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Biodiversity of the epiphyllous algae in a *Chamaecyparis* forest of northern Taiwan

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ABSTRACT. Yellow cypress, *Chamaecyparis obtusa* var. *formosana*, is a relic species and one of the most important trees in Taiwan. This is the first study of the epiphyllous algae in a yellow cypress forest. The forest surrounding Yuan-Yang Lake (YYL) in northern Taiwan was chosen for this study. Algal samples collected from leaves and trunks were isolated and cultivated under laboratory conditions. The species were identified with the aid of 23S rDNA sequence analysis. Six species of green algae, including *Chloroidium saccharophilum*, *Ettlia pseudoalveolaris*, *Klebsormidium flaccidum*, *Prasiococcus calcarius*, *Rosenvingiella radicans* and *Trebouxia* sp., and one cyanobacterial species, *Leptolyngbya* sp., were identified in the forest. This is the first instance of any of them being reported in Taiwan. On yellow cypress leaves, four coccoid species, namely *C. saccharophilum, E. pseudoalveolaris, P. calcarius*, and *R. radicans* were found. On broadleaf trees, in contrast, both the filamentous species, *Leptolyngbya* sp. and *K. flaccidum*, and the coccoid species were identified. However, there is no strong host-specificity for these epiphyllous algae. The influence of environmental factors on the microhabitat, distribution and population size of epiphyllous algae in the forest is discussed here.

Keywords: Biodiversity; *Chamaecyparis*; Environmental factor; Epiphyllous algae; Yuan-Yang Lake; Yellow cypress forest.

INTRODUCTION

In forests, the leaf surface (phyllosphere) may be colonized by a range of different organisms, including lichens, liverworts, fungi, algae and bacteria (Wylie and Schlichting, 1973; Andreoli and Paoletti, 1993). Epiphyllous algae inhabit both leaves and trunks (Schlichting, 1975). Leaf surfaces provide the ecologically appropriate landscapes for the organisms that encounter or colonize the leaf surface. Due to their considerable diversity, the role that epiphylls play in ecosystem functioning varies widely. The epiphyllous microorganisms might not only act as parasites or symbiotes (Garcia-Guzman and Dirzo, 2004), they may also affect the nutrient cycle and the loss of water by evaporation linked to their host (Bentley and Carpenter, 1984; Fürnkranz et al., 2008).

The nucleic acid sequences of 5S, 16S, 18S or internal transcribed spacer (ITS) regions are commonly used in studies of microorganisms. Recently, sequence data of 23S rDNA become available for a few bacterial, cyanobacteria and algal species (Ludwig et al., 1995; Anthony et al., 2000; Sherwood and Presting 2007; Sherwood et al., 2008). This 23S rDNA sequence is a useful tool for establishing the taxonomy to genus level for taxonomically doubtful strains in environmental samples. The identification of microorganisms in the natural ecosystem is difficult. Many cases of misidentification of strains, inadequate culture conditions leading to malformation or loss of critical features, and growth intricacies all make it difficult to apply taxonomic principles to environmental samples (Burja et al., 2001). In recent years, molecular techniques have provided excellent complementary data to address the limitations of taxonomic, physiological and biochemical approaches in biodiversity assessment (Srivastava et al., 2007). Recently, Sherwood and Presting (2007) identified a set of "universal primers", about 450 bp in length, flanking Domain V of the 23S plastid rDNA gene fragment in eukaryotic algae and cyanobacteria. Later, Sherwood et al., (2008) demonstrated the amplification of the 23S rDNA sequences corresponding to algae; this illustrated the utility of the 23S rDNA sequence of a primer for analysis of environmental samples. This "universal" primer pair allows a more convenient and effective molecular analysis of algae, based on either cultured or environmental samples. In the present study, we employed 23S rDNA sequence analysis for the identification of species.

Yuan-Yang Lake (YYL) is a subalpine lake situated in a natural reserve area in northern Taiwan that has been virtually undisturbed by human activity for a long time (Chen and Wu, 1999). It is surrounded by a cloud belt forest of

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Taiwan yellow cypress (*Chamaecyparis obtusa* var. *formosana*), an important timber tree and a relic species. A long-term ecological research site was established to study this unique environment in 1992 by the government (Chang et al., 2002).

Most previous studies of the YYL site have concentrated on the floristic diversity of the community (Liao et al., 2003) or on the relation of changes in floristic composition to seasonality, soil conditions (Huang et al., 2005) and environmental factors (Wu et al., 2001). There have been no studies of epiphyllous algae and its role in the ecosystem. As a matter of fact, there is very little information available in the literature on the ubiquitous epiphyllous algae in the subtropical forest. The YYL is a unique site for such a study, because of its long-lasting high humidity, which is a particularly suitable environment for the growth of epiphyllous algae. The purpose of this study is to estimate the diversity of epiphytic algae grown on the leaves of the dominant vascular plants in the yellow cypress forest of the YYL area. It aims to understand the existing circumstances and the diversity of the epiphyllous algae on both coniferous and broadleaf trees.

MATERIALS AND METHODS

Study Site

The Yuan-Yang Lake Natural Reserve Area (YYL) is located in the Chi-lan Mountains of north-eastern Taiwan (24°35' N, 121°24' E) (Figure 1). It is a typical subtropical mountainous cloud forest, with an area of 374 ha and an altitude range of 1650-2432 m (Table 1) (Hwang et al., 1996; Chou et al., 2000). It is composed of a mixed coniferous-broadleaf forest that extends from the lakeside to the east slope of the Hsueh-shan Mountain Range. Within this forest, two major vegetation communities can be recognized: a coniferous-hardwood community, dominated by *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (Taiwan yellow cypress) and *Rhododendron formosanum* Heiml; and a swamp community characterised by *Miscanthus transmorrisonensis* Hayata, *Schoenoplectus mucronatus* (L.) Palla subsp. *robustus* (Miq.) T. Koyama and *Sparganium fallax* Graedner (Chou et al., 2000). In addition, the forest is characterised by plenty of epiphytic bryophytes, mainly mosses, representing the unique physiognomy of the ecosystem (Liu and Hsu, 1973).

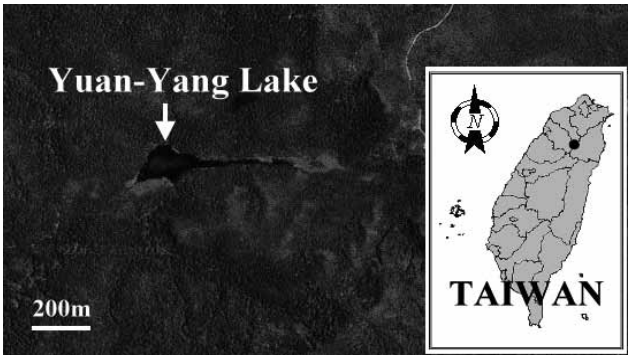

Figure 1. The map of Taiwan, showing the site of Yuan-Yang Lake area.

The annual air temperature at YYL ranges between 5 and 17.5°C, with an average of about 13°C. The annual rainfall is ca. 4,000 mm, but varies according to the number and strength of the summer typhoons. The forest is strongly influenced by the East Asia monsoon. The rainfall in summer is short but moderate to heavy, while in winter it is long-lasting but light. The relative humidity is usually higher than 80% (Hwang et al., 1996; Wu et al., 2001; Liao et al., 2003; Lai et al., 2006).

Sampling

The epiphyllous algae that grow on four of the dominant tree species in the cypress forest were sampled in April, June, August, and October in 2008 and 2009. Two species of coniferous trees, *Chamaecyparis obtusa* var. *formosana* and *Calocedrus formosana* (Florin) Florin, and two species of broadleaf trees *Eurya crenatifolia* (Yamam.) Kobus. and *Illicium philippinense* Merr were sampled. To avoid contamination from other algae populations that grow near the ground, a step ladder was used to sample the leaves from the upper part of the targeted trees. An appropriate amount of leaf pieces were cut, placed in sterile plastic boxes, and immediately stored in the portable icebox. They were transferred to the laboratory for further processing.

Isolation, cultures, and morphological observations

In the lab, a small piece of leaf with visible greenish or brown spots was excised under sterile conditions and cultured in both a liquid and an agar (1.5%) medium of NC (Kuhl, 1962) or BG11 (Stanier et al., 1971). The conditions were 25°C and 12:12 h light-dark cycle illuminated with cool-white fluorescent light (20 μmol m^{-2} s^{-1}). During incubation, the small colonies that grew on the agar were transferred to a liquid medium in 250 mL sterile flasks under the same conditions for further study.

Algae were examined under an AxioStar microscope (Zeiss, Germany) equipped with transmitted light, phase contrast and differential interference contrast (DIC, Nomarski) illumination and an AxioCam digital camera (Japan). The isolates were maintained in the Biodiversity Research Center, Academia Sinica, Taiwan. Previous studies such as those of Anagnostidis and Komárek (1988), John et al., (2002) and Rindi et al. (2004, 2007, 2008) were used for morphological identification.

DNA extraction and PCR amplification

The DNA of the algae was extracted using the phenol-chloroform protocol (Saunders, 1993). Amplification was carried out by means of PCR as described by Sherwood and Presting (2007), using PCR primers pair p23SrV_f1 (5' GGA CAG AAA GAC CCT ATG AA 3') and p23SrV_

r1 (5' TCA GCC TGT TAT CCC TAG AG 3'). The PCR amplification was performed in an Eppendorf Mastercycler gradient S thermal cycler (Eppendorf, Hamburg, Germany), using a 50 μL mixture solution consisting of 5.0 μL of 10X PCR buffer, 4.0 μL of 2.0 mM $MgCl_2$ (Promega), 3.0 μL of 1.0% BSA solution, 2.0 μL of each primer (0.4 mM), 2.0 μL (20 mM) of each dNTP, 0.2 μL of *Taq* polymerase (Promega), 26.0 μL of H_2O (bidist.), and 1.0 μL of genomic DNA. The cycling conditions for the p23SrV primers consisted of heating at 94°C for 2 minutes, followed by 35 cycles of 94°C for 20 s, 55°C for 30 s, 72°C for 30 s and a final extension time of 72°C for 10 minutes. The PCR products were visualized on 1% agarose gel stained with ethidium bromide (EtBr), further purified using the Qiagen PCR purification kit (Valencia, CA, USA).

Phylogenetic affiliations and morphotype identification

The PCR products were sequenced commercially in both directions. The forward and reverse-complementary of the reverse sequences obtained were aligned against each other to check the quality of the sequences. Ambiguous bases were checked and altered using the BioEdit program (Hall, 1999). Sequences were compared to known cultured and environmental sample sequences using the BLAST search tool (Altschul et al., 1997) on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov). The seven consensus sequences were then deposited at NCBI under the accession numbers: HQ404376 to HQ404382.

Phylogenetic analysis was performed using the Lasergene 7.1 software package. The sequences from this study were compared with close reference sequences obtained from the NCBI database by BLAST analysis. Sequences were automatically aligned using MEGA v5.0, and then corrected manually. Phylogenetic trees of reference sequences (>270 bp) were calculated by the neighbour-joining (NJ), the maximum likelihood (ML), and the maximum-parsimony (MP) methods (1000 bootstraps). The 23S rDNA sequences of *Fucus ceranoides* (GQ385189) were used as the out-group.

RESULTS

Biodiversity of epiphyllous algae in YYL area

Seven species of epiphyllous algae were identified on the leaves of the targeted trees. Of these, six were Chlorophyta and one was a cyanobacteria (Table 1). Morphologically, there were three filamentous species, namely *Leptolyngbya* sp. *Klebsormidium flaccidum,* and *Rosenvingiella radicans.* The other species were coccoid or oval, namely *Chloroidium saccharophilum*, *Ettlia pseudoalveolaris*, *Prasiococcus calcarius*, and *Trebouxia* sp. All of the coccoid species were small in size (less than 10 μm) (Table 1). Among these species, *E. pseudoalveolaris,* and *R. radicans* were the most common species and were found on every types of tree sampled.

Table 1. The species of epiphyllous algae revealed on the leaves of the studied coniferous trees (*Calocedrus formosana* and *Chamaecyparis obtusa* var. *formosana*) and broadleaf trees (*Eurya crenatifolia, Illicium philippinense,* and *Rhododendron formosanum.*) in YYL area.

Species	Cell width × length (μm)[a]	Accession numbers	Closest relative taxon (% identity)	Host plant[b]				
				Ca. formosana	*Ch. obtusa* var. *formosana*	*E. crenatifolia*	*I. philippinense*	*R. formosanum*
Cyanobacteria								
Leptolyngbya sp.	1.8±0.8×9.6±3.7	HQ404376	*L. boryana* PCC 6306 (93%)	-	-	+	–	-
Chlorophyta								
Chloroidium saccharophilum	3.8±1.5×4.3±0.8	HQ404377	*Chlorella saccharophila* strain 211-1d (99%)	+	+	+	–	+
Ettlia pseudoalveolaris	4.0±1.4×5.1±1.6	HQ404379	*Parietochloris pseudoalveolaris* (93%)	+	+	+	+	+
Klebsormidium flaccidum	8.9±2.6×16.1±6.8	HQ404378	*K. flaccidum* (99%)	-	-	+	+	+
Prasiococcus calcarius	12.6±4.9×11.9±3.8	HQ404380	*Pr. calcarius* isolate G00023 (95%)	+	-	+	+	+
Rosenvingiella radicans	9.6±3.6×17.8±5.9	HQ404381	*R. radicans* isolate G00005 (96%)	+	+	+	+	+
Trebouxia sp.	1.9±0.8×5.3±0.7	HQ404382	*T. australis* strain SAG 2205 (91%)	-	-	-	+	-

[a]Dimension expressed as mean ± SD. [b]+: Presence on the leaves of the host; -: Absence on the leaves of the host.

Some of isolates exhibited some degree of specificity to host plants. The filamentous species, *Leptolyngbya* sp. and *K. flaccidum*, only occurred on broadleaf trees (*E. crenatifolia* and *I. philippinense*), whereas other species displayed an extensive distribution on both the broadleaf and coniferous trees (Table 1). Nevertheless, a higher diversity of algae was found on broadleaf trees, than on coniferous species. On yellow cypress trees, three chlorophytes species, namely *C. saccharophilum*, *E.pseudoalveolaris*, and *R. radicans* were identified.

Phylogenetic analysis

The phylogenetic affiliation of each isolate was first assessed through the BLAST search, which related them to complete or partial 23S Domain V plastid rDNA sequence published in the Genbank. Seven OTUs (operational taxonomic unit) were found (Figure 2, Table 1), using a threshold of 97.5% identity (Taton et al., 2003). The closest affiliations for the DNA sequences obtained in this study varied between 99% to less than 91% (Table 1). For constructing phylogenetic tree, an aligned set of about 300 bp from the known sequences after elimination of sequence gaps was compared. A phylogram was constructed to provide a phylogenetic perspective for six studied epiphyllous algae in the YYL area with respect to the 17 relevant strains available in GenBank within 13 major genera in the division Chlorophyta. The NJ analysis provided a tree with similar topology as that obtained from the ML and MP analysis, with nearly all nodes supported at greater than 50% bootstrap values (1000 replicates). Excluding the prokaryote sequence of *Leptolyngbya* sp., 24 eukaryote sequences from the libraries were clustered together into 5 distinct lineages in a phylogenetic tree, supported by high bootstrap values (Figure 2).

Cluster I (Chlorococcaceae) contained coccoid species, including *Chlorella* (HM070293, GU939612), *Ettlia* (HQ404379) and *Trebouxia* (EU352795, EU725861, FJ687228, HQ404382) strains. The sequence identity be-

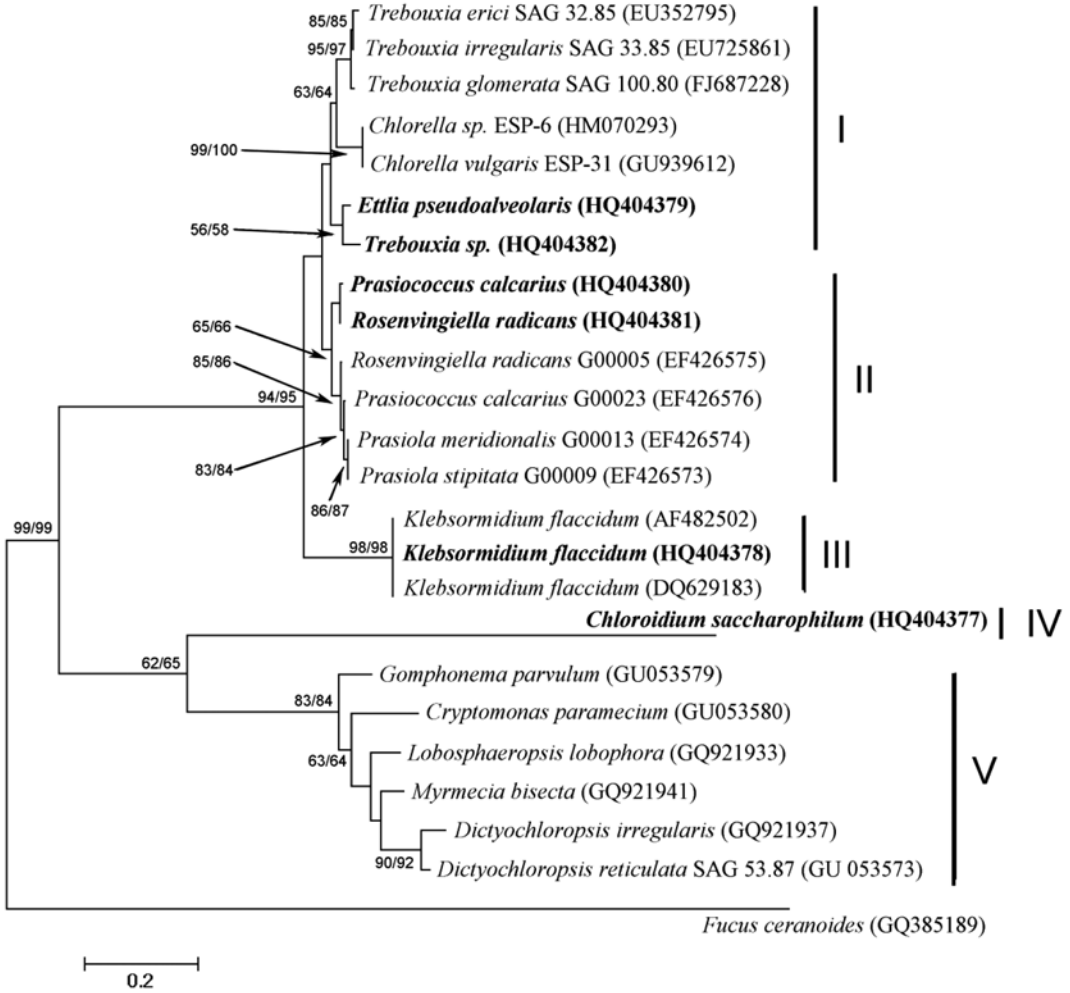

Figure 2. The Neighbour-Joining tree inferred from 23S rDNA sequences among the eukaryotic species of epiphyllous algae from the YYL area and their closest relatives. Sequences generated by the present study are indicated in bold. The *Fucus ceranoides* (GQ385189) sequence is designated as an out-group. GenBank accession numbers are in brackets. Numbers near nodes indicate bootstrap values (1000 replicates) superior or equal to 50% for neighbour-joining (NJ), and maximum-likelihood (ML) analyses. The scale marker represents 0.2 nucleotide substitution per sequence position.

tween the *Ettlia pseudoalveolaris* and *Trebouxia* sp. from the YYL area was about 96%, which did not fit in with the definition of an OTU. In another paraphyletic branch of the phylogenetic tree, the strains of *Trebouxia* and *Chlorella* belonged to two different groups. The sequence divergence of *Trebouxia* sp. (HQ404382) from other *Trebouxia* strains in Cluster I was 8-9% (cf. Figure 2).

The species in Cluster II (Prasiolaceae) have the potential to grow complicated thallus, such as *Prasiococcus* (HQ404380, EF426576), *Prasiola* (EF426573, EF426574), *Rosenvingiella* (HQ404381, EF426575) strains. The strains *Prasiococcus calcarius* and *Rosenvingiella radicans* of the present study exhibit high DNA sequence identity (99%). Notably, the strains of *Rosenvingiella* (EF426575), *Prasiococcus* (EF426576) and *Prasiola* (EF426573, EF426574) from USA were clustered into a group whose ecology was not disclosed (Sherwood and Presting, 2007). The pairwise identity of the strains from YYL with these strains ranged from 94% to 96%. However, the relationships among the strains of *Prasiococcus*, *Prasiola,* and *Rosenvingiella* are doubtful, because they would not form a well-supported monophyletic taxon (Figure 2).

Cluster III (Klebsormidiaceae) was a filamentous Chlorophyta, *Klebsormidium* cluster. The epiphyllous *Klebsormidium* taxa from YYL was well-fitted into this cluster (98%), including the type species *K. flaccidum* from terrestrial habitat (DQ629138, AF482502) (Turmel et al., 2002; Qiu et al., 2006). *Chloroidium saccharophilum* (HQ404377) deviated from the strain of *Chlorella* (Cluster I) and was located instead in Cluster IV, which had a sequence divergence of >10% from the strain of *Chlorella* (Figure 2).

Finally, some strains associated with other terrestrial algae downloaded from Genbank were clustered together in Cluster V, including Chlorophyta *Lobosphaeropsis* (GQ921933), *Myrmecia*, *Dictyochloropsis* (GQ921937, GU053573), Chrysophyta *Cryptomonas* (GU053580) and *Gomphonema* (GU053579) species. Those species contained in Cluster V obviously dissimilar to others in gene sequence with 99% supposed (Figure 2).

DISCUSSION

The epiphyllous flora in the yellow cypress forest is dominated by coccoid species belonging to Chlorophyta. This study identified six species of chlorophytes in the cypress forest, of which four are very common species on the trees' leaves. While two of three filamentous species occurred only on the broadleaf trees, the coccoid species inhabited both the broadleaf and coniferous trees. The highest diversity of epiphyllous algal species was found in the broadleaf tree *E. crenatifolia*.

The forest canopy of the YYL area is dominated by the relic yellow cypress, *C. obtusa* var. *formosana* and the understory by the shrubs *Rhododendron formosanum* and *I. phillippinense* (Chou et al., 2000). Basically, the leaf surfaces of these plants provide a microhabitat for all kinds of microbes. The tips, bases and edges of the leaves were usually the first site for colonization of microbes. As the age of the leaves increased, the complexity and density of the epiphyllous community increased and the structure of the community changed (Paterson and Wright, 1986). However, our study shows that cyanobacterium *Leptolyngbya* sp. and green alga *Trebouxia* sp. are only found on *Eurya crenatifolia* and *Illicium philippinense* trees, respectively, displaying a high host-specificity. In contrast, the coccoid species including *C. saccharophilum*, *R. radicans*, *P. calcarius,* and *E. pseudoalveolaris* are distributed on both broadleaf and coniferous trees (cf. Table 1). Apparently, they are weakly or not at all species-specific for host plants, as indicated by Monge-Najera and Blanco (1995). The reason why it exists such a difference in host-specificity for these species is still unclear. A further study is necessary.

Morphologically, four of the epiphyllous isolates are coccoid forms. The morphological identification of coccoid species is difficult because of the incomplete nature of existing trait descriptions (Richert et al., 2006). In addition, the morphology of these algae might change with environmental conditions. For such organisms, molecular information is necessary for accurate identification. For this, the reference sequences provided by the GenBank (NCBI) BLAST allow a comparison. However, there is still insufficient information available for epiphyllous or terrestrial algae. Thus, a recheck of the taxa from YYL is necessary when there are more data available in the future.

In the cluster analysis, the sequence divergence between *E. pseudoalveolaris* (HQ404379) and *Trebouxia* sp. (HQ404382) was 4%. They formed a moderately supported cluster (58% bootstrap support) and aligned with that of the *Trebouxia* (FJ687228, EU352795, EU725861) and the *Chlorella* (HM070293, GU939612) sequences cluster, but with a weak similarity (<50%) in Cluster I (Figure 2). Morphologically, these three genera are simple coccoid to oval-shaped, solitary and less than 10 μm diameter (Burja et al., 2001; Piercey-Normore, 2006). Due to shortage in distinct character, they are notorious for their taxonomic problems. Apparently, a revision of this group of Chlorococcaceae is required.

There was a similar situation in Cluster II; *Prasiococcus calcarius* (HQ404380) and *Rosenvingiella radicans* (HQ404381) formed a moderately supported cluster (66% bootstrap support), sister to that of the *Prasiococcus* (EF426576), *Rosenvingiella* (EF426575) and *Prasiola* (EF426573, EF426574) strains cluster (Figure 2). In fact, all the strains of *Prasiococcus*, *Prasiola,* and *Rosenvingiella* available in GenBank share a high DNA sequence similarity, ranging between 96% and 99%, and could be a "cosmopolitan OTU". Basically, these species belong to Trebouxiophyceae and are macroalgal genus (Neustupa, 1998; Rindi et al., 2004, 2007; Menéndez et al., 2006) with coccoid, filamentous, foliose thallus, and various transitional forms (Rindi et al., 1999). Intensive studies of

this group of algae by Rindi et al. (2004) suggest that there are still several unsolved problems in the systematics of these strains.

Genus *Klebsormidium* is a cosmopolitan green alga characterized by cells that have a parietal chloroplast with a single pyrenoid, asexual reproduction by biflagellate zoospores, and flagellar apparatus with unilateral construction (Silva et al., 1972). It is one of the most widespread taxa, distributed from polar to tropical regions and occurring in a wide range of terrestrial and freshwater habitats (Rindi et al., 2008). The specimens of *K. flaccidum* in our study (HQ404378) have a high similarity (99%) to those from Switzerland (AF482502) and the USA (DQ629183) (cf. Figure 2). Nevertheless, this species exhibits polymorphic features. The analysis of *rbcL* phylogeny shows that there was no consistency between the morpho-type and the molecular data (Rindi et al, 2008). On the basis of the taxonomic information available in the literature, most *Klebsormidium* strains could not be identified unambiguously at the species level, particularly the type species *K. flaccidum*.

A rather bizarre sequence was apparent in our phylogenetic analysis of *C. saccharophilum* (HQ404377) in Cluster IV (cf. Figure 2). Ellipsoidal *Chlorella*-like species without any special morphological features, such as spines or bristles, are very common in all kinds of aquatic and terrestrial habitats and have often been assigned as genus *Chlorella*. However, our *Chlorella*-like (namely *Chloroidium*, HQ404377) sequence was not grouped into the coccoid Cluster I, which includes the *Chlorella* strain (HM070293, GU939612). Darienko et al. (2010) investigated *Chlorella*-like strains using a polyphasic approach (i.e. morphology, reproduction, eco-physiology, and combined SSU and ITS rDNA sequences), and in their results transferred all *Chlorella*-like strains, such as *C. saccharophila* and *C. ellipsoidea* to the genus *Chloroidium*. Our data from the 23S rDNA sequence supports this rearrangement of *Chlorella* and *Chloroidium*.

The genus *Leptolyngbya* has been found in a variety of environments (Bellezza et al., 2003; Bruno et al., 2009;

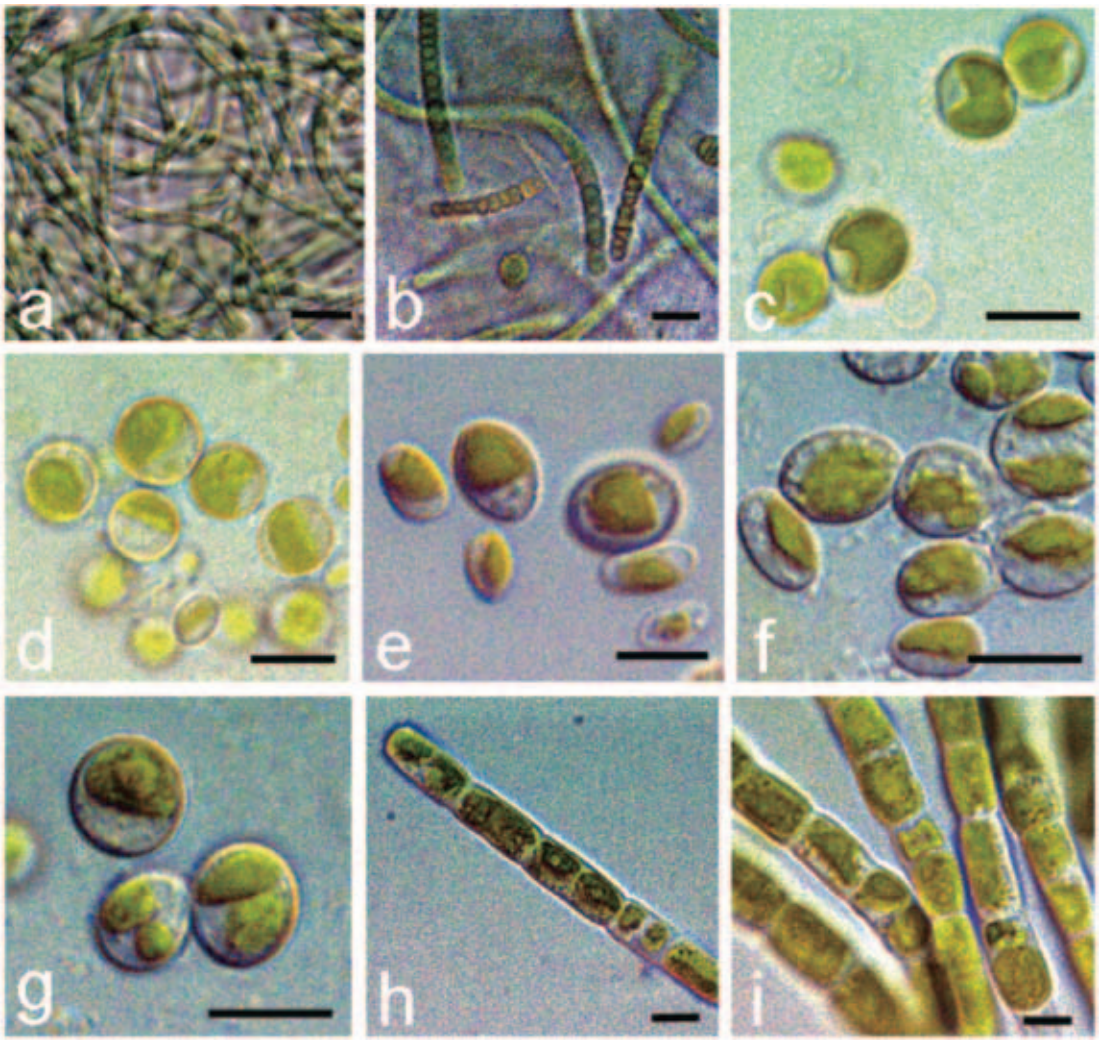

Figure 3. Light micrographs of the epiphyllous algae from the YYL area. a-b: *Leptolyngbya* sp.; c-d: *Chloroidium saccharophilum*; e-g: *Ettlia pseudoalveolaris*; h-i: *Klebsormidium flaccidum*. Scale bar = 5 μm.

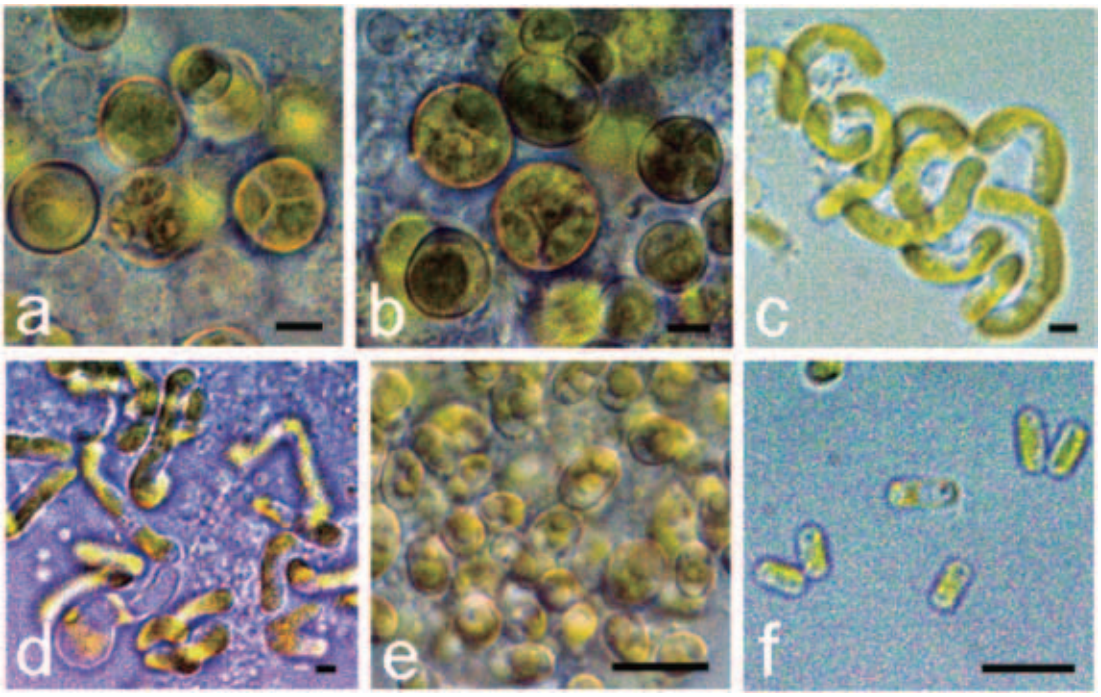

Figure 4. Light micrographs of the epiphyllous algae from the YYL area. a-b: *Prasiococcus calcarius*; c-d: *Rosenvingiella radicans*; e-f: *Trebouxia* sp. Scale bar = 5 μm.

Moro et al., 2010), particularly extreme environments. The features of *Leptolyngbya sp.* in YYL are morphologically similar to those described in a variety of aquatic and sub-aerial environments (Albertano and Kovacik, 1994; Komárek and Anagnostidis, 2005; Li and Brand, 2007). The phenotypic simplicity of this genus makes identification difficult (Komárek, 2003; Bruno et al., 2009). Thus, it belongs to the most taxonomically problematic and poorly defined genus (McGregor and Rasmussen, 2008). To clarify this genus, further study of samples from various environments is necessary.

Sonnleitner et al. (2009) mentioned that undisturbed forest canopies and their control of the microclimate may be essential for the development of epiphyll communities. In addition, factors such as micro-habitat humidity, light and nutrients transported by rainwater, animals and falling dust were believed to contribute to the possible colonization of epiphyllous algae (Monge-Najera and Blanco, 1995). Nevertheless, Freiberg (1999) suggested that within the understory of the forest, the relative abundance of epiphyllous algae was more influenced by air humidity than by light. The same case is observed for yellow cypress forest at YYL. The YYL nature preserve is situated in the cloud belt regions where the relative humidity is high throughout the year (always >80%), annual precipitation ranges between 2500-4500 mm, and low irradiation because as many as ca. 94% days per year is foggy (Wu et al., 2001; Lai et al., 2006). Fog deposition has been recognized as an important component of the hydrological and nutrient cycles in the ecosystem (Chang et al., 2002). All of these data suggest that the humid condition of the cypress forest at YYL is favourable for the development of epiphyllous algae.

The yellow cypress forest in the YYL nature preserve is characterized by a plenty of epiphytes such as lichens, liverworts and mosses in the understory. These plants, along with acid rainfalls, have contributed a part to the acidity of stem-flow (as low as pH 3.5~4.0) and the epiphyllous environment (Wu et al., 2001; Chang et al., 2002). Obviously, this might be the reason why acid-tolerant organisms such as *Leptolynbgya* and *C. saccharophilum* become dominant in the epiphyllous flora, as the cases indicated by Gehl and Colman (1985) and John et al. (2002). It is presumed that variation in the acidity of stems and epiphyllous microenvironment has served as a selection factor for the development of epiphyllous flora.

CONCLUSION

The results of the present study of epiphyllous algae in the YYL area support the polyphasic approach to microbial ecology, which combines culture, morphology and 23S rDNA phylogenetic characterizations. The epiphyllous algal flora in the YYL yellow cypress forest is dominated by green algae. On yellow cypress leaves, three species of green algae were identified, although seven species were found in the forest. The epiphyllous algae exhibit some degrees of host-specificity, depending on the species of algae. This study is the first time the epiphyllous algal species have been reported in Taiwan.

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Appendix. Morphology of epiphyllous algae.

(1) ***Leptolyngbya*** sp.
Cells are filamentous, long, flexuous, arcuated, waved or intensely coiled into cluster and mats, enveloping in thin, firm and colourless sheaths opened at the apical end. Filament is immotile, and usually slightly attenuated to the ends or not, with rounded or conical apical cells, usually constricted at the cross walls, and very rarely false branching. Cells is pale blue-green or olive-green cylindrical-shaped, 0.5-3 μm wide, length is longer than wide up to several times, rarely with prominent granules, without heterocytes and akinetes (Figure 3a-b).

(2) ***Chloroidium saccharophilum*** (W. Krüger) Darienko, Gustavs, Mudimu, Menendez, Schumann, Karsten, Friedl & Proschold 2010.
Basionym: *Chlorothecium saccharophilum* W. Krüger 1906.
Synonym: *Chlorella saccharophila* (Krüger) Migula 1907; *Chloroidium kruegeri* Nadson 1906; *Jaagia aquatica* Vischer 1955; *Kruegera saccharophila* (Krüger) Heering 1906; *Palmellococcus saccharophilus* (W. Krüger) R. H. Chodat 1909; *Pseudochlorella aquatica* (Vischer) Vischer 1955.
Description: Cell is unicellular, with cylindrical, ellipsoidal to ovoid in shape, and broadly rounded apices. Cell is 2-6 μm wide, 3-7 μm long. Chloroplast is trough-, cup-, or band-shaped, and filling about/over one half of cell. Pyrenoid is indistinctly, without starch sheath (Figure 3c-d).

(3) ***Ettlia pseudoalveolaris*** (T. R. Deason & H. C. Bold) J. Komárek 1989.
Basionym: *Neochloris pseudoalveolaris* Deason & Bold.
Synonyms: *Parietochloris pseudoalveolaris* (T. R. Deason & H. C. Bold) S. Watanabe & G. L. Floyd 1991.
Description: Cell is solitary, coccoid to oval-shaped, 3-8 μm long and 2-6 μm wide, with smooth and colorless cell wall. Chloroplast is cup- or plate-shaped, 1 or 2, and locating at the middle or side of the cell, with an indistinct pyrenoid (Figure 3e-g).

(4) ***Klebsormidium flaccidum*** (Kützing) P. C. Silva, K. R. Mattox & W. H. Blackwell 1972.
Basionym: *Ulothrix flaccida* Kützing 1849.
Synonym: *Chlorhormidium flaccidum* (Kützing) Fott 1960; *Hormidium flaccidum* (Kützing) A. Braun 1876; *Hormiscia flaccida* (Kützing) Lagerheim 1888; *Hormidium gaditanum* González-Guerrero 1946; *Hormococcus flaccidus* (Kützing) R. Chodat 1902; *Stichococcus flaccidus* (Kützing) Gay 1891.
Description: Cells are filaments, long, >150 cells, usually bent or twisted, easily dissociating with age, especially when cultured, and devoid of H-shaped pieces, biseriate parts, and false branches. Cell is thin- and smooth-walled cylindrical to barrel-shaped, or somewhat laterally swelled, 6-11 μm wide, 8-25 μm long, slight constrictions between adjacent cells. A plate-like chloroplast, often kidney-shaped in side view, is smooth margin encircling for 2/3 to the whole length of the cell, and covering about a half of the lateral wall, with a pyrenoid (Figure 3h-i).

(5) ***Prasiococcus calcarius*** (J. B. Petersen) Vischer 1953.
Synonym: *Pleurococcus calcarius* J. B. Petersen 1915.
Description: Cells are both as solitary cells and irregular colonies possibly containing hundreds of cells. Cell is 7-18 μm in diameter, with smooth wall and not stratified, but the younger ones often confluent with the older. A large chloroplast is stellate or multi-plates in shape, with a central pyrenoid. Some large cells contained a number of non-motile, oval or elliptical endospores, 0.5-1μm in diameter (Figure 4a-b).

(6) ***Rosenvingiella radicans*** (Kützing) Rindi, L. McIvor & Guiry 2004
Basionym: *Ulothrix radicans* Kützing 1849.
Synonym: *Lyngbya muralis* (Dillwyn) C. Agardh 1824; *Hormidium murale* (Dillwyn) Kützing 1845; *Oscillatoria muralis* (Dillwyn) C. Agardh 1812; *Prasiola crispa* f. *submarina* Wille 1901; *Prasiola crispa* subsp. *marina* Børgesen 1902; *Rhizoclonium murale* (Dillwyn) Kützing 1843.
Description: Cells are filaments, uniseriate, unbranched, usually bent or twist, however, adult specimens consist of a limited system of uniseriate prostrate axes, from which pluriseriate erect axes are produced. Filament is 7-17 μm in diameter, 3 to 5 times as wide as long, of 4- or more-celled packets, attached at intervals by pairwise rhizoids, or forming a cylindrical parenchmatous structure and sometimes constricted. Chloroplast is star-shaped in the middle of the cell, with 1 pyrenoid. Asexual reproduction is by non-motile spores. Sexual reproduction is by oogonia and biflagellate male gametes (Figure 4c-d). In culturing, *R. radicans* usually keep in the early stages of development consisting of a uniseriate filament, which initially attached by an individual rhizoid from the original germling.

(7)***Trebouxia*** sp.
Description: Cell is solitary, coccoid, oval, elongate-triangular to tetrahedral, with thin and colorless wall, and sometimes enclosed within a mucilaginous envelope. Cell is 3-8 μm long, 1-3 μm wide. 1-2 chloroplast is spreading cell surface, with 2 distinctly pyrenoids (Figure 4e-f).

北台灣一檜木林之葉附生藻的多樣性

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檜木為台灣珍有林木，本研究首次研究位於鴛鴦湖檜木林之葉附生藻，從 2008-2009 年在林內採集樹葉和樹幹上之藻類樣本，經形態鑑定和分離培養，並以 23S rDNA 分析以鑑定藻種。結果發現，此森林中共計有 *Chloroidium saccharophilum, Ettlia pseudoalveolaris, Klebsormidium flaccidum, Prasiococcus calcarius, Rosenvingiella radicans* 和 *Trebouxia* sp. 等六種綠藻及一種藍綠菌 *Leptolyngbya* sp.。在檜木葉上可發現 *C. saccharophilum, E. pseudoalveolaris*, *P. calcarius* 和 *R. radicans* 等四種綠藻。這些葉附生藻種均為首次在台灣被報導。在形態上，葉附生藻有五種為球狀，二種為絲狀，它們對所附生之植物的專一性程度不一。本文並探討濕度、酸性和光度等環境因子對這些葉附生藻的分布和族群大小之影響情形。

關鍵詞：鴛鴦湖；檜木林；葉附生藻；生物多樣性；環境因子。