Hyphomycetes from Taiwan－*Endophragmia* and Allied Species

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(Manuscript received 20 August 2009; accepted 28 October 2009)

ABSTRACT: Four hyphomycetous fungi, including *Endophragmia dimorphospora*, *Epicoccum purpurascens*, *Helicoma dennisii* and *Helicoma palmgenum,* were newly recorded from Taiwan. These taxa were isolated from air, soil or decaying wood stem or litter covertime by single or mass spores isolation techniques, respectively. The morphological characteristics of the four taxa were closely examined, diagnosed and illustrated. Comparison of the taxa with conspecific isolates from varied geographic regions revealed a slight difference in sporulating structures. The distinctions among them were briefly discussed.

KEY WORDS: Hyphomycetes, Taiwan, Taxonomy, Biodiversity, Mitosporic fungi.

INTRODUCTION

In traditional classification scheme, fungi without accessed sexual stage usually were being classified in the Sub-phylum Deuteromycotina. Mainly based on the macro- and micro-morphological characteristics, Saccardo and allied workers categorized the members of this group of fungi into four Classes: Hyphomycetes, Blastomycetes, Coelomycetes and Agonomycetes (mycelia sterile) (Saccardo, 1880, 1886; Talbot, 1971). Of the Hyphomycetes, Moniliales is the key order which encompassed anamorphic fungi with sporulating structures born on the discrete conidiophores or aggregated synematous or cushion-shaped sporodochia (Barnett and Hunter, 1998). Furthermore, Moniliales was further divided into two families: Moniliacece and Dematiaceae, the latter characterized with pigmented mycelium, conidiophore or conidium (Barnett and Hunter, 1998). Actually, except for the morphological features, the conidiogenesis patterns defined and implemented by Hughes (1953), Subramanian (1962), Tsubaki (1958) and Barron (1968) were extremely valuable for more rapid and precise distinction and classification of the Hyphomycetes. The themes, in terms of macro-sporulating structure and micro-scopic conidiogenesis characteristics for taxonomic systematics, later have even been extended and applied to Coelomycetes by Sutton (1980). More recently, the phylogenetic relatedness of these fungi imperfect with respect to their Ascomycetes or Basidiomycetes counter partners were being established via the molecular systematic approaches derived from the genetic evolutionary hierarchy (Loutozoni, et al., 2004). Evidently, by combination of the genotypic and phenotypic traits, the biological entity for each taxon in nature will be established eventually.

In our laboratory, survey and construct the inventory of the anamorphic fungi originated from Taiwan to illustrate their biodiversity has been setup as one our long-term goals since the early 1980 onwards. A series of papers in this regard were published and documented in the more recently published "Fungal Flora of Taiwan" (Tzean et al., 2005). Our major approach for identification of the members of the mitosporic fungi were primarily based on the system proposed by the eminent mycologists mentioned above (Hughes, 1953; Subramanian, 1962; Tsubaki, 1958; Barron, 1968).

In this communication, we reported four newly recorded hyphomycetous fungi: *Endophragmia dimorphospora* Awao et Udagawa, *Epicoccum purpurascens* Ehreb. ex Schlecht., *Helicoma dennisii* M.B. Ellis and *Helicoma palmgenum* (Penzig & Saccardo) Linder. These taxa were isolated from the decayed stems and air in several localities of Taiwan overtime, by using the single or mass spore techniques. The collection, identification and preservation of the anamorphic fungi may not only enrich and enlighten our understanding of their biodiversity, but also provide the precious microbial resources for potential application for the betterment of human welfare.

MATERIALS AND METHODS

Samples collected from various rotten vegetations from several locations in Taiwan were incubated in moist chambers (plastic boxes, 30 x 20 x 12 cm, with three layers of moistened papers) to facilitate the sporulation of the fungi associated on the collected samples. The axenic cultures were obtained by single or mass spores isolation technique. The isolation was performed under a stereomicroscope using a sterile micro-glass needle to move the spores, and also separated the possible

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contaminants. The agar disc bearing single or mass spores but visualized to be identical were transferred to oat meal agar (OMA), V8 juice agar or corn meal agar (CMA) slants or plates depending for their nutrient preference. The practice served for isolation and also for identification purpose. For soil-borne hyphomycetes, soil samples collected from varied niches were sprinkled onto water agar, incubated at room temperature for adequate time period to allow the hyphal growth or limited sporulation. Pure cultures were secured by the same single or mass spores isolation techniques aforementioned, or by single hyphal-tip isolation techniques, which was also performed under a stereomicroscope with a sterile sharp scaple. For collection and isolation of air-borne fungi, Burkard spore trap (Burkard Manufacturing Co. Ltd., UK) was used. Details of morphological characteristics and conidiogenesis were illustrated and photographed with an Olympus light microscope (BH-2) built with a drawing tube. The taxonomic systems of Barron (1968), Barnett and Hunter (1998), Ellis (1971), Hughes (1953), Kendrink (1971), Saccardo (1880), Subramanian (1962) and Tubaki (1958) were followed for identification. Dried voucher specimens were deposited in the Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan, R.O.C., for further comparative study purpose.

TAXONOMIC TREATMENTS

Endophragmia dimorphospora Awao et Udagawa, 1974. Trans. mycol. Soc. Jap. 15: 99. Figs. 1 & 2

Colonies diameter on Oat Meal Agar 50 mm in 50 days at 25°C, effuse, inconspicuous, olive to olive brown or dark brown; reverse olive brown. Mycelium mostly immersed, composed of branched, septate, subhyaline to dark reddish brown, smooth hyphae. Conidiophores macronematous, mononematous, simple, flexuous, elongated-proliferation, pale brown to dark reddish brown, smooth, 71.4-317.4 X 5.8-7.5 µm, occasionally with very short conidiophore 6.7 µm long, 4.2 µm wide. Conidiogenous cells monoblastic, perccurent. Conidia obovoid or pyriform, 1-septate, apical cell dark-black or dark brown and basal cell conico-truncate, pale brown to middle brown, 14.2-27.1 x 9.6-18.3 µm.

Specimens examined: Taiwan, Wulai, Taipei County, on rotten stem, May 15 1990. leg. J.L. Chen. TNTU 843.

Distribution: Taiwan, U.S.A., Kenya, Australia, Hong Kong, USSR, Cuba, Mexico.

Notes: The genus *Endophragmia* was established by Duvernoy and Maire (1920) to accommodate a single species, *E. mirabilis* Duvernoy & Maire. Thereafter twelve allied species have been described and listed in CABI Bioscience Databases (2009). *Endophragmia dimorphospora* was first described by Awao et Udagawa (1974) from soil in Japan. Hughes (1979) who treated *Endophragmia dimorphospora* Awao et Udagawa as a basionym, and transferred it to the genus *Endophragmiella B. Sutton* as *Endophragmiella dimorphospora* (Awao et Udagawa) S. Hughes, however, the revision appears not accepted. This species is easily distinguished by its macronematous, mononematous, simple, flexuous, elongated- proliferation conidiophores with monoblastic, perccurent conidiogenous cells, and obovoid or pyriform, 1-septate, apical cell dark-black or dark brown and basal cell conico-truncate, pale brown to middle brown conidia. The Taiwanese's isolate was very similar to the type species of *E. dimorphospora* in shape and pigmentation of its conidia, but differed in having larger and broader conidia (up to 27.1 µm long and 18.3 µm wide).

Epicoccum nigrum Link, 1815. Magazin Ges. naturf. Freunde, Berlin 7: 32. Figs. 3 & 4

Colonies diameter on Corn Meal Agar larger than 65 mm in 48 days at 25°C, effuse, velvety to moderately floccose, white to olive brown; reverse yellowish white to yellowish brown or brown. Sporodochia present. Mycelium partly superficial, partly immersed, composed of branched to anastomosis, septate, smooth to verruculose or verrucose, hyaline to dark brown, 1.5-6.4 µm wide hyphae. Conidiophores macronematous, mononematous, often dense clusters, sporodochia, short, erect or curved, unbranched or branched, pale yellowish brown to yellowish brown, smooth or roughened, 9.6-20.0 x 4.8-8.0 µm. Conidiogenous cells monoblastic. Conidia acrogenous, globose to subglobose, 13.0-23.8 µm, muriform, verruculose to verrucose, yellowish brown to dark brown, often with a paler protuberant basal cell.

Specimens examined: Taiwan, Taipei Main Train Station, Taipei City, air-borne, Oct. 25 1992. leg. J.L Chen. TNTU 1038.

Distribution: Taiwan, Japan, Uruguay, China, Scotland South Africa, U.S.A., Papua New Guinea, Ukraine, Kenya, Brazil, Canada, Switzerland, Western Cape, Ethiopia, Puerto Rico, Virgin Islands, Mauritius, Malawi, Spain, India, Scotland, Malaysia, Australia, Poland, Italy, Zimbabwe, Ecuador, Czech Republic, Ukraine, United Kingdom, New Zealand, Greece, Malawi, Zambia, Germany, Russia, Nepal, Venezuela, Mexico, Czechoslovakia, Kerea, Cuba, Russia, Tanzania.

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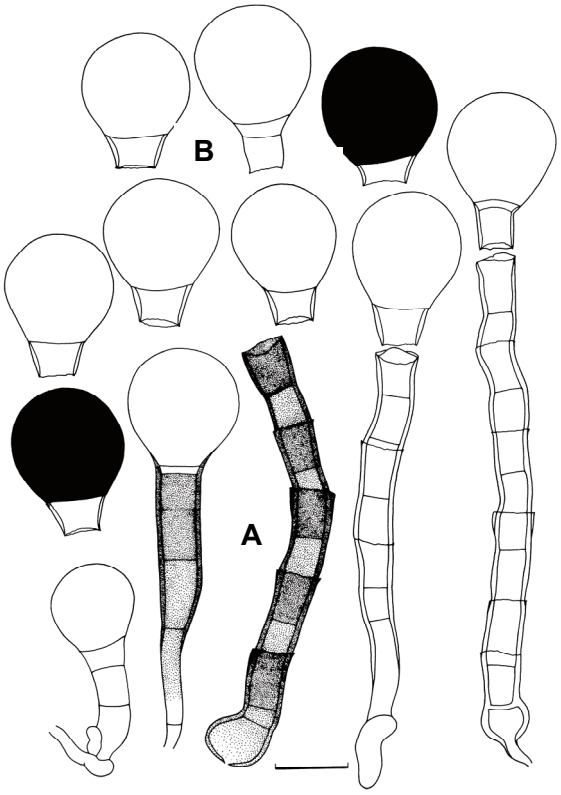


Fig. 1. ***Endophragmia dimorphospora.*** **A: Conidiophores. B: Conidia. Scale bar = 10 µm.**

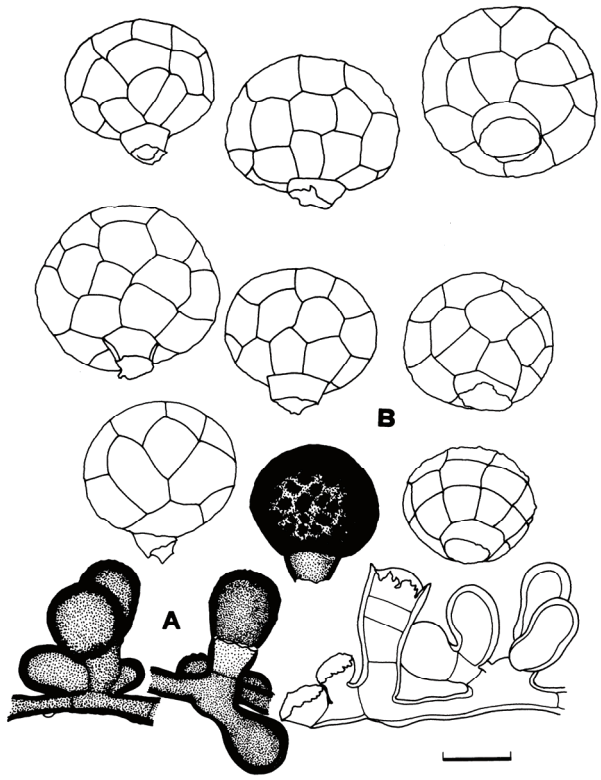
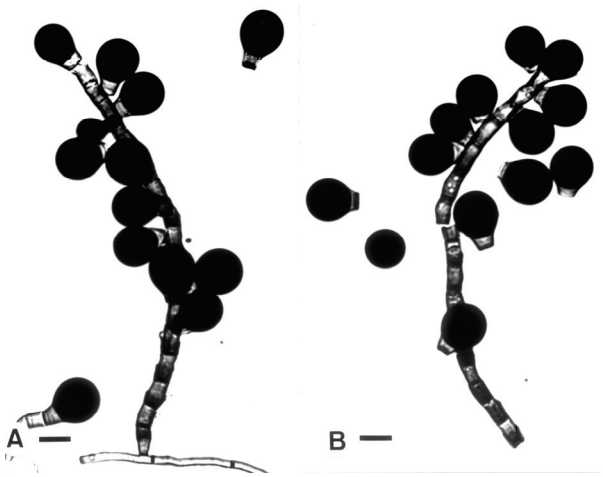


Fig. 3. ***Epicoccum nigrum.*** **A: Conidiophores. B: Conidia. Scale bar = 10 µm.**



Figs. 2. ***Endophragmia dimorphospora.*** **A & B: Simple conidiophores with monoblastic, perccurent conidiogenous cells and obovoid or pyriform conidia. Scale bars A & B = 10 µm.**

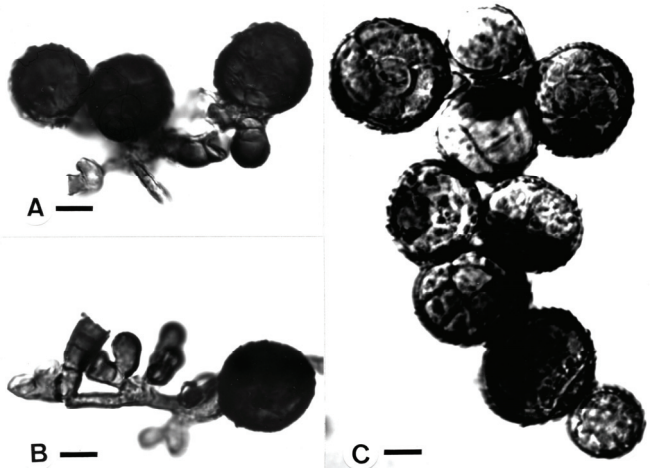


Fig. 4. ***Epicoccum nigrum.*** **A: Conidia arising from conidiophores. B: Short and branched conidiophores. C: Globose, muriform and verruculose conidia. Scale bars A-C = 10 µm.**

Notes: Since the establishment of the genus *Epicoccum* typed with *E. nigrum* by Link in 1815, thereafter about one hundred allied species have been described (CABI Bioscience Databases, 2009). It is easily distinguished by its macronematous, mononematous, often dense clusters, sporodochia, short, erect or curved, unbranched or branched, pale yellowish brown to yellowish brown conidiophores with monoblastic

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conidiogenous cells, and globose to subglobose, muriform, verruculose to verrucose, yellowish brown to dark brown conidia. Our collection in a global view was very similar to the type species of *Epicoccum nigrum* in shape and pigmentation of its conidiophores and conidia, but differed in having longer conidiophores (up to 20.0 µm) and smaller conidia (up to 23.8 µm wide).

Helicoma dennisii M.B. Ellis, 1963. Mycol. Pap. 87: 23-24. Figs. 5 & 6

Colonies growing slowly, on Oat Meal Agar attaining 40 mm in 57 days at 25°C, effuse, velvety to hairy, present fasiculate, olive brown to yellowish brown; reverse olive brown to dark brown. Mycelium mostly superficial, composed of branched or anastomosis, septate, smooth or verrucose, subhyaline to pale yellowish brown hyphae. Conidiophores macronematous, mononematous, straight or flexuous, septate, often in dense fascicles or directly raising from the creeping hyphae, simple or branched, subhyaline to pale yellowish brown or reddish brown, 24.0-208.0 x 5.0-8.4 µm; sometimes swollen at the base. Conidiogenous cells sympodial, with short denticle scattered. Conidia acropleurogenous, helicospore, hyaline to subhyaline or pale yellowish brown, smooth, rounded at the end, conico-truncate at the base, coiled into 1 1/4 spirals, (4)5-8-septate, 19.2-24.2 µm in diameter, 7.9-9.2 µm wide.

Specimens examined: Taiwan, Chihpen, Taitung County, on rotten stem, Mar. 30 1986. leg. J.L. Chen. TNTU 795.

Distribution: Taiwan, Russia, U.S.A., Japan, India, South Africa, Cuba, Venezuela.

Notes: The genus *Helicoma* was established by Corda (1837) to accommodate a single species, *H. muelleri* Corda. Thereafter fifty seven allied species have been described (CABI Bioscience Databases, 2009). *Helicoma dennisii* was first described by M.B. Ellis (1963) from dead wood in Venezuela. It was easily recognized by its macronematous, mononematous, straight or flexuous, septate, simple or branched conidiophores with sympodial conidiogenous cells and acropleurogenous, helicospore, hyaline to subhyaline or pale yellowish brown, smooth conidia. The type species of *Helicoma dennisii* was very similar to our collection in shape and pigmentation of its conidiophores and conidia, but differed in having longer conidiophores (up to 470.0 µm) and smaller conidia (up to 22.0 µm in diameter). The Taiwanese's isolate was comparable to those of Papua New Guinea's collection described by Matsushima (1971) in shape, septate, pigmentation and ontogeny of its conidia and sympodial conidiogenous cells. However, the conidia in the former (up to 24.2µm in diameter) were smaller contrast to those in the latter (up to 25.0 µm).

Helicoma palmigenum (Penzig & Saccardo) Linder, 1929. Ann. Mo. Bot. Gdn. 16: 306. Figs. 7 & 8

Colonies diameter on Oat Meal Agar larger than 60 mm in 38 days at 25°C, effuse, plane, with more or less floccose at the center, brownish grey to greyish brown; reverse greyish brown to brown or dark brown. Mycelium mostly immersed, composed of branched, septate, smooth, subhyaline to pale brown or middle brown, 1.4-7.0 µm hyphae. Conidiophores macronematous, mononematous, single or cluster, simple or branched, straight or slightly curved, septate, smooth, brown, paler towards the apex, 39.0-210.0 X 5.4-9.0 µm, often elongated-proliferation. Conidiogenous cells monoblastic, sympodial. Conidia helicospore, 9-17-septate, smooth, pale brown to yellowish brown, 28.0-35.0 µm in diameter; indivudial threads 8.0-11.0 µm wide, often with a conspicuously darker scar at the base.

Specimens examined: Taiwan, Chilan, Ilan County, on rotten stem, Sep. 20, 1993. leg. J.L. Chen. TNTU 1146.

Distribution: Taiwan, Australia, New Guinea, Seychelles, U.S.A., Japan, Cuba.

Notes: This fungus was originally recorded by Linder (1929), who treated *Helicosporium intermedium* var. *palmigenum* Penzig and Saccardo as a basionym, and transferred it to the genus *Helicoma* Corda as *H. palmigenum* (Penzig & Saccardo) Linder. It was easily recognized by its macronematous, mononematous, single or cluster, simple or branched, straight or slightly curved, septate conidiophores with monoblastic, sympodial conidiogenous cells and helicospore, septate, smooth, pale brown to yellowish brown conidia. The Taiwanese's isolate was comparable to those of Papua New Guinea's collection from rotten leaf of *Cocos nucifera* by Matsushima (1971) in shape, septation, pigmentation of its conidia. However, the conidia in the former (up to 35.0 µm in diameter) were smaller than in the latter (up to 42.0 µm). In addition, our collection also showed similarity to those of Indonesia, New Guinea, Trinidad and Hawaii's collections from decaying palm litters by Goos (1986) in shape of its conidiophores and conidia, but conidiophores were shorter and conidia smaller.

ACKNOWLEDGEMENTS

The study was supported by grant (95-2621-B-002-015-MY3) from National Science Council (NSC), Executive Yuan, Taiwan, R.O.C. to S.S. Tzean. The authors are indebted for the long lasting support from the NSC.

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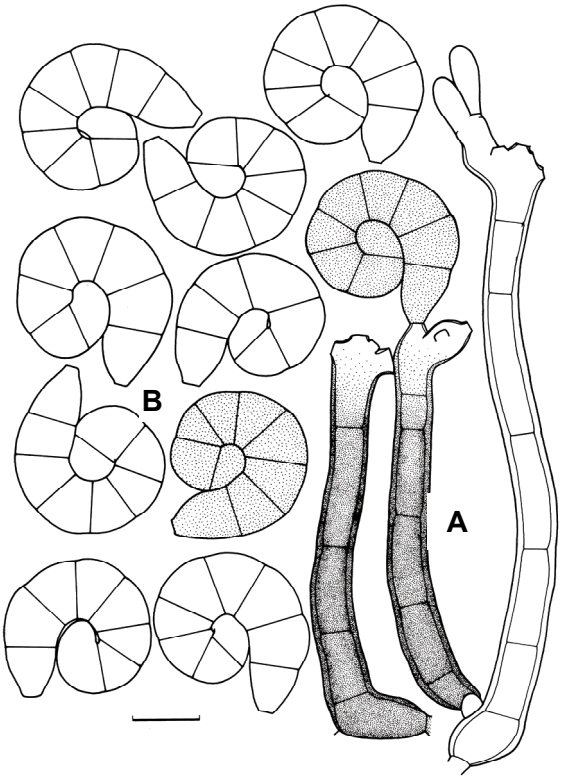


Fig. 5. *Helicoma dennisii*. A: Simple conidiophore with sympodial, short denticle conidiogenus cell on the top. B: Helical form conidia. Scale bar = 10 µm.

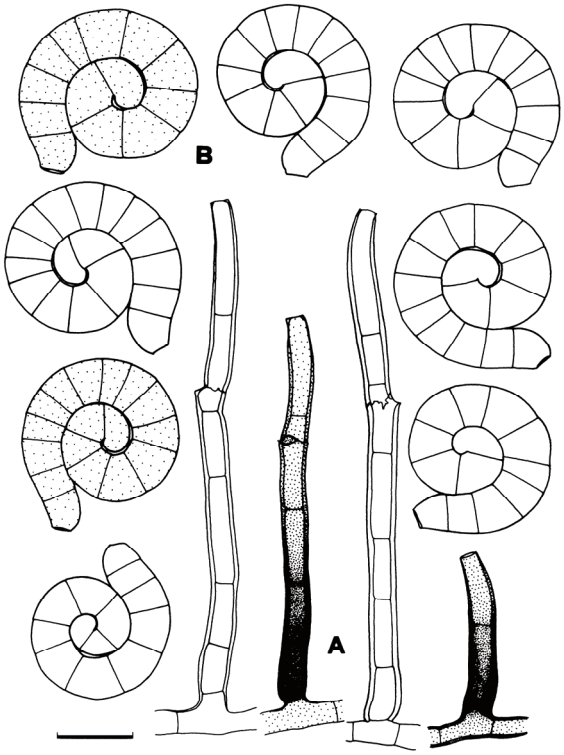


Fig. 7. *Helicoma palmigenum*. A: Simple conidiophore with sympodial, denticle conidiogenus cell. B: Helical form conidia. Scale bar = 20 µm.

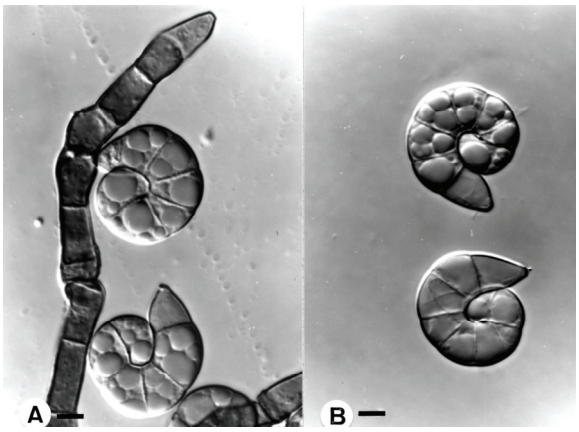


Fig. 6. *Helicoma dennisii*. A: Conidiophore and conidia. B: Conidia. Scale bars A & B = 10 µm.

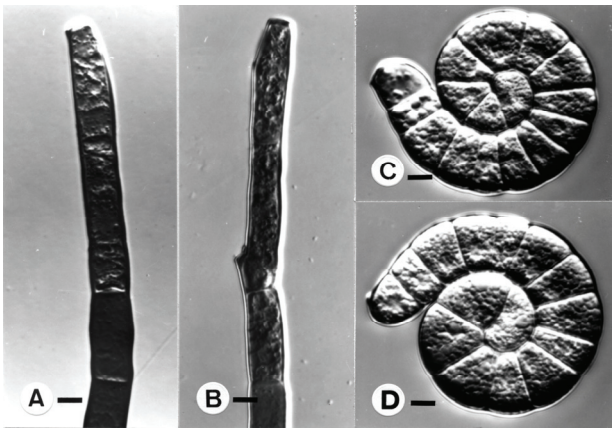


Fig. 8. *Helicoma palmigenum*. A & B: The upper part of conidiophores with monoblastic, sympodial conidiogenous cells. C & D: Helical form conidia. Scale bars A-D = 5 µm.

LITERATED CITED

Awao, T. and S. I. Udagawa. 1974. *Endophragmia dimorphospora*, a new Hyphomycetes. Trans. Mycol. Soc. Jap. **15**: 99-104.

Barnett, H. L. and B. B. Hunter. 1998. Illustrated Genera of Imperfect Fungi. APS press, Minnesota, USA. 218pp.

Barron, G. L. 1968. The Genera of Hyphomycetes from Soil. Williams & Wilkins, Baltimore, Maryland, USA. 364pp.

CABI Bioscience Databases. 2009. *Index fungorum*. [Online] Available: http://www.speciesfungorum.org/Names/Names.asp

Corda, A. C. J. 1837. Icones Fungorum hucusque cognitorum. **1**: 15.

臺灣大學學術期刊資料庫

Duvernoy, A. and R. Maire. 1920. Une nouvelle Dématiée à conidies pseudo-endogènes. Bulletin de la Société mycologique de France **36**: 86-89.
Ellis, M. B. 1963. Dematiaceous Hyphomycetes. IV. Mycol. Pap. **87**: 1-42.
Ellis, M. B. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew. 608pp.
Goos, R. D. 1986. A review of the anamorph genus *Helicoma*. Mycologia **78**: 744-761.
Hughes, S. J. 1953. Conidiophores, conidia and classification. Can. J. Bot. **31**: 577-659.
Hughes, S. J. 1958. Revisiones Hyphomycetum aliquot cum appendice de nomimibus rejiciendis. Can. J. Bot. **36**: 727-836.
Hughes, S. J. 1979. Relocation of species of *Endophragmia* auct. With notes on relevant generic names. N. Z. J. Bot. **17**: 139-188.
Kendrick, B. (ed.). 1971. Taxonomy of Fungi Imperfecti. Univ. of Toronto Press, Toronto, Ontario, Canada. 309pp.
Linder, D. H. 1929. A monograph of the helicosporous Fungi Imperfecti. Ann. Mo. Bot. Gard. **16**: 227-388.
Lutozoni, F., F. Kaukk, C. J. Cox, D. McLanghlin, G. Celio, B. Dentinger, M. Padamsee, D. Hibbett, T. Y. James, E. Baloch, M. Grube, V. Reeb, V. Hofstetter, C. Schoch, A. E. Arnold, J. Miadlikowska, J. Spatafora, D. Johnson, S. Hambleton, M. Crockett, R. Shoemarker, G. H. Sung, R. L Ücking, T. Lumbsch, K. O'Donnell, M. Binder, P. Diederich, D. Ertz, C. Gueidan, K. Hansen, R. C. Harris, K. Hosaka, Y. W. Lim, B. Matheny, H. Nishida, D. Pfister, J. Rogers, A. Rossman, I. Schmitt, H. Sipman, J. Stone, J. Sugiyama, R. Yahr, and R. Vilgalys. 2004. Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. Amer. J. Bot. **91**: 1446-1480.
Matsushima, T. 1971. Microfungi of the Solomon Islands and Papua-New Guinea, Matsushima, Kobe. 78pp.
Saccardo, P. A. 1880. Conspectus generum italiae inferiorum nempe ad Sphaeropsideas, Melaconieas et Hyphomyceteas pertinentium, systemate sporologico dispositoru. Michelia **2**: 1-38.
Saccardo, P. A. 1886. Sylloge Fungosum Omnium Hucusque Cognitorum. Vol. IV. Hyphomyceteae. Published by the author, Pavia, Italy. 807pp.
Subramanian, C. V. 1962. The classification of the Hyphomycetes, Bull. Bot Surv. India **4**: 249-259.
Sutton, B. C. 1980. The Coelomycetes, CMI, Survey, England. 696pp.
Talbot, P. H. B. 1971. Principles of Fungal Taxonomy, New York, USA. 273pp.
Tubaki, K. 1958. Studies on Japanese hyphomycetes V. Leaf and stem group, with a discussion of the classification of hyphomycetes and their perfect stages. Jour. Hattori Bot. Lab. **20**: 142-244.
Tzean, S.-S., W.-R. Hsieh, T.-C. Chang and S.-H. Wu. 2005. Fungal of Taiwan, Nation Science Council Press, Taipei, Taiwan. 2422pp.

臺灣產絲孢綱不完全菌 *Endophragmia* 和近似種之探討

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(收稿日期：2009 年 8 月 20 日；接受日期：2009 年 10 月 28 日)

摘要：本文詳細繪圖、描述四種臺灣產隸屬於不完全菌絲孢綱 (Hyphomycetes) 之新紀錄種真菌：*Endophragmia dimorphospora*, *Epicoccum purpurascens*, *Helicoma dennisii* 以及 *Helicoma palmgenum*，並簡扼比較討論此類真菌之形態鑑定特徵，以及和分布於其它不同地域之同種真菌之異同。

關鍵詞：絲孢綱、不完全菌、分類學、生物多樣性、臺灣。

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