – monitorium, Pe – peristome.

arrangement of the buccal apparatus in which the peristome and the infundibulum are oblique and joint orthogonally. *N. polymorphus* differs from the other species described by his relatively small size and the pear shape. Additionally, *Nyctotherus orthostomatus* also shows some similarity with *Nyctotherus dupouyi* Ngassam, 1983 (anterior and posterior ends rounded, with the anterior one third budding from the posterior two thirds of the cell). The distinguishing characteristics of the two species reside in the organization of their buccal and nuclear apparatus: *Nyctotherus dupouyi* possesses a peristome that begins from the apex of the cell and extends to the equatorial region where it is continuous with the infundibulum entering the endoplasm and ending posteriorly with a long curved cytopharynx. The peristome of *N. orthostomatus* is oblique and starts far from the apex of the cell; slightly above the equatorial plane it joins the infundibulum at a right angle; the infundibulum, in its turn, ends with a short

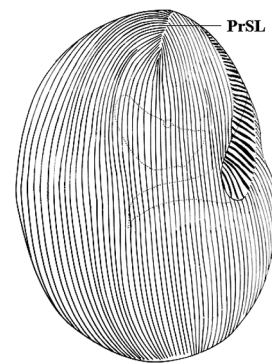


Fig. 9. Ciliary pattern of *Nyctotherus atunibaensis* n. sp. Abbreviations: PrSL – preoral suture line.

cytopharynx creating an angle of about 90° . The macronucleus of *N. dupouyi* is horseshoe-shaped, while that of *N. orthostomatus* is transversal and kidney-shaped. For all these reasons, we believe that the ciliate described above can be considered as a new species of the genus *Nyctotherus* Leidy, 1849.

Nyctotherus ndoumeleleensis reminds the previous species by its general morphology, even though the posterior pole is less wide. Another prominent feature of similarity is the slight torsion of the anterior region, consecutive to a slight spiralisation of the kineties in the anterior part of the cell. The trapezoidal macronucleus resembles that of *N. polymorphus*, but the location of the micronucleus is different – lateral and posterior. *N. ndoumeleleensis* actually presents enough variability in terms of the shape of the nuclear apparatus and the infundibulum which extends almost to the other side of the cell, ending with a curved hook-like cytopharynx. *N. ndoumeleleensis* could also be confounded with *Nyctotherus inflatus* described by Tuzet and Manier, 1858 (see: Albaret, 1975), due to its general morphology and orientation of the nuclear apparatus.

Table 4. Morphometric characters of *Nyctotherus atunibaensis* n. sp.

	Cell length (μm)	Cell width (μm)	Mn length (μm)	Mn width (μm)	Mi diam. (μm)	Kin.	Pe length (μm)	I length (μm)	A I P ($^\circ$)
Max	253	162	32	44	4	122	40	22	108
Mean	194.2	125.0	18.9	36.1	3.3	96.1	34.0	18.6	102.2
Min	145	95	20	26	2	108	25	14	89
SD	16.9	7.3	1.6	1.4	0.8	9.8	4.3	4.6	6.5

Notes. AIP – angle infundibulum-peristome in degree, Kin – kineties, Max – maximum, Min – minimum, Mi diam. – micronucleus diameter, Mn – macronucleus, Pe – peristome, SD – standard deviation; N=30.

Table 5. Comparative characters between the four species of *Nyctotherus* (N=30)

Characters		Specimens	<i>Nyctotherus orthostomatus</i>	<i>Nyctotherus ndoumeleleensis</i>	<i>Nyctotherus ngassami</i>	<i>Nyctotherus atunibaensis</i>
Cell	Length		143.5 (128 – 152)	158.0 (151 – 163)	204.3 (153 – 269)	194.2 (145 – 253)
	Width		94.8 (85 – 104)	89.7 (87 – 92)	122.5 (102 – 148)	125.0 (95 – 162)
	Shape		Pear shape	ovoid	ovoid	ovoid
Macronucleus			Transversal and kidney shaped.	Transversal and trapezoidal	Transversal and crescent in shape	Transversal and arc shape
Peristome			Oblique; start far from the apex	Vertical; start far from the apex	Curved; start far from the apex	Curved; start far from the apex
Infundibulum			Rectilinear; right angle with the peristome	Curved	Rectilinear; about 150° with the peristome	Curved
Secant systems	Preoral		+	+	+	+
	Postoral		–	–	–	–
	Caudal		–	–	+	–
Spacing of kineties			3 µm	3 µm	2 µm	2 µm

Notes: + present; – absent

Nyctotherus ngassami differs from the other species of the ovoid shape by the presence of a caudal secant system (Table 5) and a somewhat rectilinear disposition of the buccal apparatus. Although the general morphology of *N. ngassami* reminds the one of *N. dupouyi* Ngassam, 1983 and the ovoid form of *N. polymorphus* Ngassam, 1983, the area occupied by the infundibulum differs in these two later species. Besides, *N. dupouyi* and *N. polymorphus* have a massive macronucleus hiding the micronucleus while in *N. ngassami* the macronucleus is crescent in shape with a micronucleus situated anteriorly in its concavity.

By its general morphology, *Nyctotherus atunibaensis* is similar to *Nyctotherus cordiformis* Stein, 1862, living in the rectum of anuran amphibians, but it differs from the latter by the relative reduction in size. Moreover, the macronucleus of *N. atunibaensis* has a crescent shape bearing a micronucleus in a concavity oriented anteriorly, while in *N. cordiformis* the massive macronucleus is kidney shaped, bearing a micronucleus in a concavity oriented posteriorly. Both species have an apical suture line; however, the caudal suture line is present in *N. cordiformis* but absent in *N. atunibaensis*. These particularities, added to the difference in host prompted us to consider this specimen as a new representative of the genus *Nyctotherus* Leidy, 1849.

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