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-hypotrichous 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 *Trithigmnostoma* 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.