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## Description of two new species of Sicutophoridae and Nyctotheridae (Heterotrichina), endocommensal in the rectal ampulla of *Bufo regularis* (Amphibia: Anura) from the Northwest of Cameroon

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### Summary

*Prosicutophora cyclostomatus* n. sp. and *Nyctotheroides bambuiensis* n. sp. are two new ciliate species isolated from the rectums of *Bufo regularis* collected in the vicinity of Bambili and Bambui. The morphological characteristics of these Protozoa are described and their relation to similar species is discussed.

**Key words:** Bambili, Bambui, *Bufo regularis*, *Nyctotheroides bambuiensis* n. sp., *Prosicutophora cyclostomatus* n. sp.

### Introduction

The amphibian digestive tract harbors in its posterior part a diverse fauna of ciliated Protozoa, among others including a great number of heterotrichs that have been examined by many investigators (Mackinnon and Hawes, 1961; Albaret, 1975; de Puytorac and Öktem, 1967; Shorr et al., 1990; Vojtkova and Roca, 1993).

The first study of heterotrich ciliates in central Africa was conducted by de Puytorac and Öktem

(1967) on nyctotherans of the genera *Nyctotherus* (Leidy, 1849) and *Prosicutophora* (de Puytorac and Öktem, 1967) collected in anuran amphibians from Gabon. In Cameroon, similar studies have been carried out by Affa'a (1980, 1988), Affa'a and Amiet (1994). However, works on the nyctotheran ciliates in amphibians in Cameroon are scarce. For this reason, surveys and comparisons of the ciliate fauna of the amphibian digestive system from the hosts collected in different regions should provide new information about the ciliated Protozoa

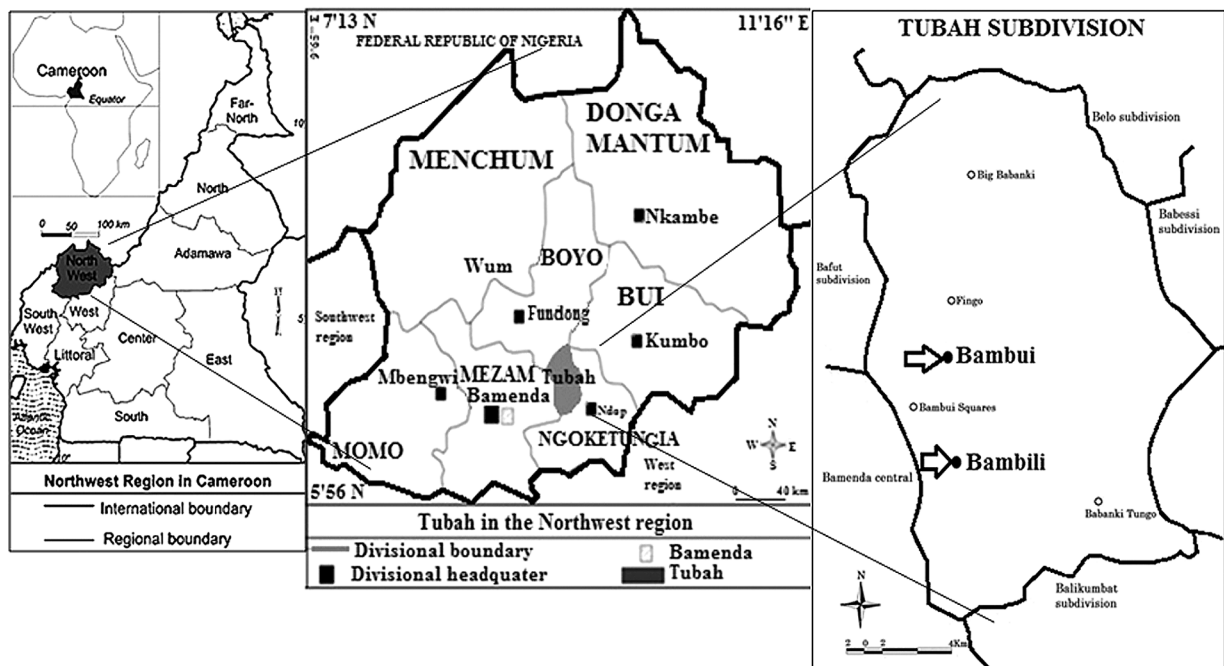


Fig 1. The study area (Helvetas Cameroon, 2002; modified, 2013).

fauna of Cameroon. Here we describe two new species, *Prosciuophora cyclostomatus* n. sp. and *Nyctotheroides bambuiensis* n. sp., obtained from the *Bufo regularis* in Tubah district of the Northwest region of Cameroon.

## Material and methods

Our study areas in the north west of Cameroon (Bambili and Bambui in Tubah district) were situated within the latitude  $5^{\circ} 59' 0''$  N, and the longitude  $10^{\circ} 15' 0''$  E, at an altitude of about 1,350 m (Fig. 1).

Rectal ampulla samples were dissected from *Bufo regularis* collected in the vicinity of Bambili and Bambui in 2012. Portions of the rectal ampulla were opened either in physiological Ringer's solution or in commercial mineral water (Volvic™ in France or Supermont™ in Cameroon). Ciliates were observed under a stereomicroscope and the actively swimming ones were selected by micropipette and first examined *in vivo*. Then the ciliates were stained with Fernandez Galiano's pyridinated silver impregnation (1994). All of the cell measurements were made with a calibrated ocular micrometer. To obtain morphometric data, groups of 30 ciliates of each species were examined. Drawings of the cells were made 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

### *PROSCIUOPHORA CYCLOSTOMATUS* SP. N.

This species occurs in the rectal ampulla of *Bufo regularis* where it lives in cohabitation with various species of the genera *Nyctotheroides*, *Opalina* and *Protoopalina*.

**Diagnosis.** Size about  $101 \times 74 \mu\text{m}$ . Body ovoid. Anterior pole pointed, posterior pole rounded. Peristome starts near the anterior pole as a spiral, extends posteriorly, and joints the infundibulum in the equatorial part of the body. Infundibulum bends inwards forming nearly one spire in the central part of the cell and ends with a forked cytopharynx. Macronucleus elongated, with a pointed posterior end and a wide anterior part, located in the anterior half of the body, close to the infundibulum. Globulous micronucleus surmounts the macronucleus. The cilia pattern shows at the poles two suture lines extending both to the left and right surfaces and forming two secant systems.

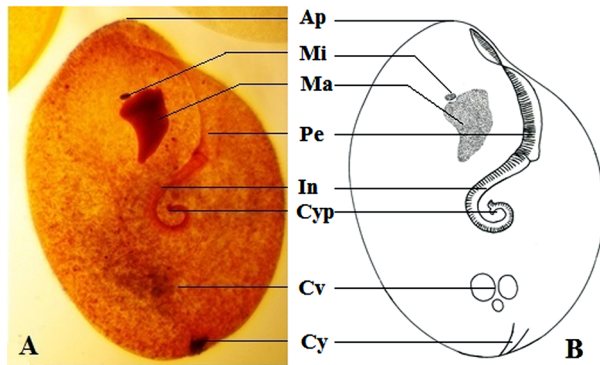
**Type host.** *Bufo regularis* Reuss, 1833.

**Type locality.** Bambili and Bambui, Tubah District, Bamenda, Cameroon.

**Habitat.** Rectal ampulla.

**Type specimens.** Slides with paratypes are deposited at the Department of Biology (Higher Teacher Training College, The University of Bamenda, Cameroon).

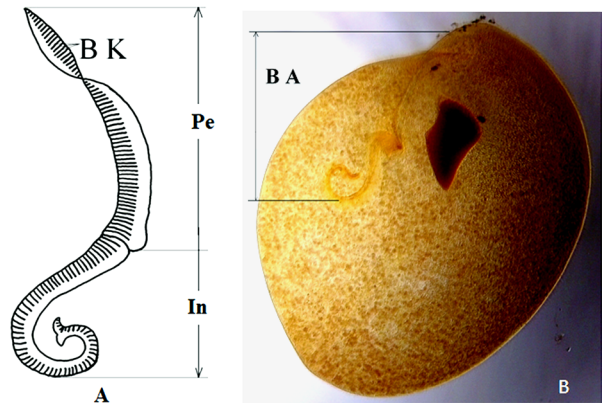
**Description.** The ciliate ovoid with a broadened posterior end; anterior end slightly pointed (Fig.



**Fig. 2.** General morphology of *Prosicuophora cyclostomatus* n. sp. A – light micrograph after silver staining ( $\times 400$ ), B – drawing; Ap – apical pole, Cv – contractile vacuoles, Cy – cytopyge, Cyp – cytopharynx, In – infundibulum, Ma – macronucleus, Mi – micronucleus, Pe – peristome.

2). Body measurements and proportions: length,  $101 \pm 13.4 \mu\text{m}$ ; width,  $74.6 \pm 6.3 \mu\text{m}$  (Table 1). Nuclear apparatus in the anterior part of the body. Macronucleus,  $22.3 \pm 3.49 \times 14.4 \pm 4.2 \mu\text{m}$ , major axis parallel to the axis of the cell. Macronuclear shape, irregular lozenge-like with a tapered posterior end, typically characteristic of the species. Micronucleus spherical, located in the concavity at the anterior side of the macronucleus (Fig. 2).

Buccal apparatus begins externally with a well-developed oblique peristome ( $34.5 \pm 0.1 \mu\text{m}$ ) and continues internally with the infundibulum ( $26.8 \pm 1.4 \mu\text{m}$ ). The terminal part of the infundibulum is prolonged by a spiral cytostome and a forked cytopharynx (Fig. 3A). In most of the specimens studied, the peristome and infundibulum are linked by a joint forming an obtuse angle ( $130.1 \pm 13.4^\circ$ ). However, in a few cases this angle is flattened instead (Fig. 3B). The vestibular opening (oral opening) is



**Fig. 3.** Buccal apparatus (BA) of *Prosicuophora cyclostomatus* n. sp. A – detail, B – linear disposition ( $\times 400$ ); BK – buccal kineties, In – Infundibulum, Pe – peristome.

close to the equatorial plane of the cell. The buccal kinetosomes form adoral membranelles composed of short striated kineties covering the peristome and the infundibulum.

The 178–206 somatic kineties are arranged in meridian and tight bipolar rows throughout the body. The ciliary topography shows on the right side two suture lines prolonged by secant systems (Fig. 4). The apical suture line is shorter and located in front of the peristome. Posteriorly, the caudal suture line is slightly discrete.

*NYCTOTHEROIDES BAMBUIENSIS* SP. N.

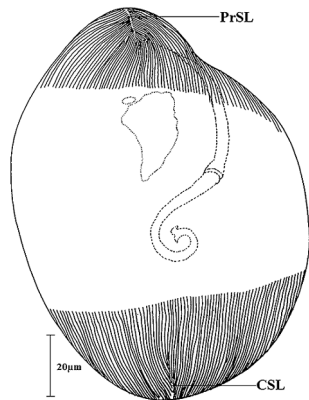
This ciliate co-inhabits with the previous species the rectum of *Bufo regularis*.

**Diagnosis.** Body shape oval. Both poles rounded, but the posterior slightly larger. Peristome begins at a short distance from the apex and extends backward in a continuous circling flat curve leading to the oral opening. Infundibulum, very long, runs laterally in the median plane of the cell, then twists and

**Table 1.** Morphometric characters of *Prosicuophora cyclostomatus* n. sp.

	Cell length ( $\mu\text{m}$ )	Cell width ( $\mu\text{m}$ )	Mn length ( $\mu\text{m}$ )	Mn width ( $\mu\text{m}$ )	Pe length ( $\mu\text{m}$ )	In length ( $\mu\text{m}$ )	MnPA ( $^\circ$ )	AIPe ( $^\circ$ )
Max	140	103	31	22	45	38	48	148
Mean	101	74.6	22.3	14.4	34.5	26.8	41.7	130.1
Min	70	52	15	8	22	17	27	104
SD	13.4	6.3	4.9	4.2	0.1	1.4	0.7	13.4

**Notes.** AIPe – angle infundibulum-peristome in degree, In – infundibulum, Max – maximum, Min – minimum, Mn – macronucleus, MnPA – macronucleus posterior angle in degree, Pe – peristome, SD – standard deviation; N=30.



**Fig. 4.** Ciliary pattern of *Prosciuophora cyclostomatus* n. sp. CSL – caudal suture line, PrSL – preoral suture line.

turns back, its bend reinforced by a monitorium. Posterior part of the infundibulum is prolonged by a cytopharynx. Orientation of the cytopharynx varies. Macronucleus, shaped an overturned kidney, bears a micronucleus in its ventral depression. The somatic kineties are quite homogenous on the two surfaces, their pattern showing two suture lines. Caudal secant systems very reduced or, in some cases, quite absent.

**Type host.** *Bufo regularis* Reuss, 1833.

**Type locality.** Bambili and Bambui, Tubah District, Bamenda, Cameroon.

**Habitat.** Rectal ampulla.

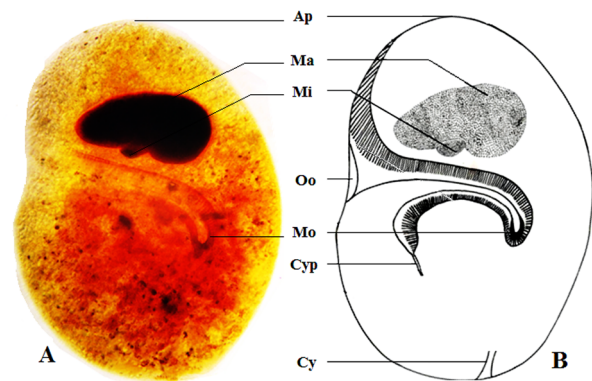
**Type specimens.** Paratype slides are kept in the Department of Biology (Higher Teacher Training College, The University of Bamenda, Cameroon).

**Description.** Body shape oval, the apical part narrower than the bottom. Cell size:  $146.50 \pm 4.24 \mu\text{m}$  long and  $108.90 \pm 24.75 \mu\text{m}$  in the widest part (Fig. 5, Table 2). Left side flattened with a hollow structure constituting an oral groove; right side convex.

**Table 2.** Morphometric characters of *Nyctotheroides bambuiensis* n. sp.

	Cell length (µm)	Cell width (µm)	Mn length (µm)	Mn width (µm)
Max	175	133	68	35
Mean	146.5	108.9	58.1	29.1
Min	60	45	25	11
SD	4.24	24.75	5.66	3.53

**Notes.** Max – maximum, Min – minimum, SD – standard deviation; N=30.



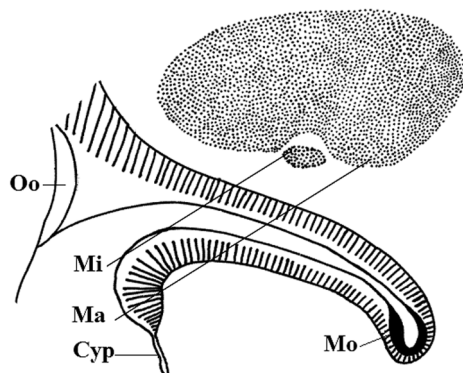
**Fig. 5.** General morphology of *Nyctotheroides bambuiensis* n. sp. A – light micrograph after silver staining ( $\times 400$ ), B – drawing; Ap – apical pole, Cy – cytopyge, Cyp – cytopharynx, Ma – macronucleus, Mi – micronucleus, Oo – oral opening.

The buccal apparatus starts close to the apex with a curved peristome (Figs 5, 6). The peristome is largely opened in the equatorial part of the cell by an oral opening. The oral groove, relatively long, appears as a U-shaped infolding forming the infundibulum (Fig. 6).

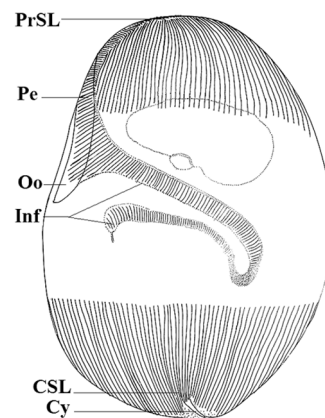
Nuclear apparatus located in the anterior half of the body, above the infundibulum. Macronucleus, kidney-shaped, about  $58.10 \pm 5.66 \mu\text{m}$  long and  $29.12 \pm 3.53 \mu\text{m}$  in the widest part, left side slightly pointed, right side broader. Macronucleus oriented transversally to the cell axis with the convex side facing the top of the body and the flattened side facing the bottom. Micronucleus ellipsoid, located in a depression in the middle of the flattened side (Figs 5, 6).

Buccal apparatus divided into two parts: the peristome and infundibulum. Peristome begins close to the apex on the left lateral face and ends with an oral opening in the mid-body. Infundibulum U-shaped, begins at the oral opening, plunges into the cytoplasm, bends and runs backwards parallel to its anterior part, ends with a cytostome prolonged by a short cytopharynx (Fig. 6). Coiled thick structure, the monitorium, supports the internal base of the infundibulum bend.

Ciliary topography: about 126-162 bipolar kineties, unequally distributed throughout the body. The preoral secant system and the caudal secant system are extensions of the anterior and posterior suture lines respectively. In the axial region of the left side, the confrontation of kineties forms posteriorly, a very short caudal secant system (Fig. 7).



**Fig. 6.** Detail of the buccal and nuclear apparatus of *Nyctotheroides bambuiensis* n. sp. Cyp – cytopynx, Ma – macronucleus, Mi – micro-nucleus; Oo – oral opening.



**Fig. 7.** Ciliary pattern of of *Nyctotheroides bambuiensis* n. sp. CSL – caudal suture line, Cy – cytopynx, Inf – infundibulum, Pe – peristome, PrSL – preoral suture line, Oo – oral opening.

## Discussion and conclusion

According to its general morphology (shape, buccal and nuclear apparatus), the first ciliate described in this work is typical to the genus *Prosciuophora* with *P. basoglui* as a typical species described by de Puytorac and Oktem (1967). The pattern of the kineties is classical, with two secant systems, the preoral and the caudal one. However, the cell described here differs drastically from *P. basoglui*, as it lacks a karyophore (a sort of a sac-like membrane enclosing the macronucleus). Additionally, the infundibulum of *P. basoglui* bears numerous short fibers, distinguishing it from our specimen.

The first specimen also shows some similarities with *Nyctotheroides spirotomatus* examined by Albaret (1975) in terms of morphology (the infundibulum structure). However, our ciliate differs from *N. spirotomatus* in the structure of the nuclear apparatus, notably the absence of karyophore. Presumably, the differences mentioned above may be due to the difference in hosts, since *P. basoglui* has been recorded in *Bufo superciliaris*.

*Nyctotheroides bambuiensis* possesses typical characteristics of the genus *Nyctotheroides* (general morphology, pattern of the buccal and somatic kineties) as stated by Affa'a (1988). This ciliate shares certain features with the species *N. heterotomus* described by Affa'a (1980), namely, the shape of the cell, the position of the oral opening, the form of the buccal apparatus and the nuclear apparatus. However, the presence of a monitorium, a long and curved infundibulum, a voluminous cytostome and a short cytopynx set these two species apart.

The second studied ciliate also resembles *N. cordiformis* Albaret (1975) in the body shape, the position of the nuclear apparatus and its shape, the position of the oral opening, the buccal apparatus, and buccal and somatic ciliature, determining two suture lines (apical and caudal). Alongside with these similarities, there are certain distinctions between our specimen of *Nyctotheroides* and *N. cordiformis*. The oral or vestibular opening, which is in the median part of the cell in *N. bambuiensis*, is closer to the posterior end in *N. cordiformis*. The area occupied by infundibulum in *N. bambuiensis* is larger than in *N. cordiformis*. The curve formed by the infundibulum is wider in *N. cordiformis*; in this species the infundibulum, after passing the middle part of the cell and forming a wide curve, turns to the posterior end. However, in our ciliate, the infundibulum forms a small curve somewhere near the dorsal side of the cell and thereafter turns towards the ventral side of the cell, where it is extended by a voluminous cytopynx. Another distinct feature to discriminate the two species is the presence of a monitorium supporting the curve of the infundibulum in *N. bambuiensis* and its absence in *N. cordiformis*. One of the main distinguishing characters of *N. cordiformis* is the karyophore, which is absent in *N. bambuiensis*.

The differences we observed could be explained by some adaptations or resistance to the environmental factors of the hosts, and from evolutionary perspective, such situation can create adaptive radiation leading to diversification in the same genus. This diversification can also be explained by the difference of hosts, since *N. heterotomus* is

a commensal ciliate of *Hylarana albolaris* and the specimen described here resides in *Bufo regularis*. Besides that, we can hypothesise that morphological differences observed in *N. bambuiensis* may have developed as a climatic adaptation.

## Aknowledgements

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