Research Article

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Polymorphic stages of the fresh water blue-green alga, *Gomphosphaeria aponina*

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The natural growth of a population of *Gomphosphaeria aponina* Kützing (Chroococcales, Cyanoprocaryota) was studied in a cemented freshwater tank in Allahabad, India. This population appeared to be a polymorphic species. Different species of the genus *Gomphosphaeria* have been segregated based on morphological features of colonies, cells and mucilage. However, these features are not well defined for different species. Our observations revealed many feature variations and, interestingly, certain features that have been described for different *Gomphosphaeria* species were seen in a single population. In this study, records of such variable morphological features were possible due to the availability of numerous specimens and continuous observations for more than two years. Further, this study revealed two points: (i) more detailed morphological studies are required both from nature as well as in culture to identify critical differences among the species, and (ii) molecular characterization of taxa appears to be necessary for final species settlement.

Key Words: Cyanoprocaryota; freshwater; *Gomphosphaeria aponina*; morphological features; polymorphic stages

INTRODUCTION

Current taxonomic criteria of Cyanobacteria include polyphasic data from morphology, ecology, biochemistry and molecular studies. However, most researchers have to rely on morphological features described in classical literature, so long as complete information remains unavailable for taxa identification. Classical information is mostly based on single collections and is limited in the information that early researchers could initially develop. We need to study the morphology of individual taxa or populations in more detail and, of course, without ignoring data from other sources. Komárek (2005) suggested that morphological and ecological descriptions of Cyanoprocaryotes are as important as their molecular studies. Even if molecular studies are considered the base line for tracing the evolutionary series, the value of serious morphological studies are important and useful in understanding structure and function relationships of the organisms.

This study is concerned with the coccoid genus, *Gomphosphaeria*. Among Chroococcales, the family Merismopediaceae is known to have two plane divisions at right angles to each other. It also includes two sub families: Merismopedioideae and Gomphosphaerioideae. Merismopedioideae has solitary cells or irregular or tabular, one-layered colonies. Gomphosphaerioideae includes the genus *Gomphosphaeria* and has spherical colonies with cells arranged peripherally or radially attached on mucilaginous stalks (Komárek and Anagnostidis 1998). At the global level, *Gomphosphaeria* is known to have eight species, *G. aponina, G. salina, G. lilacea,*

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G. virieuxii, G. natans, G. multiplex, G. okawangae and *G. semen-vitis* (Komárek and Anagnostidis 1986, 1998, Komárek 1989, Tiwari et al. 2007, 2009). In this study, a population of *G. aponina* is studied in detail and various polymorphic stages are observed and recorded.

MATERIALS AND METHODS

A natural population of *Gomphosphaeria* was found growing in a small cement tank (8' W × 12' L × 5' D) in Allahabad, India. Growth of the organism was kept under observation for more than two years (March 2007-December 2009). Microscopic observations were made with a Leica DMLB microscope with a DC 300 camera (Leica Microsystems, Plymouth, MN, USA). All photo-micrographs were taken of fresh specimens collected from the cement tank, mounted in water without any staining. During the study of *Gomphosphaeria,* weekly collections were made from the natural pond habitat, and numerous colonies (more than 15,000) were observed microscopically. Additionally, more than 1,000 photomicrographs were taken into record. When tank water was centrifuged, numerous colonies settled on the bottom and could easily be separated for observations. During May and June of 2009, the pond dried due to a failure of the tube well water supply. It was then cleaned and restored in July 2009, but colonies of *Gomphosphaeria* did not reappear until May 2010. Efforts were made to develop a clonal culture in BG-11 medium under laboratory conditions (temperature, 28 ± 2°C; light intensity, 3 K lux; and 14 h light : 10 h dark cycle) from the growth of a single 8-celled colony, but it did not survive for long.

RESULTS

Occurrence: We found the colonies of *G. aponina* growing metaphytically or planktically in a cement tank in Allahabad, India. The colonies were found free floating or infested with filamentous green algae. Many perennating colonies were also found settled on the bottom of the tank.

Colonies: Colonies were 25-125 μm in diameter, multicellular (8-256), solitary and often composed of sub-colonies. In shape, they appeared spherical, oblong, ellipsoidal, rectangular or irregular. Colonies appeared blue-green, olive green, pale-green, and, occasionally, pinkish-violet, grey, orange or greenish. Most colonies were found to be 32-celled, but young colonies with 8 cells were frequently observed. Larger colonies had 64 or 128 cells. However, compound colonies, having 128 or 256 cells, with lobed appearance were also present during July and December. The cells in a colony are always multiples of four and may vary from 8, 16, 32, 64, 128 or 256 cells (Pl. 1, figs 1-6).

Cells: Cells were widely obovoid, clubshaped, and, after division, cordiform in shape. They appeared olive-green, grey blue-green, bright blue-green, pale blue-green, yellow green, or pinkish-violet in color. The content varied from homogeneous to granulate at different stages of growth. Cells measured 3-12 μm in width and 5-15 μm in length. Cells were commonly 4-7 μm in width and 5-10 μm in length.

Mucilage and envelopes: During March and October, growing colonies had thin layers of mucilage, but mature and old colonies showed conspicuous and fibrillar gelatinous sheath matrices during July and December. When colonies were pressed under cover slips, central dichotomizing thin tubular stalks were revealed (Pl. 1, fig. 1; Pl. 2, figs 7 & 12; Pl. 3, figs 18 & 21). Also, thread like-structures in different focus appeared (Pl. 1, fig. 2; Pl. 2, fig. 11; Pl. 3, figs 14 & 15). Mature and senescent colonies developed structured envelopes around individual cells as well as a distinct central dichotomizing tubular system (Pl. 2, fig. 12).

Cultures: Efforts were made to isolate a culture in BG-11 medium, but cells did not survive for long. However, colonies grew and reproduced in water of the natural pond habitat for up to three months under laboratory conditions. Then, the cells gradually became discolored and developed more colonial mucilage. In certain colonies, individual cell envelopes with firm dichotomizing stalks were also found in colonies collected from the natural pond habitat. When such colonies were supplemented with BG-11 medium at a 1 : 1 ratio, the colonies became rejuvenated for about two months, and then cells deteriorated and died.

Development of colonies: The young and healthy colonies that were seen mostly in the month of March and October contained eight bi-lobed cells (Pl. 1, fig. 1). The cells were cuneate at the base (towards the center) and broadly rounded towards the outer side. All of these cells appeared bi-lobed due to initial cell division from the outer end (Pl. 1, figs 1 & 2). The cell division process is slow. It appears that, by the time the first series of divisions are completed, the second series of divisions at right angles to the first divisions is already initiated. Then, the colonies possess 16 bi-lobed cells (Pl. 1, fig. 2). Gradually, divisions continue and, by November-De-

cember, the colonies have increased in size, and may be composed of sub-colonies and produce up to 256 cells (Pl. 1, figs 1-6). When cell number in a colony increases, the colony may become 2-lobed, 4-lobed or more (Pl. 1, figs 5 & 6), with each lobe containing up to 64 cells. The diameter of the colony may reach up to 125 μm. The complementary multiple numbers of cells (16, 32, 64, 128 and 256) in a colony (Pl. 1, figs 1-6) often get reduced due to degeneration of one or more cells at any stage of colony development. Many unique and different colo-

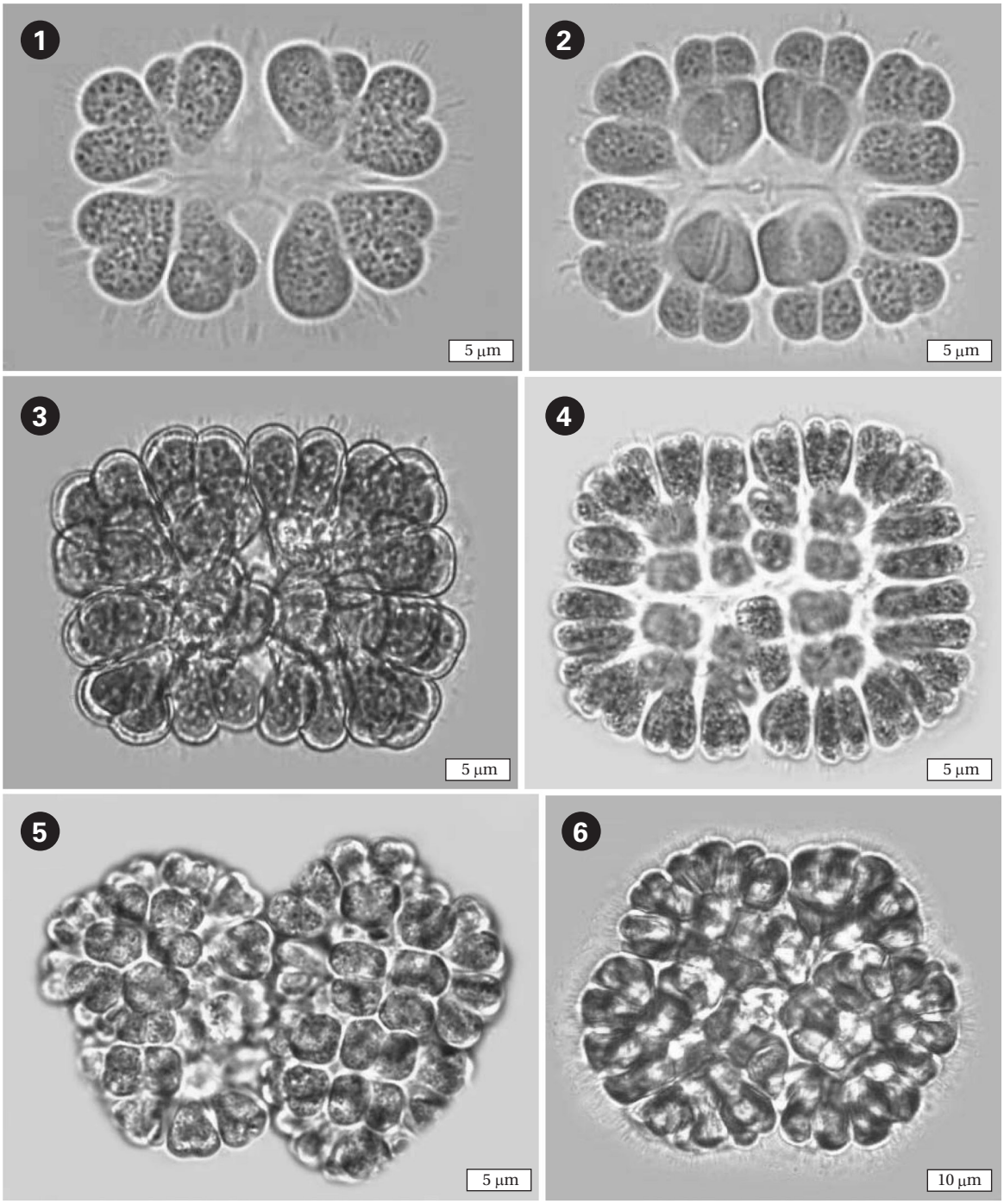

Plate 1. *Gomphosphaeria aponina* Kützing. **fig. 1.** A young, eight-celled colony. All cells look cordiform with initiation of division from the outer end and granulated cell content. **fig. 2.** A 16-celled colony. **fig. 3.** A 32-celled colony. Cells have thick envelopes. **fig. 4.** An actively growing 64-celled colony. Cells look thin and long compared to the cells of Figs 1-3. **fig. 5.** A 128-celled, bi-lobed colony. **fig. 6.** A 256-celled, multi-lobed colony.

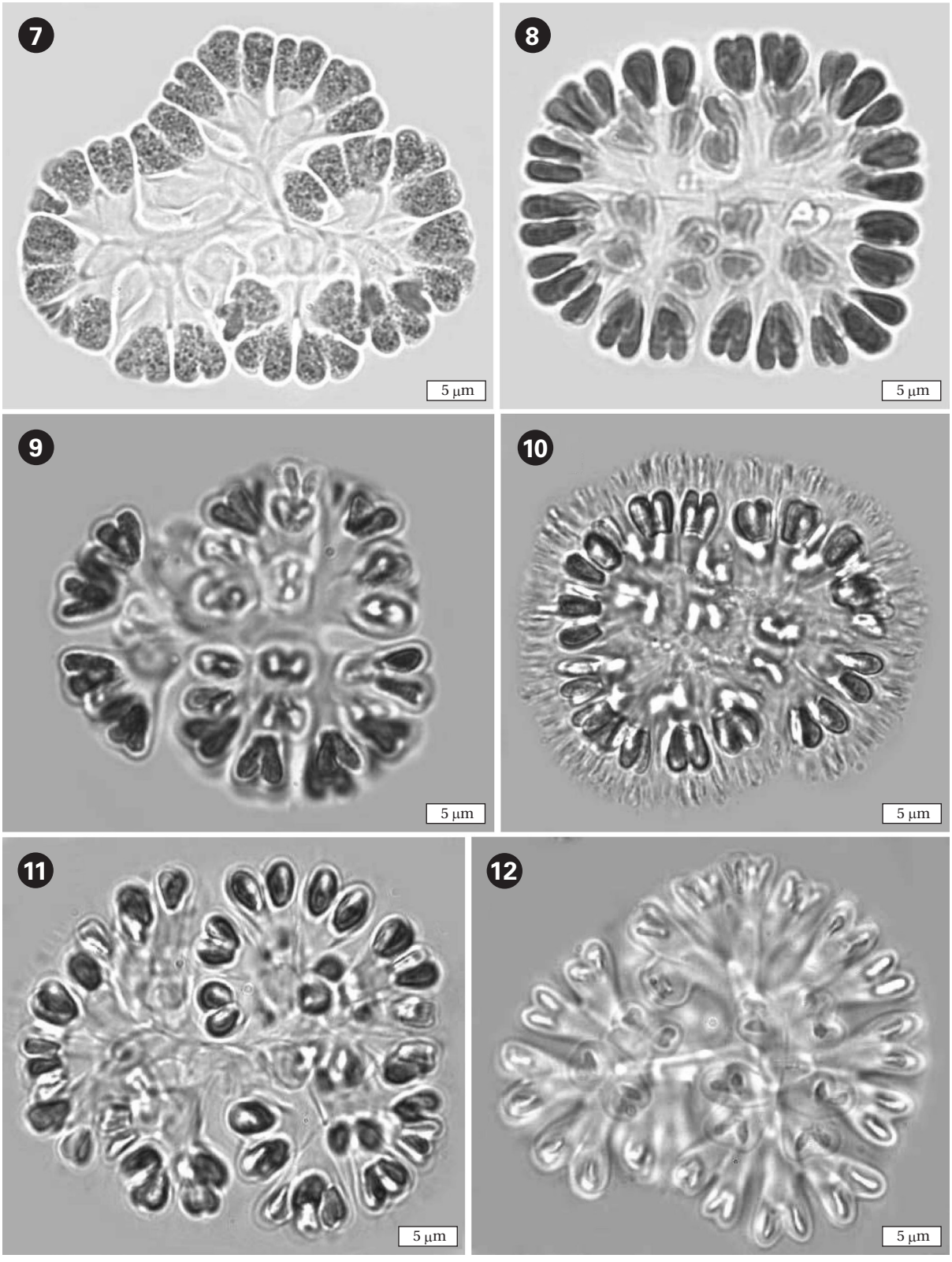

Plate 2. *Gomphosphaeria aponina* Kützing. **fig. 7.** A pressed colony with cordiform cells and distinctly dichotomized central stalk system. **fig. 8.** A colony with dark blue-green coloured cells. **fig. 9.** A colony formed from liberating a fragmented part from the parent colony. Cells have distinct individual envelopes. **fig. 10.** An old colony with a distinct fibrillar gelatinous matrix of the colony. **fig. 11.** A colony with dark blue-green, elliptical cells and a dichotomizing network of interconnecting threads. **fig. 12.** A senescent colony with thick, gelatinous, tubular connections covering up the cells.

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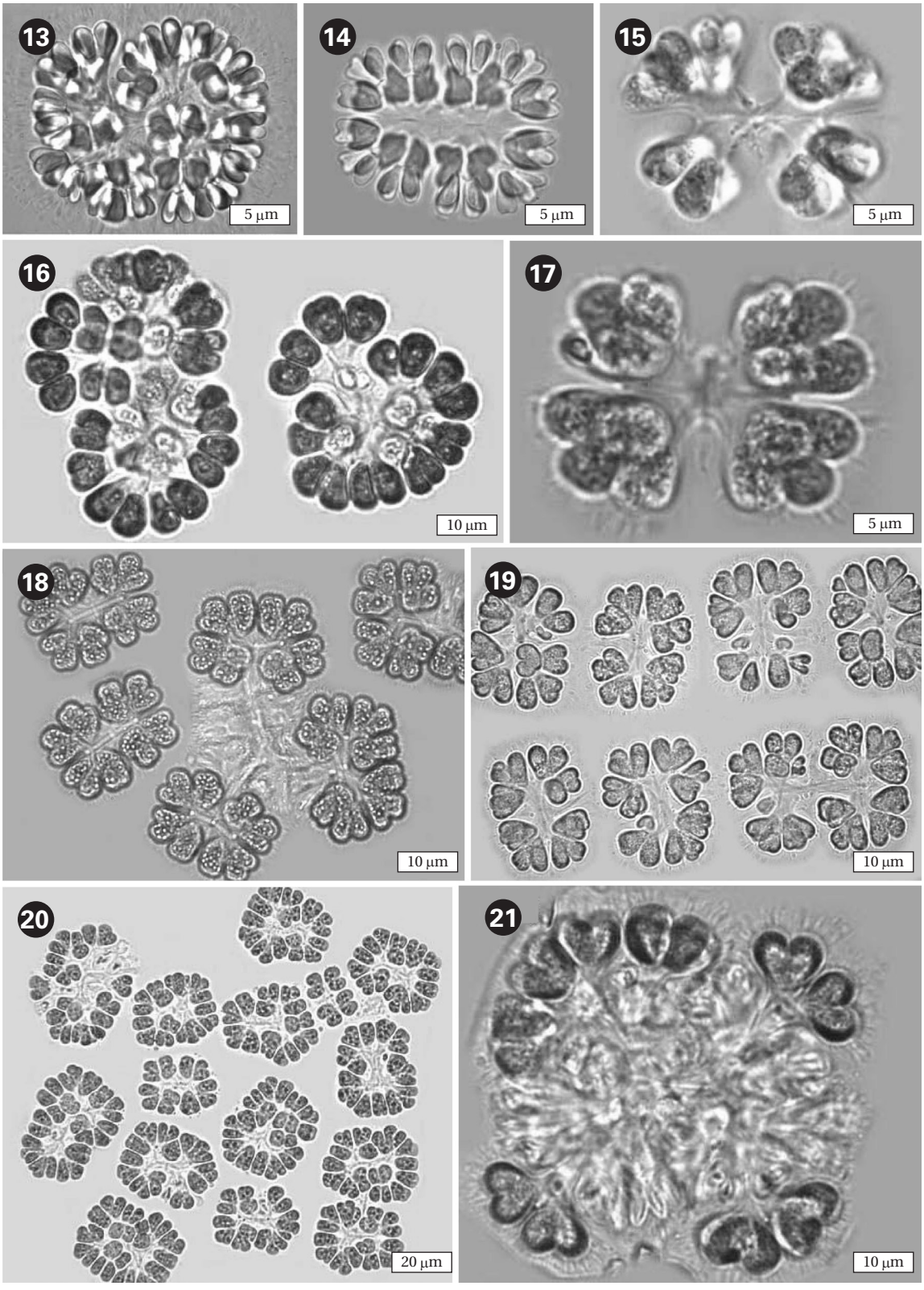

Plate 3. *Gomphosphaeria aponina* Kützing. **fig. 13.** A homogenous colony with cells arranged in pairs. **fig. 14.** A 32-celled colony. Cells have deeply bifurcated apical ends. **fig. 15.** A perennating colony from the bottom of the tank. **fig. 16.** Young colonies with dark and granulated cells. **fig. 17.** Perennating colony from the bottom of the tank. **fig. 18.** When a large colony was pressed, it liberated several small clusters (colonies). Thick remains of mucilage are seen in the center. **fig. 19.** When a large colony was pressed, it liberated eight pieces of eight-cells. **fig. 20.** Fragments of a colony when pressed. **fig. 21.** Old perennating colony, which has liberated many cells and shows remains of old sheath layers.

nies with cells of different shapes (Pl. 3, figs 13 & 14) and dark pigmentations (Pl. 2, figs 8 & 9) were observed during December and January. In many colonies, cells were surrounded by distinct and thick hyaline envelopes and had dichotomizing tubular stalks (Pl. 2, figs 9 & 12). These colonies appeared to be senescent and looked so different that it was possible to identify such populations as new species and relate certain of them (Pl. 2, fig. 12) with the evolution of colonies similar to that found in the genus *Siphonosphaera*, described by Hindak (1988).

Reproduction: When the number of cells in a colony increases, cells grow and secrete a great deal of mucilage. Apparently, pressure is generated, and certain clusters of cells may slip away on slight jerks (Pl. 3, fig. 16). During this study, when a mature colony was pressed, they got separated into four or eight small colonies each containing usually 16 to 32 cells (Pl. 3, figs 18-20). The most interesting feature was that whenever a large colony was pressed, it released several clusters and all were well organized in shape and cell number. This indicated that the pattern of cell division is perfectly synchronized and symmetrical and why clusters give the appearance of autocolonies. Frequently, thick and layered remains of parent mucilage and stalks were clearly seen (Pl. 3, figs 18-21). During June and December, many colonies with enlarged, brownish, granulated cells are covered by mucilage, get settled on the bottom of the tank, and behave as perennating colonies (Pl. 3, figs 15, 17 & 21). During September and March, they come up to the surface and form new colonies.

Taxonomic status: The genus is distinguished from its allied genera of Gomphosphaerioideae by its characteristic paired cordiform cells. The criteria are not well defined for distinctions between species. Species are categorized based on colony size and shape; cell measurement, color and content; thin or thick gelatinous colonial matrix or presence of structured envelope around individual cells; and the presence of a central dichotomizing tubular system. Our two-year observation on enumerable colonies of a single population revealed many variations and structures that have been described for different species (Komárek 1989). In fact, certain completely new shapes of colonies, cells and mucilage envelopes were seen and cannot be compared with any known species (Pl. 2, figs 10-12). One of the researchers (GLT), who has been observing Cyanoprocaryotes for the last over four decades, has never seen such a rich growth of this organism. Previous to this study and on rare occasions, only one or two specimens could be seen on a slide. In this study, records of such wide variations in terms of shape and size, color and mucilage were possible due to the availability of numerous specimens from the natural habitat and continuous observations for more than two years. Further, we searched colonies, including the bottom, lighted, shaded, free-floating, or infested with other filamentous green algae or *Hydrilla* plants, and at different depths, from every possible microhabitat of the cement tank. Since all stages were so gradual, it could not be considered that a combination of different species was growing simultaneously in the same pond. Comparison of the present population with other known species of the genus *Gomphosphaeria* indicate that, at best, it can be identified as *G. aponina. G. aponina* happens to be the type of species that is cosmopolitan in distribution and most frequently recorded. Since descriptions of other species are brief and therefore, any comparison in terms variability can not be made, it remain for future either to have full details or to have DNA sequencing of different species to understand the distinctions of species at morphological as well as at molecular level.

REFERENCES

Hindak, F. 1988. Contribution to the taxonomy of some cyanophyte genera. Preslia 60:289-308.

Komárek, J. 1989. Studies on cyanophytes of Cuba 4-6. Folia Geobot Phytotaxon 24:57-97.

Komárek, J. 2005. Studies on the cyanophytes (Cyanobacteria, Cyanoprokaryota) of Cuba 11: fresh water *Anabaena* species. Preslia 77:211-234.

Komárek, J. & Anagnostidis, K. 1986. Modern approach to the classification system of cyanophytes: 2. Chroococcales. Algol. Stud. 43 Suppl:157-226.

Komárek, J. & Anagnostidis, K. 1998. *Cyanoprokaryota: Chroococcales-Süßwasserflora von Mitteluropa*. Spektrum Akademischer Verlag, Heidelberg, 548 pp.

Tiwari, G. L., Dwivedi, V. K., Tandon, R., Tiwari, O. N. & Kant, R. 2009. Morpho-taxonomy of Coccoid Cyanobacteria. *In* Khattar, J. I. S., Singh, D. P. & Kaur, G. (Eds.) *Algal Biology and Biotechnology*. I. K. International Publishing House, New Delhi, pp. 1-26.

Tiwari, G. L., Kant, R., Tiwari, O. N., Tandon, R. & Kushwaha, L. L. 2007. Distribution, diversity and characterization of Cyanobacteria of rice-fields. Proc. Natl. Acad. Sci. India Sect. B (Biol. Sci.) 77:387-402.