CILIATED PROTISTS FROM SIKKIM, A BIODIVERSITY HOTSPOT; DESCRIPTIONS OF SOME CILIATE TAXA WITH PHYLOGENETIC NOTES USING CLASSICAL AND MOLECULAR METHODS

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CERTIFICATE

This is to certify that the research work embodied in this thesis entitled **"Ciliated Protists from Sikkim, a Biodiversity Hotspot; Descriptions of some Ciliate Taxa with Phylogenetic Notes using Classical and Molecular Methods"** is original and has been carried out by the author. It has not been submitted in full or in part for any other degree or diploma of this or any other University.

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CONTENTS

PREFACE

Introduction to Ciliated Protists

Ciliates are one of the major groups of protists. These are unicellular eukaryotes remarkably diverse in shape and size. Possession of ciliary structures is a fundamental property of ciliated protists. They are found in a variety of microhabitats, with a majority of species likely cosmopolitan. Ciliates are usually free-living; some live in symbiotic relationships, generally as harmless ecto- or endocommensals. Their very wide geographic and climatic distribution is due to their remarkable tolerance or adaptability to wide ranges of physico-chemical conditions of the environment. There are 7500–8000 known species of ciliates, but the true number may be as many as 30,000 (Lynn 2008; Simpson et al. 2005). Knowledge of their actual biodiversity lags behind that of other groups of organisms due to the fact that they are small in size, are encysted for most part of their life cycle and very few trained ciliate taxonomists are available (Foissner 1999; Hausmann and Bradbury 1996).

While the study of macroscopic, multicellular communities is progressing using well-established approaches, analyses of the microbial domain of the various ecosystems are still fragmentary. Knowledge of biodiversity of ciliates is becoming increasingly important for modeling and managing ecosystems as their significant ecological role has become better known. Ciliates play a wide array of ecological roles in a variety of freshwater, marine, and terrestrial ecosystems (Azam et al. 1983; Bartošová & Tirjaková 2008; Bartsch and Dovgal IV 2010; Dopheide et al. 2008; Dorigoa et al. 2005; Li J et al. 2010; Petz 2003; Vickerman 1992). In particular, ciliates are widely recognized as critical members of the 'microbial loop' of both marine and freshwater systems, where they prey on bacteria and smaller protists, linking these tiny primary decomposers and producers to larger consumers such as rotifers, copepods, fish larvae that feed on ciliates (Meyer 1994; Pierce and Turner 1992; Porter 1985; Sherr et al. 1986; Weisse and Rammer 2006). Likewise, ciliates form an essential part of soil ecosystems, where they release nutrients from bacteria to be made available to plants. Therefore, ciliates have been identified as a major microbial pathway for the transfer of carbon and phosphorus to higher trophic levels.

Ciliates are a significant part of artificial ecosystems. In wastewater biological treatment, they graze on bacteria to help clarify waste water (Chen et al. 2009; Fried and Lemmer 2003; Morishita 1976; Sasahara and Ogawa 1983**)** and maintain a healthy trophic web in those

artificial ecosystems; ciliates can also be used as bioindicators of water quality in both artificial and natural ecosystems (Bare´ et al. 2009; Chen et al. 2008; Fukui and Morishita 1961; Henebry and Cairns 1980; Lynn and Gilron 1992; Madoni 1994; Madoni 2003; Ruthven and Cairns 1973).

Autapomorphies of Ciliated Protists

Despite their great diversity, ciliates as a group are characterised by the following autapomorphies:

1. Presence of cilia at some stage in their life history, variable in number and arrangement, distributed over the body surface in a species specific manner and derived from an infraciliature consisting of the kinetosomes (basal bodies) with three characteristic fibrillar structures in the cytoplasm – the kinetodesmal fibril, the post ciliary microtubular ribbon, and the transverse mircotubular ribbon.
2. Nuclear dimorphism, a small (germinal) diploid micronucleus and a large (vegetative) polypoid DNA-rich macronucleus.
3. Conjugation, the basic sexual process in ciliates, involves the temporary union of two cells of complementary mating types to exchange gametic nuclei.

Ciliates: Developmental strategies

Because of complex oral and somatic kinetid patterns in ciliates, their development also shows diversity and complexity. However, these characteristics have made ciliates useful models for developmental biologists and the systematists. Ciliates undergo asexual reproduction by transverse binary fission if provided with sufficient food. This may go on for several division cycles. The anterior filial product is the proter and the posterior the opisthe. The parental ciliature is usually completely renewed during division morphogenesis except in a few cases where some part of the parental ciliature is not resorbed after division and is thus retained as a part of the new ciliature (Kamra and Sapra 1990). Under some circumstances, a ciliate may undergo reorganization or regeneration, which entails replacement of the parental ciliary structures; only one ciliary set is formed.

Most ciliates form cysts to tide over unfavourable conditions. During the encystment process, they usually lose most or all of their ciliature and are covered with a multi-layered cyst wall. Once favourable conditions return, the cell excysts with a full complement of the species specific ciliature formed while the cell is still within the cyst wall.

Sexually mature cells transform into pre-conjugants and undergo sexual reproduction called conjugation. During sexual reproduction, conjugating pairs are formed. Meiosis in each preconjugant results in the formation of haploid micronuclei which are then exchanged

between the pairs. After fusion of two haploid micronuclei in each ex-conjugant, the zygote nucleus divides mitotically; one of the daughter nuclei develops into a new micronucleus and the other into a new macronucleus. Most of the DNA in the micronucleus is of repetitive type. The development of the macronucleus involves only 1/3rd of the chromosomes which in the first replication phase are polytenized in probably 5 replication steps and appear as giant chromosomes. At this developmental stage considerable amounts of repetitive DNA are still present in the chromosomes. During the subsequent disintegration phase more than 90% of the DNA is eliminated from the macronucleus anlage. The remainder is further replicated five times, and finally the new vegetative macronucleus is formed. Replication bands become visible during the second DNA synthesis phase (Ammermann et al. 1974; Prescott 1994; Lipps and Eder 1996).

As in most ciliates analyzed so far, rDNA occurs as a single-copy gene in the micronucleus but is highly amplified in the vegetative macronucleus (Steinbrück 1990). This amplification of rDNA is accompanied by the formation of many new nucleoli in the course of macronuclear development.

Study of free-living ciliate fauna from Indian subcontinent:

Some studies were carried out on protozoa significantly in the pre-independence period (Bhatia 1936) and have been mentioned in the Fauna of Undivided British India which lists 310 ciliate species belonging to 104 genera. Noteworthy studies were conducted on free living protozoa by groups of Indian scientists namely, S.C. Pillai, T.K. Wadhwani, M.I. Gurbaxani and V. Subramanyan in the years 1942, 1947 and 1948 including a research article in Nature by Pillai, S .C. and Subramanian, V (1942) on the “Role of Protozoa in the Activated Sludge Process” (Nature, 150, 3809. p. 525). Sharma and his associates have done good piece of work on cytogenetical and behavioural study of oxytrichids (Sharma et al. 1986a, 1986b). More recently, few more commendable studies were carried out on free living Protists/Ciliates; these include: (i) Taxonomy and Ecology of Ciliated Protozoa from Marginal Marine Environments of East Coast of India (Kalavati and A.V. Raman, Zoological Survey of India, 2008). (ii) Distribution and Abundance of Marine Benthic Ciliates in a Bay-Mangrove Complex in the Godavari Delta, East Coast of India (Padma et al. 2007) (iii) Experimental Laboratory Oxidation Ponds for Assessing Chromium Toxicity on Algae and Protozoa (Tharavathy N. C., Krishnamoorthy, M., Bull Envir Sci. 2004, 22 (1), 93-9). Creditable contributions to parasitic ciliates have been made by P.K. Bandyopadhyay and his associates.

In the last three decades, investigation of fresh water ciliates from several sites in and around

Delhi has been carried out (Kamra and Sapra 1990, 1991, 1993a, 1993b, 1994a, 1994b; Arora et al. 1999; Gupta et al. 2001, 2002, 2003, 2006). Very recently, studies on the ciliate fauna from the Valley of Flowers, Silent Valley and sulphur springs from Dehradun were conducted by Santosh Kumar and K. Kamra (Kamra et al. 2008; Kamra and Kumar 2010; Kumar et al. 2010).

These studies have proved to be very fruitful in terms of ciliate diversity and systematics. Therefore, it was desirable that such a study on biodiversity of ciliated protists be conducted in a systematic manner from different places in India, especially from biodiversity hotspot in the Eastern Himalayas. Their precise cataloguing is of utmost importance as a national genetic resource.

Study of ciliate fauna from select biotopes of the Himalayan region:

The Eastern Himalayas includes a vast variety of distinct thriving ecozones which are supporting distinctive ciliate fauna. A detailed cataloguing of ciliated protists from this Himalayan state of India was undertaken and the results are given in the present thesis. The ciliates reported from Sikkim belong to diverse groups among ciliated protists. A large number among them belong to the group Hypotricha. Most of the earlier descriptions of hypotrichous ciliates lack details of the dorsal ciliature and this has remained a major lacuna. This group was therefore selected for detailed taxonomic and systematic analysis. The present study has provided new knowledge on their occurrence, distribution and systematics. The work includes descriptions of selected hypotrichous ciliates from various ecozones covering sanctuaries, lakes and hot springs of Sikkim and a substantial part of the work is dedicated to systematics of oxytrichid hypotrichs using contemporary scheme of classification (dorsal infraciliature).

Eleven collection sites from the four districts of Sikkim were identified and explored for their ciliate fauna. Over 100 ciliate species/populations were isolated from various collection sites; 90 ciliate species could be identified up to genus/species level. Classical and molecular data were used for the identifications. In most cases these corroborated each other.

In **chapter 1**, a review of literature on systematics of hypotrichous ciliates has been given.

In **chapter 2**, materials and methods followed for collection, culturing and specimen preparation for studying cells through classical (live cell observation, infraciliature observation with silver-impregnation and nuclear preparation through Feulgen reaction employed for morphostatic and morphogenetic stages) and modern (molecular systematics based on 18S rDNA) tools have been detailed.

In **chapter 3**, a catalogue of ciliated protists from different parts of Sikkim is provided along with a note on the scheme of classification followed for the selected hypotrichous ciliates discussed in the subsequent chapters.

In **chapter 4**, *Gonostomum strenuum*, a representative of simple dorsal kinety formation pattern has been described in detail as it is morphologically and morphometrically strikingly different from the only well described population of the species from Qingdao, China.

In **chapter 5**, the content has been divided in to three sections to explain the three character states with respect to fragmentation of dorsal infraciliature.

In Section A, three species with simple fragmentation of dorsal kinety 3 have been described- (i) *Oxytricha granulifera* is documented for its dorsal kinety fragmentation pattern typical of family Oxytrichidae. The Sikkim population has also been compared with another morphometrically closely related population from Baumgarten, Austria. (ii) *Cyrtohymena citrina* has been described in detail as the present study has clarified a very important trait in the ventral morphogenesis with respect to formation of anlagen V and VI for the proter. This feature is considered an autapomorphy for the genus *Cyrtohymena*. Sikkim population has also been compared with another morphometrically closely related population from Peloponnesus, Greece. (iii) *Stylonychia ammermanni* isolated from a water sample is compared with another Indian population from the Delhi region of river Yamuna; The Sikkim population shows some variations from the Delhi population with respect to the size of cells, generation time, ventral infraciliature and more importantly dorsal infraciliature. Significantly, these characters are considered important for species separation within the genus *Stylonychia*. At the end of Section A of chapter 5, fourteen distinctive populations of *Sterkiella* belonging to three different species collected from different locations across Sikkim have been described. These populations show remarkable variation in morphology and morphometry. In the phylogenetic analysis using their 18S rDNA sequences, different populations of *Sterkiella* showed a tendency to cluster together with respect to number and placement of transverse cirri; this was noticeably better than the clustering with respect to the number of macronuclei. The results support the idea that the number and placement of cirral structures have a greater systematic value than the nuclear apparatus at least in this group of ciliates.

In Section B, *Cyrtohymena* (*Cyrtohymenides*) *shii*, a representative of multiple fragmentation of dorsal kinety 3 has been detailed for its unique patterns of ventral and dorsal

morphogenesis as compared to other members of the family Oxytrichidae. The phylogenetic analysis of this taxon has clarified the systematic status of flexible bodied oxytrichid ciliates with multiple fragmentation of dorsal kinety 3 within Dorsomarginalia; the overall details that emerged led to a revision of the subgenus *Cyrtohymena* (*Cyrtohymenides*). Additionally, the taxon has been identified as one of the examples to explain convergent evolution among hypotrichous ciliates (research article published in European Journal of Protistology).

In Section C, *Paraurosomoida indiensis* gen. nov., sp. nov., a representative of oxytrichids which lack fragmentation of dorsal kinety is described for its unique ventral infraciliature lacking pretransverse ventral and transverse cirri. Additionally, the taxon has clarified systematic status of flexible bodied oxytrichid ciliates lacking fragmentation of dorsal kinety within Dorsomarginalia whose positions were hitherto unclear because of absence of molecular markers (research article accepted by European Journal of Protistology, a revised version submitted).

In chapter 6, the molecular phylogeny based on 18S rDNA sequences of selected hypotrichs from Sikkim along with related sequences available in the GenBank has been derived and analysed. In most of the cases, the identity of the species ascertained by molecular data corroborated the identifications determined through classical methods. Some species clustered with other populations of the same species reported elsewhere and others showed affinity with related species giving clues to look into the possible similarities between them.

The use of classical and molecular tools in ciliate systematics gives a thorough insight into the relationships within the group.

CHAPTER 1

SYSTEMATICS OF CILIATED PROTISTS WITH SPECIAL EMPHASIS ON HYPOTRICHOUS CILIATES: A REVIEW

Basis for Systematics

Ciliate taxonomy and systematics have undergone a number of revisions both as a group as well as for sections within the group necessitated by new information based on old and new technologies. These revisions continue to occur as more ciliates are described and new criteria gain importance. It is obvious that systematics does become subjective at some point of time, different groups of researchers relying on different criteria.

Classification of a particular group of organisms typically begins at the bottom with an examination of the variation in characters of species – the genus-species level. A number of methods have been used to determine species of ciliates, from the interbreeding criterion of the biological species concept to a variety of traits related to morphology, life history, ecology, behaviour, size and shape of a variety of structures revealed by observation of living and stained cells.

The primary character used to distinguish ciliate taxa is, the cortex, although some non cortical characters, such as nuclear features, are also important. The cortex which is the main interface between the organism and its environment can be divided into the somatic region and the oral region. The somatic region functions in locomotion, provides protective covering and defence responses, and enables attachment to the substrate. The oral region functions in the acquisition and ingestion of nutrients. Features of both regions, and particularly the kinds and arrangement of ciliary structures, are important in the characterisation of major groups of ciliates.

Genetic approaches are becoming increasingly more popular in ciliate systematics, especially those using molecular genetics. These have included the use of randomly amplified polymorphic DNA (RAPD), isoenzymes and restriction fragment length polymorphisms (RFLP). The current cutting edge approaches are the sequencing of genes, such as those for the small and large subunits of rRNA, actin, histones, tubulins, heat shock proteins and translation factors. More recently (Chantangsi et al. 2007; Lynn and Strüder-Kypke 2006),

the mitochondrial cytochrome c oxidase 1 (*cox1*) gene has been chosen by some as a species "barcode".

Above the genus-level, establishing groups is more intricate, but this should always be based on the establishment of monophyly using synapomorphies or shared-derived characters. These characters can be ultrastructural features of the somatic and oral kinetids, patterns of morphogenesis, and gene sequences. Taxonomy ultimately uses nomenclature and its rules to establish priority, ensure consistency, and maintain stability.

Briefly, in the 18th and 19th centuries, overall ciliary patterns and the dominance of "spirotrich" oral region divided the ciliates into "holotrichs" and "spirotrichs". In the 20th century, ontogenetic patterns, particularly revealed by silver stained organisms undergoing cell division, received greater weightage; this resulted in the alignment/clustering of taxa that had previously been distantly separated and vice versa (Borror 1972, 1979; Borror and Wicklow 1983; Grimes 1972; Jerka-Dziadosz 1972, 1964; Jerka-Dziadosz & Frankel 1969; Wirnsberger et al. 1985a, 1985b, 1986). Later, the transmission electron microscopy revealed a whole new set of cytoskeletal characters, particularly the somatic kinetid patterns. The diversity of these somatic kinetid patterns initially suggested eight major clades or classes (Small and Lynn 1981, 1985). In the 1970s, small subunit (SSU) rRNA genes were used to resolve relationships among the groups whose members were not rich in morphological features (Stackebrandt and Woese 1981). By the mid-1980s, several research groups began sequencing SSU rRNA genes of ciliates (Elwood et al. 1985; Sogin et al. 1986), demonstrating that ciliates, even with the small sampling of species, appeared to be monophyletic and yet showed very deep divergences, equivalent to the genetic distances between the classical plant and animal "kingdoms". The first thorough investigation of species, using both the SSU rRNA gene (Lynn and Sogin 1988; Sogin and Elwood 1986) and the large subunit (LSU) rRNA gene (Baroin et al. 1988), provided enough data for testing the deeper relationships predicted by ultrastructural research.

The molecular phylogenetic approach is now a recognized method for testing and establishing phylogenetic relationships among organism. However, it rests on the basic assumption that phylogenetic trees based on genes truly represent the phylogeny of the organisms. Ultimately, confidence in "gene trees" increases when multiple and unlinked genes show patterns congruent with each other and with phylogenies constructed on other

features, such as morphology. The basic approach for gene sequencing remains the same, but has become more efficient since the days of cloning genes into vectors in the 1980s. In brief, conserved regions of genes are used to design polymerase chain reaction (PCR) primers, which enable amplification of genes of interest (Bernhard and Schlegel 1998; Medlin et al. 1988). The PCR-amplified genes may then be cloned into a plasmid vector, amplified in bacteria, purified, and then sequenced (Baroin-Tourancheau et al. 1998; Greenwood et al. 1991; Hirt et al. 1995). As is often the case now, the PCR-amplified genes are directly sequenced (Lynn and Struder-Kypke 2005). In either case, both strands of the DNA should be sequenced to corroborate the sequence reads.

In general, one can say that genera should be differentiated on the basis of significant qualitative characters. Modern description of new species of ciliates should, wherever possible, include data provided by observation of living organisms, stained organisms for body shape, pattern of somatic kineties and organization of oral structures and gene sequence (Agatha et al. 2005; Modeo et al. 2003; Rosati et al. 2004). Somatic kineties have proved very diagnostic as they exhibit universality, constancy, and consistency within groups. Thus, to identify and establish relationships within or in between groups, one must ultimately search for characters that are highly conserved over time.

Hypotrichous ciliates (Ciliophora, Hypotricha)

(Details of terminology used to describe hypotrichous ciliates, especially the ciliary structures in morphostatic cells and during morphogenesis is given in the inset at the end of chapter).

The ciliates included in Hypotricha are dorsoventrally flattened and possess a rigid pellicle. The oral cavity is ventrally placed, guarded by an adoral zone of membranelles and the undulating membranes. Besides the oral ciliature, the ventral surface has fused bundles of cilia called cirri located in a species specific pattern. On the dorsal surface, there are dorsal kineties and dorsomarginal rows; they are longitudinal rows of more or less widely separated short cilia, which function as sensory bristles. Dorsal kineties develop from the old rows by within rows proliferation of certain ciliary units. Dorsomarginal rows are kineties on the right margin of dorsal surface that arise on the ventral surface and later shift to the dorsal surface. The bristle rows usually terminate posteriorly by caudal cirri. Like all ciliates hypotrichous` ciliates reproduce by conjugation and by transverse binary fission and follow a well defined developmental strategy including morphogenesis of ciliature prior to fission, as well as

during regeneration and reorganization. Under unfavourable conditions, they encyst, a process wherein all visible ciliature dedifferentiates and the cell is surrounded by a multi-layered cyst wall; with the onset of favourable conditions, the whole ciliature redifferentiates prior to emergence from the cyst wall.

The systematics and taxonomic position of hypotrichous ciliates has remained the central object for several researchers world over working in the field of ciliated protists (Corliss 1979; Faure-Fremiet 1961; Fleury et al. 1985; Lynn 2008; Lynn & Corliss 1991; Small & Lynn 1981; Small & Lynn 1985; Tuffrau 1987). The following summary pertains to few significant contributions in the field.

Faure-Fremiet (1961) was the first to propose division of the order Hypotrichida (into the suborders Stichotrichina and Sporadotrichina) and was subsequently supported by Corliss (1979). With the concept of "structural conservatism", Small & Lynn (1981) proposed that "the conservation of structure through time is inversely related to the level of biological organization". The idea, together with several observations of hypotrich cortical ultra structure led to remove the hypotrichs from the Class Spirotrichea and include them in a new class, the Nassophorea. Small & Lynn (1981) also pointed out the hypotrichs should be split into two groups: *Euplotes* and other related genera, and hypotrichs *sensu stricto*. Thus Small & Lynn later, in their monograph on ciliates in the "Illustrated Guide to the Protozoa" (1985) clearly split the hypotrich group, keeping the euplotids in the Class Nassophorea (subclass Hypotrichia) and removing the rest to the Class Spirotrichea along with heterotrichs and others. Fleury et al. (1985), based on the study of cortical morphogenesis, divided the order Hypotrichida into the suborders Euhypotrichina and Pseudohypotrichina. This proposition was criticised by Tuffrau (1987), who disagreed with the new suborder name, instead proposed a new classification, based on the heterogenic nature of the group Pseudohypotrichina, in an attempt to reconcile morphogenetical data. Tuffrau (1987) divided the order Hypotrichida into four suborders, keeping the classic Stichotrichina and Sporadotrichina and adding the Euplotina and Discocephalina. Lynn & Corliss (1991) presented a new scheme in which the Euplotidae were again placed with the heterotrichs, oligotrichs, and stichotrichs in the Class Spirotrichea. This was different from the earlier work (Small & Lynn 1981) in which hypotrichs were transferred into the Class Nassophorea. The conclusion drawn by (Lynn & Corliss 1991) was accepted as correct by many workers. Lynn (2008) elevated the order Hypotrichida as Hypotrichia, one of seven subclasses under

the class Spirotrichea, following Lynn and Small (1997). However, he placed dorsoventrally flattened ciliates belonging to group Hypotricha (Stein 1859; Berger 1999, 2006, 2008) under subclass Stichotrichia. Ciliates like *Euplotes* and *Diophrys* were placed under subclass Hypotrichia.

Although the classification given by Lynn (2008) has been accepted by many workers, as this has been proposed keeping in view the information gathered mainly from last two decades based primarily on classical as well as molecular data, but according to him, 'The taxonomy of stichotrichians is one of the most confusing in the phylum. The revision relies heavily on the morphology of the differentiated individual as there is trend in recent years to rely heavily on the similarities in the pattern of division morphogenesis. Stability may only be achieved when complete division morphometric patterns and molecular information for several genes are available on the majority of genera'.

Wilhelm Foissner and Helmut Berger are among the most prolific taxonomists and systematists among contemporary world's leading ciliatologists. They mainly deal with alpha taxonomy, systematics and/or ecology of ciliated protists especially of hypotrichs. Foissner has maximum number of descriptions to his credit. Till now he has contributed more than 180 genera and 555 species, largely belonging to Hypotricha. He has also made marked contribution at the levels of higher taxonomic ranks (classes, subclasses, orders, suborders, family and subfamilies). His work mostly included making new combinations, redescriptions/reviews of taxa, changing status of taxa, introducing replacement names, correcting names and defining ciliate communities. In the recent years, Weibo Song and his associates, mainly from China have done remarkable work on hypotrichous ciliates largely including marine ciliates.

Berger has contributed monumental work to summarize the morphological, morphogenetic, faunistic, and ecological data of hypotrichs from the past 220 years in the well documented series of monographs namely Monographs of the Oxytrichidae (Ciliophora, Hypotrichia), Urostyloidea (Ciliophora, Hypotricha); Amphisiellidae and Trachelostylidae (Ciliophora, Hypotricha); Gonostomatidae and Kahliellidae (Ciliophora, Hypotricha); Uroleptidae (Ciliophora, Hypotricha); Hypotricha (Ciliophora) - Monographiae Biologicae. This is the first thoroughly compiled literary work after those of Ehrenberg (1838) and Kahl (1930, 1931, 1932 and 1935).

Berger (1999, 2006, 2008), Foissner and Stoeck (2011) and many other leading workers in the field agree with Lynn (2008) that the taxonomy of stichotrichians is still in the premature stage. Therefore the monographs of Berger (1999, 2006, 2008) are still being pragmatically used. Thus the term Hypotricha (~Stichotrichs) has been in use, which comprises all non euplotid hypotrichs (Berger 2006).

Classification and terminology

Classification is according to Berger (1999, 2006, 2008) and Lynn (2008). General terminology is according to Berger (1999, 2006, 2008), Borror (1972), Martin (1982) and Wallengren (1900).

INSET: HYPOTRICH CILIATURE

Oral Ciliature: On the ventral surface, prominent ciliary structures surround the oral groove: the adoral zone of membranelles and two undulating membranes.

Adoral zone of membranelles (AZM): The AZM is positioned along the left peristomial border. The adoral zone extends along the left body margin from the anterior end diagonally to about mid-body. It is an orderly arrangement of parallel units or membranelles each consisting of two complete and two incomplete rows of ciliated kinetosomes.

Undulating membranes (UM): Bordering the right side of the oral groove, there are two parallel UMs. The shapes and lengths of UMs show variation within and between hypotrich families. The two UMs are also called paroral and endoral. The paroral usually borders the outer margin of the buccal cavity which is on the cell surface, while the endoral is on the inner wall of the cavity. Usually, the endoral consists of a single row of ciliated kinetosomes and the paroral of four rows of kinetosomes. The cilia in each membrane move in unison and aid the movement of food into the peristome.

Somatic Ciliature: The somatic ciliature consists of fused bundles of cilia called cirri located on the usually flattened ventral surface and rows of short cilia, which function as sensory bristles on the vaulted dorsal surface. Furthermore, bristle rows usually terminate posteriorly in caudal cirri.

Cirri: Each cirrus is a composite structure made up of varying number of ciliary units, arranged in diagonal files interlinked by connecting fibres and microtubules. Cirri are named according to their location and on the mode of development. Their arrangement on the ventral surface is species specific and is of high systematic importance.

Dorsal bristles: These are longitudinal rows of kinetosomal pairs; the anterior kinetosome bears a long stiff cilium, while the posterior kinetosome has a ciliary stub. Each pair is associated with microtubular fibrillar elements. The kinetosomes often lie in cortical pits. The non-motile bristles are sensory in function.

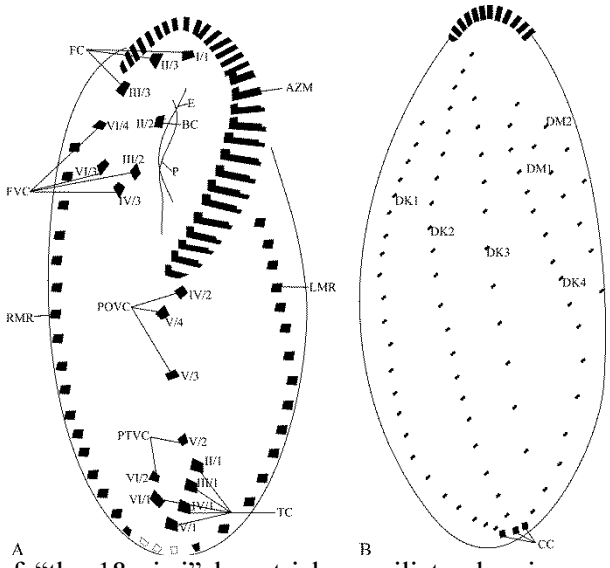

Fig. 1.1: Line diagram of "the 18 cirri" hypotrichous ciliate showing ventral (A) and dorsal (B) infraciliature; naming and numbering of ciliature as per Borror (1972), Martin (1982) and Wallengren (1900).

Types of Cirri

A variety of terms have been used for analogous and homologous cirri. They are named according to their final position on the cell surface and also according to Wallengren's classification (Wallengren 1900) defining the origin within cirral anlagen. Cirri present in the typical '18 cirri' hypotrichs are given below. The structure and positions of these cirri vary in different groups.

Frontal cirri (FC): They are arranged at the anterior end of the cell, invariably consists of three cirri, namely I/1, II/3, III/3 in "18 cirri" hypotrichs. In some species the number is increased secondarily. Usually the frontal cirri are slightly to distinctly enlarged as compared with frontoventral cirri.

Buccal cirrus (BC): This cirrus, II/2, is invariably close to the right and often near the anterior end of the paroral.

Frontoventral cirri (FVC): This group (mostly positioned in a V-shape) comprises four cirri (III/2, IV/3, VI/3, VI/4) located between the anterior portion of the right marginal row and the paroral.

Postoral ventral cirri (POVC): These cirri are located behind the proximal end of adoral zone. These three cirri (IV/2, V/3, and V/4) form a narrow group close to the buccal vortex.

Pretransverse ventral cirri (PTVC): These cirri (V/2 and VI/2) are located in front of the transverse cirri.

Transverse cirri (TC): This group is in the posterior quarter of the cell and usually comprises five cirri (secondarily increased or decreased in some cells), namely I/1, II/1, III/1, IV/1, and VI/1. They are usually enlarged as compared with pretransverse ventral cirri.

Frontal-ventral-transverse cirri (FVT): They are the complete set of cirri present on the ventral surface, usually 18 in number in oxytrichid ciliates. In some species the number is secondarily increased or decreased. The term frontal ciliature is interchangeably used for FVT cirri.

Marginal cirri (MC): These cirri run along the left and right body margins. Most hypotrichs have one left (left marginal row; LMR) and one right (right marginal row; RMR) row.

Ciliature on the Dorsal Surface

Dorsal kineties (DK): The ciliature of the dorsal surface is composed of longitudinal rows of widely separated paired ciliary units (dorsal bristles). The number and placement of these rows is species-specific. They develop during morphogenesis from old rows by within rows proliferation of certain ciliary units.

Dorsomarginal rows (DM): They are dorsal kineties on the right margin that arise from the right marginal cirral row on the ventral surface during morphogenesis and later shift to the dorsal surface. They are absent in some hypotrichs.

Caudal cirri (CC): The caudal cirri are located at the posterior margin of the dorsal surface. They are absent in some hypotrichs.

Formation of Hypotrich Ciliature during Development:
During cell division the developmental process involves a species specific sequence of cortical events leading to the formation of two sets of identical structures. Morphogenesis of ciliature thus occurs prior to fission, as well as during regeneration and reorganization. Development of new ciliary organelles for the daughter cells occurs from various anlagen/primordia.

An "anlage/primordium" is an aggregation of kinetosomes which proliferates and differentiates from the bases for new ciliary complexes. The term anarchic field is assigned to a group of non-ciliated kinetosomes arranged arbitrarily giving rise to infraciliary bases (Corliss 1979). A streak is a ciliated anlage.
The mode of anlagen formation may vary amongst various hypotrichs. The origin of an anlage may be *de novo* or from kinetosomes that were earlier a part of the parental ciliature. Proliferation, a process of addition of kinetosomes, increases the size of an anlage. The alignment of kinetosomes in the anlage develops to form the base of a ciliary organelle, a process called "differentiation". The alignment pattern is a characteristic of the developing organism. Along with alignment of kinetosomes, the associated structures like microfibrils and microtubules are also formed.

The morphogenetic events thus involve the following steps:

i. Anlage formation by appearance of scattered kinetosomes
ii. Kinetosome proliferation and alignment
iii. Formation of accessory structures and growth of cilia.

Various anlagen and their differentiation
Oral anlage/primordium (OA/OP): It is the field of kinetosomes which leads to the formation of AZM primordium and UM primordium. In the proter of most ciliates, the parental AZM is retained only a UM primordium is formed. For the opisthe, the OP cleaves longitudinally into an AZM and a UM primordium. The UM primordium splits longitudinally to form the inner and the outer Ums, often called the endoral and paroral respectively.

Cirral anlage/primordium (CA/CP): Adjacent to the OP, cirral streaks are formed from which develop the frontal ciliature. Development of cirri occurs within the streaks after lateral proliferation. The mode of differentiation and the types of cirri formed varies in different groups of hypotrichs and has been the basis of classification within the group.

Marginal anlage/primordium (MA/MP): These are primordia for the right marginal row (RMR) and left marginal row (LMR). The marginal anlagen for the two daughter cells arise at two levels by ''within-row'' anlagen formation utilizing few of the parental cirri at each level. The marginal anlagen elongate and differentiate into new marginal rows. The rest of the parental marginal cirri are resorbed. In some species marginal primordia may form *de novo.*

Dorsal anlage/primordium (DA/DP): The new dorsal kineties are formed from the dorsal primordia which also develop within parental rows. The posterior ends of some of the developing dorsal kineties proliferate laterally to form the caudal cirri.

Dorsomarginal anlage/primordium (DMA/DMP): The right dorsal kineties (dorsomarginal rows) are formed from the dorsomarginal primordia arising on the ventral surface, originating at the anterior end of the right marginal primordia and later shifting to the dorsal surface.

CHAPTER 2

MATERIALS AND METHODS

The sites for collection of water and soil samples as well as the collection regimen were planned to cover the entire state according to accessibility during different seasons. Samples were collected from protected areas like wildlife sanctuaries and areas of extreme climatic conditions such as hot sulphur springs. Multiple sampling was done wherever feasible. Physico-chemical parameters were recorded on location. Macro floral and faunal data were also obtained.

Advantages of protected areas

There are several scientific benefits that can be derived by studying ecosystems in protected areas particularly areas such as wildlife sanctuaries. Such places provide the best sampling sites with natural communities along with their preserved ecosystems. Furthermore, undisturbed communities are important to monitor the population of species living in the area as the sites can be revisited as and when required. Wildlife sanctuaries were selected in the regions of high altitudes that provided cold climatic conditions. Similarly hot springs provided warm to high temperature conditions with high sulphur content enabling study of ciliates of varied climatic conditions in the present study.

COLLECTION, CULTURING AND SPECIMEN PREPARATION

Sample collection

Water samples: Water bodies with abundant aquatic plants and weeds generally provide good source of ciliate fauna. From the water samples, larger crustaceans, debris and other unwanted materials were filtered out by using Nytex nets of decreasing mesh sizes in series. Temperature and pH of water were recorded on sites for each collection. Field collections were transferred to the laboratory. Mixed planktonic cultures were grown at temperatures close to their natural temperatures with bacteria and flagellate *Chlorogonium elongatum* as the food source and examined for the presence of ciliates. Such water samples were subjected

to periodic microscopic examination for about two weeks. Single cells were isolated to raise clonal cultures for various species.

Soil samples: Soil samples were collected from various locations within protected areas and areas around hot sulphur springs as well as from soil water interface of water bodies. Temperature and pH of soil were recorded on sites for each collection. Soil samples were collected along with partially decomposed leaves and small roots. Samples were stored in sterile plastic bags and were brought to the laboratory and were processed immediately to record the presence of ciliate fauna. Moist soils were analyzed with the non-flooded petridish method (Foissner 1987). Briefly, this method involves placing 50–100g of soil sample in a petridish (10–15cm in diameter) and saturating but not flooding it with distilled water. Two to three millilitre of run-offs are drawn from the petridishes on days 2, 7, 14, 21, and 28, and the ciliates that excyst are isolated for raising clones. The soil samples were later air-dried for about a month and then stored in sterile plastic bags. In the present study, more cells were obtained with moist soil that was processed immediately after collection than the air dried soil. Thus the number of cells that excysted decreased rapidly with the drying of soil.

Laboratory culturing

Ciliates were cultured in Pringsheim medium (Chapman-Andresen 1958) or its modifications containing the following constituents in distilled water:

$Ca(No_3)_2.4H_2O$	-	0.85 mM
KCl	-	0.35 mM
$MgSO_4.7H_2O$	-	0.08 mM
$Na_2HPO_4.2H_2O$	-	0.11 mM

A majority of ciliate species were grown by providing the green algae *Chlorogonium elongatum* as food organism. Some of the ciliate species were raised on bacteria grown on boiled wheat grain added to Pringsheim medium.

***Chlorogonium elongatum* was cultured in the laboratory by the following method –**

0.1g of yeast extract powder was dissolved in 100ml of Pringsheim medium. The yeast solution was autoclaved and 5ml of it was added to 250ml conical flasks each containing 100ml autoclaved Pringsheim medium. Log phase culture of *Chlorogonium* was washed twice with autoclaved Pringsheim medium by centrifugation and the pellet was used to inoculate the medium. The flasks were kept in incubator at 23 $\pm$ 1^0C under white light with a photoperiod of 14hrs alternating with a dark period of 10hrs. Optimum density of *Chlorogonium* culture was obtained in 4–5 days under these conditions. Freshly harvested *Chlorogonium* suspended in Pringsheim medium was used for feeding the ciliates.

Live-cell Imaging

Observations of live cells were made using differential interference contrast microscopy. The most important technical challenge for performing successful live-cell imaging is to maintain the cells in a healthy state with normal function on the microscope stage while being illuminated. Cells that appeared even slightly unhealthy were not used for imaging and data collection. Cells were picked out from cultures with the help of a micropipette while observing them under the stereo zoom microscope and transferred onto a clean slide. A thin film of petroleum jelly was applied to the outer edges of the cover slip. Keeping the cells in minimal culture fluid, the jelly smeared cover slip was gently placed on the slide with the smeared edges down facing cells. Applying petroleum jelly creates a seal therefore cells can be kept alive and healthy for up to few hours, allowing observation and live-imaging.

Calculation of generation time

From the log phase cultures 15–20 cells that showed division furrows were isolated within a span of a few minutes and placed as one cell per cavity block. Cells were kept at a temperature which is optimum for them with sufficient food until the next binary fission. Time of division into two daughter cells was noted. Durations of the division cycles of all the daughter cells were noted and the average generation time was calculated.

Staining surface structures

Protargol staining technique (Tuffrau 1967; Jerka-Dziadosz and Frankel 1969) was used with some modifications (Kamra & Sapra 1990). This technique was employed to study vegetative cells as well as cells in different morphogenetic stages. Staining by the earlier methods stains

the macronucleus, which obstructs observation of the early stages of cortical development. To avoid this, slow developing method of Jarĕno (1984), modified by Kamra and Sapra (1990) was used. The method was modified and standardized for different ciliate groups.

Details of the procedure

i) **Fixation:** A mixture of equal volume of modified Bouin's fluid (9ml saturated aqueous picric acid + 1ml formalin + 6-8 drops of glacial acetic acid) and saturated solution of mercuric chloride was used as the fixative. 1ml of a concentrated clean culture was transferred to 5ml of the fixative. Cells were fixed for 20-30 minutes and then gently centrifuged to make a pellet. The fixed cells were washed 6–7 times with tap water until the yellow colour disappeared and were finally washed with distilled water. Some cells showing slight resistance to lose yellow colour of picric acid especially urostylids were washed with 5ml of acetone for 5–10 minutes to allow the extraction of coloured matter before finally washing them with distilled water.

ii) **Enrobement:** Meyer's Albumin was prepared by mixing equal volumes of egg albumen and glycerol and stirring for 30 minutes and then filtering though a cotton filter. The mixture can be stored at 4^{o}C and used within 20 days. A drop of concentrated fixed cells suspended in distilled water and a drop of Meyer's albumin was put in the centre of a clean, grease free slide. Drops were mixed with a mounted needle spread over the middle third. Sand, grains or any other debris were removed gently with the help of a needle. Excess of albumin was removed with a fine bore pipette so that the thickness of the albumin layer should be equal to that of the organisms. The slide was kept at a slant to drain of the excess fluid. Slides thus prepared were stored in an air-tight slide box until further processing. The slides were allowed to dry at least 12h (overnight) at room temperature and were processed further within 2–3 days. Over dried slides usually gave staining of poor quality.

iii) **Fastening of the film:** Fastening of the albumin film covering the fixed cells was done by dipping the slides sequentially in absolute methanol (5 minutes), 1:1 formaldehyde: methanol (15 minutes) and 50% methanol (2 minutes). After a rinse in distilled water, the slides were washed thoroughly in tap water to remove the formalin. Slides were then rinsed in distilled water.

iv) **Bleaching:** Slides were dipped in 4 % commercial sodium hypochlorite (Merck: free chlorine 4.0% w/v) for 3–5 minutes and then washed well in distilled water to remove all traces of sodium hypochlorite.

v) **Staining:** The cells were impregnated with 0.7% protargol (Merck/Roque/Sigma), freshly prepared by sprinkling 0.35g of the powder over 50ml of distilled water at 45^{o}C for 20 minutes. The stained slides were washed well by swirling them in a beaker of distilled water.

vi) **Developing:** Slow developer was prepared as follows: 1.4g boric acid, 0.3g hydroquinone, 2g sodium sulphate and 15ml acetone were mixed and made up to 100ml with distilled water. The prepared solution was kept at dark place for 48 hours for maturation in a tight stoppered bottle wrapped with black paper. The matured developer can then be stored in the refrigerator and used within 20 days. Stained slides were dipped in the developer for 5–10 seconds. Slight exposure to light was given and developing of stain was monitored under the microscope until the cells began to show infraciliature. After achieving the desired staining of cells, slides were washed thoroughly by swirling in a beaker of distilled water and then dipped in 2.5% oxalic acid for one minute to stop the developing process. Slides were again washed in distilled water.

vii) **Fixation of silver staining**: The slides were dipped in 5% Sodium thiosulphate for 5 minutes for fixation of silver staining and were then washed well in distilled water.

viii) **Dehydration, clearing and mounting**: The slides were dehydrated in an ethanol series, 50% → 70% → 90% for 5 minutes each. After a final dehydration in absolute ethyl alcohol (10 minutes), the slides were cleared in xylene (15 minutes) and then mounted in Canada balsam.

Staining nuclear structures

Observations on nuclei were made from Feulgen stained cells (Chieco and Derenzini 1999; Feulgen 1914). Feulgen stain was used for examining the features of nuclear components during growth and cell division cycle. Prior to staining, cells were fixed in a fixative of ethanol and glacial acetic acid in the ratio of 3:1. One ml of a concentrated clean culture was transferred to 5ml of the fixative. Cells were fixed for 10–15 minutes and then gently centrifuged to make a pellet. The Feulgen reaction consists of two steps. Initially, acid hydrolysis was performed, usually for 8–12 minutes at 60^{o}C, resulting in the cleavage of the

purines exposing the aldehyde groups. The preparations were then placed in light yellow Schiff reagent (fuchsine-sulfurous acid), which forms bonds with these groups. The red-violet product formed in this step stained macronuclear nodules and micronuclei and thus enabled counting and measurement of macronuclear nodules and micronuclei in each cell.

Photomicrography, morphometric measurements, and preparation of line diagram

Leica camera DFC320 was used for photomicrography. Biometric characterization of captured images was done at a magnification of 1000× using the Leica software IM50 image manager. Line diagrams were prepared using CorelDRAW(R) Graphics Suite–Version 12.0 software.

MOLECULAR METHODS

Genomic DNA Isolation, amplification of 18S rDNA and sequencing

To extract genomic DNA, about 30–50 specimens were picked with a micropipette and transferred into microfuge tube. 180µl ATL buffer (QIAGEN) and 20µl Proteinase K (20mg/ml) were added. Subsequently, the genomic DNA was extracted using the protocol for cultured animal cells using DNeasy Blood and Tissue Kit (QIAGEN GmbH, Hilden, Germany).

The 18S rDNA was amplified using the universal eukaryotic primers EukA and EukB (Medlin et al. 1988). The amplification reaction contained 10–20 ng of DNA template, 2.5 U HotStar Taq DNA polymerase (QIAGEN) in the reaction buffer provided, 1.5mM $MgCl_2$, 200µM of dNTPs, and 0.5µM of each oligonucleotide primer. The final volume was adjusted to 50µl with sterile distilled water. The PCR protocol for 18S rDNA gene amplification consisted of an initial hot start incubation of 15 minutes. at 95°C followed by 30 identical amplification cycles (i.e., denaturing at 95°C for 45seconds, annealing at 55°C for 1minutes., and extension at 72°C for 2.5 minutes), and a final extension at 72°C for 7minutes. Negative control reactions included *Escherichia coli* DNA as a template.

The resulting PCR products were checked by electrophoresis on 1% (w/v) TBE agarose gel. The bands on the gel containing the amplified products were sliced from the gel with a clean scalpel and purified with the QIAquick PCR Purification Kit (QIAGEN GmbH, Hilden, Germany) and sequenced either directly or alternately, cloned into a plasmid vector,

amplified in bacteria, purified, and then sequenced with vector primers. In either case, both strands of the DNA should be sequenced to corroborate the sequence reads.

Direct sequencing was done through ABI 3730 automated sequencer by using following five primers (Fig. 2.1):

Two universal primers (Medlin et al. 1988), EukA, the forward (5'–AACCTGGTTGATCCTGCCAGT–3') and EukB, the reverse (5'–TGATCCTTCTGCAGGTTCACCTAC–3') and three internal primers, two forward (5'–ATTGGAGGGCAAGTCTGGTG–3' and 5'–ACACTGACGCATACAGCGAG–3') and one reverse (5'–GCCCATGCGATTCGATCAGT–3') were used for direct sequencing.

The internal primers were designed by using primer designing tool, the Primer Blast (www.ncbi.nlm.nih.gov/tools/primer-blast). Additionally, two more internal primers, the forward 5'–TTAGATGTCCTGGGCCGCA–3' and the reverse (5'–GCCGTTTCTCAGGCTCCCTCT–3' were designed and were found to be giving better read lengths and could be used with more number of cell isolates.

Sequencing was also done after cloning 18S rDNA into *E. coli* DH5α using pGEM-T Easy vector, a TA-Cloning kit (Promega Cloning kit). Colony PCR was done using M13F and M13R primers to screen for inserts of the expected size (about 1.8 kb). Plasmids were isolated and purified with Qiaprep Spin Miniprep Kit (QIAGEN) from overnight cultures. Three clones were sequenced bidirectionally using M13 sequence primers on an ABI 3730 automated sequencer.

18S rDNA gene sequence analysis and phylogenetic tree construction

18S rDNA sequences were submitted to a BLAST search (http://www.ncbi.nlm.nih.gov) to find closely related sequences; 18S rDNA sequences of a total of 20–30 taxa were retrieved including 18S rDNA sequences of one or two taxa to be used as outgroup. Sequences were aligned using CLUSTAL X2 sequence analysis software (Larkin et al. 2007). The resulting alignments were checked and corrected manually to remove ambiguous nucleotide positions at the beginning and end of the fragments. Phylogenetic analysis was performed with the MEGA (Molecular Evolutionary Genetics Analysis) software package version 5.0 (Tamura et al. 2011) available at http://www.megasoftware.net and with PAUP*

Version 4b10 (Swofford 2003). Molecular phylogeny in each case was inferred from trees constructed by means of Maximum-likelihood, Maximum parsimony, and Neighbour-joining analyses. In specific cases, different types of methods were used in latter three types of analyses and have been specified in the respective chapters.

CHAPTER 3

BIODIVERSITY OF CILIATED PROTISTS FROM SIKKIM

Sikkim-a biodiversity hot spot

Eastern Himalayas are known to be a biodiversity hotspot (Conservation International, Virginia). The Indian state of Sikkim is in the western segment of Eastern Himalayas. The present report attempts to catalogue ciliate diversity from this state. The ciliate fauna of Sikkim was expected to be rich and diverse as the state has very diverse ecozones supporting enormous genetic diversity.

The introductory information about Sikkim given in the chapter has largely been derived from "Eco-destination of India" the information compiled by Environment Information Systems (ENVIS), Ministry of Environment and Forests, Government of India. Other sources include other governmental agencies such as the Department of Tourism. These details about the physical and ecological parameters of collection sites are essential for any study on biodiversity and are useful in planning and execution of collections.

Sikkim is situated between the coordinates 27°03'47" to 28°07'34"N and 88°03'40" to 88°57'19"E; on its north west boundary is Mount Khanchendzonga with a height of 8598 meters, the third highest mountain peak in the world. Sikkim has a total area of 7,096 square kilometres, 114 km from north to south and 64 km from east to west. Despite its small size, Sikkim is geographically diverse, owing to its location on the Himalayas. The entire state has a mountainous terrain with altitudes ranging from 300 to 8,586 metres interspersed with streams, lakes, and waterfalls. The climate of the state varies from cold temperate and alpine in the northeast to subtropical in the south. Agro climatically, the state is divided into four zones, *viz*., the subtropical zone (below 1,000 meters); the humid zone (1,000–1,600 meters); the mid-hill dry zone (again in altitudes ranging from 1,000–1,600 meters); and high hill temperate zone (with an altitude of above 1,600 meters). Being bounded by such formidable features, the state has remained ecologically untouched and therefore has provided natural protection to its flora and fauna.

The northern portion of the state is deeply cut into steep slopes or long cliffs. Southern Sikkim is lower, more open, and fairly well cultivated. The trend of the mountain system is in

a general east-west direction; however, chief ridges run in a more or less north south direction. The Rivers Rangeet and Teesta form the main channels of drainage and run from north to south. The valleys cut by these rivers and their chief feeders are very deep. There are many perennial lakes at different altitudes and several hot water springs in the state. The perpetual snow line in Sikkim is approximately at 5500 meters.

Sikkim is the wettest part of the north eastern region of our country. The relative humidity remains above 70 percent throughout the year at most places. The state experiences heavy rainfall due to its proximity with the Bay of Bengal. The rainfall in North district is comparatively less than that of the other districts. Pre-monsoon rain occurs in April-May and south–west monsoon rainfall occurs normally from May and continues up to early October. The intensity of rainfall during south–west monsoon season decreases from south to north, while the distribution of winter rainfall is in the opposite order. Annual rainfall ranges from 82 mm–3494 mm. The area in the north-west has mainly snow-covered mountains with well distributed rain during the months from May to early October.

Temperature varies with altitude and slope. The mean temperature in the lower altitudinal zones varies from 4 to 18°C, whereas at higher altitudinal zones, it varies from 1 to 9°C. The maximum temperature is recorded usually during July-August and minimum during December-January. Fog is a common feature in the entire state from May to September.

From the above discussion it is obvious that Sikkim possesses climatic conditions suitable to support an extensive range of flora and fauna, providing a ciliatologist an enriching place to explore for biodiversity of ciliates. Biodiversity of ciliate fauna from diverse regions of Sikkim was explored in detail mainly emphasizing areas under protection and with extreme climatic conditions.

Ten collection sites from the four districts of Sikkim (Fig. 3.1) were identified and explored for their ciliate fauna. These included soil and aquatic habitats. Physico-chemical parameters were recorded on location. Macro floral and faunal data was also obtained. Over 105 ciliate species were isolated from various collection sites; 90 ciliate species could be identified up to genus/species level. Classical and molecular (18S rDNA sequence) data were used for identifications. In most cases these corroborated each other. About 60 of these could be raised as sustained clonal cultures. Accession numbers for 18S rDNA sequences of 26 taxa have been obtained.

Following is the district wise detail of ciliate fauna collected from Sikkim.

South Sikkim

Spread over an area of 750 square kilometres, South district of Sikkim includes snow laden mountains and vast stretches of deep valleys. Samples were collected from Maenam Wildlife Sanctuary.

Maenam Wildlife Sanctuary (Figs 3.2, 3.3; Tables 3.1, 3.2)

Situated just above the Tendong Hill in the northern corner of the South district, Maenam Wildlife Sanctuary (27° 18' 50" N; 88° 23' 35" E) is spread over an area of 36.34 square kilometres. The terrain is largely hilly with 3231 meters as the highest point at sanctuary. The term "Maenam-la"- means a treasure house of medicines. As the name suggests, a rich collection of different medicinal plants are available in the region. The flora of the area consists of dense sub-tropical and temperate forests with liverworts, mosses and ferns, a variety of rhododendrons, and berries. Macro-fauna include Red Panda, Goral, Serow, Barking Deer, Marbled Cat, Leopard Cat, Civet Cat, Blood Pheasant, Hill Partridge, Magpies, Black Eagle, Blue Necked Pitta, Sunbirds.

Sampling was done twice, once during summer (April-July) and once during winter (December-January). During sampling I weather conditions were cloudy; temperature ranged between 19–22°C, and the soil pH was mostly acidic. During the second sampling, weather conditions were clear sky; temperature ranged between 5–13°C, and the soil pH was mostly acidic.

Among the ciliate fauna reported from the sanctuary, hypotrichous ciliates (Table 3.1) were in abundance along with non-hypotrichous ciliates (Table 3.2) like *Colpidium*, *Colpoda*, *Paracolpidium*, *Spathidium*, *Frontonia* and *Vorticella.*

West Sikkim

The district is spread over an area of 1166 square kilometres. Most parts of the district lie at a high altitude and is widely known for its vast range of flora and macro-fauna.

Water and soil samples were collected from the Barsey Rhododendron Sanctuary, Khecheopalri Lake, Legship Hot Spring and Borang Hot Spring.

Barsey Rhododendron Sanctuary (Figs 3.4, 3.5; Tables 3.3, 3.4)

The Sanctuary lies in the south-west corner of the West Sikkim district (27° 11' 39" N; 88° 07' 06" E). It is spread over an area of 104 square kilometres. The altitude ranges between 2774–3200 meters. The Sanctuary receives regular snowfall in winter and has over 40 different varieties of Rhododendron during full bloom.

Samples were collected from various sites across the sanctuary below different types of shrubs and trees and from a water catchment area surrounded by a vast array of dense sub-alpine vegetation, the dominant among them being *Tsuga*, *Abies*, *Juniperus*, *Quercus*, *Rhododendron*, *Aspelinium*, *Pteris*, *Digitaria*, *Polygonatum*, reeds and various types of bamboo grasses. Water body of the area was found to be very rich in ciliate fauna.

Sampling (both water and soil) was done twice, once during summer (April-July) and once during winter (December-January). During sampling I weather conditions were cloudy; temperature ranged between 16–18°C, and the soil pH was mostly acidic. During the second sampling, weather conditions were clear; temperature ranged between 3–7°C (snowfall two days prior to collection day), and the soil pH was mostly acidic.

Among the ciliate fauna reported from the sanctuary, hypotrichous ciliates (Table 3.3) were in abundance along with non-nypotrichous ciliates (Table 3.4) like *Paramecium*, *Tetrahymena*, *Spathidium*, *Stombilidium*, *Colpoda*, *Dileptus* and *Blepharisma.*

Khecheopalri Lake (Fig. 3.6; Table 3.5)

Khecheopalri Lake (27°22'24" N; 88°12' 30" E) is spread over an area of 3.79 sq km and is located at an altitude of 1700 meters. This Lake is surrounded by dense forest and is considered as one of the sacred lakes of Sikkim. No water sport or other activities besides prayers are allowed around the lake. The lake is enveloped in a dense cover of a variety of Bamboo, *Typha*, *Canna* and Alligator weed.

Sampling (both water and soil) was done twice, once during summer (April-July) and once during winter (December-January). During sampling I weather conditions were cloudy; temperature was ~20°C, and the soil pH was mostly acidic. During the second sampling, weather conditions were clear; temperature was ~8°C and the soil pH was mostly acidic.

Among the ciliate fauna (Table 3.5) reported from the lake, hypotrichous ciliates were in abundance along with non-hypotrichous ciliates like *Bresslaua*, *Colpoda*, *Pleuronema*, *Stombilidium*, *Dileptus*, *Vorticella* and *Spathidium*.

Legship Hot Spring (Fig. 3.7; Table 3.6)

Legship hot spring (27°27' N; 88°27' E) is located at an altitude of 318 meters at the eastern bank of Rangeet river. The hot water point source is traceable only when water recedes away from river bank especially during summers. The very hot water is inaccessible as the point source is surrounded by huge rocks and is slightly below the water level, the oozing out hot water mixes with the overflowing river water. The samples were collected from the sand soil in the area around hot spring with high sulphur content.

Sampling (both water and soil) was done twice, once during summer (April-July) and once during winter (December-January). During sampling I weather conditions were cloudy; temperature was ~20°C, and the soil pH was fairly acidic. During the second sampling, weather conditions were clear; temperature was ~5°C and the soil pH was fairly acidic. Ciliate fauna from area around Legship Hot Spring is listed in Table 3.6.

Borang Hot Spring (Fig. 3.8; Table 3.7)

Borang Hot Spring (27° 22' 1"N; 88° 19' 40"E) is located at the western bank of river Rangeet at an altitude of 2286 meters. There are many point sources from where hot water oozes out and mixes with the adjoining river water. Water sample was collected at the source where water enriched with heavy sulphur was seeping down. The temperature of the water was ~ 55°C. The pH recorded was ~ 6. The samples collected from the point source were analyzed many times to find ciliates. Only one ciliate *Sterkiella cavicola* could be recovered in the laboratory conditions from the cysts present in the hot water with high sulphur content. Many attempts were made to grow the isolated cells of ciliate *Sterkiella cavicola* at high temperature but the cells showed encystment with rise in temperature.

East Sikkim

East Sikkim occupies the south-east corner of the state. The East district is situated between 88°36′ to 88° 56'E and 27° 9' to 27° 25'N with a total geographical area of 945 square kilometres; it is entirely mountainous, covered with dense forest of sal, simul, bamboo and many other varieties of plant especially rhododendrons and orchids.

Samples were collected from Fambong Lho Wild life Sanctuary, Kyongnosla Alpine Sanctuary and Changu/Tsomgo Lake.

Fambong Lho Wildlife Sanctuary (Fig. 3.9; Table 3.8)

The sanctuary (27° 27’ 38’’ N; 88° 46’ 57’’ E) is spread in an area of 51.76 square kilometres, at an altitude of 1280-2652 meters. The flora of the Sanctuary includes dense forests of oak, bamboo, rhododendrons, lichen laden conifers, ferns and plenty of wild orchids. The macro-fauna include different species of wild animals like Himalayan Black Bear, Red Panda, Civet cat or Bear cat.

Sampling (soil) was done twice, once during summer (April-July) and once during winter (December-January). During sampling I weather conditions were cloudy; temperature ranged between 19–24°C, and the soil pH was fairly acidic. During the second sampling, weather conditions were clear; temperature ranged between 3–7°C and the soil pH was fairly acidic.

Among the ciliate fauna (Table 3.8) reported from the sanctuary, hypotrichous ciliates were in abundance along with non-hypotrichous ciliates like *Bresslaua*, *Plytyophryideslatus*, *Spathidium* and *Frontonia*.

Changu/Tsongmo Lake (Fig. 3.10; Table 3.9)

The lake is situated (27°37’53’’ N; 88°76’39’’ E) at an altitude of 3658 meters on the Gangtok Nathu La highway. It is about 1 km long, oval in shape, 15 meters deep and remains frozen during the winter months up to mid May.

The soil samples were collected from the area surrounding the lake. Sampling (both water and soil) was done once during summer (April-July). During the sampling, weather conditions were clear; temperature was ~5°C and the soil pH was mostly acidic.

Among the ciliate fauna (Table 3.9) reported from the lake, hypotrichous ciliates were in abundance along with non-hypotrichous ciliates like *Dileptus*, *Spathidium* and *Colpoda.*

Kyongnosla Alpine Sanctuary (Fig. 3.11; Table 3.10)

It is spread (27°22' to 33"N; 88°44' to 13"E) in an area of 31 square kilometres at an altitude of 3350 meters, situated in the area adjoining the Tsongmo (Changu) lake along the Nathula Road. The flora of the sanctuary includes a variety of alpine flowers like Poppies, Primulas and Rhododendron and macro-fauna includes Musk Deer, Serow, Himalayan Black Bear, Red Panda, Lesser Cats, Blood Pheasant, Satyr Tragopan, Impeyan Pheasant. Many rare and highly endangered plants, some of them with great medicinal value, are found in the sanctuary. Many migratory birds also use Kyongnosla Alpine Sanctuary as a stopover before going down to the Indian plains or back to Siberia.

Sampling (soil) was done once during summer (April-July). During the sampling, weather conditions were cloudy; temperature was ~5°C and the soil pH was mostly acidic.
Among the ciliate fauna (Table 3.10) reported from the sanctuary, hypotrichous ciliates were in abundance, significantly including a new genus, along with non-hypotrichous ciliates like *Tetrahymena* and *Colpoda.*

North Sikkim

North Sikkim is the northern district of Sikkim state and is spread in the 4226 square kilometres area. The whole district is mountainous with steep valleys all the way up to the alpine altitude. The elevation increases as one goes from south to north and the area turns from temperate to alpine in the northern most regions to tundra. In North Sikkim, samples were collected from Yumthang flower valley, Yumesamdong famously known as zero-point, and the area around hot springs in the region.

Yumthang Flower Valley (Fig. 3.12; Table 3.11)
Yumthang valley (27° 50' 28" N; 88° 44' 21" E) is situated at an altitude of 3597 meters. It is famously called Valley of Flowers because it is a natural home to diverse varieties of flowers. The valley has a large variety of Rhododendrons and the valley appears red in spring. The valley is home to the Shingba Rhododendron Sanctuary. With in the valley there are many hot springs particularly in Yumesamdong.
Samples were collected from various regions across the valley and from sulphur rich areas in the immediate surroundings of two hot springs. Sampling (both soil and water) was done once during summer (April-July). During the sampling, weather conditions were cloudy; temperature was ~5°C and the soil pH was mostly acidic. Temperature of Hot spring water was 50-60°C. The pH of water recorded was ~ 6. Among the ciliate fauna (Table 3.11) reported from the valley and area surrounding Yumesamdong Hot Spring, hypotrichous ciliates were in abundance along with non-hypotrichous ciliates like *Trithigmostoma* and *Prostomata*.

Yumesamdong /Zero point (Fig. 3.12; Table 3.11)
Yumesamdong (27° 57'25"N; 88° 46'2"E) is another valley near Yumthang valley with green grazing patches and is situated at an altitude of 4572 meters. Yumesamdong remains snow laden

throughout the year and is famously known as zero-point because of its sub-zero temperature. During peak summer, ephemerals, the plants which grow and complete their life cycle during short period of time of two to three months grow abundantly in the region.

Sampling (soil) was done once during summer (April-July). During the sampling, weather conditions were cloudy; temperature was ~5°C and the soil pH was mostly acidic.

Among the ciliate fauna (Table 3.11) reported from the area, hypotrichous ciliates were in abundance along with non-hypotrichous ciliates like *Curimostoma* and *Chilodonella.*

SYSTEMATIC NOTE

The ciliates catalogued above belong to diverse groups among ciliated protozoa. Majority of them belong to the group Hypotricha (Phylum Ciliophora). The systematics within this group is largely based on the positioning of surface structures on the cortex, especially the placement of ciliature. With the continuous improvement of techniques to study infraciliature especially silver staining techniques, the dorsal profile of hypotrichous ciliates has become an important decisive attribute for systematists. This has significant support from molecular data. Based on the dorsal infraciliature the hypotrichous ciliates are broadly classified (Fig. 3.13) into two groups (Berger 2006):

i. Non-dorsomarginalian Hypotricha
ii. Dorsomarginalian Hypotricha

Non-dorsomarginalian Hypotrichs are ciliates with simple dorsal kinety pattern which lack dorsomarginal rows. These include members of the following families/groups: Gonostomatidae, Urostyloidea, Amphisiellidae and Trachelostylidae. Among the non-dorsomarginalians, it has been hypothesized that the simple dorsal kinety formation pattern (Fig. 3.14A) seen in *Gonostomum* (family Gonostomatidae) represents the state present in the last common ancestor of Hypotricha (Berger 2008). A representative of this group is described in Chapter 4.

The group **Dorsomarginalia** has probably resulted from evolution of a dorsomarginal kinety in a stem line within Hypotricha (Berger 2006). Thus dorsomarginalians are hypotrichs which possess dorsomarginal row(s). Dorsomarginalian hypotrichs include ciliates of the following families: Kahliellidae, Uroleptidae and Oxytrichidae. The family Oxytrichidae has been a grey area for systematists for varied reasons. Until 2006 the family was defined mainly

through its ventral infraciliature 'the 18-cirri pattern' (Berger and Foissner 1997, Berger 1999). With constant addition in the knowledge of dorsal profile and of molecular data, the family has been redefined (Berger 2006) based on the dorsal kinety fragmentation pattern as its major morphological apomorphy. Thus, according to the contemporary scheme of classification, the dorsal pattern which includes one or two dorsomarginal rows and simple fragmentation of kinety 3 is very likely an autapomorphy of the family Oxytrichidae (Fig. 3.14 B).

A large number of ciliates collected from various sites in Sikkim belong to the family Oxytrichidae. This provided an excellent opportunity to study them in detail especially with respect to their dorsal infraciliature; this led to the clarification of the systematic status of some taxa. Detailed account of descriptions of some select oxytrichids is given in chapter 5.

Classical and molecular data of two oxytrichid hypotrichs from Sikkim – *Cyrtohymena* (*Cyrtohymenides*) *shii* and *Paraurosomoida indiensis* (a new genus) have contributed significantly in clarifying the systematic status of flexible bodied oxytrichid ciliates which possess the following two dorsal patterns (Fig. 3.14 C, D) within Dorsomarginalia whose positions were hitherto unclear because of the absence of molecular markers.

1) The flexible bodied oxytrichid ciliates which lacked fragmentation in dorsal kinety 3, (e. g. *Paraurosomoida. indiensis*; Fig. 3.14 C).
2) The flexible bodied oxytrichid ciliates with multiple fragmentation in dorsal kinety 3, (e. g. *Cyrtohymena* (*Cyrtohymenides*) *shii*; Fig. 3.14 D).

It is clear from the present study that the flexible bodied taxa with such dorsal patterns (Fig. 3.14 C, D) are also well placed within Oxytrichidae contrary to the assumption that hypotrichous ciliates with only simple fragmentation of dorsal kinety 3 are oxytrichids.

In the context of the above, hypotrichous ciliates with usually 18 frontal-ventral-transverse cirri and/or at least one fragmenting dorsal kinety (primarily dorsal kinety 3; fragmentation sometimes secondarily lost or multiple) have been described in the present work under family Oxytrichidae. According to (Berger and Foissner 1997; Berger 1999) the family Oxytrichidae are broadly categorized in to two primarily based on the texture of the body, the flexible bodied oxytrichids belonging to subfamily Oxytrichinae Ehrenberg, 1838 and the firm bodied oxytrichids belonging to subfamily Stylonychinae Berger and Foissner 1997. Later on the basis of molecular data (Foissner et al. 2004; Schmidt et al. 2007; Paiva et al. 2009; Hu et al. 2011) the subfamily Oxytrichinae proved to be a non-monophyletic assemblage resulting in

the deactivation of the subfamily by Berger (2008). The non-monophyly of this group is very likely due to the use of the 18-cirri pattern as the main apomorphy for the Oxytrichidae (Berger 2008; Shao et al.2012).

Since no group name has yet been assigned to the flexible bodied oxytrichids, the name Oxytrichinae has been retained throughout this report.

Members of the family Oxytrichidae exhibit the following three character states (Fig 3.14) with respect to fragmentation of dorsal infraciliature:

1) Simple fragmentation of dorsal kinety 3 (Fig 3.14 B) e. g. *Oxytricha* and *Stylonychia*.
2) No fragmentation of any dorsal kinety (Fig 3.14 C) e. g. *Urosomoida* and *Paraurosomoida*.
3) Multiple fragmentation of dorsal kinety 3 (Fig 3.14 D) e. g. *Cyrtohymena* (*Cyrtohymenides*) and *Pattersoniella*.

Some selected species of oxytrichids exhibiting the above three character states have been described in three Sections A, B and C of chapter 5. Chapter 6 gives an insight into the molecular phylogeny based on 18S rDNA sequences of selected hypotrichous ciliates from Sikkim and their related sequences available in the GenBank.

CHAPTER 4

NON-DORSOMARGINALIAN HYPOTRICHA

The chapter includes description of one representative non-dorsomarginalian hypotrich with simple dorsal kinety pattern consisting of 3 dorsal bipolar kineties (due to lack of fragmentation of kinety 3) and lacking dorsomarginal rows (Fig. 3.14A). A hypothesis substantiated by molecular data (Foissner et al. 2004; Hewitt et al. 2003; Schmidt et al. 2007; Shao et al. 2007) postulates that the '18-cirri' pattern in the ventral ciliature evolved in the last common ancestor of the hypotrichs and therefore it appears throughout the Hypotricha tree. Non-dorsomarginalians represent a state present in the last common ancestor of Hypotricha (Berger 2006). Some genera that comprise this group are *Gonostomum*, *Paragonostomum*, *Wallackia* and *Cladotricha. Gonostomum* is a typical example of such ciliates.

The genus *Gonostomum* is one of the most common hypotrichs (Foissner 1997) especially in terrestrial habitats. *Gonostomum* was found in most of the samples investigated from various parts of Sikkim; *G. affine* was the most common species among them. A terrestrial *Gonostomum* species, *G. strenuum* was widely distributed in the soils around the Tsongmo or Changu lake in the East District of Sikkim. This species has been well described (Song 1990); however, the Sikkim population of *G. strenuum* was remarkably different in its morphology and morphometry. There were also significant molecular differences in the 18S rDNA sequence when compared to the only sequence available (AJ310493; Bernhard et al. 2001) on GenBank. A detailed description of *G. strenuum* Sikkim population is given below with an introductory description of the genus.

Gonostomum strenuum (Engelmann 1862) Sterki, 1878; Sikkim population (Fig. 4.1A–L; Tables 4.1, 4.2)

The genus *Gonostomum* Sterki, 1878 is characterised by the following traits: Adoral zone of membranelles typically in *Gonostomum* pattern (Berger and Foissner 1997). The paroral consists of few widely spaced cilia and extends far beyond the anterior end of the endoral.

The genus was previously included in family Oxytrichidae, but has now been separated at the family level to be a constituent of the family Gonostomatidae which include other genera such as *Paragonostomum*, *Wallackia* and *Cladotricha*.

The Sikkim population of *G. strenuum* markedly shows further increase in number of cirri in the frontoventral row, and also in the number of migratory cirri and post oral ventral cirri. Following is a description of the population found in the soil around Tsongmo or Changu Lake.

Gonostomum strenuum is diagnosed by the following autapomorphies compared to its congeners: long frontoventral row; increased number of migratory cirri and postoral ventral cirri, forming an oblique row; left marginal row typically terminates behind transverse cirri; right marginal row extends posteriad up to the posterior end of transverse cirri.

Table 4.1. Morphometric characterization of *Gonostomum strenuum* Sikkim population[a].

Character	Mean	SD	CV	Min	Max	n
Body length	68.3	3.75	5.50	63.1	74.6	25
Body width	21.6	2.54	11.75	17.4	24.3	25
AZM length	36.3	2.15	5.92	32.0	38.9	25
Adoral membranelles, number	27.9	1.20	4.29	26.0	30.0	25
AZM / body length, %	53.1	0.03	0.05	47.9	56.5	25
Macronuclear nodules, number	2.0	0.00	0.00	2.0	2.0	25
Macronucleus length	18.8	1.44	7.63	15.8	20.5	25
Macronucleus width	6.1	0.92	15.07	4.3	6.9	25
Micronuclei, number	2.2	0.42	19.17	2.0	3.0	25
Micronucleus diameter	2.4	0.30	12.24	2.0	2.9	15
Buccal cirri, number	1.0	0.00	0.00	1.0	1.0	25
Number of cirri formed from streak II	2.0	0.00	0.00	2.0	2.0	25
Number of cirri formed from streak III	3.7	0.48	13.06	3.0	4.0	25
Number of cirri formed from streak IV	4.1	0.32	7.71	4.0	5.0	25
Number of cirri formed from streak V	8.7	0.67	7.76	8.0	10.0	25
Number of cirri formed from streak VI	7.5	1.65	22.00	5.0	9.0	25
Pretransverse ventral cirri, number	1.7	0.48	28.41	1.0	2.0	25
Transverse cirri, number	3.9	0.32	8.11	3.0	4.0	25

Right marginal row, number of cirri	22.4	2.22	9.92	18.0	27.0	25
Left marginal row, number of cirri	20.1	1.97	9.80	16.0	22.0	25
Dorsal kineties, number	3.0	0.00	0.00	3.0	3.0	15
No. of bristles in DK_1	24.1	3.18	13.19	20.0	28.0	15
No. of bristles in DK_2	22.0	2.58	11.74	18.0	26.0	15
No. of bristles in DK_3	23.9	2.64	11.06	19.0	26.0	15
Caudal cirri, number	3.0	0.00	0.00	3.0	3.0	15

[a] Data based on protargol-impregnated specimens. All measurements in μm. AZM, adoral zone of membranelles; CV, coefficient of variance (in %); DK, dorsal kinety; Max, maximum; Mean, arithmetic mean; Min, minimum; n, sample size; No., number; SD, standard deviation.

Description: Body is flattened, flexible with anterior end somewhat pointed and posterior end rounded. Mean body size *in vivo* is 71μm × 22μm and 68μm × 22μm when impregnated with protargol. The length:width ratio is 3:1. Cortical granules are present. Contractile vacuole is present nearly in mid-body.

AZM is typically in *Gonostomum* pattern with middle portion straight, extending along the left margin of the cell, proximal part is abruptly bent towards the centre of the body, length averages 53% of body length, consists of about 28 membranelles; paroral made up of few, widely spaced cilia and extends far beyond the anterior end of the endoral. Frontal ciliature consists of >18 frontal-ventral-transverse cirri arranged in a species specific manner (Fig.4.1 and Table 4.1). There are one left and one right marginal row, with about 20 and 22 cirri respectively, left marginal row typically terminates behind transverse cirri, right marginal row finishes at the level of transverse cirri.

There are three dorsal bipolar kineties with about 20–28, 18–26 and 19–26 bristles respectively, with no fragmentation of the dorsal kinety and no dorsomarginal row typical of the family. Three caudal cirri are present one each at the posterior tip of the three dorsal kineties.

The cells encyst often making cysts. Each cell has invariably two ellipsoidal macronuclear nodules with average size 19μm × 6μm and 2–3 spherical micronuclei with average diameter 2.4 μm each.

Divisional morphogenesis: Morphogenesis of Sikkim population of *Gonostomum strenuum* is similar to that of the earlier described population (Song 1990).

Molecular phylogeny: 18S rDNA sequence of *Gonostomum strenuum* Sikkim population determined in this study has been submitted in GenBank database under accession number JX946277.

Occurrence and ecology: Terrestrial, widely distributed in the soils around Tsongmo or Changu lake (27° 22' 31'' N and 88° 45' 50''23' E) located in the East Sikkim.

Table 4.2. Morphometric comparison of *Gonostomum strenuum* Sikkim population (Si) with *Gonostomum strenuum* Qingdao, Chinese population (Qi; Song 1990)[a].

Character	Population	Mean	SD	CV	Min	Max	n
Body length	Si	**68.3**	3.75	5.50	63.1	74.6	25
	Qi	**103.8**	7.90	7.60	88.0	119.0	11
Body width	Si	**21.6**	2.54	11.75	17.4	24.3	25
	Qi	**37.0**	5.00	13.40	28.0	44.0	11
AZM length	Si	**36.3**	2.15	5.92	32.0	38.9	25
	Qi	**54.5**	7.20	13.20	41.0	69.0	11
Adoral membranelles, number	Si	27.9	1.20	4.29	26.0	30.0	25
	Qi	29.5	2.20	7.30	26.0	33.0	11
AZM / body length, %	Si	53.1	0.03	0.05	47.9	56.5	25
	Qi	52.5	-	-	-	-	-
Macronuclear nodules, number	Si	2.0	0.00	0.00	2.0	2.0	25
	Qi	2.0	0.00	0.00	2.0	2.0	17
Macronucleus length	Si	18.8	1.44	7.63	15.8	20.5	25
	Qi	17.1	-	-	-	-	17
Macronucleus width	Si	**6.1**	0.92	15.07	4.3	6.9	25
	Qi	**9.1**	-	-	-	-	16
Micronuclei, number	Si	2.2	0.42	19.17	2.0	3.0	25
	Qi	2.1	-	-	2.0	3.0	-
Micronucleus diameter	Si	2.4	0.30	12.24	2.0	2.9	15
	Qi	2.3	-	-	1.9	2.8	16
Buccal cirri, number	Si	1.0	0.00	0.00	1.0	1.0	25
	Qi	1.0	-	-	-	-	17
Number of cirri formed from streak II	Si	2.0	0.00	0.00	2.0	2.0	25
	Qi	2.0	-	-	-	-	17
Number of cirri formed from streak III	Si	**3.7**	0.48	13.06	3.0	4.0	25
	Qi	**2.1**			2.0	3.0	17
Number of cirri formed from streak IV	Si	**4.1**	0.32	7.71	4.0	5.0	25
	Qi	**3.3**	-	-	3.0	4.0	17
Number of cirri formed from streak V	Si	**8.7**	0.67	7.76	8.0	10.0	25
	Qi	**7.2**	-	-	6.0	8.0	17
Number of cirri formed from streak VI	Si	**7.5**	1.65	22.00	5.0	9.0	25
	Qi	**4.4**	-	-	4.0	6.0	17
Pretransverse ventral cirri and transverse	Si	5.6	0.52	9.22	5.0	6.0	25
	Qi	5.8	0.80	13.10	4.0	7.0	17
Right marginal row, number of cirri	Si	22.4	2.22	9.92	18.0	27.0	25

	Qi	25.8	2.00	7.60	23.0	29.0	13
Left marginal row, number of cirri	Si	20.1	1.97	9.80	16.0	22.0	25
	Qi	17.7	5.20	29.60	17.0	21.0	13
Dorsal kineties, number	Si	3.0	0.00	0.00	3.0	3.0	15
	Qi	3.0	-	-	3.0	3.0	17
No. of bristles in DK_1	Si	24.1	3.18	13.19	20.0	28.0	15
	Qi	24.0	-	-	-	-	-
No. of bristles in DK_2	Si	22.0	2.58	11.74	18.0	26.0	15
	Qi	23.0	-	-	-	-	-
No. of bristles in DK_3	Si	23.9	2.64	11.06	19.0	26.0	15
	Qi	25.0	-	-	-	-	-
Caudal cirri, number	Si	3.0	0.00	0.00	3.0	3.0	15
	Qi	3.0	0.00	0.00	3.0	3.0	17

[a] Data based on protargol-impregnated specimens. All measurements in μm. AZM, adoral zone of membranelles; CV, coefficient of variance (in %); DK, dorsal kinety; Max, maximum; Mean, arithmetic mean; Min, minimum; n, sample size; No., number; SD, standard deviation. The highlighted numericals point to significant morphometric differences. The sign – depicts data not available.

Discussion

Gonostomum strenuum reported in the present study was studied in detail as it exhibits significant morphological and morphometric differences from the only thoroughly described population from Qingdao, China (Song 1990). The Sikkim population is smaller with proportionally larger macronuclear nodules than the Chinese population and shows variation in the number and position of postoral ventral cirri, migratory cirri and cirri in the frontoventral row (Table 4.2). Present population shows noticeable increase in the number of cirri in all these positions.

The 18S rDNA sequence of *Gonostomum strenuum* of present population matches more or less with that of sequence (AJ310493; Bernhard et al. 2001), the only sequence available for the species.

Finally, it can be concluded that *Gonostomum strenuum* Sikkim population shows marked morphological, morphometric and molecular variations from the described populations.

CHAPTER 5

DORSOMARGINALIAN HYPOTRICHA

Hypotrichous ciliates of the family Oxytrichidae which possess dorsomarginal row(s) belong to the group Dorsomarginalia. This chapter includes descriptions of some species from different parts of Sikkim that belong to this group. The content of the chapter has been divided in to three sections, each describing representative species showing one of three character states with respect to dorsal kinety fragmentation (Fig. 3.14B, C, D) during morphogenesis - species with simple fragmentation of dorsal kinety 3, species with multiple fragmentation of dorsal kinety 3, and species with no fragmentation of any dorsal kinety.

Section A

The section includes descriptions of three typically "18 cirri" oxytrichids – *Oxytricha granulifera*, *Cyrtohymena citrina* and *Stylonychia ammermanni* and species of a genus *Sterkiella* with ≤18 cirri found from various collection sites in Sikkim. While *Oxytricha granulifera* and *Cyrtohymena citrina* belong to the sub family Oxytrichinae which are flexible bodied, *Stylonychia ammermanni* and *Sterkiella* spp. belong to the sub family Stylonychinae which are firm bodied and are considered to be evolutionarily advanced as compared to the former since they incorporate lesser number of parental cirri during anlage formation. The Sikkim populations of these species are described along with short notes on their sub families and genera.

Oxytrichids with 18 frontal-ventral-transverse cirri

Subfamily Oxytrichinae

Majority of the oxytrichines are characterized by the following features: body flexible; cortical granules present; adoral zone of membranelles usually ≤ 40% of body length; primarily 18 frontal-ventral-transverse cirri clustered in to 6 distinct groups; postoral ventral cirrus V/3 participates in anlagen formation.

A description of *Oxytricha granulifera* and *Cyrtohymena citrina* from Sikkim follows.

Oxytricha granulifera Foissner and Adam, 1983; Sikkim population (Fig. 5.1A-J; Tables 5.1, 5.2)

Genus *Oxytricha* Bory De Saint-Vincent in Lamouroux, Bory De Saint-Vincent and Deslongchamps, 1824 includes species characterized by the following traits: undulating membranes in *Oxytricha* pattern (Berger and Foissner 1997), adoral zone formed like a question mark, frontoventral cirri in V-shaped pattern, one left and one right marginal row, four dorsal kineties with simple kinety 3 fragmentation during morphogenesis, one or two dorsomarginal row(s), caudal cirri present on dorsal kineties 1, 2, and 4, anlagen V and VI of proter originate from cirrus V/4 and V/3 respectively.

Oxytricha granulifera was found from two sites in Sikkim. Following is a description of the population found from a soil sample in Barsey Rhododendron Sanctuary

Oxytricha granulifera is diagnosed by: body oval, anterior portion usually distinctly narrowed, posterior end usually rounded, right margin generally slightly concave, left always distinctly convex; two macronuclear nodules and often 2 micronuclei; cortical granules present; right marginal row beginning at the level of the right frontal cirrus, terminating behind transverse cirri; anterior pretransverse ventral cirrus distinctly separated from the posterior one; the latter two characters considered autapomorphies of *O. granulifera*.

Table 5.1. Morphometric characterization of *Oxytricha granulifera* Sikkim population[a].

Character	Mean	SD	CV	Min	Max	n
Body length	76.5	8.10	10.58	58.7	88.3	20
Body width	24.1	2.75	11.41	18.7	27.8	20
AZM length	26.2	1.69	6.45	23.7	28.8	20
Adoral membranelles, number	28.5	2.33	8.15	23.0	32.0	20
AZM / body length, %	35.0	0.05	14.55	27	46.0	20
Macronuclear nodules, number	2.0	0.00	0.00	2.0	2.0	20
Macronucleus length	15.9	1.11	7.00	14.5	17.8	20
Macronucleus width	7.1	0.64	9.07	6.0	7.9	20
Micronuclei, number	2.3	0.46	20.19	2.0	3.0	20
Micronucleus diameter	3.6	0.27	7.63	3.1	4.0	20
Frontal cirri, number	8.0	0.00	0.00	8.0	8.0	20

Ventral cirri, number	5.1	0.26	5.10	5.0	6.0	20
Transverse cirri, number	4.9	0.26	5.23	4.0	5.0	20
Right marginal row, number of cirri	31.7	2.63	8.32	25.0	36.0	20
Left marginal row, number of cirri	30.6	2.30	7.49	25.0	34.0	20
Dorsal kineties, number	5.0	0.00	0.00	5.0	5.0	10
No. of bristles in DK1	22.1	1.79	8.11	19.0	25.0	10
No. of bristles in DK2	18.4	1.71	9.31	16.0	21.0	10
No. of bristles in DK3	14.9	1.97	13.22	12.0	18.0	10
No. of bristles in DK4	7.1	0.99	14.01	6.0	9.0	10
No. of bristles in DM1	7.9	1.29	16.29	6.0	10.0	10
Caudal cirri, number	3.0	0.00	0.00	3.0	3.0	10

[a] Data based on protargol-impregnated specimens. All measurements in μm. AZM, adoral zone of membranelles; CV, coefficient of variance (in %); DK, dorsal kinety; DM, dorsomarginal row; Max, maximum; Mean, arithmetic mean; Min, minimum; n, sample size; No., number; SD, standard deviation.

Description: Body is oval, dorso-ventrally flattened, flexible, measures about 80μm × 25μm *in vivo* and 77μm × 24μm when impregnated with protargol. Length:width ratio is 3:1 in protargol impregnated cells. The cells show slow movement. Cortical granules, slightly yellowish in colour, are abundantly present. Contractile vacuole is present slightly above mid-body near the left margin of the cell.

Adoral zone averages 35% of cell length, with about 29 membranelles; the paroral and the endoral are in *Oxytricha* pattern (Berger and Foissner 1997). Frontal ciliature typically consists of 18 frontal-ventral-transverse cirri: anterior three hypertrophied frontal cirri, one buccal cirrus, four frontoventral cirri arranged in V-shaped pattern, three postoral ventral cirri behind the buccal vertex, two pretransverse ventral cirri, anterior pretransverse ventral cirrus distinctly separated from the posterior one, five transverse cirri arranged in tick mark shape. Right marginal row begins at the level of the right frontal cirrus and terminates behind the transverse cirri; left marginal row is J-shaped, surrounding the transverse cirri at the posterior end of the cell.

There are four dorsal kineties with about 19–25, 16–21 12–18 and 6–9 bristles respectively and one dorsomarginal row with about 6–10 bristles; dorsal kinety 4 begins somewhat behind mid-body. Invariably, three caudal cirri are present, one each at the posterior end of dorsal kineties 1, 2 and 4.

Each cell has two ellipsoidal macronuclear nodules with average size 16μm × 7μm and usually spherical micronuclei with average diameter 3.6μm each.

Occurrence and ecology: The cells were isolated from a soil sample collected below a small bamboo shoot in Barsey Rhododendron Sanctuary.

18S rDNA sequence: 18S rDNA sequence of *Oxytricha granulifera* Sikkim population obtained in this study has been submitted in GenBank (http://www.ncbi.nlm.nih.gov) database under accession number JX899421.

Discussion

According to (Berger 1999) *Oxytricha* is the most voluminous taxon within oxytrichids, comprising about 55 valid species and about the same number of species designated as indeterminate because of insufficient descriptions.

Oxytricha is one of the most difficult hypotrich genera and is a melting pot (*inter alia* because no well defined type species is fixed) for flexible 18-cirri hypotrichs with caudal cirri. However, Foissner (1989) proposed *Oxytricha granulifera* as the type species which is still believed to be the standard species representing a typical *Oxytricha* (Shao et al. 2012).

Table 5.2. Morphometric comparison of *Oxytricha granulifera* Sikkim population (Si) with *Oxytricha granulifera* Baumgarten, Austria population (Bau; Foissner and Adam1983).[a]

Character	Population	Mean	SD	CV	MIN	MAX	n
Body length	Si	76.5	8.10	10.58	58.7	88.3	20
	Bau	80.6	8.90	11.10	70.0	103.0	25
Body width	Si	**24.1**	2.75	11.41	18.7	27.8	20
	Bau	**33.6**	4.60	13.80	28.0	43.0	25
AZM length	Si	26.2	1.69	6.45	23.7	28.8	20
	Bau	26.5	1.90	7.20	24.0	32.0	25

Adoral membranelles, number	Si	28.5	2.33	8.15	23.0	32.0	20
	Bau	31.2	1.30	4.10	29.0	32.0	25
AZM / body length, %	Si	35.0	0.05	14.55	27.0	46.0	20
	Bau	32.8	-	-	-	-	25
Macronuclear nodules, number	Si	2.0	0.00	0.00	2.0	2.0	20
	Bau	2.0	0.00	0.00	2.0	2.0	25
Macronucleus length	Si	15.9	1.11	7.00	14.5	17.8	20
	Bau	14.4	-	-	-	-	25
Macronucleus width	Si	7.1	0.64	9.07	6.0	7.9	20
	Bau	7.7	-	-	-	-	25
Micronuclei, number	Si	2.3	0.46	20.19	2.0	3.0	20
	Bau	2.0	0.00	0.00	2.0	2.0	25
Micronucleus diameter	Si	3.6	0.27	7.63	3.1	4.0	20
	Bau	4.0	-	-	-	-	25
Frontal cirri, number	Si	8.0	0.00	0.00	8.0	8.0	20
	Bau	8.0	0.00	0.00	8.0	8.0	25
Ventral cirri, number	Si	5.1	0.26	5.10	5.0	6.0	20
	Bau	5.0	0.00	0.00	5.0	5.0	25
Transverse cirri, number	Si	4.9	0.26	5.23	4.0	5.0	20
	Bau	5.0	0.00	0.00	5.0	5.0	25
Right marginal row, number of cirri	Si	31.7	2.63	8.32	25.0	36.0	20
	Bau	32.1	2.50	7.70	29.0	41.0	25
Left marginal row, number of cirri	Si	30.6	2.30	7.49	25.0	34.0	20
	Bau	34.0	2.60	7.60	30.0	43.0	25
Dorsal kineties, number	Si	5.0	0.00	0.00	5.0	5.0	10
	Bau	5.0	0.00	0.00	5.0	5.0	25
Caudal cirri, number	Si	3.0	0.00	0.00	3.0	3.0	10
	Bau	3.0	0.00	0.00	3.0	3.0	25

[a] Data based on protargol-impregnated specimens. All measurements in µm. AZM, adoral zone of membranelles; CV, coefficient of variance (in %); Max, maximum; Mean, arithmetic mean; Min, minimum; n, sample size; No., number; SD, standard deviation. The highlighted numericals point to significant morphometric differences. '–' depicts data not available.

Sikkim population of *Oxytricha granulifera* was found to be morphologically, morphometrically and morphogenetically similar to the Baumgarten population (Foissner and Adam1983) except for some minor morphometric differences. Molecular analysis shows that 18S rDNA sequence matches with those of *Oxytricha granulifera* sequences available in GenBank.

Cyrtohymena citrina (Berger and Foissner, 1987) Foissner, 1989; Sikkim population (Fig. 5.2 A–J; Tables 5.3, 5.4)

Genus *Cyrtohymena* Foissner, 1989 is a typical oxytrichine with the following distinctive traits: a distinctive hook-shaped paroral, deep, transparent buccal cavity, body more or less flexible.

A description of Sikkim population of *Cyrtohymena citrina* is given below highlighting marked differences from the described species in morphogenesis for proter, an important trait used to characterize the genus *Cyrtohymena.*

Cyrtohymena citrina is diagnosed by: body oblong; citrine coloured cortical granules; transverse cirri in a curved row, located near posterior end, not displaced markedly anteriad; two macronuclear nodules; 2–4 micronuclei.

Table 5.3. Morphometric characterization of *Cyrtohymena citrina* Sikkim population[a].

Character	Mean	SD	CV	Min	Max	n
Body length	104.9	11.33	10.81	85.5	128.8	25
Body width	30.4	6.67	21.96	21.8	44.5	25
Adoral membranelles, number	38.8	2.40	6.19	35.0	43.0	25
AZM length	38.2	5.15	13.48	29.4	48.3	25
AZM / body length, %	37	0.04	10.99	30	46	25
Macronuclear nodules, number	2.0	0.00	0.00	2.0	2.0	25
Macronucleus length	14.0	2.15	15.34	11.6	18.2	20
Macronucleus width	7.9	2.04	25.87	5.1	12.0	20
Micronuclei, number	2.8	0.75	26.68	2.0	4.0	25
Micronucleus length	3.8	0.82	21.22	2.2	4.9	20
Micronucleus width	3.2	0.74	22.84	2.1	4.7	20
Frontoventral cirri, number	8.0	0.22	2.71	8.0	9.0	25
Ventral cirri, number	5.1	0.30	5.90	5.0	6.0	25

Transverse cirri, number	5.1	0.30	5.90	5.0	6.0	25
Right marginal row, number of cirri	24.0	1.99	8.30	20.0	27.0	25
Left marginal row, number of cirri	25.2	1.54	6.10	23.0	29.0	25
Dorsal kineties, number	5.8	0.39	6.67	5.0	6.0	12
No. of bristles in DK_1	26.3	2.35	8.92	23.0	30.0	12
No. of bristles in DK_2	21.3	4.31	20.28	10.0	27.0	12
No. of bristles in DK_3	19.4	3.03	15.60	15.0	26.0	12
No. of bristles in DK_4	16.1	4.17	25.90	9.0	23.0	12
No. of bristles in DM_1	18.1	3.58	19.79	10.0	23.0	12
No. of bristles in DM_2	9.0	1.71	18.95	6.0	11.0	12
Caudal cirri, number	3.0	0.00	0.00	3.0	3.0	20

[a]Data based on protargol-impregnated specimens. All measurements in µm. AZM, adoral zone of membranelles; CV, coefficient of variance (in %); DK, dorsal kinety; Max, maximum; Mean, arithmetic mean; Min, minimum; n, sample size; No., number; SD, standard deviation.

Description: Body is oblong, dorso-ventrally flattened, flexible, about 108µm × 32µm *in vivo* and 105µm × 30µm on average when impregnated with protargol. Length:width ratio is 3.5:1. The cells show slow movement. Brilliant citrine cortical granules are abundantly present, scattered singly or in small clusters which make cells appear slightly yellowish green at low magnification. Contractile vacuole is present slightly above mid-body near the left margin of the cell.

Adoral zone is about 37% of cell length, bordering the deep and wide buccal cavity on the left, with about 40 membranelles, paroral and endoral in *Cyrtohymena* pattern (Berger and Foissner 1997). Frontal ciliature typically consists of 18 frontal-ventral-transverse cirri: anterior three frontal cirri hypertrophied, one buccal cirrus, four frontoventral cirri arranged in V-shaped pattern, three postoral ventral cirri behind the buccal vertex, two pretransverse ventral cirri, five transverse cirri arranged in a curved row located near the posterior end not displaced markedly anteriad. Right marginal row is almost straight, finishes posteriorly just at the level of transverse cirri; left marginal row is J-shaped, goes up to posterior end surrounding the transverse cirri.

There are four dorsal kineties with about 23–30, 10–27 15–26 and 9–23 bristles respectively and usually two dorsomarginals with about 10–23 and 6–11 bristles; dorsal kinety 4 is

shortened and begins at mid-body. Invariably three caudal cirri are present, one each at the posterior end of dorsal kineties 1, 2 and 4.

Each cell has two ellipsoidal macronuclear nodules with average nodule size 14µm × 8µm and 2–4 spherical micronuclei 3.8µm × 3.2µm each.

Divisional morphogenesis: Morphogenesis is similar to the described population (Berger and Foissner 1987) except it showed striking difference with its congeners regarding origin of anlagen V and VI for protor, a character importantly used as an autapomorphy for the genus.

18S rDNA sequence: 18S rDNA sequence obtained in this study has been submitted in GenBank database under the accession number KC182574. 18S rDNA sequence matches with those of *Cyrtohymena citrina* sequences available on GenBank

Occurrence and ecology: Soil sample containing cells and cysts of *Cyrtohymena citrina* was collected from an area inhabited by small bamboos and rhododendron trees in the Barsey Rhododendron Sanctuary (27° 15' to 27° 27' N and 88° 01' to 88° 23' E).

Discussion

Cyrtohymena citrina Sikkim population by and large matches (Table 5.4) with already described Greece population (Berger and Foissner 1987). However, the present study has clarified a very important point in ventral morphogenesis with respect to formation of anlagen V and VI for proter. The origin of anlagen V and VI for proter has thus far been considered *de novo*, an autapomorphy of the genus *Cyrtohymena*. But the present study reports their origin from cirri V/4 and V/3 of opisthe. Consequently, the study on Sikkim population of *Cyrtohymena citrina* necessitates either review of the already described populations or revision of the morphogenesis of the genus *Cyrtohymena*.

Table 5.4. Morphometric comparison of *Cyrtohymena citrina* Sikkim population (In) with *Cyrtohymena citrina* Peloponnesus, Greece population (Pe; Berger and Foissner 1987).[a]

Character	Population	Mean	SD	CV	Min	Max	n
Body length	Si	104.9	11.33	10.81	85.5	128.8	25
	Pe	99.0	11.30	11.30	75.0	112.0	11
Body width	Si	30.4	6.67	21.96	21.8	44.5	25
	Pe	33.5	4.80	14.20	27.0	41.0	11
Adoral membranelles, number	Si	**38.8**	2.40	6.19	35.0	43.0	25

	Pe	**33.6**	2.00	6.00	31.0	37.0	11
AZM length	Si	38.2	5.15	13.48	29.4	48.3	25
	Pe	35.0	2.70	7.80	31.0	39.0	11
AZM / body length, %	Si	37.0	0.04	10.99	30.0	46.0	25
	Pe	35.0	-	-	-	-	11
Macronuclear nodules, number	Si	2.0	0.00	0.00	2.0	2.0	25
	Pe	2.0	0.00	0.00	2.0	2.0	11
Macronucleus length	Si	14.0	2.15	15.34	11.6	18.2	20
	Pe	15.0	3.20	21.30	11.0	21.0	11
Macronucleus width	Si	7.9	2.04	25.87	5.1	12.0	20
	Pe	7.8	1.20	14.90	7.0	10.0	11
Micronuclei, number	Si	2.8	0.75	26.68	2.0	4.0	25
	Pe	2.2	0.80	34.40	1.0	4.0	11
Micronucleus length	Si	3.8	0.82	21.22	2.2	4.9	20
	Pe	3.5	0.70	19.20	2.0	4.0	11
Micronucleus width	Si	3.2	0.74	22.84	2.1	4.7	20
	Pe	2.7	0.40	16.50	2.0	3.0	11
Frontoventral cirri, number	Si	8.0	0.22	2.71	8.0	9.0	25
	Pe	8.0	0.00	0.00	8.0	8.0	11
Ventral cirri, number	Si	5.1	0.30	5.90	5.0	6.0	25
	Pe	5.0	0.00	0.00	5.0	5.0	11
Transverse cirri, number	Si	5.1	0.30	5.90	5.0	6.0	25
	Pe	4.9	0.50	11.00	4.0	6.0	11
Right marginal row, number of cirri	Si	**24.0**	1.99	8.30	20.0	27.0	25
	Pe	**21.1**	2.20	10.30	18.0	25.0	11
Left marginal row, number of cirri	Si	**25.2**	1.54	6.10	23.0	29.0	25
	Pe	**21.5**	2.10	9.60	17.0	24.0	11
Dorsal kineties, number	Si	5.8	0.39	6.67	5.0	6.0	12
	Pe	5.8	0.40	6.90	5.0	6.0	11
Caudal cirri, number	Si	3.0	0.00	0.00	3.0	3.0	20
	Pe	3.0	0.00	0.00	3.0	3.0	11

[a] Data based on protargol-impregnated specimens. All measurements in μm. AZM, adoral zone of membranelles; CV, coefficient of variance (in %); Max, maximum; Mean, arithmetic

mean; Min, minimum; n, sample size; No., number; SD, standard deviation. The highlighted numericals point to significant morphometric differences. '–' depicts data not available.

Subfamily Stylonychinae

A majority of members of the sub family Stylonychinae are characterized by the following features: body rigid; cortical granules lacking; adoral zone of membranelles usually > 40% of body length; primarily 18 frontal-ventral-transverse (FVT) cirri clustered in to 6 distinct groups; postoral ventral cirrus V/3 positioned away from other two postoral ventral cirri and not involved in anlagen formation.

There are few stylonychines with slightly to distinctly increased or slightly decreased number of FVT cirri than the regular eighteen and show simple/multiple fragmentation of dorsal kinety 3. Few of them have been described in next chapters.

A detailed description of *Stylonychia ammermanni* Sikkim population is given below with an introductory description of the genus because of its marked differences from another Indian population (Gupta et al. 2001) with respect to morphometry and dorsal infraciliature; characters decisively used to distinguish different species of the genus *Stylonychia.*

Stylonychia ammermanni Gupta et al., 2001; Sikkim population (Figs 5.3; Tables 5.5, 5.6, 5.7)

Genus *Stylonychia* Ehrenberg, 1830 is characterized by the following features: paroral and endoral in *Stylonychia* pattern (Berger and Foissner 1997); one right and 1 left row of marginal cirri, noticeably separate posteriorly; usually four dorsal kineties and two dorsomarginal rows; caudal cirri often distinctly elongated; cortical granules lacking; anlagen V and VI of proter originate either from frontoventral cirrus IV/3 or from postoral ventral cirrus V/4; anlage IV of opisthe originates from postoral ventral cirrus V/4; dorsal kinety 3 undergoes simple fragmentation.

Stylonychia is largely confined to freshwater. Nearly half of a dozen species have been described thoroughly. *Stylonychia mytilus and S. lemnae* are two well described species of the genus (Wirnsberger et al. 1986). *Stylonychia ammermanni* was for the first time described

from Delhi region of Yamuna River by Gupta et al. in 2001 and belongs to the *S. mytilus-lemnae* complex.

Stylonychia ammermanni is diagnosed by the following traits: body markedly broader anteriorly than posteriorly, tapering backwards from centre, posterior end is usually truncated sometimes broadly rounded, left margin is generally concave, right is slightly to distinctly convex; caudal cirri widely separated; cortical granules lacking. Anlagen V and VI of proter originate from frontoventral cirrus IV/3.

A description of Sikkim population of *Stylonychia ammermanni* is given below.

Table 5.5. Morphometric characterization of *Stylonychia ammermanni* Sikkim population[a].

Character	Mean	SD	CV	MIN	MAX	n
Body length	119.6	7.53	6.29	108.0	138.0	25
Body width	50.7	8.11	16.02	36.0	62.0	25
AZM length	57.9	3.87	6.69	50.0	67.0	25
Adoral membranelles, number	42.0	3.69	8.80	33.0	47.0	25
AZM / body length, %	50	0.03	5.40	44	54	25
Macronuclear nodules, number	2.0	0.00	0.00	2.0	2.0	25
Macronucleus length	20.3	3.18	15.69	14.9	25.2	25
Macronucleus width	9.3	1.91	20.44	6.6	13.0	25
Micronuclei, number	3.1	0.55	17.82	2.0	4.0	20
Micronucleus diameter	3.7	0.41	11.06	3.0	4.3	20
Frontal cirri, number	8.1	0.22	2.78	8.0	9.0	25
Ventral cirri, number	5.1	0.22	4.43	5.0	6.0	25
Transverse cirri, number	4.9	0.49	10.09	3.0	5.0	25
Right marginal row, number of cirri	23.3	2.07	8.92	18.0	26.0	20
Left marginal row, number of cirri	16.0	2.18	13.60	13.0	20.0	20
Dorsal kineties, number	6.0	0.00	0.00	6.0	6.0	10
No. of bristles in DK1	32.6	2.64	8.11	28.0	36.0	10
No. of bristles in DK2	30.7	2.91	9.48	24.0	34.0	10
No. of bristles in DK3	21.3	2.77	13.03	17.0	28.0	10
No. of bristles in DK4	9.8	0.97	9.91	8.0	12.0	10

No. of bristles in DM1	21.3	1.89	8.89	17.0	25.0	10
No. of bristles in DM2	11.9	1.68	14.14	9.0	15.0	10
Caudal cirri, number	3.0	0.00	0.00	3.0	3.0	15

[a] Data based on protargol-impregnated specimens. All measurements in µm. AZM, adoral zone of membranelles; CV, coefficient of variance (in %); DK, dorsal kinety; Max, maximum; Mean, arithmetic mean; Min, minimum; n, sample size; No., number; SD, standard deviation.

Description: Body is firm, dorsoventrally flattened, about 124 × 52 µm *in vivo* and 120 × 51 µm on average when impregnated with protargol. Length:width ratio is 2.5:1. Body margins are slightly to distinctly converging posteriorly, left margin is usually concave, right margin is slightly to distinctly convex. Posterior end is truncated, sometimes is broadly rounded. Posterior portion of the cell usually appears translucent. The cells show rapid movement. Cortical granules are absent. Contractile vacuole is present slightly above mid-body near the left margin of the cell. Marginal rows are distinctly separate posteriorly.

Adoral zone is about 50% of cell length, consisting of about 42 membranelles; the paroral and endoral are in *Stylonychia* pattern (Berger and Foissner 1997). *Stylonychia ammermanni* has a typically large bowl shaped buccal area with a striking bulge in the region of the peristome. Frontal ciliature typically consists of 18 frontal-ventral-transverse cirri: anterior three hypertrophied frontal cirri, one buccal cirrus, four frontoventral cirri arranged characteristically, three postoral ventral cirri, two near the posterior end of the AZM arranged in a manner where V/4 is in front of IV/2, the third displaced posteriorly, two pretransverse ventral cirri, five markedly hypertrophied transverse cirri invariably in two groups of three and two.

There are four dorsal kineties and usually two dorsomarginals; dorsal kinety 4 is shortened and begins at mid-body (feature seen in the present population only, see discussion). Invariably, three caudal cirri are present, one each at the posterior end of dorsal kineties 1, 2 and 4.

Each cell has two ellipsoidal macronuclear nodules with average size 20µm x 9µm and 2-4 spherical micronuclei with average diameter 3.7µm each.

Occurrence and ecology: Sample containing cells of *Stylonychia ammermanni* was collected from soil water interface of a permanent water catchment area in the Barsey Rhododendron Sanctuary. The pool receives water from precipitation (rain and snow) and the water remains clean and clear despite the surrounding dense sub alpine foliage which consists of a vast array of vegetation the dominant of them included species of Hemlock, Junipers, Reed, Oak, Rhododendron and bamboo grass which are a constant source of organic material to the water.

18S rDNA sequence: 18S rDNA sequence of *Stylonychia ammermanni* Sikkim population obtained in this study has been submitted in GenBank database under accession number JX885703.

Discussion

Morphology: *Stylonychia ammermanni* Sikkim population shows following variations from Delhi population (Tables 5.6 and 5.7):

1) Sikkim population is smaller than Delhi population.
2) Ventral infraciliature shows some differences.
3) Dorsal infraciliature shows differences with respect to third and fourth dorsal kinety and number of bristles in each row.

Divisional morphogenesis: The generation time of Sikkim population is longer than that of the Delhi population (Table 5.6). The overall divisional morphogenesis is similar to that of *Stylonychia ammermanni* (Gupta et al. 2001) and *S. mytilus-lemnae* complex (Wirnsberger et al. 1986).

Table 5.6. Comparison of *Stylonychia ammermanni* Sikkim population with *Stylonychia ammermanni* Delhi population (Gupta et al. 2001).

Character	*Stylonychia ammermanni* (Sikkim population)	*Stylonychia ammermanni* (Delhi population)
Body appearance	No bulge in peristomial region	No bulge in peristomial region
Posterior frontoventral cirri	Arranged in J shaped manner	Arranged in two pairs, posterior pair close to cytostome
Left marginal cirri	Anterior 2-3 cirri, curved inward	Straight?
Dorsal kinety 3	Reaches up to posterior end of the cell	Finishes short of posterior end of the cell
Dorsal kinety 4	Total length is in the posterior 1/3rd of the cell	Total length is in the posterior 3/4th of the cell
Duration of cell cycle	13-15 hrs	9 hrs

Table 5.7. Morphometric comparison of *Stylonychia ammermanni* Sikkim population (Si) with *Stylonychia ammermanni* Delhi population (De; Gupta et al. 2001)[a].

Character	Population	Mean	SD	CV	MIN	MAX	n
Body length	Si	**119.6**	7.53	6.29	108.0	138.0	25.0
	De	**134.0**	5.00	3.73	125.7	146.5	25.0
Body width	Si	50.7	8.11	16.02	36.0	62.0	25.0
	De	52.1	3.01	5.78	47.3	57.8	25.0
AZM length	Si	**57.9**	3.87	6.69	50.0	67.0	25.0
	De	**70.8**	3.95	5.58	65.1	80.5	25.0
Adoral membranelles, number	Si	**42.0**	3.69	8.80	33.0	47.0	25.0
	De	51.4	2.24	4.35	46.0	55.0	30.0
AZM / body length, %	Si	50	0.03	5.40	44	54	25.0
	De	53	0.03	5.05	48	61	25.0
Macronuclear nodules, number	Si	2.0	0.00	0.00	2.0	2.0	25.0
	De	2.0	0.00	0.00	2.0	2.0	25.0
Macronucleus length	Si	20.3	3.18	15.69	14.9	25.2	20.0
	De	25.3	0.61	2.41	24.3	26.1	12.0
Macronucleus width	Si	9.3	1.91	20.44	6.6	13.0	20.0
	De	10.3	0.53	5.72	10.0	10.7	12.0

Micronuclei, number	Si	**3.1**	0.55	17.82	2.0	4.0	20.0
	De	**2.0**	-	-	2.0	4.0	25.0
Micronucleus diameter	Si	**3.7**	0.41	11.06	3.0	4.3	20.0
	De	**2.6**	0.20	7.91	2.5	3.0	12.0
Frontal cirri, number	Si	8.1	0.22	2.78	8.0	9.0	25.0
	De	8.0	0.00	0.00	8.0	8.0	25.0
Ventral cirri, number	Si	5.1	0.22	4.43	5.0	6.0	25.0
	De	5.0	0.00	0.00	5.0	5.0	25.0
Transverse cirri, number	Si	5.0	0.49	10.09	3.0	6.0	25.0
	De	5.0	0.00	0.00	5.0	5.0	25.0
Right marginal row, number of cirri	Si	23.3	2.07	8.92	18.0	26.0	20.0
	De	24.2	1.45	5.98	22.0	27.0	30.0
Left marginal row, number of cirri	Si	16.0	2.18	13.60	13.0	20.0	20.0
	De	17.2	1.12	6.51	13.0	18.0	30.0
Dorsal kineties, number	Si	6.0	0.00	0.00	6.0	6.0	10.0
	De	6.0	0.00	0.00	6.0	6.0	12.0
No. of bristles in DK1	Si	32.6	2.64	8.11	28.0	36.0	10.0
	De	52.7	4.19	7.95	44.0	58.0	12.0
No. of bristles in DK2	Si	30.7	2.91	9.48	24.0	34.0	10.0
	De	40.6	3.20	7.89	36.0	45.0	12.0
No. of bristles in DK3	Si	21.3	2.77	13.03	17.0	28.0	10.0
	De	28.8	2.66	9.22	24.0	33.0	12.0
No. of bristles in DK4	Si	9.8	0.97	9.91	8.0	12.0	10.0
	De	20.8	2.60	9.22	19.0	25.0	12.0
No. of bristles in DM1	Si	21.3	1.89	8.89	17.0	25.0	10.0
	De	29.3	2.10	7.17	26.0	32.0	12.0
No. of bristles in DM2	Si	11.9	1.68	14.14	9.0	15.0	10.0
	De	17.0	1.86	10.93	15.0	20.0	12.0
Caudal cirri, number	Si	3.0	0.00	0.00	3.0	3.0	15.0
	De	3.0	0.00	0.00	3.0	3.0	25.0

[a] Data based on protargol-impregnated specimens. All measurements in µm. AZM, adoral zone of membranelles; CV, coefficient of variance (in %); DK, dorsal kinety; DM, dorsomarginal row; Max, maximum; Mean, arithmetic mean; Min, minimum; n, sample size; No., number; SD, standard deviation. The highlighted numericals point to significant morphometric differences. The sign – depicts data not available.

Molecular phylogeny: 18S rDNA sequence matches exactly to the only sequence available of another population of *Stylonychia ammermanni* from Germany. This shows, there is hardly any difference between two populations (Sikkim and Germany) as for 18S rDNA sequences are concerned, even though, the differences given in the Table 5.6 are considered important to separate such populations of genus *Stylonychia* at species level. *Stylonychia ammermanni* also matches close to *S. mytilus and S. lemnae* as expected because of their overall similarity in the morphology.

Oxytrichids with ≤ 18 number of frontal-ventral-transverse cirri

There are a few oxytrichid genera which include species with <18 number of frontal-ventral-transverse cirri; the reduction is usually due to reduced number of transverse cirri. Genus *Sterkiella* has some species with such a reduction; additionally it depicts simple fragmentation of dorsal kinety 3.

Sterkiella spp. were found from the maximum number of sites explored in Sikkim and therefore surfaced as a dominant taxon among the hypotrichous ciliates in this region of Eastern Himalayas. *Sterkiella* was found from nineteen (Fig. 5.4) collection sites; fourteen out of them could be cultured in the laboratory and examined in detail. *Sterkiella histriomuscorum* was the most dominant species and was found from ten places followed by *S. cavicola* which was found from six and *S. tricirrata* from three sites. All fourteen distinctive populations belonging to three different species *S. tricirrata*, *S. histriomuscorum* and *S. cavicola* have been characterized by classical and molecular methodologies. Significantly, they differ in the number and arrangement of transverse cirri.

Following is the comparative account of these 14 populations with a detailed description of *Sterkiella tricirrata* beginning with an introductory note on the genus.

Sterkiella Foissner, Blatterer, Berger and Kohmann, 1991; Sikkim population (Figs. 5.4–5.13; Tables 5.8–5.12)

Sterkiella belongs to sub-family Stylonychinae. The genus can be characterised by the following autapomorphies: body slightly flexible contrary to other firm bodied stylonychines; paroral and endoral intersect virtually in the *Oxytricha* pattern (Berger and Foissner 1997); the postoral ventral cirrus V/3 distinctly displaced posteriorly; one right and one left row of marginal cirri, distinctly separate posteriorly; six dorsal kineties; caudal cirri present.

Table 5.8. Various populations of *Sterkiella* from Sikkim with name and type of collection site (soil; FW: fresh water), and number and arrangement of transverse cirri (TC).

Population	Collection Site	Species Name (number and arrangement of transverse cirri in parenthesis)
A	Barsey Rhododendron Sanctuary (soil)	*Sterkiella histriomuscorum* (4-5TC, tick mark shape)
B	Khecheopalri lake (FW)	*Sterkiella histriomuscorum* (5TC, tick mark shape)
C	Changu Lake (FW)	*Sterkiella histriomuscorum* (4TC, in a row)
D	Changu Lake (FW)	*Sterkiella histriomuscorum* (5TC, tick mark shape)
E	Barsey Rhododendron Sanctuary (FW)	*Sterkiella histriomuscorum* (5TC, tick mark shape)
F	Barsey Rhododendron Sanctuary (FW)	*Sterkiella histriomuscorum* (5TC, tick mark shape)
G	Barsey Rhododendron Sanctuary (FW)	*Sterkiella cavicola* (5TC, tick mark shape)
H	Barsey Rhododendron Sanctuary (soil)	*Sterkiella cavicola* (5TC, tick mark shape)
I	Kyongnosla Alpine sanctuary (soil)	*Sterkiella cavicola* (4TC, in a row)
J	Barsey Rhododendron Sanctuary (soil)	*Sterkiella cavicola* (4TC, in a row)
K	Kyongnosla Alpine Sanctuary (soil)	*Sterkiella cavicola* (4TC, in a row)
L	Borang/Polok Hotspring (FW)	*Sterkiella cavicola* (4TC, tick mark shape)
M	Changu Lake (FW)	*Sterkiella tricirrata* (3TC, in a row)
N	Kyongnosla Alpine Sanctuary (soil)	*Sterkiella tricirrata* (3TC, in a row)

Sterkiella histriomuscorum Foissner, Blatterer, Berger and Kohmann, 1991; Sikkim populations (Figs 5.5–5.8 and Table 5.9):

Sterkiella histriomuscorum is diagnosed by the following traits: two macronuclear nodules; primarily 5 transverse cirri (sometimes 4); four dorsal kineties and two dorsomarginal rows.

Ten populations of *Sterkiella histriomuscorum* were found from various collection sites in Sikkim. Out of these, six populations could be cultured and studied in detail. Among these populations considerable variation with respect to morphometry and importantly the number and placement of transverse cirri was noticed. Population A possessed 4 or 5 (Table 5.9) transverse cirri arranged in a tick mark shape and population C invariably had 4 transverse cirri arranged in a row; in the rest of the populations, the transverse cirri were typically 5 in number and were arranged in a tick mark shape. The average length of protargol-impregnated cells ranged from 62–122 μm and average width from 28–57 μm. The range is moreover similar to the described populations from other regions, however, such wide range in the size of cells and variation in number and arrangement of transverse cirri belonging to the same species obtained in a small geographical area is worth a mention, indicating diversity in ecozones in a small area.

Sterkiella tricirrata Buitkamp 1977; Sikkim populations (Fig. 5.9 and Table 5.10):

Sterkiella tricirrata is diagnosed by the following traits: two macronuclear nodules, primarily 3 transverse cirri (4 in some cells), four dorsal kineties and two dorsomarginal rows.

Data on *Sterkiella tricirrata* is very scarce; very less information is available about the only description available (Buitkamp 1977). In Sikkim, three populations of *Sterkiella tricirrata* were found from various collection sites. Out of these, two populations could be cultured and studied in detail. Molecular data of only one population (N) is available; population N is described below:

Description: Body is oval, slightly flexible with both anterior and posterior end rounded. Mean body size *in vivo* is 69μm × 31μm and 68μm × 30μm when impregnated with protargol. The length:width ratio is 2:1. Cortical granules are present. Contractile vacuole is present nearly in mid-body.

AZM averages 43% of body length, with about 29 membranelles; paroral and endoral are typically in *Oxytricha* pattern (Berger and Foissner 1997). Frontal ciliature consists of <18 frontal-ventral-transverse cirri: anterior three slightly hypertrophied frontal cirri, one buccal cirrus, four frontoventral cirri arranged in V-shaped pattern, three postoral ventral cirri positioned behind the buccal vertex, V/3 cirrus distinctly posteriorly displaced, two pretransverse ventral cirri, three transverse cirri arranged in a row. Right marginal row terminates behind the transverse cirri, left marginal row terminates slightly ahead of posterior most transverse cirrus.

There are four dorsal kineties with about 14–18, 13–18, 9–14 and 8–14 bristles respectively and two dorsomarginal rows with about 7–11 and 4–5 bristles respectively. There are invariably three caudal cirri, one each at the posterior end of dorsal kineties 1, 2 and 4.

Each cell has two ellipsoidal macronuclear nodules with average nodular size 10μm × 7μm and usually spherical micronuclei with average diameter 2 μm each.

Divisional morphogenesis: Morphogenesis of Sikkim population of *Sterkiella tricirrata* is similar to that of other species of *Sterkiella*.

Molecular phylogeny: 18S rDNA sequence of *Sterkiella tricirrata* Sikkim population determined in this study has been submitted in GenBank database under accession number JX946275.

Occurrence and ecology: Soil sample collected from under a rhododendron tree in Kyongnosla Alpine Sanctuary (27°22' to 33"N; 88°44' to 13"E), Sikkim.

The cells of two populations of *Sterkiella tricirrata* (M and N) are comparatively smaller in size than those of *S. cavicola* and from a majority of populations of *S. histriomuscorum.* Average length of protargol-impregnated cells ranged from 63–68 μm and average width from 28–30 μm. Average cell length of Sikkim populations is similar to the earlier described population, but there are marked differences in the body shape, number of cirri in the marginal rows and number of membranelles in the adoral zone (the only information available of the earlier described population). The Ivory Coast population (Berger 1999, Buitkamp 1977) is longer and less wider than the Sikkim populations. Further differences between Ivory Coast population and Sikkim populations with respect to morphology are: the

number of right marginal cirri ranged 16–21 versus 11–13, left marginal cirri ranged 14–18 versus 10–13 and number of membranelles in adoral zone ranged 26–33 versus 23–25. The Ivory Coast population was presumed to be belonging to *Sterkiella* because of distinctly posteriorly displaced postoral ventral cirrus V/3, but in the Sikkim population its placement in *Sterkiella* can be confirmed from the dividers (Fig. 5.9) that cirrus V/3 is not used in morphogenesis and is therefore posteriorly displaced.

Sterkiella cavicola (Kahl 1935) Foissner, Blatterer, Berger and Kohmann, 1991; Sikkim populations (Figs 5.10, 5.11 and Table 5.11):

Sterkiella cavicola is diagnosed by the following traits: four macronuclear nodules; primarily 5 transverse cirri (sometimes 4); four dorsal kineties and two dorsomarginal rows.

Cells of six distinctive populations of *Sterkiella cavicola* were obtained from different collection sites in Sikkim. Out of these, six populations could be cultured and studied in detail. Cells of *S. cavicola* are larger in size in comparison to a majority of *S. histriomuscorum* populations of the region. Supernumerary cirri in the postoral region were present in a few cells, especially those populations which had 4 transverse cirri, indicating their affinity to *Gastrostyla steinii*, a member of the oxytrichid *sensu lato* group (Berger and Foissner 1997). Average length of protargol impregnated cells ranged from 84–126 µm and average width from 32–55 µm. All the populations of *S. cavicola* were slightly smaller in size than the earlier described populations; one population L was significantly smaller. Note that population L was collected from the point source of Borang Hot Spring which was very rich in sulphur.

The following discussion is based on observations made on all fourteen populations of *Sterkiella.*

Discussion

Morphological analysis

Species of *Sterkiella* show variation in number of nuclei and number and placement of transverse cirri. In the present study too, various populations of *Sterkiella* spp. were found to be showing noticeable variation with respect to these two characters.

Table 5.9. Morphometric characterization of six populations of *Sterkiella histriomuscorum* reported from Sikkim[a]

Character	Population	Mean	SD	CV	Min	Max
Body length	A	**61.6**	5.13	8.32	54.4	70.1
	B	113.6	7.01	6.18	102	121.8
	C	87.0	8.37	9.63	73.8	100.1
	D	**121.6**	15.13	12.44	99.8	143.0
	E	71.1	7.90	11.11	60.3	84.7
	F	105.8	7.20	6.80	91.0	120.0
Body width	A	**27.7**	2.14	7.70	24.1	32.1
	B	47.3	4.36	9.23	37.7	53.5
	C	31.7	3.86	12.17	27.3	38.2
	D	**56.8**	8.65	15.22	45.5	70.8
	E	30.6	2.83	9.27	27.4	36.7
	F	45.7	5.14	11.27	33.0	55.0
AZM length	A	**28.3**	1.21	4.26	26.6	30.7
	B	48.1	3.38	7.02	41.6	54.1
	C	36.0	4.30	11.95	30.8	41.6
	D	**49.2**	8.38	17.20	35.1	63.4
	E	30.2	2.26	7.50	27.0	35.1
	F	45.8	2.89	6.32	40.0	50.0
Adoral membranelles, number	A	**28.6**	2.21	7.75	25.0	32.0
	B	**44.4**	3.44	7.74	39.0	50.0
	C	32.6	2.99	9.17	30.0	38.0
	D	43.0	5.94	13.82	32.0	50.0
	E	28.2	2.70	9.57	25.0	33.0
	F	37.9	2.86	7.56	34.0	44.0
AZM / body length, %	A	**46**	0.03	6.63	41	54
	B	42	0.02	5.40	38	45
	C	41	0.03	7.15	35	45
	D	**40**	0.04	10.42	34	46
	E	43	0.04	10.00	35	50
	F	45	0.04	7.86	35	50
Macronuclear nodules, number	A	2.1	0.22	10.91	2.0	3.0
	B	2.1	0.32	15.06	2.0	3.0
	C	**2.7**	0.98	36.60	2.0	4.0
	D	2.0	0.00	0.00	2.0	2.0
	E	2.0	0.00	0.00	2.0	2.0
	F	2.0	0.00	0.00	2.0	2.0
Macronucleus length	A	**11.1**	1.09	9.79	9.7	13.8
	B	21.4	1.28	5.97	19.4	23.4
	C	14.9	2.78	18.58	11.4	19.4
	D	**22.9**	2.47	10.82	17.5	26.5
	E	12.2	1.03	8.47	10.8	14.4
	F	19.2	1.29	6.72	16.4	21.1
Macronucleus width	A	**5.7**	0.45	7.96	5.1	6.8
	B	7.8	1.51	19.37	5.6	9.9
	C	8.5	0.82	9.65	7.2	9.7
	D	**14.4**	2.14	14.86	12.4	18.2
	E	6.1	0.71	11.57	5.2	7.1
	F	7.6	0.49	6.37	6.5	8.2
Micronucleus diameter	A	**1.8**	0.17	9.37	1.6	2.2
	B	3.1	0.25	7.94	2.8	3.6
	C	2.5	0.27	11.05	2.0	2.8
	D	**3.8**	0.21	5.67	3.4	4.1
	E	3.3	0.23	6.94	3	3.8
	F	2.8	0.32	11.11	2.0	3.4

Frontal cirri, number	A	8.0	0.22	2.81	7.0	8.0		F	21.2	1.53	7.24	19.0	26.0
	B	8.0	0.00	0.00	8.0	8.0	Dorsal kineties, number	A	6.0	0.00	0.00	6.0	6.0
	C	8.0	0.00	0.00	8.0	8.0		B	6.0	0.00	0.00	6.0	6.0
	D	8.0	0.00	0.00	8.0	8.0		C	6.0	0.00	0.00	6.0	6.0
	E	8.0	0.00	0.00	8.0	8.0		D	6.0	0.00	0.00	6.0	6.0
	F	8.0	0.00	0.00	8.0	8.0		E	6.1	0.32	5.18	6.0	7.0
Ventral cirri, number	A	**5.7**	0.99	17.49	5.0	8.0		F	5.9	0.32	5.36	5.0	6.0
	B	5.0	0.00	0.00	5.0	5.0	No. of bristles in DK1	A	**18.8**	1.82	9.70	15.0	22.0
	C	5.0	0.00	0.00	5.0	5.0		B	33.7	2.98	8.85	29.0	38.0
	D	5.0	0.00	0.00	5.0	5.0		C	19.3	1.70	8.82	17.0	22.0
	E	5.1	0.32	6.20	5.0	6.0		D	**43.3**	5.77	13.34	33.0	52.0
	F	4.8	0.50	10.36	3.0	5.0		E	23.5	2.17	9.23	21.0	27.0
Transverse cirri, number	A	**4.5**	0.83	18.55	4.0	7.0		F	29.9	2.85	9.53	25.0	34.0
	B	5.0	0.00	0.00	5.0	5.0	No. of bristles in DK2	A	**17.3**	1.56	9.01	15.0	21.0
	C	**4.0**	**0.00**	**0.00**	**4.0**	**4.0**		B	29.9	3.75	12.56	25.0	35.0
	D	4.9	0.32	6.45	4.0	5.0		C	21.0	1.05	5.02	19.0	22.0
	E	5.2	0.42	8.11	5.0	6.0		D	**34.9**	3.60	10.33	29.0	42.0
	F	5.4	1.10	20.29	5.0	9.0		E	20.7	2.07	9.99	19.0	24.0
Right marginal row, number of cirri	A	**19.0**	1.81	9.51	15.0	22.0		F	19.9	1.54	7.73	18.0	22.0
	B	23.9	1.37	5.73	22.0	26.0	No. of bristles in DK3	A	**13.8**	1.85	13.42	11.0	18.0
	C	23.3	2.11	9.06	20.0	26.0		B	22.7	1.95	8.57	20.0	27.0
	D	**26.0**	2.98	11.47	21.0	29.0		C	18.3	2.11	11.53	15.0	22.0
	E	23.5	1.96	8.33	21.0	27.0		D	**25.5**	4.38	17.17	19.0	30.0
	F	24.6	2.56	10.45	20.0	28.0		E	17.5	1.87	10.69	15.0	20.0
Left marginal row, number of cirri	A	**14.8**	1.41	9.56	13.0	18.0		F	16.8	2.05	12.21	14.0	20.0
	B	19.9	1.37	6.89	18.0	23.0	No. of bristles in DK4	A	**13.1**	1.61	12.30	10.0	16.0
	C	20.3	1.16	5.71	19.0	22.0		B	25.2	2.53	10.04	20.0	29.0
	D	**22.8**	2.70	11.84	19.0	27.0		C	13.5	1.51	11.18	11.0	16.0
	E	20.5	1.90	9.27	18.0	23.0		D	**25.3**	5.64	22.29	15.0	34.0

	E	13.3	2.07	15.49	11.0	16.0
	F	15.0	2.40	15.99	11.0	19.0
No. of bristles	A	**8.0**	0.79	9.93	7.0	9.0
in DM1	B	13.0	3.62	27.85	9.0	19.0
	C	8.8	0.92	10.44	8.0	10.0
	D	**13.2**	2.10	15.89	9.0	16.0
	E	8.8	1.47	16.66	7.0	11.0
	F	11.6	1.67	14.42	9.0	14.0
No. of bristles	A	**4.1**	0.55	13.48	3.0	5.0
in DM2	B	**8.8**	1.62	18.40	6.0	11.0
	C	7.0	1.25	17.82	5.0	9.0
	D	8.1	1.37	16.92	6.0	10.0
	E	5.8	0.75	12.90	5.0	7.0
	F	6.3	1.12	17.65	5.0	8.0
Caudal cirri,	A	3.0	0.00	0.00	3.0	3.0
number	B	3.0	0.00	0.00	3.0	3.0
	C	3.0	0.00	0.00	3.0	3.0
	D	**3.4**	0.52	15.19	3.0	4.0
	E	3.0	0.00	0.00	3.0	3.0
	F	3.0	0.39	13.36	2.0	4.0

[a] Data based on protargol-impregnated specimens. All measurements in µm. AZM, adoral zone of membranelles; CV, coefficient of variance (in %); DK, dorsal kinety; DM, dorsomarginal row; Max, maximum; Mean, arithmetic mean; Min, minimum; No., number; SD, standard deviation. Sample size: for dorsal ciliature, 10; for rest of the characters, 20. The highlighted numericals point to significant morphometric differences.

Table 5.10. Morphometric characterization of two populations of *Sterkiella tricirrata* reported from Sikkim[a]

Character	Population	Mean	SD	CV	Min	Max	n
Body length	M	63.4	7.31	11.53	52.5	77.7	20
	N	67.8	4.43	6.53	62.1	76.2	20
Body width	M	27.9	4.30	15.43	21.2	34.4	20
	N	30.1	1.67	5.55	27.9	32.9	20
AZM length	M	27.6	3.14	11.39	20.7	31.3	20
	N	29.3	2.27	7.75	26.6	34.0	20
Adoral membranelles, number	M	28.8	2.70	9.37	25.0	32.0	20
	N	28.8	2.30	7.99	26.0	33.0	20
AZM / body length, %	M	44	0.03	7.46	37	48	20
	N	43	0.03	5.87	40	46	20
Macronuclear nodules, number	M	2.0	0.00	0.00	2.0	2.0	20
	N	2.0	0.00	0.00	2.0	2.0	20
Macronucleus length	M	10.8	0.80	7.41	9.1	11.7	20
	N	9.8	0.94	9.61	8.7	10.9	20
Macronucleus width	M	8.0	1.27	15.82	5.8	10.7	20
	N	6.9	0.63	9.13	6.0	7.9	20
Micronucleus diameter	M	2.5	0.29	11.53	2.1	2.9	10
	N	2.0	0.19	9.22	1.8	2.3	10
Frontal cirri, number	M	7.8	0.44	5.67	7.0	8.0	20
	N	8.0	0.00	0.00	8.0	8.0	20
Ventral cirri, number	M	5.1	0.32	6.20	5.0	6.0	20
	N	5.0	0.00	0.00	5.0	5.0	20
Transverse cirri, number	M	3.0	0.00	0.00	3.0	3.0	20
	N	3.2	0.63	19.76	3.0	5.0	20
Right marginal row, number of cirri	M	20.4	1.65	8.07	18.0	23.0	20
	N	18.7	1.42	7.58	16.0	21.0	20
Left marginal row, number of cirri	M	17.8	2.10	11.78	15.0	21.0	20
	N	16.0	1.15	7.22	14.0	18.0	20
Dorsal kineties, number	M	6.1	0.32	5.18	6.0	7.0	10
	N	6.0	0.00	0.00	6.0	6.0	10
No. of bristles in DK1	M	15.8	1.23	7.78	14.0	18.0	10
	N	15.5	1.35	8.74	14.0	18.0	10
No. of bristles in DK2	M	17.5	1.27	7.25	16.0	20.0	10
	N	15.6	1.58	10.11	13.0	18.0	10
No. of bristles in DK3	M	14.3	1.64	11.44	12.0	17.0	10
	N	11.0	1.41	12.86	9.0	14.0	10
No. of bristles in DK4	M	11.2	2.20	19.65	8.0	15.0	10
	N	13.1	1.52	11.63	8.0	14.0	10
No. of bristles in DM1	M	8.1	0.88	10.81	7.0	10.0	10
	N	8.7	1.49	17.18	7.0	11.0	10
No. of bristles in DM2	M	4.6	0.97	21.00	4.0	7.0	10
	N	4.6	0.52	11.23	4.0	5.0	10
Caudal cirri, number	M	3.0	0.00	0.00	3.0	3.0	10
	N	3.0	0.00	0.00	3.0	3.0	10

[a] Data based on protargol-impregnated specimens. All measurements in µm. AZM, adoral zone of membranelles; CV, coefficient of variance (in %); DK, dorsal kinety; DM, dorsomarginal row; Max, maximum; Mean, arithmetic mean; Min, minimum; n, sample size; No., number; SD, standard deviation.

Table 5.11. Morphometric characterization of six populations of *Sterkiella cavicola* reported from Sikkim[a]

Character	Population	Mean	SD	CV	Min	Max
Body length	G	**126**	7.92	6.28	115.3	139.2
	H	102	7.90	7.70	89.9	113.7
	I	121	13.98	11.60	106.2	148.1
	J	122	9.18	7.50	109.4	139.3
	K	109	10.85	9.95	90.6	121.9
	L	**83.8**	9.40	11.22	70.9	98.0
Body width	G	44.8	4.47	9.96	37.4	53.3
	H	37.3	3.47	9.31	31.9	42.0
	I	52.5	5.42	10.31	41.0	61.2
	J	**54.5**	6.45	11.84	45.2	66.9
	K	44.7	4.00	8.95	38.6	51.1
	L	**32.4**	4.89	15.09	26.5	42.5
AZM length	G	**66.1**	5.20	7.86	59.6	74.8
	H	48.2	4.26	8.84	40.9	53.6
	I	45.3	5.40	11.91	39.4	26.5
	J	47.6	2.87	6.03	42.5	51.9
	K	42.2	6.56	15.54	32.7	50.4
	L	**35.5**	2.94	8.29	31.0	40.4
Adoral membranelles, number	G	40.3	6.02	14.94	33.0	51.0
	H	**44.2**	5.79	13.10	34.0	53.0
	I	38.9	4.65	11.96	33.0	47.0
	J	40.9	4.04	9.88	36.0	47.0
	K	37.6	4.60	12.23	31.0	45.0
	L	**30.8**	3.22	10.47	26.0	37.0
AZM / body length, %	G	**52**	0.01	2.28	51.0	54.0
	H	47	0.03	6.97	43.0	52.0
	I	**38**	0.02	2.30	35.0	40.0
	J	39	0.02	2.17	37.0	42.0
	K	39	0.03	8.86	31.0	43.0
	L	42	0.02	4.46	40.0	46.0
Macronuclear nodules, number	G	4.0	0.00	0.00	4.0	4.0
	H	3.9	0.32	8.11	3.0	4.0
	I	4.0	0.00	0.00	4.0	4.0
	J	4.1	0.32	7.71	4.0	5.0
	K	4.0	0.00	0.00	4.0	4.0
	L	4.0	0.00	0.00	4.0	4.0
Macronucleus length	G	12.8	2.78	21.82	9.4	17.9
	H	11.9	1.56	13.15	9.6	14.0
	I	**14.1**	1.81	12.86	11.7	17.7
	J	**14.1**	2.79	19.76	9.9	18.7
	K	10.8	1.24	11.50	8.2	12.6
	L	**9.8**	1.59	16.24	7.8	12.0
Macronucleus width	G	**9.2**	1.35	14.67	7.2	10.9
	H	7.9	0.55	7.05	7.0	9.0
	I	7.6	0.78	10.28	6.4	9.0
	J	8.0	1.60	20.03	5.5	10.1
	K	**6.5**	1.14	17.55	4.5	8.4
	L	7.5	1.23	16.55	5.6	9.4
Micronucleus diameter	G	2.6	0.20	7.90	2.2	2.9
	H	3.2	0.38	11.93	2.6	3.7
	I	**3.7**	0.29	7.90	3.6	4.2
	J	2.5	0.22	8.96	2.1	2.8
	K	**2.4**	0.26	10.74	2.0	2.9
	L	**2.4**	0.25	10.79	2.0	2.7

Frontal cirri, number	G	8.0	0.00	0.00	8.0	8.0		L	**22.0**	1.83	8.30	19.0	25.0
	H	8.0	0.00	0.00	8.0	8.0	Dorsal	G	6.0	0.00	0.00	6.0	6.0
	I	8.0	0.00	0.00	8.0	8.0	kineties,	H	6.0	0.00	0.00	6.0	6.0
	J	8.0	0.00	0.00	8.0	8.0	number	I	6.0	0.00	0.00	6.0	6.0
	K	8.0	0.00	0.00	8.0	8.0		J	6.0	0.00	0.00	6.0	6.0
	L	**7.8**	0.42	5.41	7.0	8.0		K	6.0	0.00	0.00	6.0	6.0
Ventral cirri, number	G	5.0	0.00	0.00	5.0	5.0		L	6.0	0.00	0.00	6.0	6.0
	H	5.1	0.33	6.52	5.0	6.0	No. of bristles	G	41.1	4.61	11.21	36.0	51.0
	I	5.0	0.00	0.00	5.0	5.0		H	**43.8**	6.41	14.63	37.0	57.0
	J	**5.7**	0.95	16.64	5.0	7.0		I	31.2	4.64	14.87	23.0	41.0
	K	**6.1**	0.88	14.35	5.0	7.0		J	40.8	3.39	8.32	35.0	46.0
	L	5.1	0.32	6.20	5.0	6.0		K	29.4	2.91	9.91	26.0	34.0
Transverse cirri, number	G	**5.0**	0.00	0.00	5.0	5.0		L	**27.5**	3.24	11.78	23.0	32.0
	H	**5.0**	0.00	0.00	5.0	5.0	No. of bristles	G	32.8	3.58	10.93	27.0	39.0
	I	**4.0**	0.00	0.00	4.0	4.0		H	**36.5**	3.98	10.90	32.0	44.0
	J	**4.0**	0.00	0.00	4.0	4.0		I	28.2	2.70	9.57	25.0	32.0
	K	4.1	0.32	7.71	4.0	5.0		J	34.8	2.20	6.32	31.0	38.0
	L	4.3	0.48	11.23	4.0	5.0		K	27.1	4.15	15.31	20.0	32.0
Right marginal row, number of cirri	G	27.3	1.70	6.24	25.0	31.0		L	**24.5**	3.31	13.50	20.0	29.0
	H	25	2.62	10.50	21.0	30.0	No. of bristles	G	22.3	1.70	7.64	20.0	25.0
	I	27.2	1.81	6.67	25.0	30.0		H	**25.2**	1.99	7.89	21.0	28.0
	J	**31.1**	1.20	3.85	28.0	32.0		I	22.7	2.87	12.64	19.0	27.0
	K	27.9	2.02	7.26	25.0	31.0		J	26.6	1.96	7.35	23.0	29.0
	L	**24.1**	2.73	11.31	21.0	30.0		K	20.2	2.25	11.14	17.0	24.0
Left marginal row, number of cirri	G	26.2	1.75	6.68	25.0	29.0		L	**19.3**	2.71	14.04	16.0	23.0
	H	23.5	1.96	8.33	22.0	27.0	No. of bristles	G	19.0	2.67	14.04	14.0	22.0
	I	24.5	1.84	7.51	22.0	27.0		H	24.8	2.44	9.84	22.0	29.0
	J	**27.5**	1.65	6.00	25.0	31.0		I	22.3	1.77	7.92	20.0	25.0
	K	25.8	1.75	6.79	23.0	29.0		J	**29.8**	4.21	14.13	23.0	34.0

	K	21.7	3.77	17.39	17.0	28.0
	L	**18.3**	2.16	11.82	15.0	22.0
No. of bristles	G	15	2.98	19.88	10.0	19.0
	H	16.7	3.02	18.09	10.0	20.0
	I	13.5	2.22	16.47	11.0	17.0
	J	15.2	1.75	11.52	12.0	17.0
	K	12.2	2.20	18.04	10.0	15.0
	L	12.2	1.62	13.27	10.0	15.0
No. of bristles	G	9.2	2.90	31.50	5.0	13.0
	H	**11.2**	3.22	28.79	7.0	16.0
	I	**7.6**	1.71	22.54	5.0	10.0
	J	8.3	1.16	13.97	7.0	11.0

	K	6.7	1.49	22.30	5.0	9.0
	L	7.8	1.69	21.62	6.0	11.0
Caudal cirri,	G	3.1	0.32	10.20	3.0	4.0
	H	3.0	0.00	0.00	3.0	3.0
	I	3.0	0.00	0.00	3.0	3.0
	J	3.0	0.00	0.00	3.0	3.0
	K	3.0	0.00	0.00	3.0	3.0
	L	3.0	0.00	0.00	3.0	3.0

[a] Data based on protargol-impregnated specimens. All measurements in µm. AZM, adoral zone of membranelles; CV, coefficient of variance (in %); DK, dorsal kinety; DM, dorsomarginal row; Max, maximum; Mean, arithmetic mean; Min, minimum; No., number; SD, standard deviation. Sample size: for dorsal ciliature, 10; for rest of the characters, 20. The highlighted numericals point to significant morphometric differences.

One population (C) of *Sterkiella histriomuscorum* invariably possessed four transverse cirri in a row and is therefore different from those populations having few cells with 4 transverse cirri. Similarly in *Sterkiella cavicola*, four populations (I, J, K and L) invariably possessed four transverse cirri in a row and appear to be similar to another species *Sterkiella quadrinucleata*, but the latter is a marine species and is very large as compared to the *Sterkiella cavicola* cells of Sikkim populations. As of now these populations have been included in *S. histriomuscorum* and *S. cavicola* respectively; however, they may be assigned new species status keeping in view the separate species status assigned to *S. tricirrata* because of presence of three cirri in the transverse region.

According to the present study, the important feature of absence of cortical granules in the members of sub-family Stylonychinae as against their presence in the other sub-family, Oxytrichinae (Berger and Foissner 1997) needs reinvestigation as cortical granules have been found in some populations of *Sterkiella* from Sikkim. Additionally, the placement of *Sterkiella* spp. in the sub-family Stylonychinae can also be questioned as their body texture is not rigid but slightly flexible and the shape of undulating membranes matches with the members of sub-family Oxytrichinae.

Molecular analysis (Fig. 5.12)

18S rDNA sequences of *S. histriomuscorum* and *S. cavicola* matched more or less with those available on GenBank database for their respective species; interestingly, each of them showed affinity towards *Gastrostyla steinii* and *Pattersoniella vitiphila*, members of the oxytrichid *sensu lato* group. 18S rDNA sequences of four populations (I, J, K and L) of *S. cavicola* with 4 transverse cirri in a row showed affinity to that of *Gastrostyla steinii* and others with 5 transverse cirri in tick mark shape matched more with that of *Pattersoniella vitiphila.* These results further substantiate the hypothesis made by earlier authors (Foissner et al. 2004) that there is a stronger relationship between *Gastrostyla*, *Sterkiella* and *Pattersoniella.* According to their study, this observation matches the morphological and ontogenetical data because *Pattersoniella*, like, *Gastrostyla*, has an increased number of FVT cirri and the individual cirral groups of *Sterkiella*, *Pattersoniella*, and *Gastrostyla* can be homologized (Fig. 5.13; Foissner et al. 2004). They further added that there are no morphological objections to derive the *Gastrostyla-Pattersoniella* group from a *Sterkiella*-like ancestor because all have the same type of oral apparatus and dorsal kinety pattern, and include species with more than the usual two macronucleus nodules. The study on Sikkim

populations of *Sterkiella* further substantiate the above hypothesis as many populations of *Sterkiella* have supernumerary cirri and therefore shows their affinity with *Gastrostyla.* This observation needs further exploration.

Table 5.12: Accession numbers of 18S rDNA sequences of fourteen populations of *Sterkiella* (submitted to GenBank)[a]:

Population	Accession number
A	JX946274
B	JX893369
C	KC404828
D	KC193246
E	KC193240
F	KC193247
G	KC193241
H	KC193242
I	KC193243
J	JX893370
K	KC193244
L	KC182573
M	-
N	JX946275
O	KC193245

[a]18S rDNA sequence of *S. tricirrata* was not available on GenBank data and has been submitted to the GenBank with accession number JX 946275. The molecular data of the population M and morphometric data of population O could not be obtained. Most of the above sequences have been submitted under the name of *Sterkiella* sp. as in these cases the alignment score was variable indicating differences within species; this is in accordance with the results obtained through classical methodology. In future, these accession numbers can be designated to new species if required.

More interestingly, in the phylogenetic analysis, populations of *Sterkiella* for which 18S rDNA sequences could be derived showed tendency to segregate and club in groups with respect to number and placement of transverse cirri. Additionally, their tendency to cluster together with respect to number and placement of transverse cirri was noticeably more than with respect to number of macronuclei. Thus results are supportive to the idea that the number and placement of cirral structures have more systematic value than the nuclear apparatus at least in this particular group.

SECTION B

Multiple fragmentation of dorsal kinety 3 during morphogenesis is seen in some members of the family Oxytrichidae. Some oxytrichids collected from Sikkim exhibit this character; these are: *Cyrtohymena* (*Cyrtohymenides*) *shii* (member of the sub family Oxytrichinae) and *Pattersoniella vitiphila* (member of the sub family Stylonychinae). Of these *Cyrtohymena* (*Cyrtohymenides*) *shii* Sikkim population has been studied in detail because of its unique morphogenetic pattern. The phylogenetic analysis of this taxon has clarified the systematic status of flexible bodied oxytrichid ciliates with multiple fragmentation of dorsal kinety within Dorsomarginalia; additionally, it has helped in revising subgenus *Cyrtohymena* (*Cyrtohymenides*) and has been identified as one of the examples to explain convergent evolution among oxytrichid ciliates.

Cyrtohymena* (*Cyrtohymenides*) *shii (Shi et al., 1997) Shao et al., 2012; Sikkim population (Figs 5.14–5.23; Tables 5.12–5.15)

Cyrtohymena (*Cyrtohymenides*) *shii* Sikkim population was found from a water catchment area at the entry point of the Barsey Rhododendron Sanctuary and is described below.

Description (Figs 5.14A–C, 5.15A, B, 5.16A–C; Table 5.12)**:** Body is oblong, right margin is usually slightly concave, left margin is slightly convex, widest in midline, gradually narrowing posteriorly, dorso-ventrally flattened, slightly flexible, about 150 μm × 50 μm *in vivo* (Figs 5.14A, 5.16A) and 138 × 51 μm on average when impregnated with protargol (Figs 5.15A, B, 5.16B, C). Length:width ratio is 2.5:1. The cells show rapid movements; they prey upon smaller encysting or excysting cells (Fig. 5.17B). Presence of abundant brilliant citrine cortical granules make cells appear slightly yellowish green at low magnification; individual granules are about 1 μm in cross section, scattered singly or in small clusters (Fig. 5.14B, C). The cytoplasm is colourless with many lipid droplets about 3 μm in diameter. Contractile vacuole is present slightly ahead of mid-body near left cell margin with two collecting canals (Fig. 5.16A). Each cell has two ellipsoidal macronuclear nodules, about 21 μm × 10 μm, 3–8 spherical micronuclei about 3 μm in cross section in protargol-impregnated interphasic cells.

Adoral zone of membranelles is about 37% of cell length, composed of 50 membranelles on

average, buccal cavity is deep and wide, undulating membranes are in typical *Cyrtohymena* pattern (Berger and Foissner 1997; Figs 5.15A, 5.16B, 5.17D). 18 frontal-ventral-transverse cirri include three hypertrophied frontal cirri, one buccal cirrus, four frontoventral cirri arranged in V-shaped pattern, three postoral ventral cirri behind the buccal vertex, two pretransverse ventral cirri, five markedly hypertrophied transverse cirri invariably in two groups of three and two displaced anteriad 14.5 μm on average from posterior tip of cell. One left and one right marginal row are with about 30 and 29 cirri respectively; rows are not confluent posteriorly: right row ends at level of posterior-most transverse cirrus, left row at tip of cell (Figs 5.15A, 5.16B). There are five or six, rarely seven, dorsal kineties (Figs 5.15B, 5.16C): first and second bipolar with 36–48 and 29–37 bristles respectively, next two or three shorter, composed of 11–23, 8–20, 0–21 bristles respectively; last kinety (kinety 6 in Fig. 5.16C) longer with 13–32 bristles. Two or three dorsomarginal rows are with about 11–28, 6–16, 0–8 bristles (10% of cells have two; Figs 5.15B, 5.16C). Three caudal cirri are present, one each at the posterior end of dorsal kineties 1, 2, and last before the dorsomarginal rows (Figs 5.15B, 5.16C).

Average length of cirri and bristles after protargol impregnation is as follows: frontal and marginal, 12 μm; ventral, 8 μm; transverse, 27 μm; caudal, 14 μm; dorsal cilia, 4 μm long.

Cells often encyst; cysts are with irregular wall, 40–60 μm in diameter (Fig. 5.17E). Conjugation occurs frequently under laboratory conditions. Sometimes homopolar doublets are seen in a normally growing culture (Fig. 5.17C).

Table 5.12. Morphometric characterization of *Cyrtohymena* (*Cyrtohymenides*) *shii* Sikkim population[a].

Character	Min	Max	Mean	SD	CV	n
Body length	121.3	155.8	138.3	8.41	6.1	20
Body width	36.8	60.1	51.2	6.48	12.7	20
Adoral membranelles, number	44	57	50.4	3.08	6.1	20
AZM length	37.6	61.9	50.6	5.74	11.4	20
AZM / body length %	28	42	37.0	0.04	10.6	20
Macronuclear nodules, number	2	2	2.0	0.00	0.0	20
Macronucleus length	16.8	24.4	20.5	2.21	10.8	20
Macronucleus width	8.4	11.5	9.7	0.88	9.1	20

Micronuclei, number	3	8	4.9	1.57	32.3	20
Micronucleus diameter	2.6	3.6	3.1	0.29	9.5	20
Frontal cirri, number	3	4	3.1	0.22	7.3	20
Buccal cirri, number	1	1	1.0	0.00	0.0	20
Frontoventral cirri, number	4	5	4.2	0.37	8.8	20
Postoral ventral cirri, number	3	5	3.2	0.52	16.4	20
Pretransverse ventral cirri, number	2	3	2.1	0.22	10.9	20
Transverse cirri, number	5	6	5.1	0.31	6.0	20
Right marginal row, number of cirri	24	33	29.1	2.16	7.5	20
Left marginal row, number of cirri	25	35	29.6	2.50	8.5	20
Caudal cirri, number	3	3	3.0	0.00	0.0	20
Dorsal kineties, number	5	6	5.7	0.47	8.3	20
No. of bristles in DK_1	36	48	41.0	3.04	7.4	20
No. of bristles in DK_2	29	37	32.3	2.22	6.9	20
No. of bristles in DK_3	11	23	15.9	2.98	18.8	20
No. of bristles in DK_4	8	20	14.4	3.28	22.9	20
No. of bristles in DK_5	0	21	11.6	5.25	45.3	10
No. of bristles in DK_6	13	32	22.8	4.77	21.0	20
Dorsomarginal rows, number	2	3	3.0	0.22	7.6	20
No. of bristles in DM_1	11	28	20.4	4.74	23.3	20
No. of bristles in DM_2	6	16	11.8	2.61	22.1	20
No. of bristles in DM_3	0	8	4.2	2.14	51.0	20
Dorsal bristle length	3.5	4.2	3.9	0.26	6.6	10
Distance between posterior end of cell to posterior-most transverse cirrus	10.9	17.1	14.5	1.75	12.0	20

[a] Data based on protargol-impregnated specimens. All measurements in μm. AZM, adoral zone of membranelles; CV, coefficient of variance (in %); DK, dorsal kinety; DM, dorsomarginal row. Max, maximum; Mean, arithmetic mean; Min, minimum; n, sample size; No., number; SD, standard deviation. It is not uncommon to find cells with more than eighteen frontal-ventral-transverse cirri (20 percent of cells scored). Five percent cells scored had additional complete or incomplete left marginal rows.

Voucher material: A slide (accession number: NHMUK 2012.7.19.1) containing several protargol-impregnated cells of *C.* (*C.*) *shii* Sikkim population has been deposited in the

British Natural History Museum, Cromwell Road, London SW7 5BD, UK.

Divisional morphogenesis (Figs 5.18–5.22; Table 5.13): One divisional cycle takes 19 ± 1 h at an optimum temperature of 18 ± 2 °C (s). Divisional morphogenesis begins with the appearance of a small field of kinetosomes (Figs 5.18A, 5.21A) arising close to the left transverse cirrus; proliferation of this anarchic field forms the oral primordium (Figs 5.18B, 5.21B). The adoral zone of membranelles for the opisthe is formed from the oral primordium while that of the parental cell is retained unchanged for the proter.

Table 5.13. Parental ciliary structures associated with the formation of anlagen for frontal ciliature in *Cyrtohymena* (*Cyrtohymenides*) *shii* Sikkim population[a].

Daughter cell	Anlagen number	Parental structure associated with origin of anlagen
Proter	I	Parental paroral membrane
Proter and Opisthe	II[b]	II/2 + oral primordium
	III[b]	III/2 + oral primordium
	IV[b]	IV/3 + V/4
	V[b]	V/4
	VI[b]	V/4
Opisthe	I	From the base of the five streaks arising from oral primordium

[a] Cirri are named according to Wallengren (1900). IV/2 and V/3 possibly incorporated into the field of kinetosomes above the newly forming adoral membranelles for the opisthe.
[b] These anlagen split transversely to form one set each for proter and opisthe.

The anlagen for the frontal ciliature are formed as follows: Two streaks arise from the anterior right side of the oral primordium and move anteriad (Figs 5.18E, 5.21E). Cirri V/4 and IV/3 disaggregate followed by III/2 and later II/2 to give rise to cirral streaks (Figs 5.18C–E, H, 5.21C–E, H). Cirrus V/4 gives rise to three streaks consecutively (Figs 5.18F, 5.21F). Anlage I for the opisthe develops from loose kinetosomes present just ahead of new membranelles while that for the proter is formed from the partially disaggregated parental paroral (Figs 5.18I–L, 5.19, 5.21I–L, 5.22A–C). The paroral, endoral and the first frontal cirrus are formed from this anlage. The rest of the anlagen for the two daughter cells are

formed in conjunction by the resorption of parental cirri. Anlage II for the proter is formed from the streak from II/2 (Figs 5.18H, 5.21H) and anlage II for the opisthe from the oral primordium (Figs 5.18E, 5.21E). Anlage III for the proter develops from the streak from III/2 and anlage III for the opisthe from the oral primordium (Figs 5.18E, G, 5.21E). Anlage IV for the proter and opisthe forms from the streak from IV/3 and the first streak from the cirrus V/4 (Figs 5.18G, 5.21G). Anlagen V and VI for the proter and opisthe are formed from the remaining two streaks coming from the disaggregated cirrus V/4 (Figs 5.18I, 5.21I). Cirri IV/2 and V/3 disaggregate and their kinetosomes possibly contribute to the frontal anlagen (Figs 5.18H, I, L, 5.21H, I, L). Thus four parental cirri are directly involved in anlagen formation and two parental cirri possibly contribute to the already formed anlagen (Table 5.13). These anlagen for both daughter cells are joined at one point or the other in time. Later, each of these anlagen split transversely into two segments (Figs 5.18J–L, 5.19A, 5.21J–L, 5.22A), which move anteriad and posteriad respectively. Eighteen frontal-ventral-transverse cirri arise from these anlagen, splitting in the typical oxytrichid pattern of 1, 3, 3, 3, 4, 4 (Figs 5.19C, D, 5.22C, D).

The marginal anlagen arise at each of two levels by ‘‘within-row’’ anlagen formation (Figs 5.19A–C, 5.22A–C) utilizing one or two of the parental cirri at each level. The marginal anlagen elongate utilizing ten to twelve parental cirri and differentiate into new marginal rows. The rest of the parental marginal cirri are resorbed.

On the dorsal surface, three anlagen are formed at two levels within the three dorsal kineties on the left for the proter and opisthe. The first two give rise to two bipolar dorsal kineties while the third undergoes multiple fragmentation to give rise to three or four and rarely five dorsal kineties (Figs 5.20B–D, 5.22E, G) for each daughter cell. The three dorsomarginals arise from the anterior end of the new right marginal row (Figs 5.19D, 5.22D). Three caudal cirri are formed, one each at the posterior ends of the two bipolar kineties and the rightmost kinety (Figs 5.20D, 5.22G). The fragmentation of dorsal kineties is a very quick process, commencing when the formation of two complete sets of cirri on the ventral surface begins and finishing as the division furrow deepens (Figs 5.20A–F, 5.22E–H). The fragmentation process takes not more than half an hour. No parental bristles are retained for the daughter cells.

Physiological reorganization (Fig. 5.17A): 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.

18S rDNA gene sequence analysis and phylogenetic tree construction

The nucleotide sequence of *Cyrtohymena* (*Cyrtohymenides*) *shii* Sikkim population has been deposited in GenBank database under accession number JQ513386.

18S rDNA sequence determined in this study was submitted to a BLAST search (http://www.ncbi.nlm.nih.gov) to find closely related sequences; 18S rDNA sequences of a total of 26 taxa were retrieved. 18S rDNA sequence of *Urostyla grandis* of family Urostylidae was retrieved to be used as outgroup. Sequences were aligned using CLUSTAL X2 sequence analysis software (Larkin et al. 2007). The resulting alignments were checked and corrected manually to remove ambiguous nucleotide positions at the beginning and end of the fragments. Phylogenetic analysis was performed with the MEGA (Molecular Evolutionary Genetics Analysis) software package version 5.0 (Tamura et al. 2011) available at http://www.megasoftware.net. The evolutionary distances were computed using the GTR+G+I nucleotide substitution model (Saitou and Nei 1987) in Maximum-likelihood tree. A Neighbour-joining tree using Maximum Composite Likelihood method (Tamura et al. 2004) and a Maximum parsimony tree were also constructed to check the relative stability of tree topologies. In each case tree construction was done using 1,000 bootstrap replicates. The analysis involved 27 nucleotide sequences. There were a total of 1,721 positions in the final dataset. Distance matrix was constructed by Maximum Composite Likelihood method for nine taxa that clustered with *C.* (*C.*) *shii* Sikkim population in Maximum-likelihood, Maximum parsimony, and Neighbour-joining trees.

In Maximum-likelihood and Maximum parsimony trees, the topologies are congruent and therefore are merged together in Fig. 5.23A. In Neighbour-joining tree the topology is different from the other two trees and hence has been given separately (Fig. 5.23B). The Distance matrix constructed by Maximum Composite Likelihood method for nine taxa that clustered with *C.* (*C.*) *shii* Sikkim population is given in Table 5.14: the minimum genetic distance of 0.001 was found to be between *C.* (*C.*) *shii* Sikkim population and both populations of *C. citrina*. The genetic distance between *C.* (*C.*) *shii* Sikkim population and *Afrokeronopsis aurea* was 0.018. In this cluster, the maximum genetic distance of 0.029 was found to be between *C.* (*C.*) *shii* Sikkim population and *Rubrioxytricha ferruginea*.
In all three trees, 18S rDNA sequence of *C.* (*C.*) *shii* Sikkim population matches well with

those of two populations of *C. citrina*. It also clusters with that of *Afrokeronopsis aurea*, a neokeronopsid.

Table 5.14. Genetic distances between the 18S rDNA sequences of nine taxa[a] highlighted in the trees.

Species	*O. flexilis*	*P. weissei*	H. sp.	*O.* sp.	*R. ferruginea*	*C. citrina* (Pop. 1)	*C. citrina* (Pop. 2)	*C.* (*C.*) *shii* Sikkim pop.	*A. aurea*
P. weissei	0.009								
H. sp.	0.011	0.014							
O. sp.	0.011	0.014	0.000						
R. ferruginea	0.021	0.022	0.022	0.022					
C. citrina	0.017	0.019	0.018	0.018	0.028				
C. citrina	0.017	0.019	0.018	0.018	0.028	0.000			
C. (*C.*) *shii* Sikkim pop.	0.018	0.020	0.019	0.019	0.029	0.001	0.001		
A. aurea	0.026	0.027	0.024	0.024	0.032	0.018	0.018	0.018	

[a] Full names of species are as follows: *O. flexilis*, *Onychodromopsis flexilis*; *P. weissei*, *Paraurostyla weissei*; H. sp., Hypotrichida sp.; *O.* sp., *Oxytricha* sp.; *R. ferruginea*, *Rubrioxytricha ferruginea*; *C. citrina* (Pop. 1), *Cyrtohymena citrina* (Population 1); *C. citrina* (Pop. 2), *Cyrtohymena citrina* (Population 2); *C.* (*C.*) *shii* Sikkim pop., *Cyrtohymena* (*Cyrtohymenides*) *shii* Sikkim population; *A. aurea*, *Afrokeronopsis aurea.* The minimum and maximum genetic distances between *C.* (*C.*) *shii* Sikkim population and other nine taxa have been boxed.

Ecology and Occurrence

Sample was collected from soil water interface of a permanent water catchment area close to the entry point of the Barsey Rhododendron Sanctuary (27°15' to 27°27'N; 88°01' to 88°23'E), Sikkim. Other ciliates observed in the samples from the same water body were *Oxytricha longigranulosa*, *Pattersoniella vitiphila*, *Stylonychia ammermanni*, *Sterkiella histriomuscorum*, *S. cavicola*, *Frontonia* sp., *Colpoda* sp., *Paramecium caudatum* and *Tetrahymena* sp.

Discussion

So far 17 species belonging to genus *Cyrtohymena* have been reported (Berger 1999; Foissner 2004; Shao et al. 2012; Shi et al. 1997) with *C. muscorum* as the type species.

Foissner (2004) and Shao et al. (2012) reassigned species with multiple dorsal kinety fragmentation, namely *C. australis* Foissner, 1995, *C. aspoecki* Foissner, 2004, and *C. shii* Shi et al., 1997 to the subgenus *Cyrtohymena* (*Cyrtohymenides*) Foissner, 2004.

The general morphology of *C.* (*C.*) *shii* Sikkim population resembles that of *C.* (*C.*) *shii* Heilmgjang, China population with minor differences in morphometry (Table 5.15) and distribution of cortical granules (Shi et al. 1997, 1998). Some vital information regarding the colour of cortical granules, detailed morphogenesis and molecular data are not available for the Heilmgjang population to make a more detailed comparison.

Cortical granules in *C.* (*C.*) *shii* Sikkim population are brilliant citrine, matching well with those of *C. citrina* (Berger and Foissner 1987) and *C.* (*C.*) *australis* (Foissner 1995); their irregular arrangement in *C.* (*C.*) *shii* Sikkim population differs considerably from that in *C. citrina, C.* (*C.*) *australis* and *C.* (*C.*) *shii* Heilmgjang Population (Berger and Foissner 1987; Shao et al. 2012; Shi et al. 1997).

Table 5.15. Morphometric comparison of *Cyrtohymena* (*Cyrtohymenides*) *shii* Sikkim population (Si) with *C.* (*Cyrtohymenides*) *shii* Heilmgjang, China population (He; Shao et al. 2012; Shi et al. 1997)[a].

Character	Population	Min	Max	Mean	SD	CV	n
Body length	Si	121.3	155.8	138.3	8.41	6.1	20
	He	137.0	210.0	175.0	19.00	10.9	20
Body width	Si	36.8	60.1	51.2	6.48	12.7	20
	He	50.0	75.0	62.6	8.60	13.7	20
Adoral membranelles, number	Si	44.0	57.0	50.4	3.08	6.1	20
	He	49.0	58.0	51.7	2.10	4.0	20
AZM length	Si	37.6	61.9	50.6	5.74	11.4	20
	He	55.0	71.0	66.5	4.70	7.0	20
AZM / body length %	Si	28.0	42.0	37.0	0.04	10.6	20
	He	-	-	38.0	-	-	-
Macronuclear nodules, number	Si	2.0	2.0	2.0	0.00	0.0	20
	He	2.0	2.0	2.0	0.00	0.0	20
Micronuclei, number	Si	3.0	8.0	4.9	1.57	32.3	20
	He	2.0	6.0	4.0	1.00	25.0	20

Frontal cirri, number	Si	3.0	4.0	3.1	0.22	7.3	20
	He	3.0	3.0	3.0	0.00	0.0	20
Posterior frontal cirri, number	Si	4.0	5.0	4.2	0.37	8.8	20
	He	4.0	4.0	4.0	0.00	0.0	20
Buccal cirri, number	Si	1.0	1.0	1.0	0.00	0.0	20
	He	1.0	1.0	1.0	0.00	0.0	20
Postoral ventral cirri, number	Si	3.0	5.0	3.2	0.52	16.4	20
	He	3.0	3.0	3.0	0.00	0.0	20
Pretransverse ventral cirri, number	Si	2.0	3.0	2.1	0.22	10.9	20
	He	2.0	2.0	2.0	0.00	0.0	20
Transverse cirri, number	Si	5.0	6.0	5.1	0.31	6.0	20
	He	5.0	5.0	5.0	0.00	0.0	20
Right marginal row, number of cirri	Si	24.0	33.0	29.1	2.16	7.5	20
	He	30.0	33.0	30.9	1.10	3.6	20
Left marginal row, number of cirri	Si	25.0	35.0	29.6	2.50	8.5	20
	He	30.0	35.0	32.8	1.30	4.0	20
Dorsal kineties and dorsomarginal row, number	Si	8.0	9.0	8.7	0.49	5.7	20
	He	8.0	9.0	8.4	0.50	5.9	20
Caudal cirri, number	Si	3.0	3.0	3.0	0.00	0.0	20
	He	3.0	3.0	3.0	0.00	0.0	20

[a] Data based on protargol-impregnated specimens. All measurements in µm. AZM, adoral zone of membranelles; CV, coefficient of variance (in %); Max, maximum; Mean, arithmetic mean; Min, minimum; n, sample size; SD, standard deviation.

Morphogenesis: Ventral morphogenetic events have been described within the genus *Cyrtohymena* only for the type species *C. muscorum* (Voss 1991). In this regard, *C.* (*C.*) *shii* Sikkim population shows several distinct differences in the following ways: (1) The oral primordium in *C.* (*C.*) *shii* Sikkim population originates near the anterior most left transverse cirri, while in *C. muscorum* it originates near the postoral ventral cirri. (2) There is no de novo origin of frontal anlagen V and VI for the proter as reported for *C. muscorum* (Voss 1991). (3) Only four parental cirri – II/2, III/2, IV/3 and V/4 – act as origins for formation of new frontal anlagen while parental cirri IV/2 and V/3 disaggregate possibly to contribute to the new anlagen. However, in *C. muscorum* six parental cirri act as origins of anlagen. (4)

Frontal anlagen II–VI for the proter and the opisthe are conjoined at some stage and split transversely later; in *C. muscorum*, the frontal anlagen for proter and opisthe are likely not conjoined at any stage.

Subgenus *Cyrtohymena* (*Cyrtohymenides*) Foissner, 2004: This subgenus was erected to include species of the genus *Cyrtohymena* which have three or more dorsomarginal rows. Additionally, Foissner (2004) stated that members of the subgenus tend to have a large body size with an increase in the number of cirri in the frontal ciliature, increased number of dorsal kineties perhaps due to increase in the fragmentation during ontogenesis and partial retention of parental dorsal kineties. Unfortunately the last two features were doubtful according to Foissner (2004). He designated *C*. (*C.*) *aspoecki* as the type species and also assigned *C*. *australis* Foissner, 1995 to the subgenus, although *C*. *australis* has two dorsomarginal rows (Foissner 2004). Shao et al. (2012) transferred *Oxytricha shii* Shi et al., 1997 to *Cyrtohymena* (*Cyrtohymenides*). The Sikkim population of *C.* (*C.*) *shii* fits well within the subgenus except for the large size trait. In the Sikkim population 20% of the cells possess an increased number of frontal, ventral and transverse cirri arising from an increased number of frontal anlagen. *Cyrtohymena* (*Cyrtohymenides*) *aspoecki* has a higher number of frontal, ventral, and transverse cirri but the arrangement of the frontal ciliature is different in the two species.

The subgenus *Cyrtohymenides* comprises three species, however, little or no morphogenetic data have been available. The above description of *C.* (*C.*) *shii* reveals that the increase in the number of dorsal rows is due to multiple fragmentation of the third dorsal anlage during ontogenesis and the presence of three dorsomarginal rows; this may be a characteristic feature of the subgenus. No retention of parental dorsomarginal rows has been found in *C.* (*C.*) *shii* Sikkim population; additionally the body size of the Sikkim population is smaller than that of the other two species assigned to the subgenus necessitating an improved diagnosis of the subgenus. The following improved diagnosis of the subgenus is based on the data of the species *C.* (*C.*) *shii* by Shi et al. (1997, 1998) and the present study, and data available of the other two species *C*. (*C.*) *aspoecki* and *C*. (*C.*) *australis*; the diagnosis can be improved further when more information on *C*. (*C.*) *aspoecki* and *C*. (*C.*) *australis* is available. An improved diagnosis of the species *C.* (*C.*) *shii* is also provided based on the data by Shi et al. (1997, 1998) and our observations.

Improved diagnosis of the subgenus *Cyrtohymena* (*Cyrtohymenides*): Cyrtohymenids with a classical cyrtohymenid oral apparatus (paroral membrane strongly curved anteriorly) and

general body organization (cortex flexible with coloured granules); relatively large body size (≥150 μm); slightly increased number of frontal-ventral-transverse cirri; marginal rows not confluent posteriorly; multiple fragmentation of the third dorsal kinety during ontogenesis; three or more dorsomarginal rows.

Improved diagnosis of *Cyrtohymena* (*Cyrtohymenides*) *shii*: Size *in vivo* about 125–230 × 40–75 μm; small (about 1 μm across), brilliant citrine cortical granules randomly arranged singly and in small clusters; two macronuclear nodules and 2–8 micronuclei; adoral zone one third of body length; five transverse cirri arranged in two groups of three and two; right marginal row of cirri not extending to posterior tip of cell, left row terminates at rear end of cell; five or six dorsal kineties: first two bipolar, next three or four shorter, the latter arise by multiple fragmentation of rightmost dorsal anlage, usually three dorsomarginal rows; three caudal cirri, one each at posterior tip of the two bipolar kineties and the rightmost kinety, linearly arranged along right posterior margin; six parental cirri involved in anlagen formation for frontal ciliature: four parental cirri disaggregate to form frontal anlagen while two others disaggregate to perhaps contribute to existing anlagen, all anlagen for proter and opisthe conjoined at some stage before separating reflecting primary primordia.

Phylogeny: The sequence of 18S rDNA of *C.* (*C.*) *shii* Sikkim population matches well with those of two populations of *C. citrina*, the only species of the genus for which sequences are available (Fig. 5.23). However, the two species differ in several morphological, morphometric and morphogenetic characters (Berger and Foissner 1987; Berger 1999).

18S rDNA sequences of *C.* (*C.*) *shii* Sikkim population and *C. citrina* cluster close to that of *Afrokeronopsis aurea* in the phylogenetic tree even though the latter was assigned to a different family, the Neokeronopsidae (Foissner and Stoeck 2008; Foissner et al. 2010). Interestingly, *Afrokeronopsis aurea* shares some features with *C.* (*C.*) *shii* Sikkim population – the shape of the undulating membranes, the dorsal ciliature, and the shape of the marginal rows (acute with distinct indentation at the site of caudal cirri). Moreover, about 20% of *C.* (*C.*) *shii* Sikkim population possess an increased number of frontal, ventral, and transverse cirri arising from an increased number of frontal anlagen. These features perhaps indicate an evolutionary tendency in *C.* (*C.*) *shii* Sikkim population towards acquiring ciliature similar to the *Afrokeronopsis aurea* type. This proposition is in accordance with the CEUU (Convergent Evolution of Urostylids and Uroleptids) hypothesis (Foissner et al. 2004), which suggests that a midventral pattern evolved at least two times: the first, older event caused the

ancestor to split into an oxytrichid and a urostylid lineage, while the second, more recent event caused the development of a midventral pattern in several oxytrichid lineages. The similarities of *C.* (*C.*) *shii* and *Afrokeronopsis aurea* in this context are significant.

Pattersoniella vitiphila Foissner, 1987; Sikkim population (Figs 5.24; Tables 5.16, 5.17)

Pattersoniella vitiphila is diagnosed by the following traits: Paroral and endoral in *Oxytricha* pattern; number of frontal-ventral-transverse cirri markedly increased from the usual 18 in oxytrichids; one right and 1 left row of marginal cirri; multiple dorsal kineties due to multiple fragmentation of kinety 3 and retention of some parental kineties; usually three caudal cirri.

Cells of *Pattersoniella vitiphila* populations were reported from four places in Sikkim, two from Barsey Rhododendron Sanctuary, one from Maenam Wildlife Sanctuary and one from Legship Hot Spring. Out of these, three populations (A & B from Barsey, and C from Maenam) could be studied in detail. Sikkim populations showed some morphological, morphometric and morphogenetic peculiarities from the earlier described population.

One population (A) found in the sample collected from Barsey Rhododendron Sanctuary is described below.

Description: Cells are oval in shape with left margin slightly concave than right, anterior and posterior ends are broadly rounded. Body is firm, dorsoventrally flattened with ventral side slightly concave and dorsal side distinctly convex. Average body size is 190 × 88 µm *in vivo* and 180 × 84 µm in protargol-stained preparations.

Table 5.16. Morphometric characterization of *Pattersoniella vitiphila* (Population A) Sikkim population[a]

Character	Mean	SD	CV	Min	Max	n
Body length	181.5	23.53	12.96	141.1	215.4	25
Body width	84.2	13.05	15.50	62.2	104.0	25
AZM length	88.7	19.31	21.77	62.9	114.1	25
Adoral membranelles, number	78.4	17.50	22.34	57.0	105.0	20
AZM / body length, %	0.5	0.06	11.47	0.4	0.6	25
Macronuclear nodules, number	17.9	3.23	18.11	13.0	24.0	20
Macronucleus length	8.1	1.07	13.24	6.6	10.3	20

Macronucleus width	7.1	0.97	13.69	5.7	8.9	20
Micronuclei, number	6.1	2.39	39.58	3.0	12.0	20
Micronucleus diameter	2.9	0.36	12.35	2.0	3.3	10
Frontal cirri, number	8.0	0.73	9.07	7.0	9.0	25
Ventral cirri, number	22.9	3.01	13.13	18.0	28.0	25
Transverse cirri, number	10.2	1.20	11.73	8.0	13.0	25
Right marginal row, number of cirri	37.1	4.77	12.88	28.0	46.0	25
Left marginal row, number of cirri	33.2	2.66	8.03	28.0	37.0	25
Dorsal kineties, number	10.2	1.09	10.67	9.0	12.0	10
Caudal cirri, number	3.0	0.00	0.00	3.0	3.0	10

[a] Data based on protargol-impregnated specimens. All measurements in μm. AZM, adoral zone of membranelles; CV, coefficient of variance (in %); DK, dorsal kinety; Max, maximum; Mean, arithmetic mean; Min, minimum; n, sample size; No., number; SD, standard deviation.

Discussion

Pattersoniella vitiphila has erstwhile been reported from the Southern hemisphere; its presence at some places in Sikkim shows its significance with respect to dispersal patterns. *Pattersoniella vitiphila* shows important modifications from typical *Oxytricha* pattern with respect to ventral and dorsal morphogenesis. Dorsal morphogenesis proceeds with the anlagen formation in kineties 1-3, later the kinety 3 divides by multiple fragmentation to give rise to 4-5 kineties. Furthermore, the formation of > 2 dorsomarginal rows leads to multiple kineties. However non dividing specimens have more than 10 kineties indicating retention of parental dorsal kineties.

The earlier described population of *Pattersoniella vitiphila* was reported in the terrestrial habitats of Fiji island Viti Levu. In Sikkim, population (A) was isolated from water sample collected from soil water interface of a permanent water body located near entry point of Barsey Rhododendron Sanctuary, population (B) was isolated from soil sample collected below a rhododendron tree in Barsey Rhododendron Sanctuary, population (C) was isolated from the soil sample collected from Maenam Wildlife Sanctuary.

Table 5.17. Morphometric comparison of four populations of *Pattersoniella vitiphila*, three (A, Barsey, water; B, Maenam, soil; C, Barsey, soil) from Sikkim and one (D; soil) from Viti, Fiji Island (Foissner 1987) [a].

Character	Pop.	Mean	SD	CV	Min	Max	n
Body length	A	181.5	23.53	12.96	141.1	215.4	20
	B	160.6	22.57	14.06	113.7	210.3	20
	C	151.2	19.18	12.68	123.3	181.7	20
	D	230.5	31.00	13.50	170	295	25
Body width	A	84.2	13.05	15.50	62.2	104.6	20
	B	79.5	9.69	12.18	61.0	98.3	20
	C	79.6	8.13	10.22	67.0	100.2	20
	D	108.8	16.66	15.30	70.0	140	25
AZM length	A	88.7	19.31	21.77	62.9	114.1	20
	B	75.3	11.55	15.33	54.0	111	20
	C	72.9	9.19	12.61	57.0	88	20
	D	107.4	21.10	19.60	70.0	140	25
Adoral membranelles, number	A	78.4	17.5	22.34	57	105	20
	B	64.6	8.76	13.56	51	88	20
	C	61.6	7.52	12.22	49	76	20
	D	60.4	8.40	14.00	44	83	25
AZM / body length, %	A	48	0.06	11.40	40	56	20
	B	47	0.03	6.54	41	53	20
	C	48	0.03	5.20	45	53	20
	D	46	-	-	-	-	-
Macronuclear nodules, number	A	17.9	3.23	18.11	13	24	20
	B	21.4	3.07	14.33	18	30	20
	C	17.4	3.83	22.0	10	24	20
	D	15.6	1.10	7.20	13	18	25
Macronucleus length	A	8.1	1.07	13.24	6.6	10.3	20
	B	7.8	0.97	12.47	6.0	10	20
	C	7.7	0.85	11.01	6.0	6	20
	D	17.4	4.30	24.60	11.0	27	25

Macronucleus width	A	7.1	0.97	13.68	5.7	8.9	20
	B	6.2	0.62	9.92	5.0	7.0	20
	C	6.1	0.66	10.87	5.0	7.0	20
	D	13.2	2.10	16.10	9.0	20	25
Micronuclei, number	A	6.0	2.39	39.58	3	12	20
	B	5.8	2.12	60.60	4	10	20
	C	5.4	2.79	45.60	3	9	20
	D	6.0	3.00	50.00	2	12	25
Micronucleus diameter	A	2.9	0.36	12.35	2	3.3	20
	B	3.0	1.41	47.14	2	4.0	20
	C	1.8	0.78	44.30	1	3.0	20
	D	4.1	0.50	12.30	3.5	5.6	25
Frontal cirri number	A	8.0	0.73	9.07	7	9	20
	B	8.0	1.30	10.08	7	9	20
	C	8.0	1.90	15.06	7	9	20
	D	5.8	0.70	11.50	5	8	25
Ventral cirri, number	A	22.9	3.01	13.13	18	28	20
	B	17.4	3.70	12.98	14	25	20
	C	19.7	2.60	10.60	16	24	20
	D	14.6	1.20	8.20	13	18	25
Transverse cirri , number	A	10.2	1.20	11.73	8	13	20
	B	9.6	1.90	13.60	8	11	20
	C	10.1	1.60	9.70	8	12	20
	D	9.0	0.80	9.40	8	11	25
Right marginal row, number of cirri	A	37.1	4.77	12.88	28	46	20
	B	33.5	2.70	8.08	28	38	20
	C	33.1	1.68	5.08	31	37	20
	D	31.5	2.5	8.00	27	36	25
Left marginal row, number of cirri	A	33.2	2.66	8.02	28	37	20
	B	32.0	2.85	8.60	27	40	20
	C	31.6	3.02	9.56	23	36	20
	D	29.1	2.90	9.90	22	34	25

Dorsal kineties, number	A	10.2	1.09	10.67	9	12	10
	B	10.1	1.13	12.93	9	12	10
	C	9.9	0.95	9.67	9	12	10
	D	-	-	-	9	12	-
Caudal cirri, number	A	3.0	0.00	0.00	3.0	3.0	10
	B	3.0	0.00	0.00	3.0	3.0	10
	C	3.0	0.00	0.00	3.0	3.0	10
	D	3.0	0.00	0.00	3.0	3.0	-

[a] Data based on protargol-impregnated specimens. All measurements in µm. AZM, adoral zone of membranelles; CV, coefficient of variance (in %); DK, dorsal kinety; Max, maximum; Mean, arithmetic mean; Min, minimum; n, sample size; No., number; SD, standard deviation.

Cells of Sikkim populations are different from Viti population in the following respects:

1. Cells of Sikkim populations are smaller than Viti population.
2. Number of frontal- ventral-transverse cirral streaks in Sikkim populations is distinctly increased leading to further increase in the frontal- ventral-transverse cirri number.
3. There are >2 dorsomarginal rows in Sikkim populations as compared with Viti population where the cells show invariably two dorsomarginals.

The detail pertaining to *Cyrtohymena* (*Cyrtohymenides*) *shii* has been compiled in the form of a research article entitled **"Morphology, ontogenesis, and molecular phylogeny of an Sikkim population of *Cyrtohymena* (*Cyrtohymenides*) *shii*, including remarks on the subgenus"** (available on line in the section, Articles in press in the European Journal of Protistology, Singh, J., et al., Eur. J. Protistol. (2012).

SECTION C

There are a few members of family Oxytrichidae which do not show fragmentation of any dorsal kinety during morphogenesis. Based on the number of cirri in their frontal ciliature, they can further be grouped as follows (i) species with invariably full set of 18 frontal-ventral-transverse cirri e. g. *Oxytricha islandica*, *O. lanceolata*, *O. pseudosimilis* and *O. setigera* and (ii) species with '<18 frontal-ventral-transverse cirri'. The first group of oxytrichids has not yet been assigned a separate generic status; they have preliminarily been included in Genus *Oxytricha* as *incertae sedis* (Berger 1999; Shao et al. 2012). Oxytrichids of the second type were excluded from Genus *Oxytricha* and assigned a separate genus *Urosomoida* (Foissner 1982, Hemberger 1985).

A cell type that clearly belonged to the second category was isolated from a soil sample collected from Kyongnosla Alpine Sanctuary in the Eastern Himalayas. The distinctive traits, especially the unique ventral infraciliature completely lacking pretransverse ventral and transverse cirri, the only known oxytrichid with this character, warrants its placement into a new genus *Paraurosomoida indiensis* gen. nov., sp. nov.

***Paraurosomoida indiensis* gen. nov., sp. nov.** (Figs 5.25, 5.34; Tables 5.18 and 5.19)

***Paraurosomoida* gen. nov.**

Diagnosis: Body very flexible; cortical granules present; adoral zone of membranelles formed like a question mark; undulating membranes nearly in *Oxytricha* pattern. Three frontal cirri; one buccal cirrus; four frontoventral cirri arranged in V-shaped pattern; postoral ventral cirri form a cluster behind buccal vertex; pretransverse ventral cirri and transverse cirri absent. One right and 1 left marginal rows of cirri. Three bipolar dorsal kineties with one caudal cirrus each; usually one dorsomarginal row. Six parental cirri involved in anlagen formation for frontal ciliature; no fragmentation of dorsal kinety 3 during morphogenesis.

Etymology: The name *Paraurosomoida* is a composite of the Greek prefix *para*+ (beside, at, along, during) and the name of the genus *Urosomoida* and refers to the similar ciliature of *Urosomoida* and *Paraurosomoida*. Like *Urosomoida*, feminine gender (Aescht 2001: 304).

Species assignable: *Paraurosomoida indiensis* sp. nov.

***Paraurosomoida indiensis* sp. nov.**

Diagnosis: Body oblong with slightly less wide posterior end, with mean body size *in vivo* about 90 × 25 μm; small (about 0.8 μm in diameter), brilliant citrine cortical granules randomly arranged singly and in small linear groups; two macronuclear nodules and 2–4 micronuclei; adoral zone 25%–35% of body length, composed of 25 membranelles on average; marginal rows of cirri not confluent posteriorly, the gap between them often difficult to recognise; left and right marginal row, with about 28 and 30 cirri respectively; three bipolar dorsal kineties (Figs 5.25E, 5.26E) with about 10–17, 14–20 and 9–19 bristles respectively; 9–15 (on average 11) cirri in frontal ciliature.

Type locality: Soil sample from the base of a rhododendron tree in Kyongnosla Alpine Sanctuary (27°22' to 33"N; 88°44' to 13"E), India.

Type material: Two protargol slides containing the holotype specimen and several paratypes are being deposited in the British Natural History Museum, Cromwell Road, London SW7 5BD, UK.

Etymology: The species group name *indiensis* refers to the country (India) where this species was discovered.

Description: Body is oblong, gradually narrows posteriorly with rounded posterior end, dorsoventrally flattened and highly flexible, about 90 × 25 μm *in vivo* (Figs 5.25A, 5.26A) and 86 × 23 μm on average when impregnated with protargol (Figs 5.25D, 5.26B). Length:width ratio is 3.7:1 in protargol impregnated cells. Cells show slow movement. Body shows light brown pigmented patches at anterior and posterior end of cell. Cortical granules are citrine coloured, scattered singly and in small linear groups, individual granule is about 0.8 μm in diameter, (Fig. 5.25B). Cytoplasm is colorless with many lipid droplets about 2.5 μm in diameter. The contractile vacuole is with two collecting canals positioned slightly above mid-body near left margin of cell (Fig. 5.26A).

Adoral zone of membranelles is about 25–35% of cell length, composed of 25 membranelles on average. Undulating membranes are nearly in *Oxytricha* pattern (Berger and Foissner 1997). Buccal cavity is narrow and flat. There are nine to fifteen (on average 11) cirri in

frontal ciliature: three slightly hypertrophied frontal cirri, four frontoventral cirri positioned in V-shape, one buccal cirrus, 1–7 (on average 3) postoral ventral cirri, remarkably no pretransverse ventral cirri and transverse cirri (one to five cirri are seen in the posterior end in less than 5 % of the cells aligned to the right marginal row of cirri but not at the location usually occupied by transverse cirri; Fig. 5.26D). There is one left and one right marginal row, with about 28 and 30 cirri respectively, rows are not confluent posteriorly, the small gap is usually occupied by left caudal cirrus (Figs 5.25D, 5.26B). Three bipolar dorsal kineties are (Figs 5.25E, 5.26E) with about 10–17, 14–20, and 9–19 bristles respectively; one dorsomarginal row with about 6–13 bristles (Figs 5.25E, 5.26E, 5.28B, D, 5.32C, E). 20% cells have a second row with about 2–5 bristles (Figs 5.29A, B, 5.30A, B). There are three caudal cirri, one each at the posterior end of dorsal kineties; 20% of cells investigated have two or four caudal cirri. Average length of cirri and bristles after protargol impregnation is as follows: frontal and ventral, 8 μm; marginal, 9 μm; caudal, 11 μm; dorsal cilia, 3 μm. Cysts are with irregular wall, the size in life is 33–40 μm in diameter, cytoplasm is heavily granulated (Fig. 5.25C).

Each cell has two ellipsoidal macronuclear nodules with average size 15 × 7 μm and 2–4 spherical micronuclei with average size 2.6 × 2.1 μm in protargol-impregnated interphasic cells. Cells show conjugation under laboratory conditions in raw cultures.

Divisional morphogenesis (Figs 5.27A–H, 5.28A–D, 5.31A–G, 5.32A–E; Table 5.19): One divisional cycle takes 24 ± 1 h at optimum temperature 15 ± 2 °C (s). Divisional morphogenesis begins with the appearance of a small anarchic field of about 3–5 kinetosomes in a region about 37% of cell length from the posterior tip (Figs 5.27A, 5.31A); four or five more such clusters with increased number of kinetosomes develop in a row up to the postoral ventral cirri; proliferation in these anarchic fields and their merging forms the oral primordium (Figs 5.27B, 5.31B). The adoral zone of membranelles for the opisthe is formed from the oral primordium while that of the parental cell is retained unchanged for the proter.

The anlagen for the frontal ciliature are formed as follows: Two streaks arise from the anterior right side of the oral primordium and move anteriad (Figs 5.27D, 5.31D). Disaggregation of cirri V/4 followed by V/3, IV/3, IV/2, III/2 and later II/2 give rise to cirral streaks (Figs 5.27C–H, 5.31C–G, 5.32A; Table 5.19). Anlage I for the opisthe develops from loose kinetosomes present just above the new membranelles while that for the proter is

formed from the partially disaggregated parental paroral and endoral; the paroral, endoral and the first frontal cirrus are formed from this anlage. Anlagen II–IV for the two daughter cells are formed as follows: for the proter, anlage II is formed from the streak from cirrus II/2, anlage III develops from the streak from cirrus III/2, anlage IV forms from the streak from cirrus IV/3 (Figs 5.27E–H, 5.31E–G, 5.32A); for the opisthe, anlagen II and III are formed from streaks that arise from the oral primordium and anlage IV is formed from the streak from cirrus IV/2 (Figs 5.27F, G, 5.31F, G). Anlagen V and VI for both daughter cells are formed from the two primary primordia originating from disaggregated cirri V/4 and V/3 respectively (Figs 5.27C–H, 5.31C–G, 5.32A). Thus six parental cirri are directly involved in anlagen formation. Later, two sets of cirri arise from these anlagen to form eleven cirri in the frontal ciliature, splitting in the pattern of 1, 2, 2, 2, 2, 2 (Figs 5.28A–C, 5.32B–D; Table 5.19).

The marginal anlagen for the two daughter cells arise at two levels by ‘‘within-row’’ anlagen formation (Figs 5.28A, 5.32B) utilizing few of the parental cirri at each level. The marginal anlagen elongate and differentiate into new marginal rows. The rest of the parental marginal cirri are resorbed.

On the dorsal surface, three anlagen are formed at two levels within the three dorsal kineties to give rise to three bipolar dorsal kineties for each daughter cell. There is no fragmentation of dorsal kinety 3. The dorsomarginal row arises from the anterior end of the new right marginal row (Figs 5.28B, 5.32C). Three caudal cirri are formed, one each at the posterior ends of the three new dorsal kineties for each daughter cell (Figs 5.28D, 5.32E). No parental bristles are retained for the daughter cells. Macronuclear nodules divide amitotically while the micronuclei undergo mitosis in a typical oxytrichid pattern.

Physiological reorganization (Figs 5.29, 5.30): Cells undergo reorganization when culture conditions are not ideal (usually, starvation). The reorganizers make a single set of ciliature which then replaces the old one. The eleven cirri in the frontal ciliature arise in the pattern of (1, 2, 2, 2, 2, 2) from the anlagen I–VI.

18S rDNA gene sequence analysis and phylogenetic tree construction (Figs 5.33 and 5.34): The nucleotide sequence obtained in this study has been deposited in GenBank database under accession number JX139117.

18S rDNA sequence of *Paraurosomoida indiensis* was submitted to a BLAST search (http://www.ncbi.nlm.nih.gov) to find closely related sequences; 18S rDNA sequences of 30 different taxa with complete gene sequence lengths were retrieved. 18S rDNA sequences of *Bergeriella ovata* and *Urostyla grandis* of family Urostylidae were used as outgroup. Sequences were aligned using CLUSTAL X2 sequence analysis software (Larkin et al. 2007). The resulting alignments were checked and corrected manually to remove ambiguous nucleotide positions at the beginning and end of the fragments. Phylogenetic analyses were performed with the MEGA (Molecular Evolutionary Genetics Analysis) software package version 5 (Tamura et al. 2011) available at http://www.megasoftware.net and with PAUP* Version 4b10 (Swofford 2003). A Neighbour-joining tree using Maximum Composite Likelihood method (Tamura et al. 2004) was constructed using MEGA version 5. The 50% majority-rule consensus distance tree (minimum evolution) was also constructed using the tree bisection and reconnection (TBR) branch swapping algorithm with PAUP* Version 4b10 based on the General Time Reversible model (Saitou and Nei 1987) to check the relative stability of tree topologies. In each case tree construction was done using 1,000 bootstrap replicates. The analysis involved 31 nucleotide sequences. There were a total of 1,746 positions in the final dataset. In both trees, the topology that emerged for members of the subfamily Stylonychinae has been merged together; accession numbers of their 18S rDNA sequences are as follows: AF164123, AF164124, AF164128, AF164133, AF396973, AF508754, AF508770, AF508772, AJ310486, AJ310487, AJ310494, AJ310495.

Phylogenetic trees constructed by different methods using 18S rDNA sequence of *Paraurosomoida indiensis* places it consistently among the flexible oxytrichids, close to *Cyrtohymena citrina* and *Paraurostyla weissei.* This clarifies the systematic status of flexible bodied oxytrichid ciliates lacking fragmentation of dorsal kinety within Dorsomarginalia; their positions are hitherto unclear because of the absence of any molecular data.

Table 5.18. Morphometric characterization of *Paraurosomoida indiensis* [a].

Character	Min	Max	Mean	SD	CV	n
Body length	74.8	101.7	85.7	7.41	8.7	20
Body width	18.8	32.8	22.9	3.43	15.0	20
Adoral membranelles, number	21.0	30.0	25.1	2.06	8.2	20
AZM length	22.6	28.4	25.6	1.61	6.3	20
AZM / body length %	26.0	35.0	30.0	0.03	8.4	20

Macronuclear nodules, number	2.0	2.0	2.0	0.00	0.0	20
Macronucleus length	12.1	20.0	15.1	2.08	13.7	20
Macronucleus width	5.1	8.5	6.5	0.87	13.5	20
Micronuclei, number	2.0	4.0	2.5	0.70	27.3	50
Micronucleus length	2.3	3.0	2.6	0.19	7.6	20
Micronucleus width	1.8	2.4	2.1	0.20	9.3	20
Frontal cirri, number	3.0	3.0	3.0	0.00	0.0	50
Buccal cirri, number	1.0	1.0	1.0	0.00	0.0	50
Frontoventral cirri, number	4.0	4.0	4.0	0.00	0.0	50
Postoral ventral cirri, number	1.0	7.0	3.4	1.15	33.2	50
Number of cirri in frontal ciliature of opisthe	9.0	15.0	11.3	1.30	11.5	50
Right marginal row, number of cirri	23.0	35.0	29.6	3.10	10.5	20
Left marginal row, number of cirri	23.0	32.0	27.6	2.48	9.0	20
Caudal cirri, number	2.0	4.0	3.0	0.46	15.3	20
Dorsal kineties, number	3.0	3.0	3.0	0.00	0.0	10
Number of bristles in dorsal kinety 1	10.0	17.0	12.6	2.04	16.2	10
Number of bristles in dorsal kinety 2	14.0	20.0	16.7	1.81	10.9	10
Number of bristles in dorsal kinety 3	9.0	19.0	13.4	2.39	17.9	10
Dorsomarginal rows, number	1.0	2.0	1.2	0.37	31.9	10
Number of bristles in dorsomarginal row 1	6.0	13.0	9.0	2.00	22.2	10
Number of bristles in dorsomarginal row 2	2.0	5.0	3.8	1.30	34.3	05

[a] Data based on protargol-impregnated specimens. All measurements in μm. AZM, adoral zone of membranelles; CV, coefficient of variance (in %); Max, maximum; Mean, arithmetic mean; Min, minimum; n, sample size; SD, standard deviation. Five percent of cells scored had additional complete or incomplete left marginal rows.

Occurrence and ecology: As yet found only in one of the soil samples collected from the Kyongnosla Alpine Sanctuary located at an altitude of 3290–4116 m. The sanctuary remains covered with the snow for most part of the year and was accessible for sample collection from April to October. The sample was collected from a small portion of land exposed due to melting of snow. Flora surrounding the collection site includes conifers, variety of rhododendrons, ground orchids and wild strawberries. Cells of *P. indiensis* were found in the

soil sample collected below a rhododendron tree. The soil was slightly acidic (pH 6.5). The temperature of the soil from where the collection was made was around 7°C. The other ciliates observed in the same sample were *Pseudouroleptus caudatum*, *Sterkiella histriomuscorum*, *S. tricirrata*, *Gonostomum affine*, *Colpoda* sp., and *Tetrahymena* sp.

Table 5.19. Parental ciliary structures associated with the formation of anlagen for frontal ciliature in *Paraurosomoida indiensis*.

Daughter cell	Anlagen number	Parental structure associated with origin of anlagen
Proter	I	Parental paroral membrane
	II	II/2
	III	III/2
	IV	IV/3
	V	V/4
	VI	V/3
Opisthe	I	From the base of the 3 streaks arising from oral primordium
	II	From the base of the 3 streaks arising from oral primordium
	III	From the base of the 3 streaks arising from oral primordium
	IV	IV/2
	V[a]	V/4
	VI[a]	V/3

[a] These anlagen split transversely to form one set each for proter and opisthe.

Discussion

Justification for creation of a new genus: *Paraurosomoida indiensis* is a typical oxytrichid ciliate; however, it is the only member of the family which lacks pretransverse ventral cirri and transverse cirri. All oxytrichids except the stylonychines (sub family Oxytrichinae *sensu* Berger and Foissner 1997) are characterized by a flexible body, cortical granules frequently present, typically 18 cirri in the frontal ciliature, usually two dorsomarginal bristle rows, postoral ventral cirrus V/3 participating in primordia formation, fragmentation of the third dorsal kinety and frontal-ventral-transverse cirri arising in a pattern of 1, 3, 3, 3, 4, 4 from six anlagen. There are a few oxytrichid genera such as *Urosoma* and *Urosomoida* which have less than 18 cirri in the frontal ciliature, one dorsomarginal row and show no fragmentation of the third dorsal kinety. *Paraurosomoida indiensis* markedly differs from *Urosoma*. *Urosoma* has usually 2 pretransverse ventral cirri and 5 transverse cirri; adoral zone of membranelles and undulating membranes in the *Gonostomum* pattern; and the frontoventral cirri arranged in a row. *Urosomoida* shows a marked reduction in the number of frontal-ventral-transverse cirri (14–17) and most species are either tailed or tapered posteriorly. But all species of

Urosomoida have some pretransverse ventral cirri and transverse cirri. The number of cirri in the frontal ciliature in *P. indiensis* (9–15, usually 11, extra cirri in some cells are additional postoral ventral cirri; Fig. 5.26C) is markedly less than in *Urosomoida* and significantly there is a complete loss of pretransverse ventral cirri and transverse cirri despite that the cells of *P. indiensis* are only slightly narrow posteriorly. The complete loss of pretransverse ventral cirri and transverse cirri makes a firm ground for the separation of *P. indiensis* from *Urosomoida* at the generic level. Further differences between *Urosomoida* and *P. indiensis* with respect to morphogenesis are as follows: the frontal anlagen V and VI for the proter in *Urosomoida* arise de novo (Buitkamp 1975; Foissner and Adam 1983) but in *P. indiensis,* these anlagen originate from the two right postoral ventral cirri (Figs 5.27C–H, 5.31C–G, 5.32A; Table 5.19). The oral primordium in *Urosomoida* originates in the middle of the cell while in *P. indiensis* it arises in a region about one third of cell length from the posterior tip.

The dorsal ciliature and reduced frontal ciliature in *P. indiensis* bears similarity to that in two 'non oxytrichid Dorsomarginalia' genera – *Erimophyra* Foissner et al., 2002 and *Apourosomoida* Foissner et al., 2002; however, there are significant differences: in both the latter species, anlage V is absent. *Erimophyra* also lacks cortical granules and invariably possesses two postoral ventral cirri and one transverse cirrus. The postoral ventral cirri in *P. indiensis* are formed from anlagen IV and V as against in *Erimophyra* in which they are formed from anlage IV only (Berger 2008). *Apourosomoida* possesses only two dorsal kineties and lacks dorsomarginal rows. Another characteristic feature of *Apourosomoida* is a gap in the adoral zone of membranelles (Berger 2008).

Paraurosomoida indiensis is markedly different from many amphisiellids with similar ventral and dorsal infraciliature due to absence of a median cirral row which is the most important character of amphisiellids (Berger 2008). Additionally amphisiellids also lack postoral ventral cirri which are present in *P. indiensis.*

In the light of the above differences, the assignage of a separate genus status to the new taxon is fully justified; it is the only reported oxytrichid without pretransverse ventral cirri and transverse cirri.

Molecular phylogeny: There is no molecular data available for species that appear to be close to *Paraurosomoida indiensis* in morphology and morphogenesis. The 18S rDNA sequence of *P. indiensis* clusters with those of *Cyrtohymena citrina* and *Paraurostyla weissei*

(Figs 5.33 and 5.34), both of which are morphologically not very close to *P. indiensis*. The similarity in the cortical granules could be one of the reasons for these three taxa to cluster together and considering this similarity, the more closeness shown by *P. indiensis* towards *Cyrtohymena citrina* seems reasonable.

It has been hypothesized that those species which possess dorsomarginal rows but show no fragmentation of the third dorsal kinety must be separated from the oxytrichids as non oxytrichid Dorsomarginalia (Berger 2008). However, *Parasterkiella thompsoni*, a taxon which has dorsomarginal row but lacks dorsal kinety 3 fragmentation is well placed within the family Oxytrichidae (Küppers et al. 2011) refuting the above hypothesis. The 18S rDNA sequence analysis of *P. indiensis* further corroborates the above observation. More molecular data on non oxytrichid Dorsomarginalia sensu Berger (2006) are, however, essential for any further comment.

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CHAPTER 6

MOLECULAR PHYLOGENY BASED ON 18S RDNA SEQUENCES OF SELECTED HYPOTRICHOUS CILIATES FROM SIKKIM

For the past few decades (Baroin et al. 1988; Elwood 1985; Lynn and Sogin 1988; Stackebrandt and Woese 1981; Sogin and Elwood 1986; Sogin et al. 1986), ciliatologists have employed a variety of molecular techniques to address questions on phylogeny and population diversity. The reliability of molecular data has increased over the years with improvement of techniques. Their acceptance becomes imperative when in many cases congruent results are obtained by classical methodologies and vice versa. For testing and establishing phylogenetic relationships among organisms, the whole genome sequence can be used, but, it is almost impossible to deduce and use it for every organism. Therefore, certain phylogenetic marker genes which act as surrogate for the whole genome sequence can be used to infer phylogeny of related organisms. The genes which can be used as phylogenetic markers should have the following characteristics:

1. Significant genetic variability and divergence at the species level.
2. An appropriately short sequence length so as to facilitate DNA extraction, amplification and sequencing (Too short sequences e. g. 5S and 5.8S rRNA are highly conserved and too long give errors during amplification and sequencing).
3. Presence of highly conserved flanking sites for developing universal primers.

18S rDNA (18S rRNA gene) as phylogenetic marker

The analysis based upon 18S rDNA sequences offers the potential for more accurate and efficient method for identifying ciliates and characterizing culturable and unculturable ciliate communities. Comparison of 18S rDNA sequences of species is an excellent taxonomic tool and is one of the most widely used universal markers in phylogenetic studies and has become the “gold-standard” for molecular taxonomy and biodiversity analysis.

18S rRNA is a constituent of the ribosomal RNA. Ribosomal RNA must have been present since very early in the development of life, because it is essential for protein synthesis. Any mutation in the 18S rRNA gene can affect directly the proper functioning of the ribosome and

lead to the elimination of less efficient organisms through selection. One may assume, therefore, that the rRNA genes have a large number of highly conserved sequence patterns. Nevertheless, the process of evolution allowed a number of sequence changes which did not impair the functioning of the ribosome, and which were maintained over evolutionary times. These sequence differences along with a large number of highly conserved sequence patterns enable 18S rDNA/18S rRNA gene to be used to derive phylogenetic relationships among different organisms.

Additionally, the presence of many copies of 18S rDNA per genome provides large amounts of templates DNA for amplification through Polymerase Chain Reaction (PCR) using thermo cycler. In general, 18S rDNA sequences are easy to amplify due to highly conserved flanking regions allowing for the use of universal primers. In addition, the 18S rDNA sequence has hyper-variable regions, where sequences have diverged over evolutionary time. Highly conserved regions often flank these hyper-variable regions. Primers are designed to bind to conserved regions and amplify variable regions.

There are few glitches, however, in using 18S rDNA as an ideal phylogenetic marker gene. There is always a question as how many point mutations might have occurred at one base position to date, for instance from A to G to U and back to A for instance. This uncertainty, and the fact that no one knows when the particular mutation took place, makes it difficult to use the 18S rDNA as an ideal evolutionary clock. Nevertheless, it rests on a basic assumption that phylogenetic trees based on genes are close to accurate. The confidence in phylogenetic analysis based on molecular data increases when multiple and unlinked genes reveal patterns corroborating each other and with phylogenies constructed on other features, such as morphology.

In ciliated protists rDNA occurs as a single-copy gene in the micronucleus but is highly amplified in the macronucleus (Steinbrück 1990). The rDNA in the nucleolar organizer regions (NOR) consists of tandem repeats of the transcriptional unit for the precursor of 18S, 5.8S, and 28S ribosomal RNA (rRNA), separated by an intergenic spacer (IGS). Two internal transcribed spacers, known as ITS 1 and ITS 2, are located between the regions coding for 18S and 5.8S rRNA, and between the latter and the 28S coding region. In addition, an external transcribed spacer (ETS) occurs upstream to the 18S gene and downstream to 28S gene (Long and Dawid, 1980; Roiha et al. 1981).

DNA of the micronucleus is large and is contained in chromosomes; in the macronucleus, it is in the form of many millions of gene-sized molecules that are aggregated in groups to produce the chromatin granules. Micronuclear and macronuclear DNA can be resolved on agarose gels in the form of a band and into a continuum of sizes in the form of a smear respectively (Lipps 1980, Lawrence et al. 1981, Prescott 1994). Figure 6.1 shows gel electrophoresis of genomic DNA and amplified 18S rDNA respectively, of hypotrichous ciliates from Sikkim.

18S rDNA sequences of selected hypotrichous ciliates from Sikkim were obtained and were used to find out their phylogenetic positions with respect to each other and their related taxa reported elsewhere. In most of the cases, the identity of the species ascertained by molecular data corroborated the identifications determined through classical methods. 18S rDNA sequences of some species clustered with those of the earlier described populations of the same species and others showed affinity with those of related species giving clues to look into the possible similarities between them.

Procedures followed for obtaining molecular data and their analysis have been described in detail in chapter 2 (Materials and Methods). Figure 6.2 shows a phylogenetic tree constructed by using MEGA (Molecular Evolutionary Genetics Analysis) software package version 5.0 (Tamura et al. 2011) available at http://www.megasoftware.net.

CONCLUDING REMARKS

The present work documents biodiversity of ciliate fauna especially of hypotrichous ciliates of Sikkim, the western segment of Eastern Himalayas, and includes description of some taxa with phylogenetic notes. The results also gave an insight into ciliate systematics.

Cataloguing and describing ciliate community structure of Sikkim is a first ever effort to investigate ciliate biodiversity of the region. The community of ciliated protists of the region is undoubtedly very rich and distinct. The distinctively diverse populations of a genus *Sterkiella* from a variety of ecozones is itself an indicative of the wide scope of the enormous diversity. Members of the family Oxytrichidae are predominant among the hypotrichous ciliates of the region. Few populations are strikingly similar to the earlier described populations while others show remarkable differences with respect to morphology, morphometry, morphogenesis and molecular characteristics. Detailed study of some cell types provided important data that helped to fill gaps in ciliate systematics. The study on *Sterkiella* populations gives a prospect to explore them further for important answers of systematic significance.

The presence of *Pattersoniella vitiphila*, erstwhile described only from few locations in the southern hemisphere and thought to be located only in that region, in four places in Sikkim showed that unless extensive ciliate cataloguing is done, no conclusions must be drawn on their distribution. Such studies can also throw light on the dispersal patterns of ciliated protists.

The presence of related species in extreme conditions as well as in non extreme conditions shows the tolerance and adaptability of ciliated protists to such wide ranges of climatic conditions.

In summary, the present study gave some very important results:

- The data catalogued has provided new knowledge on the occurrence and distribution of ciliate fauna across Sikkim.

- The study of some selected cases provided literary information of significance.
- The detailed study of two species, *Cyrtohymena* (*Cyrtohymenides*) *shii* and *Paraurosomoida indiensis* gen. nov., sp. nov. helped in filling gaps and clear anomalies in the Hypotrich Systematics.
- The phylogenetic study using classical and molecular methods on *Sterkiella* further substantiated the separation of species solely based on slight variation in the ventral ciliature at the posterior region and gave an important clue about the relative significance of two important attributes (number and placement of cortical ciliature and number of macronuclei) used in the systematics of oxytrichid ciliates.

The importance of in depth biodiversity studies is the highlight of the present work. More such endeavours are the need of the hour as cataloguing diversity is the first step towards maintaining biodiversity.

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Fig. 2.1. 18S rDNA sequence with detail of primer positions and orientations: Two universal primers (EukA and EukB; Medlin et al. 1988) and three internal primers; two forward, IFP–1and IFP–2 and one reverse, IRP–1, designed by using primer designing tool, the Primer Blast (www.ncbi.nlm.nih.gov/tools/primer-blast). All primers are shown in 5'→3' orientation.

AACCTGGTTGATCCTGCCAGT (EukA)

>>>>>>>>>>>>>>>>>>>>>>>>

AACCTGGTTCATCCTGCCAGTAGTCATATGCTTGTCTCAAAGACTAAGCCATGCATGTCTAA
GTATAAATGTTATACAGTGAAACTGCGAATGGCTCATTAAAACAGTTATAGTTTATTTGATAA
TCAAATTTACATGGATAACCGTGGTAATTCTAGAGCTAATACATGCTGTTAAGCCTGACTTTT
AGGAAGGGCTGTATTTATTAGATAACAAATCAATATTCCTTGTGTCTATTGTGACGACTCATA
ATA**ACTGATCGAATCGCATGGGC**TTTGCTCGCGATACATCATTCAAGTTTCTGCCCCATCA

<<<<<<<<<<<<<<<<<<<<<<<<

GCCCATGCGATTCGATCAGT (IRP-1)

GCTCGATGGTAGTGTATTGGACTACCATGGCGTTCACGGGTAACGGAGGATTAGGGTTCGATT
CCGGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGTAAATTA
CCCAATCCTGACTCAGGGAGGTAGTGACAAGAAATAACGGACCGAAGCCTATGTTTCGGGAT
TGCAATGAGTAGAATTTAAACCCCTTTACGAGGATCA**ATTGGAGGGCAAGTCTGGTG**

>>>>>>>>>>>>>>>>>>>>>>>>

ATTGGAGGGCAAGTCTGGTG (IFP-1)

CCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTC
GTAGTTGGATTTCTGGGAGGGTGCCAATGTCGTCTTGTTGATTGTGCAGAGGCGCCCTTCCAT
CCTTCTGTTAACGTTTCTGATATTTATTTATTGGTCTCGGGCTCAGATATTTTACCTTGAGAAA
ATTAGAGTGTTCCAGGCAGGCTCGCGCCGGAATACATTAGCATGGAATAATAGAATAGGACT
TTAGTCTCCTTTGTTGGTTTGAGGGACTGAAGTAATGATTAATAGGGATAGTTGGGGGCATTA
GTATTTAATTGTCAGAGGTGAAATTCTCGGATTTGTTAAAGACTAACTTATGCGAAAGCATTT
GCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCAAAGACGATCAGATACCGT
CCTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGAGGCGCGCCTTATCCGCCTTCGGCA
CCTTATGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAA
AGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCTTGCGGCTCAATTTGACTCAACACGGG
AAAACTTACCAGGTCCAGACATAGTGAGGATTGACAGATTGATAGCTCTTTCTTGATTCTATG
GGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAA
CGAGACCTTAGCCTACTAACTAGTCGATCCGTTGCTTAGCGGACACGACTTCTTAGAGGGACT
TTGTGACTAAACACAAGGAAGTTTGAGGCAATAACAGGTCTGTGATGCCCTTAGATGTCCTG
GGCCGCACGCGTGCT**ACACTGACGCATACAGCGAG**TACTTCCCAGCTCCGTGAGGCAGCTG

>>>>>>>>>>>>>>>>>>>>>>

ACACTGACGCATACAGCGAG (IFP-2)

GTAATCAGCAATATGCGTCGTGATGGGGATAGATCTTTGGAATTATAGATCTTGAACGAGGA
ATTCCTAGTAAGCGCAAGTCATTAGCTTGCGCTGATTAAGTCCCTGCCCTTTGTACACACCGC
CCGTCGCTCCTACCGATTTCGAGTGATCCGGTGAACCATTTGGACCGCGATGCCCTTGTGGTG
TTGTGGAAAATCTAGTAAACCATATCACTTAGAGGAAGGAGAAGTCGTAACAAGGTTTCC
GTAGGTGAACCTGCGGAAGGATCA

<<<<< <<<<<<<<< <<<<<<<<<<<<<

TGATCCTTCTGCAGGTTCACCTAC (EukB)

Fig. 3.1. Map of Sikkim (not to scale) showing district wise location of collection sites (South Sikkim: Maenam Wildlife Sanctuary; West Sikkim: Barsey Rhododendron Sanctuary, Khecheopalri Lake, Leg ship Hot Spring, Borang Hot Spring; East Sikkim: Kyongnosla Alpine Sanctuary, Fambong Lho Wildlife Sanctuary, Changu Lake/Tsongmo Lake; North Sikkim: Yumthang Flower Valley, Yumesamdong Hot Spring, Yumesamdong/Zero point (Shingba Rhododendron Sanctuary).

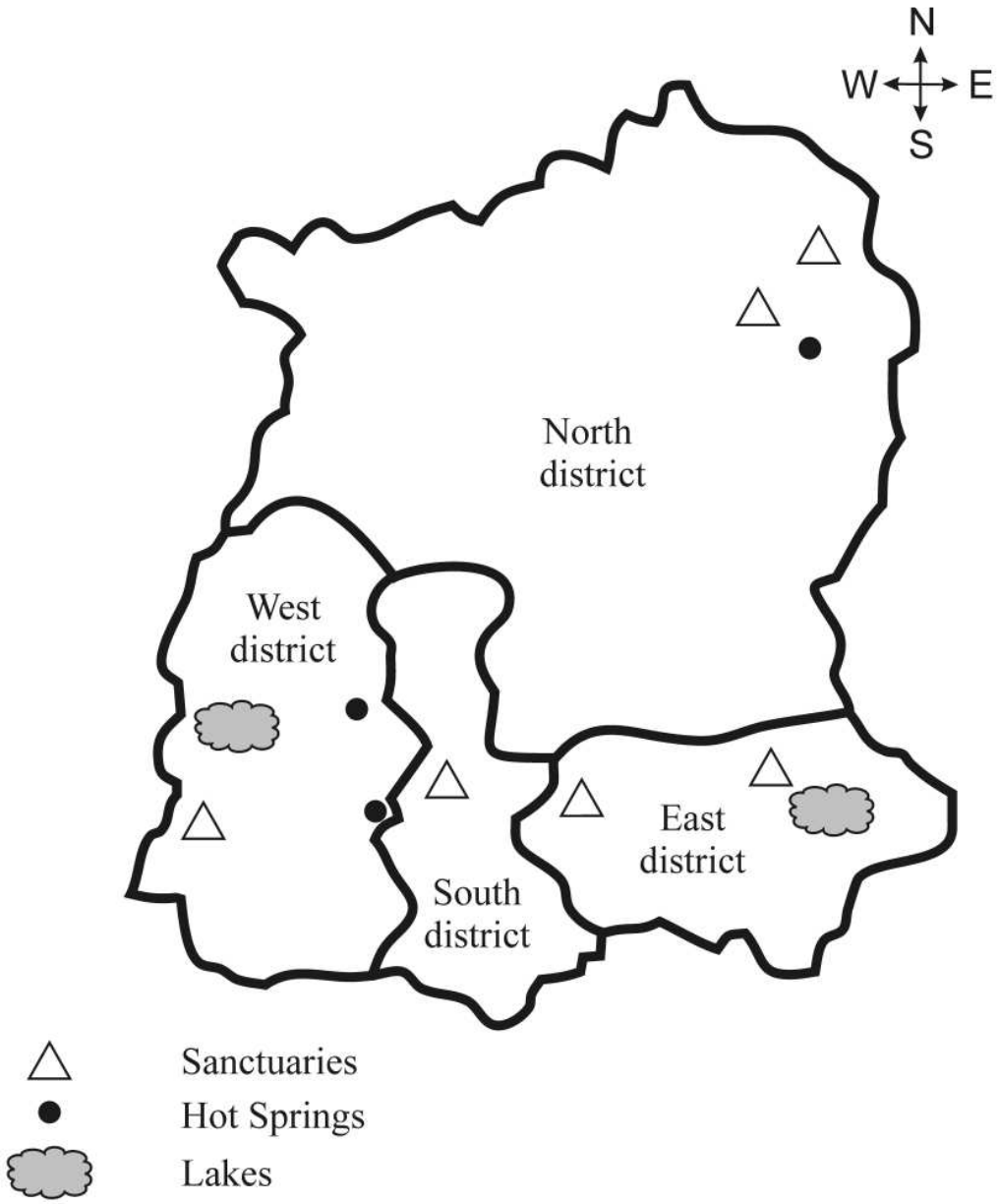
N
W
E
S
North district
West district
East district
South district
Sanctuaries
Hot Springs
Lakes

Table 3.1. Hypotrichous ciliate fauna of Maenam Wildlife Sanctuary, Sikkim.

S. No.	Name of the species
A.	*Gonostomum affine*
B.	*Gonostomum singhii*
C.	*Cotterillia bromelicola*
D.	*Sterkiella cavicola*
E.	*Sterkiella histriomuscorum* (with 5 transverse cirri)
F.	*Sterkiella histriomuscorum* (with 4 transverse cirri)
G.	*Gastrostyla minima*
H.	*Caudiholosticha* sp. 1
I.	*Gastrostyla dorsicirrata*
J.	*Pattersoniella vitiphila*
K.	*Anteholosticha intermedia*
L.	*Bakuella edaphoni*
M.	*Caudiholosticha* sp. 2

Table 3.2. Non-hypotrichous ciliate fauna of Maenam Wildlife Sanctuary, Sikkim.

S. No.	Name of the species
A.	*Colpidium* sp.
B.	*Colpoda* sp.
C.	*Paracolpidium* sp.
D.	*Colpoda* sp.
E.	*Bresslaua* sp.
F.	*Spathidium* sp.
G.	*Frontonia* sp. 1
H.	*Frontonia* sp. 2
I.	*Frontonia* sp. 3
J.	*Vorticella* sp.

Table 3.3. Hypotrichous ciliate fauna of Barsey Rhododendron Sanctuary, Sikkim

S. No.	Name of the species
A.	*Oxytricha granulifera*
B.	*Oxytricha* sp.
C.	*Oxytricha longigranulosa*
D.	*Oxytricha* sp.
E.	*Cyrtohymena citrina*
F.	*Urosomoida* sp.
G.	*Cyrtohymena* (*Cyrtohymenides*) *shii*
H.	*Sterkiella cavicola* sub sp. 1
I.	*Sterkiella cavicola* sub sp. 2
J.	*Sterkiella histriomuscorum* sub sp. 1
K.	*Sterkiella histriomuscorum* sub sp. 2
L.	*Sterkiella histriomuscorum* sub sp. 4
M.	*Stylonychia ammermanni*
N.	*Pattersoniella vitiphila*
O.	*Gastrostyla* sp. 1
P.	*Gastrostyla* sp. 2
Q.	*Bakuella* sp.
R.	*Caudiholosticha* sp.
S.	*Anteholosticha* sp.

Table 3.4. Non-hypotrichous ciliate fauna of Barsey Rhododendron Sanctuary, Sikkim

S. No.	Name of the species
A.	*Paramecium* sp. 1
B.	*Paramecium* sp. 2
C.	*Bresslaua* sp.
D.	*Tetrahymena* sp.
E.	*Spathidium* sp.
F.	*Stombilidium* sp.
G.	*Colpoda* sp. 1
H.	*Colpoda* sp. 2
I.	*Dileptus* sp.
J.	*Blepharisma* sp.

Table 3.5. Hypotrichous and non-hypotrichous ciliate fauna of Khecheopalri Lake, Sikkim

S. No.	Name of the species
A.	*Gonostomum kuehnelti*
B.	*Gonostomum affine*
C.	*Oxytricha longigranulosa*
D.	*Notohymena antarctica*
E.	*Sterkiella cavicola*
F.	*Rigidohymena tetracirrata*
G.	*Steinia sphagnicola*
H.	*Laurentiella strenua*
I.	*Pseudouroleptus caudatum*
J.	*Euplotes encysticus*
K.	*Bresslaua* sp.
L.	*Colpoda inflata*
M.	*Pleuronema* sp.
N.	*Stombilidium* sp.
O.	*Dileptus* sp.
P.	*Vorticella* sp.
Q.	*Spathidium* sp.

Table 3.6. Hypotrichous ciliate fauna of Leg ship Hot Spring, Sikkim.

S. No.	Name of the species
A.	*Gonostomum gonostomoida*
B.	*Oxytricha* sp. 1
C.	*Oxytricha* sp. 2
D.	*Urosomoida longa*
E.	*Rigidohymena tetracirrata*
F.	*Stylonychia* sp.
G.	*Pattersoniella vitiphila*
H.	An Amphisiellid sp.
I.	*Hemiamphisiella terricola*
J.	*Euplotes* sp.

Table 3.7. The only hypotrichous ciliate reported from Borang Hot Spring, Sikkim.

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Name of the species
Sterkiella cavicola

Table 3.8. Hypotrichous and non-hypotrichous ciliate fauna of Fambong Lho Wildlife Sanctuary, Sikkim.

S. No.	Name of the species
A.	*Gonostomum* sp.
B.	*Oxytricha granulifera*
C.	*Notohymena* sp.
D.	*Urosomoida* sp.
E.	*Sterkiella histriomuscorum*
F.	*Pseudouroleptus caudatus*
G.	*Caudiholosticha* sp.
H.	*Bresslaua* sp.
I.	*Plytyophryideslatus* sp.
J.	*Spathidium* sp. 1
K.	*Spathidium* sp. 2
L.	*Frontonia* sp.

Table 3.9. Hypotrichous and non-hypotrichous ciliate fauna of Changu Lake/Tsongmo Lake, Sikkim.

S. No.	Name of the species
A.	*Gonostomum strenuum*
B.	*Gonostomum* sp.
C.	*Urosomoida agilis* 1
D.	*Urosomoida agilis* 2
E.	*Oxytricha* sp.
F.	*Sterkiella histriomuscorum*
G.	*Sterkiella tricirrata*
H.	*Dileptus* sp.
I.	*Spathidium* sp.
J.	*Colpoda steinii*

Table 3.10. Hypotrichous and non-hypotrichous ciliate fauna of Kyongnosla Alpine Sanctuary, Sikkim.

S. No.	Name of the species
A.	*Gonostomum affine*
B.	*Notohymena* sp.
C.	*Oxytricha* sp. 1
D.	*Oxytricha* sp. 2
E.	***Paraurosomoida indiensis* gen. nov., sp. nov.**
F.	*Sterkiella cavicola*
G.	*Sterkiella tricirrata*
H.	*Territricha* sp.
I.	*Pseudouroleptus caudatum*
J.	*Tetrahymena* sp.
K.	*Colpoda cucullus*

Table 3.11. Hypotrichous and non-hypotrichous ciliate fauna of Yumthang Flower Valley, the area around Yumesamdong Hot Spring and Yumesamdong/Zero point, Sikkim.

S. No.	Name of the species
A.	*Notohymena granulifera*
B.	*Urosomoida agilis* subspecies 1
C.	*Urosomoida agilis* subspecies 2
D.	*Sterkiella histriomuscorum*
E.	*Sterkiella tricirrata*
F.	A *Stichotrichine*
G.	*Gonostomum affine* subspecies 1
H.	*Gonostomum affine* subspecies 2
I.	*Urosomoida* sp.
J.	*Sterkiella histriomuscorum*
K.	*Parakahiella haideri*
L.	*Cyrtohymena citrina*
M.	Unidentified
N.	*Trithigmostoma* sp.
O.	*Prostomata* sp.
P.	*Curimostoma* sp.
Q.	*Chilodonella* sp.
R.	Unidentified

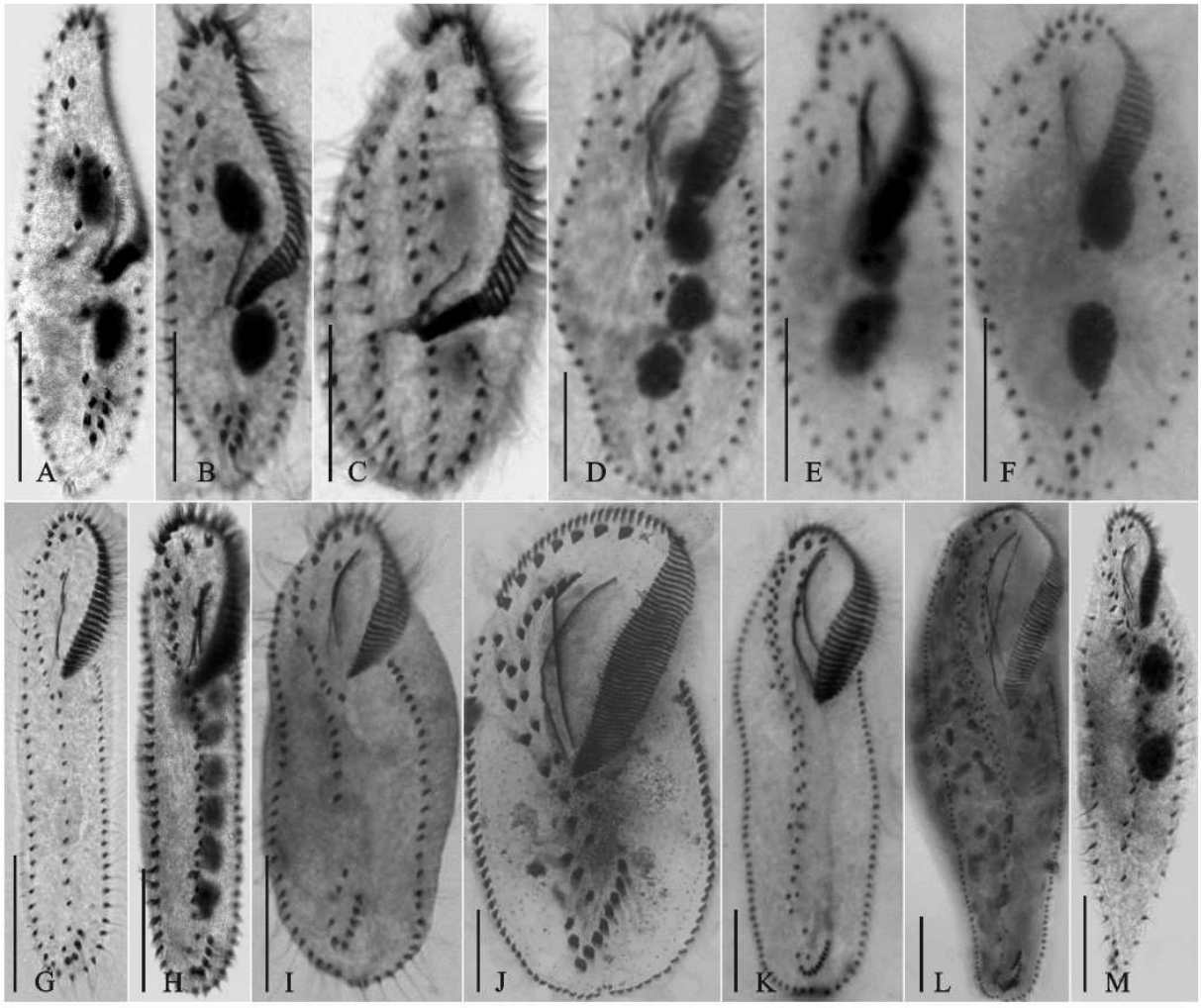


Fig. 3.2. Photomicrographs of hypotrichous ciliate fauna from Maenam Wildlife Sanctuary, Sikkim. Bars: 25µm.

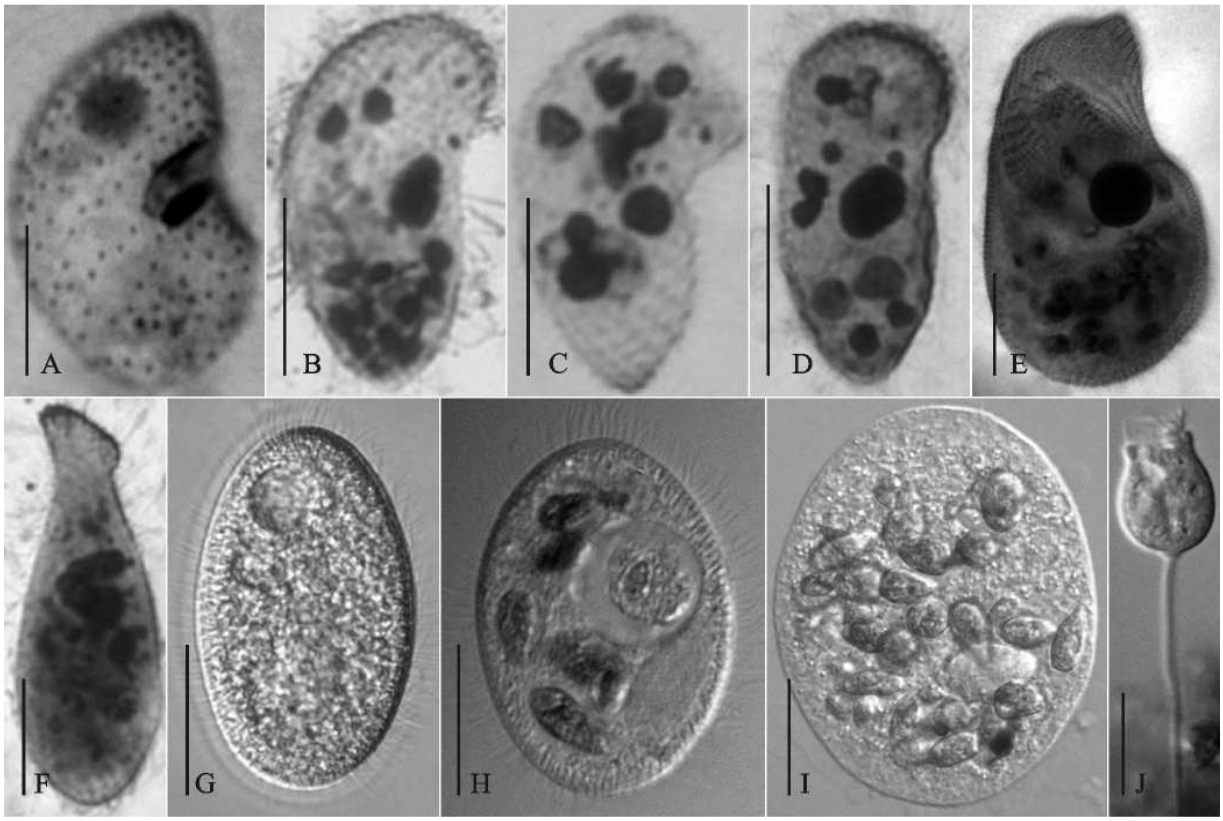


Fig. 3.3. Photomicrographs of non-hypotrichous ciliate fauna from Maenam Wildlife Sanctuary, Sikkim. Bars: 25μm.

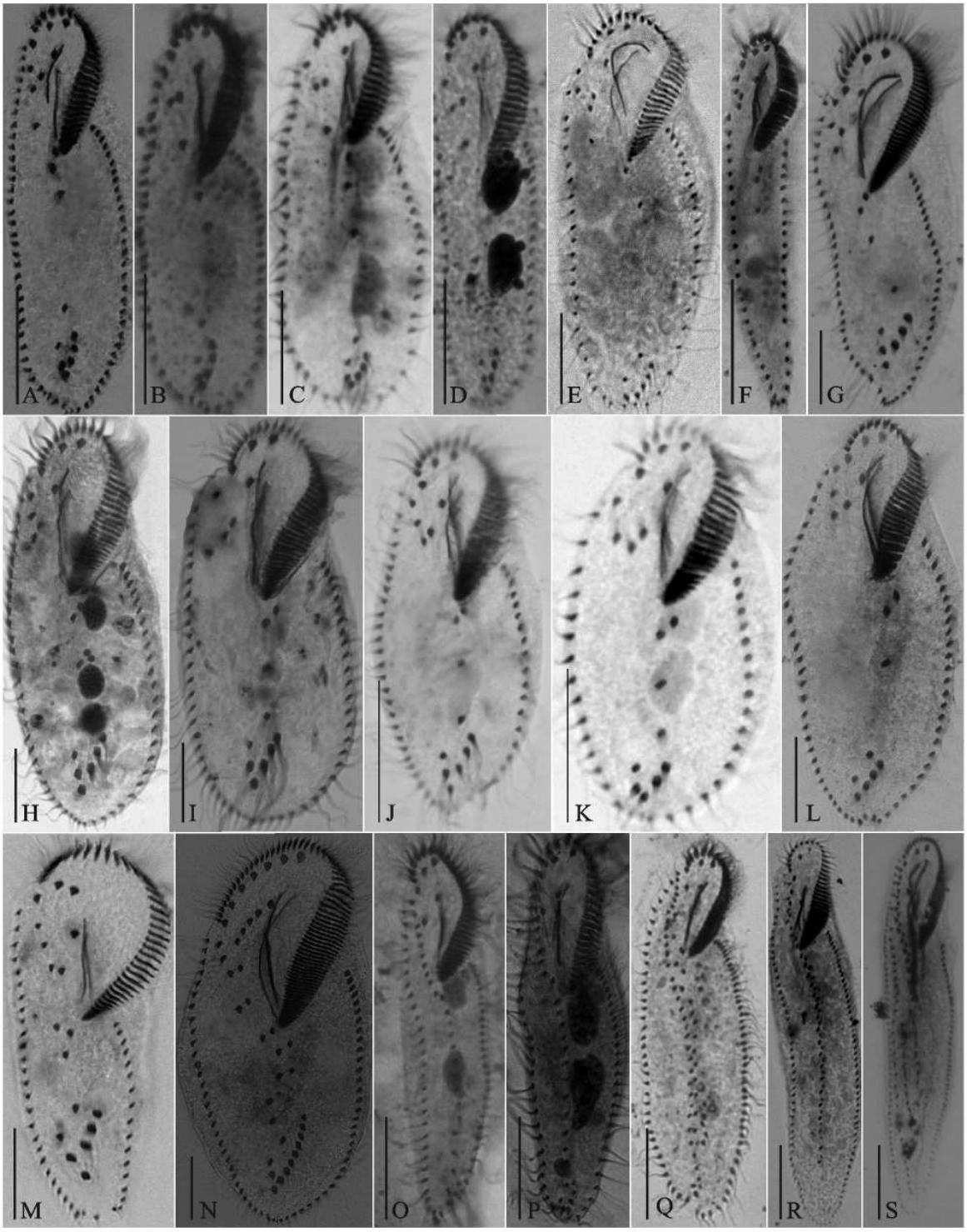


Fig. 3.4. Photomicrographs of hypotrichous ciliate fauna from Barsey Rhododendron Sanctuary, Sikkim. Bars: 25µm.

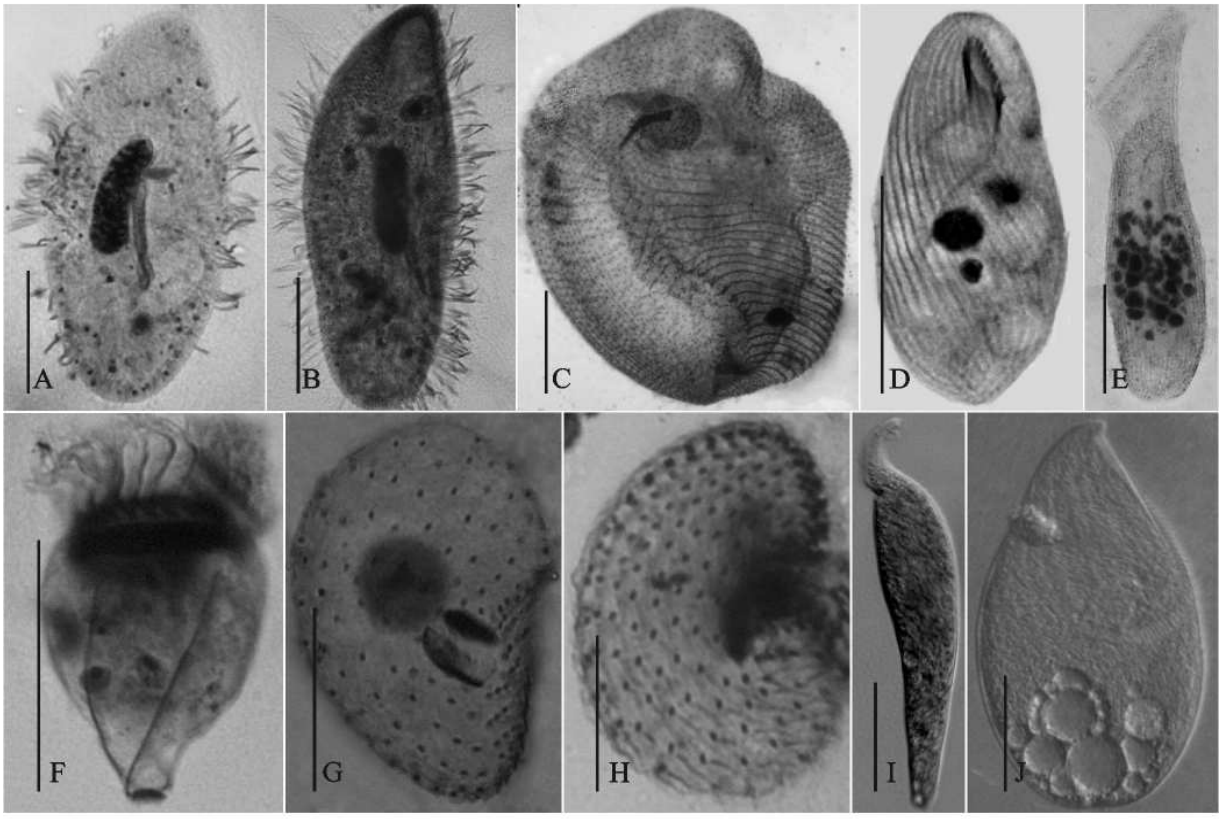


Fig. 3.5. Photomicrographs of non-hypotrichous ciliate fauna from Barsey Rhododendron Sanctuary, Sikkim. Bars: 25µm.

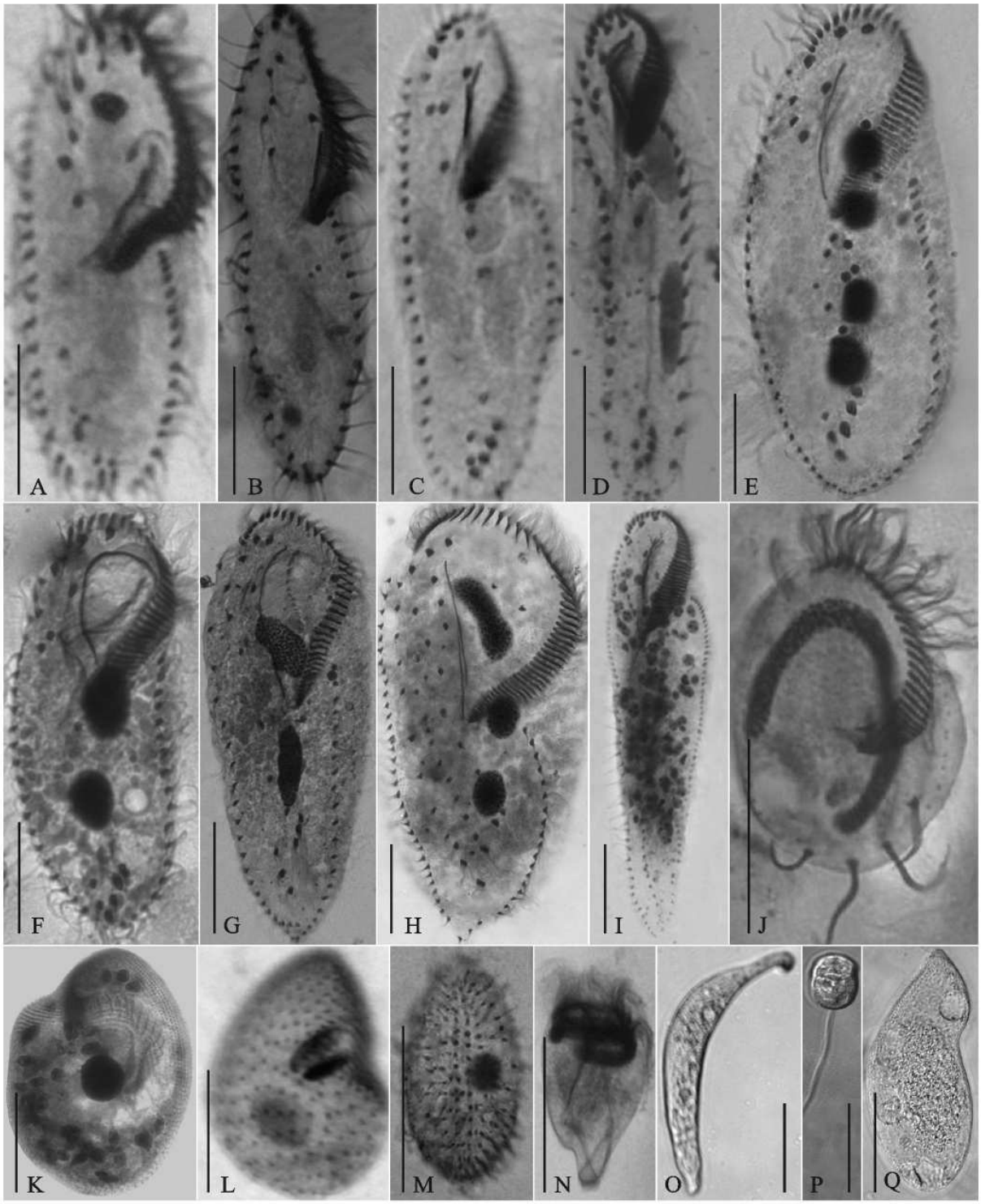


Fig. 3.6. Photomicrographs of ciliate fauna from Khecheopalri Lake, Sikkim. Bars: 25µm.

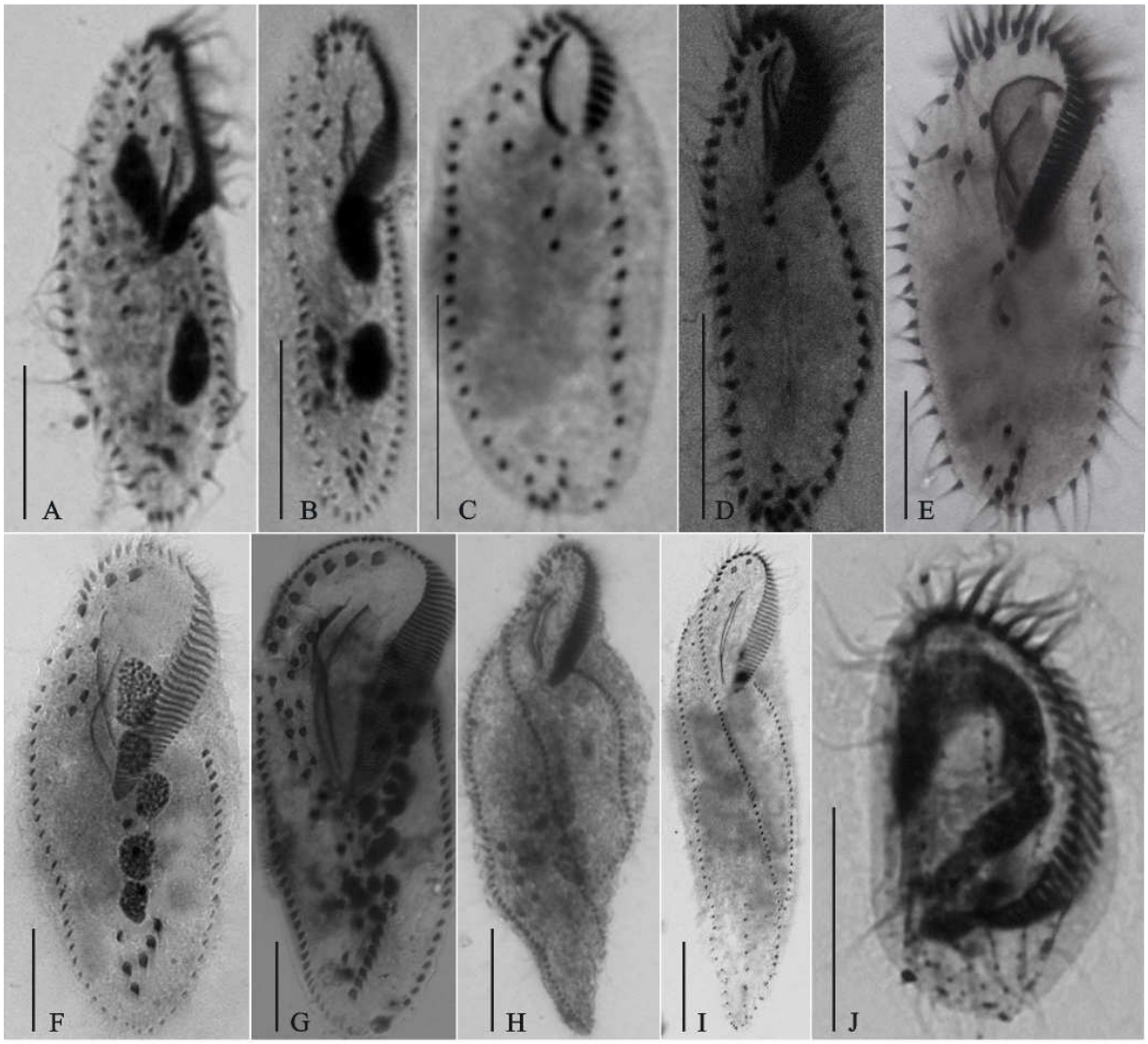


Fig. 3.7. Photomicrographs of ciliate fauna from Legship Hot Spring, Sikkim. Bars: 25µm.

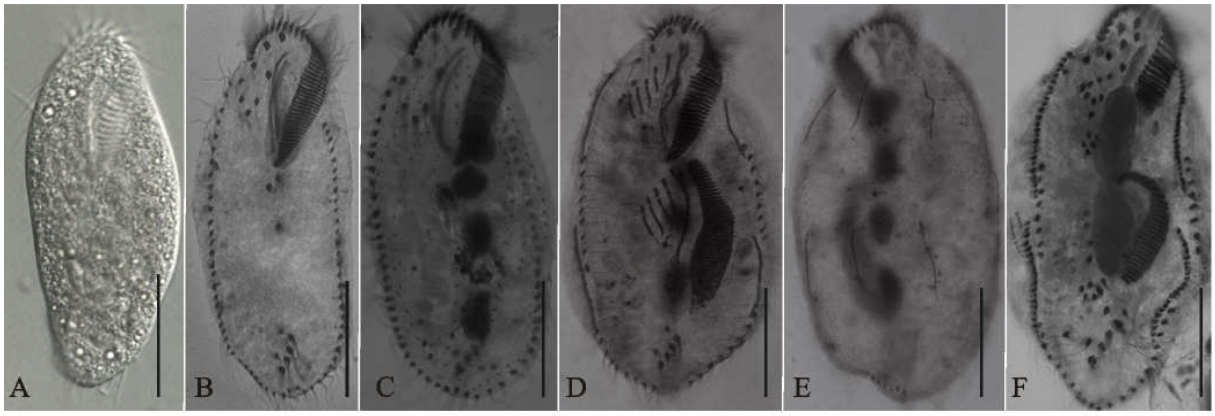


Fig. 3.8. Photomicrographs of *Sterkiella cavicola* Borang Hot Spring Population (A; in vivo, Differential Interference Contrast and B-F; after protargol impregnation). **A, B**. Infraciliature of interphasic cells; A, ventral view; B, dorsal view. **C-F.** Infraciliature of morphogenetic stages. Bars: 25µm.

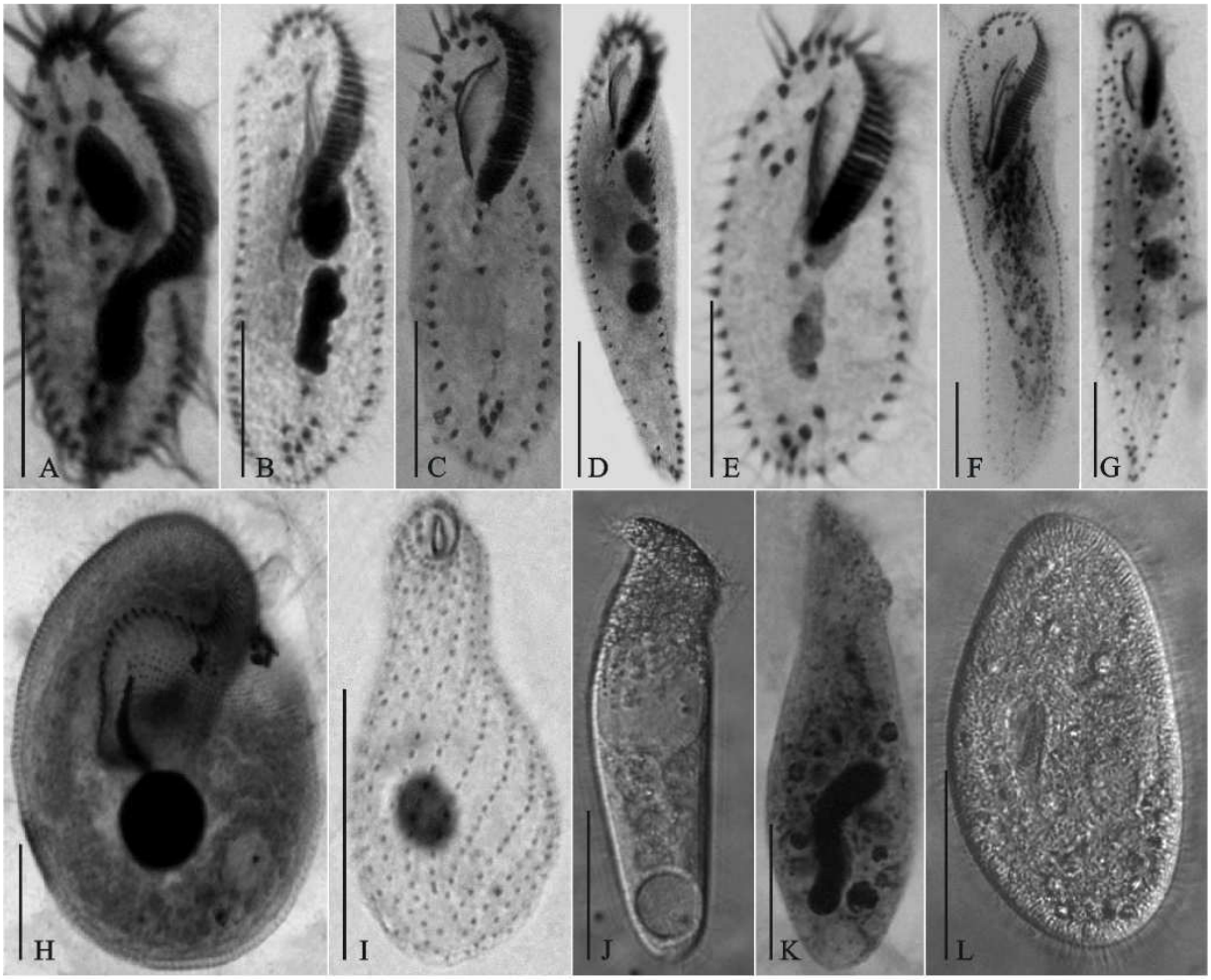


Fig. 3.9. Photomicrographs of ciliate fauna from Fambong Lho Wildlife Sanctuary, Sikkim. Bars: 25µm.

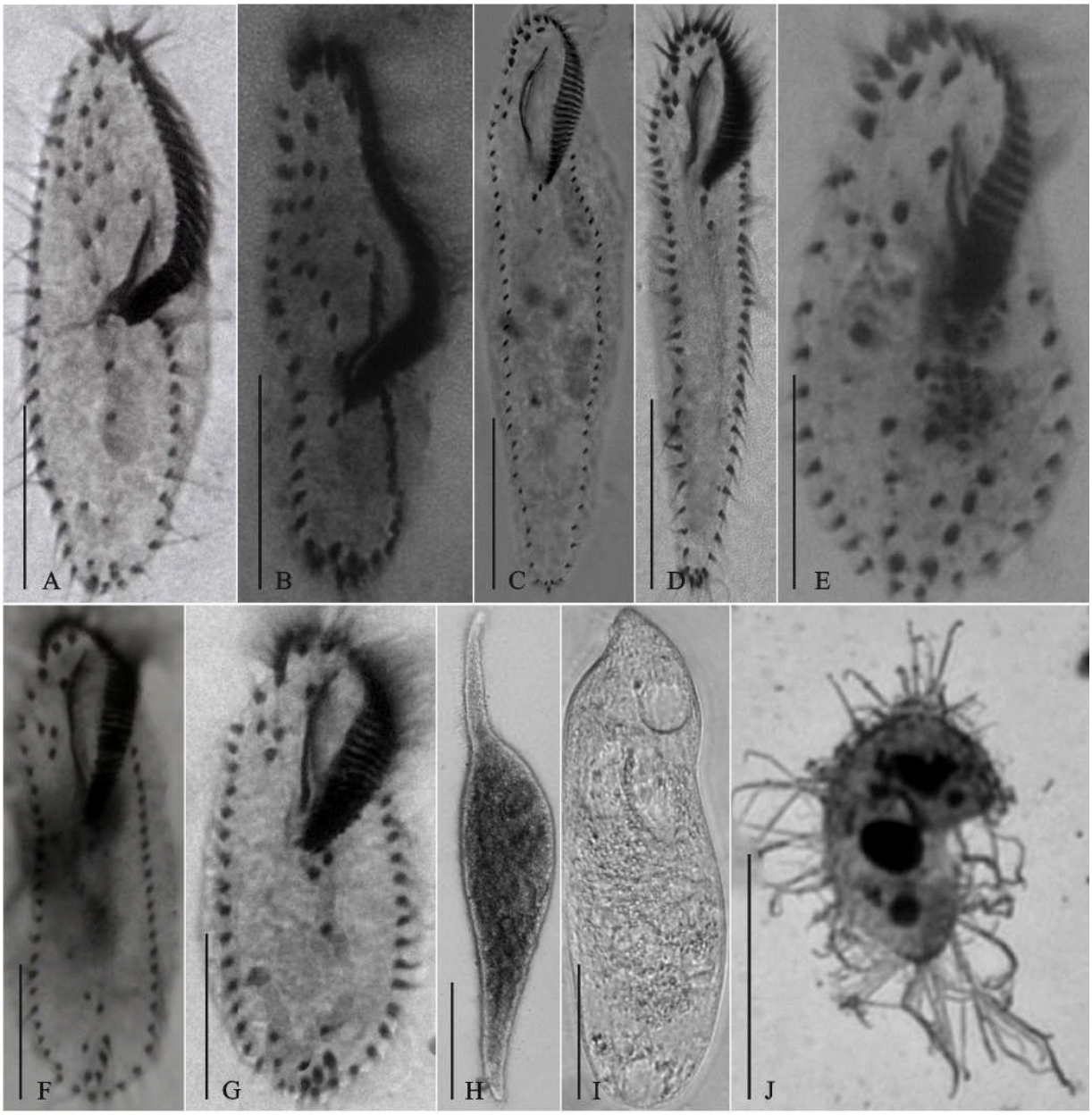


Fig. 3.10. Photomicrographs of ciliate fauna from Changu Lake, Sikkim. Bars: 25µm.

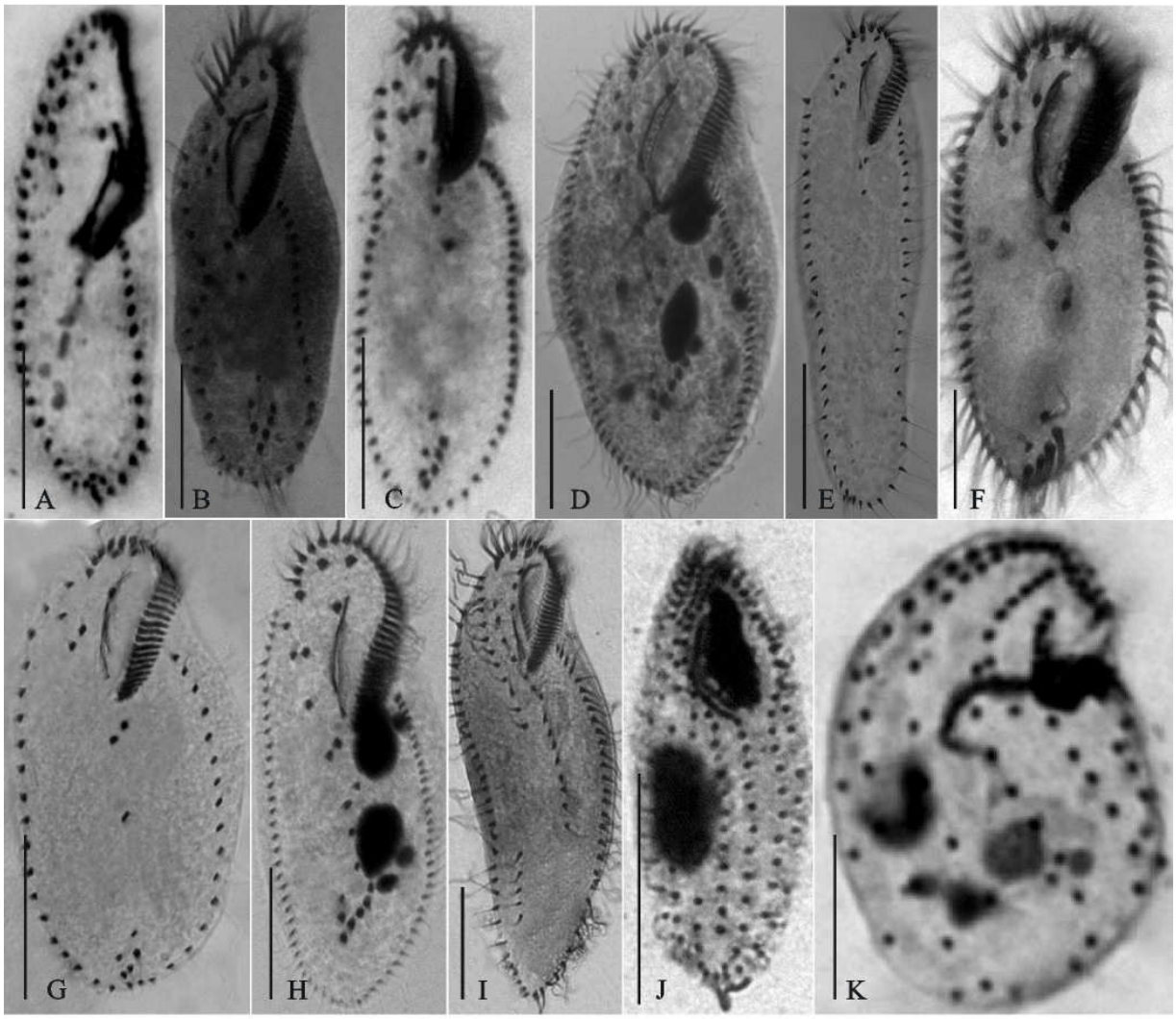


Fig. 3.11. Photomicrographs of ciliate fauna from Kyongnosla Alpine Sanctuary, Sikkim. Bars: 25µm.

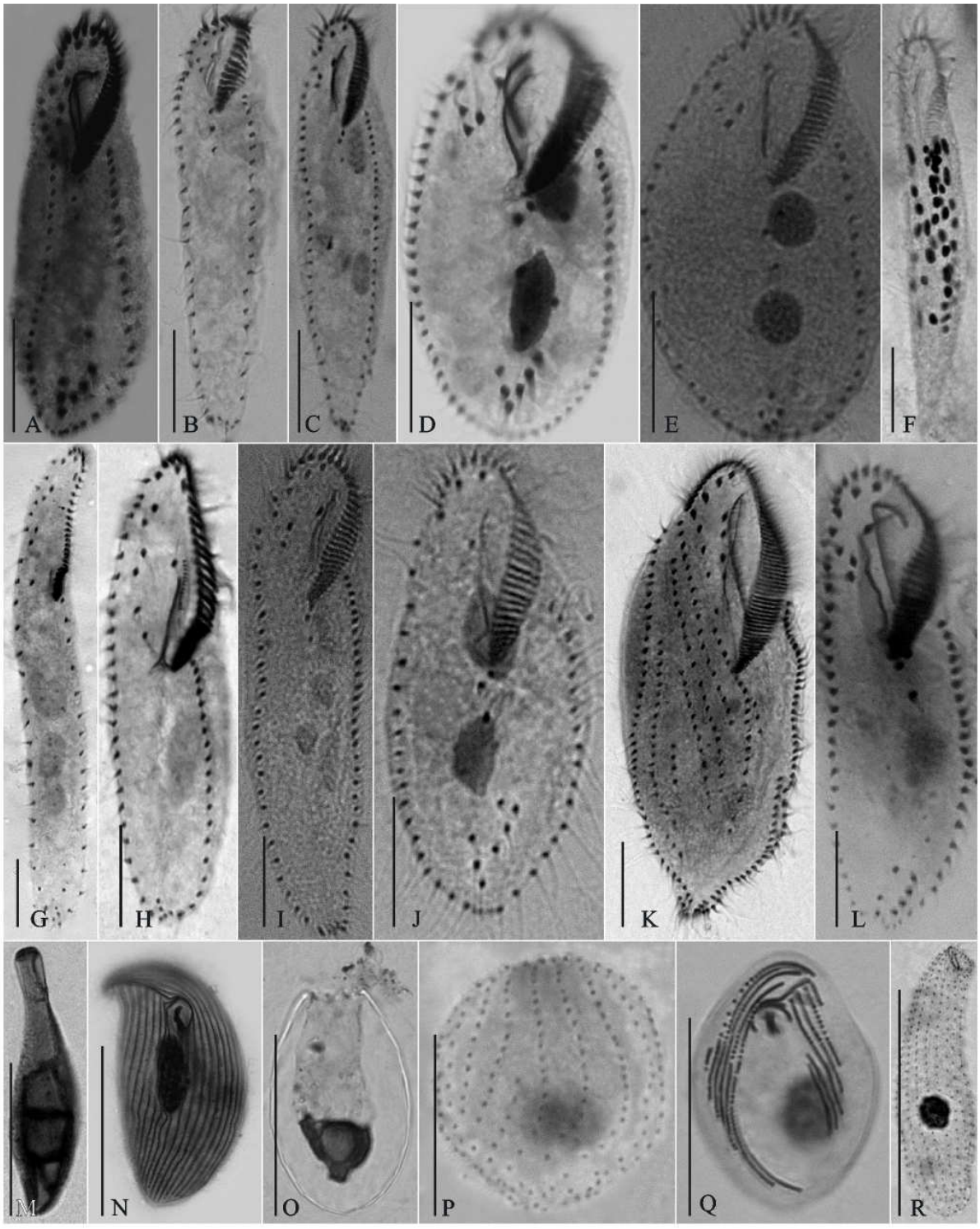


Fig. 3.12. Photomicrographs of ciliate fauna from Yumthang Flower Valley, Yumesamdong Hot Spring and Yumesamdong/Zero point, Sikkim. Bars: 25µm.

Fig. 3.13. Diagram suggesting proposed phylogenetic relationships within the Hypotricha. Paraphyletic groups are marked by quotation marks. The question marks indicate uncertainty in the classification. At the present state of knowledge it is impossible to estimate which taxa branched off first within a certain group. Main morphological autapomorphies (circles 1-4): **1** □ 18 frontal-ventral-transverse cirri; three dorsal kineties; three caudal cirri; contractile vacuole at left body margin; high support by molecular data. **2** □ dorsomarginal kineties present. **3** □ fragmentation of dorsal kinety 3. **4** □ body rigid; adoral zone of membranelles ≥ 40% of body length; cortical granules lacking; high support by molecular data (Adapted from Berger 2006).

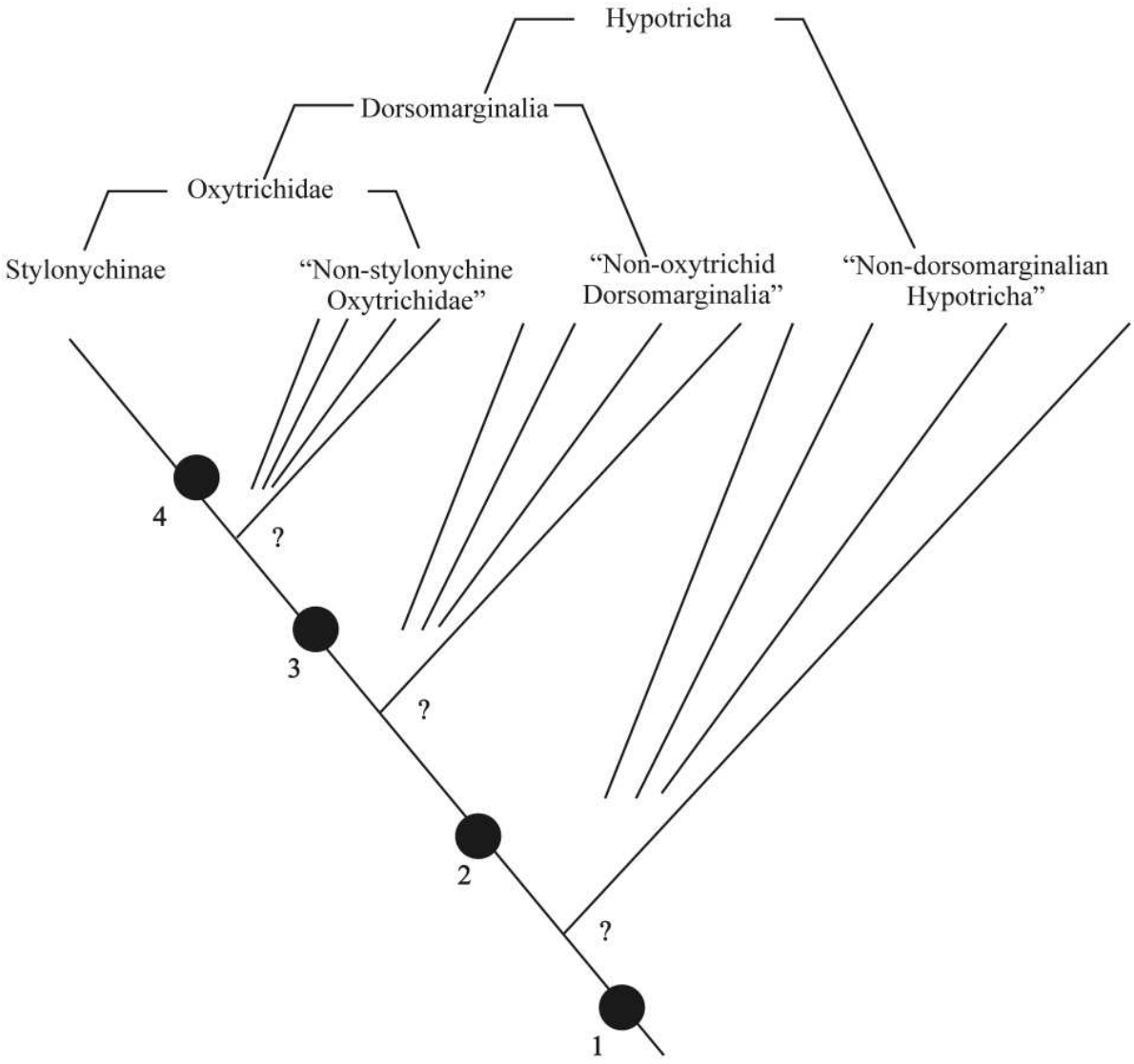
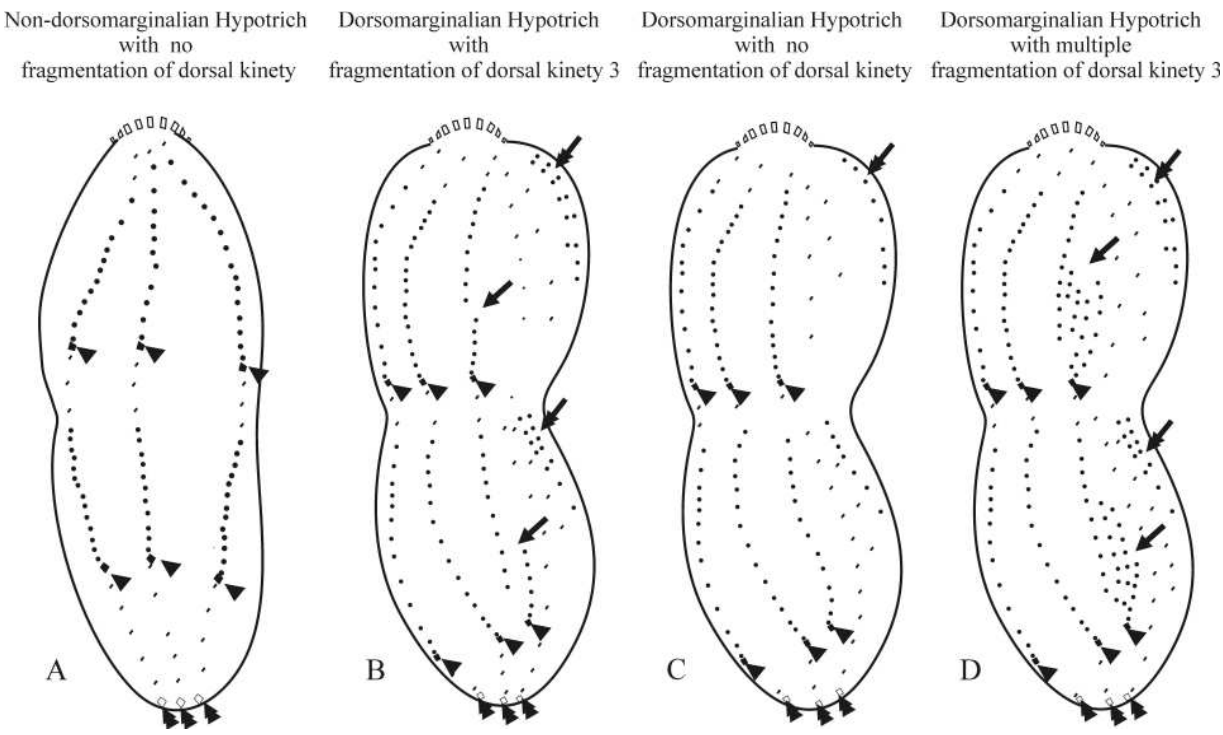
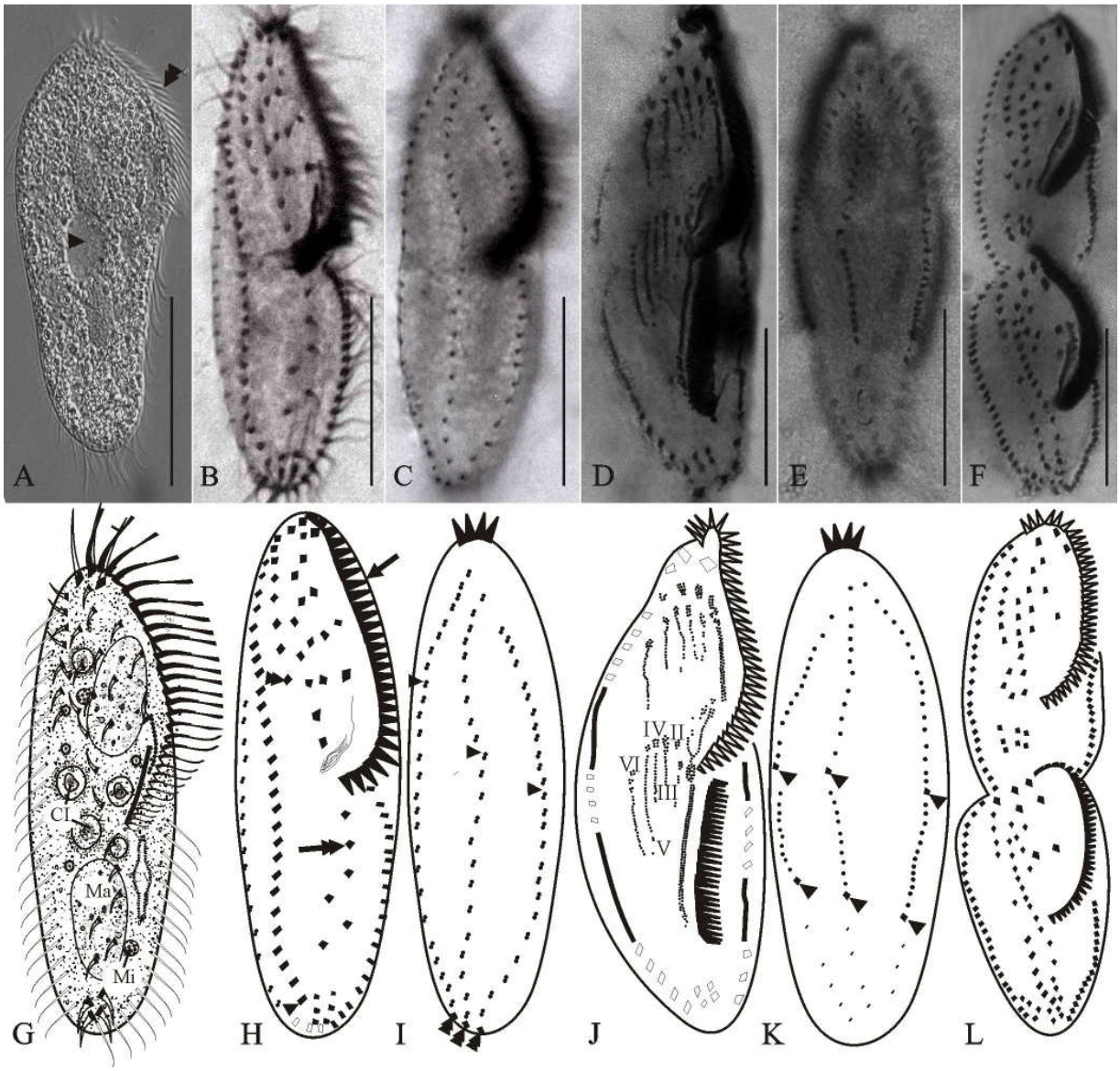

Hypotricha
Dorsomarginalia
Oxytrichidae
Stylonychinae
"Non-stylonychine Oxytrichidae"
"Non-oxytrichid Dorsomarginalia"
"Non-dorsomarginalian Hypotricha"
4
?
3
?
2
?
1

Fig. 3.14. Schematic illustration of dorsal infraciliature, non dorsomarginalian hypotrich e.g. *Gonostomum* (A); three states of fragmentation of dorsal kinety 3 in dorsomarginalian oxytrichid hypotrichs (B-C), arrows show fragmentation of dorsal kinety; double arrows show dorsomarginal rows; arrowheads show caudal cirri formed at the posterior end of each (in A; all three bipolar DK $_{1,2 \text{ and } 3}$; in B and C, DK $_{1,2 \text{ and } 4}$ and in D, DK $_{1,2 \text{ and right most one}}$) new dorsal kinety. Double arrowheads show parental caudal cirri which will are resorbed later.


Non-dorsomarginalian Hypotrich with no fragmentation of dorsal kinety
Dorsomarginalian Hypotrich with fragmentation of dorsal kinety 3
Dorsomarginalian Hypotrich with no fragmentation of dorsal kinety
Dorsomarginalian Hypotrich with multiple fragmentation of dorsal kinety 3
A
B
C
D

Figs 4.1 A–L. Photomicrographs and line diagrams of *Gonostomum strenuum* Sikkim population *in vivo* (A; Differential Interference Contrast and G; line diagram) and after protargol impregnation (B–F; photomicrographs and H–L; line diagrams). **A.** Complete interphasic cell showing, contractile vacuole (arrow head) and characteristic AZM (double arrow head) in *Gonostomum* pattern. **B and C.** Infraciliature of interphasic cells; B, ventral and C, dorsal surface. **D and E.** Infraciliature of middle dividers; D, ventral and E, dorsal surface. **F.** Ventral infraciliature of a very late divider. **G.** Ventral view of a complete cell. CI, cytoplasmic inclusions; CV, contractile vacuole; Ma, macronuclear nodules; Mi, micronuclei. **H.** Infraciliature of ventral surface, arrow shows AZM characteristic in *Gonostomum* pattern; right marginal row (arrowhead) finishes just at the level of transverse cirri, left marginal row goes up to posterior end surrounding transverse cirri; long frontoventral row (double arrow) with increased number of cirri, both the characters typical of *G. strenuum*; migratory cirri (double arrowhead) and other frontoventral cirri also shows increase in number. **I.** Dorsal side, arrow head depicts three dorsal kineties with no fragmentation again characteristic of *Gonostomum*; double arrowheads show caudal cirri. **J.** Ventral infraciliature of a middle divider, anlagen in the form of cirral streaks are marked II-VI. **K.** Dorsal infraciliature, showing new kineties formed only by within row proliferation and not by fragmentation of dorsal kinety seen in other oxytrichids; arrowheads show caudal cirri formed at the posterior most end of each new dorsal kinety in proter and opisthe. **L.** Ventral infraciliature of very late divider, new AZM (dark) is formed for opisthe and parental AZM (light) is retained for proter. Bars: 25μm.


A
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C
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K
L
Cl
Ma
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IV
II
VI
III
V

Fig 5.1A–J. Photomicrographs and line diagrams of *Oxytricha granulifera* Sikkim population *in vivo* (A; Differential Interference Contrast and F; line diagram) and after protargol impregnation (B–E; photomicrographs and G–J; line diagrams). **A.** Complete interphasic cell. **B and C.** Infraciliature of interphasic cells; B, ventral and C, dorsal surface. **D and E.** Infraciliature of late dividers; D, ventral and E, dorsal surface. **F.** Complete interphasic cell. CI, cytoplasmic inclusions; CV, contractile vacuole; Ma, macronuclear nodules; Mi, micronuclei. **G.** Infraciliature of ventral surface; arrow showing paroral and endoral characteristically in *Oxytricha* pattern. **H.** Dorsal side with bristle rows, arrow shows short fourth dorsal kinety formed as result of fragmentation of third dorsal kinety; arrowheads show caudal cirri formed at the posterior most end of each new dorsal kinety (DK $_{1,2 \text{ and } 4}$) in proter and opisthe. **I.** Ventral infraciliature of very late divider, arrowheads show newly formed dorsomarginal which later shift to dorsal surface. **J.** Dorsal infraciliature of very late divider, arrows show simple fragmentation of dorsal kinety 3; arrowheads show caudal cirri. Bars: 25μm.

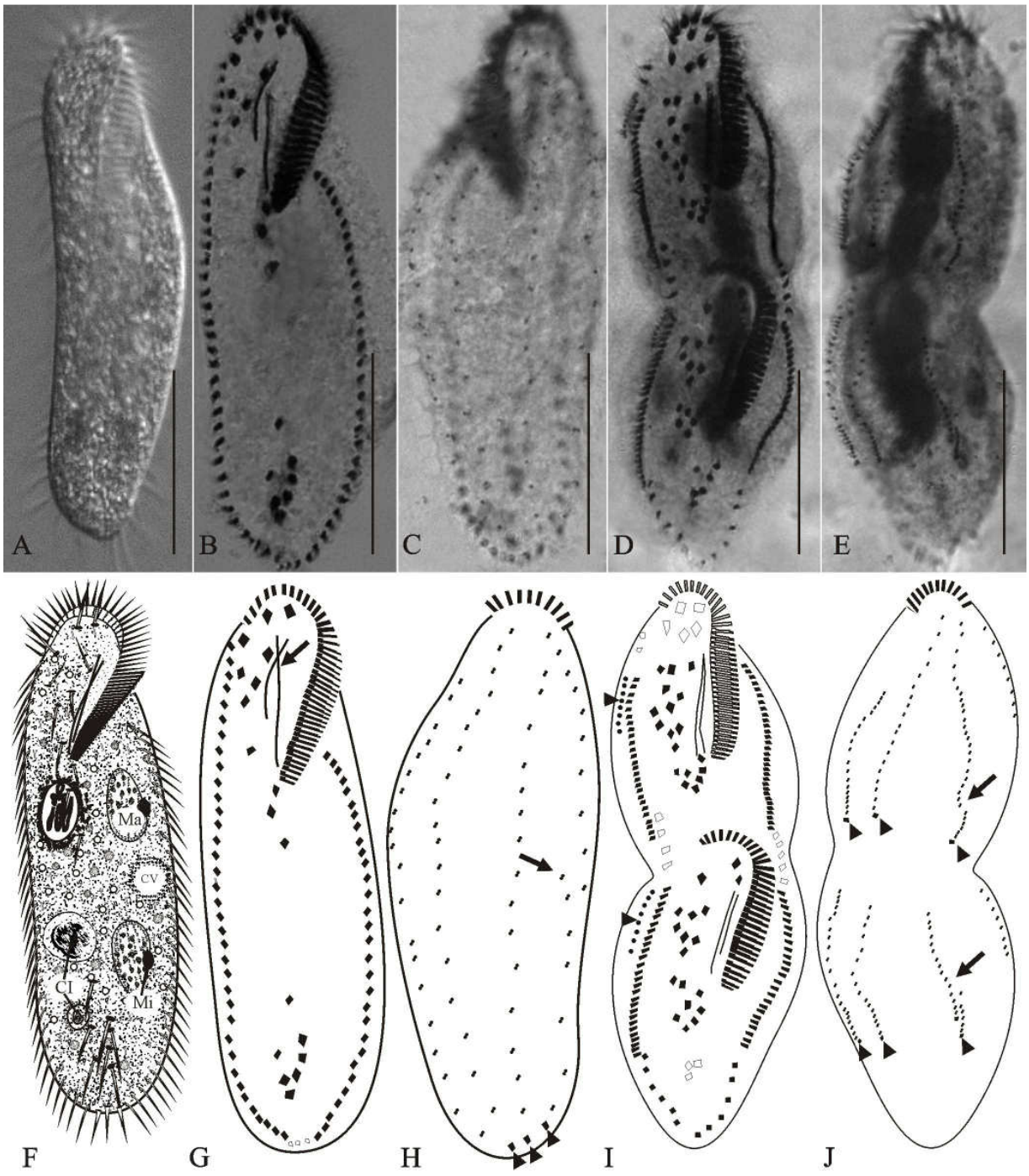
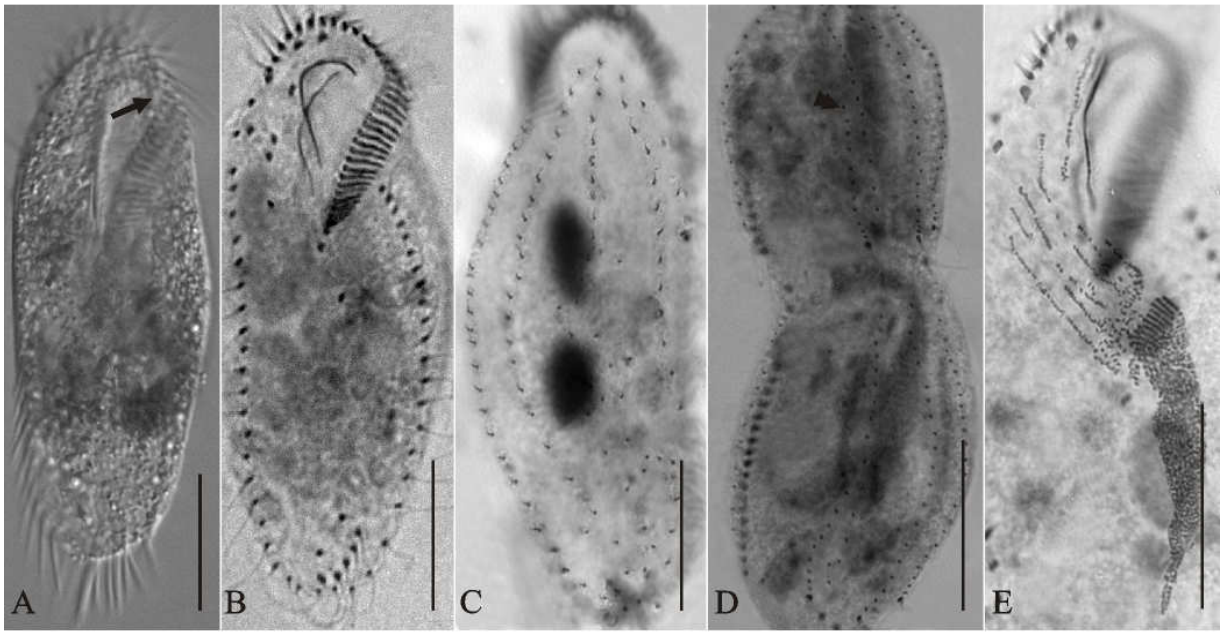

A
B
C
D
E
Ma
CV
CI
Mi
F
G
H
I
J

Fig 5.2A–J. Photomicrographs and line diagrams of *Cyrtohymena citrina* Sikkim population *in vivo* (A; Differential Interference Contrast and F; line diagram) and after protargol impregnation (B–E; photomicrographs and G–J; line diagrams). **A.** Complete interphasic cell, arrow shows paroral typically in *Cyrtohymena* pattern. **B and C.** Infraciliature of interphasic cells; B, ventral and C, dorsal surface. **D.** Dorsal infraciliature of late dividers. **E.** Divider with streaks. **F.** Complete interphasic cell. CI, cytoplasmic inclusions; CV, contractile vacuole; Ma, macronuclear nodules; Mi, micronuclei. **G.** Infraciliature of ventral surface; arrow showing transverse cirri in a row characteristically seen in *C. citrina*; right marginal row (double arrow) almost straight, finishes just at the level of transverse cirri; left marginal row J-shaped goes up to posterior end surrounding transverse cirri from behind; arrowheads show caudal cirri mostly visible from ventral side also **H.** Dorsal side with bristle rows, arrow shows short fourth dorsal kinety formed as result of fragmentation of third dorsal kinety **I.** Dorsal infraciliature of very late divider, arrows show simple fragmentation of dorsal kinety 3, arrowheads show caudal cirri formed at the posterior most end of each new dorsal kinety (DK $_{1,2 \text{ and } 4}$) in proter and opisthe. **J.** Divider with streaks, arrow shows origin of anlagen VI for proter from anlagen VI of opisthe and is thus not originating *de novo* as reported for genus *Cyrtohymena*. Bars: 25µm.


A
B
C
D
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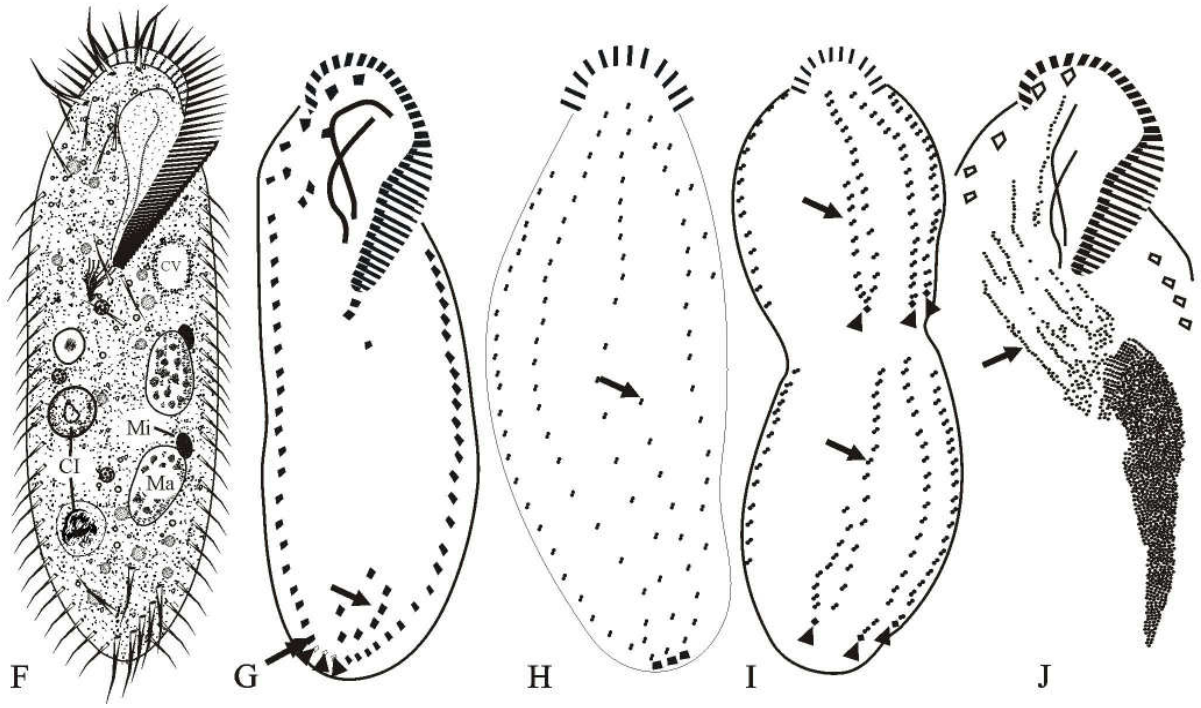
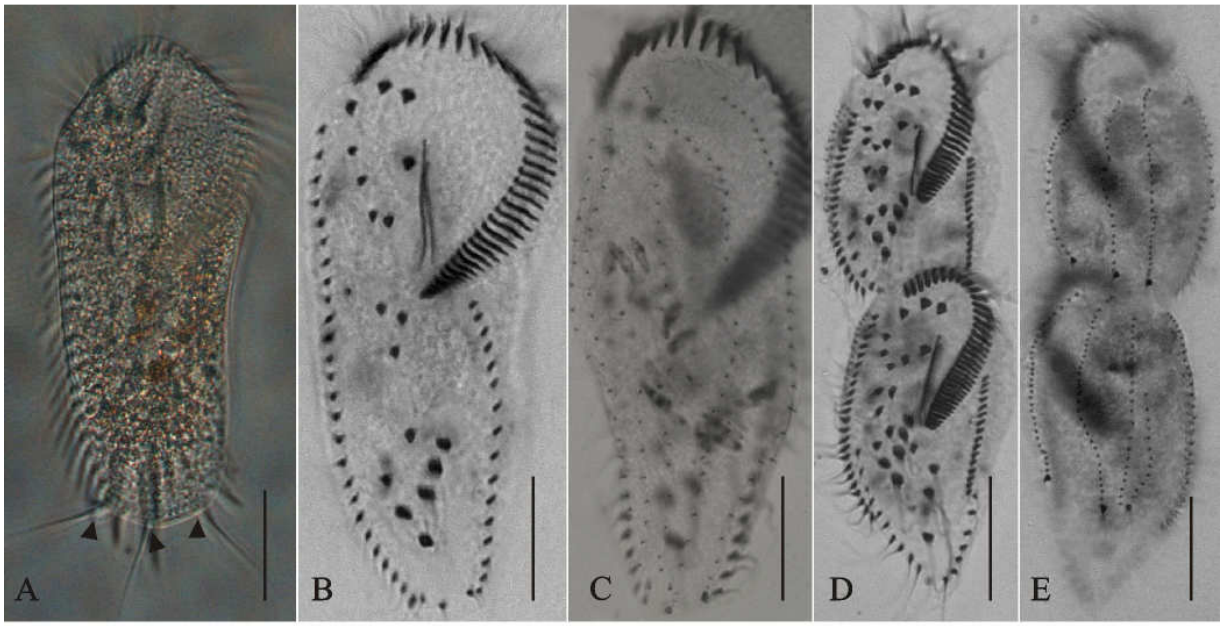

cv
Mi
Cl
Ma
F
G
H
I
J

Fig 5.3A–J. Photomicrographs and line diagrams of *Stylonychia ammermanni* Sikkim population *in vivo* (A; Differential Interference Contrast and F; line diagram) and after protargol impregnation (B–E; photomicrographs and G–J; line diagrams). **A.** Complete interphasic cell, arrowheads showing caudal cirri. **B and C.** Infraciliature of interphasic cells; B, ventral and C, dorsal surface. **D and E.** Infraciliature of late dividers; D, ventral and E, dorsal surface. **F.** Complete interphasic cell. CI, cytoplasmic inclusions; CV, contractile vacuole; Ma, macronuclear nodules; Mi, micronuclei. **G.** Infraciliature of ventral surface; AZM > 40%; paroral and endoral (arrow) characteristically in *Stylonychia* pattern; transverse cirri (double arrow) typically in 3+2 pattern; posterior end of cell truncated with right and left marginal rows distinctly separate and caudal cirri located in a space between two rows. **H.** Dorsal infraciliature with bristle rows, shows fourth bristle row (arrow) short and third row complete; arrowheads show caudal cirri formed at the posterior most end of each new dorsal kinety ($DK_{1,2\ and\ 4}$). **I.** Ventral infraciliature of very late divider, arrow heads show newly formed dorsomarginal which later shift to dorsal surface. **J.** Dorsal infraciliature of a late divider, morphogenesis proceeds with simple fragmentation of dorsal kinety 3, arrows depict the breaking point of fragmentation of third dorsal kinety to give rise to fourth dorsal kinety, in opisthe the process is advanced therefore shows separate third and fourth dorsal kinety; arrowheads show caudal cirri; double arrowheads show dorsomarginal rows. Bars: 25µm.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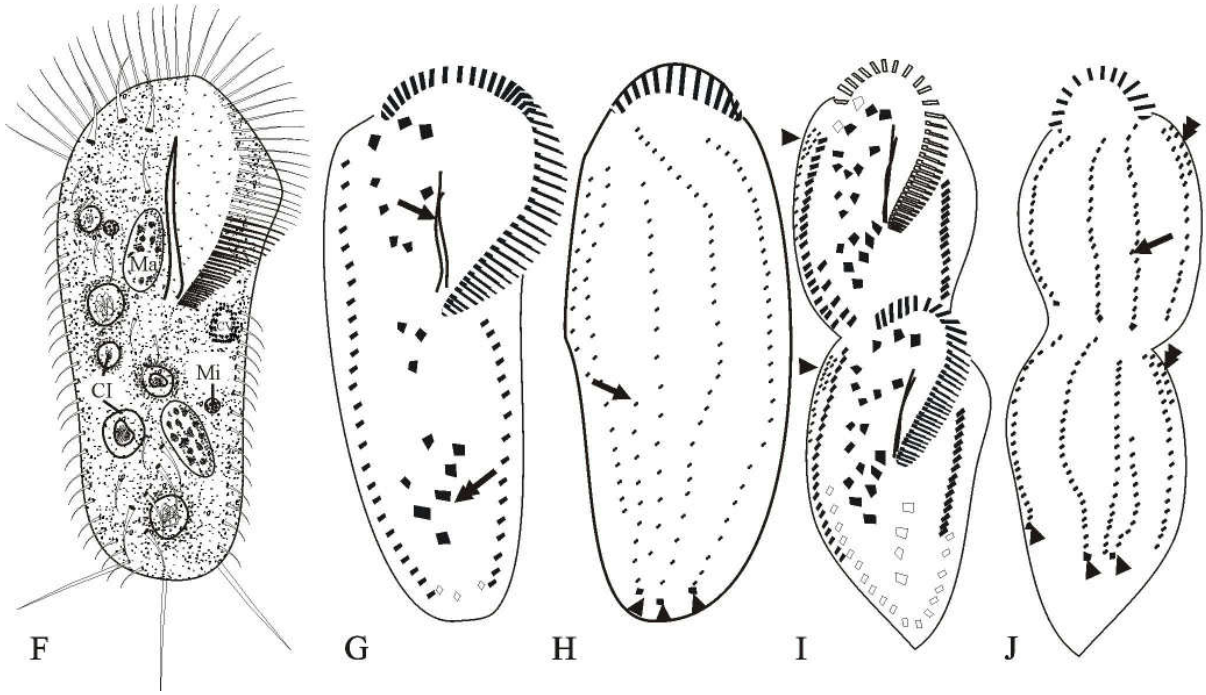
Ma
Mi
Cl
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G
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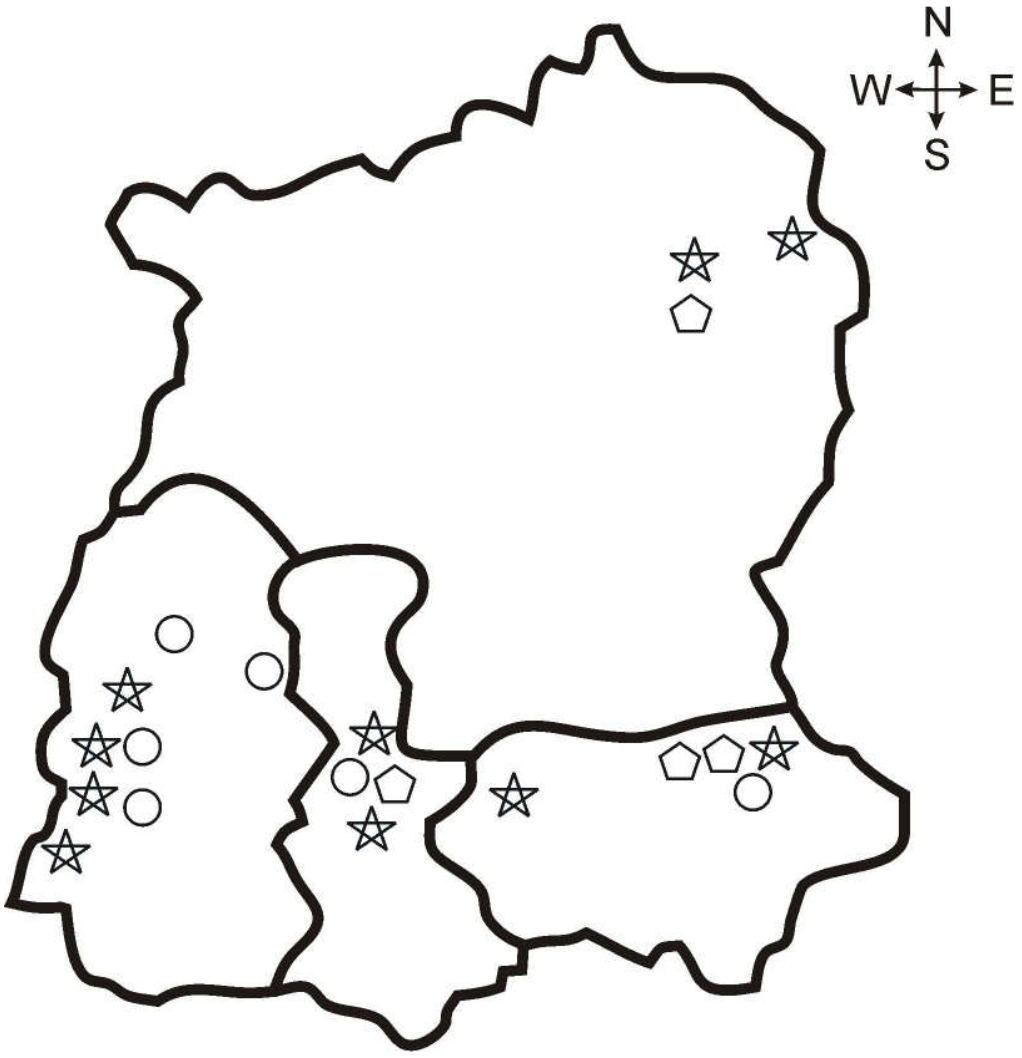
J

Fig. 5.4. Map of Sikkim (not to scale) showing district wise location of collection sites; star represents *Sterkiella histriomuscorum,* circle represents *Sterkiella cavicola* and pentagon represents *Sterkiella tricirrata.*

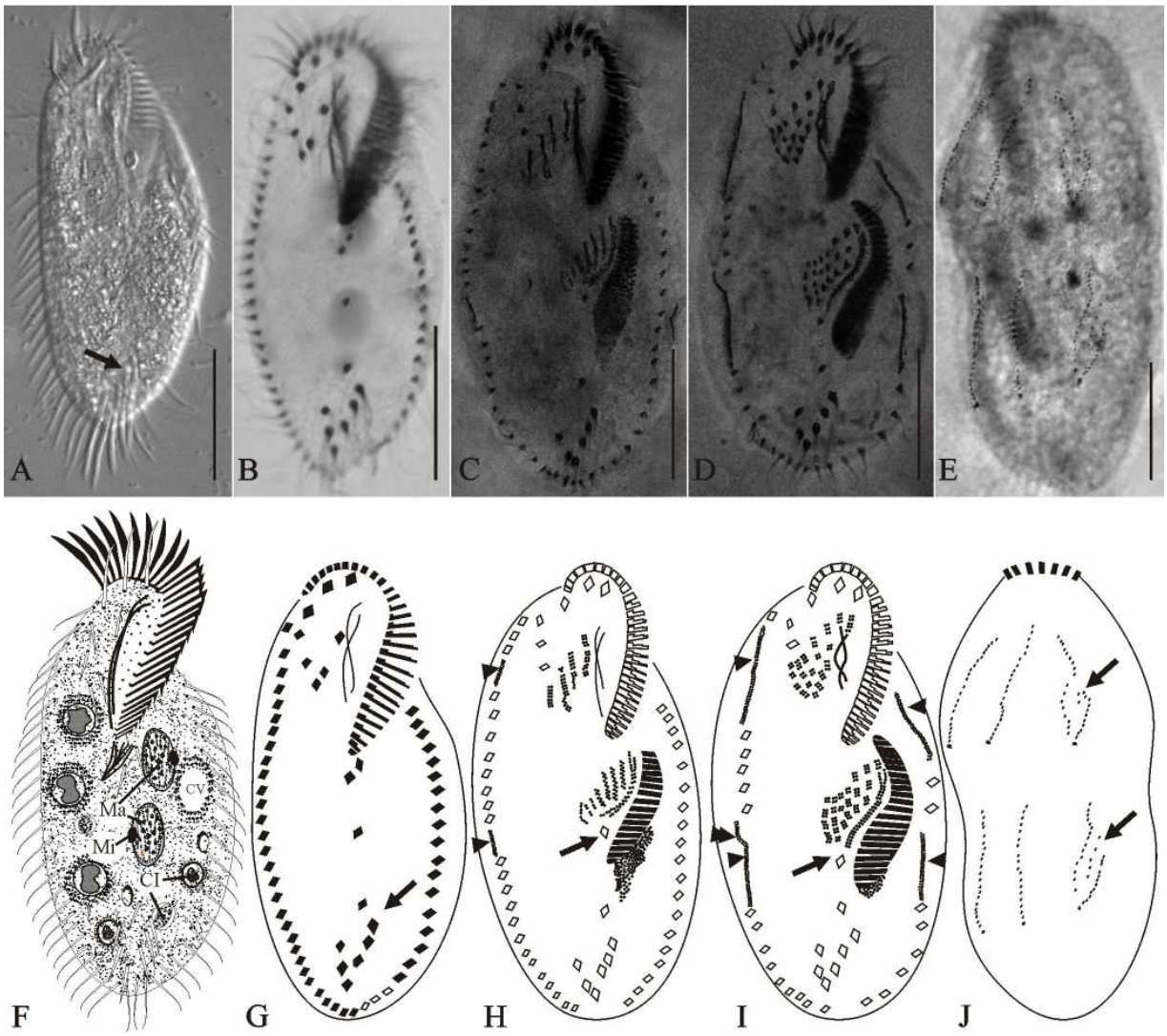


Sterkiella histriomuscorum

Sterkiella cavicola

Sterkiella tricirrata

Fig 5.5A–J. Photomicrographs and line diagrams of *Sterkiella histriomuscorum* Sikkim (E) population *in vivo* (A; Differential Interference Contrast and F; line diagram) and after protargol impregnation (B–E; photomicrographs and G–J; line diagrams). **A.** Complete interphasic cell, arrow showing five transverse cirri in 3+2 pattern. **B.** Infraciliature of interphasic cell. **C. D and E.** Infraciliature of dividers; C and D, ventral and E, dorsal surface. **F.** Complete interphasic cell. CI, cytoplasmic inclusions; CV, contractile vacuole; Ma, macronuclear nodules; Mi, micronuclei. **G.** Infraciliature of ventral surface; arrow showing five transverse cirri in 3+2 pattern. **H.** Ventral infraciliature of a early divider, arrow shows cirrus V/3 not involved in anlagen formation during morphogenesis; arrowheads show marginal anlagen formed initially in right marginal row by within-row formation **I.** Ventral infraciliature of a middle divider, arrowheads show marginal anlagen formed in each row at two levels, these give rise to marginal rows for the two daughter cells which replace the parental rows; double arrowhead shows the origin of dorsomarginal anlagen from the anterior end of the right marginal anlagen to form the dorsomarginal row(s) which later shift to the dorsal surface; arrow shows cirrus V/3 not involved in anlagen formation during morphogenesis **J.** Dorsal infraciliature of a divider, arrows show simple fragmentation of dorsal kinety. Bars: 25μm.


A
B
C
D
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Ma
Mi
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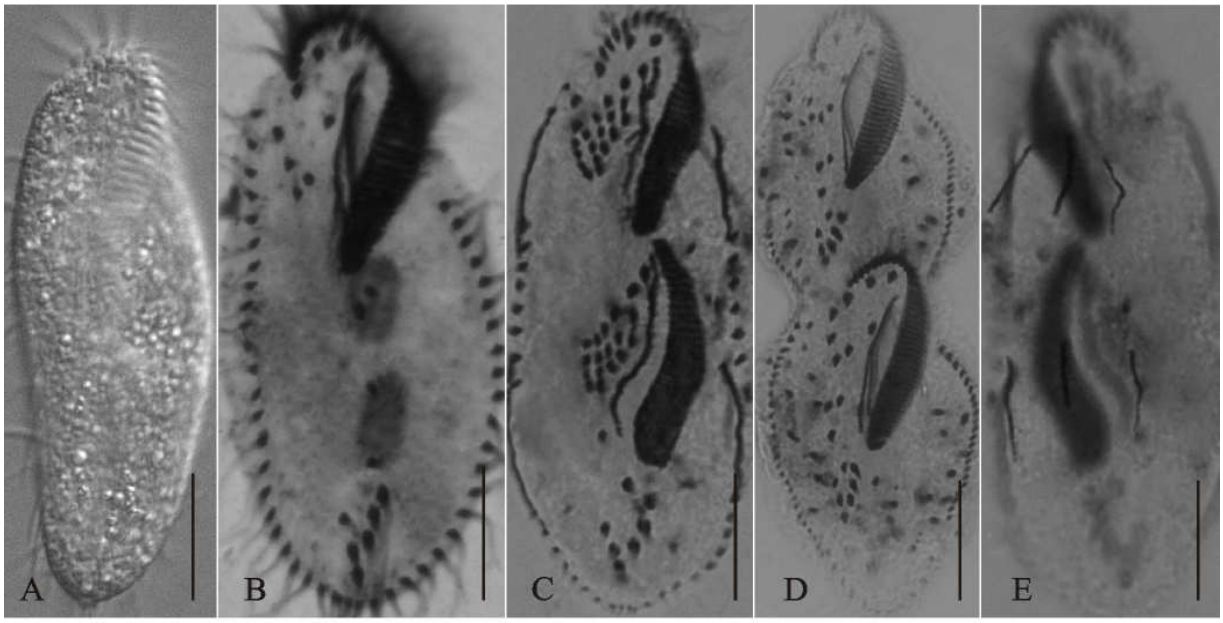
Fig 5.6A–J. Photomicrographs and line diagrams of *Sterkiella histriomuscorum* Sikkim (B) population *in vivo* (A; Differential Interference Contrast and F; line diagram) and after protargol impregnation (B–E; photomicrographs and G–J; line diagrams). **A.** Complete interphasic cell. **B.** Infraciliature of interphasic cell. **C. D and E.** Infraciliature of dividers; C and D, ventral and E, dorsal surface. **F.** Complete interphasic cell. CI, cytoplasmic inclusions; CV, contractile vacuole; Ma, macronuclear nodules; Mi, micronuclei. **G.** Infraciliature of ventral surface; arrow showing five transverse cirri in 3+2 pattern. **H.** Ventral infraciliature of a divider, arrow shows cirrus V/3 not involved in anlagen formation during morphogenesis. **I.** Ventral infraciliature of a very late divider, arrowheads show newly formed dorsomarginals which later shift to dorsal surface. **J.** Dorsal infraciliature of a divider, arrow shows beginning of simple fragmentation of dorsal kinety 3. Bars: 25µm.


A
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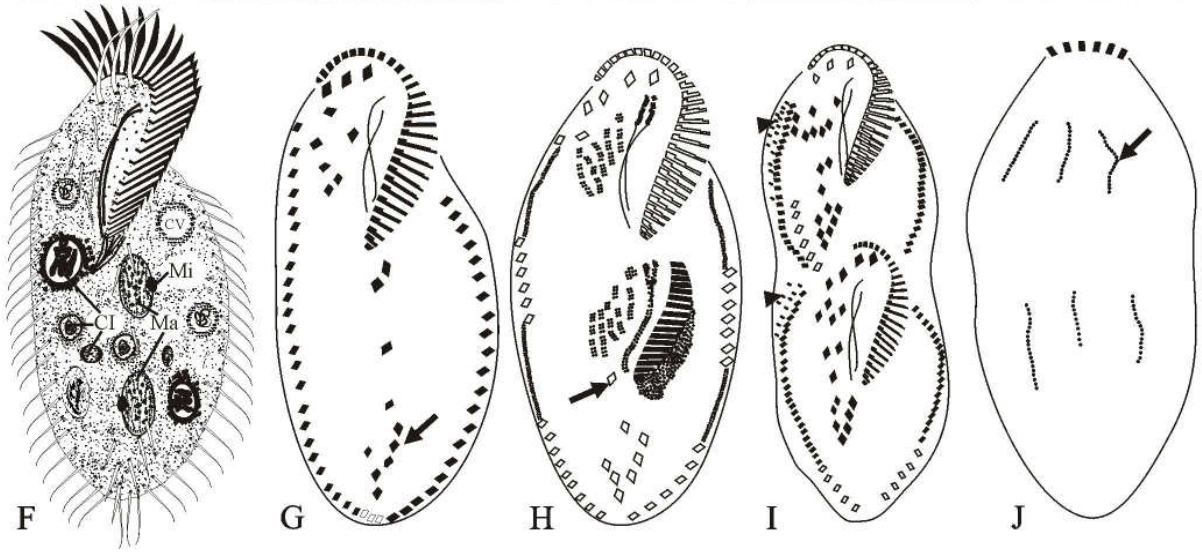
CV
Mi
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Fig 5.7A–J. Photomicrographs and line diagrams of *Sterkiella histriomuscorum* Sikkim (A) population *in vivo* (A; Differential Interference Contrast and F; line diagram) and after protargol impregnation (B–E; photomicrographs and G–J; line diagrams). **A.** Complete interphasic cell showing, contractile vacuole (arrowhead). **B and C.** Infraciliature of interphasic cells; B, ventral and C, dorsal surface. **D and E.** Infraciliature of dividers; D, ventral and E, dorsal surface. **F.** Complete interphasic cell. CI, cytoplasmic inclusions; CV, contractile vacuole; Ma, macronuclear nodules; Mi, micronuclei. **G.** Infraciliature of ventral surface; arrow showing four transverse cirri in tick mark shape; right marginal row and left marginal row distinctly separate posteriorly. **H.** Dorsal side with bristle rows, arrowheads show caudal cirri formed at the posterior most end of each new dorsal kinety (DK $_{1,2 \text{ and } 4}$); double arrowheads show two dorsomarginal rows. **I.** Ventral infraciliature of a divider, arrow shows cirrus V/3 not involved in anlagen formation during morphogenesis; double arrow shows partial disintegration of paroral and endoral. **J.** Dorsal infraciliature of very late divider, arrows show simple fragmentation of dorsal kinety 3. Bars: 25µm.

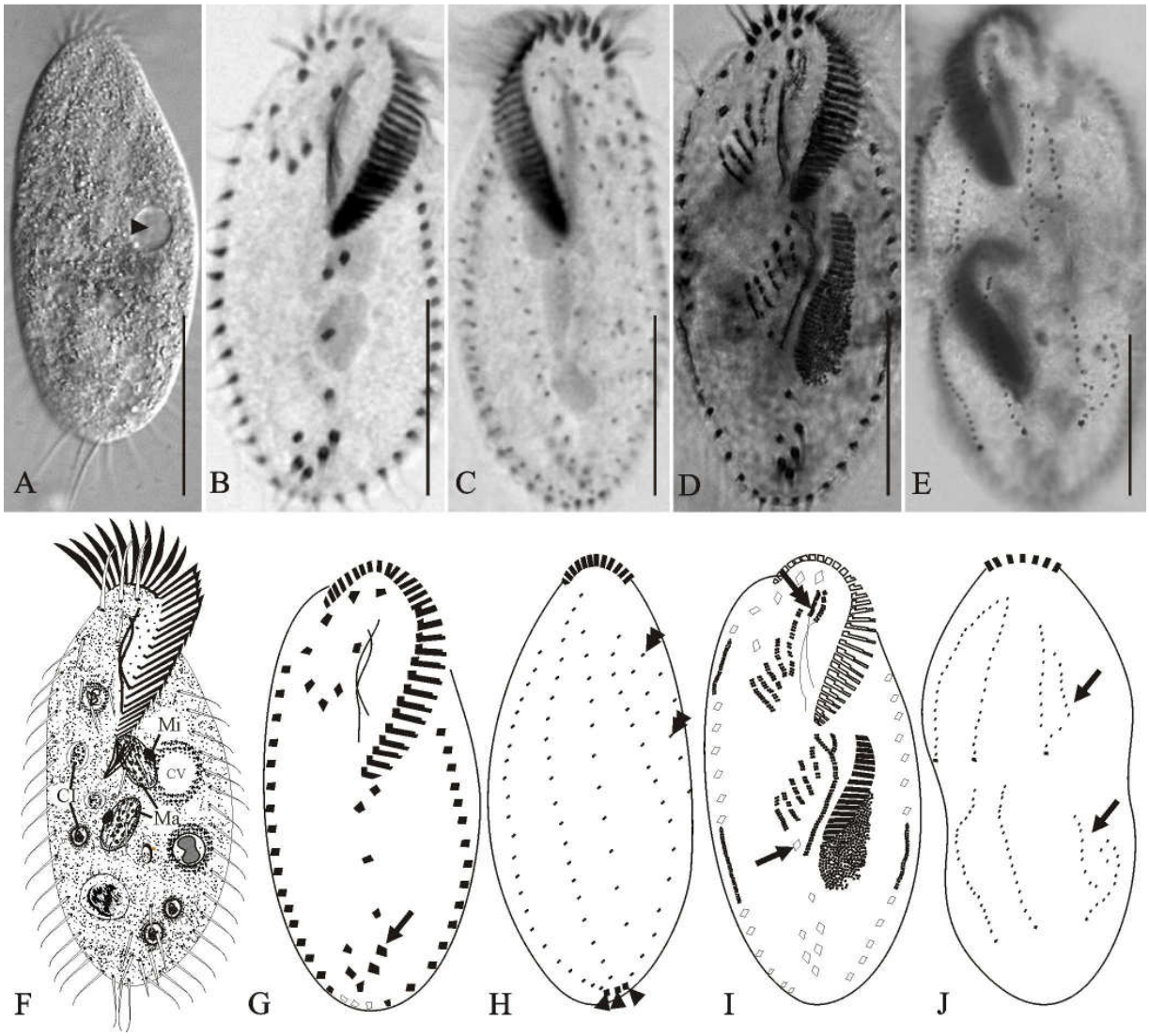
A
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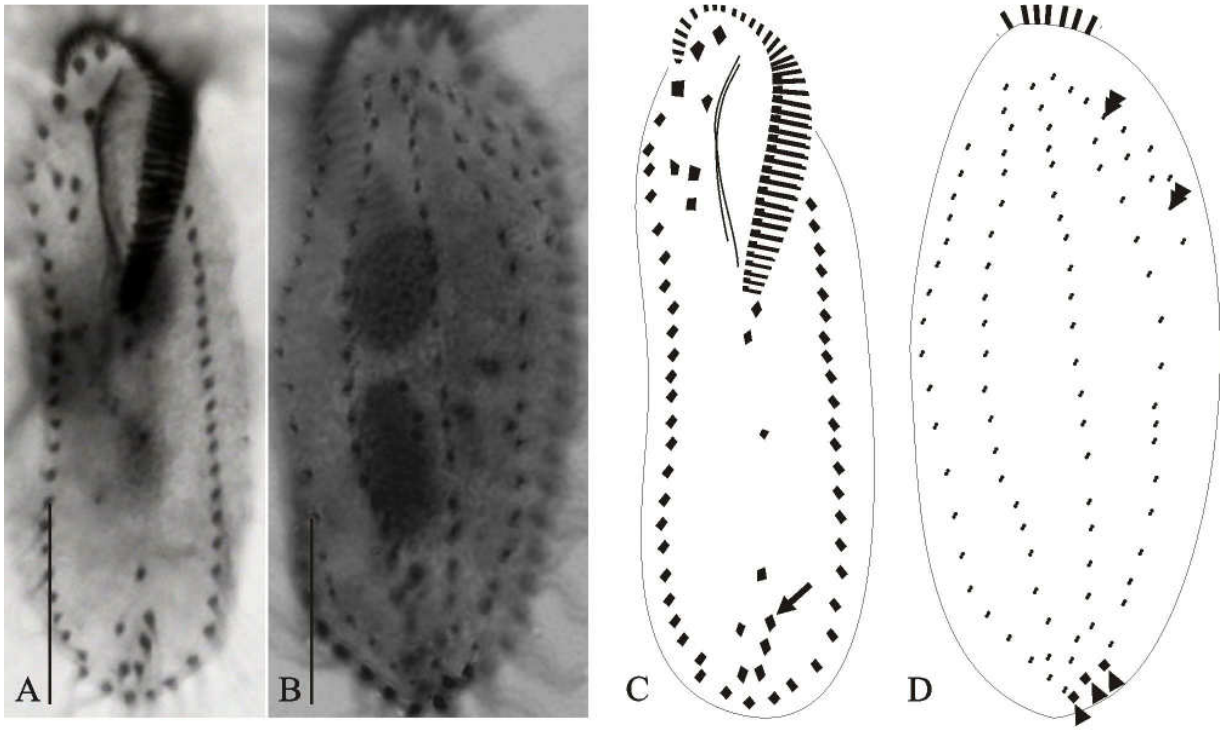
Fig 5.8A–D. Photomicrographs and line diagrams of *Sterkiella histriomuscorum* Sikkim (C) population (A, B, photomicrographs after protargol impregnation; C, D, line diagrams). **A, B.** Infraciliature of interphasic cells; A, ventral surface showing adoral zone of membranelles and frontal ciliature; B, dorsal surface showing six bristle rows, four dorsal kineties and two dorsomarginal rows. **C.** Infraciliature of ventral surface; arrow showing four transverse cirri in a row. **D.** Infraciliature of dorsal surface; three caudal cirri (arrows) present at posterior most end of dorsal kinety 1, 2, and 4; double arrowheads show two dorsomarginal rows. Bars: 25µm.


A
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D

Fig 5.9A–L. Photomicrographs and line diagrams of *Sterkiella tricirrata* Sikkim (N) population *in vivo* (A; Differential Interference Contrast and G; line diagram) and after protargol impregnation (B–F; photomicrographs and H–L; line diagrams). **A.** Complete interphasic cell. **B and C.** Infraciliature of interphasic cells, B, ventral and C dorsal surface. **D. E and F.** Infraciliature of dividers; D and E, ventral and F, dorsal surface. **G.** Complete interphasic cell. CI, cytoplasmic inclusions; CV, contractile vacuole; Ma, macronuclear nodules; Mi, micronuclei. **H.** Infraciliature of ventral surface; arrow showing three transverse cirri in a row. **I.** Dorsal infraciliature of a interphasic cell, arrowhead shows caudal cirri; double arrowhead shows dorsomarginal rows. **J.** Ventral surface of a divider, arrow shows V/3 not involved in anlagen formation during morphogenesis. **K.** Ventral surface of a divider, arrowheads show newly formed dorsomarginal which later shift to dorsal surface (double arrowheads in L). **L.** Dorsal infraciliature of a late divider, arrows show simple fragmentation of dorsal kinety 3. Bars: 25μm.

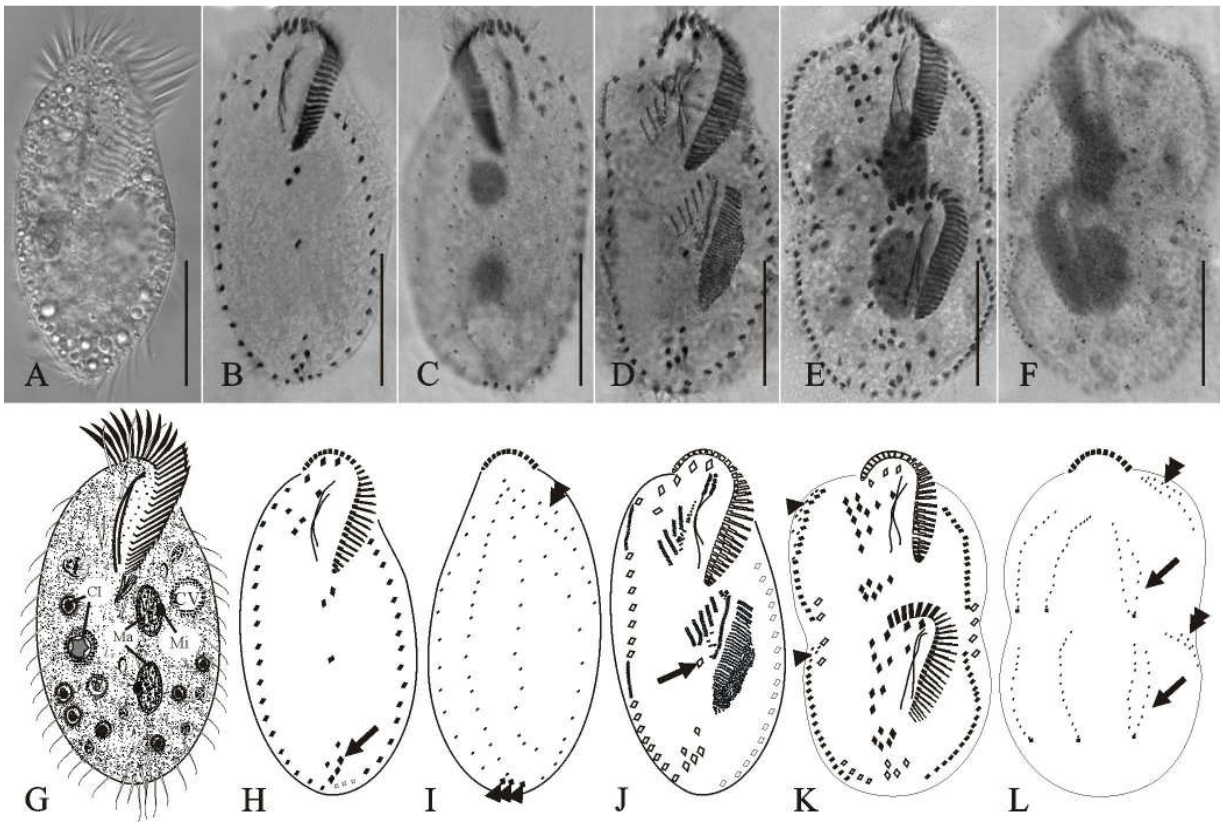
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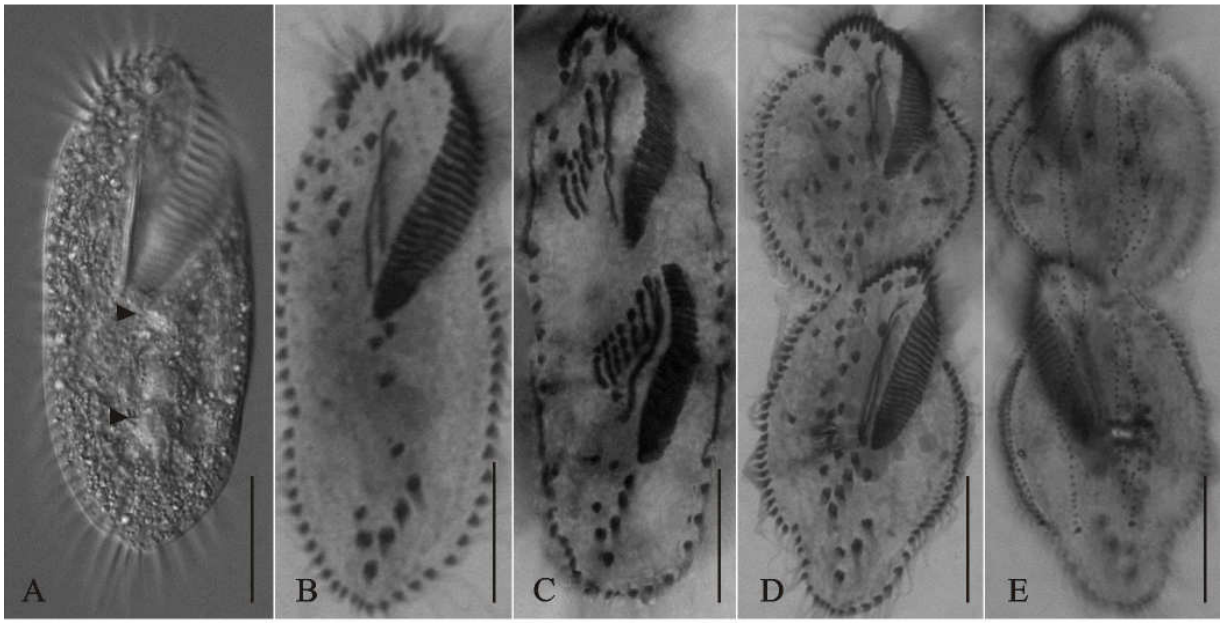
Fig 5.10A–J. Photomicrographs and line diagrams of *Sterkiella cavicola* Sikkim (H) population *in vivo* (A; Differential Interference Contrast and F; line diagram) and after protargol impregnation (B–E; photomicrographs and G–J; line diagrams). **A.** Complete interphasic cell showing, four macronuclear nodules (arrowheads). **B.** Infraciliature of interphasic cell. **C. D and E.** Infraciliature of dividers; C and D, ventral and E, dorsal surface. **F.** Complete interphasic cell. CI, cytoplasmic inclusions; CV, contractile vacuole; Ma, macronuclear nodules; Mi, micronuclei. **G.** Infraciliature of ventral surface, arrow shows undulating membrane in Oxytricha pattern; transverse cirri (double arrow) in 3+2 pattern. **H.** Ventral infraciliature of a dividing specimen; arrow shows cirrus V/3 not involved in anlagen formation during morphogenesis. **I.** Ventral infraciliature of very late divider, arrow head in proter shows newly formed dorsomarginal which later shift to dorsal surface. **J.** Dorsal infraciliature of a late divider, arrows showing simple fragmentation of dorsal kinety 3. Bars: 25µm.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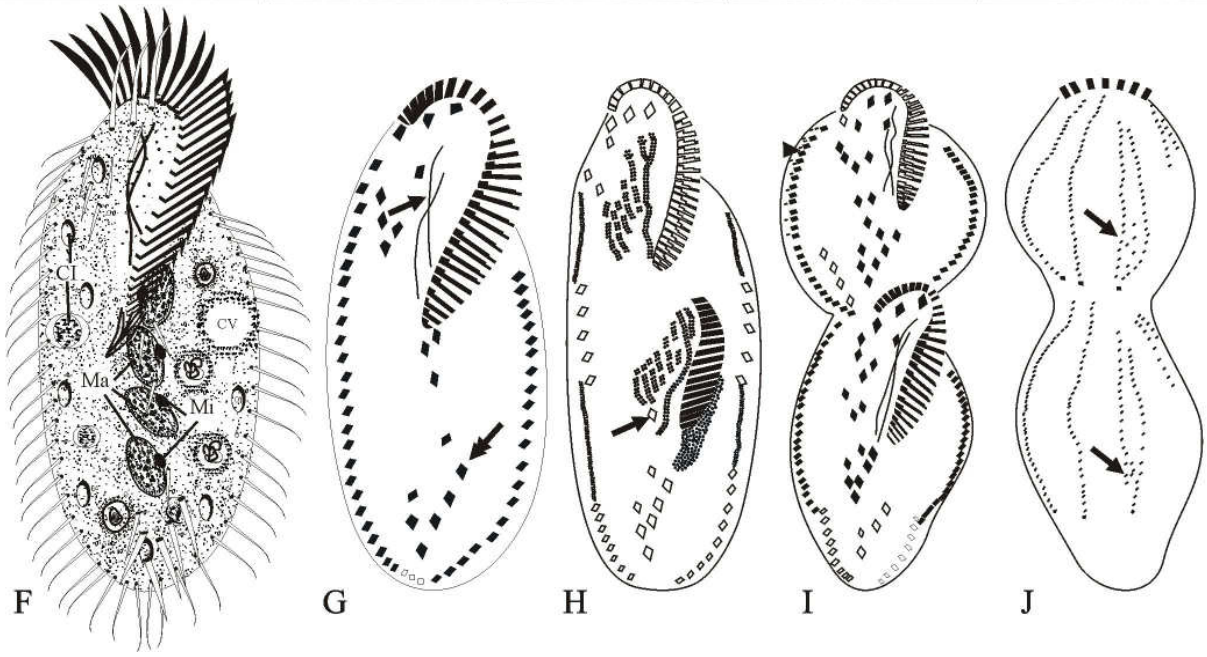
CI
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Mi
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Fig 5.11A–J. Photomicrographs and line diagrams of *Sterkiella cavicola* Sikkim (J) population *in vivo* (A; Differential Interference Contrast and F; line diagram) and after protargol impregnation (B–E; photomicrographs and G–J; line diagrams). **A.** Complete interphasic cell, arrowhead showing AZM in question mark. **B.** Infraciliature of interphasic cell. **C. D and E.** Infraciliature of dividers; C and D, ventral and E, dorsal surface. **F.** Complete interphasic cell. CI, cytoplasmic inclusions; CV, contractile vacuole; Ma, macronuclear nodules; Mi, micronuclei. **G.** Infraciliature of ventral surface, arrow shows four transverse cirri in row. **H.** Ventral infraciliature of a dividing specimen; arrow shows cirrus V/3 not involved in anlagen formation during morphogenesis. **I.** Ventral infraciliature of very late divider, arrowheads show newly formed dorsomarginals which later shift to dorsal surface. **J.** Dorsal infraciliature of a late divider, arrows showing simple fragmentation of dorsal kinety 3.

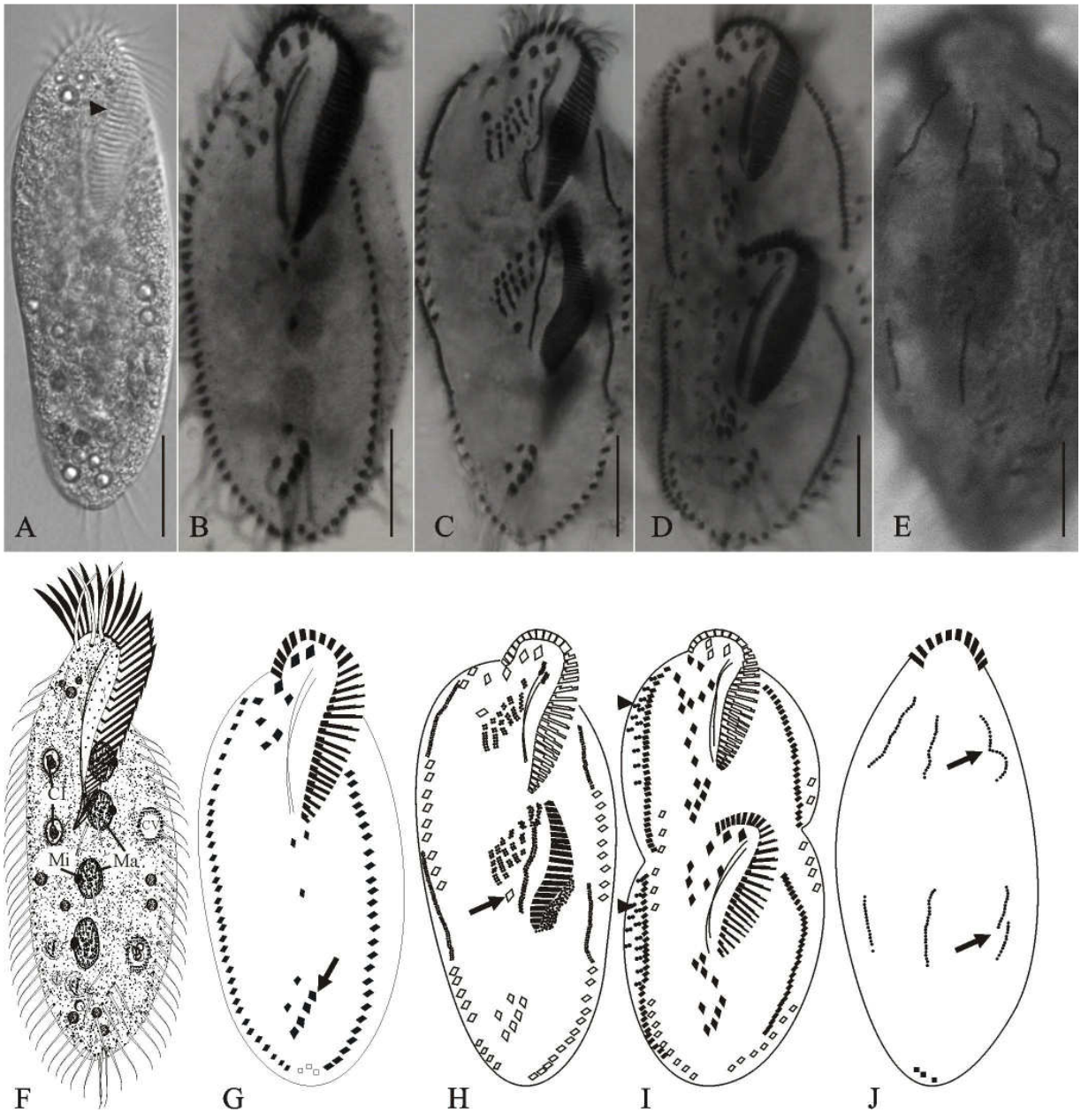
A
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Fig 5.12. A Neighbour-joining tree constructed by using 18S rDNA sequences of fourteen populations belonging to three different species of *Sterkiella* and their related sequences, applying the Kimura 2-parameter model. There were a total of 1765 positions in the final dataset. The numbers at nodes represent bootstrap values in order: Maximum likelihood (ML), Maximum parsimony (MP) and Neighbour-joining (NJ). ML tree was constructed by applying the GTR+G+I nucleotide substitution model. MP tree was obtained using the Close-Neighbour-Interchange algorithm. Clades with different topologies in MP and ML trees relative to NJ tree are indicated with asterisks. In each case tree construction was done using 1,000 bootstrap replicates. The codes following the names are GenBank accession numbers. Bootstrap values lower than 40% are replaced with hyphens. The trees are drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer phylogenetic trees.

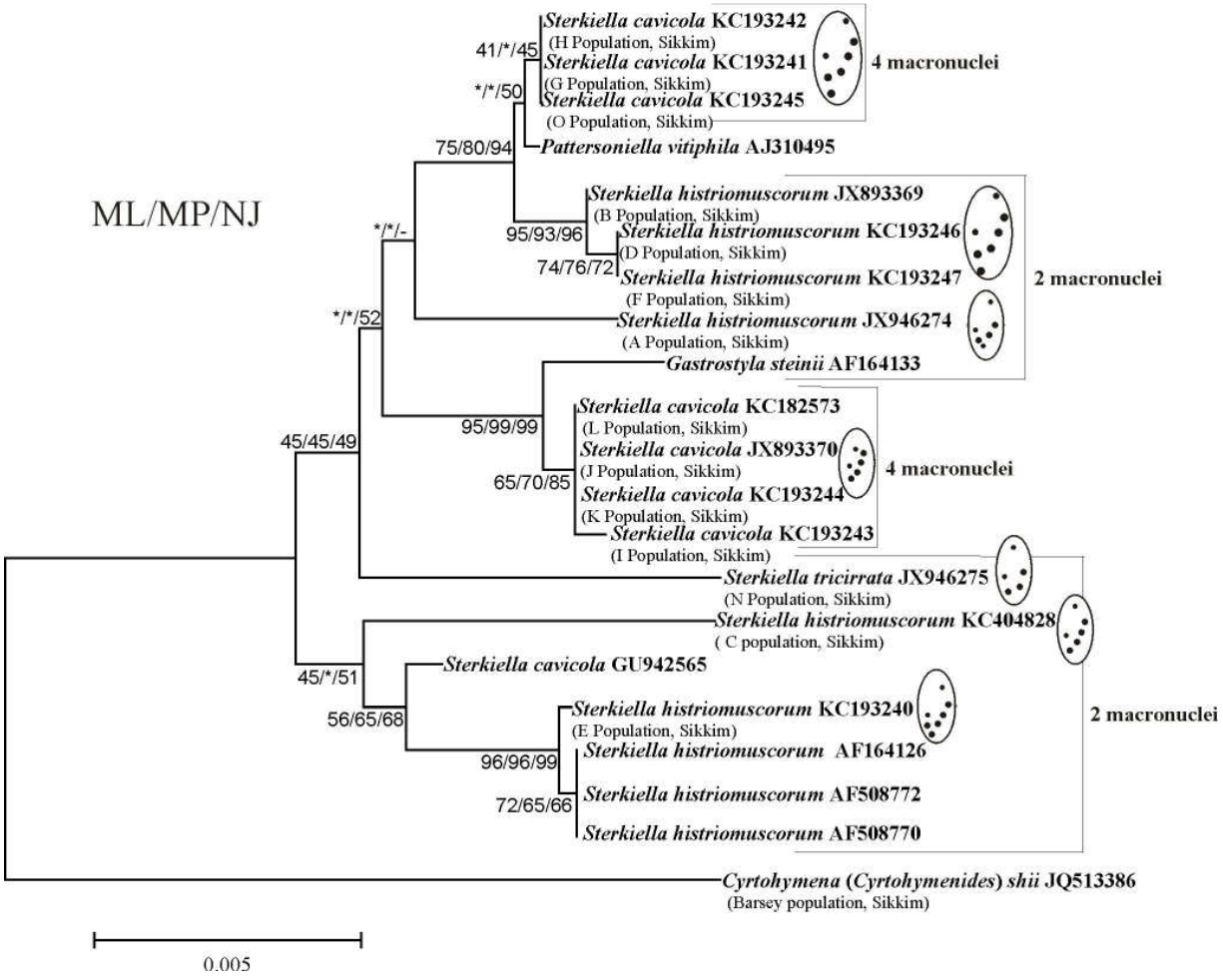

ML/MP/NJ
Sterkiella cavicola KC193242
(H Population, Sikkim)
Sterkiella cavicola KC193241
(G Population, Sikkim)
Sterkiella cavicola KC193245
(O Population, Sikkim)
4 macronuclei
Pattersoniella vitiphila AJ310495
Sterkiella histriomuscorum JX893369
(B Population, Sikkim)
Sterkiella histriomuscorum KC193246
(D Population, Sikkim)
Sterkiella histriomuscorum KC193247
(F Population, Sikkim)
Sterkiella histriomuscorum JX946274
(A Population, Sikkim)
2 macronuclei
Gastrostyla steinii AF164133
Sterkiella cavicola KC182573
(L Population, Sikkim)
Sterkiella cavicola JX893370
(J Population, Sikkim)
Sterkiella cavicola KC193244
(K Population, Sikkim)
Sterkiella cavicola KC193243
(I Population, Sikkim)
4 macronuclei
Sterkiella tricirrata JX946275
(N Population, Sikkim)
Sterkiella histriomuscorum KC404828
(C population, Sikkim)
Sterkiella cavicola GU942565
Sterkiella histriomuscorum KC193240
(E Population, Sikkim)
Sterkiella histriomuscorum AF164126
Sterkiella histriomuscorum AF508772
Sterkiella histriomuscorum AF508770
2 macronuclei
Cyrtohymena (Cyrtohymenides) shii JQ513386
(Barsey population, Sikkim)
41/*/45
//50
75/80/94
//-
95/93/96
74/76/72
//52
95/99/99
45/45/49
65/70/85
45/*/51
56/65/68
96/96/99
72/65/66
0.005

Fig. 5.13. Homology of cirri in *Sterkiella*, *Gastrostyla* and *Pattersoniella*. Numbering of frontal-ventral-transverse cirral anlagen and cirri according to Wallengren (1900) and Berger (1999). Cirri originating from same anlage are connected by broken lines. Pretransverse cirri (cirri V/2 and VI/2) encircled by dotted line. Frontoterminal cirri (cirri VI/3 and VI/4; in *Gastrostyla* number slightly increased) encircled by broken line. *Sterkiella* has the plesiomorphic set of 18 frontal-ventral-cirri. The anlagen V and VI of *Gastrostyla* produce an increased number of cirri which form the frontoventral row. The postoral cirri V/3 and V/4 cannot be homologised unequivocally and are thus marked by "?". In *Gastrostyla steinii* anlage II does not produce a transverse cirrus; thus, it has only four transverse cirri. *Pattersoniella* has some additional cirral anlagen whose cirri are connected by dotted lines (corresponding transverse cirri marked by arrows). I-VI – frontal-ventral-transverse cirral anlagen, 1-4 – cirri within anlage (Adapted from Foissner et al. 2004).

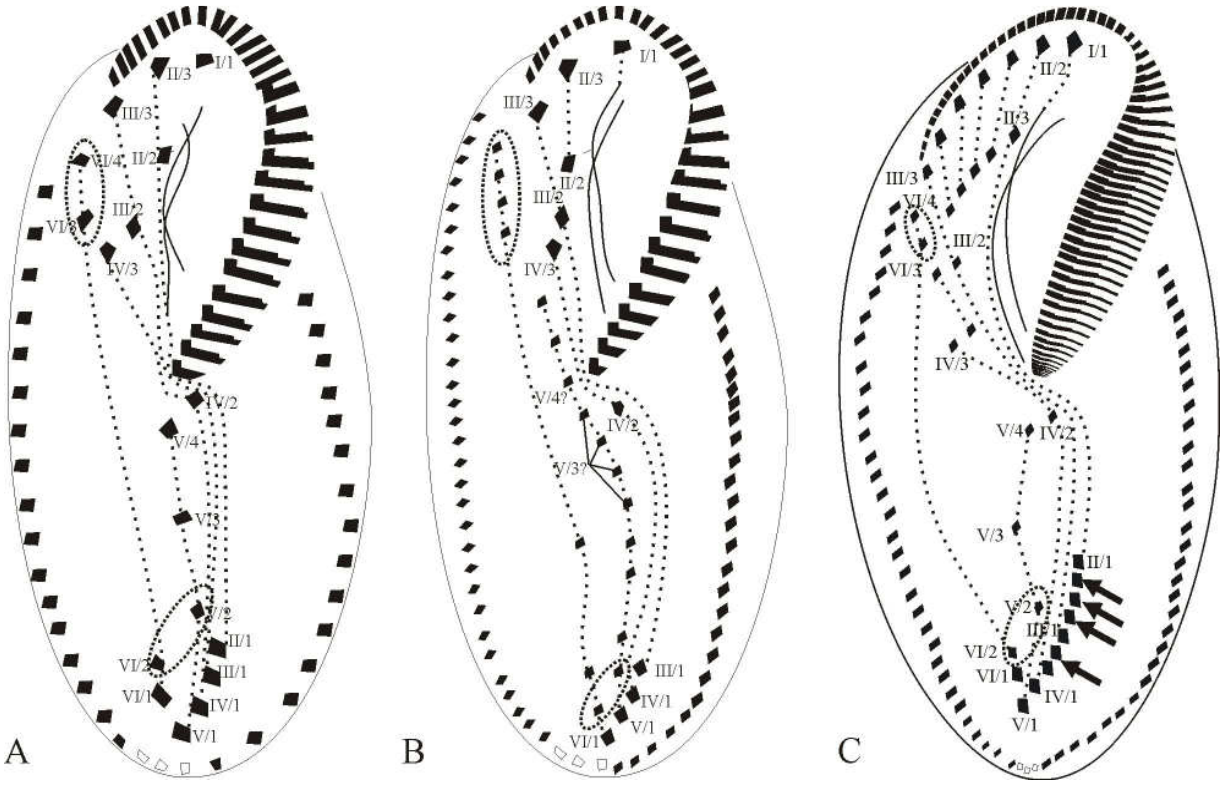
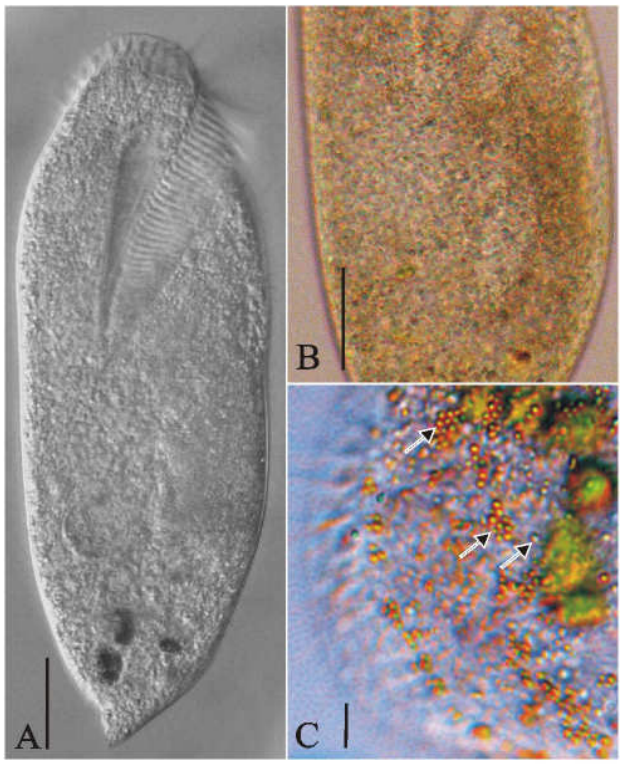

A
I/1
II/3
III/3
II/4
II/2
III/2
VI/3
IV/3
IV/2
V/4
V/3
V/2
II/1
VI/2
III/1
VI/1
IV/1
V/1
B
I/1
II/3
III/3
II/2
III/2
IV/3
V/4?
IV/2
V/3?
III/1
IV/1
V/1
VI/1
C
I/1
II/2
II/3
III/3
VI/4
III/2
VI/3
IV/3
V/4
IV/2
V/3
II/1
V/2
III/1
VI/2
VI/1
IV/1
V/1

Fig. 5.14A–C. *Cyrtohymena (Cyrtohymenides) shii* Indian population in vivo (Differential Interference Contrast). **A.** Complete cell showing, inter alia, the short tail and the distinctive hook-shaped paroral and the deep transparent buccal cavity. **B, C.** Details of cell (B, slightly squeezed) to show brilliant citrine cortical granules randomly arranged singly and in small clusters (arrows in C). The color of cortical granules appears slightly reddish in (C) due to DIC effect. Bars: 25 μm (A, B), 10 μm (C).

Fig. 5.15A, B. *Cyrtohymena (Cyrtohymenides) shii* Indian population after protargol impregnation. Ventral and dorsal view. For ciliature details, see Figs 3B, C. Bars: 25 μm.


A
B
C

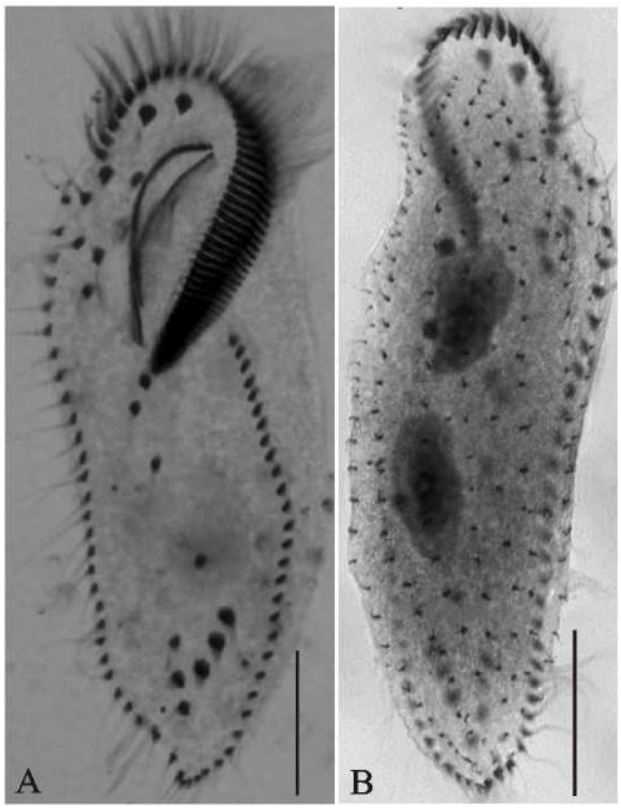

A
B

Fig. 5.16A–C. *Cyrtohymena* (*Cyrtohymenides*) *shii* Sikkim population *in vivo* (A) and after protargol impregnation (B, C). **A.** Ventral view. **B, C.** Infraciliature of ventral and dorsal side. AZM, adoral zone of membranelles; BC, buccal cirrus; CC, caudal cirri; CI, cytoplasmic inclusions; CV, contractile vacuole; $DK_{1–6,}$ dorsal kineties; $DM_{1–3,}$ dorsomarginal rows; E, endoral membrane; FC, frontal cirrus; FVC, frontoventral cirrus; LMR, left marginal row; Ma, macronuclear nodules; Mi, micronuclei; P, paroral membrane; POVC, postoral ventral cirrus; PTVC, pretransverse ventral cirrus; RMR, right marginal row; TC, transverse cirrus. Bars: 25 μm.

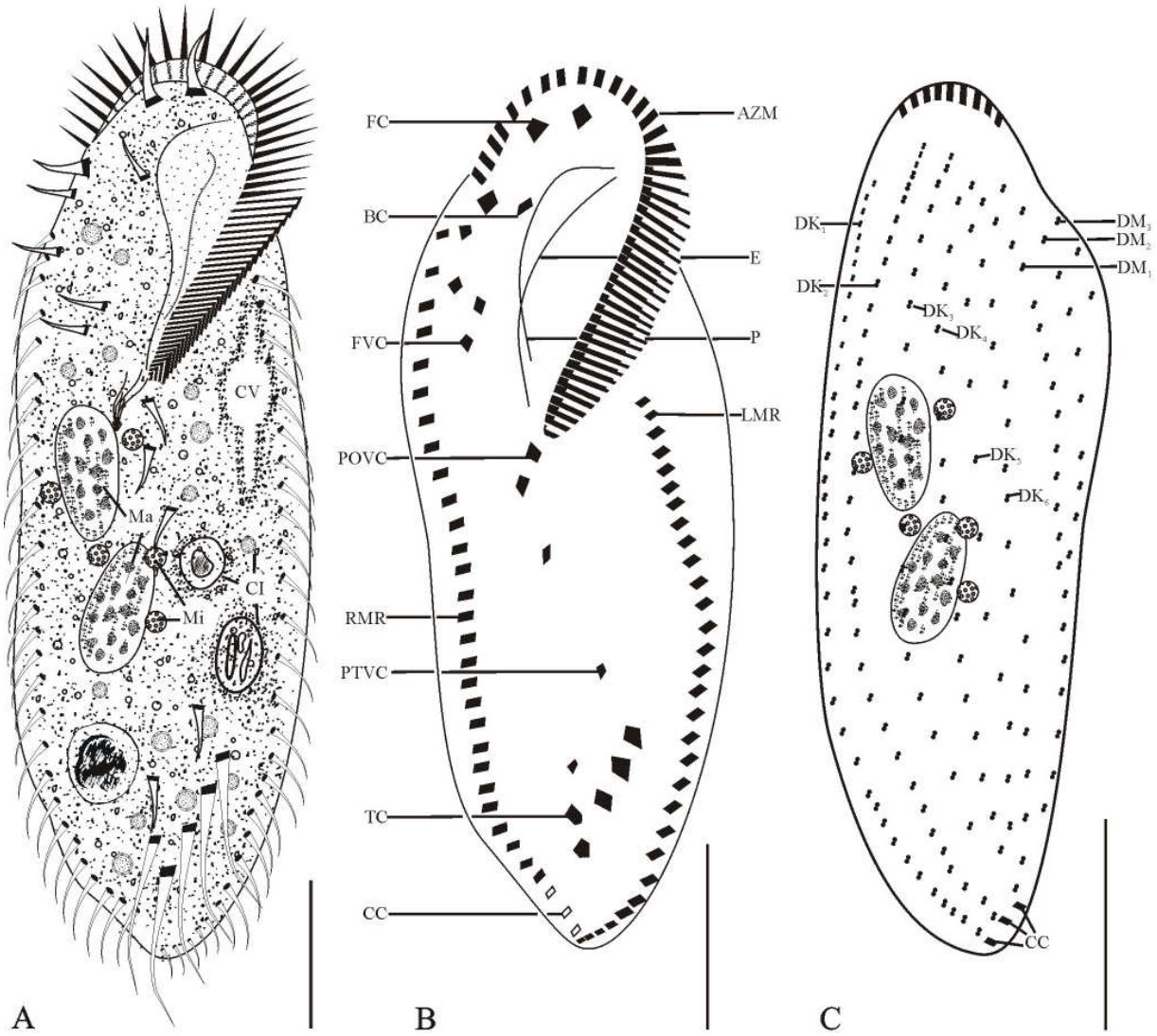

A
CV
Ma
Mi
CI
B
FC
BC
FVC
POVC
RMR
PTVC
TC
CC
AZM
E
P
LMR
C
DK1
DK2
DK3
DK4
DK5
DK6
DM3
DM2
DM1
CC

Fig. 5.17A–E. Photomicrographs of protargol-impregnated *Cyrtohymena* (*Cyrtohymenides*) *shii* Sikkim population. **A.** Infraciliature of ventral surface of a late reorganizer. **B.** A cell engulfing an encysting cell of the same species. **C.** Homopolar doublet. **D.** The anterior segment of the cell (ventral view) to show the undulating membranes in the *Cyrtohymena* pattern (arrow points to the ciliary ends of the endoral forming a linear structure seen in most cells). **E.** Mature cyst. Ma, macronuclear nodules; Mi, micronuclei; Bars: 25 μm (A–C), 10 μm (D, E).

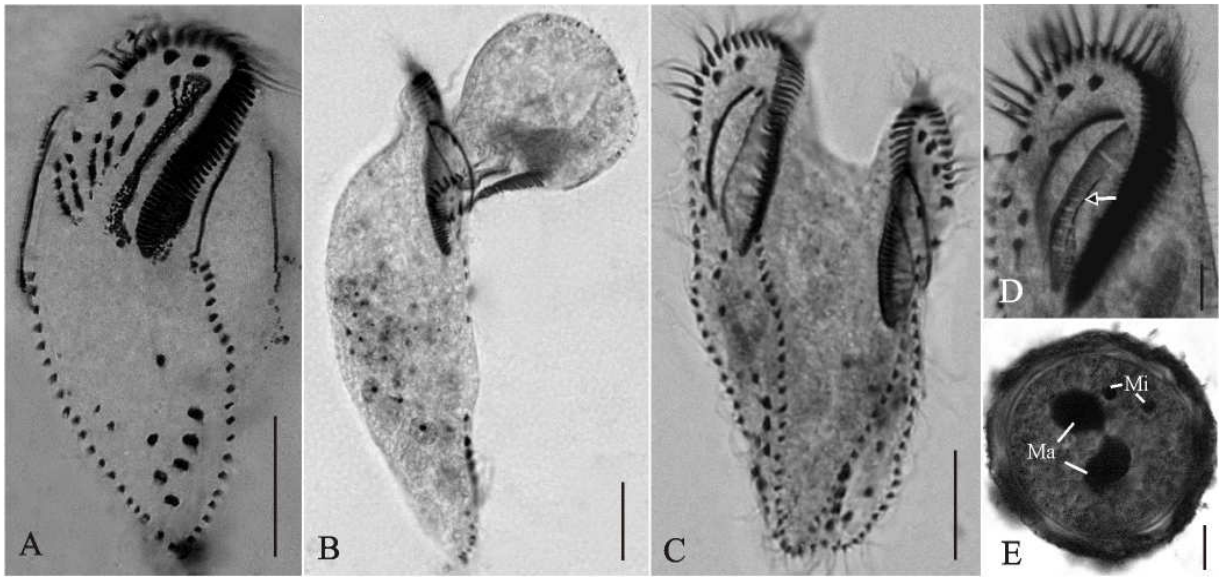

A
B
C
D
E
Mi
Ma

Fig. 5.18A–L. Line diagrams of mid-segments of protargol-impregnated early and middle dividers of *Cyrtohymena* (*Cyrtohymenides*) *shii* Sikkim population depicting morphogenetic events on the ventral surface (for documentation, see Figs 8A–L). **A.** Stomatogenesis begins close to cirrus II/1 (arrow). **B.** Proliferation of this field gives rise to oral primordium (arrow). **C–E.** Three parental cirri disaggregate (arrows) in the order V/4, IV/3, III/2 to give rise to cirral streaks (C–E), and two streaks (double arrows) proceed anteriad from the right anterior face of the oral primordium (E). **F.** Three streaks (arrows) coming from cirrus V/4 become clearly visible. **G.** The left streak (arrow) from V/4 merges with the streak coming from cirrus IV/3, the streak on the right (double arrow) formed from the oral primordium joins the one arising from cirrus III/2. **H.** As the membranelles begin to form in the oral primordium (arrow), cirrus II/2 disaggregates (double arrow) to give rise to a streak. **I.** Cirrus IV/2 (arrow) disaggregates and merges with the loose kinetosomes in front of the oral primordium. The streak arising from cirrus II/2 joins (double arrow) the left streak from the oral primordium. The parental paroral disaggregates partially (arrowhead). **J, K.** Five streaks thus developed begin to split transversely to give two sets, one for each daughter cell. **L.** Cirrus V/3 disaggregates (arrow) and merges with the loose kinetosomes in front of the oral primordium. Bars: 25 μm.

I/1
II/3
III/3
II/2
VI/4
VI/3
III/2
IV/3
IV/2
V/4
V/3
V/2
VI/2
II/1
III/1
VI/1
IV/1
V/1
A
B
C
D
E
F
G
H
I
J
K
L

Fig. 5.19A–D. Line diagrams of protargol-impregnated late dividers of *Cyrtohymena* (*Cyrtohymenides*) *shii* Sikkim population depicting morphogenetic events on the ventral surface. The loose kinetosomes close to the oral primordium begin to align to form the paroral, endoral and cirrus I/1 for the opisthe (arrows in A–D) while the disaggregated paroral and later endoral undergo proliferation to form the paroral, endoral and cirrus I/1 for the proter (double arrows in A–D). Arrowheads in (A–C) mark marginal row anlagen. Dorsomarginal anlagen arise at the anterior ends of the right marginal anlagen to form the dorsomarginal rows which shift to the dorsal surface (double arrowheads in D). Bars: 25 μm.

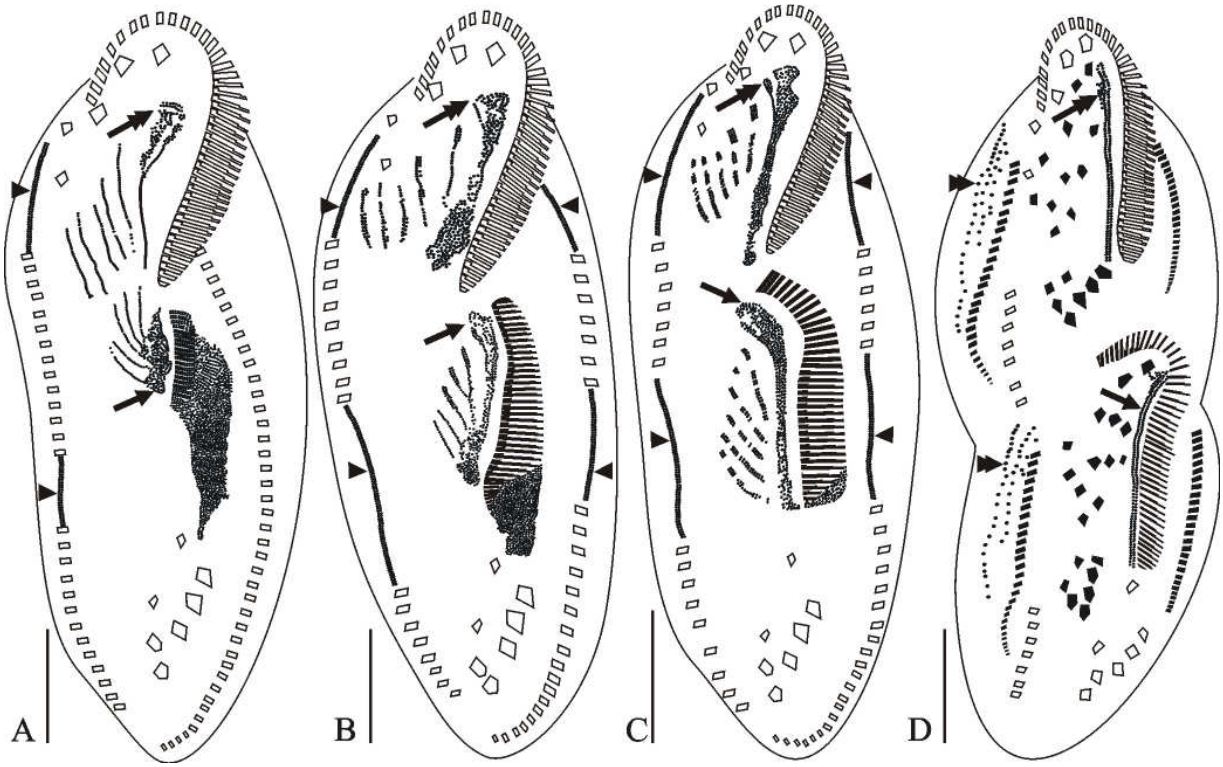

A
B
C
D

Fig. 5.20A–F. Line diagrams of protargol-impregnated dividers of *Cyrtohymena* (*Cyrtohymenides*) *shii* Sikkim population depicting morphogenetic events on dorsal (A–D) and ventral (E, F) surface. **A–D.** Two sets of three dorsal anlagen each arise within row for the two daughter cells in DK_{1-3}; while the left two give rise to kineties 1 and 2, the third undergoes fragmentation to give rise to three or four kineties (arrows in B–D). Three caudal cirri, one each at the posterior ends of the new dorsal kineties 1, 2, and the rightmost one (C, D), are formed in both the proter and the opisthe (double arrows). **E, F.** Line diagram of the divider (ventral surface) depicted in (B), and (F) is a line diagram of the divider (ventral surface) depicted in (D) to show that fragmentation begins when the cirral split and some movement has already occurred on the ventral surface and is complete even before the daughter cells split. The three dorsomarginal rows for both daughter cells shift to the dorsal surface (arrowheads in D). Bars: 25 μm.

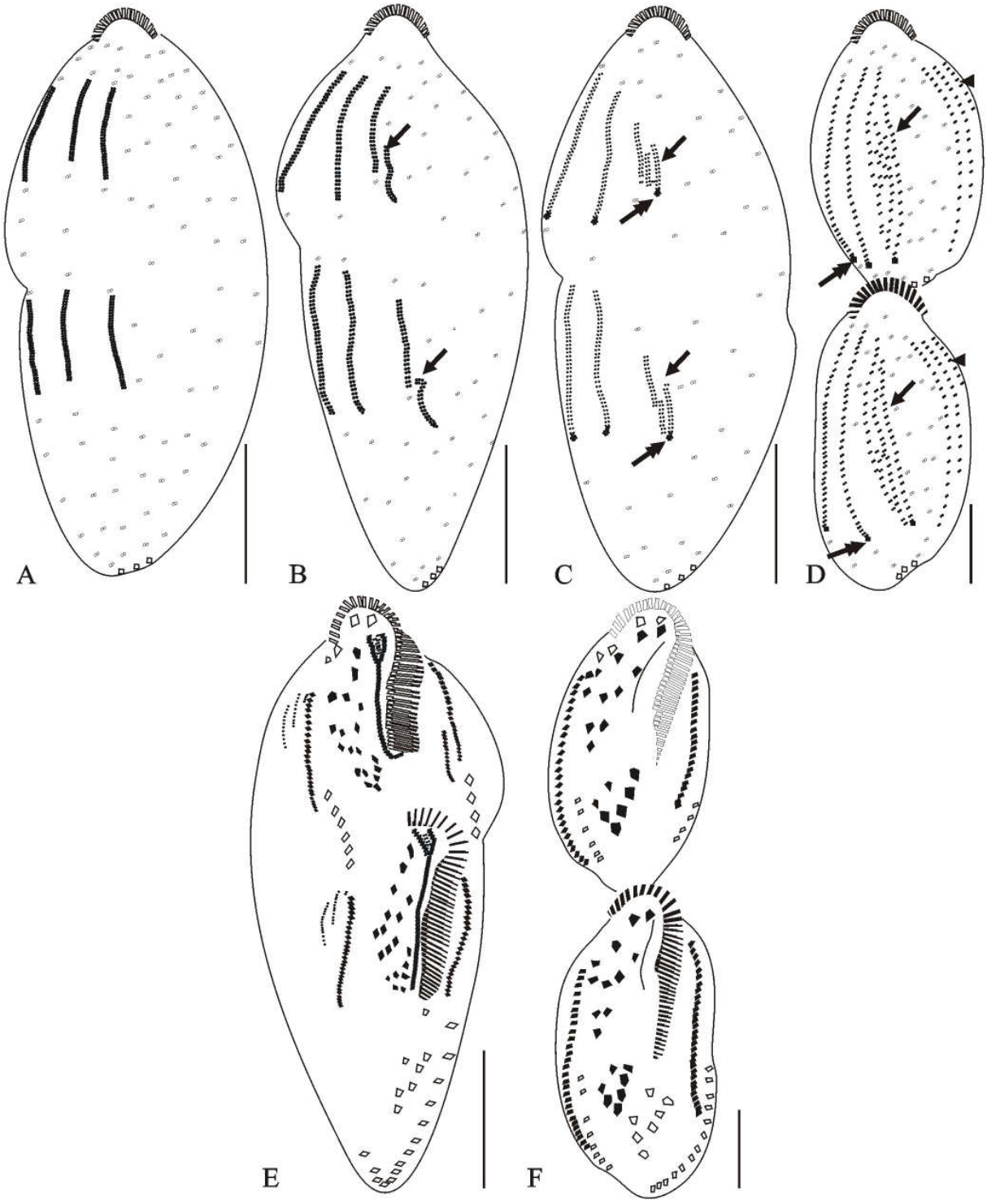

A
B
C
D
E
F

Fig. 5.21A–L. Photomicrographs of protargol-impregnated early and middle dividers of *Cyrtohymena* (*Cyrtohymenides*) *shii* Sikkim population depicting morphogenetic events on the ventral surface. For details see Figs 5.18 A–L. Bars: 25 μm.

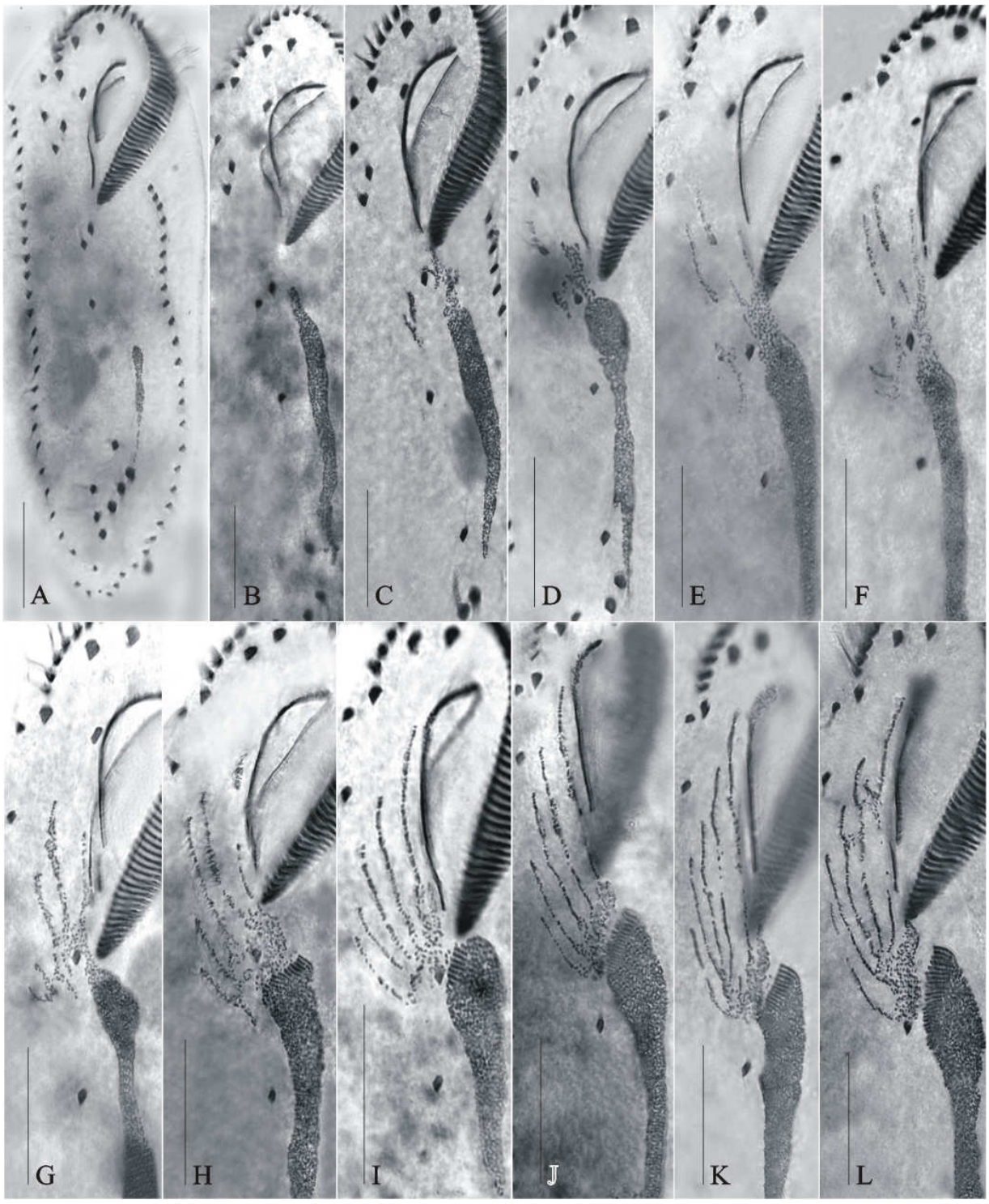

A
B
C
D
E
F
G
H
I
J
K
L

Fig. 5.22A–H. Photomicrographs of protargol-impregnated late dividers of *Cyrtohymena* (*Cyrtohymenides*) *shii* Sikkim population showing morphogenetic events on the ventral (A–D, F, H) and dorsal surface (E, G). The frontal anlagen for both daughter cells separate (A) and new cirri are formed and take their positions in the elongating divider (B–D). The marginal anlagen are formed within row and the cirri formed therein replace the parental rows (A–D). Arrows in (C) and (D) point to the new dorsomarginal rows. Three dorsal anlagen form within row; the third anlage splits (E) when the new cirri of the frontal ciliature have traversed a considerable distance (F). Multiple fragmentation is over at the stage (G, arrow) when the daughter cells are about to split apart (H). Bars: 25 μm.

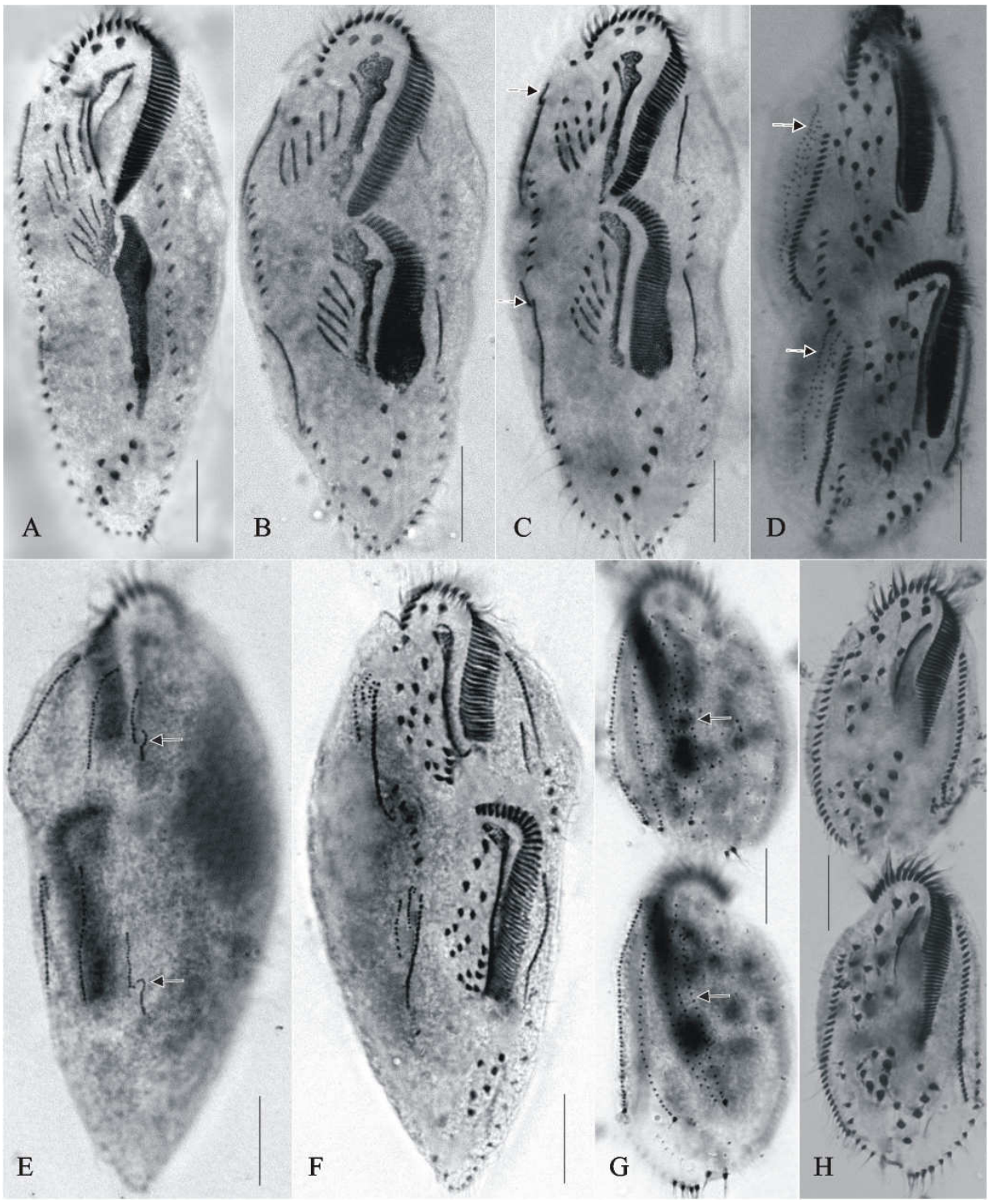
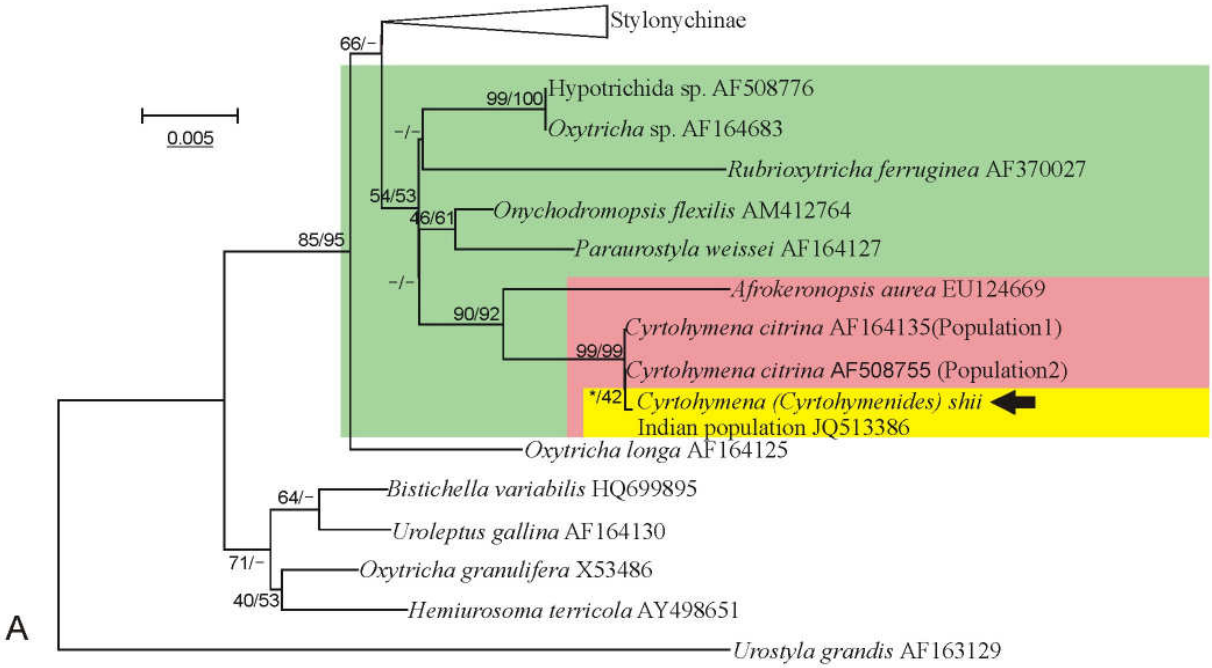

A
B
C
D
E
F
G
H

Fig. 5.23A, B. Phylogenetic trees of 18S rDNA sequences of 27 taxa, showing the position of *Cyrtohymena* (*Cyrtohymenides*) *shii* Sikkim population (arrow). **A.** Maximum-likelihood tree constructed by applying the GTR+G+I nucleotide substitution model. The bootstrap values emerged in Maximum parsimony tree are shown at nodes in order of Maximum parsimony (MP) and Maximum-likelihood (ML). Clades with different topologies in the MP tree relative to ML tree are indicated with asterisks. **B.** Neighbour-joining tree constructed by using Maximum Composite Likelihood nucleotide substitution model. The numbers at nodes represent bootstrap percentages. In each case tree construction was done using 1,000 bootstrap replicates. The codes following the names are GenBank accession numbers. Bootstrap values lower than 40% are replaced with hyphens. The trees are drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer phylogenetic trees. The topology that emerged for members of the subfamily Stylonychinae has been merged together; accession numbers of their 18S rDNA sequences are as follows: AF164123, AF164124, AF164128, AF164133, AF396973, AF508770, AF508771, AJ310486, AJ310487, AJ310494, AJ310495, GU942565.


Stylonychinae
66/–
99/100
Hypotrichida sp. AF508776
0.005
–/–
Oxytricha sp. AF164683
Rubrioxytricha ferruginea AF370027
54/53
46/61
Onychodromopsis flexilis AM412764
85/95
Paraurostyla weissei AF164127
–/–
Afrokeronopsis aurea EU124669
90/92
Cyrtohymena citrina AF164135(Population1)
99/99
Cyrtohymena citrina AF508755 (Population2)
*/42
Cyrtohymena (Cyrtohymenides) shii
Indian population JQ513386
Oxytricha longa AF164125
64/–
Bistichella variabilis HQ699895
Uroleptus gallina AF164130
71/–
Oxytricha granulifera X53486
40/53
Hemiurosoma terricola AY498651
A
Urostyla grandis AF163129

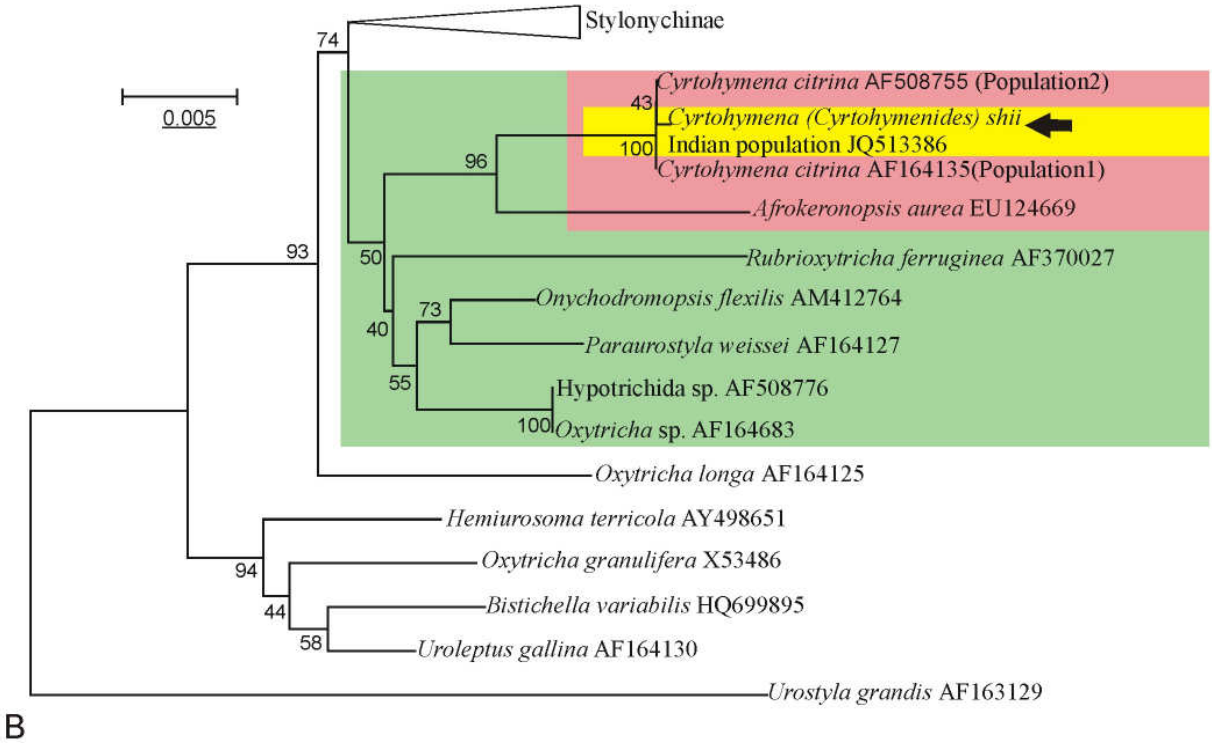

Stylonychinae
74
0.005
Cyrtohymena citrina AF508755 (Population2)
43
Cyrtohymena (Cyrtohymenides) shii
100
Indian population JQ513386
96
Cyrtohymena citrina AF164135(Population1)
Afrokeronopsis aurea EU124669
93
50
Rubrioxytricha ferruginea AF370027
73
Onychodromopsis flexilis AM412764
40
Paraurostyla weissei AF164127
55
Hypotrichida sp. AF508776
100
Oxytricha sp. AF164683
Oxytricha longa AF164125
Hemiurosoma terricola AY498651
94
Oxytricha granulifera X53486
44
Bistichella variabilis HQ699895
58
Uroleptus gallina AF164130
Urostyla grandis AF163129
B

Fig. 5.24 A–L. Photomicrographs and line diagrams of *Pattersoniella vitiphila* Indian population *in vivo* (A; Differential Interference Contrast and G; line diagram) and after protargol impregnation (B–F; photomicrographs and H–L; line diagrams). **A.** Complete interphasic cell. **B and C.** Infraciliature of interphasic cells; B, ventral and C, dorsal surface. **D, E and F.** Infraciliature of late dividers; D, ventral, E and F, dorsal. **G.** Complete interphasic cell. CI, cytoplasmic inclusions; Ma, macronuclear nodules; Mi, micronuclei. **H.** Infraciliature of ventral surface; arrow showing increased number of transverse cirri characteristically seen in *Pattersoniella* **I.** Dorsal side with bristle rows, arrow shows increased number of dorsal kineties formed as result of multiple fragmentation of dorsal kinety 3. **J.** Ventral infraciliature of late divider, arrowhead shows three dorsomarginal rows unusually seen in oxytrichids. **K.** Arrow shows multiple fragmentation of dorsal kinety 3. **L.** arrowheads show caudal cirri formed at the posterior most end of each new dorsal kinety 1, 2 and last most before dorsomarginal rows. Bars: 25μm.

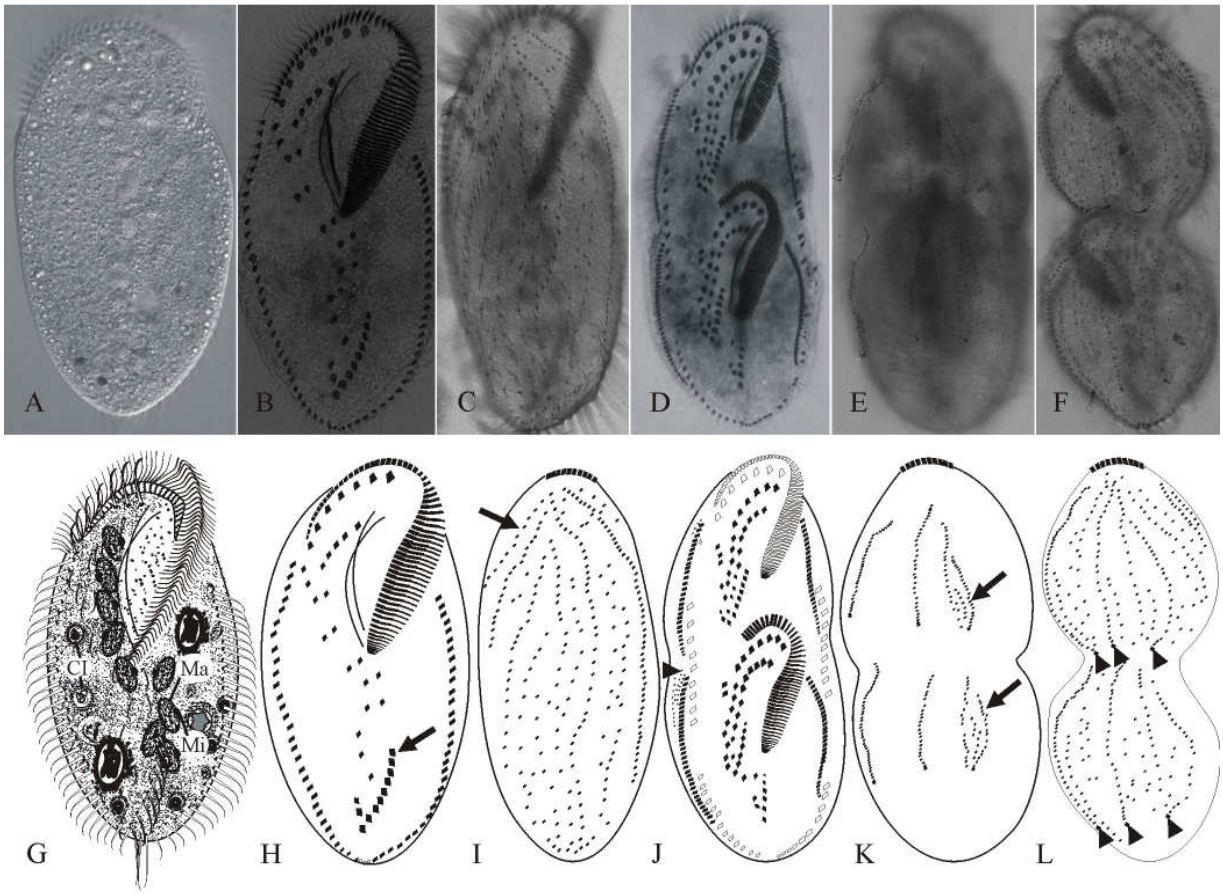

A
B
C
D
E
F
Cl
Ma
Mi
G
H
I
J
K
L

Fig. 5.25A–E. *Paraurosomoida indiensis* in vivo (A–C, Differential Interference Contrast) and after protargol impregnation (D, E). **A.** Ventral view showing outline and light brown pigmented patches at the lateral and posterior end. **B.** Details of cell (slightly squeezed) to show citrine (somewhere appear slightly reddish due to DIC effect) cortical granules randomly arranged singly and in small linear groups. **C.** Mature cyst. **D, E.** Infraciliature of ventral and dorsal side. Inset: posterior end of cell, caudal cirri marked with arrows. For details see Figs 5.26B, E. Bars: 25 μm.

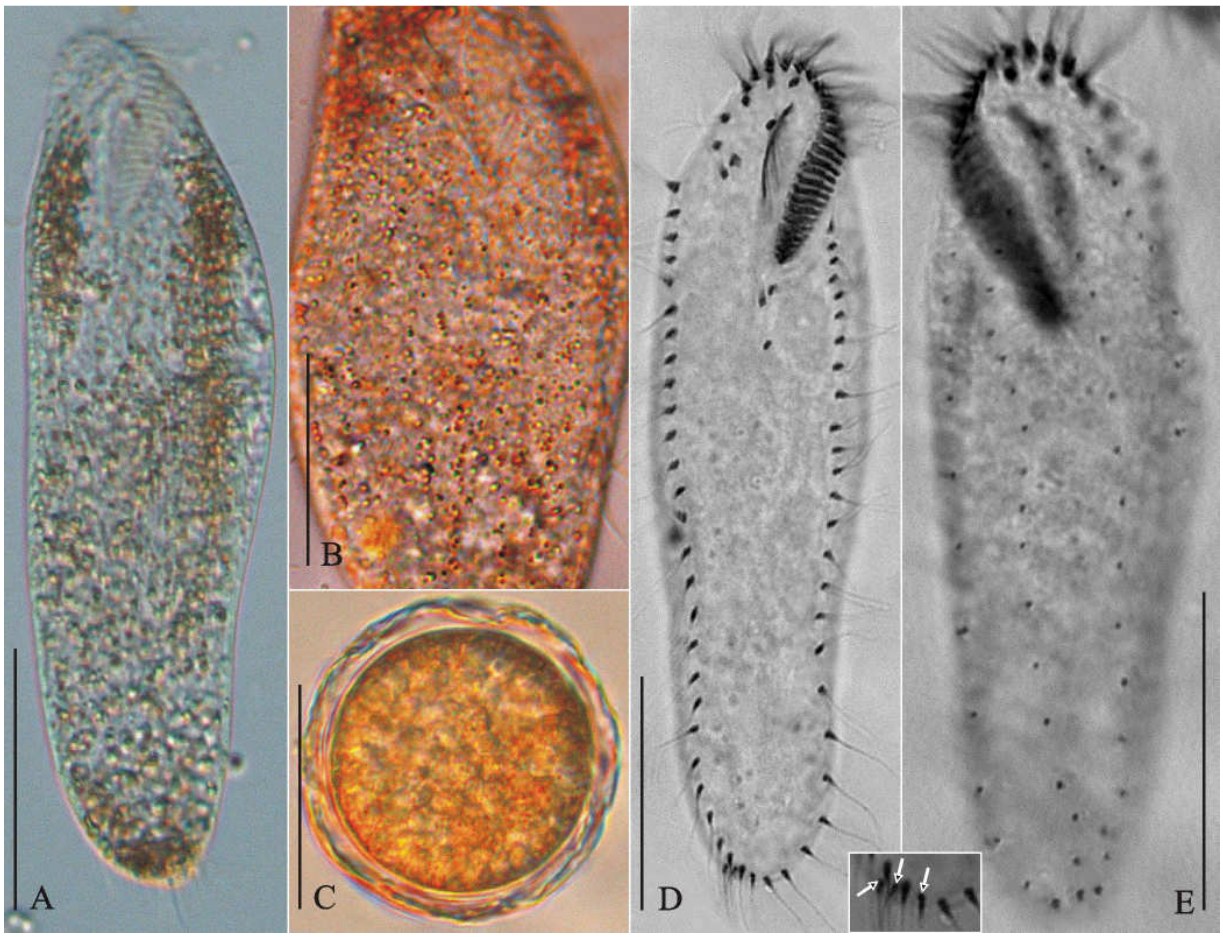

A
B
C
D
E

Fig. 5.26A–E. *Paraurosomoida indiensis* in vivo (A) and after protargol impregnation (B–E). **A.** Ventral view; inset shows arrangement of cortical granules. **B.** Infraciliature of ventral side; arrow points to rearmost left marginal cirrus. **C.** Part of the cell showing an increase in the number of postoral ventral cirri unusually present in few cells. **D.** Posterior part of the cell showing extra cirri sometimes present aligned to the marginal row. **E.** Infraciliature of dorsal surface with nuclear apparatus. AZM, adoral zone of membranelles; BC, buccal cirrus; CC, caudal cirri; CI, cytoplasmic inclusions; CV, contractile vacuole; $DK_{1–3,}$ dorsal kineties; DM, dorsomarginal row; E, endoral; FC, frontal cirrus; FVC, frontoventral cirrus; LMR, left marginal row; Ma, macronuclear nodules; Mi, micronucleus; P, paroral; POVC, postoral ventral cirrus; RMR, right marginal row. Bars: 25 μm.

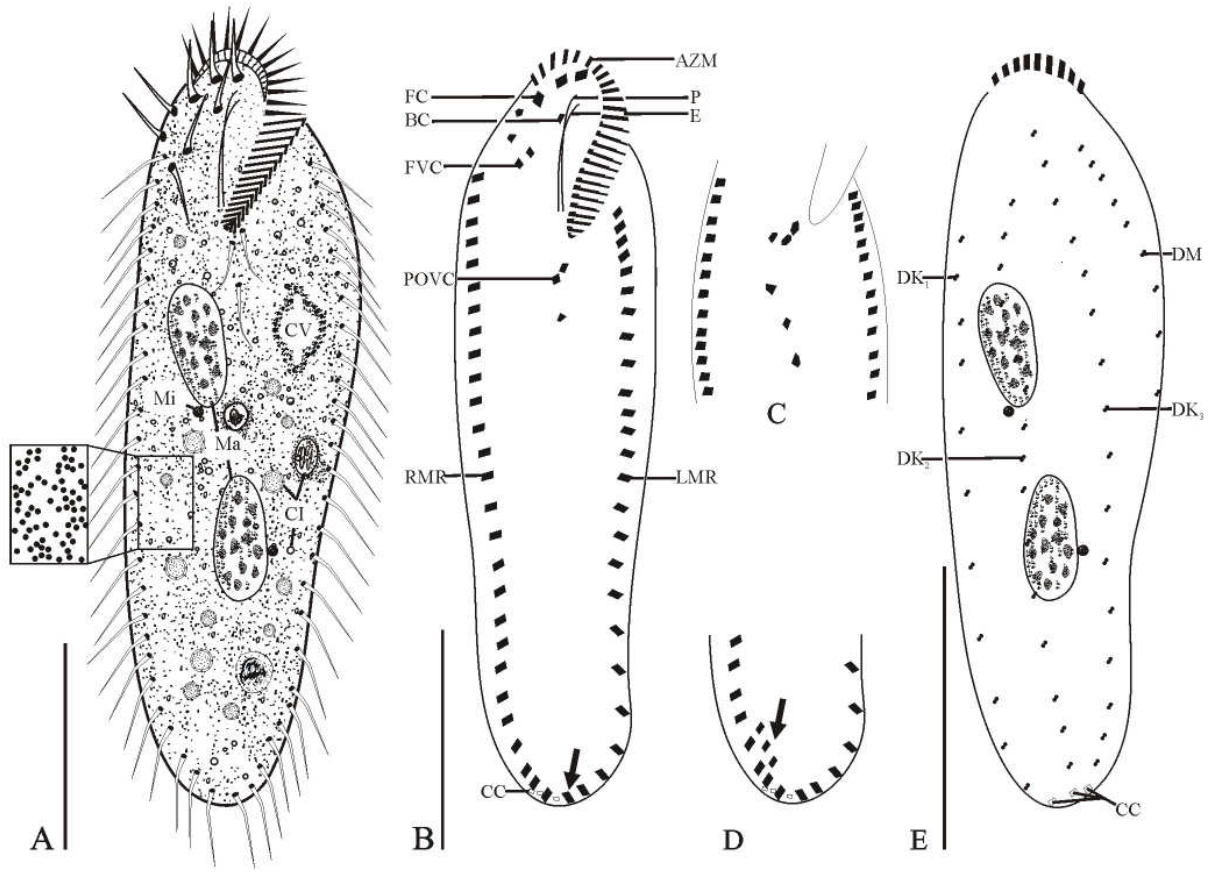
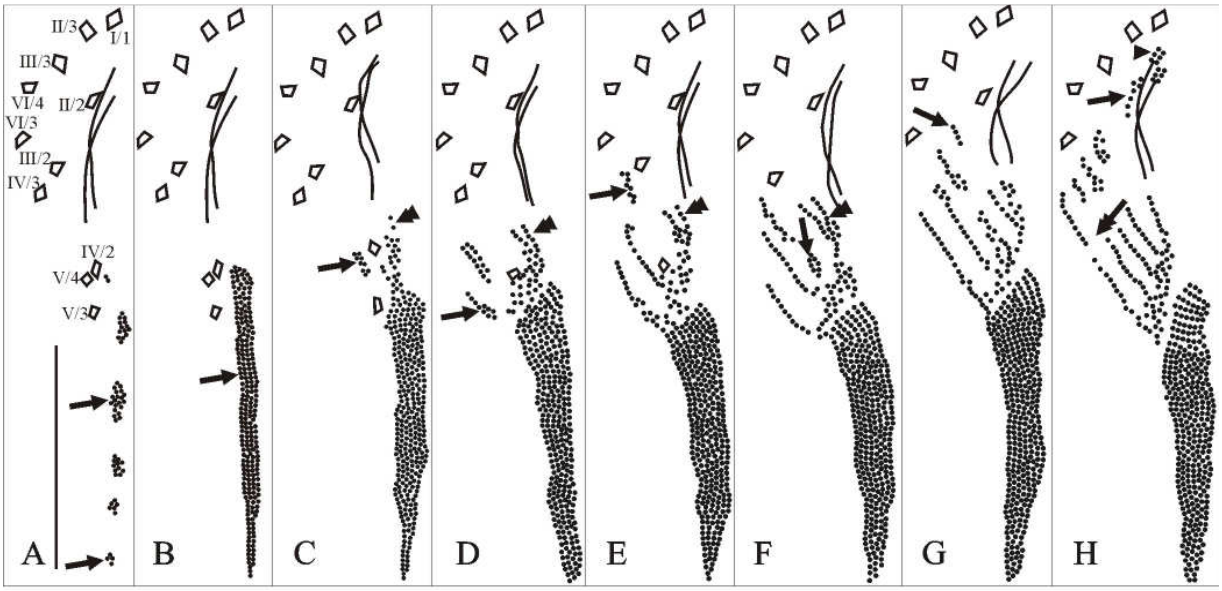

A
Mi
Ma
CV
CI
B
AZM
FC
BC
P
E
FVC
POVC
RMR
LMR
CC
C
D
E
DM
DK1
DK3
DK2
CC

Fig. 5.27A–H. Line diagrams of mid-segments of protargol-impregnated early and middle dividers of *Paraurosomoida indiensis* depicting morphogenetic events on the ventral surface (for documentation, see Figs 5.31A–G, 5.32A). **A.** Stomatogenesis begins (arrows) with the formation of four or five small assemblages of kinetosomes developed in a row up to the level of the postoral ventral cirri. **B.** Proliferation and joining of these small fields give rise to oral primordium (arrow) **C–H.** For the opisthe, two streaks (double arrowheads in C–F) arise from the right anterior face of the oral primordium while the other three arise from disaggregated parental cirri V/4, V/3 and IV/2 (arrows in C, D and F respectively); as membranelles form in the oral primordium (D–H), loose kinetosomes in front align to form anlage I which develops into the paroral, endoral and cirrus I/1. For the proter, the parental paroral and endoral disaggregate partially (arrowhead in H) and undergo proliferation to form the paroral, endoral and cirrus I/1; cirri IV/3, III/2 and II/2 disaggregate (arrows in E, G and H respectively) to give rise to cirral streaks. The streaks arising from cirri V/4 and V/3 split transversely (double arrow in H) to form V and VI anlagen for both proter and opisthe. Bars: 25 μm.

Fig. 5.28A–D. Line diagrams of protargol-impregnated middle and late dividers of *Paraurosomoida indiensis* depicting morphogenetic events **A.** Ventral view of middle divider showing, two sets of anlagen, one for each daughter cell. Marginal anlagen form in each row at two levels by within-row formation (arrowheads). **B.** Ventral view of a late divider. Eleven cirri arise from the frontal anlagen in each set, splitting in a 1, 2, 2, 2, 2, 2 pattern. Dorsomarginal anlagen arise at the anterior ends of the right marginal anlagen (arrowheads). **C, D.** Ventral (C) and dorsal (D) view of a late divider. Arrowheads in D point to the new dorsomarginal rows. In (B) and (C), the new postoral ventral cirri have been encircled. Bars: 25 μm.


II/3
I/1
III/3
VI/4
II/2
VI/3
III/2
IV/3
IV/2
V/4
V/3
A
B
C
D
E
F
G
H

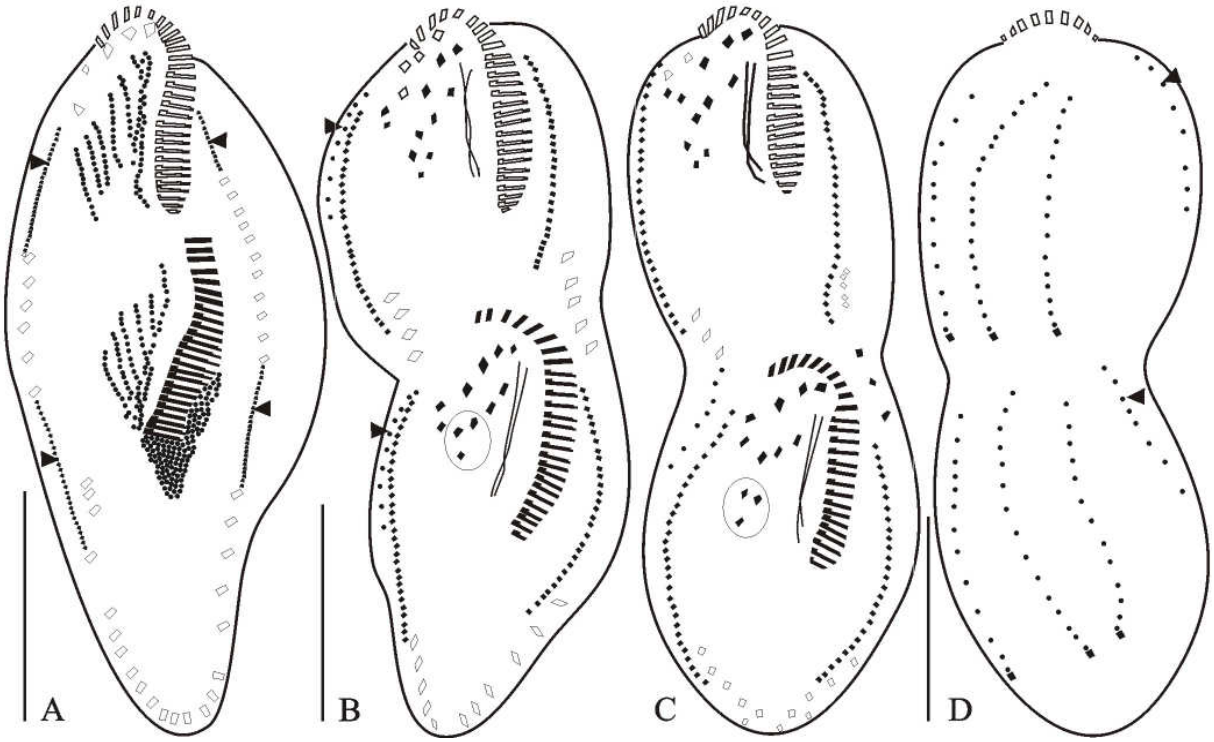

A
B
C
D

Fig. 5.29A, B. Line diagrams of protargol-impregnated *Paraurosomoida indiensis.* Infraciliature of ventral and dorsal side of a late reorganizer. Bars: 25 µm.

Fig. 5.30A, B. Photomicrographs of protargol-impregnated *Paraurosomoida indiensis*. Infraciliature of ventral and dorsal side of a late reorganizer. Bars: 25 µm.

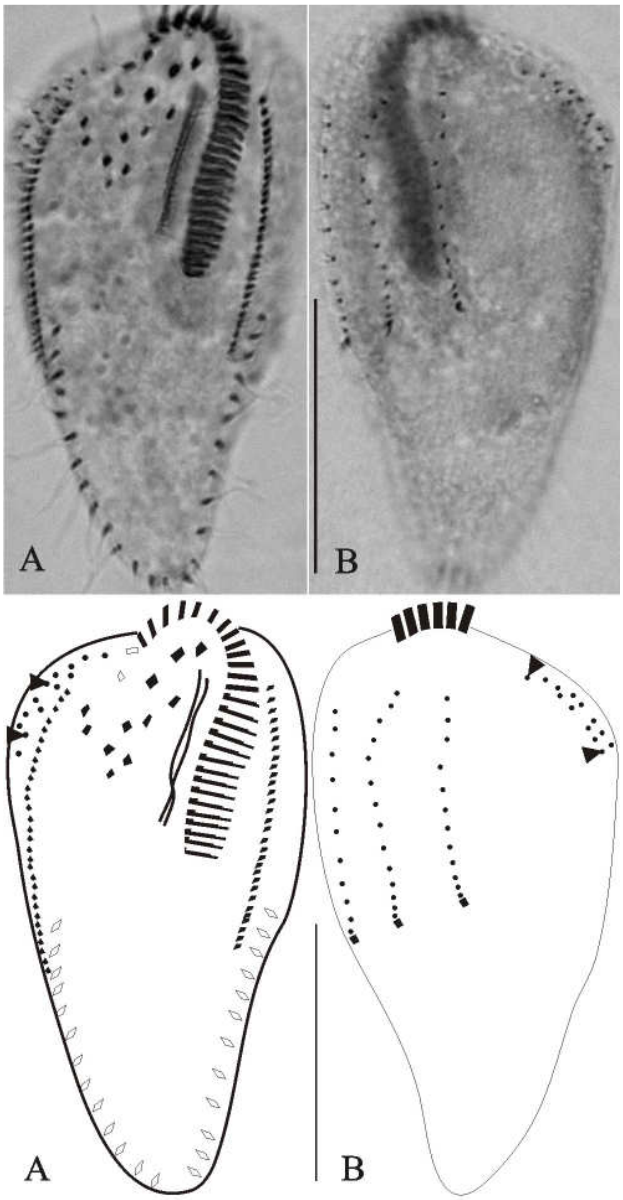
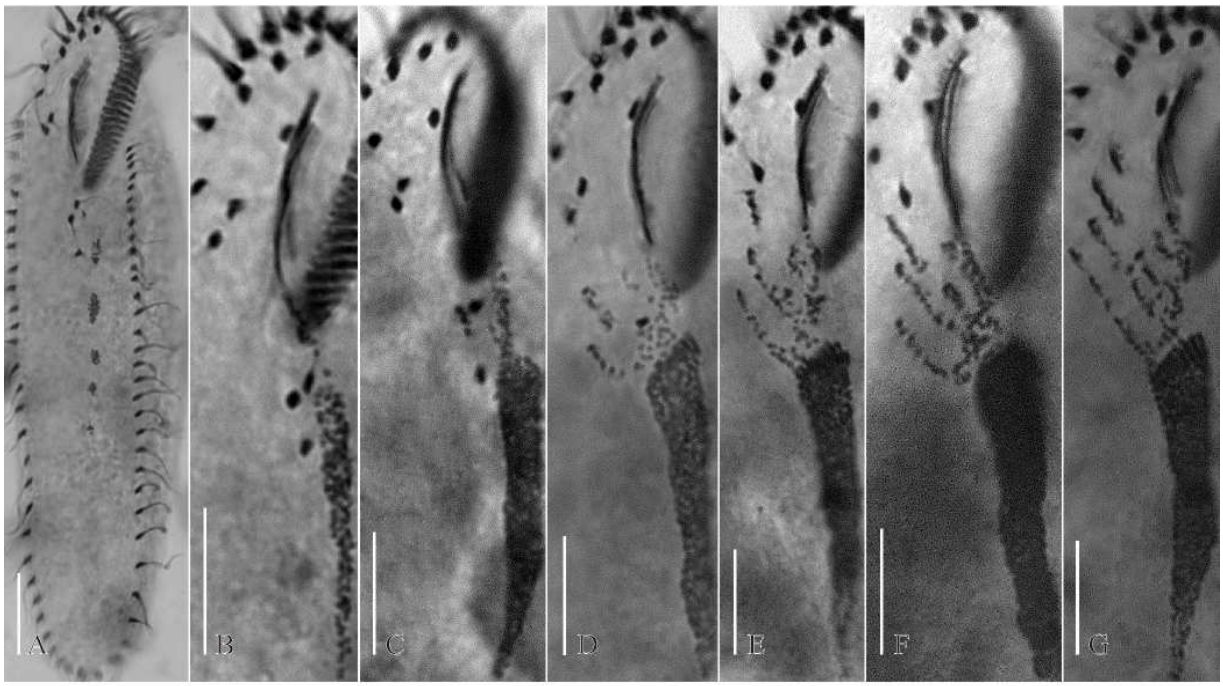

A
B
A
B

Fig. 5.31A–G. Photomicrographs of protargol-impregnated early and middle dividers of *Paraurosomoida indiensis* depicting morphogenetic events on the ventral surface. For details see Figs 5.27A–G. Bars: 10 μm.

Fig. 5.32A–E. Photomicrographs of protargol-impregnated middle and late dividers of *Paraurosomoida indiensis* depicting morphogenetic events on the ventral surface (A–D), dorsal surface (E). For details see Figs 5.27H, 5.28A–D. Bars: 10 μm.


A
B
C
D
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F
G

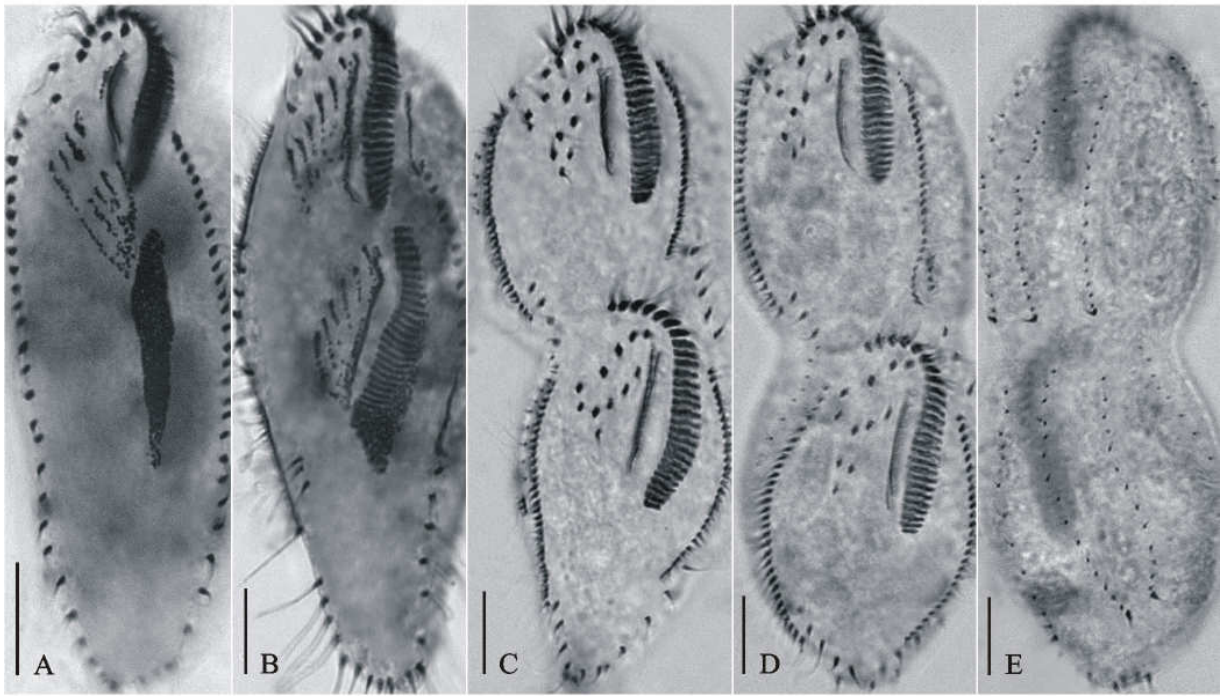

A
B
C
D
E

Fig. 5.33. Neighbour-joining tree showing the position of *Paraurosomoida indiensis* (arrow), constructed with software MEGA 5 using Maximum Composite Likelihood nucleotide substitution model. Bootstrap values are shown at nodes. The codes following the names are GenBank accession numbers. Bootstrap values lower than 40% are replaced with hyphens. The scale bar corresponds to a distance of 5 substitutions per 1,000 nucleotide positions. For details about topology of Stylonychinae, see "Materials and Methods" section.

Fig. 5.34. The 50% majority-rule consensus distance tree (minimum evolution) showing the position of *Paraurosomoida indiensis* (arrow), constructed using the "tree bisection and reconnection (TBR)" branch swapping algorithm with PAUP* Version 4b10 based on the General Time Reversible model. Gaps were treated as missing data. The support for the internal branches was estimated using the bootstrap method with 1,000 replicates. The codes following the names are GenBank accession numbers. For details about topology of Stylonychinae, see "Materials and Methods" section.

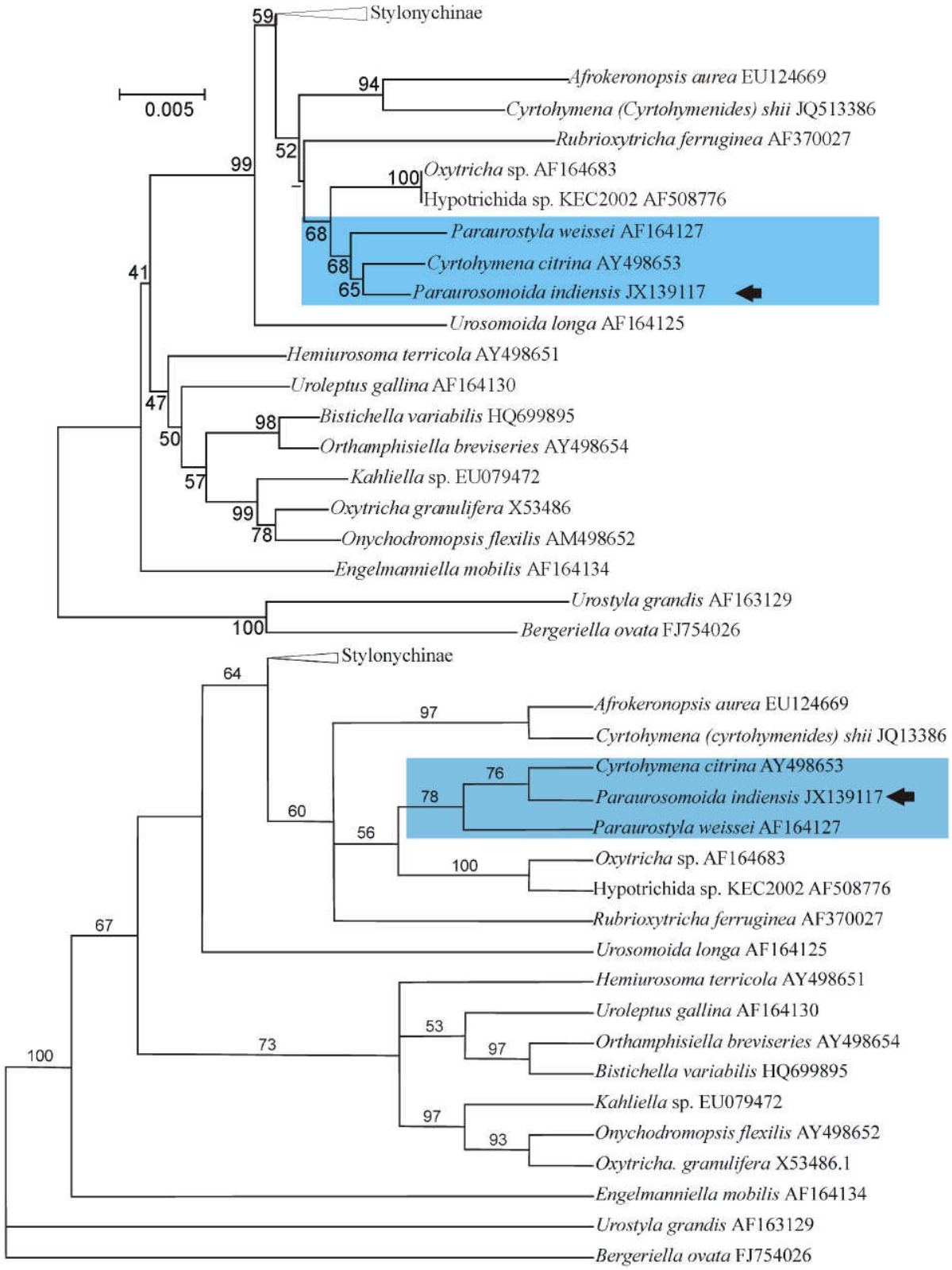

0.005
Stylonychinae
Afrokeronopsis aurea EU124669
Cyrtohymena (Cyrtohymenides) shii JQ513386
Rubrioxytricha ferruginea AF370027
Oxytricha sp. AF164683
Hypotrichida sp. KEC2002 AF508776
Paraurostyla weissei AF164127
Cyrtohymena citrina AY498653
Paraurosomoida indiensis JX139117
Urosomoida longa AF164125
Hemiurosoma terricola AY498651
Uroleptus gallina AF164130
Bistichella variabilis HQ699895
Orthamphisiella breviseries AY498654
Kahliella sp. EU079472
Oxytricha granulifera X53486
Onychodromopsis flexilis AM498652
Engelmanniella mobilis AF164134
Urostyla grandis AF163129
Bergeriella ovata FJ754026
Stylonychinae
Afrokeronopsis aurea EU124669
Cyrtohymena (cyrtohymenides) shii JQ13386
Cyrtohymena citrina AY498653
Paraurosomoida indiensis JX139117
Paraurostyla weissei AF164127
Oxytricha sp. AF164683
Hypotrichida sp. KEC2002 AF508776
Rubrioxytricha ferruginea AF370027
Urosomoida longa AF164125
Hemiurosoma terricola AY498651
Uroleptus gallina AF164130
Orthamphisiella breviseries AY498654
Bistichella variabilis HQ699895
Kahliella sp. EU079472
Onychodromopsis flexilis AY498652
Oxytricha. granulifera X53486.1
Engelmanniella mobilis AF164134
Urostyla grandis AF163129
Bergeriella ovata FJ754026

Fig. 6.1. Agarose gel electrophoretic images of genomic DNA and amplified 18S rDNA of different isolates of hypotrichous ciliates from Sikkim (A, genomic DNA; B, amplified 18S rDNA). **A.** Lanes 2 and 3 show resolution of genomic DNA extracted from two isolates; micronuclear DNA visible in the form of distinct band (chromosomal DNA) and macronuclear DNA in the form of smear (gene sized DNA). **B.** Lanes 2-8 show amplified 18S rDNA segments of seven different isolates. The DNA samples were electrophoresed in 1% agarose gel in parallel (lane 1 in A, B) with enzyme digested products of lamda DNA (200 bp DNA ladder).

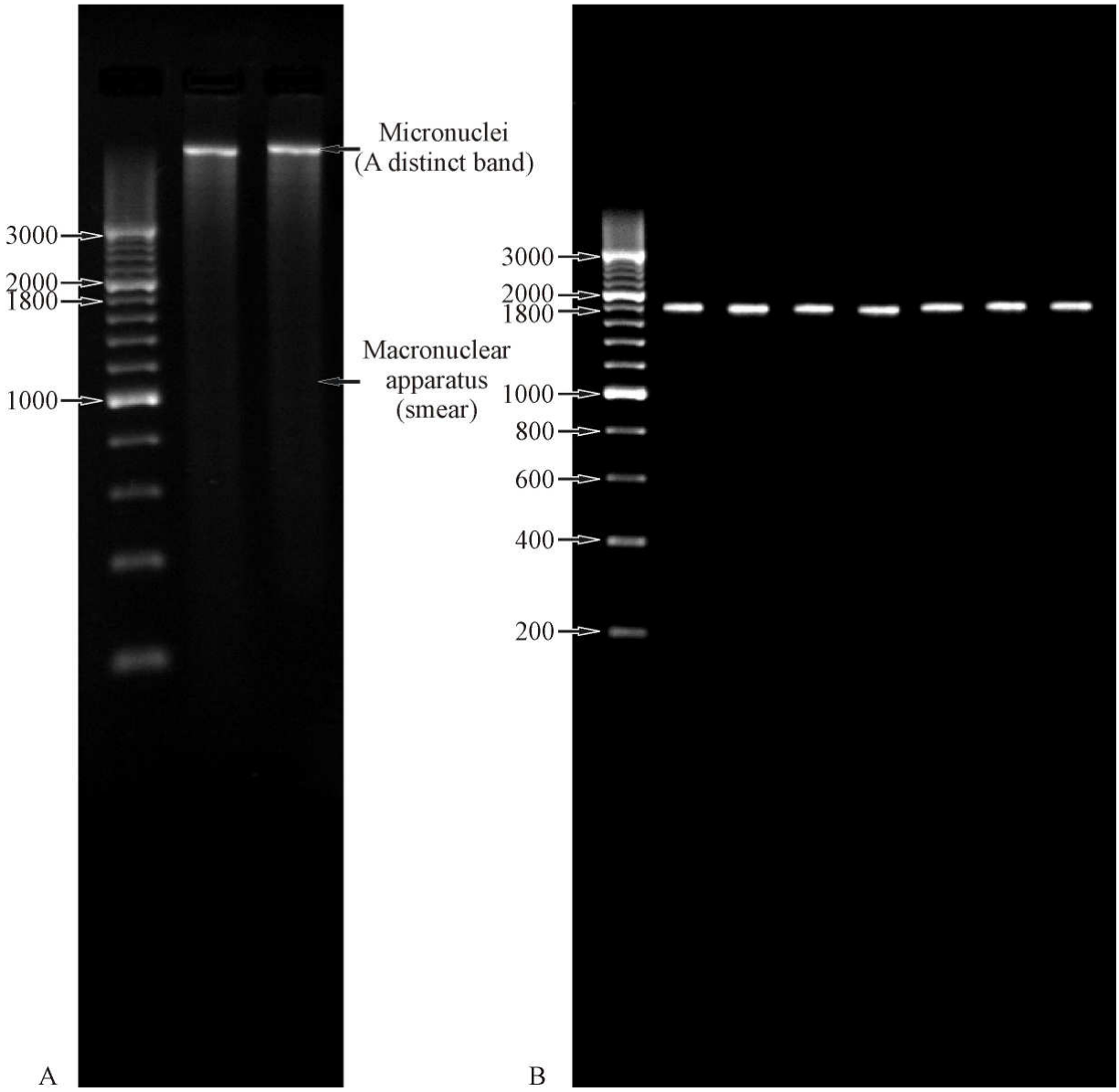
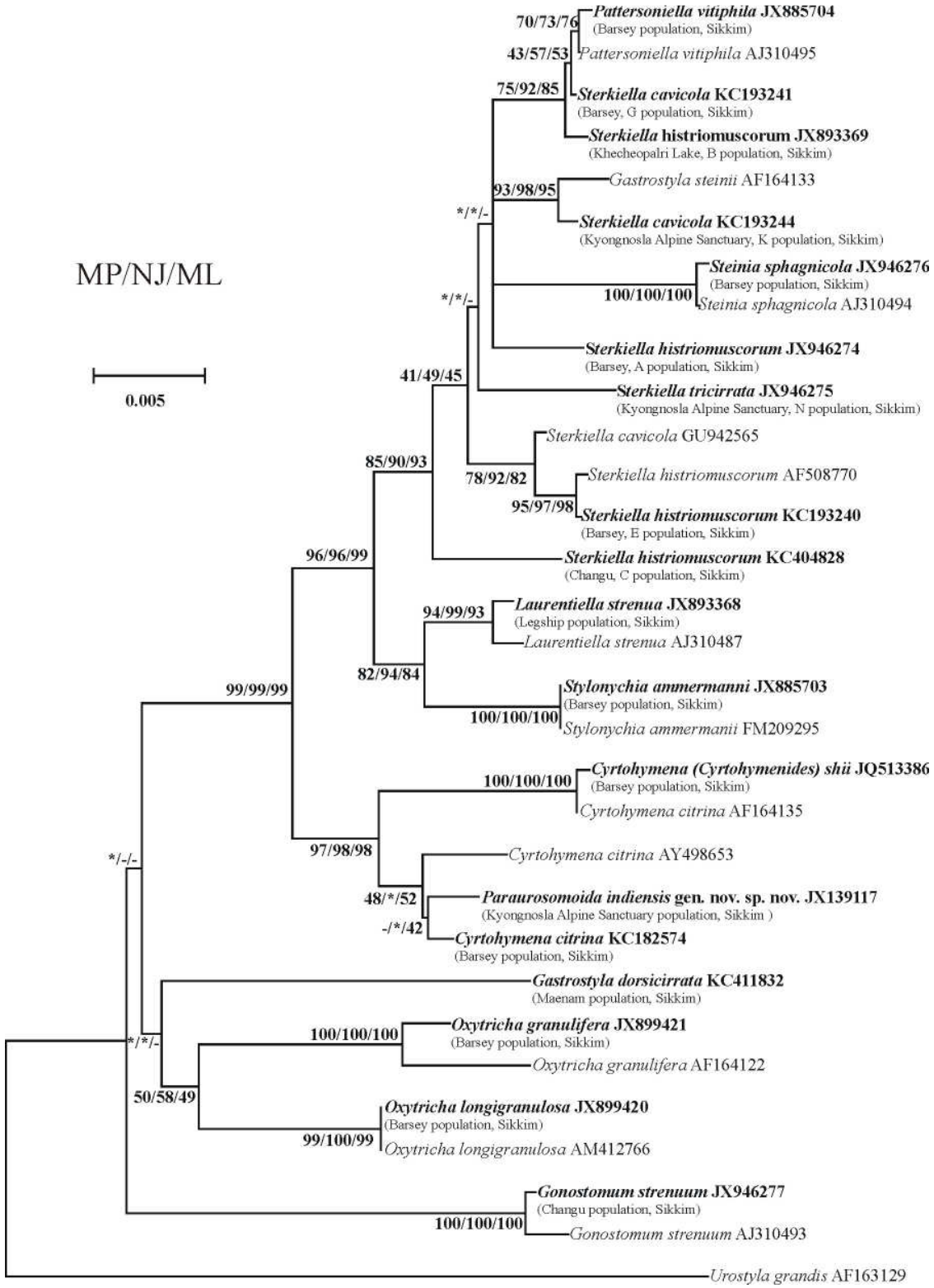

Micronuclei
(A distinct band)
3000
2000
1800
1000
Macronuclear
apparatus
(smear)
A
3000
2000
1800
1000
800
600
400
200
B

Fig. 6.2. A Maximum-likelihood tree of 31 taxa, constructed by using 18S rDNA sequences applying the Kimura 2-parameter model. There were a total of 1762 positions in the final dataset. The numbers at nodes represent bootstrap values in order of Maximum parsimony (MP), Neighbour-joining (NJ) and Maximum likelihood (ML). Maximum parsimony tree was obtained using the Close-Neighbour-Interchange algorithm. Neighbour-joining tree was constructed using Kimura 2-parameter model. Clades with different topologies in MP and NJ trees relative to ML tree are indicated with asterisks. In each case tree construction was done using 1,000 bootstrap replicates. The codes following the names are GenBank accession numbers. Bootstrap values lower than 40% are replaced with hyphens. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer phylogenetic trees. In case of *Sterkiella*, data from representative populations have been used.


MP/NJ/ML
0.005
70/73/76
Pattersoniella vitiphila JX885704
(Barsey population, Sikkim)
43/57/53
Pattersoniella vitiphila AJ310495
75/92/85
Sterkiella cavicola KC193241
(Barsey, G population, Sikkim)
Sterkiella histriomuscorum JX893369
(Khecheopalri Lake, B population, Sikkim)
Gastrostyla steinii AF164133
93/98/95
//-
Sterkiella cavicola KC193244
(Kyongnosla Alpine Sanctuary, K population, Sikkim)
Steinia sphagnicola JX946276
(Barsey population, Sikkim)
100/100/100
Steinia sphagnicola AJ310494
//-
Sterkiella histriomuscorum JX946274
(Barsey, A population, Sikkim)
41/49/45
Sterkiella tricirrata JX946275
(Kyongnosla Alpine Sanctuary, N population, Sikkim)
Sterkiella cavicola GU942565
85/90/93
78/92/82
Sterkiella histriomuscorum AF508770
95/97/98
Sterkiella histriomuscorum KC193240
(Barsey, E population, Sikkim)
96/96/99
Sterkiella histriomuscorum KC404828
(Changu, C population, Sikkim)
94/99/93
Laurentiella strenua JX893368
(Legship population, Sikkim)
Laurentiella strenua AJ310487
82/94/84
99/99/99
Stylonychia ammermanni JX885703
(Barsey population, Sikkim)
100/100/100
Stylonychia ammermanni FM209295
Cyrtohymena (Cyrtohymenides) shii JQ513386
(Barsey population, Sikkim)
100/100/100
Cyrtohymena citrina AF164135
97/98/98
*/-/-
Cyrtohymena citrina AY498653
48/*/52
Paraurosomoida indiensis gen. nov. sp. nov. JX139117
(Kyongnosla Alpine Sanctuary population, Sikkim)
-/*/42
Cyrtohymena citrina KC182574
(Barsey population, Sikkim)
Gastrostyla dorsicirrata KC411832
(Maenam population, Sikkim)
Oxytricha granulifera JX899421
(Barsey population, Sikkim)
100/100/100
//-
Oxytricha granulifera AF164122
50/58/49
Oxytricha longigranulosa JX899420
(Barsey population, Sikkim)
99/100/99
Oxytricha longigranulosa AM412766
Gonostomum strenuum JX946277
(Changu population, Sikkim)
100/100/100
Gonostomum strenuum AJ310493
Urostyla grandis AF163129