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Endosporoideus gen. nov., a mitosporic fungus on *Phoenix hanceana*

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Abstract: *Endosporoideus pedicellata* gen. et sp. nov. is described and illustrated from decaying petioles of *Phoenix hanceana* collected from grassland in Tai Mo Shan, Hong Kong. The genus is unique in producing solitary, phragmosporous conidia. The conidia comprise a brown to dark brown inner-wall layer and thick, hyaline outer-wall layer and are produced holoblastically from determinate conidiogenous cells on micronematous, mononematous conidiophores. Cells of conidia may disarticulate at the septa. Rep-

resentative steps in conidiogenesis of *E. pedicellata* are illustrated with light micrographs, and details of the conidiogenous events are interpreted schematically.

Key words: palmicolous fungi, saprobes, systematics

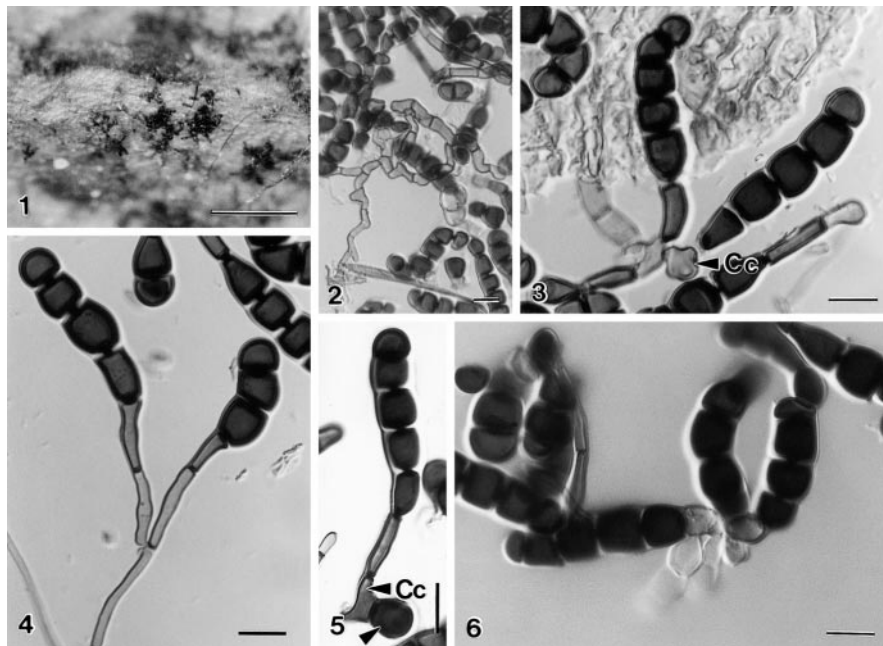
INTRODUCTION

In a continuing study of palm fungi in the tropics (Yanna et al 1997, 1998a, b, 1999, 2000a, b, 2002; Hyde et al 2002), an undescribed dematiaceous mitosporic fungus was found on decaying fronds of *Phoenix hanceana* Naud., growing in grassland mountain areas in Hong Kong. The fungus is reminiscent of genera such as *Janetia*, *Pithomyces* and *Trichocladium* (Carmichael et al 1980, Goh and Hyde 1996) in producing micronematous, mononematous conidiophores, and solitary, holoblastic, dematiaceous, phragmosporous conidia.

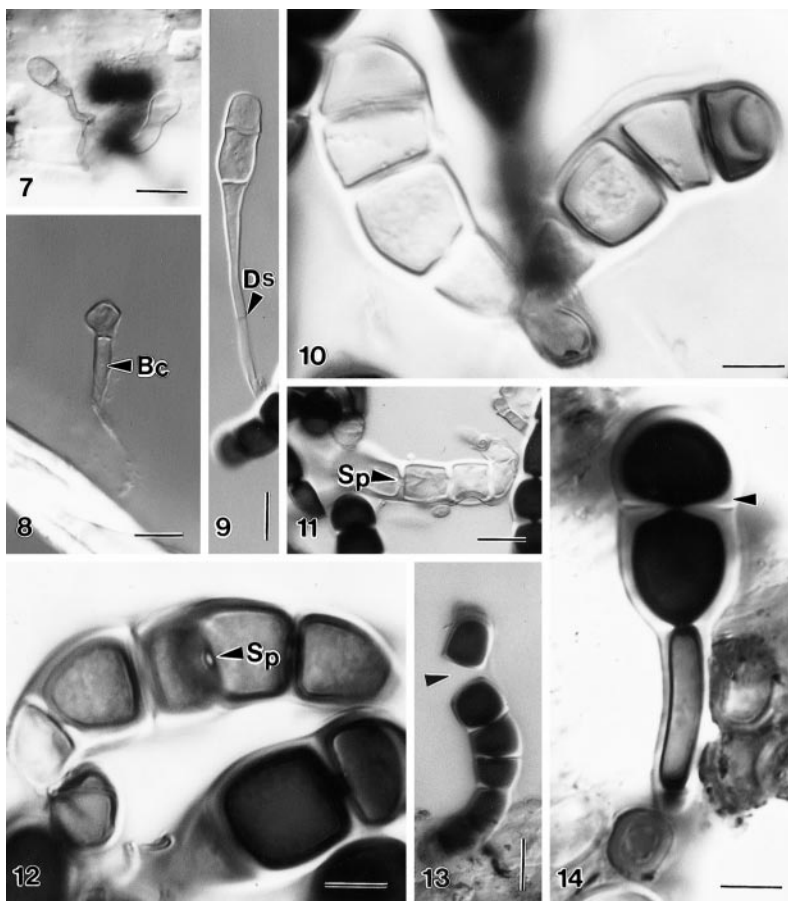
The fungus is unique in producing conidia with an

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FIGS. 1–6. Light micrographs of *E. pedicellata* (from holotype). Cc = conidiogenous cells. 1. Colonies on natural substratum. 2. Hyphae and conidia. 3, 4. Conidiogenous cells and conidia. 5. Conidiogenous cell with a mature conidium and a developing conidium (arrowhead). 6. Conidia in fascicles. Note the immature conidia produced from the conidiogenous cell (arrowhead). Scale bars: 1 = 500 μ m; 2 = 25 μ m; 3–6 = 10 μ m.



FIGS. 7–14. Light micrographs of representative steps in the conidiogenesis of *E. pedicellata*. Bc = conidial basal cell, Ds = conidial delimiting septum, Sp = septal pore. 7. Immature conidia on natural substratum. 8. Immature conidium with a swollen conidial cell and a basal cell. 9. Immature conidium with two swollen conidial cells and a basal cell. Note the delimiting septum. 10. Immature conidia with thickened, hyaline wall and septa. 11. An immature conidium with a septal pore in side view. 12. Conidial septal pore viewed from above. 13. Mature conidium with cells disarticulate at the septum (arrowhead). 14. Mature conidium with two swollen conidial cells and a cylindrical basal cell. Note the thick hyaline conidial wall and septa (arrowhead). Scale bar: 7–9, 11, 13 = 10 μm ; 10, 12, 14 = 5 μm .

extensively thick, hyaline outer-wall layer and brown inner-wall layer. The conidia appear to resemble isthospores that are formed endogenously in thick sheaths, but they are in fact phragmosporous. Cryo-scanning electron microscopy was used to confirm these observations. A new genus, *Endosporoideus*, is introduced and a new species, *E. pedicellata*, is described to accommodate the unidentified fungus. Representative steps in conidiogenesis of *E. pedicellata* are illustrated using interference contrast microscopy, and the interpreted conidiogenesis events are illustrated with schematic diagrams.

METHODS

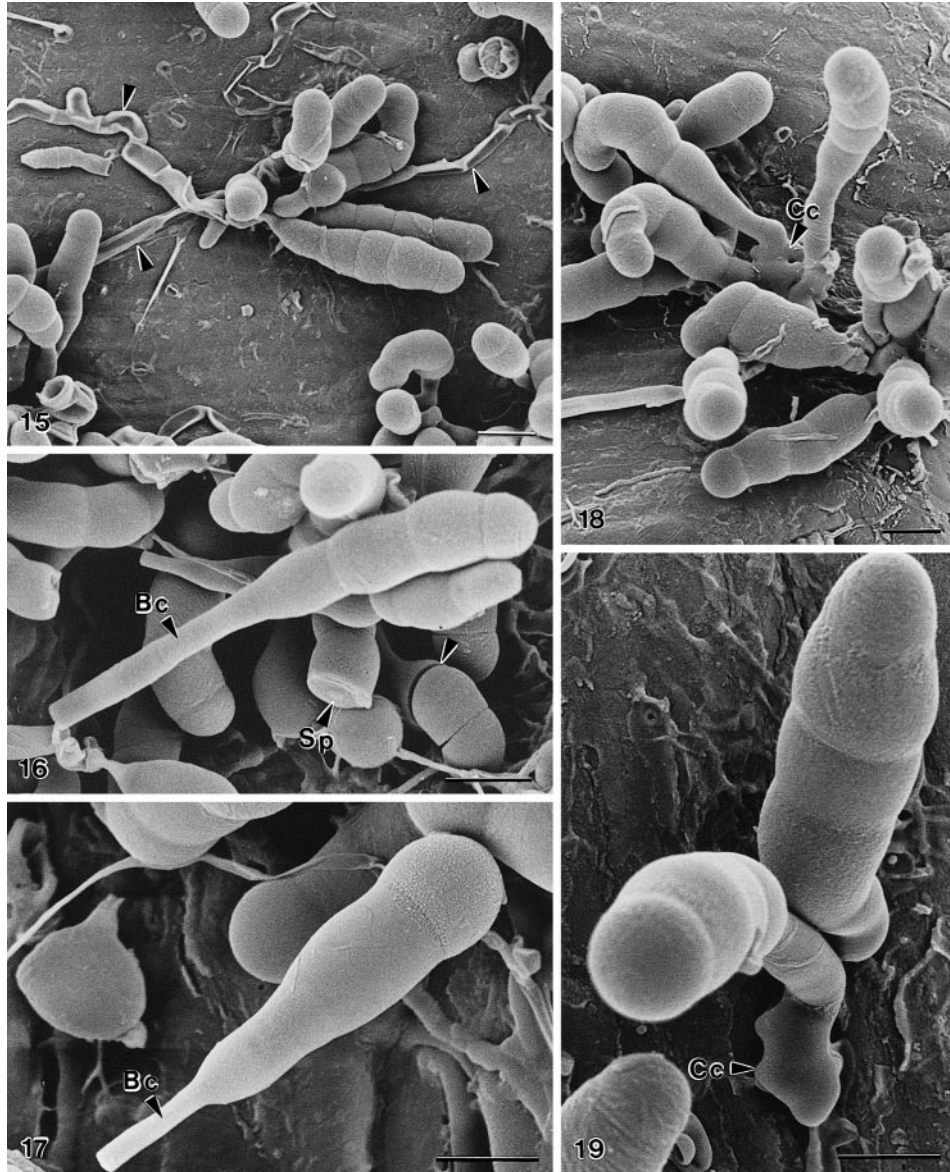
Decaying fronds of *Phoenix hanceana* were collected in Hong Kong. Fungi growing on the substrata were examined with light and cryoscanning electron microscopy as described in Ho et al (1998). All measurements provided in

this paper were made from material mounted in water. Material has been deposited in the herbarium of The Centre for Research in Fungal Diversity, University of Hong Kong (HKU[M]).

TAXONOMY

Endosporoideus W.H. Ho, Yanna, K.D. Hyde et Goh, gen. nov.

Coloniae in substrato naturali effusae, dematiaceae. *Mycelium* partim in substrata immersum vel partim superficiale, ex hyphis ramosis, septatis, pallide brunneis, laevibus, tenuitunicatae, flexuosae compositum. *Stromata*, *setae* et *hyphopodia* absentia. *Conidiophora* micronemata, mononemata. *Cellulae conidiogenae* terminales vel intercalares, polyblasticae, persistentes, dematiaceae, laevibus. *Conidia* holoblastica, solitaria, multi-euseptata, clavata vel cylindrica-clavata, flexuosa, dematiaceae; *cellula basilare* angustum, productum; *tunica* et *septa* hyalina, tenuitunicata cum immaturitas, crassitunicatae cum maturesca, endosporium



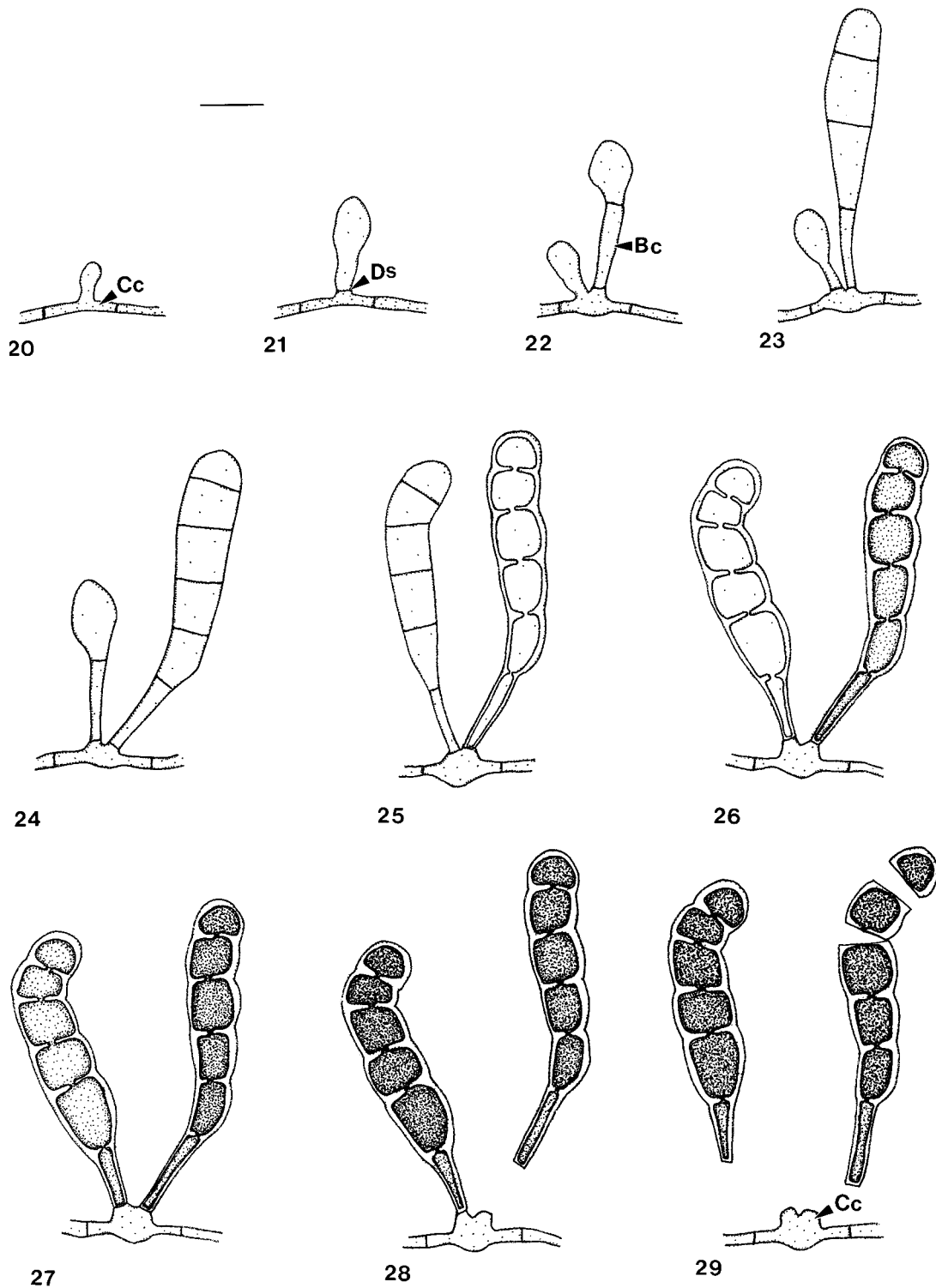
FIGS. 15–19. Scanning electron micrographs of *E. pedicellata* (from holotype). Bc = basal cells, Cc = conidiogenous cells, Sp = conidial septal pore. 15. Colony on natural substratum illustrating fascicles of conidia and superficial hyphae (arrowheads). 16, 17. Phragmoid conidia. Note the elongated basal cells, disarticulation of conidial cells (arrowhead) and the exposed conidial septal pore. 18. Fascicles of conidia produced from lobed conidiogenous cells. 19. Higher magnification of the lobed conidiogenous cell at the base of the conidia. Scale bars: 15, 16 = 10 μm ; 17, 18 = 5 μm ; 19 = 15 μm .

brunneis cum maturitas, similis sum dematiaceae isthmospora. *Conidiorum secessio* schizolytica.

Species typica *Endosporoideus pedicellata* W.H. Ho, Yanna, K.D. Hyde & Goh.

Colonies on natural substratum effuse, dematiaceous. *Mycelium* partly immersed and partly superficial, comprising branched, septate, pale brown, smooth, thin-walled, flexuous or interwoven hyphae. *Stromata*, *setae* and *hyphopodia* absent. *Conidiophores* micronematous, mononematous. *Conidiogenous cells* terminal or intercalary, polyblastic, persistent, dematiaceous, smooth-walled, proliferating; *conidiogenous*

loci appearing successively at the same level without elongation of the conidiogenous cells, giving rise to a cluster of conidia. *Conidia* holoblastic, solitary, transversely multi-euseptate, with prominent central septal pores, clavate or cylindrical-clavate, flexuous, dematiaceous, broadly rounded at the apex, truncate at the base, frequently with a narrow, elongated basal cell; *conidial walls* and *septa* hyaline, thin when immature, thickening during maturation, the inner wall becoming brown at maturity, resembling dematiaceous endogenous isthmospores surrounded with thick hyaline membranes; *conidial cells* occasionally



FIGS. 20–29. Diagrammatic representation of conidial development and dehiscence in *E. pedicellata*. Bc = basal cell, Cc = conidiogenous cell, Ds = delimiting septum. 20. Holoblastic ontogeny of the first conidium. 21. Conidial delimitation. 22. Formation of the first transverse conidial septum in the first conidium, and ontogeny of the second conidium. 23, 24. Conidial maturation with apical wall-building, to form several transverse septa as the conidia elongate. 25. Conidial maturation with diffuse wall-building in the first conidium, to form thick conidial wall and septa. 26, 27. Deposition of an inner brown to dark brown wall layer. 28. Schizolytic conidial secession of the first conidium. 29. Schizolytic conidial secession of the second conidium and cells disarticulate at the septa in the first conidium. Note the lobed appearance of the conidiogenous cells after conidial secessions. Scale bar = 10 μm .

TABLE I. Diagnostic characteristics of *Endosporoideus* and similar genera

	<i>Endosporoideus</i> W.H. Ho, Yanna, K.D. Hyde & Goh	<i>Antennathula</i> Fr. ex. F. Strauss (Ellis 1971)	<i>Henicospora</i> P.M. Kirk & B. Sutton (1980)	<i>Janetia</i> M.B. Ellis (Goh and Hyde 1996)
Conidiophores	Micronematous	Micronematous	Micro- or semi- macronematous	Micronematous
Conidiogenous cells	Mononematous	Mononematous	Mononematous	Mononematous
	Integrated	Integrated	Integrated	Integrated
	Holoblastic	Holoblastic	Holoblastic	Holoblastic
	Polyblastic	Monoblastic	Mono- or polyblastic	Polyblastic
Conidia	Cylindrical when unilocal, lobed when multilocal	Cylindrical or doliform	Cylindrical	Denticulate
	Solitary, mostly in fascicles	Solitary	Solitary	Solitary
	Phragmosporous	Phragmosporous	Phragmosporous	Phragmosporous
	Euseptate	Euseptate	Euseptate	Euseptate, with or without distoseptata
Chlamydo­spores	Schizolytic	Schizolytic	Rhexolytic	Schizolytic
	Absent	Absent	Absent	Absent
Teleomorph	Unknown	<i>Euantennaria</i> Speg.	Unknown	Unknown

disarticulate at the septa. *Conidia* formed holoblastically by apical wall building, followed by delimitation by a transverse septum, and schizolytic secession; conidiogenous cells proliferate without elongation, forming conidia successively from other conidiogenous loci; conidia mature by (i) apical wall-building, to form several transverse septa as the conidia elongate, (ii) diffuse wall-building, to form thick conidial wall and septa, and subsequently (iii) deposition of an inner brown to dark brown wall layer.

Etymology. *Endosporoideus* refers to the endospore-like conidia that comprises a hyaline outer wall layer and brown inner wall layer.

Endosporoideus pedicellata W.H. Ho, Yanna, K.D. Hyde et Goh, sp. nov. FIGS. 1–19

Coloniae in substrato naturali effusae, fuscus. *Mycelium* partim in substrato immersum vel partim superficiale, ex hyphis ca. 12 µm lata, ramosis, septatis, pallide brunneis, laevibus, tenuitunicatae, flexuosae compositum. *Cellulae conidiogena*e pallide brunneis vel brunneis, initio cylindrica, tum lobatus, laevibus. *Conidia* (16–)24–74 × 10–12 µm, phragmospora, solitaria, 1–7-euseptata, clavata vel cylindrica-clavata, recta vel flexuosa, brunneis vel fuscus; *pori septum* centrale, conspicua, 2–3 µm lata, 1.5–2 µm longa; *cellula basilare* angustum, productum; *tunica* et *septa* hyalina, cum

immaturitas tenuitunicata, cum maturesca crassitunicatae, usque ca. 1 µm crassa, cum maturitas endosporium brunneis vel fuscus, similis sum dematiaceae isthmospora. *Conidiorum secessio* schizolytica.

Colonies on natural substratum effuse, dark brown (FIG. 1). *Mycelium* partly immersed and partly superficial, comprising branched, septate, pale brown, smooth, thin-walled, flexuous or interwoven hyphae, ca. 12 µm wide (FIG. 2). *Conidiogenous cells* pale brown to brown, cylindrical initially, becoming lobed after proliferation, smooth-walled (FIGS. 3–6); *conidiogenous loci* appearing successively at the same level without elongation of the conidiogenous cells, giving rise to a cluster of conidia (FIGS. 3, 5, 6). *Conidia* (16–)24–74 × 10–12 µm (\bar{x} = 58.3 × 11.5 µm, n = 25), phragmosporous, solitary, 1–7-euseptate, with prominent central septal pores, viewed as channels in side view, 2–3 µm wide, 1.5–2 µm long, clavate or cylindrical-clavate, straight or flexuous, brown to dark brown, broadly rounded at the apex, truncate at the base, occasionally with a narrow, elongate basal cell (FIGS. 3–6, 10–12, 14); *distal conidial cells* 6–8 × 5–9 µm (\bar{x} = 7.5 × 7.1 µm, n = 25); *central conidial cells* 8–12 × 7–10 µm (\bar{x} = 10 × 9 µm, n = 25); *proximal conidial cells* elongate, cylindrical, 7–34 × 2–6 µm (\bar{x} = 17.3 × 3.5 µm, n = 25); *conidial walls*

TABLE I. Extended

<i>Pithomyces</i> Berk. & Broome (Ellis 1971)	<i>Polyschema</i> H.P. Upadhyay (Ellis 1976)	<i>Sanjuanomyces</i> R.F. Castañeda & W.B. Kendr (1991)	<i>Trichocladium</i> Harz (Goh and Hyde 1999)
Micro- or semi- macronematous	Micro- or semi- macronematous	Micronematous	Micro- or semi- macronematous
Mononematous	Mononematous	Mononematous	Mononematous
Integrated	Integrated or discrete	Integrated	Integrated
Holoblastic	Enteroblastic tretic	Enteroblastic tretic	Holoblastic
Mono- or polyblastic	Mono- or polytretic	Monotretic	Mono- or polyblastic
Cylindrical, denticulate	Spherical or subspherical	Cylindrical	Pyriform to clavate
Solitary	Solitary	Solitary	Solitary
Amero-, didymo-, phragmo- or dictyosporous	Didymo- or phragmosporous	Phragmosporous	Didymo- or phragmosporous
Euseptate	Euseptate	Euseptate	Euseptate
Rhexolytic	Schizolytic	Schizolytic	Schizolytic
Absent	Sometimes present	Absent	Absent
<i>Leptosphaerulina</i> McAlpine	Unknown	Unknown	Unknown

and *septa* hyaline, thin when immature, thickening during maturation, up to ca. 1 μ m thick, and the inner wall becoming brown to dark brown at maturity, resembling dematiaceous endogenous isthmospores surrounded by thick hyaline membranes (FIGS. 7–11, 14). The conidia secede by schizolysis, and occasionally the individual conidial cells may disarticulate at the septa (FIG. 13).

HOLOTYPE. HONG KONG. New Territories, Tai Mo Shan, Twisk, on dead petiole of *Phoenix hanceana* Naud. (Arecaceae), 21 Feb. 1998, Yanna YAN237 (HKU[M] 10066).

Teleomorph. unknown.

Known distribution. Hong Kong.

Etymology. *pedicellata* refers to the elongate base of the conidia.

Cryo-scanning electron microscopy.—*Superficial* hyphae were smooth-walled, flexuous or interwoven (FIG. 15). Conidia were produced in loose fascicles from lobed conidiogenous cells (FIGS. 18, 19), and the conidia seceded schizolytically, with a flat base (FIGS. 16, 17). Conidia were constricted at the septa and sometimes disarticulated at the septa. The central septal pores were visible when the conidial fragments were viewed at an angle (FIG. 16). A mucilaginous sheath

or membrane surrounding conidia was not observed (FIGS. 16–19).

DISCUSSION

Conidiogenesis of Endosporoideus.—This sequence of conidium development in *E. pedicellata* was observed: (i) holoblastic conidial ontogeny (FIG. 20); (ii) delimitation of new conidium by a transverse septum (FIG. 21); (iii) proliferation of conidiogenous cell and a new conidium forms from a second conidiogenous locus at the same level (FIG. 22); (iv) maturation by (a) apical wall-building with several transverse septa forming as the conidium elongates (FIGS. 23, 24), (b) diffuse wall-building forming thick conidial wall and septa (FIG. 25), and subsequently by (c) deposition of an inner brown to dark brown wall layer (FIGS. 26, 27); (v) schizolytic conidium secession (FIG. 28); (vi) frequent disarticulation of cells of conidia at the septa (FIG. 29). The conidiogenous events of *Endosporoideus* are similar to those of *Janetia* in the mode of proliferation. Both genera form conidiogenous loci successively at the same level without elongation of the conidiogenous cells (Goh and Hyde 1996).

Another collection.—Our collection is similar to Matsushima's MFC3377 as described in Matsushima (1975, p 167, entry 575, plate 365, figs. 1, 2) on dead stems of *Miscanthus sinensis* Anders. in Japan. In MFC3377, conidia (or "fertile hyphae" in Matsushima 1975) are in loose fascicles, cylindrical, straight to curved, 40–100 × 8–11 μm; the conidial cells (or "conidia" in Matsushima 1975) are rectangular, 7–13 × 6–8 μm, thick-walled, black (Matsushima 1975). The morphological characters of both collections are similar. We unsuccessfully requested loan of MFC3377.

Comparison of Endosporoideus with similar genera.—*Endosporoideus* is morphologically most similar to *Trichocladium*, whose species produce solitary, oval to clavate, euseptate, pigmented phragmosporous conidia from micronematous, mononematous conidiophores in effuse colonies. *Trichocladium* recently was reviewed by Goh and Hyde (1999) who accepted 18 species. *Trichocladium melhae* since has been described from marine habitat (Jones et al 2001). *Trichocladium* differs from *Endosporoideus* mainly in producing mid- to dark brown conidia that lack a thick, hyaline outer-wall layer and never assemble in fascicles. The thick hyaline outer wall layer in the conidia of *Endosporoideus* is unique among mitosporic fungi, to our knowledge.

Endosporoideus also resembles *Janetia* in producing solitary, clavate to cylindrical-clavate phragmosporous conidia from micronematous, mononematous conidiophores in effuse colonies. However, *Janetia* differs from *Endosporoideus* mainly in producing brown euseptate conidia, with or without distosepta, on denticulate, densely pigmented conidiogenous cells.

Other genera that are characterized by solitary phragmosporous conidia produced from micronematous conidiophores in effuse colonies include *Antennatula*, *Henicospora*, *Janetia*, *Pithomyces*, *Polyschema* and *Sanjuanomyces* (Ellis 1971, 1976; Sutton 1980; Rao and de Hoog 1986; Castañeda Ruiz and Kendrick 1990a, b, 1991; Goh and Hyde 1996). *Endosporoideus* is unique because the pigmented conidia have a thick, hyaline outer-wall layer. The important morphological characters of *Endosporoideus* and the above seven genera, including *Trichocladium*, are compared (TABLE I).

Endosporoideus is comparable to genera such as *Anellodentimyces* Matsush. and *Arthrocladium* Papendorf, which are characterized by the production of clusters of dematiaceous, phragmosporous conidia on micronematous conidiophores (Carmichael et al 1980, Matsushima 1985). These genera differ from *Endosporoideus* in producing clusters of conidia by sympodial or percurrent proliferation, which results

in elongation of the conidiogenous cells. *Endosporoideus* also is comparable to genera such as *Cirrenalia* Meyers & R.T. Moore and *Curculiospora* Arnaud (Carmichael et al 1980, Goos et al 1985). *Cirrenalia* and *Curculiospora*, however, differ from *Endosporoideus* because the conidiogenous cells are unilocal and the conidia are helical.

Conidial wall and septa in Endosporoideus.—Matsushima (1975) described the ontogeny of the conidia of MFC3377 as endogenous. In *E. pedicellata*, the conidia are evidently hologenous. We have illustrated that the conidia are initially hyaline and thin-walled, later becoming thick-walled. At maturity a brown layer is formed beneath the thick, hyaline wall layer. It is thus evident that the conidia are phragmosporous, with a thick hyaline outer-wall layer. The brown conidial cells with a thick, hyaline outer-wall layer in *Endosporoideus* are unique among mitosporic fungi.

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