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Research Article

Morphology and Morphogenesis of *Pseudourostyla levis* (Ciliophora, Hypotrichia) from River Yamuna, Delhi, India.

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Abstract

The ciliated protozoa, *Pseudourostyla levis* was collected from river Yamuna in Delhi (India). Morphometric characterization and cortical morphogenesis during the division cycle was investigated. The ciliate *Pseudourostyla* measures 274µm × 80 µm in protargol stained preparations. Cortical structures include buccal, frontal and somatic ciliature. The main morphogenetic events during division cycle in *P. levis* includes, partial retention of parental adoral membranelles, dedifferentiation of parental midventral cirri to form frontoventral complex and formation of marginal rows on each side from a common marginal primordium. In the present study, the morphometric comparison of the present isolate with congeners is also presented.

Keywords: *Pseudourostyla levis*, ciliated protozoa, morphometric characterization, cortical morphogenesis.

Introduction

Hypotrichous ciliates have successfully exploited varied ecosystems, leading to a diverse species ensemble. The complex, diverse and highly organized cortical structures of these cilioprotist microorganisms reflect the adaptations for locomotion, feeding, reproduction and to survive in fluctuating environmental conditions [1]. Hypotrichs display distinct morphology and morphogenetic patterns during binary fission, conjugation and regeneration. The sites at which different primordia appear are relatively conservative [2,3]. Systematics of ciliates have emphasized that besides morphological criteria [4], the morphogenetic characters must also be taken into account [5-8]. A combination of morphological, morphogenetic and molecular phylogenetic data is used to assign taxonomic position and understanding the evolutionary relationship within the diverse and complex group of hypotrichous ciliates [9,10]. Morphogenetic studies provide an important tool to study three aspects of biodiversity: namely; taxonomy, evolutionary relationships and ecology of ciliates [11]. Many distinct morphogenetic patterns have been described amongst hypotrichs. Wicklow (1982) has compiled them under four patterns, viz., Euplotine, Sporadotrichine, Stichotrichine and Urostyline patterns [12]. On the basis of frontal ciliature and midventral complex, Berger (2006) grouped Urostylids into four major taxa, namely, Holostichidae, Bakuellidae, Urostylidae and Epiclintidae [13].

Urostylids are most diverse and complex groups of hypotrichs but due to lack of sufficient morphogenetic data, their evolutionary relationships are still not clear [13-18]. *Pseudourostyla was separated from other urostylids on the basis of frontal ciliature, mid ventral complex and presence of two or more rows of left and right marginal cirri, originating from a common primordium which arises within the rightmost row* [13,19, 20]. On the basis of morphology and morphogenesis, nine morphospecies have been assigned to the genus *Pseudourostyla* [21-30].

The present study has been performed on the cells of *Pseudourostyla*, collected from Delhi, India, with the aim for proper systematic assignment to the ciliate and to compare the Indian population with congeners.

Materials and methods

Sample collection and culturing of cells

Water samples were collected from stagnant water bodies near the river Yamuna and water pools near Najafgarh area (28° 34' N, 76° 07' E), Delhi, India. Water temperature and pH at the time of collection was 27-28°C and 7.4 respectively. Cultures were maintained in the at 24℃± 1℃, in the modified Pringsheim's medium and were fed with *Chlorogonium elongatum* [31].

Morphology and morphogenesis

Live cells were observed under high power oil emersion objective with bright field Nomarski phase contrast microscope. To study the infraciliature, cells were stained with protargol [32]. Measurements were done in arbitrary units by an ocular micrometer (Leitz) and converted into metric units with the help of a stage micrometer. Drawings of the impregnated specimens were made by camera lucida. Statistical analysis of the data was performed by methods described in Sokal and Rohlf (1969) [33]. Classification follows Berger (2006) [13] and the terminology for the cirri is according to Berger (2001, 2006), Borror (1972), Jerka-Dziadosz (1972) [34,13,19,21].

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Results

Cortical morphology of the morphostatic cells of *Pseudourostyla levis* (Figs. 1,12)

The ciliate is elongate and dorsoventrally flattened cell, measuring 274 µm ± 13.4 µm in length and 80.02 µm ± 11.69 µm in width (mean ±S.D., n= 100). Nuclear apparatus consists of 23-59 macronuclei and 3-11 micronuclei. Biometric characterization of *P. levis* (India) is presented in Table - 1. Cortical structures can be defined as buccal, frontal or frontoventral (FVT) complex and somatic ciliature, which are distinct ontogenetically and spatially [5].

Table 1: Biometrical characterization of *Pseudourostyla levis*, collected from Delhi (India)

Character	Mean ($\bar{X}$)	Standard deviation	Coefficient of variation (CV)	Range		Sample size
				Minimum	Maximum	
Body length (µm)	274.31	13.42	4.89	252.6	296.6	100
Body width (µm)	80.02	11.69	14.6	64.3	115.9	100
Distance from the anterior end of the body to the end of AZM (µm)	105.0	11.65	11.09	77.3	135.3	26
Number of membranelles in AZM	95.6	7.03	7.35	84	113	25
Number of cirri in -						
RFR	12.15	0.88	7.24	11	13	25
LFR	10.2	0.86	8.43	9	12	25
RVR	19.72	2.01	10.91	15	23	25
LVR	18.04	2.77	15.35	12	23	25
LMR_1	33.48	6.2	18.51	22	46	25
LMR_2	36.52	5.48	15.0	28	54	25
LMR_3	30.28	4.78	15.78	22	41	25
LMR_4	23.32	7.67	32.89	17	40	25
LMR_5	11.5	4.2	36.52	5	20	25
RMR_1	29.04	8.12	27.96	18	51	25
RMR_2	37.24	6.32	16.97	25	53	25
RMR_3	39.64	4.98	12.56	29	51	25
RMR_4	36.04	10.71	29.71	18	58	24
Number of transverse cirri	7.62	0.87	11.41	6	10	25
Number of dorsal kineties	7.0	0	0	7	7	25
Number of macronuclei	37.4	5.3	14.17	23	59	225
Number of micronuclei	5.9	1.73	29.3	3	11	225

Abbreviations in table: AZM – Adoral Zone of Membranelles; RFR – Right Frontal Row; LFR - Left Frontal Row; RVR – Right Ventral Rows; LVR – Left Ventral Rows; RMR – Right Marginal Rows; LMR – Left Marginal Rows

Buccal ciliature

It includes the adoral zone of membranelles (AZM), undulating membranes (UMs) and the paroral cirrus. The AZM extends up-to 1/3-1/4 of the body length and consists of 84-113 membranelles, arranged in parallel rows. On the right side of AZM, two undulating membranes are present, the outer paroral membrane and the inner endoral membrane. The first cirrus of the right frontal row is called the paroral cirrus and is considered as a part of the buccal ciliature because of its developmental origin. It differs from other frontal cirri in being differentiated from the anterior terminus of the developing undulating membranes [8].

Frontal ciliature (FVT COMPLEX)

The frontal ciliature also called the FVT complex, includes frontoventral cirri, transverse cirri and an isolated malar cirrus. There are two rows of frontoventral cirri, along the central meridian of the cell, starting in front of the buccal cavity and terminating in front of an oblique row of transverse cirri (TC). The anterior most cirri of the frontoventral rows are hypertrophied and therefore, can be further categorized as frontals and midventrals [5]. The number of transverse cirri varies between 6 and 10. Very rarely, one or two very small cirri in front of transverse cirri are observed. These are probably comparable to the post-ventral cirri described by Takahashi (1988) [23].

Somatic ciliature

It includes marginal rows and dorsal kineties. On either side of FVT complex, there are parallel rows of marginal cirri (MC), five rows on the left and four on the right. Dorsal surface is covered with seven longitudinal ciliary rows or dorsal kineties (Fig.8)

Division morphogenesis in *Pseudourostyla levis* (India) [Figs.1-25]

During division, the cortical development involves a species-specific sequence of events leading to the assembly of two sets of identical structures *i.e.*, in the proter and the opisthe. Various primordia formed during the cortical morphogenesis are, oral primordia (including primordia for AZM and UMs) and primordia for FVT complex i.e., FVT primordia, marginal cirral primordia for marginal rows (all on the ventral surface) and somatic primordia for dorsal kineties (on the dorsal surface).

Division morphogenesis

Division cycle of P. levis has been divided into six stages depending upon the appearance and extent of development of different primordia on the ventral surface.

Stage 0 (**Figs.1,12**)

This stage represents the non-dividing cell, whose morphology has already been described above.

Stage 1 (Figs.2,13)

The first morphogenetic event of the cortical morphogenesis is the appearance of the AZM primordia for the opisthe, (AZM''). Small fields of kinetosomes are formed to the immediate left of the parental ventral cirri situated just posterior to the parental AZM.

Stage 2 (Figs. 3, 14,15,16)

During this stage, morphogenetic events begin for the proter cell also, where primordia for the undulating membranes (UM') and the FVT complex (FVT') appear. UM' is formed by the disintegration of the parental undulating membranes (Figs.14,15).The malar cirrus also disintegrates to form FVT' (Fig. 16). Simultaneously, extensive kinetosome proliferation occurs in the AZM'' primordium and results in the formation of an anarchic, longitudinal field of kinetosomes (Fig.16).

Stage 3 (Figs.4,17,18,19)

A group of kinetosomes appear at the posterior end of the parental AZM (fig 4). AZM primordia for the proter (AZM') appears at the posterior end of parental AZM (Fig.17). Proliferation of kinetosomes occur in the FVT'. In the opisthe, UM'' appears. During late stage 3, marginal primordia appear for future proter and opisthe (Fig.18). The primordia for new marginal rows are developed within only one of the several old marginal rows. FVT' and FVT'' primordia get organized in the form of short diagonal steaks (Fig.19).

Stage 4 (Figs. 5, 20,21,22)

From this stage onwards, the further development is almost similar in the proter and the opisthe. Membranelles begin to differentiate in both AZM' and AZM''(Fig.4). In the FVT' and FVT'', the process of streak formation is completed (Fig.20). However, an important morphogenetic feature is observed at this stage. Membranelles of the old lapel region of the parental AZM, divide into two segments (Fig.21). All the divided left segments get resorbed (Fig.22), while the remaining part of the old AZM is added on to the top of the developing AZM'. Differentiation begins in both MC' and MC'' forming streaks which represent the future marginal rows.

During stage 4 (Fig.22), further differentiation of structures from their respective primordia takes place. Differentiation of UM' and UM'' is marked by the appearance of a fork at their anterior end, the right branch of which later develops into the paroral cirrus or the first frontal cirrus. In the FVT' and FVT'', the anterior most streak splits into two parts: the inner part develops into the malar cirrus, while the other segment forms a frontal cirrus during stage 5.

Stage 5 (Figs. 6,7, 23)

The cortical development reaches near completion during this stage (Figs. 6,7). Differentiation of new cirri

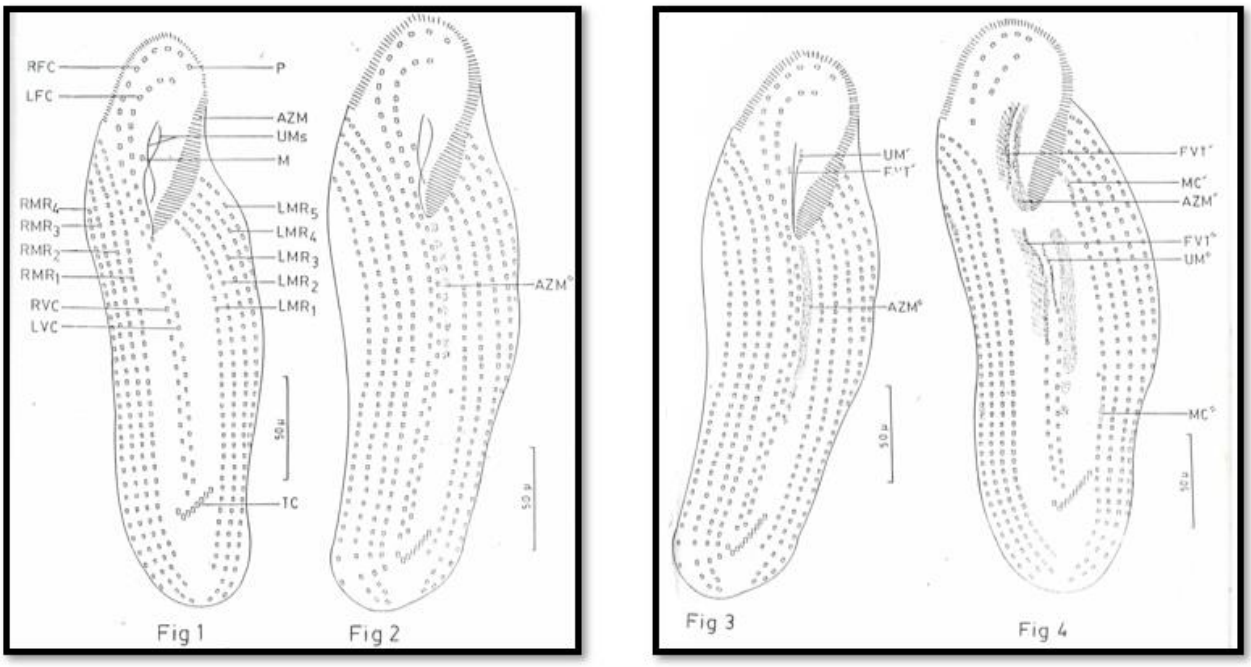
for the FVT complex from their respective primordia takes place and new cirri are formed in both MC' and MC"(Fig.23). The old marginal rows are resorbed along with the left-over frontal ciliature.

Development of dorsal ciliature (Figs. 8-11,24)

The dorsal suface of morphostatic cell has seven rows of dorsal kineties (Fig.8). The dorsal kineties develop from the somatic primordia, formed at two zones within each kinety (Fig.9-11). Subsequently, kinetosomal proliferation occurs and the new kineties elongate and expand in both directions i.e., in the anterior as well as in the posterior direction (Fig. 24). The old kineties get resorbed and are replaced by new kineties.

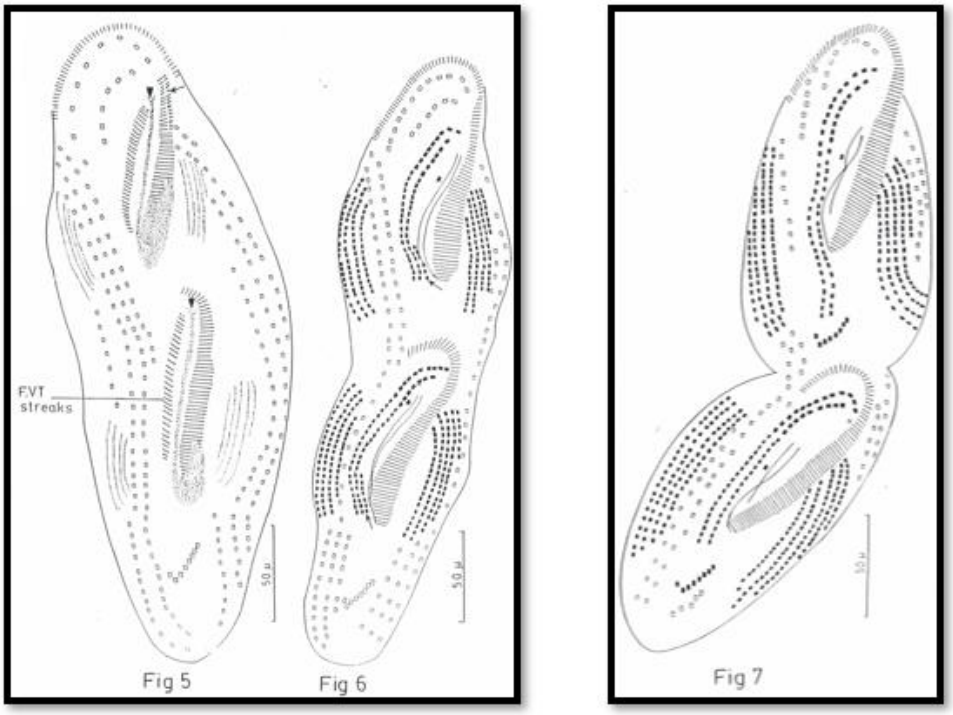
With the commencement of cytokinesis, the new cirri arrange themselves according to their species-specific pattern and the remaining parental ciliature gets resorbed.

A similar sequence of morphogenetic events is observed during the reorganization process (Figs.25-27). During re-organization, a single set of primordia is formed.



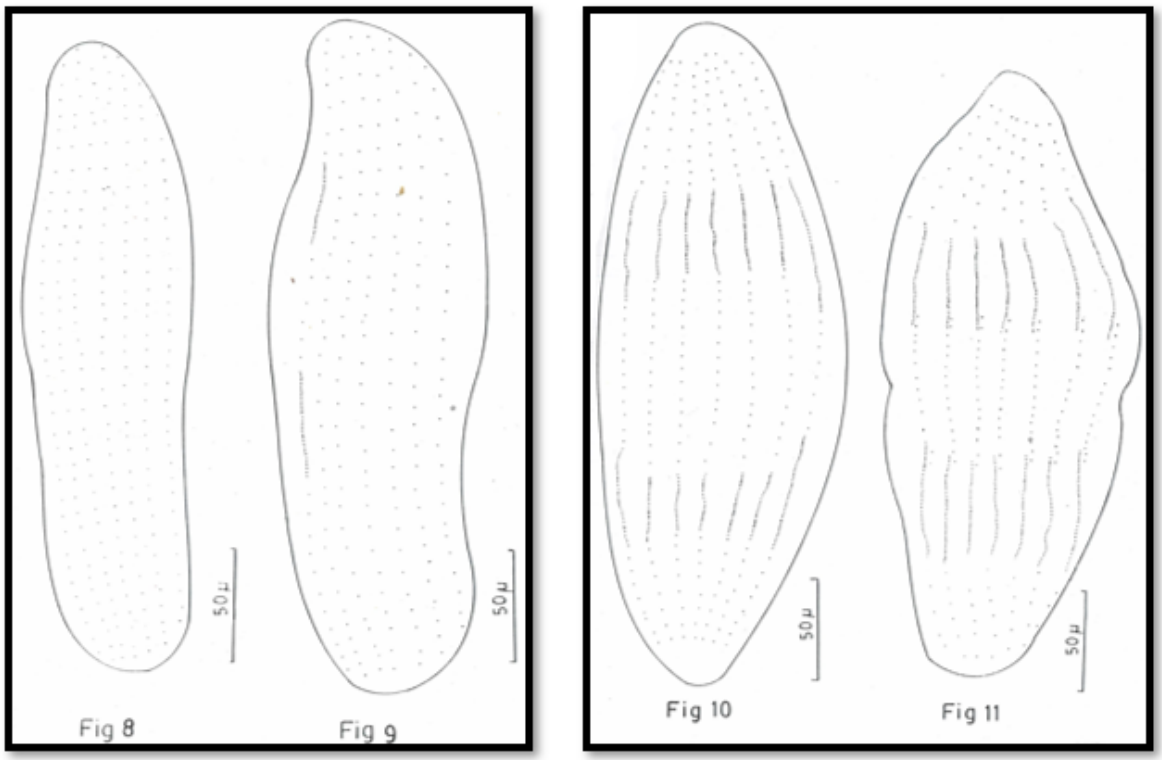
Figures 1-4: Line diagrams of ventral surface of protargol impregnated cells of *Pseudourostyla levis*, at different stages of Morphogenesis (ventral surface).

Fig.1: morphostatic cell; **Fig. 2**: Cell at stage 1 showing the formation of AZM primordium for the opisthe (AZM") from the left mid ventral cirri; **Fig. 3**: Cell at stage 2 showing the formation of primordium UM', FVT' for the proter and proliferation of kinetosomes in AZM"; **Fig. 4**: Cell at stage 3 showing formation of AZM primordia for the proter (AZM') and marginal cirral primordia for both proter and opisthe (MC', MC"); streak formation begins in both FVT' and FVT".



Figures 5-7: Line diagrams of protargol impregnated cells of *Pseudourostyla levis* during morphogenesis.

Fig. 5: Cell at stage 4 showing membranelle formation in AZM', AZM'' and completion of streak formation in FVT' and FVT''. Arrow indicates resorbing left segments of membranelle of parental AZM; **Fig. 6**: Cell at stage 5. Differentiation of cirri in their respective primordia is completed; **Fig. 7**: Cell at late stage 5. Transverse cirri (TC) acquire their final position and space between individual cirri enlarges.



Figures 8-11: Line diagrams of protargol impregnated cells of *Pseudourostyla levis* (dorsal surface). **Fig.8**: Seven rows of dorsal kineties in morphostatic cell. **Figs. 9-11**: Dorsal surface of dividing cells showing formation and proliferation of somatic primordia in dorsal kineties

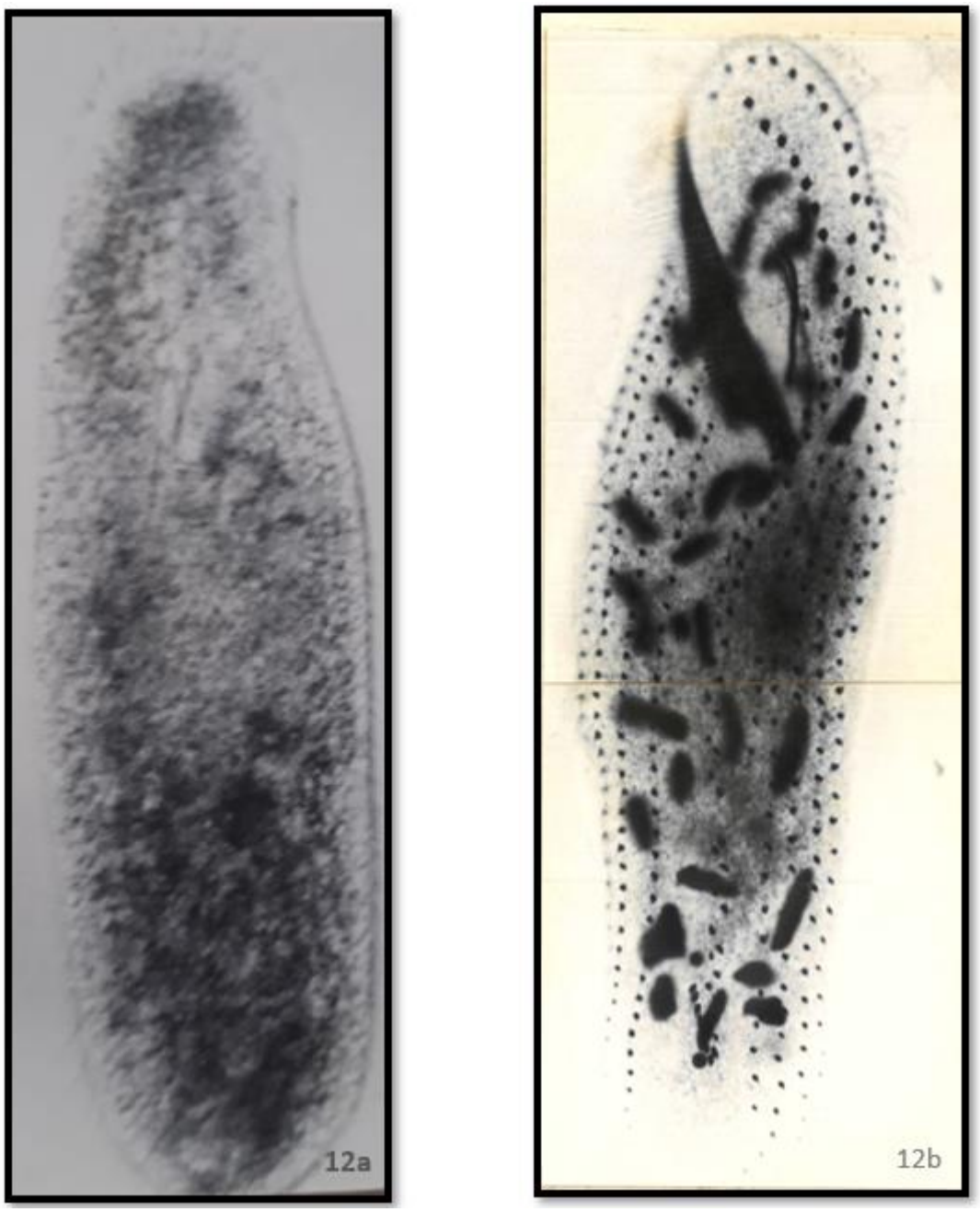
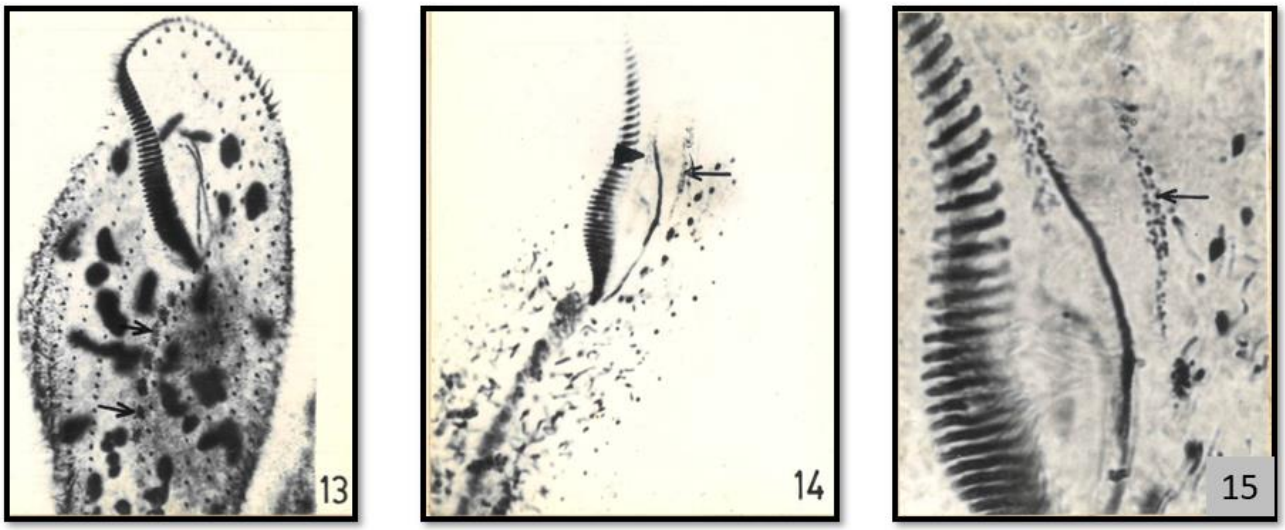
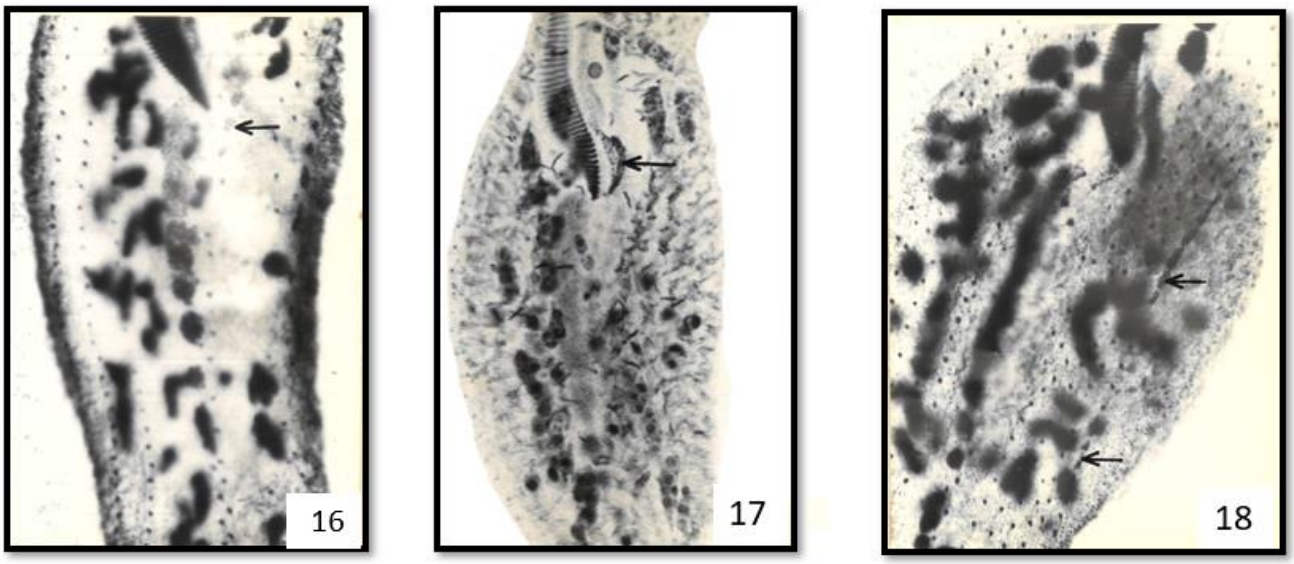


Fig.12a: Photomicrograph of live cell under Nomarski Phase contrast microscope.

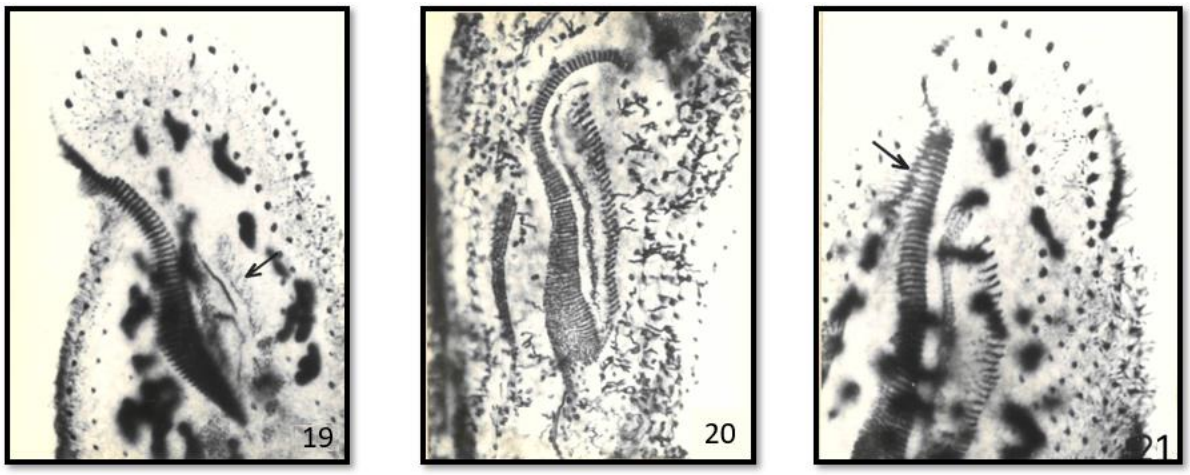
Fig.12b: Photomicrograph of protargol impregnated cell of *Pseudourostyla levis*, showing the ciliature on the ventral surface. ×450.



Figures13-15: Photomicrographs of protargol impregnated cells of *P. levis* showing morphogenetic events. × 450. **Fig.13**: Cell at stage 1 showing formation of oral primordium (arrow) for the opisthe (AZM"); **Fig.14**: Cell at stage 2 showing disaggregating malar cirrus which forms FVT'(arrow) and arrowhead indicates disaggregating Ums; **Fig.15**: a cell at stage 2 showing disaggregating parental UMs. ×1000.

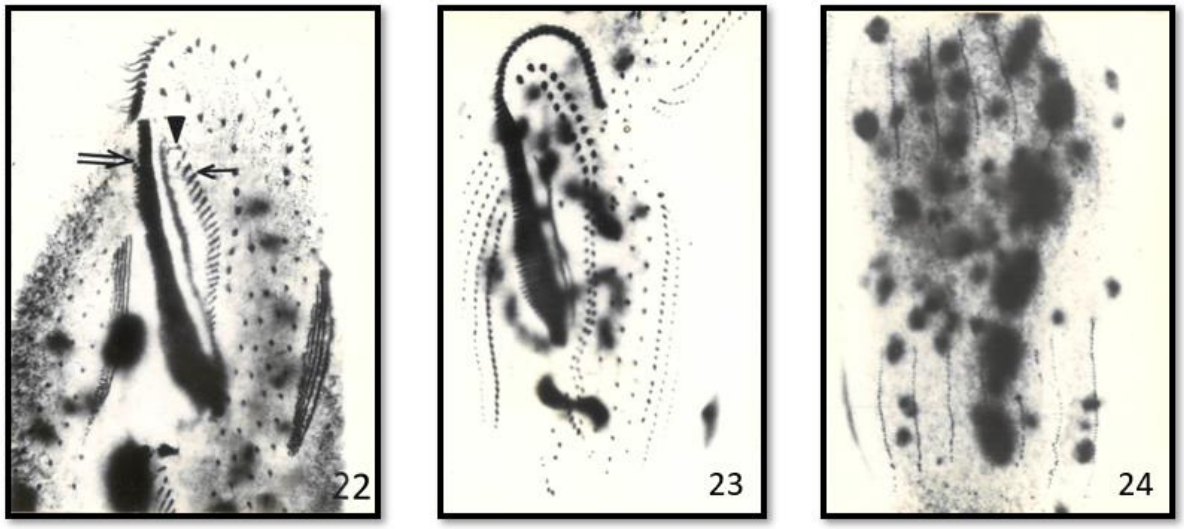


Figures 16-18: photomicrographs of protargol impregnated cells of P. levis. ×450. **Fig 16**: Cell at stage 2 showing formation of FVT"; **Fig. 17**: Cell at early stage 3 showing proliferation of kinetosomes in FVT' and FVT". AZM primordium for the proter (AZM') is formed (arrow); **Fig.18**: Cell at late stage 3 showing appearance of marginal primordia for the proter (MC') and for the opisthe (MC").



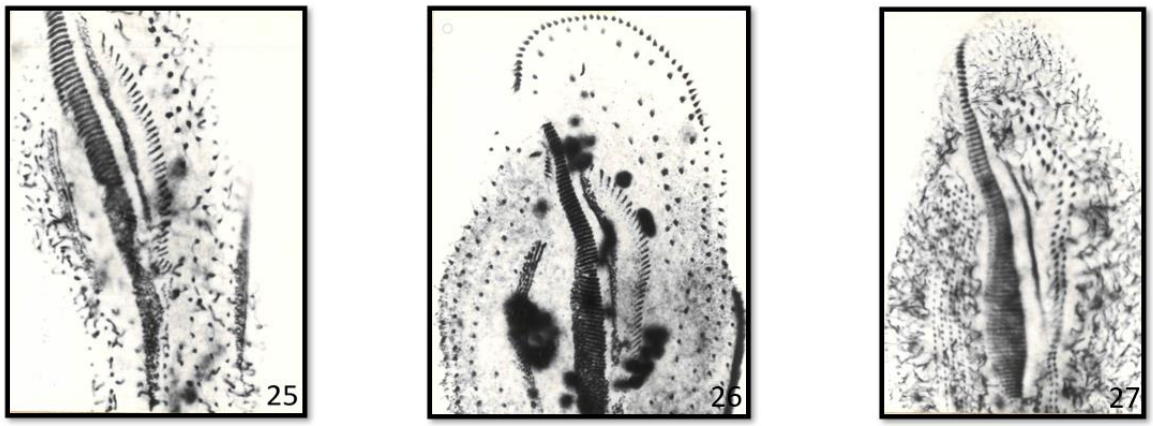
Figures 19-21: Photomicrographs of protargoal impregnated cells of *P. levis.* ×450.

Fig. 19: Cell at late stage 3 showing alignment of basal bodies into short diagonal streaks (arrow); **Fig. 20**: Cell at stage 4 showing streaks formation in the FVT" and membranelles are formed for the future opisthe; **Fig. 21**: Cell at late stage 4 showing splitting of membranelles of the lapel part of the parental AZM (arrow).



Figures 22-24: photomicrographs of protargol impregnated cells of *P. levis*. ×450

Fig. 22: Cell at late stage 4 showing resorption of the left segment of the split membranelles of the parental AZM (double stem arrow). The arrowhead indicates the fork-like branching in the UM', the first diagonal streak showing a split (single stem arrow); **Fig. 23**: Cell at stage 5 showing the completion of the differentiation of cirri in their respective primordia; **Fig. 24**: kinetosome proliferation in each dorsal kinety on the dorsal surface.



Figures 25-27: Photomicrographs of protargol impregnated cells of *P. levis* in various stages of reorganization. ×450.

Discussion

Historically, Pseudourostyla was separated from other urostylids and given a generic status by Borror (1972) [19], *on the basis of possession of midventral cirri.* Subsequently, presence of midventral cirri was reported in *Urostyla grandis* [21] and its separation on this character was no longer valid [5]. On the basis of other structural and morphogentic differences, (primarily the development of marginal rows from groups of longitudinal streaks in a common field on each side, rather than in streaks associated with each row), Borror (1979) suggested that it should be separated from *Urostyla* [5]. However, Wicklow (1981) [35] raised it to the level of superfamily on the grounds of the differences in the ontogeny of marginal rows and this was retained in the subsequent revision by Borror and Wicklow (1983) [8]. The presence of mid ventral cirri is considered to be a diagnostic feature of Urostylina Jankowski, [8,35,36]. On the basis of the presence of hypertrophied frontal cirri, presence of more than two rows of left and right marginal cirri and the mode of formation of marginal cirri from a common primordium to the rightmost row of each group, suggests that the present isolate from India belongs to the genus *Pseudourostyla* [5, 22-30].

Present morphometric study shows that *P. levis* (India) differs from *P. levis*(Japan) [22,23] in the following respects :

1. The lower limits of the size range of *P. levis* (India) are slightly higher.

2. Takahashi (1988) [23] reported the presence of two post-ventral cirri (PVC) in front of the transverse cirri. In Indian strain, rarely a cell shows either one or two small cirri in the same region, which may or may not correspond to the PVC.

3. Number of dorsal kineties also varies in the two strains *i.e.*, in *P. levis*, from Japan [23], there are 7-8 rows of dorsal cilia, while in the Indian strain, all the cells show 7 rows.

From the present study on *P. levis*, it can be inferred that although minor differences in morphology and morphogenesis are observed in the Indian and Japanese strain, but these differences do not appear significant, to assign a new species to the present isolate.

Morphometric comparison of *P. levis* (India) with congeners [Table-2]

Various species of *Pseuodourostyla* reported so far include *P. cristata* (=*Urostyla cristata*, Jerka-Dsiadosz,1964) Borror,1972[37,19]; *P. muscorum* (Kahl,1932) Borror (1972) [38,19]; *P. levis* Takahashi,1973, [22]; *P. nova* (Wiackowski,1988) [24]; *P. pelontensis* Paiva *et al*, 2006 [25], *P. cistatoides* Jung *et al.*, ,2012 [27]; *P. subtropica* Chen et al.,2014 [28]; *P. dimorpha* Foissner 2016[39] and *P. guizhouensis* sp.nova Li *et al.*,2017[30].

A morphometric comparison of *Pseuodourostyla levis* (India) with congeners [Table-2], shows that the main characters like, hypertrophied frontal cirri, number of frontoventral rows are relatively conserved features. However, some traits like cell size, number of left and right rows of marginal cirri, number of adoral zone of membranelles and transverse cirri and number of macronuclei and micronuclei show variability in different species. Number of dorsal kineties also varies in different described species. Although the dorsal ciliary pattern is considered to be conservative and stable character, but variation in the number of dorsal kineties is known to occur among population of ciliates [40,41]. These variations can be regarded as population dependent and can be considered as a function of variety of environmental and genetic mechanisms [8,26]. In *P. nova*, (population from Polland), a pair of frontoterminal cirri or migratory cirri are present on the right side of the midventral cirri, close to AZM [24]. In *P. cristata*, collected from Japan, similar frontoterminal cirri are present between distal end of AZM and anterior end of innermost right marginal row [26]. These frontoterminals reported in almost all reported species of *Pseudourostyla* but in *P.levis* (present study) frontoterminal cirri are not observed and neither reported in Japanese population [22,23].

Table 2: Comparison of characteristics of *Pseudourostyla levis* (India) with those of published data of different species of genus

Character	*P. levis* (India)	*P. levis* (Japan)	*P. nova* (Polland)	*P. cristata* (Polland)	*P .cristata* (Japan)	*P. pelotensis* (Brazil)	*P. subtropica* (China)	*P. cristatoides* (South Korea)
Body length(µm)								
Range	252.6-296.6	162.5-266.3	168-324	300-450	275-378	145-195	300-600	210- 290
Mean± S.D	274.31± 13.42	213.1± 19.6	232.21±36.18	-	325.9 ± 26.98	163.1 ±15.5	422.2 ± 88.8	-
CV %	4.89	-	15.58	-	8.3	9.5	21	-
Body width (µm)								
Range	64.3 -115.9	25 -100	46 -80	120 -180	108 -224	51 -70	160- 360	65 -125
Mean± S.D	80.2 ± 11.69	-	64.56 -7.85	-	156.6 ±32.7	58.9 ± 6.4	225 -63.5	-
CV %	14.6	-	12.16	-	20.9	10.8	28.2	-
Percentage of body length occulied by AZM	25 -33	25 -33	Approx.45 *	Approx. 25 *	40	Approx.33 *	25 -33	33
Number of AZM								
Range	84 -113	Appox.83	45-57	93- 130	80-94	38 -54	104 -173	84 -115
Mean± S.D	95.6 ± 7.03	-	50.33 ± 3	-	88.6 ±13.37	47.4 ± 3.9	131.4 ± 23.1	-
CV %	7.35	-	5.96	-	3.8	8.3	17.6	-
Number of FT cirri	0	-	2	2	2	2	2	2
Number of frontoverntral rows	2	2	2	2	2	2-3	2	2
Number of RMR								
Range	4	-	2	7	5	5- 8	5 -9	4 -5
Mean± S.D	4 ± 0	4.1± 0.4	2 ± 0	-	5± 0	5.7 ± 0.9	6.6 ± 1.6	-
CV %	0	-	0	-	0	16.1	24.4	-
Number of LMR								
Range	5	-	2	7	5 -6	4 -6	7 -13	5 -7
Mean± S.D	5 ± 0	5.5 ± 0.5	2± 0	-	5.5 ± 0.51	5.4 ± 0.6	9.0 ± 1.9	-
CV %	0	-	0	-	9.2	11.9	20.8	-
Number of TC								

Character	*P. levis* (India)	*P. levis* (Japan)	*P. nova* (Polland)	*P. cristata* (Polland)	*P .cristata* (Japan)	*P. pelotensis* (Brazil)	*P. subtropica* (China)	*P. cristatoides* (South Korea)
Range	6 -10	6 -10	7 -9	8 -12	7 -10	5 -9	7 -12	6 -12
Mean± S.D	7.62 ± 0.87	8 ± 0.9	7.73 ± 0.694	-	8.4 ± 0.99	6.3 ± 1.14	9.9 ± 1.3	-
CV %	11.41	-	8.94	-	11.9	16.9	13	-
Number of dorsal kineties								
Range	7	7 -8	7	8	8 -10	7 -11	8 -14	10 -13
Mean± S.D	7 ± 0	7.8 ± 0.5	7± 0	-	8.5 ± 0.59	8.4 ± 1.3	10.9 ± 1.8	-
CV %	0	-	0	-	6.9	15.1	16.7	-
Number of macronuclei								
Range	23 -59	17 -73	14 -28	44 -83	15-36	62 -114	68-219	30-106
Mean± S.D	37.4 ± 5.3	-	17.97 ± 4.29	-	26.6±5.56	88.2 ± 11.7	114.4± 37.2	-
CV %	14.17	-	23.78	-	20.9	13.1	32.5	-
Number of micronuclei								
Range	3- 11	0- 16	5 -8	6 -8	2 -9	-	2 -6	-
Mean± S.D	5.9 ± 1.73	-	6.4 ± 0.87	-	5.4 ± 1.9	-	3.7 ± 1	-
CV %	29.3	-	13.57	-	35.9	-	28	-
References	Present study	[23]	[24]	[21]	[26]	[25]	[28]	[27]

Dash (-) denotes data not available

Abbreviations: AZM – Adoral Zone of Membranelles; FT – Frontoterminal Cirri; RMR – Right Marginal Row; LMR – Left Marginal Row; TC – Transfer Cirri

Conclusion

P. levis (India) closely resembles *P. levis* (Japan). The prevailing ambiguity in the morphometric and morphogenetic characters encountered in different morphospecies of *Pseudourostyla* suggests that the taxonomic problem of species discrimination requires further study of morphological, biochemical and physiological variations. A comparative molecular analysis, based on small subunit of ribosomal DNA (SSSUrDNA), is required to be done in the Indian isolate, to establish the evolutionary relationship with other urostylids.

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Conflict of interest

The author declares no competing interests.

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