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# Morphology and morphogenesis of *Rubrioxytricha indica* n. sp. (Ciliophora: Hypotrichida)

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**Summary.** *Rubrioxytricha indica* n. sp. 80 × 30 µm *in vivo* was isolated from a pond in New Delhi, India. It possesses a single caudal cirrus and numerous brown coloured crystalloids in the cytoplasm and three contractile vacuoles. Uniformly scattered dark granules are present either as single entities or in clusters of 2-5. The ventral ciliature consists of 18 frontal-ventral-transverse cirri, one row each of right and left marginal cirri, three long dorsal kineties, two dorsomarginal rows, two undulating membranes (UMs) in *Cyrtohymena* pattern (Berger and Foissner 1997) and a question mark shaped adoral zone of membranelles. The cirrus V/3 participates in morphogenesis at very late stages of division; its kinetosomes merging with the posterior part of the developing UMs of the opisthe. There is no splitting of the third dorsal primordium. Consequently three dorsal kineties ($DK_{1-3}$) are formed and only one caudal cirrus develops at the end of $DK_3$.

**Key words:** diagnosis, morphogenesis, morphometry, *Rubrioxytricha indica* n. sp.

**Abbreviations:** AZM - adoral zone of membranelles, BL - body length, BW - body width, CC - caudal cirrus, CV - coefficient of variance, DK - dorsal kinety, DM - dorsomarginal, FVT - frontal-ventral-transverse, LMC - left marginal cirri, n - number of cells, OP - oral primordium, RMC - right marginal cirri, SD - standard deviation, UMs - undulating membranes.

## INTRODUCTION

The genus *Rubrioxytricha* was erected by Berger (1999) by separating it from the genus *Oxytricha* on the basis of the presence of one or two caudal cirri and distinctly or slightly homogeneously coloured cytoplasm. Other features of the genus include, highly flexible body, question mark shaped adoral zone of membranelles, 18 frontal-ventral-transverse (FVT) cirri wherein posterior frontal cirri are arranged in a 'V' shaped pattern, 5 ventrals and transverse each and one row each of left marginal (LMC) and right marginal cirri (RMC). Two undulating membranes are arranged in the *Oxytricha* pattern (Berger and Foissner 1997). Cells also possess cortical granules. Berger (1999) has listed two species of this genus; *R. haematoplasma* comb. n. and *R. ferruginea* comb. n., originally reported as

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*O. haematoplasma* (Blatterer and Foissner 1990) and *O. ferruginea* (Stein 1859) respectively.

In the present study, we report the morphometric and morphogenetic data of *Rubrioxytricha indica* n. sp. and its comparison with the two described species of *Rubrioxytricha*, similar sized species of *Cyrtohymena* and similar sized species of *Oxytricha* possessing cortical granules.

## MATERIALS AND METHODS

*Rubrioxytricha indica* n. sp. was isolated from a small pond in New Delhi (28°34'N, 76°07'E). Cells were acclimatized to the laboratory conditions and then grown at 23 ± 1°C with axenically cultured *Chlorogonium elongatum* as the food organism (Ammermann *et al.* 1974).

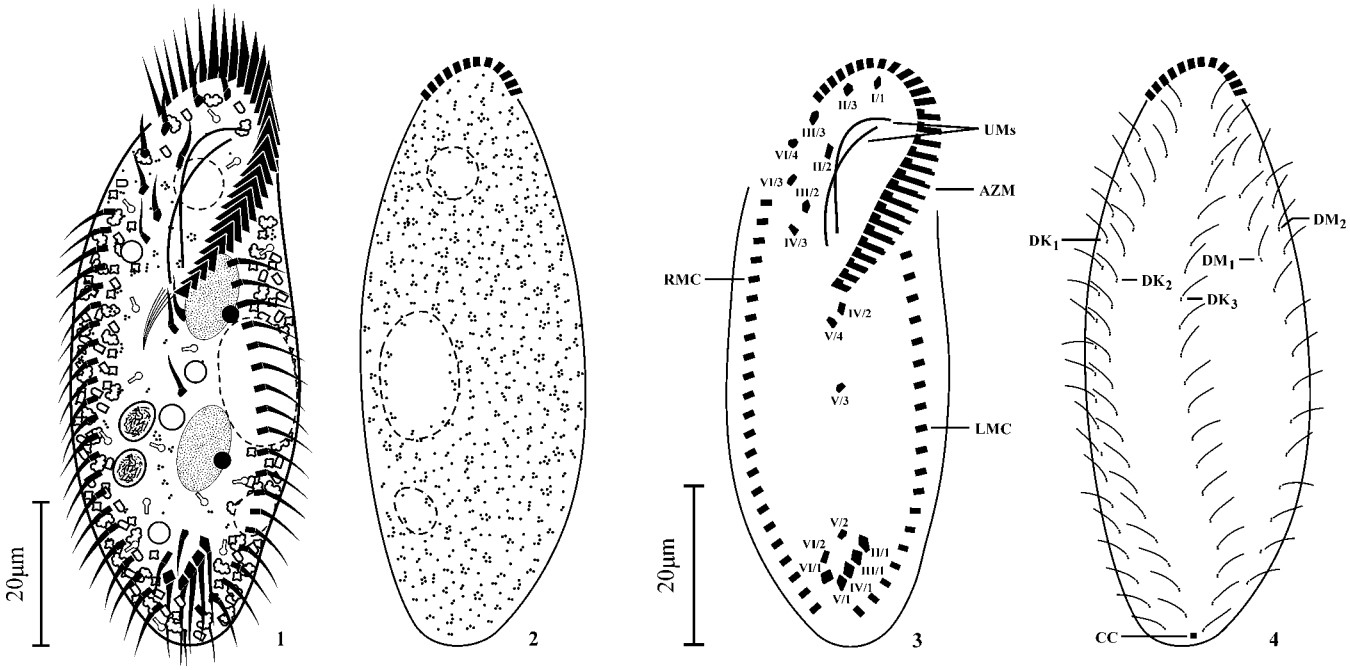
Morphometric characterization of non-dividing cells was performed after staining with the modified protargol method (Kamra and Sapra 1990). The enrobed cells were bleached for 4 minutes in a 5% dilution of commercial sodium hypochlorite solution before staining in 0.5% protargol (Roque). All measurements were performed using an ocular micrometer (Jena) with one unit = 32.5 µm. Measurements and counts were done at the magnification of 400× and 1000× respectively. Live cells were observed under differential interference contrast microscope at the magnification of 1000×. Observations on cortical granules and cytoplasmic crystalline inclusions were made under bright field microscope for confirming their colour etc. Magnification was calculated using a micrometer slide (E. Leitz). The general terminology followed was according to Wallengren (1900), Borror (1972) and Berger (1999).

For making schematic illustrations, protargol impregnated cells were projected on a table using a slide projector. The diagrams were further reproduced by scanning and using the computer program coral draw (version 8.0).

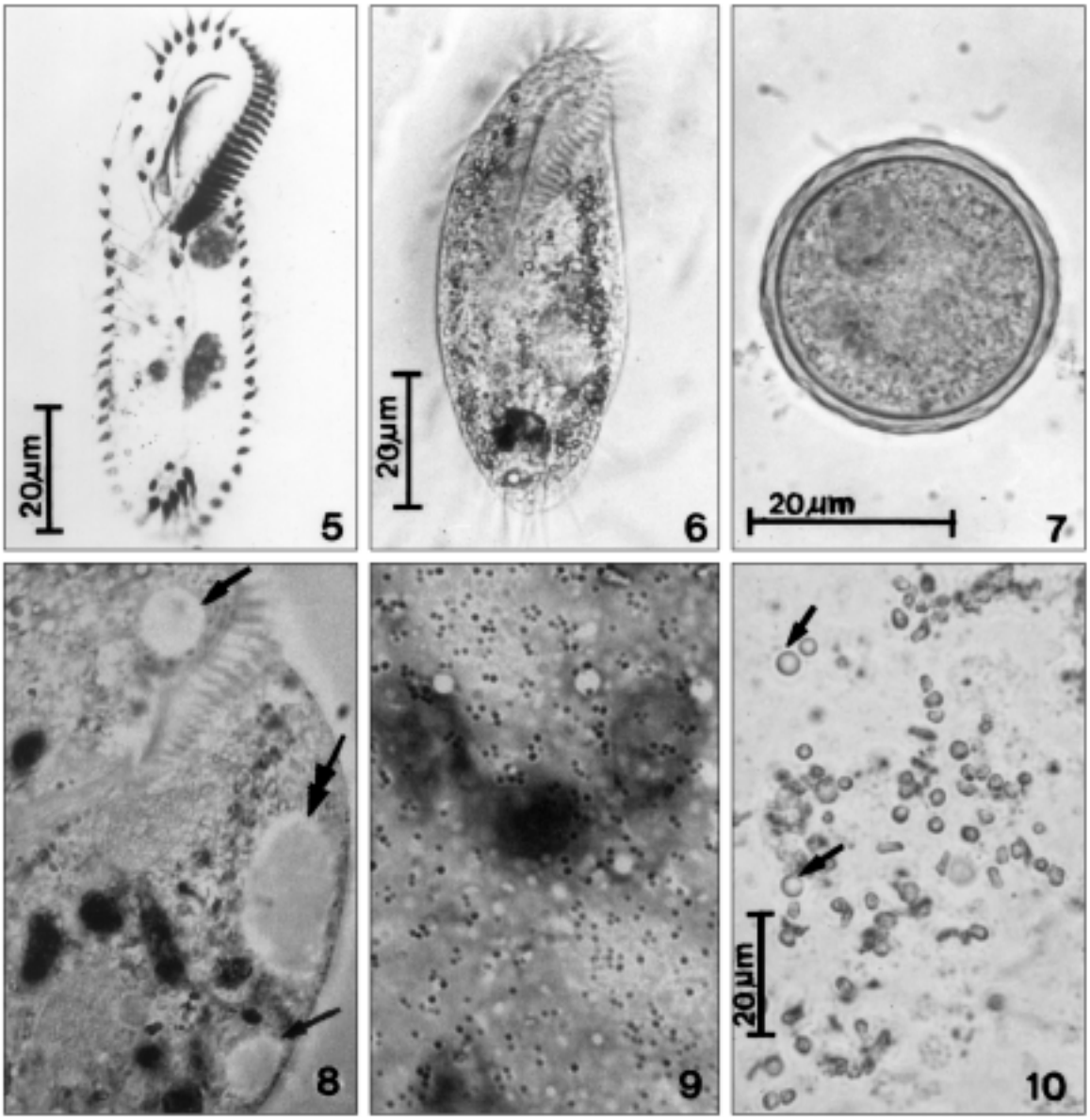
## RESULTS

### *Rubrioxytricha indica* n. sp.

**Diagnosis:** Size about 69 × 27 µm in protargol preparation and 80 × 30 µm *in vivo*; highly flexible and elliptical in shape. 2 macronuclear nodules and 2-3 micronuclei. Cytoplasm shows numerous dark brown crystalline inclusions, present in scattered dense aggregates. Cortical granules spherical and dark green in colour, uniformly distributed, arranged either singly or in clusters of 2-5. Adoral zone of membranelles (AZM) about 40% of body length. Two undulating membranes




**Figs 1-4.** *Rubrioxytricha indica* n. sp. **1** - ventral view *in vivo*; **2** - dorsal surface showing cortical granules; **3** - infraciliature of ventral surface: adoral zone of membranelles (AZM), undulating membranes (UMs), frontal cirri (II/2, I/1, II/3, III/3, VI/4, VI/3, IV/3 and III/2), ventral cirri (IV/2, V/4, V/3, V/2 and VI/2), transverse cirri (II/1, III/1, IV/1, V/1 and VI/1), right marginal cirral row (RMC), left marginal cirral row (LMC); **4** - dorsal surface: dorsal kineties ($DK_{1-3}$), dorsomarginal rows ($DM_{1\&2}$) and caudal cirrus (CC).




**Figs 5-10.** *Rubrioxytricha indica* n. sp. (5 - protargol impregnation; 6-10 *in vivo*). **5 -** infraciliature of ventral surface and nuclear apparatus of the holotype specimen. For detailed labeling of infraciliature see Fig. 3; **6 -** ventral view showing characteristic body outline; **7 -** resting cyst; **8 -** three contractile vacuoles in diastole in slightly squeezed (flattened) specimen; second contractile vacuole (double arrow) largest in size, the other two are smaller (arrows) (Mag. 1000×); **9 -** dorsal surface showing uniformly scattered spherical cortical granules arranged either singly or in clusters of 2-5 (Mag. 1000×); **10 -** crystalline inclusions of various shape and size and spherical lipid droplets (arrows) of squashed cell.

in *Cyrtohymena* pattern (See morphology and morphometry section for further details). On average about 22 cirri in right and 19 in left marginal row, 8 frontal, 5 ventral and 5 transverse cirri. Three dorsal kineties, 2 dorsomarginals and 1 caudal cirrus. Participation of cirrus V/3 in morphogenesis at very late stages and its kinetosomes merge with undulating membranes of opisthe.

**Etymology:** Species named after the country from where it has been isolated.

**Slide deposition:** Two slides have been deposited in the Oberösterreichische Landes-museum in Linz (LI), Austria.

**Morphology and morphometry (Figs 1-10, 28; Table 1):** Size about 69 × 27 µm in protargol preparations and about 80 × 30 µm *in vivo*, average length to width ratio 2.6:1. Shape elliptical, very flexible, dorsoventrally flattened. Generally bottom dwellers showing sluggish creeping movements. Two macronuclear nod-

**Table 1.** Morphometric characteristics of *Rubrioxytricha indica* n. sp. (Data from protargol impregnated non-dividing cells, n=20).

| Characters | Mean | Min | Max | SD | CV |
|---|---|---|---|---|---|
| Body length (µm) | 69.1 | 64.7 | 74.1 | 3.30 | 4.77 |
| Body width (µm) | 26.6 | 23.5 | 31.8 | 2.80 | 10.53 |
| Body length/ Body width | 2.6 | 2.2 | 3.0 | 0.22 | 8.46 |
| Macronuclear nodules, number | 2.0 | 2 | 2 | 0.00 | 0.00 |
| Macronuclear nodules, length (µm) | 11.8 | 10.8 | 12.7 | 0.63 | 5.34 |
| Macronuclear nodules, width (µm) | 7.8 | 6.9 | 8.4 | 0.46 | 5.89 |
| Micronuclei, number | 2.3 | 2 | 3 | 0.47 | 0.00 |
| Micronuclei, diameter (µm) | 3.2 | 3.1 | 3.3 | 0.10 | 3.13 |
| Adoral Membranelles, number | 29 | 27 | 31 | 1.36 | 4.69 |
| Adoral zone length (µm) | 27.6 | 23.5 | 30.6 | 2.14 | 7.75 |
| Adoral zone length/ Body length | 0.4 | 0.3 | 0.4 | 0.03 | 7.50 |
| Frontal cirri, number | 8.0 | 8 | 8 | 0.00 | 0.00 |
| Ventral cirri, number* | 5.2 | 5 | 7 | 0.64 | 0.12 |
| Transverse cirri, number* | 5.2 | 5 | 7 | 0.52 | 0.10 |
| Number of cirri in left marginal cirral row** | 19.2 | 17 | 21 | 1.25 | 6.51 |
| Number of cirri in right marginal cirral row | 22.3 | 21 | 24 | 1.09 | 4.89 |
| Number of dorsal kineties (DKs) | 3.0 | 3 | 3 | 0.00 | 0.00 |
| Number of dorsomarginal rows (DMs) | 2.0 | 2 | 2 | 0.00 | 0.00 |
| Dorsal bristles, number in | | | | | |
| $DK_1$ | 19.6 | 18 | 21 | 1.07 | 0.05 |
| $DK_2$ | 20.7 | 20 | 22 | 0.67 | 0.03 |
| $DK_3$ | 19.8 | 18 | 23 | 1.47 | 0.07 |
| $DM_1$ | 15.1 | 12 | 17 | 1.38 | 0.09 |
| $DM_2$ | 4.6 | 3 | 7 | 1.56 | 0.34 |
| Dorsal bristles, total number | 79.0 | 76 | 88 | 3.33 | 0.04 |
| Caudal cirri, number | 1.0 | 1 | 1 | 0.00 | 0.00 |

*Nearly 14 % cells show one or two supernumerary ventral/ transverse cirri (n=400),** 12% cells show a second LMC with few cirri (n=100)

**Table 2.** Parental structures associated with the origin of FVT primordia and undulating membranes during division morphogenesis of *Rubrioxytricha indica* n. sp.

| Daughter cell | Parental structure | Primordium number/structure |
|---|---|---|
| Proter | Parental undulating membranes | I |
| | II/2 + kinetosomes of streak II of opisthe | II |
| | III/2 | III |
| | IV/3 | IV |
| | Streak V of opisthe | V |
| | Streak VI of opisthe | VI |
| Opisthe | Oral primordium | I |
| | Oral primordium | II |
| | Oral primordium | III |
| | IV/2 | IV |
| | V/4 | V |
| | V/4 | VI |
| | V/3 | Posterior part of undulating membranes of opisthe |

**Table 3.** Morphometric comparison of three species of *Rubrioxytricha.* All dimensional measurements are averages in µm.

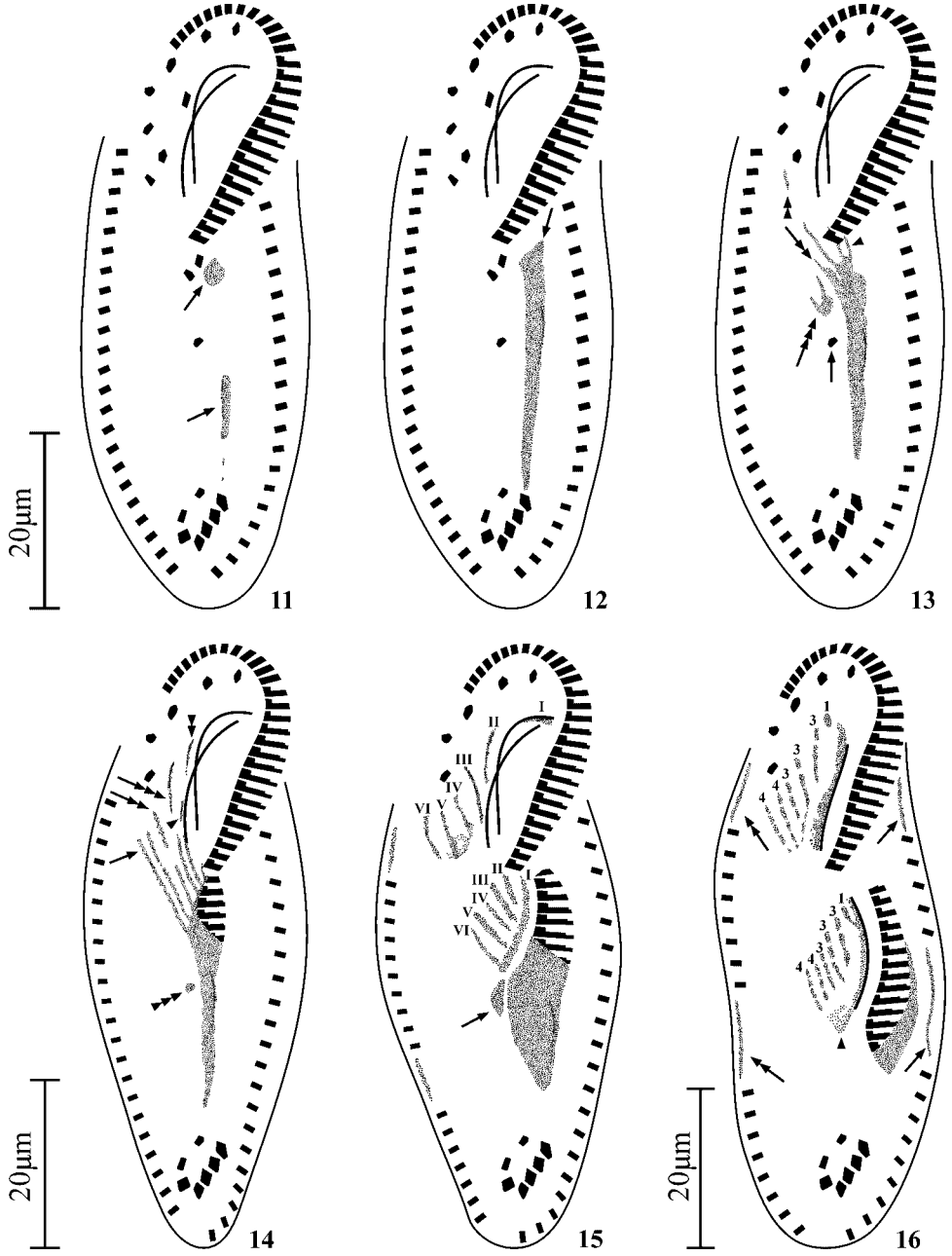
| Characters | *R. indica* n. sp. (Present report) | *R. haematoplasma* (Blatterer and Foissner 1990, Berger 1999) | *R. ferruginea* (Stein 1859, Song and Wilbert 1989, Berger 1999) |
|---|---|---|---|
| Body shape | Elliptical, both ends rounded | * | Both ends rounded with concave margins |
| Body length (BL) | 69.1 | 113.3 | 143.4 |
| Body width (BW) | 26.6 | 41.4 | 47.2 |
| Body length/ Body width | 2.6:1 | 2.7:1 | 3.0:1 |
| Macronuclear nodules, length | 11.8 | 15.9 | * |
| Macronuclear nodules, width | 7.8 | 8.6 | * |
| Micronuclei, number | 2-3 | 2 | 2 |
| Adoral membranelles, number | 29 | 38.4 | 38.3 |
| Number of cirri in left marginal cirral row | 19.2 | 35.8 | 33.0 |
| Number of cirri in right marginal cirral row | 22.3 | 33.4 | 32.7 |
| Dorsal rows | 5.0 | 4.0 | 5.0 |
| Caudal cirri | 1 | 1 | 1-2 |
| OP origin | Dual; in two parts | * | * |
| Colour of cortical granules | Dark green | Lemon yellow to green | Brownish |
| Arrangement of cortical granules | Uniformly scattered | In rows | In rows |
| Colour of the cytoplasm | More or less colourless | Usually distinctly or rarely slightly homogeneously orange to reddish | Homogeneously rusty brown |
| Cytoplasmic inclusions | Numerous sharp dark crystalline bodies in groups | * | * |

* Not mentioned by the authors.

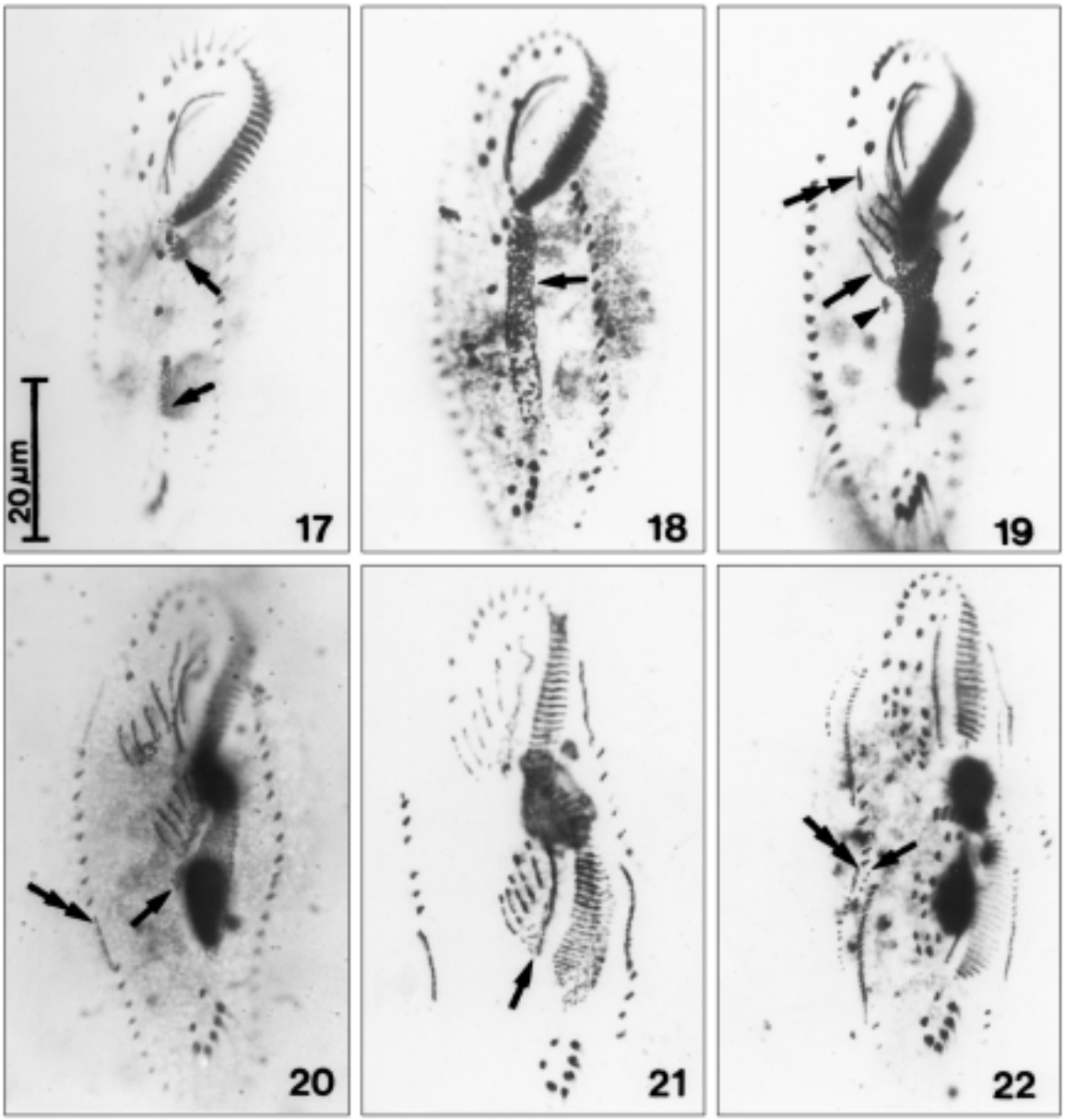
ules situated slightly left of mid line of the cell, ellipsoidal, about 12 × 8 µm. Two to three micronuclei, spherical, about 3.2 µm, attached to the macronuclear nodules at variable positions. Three contractile vacuoles consistently seen in live, swimming specimens (Fig. 8). One (first) contractile vacuole present in the anterior region near the tip of UMs, the second in the mid region below the AZM near the left cell margin and the third one below the second. The second contractile vacuole is the largest whereas the other two are smaller and almost similar in size. All contractile vacuoles show synchronous systolic activity. The diastolic activity of the third contractile vacuole is delayed in relation to the other two. Encystment and excystment frequent. Mature cyst with smooth cyst wall (Fig. 7), 28 µm in protargol stained preparations. Cortical granules (1-1.5 µm), dark green in color, uniformly distributed, and arranged either singly or in clusters of 2-5 (Figs 2, 9), more abundant on the dorsal surface, do not impregnate with protargol.

Cytoplasm with lipid droplets (3-5 µm) and numerous small crystalline inclusions of different shapes roughly spherical (2-2.4 µm) or irregular, rusty brown in color (Fig. 10). Morphometric features of cells are shown in Table 1.

Adoral zone occupies 40% of the body length with about 29 membranelles. Buccal cavity large and deep, undulating membranes (UMs) in *Cyrtohymena* pattern (one may however note that the curvature of the paroral membrane in *R. indica* n. sp. is not as pronounced as that seen in the *Cyrtohymena* pattern). Cirral pattern with 18 FVT cirri (Figs 3, 5). Anterior frontals (II/2, I/1, II/3 and III/3) slightly thicker than the posterior ones. Three postoral ventral cirri (IV/2, V/4 and V/3) near the cytostome, with IV/2 and V/4 appearing as a pair while V/3 placed at a distance from them. About 14% cells (n=400) show one or two supernumerary cirri, generally in the group of ventrals and occasionally as additional transverse cirri. Right marginal row starts at the level of III/2 and terminates below the level of VI/1. Left marginal row 'J' shaped and terminates at the midline. About 12% cells show a second LMC row with few cirri (n=100).



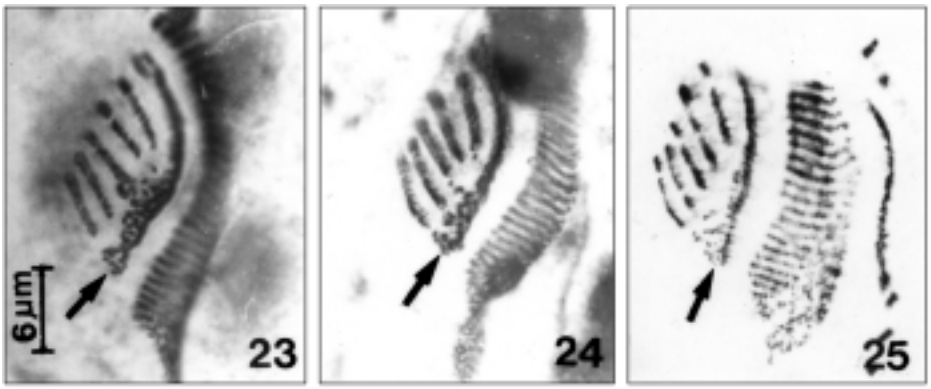

**Figs 11-16.** Division morphogenesis of *Rubrioxytricha indica* n. sp. on the ventral surface. **11 -** OP in parts (arrows); **12 -** long OP after joining of both the parts (arrow); **13 -** origin of streaks of opisthe: I, II and III from OP (arrow head), streaks IV from IV/2 (double arrow), streaks V and VI for opisthe from V/4 (triple arrow), intact V/3 (arrow), disaggregating IV/3 (double arrow head); **14 -** movement of streaks V and VI (arrow), disaggregated IV/3 (double arrow) and III/2 (triple arrow), migrated kinetosomes of streak II of opisthe (arrow head) to join disaggregated II/2 (double arrow head), disaggregating V/3 (triple arrow head); **15 -** two sets of six FVT ciliary streaks (I-VI), disaggregated V/3 (arrow); **16 -** cirri differentiation of FVT streaks in proter and opisthe in 1,3,3,3,4,4 pattern, LMC primordia (arrows), RMC primordia (double arrows), kinetosomes of V/3 merging with the posterior part of UMs of opisthe (arrow head). FVT - frontal-ventral-transverse, LMC - left marginal cirri, OP - oral primordium, RMC - right marginal cirri, UMs - undulating membranes.




**Figs 17-22.** Division morphogenesis of *Rubrioxytricha indica* n. sp. after protargol impregnation on ventral surface. **17 -** origin of OP in parts (arrows); **18 -** long OP (arrow); **19 -** anterior movement of streaks V and VI (arrow), disaggregating IV/3 (double arrow) and V/3 (arrow head); **20 -** disaggregated V/3 (arrow), RMC primordium (double arrow); **21 -** kinetosomes of V/3 merging with the posterior part of developing UMs of opisthe (arrow); **22 -** very long $DM_1$ (double arrow), short $DM_2$ (arrow). DM - dorsomarginal, OP - oral primordium, RMC - right marginal cirri,UMs - undulating membranes.

Dorsal bristles about 4-5 µm long *in vivo*, arranged in 5 rows ($DK_{1-3}$ and $DM_{1,2}$) (Figs 4, 28). $DK_{1-3}$ complete rows and curved in the middle, a few posterior bristles of $DK_3$ row arranged in a curvature that runs opposite to that of $DK_{1,2}$. $DM_1$ long with about 15 bristles while $DM_2$ short with only 3-7 bristles. Single inconspicuous caudal cirrus present at the end of $DK_3$.

**Divisional morphogenesis (Figs 11-27, 29; Table 2):** Stomatogenesis starts with the de novo appearance of kinetosomes between the left marginal cirral row and the post oral ventral cirrus IV/2 forming an oral primordium (OP). The kinetosomes proliferate and extend the field posteriorly. Simultaneously a small field of kinetosomes also appears near the transverse cirrus II/1 (Figs




**Figs 23-25.** The oral region of opisthe of *Rubrioxytricha indica* n. sp. after protargol impregnation. Kinetosomes of disaggregated V/3 merge with the posterior part of developing UMs (arrows). UMs - undulating membranes.

**Table 4.** Morphometric comparison of *R. indica* n. sp. with *Cyrtohymena* species. All dimensional measurements are averages in µm.

| Characters | *R. indica* n. sp. (Present report) | *C. citrina* (Foissner 1989) | *C. primicirrata* (Foissner 1984) | *C. quadrinucleata* (Foissner 1984) |
|---|---|---|---|---|
| Body shape | Elliptical, both ends rounded | Slender, slightly 'S' shaped, both ends rounded | Elliptical, both ends rounded | Orthogonal, both ends rounded |
| Body length (BL) | 69.1 | 99.0 | 84.7 | 86.6 |
| Body width (BW) | 26.6 | 33.5 | 36.0 | 40.8 |
| Body length/ Body width | 2.6:1 | 2.9 | 2.3 | 2.1 |
| Macronuclear nodules, length | 11.8 | 7-10 | 10-15 | 9-16 |
| Macronuclear nodules, width | 7.8 | * | 7-9 | 7-10 |
| Macronuclear nodules, number | 2 | 2 | 2 | 4 |
| Micronuclei, number | 2-3 | 1-4 | 2-3 | 2-4 |
| Adoral membranelles, number | 29 | 33.6 | 30.2 | 40.0 |
| Number of cirri in left marginal cirral row | 19.2 | 21.5 | 15.6 | 18.3 |
| Number of cirri in right marginal cirral row | 22.3 | 21.1 | 20.3 | 20.3 |
| Dorsal rows | 5.0 | 5.8 | 6.0 | 6.0 |
| Caudal cirri | 1 | 3 | 3 | 3 |
| OP origin | Dual; in two parts | * | * | * |
| Colour of cortical granules | Dark green | Orange yellow | Shiny yellow | Absent |
| Arrangement of cortical granules | Uniformly scattered | Arranged around cirri and dorsal bristles | Arranged in groups of 3-10 along cirri, AZM, and dorsal bristles | Absent |
| Colour of the cytoplasm | More or less colourless | * | Colourless | Colourless |
| Cytoplasmic inclusions | Numerous sharp dark crystalline bodies in groups | * | * | * |

*Not mentioned by the authors.

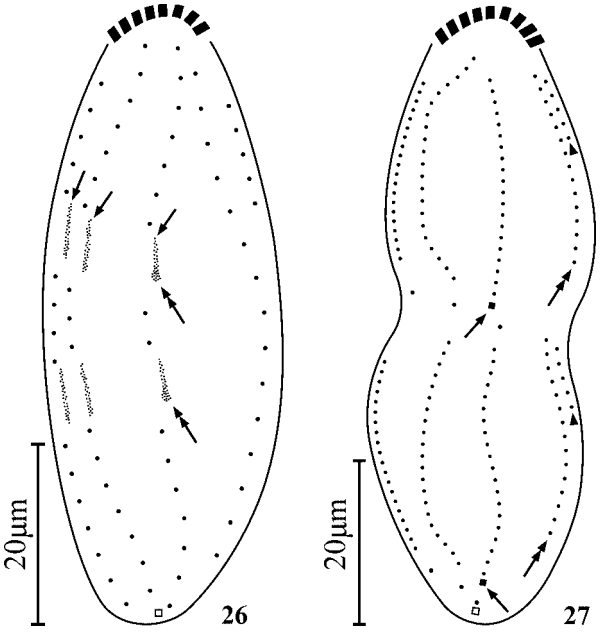
11, 17); it extends anteriorly becoming continuous with the anterior kinetosomal field (Figs 12, 18). As the posterior field of kinetosomes moves anteriorly, its continuity with the transverse cirrus is broken.

Two sets of six FVT ciliary streaks originate from the OP and six parental cirri II/2, IV/3 and III/2 and IV/2, V/4 and V/3. In the proter, the parental UMs function as streak I (Fig. 15). The streak II shows composite origin

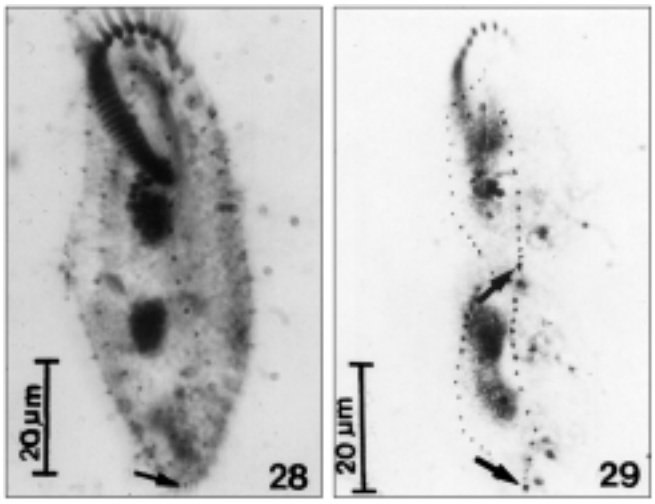
**Table 5.** Morphometric comparison of *R. indica* n. sp. with *Oxytricha* species possessing cortical granules. All dimensional measurements are averages in µm.

| Characters | *R. indica* n. sp. (Present report) | *O. tenella* (Song and Wilbert 1989) | *O. granulifera* (Foissner and Adam 1983) | *O. longigranulosa* (Berger and Foissner 1989) | *O. procera* (Kahl 1932) | *O. durhamiensis* (Kahl 1932, Berger 1999) | *O. oxymarina* (Kahl 1932, Berger 1999) |
|---|---|---|---|---|---|---|---|
| Body shape | Elliptical, both ends rounded posterior end broadly rounded | Anterior end narrowly and with parallel margins | Broad or slender oval | Elliptical | Slightly spindle shaped | Elliptical | Both ends broadly rounded |
| Body length (BL) | 69.1 | 50.9 | 80.6 | 87.6 | * | * | * |
| Body width (BW) | 26.6 | 26.3 | 33.6 | 31.9 | * | * | * |
| Body length/ Body width | 2.6:1 | 1.9 | 2.4 | 2.7 | * | * | * |
| Body length (BL) *in vivo* | 80 | 50-70 | 80-130 | 80-135 | 100-120 | 60-100 | 80-120 |
| Body width (BW) *in vivo* | 30 | 30-40 | 35-50 | 35-55 | * | 33-45 | * |
| Macronuclear nodules, length | 11.8 | 10.9 | 14.4 | 14.8 | * | * | 14-48 |
| Macronuclear nodules, width | 7.8 | 8.2 | 7.7 | 7.6 | * | * | * |
| Micronuclei, number | 2-3 | 2-3 | 2 | 1-2 | 2 | 2 | 2-3 |
| Adoral membranelles, number | 29 | 26.0 | 31.2 | 26.5 | * | 25-30 | * |
| Number of cirri in left marginal cirral row | 19.2 | 22.3 | 34.0 | 22.9 | * | * | 31-33 |
| Number of cirri in right marginal cirral row | 22.3 | 25.6 | 32.1 | 25.9 | * | 24 | 33-39 |
| Dorsal rows | 5.0 (3DKs and 2 DMs) | 5 (4DKs and 1 DM) | 6 | 6 | * | 5-6 | * |
| Caudal cirri | 1 | 3 | 3 | 3 | 3 | 3-4 | * |
| OP origin | Dual; in two parts | * | Between POVCs and LMC | Dual; in parts | * | * | * |
| Colour of cortical granules | Dark green | * | Colourless to yellowish | Colourless | Dark granules in posterior part | * | Dark granules in posterior part |
| Arrangement of cortical granules | Uniformly scattered | Irregularly arranged | Absent only along DKs | In groups; arranged in rows | * | In groups of 2-5, further arranged in rows | * |
| Colour of the cytoplasm | More or less colourless | Slightly yellowish | Colourless | Colourless | * | * | Colourless |
| Cytoplasmic inclusions | Numerous sharp dark crystalline bodies in groups | Often dark grayish inclusions | Yellowish crystals | Greasy shiny globules | * | * | * |

*Not mentioned by the authors.




**Figs 26-27.** *Rubrioxytricha indica* n. sp. showing division morphogenesis on the dorsal surface. **26 -** within row primordia formation ($DP_{1-3}$; arrows), Proliferation of kinetosomes at the posterior ends of $DP_3$ (double arrows); **27 -** presence of one caudal cirrus each at the ends of newly formed $DK_3$ (arrows), long $DM_1$ (double arrows), Short $DM_2$ (arrow heads). DK - dorsal kinety, DM - dorsomarginal, DP - dorsal primordium.




**Figs 28-29.** *Rubrioxytricha indica* n. sp. showing vegetative and division morphogenesis on the dorsal surface after protargol impregnation. **28 -** dorsal surface showing the presence of one caudal cirrus at the end of $DK_3$ (arrow); **29 -** newly formed caudal cirrus at the end of each $DK_3$ (arrows). DK - dorsal kinety.

from II/2 and streak II of opisthe (Fig. 14). The streaks III and IV originate from the disaggregating III/2 and $F_7$ IV/3 respectively (Figs 13, 14, 19). The streaks V and VI of the proter originate from advancing V and VI streaks of the opisthe (Figs 14, 19). In the proter, outer UM reorganizes completely (Fig. 16). In the opisthe, streaks I, II and III originate from the OP (Fig. 13). The streaks IV, V and VI are formed from the parental IV/2 and V/4 respectively (Fig. 13). The parental cirrus V/3 participates at very late stages of morphogenesis (Figs 14, 15, 19, 20); its participation is noticed subsequent to formation of two sets of six streaks. The kinetosomes of V/3 merge with those of the posterior part of developing UMs of the opisthe (Figs 16, 21, 23-25; Table 2).

The FVT streaks differentiate in the usual pattern of 1, 3, 3, 3, 4, 4 forming 18 FVT cirri, one set each for the two daughter cells (Fig. 16). The development of the marginal cirri occurs by within row primordia formation. Initiation of RMC primordia formation occurs earlier than LMC primordia (Figs 15, 20).

On the dorsal surface, two sets of three primordia arise in the three dorsal kineties by within row proliferation of kinetosomes (Fig. 26). These primordia elongate and differentiate into new dorsal rows ($DK_{1-3}$). The posterior most kinetosomes of $DK_3$ proliferate to develop into one caudal cirrus each in the proter and the opisthe (Figs 27, 29). The two dorsomarginal rows are formed from two primordia, arising near the right marginal primordia and later shift to the dorsal surface (Fig. 22).

## DISCUSSION

### Generic assignment of the new species

*Rubrioxytricha indica* n. sp. possesses a question mark shaped AZM, curved and intersecting UMs; 18 FVT cirri, posterior frontal cirri in "V" shaped pattern, postoral ventral cirri in dense cluster behind buccal vertex, two pretransverse ventrals and 5 transverse cirri; one right and left marginal row of cirri; three dorsal kineties, two dorsomarginal rows and one caudal cirrus. Brown coloured crystalline inclusions are present in cytoplasm. Green coloured cortical granules are uniformly distributed in clusters.

### Comparison with related and similar species

*Rubrioxytricha indica* n. sp. shows marked differences from the already two described species namely

*R. haematoplasma* (Blatterer and Foissner 1990, Berger 1999) and *R. ferruginea* (Song and Wilbert 1989, Berger 1999).

It is much smaller, therefore shows corresponding differences in its morphometric features from the other two species (Table 3). It is also distinct by the presence of dark brown crystalline inclusions, which are present in substantial number in the cytoplasm.

The cortical granules in *R. indica* n. sp. are uniformly distributed in clusters, whereas in case of other two reported species, these are arranged in longitudinal rows.

*Rubrioxytricha indica* n. sp. possesses three dorsal kineties and two dorsomarginal rows. No variation was noticed in the population in this regard. In contrast, *R. haematoplasma* possesses either three dorsal kineties and one dorsomarginal row (Blatterer and Foissner 1990, Berger 1999) or four dorsal kineties and one dorsomarginal row (Shin and Kim 1993). Likewise, it also differs from *R. ferruginea*, which has four dorsal kineties and one dorsomarginal row (Song and Wilbert 1989, Berger 1999). One may also mention that out of several hundred specimens examined no variation was seen in the number of caudal cirri i.e. presence of a single caudal cirrus at the end of $DK_3$. In *R. ferruginea* one or two caudal cirri are present.

The *Cyrtohymena* species differ from *R. indica* n. sp. in most of the morphometric features, colour and arrangement of cortical granules, nature of crystalline cytoplasmic inclusions and most importantly in number of caudal cirri (Table 4).

The *Oxytricha* species are distinct from *R. indica* n. sp. by the pattern of UMs (Berger and Foissner 1997), dorsal rows, number of caudal cirri and arrangement of cortical granules (Table 5).

## Variability of cirral pattern

In *R. indica* n. sp. morphometric analysis of a large sample size (n=400) shows ~14% cells possess one or two extra ventral or transverse cirri. Furthermore, nearly 12% of the clonal population of *R. indica* n. sp. shows a second LMC row with few cirri. Development of the second row shows that it is formed by splitting of the primordium from the first LMC row. Thus the development pattern of the second LMC row in the above mentioned cases is similar to that seen in *Pleurotricha curdsi* (Gupta *et al.* 2003).

It is not certain whether these variations are intrinsic traits of the isolate or have arisen due to the influence of other factor (s) such as the clonal age or presence of intracellular symbionts/parasites. Morphometric variations are also known to arise in oxytrichids by the presence of metals in the medium (Arora *et al.* 1999, Machwe *et al.* 2001). Since the present data has been obtained from the laboratory grown cells, the role of the last mentioned factor is ruled out.

## Morphogenesis

Morphogenetic data of *R. indica* n. sp. clearly shows the involvement of cirrus V/3. The cirrus V/3 disaggregates much later in the course of development after formation of two sets of six FVT streaks when its kinetosomes merge with the posterior part of the undulating membranes of the opisthe. In an earlier study of morphogenesis in *R. haematoplasma* (Blatterer and Foissner 1990, Berger 1999), involvement of cirrus V/3 in morphogenesis could not be seen with certainty.

The divisional morphogenesis of *R. indica* n. sp. is essentially similar to that of *Oxytricha* except the nature of involvement of V/3 wherein it takes part in the formation of streaks IV, V and VI along with the participation of cirri IV/2 and V/4.

It is noteworthy that cirrus V/3 remains intact till late stages of development, a situation not seen in other genera of oxytrichinae. Thus this feature could well be considered specific for the genus *Rubrioxytricha*.

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## REFERENCES

Ammermann D., Steinbrück G., Berger L. V., Hennig W. (1974) The development of the macronucleus in the ciliated protozoan *Stylonychia mytilus*. *Chromosoma (Berl.)*. **45:** 401-429

Arora S., Gupta R., Machwe S., Sapra G. R. (1999) Influence of cadmium on development of surface ciliary structures in the ciliate *Stylonychia mytilus* (Ciliophora, Oxytrichidae). *Eur. J. Protistol.* **35:** 281-289

Berger H. (1999) Monograph of the Oxytrichidae (Ciliophora, Hypotrichida). Kluwer Academic Publishers. Dordrecht/ Boston/ London. *Monograph. Biol*. **78:** 1-1079

Berger H., Foissner W. (1989) Morphology and biometry of some soil hypotrichs (Protozoa, Ciliophora) from Europe and Japan. *Bull. Br. Mus. Nat. Hist. (Zool.).* **55:**19-46

Berger H., Foissner W. (1997) Cladistic relationships and generic characterization of oxytrichid hypotrichs (Protozoa, Ciliophora). *Arch. Protistenk.* **148:** 125-155

Blatterer H., Foissner W. (1990) Beiträge zur Ciliatenfauna (Protozoa: Ciliophora) der Amper (Bayern, Bundesrepublik Deutschland). *Arch. Protistenk.* **138:** 93-115

Borror A. C. (1972) Revision of the order Hypotrichida (Ciliophora, hypotrichida). *J. Protozool*. **26:** 51-55

Foissner W. (1984) Infraciliatur, Silberliniensystem and Biometric einiger neuer und wenig bekannter terrestrischer, limnischer und mariner Ciliaten (Protozoa: Ciliophora) aus den Klassen Kinetofragminophora, Colpodea und Polyhymenophora. *Stapfia* **12:**1-165

Foissner W. (1989) Morphologie und infraciliatur einiger neuer und wenig bekannter terrestrischer und limnischer Ciliaten (Protozoa, Ciliophora). - *Sber. Ost. Akad. Wiss., Math.-naturwiss. Klas., Abt. I*, **196 (year 1987):** 173-247

Foissner W., Adam H. (1983) Morphologie and Morphogenese des Bodenciliaten *Oxytricha granulifera* sp. n. (Ciliophora, Oxytrichidae). *Zool. Scr.* **12:**1-11

Gupta R., Kamra K., Arora S., Sapra G. R. (2003) *Pleurotricha curdsi* (Shi, Warren and Song 2002) nov. comb. (Ciliophora: Hypotrichida): morphology and ontogenesis of an Indian population; redefinition of the genus. *Eur. J. Protistol.* **39:** 275-285

Kahl A. (1932) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. *Tierwelt Dtl*. **25:** 399-650

Kamra K., Sapra G. R. (1990) Partial retention of parental ciliature during morphogenesis of the ciliate *Coniculostomum monilata* (Dragesco *et* Njiné, 1971) Njiné, 1978 (Oxytrichidae, Hypotrichida). *Eur. J. Protistol.* **25:** 264-278

Machwe S., Arora S., Gupta R., Sapra G. R. (2001) Cisplatin induces modifications in the development of cell surface patterns of ciliates. *Cell Biol. Internat.* **25:** 1131-1138

Shin M. K., Kim W. (1993) New records of three oxytrichid hypotrichs (Ciliophora: Hypotrichida: Oxytrichidae) from the Han river in Seoul, Korea. *Korean J. Zool.* **36:** 223-230

Song W., Wilbert N. (1989) Taxonomische Untersuchungen an Aufwuchsciliaten (Protozoa, Ciliophora) im Poppelsdorfer Weiher, Bonn. *Lauterbornia* **3:** 2-221

Stein F. (1859) Der Organismus der Infusionsthiere nach eigenen Forschungen in systematischer Reihenfolge bearbeitet. I. Abtheilung. Allgemeiner Theil und Naturgeschichte der hypotrichen Infusionsthiere. W. Engelmann, Leipzig.

Wallengren H. (1900) Zur Kenntnis der vergleichenden Morphologie der hypotrichen Infusorien. *Bih. K. Svenska Vetensk Akad. Handl*. **26:** 1-31

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