



International Journal of Botany

ISSN: 1811-9700

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Two New Records of Coleochaetalean Algae (Coleochaetales, Chlorophyta) from Northeast Thailand

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Abstract: This study is an attempt to contribute and add information on the freshwater algal flora in Thailand. In this study, two members of coleochaetalean green algae, viz., *Chaetosphaeridium globosum* (Norstedt) Klebahn and *Coleochaete nitellarum* Jost are reported for the first time for Thailand. Both of them were found as epiphytes on charophyte algae in a mesotrophic reservoir, namely Hui Nam Sab from Amnat Charoen province, Northeast Thailand. Illustrated descriptions of the newly recorded species along with a short note on the species and its distribution are provided. In addition, some ecological aspects of the studied reservoir and coleochaetalean algae are discussed.

Key words: Coleochaetales, green algae, diversity, new record, water quality, Thailand

INTRODUCTION

Coleochaetalean algae (Coleochaetales) are a small group of freshwater epiphytic species in the Chlorophyta (Graham and Wilcox, 2000). They show considerable morphological variation but are united by a suite of structural features, including a characteristic hair (setae) composed of a sheath that forms as an outgrowth of the cell wall and an inner bristle that is apparently continuously extruded from the base (Graham, 1990). The setae probably function as an anti-herbivore defence or nutrient assimilation (Marchant and Pickett-Heaps, 1973; Graham and Wilcox, 2000). Coleochaetalean algae can reproduce asexually by producing scaly biflagellate zoospores which leave to begin a new plant, while sexual reproduction of them is oogamous, with a large non-motile egg and smaller free-swimming sperm (Graham, 1990). Coleochaetalean algae can be found worldwide as epiphytes on submerged portions of aquatic macrophytes and also grow on nonliving inorganic substrates such as beer cans, plastic bags and pebbles near the shallow littoral zone of oligotrophic ponds and lakes (Graham, 1984; Graham and Wilcox, 2000).

Up to now, three genera of coleochaetalean algae are recorded: *Coleochaete*, with about 15 species (Szymanska, 1989; Cimino and Delwiche, 2002; Delwiche *et al.*, 2002); *Chaetosphaeridium*, with 4-5 described species (Thompson, 1969); and *Awadhiella* considered as rare genus (Prasad and Asthana, 1979). Although the *Coleochaete* and the allied genus

Chaetosphaeridium have no particular economic importance, in view of their close relationship to land plants, they provide well excellent simple systems for the study of more complex physiological and reproductive process of plants (Graham, 1993; Graham *et al.*, 2000). Recently, members of coleochaetalean algae have been considered as key algal group for studying evolution and phylogenetic relationship of early land plants (Delwiche *et al.*, 2002; Lewis and McCourt, 2004; Becker and Marin, 2009).

Thailand is the country situated in the tropical and humid climatic zone and supports a variety of freshwater habitats. Northeastern part of the country is known to have a large number of small ponds, swamps, rivers and natural lakes, but little is known about the freshwater algal flora in the region. In order to fill this gap, we attempt to make a contribution on freshwater algae in northeastern part. Survey of phycological literatures reveals few works (Patel, 1968; Prasad and Srivastava, 1965; Patel, 1970; Nishihama, 1970; Islam, 1974) dealing with Asian coleochaetalean algae and there is no previous information of this algal group in Thailand. Therefore, we aim to investigate the coleochaetalean algae to add new information to our freshwater algal flora. During the course of extensive survey of freshwater green algae in Northeast Thailand, we came across two species in the Coleochaetales growing on the thalli of charophytes. They are reported here for the first time and treated as new records for algal flora of Thailand.

MATERIALS AND METHODS

Hui Nam Sab reservoir is located approximately 5 km northeast of Mueang Amnat Charoen city center, 500 m away from Mueang Amnat Charoen School. Geographically, it lies between 15°52' 38.4 N latitude and 104°37' 7.5 E longitude and at altitude of 540 m from sea level (Fig. 1). The reservoir is about 0.5 km² in area and with average depth of about two meters during postmonsoon period. The water of this reservoir is used for different purposes especially agricultural irrigation and fisheries. The reservoir is also used by animals for drinking purpose and by local residents for bathing and washing.

Samples of aquatic macrophytes including charophytes were taken on 4 sites from the reservoir at the winter season (December) in 2008. Some water variables, temperature, conductivity and pH were measured using a Horiba Water Quality Checker (U-10)

(Table 1). To assess the trophic status of the reservoir, total phosphorus and chlorophyll a were also determined according to standard method of APHA/AWWA/WEF (1998) and data were then used to calculate Trophic State Index (TSI) according to the method of Carlson (1977) and Carlson and Simpson (1996).

Observations on algal material was performed under both dissecting and light compound microscopes to see microscopic forms of coeleochaetalean algae attached on pieces of aquatic macrophytes. Drawings were made with the aid of camera lucida. Photographs were taken under an Olympus BX51 microscope with Olympus digital camera (DP12) apparatus. Voucher material was kept at Applied Taxonomic Research Center, Faculty of Science, Khon Kaen University. The algal material was identified based on the following systematic papers: Prescott (1962), Thompson (1969), Prasad and Srivastava (1965), Patel (1968) and John (2002).

Table 1: Environmental variables measured from each site in Hui Nam Sab reservoir

Variables	Site 1	Site 2	Site 3	Site 4	Mean	TSI	Trophic state*
Temperature (°C)	29.6	30.5	30.2	30.8	30.3	-	-
Conductivity (μS cm ⁻¹)	78.0	72.0	80.0	77.0	76.8	-	-
pH	6.0	5.8	5.9	6.0	5.9	-	-
Total phosphorus (μg L ⁻¹)	16.0	14.0	14.0	13.0	14.3	42.5	Mesotrophy
Chlorophyll a (μg L ⁻¹)	4.2	3.8	4.6	4.5	4.3	44.9	Mesotrophy

*Trophic state of the water is classified according to Carlson and Simpson (1996)

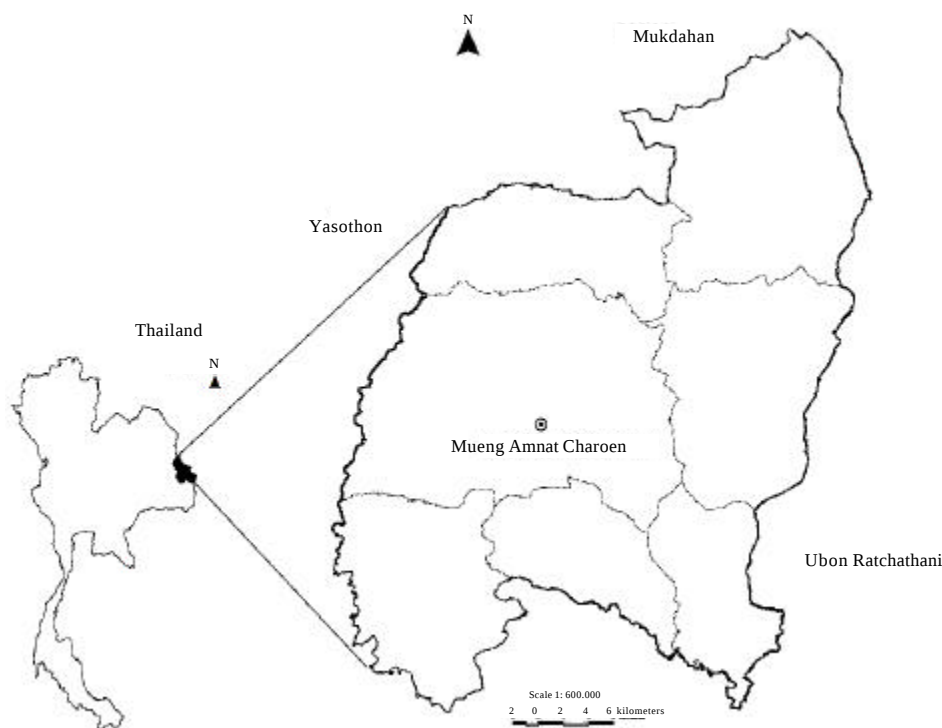


Fig. 1: Location of sampling area at Mueng Amnat Charoen, Northeast Thailand

RESULTS AND DISCUSSION

Two species in the Coleochaetales, *Chaetosphaeridium globosum* and *Coleochaete nitellarum* were found as epiphytes on the thalli of two charophytes, *C. corallina* Klein ex Willd., em. and *Nitella* sp. Both *C. globosum* and *C. nitellarum* are new records for Thailand and described below.

Taxonomic enumeration

Family Chaetosphaeridiaceae

Genus *Chaetosphaeridium* Klebahn

Chaetosphaeridium globosum (Norstedt) Klebahn

(Fig. 2a): Cell is solitary or clustered. Cell shape is globose. Clustered cells are without intercellular tubular connection. Each cell is about 15 µm in diameter, bearing a long seta, up to 160 µm in length. Basal sheath of seta is 18-20 µm in long and up to 4 µm in width. Cell contains a parietal shaped chloroplast and containing usually a single central pyrenoid (Fig. 2a). Reproductive structure was not seen in the material.

The genus *Chaetosphaeridium* comprises 4-5 species: *C. pringsheimii* Klebahn, *C. ovalis* Smith, *C. globosum* (Nordst.) Klebahn, *C. gemmatum* Thompson and *C. huberi* (*nomen dubium*) (Thompson, 1969). *C. globosum* is one of well known species of the genus which is often epiphytic on the stems, leaves or pieces of some aquatic macrophytes, such as water-lily leaves (*Nuphar*, *Nymphaea*), the submerged leaves of *Potamogeton* and *Najas* and petioles and culms of emergent aquatic plants, e.g. *Typha*, *Nelumbo*, *Sagittaria* and some filamentous and macrophytic algae (Thompson,

1969; Patel, 1970; Sarma, 1986; John, 2002). In agreement with previous reports, we also found *C. globosum* as epiphytes growing randomly on two macrophytic algae, *Chara corallina* and *Nitella* sp. The general characteristics of Thai *C. globosum* follow the description by Prescott (1962), Thompson (1969), Patel (1970), Sarma (1986) and John (2002) indicating there is less morphological variation among Thai material and material from other countries.

According to the taxonomic keys provided by Thompson (1969) and John (2002), *C. globosum* can be distinguished from the closely related species, *C. ovalis* by having globose-shaped cell and is clearly different from *C. pringsheimii* by having shorter seta and without interconnected tubular outgrowth connecting between the cells.

Distribution: It seems that *C. globosum* can occur widely throughout the world. It has been reported in Europe (John, 2002), North America (Prescott, 1962; John, 2003), Africa and South Africa (Sarma, 1986) Australia and New Zealand (Day *et al.*, 1995). In Asia, it was reported from China (Sarma, 1986), Japan (Sarma, 1986), India (Patel, 1970), Bangladesh (Islam and Irfanullah, 2005), Burma (Sarma, 1986) and Thailand (this study).

Family Coleochaetaceae

Genus *Coleochaete* Bréb.

Coleochaete nitellarum Jost (Fig. 2b-c, 3a-d): Thallus consists of numerous irregularly branched prostrate filamentous, growing diffusely along the surface of the host plant (Fig. 2b, 3a). The filaments are composed of

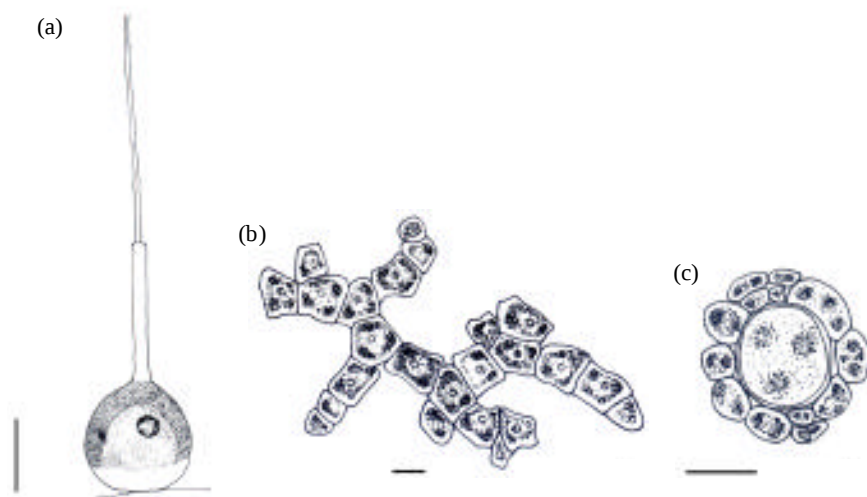


Fig. 2: Drawings of coleochaetalean algae from Thailand: (a) *Chaetosphaeridium globosum* (Norstedt) Klebahn, (b) Vegetative thallus of *Coleochaete nitellarum* Jost and (c) Spermatocarp of *C. nitellarum*, (scale bar: a = 10 µm, b, c = 20 µm)

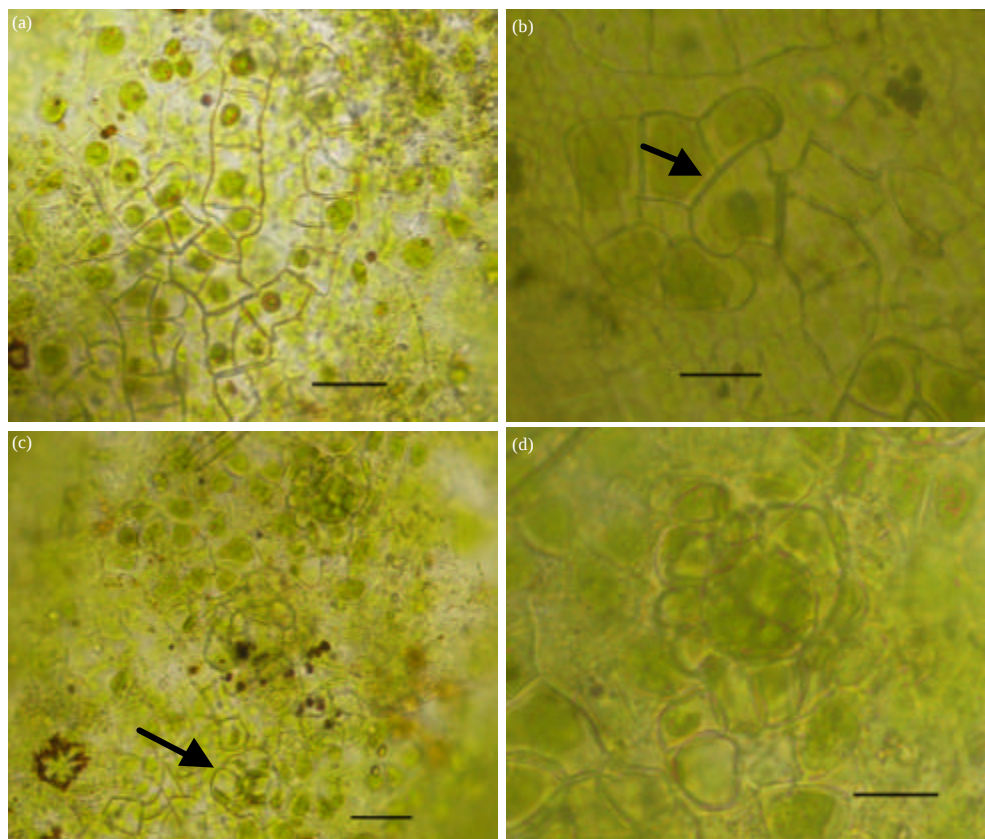


Fig. 3: Photomicrographs of *C. nitellarum*: (a) vegetative thallus, (b) characteristic of T-shaped cell division (indicated by arrow), (c) developing spermocarps (arrow indicates the young spermocarp), (d) fully developed spermocarp (scale bar: a, c = 30 μ m, b, d = 20 μ m)

sub-rectangular, squar or polygonal cells. Few cells bear setae, which project out of the host cell. Each cell contains a single plate-like chloroplast filling the greater part of the cell and containing a single pyrenoid. A single nucleus is present in the cell. The cells measure from 15-30 μ m in length and 15-27 μ m in width.

Zoospores and sexual reproductive structures, antheridia and oogonia were not seen in the material. It, however, showed the fertilized zygotes (oospores) enclosed with cortication or spermocarp structure (Fig. 2c). Spermocarps produced laterally on the filaments are clearly distinguishable from other vegetative cells by having cortication and larger morphological structure (Fig. 3c). Oospores are about 27-33 μ m in diameter. Spermocarps (oospore with cortical cells) are spherical in apical view and measured about 45-56 μ m in diameter (Fig. 3d).

Among approximately 15 species of the genus *Coleochaete* (Szymanska, 1989; Szymanska and Spalik, 1993), *C. nitellarum* has a distinctive morphology which is distinctly different from other species by having

irregularly branching filament, while other *Coleochaete* species have morphology of pseudoparenchymatous or parenchymatous-like thallus, except *C. irregularis* showing monostromatic expansion of loosely arranged cells in filaments (Szymanska and Spalik, 1993; Delwiche *et al.*, 2002). *C. nitellarum* has been reported as endophytes or epiphytes on charophyte algae (Prescott, 1962; Prasad and Srivastava, 1965; Cimino and Delwiche, 2002). In this study, *C. nitellarum* was found epiphytic on charophyte algae, namely *Chara corallina* and *Nitella* sp. Our material has general characteristics (size of vegetative cells and dimension of oospores and spermocarps) that are rather similar to Bangladesh material reported by Islam (1974) and Islam and Irfanullah (2005). However, it is noticeable that Thai material is slightly different from Indian material (Prasad and Srivastava, 1965; Patel, 1968) and North American material (Prescott, 1962; Delwiche *et al.*, 2002) in having larger size of the vegetative cells. Recently, Cimino and Delwiche (2002) reported a molecular phylogenetic analysis of *Coleochaete* and suggested that there were at least one

more species (cryptic species complex) existing within endophytic members of *Coleochaete*, especially *C. nitellarum*. Therefore, the variation of vegetative cell size between Thai material and other material mentioned above may be due to the difference in cryptic form of *C. nitellarum*. However, the presence of T-shaped cell division (Fig. 3b) being the unique characteristic of *C. nitellarum* observed in this study confirmed correct identification of the material as *C. nitellarum*.

Distribution: *C. nitellarum* is one of the most common species of *Coleochaete* occurring frequently on charophytes, especially *Nitella* and *Chara* (Prescott, 1962; Prasad and Srivastava, 1965; Islam, 1974; Cimino and Delwiche, 2002). However, it can be found occasionally on stem of some aquatic macrophytes (Cattaneo, 1978). *C. nitellarum* was reported as common species in North America (Prescott, 1962) including Canada (Cattaneo, 1978), but it seems to be doubtful species recorded on only few occasions in the British Isles (John, 2002). *C. nitellarum* can be found in Australia and New Zealand (Day *et al.*, 1995; Entwisle and Skinner, 2001) and Asia: Bangladesh (Islam, 1974; Islam and Irfanullah, 2005), India (Prasad and Srivastava, 1965) and Thailand (this study).

Key to the species occurring epiphytically on charophytes in Hui Nam Sab reservoir:

Cell solitary with sheath hair (setae), 2 or more cells clustered in some groups, not interconnected by tubular outgrowth

..... *Chaetosphaeridium globosum*
Thallus irregular branching filaments, some cells bearing setae, oogonia or spermatocarp present
..... *Coleochaete nitellarum*

Ecological condition of the studied reservoir: The water of Hui Nam Sab reservoir was rather clear (seen by eyes), slightly acidic (pH 5.8-6.0) and low in conductivity (72-78 $\mu\text{S cm}^{-1}$) (Table 1). Normally, conductivity is an indicator of the concentration of dissolved electrolyte ions in the water and significant increases in conductivity may be an indicator that polluting discharges have entered the water (Behar, 1997). Thus, the water quality of the studied reservoir may be interpreted as unpolluted water.

In addition to data mentioned above, we also determined the trophic state of the reservoir based on Carlson (1977)'s Trophic State Index (TSI). TSI values calculated based on the basis of total phosphorus and chlorophyll a revealed that the reservoir was in a slightly mesotrophic state (Table 1). According to Carlson (1977) and Carlson and Simpson (1996), the mesotrophic

condition of the water reflects intermediate primary productivity associated with moderate level of nutrients, especially phosphorus and nitrogen contents. This mesotrophic condition is not harmful effect of water pollution. From this data, it may be interpreted that the studied reservoir has water quality in good condition.

Generally, the mesotrophy is the intermediate status between oligotrophy (low nutrients) and eutrophy (high nutrients) (Carlson and Simpson, 1996). Depending on the degree of eutrophication, subsequent negative environmental effects such as loss of oxygen (anoxia), toxic algal blooms, severe reduction in water quality, fish kills and loss of aquatic biodiversity including species important to commercial and fisheries may occur (Smith *et al.*, 1999; Khan and Ansari, 2005). Although during the studied period no serious event of eutrophication occurred, such as phytoplankton bloom and floating macroalgae, there were some situations of nutrient enrichment into the reservoir due to human activities such as agriculture, fisheries, bathing and washing in the reservoir area which may be the initiative of eutrophic development. One signal that may involve nutrient enrichment occurred in the studied area; around littoral zone of the reservoir was abundant with hydrophytes, for example, *Typha* sp., *Ceratophyllum* sp., *Najas* sp., *Hydrilla* sp., *Lymncharis* sp., *Potamogeton* sp., *Utricularia* sp., *Chara corallina* and *Nitella* sp. The signal of abundance in aquatic macrophytes generally reflects the development of eutrophic status of water causing the reduction in number of species diversity (Brown, 1987; Khan and Ansari, 2005). Therefore, the information observed in this study will be useful to assess environmental problem and to protect aquatic biodiversity loss in the studied reservoir.

During the course of extensive survey of algae in several freshwater bodies in Thailand, we never found coleochaetalean algae in unclean or eutrophic freshwater ponds and lakes. They were found in a slightly mesotrophic water as shown in this study. Our information agrees with previous information; some authors (Smith, 1950; Graham, 1984; Cimino *et al.*, 2003) mentioned that most species-rich localities of coleochaetalean algae appeared to be clean and oligotrophic freshwater lakes and ponds. Besides, Graham and Wilcox (2000) mentioned that most species of coleochaetalean algae are sensitive to the effects of cultural eutrophication and they disappeared from polluted lakes. According to our water quality analysis, overall data supports that water quality of the studied reservoir is unpolluted and good condition which is suitable to be habitat for coleochaetalean algae. Therefore, ecological condition of freshwater ponds and

lakes ranging from oligotrophy to mesotrophy may be used as tentative indicator for surveying coleochaetalean algae in other water bodies of Thailand. It should be borne in mind that other coleochaetalean algae, especially *Coleochaete* species which are not reported in this study are still waiting to be discovered in the future.

ACKNOWLEDGMENT

We would like to thank Prof. Srisumon (Chantanachai) Sitathani for her encouragement and advice in our initiation into work on algae. This work was supported financially by the Applied Taxonomic Research Center, Khon Kaen University.

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