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Morphology and infraciliature of the soil ciliate *Metopus hasei* Sondheim, 1929 (Ciliophora, Armophorida) from Biele Karpaty Mountains (Slovakia)

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Summary

The morphology and infraciliature of the ciliate *Metopus hasei* Sondheim, 1929 isolated from soil in Moravské Lieskové, Biele Karpaty Mountains, Slovakia, were investigated using live observation and protargol impregnation. Carpathian isolate is distinguished by body size of 90 × 25 µm in vivo, 5 perizonal and 10-13 somatic kineties, adoral zone composed of 16 membranelles and occupying 34% of body length on average, and cortical granules arranged in 4-6 loose rows between each two adjacent somatic kineties forming a regular pattern on both dorsal and ventral side of cell, but a blank area appears on ventral side underneath preoral dome beside the adoral zone of membranelles and between each two perizonal kineties. *Metopus hasei* is characterized by an unusually high inter-population variability, so biological species formation hardly recognizable at morphological level could be supposed.

Key words: *Metopus hasei*, morphometry, Slovakia's soil ciliates

Introduction

Metopids are bacterivorous ciliates with an oral apparatus composed of an adoral zone of membranelles as well as paroral membrane, and with somatic kineties built from dikinetids. Further they are characterized by the presence of endosymbiotic bacteria. Species of the genus *Metopus* Claparède et Lachmann, 1858 inhabit anaerobic habitats, such as polluted waters and flooded soils with oxygen deficiency (Jankowski, 1964; Foissner et al., 2002). *Metopus hasei* Sondheim, 1929 is a common soil inhabitant found all over the world except Antarctica (Foissner, 1998). However, only three populations of this species have been described in detail (Foissner, 1981; Foissner and Agatha, 1999). Strong variability in cell morphology, namely in morphometric characteristics, was shown between populations from various biogeographical regions.

In this paper, I describe the morphology of a Carpathian isolate of *Metopus hasei,* differing from other described populations of this species in several important cytological features, viz., the pattern of cortical granules and the length of the adoral zone and the perizonal stripe.

Material and Methods

Metopus hasei was isolated from a soil sample collected on October 31, 2004 in an oak-hornbeam forest in Biele Karpaty Mts., Slovakia (48°50´N, 17°46´E; 350 m above sea level). The sample consisted of soil mixed with leaf-litter (upper 0-4 cm layer); pH was 6.8.

The sample was processed with the non-flooded Petri dish method as described in Foissner (1987) and Foissner et al. (2002). The investigation was based on live observation and protargol impregnation, protocol A (Foissner, 1991). The drawing of live ciliates, based on free-hand sketches, represents a summary of observations of live and fixed cells. Illustrations of prepared cells were made with a drawing device. Morphometry is based on 10 well-impregnated specimens, derived parameters were calculated according to statistics textbooks. Terminology is given according to Foissner and Agatha (1999).

Results

***Metopus hasei* Sondheim, 1929** (Figs 1a-l, 2a-c; Table 1).

Description of the Carpathian isolate: Body size 80-95 × 20-30 µm in vivo, usually about 90 × 25 µm, body length:width ratio 3.0-4.3:1, on average 3.6:1 in protargol preparations (Table 1), where the ciliates are stouter than when alive, preoral:postoral body portion an average of 0.5:1 in silver-impregnated specimens. Body outline elongate ellipsoidal, cells widest in mid-body (Figs 1a, f-l). Preoral dome only slightly sigmoidal, distinctly curved and inclined about 45° to main body axis, projecting above anterior left body margin, dorsoventrally flattened about 2:1. Postoral body portion cylindrical, with posterior body end broadly rounded. Macronucleus elongate ellipsoidal (length:width ratio an average of 3.1:1), rarely reniform or indistinctly tortuous, usually about 24 × 8 µm in protargol preparations, contains numerous nucleoli. Anterior macronuclear end situated underneath or slightly above left of adoral zone of membranelles, posterior end not overlapping anterior body half (Figs 1a, c, f-l, 2a-c). Micronucleus globular to ellipsoidal, conspicuously large (on average, 4.9 µm in largest diameter), usually near or attached to anterior portion of macronucleus. Contractile vacuole in posterior body end, excretory pores not recognizable (Fig. 1a). Cytoplasm colourless, hyaline, packed with bacterial rods (on average 5 µm long), which impregnate well with protargol, and with food vacuoles, 5-15 µm in diameter, containing bacteria.

Cortical granules are difficult to recognize in vivo, because they are colourless and minute (approximately 0.3 µm in diameter), but perfectly distinct in not heavily impregnated specimens. Granules are arranged in 4-6 loose rows between each two adjacent somatic kineties, 3-8 granules (on average 6 in mid-body) are situated between each two somatic dikinetids of an individual ciliary row (Fig. 1e). Cortical granules form a distinctly regular pattern on both dorsal and ventral cell side, but a blank area appears on ventral side underneath the preoral dome beside the adoral zone of membranelles and between each two perizonal kineties (Fig. 1b).

Somatic cilia are about 10 µm long, those of perizonal stripe a little longer. The number of caudal cilia, whose length is about 30 µm, is from 4 to 6. Dikinetids are generally arranged in 10-13 meridional rows (Figs 1 c, d, 2 a-c), leaving a blank posterior pole centre. Perizonal ciliary stripe, of a usual structure, occupies about 25% of body length (Table 1) and is slightly shorter than the adoral zone of membranelles.

The adoral zone of membranelles is more or less spiralized, hardly roofed by preoral dome. It begins about 3.7 µm behind the anterior left margin of the preoral dome and consists of 13-18 membranelles occupying about 34% of the body length. The paroral membrane is short (about 7.8 µm) and almost straight.

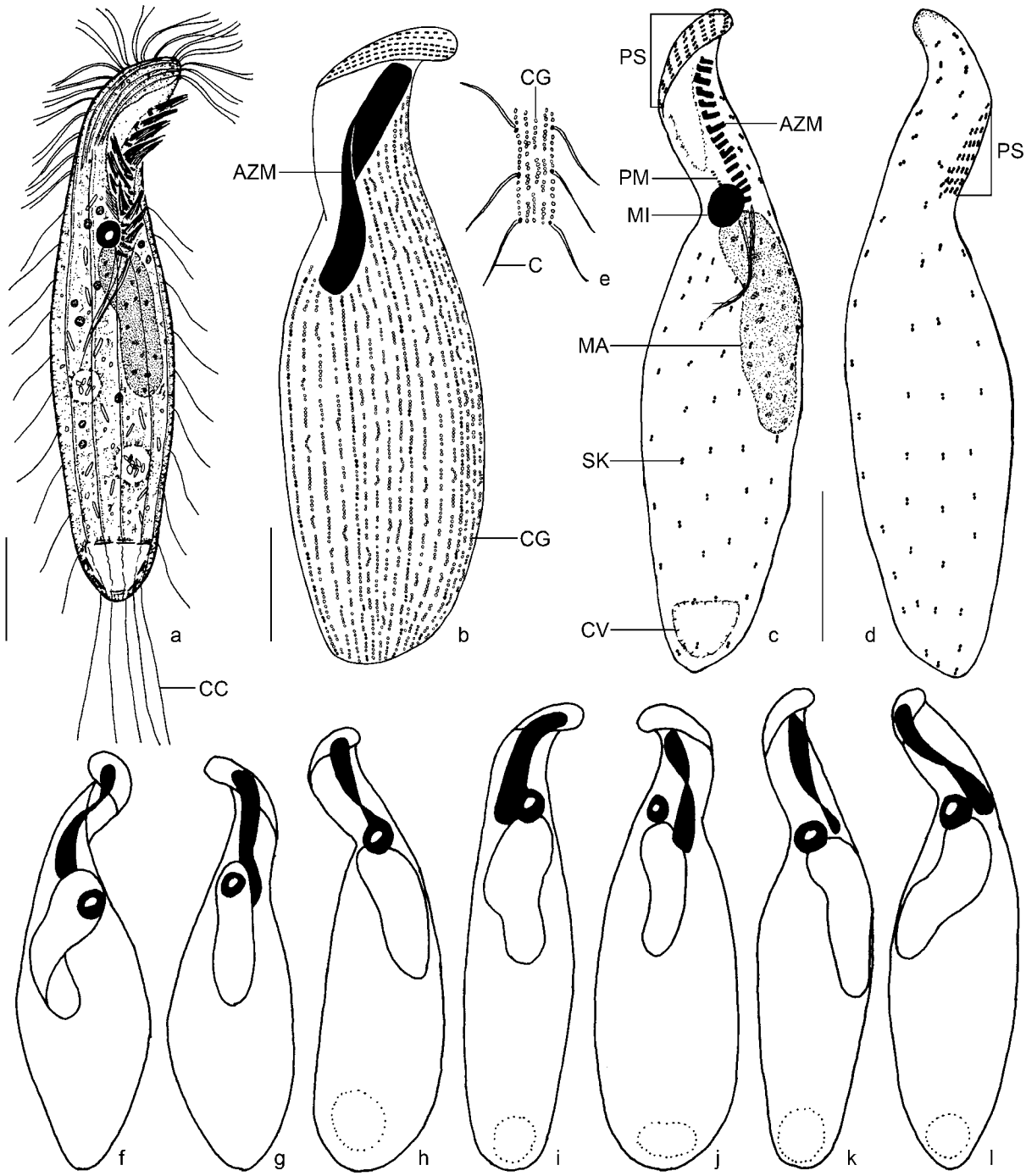
The carpathian isolate of *M. hasei* occurred in leaf-litter and soil in the bottom of Petri dishes where it developed in moderate abundance on the 10th day after saturation and remained until the end of investigation (21st day). This species feeds on bacteria and their spores.

Discussion

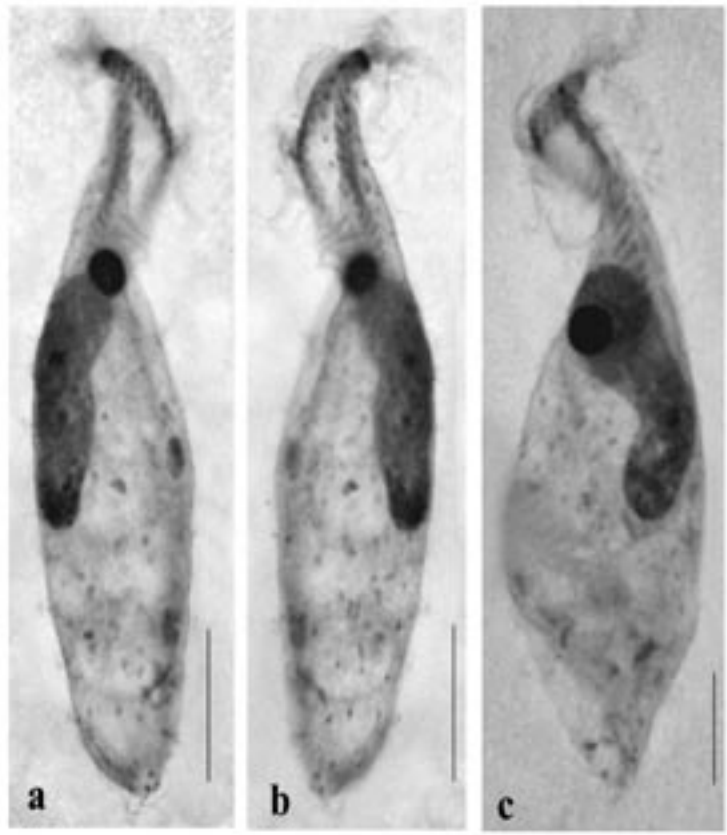
Metopus hasei was discovered in mud from Madagascar (Sondheim, 1929) and since then has been reported from terrestrial habitats of all main biogeographical regions except Antarctica (Foissner, 1998; Foissner and Agatha, 1999). Re-descriptions of this species were based on populations from Austria (Holoarctic region) (Foissner, 1981), Namibia and South Africa (Paleotropic region) (Foissner and Agatha, 1999). The population isolated from Biele Karpaty Mountains obviously differs from those described before in cortical granulation. The carpathian isolate of *M. hasei* has a regular pattern on both dorsal and ventral cell side, while the other populations investigated so far in detail are characterized by inconspicuous densely irregularly arranged cortical granules (Foissner and Agatha, 1999).

Though cortical granulation is widely used in taxonomy of spirotrich ciliates, its importance for metopid taxonomy has not yet been assessed.

The Carpathian population matches the Austrian one in several morphometric characteristics (body size, number of ciliary rows) better than it do both African populations investigated. In particular, the European populations differ from the African ones in



Figs 1a-l. *Metopus hasei* from life (a, e, f-l) and after protargol impregnation (b, c, d). **a:** Ventral view of representative specimen. **b:** Ventral view showing cortical granulation. The blank area is underneath preoral dome beside the adoral zone of membranelles. **c, d:** Infraciliature of ventral and dorsal side and nuclear apparatus. **e:** Surface view showing loosely arranged cortical granules in 4-5 rows between each two kineties. **f-l:** Shape and nuclear variants from various aspects. AZM – adoral zone of membranelles, C – cilia, CC – caudal cilia, CG – cortical granules, CV – contractile vacuole, MA – macronucleus, MI – micronucleus, PM – paroral membrane, PS – perizonal stripe, SK – somatic kinety. Scale bars 10 μm.



Figs 2a-c. *Metopus hasei*, infraciliature of ventral (a, c) and dorsal side (b) and nuclear apparatus after protargol impregnation. Preoral dome is distinctly curved, inclined about 45° to the main body axis and projecting above anterior left body margin. Anterior macronuclear end is situated above left of adoral zone of memranelles and posterior end does not overlap anterior body half. Scale bars 10 µm.

ratio values; the adoral zone of membranelles occupies about 34% of body length in both the Carpathian and the Austrian population, and about 42% of body length in the African populations. Perizonal stripe occupies about 25% of the body length in the Carpathian population, and about 34% of body length in the African populations. Unfortunately, morphometric data on perizonal stripe of the Austrian isolate (Foissner, 1981) are not available. However, the Carpathian isolate differs from the Austrian one in number of adoral membranelles (13-18 vs. 17-20) and in absence of hyaline membrane around micronucleus, which also lacking in both African populations investigated. Interestingly, the Carpathian isolate corresponds to the Namibian one in some morphometric features (macronuclear size, micronuclear diameter, number of adoral membranelles) much better than to the Austrian isolate. Most of morphometric discrepancies were found between the Carpathian and the South African isolate, they concerned body size (67 × 19 µm vs. 113 × 24 µm), macronuclear size (24 × 8 µm vs. 37 × 8 µm), the number of ciliary rows (10-13 vs. 14-16) and the number of adoral membranelles (16 vs. 24).

Differences between populations of *Metopus hasei* from various habitats and regions (alpine soil in Austria, soil mixed with leaf-litter in Slovakia, dry mud of a temporary mountain river in Namibia, and soil in South Africa) are probably due to the adaptations evolved under conditions of various ecological constraints. These distinct, geographically isolated populations are a good example of microspecies formation and lend support to the theory of the biogeography and endemism in ciliates (Foissner, 2006). They may even represent different species, not yet recognizable at the morphological level. To test this supposition, an investigation with the use of molecular methods is necessary.

Table 1. Morphometric data on *Metopus hasei*
(data based on protargol-impregnated specimens from non-flooded Petri dish cultures)

Characteristics	Mean	M	SD	CV	SE	Min	Max	n
Body, length	67.4	67.2	5.1	7.6	1.6	60.9	73.4	10
Body, width	19.1	19.1	1.3	6.6	0.4	17.2	20.3	10
Body, length:width, ratio	3.6	3.4	0.5	13.5	0.2	3.0	4.3	10
Anterior body end to posterior end of perizonal stripe, distance	17.1	17.2	3.5	20.2	1.1	10.9	21.9	10
Anterior body end to posterior end of adoral zone of membranelles, distance	22.8	23.4	2.6	11.5	0.8	17.2	26.6	10
Distance anterior end to end of perizonal stripe:body length, ratio in %	25.3	26.9	4.3	16.9	1.4	17.3	29.8	10
Distance anterior end to end of adoral zone of membranelles:body length, ratio in %	33.9	33.0	4.6	13.5	1.4	27.6	42.0	10
Posterior end of adoral zone of membranelles to posterior body end, distance	43.7	44.5	6.1	14.0	1.9	35.9	50.8	10
Macronucleus, length	23.7	23.4	1.8	7.8	0.6	20.8	26.6	10
Macronucleus, width	7.7	7.7	1.0	13.5	0.3	6.3	9.4	10
Anterior body end to anterior end of macronucleus, distance	17.6	17.2	2.9	16.2	0.9	14.1	22.7	10
Micronucleus, largest diameter	4.9	4.8	0.5	10.9	0.2	4.2	6.1	10
Macronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	10
Micronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	10
Somatic ciliary rows, number	11.5	11.5	0.9	8.1	0.3	10.0	13.0	10
Dikinetids in a right lateral somatic kinety, number	16.8	17.0	2.4	14.5	0.8	12.0	19.0	10
Adoral membranelles, number	15.6	15.5	1.9	12.3	0.6	13.0	18.0	10
Perizonal ciliary rows, number	5.0	5.0	0.0	0.0	0.0	5.0	5.0	10
Paroral membrane, length	7.8	7.0	2.7	34.5	0.8	4.7	12.8	10

Measurements in µm. CV, coefficient of variation in %; M, median; Max, maximum; Min, minimum; n, number of individuals investigated; SD, standard deviation; SE, standard error of arithmetic mean; Mean, arithmetic mean.

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