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Protistology

ORIGINAL ARTICLE

Morphology, morphometry and infraciliature of four new species of Clevelandellida de Puytorac and Grain, 1976 (Ciliophora: Armophorea) from the digestive tube of hydromorphic earthworms in the Cameroonian coastal zone

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Summary

Based on morphological, morphometric and infraciliature criteria, as evidenced by the ammoniacal pyridinated silver carbonate technique and 4',6-diamidino-2-phenylindole (DAPI) staining, we contribute to the knowledge of four new ciliated protozoa species from the digestive tract of earthworms (Oligochaete: Glossoscolecidae), collected on the banks of the Nkam River (Cameroon). These new species belong to the Class Armophorea, the genera *Nyctotherus* and *Nyctotheroides*. In the genus *Nyctotherus*, three species are described: *N. nkamensis* n. sp. (ovoid, 85–138 µm × 52–115 µm), *N. emini* n. sp. (ovoid, 66–185 µm × 50–95 µm), and *N. renimorphus* n. sp. (kidney form, 125–220 µm × 75–180 µm). They have in common the presence of an apical secant system on their right side. In the genus *Nyctotheroides*, the species *N. ndogbeleensis* n. sp. (elongated form, 75–120 µm × 25–40 µm) is characterized by the existence of three secant systems on both sides: an apical secant system on the right side and two secant systems (the apical and the caudal) on the left side. Contrary to known congeners, the species described in this study have a very pronounced polymorphism.

Key words: Ciliophora, earthworms, Nkam, *Nyctotherus, Nyctotheroides*

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Introduction

Protists are the dominant eukaryotes in the biosphere where they play key functional roles. Protozoan ciliates constitute a branching of the protists widely distributed in the most diverse biotopes, both in the fresh and salt waters as well as in the bodies of many animals. Free-living ciliates are either pelagic and planktonic (Sanchez Rodriguez et al., 2011; Santoferrara and McManus, 2017) or littoral (Wang et al., 2009; Doherty et al., 2010). They are either attached to algae and aquatic metazoans (Šimek et al., 2016; Sparvoli et al., 2018) or float on the bottom of dead leaves or in the middle of algal agglomerations (Šimek et al., 2016). Some are catharobes and live in clear water, clean and well oxygenated; others are more or less saprobionts, common in putrid waters that are very changable 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 results have shown that there is a huge biomass of ciliates in soil and sediments in edaphic form (Dong et al., 2016; Li et al., 2018). Ectoparasites forms have been found in marine molluscs (Xu et al., 2000; Xu and Song, 2008). In Cameroon, the commensal forms of ciliated protists were found in the rectal bulb of the Batrachians and in the digestive tract of the Oligochaete Annelids (Fokam et al., 2013; Fokam et al., 2014; Nana et al., 2018). This category of protists, identified in the digestive tract of oligochaetes, is very variable and can be classified in three groups. The first group is the Clevelandellida that have a buccal apparatus housed in the anterior pole of the cell (Ngassam, 1983; Fokam et al., 2014). The second group, Hysterocinetidae, 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., 2012). The third group, Astomes, are ciliates without buccal apparatus (Nana et al., 2010; Fokam et al., 2012; Obert and Vďačný, 2021). The group, which interests us in this study, is the Clevelandellida that colonize and cohabit the rectal bulb of the Batrachians, but also are found in the digestive tract of oligochaetes Glossoscolecidae and Megascolecidae. Knowledge about the diversity of this group of ciliated protozoa remains rudimentary. The existing data are those of Ngassam (1983), Affa'a et al. (2004), and Fokam et al. (2014). Based on morphological, morphometric and infracilliary analyses, our study aims at contributing to the knowledge of biodiversity of this poorly known and largely underestimated protozoan group.

Material and methods

Study region, collection and identification of earthworms

Earthworms were collected on the banks of the Nkam River in Ndogbélé (4°45'11''N; 9°96'64''E) at Yabassi, the administrative zone of Littoral Region in Cameroon (Fig. 1). This locality extends on a surface area of approximately 3080 km^2 between 4°27' and 4°40' of latitude North and between 9°57' and 10°10' of longitude East. This area is one of the least populated districts in the Littoral region with 4.77 inhabitants per km^2. The 85% of the surface area are occupied by a dense primary forest composed of large trees. Yabassi is watered by an equatorial climate of the Guinean type and the Cameroonian coastal sub-type. The climate is of two seasons, including a long rainy season, from March to November, with rainfall generally ranging between 4000 mm and 5000 mm per year, and a short dry season, from December to February. Air temperatures vary between 23 °C to 33.5 °C (Suchel, 1972).

Worms were identified as belonging to the family Glossoscolecidae, the genus *Alma*, according to the keys presented by Sims and Gerard (1999).

Collection and staining 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

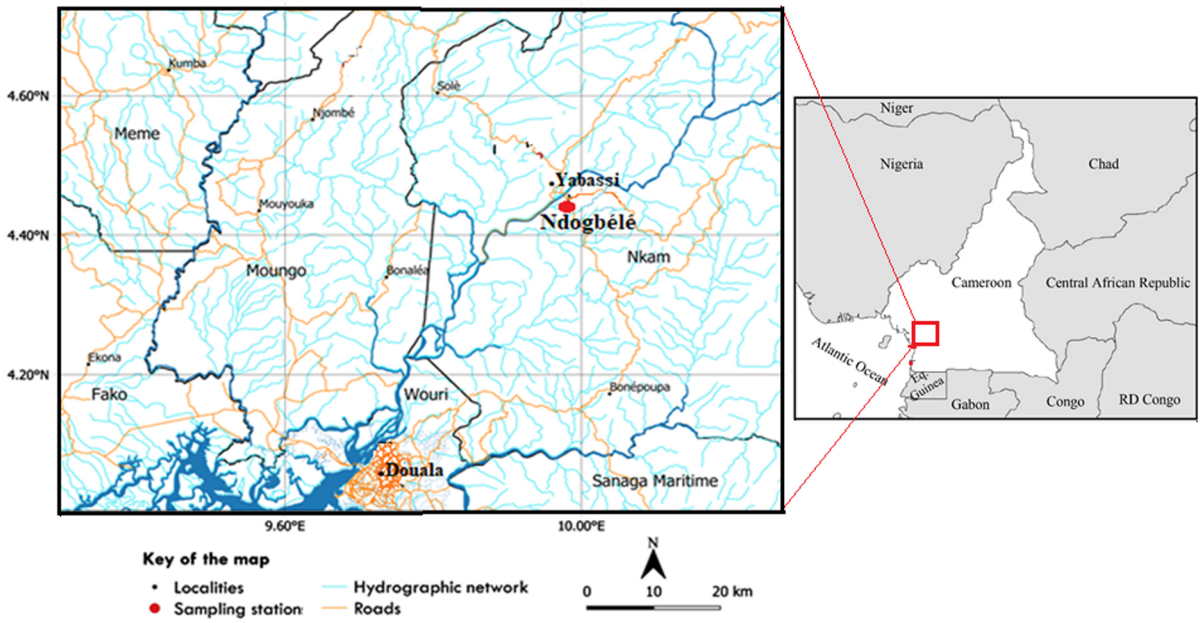

Fig. 1. Collection site for earthworms.

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 30 stained cells of each species. Abundance and frequency of infestation was obtained from the 30 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. Ciliate classification follows Lynn (2008).

Results

Taxonomic description

Phylum Ciliophora Doflein, 1901
Sub-phylum Intramacronucleata Lynn, 2008
Class Armophorea Lynn, 2008
Order Clevelandellida de Puytorac and Grain, 1976
Family Nyctotheridae Amaro, 1972
Genus *Nyctotherus* Leidy, 1849

In this genus, while grouping ciliates characterized by the presence of an apical secant system on their right side, we found three new species.

***Nyctotherus nkamensis* n. sp.**

General morphology and morphometry (Table 1). *Nyctotherus nkamensis* n. sp. cohabits with the Hysterocinetidae ciliates in the midgut of earthworms, *Alma nilotica* and *A. emini*. On average, there are 10 cells of ciliates per infested worm. The cells are ovoid, with the posterior pole less rounded than the anterior pole (Fig. 2). Cell measurements are 85-138 μm in length and 52-115 μm in width. The macronucleus has an irregular shape measuring 20-25 μm in length and 10-14 μm in width, located in the first half of the cell and arranged obliquely towards the dorsal side. This macronucleus is surmounted by a spherical micronucleus of about 3 μm in diameter. The buccal apparatus consists of a peristome almost as long as the infundibulum. The peristome starts behind the apex and continues along a curvilinear trajectory. The infundibulum, very characteristic, is heart-shaped. The adoral fringe has an average of 22 adoral fibers at the peristome and 33 – at the infundibulum.

Infraciliature. There are 95-110 kineties unevenly distributed on the two lateral faces of the ciliate. On the right side, 45-50 tightly packed kineties cover the cell. The apical secant system is well marked, the preoral one is very well developed and is characterized by the presence of short ciliary striae (Fig. 2, C). On the left side, there are 35-40 regularly spaced kineties (Fig. 2, D).

Table 1. Main characteristics of the new and some known species of the genus *Nyctotherus*.

	Nyctotherus nkamensis n. sp.	*Nyctotherus emini* n. sp.	*Nyctotherus renimorphus* n. sp.	*Nyctotherus ngassami* Fokam et al., 2014	*Nyctotherus hoyoi* Tuzet, Manier et Jolivet, 1957	*Nyctotherus ovalis* Leidy, 1850
Cell form	Ovoid	Ovoid	Kidney	Kidney	Globular, Ovoid	Ovoid
Cell length (µm)	85-138	66-185	125-220	153-269	85-280	57-185
Cell width (µm)	52-115	50-95	75-180	102-148	48-230	48-100
Kineties left side	35-40	30-35	39-55	32-43	43-45	54-61
Kineties right side	45-50	32-38	45-60	32-43	43-45	55-62
Macronucleus length (µm)	20-25	23-27	32-36	16-31	37	40
Macronucleus width (µm)	10-14	12-16	12-17	36-53	20	15
Micronucleus diameter (µm)	3-4	3-4	3-5	3-5	4-5	2-3
Peristome (fibers)	22	36	55	29	30	25
Infundibulum (fibers)	33	23	20	48	50	34
Frequency (%)	14	12	10	–	–	–
Average abundance	10±3	7±1	15±5	–	–	–
Type host	*Alma nilotica* and *A. emini* (Earthworms)	*Alma emini* (Earthworms)	*Alma nilotica* and *A. emini* (Earthworms)	*Eupolytoreutus* sp. (Earthworms)	*Spirostreptus virgalor*, *Spirostreptus multisulcalus*, *Ophislreplus digitulatus occiduus*, *Scaphiostreptus* *Aculiconus* (Myriapods diplopods)	*Blatta orientalis* (Insect)
Type locality	Ndogbélé (Littoral Cameroon)	Ndogbélé (Littoral Cameroon)	Ndogbélé (Littoral Cameroon)	Bambili and Bambui (North-West Cameroon)	Zaïre (Democratic Republic of Congo) and Maboké (Central African Republic)	Moroccan strain in breeding in Rennes

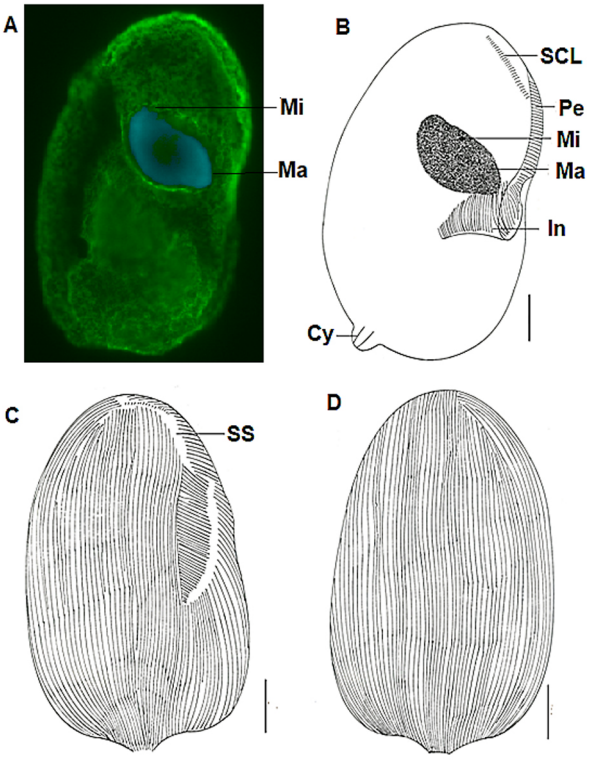

Fig. 2. *Nyctotherus nkamensis* n. sp. A – General morphology after double staining by DAPI (blue) and immunofluorescence microscopy (IF) using FITC-conjugated anti-tubulin antibody (green); B – general morphology (drawings after silver staining); C – ciliature of the ventral face; D – ciliature of the dorsal face. *Abbreviations*: Cy – cytopyge, In – infundibulum, Ma – macronucleus, Mi – micronucleus, Pe – peristome, SCL – short ciliary lines, SS – secant system. Scale bars: 20 μm.

Diagnosis. Commensal of the digestive tract of *Alma nilotica* and *A. emini*; ovoid cell with the posterior pole less rounded than the anterior pole: 85-138 ×52-115 μm; 95-110 kineties unevenly distributed on both sides; peristome: 22 fibers; infundibulum: 33 fibers; frequency: 14%; average abundance: 10 cells per infested worm; locality of collection of the host: Ndogbélé at Yabassi.

Type host. Hindgut in earthworms *Alma nilotica* and *A. emini*.

Type locality. Ndogbélé (4°45'11''N; 9°96'64''E) at Yabassi, Littoral Region, Cameroon.

Etymology. The species is named "nkamensis" in reference to the "Nkam" river, place of collection of the hosts.

Type material. Slides of the holotype (MNHN-IR-2017-0002) are deposited to the protist collection of the National Museum of Natural History, Paris, France.

Remarks. *Nyctotherus nkamensis* n. sp. differs from the other ovoid-shaped species by the presence of a caudal secant system and a somewhat rectilinear disposition of the buccal apparatus. Although the general morphology of *N. nkamensis* reminds the one of *N. ngassami* Fokam, Nana, Ngassam, Bricheux, Bouchard, Vigues and Sime-Ngando, 2014 and the ovoid form of *N. hoyoi* Tuzet, Manier and Jolivet, 1957, the area occupied by the infundibulum differs in these two latter species. Beside, *N. dupouyi* Ngassam, 1983 and *N. hoyoi* present a massive macronucleus hiding the micronucleus while in *N. nkamensis*, the macronucleus is crescent in shape with a micronucleus situated anteriorly in it concavity.

Nyctotherus emini n. sp.

General morphology and morphometry (Table 1). *Nyctotherus emini* n. sp. lives together with *Ptychostomum elongatum* Njiné and Ngassam, 1993 and *Proptychostomum commune* Ngassam and Grain, 1997 (Hysterocinetidae) in the midgut of earthworms *Alma emini*. The cell is ovoid, with the anterior pole more rounded than the posterior pole (Fig. 3). It measures on average 110 μm (66-185 μm) in length and 75 μm (50-95 μm) in width. The long macronucleus, located close to the anterior pole of the cell, is transversal. It measures on average 14 μm in length and 25 μm in width, and is surmounted by a micronucleus averaging 3 μm in diameter. The peristomal gutter starts above the apex and is 35 μm long. The infundibulum, slightly curved, is 24 μm in length; it opens at the level of the equatorial plane. The adoral fringe has on average 36 membranelles at the peristome and 23 – at the level of the infundibulum. Cytopyge is a well-marked slit.

Infraciliature. The somatic ciliature consists of 62-73 kineties covering the cell. There are 32-38 kineties arranged longitudinally on the right side where the secant system is well marked (Fig. 3, C). On the left side, 30-35 kineties are arranged obliquely (Fig. 3, D).

Diagnosis. Commensal of the digestive tract of *Alma emini*; ovoid cell with anterior pole more rounded than the posterior pole: 66-185×71-76 μm; 50-95 kineties evenly distributed on both sides; peristome: 36 fibers; infundibulum: 23 fibers; frequency: 12%; average abundance: 7 cells per infested worm; locality of collection of the host: Ndogbélé at Yabassi.

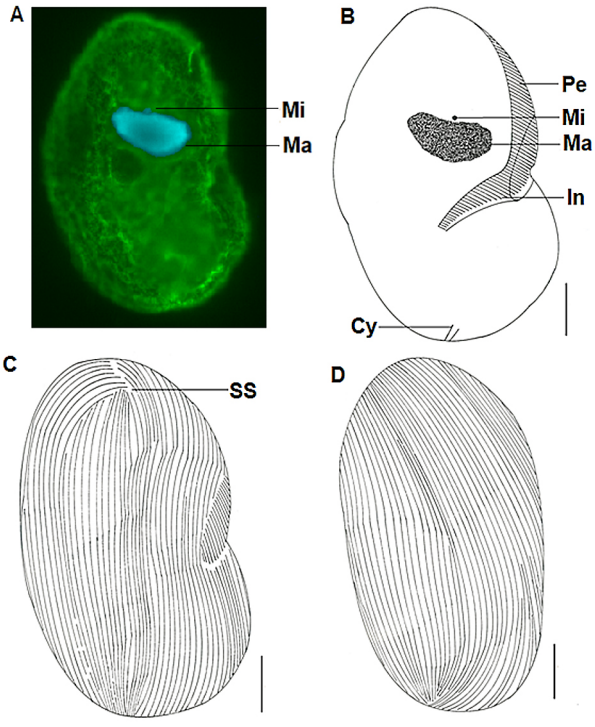

Fig. 3. *Nyctotherus emini* n. sp. A – General morphology after double staining by DAPI (blue) and immunofluorescence microscopy (IF) using FITC-conjugated anti-tubulin antibody (green); B – general morphology (drawings after silver staining); C. – ciliature of the ventral face; D – ciliature of the dorsal face. *Abbreviations*: Cy – cytopyge, In – infundibulum, Ma – macronucleus, Mi – micronucleus, Pe – peristome, SS – secant system. Scale bars: 20 μm.

Type host. Midgut, in earthworms *Alma emini*.

Type locality. Ndogbélé (4°45'11''N; 9°96'64''E) at Yabassi, Littoral Region, Cameroon.

Etymology. The species is named "emini" in reference to the specificity of the host.

Type material. Slides of the holotype (MNHN-IR-2017-0004) are deposited to the protist collection of the National Museum of Natural History, Paris, France.

Remarks. By its general morphology, *Nyctotherus emini* n. sp. could at first sight be confused with *N. ovalis* Leidy, 1850, if it were not for the number and very tight arrangement of the kineties on its two faces. This species differs from *N. hoyoi* and *N. dupouyi* by its slightly curved infundibulum and the presence of oblique cinetia on the left side.

Nyctotherus renimorphus n. sp.

General morphology and morphometry (Table 1). This ciliate is commensal of the digestive tract of *Alma nilotica* and *A. emini* where it cohabits the midgut and hindgut with the Hysterocinetidae of the genus *Ptychostomum* Stein, 1860. This species has a frequency of 10% and an average abundance of 15 ciliates per worm. The cell is kidney-form with the anterior pole more rounded than the posterior pole (Fig. 4). It averages 185 μm (125-220 μm) in length and 98 μm (75-180 μm) in width. The macronucleus is irregularly shaped and obliquely arranged, straddling the first and second upper thirds of the cell. It measures on average 34 μm in length and 15 μm in high. The micronucleus, located above the macronucleus near the right side, measures between 3 and 5 μm in diameter. The buccal apparatus is always constituted by a peristome and an infundibulum. The peristome begins near the right face; it is 80 μm in length on average and made up of 55 adoral organelles. The infundibulum carries about 20 fibers. The cytopharynx is curvilinear. The cytopyge is presented by a well-marked V-shaped piece.

Infraciliature. The somatic ciliature consists of 95-110 kineties unevenly distributed on the two la-teral faces of the ciliate (Fig. 4, C-D). On the right side, these kineties are less dense and arranged longitudinally. On the left side, they are more dense and arranged obliquely.

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

Type locality. Ndogbélé (4°45'11''N; 9°96'64''E) at Yabassi, Littoral Region, Cameroon.

Etymology. The species is named "*renimorphus*" in reference to the kidney shape of the cells.

Type material. Slides of the holotype (MNHN-IR-2017-0006) are deposited to the protist collection of the National Museum of Natural History, Paris, France.

Remarks. *Nyctotherus renimorphus* n. sp. is similar in its general somatic ciliature to *N. neocurtillae* Carini, 1938, a commensal of *Neocurtilla hexadactyla* Earl, 1972, and *Neocurtilla alpha* Earl, 1972, a commensal of the hindgut of *Gryllotalpa gryllotalpa*. However, it differs from them by the reniform morphology and its V-shaped cytopyge, bringing our ciliate closer to *Nyctotherus hoyoi* Tuzet, Manier and Jolivet, 1957. The described ciliate is morphologically close to *Pronyctotherus dragescoi* Albaret and

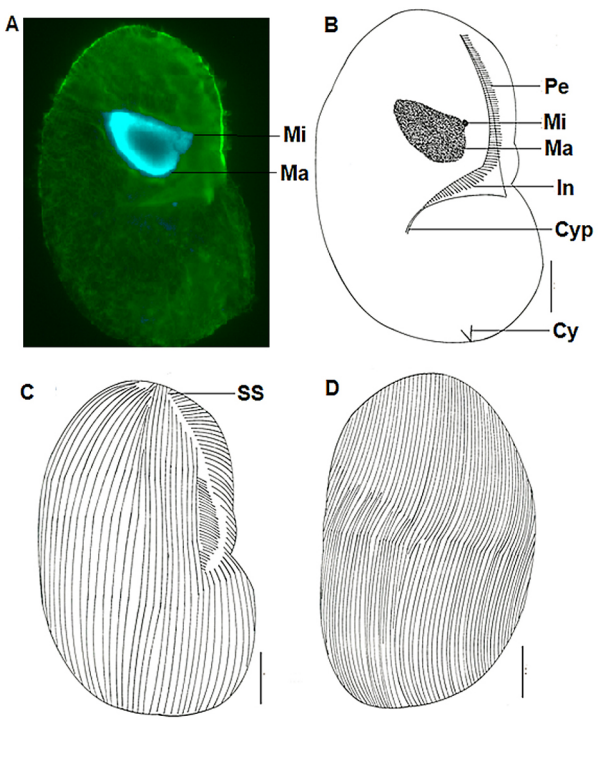

Fig. 4. *Nyctotherus renimorphus* n. sp. A – General mor-phology after double staining by DAPI (blue) and immunofluorescence microscopy (IF) using FITC-conjugated anti-tubulin antibody (green); B – general morphology (drawings after silver staining); C. – ciliature of the ventral face; D – ciliature of the dorsal face. *Abbreviations*: Cy – cytopyge, In – infundibulum, Ma – macronucleus, Mi – micronucleus, Pe – peristome, SS – secant system. Scale bars: 20 μm.

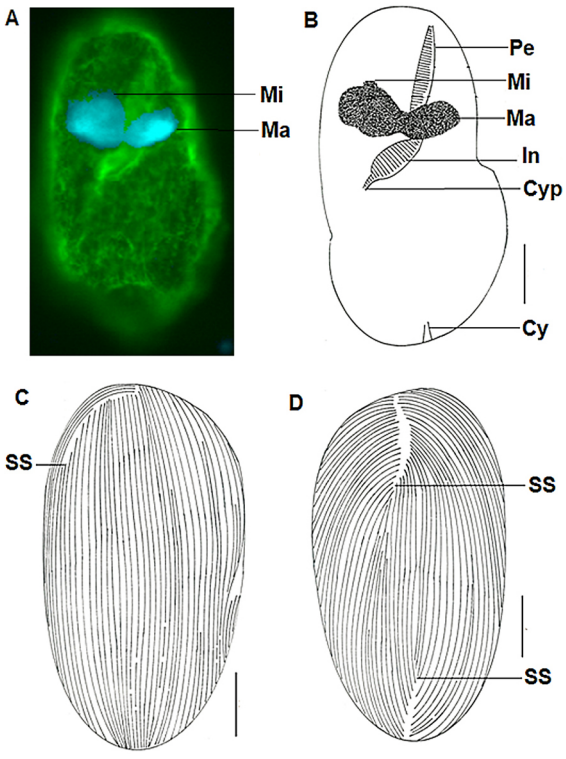

Fig. 5. *Nyctotheroides ndogbeleensis* n. sp. A – General morphology after double staining by DAPI (blue) and immunofluorescence microscopy (IF) using FITC-conjugated anti-tubulin antibody (green); B – general morphology (drawings after silver staining); C. – ciliature of the ventral face; D – ciliature of the dorsal face. *Abbreviations*: Cy – cytopyge, In – infundibulum, Ma – macronucleus, Mi – micronucleus, Pe – peristome, SS – secant system. Scale bars: 20 μm.

Njiné, 1975, with the difference that in the latter, the somatic ciliature is dense. It presents an apical secant system on the right side, which is a typical character of *Nyctotherus*.

Genus *Nyctotheroides* Albaret, 1975

Nyctotheroides groups the ciliates characterized by the existence of three secant systems on their two faces (Albaret, 1975): on the right face, we note an apical secant system and on the left face – two secant systems (one apical and the other caudal). In this genus, we found a new species.

***Nyctotheroides ndogbeleensis* n. sp.**

General morphology and morphometry (Table 2). This ciliate is commensal in the digestive tract of *Alma nilotica* and *A. emini* collected at Ndogbélé where it cohabits in the hindgut with the Hysterocinetidae ciliates of the genus *Ptychostomum* and *Preptychostomum* de Puytorac, 1968. *Nyctotheroides ndogbeleensis* n. sp. has a frequency of 25% and an average abundance of 23 ciliates per worm. The cell is elongate and averages 95 μm (75-120 μm) in length and 32 μm (25-40 μm) in width (Fig. 5). The constriction of the right side is more or less marked. The macronucleus is bilobed and forms two dense asymmetric masses located perpendicular to the curvature of the peristome. It measures 15-22 μm in length and 7-10 μm in width. The cytopyge, a narrow and oblique channel, opens on the ventral side of the posterior pole of the cell. The buccal apparatus comprises on average 45 adoral fibres.

Infraciliature. The somatic kineties are almost equally distributed on the two lateral faces (Fig. 5,

Table 2. Main characteristics of the new and some known species of the genus *Nyctotheroides*.

	Nyctotheroides ndogbeleensis n. sp.	*Nyctotheroides chiromantisi* Albaret, 1975	*Nyctotheroides njinei* Albaret, 1975
Cell form	Elongated	Kidney	Kidney
Cell length (µm)	75-120	148-181	95-125
Cell width (µm)	25-40	72-89	59-81
Kineties left side	10-15	45-50	32-36
Kineties right side	25-30	40-45	32-36
Macronucleus length (µm)	15-22	43	36
Macronucleus width (µm)	7-10	18	17
Micronucleus diameter (µm)	2-4	5-6	4-5
Peristome (fibers)	30	65	35-40
Infundibulum (fibers)	22	70	85-95
Frequency (%)	25	100	60
Average abundance	23 ± 6	–	–
Type host	*A. nilotica* and *A. emini* (Earthworms)	*Chiromantis rufescens* (Amphibians)	*Phrynobatrachus batesi* (Amphibians)
Type locality	Ndogbélé (Littoral Cameroon)	Maboké (Central African Republic)	Cameroon

C-D). The right side bears 25-30 kineties; 10-15 of these kineties form, together with the single dorsal kineties that terminate above the peristome, a right dorsal apical secant system. The left lateral side shows a reduced preoral secant system, formed by the confrontation of left dorsal kineties and the ends of right ventral kineties. The left apical secant system is formed by the chevron-like confrontation of 3 to 4 latero-ventral kineties at the end parallel to the peristomal groove and as many lateral kineties. This secant system is almost median (Fig. 5, D).

Diagnosis. Commensal of the digestive tract of *Alma nilotica* and *A. emini*; elongated cell: 75-120 µm Ч 25-40 µm; bilobed macronucleus forming two dense dissymmetrical masses: 15-22×7-10 µm; buccal apparatus: 45 adoral fibers; frequency: 25%; mean abundance: 23±5 ciliates per infested worm; locality of host collection: Ndogbélé in Yabassi.

Type host. Hindgut, earthworm (*Alma emini* and *A. nilotica*).

Type locality. Ndogbélé (4°45’11’’N; 9°96’64’’E) at Yabassi, Littoral Region, Cameroon.

Etymology. The species is named “ndogbeleensis” in reference to Ndogbélé, the collection place of the hosts of the ciliates.

Type material. Slides of the holotype (MNHN-IR-2017-0007) are deposited to the protist collection of the National Museum of Natural History, Paris, France.

Remarks. *Nyctotheroides ndogbeleensis* n. sp. has a general appearance quite similar to that of *N. chiromantisi* Albaret, 1975, a commensal of the rectum of *Chiromantis rufescens*, of *N. njinei* Albaret, 1975, a commensal of the rectum of *Phrynobatrachus batesi,* and of *N. faberi* Carini, 1939, a parasite of *Hyla faber*. It differs, however, by the bilobed shape of its macronucleus located perpendicular to the anteroposterior axis of the cell. Additionally, it differs notably from *N. tejerai* Pinto, 1926 (a commensal of the rectum of *Leptopelis notatus*) by its elongated shape, but also by its peristome, which is clearly longer than the infundibulum and located behind the nuclear apparatus.

Discussion

The described ciliated protists, collected for the first time in the digestive tract of oligochaetes of the genus *Alma*, are thus distinguished from known species by various characters. With respect to their morphology and the pattern of their ciliature (absence of a postoral secant system), the first three species described above belong to the genus *Nyctotherus* as originally described by Leidy (1849) and some later authors (Grassé, 1928; de Puytorac and Öktem, 1967; Albaret and Njiné, 1975; Ngassam, 1983; Fokam et al., 2014).

Ecologically, *Nyctotherus nkamensis* n. sp., *Nyctotherus renimorphus* n. sp., and *Nyctotheroides ndogbeleensis* n. sp. proliferate mostly in the hindgut of earthworms. It has been clearly demonstrated that the hindgut portion of the digestive tract of *Alma emini* and *A. nilotica* is alkaline, less rich in mineral elements and less viscous than the foregut (Nana et al., 2014). It is also noted that in the foregut,

the ciliated mesofauna is very mobile and active unlike in the hindgut populated by Hysterocinetidae (Nana et al., 2014). De Puytorac and Mauret (1956) had already shown a stratification of Astome, endocommensal ciliates of the digestive tract of *Allolobophora savignyi*, related to physicochemical variables. The cohabitation of *Nyctotherus emini* n. sp. and some Hysterocinetidae in the midgut confirms the theory of "species association", characterized by a distribution in distinct compartments. Indeed, species that enter into various associations, either ecological or trophic, in various compartments, inevitably show similar reactions to variations in the conditions of their environment (Pinel-Alloul et al., 1990).

Let us note that the variation in abundance is not the only characteristic fact of the Nyctotheridae of the digestive tract of earthworms. Gohre (1943) highlighted an analogous fact for the three species of *Gregarina*, parasites of the larva of the insect *Tenebrio molitor*. Adam (1951), studying the distribution of ciliates in the large intestine of the horse, showed that two successive faunas could be recognized there. One, with *Blepharocorys uncinata* and *Cycloposthium bipalmatum*, was characteristic of the cecum and colon up to the pelvic curvature; the other, with *Bundleia postciliata* and *Blepharocorys curvigula*, was present from there to the rectum. In all the similar cases, the variation of abundance and the stratification of the described species would obviously depend on the varied physico-chemical and biotic conditions, successively offered by the biological environment to the ciliates, in relation to the needs of each of them. In such a seriation, the external environment would intervene additionally, promoting a competition between the various groups of protists. If there is such an antagonism in the case of the ciliates that are the objects of this study, its importance does not seem considerable under normal conditions. Indeed, when one of the species is missing, the place it would occupy in the portion of the digestive tract generally remains free. These observations do not exclude the possibility of such competition at the origin of the settlement of the ciliates in the digestive tract. If this was so, as is probable, selection might have subsequently sorted out the forms so closely adapted to certain portion of the digestive tract as to render the antagonism between them unnecessary.

Schmidt with coauthors (2010, 2011) showed that the enzymes (cellulase, chitinase, and hydrogenase) were nearly absent in the pharynx, esophagus, crop, and gizzard of many worms, whereas they reach their highest concentration in the anterior part of the intestine. While the Hysterocinetidae that populate the hindgut seem to be able to do without such diastases, it is rather possible that the Nyctotheridae have a great need for them. A similar case has been proved in *Diplodinium* and *Entodinium* of the rumen of ruminants: the representatives of the former have cellulase and cellobiase that enable them to transform cellulose into glucose, whereas species of the latter genus lack them (Tracey, 1951).

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