

First Record of Five Soil Ciliates (Ciliophora, Hypotricha) from Saudi Arabia

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Abstract: The morphology and infraciliature of five soil hypotrichous collected from the farm-land at Zulfi city, Saudi Arabia were investigated using living observation and silver impregnation methods. *Gastrostyla steinii* was found to have 4-8 macronuclei and thus its congener, *G. muscorum* which was characterized by eight macronuclei is likely a synonym with *G. steinii*. Redescriptions on *Hemiamphisiella granulifera*, *Urostyla grandis* Ehrenberg, 1830 and *Nudiamphisiella interrupta*, revealed that Saudi Arabia populations were morphologically identical with previous studies. A *Nudiamphisiella* species which is quite similar with *N. illuvialis* was also discussed. The above mentioned species shows first records in Saudi Arabia and extend the known distribution areas of these species.

Key words: Hypotrichous, infraciliature, soil ciliates, first record, congener, Saudi Arabia

INTRODUCTION

Ciliate protozoans are considered as the dominant protistan group in the interstitial system of marine sediments. They play an important role in the processes and evolution of the benthic ecosystem (Fenchel, 1969; Sorokin, 1999), Soil protozoa and ciliates in particular, represent a microbial group abundant in the rhizosphere with an influential role on nutrient cycling (Acosta-Mercado and Lynn, 2004). Under laboratory conditions, ciliates regulate the size and the composition of bacterial communities (Acosta-Mercado and Lynn, 2004) on soil ciliates are quite few (Berger *et al.*, 2006; Foissner *et al.*, 2008). In the list of soil ciliates, a total of 964 soil ciliate species (644 described and 320 undescribed) are recorded in world wild (Chao *et al.*, 2006). Studies of ciliate biodiversity in Saudi Arabia concentrated mainly on marine groups (AL-Rasheid, 1996, 1997; AL-Rasheid *et al.*, 2001; Al-Farraj, 2008). Very little information exists about soil ciliates in Saudi Arabia (Foissner and AL-Rasheid, 2007).

MATERIALS AND METHODS

All species were collected from the farm land at Zulfi city which is located northwest of Riyadh, Saudi Arabia in October, 2009. Soil samples were taken directly from the upper 5 cm surface and then were processed with the non-flooded petri dish method in laboratory (Foissner, 1987a). Cells were isolated and observed *in vivo* using differential interference contrast microscopy. Staining with protargol method (Wilbert, 1975) was performed in order to reveal the infraciliature. Drawings were made with the help of a camera lucida. Measurements were made under 100-1250× magnification.

Terminology mainly follows Berger (1999, 2006, 2008). The examined material is deposited in Department of Sciences, Teachers College, King Saud University.

RESULTS AND DISCUSSION

***Gastrostyla steinii*:** Cell size measuring 130-250×50-100 μm *in vivo* (Fig. 1a-o; Table 1) (Engelmann, 1862). Body elliptical with posterior part slightly broader than anterior end (Fig. 1a, b, m). Cortical granules absent. Cell opaque due to numerous granules (2-5 μm) (Fig. 1a, b). Single Contractile Vacuole (CV) about 15 μm in diameter, positioned in equatorial region near left margin (Fig. 1b). Macronucleus moniliform, composed of 4-8 nodules each nodule globular to elliptical in shape and about 25×12 μm in size (Fig. 1c, d, g, h, o); Micronuclei (Mi) numbering 1-3, ovoid in shape, adjacent to macronucleus nodules (Fig. 1g, h, o). Buccal field about 1/3 of body length. Adoral Zone of Membranelles (AZM) composed of 37-65 membranelles, cilia of which measure about 12 μm long *in vivo* (Fig. 1a, b, m). The 19-23 frontoventral (three frontal one buccal and 16-19 ventral) cirri two to three of which located near Transverse Cirri (TC) (Fig. 1d, e, i, n). The 5-7 transversal cirri, each ca. 18 μm long, positioned in the posterior of the body (Fig. 1h, n). Right Marginal Row (RMR) and Left Marginal Row (LMR) composed of ca. 39 and 35 cirri, respectively (Fig. 1i, n). Invariably 3 caudal cirri (Fig. 1j, o). Seven dorsal kineties with 3 posteriorly shortened ones (Fig. 1f, j, o).

Gastrostyla steinii has been frequently reported in the past years (Engelmann, 1862; Dragesco, 1966; Foissner, 1982; Berger, 1999). This Saudi Arabia population corresponds well with the population described by Foissner (1982) in the body shape and the ciliary pattern but differs from the later in the number of

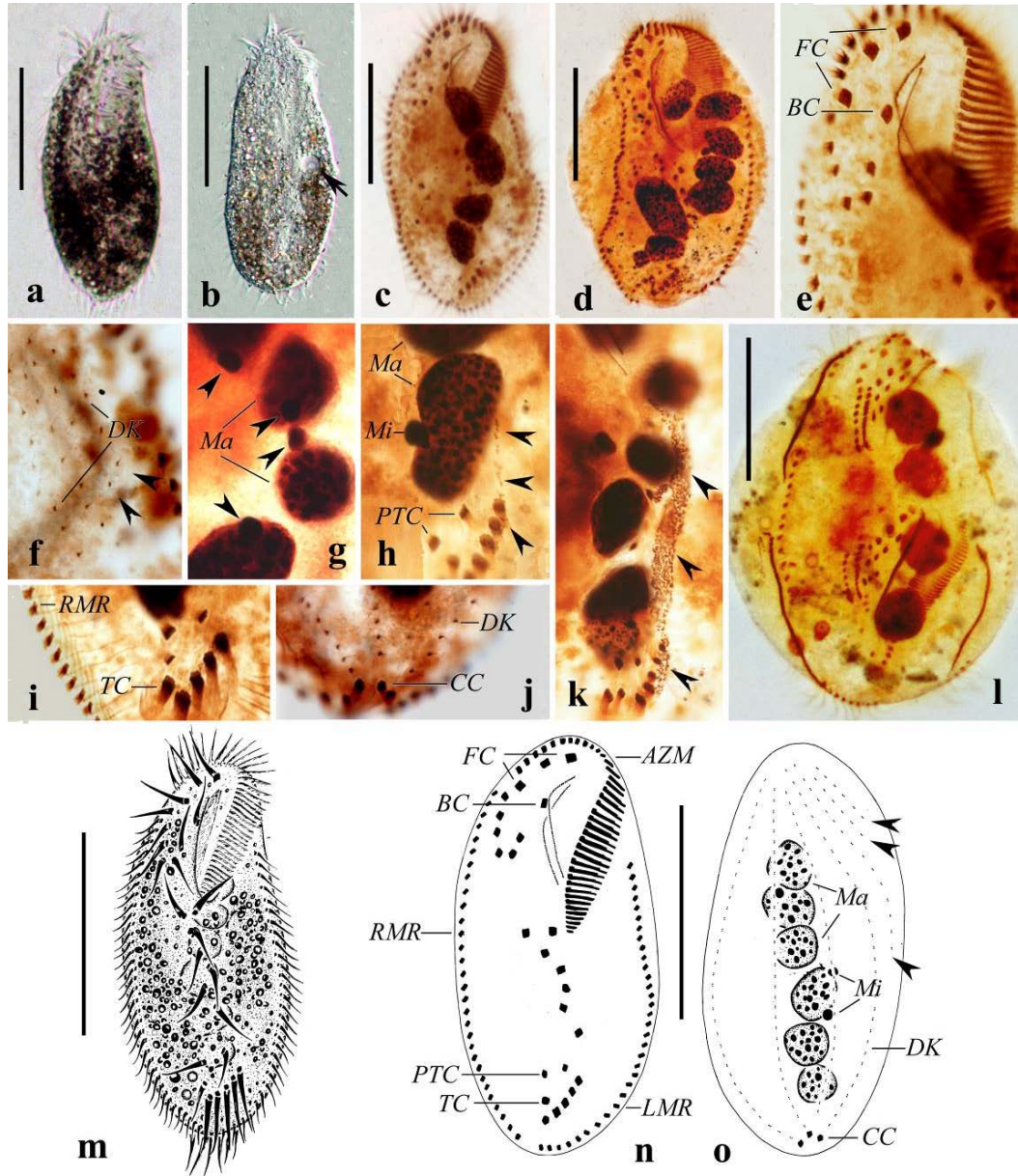


Fig. 1: *Gastrostyla steinii* from life (a, b, m) and after protargol impregnation (c-l, n, o). The a, b) typical individuals, arrow in (b) marks the contractile vacuole; c, d) ventral views of infraciliature; e) detail view of anterior end to show frontal cirri and buccal cirrus; f) part of dorsal view, arrowheads indicate posteriorly shortened dorsal kineties; g) detail view of macronuclei and micronuclei (arrowheads); h) early morphogenetic stage, arrowheads mark the origination of opisthe's oral primordium which is near anterior transverse cirri; i) posterior end to show transversal cirri and right marginal row; j) dorsal view of posterior end, noting the caudal cirri; k) early morphogenetic stage, indicating the development of opisthe's oral primordium (arrowheads); l) late morphogenetic stage, marking the completion of development of frontal-midventral-transverse cirral anlagen; m) ventral view of a typical individual; n, o) ventral and dorsal views of infraciliature, arrows in (o) indicate the posteriorly shortened dorsal kineties. AZM: Adoral Zone Membranelles; BC: Buccal Cirrus; CC: Caudal Cirri; DK: Dorsal Kinety; FC: Frontal Cirri; LMR: Left Marginal Row; Ma: Macronuclear nodules; Mi: Micronuclei; PTC: Pretransverse Cirri; RMR: Right Marginal Row; TC: Transverse Cirri; Scale bars = 100 μ m in (a, b, m); 65 μ m in (c, d, l, n, o)

macronuclei (4-8 vs. 4-6) and the number of dorsal kineties (7 vs. 6). These differences could be population dependent. Moreover, no caudal cirri were reported in original article (Engelmann, 1862) however, Foissner (1982) indicated that the last 3-5 cirri of left marginal row are smaller and slightly separated and thus were presumable caudal cirri. While in present study, three caudal cirri were clearly observed and this proves Foissner's speculation. The misleading original description might be explained by unadvanced technics in 19th century. *Gastrostyla muscorum* was separated

from *G. steinii* only by the number of macronuclei (8 vs. 4-6 in original population) (Kahl, 1932; Berger, 1999). In the present population, number of macronuclei of *G. steinii* may ranges from 4-8. Thus, *G. muscorum* should be synonymized with *G. steinii*.

***Hemiamphisiella granulifera*:** Body size *in vivo* about 150-250×35-50 μm with ratio of length to width about 5:1 (Foissner, 1987b, 1988) (Fig. 2a-f), body dorsoventrally flattened and flexible with a long pointed posterior end (Fig. 2a-d). Contractile vacuole, 15 μm in diameter, ahead of the equator and near left cell margin (Fig. 2b, c). Cortical granules colorless and globular, ca. 0.8 μm in diameter. Two or three macronuclear nodules, ca. 35×12 μm, slightly left of midline (Fig. 2e, f). One or two micronuclei globular, closely attached to macronuclear nodules (Fig. 2f). Adoral zone of membranelles composed of about 32 membranelles, three frontal cirri and one buccal cirrus. One cirrus behind right frontal cirrus and one post-peristomial cirrus. Amphisiellid median cirral row composed of ca. 35 cirri. Right and left marginal row composed of ca. 44 and 38 cirri, respectively. Always three dorsal kineties and two caudal cirri. This species was originally found in Kenya, East Africa and well described under the name of *Strongylidium granuliferum* (Foissner, 1987b). Then, it was combined with

Table 1: Morphometric data on *Gastrostyla steinii*

Character	Min.	Max.	Mean	SD	CV	n
Body length	117	200	157.2	18.6	11.9	19
Body width	60	110	82.6	13.3	16.1	19
Length of buccal field	55	90	74.1	10.7	14.4	19
No. of adoral membranelles	37	65	52.2	6.5	12.5	21
No. of frontoventral cirri	19	23	20.8	1.1	5.2	19
No. of transverse cirri	5	7	5.6	0.7	12.4	19
No. of left marginal cirri	29	44	35.4	3.7	10.3	19
No. of right marginal cirri	32	44	38.7	3.2	8.4	19
No. of caudal cirri	3	3	3.0	0.0	0.0	19
No. of dorsal kineties	7	7	7.0	0.0	0.0	19
No. of macronuclei	4	8	5.2	1.1	20.8	19
No. of micronuclei	1	4	2.7	0.7	23.9	19

All data are based on protargol-impregnated specimens; Measurements in μm; CV: Coefficient of Variation in %; Max.: Maximum; Mean, median; Min.: Minimum; N: no. of specimens investigated; SD: Standard Deviation

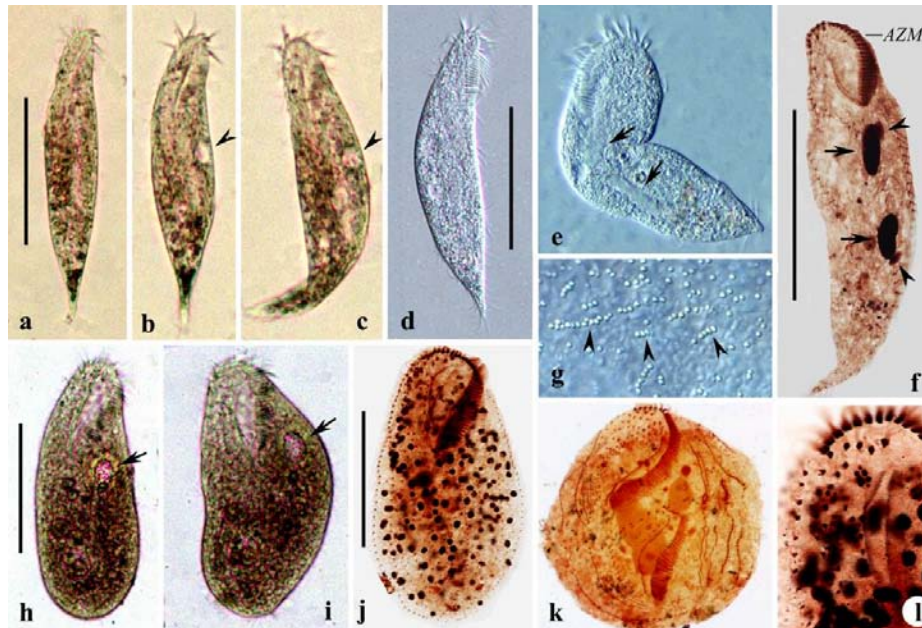


Fig. 2: Photomicrographs of *Hemiamphisiella granulifera* (a-f) and *Urostyla grandis* (g-l) from life (a-e, g, h, i) and after protargol (f, j-l). From a-d) typical individuals, arrow in b, c) marks the contractile vacuole; e) arrows show two macronuclei; f) general view of infraciliature, arrows show macronuclei and arrowheads indicate micronuclei; g) the distribution of cortical granules (arrowheads); h, i) typical individuals, arrow shows contractile vacuole; j) general view of infraciliature; k) ventral view of middle morphogenetic stages; l) infraciliature of anterior portion. AZM: Adoral Zone Membranelles. Scale bars = 100 μm in (a, d, g); 130 μm (h, j)

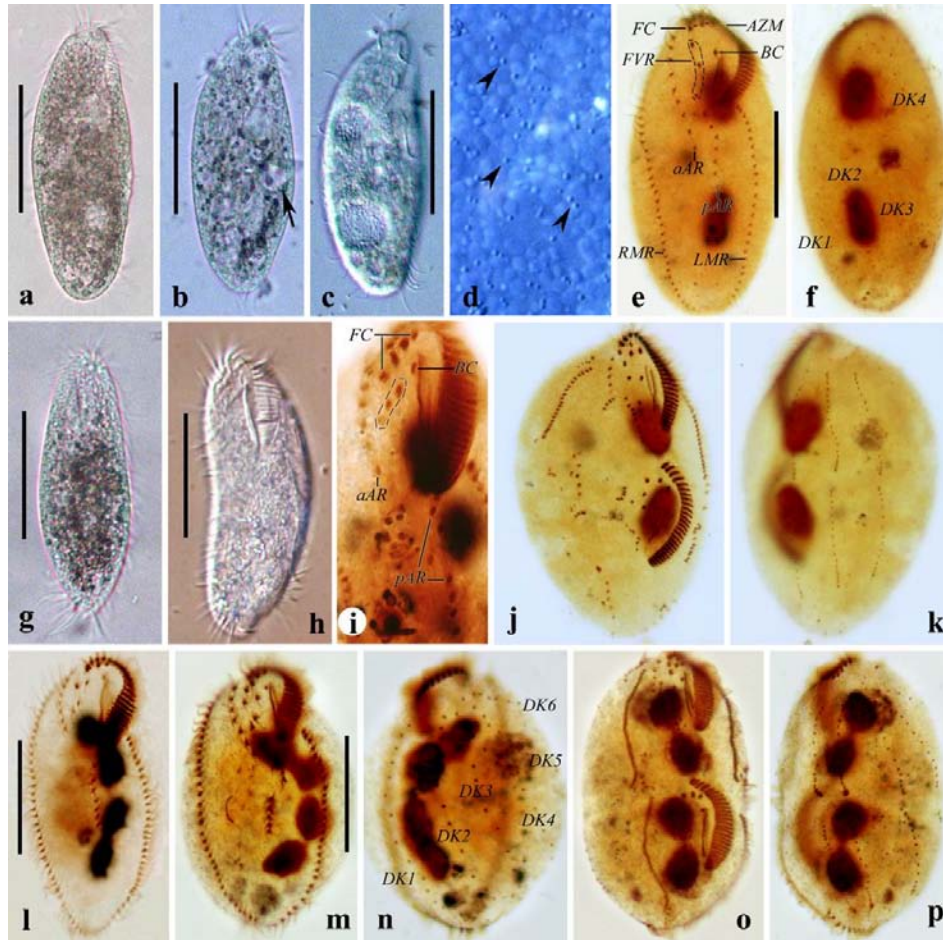


Fig. 3: Photomicrographs of *Nudiamphisiella interrupta* (a-f, j, k) and *Nudiamphisiella cf. illuvialis* (g-i, l-p) from life (a-d, g, h) and after protargol (e, f, i-p); a-c) ventral views of typical individuals, arrow in (b) marks contractile vacuole; d) cortical granules (arrowheads); e, f) ventral and dorsal view of infraciliature; g, h) typical individuals; i) infraciliature of anterior portion, broken line circled the frontoventral row; j, k) ventral and dorsal view of late morphogenetic stages; l, m) ventral view of infraciliature; n) dorsal view of infraciliature; o, p) ventral and dorsal view of late morphogenetic stages. aAR: Anterior Piece of the Amphisiellid Cirral Row; AZM: Adoral Zone Membranelles; BC: Buccal Cirrus; DK: Dorsal Kinetid; FC: Frontal Cirri; FVR: Frontoventral Row; LMR: Left Marginal Row; pAR: Posterior Piece of the Amphisiellid Cirral Row; RMR: Right Marginal Row. Scale bars = 100 µm in (a-c); 65 µm in (e-h, l, m)

Hemiamphisiella by Foissner (1988). The population corresponds well with previous one, so the identification is no doubt.

Urostyla grandis: Cell *in vivo* about 150-300×60-90 µm, light brown in colour. Body flexible and contractile (Fig. 2g-l) elliptical in shape with anterior and posterior end broadly rounded (Fig. 2h, i). Cytoplasm packed with numerous granules, about 1-3 µm across. Contractile vacuole near left body margin before the equator (Fig. 2h, i). Cortical granules colorless, 1 µm in diameter (Fig. 2g). About >100 macronuclear nodules scattered

throughout cytoplasm (Fig. 2j). Adoral Zone of Membranelles (AZM) composed of 40-47 membranelles. 5-9 buccal cirri (Fig. 2l). About 7-11 transversal cirri. The 4-6 left and 4-5 right marginal rows (Fig. 2j). Always three dorsal kineties. This species has been redescribed by Song and Wilbert (1989). The population corresponds well with previous studies.

Nudiamphisiella interrupta: Cell *in vivo* about 150-250×40-50 µm. Body elongate elliptical with posterior end broadly rounded (Foissner *et al.*, 2002) (Fig. 3a-f, j, k). Two macronuclear nodules slightly left of midline and

1-3 micronuclei (Fig. 3e, f). Cortical granules, >0.5µm in diameter, widely spaced (Fig. 3d). Adoral Zone of Membranelles (AZM) composed of about 27 membranelles, three Frontal Cirri (FC) and one Buccal Cirrus (BC).

Frontoventral Row (FVR) behind the right frontal cirrus, composed usually of three or four cirri. Ventrally a median Amphisiellid cirral Row (AR) composed of two pieces, the anterior piece of ca. 8 cirri located right of frontoventral cirri and the posterior piece of 6-10 cirri commenced left of the posterior end of anterior piece. The right and left marginal row composed of ca. 28 and 21 cirri, respectively with three caudal cirri and four dorsal kineties (Fig. 3e, f).

This original population was reported in Namibia, Africa and well described mainly on morphology and infraciliature (Foissner *et al.*, 2002). Morphogenesis study was then performed based on a Brazilian strain (Paiva and Silva-Neto, 2009). The population corresponds well with the previous descriptions, so the identification is no doubt.

Nudiamphisiella cf. illuvialis: Cell size *in vivo* about 100-200×30-50 µm. Body lanceolate with anterior and posterior end distinctly tapering (Eigner and Foissner, 1994) (Fig. 3g-i, l-p). Two to four macronuclear nodules left of midline, ellipsoidal (Fig. 3l, m). Contractile vacuole, 10 µm in diameter, slightly ahead of mid-body and near left cell margin. Adoral Zone of Membranelles (AZM) composed of 24-27 membranelles.

Three or Four Frontal Cirri (FC) and One Buccal Cirrus (BC). Frontoventral Row (FVR) behind the right frontal cirrus, composed of 2 or 3 cirri. Ventrally a median Amphisiellid cirral Row (AR) composed of two pieces, the anterior piece of about 7 cirri located right of frontoventral cirri and the posterior piece of 5 or 6 cirri commenced left of the posterior end of anterior piece. The right and left marginal row composed of 35-46 and 27-34 cirri, respectively. The 3-5 caudal cirri and five or six dorsal kineties (Fig. 3i-n).

This specie is fairly similar to *Nudiamphisiella illuvialis* in the general morphology. While the significant difference is no cirri row (transverse cirri *sensu*) between marginal rows at rear portion present in the organism as in *N. illuvialis*. For accurate comparison, more well prepared samples and molecular information are needed in future investigations (Eigner and Foissner, 1994).

CONCLUSION

Thus, a survey on the soil ciliates from Saudi Arabia was carried out and consequently five hypotrichous

ciliates were collected and morphological studied. All of them are first records of Saudi Arabia and descriptions were supplied here.

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