CHAPTER 6

FUNGAL COMMUNITY VARIATION ON DECAYING FRONDS OF THAI DWARF FISHTAIL PALM

6.1. Introduction

In ecology, the term "*community*" has traditionally been defined as "any naturally occurring group of different organisms inhabiting a common environment" (Abercrombie *et al.*, 1957). In the microbiological glossaries, including microfungi, the term is defined more narrowly as "any phytosociological taxon" (Kirk *et al.*, 2001). However, the definition of "*community*" of Whittaker (1975) "an assemblage of population of plants, animals, bacteria and fungi that live in an environment and interact with one another, forming together a distinctive living system with its own composition, structure, environmental relations, development and functions" is probably the most appropriate definition to be applied to almost all of organisms. Using this definition, it seems impossible to consider the structure and development of fungal communities without investigating their interactions with other organisms and the environment.

Fungal community structure plays major roles in determining both above- and belowground biodiversity within ecosystem (Bever *et al.*, 2001). Fungi control many regulatory steps in ecosystems (Morris and Robertson, 2005), for example, as saprophytes, they control the rate at which organic matter is returned as inorganic nutrients available for plant uptake. As mutualists, they provide nutrients and water to plants to increase net primary productivity. As pathogens, they cause mortality and

affect community composition and turnover. Yet, we know relatively little information about the size and component parts of the fungal networks that contribute to each function. The comment of Waksman (1916) who suggested that "the question is not how many numbers and types of fungi can be found in specific ecosystem, but what organisms lead an active life in such ecosystem" is still relevant to be applied, nowadays, to the fungal community on different habitats such as decaying woods, freshwater, marine, etc., because ecosystem function is not governed by the species of fungi present, but by the role that each fungus plays in carrying out certain tasks and the rates at which tasks are accomplished.

How do we analyze and characterize communities in fungi? This is an issue that yet be appropriately answered until now due to the complexity in fungal living states. Fungi have widely been known to be able to change their living states, for example, from endophyte to saprophyte or pathogen to saprophyte, depend on the environmental conditions. In general, there are two methods, classical and molecular, have been widely used in measuring fungal community on different ecosystems or habitats. In classical method, Schmit and Lodge (2005) examined and summarized a wide variety of this method, including direct sampling of fungal fruiting bodies, incubation of substrata in moist chambers, culturing of endophytes and particle plating. In molecular method, the use of DNA extraction technique through PCR amplification and sequencing, followed by comparison with known DNA libraries has become a common method in analysis fungal communities on soil or other substrata (Bidartondo and Gardes, 2005). One of the advantages of a classical study is a list of species found during the study, wherein it is often impossible for a molecular-based study to present a similar list. Assembling a species list enables researchers to

compare data across sites and studies and among different taxonomic or ecological groups. By combining species lists from multiple studies, researchers can determine basic information about individual species, such as geographic range, host relationships and ecological distribution. The fungal communities of different areas can be compared to determine patterns of species diversity. Classical methods are also the only methods that can be used to demonstrate which fungi are reproducing in a particular environment or on a given substratum, as opposed to which fungi are present but cannot reproduce. One final advantage of classical methods is that compared with molecular methods, they are generally less expensive for many investigators, especially those in developing countries. Despite their widespread use, the classical methods have also certain disadvantages when compared with sampling using molecular techniques. Some species may not grow or produce reproductive structures in culture and may reproduce rarely in natural settings. These species will be missed by traditional sampling methods, even though they could be important members of the fungal community. The fact that some species will not be detected clearly has the potential to bias classical studies. Unfortunately, it is difficult to assess how many species are missed by classical techniques or to determine if this can bias the results of any particular study. While molecular-based studies of fungal diversity can provide an independent assessment of the fungal community, they are limited to sampling a small area, which can result in a different set of biases (Schmit and Lodge, 2005; Bidartondo and Gardes, 2005).

In the study of palmicolous fungi communities, the utilization of molecular approach has not been widely applied yet. This problem is probably caused by an expensive cost of the method, very small samplings required for analysis and very

limited available data in the fungal genome database to be compared with the analysis. Therefore, most studies in the palmicolous fungal community used only classical methods by using direct sampling of fungal fruiting bodies, incubation of substrata in moist chambers or culturing of endophytes (Fröhlich and Hyde, 2000; Yanna, 2001, Yanna *et al.*, 2001; Taylor and Hyde, 2003; Pinnoi *et al.,* 2004; Pinruan *et al.*, 2007). Their studies revealed that several taxa such as *Anthostomella*, *Arecomyces*, *Astrosphaeriella, Linocarpon* and *Oxydothis* were commonly found in associated with terrestrial palms in various localities, as well as Xylariaceae, Hyponectriaceae and Lophiostomataceae at families level (Fröhlich and Hyde, 2000; Taylor and Hyde, 2003). In contrast, however, Pinruan *et al.* (2007) reported a distinct fungal community composition on the *Licuala longicalycata* (a peat swamp palm) fronds. Taxa such as *Annulatascus velatisporus*, *Microthyrium* sp., *Phaeoisaria clematidis*, *Massarina bipolaris*, *Phruensis brunneispora*, *Thailiomyces setulis* and *Solheimia costaspora*, which were rarely to be found on other studies, were surprisingly appeared as the dominant taxa (Pinruan *et al.*, 2007). These results have indicated that fungal community structure is likely affected by host or biogeography.

In this study, the classical method was applied by using direct observation of fungal fruiting bodies on palm fronds of *Wallichia siamensis* in order to test the hypothesis that fungal community on palms is affected by host and tissue types. In addition, the fungal community of this study was also compared with the previous studies.

6.2. Materials and Methods

Collection and Experimental Design

Decaying fronds from five trees of *Wallichia siamensis* were chosen randomly without considering the age of decaying stage. Three microhabitats being primary rachis, secondary rachis and pinna, were selected. Ten samples of each microhabitat were performed and examined. The dimension of primary rachis length was *ca* 10 cm; whereas the pinna and secondary rachis were performed following its natural size. The total 150 samples (50 samples from each microhabitat) were also examined. All field samples were put in 11.5" x 16.5" resealable bags. Collecting bags were sealed and labeled as follows: *Name of the palm, Collecting site, Collector/s, Date.*

Examination of Materials

On returning to the laboratory, the materials were immediately incubated and examined periodically over the next month. The decaying and senescent materials were examined for saprophytic Ascomycetes, Coelomycetes, Hyphomycetes and Basidiomycetes. The materials were examined using an Olympus SZ H10 dissecting microscope to determine the presence of the fungal fruiting structures and Olympus BX51 to determine microscopic structures. Once fully examined, the piece of material was air dried and placed in a resealable envelope with the following information: *Herbarium number, Host name, Collection site, Collector/s, Date*. Dried herbarium specimens were deposited at CMU Herbarium (CMU), Faculty of Science, and Molecular of Plant Pathology Laboratory, Department of Plant Pathology, Faculty of Agriculture, Chiang Mai University, Thailand.

Data Analysis

The total number of species and the number of fungi per sample were recorded and calculated. Species-area curves were plotted for each collection to examine the sample size (Begon *et al.*, 1992). Dominance diversity curves were plotted as a reflection of the relative abundance of species in each microhabitat sample (Kent and Coker, 1992). Percentages abundance, recurrence and frequency of occurrence of each species were employed in order to compare the dominance of fungi among different collections (Cai *et al.*, 2006) as follow:

$$\% \text{ abundance of a taxon } X_a = \frac{\Sigma \text{ records of taxon } X_a}{\Sigma \text{ records of all taxa}_a} \times 100$$

$$\% \text{ recurrence of a taxon } X_a = \frac{\Sigma \text{ records of taxon } X_a}{\Sigma \text{ records of taxon X}} \times 100$$

$$\% \text{ occurrence of a taxon } X_a = \frac{\Sigma \text{ records of taxon } X_a}{\text{Number of plant parts investigated}} \times 100$$

The taxa with a percentage of occurrences higher than or equal to 10% are considered to be common species.

Shannon-Weiner diversity indexes (H'), which incorporate species richness and species evenness (Begon *et al.*, 1992), was applied to evaluate the diversities of

fungal communities. Species richness refers to the number of species in a community and species evenness refers to the contribution (relative abundance or equability).

Shannon diversity index $\mathbf{H= -\sum p_i \, . \, ln \, p_i}$ ($\mathbf{p_i}$: proportion of species $\mathbf{i^{th}}$)

(The higher of the Shannon diversity index is, the more diverse is the community)

Species evenness $\mathbf{E = H/lnS}$ (**S**: total species number)

(Shannon evenness accounts the equability of species present (E) (Gotelli and Colwell, 2001). Shannon evenness ranges from 0 to 1. If one community with Shannon evenness index equal to 1, it means that distribution of every species in the community is equal)

Similarities among the fungal communities from different part of fronds was calculated by using Sørensen's index of similarity (S') with values between 0 (no similarity) and 1 (absolute similarity) (Magurran, 1988). A *t*-test was also performed to compare the Shannon-Weiner indices between different fungal communities (Hutcheson, 1970).

Two types of Hierarchical Cluster Analysis (HCA) were employed to classify species recorded on the palms fronds and sites collection, respectively. In the first HCA, species with total recorded (>10) were selected as cases in the analysis. Ward method was used as a clustering method; Squared Euclidean Distance was selected as an interval measurement and Z-scores was used to standardize the transform value of variable group. In the second HCA, Centroid method was selected as a clustering method instead of Ward method, sites were selected as cases, and other parameters

were set as the first HCA analysis. The second HCA analysis was also used as a site topology for Indicator Species Analysis (ISA).

In order to constructs a configuration/map of the fungal community on specimens collected, Multi Dimensional Scaling (MDS) ALSCAL analysis using Euclidian model distance was performed based on dissimilarity matrices and down-weighting of rare taxa (with frequency of occurrence < 10%). The relationship between assemblage of the fungal community (with frequency of occurrence > 10%) and different type of palm tissues was also analysed using a simple correspondence analysis. Two dimensional plots were generated from the MDS and Correspondence analyses.

Indicator species analyses (ISA) was carried out to examine the indication of taxa occurred on the palms fronds. The Indicator value (IV) for a species was determined by combining relative frequency and relative abundance in a given group and can range from 0 (no indication) to 100% (perfect indication, meaning the species is present in all samples in the group and absent from all samples in other groups) (Dufrêne and Legendre, 1997). Indicator species was assigned if they had an indicator value (InVal) higher than 25% (Dufrêne and Legendre, 1997). All analyses were performed using SPSS 16.0 software (Anonymous, 2007) and XLSTAT-Pro version 7.5 (Anonymous, 2004).

6.3. Results

A total of 150 specimens of palm fronds (50 from each tissue type/microhabitat) were examined for the presence of fungal fruiting bodies, yielding 35 ascomycetes (representing 63.7% of all taxa), 8 coelomycetes (14.5%) and 12

hyphomycetes (21.8%). All taxa recorded along with the information of Σ records, total frequency of occurrence and % abundances are presented in appendix 5. A more detail information of Σ records, total frequency of occurrence, % recurrence and % abundances of all taxa at each site and tissue type is also given in appendix 6.

Several common palmicolous fungi genera as previously reported (Fröhlich and Hyde, 2000; Taylor and Hyde, 2003), such as *Lophiostoma*, *Oxydothis*, *Diaporthe*, *Arecomyces*, *Rachidicola* and *Terriera* were encountered in this study. Overall, the most common taxa with frequency of occurrence (FO) more than 30% were *Lophiostoma macrostomum* (44%), *Chaetospermum chaetosporum* (34%) and *Oxydothis daemonoropsicola* (30.7%). When the frequency of occurrence value of all taxa was calculated based on tissue types, it was found that *Pestalotiopsis guepinii*, *Mycosphaerella wallichiae* and *Oxydothis wallichianensis*, appeared as dominant taxa on pinna with frequency of occurrences of 84%, 74% and 54%, respectively (appendix 6). On primary rachis, the most common taxa were dominated by *O. daemonoropsicola*, *Morenoina palmicola* and *Lophiostoma macrostomum* with frequency of occurrences of 92%, 84% and 66%, respectively (appendix 6). On the other hand, *Myelosperma tumidum* (FO = 68%), *Lophiostoma macrostomum* (FO = 66%) and *Chaetospermum chaetosporum* (FO = 46%) appeared as the common species on secondary rachis (appendix 6).

Species area curve was plotted to indicate the increasing number of fungi with the number of samples examined (fig. 6.1). The asymptotes were reached at around 40 samples (fig. 6.1). Therefore, a total 150 samples size (50 samples for each tissue type) used in this investigation could provide a reasonable estimates of the fungal community in this study.

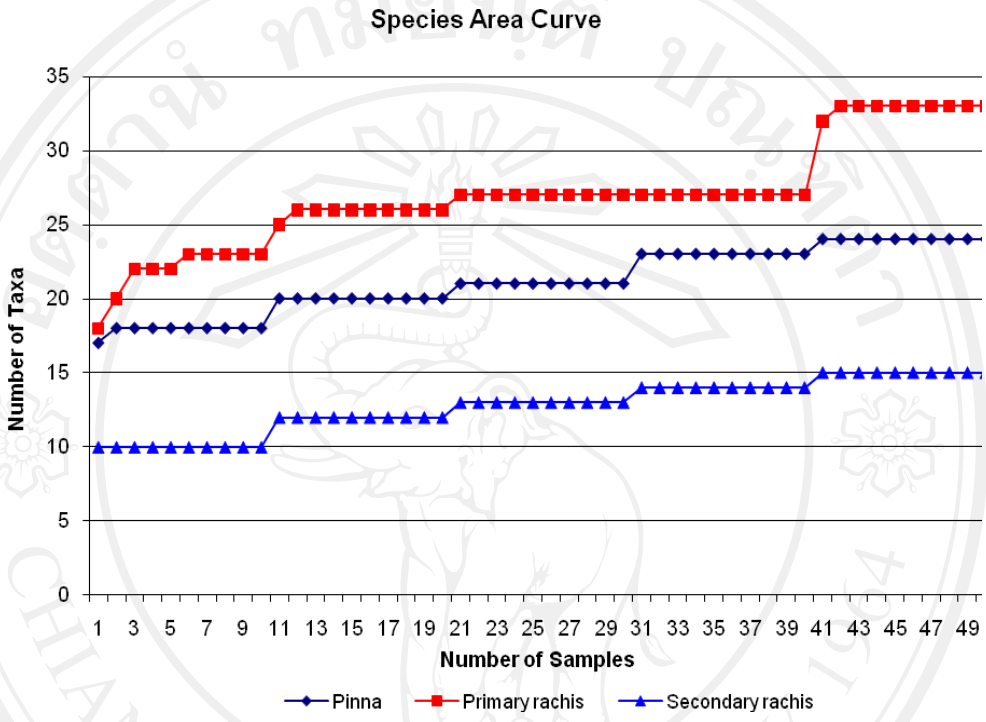

Fig. 6.1 Species area curve of fungal number identified against number of samples examined.

A proportion of fungal abundance was plotted and shown in figure 6.2. There were only about three species of fungi with more than 30 abundances appeared on all tissue types (primary rachis, secondary rachis and pinna), two species with more than 40 abundances (one species from both pinna and primary rachis) and one species with total abundance more than 45 (from primary rachis) (fig. 6.2).

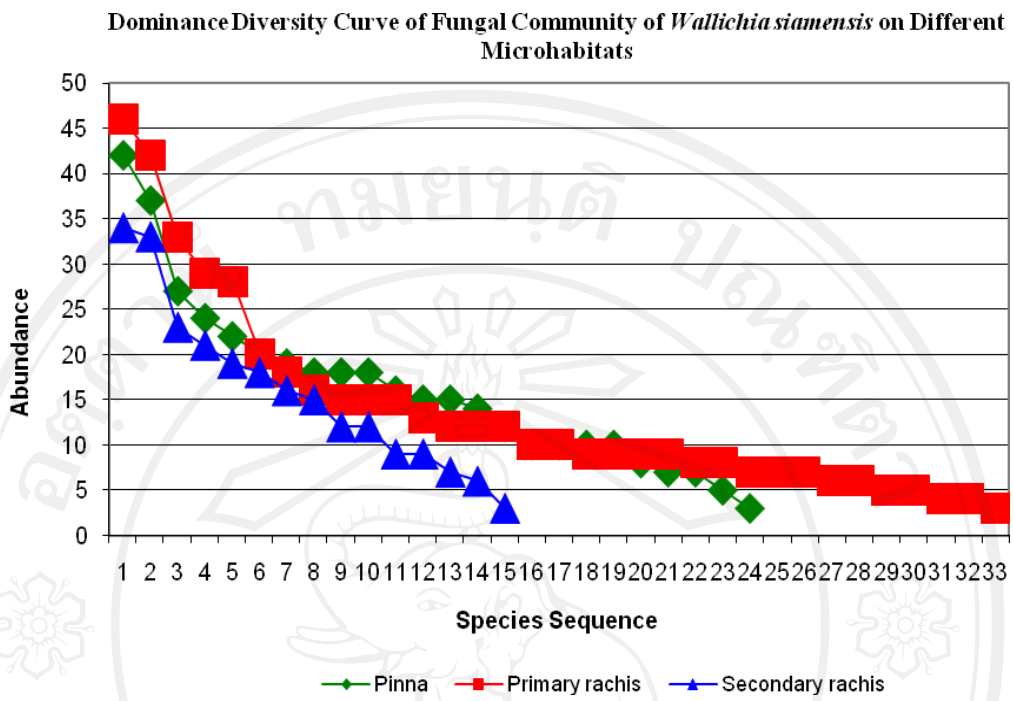

Fig. 6.2 Dominance diversity curve showing the proportion of fungal abundance on different frond types of *Wallichia siamensis*.

Based on a calculation of total specimen dataset, ascomycetous taxa were found to have highest species richness and abundance, with 35 species and 697 total numbers of records (abundance), respectively (fig. 6.3). Members of Hyphomycetes appeared as a second highest in species richness with 12 taxa recorded, but showed to be the lowest one in abundances with only 180 number of individual recorded from all total samples examined (fig. 6.3).

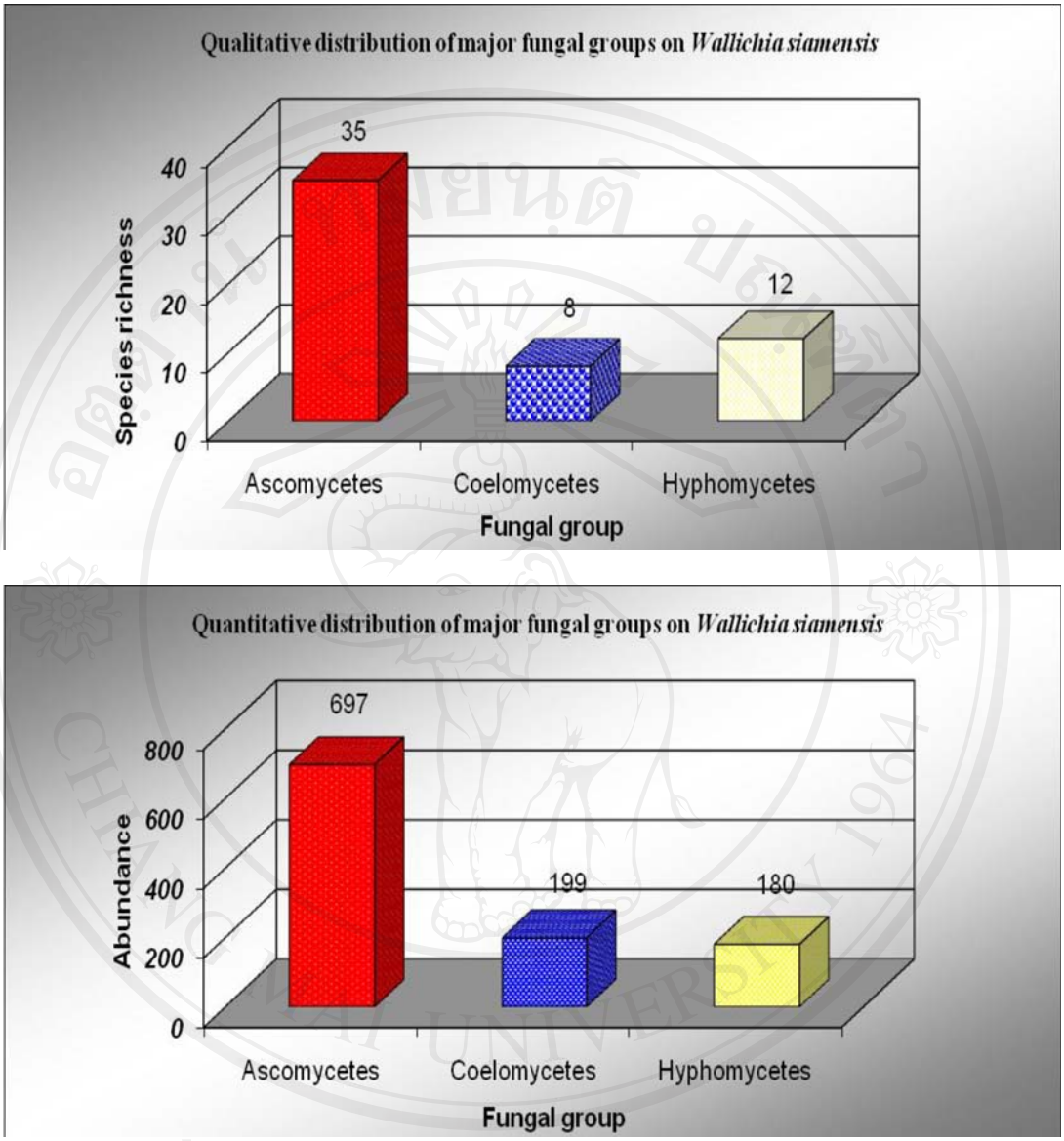

Fig. 6.3 Distribution of all taxa calculated from total specimens of *Wallichia siamensis*. The graphics are presented based on qualitative (number of species/species richness) and quantitative (total abundance) data. Taxa are grouped based on their pseudo-systematic position.

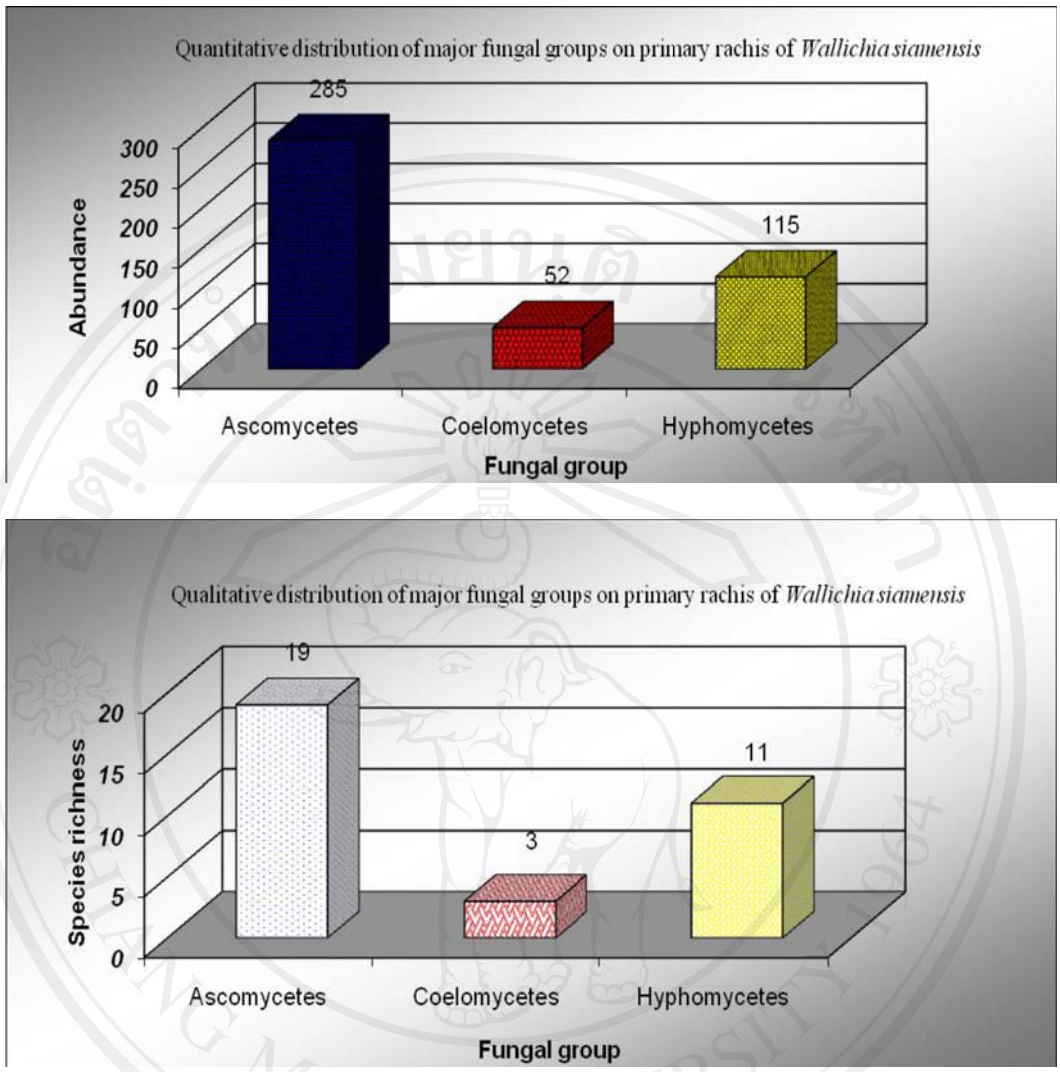

Fig. 6.4 Distribution of all taxa recorded from primary rachis of *Wallichia siamensis*. The graphics are presented based on qualitative (number of species/species richness) and quantitative (total abundance) data. Taxa are grouped based on their pseudo-systematic position.

On primary rachis, taxa of Ascomycetes also appeared as the highest group in species richness (19 species) and abundances (265 number of records), followed by hyphomycetous taxa (11 species and 115 number of records) and coelomycetous taxa (3 species and 52 number of records) (fig. 6.4). Ascomycetous taxa also appeared as common systematic group of fungi found on secondary rachis (8 species and 164 number of records) and pinna (15 taxa and 251 number of records) (figs. 6.5 and 6.6).

However, hyphomycetous taxa that exposed as the second highest pseudo-systematic group in species richness and abundances based on total specimens calculation, appeared as the lowest group of fungi occurs on secondary rachis (3 species and 22 number of records) and pinna (4 species and 43 number of records), respectively (figs. 6.5 and 6.6).

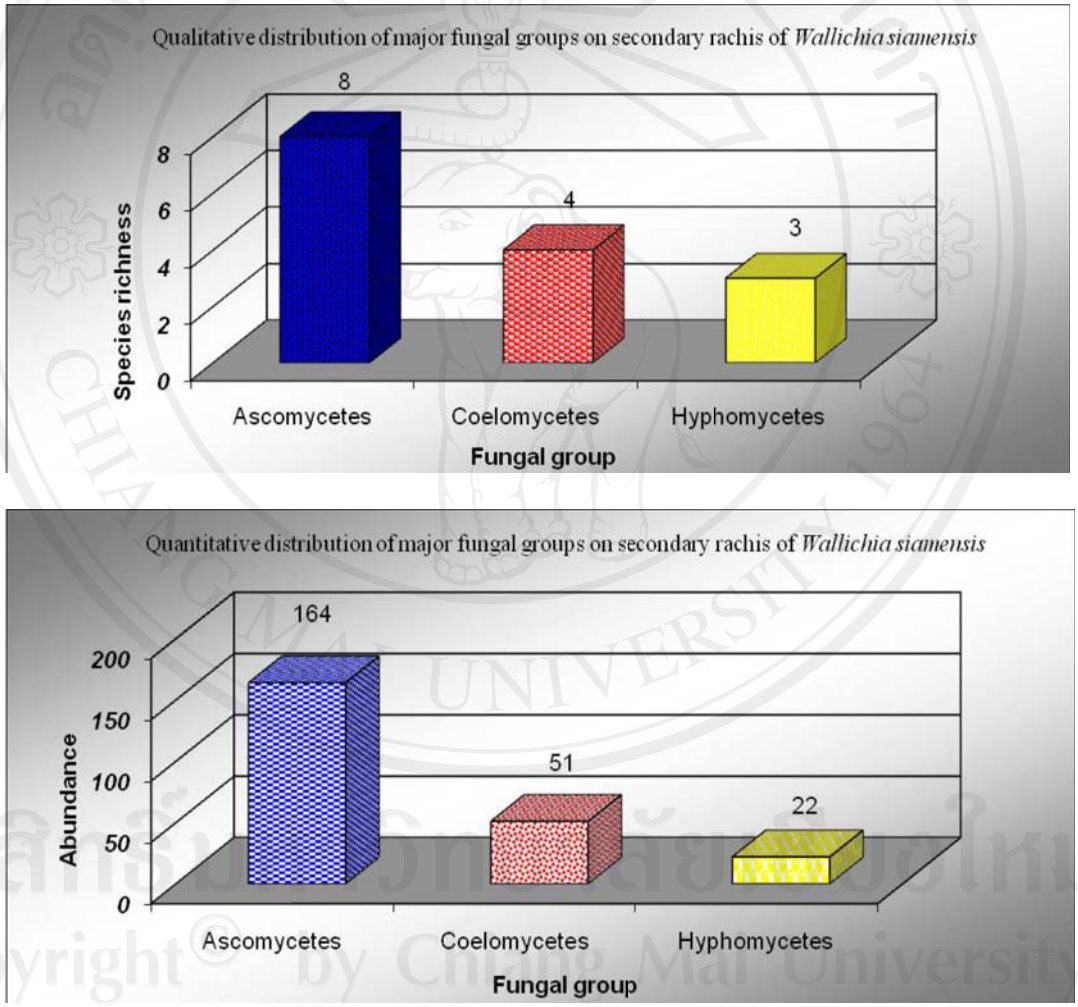

Fig. 6.5 Distribution of all taxa recorded from secondary rachis of *Wallichia siamensis*. The graphics are presented based on qualitative (number of species/species richness) and quantitative (total abundance) data. Taxa are grouped based on their pseudo-systematic position.

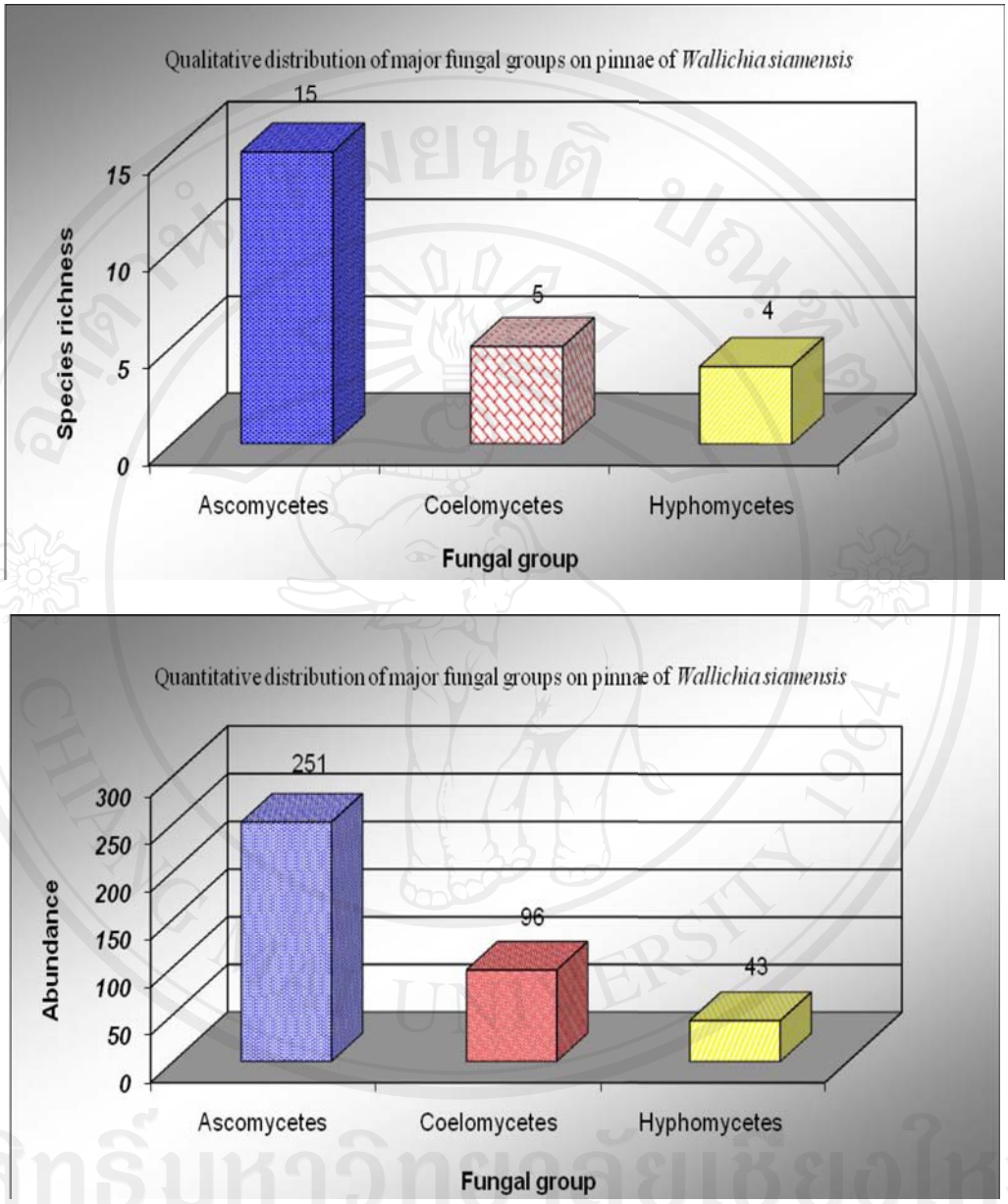

Fig. 6.6 Distribution of all taxa recorded from pinna of *Wallichia siamensis*. The graphics are presented based on qualitative (number of species/species richness) and quantitative (total abundance) data. Taxa are grouped based on their pseudo-systematic position.

Shannon-Weiner diversity indices (H') illustrated in figs. 6.7 and 6.8, apparently showed the highest fungal community diversity occurred on primary rachis followed by fungal community on pinna and secondary rachis, with H' indices of 3.3, 3 and 2.6, respectively. Shannon-Weiner evenness index (E', indicates how evenly the individuals are distributed among the different species) of fungal community on pinna was the highest one (E' = 1) while the fungal community evenness on primary rachis and secondary rachis were equal (E' = 0.9) (fig. 6.7).

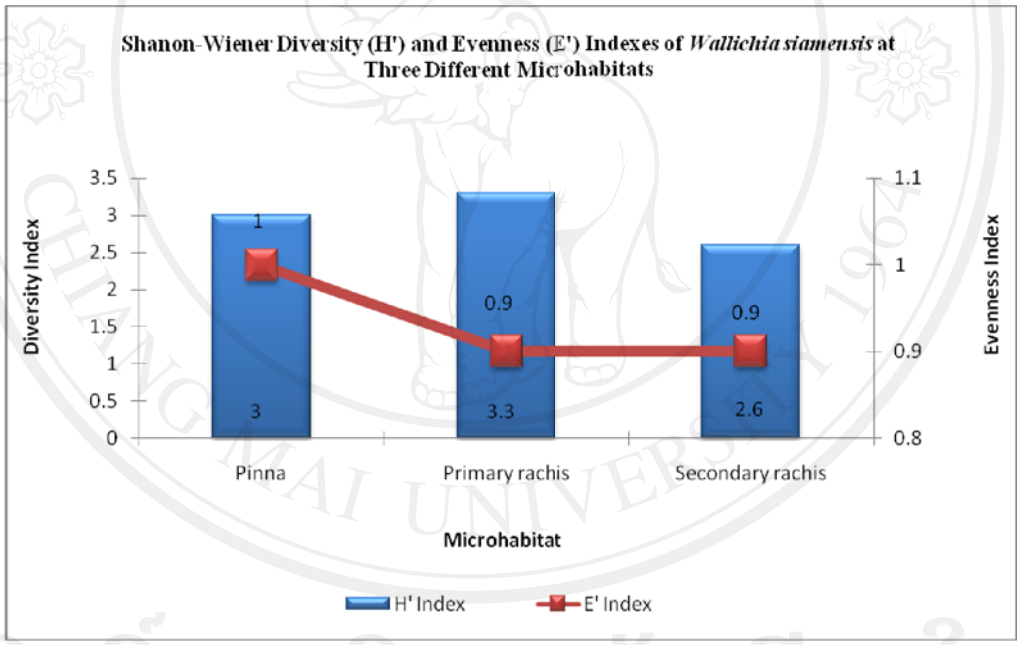

Fig. 6.7 Histogram of Shannon-Weiner diversity index (H') and evenness index (E') showing the diversity and evenness levels of fungal community on different tissue types of *Wallichia siamensis*.

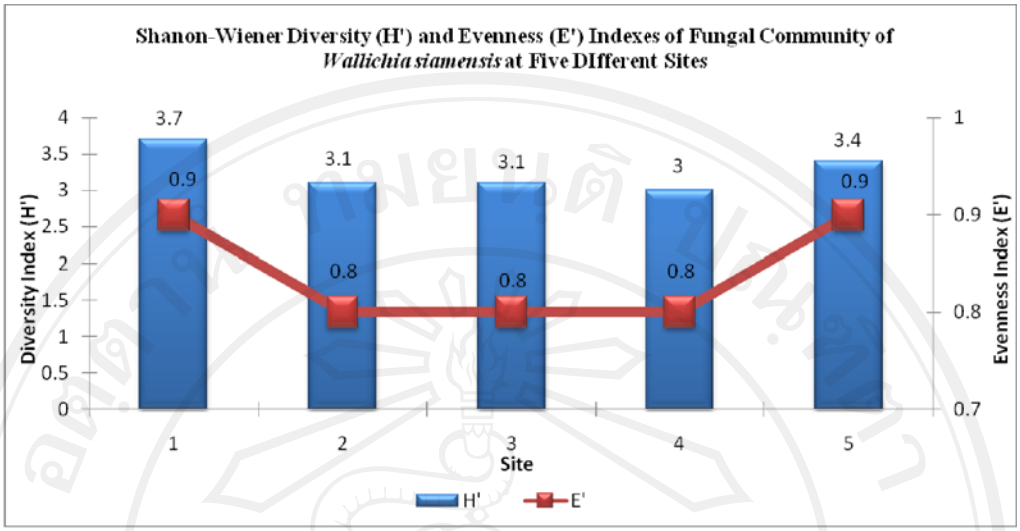

Fig. 6.8 Histogram of Shannon-Weiner diversity index (H') and evenness index (E') showing the diversity and evenness levels of fungal community on different sites.

The level of fungal community diversity and evenness at each tissue type of *W. siamensis* as microhabitat was also illustrated using box plots diagram (figs. 6.9 and 6.10). The box plots diagram showed that the median value of the Shannon-Weiner diversity index (H') distribution of fungal community on *W. siamensis* generated from total specimens collected is 3.1 (fig. 6.9). When analyzing the Shannon-Weiner index diversity of fungal community at each tissue type, secondary rachis appeared as the lowest one (1.9) compared to pinna (2.3) and primary rachis (2.7), respectively (fig. 6.10). In this diagram, outlier was found at the the fungal community on pinna, and extreme values were found on secondary rachis (fig. 6.10) which indicated the presence of extremely high and low diversities at both tissue types.

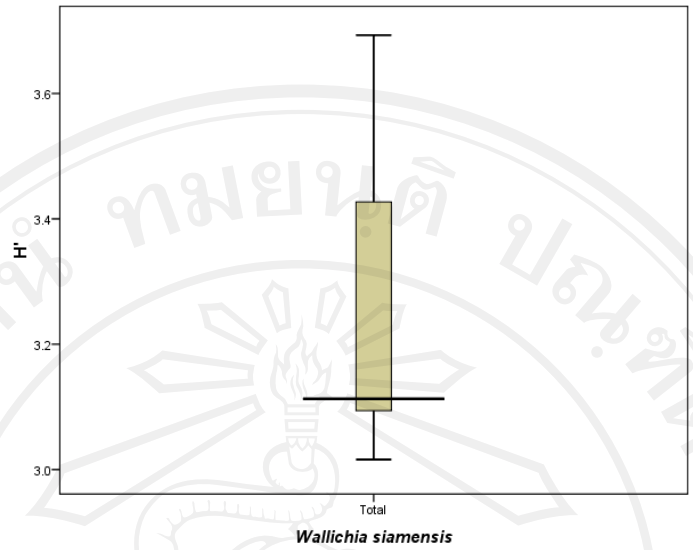

Fig. 6.9 Box plot showing diversity level of fungal community on total samples collected of *Wallichia siamensis*. The lower and upper boundaries of each box enclose 25-75% of the data. The line within the box shows the median values, the bar lines above and below the boxes indicate minimum and maximum values.

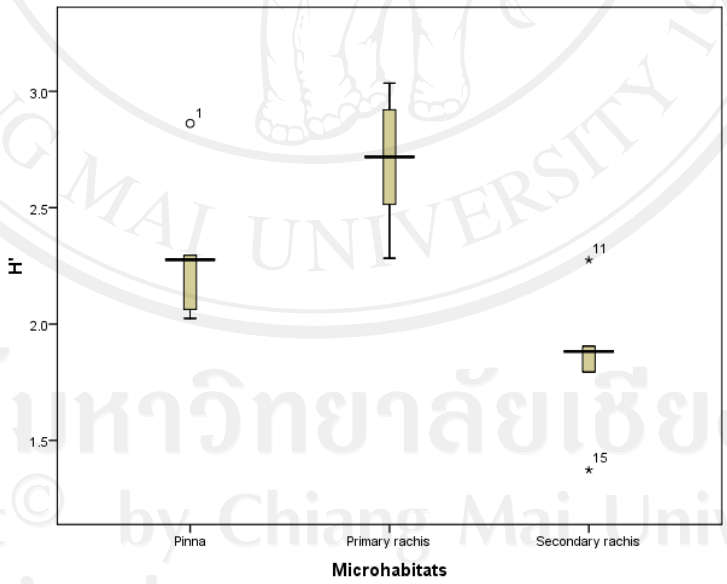

Fig. 6.10 Box plots showing proportion of diversity level of fungal community on samples collected from different tissue types of *Wallichia siamensis*. The lower and upper boundaries of each box enclose 25-75% of the data. The line within the box shows the median values, the bar lines above and below the boxes indicate minimum and maximum values, and ° indicates outliers.

Box plots diagram based on abundance dataset were also performed (fig. 6.11). According to the analysis generated from this dataset, it was clear that the median value of diversity index of fungal community on *W. siamensis* is 19.5. Outliers and extreme values were not found at all of the sampling locations (figs. 6.11 and 6.12), which indicated the absence of extremely high or low fungal abundances at several sites. Average abundance of fungal community on secondary rachis (< 5) is the lowest one compared to primary rachis and pinna (fig. 6.12). The median value of abundance distribution among microhabitats is varying as follow: pinna (7), primary rachis (8) and secondary rachis (4), respectively (fig. 6.12). Outliers were found at the three locations of the fungal community at pinna and two on secondary rachis which indicated a very high abundance from several sample sites of pinna and secondary rachis (fig. 6.12). No extreme values were found on total samples (figures 6.11 and 6.12).

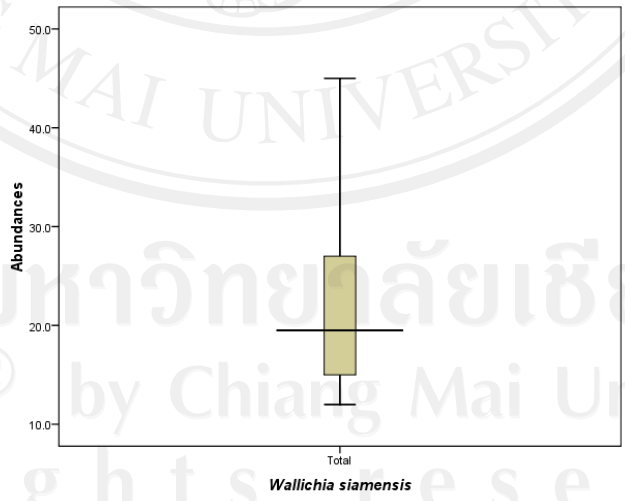

Fig. 6.11 Box plot showing abundance of fungal community on total samples collected of *Wallichia siamensis*. The lower and upper boundaries of each box enclose 25-75% of the data. The line within the box shows the median values, the bar lines above and below the boxes indicate minimum and maximum values, and ° indicates outliers.

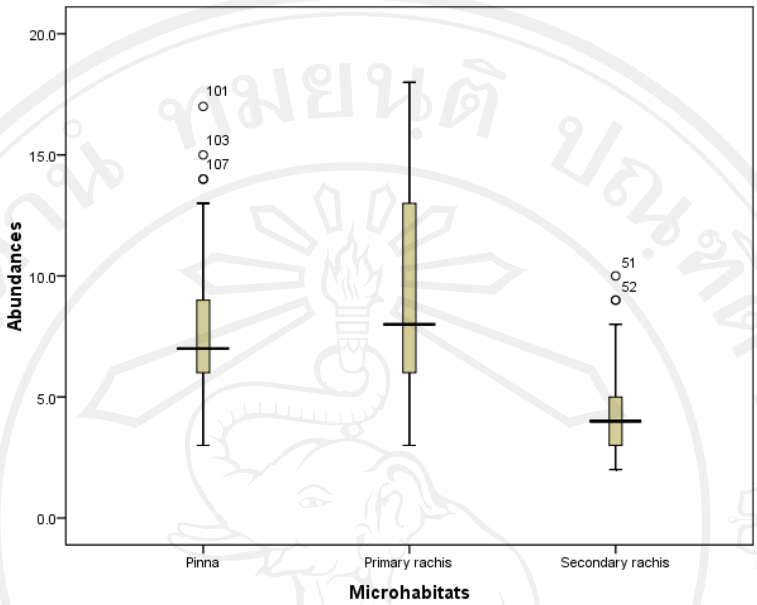

Fig. 6.12 Box plots showing abundance of fungal community on samples collected from different tissue types of *Wallichia siamensis*. The lower and upper boundaries of each box enclose 25-75% of the data. The line within the box shows the median values, the bar lines above and below the boxes indicate minimum and maximum values, and ° indicates outliers.

By evaluating the Sørensen index of similarity (S') among fungal community on different tissue types of *W. siamensis*, it was apparent that the fungal community on primary rachis was the most similar to the fungal community occurs on secondary rachis (S' qualitative = 0.6, S' quantitative = 0.5), followed by the similarity between fungal community on pinna-secondary rachis (S' qualitative = 0.5, S' quantitative = 0.2) and pinna-primary rachis (S' qualitative = 0.2, S' quantitative = 0.1), respectively (figure 6.13).

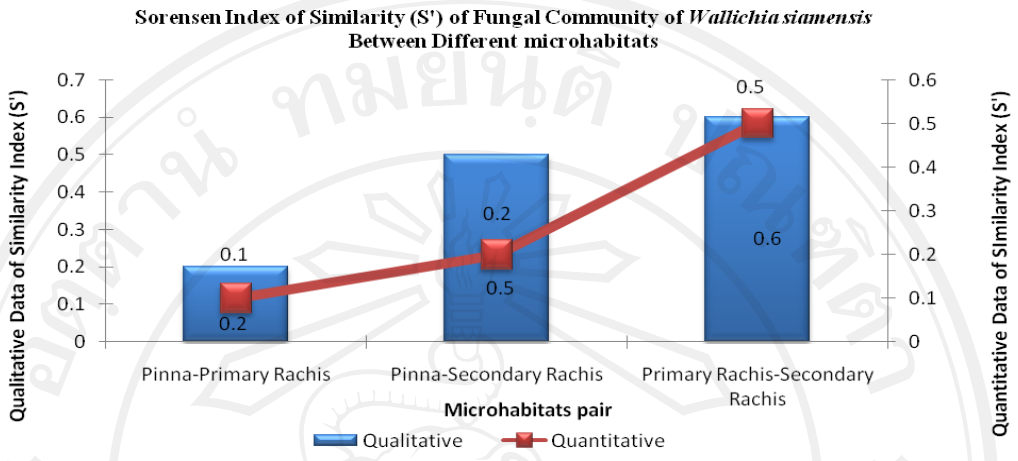

Fig. 6.13 Histogram showing similarity of fungal community occurs on different tissue types (as microhabitat) of *Wallichia siamensis*. Sørensen index of similarity based on qualitative (species richness) and quantitative (abundance) data is plotted against tissue types.

A *t*-test that performed in order to compare the Shannon-Wiener indices (H') in the analysis, apparently showed that the fungal diversity at pinna (mean = 2.3; standard deviation = 0.3) is lower but more variable than primary rachis (mean = 2.7; standard deviation = 0.3) (table 6.1). At 0.5, the Pearson correlation between the fungal communities on pinna and primary rachis is statistically significant. Therefore, since the significance value for change in fungal diversity of pinna and primary rachis is 0.04 (< 0.05), therefore, the higher average of diversity index of 0.3 point per single tree at primary rachis is not due to chance variation, but can be attributed to the specificity on the palm tissue.

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pinna	2.30400	5	.334929	.149785
	Primaryrachis	2.69420	5	.304018	.135961

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Pinna & Primaryrachis	5	.580	.305

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Pinna – Primary rachis	-0.39	0.29	0.13	-0.76	-0.03	-2.968	4	.041

Table 6.1 Two tail *t*-test tables showing comparison of mean data between fungal diversity indices on pinna and primary rachis of *Wallichia siamensis*.

Across all of the 5 trees, H' index between fungal communities on pinna and secondary rachis is different about 0.5 points on average at natural decomposition, and the average H' index on pinna was higher overall and consistent across the 5 trees. (table 6.2) According to STDEV value, the diversity on pinna is more variable than

secondary rachis. At 0.5, the Pearson correlation between the fungal communities on pinna and secondary rachis is statistically significant, therefore, since the significance value for change in fungal diversity of pinna and secondary rachis is 0.03 (< 0.05), it was clear that the higher average of diversity index of 0.5 point per single tree on pinna can be attributed to the specificity of fungal community on the palm tissue.

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pinna	2.30400	5	.334929	.149785
	Secondary rachis	1.84580	5	.322114	.144054

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Pinna & Secondary rachis	5	.510	.380

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Pinna – Secondary rachis	.458200	.325361	.145506	.054211	.862189	3.149	4	.035

Table 6.2 Two tail *t*-test tables showing comparison of mean data between fungal diversity indices on pinna and secondary rachis of *Wallichia siamensis*.

A similar result was also shown in the table 6.3. Since the significance value for change in fungal diversity of primary and secondary rachis is 0.01 (< 0.05), the

difference of fungal communities on primary and secondary rachis is also statistically significant and could be attributed to the specificity of the plant tissue in supporting the fungal community diversity. The STDEV value also showed that the fungal diversity on secondary rachis is more variable than primary rachis.

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Primaryrachis	2.69420	5	.304018	.135961
	Secondaryrachis	1.84580	5	.322114	.144054

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Primaryrachis & Secondaryrachis	5	.140	.822

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Primaryrachis - Secondaryrachis	.848400	.410839	.183733	.338276	1.358524	4.618	4	.010

Table 6.3 Two tail *t*-test tables showing comparison of mean data between fungal diversity indices on primary and secondary rachis of *Wallichia siamensis*.

A Hierarchical cluster analysis (HCA), an exploratory tool designed to reveal natural groupings (or clusters) within a data set, showed that two distinct clusters were performed (fig. 6.14). The first cluster contains a group of taxa with frequency of

occurrence less than 25%, and the second cluster contains a group of taxa with frequency of occurrence over 25% (fig. 6.14).

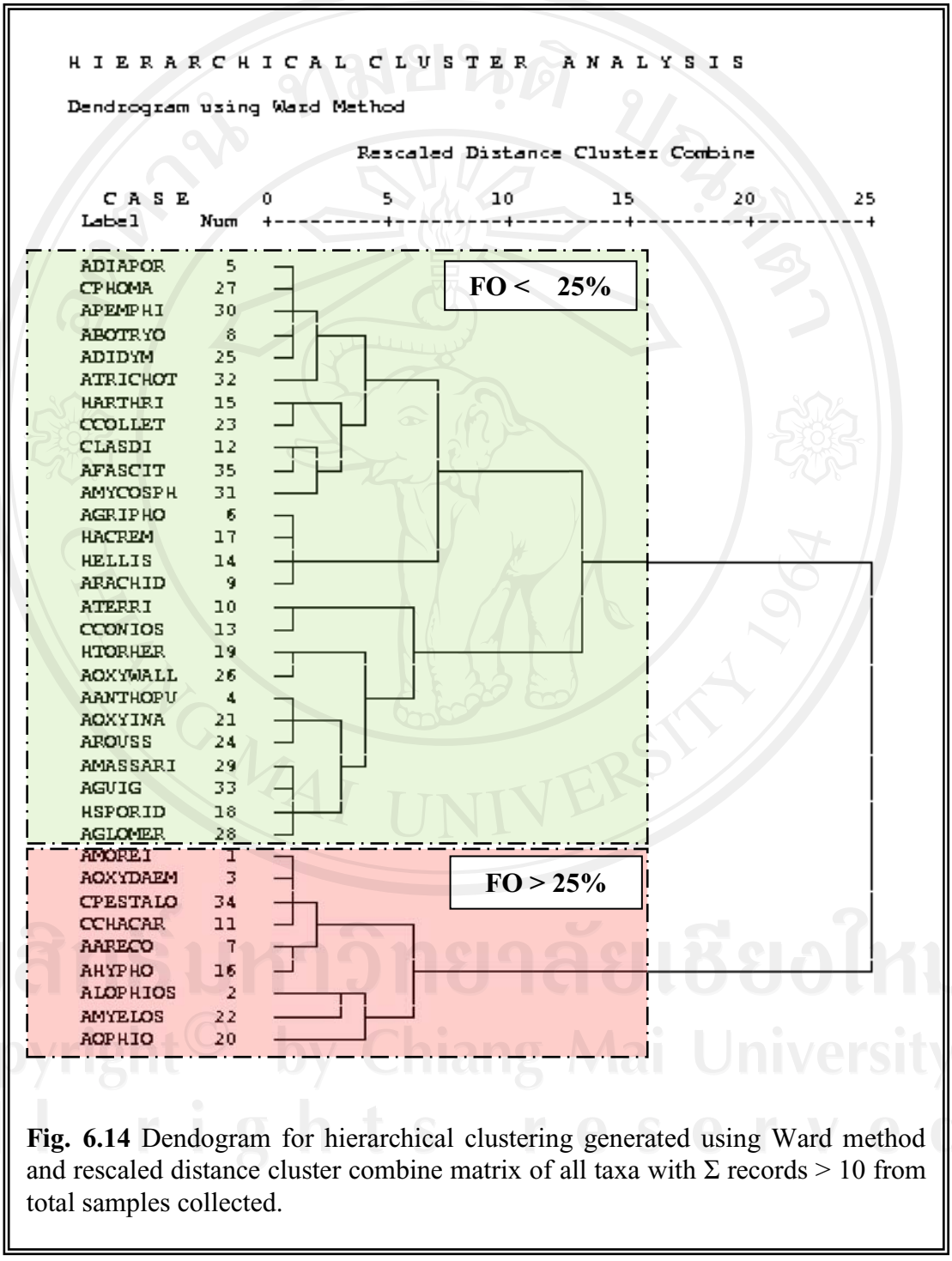

Fig. 6.14 Dendogram for hierarchical clustering generated using Ward method and rescaled distance cluster combine matrix of all taxa with Σ records > 10 from total samples collected.

Among the species within the group of taxa with frequency of occurrence over than 25%, three species, namely, *Ophioceras tenuisporum*, *Lophiostoma macrostomum* and *Morenoina palmicola*, were attributed as indicator species of fungal community on decaying fronds of *W. siamensis* due to they have highest Indicator Value (InVAl) with 65%, 61.3% and 56%, respectively (fig. 6.15). A detail InVAl of each species within the second cluster (FO > 25%) is presented in the table 6.4.

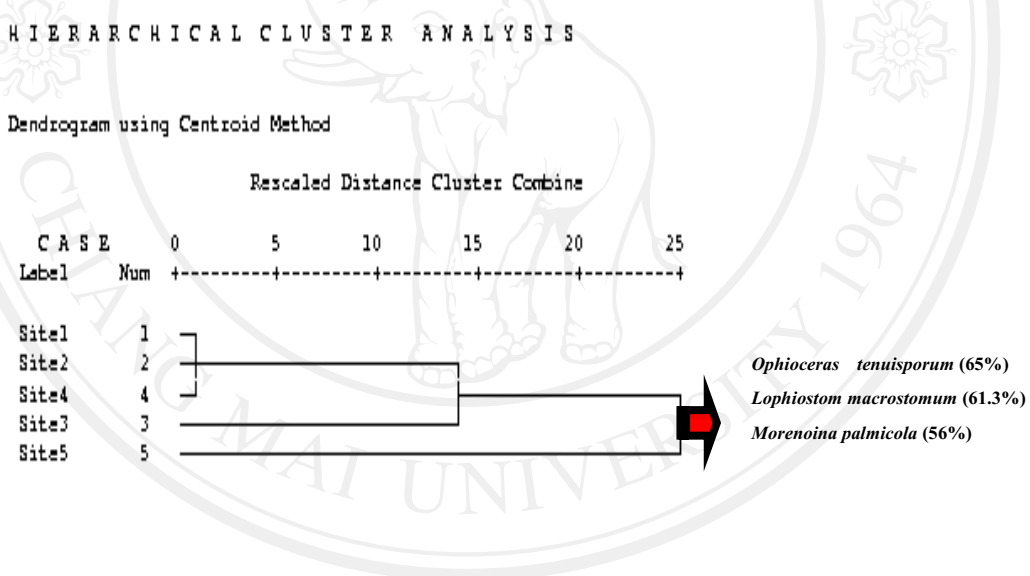

Fig. 6.15 Dendogram representing hierarchical clustering (using Centroid method) of the pooled data from all specimens collected at five sites. Only species with the highest indicator values (>50%) at the main cluster are presented.

Table 6.4 Percentage indicator values (*IndVal* percentage) for all taxa with frequency of occurrence (FO) > 25%.

No.	Taxa	INVAL (from Centroid hierarchical cluster)					
		Cluster 1		Cluster 2		Cluster 3	
		1	2	1	2	1	2
1	***Morenoina palmicola***	**56%**	35.2	32%	45%	30.6%	50.6%
2	***Lophiostoma macrostomum***	16.9%	**61.3%**	43%	45.3%	49%	39.1%
3	*Oxydothis daemonoropsicola*	53%	42.3%	36.9%	50.2%	35.5%	56%
4	*Arecomyces frondicola*	34%	35.7%	10.5%	66.9%	59.5%	30.4%
5	***Ophioceras tenuisporum***	0%	**65%**	0%	86.7%	59.3%	32.6%
6	*Myelosperma tumidum*	49%	42%	26.3%	54.2%	29.8%	56.5%
7	*Chaetospermum chaetosporum*	41.9%	36.9%	40%	38.3%	30.3%	45.4%
8	*Pestalotiopsis guepinii*	47%	39.5%	47.6%	37.6%	40%	40%
9	Unidentified Hyphomycetes 1	34.4%	33%	26.3%	37.4%	24.8%	41%

Box plots diagram was also performed to analyse a distribution of the three indicator species on different tissue types. The box plots diagram showed that *Ophioceras tenuisporum* (fig. 6.16) and *Lophiostoma macrostomum* (fig. 6.17) occurred more frequently on secondary rachis than on pinna and primary rachis, respectively. Another indicator species, *Morenoina palmicola* (fig. 6.18) occurred more frequently on primary rachis than on pinna and secondary rachis. These results are also clearly illustrated in figure 6.19 by using Correspondence Analysis (CA).

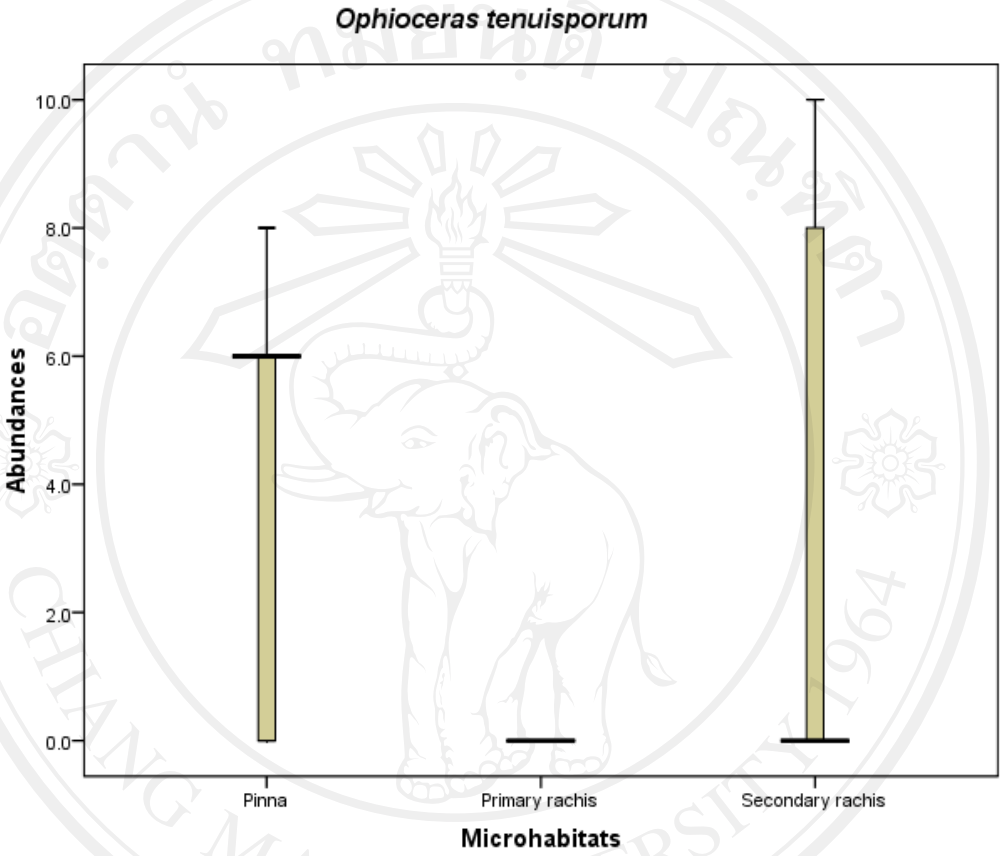

Fig. 6.16 Box plots showing distribution of *Ophioceras tenuisporum* on *Wallichia siamensis* based on tissue types. The lower and upper boundaries of each box enclose 25-75% of the data. The line within the box shows the median values, the bar lines above and below the boxes indicate minimum and maximum values, and ° indicates outliers.

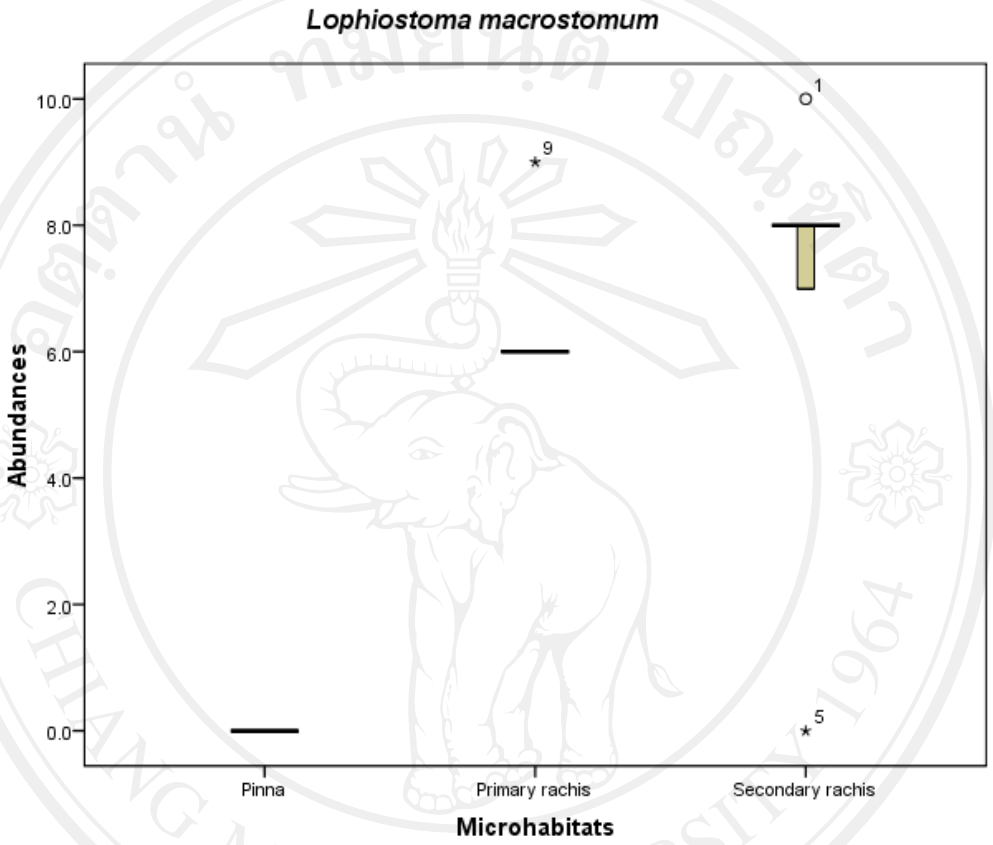

Fig. 6.17 Box plots showing distribution of *Lophiostoma macrostomum* on *Wallichia siamensis* based on tissue types. The lower and upper boundaries of each box enclose 25-75% of the data. The line within the box shows the median values, the bar lines above and below the boxes indicate minimum and maximum values, and ° indicates outliers.

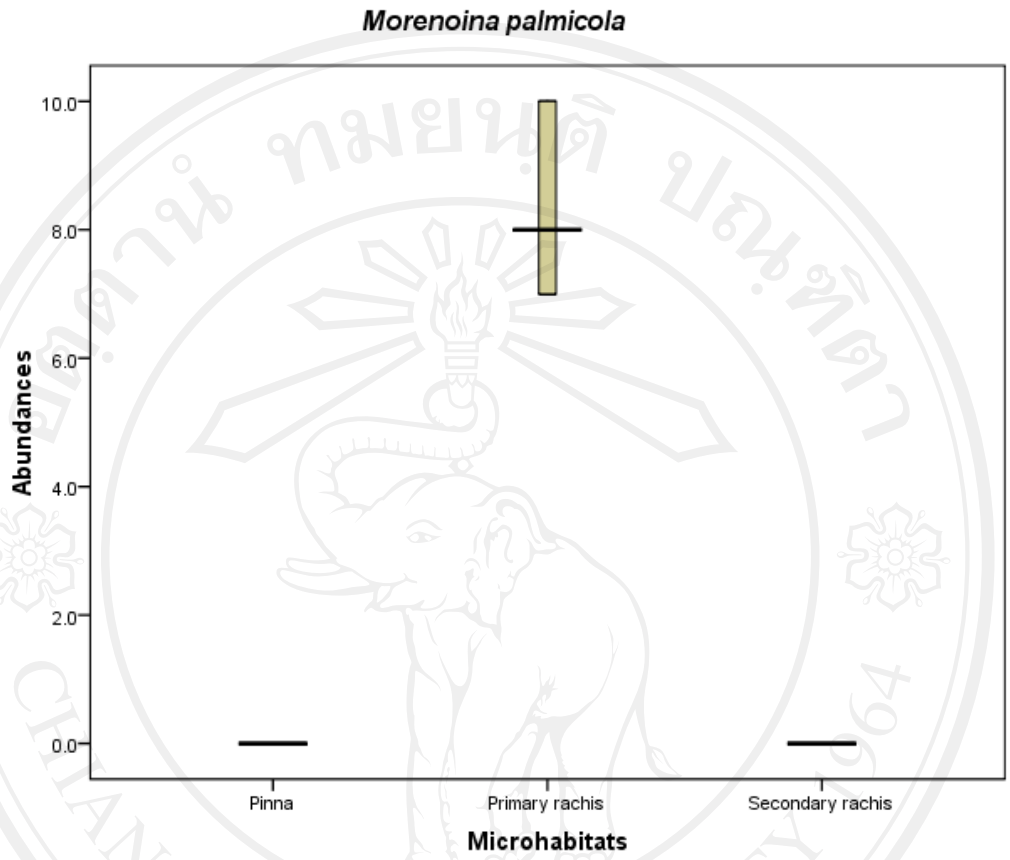

Fig. 6.18 Box plots showing distribution of *Ophioceras tenuisporum* on *Wallichia siamensis* based on tissue types. The lower and upper boundaries of each box enclose 25-75% of the data. The line within the box shows the median values, the bar lines above and below the boxes indicate minimum and maximum values, and ° indicates outliers.

By performing Correspondence Analysis (CA) in order to analyze the relationship between taxa and tissue types (microhabitat), it is apparent that several taxa were well separated in concordance with tissue types (fig. 6.19). On pinna, taxa such as *Phoma* sp., *Colletotrichum gloeosporioides*, *Glomerella cingulata*, *Massarina palmicola* and *Oxydothis wallichianensis*, appear as common species to inhabit this

tissue type (fig. 6.19). On primary rachis, taxa such as *Oxydothis daemonoropsicola*, *Morenoina palmicola*, *Astrosphaeriella fronsicola*, *Anthostomella puigarii* and *Arecomyces frondicola*, were very frequently found (fig. 6.19). On secondary rachis, taxa such as *Myelosperma tumidum*, *Didymosphaeria calamicola*, *Roussoëlla palmicola*, *Oxydothis inaequalis*, *Lasiodiplodia theobromae*, *Terriera brevis*, *Lophiostoma macrostomum*, *Rachidicola obclavatum* and *Sporidesmium* sp., were frequently recorded (fig. 6.19).

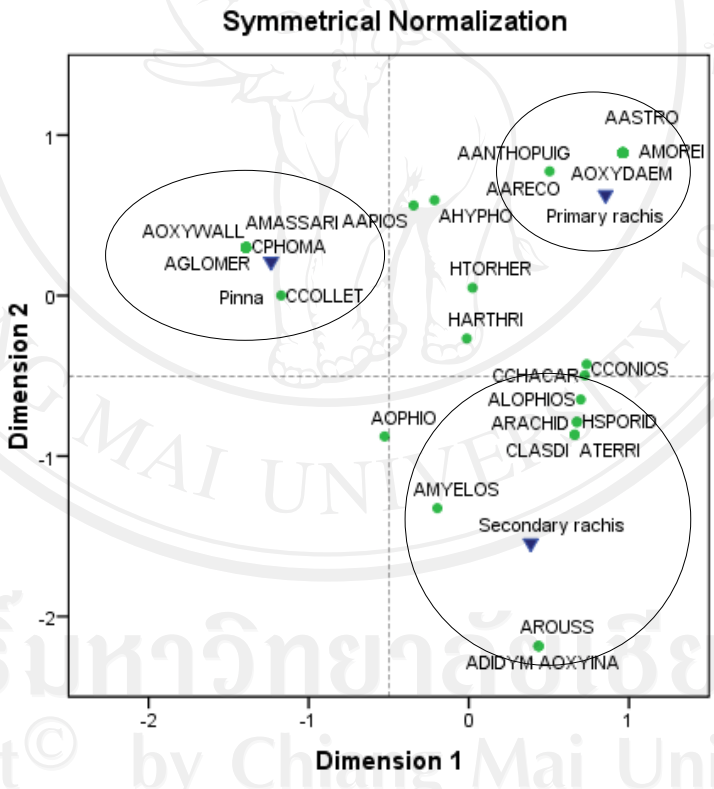

Fig. 6.19 Correspondence Analysis (CA) showing a relationship between taxa (FO > 10%) and tissue types (as microhabitat). Horizontal axis is dimension 1 with inertia = 0.8, vertical axis is dimension 2 with inertia = 0.5. Taxa showed by acronyms.

6.4. Discussion

Fungal Community Composition

The present study apparently showed that taxa belong to ascomycetes (teleomorphic fungi) was more frequently found on decaying fronds of *W. siamensis*. It was shown by the proportion of fungal communities on different tissue types in which exposed the ascomycetous taxa being the highest fungal group in diversity and abundance. The dominance of ascomycetous taxa was probably due to the nature of substratum collected during this study which was mostly at the middle stage of decomposition processes (Dix and Webster, 1985; Gessner *et al*., 1993), and environmental factors (Kane *et al*., 2002). Suzuki (2002) and Kane *et al*. (2002) reported the indication of anamorphic taxa, in particular Hyphomycetes, at early stage of decomposition, followed by ascomycetous and coelomycetous taxa at the later stage, and finally, basidiomycetous taxa. The fact that specimens collected in the present study mostly decayed at the middle or late stage of decomposition, it was not unexpected that the ascomycetous taxa were dominant. This group of fungi is regarded as slow-growing fungi. In addition, competitive interactions among the fungi inhabiting wood substrates seem to be another factor affected the dominance of the ascomycetous fungi on palm tissues as previously reported by Shearer and Zare (1988). Slow-extending fungi such as Ascomycetes were found to be more competitive than fast-extending fungi like Hyphomycetes (Shearer and Zare, 1988; Hyde *et al*., 2005). It was also illustrated by Yuen *et al*. (1999) who indicated that persistent and late colonizers are more likely to produce antagonistic substances so as to inhibit the growth of early colonizers.

The dominance of Ascomycetes was also reported from other palms, namely, *A. alexandrae*, *C. nucifera*, *L. longicalycata* and *N. fruticans*, while, Basidiomycetes were poorly represented in all studies, and this probably reflects the fact that few Basidiomycetes occur on decaying palms (table 6.5).

Table 6.5 A proportion of fungal community diversity on various species of palms.

Host	Ascomycetes	Basidiomycetes	Anamorphic fungi	Total	References
Archontophoenix alexandrae	91 (60%)	1 (1%)	59 (39%)	151	Taylor (1997)
Cocos nucifera	44 (57%)	1 (1%)	32 (42%)	77	Taylor (1997)
Eleiodoxa conferta	45 (48%)	2 (2%)	47 (50%)	94	Pinnoi *et al*. (2006)
Licuala longicalycata	79 (54%)	3 (2%)	65 (44%)	147	Pinruan *et al*. (2007)
Livistona australis	15 (47%)	-	17 (53%)	32	Yanna (2001)
Nypa fruticans	35 (85%)	2 (5%)	4 (10%)	41	Hyde and Alias (2000)
Oraniopsis appendiculatum	24 (43%)	-	32 (57%)	56	Yanna (2001)
Salacca affinis	26 (40%)	3 (5%)	36 (55%)	65	Yanna (2001)
Wallichia siamensis	35 (64%)	-	20 (36%)	55	This study

In regard to host-specificity, Shivas and Hyde (1997) noted that there is a high degree of host-specificity in plant pathogens (parasites) and endophytes, while saprobes are considered the least host-specific fungi (Fröhlich and Hyde, 2000: Taylor and Hyde, 2003). Even though there was little evidence of host specificity within saprobic fungi, however, the result from the present study indicated that at some degree, palmicolous fungi showed specificity on host. Approximately 11 species, viz, *Arecomyces frondicola*, *Diaporthe palmarum*, *Fasciatispora petrakii*, *Lachnum palmae*, *Morenoina palmicola*, *Myelosperma tumidum*, *Oxydothis daemonoropsicola*, *O. inaequalis*, *O. wallichianensis*, *Pemphidium rattanicola* and *Rachidicola obclavatum* have only been found on palm family (Fröhlich and Hyde, 2000: Taylor and Hyde, 2003; Hidayat *et al*., 2006). Of them, *O. inaequalis* and *O. wallichianensis* have only been found on *W. siamensis* (Hidayat *et al.*, 2006). Both *Oxydothis* species are probably host specific at least at family level of the host as Taylor and Hyde (2003) also insisted that the genus *Oxydothis* is probably exclusive palm endophytes and switch to be saprobes once their host is dead.

Indicator Species

Of the three species with the highest indicator species values, only *Morenoina palmicola* and *Lophiostoma macrostomum* were recognized from the previous study as common taxa associated with palms (Fröhlich and Hyde, 2000). Both species were commonly found on various palm species distributed in Australia, Brunei Darussalam, Ecuador and Hong Kong (Fröhlich and Hyde, 2000). Another species, *Ophioceras tenuisporum*, was less common found as dominant species on various species of palms (Fröhlich and Hyde, 2000; Taylor and Hyde, 2003). *Ophioceras tenuisporum*

was commonly reported as saprobic on decaying woody substrates in freshwater habitat (Shearer *et al*., 1999), never as endophyte, however, it was found as an endophyte in the present study (chapter 8). According to this result, therefore, the nature of this species is probably endophyte to woody substrates of plants tissue, and finnaly become saprobes after the death of their hosts. It was also shown by the fact that *O. tenuisporum* restricted to the woody tissues (primary rachis and secondary rachis) on which theywere found and isolated as endophytes.

Two hyphomyceteous fungi, *Arthrinium phaeospermum* and *Torula herbarum*, were encountered on all tissue types, however, they were not categorized as indicator species within fungal community on *W. siamensis* as only few records recorded on each tissue type. It is possible that these taxa only inhabited accidentally on each tissue type. Both taxa are commonly recognized as saprobes on a wide variety of decaying plants (Ellis, 1971, 1976). These species were also frequently isolated from water and soil, and also recognized as cosmopolitan, found in tropical and temperate regions (Ellis, 1971; 1976). On palms, *Arthrinium* was reported as saprobe and endophyte on *Licuala longicalycata* (Pinruan *et al*., 2007), *Cocos nucifera* and *Trachycarpus fortunei* (Taylor and Hyde, 2003), *Rhopalostylis* sp. (McKenzie *et al*., 2004), *Livistona chinensis* (Zhuang, 2001) and *Calamus* sp. (Thaung, 2008). On the other hand, *Torula herbarum* was frequently found on *Archontophoenix alexandrae* and *Trachycarpus fortunei* (Taylor and Hyde, 2003), *Areca catechu* (Matsushima, 1980) and *Cocos nucifera* (Taylor, 1997).

Is fungal community on Wallichia siamensis tissue preferences?

The recurrence of fungi on different tissue types was also examined and analyzed in order to reveal the specificity of palmicolous fungal communities with palm tissues. It was apparent that about 21 species of fungi on *W. siamensis* were found exclusively on primary rachis (fig. 7.18); three species, namely, *Roussoëlla palmicola*, *Didymosphaeria calamicola* and *Oxydothis inaequalis* found exclusively on secondary rachis; and seven species, namely, *Oxydothis wallichianensis*, *Phoma* sp., *Glomerella cingulata*, *Massarina palmicola*, *Pemphidium rattanicola*, *Lachnum palmae* and *Eriosporella calami*, were only found on pinna (fig. 6.20). On the other hand, two hyphomycetous fungal species, *Arthrinium phaeospermum* and *Torula herbarum*, were found on all tissue types (fig. 6.20). Distinct fungal community composition occurs on different tissue types of *W. siamensis* indicated the specificity or exclusive association between the palmicolous fungi to different palm tissue. The differences among fungal community structures colonising pinna, primary rachis and secondary rachis were also significantly supported by two tail *t*-test analysis. This result supports the previous studies reported by Taylor and Hyde (2003) and Yanna (2001).

The specificity of fungal community on palms tissues is probably related to the difference in nutrients available and physical structures of each tissue. For examples, thin-walled parenchymatous cells in leaflets and thick-walled cells in petioles and rachides; cellulose and lignin substrates availability in trunks, petioles and leaflets; abundant vascular bundles and associated sclerenchyma in trunks and petioles which could retain more moisture and dry out more slowly (Tomlinson, 1990). All these factors enable more complex fungal communities to develop and

colonize the different substrates. Petrini *et al*. (1992) and Hyde *et al*. (2005) also remarked that different plant tissues and organs may, in fact, resemble distinct microhabitats, and there are a number of different microhabitats, even within a single plant. Therefore, there should be a large number of fungi in their specific microhabitats which have not yet been explored as whole plants have rarely been examined for fungi. These missing fungi may provide potential genetic resources for humankind such as antibiotic, biocontrol agents, enzymes and so forth.

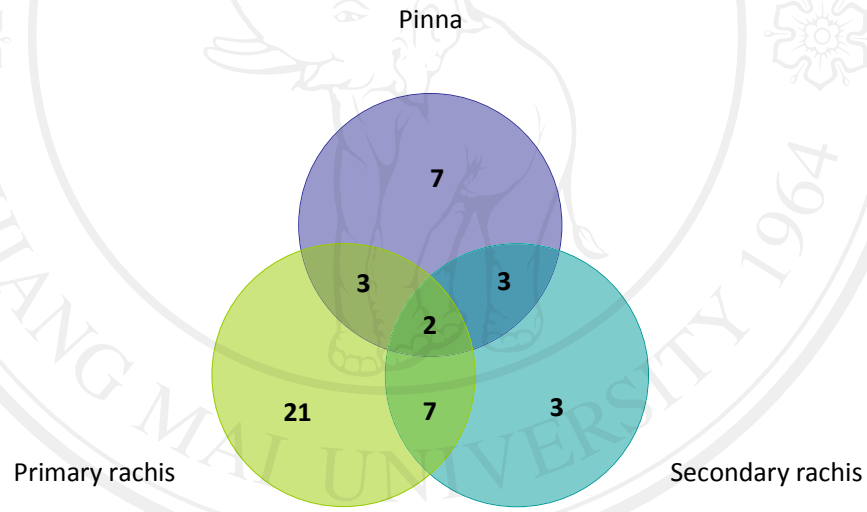

Fig. 6.20 Proportion number of fungi overlapping among different tissue types of *Wallichia siamensis*.

The recurrence of distinct saprobic fungi on different tissue types was also reported in other habitats and plants (Adaskaveg *et* al., 1991; Yanna *et al.*, 2001; Yanna, 2001; Cai *et al*., 2006). The repeated occurrence of certain fungi on different

tissue types might be due to the differences in nutritional requirements of the fungi, or the ability of fungi to utilize different substrates (Adaskaveg *et al*., 1991). For example, several polyporous fungi such as *Ganoderma zonatum* and *Phanerochaete chrysosporium* showed their ability to utilize lignin in fibers near vascular tissues in palm fronds of *Phoenix canariensis* (date palm) (Adaskaveg *et al*., 1991). The petioles of palms differ from the leaflets as they have more concentrated supportive tissue, and the texture of rachis-bases also differs from rachis-tips and mid-rachides (Tomlinson, 1990). It was clearly shown by this study in which fungal community on primary rachis was much closer/more similar to the fungal community on secondary rachis.