Species of *Gonostomum* and *Paragonostomum* (Ciliophora, Hypotrichida, Oxytrichidae) from the Valley of Flowers, India, with descriptions of *Gonostomum singhii* sp nov, *Paragonostomum ghangriai* sp nov and *Paragonostomum minuta* sp nov

Komal Kamra · Santosh Kumar · G.R. Sapra

Received: 30 July 2007 / Accepted: 21 February 2008

Abstract Soil samples taken from the Valley of Flowers, a component of the Nanda Devi Biosphere Reserve in the Himalayan regions of India showed the presence of twenty two free living species of ciliates. There is a preponderance of species which exhibit oral ciliature and ontogenesis in the *Gonostomum* pattern. Of the four species of the genus *Gonostomum*, three viz., *G. affine*, *G. gonostomoida* and *G. kuehnelti* are similar to described species; *Gonostomum singhii* is new. The two species of genus *Paragonostomum* viz., *P. minuta* and *P. ghangriai* are new. The three new species are described in the present paper. All these species show prominent hypertrophied ciliary structures. Their paroral membranes reveal characteristic differences with respect to their position, number of constituent cilia and the distance between adjacent cilia. It is proposed that such species specific features of the paroral membrane have a bearing in exercising different food organism preferences as they co-exist at many sites. This single factor has possibly

played an important role in species diversification of this group of hypotrichs in this isolated habitat.

Keywords Soil ciliates · Valley of flowers · *Gonostomum-Paragonostomum* · Paroral differences · Evolutionary significance

Introduction

Ciliated protozoa are almost cosmopolitan and are being catalogued from varied biomes [1–4]. These single celled organisms form an important component of soil biota playing a major ecological role in the food web by forging links between heterotrophic bacteria and primary consumers [5]; they are increasingly being used as bio-indicators of soil pollutants [2]. Soil ciliates are ubiquitous but poorly explored. Based on the data from five subcontinents and 359 soil samples, Chao et al [4]. have compiled a list of 964 soil ciliates, 644 of which are described. Applying various statistical methods, the above authors predict that nearly half of the surviving soil ciliates are yet to be discovered. This estimate is likely to undergo a substantial upward revision, when one considers that the large Indian subcontinent with diverse and favourable tropical conditions has not been explored. The present study is a part of a project to fill this void, carried out on the soil ciliates from the world famous Valley of Flowers. It is one of the two core zones in the Nanda Devi Biosphere Reserve (UNESCO Man and Biosphere Programme) in the Himalayan regions of India.

K. Kamra¹ (✉) · Santosh K¹ · G.R. Sapra²

¹Sri Guru Tegh Bahadur Khalsa College,
University of Delhi,
Delhi-110007

²Professor (retired),
Department of Zoology,
University of Delhi, Delhi - 110 007,
India

e-mail: komalkamra@yahoo.com

Fig. 1. Map of the Valley of Flowers National Park. The sampling sites were along the permitted trek route (arrow). 1-6: Summits surrounding the Valley, 1: Nar Parbat, 2: Nilgiri Parbat, 3: Rataban, 4: Gauri Parbat, 5: Saptasring, 6: Kunt Khal; 7: Ghangria (a temporary establishment at the periphery of the protected park. Source – Office of the Divisional Forest Officer, Nanda Devi National Park, Joshimath.

The valley lies between $30^{\circ} 41'$ to $30^{\circ} 48'N$ and $79^{\circ} 33'$ to $79^{\circ} 46'E$. Situated in the far interior of the Chamoli Garhwal Himalayas at a height of 3250m–6750m above the sea level, the Valley of Flowers is spread over an area of 87.5 sq Kms [6]. It is nestled among snow-clad summits: Nar Parbat (5,245 m) to the northwest, Nilgiri Parbat (6,479 m) to the north, Rataban (6,126 m) and Gauri Parbat (6,708 m) to the east Saptasring (5,038 m) to the south and Kunt Khal, (5,855 m) to the south west. The valley forms the upper catchments of the river Bhyundar, which drains into the river Alaknanda, a major tributary of the river Ganga (Fig. 1).

The well exposed bedrock comprises crystallines of the Vaikrita group with sedimentary, mica schist and shale rocks. The Valley of Flowers has the microclimate of an enclosed inner Himalayan valley. There is often dense fog and rain especially during the late summer monsoon. The valley has an unusually rich flora of over 600 species with many rarities such as orchids, poppies, primulas, calendulas, daisies and anemones; it has many plants of medicinal value. The density of wild animals in the Valley is not high but all the animals found are nationally rare or endangered. There are 13 species of mammals, 114 species of birds, and many species of bees and butterflies. The valley itself is devoid

of human habitation. There is no record of the microfauna except for one study on diatoms [7].

The valley is covered with permafrost for eight months [6]. From May to August, it erupts with several flowering communities, the dominant forms replacing one another. During this period, the mean daily temperature of the valley ranges from $8.8-22^{\circ}C$ (max) and $3.0-10.5^{\circ}C$ (min). The relative humidity varies between 80–100%. The soil is acidic, pH ranging from 3.8 to 6.2, and has high humus content. The soil nitrogen ranges from 0.7% to 1.75% and the organic carbon ranges from 8.92% to 33.87%.

The soil samples showed presence of twenty two free living ciliate species of which seventeen species were hypotrichs. Among these, the preponderance of several species exhibiting oral ciliature and ontogenesis in the *Gonostomum* pattern, six in all, was striking. The present paper describes these six species belonging to two genera, viz., *Gonostomum* [8] and *Paragonostomum* [9].

Material and methods

Soil samples were collected from 24 sites along the accessible areas of the valley taken from 0–10 cms depth. Sam-

Fig. 2. Line diagrams of protargol impregnated non-dividers of *Gonostomum affine* Indian population, a) ventral surface b) dorsal surface c) nuclear apparatus (macronuclei are outlined while micronuclei are filled in). Abbreviations (for all line diagrams in the text): AZM – adoral zone of membranelles, PM – paroral membrane, EM – endoral membrane, BC – buccal cirri, FC – frontal cirri, FV – fronto-ventral cirri, FT – fronto-terminal cirri, POVC – post-oral ventral cirri, PTV – pre-transverse ventral cirri, TC – transverse cirri, LMC – left marginal cirri, RMC – right marginal cirri, DK₁₋₃ – dorsal kineties 1-3, CC₁₋₃ – caudal cirri, Ma – macronuclear nodule, Mi – micronucleus. Bar represents 10 μ m.

ples were obtained during the early, mid and late flowering periods of a season.

All collections were analyzed in the laboratory with the non-flooded petridish method [2, 10–12]. Briefly, this simple method involves placing 100 g terrestrial material in a petridish (10–15 cm in diameter) and saturating but not flooding it with distilled water. About 2 ml of the run-offs were drawn from the petridish on days 2, 7, 14, 21, and 28 and were analyzed for ciliates. It was not possible to culture these ciliates in the laboratory despite varying culturing conditions: temperature, pH, food organisms. The data presented here are from mixed samples.

Live observations were made using differential interference contrast microscope. Protargol staining [13] was used for visualizing surface structures including ciliature. Biometric characterization was done at a magnification of 1000 X directly from the Leica software IM50 image manager; Leica camera DFC320 was also employed for photography. Line diagrams were prepared using Corel graphics software.

Nomenclature and terminology is based on Borror [14] and Foissner [9, 15].

Results and discussion

The 22 free living soil ciliate species belonging to 17 genera are listed in table 1; these include 5 non – hypotrichs and 17 hypotrichs.

The genera *Gonostomum* and *Paragonostomum* share the following synapomorphies: a characteristically shaped adoral zone of membranelles (AZM), a peculiar paroral membrane (PM) consisting of few, well spaced cilia, endoral membrane (EM) made of tightly packed cilia, flat and small buccal cavity, morphogenesis characterized by the presence of primary primordial [9, 16–20]. However, *Paragonostomum* lacks transverse cirri, the only reported oxytrichid genus to have this feature [9]. The frontal ciliature of these genera is species specific being present in cirral rows (FVs) or in groups of cirri: buccal (BC), frontal (FC), fronto-ventral (FV), fronto-terminal (FT), post-oral ventral* (POVC), pre-transverse ventral (PTVC) and

*Berger [16] suggests the term “post-oral ventral” for these cirri even though they do not go beyond the AZM tip because of their morphogenetic affiliation to the post-oral ventral cirri in the family Oxytrichidae.

transverse (TC). All reported species of these genera possess one row each of left marginal cirri (LMC) and right

marginal cirri (RMC), three dorsal kinetics (DKs) and three caudal cirri (CCs).

Table 1. List of 22 free living soil ciliate species from the Valley of Flowers.

S. No.	GROUP	SPECIES
1	Non - hypotrichs	<i>Arcuospathidium</i> sp
2		<i>Platyophrya</i> sp
3		<i>Colpoda inflata</i>
4		<i>Tetrahymena rostrata</i>
5		<i>Vorticella astyliiformis</i>
6	Hypotrichs	a stichotrichine (yet to be identified)
7		<i>Anteholosticha australis</i>
8		<i>Caudiholosticha</i> sp
9		<i>Birojimia</i> sp
10		<i>Fragmocirrus</i> sp
11–16		4 species of <i>Gonostomum</i> and 2 species of <i>Paragonostomum</i>
17, 18		<i>Oxytricha setigera</i> , <i>Oxytricha</i> sp
19		<i>Cyrtohymena citrina</i>
20		<i>Notohymena</i> sp
21		<i>Urosomoida agilis</i>
22		<i>Stylonychia</i> sp

Of the six species of this group of oxytrichids found in the soil samples from the Valley of Flowers, four belong to the genus *Gonostomum* and two to the genus *Paragonostomum*. The synapomorphies of the two genera prompted the authors to describe these six species in the present paper. Figure 2 gives line diagrams of protargol impregnated *G. affine* cells from the Valley of Flowers as an example.

Most of our collection sites showed the presence of two or more species of the genera *Gonostomum* and *Paragonostomum*; one particular site had four species. Protargol preparations of mixed cultures showed sufficient number of dividers to reveal morphogenetic stages and the data were conclusive enough to designate these species to the *Gonostomum* pattern of ontogenesis.

One new species of *Gonostomum*, viz., *G. singhii* sp nov, and two new species of *Paragonostomum*, viz., *Paragonostomum ghangriai* sp nov and *Paragonostomum minuta* sp nov are described below. Morphometric comparisons of three species of *Gonostomum*, viz., *G. affine*, *G. gonostomoida* and *G. kuehnelti* have been made with published data.

Fig. 3. a) live cell (dorsal surface) and b–d) line diagrams of a protargol impregnated non-divider of *Gonostomum singhii* sp nov, b) ventral surface c) dorsal surface d) nuclear apparatus. For labelling, refer Fig. 2. Bar represents 10 μ m.

Table 2. Morphometric data* on *Gonostomum singhii* sp nov.

Characters	Mean	Max	Min	SD	CV
Body length, μm	66.2	76.3	60.8	4.93	7.44
Body width, μm	22.8	27.0	18.0	2.58	11.34
Length: width ratio	2.9	3.5	2.3	0.34	11.82
Macronucleus no	12	2	2	0	0
Macronucleus length, μm	10.6	12.9	8.6	1.28	12.07
Macronucleus width, μm	5.8	7.0	4.5	0.87	15.03
Micronucleus no	2	2	2	0	0
Micronucleus diameter, μm	2.0	2.4	1.4	0.26	13.28
Length of AZM, μm	32.5	35.7	29	2.28	7.00
AZM: body ratio percent	49.2	56.3	40.5	4.23	8.6
AM no	26.5	28	25	1.15	4.35
Anterior end to beginning of PM, μm	17.1	20.0	14.6	1.34	7.83
Anterior end to end of PM, μm	27.8	30.9	24.7	1.46	5.26
Length of PM, μm	10.6	11.2	10.1	0.26	2.45
Number of kinetosomes in PM	14.8	16	14	0.79	5.32
Gap between kinetosomes in PM	0.8	0.9	0.7	0.81	10.13
Anterior end to beginning of EM, μm	22.9	25.6	20.3	1.52	6.66
BC, no	1	1	1	0	0
FC, no	3	3	3	0	0
FT, no	2	2	2	0	0
FV, no	2	2	2	0	0
POVC, no	3	3	3	0	0
PTVC, no	2	2	2	0	0
TC, no	4	4	4	0	0
RMC, no of rows	1	1	1	0	0
RMC, no of cirri	16.6	18	15	0.94	5.63
LMC, no of rows	1	1	1	0	0
LMC, no of cirri	11.5	14	10	1.25	10.84
DKs, no	3	3	3	0	0
Bristles in DK ₁	18	22	16	1.56	8.68
Bristles in DK ₂	15.5	16	15	0.53	3.40
Bristles in DK ₃	21.8	25	19	1.94	8.89
CC, no	3	3	3	0	0

* Data based on protargol stained non-dividers obtained from a non-flooded petridish culture; n =10, Max=maximum, Min=minimum, SD=standard deviation, CV=coefficient of variation (%).

Gonostomum singhii sp nov (Figs. 3, 9 a–e, table 2)

Diagnosis: Average size of non dividers *in vivo* 71 X 26 μm , protargol stained cells 66 X 23 μm ; cortical granules colourless and scattered; 1 contractile vacuole; 2 macronuclei, 2 micronuclei; AZM 50% of body length, PM made of 14–16 widely spaced cilia, EM with tightly packed cilia; 17 FVT cirri – 1 BC, 3 FC, 2 FV, 2 FT, 3 POVC, 2 PTVC, 4 TC; 1 LMC row and 1 RMC row; 3DKs and 3CCs; cirri

long and highly hypertrophied; ontogenesis in *Gonostomum* pattern

Type location: Soil samples from the Valley of Flowers National Park, India, 30° 41' to 30° 48'N and 79° 33' to 79° 46'E.

Etymology: The species name is derived from the term *singhi*, the local name of the common Indian food

fish *Heteropneustus fossilis*, as the anterior membranelles of the AZM of the ciliate resemble the long barbules of the fish.

Type Material: One protargol impregnated slide containing well stained specimens has been sent to The Natural History Museum, Cromwell Road, London SW7 5BD, UK.

Description: Body length and width of live cells averages $71 \times 26 \mu\text{m}$ and those of protargol impregnated non dividers range between $60.8\text{--}76.3 \mu\text{m}$ and $18.0\text{--}27.0 \mu\text{m}$ respectively. The cortex is scattered with colourless granules that are present in no particular arrangement. They do not stain with protargol. The single contractile vacuole is present just below the AZM tip along the left side of the body. The nuclear apparatus consists of 2 oblong macronuclei and 2 spherical micronuclei. The AZM occupies 50% of the body length; the middle segment of the AZM is straight and situated along the left margin of the cell and the proximal portion curves inwards towards the centre of the body. The PM is long (16% of body length) composed of 14–16 widely spaced cilia ($0.8 \mu\text{m}$ between two adjacent cilia) while the EM consists of tightly packed cilia; the PM extends far beyond the anterior end of the EM. The FVT cirri are as follows: 1 BC, 3 FC, 2 FT, 2 FV, 3 POVC, 2 PTVC, 4 TC. There is one row each of LMC

with 10–14 cirri and RMC with 15–18 cirri. Dorsal ciliature consists of 3 DKs and 3 CCs. Morphogenesis is typified by the presence of primary frontal-ventral-transverse (FVT) primordia.

A notable feature of *G. singhii* is the presence of very long and highly hypertrophied cirri: frontal cirri $17\text{--}18 \mu\text{m}$, fronto-ventral, fronto terminal and post orals $11\text{--}13 \mu\text{m}$, pre-transverse ventral cirri $10\text{--}11 \mu\text{m}$, transverse cirri $16\text{--}18 \mu\text{m}$, marginal cirri $10\text{--}13 \mu\text{m}$, and the caudal cirri $11\text{--}13 \mu\text{m}$. The transverse cirri differ from the pre-transverse ventral cirri in the extent of hypertrophy, the former being thicker. The dorsal cilia are $2\text{--}3 \mu\text{m}$ long. For a cell which is, on an average, $66 \times 23 \mu\text{m}$ in size, the ciliary structures are strong.

Comparison with related species: *G. singhii* sp nov differs from the other recorded species of the genus by the presence of invariably 2 pre-transverse ventral cirri and 4 transverse cirri; the 4 transverse cirri are very long and hypertrophied. This configuration is also seen in *G. namibiense* [9] which is a tailed form. Arrangement of the FVT cirri and the presence of long, hypertrophied cirri are similar to that seen in *G. kuehnelti* [10] but, the latter has multiple macronuclear nodules. Eigner [21] reported an Austrian population designated as clone IV of *G. affine* which appears to be quite similar to *G. singhii*; however, in the absence of

Fig. 4. a) live cell (ventral surface) and b–d) line diagrams of a protargol impregnated non-divider of *Paragonostomum ghangriai* sp nov, b) ventral surface c) dorsal surface d) nuclear apparatus. For labelling, refer Fig. 2. Bar represents $10 \mu\text{m}$.

Table 3. Morphometric data* of *Paragonostomum ghangriai* sp nov.

Characters	Mean	Max	Min	SD	CV
Body length, μm	72.2	77.9	66.6	3.99	5.52
Body width, μm	21.2	23.8	18.7	1.50	7.07
Length: width ratio	3.4	3.6	2.9	0.22	6.51
Macronucleus, no	18.0	21	13	3.02	16.77
Macronucleus diameter, μm	4.1	5.4	3.2	0.79	19.20
Micronucleus, no	4.9	6	4	0.74	15.04
Micronucleus diameter, μm	1.5	1.8	1.2	0.15	10.27
Length of AZM, μm	32.6	34.0	29.5	1.53	4.70
AZM: body ratio percent	45.1	50.9	42.6	2.92	6.47
AM no	29.4	37	24	3.78	12.85
Anterior end to beginning of PM, μm	16.3	17.3	15.6	0.56	3.42
Anterior end to end of PM, μm	17.9	18.7	17.1	0.52	2.89
Length of PM, μm	1.6	2.1	1.3	0.19	11.71
Number of kinetosomes in PM	3	4	2	0.47	15.70
Gap between kinetosomes in PM, μm	0.3	0.3	0.2	0.03	10.36
Anterior end to beginning of EM, μm	18.3	19.3	17.8	0.47	2.57
FVs, no	5	5	5	0	0
FV ₁ , no of cirri	1	1	1	0	0
FV ₂ , no of cirri	4	4	4	0	0
FV ₃ , no of cirri	7	8	6	0.47	6.73
FV ₄ , no of cirri	11	12	10	0.67	6.05
FV ₅ , no of cirri	21.9	23	21	0.57	2.59
RMC row, no	1	1	1	0	0
RMC, no of cirri	23.5	25	21	1.35	5.76
LMC row, no	1	1	1	0	0
LMC, no of cirri	19.1	22	15	1.97	10.31
DKs, no	3	3	3	0	0
Bristles in DK ₁	11.4	18	9	2.91	25.55
Bristles in DK ₂	14.5	16	12	1.35	9.34
Bristles in DK ₃	16.3	21	13	2.16	13.26
CC, no	3	3	3	0	0

* Data based on protargol stained non-dividers obtained from a non-flooded Petridish culture; n=10, Max=maximum, Min=minimum, SD=standard deviation, CV=coefficient of variation (%).

morphometric data of the Austrian ‘clone’ a comparison between the two is not possible. When further data are obtained for this population, it may be assigned to the species *G. singhii* if found similar. *G. singhii* and *G. affine* differ with respect to their FVT cirri. The number and position of the FVT cirri in *G. singhii* is an invariable character in contrast to the variability of these cirri within *G. affine*. Additionally, the presence of 2 pre-transverse ventral cirri and 4 transverse cirri in *G. singhii* is a stable characteristic feature of the species.

***Paragonostomum ghangriai* sp nov** (Figs. 4, 11a–h, table3)

Diagnosis: Body elongated without a tail; mean size *in vivo* of non dividers $75 \times 22 \mu\text{m}$, protargol stained cells range between $67\text{--}78 \mu\text{m} \times 19\text{--}24 \mu\text{m}$; cortical granules colourless and scattered; extrusomes present; single contractile vacuole, 13–21 macronuclei, 4–6 micronuclei; AZM 40% of body length, PM 2–4 cilia, sometimes bipartite, EM made of tightly packed cilia; 5 fronto-ventral rows (FV_{1–5}), transverse cirri absent; 1 LMC row and 1 RMC

row; 3DKs and 3CCs; cirri long; ontogenesis in *Gonostomum* pattern

Type location: Soil samples from the Valley of Flowers National Park, India, 30° 41' to 30° 48'N and 79° 33' to 79° 46'E.

Etymology: The species was present in abundance in the samples taken closest to the small temporary settlement named *Ghangria* located at the periphery of the protected region of the valley.

Type material: One protargol impregnated slide containing well stained specimens has been sent to The Natural History Museum, Cromwell Road, London SW7 5BD, UK.

Description: The body is elongated and slightly sigmoidal, with a rounded posterior end, and lacks a tail. Length and width of live cells averages $75 \times 22 \mu\text{m}$. The cells move fast forwards with jerks, sometimes rolling over sideways and length – wise. Scanty colourless granules are dispersed in the cortex; they do not stain with protargol. Fat globules are present, more in the posterior region of the cell. The single contractile vacuole is present just below the AZM tip, a little left of the mid – body. The species is characterized by the presence of extrusomes throughout the body in no particular arrangement. Protargol impregnated non dividers range between 66.6–77.9 μm long and 18.7–23.8 μm broad. Extrusomes measure $1.6\text{--}2.1 \times 0.6\text{--}0.7 \mu\text{m}$. The nuclear apparatus consists of 13–21 macronuclei and 4–6 micronuclei. The AZM occupies 45% of the body length; the middle segment of the AZM is straight and situated along the left margin of the cell and the proximal portion curves inwards towards the centre of the body. The PM is usually composed of 2–4 cilia, sometimes in a bipartite arrangement (six cilia seen in one cell), while the EM consists of tightly packed cilia. There are five fronto-ventral rows (FV_{1–5}) with 1, 4, 7, 11, and 22 cirri on an average; transverse cirri are absent. There is one row each of LMC and RMC. Dorsal ciliature consists of 3 DKs and 3 CCs. For biometry, refer table 3. Morphogenesis is typified by the presence of primary primordia for the frontal ciliature.

Comparison with related species: The genus *Paragonostomum* was erected by Foissner [9] for four tailed species which shared two synapomorphies with the genus *Gonostomum*: a typical oral ciliature [18] and the mode of ontogenesis in the *Gonostomum* pattern [16]. However, they all lacked transverse cirri, significantly the only oxytrichids to have this feature. Subsequently, the report of a tail-less species of the genus, *Paragonostomum simplex* [17], established the lack of transverse cirri as the landmark feature of

the genus. *P. ghangriai* sp nov is thus placed in the genus *Paragonostomum*.

P. ghangriai sp nov differs from the only species of the genus without a tail, *P. simplex* [17] in the following ways – the PM in *P. ghangriai* sp nov is distinctively made of 2–4 cilia sometimes in a bipartite arrangement whereas in *P. simplex* the PM is made up of a continuous row of an average of 9 cilia; there are five fronto-ventral rows in *P. ghangriai* sp nov with 1, 4, 7, 11, and 22 cirri on an average as against 16 cirri in the oral region (1 BC, 3 FC, 8 FV, 4 FT) in *P. simplex*, and the nuclear apparatus in *P. ghangriai* sp nov consists of a larger number (~18) of almost spherical macronuclear nodules and 4–6 micronuclei as against (~10) macronuclei and 1–5 micronuclei in *P. simplex*. Additionally, in *P. ghangriai* sp nov, the cirri of the left marginal row become compacted posteriorly and tend to merge with the caudal cirri. The most prominent feature of the species is the presence of extrusomes which stain with protargol.

Paragonostomum minuta sp nov (Figs. 5, 10 a–f)

Diagnosis: Body oblong without a tail, pointed posterior end; mean body size of protargol stained non dividers $33 \times 12 \mu\text{m}$; 7–8 oval macronuclei, 2 spherical micronuclei; AZM 50% of body length, PM with 2–3 cilia, EM made of tightly packed cilia; frontal ciliature lacks transverse cirri, 7 cirri in the frontal (oral) region and one fronto-terminal row with 12 cirri; one row each of LMC and RMC with 4 and 17 cirri on an average; 3DKs and 3CCs; all cirri highly hypertrophied and long

Type location: Soil samples from the Valley of Flowers National Park, India, 30° 41' to 30° 48'N and 79° 33' to 79° 46'E.

Etymology: This is the smallest reported species of the genus, hence the species name *minuta*.

Type material: One protargol impregnated slide containing well stained specimens has been sent to The Natural History Museum, Cromwell Road, London SW7 5BD, UK.

Description: The body is oblong with a pointed posterior end; the right margin of the body is markedly convex. The cells constantly roll over soil particles; it is difficult to dislodge them from the latter without breaking the cells due to the small body size and large ciliary structures. Live cell photomicrography could only be done taking soil particles along with the cells on the slide, giving slightly hazy pictures. Cortical granules are few and scattered. One contractile vacuole is positioned near the mid body, close to the

Fig. 5. a) live cell (ventral surface) and b–d) line diagrams of a protargol impregnated non-divider of *Paragonostomum minuta* sp nov, b) ventral surface c) dorsal surface d) nuclear apparatus. For labelling, refer Fig. 2. Bar represents 10 μ m.

AZM. Body size of protargol impregnated non dividers averages $33 \times 12 \mu$ m. The nuclear apparatus consists of 7–8 macronuclei and usually 2 micronuclei. The AZM occupies 50 % of body length with about 20 membranelles, middle segment of the AZM straight and situated along the left margin of the cell and the proximal portion curves inwards towards the centre of the body. The PM is composed of 2–3 cilia beginning 9.4μ m from the anterior end of the cell, with closely spaced cilia (0.3μ m between two adjacent cilia). The EM consists of tightly packed cilia beginning about 10μ m from the anterior end of the cell. There are seven cirri in the frontal (oral) region, one of them close to the PM. In addition there is one fronto-terminal row with 12 cirri which extends up to $3/4$ of the body length. Transverse cirri are absent. There is one row of LMC made of 4 cirri extending up to the level of the posterior end of the fronto-terminal row and one right marginal row with 17 cirri extending up to the posterior tip of the cell. The dorsal ciliature consists of 3 DKs with 6, 8 and 8 bristles respectively, and 3 CCs.

The ciliary structures are very long and highly hypertrophied. For a cell which is, on an average, $33 \times 12 \mu$ m, the lengths of various ciliary structure are as follows – membranelles of the AZM 5.4μ m, cirri of the frontal ciliature 5.4 – 8.1μ m, marginal cirri 7.3 – 12.5μ m, dorsal cilia 2.8μ m and caudal cirri 8.2 – 11.8μ m.

This species was found only at one site. The cells are small and cling to soil particles using their long and highly hypertrophied cirri. Attempts to dislodge the cells failed to

give complete cells. Protargol stained mixed cultures including soil particles, therefore, do not yield flat cells for proper measurements. Suitably stained cells were too few to make an appropriate biometric analysis. The species, however, is unique and clearly distinct from other species of the genus by virtue of the extremely small size, a tail-less pointed posterior end and a characteristic ciliature. It was impossible to ignore their presence and not report them just because of the absence of biometric data.

Comparison with related species: Among the reported species of *Paragonostomum*, *P. minuta* sp nov is the smallest; in fact it is less than half the size. There is no tail though the posterior end is distinctly pointed. The body shape of *P. minuta* sp nov comes close to that of *P. binucleatum* [9] which definitely possesses a tail like narrow posterior portion. The arrangement of frontal ciliature in *Paragonostomum minuta* is similar to that in *Gonostomum gonostomoida* [22, 23] but the latter possess transverse cirri.

***Gonostomum affine* VoF population** (Figs. 2, 12a–h, table 4)

Description: Body length and width of live cells averages $64 \times 19 \mu$ m and those of protargol impregnated non dividers range between 56.4 – 70.1μ m and 15.1 – 21.3μ m respectively. The cortical granules are colourless and scattered in the cortex. They do not stain with protargol. The single contractile vacuole is present just below the

Table 4. Morphometric data* of *Gonostomum affine* (VoF population).

Characters	Mean	Max	Min	SD	CV
Body length, μm	60.6	70.1	56.4	3.70	6.10
Body width, μm	17.7	21.3	15.1	2.03	11.44
Length: width ratio	3.4	3.8	2.7	0.45	13.30
Macronucleus, no	2	2	2	0	0
Macronucleus length, μm	12	14.8	10	1.30	10.77
Macronucleus width, μm	4.9	6.0	4.1	0.51	10.45
Micronucleus, no	2	2	2	0	0
Micronucleus diameter, μm	2.4	2.6	2.2	0.02	1.20
Length of AZM, μm	31.7	34.6	28.5	2.67	8.42
AZM: body ratio percent	52.5	60.2	44.9	3.91	7.45
AM no	27.8	31	26	1.55	5.57
Anterior end to beginning of PM, μm	16.2	18.8	13.4	1.31	8.11
Anterior end to end of PM, μm	25.4	28.6	23.7	1.42	5.61
Length of PM, μm	10.2	12.3	8.2	0.98	9.61
Number of kinetosomes in PM	13.8	15.0	12.0	1.30	9.44
Gap between kinetosomes in PM, μm	0.5	0.6	0.5	0.04	8.08
BC, no	1	1	1	0	0
FC, no	3	3	3	0	0
FT, no	2	2	2	0	0
FV, no	2	2	2	0	0
POVC, no	3	3	3	0	0
PTVC, no	1.6	2	1	0.52	32.50
TC, no	2	2	2	0	0
RMC row, no	1	1	1	0	0
RMC, no of cirri	19.6	21	15	2.61	13.30
LMC row, no	1	1	1	0	0
LMC, no of cirri	12.6	15	10	2.30	18.27
DK, no	3	3	3	0	0
Bristles in DK_1	17.0	21	14	2.36	13.86
Bristles in DK_2	15.6	18	12	1.54	9.88
Bristles in DK_3	17.9	24	14	2.85	15.90
CC, no	3	3	3	0	0

* Data based on protargol stained non-dividers obtained from a non-flooded petridish culture; n=10, Max=maximum, Min=minimum, SD=standard deviation, CV=coefficient of variation (%).

AZM tip along the left side of the body. The nuclear apparatus consists of 2 oval macronuclei and 2 spherical micronuclei. The AZM: body length ratio varies between 45–60%; the middle segment of the AZM is straight and situated along the left margin of the cell and the proximal portion curves inwards towards the centre of the body. The PM length ranges between 8.2–12.3 μm composed of 12–15 with a gap between adjacent cilia being 0.5 μm . The EM consists of tightly packed cilia. The PM extends far beyond the anterior end of the EM. The FVT cirri are as follows: 1 BC, 3 FC, 2 FT, 2 FV, 3 POVC,

2 PTVC, 2 TC. There is one row each of LMC with 10–15 cirri and RMC with 15–21 cirri. Dorsal ciliature consists of 3 DKs and 3 CCs. Morphogenesis is typified by the presence of primary frontal-ventral-transverse (FVT) primordia.

*Comparison with other populations of **Gonostomum affine** (Stein, 1859) Sterki, 1878:* The genus *Gonostomum* was first defined unambiguously by Sterki [8] for this species. This species has been described from a variety of places [24, 25] and a large variation exists in the biometric

Fig. 6. Line diagrams of a protargol impregnated non-divider of *Gonostomum gonostomoida* VoF population, a) ventral surface b) dorsal surface c) nuclear apparatus. The fronto-terminal cirri are present in a row (arrow). For the rest of the labelling, refer Fig. 2. Bar represents 10 µm.

characters within the species; for example, the body length varies in different populations from 50 µm to 160 µm. This has prompted Foissner [24] to suggest that *Gonostomum affine* may, in fact, be a group of sibling species. For a review of the known populations, refer Berger [16]

Gonostomum gonostomoida VoF population (Figs. 6, 13a–d)

Description: Very few cells excysted from one of the soil samples and data collected were not enough for a proper biometric analysis (Foissner [24] has reported that the non-flooded petridish method, although being the best method available for soil ciliates, may not be good enough for soils that remain permanently wet as is the case with our samples).

The mean body size of protargol impregnated non-dividers is 78×23 µm. There are 2 macronuclei, each 15.5×8.5 µm, and 3–4 micronuclei each with a diameter of 2.2 µm. The AZM shape is typical of *Gonostomum* species and occupies 50% of the cell length, with 27 membranelles on an average. The PM is composed of about 14 cilia with 0.5 µm space between adjacent cilia; EM consists of tightly packed cilia beginning about 25.7 µm from the anterior end of the cell. The FVT ciliature consists of 1 BC, 3 FC, 2 FV, 3 POVC, one FT row with an average of 16 cirri, and 3

Fig. 7. Line diagrams of a protargol impregnated non-divider of *Gonostomum kuehnelti* VoF population, a) ventral surface b) dorsal surface c) nuclear apparatus. For labelling, refer Fig. 2. Bar represents 10 µm.

transverse cirri. The RMC row has 22 cirri and the LMC row has 14 cirri; The 3 DKs are composed of 22, 21 and 31 bristles respectively; there are 3 CCs.

Comparison with *Gonostomum gonostomoida* (Hemberger, 1985) Berger, 1999 comb nov: The species was first described by Hemberger [22, 23] as *Trachelochaeta gonostomoida* and later transferred to genus *Gonostomum* by Berger [16]. The body size of Hemberger's population from Germany is bigger ($110\text{--}200 \times 30\text{--}50$ µm). Hemberger also reported the presence of 2 macronuclei and 2 micronuclei in cells of the German population. The AZM occupies 40% of the cell length in his population. The FVT number and their placement in the cell is, however, the same as in the VoF population and we feel justified in placing these cells in the *Ggonostomoida* species even though the cell size is smaller in the VoF population.

Gonostomum kuehnelti VoF population (Figs 7, 14a–g, table 5)

Description: Body length and width of protargol impregnated non dividers range between 55.2–70.0 µm and 15.3–26.4 µm respectively. The cortical granules are colourless and do not stain with protargol. The single contractile vacuole is present just below the AZM tip along the left side of the body. The nuclear apparatus consists of 2 groups of macronuclear nodules, 6–9 in the anterior group and

Table 5. Morphometric data* of *Gonostomum kuehnelti* (VoF population).

Characters	Mean	Max	Min	SD	CV
Body length, μm	61.6	70.0	55.2	5.16	8.37
Body width, μm	18.6	26.4	15.3	3.32	17.82
Length: width ratio	3.3	4.1	2.5	0.49	14.67
Macronuclear nodules, no	14.0	19	13	1.94	13.88
Macronuclear nodules, no in anterior group	6.5	9	6	0.97	14.94
Macronuclear nodules, no in posterior group	8.1	10	6	0.99	12.27
Macronuclear nodules, diameter, μm	4.0	4.6	3.4	0.40	10.04
Micronucleus no	2	2	2	0	0
Micronucleus diameter, μm	1.1	1.4	1.0	0.13	11.75
Length of AZM, μm	29.2	31.6	26.5	1.61	5.52
AZM: body ratio percent	47.6	52.8	40.5	4.46	9.36
AM no	25.0	28	22	1.83	7.30
Anterior end to beginning of PM, μm	15.3	17.2	14.3	0.78	5.11
Anterior end to end of PM, μm	20.4	22.1	19.0	0.90	4.43
Length of PM, μm	5.4	5.5	4.9	0.18	3.44
Number of kinetosomes in PM	10.7	12	8	1.16	10.83
Gap between kinetosomes in PM, μm	0.5	0.6	0.5	0.05	9.06
Anterior end to beginning of EM, μm	17.6	19.1	17.0	0.69	3.90
BC, no	1	1	1	0	0
FC, no	3	3	3	0	0
FT, no	2	2	2	0	0
FV, no	2	2	2	0	0
POVC, no	3	3	3	0	0
TC, no	4	4	4	0	0
RMC row, no	1	1	1	0	0
RMC, no of cirri	20.9	22	19	0.99	4.76
LMC row, no	1	1	1	0	0
LMC, no of cirri	15.1	16	13	1.10	7.28
DK, no	3	3	3	0	0
Bristles in DK ₁	15.6	18	14	1.07	6.88
Bristles in DK ₂	14.4	16	13	0.97	6.71
Bristles in DK ₃	18.6	20	17	0.84	4.53
CC, no	3	3	3	0	0

* Data based on protargol stained non-dividers obtained from a non-flooded Petridish culture; n =10, Max=maximum, Min=minimum, SD=standard deviation, CV=coefficient of variation (%).

6–10 in the posterior group. Each nodule measures 2.8–4.6 μm ; there are 2 spherical micronuclei with a diameter of 2.2 μm . The AZM: body length ratio is ~ 47.6%. The middle segment of the AZM is straight and situated along the left margin of the cell and the proximal portion curves inwards towards the centre of the body. The PM length averages 5.4 μm and is composed of 8–12 cilia with a gap between adjacent cilia being 0.5 μm . The FVT cirri are as follows: 1 BC, 3 FC, 2 FT, 2 FV, 3 POVC, 4 TC. There is one row each of LMC with 13–16 cirri and RMC with 19–22 cirri.

Dorsal ciliature consists of 3 DKs and 3 CCs. Morphogenesis is typified by the presence of primary frontal-ventral-transverse (FVT) primordia.

Comparison with *Gonostomum kuehnelti* Foissner, 1987: The population of *Gonostomum kuehnelti* from the Valley of Flowers compares well with the description of the species from the upper soil layer of farm fields in Salzburg, Austria [10]. A characteristic feature of the species appears to be the presence of macronuclear nodules in two groups.

In the VoF population, the anterior group has 6–9 nodules and the posterior group has 6–10 nodules. The number and placement of FVT cirri resembles that in *G affine* but is almost invariable in the VoF population as well as in the Austrian population; this is in contrast to the situation in *G affine* where the number is variable. Recently, Vd'ačný and Tirjakova [26] have described a soil ciliate, *G albicarpathicum*, which resembles *G kuehnelti*, but differs from the latter in having lesser (3–6) macronuclear nodules and in the arrangement of frontal ciliature.

A notable feature of *G kuehnelti* is the presence of very long cirri; the lengths in our cells are as follows: frontal cirri 14–16 μm , fronto-ventral, fronto terminal and post orals 10–12 μm , transverse cirri 17–19 μm , marginal cirri 11–13 μm , and the caudal cirri 14–15 μm . The dorsal cilia are 2–3 μm long. The cell resembles *Gonostomum singhii* sp nov in this regard; however, the macronuclear peculiarity is notable.

Further comments

The structure and position of the paroral membranes in the genera *Gonostomum* and *Paragonostomum* appear to be species defining characters (Fig. 8). In the *G affine* population of the Valley of Flowers, the paroral is long, made up of 16–17 kinetosomes. In *G singhii* sp nov too, the paroral is long and consists of 14–16 kinetosomes. Similarly, in the other two species, namely *G gonostomoida* and *G kuehnelti*, the paroral has 14 and 9 kinetosomes respectively. The average gap between the kinetosomes of the paroral in *G affine*, *G gonostomoida* and *G kuehnelti* is 0.5 μm whereas that in *G singhii* it is 0.8 μm . The length of the paroral with respect to body length is thus increased to 16 % in *G singhii* whereas in the other three species, it occupies 6–8% of the body length. The two *Paragonostomum* species – *P ghangriai* sp nov and *P minuta* sp nov – possess a highly reduced paroral with 2–4 kinetosomes only, sometimes present in a bipartite distribution.

Table 6. Lengths of various ciliary structures in the six species of the *Gonostomum-Paragonostomum* from the Valley of Flowers.

Species with body size →	<i>Paragonostomum</i> <i>minuta</i> sp nov	<i>Gonostomum</i> <i>affine</i>	<i>Gonostomum</i> <i>kuehnelti</i>	<i>Gonostomum</i> <i>singhii</i> sp nov	<i>Paragonostomum</i> <i>ghangriai</i> sp nov	<i>Gonostomum</i> <i>gonostomoida</i>
Character, μm ↓	(33 × 12 μm)	(60 × 17 μm)	(61 × 18 μm)	(66 × 23 μm)	(72 × 21 μm)	(78 × 23 μm)
AZM ciliary length	5.4	9.3	11.6	10.4	11–14	10.5
F,FV,FT, POVC cirri	5.4–8.1	7.4–12.1	10.3–16.5	11.2–18	10–14	10.0
Transverse cirri	–	14	15.2	16.8	–	19.3
Cirri in LMC and RMC	7.3–12.5	10.2–11.2	11.1–12.2	10.3–11.5	11–14	14.3–14.5
Cilia in dorsal kineties	2.8	3.0	2.8	3.2	3.5	3.4
Caudal cirri	8.2–11.8	8.2	14.2	11–12	14.1	14–15

Fig. 8. Line diagrams of four species of *Gonostomum* and two species of *Paragonostomum* from the Valley of Flowers showing only the oral ciliature (the anterior half of each cell drawn), showing differences in their paroral membranes with regard to their size, number of constituting kinetosomes, distances between adjacent kinetosomes, and the spacing with respect to the anterior end of the cell. Bar represents 10 μm .

a

b

c

d

e

a

b

c

d

e

f

Fig. 10 a–f: *Paragonostomum minuta* sp nov

b

c

d

e

f

g

h

Fig. 11 a–h: *Paragonostomum ghangriai* sp nov

Fig. 9–11. Photomicrographs of protargol impregnated cells of one new species of *Gonostomum* and two new species of *Paragonostomum* from the Valley of Flowers; 9 a–e: *Gonostomum singhii* sp nov, a – ventral view, b – paroral membrane (arrow), c,d – two pre-transverse ventral cirri and four transverse cirri showing long cirri (c) and only cirral bases (d), e – dorsal view; 10 a–f: *Paragonostomum minuta*, a–e – ventral view, a – an entire cell, b – fronto-terminal (arrow) and LMC (arrowhead) rows in focus, c – right marginal row in focus, d – fronto-ventral cirri, e – paroral membrane (arrow), f – dorsal view; 11a–h: *Paragonostomum ghangriai* sp nov, a – ventral view, b,c,d – paroral membranes (arrows) in three different cells with 2,3 and 6 cilia respectively, e – FV rows in the oral region, f – three caudal cirri in close proximity to the compacted posterior cirri of the LMC row, g – dorsal view, h – randomly scattered extrusomes (arrows).

Fig. 14 a–g: *Gonostomum kuehnelti*

Fig. 12–14. Photomicrographs of protargol impregnated cells of the three described species of *Gonostomum* from the Valley of Flowers; 12 a–f: *Gonostomum affine*, a – ventral view, b – two pre-transverse and two transverse cirri (highlighted), c – oral region of a cell showing a larger number of fronto-ventral cirri (arrows show additional cirri), d – paroral membrane (arrow), e – dorsal view, f – caudal cirri magnified; 13 a–d: *Gonostomum gonostomoida*, a – ventral view (arrow showing the paroral membrane), b – three transverse cirri in high power, c – dorsal view, d – three caudal cirri in high power; 14 a–g: *Gonostomum kuehnelti*, a – ventral view, b – four transverse cirri in a typical quadrangular position, c – dorsal view, d – three caudal cirri, e – paroral membrane (arrow), f – fronto-ventral cirri in high magnification, g – a late divider.

Gonostomum and *Paragonostomum* sps, by and large, feed on bacteria. The oral ciliature plays a major role in preferential food gathering among the filter feeding ciliates [27]. The species specific variations in the paroral membranes of the group may have been a result of evolutionary diversification allowing them to co-exist and forge on different types of bacteria (recall Gause's exclusion principle and character displacement).

The large and highly hypertrophied ciliature with respect to body size (table 6) in the six species described above are worth a mention in this regard. Notable among these is the size of marginal cirri and dorsal bristles in *P minuta*. This character is probably highly useful in locomotion for these soil ciliates which spend most of their active time moving around soil particles that remain wet throughout the year.

In general, species of *Gonostomum* and *Paragonostomum* have a concentration of their cirri in the oral (anterior) region. The anterior end is also variably pointed. These appear to be adaptive features for facilitating movements in their rough surroundings.

Multi-macronuclearity is a common feature among the hypotrichs of the Valley of Flowers; nine of the seventeen species are multi-macronuclear. Three out of six species described in the present paper are also multi-macronuclear. *G kuehnelti* has ~14 macronuclear nodules in two sets; *Paragonostomum ghangriai* and *P minuta* have ~18 and ~7 macronuclear nodules respectively. Significance of this phenomenon is not clear.

Acknowledgments The above work is a part of the research conducted under the Project SR/SO/AS-04/2004 awarded to the corresponding author by the Department of Science and Technology, New Delhi, India. The authors are grateful to the host institution, Sri Guru Tegh Bahadur Khalsa College for providing the necessary infrastructural facilities to carry out the research work. The constant guidance of Prof Rup Lal, Head, Department of Zoology, University of Delhi, is greatly acknowledged. The authors are grateful to the editor and anonymous reviewers for several constructive suggestions for improvement of the manuscript.

References

- Song W, Warren A, Hu X, Chen Z, Wang M, Zhu M, Ma H, Gong J, Lin X and Ji D (2007) On the biodiversity, cell development and phylogeny of marine free-living ciliates: researches carried out in China. International Symposium on Ciliate Biology, India
- Foissner W (1987) Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. Progress in Protistol 2:69–212
- Dragesco J and Dragesco-Kernéis A (1963) Ciliés libres de l'Afrique intertropicale, Faune Tropicale XXVI, France
- Chao A, Li PC, Agatha S and Foissner W (2006) A statistical approach to estimate soil ciliate diversity and distribution based on data from five continents. Oikos 114: 479–493
- Bonkowski M and Schaefer M (1997) Interactions between earthworms and soil protozoa: a trophic component in the soil food web. Soil Biol and Biochem 29:499–502
- Kala CP (2004) The Valley of Flowers: Myth and Reality. International Book Distributors
- Nautiyal P (2004) Diatom Biodiversity in the Himalayan Lotic Systems, Vedams eBooks (P) Ltd., India
- Sterki V (1878) Beiträge zur Morphologie der Oxytrichen. Zeit Wiss Zool Leipzig (ser. 3): 31:29–58
- Foissner W, Agatha S and Berger H (2002) Soil ciliates (Protozoa, Ciliophora) from Namibia (Southwest Africa), with emphasis on two contrasting environments, the Etosha region and the Namib Desert. Denisia 5:1–1459
- Foissner W (1987) Neue terrestrische und limnische Ciliaten (Protozoa, Ciliophora) aus Österreich und Deutschland. Sitzungsberichte der österreichischen Akademie der Wissenschaften, Wien 195:217–268
- Foissner W (1992) Estimating the species richness of soil protozoa using the “non-flooded petridish method”. In: Lee JJ and Soldo AT (eds) Protocols in Protozoology. Allen Press, Lawrence, Kansas, pp. B–10.1–10.2
- Buitkamp U (1979) Vergleichende Untersuchungen zur Temperaturadaptation von Bodenciliaten aus klimatisch verschiedenen Regionen. Pedobiologia 19:221–236
- Kamra K and Sapra GR (1990) Partial retention of parental ciliature during morphogenesis of the ciliate *Coniculostomum monilata* (Dragesco and Njiné, 1971) Njiné, 1978 (Oxytrichidae, Hypotrichida). Europ J Protistol 25:264–278
- Borror AC (1972) Revision of the order Hypotrichida (Ciliophora, Protozoa). J Protozool 19:1–23
- Foissner W (1998) An updated compilation of world soil ciliates (Protozoa, Ciliophora), with ecological notes, new records, and descriptions of new species. Europ J Protistol 34:195–235
- Berger H (1999) Monograph of the Oxytrichidae (Ciliophora, Hypotrichia). Kluwer Academic Publishers, Dordrecht, The Netherlands
- Foissner W, Berger H, Xu K and Zechmeister-boltenstern S (2005) A huge, undescribed soil ciliate (Protozoa: Ciliophora) diversity in natural forest stands of Central Europe. Biodiversity and Conservation 14:617–701
- Berger H, Foissner W (1997) Cladistic relationships and generic characterization of Oxytrichid Hypotrichs (Protozoa, Ciliophora). Arch. Protistenkd 148:125–155
- Foissner W (1983) Die Morphogenese von *Urosoma macrostyla* (Wrześniowski, 1870) (Ciliophora: Oxytrichidae). Arch Protistenk 127:413–428
- Song W (1990) A comparative analysis of the morphology and morphogenesis of *Gonostomum strenua* (Engelmann, 1862) (Ciliophora, Hypotrichida) and related species. J Euk Microbiol 37:249–257

21. Eigner P (1999) Comparison of divisional morphogenesis in four morphologically different clones of the genus *Gonostomum* and update of the natural hypotrich system (Ciliophora, Hypotrichida). *Europ J Protistol* 35:34–48
22. Hemberger H (1982) Revision der ordnung Hypotrichida Stein (Ciliophora, Protozoa) an hand von protargolpräparaten und morphogenesedarstellungen. Dissertation Universitat Bonn. I-IV and p 296
23. Hemberger H (1985) Neue Gattungen und Arten hypotricher Ciliaten. *Arch Protistenk* 130:397–417
24. Foissner W (2000) Notes on ciliates (Protozoa, Ciliophora) from *Espeletia* trees and *Espeletia* soils of the Andean Páramo, with descriptions of *Sikorops espeletiae* nov spec and *Fragmocirrus espeletiae* nov gen, nov spec. *Studies on Neotropical Fauna and Environment* 35:52–79
25. Foissner W, Stoeck T, Schmidt H and Berger H (2001) Biogeographical differences in a common soil ciliate, *Gonostomum affine* (Stein), as revealed by morphological and RAPD-fingerprint analysis. *Acta Protozool* 40:83–97
26. Vd'áčný P and Tirjakova E (2006) A new soil hypotrich ciliate (Protozoa, Ciliophora) from Slovakia: *Gonostomum albicarpaticum* nov spec. *Europ J of Protistol* 42:91–96
27. Hausmann K, Hülsmann N and Radek R (2003) *Protistology*. E Schweizerbart'sche Verlagbuchhandlung, Germany