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## Diversity of Freshwater Green Macroalgae in Phu Wua Wildlife Sanctuary

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### Abstract

Green freshwater macroalgae are known as primary producers in aquatic ecosystems. They are also used as a traditional food in various parts of Thailand. However, knowledge of their taxonomy, especially their morphological data are rare. Therefore, this study was aimed to investigate the diversity and specific morphology of green macroalgae from Phu Wua Wildlife Sanctuary. Specimens were collected from five waterfalls, Ched Sii, Tham Fhun, Cha Nan, Tad Nok Khian and Tham Phra Waterfalls. Six genera consisting of eighteen species were found. The most diverse genus was *Spirogyra*. The next most common were *Mougeotia* and *Oedogonium*. Ched Sii Waterfall revealed the highest diversity of macroalgae, with nine species. *Zygnema ralfsii* (Hassall) de Bary was a common species found at the sampling sites. Alternatively, *Draparnaldia mutabilis* (Roth) Cedergren was a rare species as it was found only at Tham Fhun Waterfall. This report is the first description of the characteristics of freshwater green macroalgae from Phu Wua Wildlife Sanctuary.

**Keywords:** macroalgae, Phu Wua, *Spirogyra*, *Draparnaldia*

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## 1. Introduction

Algae are important and well known primary producers in aquatic ecosystems. Their photosynthetic activities absorb carbondioxide and generate oxygen. These are important functions of algae as primary producers [1]. Macroalgae are a group of algae that can be viewed in the field without the need for magnification. They form slimy filamentous colonies and mats of various colours. Some macroalgae have well developed structures that are similar to those of higher plants [2]. Most freshwater macroalgae are usually found attached to substrates, e.g., cobble stones, gravel, tree branches and roots [3].

Phu Wua Wildlife Sanctuary was established in 1975 by the Forestry Department with an area of 186.5 km<sup>2</sup>. It is situated in northeastern Thailand. Its geography is considered a sandstone mountain. During the wet season, there are many streams flowing down from mountain that form five waterfalls [4]. However, during the dry season, these streams and waterfalls are mostly dry. These extreme conditions form the habitat of some very interesting macroalgae.

Green freshwater macroalgae have been reported in various parts of Thailand and they are widely used for purposes such as traditional foods, supplementary foods and as medicinal products [5-6]. However, their taxonomy has not been reported. Only some specimens have been identified on the basis of specific morphology since reference data is inadequate [7-17].

Therefore, this study aimed to investigate the diversity of green macroalgae and describe their specific morphology and the characters of vegetative cells including the reproductive structures of each species. This report provides helpful data that will be useful in future research. Moreover, this is the first report of algae in Phu Wua Wildlife Sanctuary.

## 2. Materials and Experiments

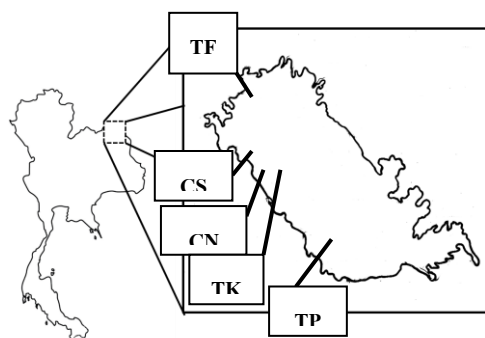
### 2.1 Study sites

Specimens were collected from five waterfalls, Ched Sii (18°09'37.37"N 103°56'56.44"E), Tham Phun (18°15'53.57"N 103°53'49.37"E), Cha Nan (18°13'30.2"N 103°53'32.9"E), Tad Nok Khian (18°09'53.43"N 103°54'14.00"E) and Tham Phra Waterfalls (18°08'13.68"N 103°59'32.81"E). All sampling sites were in Phu Wua Wildlife Sanctuary, Bung Khla District, Bueng Kan Province (Figure 1). The sample collection was done between June and October 2015.

### 2.2 Macroalgae collection and investigation

Green macroalgae samples were individually collected from free flowing water and substrates, i.e., rocks, cobblestones, and tree branches found immersed in the water and from the banks of the streams in the study area. All specimens were divided into two subsamples, fresh samples for identification and a second specimen that was preserved with 2.0% glutaraldehyde. Both subsamples were kept in plastic boxes at low

temperature (0-4°C) during transport. All specimens were imaged to record their characteristic filaments using an Olympus CX21 microscope. The width and the length of individual cells, branch patterns, the form of their chloroplasts and the size of their zygotes were recorded.



**Figure 1** The study sites in the area of Phu Wua Wildlife Sanctuary, TF: Tham Fhun Waterfall, CS: Ched Sii Waterfall, CN: Cha Nan Waterfall, TK: Tad Nok Khian Waterfall, and TP: Tham Phra Waterfall

### 3. Results and Discussion

Green macroalgae from Phu Wua Wildlife Sanctuary were collected and identified. Six genera, comprising eighteen taxa were discovered. The most diverse genus was *Spirogyra* which accounted for 39% of all taxa. The next most common were *Mougeotia* (28%) and *Oedogonium* (17%). A single species each of *Bulbochaete*, *Draparnaldia* and *Zygnema* were found which comprised 5% of material collected during this study. The sampling site that revealed the highest species diversity was Ched Sii

Waterfall, where nine species were found (Table 1). The descriptions of the discovered taxa are as follows.

*Bulbochaete quadrata* Wittrock ex Hirn 1900

Filaments consisted of rich of branches with non-wavy edges of vegetative cells. Each cell had a long hair with abulbous base. Chloroplasts were parietal and net-like. Cells were 14-25 µm wide and 54 µm long, 2.2-3.8 times as long as wide (Figure 2; 1).

*Bulbochaete* was reported in several parts of the world including Thailand. This taxon was found in the central and north part of the country [9], [24]. The current study is the first report of *Bulbochaete* in northeastern Thailand, particularly in Phu Wua Wildlife Sanctuary.

*Draparnaldia* Bory 1808 is well known for its uniseriate filaments with a soft mucilaginous envelope. Barrel-shaped or cylindrical cells are aligned along the main axes. Secondary branches are laterally placed on the main axes. *Draparnaldia* is classified into the order Chaetophorales, and family Chaetophoraceae [18], [22]. In this sanctuary, only one specimen of *Draparnaldia* was collected and identified as *D. mutabilis* (Roth) Cedergrén 1920. This taxon was reported in several parts of the world [18], [25]. However, in Thailand, this genus has rarely been reported. Our finding expands the biogeography of this rare taxon to Thailand. Moreover, we described the characteristics at the species level as follows.

*Draparnaldraia mutabilis* (Roth) Cedergrén

1920

The thalli were bright green and slippery with a mucilaginous envelope. The main axes consisted of cylindrical cells in which the width was greater than the length, 36-47  $\mu\text{m}$  wide and 16-20  $\mu\text{m}$  long. A single parietal chloroplast laid transversely along the main axes. The primary branches formed in an opposing manner. Cylindrical and barrel-shape cells were found in primary branches. The secondary branches were narrow and formed a lance-like outline. The cells of secondary branches were 5-7  $\mu\text{m}$  wide and 25-30  $\mu\text{m}$  long (Figure 2; 2).

Three *Oedogoniales* species were found, *Oedogonium calcareum* Cleve et Wittrock 1870, *O. cf. curtum* Wittrock et Lundell in Wittrock 1870, and *Oedogonium* sp.1. *Oedogonium calaceum* was found at two waterfalls, Cha Nan and Tad Nok Khian. *Oedogonium* sp.1 was also found at two waterfalls, Ched Sii and Tham Fhun. *Oedogonium cf. curtum* was found only at Tham Fhun Waterfall. However, *Oedogonium* was not found at Tham Phra Waterfall. The vegetative cells of *Oedogonium* sp.1 are cylindrical shape, 24-38  $\mu\text{m}$  wide and 42-175  $\mu\text{m}$  long. Sexual reproduction of *Oedogonium* sp.1 was not observed (Figure 2; 11). The characteristics of each taxa are as follows.

*Oedogonium cf. curtum* Wittrock et

Lundell in Wittrock 1870

Vegetative cells were distinctly swollen at the distal ends. There was a row of puncta at the end of cells without spiral orientation. Cells were 15-24  $\mu\text{m}$  wide and 78-91  $\mu\text{m}$  long (Figure 2; 10).

*Oedogonium calareum* Cleve et Wittrock

1870

Cylindrical cells were 11  $\mu\text{m}$  wide and 31  $\mu\text{m}$  long. Cells were not swollen at distinct ends. Oospores were depressed-spherical, 24  $\mu\text{m}$  wide and 20  $\mu\text{m}$  long. The spore walls were smooth. The suffultory cells were similar to adjacent vegetative cells (Figure 1; 9). *Oedogonium calareum* has been reported in Africa, Asia, New Zealand and various parts of Europe [26].

The genus *Oedogonium* was reported in various parts of Thailand. A single species of *Oedogonium* was reported at Mae Ngat Somboonchol Dam Reservoir [9], Pra Wiharn National Park [16], Huai Kha Khang Wildlife Sanctuary [7]. Additionally, six species of *Oedogonium* were reported in the Thong Pha Phum District of Southern Thailand [10], but their reported taxa were *Oedogonium* sp.1-6. Neither oospores nor any sexual reproduction details were described.

**Table 1** Distribution of green macroalgae in Phu Wua Wildlife Sanctuary

Taxa	Ched	Tham	Cha	Tad Nok	Tham
	Sii	Fhun	Nan	Khian	Phra
<i>Bulbochaete quadrata</i> Wittrock	-	-	+	+	-
<i>Draparnaldia mutabilis</i> (Roth) Cedergren	-	+	-	-	-
<i>Mougeotia parvula</i> Hassall	+	+	+	+	-
<i>Mougeotia laevis</i> (Kutzing)W Archer	-	-	-	-	+
<i>Mougeotia</i> sp.1	+	-	-	-	-
<i>Mougeotia</i> sp.2	-	-	+	-	-
<i>Mougeotia</i> sp.3	-	-	+	-	-
<i>Oedogonium calareum</i> Cleve et Wittrock	-	-	+	+	-
<i>Oedogonium</i> cf. <i>curtum</i>	-	+	-	-	-
<i>Oedogonium</i> sp.2	+	+	-	-	-
<i>Spirogyra dubia</i> Kutzing	-	+	+	+	-
<i>Spirogyra</i> cf. <i>inflata</i> (Vaucher) Kützing	+	+	+	-	+
<i>Spirogyra grossii</i> Schmittle	-	+	-	-	-
<i>Spirogyra schmidtii</i> West&West	+	-	-	-	-
<i>Spirogyra shantungensis</i> Li	+	-	-	+	-
<i>Spirogyrarhizobanchialis</i> Jao	+	-	-	-	-
<i>Spirogyra</i> sp.	+	-	-	-	-
<i>Zygnema ralfsii</i> (Hassall) de Bary	+	+	+	+	+

- : not found, + : found

The genus, *Mougeotia*, is uniseriate unbranched filaments with mucilaginous layers at the outer wall containing a single plate-like chloroplast with an axial row of pyrenoids. *Mougeotia* was classified into the order Zygnematales and family Zygnemaceae [18], [22]. From this study, five morphological types of *Mougeotia* were found, *Mougeotia laevis* (Kutzing) W. Archer 1867, *M. parvula* Hassall

1847 and *Mougeotia* sp. 1-3. Unfortunately, the sexual reproduction of *Mougeotia* sp.1-3 was not observed. However, these taxa revealed obviously differences in morphology, such as in their cell width, cell length and length/width ratio (Figure 2; 3-8 and Table 2).

*Mougeotia laevis*, with long cylindrical cells (8.9 L/W ratio), showed an oospore in the middle of a short conjugation tube. The oospores

of this taxon were ellipsoidal with shallow depressions or scrobiculate. Oospores of *M. parvula* were almost spherical and formed wholly in very short conjugation tubes (Figure 2; 8).

*Mougeotia* were cosmopolitan, mostly found at the margins of the small ponds and lakes [18]. In Thailand, this genus was reported (in 1979) in a rice field in the northeastern part of the country [27]. Additionally, *Mougeotia* were continuously reported in various parts of the country, such as Na Thap River, Songkhla Province [15], Khao Pra Wiharn National Park, Srisaket Province [16], the area of the Golden Jubilee Thong Pha Phum Project, Thong Pha Phum District, Kanchanaburi Province [10] and Huay Kha Khang Wildlife Sanctuary [7]. However, the details of *Mougeotia* were described only at the genus level. The sexual reproduction and oospore characters of this taxa in Thailand are ambiguous. In the present study, we described the sexual reproduction and oospore characteristics of *M. parvula* and *M. laevis*.

*Spirogyra* Link 1820 consists of unbranched long filaments, cylindrical cells with an outer mucilage and is the largest genus among Zygnemaceae. Their major characteristics are spiral chloroplasts and scalariform conjugation. In

this investigation, *Spirogyra* was the most diverse taxa, as seven species were found. *Spirogyra* cf. *inflata* (Vaucher) Kützing was a common species, found in four of the five waterfalls (Table 1). The next most common was *Spirogyra dubia* Kützing, which was found in three waterfalls. The highest species diversity (five species) of this genus was at the Ched Sii Waterfall. The description of each taxon is as follows.

#### *Spirogyra dubia* Kützing

Thallus were filamentous, unbranched and uniseriated. Cells were cylindrical, 43 µm wide and 286 µm long, with a 6.65 L/W ratio. There were three chloroplasts with some pyrenoids along the chloroplast bands. Each chloroplast made 2.5 turns in a cell. Sexual reproduction by conjugation forms as scalariform. Zygospores were ellipsoidal, 69 µm wide and 83 µm long, with a 1.2 L/W ratio (Figure 2; 13).

#### *Spirogyra* cf. *inflata* (Vaucher) Kützing 1843

Cells were cylindrical, 15-22 µm wide and 128-192 µm long, with a 5.8-12.8 L/W ratio. A single chloroplast with some pyrenoids made six turns in a cell. The end walls were ring-like or infolded (replicate). Sexual reproduction was not observed (Figure 2; 14.-15).

**Table 2** Characteristics of *Mougeotia* in Phu Wua Wildlife Sanctuary

Taxa	Vegetative cells			Oospores		
	Width ( $\mu\text{m}$ )	Length ( $\mu\text{m}$ )	L/W	Width ( $\mu\text{m}$ )	Length ( $\mu\text{m}$ )	L/W
<i>Mougeotia parvula</i> Hassall	11	123-151	11.1-13.7	29	31	1.1
<i>Mougeotia levis</i> (Kutzing)W Archer	20	117-178	5.8-8.9	33	47	1.42
<i>Mougeotia</i> sp.1	22	277	12.6	*	*	*
<i>Mougeotia</i> sp.2	30	225	7.5	*	*	*
<i>Mougeotia</i> sp.3	23	102-108	4.43-4.69	*	*	*

\* : not found

*Spirogyra grossii* Schmiedle

Cells were cylindrical, 45  $\mu\text{m}$  wide and 118  $\mu\text{m}$  long, with a 2.62 L/W ratio. There were three chloroplasts with globular pyrenoids along the chloroplast bands. Each chloroplast made 2.5-3 turns in a cell. Sexual reproduction by conjugation formed as scaliform. The conjugation tubes were formed by both gametangia, male and female gametes. Sterile cells were slightly swollen. Zygospores were ellipsoidal, 50  $\mu\text{m}$  wide and 70  $\mu\text{m}$  long, with a 1.4 L/W ratio (Figure 2; 16).

*Spirogyra schmidtii* West&West

Cells were cylindrical, 26  $\mu\text{m}$  wide and 173  $\mu\text{m}$  long, with a 6.65 L/W ratio. There were three chloroplasts with some pyrenoids along the chloroplast bands. Each chloroplast made 2-2.5 turns in a cell. Sexual reproduction by conjugation formed as scaliform. The conjugation tubes were formed by both male and female gametangia. The tubes were 16.7  $\mu\text{m}$  wide and 11  $\mu\text{m}$  long. Sterile cells were slightly swollen. Zygospores were long

ellipsoids, 34  $\mu\text{m}$  wide and 61  $\mu\text{m}$  long, with a 1.8 L/W ratio (Figure 2; 19-20).

*Spirogyra shantungensis* Li

Cells were cylindrical, 67  $\mu\text{m}$  wide and 125  $\mu\text{m}$  long, with a 1.87 L/W ratio. There were three chloroplasts with globular pyrenoids along the chloroplast bands. Each chloroplast made 2-2.5 turns in a cell. Sexual reproduction by conjugation formed as scaliform. The conjugation tubes were formed by both gametangia. Sterile cells were swollen and contained ellipsoid zygospores that were 51  $\mu\text{m}$  wide and 80  $\mu\text{m}$  long, with a 1.6 L/W ratio (Figure 2; 21-22).

*Spirogyra rhizobrachialis* Jao

Cells were 53  $\mu\text{m}$  wide and 225  $\mu\text{m}$  long with a 4.24 L/W ratio. There were five chloroplasts with small pyrenoids along the chloroplast bands. Each chloroplast made 2.5 turns in a cell. Sexual reproduction by conjugation formed as scaliform. The conjugation tubes were formed by both gametangia. Fertilized cells were cylindrical.

Zygospores were long-ellipsoid, 50  $\mu\text{m}$  wide and 100  $\mu\text{m}$  long, with a 2.0 L/W ratio. The attached part formed a rhizoid-like shape (Figure 2; 17-18).

*Spirogyra* sp.

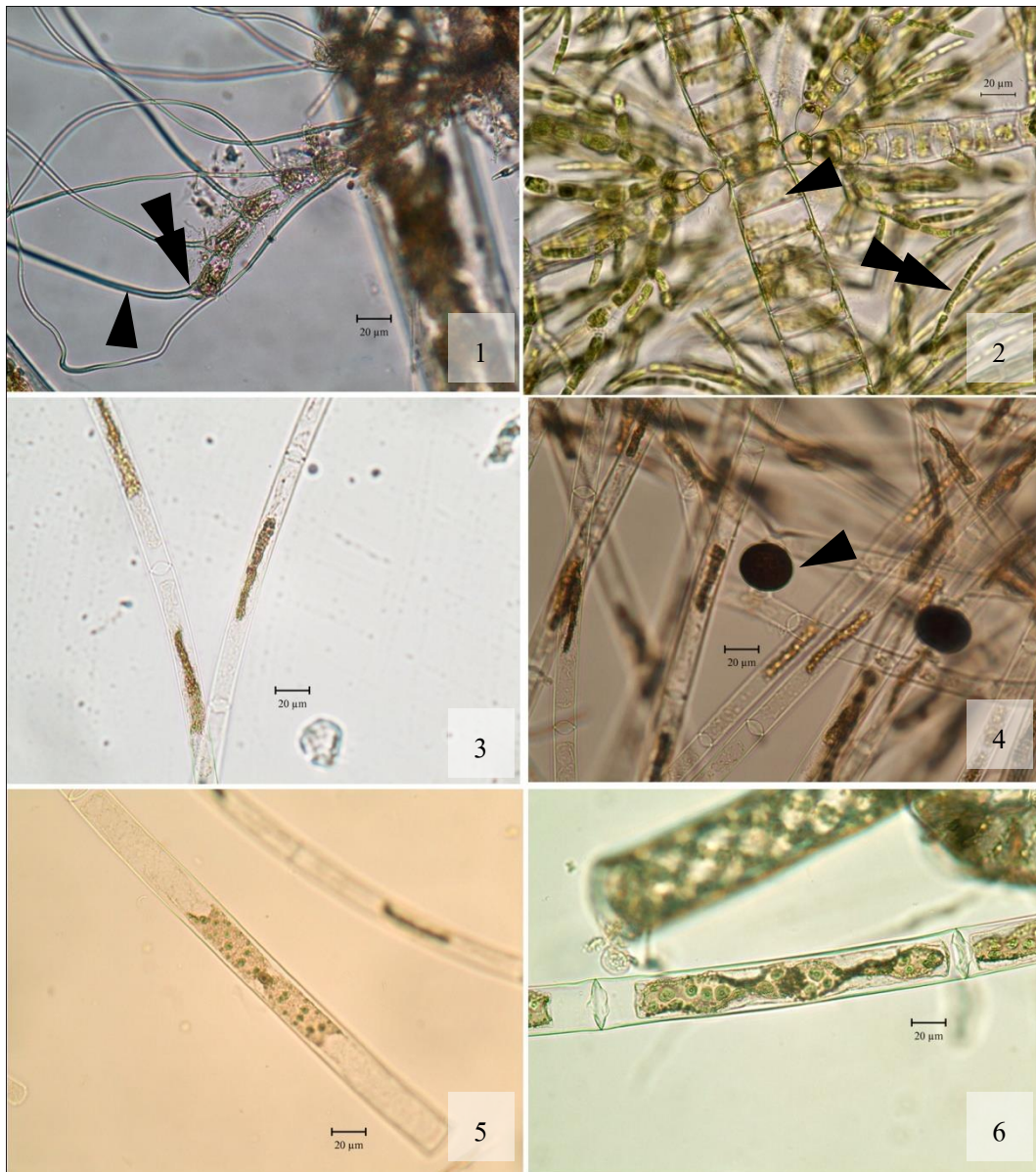
Cells were 54  $\mu\text{m}$  wide and 223  $\mu\text{m}$  long with a 4.13 L/W ratio. There were three chloroplasts with some pyrenoids along the chloroplast bands. Each chloroplast made 1.5-2 turns in a cell. Sexual reproduction by conjugation formed as scaliform. The conjugation tubes were formed by male gametangia. Male gametangia were flat, while the female gametangia were swollen. Fertilized cells were inflated. Zygospores were ellipsoidal, 46  $\mu\text{m}$  wide and 76  $\mu\text{m}$  long, with a 1.65 L/W ratio. The outer walls of zygospores were thick and clear (Figure 2; 23.-24). Although sexual reproduction characteristics were observed, there were no previous reports of this. Consequently, identification at the species level and molecular studies are still needed.

*Spirogyra* were studied and reported from various parts of the world. More than a hundred species were described in the USA since 1986 [28-30]. In the Netherlands, 82 species were discovered and characterised [31-32]. In Thailand, *Spirogyra* has been reported from various parts of the country, including Mae Cheam Stream, Chiang Mai Province, the area of the Golden Jubilee

Thong Pha Phum Project, Thong Pha Phum District, Kanchanaburi Province, the Ping and Nan Rivers, the Kham Watershed, Chiang Rai Province, Na Ku Ha Village, Phrae Province, the Kok River, Chiang Rai Province, the Huerng River, Leoi Province, Songkram River and Nakhon Phanom Province [8-12], [14]. However, all of these studies recorded data and described morphology only at the genus level. Additionally, Thiamdao [17] reported 11 species from various places around Thailand, seven of which were newly recorded in the country. However, the sexual reproduction characteristics were not clearly described. From our investigation, up to seven characteristics of both sexual reproduction and zygospores are described.

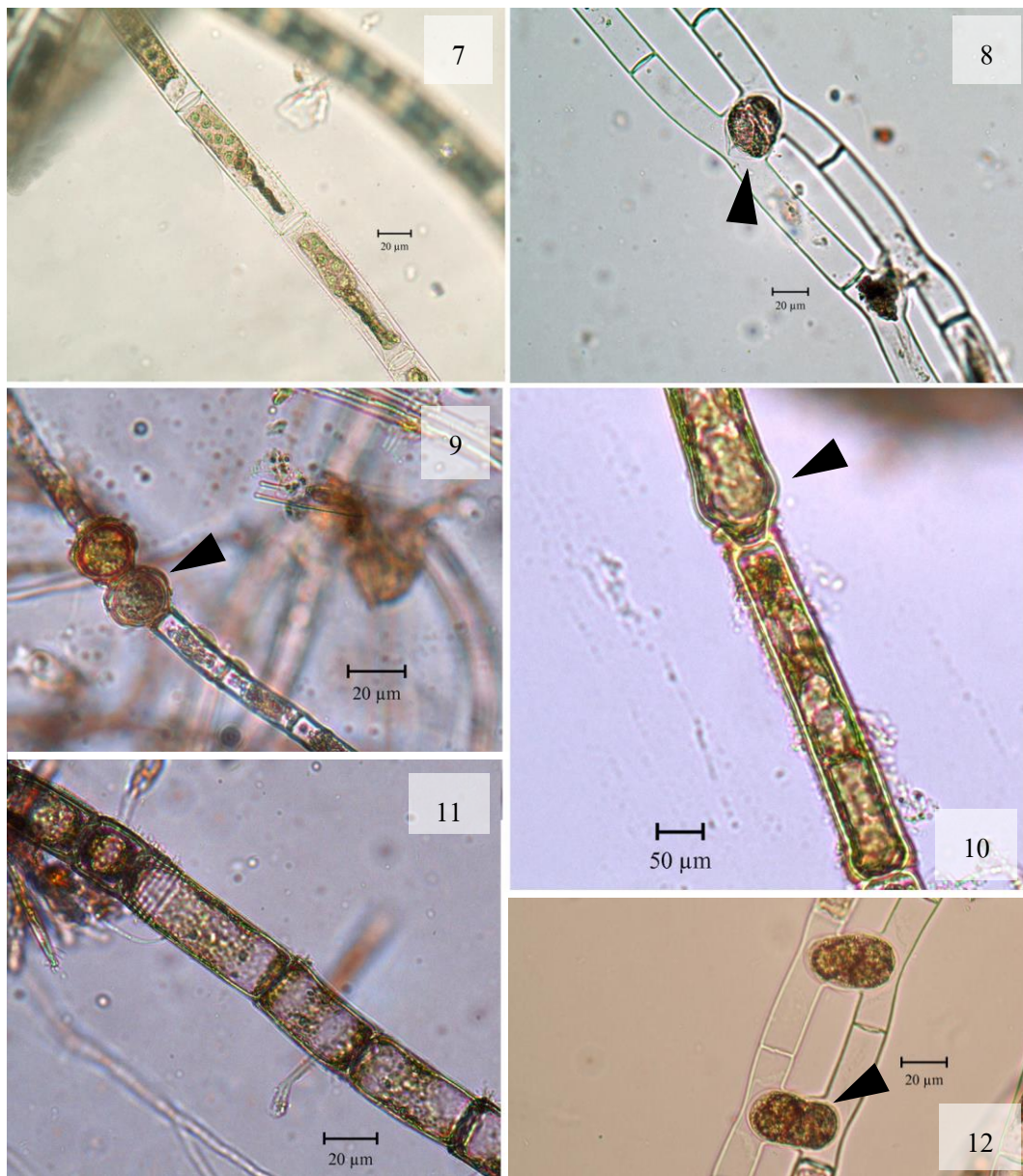
*Zygnema*, forms unbranched filaments, but occasionally reveals an attachment forming a rhizoid-like structure. Thalli are slimy, often surrounded by mucilage. Star-shape chloroplasts, 2 or up to 4, are contained in a cell. Conjugation tubes are developed by sexual reproduction in which the zygospores are developed in the conjugation tubes [18]. This genus is classified into the order Zygnematales and family Zygnemaceae [18], [22]. In our investigation, a single species of *Zygnema* was





**Figure 2** Macroalgae in Phu Wua Wildlife Sanctuary

1. *Bulbochaete quadrata*, long hair projected from each cell (arrowhead) with bulbous base (double arrowhead)
2. *Draparnaldrai mutabilis*, a single chloroplast laid transversely in main axes cells (arrowhead), secondary branches were narrow (double arrowhead)
- 3-4. *Mougeotia parvula*, zygospore formed in the conjugation tube (arrowhead)
5. *Mougeotia* sp.1
6. *Mougeotia* sp.2



**Figure 2** Macroalgae in Phu Wua Wildlife Sanctuary (continued)

7. *Mougeotia* sp.3

8. *Mougeotialevis*, ellipsoid oospore with shallow depressions laid into the middle of the conjugation tube (arrowhead)

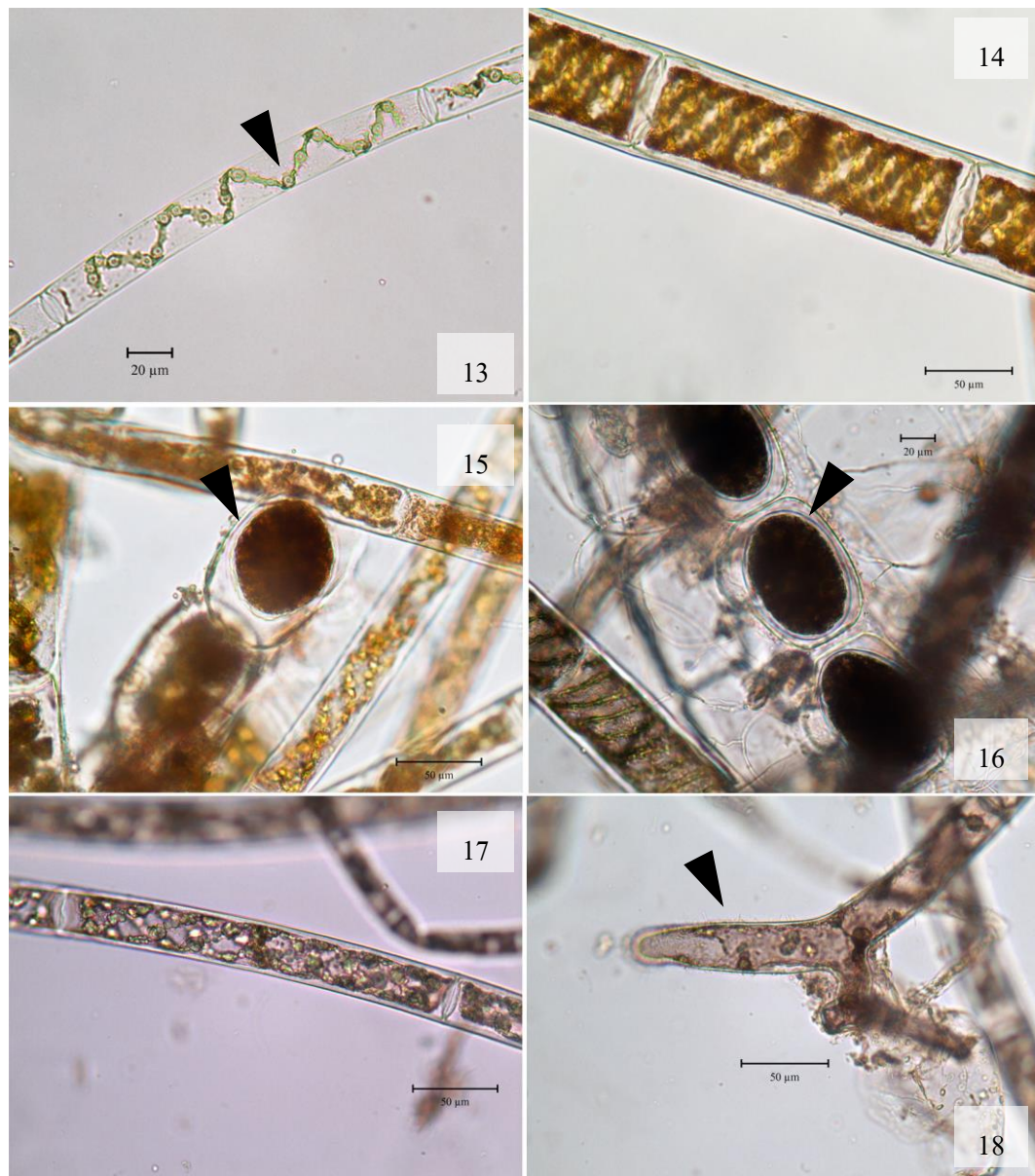
9. *Oedogonium calaceum*, depressed-spherical oospores (arrowhead)

10. *Oedogonium* cf. *curtum*, vegetative cells with distinctly swollen at the distal ends (arrowhead)

11. *Oedogonium* sp.1

12. *Zygnema ralfsii*, yellow-brown median spore wall formed in conjugation tubes (arrowhead)





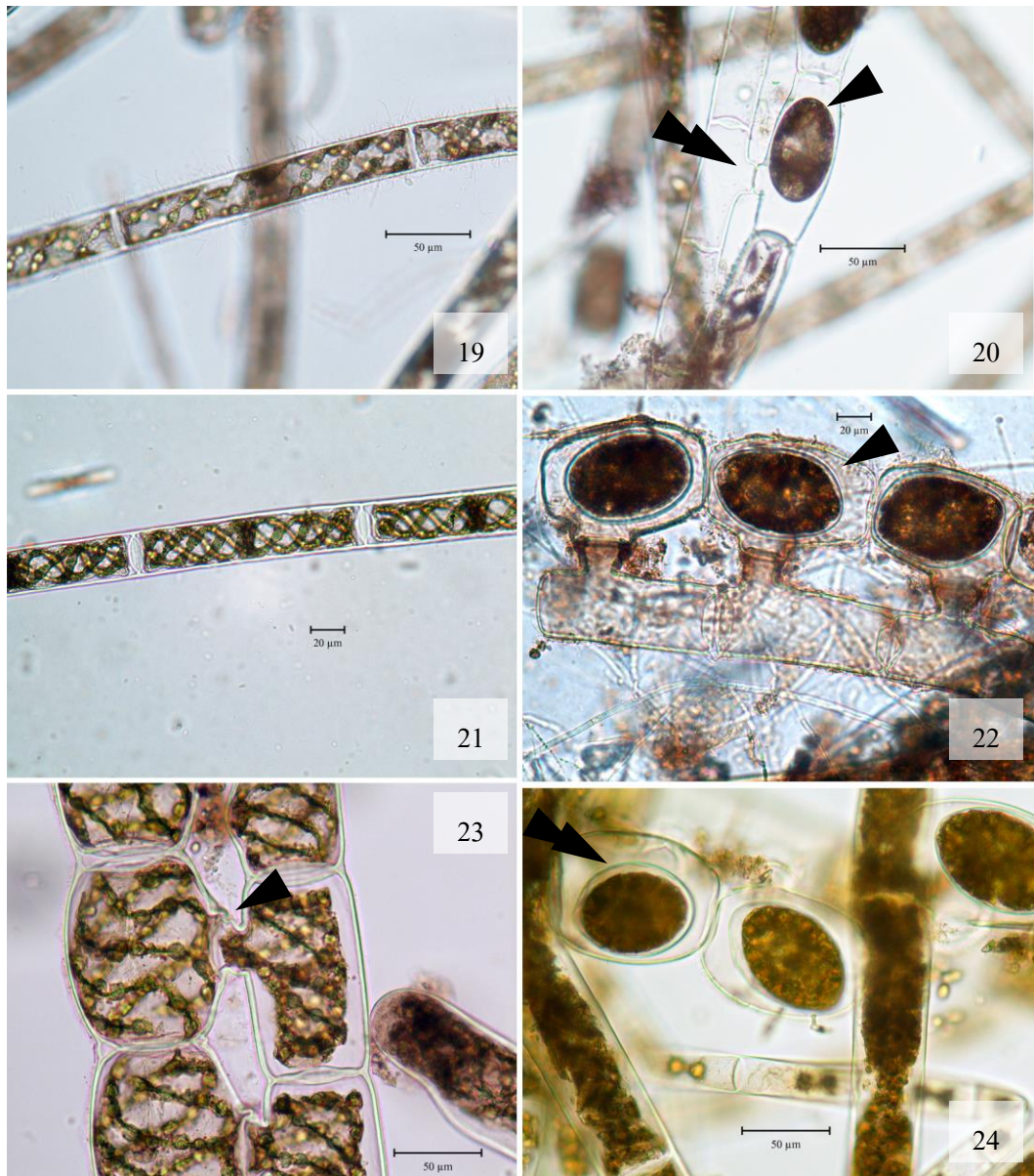
**Figure 2** Macroalgae in Phu Wua Wildlife Sanctuary (continued)

13. *Spirogyra* cf. *inflata*, the single chloroplast in a cell (arrowhead)

14-15. *Spirogyra dubia*, ellipsoid zygospore (arrowhead)

16. *Spirogyra grossii*, ellipsoid zygospore in slightly swollen cell (arrowhead)

17-18. *Spirogyra rhizobranhialis*, rhizoid-like shape of attached part (arrowhead)



**Figure 2** Macroalgae in Phu Wua Wildlife Sanctuary (continued)

- 19-20. *Spirogyra schmidtii*, slightly swollen sterile cells with long-ellipsoid zygospore (arrowhead), conjugation tubes formed by both gametangia (double arrowhead)
- 21-22. *Spirogyra shantungensis*, ellipsoid zygospores in swollen sterile cells (arrowhead)
- 23-24. *Spirogyra* sp., conjugation tubes formed by male gametangia (arrowhead), thick and clear outer wall of zygospores (double arrowhead)

found. Particularly, sexual reproduction was observed and identified as *Zygnema ralfsii* (Hassall) de Bary. This taxon is considered a common species and was found in all the waterfalls of the Phu Wua Wildlife Sanctuary.

#### *Zygnema ralfsii* (Hassall) de Bary

Vegetative cells were 17 µm wide and 55-75 µm long. There were 2 star-shape chloroplasts in a cell. The median spore wall was yellow-brown. Zygospores were formed in conjugation tubes. Nevertheless, in most of *Zygnema*'s species, the zygospores were formed in gametangia [18]. The zygospores of *Z. ralfsii* were 26 µm wide and 42 µm long (Figure 2; 12).

From previous reports, one morphotype of *Zygnema* was found in Pra Wiharn National Park [16]. Suphan and Peerapornpisal [10] reported three morphotypes of *Zygnema* from Thong Pha Phum District, in Southern Thailand. Although only one such species was found in the present study, its sexual reproduction, especially its zygospore characteristics, are described.

The characteristics of green macroalgae, mentioned above, were the first morphological descriptions from this region. Particularly, the sexual reproduction structures, which are the most important details for identification at the species level, were also shown. These results can be used as reference data for the future studies.

## 4. Conclusions

From our investigation, 18 species classified into six genera were found and described. *Spirogyra* was the most diverse genus, with seven species. *Mougeotia* and *Oedogonium* were found the next most common, with five and three species, respectively. A single species of *Bulbochaete*, *Draparnaldia* and *Zygnema* were each found. The most common species of this study, found in all sampling sites, was *Zygnema ralfsii* (Hassall) de Bary. *Draparnaldia mutabilis* (Roth) Cedergrén was considered a rare species since this genus was found and described infrequently, especially in Thailand.

Ched Sii Waterfall showed the highest diversity of macroalgae as nine species were found. Eight species were found in Tham Fhun Waterfall. Seven and six species were found at Cha Nan and Tad Nok Khian Waterfalls, respectively. At Tham Phra Waterfall, only three species of green macroalgae were observed.

Characteristics of each taxa described above were categorized into two groups, branched and unbranched filamentous macroalgae. The branched filamentous groups were *Bulbochaete* and *Draparnaldia*. *Bulbochaetes* having long hairs with a bulbous base. *Draparnaldia* were bright green and slippery. The thallus of this genus consisted of main, primary and secondary branches. In the unbranched filamentous group, the characteristics

of each genus revealed an obvious difference in the chloroplast pattern. A plate-like or twisted-plate chloroplast characterised *Mougeotia*. Algae with a spiral ribbon-like chloroplast were *Spirogyra*. Perforate net-like chloroplasts indicated the genus *Oedogonium*. Stellate or star-shape chloroplasts were characteristic of *Zygnema*.

Identification of some specimens were incomplete by the reason of their unobserved sexual reproduction. However, *Spirogyra* sp. was a specimen that showed a conjugation tube including a characteristic of zygospore. Unfortunately, the data of the current study did not match any previous descriptions. A molecular approach will be needed to clarify the morphological plasticity of green macroalgae in this tropical zone.

## 5. Acknowledgements

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