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MORPHOLOGY AND REPRODUCTION OF *OOCYSTAENIUM ELEGANS* GONZALVES ET MEHRA (CHLORELLALES, TREBOUXIOPHYCEAE, CHLOROPHYTA)

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The present study reports about the occurrence of *Oocystaenium elegans* being reported for the first time from northern part of India. It is shown that male and female cells can be identified only when they produce antherozoids and eggs and not on the basis of shape and size of cells. Nuclear stages observed by iron alum acetocarmine method indicated that uninucleate stage was never observed in vegetative cells, minimum number of nuclei was 16 in young autospores and maximum was 128 in male cells producing antherozoids. It appeared to be a rare alga and presently restricted to Indian sub-continent.

Key words: Autospores, *Oocystaenium elegans*, Sexual Reproduction

The *Oocystaenium elegans* Gonzalves and Mehra is a monotypic green alga. It appeared to be a rare green alga as it is reported from Indian subcontinent only (Guiry and Guiry 2021). It was first described by Gonzalves and Mehra (1959) from a small water accumulation in a field of Goregaon near Mumbai (Maharashtra), India. Thereafter, this species was again reported by Kamat (1969) from pools of Nagpur. Pandey and Pandey (1979) reported a new species as *O. mitrae* from open drain of Allahabad. Some cultural observations have also been made by Ichimura (1976) from soil samples collected from Nepal. The taxonomic position of the genus *Oocystaenium* has been very complicated. Previously alongwith other several genera, *Oocystaenium* was classified into the Family Oocystaceae, Order Chlorococcales, class-Chlorophyceae under the Division Chlorophyta (Philipose, 1967), but later on it has been transferred to the Order Chlorellales, Class Trebouxiophyceae, Division-Chlorophyta (Lewis and Mccourt, 2004) and in Algaebase (Guiry and Guiry 2021). Present authors found its abundant growth in Prayagraj, India and described the species in detail with some new observations. Present authors found its abundant growth in Prayagraj, India and described the species in detail with some new observations

MATERIAL AND METHODS

Growth of *Oocystaenium elegans* Gonzalves *et* Mehra was observed growing in a temporary ditch filled with one foot depth water from July to September, 2019 in Jhunsi, Prayagraj, India. The algal growth containing greenish water samples were collected in 50 ml vials (Polylab) and brought to M.O.P. Iyengar Algae Lab, Department of Botany, University of Allahabad, Prayagraj, U.P., India and 45 ml natural samples was preserved in 4% formaldehyde and 5 ml of freshly collected sample was thoroughly mixed by homogenizer (RQT-127A/D, REMI) and 1 ml algal sample was transferred into the petridishes filled with liquid Bold's Basal medium (Nichols 1973). Enrichment and pure cultures were developed by pour plate and streaking method (Kaushik 1987) and their unialgal cultures were developed by repeated culturing and sub

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culturing techniques (Kant *et al*. 2005). The alga was maintained in Bold's Basal medium under 16:8 LD regiment of about 2KLux with temperature 28±2ºC. The morphological details of various developmental stages were observed from the material collected from nature as well as from enrichment cultures. Photographs were taken under the Trinocular Research Microscope (Leica-DMLB) attached with digital camera (DC-300). All the morphological observations were recorded and cells size of the alga was measured with the help of micron scale bar.

OBSERVATIONS

In nature, the cells of the alga were found free floating or attached and also in mucilaginous accumulations of various shapes on submerged soil surface (Pl. 1, Fig. 1). Cells growing in liquid BBM medium in petridishes under undisturbed condition showed formation of soft mucilaginous trail (Pl. 1, Fig. 2) at one or occasionally at both ends and indicated slight displacement of cells. Individual cells were ellipsoidal in shape, thin walled and without any polar thickening. Since all sort of cell sizes were available at all the time during vegetative as well as at reproductive phase of growth and therefore it was difficult to identify as male or female cells before actual differentiation of contents into eggs or antherozoids. Each cell possessed a few to numerous elongate spindle shaped green chloroplasts (Pl. 1, Fig. 3; Pl 2. Figs. 1 & 2) situated at periphery of cells and each with a distinct pyrenoid (Pl 2. Figs. 3-5). Cells vary in diameter (30-100µm in diameter at mid point of the cell) and in length (115-235µm from pole to pole). When cells were stained by iron-alum acetocarmine squash technique (Godward 1948) cells contained 16 to more than a hundred nuclei in different vegetative cells depending on cell size (Pl. 1, Figs. 13-15). In general uninucleate cells were never observed except during egg formation in female cells. It appears that during egg formation in female cells except one nucleus, the rest were degenerated. Minimum number of nuclei per cell was found to be 16 in young cells and up to 128 in mature cells. In reproductive cells where protoplasm was getting segregated usually had more than 32 nuclei, where as in oogonial cell only one nucleus was found. The number of nuclei varied 16-64 in autospores.

During asexual reproduction, 2-4 autospores were formed by simultaneous segregation of protoplast into 2-4 walled autospores (Pl. 1, Figs. 7 & 8; Pl 2. Fig. 6). Both narrower and broader cells may produce two or four autospores in any cell. Formation of autospores was quite prevalent in broader cells. Autospores get separated by breaking of parent cell wall at any place (Pl. 2., Figs. 7 & 8). The number of nuclei in each young autospores may vary from 16-64. Although the both types of materials collected from nature as well grown in culture were observed continuously, but the sexual reproduction was observed only in the material collected from natural habitat in September as the water was receding in the ditch. In culture apparently due to unsuitable conditions cells gradually degenerated and disappeared after two months of slow growth and sexually reproducing cells were not seen. During our continuous observations from the natural material, we could never visualize or distinguish cells as male and female cells because eggs and antherozoids were produced by both narrower and broader cells. The onset of sexual reproduction was sudden and simultaneous to produce antherozoids and eggs. On an average of ten observations ca 50% cells remained vegetative, and ca 30% turned to antheridial and ca 20% to oogonial cells. It was not possible to make any specific boundary of measurements to distinguish male or female cells on the basis of size and shape of vegetative cells. Eggs are spherical and measure 50-90 µm in diameter (Pl. 1, Figs. 4 & 5). However, number of antherozoids varied from 16-128 (Ichimura 1976), 36-48 (Gonzalves and Mehra 1959, Kamat 1969). In the present alga number of antherozoids also varied from 16-128, but most common number

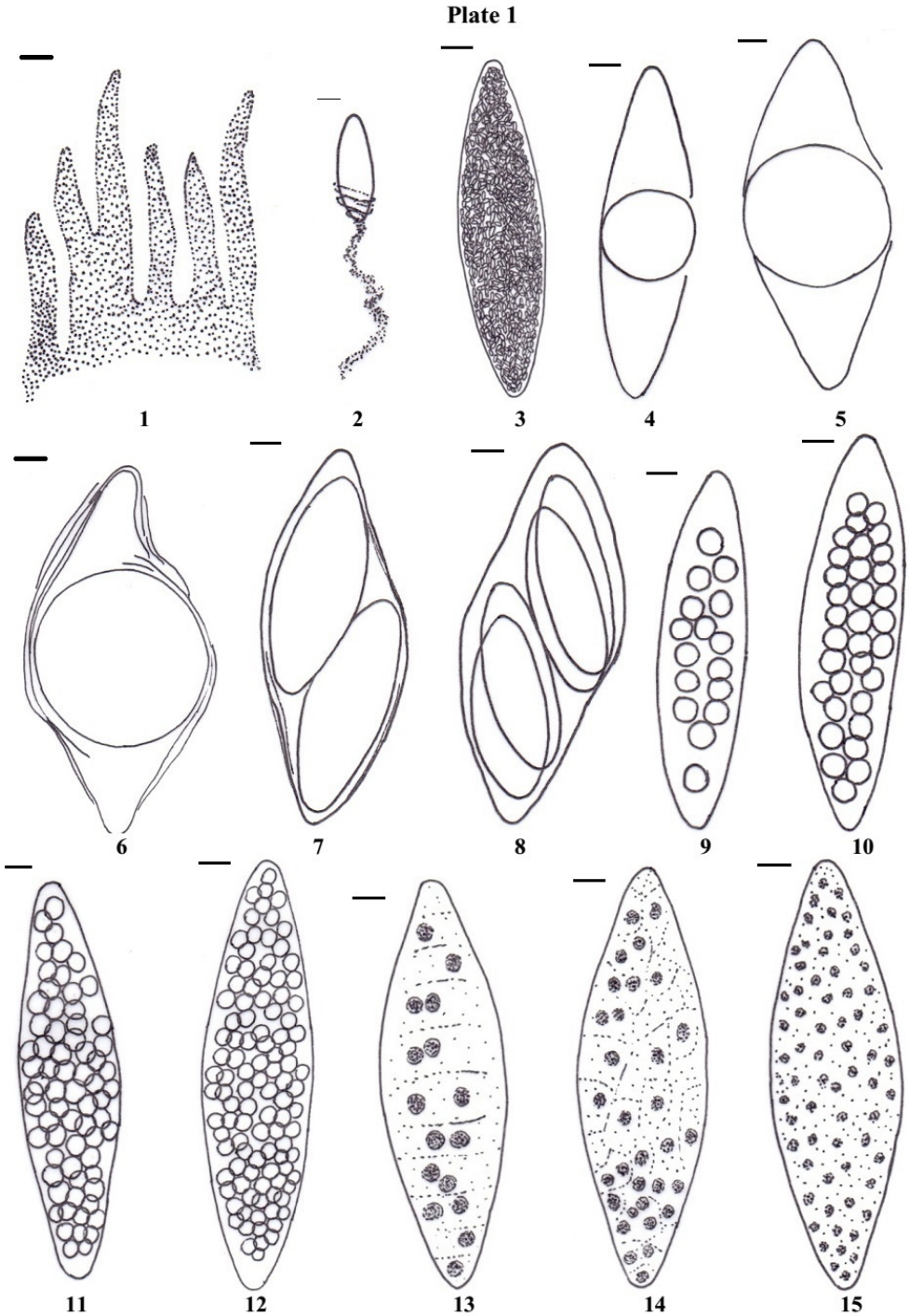

Plate 1. Figs 1-15, Line diagrams of *Oocystaenium elegans* Gonzalves et Mehra 1. Finger like projections of thalli with numerous cells **2.** Solitary cell with their individual stalk **3**. A vegetative cell with numerous chloroplast **4 & 5**. Successive stages in the development of female cells leading to production of spherical egg **6.** Formation of oospore **7 & 8**. Stages in production of autospores **9-12**. Stages in development of male sex cells **13**. Male cell showing 16 nuclei **14**. Male cell showing 32 nuclei **15**.Male cell showing 64 nuclei **Scale bar: Fig.1=10mm; fig.2=30µm; figs. 4-15=20 µm**

Plate 2 (1-15): *Oocystaenium elegans* Gonzalves et Mehra 1 & 2. Vegetative cells with numerous spindle shaped chloroplast **3-5**. Cells showing a peripheral arrangement of plastids **6.** Formation of two autospore **7.** Showing cell division **8.** Showing liberation of two autospores **9-11**.Showing different stages of antherozoid formation **12.** Showing empty male cell **13.** Biflagellated antherozoids **14 & 15**. Formation of oospores

was found to be 32 and 64 (Pl. 1, Figs. 9-12; Pl. 2. Figs. 9-11). Liberation of antherozoids occurred by gelatinization and formation of irregular pore at any one end of the cell (Pl. 2. Fig. 12). Antherozoids are 10-15 µm broad and 15-20µm long, slightly pale in colour and have somewhat deformed spherical plastids (Pl. 2. Fig. 13). After fertilization thick walled and somewhat irregularly ornamented oospore were formed (Pl. 1, Fig. 6; Pl. 2., Figs. 14 & 15).

DISCUSSION

Survey of literatures indicated that *O. elegans* is a rare alga. It is apparently restricted to Indian sub-continent and known globally as only single species in Algaebase (Guiry and Guiry 2021). Previously *O. elegans* was classified under order Chlorococcales (Philipose, 1967), but later on the alga was transferred to the Order Chlorellales, under class-Trebouxiophyceae of Division Chlorophyta (Lewis and Mccourt, 2004, Guiry and Guiry 2021). The *O. elegans* complete its life cycle in short span of time (within three weeks) and reproduce mainly by oogamous sexual reproduction. Observations by earlier workers are mostly similar except that male and female cells as described by Gonzalves and Mehra (1959) and Kamat (1969) and also in *O. mitre* (Pandey and Pandey 1979) are found not to be distinguishable in vegetative stage but only at the stage when antherozoids and eggs are formed. Nuclear stages by acetocarmine squash method revealed that cells are always multinucleate and possessed 16 or more nuclei per cell. Ichimura (1976) has also observed that male and female cells appeared in the same clonal population and confirmed homothallic behaviour of the alga for the first time. Further, he also observed that oospores were produced in all individual clones and sexual reproduction was triggered under high light conditions. All previous reports have indicated that eggs were always produced singly in any female cell.

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