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Clavulicium extendens sp. nov (Corticiaceae), a Fungus Spreading on Twigs in Queensland Rainforests

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Abstract

Clavulicium extendens sp. nov., is newly described from Queensland. It occurs in rainforest and wet sclerophyll forest where it forms an extensive, white, external, mycelial membrane on dead and living twigs and stems, and spreads aerially between branches by means of attachment pads. No evidence was found that *C. extendens* is able to penetrate or injure living plant tissue, but it is not yet possible to conclude that this fungus is non-pathogenic.

Introduction

Among the basidiomycete fungi present in rainforests in Queensland is a distinctive species forming an extensive, white, adherent, mycelial membrane that spreads on the surface of dead and living twigs and stems of shrubs, saplings and vines. Adjacent stems that touch become united by means of attachment pads resulting from vigorous hyphal growth. Most collections of this fungus are sterile, but a fertile collection received in July 1995, from Fraser Island in south-eastern Queensland, enabled it to be identified as a previously undescribed species of *Clavulicium*. This specimen was studied along with a number of sterile collections in order to ascertain the morphology and development of the fungus and to formally describe it. Isolates from the fresh collections were also examined and used to determine the optimum temperature range for growth in culture. The possible pathogenicity of the fungus to rainforest understorey plants and adjacent plantation species was explored by treating potted seedlings with natural inoculum.

Materials and Methods

Six fresh collections of the white, membrane-forming basidiomycete received from different parts of Queensland were studied microscopically in order to compile a description. Locations of collections are identified here according to Queensland pastoral districts as depicted by Stanley and Ross (1983). Sections were mounted in 2% phloxine in 4% potassium hydroxide solution, or in Melzer's Reagent for examination. Colours were determined using the charts of Rayner (1970) and Methuen (Kornerup and Wanscher 1978). Collections were dried and lodged in the Pathology herbarium of the Queensland Forestry Research Institute, Indooroopilly, Brisbane (QFRI), or herbarium BRIP (Queensland Department of Primary Industries Agricultural Production, Indooroopilly). Cultures isolated from mycelial membrane or fruit-body subiculum in three of the fresh collections were examined on 2% malt agar and described using an adaptation of the method of Nobles (1965; α-naphthol was used to test for laccase, Stalpers 1978). Two of the isolates were grown simultaneously at different temperatures in a multi-range incubator on plates of 2% malt agar and potato dextrose agar in order to determine the temperature–growth profile of the fungus. Plates were inoculated at the edge and radial growth was recorded when the fastest colonies nearly covered the plates.

An attempt was made to investigate the possible pathogenicity of the fungus by microscopically examining stem sections cut from naturally colonised, host-plant material for evidence of hyphal penetration, and by treating young potted seedlings of *Elaeocarpus grandis* F.Muell. and *Eucalyptus*

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pellita F.Muell. with natural inoculum. Colonised twigs of *Backhousia myrtifolia* Hook.f. & Harv. freshly collected from Central Station, Fraser Island, were partly inserted into the soil allowing the mycelial membrane to lie in contact with the stem of treated plants just above soil level. Six treated plants of each species were kept in an environment of 25°C, 80% relative humidity, and a 12-h day–night photoperiod, while four treated plants of each host were each loosely covered in a clear polythene bag and maintained at about 25°C in a glasshouse using natural daylight. Untreated control plants of each species were kept simultaneously under the same conditions. Plants were inspected regularly, and two with evidence of fresh surface mycelial growth were examined microscopically after 12 weeks.

Results

Taxonomic Treatment

Clavulicium extendens Hood sp. nov. (Figs 1 and 2)

Fructificatio resupinata, membranacea, irregulariter orbicularis, 1.5–2.5 mm diametro, tenuis vel crassiuscula (0.1–0.7 mm), confluens ex parte vel discreta; margo distinctus, erigens in fructificationibus discretis; hymenium superficie levi, compactum, luteum, in sicco luteolum vel salmoneum; subiculum friabile, album. Systema hypharum monomiticum; hyphae laxae intertextae autem arctae versus subhymenium et parallelae ad substratum basi, fibulatae, ramificantes, 2.5–5 µm latae, hyalinae, parietibus tenuibus vel incrassatis. Cystidia rara, plerumque non projecta, angusta, fusiformi-subulata, strangulata apicem versus, fibula basi, contentiis uniformibus, 33–37 × 4–5 µm, hyalina, parietibus tenuibus. Basidia non projecta, clavata vel parum subutriformia, fibula basi, sterigmatibus 2 prominentibus (6.5–10 µm longis), cum vel sine guttulis, 33–51 × 6–9 µm. Basidiosporae liberae vel adhaerentes in paribus, obovatae vel ellipsoideae, apiculis prominentibus, leves, aliquae guttulis, (7–) 8–11 (–12) × 4.5–6.5 µm, non amyloideae. Thallus (pars externa) in formis duabus: extensus circum et secus (> 1 m) ramulos vel radians in caulibus grandibus ex locis conjunctivis, adhaerens, membranaceus, levis, margine distincto, tenuis (50–80 µm), opacus, albus; iterum adhaerens, funicularis, ramificatione sympodiali, teres (≤ 1 mm latus) vel complanatus et transiens forma membranacea, levis, albus. Hyphae in ambabus formis parallelae ad substratum, fibulatae, ramificantes, 2–5 µm latae, hyalinae, parietibus tenuibus. Holotypus BRIP 24004.

Fruitbody resupinate, membranaceous, irregularly orbicular, 1.5–2.5 mm diam., thin or moderately thick (0.1–0.7 mm), confluent in part or discrete; margin distinct, lifting on discrete fruitbodies; hymenium smooth, compact, orange-yellow (Methuen) or luteous (Rayner), when dry pale orange (Methuen) or salmon (Rayner); subiculum friable, white. *Hyphal system* monomitic; hyphae loosely interwoven but tight towards subhymenium and parallel to substratum at base, nodose-septate, branching, 2.5–5 µm wide, hyaline, thin- to thick-walled. *Cystidia* uncommon, generally not projecting, narrow, fusiform-subulate, strangulated towards the apex, with clamp at base, with uniform contents, 33–37 × 4–5 µm, hyaline, non-staining in sulphovanillin, with thin walls. *Basidia* not projecting, clavate or slightly subutriform, with clamp at base, with 2 prominent sterigmata (6.5–10 µm long), with or without guttules, 33–51 × 6–9 µm. *Spores* free or adhering in pairs, obovoid or ellipsoid, with prominent apiculus, smooth, some with guttules, (7–) 8–11 (–12) × 4.5–6.5 µm, non-amyloid.

External thallus in two forms: extended around and (> 1 m) along branchlets or radiating from contact points on large stems, adherent, membranous, smooth, with distinct margin, thin (50–80 µm), opaque, white; also adherent, cord-like, with sympodial branching, terete (≤ 1 mm wide) or flattened and merging with membranous form, smooth, white. Hyphae in both forms parallel to substratum, nodose-septate, branching, 2–5 µm wide, hyaline, thin-walled.

Cultures from fruitbody subiculum (holotype) and external thallus identical, slow growing at optimum temperature range (20–27°C, Fig. 3), slower on malt than on potato dextrose agar, at 6 weeks on 2% malt agar sub-felty, colourless, translucent; hyphae nodose-septate, with occasional hyphal swellings, hyaline, thin-walled, lacking propagules or spores. Culture code: 2, 3,7/(26), 32, 36, 38, 47, 54.

Habitat

On living and dead angiosperm host species in rainforest and wet sclerophyll forest, associated with a white rot. *Distribution*: Queensland.

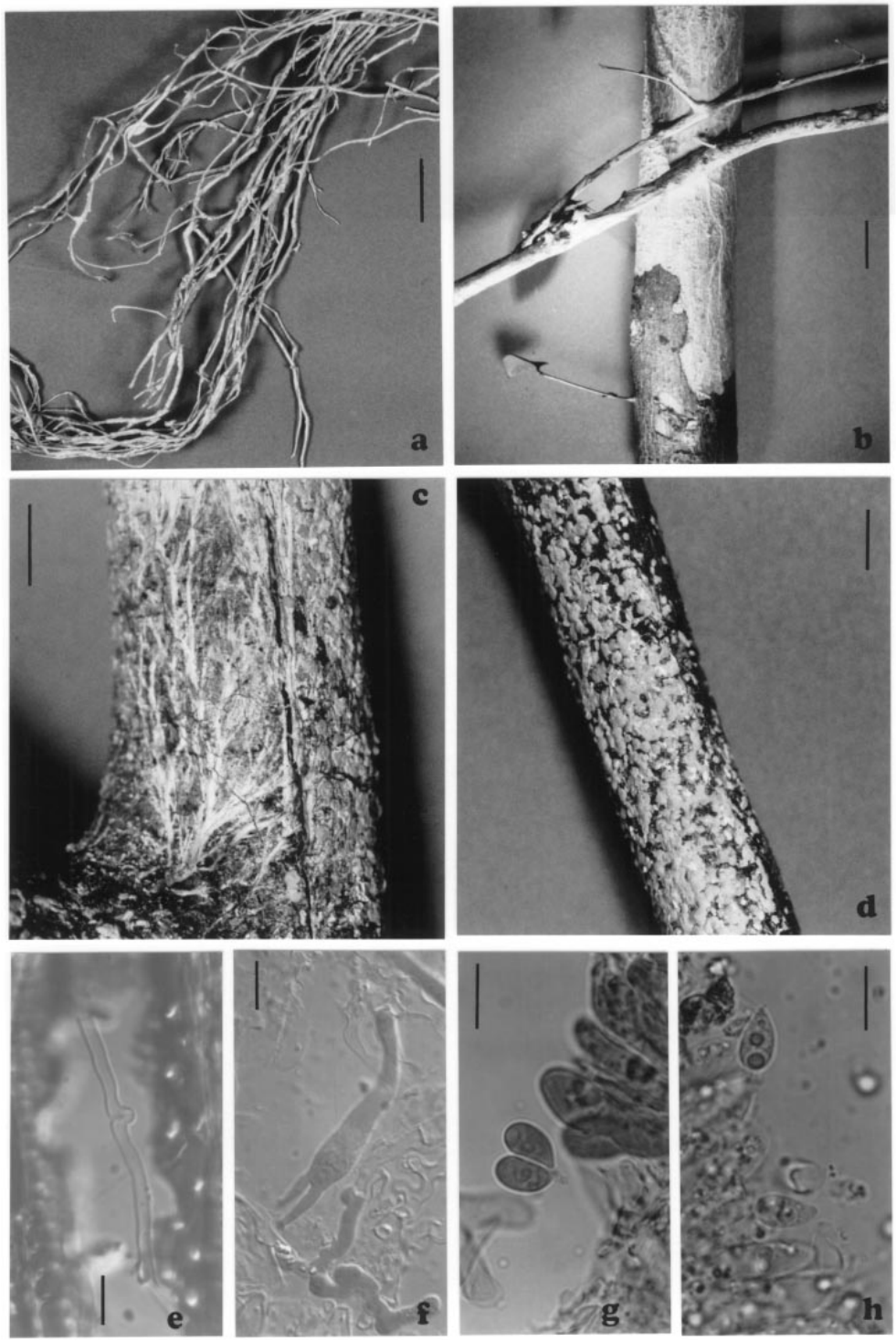

Fig. 1. *Clavulicium extendens*, (*a*) dead shoots fully coated in white mycelial membrane, (*b*) live stems, with green leaves, connected by attachment pads and coated in mycelial membrane, (*c*) branching mycelial cords, (*d*) fruitbodies, (*e*) clamped hypha in a vessel lumen within a dead twig coated externally in mycelium membrane, (*f*) basidium with basal clamp and clamped subiculum hyphae, (*g*) and (*h*) basidiospores. Scale bar = 20 mm (*a*), 10 mm (*b–d*), 10 µm (*e–h*).

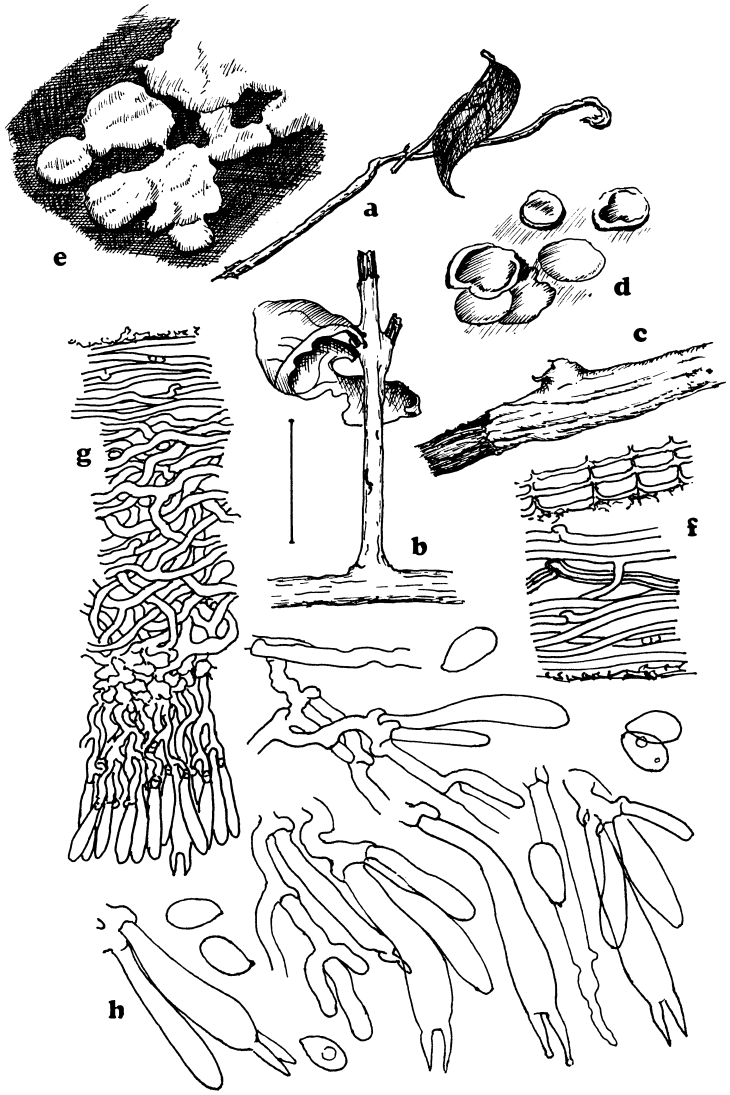

Fig. 2. *Clavulicium extendens*, (*a*) dead shoot fully coated in white mycelial membrane, with an attached broken living twig, (*b*) living twig of *Backhousia myrtifolia* with stem mostly coated by mycelial membrane, (*c*) dead twig partly coated by mycelial membrane, (*d*) discrete fruitbodies, three with margins lifted exposing white subiculum beneath, (*e*) partly confluent fruitbodies, (*f*) section of mycelial membrane with abutting host cork cells (diagrammatic), (*g*) section of fruitbody (diagrammatic), (*h*) fruitbody hyphae, paraphyses, cystidia, basidia and basidiospores. Scale bar = 6 cm (*a*), 8 mm (*b*), 4 mm (*c, d, e*), 40 µm (*f, g*), 20 µm (*h*).

Etymology

From Latin *extendens*, spreading, in reference to the growth habit of the external thallus.

Type

Australia: Queensland: Wide Bay: Fraser Island, Central Station, on twigs and branches of *Backhousia myrtifolia* in satinay forest (*Syncarpia hillii* F.M.Bailey, with *Lophostemon confertus* (R.Br.) Peter G.Wilson & J.T.Waterh.), S. Masterson, 21 July 1995 (holotype BRIP 24004, isotype QFRI 8695).

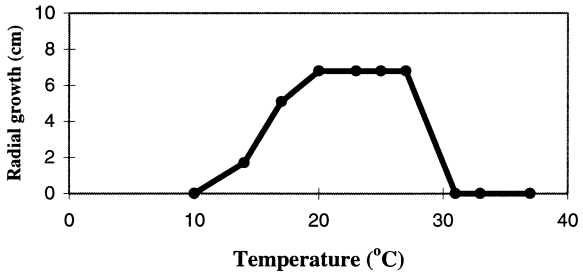

Fig. 3. Growth profile shown by *Clavulicium extendens* (isolates QFRI 8582.8 and QFRI 8588.3) after 9 weeks at different temperatures on potato dextrose agar.

Additional Specimens Examined (non-fertile)

Australia: Queensland: Cook: Atherton Tableland: A. F. Wright, 27 July 1994 (QFRI 8577); Danbulla State Forest, unidentified vine species in rainforest, A. F. Wright, 2 Aug. 1994, cultured (QFRI 8582); Malanda, on rainforest tree, A. F. Wright, 29 Aug. 1994, cultured (QFRI 8588). North Kennedy: Paluma, in rainforest, W. Craven, 19 Feb. 1995 (QFRI 8650). Wide Bay: Fraser Island, Poyungan Valley, in 'scrub', S. Rohner, 29 Dec. 1994 (QFRI 8675).

Remarks

Cunningham (1963) described a large number of species of resupinate, non-poroid fungi from Australasia, some of which have since been reallocated to other genera such as *Hyphoderma* Wallr. (e.g. Stalpers 1985). However, the species discussed here was not included among those featured by Cunningham. It belongs within *Clavulicium* Boidin which comprises species with resupinate fruitbodies, monomitic hyphal systems, and spores containing oil droplets or granular contents. Most species, including the type (*C. macounii* (Burt) J. Erikss. & Boidin ex Parmasto), have basidia with two sterigmata. Five species have previously been described, all from temperate Europe, America, or Asia: *C. delectabile* (Jacks.) Hjortstam, *C. macounii*, *C. spurium* (Bourdot) J. Erikss. & Hjortstam, *C. venosum* (Berk. & Ravenel) Ginns, and *C. vinoscabens* (Burt) Pouzar. *Clavulicium extendens* is distinguished from others in this genus, and from the similar *Hyphoderma multicystidium* var. *disporum* (M. Dueñas & Tellería) Hjortstam & Tellería from Europe, by its small, discrete or partially fused fruitbodies, an extensive external membranous thallus, and in the nature of its cystidia which are thin walled, aseptate, not projecting, and uncommon. Oil droplets are present in some, but not all basidiospores.

Pathogenicity

Under the microscope hyaline, branching, thin-walled, clamped hyphae 2.5–3.5 µm wide were present within the lumens of xylem vessels 350 µm and 700 µm, respectively, beneath the bark surface of a dead vine segment (QFRI 8582) and a 3-mm-diameter dead twig (BRIP 24004). These hyphae were identical to those in the white mycelial membrane of *C. extendens* present on the surface of both specimens. In the second specimen (BRIP 24004) vessels nearer the surface were packed with thick-walled, clamped hyphae, and tissues external to the xylem were all but replaced by similar, close-packed, thick-walled hyphae. In living twigs still holding green leaves in each of these collections clamped hyphae were also often plentiful within the cork cells directly beneath the white mycelial membrane. However, such hyphae were not observed deeper than the phelloderm in these living specimens.

In the potted seedling experiment mycelial connecting pads formed between the inoculum twigs and the stems of two treatment seedlings of *Elaeocarpus grandis* in the glasshouse, and two treatment seedlings of *Eucalyptus pellita* held under controlled environmental conditions. White mycelial membrane of *C. extendens* spread from a few millimetres to *c*. 2 cm along the stems of these seedlings which remained healthy and

showed no signs of necrosis beneath the surface mycelium. In one seedling of *E. pellita* the white surface mycelium was identical to the naturally produced membrane of *C. extendens*, and was composed of subparallel, clamped, hyaline, thin-walled hyphae forming a layer 30–90 µm thick, which penetrated only to the outermost cork cells. In the other *E. pellita* seedling such hyphae (2.5 µm wide) were found in external cortex cells, but also did not penetrate further below a suberised cork zone.

Discussion

A number of basidiomycetes belonging to several different families spread vegetatively above ground by forming mycelial connecting pads that bond adjacent host stems together at points of contact (e.g. Gilmour 1959, 1966; Ainsworth and Rayner 1990; Stenlid and Holmer 1991; Hedger *et al*. 1993; Nuñez 1996). Some of these fungi are pathogenic on living stems. *Hymenochaete tabacina* (J.Sowerby: Fr.) Lév. is a mild invader of suppressed living hosts (Stenlid and Holmer 1991), while *Dextrinocystidium sacratum* (G.Cunn.) Sheng H.Wu is a disease agent able to kill its hosts (Gilmour 1959, 1966, as *Peniophora sacrata* G.Cunn.). *Clavulicium extendens* is particularly vigorous among this group. Besides forming typical mycelial connecting pads it also spreads many decimetres over stems, twigs, and fallen branches by means of superficial mycelial membranes and cords. As dead host twigs decay and collapse they remain linked together by the mycelium in dense white net-like hanging tangles. However, although *C. extendens* is sometimes popularly known as ‘white death’ fungus, its pathogenicity has not yet been demonstrated. This study found no indication that *C. extendens* injures the plants on which it grows, suggesting that it may be able to exploit only dead wood. There was no evidence that the surface mycelium penetrated living stems either naturally or when potted seedlings were treated with inoculum. Nevertheless, additional confirmatory study is needed, especially as the conditions chosen for the seedling experiment may not have been conducive to vigorous mycelial growth. Besides using natural inoculum, several additional seedlings of *Elaeocarpus grandis* and *Eucalyptus pellita* were treated with black bean (*Castanospermum australe* A.Cunn. & C.Fraser ex Hook.) woodblock cultures of *C. extendens* (I. A. Hood and M. Ramsden, unpublished results), but although inoculum was fresh when applied it eventually failed to grow further, possibly due to difficulties in maintaining a controlled temperature and humidity regime. Apart from this, the possibilities that *C. extendens* may be able to penetrate immature unsuberised tissues or even smother living shoots require consideration. Dead shoots enveloped in mycelial membrane right to the young tips (Fig. 2*a*) or still holding shrivelled brown leaves were observed, implying rapid death and early colonisation before sufficient time for significant tissue deterioration had elapsed. If *C. extendens* is found to occur significantly in new plantations of species such as *Eucalyptus pellita* established on sites near residual rainforest, it will still be necessary to confirm that the fungus is not pathogenic to these hosts.

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