Turk J Zool
24 (2000) 245-252

The Ciliate Fauna in the Digestive System of *Rana ridibunda* (Amphibia: Anura)-II *Nyctotherus* (Nyctotheridae: Heterotrichida)

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Received: 29.01.1999

Abstract: A new ciliate species, *Nytotherus oektemae* n. sp. and common rectal ciliate of anuran amphibians, *Nyctotherus cordiformis* were isolated from the rectums of Rana ridibunda collected in the vicinity of Van. The morhological characteristics of these Protozoa were described and their similarities and differences were discussed.

Key Words: *Rana ridibunda*, Turkey, Nyctotherans, *Nyctotherus oektemae* n.sp.

Rana ridibunda'nın (Amphibia: Anura) Sindirim Sistem Siliyat Faunası-II *Nyctotherus* (Nyctotheridae: Heterotrichida)

Özet: Van'daki *Rana ridibunda'nın* rektumunda yeni bir siliyat türü, *Nyctotherus oektemae* ve kuyruksuz amfibilerin yaygın rektal siliyatı olan *Nyctotherus cordiformis* izole edilmiştir. Bu iki türün morfolojik özellikleri belirlenerek benzerlik ve farklılıklar tartışılmıştır.

Anahtar Sözcükler: *Rana ridibunda*, Türkiye, Nyctoterler, *Nyctotherus oektemae* n. sp.

Introduction

There is no agreement among researchers about defining and naming individuals belonging to Nyctotherans. *Nytotherus* was first described as an entozoic protist from anuran amphibians and various inevertebrates by Leidy (1-3). In 1928, Grassé classified this genus into two groups depending on the absence and presence of a caryophore: *Nyctotherus* and *Nyctotherioides*. Later, several researchers such as Corliss, Earl, Amaro and Sena gave a list of species of the genera *Nyctotherus* and *Nyctotheroides* (3). The starting point of researchers was to determine if the species possessed a caryophore. Putytorac and Öktem (4) reported that the caryophore structure lack phylogenetic significance. On the other hand, Albaret (2), having described the different ciliary topography of the *Nyctotherus* species, devided these species into genera *Nyctotherus* and *Nyctotheroide*s, and accordingly, prepared a list of these species. However, most researchers still believe that the species belong merely to the genus *Nyctotherus* (1, 2, 4-8).

Nyctotherans associated with anuran amphibians live endocommensally only in the posterior part of the amphibians' digestive tract. Nyctotheran species have been examined by many investigators (3-7, 9-16). In Cameroon, 45 Nyctotherean species were found in 41 anuran species (16). In studies carried out in many countries of Europe, it was also reported that *Nyctotherus cordiformis, Nyctotherus hylae* and *Nyctotherus magnus* exist in different anuran amphibians (6, 7). The first study on Nyctotheran ciliates in Turkey was conducted by Öktem (17). Öktem examined *Nyctotherus cordiformis* and *Nyctotherus hylae* with a light microscope and electron microscope comparatively.

However, there are few publications on the Nyctotheran ciliates in amphibians in Turkey. For this reason, surveys and comparisons of digestive system ciliate fauna of amphibians in different regions should provide information about the Protozoa fauna of Turkey.

The present paper deals with *Nyctotherus* species obtained from the *Rana ridibunda* in Van and includes the description of a new species.

Materials and Methods

Rectal fluid samples were taken from *Rana ridibunda* collected in the vicinity of Van from 1992-1998. Ciliates in Ringer solution selected by micropipette were first examined in vivo. Then, the ciliates were stained with Mayer's hematoxylin, Chatton-L woff's silver

impregnation and Feulgen's nucleal reaction techniques and prepared as permanent slides (18, 19). Specimens were examined by Euromex stereomicroscope, Nikon research microscope and Prior phase-contrast microscope. All of the cell measurements were made with a calibrated ocular micrometer and all measurements given in the study are in micrometers. The original figure in the article was drawn by camera lucida. The system of classification adopted here is that proposed by Levine et al. (8). Comparative data of various morphological characteristics were analyzed by one-way variance analyis using NINITAB software.

Results

Nyctotherus cordiformis Stein, 1862

(Figure 1a, b; Table 1,2)

This well-known species occurs in the rectum of anuran amphibians in addition to *Balantidum* and *Opalina.*

The body is kidney-shaped. Peristome begins anteriorly and then plunges into prolonged internal passage, infundibulum (the funnel-shaped organelle), ends at the cytostome posteriorly; cytopharynx is short. In somatic ciliature, ciliary rows (kineties) are arranged in

Table 1. Comparative data between the two species of *Nyctotherus* (N=25). L. body length; W. body width; ML. macronucleus length; MW. macronucleus width; PL. peristome length; a. distance of the peristome from apex; OO.distance of the oral opening from apex; b. infundibulum part which is perpendicular to the body axis; IL. infudibulum length; c. distance between oral opening and ending point of the ifundibulum. SD. standard deviation; SE. standard error.

	Nyctotherus cordiformis				*Nyctotherus oektemae* n.sp			
Characteristics	Mean	Range	SD	SE	Mean	Range	SD	SE
L	116.70	97.50-132.50	9.65	1.93	117.20	95.00-125.00	6.51	1.30
W	80.20	57.50-95.00	8.38	1.68	74.50	62.50-82.50	5.68	1.14
W/L x 100	68.71	58.97-65.67	4.47	0.89	63.57	56.82-68.89	3.39	0.68
ML	49.80	40.00-65.00	6.29	1.26	45.90	37.50-50.00	3.45	0.69
MW	17.40	12.50-22.50	2.55	0.51	13.00	10.00-15.00	1.44	0.29
MW/ML X 100	35.33	21.74-44.74	5.79	1.16	28.41	22.22-33.33	3.24	0.65
A	5.80	2.50-7.50	1.57	0.31	6.60	5.00-10.00	1.59	0.32
PL	51.20	40.00-57.50	4.57	0.91	59.00	50.00-65.00	3.95	0.79
PL/L	43.93	37.78-50.00	2.91	0.58	50.39	45.83-55.56	2.96	0.59
OO	58.60	45.00-65.00	4.40	0.88	66.00	55.00-70.00	3.54	0.71
OO/L	50.34	45.00-57.14	3.27	0.66	56.36	52.08-60.00	2.15	0.43
B	36.50	25.00-47.50	7.22	1.44	46.70	37.50-55.00	4.88	0.98
b/G x 100	45.77	30.30-63.33	9.18	1.84	62.66	55.56-73.33	4.28	0.86
IL	57.60	40.00-67.50	6.63	1.33	85.30	72.50-97.50	7.61	1.52
C	33.60	15.00-40.00	5.55	1.11	47.50	40.00-52.50	3.23	0.65
C/L x 100	28.85	13.33-40.00	4.72	0.94	40.59	33.33-56.67	2.70	0.54

Table 2. Number of cilary rows of *Nyctotherus cordiformis* (N=16) and *Nyctotherus oektemae* n. sp. (N=30). SD standard deviation; SE. standard error.

	Nyctotherus cordiformis				*Nyctotherus oektemae* n.sp			
Characteristics	Mean	Range	SD	SE	Mean	Range	SD	SE
Left side	37	32-43	3.28	0.82	37	30-42	2.85	0.52
Right side	32	30-50	1.67	0.42	31	28-37	2.07	0.38

longitudinal lines on the dorsal and right sides of the ciliate. But, on the left there is an apical suture (system secan) line in the anterior and a caudal suture line in the posterior. Massive macronucleus is kidney-shaped and carries micronucleus in its posterior depression. There is a contractile vacuole discharging through a small canal called the anal tube to the cytoproct.

Body measurements and proportions are summarized in Table 1 and the number of ciliary rows is given in table 2.

Nyctotherus oektemae n.sp.

(Figure, 2a, b, c, 3a, b; Table 1, 2)

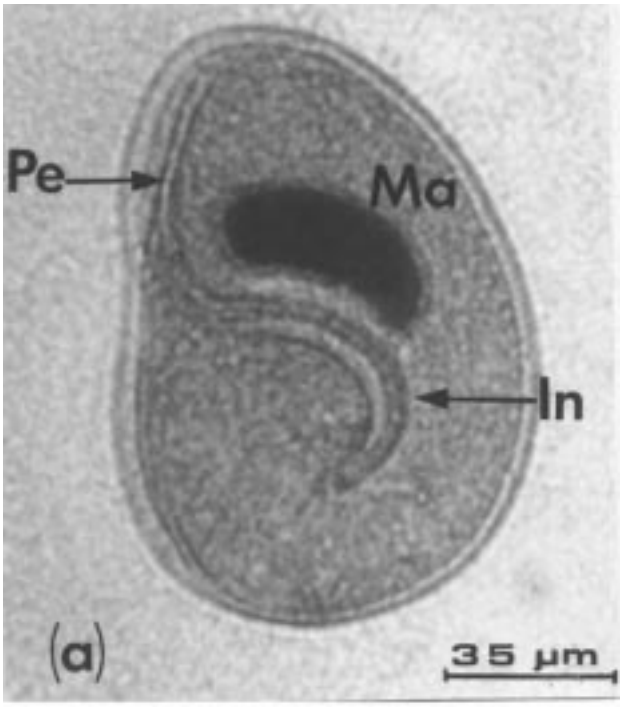
Diagnosis: The body is ovoid. Anterior end is more pointed than the posterior end. The peristome begins near the anterior end of the body and extends nearly straight backwards. After it passes to the middle of the body, it bends inwards, leading into the infundibulum which is shaped like a long curved channel. The area which the curve of infundibulum occupies in the cell is quite large. Infundibulum passes through the middle part of the cell more or less obliquely to the transverse body axis. At this point, the infundibulum makes a wide curvature that almost extends up to the dorsal edge and then runs posteriorly. Macronucleus is located in the anterior half of the body, close to the infundibulum. It is mostly depressed in the middle. This depression is more prominent on the side of the infundibulum. The side facing the ventral is flat. The structure has coarse granules. Right side ciliature of the cell consists of bipolar ciliary rows. On the left side an apical suture runs up to the middle of the cell. Posterior part contains a caudal suture which is shorter and less marked compared with the apical suture line.

Body measurements and proportions are summarized in Table 1 and the number of ciliary rows is given in Table 2.

Description: Since the anterior half is not as wide as the posterior half, a pointed shape is observed in the anterior part. For this reason, the appearance of the body is oval. In the posterior end, the body seems to be composed of two layers placed over one another; but with one of them projecting at the posterior end.

Body length is 117.20 (95.00-125.00) μm, its width is 74.50 (62.50-82.50) μm. Width of the body is 63.57% of its length.

Extended macronucleus is located in the anterior of the body, close to the infundibulum. Its length and width are 45.90 (37.50-50.00) μm and 13.00 (10.00-15.00) μm respectively. Width of the macronucleus is 28.41%



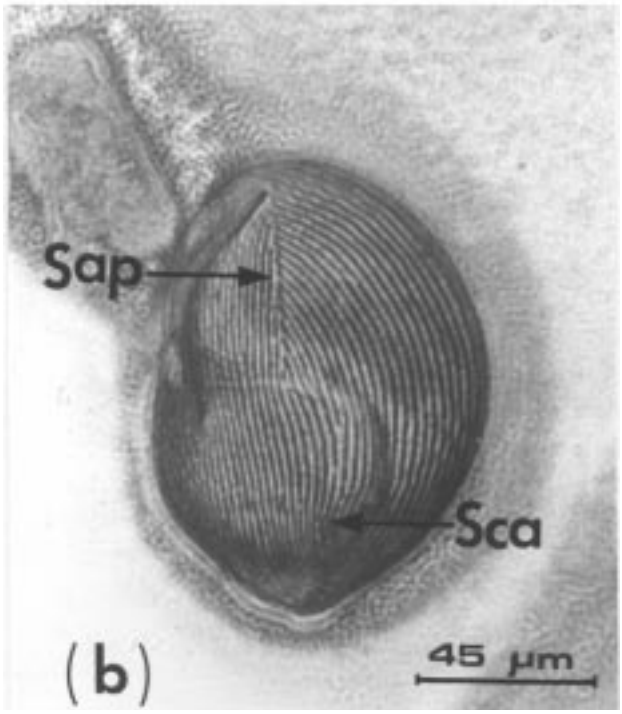


Figure 1. Light micrographs of *Nyctotherus cordiformis.*
a. General morphology (stained Mayer's hematoxylen) b. Left surface ciliature (silver impregnation) Pe. peristome; In. infundibulum; Ma. macronucleus; Sap. apical suture line; Sca. Caudal suture line.

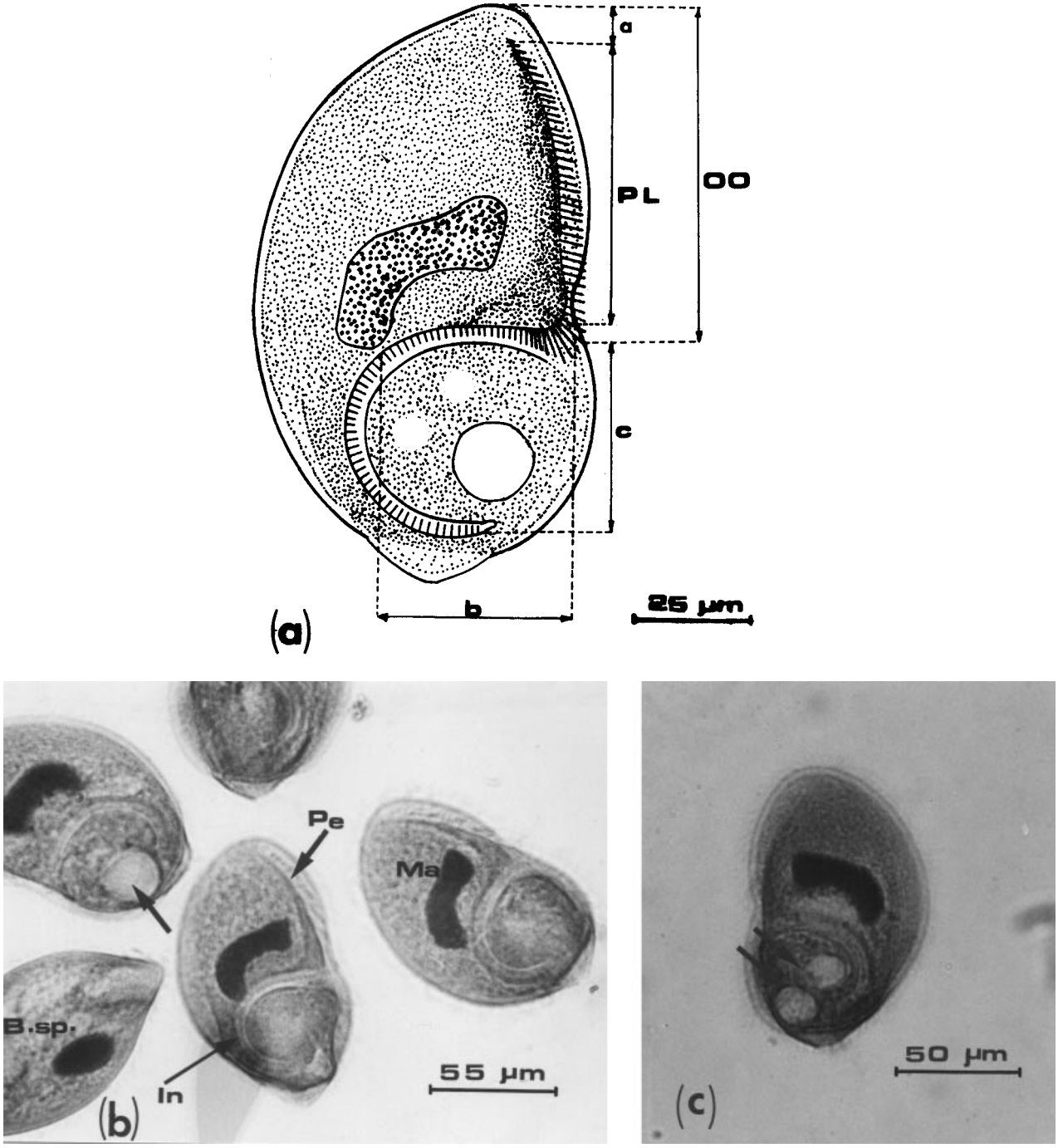


Figure 2a,b,c. Light micrographs and drawing belonging to general morphology of *Nyctotherus oektemae* n. sp. (stained with Mayer's hematoxylen). Pe. peristome; In. infundibulum; ma. macronucleus; Arrows. contractile vacuoles; *B.* sp. Balantidium sp. See table 1 for a, b, c, PL, OO.

of its length. The side facing the infundibulum is caved-in whereas the other side is almost flat despite a slight depression. The side toward the dorsal edge is almost round while the side facing the ventral is quite flat. In certain samples, the macronucleus looks like the wing of a butterfly. On the other hand, the micronucleus is spherical and laid in the concavity of macronucleus at its infundibulum side.

Oral apparatus which is characteristic of these protists begins with peristome and continues with infundibulum. Cytostome, which is located in the end part of the infundibulum is followed by a short cytopharynx. The distance of the peristome from the anterior end is 6.60 (5.00-10.00) μm and its length is 59.00 (50.00-65.00) μm. The length of the peristome is 50.39% of cell length. Vestibular opening (oral opening) which is the beginning part of the infundibulum is closer to the posterior end. Its distance from the anterior end is 66.00 (55.00-70.00) μm. This distance corresponds to 56.36% of the cell length on average. Infundibulum occupies a large area in the cell. Perpendicular length of the infundibulum to the longitudinal axis of the cell is 46.70 (37.50-55.00) μm (b) (Table 1; Figure 2a). Infundibulum, after passing through the middle parts of the body, makes a wide curvature which measures approximately 63% of the cell width. In many samples, the curvature comes near the dorsal edge. Then, it turns towards the posterior and extends to the vicinity of the cytopyge. Total length of the infundibulum is 85.30 (72.50-97.50) μm. To determine the distance along which the infundibulum proceeds in the posterior half, the span between the oral opening and the ending point of the infundibulum was measured throughout a line parallel to the longitudinal axis of the protist (c) (Table 1; Figure 2a). This distance is 47 .50 (40.00-52.50) μm and corresponds to 40.59% of the total cell length. Membranelle fimbriae called adoral zone membranelles (AZM) covering the peristome and infundibulum with paroral membrane (PM) were found in the infundibulum comprising oral ciliature of the protist. Adoral zone membranelles located in the peristome are short. However, these membranelles become longer as they approach the vestibular opening and become shorter as they approach the cytostome. There are no membranelles in the cytostome and cytopharynx. Two paroral membranes (anterior and posterior) which are nearly as long as the infundibulum begin from the oral opening and extend up to the cytostome.

Contractile vacuole is connected to the cytopyge in the posterior part. In certain samples, a second contractile vacuole has been observed in the same zone.

Somatic ciliature resembles the general organization of nyctotheran ciliates (Figure 1a, b). In the right surface of the body are found bipolar ciliary rows. Some of the ciliary rows in the left surface form a suture line (apical suture line) in the anterior half. The apical suture line in no case surpasses the middle part of the cell. Its length is 42 μm on average. An important point that attracts attention is that in the left lateral there is another short

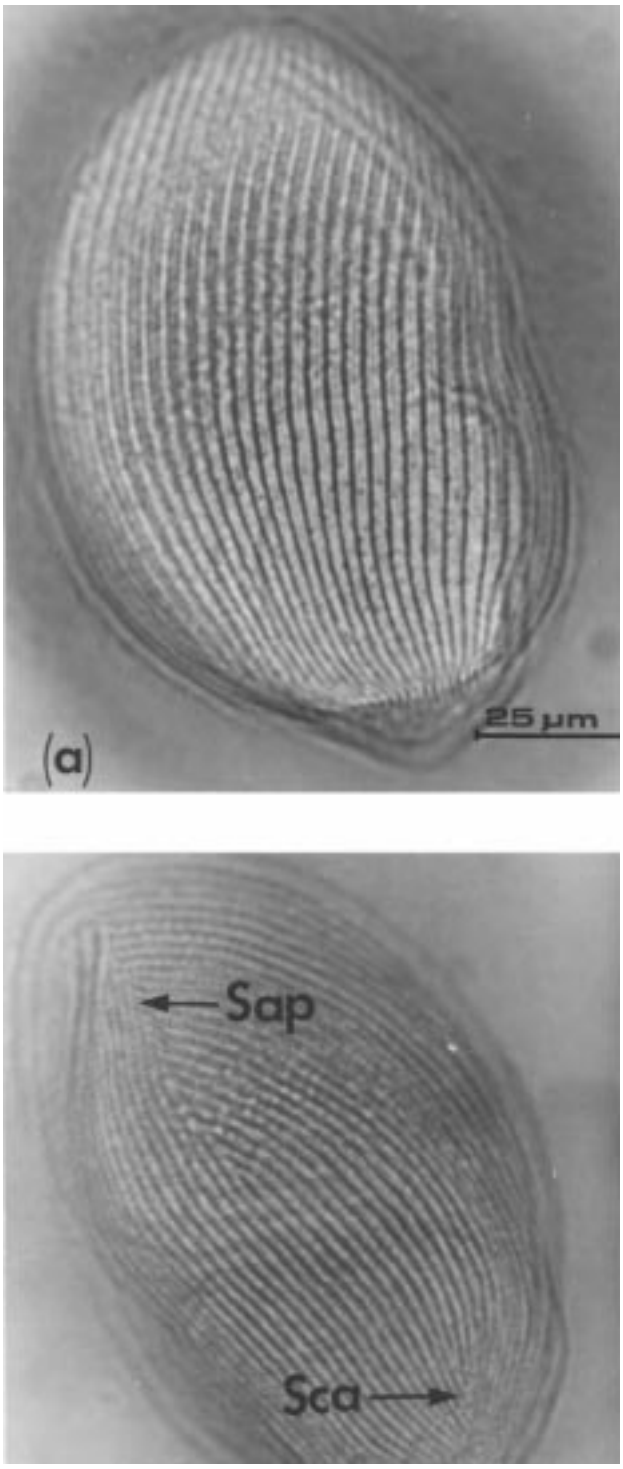


Figure 3. Light micrographs of *Nyctotherus oektemae* n. sp. (silver impregnation).
a. Right surface ciliature b. Left surface ciliature. Sap. apical suture line; Sca. caudal suture line.

suture (caudal suture) line which is located near the posterior end.

On average, there are 31 cilary rows on the right surface and 37 cilary rows on the left surface.

Type host: *Rana ridibunda* Pallas, 1771

Type locality: Van, Turkey

Habitat: Rectum

Type specimens: Permanent preparations belonging to this species and fixed material preserved in formol saline are kept in the Department of Biology, Faculty of Arts and Sciences, Yüzüncü Yıl University.

Derivatio nominis: The name *"Nyctotherus oektemae"* was derived from the surname of my esteemed lecturer, Prof. Dr. Nimet ÖKTEM who is the leading protozoologist in Turkey having made international contributions in this field.

Remarks: This species is similar to *Nyctotherus cordiformis* in terms of general morphology. However, *Nytotherus oektemae* n. sp. is distinguished from *Nyctotherus cordiformis* by the following points:

(a) the beginning part of the infundibulum (oral opening) is nearer to the posterior end of the body

(b) infundibulum is longer and its curvature is wider

(c) the infundibulum part which is perpendicular to the body transverse axis makes a curvature after the middle part of the body is passed

(d) shape of the macronucleus is different: macronucleus is flatter and slender

Discussion and Conclusion

When the present results were compared to those by Öktem (17), it was found that *Nyctotherus cordiformis* specimens were relatively smaller only in terms of body and macronucleus lengths and widths. However, in our study, we did not encounter ciliary topography on the left surface of *Nytotherus cordiformis* shown by Öktem (17). We determined two suture lines (apical and caudal suture lines) in our specimens as in the specimens obtained from *Leptopelis calcaratus* (4). Puytorac and Öktem (4) suggested that the difference in the somatic ciliature of the left lateral of nyctotherans could be the difference among the species of their host. Since *Nytotherus cordiformis* obtained from the same host species was examined by Öktem (17) in detail by light and electron microscopy, it is not discussed further here. However, we compared our specimens of *Nyctotherus cordiformis* with *Nyctotherus oektemae* n. sp.

As stated above, *Nyctotherus oektemae* n. sp. resembles *Nyctotherus cordiformis* which was detected in various anuran amphibians (4, 9, 10, 13, 17), in certain basic characteristics. When Öktem's (17) definitions and figures are considered together with our findings concerning *Nyctotherus cordiformis,* it may be suggested that *Nyctotherus oektemae* n. sp. has different characteristics. These differences are explained below.

In *Nyctotherus cordiformis,* the length of the peristome corresponds to 43.93% of the cell length. However, it was found to be 59.39% in *Nyctotherus oektemae* n. sp. According to variance analysis results, the difference found was considered significant ($P<0.001$).

Although the oral or vestibular opening is in the middle part of the cell in *Nyctotherus cordiformis,* it is closer to the posterior end in *Nyctotherus oektemae* n. sp. The distance of the vestibular opening of *Nyctotherus cordiformis* from the anterior end is 50.34% of the cell length. On the other hand, this distance is 56.36% in *Nyctotherus oektemae* n. sp. This difference was found to be significant ($P<0.001$).

The area occupied by infundibulum differs in these two species. When compared with other species, this area is larger in *Nyctotherus oektemae* n. sp. Length of the infundibulum part which is perpendicular to the longitudinal body axis is 36.50µm in *Nyctotherus cordiformis* and 46.70µm in *Nyctotherus oektemae* n. sp. In *Nyctotherus cordiformis,* the infundibulum bends by forming a curve in the middle of the cell. However in the other form, the curve is formed after the middle part of the cell is passed. Thus its length is 45.77% of the cell width in *Nyctotherus cordiformis* and 62.66% in *Nyctotherus oektemae* n. sp. Therefore, there is a significant difference between these two species ($p<0.001$).

The curve the infundibulum forms is wider in *Nyctotherus oektemae* n. sp. ınfundibulum, after passing the middle part of the cell, forms a wide curve which runs near the dorsal side, and turns to the posterior. However, in *Nyctotherus cordiformis,* it forms a curve somewhere near the middle of the cell and thereafter turns toward the posterior. In *Nyctotherus oektemae* n. sp., the infundibulum extend to the vicinity of the cytopyge. In constrast, it ends immediately after it turns towards to the posterior in *Nyctotherus cordiformis.* The distance of the infundibulum in the posterior half of the cell differs significantly between the two species ($P<0.001$). This value corresponds to 28.85% of the cell length in *Nyctotherus cordiformis* and 40.59% in *Nyctotherus*

oektemae n. sp. Total length of the infundibulum is 57.60 µm and 85.30 µm in *Nyctotherus cordiformis* and *Nyctotherus oektemae* n. sp., respectively.

Macronucleus of *Nyctotherus cordiformis* is massive and kidney-shaped. Its side facing the anterior is domed. On the other hand, the macronucleus of *Nyctotherus oektemae* n. sp. is flatter and especially its side facing the infundibulum is depressed. Although the difference in the length of the macronucleus is insiginificant, their macronucleus with is different. In *Nyctotherus cordiformis,* the width of the macronucleus is 35.33% of its length. This value is 28.41% in the other species ($P<0.001$). Therefore, the macronucleus of *Nyctotherus oektemae* n. sp. is slender. In terms of shape, it is very similar to the macronucleus of *Nyctotherus hylae* rather than *Nyctotherus cordiformis.*

The difference between the two species with regard to body shape is insignificant. *Nyctotherus cordiformis* is kidney-shaped. On the other hand, *Nyctotherus oektemae* n. sp. is ovoid since its anterior end is more pointed than the posterior. Even though we have not found a significant difference between cell length and width, it has been determined that the width of *Nyctotherus cordiformis* corresponds to 68.71% of its length whereas this value is 63.57% in *Nyctotherus oektemae* n. sp. The difference between these values was considered significant ($P<0.001$).

The morphological features of the body and macronucleus of *Nytotherus oektemae* n. sp. closely resemble those of *Nyctotherus hylae.* This species have been detected mainly from frogs belonging to the genus *Hyla.* However, *Nyctotherus oektemae* n. sp. differs from *Nyctotherus hylae* by the size of the body and macronucleus, ciliary rows number and, *Nyctotherus hylae* has a hyaline mass in the anterior part of its body (11, 17). *Nyctotherus oektemae* n. sp. differs from the other members of the genus *Nyctotherus* in body size and shape, number of ciliary rows, size and shape of the macronucleus and localization of the contractile vacuole (3, 9, 10, 12). *Nyctotherus reniformis* (9), *Nyctotherus nankingensis, Nyctotherus pyriformis* (10), *Nyctotherus magnus* (12) have been described insufficiently. There is little published information about these species. However, from their general appearance and body measurements it seems to be reliable. *Nyctotherus magnus* is a giant species and its large body measurements help to distinguish *Nyctotherus magnus* form other species (12). *Nyctotherus macropharyngeus* can easily be distinguished from the new species by its long cytopharynx spirally coiled (13).

In this study, our aim was to determine and describe the ciliate fauna of *R. ridibunda* of Turkey. Accordingly, the species of genus *Nyctotherus* living in the rectum of *R. ridibunda* in the vicinity of Van were determined, a new species was described, and its relationships to similar species were discussed.

Acknowledgments

The authors are indebted to Professor Nimet ÖKTEM and Dr. Byram GÖÇMEN (Department of Zoology, Ege University) for their helpful suggestions on this research and their assistance in taking the photographs of the ciliates.

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