
Spirodecospora gen. nov. (Xylariaceae, Ascomycotina), from Bamboo in Hong Kong

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Monographic studies on *Anthostomella* and related genera have yielded an interesting taxon having immersed, obpyriform ascomata with a central ostiole. The ascospores are relatively large, olivaceous, with conspicuous spiral wall ornamentations, and are surrounded by a hyaline mucilaginous sheath. At the transmission electron microscopic level, the ascospore wall comprises a thin, electron-dense episporium and a thick, electron-transparent mesosporium, with verrucose ornamentations arising from the episporium. The sheath surrounding the ascospores comprises fibrillar material. The subapical ring observed in the ascus is typically xylariaceous. *Spirodecospora* gen. nov., with one species, *S. bambusicola*, is described, and compared with other genera in the Xylariaceae.

Introduction

Monographic studies of species of *Anthostomella* Sacc. and related genera have revealed an interesting taxon from bamboo in Hong Kong. Ascospores were relatively large and olivaceous, surrounded by conspicuous spiral wall ornamentations, and a hyaline mucilaginous sheath. The taxon could not be suitably placed in *Anthostomella* or related genera. *Spirodecospora* B.S. Lu, K.D. Hyde and W.H. Ho gen. nov. is therefore introduced to accommodate this taxon with *S. bambusicola* B.S. Lu, K.D. Hyde and W.H. Ho as the type and only known species.

Materials and methods

Bamboo samples were collected from Victoria Peak in Hong Kong and returned to the laboratory, where they were incubated in plastic boxes, lined with moistened tissue, and periodically examined for fungi.

Scanning electron microscopy (SEM) preparation

An ascospore suspension was prepared and pipetted onto a polycarbonate membrane and the ascospores were allowed to settle for 10 min. The membrane

was fixed in 2 % (w/v) OsO₄ at 4 C overnight and washed twice in distilled water. Fixed material was dehydrated through a graded ethanol series from 10 % to 90 % (in 10 % steps), to 95 %, and finally 100 % (three times). Ethanol was replaced by acetone in 2:1, 1:1 and 1:2 (ethanol: acetone) steps, and finally absolute acetone (three times). The dehydrated material in acetone was dried in a Polaron E3000 critical point drying apparatus and mounted on aluminum stubs. Mounted specimens were coated with gold and examined in a JEOL T20 at 20 kV.

Transmission electron microscopy (TEM) preparation

An ascospore suspension was mixed with an equal volume of 2 % (w/v) molten, cooled Ion Agar. Solidified and cut specimens were fixed in 2 % (w/v) KMnO₄ for 10 min and dehydrated following the same procedure as SEM. The material was embedded in Möllenhauer's resin (Möllenhauer, 1964). The pre-trimmed material was sectioned on a LKB ultramicrotome and followed by staining with lead citrate for 10 min and saturated uranyl acetate solution for 30 min. Specimens were examined in a JEOL 100SX operated at 80 kV.

Results

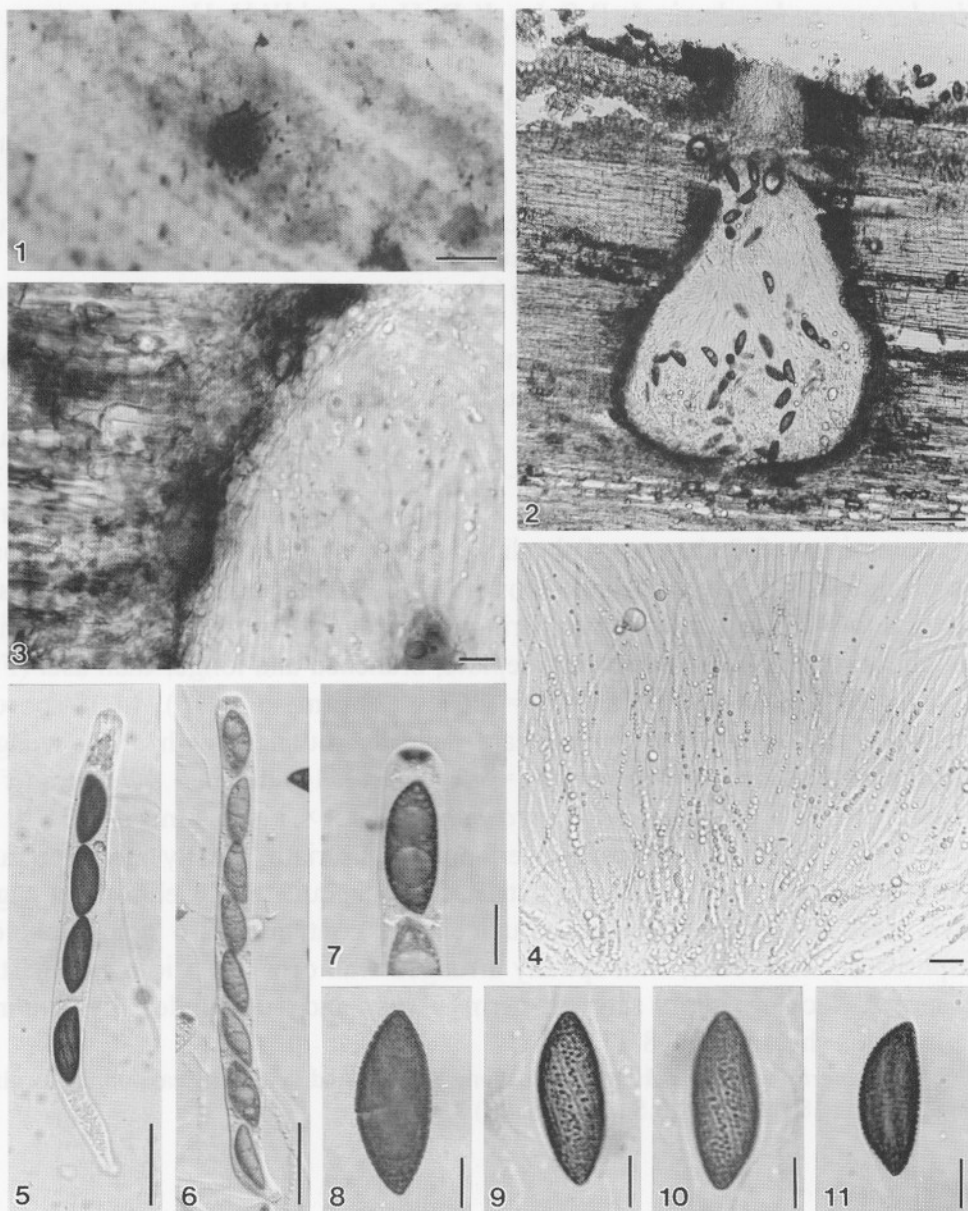
Spirodecospora B.S. Lu, K.D. Hyde and W.H. Ho, gen. nov.

Etymology: from the Latin *Spiro* meaning "spiral" and *deco* meaning "decoration" in reference to the spiral ornamentations on the ascospores.

Ascomata immersa, obpyriformia, ostiolata, paraphysata. *Asci* (4-)8-spori, cylindrici, unitunicati, ad apicem rotundati, jodi ope azurescens praediti. *Ascosporae* inequilateraliter late ellipsoideae, viridifuscae vel brunneae, cum tuberculis spiraliter ornamentatae, fissuris et pons germinum non praeditae.

Typus generis: *Spirodecospora bambusicola* B.S. Lu, K.D. Hyde and W.H. Ho.

Ascomata deeply immersed in host, obpyriform, ostiolate, solitary. *Peridium* comprising two layers, an inner layer of hyaline, flattened cells, and an outer layer of dark brown, thick-walled, angular or compressed cells, fusing with host cells at the periphery. *Paraphyses* filamentous, numerous, septate, branched and longer than asci. *Asci* (4-)8-spored, cylindrical, pedicellate, unitunicate, apically rounded, with a J⁺, wedge-shaped, subapical ring. *Ascospores* uniseriate or partly overlapping uniseriate, variable in shape, mostly inequilaterally broadly ellipsoidal, with one side flattened, or inequilaterally ellipsoidal, brown to olivaceous, unicellular, lacking germ slits or germ pores, with conspicuous warts which are arranged spirally around the ascospores and surrounded by mucilaginous sheath.



Figs. 1-11. *Spirodecospora bambusicola* (from holotype). **1.** Appearance of ascoma ostiole on host surface. **2.** Section of ascoma. **3.** Peridium. Note the inner part with hyaline, flattened cells and the outer part with dark brown, thick-walled, angular or compressed cells. **4.** Paraphyses. **5-6.** Asci with 4 or 8 ascospores. Note the J+, subapical ring. **7.** Apex of ascus with J+, wedge-shaped subapical ring. **8-11.** Ascospores with conspicuous, spirally arranged wall ornamentations. Bars: 1 = 1 mm, 2 = 100 μ m, 5-6 = 50 μ m, 7 = 20 μ m, 3, 4, 8-11 = 10 μ m.

Spirodecospora bambusicola B.S. Lu, K.D. Hyde and W.H. Ho, sp. nov.

(Figs. 1-18)

Ascomata 300-500 μm in diametro, 500-600 μm alta, obpyriformia, immersa, solitaria. *Asci* 200-330 \times 18-25 μm , (4-)8-sporei, cylindrici, subapicale rotundati, cum annulis subapicalis, J+, cuneatis praediti, 8.8-10 μm in diametro, 3.8-7.5 μm alti. *Ascospores* 28-45 \times 11-15 μm , inequilateraliter late ellipsoideae, viridifuscae vel brunneae, cum tuberculis spiraliter ornamentatae, fissuris et poris germinum non praeditae.

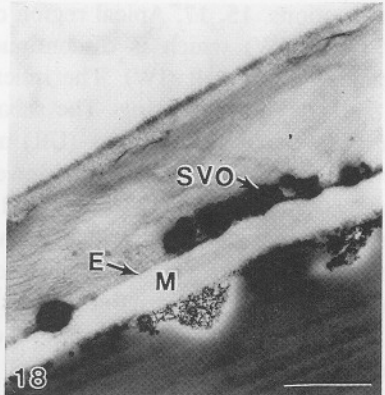
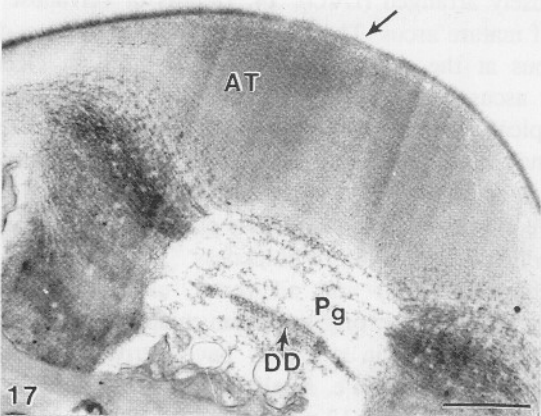
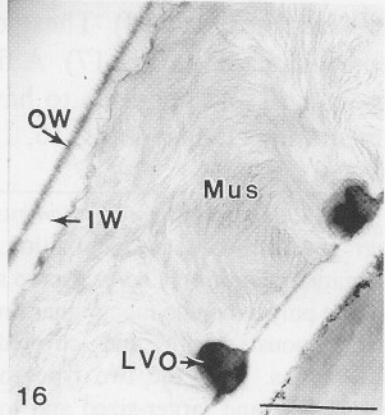
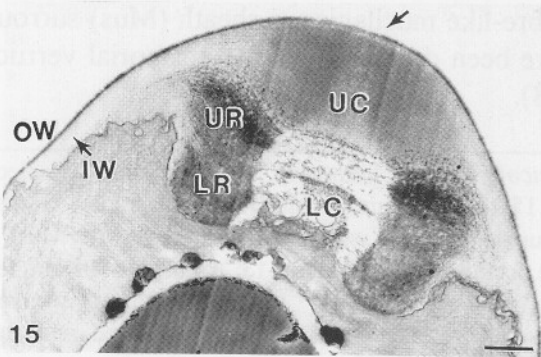
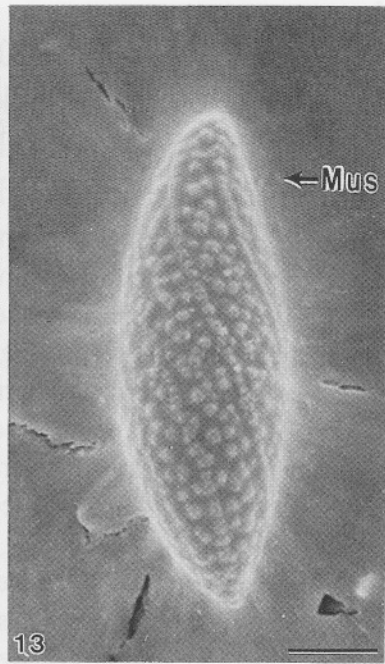
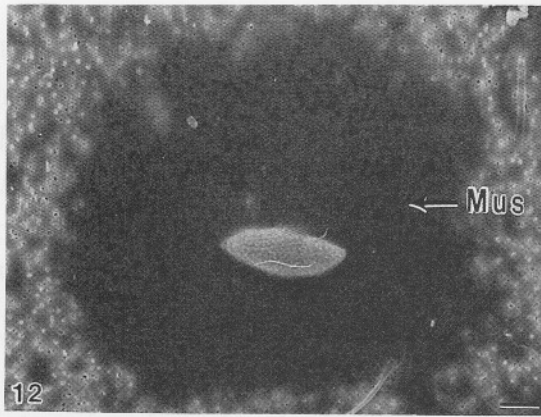
Holotypus: HONG KONG, Hong Kong Island, Victoria Peak, on dead culms of *Bambusa* sp., 18 July 1997, B.S. Lu (HKU(M) 7303).

Ascomata deeply immersed in host, visible as blackened, raised ostiolar dots, many surrounded by black clustered ascospores, solitary (Fig. 1); in vertical section 300-500 μm diam., 500-600 μm high, obpyriform, with a central, periphysate ostiolar canal, 70-90 μm diam., 180-280 μm high (Fig. 2). *Peridium* 15-25 μm (\bar{x} = 20 μm , n = 10) wide, comprising two layers, an inner part comprising 2-3 layers of hyaline, flattened cells, and an outer part comprising several layers of dark brown, thick-walled, angular or compressed cells, fusing with host cells at the periphery (Fig. 3). *Paraphyses* 2-4 μm wide throughout, filamentous, numerous, septate, branched and longer than asci (Fig. 4). *Asci* 200-330 \times 18-25 μm (\bar{x} = 267 \times 21 μm , n = 15), (4-)8-spored, cylindrical, short-pedicellate, unitunicate, apically rounded, with a J+, wedge-shaped, subapical ring, 8.8-10 μm (\bar{x} = 9.4 μm , n = 10) diam., 3.8-7.5 μm (\bar{x} = 5.6 μm , n = 10) high (Figs. 5-7). *Ascospores* 28-45 \times 11-15 μm (\bar{x} = 36 \times 14 μm , n = 20), uniseriate or partly overlapping uniseriate, mostly inequilaterally broadly ellipsoidal, with one side flattened, or inequilaterally ellipsoidal, light brown, dark brown, or olivaceous, unicellular, containing 2-6 lipid globules, conspicuously verrucose, with spirally arranged wall ornamentations around the ascospores, lacking germ slits or germ pores, and surrounded by hyaline, mucilaginous sheath (Figs. 8-11).

Other material examined: Hong Kong, Hong Kong Island, Victoria Peak, on dead culms of *Bambusa* sp., 18 July 1997, B.S. Lu (HKU(M) 7314).

SEM

The wall of the ascospores is covered with numerous, spirally arranged, semi-spherical ornamentations. These ornamentations comprise 3-4 rows of tightly packed, spirally arranged, small verrucose ornamentations (SVO) (ca 270 nm diam. at the base), 4 μm apart. Relatively large ornamentations (LVO) (ca 500 nm diam. at the base) are irregularly interdisposed between the lines of smaller ornamentations. Ascospores are surrounded by thick mucilaginous sheath (Mus) (ca 400 μm thick) (Figs. 12-14).



TEM

The mature ascus wall is 300 nm thick, comprising a thin, electron-dense, outer wall (OW; *ca* 40 nm thick) which is discontinuous at the ascus apex (arrowed in Figs. 15, 17) and thicker, electron-transparent, inner wall (IW) which is irregular in thickness (100-250 nm) (Figs. 15, 16). The inner wall differentiate into the apical thickening (*ca* 2 μ m thick) and a subapical ring (*ca* 6 μ m diam., 3 μ m high, rim 1.6 μ m diam.) at the ascus apex. The subapical ring comprise an upper, electron-dense part (UR, *ca* 2 μ m high) and a lower, less electron-dense part (LR, 1 μ m high). The upper part of the channel (UC) of the apical thickening is rough and narrow, while the lower part (LC) is smooth and wider (Fig. 15). A plug (Pg) is located mid-way in the channel and contains electron-dense, transverse, fibrillar deposits (DD) (Fig. 17).

The mature ascospore wall comprises an outer, electron-dense episporium (30 nm thick) (E) and an inner, electron-transparent mesosporium (320 nm thick) (M) and is covered with electron-dense verrucose ornamentations, which comprise two types, i. e. smaller-sized, tightly packed (SVO) (*ca* 270 nm diam. at the base, Fig. 18), and larger-sized, loosely arranged (LVO) (*ca* 500 nm diam. at the base, Figs. 16, 18). These ornamentations have probably originated from the episporium (Figs. 16, 17). A fibre-like mucilaginous sheath (Mus) surrounds the ascospore and appears to have been derived from the episporial verrucose ornamentations (Mus) (Figs. 16, 18).

Figs. 12-18. *Spirodecospora bambusicola* (from holotype; 12-14. Scanning electron micrographs, osmium tetroxide fixation; 15-18. Transmission electron micrographs, potassium permanganate fixation). **12.** Ascospore surrounded by a wide mucilaginous sheath (Mus). The pore of the polycarbonate membranes are occluded by the sheath. **13.** Ascospore surrounded by a mucilaginous sheath and covered with conspicuous, spirally arranged, verrucose ornamentations. Note the two types of the ornamentations, i.e. smaller-sized and tightly packed (SVO), and larger-sized and loosely arranged (LVO). **14.** Details of verrucose wall ornamentations. **15, 17.** Apical region of mature ascus. The ascus wall has an electron-dense outer wall (OW) which is discontinuous at the ascus apex (arrowed), and an electron-transparent inner wall (IW). The inner ascus wall differentiates into the apical thickening (AT) and the subapical ring. The subapical ring below the apical thickening comprises an upper, less electron-dense part (UR) and a lower, electron-dense part (LR). A channel is present in the upper part (UC) and lower part of the ring structure. A plug (Pg) occurs in the channel of ring. **16, 18.** The ascus wall comprises an electron-dense outer wall (OW) and an electron-transparent inner wall (IW) which is irregular in thickness. The ascospore wall comprises an outer, electron-dense episporium (E) and an inner, electron-transparent mesosporium (M). Wall ornamentations which probably originate from episporium and fibrous mucilaginous sheath (Mus) occur in close association and may have derived from these ornamentations. Note the two types of ornamentations, i.e. smaller-sized and tightly packed (SVO), and larger-sized and loosely arranged (LVO). Bars: 12 = 10 μ m, 13 = 5 μ m, 14-18 = 1 μ m.

Table 1. Comparison of *Spirodecospora* with *Anthostomella* and some related genera in Xylariaceae.

	<i>Spirodecospora</i>	<i>Anthostomella</i> (Francis, 1975)	<i>Amphisphaerella</i> (Kirschstein, 1934)	<i>Lopadostoma</i> (Rappaz, 1995)	<i>Pandanicola</i> (Hyde, 1994)
Ascomata	Deeply immersed in host	Immersed often under a clypeus	Immersed becoming superficial with only the base immersed	Few ascomata grouped in valsoid configuration in a small stroma that may be delimited by black margins	Immersed under a clypeus
	Obpyriform with an oblique or central ostiole	Globose, subglobose, or conical, with a central ostiole	Globose with a central ostiole	Globose with a central ostiole	Subglobose with a flattened base and a central ostiole
Asci	Cylindrical, with a J+, wedge-shaped, subapical ring	Broad cylindrical, with a J+/J- discoid, or wedge-shaped, subapical ring	Cylindrical, with a J+/J-, discoid or wedge-shaped subapical ring	Cylindrical, with a J+, discoid or wedge-shaped, subapical ring	Broadly cylindrical, lacking an apical ring
Ascospores	One-celled, olivaceous, brown	Mostly one-celled, some with one or two hyaline dwarf cell(s), light brown, brown, dark brown, olivaceous, or black	One-celled, brown	Young spores one-celled, with a thin epispore, olivaceous to dark brown	One-celled, reddish brown
	Conspicuous spirally arranged verrucose wall ornamentations, surrounded by hyaline, fibrous mucilaginous sheath	Mostly smooth-walled, surrounded by a sheath, or with mucilaginous appendages	Smooth-walled, often surrounded by a sheath	Smooth-walled, lacking a sheath	Very thick and smooth-walled, no sheath
	Lacking a germ slit or germ pore	Mostly with a germ slit	Equatorial germ pores	Germ slit circular, straight, or absent	Polar germ pores

Discussion

Anthostomella is a species-rich genus with more than 250 names (Francis, 1975). There is considerable variability in ascus and ascospore shape and in the morphology of the apical ring (Hyde, 1996). Asci may contain 2, 4 or 8 ascospores and ascospores may vary from light brown, to olivaceous, or even black, and in having dwarf cell(s), appendages or mucilaginous sheaths, and/or germ slits or germ pores. The genus was partially monographed by Francis (1975) who examined the species on stems and leaves of herbaceous plants and gymnosperms in Europe. Rappaz (1995) studied the material collected on hard wood in Europe and North America, while Hyde (1996) accepted 42 species (of which ten were new) occurring on palms and *Pandanus* in tropical areas. However, there is no major revision of *Anthostomella*, the genus is poorly understood and it is certainly heterogeneous.

Spirodecospora bambusicola is distinct from *Anthostomella* as ascospores are covered in very conspicuous, spirally arranged, wall ornamentations and surrounded by a fibrous mucilaginous sheath (Francis, 1975). Ascospores are also relatively larger than those found in most *Anthostomella* species, and lack germ slits. *S. bambusicola* also differs from *Anthostomella* species as the ascomata are relatively large.

Spirodecospora bambusicola also differs from species of *Amphisphaerella* (Sacc.) Kirschst. as the ascospores lack germ pores (Hyde, 1995). It is distinctive from the type of *Pandanicola*, *P. calocarpa* (Syd. and P. Syd.) K.D. Hyde as the ascospores lack polar germ pores and have a J+ subapical ring in the ascus (Hyde, 1994). The genus is also distinguished from *Lopadostoma* Rappaz in having single ascoma, and in lacking both a stroma and a germ slit (Rappaz, 1995).

The ascus ring in *Spirodecospora* is similar to xylariaceous taxa at the TEM level. Griffiths (1973) and Greenhalgh and Evans (1967) have illustrated the ascus apex of *Hypoxylon multiforme* (Fr.) Fr. and *H. fragiforme* (Pers. ex Fr.) Kickx., respectively, at the TEM level. Beckett, Heath and Mclaughlin, (1974) have also illustrated the apical ring in *Xylaria longipes* Nitschke at TEM level. The mature asci in these species have a thin, electron-dense outer wall which is absent at the ascus apex (channel of ring), and an inner thicker wall which is irregular in thickness. The inner wall differentiates at the apex into the apical thickening and subapical ring. The subapical ring comprises an upper, less electron-dense, and a lower, electron-dense part, although the length of the two parts may vary (Greenhalgh and Evans, 1967; Griffiths, 1973; Beckett *et al.*, 1974). Ascus morphology is a very important character at the family level

(Greenhalgh and Evans, 1967), and therefore, *Spirodecospora*, which has a similar ascus ultrastructure, is included in the Xylariaceae.

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