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ORIGINAL ARTICLE

Morphological and molecular profiling of *Spirogyra* from northeastern and northern Thailand using inter simple sequence repeat (ISSR) markers

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Abstract Green algae, *Spirogyra* (Chlorophyta), are found in a wide range of habitats including small stagnant water bodies, rivers, and streams. Species identification of *Spirogyra* based on morphological characteristics has proven to be a difficult process. An accurate identification method is required to evaluate genetic variations. This study is aimed at investigating the molecular profiling of 19 samples of *Spirogyra* from northern and northeastern Thailand. The morphological characteristics of each sample were recorded, viz. cell dimensions (width and length), along with the number and arrangement of chloroplast spirals/pyrenoids. With regard to a correlation of the biological and ecological parameters, conductivity was clearly significantly related to the number of pyrenoids. While DO is negatively related to the number of chloroplast spirals. Molecular studies with 10 ISSR primers were amplified to examine the DNA fingerprints. Morphological characters were determined to be significantly different by revealing 5 traits ($P < 0.05$) for all specimens. In addition, the DNA markers of all specimens were investigated using 10 ISSR primers. The results show that the PCR technique amplified 108 fragments. An analysis of the DNA fragments grouped all samples by ISRR-PCR, which were then separated into two groups according to their distribution.

1. Introduction

Spirogyra is consumed in an uncooked form in the north and northeast of Thailand where *Spirogyra* is locally known as tao, thao and phakkai. *Spirogyra* is a filamentous type of freshwater green algae, of which the most easily recognized genus in Zygnemaceae due to its spirally coiled chloroplasts. *Spirogyra* spp. are filamentous, unbranched algae that have a unique mode of sexual reproduction. There are more than 400 species in the world. *Spirogyra* records remain limited to the generic level in floristic checklists and biodiversity inventories because

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of certain identification problems. The taxonomy of *Spirogyra* for vegetative growth consists of three characteristics: (i) type of cross walls (plane, replicate semi-replicate or colligate), (ii) cell width, and (iii) chloroplast number (Berry and Lembi, 2000; Hainz et al., 2009). The process of conjugation has to be included in species identification. Samples in sexually reproductive stages have rarely been collected. Stress from temperature, drought, and pH to the *Spirogyra* could induce the formation of conjugation tubes for the fertilization of male and female gametes. The morphology of this conjugation tube and zygote is also often used for identification. Little is known of its ecology and the effects it has on the morphologically distinct filamentous forms (Hainz et al., 2009). The morphology of some species in the genus *Spirogyra* and some related species, such as *Zygnema* and *Cladophora*, revealed them to be cell-shaped with a spiral chloroplast. Reports on the diversity of *Spirogyra* spp. in Thailand have been limited. Lewmanomont et al. (1995) recorded 8 *Spirogyra* spp. in Thailand as follows; *Spirogyra crassa* Kutz., *Spirogyra decimina* (Mull.) Kutz., *Spirogyra dubia* Kutz., *Spirogyra fluviatilis* Hilse., *Spirogyra gracilis* Kutz., *Spirogyra neglecta* Kutz., *Spirogyra schmidii* West & G. S. West and *Spirogyra stictica* (Engl. Bot). While, Thiamdao and Peerapornpisal (2011) have investigated the morphology of *Spirogyra ellipsospora* Transeau in northern Thailand. The vegetative cells were 118–200 × 240–600 μm. Three to five parietal chloroplast bands made 4–5 turns in each cell with numerous circular pyrenoids placed in the middle of the chloroplast band. A rough margin of chloroplast bands was observed.

In some cases, the identification of *Spirogyra* is mainly based on the conjugation tube process and the zygospores. However, this genus is mostly found in its vegetative stage, which complicates the studies on the ecological demand for individual species. The species identification of related *Spirogyra* based on the morphological characteristics can be difficult. However, in addition, *Spirogyra* can respond to the environmental conditions through the expression of different filament type groups (morphotypes), cell length/width and the number of chloroplast spirals which are related to the physico-chemical parameters of the water resource. At the same time, environmental stresses such as those related to temperature, drought and pH could stimulate the induction of the formation of a conjugation tube and gametes. The morphology of the conjugation tube and zygote is required for specific identification. Yoshida et al. (2003) reported that within their habitat, they are divided into two groups. One group floats in still water, and the other group lives in running water, and forms rhizoids for the purpose of anchoring to the substratum.

Table 1 Morphological characteristic of each *Spirogyra* specimens in each trait.

Details	Trait 1	Trait 2	Trait 3	Trait 4	Trait 5
Vegetative cell width (μm)	41–92	40–56	35–60	41–50	43–63
Vegetative cell length(μm)	115–223	80–185	90–193	127–164	93–195
L/W ratio vegetative cell	2.4–2.8	2.0–3.3	2.57–3.21	3.09–3.28	2.16–3.09
Number of chloroplasts	2–3	2	3	2	4–5
Shape of zoospore	Ellipsoid	ns	ns	ns	ns
Zoospore width	60–73	ns	ns	ns	ns
Zoospore length	75–95	ns	ns	ns	ns
L/W ratio zoospore	1.25–1.3	ns	ns	ns	ns
Shape of pyrenoid	Discoid				

Remark: ns = not seen.

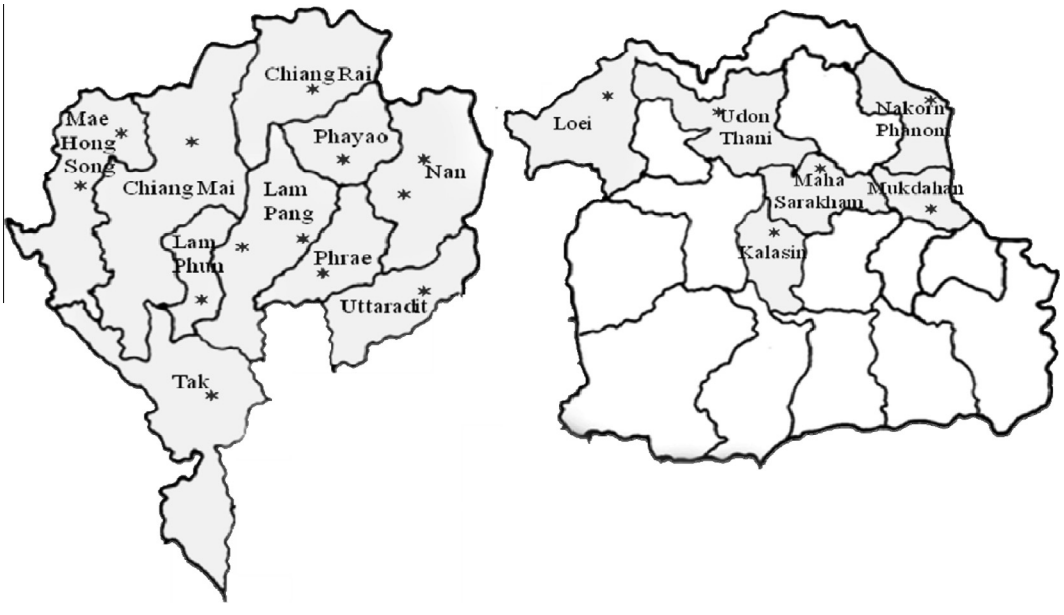

Figure 1 Location of 19 sampling sites (★) where the samples of *Spirogyra* spp. were collected.