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Morphology and Morphometrics of Two Anaerobic Ciliates *Metopus minor* (Kahl, 1927) and *Metopus hasei* (Sondheim, 1929) from Göksu Delta with Ecological Notes

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Abstract

Genus *Metopus*, a bioindicator of anaerobic conditions was isolated from the different stations of Göksu Delta, Environmental Ministry of Special Protected Areas, Silifke, Mersin. Cultivation and isolation were done by non-flooded petri dish method; morphology of the specimens was worked by live observation and after protargol impregnation by the aid of DIC microscopy. Illustrations of the live specimens were based on free sketches and/or micrograph prints, micrographs and measurements were performed digitally by IM50 image manager system and Q-Win measurement program. All the data was evaluated and species description, original drawings, morphometric measurements have been prepared. Additionally, Göksu population was compared with other populations and distinguishing characters of two species with habitat knowledge were given. *Metopus hasei* and *Metopus minor* were new contributions to Turkish Ciliate Fauna

Key Words: Anaerobic, Ciliophora, Heterotrichida, Wetland.

Introduction

Systematic and ecological studies are important in determining natural sources and evaluate the dispertion changes of established living organisms. Much of the literature regarding soil protozoa as bioindicators in ecosystems has already been extensively reviewed (Foissner 1987, 1994, 1997, 1999; Laybourn-Parry, 1992) and there are some protozoa which are uniquely important in anoxic environments (Finlay and Fenchel 1992). Protozoa with their rapid growth and fragile external membranelles, can react more quickly due to environmental changes (Foissner,1994). Certain ciliates are excellent and simple bioindicators in showing oxygen regime of soil. Metopid ciliates are the ones which can live and reproduce only under microaerobic or anaerobic conditions. The only reason for such an exception is, they lack mitochondria (Finlay and Fenchel 1992; Foissner, 1987; 1999).

In this study two anaerobic ciliate, *Metopus minor* and *Metopus hasei*, which are isolated from an inundation zone of the Göksu Delta, investigated taxonomically.

Material and method

Study area

Göksu Delta is a special protected Ramsar area which is located at Mersin province in the south of Turkey, Mediterranian coastal zone with 33 54' 09"N - 36 13' 16"E coordinates (Figure 1). The Göksu Delta Wetlans System is fed by continuously flowing springs, by seasonal floods and by the spring-winter rains. The entire delta measures 15ha of which 5500 ha is true wetland habitat. The wetland area includes fresh water lake and a lagoon. (Anonymous, 1994; 1998). The majority of the soils in the delta are very young. The pH of these soils ranges from 7 to 9 (Çapar, 2005). As the sediments are being carried from distant and many different areas their chemical and mineralogical combinations are varied. Since the

Taurous mountains mainly consist of limestone, the aluvial sediment in the Göksu Delta Soils are also mostly limestone (Anonymous, 1991; 1994; DHKD, 1993; Özus, 1988).

Sampling and sample processing

Samples were collected seasonally during 2000-2001 from twelve stations of the Göksu Delta. Each sampling station consisted of five sub-samples which were taken in an area of about 100 m^2 and a depth 5 to 10 cm. All the material was collected by the help of a shovel and usually involved mineral top soil with fine plant roots, leafs and humic layer from the soil surface.

The samples were air-dried at laboratory condition and then treated with the non-flood Petri dish method, as described by Foissner (1987, 1991). By this method, the petri dish cultures were investigated for ciliate species by inspecting 2 ml of run-off on days 2, 7, 14, 21 and 28. During this time, a few cells were isolated for each species with a fine pipette and cultivated in poor cultures for staining. After this process many specimens were obtained for each species.

Species identification and morphometric measurements

All the determinations were done mainly on live specimen using a high power oil immersion objective and differential interference contrast microscopy. The ciliary pattern was revealed by protargol impregnation, which has been described by Foissner (1991, 1993) and Foissner et al. (2002). Morphology of the species followed the literatures in Corliss, 1979; Corliss and Lom 1985; Foissner, 1980, 1998; Foissner and Agatha, 1999; Foissner et al 2002; Kahl, 1932.

Measurements were performed digitally by IM50 image manager system and Q-win measurement program in μm on randomly selected individuals. Statistics were calculated according to text books.

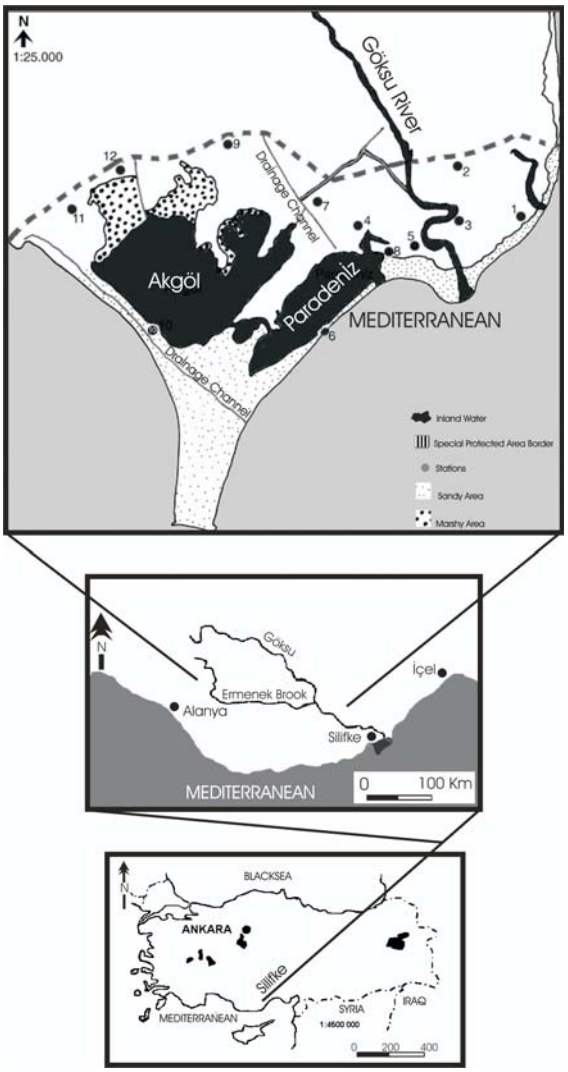

Figure 1. Study area.

Results and Discussion

Morphometric data of the two species are shown on table 1and 2. Species definitions were carried out according to Corliss, 1979; Corliss and Lom 1985; Foissner, 1980, Foissner, 1998; Foissner and Agatha, 1999; Foissner et al., 2002; Kahl, 1932. The species descriptions and original drawings are performed below.

Class: Spirotricha Bütschli, 1889
Ordo: Heterotrichida Stein, 1859
Family: Metopidae Kahl, 1927
Genus: *Metopus* Claparede & Lachmann, 1858

***Metopus minor* Kahl, 1927 (Table 1; Figure 2, 3)**

Description:

Size in vivo 35-52 X 20-28 μm; anterior top of the body elongated by nose-like preoral projection, posterior side

broadly rounded.

Macronucleus 10 X 7 µm, globular to slightly elipsoidal, located dorsally at the same level of adoral zone; single micronucleus 23 µm across.

Cortex flexible, not furrowed by ciliary rows; cytoplasm colourless, full with granules. Food vacuoles 5-6 µm across; 5-10 µm sized contractile vacuole in posterior end.

Oral opening laterally located and covered by adoral zone of membranelles. Adoral zone of membranelles distinctly spiraled, come up to 60% of body lenght and composed of approximately 15 adoral ciliary membranelles.

Somatic ciliature dikinetidly ciliated on ventral side and around the postoral projection. On dorsal side only posterior basal body ciliated. Somatic cilia 10-12 µm; caudal cilia 30-35 µm lenght.

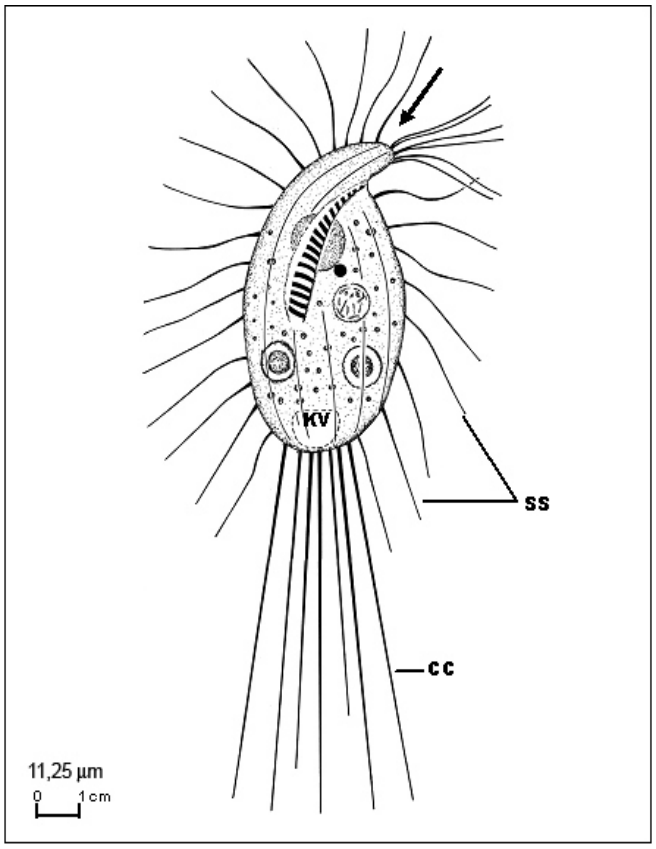

Figure 2. *Metopus minor*, in vivo, ventral view, anteriorly located elongate preoral dome and nose-like projection **(arrow)**, dorsally located macronucleus and adoral ciliary zone, **CC:** Caudal cilia, **KV:** Contractile vacuole, **SS:** Somatic cilia.

Table 1. Morphometric data from Göksu population of *Metopus minor.*

Characters[a]	X	M	SD	SE	CV	MIN	MAX	n
Body, length	44.78	47.34	6.09	2.03	33.02	35.7	52.14	9
Body, width	24.76	26.11	2.86	0.95	7.29	20.26	27.98	9
Body, maximum postoral width	23.43	23.28	3.17	1.05	8.95	19.5	27.42	9
Body, length:width ratio	1.83	1.83	0.18	0.06	0.02	1.44	2.04	9
Anterior cell end to proximal end of adoral zone of membranelles, distance	27.41	28.02	4.07	1.35	14.7	21.5	32	9
Macronucleus, length	10.18	10	1.72	0.57	2.64	8	13	9
Macronucleus, width	7.65	7.08	1.45	0.48	1.88	5.87	10	9
Macronucleus, number	1	1	0	0	0	1	1	9
Micronucleus, number	1	1	0	0	0	1	1	9
Somatic ciliary rows, number	6.33	6	1	0.33	0.88	5	8	9
Caudal cilia, number	7	7	0.70	0.23	0.44	6	8	9
Adoral zone of membranelles, number	14.55	15	2.40	0.80	5.13	10	18	9
Contractile vacuole, diameter	7.7	8	1.92	0.6	3.28	5	10	9

[a]Measurements on µm. CV- coefficicent of variation; M- median; Max-maximum; Min- minumum; n- number of individuals investigated; SD- standart deviation; SE- standart error; X- arithmetic mean.

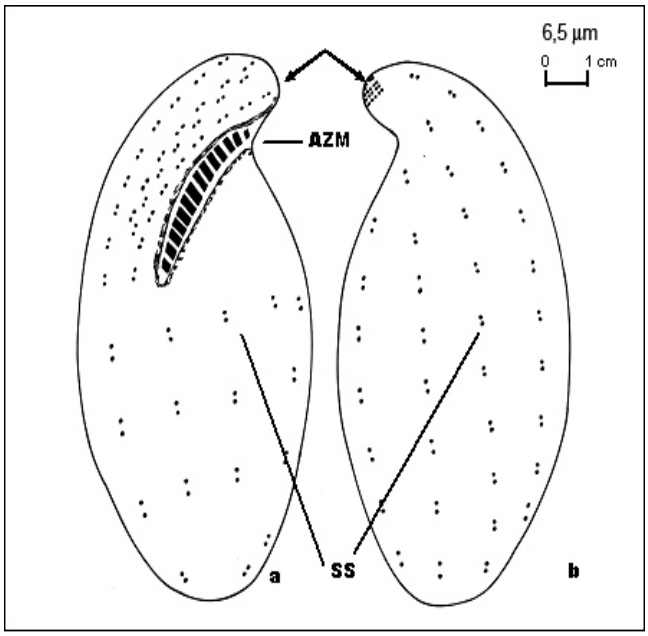

Figure 3. *Metopus minor*, after protargol impregnation, **a, b)** Ventral ve dorsal view; preoral dome with nose-like projection **(arrows)**, **AZM:** Adoral ciliary zone, **SS:** Somatic kineties.

***Metopus hasei* Sondheim, 1929** (Table 2; Figure 4, 5)

Description

Size in vivo 59-81 X 17-21 µm; body shape cylindroidal; preoral dome slightly sigmoidal and distinctly curved and slope to almost 45 degrees to main body axis, projecting knop-like above the left body margin. Postoral body portion cylindroidal with backward slightly narrowed and rounded.

Macronucleus about 22 X 7 µm, elipsoidal, located underneath of adoral zone, contains one 2-3 µm sized micronucleus attached to macronucleus.

Cortex flexible, slightly furrowed by ciliary rows; cytoplasm colourless, full with granules. Food vacuoles 5-10 µm across, contain bacteria; contractile vacuole in posterior end.

Oral opening laterally located and covered by adoral zone of membranelles, adoral zone 35-45% of the body length, slightly sigmoidal, hardly roofed by preoral dome, and composed of 15-17 adoral ciliary membranelles.

Somatic cilia 10 µm, dikinetidal, only posterior basal body ciliated. Caudal cilia 40 µm long.

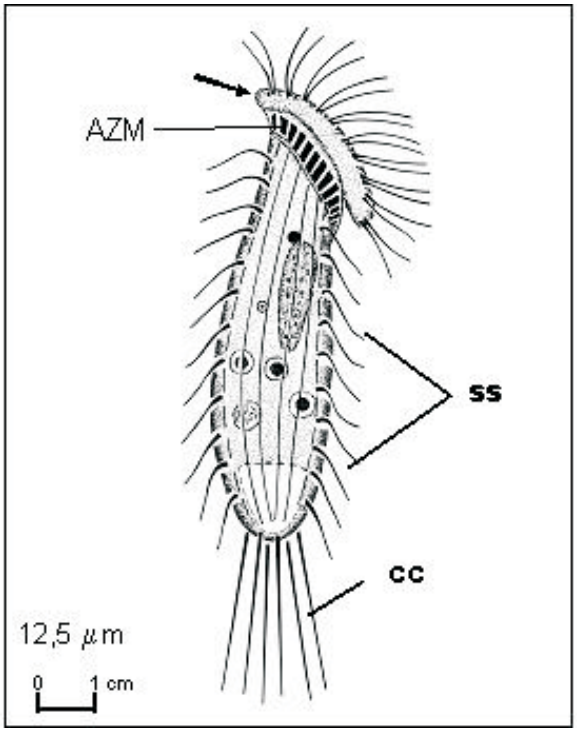

Figure 4. *Metopus hasei*, in vivo, ventral view, preoral projection with petal cover **(arrow)**, **AZM:** Adoral ciliary zone, **CC:** Caudal cilia, **SS:** Somatic cilia.

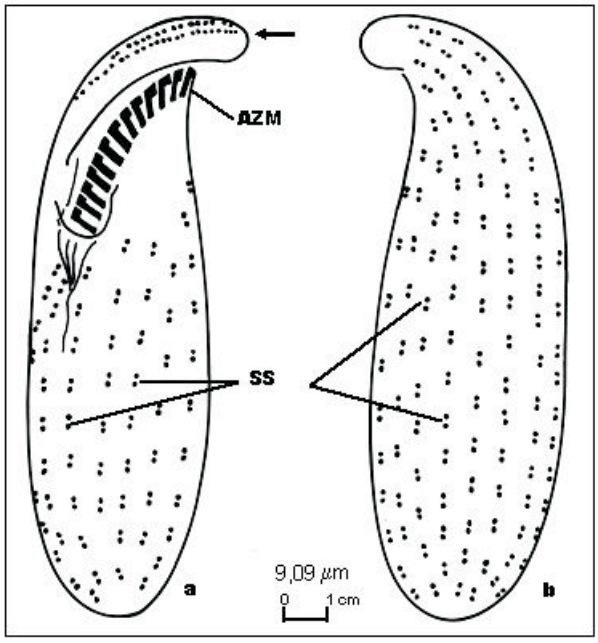

Figure 5. *Metopus hasei*, after protargol impegnation, **a)** Ventral view, preoral projection with petal cover **(arrow)** and dikinetidal somatic kineties, **b)** Dorsal view, dikinetidal somatic kineties, **AZM:** Adoral ciliary zone, **SS:** Somatic kinety.

Table 2. Morphometric data from Göksu Population of *Metopus hasei.*

Characters[a]	X	M	SD	SE	CV	MIN	MAX	n
Body, length	69,17	68,79	7,27	2,42	47,03	58,63	80,94	9
Body, width	18,65	18,9	1,22	0,40	1,32	16,79	20,49	9
Body, maximum postoral width	17,17	17,5	2,15	0,71	4,11	13,6	20,43	9
Body, length:width ratio	3,694	3,67	0,15	0,05	0,02	3,49	3,95	9
Anterior cell end to proximal end of adoral zone of membranelles, distance	25,67	25,89	2,73	0,91	6,66	22,5	31,04	9
Macronucleus, length	21,30	22	2,71	0,90	6,57	17,21	24,7	9
Macronucleus, width	7,45	7,5	0,34	0,11	0,10	6,9	8	9
Anterior cell end to posterior end of macronucleus, distance	41,09	40,8	1,82	0,60	2,96	38,9	43,68	9
Micronucleus, length	2,69	2,86	0,35	0,11	0,10	2	3	9
Micronucleus, width	2,61	2,6	0,24	0,08	0,05	2,1	2,9	9
Macronucleus, number	1	1	0	0	0	1	1	9
Micronucleus, number	1	1	0	0	0	1	1	9
Somatic ciliary rows, number	10,33	10	0,5	0,16	0,22	10	11	9
Caudal cilia, number	5,44	5	0,52	0,17	0,24	5	6	9
Adoral zone of membranelles, number	15,77	16	0,83	0,27	0,61	15	17	9
Contractile vacuole, diameter	12,11	12	1,36	0,45	1,65	10	14	9

[a]Measurements on µm. CV- coefficicent of variation; M- median; Max-maximum; Min- minumum; n- number of individuals investigated; SD- standart deviation; SE- standart error; X- arithmetic mean.

Wetlands are characterized by permanent or temporal flooding. Flooded soils occur naturally in humid climates, in floodplains, and in coastal areas, or are created in wetland rice agriculture. Wetland soils become largely anoxic soon after flooding (Lehmann-Richter et al., 1999; Bodelier, et al., 2000). Hereupon metopids occur only in the anaerobic or microaerobic soils. The eutrophic pasture is a special case which might be useful for detecting oxygen deficits that are not particularly obvious (Foissner, 1999).

Metopus as a heterotrich ciliate has an adoral zone of membranelles for feeding and conventional cilia for locomotion. The locomotor cilia are arrayed in kineties,

only visible as they pass over the macronucleus. The genus is distinctive because of the twist of the anterior end of the cell. A collection of granules is visible at the anterior end, and there is a posterior contractile vacuole. Most species in this genus are encountered in anoxic or reduced habitats (Schwarz and Frenzel, 2003, 2005).

Representatives of the genus seem to be ubiquitous filter-feeders of bacteria in sediments, landfills and anoxic water. All well-studied *Metopus* species have been shown to be anaerobic organisms, with a variable tolerance of dissolved oxygen (Curds, et al., 1983).

In the form hereinafter set forth, the two metopid ciliates of Göksu Delta are compared, concerning morphology and morphometrics including the habitat knowledge, with other populations.

M. minor was discovered for the first time in a sapropelic pond of Hamburg, Germany (Kahl, 1927). In 1980, Foissner found it in Austrian Central Alps about 1800m. above sea level and Estaban et al., (2000) recorded again from a brakish water in a crater lake. It was reliably recorded from moor land and mud rock pools of Venezuella and from rice fields in Italy (Foissner et al., 2002; Schwarz and Frenzel, 2003). Besides these, there are records from Iceland and Namibia (Foissner et al., 2002).

M. minor easily seperates from the congeners due to its small body size with a proeoral dome projection and distinctly long caudal cilia (Figure 2). In none of the investigations morphometric measurements of populations are given in detail except the Iceland population. However, all the descriptions have some basic data such as body lenght, adoral ciliary membranelles and somatic kinety number.

Our population fits within the original description of Kahl 1927 and additionally with Esteban et al., 2000, especially in considering body length and somatic kinety number. When compared with Austrian and Iceland populations, Göksu population is more or less 5 µm longer in body length (35-52 vs. 28-35µm) and has more adoral zone number (10-18 vs. 6-10).

Metopus hasei is the most common soil metopid (Foissner, 1999) which was discovered by Sonheim, in a rewetted mud from an unknown locality in Madagastar (Foissner and Agatha, 1999). Afterwards, it was recorded in Austria and Australia (Foissner, 1995; Blatterer and Foissner, 1988). Later on, *M. hasei* was found from a trophical dry forest of Costa Rica-Central America and flooded zone of Amazonia rain forest. The later descriptions were obtained from wet mosses of marshy areas in Sheldrick waterfalls and a forest near Sheldrick waterfall in Kenya (Foissner, 1995; 1999). Finally it was recorded from the city park of Hamburg, collected from leaf litter, and from sewedge irrigation fields of Berlin and from Namibia (Foissner, 2000). *M. hasei* has since been found from all biogeographical regions except Antartica (Foissner, 1998).

At first glance, *M. hasei* resembles *M. palaeformis* because of their ourline similarities but because of having distinct caudal cilia, *M. hasei* differs from *M. palaeformis* immediatedly (Figure 4). According to available data, our population fits with the Austria and Namibia populations taking into account the body measurements and somatic ciliature. However, South Africa population has longer body length (113 vs. 60-65), more adoral zone of membranelles (23 vs. 15-18) and bigger macronucleus (49 vs. 22-25) compared with the rest of the populations.

In Göksu Delta metopid species are found in the second, third, fifth, eighth and ninth stations (Figure 1). When these regions are characterised, the stations are at the flooded zone of the river bed around rice fields; in the severe saline and highly vegetated marshy area where the river and the sea unite; marshy areas close to estuary lagoon which are upon the alluvial delta basin and finally from meadow where the base water is high.

Such habitats potentially become anoxsic from time to time where there are favourite living quarters for metopid ciliate such as *M. hasei* and *M. minor*. Additionally, the characteristics of the stations where metopid ciliates are found, match with the previous habitats mentioned above. According to the live observation results of the food vacuoles, both species feed on bacteria.

Up till now, genus *Metopus* has been only recorded as *M. es* and *M. striatus* from a pond in Van province which is covered with *Lemna* (Şenler and Yıldız, 2004). The first records of *M. hasie* and *M. minor* to Turkish Ciliate Fauna were given in this study.

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