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THE GENUS *LICHINELLA NYLANDER*, CYANOLICHEN: A NEW REPORT FROM INDIA

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In present study, *Lichinella stipatula* a member of Lichinaceae, cyanolichen is being reported and described for the first time from India. The genus *Lichinella* Nylander of the family Lichinaceae is characterized by thallinocarpous fruiting body and the presence of unicellular cyanoprokaryotic photobiont. The growth of *Lichinella stipatula* was observed as black crust growing on exposed surface of old building of Allahabad. Continuous close morphological and anatomical observation of growth and thallus resulted as observation of fruiting body on the thallus of organism.

Key words: Cyanolichen, Lichinaceae, *Lichinella,* photobiont

Lichens are the most successful alliance between two dissimilar autotrophic and heterotrophic members, the photobiont and the mycobiont. The members of two major groups of algae (blue-green algae /cyanobacteria and green) are considered as photobiont partners. However, mycobiont members may be from Basidiomycetes, Ascomycetes or Deuteromycetes. In Cyanolichens, the symbiotic photosynthetic partners (photobiont) may belong to the cyanoprokaryotic group. Cyanolichens are among the primary colonizers on exposed surface of highly inaccessible environments. Out of total known lichens, about 10%, have blue-green algae (Cyanobacteria) as the main photosynthetic partner. Mycobiont of the lichens are known to secrete a great number of organic compounds and contribute for supplying minerals from the substrate to lichen. The cyanolichens contribute a lot as primary colonizer in the harsh arid environment. The biological weathering of rocks of harsh localities is caused by organic acids produced by cyanolichens as a by product of metabolism. In this way cyanolichens contribute in environment by creating suitable conditions even on harsh arid environment for growth of other organism. The *Lichinella stipatula* is being reported as a new record to the lichen diversity of India and is briefly described here.

MATERIALS AND METHODS

Study area: The collection site of cyanolichen specimen was exposed surface of an old monument, situated at Allahabad city of Uttar Pradesh province, India, 25º30 N Latitude, 81º55 E Longitude and 108 m Altitude.

Climatic condition of study area:

Temperature: Lowest 0±2º C during extreme cold season and highest 60±2º C during extreme hot season

pH: Slightly acidic (6.2)

Sunlight exposure: Maximum 14 h during summer season and minimum 10 h during winter season.

Darkness exposure: Maximum 14 h during winter season and 10 h during summer season

Exposed surface crust: Silica and calcium rich

Sampling: Samples of cyanolichen were collected during all season's upto four years (2008 to 2012) during the study period from the different spots of the same locality

Isolation and culturing of Cyanobiont:

The material of cyanolichen used in the present study was available in plenty on exposed rock surface of an old and abandoned building at Allahabad city and it was studied in nature as well as culture (Kant *et al.* 2008). For the growth of cyanobiont of cyanolichen, specimens were put into dishes containing BG 11 culture medium (Stanier *et al.* 1971). The process of isolation of cyanobiont partner from liberated perennating cells was repeated many times till the pure culture of cyanobiont was isolated.

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Finally three cloned strains were developed from discrete colonies developed from single cells and have been deposited in Allahabad Culture Collection, University of Allahabad, India as as *Cyanoarbor rupestris* ACC10651, ACC10654 and ACC10655. All the three strains behave exactly in the same way.

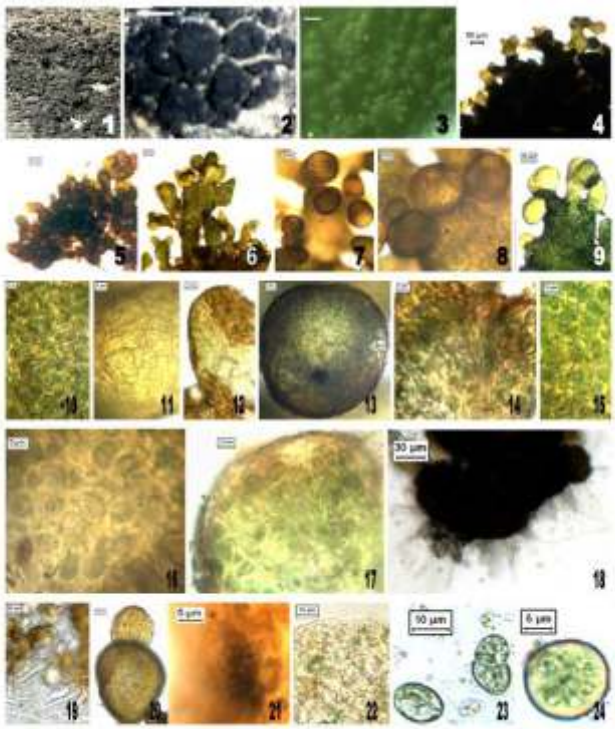
Microscopic observations of *Lichinella stipatula* were made with the help of Leica DMLB microscope attached with digital camera DC 300 with Quin Imaging System and field growth observation were made with the help of Nikon digital Camera (Coolpix 8400). Morphological and anatomical observations were made from the three types of the materials 1. cyanolichen material collected upto four consecutive years from natural habitat during four seasons viz, rainy, autumn, winter and summer seasons collected (2008-2012),

2. Cyanolichen grown in enrichment upto one week and 3. Pure culture of cyanobiont. The specimens of *Lichinella stipatula* are deposited in Allahabad Culture Collection, Department of Botany, University of Allahabad, Allahabad as Voucher Nos. *Lichenella* 2015, 3029 and 4289.

Specimen Examined: INDIA, Uttar Pradesh: Allahabad, 25/06/2008, *Lichinella*, R. Kant, 2015, 3029

RESULTS AND DISCUSSION

The growth of *Lichinella stipatula* was inconspicuous and often ignored from the site and was first time observed during June 2008 and was found growing as dark brown to blackish crust on exposed surface of old and abandoned building of Allahabad. Earlier the growth was confused with *Cyanoarbor rupestris,* cyanoprokaryote, but due to repeated appearance of fungal mycelium during culturing and isolation process of *Cyanoarbor,* created the confusion of the material. But due to absence of fruiting body, the organism could not be described as Cyanolichen. Continuous close morphological and anatomical observation of growth pattern, thallus morphology and anatomy of cyanolichen as well as both partners photobiont and



Figures. **1-24**: showing different stages of *Lichinella stipatula*

1. Crustose growth of *Lichinella stipatula* in nature (bar= 1 M), **2.**Cracked Crustose growth (bar= 1cm), Crustose growth showing granular appearance with small fruiting body (bar= 0.1 mm), **3.** Collaroid growth with swollen tips, **4.** Dichotomously branched thallus with basal region (arrow marked) during dry season, **5**.Showing spherical large granular appearance (upper view), **6.** Showing globose propugules with nipple shaped tip of trichogyne, **7.** Single dichotomously branched Greenish appearance during rainy season, **8.**Peripheral photobiont cells growth with fungal mycelium during rainy season, **9.** Compactly arranged photobiont cells on the peripheral region of Lichinella under dry conditions, **10.** Broken Thallus showing glistening internal region filled with loosely arranged fungal mycelia, **11.** T.S. of Thallus showing intervened loosely arranged fungal mycelium, **12.**T.S. of Thallus showing homoiomerous type of hymenium with loosely arranged fungal mycelium and photobiont cells, **13.** T.S. of thallus with mycobiont mycelium and greenish photobiont cells,**14-16**.Photobiont cells filled with angular mycobiont haustoria, **17.**Loosely arranged intervened fungal mycelia with photobiont cells, **18.**Showing protruding fungal mycelia in enriched medium, **19.** Large protruded fungal mycelial growth from the *Lichinella* thallus in medium, **20.** Development of new propogules from the old colony, **21.** Showing polyporous asci, **22.** Showing empty dried mucilaginous and mycelial strand, **23.** Single and two celled stages of photobiont during dry season,

24. Single celled photobiont in medium

mycobiont upto four consecutive years (June 2008 to May 2012) resulted in tracing of fruiting body on the thallus of crustose cyanolichen. The *L. stipatula* belongs to the cyanolichen family Lichinaceae (Lichinales, Ascomycota).

So far roughly total 39 genera are described from the family Lichinaceae, Lichinales, cyanolichen by various workers (Büdel, 1987; Büdel and Henssen 1983, 1988; Büdel *et al.* 1994, 1997; Henssen 1963, 1979, 1973, 1990, 1994; Henssen and Büdel, 1984; Henssen et al., 1981, 1985, 1987; Moreno and Egea 1991, 1992; Rikkinen 2002; Schultz, 1998, 2005; Schultz and Büdel 2002; Schultz *et al.* 1999, 2001). Presently known genera of Lichinaceae include viz. *Anema, Calotrichopsis, Cryptothele, Digitothyrea, Edwardiella, Ephebe, Euopsis, Finkia, Gyrocollema, Jenmania, Lecidopyrenopsis, Lemmopsis, Lempholemma, Leprocollema, Lichina, Lichinella, Lichinodium, Mawsonia, Metamelanea, Paulia, Peccania, Phloeopeccania, Phylliscidiopsis, Phylliscidium, Phyllisciella, Phylliscum, Porocyphus, Pseudopaulia, Psorotichia, Pterygiopsis, Pyrenocarpon, Pyrenopsis, Stromatella, Synalissa, Thelignya, Thermutis, Thermutopsis, Thyrea and Zahlbrucknerella.*

In general all types of growth forms are reported in the Lichinaceae and the members of Lichinaceae exhibits a wide range of growth forms from dwarf fruticose to squamulose, squamulose-crustose to crustose, crustose-granulose to rimose-areolate and irregularly areolate type (Schultz 2005). For the identification of cyanolichen members of Lichinaceae (Lichinales), a taxonomic key of 35 genera and 250 species was developed by Schultz and Büdel (2002). Thallus growth forms create confusion on identification but they are different in thallus margin, fruting body, thallus anatomy and photobiont partner. Cyanolichen genera viz. *Lichinella*, *Metamelanea, Phylliscidium* and *Pyrenopsis* of Lichinaceae closely resemble in morphological appearance as their thallus looks like dwarf fruticose to squamulose or squamulose-crustose to crustose, crustose-granulose to rimose-areolate and irregularly areolate type and many times creates confusion on identification, due to which correct identification is more problematic, but they are different in thallus margin, fruiting body, thallus anatomy and photobiont partner.

As far as the photobiont partners are concerned roughly 40% genera of Lichinaceae prefer the association with *Gloeocapsa* as the photobiont partner, 20% with *Cyanobacterium* and 10% with *Chroococcidiopsis* (Rikkinnen, 2002). The cyanobiont partners of rest genera of Lichinaceae are doubtful and they were identified as *Chroococcales*. Cyanolichen genera viz. *Anema, Cryptothele, Edwardiella, Euopsis, Jenmania, Lecidopyrenopsis, Phloeopeccania, Phylliscidium, Pyrenopsis, Synalissa and Metamelanea* known for the association with *Gloeocapsa; Finkia, Gyrocollema, Lemmopsis, Leprocollema, Mawsonia* and *Phylliscidiopsis* with *Cyanobacterium; Lichinella, Peccania* and *Psorotichia* with *Chroococcidiopsis / Cyanoarbor / Myxosarcina. Photobiont members of the rest genera of Lichinaceae belong to the order Chroococcales.* In fact most of the genera of Lichinaceae were described by mycologists and they could not identify the proper photobiont partner of cyanolichens.

The genus *Lichinella* is characterized by squamulose-crustose to crustose-granulose thallus growth forms; homoiomerous and unstratified thallus anatomy, loosely or densely arranged hyphae and if fertile, fruiting body is thallinocarpous type. Identification of *Lichinella* is very difficult in sterile conditions due to absence of fruiting bodies.

Currently, approximately 18 species of genus *Lichinella* are recognized viz. *Lichinella stipatula, L. americana* Henssen, *L. flexa, L. intermedia, L. robustoides* Henssen, Büdel and Nash, *L. minnesotensis* (Fink) Essl. and Egan, *L. iodopulchra* (Croz.), *L. nigritella* (Lettau), *L. sinaica,* (Marton and Galun), *L. cribellifera* Moreno and Egea, *L. myriospora* (Zahlbr.) Moreno and Egea and *L. granulose* Schultz. Cyanolichen specimen collected from

Allahabad revealed close similarity with the *L. stipatula* in thallus morphology, anatomy and fruiting body but different from *L. stipatula* in presence of photobiont partner, as photobiont was identified as unicellular member of family Chroococcaceae. However, photobiont of cyanolichen specimen collected from Allahabad, India is identified as *Cyanoarbor rupestris* Wang, Entophysalidaceae, Chroococcales (Komárek and Anagnostidis, 1998, Wang 1989).

Although a lot work have been carried out on lichen flora in India, but it is either limited upto floristic account or check list of Indian lichens (Patwardhan and Prabhu 1977; Singh 1980; Awasthi 1991; Lal and Upreti 1995; Diwakar *et al.* 2003; Sinha and Elix, 2003) or association of fungal and photobiont and their physiological aspects (Rai 1990; Rai *et al.* 1980, 1881, 1983, 2000). There was no earlier report on occurrence of genus Lichinella from India. So far total roughly 39 genera of cyanolichen family Lichinaceae (Lichinales) are recognized (Büdel 1987, Büdel and Henssen 1983, 1988; Büdel *et al.* 1994, 1995; Henssen 1979, 1990, 1994, Henssen and Büdel 1984; Henssen *et al.* 1981, 1985, 1987, Moreno and Egea 1991, 1992; Nylander 1873, Schultz 1998, 2005; Schultz and Büdel, 2002; Schultz *et al.* 1999, 2001).

The genus *Lichinella* (Lichinaceae, Lichinales) was established by Nylander (1873) with its type species *Lichinella stipatula* Nyl. So far approximately 18 species of the genus *Lichinella* were reported and described by different lichenologists from all over the Globe (Büdel and Nash 1985; Essl. and Egan 1995; Henssen 1963; Moreno and Egea 1991, 1992; Nylander 1873; Schultz 2005; Zahlbruckner, 1925). They include *Lichinella algerica* (Steiner) Moreno and Egea, *L. americana* Henssen, *L. applanata* Henssen, *L. cribellifera* Moreno and Egea, *L. flexa* (Henssen) Büdel and Nash, *L. granulosa* Schultz., *L. hondoana* (Zahlbr.) Moreno and Egea, *L. inflata* (Henssen) Moreno and Egea, *L. intermedia* (Henssen), Büdel and Nash, *L. iodopulchra* (Croz.), *L. lojkana* (Hue) Zahlbruckner, *L. mauritanica* (Lange) Moreno and Egea, *L. minnesotensis* (Fink) Essl. and Egan, *L. myriospora* (Zahlbr.) Moreno and Egea, *L. nigritella* (Lettau) Moreno and Egea, *L. robustoides* Henssen, *L. sinaica* (Marton and Galun) Moreno and Egea and *L. stipatula* Nylander.

The occurrence of *Lichenella* sp. was often ignored due to inconspicuous growth and inaccessibility of the growing site. A detailed critical and comparative study revealed that this specimen is closely approach to the *Lichinella stipatula*. Hence it is reported first time from India, *Lichinella stipatula* is new to the India. Lichinella *stipatula* was found growing on inaccessible habitat of harsh environment of an old and abandoned building at Allahabad and was first time observed during June 2005. The cyanolichen was found as scattered blackish patches of 2-5 (-10) cm in diameter and of 0.5-4 mm in height (Figs.1-3). Under low magnification they appear as dense coralloid growth (Fig. 4). It consists of irregularly lobed or pseudo-dichotomously branched cylindrical, terete and radial structures (Figs. 5, 6.). Initially they appear to have lobed growth of a colonial cyanobacterium (photobiont) with compact arrangement of cells (Fig. 12). Gradually, a fungus gets associated and slowly a lichenized structure grow erect more to reproductive structure of lichen. However, close observations revealed that it contained peripheral photobiont and internal fungal hyphae (Figs.10-12). When green axes of cyanolichen of rainy season were kept on growth medium, large hyphae protrude out from the thallus (Fig. 18,19). However photobiont also started growing and it was repeatedly separated and isolated in axenic cultures. A detailed comparative study of the cyanolichen specimen with allied species of the genus *Lichinella* have revealed its affinity with *Lichinella stipatula* in morphological and anatomical appearance. During study it appeared that 4 mm growth of lichen was result of many years.

Morphology

Cyanolichen thallus crustose -granulose to

granulose-areolate with 0.5-3 mm height and 0.1-0.5 mm width, with or without definite crenate margins, dichotomously branched, branches elongated with swollen spherical tips showing ascogones and trichogyne (Fig. 7), branches 0.1-2 mm long and 0.02-0.5 mm thick. Entire surface of cyanolichen specimen is covered by cells of photobiont and arranged in a single or occasionally in more layers (Fig. 18). On breaking of the dry axes of thallus, revealed the internal lumen as filled with white glistening material of loosely arranged fungal mycelia (Fig. 12). During dry weather the thalli look brown or black crust (Figs. 1,2) and this might be due to presence of scytonemin pigment but during rainy season they become greenish (Fig. 6) due to production of photosynthetic pigments and cells start dividing the growth appears more concentrated on tips (Figs. 9,10). The tips often branched and may have globular bodies which may get detached and act as propagating structures (Fig. 7,8,20).

Anatomy

Cyanolichen anatomy homeoiomerous type, not stratified, fungal hyphae loosely arranged with or without thalinocarpous fruiting bodies. (Figs.13-15). Photobiont cells surrounded by fungal mycelia (Fig. 22) and filled with entered, angular haustoria (Figs. 16,17).

Mycobiont: Cells short to elongated, apical cells angular or rounded in shape, asci polyporous (Fig. 21).

Photobiont: The photobiont partner form spherical or flattened colonies. The cells are closely packed and protrude to form dome shaped structures (Fig. 11) but they did not grow to form larger thalli, the cells are 5-10 µm in diameter, rounded or polygonal in shape and filled with homogenous blue-green content (Figs. 23,24).

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