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## Occurrence of cyanobacteria–diatom symbiosis in the Bay of Bengal: implications in biogeochemistry

Diazotrophs ( $N_2$ -fixing organisms), mainly belonging to cyanobacteria, form a major group of phytoplankton in nutrient-poor oligotrophic oceans<sup>1</sup>. These organisms support the non-diazotrophic phytoplankton community and primary production by the release of fixed nitrogen ( $N_2$ ) into the marine environment and thereby play an important role in the biogeochemistry of oligotrophic environments<sup>2</sup>. *Trichodesmium* spp., a colony-forming cyanobacterium and *Richelia intracellularis* Schmidt, a cyanobacterial endosymbiont of several diatom genera, are the most important diazotrophs accounting for ~63% of pelagic  $N_2$ -fixation in the world's oceans<sup>3</sup>. However, information on *R. intracellularis* is lacking<sup>3</sup>, even though its distribution is widespread.

*R. intracellularis* is mostly associated with the diatoms *Hemiaulus* (Atlantic Ocean) and *Rhizosolenia* (North Pacific central gyre). *R. intracellularis* has been reported earlier along the east coast of India<sup>4,5</sup> and recently in northern Arabian Sea<sup>6</sup>. In both these reports from the Indian region, the endosymbiont *R. intracellularis* was reported along with *Rhizosolenia*. In a phytoplankton-monitoring programme, under the Indian Expendable bathythermographic (XBT) programme along the Chennai–Port Blair (12 stations) and Port Blair–Kolkata transects (10 stations) in the Bay of Bengal, we have observed the presence of considerable number of diazotrophs as endosymbionts of diatoms *Rhizosolenia*,

*Hemiaulus* and *Guinardia* from preserved samples. Each *R. intracellularis* trichome is composed of 8–10 vegetative cells with one terminal heterocyst (site of nitrogen fixation). Length of the trichomes varied between 34 and 40  $\mu$ m and diameter was ~4  $\mu$ m.

*R. intracellularis* is easily distinguishable if the host diatoms are *Rhizosolenia* and *Guinardia* (Figure 1 a and c). In case of live samples of *Hemiaulus*, *R. intracellularis* can be seen easily under epifluorescence, irrespective of the host species<sup>7</sup>. However, Ferrario *et al.*<sup>8</sup> were able to take a photomicrograph of *Hemiaulus membranaceus* Cleve with *R. intracellularis* under light microscope, but found it difficult to recognize it with *Hemiaulus hauckii* Grunov using standard light microscope. In the present

study, *R. intracellularis* was identified from *H. membranaceus* based on the presence of heterocyst (Figure 1 b). However, *R. intracellularis* was not clearly visible as it was seen in *Rhizosolenia* and *Guinardia*. Microscopic examinations revealed that *R. intracellularis* trichomes in *Rhizosolenia* and *Guinardia* were found to be located at the ends of the cell and heterocysts oriented terminally. In *Hemiaulus*, *R. intracellularis* was located at the centre. *Rhizosolenia* generally have 1–4 trichomes of *R. intracellularis* host per cell. *R. intracellularis* trichomes varied between 2 and 4 per *Hemiaulus* cell and > 2 per *Guinardia* cell.

The trichome size of *R. intracellularis* varied depending upon the host diatoms, i.e. those inside *Rhizosolenia* were larger in size compared to the ones present

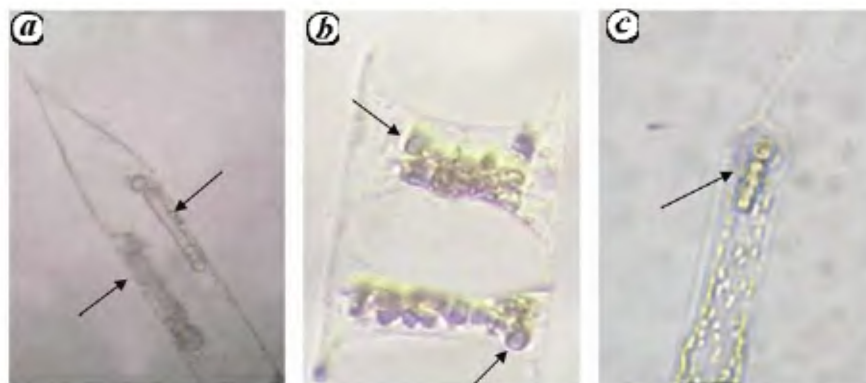


Figure 1 a–c. Photomicrographs of diatoms (*Rhizosolenia*, *Hemiaulus* and *Guinardia*) containing trichomes of *Richelia intracellularis*. Arrows indicate location of the trichomes.

in *H. membranaceus* and *Guinardia* (Figure 1). These observations are similar to the results obtained in the Pacific Ocean<sup>8</sup>. Ferrario *et al.* opined that *Richesthia* could have more than one species or belong to different genera<sup>8</sup>. Recently, it has also been observed that the lifecycle stage might also determine the number and size of the vegetative cells<sup>9</sup>. It is also relevant to note that net collected samples preserved glutaraldehyde and stored at 4°C in dark until further analysis through epifluorescence microscope, will be a better option to quantify their numbers<sup>9</sup>.

The observation of *R. intracellularis* from the collected samples indicates that it is widespread in the Bay of Bengal; the maximum abundance observed was 125 trichomes per litre. Therefore, on the basis of previous reports<sup>4-6</sup> and our observations, it can be stated that *R. intracellularis* is widely distributed in the northern Indian Ocean, thereby indicating its important role in the biogeochemistry. A recent report indicates that the large number of diazotrophic diatoms in subtropical waters to the east of Madagascar may have important implications

for the biogeochemistry of the austral phytoplankton bloom in the region<sup>10</sup>. In view of this, further studies are needed to explore *R. intracellularis* distribution and its implications in ocean biogeochemistry.

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