SHORT COMMUNICATION

Taxonomic studies of some rare species of Genus *Sirogonium* Kützing and *Zygnema* Agradh from Darbhanga, North Bihar

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Abstract

The freshwater algal flora is a primary producer in the aquatic ecosystem. But due to, changes in climatic conditions and pollution in water bodies affected the algal diversity. These algal floras, because of their being rich in nutritional contents such as protein, carbohydrate, lipid, vitamins, and folic acid, can be used as alternative sources of food in the future. Besides, it also helps in nitrogen fixation, acts as a growth-promoting substance, and is used as food for aquatic animals. The author describes two species of the genus *Sirogonium* Kützing and three algal species of the genus *Zygnema* Agardh belonging to the family Zygnemataceae in the present paper. Out of 5 species, *Z. excoummne* is being reported for the first time from the Indian subcontinent, while *S. floridanum* for the first time from Bihar. The remaining three species, *S. megasporum*, *Z. substellinum* and *Z. tenue,* are being observed for the second time from Darbhanga, North Bihar, after Kargupta & Jha (2004).

Keywords: Genus, Rare species, Zygnemataceae.

Highlights

- *Z. excoummne* is being reported for the first time from the Indian subcontinent.
- *S. floridanum* reported for the first time from Bihar.
- *S. megasporum*, *Z. substellinum* and *Z. tenue,* are being observed for the second time from Darbhanga, North Bihar,

These rare species are reported from the freshwater habitat.

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Introduction

Darbhanga earlier known as the city of ponds, located at 26°6'42.72"N latitude and 85°53'45.61"E longitude, is a habitat of the multitude of filamentous freshwater green algae. These green algae release approximately 50% of total oxygen, the most important source of life in the environment. It can convert solar energy into chemical energy by carbon dioxide fixation. These aquatic algal florae are more efficient than terrestrial bodies. The algal species has attracted researchers regularly for the production of biofuels like biohydrogen, bio-methanol, biodiesel, etc. Besides, algal species can also be used as food, soil additives, and health supplements for the bioremediation of deadly toxic metal ions, chemicals, wastewater treatments and energy sources. They can produce varieties of secondary metabolites with antioxidant, antibacterial, antifungal, antiviral, antitumor and anti-inflammatory activities. The algal strains are rich in nutritional value as they contain organic and inorganic substances benefiting the human population (Kuda 2002). The algal strains are abundant in carbohydrates, sugar, flavonoids, protein, phenolic compounds, etc. The phenolic compounds play a defensive role against dangerous UV radiation, intruding fungi, viruses, bacteria, etc. Carbohydrate, one of the energy rich compounds and structural and storage components of the cell, helps the algae to adjust themselves according to the environmental condition. Algae is considered as one of the major sources of protein other than vegetables, meat, pulse, etc. The mortality rate is increasing day by day due to the scarcity of food to feed the growing population and malnutrition. Hence, in the future algal strains can be used as one of the alternative sources of food to feed the population. The knowledge of algae is valuable in the field of environmental science and ecology. It can act as a sensitive bioindicator to assess water quality. Its availability and composition can provide information regarding pollution, the overall health of the ecosystem, etc (Omar 2010). These microalgae are photosynthetic organisms that contribute to carbon sequestration by converting CO_2 present in the environment into organic biomass. This helps in mitigating the harmful effects of climate change (Peter *et al.,* 2021). Microalga serve as natural biofertilizers that enrich the soil by nitrogen fixation, promote plant growth and reduce the reliance on synthetic fertilizers (Ammar *et al*., 2023). These practical applications of algal strains in numerous areas generate interest in identifying the algal strains.

The filamentous freshwater green algae grow as an algal mat in shallow water like ditches, puddles, ponds, canals etc. Algae are classified by several phycologists like Smith (1951), Chapman (1962), Graham *et al.,* (2008), Fritsch (1935) etc. Among all these, the most authoritative and comprehensive classification of algae was proposed by Fritsch (1935). The present study is based on

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the Fritsch classification. The genera *Sirogonium* and *Zygnema* belong to the class Chlorophyceae, order- Conjugales and family- Zygnemataceae. The taxa of the family Zygnemataceae are unicellular and uniseriate, containing spiral, stellate and ribbon-shaped chloroplasts. Some of the genus, like *Sirogonium*, *Zygnema, Debarya*, *Zygnemopsis* etc., occurs rarely. These algae exchange their genetic material through the process of conjugation and are termed as "Conjugatophyceae". These rarely occurring species have been less studied in Bihar. Among these genera, two of the genera, *Sirogonium* Kützing and *Zygnema* Agardh, have been studied in the present paper. The rare taxon prefers to grow mostly during the winter season at pH between 6.5 to 7.5 and temperature between 18 to 34°C.

The genus *Sirogonium* Kützing are filamentous freshwater green algae with unbranched thallus. It contains straight or slightly curved chloroplasts in numbers between 2 and 10. It lacks conjugation tubes and conjugation between the filaments happens directly through geniculation. Zygospores of the genus *Sirogonium* Kützing vary in shape from ellipsoid to ovoid and in color from yellow to brown. A total of 39 species worldwide represent *Sirogonium* Kützing. Out of these 39 species, only 10 species are reported from different regions of India (Randhawa 1959; Sarma & Khan 1980). In the monograph on Zygnemataceae by Randhawa (1959), a total of 15 species of *Sirogonium,* including eight species from Indian habitat, were reported. These species were reported by Martens (1869a), Dixit (1937), Iyengar (1963), Prasad (1964), Rattan (1964), Srivastava (1981a, b), Usha devi & Panikkar (1994), Halder (2016), etc. Kamat (1968) did tremendous work on freshwater green algae. During his work, Kamat reported 2 species of *Sirogonium*. Agarkar & Agarkar (1972), while exploring Kanpur algal flora, identified one species of *Sirogonium*. Mahato *et al.,* (1996) explored the Zygnemataceae from Chota Nagpur Plateau of Jharkhand and discovered a new taxon of *Sirogonium* (*S. pseudocanalis*). Rai & Misra (2007) reported two species of *Sirogonium* (S. *illinoiense* (Transeau) G M Smith & *S. sticticum* (Engl. Bot.) Kutzing from Nepal. Jha (2010), while exploring the Kosi River basin, reported *S. sticticum* Kützing for the first time from the studied area. Tagad (2016) studied the planktonic algae from Pune and recorded 344 species belonging to 112 genera. These 344 species include one species of *Sirogonium* (*S. tenuis* (Nordstedt) Transeau/Verma *et al.,* (2021) published a checklist of Chlorophyceae from Uttar Pradesh. Their checklist includes 465 taxa of Zygnematophyceae. Among these taxa, they reported eight taxa of *Sirogonium*. Jha & Jha (2023) identified three species of *Sirogonium* from the Supaul district of Bihar. Among these three taxa, *S. megasporum* and *S. melanosporum* were identified for the first time from Bihar.

The genus *Zygnema* Agardh contains filamentous thallus and unbranched and unattached filaments. Its vegetative cell length is either equal to or several times its diameter. *Zygnema* Agardh*'s* width is 10 to 50 µm, containing two or rarely four stellate chloroplasts with a large pyrenoid at the center. Reproduction in *Zygnema* Agardh is mainly by zygospores or akinetes. Zygospores, which are ellipsoid, ovoid, or compressed globose in shape, develop either in the conjugation canal or in one of the filaments. Mesospore of *Zygnema* Agardh is smooth, sorbiculate, or variously ornamented.

The genus *Zygnema* Agardh is represented by a total of 204 species across the world (Kadlubowska 1984; Guiry & Guiry 2016). Out of these, only 76 species of *Zygnema* Agardh have been identified by Randhawa (1959), Gupta (2012) and Halder (2017). Some other quantum research works on *Zygnema* Agardh were presented by Martens (1869a), Singh (1959), Kothari (1971), Prasad & Misra (1983, 1984), Sarma & Kargupta (1986,1987), Pandey & Habib (1995) from Siliguri, Manipur, Assam, Uttar Pradesh, Punjab, West Bengal. Pandey & Habib (1995) described 5 taxa of *Zygnema* from Bareilly. Jha (2010), while exploring Kosi River basin in Bihar and Nepal, for the first time identified *Z. chalybeospermum* and *Z. insigne*. Kumar & Sahu (2012), during their survey of the algal flora of paddy fields of Lalgutwa, Jharkhand, described *Z. stellinum*. Jadhavar (2016) explored the Zygnemataceae of Mehekari Lake of Maharashtra and reported 12 species of Zygnemataceae. Among these 12 species, he identified one species of *Z. micropunctatum.* Satpati & Pal (2016) studied filamentous algae from Sundarban, India, and reported 31 new taxa, including two taxa of *Zygnema* (*Z. collinsianum* and *Z. oudhense*). Halder (2017) analyzed two species of *Zygnema* (*Z. cruciatum* and *Z. czurdae*) from Hooghly, West Bengal. Yadav and Sabale (2019), while working on the Bhandarwadi minor irrigation project of Maharashtra, identified five species of *Zygnema* (*Z. cyanosporum, Z. czurdae, Z. melanosporum, Z. mucigenum* and *Z. pectinatum*). Verma *et al.,* (2021) in their checklist of Chlorophyceae from Uttar Pradesh, included 17 species of *Zygnema*. Sinha (2022) for the first time, observed *Z. globosum* from the Kalimpong hills, West Bengal.

Classical taxonomic studies of *Sirogonium* and *Zygnema* genera provide a crucial foundation for biodiversity research, ecological monitoring, evolutionary studies, and practical applications in agriculture and industry. Zygnema species are rich in starch content, i.e., 24.29 ± 0.56 % dry weight and can be used as feedstock in biodiesel and in bioethanol production (Zhang *et al.* 2016). The species of *Sirogonium* and *Zygnema* are capable of efficiently remediating water contaminated with organochlorine and pyrethroid pesticides (Riaz *et al.,* 2017). The identified algae, which I found, belong to *Sirogonium* and *Zygnema* genera and, therefore, will have the same applicative value, although in lesser-known geography before, Darbhanga, Bihar.

Keeping in mind the paucity of morphological information about the genus from the North Bihar region, the floristic survey of the genus *Sirogonium* Kützing and *Zygnema* Agardh was aimed. The current study explores two taxons of *Sirogonium* Kützing and three taxons of *Zygnema* Agardh from different unexplored localities of Darbhanga, Bihar.

Material and Methods

Study area

Darbhanga is a landlocked state bounded by Madhubani, Samastipur, Saharsha, Sitamarhi and Muzaffarpur districts in North, South, East and West directions, respectively. The eastern portion of Darbhanga consists of Ghanshyampur, Biraul, and Kusheshwar Asthan. The algal samples were randomly collected from April 2023 to November 2023 from different locations of Biraul and Kusheshwar Asthan of Darbhanga district and were stored in a sterile glass or plastic container. Some algal mats from different collection sites are shown in the figure below.

The pH and temperature of the specimen were recorded at the time of collection.

Sampling and Identification

The specimens were preserved in 4% (v/v) formaldehyde solution. The slides of the specimen were prepared using glycerine (5%) and observed using a binocular compound microscope (Olympus). The algal specimens were treated with 8% KOH and lactic acid and were conserved for 24 hours to observe the clear sculpturing of the zygospores. Morphological parameters of the Zygnematacean taxa were observed and recorded for taxonomic evaluation using an ocular micrometer. The camera lucida diagrams (CLD) were drawn using the prism-type camera lucida, and the microphotograph (MP) of the specimen was taken. The taxa identification was accomplished using authentic literature and monographs (Transeau 1951, Randhawa 1959).

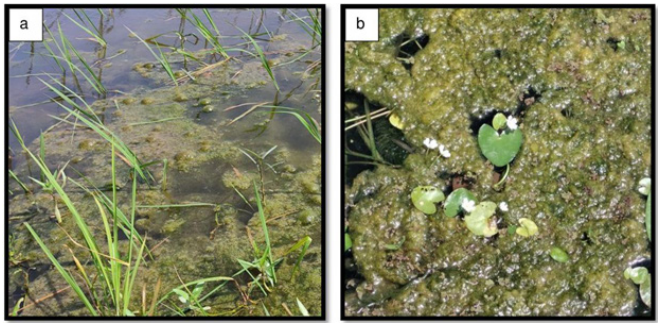


Fig. 1: (a) algal mats from Durga sthan pond, Biraul with coordinates 25°56'39.5"N 86°15'06.0"E Durga Sthan Pond; (b) algal mats from canal of Hirni, Kusheshwar Asthan with coordinates 25°49'20.5"N 86°17'50.9"E Asma bridge

Results

In the present taxonomic study, two species of *Sirogonium* Kützing and three species of *Zygnema* Agardh were reported. These taxons were described using the camera lucida diagram (CLD) and microphotograph (MP). Among these two species of *Sirogonium* Kützing, *S. floridanum* is reported for the first time from Bihar, whereas *S. megasporum* is observed for the second time from Bihar since Jha & Jha (2023). Out of the three species of *Zygnema* Agardh, *Z. excommune* Transeau is observed for the first time from India. Whereas, *Z. substellinum* and *Z. tenue* are observed for the second time from Bihar after Kargupta & Jha (2004).

Sirogonium floridanum (TRANSEAU) G. M. SMITH

Vegetative cells 52 to 60 µm × 112 to 272 µm long; chloroplasts 5; more or less straight or slightly curved; conjugation between the gametangia is direct; gametangia shortened and reflexed; inflation in receptive gametangia up to 88 to 112 µm; zygospores ellipsoid; 72 to 96 µm in diameter; 100 to 128 µm long; smooth and yellow-brown mesopore. (Randhawa 1959) (Fig.2: (a-f); Fig.4: (a-e))

The current specimen bears a resemblance to the type species.

Habitat

Sample No. AK-35; collected on 12.12.2021; from a pond of L. N. Mithila University campus (Dist. Darbhanga) University Lake at pH-7.3 and Temperature 20°C.

Distribution

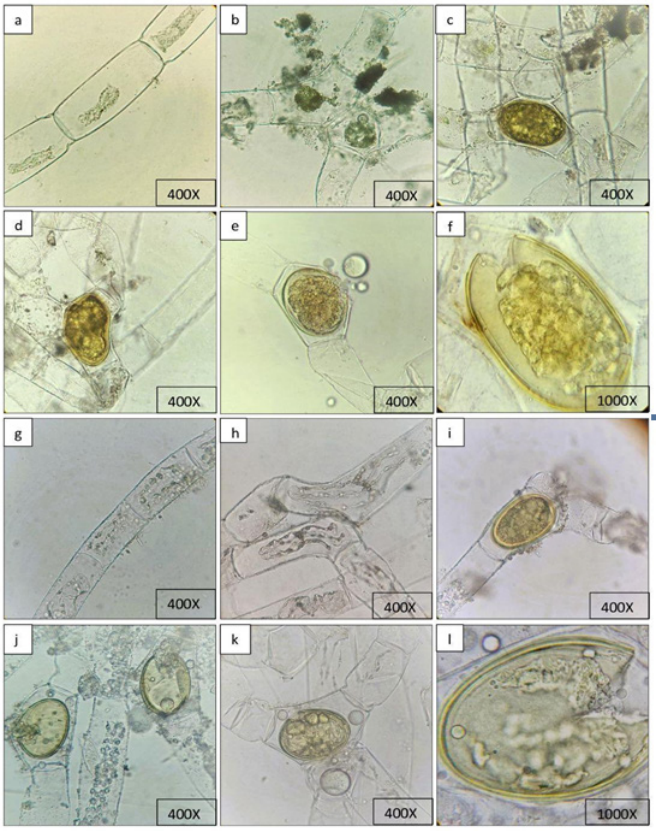
Uttar Pradesh (Gupta 2012).

This species is recorded for the first time from Bihar.

S. megasporum (Jao) Transeau

Vegetative cells 44 to 52 µm in diameter and 108 to 268 µm long; chloroplasts 4; straight or slightly curved; conjugate directly between the gametangia; gametangia shortened and reflexed; inflation on the inner side up to 80 µm; ellipsoid shaped zygospores; rarely ovoid; 60 to 80 µm broad; 76 to 124 µm in length; smooth and yellow to brown median spore walls. (Randhawa 1959) (Fig.2: (g-l); Fig.4: (f-g); Fig.5: (h-k))

The present specimen takes after the type species.



The specimen's moisture was maintained using glycerine and was observed under controlled lab conditions.

Fig. 2: (**a-e)** Sirogonium *floridanum* (TRANSEAU) G. M. SMITH**,** captured using a binocular microscope at magnification of 400x, and **(f)** at magnification of 1000x. **(a)** represents vegetative cells containing 5 straight or slightly curved chloroplasts, **(b-d)** represents direct conjugation between the male and receptive gametangia and formation of zygospore as a result of conjugation, and **(e-f)** represents the ornamentation of the cell. **(g-l)** is *S. megasporum* (Jao) Transeau, **(g-k)** is captured using a binocular microscope at magnification of 400x, and **(l)** at magnification of 1000x, **(g)** represents vegetative filament containing 4 straight or slightly curved chloroplasts, **(h)** shows the initial stage of conjugation between two filaments, **(i-k)** represents formation of zygospore as a result of conjugation between two filaments, and **(l)** represents the ornamentation of the cell

Habitat

Sample No. AK-21; collected on 30.03.2022; from Anandbag Pond (behind Zoology Department) L. N. Mithila University (Dist. Darbhanga) at pH-7.3 and Temperature 31°C.

Distribution

USA (Randhawa 1959); India: Bihar (Kargupta & Jha 2004); Uttrakhand, Kolkata (Gupta 2012).

The type species is recorded for the second time from Bihar.

Zygnema excommune Transeau

Vegetative cell 28 to 32 µm × 40 to 84 µm; 2 stellate chloroplasts with single pyrenoid at the center; conjugation scalariform type; fertile gametangia tubular and a bit enlarged on the conjugation side; globose to ovoid zygospores; Zygospore 32 to 36 µm × 32 to 48 µm; median spore walls sorbiculate and brown; pits 2 µm in diameter; 3 to 4 µm apart. (Randhawa 1959) (Fig.3: (m-p); Fig.5: (l-o))

The present specimen takes after the type species.

Habitat

Sample No. AK-98; collected on 5.04.2023, from a canal of Biraul (Dist. Darbhanga) Durga Sthan Pond growing at pH-6.6 & Temperature 31°C.

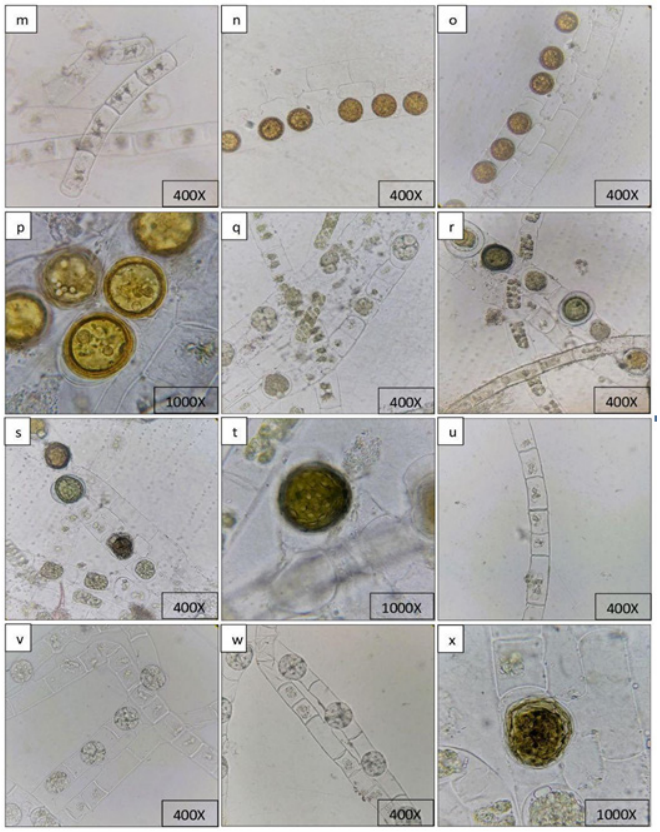
Distribution

Bohemia.

This species is recorded for the first time in India.

Zygnema substellinum TAFT

Vegetative cells 20 to 28 µm × 36 to 60 µm; stellate chloroplasts 2; scalariform type conjugation; zygospore in one of the gametangia; inflation in receptive gametangia greatly on



The specimen's moisture was maintained using glycerine and was observed under controlled lab conditions.

Fig. 3: (**m-o)** is *Zygnema excommune* TRANSEAU, is captured using a binocular microscope at magnification of 400x, and **(p)** at magnification of 1000x represents sorbiculate spore walls as a result of ornamentation of the cell. **(m)** represents vegetative filament containing 2 stellate shaped chloroplasts, **(n-o)** represents the formation of globose to ovoid shaped zygospore as a result of conjugation between the filaments. **(q-t)** is *Zygnema substellinum* TAFT, **(q-s)** is captured using a binocular microscope at magnification of 400x, and **(t)** at magnification of 1000x, **(q)** represents both the vegetative and conjugative filament, **(r-s)** shows the conjugation between the male and receptive gametangia and the formation of ovoid shaped zygospores, and **(t)** represents sorbiculate median spore wall, **(u-x)** is *Zygnema tenue* KÜTZING, wherein fig (u-w) is observed using a binocular microscope at magnification of 400x, and **(x)** at magnification of 1000x represents sorbiculate spore walls with 2-3µm pits **(u)** represents the vegetative filaments with 2 stellate shaped chloroplasts and pyrenoid at the center, **(v-w)** shows conjugation between the two filament and the inflation near the zygospore

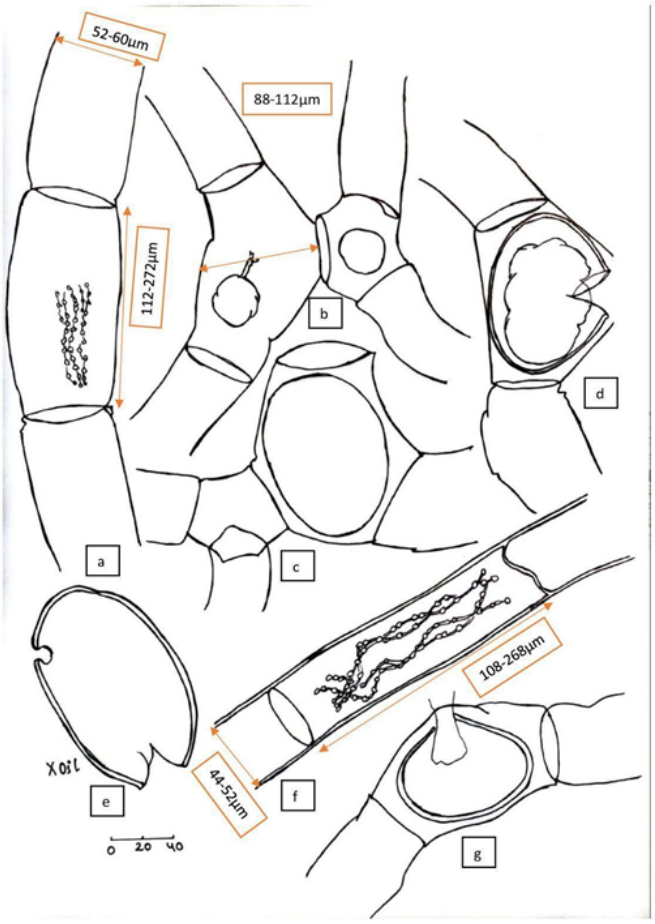


Fig. 4 : (a-d) are camera lucida diagrams (CLDs) of *Sirogonium floridanum* (TRANSEAU) G. M. SMITH, observed at magnification of 400x, represent vegetative and conjugative filaments drawn at a scale of 1 unit equal to 20µm and **(e)** is the CLD observed at magnification of 1000x, represent ornamentation drawn at approximately double of the scale used for the former. **(f-g)** are camera lucida diagrams (CLDs) of *S. megasporum* (Jao) Transeau, observed at magnification of 400x represents vegetative and conjugative cells drawn at a scale of 1 unit equal to 20µm

the conjugation side and slightly inflated on opposite side; zygospores ovoid; 32 to 44 µm × 36 to 44 µm in dimension; shiny pectic cellulose material surrounding the zygospores; median spore wall sorbiculate; brown to blue; pits 3 to 4 µm in diameter.

(Randhawa 1959) (Fig.3: (q-t); Fig.6: (p-r))

The taxon described above bears resemblance to the earlier identified species.

Habitat

Sample No. AK-111; collected on 02.05.2023; from a canal of Hirni, Kusheshwar Asthan (Dist. Darbhanga) Asma bridge growing next to *Oedogonium* and *Spirogyra* species at pH-7.5 & Temperature 34°C.

Distribution

USA (Randhawa 1959); India: Maharashtra; Punjab (Gupta 2012), Bihar (Kargupta & Jha 2004).

The type species is recorded for the second time after Kargupta & Jha (2004).

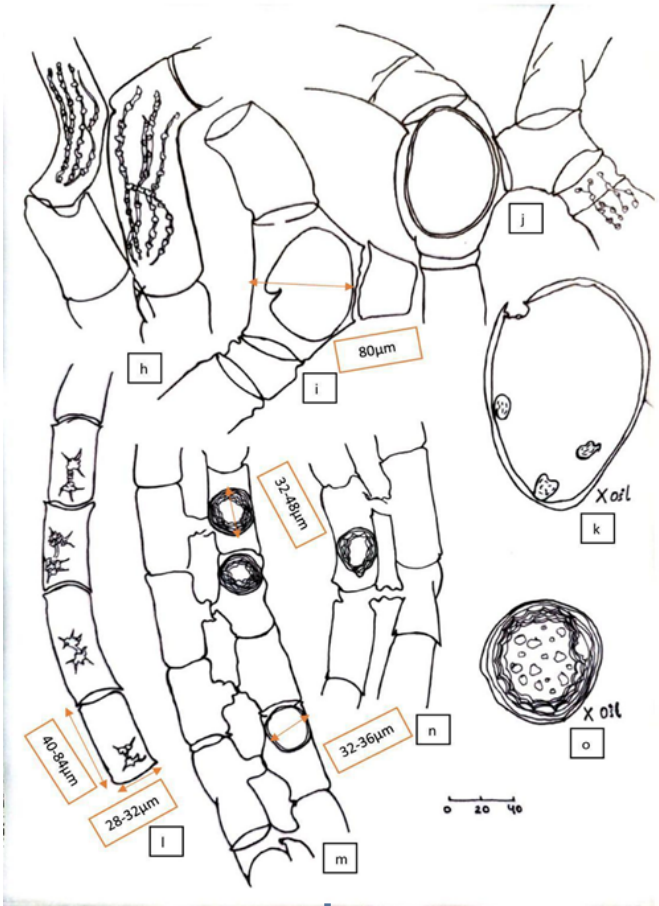


Fig. 5 : (h-j) are CLDs of *S. megasporum* (Jao) Transeau observed at magnification of 400x, drawn at a scale of 1 unit equal to 20 µm and **(k)** is the CLD of specimen observed at magnification of 1000x represent ornamentation drawn at approximately double of the scale used for the former. **(l-n)** are CLDs of *Zygnema excommune* TRANSEAU, observed at magnification of 400x, represent vegetative and conjugative cells drawn at a scale of 1 unit equal to 20µm and (o) is the CLD observed at magnification of 1000x represents ornamentation drawn at approximately double of the scale used for the former

Zygnema tenue KÜTZING

Vegetative cells 28 to 32 µm × 32 to 80 µm; with 2 stellate chloroplasts; scalariform type conjugation; zygospore develops in one of the gametangia; receptive cell swollen on both sides; globose to ovoid shaped zygospores; 32 to 40 µm in diameter and 32 to 36 µm long; mesospore brown and sorbiculate; pits diameter 2 to 3 µm; sometimes aplanospores observed.

(Randhawa 1959) (Fig.3: (u-x); Fig.6: (s-v))

The present specimen takes after the type species.

Habitat

Sample No. 115; collected on 22.02.2023, from a ditch near the railway station of Biraul (Dist. Darbhanga) Railway Quarters growing parallel to *Spirogyra* species at pH- 7.1 & Temperature 29°C.

Distribution

USA (Randhawa 1959); China; India: Punjab, Kolkata (Gupta 2012), Bihar (Kargupta & Jha 2004).

This species is recorded for the second time from Bihar after Kargupta & Jha (2004).

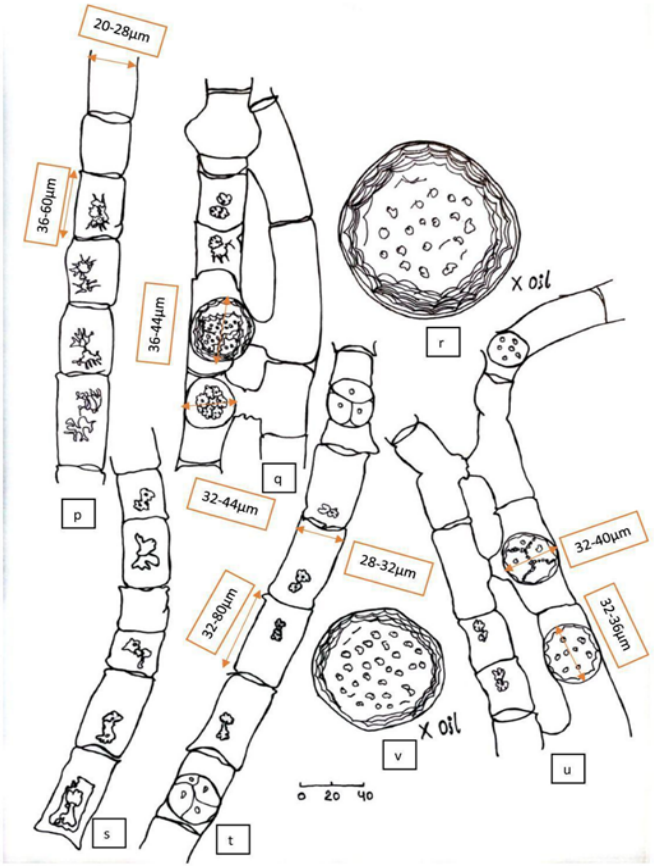


Fig. 6 : (p-q) CLDs of *Z. substellinum* TAFT drawn using a camera lucida device observed at a magnification of 400X, represents vegetative and conjugative cells of the species drawn at a scale of 1 unit equal to 20 µm and **(r)** at a magnification of 1000X, is the sculptured cell for observation of median spore walls drawn at approximately double of the scale used for the former. **(s-u)** are CLDs of *Z. tenue* KÜTZING drawn with the help of camera lucida device observed at a magnification of 400X, represents vegetative and conjugative cells of the species drawn at a scale of 1 unit equal to 20 µm and (v) is the CLD observed at a magnification of 1000X, is the ornamentation of the median spore wall drawn at approximately double of the scale used for the former

Conclusion

The current taxonomic study reveals that species of the genus *Sirogonium* Kützing and *Zygnema* Agardh prefer canals, ditches, and temporary water bodies as their habitat. Its reproductive or fruiting stage is observed at pH 6.5 to 7.5 and temperature 29 to 34°C. The species *S. floridanum* (TRANSEAU) G. M. SMITH, *S. megasporum* (Jao) Transeau, *Z. substellinum* Taft and *Z. tenue* Kützing generally found within as well as outside India. Whereas *Z. excommune* Transeau occurs for the very first time in India. The species *S. floridanum* was observed growing in Bihar for the first time. The species *S. floridanum* (TRANSEAU) G. M. SMITH, *S. megasporum* (Jao) Transeau and *Z. excommune* Transeau were observed growing in pure form. Therefore, it can be used for further studies like biochemical analysis, etc. It was established that the Bihar region continues to possess algal wealth. So, we require expertise to investigate further the flora of Bihar.

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Authors' Contributions

Anuradha Kumari: designs the experiment, execution, methodology, experimentation, data collection, analysis, and ms writing.

Ankit Kumar Singh: direction in writing of MS and compilation. All authors have approved the final manuscript.

Conflict of Interest

No potential conflict of interest was reported by the authors.

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