CHAPTER 5

PHYLOGENETIC RELATIONSHIP OF *Dictyochaeta wallichianensis* WITHIN CHAETOSPHAERIACEAE

5.1. Introduction

Chaetosphaeria Tul. and Tul. and its allied genera are commonly found worldwide occurring as saprobic pyrenomycetous ascomycetes which reproduce both sexually and asexually on extensively decomposed plant substrates. Since its original description (Tulasne and Tulasne, 1863), the genus has been repeatedly redefined (Saccardo, 1883; Booth, 1957; Müller and von, Arx 1962; Gams and Holubová-Jechová, 1976; Réblová, 1999, 2000). The circumscription and phylogeny of the genus recently has been reviewed (Réblová, 2000; Réblová and Winka, 2000). It currently encompasses 30 species distributed in four natural groups which also include morphological entities in 11 anamorphic genera (Réblová, 2000). Taxonomic opinions at the family level have placed the genus in the Trichosphaeriaceae (Dennis, 1978), Lasiosphaeriaceae (Barr, 1990) and Chaetosphaeriaceae (Réblová *et al.*, 1999). The recent phylogenetic analysis by Fernández *et al.* (2006) insisted the monophyletic of family Chaetosphaeriaceae which consists of *Chaetosphaeria* s. lat. and its anamorphs.

In general, *Chaetosphaeria* teleomorphs are simple and relatively homogeneous, while their anamorphs are complex and diverse. Therefore, species identification is based primarily on characters of the anamorphs (i.e. in genera such as *Chloridium* Link, *Codinaea* Maire, *Dictyochaeta* Speg., *Dictyochaetopsis* Aramb. &

Cabello) resulting in species with almost indistinguishable teleomorphs in many instances. Species identification can become even more challenging when morphological information on anamorphs is not available. When anamorph data are available from culture, there is the possibility of encountering altered or aberrant morphologies. In addition, some anamorphic taxa connected to *Chaetosphaeria* are monophyletic (Fernández *et al*., 2006).

The genus *Dictyochaeta*, one of the anamorphic genus in family Chaetosphaeriaceae, was erected by Spegazzini in 1923 and it is considered as an earlier name for *Codinaea* (Gamundi *et al.*, 1977). The type species is *D. fuegiana* Speg., and it was originally described with sterile setae accompanied by shorter fertile hyphae, and conidia curved, hyaline, non-septate, non-setulate. In *Dictyochaeta*, the conidiogenous cells arise terminally and singly on simple conidiophores, in several species, setae are absent. Therefore, genus *Codinaea*, also characterized by having sterile setae and shorter fertile hyphae, was closely related to the earlier genus. Later, Arambarri and Cabello (1990) erected another closely related genus, *Dictyochaetopsis* (syn. *Codinaeopsis* Morgan-Jones). The later genus is distinct from *Dictyochaeta* by having lateral phialides.

Arambarri and Cabello (1989) attempted to transfer 21 species of *Codinaea* to *Dictyochaeta.* However, they failed to provide a reference to the basionyms, thus contravening article 33.2 of the *International Code of Botanical Nomenclature* that notes "*Before 1 January 1953 an indirect reference to a basionym or replaced synonym is sufficient for valid publication of a new combination, a new generic name with a basionym, or a nomen novum. Thus, errors in the citation of the basionym or replaced synonym, or in author citation, do not affect valid publication of such*

names". Two of those 21 species, *Dictyochaeta heteroderae* (Morgan-Jones) Carris & Glawe and *D. parva* (S. Hughes & W. B. Kendr.) Hol.-Jech. had been validly transferred by earlier authors (Carris and Glawe, 1988; Holubová-Jechová, 1988). The other 19 species of *Codinaea* are transferred to *Dictyochaeta* by Whitton *et al.* (2000), along with a few other remaining species within *Codinaea* which are transferred to *Dictyochaeta* or *Dictyochaetopsis.* They also provided a key to those species of *Dictyochaeta* which were not included by Kuthubutheen and Nawawi (1991a), and a second key to all species of *Dictyochaetopsis.*

Recently, about 10 species of *Dictyochaeta* have been reported associated with various species of palms, namely, *D. assamica* (Agnihothr.) Aramb., Cabello & Mengasc., *D. coffeae* (Maggi & Persiani) Aramb. & Cabello, *D. fertilis* (S. Hughes & W.B. Kendr.) Hol.-Jech. and *D. parva* (S. Hughes & W.B. Kendr.) Hol.-Jech. on *Archontophoenix alexandrae*, *D. gyrosetula* Kuthub., Nawawi & G.M. Liew and *D. ramulosetula* Kuthub. on *Licuala longicalycata*, *D. intermedia* Gusmão & S.M. Leão and *D. simplex* (S. Hughes & W.B. Kendr.) Hol.-Jech. on *Phoenix hanceana*, *D. minutissima* A. Hern. Gut. & J. Mena on *Coccothrinax miraguama*, *D. stipitocolla* Kuthub. & Nawawi on *Pinanga malaiana*, *D. tumidoseta* Kuthub. & Nawawi on *Oncosperma tigillaria* and *D. variabilis* Kuthub. & Nawawi on *Oncosperma horridum*. Of them, only two species, namely, *D. gyrosetula* and *D. ramulosetula* on *Licuala longicalycata* have been reported from Thailand. In this chapter, the phylogenetic relationship of *Dictyochaeta wallichianensis*, a novel and dominant species found during the ecological study of this thesis, is described and elucidated.

5.2. Materials and Methods

Collecting Protocols, Site Description and Materials Examinations

The specimen of *Dictyochaeta wallichianensis* was collected at Huay Kog Ma, Doi Suthep-Pui national park, Chiang Mai, Thailand. Other details of the collection methods were described earlier in the chapter 2.

The specimens were observed using an Olympus BX50 photomicroscope system with differential interference contrast microscopy. Water is the medium used for all examinations, spore measurements and most of the illustrations. Measurements are given as (minimum) mean ± standard deviation (maximum) (n = sample size). Specific reagents were used when necessary as follows: Melzer's reagent was used to investigate any reactions in the ascus. Ascomata sections of rehydrated fruiting structures were made with a Micron HM505E cryostat microtome or by hand. Lactophenol was added to the slides for permanent fixation. Dried herbarium specimens were deposited at Mushroom Research Center Herbarium, Chiang Mai, Thailand, and CMU Herbarium (CMU), Faculty of Science, Chiang Mai University, Chiang Mai, Thailand. Cultures were obtained in this study according to the method of Choi *et al.* (1999). An ex-type culture is maintained in the Molecular of Plant Pathology Culture Collection, Department of Plant Pathology, Chiang Mai University, Chiang Mai, Thailand.

Molecular Characterization

Total genomic DNA was extracted from mycelial cultures grown on malt extract agar (Difco) following a 2 × cetyltrimethylammoniumbromide (CTAB) protocol (Rogers and Bendich, 1994). DNA amplification of internal transcribed

spacer (ITS) nrDNA region was performed by polymerase chain reaction (PCR) using ITS4 and ITS5 primers (White *et al.*, 1990) to generate about 612 nucleotides from the complete ITS region. The amplification conditions were performed in a 50 µl reaction volume as follows: 1 × PCR buffer, 0.2 mM each dNTP, 0.3 mM of each primer, 1.5 mM $MgCl_2$, 0.8 units Taq polymerase and 10 ng DNA. PCR parameters for all the regions were performed as follows: initial denaturation at 94ºC for 3 min, 30 cycles of 94ºC for 1 min, 52ºC for 50 s and 72ºC for 1 min and final extension of 72ºC for 10 min.

The characterization of PCR products was performed via agarose gel electrophoresis on a TAE 1% agarose gel containing ethidium bromide (EtBr) as the staining agent. The PCR product was purified using Qiaquick purification kit (Qiagen) and DNA concentration of the PCR products was subjected to automatic sequencing (ABI PRISM Dye Terminator Cycle Sequencing and ABI PRISM Sequencer model 377, Perkin Elmer). The GenBank accession numbers of the sequences and taxa used to construct the phylogenetic trees are shown in appendix 4.

Sequences were aligned in ClustalX version 2.0.3 (Larkin *et al.*, 2007) and BioEdit (Hall, 1999) using default parameters. The sequences alignments were checked and manual adjustment were made where necessary. Regions designated as ambiguously aligned were excluded from the analyses. Gaps were treated as missing data. Phylogenetic analyses were performed in PAUP version 4.0b10 (Swofford, 2002).

Unweighted Maximum Parsimony (UMP) analysis was performed in this study. Trees were inferred using the heuristic search option with 1000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed

and all multiple parsimonious trees were saved. Descriptive tree statistics (tree length [TL], consistency index [CI], retention index [RI], related consistency index [RC], homoplasy index [HI] and log likelihood [-ln L]) were calculated for trees generated under different optimality criteria. The Kishino-Hasegawa (KH) likelihood test (Kishino and Hasegawa, 1989) was carried out using PAUP to compare the best tree topology obtained by the nucleotide sequence data with a constrained tree. Clade stability was assessed in bootstrap analyses with 1000 replicates, each with 1000 replicates of random stepwise addition of taxa. Random sequence addition was used in the bootstrap analyses. Trees were figured in TreeView (Page, 1996). Other details are outlined in Cai *et al.* (2005).

5.3. Results

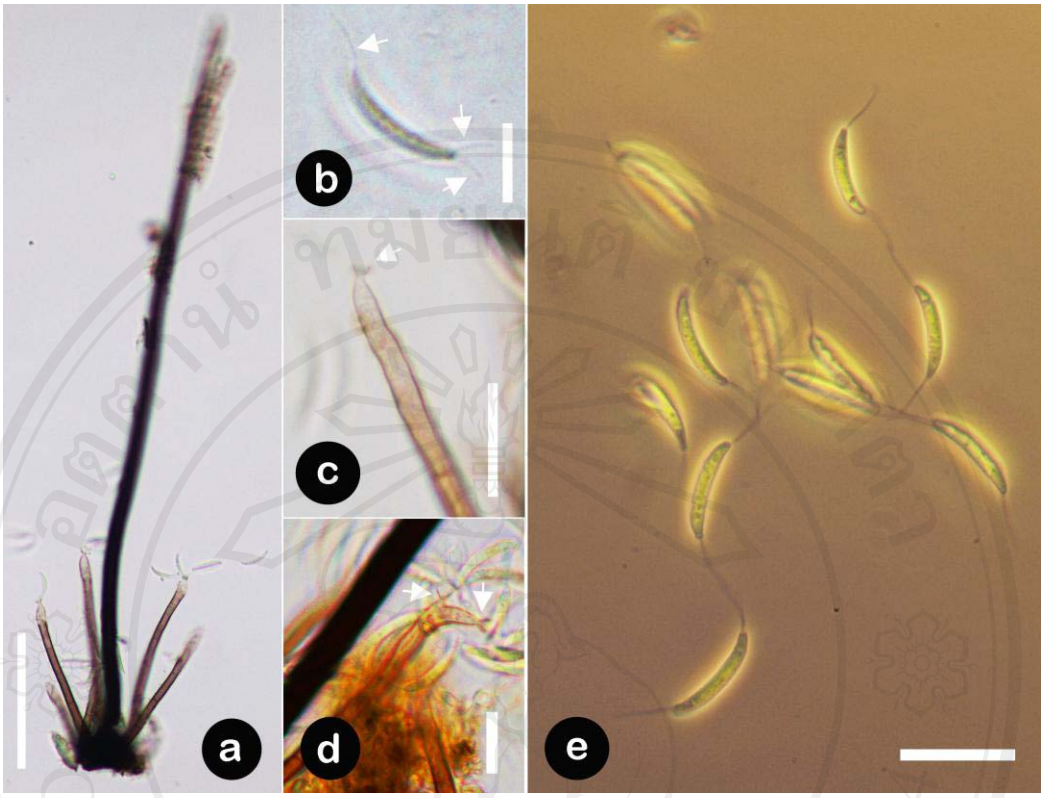
Taxonomy

Dictyochaeta wallichianensis Hidayat & To-anun**, sp. nov.**

(Figs. 5.1; 5.2)

Differt a D. assamica, D. gamundii conidia diminution et setula decretus, et D. fertilis constitus stromata et 1-2-phialidis, et D. plovercovensis conidia symmetrica.

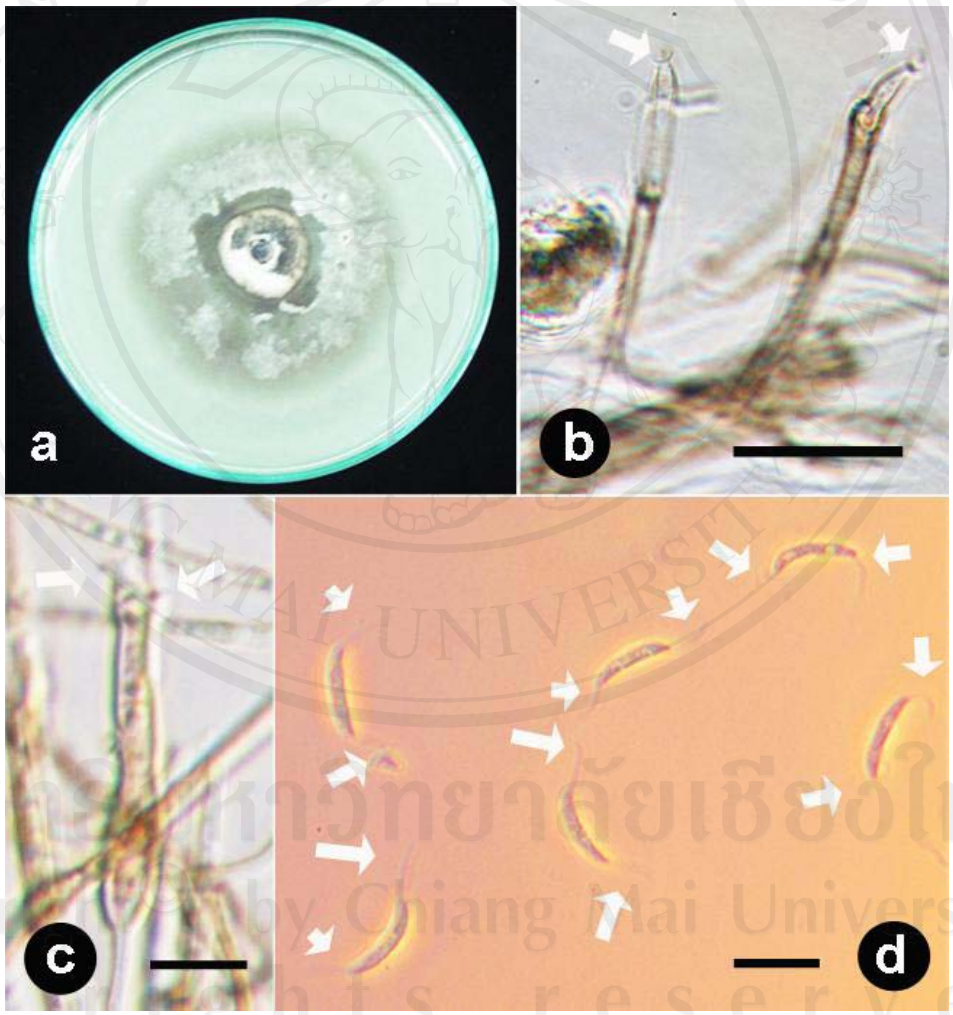
Etymology: Refers to the genus name of its host, *Wallichia*.



Figs. 5.1 Micrographs of *Dictyochaeta wallichianensis* **sp. nov.** (from holotype). a. Setae and conidiophores. b. Conidium with single setula at one end and double setulae at another end (Arrows). c. Conidiogenous cell with single funel-shaped collarette (arrow). d. Conidiogenous cell with two funel-shaped collarettes. e. Conidia aggregates. Bars: a = 40 µm; b,d = 7 µm; c = 10 µm; e = 15 µm.

Colonies on natural substratum effuse, brown to dark brown. ***Mycelium*** immersed, pale to light brown, (2.5) 3.3 ± 0.6 (3.8) µm (n = 30) wide, smooth, septate, branched. ***Stromata*** composed of 4-10 globose to subglobose, brown to blackish cells, (12.5) 18.8 ± 4.5 (25) µm (n = 30) diam. ***Setae*** fertile, thick-walled, 10-12-septate, dark brown to blackish, smooth, erect, straight, gradually becoming paler towards the apex, with blunt end, 282 ± 36.9 (340) × (2.5) 3.4 ± 0.6 (3.8) µm (n = 30), cells of the setae (12.5) 21 ± 6.5 (30) µm (n = 30) long. ***Conidiophores*** distinct, erect, cylindric, 3-6 fasciculates around the base of a seta, simple, pale yellowish brown, paler at the apex, 5 to 10-septate, narrowly clavate, (45) 86.5 ± 48.6 (225) × (2) 2.6 ± 1 (2.5) µm

(n = 30). ***Conidiogenous cells*** cylindric to narrowly clavate, pale yellowish brown, phialides, monoblastic or polyblastic, with one or two funnel-shape collarettes, (5) 10.3 ± 4.3 (20) µm (n = 30). ***Conidia*** hyaline, aggregated in hyaline slimy masses, (14) 15.3 ± 0.7 (17) × (1) 1.9 ± 0.3 (2.5) µm (n = 30), aseptate, slightly curved, symmetrical, tapering at both ends, with a simple, often single setula at each end, sometimes two setulae at one end, (7) 8.3 ± 0.8 (10) µm (n = 30) long.



Figs. 5.2 Micrographs of *Dictyochaeta wallichianensis* **sp. nov.** on PDA (from holotype). a. Appearances of 2 months culture. b-c. Conidiogenous cells with funel-shaped collarette (arrows). d. Conidia. Bars: b = 30 µm; c = 7.5 µm; d = 10 µm.

Colonies on PDA slow growing, pale to light brown, darker near the margin, attaining diameter of 1-1.5 cm after 14 days. ***Mycelium*** septate, hyaline to light brown, smooth, branched, (1) 1.6 ± 0.4 (2) µm (n = 30) diam. ***Conidiophores*** straight, 90 degree from mycelium, 40-100 µm, light brown to brown, 2-6 septate. ***Conidiogenous cells*** hyaline to light brown, phialides, discrete, monoblastic or polyblastic, consists of 1-4 funnel-shaped collarettes, (30) 33.7 ± 2.1 (36) × (3) 3.9 ± 0.5 (4.5) µm (n = 30). ***Conidia*** hyaline, fusoid, symmetric, tapering at both ends, aseptate, smooth, (9) 11.7 ± 1.4 (15) × (1.5) 2 ± 0.3 (2.5) µm (n = 30). ***Setula*** (7) 7.8 ± 9 (0.8) µm (n = 30) long at both ends.

Material examined: THAILAND, Chiang Mai province, Huay Kog Ma, Doi Suthep-Pui national Park, on decaying fronds *Wallichia siamensis* Becc.(Arecaceae), 17 November 2006, Iman Hidayat (**Holotype**: 154).

Habitat: Decaying fronds of *W. siamensis*.

Distribution: Only known from the type locality.

Molecular Phylogenetic Analysis

The ITS dataset consisted of 44 taxa and covered the ITS and 5.8S region of nrDNA. *Cercophora sparsa* (Sacc. & Fairm.) R. Hilber and *Lasiosphaeria glabrata* (Fr.) Munk were the designated as outgroup. One hundred and thirty-two characters (ambiguous regions) were excluded, therefore, the final dataset comprised 418 characters in which 78 are constant, 101 parsimony-uninformative and 239 parsimony-informative. Nine parsimony trees were generated from the parsimony analyses. The best parsimonious tree selected by KH test ($P < 0.05$) was generated in

1664 steps (CI = 0.4, RI = 0.5, RC = 0.2, HI = 0.6, -ln L = 7889.4). The best parsimonious tree is shown in figure 5.3.

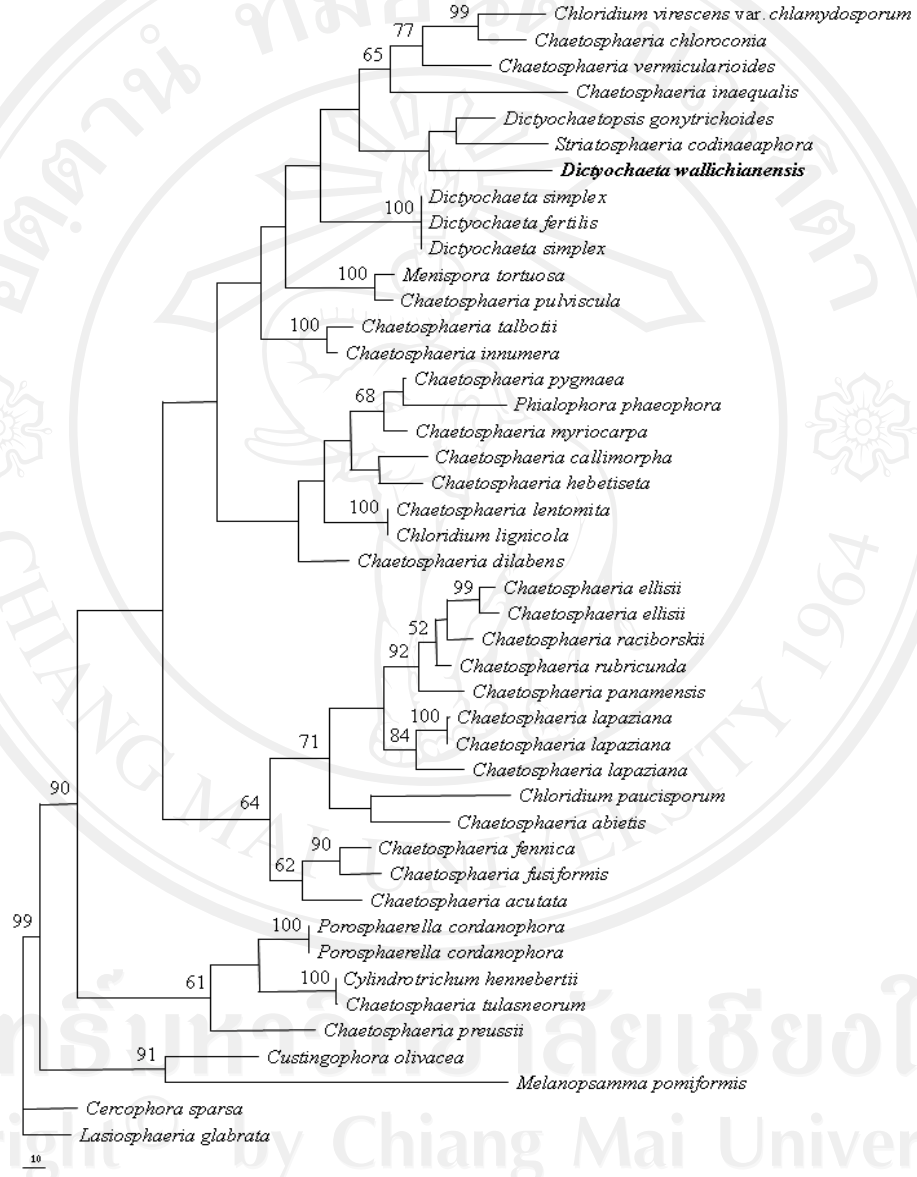


Fig. 5.3 The best parsimonious tree based on ITS nrDNA sequence data representing placement of *Dictyochaeta wallichianensis* within representatives of the family Chaetosphaeriaceae. The tree is obtained from heuristic search with 1000 random taxon addition of the sequences alignment. Bootstrap values (>50%) from 1000 replicates of Unweighted Maximum Parsimony (UMP) analyses are shown above internodes.

5.4. Discussion

Kuthubutheen and Nawawi (1991b) were the first published the dichotomous key to *Dictyochaeta* species which consists of 59 species, some of them transferred from genus *Codinaea* (Gamundi *et al.,* 1977). Since then, a further six species have been described by several authors (Castañeda Ruiz and Kendrick, 1990a, b; Bhat and Kendrick, 1993; Hernández-Gutiérrez and Mena, 1996; Castañeda Ruiz *et al.*, 1998). Whitton *et al.* (2000) further discussed genera *Dictyochaeta* and *Dictyochaetopsis*, and transferred 26 species of *Codinaea* to *Dictyochaeta* and four species to *Dictyochaetopsis*. Whitton *et al.* (2000) also provided the key to species of both genera.

The novel species found in Thailand, *Dictyochaeta wallichianensis*, morphologically, is most similar to *D. plovercovensis* Goh & K. D. Hyde (Goh and Hyde, 1999)*, D. assamica* (Agnihothr.) Aramb. Cabello & Mengasc. (Kuthubutheen and Nawawi, 1991a), *D. fertilis* (S. Hughes & B. Kendr.) Hol.-Jech. (Holubová-Jechová, 1984) and *D. gamundii* Aramb. & Cabello (Arambarri *et aI.,* 1987), in having long fertile setae, fasciculate conidiophores and unicellular bisetulate conidia. However, *D. wallichianensis* is distinct from those of four similar species by having two setulae at one end of conidia. In details, *D. wallichianensis* differs from *Dictyochaeta assamica* and *D. gamundii* in having smaller conidia with shorter setulae (Kuthubutheen and Nawawi, 1991a; Arambarri *et aI.,* 1987). On the other hand, *Dictyochaeta fertilis* differs to *D. wallichianensis* in lacking of stroma but having polyphialides conidiophores (with up to 8 successive proliferations) (. (Holubová-Jechová, 1984). *Dictyochaeta plovercovensis* is also distinct from *D. wallichianensis* due to setae having one or two phialides with a funnel-shaped

collarette and asymmetric conidia (Goh and Hyde, 1999). The following modification dichotomous key to *Dictyochaeta* species is presented in order to help for the identification of the members within this genus.

Key to species of *Dictyochaeta* (Modification from Whitton *et al.*, 2000)

1a. Setae present ... **2**

1b. Setae absent ... **8**

2a. Conidia filiform, aseptate, curved, 20-25 x 1 µm; conidiophores 40-130 µm long; setae always sterile, 275-300 µm long ... ***D. uncinata***

2b. Conidia not filiform ... **3**

3a. Conidia cylindrical ... **4**

3b. Conidia falcate ... **5**

4a. Conidia cylindrical, 1-septate, asetulate, 12-18 x 1.8 µm; setae 35-55 µm long; conidiophores 3.5-18 µm long ... ***D. fruticola***

4b. Conidia cylindrical, aseptate, asetulate, 4.8-7.2 x 1.1-1.5 µm; setae 90-145 µm long; conidiophores 32-59 µm long ... ***D. microcylindrospora***

5a. Conidia falcate, 1-septate, asetulate, 12-18 x 1.8 µm; setae up to 100 µm long; conidiophores up to 20 µm long ... ***D. ixorae***

5b. Conidia falcate, aseptate ... **6**

6a. Conidia falcate, aseptate, asetulate, 6-9 x 1.8-2 µm; setae up to 120 µm long; conidiophores up to 30 µm long ... ***D. falcatispora***

6b. Conidia falcate, aseptate, one setula at each end of conidium, 13-15 x 1.5-2 µm; setae up to 480 µm long; conidiophores up to 90 µm long; conidiogenous cells usually monophialidic ... **7**

7a. Setae with one or two phialides near the apex, conidia asymmetric, one setula at each end of conidium, ... ***D. plovercovensis***

7b. Setae lacking of phialides, conidia symmetric, sometimes having two setulae at one end of conidium ... ***D. wallichianensis***

8a. Conidia asetulate, or with rudimentary setulae only ... **10**

8b. Conidia with setulae ... **12**

9a. Conidia falcate, (0-)1(-2) septate, one rudimentary setula at the apex, base acute, 17-23 x 2.5-3 µm; conidiophores 50-110 µm long ... ***D. zapatensis***

9b. Conidia aseptate, no rudimentary setulae ... **9**

10a. Conidia fusoid, base acute, apex obtuse, 5.5-9 x 0.6-1 µm; conidiophores 15-75 µm long, sometimes branched ... ***D. minutissima***

10b. Conidia falcate, both ends attenuated and obtuse ... **11**

11a. Conidia 15-21.5 x 1.2-2 µm; conidiophores 155-310 µm ... ***D. seychellensa***

11b. Conidia 24-32 x 3-4 µm; conidiophores 70-100 µm ... ***D. occidentalis***

12a. Conidiogenous cells with cylindrical collarette; conidia broadly ellipsoid, aseptate, 6.5-10.5 x 6-8 μm; conidiophores up to 160 μm long, apex coarsely *verrucose* ... ***D. ciliata***

12b. Conidiogenous cells with funnel-shaped collarette; conidia otherwise ... **13**

13a. Number of setulae variable ... **14**

13b. One setula at each end of conidium ... **15**

14a. Conidia with one apical setula and a fringe of basal setulae, broadly ellipsoid, aseptate, 14-19.5 x 8-11 μm; conidiophores 310-550 /lm long ... ***D. fimbriaspora***

14b. Conidia with variable number of setulae at each end, irregularly ellipsoid, aseptate, 14-18.5 x 5-6.5 μm; conidiophores 365-445 μm long ... ***D. multisetula***

15a. Conidia ellipsoid to obclavate, base obtuse to truncate, apex acute, aseptate, 7.5-9.5 x 3-5 μm; conidiophores 90-130 μm long ... ***D. tropicalis***

15b. Conidia reniform, both ends rounded, aseptate, 6-8.5 x 3-4.5 μm; conidiophores 95-220 μm long ... ***D. renispora***

The phylogenetic tree generated from ITS+5.8S rDNA sequence analysis indicated the monophyletic of family Chaetosphaeriaceae, particularly some anamorph states connected to *Chaetosphaeria* teleomorph with 99% bootstrap support (fig. 5.3). It also supported the previous study published by Fernández *et al*. (2006) who remarked that some anamorphic taxa connected to *Chaetosphaeria* are

monophyletic. The phylogenetic tree resulted in this analysis also supports the proposal of *Dictyochaeta wallichianensis* sp. nov., as a new species due to this taxon is separated from other *Dictyochaeta* species. It is also confirmed the polyphyletic of *Dictyochaeta*, even though with limited number of taxa included in the analysis. The present study also showed a close relationship of *D. wallichianensis* with *Dictyochaetopsis gonytrichoides* (Shearer & J. L. Crane) Whitton, McKenzie & K. D. Hyde (in the tree as *Codinaeopsis gonytrichoides*), but with a low bootstrap support (< 50%).

The genus *Dictyochaetopsis* Aramb. & Cabello is morphologically separated from *Dictyochaeta* in having lateral phialides (Arambarri and Cabello, 1990. The low statistical support among these two genera indicated insufficient morphological delimitation such as lateral phialides in separating taxa at generic level. However, due to a small number of representative taxa included in this analysis, therefore, further analysis that includes large number of taxa is quite necessary in order to clarify the relationship among these three genera, particularly, and the anamorph genera within *Chaetosphaeria* teleomorph.

Anamorph-teleomorph connection based on the phylogenetic tree generated from ITS sequence dataset showed that members of genus *Dictyochaeta* are linked with two teleomorphic states, namely, *Chaetosphaeria* and *Striatospora*. The two teleomorphic genera are separated morphologically based on the presence of ascospores ornamentation of genus *Striatospora* (Fernández *et al.*, 2006). The preliminary molecular phylogenetic studies of *Chaetosphaeria* and allied taxa reported by Fernández *et al.* (1998) determined that ascospore ornamentation and pigmentation are homoplasious morphological character in the group. Furthermore,

relevant data have revealed ascospores morphology to be phylogenetically informative only at the species level (Fernández *et al.*, 2006). However, it is unclear whether genus *Striatospora* should be maintained as separate genus to the *Chaetosphaeria* within the family Chaetosphaeriaceae as the sequences available are very limited.