Phylogenetic evaluation of species nomenclature of *Pestalotiopsis* in relation to host association

R. Jeewon[2*], E.C.Y. Liew[2] and K.D. Hyde[1]

[1]Centre for Research in Fungal Diversity, Department of Ecology & Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, PR China
[2]School of Land, Water and Crop Sciences, McMillan Building A05, The University of Sydney, NSW 2006, Australia

Jeewon, R., Liew, E.C.Y. and Hyde K.D. (2004). Phylogenetic evaluation of species nomenclature of *Pestalotiopsis* in relation to host association. Fungal Diversity 17: 39-55.

DNA data from a number of *Pestalotiopsis* isolates were analysed to investigate whether isolates from the same host are phylogenetically related. The validity of naming species of *Pestalotiopsis* based on host association was investigated. Regions of the ITS and 5.8S of the rDNA gene were amplified from genomic DNA using PCR. DNA characters were analysed using maximum parsimony (weighted and unweighted) and maximum likelihood criteria. Isolates from the same host were not phylogenetically closely related. A close phylogenetic relationship between isolates possessing similar morphological characters was apparent. Results indicate that the naming of species based on host association is unwise and dispute the assumption that species are host-specific. When new *Pestalotiopsis* species are described, morphological characters should be taken into account rather than host association. The implications of the results on fungal biodiversity studies are discussed.

Key words: host association, morphology, nomenclature, *Pestalotiopsis*, phylogeny, rDNA gene.

Introduction

Species of *Pestalotiopsis* commonly cause diseases on a variety of plants (Hyde and Fröhlich, 1995; Swart *et al*., 1999; Hopkins and McQuilken, 2000; Rivera and Wright, 2000), are commonly isolated as endophytes (Brown *et al*., 1998; Suryanayaranan *et al*., 2000; Toofanee and Dulymamode, 2002; Worapong *et al*., 2002; Wei and Xu, 2004) or occur as saprobes (Yanna *et al*., 2002). *Pestalotiopsis agallochae* from *Excoecaria agallocha* and numerous other isolates from *Leucospermum* sp. have been reported to cause leaf spots (Pal and Purkayastha, 1992; Taylor *et al*., 2001). *Pestalotiopsis longisetula* has been observed to be the major pathogen causing rot lesions on strawberry fruit

*Corresponding author: R. Jeewon; e-mail: rjeewon@graduate.hku.hk.

(Howard and Albregts, 1973). The same species and *P. theae* have been described as pathogenic on tea, *Camellia sinensis* (Koh *et al*., 2001).

Pestalotiopsis contains about 205 named species (Funindex, http://194.131.255.3/cabipages/names/Names.asp), many named after the host on which they were first observed. The understanding of species relationships within this weakly parasitic genus has been complicated because of inadequate morphological characters available to differentiate species. The conidia (asexual spores) are usually fusiform, straight or slightly curved, and 3-4 euseptate. Median cells are pigmented (either concolourous or versicolourous). Apical appendages are mostly filiform, either knobbed or not, one to many (most often 2-3), branched or unbranched. Basal appendages of 4-9 μm are usually present, and arise from a basal cell (Guba, 1961; Worapong *et al*., 2002; Wei and Xu, 2004).

In many instances, the association with specific hosts has provided a convenient means for species separation as well as describing new species. This practice has been common and has ultimately led to the assumption that some fungi are generally host-specific (Zhou and Hyde, 2001). Pal and Purkayastha (1992) described *Pestalotiopsis agallochae*, which causes leaf spots on *Excoecaria agallochae* based on the host association. Venkatasubbaiah *et al*. (1991a) reported that a correlation exists between the host plant *Oenothera laciniata* and *Pestalotiopsis oenothera* as this species was found to produce some important secondary metabolites. Despite the fact that the latter species bears morphological resemblances to many other *Pestalotiopsis* species, it was described as a new species because it was the first time that a *Pestalotiopsis* species was isolated from *Oenothera laciniata* (Venkatasubbaiah *et al*., 1991b). Kohlmeyer and Kohlmeyer (2001) described a new species of *Pestalotiopsis*, *P. juncestris,* which is very similar to *P. versicolor* and other similar species described in Guba (1961), but appears to have been described as a new species based on the fact that this species was recorded on the host *Juncus roemerianus*. A similar approach was used by Patil and Thite (1977), Sohi and Prakash (1979), Singh (1981), and Yuan (1996) who introduced *Pestalotiopsis embeliae*, *P. chethallensis*, *P. arborei*, and *P. acaciae* respectively, based on host associations rather than unique morphological characters. Nevertheless, some new species have been named based on morphological characters as well as host. For instance, Zhu *et al*. (1991) observed the anamorph of *Pestalosphaeria janggangensis* and named it *Pestalotiopsis podocarpi* based on the host *Podocarpus nagi* as well as on morphological and cultural evidence.

Although many species names of *Pestalotiopsis* refer to the host species from which they were recovered, some investigations have shown that

Pestalotiopsis species are not highly host-specific. In addition, morphologically distinctive taxa, usually considered to be host-specific, have been recorded on a wide variety of hosts (Mordue and Holliday, 1971; Mordue, 1976). The fact that a large number of *Pestalotiopsis* species have been recorded on one host only does not mean that they are host-specific. These *Pestalotiopsis* species described from one specimen, are probably doubtful species. In addition, it is highly probable that many taxa cited in the literature are based on misinterpretations or synonyms of species with wide host ranges.

Several species have already been reassessed, redescribed and synonymised since their initial identification (Nag Rag, 1985, 1986, 1989, 1993). El Sayed *et al*. (1985) demonstrated that spore inocula of *P. mangiferae* produced disease symptoms on *Eucalyptus camaldulensis* and *E. torquata*. *Pestalotiopsis mangiferae* has also been reported to occur on multiple hosts such as *Elaeis guineensis*, *Hyphaene thebaica*, *Mangifera indica*, *Vitis vinifera* and other unrelated hosts (Guba, 1961; Mordue, 1980). La Rue and Bartlett (1921) isolated strains of *Pestalotiopsis guepini* from different hosts and stated that these strains did not appear to be confined to particular hosts. Suto and Kobayashi (1993) examined herbarium specimens of *Pestalotiopsis* species reported to infect conifers in Japan and synonymised several species based on morphology. They stated that difference of host plant should not be used as a criterion for distinguishing species. Recently, Hopkins and McQuilken (2000) assessed the pathogenicity of 18 isolates of *Pestalotiopsis sydowiana* and demonstrated that the isolates were not host-specific and infected other species of hardy ornamentals other than from those they were originally isolated from. There is little direct evidence to support host-specificity of *Pestalotiopsis* species and additional work is needed to understand host range and host range of these species.

The concept of naming *Pestalotiopsis* species, especially those which are weakly parasitic, based on host and disease symptoms, is probably an inaccurate means of naming a particular species. Whether genetic differences among morphologically indistinguishable species from different hosts (those assumed to be host-specific) reflect an evolutionary adaptation to different hosts remains to be investigated.

This study reports a molecular phylogenetic approach to determine phylogenetic relationships among morphologically indistinguishable species from different hosts. *Pestalotiopsis* isolates from the host *Scaevola hainanensis*, *Leucospermum, Protea mellifera* and several other hosts were made in an attempt to determine whether host is phylogenetically predictive. DNA sequences from the rDNA gene region were used in the phylogenetic analyses.

Materials and methods

Fungal strains and DNA extraction

Fungal strains used for this study are listed in Table 1. *Pestalotiopsis gracilis, Pestalotiopsis* sp. EN2*, Pestalotiopsis* sp. EN4*, Pestalotiopsis* sp. EN5, *Pestalotiopsis* sp. EN6*, Pestalotiopsis* sp. EN7*, Pestalotiopsis* sp. EN8*, Pestalotiopsis* sp. EN9, *Pestalotiopsis* sp. EN10 *and Pestalotiopsis* sp. EN12 were obtained from the same host, *Scaevola hainanensis*, which was collected in Hong Kong. *Pestalotiopsis theae* and *P. sydowiana* isolates were obtained from *Protea mellifera* while *Pestalotiopsis aquatica, P. longisetula, Pestalotiopsis vismiae, Pestalotiopsis* sp.7*, Pestalotiopsis* sp.8*, and Pestalotiopsis* sp.9 were isolated from *Leucospermum* sp. Taxa used were identified based on the keys provided by Steyaert (1949), Guba (1961) and Nag Rag (1993). All fungi used in this experiment were cultured on Potato Dextrose Agar and total genomic DNA was extracted following a 2X CTAB protocol as outlined by Jeewon *et al*. (2003) and Lacap *et al*. (2003).

Amplification and sequencing of the nuc ITS rDNA

Polymerase chain reaction amplification products spanning approximately 600 bp of the ITS regions and 5.8S gene of the rDNA molecule were amplified with primers ITS 4 and ITS 5 (White *et al*., 1990; Worapong *et al*., 2002). The amplification reaction included 5 ng of fungal genomic DNA as template, enzyme buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.3 µM of each primer and 1.5 units of Taq DNA Polymerase (total volume of 50 µL). Thermal cycling parameters included an initial denaturation of 94ºC for 3 min, followed by 29 cycles consisting of denaturation at 94ºC for 1 min, annealing at 54ºC for 50 sec, and extension of 72ºC for 1 min. A final extension at 72ºC for 10 mins was included at the end of the thermal cycling. Amplified products were visualised on 1% agarose gel to check for product size and purity. PCR products were purified using minicolumns containing purification resin according to the manufacturer's protocol (Promega Wizard PCR Preps DNA Purification System). Primers ITS 2, ITS 3, ITS 4 and ITS 5 (White *et al*., 1990) were used to sequence both strands of the DNA molecule in an automated sequencer (ALF Express, Pharmacia-Biotech, Piscataway, NJ, USA) following the manufacturer's protocols.

Table 1. Representative strains of *Pestalotiopsis* used in this study, their accession numbers, host and geographical origin.

Species	Source of Culture[a]	Host/ Geographic Origin/ Disease	GenBank Accession No
Pestalotiopsis adusta	ICMP 5434	*Digitalis purpurea*, New Zealand, leaf spot	AF409955
Pestalotiopsis aquatica	HKUCC 8311	*Leucospermum* sp., S. Africa, leaf spot	AF409956
Pestalotiopsis bicilia	BRIP 25718	*Xanthorrhoea* sp., Australia, leaf spot	AF409973
Pestalotiopsis dichaeta	BRIP 25627	*Bletia* sp., Australia, Grey Blight	AF409987
Pestalotiopsis funerea	ICMP 7314	*Cupressocyparis leylandii,* New Zealand, Canker	AF405299
Pestalotiopsis gracilis	HKUCC 8320	*Scaevola hainanensis,* Hong Kong, China, NA	AF409962
Pestalotiopsis longisetula	STE-U 1771	*Leucospermum* sp., Cecily, Leaf spot	AF409971
Pestalotiopsis sinensis	BRIP 25617	*Chrysalidocarpus lutescens,* Australia, NA	AF409966
Pestalotiopsis sydowiana	HKUCC 8326	*Protea mellifera*, S. Africa, NA	AF409970
Pestalotiopsis theae	HKUCC 7982	*Protea mellifera*, S. Africa, NA	AF405297
Pestalotiopsis uvicola	BRIP 25613	*Verticordia* sp., Australia, Collar rot	AF409994
Pestalotiopsis versicolor	BRIP 14534	*Psidium guajava*, Australia	AF405298
Pestalotiopsis virginiana	HKUCC 8380	*Polygonum multiflorum*, Hong Kong, NA	AF409959
Pestalotiopsis vismiae	HKUCC 8328	*Leucospermum* sp., Hawaii, USA, leaf spot	AF409977
Pestalotiopsis sp. EN2	HKUCC 8370	*Scaevola hainanensis,* Hong Kong, China, NA	AF409964
Pestalotiopsis sp. EN4	HKUCC 8372	*Scaevola hainanensis,* Hong Kong, China, NA	AF409983
Pestalotiopsis sp. 4	BRIP 25624	*Nepenthes khasiana*, Australia	AF409989
Pestalotiopsis sp. EN5	HKUCC 8373	*Scaevola hainanensis,* Hong Kong, China, NA	AF409960
Pestalotiopsis sp. EN6	HKUCC 8374	*Scaevola hainanensis,* Hong Kong, China, NA	AF409981
Pestalotiopsis sp. 7	HKUCC 8325	*Leucospermum* sp., S. Africa, NA	AF409979
Pestalotiopsis sp. EN7	HKUCC 8375	*Scaevola hainanensis,* Hong Kong, China, NA	AF409982

Table 1 continued. Representative strains of *Pestalotiopsis* used in this study, their accession numbers, host and geographical origin.

Species	Source of Culture[a]	Host/ Geographic Origin/ Disease	GenBank Accession No
Pestalotiopsis sp. 8	HKUCC 8324	*Leucospermum* sp., S. Africa, leaf spot	AF409961
Pestalotiopsis sp. EN8	HKUCC 7984	*Scaevola hainanensis,* Hong Kong, China, NA	AF405294
Pestalotiopsis sp. 9	STE-U 1755	*Leucospermum* sp., S. Africa, leaf spot	AF409980
Pestalotiopsis sp. EN9	HKUCC 8319	*Scaevola hainanensis,* Hong Kong, China, NA	AF409963
Pestalotiopsis sp. EN10	HKUCC 7985	*Scaevola hainanensis,* Hong Kong, China, NA	AF409978
Pestalotiopsis sp. EN 12	HKUCC 8321	*Scaevola hainanensis,* Hong Kong, China, NA	AF409994
Pestalotiopsis sp. EN 14	HKUCC 3183	*Kandelia candel*, Hong Kong, China, NA	AF409965
Bartalinia robillardoides	BRIP 14180	*Macrotyloma daltonii,* Australia	AF405301

[a]BRIP: Queensland Department of Primary Industries Plant Pathology Herbarium; HKUCC: The University of Hong Kong Culture Collection; ICMP: International Collection of Microorganisms from Plants; STE-U: University of Stellenbosch Culture Collection.

Phylogenetic analysis

Nucleotide sequences of the 5.8S gene and the internal transcribed spacers (ITS 1 and ITS 2) generated from the four primers were assembled and aligned using Clustal X (Thompson *et al*., 1997) and SeqPup (Gilbert, 1996). Manual gap adjustments were made to improve the alignment. Sequence data for all the isolates were deposited in GenBank (see Table 1 for accession numbers). The DNA matrix for phylogenetic analyses consisted of 600 sites within the boundaries of ITS1 and ITS 2 including the 5.8S gene. Phylogenetic analyses were conducted employing three optimality criteria in PAUP*4.0b10 (Swofford, 2001): Maximum Parsimony (MP), Weighted Parsimony (WP) and Maximum Likelihood (ML).

ITS data were analysed initially using MP and WP. All characters were unordered and heuristic searches were conducted under different conditions (different transition transversion ratios and treating gaps as missing data or fifth state) to test the phylogenetic relationships among the taxa and to determine the most appropriate parameters giving the best trees for subsequent analyses. The heuristics search option was used, ignoring invariant and uninformative characters. Random addition of sequences for 10 replicates with

tree bisection-reconnection (TBR) branch swapping on 5000 trees per replicate, and 100 replicates with TBR swapping on 1000 trees per replicate were performed using the following settings: multrees, steepest descent, maxtrees = 30,000. When gaps were treated as newstate, a region of the ITS 1 region consisting of 30 nucleotides (nucleotide 117-146 of the aligned data) were excluded in the analysis because of a number of gaps which created ambiguous alignment. This treatment optimises the order of sequences and reduces the effect of false positive results. To increase the probability of finding all most parsimonious trees, heuristic searches using 1000 rounds of random addition with tree bisection-reconnection (TBR) branch swapping was implemented to generate parsimony trees. Bootstrap analysis was based on 1000 resampled data sets analysed with random addition of taxa to assess clade stability. Tree scores, including consistency index, retention index, rescaled consistency index and homoplasy index (CI, RI, RC and HI) were also calculated for all the trees generated under different conditions as measures of homoplasy in the data. *Bartalinia robillardoides* was used as the outgroup to determine character polarity based on its phylogenetic relatedness to *Pestalotiopsis* (Jeewon *et al*., 2002).

Transition transversion ratio, shape parameter and base frequencies were estimated to find the maximum likelihood tree. These parameters were estimated before the ML analysis by using a strict consensus tree derived from an earlier MP analysis as a reasonable estimate of the phylogeny (Swofford *et al*., 1996). The gamma model of site rate variation with a shape parameter of 0.5 and transitions weighted two times over transversions was also used with no enforcement of a molecular clock. Initial branch lengths were obtained using Rogers-Swofford approximation methods and two models of nucleotide substitution- the HKY (Hasegawa *et al*., 1985) and F84 (Felsenstein, 1984) were used.

Kishino-Hasegawa tests (Kishino and Hasegawa, 1989) and Templeton tests (Templeton, 1983), as implemented in PAUP*, were used to estimate topological differences between trees and to determine whether the trees inferred from the different optimality criteria were significantly different. Trees were viewed in Treeview (Page, 1996).

Results

The MP heuristic search treating gaps as missing data with transitions and transversions weighted equally resulted in 3246 trees of 129 steps, consistency index = 0.853, retention index =0.963, rescaled consitency index = 0.821, homoplasy index = 0.147 and - ln likelihood = 1542.4023. All the trees had essentially similar branching patterns and the strict consensus tree is shown

in Fig. 1. The data set could not be bootstrapped as the process was computationally too demanding. The analysis had to be aborted after 20 replicates. When gaps were treated as newstate in the unweighted parsimony analysis, 4 equally most parsimonious trees were obtained (TL = 173 steps, consistency index = 0.850, retention index = 0.970, rescaled consitency index = 0.824, homoplasy index = 0.150 and – ln likelihood = 1460.2544). The topology of the tree was identical to the tree shown in Fig. 1 (results not shown).

Analysis of the same data set with weighted parsimony (a transition transversion ratio of 1.5:1, as related to the estimated values from likelihood tests) and treating gaps as new state, yielded only two most parsimonious trees, the consensus of which is shown in the Fig. 2 (TL = 190 steps, consistency index = 0.845, retention index = 0.968, rescaled consistency index = 0.818, homoplasy index = 0.155 and - ln likelihood = 1458.9744). In contrast, weighted parsimony analysis with gaps treated as missing data resulted in trees identical to that in Fig. 1.

ML searches under the HKY model, transition weighted two times over transversion, shape parameter of 0.5 and estimated base frequencies and yielded one tree identical to Fig. 1 (results not shown). Tree length was 129 steps, consistency index = 0.853, retention index = 0.963, rescaled consitency index = 0.821, homoplasy index = 0.147 and - ln likelihood = 1525.8107. Estimated base frequencies were as follows: A = 0.2571, C = 0.2251, G = 0.2108 and T = 0.3068. When transition-transversion ratio and shape parameter estimated, the ML analyses produced 2 trees, which was also identical in topology to that of Fig. 1 (results not shown). Estimated transition-transversion ratio and shape parameter was 2.32971 and 0.143189, respectively.

The Kishino-Hasegawa and non-parametric tests (Templeton test) showed that trees generated by different search strategies under different optimality criteria were not significantly different ($P< 0.05$) than the optimal tree (Table 2). In addition, all the phylogenetic inference tools applied to the sequence data gave trees of almost similar topologies.

To infer phylogenies among isolates in this study, the WP tree (Fig. 2) has been selected because the robustness of the clades could be statistically assessed by bootstrapping and it also shows relationships which are more resolved. Fig. 2 identifies two notable taxonomic groupings (Clades A and B) similar to those of Fig. 1 but Clade A is slightly more resolved. Most of the clades are supported by high bootstrap values (> 65%) except for three of them with 56%, 58% and 54% bootstrap support. Clades A and B consist of isolates that are closely related to each other and supported by a 100% bootstrap confidence.

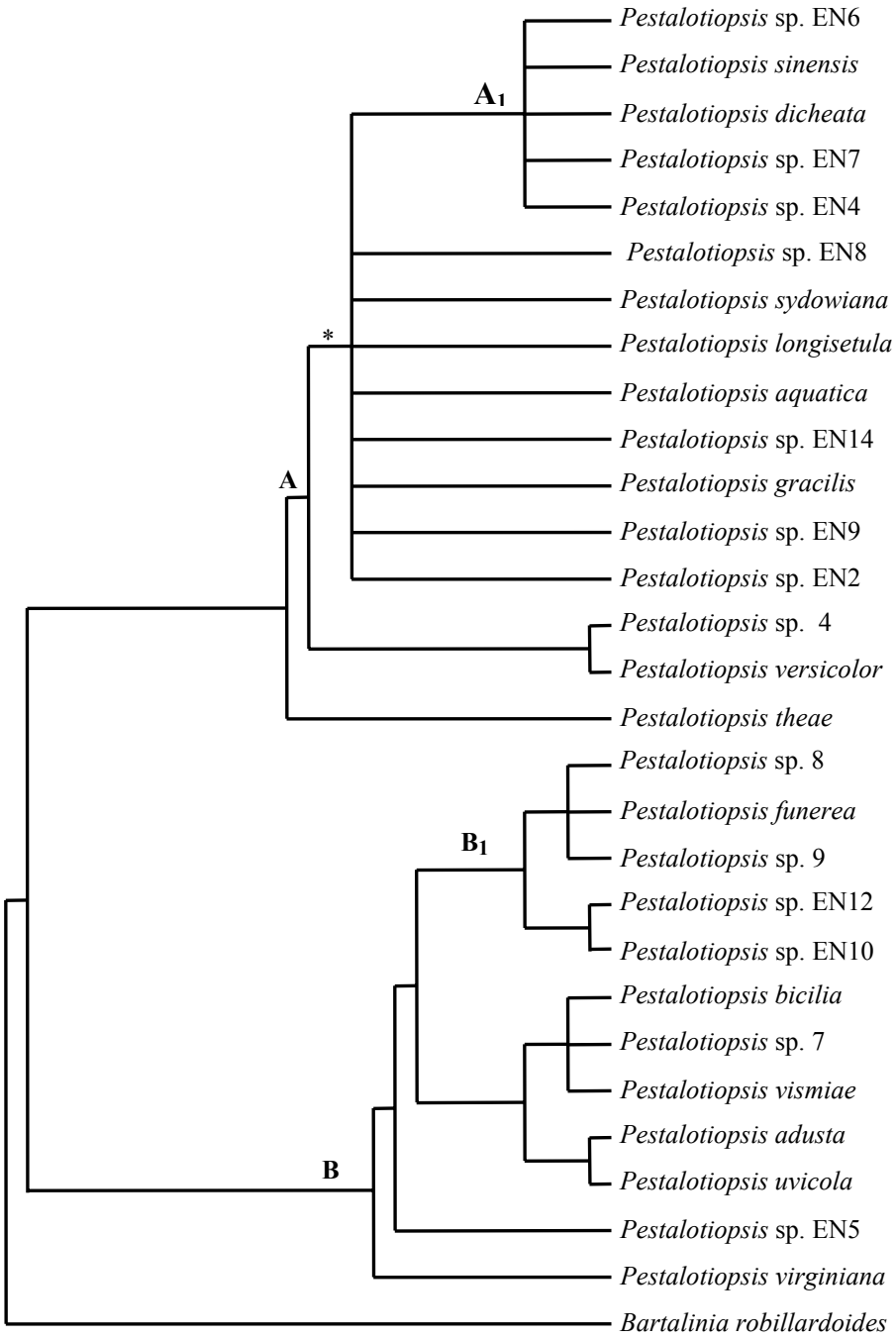

Fig. 1. Strict consensus of 3246 trees generated by equally weighted parsimony and treating gaps as missing data. TL= 129 steps, CI= 0. 853, - Log likelihood =1542.4023. * shows the clade which was partially resolved in Fig. 2. Letters A and B above the branches represent the different monophyletic groups possessing distinct morphological characters. *Bartalinia robillardoides* is the outgroup taxa.

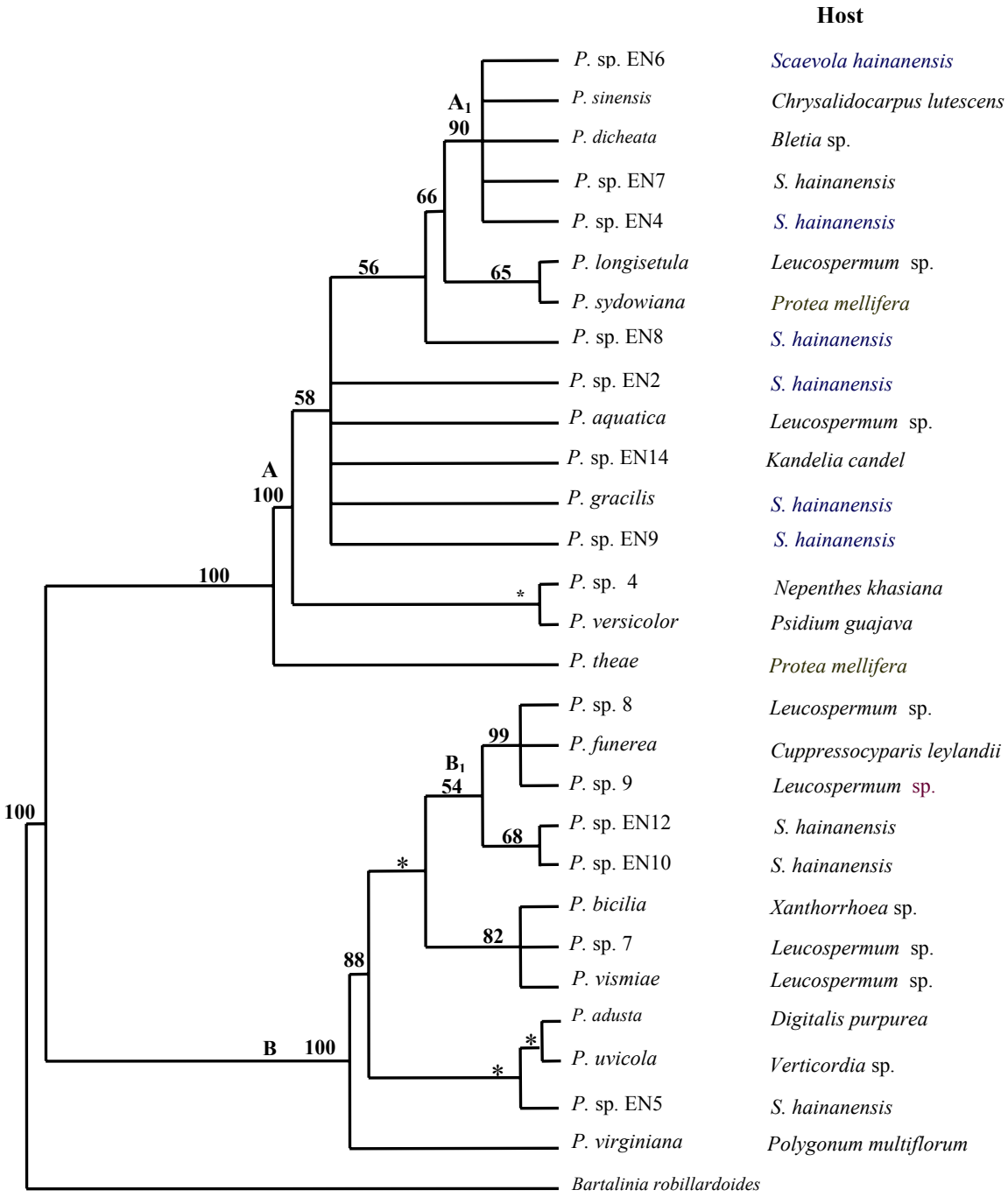

Fig. 2. Consensus of 2 trees obtained from a weighted parsimony (Transition Transversion ratio=1.5:1) and treating gaps as newstate. TL= 190 steps, CI= 0.845, -Log likelihood= 1458.9744. Numbers above branches are parsimony bootstrap percentages from 1000 replicates. * shows the clades which had < 50% bootstrap support. Letters A and B above the branches represent the different monophyletic groups possessing distinct morphological characters. *Bartalinia robillardoides* is the designated outgroup taxa.

Table 2. Results of the Kishino-Hasegawa (KH) and Templeton Test for the trees generated by different optimality criteria.

	MP (Fig. 1) [a]	WP (Fig. 2) [b]	ML [c]	ML [d]
CI	0.853	0.845	0.853	0.840
Tree length	129	190	129	131
- Ln Likelihood	1542.4023	1458.9744	1521.6173	1525.8107
KH Test[e]	P=0.3177	Best	P= 0.5641	P=0.3177
Templeton Test[e]	P= 0. 3173	Best	P= 0.5637	P= 0.3173

[a]MP tree treating gaps as missing data and transition transversion given equal weight.
[b]WP tree treating gaps as fifth state and a transition transversion of 1.5:1.
[c]ML tree generated by weighting transition 2 times over transversions and shape parameter of 0.5.
[d]ML tree obtained by estimating transition transversion ratio and shape parameter.
[e]Probability of getting a more extreme T-value under the null hypothesis of no difference between the two trees (two tailed test) with significance at $P< 0.05$.

The hosts from which the strains have been isolated are shown in Fig. 2. Phylogenetic analyses do not support a close relationship between species isolated from the same host. Isolates from *Scaevola hainanensis* intermingle with other isolates obtained from other hosts in all the clades. *P. sydowiana* and *P. theae*, which were both isolated from *Protea mellifera* did not cluster together. In addition, *P. aquatica*, *P. longisetula*, *P. vismiae* and *Pestalotiopsis* sp.9, isolated from *Leucospermum sp*. do not appear to be phylogenetically close to each other. It can be observed that species relationships are independent of host and that isolates that group together in the phylogenetic tree are similar in morphology. For instance, *P. dichaeta*, *P. sinensis*, *Pestalotiopsis* sp. EN4, *Pestalotiopsis* sp. EN6 and *Pestalotiopsis* sp. EN7, which were isolated from *Bletia* sp, *Chrysalidocarpus lutescens* and *Scaevola hainanensis* are closely related to each other and form a well supported group with high bootstrap support (90%). All these isolates are characterised by: brown median cells (with a total conidial length of >25 31.µm) which are slightly constricted at the septa; short, hyaline and conic apical cell with mostly 3 apical appendages (< 20 µm in length); basal cells that are conic bearing basal appendages of 4-7 µm. Similarly, *P. longisetula*, *P. sydowiana* and *Pestalotiopsis* sp. EN8, isolated from *Leucospermum* sp., *Protea mellifera* and *Scaevola hainanensis* respectively are phylogenetically related. The conidial morphologies of these species are similar and characterised by versicolorous median cells, conidial length of greater than 20 µm and apical appendage length of 20-25 µm.

Species in Clade A (excluding *P. theae* and Clade A_1) are characterised by the presence of versicolorous median cells, conidial length greater than 20

μm and apical appendages ranging from 20-35 μm whereas species in Clade B are characterised by concolorous median cell with diverse spore sizes. These clades are based on morphological similarities rather than relatedness to host.

Discussion

In the present study, rDNA sequences from morphologically similar and dissimilar taxa from the same and different hosts were analysed in order to establish whether species nomenclature based on host association has any phylogenetic significance. Phylogenies generated here indicate a close relationship among morphologically related species rather than association with host, and hence provide new insights to the concept of host-based nomenclature in *Pestalotiopsis* species.

A noteworthy observation is the relationship of *Pestalotiopsis* sp. EN5, *Pestalotiopsis* sp. EN6, *Pestalotiopsis* sp. EN8, *Pestalotiopsis* sp. EN9 and *Pestalotiopsis* sp. EN10. All of these isolates were from *Scaevola hainanensis* but are phylogenetically distant from each other. Each of these isolates clusters with others with similar conidial morphology, independent of the host from which they were isolated. In addition the two endophytic isolates *Pestalotiopsis sydowiana* and *P. theae* from *Protea mellifera* do not appear to be closely related. The latter is characterised by three brown concolorous median cells with long fusiform conidia (>25 μm) and apical appendages (25-40 μm) with apical knobs. *Pestalotiopsis sydowiana* possesses similar morphology except that median cells are versicolorous and apical appendages not knobbed. Morphological characters of some of the isolates used in this study have already been detailed in Jeewon *et al*. (2002, 2003) and not discussed here.

Pestalotiopsis sydowiana isolated from *Protea mellifera* was closest to *P. longisetula*, which was isolated from *Leucospermum* sp. Both species are characterised by versicolorous median cells, conidial length of 20-25 μm and apical appendage of >25 μm. Another interesting example is the relationship of 3 isolates: *Pestalotiopsis aquatica*, *P. gracilis* and *Pestalotiopsis* sp. EN14, which were isolated from unrelated hosts, *Leucospermum* sp. (*Proteaceae*), *Kandelia candel* (*Rhizophoraceae*) and *Scaevola hainanensis* (*Goodeniaceae*), respectively. They have 99% similarity in their nucleotide sequences within the ITS and 5.8S region of the rDNA gene. There is a high probability that these 3 isolates are conspecific. The same applies to *Pestalotiopsis sinensis*, *P. dicheata* and *Pestalotiopsis* sp. EN6, which were isolated from *Chrysalidocarpus lutescens* (*Arecaceae*), *Bletia* sp. (*Orchidaceae*) and *Scaevola hainanensis* (i), respectively. These isolates share 98% sequence similarity within the ITS and 5.8S region of the rDNA gene. High percentage

of similarities of the ITS sequences was also observed among isolates found in Clade B_1. The inferred phylogenetic results are largely concordant with previous studies where phylogenetically significant characters were established to delineate *Pestalotiopsis* species (Jeewon *et al*., 2003).

Several authors have erroneously named new *Pestalotiopsis* species based on host-specificity, despite having similar morphological characteristics to previously described species (e.g. Sohi and Prakash, 1979; Venkatasubbaiah *et al*., 1991b). There has been debate over naming species based on host and their presumed host-specificity. In many cases, mycologists have failed to provide a comprehensive and accurate description of new species described based on host (La Rue and Bartlett, 1921; Mordue, 1986). The results of the present study indicate that there is little justification in naming *Pestalotiopsis* species based on host association. This is in agreement with the suggestions made by La Rue and Bartlett (1921) and Mordue (1986) that morphologically similar species isolated for the first time from a particular host should not be described as new species. If nomenclature based on host is valid, then all the isolates from the same host would presumably form monophyletic groups corresponding to host. As shown in this study, isolates from *Leucospermum* sp., *Scaevola hainanensis* and *Protea mellifera* were polyphyletic and do not represent natural groups associated with their hosts. The results herein indicate that these isolates possess considerable diversity in morphology and isolates group together based on similarities in conidial morphology as elaborated by Jeewon *et al*. (2002, 2003).

Even though taxonomic sampling in this study was restricted to a limited number of isolates from the same and different hosts, there is evidence to dispute the classical host species-based nomenclature of *Pestalotiopsis* as frequently followed by numerous researchers. Host relationships and the use of host names for species nomenclature are poor taxonomic indicators and reflect inappropriate taxonomic methods. Morphologically indistinguishable species from different hosts should have the same name. A uniform and informative system independent of host association is needed to name new *Pestalotiopsis* species.

Results from this study have important implications for fungal biodiversity studies, in particular in the estimation of globally extant fungal species. *Pestalotiopsis* species are frequently isolated as pathogens, saprobes or endophytes from host plants in biodiversity and ecological studies (Wu *et al*., 1982; Hyde and Fröhlich, 1995; Brown *et al*., 1998; Hopkins and McQuilken, 2000; Rivera and Wright, 2000; Tang *et al*., 2003; Wei and Xu, 2004). Given that naming of species based on host is not valid, the high number of species reported in the literature is an overestimate. Many taxa may be conspecific. If

other ubiquitous genera (e.g. *Colletotrichum*, *Phoma*, *Phomopsis*) share similar nomenclatural problems, fungal numbers based on previously described taxa may have been overestimated.

It has been shown in this and other studies that several *Pestalotiopsis* species or multiple strains of the same species can occur on an individual host (La Rue and Bartlett, 1921; Suto and Kobayashi, 1993; Kim *et al*., 1997; Hopkins and McQuilken, 2000) It is unclear whether the these fungi have been described as different hosts or have been recorded as the same species. Because of this, estimates of extant *Pestalotiopsis* species could be inaccurate and mycologists concerned with these areas of research should be aware of these taxonomic problems.

The fact that many *Pestalotiopsis* species produce important biochemical compounds is of considerable interest to researchers involved in screening for secondary metabolites (Strobel *et al*., 1996; Li *et al*., 2001; Worapong *et al*., 2002; Tomita, 2003). In this context, when taxonomic identification is made only to genus level, it would be wise to use as many isolates of the same genus as possible for screening as one host can be infected by different species that have distinct evolutionary lineages.

Our study has addressed the nomenclatural problems of the genus *Pestalotiopsis*. Results of this investigation have important implications in several major fungal research areas, as discussed. It would be remiss to regard these problems as restricted to this one genus. It is timely and indeed necessary that other genera, especially those that are ecologically ubiquitous, be considered in future studies.

Acknowledgements

We are grateful to the Hong Kong Research Grants Council for funding. The University of Hong Kong is thanked for providing an Honorary Assistant Professorship to the first author. We also thank several colleagues, P. Alva, A. Aptroot, E.H.C. Mckenzie, R.G. Shivas, J.E. Taylor, P.W. Crous and M.J. Wingfield for providing cultures and specimens for this study as well as other studies on *Pestalotiopsis*. Heidi Kong and Helen Leung are thanked for laboratory assistance. R. Dulymamode and Rajeeta Jeewon are thanked for their continuous support during the course of the study.

References

Brown, B.B., Hyde, K.D. and Guest, D.I. (1998). Preliminary studies on endophytic fungal communities of *Musa acuminata* species complex in Hong Kong and Australia. Fungal Diversity 1: 27-51.

El Sayed, A.B., Salem, M.A., Seif El Din, A.A., Omar, A.A., Mikhail, S.H. (1985). Reaction of *Eucalyptus* species to *Pestalotiopsis magniferae* in Egypt. Australian Forest Research 15: 463-468.

Felsenstein, J. (1984). Distance methods for inferring phylogenies. A justification. Evolution 38: 16-24.

Gilbert, D.G. (1996). SeqPup biosequence editor and analysis software for molecular biology. Bionet. Software July 1996.

Guba, E.F. (1961). Monograph of *Pestalotia* and *Monochaetia*. Harvard University Press. Cambrigde Masssachusetts USA.

Hasegawa, M., Iida, Y., Yano, T.A., Takaiwa, F. and Iwabuchi, M. (1985). Phylogenetic relationships among eukaryotic kingdoms inferred from ribosomal RNA sequences. Journal of Molecular Evolution 22: 32-38.

Hopkins, K.E. and McQuilken M.P. (2000). Characteristics of *Pestalotiopsis* associated with hardy ornamental plants in the UK. European Journal of Plant Pathology 106: 77-85.

Howard, C.M. and Albregs, E.E. (1973). A strawberry fruit rot caused by *Pestalotia longisetula*. Phytopathology 63: 862-863.

Hyde, K.D. and Fröhlich, J. (1995). *Mycosphaerella palmicola* associated with leaf spots of *Cocos nucifera* in Australia Iran Jaya and Papua New Guinea. Mycological Research 99: 704-706.

Jeewon, R., Liew, E.C.Y. and Hyde, K.D. (2002). Phylogenetic relationships of *Pestalotiopsis* and allied genera inferred from ribosomal DNA sequences and morphological characters. Molecular Phylogenetics and Evolution 25: 378-392.

Jeewon, R., Liew, E.C.Y., Simpson, J.A., Hodgkiss, I.J. and Hyde, K.D. (2003). Phylogenetic significance of morphological characters in the taxonomy of *pestalotiopsis* species. Molecular Phylogenetics and Evolution 27: 372-383.

Kim, K.S., Choi, H.S., Lim, T.H., Chang, T.H., Chung, B.K., Kim, M.J. and Lee, Y.S. (1997). Genetic diversity of leaf blight pathogen of sweet persimmon *Pestalotiopsis* species with random amplified polymorphic DNA (RAPD). Korean Journal of Mycology 13: 311-316.

Kishino, H. and Hasegawa, M. (1989). Evaluation of the maximum likelihood model estimates of the evolutionary tree topologies from sequence data, and the branching order in Homonoidea. Journal of Molecular Evolution 29: 170-179.

Koh, J.Y., Shin, G.H. and Hur, J.S. (2001). Seasonal occurrence and development of grey blight of tea plants in Korea. Plant Pathology Journal 17: 40-44.

Kohlmeyer, J. and Kohlmeyer, V.B. (2001). Fungi on *Juncus roemerianus* 16. More new coelomycetes, including *Tetranacriella* gen. nov. Botanica Marina 44: 147-156.

La Rue, C.L. and Bartlett, H.H. (1921). A demonstration of numerous distinct strains within the nominal species *Pestalozzia guepini* Desm. American Journal of Botany 9: 79-92.

Lacap, D.C., Hyde, K.D. and Liew, E.C.Y. (2003). An evaluation of the fungal 'morphotype' concept based on ribosomal DNA sequences. Fungal Diversity 12: 53-66.

Li, J.Y., Harper, J.K., Grant, D.M., Tombe, B.O., Bashyal, B., Hess, W.M. and Strobel, G.A. (2001). Ambuic acid, a highly functionalised cyclohexenone with antifungal activity from *Pestalotiopsis* spp. and *Monochaetia* sp. Phytochemistry 56: 463-468.

Mordue, J.E.M. (1976). *Pestalotiopsis psidii*. C.M.I. Descriptions of Pathogenic Fungi and Bacteria No 515.

Mordue, J.E.M. (1980). *Pestalotiopsis mangiferae*. C.M.I. Descriptions of Pathogenic Fungi and Bacteria No 676.

Mordue, J.E.M. (1986). Another unusual species of *Pestalotiopsis*: *P. montellicoides* sp. nov. Transactions of the British Mycological Society 84: 665-668.

Mordue, J.E.M and Holliday, P. (1971). *Pestalotiopsis theae*. C.M.I. Descriptions of Pathogenic Fungi and Bacteria No 318.

Nag Rag, T.R. (1985). Redisposals and redescriptions in the *Monchaetia- Seiridium Pestalotia-Pestalotiopsis* complexes. I. The correct name for the type species of *Pestalotiopsis*. Mycotaxon 22: 43-51.

Nag Rag, T.R. (1986). Redisposals and redescriptions in the *Monchaetia- Seiridium Pestalotia-Pestalotiopsis complexes*. VII. *Pestalotia citrini P. maura* and *Pestalotiopsis uvicola*. Mycotaxon 26: 211-222.

Nag Rag, T.R. (1989). Redisposals and redescriptions in the *Monchaetia- Seiridium Pestalotia-Pestalotiopsis complexes*. IX. On the status of *Pestalotia eupyrena Pestalotia gastrolobi* and *Pestalotia veneta*. Mycotaxon 35: 287-296.

Nag Raj, T.R. (1993). Coelomycetous anamorphs with appendage bearing conidia. Mycologue Publications, Waterloo, Ontario, Canada.

Page, R.D.M. (1996). Treview: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12: 357-358.

Pal, A.K and Purkayastha, R.P. (1992). New Parasitic fungi from Indian mangrove. Journal of Mycopathological Research 30: 173-176.

Patil, M.S. and Thite, A.N. (1977). Some Deutoromycetous fungi from Maharashtra. I. Maharashtra Vidnyan Mandir Patrika 12: 28-35.

Rivera, M.C. and Wright, E.R. (2000). First report of azalea petal blight caused by *Pestalotiopsis guepini* in Argentina. Plant Disease 84: 100.

Singh, N.I. (1981). Some new host records for India. Indian Phytopathology 34: 233-234.

Sohi, H.S and Prakash, O. (1979). A new species of *Pestalotiopsis chetallensis* on banana leaf spot. Indian Phytopathology 31: 252-254.

Steyaert, R.L. (1949). Contributions à l'étude monographique de *Pestalotia* de Not. et *Monochaetia* Sacc. (*Truncatella* gen. nov. et *Pestalotiopsis* gen. nov.). Bulletin Jardin Botanique État Bruxelles 19: 285-354.

Strobel, G., Yang, X., Sears, J., Kramer, R., Sidhu, R.S. and Hess, W.M. (1996). Taxol from *Pestalotiopsis microspora* of *Taxus wallachiana*. Microbiology 142: 435-440.

Suryanayaranan, T.S., Senthilarasu, G. and Muruganadam, V. (2000). Endophytic fungi from *Cuscuta reflexa* and its host plants. Fungal Diversity 4: 117-123.

Suto, Y. and Kobayashi, T. (1993). Taxonomic studies on the species of *Pestalotiopsis*, parasitic on conifers in Japan. Transactions of the Mycological Society of Japan 34: 323-344.

Swart, L., Taylor, J.E., Crous, P.W. and Percival, K. (1999). *Pestalotiopsis* leaf spot disease of Proteaceae in Zimbabwe. South African Journal of Botany 65: 239-242.

Swofford, D.L. (2001). PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts USA.

Swofford, D.L., Olsen, G.J., Waddell, P.T. and Hillis, D.M. (1996). Phylogenetic inference. Pages 407-425. In: *Molecular Systematics*, 2nd edn. (eds. D.M. Hillis, C. Moritz and B.K. Mable). Sinauer Associates, Sunderland, Massachusetts, USA.

Tang, A.M.C., Hyde, K.D. and Corlett, R.T. (2003). Diversity of fungi on wild fruits in Hong Kong. Fungal Diversity 14: 165-185.

Taylor, J.E., Crous, P.W. and Palm, M.E. (2001). Foliar and stem fungal pathogens of Proteaceae in Hawaii. Mycotaxon 78: 449-490.

Templeton, A.R. (1983). Phylogenetic inference from restriction endonuclease cleavage sites maps with particular reference to the evolution of humans and the apes. Evolution 37: 269-285.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997). The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24: 4876-4882.

Tomita, F. (2003). Endophytes in Southeast Asia and Japan: their taxonomic diversity and potential applications. Fungal Diversity 14: 187-204.

Toofanee, S.B and Dulymamode, R. (2002). Fungal endophytes associated with *Cordemoya integrifolia*. Fungal Diversity 11: 169-175.

Venkatasubbaiah, P., Grand, L.F. and Gerald Van Dyke, C. (1991a). A new species of *Pestalotiopsis* on *Oenothera.* Mycologia 83: 511-513.

Venkatasubbaiah, P., Gerald Van Dyke C, Chilton W.S. (1991b). Phytotoxins produced by *Pestalotiopsis oenothera* a pathogen of evening primrose. Phytochemistry 30: 471-1474.

Wei, J.G. and Xu, T. (2004). *Pestalotiopsis kunmingensis*, sp. nov., an endophyte from *Podocarpus macrophyllus*. Fungal Diversity 15: 247-254

White, T.J., Bruns, T.L., Lee, S. and Taylor, J.W. (1990). Amplication and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: a Guide to methods and Applications* (eds. M. Innis, D.H. Gelfand, J.J. Sninsky and J.T. White). Academic press, San Diego, USA.

Worapong, J., Ford, E., Strobel, G. and Hess, W. (2002). UV light induced conversion of *Pestalotiopsis microspora* to biotypes with multiple conidial forms. Fungal Diversity 9: 179-193.

Wu, C.G., Tseng, H.Y. and Chen, Z.C. (1982). Fungi inhabiting on *Schoenoplectus triqueter* L. Palla (I). Taiwania 27: 35-38.

Yanna, Ho, W.H. and Hyde, K.D. (2002). Fungal succession on fronds of *Phoenix hanceana* in Hong Kong. Fungal Diversity 10: 185-211.

Yuan, Z.Q. (1996). Fungi and associated tree diseases in Melville Island, Northern territory, Australia. Australian Systematic Botany 9: 337-360.

Zhou, D.Q. and Hyde, K.D. (2001). Host-specificity, host-exclusivity and host-recurrence in saprobic fungi. Mycological Research 105: 1449-1457.

Zhu, P., Ge, Q. and Xu, T. (1991). The perfect stage of *Pestalosphaeria* from China. Mycotaxon 50: 129-140.

(Received 1 July 2004; accepted 20 July 2004)