Mycoscience 41: 533–537, 2000

A new species of *Embellisia* from soil with high levels of heavy metals

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Accepted for publication 28 August 2000

Embellisia thlaspis **is described as a new species from the roots of the crucifer *Thlaspi caerulescens*. The host plant was growing in soil in an area of disused zinc and lead mines where high levels of heavy metals have been recorded. Examining this novel fungus using SEM, the detailed structure of the conidiogenous loci confirms earlier observations of this genus.**

Key Words——*Embellisia*; endophytes; heavy metals; SEM; *Thlaspi.*

As part of a project to determine the role of fungi in plants that can tolerate high levels of heavy metals, the fungal components of the leaves, roots, rhizoplane and rhizosphere of *Thlaspi caerulescens* J. S. & C. Presl. were isolated and sent to CABI Bioscience for identification. The plants were collected from Charterhouse Nature Reserve, N. Somerset, UK in an area of disused zinc and lead mines. This reserve has a distinctive flora and fauna and is a UK Site of Special Scientific Interest (SSSI). The plants were gathered from a 1 m^2 plot overlying a smelter tip. Twenty one different fungi were found (Coles et al., 1999) amongst which was one that was provisionally identified as *Embellisia* sp. cf. *indefessa* E. G. Simmons. Species of the genus *Embellisia* are readily recognized by the predominantly transversely septate conidia with distinctly thickened and pigmented septa. This fungus was only isolated from the roots of one plant and is likely to be an endophyte although its actual role was not proven. The isolate was sent to Professor E. G. Simmons, who determined it to be a new species.

While the better known species of *Embellisia* are plant pathogens, such as *E. allii* (Campan.) E. G. Simmons and *E. hyacinthi* de Hoog & P. J. Mull. bis (Simmons, 1983, 1990; David, 1991), others are known to occur in soil and in relatively extreme environments. An example of the latter is *E. phragmospora* (Emden) E. G. Simmons, isolated from the soil of the Zuider Zee polders (van Emden, 1970) and *E. tellustris* E. G. Simmons, originally described from the grasslands of Wyoming but also known from soil of a salt marsh in Kuwait and from Antarctica (Simmons, 1983). Added to this is the hyphomycete currently known as *Dendryphiella salina* (G. K. Sutherl.) G. J. F. Pugh & Nicot which actually belongs in *Embellisia* and has been isolated from sea water (Kohlmeyer and Kohlmeyer, 1979; David and Onofri, in prep.).

Taxonomy

Embellisia thlaspis E. G. Simmons & J. C. David, sp. nov. Figs. 1, 2

Ex cultura in PCA descripta. Conidiophora aeria simplicia vel 1-10-ramosa. Conidia catenata, in quoque catena usque ad 20; conidia prima longe angusta, usque ad 34–45 × 5–7 μm, transverse 4-8-septata; conidia secundaria plerumque subcylindrica vel longianguste ovoidea, usque ad 21–31 × 5.5–8.0 μm, transverse 4-5 (-7)-septata, rarissime 1 longisepto praedita, modice brunnea, levia, septis fuscatis.

Habitatio typi: in radicibus *Thlaspis caerulescentis* J. S. & C. Presl., Charterhouse Nature Reserve, Mendip Hills, Somerset, UK. Typus: partes ex EGS 45-069 (ex IMI 374287, K. Coles, xii.1996 lectus) desiccatae et in IMI (IMI 380483–holotypus) et in BPI isotypus conservatae.

Embellisia thlaspis grows moderately well on Potato Carrot Agar (PCA), ca. 1.5 cm radially in 1 wk, in comparison with *E. indefessa* E. G. Simmons (Simmons, 1983), ca. 2.7 cm, and with *E. dennisii* (M. B. Ellis) E. G. Simmons (Simmons, 1990), less than 1.0 cm. Sporulation is abundant, even dense, in colonies that are not appreciably concentrically zonate, whereas cultures of *E. indefessa* produce 4-5 conspicuous zones of sporulation under the same growth conditions. Essentially all hyphae at the surface of the black colonies produce ascending or erect conidiophores and chains of conidia.

Conidiophores may be simple, with a single simple or branched chain of conidia. More commonly, primary conidiophores produce 3-5 lateral and subapical branches that support an open tuft of narrow chains. Continued development around the apex may yield a cluster of 10–12 secondary conidiophore branches and branching

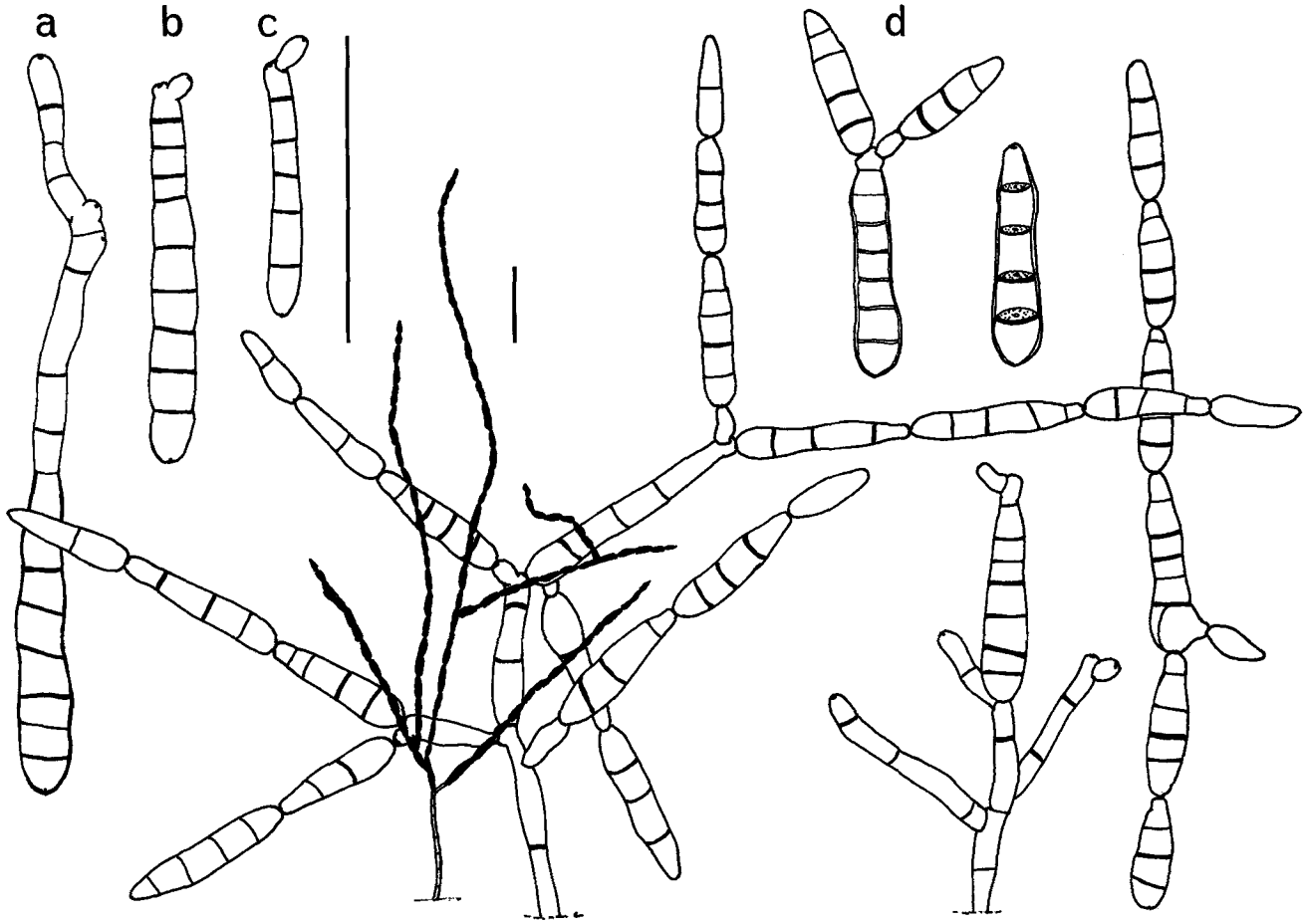

Fig. 1. *Embellisia* species. a-c. Typical primary conidia of *E. dennisii* (a), *E. indefessa* (b), *E. thlaspis* (c). d. Conidia, conidiophores, and sporulation habit of *E. thlaspis* (holotype). Bars = 50 μm (short bar for habit and long bar for the others).

chains of conidia. Primary conidiophores may be as long as 250–300 μm, but usually are considerably shorter when they initiate sporulation.

Chains consist of up to ca. 30 conidia. An apical tuft of chains may consist of as many as 60–100 conidia in young growth or many more in older, well-branched clusters. Primary conidia, those that terminate the main conidiophore axis, typically are longer than those produced later in the chain. The apex of these initial conidia differentiates into a secondary conidiophore that usually is a single short cell only slightly narrower than the conidium body cells. A short 1-cell geniculate, conidiogenous branch commonly is produced from this secondary conidiophore. Primary conidia reach a size range of 34–45 × 5–7 μm and have 4–8 transverse septa; longitudinal septa have not been seen in samples of primary conidia from 7-d PCA cultures. Most secondary conidia in the chains, like primary ones, are subcylindrical or long narrow-ovoid; significant numbers are short ovoid, and others are ellipsoid with smoothly incurved median walls without being sharply constricted. The upper size range for most of the population is 21–31 × 5.5–8.0 μm. Most conidia have 4–5 transverse septa, with 6–7 being unusual; longitudinal septa are extremely rare.

Each subterminal conidium in a chain generates a poorly defined apical region, a secondary conidiophore, which may or may not be separated from the spore body by a transverse septum; this conidiogenous region or cell usually is slightly smaller in diameter, paler, and thinner walled than the spore body. Septa mature with the generically typical surface appearance of flat rings that abut the subhyaline inner face of the conidium wall without merging with it. When septa of older conidia are viewed at a tilted angle, they are seen to be pigmented discs with a distinct, translucent central pore. Conidium colour is medium brown, with outer wall and septa in darker contrast. Conidium walls are smooth. The kinds of hyphal microchlamydospores and various vegetative cell enlargements and distortions found in some species of *Embellisia* have not been seen in 1-2 wk old PCA cultures of *E. thlaspis*.

The key to 13 *Embellisia* species described and illustrated by Simmons (1983) plus characters of a few additions (Simmons, 1990) suggest comparison of *E. thlaspis* with *E. indefessa* and *E. dennisii*. Aside from growth rate differences noted above, the diagnostic features of these species are summarized in Table 1 and illustrated in

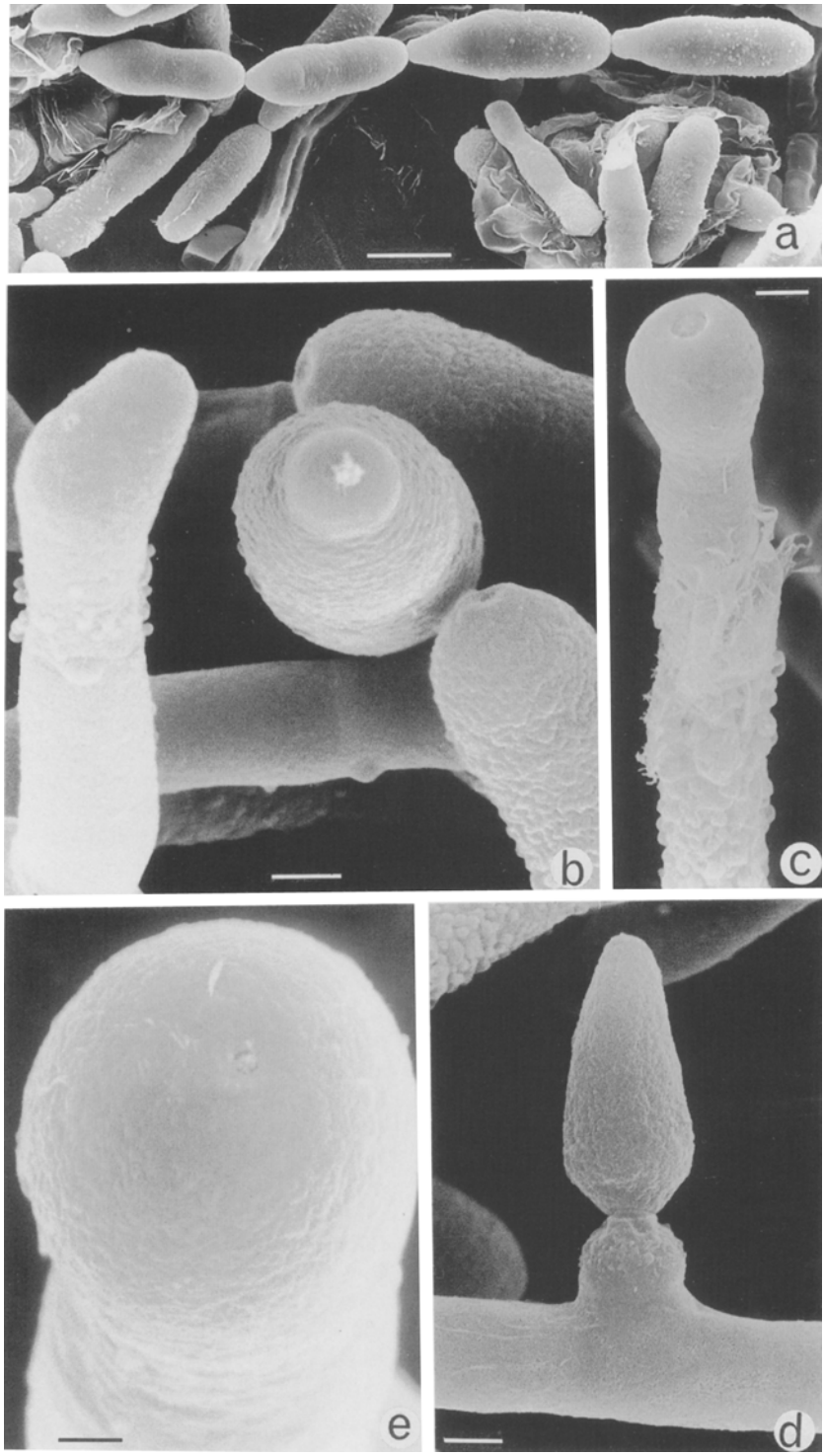

Fig. 2. Scanning electron micrographs of *Embellisia thlaspis* (IMI 374287). a. Conidial chain, with secondary conidiophore indicated by an arrow. b. Conidia and conidiophore showing conidiogenous loci. c. Conidiophore showing regrowth and the distinct crater around the pore. d. Young conidium still attached to the conidiogenous cell. e. Detail of conidiogenous cell with pore. Scale bars: a = 10 μm; b-d = 2 μm; e = 1 μm.

Table 1. Comparative morphological data for the conidia of three similar species of *Embellisia*.

Species	*E. thlaspis*	*E. indefessa*	*E. dennisii*
Primary conidia (μm)	21−31×5.5−8	20−40×8−10	(20−)30−40×6−8
Secondary conidia (μm)	34−45×5−7	48−83×8−10	50−100×7−8
Transverse septa	4−5(−7)	3−4	3−12
Longitudinal septa	Very rare	0−1(−2)	0−3
Shape	Long, narrowovoid	Predominantly short-ovoid	Cylindrical obclavate
Chains	Long, thread-like (ca. 30 units)	Fairly long (ca. 10 units)	Short and branched
Secondary conidiophores	Short (1-celled or 1–2 geniculate)	Short (1-celled or 1–2 geniculate)	Long (3–4 geniculate)

Fig. 1.

Electron microscopy

During the course of the investigation of this new species we were able to make detailed examinations of the conidiogenous structures using the SEM. The results are given in Fig. 2.

The general view (Fig. 2a) shows the conidia occurring in chains: the oldest conidia have visible surface ornamentation and the youngest are smooth-walled. Although not obvious, the chains are branched and a conidium with a geniculate apical secondary conidiophore can be seen in Fig. 2a (see arrow in bottom left hand corner) and in Fig. 1d. Figure 2b gives more detail of the conidia, with slightly roughened walls and craters at the end where the conidia were attached to the conidiogenous cell or each other. The middle conidium has a raised area at the end which is likely to be the initial of a secondary conidiophore. A general view of a conidiophore is given in Fig. 2c. The lower part of the conidiophore wall is distinctly tuberculate, the middle section appears shredded and the upper part including the conidiogenous cell is smooth. The conidiogenous cell is slightly swollen and has an apical declivity around the conidiogenous locus. The relationship between the conidium and the conidiogenous cell can be seen in Fig. 2d where a young conidium is still attached although the dehiscence line is clearly visible. Figure 2e specifically shows a conidiogenous cell with a small pore at the centre of the conidiogenous locus.

Discussion

Simmons (1971), when he introduced the new genus *Embellisia,* remarked that, "the conidiogenous site in *Embellisia*, though referred to as a 'pore', has the appearance of an umbilicus or crater developed when circumferential growth of the conidiophore tip continues upward around the connective to a young conidium. After the conidium dislodges, the actual point of conidium origin remains visible as a short cone (the base of the connective) surrounded by the higher walls of the umbilicate conidiophore tip." Although this observation was made using light microscopy it cannot normally be seen clearly. When viewed using a light microscope the conidiogenous locus is distinctly thickened and pigmented and this is evident in the SEM micrograph (Fig. 2c) showing the swollen conidiogenous cell that appears to have grown up around the conidiogenous locus. The conidiogenous locus thus has a distinct crater where the conidium was attached, as noted by Simmons (1971).

Conidium ontogeny in *Embellisia* is usually referred to as tretic (Ellis, 1971) or porogenous (Cole and Samson, 1979; Cole, 1981), and this pattern also occurs in the similar and related genera, *Alternaria* and *Ulocladium*. Both terms imply the extrusion of an inner wall layer of the conidiogenous cell through a preformed pore or channel to form the conidium. This is an enteroblastic method of conidiogenesis but evidence from SEM (Cole and Samson, 1979) and TEM (Carroll and Carroll, 1974) indicate that this method of conidiogenesis involves all the wall layers. The micrographs of the conidiogenous cells in Fig. 2 show the existence of a well-delimited narrow pore which is visible after conidial secession. Fig 2d does not appear to support the enteroblastic interpretation of porogenous conidiogenesis as there is clearly a broad area of contact between the base of the conidium and the conidiogenous cell.

The difference of surface ornamentation along the conidiophore in Fig. 2c represents two growth phases. At some stage the conidiophore ceased extension growth and the outer wall became hardened and ornamented. Subsequently, extension growth began again, breaking out through the old wall, to produce a new conidiogenous cell. This is an example of what Hennebert and Sutton (1994) term enterogenous erumpent proliferation of the conidiophore.

The results (Fig. 2) are significant since they confirm Simmons's observation and support its value as one of the distinguishing characteristics of the genus. These observations also underline the importance of observing the details of conidiogenesis using SEM, particularly in the search for characters that may define anamorphic taxa more reliably.

Acknowledgements——The authors are indebted to Professor Emory Simmons (Crawfordsville, Indiana) for examining the isolate and providing the description and to Dr P. F. Cannon for his helpful comments on the manuscript.

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