Notohymena saprai sp. nov, a new oxytrichid ciliate (Protozoa, Ciliophora) from the Valley of Flowers, a Himalayan bioreserve region; description and morphogenesis of the new species

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Abstract A new oxytrichid ciliate *Notohymena saprai* sp. nov was isolated from the Valley of Flowers, an isolated bioreserve in the upper Himalayas. The ciliate was found in the soil samples collected from the peripheral areas of the flowering region of the valley which is covered with permafrost for nearly eight months in a year. Cells were excysted from the soil samples by the non-flooded petridish method. Species identification was performed by observations on live and silver-impregnated cells. *Notohymena saprai* sp. nov is highly flexible, measures $152 \times 49 \mu\text{m}$ and shows abundant presence of dark green sub-pellicular granules. The species is distinct by virtue of its new combination of morphometric and developmental characters. Prominent distinguishing features of the new species are its relative large size and presence of four macronuclei (other species of this genus have two macronuclei). The study also reports morphogenetic peculiarities which separate it from the other known species of the genus.

Keywords *Notohymena saprai* sp. nov · Ciliated protozoa · Valley of Flowers · Systematics · Morphogenesis

Introduction

The Valley of Flowers is a protected national park in India situated in the upper Himalayas at a height of 3250–6750 m above sea level [1, 2] and has been isolated for long from the neighboring regions. The Valley is covered with permafrost for nearly eight months of the year. Three hundred recorded species of flowers grow in the four summer months, one dominant form replacing another. In the first ever survey of the microbial fauna, 22 ciliate species were found in soil samples from the Valley. Among these, 11 species appear to be new, indicating a high level of endemism in the ciliate fauna of the Valley [3]. Among them 17 are hypotrichs and 9 are multi-macronucleated. The authors have recently reported that six species belong to the *Gonostomum*–*Paragonostomum* group, three of which are new [3]. We report the presence of a new species of the genus, *Notohymena*, found in the soil samples. It is different and distinct from the five known species of the genus [3], showing a new combination of morphological, morphometric and morphogenetic characteristics. It is clearly distinguishable by the presence of four macronuclei as against two in other known species of the genus.

Materials and methods

Soil samples were collected in the early, mid and late flowering periods during 2006–2007 from a depth of 0–10 cm from 24 sites along the permitted trekking trail of the valley (Fig. 1). Ciliates were obtained from these samples employing the non-flooded petridish method [4, 5].

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Clonal cultures of the new *Notohymena* species were raised from excysted cells. The cells thrive well in Pringsheim's medium with the green alga *Chlorogonium elongatum* as the food organism [6] exhibiting optimum growth at a temperature of $18^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

Live observations of cells were made using differential interference contrast microscopy. Protargol staining [7] was used for visualizing surface ciliature. Nuclear observations were made using Feulgen stained cells. Biometric characterization was done at a magnification of 1000X directly from the Leica software IM50 image manager; Leica camera DFC320 was employed for photography. Line diagrams were prepared using Corel graphics software.

Nomenclature and terminology is based on Borror [8], Foissner [9], Martin [10] and Wallengren [11].

Results

Cells of a new species of the genus *Notohymena* were found in soil samples collected from three sites near the periphery of the protected flowering region, close to the temporary settlement, Ghangria (squares in Fig. 1). A description of the new species, *Notohymena saprai* sp. nov, is given below.

Diagnosis

Body size in vivo $149 \times 49 \mu\text{m}$; cytoplasm colorless with irregularly arranged small, dark, green, sub-pellicular cortical granules and numerous fat globules; four macronuclei

and 4–6 micronuclei; oral ciliature in *Notohymena* pattern, adoral zone of membranelles (AZM) occupying 38% of body length, paroral membrane (PM) shorter than the endoral membrane (EM); frontal-ventral-transverse (FVT) ciliature with 8 frontals (including one buccal cirrus), three post-oral ventral cirri (POVC), two pre-transverse ventral cirri (PTVC) and five transverse cirri (TC); marginal cirri in one row on the left (LMC) and one row on the right (RMC) non-confluent posteriorly, additional incomplete marginal rows often present; four dorsal kineties (DKs) and two dorso-marginal rows (DMs), three caudal cirri (CCs); *de novo* formation of the oral primordium (OP) close to the POVC; FVT primordia V and VI for both daughter cells arise from the OP.

Type location

Soil samples from the Valley of Flowers (30° 41' to 30° 48'N and 79° 33' to 79° 46'E), Nanda Devi Bioreserve, India.

Type material

The holotype slide has been deposited (Accession no 2008:12:4:1) in the British Natural History Museum, Cromwell Road, London SW7 5BD, UK.

Etymology

The species has been named after Professor G. R. Sapra as an acknowledgment of his contributions to the field of ciliate biology.

Fig. 1 Outline map of the Valley of Flowers National Park: Mt. Kunt Khal and Mt. Saptasring flank the entrance to the protected region; Mt. Nilgiri Parbat is to the north of the valley. Ghangria, a temporary establishment outside the protected park, is the entrance point of the trek track. Arrow indicates the beginning of the flowering region. The sampling sites where *Notohymena saprai* sp. nov was found are marked by squares at the periphery of the flowering region. Source – Office of the Divisional Forest Officer, Nanda Devi National Park, Joshimath

Description (Fig. 2, Plate I, III)

Cells of *Notohymena saprai* sp. nov are oblong, dorso-ventrally flattened and very flexible, extending up to 152 μm in length. Protargol-stained cells measure $149 \times 49 \mu\text{m}$ on an average with a length:width ratio of 3:1. Cells show abundant presence of dark green, sub-pellicular cortical granules $1.2 \mu\text{m}$ in diameter each not arranged in any particular manner. The cytoplasm is colorless with many lipid droplets about $2.2 \mu\text{m}$ in diameter each. A single contractile vacuole is present far anterior to the midline near the left margin of the cell. At optimum temperature conditions, i.e. $18^\circ\text{C} \pm 2^\circ\text{C}$, cells divide every 17 h. The cells

encyst frequently under laboratory conditions but were not seen to conjugate. Sometimes doublets may be formed in a normally growing culture (Plate III f). Each cell has four ellipsoidal macronuclei and 4–6 spherical micronuclei.

Biometric characterization of a population is given in Table 1.

A non-divider shows the following features: The AZM extends to ca 38% of cell length, PM of the *Notohymena* type with a characteristic hook at the distal end, 18 FVT cirri (F_{1-8} , V_{1-5} and T_{1-5}); the transverse cirri are usually present in a curved row, sometimes in a tick mark arrangement. The FVT cirri are numbered in this form in Fig. 2 to distinguish frontal and ventral cirri from the transverse ones, but these

Fig. 2 Line diagrams of protargol impregnated vegetative cells of *Notohymena saprai* sp. nov, 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, F_{1-8} – frontal cirri, V_{1-3} – post-oral ventral cirri (POVC), $V_{4,5}$ – pre-transverse ventral cirri (PTVC), T_{1-5} – transverse cirri, LMC – row of left marginal cirri, RMC – row of right marginal cirri, DK_{1-3} – dorsal kinetics 1-3, CC_{1-3} – caudal cirri, Ma – macronuclear nodule, Mi – micronucleus. According to the numbering system for FVT cirri introduced by Wallengren (11), the cirri numbered in figure 2a have the following designations: F_1 – II/2, F_2 – I/1, F_3 – II/3, F_4 – III/3, F_5 – VI/4, F_6 – VI/3, F_7 – IV/3, F_8 – III/2, V_1 – IV/2, V_2 – V/4, V_3 – V/3, V_4 – V/2, V_5 – VI/2, T_1 – II/1, T_2 – III/1, T_3 – IV/1, T_4 – V/1, T_5 – VI/1.

Table 1 Biometric characterization of *Notohymena saprai* sp. nov

Character	Mean	Max	Min	Median	SE	SD	CV
Body length, μm	149.2	167.9	128.6	150.6	2.48	11.09	07.43
Body width, μm	48.8	58.8	39.6	46.9	1.06	04.73	09.69
Adoral membranelles, no	52.7	58	48	52.5	0.53	02.39	04.54
AZM / body length %	38.5	41	34	39.0	0.55	02.46	06.39
Macronucleus no	4	4	4	4	0	0	0
Macronucleus length, μm	13.8	16.5	10.5	14.1	0.34	01.53	11.07
Macronucleus width, μm	9.1	11.8	7.1	9.0	0.26	01.18	12.97
Micronucleus no	3.7	5	2	4	0.26	00.82	22.24
Micronucleus diameter, μm	2.0	2.2	1.8	2.1	0.06	0.18	8.82
Buccal cirrus	1	1	1	1	0	0	0
Frontal cirri, no*	8	8	8	8	0	0	0
Ventral cirri, no*	5	5	5	5	0	0	0
Transverse cirri, no*	5	5	5	5	0	0	0
RMC row (s)	1	1	1	1	0	0	0
RMC, no of cirri	43.1	59	36	42.5	1.16	05.20	12.06
LMC row (s)*	1	1	1	1	0	0	0
LMC, no of cirri	43.9	51	39	43	0.78	03.50	07.98
Dorsal kineties, no	6	6	6	6	0	0	0
Dorsal kinety 1	30.9	35	25	31.5	1.1	03.48	11.26
Dorsal kinety 2	31.1	34	27	32	0.74	02.33	07.49
Dorsal kinety 3	21.0	26	17	20.5	0.88	02.79	13.27
Dorsal kinety 4	12.7	17	10	12	0.62	01.95	15.32
Dorso-marginal 1	23.1	27	16	24.5	1.05	03.31	14.35
Dorso-marginal 2	11.5	18	8	11	0.96	03.03	26.32
Caudal cirri, no*	3	3	3	3	0	0	0

* It is not uncommon to find cells with more than eighteen FVT cirri (18 out of 100 cells scored). Often, cells have additional complete or incomplete LMC rows (15 out of 100 cells scored). Sometimes, additional LMCs and RMCs are present in blocks of 2–6 cirri.

Table 2 Parental ciliary structures associated with the origin of FVT primordia in *Notohymena saprai* sp. nov. Cirri are named according to Wallengren [11]. Abbreviations in parentheses are as given in Fig. 2

Daughter cell	Primordium no.	Parental structure associated with origin of primordium	
Proter	I	Parental paroral membrane	
	II	II/2* (F_1)	
	III	III/2* (F_8)	
	IV	IV/3* (F_7)	
	V	OP (anterior segments of streak 4, streak 5)**	
	VI		
Opisthe	I	From the base of the 5 streaks arising from OP	POVC cirri:
	II	OP (streak 1)	IV/2 (V_1),
	III	OP (streak 2)	V/4 (V_2),
	IV	OP (streak 3)	V/3 (V_3),
	V	OP (posterior segments of streak 4, streak 5)**	incorporated in the OP
	VI		

*Streaks 1, 2 and 3 originating from the OP proceed anteriorly to align with these primordia; however, whether they contribute to them is unclear. ** Streak 4 and streak 5 split transversely

Figs. 3–10 Line diagrams of protargol impregnated dividers of *Notohymena saprai* sp. nov showing OP formation; Figs. 3–4 – groups of kinetosomes arise apokinetally close to the POVCs – IV/2, V/4 and V/3; Figs. 5–8 – blocks of kinetosomes appear (arrow in fig 7) in the region below the POVC which finally merge (Fig. 8); Figs. 9–10 – disaggregation of POVCs (arrows) to merge with the OP. Arrowheads in fig 10 show early stages of FVT streaks 1–2.

numbers are not necessarily homologous with similarly numbered cirri in other hypotrichs. A key relating these numbers to numbers based on the ontogenetic origins of the cirri (as devised by Wallengren 1900) is given in the legend to Fig. 2; Wallengren's nomenclature is used hereafter since this facilitates ontogenetic comparisons. The LMC row has ca 44 cirri and the RMC row has ca 43 cirri. The marginal

rows are not confluent posteriorly. The dorsal ciliature is of the typical oxytrichid type. Three dorsal kineties (DK_{1-3}) are long, running the entire length of the cell; DK_4 is short, composed of 10–17 bristles, placed in the posterior one third of the body. There are 2 dorsomarginal rows ($DM_{1,2}$), DM_1 is long and DM_2 is short. Three caudal cirri (CC_{1-3}) are present, one each at the posterior ends of $DK_{1,2}$ and 4.

The average lengths of cirri are as follows: frontal-14.2 μm , ventral-14.0 μm , transverse-17.4 μm , marginal-13 μm , caudal-13.9 μm . The dorsal cilia measure 6.1 μm on an average.

Division morphogenesis (Figs. 3–20, Plate I–III)

Oral ciliature

Stomatogenesis begins with the appearance of a small field of kinetosomes arising close to the posterior edge of the three post-oral ventral cirri V_3 (V/3), V_2 (V/4) and then V_1 (IV/2). This kinetosomal field is joined by several groups of kinetosomes that appear apokinetally in the space between V_3 (V/3) and the transverse cirri to form the oral primordium (OP). The cirri V_3 (V/3), V_2 (V/4) and V_1 (IV/2) later disaggregate to yield kinetosomes to the proliferating OP. There is no contribution of the transverse and POVC in the formation of the OP. A new AZM for the opisthe develops from the OP, while the parental AZM is retained unchanged for the proter.

FVT primordia for the proter (Ip–VIp) and the opisthe (Io–VIo)

The PM disaggregates completely forming the primordium Ip which reconstitutes to form the PM and the F_2 (I/1) for the proter. The EM also reorganizes after disaggregating completely. Two streaks arise at the anterior face of the OP and proceed antieriad; three more streaks arise one after another also proceeding to the oral segment of the cell (streaks 1–5). The cirrus II/2 (buccal cirrus) disaggregates to form IIp. Some kinetosomes from streak1 appear to merge with IIp posteriorly, but whether they finally do so is unclear; the rest of the streak 1 gives rise to primordium IIo. Cirrus F_8 (III/2) and later F_7 (IV/3) disaggregate to form primordia IIIp and IVp respectively; streaks 2 and 3 from OP align themselves close to the posterior ends of these primordia but remain separate to later give rise to IIIo and IVo respectively. Some kinetosomes from these streaks (2 and 3) remain in the anterior region but whether they merge with IIIp and IVp posteriorly is unclear; perhaps these kinetosomes are resorbed. Streaks 4 and 5 proceed far antieriad and split to form primordia Vp and VIp as well as primordia

Figs. 11–14 Line diagrams of protargol impregnated dividers of *Notohymena saprai* sp. nov showing early and mid morphogenetic events in division; Fig. 11 – beginning of disaggregation of PM (double arrowhead), cirri II/2 and III/2 (arrowheads) to form FVT primordia Ip, IIp and IIIp, alignment of membranelles in OP (double arrow), formation of the first three streaks from OP proceeding antieriad to give rise to FVT primordia Ilo, IIlo and IVo (arrows), note the complete merging of kinetosomes from the disaggregated POVC with OP; Fig. 12 – beginning of disaggregation of EM (double arrowhead) to later merge with disaggregating PM to form primordium Ip, disaggregation of cirrus IV/3 (arrowhead) to form FVT primordium IVp, formation of FVT primordia Vp, Vo and VIp, VIo from two streaks emerging from OP which split transversely (double arrows); Fig. 13 – transverse split clearly visible between Vp and Vo as well as between VIp and VIo (double arrow), some kinetosomes proceed antieriad from streaks 1-3 and appear to merge with the proter's FVT primordia IIp, IIp and IVp (arrowheads) but whether they do so or get resorbed later is unclear (see inset 13a); Fig. 14 – complete complement of FVT primordia for proter and opisthe, within-row formation of primordia for RMC and LMC rows for both daughter cells (arrowheads). Arrow shows AZM formation for opisthe while double arrow shows formation of Io from the base of the FVT streaks for the opisthe.

Table 3 Comparison of characteristics of *Notohymena saprai* sp. nov with those of published data from other species of the genus (figure denote mean values)

Character	<i>N. saprai</i> sp. nov	<i>N. australis</i> [13]	<i>N. rubescens</i> [14]	<i>N. antarctica</i> [15]	<i>N. selvatica</i> [12]	<i>N. pampasica</i> [16]
Body length, μm	149.2	81.0	84.1	85.5	170-190 L	83.8
Body width, μm	48.8	31.4	33.7	40.1	60-70 L	35.3
AM no	52.7	39	26.6	30.2	-	-
AZM / body length %	38.5	-	-	-	27	-
Macronucleus, no	4	2.0	2	2	2	2
Macronucleus length, μm	13.8	11.7	11.8	12.9	-	13.3
Macronucleus width, μm	9.1	8.4	7.3	7.1	-	9.7
Micronucleus, no	3.7	3.1	2.3	2.4	2-3	2.1
Micronucleus length, μm	2.0,	2.9,	3,	3.2,	-	3.2,
Micronucleus width, μm	2.0	2.8	3	2.8	-	2.8
Buccal cirrus	1	1	1	1	1	1
Frontal cirri, no	7	-	7	7	7	7
Ventral cirri, no	5	-	5	5	5	5
Transverse cirri, no	5	5	5	5	4	5
RMC row (s)	1	1	1	1	1	1
RMC, no of cirri	43.1	29.5	18.3	16.8	-	17.4
LMC row (s)	1	1	1	1	1	1
LMC, no of cirri	43.9	28.2	17.0	17.9	-	17.1
DK, no	6	6.3	6	6.0	-	6
Caudal cirri, no	3	7.5	3	3.0	3	3

Dash (–) denotes data not available.

Note: The figures in bold highlight the following in comparison to the rest of the species:

1. The bigger size of *N. saprai* sp. nov
2. The presence of four macronuclei in *N. saprai* sp. nov
3. The high number of RMC and LMC in *N. saprai* sp. nov
4. The presence of a higher number of caudal cirri in *N. australis*
5. The presence of four transverse cirri in *N. selvatica*

Vo and VIo. Primordia IIo to VIo remain connected posteriorly and primordium Io separates from their posterior ends. Io then gives rise to the PM, EM and the cirrus F_2 (I/1) for the opisthe.

The cirri V_1 (IV/2), V_2 (V/4) and V_3 (V/3) disaggregate to merge with the proliferating OP and it is unclear whether the streaks arising from the OP come separately from any of these cirri; it is thus difficult to say whether these cirri specifically form any of the opisthe primordia.

Thus 6 parental cirri and the PM are involved in primordial formation (Table 2). The 18 FVT cirri arise from these

primordia, splitting in the typical oxytrichid pattern of 1, 3, 3, 3, 4, 4.

Development of marginal rows

During division morphogenesis marginal primordia arise at each of 2 levels by “within-row” primordia formation utilizing 1–2 of the parental cirri at each level. The marginal primordia elongate utilizing 10–12 parental cirri and differentiate into new marginal rows. The rest of the parental marginal cirri are resorbed. Occasionally, additional short primordia develop alongside the regular ones to give rise to the extra marginal cirri (Plate III c).

Fig. 15–17 Line diagrams of protargol impregnated dividers of *Notohymena saprai* sp. nov showing late morphogenetic events in division. The parental AZM is retained for the proter after reorganization, while a new AZM is formed for the opisthe from the OP. The PM, EM and cirrus II/2 for the proter are formed from the completely disaggregated parental PM and EM while those for the opisthe are generated from the primordium Io (double arrowhead in fig 15). The new PM and EM are parallel to each other initially (arrow in fig 17). The FVT cirri are cut off in a 1,3,3,3,4,4 pattern from the primordia Ip-Vip and Io-IVo for both daughter cells (figs 15–17). The primordia for LMC and RMC rows for both daughter cells are formed by within-row primordia formation (arrowheads in figs 15, 17). Two streaks arise from the anterior ends of the RMC primordia (double arrowheads) one after another to give rise to the two dorsomarginals rows. The parental marginal cirri (arrow in fig 16) and the parental FVT which are not used for primordia formation are resorbed. The parental cirri are outlined while the new cirri are filled in.

Figs. 18–20 Line diagrams of protargol impregnated dividers of *Notohymena saprai* sp. nov showing morphogenetic events on the dorsal surface. Fig 18 – Formation of three dorsal primordia by within-row primordia formation at two levels, one for the proter (arrows) and the other for the opisthe (double arrows); Figs. 19, 20 – Posterior thickening of dorsal primordia by kinetosome proliferation (arrows in Fig. 19) to form the caudal cirri (arrowheads in Fig. 20); unequal split in the third primordium from the left (of the cell) to form the fourth dorsal primordia (arrows in Fig. 20); the four dorsal primordia give rise to the four dorsal kineties; the newly formed dorsomarginals shift to the dorsal surface (double arrows). The parental bristles that are not incorporated in primordia formation are resorbed.

Development of dorsal ciliature

The dorsal ciliature is formed as in most other oxytrichids. Three dorsal primordia DP_{1-3} are formed at 2 levels within DK_{1-3} . DK_4 is formed by an unequal fragmentation of the newly formed DK_3 . The 2 dorsomarginals originate close to the anterior ends of the outer RMC row primordia. Three CCs differentiate, one each at the posterior ends of the new kineties $DK_{1, 2 \text{ and } 4}$.

As cytokinesis commences, the new cirri arrange themselves according to their species-specific pattern while the remaining parental ciliature undergo resorption.

Reorganization morphogenesis

Cells undergo reorganization when starved or when culture conditions are not ideal. The reorganizers make a single set of ciliature which then replaces the old one (Plate III d,e).

Plate I a–d Photomicrographs of live cells of *Notohymena saprai* sp. nov. a) an entire cell in ventral view (arrow marks the hook of PM); b, c: segments of a cell highlighting cortical granules (b) mostly randomly distributed (as within the square) but sometimes in rows (arrow in b) or in groups (double-arrow in c), and fat globules (arrows in c); d) the anterior one third of cell showing a single contractile vacuole (arrow) along the left margin of the cell; **e–j**: Photomicrographs of protargol-impregnated cells of *Notohymena saprai* sp. nov. showing the ciliature on the ventral (e) and dorsal (f) surface in a vegetative cell; for explanation, see legends to fig 2; g–j: early division stages; for explanation, see legends to figs 3–7

Plate II a–f Photomicrographs of protargol-impregnated dividers of *Notohymena saprai* sp. nov. showing morphogenetic events on the ventral surface; for explanation, see figs 8–17

Discussion

Justification of new species status

- 1) Morphology and morphometry: *N. saprai* sp. nov is distinct from the other five species of the genus (Table 3) that have been described thus far in having a new combination of characters. The most promi-

nent distinguishing feature is the presence of four macronuclei as against two in all other species. The size of *N. saprai* sp. nov is one and half times larger than four of the reported forms; *N. selvatica* [12] is much larger. The cortical granules are dark green in colour. *N. saprai* sp. nov is characterized by a very large number of marginal cirri in both the LMC and the RMC rows in proportion to the cell size.

Plate III a–f Photomicrographs of protargol-impregnated cells of *Notohymena saprai* sp. nov showing morphogenetic events on the dorsal surface (a, b), for explanation, see figs 18–20; c: streaks for extra RMC rows (arrows) in a divider; d–e: a reorganizer showing morphogenetic events on the ventral surface (d) and on the dorsal surface (e), one set of newly formed ciliature replacing the parental set; f: a homopolar doublet

- 2) Morphogenesis: The morphogenesis of *N. saprai* sp. nov. presents several peculiarities and places it apart from the co-generes:
- The OP arises as small clusters of kinetosomes close to the three POVCs and not close to the transverse cirrus II/1 as in all other species of *Notohymena*. Several other clusters of kinetosomes form apokinetally in the space between the transverse cirri and the POVC and align to merge with the primordium. The POVC disaggregate and merge with the proliferating OP. Such a pattern of OP formation also occurs in several members of the sub family Oxytrichinae [17] but has not been reported from any of the other species of *Notohymena*.
 - The origin of the FVT primordia, especially of those for the opisthe, is unique in the family Oxytrichidae. The streaks Ilo-IVo arise from the OP and their origin from any particular POVC cannot be traced as the latter disaggregate and join the OP prior to the formation of these streaks.
 - The FVT primordia Vp, VIp, Vo and VIo arise from the OP as two streaks which later split to form four. This is in contrast to the *de novo* origin of the Vp and VIp in the other species of *Notohymena*. The split of the two right-most FVT streaks is a feature seen in some members of both the sub families of family Oxytrichidae [17], but in such cases, these streaks arise distinctly from the specific POVCs.
 - The origin of Ip from the reorganizing paroral membrane, Iip from cirrus II/2, IIIp from cirrus III/2, and IVp from cirrus IV/3 occurs as in most members of the family Oxytrichidae. However, in *N. saprai* sp. nov. they may be incorporating kinetosomes from the streaks formed for the opisthe as the latter align very close to the proter streaks. At this stage, the FVT streaks remind one of the primary primordia in *Gonostomum* pattern [18, 19].

The presence of supernumerary cirri/ cirral rows is a common feature of *N. saprai* sp. nov. These are not retained parental cirri but are formed from extra primordia during division.

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References

- Kala CP (2004) The valley of flowers: myth and reality. International Book Distributors, ISBN 81-7089-311-9
- Nautiyal P (2004) Diatom biodiversity in the Himalayan lotic systems. Vedams eBooks (P) Ltd., India
- Kamra K, Santosh K and Sapra GR (2008) 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. Ind J Microbiol 48:372–388
- Foissner W (1987) Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. Progress in Protistology 2:69–212
- Foissner W (1992) Estimating the species richness of soil protozoa using the “non-flooded petri dish method”. In: Lee JJ and Soldo AT (eds) Protocols in protozoology. Allen Press, Lawrence, Kansas, pp. B–10.1–10.2
- Ammermann D, Steinbrück G, von Berger L and Hennig W (1974) The development of the macronucleus in the ciliated protozoan *Stylonychia mytilus*. Chromosoma (Berl.) 45:401–429
- 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
- Martin J (1982) Évolution des patrons morphogénétiques et phylogénèse dans le sous-ordre des Sporadotrichina (Ciliophora, Hypotrichida). Protistologica 18:431–447
- Wallengren H (1900) Zur Kenntnis der vergleichenden Morphologie der hypotrichen Infusorien. Bih K svensk Vetensk Akad Handl 26:1–31
- Hemberger H (1985) Neue Gattungen und Arten hypotricher Ciliaten. Arch Protistenk 130:397–417
- Foissner W and O'Donoghue PJ (1990) Morphology and infraciliature of some freshwater ciliates (Protozoa: Ciliophora) from western and south Australia. Invertebr Taxon 3:661–696
- Blatterer H and Foissner W (1988) Beitrag zur terricolen Ciliatenfauna (Protozoa: Ciliophora) Australiens. Stapfia 17:1–84
- Foissner W (1996) Faunistics, taxonomy and ecology of moss and soil ciliates (Protozoa, Ciliophora) from Antarctic

- ca, with description of new species, including *Pleuroplithoides smithi* gen n, sp. n Acta Protozool 35:95–123
16. Küppers GC, Claps MC and Lopretto EC (2007) Description of *Notohymena pampasica* n sp. (Ciliophora, Stichotrichia). Acta Protozool 46:221–227
 17. Berger H (1999) Monograph of the Oxytrichidae (Ciliophora, Hypotrichia). Monographiae Biol 78:i–xii, 1–1080
 18. Foissner W (1983) Die Morphogenese von *Urosoma macrostyla* (Wrześniowski, 1870) (Ciliophora: Oxytrichidae). Arch Protistenk 127:413–428
 19. 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