CHAPTER 3

THE GENUS *Oxydothis* AND ITS PHYLOGENETIC RELATIONSHIP WITHIN THE XYLARIALES

3.1. Introduction

Oxydothis Penz. and Sacc. (Xylariales) includes more than 70 ascomycete species, which are saprobic, endophytic or parasitic on members of the Gramineae, Liliaceae, Palmae and Pandanaceae (Penzig and Saccardo, 1897; Hyde *et al.,* 2000). The genus is typified by *Oxydothis grisea* Penz. and Sacc. (Penzig and Saccardo, 1897) and delimited based on morphological features such as long cylindrical asci with a J+ subapical apparatus; and long fusiform to filiform, hyaline, bicelled ascospores, which taper from the centre to spine–like ends, pointed or rounded processes (Penzig and Saccardo, 1897; Hyde, 1994a, b).

The familial placement of *Oxydothis*, however, is still tentative. There is uncertainty as to which morphological characters should be used to delimit the genus as its taxonomic and phylogenetic relationships with other members of the Xylariales are still not resolved. Based on morphological similarities with *Leiosphaerella* Höhn., and presence of horizontal and clypeate ascomata, *Oxydothis* was placed within the Amphisphaeriaceae (Müller and Arx, 1962, 1976; Wehmeyer 1975; Samuels and Rossman, 1987). The genus was, however, referred to the Physosporellaceae (Phyllacorales) by Barr (1976) and later transferred to the Hyponectriaceae based on the perithecial ascomata with papillate ostiole and cylindrical asci (Hawksworth *et al.*, 1995). A scanning Electron Microscopy (SEM) study of the ultrastructure of the

Oxydothis ascus could not resolve the generic and familial affiliations of *Oxydothis* (Wong and Hyde, 1999). Wang and Hyde (1999) excluded *Oxydothis* from the Hyponectriaceae based on the arrangement of the ascomata, structure of the asci and ascospores which are unlike *Hyponectria buxi*. Phylogenetic analyses of 5.8S nrDNA and internal transcribed spacer (ITS2) sequence data have revealed that *Oxydothis* has evolutionary affiliations with members of the Clypeosphaeriaceae (Kang *et al.*, 1998). Though Clypeosphaeriaceae was found to be heterogeneous, Kang *et al.* (1999b; 2002) placed *Oxydothis* within the family. Phylogenies analyses of 28S and 18S nrDNA revealed closer affiliations of *Hyponectria* with *Oxydothis* and *Appendicospora*, although these relationships had poor statistical support (Smith *et al.*, 2003).

During the present study on diversity of palmicolous fungi in northern Thailand, four species of *Oxydothis* were collected. Three are new species. *Oxydothis cyrtostachicola* sp. nov. is described from decaying fronds of *Cyrtostachys renda* (Arecaceae), while *Oxydothis wallichianensis* sp. nov. and *O. inequalis* sp. nov. are described from decaying leaves and fronds of *Wallichia siamensis* (Arecaceae), respectively. The new species are described and illustrated here. In addition the phylogenetic analyses of partial 28S and ITS+5.8S nrDNA sequence data were performed in order to understand possible familial placement of *Oxydothis* within the Xylariales.

3.2. Materials and methods

Microscopic examination

Decaying fronds of *Wallichia siamensis* and *Cyrtostachys renda* were collected in northern Thailand during July and October 2005. The microscopic observation of *Oxydothis* spp. was performed as outlined in Shenoy *et al.* (2005).

DNA extraction, amplification and sequencing

The total genomic DNA from the fungal species was extracted directly from the ascomata growing on the palm substrate, following a modified protocol of Hirata and Takamatsu (1996). Ascomata were picked up using fine forceps, and suspended in 500 µl of 5% Chelex solution (Bio-Rad, Richmond, Calif.). Ascomata were disrupted by means of a micropestle and content vortexed thoroughly for 1 min. Tube was incubated at a temperature of 100 ˚C for 15 mins and vortexed again for 1 min to allow maximum disruption. The contents were centrifuged at a speed of 14000g for 30 s and the supernatant was transferred to a new tube. This sample was directly used as templates for PCR.

DNA amplification was performed by polymerase chain reaction (PCR). For partial 28S nrDNA amplification, LROR and LR5 primers (Vilgalys and Hester, 1990) were used, while ITS5 and ITS4 primers (White *et al.*, 1990) were used for ITS nrDNA amplification. The amplification procedure followed the protocol outlined in Jeewon *et al.* (2004) with total reaction volume of 25 µl. The PCR products spanning approximately 850 bp (partial 28S nrDNA) and 550 bp (ITS nrDNA), were checked on 1% agarose electrophoresis gels stained with ethidium bromide. The amplified PCR products were purified using minicolumns, purification resin and buffer

according to the manufacturer's protocol (Amersham Biosciences, Catalog no. 27–9602–01). DNA sequencing was carried out using the above-mentioned primers in an Applied Biosystems 3730 DNA Analyzer at the Genome Research Centre, The University of Hong Kong.

Sequence alignment and phylogenetic analyses

For each species of *Oxydothis*, sequences obtained from the respective primers (LROR and LR5; ITS5 and ITS4) were aligned in Clustal X (Thomson *et al.*, 1997) and Bioedit (Hall, 1999). In total, two datasets were analysed: dataset based on 28 nrDNA sequences (Dataset I) and dataset based on 5.8S nrDNA (Dataset II). All sequences used in the analysis are listed in appendix 1.

Phylogenetic analyses were performed in PAUP* (Swofford, 2002). Ambiguously aligned sites were excluded from the all analyses. Unweighted parsimony (UP) and weighted parsimony (WP) analyses were performed. Gaps were treated as missing data. WP analyses were also performed using a symmetric step matrix generated with the program STMatrix version 2.2 (François Lutzoni and Stefan Zoller, Department of Biology, Duke University, Durhan, NC).

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. Clade stability was assessed in bootstrap analyses with 1000 replicates, each with 10 replicates of random stepwise addition of taxa.

Random sequence addition was used in the bootstrap analyses. Kishino-Hasegawa tests (Kishino and Hasegawa, 1989) were performed in order to determine whether trees were significantly different. Trees were figured in Treeview (Page 1996). Other details are outlined in Cai *et al.* (2005) and Kodsueb *et al.* (2006).

The best-fit model of evolution was determined by MrModeltest2.2 (Posada and Crandall, 1998). Posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes 3.0b4 (Huelsenbeck and Ronquist, 2001), using above estimated model of evolution. Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generations (resulting 10,000 total trees). The first 1,000 trees that represented the burn-in phase of the analyses were discarded and the remaining 9,000 were used for calculating posterior probabilities (PP) in the majority rule consensus rule tree. The analyses were repeated five times starting from different random trees to ensure trees from the same tree space were being sampled during each analysis. Posterior probabilities equal to and above 95% were regarded as significant.

3.3. Results

Taxonomy

Oxydothis wallichianensis Hidayat, To-anun and K.D. Hyde, *Fungal Diversity* **23**: 167 (2006)

MycoBank MB 510053 **(Fig. 3.1)**

Ascomata 70–150 µm diam, 55–100 µm alta, subglobosa, ostiolata. Asci 87.5–125 × 10–15 µm, 8-spori, unitunicati, pedunculati, aparatu apicale J+, 2–2.5 µm alti, 3–3.5 µm diam. praediti. Ascosporae 32.5–55 × 6.3–7.5 µm, 1-2 seriate, fusiformis, hyaline, bicellulares.

Etymology: In reference to the host genus, *Wallichia*.

Stromata 2–3 mm long × 1.5–2.5 mm wide, surrounded by ellipsoidal, brown borders. ***Ascomata*** 70–150 µm diam. × 55–100 µm high, stromata domes on the host surface, mostly clustered in groups of up to 18; in section immersed to erumpent, subglobose, papilla at one end curving upwards to the host surface. ***Peridium*** 5–8.8 µm thick comprised of 2–3 layers outer layers of oblong, dark–brown cells. ***Asci*** 87.5–125 × 10–15 µm, 8-spored, unitunicate, cylindrical, pedicellate, with a J+, 2–2.5 µm high × 3–3.5 µm diam., wedge-shaped, subapical ring. ***Ascospores*** 32.5–55 × 6.3–7.5 µm, 1–2-seriate, fusiform, 1-septate, hyaline, tapering abruptly near the ends to form long spine-like processes.

Material examined: THAILAND, Chiang Mai, Doi Suthep-pui national park, on decaying leaflets of *Wallichia siamensis* Becc. (Arecaceae), 21 July 2005, Iman Hidayat FIH 010 (**Holotype**: MRC 0002). **Isotype**: *ibid.,* HKU (M) 17174.

Habitat: Saprobic on *Wallichia siamensis* leaflets.

Distribution: Only known from the type locality.

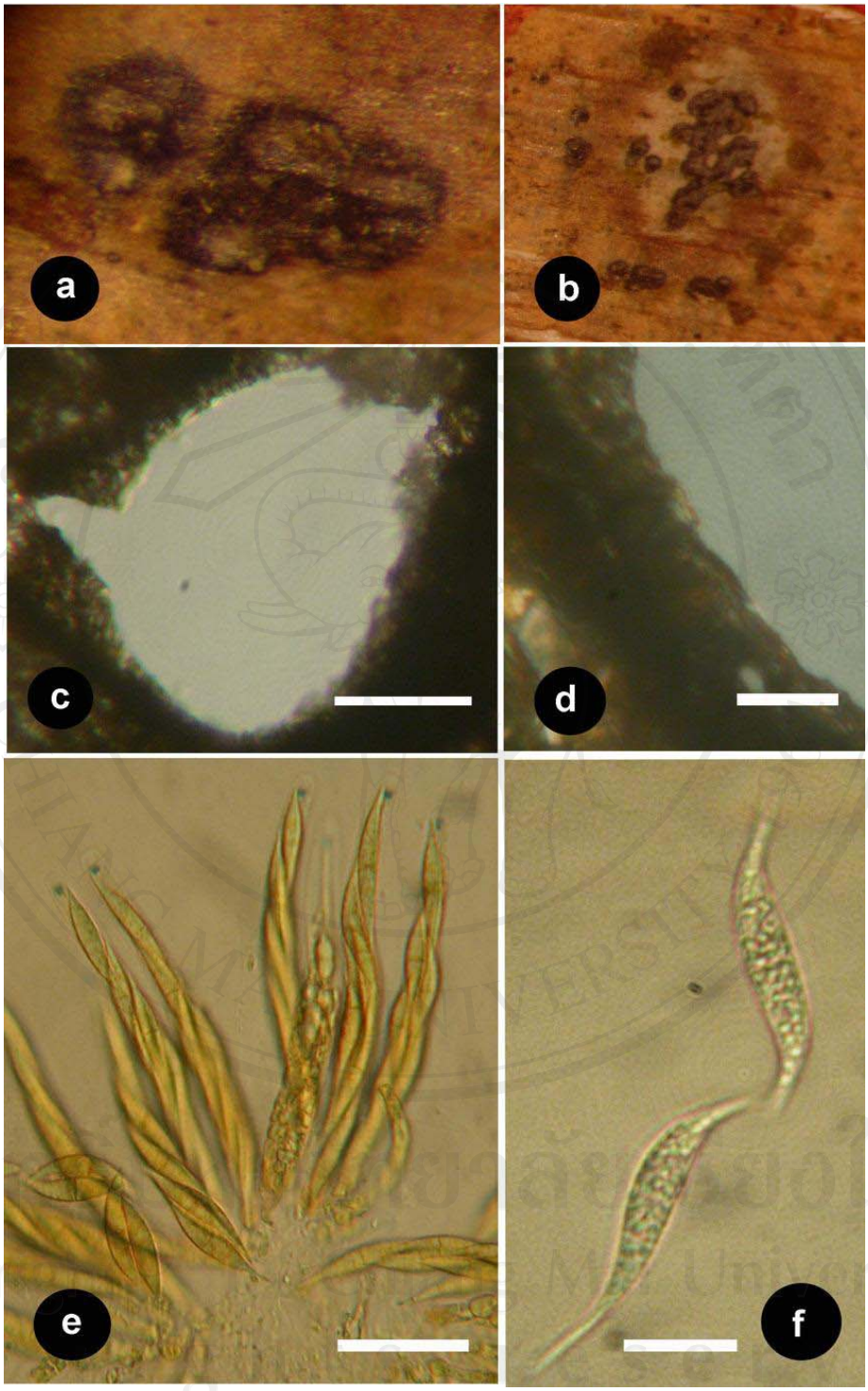

Fig. 3.1 Micrographs of *Oxydothis wallichianensis* (from the holotype). a-b. Ascomata on the host surface. c. Vertical section through the ascoma. d. Peridium. e. Asci and ascal ring. f. Ascospores. Bars: c = 50 µm; 13, d = 5 µm; e = 50 µm; f = 10 µm.

Oxydothis inaequalis Hidayat, To-anun and K.D. Hyde, *Fungal Diversity* **23**: 165 (2006)

MycoBank MB 510054 **(Fig. 3.2)**

Ascomata 110–120 µm diam, 22–30 µm alta, immersa, ellipsoida, ostiolata. Asci 200–285 × 11.3–12.5 µm, 8-spori, unitunicati, pedunculati, aparatu apicale J+, 3.5–4 µm alti, 3–3.5 µm diam. praediti Ascosporae 75–100 × 5–7.5 µm, 1-2 seriate, fusiformis, hyalinae, bicellulares.

Etymology: From the Latin *inaequalis* meaning "unequal, various" in reference to the ascospore processes that appear slightly unequal in length.

Stromata 5–40 mm long × 5–10 mm wide, visible as blackened ellipsoidal regions on the host surface, lacking borders. ***Ascomata*** 110–120 µm diam. × 22–30 µm high, forming slightly raised domes, singly or clustered in groups up to 10 (mostly 2–3); in section immersed, ellipsoid, long axis horizontal to that of the host surface, papilla at one end curving upwards to the host surface. Stromatic tissue surrounds the ascomata within the host hypodermis. ***Peridium*** 10–12.5 µm thick, comprised of 2–3 layers; outer layers of oblong, dark-brown cells and sometimes with an additional inner layer of oblong, hyaline cells. ***Paraphyses*** deliquescent early, septate, *ca* 2.5 µm in diam. ***Asci*** 200–285 × 11.3–12.5 µm, 8-spored, unitunicate, cylindrical, short pedicellate, J+, 4–6(–7) µm high, 3–4 µm diam., wedge-shaped, subapical ring, apically truncate. ***Ascospores*** 75–100 × 5–7.5 µm, 1–2 seriate, fusiform, 1-septate,

hyaline, tapering gradually to form long pointed processes. The ascospores processes are sometimes uneven which may make the septum appear slightly eccentric.

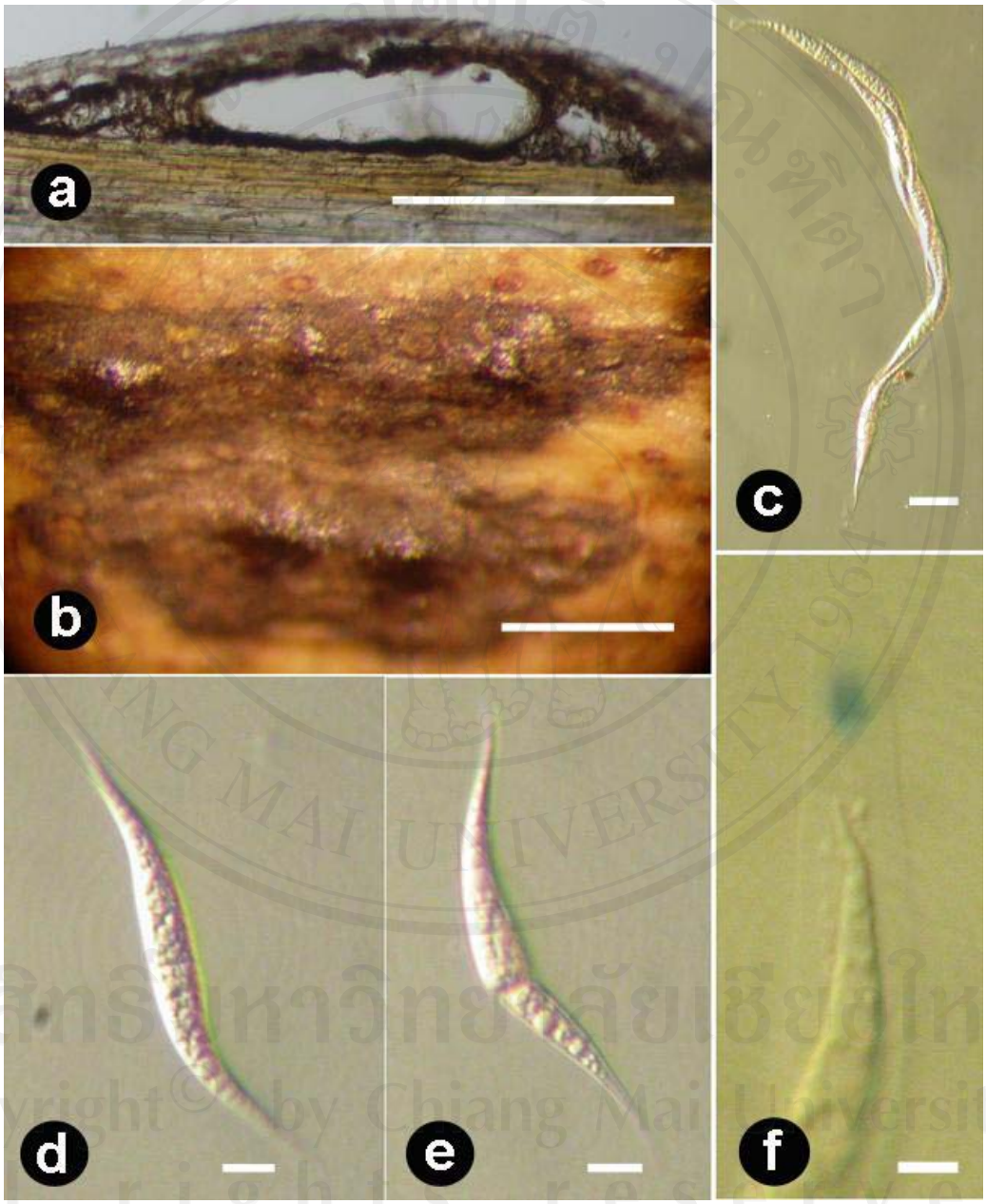

Fig. 3.2 Micrographs of *Oxydothis inaequalis* (from holotype). a. Vertical section through the ascoma. b. Ascomata on the host surface. c. Ascus. d-e. Ascospores. f. Amyloid positive of ascal ring. Bars: a = 110 µm; b = 10 mm; c = 12 µm; d = 7 µm; e = 7.5 µm; f = 4 µm.

Material examined: THAILAND, Chiang Mai, Doi Suthep-pui national park, decaying rachis of *Wallichia siamensis* Becc. (Arecaceae), 21 July 2005, Iman Hidayat FIH 018 (**Holotype**: MRC 0004). **Isotype**: *ibid.*, HKU (M) 17169.

Habitat: Saprobic on the *Wallichia siamensis* fronds.

Distribution: Only known from the type locality.

Oxydothis daemonoropsicola J. Fröhl. and K.D. Hyde, *Fungal Diversity Research Series* **3**: 183 (2000)

Material examined: THAILAND: Chiang Mai, Doi Suthep-pui national park, decaying rachis of *Wallichia siamensis* Becc. (Arecaceae), 21 July, 2005, Iman Hidayat FIH 019 (MRC 0005, 0006).

Habitat: Saprobic on fronds of *Archontophoenix alexandrae*, *Daemonorops margaritae*, *Wallichia siamensis* (Taylor and Hyde, 2003).

Distribution: Australia, Hong Kong, Malaysia and Thailand (Taylor and Hyde, 2003).

Oxydothis cyrtostachicola Hidayat, To-anun and K.D. Hyde, *Fungal Diversity* **23**: 164 (2006)

MycoBank MB 510055 **(Fig. 3.3)**

Ascomata 130–155 µm diam, 20–34 µm alta, immersa, subglobosa, ostiolata. Asci 102–120 × 12–13 µm, 8-spori, pedunculati, aparatu apicale J–, praediti Ascosporae 48–52 × 5–6 µm, hyalinae, fusiformis, bicellulares.

Etymology: In reference to the host genus, *Cyrtostachys*.

Ascomata forming under slightly raised, ellipsoidal regions on the host surface, black border, solitary or in groups 2–3; in section immersed, subglobose, ostiole eccentric, long axis horizontal to that of the host surface with neck at one end, forming *ca* 130–155 µm diam. × 20–34 µm high. ***Peridium*** comprised of 2–3 layers outer layers of oblong, dark-brown cells and with an additional inner layer of oblong, hyaline cells. *Paraphyses* deliquesce early. ***Asci*** 102–120 × 12–13 µm, 8-spored, unitunicate, clavate, short pedicellate, J-, refractive subapical ring, has a canal leading to the apex. ***Ascospores*** 48–52 × 5–6 µm, fusiform, 1-septate, hyaline, tapering gradually from the central septum to pointed processes, without spine-like form.

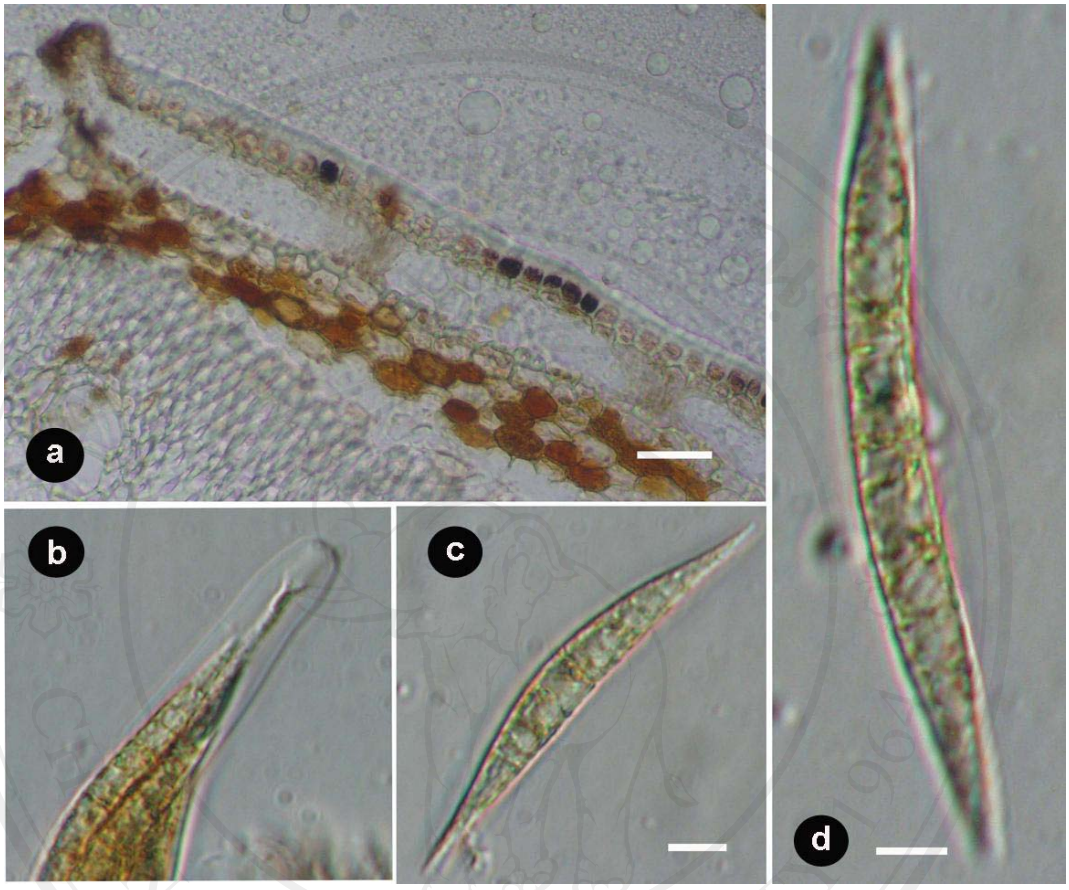

Fig. 3.3 Micrographs of *Oxydothis cyrtostachicola* (from holotype). a. Vertical section through the ascoma. b. Asci and ascal ring. c-d. Ascospores. Bars: a = 20 μm; c-d = 5 μm.

Material examined: THAILAND, Chiang Mai, Chiang Mai University garden, on petioles of *Cyrtostachys renda* Blume (Arecaceae), October 30, 2005, FIH 151 (**Holotype**: MRC 0007). **Isotype**: *ibid*., HKU (M) 17170.

Habitat: Saprobic on fronds of *Cyrtostachys renda*.

Distribution: Only known from the type locality.

3.4. Discussion

Oxydothis is a common ascomycete genus, with 72 species which have been reported from monocotyledons and especially on palms (Hyde, 1994a, b; Shenoy *et al.*, 2005). Fröhlich and Hyde (2000) reported that *Oxydothis* was the most common ascomycete genus occurring on palms. The basic taxonomic features of *Oxydothis* were discussed by Hyde (1994a), who emphasized on several morphological features to delimit the species, i.e., ascoma orientation, ascal ring size and shape and ascospore apex structure.

The three novel species differ from other morphologically similar *Oxydothis* species in ascomata shape and ostiole position, ascal ring and ascospore morphology. The ascospores of *Oxydothis wallichianensis* are similar to those of eight other species and these taxa are compared in table 3.1. *Oxydothis elaeidis* and *O. sabalensis* are the most similar species. The character that distinguishes *O. wallichianensis* from *O. elaeidis* is the ascal ring size, which is larger in the former (2–2.5 x 3–3.5 µm vs 1.6 x 2.4–3.2 µm). The grouping of ascomata (up to 20) under brown regions is also specific to *O. wallichianensis* and distinguishes it from other similar *Oxydothis* species, such as *O. parvula, O. sabalensis* and *O. batuapoensis* where ascomata are mostly solitary.

Table 3.1 Comparison of *Oxydothis wallichianensis* with similar *Oxydothis* species (measurements from Hyde, 1994b and Fröhlich and Hyde, 2000).

Taxa	Ascus		Ascal ring		Ascospores	
	Length (µm)	Width (µm)	Height (µm)	Width (µm)	Length (µm)	Width (µm)
O. wallichianensis	87.5–125	10–15	2–2.5	3–3.5	32.5–55	6.3–7.5
O. batuapoensis	83.5–117.5	(4.9–) 5.3–7.2	0.2–0.4	1.3–1.55 (–2)	33.9–48.8	2.9–3.4 (–4.1)
O. dispariapicis	224.2–277	14.1–17	2–3.4	2.4–3	(98–) 115–132.5	(6–) 7.3–8.8
O. licualicola	155–205	(10.5–) 12.5–15	1.8–2.5	3	60–80	5–7.5
O. livistonicola	*ca* 260	*ca* 10	1.2–1.6	2.4–3.2	74–96	6–8
O. parvula	110–130	8–10	1–1.6	2–2.8	49–62	4–6
O. perangusta	96–130	6.4–8	0.96–1.5	1.1–1.45	48–64	3–3.8
O. elaeidis	95–130	14–16	1.6	2.4–3.2	44–56	6–8
O. sabalensis	95–130	14–16	0.8–1	2.6–4	44–56	4–6

Oxydothis asymmetrica is the only *Oxydothis* species with similar ascospores to those of *O. inaequalis*, both in size and shape (table 3.2). The significant characters that distinguish it from *O. inaequalis* are ascus size (200–285 × 11.25 µm vs. (6.8–) 8.1–11.9 (–12) µm) and ascal ring size (3.5–4 × 3–3.5 µm vs. 0.8–1.55 × 2.1–2.5 µm). *Oxydothis asymmetrica* also differs in having lenticular ascomata in section with a central pore, while *O. inaequalis* has ellipsoidal ascomata with an eccentric orientation.

Table 3.2 Comparison of *Oxydothis inaequalis* with *O. asymmetrica* (measurements from Hyde, 1994b; Fröhlich and Hyde, 2000).

Taxa	Ascus		Ascal ring		Ascospores	
	Length (µm)	Width (µm)	Height (µm)	Width (µm)	Length (µm)	Width (µm)
O. inaequalis	200–285	11.3–13.8	3.5–4	3–3.5	78–100	5–6.3
O. asymmetrica	157.1–208.8	(6.8–) 8.1–11.9 (–12)	0.8–1.6 (–7)	2.1–2.5	67.5–81.5	4.7–5.5(–7)

The third *Oxydothis* species from *Wallichia siamensis* is *Oxydothis daemonoropsicola.* The characteristics of ellipsoidal ascomata, long cylindrical asci, J+ ascal ring and fusiform ascospores are typical of *O. daemonoropsicola.* The size of asci, ascal ring and ascospores are also close to those of the type as reported by Fröhlich and Hyde (2000) (table 3.3).

Table 3.3 Comparison of *Oxydothis* sp. FIH 019 with similar *Oxydothis* species (measurements from Hyde, 1994b; Fröhlich and Hyde, 2000).

Taxa	Ascus		Ascal ring		Ascospores	
	Length (µm)	Width (µm)	Height (µm)	Width (µm)	Length (µm)	Width (µm)
Oxydothis sp. FIH 019	225–255	12.5–13.8	2–2.3	2–3	95–105	5–6.3
O. daemonoropsicola	222.2–282.8	12.1–18.1	2.6–3.8	2.6–3.8	91.8–112.2	5.1–7.7
O. megalospora	224.5–292.8	11.7–16.6	3–7.5	3–5	103.7–126.9	5.6–7.6

Only four species of *Oxydothis* are characterized by a J– subapical ring: *O. ianei* (Taylor and Hyde, 2003), *O. livistonae*, *O. nonamyloidea* and *O. nontincta* (Fröhlich and Hyde, 2000). Species with J- subapical rings are distinguished by ascospore shape, size and in having or lacking mucilage at the ends. *Oxydothis cyrtostachicola* is characterized by fusiform ascospores, which tapering gradually from the central septum to pointed processes (not spine–like) and without mucilaginous drops at the ends. *O. cyrtostachicola* is closer to *O. ianei* and *O. nonamyloidea*; however, the asci and ascospores in *O. cyrtostachicola* are distinct from *O. ianei* and *O. nonamyloidea* in size. These taxa are compared in table 3.4.

Table 3.4 Comparison of *Oxydothis cyrtostachicola* with J– *Oxydothis* species (measurements from Hyde, 1994b; Fröhlich and Hyde, 2000; Taylor and Hyde, 2003)

Taxa	**Ascus**		**Ascospores**	
	Length (µm)	**Width (µm)**	**Length (µm)**	**Width (µm)**
O. cyrtostachicola	102–120	12–13	48–52	5–6
O. ianei	108–174	7.2–9.6	56–68	2.6–3.8
O. nonamyloidea	205–260	18–22	94–115	3.5–4.5
O. nontincta	(136–)146–200	11–14	68–96	5–6.4(–7)
O. livistonae	*Ca* 300	11–14	150–170	4–5.5

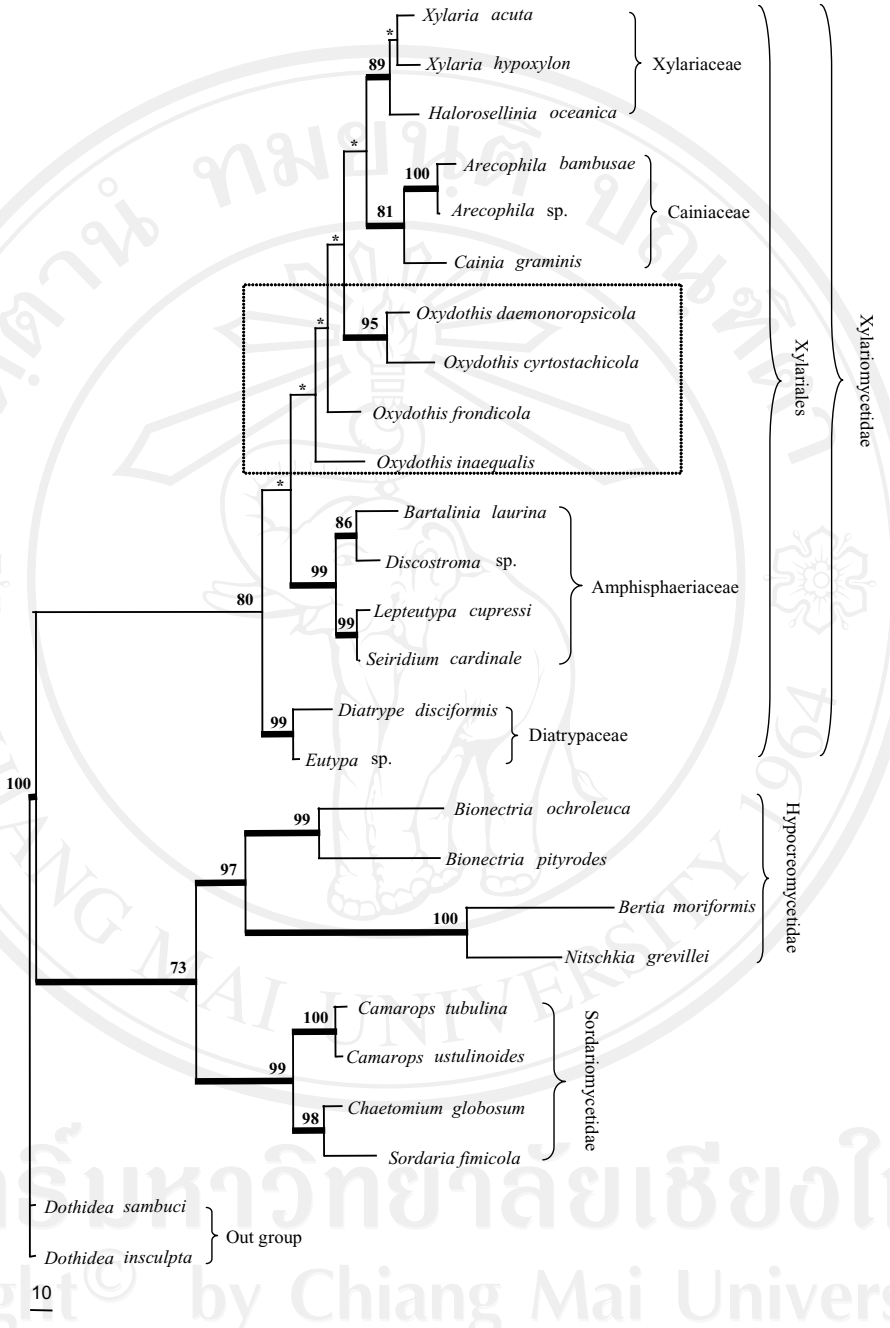

Fig. 3.4 Phylogenetic tree based on partial 28S nrDNA sequence data representing placement of members of the *Oxydothis* within class Sordariomycetes. Bootstrap values ≥ 50% from maximum parsimony analyses are shown above internodes and thickened branches indicate Bayesian analyses when posterior probabilities values ≥ 95%. Bootstrap values which are less than 50% shown by asterisk.

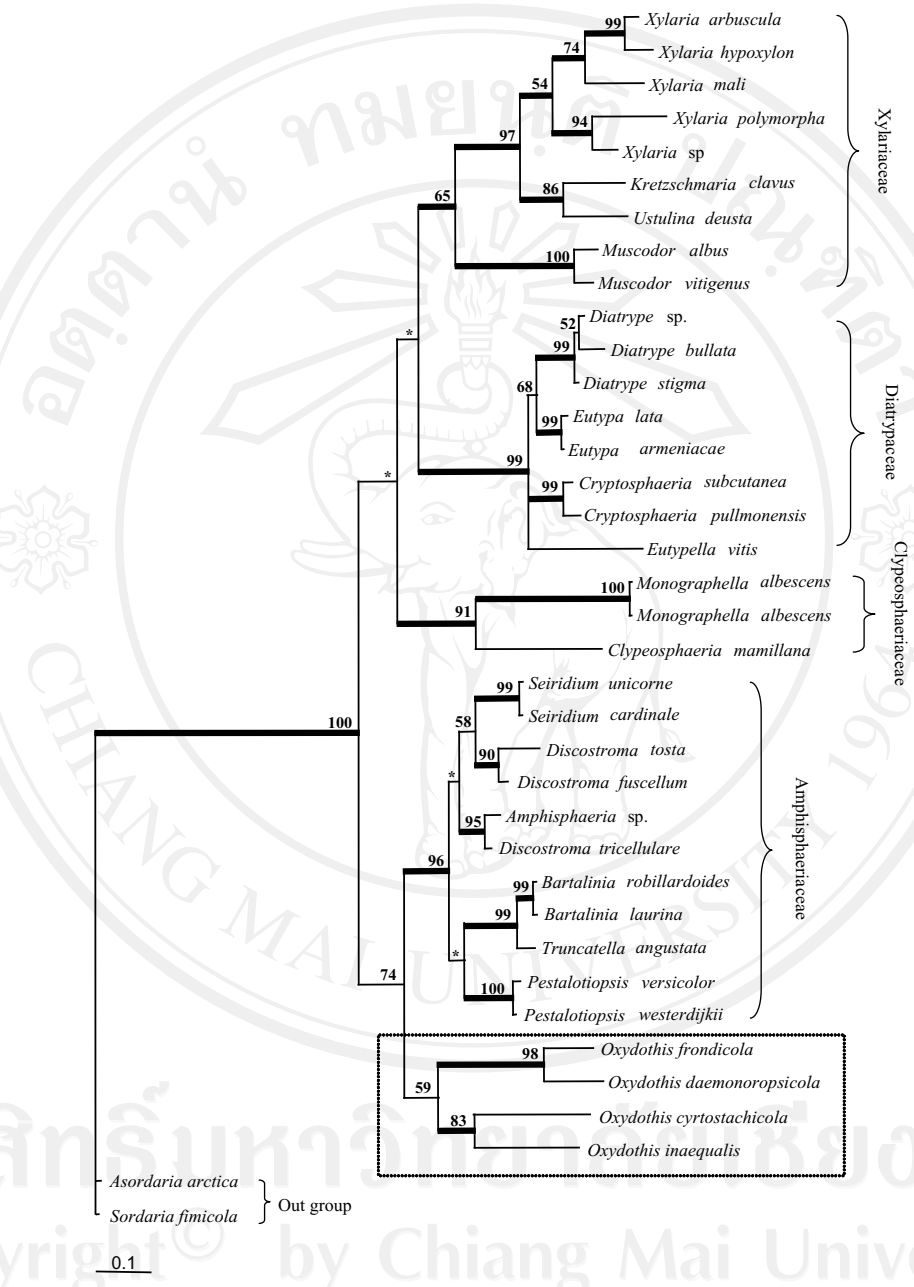

Fig. 3.5 Phylogenetic tree based on ITS nrDNA sequence data representing placement of members of the *Oxydothis* within the Xylariales. Bootstrap values ≥ 50% from maximum parsimony analyses are shown above internodes and thickened branches indicate Bayesian analyses when posterior probabilities values ≥ 95%. Bootstrap values which are less than 50% shown by asterisk.

The 28S nrDNA dataset (Dataset I) consisted of 26 taxa. The other reference taxa included members of known ascomycete families of the Sordariomycetes. *Dothidea sambuci* and *D. insculpta* were the designated outgroups. The final dataset comprised 913 characters. Likelihood-ratio test in MrModeltest2.2 suggested that the best fit model of evolution for this dataset was SYM+I+G. Thirty-nine characters (ambiguous regions) were excluded in the analyses. In parsimony analyses, when gaps were treated as missing data, there were 505 constant characters, 95 parsimony-uninformative characters, and 274 parsimony–informative characters for both UP and WP. Two trees were obtained from UP and WP analyses. Based on K-H test ($P^* \geq 0.05$), these four trees were not significantly different (details not shown). The single parsimonious tree (TL= 9, CI = 0.6, RI = 0.7, RC = 0.4, HI = 0.4, -ln L= 5890.3) generated from WP analyses with *Dothidea sambuci* and *D. insculpta* as outgroups and treating gaps as missing data is shown in fig. 3.4. Bootstrap values (equal to or above 50%) based on 1000 replicates are shown on the upper branches, while values of the posterior probabilities (PP) resulted from BMBMC analyses are represented on the lower branches.

The ITS dataset consisted of 37 taxa and analyses covered the ITS and 5.8S region. *Sordaria fimicola* and *Asordaria arctica* were the designated outgroups. The final dataset comprised 612 characters. Likelihood-ratio test in MrModeltest2.2 suggested that the best-fit model of evolution for this dataset was GTR+I+G. One hundred and six characters (ambiguous regions) were excluded in the analyses. In parsimony analyses, when gaps were treated as missing data, there were 199 constant characters, 36 parsimony-uninformative characters and 271 parsimony-informative characters for both UP and WP. Four trees were generated from UP and WP analyses

and they were not significantly different. The single parsimonious tree (TL = 1284, CI = 0.5, RI = 0.7, RC = 0.3, HI = 0.5, -ln L = 6699.4) generated from WP analyses and treating gaps as missing data is shown in fig. 3.5. Bootstrap values (equal to or above 50%) based on 1000 replicates are shown on the upper branches, while values of the posterior probabilities (PP) resulted from BMBMC analyses are represented on the lower branches.

Barr (1990) placed *Oxydothis* in the Hyponectriaceae based on its similarity with *Pemphidium*. *Oxydothis* is very similar to *Pemphidium* Mont. in ascomata, asci and ascospore morphology. The latter, however, differs from *Oxydothis* in having a non-amyloid subapical ring and 1-celled, cylindrical ascospores with unusual polar appendages (Hyde *et al.*, 2000). Wehmeyer (1975) placed *Pemphidium* in the Amphisphaeriaceae and this placement was accepted by Eriksson and Hawksworth (1991), who emphasized more on ascus and paraphyses morphology for it inclusion in the family (non–amyloid apical ring in asci is not typical of the Amphisphaeriaceae). Wang and Hyde (1999) later excluded *Oxydothis* from the Hyponectriaceae based on arrangement of ascomata, structure of asci and ascospores. They also excluded *Pemphidium* from the Hyponectriaceae due to few similarities with *Hyponectria*.

The non–amyloid subapical ring is not rare in *Oxydothis* since four reported *Oxydothis* species, namely, *O. ianei* (Taylor and Hyde, 2003), *O. livistonae*, *O. nonamyloidea* and *O. nontincta* (Fröhlich and Hyde, 2000) have non-amyloid subapical rings.

Samuels and Rossman (1987) reported a *Selenosporella* anamorph for *Oxydothis selenosporellae*, however, *Selenosporella* is also a synanamorph of *Iodosphaeria* (Lasiosphaeriaceae; Sordariales) (Samuels *et al.*, 1987). This may

probably explain the distinctive evolutionary history of *Oxydothis* in relation to the members of the Amphisphaeriaceae, which are known to produce *Pestalotiopsis*-like anamorphs (Nag Raj, 1977; Kang *et al.*, 1999a; Jeewon *et al.*, 2002; 2003). An ultrastructure of asci and ascospores of *Oxydothis* also suggested that *Oxydothis* is closer to the Diatrypaceae than the Amphisphaeriaceae (Wong and Hyde, 1999).

The 28S nrDNA sequence data was also analysed to confirm the placement of *Oxydothis* within the Xylariales. Molecular phylogenies in previous studies (Kang *et al.*, 1998, 1999b, 2002; Smith *et al.*, 2003) supported the placement of *Oxydothis* within the Xylariales (Sordariomycetes). Morphological characters such as presence of pseudostroma, ascoma perithecial, papillate ostiole, periphysate, cylindrical asci with J+ ring and transversely septate ascospores also support its placement within the Xylariales. Although *Oxydothis* species are nested in the Xylariales with 80% bootstrap support, there is insufficient evidence to place *Oxydothis* within any known families of the Xylariales. It was also clear that a molecular analysis does not support *Oxydothis* bears a monophyletic group and in addition *O. frondicola* did not cluster with other *Oxydothis* as would be expected based on morphological evidence. The association of *Oxydothis* with other known xylariaceous taxa receives very weak statistical support and most clades are not phylogenetically resolved. Therefore it was difficult to making any systematic conclusions based on our 28S nrDNA sequence dataset. Another major observation in this study was the lack of statistical support for all the major nodes within the Xylariales. Even addition of more taxa with broader taxon sampling from all families failed to resolve some of the major clades. Similar results were obtained from previously published nrDNA phylogenies (Smith *et al.*, 2003; Duong, *et al.*, 2004; Bahl *et al.*, 2005).

Parsimony analyses of the ITS dataset supported the monophyly of *Oxydothis* with 59% bootstrap support. *Oxydothis frondicola* and *O. daemonoropsicola* are closely related to each other, while *O. cyrtostachicola* and *O. inaequalis* share close phylogenetic affinities and both of these relationships are strongly supported. This is in accordance with their ascospore morphology. *Oxydothis frondicola* and *O. daemonoropsicola* produce filiform ascospores, while *O. cyrtostachicola* and *O. inaequalis* posess fusiform ascospores which tapering gradually from the central septum to pointed processes. Phylogenetic results also indicate that *Oxydothis* share close phylogenetic affinities to the Amphisphaeriaceae (74% bootstrap support). It is therefore highly plausible that *Oxydothis* is more closely related to members of the Amphisphaeriaceae, but whether it should be accomodated within this family as have been postulated by Muller and Arx, (1962, 1976), Wehmeyer (1975), Samuels and Rossman (1987) and Samuels *et* al., (1987) based on morphology is still doubtful. On the other hand, whether *Oxydothis* should be accommodated in a new family will definitely need further detailed study with more species (including those from *Pemphidium, Leiosphaerella,* and *Iodosphaeria)* and sequence analyses based on protein-coding genes. Single gene phylogeny based on nrDNA was insufficient and inefficient in resolving phylogenetic relationships within the Xylariales (Smith *et al.*, 2003; Bahl *et al.*, 2005). Several attempts to promote the formation of the anamorph of *Oxydothis* in culture and amplify partial RPB2 gene from a number of specimens were carried out but were unsuccessful.

Although the phylogeny of the Hyponectriaceae is still obscure, this result suggests that *Oxydothis* should be excluded from the Hyponectriaceae and Amphisphaeriaceae. Morphological characters such as ellipsoidal ascomata with long

axis parallel to the host surface, long cylindrical asci with canal leads to the apex, variability of apical apparatus shape (discoid, wedge shape and cylindrical) and size, ascospores with long fusiform or filiform with gradually tapering from the centre to pointed processes, support the exclusion from those families (table 3.5). Furthermore, the discovery of *Oxydothis* anamorph will be the key factor in resolving its affinities.

Table 3.5 Morphological comparison of *Oxydothis* with Amphisphaeriaceae and Hyponectriaceae.

Morphological characters	***Oxydothis***	**Amphisphaeriaceae**	**Hyponectriaceae**
Stromata	Pseudostroma	Crustose often clypeate	Reduced (clypeus absent)
Ascomata	Often ellipsoidal and long axis parallel to the host surface, rarely subglobose	Globose	Subglobose
Asci shape	Long cylindrical with canal leads to the apex	Cylindrical	Cylindric–clavate to clavate
Apical apparatus	J+ or J– subapical ring, variable in shapes (discoid, wedge shape, and cylindrical) and sizes	Usually J+, small	Small J+ or J–, apical ring
Ascospores colour	Hyaline	Hyaline to brown	Hyaline to pale brown

(Table continued)

Morphological characters	***Oxydothis***	**Amphisphaeriaceae**	**Hyponectriaceae**
Ascospores shape	Long fusiform or filiform, gradually tapering from the centre to pointed processes, which may be spine–like, or with round ends	Ellipsoidal to fusiform	Various
Ascospores ornaments	Often with small amounts of mucilage without germ pores	Rarely ornamented; however often with germ pores	Lacking ornaments