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REGULAR PAPER

Diversity of subaerial algae and cyanobacteria growing on bark and wood in the lowland tropical forests of Singapore

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Background and aims – Knowledge on diversity and distribution of algae and cyanobacteria in subaerial habitats still lags behind those of freshwater and marine environments. Notably, data on diversity of microalgae in tropical corticolous habitats are still scarce. We investigated species composition of subaerial epixylic algae and cyanobacteria from two Singaporean rainforest localities. We asked whether there are differences in species composition and alpha-diversity of samples taken in different areas and in different habitat types (bark vs. decaying bare wood). In addition, we asked whether there are differences in species turnover (beta-diversity) among different habitat types and areas.
Methods – The cultivation-based approach and the microscopic analysis of populations were used. In total, 20 samples of bark and decaying wood from two forested areas were analyzed. Statistical analyses involved the non-metric multidimensional scaling (NMDS) of species data. Significance of differences in algal composition between groups of samples was evaluated by the non-parametric two-way ANOSIM (Analysis of Similarities) using the crossed design with permutations in blocks. The SIMPER method was used to identify species that characteristically discriminate between habitat types and sampling areas.
Key results – In total, 57 species were identified. Green algae (Trebouxiophyceae, Chlorophyceae, Trentepohliales) were dominant, and Cyanobacteria were the second most frequent group. The dominants of the subaerial assemblages differed from corresponding temperate habitats and, in addition, their alpha-diversity was considerably higher. Several green algal morphospecies were characteristic for the bark localities (e.g. *Dictyochloropsis* spp., *Pseudomarvania aerophytica*, *Printzina effusa* and *Printzina lagenifera*). The alpha-diversity was similar in both habitat types, but the species turnover among samples (beta-diversity) was significantly higher in the decaying wood samples.
Conclusions – Tropical corticolous habitats probably harbour higher diversity than corresponding temperate habitats. High beta-diversity of decaying wood illustrates general importance of this substrate for biodiversity of subaerial algae in the tropics.

Key words – Chlorophyceae, Cyanobacteria, Singapore, subaerial algae, Trebouxiophyceae.

INTRODUCTION

Knowledge on diversity and distribution of algae and cyanobacteria in subaerial habitats still lags considerably behind those of freshwater and marine environments. Hoffmann (1989) pointed to some obstacles complicating studies on algal species composition in terrestrial localities. Particularly, morphological simplicity of many aero-terrestrial microalgae, resulting from selection pressure for low surface-to-volume ratios of cells that leads to globular or elliptical cell shapes in many unrelated species, was mentioned. Consequently, morphology-based identification of terrestrial algal taxa requires considerable effort in distinguishing minute differences in structure and variation of form in populations that would be completely unidentifiable or even undetectable in the field material. However, there has still been considerable progress in knowledge on distribution and taxonomic structure of subaerial algal micro-communities in the past twenty years (Ettl & Gärtner 1995). Indeed, using the morphology-based methods individual morphotypes often cannot be identified into the species level, but still, they can be discerned and used as the operational taxonomic units (OTU's) in floristic accounts or in diversity assessment (e.g. Handa & Nakano

1988, Nakano et al. 1991, Freystein et al. 2008, Mikhailyuk 2008, Büdel et al. 2009).

The omnipresent algal growths in epiphytic subaerial microhabitats, i.e. tree bark, leaves or bare wood surface, contain characteristic and diverse assemblages consisting usually mainly of coccoid green algae and different cyanobacterial morphotypes (Hoffmann 1989, Ettl & Gärtner 1995). Major differences in species structure of these microhabitats between tropical and temperate ecosystems were already recognized by Printz (1939). The tropical algal epiphytic assemblages, macroscopically dominated mostly by conspicuous species of Trentepohliales, markedly differ from *Apatococcus*- and *Desmococcus*-dominated bark growths in temperate ecosystems (Brand & Stockmayer 1925). Therefore, most floristic and taxonomic studies on tropical subaerial algae were concentrated on Trentepohliales (e.g. Hariot 1889, Thompson & Wujek 1997, Neustupa 2003, Rindi et al. 2006a, Rindi & López-Bautista 2008). On the other hand, data on distribution and diversity of other algal groups from tropical subaerial habitats are still extremely scarce. Several recent taxonomic studies reported new species of microscopic green algae from the bark-inhabiting tropical assemblages (Neustupa & Šejnohová 2003, Rindi et al. 2006b, Neustupa et al. 2007). Neustupa & Škaloud (2008) reported species richness dynamics of bark algae and cyanobacteria in rainforest mountainous habitats of South-East Asia. They noted higher species richness in the bark samples (on average 4.8. to 7.8 species in a bark sample taken from a single tree) in comparison with similar studies conducted in temperate or subtropical ecosystems – about 1.7 to 5.9 species in the single bark sample (Cox & Hightower 1972, Handa & Nakano 1988, Nakano et al. 1991, Mikhailyuk et al. 2001). In addition, the effect of habitat type on species composition was detected, as the samples from the closed forest undergrowth differed considerably from the samples taken from trees growing in the synanthropic habitat. On the other hand, they did not detect effects of a host tree species or bark roughness on algal species composition. These results indicate that the microhabitat conditions, especially humidity and illumination, may play a crucial role in determining diversity and species composition of tropical bark-growing algal assemblages. Apart from Trentepohliales that dominated primarily in open space samples, the other frequent taxa belonged to the genera *Pseudococcomyxa*, *Dictyochloropsis* and *Nostoc*. The sarcinoid *Apatococcus*-like green algae that very often dominate temperate bark-growing algal assemblages (Ettl & Gärtner 1995) were missing from tropical samples studied by Neustupa & Škaloud (2008). Almost 43% of morphotypes were only present in a single sample and just two species (*Pseudococcomyxa simplex* and *Dictyochloropsis sp.*) occurred in more than 50% of samples. This indicates possibly much higher overall diversity, and (in notable difference from temperate ecosystems – see e.g. Mikhailyuk 1999) high variation of species composition among samples, with few "core species" inhabiting most of the available micro-localities. Thus, tropical ecosystems may possibly harbor an important part of the global pool of bark-inhabiting species of algae and cyanobacteria. Therefore, knowledge on their taxonomic composition and their distribution within the tropical forest habitats is of special interest, particularly in the context of recent worldwide deforestation of tropical ecosystems (Wright 2005).

In this study, we investigated species composition and diversity from twenty samples of bark- and wood-growing microalgal assemblages from lowland rainforest habitats of Singapore. Two forested areas with the area of about 1 ha were sampled and ten samples from a bark of living trees and ten samples of algal assemblages growing on the bare decaying tree wood were analyzed. Primarily, we posed the following questions:

1) What are the dominants of subaerial assemblages and does their species composition differ from the tropical mountainous bark samples reported in the previous study of Neustupa & Škaloud (2008)?

2) Are there differences in species composition and alpha-diversity of samples taken in different areas (c. 10 km apart; old-growth forest vs. secondary forest) and of samples taken from the different habitat types (bark vs. bare wood)? Which species eventually characterize these habitat types?

3) What are the beta-diversity indices (indicating change in species composition) between different habitat types and areas?

The species data were acquired using cultivation and morphology-based identification of strains. Regarding high number of probably undescribed species and confusing state of taxonomy in many terrestrial algae (Ettl & Gärtner 1995), we did not attempt to identify all the morphotypes. Rather, we concentrated on their documentation and delimitating their occurrence in individual samples, in order to acquire the taxa-in-samples dataset for the diversity assessment.

MATERIAL AND METHODS

The samples were collected in two forested areas of about 1 ha in Singapore:

1) Bukit Timah Nature Reserve (geographical coordinates 1°21'11''N and 103°46'42''E; altitude 100–120 m a.s.l.), collected on 25 Jan. 2008. The sampling area was located in the old-growth dipterocarp rainforest stand with shaded conditions in the undergrowth provided by multiple stratified tree canopies. The samples from this area were designated as TB – bark samples, TW – wood samples.

2) Central Catchment Nature Reserve, MacRitchie Reservoir (geographical coordinates 1°21'27''N and 103°48'32''E; altitude 50–58 meters a.s.l.), collected on 28 Jan. 2008. This sampling area was located at the eastern part of the reserve in about forty to fifty years old secondary forest stand. The samples from this area were designated as MB – bark samples, MW – wood samples.

In total, twenty samples were analyzed. The bark of ten randomly chosen trees (five in each sampling area) with a trunk diameter of more than 30 cm was sampled for surface microbial growths at the height of 120–140 cm above the soil level. Each sample from an investigated tree was taken by sterile dissector as a composite sample of bark, all around the trunk perimeter. In addition, the microbial growths covering the upper surface of ten decaying bare woods lying on the forest floor were collected (five in each sampling area).

The samples were incubated on agar plates in laboratory at Prague within 48 hours after the collections had been made in Singapore. They were homogenized by mixing the bark samples with glass microspheres (diameter 0.5 mm) in sterile liquid medium in Eppendorf tubes. Subsequently, they were cultivated on BBM (Bischoff & Bold 1963) agar me-

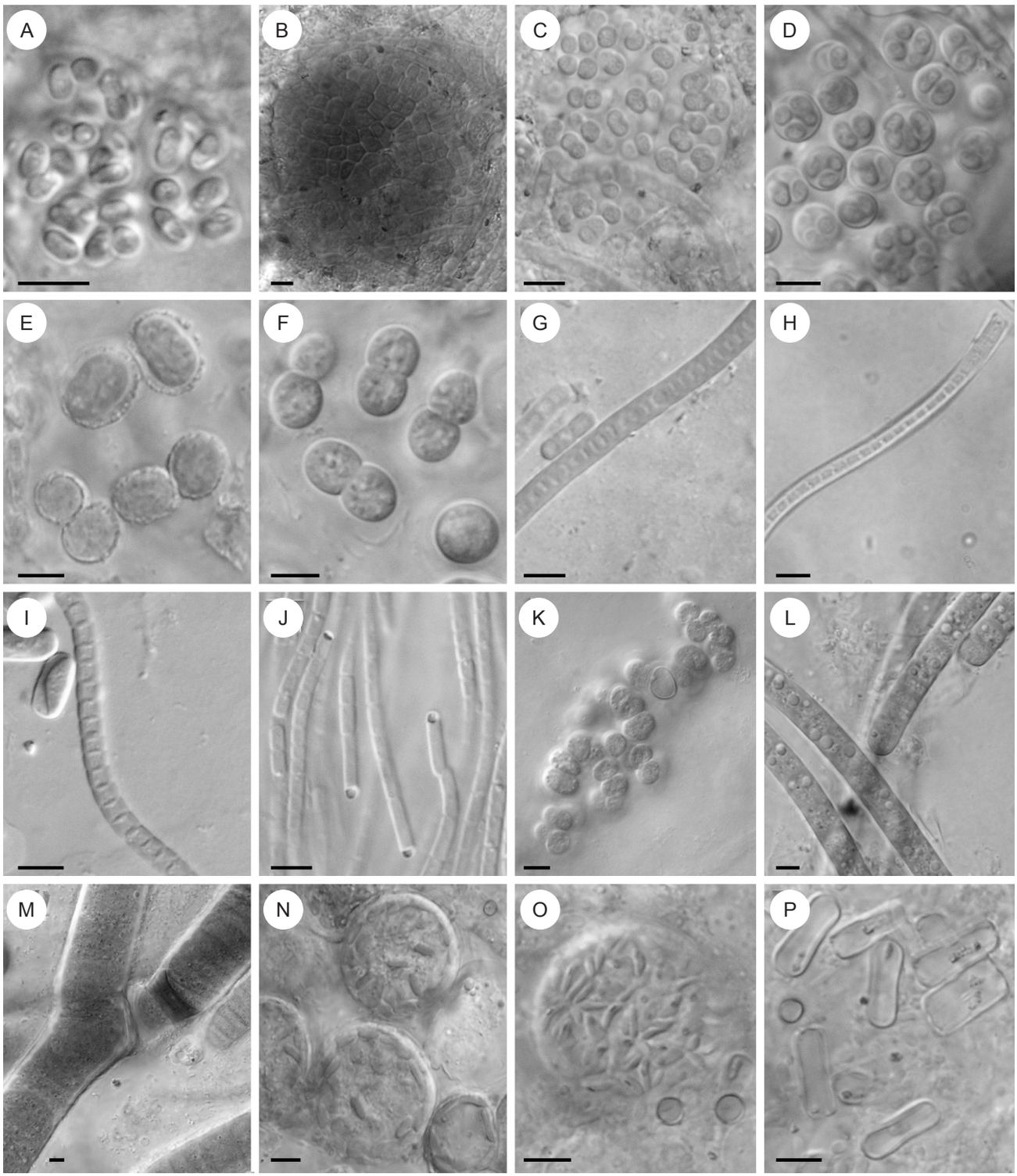

Figure 1 – A, *Aphanothaece* cf. *conglomerata*; B, *Cyanosarcina* sp.; C, *Gloeocapsa* sp.; D, *Gloeocapsopsis* sp.; E, F, cf. *Gloeothaece* sp.; G, *Leptolyngbya* cf. *cebennensis*; H, *Leptolyngbya* sp. 1; I, *Leptolyngbya* sp. 2; J, *Leptolyngbya* sp. 3; K, *Nostoc* sp.; L, *Phormidium* cf. *libidum*; M, *Scytonema* sp.; N, O, *Botrydiopsis* cf. *intercedens*; P, *Diadesmis* cf. *contenta*. Scale bars indicate 5 µm.

Table 1 – The list of species at the investigated localities. MB, Central Catchment Nat. Res., MacRitchie Reservoir, bark samples; MW, Central Catchment Nat. Res., MacRitchie Reservoir, wood samples; TB, Bukit Timah Nat. Res., bark samples; TW, Bukit Timah Nat. Res., wood samples.

	Fig.	MB1	MB2	MB3	MB4	MB5	MW1	MW2	MW3	MW4	MW5	TB1	TB2	TB3	TB4	TB5	TW1	TW2	TW3	TW4	TW5
CYANOBACTERIA																					
Aphanothaece cf. *conglomerata*	1a			1									1								
Cyanosarcina sp.	1b			1																	
Gloeocapsa sp.	1c			1																	
Gloeocapsopsis sp.	1d			1																	
cf. *Gloeothaece* sp.	1e, f			1																	
Leptolyngbya cf. *cebennensis*	1g			2																	
Leptolyngbya sp. 1	1h									3											
Leptolyngbya sp. 2	1i																	3			
Leptolyngbya sp. 3	1j														1			2	2		
Nostoc sp.	1k												1	1	1	1			3	1	
Phormidium cf. *libidum*	1l			2																	
Scytonema sp.	1m			1														2			
HETEROKONTOPHYTA																					
Botrydiopsis cf. *intercedens*	1n, o			1																	
Diadesmis cf. *contenta*	1p			1								1									
Eustigmatos cf. *vischeri*	2a			1																	
Eustigmatos sp.	2b									2											
Pleurogaster sp.	2c, d											1	2		1					1	2
CHLOROPHYTA																					
Apatococcus sp.	2e	1	2	1							1			1							
"*Avernensia*" sp.	2f	2	3		3	2	1	1				2			1		1			1	
Chlamydomonas sp.	2g																	1			
Chlorella cf. *angusto-ellipsoidea*	2h					3	1		1	2	1			2							
Chlorella cf. *luteoviridis*	2i	1						2	1									2			
Chlorella cf. *saccharophila* 1	2j										1										
Chlorella cf. *saccharophila* 2	2k									1											
Chlorella cf. *sphaerica*	2l	1			1	1															
Chlorella sp. 1	2m				1																
Chlorella sp. 2	2n						1														
Chlorella sp. 3	2o									2											
Chlorella sp. 4	2p			2																	
Chlorella sp. 5	3a											1	3	2	3		3		1	2	3
Chlorella sp. 6	3b													2							
cf. *Chlorella rugosa*	3c			1																	
Chlorolobion cf. *braunii*	3d												1								
Coenochloris sp. 1	3e	1																			
Coenochloris sp. 2	3f				1			2													
Dictyochloropsis irregularis	3g						1							2					1		
Dictyochloropsis cf. *symbiontica*	3h									1											
Dictyochloropsis sp.	3i, j	3	3	1	1	3			1			2	2	3	2		1		2	2	1
Elliptochloris cf. *subsphaerica*	3k, l		1					2	2		2										
Elliptochloris sp.	3m, n						3		1			2	2	3		3		2	2	1	
Klebsormidium cf. *nitens*	3o																	1			
Mychonastes homosphaera	4a																				1
Mychonastes sp.	4b		1																		
Myrmecia cf. *globosa*	4c							1													
Myrmecia cf. *irregularis*	4d													1							
Myrmecia sp.	4e					1															
Podohedra cf. *saltans*	4f		1								1										
Podohedra cf. *tropica*	4g							2													
Podohedra sp.	4h			1						1											
Printzina effusa	4i				2	2						2	2	3	2	2	1			2	2
Printzina lagenifera	4j											3	3	2						1	
Pseudococcomyxa sp.	4k						1	3	3				1						1		
Pseudomarvania aerophytica	3p	1		1			1					1	1	3	3	2					
Scenedesmus cf. *rubescens*	4l								1												
Spongiochloris cf. *spongiosa*	4m																	1			
Stichococcus sp.	4n										1	1	1	2	2	3	1	2		3	2
Watanabea cf. *reniformis*	4o,p										1										

dium at a temperature of 23 °C and an illumination of 40 µmol m^{-2} s^{-1} provided by 18W cool fluorescent tubes (Philips TLD 18W/33). Microphotographs were taken with the Olympus B×51 light microscope and the Olympus Z5060 digital equipment using Nomarski differential interference contrast optics. Quantities of individual species were estimated as

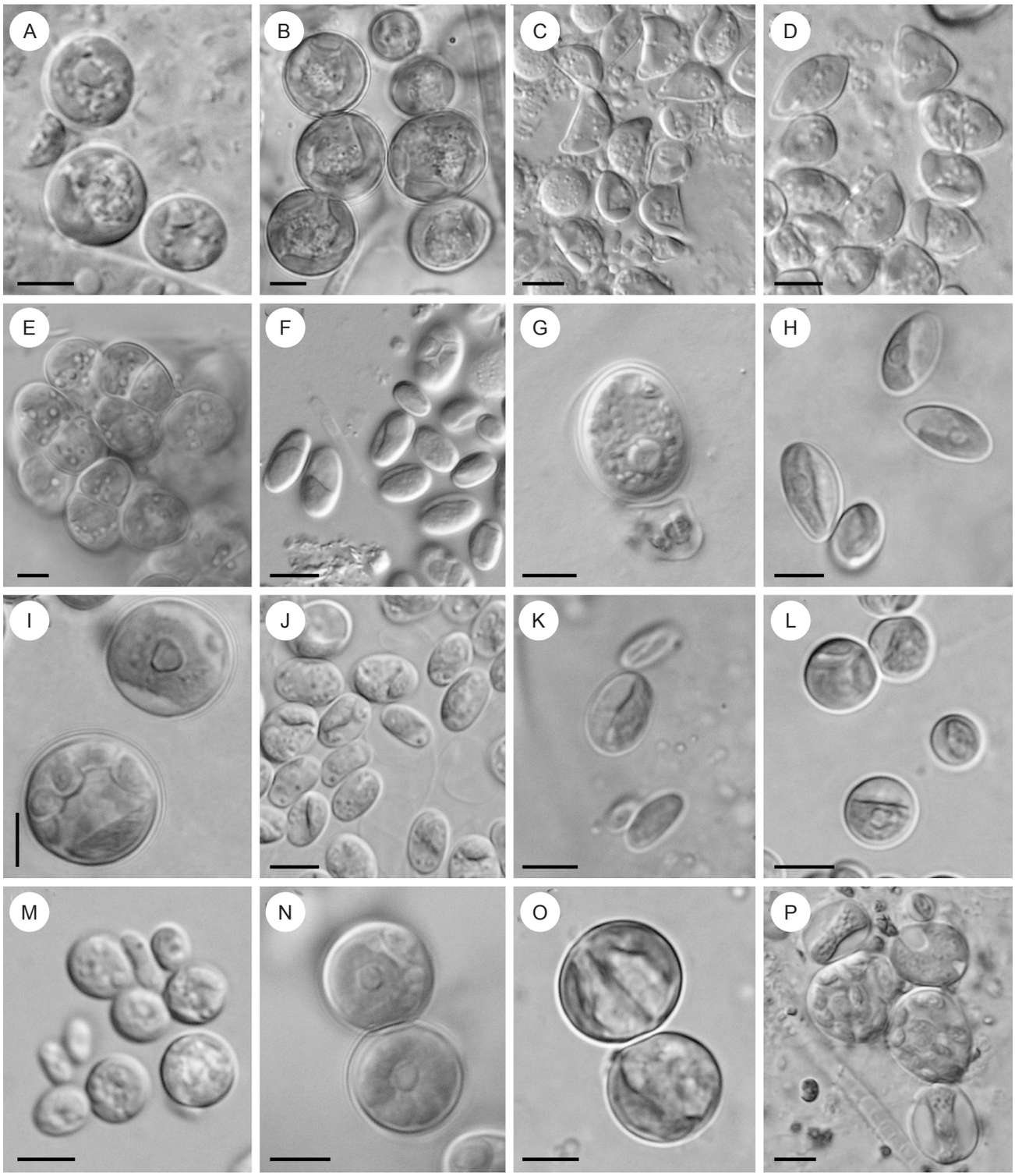

Figure 2 – A, *Eustigmatos* cf. *vischeri*; B, *Eustigmatos* sp.; C, D, *Pleurogaster* sp.; E, *Apatococcus* sp.; F, "*Avernensia*" sp.; G, *Chlamydomonas* sp.; H, *Chlorella* cf. *angusto-ellipsoidea*; I, *Chlorella* cf. *luteoviridis*; J, *Chlorella* cf. *saccharophila* 1; K, *Chlorella* cf. *saccharophila* 2; L, *Chlorella* cf. *sphaerica*; M, *Chlorella* sp. 1; N, *Chlorella* sp. 2; O, *Chlorella* sp. 3; P, *Chlorella* sp. 4. Scale bars indicate 5 µm.

Table 2 – The results of SIMPER analyses illustrating species that differentiate individual habitat types or sampling areas.

Habitat types (across areas)	**Bark** (average abundance)	**Wood** (average abundance)	**Contribution** (%)
Dictyochloropsis sp.	2.0	0.7	8.8
"*Arvensenia*" *sp.*	1.3	0.4	7.4
Elliptochloris sp.	1.0	0.9	6.0
Pseudomarvania aerophytica	1.2	0.1	5.9
Printzina effusa	1.5	0.5	5.3
Pseudococcomyxa sp.	0.1	0.8	4.9
Areas (across habitat types)	**Central Catchment** (average abundance)	**Bukit Timah** (average abundance)	**Contribution** (%)
Chlorella sp. 5	0.0	1.8	8.7
Stichococcus sp.	0.1	1.7	7.7
Elliptochloris sp.	0.4	1.5	7.2
Dictyochloropsis sp.	1.2	1.5	5.6
Printzina effusa	0.4	1.6	5.5
"*Avernensia*" sp.	1.2	0.5	4.8

three categories (rare species – one or two colonies on Petri dishes, more frequent species – up to 25% of the colonies, dominant species – more than 25% of the colonies). For identification we used the relevant taxonomic and identification monographs and papers (Printz 1939, Komárek & Fott 1983, Sarma 1986, Ettl & Gärtner 1995, Komárek & Anagnostidis 2005, Rindi et al. 2005). The taxonomically confusing green algal genus *Pseudococcomyxa* was tentatively identified into two morphologically discernible groups according to Friedl et al. (2007), and designated as *Pseudococcomyxa* and "*Avernensia*".

Statistical analyses involved the non-metric multidimensional scaling (NMDS) of species composition data in samples using the Manhattan distance measure. Significance of differences in algal composition between groups of samples was evaluated by the non-parametric two-way ANOSIM (Analysis of Similarities) using the Bray-Curtis quantitative distance measure in PRIMER, v. 6.1.6 (Clarke & Gorley 2006). ANOSIM is based on comparisons of ranked distances between groups with ranked distances within groups. The test statistic R is defined as

$$R = (rB - rW)/[0.25.N.(N - 1)]$$

where rB is the mean rank of all distances between groups, rW the mean rank of all distances within groups and N is the total number of samples. We used the two-way crossed design with permutations in blocks (Clarke & Gorley 2006) for testing the effect of the habitat type (bark vs. wood) on species composition with the effect of different areas taken into account. Conversely, the difference in species composition in relation to the sampling area (Bukit Timah Reserve vs. Central Catchment Reserve) was tested with the effect of different habitat types controlled. In both tests, all the 15,876 possible permutations were used for the computation of the p-values.

Species that characteristically discriminate between the individual habitat types and sampling areas were identified using SIMPER (Similarity Percentage) method based on Bray-Curtis measure in PRIMER, v. 6.1.6. The SIMPER method assesses which taxa are primarily responsible for an observed difference between groups of samples (habitat type, sampling area), and computes the overall percentage contribution of each species made to dissimilarity between groups (Clarke & Gorley 2006). We used the two-way design analogous to those used in ANOSIM so that only similarities and dissimilarities between samples within the same level of the second factor were considered. The differences in alpha-diversity were evaluated by permutation tests on the Menhinick diversity index (Magurran 2004) defined as

$$I = S/\sqrt{N}$$

The Menhinick diversity indices for each sample were computed, and their between-group differences assessed by 1000 permutation replicates in PAST, v. 1.80 (Hammer et al. 2001). The turnover diversity (beta-diversity) between the groups of samples was illustrated by the Bray-Curtis distance measure of species composition data (Magurran 2004). The differences in beta-diversity between groups defined by the sampling area and the habitat type were evaluated by the two-group permutation tests (1000 replicates).

RESULTS

In total, we identified 57 taxa of algae and cyanobacteria in twenty investigated samples (table 1 & figs 1–4). In most morphospecies, the exact identification into traditional morphologically defined species was not possible. Consequently, most taxa were designated by names indicating their taxonomic affinity according to their morphological characteristics. Their future exact taxonomic evaluation, including eventual descriptions of new taxa or nomenclatural changes should be made by parallel analysis of molecular data (Rindi et al. 2006b). Nevertheless, we could clearly distinguish several characteristic taxa that were dominant in multiple samples, often in both investigated areas (table 1). However, there still were 60.7% of species that were only once encountered in in-

vestigated samples. The NMDS ordination illustrated clustering of the groups of samples according to their sampling area (along the first axis) and according to the habitat type (along the second axis), respectively (fig. 5). Significance of these differences in species composition was evaluated using the two-way ANOSIM. The sampling area effect was strongly

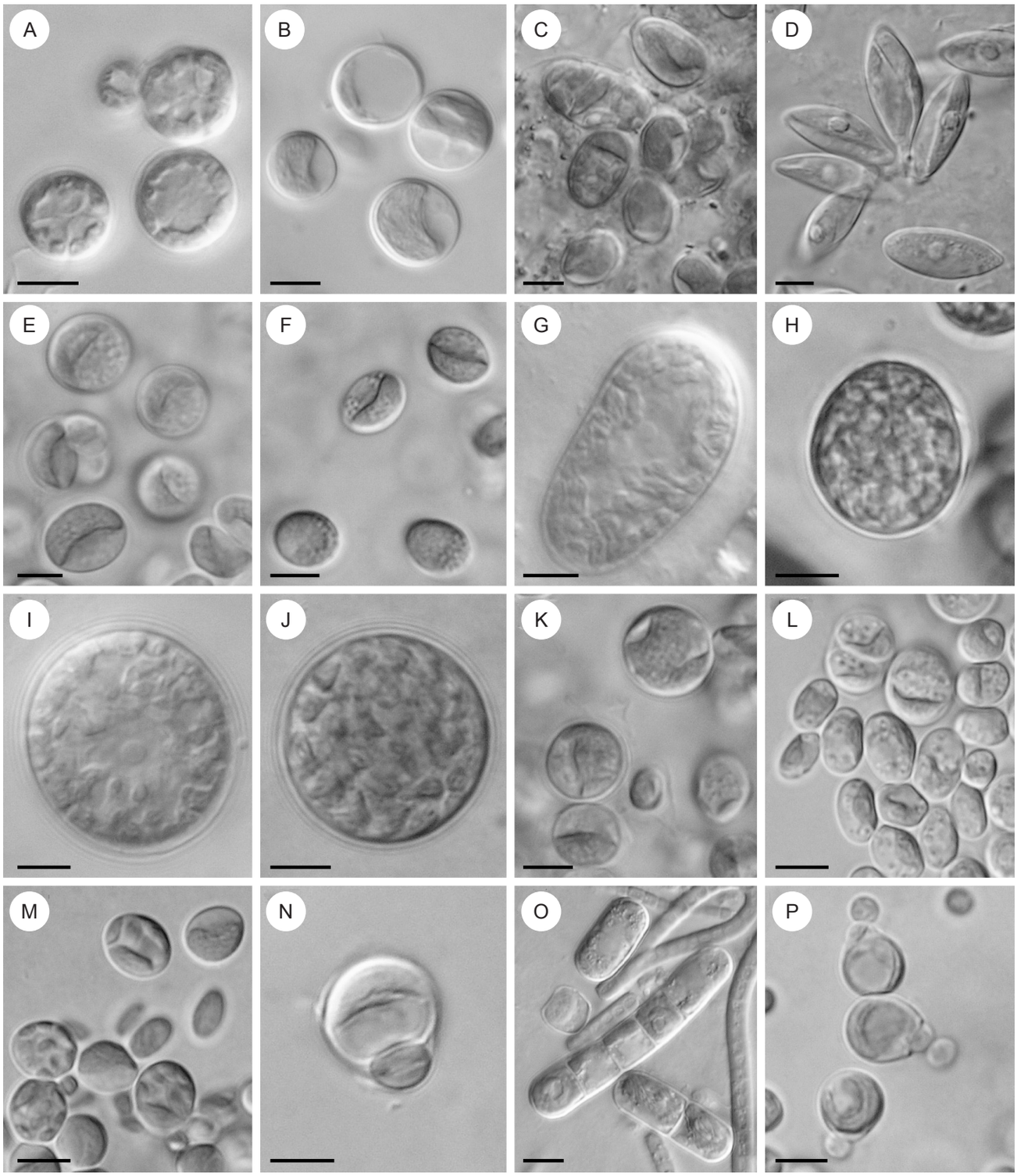

Figure 3 – A, *Chlorella* sp. 5; B, *Chlorella* sp. 6; C, cf. *Chlorella rugosa*; D, *Chlorolobion* cf. *braunii*; E, *Coenochloris* sp. 1; F, *Coenochloris* sp. 2; G, *Dictyochloropsis irregularis*; H, *Dictyochloropsis* cf. *symbiontica*; I, J, *Dictyochloropsis* sp.; K, L, *Elliptochloris* cf. *subsphaerica*; M, N, *Elliptochloris* sp.; O, *Klebsormidium* cf. *nitens*; P, *Pseudomarvania aerophytica*. Scale bars indicate 5 µm.

significant (R = 0.64, none of the permuted 15,875 statistics had the higher R-value than was the original one). Thus, the permutation *p*-value was 0.00006 – the lowest possible in this data design. The effect of habitat type (bark vs. wood) on species composition was also strong, even if not so pronounced as the sampling area effect. The R-value was 0.299 and there were 18 higher permuted statistics out of the total 15,875, resulting in still highly significant *p*-value of 0.0012.

The results of the two-way SIMPER analysis indicated species that were primarily responsible for discriminating the individual groups (table 2). The bark habitat in our localities was primarily distinguished by the conspicuous presence

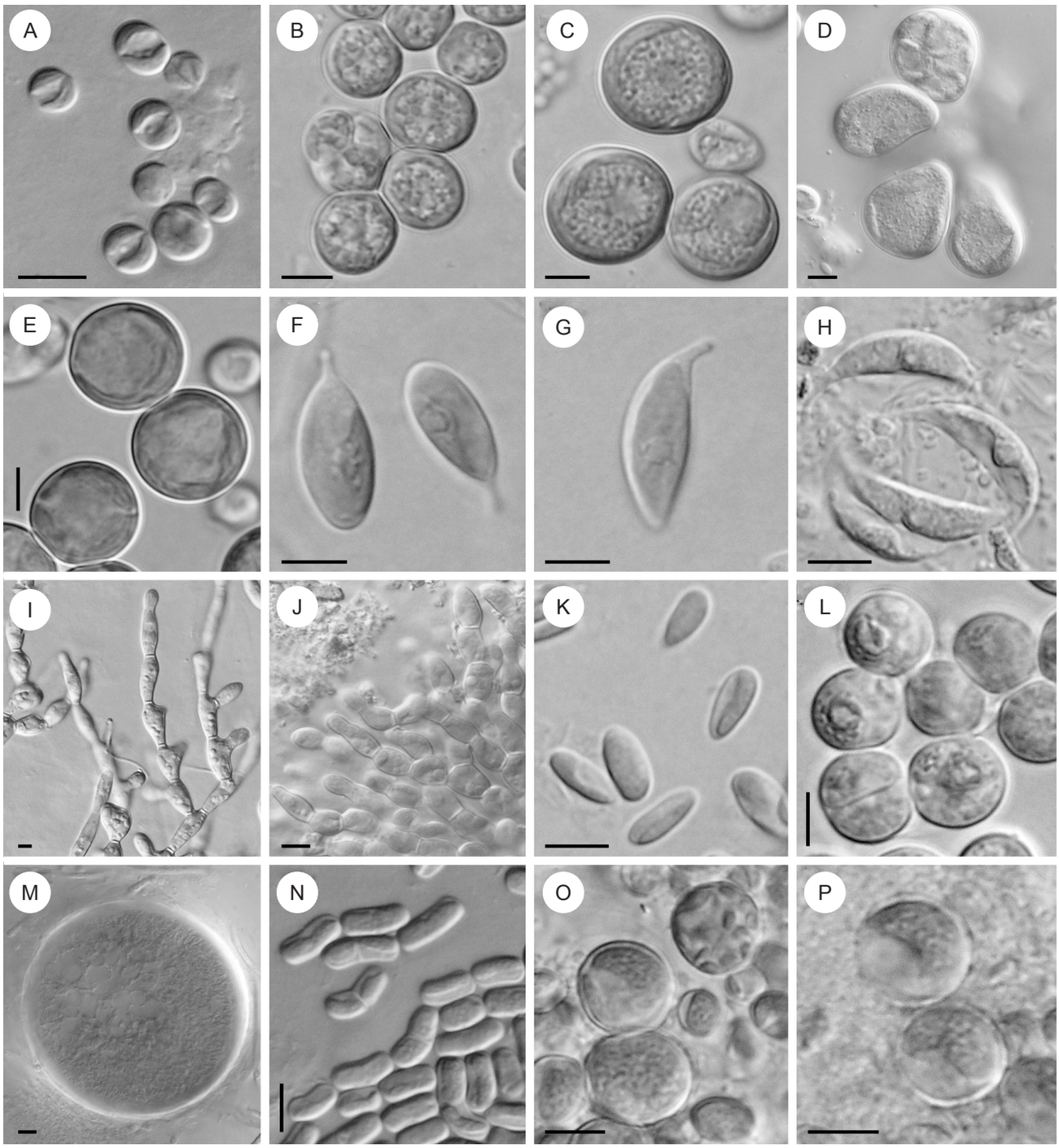

Figure 4 – A, *Mychonastes homosphaera*; B, *Mychonastes* sp.; C, *Myrmecia* cf. *globosa*; D, *Myrmecia* cf. *irregularis*; E, *Myrmecia* sp.; F, *Podohedra* cf. *saltans*; G, *Podohedra* cf. *tropica*; H, *Podohedra* sp.; I, *Printzina effusa*; J, *Printzina lagenifera*; K, *Pseudococcomyxa* sp.; L, *Scenedesmus* cf. *rubescens*; M, *Spongiochloris* cf. *spongiosa*; N, *Stichococcus* sp.; O, P, *Watanabea* cf. *reniformis*. Scale bars indicate 5 µm.

of *Dictyochloropsis* sp., *"Avernensia"* sp., *Pseudomarvania aerophytica*, *Printzina effusa* and *Printzina lagenifera*. On the other hand, the bare wood habitat of both localities contained considerably more populations of *Pseudococcomyxa* sp. and *Elliptochloris* cf. *subsphaerica* that were rare or even absent from the bark samples. The sampling areas differed primarily by the characteristic presence of *Chlorella* sp. 5, *Stichococcus* sp., *Elliptochloris* sp. and *Printzina effusa* in Bukit Timah, whereas *Chlorella* cf. *angusto-ellipsoidea*, *Pseudococcomyxa* sp. and *Elliptochloris* cf. *subsphaerica* were characteristic for the Central Catchment Reserve secondary forest area.

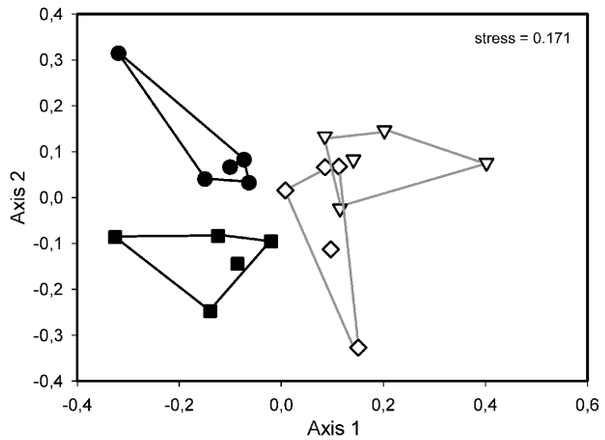

Figure 5 – The non-metric multidimensional scaling (NMDS) ordination diagram of samples according to their species composition. MB samples - ●, MW samples - ■, TB samples - ∇, TW samples - ◊.

The average species number was 8.9 per sample in the bark samples and 7.1 in the bare wood samples. There were no differences in alpha-diversity, evaluated by the Menhinick index, between groups of samples defined by the sampling area and the habitat type, even if the mean was slightly higher in bark samples (fig. 6). This difference was not significant even in groups of bark and wood samples combined from both sampling areas. On the other hand, the beta-diversity (turnover diversity in groups of samples) based on the Bray-Curtis distance of species composition between samples was consistently higher in the wood samples than in the bark samples (fig. 7), i.e. the wood samples had more variable species composition than the bark samples. This difference was significant in the Bukit Timah samples (permutation *p*-value = 0.009), but it was slightly insignificant in the Central Catchment samples (permutation *p*-value = 0.074), even if the mean was also higher in the wood habitat from this sampling area. However, this difference was strongly significant in groups of bark and wood habitats combined from both sampling areas (permutation *p*-value = 0.0047). The species turnover (beta-diversity) was higher in the Central Catchment area than in the Bukit Timah forest. However, this difference was not significant in permutation tests on the Bray-Curtis distances between samples from bark habitats in both areas and in samples from wood habitats, respectively. Nevertheless, it was significant in samples from the Bukit Timah and the Central Catchment areas combined from both habitat types (permutation *p*-value = 0.0098).

DISCUSSION

Certainly, we are still far from the sufficient knowledge of structure and diversity of subaerial tropical algal assemblages. Our recent study (Neustupa & Škaloud 2008), based on the investigation of bark algal assemblages from the tropical mountainous habitats, demonstrated that the taxonomic structure of the autotrophic micro-communities differed from those found in comparative temperate and subtropical habitats. Whereas the non-tropical samples were usually dominated by *Apatococcus lobatus*, *Desmococcus* spp., *Klebsormidium* spp., *Trentepohlia umbrina* or *Prasiola crispa* (Brand & Stockmayer 1925, Laundon 1985, Gärtner & Ingolić 2003, Rindi & Guiry 2004), the tropical species composition differed considerably. In mountainous South Asian samples the dominant and frequently occurring taxa were the morpho-

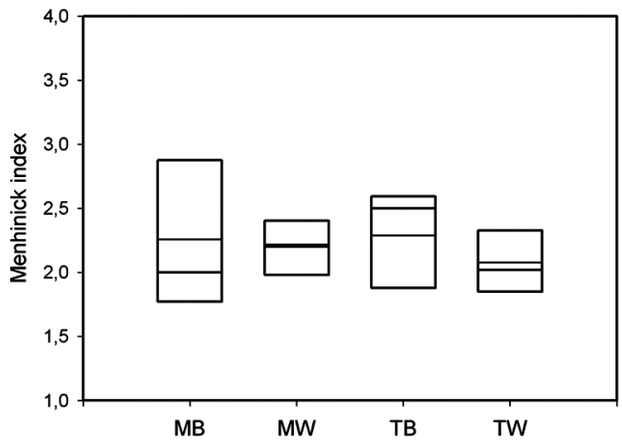

Figure 6 – The box-plots of diversity measures of individual sets of samples. Alpha-diversity of groups of samples evaluated by the Menhinick index.

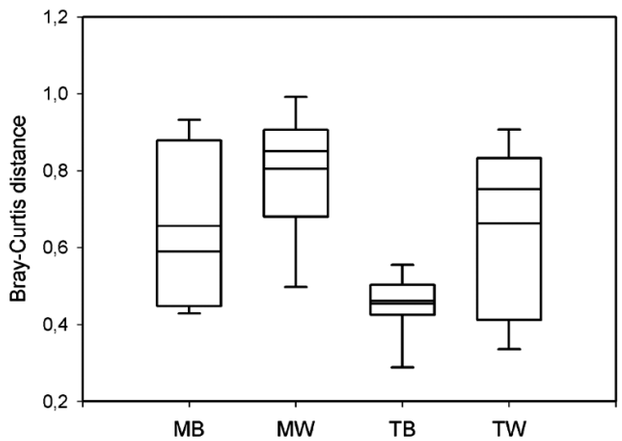

Figure 7 – The box-plots of diversity measures of individual sets of samples. Beta-diversity of groups of samples evaluated by the Bray-Curtis distances between individual samples.

types of *Pseudococcomyxa* spp., *Nostoc* cf. *entophytum*, *Dictyochloropsis* sp., *Printzina effusa* and *Trentepohlia* cf. *aurea* (Neustupa & Škaloud 2008). In addition, the characteristic temperate species were completely absent from the mountainous tropical samples.

In this study, the dominants of individual samples and the sampling areas differed, but there were still several frequent species that occurred in at least 40% samples: *Pseudococcomyxa* and "*Avernensia*" morphotypes, *Dictyochloropsis* sp. (morphologically identical with those from the previous study), *Chlorella* sp. 5, *Pseudomarvania aerophytica, Elliptochloris* sp. and *Printzina effusa* (table 2). Most notably, *Dictyochloropsis* sp., *Printzina effusa* and morphotypes attributable to the traditionally defined genus *Pseudococcomyxa* occurred frequently in bark-growing assemblages in both the main altitudinal vegetation formations of the equatorial tropics – the lowland and mountainous rainforests. On the other hand, some interesting differences between the lowland and mountainous samples should be noted. *Trentepohlia* cf. *aurea* was completely missing from the samples investigated in this study, even if it was macroscopically dominant in some mountainous algal assemblages (Neustupa & Škaloud 2008). On the contrary, morphotypes corresponding to *Pseudomarvania aerophytica*, the conspicuous green algal species with the budding-like reproduction, were frequently found in the lowland Singaporean samples, but it was missing from the mountainous assemblages. In addition, this species was described by Neustupa & Šejnohová (2003) from the bark in the Malaysian secondary rainforest. This seemingly predictable species of lowland forest bark-growing algal assemblages is possibly missing from temperate bark-growing algal assemblages. Handa et al. (2003) described morphologically closely similar green alga *Stichococcus ampulliformis*, growing on the tree bark in subtropical climate of South-West Japan. Eliáš & Neustupa (2009) illustrated close relationship of these two organisms. Subsequently, they were reclassified into a single genus *Pseudomarvania*. This genus may now be considered one of the "flagship" bark-inhabiting algae of South-East and East Asian tropical and subtropical regions. The dominant temperate bark-growing species were either not at all found in the investigated tropical samples (i.e. *Prasiola* spp., *Desmococcus* spp. or *Trentepohlia umbrina*), or they were only rarely present as components of tropical assemblages (*Apatococcus* sp., *Klebsormidium* spp.).

The samples from bark and decaying wood microhabitats differed significantly as well as the samples from both the sampling areas. Actually, the sampling area was identified as the prime factor influencing variation in species composition (fig. 5). Regarding the 78.6% of taxa occurring in only one of the sampling areas, we can presume that there may be high variability in algal subaerial assemblages at the regional level. However, the effect of the habitat type was still clearly significant and we were able to identify taxa with strong affinity to a particular habitat type. *Dictyochloropsis* sp., *Pseudomarvania aerophytica, Printzina effusa* and *Printzina lagenifera* were probably the most conspicuous species that clearly preferred the bark habitat and rarely occurred in the wood samples. Species of the genus *Dictyochloropsis* were typically found on bark in other ecosystems, too (Geitler 1966, Ettl & Gärtner 1995, Škaloud et al. 2005). The *Trentepohlia* and *Printzina* species are usually most frequent in bark- and rock-inhabiting subaerial algal growths (Printz 1939, Rindi et al. 2005). Whether the bark-inhabiting tropical trentepohliacean species locally differ also from their epilithic counterparts should be investigated in the future.

The average species richness of 8.9 species per a single sample, detected in this study, was higher than 4.8 and 7.8 species found in bark samples from the mountainous tropical rainforest and mountainous tropical garden habitat, respectively (Neustupa & Škaloud, 2008). In addition, this number is considerably higher in comparison with the data reported in the other cultivation-based studies conducted either in temperate or subtropical ecosystems. There were in average 1.7 species per a bark sample detected by Mikhailyuk (1999) from temperate Ukraine. In subtropical ecosystems, there were 4.0 species reported by Mikhailyuk et al. (2001) from Israel, 1.9 species on the tree bark of subtropical forests from Miyajima Island in Japan, average 5.9 bark-inhabiting species per sample in south-western Japan (Nakano et al. 1991) and average 3.8 species reported by Cox & Hightower (1972) from Tennessee., U.S.A. We certainly need to take into account possible differences in species richness caused by the different methods used in the above mentioned studies. However, they all were based on cultivation of corticolous microalgal growths on BBM (or BBM-like) agar-solidified media, so that we still may consider their results as broadly comparable. Certainly, the greatest possible artificial differences may result from different "identification strategies" of individual investigators. Therefore, the comparisons among such studies must still be considered cautiously.

Nevertheless, the lowland tropical ecosystems possibly seem to have higher alpha-diversity of microalgae on a tree bark than similar microhabitats in comparatively drier and colder ecosystems. In addition, the real algal diversity of these localities is certainly higher than our species numbers, as the extremely rare species might still be overlooked and the cultivation-based approach do not capture the non-cultivable cyanobacteria and algae. In this respect, the environmental sequencing approaches may provide extremely useful comparative data sets in the future. However, we also must notice that the indirect approaches (as those based on cultivation of samples on agar plates) cannot identify, which species actually grow on the substrate, and which may only be present in inactive stages. However, unicellular microalgae clearly cannot be determined from natural samples (Ettl & Gärtner 1995) so that the culturing methods are essential for obtaining actively dividing populations of individual species. The environmental sequencing approaches also suffer from this inevitable noise in data, and this must, therefore, always be taken in mind, when interpreting seemingly ubiquitous occurrence of some small, easily dispersing species.

Whereas we detected significant differences in species composition of samples from different sampling areas and habitat types, their alpha-diversity did not differ. In other words, the bark samples alone cannot sufficiently represent the diversity of subaerial epiphytic algae of a locality. On the other hand, algal diversity of decaying wood was not only comparable to the bark habitat, but it was also composed of different species. Therefore, importance of these microhabitats, especially abundant in old-growth, primary rainforests, for the overall biodiversity of subaerial algae must be

stressed. This argument is further supported by the higher species turnover among samples (beta-diversity) of the wood habitat over the bark assemblages, possibly caused by higher substrate differentiation of decaying wood samples in comparison to bark of living trees.

There is still little knowledge on geographic distribution of subaerial microalgae. While limitations of morphology-based identification of subaerial algae should certainly be regarded, similar investigation of comparable habitats in other tropical regions would still be of much use. At least, dominant or easily identifiable morphospecies could be compared and their distribution patterns established. Nevertheless, wide application of molecular-based methods of identification will probably facilitate more studies and projects aimed at the investigation of diversity patterns of tropical subaerial algae.

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