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Studies on four araphid taxa (Bacillariophyta) from Srikhola River, Eastern Himalaya

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Abstract

Diatoms were collected from the Srikhola River of Darjeeling district, West Bengal in Eastern Himalaya. Four taxa, namely, *Diatoma mesodon* (Ehrenberg) Kützing, *Fragilaria capucina* var. *vaucheriae* (Ehrenberg) Kützing, *Punctastriata linearis* Williams & Round and *Ulnaria ulna* (Nitzsch) Compère were observed using differential interference contrast (DIC) and field-emission scanning electron microscopy (FE-SEM) and identified in light of modern diatom taxonomic trends. Limnological analysis of Srikhola River indicates its water is clean and not polluted. *Diatoma mesodon* and *Punctastriata linearis* are strictly confined to hilly streamline as epilithic form. *Fragilaria capucina* var. *vaucheriae* and *Punctastriata linearis* are reported for the first time from West Bengal.

Key Words: Araphid diatoms, River, microscopy, new records, West Bengal, Eastern Himalaya.

Introduction

Diatoms are siliceous algae that are well documented from aquatic systems in terms of richness and abundance (Hoek *et al.*, 1995). According to Stoermer and Smol, (1999) identification of this group of algae are complicated due to variations in the forms and frustule ornamentations. Studies on diatoms in India were initiated more than one and a half century back by Ehrenberg, (1845). After that many scientists surveyed diatom flora in India. Among them Gonzalves and Gandhi, (1952, 1953, 1954); Krishnamurthy, (1954); Gandhi, (1955, 1957a-b, 1958a-b, 1959a--d, 1960, 1961, 1962, 1964, 1966, 1967, 1998); Sarode and Kamat, (1979, 1980, 1983, 1984); Venkataraman, (1957) and Desikachary, (1988, 1989) have made significant contribution. India is rich in biodiversity having two extremely important biodiversity hot spots namely Eastern Himalaya and Western Ghats. We know that biodiversity hot spots are more prone to endemic species so there is always greater chance to find new endemic diatom taxa as suggested by works of Karthick and Kociolek, (2011, 2012a, 2012b). In general Indian rivers were not so much studied for diatom flora exploration. Some notable works were done in Cooum River (Iyengar and Venkataraman, 1951), Ganga and Barna River (Singh, 1963), Tapti River (Barhate and Tarar, 1981; Ragothaman and Jaisawal, 1995), Cauvery River (Somashekar, 1983) and Alaknanda River (Nautiyal *et al.*, 1995, 1996a, 1996b; Nautiyal & Nautiyal, 1996). In West Bengal both taxonomic and ecological studies on diatoms are very scanty such as by Ehrenberg, (1845); Skvortzow, (1935); Das and Santra, (1982); Prasad *et al.*, (1988); Pal and Santra, (1990); Chowdhury and Pal, (2008); Bhattacharya *et al.*, (2011); Roy and Keshri, (2015a, 2015b); Keshri *et al.*, (2016) and Paul *et al.*, (2016).

Documentation on diatom flora of Srikhola river, West Bengal, India was not done earlier. The aim of this study was to investigate the diatoms in the Srikhola River, at the foothill of Singalila National Park, thereby expanding the knowledge on the diatom flora of the state West Bengal in Eastern Himalayan region.

Materials and Methods

Study Area

The study area located at the Srikhola River which is situated in the foothills of Singalila National Park of West Bengal, India. Singalila National Park is located at an altitude of more than 7000 feet above sea level, in the Darjeeling subdivision of Darjeeling district, West Bengal. The Park is the part of Eastern Himalayas or falls under Darjeeling Himalayan region. Srikhola River flows through the Park (Das and Ghosh, 2011). Srikhola is a beautiful small village in the Darjeeling Pulbazar block, Darjeeling Sadar subdivision of Darjeeling district of West Bengal which falls in the trekking route from Phalut and Sandakphu towards Darjeeling and Siliguri. In lepcha dialect “Sri” means “little” and “Khola” means “waterway” that implies the name Srikhola. The latitude and longitude of the study area are respectively N 27°07'82" and E 88°04'49" and situated at an altitude of 6184 feet above sea level.

Sample Collection

Diatom materials were collected from two sampling stations on 27th & 28th February, 2015 (Plate 1, Figs. 19 & 20). Epiphytic samples were collected by crushing the submerged aquatic roots and stems and resulting suspension was transferred into a glass vials. Epilithic samples were collected by vigorously scrubbing submerged and semi-submerged stones with a tooth brush and the liquid containing diatoms transferred into amber coloured glass vials. Episammic samples also were collected by using dropper. Total 16 samples were preserved in 70% ethanol and pH, temperature, TDS, electrical conductivity and salinity of the spots were measured using PCS multiparameter tester 35 series device.

Plate 1. Figs. 19-20: Srikhola River of Darjeeling district, West Bengal; India.

Cleaning Techniques

Sub-samples were cleaned using 30% hydrogen peroxide solution. These procedures are modified from the techniques of Krammer and Lange-Bertalot, (2000); Taylor *et al.*, (2005) and Karthick *et al.*, (2010). The organic coating removed and clean samples were then repeatedly centrifuged at 3000-3200 rpm and alternatively rinsed with distilled water for 3-4 times.

Slide preparation & Microscopy

Small drop of cleaned sample was mounted onto glass slides using MeltMount™ (R.I. 1.704) mounting medium and subsequently observed under an Olympus IX 81 Confocal LS microscope equipped with 100X DIC (oil) optics and photographs were taken with IPP software.

Scanning electron microscopy was performed using aliquots of the cleaned material air dried on cover slip and mounted on aluminium stub using double sided carbon tape. Stub was sputter coated with gold by Quorum Q 150R model and observed under Zeiss SUPRA 55 FE-SEM using accelerating voltage in between 4 - 5 kv and a working distance of 2.5 – 2.7 mm.

The collected preserved materials along with the permanent slides are stored in the Herbarium of Phycology laboratory in the department of Botany, The University of Burdwan for future study and reference purpose (BURD-JPK SRD 1-SRD 15 & SRD 19 dated: 27.02.2015 & 28.02.2015).

MORPHO-TAXONOMIC RESULTS

Both sites had slight alkaline waters (pH 8.42- 8.45) and other limnological parameters of those spots were measured and given as below:-

Water temperature: 8.5-11.8°C; Electrical Conductivity (EC): 28.2-34.1 µS/cm; Total Dissolved Solids (TDS): 20-24.4 ppm; Salinity: 14.3-18.5 ppt.

Systematic descriptions and identification of the diatoms have been done using standard literature including Rumrich *et al.*, (2000); Wu and Wang, (2002) and Kobayasi *et al.*, (2006).

In this study, 4 araphid diatom taxa under 4 genera have been thoroughly studied with the help of DIC (Differential interference contrast) microscopy and FE-SEM (Field-emission scanning electron microscopy). These 4 diatom taxa fall into Subdivision Bacillariophytina.
Classification followed as proposed by Round *et al.*, (1990); Medlin and Kaczmarska, (2004) and Cox, (2015).

Description of the taxa:

Family Tabellariaceae Kützing

Diatoma mesodon (Ehrenberg) Kützing (Plate 2, Figs. 1-4; Plate 3, Figs. 10-13)
Kobayasi *et al.*, 2006, pl. 68, figs. 1, 3-5, 7.

(Synonyms: *Diatoma vulgaris* var. *mesodon* (Ehrenberg) Grunow; *Fragilaria mesodon* Ehrenberg; *Diatoma hiemalis* var. *mesodon* (Ehrenberg) Kirchner; *Diatoma hiemalis* f. *mesodon* (Ehrenberg) Forti).

LM & SEM morphology: Valve length = 17.7-33.9 µm, Valve breadth = 7.4-16.3 µm, Striae density = 27-30/ 10 µm. In valve view the frustules are rectangular in shape; valves are short, elliptical - lanceolate to elliptical with broadly rounded apices. Transapical costae [Fig. 4 (e)] widely spaced and 2-3 in 10 µm, which can be parallel or oblique and sometimes incomplete. Striae fine, uniseriate and consisting of small poroid areolae weakly radiating in centre and more strongly to the extremities. The axial area is linear and very narrow. Rimoportula [Figs. 1 (a) & 3(c)] is single and present near one valve apex. Apical pore fields [Fig. 3 (d)] are present at both apices. Sometimes very small spines [Fig. 2 (b)] are present in the periphery regions of the valve face.
Notes: Collection No. – BURD-JPK SRD 1, 3, 5, 8. Habitat- epilithic.
Distribution in India: Reported from Bundelkhand region of Central India (Verma and Nautiyal, 2010), Peninsular India (Karthick *et al.*, 2013) as *Diatoma mesodon*; Kashmir & Ladakh (Compère, 1983), Kalimpong, West Bengal (Prasad *et al.*, 1988) and Sikkim (Das and Adhikary, 2014) as *Diatoma hiemale* var. *mesodon*.

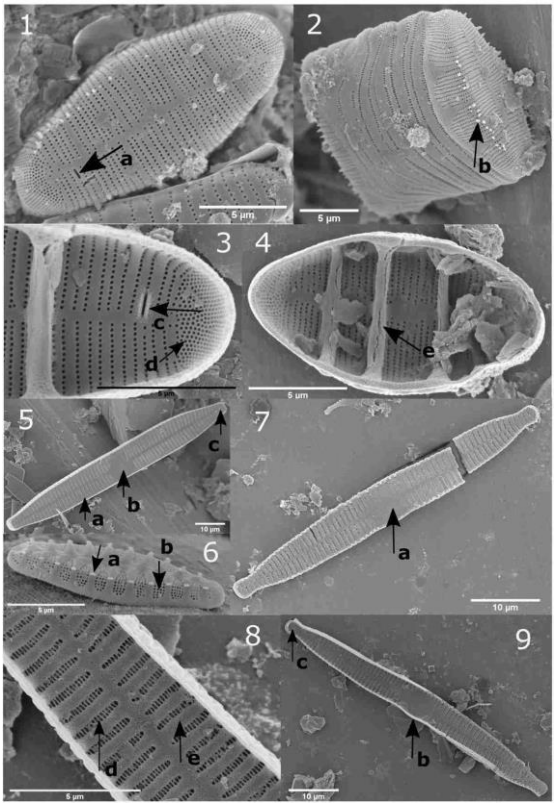

Plate 2. Figs. 1-4: *Diatoma mesodon* (Ehrenberg) Kützing [FE-SEM images]. Fig. 5: *Ulnaria ulna* (Nitzsch) Compère [FE-SEM images]. Fig. 6: *Punctastriata linearis* Williams & Round [FE-SEM images]. Figs. 7-9: *Fragilaria capucina* var. *vaucheriae* (Ehrenberg) Kützing [FE-SEM images]. (Scale bar shown in each figure individually)

Family Fragilariaceae Greville
Fragilaria capucina var. ***vaucheriae*** (Ehrenberg) Kützing (Plate 2, Figs. 7-9; Plate 3, Figs. 15-16)

Rumrich *et al.*, 2000, pl. 3, figs. 7-13. (Synonyms: *Fragilaria capucina* var. *vaucheriae* (Kützing) Lange-Bertalot; *Ceratoneis vaucheriae* (Kützing) H. Kobayasi; *Fragilaria vaucheriae* var. *parvula* (Kützing) Cleve-Euler; *Ctenophora vaucheriae* (Kützing) Schönfeldt; *Fragilaria intermedia* Grunow Van Heurck; *Synedra rumpens* var. *meneghiniana* Grunow; *Synedra vaucheriae* (Kützing) Kützing).

LM & SEM morphology: Valve length = 61.4-86.7 µm, Valve breadth = 6.2-12.7 µm, Striae density = 14-15/ 10 µm. Valves are lineo - lanceolate to linear, becoming elliptical in case of small frustules; valve face is somewhat flat or slightly undulate due to raised costae. Apex usually slightly capitate in large forms, rather sub - beaked to beaked among small frustules; central margin of valve [Fig. 9 (b)] expanded to one side, narrow and linear longitudinal area, forming a visible pseudo- raphe. Striae are broad and widely spaced, distinct, composed of long lineolae [Fig. 8 (d)]. The striae are interrupted by an expanded central area [Fig. 7 (a)] which is expanded to one side of the valve or sometimes to almost both sides of the valve. A single rimoportula [Fig. 9 (c)] is present on each valve near the valve apex or just off the central sternum.
Notes: Collection No. – BURD-JPK SRD 1, 3, 8. Habitat- epilithic.

Distribution in India: Reported from Kashmir & Ladakh (Compère, 1983) as *Fragilaria capucina* var. *vaucheriae*; Kodaikanal, Tamil Nadu (Suxena, 1983), Dehradun, Uttarakhand (Gupta, 2005) as *Fragilaria vaucheriae*; Bombay, Maharashtra (Gonzalves and Gandhi, 1952) as *Synedra rumpens* var. *meneghiniana*; Ahmedabad, Gujarat (Gandhi, 1964) as *Fragilaria rumpens* var. *meneghiniana*; Uttar Pradesh (Singh, 1963; Tripathi *et al.*, 2012), Radhanagari, Maharashtra (Gandhi, 1957b), Kolhapur, Maharashtra (Gandhi, 1958a), Maharashtra (Sarode and Kamat, 1979, 1980, 1983) as *Fragilaria intermedia*; Odisha, Assam and Sikkim (Das and Adhikary, 2014) as *Fragilaria intermedia* var. *robusta*.
This is new record of the taxa from West Bengal.

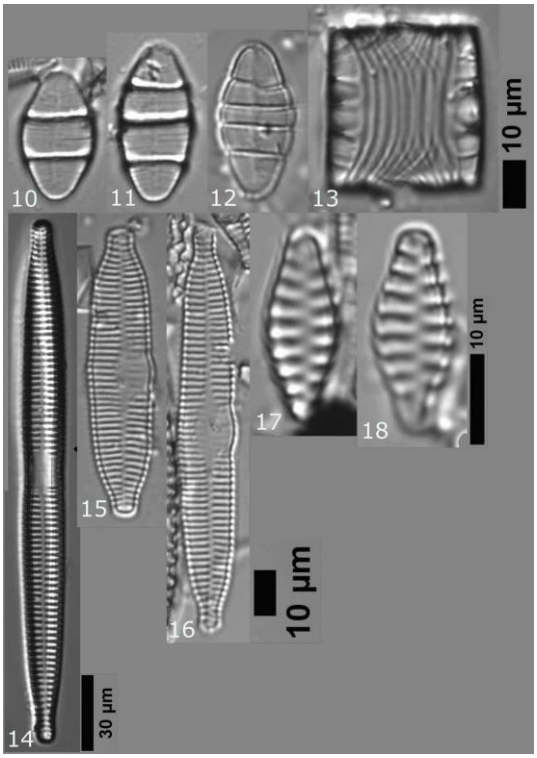

Plate 3. Figs. 10-13: *Diatoma mesodon* (Ehrenberg) Kützing [DIC images]. Fig. 14: *Ulnaria ulna* (Nitzsch) Compère [DIC images]. Figs. 15-16: *Fragilaria capucina* var. *vaucheriae* (Ehrenberg) Kützing [DIC images]. Figs. 17-18: *Punctastriata linearis* Williams & Round [DIC images]. (Different 10 µm scale bar present beside each taxa)

Family Fragilariaceae Greville
Punctastriata linearis Williams & Round (Plate 2, Fig. 6; Plate 3, Figs. 17-18). Wu and Wang, 2002, figs. 6 (G-I).

(Synonyms: *Odontidium mutabile* W. Smith; *Fragilaria pinnata* Ehrenberg; *Fragilaria mutabilis* var. *subsolitaris* Grunow; *Fragilaria elliptica* Schumann; *Fragilaria pinnata* var. *subrotunda* Mayer).
LM & SEM morphology: Valve length = 13.6-23.1µm, Valve breadth = 2-7.9 µm, Striae density = 12-14/ 10 µm.

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Valves elliptical to broadly elliptical, sometimes with central inflation, ends rounded, sometimes heteropolar with depression at one end. Striae [Fig. 6 (b)] level slightly depressed the valve surface. Striae consisting of a distinctive, high silicified, regular net of transapical and apical bars. Small spines [Fig. 6 (a)] situated along the valve/mantle junction on the interstriae.
Notes: Collection No. – BURD-JPK SRD 1, 3, 14. Habitat- epilithic.

Distribution in India: Reported from Nagpur, Maharashtra (Sarode and Kamat, 1980) as *Fragilaria pinnata* f. *subrotunda*.
This is new record of the taxa from West Bengal.
Family Ulnariaceae Cox
Ulnaria ulna (Nitzsch) Compère (Plate 2, Fig. 5; Plate 3, Fig. 14)
Kobayasi *et al.*, 2006, pl. 107, figs. 1-2. (Synonyms: *Fragilaria ulna* (Nitzsch) Lange-Bertalot; *Synedra ulna* (Nitzsch) Ehrenberg; *Frustulia ulna* (Nitzsch) C. Agardh).

LM & SEM morphology: Valve length = 81.3-205.8 µm, Valve breadth = 9.5-13.3 µm, Striae density = 8-9/ 10 µm. Valves are linear with cuneate poles, margins are parallel, tapering to protracted apices. Central sternum is narrow and straight. Central area [Fig. 5 (b)] transversely expands and forming a rectangular area, there may be "ghost" striae seen in central area. Striae [Fig. 5 (a)] are parallel. Single labiate process [Fig. 5 (c)] is present in near the apex of the valve.

Notes: Collection No. – BURD-JPK SRD 4, 7. Habitat- epiphytic.

Distribution in India: Reported from Manipur (Biswas, 1936), South India (Venkataraman, 1939), Cooum river, Madras (Iyengar and Venkataraman, 1951), Bombay & Salsette, Maharashtra (Gonzalves and Gandhi, 1952), West Bengal (Dutt *et al.*, 1954), Madras (Chacko and Krishnamurthy, 1954), Rajasthan (Gandhi, 1955), Gersoppa falls (Jog-falls) (Gandhi, 1957a, 1966), Kolhapur, Maharashtra (Gandhi, 1957b), Hirebhsagar Dam, Mysore (Gandhi, 1958b); Delhi (Singh, 1958), Mysore, Andhra Pradesh (Gandhi, 1959b), Kolhapur, Maharashtra (Gandhi, 1959a), Dharwad, Karnataka (Gandhi, 1959d), Ahmedabad, Gujarat (Gandhi, 1959c, 1960, 1961, 1964, 1967), Uttar Pradesh (Singh, 1961, 1963), Lonavla, Maharashtra (Gandhi, 1962), Kanpur, Uttar Pradesh (Ahmad, 1972), Nainital (Kamat and Aggarwal, 1975), Mansar Lake, Jammu (Anand and Kant, 1976), Marathwada, Maharashtra (Sarode and Kamat, 1979), Allahabad, Uttar Pradesh (Pandey and Pandey, 1980), South India (Rao *et al.*, 1982), Tapti river (Barhate and Tarar, 1983), River Moosi (Venkateswarlu, 1983), Vidarbh, Maharashtra (Sarode and Kamat, 1983, 1984), Andhra Pradesh (Sarojini and Subbarangaiah, 2000), Chennai, Tamilnadu (Eswari and Ramani Bai, 2002), South India (Chandrasekaran, 2000) [All as *Synedra ulna*]. Himachal Pradesh (Dwivedi and Mishra, 2014) as *Fragilaria ulna*.

Discussion

Srikhola River is a small waterway of Darjeeling Himalayan region. From the two sampling stations we found lots of centric, monoraphid, biraphid and eunotioid diatom taxa but araphid taxa were less recorded in proportion. Comparing with previous works (Pal and Santra, 1990; Chowdhury and Pal, 2008; Bhattacharya *et al.*, 2011; Roy and Keshri, 2015a, 2015b; Keshri *et al.*, 2016) on fresh water low altitudinal dams or ponds, we found that some diatom taxa are strict inhabitants of high altitudinal fresh waters viz. *Diatoma* spp. and *Punctastriata* spp.

The limnological condition of the sampling stations in winter season showed low conductivity (28.2-34.1 µS/cm) and salinity (14.3-18.5 ppt) as most freshwater streams and lakes water has low salinity and conductivity. In "clean" water, TDS (total dissolved solids) is approximately equal to salinity and water should be slightly alkaline (Allan, 1995). So from the limnological data (pH 8.42- 8.45; TDS 20-24.4 ppm) availed we can conclude that the Srikhola River water is definitely clean. And therefore the occurred diatoms in Srikhola River show their preference over clean water.During this study *Punctastriata linearis* created problem in identification when we observe it with only light microscope, even DIC microscope is unable to differentiate between closely related species like *Punctastriata lancettula, Punctastriata mimetica* and sometimes genus like *Staurosirella pinnata*. But electron microscope (FE-SEM) has been found only to reveal the minute ultra structural details of *Punctastriata linearis* as it shows small spines, striae made of net like areolae, elliptical striae not terminated at the valve mantle in acute form.

In the present assemblage all the taxa except *Punctastriata linearis*, are more or less commonly reported from different parts of India. However, all the taxa are newly reported from this Darjeeling Sadar subdivision. *Fragilaria capucina* var. *vaucheriae* and *Punctastriata linearis* are new reports from West Bengal. Quantitatively amongst those diatoms, *Ulnaria ulna* is most commonly occurring taxa in India and it is almost cosmopolitan in distribution.

Diatoma mesodon is also commonly occuring in hilly Rivers and streams but still there are scanty reports in different parts of India including West Bengal.

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