STUDIES IN MYCOLOGY 52: 1–144. 2005.

Phylogenetic relationships and morphology of *Cytospora* species and related teleomorphs (*Ascomycota*, *Diaporthales*, *Valsaceae*) from *Eucalyptus*

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Abstract: *Cytospora* species and their *Valsa* teleomorphs are commonly found on *Eucalyptus* trees and some of these have been associated with stem canker diseases. The taxonomy of these fungi has been confused and has in many cases hindered pathology studies. This study was based on extensive collections of *Cytospora* species and their teleomorphs from *Eucalyptus* trees in Africa, Australia, Central and South America, Southeast Asia and California. Sixty-two *Cytospora* and three *Cytospora*-like isolates from *Eucalyptus*, yielding 33 unique ITS-rDNA sequences, were compared for homology to *Cytospora* species from other hosts. Phylogenetic analysis clustered isolates of *Cytospora* from *Eucalyptus* into at least 15 unrelated groups. The *Cytospora*-like isolates that morphologically resembled *Cytospora* clustered in a separate group, which is related to *Phomopsis*. Morphology of the fungi was examined on natural subtrates and in culture in order to identify distinctive characters linked to the phylogenetic lineages emerging from DNA sequence analyses. The specimens from *Eucalyptus* included morphological features encompassing the *Cytospora* infrageneric sections *Cytospora*, *Lamyella*, *Leucocytospora*, and *Torsellia* with the majority residing in sect. *Lamyella*. Several species exhibited morphological characteristics of more than one section and other species had unique characteristics not represented in the established sections. Phylogenetic inference did not support the sections that have been established based on morphological characteristics. The concepts underlying the establishment of sections in *Valsa* and *Cytospora* were, therefore, discarded and descriptive terms have been introduced to distinguish between ascostroma and locule forms. Descriptions of *Cytospora australiae*, *C. eucalyptina*, *C. eucalypticola*, and the anamorphs of *Valsa eucalypti* and *Leucostoma sequoiae* have been emended based on morphological studies of cross-sections of holotype and isotype specimens. Teleomorphs associated with *Cytospora* specimens on *Eucalyptus* have been described from Australia, California, Chile, Congo, Hawaii, India and Uganda. Each teleomorph had unique morphological characteristics and DNA sequence but several conformed to the broad description of *Valsa ceratosperma*, even though they resided in separate phylogenetic lineages. Additionally, sequences for *V. ceratosperma* on hosts other than *Eucalyptus* resided in separate lineages and were different from all of the isolates from *Eucalyptus*. One of the lineages, commonly found on *Quercus*, was recognised as corresponding to the original species concept and was designated as *V. ceratosperma sensu stricto* (= *V. ceratophora*). Results of this study have shown that numerous genetically distinct lineages of *Cytospora* and *Cytospora*-like fungi occur on *Eucalyptus*, and that the current description of *V. ceratosperma* encompases several distinctly different fungi. *Leucostoma sequoiae* and *V. eugeniae* were found on *Eucalyptus*, and *V. eucalypti* and *L. sequoiae* have been synonymised. Cultural characteristics including colony colour, pycnidium structure, cardinal temperatures for growth, and tolerance to cycloheximide have been described for the species. Several new species of *Valsa* and *Cytospora* from *Eucalyptus* have also been delimited based on morphological characteristics. This wide-ranging study should contribute to a better understanding of the taxonomy of *Cytospora* spp. and their teleomoprhs, particularly on *Eucalyptus*. It is also hoped that this will lead to improved management strategies for diseases associated with these fungi.

Taxonomic novelties: *Cytospora abyssinica* G.C. Adams & Jol. Roux & Gezahgne sp. nov., *C. austromontana* G.C. Adams & M.J. Wingf. sp. nov., *C. berkeleyi* G.C. Adams sp. nov., *C. diatrypelloidea* G.C. Adams & M.J. Wingf. sp. nov., *C. disciformis* G.C. Adams & M.J. Wingf. sp. nov., *C. nitschkii* G.C. Adams, Jol. Roux & Gezahgne sp. nov., *C. valsoidea* G.C. Adams & M.J. Wingf. sp. nov., *C. variostromatica* G.C. Adams & M.J. Wingf. sp. nov., *Valsa brevispora* G.C. Adams & Jol. Roux sp. nov., *V. cinereostroma* G.C. Adams & M.J.Wingf. sp. nov., *V. fabianae* G.C. Adams, M.J. Wingf. & Jol. Roux sp. nov. (anamorph *C. eucalypticola* van der Westh.), *V. myrtagena* G.C. Adams & M.J. Wingf. sp. nov.
Key words: canker, endophyte, gene tree, *Phomopsis*, phylogeny, plantation forestry.

INTRODUCTION

Valsa Fr. species and their *Cytospora* Ehrenb. anamorphs are amongst the fungi most commonly isolated from *Eucalyptus* bark and leaves. The taxonomy of these fungi has never been treated in any detail and it has been seriously confused. Most studies where in *Cytospora* or *Valsa* species are found on *Eucalyptus* have been linked to pathology problems, and available names have apparently been used without treatment of the taxonomy of the fungi.

Several species of *Cytospora* and their teleomorphs in *Valsa* have been reported on *Eucalyptus*. The most commonly cited species on this widely planted and important tree genus is *Cytospora eucalypticola* van der Westh. This fungus was first described as a pathogen causing cankers and death of *Eucalyptus saligna* in Tzaneen, Limpopo province, South Africa (van der Westhuizen 1965a, b). The disease continues to be reported in this area, particularly on seedlings of *Eucalyptus grandis* under closed canopies. In South Africa, *Eucalyptus* species, hybrids and clones occasionally suffer bark lesions, branch dieback and stem cankers caused by *Cytospora* spp. following stresses. For example, plantations of *Eucalyptus dunnii* can collapse from *Cytospora* stem canker following combined drought and frost injury. *Cytospora* occurs on stems of young hybrid *E. grandis* clones suffering from bacterial wilt [*Ralstonia solanacearum* (Smith) Yabuuchi *et al.*] or root rot diseases, termite damage or machete wounds (Roux *et al.* 2000). The teleomorph of *Cytospora* is seldom associated with fresh cankers. It has, however, been found sporulating profusely on bark lesions at the bases of *E. grandis* × *E. tereticornis* hybrids in the Congo following fire injury (Roux *et al.* 2000) and on *E. grandis* in Ugandan wetlands with poor drainage (Roux *et al.* 2001).

Cytospora spp. and their teleomorphs accounted for 33 % of fungi associated with canker diseases in a survey of *Eucalyptus* stems in natural forests and plantations in Tasmania (Yuan & Mohammed 2001). Cytospora canker is an occasional disease wherever *Eucalyptus* is grown in commercial plantations in the Southern Hemisphere, including Australia (Davison & Tay 1983, Fraser & Davison 1986), Africa, Indonesia, Thailand, South and Central America (M.J.Wingfield, unpubl. data), India (Soni *et al.* 1983, Sharma *et al.* 1985) and rarely in the Northern Hemisphere in California (Cooke & Harkness 1881), Florida (Alfieri *et al.* 1994), Spain (Sankaran *et al.* 1995), Japan (Old *et al.* 1991), and the former Soviet Union (Gvritishvili 1982). Additionally, *C.eucalypticola* is an endophyte in xylem and in leaves of *Eucalyptus* (Bettucci & Saravay 1993, Fisher *et al.* 1993, Smith *et al.* 1996).

Old *et al.* (1991) considered that the *Cytospora* species on *Eucalyptus* in Australia fit the description of *C. eucalypticola*, while the teleomorphs in Australia and Japan resemble *Valsa ceratosperma* (Tode) Maire [anamorph = *Cytospora sacculus* (Schwein.) Gvrit.]. He suggests retaining the anamorph name *C. eucalypticola* rather than *C. sacculus* because uncertainty remained as to whether the *Cytospora* and *Valsa* from *Eucalyptus* in Australia were the same as those from *Eucalyptus* in Japan and elsewhere in the Northern Hemisphere. *Cytospora eucalypticola* from South Africa and Australia has small narrow conidia (3–4 × 0.7–1 µm), unbranched conidiophores and locules of variable size and irregular arrangement in the pycnidium (van der Westhuizen 1965a, Old *et al.* 1991). The *Cytospora* on *Eucalyptus* in Japan has small, narrow conidia but locules are uniform in size and arranged radially. Additionally, the Japanese isolates differ consistently and distinctly in culture characteristics (pale brown with sparse fruiting) from the Australian and South African isolates (dark olivaceous with abundant fruiting) (Old *et al.* 1991).

Valsa fruiting bodies from *Eucalyptus* in Australia and Japan are similar to *V. ceratoperma* from other hosts (Kobayashi 1970, Old *et al.* 1991). The teleomorphs from *Eucalyptus* in the former U.S.S.R. are also described as *V. ceratosperma* by Gvritishvili (1982) but those in California and Florida are described as *Valsa eucalypti* Cooke & Harkn. (Cooke & Harkness 1881, Alfieri *et al.* 1994). The latter is thought to be a species of *Leucostoma* (Nitschke) Höhn. by Spielman (1985). *Valsa ceratosperma*, in the studies of Old *et al.* (1991), varies considerably in ascospore size on different continents. Australian specimens from *Eucalyptus* have spores that ranged from 7–8 × 1.5–1.8 µm, compared to Japanese specimens at 5.6–7 × 1.8–2 µm. Spores of *V. ceratosperma* from other hosts in Japan range from 5.5–9 × 1–2 µm whereas those from England have a mean of 10.5 × 2.5 µm. The breadth of the size range seems inordinately liberal to precisely circumscribe a species of *Valsa*.

Two species of *Cytospora* have been described from *Eucalyptus* in Argentina. Of these *Cytospora australiae* Speg. has longer and wider conidia (4–6 × 2–3 µm) and regular radially arranged locules compared to *C. eucalypticola* (van der Westhuizen 1965a). The other species, is *Cytospora eucalyptina* Speg., which van der Westhuizen (1965a) considered a synonym of *C. australiae*. A third species, *Cytospora agarwalii* Soni, Dadwal & Jamaluddin, has been described from India (Soni *et al.* 1983). This fungus also has long conidia (5 × 1.2 µm) and regular radially arranged locules as well as white stromata.

The anamorphs of *Valsa* vary with respect to the configurations of locules with chambers and ostioles. Historically, different locule types have been placed in separate sections or subgenera and sections. In the present study, the infrageneric rankings used by

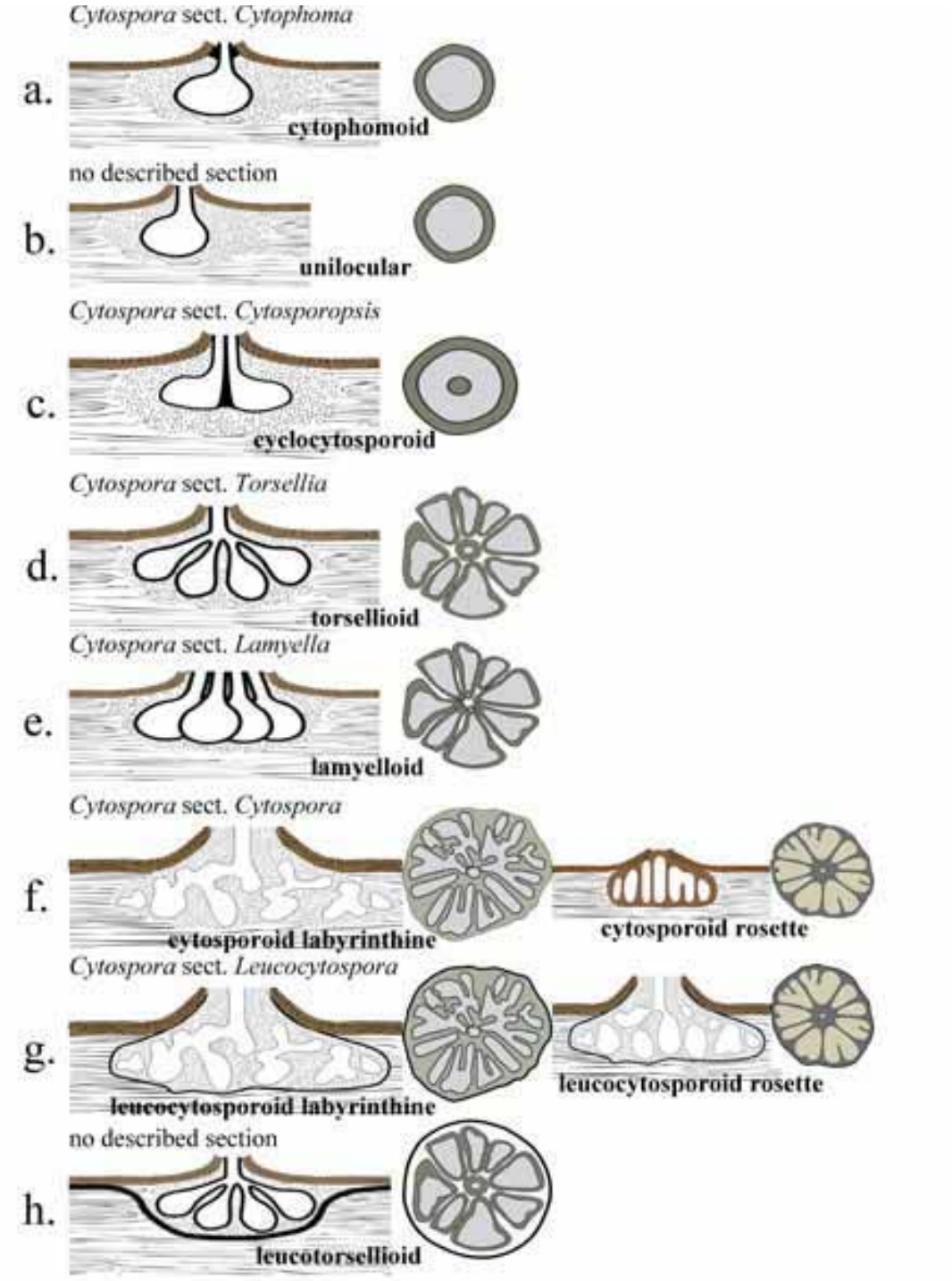

Fig. 1. Locule forms that are the basis of the former infrageneric sections of *Cytospora*, and the new descriptive terms (bold) that are substituted for section features, as illustrated with tangential and longitudinal cross-sections of conidiomata. A. Former sect. *Cytophoma* and descriptive term "cytophomoid", refers to an undivided locule and wing-like ectostroma around the ostiole (monospecific for *C. pruinosa*). B. The descriptive term "unilocular", refers to an undivided locule without wing-like ectostroma around the ostiole; may be with or without disc. C. Former sect. *Cytosporopsis* (originally named sect. *Cyclocytospora*) and descriptive term "cyclocytosporoid", refers to a toruloid locule with a central column of ostiolar tissue (monospecific for *C. umbrina*). D. Former sect. *Torsellia* and descriptive term "torsellioid" refers to multiple independent locules sharing one ostiole. E. Former sect. *Lamyella* and descriptive term "lamyelloid", refers to multiple independent locules with multiple ostioles. F. Former sect. *Cytospora* and descriptive term "cytosporoid", refers to a divided locule and shared walls. The two at left are "labyrinthine cytosporoid" and the two at right are "rosette cytosporoid". G. Section or subgenus *Leucocytospora* and descriptive term "leucocytosporoid", refers to divided locule and shared walls delimited by black conceptacle. H. Descriptive term "leucotorsellioid", refers to independent locules delimited by black conceptacle.

Spielman (1985) and Gvritishvili (1982), sections *Cytophoma* (Höhn.) Gvrit., *Cytosporopsis* (Höhn.) Gvrit., *Cytospora*, *Torsellia* (Fr.) Gvrit., *Lamyella* (Fr.) Gvrit., and *Leucocytospora* (Höhn.) Gvrit., are discussed but they are discarded in favour of using new and hopefully less confusing descriptive terms for locule forms. The key morphological features of the former sections and the new descriptive terms are illustrated in Fig. 1.

A problem arises in interpreting descriptions of *Cytospora* species in historic and more current literature. Most descriptions fail to clarify the key characteristics of the infrageneric sections, including the configurations of the locules, chambers, and ostioles of the conidiomatal stromata. The scope of this problem becomes evident when one considers that 334 species of *Cytospora* were described by Saccardo (1882–1931), and that subsequently many other species have been described. Accurate identification of species of *Cytospora*, therefore, generally has required re-consideration of type specimens.

Testing isolates of *Cytospora* from *Eucalyptus* spp. for pathogenicity and host range has not been accomplished because disease is not readily induced (Old *et al.* 1986, Shearer *et al.* 1987, Old & Kobayashi 1988, Yuan *et al.* 1995). It is not unusual to experience difficulty in inducing disease with *Cytospora* species (Schoeneweiss 1983). For example, to initiate Cytospora canker formation on *Populus* spp., the moisture content of the bark and xylem of a cutting needed to be reduced to approximately 30% of saturation in the living branch (Bloomberg 1962a). Following initiation of disease, canker growth can cease when the water potential changes to levels more favourable to plant growth.

Characters other than morphology and pathogenicity are needed to differentiate species of *Cytospora* and *Cytospora*-like pathogens of *Eucalyptus*. Host preference is not a verifiable characteristic for this genus and the anamorphs that form in nature vary considerably in morphology from those that form in culture. Teleomorphs are rare, do not form in culture, and the morphology of these structures is often not taxonomically useful at the species level (see *V. ceratosperma* below). DNA sequences can provide a large number of characters useful in assessing the relationships among isolates and species of *Valsa*, the *Cytospora* anamorphs, and other *Cytospora*-like pathogens. The large number of characters arising from this taxonomic approach also permits the statistical testing of hypothesized phylogenetic relationships among these organisms.

Cytospora species are commonly isolated from cankers on symptomatic *Eucalyptus*. Cultures are occasionally stored for pathology studies but natural materials with fruiting structures (voucher specimens) are seldom accessioned with the corresponding cultures. The objectives of this study have thus been to determine relationships among cultures of *Cytospora* and *Cytospora*-like fungi from *Eucalyptus*, to describe cultural characteristics, and to describe the corresponding morphological features of anamorphs and teleomorphs on natural material. Furthermore, our goal has been to relate morphological characters to distinct lineages on a gene tree arising from the analysis of DNA sequence data. Descriptions of type and isotype specimens are emended to include locule forms in the conidiomatal stromata. Collections of unique anamorphs and teleomorphs are described as new species.

ECOLOGY AND PATHOLOGY

Disease symptoms

Cytospora canker is a problem on angiosperm and gymnosperm woody plants throughout the world. *Cytospora* species predominantly infect woody plants but have occasionally been described on annuals, for example *Cytospora tritici* Punith. on *Triticum aestivum* and *Cytospora sacchari* E.J. Butler on *Saccharum officinarum*. These fungi have been reported to cause diffuse cankers on over 85 species of woody hosts (Farr *et al.* 1989, Sinclair *et al.* 1987, and herein). The disease is usually referred to as Cytospora canker, but is sometimes called Valsa canker, Leucostoma canker and perennial canker.

Some tree species respond to infection by producing copious gummosis or resin at the site of active canker growth. The pathogens infect the inner bark and in the conifers no discolouration of the adjacent cambium is observed even though the fungus can be isolated from nearby xylem (Schoeneweiss 1983). In hardwood trees, particularly stone fruit, poplar and maple trees, the bark canker pathogens discolour the sapwood (Fig. 2). Biggs *et al.* (1983) reported that dense wedges of wide hyphae colonise the periderm. The cells behind the advancing front are colonised both inter- and intracellularly by narrower hyphae, and the inner bark turns dark while the underlying sapwood is stained a pale brown colour. During the discolouration of the sapwood the hydraulic conductivity of the xylem vessels in the new wood is also disrupted (Hampson & Sinclair 1973, Chang *et al.* 1991b).

Chang *et al.* (1991b) measured hydraulic conductivity in inoculated *Prunus persica* in eight open-pollinated families that vary in relative susceptibility to Cytospora canker. Safranin dye movement indicated that the sapwood in the inoculated branch segments of seedlings from the susceptible *Prunus persica* families was almost completely blocked. In the disease tolerant families, 15–30 % of the water transport capabilities

Fig. 2. Cankers and external signs of *Cytospora* infection. A. Canker on *Prunus persica* with stained cambium and gummosis. B. Canker on *Acer* sp. with stained cambium. C. Diffuse canker on *P. persica* shows circadian rings of growth following inoculation in October and examination in May. D. Healing canker in *P. persica* following inoculation in May and examination in September. E. Crystallised resin exuded over canker on *Picea pungens*. F. Yellow tendrils (cirrhi) of conidia exuding from conidiomata. G. Discrete ostiolar beaks of conidiomata visible on bark.

of the controls was maintained following inoculation. Disfunctional xylem was correlated with reduction in hydraulic conductance per pressure gradient, and the seedlings from tolerant families were better able to maintain water transport through the region of the wood invaded by the fungus. Furthermore, inoculation experiments indicated deeper penetration of the fungus into the xylem of seedlings from susceptible as opposed to tolerant families. In the susceptible families, *Cytospora* could penetrate past the active xylem and deep into the pith of the branches where no symptoms of disease were evident (Chang *et al.* 1991b). *Cytospora* colonised bark where no necrosis or obvious injury was apparent, some distance from the canker (Schoeneweiss 1983).

In temperate regions of the world, the common result of infection by *Cytospora* spp. is wilting during leaf expansion in the spring. The diffuse cankers can spread rapidly and extensively during the period when a dormant tree begins active spring growth with bud break and shoot elongation. Removal of the bark and examination of the cambium may reveal regularly variable shades of darkened cambium at the apex and base of the canker, which appear to be diurnal expansion patterns of fungal growth (Fig. 2). This rapid expansion of the canker contrasts with disease symptoms on the same individual tree when inoculation occurs during the later part of the growing season and up until autumn. Inoculations during summer result in cankers that are usually limited by host defensive responses (Fig. 2). In summer stone fruit trees form copious gum (gummosis) at the site of infection but growth of the canker is restricted to a few centimeters (Chang *et al.* 1989b). In coniferous trees, copious resin soaks the branch and then the resin typically dries. This dried resin is often used as a visual clue to the occurrence and location of a diffuse branch canker (Fig. 2).

In many tree species, Cytospora cankers become perennial and canker expansion increases when tree defenses are compromised, usually by seasonal dormancy but also by drought, cold injury of wood, sun scald of bark, flooding of roots, hail, freezing or other stresses (Schoeneweiss 1981a, b). In plum, *Prunus domestica* (and related species), cultivars vary in whether the cankers are annual or perennial (DeVay *et al.* 1974, English *et al.* 1982). Infections are initiated through cracks and wounds to the bark. Wounds can be caused by mechanical injury including pruning wounds, cold injury, leaf scars and shade weakened twigs (Biggs 1989b). Many perennial cankers originate at pruning wounds and these cankers can be highly destructive if they occur at locations critical to the architectural strength of the tree (Biggs 1989b).

Blackstem of poplar cuttings and seedlings is a unique symptom of the diffuse Cytospora canker that primarily affects *Populus deltoides*. The common name for the disease is based on the necrosis that blackens the inner bark. The cambium darkens to a reddish brown colour, appears water soaked, and emits a foul odor. The disease is caused by *Cytospora chrysosperma* (Pers.) Fr. and it is an economically significant problem that may result in stock losses of up to 50 % (Walla & Stack 1980). Poplars are vegetatively propagated in nurseries by planting dormant cuttings directly into soil. In the autumn, inconspicuous lesions may occur at the ends of cuttings or at lenticels and leaf scars. The cuttings and seedlings are placed in cold storage over winter at approximately 8 ºC for up to 6 mo. Lesions enlarge during storage and, also following planting.

A highly destructive disease known as sudden death of clove trees (*Syzygium aromaticum*, syn. *Eugenia caryophyllus*), is reported to be caused by *Valsa eugeniae* Nutman & F.M. Roberts (Nutman & Roberts 1953). The pathogen may also cause death of cashew trees (Sivanesan & Holliday 1970). Sudden death is closely related with water stress and wilting can be arrested by irrigation. The acute disease occurs in Zanzibar, Tanzania, Malaysia, Indonesia, Thailand and Jamaica. Sudden death of clove occurs only on mature trees, generally 12–15 yr or older. A mild form of the disease begins with recently expanded leaves turning chlorotic, then the canopy, followed by a dramatic abscission of greenish leaves and the remaining attached leaves wilt, wither and turn a russet brown. The affected trees die in 8–20 wk. A severe form begins with a pronounced drooping and subsequent withering of the entire canopy. The trees die in 7–14 d (Dabek & Jones 1985). In both forms, tyloses plug the vessels and the sapwood is eventually stained a bright yellow with distinctive bluish black zone lines separating healthy from stained wood. The yellow staining of the wood begins in the cambium at the collar of the tree or below ground then spreads upward over 9 mo. The disease may be transmitted through natural grafting of roots.

The etiology of sudden death does not suggest a typical *Valsa* species as the primary agent unless a powerful diffusable toxin is being formed. Dabek & Jones (1985) have investigated the occurrence of phytoplasma in the declining trees. Phytoplasma may possibly have a role as part of a disease complex.

Losses from sudden death have exceeded 50 % of trees during a 10–15 yr period. The primary pathogen acts as a soilborne root disease agent. Replacement seedlings planted in the soil of an affected site show slow decline and progressive root disease. Seedlings are immune and plants up to 4 yr have resistance. Ascostromata form in masses of more than 100 perithecia along bark cracks beginning near the ground and continuing upward for several years. The ascostromata are the largest formed by species of *Valsa*. The conidiomata are formed before the ascostromata

but are generally sparse or rare. Sudden death is the most destructive disease known to be associated with a species of *Valsa*. If the symptoms are a reflection of relative virulence of the species, then the report herein of *V. eugeniae* on *Eucalyptus* species may be of significance.

Signs of the Cytospora canker include fruiting bodies that often are noted due to the spiral red, orange or cream coloured tendrils (cirrhi) of conidia extending from the bark over the canker (Fig. 2). In other instances, the erumpent discs or long beaks of the pycnidia or perithecia are evident extending from the bark over the cankers (Fig. 2). Fruiting bodies are often found in dead twigs and damaged bark on trees, and whether the fungus is the causal agent or a saprophyte is seldom clear, unless the cambium shows an advancing margin of infection.

Cytospora species are also found occasionally in lumber causing wood stain, particularly *Valsa pini* (Alb. & Schwein.) Fr. and *Valsa friesii* Sacc. in coniferous wood. *Valsa ambiens* subsp. *leucostomoides* (Peck) Spielman has been reported to produce a purple pigment in culture (Spielman 1983). It also has been reported to discolour wood of *Acer saccharum* following wounding during harvesting of sugar sap.

Host stress

It has been recognised for many years that drought stress is implicated as a predisposing factor in Cytospora canker of trees (Schmidle 1953, Wright 1957). This association has been based on field observations but in some cases, has also been supported by correlations between reduction in growth rings and initiation of canker in diseased compared to healthy trees (Jorgensen & Cafley 1961). Canker growth has been demonstrated to vary inversely with shoot moisture content and soil moisture content (Bloomberg 1962b). Additionally, canker growth becomes significantly slower and eventually stops when drought stressed poplar cuttings are subjected to abundant soil moisture (Bloomberg 1962a). Schoeneweiss (1983) provided experimental evidence to show that drought stress predisposes trees to Cytospora canker. Bier (1958) suggested that drought stress predisposes trees to facultative parasites when bark moisture falls below a critical level. Schoeneweiss (1983) described this as a threshold level of stress that must be exceeded for woody stems to become predisposed to the canker disease. In *Picea pungens*, bark cankers, caused by *Cytospora kunzei* Sacc. (= *C. halesiae* Ellis & Everh.), appear when plants reach a threshold deficit level of water potential of –20 to –30 bars. Likewise, Bertrand *et al.* (1976) reported that cankers caused by *Cytospora leucostoma* Fr. are significantly larger on *Prunus* trees growing on sites where tree water potentials are under –1.5 mega pascals (Mpa), a threshold deficit level. Guyon *et al.* (1996) reported susceptibility of *Populus tremuloides* trees to canker caused by *C. chrysosperma* occurs when water potential drops below the threshold deficit level of –1.6. Additionally, susceptibility to blackstem is associated with predisposition caused by drought stress (Schoeneweiss 1967). Defoliation stress has also been correlated with larger canker size when the threshold of 75 % is exceeded (Guyon *et al.* 1996). Host resistance mechanisms such as periderm formation (Puritch & Mullick 1975) and rate of lignification (Biggs *et al.* 1983) are reduced by drought stress. Additionally, tree wounds are susceptible to infection by *C. chrysosperma* for a longer period when trees are drought stressed compared to non-stressed trees (Butin 1955, McIntyre *et al.* 1996).

Urban trees are often subjected to restricted growth conditions that predispose them to Cytospora canker (Smiley *et al.* 1986). Soil compaction is a primary stress factor in urban environments and can also be a problem in plantation sites. Soil compaction affects tree root development because the increased bulk density reduces the percentage of large pores with consequent reduction in aeration, moisture infiltration and movement of nutrients and salts. Reduced water percolation causes waterlogging in wet seasons and physically prevents root penetration to deeper levels for water absorption during drought periods. Thus cycling drought and flooding events stress the trees. Studies of hybrid poplars at two poorly-drained plantation sites revealed that root development was reduced at both sites by a discontinuous ortstein layer of cemented B-horizon soil high in accumulated Fe, Mn and Al (Abebe & Hart 1990). At these sites the increasing number and size of Cytospora cankers has been correlated to increasing Fe and Al plant nutrient levels (Abebe & Hart 1990). Flooding stress alone has not been correlated with predisposition to cankers in poplars. In experimentally induced flooding conditions (–0.4 Mpa water potential), Guyon *et al.* (1996) found no significant difference in canker size on *Populus tremuloides* as opposed to those of control treatments.

Different types of stress can give rise to similar predisposition of plant hosts to Cytospora canker. Low temperature injury to buds and dormant twigs predisposes trees to Cytospora canker (Helton 1961a, Tekauz & Patrick 1974, Dhanvantari 1978) as does cold injury to wood. Genetic factors associated with increased cold hardiness in *Prunus persica* also are associated with increased tolerance to *Cytospora* (Chang *et al.* 1989b). Frost injury during the growing season predisposes *Pseudotsuga menziesii* to Cytospora canker caused by *Valsa kunzei* Nitschke (= *Leucostoma kunzei* (Fr.) Munk ex H. Kern) (Reich & van der Kamp 1993). Trees planted in frost prone areas of a plantation can suffer severe dieback damage ranging from occasional death of branch segments to

progressive loss of stems eventually resulting in death. Severe damage is correlated with early frosts that occur when buds expand beyond the bud scales. Trees outside of the frost pockets normally show only small latent branch cankers that cause no further damage and, therefore, dieback-prone areas can be identified by landform (Reich & van der Kamp 1993).

Temperature stress, such as sun scald on thin barked trees, predisposes trees to Cytospora canker generally when dying cambium causes disruption of water relations distal to the damaged area. Water potential stresses are thus produced above the canker site despite soil moisture levels. Additionally, bark moisture content falls to a minimum during the winter predisposing trees to Cytospora canker (Butin 1957, Gibbs 1957). Fire injury causes many tree species to succumb to Cytospora canker. Dearness & Hansbrough (1934) reported that following a light ground fire, one species of *Cytospora*, *C. pulcherrima* Dearn. & Hansbr. (= *Valsella pulcherrima* (Ellis & Everhart) Berl.), infected the heat-injured bark of 11 hardwood species within a 1000 m^2 area, while *Cytospora pinastri* Fr. : Fr. infected the one conifer. Spielman (1983) reexamined herbarium specimens from the report of Dearness & Hansbrough (1934) and concluded that most of the infections had been caused by *C. chrysosperma*.

Tree species planted outside their native range or "off-site" are often susceptible to Cytospora cankers. In the forest, Cytospora cankers occur on suppressed trees and trees exposed at the forest edge. This is particularly evident on *Prunus serotina* in the northern hardwood forests in Michigan. In conifer species, Cytospora canker is generally seen on the lowermost branches of mature trees and the canker stops expansion at the trunk, causing branch pruning. This is considered a damaging stress-related disease on *Picea pungens* associated with infection by *C. kunzei*, but is not considered important on *Pinus strobus* infected by *Cytospora abietis* Sacc., or *Pinus sylvestris* with infections by *Cytospora pini* Desm. The three diseases are uniquitous in Michigan. The difference in perspective is that *Picea pungens* has greater value as an ornamental tree. The disease is progressive with each subsequent lowermost branch dying, until after a decade trees have very few branches remaining. Soil compaction is generally assumed to be the stress factor associated with this disease.

The severity of Cytospora canker and its impact varies from species to species and clone to clone in poplars and eucalypts. Both plants have wide genetic bases for adaptability to site and harsh environments. An integrated pest management approach aimed at limiting the damage by Cytospora canker must necessarily place emphasis on site factors. Plantations should be established matching the most suitable species or clone to each particular site. To fully benefit from the fast growing habits of these trees and in order to minimise yield losses, resistant species or clones should be obtained through local screening trials including widespread clone-site trials over a broad range of sites and over the entire rotation period. In the case of poplar plantations, it has been recommended that more than two clones are planted on each site to reduce disease losses. Also, planting clonal blocks in a plantation is recommended, such that each block is no more than five hectares (Pinon 1984). This produces a geographic mosaic where risks are reduced, disease problems can be rapidly recognised and seriously damaged trees can be eliminated.

Host preference and host resistance

Some species of *Cytospora* are found on numerous species and genera of host plants while others occur only on hosts of one plant family or one genus (Farr *et al.* 1989). Collections of *Cytospora* species and strains have been tested for host preference on a range of plants in various studies (Helton 1961b, Kepley & Jacobi 2000). Even the most carefully designed studies of host preference in *Cytospora* are troubled by the dependence on morphological characteristics to identify species. For example, Kepley & Jacobi (2000) considered host preference in several *Cytospora* spp. that occur in close proximity on several hosts in the local urban and riverine forests. The question they addressed was whether a *Cytospora* sp. on one host species could serve as inoculum for Cytospora canker on the different hosts in the forest. They isolated local strains identified as *Cytospora umbrina* (Bonord.) Sacc. from *Alnus tenuifolia*, *Cytospora pruinosa* (Fr.) Sacc. from *Fraxinus pennsylvanica*, *C. chrysosperma* from *Populus deltoides*, *C. chrysosperma* from *Populus tremuloides*, "*C. sacculus*" from *Ulmus pumila*, and *Cytospora fugax* Fr. from two unidentified species of *Salix*. Thereafter, they tested the isolates for pathogenicity on a host range consisting of the tree species listed above. They also applied drought stress to the plants by withholding moisture prior to, and for 4 wk following, inoculation. *Cytospora sacculus* isolates were pathogenic on *Ulmus* and *Populus deltoides*, but not on *Populus tremuloides*. These isolates were considered as host specific on two of six hosts. *Cytospora fugax* isolates appeared specific to the different species of *Salix* from which they originated (Kepley & Jacobi 2000). Jacobi & G.C. Adams (unpubl. data) later found that the morphological species *C. fugax* used in these experiments is composed of two genetically distinct fungi. The same was true of *C. sacculus*, as inferred from differences in ITS-rDNA sequence. There is now a need to genetically identify *Cytospora* species, because the morphological species concepts likely overlap a range of biological species, even in one locale.

Genetic studies of resistance to Cytospora canker in fruit trees have calculated the heritability of resistance without knowledge of the mechanism of resistance. These estimates consider the relative contribution of genetic and environmental variability as a guide to breeders in maximising breeding efficiency in improving tree crops. Statistically, removing the yearly environmental effect will increase the selection efficiency and the rate of genetic gain. Heritability is often estimated by regressing average performance of progeny on the performance of their maternal parent (parent-offspring linear statistical model for quantitative traits). In Cytospora canker resistance, the heritability of the genetic variance is highly significant among diverse *Prunus persica* genotypes (Chang *et al.* 1991a). Thus, it is possible to select resistant individuals and to maintain them in a breeding programme without the masking effect of year to year variation. The heritability estimate and standard deviation in the studies are 0.72 and 0.11, respectively (Chang *et al.* 1991a).

Butin (1955) showed that not as much suberin is produced by drought stressed poplars compared to those with higher moisture content. He also suggested this phenomenon is correlated with resistance to Cytospora canker. The rate of suberin formation in wounds in bark tissues is correlated with resistance to Cytospora canker in other hardwoods (Biggs & Miles 1988, Biggs 1989a). Biggs & Scorza (1997) demonstrated that suberin formation in wounds on *Prunus persica* is highly correlated for sib-families (r = 0.93–0.96) and moderately correlated (r = 0.47–0.50) in individual trees. However, they caution that suberin accumulation is difficult to determine and no field characteristics are associated with it. Neither the thickness of the layer of suberised tissues nor the average number of phellem cells at 10 d following wounding are correlated with suberin accumulation (Biggs & Scorza 1997).

Bloomberg (1962a) demonstrated that lowering the moisture content in bark and wood of poplars causes an increase in susceptibility to Cytospora canker. He compared poplar parents and hybrids with different levels of resistance to canker. Two hybrid varieties that have slower rates of moisture loss during dormancy exhibit greater resistance to canker. He further reported that the more resistant hybrids have anatomical differences that relate to their resistance, including relatively wider vessels, longer phloem rays, wider sieve tube zones, thicker periderm and larger piths.

Hodges & Lorio (1969) have stated that an increase in sugars and a decrease in starch in the inner bark of *Pinus taeda* under moisture stress might be involved in predisposition to Cytospora canker. Additionally, Schoeneweiss (1975) mentioned that specific amino acids might have inhibitory or stimulatory effects on pathogenicity levels, and that free amino acids within a plant host vary in response to stress. Likewise, defoliation alters host physiology by changing levels of synthesis of phenolic compounds and amino acids (Schoeneweiss 1975, 1981b).

Virulence

Cytospora species are endophytes encountered during isolations from asymptomatic sound bark and xylem of many tree species (Bills 1996), and lesion-free leaves and xylem of *Eucalyptus* (Bettucci & Saravay 1993, Fisher *et al.* 1993, Smith *et al.* 1996). The term endophyte is used here in the sense of Chapela (1989) to refer to any fungus inhabiting the internal environment of a living plant. *Cytospora* species have been found to be the dominant fungi in the xylem of *E. globulus* in Uruguay where they are confined to the xylem (Bettucci & Saravay 1993). In a study of endophytic fungi in *E. nitens* and *E. grandis* from two distinct geographic and climatic areas of South Africa (Smith *et al.* 1996), *Cytospora* was found to be the dominant species in the xylem, however, in both tree species *Cytospora* also occurred in the leaves. In the colder and wetter region, *Cytospora* was six times more common in xylem of *E. nitens* than in leaves, whereas, in *E. grandis* the fungus was six times more common in the leaves than in the xylem.

One of the earliest demonstrations of the endophytic nature of *Cytospora* was provided by Christensen (1940). He demonstrated that saplings of *Populus*, *Salix* and *Sorbus aucuparia* when carefully surface sterilised become parasitised by *Cytospora* following excision, coating in hot wax, and incubation in a cold room for a few weeks. The parasitic nature of *Cytospora* appears to fit the definition of Petrini (1991) of latent pathogens existing as symptomless endophytic infections. Chapela (1989) referred to *Cytospora* species as xylotropic endophytes. He argued that previous studies of endophytes in trees have not excluded the possibility that the isolated fungi are derived from spores on the bark whereas the methods used in his study exclude that possibility (Chapela & Boddy 1988). Xylotropic endophytes differ from general endophytes by growing into secondary xylem upon drying of the wood. The initial colonisation occurs on the healthy tree well before any symptoms appear. The xylotropic endophytes are inconspicuous fungi in healthy tree organs but rapidly colonise large volumes of wood when water content of branches and stems decreases below a certain threshold (Boddy & Rayner 1983). Such drying is a common feature of pruning, abscission and senescence of any plant tissue (Chapela & Boddy 1988). Xylotropic endophytes show a degree of host preference with the existence of family-family associations between fungus and plant (Chapela 1989). Recently, however, McIntyre *et al.*

(1996) have argued that in Colorado *C. chrysosperma* is epiphytic on the bark of *Populus tremuloides* and not endophytic. Because Chapela's studies (1989) also included *C. chrysosperma* in *Populus tremuloides*, a re-examination of this issue is needed.

Cytospora species are often considered facultative wound parasites that attack weakened trees, but some may be strictly saprophytic on dying trees (Christensen 1940). Pathogenicity may be a species-specific character that is particularly difficult to determine. For example, *Valsa sordida* Nitschke (anamorph *C. chrysosperma*) is highly virulent in inoculations of *Populus*, whereas, *C. nivea* Hoffm. : Fr. is not virulent in the same tests. Both of these species are ubiquitous endophytes in *Populus tremuloides*. Fruiting bodies of *Valsa* or *Cytospora* are often found on branches that have fallen to the ground. In our experience, these fungi are sometimes species capable of showing pathogenicity in inoculations on the host, but other times they are not. Where they are non-pathogenic, they are assumed to have seized an opportunity to colonise senescing tissues as saprophytes or xylotropic endophytes of low virulence.

A routine test for pathogenicity is to excise branches from the dormant host, seal the cut ends with hot wax, and to inoculate these stem pieces. Strains that are pathogenic produce cankers that expand extensively as the branches slowly dry over several months while those that are saprophytic on the host species do not expand. Water potential can be set in small diameter excised branches to a predetermined level approximating the condition of drought-stress with the use of a pressure bomb. This method provides for a more rigorous test of relative pathogenicity.

Comparing the virulence of *Cytospora* isolates *in situ* on tree species must be approached cautiously due to variability of response of individual trees to environmental stresses. A first step in reducing experimental errors is to select hosts of one genotype, full-sibfamily or half-sib family. An additional step is needed to select branches that are uniform in age and diameter. A pre-set and locked caliper is useful for selecting branches, and a branch of the previous year's growth is optimal for inoculation.

Virulence experiments need to be designed statistically to decrease experimental errors associated with sampling and replication. Sampling errors usually involve variations in canker size measurements on inoculated branches of a given tree per strain (within tree variation), while replication errors involve variation in canker size on different inoculated trees (between tree variation). Increasing replications influences experimental errors variably, and replications can be branches inoculated per strain per tree, or trees inoculated per strain. In our studies with genetically identical scions, inoculating more than one branch per tree (per strain) has a minimal effect on reducing experimental error, but inoculating more than six trees significantly reduces the statistical variance (Adams *et al.* 1989). Inoculating 6–9 trees, one branch per tree gives high levels of precision in the detection of differences in strain virulence while this also requires the minimum number of trees and reduces labor. In our studies, this experimental design can detect a minimum difference in canker size (virulence) at $P = 0.05$ between two isolates with 90 % assurance that the difference in virulence is genuine (Adams *et al.* 1989). The variation in response (susceptibility) among individual genetically identical trees is significant and this is apparently due to differences in root structure, soil type, root infection (English *et al.* 1982), or other unexpected environmental factors.

Comparisons of relative virulence among *Cytospora* isolates inoculated onto several host species and replicated over several years can result in complex, statistically significant interactions. These interactions often prevent investigators from arriving at statistically supported comparisons. A new mathematic analysis known as Ammi analysis, has been developed for handling genotype by environment interactions in such complex experimental designs so that results can be interpreted in a meaningful way (Gauch 1992, 1993, Gauch & Zobel 1996). Examples where this method has been used in forest pathology studies are rare (Adams 2002, Adams *et al.* 2002b).

Nitrogen fertilisation affects Cytospora canker development. Burks *et al.* (1998) showed that nitrogen deficiency contributes to significantly larger cankers caused by *C. chrysosperma* on *Populus tremuloides* under controlled conditions in hydroponic culture. Abnormally high nitrogen rates (333 ppm) also caused larger cankers but differences were not statistically significant. They concluded that proper management of nitrogen fertilisation favours the plant's capability to mobilise effective defense responses. In fungi, the quantity and form of nitrogen nutrition affects pathogenicity (van Ardel 1966, Huber & Watson 1974). Specific amino acids can have inhibitory or stimulatory effects on pathogenicity (Schoeneweiss 1975). Additionally, different nitrogen catabolic abilities particularly nitrate utilisation are noted in strains of a fungal species that differ in host preference (Pearson *et al.* 1987). Thus, the size of cankers caused by *C. leucostoma* Fr. and *Cytospora cincta* Sacc. was reduced following pregrowth on peptone, and increased following pregrowth on serine, at 50 mM nitrogen equivalents (Jensen & Adams 1995).

Phytotoxins and oxalic acid production by *Cytospora* species may have roles in virulence (Défago 1942, Kern 1957, Hubbes 1960a, Rozsnyay & Barna 1974, Traquair 1987, Svircev *et al.* 1991), but further studies are needed and more species should be studied.

Traquair (1987) demonstrated that *C. cincta* and *C. leucostoma* produce oxalic acid in the form of calcium oxalate crystals in inoculated *Prunus persica* bark and in agar culture. Calcium oxalate alone causes necrosis of the plant tissues. It was hypothesized to have a role in the early stages of pathogenesis by acting synergistically with polygalacturonases. It lowers the pH of the infected tissues to favour enzyme activity and chelate calcium ions in the middle lamellae of cell walls causing maceration. Hubbes (1960a) suggested that mycelial weight and toxin production by several *Cytospora* species grown in bark extract broth in still culture is correlated with toxicity symptoms on tomato sprouts but not with virulence of the *Cytospora* strains on *Salix* hosts. Rozsnyay & Barna (1974) demonstrated that culture filtrates of *C. cincta* induce wilting in tomato and other plants. They tentatively identified the unknown phytotoxin as a heat stable molecule, with a protein component of *ca* 5000 daltons, that had no lipid component. Svircev *et al.* (1991) re-examined phytotoxins in *C. cincta* and *C. leucostoma*. After removing the influence of calcium oxalate, they were unable to repeat the results of Rozsnyay & Barna (1974). However, they isolated and partially purified a heat labile cold acetone precipitate from the peptide fraction that is less than 1000 d. This fraction has phytotoxic properties in *Prunus persica*. Analytical polyacrylamide and preparative granulated bed isoelectric focusing in the pH range 3.5–6 yielded two sub-fractions that contained small peptides that cause stem necrosis in *Prunus persica* (Svircev *et al.* 1991). These phytotoxins could now be used in screening for disease resistance in breeding. Phytotoxic compounds may influence host preference and virulence in *Cytospora*. So, it may be worthwhile to determine the presence and nature of phytotoxic compounds produced by many species of *Cytospora*. Additionally, determining whether lactone secondary metabolites (see below) have a role in pathogenesis could be useful and interesting.

Inoculation of host material

Most studies report that conidia, ascospores and hyphae are equally infective when used in inoculations. The efficacy of the conidia as infective propagules has been questioned occasionally because they are unusually small and narrow and resemble spermatia such as those produced in *Diatrype* Fr. A few reports claim that ascospores but not conidia are infective in inoculations. Kamiri & Laemmlen (1981a) inoculated drought-stressed and wounded *Picea pungens* with conidia or ascospores of *V. kunzei* in greenhouse trials and only ascospores induced canker formation. Waterman (1955) also reported that conidia of *V. kunzei* are not infective. Controversy remains because in other studies conidia of *V. kunzei* are infective (Wehmeyer 1925).

Epidemiological studies on ascospore and conidia dispersal in *V. kunzei*, indicate that ascospores are the major source of primary inoculum and that most infection of *Picea* spp. occurs in spring during peak ascospore release (Kamiri & Laemmlen 1981b). Conidia exude from conidiomata in gelatinous tendrils or cirrhi during temperatures above freezing when relative humidity is 100 % for *ca* 48–72 h or when free water is present for *ca* 6–24 h (Barakat *et al.* 1995). A large proportion of the conidia in dried cirrhi remain viable throughout the summer, in the field. Conidia and ascospores can germinate and infect plants at temperatures ranging from *ca* 5–28 ºC, if conditions are sufficiently wet (Barakat *et al.* 1995). Conidia and ascospores require a saturated atmosphere and a carbon source for germination (Kamiri & Laemmlen 1981b).

In areas having climates with long, cold winters, it is best to inoculate established trees in autumn (September–October) for maximum canker expansion as measured in spring. Canker expansion may be of considerable length, greater than 5 cm and up to half a meter, by spring (May) on *Prunus persica*. Conidiomata often are abundantly produced. Excision of branches inoculated with various strains in spring or early summer and storing branches on the ground for 2 yr has given rise to ascostromatal formation in some instances (Wang *et al.* 1998). In warmer regions such as Washington State, Barakat & Johnson (1997) report that canker enlargement is greatest for inoculations in mid summer (July). In regions where host plants are not subject to a significant cold dormancy period such as South Africa, inoculation tests have not been satisfactory for critical evaluations of comparative pathogenicity or virulence. This has also been the situation with inoculations in glasshouse environments. Exposing glasshouse trees to prolonged drought stress, repeated girdling, or transfer to several days of near freezing dark incubation, have not been sufficient treatments to encourage significant canker expansion with *Eucalyptus*, *Rhus* and *Pinus* (G.C. Adams, unpubl. data). Occasionally, limited formation of conidiomata on bark damaged by the inoculation method may occur on cankers that are only 1–2 cm in length.

Inoculation of dormant cuttings of *Populus trichocarpa*, with the cut ends sealed with wax, is a method that we have found useful for assessment of pathogenicity. Comparisons of relative virulence among species that occur on *Populus* are readily accomplished following approx. 4 mo of incubation at room temperature. Conidiomata with natural morphology are often also produced on the inoculated stems. Wrapping the cuttings in household plastic film

distorts the morphology of the ostioles and discs of the fungi. Additionally, such wrapping favours surface colonisation by non-pathogenic strains. Dormancy with cold hardiness appears to be essential when using this method.

We have adapted the method of Scorza & Pusey (1984) for routine inoculations of all host substrates. Branches of equal diameter are selected using a caliper and a spot of bark is wiped with 70 % ethanol and frozen for about 15 s with a chemical aerosol that forms visible frost on the inoculation site. An empty staple gun is pressed firmly against the frosted bark and the trigger activated. This bruises the inner bark to a depth of approximately 2 mm and a length of 1 cm and often cracks the bark. Inoculant in the form of spores, or a 1 cm diam plug of agar culture, is placed on the bruised spot and the treated area is wrapped with Parafilm®. Control inoculations may show some persistent damage of approx. 1 cm in length but generally heal. Another useful method for inoculation of hosts with *Cytospora* species is to cut the bark so that a rectangular flap is formed that remains attached at one side. The wound exposes inner bark, cambial tissues and xylem. An agar disc from an actively growing culture is placed beneath the bark flap and the flap is pressed into place and wrapped with tape (Wysong & Dickens 1962, Spielman 1983).

An excised shoot assay has been developed that is sufficiently related to field-inoculated reactions as to be reliable in screening for tolerance to Cytospora canker in the breeding of peach cultivars (Chang *et al.* 1989a). The assay is a laboratory procedure where the pathogen is grown on acidified oatmeal agar with 200 ppm Streptomycin for 14 d in a sterile plastic tissue culture container (Nalgene® boxes of 6 × 6.5 × 9.5 cm). Dormant shoots of current year's growth are surface disinfested with 0.5 % sodium hypochlorite, rinsed, and placed upright in the containers with *ca* 1 cm of shoot pushed into the agar. The containers are incubated at room temperature under fluorescent lighting for *ca* 1 mo. The shoots must be dormant for assaying relative tolerance of the plant germplasm or relative virulence of fungal strains. The excised shoot assay can significantly accelerate the breeding process (1 mo versus 7 mo for field tests in Michigan) and it utilises significantly less shoot material than other techniques. Shoot material can be a limiting factor in testing young seedlings for canker tolerance.

Associated pathogens and microorganisms

In certain disease situations, *Cytospora* species are associated with other pathogens. For example, in Michigan, when *Sorbus aucuparia* is severely infected by the fire blight bacterium, *Erwinia amylovora*, the trees blister with the ascocarps of *Biscogniauxia marginata* (Fr.) Pouzar and *V. massariana* De Not. Fire blight is believed to cause the mortality. In *Populus tremuloides*, *C. nivea* is associated with *Hypoxylon mammatum* (Wahlenb.) P. Karst. and often also with *Peniophora rufa* (Pers.) Boidin. *Hypoxylon mammatum* is considered to be the causal agent of the subsequent mortality. Cytospora canker is often present on parts of *Eucalyptus* trees above parts that have cambium killed by *Botryosphaeria dothidea* (Moug. : Fr.) Ces. & De Not. *Cytospora* species often cause aggressively expanding cankers on the upper trunks and branches of various tree species, particularly *Acer* spp., that have disrupted water relations caused by a crown rot or localised root rot. A target canker, caused by a *Neonectria galligena* (Bres.) Rossman & Samuels or other pathogens, can also predispose the upper stem to Cytospora canker due to disrupted water relations. *Cytospora abietis* cankers are common on *Abies magnifica* and *Abies concolour* in areas of infection by Dwarf Mistletoe (*Arceuthobium abietina*) (Scharpf 1969).

In our experience, the mycoparasite *Phoma glomerata* (Corda) Wollenw. & Hochapfel is occasionally isolated along with *Cytospora* species in South Africa and can reside within mycelium of the *Cytospora* as a mycoparasite. *Phoma glomerata* is also an endophyte in leaves and xylem of *Eucalyptus* (Smith *et al.* 1996). Cultures of *C. cincta* from *Malus* hosts, which are distinctly different than other *C. cincta* from *Prunus* hosts (Proffer & Jones 1989, Surve-Iyer *et al.* 1995), invariably become overgrown by a species of *Penicillium* which very slowly and imperceptibly cover the colony surface. *Cytospora cincta* from *Prunus* in the same lab does not show such contamination. The origin of this contaminant is apparently within *C. cincta*, as all efforts to eliminate it have failed repeatedly.

Biochemistry/biotechnology

Unidentified species of *Cytospora* produce unique and valuable antibiotic agents and chemotheropeutics. Three *Cytospora* species are subjects of U.S. patents involving drug discovery, and two of these discoveries arose through efforts to screen endophytes for useful products. These discoveries have increased interest in endophytes for their previously unrecognised value as potential producers of unique secondary metabolites (Bills 1996, Bills *et al.* 2002).

Recent natural products discovered from a tropical and endophytic *Cytospora* sp. are the cytosporones, lactones with potent antibacterial and antifungal antibiotic properties (Brady *et al.* 2000). Cytosporones D and E are trihydroxybenzene lactones and octaketides. They are produced by a *Cytospora* sp. isolated from a plant (*Conocarpus erecta*) growing in the Guanacaste Conservation Area of Costa Rico. The trihydroxybenzene is stated as

the likely source of antibiotic activity. The minimum inhibitory concentration for Cytosporone D against strains of *Candida albicans* (C.P. Robin) Berkhout, *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli* is 4, 8, 8, and 64 ppm. This compares to Gentamicin with > 128, 2, 16, and 2 ppm, respectively.

A *Cytospora* sp. produces three dilactones, grahamimycins (Grahamimycin A, A1, and B), with broad-spectrum antibiotic properties (Gurusiddaiah *et al.* 1980a, b). At the time of this discovery the lactones represented a new class of antibiotic structures. The *Cytospora* sp. (ATCC 20502), isolated at the Western Forest Products Laboratory in British Columbia, occurs as a saprophyte in the heartwood of a *Pinus contorta* (lodgepole pine). The strain and its unique lactones have been patented (U.S. Patents 4.220.718 and 4.239.690). Grahamimycins exhibit antibiotic activity against 36 species of gram positive and gram negative bacteria, eight species of cyanobacteria, two green algae and five fungi included in the screening process (Gurusiddaiah & Ronald 1981). A high level of antibiotic production is achieved both in the cell-free broth as well as in the cells. Production is highest with lactose as a carbon source. The patent mentions the potential of using the compounds as topical ointments in healing of skin wounds including those caused by *Staphylococcus aureus*.

Bills (1994) isolated an endophytic *Cytospora* sp. from the living bark of *Betula alleghaniensis* in West Virginia (Stevens-Miles *et al.* 1996). He later filed U.S. Patent 5.278.068 on the fungal strain (ATCC 74091, also listed as 74901) and the discovery of Cytosporin A, B & C (Bills 1994). Cytosporins are angiotensin II antagonists, binding inhibitors. Angiotensin II is a powerful vasoconstrictor of arteries that reacts with receptors on the surfaces of major organs such as the brain and adrenal glands. Angiotensin II is the active hormone of the renin-angiotensin system (RAS), which regulates blood pressure and is, therefore, important in hypertension, congestive heart failure, and cirrhosis in mammals.

The *Cytospora* isolates that have been listed in patents are not identifiable to species because the current identification system places emphasis on crucial morphological features that form on bark of living trees in nature. No workable system exists for identification in culture or on inoculated host tissues *in vitro*. Clarifying the morphological species concepts in *Cytospora*, constructing a molecular phylogeny, then describing the species in culture, should aid in drug discovery efforts.

Cytospora rhizophorae Kohlm. & E. Kohlm. (ATCC 38475) is the only identified species that is reported to form a patented natural product. During a continuing search for promising lead compounds for development as novel antibiotic and anticancer drugs, *C. rhizophorae* was found to produce a compound, Cytosporacin, with modest activity against Gram positive bacteria. Cytosporacin is a novel polyketide possessing an unprecedented carbon skeleton of two condensed heteronuclear aromatic systems, naphthopyranone and isochromandione moieties, linked by a C–C bond (He *et al.* 2003). *Cytospora rhizophorae* is a halotolerant species that occurs on roots of two species of mangrove, *Rhizophora mangle* and *R. racemosae*, and it has been isolated from Florida, Hawaii, Bahamas, Mexico, Guatemala, New Guinea, Australia and Liberia (Kohlmeyer & Kohlmeyer 1971, Shaw 1989).

HISTORICAL OVERVIEW

A thorough review of the history of classification and nomenclature of the genus *Valsa* and anamorph genus *Cytospora*, and their segregates was presented by Spielman (1983). This was briefly summarised and updated two years later (Spielman 1985). Little has changed since these two documents were published.

Ehrenberg (1818) described the genus *Cytospora* but when Fries (1823) adopted the genus he misspelled it as *Cytispora*, an orthographic variant (Spielman 1980). Thus, much of the early mycological literature treats the species under *Cytispora*. Saccardo, in *Syll. Fung.* 3: 252, 1884, uses the original spelling, and this tradition has continued in most published works. Today, the genus *Cytospora* contains the anamorphs of the genera *Valsa*, *Leucostoma*, *Valsella* Fuckel and *Valseutypella* Höhn. *Valsa* was erected as a genus in 1849 by Fries (1849). Tulasne & Tulasne (1863) were the first authors to postulate that *Valsa* and *Cytospora* represent two forms of the same organism. However, the name *Valsa* persisted for some time as a genus that contains species of ascomycetous fungi with many different centrum types. Saccardo (1882–1931) narrowed the circumscription of the genus by restricting it to species with hyaline allantoid ascospores. This concept of *Valsa* was retained in the works of Winter (1881–1887) and Ellis & Everhart (1892) in which species were included that have subsequently been classified in distantly related genera such as *Eutypa* Tul. & C. Tul. and *Eutypella* (Nitsche) Sacc.

Modern concepts of *Valsa*, *Cytospora* and allied fungi begin with the many works, spanning 1906–1928, of von Höhnel (1906, 1910, 1914a, b, 1917, 1918a, b, 1919, 1923, 1927, 1928a, b). Von Höhnel (1919) was the first to begin separating species based on centrum characteristics. He removed many species that belong to different genera and relegated many names to synonymy based on studies of type

specimens. Nannfeldt (1932) further removed species that belonged to different genera.

Order and family relations

Von Höhnel (1917) proposed the family *Diaporthaceae* Höhn. : Wehm. He separated the genera in the *Diaporthaceae* into two subfamilies, Eu-Diaportheen and Valseen. *Valsa*, *Leucostoma*, and *Valsella* were placed in the Valseen sub-family. Luttrell (1951) circumscribed the family *Diaporthaceae*, describing the *Diaporthe* centrum type based on the *Diaporthe* perithecium and the *Endothia* ascus type. *Valsa* shared these family characteristics. Nannfeldt (1932) elevated the family *Diaporthaceae* to the order *Diaporthales* Nannf.

Several authors have segregated families in the *Diaporthales* based on various morphological criteria and a consensus has not been reached. Early segregation included two families, the *Diaporthaceae* for the Eu-Diaportheen and *Valsaceae* for the Valseen (von Höhnel 1917, von Arx & Müller 1954, Gäumann 1964). *Valsa* and its segregate genera were placed in the *Valsaceae*, a family characterised as having allantoid ascospores. The family *Diaporthaceae* was characterised as having non-allantoid ascospores. We consider the latter families artificial because little difference exists in ITS-rDNA sequence between the type genera *Diaporthe* Nitschke and *Valsa*. Eriksson & Hawksworth (1993) proposed that *Diaporthales* be segregated into the families *Valsaceae* (including the *Diaporthaceae*) and *Melanconidaceae*. This system places *Diaporthe* and *Valsa* in the same family and, therefore, it may represent a more natural classification. Current classifications retain these families without segregating the genera (Eriksson *et al.* 2001, Kirk *et al.* 2001). Vasilyeva (1994) referred to the *Diaporthales* as the *Valsales* and placed 7 tribes and 33 genera in the family *Valsaceae*. Undoubtedly, different judgements regarding the importance of correlated morphological characters and different taxonomic hypotheses will be presented in the future. Major revisions of order and family structure in the *Diaporthales* based on phylogenetic analyses are certain to occur in the future (Zhang & Blackwell 2001).

Diaporthales: ***Valsaceae*** Tul. & C. Tul., Sel. Carp. Fung. 1: 180. 1861.

Members of the *Valsaceae* have centrum characteristics including unitunicate inoperculate asci that loosen from the subhymenia and float freely in the perithecia. *Asci* are ellipsoidal to clavate, mostly octosporous, with an apical apparatus that is shallow, ring shaped, refractive, non-amyloid, and chitinoid (staining blue black with nigrosin). *Paraphyses* may be lacking at maturity or present, and the ostioles are periphysate. *Ascospores* are hyaline, yellowish to brown, with various shapes and septation. Secondary characteristics include presence or absence of stromatic tissues and characteristics of the stromata. *Stromata* are immersed to erumpent in tissues of vascular plants, usually pseudostromata (including host cells), prosenchymatous or pseudoparenchymatous, and containing several perithecia in various configurations. Often the stromata form a disc (clypeus) that surrounds the apex of the ostiolar neck(s). Stromata may form blackened marginal zones abruptly circumscribing the stromata in the host tissues, the conceptacle. *Perithecia* usually have necks and have walls that are thin, bilayered, brown externally, of compressed cells, and *textura epidermoidea* to *textura angularis*. Necks are usually laterally inclined to eccentric but may be central.

Anamorphs usually are *conidiomata* varying from acervular to pycnidial or stromatic. *Conidiogenous cells* are generally phialidic and conidia vary in shape, septation and pigmentation.

Classification of holomorphs

Genera, subgenera and sections: Adanson (1763) proposed a genus *Valsa*, but Fries (1849) placed the species in *Valsa* Adanson into the modern genus *Diatrype*, and other fungi were placed in *Valsa*. For many years the International Code of Botanical Nomenclature (ICBN) accepted Fries (1849) as the starting date for ascomycetous fungi and Fries' concept of *Valsa* is used throughout the literature. However, in 1981 the ICBN moved the starting date for fungi back to Linnaeus (1753). This change invalidated the genus *Diatrype* and changed the genus *Valsa* to having an unfamiliar concept, and resulted in confusion particularly regarding the literature concerning plant pathology. Therefore, a successful effort was undertaken to propose conservation of the modern generic concepts of *Valsa* Fr. and *Diatrype* Fr. *Valsa* Adanson was rejected as *nomen rejiciendum propositum*, while *Valsa* Fr. was conserved, *nomen conservandum propositum*. The 1849 book by Fries retains the status of a sanctioned work in the Saint Louis Code (Greuter *et al.* 2000).

The modern teleomorphic genera *Valsa*, *Leucostoma*, *Valsella* and *Valseutypella* (Fig. 3) form anamorphs in the genus *Cytospora*. These genera are in current usage in literature, however, critical studies have questioned whether they are suitably distinct from the genus *Valsa* to warrant separation at the genus level (Gilman *et al.* 1957, Munk 1957, Gvritishvili 1982, Vasilyeva 1988, 1994). *Leucostoma* has been distinguished from *Valsa* by the presence of a dark conceptacle; a dish-shaped barrier of darkly pigmented fungal tissue at the periphery of the ascostroma tissues demarcating with a black line the ascostroma from the host tissues. This barrier is usually visually evident

in the field because the majority of the ascostromatal tissues are pale grey to white. Nitschke (1867–1870) originally proposed *Leucostoma* as a subgenus of *Valsa*; von Höhnel (1917) raised it to generic rank. Vasilyeva (1988, 1994) argued that in the *Diatrypales*, the presence or absence of the conceptacle is considered significant only at the species level and, therefore, that the character should be given the same significance in the *Valsaceae*. Vasilyeva (1988) argued for the return of *Leucostoma* to subgeneric rank in order to reflect the weight assigned to the presence or absence of the conceptacle in other genera, such as in genera of the *Diatrypaceae*.

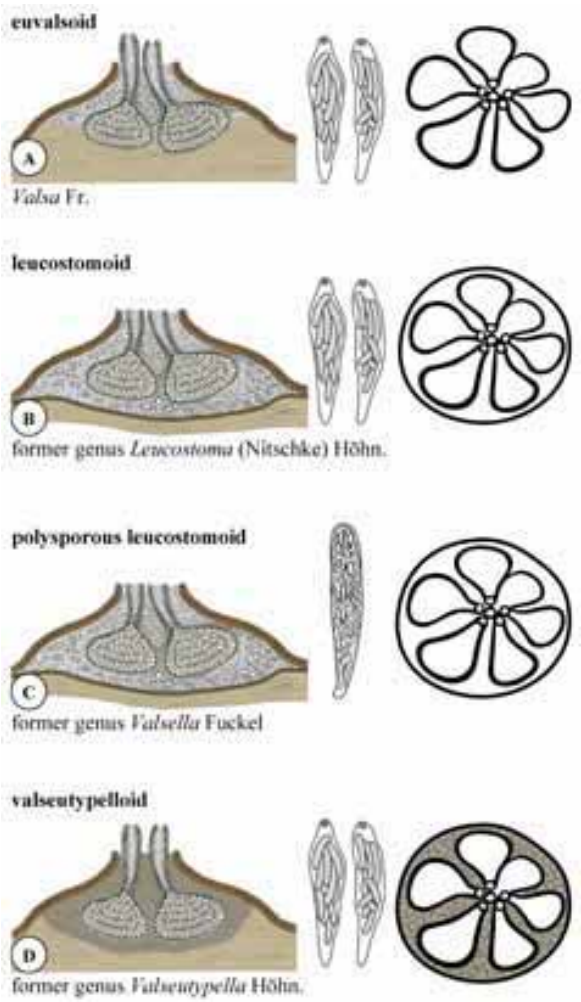

Fig. 3. Ascostromatal forms of *Valsa* and new descriptive terms (bold) that are substituted for subgeneric features, as illustrated with longitudinal and tangential cross-sections. The forms are the basis of the former subgeneric divisions in *Valsa*, and genera now transferred into *Valsa*. A. *Valsa* Fr. and descriptive term "euvalsoid", refers to ascocarps with or without stroma, not delimited by conceptacle. B. Former genus *Leucostoma* and descriptive term "leucostomoid", refers to ascostromata delimited by dark conceptacles. C. Former genus *Valsella* and descriptive term "polysporous leucostomoid", refers to ascostromata with polysporous asci and delimited by dark conceptacles. D. Former genus *Valseutypella* and descriptive term "valseutypelloid", refers to ascostromata with parenchymatous stroma surrounding perithecia.

The *Diatrypales* is an order having great parallelism with the *Diaporthales* in ecology, substrate, and stroma structure. Apparently, Gvritishvili (1982) also viewed the conceptacle as a character of significance at the subgeneric level because *Leucocytospora*, the anamorph of *Leucostoma*, was treated as a subgenus of *Cytospora* in his monograph. *Leucostoma auerswaldii* (Nitschke) Höhn. and *Leucostoma curreyi* (Nitschke) Défago often lack the conceptacle in certain collections or the black basal zones are only partially formed. Also, *Valsa* species are sometimes found with partial or complete black zones (Spielman 1983). Vasilyeva (1994) removed *L. curreyi*, but retained *L. auerswaldii*, in the portion of her dichotomous key that states that the black zone is present. The black line is often absent in specimens of the "*Leucocytospora*" anamorphs of *Leucostoma* species where the teleomorphs show the character on the same branch. The presence of the conceptacle is variable in many anamorphs of *Leucostoma* but is consistently present in others, such as in *Leucocytospora nivea* (Hoffm. : Fr.) Höhn.

Valsella was recognised as a *Leucostoma* that forms more than eight ascospores in an ascus (Petrak 1919, Barr 1978). Petrak (1919, 1969) and Müller and von Arx (1973) did not believe that the polysporous nature of *Valsella* was sufficient to separate species of *Valsella* from *Leucostoma*. Petrak (1969) argued that *Valsella polyspora* Nitschke and *Valsella adhaerens* Fuckel are polysporous forms of *Valsa auerswaldii* (subgenus *Leucostoma*), and that *Valsella salicis* Fuckel, *Valsella fertilis* (Nitschke) Sacc., and *Valsella nigro-annulata* Fuckel are polysporous forms of *Valsa translucens* (De Not.) Ces. & De Not. (subgenus *Leucostoma*). Surprisingly, Vasilyeva (1994) retained *Valsella* at the generic rank.

Von Höhnel (1918a) introduced the genus *Valseutypella* for the species *Valseutypella tristicha* (De Not.) Höhn. Three species were described including *Valseutypella multicollis* Checa, G. Moreno & M.E. Barr and *Valseutypella khandalensis* Vaidya (Vaidya 1981, Checa *et al.* 1986). *Cytospora* anamorphs were described for two of the species (Hubbes 1960b, Checa & Martinez 1989). Barr (1990) retained *Valseutypella*, *Valsella*, and *Leucostoma* as separate genera within the *Valsaceae* while reforming her earlier concepts (Barr 1978) of tribes. However, Vasilyeva (1994) placed *Valseutypella tristicha* into the genus *Valsa* as *Valsa tristicha* (De Not.) Lar. N. Vassilyeva. The ITS-rDNA sequence of *V. multicollis* ATCC 66780 is that of a typical member of *Valsa* (G.C. Adams, unpubl. data) and the LSU sequence of *V. tristicha* CBS 465.59 is

apparently also within the *Valsa* clade (A.Y. Rossman, pers. comm.).

Various sectional and subgeneric divisions are in use for the holomorphs, including sections of the genus *Valsa*: sections *Cypri* Z. Urb., *Monostichae* (Nitschke) Z. Urb., and *Valsa* (Spielman 1985), and subgen. *Leucostoma* Nitschke (Gilman *et al.* 1957, Munk 1957, Gvritishvili 1982). The sections of *Valsa* are based on morphological features of ascostromata (Fig. 4). *Valsa* sect. *Valsa* has large asci (30–80 × 6–12 µm) and ascospores (8–30 × 1.5–8 µm), few (3–15) large perithecia (300–650 µm diam) arranged circinately and inclined (laying on their sides rather than upright), and with little entostromatic tissue. The ostiolar necks converge laterally to a well-developed ectostromatic disc. Section *Valsa* was originally named sect. *Circinatae* (Nitschke) Z. Urb. Section *Monostichae* has small asci (30–45 × 5–6 µm) and ascospores (3–12 × 0.75–2.5 µm), many (10–40) smaller perithecia (150–300 µm diam) clustered and nearly upright, and well-developed (massive) entostromatic tissue. Ostioles fill the poorly-developed ectostromatic disc. Section *Cypri* has large asci and ascospores, few large perithecia clustered, and well-developed entostromatic tissue. The ostioles unite and fuse into a mass and no disc is present. Subgenus *Leucostoma* has large asci and ascospores, few large perithecia arranged circinately and inclined, and well-developed entostromatic tissue delimited by a dark dish-shaped conceptacle. The ostioles converge laterally to a well-developed ectostromatic disc. Subgenus *Valsella* is similar to subgen. *Leucostoma* but differs in the asci being polysporous. Subgenus *Valseutypella* has perithecia entirely surrounded by a stroma of pseudoparenchymatous (or sclerotial) cells.

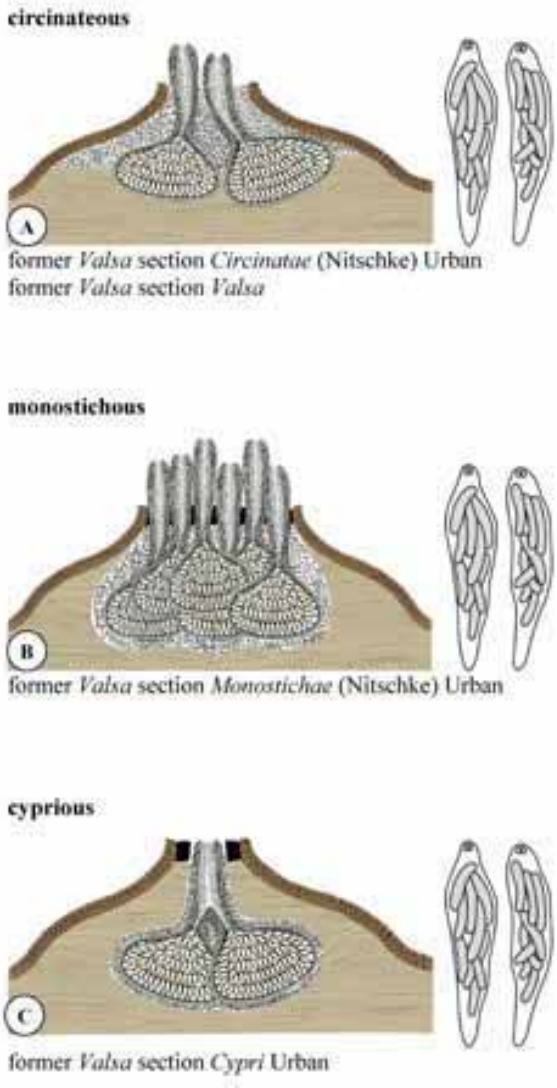

Fig. 4. Ascostromatal forms of *Valsa* that are the basis of the former infrageneric sections, and new descriptive terms (bold) that are substituted for section features, as illustrated with longitudinal cross-sections. A. Former sect. *Valsa* and descriptive term "circinateous", refers to ascostroma with few large inclined circinate perithecia and large ascospores. B. Former sect. *Monostichae* and descriptive term "monostichous", refers to ascostroma with many small, crowded, upright to inclined, perithecia and small ascospores. C. Former sect. *Cypri* and descriptive term "cyprious", refers to ascostroma with fused ostiolar necks (monospecific for *V. cypri*).

Saccardo (1882–1931) did not use the sections for *Valsa*. Rather, he emphasized ascospore size and separated *Valsa* species in to two common groups. These were the *Macrosporae* with ascospores greater than 12 µm long, and the *Microsporae* with ascospores less than 8 µm long. Ellis & Everhart (1892) employed this system but added an intermediate group, *Mesosporae* with ascospores 8–12 µm long.

Valsa Fr., Syst. Orb. Veg.: 107. 1825; emend. Saccardo, Atti Accad. Sci. Veneto-Trentino-Istriana Padua, 4: 4. 1875; Syll. Fung. 1: 108. 1882; *nomen conservandum propositum*, non *Valsa* Adanson 1763, *nomen rejiciendum propositum*.

= *Leucostoma* (Nitschke) Höhn., Ber. Deutsch. Bot. Ges. 35: 637. 1917; Ann. Mycol. 16: 134. 1918.

= *Valsella* Fuckel, Symb. Mycol. in Jahrb. Nas sauischen: 203. 1869.

= *Valseutypella* Höhn., Ann. Mycol. 16: 224. 1918.

Stromatic in vascular plants, immersed, slightly erumpent to strongly erumpent. Stromatic tissues prosenchymatous or pseudoparenchymatous, forming a disc, an immersed stroma, or an erumpent stroma, at times delimited by blackened marginal lines. *Ectostroma* of dense thick-walled cells with or without amorphous mealy tissue at the disc surface. *Entostroma* of pseudostromatic, thin-walled hyphal cells, loosely packed and interwoven. *Ascomata* perithecial, perithecia inclined to upright, in valsoid or diatrypelloid configurations, immersed, usually

embedded in entostroma, with beaks converging at disc or surface. *Ostioles* many per disc, periphysate; walls of perithecia bilayered, narrow (15–30 µm), outer layer of *textura epidermoidea* to *textura angularis*. *Paraphyses* may be lacking at maturity but usually present, often collapsed and broad. *Asci* free, ellipsoid to clavate, apical ring refractive, narrow, non-amyloid, chitinoid. *Ascospores* hyaline, allantoid, one-celled, thin-walled, smooth, biseriate, crowded, 4, 8, or polysporous per ascus.

Lectotype species: *Valsa ambiens* (Pers.) Fr. (*fide* von Höhnel 1917), (PR) 163781, selected by Urban 1957. *Neotype*: *Valsa ambiens* subsp. *ambiens* (Pers.) Fr., (PR) 163781, selected by Urban (1957) and Spielman (1985); teleomorph of *Cytospora leucosperma* Fr. (UPS) Sweden, Fries, Scler. Suec. 156, as *Cytispora leucosperma*, selected by Spielman (1985).

Anamorph: *Cytospora* Ehrenb.

Classification of anamorphs

Grove (1935) and Gutner (1935) published useful compilations of descriptions of the known species of *Cytospora*. The most recent monographic works include those of Kobayashi (1970) for species in Japan; Gvritishvili (1982) for species in the former U.S.S.R., and Spielman (1983, 1985) for North American species on hardwoods. These works erect new species and synonymise many others based on morphology.

Form-genera, subgenera and sections: Von Höhnel (1914a, 1917, 1918a, 1923) recognised that the locules of *Cytospora* species vary in configuration and proposed several genera for distinguishing these types, including the genera *Cytophoma* Höhn., *Cytospora*, *Cytosporopsis* Höhn., *Lamyella* Fr., *Leucocytospora* (Höhn.) Höhn. and *Torsellia* Fr. The monographic studies on *Cytospora* by Gutner (1935) and Urban (1958) recognised most of these genera. Sutton (1977, 1980) submerged these genera into the single genus *Cytospora*. More recent monographs by Spielman (1983, 1985) and Gvritishvili (1982) treated the anamorph types recognised by Höhnel as infrageneric rankings of section or subgenus. Gvritishvili (1982) used the following sections of the genus *Cytospora*: *Cytophoma*, *Cytospora*, *Cytosporopsis*, *Lamyella* (Fr.) Gvrit., *Leucocytospora* (Höhn.) Gvrit., and *Torsellia* (Fig. 1). However, he placed sections *Cytophoma* and *Cytospora* in subgen. *Cytospora*, sections *Cytosporopsis* and *Leucocytospora* in subgen. *Leucocytospora* Höhn. and sections *Lamyella* and *Torsellia* in subgen. *Torsellia* (Fr.) Gvrit.

Cytophoma has undivided often globose to compressed discrete locules, like *Phoma* Sacc., and discrete ostioles with ectostromata forming rings or collars around the ostiolar regions below the bark, which in median section appear as two "wings". *Cytosporopsis* has discrete locules shaped like flattened torus or toroid encircling a pillar of stromatic tissue. *Cytospora* has complex locules with multiple invaginations. These invaginations create a labyrinthiform locule with multiple chambers. *Torsellia* has multiple locules, each with independent walls. The beaks of the locules merge into a discrete ostiole. *Lamyella* has multiple locules and each locule has independent walls. The beaks of the locules merge separately to the surface of the bark or into an ectostromatic disc. Thus, species in *Lamyella* differ from those in sect. *Torsellia* in having numerous ostioles. *Leucocytospora* has complex locules with multiple invaginations forming a labyrinthiform locule of multiple chambers surrounded by entostromatic tissue and delimited by a dark dish-shaped conceptacle. Thus, *Leucocytospora* differs from sect. *Cytospora* in being surrounded by a dark conceptacle.

Cytospora Ehrenb., Sylv. Mycol. Berol.: 28. 1818.
- = *Cytispora* Fr., Syst. Mycol. 2(2): 540. 1823.
- = *Cytophoma* Höhn., Sitzungsber. Kaiserl. Akad. Wiss., Math.-Naturwiss. Kl., Abt. 1, 123: 133. 1914.
- = *Cytosporopsis* Höhn., Ann. Mycol. 16: 123. 1918.
- = *Cyclocytospora* Höhn., Mitt. Bot. Inst. TH, Wien 5: 17. 1928.
- = *Lamyella* Fr., Summa Veg. Scand.: 410. 1849.
- = *Leucocytospora* (Höhn.) Höhn., Ann. Mycol. 16: 130. 1918; Mitt. Bot. Lab. TH, Wien 4: 73. 1927.
- = *Torsellia* Fr., Summa Veg. Scand.: 412. 1849.

Coelomycetous, stromatic to pycnidial, ostiolate, immersed in vascular plant tissues, slightly to strongly erumpent, at times delimited by blackened marginal lines. *Discs* (clypeus) prominent or lacking, one to few ostioles per disc. *Locules* single undivided to multiple undivided, to single multi-chambered with invaginations, globoid to flattened toroid, in ectostroma or embedded in entostroma; wall bilayered, outer layer prosenchymatous, ultimately sclerenchymatous. *Conidiophores* present or absent, hyaline, branched or not branched, thin walled, filamentous. *Conidiogenous cells* enteroblastic phialidic. *Conidia* hyaline, allantoid, one-celled, thin-walled, relatively small and narrow (3–8 × 0.75–1.5 µm).

Lectotype species: *Cytospora chrysosperma* (Pers.) Fr. (UPS) Sweden, Fries, Scler. Suec. 154, selected by Donk (1964).

Ascostromatal forms corresponding to conidiomatal locule types

The important morphological differences among the sections of *Cytospora* are said to correlate with important morphological differences among the teleomorphs

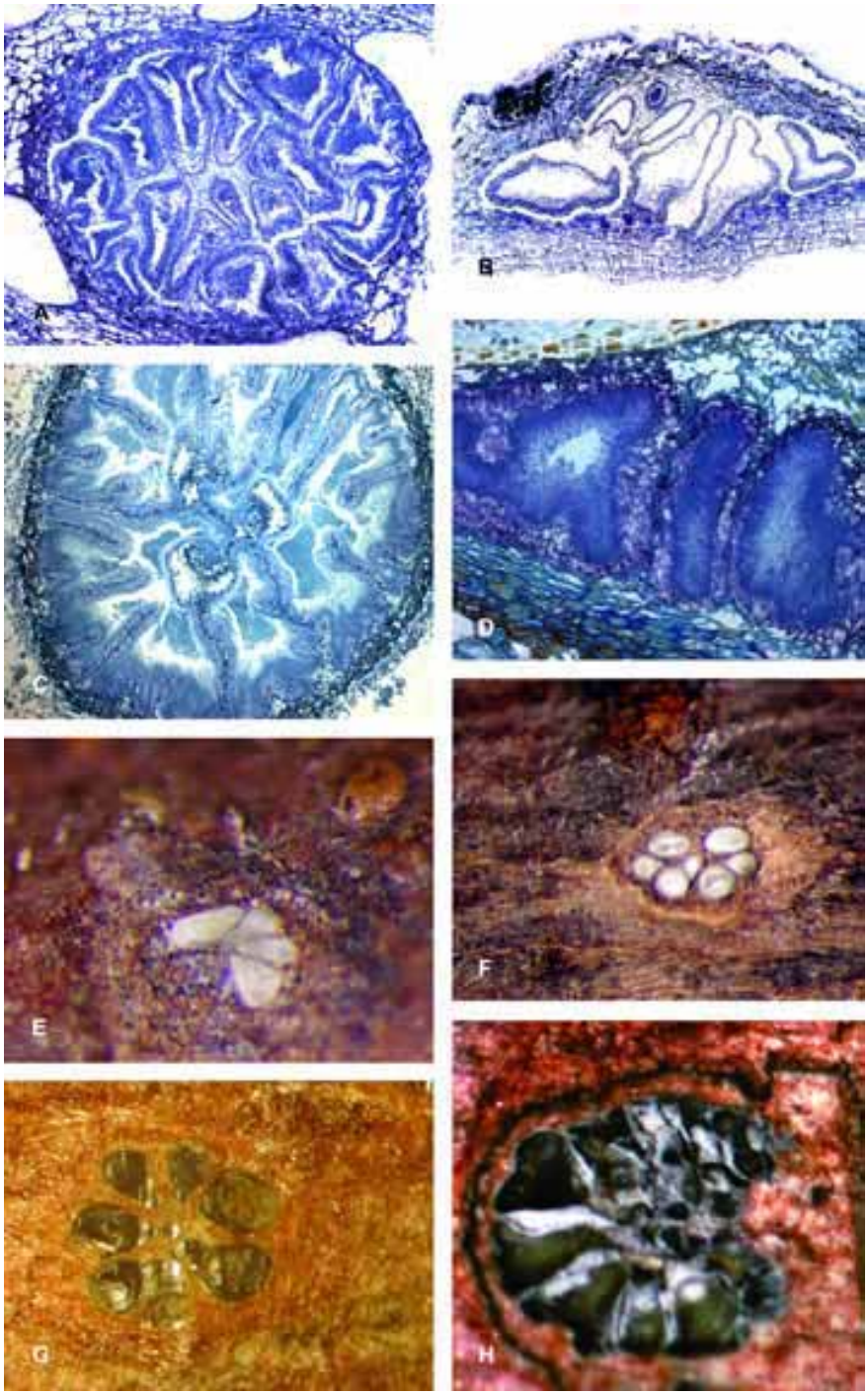

Fig. 5. Confusing locule morphologies in *Cytospora*. A–B. tangential and longitudinal cross sections of a conidioma of *C. australiae* that appears to have a multi-chambered locule divided by shared walls characteristic of a labyrinthine cytosporoid form but the conidioma actually has many crowded and compressed independent locules with separate walls characteristic of a torsellioid form. C–D. tangential and longitudinal cross sections of a conidioma of *V. eucalypti* that appears to have a multi-chambered locule divided by shared walls and delimited by a conceptacle characteristic of a leucocytosporoid conidioma but the conidioma actually has independent locules with unshared walls delimited by a conceptacle characteristic of a leucotorsellioid conidioma. Each independent locule wall is parallel and adhering to the separate wall of an adjacent locule (double walls). E. A rosette cytosporoid conidioma of *C. nitschkii* having shared walls between divided chambers of a locule. F. A conidioma of *C. nitschkii* that resembles the rosette cytosporoid form but each locule has separate and independent walls characteristic of a rosette torsellioid form. G. A conidioma of *V. eucalypti* that illustrates a rosette leucotorsellioid form (conceptacle is not apparent). H. A second conidioma of *V. eucalypti* that mimics the rosette leucocytosporoid form but is a leucotorsellioid form because each locule has an independent wall. The paired-walls are difficult to discern because each independent wall is parallel and adhering closely to the separate wall of an adjacent locule.

(Urban 1957, 1958, Gvritishvili 1982, Spielman 1985). Spielman (1983) believed that *Valsa* sect. *Valsa* forms cytosporoid conidiomata (sect. *Cytospora*), *Valsa* sect. *Monostichae* forms torsellioid (one ostiole) and lamyelloid (multiple ostioles) conidiomata (sect. *Torsellia* inclusive of sect. *Lamyella*) and *Valsa* sect. *Cypri* forms anamorphs in sect. *Cytophoma*. Spielman (1985) stated that there is a 1 : 1 correspondence between teleomorph morphology, the basis of holomorph sections, and anamorph morphologies, the basis of anamorph sections, except for *Valsa melanodiscus* Otth. This species was placed in sect. *Monostichae* (with reservations) in Spielman (1985) and in sect. *Valsa* in Urban (1958). Both researchers comment that the teleomorph needs a new section to accomodate it. It forms a unique anamorph for which the sect. *Cytosporopsis* was erected. Two sections of *Cytospora* are monospecific, only *Valsa cypri* (Tul.) Tul. & C. Tul. forms anamorphs in sect. *Cytophoma*, and only *V. melanodiscus* forms anamorphs in sect. *Cytosporopsis*. Teleomorphs in subgen. *Leucostoma* and *Valsella* form anamorphs in sect. *Leucocytospora*. The teleomorph morphologies that are proposed to correspond with particular anamorph morphologies, including new combinations from this study are illustrated in Fig. 6.

In this study, we have concluded that the sections proposed for *Cytospora* do not correspond well with particular holomorph sections. Proposed associations in Fig. 6 were useful only as generalisations. Some anamorphs did not conform with features of the described anamorph sections. For example, there was no appropriate section for uniloculate conidiomata without invaginations. Some species in subgenera *Leucostoma* formed anamorphs distinct from those of the assigned section *Leucocytospora*. Some species formed a range of conidiomatal morphologies that overlapped a few anamorph sections. The occurrence of species with characteristics of two or more sections led to rejection of the infrageneric ranks. However the conidiomal forms were of practical value for describing the morphology of species. We have, therefore, substituted descriptive terms for features common to former subgenera and sections.

Confusing locule types

Misinterpretation of the locule type of *C. australiae* has led to erroneous reports of the species distribution and to misidentified specimens in herbaria (UC, PREM). *Cytospora australiae* has many compressed locules with independent walls, the locule type of sect. *Torsellia*. However, crowding and compression of the locules distorts the shape and orientation so that it is difficult to recognise that each locule has separate walls from those of adjacent locules (Fig. 5). The resulting contorted morphology is occasionally interpreted as a locule divided by invaginations into multiple chambers sharing common walls, the locule type of sect. *Cytospora*. Distinguishing the locule type of the conidioma in sect. *Torsellia* from that of sect. *Cytospora* is often difficult. *Cytospora australiae* at low magnification (×10) resembles common North Temperate species like *C. chrysosperma* in sect. *Cytospora*; at high magnification (×100) the independent walls are revealed to form the locule type of sect. *Torsellia*.

The locule morphology of the *Cytospora* anamorph of *V. eucalypti* (≡*L. sequoiae*) may also be misinterpreted. The conidiomata of *V. eucalypti* have less compressed locules with fewer chambers than those of *C. australiae*. However, the independent walls of adjacent locules are parallel and firmly pressed together so two walls appear as one. The double wall between adjacent locules is repeated throughout the conidiomata. On the *Eucalyptus* host the locules are crowded, compressed and confusing at low magnification, like *C. australiae* (Fig. 5). On the *Sequoia* host, the locules are simple, uncrowded and clearly have independent walls (Fig. 5).

The problem of distinguishing two closely adhering walls, a double wall, from a shared single wall is often problematic for species of *Cytospora*. Even in the most common conidioma type, a locule divided into *ca* 6 chambers arranged radially to form a rosette shape, it may be difficult to determine whether a thick shared wall or a double wall is present. The difference between these two forms is crucial when assigning species to the sections *Cytospora* or *Torsellia*. Examples of rosette conidiomata that confound interpretation are shown in Fig. 5. A disturbing conclusion from our study is that species in sections *Torsellia* and *Lamyella* do form rosette conidiomata with chambers having shared walls in culture. Furthermore, in nature particular species, such as *C. berkeleyi*, form rosette conidiomata with some chambers having shared walls and others having independent walls (i.e., independent locules). Maintaining the current concept of sections of *Cytospora* requires such species to be placed in two sections. Based on these observations we argue below that the rosette conidioma is the archetypal morphology of the genus *Cytospora*.

Ancestral morphology and the conidioma

The complex multi-chambered labyrinthine conidiomatal stroma, typical of *C. chrysosperma* and other North Temperate *Cytospora* species was a rare form on *Eucalyptus*. The species described from *Eucalyptus* were mostly from the Southern Hemisphere. They ranged in form from species with a simple globoid locule to those with labyrinthiform conidiomata. This led us to question what form might represent the ancestral morphology in *Cytospora*. The simple globoid locule

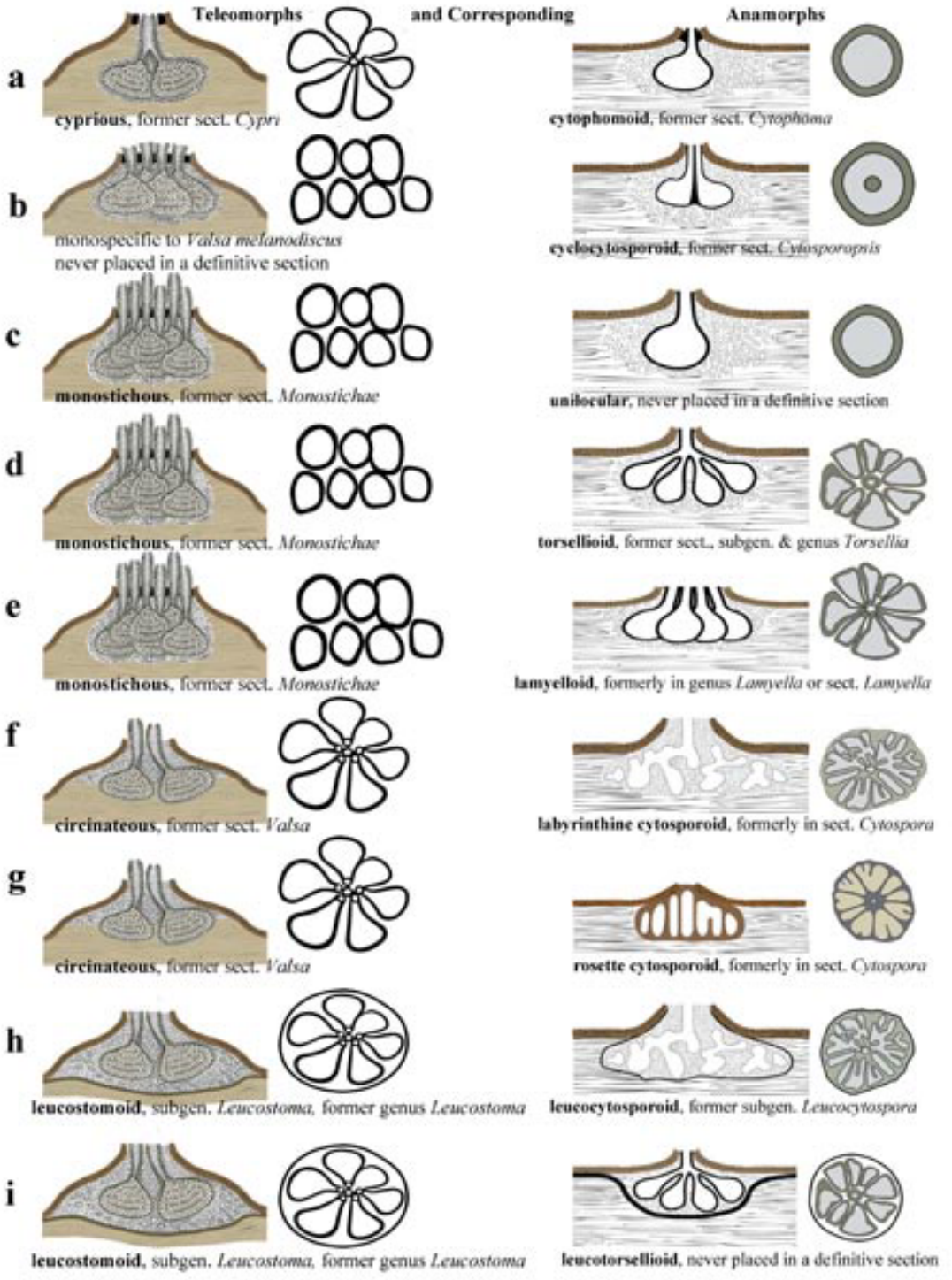

Fig. 6. Previously hypothesized correlations between the morphologies of the teleomorphs (former holomorph sections of *Valsa*) and morphologies, i.e., locule forms, of the anamorphs (former sections of *Cytospora*). Descriptive terms for the stroma features of the teleomorph and the anamorph (locule forms) are in bold. A. *Valsa* sect. *Cypri* and *Cytospora* sect. *Cytophoma*. B. *Valsa melanodiscus* and *Cytospora* sect. *Cytosporopsis* (monospecific). C. *Valsa* sect. *Monostichae* and unilocular conidioma. D. *Valsa* sect. *Monostichae* and *Cytospora* sect. *Torsellia*. E. *Valsa* sect. *Monostichae* and *Cytospora* sect. *Lamyella*. F. *Valsa* sect. *Valsa* and *Cytospora* sect. *Cytospora* with labyrinthine locule. G. *Valsa* sect. *Valsa* and *Cytospora* sect. *Cytospora* with rosette locule. H. *Valsa* subgen. *Leucostoma* or *Valsa* subgen. *Valsella* and *Cytospora* sect. *Leucocytospora*. I. *Valsa* subgen. *Leucostoma* or *Valsa* subgen. *Valsella* and leucotorsellioid form (newly described conidioma form).

with rarely a single invagination of the wall could represent the ancestral morphology. However, we argued that this was a derived and reduced morphology. Rather the rosette conidioma, the simple conidioma with *ca* six chambers arranged radially sharing common walls with adjacent chambers, was shared by many species of *Cytospora* in nature and most species in culture. Several species of *Cytospora* appeared to occasionally produce this locule type despite regular production of more elaborate types. While the rosette conidioma was a more complex morphology than the simple globoid locule, we believe the rosette form was the fundamental blueprint for the conidiomata of *Cytospora*. For example, the rosette was the simpler form seen in *Cytospora* sect. *Cytospora*, yet it was also formed by species from sections *Torsellia* and *Lamyella*. The rosette form was produced by *C. eucalypticola* when a conidioma formed on the surface of the bark whereas the globoid locule was produced deeper in the bark.

The more complex locule types would be envisioned to have arisen from the rosette form following further invaginations of the wall. Formation of partial invaginations led to the more elaborate rosette form represented in *C. disciformis* (Fig. 5). Further elaboration resulted in the familiar morphology of *C. chrysosperma* (Fig. 5). It was more difficult to envision the modification of the rosette to the form with multiple locules, of sections *Torsellia* and *Lamyella*. We envisioned shared walls splitting lengthwise into two parallel walls. The split walls bounding a chamber then ballooned out with expansion of the chamber resulting in the *Torsellia* locule type. The *Lamyella* locule type probably proceeded from the *Torsellia* locule type. Other workers had linked the *Lamyella* and *Torsellia* locule types and viewed the *Lamyella* type as a normal variant of the *Torsellia* type. For example, Spielman (1985) rejected the use of sect. *Lamyella* and submerged the locule type into sect. *Torsellia*. The development of the *Lamyella* locule type necessitated that ostiolar necks formed independently and then failed to converge and fuse near the central point of the stroma. The *Lamyella* locule type would arise by the meristematic ostiolar regions being more strongly directed toward the bark surface in *Lamyella* than toward convergence, as in *Torsellia*.

TAXONOMY: TREATMENT OF GENERA, SUBGENERA, AND SECTIONS

Generic concepts

Ever since the works of von Höhnel (1906–1928), it has been debated whether the genera *Valsa*, *Leucostoma*, *Valsella*, and more recently *Valseutypella* are distinct (supported by Défago 1942, Urban 1957, 1958, Barr 1978, 1990) or whether they represent variations among species in *Valsa* (supported by Petrak 1919, 1969, Hubbes 1960a, Vasilyeva 1988, 1994). The latter view submerges all the genera into *Valsa* either as subgenera, or as species with no additional infrageneric rank. For example, Vasilyeva (1994) included the type species of *Valseutypella* in *Valsa* with no subgeneric or section rank. The latter taxonomic hypothesis is based on morphological grounds and is outlined in part by Vasilyeva (1988).

In this study, we have accepted the hypothesis that *Leucostoma*, *Valsella* and *Valseutypella* are synonyms of *Valsa*. We have based this judgement both on morphological studies and the results of DNA sequence analyses. There was little support to infer a common lineage of descent for species of *Leucostoma*. The closest inferred relation to species of *Leucostoma* and *Valsella* were species of *Valsa* without black zone lines (conceptacles) and without polysporous asci. Molecular phylogenetic analyses based on several genes would be necessary to provide firmer support for our generic concept.

Subgeneric & section concepts

In this monograph, genera in current use in the literature, including *Valsa*, *Leucostoma*, *Valsella* and *Valseutypella*, were treated as belonging in one genus, *Valsa*. The genera *Leucostoma* and *Valseutypella* (as well as the subgenus *Leucostoma*) were viewed as collections of *Valsa* species that had specific entostromatal and ectostromatal morphologies in the teleomorph. *Valsella* was viewed as collections of multi-ascospored variants of populations of *Valsa* species that also produced octo-ascospored variants. The holomorph sections *Valsa* (= sect. *Circinatae*), *Monostichae* and *Cypri* separated species having few large perithecia circinately arranged with large asci and ascospores (sect. *Valsa*), from species having many small perithecia not circinately arranged with small asci and ascospores (sect. *Monostichae*), and from species having fused ostiolar necks/beaks (sect. *Cypri*). We recognised that the subgenera and sections are artificial and do not reflect natural relationships, or inferred phylogenetic relationships. However, the ranks of subgenus and section were useful in visualising particular generalised stromatic forms and in simplifying identification and description of species. Below, we have substituted descriptive terms for the combinations of distinguishing morphological features that had been used to separate the former subgenera and sections. Descriptive terms adequately served these purposes and further facilitated comprehension of the complex morphology of *Valsa* species.

The sections defined for *Cytospora* by Urban (1957, 1958) and Gvritishvili (1982) used locule types to separate species. The use of sections facilitated

description of *Cytospora* species and comprehension of the morphology. However, proposing subgenera and sections for anamorph morphological groups (Gvritishvili 1982) added unnecessary complexity and reduced ease of comprehension. *Cytospora* species on *Eucalyptus* exhibited the full range of variability in morphology described by von Höhnel (1906–1928). Some *Cytospora* species on *Eucalyptus* exhibited combinations of locule types characteristic of several sections. Great variation in locule form was occasionally present on one specimen, i.e., *C. variostromatica*, and in other instances only present among collections from different geographical locations, i.e., *C. eucalypticola* emend. There was little support to infer common lineages of descent for sections of *Cytospora*. In this monograph we abandoned the use of infrageneric ranks and substituted descriptive terms, defined below, for various locule arrangements in stromatal tissues.

Species concepts

Up to the early 1900s an overwhelming number of *Cytospora* and *Valsa* species were described. These species were often considered distinct because they occurred on unique hosts rather than because they had distinct morphological features. Little effort was placed on determining which species might be synonyms until after Saccardo (1882–1931). Lists of these species are present in Saccardo (1882–1931), Grove (1935) and Gutner (1935). During this period, von Höhnel (1906–1928a, b) began to incorporate modern concepts in his morphological studies of the genus. He reduced many species to synonymy. Morphological and physiological studies were combined to delimit species and to identify synonyms beginning in 1935 with Défago (1935, 1942) and continuing with Kern (1955, 1957, 1961), Urban (1957, 1958), Hubbes (1960a) and others. Morphological studies continue to relegate more species to synonymy (Barr 1978, Gvritishvili 1982, Spielman 1985, Vasilyeva 1994).

DNA sequence comparisons presented in this study provided evidence that recent efforts aimed at relegating species to synonyms had been inordinately extreme (see below, *V. ceratosperma s. str.*), and re-evaluation was again necessary before accepting synonyms and resurrecting species.

Nomenclature

In this study, we have refrained from naming anamorphs with binomials in *Cytospora* for newly described holomorphs in *Valsa*, although plant pathologists (such as ourselves) generally favour naming anamorphs. Our reason for not providing two names for a single organism was based on the likelihood that DNA sequence data would become the means for the practical identification of *Cytospora* and most other fungi. Therefore, whether conidiomata or ascomata are present would become less relevant for identifications. In this study, morphological characteristics evident on a specimen were often inadequate for accurate identification of a species and DNA sequence was crucial. Article 59, Recommendation 59A.3, of the International Code of Botanical Nomenlature (ICBN) Saint Louis Code (Greuter *et al.* 2000), discourages the use of binary names for anamorphs when the teleomorph connection is firmly established and where there is no practical need for separate names.

Preference for naming anamorphs arises because many species seldom form teleomorphs on diseased plants, and anamorphs of such species need to be distinguished from other species for which no sexual states are known. Described *Cytospora* species by far outnumber *Valsa* species. Plant pathologists have difficulty identifying pathogens based solely on anamorph characters, especially, when formal descriptions of holomorphs exclude mention of the characters of the anamorph. Such exclusion should be strongly discouraged. Many descriptions mention the size of conidia as the only character for the anamorph. The need for maintaining the dual nomenclatural system for reasons of practical identification is strongly defended (Gams 1993, 1995). However, it is argued that using duplicate names burdens the literature, and if the system of duplicate names is abandoned then the anamorph names will eventually vanish from use (Cannon & Kirk 2000, Seifert & Samuels 2000).

Type specimens of holomorphs often lack anamorphs because in *Valsa* teleomorphs often form without anamorphs present. The characters of both morphs are needed for identification of the species, and important morphological features are present only on natural material. For anamorphic characters to become fixed as characters of the holomorph, species descriptions are emended and epitype specimens of anamorphs are deposited to serve as interpretive types (Schroers 2001).

Replacing subgeneric and section divisions with descriptive terms

Descriptive terms for ascostromata:

euvalsoid = having ascostromata without conceptacles. The definition was derived from *Valsa* subgen. *Valsa*. Fig. 3.

Valsa Fr. subgen. *Valsa*: *Ascostromata* not delimited by black zone lines (conceptacle). *Perithecia* in host bark and wood or in entostroma. *Asci* with 4 or 8 ascospores per ascus. *Type species*: *Valsa ambiens* subsp. *ambiens* (Pers.) Fr.

leucostomoid = having ascostromata delimited by conceptacles. The definition was derived from *Valsa* subgen. *Leucostoma* Höhn. Fig. 3.

Valsa subgen. *Leucostoma* Höhn.: *Ascostromata* delimited by black zone lines (conceptacle). *Perithecia* surrounded by entostroma. *Asci* with 4 or 8 ascospores per ascus. *Type species*: *Valsa leucostoma* (Pers.) Fr. (= *Leucostoma persoonii* (Nitschke) Höhn.).

polysporous = having polysporous asci.

polysporous leucostomoid = having polysporous asci and ascostromata delimited by conceptacles. The definition was derived from *Valsella* Fuckel. Fig. 3.

Valsella Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 203. 1869: *Ascostromata* delimited by black zone lines (conceptacle). *Perithecia* surrounded by entostroma. *Asci* polysporous. *Type species*: *Valsa fertilis* Nitschke (= *Valsella fertilis* (Nitschke) Sacc.).

valseutypelloid = having perithecia surrounded by well-developed stromata of pseudoparenchyma tissues. The definition was derived from *Valseutypella* Höhn. Fig. 3.

Valseutypella Höhn., Ann. Mycol. 16: 224. 1918: *Ascostromata* delimited or not by black zone lines (conceptacle). Stromata well-developed, entirely of pseudoparenchymatic tissues. *Perithecia* surrounded by stromata. *Asci* with 4 or 8 ascospores per ascus. *Type species*: *Valsa tristicha* (De Not.) Lar. N. Vassilyeva.

circinateous = having few large laterally inclined and circinately arranged perithecia and large ascospores (13–)16–35 µm long. The term included more information than "circinate" or "valsoid". The definition was derived from *Valsa* subgen. *Valsa* sect. *Valsa*. Fig. 4.

Valsa subgen. *Valsa* sect. *Valsa*: *Perithecia* large, few, circinate, laterally inclined (valsoid). *Ascospores* large (13–)16–35 µm long. *Type species*: *Valsa ambiens* subsp. *ambiens* (Pers.) Fr.

monostichous = having numerous small upright to inclined crowded perithecia and small ascospores (4–)6–12 µm long. The definition was derived from *Valsa* subgen. *Valsa* sect. *Monostichae*. Fig. 4.

Valsa subgen. *Valsa* sect. *Monostichae* (Nitschke) Z. Urb.: *Perithecia* small, many, crowded, upright to inclined. *Ascospores* small (4–)6–12 µm long. *Type species*: *Valsa abietis* (Fr.) Fr. (*fide* Urban 1957).

cyprious = having perithecial ostioles that are fused. The definition was derived from *Valsa* subgen. *Valsa* sect. *Cypri*. Fig. 4.

Valsa subgen. *Valsa* sect. *Cypri* Z. Urb.: *Perithecial ostioles* crowded and fused in obscured disc. *Type species*: *Valsa cypri* (Tul.) Tul. & C. Tul. (*fide* Urban 1957).

For example, a species that had the morphological characters equivalent to the former *Valsa* subgen. *Valsa* sect. *Monostichae* was described as a monostichous euvalsoid species. *Valsella nigro-annulata* Fuckel was referred to as the polysporous leucostomoid and circinateous species *Valsa nigro-annulata* (Fuckel) G.C. Adams *et al.*, and *Valsella fertilis* (Nitschke) Sacc. was herein referred to as the polysporous leucostomoid and monostichous species *Valsa fertilis* Nitschke. The descriptive words provided flexibility for including *Valsa* species with new combinations of morphological characteristics distinct from described subgenera and sections. For example, a new species could be polysporous euvalsoid and cyprious.

Descriptive terms for conidiomata:
Historically, the anamorph sections, *Cytophoma*, *Cytosporopsis*, *Cytospora*, *Torsellia*, and *Lamyella*, had not been applied to the subgenus *Leucostoma*, or genera *Leucostoma*, *Valsella* and *Valseutypella*. The descriptive terms listed below substituted for the morphological features that characterised the Sections, and were applied to species formerly in *Leucostoma*, *Valsella* and *Valseutypella*. Some descriptive terms were proposed for newly recognised features.

cytosporoid = having a single locule subdivided by invaginations into several chambers. The definition was derived from *Cytospora* sect. *Cytospora*. Fig. 1.

Cytospora sect. *Cytospora*: *Conidiomatal stromata* not delimited by black zone lines (conceptacle). *Conidiomata* of multi-chambered locules, subdivided by invaginations, sharing common walls. *Type species*: *Cytospora chrysosperma* (Pers.) Fr. (*fide* Donk 1964).

We distinguished between two distinct forms in cytosporoid *Cytospora*. One form had few (3–7), regular, radially arranged locules in the conidiomata, which we referred to as **rosette cytosporoid**. The other form had complex labyrinthine invaginations that were irregular except at the periphery and the individual locules were difficult to enumerate. We referred to this form as being **labyrinthine cytosporoid**. The latter conidioma type generally had relatively larger conidia and conidiophores. The type species of *Cytospora* formed the latter conidioma type.

cytophomoid = having an undivided locule and a distinctive ring of ectostroma that was wing-like in longitudinal median cross section encircling the ostiole. The definition was derived from *Cytospora* sect. *Cytophoma*. Fig. 1.

Cytospora sect. *Cytophoma* (Höhn.) Gvrit.: Conidiomatal stromata not delimited by black zone lines (conceptacle). *Conidiomata* of discrete simple undivided locules with ring-like ectostromata encircling the ostiolar beak (wing-like in longitudinal median

cross section). *Type species*: *Cytospora pruinosa* (Fr.) Sacc.

unilocular = having an undivided locule with or without disc of ectostroma but lacking ring-like ectostroma encircling the ostiole. Fig. 1.

Many of the specimens examined in this study (such as the **holotype** PREM 42543 of *Cytospora eucalypticola* van der Westh. as well as specimen MSC 380718) had stromata similar to cytophomoid *Cytospora* except that they lacked the distinguishing ring-like and wing-like ectostromata encircling the ostiole. We referred to these as unilocular *Cytospora* spp.

cyclocytosporoid = having an undivided toroid locule with a central column of ostiolar tissue. The definition was derived from *Cytospora* sect. *Cytosporopsis*. Fig. 1.

Cytospora sect. *Cytosporopsis* (Höhn.) Gvrit.: *Conidiomatal stromata* sometimes delimited by black zone lines (conceptacle). *Conidiomata* of discrete undivided toroid locules (doughnut-shaped) with central columns of tissue. Columns formed in the centres of ostiolar beaks, and ostiolar openings were ring-shaped encircling the columns. *Type species*: *Cytospora umbrina* (Bonnord.) Sacc.

torsellioid = having multiple locules with separate walls and a single shared ostiole. The definition was derived from *Cytospora* sect. *Torsellia*. Fig. 1.

Cytospora sect. *Torsellia* (Fr.) Gvrit.: *Conidiomatal stromata* not delimited by black zone lines (conceptacle). *Conidiomata* of groups of simple undivided locules. *Locules* not sharing common walls. *Ostioles* converging to shared single ostioles. Type species: *Cytospora sacculus* (Schwein.) Gvrit.

lamyelloid = having multiple locules with separate walls and multiple ostioles. The definition was derived from *Cytospora* sect. *Lamyellia*. Fig. 1.

Cytospora sect. *Lamyella* (Fr.) Gvrit.: *Conidiomatal stromata* not delimited by black zone lines (conceptacle). *Conidiomata* of groups of simple undivided locules. *Locules* not sharing common walls. *Ostioles* converging independently to shared discs, or to surface. *Type species*: *Cytospora sphaerocephala* (Schwein.) Sacc.

leucocytosporoid = cytosporoid, but having a dark conceptacle. The definition was derived from *Cytospora* sect. *Leucocytospora*. Fig. 1.

Cytospora sect. *Leucocytospora* Höhn.: *Conidiomatal stromata* delimited by black zone lines (conceptacle). *Conidiomata* of locules subdivided by invaginations, sharing common walls. *Type species*: *Cytospora leucostoma* Fr.

For example, *Cytospora leucostoma* had labyrinthine leucocytosporoid conidiomata.

leucotorsellioid = torsellioid, but having a dark conceptacle. The definition was derived from *Cytospora* sections *Leucocytospora* and *Torsellia*. Fig. 1.

Specimens of *V. eucalypti* (≡ *L. sequoiae*), such as isotypes UM 15128 and MSC 11471 as well as specimen MSC 380713, "*C. eucalypti*", *C. abyssinica*, and *C. agarwalii* had conidiomatal stromata resembling a combination of features of the former sections *Torsellia* and *Leucocytospora*.

Morphology of holomorphs

Ectostroma and *entostroma*: Wehmeyer (1926) is usually cited for having defined the ectostroma and entostroma. However, Wehmeyer (1926) cites details of the stromata of *Diatrype*, whereas Wehmeyer (1933) describes that of *Diaporthe*. The latter study is more relevant to *Valsa*. Wehmeyer's concepts emphasise the particular location of hyphae in layers of bark of the host and his description is paraphrased, as follows. The ectostroma originates from hyphae in the bark phellogen. Coinciding with perithecial development, the hyphal cells of the ectostroma enlarge greatly, forming a palisade-like pseudoparenchyma tissue that bursts apart the bark periderm exposing the phelloderm where the remaining ectostroma rapidly darkens. The ectostroma may remain sterile or produce pycnidia. Just beneath the conic to pulvinate ectostroma, in the bark the entostroma occurs in which the perithecia develop (Fig. 7). Blackened tissue composed of dark-walled hyphae fuses with the bark cells forming the conceptacle which cuts off the bark from the developing entostroma, in species of leucostomoid and polysporous leucostomoid *Valsa*. The blackened zones (conceptacle) may be distinct on the surface of the bark (Fig. 7), or exposed by cutting vertically through the ascostroma (Fig. 7). The blackened zones may be faint, incomplete, or almost lacking. Hyphae proliferate within the entostroma binding together the partially degenerated bark elements into a compact mass of entostroma (Wehmeyer 1926). The perithecia develop in the mass of the entostroma. The configuration and development of the entostroma and blackened zone lines as well as the position and grouping of the perithecial primordia provide the characters of the stroma unique to a species.

Spielman (1985) described the stroma more in terms of hyphal tissue types rather than relative bark position. She describes ectostromatic tissues as composed of angular to hyphal cells with thick brown walls having cytoplasmic contents that do not stain with cotton blue. These characteristics are consistent with the disc

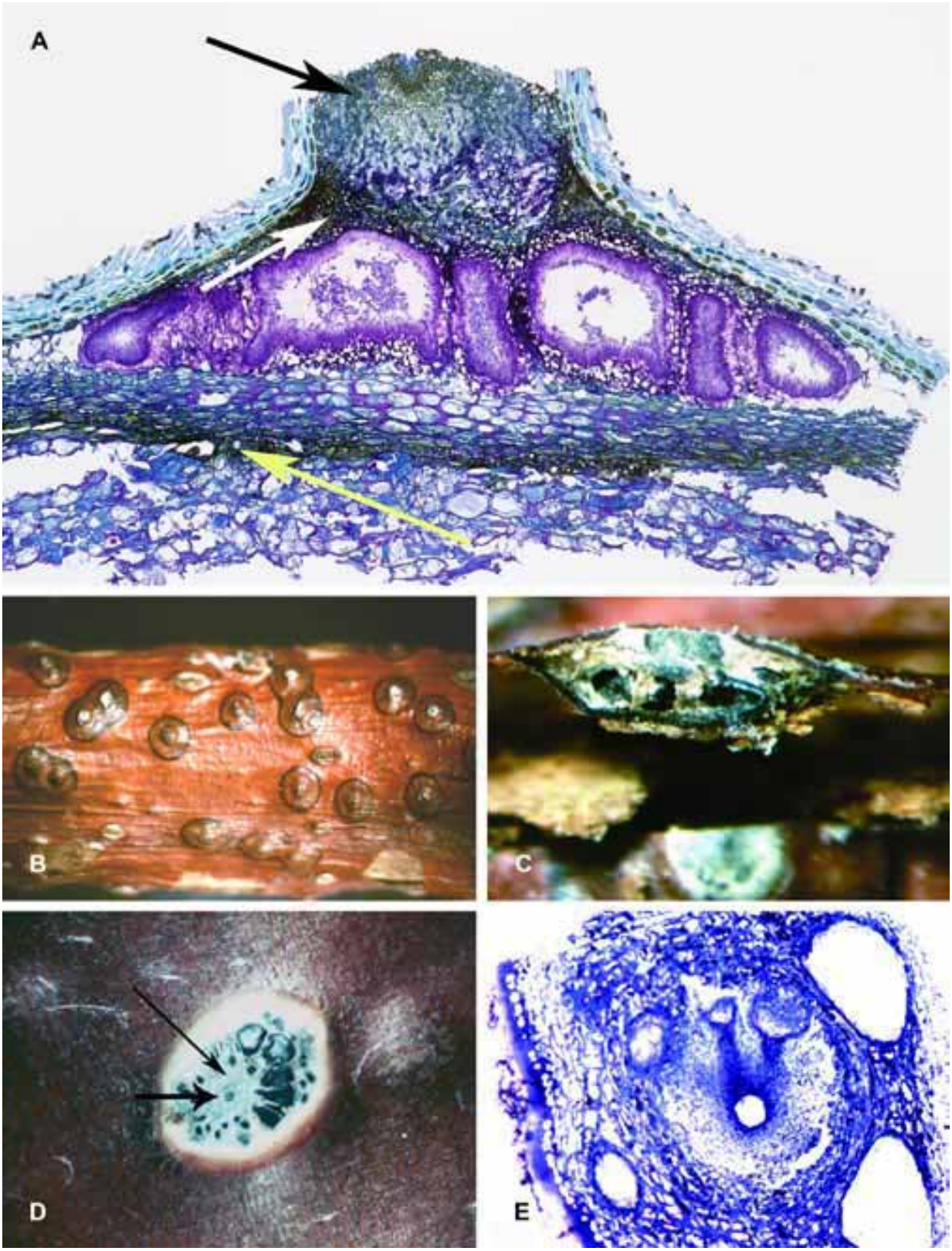

Fig. 7. Stromatal tissues. A. Longitudinal cross section through ascostroma of leucostomoid *V. eucalypti* with black arrow pointing at ectostroma, white arrow entostroma, and yellow arrow conceptacle (black zone) tissues. B. A specimen of leucostomoid *V. cincta* with the rare occurrence of an externally visible black conceptacle on the bark surface. C. Longitudinal cross section of *V. cincta* having black conceptacle delimiting sides and base of the ascostroma in bark. D. Arrow pointing to conidioma in centre of ascostroma surrounded by stroma tissue and perithecia. E. Tangential microtome section through disc of conidioma showing independent ostiolar necks converging toward disc and fusing into a discrete ostiole.

in many species of *Valsa* and *Cytospora*. However, the definition becomes confused when the cells disintegrate becoming amorphous and mealy in texture, a common occurrence. The latter characteristics are typical of the discs of leucostomoid and polysporous leucostomoid *Valsa* and their anamorphs. The disc tissue more closely resembles the tissue of the entostroma; entostroma surrounds the perithecia in these species. Spielman (1985) described entostroma as composed of loosely packed thin-walled hyphal cells with cytoplasm that stains in cotton blue.

We use the term entostroma for tissues of the stroma that surround perithecia or pycnidia regardless of the nature of the cells. For example, in the valseutypelloid species *V. tristicha* the perithecia are surrounded in a pale coloured parenchymatous tissue, which we refer to as entostroma. Externally, the stroma is of dark parenchymatous tissue, which we refer to as ectostroma. Stromatic tissues, including ectostroma and entostroma, are usually used in descriptions of the teleomorph and anamorph.

Morphology of anamorphs

Accurately identifying a species of *Cytospora* based on morphological features is recognised as a problem that is perhaps insurmountable. This is discussed in the monograph of Spielman (1983) where she states that the anamorph of *Valsa ambiens* (Pers.) Fr. is not distinguishable from anamorphs of many other species of *Valsa*. Défago (1935) quotes a worker, Chabrolin, as stating "Une détermination exacte est pratiquement impossible dans l'état actuel de la systématique de ce groupe." Spielman (1983) introduces this quote again in a discussion of the inadequacy of using morphological features alone for species delimitation. Non-morphological data were introduced to improve the accuracy of delimiting taxa beginning with Défago (1935). These studies have continued (Défago 1942, Kern 1955, 1957, 1961, Hubbes 1960a, Urban 1958, Spielman 1983, Kastirr 1985, Proffer & Hart 1988, Adams *et al.* 1990, Pluim *et al.* 1994, Surve-Iyer *et al.* 1995, Adams *et al.* 2002a). The only monographic work to introduce non-morphological data was that of Urban (1958).

Stroma or tissue supporting the conidioma: The anamorph is considered to be within the ectostroma when it forms within an ascostroma. This occurs in only a few species such as *V. cincta* Fr. and *V. massariana* (Fig. 7D). The ostiolar necks of anamorphs often converge and insert into a disc that is comprised of ectostroma. The presence and abundance of the ectostromatic disc in collections of *Cytospora* are highly variable within a collection and within a species. Discs are found absent in a conidiomatal stroma adjacent to another having a thick convex disc. Appearance of the disc may change with age of the stromata being white in young stromata and dark in older, more weathered structures. Discs may be large, hemispherical and glossy, when fully erumpent, but reduced, convex and dull when partially erumpent and partially covered by remnants of the splitting bark (Fig. 8). Characters of the disc vanish when major portions of conidiomatal stromata are exposed after erupting through the bark surface (Fig. 8). Commonly, discs are flat, level with the bark surface, and contain a discrete ostiole level with the disc surface (Fig. 8).

For conidiomata or ascostromata, we use the term ostiole for the opening of the fruiting body at the bark surface. The term neck or ostiolar neck is used for the length beneath the disc and bark, the term beak or ostiolar beak for the length above the disc and bark surface, and the term neck/beak when formed in culture. A variable character of the conidiomata is the number of ostioles and the lengths of the ostiolar beaks. In some species of *Cytospora* the characteristics of anamorph ostioles appear relatively stable. For example, the majority of the conidiomata form flat whitish discs with 2–3 ostioles at the same level as the disc surface in *V. eucalypti*. This is stable whether the species occurs on *Sequoia* or *Eucalyptus*, or in North America or India. Urban (1958) considered the number of ostioles in the discs to be diagnostic for certain species and used the character to distinguish the anamorph of *V. malicola* Z. Urb. from that of *V. ambiens*.

Spielman (1983) believed that the characters of ostiole number and arrangement in discs of conidiomata are extremely variable in species. She cites the anamorph of *V. ambiens* as exhibiting a continuous range of ostiole numbers per disc. We observed a continuous range of ostiole numbers commonly in lamyelloid *Cytospora* species. In most lamyelloid species each independent locule retains one ostiolar neck that reaches the disc surface and the ostiole number varies proportionately with the number of locules. Rarely, in torselloid species the ostiolar necks of multiple locules converge into two or three ostioles and this was observed in *C. sacculus* and the anamorphs of *V. eucalypti* and *V. eucalypti sensu* Sharma *et al.* Lamyelloid species occasionally have the ostiolar necks of several locules converge into one ostiole, while other necks remain separate, in a shared disc.

The length of the ostiolar necks below discs appears correlated with the thickness of the bark and is variable in a species due to bark variability. In some species ostiolar necks from independent locules converge deep below the disc into a single ostiole. The shared ostiolar neck, therefore, is relatively long. This characteristic is rare among species of *Cytospora* and we tentatively treat it as a stable and distinctive characteristic.

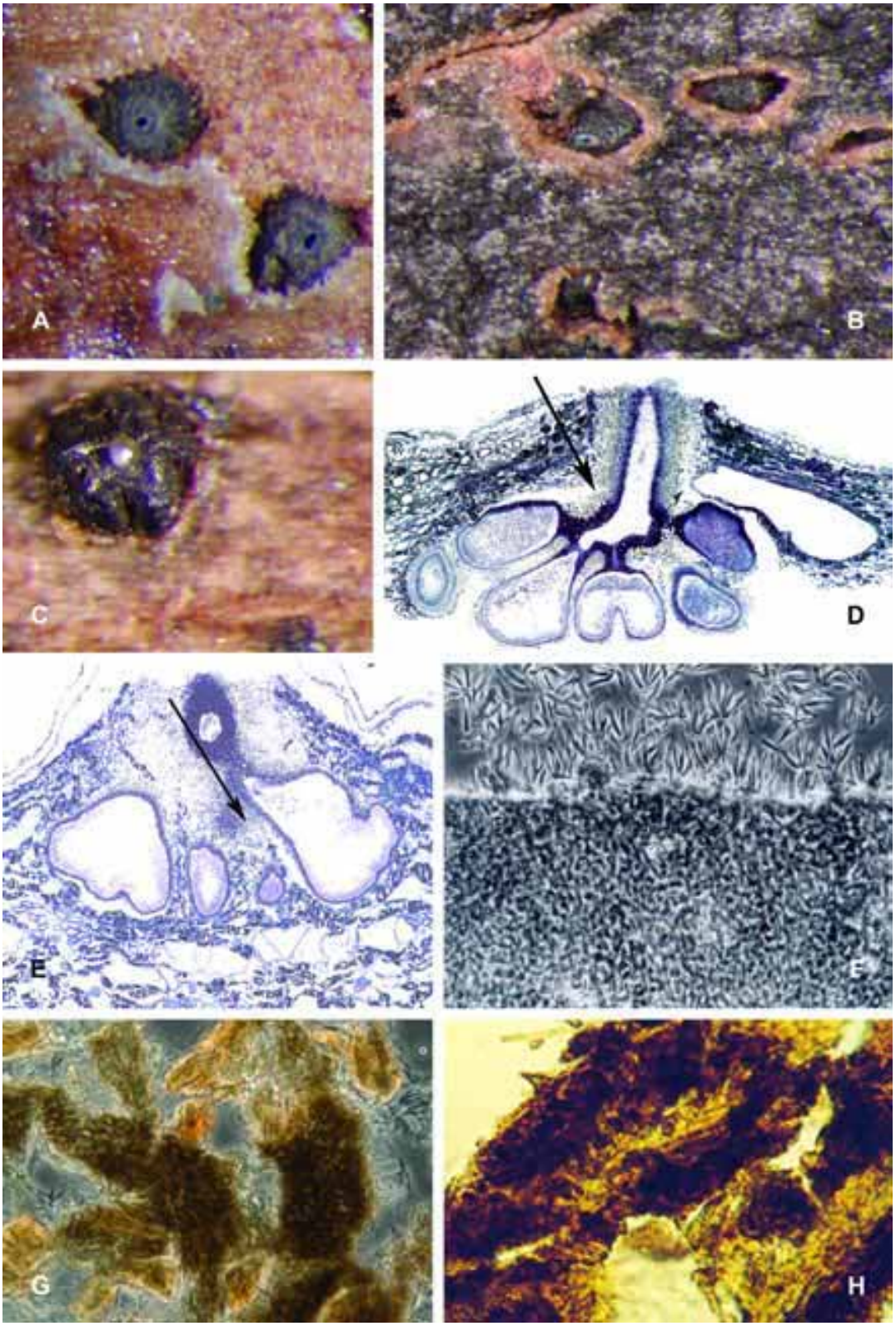

Fig. 8. Discs and stromatal tissues in conidiomata. A. Typical disc of conidioma nearly level with bark surface and with ostiole at the same level as the disc surface. B. Upper surface of conidiomata erumpent through bark surface obscuring discs. C. Fully erumpent glossy dark hemispherical disc of *C. abyssinica*. D. Longitudinal cross section through conidioma of *C. eucalyptina* with black arrow indicating stroma tissues surrounding full length of ostiolar neck. E. Longitudinal cross section through conidioma with black arrow indicating stroma tissues surrounding independent locules. F. Conidioma wall of *textura epidermoidea*. G. Conidioma wall of *textura angularis*. H. Conidioma wall with outer layer of dark crust-like sclerenchymatous tissue and inner layer of light pseudoparenchymatous *textura angularis*.

The lengths of ostiolar beaks above the discs are likely influenced by environment because Défago (1942) showed that the length of beaks of teleomorphs is directly correlated to relative humidity. However, in culture where discs do not form, we have found that particular species will dependably form long or short necks/beaks.

Spielman (1983) reported that in the anamorphs, the characters of the disc and stroma are highly variable within a species. She illustrated this for the species *C. chrysosperma* from one site and several hosts. Also, disc and stroma characters are highly variable on an individual host, depending on the thickness of bark and the stage of development. Transitions between ectostroma and entostroma are difficult to ascertain, or are abrupt. Conidiomata are surrounded by stromatic tissues or host tissue. Workers differ in whether they differentiate between ecto- and ento- stroma when describing the ascostromata and few use the terms in formal descriptions of the morphology of conidiomata. We refer to the stromatic tissue around the globe of a fruiting body as entostroma regardless of whether it surrounds a locule, conidioma or perithecium. A few species of *Cytospora* are distinguished in part by how deeply below the disc the stromatic tissues descend around the ostiolar neck or the locules (Fig. 8). Conidiomata of leucostomoid and polysporous leucostomoid *Valsa* are entirely surrounded by stromatic tissues.

Sutton (1980) considered conidiomata of *Cytospora* to be stromatic rather than pycnidial and placed *Cytospora* in the section of his key *Phialostromatineae*, rather than in *Phialopycnidiineae*. He also considered *Phomopsis* as stromatic and not pycnidial. We do not accept *Cytospora* as being only stromatic or only pycnidial. *Cytospora* species are generally stromatic in nature. However, many species of *Cytospora* form globose conidiomata having single walls in culture and are pycnidia. Uecker (1989) similarly concluded that *Phomopsis* is sometimes stromatic on host tissue and pycnidial in culture. The locules of *Cytospora* pycnidia in culture are not always divided by invaginations. A comprehensive study of many species in culture is needed to determine whether stromatic conidiomata are formed reproducibly under controlled conditions.

Orientation and number of locules in conidiomata: Usually, conidiomata occur singly and in large numbers on branches in nature. Rarely, a conidioma occurs in the centre of the ascostroma, such as in *V. cincta* (Fig. 7D). The orientation of locules of a conidioma can be circinate and upright, circinate and laterally inclined (valsoid), clustered at one level and upright (diatrypelloid), clustered at various levels and upright (monostichous), solitary and upright, or solitary and inclined. Occasionally, two to three locules will converge into a disc while neighbouring locules remain solitary. Rarely, a locule divided by invaginations into several chambers will form a conidioma with other locules that are undivided internally.

Conidiomatal centrum: Pycnidial wall tissues resemble perithecial wall tissues, but seldom are identical. They are brown and generally begin as prosenchymatous which differentiate to *textura epidermoidea* or *textura angularis*, then sclerenchymatous (Fig. 8). As the conidiomatal wall changes, cell shape becomes obscured and the tissue becomes harder. This does not occur in the perithecium wall that usually remains *textura epidermoidea*. In conidiomata, sometimes only the tissue of the ostiolar neck becomes crust-like. This type of tissue is referred to as sclerenchymatous and its evident hardness is noted by breakage of microscope cover slips during preparation of mounts using applied pressure. The thicker and more crust-like the wall, the more evident the hyaline wall layer becomes, beneath the crushed and fractured pigmented layer. The bilayered wall is described as a characteristic of the order (Barr 1990, in reference to ascomata). The internal layer is made up of a few rows of compressed cells.

Cytospora species with small conidia, 3–4.5 µm in length, have hymenia composed of discrete, long tapering phialides of approximately 10 × 1.2 µm. In cross section of a conidioma, the hymenium appears as unbranched phialides (Fig. 9). If the conidioma is thoroughly crushed then the phialides are seen to be in whorls, branching from a discrete basal cell. The basal cell is wider than the phialide and square shaped, *ca* 1.2–1.5 µm wide (Fig. 9). We describe such conidiogenous cells as unbranched or occasionally branched at the base (the view of a cross section). *Cytospora* species with larger conidia, 5–6 µm in length, have conidiophores with one or more branches at mid-length, and larger phialides, 12–30 × 1.5–2 µm, in the hymenia (Fig. 9). We describe these conidiophores as occasionally branched above bases, or branched 1–3 times with 1–2 septa. We are uncertain what other investigators have meant when they state that conidiophores are unbranched or branched.

Conidiogenesis is enteroblastic phialidic and does not vary noticeably among *Cytospora* species. Phialides are long and slender, gradually tapering to the apices. A minute collarette is present at the apex but seldom evident at 1000 × phase contrast. Additionally, periclinal thickening occurs just below the collarette but this is not resolvable with many research microscopes. Electron micrographs of phialides, collarettes and periclinal thickening show that the apex of the collarette grows thicker and flares as successive conidia are liberated (Fig. 9).

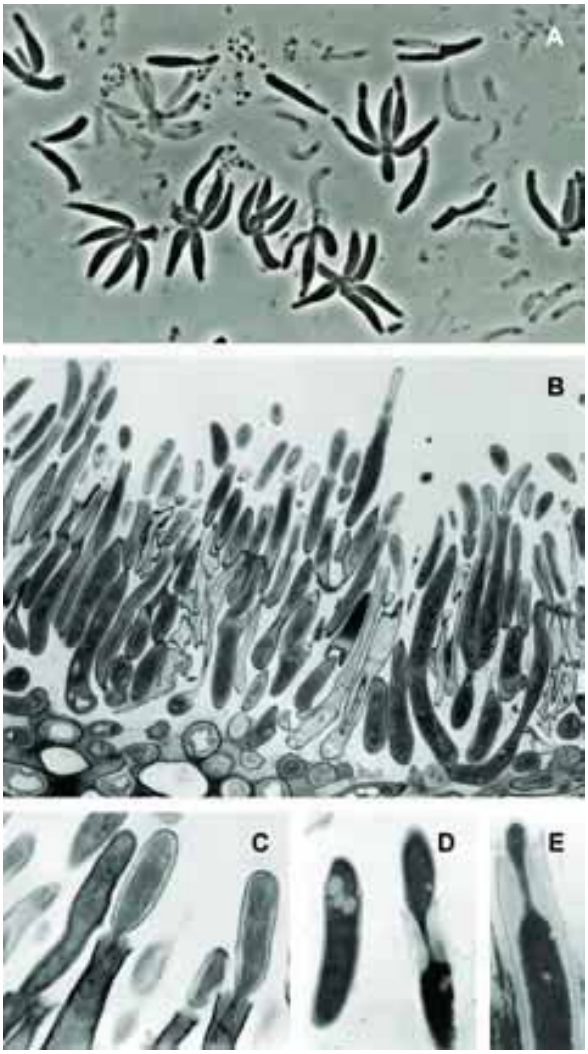

Fig. 9. Conidiophores and conidiogenous cells. A. Whorls of conidiogenous cells branch from a common basal cell. B. Transmission electron micrograph (TEM) of conidiophores in the hymenium of *C. australiae*. C. TEMs of phialides show collarettes and periclinal thickenings at the apices. Apical pores of phialides of *C. australiae* have short narrow channels (left) compared to those of *C. acaciae* that have long narrow channels (right). Conidium of *C. acaciae* (centre) has lipid globules visible at one end.

Rarely, sterile elements are seen in the hymenia of conidiomata of *Cytospora* species (Fig. 10), such as the anamorph of *L. sequoiae* (Bonar 1928) and *C. exigua* Sacc. (Gvritishvili 1982). They might be trichogyne-like elements with a function involving fertilisation. They grow into or form in a conidioma among the conidiophores. Presence of sterile elements varies among the conidiomata of a collection. Probably, the sterile elements are not a reliable character for distinguishing a species.

Conidia of *Cytospora* species are thin-walled, hyaline, aseptate and allantoid. The amount of relative curve in conidial shape does not vary noticeably among species, and no species has lunate or falcate conidia. Differences in conidial width are distinctive and useful in differentiating species. Unfortunately many descriptions in the literature are inaccurate in this measure. A difference in relative conidium width of 1 µm diam versus 1.5 µm diam is significant (a large difference) and readily distinguishes different species. Unfortunately, the width of conidia in descriptions of type material often is reported as wider than modern measurements show. Tendrils and globules of conidia oozing out of conidiomata vary in colour from beige, to yellow, orange, and red. Yellow is most common. The colour does not originate with the conidium so it must be a feature of the gelatinous matrix.

Morphology of teleomorphs

Stroma or tissue supporting the perithecia: *Valsa* species form ascostromatic tissues that contain some interspersed host cells and, therefore, the stromata are considered to be pseudostromata (Spielman 1985). Ascostromata vary distinctly among species; and subgenera and sections of *Valsa* are based on the characters of stromata. New descriptive terms are substituted for the characteristics of ascostromatic tissues that distinguish the former sections. Some characters of ascostromata are prone to be unstable. For example, Spielman (1983, 1985) observed that ascostromata vary in both presence and abundance of discs, entostromata, and ectostromata among different collections of a taxon, especially when bark thicknesses differ and when the host species differ. We report such differences for *V. eucalypti* on *Eucalyptus* versus on *Sequoia*. Additionally, Spielman (1983, 1985) reported great variability among ascostromata collected at different developmental stages from an individual tree. Ascostromata of *Valsa* on *Eucalyptus* have been reported rarely in the past, and collections described herein are rare and important compared to collections of the anamorphs. Ascostromata on *Eucalyptus* are not observed to occur on multiple hosts at a location, and the collection locations have seldom been re-visited and re-sampled.

The variable character of ascostroma that most hinders determinations is the presence of the conceptacle in a species. *Valsa eucalypti* is illustrative as only some of the specimens in the exsiccata of Ellis & Everhart (1892) exhibit the conceptacle. Among anamorphs, absence of the conceptacle is more common. Conceptacles may vary from absent to pale brown to distinct and black in a single collection.

Variable characters of ascostromata include the number and arrangement of ostioles in the disc, and the length of ostiolar beaks above the bark surfaces. *Valsa pini* and *V. malicola* have the diagnostic feature of teleomorph ostioles occurring around the periphery of the discs. Our collections of *V. malicola* on *Malus* from North America and South Africa are consistent in ostiole arrangement. Arguments concerning lack of stability in ostiole characteristics are confounded

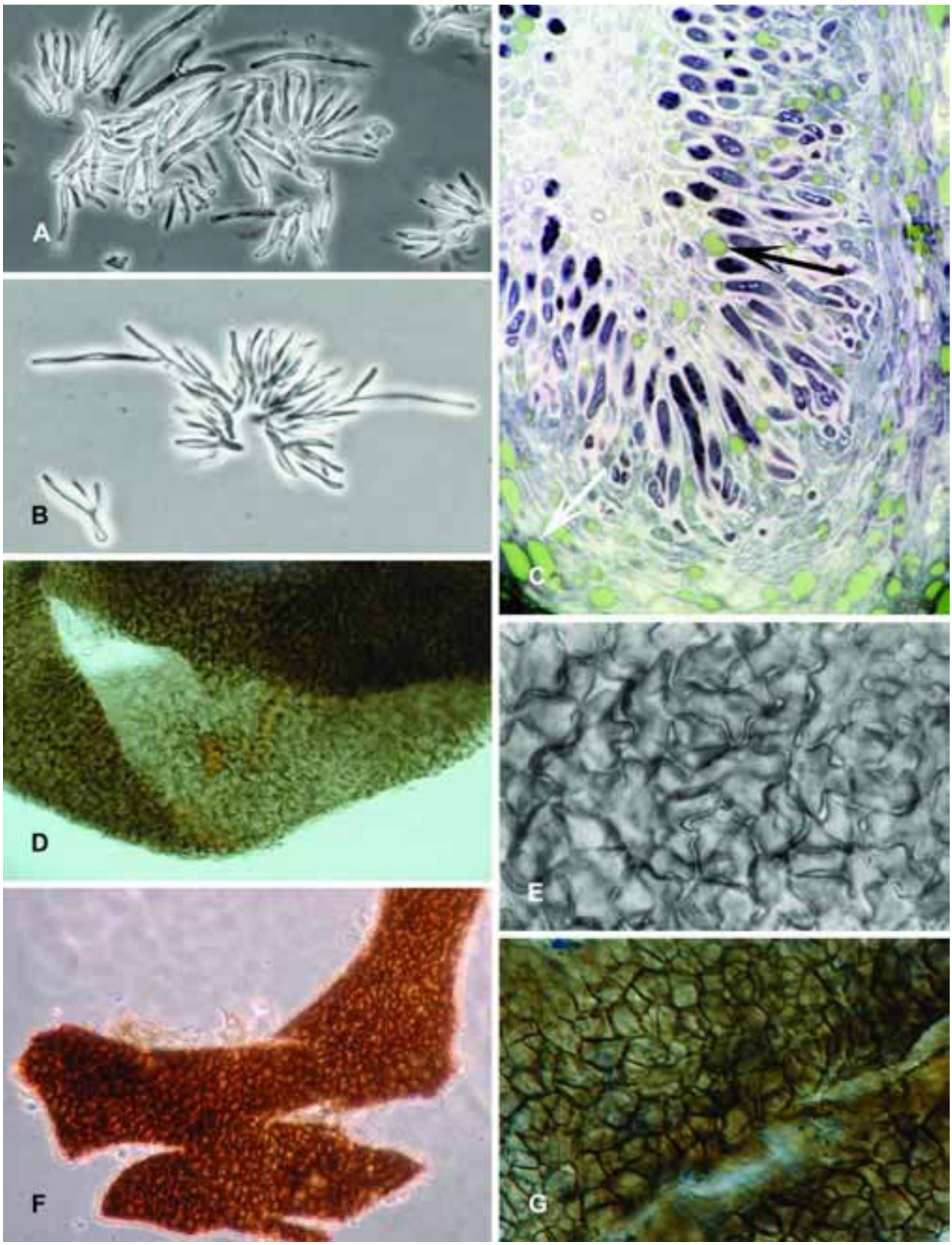

Fig. 10. Cells and tissues. A. Filamentous sterile elements with thick gelatinous coatings among conidiophores in the hymenium of *C. austromontana*. B. Thin-walled filamentous sterile elements in hymenium of *C. eucalypticola*. C. Cross section of the centrum or ébauche of *V. eucalypti* with white arrow pointing to lipid globules in the cells of the perithecial wall, black arrow pointing to lipid globules in cells of paraphyses among asci. The centre of the ébauche is filled with tightly packed paraphyses. D–E. Two views of a perithecial wall of *V. cinereostroma* with *textura epidermoidea*. F–G. Two views of a perithecial wall of *V. brevispora* with *textura angularis*.

by the difficulty of identifying biological species and the inaccuracy of determining which species might be synonyms. For example, a worker who reduces species to synonymy may argue for ostiole number and arrangement being variable characteristics in a species, whereas a taxonomist that follows a more limited species concept may argue otherwise. Now, DNA sequence homology can be utilised to test arguments regarding synonymy, and to clarify questions relating to the morphological variation inherent in a species.

The shape of discs varies from circular to lenticular in many specimens and the orientation of ostioles in the discs varies from circular, clustered, separate and crowded (Fig. 11). Sometimes, discs are obscured by the dense aggregation of the ostioles or ostiolar beaks (Fig. 11). The occurrence and thickness of discs also varies greatly. Some of the variation is due to the extent a disc is erumpent through the bark. The disc is usually white in leucostomoid and polysporous leucostomoid *Valsa*. Collections may show variation in disc colour, with one specimen having white discs and entostromata, while another has dull grey discs and entostromata. This variation often is interpreted as being due to the more advanced age of the second specimen at the time of collection.

Length of the beak on host tissue does not appear to be a reliable diagnostic character because collections with long beaks invariably have nearby stromata with ostioles at the same level as the disc surface (without beaks). However, sometimes the length of beaks has been impacted by breakage. Beak length may vary in neighbouring stromata from 3–8 fold (see *V. eugeniae*). However, in culture under controlled environmental conditions presence or absence of long necks/beaks is a relatively stable character. Certain species reliably form long necks/beaks in culture for isolates from variable locations.

A useful character for differentiating species of *Valsa* is the abundance and extent of stromatal tissue occurring from beneath the disc downward to the globe of the perithecium. Ectostroma may extend from the disc to surround the ostiolar necks for a portion of their length. Entostroma may extend for the entire length of the neck or may encompass part or all of the perithecial globes. Entostroma has been described as poorly-developed in circinateous *Valsa* species while generally well-developed in monostichous species. The extent of the stromatic tissues is considered a significant morphological trait but the reliability of this trait has not been experimentally determined.

Orientation and number of perithecia in ascostromata: Studies of *Valsa* species from North Temperate regions generally report that species cluster into two morphological groups based on size of perithecia, asci, ascospores, and arrangement of perithecia. Nitschke (1867–1879) referred to these groups as *Circinatae* and *Monostichae* emphasizing the arrangement of perithecia. The species in *Circinatae* have inclined perithecia oriented in a circular pattern, at one level in the bark or wood. The species in *Monostichae* have upright perithecia oriented in crowded clusters and at variable levels in the bark or wood (see above for more detail). Leucostomoid species usually form ascostroma with inclined perithecia arranged circinately. However, orientation may differ on different host substrates. For example, the perithecia of *V. eucalypti* are upright on *Eucalyptus* and the ascostromata approach a conical form, whereas on *Sequoia* the perithecia are dramatically inclined and the ascostromata are discoid. Additionally, perithecia are less crowded in the ascostromata on *Sequoia*.

Ascomatal ontogeny and the hamathecium: The term hamathecium refers to the interascal tissues and all hyphal elements and tissues projecting into the cavity and ostiole of ascomata (Eriksson 1981). The hamathecium or centrum of the *Diaporthales* was originally described by Luttrell (1951) as the *Diaporthe* centrum type, based on the perithecia of *Diaporthe* and the asci of *Endothia* Fr. Luttrell conceived centrum types as characteristic of various phylogenetic groups. Traditionally, the *Diaporthe* centrum type is viewed as follows: a pseudoparenchymatous centre lysed by the developing asci and lacking paraphyses; asci that deliquesce from the hymenium and float freely in the cavity; asci that do not forceably discharge spores; and, asci with a non-functional, chitinoid, non-amyloid, refractive apical ring. However, paraphysis-like cells have been noted by workers, such as Tulasne & Tulasne (1863), in *Valsa* and in other species of *Diaporthales*. Paraphysis-like broad bands of cells have been reported as present in the centrum but typically absent in mature perithecia (Barr 1978), but Munk (1957) challenged this view and argued that all fresh collections of diaporthaceous fungi contain paraphyses.

Concepts slowly changed during the 1980's consistent with careful study of perithecial development in *Diaporthe* (Jensen 1983, Uecker 1988) and *Gnomonia* Ces. & De Not. (Fayret & Parguey-Leduc 1975, Huang & Luttrell 1982). Paraphyses are now recognised as common in the *Diaporthales* (Jensen 1983, Letrouit-Galinou *et al.* 1994). Jensen (1983) noted that uninucleate paraphyses form at the centre of the perithecial initial and are surrounded by several layers of circumferentially oriented multinucleate pseudoparenchymatous cells. Paraphyses may arise from mixed origins, traceable to both ascogenous cells in the hymenium and non-ascogenous envelope hyphae beyond the hymenium, which challenges the long-held view of the separation of sexual and somatic

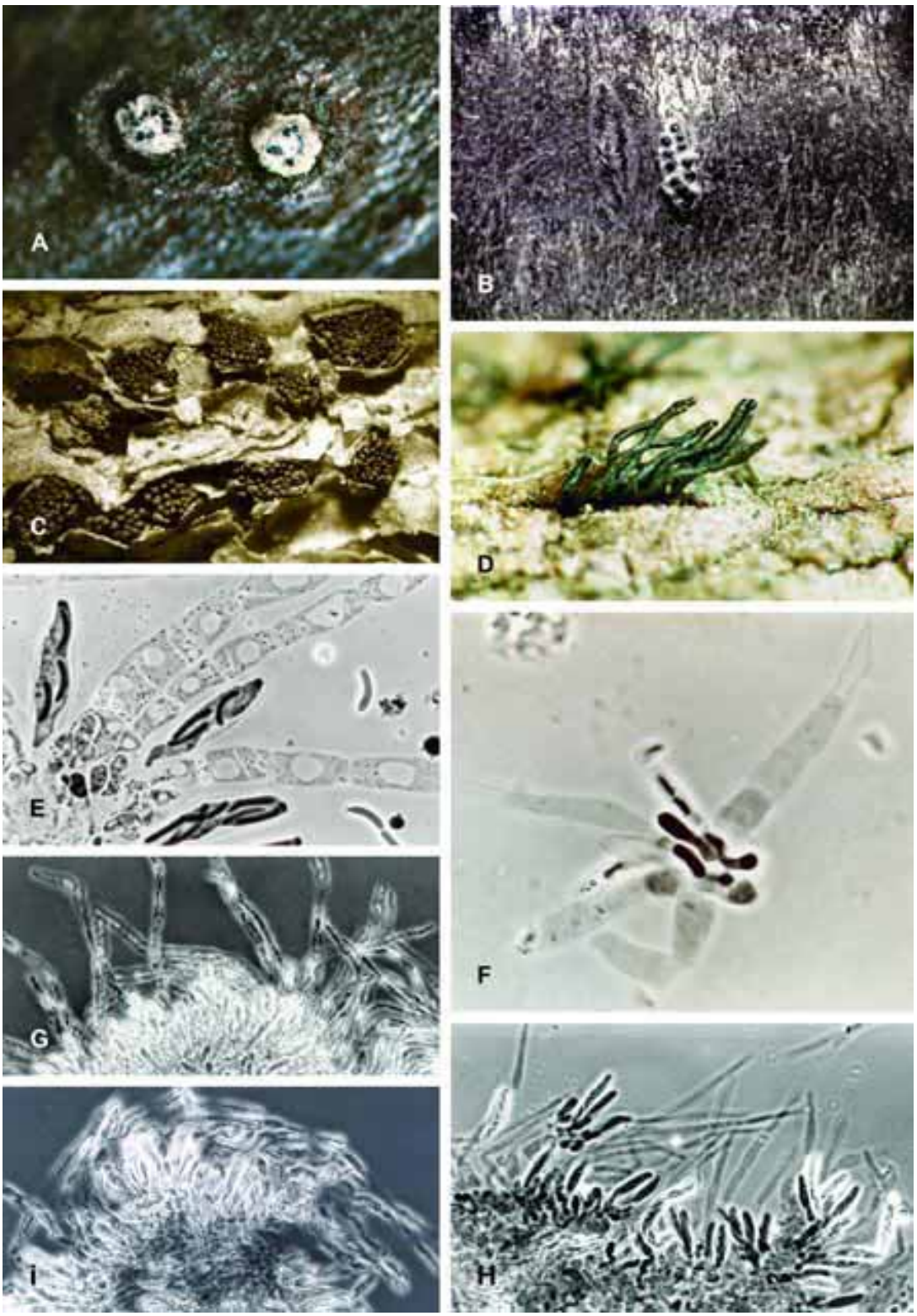

Fig. 11. Discs and paraphyses of ascostromata: A. White circular discs and circular arrangement of spaced black ostioles typical of *V. cincta*. B. Lenticular white disc with linear arrangement of black ostioles typical of *V. leucostoma*. C. Tightly packed ostioles obscuring discs in an undescribed species of *Valsa* from *Pinus radiata*. D. Clustered beaks in obscured disc typical of *V. eugeniae*. E. Long and wide septate paraphyses with 5–6 cells, each cell having a lipid globule, attached to hymenial cells among mature and immature asci of *V. cincta*. F. Wide tapering paraphyses with 2–3 cells arising from the same ascogenous cells as the asci in *V. myrtagena*. G. Filamentous paraphyses with disorganised cellular contents in hymenium of *V. cinereostroma*. H. Filamentous paraphyeses with uniform cytoplasmic contents in hymenium of "*V. eucalypticola*". I. Filamentous paraphyses with disorganised cellular contents in hymenium of *V. eugeniae*.

systems (Jensen 1983). Jensen (1983) reported that during ascus maturation in the ébauche (the perithecial primordium with differentiated peridium), the centrum pseudoparenchyma and the paraphyses become compressed against the peridium or pericentral envelope. The compressed pseudoparenchyma form a subhymenial layer that is reminiscent of an inner hyaline layer of a two-layered perithecial wall. Uecker (1989) concluded that paraphyses degenerate at maturity. Perhaps a reason why the paraphyses generally are not noticed is that they do not become free from the hymenium with the asci.

The ontogeny of *Valsa* can be extrapolated from the study of *Diaporthe phaseolorum* by Jensen (1983). His description is reorganised, paraphrased and presented as follows: Ascospores or conidia germinate to form hyphae of several types, generally narrow hyphae with few nuclei and wide hyphae with many nuclei. Stromatal initials are the first recognisable structures with ectostroma composed of hyphae of *ca* 15 µm wide forming multinucleate (*ca* 15 nuclei per cell) pseudoparenchymatous cells. Entostroma underlying the ectostroma is composed of uniformly narrow hyphae (*ca* 2–3 µm wide) that subsequently form uninucleate prosenchymatous cells. Conidiomatal initials form in the ectostroma by numerous hyphae entwining at the surface of the substratum. Perithecia are initiated in the prosenchymatous entostroma near the periphery of the ectostroma. The stroma is delimited eventually by compact prosenchymatous cells forming a dark-celled zone line. The loosely coiled ascogonia arise from typical uninucleate entostromatal cells. Groups of intensely staining ascogonial coils enlarge and become multinucleate. Many ascogonial cells are soon directly connected to the conidioma by long intensely staining hyphae. These hyphae intercalate between the pseudoparenchymatous cells of the ectostroma and in some cases terminate at the conidial hymenium. Around the ascogonial coils, a perithecial primordium begins to develop. In the earliest stages the primordium has a centre of uninucleate cells that gives rise to paraphyses surrounded by multiple layers of multinucleate circumferentially oriented cells. External multinucleate cells become thick-walled, *textura epidermoidea* to *textura angularis* and form the peridium as the primordium begins to differentiate, becoming the ébauche. Synchronously with enlargement of the primordium, binucleate ascogonial cells are produced by the septation of the inner coil cell of the ascogonium. The ascogenous cells become distributed as a layer (i.e., do not ramify as branching) of easily separable cells forming a bowl-shaped hymenium in the lower portion of the ébauche. Paraphyses continue to originate and enlarge from chains of uninucleate cells that are associated with both binucleate ascogenous cells in the lower hymenium and multinucleate pseudoparenchyma cells beyond the hymenium. The free terminal cells of the paraphyses become binucleate. The apical region of the ébauche develops to form an incipient neck that is produced exclusively from peridial cells. Uninucleate periphyses are produced from the multinucleate peridial cells in the neck and from the uppermost centrum pseudoparenchyma cells. Cells of the ascogenous system produce croziers near the cessation of perithecial enlargement. Asci begin to enlarge and detach by schizogenous separation (not by deliquescence) at a septum between ascus and crozier before maturity, and spores continue to mature in the free asci. During this development, the paraphyses and centrum pseudoparenchyma are compressed. Some paraphyses are obliterated but many compressed paraphyses remain at maturity. Pressure developed inside the perithecium forces intact asci up the neck then asci forceably eject the ascospores.

Uecker's (1989) account of perithecial development provides several unique observations. Uecker reported that the perithecium begins as ascogonia wrapped in 2–3 layers of narrow hyphae. A cavity is obvious at the time that paraphyses reach their maximum length and when croziers and young *Asci* are being produced in increasing numbers. The centrum pseudoparenchyma still occupies a major portion of the centre at that time. The centrum pseudoparenchyma was viewed as a nutritive tissue that is exhausted at maturity (Wehmeyer 1933, Parguey-Leduc & Chadefaud 1963, Uecker 1989).

Uecker (1989) noted that the conidiomata begin as a ball of radially oriented hyphae. Enlargement occurs by addition of cells to the periphery and also expansion of cells already present, until the mass becomes erumpent through the plant epidermis. He believed that conidiomata sometimes form as pycnidia and are sometimes stromatic. Uecker (1989) did not believe that trichogyne-like hyphae enter the conidiomatal hymenium. However, he reported that hyphae form connections between the ascoma and conidioma, and hyphal elements are rarely seen in the conidiomatal hymenium. Trichogyne-like elements in conidiomata that we observe appear connected to the hymenial cells alongside the conidiophores (Fig.10).

The development of the *Diaporthe* type centrum is unusual because uninucleate systems are found to arise from multinucleate, binucleate and uninucleate cells. The sterile hyphae surrounding the ascogonium and forming the primordium of the ascoma of *Valsa* branches in an arbuscula, rather than a palisadic or plectenchymatous pattern (Parguey-Leduc 1967, 1973, Parguey-Leduc & Janex-Favre 1981). Ascogenous cells multiply by septation rather than by branching and ramification. Paraphyses are produced from both ascogenous cells and somatic cells. Additionally,

asci discharge ascospores in a united mass. Jensen (1983) concluded that the differentiation of cells in the perithecium appears to depend on the final location of the differentiated cells rather than from the cell type of origin.

The Luttrellian concept of the *Diaporthe* centrum type has been modified based on understanding that; 1) the refractive apical annulus of the ascus is functional in forcible discharge of the ascospores, 2) the bases of asci detach by schizogenous separation at a septum between ascus and crozier, and 3) paraphyses are present, and that paraphyses commonly are more filamentous than broad bands of paraphysis-like cells (Fig. 11). The *Diaporthe* centrum type is now viewed as less phylogenetically unique. Rather, it appears to represent part of a continuum of similar developmental types, the *Xylaria*-, *Sordaria*-, and *Diaporthe*-type (Jensen 1983, Spatafora & Blackwell 1994). Additionally, the *Diaporthe* centrum type displays some similarities to that of *Ophiostoma* (van Wyk & Wingfield 1991a, b). Both centrum types share centrum pseudoparenchyma, a basal hymenium, asci that become free in the cavity, and broad paraphysis-like elements.

In *Valsa*, perithecial walls appear bilayered, olivaceous to medium brown in the external layer, and golden to hyaline in the inner layer. The external wall is *textura epidermoidea*, with cells resembling jigsaw puzzle pieces, or *textura angularis* (Fig. 10). Rarely, a species has cells that are more square in shape. The wall of the beak approaches *textura porrecta*. Perithecial walls do not become crust-like or sclerenchymatous, as do the conidiomal (pycnidial) walls.

On host tissue, perithecia within a single ascostoma are generally at the same level of developmental maturity but perithecia on smaller diameter branches can reach maturity before those on larger branches. Careful examination of a specimen with predominantly empty perithecia may occasionally uncover a less mature stromata more deeply embedded in the host tissues.

The ascus: The asci of *Valsa* spp. are infissitunicate, inoperculate, unitunicate annellasce with a refractive apical ring that is chitinoid and non-amyloid. Dughi (1957) provided the term infissitunicate to characterise the manner of ascus dehiscence without prejudicing the concept of the ascus wall construction. The ring in the apical apparatus of the ascus of *Valsa* appears to be composed of the upper and lower rings of the two-ringed annellasce (Fig. 12) envisioned by Chadefaud (Parguey-Leduc & Chadefaud 1963, Chadefaud 1982, Parguey-Leduc *et al.* 1994), except in a few species such as *Valsa microstoma* (Pers. : Fr.) Fr. (Parguey-Leduc & Chadefaud 1963). A subapical region that stains with chlorazol-black is present below the ring (Fig. 12) in many preparations. A distinctive thin concave zone also occurs immediately below the ring in TEM images of the ascus apical apparatus (Fig. 13). Parguey-Leduc & Chadefaud (1963) believe that asci show a central strand or string-like structure. The string structure is attached to the base of the refractive ascus ring and winds among the ascospores. This structure was not seen in our TEM images of asci (Fig. 13). Parguey-Leduc & Chadefaud (1963) believed that the ascus tip ruptures during forcible discharge and that the ring and the spores united by the string are ejected together, in mass. This mode of discharge is referred to as "pseudo-jack-in-the-box" and the string-like structure is called the "tractus" (Parguey-Leduc & Chadefaud 1963). The release of the refractive apical ring from the ascus, as described in the pseudo-jack-in-the-box ejection, is seen occasionally in microscope mounts (Fig. 12). However, spore dehiscence following pressure applied to a slide mount is unlikely to accurately represent natural spore ejection. The release of the ascus ring accompanying the ascospores, presence of a tractus, and the "pseudo-jack-in-the-box" discharge are difficult to accommodate in current concepts of ascus function. They are likely artifacts of preparation of slide mounts and staining treatments. The mechanism of ascospore ejection in *Valsa* deserves investigation.

Isolation and baiting

Single ascospore colonies can be obtained by soaking ascocarps in water and suspending them above agar media as the asci forcibly discharged their spores after they ooze out in a cirrhus. Commonly, cultures of *Valsa* species are obtained by cutting a fruiting body in half horizontally and applying a drop of water or wetting agent to the exposed chambers. Once swelling of the gelatinous matrix occurs a portion of the spore mass can be lifted out and spread across the surface of an acidified or antibacterial antibiotic amended agar medium. Conidia and ascospores of the fungus on host tissue generally lose viability following 2 yr at room temperature, but freezing at –20 ºC extends viability.

Standard forest pathology methods are usually successful for isolating the pathogen, including brief flaming of branch surfaces followed by cutting to expose the canker margin and isolating tissue pieces from the margin onto malt extract agar (MEA). Standard plant pathology methods for isolation where *ca* 0.5 % sodium hypochlorite is used to surface disinfest the tissue prior to culturing on acidified potato-dextrose agar (PDA) are also effective. Isolation of endophytic *Cytospora* spp. from xylem usually involves removal of bark, brief alcohol treatment followed by 5 % sodium hypochlorite (full strength household bleach) treatment for 5 min, a wash in sterile distilled water and isolation onto MEA with or without antibiotics such as 50 ppm chloramphenicol (Bettucci & Saravay 1993,

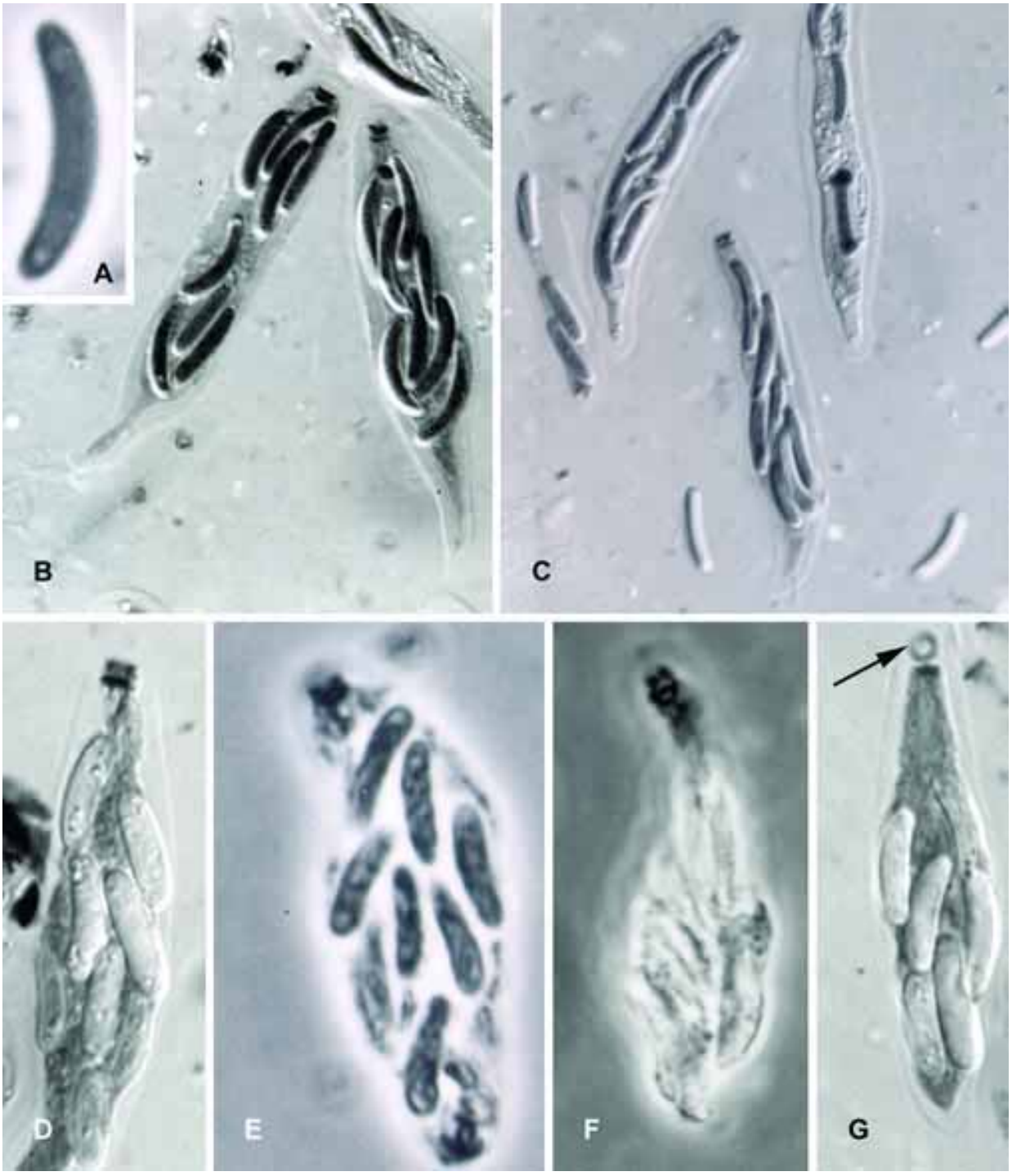

Fig. 12. Asci, croziers, and ascospores of *Valsa* species: A. Hyaline allantoid ascospore of *V. cincta* with one lipid globule at each end. B–C. Asci of *V. cincta* with apical apparatus stained with chlorazol-black. An area corresponding to the refractive ring and a second lower area at the topmost point of the cytoplasm stain black. D. Ascus of *V. sordida* with stained apical apparatus. E. Ascus of *V. eugeniae* with stained apical apparatus and ascospores with lipid globules. F. Ascus of *V. cinereostroma* with stained apical apparatus. G. Ascus with ring apparatus broken free and fully visible (arrow). The lower area of staining remains intact at the top of the cytoplasm.

Smith *et al.* 1996), and 400 ppm chloramphenicol with 50 ppm cycloheximide (Bills & Polishook 1991).

Employing fungitoxicants in media to improve isolation of a genus or species depends on differential sensitivity among genera or species to the toxicant. A comparative study by Micales & Stipes (1986) of sensitivities of *Endothia* and *Cryphonectria* species (*Diaporthales*) to 21 fungitoxicants showed no differences in sensitivities except for differential responses to cycloheximide. Differential sensitivities are typically expressed at the species level. Because literature on *Valsa* reports contradictory values for sensitivity to cycloheximide (see below) this fungitoxicant needs to be further investigated for work with *Valsa* spp. A break point of 1–2 ppm cycloheximide revealed differential sensitivities among species of *Endothia* and *Cryphonectria*, including *Endothia viridistoma* Wehm. (Micales & Stipes 1986). The latter species is now known to be a *Valsa* (Myburg *et al.* 2004, G.C. Adams & M. Gryzenhout, unpubl. data).

Green apple fruit, usually cv. "Granny Smith", can be used for baiting to improve isolation of the pathogen from stone fruit cankers. This method has yielded a greater proportion of *C. cincta* isolates than standard direct isolation from cambium onto agar medium (Wang *et al.* 1998). Additionally, apple fruit are useful in virulence comparisons among isolates of some *Cytospora* species, such as *C. leucostoma* (Hammar *et al.* 1989, Jensen & Adams 1995, Wang *et al.* 1998). Mean lesion size and colour varies between species. For example, lesions have a mean diameter of 19 mm (range 10–26 mm) and are dark brown in colour for 50 isolates of *C. cincta*, whereas, lesions have a mean diameter of 43 mm (range 31–61 mm) and are non-pigmented for 50 isolates of *C. leucostoma* (Jensen & Adams 1995, Wang *et al.* 1998). The lesions have distinctive characteristics that are helpful in isolations of *Cytospora* from cankers on fruit trees. In this instance lesion colour and size can be considered species-specific. However, not all species of *Cytospora* will infect green apple fruit following inoculation.

Culturing

Many standard agar media are suitable for cultivation of *Valsa* species and Leonian's medium (Leonian 1923) is excellent for this purpose. This medium includes 6.25 g malt extract, 6.25 g maltose, 0.6 g peptone, 0.625 g $MgSO_4 \cdot 7H_2O$, 1.25 g KH_2PO_4, and 20 g agar /L. The most common medium used in monographic studies of *Valsa* species is KHG medium: 20 g glucose, 5 g yeast extract, 1 g $CaNO_3$, 0.25 g KH_2PO_4, 0.25 g $MgSO_4$, 0.25 g KCl, 0.01 g $FeCl_3$, 18 g agar /L. A vitamin stock, such as that of Beadle & Tatum (1945), should be added to media, routinely, as many *Cytospora* species require several vitamins including biotin, thiamine (= vitamin B1 aneurine), inositol and pantothenic acid (= vitamin B5) (Fries 1938, Défago 1942, Hubbes 1960a, b, Lukezic *et al.* 1965, Kastirr 1985). Vitamins stimulate growth as well as fructification (Hubbes 1960b, Kastirr 1985). Kastirr (1985) found that thiamine and biotin (only in combination) stimulate pycnidium formation in several nutrient media for four species of *Cytospora*. Utilisation of carbon sources by *Cytospora* isolates was examined by Helton and Konicek (1962a). The preferred carbon source is maltose (Helton & Konicek 1962a) or starch; poorest growth is usually on xylose (Hubbes 1960a). Helton & Konicek (1962b) also considered utilisation of nitrogen sources.

Nitrogen utilisation by *C. leucostoma* and *C. cincta* is a species-specific character and in some instances, nitrate utilisation is a strain-specific character (Jensen & Adams 1995). *Cytospora leucostoma* is capable of utilising, as sole nitrogen source, all of 47 nitrogen compounds except methylated purines and pyrimidines, anthranilic acid, cysteine, homocysteine, hydroxylamine, indole, lysine, and urocanic acid. *Cytospora cincta* has the same limitations and, additionally can not utilise acetamide and the branched side-chain aliphatic amino acids (isoleucine, leucine, and valine) as sole nitrogen source. One isolate of *C. cincta* (ATCC 62910) is unable to utilise nitrate and nitrite. The sole nitrogen source that produces the greatest growth, at 50 mM nitrogen equivalents and 350 mM carbon equivalents, is allantoin followed by asparagine in *C. leucostoma*, but in *C. cincta* it is serine followed by histidine.

A strain of *C. leucostoma* infected with virus and exhibiting low virulence on *Prunus* did not utilise nitrate or nitrite as sole nitrogen source, and exhibited a global regulatory deficiency in nitrogen metabolism that could be a mechanism for the reduced virulence (Jensen & Adams 1995). Infection by double-stranded RNA mycoviruses may affect nitrogen metabolism in strains of *Cytospora* more often than is commonly realised.

Species such as *C. leucostoma* that form colonies with highly lobate margins grow poorly on weak media, particularly powdered commercial preparations of PDA. In our experience, vitamins do not affect this pattern but the margins grow nearly uniformly on clarified oatmeal agar (Adams *et al.* 1990) or V8® juice agar (Pluim *et al.* 1994), and the distinct character trait becomes masked.

Successful isolation of endophytic *Cytospora* species from bark has been reported by Bills (1994) using a specialised isolation medium (Bills & Polishook 1991). The medium contains 400 ppm cycloheximide, 50 ppm chloramphenicol and phytone (10 g/L) added to Mycosel® agar (BBL Laboritories, MD, U.S.A.). Growth of *Cytospora* species on that level of cycloheximide is surprising because, in previous studies, *C. leucostoma* did not grow on media

amended with 2 ppm (Hammar *et al.* 1989). Therefore, the relative tolerance to this antibiotic may be a character useful in distinguishing species in culture.

Most *Cytospora* species grow poorly when submerged in broth, regardless of shaking for aeration.

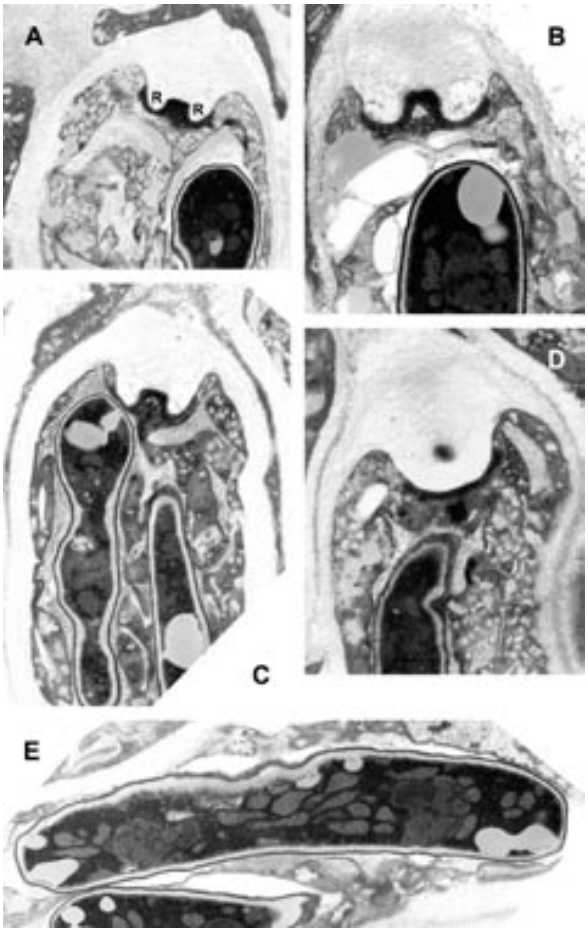

Fig. 13. Transmission electron micrographs of asci, ascospores, and apical apparatus in *V. eucalypti* stained with uranyl acetate and lead citrate. A–D. The walls, ring (R), and apical apparatus do not stain and are therefore presumably of polysaccharide. A darkly staining structure adheres to the underside of the ring. The dark structure is thin and limited in the extent that it covers the periphery of the underside of the ring. The apical ring apparatus protrudes into the lumen or cytoplasm of the ascus and the dark structure enters part of the channel in the centre of the ring. The dark structure might have a function in maintaining the shape of the ring. Lipid globules can migrate partially into the channel and are also visible in the top region of the cytoplasm. Cytoplasm of the spores stains darker than cytoplasm of the asci. The thickness of a spore is much wider than the apical pore therefore the pore must dilate considerably during ejection of spores. E. A longitudinal view of an ascospore shows two nuclei, many mitochondria, and lipid globules near both ends of the spore. The wall of ascospores is bilayered with a thick inner layer that does not stain and a thin outer layer that stains black and therefore presumably contains protein. Among the stained contents of the asci a tractus is not visible.

However, lowering the depth of the broth to the height of an inoculum plug usually permits vigorous growth in stationary culture. Surprisingly, Gurusiddaiah *et al.* (1980a, b) reported that a grahamimycin producing strain of *Cytospora* sp. grew equivalently without air (they use the term "anaerobic") as with aeration, in a fermentation vat over 10 d. The species that have been the most difficult to cultivate in our laboratory are those that formed pigments in the broth or agar, such as *V. pini*, *V. friesii*, and *V. ambiens*. Growth stagnates in agar or broth. DNA extractions are difficult to amplify with PCR and we have found that amplification is increasingly inhibited with increasing levels of pigment present.

Storage of cultures

Cultures remain viable for at least two years on agar slants, and older slants (6-yr-old) can be revived. This is true whether the cultures are stored at room temperature or at 4 ºC. Pouring molten agar medium at 50 ºC over the surface of an old slant may initiate growth where transferring a piece of the culture to new medium fails. This appears to be due to survival of the fungus in areas of the slant where the agar has dried down to the glass. We use slants in screw cap vials and tighten the caps after respiration is no longer sufficient to create pressure in the vial. Covering such cultures with sterile heavy mineral oil retains viability nearly indefinitely. We back up this storage method with cultures stored at –20 ºC in soil amended with 10 % w/w wheat bran. The soil is moist and sterile when inoculated but air-dried after growth and before freezing (Butler 1970). Cultures in such soil tubes retain viability for over 15 yr. Conidia and agar plugs also survive well in 15–20 % glycerol at –80 ºC. Storage in water is not effective as most cultures die before the second year. Storage in silica gel also has not succeeded in our experience. Fruit bodies on natural substrates are vital for 2 yr. Freezing at –20 ºC extends viability to several years.

Herbarium specimens from nature

Diagnostic characteristics of the species, whether teleomorphic or anamorphic, are only formed on living plants in nature. Critical morphological studies require such material. Teleomorphs do not readily form on inoculated hosts. Anamorphs can be produced by inoculating hosts, but often *Cytospora* strains are not virulent without the complex application of a threshold level of environmental stress. In temperate climates the period of delay is many months as inoculations are done during early dormancy (November) and harvesting of material with conidiomata is done during mid to late spring (May). Herbarium specimens from inoculation of host tissue are utilised as type material in the description of one species in this work.

Table 1. Taxa studied. Isolates arranged by cladogram code and geography for Figs 14–15.

Taxon	Geographic origin	Host *E.* = *Eucalyptus*	Specimen	Culture	Cladogram code	GenBank
Cytospora eucalypticola	Australia	*E. marginata*	–	CBS118084; ATCC56123	Australia-1	AF192314[a]
	Tasmania, Australia	*E. delegatensis*	–	ATCC96149	Australia-1	AF192314
	Batlow, NSW Australia	*E. delegatensis*	–	C.M.W. 1158[b]	Australia-1	AF192314
	Batlow, NSW Australia	*E. delegatensis*	–	CBS116847; C.M.W. 1159	Australia-1	AF192314
Cytospora disciformis	Canberra, ACT Australia	*E. globulus*	–	CBS116828; C.M.W. 6750	Australia-2	AY347357
	Canberra, ACT Australia	*E. globulus*	–	CBS118083; C.M.W. 6751	Australia-2	AY347357
Cytospora eucalypticola	Canberra, ACT Australia	*E. globulus*	–	CBS116848; C.M.W. 3374	Australia-3	AY347359
	Cam River, VIC Autralia	*E. globulus*	–	CBS116849; C.M.W. 6747	Australia-3	AY347359
	Cam River, VIC Autralia	*E. globulus*	–	CBS118085; C.M.W. 6748	Australia-3	AY347359
Cytospora austromontana	Perisher V, NSW Australia	*E. pauciflora*	MSC380693[c]	CBS116820; C.M.W. 6735	Australia-4	AY347361
Valsa eugeniae, Cytospora state	Brisbane, □LD Australia	*Tibouchina* sp.	–	CBS116838; C.M.W. 7029	Australia-5	AY347364
	Brisbane, □LD Australia	*T. heteromalla*	–	CBS116839; C.M.W. 7030	Australia-5	AY347364
Cytospora variostromatica	Orbost, VIC Australia	*E. globulus*	MSC380695	CBS116858; C.M.W. 6766	Australia-6	AY347366
	Kyogle, NSW Australia	*E. grandis* × *camaldulensis*	–	CBS116859; C.M.W. 6746	Australia-7	AY347367
Cytospora diatrypelloideae	Orbost, VIC Australia	*E. globulus*	MSC380719	CBS116826; C.M.W. 8549	Australia-8	AY347368
Cytospora austromontana	Perisher V, NSW Australia	*E. pauciflora*	MSC380694	CBS116821; C.M.W. 6736	Australia-9	AY347362
Valsa fabianae	Tasmania, Australia	*E. nitens*	DAR43948[d]	CBS116840; ATCC 96150	Australia-10	AY347358[b]
Cytospora subclypeata	Australia	*Ulmus procera*	–	CBS 116856	–	AY347326
Cytospora variostromatica	Orbost, VIC Australia	*E. globulus*	MSC380696	–		
Valsa brevispora	Tchittanga, Republic of Congo	*E. grandis* × *tereticornis*	MSC368317	CBS 116811; C.M.W. 5260	Congo-1	AF192315
	Tchittanga, Republic of Congo	*E. grandis* × *tereticornis*	MSC368318	CBS 116812; C.M.W. 5261	Congo-1	AF192315
Cytospora nitschkii	Wondo Genet, Ethiopia	*E. globulus*	MSC380699	CBS 117606; C.M.W.10184	Ethiopia-2	AY347355
Cytospora abyssinica	Wondo Genet, Ethiopia	*E. globulus*	MSC380700	CBS 116189; C.M.W.10181	Ethiopia-6	AY347353
Cytospora nitschkii	Wondo Genet, Ethiopia	*E. globulus*	MSC380701	CBS 116854; C.M.W. 10180	Ethiopia-7	AY347356
Cytospora abyssinica	Wondo Genet, Ethiopia	*E. globulus*	–	CBS 117605; C.M.W. 10179	Ethiopia-9	AY347352
	Wondo Genet, Ethiopia	*E. globulus*	MSC380702	CBS 117004; C.M.W. 10178	Ethiopia-10	AY347354
Cytospora putative species 1	Piet Retief, South Africa (SA)	*E. grandis*	–	C.M.W. 5357	South Africa-1	AF192318

Table 1. (Continued).

Taxon	Geographic origin	Host *E.* = *Eucalyptus*	Specimen	Culture	Cladogram code	GenBank
	KwaMbonambi, SA	*E. grandis*	–	C.M.W. 5358	South Africa-2	AF192319
Cytospora variostromatica	Pretoria, SA	*E. camaldulensis*	–	CBS 118086; PPRI 5297	South Africa-3	AF260264
	KwaMbonambi, SA	*E. grandis*	–	C.M.W. 1237	South Africa-4	AF260263
	KwaMbonambi, SA	*E. grandis*	–	C.M.W. 1238	South Africa-4	AF260263
	KwaMbonambi, SA	*E. grandis*	–	CBS 116860; C.M.W. 1240	South Africa-4	AF260263
Cytospora eucalypticola	Seven Oaks, SA	*E. grandis*	–	C.M.W. 940	South Africa-5	AF260265
Valsa aff. *cinereostroma*	Amsterdam, SA	*E. nitens*	–	CBS118087; C.M.W. 1514	South Africa-6	AY347378
Cytospora aff. *austromontana*	Hermanus, SA	*E. grandis*	–	CBS 116822	South Africa-7	AY347379
Valsa aff. *cinereostroma*	White River, SA	*E. grandis* Clone	PREM 50454[e]	CBS116831; C.M.W. 6501	South Africa-8	AY347376
	White River, SA	*E. grandis*	–	CBS116832; C.M.W. 6502	South Africa-8	AY347376
	Wartburg, SA	*E. nitens*	–	CBS116833; C.M.W. 6503	South Africa-8	AY347376
Valsa cinereostroma, Cytospora state	SA	*Mangifera indica*	–	CBS116830	South Africa-9	AF260267
Cytospora eucalypticola	Newcastle, SA	*E. dunnii*	MSC 380697	CBS116851	South Africa-10	AY347360
	KwaMbonambi, SA	*E. saligna*	MSC 380718	CBS116853	South Africa-11	AY347369
Cytospora chrysosperma s. lat. 'Cytospora australiae'	Wellington, SA	*E. viminalis*	PREM 13072	–	–	–
Valsa eugeniae	Tanzania	*Eugenia* sp.	–	CBS118569; IMI 044946[f]	–	AY347344
Valsa fabianae	Entebbe, Uganda	*E. grandis*	MSC 368320	CBS116818; C.M.W. 5309	Uganda-1	AF260266
	Tororo, Uganda	*E. grandis*	MSC 368319	CBS116841; C.M.W. 5308	Uganda-2	AY347371
Cytospora eucalypticola	Mishenyi-Itojo, Uganda	*E. grandis*	MSC 380698	CBS118088	Uganda-9	AY347373
Valsa fabianae	Tororo, Uganda	*E. grandis*	MSC 368321	CBS116842; C.M.W. 5315	Uganda-12	AY347372
Valsa cinereostroma	Chile	*E. globulus*	MSC 375220	CBS 117081; C.M.W. 5700	Chile-1	AY347377
	Chile	*E. nitens*	–	CBS 117082; C.M.W. 5701	Chile-1	AY347377
Cytospora eucalyptina	Cali, Columbia	*E. grandis*	MSC 375217	CBS 116853; C.M.W. 5882	Columbia-1	AY347375
	Columbia	*E. grandis*	–	CBS 117011; C.M.W. 5883	Columbia-1	AY347375
	Mexico	*E. grandis*	–	CBS 117080; C.M.W. 516	Mexico-1	AF192317
	Mexico	*E. grandis*	–	C.M.W. 517	Mexico-1	AF192317
Cytospora disciformis	Uruguay	*E. grandis*	MSC 368323	CBS 116827; C.M.W. 6509	Uruguay-1	AY347374
Valsa brevispora, Cytospora state	Acarigua, Venezuela	*E. camaldulensis*	–	CBS 116813; C.M.W. 3393	Venezuela-1	AF192321
	Acarigua, Venezuela	*E. camaldulensis*	–	CBS 116829; C.M.W. 3394	Venezuela-1	AF192321

Table 1. (Continued).

Taxon	Geographic origin	Host *E. = Eucalyptus*	Specimen	Culture	Cladogram code	GenBank
Cytospora australiae	Argentina	*E. globulus*	LPS 31746[g]	–	–	–
Cytospora eucalyptina	Argentina	*E. globulus*	LPS 11656	–	–	–
Valsa eucalypti, Cytospora state	Palo Alto, California	*E. paniculata*	MSC 380708	CBS116816	California-1	AY347365
Valsa eucalypi	Mt. Tamalpais, California	*Sequoia sempervirens*	MSC 380714	CBS116814	California-1	AY347340
Valsa eucalypi, Cytospora state	Mt. Tamalpais, California	*Sequoia sempervirens*	MSC 380713	CBS116815	California-1	AY347340
Cytospora berkeleyi	Berkeley, California	*E. globulus*	MSC 380709	CBS116824	California-2	AY347351
	Palo Alto, California	*E. globulus*	MSC 380710	CBS116823	California-3	AY347350
	Palo Alto, California	*E. globulus*	MSC 380711	CBS117005	California-3	AY347350
	Berkeley, California	*E. globulus*	MSC 380712	CBS116825	California-4	AY347349
Valsa eucalypti	California	*E. globulus*	MSC 11472 MICH 15128	–	–	–
Cytospora berkeleyi	Berkeley, California	*E. paniculata*	UC 143778[h]	–	–	–
'Cytospora australiae'	Berkeley, California	*E. globulus*	UC 275812	–	–	–
'Cytospora australiae'	Berkeley, California	*E. globulus*	UC 500550	–	–	–
Valsa sordida	Colorado	*Salix* sp.	–	G.C.A.95-94[i]	–	AY347321
Valsa myrtagena	Hilo, Hawaii	*Tibouchina urvilleana*	MSC 380715	CBS116843	Hawaii-1	AY347363
Valsa ambiens subsp. *leucostomoides*	Illinois	*Acer negunda*	CUP 060135[j]	CBS116810	–	AY347348
Valsa ceratosperma s.lat.	Michigan	*Rhus typhina*	MSC 368322	CBS118090	–	AF192324
Cytospora sacculus s.str.	Michigan	*Quercus alba*	MSC 380716	CBS116855	–	AY347334
Valsa kunzei	Michigan	*Picea pungens*	MSC 380720	CBS118093; ATCC 64880	–	AY347320
	Michigan	*Picea pungens*	MSC 380721	CBS118094; ATCC 64881	–	AY347320
Valsa massariana	Michigan	*Sorbus aucuparia*	MSC 380722	G.C.A.Lmass	–	AY347338
Diaporthe vaccinii	Michigan	*Vaccinium corymbosum*	–	CBS118571	–	AF191166
Valsa ambiens subsp. *ambiens*	New Jersey	*Acer rubrum*	CUP 069132	CBS116809; ATCC 52279	–	AY347339
Valsa ambiens subsp. *leucostomoides*	New York	*Acer rubrum*	CUP060133	CBS118089; ATCC 52280	–	AY347346
	New York	*Acer saccharum*	CUP 060137	ATCC 52281	–	AY347347
Valsa pini	New York	*Pinus strobus*	–	CBS224.52	–	AY347316
Phomopsis vaccinii	Wisconsin	*Vaccinium macrocarpon*	–	ATCC 18451	–	AF317579
Cytospora agarwalii	Jabalpur, India	*Eucalyptus* sp.	IMI 249224	–	–	–

Table 1. (Continued).

Taxon	Geographic origin	Host *E. = Eucalyptus*	Specimen	Culture	Cladogram code	GenBank
'Valsa eucalypti' sensu Sharma *et al. Cytospora eucalypti nom. inval.*	Kerala, India	*E. grandis*	IMI 261564	–	–	–
Valsa eucalypticola nom. inval.	Kerala, India	*E. grandis*	IMI 261568	–	–	–
	Kerala, India	*E. grandis*	IMI 257896	–	–	–
'Cytospora eucalypticola' sensu Sharma *et.al.*	Kerala, India	*E. tereticornis*	IMI 284046	–	–	–
Cytospora eriobotryae	Saharanpur, India	*Eriobotrya japonica*	–	CBS116846	–	AY347327
Cytospora-like species	Indonesia	*E. urophylla*	MSC 380703	CBS117015; C.M.W. 461	Indonesia-1	AF192316
	Indonesia	*E. urophylla*	MSC 385000	CBS117016; C.M.W. 462	Indonesia-1	AF192316
	Indonesia	*E. urophylla*	MSC 380704	C.M.W. 460	Indonesia-2	AF192313
Valsa myrtagena, Cytospora state	Sibisa, North Sumatra, Indonesia	*E. grandis*	MSC 380705	CBS117013; C.M.W. 4046	Indonesia-3	AY347380
	Sibisa, North Sumatra, Indonesia	*E. grandis*	MSC 380706	CBS117014; C.M.W. 4047	Indonesia-3	AY347380
Cytospora valsoidea	Sibisa, North Sumatra, Indonesia	*E. grandis*	MSC 380717	CBS117003; C.M.W. 4309	Indonesia-4	AF192312
	Sibisa, North Sumatra, Indonesia	*E. grandis*	MSC 380707	CBS116857; C.M.W. 4310	Indonesia-4	AF192312
Valsa eugeniae, Cytospora state	Suluwesi, Indonesia	*Eugenia* sp.	–	CBS116835; C.M.W. 8646	Clove	AY347341
	Suluwesi, Indonesia	*Eugenia* sp.	–	CBS116836; C.M.W. 8647	Clove	AY347342
	Suluwesi, Indonesia	*Eugenia* sp.	–	CBS116837; C.M.W. 8637	Clove	AY347343
Valsa eugeniae	Suluwesi, Utara, Indonesia	*Eugenia* sp.	MSC 380723	CBS116834	Clove	AY347343
Cytospora putative species 1	Thailand	*E. camaldulensis*	–	CBS116861; C.M.W. 464	Thailand-1	AF192320
Valsa ceratosperma s.lat.	Japan	*Malus pumila*	–	CBS117012	–	AF192326
Valsa friesii	Germany	*Abies alba*	–	CBS113.81	–	AY347318
Valsa subclypeata	Netherlands	*Rhododendron ponticum*	–	CBS117.67	–	AY347331
Cytospora decorticans	Netherlands	*Fagus sylvatica*	–	CBS116.21	–	AY347335
Cytospora mougeotii	Norway	*Picea abies*	–	ATCC 44994	–	AY347329
Valsa abietis	Switzerland	*Abies alba*	–	CBS185.42	–	AY347336
Valsa ambiens	Switzerland	*Taxus baccata*	–	CBS191.42	–	AY347330
Valsa ceratophora	Switzerland	*Taxus baccata*	–	CBS192.42	–	AY347333
Valsa friesii	Switzerland	*Abies alba*	–	CBS194.42	–	AY347328
Valsa pini	Switzerland	*Pinus sylvestris*	–	CBS197.42	–	AY347332
Valsa germanica	Switzerland	–	–	CBS195.42	–	AY347325

Table 1. (Contintued).

Taxon	Geographic origin	Host *E. = Eucalyptus*	Specimen	Culture	Cladogram code	GenBank
Valsa salicina	Switzerland	*Salix* sp.	–	CBS 203.42	–	AY347323
Valsa leucostoma	Switzerland	*Sorbus aucuparia*	–	CBS 133.76	–	AF191173
Valsa sordida	U.K.	*Populus tremula*	–	CBS 197.50	–	AY347322
Valsa auerswaldii	USSR	–	–	CBS 153.29	–	AY347337
Valsa friesii	–	*Pinus sylvestris*	–	CBS 179.70	–	AY347317
Valsa friesii	–	*Abies alba*	–	CBS 505.72	–	AY347319

[a]GenBank sequences are the authors except AF317579.

[b]Accession numbers with the prefix C.M.W. are of the culture collection of M.J. Wingfield at the Tree Protection Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

[c]Accession numbers with the prefix MSC are in the Beal-Darlington herbarium at the Michigan State University (MSC), East Lansing, MI, U.S.A.

[d]Accession number with the prefix DAR is in the herbarium of the Department of Agricultural Research (DAR), CSIRO, Australia.

[e]Accession numbers with the prefix PREM are in the National Mycological Herbarium in Pretoria (PREM), South Africa.

[f]Accession numbers with the prefix IMI are of the culture collection of the International Mycological Institute (IMI), CABI Bioscience, Egham, Surrey, U.K.

[g]Accession numbers with the prefi□

[h]Accession numbers with the prefix UC are in the mycology herbarium of the University of California (UC), Berkeley, CA, U.S.A.

[i]Accession numbers with the prefix G.C.A. are of the culture collection of G.C. Adams at Michigan State University, East Lansing, MI, U.S.A.

[j]Accession numbers with the prefix CUP are in the mycology herbarium of Cornell University (CUP), Ithaca, NY, U.S.A.

Fruiting of cultures *in vitro*

Teleomorph formation in agar culture has been described by Leonian (1921) for *C. leucostoma*. He found that addition of 2–12 % sucrose to oatmeal agar stimulates perithecial formation *in vitro*. The higher percentage sucrose gave rise to the greatest level of stimulation. He also reported that addition of NaCl to the medium stimulates perithecial formation. In both cases, single ascospore cultures fruit while conidial cultures do not.

Wehmeyer (1925) succeeded in stimulating perithecial fruiting of *V. kunzei* on sterile stems of *Thuja plicata* but not on agar. He incubated the inoculated twigs at 0–3 ºC for 4 mo *in vitro*. He also reported that single ascospore cultures form perithecia on the twigs, but not single conidia. Both species are presumed to be homothallic. However, it is difficult to understand the requirement for single ascospores versus conidia unless the ascospores contain nuclei of two mating types and both species are secondarily homothallic. Hubbes (1960b) found that perithecia of *Valseutypella tristicha* will form on sterilised twigs of its *Rosa* host, but not on twigs of *Salix* or *Populus* within 6 wk of incubation at 21 ºC.This species produces four ascospores per ascus and, therefore, will likely be secondarily homothallic. To the best of our knowledge this work has not been repeated.

MATERIALS AND METHODS

Herbarium specimens examined

The herbarium specimens examined in this study were loaned from the following institutions: the International Mycological Institute (IMI), CABI Bioscience, Egham, Surrey, U.K.; the Department of Agricultural Research (DAR), CSIRO, Australia; the Colecciones Micológicas, Universida of t Nacional de La Plata, Instituto de Botanica C.Spegazzini, La Plata (LPS), Argentina; the National Mycological Herbarium (PREM) in Pretoria, South Africa; the mycology herbarium of the University of California (UC), Berkeley, CA, U.S.A.; the mycology herbarium of Cornell University (CUP), Ithaca, NY, U.S.A.; the mycology herbarium of the University of Michigan (MICH), Ann Arbor, MI, U.S.A.; and the mycology section of the Beal-Darlington herbarium at Michigan State University (MSC), East Lansing, MI, U.S.A. The new specimens described in this study were stored in the mycology section of MSC.

The morphology of type specimens was examined, including *C. agarwalii* IMI 249224, *C. australiae* LPS 31746, *C. eucalyptina* LPS 11656, *C. eucalypticola* PREM 42543, *L. sequoiae* UC 469596, isotype *V. eucalypti* (MICH 15128, MSC 11472 exsiccate of J.B. Ellis's North American Fungi, series 1 # 871, Ellis & Everhart 1892), type specimens and other specimens of *V. ambiens* (CUP 060135, CUP 069132, CUP 060133, CUP 060137), type specimens of several *Valsa* spp. nom. inval. (IMI 257876, IMI 257896, IMI 261564, IMI 261568, IMI 284046), specimens labeled *C. australiae* (UC 275812, UC 143778, UC 500550, PREM 13072, PREM 50454), and specimens labeled *V. ceratosperma* from *Eucalyptus* (DAR 43948).

Morphology *in vitro*

All isolates used in this study (Table 1) (except the *Diaporthe vaccinii*) were plated on oatmeal agar with 12 % sucrose to stimulate sexual fruiting. Milled oats, 60 g were added to 1 L distilled water, autoclaved, homogenised in a standard blender for 3 min, and filtered through four layers of cheesecloth. Sucrose, 120 g, was added and the volume brought up to 1 L, then autoclaved. Thirty millilitres of the medium were poured into 85 mm diam Petri dishes. The agar plates were inoculated with mycelium from actively growing cultures on Leonian's medium. The resulting cultures were incubated at room temperature in diffuse light for 90 d, then examined for ascostromata or perithecial formation.

For formation of pycnidia on host tissue for morphological studies, we used the following method. *Eucalyptus* leaves were obtained from a florist and autoclaved for 20 min submerged in water, twice on successive days. Two autoclaved leaves were placed on the surface of 2 % MEA or Leonian's agar. The isolate was inoculated near the leaves and incubated at room temperature. Once hyphae colonised the tissues of the leaves, one leaf was removed and placed onto water agar. The plates were not sealed with tape until colony expansion ceased. Following *ca* 20 d incubation at 24 ºC in diffuse sunlight or under 12 h cool white fluorescent light and 12 h dark, mature conidiomata were usually formed on leaves of one or both plates and could be examined under a dissecting microscope. Similarly, the excised shoot assay method described above could be used with dormant shoots for the purpose of initiating formation of pycnidia in host tissues. Specimens were examined for locule structure, conidioma diameter, and ostiolar neck length. Phase contrast and differential interference contrast microscopes were used to examine wall structure, shape and size of conidia, and branching of conidiophores.

Culture characteristics, cardinal temperatures for growth, and tolerance to 2 ppm cycloheximide

All isolates listed in Table 1 (except the *Diaporthe vaccinii*) were placed on PDA (Difco Company, Detroit, MI, U.S.A.) in 85 mm diam plastic Petri dishes. The PDA had been acidified with one drop of 20 % lactic acid. The inoculated plates were incubated at 4, 25, 32,

and 37 ºC in the dark for 30 d. Each isolate was placed on three plates and the experiment was replicated once. Linear measurements of growth were taken at intervals from each plate until the growth reached the edge of the plate or stagnated prior to reaching the edge. Linear growth/h was determined for each isolate by calculating the mean of three plates. Then the expected diameter of growth (linear growth rate × 2) over 7 d was calculated for each isolate at each temperature. For a species, diameter of growth over 7 d at each temperature was determined by calculating the mean among the available isolates.

Colours of the colonies were determined from cultures on acidified PDA incubated at 25 ºC in diffuse fluorescent light for 30 d. Colours of the surface and reverse were compared to colour charts of the National Bureau of Standards (Kelly 1965) under bright "sunlight" fluorescent light (6400 ^{o}K, 82 CRI, 1400 lumens) by two investigators. Colony margin, texture, elevation and presence of zonal growth and pycnidia were recorded. Cultures were also grown on oatmeal agar to compare production of pycnidia to that on PDA. Oatmeal agar was made from 60 g milled oats per liter. The oats were autoclaved in distilled water, blended at high speed in a standard household blender for 1 min, then autoclaved with 20 g agar. The agar was poured into 85 mm diam plastic Petri dishes. Following cooling, the dishes were inoculated and incubated at 25 ºC in diffuse fluorescent light for 30 d.

Pycnidial density, diameter and colour of the exuding cirrhi were recorded from autoclaved *Eucalyptus* leaves on water agar. Ten pycnidia from each isolate on autoclaved leaves were hand sectioned longitudinally and the number and arrangement of locules and walls were described. Twenty measurements of all other morphological structures were taken except when scarcity of holotype material made this impossible. Minimum and maximum measurements are presented in parentheses surrounding the averages or average range.

Cycloheximide was prepared as a stock solution in absolute ethanol on a w/v basis. One mL stock solution was added to 1 L of V8® agar at *ca* 45 ºC after autoclaving. The control was V8® agar containing 1 mL absolute ethanol. The medium contained 163 mL V8® juice (Campbell Soup Comp., Camden, NJ, U.S.A.), 2 g $CaCO_3$, 1 g K_2HPO_4, 17 g agar and 837 mL distilled water. Available isolates of *Valsa* and *Cytospora*-like species were placed on three replicate plates of V8® agar amended with 2 ppm cycloheximide and on the control V8® agar. Isolates from species that showed high sensitivities (no growth) to 2 ppm cycloheximide in V8® agar were further evaluated on 2 ppm cycloheximide added to Leonian's agar and on the control (Leonian's agar) in order to check whether sensitivities were influenced by differences in basal media and nutrition. Growth rate was measured at intervals during incubation at 25 ºC in the dark. Sensitivities were reported for a species as mean diameter of growth over 7 d, as described above. The objective was to determine whether 2 ppm cycloheximide could be used as an *in vitro* means of distinguishing among species based on a assay of growth rate on amended agar.

Examination by light and electron microscopy

For examination of pycnidia and perithecia on bark, samples were washed for 30 min in 2 % KOH, rinsed with water, and placed in fixative (2 % formaldehyde, 2 % glutaraldehyde in 0.025 M phosphate buffer pH 7.4) for 1 d. The specimens were then soaked 2–3 h in 5 % HCl and neutralised in buffer. Specimens were then placed in osmium (1 % OsO_4 in 0.025 M buffer) for 3 h, followed by dehydration in a graded ethanol series. Specimens were transferred from 100 % ethanol to 100 % propylene oxide, then to a graded series of resin/propylene oxide solutions. Resin concentrations were 25, 50, 75, 87 and 100 %, and each stage was infiltrated for approximately 12 h. The resin was a mixture of Poly/Bed 812® (PolyScience, Inc., Warrington, PA, U.S.A.) and Araldite epoxy resins, and dodecenylsuccinic anhydride hardener (DDSA) in the proportions 5 : 4 : 12. The resin was cured at 60 ºC for 2 d, and sections for light microscopy were cut with a glass knife at approximately 0.75 mm, with thickness of 2–3 µm. Slides were stained with 1 % toluidine blue at 100 ºC, and mounted with the same resin used for embedding.

Conidiophores were examined by transmission electron microscopy (TEM) on ultrathin sections, 0.75–1 µm thickness, cut with a diamond knife from the same blocks as were used for light microscopy. The sections were stained with aqueous uranyl acetate and Reynold's lead citrate (1.33 g lead nitrate, 1.76 g sodium citrate, in 30 mL distilled water).

Specimens prepared on slides for light microscopy were stained with lactophenol cotton blue, mounted in polyvinyl alcohol lactoglycerol, the coverslip added, and heated for 1 h at 60 ºC to solidify mountant (Koske & Tessier 1983). For photographing asci and hymenia, specimens were stained with 0.5–1 % aqueous chlorazol-black and flattened or squashed by pressure applied to the cover slip. In the latter slides, the shapes of asci were distorted and often wider than natural. For photographing conidia, tendrils were lifted and placed in water on a slide then spread and allowed to air dry. A drop of PermaFluor™ aqueous mounting medium (ThermoShandon, Pittsburgh, PA, U.S.A.) was added and a cover slip was placed over the dried conidia and pressure was applied to remove air bubbles. Photographs were taken under Nomarski

(differential interference contrast), phase and bright field optics.

Strains for DNA sequencing

A total of 67 isolates of *Cytospora*, *Cytospora*-like species and *Valsa* as well as two *Diaporthe* species were selected for DNA sequencing (Table 1). Sequences of additional isolates from host plants other than *Eucalyptus* were included in the analysis. Many, but not all cultures were accompanied by herbarium specimens. The cultures used in this study originated from several collections including the following: the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands; the American Type Culture Collection (ATCC), Manassas, VA, U.S.A.; the International Mycological Institute (IMI), CABI Bioscience, Egham, Surrey, U.K.; the culture collection of M.J. Wingfield (C.M.W.) at the Tree Protection Cooperative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa; and the culture collection of G.C. Adams (G.C.A.) at Michigan State University, East Lansing, MI, U.S.A.

DNA extraction and amplification

Total genomic DNA was extracted from 65 isolates of *Valsa* and two isolates of *Diaporthe* (Table 1). Isolates were grown in 25 mL of 2 % malt extract broth at room temperature for 5–10 d. Mycelium was then harvested by vacuum filtration through miracloth (Calbiochem-Novobiochem Corp., La Jolla, CA, U.S.A.), lyophilised, and stored at –20 ºC. DNA was extracted from lyophilised hyphae by one of two methods of extraction, a standard sodium dodecyl sulfate (SDS) phenol method (Lee *et al.* 1988) and a cetyltrimethyl-ammonium bromide (CTAB) method (Hallen *et al.* 2003). Hyphae were ground to a fine powder in liquid nitrogen with a pestle. One to 2 mL of the extraction buffer was added and mixed. The extract was then centrifuged to remove solids, purified with phenol : chloroform : isoamyl alcohol (24 : 24 : 1) extractions and precipitated with isopropanol and centrifugation. The precipitate was air dried under vacuum and then dissolved in 50 µL TE (10 mM Tris-HCL, 1 mM EDTA, pH 8.0). Approximately 2.5 ng of the total genomic DNA was used per 100 µL reaction mixture for polymerase chain reaction amplification (PCR) (White *et al.* 1990). Various brands of prepackaged buffers and polymerases were used for PCR amplification during this research. Reaction mixtures were approximately those of White *et al.* (1990). Primers used in the amplifications included ITS1, ITS2, ITS3, and ITS4 for the ITS-rDNA (White *et al.* 1990).

The cycling reactions were performed in a DNA Thermal Cycler (Perkin-Elmer Corp., Norwalk, CT, U.S.A.) or similar machine. Thermal cycling was programmed for a 2 min hot start of 94 ºC followed by 35 cycles of 1 min 94 ºC, 1 min 50 ºC, 45 s at 72 ºC. The 45 s at 72 ºC was extended for each cycle by 4 s. The amplification ended with an additional 7 min extension of 72 ºC. PCR amplification products were separated by agarose gel electrophoresis, stained with ethidium bromide and examined under ultraviolet light. Two hundred microlitres of each product (not exposed to ultraviolet light) were purified by using the DNA binding resin and protocol of Wizard PCR Preps DNA purification system (Promega Corp., Madison, WI, U.S.A.).

Sequencing was performed using a *Taq* DyeDeoxi Terminator^{TM} cycle system, the ABI Catalyst 800, and the ABI Prism 373A or 377 fluorescence sequencer (PE Applied Biosystems, Foster City, CA, U.S.A.). Sequencing reactions were carried out using the Big Dye fluorescent labeling sequencing kit (PE Applied Biosystems). Amplified double-stranded PCR products were sequenced independently along both strands with the primers listed above.

DNA data analysis

Sequences of a strain were merged and aligned using ESEE v. 1.09e (Cabot & Beckenbach 1989), visually edited and aligned, then a composite consensus sequence was proofread. Sequences of all strains were aligned with CLUSTAL X 1.81 (Thompson *et al.* 1994, 1997), visually proofread and again aligned with CLUSTAL and proofread. The ITS1–5.8S–ITS2 rDNA sequences were analysed as uniformly weighted, unordered characters, and as interleaved blocks of aligned sequence.

Insertions/deletions (indels) and gaps introduced for alignment purposes were coded by the simple coding method of Simmons & Ochoterena (2000). All gaps that had different 5' and/or 3' termini were coded as separate presence or absence characters. The longer gaps in a set of sequences with different but completely overlapping gaps were coded as inapplicable for the shorter gap character being coded. Ambiguities in alignment of short segments of sequence were tested experimentally for their effect on topology and bootstrap indices.No segments were excluded from the analyses. Sequences have been deposited in GenBank (AF192314–21, AF260263–6, AY347316–80, Table 1).

Initially, the ITS-rDNA sequences of *Cytospora* and *Cytospora*-like isolates from *Eucalyptus* were compared in a maximum parsimony analysis (Swofford & Maddison 1987) with a total of 147 taxa in the *Valsaceae* that we have sequenced (G.C. Adams, unpubl. data). The initial analysis was computed in PAUP v. 4.0b10 (Swofford 2003) using heuristic searches with the tree bisection-reconnection (TBR) branch-swapping algorithm. A reduced set of data

containing 84 taxa was then analysed more thoroughly, as above. The equally most parsimonious tree (MPT) with the greatest LN likelihood was selected by means of the Kishino-Hasegawa test (Kishino & Hasegawa 1989) for display. To develop a consensus tree, 1000 heuristic searches (Hedges 1992) were performed by bootstrapping (Felsenstein 1985). Confidence intervals for branches on the consensus tree were inserted into the selected MPT (Fig. 14). The pruned taxa were additional members of groups already well represented in the reduced set, or entire groups distant from the relevant groups near to the groupings containing *Cytospora* and *Cytospora*-like isolates from *Eucalyptus*. The presence or absence of the pruned taxa did not affect placement of the *Cytospora* sequences in the final analysis.

Because at least one clade appeared to be evolving at an accelerated rate, based on branch lengths, a second method of phylogenetic analysis, maximum likelihood (Felsenstein 1981), which was less sensitive to unequal rates of evolution, was employed. The maximum likelihood model of substitution for the 84 taxa data set was computed using Modeltest v. 3.04 (Posada & Crandall 1998). The number of taxa prevented calculation of a maximum likelihood tree with PAUP. Therefore, the likelihood model, GTR+G+I, was used in a Bayesian phylogenetic analysis with MrBayes v. 3.0b4 (Ronquist & Huelsenbeck 2002). The Markov chain Monte Carlo convergence acceleration technique of Metropolis coupling was employed (Metropolis-Hastings algorithm, Larget & Simon 1999, Huelsenbeck & Ronquist 2001). Three million searches were performed, with a burnin of 60 000 discarded trees, to calculate the posterior probability distributions for branches on the Bayesian maximum likelihood consensus tree, BMLT (Fig. 15). The MPT and the BMLT trees were displayed using TreeView (Page 1996).

RESULTS

Phylogenetic analyses and trees

DNA sequences of *Cytospora* and *Cytospora*-like fungi from *Eucalyptus* have been deposited in GenBank (Table 1). The length of DNA sequence for each isolate, inclusive of introduced gaps to permit alignment of the entire set, was 610 nucleotides. Gap coding increased the length of the sequences to 641 characters and 220 parsimoniously informative characters.

Phylogenetic analysis of the data set of *Eucalyptus* isolates and 41 other taxa (total 84 taxa) was represented in cladograms (Figs 14–15). The italicised names in cladograms were of identified species in the *Diaporthales*. Names in Roman fonts that included a country of origin and a number represented unique DNA sequences that corresponded to one or more isolates given in Table 1. Maximum parsimony produced a tree of 1068 steps, LN = –7002.08, and consistency index of 0.464, retention index of 0.821, and a rescaled consistency index of 0.381 (Fig. 14). Bayesian analysis generated a consensus tree with a mean tree length of 4.0925 and estimated LN = –6415.68 (arithmetic mean) once the posterior probability reached P = 0.000 and the cumulative posterior probability reached P = 1.000 (Fig. 15).

In this study, a clade that included *Valsa eugeniae* Nutman & F.M. Roberts represented the root of the gene tree lineage that included *Cytospora* anamorphs of *Valsa* subgenera *Valsa*, *Leucostoma*, *Valsella*, and *Valseutypella* (*Valseutypella* spp. data not shown). Two mono-specific sections of *Cytospora* were not represented on this reduced gene tree (Fig. 14) because no isolates from *Eucalyptus* were close to these species in sequence homology, sect. *Cytophoma* represented by *C. pruinosa*, and sect. *Cytosporopsis* (sect *Cyclocytospora*) represented by *C. umbrina* (= *C. melanodiscus* Höhn.).

Many of the well-known species of *Valsa*, including species in *Leucostoma* and *Valsella* (Kobayashi 1970, Barr 1978, Spielman 1985), were remote from most *Cytospora* isolates from *Eucalyptus* including *V. ceratosperma* on *Quercus* from the U.K. and U.S.A. (as *C. sacculus*), and on *Malus* from Japan (Figs 14, 15).

Cytospora isolates from *Eucalyptus* formed several clusters, representing perhaps as many as 16 putative lineages marked by vertical bars on Fig. 14. No herbarium specimens were available for the putative lineage that includes South Africa-1, South Africa-2 and Thailand-1, and therefore the bar was labeled as unknown in morphology. This group was described from culture under "*Cytospora* putative species 1", below. *Cytospora*-like isolates, from Indonesia (Indonesia-1 and Indonesia-2), clustered in a lineage with *Diaporthe* / *Phomopsis* with 100 % bootstrap confidence level. However, these fungi formed invaginations in the locule of the conidiomata, had allantoid conidia and no beta conidia, and were *Cytospora* sp. based on morphology alone.

Ascostromata

The studied species formed ascostromata that did not readily fit into the former sections *Valsa* and *Monostichae*. They had small asci and ascospores and well-developed entostromata but often had few and circinately oriented, laterally inclined perithecia. *Valsa fabianae* was euvalsoid with perithecia generally solitary and laterally inclined but approached circinateous with increasing perithecia. *Valsa myrtagena* was euvalsoid and formed groups of four or less perithecia which approached circinateous,

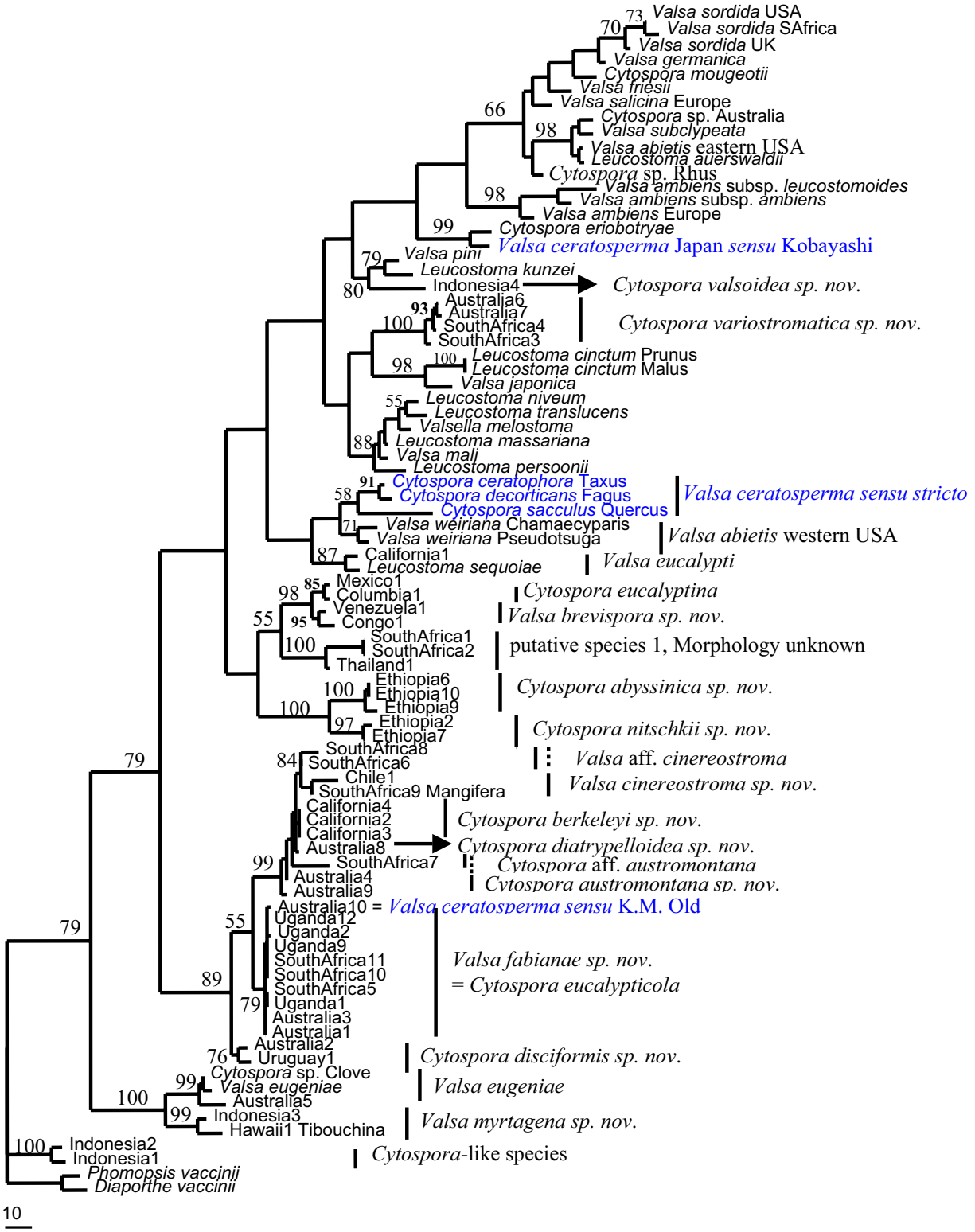

Fig. 14. Cladogram of *Cytospora* species, and their *Valsa*, *Leucostoma* and *Valsella* teleomorphs, from cankers on *Eucalyptus* trees compared to species on other hosts. The species relationships were inferred from parsimony analysis of DNA sequence of the ITS1-5.8S-ITS2 region of the nuclear ribosomal DNA operon. The single gene tree represented is from one of the most parsimonious trees with the greatest log likelihood. Branch lengths correspond to inferred genetic distances with the units of the bar equaling the number of nucleotide substitutions. Isolates from *Eucalyptus* are designated by country of origin and a number representative of a population with a distinct sequence (i.e., Indonesia-3). Numbers at nodes are percent bootstrap support (1000 replications). Scale bar = 10 nucleotide substitutions.

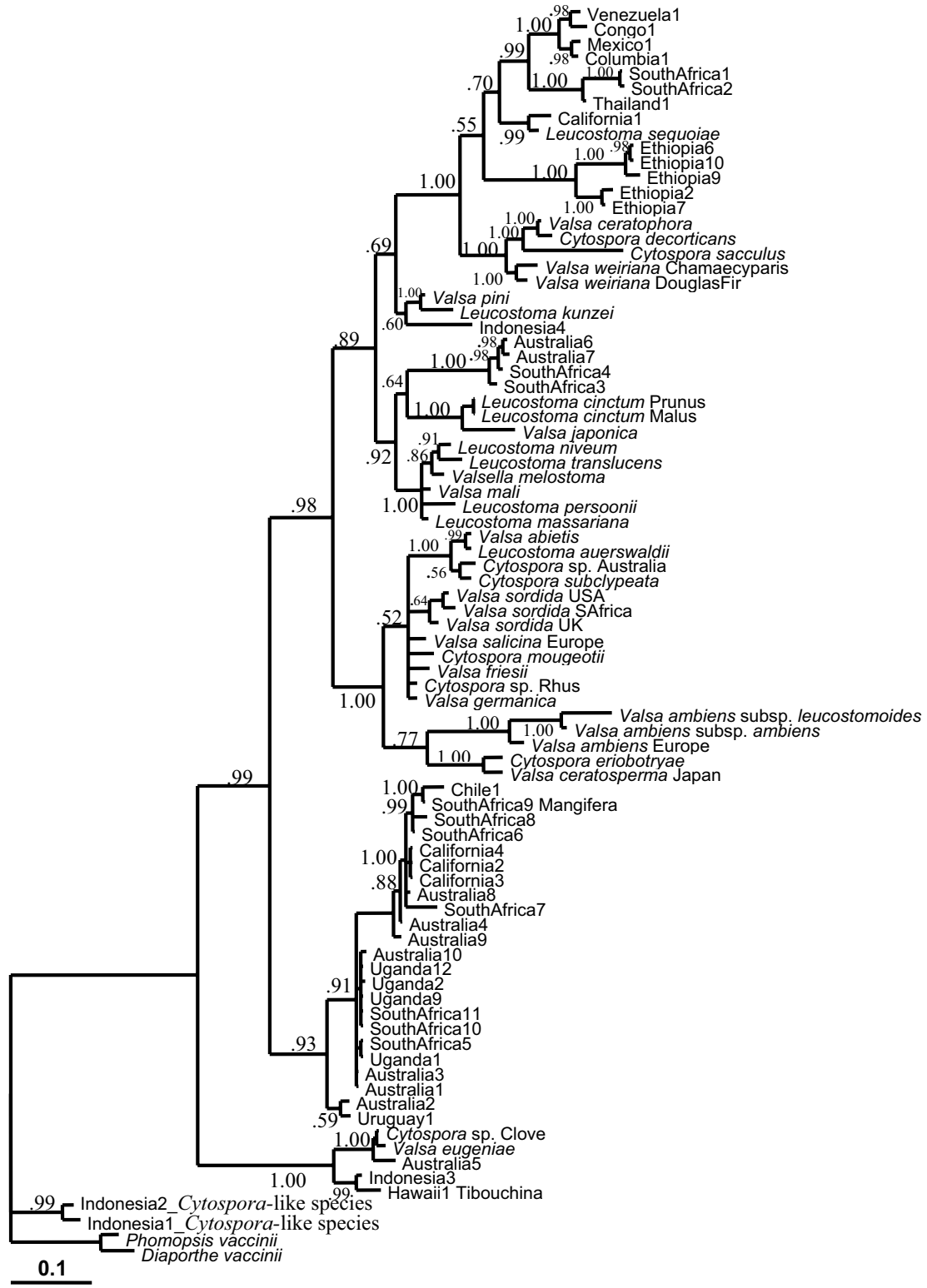

Fig. 15. Cladogram of *Cytospora* species, and their *Valsa*, *Leucostoma* and *Valsella* teleomorphs, from cankers on *Eucalyptus* trees compared to species on other hosts. The species relationships are inferred from Bayesian analysis of DNA sequence of the ITS1-5.8S-ITS2 region of the nuclear ribosomal DNA operon by means of a maximum likelihood model. The tree represented is the maximum likelihood consensus tree calculated using the Metropolis-Hastings algorithm and three million searches, following the burning of 60,000 discarded trees. Branch lengths correspond to inferred genetic distances with the units of the bar equaling the number of expected nucleotide substitutions. Isolates from *Eucalyptus* are designated by country of origin and a number representative of a population with a distinct sequence (i.e., Indonesia-3). Numbers at nodes are posterior probability distributions. Scale bar = 0.1 nucleotide substitutions per site.

although perithecia were upright. "*Valsa eucalypticola*" and *V. brevispora* were euvalsoid and had 5–16 perithecia. In "*V. eucalypticola*" the ascostromata were circinateous becoming monostichous with increasing perithecia, while ascostromata of *V. brevispora* were monostichous with upright perithecia even in the smaller stromata. Both species had well-developed entostromatal tissue. *Valsa eugeniae* was euvalsoid and monostichous. *Valsa cinereostroma* was circinateous and leucostomoid with small asci and ascospores. The ascostromata of *V. eucalypti sensu* Sharma *et al.* were leucostomoid and circinateous with uncrowded inclined perithecia, with small perithecia, asci, and ascospores of 6–6.5 µm. The size range of the asci and ascospores of the *Valsa* species on *Eucalyptus* would have placed the species into *Microsporae* in Ellis & Everhart (1892), except for *V. eucalypti*. *Valsa eucalypti* was leucostomoid and circinateous with uncrowded inclined perithecia, and small asci, however, ascospores were in the smallest extreme for sect. *Valsa* and within the range of sect. *Monostichae*. Our measurements of ascospore length in *V. eucalypti* averaged 8.5 µm which was within the range for the group *Mesosporae*. However, Ellis & Everhart (1892) placed the species in *Microsporae*. Ellis & Everhart (1892) did not recognise the leucostomoid morphology of *V. eucalypti* or they would have placed the species in *Leucostoma*. They did not include leucostomoid species in the groups based on ascospore length. *Valsa eucalypti* and *V. eucalypti sensu* Sharma *et al*. (1985) formed leucotorsellioid anamorphs. Historically, anamorphs with torsellioid features were thought to correspond to teleomorphs with the typical characters of sect. *Monostichae*.

Perithecia with ascospores were not formed by the isolates in Table 1 when grown on oatmeal agar with 12 % sucrose for 100 d at room temperature followed by 30 d at 4 ºC. However, several isolates formed fruiting bodies that were massive compared to those formed on oatmeal, PDA, KHG or Leonian's agar. For example, *V. eugeniae* formed 2–10 mm diam conidiomata, *V. eucalypti* 7–8 mm diam complex stromatal conidiomata, *V. myrtagena* 5–8.5 mm diam conidiomata with multiple long necks/beaks, *V. cinereostroma* 8 mm diam sterile stromata, *V. kunzei* 6–7 mm and *V. leucostoma* 2–5 mm diam complex stromatal conidiomata.

Ascus and ascospore morphology

Among the species we studied, the apical apparatus was not noticably variable in relative size, shape, orientation, or staining reaction with nigrosin, chlorazol-black, cotton blue or Melzer's reagent (Fig. 12). Few preparations clearly showed whether the ring was composed of two components or one. The structures that stained with chlorazol-black remained unstained in TEM preparations of asci suggesting that the ring apparati were composed of polysaccharides. In TEM images, there was a dark staining structure at the lower periphery of the unstained ring that may have provided support for the ring (Fig. 13). Lipid was present in the ascus apex. Little variation in ascus morphology was noted in *Valsa* other than size differences. The asci varied from clavate to cylindrical but this variation could be an artifact of preparation of mounts and was not consistent within a species. Occasionally asci tapered downward to terminate in a stalk-like shape of approximately 2–3 µm in length. The minute stalk was occasionally bent rather than straight. Asci appeared nearly uniform in developmental maturity within a discrete perithecium, and among perithecia in a stroma, however, this was often an artifact of viewing only the free floating asci. In one specimen studied, croziers had apparently formed but asci had not yet developed (Fig. 12).

Ascospores of *Valsa* species closely resembled conidia but were proportionately larger. They were thin-walled, hyaline, simple and allantoid. The amount of relative curve in ascospore shape did not vary among species. There was little variation in width of ascospores within a species; among species differences in relative width were distinctive and rare. Therefore, relative width of ascospores was useful as a basis for differentiating species. Some specimens of *Valsa* species had ascospores with a small guttule at each end. TEM micrographs of ascospores of *V. leucostoma* showed large lipid globules at the end of a spore (Fig. 13). In *V. eucalypti* two nuclei were present in each ascospore (Fig. 13) which could be the norm for species in *Valsa*.

Paraphyses

In many specimens, asci at different developmental stages occurred attached to the hymenium among paraphyses (Fig. 11). Paraphyses were distinctly different in width, septation and free terminal cell shape among *Valsa* species. We illustrated paraphyses for several species of *Valsa* including species with large asci, *V. cincta* and *V. leucostoma*, and species with small asci, *V. myrtagena* (Fig. 11). *Valsa cincta* and *V. leucostoma* had paraphyses that were large, broad and cylindrical with four or more septa and with each cell containing one large lipid globule. These paraphyses fit the generalised descriptions of "paraphysis-like broad bands" that have been described in the literature. *Valsa myrtagena* had long, filamentous paraphyses with tapering walls terminating in free pointed apical cells, 1–2 septa. *Valsa cinereostroma* and "*V. eucalypticola*" had narrow cylindrical paraphyses. The cytoplasmic contents of the paraphyses of *V. cinereostroma* appeared degraded while those of "*V. eucalypticola*" appeared uniformly hyaline (Fig. 11).

We found abundant asci with fully formed ascospores in many of the *Valsa* specimens that contained paraphyses. We would interpret fully formed ascospores as the indicator of maturity; so, paraphyses were present at maturity. Figure 10 illustrates the hymenium of *V. eucalypti* with mature attached asci, ascospores, and paraphyses filling the entire perithecium. The paraphyses were packed together at the centre of the perithecium and contained lipid globules. The perithecial wall also contained lipid globules. Figure 11 also illustrates immature asci, mature attached asci, ascospores, and paraphyses occurring together in hymenia. No variation in the centrum type was noted among species in this study, except in the relative size of paraphyses in relation to asci.

Locules

Conidiomata were interspersed among ascostromata in *V. brevispora*, *V. cinereostroma*, *V. eucalypti*, *V. eucalypti sensu* Sharma *et al.*, "*V. eucalypticola*", and *V. fabianae*. However, in the many collections of *V. fabianae* only the holotype specimen from Tasmania, a specimen from the type locality of the anamorph, *C. eucalypticola*, and Ugandan specimens had ascostromata interspersed with conidiomata. Locule orientation among conidiomata varied greatly for species from *Eucalyptus*. The orientation of locules in the lamyelloid conidioma of *C. valsoidea* was valsoid; locules were arranged circinately and inclined with their ostiolar necks converging towards a disc at the bark surface. In the lamyelloid conidiomata of *C. diatrypelloidea*, tightly packed locules were arranged more upright or diatrypelloid. In the leucotorsellioid anamorph of *V. eucalypti* on *Sequoia* the locules were arranged circinately and the necks converged into one ostiole, while the entire conidioma was discoid (similar to the ascostromata). On *Eucalyptus*, the locules were upright and the entire conidioma was conical (similar to the ascostromata). The unilocular conidioma of *C. eucalypticola* formed inclined or upright discrete locules. When the unilocular conidioma did not have a disc, then the locule tended to be upright.

Conidiophores, sterile elements and conidia

Conidiophores examined with TEM showed that phialides of *C. australiae* differed from *Cytospora acaciae* Oudem. in the length of the canal from the lumen to the apex (Fig. 9). Further study was needed to understand the significance of the observation. Conidiophores were obviously and frequently branched above the base in only a few species. The leucostomoid species (*C. agarwalii*, *V. eucalypti*, *V. eucalypti sensu* Sharma *et al.*, and *V. cinereostroma*) had branched conidiophores, except *C. abyssinica*. Also, "*V. eucalypticola*" and *C. variostromatica* had branched conidiophores. The majority of species had conidiophores that were branched at the base, and only occasionally to rarely branched above the base, near mid-height. In cross section through a hymenium, the latter species would appear to have conidiophores that were unbranched and the length of a phialide. Relative width by length of phialides did not vary among species. No species had uniquely shaped phialides or phialides with annellations.

Sterile elements were seen in the hymenia of conidiomata of *Cytospora* species, such as in *C. austromontana*, the *Cytospora*-like species and the anamorphs of *V. eucalypti* and of *V. fabianae* (= *C. eucalypticola*) (Fig. 10). Elements were hyaline, thin-walled, cylindrical, not clavate, and usually more than four times as long as wide. Hymenial elements of *C. austromontana* were surrounded by a thick gelatinous layer (Fig. 10). Presence of sterile elements varied among the conidiomata of a collection. Probably, the sterile elements were not a reliable character for distinguishing a species. In the hymenium of the *Cytospora*-like species clavate highly refractive cells were interspersed among conidiophores. The cells were thought to contain oil and were approximately the length of the conidiophores. We were unfamiliar with conidiomata having such cells occurring in the hymenia among coelomycetes.

Conidia of *Valsa* species closely resembled ascospores but were proportionately smaller. They were thin-walled, hyaline, simple and allantoid. The amount of relative curve in conidium shape varied rarely among species. We observed two species that had straight conidia in nature, *V. myrtagena* and *C. valsoidea*; but in culture the conidia were predominantly allantoid. We described *V. brevispora* as producing short (only 3 µm long) allantoid, tangerine-section shaped conidia in nature. However, in culture the conidia were allantoid. *Cytospora abyssinica* had allantoid conidia in nature but sometimes formed straight to tangerine-section shaped conidia in culture. Additionally, it was rare among species of *Cytospora* on *Eucalyptus* for conidia to be wider than 1 µm. Only *C. nitschkii* had conidia as wide as 1.5 µm in diam and only ascospores were as wide as 2 µm diam in this study. There was no noticeable variation in size and shape of conidia within a species, except in *C. eucalyptina*. Isolates of the species from Mexico had shorter conidia than the holotype. Occasionally within an isolate there was noticeable variation in the length of conidia. This had been reported for the *V.* aff. *cinereostroma* isolates when conidiomata from nature were examined (Crous *et al.* 1990). Surprisingly, conidia were uniform for these isolates *in vitro*.

The colour of cirrhi appeared to be uniform for a species if environmental conditions and age in culture were comparable. The cirrhi were usually yellow but

they were occasionally beige or orange. We placed no taxonomic weight on the colour because we observed that an isolate in culture could produce beige or orange cirrhi depending on unknown environmental factors. When cirrhi were beige they were generally in globules rather than tendrils. Additionally, as cultures aged cirrhi often changed from light yellow tendrils to dull orange globules.

Conidiomata *in vitro*

In culture, globose, conidiomata formed with usually 3–6 radially arranged chambers. However, except size and shape of conidia, the morphological features that were distinctive among natural specimens were often absent on autoclaved leaves. For example, herbarium specimens having unilocular cytosporoid conidiomata usually gave rise to rosette cytosporoid conidiomata in culture. Sometimes isolates from herbarium specimens showing multi-ostiolate conidiomata also formed multiple ostioles in stromata in culture, but usually they were only uni-ostiolate in culture. Necks/beaks were variable among the species but appeared uniform within a species. For example, all isolates of *V. fabianae* formed relatively long beaks in culture. Isolates of other species such as the *Cytospora*-like fungus would be uniform in forming relatively short beaks, and other species dependably formed no necks/beaks in culture. The necks/beaks of the *Cytospora*-like fungus displayed regularly distributed surface cells protruding from the tissue resembling short blunt hairs. This trait had not been observed for *Cytospora* species; but similar cells were illustrated on the beak of *Phomopsis eucalypticola* Old & Z.□. Yuan (Yuan *et al.* 1995). The structure of the wall of the beak, but not that of the globe, was of sclerenchymatal textura porrecta, and this was common for *Cytospora* species in nature.

Fig. 16. Tangential sections through conidiomata of *Cytospora* and *Cytospora*-like fungi produced on *Eucalyptus* leaves on water agar. A. *C. variostromatica*. B. *C. eucalyptina*. C. *C. sacculus*. D. *C. nitschkii*. E. *Cytospora* putative species 1. F. *Cytospora*-like fungus.

Several *Cytospora* species formed conidiomata that were glabrous on leaves on agar, while other species had wooly coatings of hyphae on the exterior of the conidiomata (Fig. 16). The globe, but not the neck, of the conidioma of the *Cytospora*-like fungus (phylogenetic lineages Indonesia-1 and Indonesia-2) had a wooly coating of hyphae on the surface which was the exterior of a thick outer wall (Fig. 16F).

Simple pycnidia were formed in culture by many *Cytospora* species that formed cytosporoid conidiomata in nature (Table 2), but relatively few species produced distinctive pycnidia. The pycnidia had a discrete wall and the locule was divided by invaginations into usually 2–6 chambers. Distinctive features were limited to the sum of the characters of the exterior surface, neck/beak length, colour, and, perhaps, relative mean diameter. The relative mean diameter varied with media but was more consistent on autoclaved leaves and stems. However, diameter of conidiomata *in vitro* was not similar to the diameter in nature and conidiomata seldom formed within the tissues of the autoclaved plants. Huge stromata formed occasionally *in vitro* and the larger the stromata, the fewer stromata were produced. Our conclusion was that more study is necessary before diameter and density of conidiomata forming *in vitro* can be accepted as reliable, reproducible characters.

Species that formed cytosporoid conidiomata in nature formed pycnidia *in vitro* on autoclaved stems and leaves. *Cytospora disciformis* was a typical representative of the cytosporoid species forming pycnidia of one tissue type. *Cytospora* putative species 1 differed among pycnidial producers in having relatively numerous (*ca* 16) chambers formed in the locule. The conidiomata of *V. brevispora* was unique in having a separate exterior wall encompassing the wall layer that formed the locule and invaginated to divide the locule into chambers. The latter type of conidioma would not fit our concept of a pycnidium, rather it would be a simple stromatal conidioma. Species that formed torsellioid, leucotorsellioid and leucocytosporoid conidiomata in nature formed complex stromatal conidiomata *in vitro*. Pycnidia had one wall, simple stromatal conidiomata had two walls, whereas complex stromatal conidiomata had locules (undivided) with independent walls surrounded by 2–3 exterior wall layers (Fig. 16B–C). Furthermore, leucotorsellioid species were most complex in the

numbers and colours of wall layers surrounding the locules and the ostioles (Fig. 16D). For example, *C. agarwalii* formed globose fruiting bodies in culture that would be called pycnidia if examined casually, but sectioning revealed three wall layers with different colours. The walls presumably corresponded to ectostroma, entostroma, and locule walls when formed on host substrate. The colours of the wall layers corresponded to the colours of various stromatic tissues of conidiomata in host substrates. Furthermore, leucostomoid species occasionally produced a whitish collar (or layer) of tissue around the apex of the ostiole, which could correspond to the whitish disc formed in host substrate. Similarly, *C. abyssinica*, *C. eucalyptina*, *C. nitschkii*, *V. eucalypti* and *V. eucalypti sensu* Sharma *et al.* produced stromatal conidiomata with several walls of different colours *in vitro*.

Differential sensitivities to cycloheximide

Differences among species in cycloheximide sensitivity are presented in Table 2. Differential sensitivity to cycloheximide was significant among species of *Cytospora* but uniform among isolates within most species. An exception was *V. myrtagena* where isolates from Hawaii, U.S.A., differed in sensitivity from isolates of Sumatra, Indonesia. Furthermore, sensitivity to cycloheximide was influenced by the growth medium in certain species. For example, sensitivity to 2 ppm cycloheximide was reduced in Leonian's agar compared to V8® juice agar in some species. The latter medium was less favourable for robust growth of *Cytospora*. Such a dramatic influence of growth media was evident for *C. disciformis* and the *Cytospora*-like species (Table 2). We did not test tolerance to cycloheximide at concentrations as high as 400 ppm.

Differential growth response to temperature

Cardinal temperatures of growth were significantly different among species (Table 2). The temperatures for growth that effectively differentiated among species were 37 ºC and 32 ºC. At least six species (*V. brevispora*, *V. eugeniae*, *V. fabianae*, *V. myrtagena*, *C. eucalyptina*, *Cytospora* putative species 1) grew well at 37 ºC, which was unexpected for the genus *Cytospora*. Two species (*V. eucalypti* and *C. valsoidea*) did not grow at 32 ºC. While several species grew more slowly at 32 ºC than at 25 ºC, many species grew faster. Growth rate in response to the cardinal temperatures was uniform among isolates within a species except in *V. myrtagena*. Isolates of *V. myrtagena* from Indonesia scarcely grew at 37 ºC, whereas isolates from Hawaii grew well.

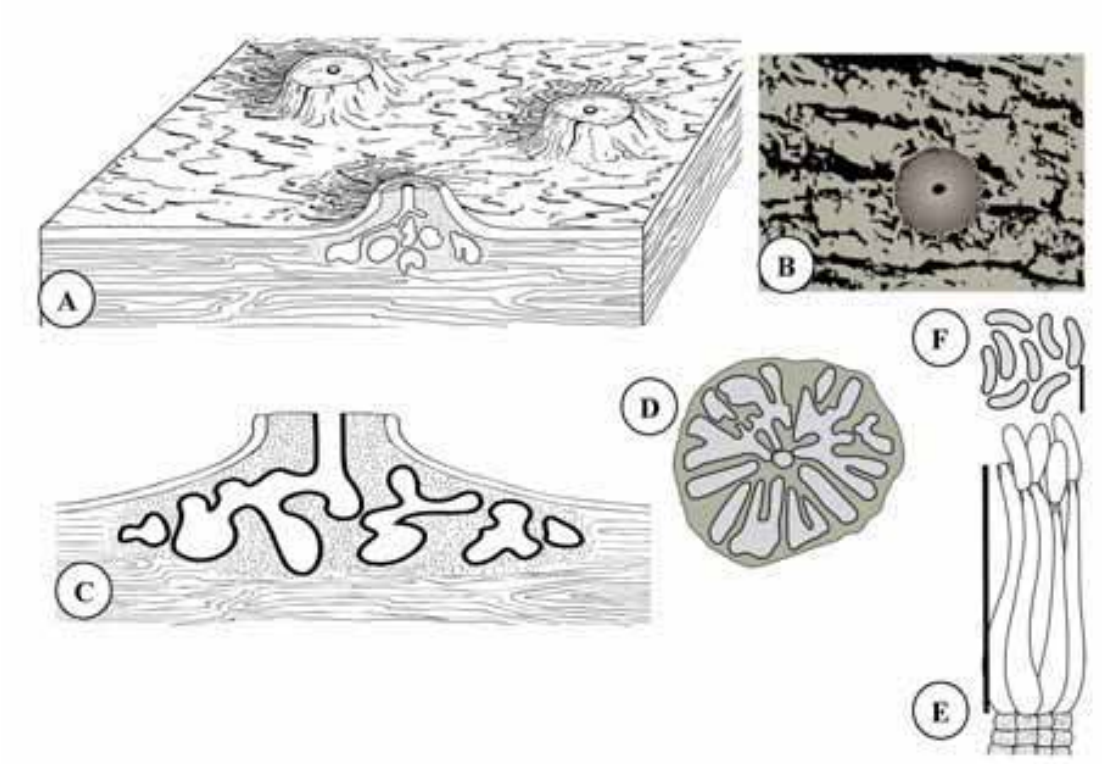

Fig. 17. Illustration of the holotype of *C. australiae*. A. Habit sketch. B. Ostiolar disc erumpent from bark. C. Longitudinal section through conidiomal stroma in plant. D. Horizontal cross section through conidiomal stroma. E. Conidiophores in hymenium. F. Conidia. Scale bars: E = 10 µm, F = 4.5 µm.

Table 2. Differential characteristics *in vitro*.

Taxon	Cycloheximide tolerance growth as % control[a]	Mean linear growth on PDA mm diam / 7d[b]			Locules yes/no[c]
	V8® agar or (Leonian's agar)	37 ºC	32 ºC	25 ºC	Leaves
Valsa brevispora	80.5	38	140	140	no
Valsa ceratosperma s.str.	0	0	2	108	yes
Valsa cinereostroma	0	0	40	138	yes
V. aff. *cinereostroma*	0	0	40	70	no
Valsa eucalypti	64	0	0	137	yes
Valsa eugeniae	64	21–42	136	136	no
Valsa fabianae	0	20	115	88	no
Valsa myrtagena (Sumatra)	0 (25)	3	114	114	no
Valsa myrtagena (Hawaii)	50	39	128	128	no
Cytospora abyssinica	42	0	14	88	yes
Cytospora austromontana	0[d]	0[d]	60[d]	3.8 (29[d])	no
C. aff. *austromontana*	0	0	220	62	no
Cytospora berkeleyi	0	0	5	54	no
Cytospora diatrypelloidea	0	0	9	30 (67[d])	no
Cytospora disciformis	0 (33)	0–3	47	60	no
Cytospora eucalyptina	83	13	183	138	yes
Cytospora nitschkii	86	0	6	94	yes
Cytospora valsoidea	82.6 (53)	0	0	50	no
Cytospora variostromatica	66	0–5	87	81	no
Cytospora putative species 1	86	28	220	145	no
Cytospora-like species	0 (25)	0	162	185	no

[a]Measurements were the ratio of mean growth with cycloheximide divided by mean growth without (control) × 100. Cycloheximide was added to media in absolute ethanol (1 mL/L) for a final concentration of 2 ppm/L.

[b]Growth rate on PDA was measured on 85 mm Petri dishes after 7 d incubation in dark. The measurements were a mean of the number of available isolates of a species listed in Table 1.

[c]yes = Conidiomata with locules having independent walls. Conidiomata were stromatal and possessed other encompassing layers (walls) often of differing colours;

no = Conidiomata with locules not having independent walls. Conidiomata were simple pycnidia with a total of one wall. Conidiomata were producted on autoclaved *Eucalyptus* leaves overlying water agar.

[d]Growth responses were recorded on oatmeal agar because growth on PDA was poor.

TAXONOMY: TREATMENT OF SPECIES

Re-interpretation of pre-existing holotypes and isotypes

1. *Cytospora australiae* Speg. var. ***australiae***, Anales Soc. Ci. Argent. 9: 189. 1880; Fungi Arg., Pug. I, 120: 189. 1899; Syll. Fung. 3: 256. 1884, **emend.** G.C. Adams & M.J. Wingf. Figs 17–18.

Teleomorph unknown. *Conidiomatal stromata* immersed in bark, erumpent, torsellioid, discoid, circular to ovoid, up to 1 mm diam, numerous crowded and compressed locules with brown entostroma above globes. Superficially resembling labyrinthine cytosporoid conidiomata. *Discs* reduced, pale brown to olivaceous, flat, circular to ovoid, 30–50 µm diam, with discrete ostioles. *Ostioles* dark reddish brown, 15–17 µm diam, at the same level as the discs, surrounded by brown entostromata of *textura globosa* below discs. *Locules* simple undivided, not sharing common walls, subgloboid to compressed, 67–98 µm diam, radially arranged, numerous, crowded and compressed, with necks converging at the discs into the discrete ostioles. *Conidiophores* hyaline, unbranched or occasionally branched at base, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes

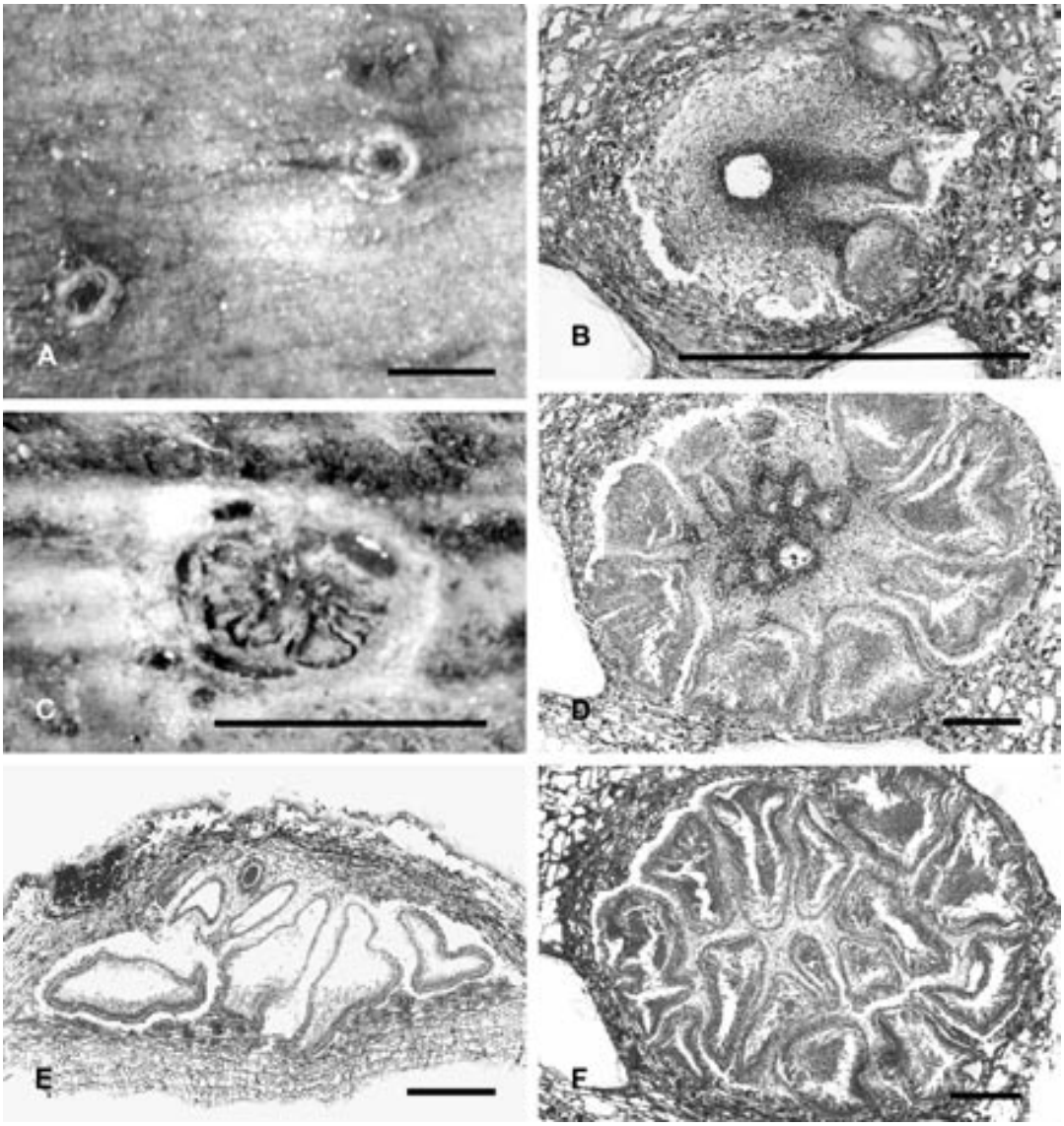

Fig. 18. Morphology of the holotype of *C. australiae*. A. Discrete ostioles in discs on *Eucalytus* branch. B. Tangential section through disc shows multiple ostioles converging into a discrete ostiole. C. Tangential section shows circinate locules with independent walls. D. Tangential microtome section shows circinate locules with independent walls. E. Longitudinal section through conidioma shows crowded locules with independent walls. F. Tangential microtome section of conidioma shows crowded independent locules resembling a discrete locule divided into multiple chambers. Scale bars: A–B = 100 µm, C = 1 mm, D–F = 100 µm.

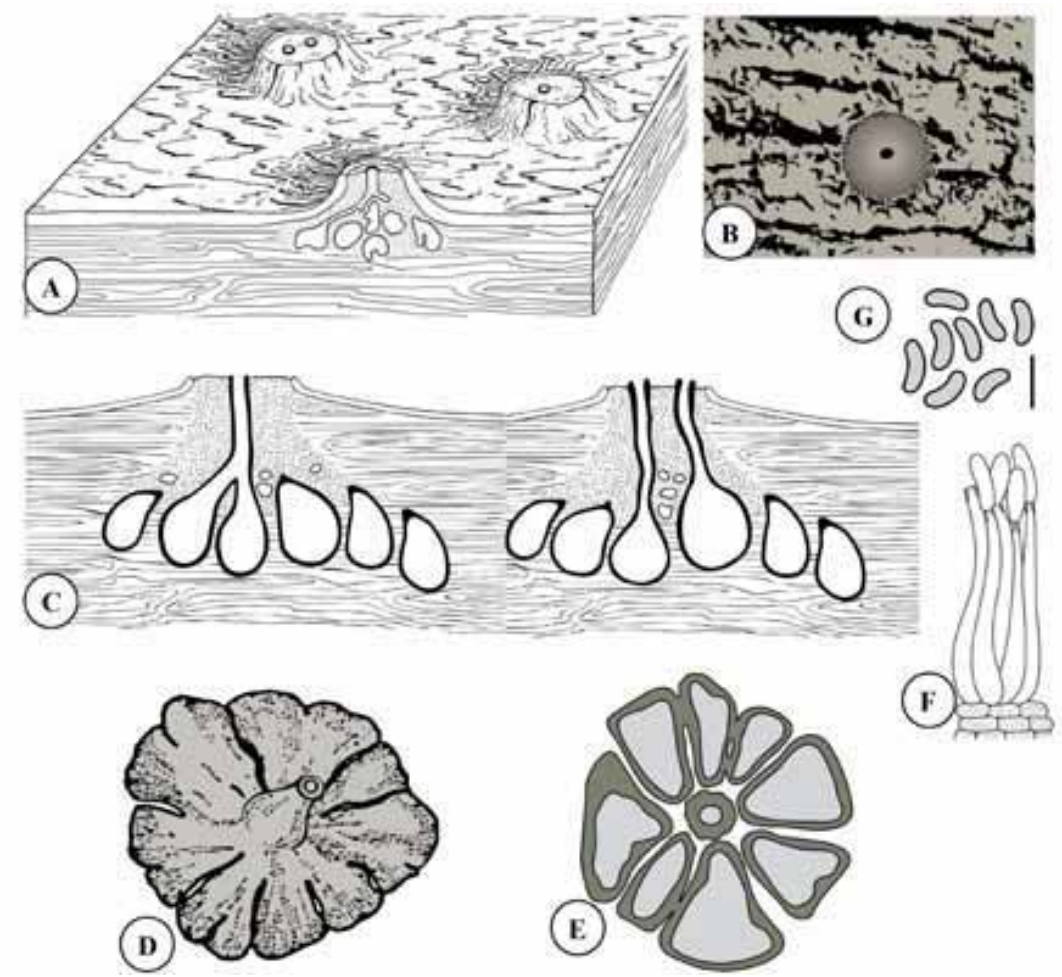

Fig. 19. Illustration of the holotype of *C. australiae* var. *foliorum*. A. Habit sketch. B. Ostiolar disc erumpent from bark. C. Longitudinal section through conidiomatal stromata in plant. D. Top view of conidioma isolated from plant tissues. E. Horizontal cross section through conidiomal stroma. F. Conidiophores in hymenium. G. Conidia. Scale bar G = 3.5 µm.

minute, 6–10 × 1 µm. *Conidia* hyaline, eguttulate, allantoid, aseptate, 4.5 × 1.2 µm.

Host: *Eucalyptus globulus*.

Distribution: Only known from the type locality.

Specimen examined: **Argentina**, Buenos Aires: Recoleta, on dead twigs of *Eucalyptus globulus*, 18 Feb. 1880, C. Spegazzini (LPS 31746, **holotype** of *C. australiae*).

Notes: This species had been mis-interpreted, causing mistaken reports of its occurrence in several nations such as U.S.A. and South Africa. The type specimen had independent locules of torsellioid conidiomata. However, the locules were so numerous, crowded and compressed that the stromatal morphology had been mistaken for that of labyrinthine cytosporoid conidiomata.

2. *Cytospora australiae* var. ***foliorum*** Gutner, Acta Inst. Bot. Acad. Sci. USSR 2: 428. 1935. Fig. 19.

(Interpreted description based on translation of Russian description and illustration)
Teleomorph unknown. *Conidiomatal stromata* immersed in leaf tissues, erumpent from top or bottom sides of leaves, torsellioid, ovoid and cone-shaped, *ca* 200 µm diam and 130 µm high, dark reddish brown, 2–3 locules in entostromata. *Discs* dark reddish brown, flat, ovoid to circular, 60–75 µm diam, 1 ostiole. *Ostioles* dark reddish brown, 60–75 µm diam, at the same level as the disc surfaces, surrounded below discs by lighter entostroma. *Locules* simple undivided, not sharing common walls, *ca* 45–100 µm diam, regularly arranged, walls dark reddish brown, pseudoparenchymatous, 7.5–12 µm thick, discrete ostioles converging acutely and diagonally considerably below discs into 1 shared ostiole. *Conidiophores* hyaline, unbranched to occasionally branched at the base or at mid-height, embedded in

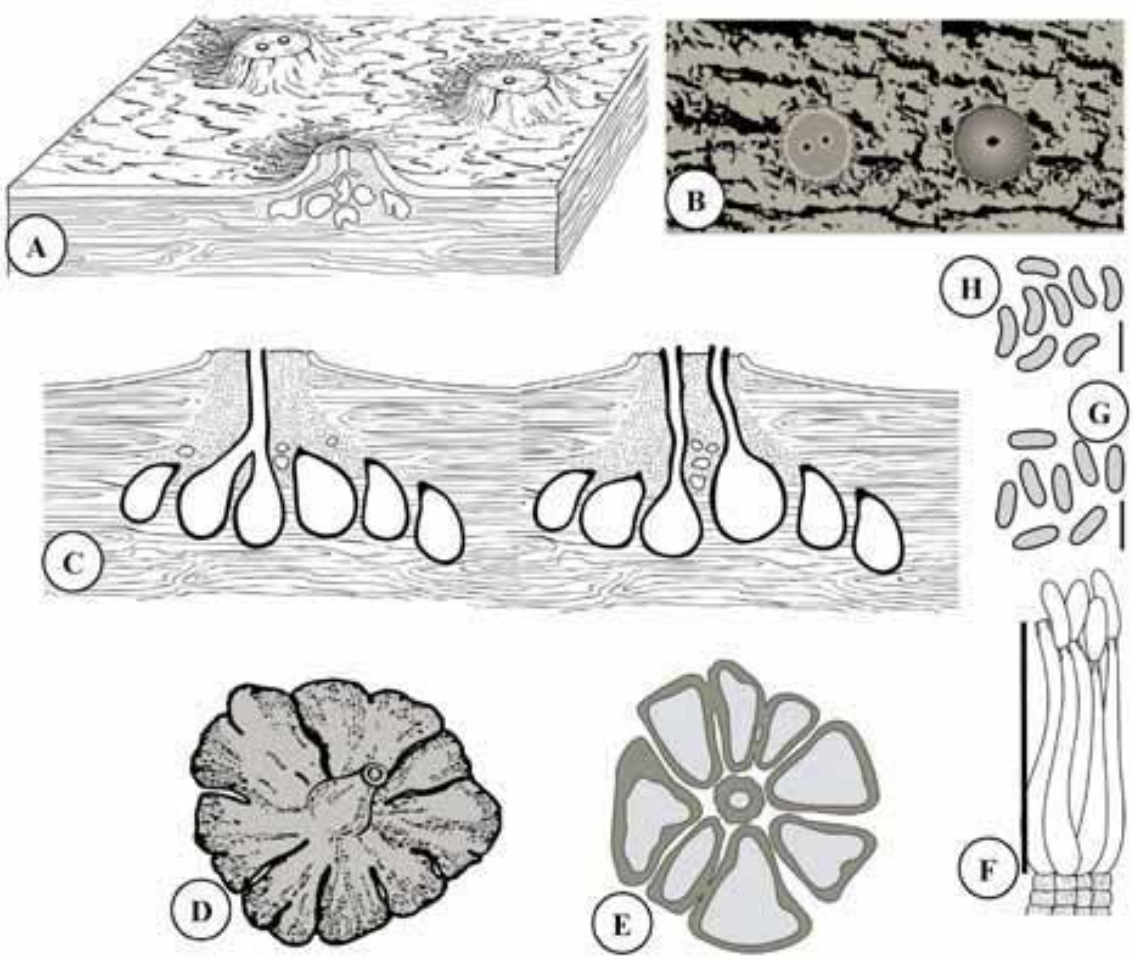

Fig. 20. Illustration of the holotype of *C. eucalyptina*. A. Habit sketch. B. Ostiolar discs erumpent from bark. C. Longitudinal sections through conidiomal stroma in plant. D. Top view of conidioma isolated from plant tissues. E. Horizontal cross section through conidiomal stroma. F. Conidiophores in hymenium. G. Conidia. Scale bars: F = 8 µm, G–H = 3.5 µm.

a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, periclinal thickenings slight. *Conidia* hyaline, eguttulate, straight to allantoid, aseptate, 3–3.75 × 1–1.5 µm.

Host: *Eucalyptus* sp.

Distribution: Georgia, U.S.S.R.

Specimens examined by Gutner: **Georgia**, on dead leaves of *Eucalyptus*, L.S. Gutner ("*Cytospora foliicola* Libert., leg. Siemanszko", no specimen number provided. If extant and adequately labeled, then the specimen would be the **holotype** of *Cytospora australiae* var. *foliorum*). We were unable to locate authentic material.

Notes: Based on the published description and illustration, the new variety was described from a single collection. The collection was Gutner's but she noted that the Polish mycologist Seimaszko had identified the specimen as *Cytospora foliicola* Lib. *Cytospora foliicola* was later transferred to *Ceuthospora* [*Ceuthospora foliicola* (Lib.) Jaap]. Gutner examined specimens of *C. foliicola* from many hosts and she rejected Seimasko's identification. *Cytospora foliicola* had much larger conidia, 12 × 2.5 µm, bearing a funnel-shaped mucoid apical appendage (DiCosmo *et al.* 1983, 1984, Nag Raj 1993). The only known species of *Ceuthospora* described from *Eucalyptus* leaves was *C. innumera* Massee and it had conidia 21 × 3.5 µm with single appendages (Nag Raj 1993).

Gutner treated several species in the genus *Torsellia* in the monograph (Gutner 1935). *Cytospora australiae* was torsellioid, and the description and illustration of the new variety agreed with *Torsellia*. However, Gutner did not place the new variety in the genus *Torsellia* nor did she recognise that *C. abietis* and *C. australiae* belonged in *Torsellia*, so we conclude that her concept of *Torsellia* was incomplete. In the diagnosis Gutner, emphasized the acute convergence of independent

ostiolar necks into a discrete neck well below the disc. She also carefully illustrated this character which we had seen only in *C. eucalyptina* (see below). Without material to study, we interpreted this variety as similar to *C. eucalyptina* but with much smaller conidia and reduced stromata. The stroma was likely reduced due to formation in a leaf rather than in bark.

3. *Cytospora eucalyptina* Speg., Fungi Arg. novi v. crit., 742: 319. 1899; Syll. Fung. 14: 903. 1902, **emend.** G.C. Adams & M.J. Wingf. Figs 20–23.

Teleomorph unknown. *Conidiomatal stromata* immersed in bark, erumpent, torsellioid, ovoid to elongate ovoid, 0.6–1.2 mm diam, medium grey, up to 13 locules in cream to pale brown entostromata below discs. *Discs* medium brown to dark grey-brown, convex to hemispherical, circular, 0.15–0.35 mm diam, 1(–3) ostiole(s). *Ostioles* dark brown, 60–196 µm diam, at the same level as the disc surfaces, surrounded below discs by pale brown entostromata of *textura globosa* or amorphous material. *Locules* simple, undivided, not sharing common walls, *ca* 100 µm diam, regularly radially arranged, walls brown, of *textura epidermoidea*, 4–6 cells, 10 µm thick, discrete ostiolar necks converging considerably below the discs, globes surrounded by bark cells. *Conidiophores* hyaline, unbranched to occasionally branched at the base or at mid-height, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, periclinal thickenings minute, 6–9.5 × 1–1.5 µm. *Conidia* hyaline, eguttulate, allantoid to nearly straight, aseptate, (3–)4–5(–6) × 1–1.3 µm.

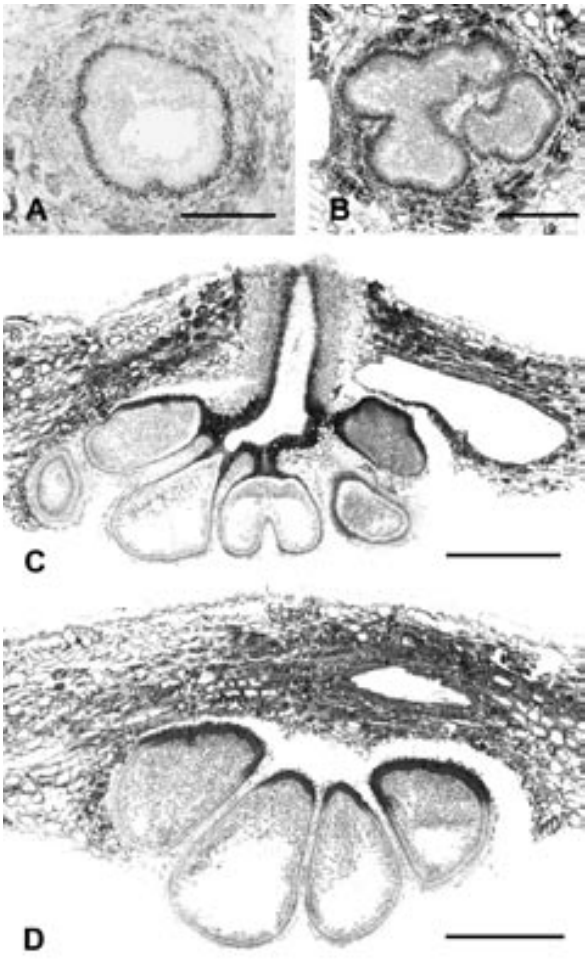

Fig. 21. Morphology of the holotype of *C. eucalyptina*. A. Tangential microtome section below disc of a single ostiole. B. Tangential microtome section of same ostiole deeper below disc where ostioles begin to converge. C. Median longitudinal section shows locules with independent walls, ostiolar necks converging into a discrete ostiole, and locules surrounded by stroma tissues. D. Longitudinal section further out on the periphery of the conidioma shows independent locules. Scale bars: A–D = 100 µm..

Cultures: Colony growth on PDA of isolates from Columbia is predominantly yellowish white (Munsell 4.5Y 9.2/1.2) on surface varying to yellowish grey,

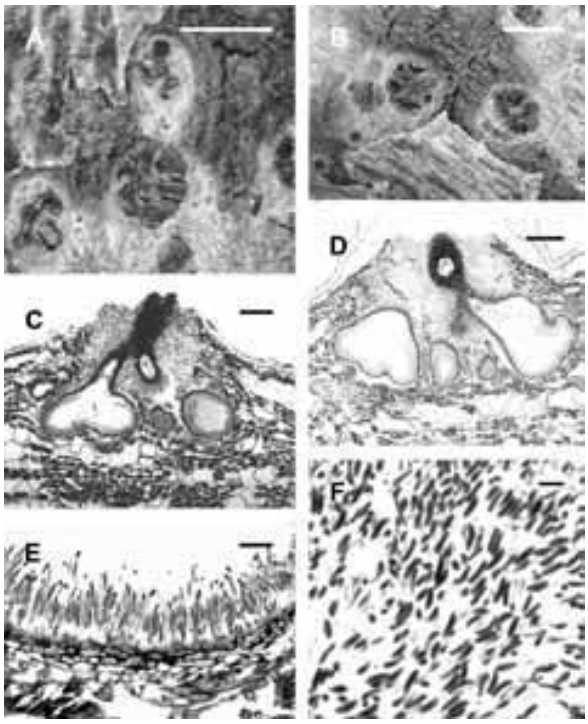

Fig. 22. Morphology of the Colombia-1 specimen of *C. eucalyptina*. A. Tangential section exposing circinately arranged locules in *Eucalyptus* bark. B. Deeper tangential cut through circinately arranged locules and nearby single ostiolar discs (lower left). C. Median longitudinal microtome section shows locules with independent walls, multiple ostiolar necks converging into a discrete ostiole, and surrounding stroma tissues. D. Another median longitudinal microtome section shows extent of stroma tissues. E. Microtome section through hymenium shows arrangement and branching of conidiophores and wall tissue. F. Conidia of the holotype. Scale bars: A–B = 1 mm, C–D = 100 µm, E = 10 µm, F = 4 µm.

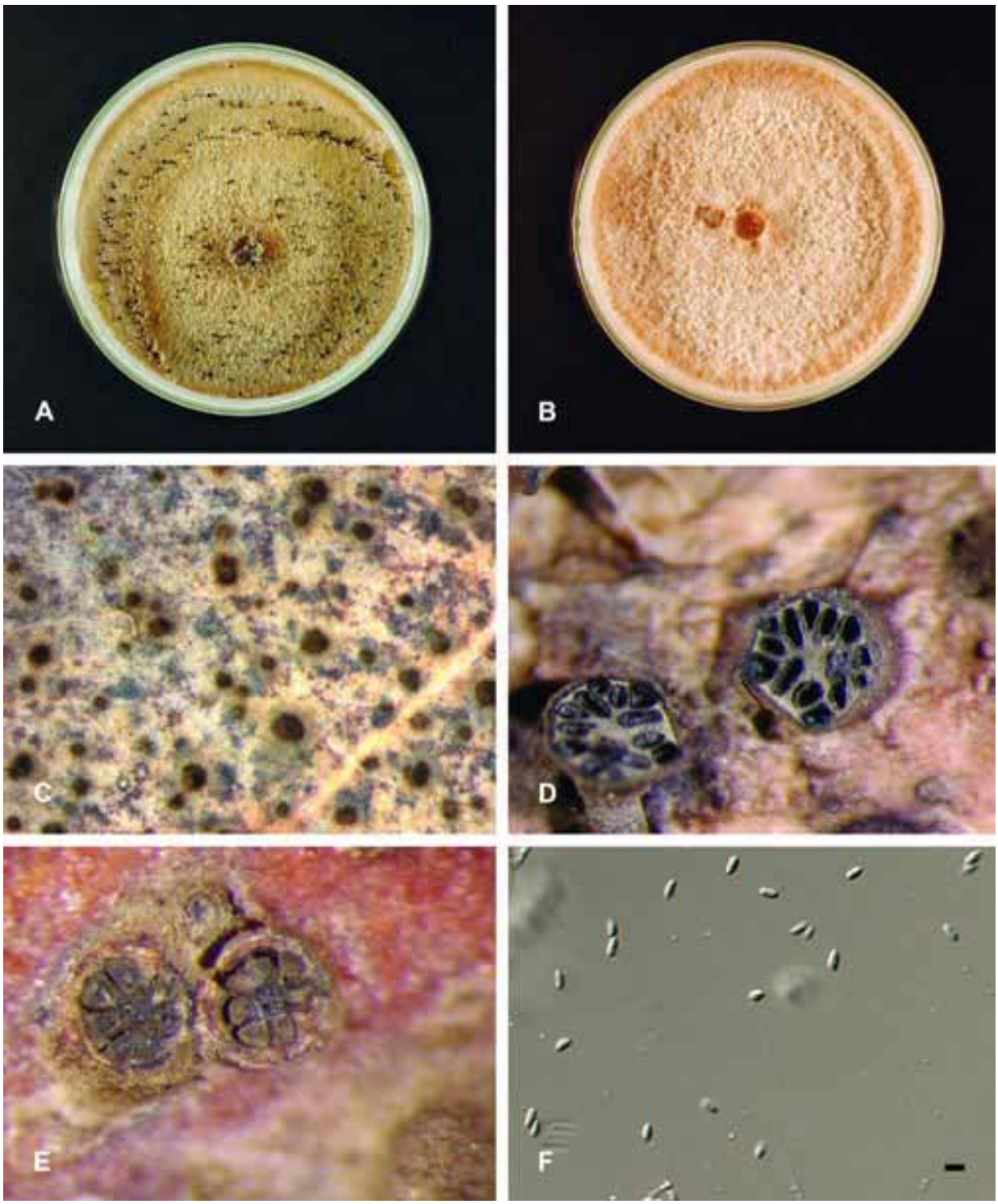

Fig. 23. Culture characteristics of *C. eucalyptina*. A, B. Colony morphology on PDA of isolates CBS 117080, Mexico-1 (left) with zonal growth and CBS 116853, Columbia-1 (right). C. Conidiomata produced on autoclaved *Eucalyptus* leaf. D. Tangential sections of leucotorsellioid conidiomata show outer wall of brown ectostroma, white entostroma, and dark brown walls of multiple independent locules. E. Tangential sections of torsellioid conidiomata show independent locules. F. Conidia. Scale bar: F = 5 μm.

greyish yellow to dark greyish yellow with age (Munsell 4.4Y 7.2/3.8, 3.8Y 5.9/4.0, 3.8Y 7.4/1.4). Colour of the reverse is predominantly dark orange yellow (Munsell 9.3YR 6.0/7.9) but varies from moderate yellow, deep yellow brown to pale and moderate olive-brown (Munsell 3.8Y 7.1/6.5, 8.8YR 3.1/5.0, 2.1Y 4.9/7.9, 2.7Y 3.6/5.5). Colony texture is felty, slightly raised with no growth zones. Pycnidia do not form on the agar. Colony growth of isolates from Mexico is predominantly pale greyish olive (Munsell 7.8Y 5.5/2.5) on the surface. Colour on the reverse is pale to dark olive-brown (Munsell 2.1Y 4.9/7.9, 2.0Y 1.9/2.2). A diffusible pigment colours the agar dark brown (Munsell 5.3YR 1.6/3.4), and influences interpretation of the reverse colony colour. Pycnidia form on the agar but no cirrhi occur. Conidia are allantoid and nearly straight. Colony texture is felty, slightly raised with no growth zones. Rosette torsellioid conidiomata with 5–8 regular locules arranged radially surrounded by a wooly brown outermost ectostromatal layer and with no apparent necks/beaks form on the autoclaved leaf. Additionally, leucotorsellioid conidiomata with 12–16 regular locules with dark walls in pale entostromata surrounded in an outermost brown wooly to glabrose ectostromatal layer and with no apparent necks/beaks form on the autoclaved leaf.

Cardinal temperatures: Colonies (mean of three isolates) obtain a mean growth on PDA at 4 ºC of 3.5 mm diam, at 25 ºC of 138 mm diam, at 32 ºC of 183 mm diam, and at 37 ºC of 13 mm diam after 7 d in the dark. Growth at 25 ºC on 2 ppm cycloheximide in V8® agar is 83 % of growth on V8® agar without the antibiotic after 7 d in the dark.

Hosts: *Eucalyptus globulus* and *E. grandis*.

Distribution: La Plata, Argentina; Cali, Colombia; Mexico.

Specimens examined: **Argentina**, La Plata, on dead twigs of *E. globulus*, 18 Aug. 1888, C. Spegazzini (LPS 11656, **holotype** of *Cytospora eucalyptina*). **Colombia**, Cali, on dead branches of *E. grandis*, May 2000, M.J. Wingfield (MSC 375217), living culture CBS 116853. **Mexico**, on cankered branches of *E. grandis*, Jul. 1996, M.J. Wingfield, living cultures CBS 117080, C.M.W. 517.

Notes: A unique feature of the torsellioid anamorph was that the independent ostiolar necks converged acutely, fusing considerably beneath the discs to a discrete ostiole. Additionally, an extensive entostroma surrounded the resulting discrete ostioles. The species was most similar to *C. australiae* var. *foliorum* where the necks also converged at an acute angle into a discrete neck well below the disc. Van der Westhuizen (1965a) considered *C. eucalyptina* a synonym of *C. australiae* but we treated them as separate species. In *Cytospora australiae* the independent beaks fused into discrete ostioles within the disc, not well below the disc, and the stromata were crowded and compressed and the conidia were larger. *Cytospora eucalyptina* was reduced to synonymy with *C. sacculus* by Gvritishvili (1969), Vasilyeva (1994) and Hayova & Minter (1998). We restricted the species concept of *C. sacculus*, below, and excluded *C. australiae* from synonymy.

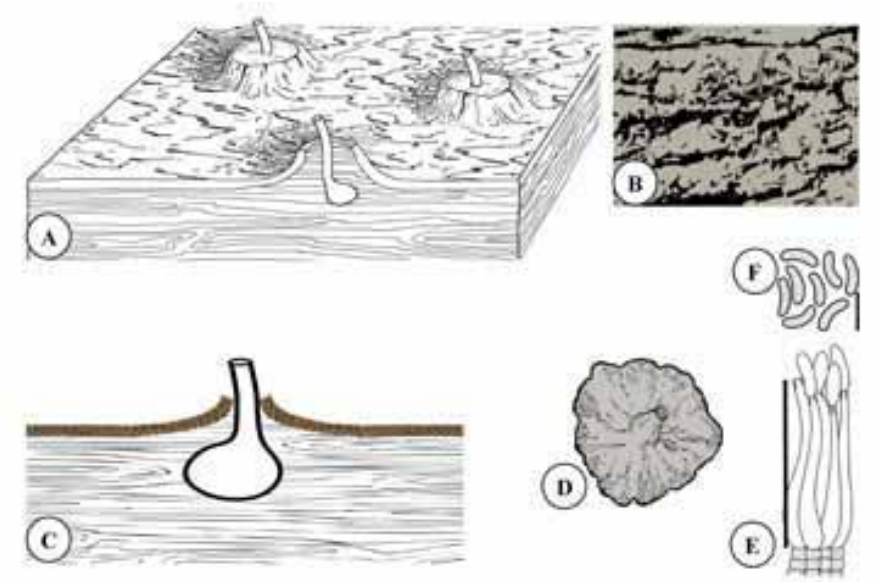

Fig. 24. Illustrations of the holotype of *C. eucalypticola*. A. Habit sketch. B. Ostiolar disc erumpent from bark. C. Longitudinal section through conidiomal stroma in plant. D. Top view of conidioma isolated from plant tissues. E. Conidiophores in hymenium. F. Conidia. Scale bars: E = 8 µm, F = 4 µm.

4. *Cytospora eucalypticola* van der Westh., S. African For. J. 54: 8–11. 1965; non *C. eucalypticola sensu* Sharma, Mohanan & Maria Florence, Kerala For. Res. Inst. Res. Rept 36: 258. 1985. Figs 24–25.

Here we reinterpret and redescribe the holotype specimen only. An emended description of this species with cultural characteristics is part of the diagnosis of the holomorph, *Valsa fabianae* below.

Conidiomatal stromata immersed in bark, erumpent, unilocular, of solitary locules, usually without entostromata. *Discs* usually absent. *Ostioles* medium to pale grey, 100–130 µm diam, at the same level

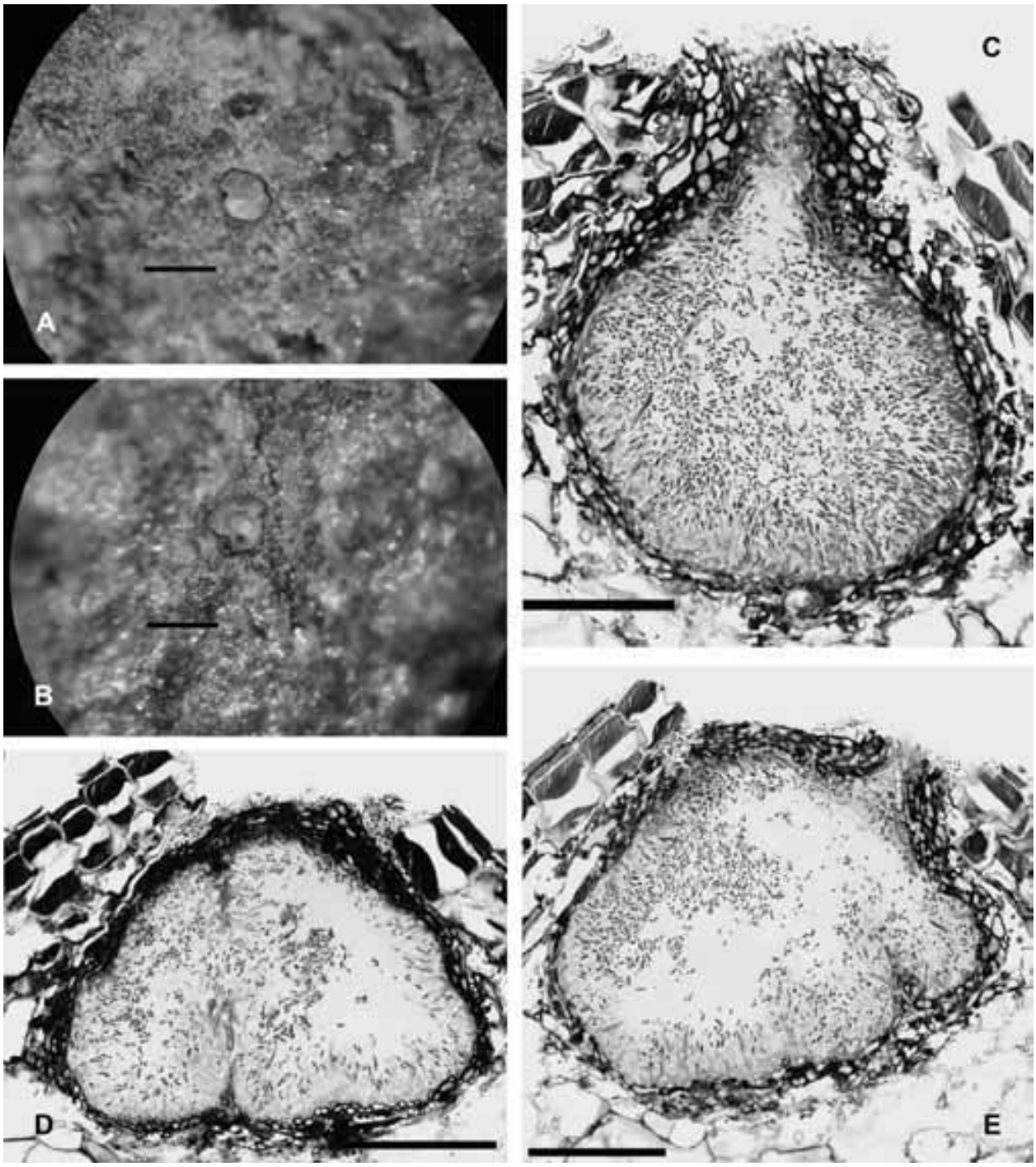

Fig. 25. Morphology of the holotype of *C. eucalypticola*. A. Tangential section through conidioma in *Eucalyptus* bark shows uniloculate character. B. Tangential section through conidioma shows incomplete invagination of wall. C. Median longitudinal microtome section shows typical simple undivided locule. D. Longitudinal microtome section shows rare divided locule. E. Median longitudinal microtome section shows simple locule with partial invagination of wall. Scale bars: A–B = 1 mm, C–E = 100 µm.

as the bark surface or rarely extending above to 200 µm length. *Locules* simple, undivided, not sharing common walls, to rarely subdivided into two chambers by invagination and sharing common walls, 200–400 µm diam, walls of *textura epidermoidea*, 4–5 cells, 10–17 µm thick. *Conidiophores* hyaline, unbranched or occasionally branched at the base, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, 6.5–9 × 1 µm. *Conidia* hyaline, eguttulate, allantoid, aseptate (3.5–)4(–4.5) × 0.8 µm.

Host: *Eucalyptus saligna*.

Distribution: Tzaneen, Northern Transvaal, South Africa.

Specimen examined: **South Africa**, Northern Transvaal, Tzaneen, Westphalia Estates, on dead branches of *Eucalyptus saligna* in plantations. 31 Mar. 1964, G.C. van der Westhuizen (PREM 42543, **holotype** of *C. eucalypticola*).

Note: The fruiting bodies on this specimen were unilocular conidiomata, often lacking discs.

5. *Cytospora agarwalii* Soni, Dadwal & Jamaluddin, Curr. Sci. 52(12): 603. 1983. Figs 26–27.

Teleomorph unknown. *Conidiomatal stromata* in culture, globose, leucotorsellioid, ectostromata and entostromata surrounding locules with independent walls, large, 1.5 mm diam. *Ectostromata* dark olivaceous to grey, thick, 30–79 µm wide. *Entostromata* beige to white, thick, 30–90 µm wide. *Discs* white to cream tissue encircling discrete dark olivaceous ostiole. *Ostioles* dark olivaceous, 73.5 µm diam, not forming beaks. *Conidiomata* globose, solitary, dark grey, 8–10 locules, regular, arranged radially, surrounded by pale entostromata. *Locules* simple, undivided, tear-shaped, 160–260 × 130–170 µm, not sharing common walls. *Conidiophores* hyaline, branched above base, 10–15 ×1.2 µm, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, 10 ×1 µm. *Conidia* hyaline, eguttulate, allantoid, aseptate (4.5–)5 × 1.2 µm.

Host: *Eucalyptus* sp.

Distribution: Only known from the type locality.

Specimen examined: **India**, Madhya Pradesh, Jabalpur, Regional Forest Research Centre, on living branches of *Eucalyptus* sp., 10 Jun. 1980, Bharat Sarkar, no. 9 (IMI 249224, **holotype** of *Cytospora agarwalii*).

Notes: Soni *et al.* (1983) stated that the species was described as new because the conidia were smaller than in known *Cytospora* species. However, the conidia of this species were longer and thicker than most other species of *Cytospora* on *Eucalyptus*. The holotype is represented by a dried agar culture and the description

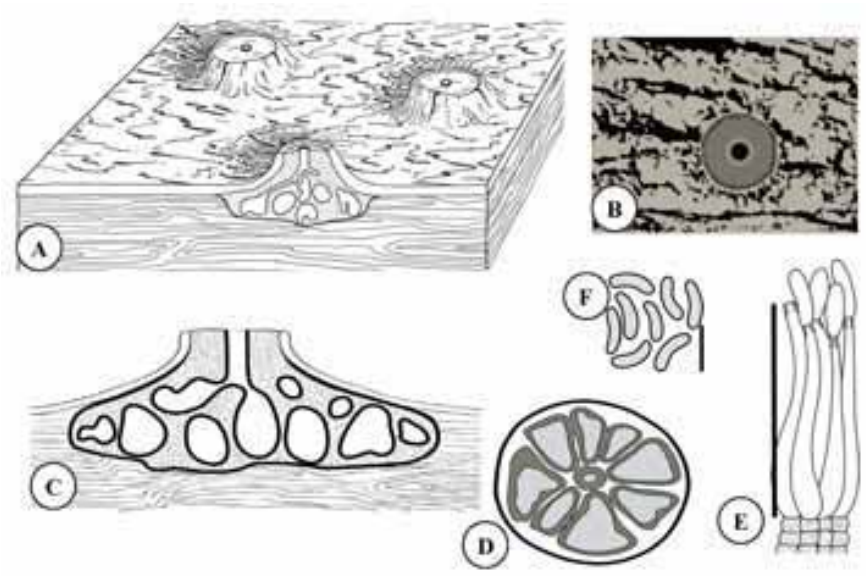

Fig. 26. Illustrations of *C. agarwalii*. A. Habit sketch. B. Ostiolar disc erumpent from bark. C. Longitudinal section through conidiomal stroma in plant. D. Horizontal cross section through conidiomal stroma. E. Conidiophores in hymenium. F. Conidia. Scale bars: E = 10 µm, F = 5 µm.

Fig. 27. Morphology of conidiomata of *C. agarwalii* in culture. A. Spherical conidioma with white fringe of entostroma around ostiole. B. Section through conidioma shows layers of dark brown ectostroma and white entostroma surrounding ostiolar neck and globe. C. Longitudinal section through leucotorsellioid conidioma shows outer layer of dark brown ectostroma, middle layer of white entostroma, and inner walls of dark brown locules. D. Tangential section shows *ca* five independent black locules per leucotorsellioid conidioma. Scale bars: A–D = 1 mm.

by Soni *et al.* (1983) was of the fungus in culture. The large conidomatal stromata with independent locules surrounded by two different coloured layers of tissue (walls) were unique. We interpreted the wall layers as ectostromata and entostromata. Entostromata also encircled the apex of the ostiole resembling a thin off-white collar. These characters occurred in leucostomoid *Valsa* in culture. We believe that *C. agarwalii* might be closely related to *V. eucalypti* Cooke & Harkn., and "*V. eucalypti*" *sensu* Sharma *et al.*

6. *Valsa eucalypti* Cooke & Harkn., Grevillea 9(51): 85. 1881. **emend**. G.C. Adams & M.J. Wingf. Figs 28–31.

≡ *Leucostoma sequoiae* Bonar, Mycologia 20: 295. 1928.

Ascostromata immersed in bark, erumpent, circular to ovoid, 0.9–2 mm diam, leucostomoid circinateous, 0.4–0.5 mm deep, 4–15 perithecia arranged circinately at one depth in the bark, conceptacles light to dark. *Discs* prominent, white to pale brown, nearly flat to convex, circular to lenticular (0.4–)0.7–0.9(–1.2) mm diam, of hyaline amorphous material (2–)4–15 ostioles (on *Eucalyptus*) to (6–)7–10(–12) (on *Sequoia*) laterally inserted. *Ostioles* dark brown, sometimes shiny, 40–120 µm diam, at the same level as the disc surfaces or just above, spaced apart to tightly clustered in discs. *Perithecia* pale to medium brown, globoid, laterally inclined, 0.35–0.5 mm diam, walls of *textura epidermoidea*, (5–)6–7(–14) cells in width, surrounded by cinereus to pale brown entostromata of *textura intricata* from below discs to globes. *Asci* free, elongate-obovoid, sub-cylindrical to clavate, 22–30 × 3–5 µm, with refractive, chitinoid ring in the non-amyloid apical apparatus, 8-spored. *Ascospores* biseriate, sometimes uniseriate, elongate allantoid, thin-walled, hyaline, aseptate (6–)8.5(–10) × 1.5–1.8 µm.

Anamorph intermixed with teleomorph or on separate bark area, discrete. *Conidiomatal stromata* immersed in bark, erumpent, leucotorsellioid, discoid, circular to ovoid, 0.6–1.6 mm diam, 1–12 locules in hyaline to cinereus entostromata below the discs, conceptacles dark. *Discs* off-white, cream to tan, nearly flat, circular to ovoid, 0.4–0.6 mm diam, 1–3(–4) ostioles. *Ostioles*

grey to medium brown, 120–200 µm diam, at the same level as the disc surface. *Conidiomata* grey-brown, of multiple independent locules. *Locules* simple, undivided, radially arranged, not sharing common walls, subgloboid, compressed, 110 µm diam, lying on their sides, with discrete ostioles converging with several ostioles into 1–4 ostioles that are inserted into reduced well-developed discs. Walls of dark grey-brown *textura epidermoidea*, 3–4 cells, 10 µm thick, surrounded by entostromata. *Conidiophores* hyaline, usually branched (0–)1–3(–4) times with 1–5 septa, 12–30 × 1–1.5 µm, inclusive of conidiogenous cells, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, periclinal thickenings slight (4–)8(–11) × 1–1.5 µm. *Conidia* hyaline, eguttulate, allantoid, thin-walled, aseptate (4–)5(–6) × 1 µm.

Cultures: Colony growth on PDA is yellowish white, yellowish grey to pale greyish yellowish brown (Munsell 84.5Y 9.2/1.2, 3.8Y 7.4/1.4, 9.7YR 6.4/2.5) on surface. Colour of the reverse is usually the same colour as the surface but occasionally is dark yellow with hints of dark yellowish brown (Munsell 3.9Y 6.0/6.4, 9.4YR 2.3/3.3). Colony texture is felty, slightly raised with no growth zones. Pycnidia form

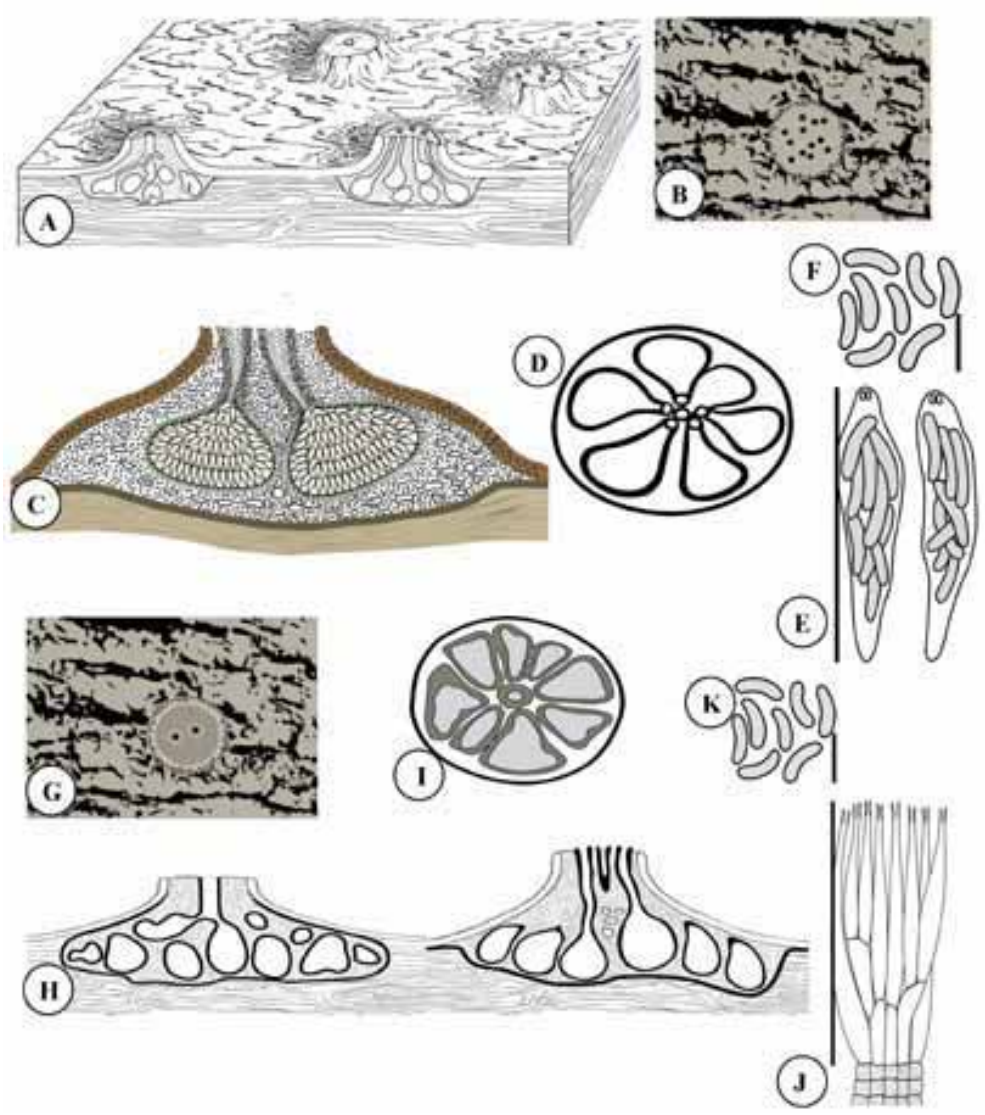

Fig. 28. Illustrations of an isotype of *V. eucalypti*. A. Habit sketch. B. Ostiolar disc of teleomorph erumpent from bark. C. Longitudinal section through ascostroma in plant. D. Tangential section through ascostroma. E. Asci. F. Ascospores. G. Ostiolar disc of anamorph erumpent from bark. H. Longitudinal sections through conidiomatal stromata in plant. I. Tangential section through conidiomal stroma. J. Conidiophores in hymenium. K. Conidia. Scale bars: E = 26 µm, F = 8.5 µm, J = 22 µm, K = 5 µm.

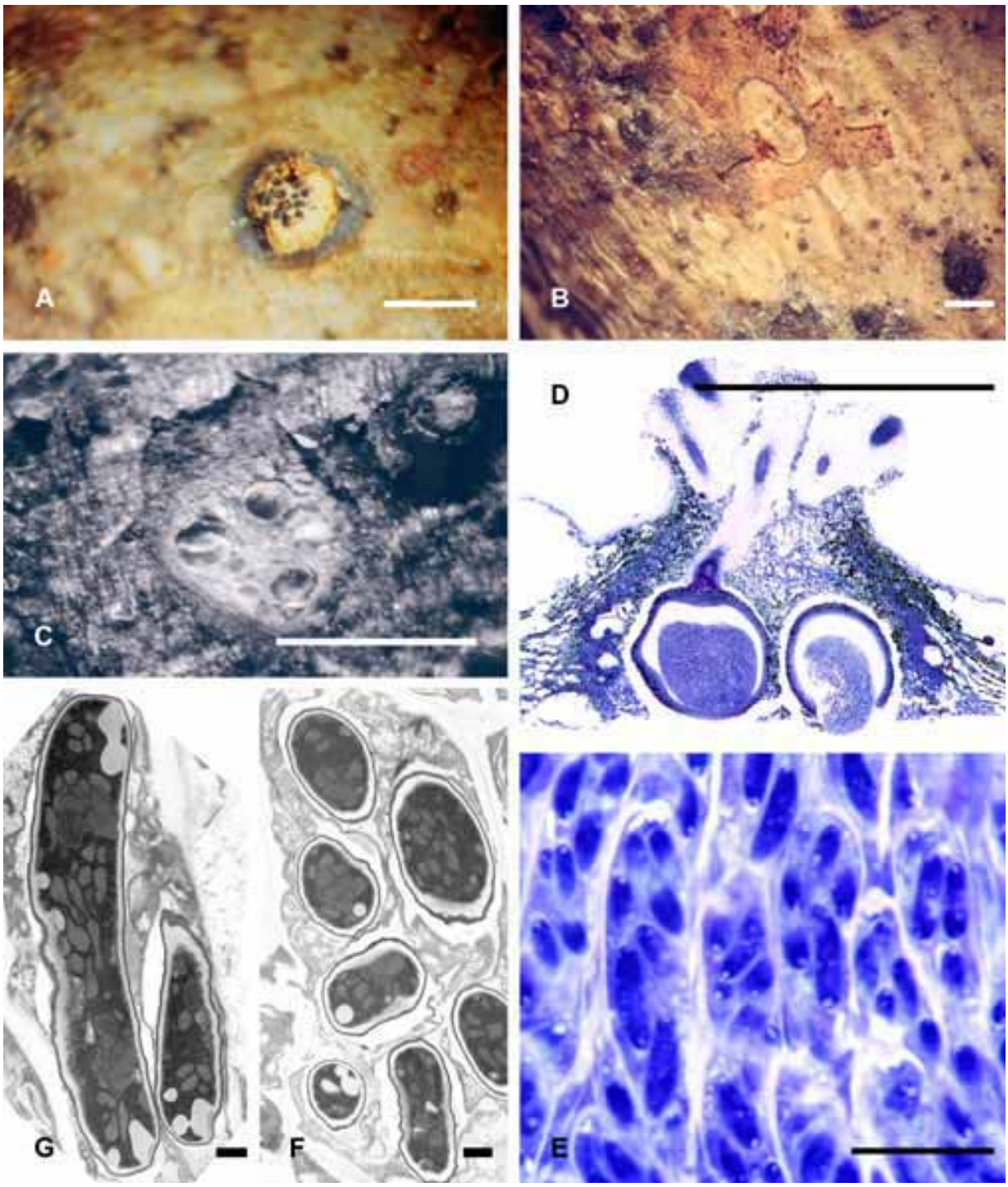

Fig. 29. Morphology of the teleomorph of an isotype of *V. eucalypti*. A. Hemispherical whitish ascostomal disc with multiple (*ca* 16) dark ostioles at the same level as the disc surface. B. Dark line of conceptacle that delimits the ascostoma. C. Tangential section through ascostroma shows cross sections of perithecia and surrounding white entostroma. Conceptacle is difficult to see. D. Median longitudinal microtome section through ascostroma shows arrangement of large globose perithecia surrounded in entostroma and containing asci. Long necks converge into the white disc. E. TEM of longitudinal microtome section through single ascospore shows two nuclei, numerous mitochondria, and lipid globules at both ends. F. TEM of longitudinal microtome section through ascus shows 7 ascospores. G. Asci and ascospores as viewed in bright field light microscopy of a microtome section. Scale bars: A–D = 1 mm, E = 1 µm, F = 1 µm, G = 10 µm.

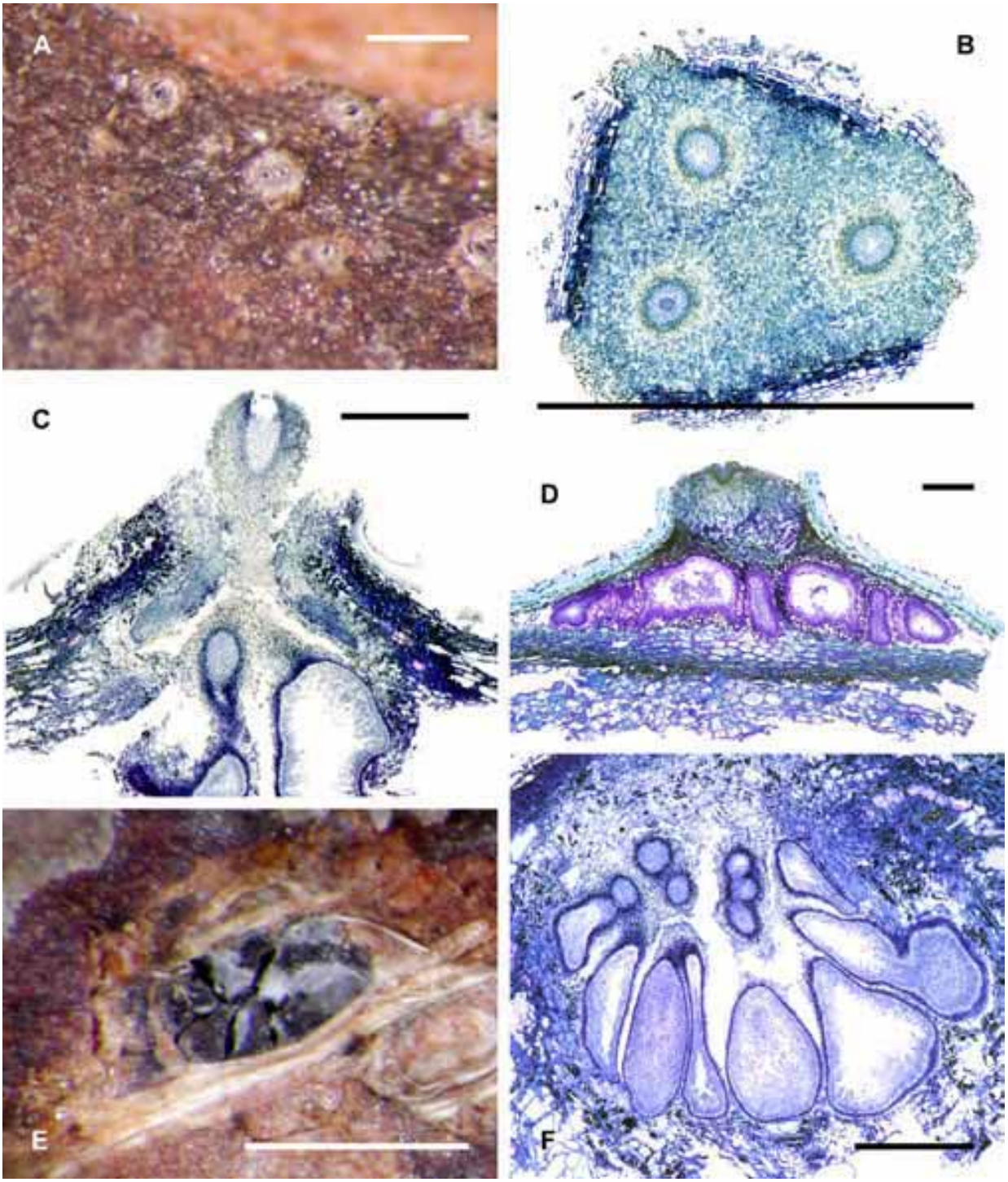

Fig. 30. Morphology of the anamorph of *V. eucalypti*. A. Conidiomatal ostioles (1–3) in discs on *Eucalyptus* bark (California-1). B. Microtome section of disc with typical three ostioles (holotype). C. Longitudinal section through conidiomatal stroma on *Eucalyptus* shows individual locules surrounded in stroma tissues (isotype). D. Longitudinal section through conidiomal stroma on *Sequoia* shows individual locules surrounded in stroma tissues and dark line of conceptacle. Conidioma is more compact and less tall in *Sequoia* bark. E. Tangential section through disc shows converging ostioles from independent locules (isotype). F. Tangential microtome section through conidioma stroma shows arrangement of independent locules and converging ostioles (isotype). G. Tangential section through conidioma shows circinate arrangement of independent locules. Scale bars: A = 1 mm, B–D, F = 100 μm, E = 1 mm.

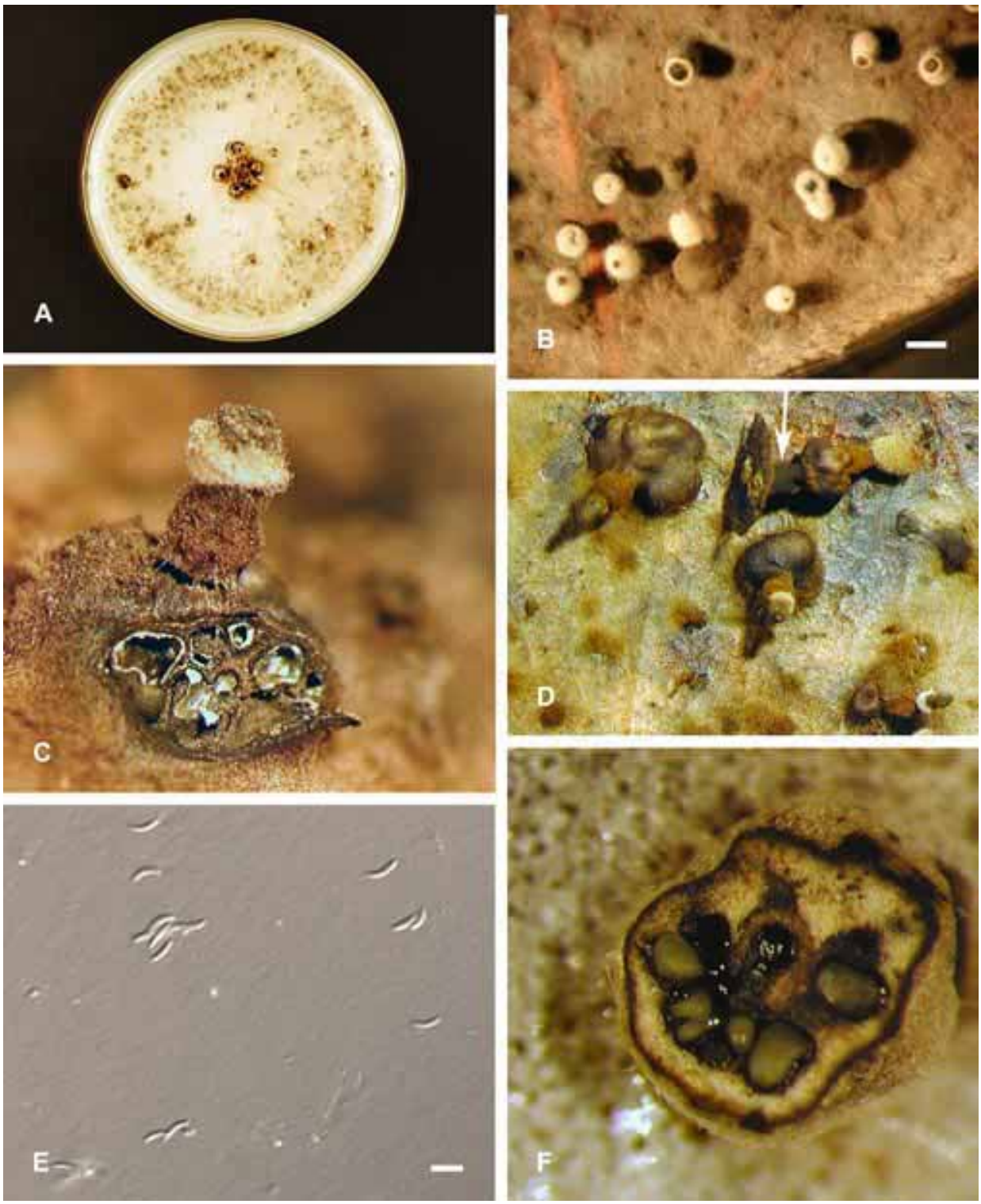

Fig. 31. Culture characteristics of *V. eucalypti*. A. Colony morphology on PDA of isolate CBS 116816, California-1. B. White entostromata surrounding ostioles of conidiomata. C. Longitudinal section through conidioma shows arrangement of locules with independent walls in light brown entostroma. D. Conidiomata produced on autoclaved *Eucalyptus* leaf. Conidiomata are often raised above the surface on a stalk-like extension (arrow). E. Allantoid conidia. F. Longitudinal section through leucotorsellioid conidioma shows dark ectostroma and light entostroma surrounding multiple independent locules with dark walls containing gelatinous masses of conidia. Scale bars: B = 1 mm, E = 5 μm.

on agar and exude yellow cirrhi. Leucotorsellioid conidiomata with 7–16 dark-walled locules in pale brown entostromata surrounded by an outermost thin layer of dark brown ectostromata and with discrete stout necks/beaks form on the autoclaved leaf. Few large glabrose conidiomata with unique lobate surface resulting from close adherence of outer wall to the shape of the inner locules. Conidiomatal ostioles are capped with cream-coloured tissue (entostromata) at the apices of thick necks/beaks.

Cardinal temperatures: Colonies obtain a mean growth on PDA at 4 ºC of 6 mm diam, at 25 ºC of 137 mm diam and no growth at 32 ºC or at 37 ºC after 7 d in the dark. Growth at 25 ºC on 2 ppm cycloheximide in V8® agar is 64 % of growth on V8® agar without the antibiotic after 7 d in the dark.
Hosts: *Eucalyptus globulus*, *E. paniculata*, *Sequoia sempervirens*.

Distribution: Marin County and Palo Alto, California, U.S.A.

Specimens examined: **U.S.A.**, California, on dead branches of *E. globulus*, 1880, Cooke and W.H. Harkness (UM 15128, MSC 11471, **isotypes** of *Valsa eucalypti*); Marin County, Mill Valley, Lake Lagunitas on Mt. Tamalpais, on twig of *Sequoia sempervirens*, 20 May 1923, L. Bonar (UC 469596, **holotype** of *Leucostoma sequoiae*). At this type locality, on fallen branch of *Sequoia* sempervirens, Dec. 1995, G.C. Adams (MSC 380713, MSC 380714), also living cultures CBS 116815 and CBS 116814, respectively; Palo Alto, campus of Stanford University, on fallen cankered branch of *E. paniculata*, 12 Jun. 2001, G.C. Adams (MSC 380708), living culture CBS 116816.

Notes: We believe that *Leucostoma sequoiae* is synonymous with *V. eucalypti* based on ITS-rDNA sequence homology and morphology, and the name *Valsa eucalypti* Cooke & Harkn. has priority. Bonar (1928) described long sterile hymenial elements interspersed among the conidiophores that extended beyond the conidiophores into the conidiomatal chambers. Such hymenial elements were not seen in collections other than the holotype, but were observed in some collections of other species including *C. exigua*, *C. austromontana* and *C. eucalypticola*. A horizontal section through a conidiomatal disc with one ostiole often revealed three converging ostioles (torsellioid), similar to *C. australiae*. The anamorphs of *V. eucalypti* and *V. auerswaldii*, and also *C. agarwalii*, and *C. abyssinica* exhibited the newly described leucotorsellioid type of conidiomata. Leucostorsellioid conidiomata were associated with some teleomorphs of leucostomoid *Valsa*. The question arises as to whether *V. eucalypti* was introduced to California on *Eucalyptus* or whether a redwood pathogen has moved onto the exotic host. Evidence below favours the former hypothesis and the origin of the species and similar species could be India.

7. *Valsa eucalypti* *sensu* J.K. Sharma, C.N. Mohanan & Florence, Kerala For. Res. Inst. Res. Rep. 36: 262. 1985. Figs 32–34.
Anamorph: ***Cytospora eucalypti*** J.K. Sharma, C. Mohanan & Florence, Kerala For. Res. Inst. Res. Rep. 36: 258. 1985 (*Nom. inval.,* Art. 37.1). Fig. 34.

Ascostromata immersed in bark, erumpent, lenticular, 1.0–2 × 0.8–1 mm, leucostomoid circinateous, 12–16 perithecia, circinately arranged, delimited by distinct brown to black conceptacle. *Discs* medium brown, grey to shiny black, circular to ovoid, nearly flat, 600–800 µm diam, 12–16 obscured ostioles. *Ostioles* at the same level as the discs or slightly above, inserted laterally, 80–100 µm diam. *Perithecia* dark brown, circinately arranged, not crowded, globoid to subgloboid, laterally inclined, 150 × 200 µm diam, surrounded in beige to tan entostromata, walls brown, of *textura angularis*. *Asci* free, subcylindrical to clavate, 18–20 × 4–5 µm, 8-spored. *Ascospores* biseriate, allantoid, thin-walled, hyaline, aseptate, 6–6.5 × 1.5 µm.

Anamorph intermixed with teleomorph or on separate bark area, discrete. *Conidiomatal stromata* immersed in bark, erumpent, leucotorsellioid, discoid, lenticular to circular, 10–17 independent locules, in beige to tan entostromata below the discs, distinct pale brown to black conceptacles (0.9–)1.3(–1.7) × (0.8–)1(–1.2) mm. *Discs* shield-like, grey to shiny black, nearly flat, circular to ovoid, 300–600 µm diam, sometimes the conceptacle is visible around the discs, 1–2 ostioles. *Ostioles* dark brown, 120–200 µm diam with 98 µm pore diam, nearly at the same level as the disc surfaces. *Locules* simple undivided, tear-shaped, radially arranged, not sharing common walls, walls medium to dark brown (265–)330(–400) × (125–)162(–200) µm, with ostioles laterally converging to 1–2 shared ostioles. *Conidiophores* hyaline, branched above bases, 12–15 × 1.2 µm, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, periclinal thickenings minute, 10 × 0.8–1 µm. *Conidia* hyaline, eguttulate, allantoid, thin-walled, aseptate, 4.8 × 0.8–1 µm.

Host: *Eucalyptus grandis*.

Distribution: Meenmutty Idukki District, Kerala, India.

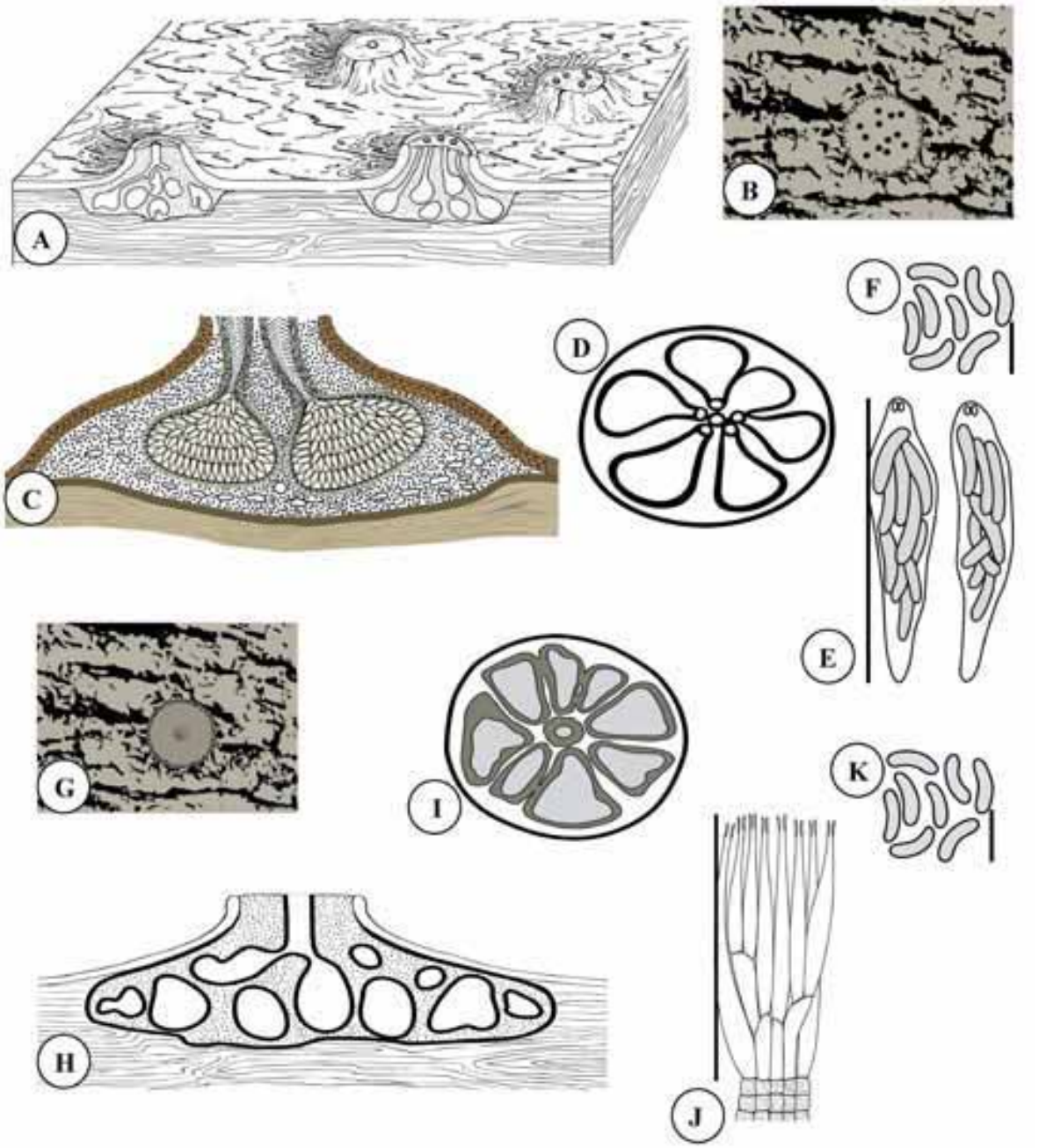

Fig. 32. Illustrations of *V. eucalypti sensu* Sharma *et al*. A. Habit sketch. B. Ostiolar disc of teleomorph erumpent from bark. C. Longitudinal section through ascostroma in plant. D. Horizontal cross section through ascostroma. E. Asci. F. Ascospores. G. Ostiolar disc of anamorph erumpent from bark. H. Longitudinal sections through conidiomatal stromata in plant. I. Horizontal cross section through conidiomal stroma. J. Conidiophores in hymenium. K. Conidia. Scale bars: E = 19 μm, F = 6 μm, J = 13.5 μm, K = 5 μm.

Specimens examined: **India**, Kerala, Meenmutty Idukki District, Kerala Forest Research Institute, on stems of *E. grandis*, 10 Aug. 1981, J.K. Sharma, No. 010 (IMI 261564 **holotype** of *Cytospora eucalypti*, no mature teleomorph present); Kerala Forest Research Institute, on stems of *E. grandis*, 2 May 1981, J.K. Sharma, KFRI-008 (IMI 257896 cited as IMI 257876, one of two **holotypes** of "*Valsa eucalypticola*", not *V. eucalypti*, no anamorph present); and Kerala Forest Research Institute, on stems of *Eucalyptus grandis*, 20 Aug. 1981, J.K. Sharma, No. 014 (IMI 261568, one of two **holotypes** of "*Valsa eucalypticola*" nom. inval., not *V. eucalypti*, no anamorph present).

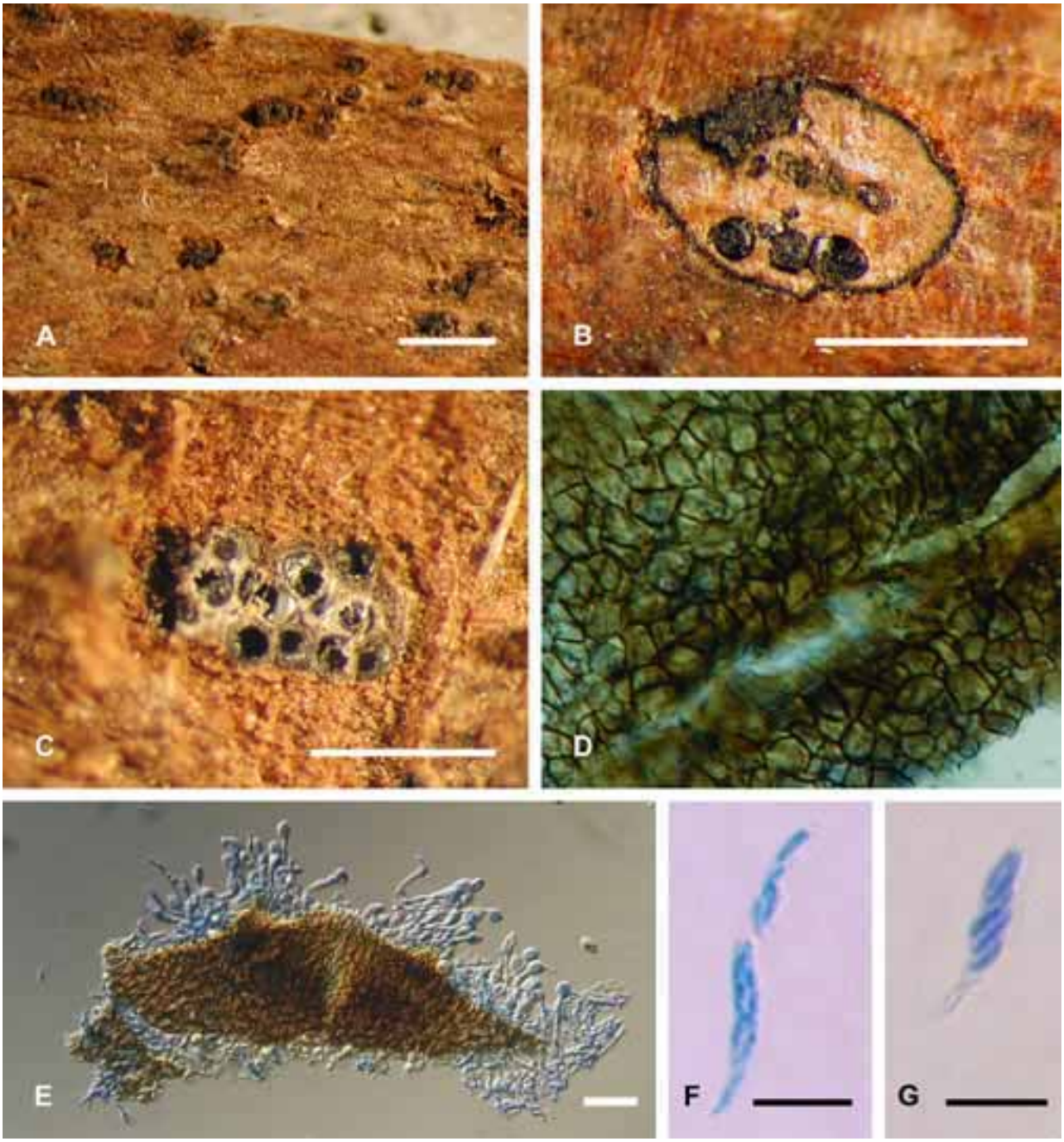

Fig. 33. Morphology of teleomorph of *V. eucalypti sensu* Sharma *et al*. A. Habit of ascostromatal discs on *Eucalyptus* bark. B. Dark conceptacle delimiting ascostroma with white entostroma surrounding globose perithecia. C. Tangential section through ascostroma below the disc shows *ca* 12 perithecia and white entostroma. D. Dark brown perithecial wall of *textura angularis*. E. Croziers or immature asci attached to perithecial wall. F–G. Asci with monoseriate and biseriate arrangement of ascospores. Scale bars: A–C = 1 mm, E–G =10 µm.

Notes: The teleomorph resembled *V. eucalypti* Cooke & Harkn., but had a darker conceptacle and smaller perithecia, asci and ascospores. The anamorph had a distinctive shield-like disc that often was shiny and black; and the conceptacle was visible around the disc. The disc was continuous with the conceptacle. The teleomorphs had distinctive dark conceptacles and, therefore, were leucostomoid like *V. eucalypti* Cooke & Harkn. "*Cytospora eucalypti*" was leucotorsellioid, therefore, *V. eucalypti sensu* Sharma *et al.* was similar to, and could be the same species as, *V. eucalypti* Cooke & Harkn., previously known only from California.

"*Cytospora eucalypti*" is invalidly published because it is published in a research report, only. It is apparently described as the anamorph of what Sharma *et al.* (1985) believed is *V. eucalypti*, but no comments concerning a connection between anamorph and

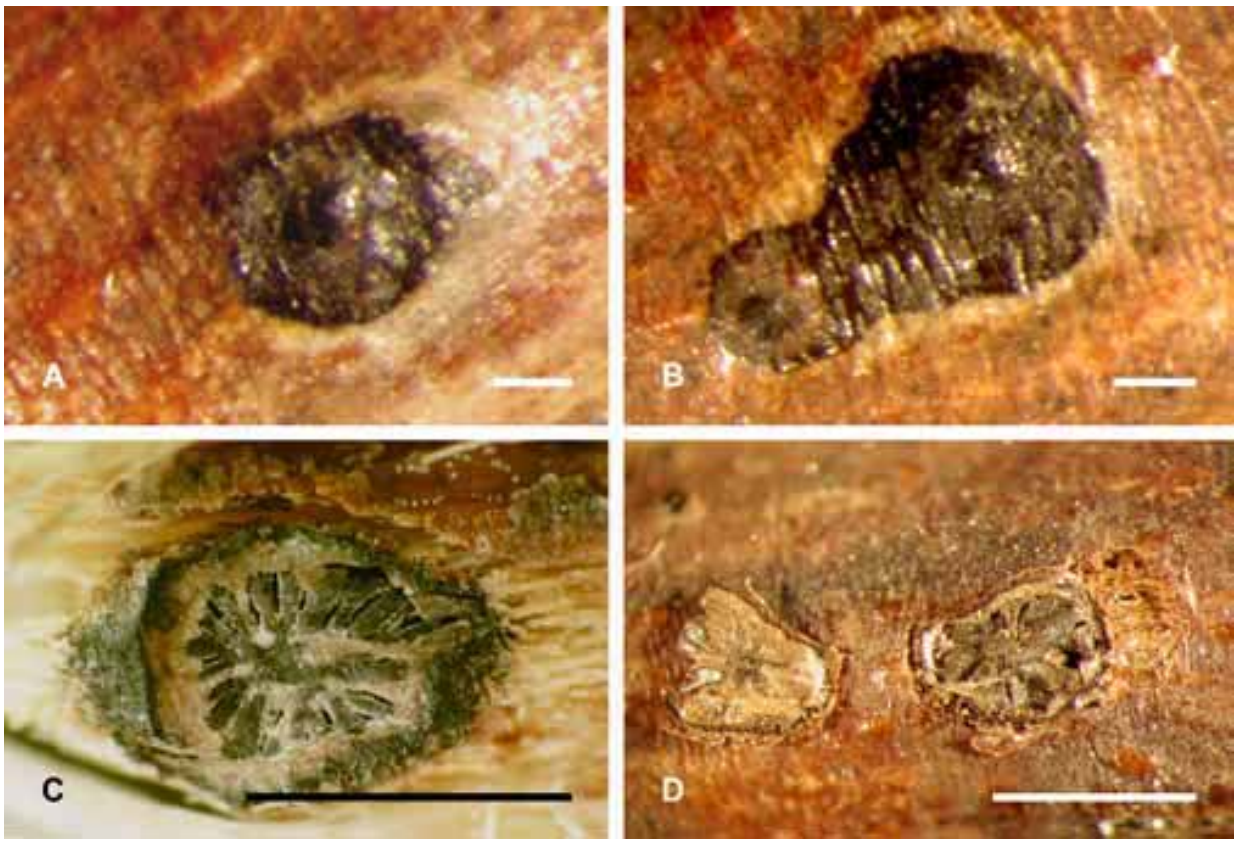

Fig. 34. Morphology of "*C. eucalypti*". A. Glossy black disc and single ostiole. B. Compound conidiomal disc with two separate ostioles. C. Tangential section through conidioma revealing arrangement of multiple (*ca* 15) locules surrounded in stroma tissue. It is difficult in this section to recognise that each locule has independent walls. D. Tangential section through two conidiomata shows circinate arrangement of independent locules. Conceptacles delimiting the conidiomata are light in colour. Scale bars: A–B = 100 µm, C–D = 1 mm.

teleomorph were provided. The description of the anamorph occurs prior to the teleomorph description and is separated by several pages of unrelated text. The two descriptions list the same specimen as "holotype", IMI 261564.

A Latin diagnosis is supplied for the anamorph. Two different "holotypes" are listed, IMI 257876 and IMI 261568, in the English version of the diagnosis of "*C. eucalypti*". Specimen IMI 261564 includes a small number of ascostromata and many conidiomata. Unfortunately, IMI 261568 is also listed as one of two "holotypes" for a distinctly different fungus, *V. eucalypticola* J.K. Sharma, C.N. Mohanan & Florence (see below). IMI 261568 is *V. eucalypticola* and does not contain any conidiomata. The description by Sharma *et al.* (1985) of the "*V. eucalypti*" teleomorph states "without dark zonations", however, a dark conceptacle is present. No cultures of "*C. eucalypti*" exist at the *Kerala Forest Research Institute*, according to K.V. Sankaran (pers. comm). New cultures and specimens will be required to resolve questions pertaining to this species.

8. *Valsa eucalypticola* J.K. Sharma, C.N. Mohanan & Florence, Kerala For. Res. Inst. Res. Rep. 36: 262. 1985 (*Nom. inval.*, Art. 37.1). Figs 35–37.
Anamorph: ***Cytospora eucalypticola*** *sensu* J.K. Sharma, C.N. Mohanan & Florence, Kerala For. Res. Inst. Res. Rep. 36: 258. 1985; non *C. eucalypticola* van der Westh.

Ascostromata immersed in bark, erumpent, circular, ovoid to lenticular, 0.6–1.2 × 0.2–0.6 mm diam, euvalsoid, circinateous becoming monostichous with increasing perithecia, 5–16 perithecia, in white to grey-brown (with age) entostromata extending below the discs. *Discs* below bark surfaces, obscured by crowded ostioles, grey-brown to medium brown, circular to lenticular, flat (0.4–)0.6(–0.8) mm diam, 3–12 inserted ostioles. *Ostioles* dark grey-brown to black, 78–98 µm diam, just above to 600 µm above disc surfaces, crowded in discs, surrounded by white to grey-brown furfuraceous (with age) entostromata, below the discs. *Perithecia* medium brown, globose (196–)235(–300) µm diam, upright to laterally inclined, surrounded by white (dark grey-brown and furfuraceous with age) entostromata from below discs to bases, walls

of *textura epidermoidea*. *Asci* free, subcylindrical to clavate, 23–25 × 3–3.5(–4) µm, with a refractive chitinoid ring in the non-amyloid apical apparatus, 8-spored. *Ascospores* biseriate, elongate allantoid, thin-walled, hyaline, aseptate (5–)5.4(–6) × 1 µm.

Anamorph interspersed amongst ascostromata, rare, discrete. *Conidiomatal stromata* immersed in bark, erumpent, rosette cytosporoid, reduced, circular, discoid, (0.3–)0.5(–1) mm diam. *Discs* below bark surfaces, grey-brown to medium brown, circular to ovoid, flat, 0.3–0.4 mm diam, 1–3 inserted ostioles. *Ostioles* dark grey-brown to black, at the same level as the disc surface, 78–98 µm diam, surrounded by white to grey-brown furfuraceous (with age) entostromata, below the discs. *Locules* with 2–5 chambers, irregularly arranged, and sharing common walls. Walls of dark brown *textura epidermoidea*, to sclerenchymatous, surrounded by white to grey-brown furfuraceous (with age) entostromata. *Conidiophores* hyaline, occasionally branched above bases, 11–15 µm, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, periclinal thickenings minute, 10 × 0.8–1.2 µm. *Conidia* hyaline, eguttulate, allantoid, thin-walled, aseptate, 4.0 × 0.8–0.9 µm.

Hosts: *Eucalyptus grandis*, *E. tereticornis*.

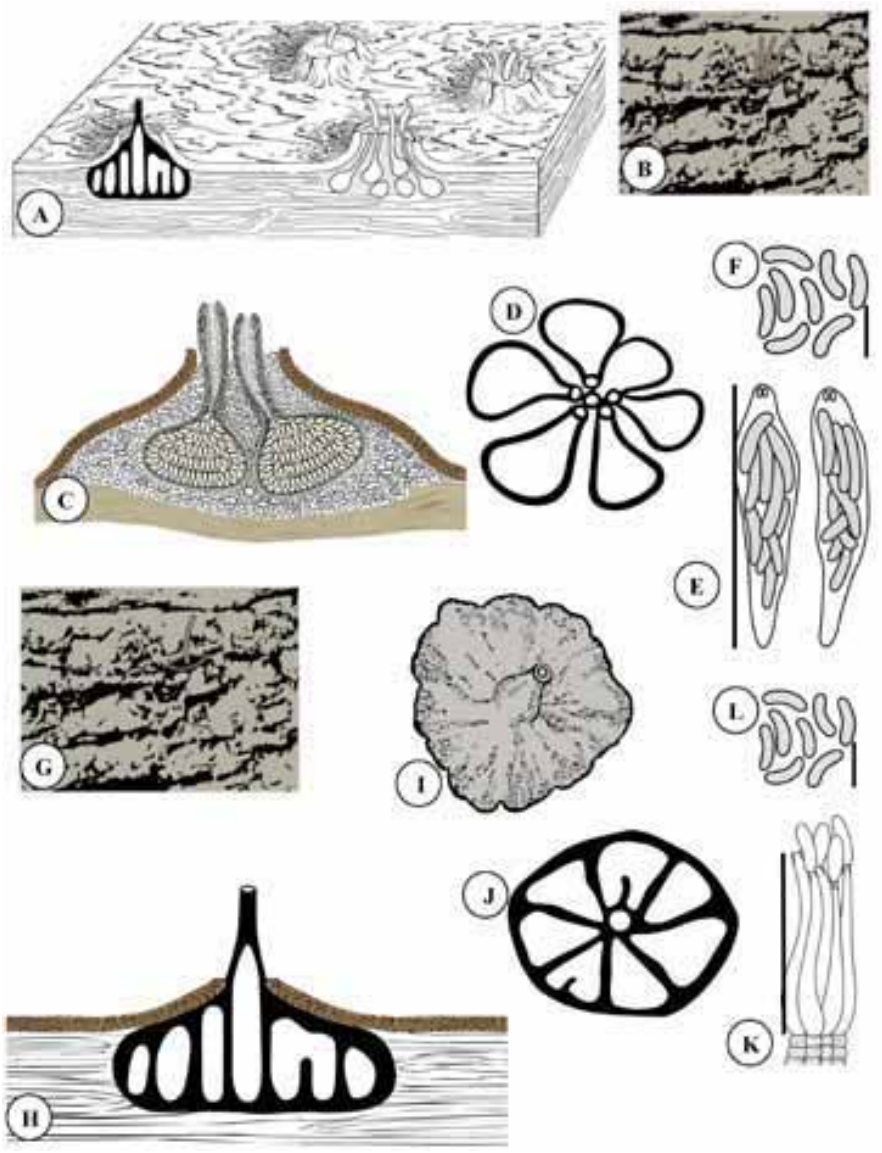

Fig. 35. Illustrations of "*V. eucalypticola*". A. Habit sketch. B. Ostiolar disc of teleomorph erumpent from bark. C. Longitudinal section through ascostroma in plant. D. Horizontal cross section through ascostroma. E. Asci. F. Ascospores. G. Ostiolar disc of anamorph erumpent from bark. H. Longitudinal section through conidiomal stroma in plant. I. Top view of conidioma isolated from plant tissues. J. Horizontal cross section through conidiomal stroma. K. Conidiophores in hymenium. L. Conidia. Scale bars: E = 24 µm, F = 5.5 µm, K = 13 µm, L = 4 µm.

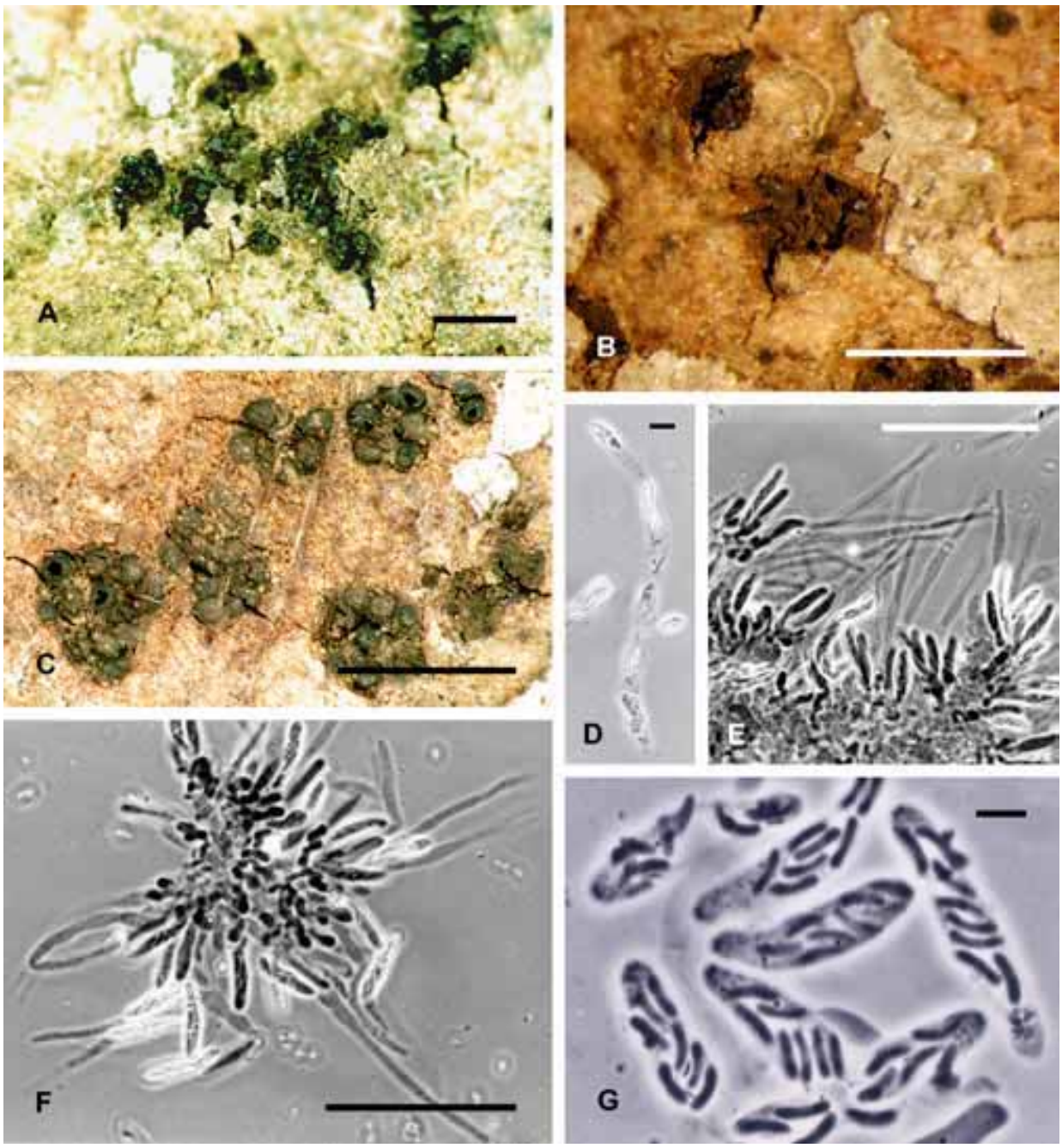

Fig. 36. Morphology of teleomorph of "*V. eucalypticola*". A. Habit of ostiolar beaks and discs of ascostromata. B. Close up of clusters of dark brown beaks extending above brown discs. C. Tangential section through ascostromata below discs shows clustered circinate arrangement of a few (*ca* 6–8) globose perithecia. D. Paraphysis shows degraded cytoplasmic contents. E. Filamentous paraphyses in hymenium among mature asci with ascospores. F. Another view of paraphyses in hymenium with mature asci. G. Asci with apical ring apparatus stained with chlorazol-black, and ascospores. Width and shape of asci are somewhat distorted by pressure used to flatten image for improving focus on the stained rings. Scale bars: A–C = 1 mm, D = 10 µm, E–F = 100 µm, G =10 µm.

Distribution: Thrissillery, Wynad District, Kerala, India.

Specimens examined: **India**, Kerala, Thrissillery, Wynad District, on stems of *E. grandis*, 20 Aug. 1981, J.K. Sharma, no. 014 (IMI 261568, one of two "holotypes" of *Valsa eucalypticola*, few anamorphs present, not *Cytospora eucalypti*); on *E. grandis*, 2 May 1981, J.K. Sharma, KFRI-008 (IMI 257896, cited as IMI 257876, one of two "holotypes" of *Valsa eucalypticola*, no anamorphs present, not *Cytospora eucalypti*); on *E. tereticornis*, 18 Feb. 1984, J.K. Sharma, KFRI-1147 (IMI 284046, dried culture of *Cytospora eucalypticola*).

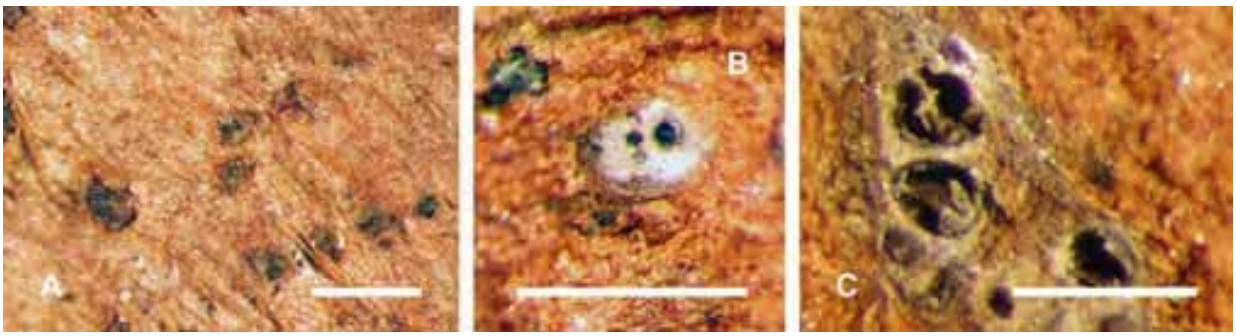

Fig. 37. Morphology of anamorph of "*V. eucalypticola*" (= *C. eucalypticola sensu* Sharma *et al*.). A. Habit of conidiomatal discs shows presence of three ostioles in a disc. B. Tangential section through disc just below bark surface shows white stroma tissue around three ostioles. C. Tangential section through group of conidiomata surrounded by white stroma tissues shows divided locules each with several chambers. Scale bars: A–C = 1 mm.

Notes: "*Valsa eucalypticola*" is invalidly published because it is published in a research report, only. However, the brief note includes a Latin diagnosis of the teleomorph, and isotype specimens are deposited at IMI. Two "holotypes" are listed, IMI 257896 and IMI 261568 in the Latin diagnosis. The two "holotypes" differ and are primarily of teleomorphs. Conidiomata are present but scarce on IMI 261568.

At the outset of this study, we believed the two specimens represented the same fungus but IMI 261568 had white entostromata and beaks that were just slightly above the surface, whereas IMI 257896 had dull grey-brown furfuraceous entostromata and beaks that were frequently 400–600 µm above the surface. The latter specimen apparently was old because few perithecia contain asci or spores. Conidiomata present on IMI 261568 were not similar to *C. eucalypticola* van der Westh. They differed in being cytosporoid rather than unilocular when deeply immersed in the host, and in being surrounded by white entostromata. A third specimen was deposited with the English diagnosis of the anamorph, IMI 284046. This specimen was a dried agar culture with conidiomata from a Petri dish.

The specimen could not be distinguished from many other *Cytospora* species on *Eucalyptus*. IMI 284046 also appeared listed as a holotype of "*C. eucalypti*" and that listing is erroneous. The written diagnosis of the Indian anamorph in Sharma *et al.* (1985) describes a more complex locular structure than the range of variation in the anamorph of *V. fabianae* sp. nov. (anamorph *C. eucalypticola*), from the worldwide collection. Based on the observed morphology of the teleomorph and anamorph, we do not believe this fungus is *C. eucalypticola* van der Westh., nor the teleomorph *V. fabianae sp. nov.* DNA sequences would be needed to accurately interpret and identify the Indian fungus. No cultures of the Indian species are available, according to K.V. Sankaran (pers. comm.) of Kerala Forest Research Institute.

LEUCOSTOMOID *VALSA*

The pre-existing holotypes of *C. agarwalii*, "*V. eucalypti*" *sensu* Sharma *et al.*, "*C. euclaypti*" *sensu* Sharma *et al.*, and isotypes of *V. eucalypti* were leucostomoid *Valsa* species. Below are other leucostomoid species on *Eucalyptus*.

9. *Valsa cinereostroma* G.C. Adams & M.J.Wingf., **sp. nov.** Figs 38–41. MycoBank MB500212.

Etymology: "*cinereostroma*" refers to the pale grey entostroma of the ascostroma.

Ascostromata in cortice immersa, erumpentia, circularia vel ovoidea (0.7–)1.2(–1.5) mm diametro, peritheciis (6–)9(–16) in entostromate pallide griseo infra discum circinatim dispositis, conceptaculo fuscato praedita. Disci prominentes, atro-brunnei, hemisphaerici, circulares vel ovoidei (0.3–)0.4(–0.6) mm diametro, ostiolis 5–16 praediti. Ostiola lateraliter inserta, atro-brunnea, superficiei disci plana, in centrum aggregata (45–)60(–70) µm diametro. Perithecia globosa (0.3–)0.4(–0.45) mm diametro, entostromate albo vel griseo e materia amorphica incohaerenti composito circumdata, parietibus fusco-brunneis e textura intricata vel epidermoidea compositis praedita. Asci liberi, clavati vel elongati, obovoidei (22–)30(–34) × (5–)7(–9) µm, annulo refractivo chitinoideo in apparatu apicali non amyloideo, 8-spori. Ascosporae biseriatae, elongato-allantoideae, tenui-tunicatae, hyalinae, unicae, 7.5–8 × 2 µm.

Anamorpha inter ascostromata interspersa, discreta. Stromata conidiomatica in cortice immersa, erumpentia, usque ad 1.5 mm diametro, conceptaculo fuscato praedita. Disci fusco-brunnei, paene plani, circulares, usque ad 0.5 mm diametro, ostiolo unico praediti. Ostiola cretaceo-cinerascentia, furfuracea. Loculi ad typum complexum multi-locellatum pertinentes, introrsum per plicas in cavernulis irregulares radiatim dispositas parietibus communalibus praeditas partiti. Conidiophora in matrice continua gelatinosa inclusae, hyalina, ad basem ramosa, supra basem usque ad 4-ramosa, 9–15 × 1 µm (phialides includentia). Cellulae

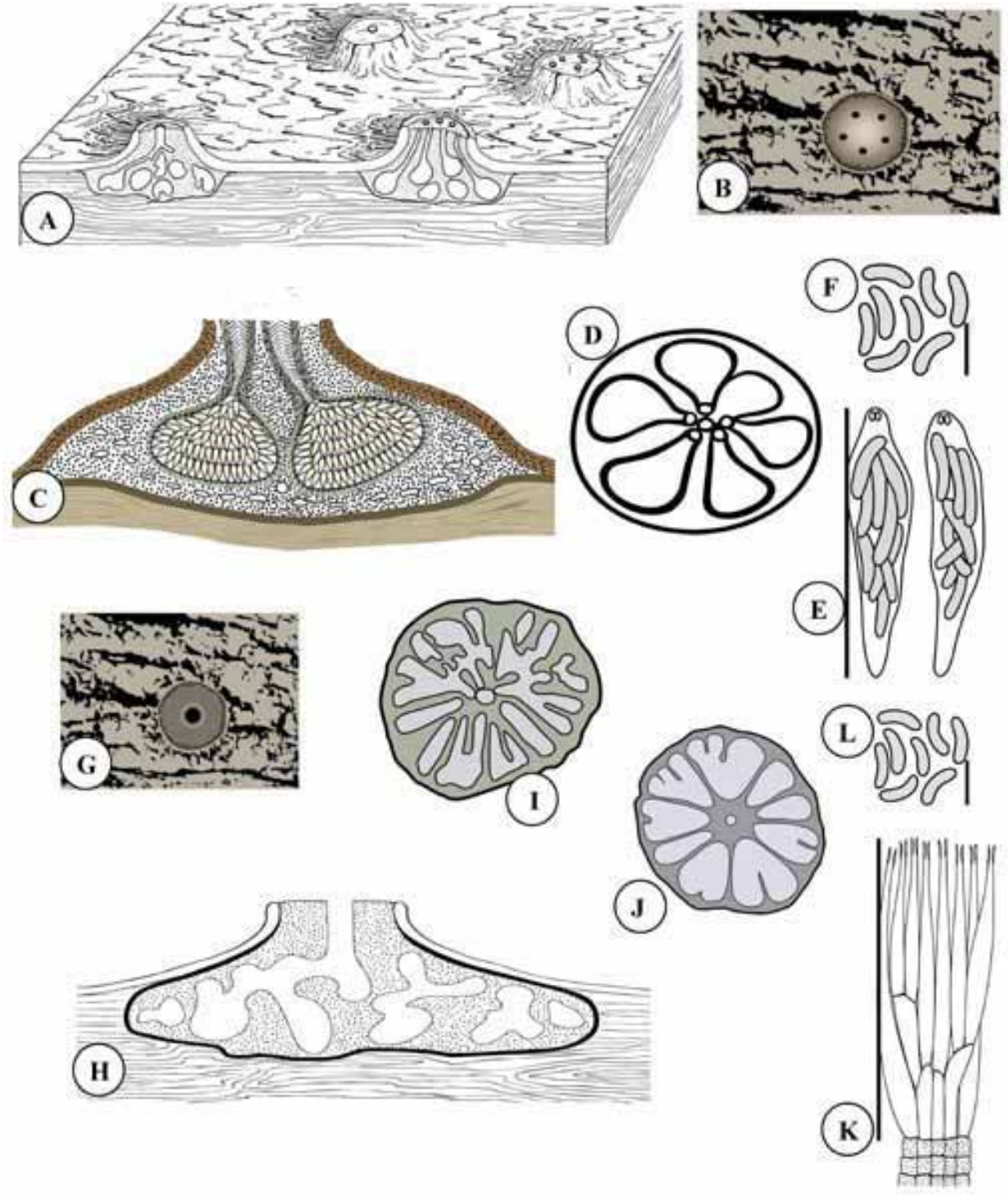

Fig. 38. Illustrations of *V. cinereostroma*. A. Habit sketch. B. Ostiolar disc of teleomorph erumpent from bark. C. Longitudinal section through ascostroma in plant. D. Horizontal cross section through ascostroma. E. Asci. F. Ascospores. G. Ostiolar disc of anamorph erumpent from bark. H. Longitudinal section through conidioma stroma in plant. I. Horizontal cross section through large conidiomal stroma. J. Horizontal cross section through small conidiomal stroma. K. Conidiophores in hymenium. L. Conidia. Scale bars: E = 30 µm, F = 8 µm, K = 12 µm, L = 5.5 µm.

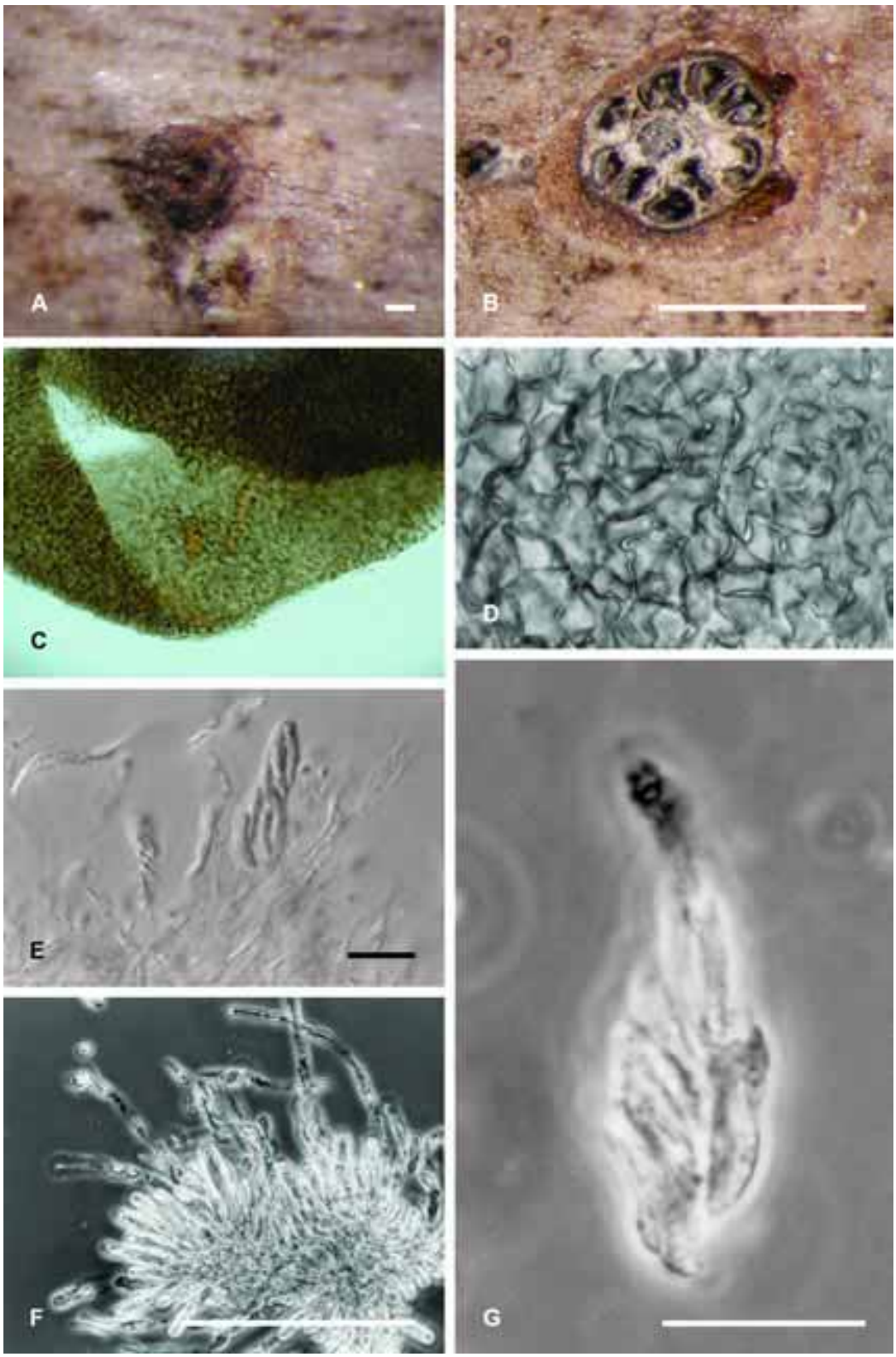

Fig. 39. Morphology of teleomorph of *V. cinereostroma*. A. Surface view of circular ascostromal disc with several ostioles at the same level as the disc surface. B. Tangential section through ascostroma shows circinate arrangement of perithecia in white entostroma delimited by black conceptacle. C. Perithecial wall of *textura epidermoidea*. D. Magnified view of *textura epidermoidea*. E. Paraphyses and mature ascus with biseriate allantoid ascospores and apical ring observed with differential interference contract microscopy. F. Hymenium with many long filamentous paraphyses among mature and immature asci (phase contrast). G. Ascus with apical apparatus stained with chlorazol-black shows two or more regions of staining with the upper region corresponding to the refractive ring. Scale bars: A = 100 µm, B = 100 µm, E, G = 10 µm, F = 100 µm.

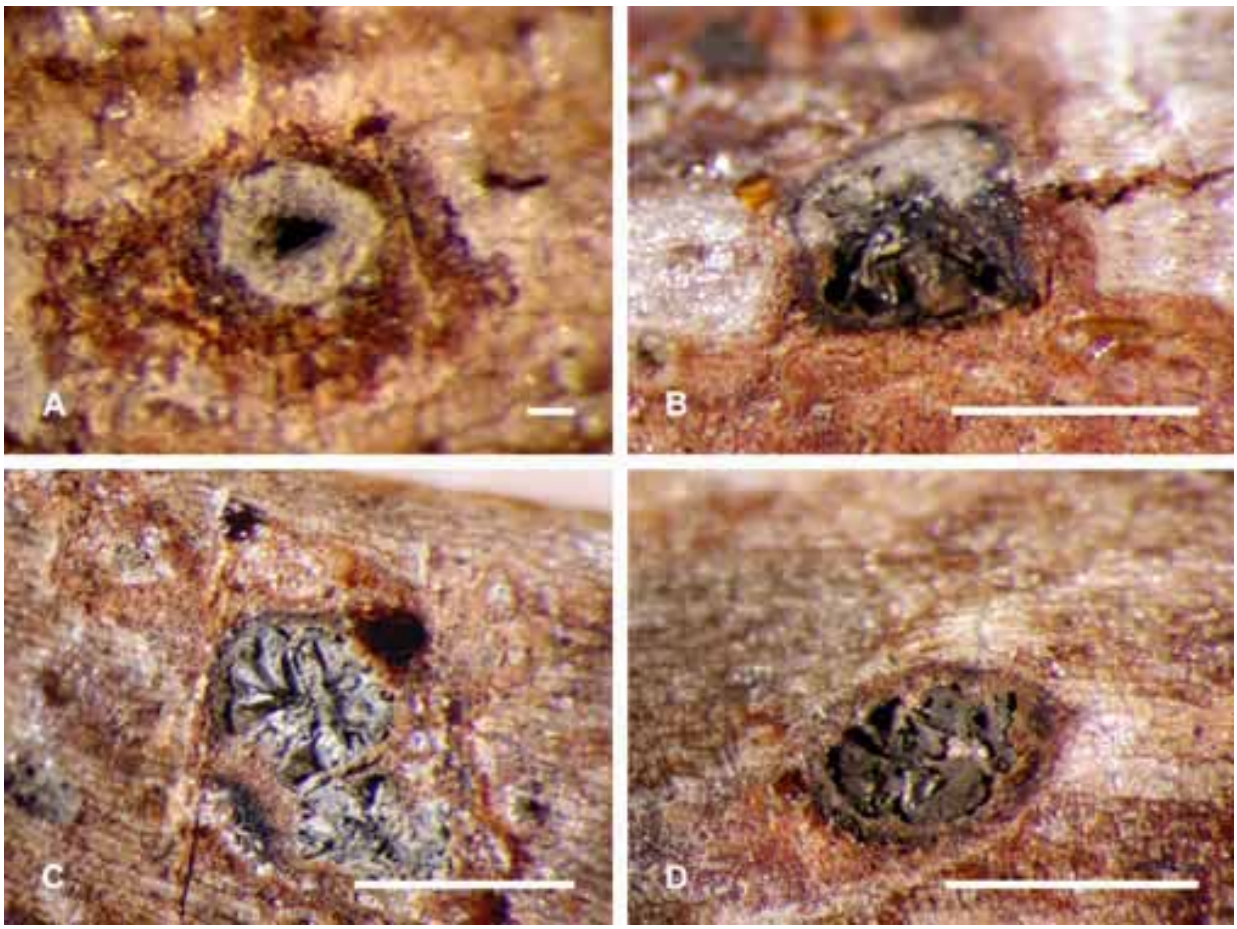

Fig. 40. Morphology of the anamorph of *V. cinereostroma*. A. Tangential section through the ostiolar disc shows stroma tissue around the ostiole. B. Longitudinal and tangential sections through a conidioma shows entostroma and multiple chambers dividing the locule delimited by a dark conceptacle. C. Tangential view of a conidiomatal stroma surrounded by entostroma and multiple chambers delimited by a light coloured conceptacle. D. Tangential section through an old conidiomatal stroma shows crowded chambers in an irregular arrangement. Scale bars: A = 100 µm, B–D = 1 mm.

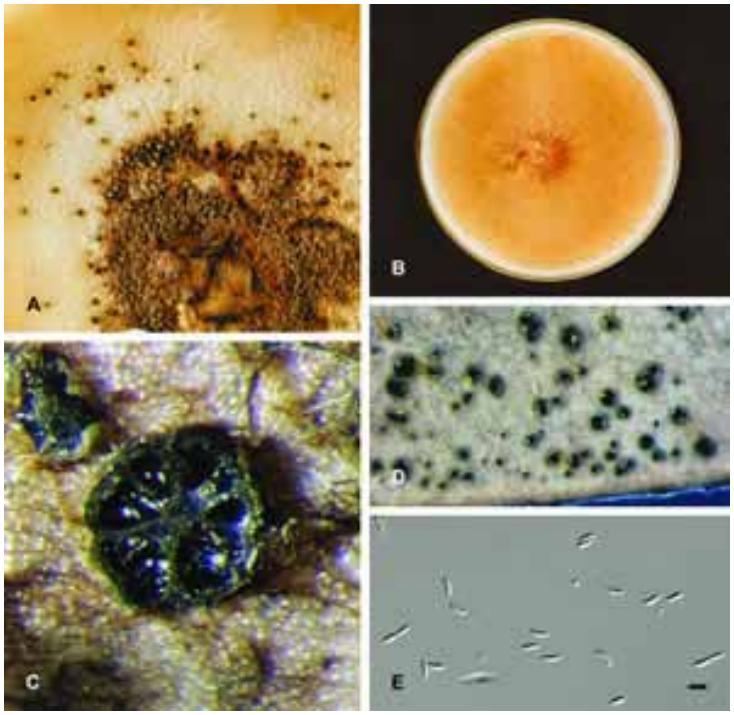

Fig. 41. Culture characteristics of *V. cinereostroma*. A. Conidiomata of isolate CBS 116830, SouthAfrica-9 produced near inoculation sites on PDA. B. Colony morphology on PDA of isolate CBS 116830. C. Tangential section through conidioma shows locule divided into multiple chambers with regular radial arrangement. D. Conidiomata produced on autoclaved *Eucalyptus* leaf. E. Allantoid conidia. Scale bar: E = 5.5 µm.

conidiogenae enteroblastice phialidicae, subcylindricae, ad apicem contractae, collaretta minuta atque parte incrassata periclini exigua praeditae, (7–)8(–10) × 1 µm. Conidia hyalina, eguttulata, elongato-allantoidea, unica (5–)5.5(–6) × 1 µm.

Ascostromata immersed in bark, erumpent, circular to ovoid (0.7–)1.2(–1.5) mm diam, leucostomoid circinateous, (6–)9(–16) perithecia arranged circinately in pale grey entostromata below the discs, conceptacles dark. *Discs* prominent, dark brown, hemispherical, circular to ovoid (0.3–)0.4(–0.6) mm diam, 5–16 ostioles. *Ostioles* laterally inserted, dark brown, at the same level as the disc surface, clustered in centres of discs (45–)60(–70) µm diam. *Perithecia* globose (0.3–)0.4(–0.45) mm diam, laterally inclined, surrounded with white to grey entostromata of loose amorphous material, walls dark brown, of *textura intricata* to *epidermoidea*. *Asci* free, clavate to elongate obovoid (22–)30(–34) × (5–)7(–9) µm, with a refractive chitinoid ring in the non-amyloid apical apparatus, 8-spored. *Ascospores* biseriate, elongate-allantoid, thin-walled, hyaline, aseptate, 7.5–8 × 2 µm.

Anamorph interspersed amongst ascostromata, discrete. *Conidiomatal stromata* immersed in bark, erumpent, rosette to labyrinthine leucocytosporoid, up to 1.5 mm diam, conceptacles dark. *Discs* dark brown, nearly flat, circular, up to 0.5 mm diam, discrete ostioles. *Ostioles* chalky pale grey, furfuraceous. *Locules* multi-chambered, subdivided by invaginations into regular to irregular radially arranged chambers sharing common walls. *Conidiophores* hyaline, branched at base, up to four branches above the base 9–15 × 1 µm, inclusive of phialides, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, minute collarettes, periclinal thickenings minute, (7–)8(–10) × 1 µm. *Conidia* hyaline, eguttulate, elongate-allantoid, aseptate (5–)5.5(–6) × 1 µm.

Cultures: Colony growth on PDA is yellowish white to yellowish grey at the margin (Munsell 4.5Y 9.2/1.2, 3.8Y 7.4/1.4) and predominantly olive-grey to olive-black (Munsell 8.1Y 3.5/0.9, 9.0Y 1.1/0.9) with tufts of yellowish grey on the surface. Colour of the reverse is olive-grey, olive-black to black (Munsell N 0.8/). Pycnidia rarely form on the agar and exude cream to pale yellow cirrhi. Colony texture is felty, slightly raised with no growth zones. Rosette cytosporoid conidiomata divided into 6–10 regular radially arranged chambers and with discrete short necks/beaks form on the autoclaved leaf. Dark glabrose conidiomata erupt from within the leaf with the globe being partially covered with leaf epidermis.

Cardinal temperatures: Colonies (mean of 5 isolates) obtain a mean growth on PDA at 4 ºC of 5.5 mm diam, at 25 ºC of 138 mm diam, at 32 ºC of 42 mm diam, and no growth at 37 ºC after 7 d in the dark. No growth at 25 ºC occurs on 2 ppm cycloheximide in V8® agar, and growth of 0–12 mm diam occurs on 2 ppm in Leonian's agar after 7 d in the dark.

Hosts: *Eucalyptus globulus*, *Mangifera indica*.

Distribution: Chile, South Africa.

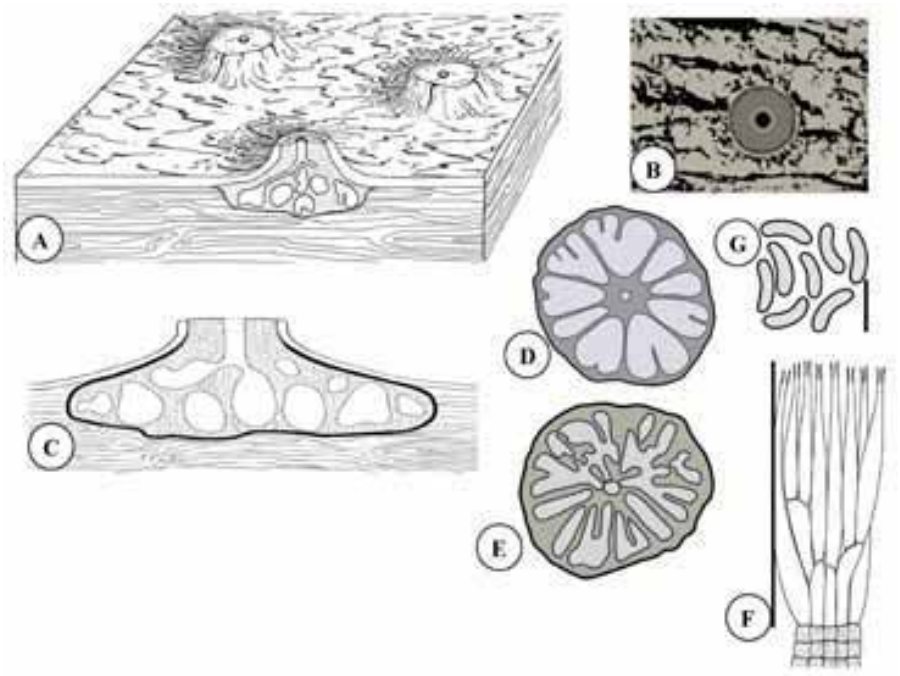

Fig. 42. (p. 77) Illustrations of *Valsa* aff. *cinereostroma*. A. Habit sketch. B. Ostiolar disc erumpent from bark. C. Longitudinal section through conidiomal stroma in plant. D. Horizontal cross section through small conidiomal stroma. E. Horizontal cross section through large conidiomal stroma. F. Conidiophores in hymenium. G. Conidia. Scale bars: F = 12 µm, G = 5.5 µm.

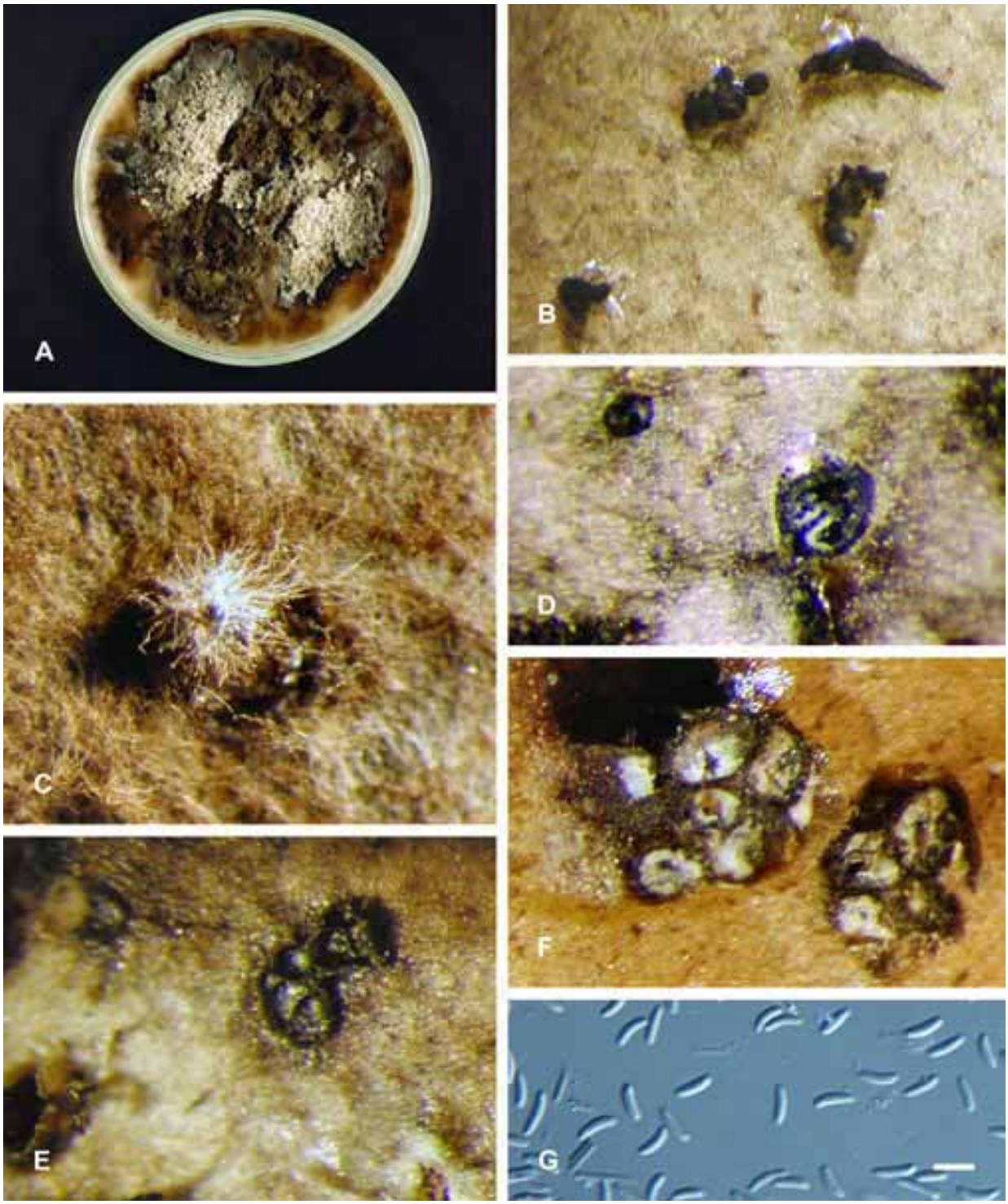

Fig. 43. Culture characteristics of *Valsa* aff. *cinereostroma*. A. Colony morphology on PDA of isolate CBS 116832, SouthAfrica-8. B–C. Conidiomata, with relatively long necks and conidioma with white frill of hyphae around short neck, on *Eucalyptus* leaf. D–F. Tangential sections through conidiomata with simple locules divided by invaginations into simple or regular, radially arranged, chambers. G. Allantoid conidia. Scale bars: G = 5.5 µm.

Specimens examined: **Chile**, on dead branches of *E. globulus*, Mar. 2000, M.J. Wingfield (MSC 375220, **holotype** of *Valsa cinereostroma*), and ex-type living culture CBS 117081. **South Africa**, from fruit of *Mangifera indica*, 1998, C. Roux, living culture CBS 116830.

Note: This species differs from the other leucostomoid species on *Eucalyptus* in having rosette leucocytosporoid conidiomata rather than leucotorsellioid conidiomata.

10. *Valsa* aff. ***cinereostroma***, reported as "*Cytospora australiae*" in South Africa, S. African J. Bot. 56(5): 584. 1990. Figs 42–43.

Teleomorph not observed. *Conidiomatal stromata* immersed in bark or in leaves, variably erumpent, rosette cytosporoid. *Locules* subdivided by invaginations into six or more regular radially arranged chambers sharing common walls. *Conidiophores* hyaline, branched at base, up to four branches above the base 9–15 × 1 µm, inclusive of phialides, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, minute collarettes, periclinal thickenings minute, (7–)8(–10) × 1 µm. *Conidia* hyaline, eguttulate, elongate-allantoid, aseptate, variable, (3.5–)5.5(–6) × 0.9–1 µm on the natural material, but uniform (5–)5.5(–6) × 1 µm *in vitro*.

Cultures: Colony growth on PDA is yellowish white to yellowish grey at the margin (Munsell 4.5Y 9.2/1.2, 3.8Y 7.4/1.4) and predominantly olive-grey to olive-black (Munsell 8.1Y 3.5/0.9, 9.0Y 1.1/0.9) with tufts of yellowish grey on the surface. Colour of the reverse is olive-grey, olive-black to black (Munsell N 0.8/). Colonies cease growth prior to reaching the plate edge (PDA, KHG, OA, Leonian's agar) and the margins are irregular and lobate. Pycnidia rarely form on PDA but form on Leonian's agar and exude cream cirrhi. Colony texture is felty, slightly raised with no growth zones. Unilocular to rosette cytosporoid conidiomata with long, tapering necks/beaks form on autoclaved leaves. Conidiomata are dark, generally glabrose and occasionally exhibit a fringe of hyphae around the necks/beaks.

Cardinal temperatures: Colonies (mean of 3 isolates) obtain a mean growth on PDA at 4 ºC of 5.5 mm diam, at 25 ºC of 71 mm diam, at 32 ºC of 40 mm diam, and no growth at 37 ºC after 7 d in the dark. No growth at 25 ºC occurs on 2 ppm cycloheximide in V8® agar, and growth on 2 ppm in Leonian's agar after 7 d in the dark. Growth at 25 ºC on 2 ppm cycloheximide in Leonian's agar is 0–33 % of growth on Leonian's agar without the antibiotic after 7 d in the dark.

Hosts: *Eucalyptus grandis*, *E. nitens*.

Distribution: Amsterdam plantation, Jessivale State Forest and White River, Barberton, South Africa.

Specimens examined: **South Africa**, Barberton, White River, Jessivale State Forest, on twig from *E. nitens*, Dec. 1988, P.W. Crous (PREM 50454, as "*Cytospora australiae* Speg."), also living cultures CBS 116831, CBS 116832, CBS 116833, and C.M.W. 1514.

Notes: These specimens could be within the population variation of *V. cinereostroma*. Colony characteristics were identical to *V. cinereostroma* except these isolates did not grow to fill the Petri dish on several media and formed irregular lobate margins. We could not locate conidiomata on PREM 50454 and had only a thin section of one stroma on a prepared slide for study. Better specimens on host tissue were needed to compare stromatal characteristics. In the original report Crous *et al.* (1990) noted that the conidiomatal stromata varied in the extent of their erumpant character on stems depending on the time of year. Less apparent variation occurred with the conidiomata on leaves. Crous *et al.* (1990) noted that the conidiomata were found associated with lesions caused by other pathogens, stresses, or wind damage.

11. *Cytospora abyssinica* G.C. Adams, Jol. Roux & Gezahgne, **sp. nov.** Figs 44–46. MycoBank MB500213.

Etymology: "*abyssinica*" refers to the ancient nation of Abyssinia, its people and culture in the upper and eastern regions of Ethiopia with high mountains.

Teleomorpha ignota. Stromata conidiomatica in cortice immersa, erumpentia, discoidea, circularia vel lenticularia, 0.6–0.75(–1.5) mm diametro, loculis 6–8 in entostromate alutaceo vel pallide brunneo infra discum inclusis, conceptaculo fuscato praeditua. Disci fusco-grisei, e materia amorphica compositi, subplani, circulares vel ovoidei, 100 µm diametro, ostiolo unico praediti. Loculi ad typum simplicem, non divisum pertinentes, radiatim dispositi, arcte contigui, parietibus non communalibus, lacrimiformes, 100 × 250 µm diametro, ex obliquo jacentes, ostiolis ad discum in ostiolo unico convergentibus, parietibus e textura epidermoidea aurea compositis. Cellulae conidiogenae in matrice continua gelatinosa inclusae, enteroblastice phialidicae, base ramosae, rosulam phialidum formantes, hyalinae, subcylindricae, ad apicem contractae, collarcula minuta praeditae (7.5–)12–14(–16) × 1.3 µm. Conidia hyalina, eguttulata, allantoidea, unica (3.3–)4(–5) × 1 µm.

Teleomorph unknown. *Conidiomatal stromata* immersed in bark, erumpent, leucotorsellioid and leucocytosporoid, discoid, circular to lenticular, 0.6–

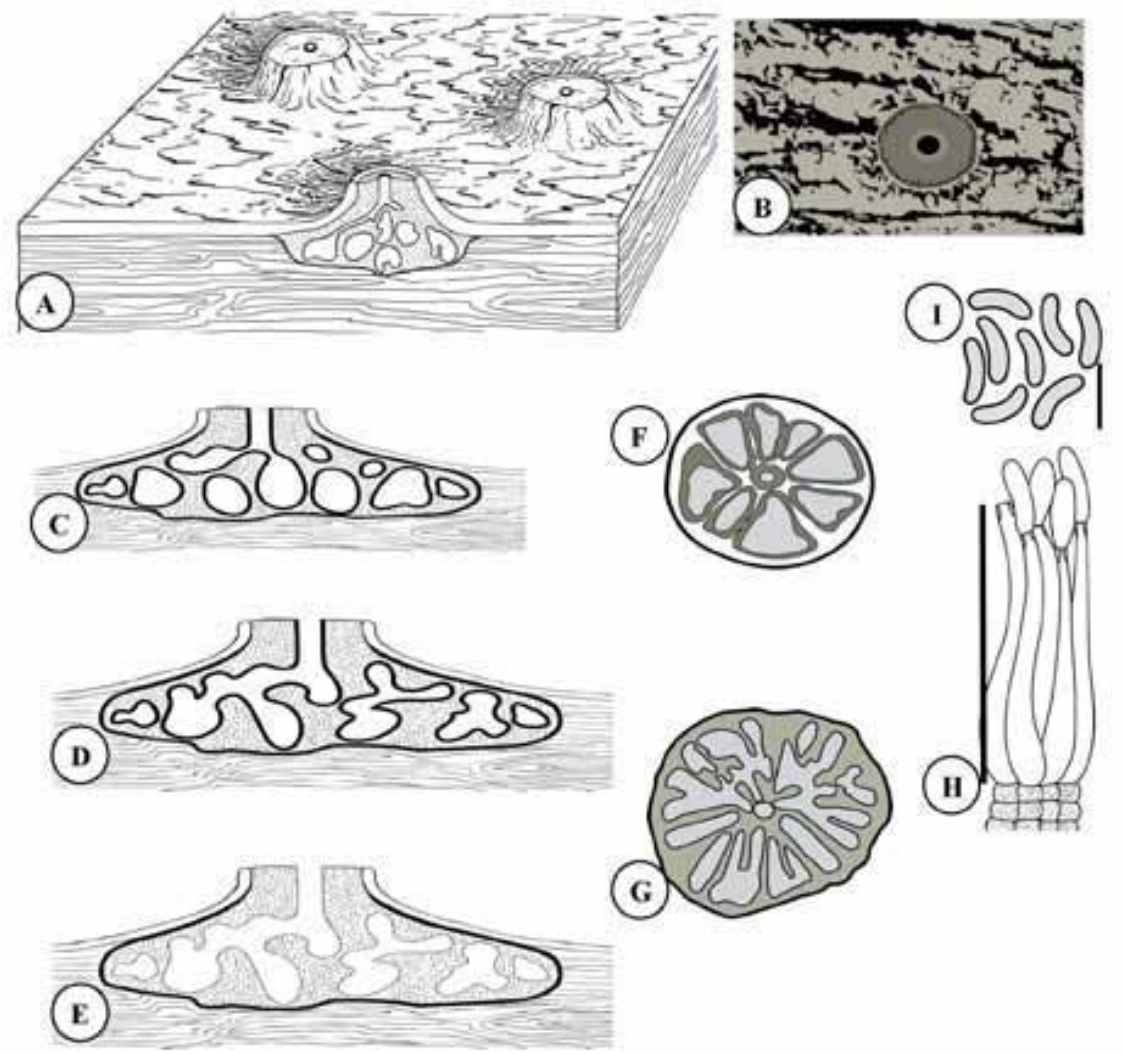

Fig. 44. *Cytospora abyssinica*. A. Habit sketch. B. Ostiolar disc erumpent from bark. C–E. Longitudinal sections through three conidiomatal stromata in plant. F–G. Horizontal cross sections through two conidiomatal stromata. H. Conidiophores in hymenium. I. Conidia. Scale bars: H = 13 µm, I = 4 µm.

0.75(–1.5) mm diam, 6–8 locules in pale to tan-brown entostromata below the discs, conceptacles dark. *Discs* dark grey, of amorphous material, nearly flat, circular to ovoid, 100 µm diam, with discrete ostioles. *Locules* simple undivided, radially arranged, closely packed, not sharing common walls, tear-shaped, 100 × 250 µm diam, lying on their sides, ostiolar necks converging into discrete ostioles at the discs, walls of golden *textura epidermoidea*. *Conidiophores* hyaline, branched at the base, a rosette of phialides, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute (7.5–)12–14(–16) × 1.3 µm. *Conidia* hyaline, eguttulate, allantoid, aseptate (3.3–)4(–5) × 1 µm.

Cultures: Colony growth on PDA is pale yellow, pale greenish yellow to pale greyish olive (Munsell 4.7Y 9.0/3.8, 9.5Y 9.0/4.2, 7.8Y 5.5/2.5) with occasional yellowish white (4.5Y 9.2/1.2) tufts and patches on the surface. Colour of the reverse is pale greyish olive to dark greyish olive (Munsell 7.8Y 5.5/2.5, 9.7Y 2.0/1.8) with the centre of the colony having the darkest colour. Growth zones are apparent especially on the reverse. Pycnidia rarely form on the agar and exude red-brown cirrhi. Colony texture is felty, slightly raised with prominant growth zones. Leucotorsellioid conidiomata with 15 or more locules with dark walls in pale brown entostromata surrounded in darker brown to grey ectostromata and with no apparent necks/beaks form on the autoclaved leaf. Few large globose to hemispherical conidiomata form with wooly brown surfaces and may coalesce into small groups having fused the outermost ectostromatal tissues.

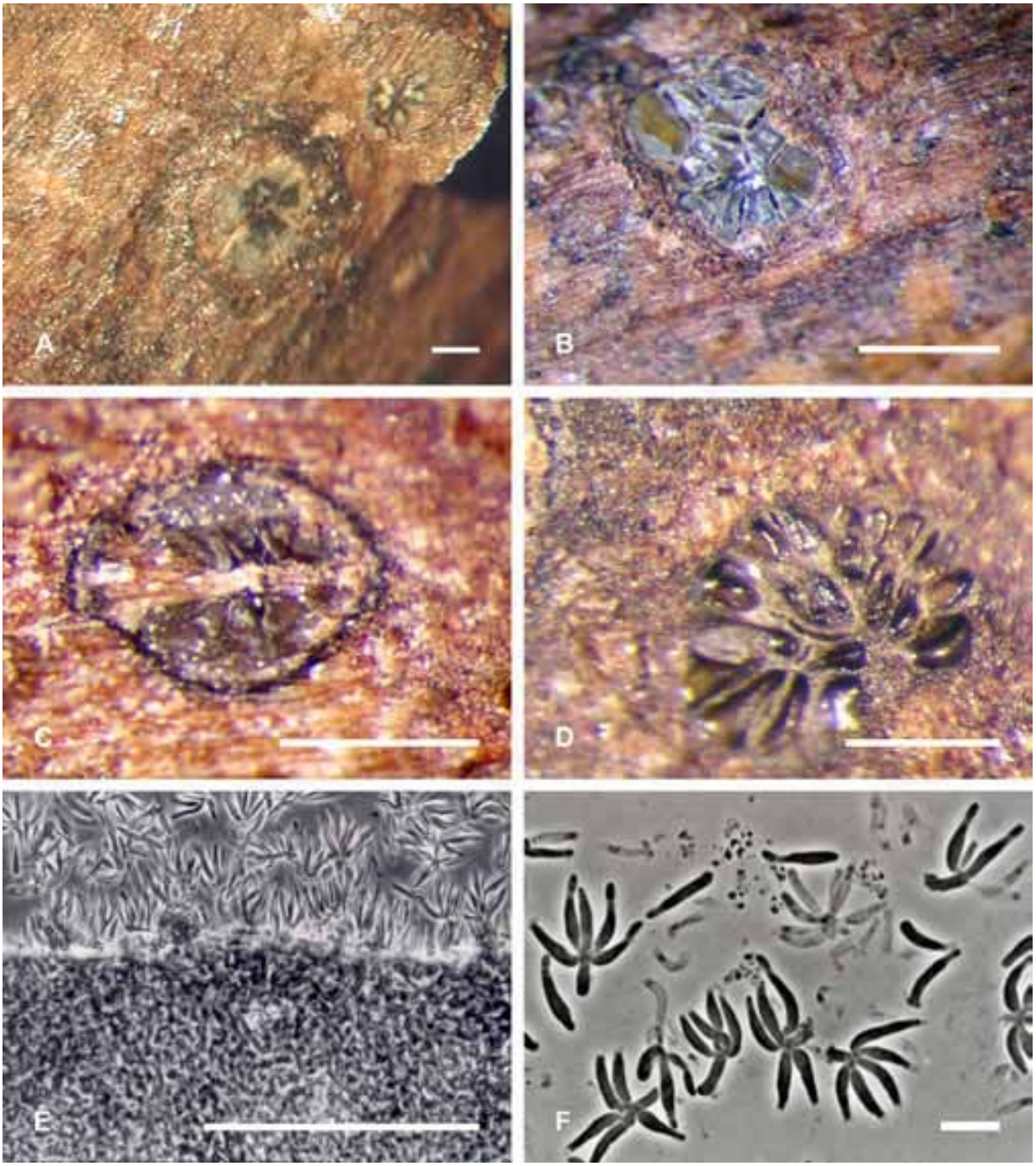

Fig. 45. Morphology of *C. abyssinica*. A. Shallow tangential cut through disc shows converging ostioles. B. Deeper tangential section through conidiomal stroma shows arrangement of locules, each locule has independent walls. C. Black line of conceptacle delimiting conidiomal stroma with multiple converging ostioles visible. D. Tangential section through conidiomal stroma shows crowded locules. E. Conidioma wall of *textura epidermoidea* and nearby conidiogenous cells. F. Whorls of conidiogenous cells branching from basal cells with collarettes visible in enlargements. Scale bars: A = 200 µm, B, C, E = 500 µm, D = 100 µm, F =10 µm.

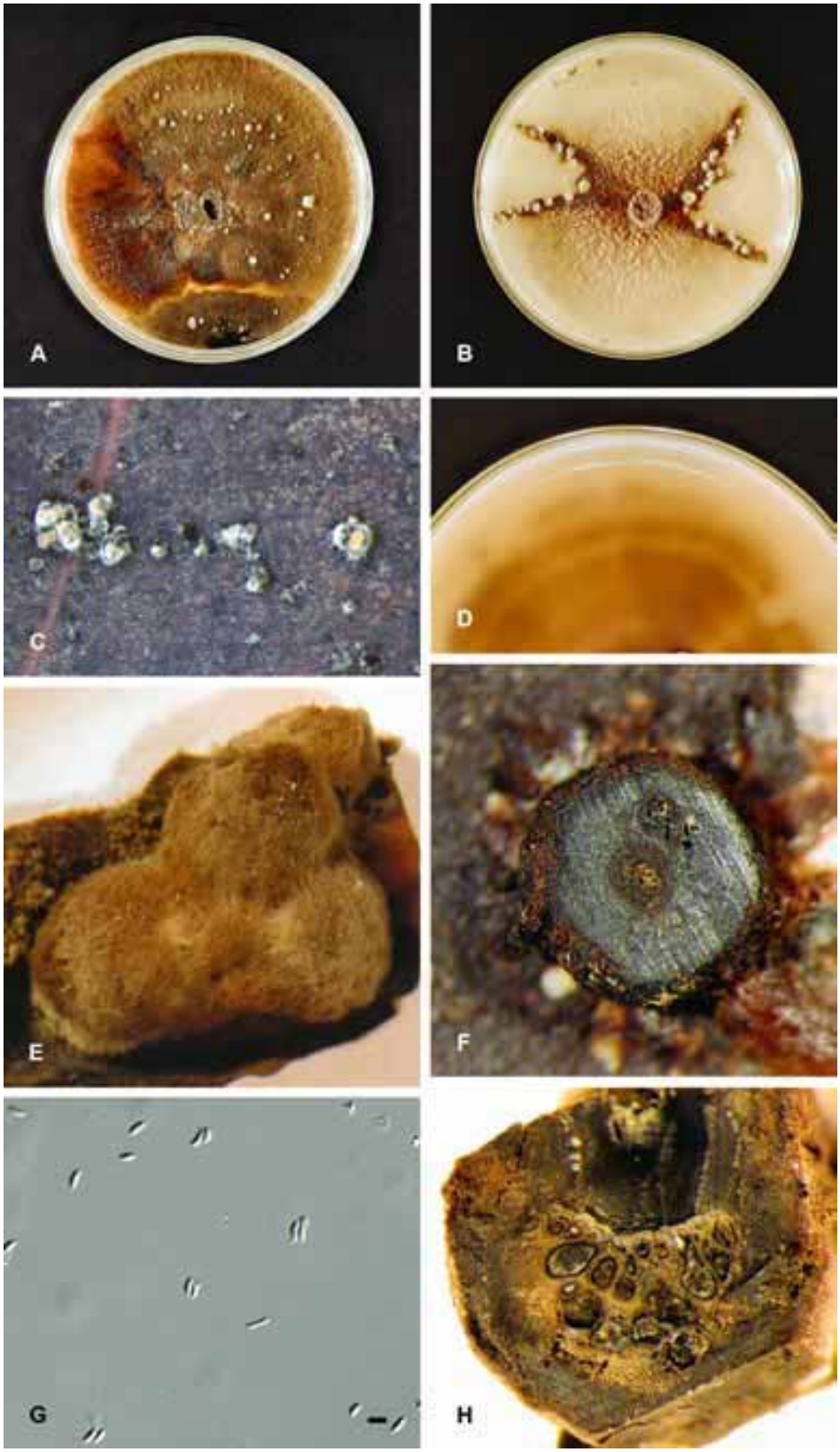

Fig. 46. Culture characteristics of *C. abyssinica*. A–B. Colony morphology on PDA of isolates CBS 117004, Ethiopia-10 (left) and CBS 116189, Ethiopia-6 (right). C. Conidiomata produced on autoclaved *Eucalyptus* leaf. D. Zonal growth on PDA of isolate CBS 117004 viewed from reverse side of Petri dish. E. Three conidiomata. F. Tangential section through upper portion of conidioma shows an ostiole in dark entostroma. G. Allantoid conidia. H. Tangential section through torsellioid conidioma shows multiple locules with dark independent walls surrounded by brown entostroma. Scale bar: G = 4 µm.

Cardinal temperatures: Colonies obtain a mean growth on PDA at 4 ºC of 8.5 mm diam, at 25 ºC of 88 mm diam, at 32 ºC of 14 mm diam, and no growth at 37 ºC after 7 d in the dark. Colonies die after 7 d at 37 ºC. Growth at 25 ºC on 2 ppm cycloheximide in V8® agar is 42 % of growth on V8® agar without the antibiotic after 7 d in the dark.

Host: *Eucalyptus saligna*.

Distribution: Wondo Genet, Ethiopia.

Specimens examined: **Ethiopia**, Wondo Genet, Forestry College Compound on dead twigs of *E. saligna*, Sep. 2002, Alemu Gezahgne (MSC 380700, **holotype** of *Cytospora abyssinica*), living ex-type culture CBS 116189; Forestry College Compound on dead twigs of *E. saligna*, Sep. 2002, Alemu Gezahgne (MSC 380702), also living cultures CBS 117004 and CBS 117605.

Note: This species closely resembles the anamorphs of *V. eucalypti* and "*V. eucalypti*" *sensu* Sharma *et al.* in stromatal characteristics but differed from them in having smaller conidia and non-branched conidiophores.

EUVALSOID *VALSA*

12. *Valsa ceratosperma* (Tode) Maire, Publ. Inst. Bot. Barcelona 3(4): 20. 1937, *s. str*.
Anamorph: ***Cytospora sacculus*** (Schwein.) Gvrit., Mikol. Fitopatol. 3: 207. 1969, *s. str*. Figs 47–48.

Ascostromata immersed in bark and wood, erumpent, circular to ovoid, conical to dome-shaped, up to 3 mm diam, euvalsoid monostichous, (5–)15–40 perithecia arranged at different depths in the entostroma or wood. *Entostromata* well-developed to massive, below the discs. *Discs* obscured by numerous ostioles to prominant, cream to dark brown, circular to ovoid, up to 2.5 mm diam, 5–40 ostioles inserted upright and closely packed. *Ostioles* dark brown, 70–150 µm diam, level to 2 mm above disc surfaces, crowded. *Perithecia* medium brown, compressed globoid, 0.17–0.45 mm diam, surrounded by pale brown entostromata of *textura globosa* from below discs to bases, walls of *textura epidermoidea*, 3–4 layers of cells, 10–15 µm thick. *Asci* free, subcylindrical to clavate, 30–45 × 5–6.5 µm, with a refractive chitinoid ring in the non-amyloid apical apparatus, 8-spored. *Ascospores* biseriate, elongate-allantoid, thin-walled, hyaline, aseptate (3–)6–12 × 1.5–2 µm.

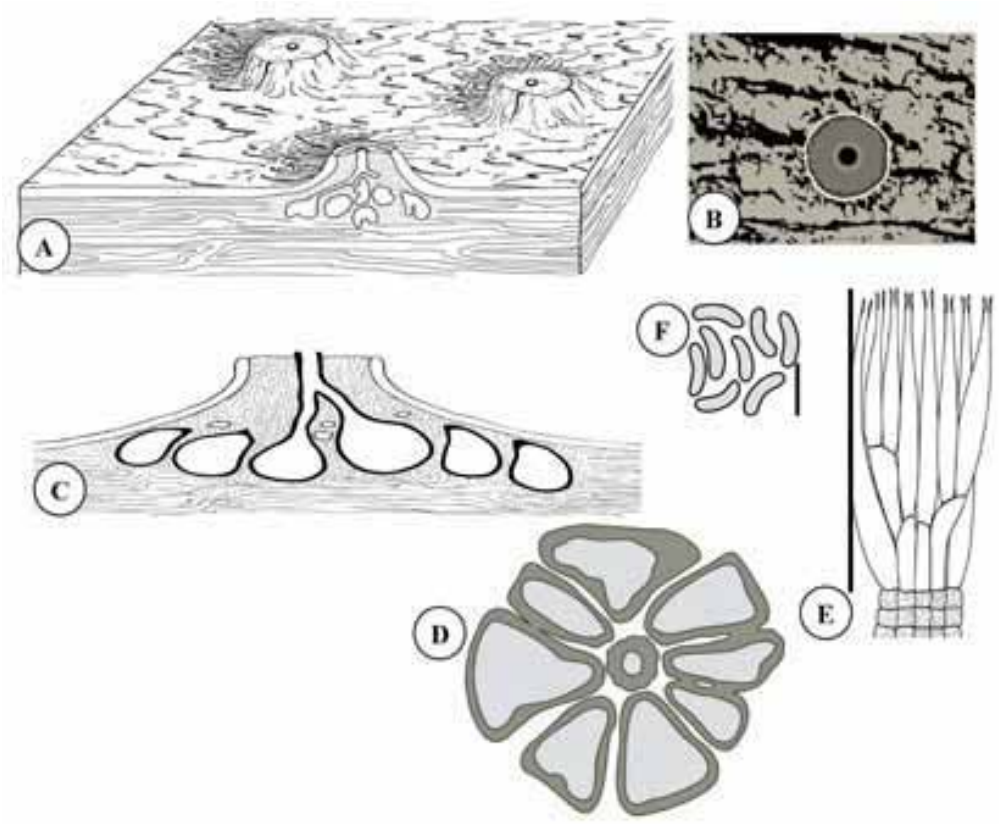

Fig. 47. Illustrations of *C. sacculus s. str.* the anamorph of *V. ceratosperma s. str*. A. Habit sketch. B. Ostiolar disc erumpent from bark. C. Longitudinal section through conidiomal stroma in plant. D. Horizontal cross section through conidiomal stroma. E. Conidiophores in hymenium. F. Conidia. Scale bars: E = 16 µm, F = 5.5 µm.

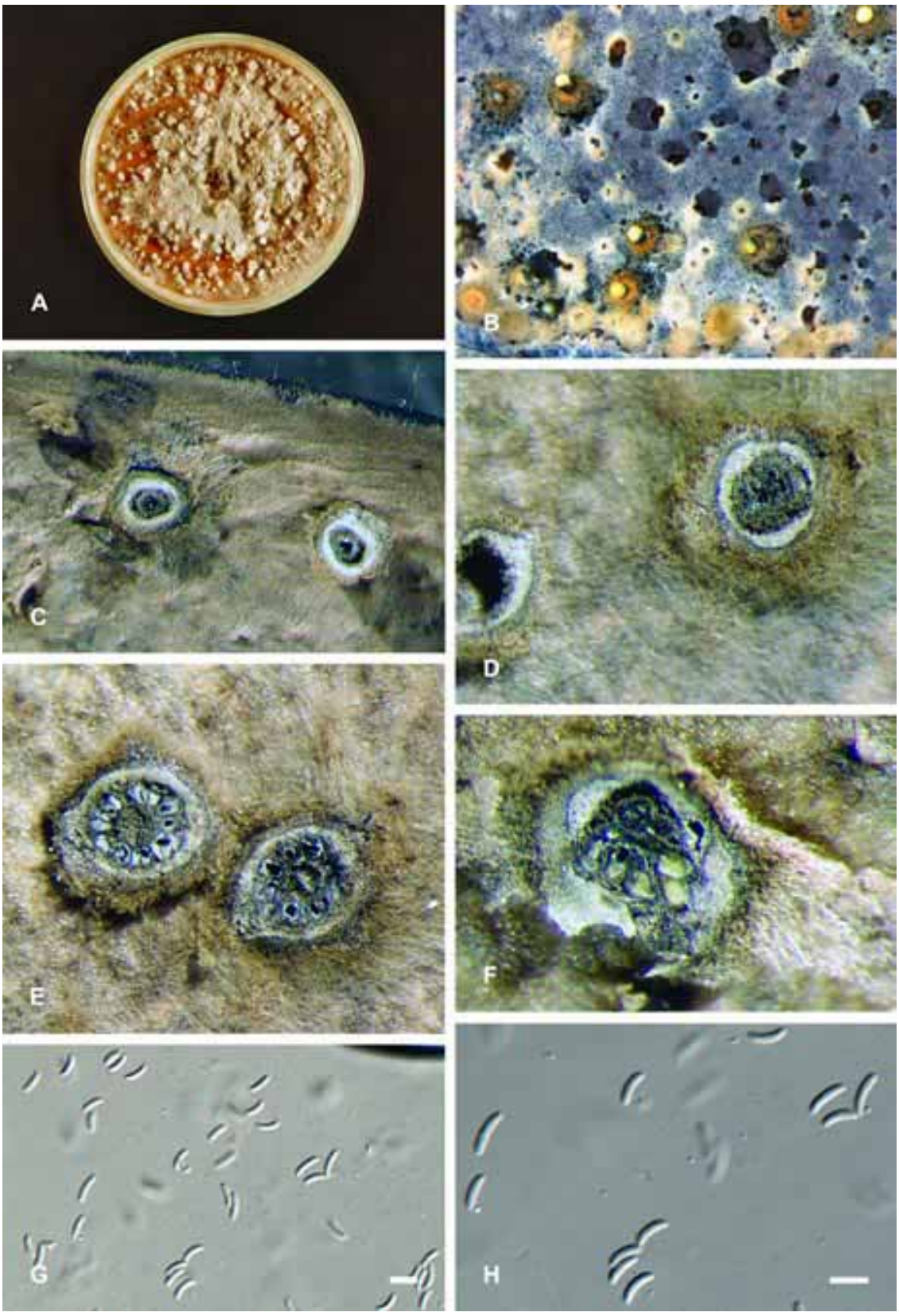

Fig. 48. Culture characteristics of *V. ceratosperma sensu stricto* (anamorph = *C. sacculus*). A. Colony morphology on PDA of isolates CBS 116855. B. Conidiomata produced on autoclaved *Eucalyptus* leaf. C–D. Tangential section through discs shows white disc tissues surrounding separate walls of ostioles. E–F. Tangential sections through torsellioid conidiomata show white ectostromata, grey entostromata, and multiple locules with independent grey walls. G–H. Allantoid conidia. Scale bars: G–H = 6 µm.

Anamorph separate from teleomorph stromata, discrete. *Conidiomatal stromata* immersed in bark, erumpent, discoid, convex to conical, torsellioid and lamyellioid, up to 1.5 mm diam. *Discs* if present dark brown or grey, nearly flat to convex, circular to ovoid, up to 0.6 mm diam, 1(–8) laterally inserted ostioles. *Ostioles* brown to grey, 40–80 µm diam, level to above the disc surfaces. *Locules* globose, 4–8 simple undivided, 100–200 µm diam, not sharing common walls, walls of *textura epidermoidea*, surrounded by entostromata, each locule with an ostiole converging toward the disc, usually to 1 shared ostiole (up to 8 ostioles) per disc. *Conidiophores* hyaline, unbranched or occasionally branched at base, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, periclinal thickenings minute, 12–20 × 1–1.5 µm. *Conidia* hyaline, eguttulate, allantoid, aseptate (3–)4–7 × 1 µm.

Types: *V. ceratosperma* (Tode) Maire (as *Sphaeria ceratosperma* Mougeot & Nestler 1818, Stirp. Vog.-Rhem, 567, in Fries 1823) (CUP) (*fide* Hubbes 1960a) **Neotype**. *Cytospora sacculus* (Schwein.) Gvrit., **holotype**, Schweinitz, Syn. Fung. 1309–164, Salem (as *Sphaeria sacculus*) (PH).

Culture: Colony growth on PDA is greyish yellow-brown and brownish grey (Munsell 9.5YR 4.6/2.1, 6YR 5.6/0.8) with an edge of dark orange-yellow and brownish yellow (9.3YR 6.0/7.9, 9YR 5.8/10.4). Colour of the reverse is deep yellow-brown and brownish orange (Munsell 8.8YR 3.1/5.0, 8.5YR 6.2/6.2). Colony texture is felty, slightly raised with no growth zones. Pycnidia form abundantly on PDA and oatmeal agar (45 d) and exude dull orange cirrhi. Leucotorsellioid conidiomata with 15 or more regular radially arranged locules in grey entostromata surrounded in white ectostromata and with indistinct discrete necks/beaks form on the autoclaved leaf. Conidiomata exuded yellow cirrhi on leaves. Conidiomata have a white ectostromatal layer and appear to have a second outermost ectostromatal layer which is grey and slightly wooly.

Cardinal temperatures: Colonies (mean of 3 isolates) obtain a mean growth on PDA at 4 ºC of 11 mm diam, at 25 ºC of 108 mm diam, at 32 ºC of 2 mm diam, and no growth at 37 ºC after 7 d in the dark. Colonies obtain a mean growth on V8® agar at 25 ºC of 37 mm diam after 7 d in the dark. No growth at 25 ºC occurs on 2 ppm cycloheximide in V8® agar after 7 d in the dark.

Hosts: *Quercus*, *Fagus*, *Taxus*.

Distribution: Worldwide.

Specimens examined: **U.S.A.**, Michigan, *Quercus alba*, 1992 (MSC 380716, *Cytospora sacculus*), also living culture CBS 116855. **The Netherlands**, *Fagus sylvatica*, 1921, living culture CBS 116.21. **Switzerland**, *Taxus baccata*, 1942, living culture CBS 192.42.

Notes: Many specimens have the morphology of *V. ceratosperma* and its anamorph *C. sacculus* but do not share close sequence homology with the specimens used as the DNA sources listed here under *V. ceratosperma s. str.* We attempted to narrow the species concept to one that we assume is closest to the neotype specimen, and to *V. ceratophora* Tul. & C. Tul. (as *Sphaeria ceratosperma* Mougeot & Nestler 1818, Stirp. Vog.-Rhem, 567, in Fries 1823; neotype in Spielman 1985) and other synonyms such as *V. decorticans* Fr. (*Sphaeria decorticans* Fr. 1823). Narrowing the species concept and basing it on DNA sequence should improve future understanding of the distinctive morphological and biological characteristics of the species.

13. *Valsa fabianae* G.C. Adams, M.J. Wingf. & Jol. Roux, **sp. nov.** Figs 49–52. MycoBank MB500214.
Anamorph: ***Cytospora eucalypticola*** van der Westh., S. African For. J. 54: 8. 1965, emend. G.C. Adams & M.J. Wingf.

Etymology: "*fabianae*" refers to the students and scientists at the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria. The epithet recognises the many dedicated mycologists at FABI.

Ascostromata in cortice atque ligno immersa, erumpentia, circularia vel ovoidea, usque ad 0.8 mm diametro, saepe in statu perithecii solitarii rostro longo ostiolari praediti, perithecii 1–4(–7) in profunditate unica in ligno dispositis, in entostromate pallide brunneo infra discos disposita. Disci interdum perithecio solitario sed plerumque perithecii 2 vel plus praediti, prominentes, saturate mediocriter brunnei vel fusco-brunnei, convexi, circulares vel ovoidei, 0.24–0.6 mm diametro, ostiolis 1–7 lateraliter insertis praediti. Ostiola mediocriter brunnea vel mediocriter grisea, 70–150 µm diametro, plana vel 0.4–0.7 supra superficiem disci, transverse in discum dispersa. Perithecia mediocriter brunnea, globosa, 0.17–0.45 mm diametro, ex area sub disco usque ad basem vel entostromate pallide brunneo e textura globosa composito circumdata, vel stromate carentia, parietibus e textura epidermoidea compositis, stratis cellularum 3–4 × 10–15 µm crassis praedita. Asci liberi subcylindrici vel clavati, in stipite brevi, 23–36 × 4–6.5 µm, in apparatu apicali non amyloideo annulo refractivo chitinoideo praediti, 8-spori. Ascosporae biseriatae elongato-allantoideae tenuitunicatae hyalinae unicae (6–)7–8.5 × 1.5 µm.

Anamorpha plerumque in teleomorphas interspersa, discreta. Stromata conidiomatica in cortice immersa, erumpentia, plerumque 1-pycnidiata, 0.15–0.5 mm diametro, raro usque 7-pycnidiata, profunde in cortice immersa; stromata ubi in superficie corticali saturate mediocriter brunnescentia hemisphaerica, usque ad 0.6 mm diametro. Disci, quum praesentes, atro- vel medio-brunnei vel grisei, convexi vel paene plani, circulares vel ovoidei, 0.1–0.3 mm diametro, ostiolis (1–)2–4(–7) lateraliter insertis. Ostiola mediocriter brunnea, 40–80 µm diametro, conspicue tenuioria quam rostra perithecialia. Loculi globosi quum profunde in cortice immersi ad typum simplicem pertinentes, raro introrsum plicis imperfectis vel quum in superficie ad typum plurilocularem, cavernulis (2–)6(–7) regularibus radiatim dispositis 100–200 µm diametro parietibus communalibus praediti; in cultura loculi pluriloculares cavernulis aequiis 3–4 regularibus radiatim dispositis parietibus communalibus praediti. Cellulae conidiogenae in matrice continua gelatinosa inclusae, simplices vel interdum ad basem ramosae, hyalinae, enteroblastice phialidicae, subcylindricae ad apicem contractae, collaretta minuta atque parte incrassata periclini exigua praeditae (6.5–)10(–14) × 1–1.5 µm. Partes hymeniales raro inter cellulas conidiogenas interspersae, elongatae vel subcylindricae, inflatae. Conidia hyalina, eguttulata, allantoidea, unica (3.5–)4(–4.5) × 1 µm.

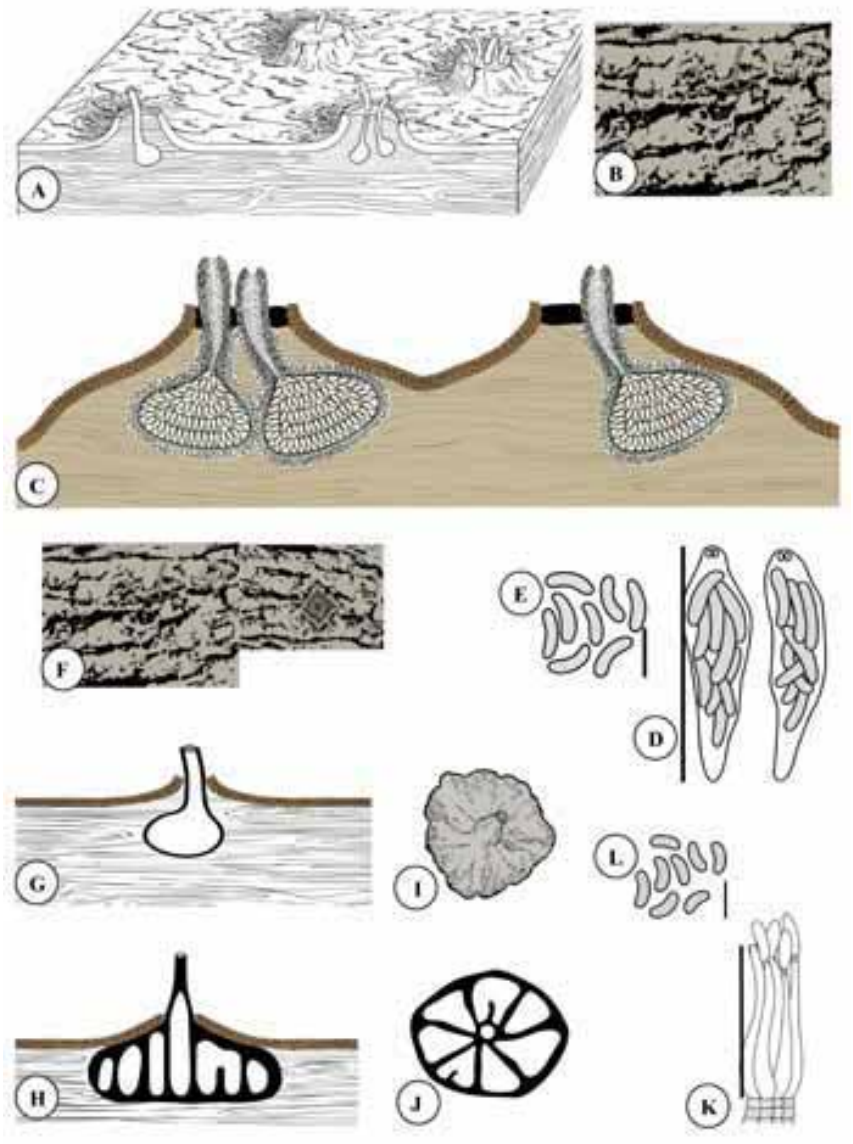

Fig. 49. Illustrations of the teleomorph and anamorph of *V. fabianae*. A. Habit sketch. B. Ostiolar disc of teleomorph erumpent from bark. C. Longitudinal sections through ascostromata in plant. D. Asci. E. Ascospores. F. Ostiolar discs of anamorph erumpent from bark. G. Longitudinal section through conidioma stroma deep in plant. H. Longitudinal section through conidioma stroma on surface in plant bark. I. Top view of conidioma isolated from plant tissues. J. Horizontal cross section through conidiomal stroma on surface of plant bark. K. Conidiophores in hymenium. L. Conidia. Scale bars: D = 30 µm, E = 8 µm, K = 10 µm, L = 4 µm.

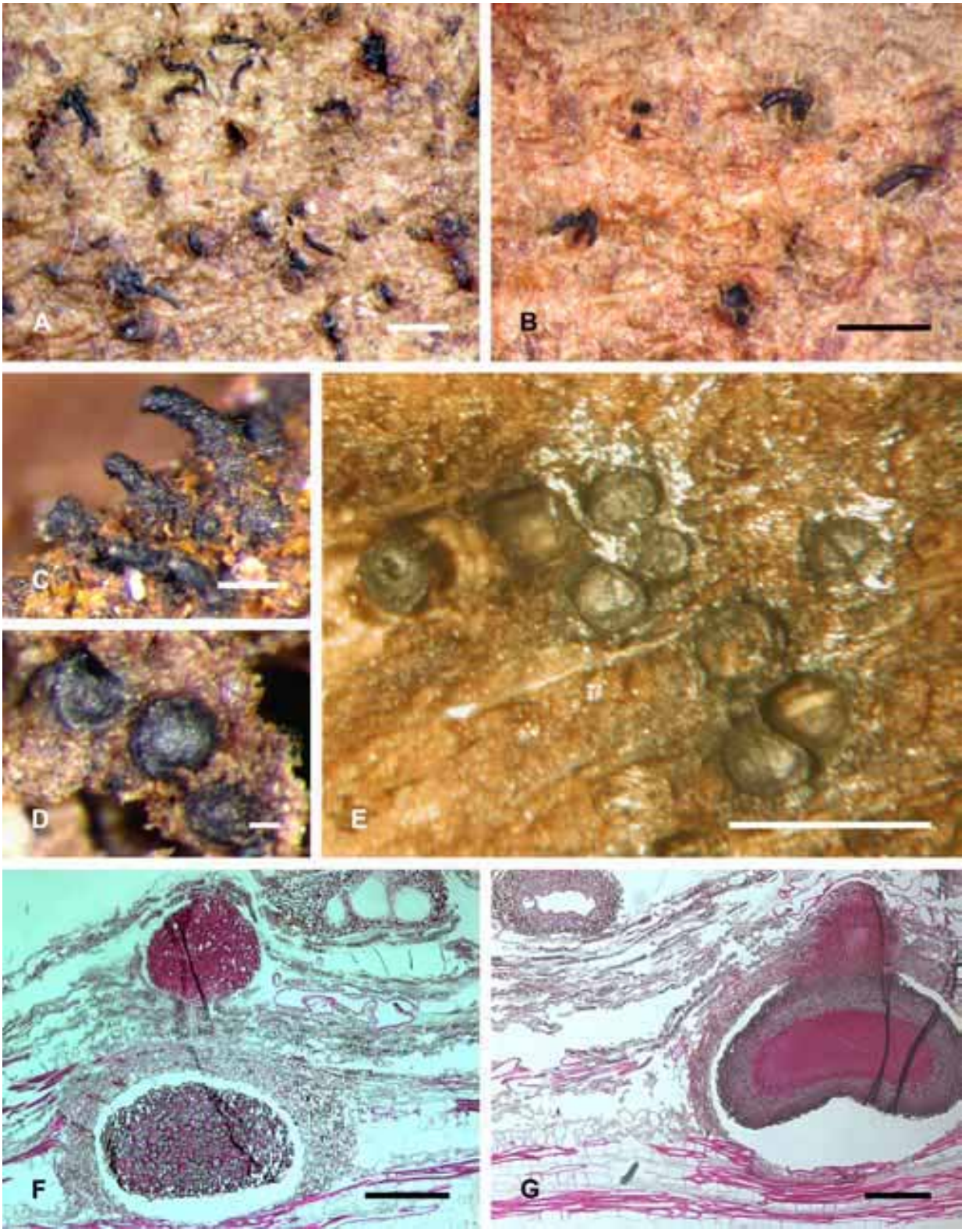

Fig. 50. Morphology of the teleomorph of *V. fabianae*. A–C. Beaks of perithecia extending above *Eucalyptus* bark surface (Uganda-12, Uganda-9). Most are discrete. D–E. Tangential sections revealing the arrangement of the perithecia (Uganda-9, Australia-10). F. Longitudinal microtome section through solitary perithecia (left) of the ascostroma and conidioma on surface (upper right) (Australia-10) with thin layer of entostroma surrounding the perithecium. G. Longitudinal microtome section through another solitary perithecia (right) and conidioma on surface (upper left) (Australia-10) with thin layer of entostroma surrounding the perithecium. Scale bars: A–B = 1 mm, C–D = 100 µm, E = 1mm, F–G = 100 µm.

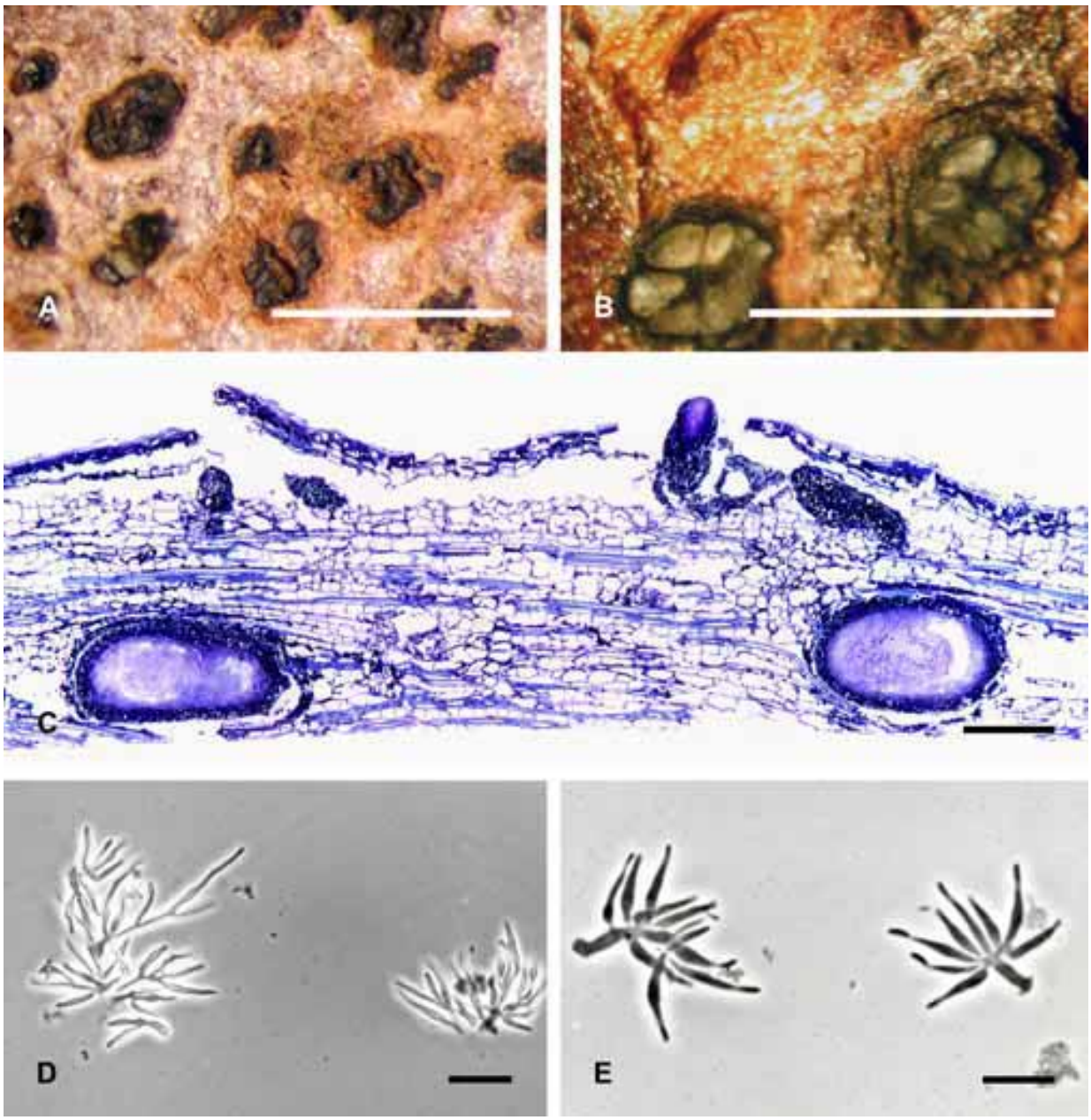

Fig. 51. Morphology of the variable anamorph of *C. eucalypticola* (compare with holotype, Figs 24, 25). A. Aggregated conidiomata formed near surface of *Eucalyptus* bark with cytosporoid characteristics of the former section *Cytospora* (SouthAfrica-10). B. Conidiomata formed near surface of bark with the rosette cytosporoid form (Australia-10). C. Longitudinal microtome section through 2 typical conidiomata formed deep in bark. If the nearby ostiolar necks do not converge then the conidiomata are simply unilocular but if the necks converge then the conidiomata are lamyelloid (SouthAfrica-10). At low magnification the conidiomata differ from ascomata in not being surrounded with a thin layer of entostroma. D. Long filamentous hymenial elements are interspersed among conidiogenous cells and arise from the same hyphae (Australia10). E. Whorls of conidiogenous cells branch from common discrete basal cells. Scale bars: A–B = 1 mm, C = 100 µm, D–E =10 µm.

Ascostromata immersed in bark and wood, erumpent, circular to ovoid, up to 0.8 mm diam, euvalsoid becoming circinateous with increasing perithecia, often solitary perithecia with long ostiolar beaks, 1–4(–7) perithecia, inclined, arranged at one depth in the wood, with pale brown entostromata extending below the discs. *Discs* occasionally present with solitary perithecia, usually present with 2 or more perithecia, prominent, rich medium brown to dark brown, convex, circular to ovoid, 0.24–0.6 mm diam, 1–7 laterally inserted ostioles. *Ostioles* medium brown to medium grey, 70–150 µm diam, projecting to 0.4–0.7 mm above disc surfaces, spaced apart from each other in discs. *Perithecia* medium brown, globose, 0.17–0.45 mm diam, surrounded by pale brown entostromata of *textura globosa* from below discs to bases, or stromata

absent, walls of *textura epidermoidea*, 3–4 layers of cells, 10–15 µm thick. *Asci* free, subcylindrical to clavate, with short stalks, 23–36 × 4–6.5 µm, with a refractive chitinoid ring in the non-amyloid apical apparatus, 8-spored. *Ascospores* biseriate, elongate allantoid, thin-walled, hyaline, aseptate (6–)7–8.5 × 1.5 µm.

Anamorph usually interspersed amongst teleomorphs, discrete. *Conidiomatal stromata* immersed in

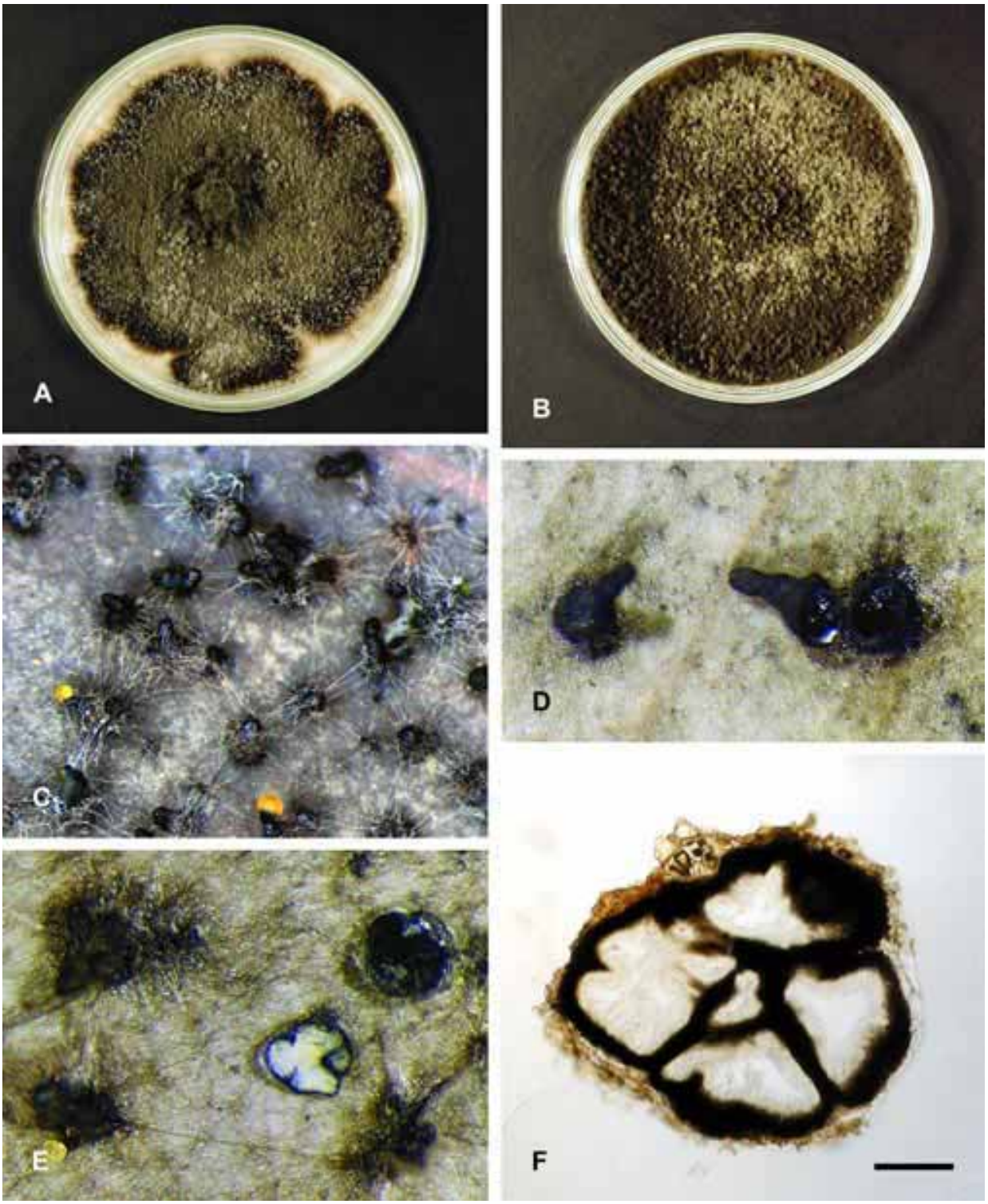

Fig. 52. Culture characteristics of *V. fabianae* (= *C. eucalypticola*). A–B. Colony morphology on PDA of isolates CBS 116852, SouthAfrica-11 (left) and CBS 116848, Australia-3 (right). C–D. Conidiomata produced on autoclaved *Eucalyptus* leaf. E. Longitudinal section through a conidioma shows single locule. F. Thin tangential section of conidioma of isolate CBS 116852. Scale bar: F = 100 µm.

bark, erumpent, unilocular, lamyelloid, and rosette cytosporoid, usually solitary and unilocular deep in bark, 0.15–0.5 mm diam, rarely to 7 pycnidia (lamyelloid); when on surfaces of bark the stromata become rich medium brown, hemispherical, rosette cytosporoid, and up to 0.6 mm diam. *Discs* if present dark to medium brown or grey, convex to nearly flat, circular to ovoid, 0.1–0.3 mm diam (1–)2–4(–7) laterally inserted ostioles. *Ostioles* medium brown, 40–80 µm diam, noticeably thinner than perithecial beaks, level to 0.15–0.3 mm above the disc surfaces. *Locules* globose, simple undivided when deep in the bark, rarely 1–2 incomplete invaginations, or rosette cytosporoid when on the surfaces with (2–)6(–7) uniform regular radially arranged chambers, 100–200 µm diam, sharing common walls. In culture, conidiomata rosette cytosporoid with 3–4 regular, radially arranged chambers sharing common walls. *Conidiophores* hyaline, unbranched or occasionally branched at base, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, periclinal thickenings slight (6.5–)10(–14) × 1–1.5 µm. Hymenial elements rarely interspersed amongst the conidiogenous cells, elongate to subcylindrical, inflated. *Conidia* hyaline, eguttulate, allantoid, aseptate (3.5–)4(–4.5) × 1 µm.

Cultures: Colony growth on PDA is predominantly olivaceous-grey (Munsell 8.1Y 3.5/0.9) on the surface, with occasional tufts of paler shades to rarely white. Colour of the reverse is olive-black (Munsell 9.0Y 1.1/0.0). Colony texture is felty, slightly raised with no growth zones. Pycnidia form on the agar and exude predominantly cream or occasionally yellow cirrhi. Unilocular conidiomata with long necks/beaks and with locules undivided to occasionally divided by invaginating walls into 1–5 irregular to regular chambers form on the autoclaved leaf. Conidiomata are dark, glabrose and may fuse in small groups with several necks/beaks.

Cardinal temperatures: Colonies (mean of 8 isolates) obtain a mean growth on PDA at 4 ºC of 7 mm diam, at 25 ºC of 88 mm diam, at 32 ºC of 115 mm diam, and at 37 ºC of 20 mm diam after 7 d in the dark. Colonies obtain a mean growth on Leonian's at 25 ºC of 37 mm diam after 7 d in the dark. No growth at 25 ºC occurs on 2 ppm cycloheximide in V8® agar or Leonian's agar after 7 d in the dark.

Hosts: *Eucalyptus nitens*, *E. saligna*, *E. grandis*, *E. marginata*, *E. delegatensis*, *E. globulus*, *E. dunnii*.

Distribution: Tasmania, Batlow NSW, Cann River VIC, and Canberra ACT, Australia; Entebbe, Tororo, and Mishenyi-Itojo, Uganda; Tzaneen, Limpopo, KwaMbonambi, Newcastle, and Seven Oaks, South Africa.

Specimens examined: **Australia**, Tasmania, Esperance Valley, Geeveston, DFR trial, teleomorph and anamorph on cankers of *E. nitens*, 4 Jun. 1987, K.M. Old (DAR 43948, **holotype** of *V. fabianae*), and ex-type living culture CBS 116840. **Uganda**, Entebbe, in swamp, teleomorph and anamorph on cankered branch of *E. grandis*, Jun. 1999, Jol. Roux (MSC 368319, MSC 368320), also living cultures CBS 116841 and CBS 116818, respectively. Mishenyi-Itojo, anamorph on cankered branch of *E. grandis*, Jun. 1999, Jol. Roux (MSC 380698), also living culture CBS 118088; Tororo, anamorph on cankered branch of *E. grandis*, Jun. 1999, Jol. Roux (MSC 368321), also living culture CBS 116842. **South Africa**, Limpopo, Tzaneen, Westfalia Estates on dead branches of *E. saligna* in plantations, 31 Mar. 1964, G.C. van der Westhuizen (PREM 42543 **holotype** of *C. eucalypticola*); Kwazulu-Natal, Homeleigh plantation house, on large fallen branch of *E. saligna*, 1999, G.C. Adams (MSC 380718), also living culture CBS 116852; Newcastle, Normandine plantation on bark of advancing canker on *E. dunnii* stressed by extreme drought and frost damage, 22 Jun. 1999, Jol. Roux & G.C. Adams (MSC 380697), also living culture CBS 116851.

Notes: The frequent solitary nature of the perithecia in this species could cause it to be mistakenly relegated to a genus other than *Valsa*, in most taxonomic schemes. DNA sequence homology supported the inference that it was a *Valsa* species, as did the *Cytospora* anamorph. Only *Valsa germanica* Nitschke shared with *V. fabianae* the unusual character of perithecia embedded singly within bark tissue and with discrete perithecial necks emerging separately and scattered about the surface (Munk 1957, Urban 1958, Kobayashi 1970). *Valsa germanica* was not close to *V. fabianae* in ITS-rDNA sequence homology. Often in *V. fabianae* only 2–4 perithecia are present in a reduced ascostromata. When perithecia are embedded in the wood, the globes appear surrounded by wood but are actually surrounded by thin stromatic tissue. Stromatal tissue surrounded each perithecium, separately, as seen in microtome sections (Fig. 50). The variable nature of the conidiomatal stroma is most similar to *C. variostromatica*.

The holotype specimen was studied earlier by Old *et al.* (1991) under the name *V. ceratosperma*, and the perithecia in this specimen are not solitary. The anamorph is most unusual in varying from specimen to specimen. Most specimens show the common morphology of unilocular conidiomata interspersed

with valsoid arrangements of lamyelloid conidiomata which appear to be the typical morphology when the conidiomata form relatively deeply in the bark. Other specimens have entirely the rosette cytosporoid morphology, which appears to be the typical morphology when the conidiomata form on or near the bark surfaces. The valsoid arrangement of the pycnidia superficially resembles a teleomorph when examined using a hand lens. Thus, the anamorphs often do not resemble the holotype of *C. eucalypticola*, the latter has only unilocular conidiomata. The specimen (MSC 380718) is most similar to the holotype of *C. eucalypticola*, and both specimens are from *Eucalyptus saligna*.

14. *Valsa brevispora* G.C. Adams & Jol. Roux, **sp. nov.** Figs 53–55. MycoBank MB500215.

Etymology: "*brevispora*" refers to the uniquely short ascospores.

Ascostromata in cortice arboris immersa, erumpentia, circularia vel ovoidea, usque ad 1.5 mm diametro, peritheciis 4–15 in profunditatibus variis in ligno dispositis, in entostromate pallide brunneo infra discum inclusis. Disci prominentes, brunnescentes, convexi, circulares vel ovoidei, usque ad 0.5 mm diametro, ostiolis (3–)4–14 lateraliter vel recte insertis praediti. Ostiola fusco-brunnea vel atro-grisea, 50–90 µm diametro, dispersa, paene plana vel 0.1–0.3 mm supra superficiem disci. Perithecia globosa (0.18–)0.2–0.25 mm diametro, ex area sub disco usque ad basem entostromate e materia amorpha composito circumdata, parietibus mediocriter brunneis e textura

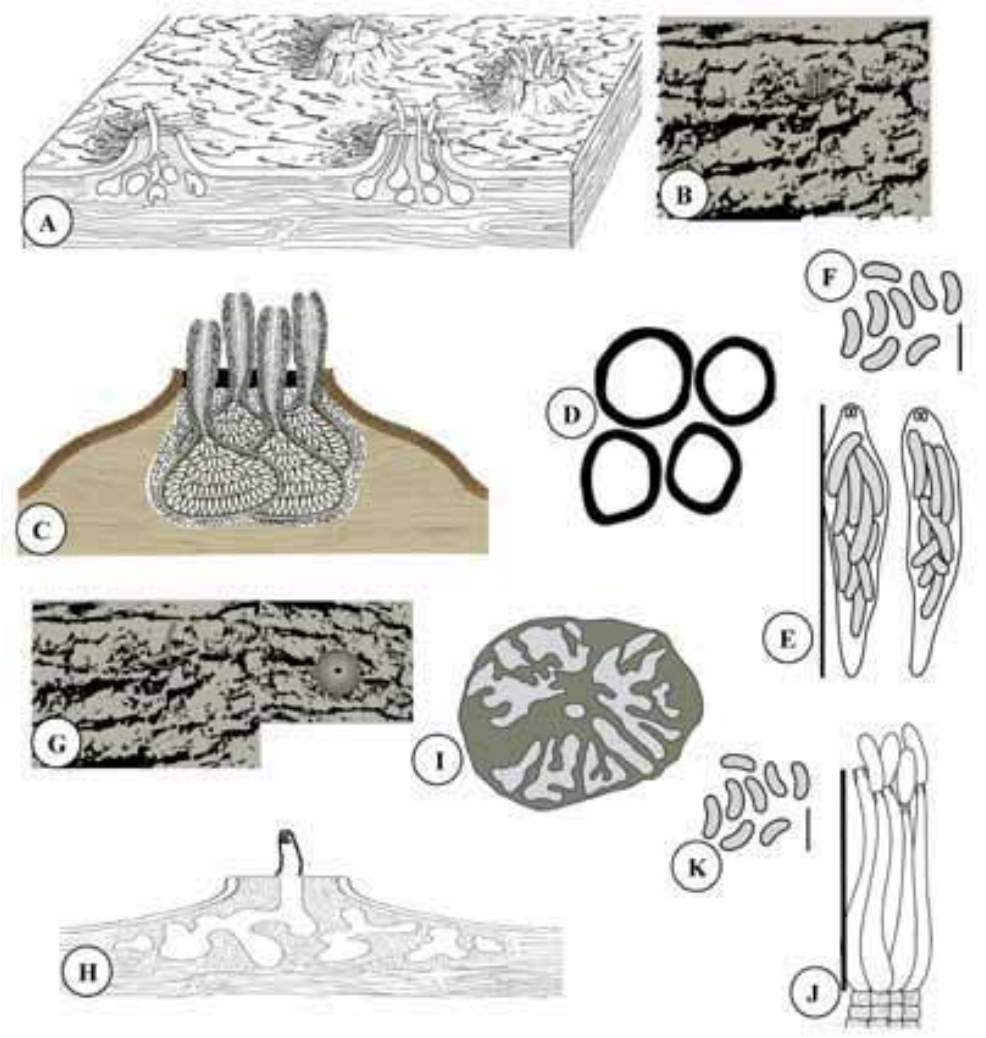

Fig. 53. Illustrations of the teleomorph and anamorph of *V. brevispora*. A. Habit sketch. B. Ostiolar disc of teleomorph erumpent from bark. C. Longitudinal section through ascostroma in plant. D. Horizontal cross section through ascostroma in plant. E. Asci. F. Ascospores. G. Ostiolar discs of anamorph erumpent from bark. H. Longitudinal section through conidioma stroma deep in plant. I. Horizontal cross section through conidiomal stroma. J. Conidiophores in hymenium. K. Conidia. Scale bars: E = 20 µm, F = 5 µm, J = 7 µm, K = 3 µm.

angularis compositis. Asci liberi, clavati in stipite brevi, 18–22 × (4–)5–6(–7) µm, annulo refractivo non amyloideo in apparatu apicali praediti, 8-spori. Ascosporae biseriatae elongato-allantoideae tenuitunicatae hyalinae unicae, 4–5.5 × 1–1.5 µm.

Anamorpha in eodem corticis fragmento, discreta. Stromata conidiomatica in cortice immersa, erumpentia, circulares vel ovoidei, usque ad 0.4–0.7 mm diametro. Disci pallide brunnei vel mediocriter grisei, plani, ovoidei, usque ad 0.25 mm diametro, ostiolo unico praediti. Ostiola atro-brunnea vel atro-grisea, paene aequa vel usque ad 0.2 mm supra superficiem disci. Loculi circulares vel ovoidei vertice compressi, ad typum complexum multi-locellatum pertinentes, introrsum per plicas in cavernulas irregulariter dispositas parietibus communalibus praeditas partiti. Cellulae conidiogenae in matrice continua gelatinosa inclusae, hyalinae, simplices, enteroblastice phialidicae, subcylindricae, ad apicem contractae, 6–8 × 0.9–1 µm. Conidia hyalina, eguttulata, brevi-allantoidea, aspectu in forma segmenti fructus citri, unica, 3(–3.5) × 1 µm.

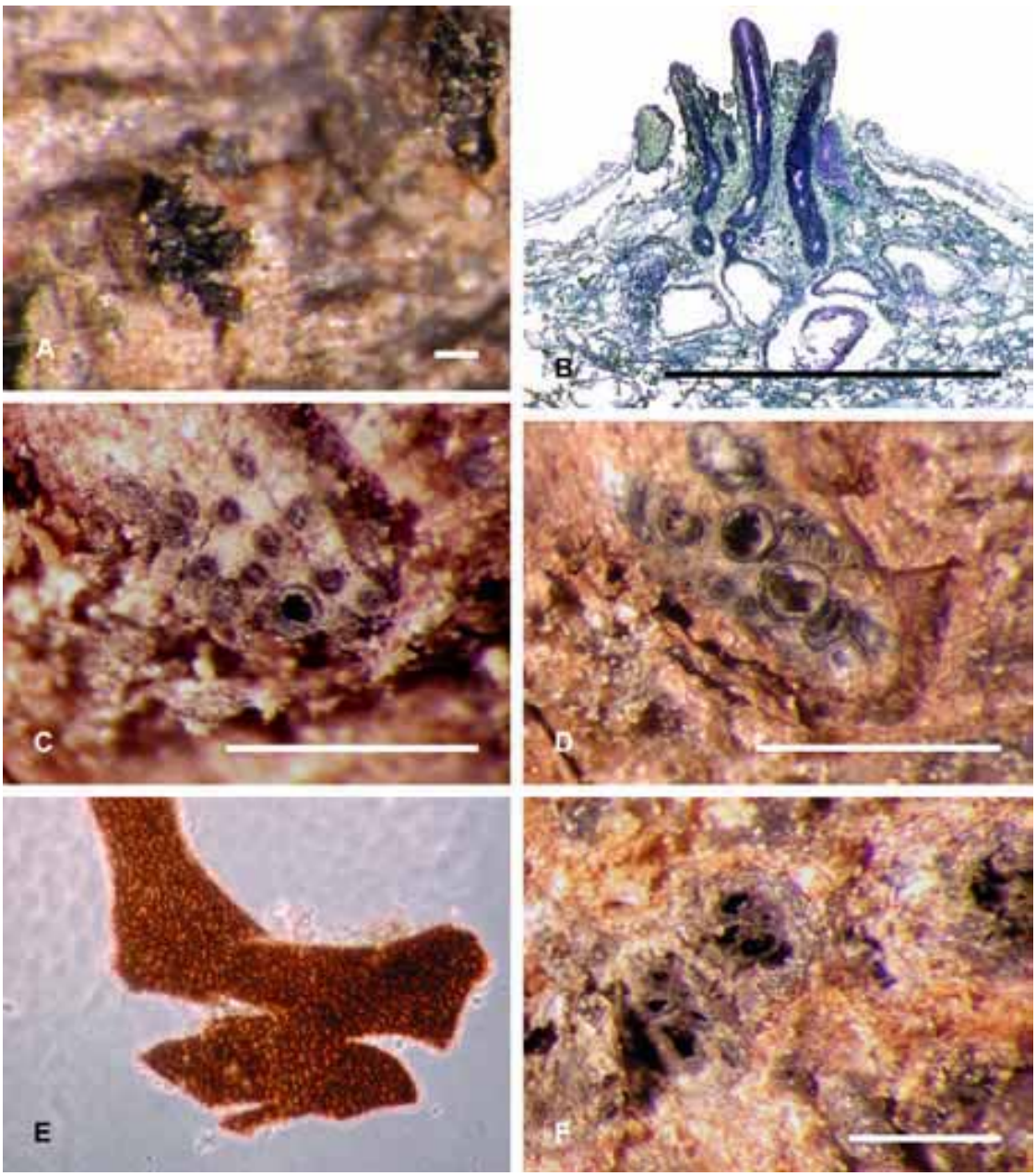

Fig. 54. Morphology of the teleomorph and anamorph of *V. brevispora*. A. Multiple tightly clustered beaks obscure the disc and extend above the surface of the bark of *Eucalyptus*. B. Median longitudinal microtome section of ascostroma shows convergent ostiolar necks surrounded by stroma tissue, and the monostichous arrangement of perithecia. C. Tangential section just below the beaks shows ostioles surrounded in stroma tissue. D. Tangential section deep below the disc shows globose perithecia surrounded by entostroma. E. Perithecial wall of *textura epidermoidea*. F. Tangential section of conidiomata shows multiple chambers dividing each locule. Scale bars: A = 100 µm, B–D, F = 1 mm.

Ascostromata immersed in bark, erumpent, circular to ovoid, up to 1.5 mm diam, euvalsoid, 4–15 perithecia, monostichous. Perithecia inclined to upright, arranged at various depths with pale brown entostromata extending below the discs. *Discs* prominent, pale brown, convex, circular to ovoid, up to 0.5 mm diam (3–)4–14 laterally to vertically inserted ostioles. *Ostioles* dark brown to dark grey, 50–90 µm diam, spaced apart from each other in discs, nearly level to 0.1–0.3 mm above disc surfaces. *Perithecia* globose (0.18–)0.2–0.25 mm diam, surrounded from below the discs to globe bases with entostromata of amorphous material, walls medium brown, of *textura angularis*. *Asci* free, clavate with a short stalk, 18–22 × (4–) 5–6(–7) µm, with a refractive chitinoid ring in the non-amyloid apical apparatus, 8-spored. *Ascospores* biseriate, elongate allantoid, thin-walled, hyaline, aseptate, 4–5.5 × 1–1.5 µm.

Anamorph intermixed with teleomorph, discrete. *Conidiomatal stromata* immersed in bark, erumpent, labyrinthine cytosporoid, circular to ovoid, 0.4–0.7 mm diam. *Discs* pale brown to medium grey, flat, ovoid, up to 0.25 mm diam with discrete ostioles. *Ostioles* dark brown to dark grey, nearly level, up to 0.2 mm above disc surfaces. *Locules* circular to ovoid, compressed vertically, complex multi-chambered, subdivided by invaginations into irregularly arranged chambers sharing common walls. *Conidiophores* hyaline, unbranched, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, 6–8 × 0.9–1 µm. *Conidia* hyaline, eguttulate, short allantoid, tangerine-section shaped, aseptate, 3(–3.5) × 1 µm.

Cultures: Colony growth on PDA is predominantly yellowish white, occasionally pale greyish olive (Munsell 4.5Y 9.2/1.2, 7.8Y 5.5/2.5) on the surface.

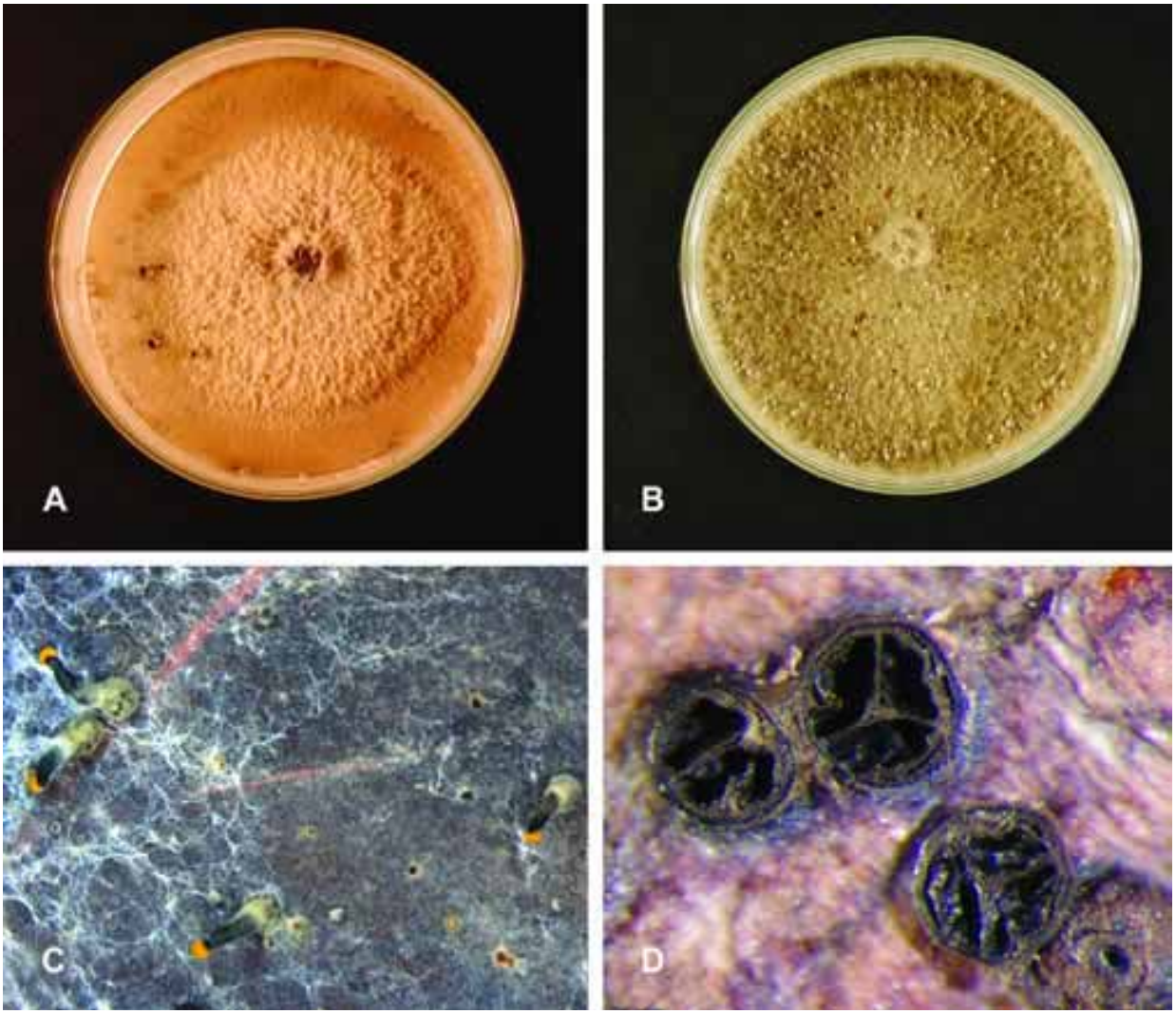

Fig. 55. Culture characteristics of *V. brevispora.* A–B. Colony morphology on PDA of isolates CBS 116813, Venezuela-1 (left) and CBS 116811, Congo-1 (right). C. Conidiomata produced on autoclaved *Eucalyptus* leaf. D. Tangential sections through conidiomata shows divided locules.

Colour of the reverse is pale to dark yellow (Munsell 4.3Y 8.8/6.8, 3.9Y 6.0/6.4) and dark greyish yellow to dark greyish brown (Munsell 3.8Y 5.9/4.0, 5.5YR 2.0/1.5). A diffusible pigment colours the agar dark brown (Munsell 5.3YR 1.6/3.4) and influences interpretation of the reverse colony colour. Only an occasional isolate forms pycnidia on the agar and exudes pale orange to yellow orange cirrhi. Colony texture is felty, slightly raised with no growth zones. Unilocular to rosette cytosporoid conidiomata with long necks/beaks and with locules divided by invaginating walls into 1–7 irregular to regular radially arranged chambers form on the autoclaved leaf. Individual chambers may be further modified by partially invaginating walls. Conidiomata have a cream, slightly wooly surface covering the globes but not the dark necks/beaks. The conidioma is unique in having a separate thin dark ectostromatal wall encompassing the wall layer that invaginates to divide the locule into chambers.

Cardinal temperatures: Colonies (mean of 4 isolates) obtain a mean growth on PDA at 4 °C of 4.5 mm diam, at 25 °C of 140 mm diam, at 32 °C of 140 mm diam, and at 37 °C of 38 mm diam after 7 d in the dark. Growth at 25 °C on 2 ppm cycloheximide in V8® agar is 80.5 % of growth on V8® agar without the antibiotic after 7 d in the dark.

Hosts: *Eucalyptus camaldulensis*, *Eucalyptus grandis* × *E. tereticornis* hybrids.

Distribution: Tchittanga, Republic of Congo and Acarigua, Venezuela.

Specimens examined: **Republic of Congo**, Tchittanga, on bark of *Eucalyptus grandis* × *E. tereticornis* infected with bacterial wilt, 20 Jun. 1998, Jol. Roux (MSC 368317 **holotype** of *V. brevispora*, living ex-type culture CBS 116811, and MSC 368318, also living culture CBS 116812). **Venezuela**, Acarigua, from bark of *E. camaldulensis*, Jun. 1997, M.J. Wingfield, living cultures CBS 116813 and CBS 116829.

Note: The species has especially small ascospores and conidia but complex labyrinthine cytosporoid conidiomata.

15. *Valsa eugeniae* Nutman & F.M. Roberts, Trans Br. Mycol. Soc. 36: 229. 1953. Figs 56–58.

Ascostromata immersed in bark, initially in the phellogen, erumpent, ovoid (0.3–)0.5(–1.4) mm diam, becoming linear when in bark cracks, up to (0.6–)1.2(–3) × 0.3 mm, euvalsoid monostichous, containing (14–)25(–70) perithecia in reddish brown entostromata below the discs. *Discs* obscured by tightly grouped ostiolar beaks. *Ostioles* crowded, dark reddish brown, 70–74 µm diam, vertically inserted, 0.2–2.5 mm above bark surfaces. *Perithecia* globose, seldom compressed, (195–)240(–295) µm diam, crowded, upright, surrounded in entostromata of *textura intricata*, walls dark reddish brown, of *textura epidermoidea*. *Asci* free, subclavate (15–)18(–20) × (3–)4.5(–5) µm with refractive chitinoid rings in non-amyloid apical apparati, 8-spored. *Ascospores* biseriate, thin-walled, hyaline, allantoid, aseptate, 5–5.8 × 1.2 µm.

Anamorph discrete, rare, usually absent or on different branch than teleomorph. *Conidiomatal stromata* reduced to absent. *Conidiomata* immersed in bark, erumpent, rosette cytosporoid, ovoid to elongate ovoid, 200–280 µm diam, dark reddish brown, subdivided by invaginations with regular radially arranged chambers sharing common walls. *Discs* absent. *Ostioles* dark reddish brown, 15–60 µm above surface. *Conidiophores* hyaline, branched, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, periclinal thickenings minute, 10–20 × 1–1.5 µm. *Conidia* hyaline, eguttulate, allantoid, aseptate, 2–4 × 0.8–1 µm. Conidia extruded in yellow globules or tendrils.

Cultures: Colony growth on PDA is predominantly yellow-white to greyish yellow and greyish olive to pale olive-grey (Munsell 4.5Y 9.2/1/2, 3.8Y 7.4/1.4, 4.4Y 7.2/3.8) on the surface. Colour of the reverse is dark yellow, pale yellow, and yellowish white (Munsell 3.9Y 6.0/6.4, 4.7Y 9.0/3.8, 4.5Y 9.2/1/2) with areas of olive-black (Munsell 9.0Y 1.1/0.0). Pycnidia form on the agar and exude yellow-orange cirrhi. Colony texture is felty, slightly raised with no growth zones. Unilocular conidiomata with locules divided by partially invaginating walls into 1–4 irregular chambers and with or without necks/beaks form on the autoclaved leaf. Conidiomata have a wooly surface of loose cream ectostromatal tissue and often coalesce into clusters through crowding or through apparent fusion of the ectostromata.

Cardinal temperatures: Colonies (mean of 4 isolates) obtain mean growth on PDA at 4 ºC of 3.8 mm diam, at 25 °C of 150 mm diam, at 32 °C of 136 mm diam, and at 37 °C of 21–42 mm diam after 7 d in the dark. Growth at 25 °C on 2 ppm cycloheximide in V8® agar is 64 % of growth on V8® agar without the antibiotic after 7 d in the dark.

Hosts: *Eucalyptus grandis*, *Eugenia caryophyllus* (syn. *Syzygium aromaticum*), *Eugenia* sp., *Tibouchina heteromalla*, *Tibouchina* sp.

Distribution: Bisbane □LD, Australia; Suluwesi, Indonesia; Tanzania; West Malaysia.

Specimen examined: **Indonesia**, Suluwesi, Utara, on *Eucalyptus grandis*, 1 Aug. 2003, M.J. Wingfield (MSC 380723 of *Valsa eugeniae*), also living culture CBS 116834.

Notes: Characteristic features of this species include its large ascostromata and large number of perithecia per ascostromata. It is reported to be associated with sudden death disease of clove trees (seedlings are immune), forming brilliant saffron-yellow water-soluble stain of affected clove wood, stain delimited from healthy wood by narrow blue-grey to black zone lines (Nutman & Roberts 1953, Sivanesan & Holliday 1970).

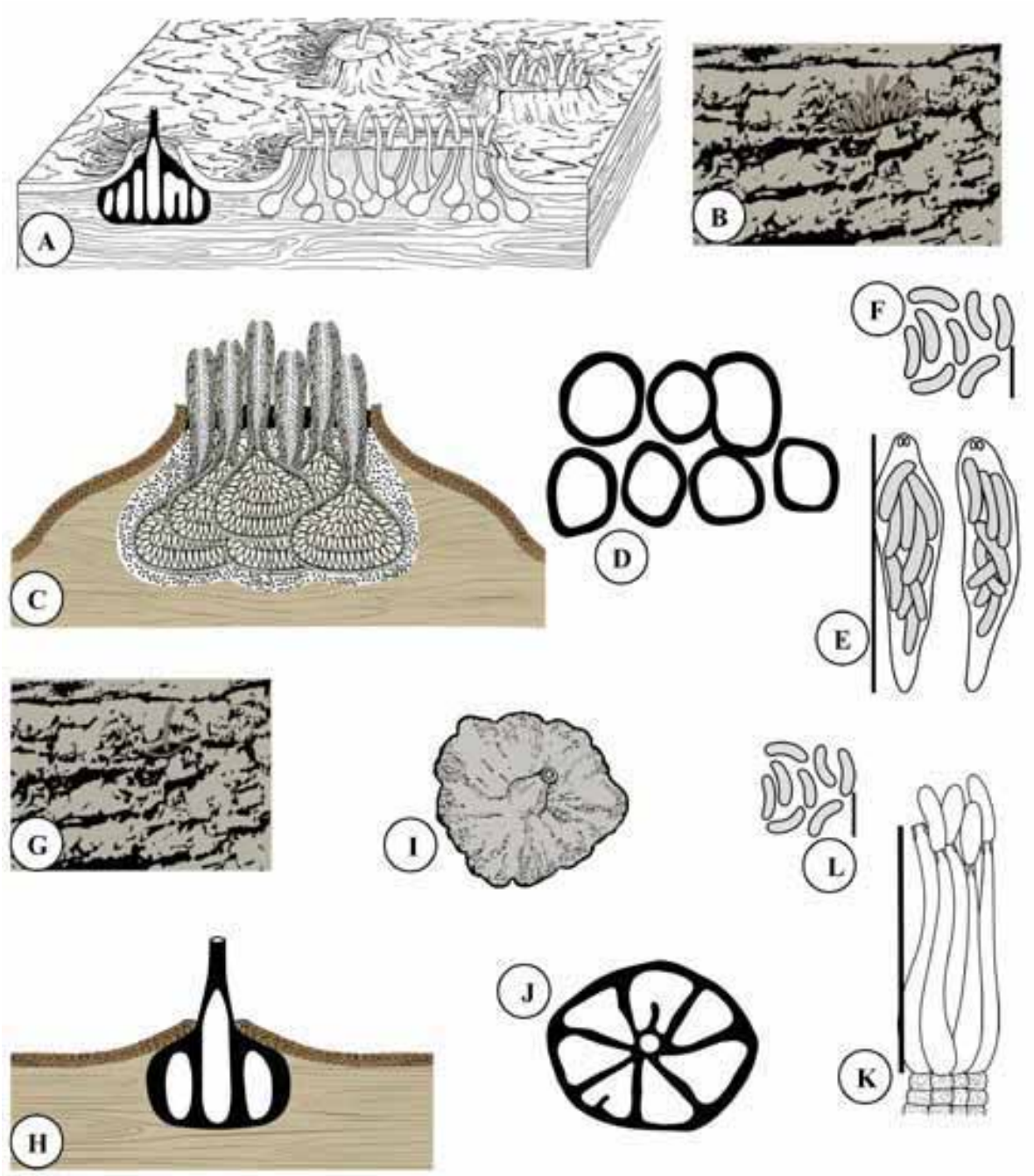

Fig. 56. Illustrations of the teleomorph and anamorph of *V. eugeniae*. A. Habit sketch. B. Ostiolar disc of teleomorph erumpent from bark. C. Longitudinal section through ascostroma in plant. D. Horizontal cross section through ascostroma in plant. E. Asci. F. Ascospores. G. Ostiolar disc of anamorph erumpent from bark. H. Longitudinal section through conidioma stroma deep in plant. I. Top view of conidioma isolated from plant tissues. J. Horizontal cross section through conidiomal stroma. K. Conidiophores in hymenium. L. Conidia. Scale bars: E = 18 µm, F = 5.5 µm, K = 15 µm, L = 3 µm.

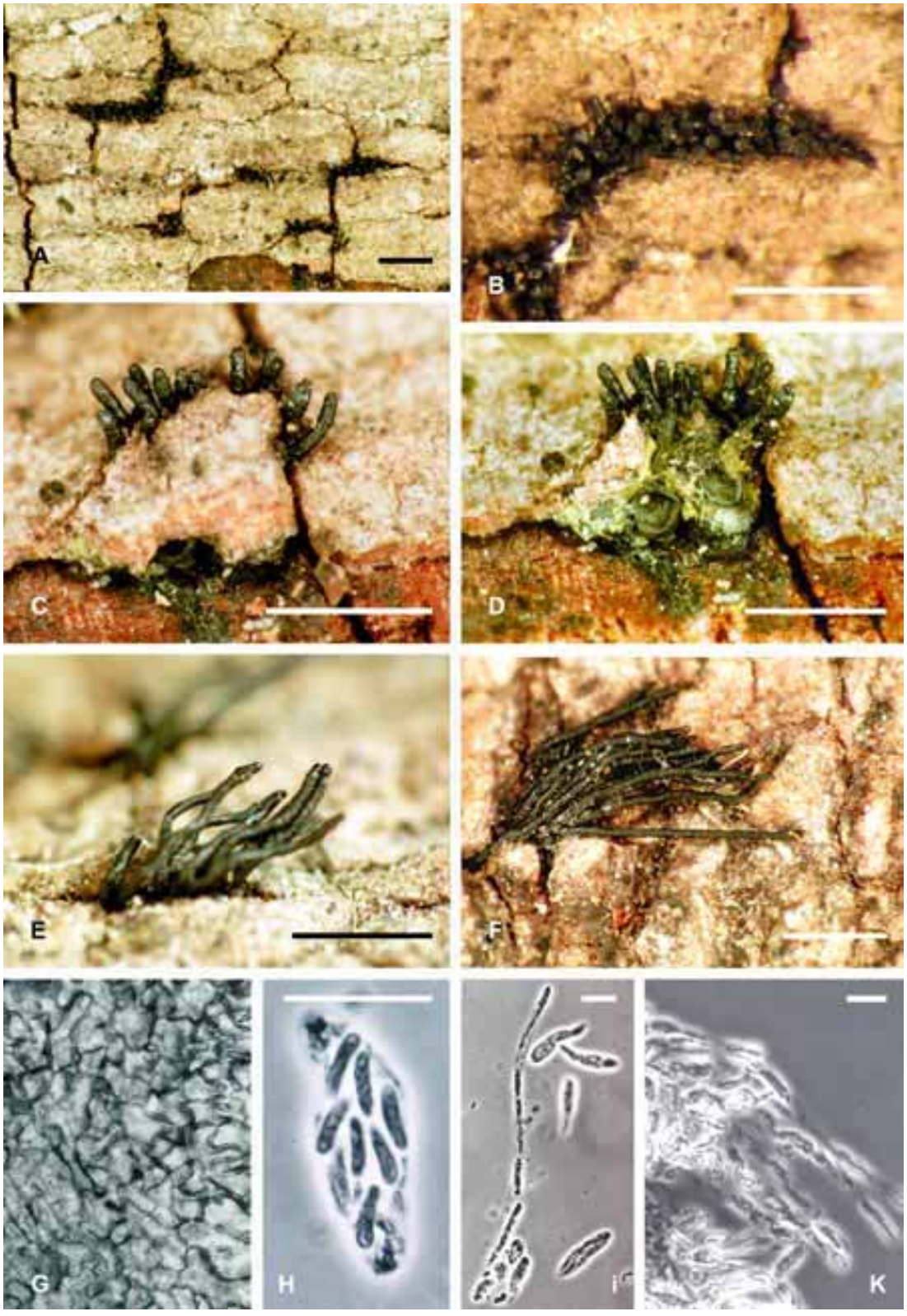

Fig. 57. Morphology of the teleomorph of *V. eugeniae*. A. Habit of massive ascostromata in cracks in the bark. B. Enlargement of massive ascostroma with crowded ostiolar beaks (*ca* 30) obscuring disc. C. Ostiolar beaks (*ca* 15) obscuring disc and extending above the bark surface. D. Exposed perithecia show monostichous arrangement below the beaks. E–F. Aggregated beaks extending great lengths above bark surface. G. A close up view of perithecial wall of *textura epidermoidea*. H. Ascus with eight ascospores and apical ring apparatus stained with chlorazol-black. Shape of the ascus is wider than natural due to pressure applied to improve focus. I–J. Filamentous paraphyses next to several mature asci show relative length. The shape of the asci is more natural in these images. Scale bars: A–F = 1 mm, I–J = 100 µm.

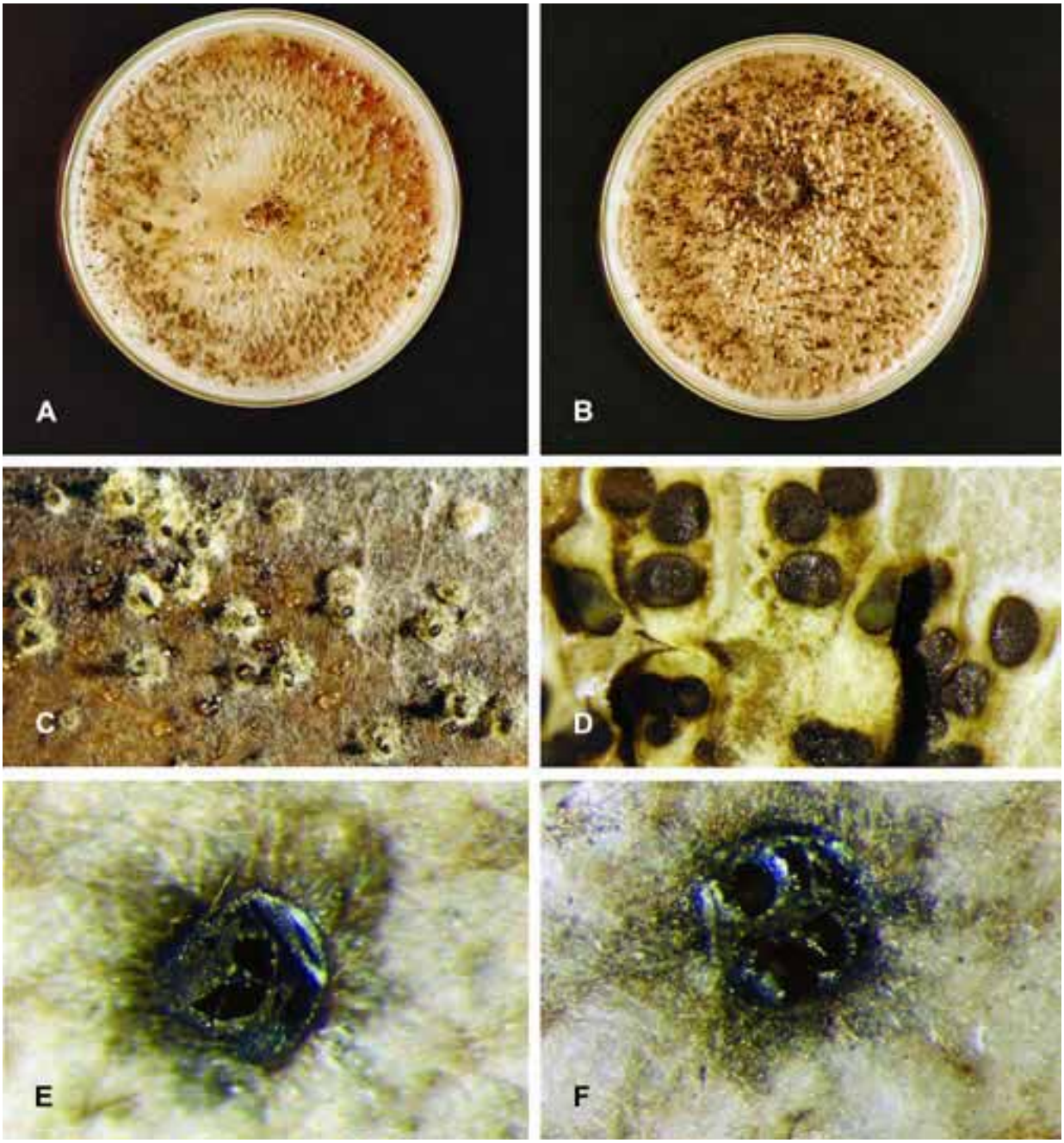

Fig. 58. Culture characteristics of *V. eugeniae*. A–B. Colony morphology on PDA of isolates CBS 116835, Clove (left) and CBS 116838, Australia-5 (right). C. Conidiomata produced on autoclaved *Eucalyptus* leaf. D. Tangential sections through groups of immature conidiomata. E–F. Tangential sections through conidiomata show divided locules.

16. *Valsa myrtagena* G.C. Adams & M.J. Wingf., **sp. nov.** Figs 59–61. MycoBank MB500216.

Etymology: "*myrtagena*" refers to the plant family *Myrtaceae* of the host species.

Ascostromata in cortice immersa, erumpentia, ovoidea vel lenticularia, usque ad 1.5 mm diametro, peritheciis (1–)4–7 in entostromate pallide brunneo infra discum inclusis. Disci prominentes, fusco-brunnei vel rubello-brunnei, onvexi, ovoidei vel lenticulares, 0.4–0.7 × 0.15–0.4 mm, ostiolis 4–7 lateraliter insertis praediti. Ostiola fusco-brunnea, 60–85 µm diametro, usque ad 0.25–0.4 mm supra superficiem disci assurgentia. Perithecia globosa, 0.15–0.3 × 0.2–0.3 mm diametro, circinatim disposita, dimidio superiore entostromate e textura intricata composito circumdato, parietibus fuscate rubrescenti-brunneis e textura epidermoidea compositis. Asci liberi, clavati in stipite brevi (14–)16(–18) × 5 µm, in apparatu apicali non amyloideo annulo refractivo chitinoideo praediti, 8-spori. Ascosporae biseriatae tenuitunicatae hyalinae allantoideae unicae (5–)6(–7) × 1.2 µm.

Anamorpha discreta, rara, plerumque absens vel in ramo alio quam teleomorpha. Stromata conidiomatica in materia nativa non visa; conidiomata in cultura pycnidialia 0.3–0.4 mm diametro, parietibus conspicuis duobus ad typum plurilocularem pertinentibus, introrsum per plicas partitis in cavernulas radiatim dispositas parietibus communalibus praedita. Cellulae conidiogenae in matrice continua gelatinosa inclusae, simplices, hyalinae, enteroblastice phialidicae, subcylindricae, ad apicem contractae, collaretta minuta praeditae, 5–7 × 1 µm. Conidia hyalina, eguttulata, stricta vel interdum allantoidea, unica, 3–3.5(–4) × 1 µm.

Ascostromata immersed in bark, erumpent, ovoid to lenticular, up to 1.5 mm diam, euvalsoid approaching circinateous, (1–)4–7 perithecia in pale brown entostromata below the discs. *Discs* prominent, dark brown to reddish brown, convex, ovoid to lenticular, 0.4–0.7 × 0.15–0.4 mm, 4–7 ostioles inserted upright. *Ostioles* dark brown, 60–85 µm diam, up to 0.25–0.4 mm above disc surfaces. *Perithecia* globose, 0.15–0.3 × 0.2–0.3 mm diam, arranged circinately, upper half surrounded by entostromata of *textura intricata*, walls dark reddish brown, of *textura epidermoidea*. *Asci* free, clavate with short stalks (14–)16(–18) × 5 µm with refractive chitinoid rings in non-amyloid apical apparati, 8-spored. *Ascospores* biseriate, thin-walled, hyaline, allantoid, aseptate (5–)6(–7) × 1.2 µm.

Anamorph discrete, rare, usually absent or on different branch than teleomorph, rosette cytosporoid. *Conidiomatal stromata* not observed on natural material. In culture, conidiomata 0.3–0.4 mm diam, with two distinct walls, rosette cytosporoid, subdivided by invaginations, up to 6 regular radially arranged chambers sharing common walls. *Conidiophores* hyaline, unbranched, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, 5–7 × 1 µm. *Conidia* hyaline, eguttulate, straight to occasionally allantoid, aseptate, 3–3.5(–4) × 1 µm.

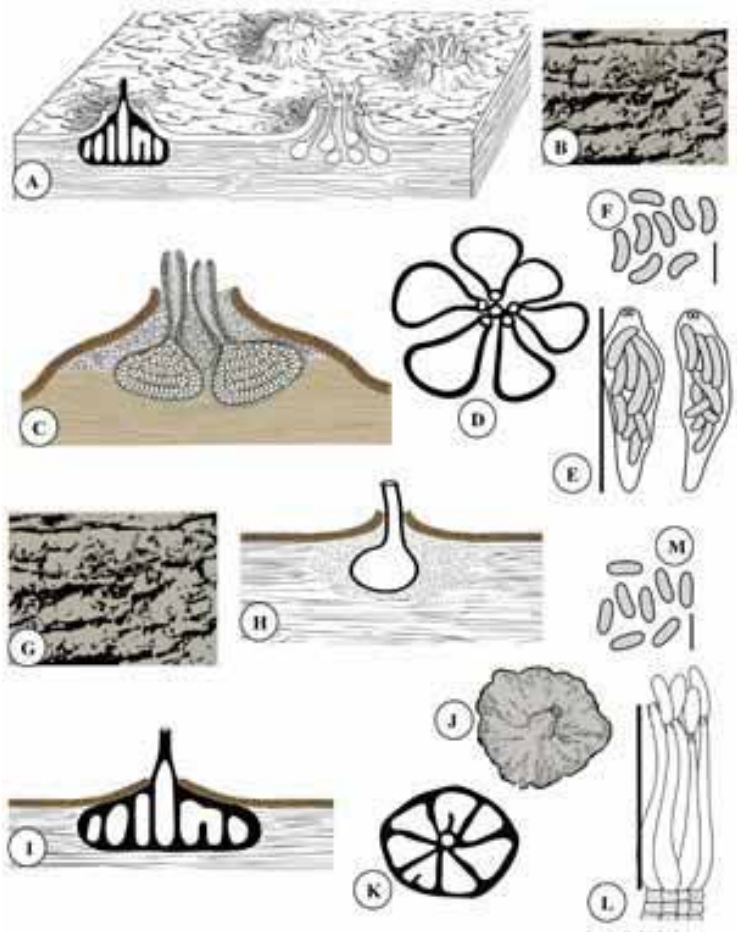

Fig. 59. Illustrations of the teleomorph and anamorph of *V. myrtagena*. A. Habit sketch. B. Ostiolar disc of teleomorph erumpent from bark. C. Longitudinal section through ascostroma in plant. D. Tangential section through ascostroma in plant. E. Asci. F. Ascospores. G. Ostiolar disc of anamorph erumpent from bark. H. Longitudinal section through small conidioma stroma in plant. I. Longitudinal section through large conidioma stroma in plant. J. Top view of conidioma isolated from plant tissues. K. Horizontal cross section through conidiomal stroma. L. Conidiophores in hymenium. M. Conidia. Scale bars: E = 16 µm, F = 6 µm, L = 6 µm, M = 3 µm.

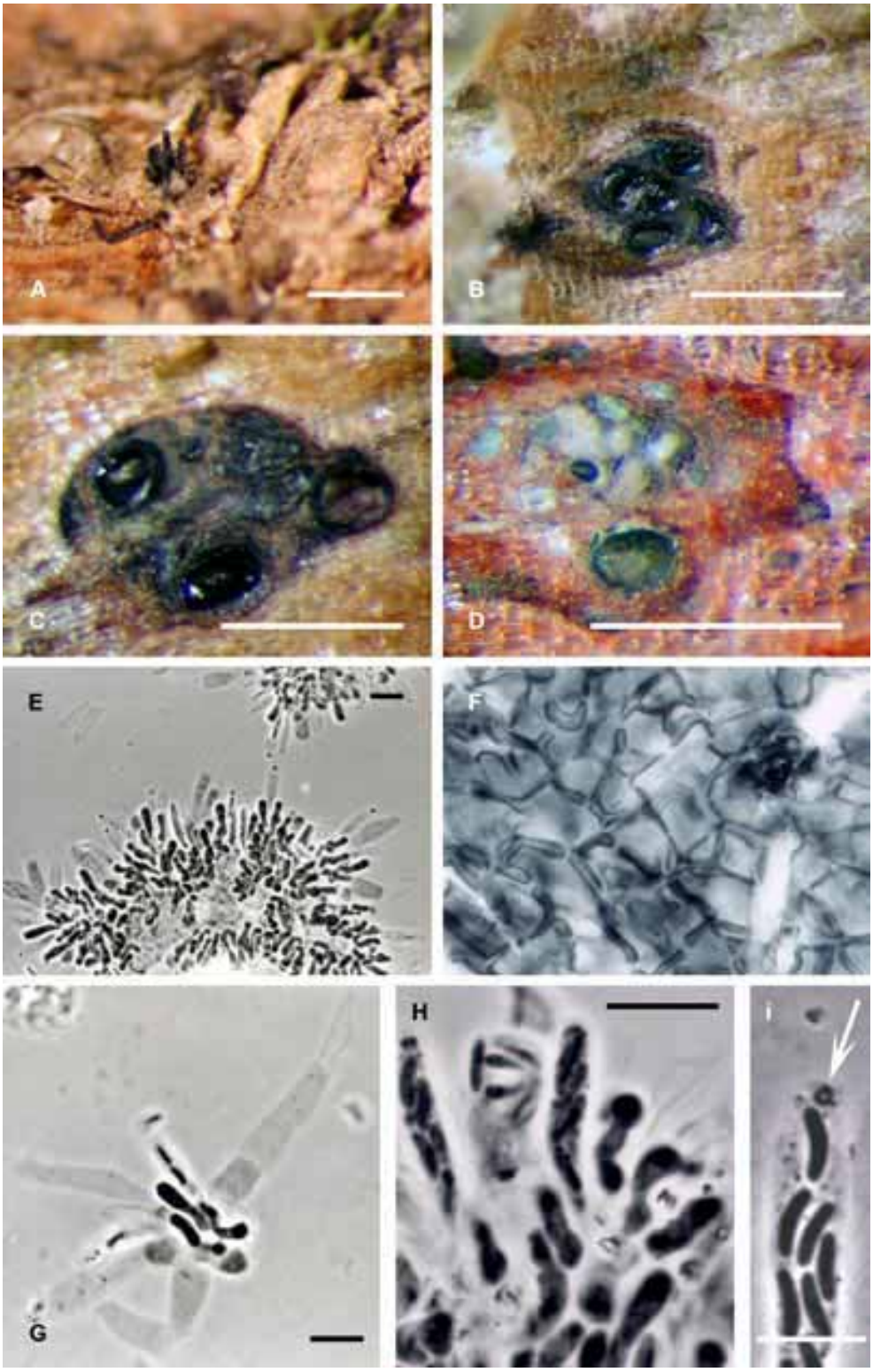

Fig. 60. Morphology of the teleomorph of *V. myrtagena*. A. A few crowded beaks obscuring the ascostroma disc and extending above the disc. B–D. Tangential sections through three ascostromata shows dark and light entostromata (and possibly stained wood) surrounding 4–5 globose perithecia. The dark zones at the peripheries of the stromata resemble conceptacles but are not interpreted as being conceptacles. E. Hymenium with short tapering paraphyses and mature and immature asci. F. Perithecial wall of *textura epidermoidea* with unusually wide and prominent cell borders, an artifact of preparation of the mount. G. Four short paraphyses, each of 2–3 cells and tapering to a pointed terminal cell, arise from the same ascogenous cells as the immature asci. The basal cells of the paraphyses appear to contain darker or denser cytoplasm than terminal cells. H–I. Asci with apical apparatus stained in chlorazol-black, dislodged ring facing forward (arrowhead), and biseriate ascospores. Scale bars: A–D = 1 mm, E = 10 µm, G–I = 10 µm.

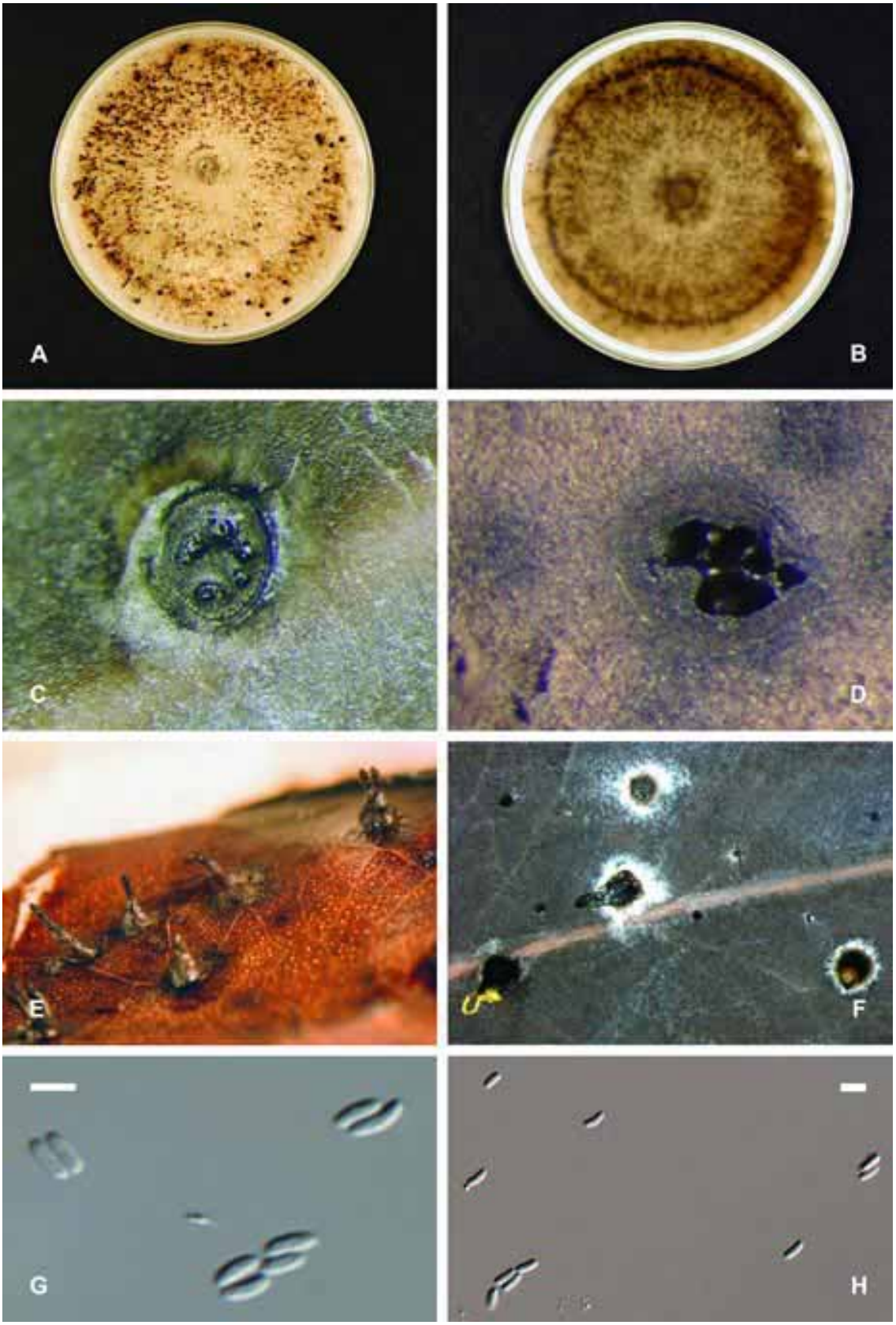

Fig. 61. Culture characteristics of *V. myrtagena*. A–B. Colony morphology on PDA of isolate CBS 116843, Hawaii-1 (left) and reverse side of Petri dish of isolate CBS 117013, Indonesia-3 (right) shows zonal growth. C–D. Tangential sections through conidiomata show divided locules. E–F. Conidiomata with long necks produced on autoclaved *Eucalyptus* leaves. G–H. Allantoid conidia. Scale bar: G–H = 3.5 µm.

Cultures: Colony growth on PDA is predominantly yellow-grey (Munsell 3.8Y 7.4/1.4) to pale greyish olive (Munsell 7.8Y 5.5/2.5) on the surface. Colour of the reverse is greyish olive to dark greyish olive (Munsell 8.0Y 3.6/2.0, 9.7Y 2.0/1.8) and moderate yellow to dark yellowish brown (Munsell 3.8Y 7.1/6.5, 9.4YR 2.3/3.3) with areas of dark olive-brown to olive-black (Munsell 2.0Y 1.9/2.2, 9.0Y 1.1/0.9). A diffusible pigment colours the agar pale olive-brown (Munsell 2.1YR 4.9/7.9) and influences interpretation of the reverse colony colour. Pycnidia form on the agar and exude dark orange cirrhi. Colony texture is felty, slightly raised with a few wide growth zones. Unilocular conidiomata with locules divided by partially invaginating walls into 1–4 irregular chambers and with 1–2 long slender necks/beaks form on the autoclaved leaf. The glabrose conidiomata are conical and taper gradually to an unusually long and slender neck/beak. A white to dark subiculum often occurs on the leaf surface encircling the bases of individual conidiomata.

Cardinal temperatures: Colonies obtain a mean growth on PDA at 4 ºC of 6 mm diam and at 25 ºC of 147 mm diam after 7 d in the dark. Indonesian isolates grow to 114 mm diam and Hawaiian isolates to 128 mm diam, at 32 ºC; and Indonesian isolates grow to 3 mm diam and Hawaiian isolates to 39 mm diam at 37 ºC, after 7 d in the dark. No growth of Indonesian isolates occurs at 25 ºC on 2 ppm cycloheximide in V8® agar. Growth at 25 ºC on 2 ppm cycloheximide in Leonian's agar is 25 % of growth on Leonian's agar without the antibiotic after 7 d in the dark. Growth of Hawaiian isolates at 25 ºC on 2 ppm cycloheximide in V8® agar is 50 % of growth on V8® agar without the antibiotic after 7 d in the dark.

Hosts: *Eucalyptus grandis*, *Tibouchina urvilleana*.

Distribution: Sumatra, Indonesia; Hilo, Hawaii, U.S.A.

Specimens examined: **U.S.A.**, Hawaii, Hilo on dead cankered branch of *T. urvilleana* in garden, 2001, M.J. Wingfield (MSC 380715, **holotype** of *Valsa myrtagena*), living ex-type culture CBS 116843. **Indonesia**, Sibisa, North Sumatra, ginger site plantation on cankered branch of *E. grandis* in plantation, 1996, M.J. Wingfield (MSC 380705, MSC 380706), also living cultures CBS 117013 and CBS 117014, respectively.

Notes: The ITS-rDNA sequence of the species was similar to that of *V. eugeniae* indicating a close relationship. However, the ascostromata were much reduced, containing 4–8 perithecia that were arranged circinately compared to groups of up to 100 arranged in tiers of different levels as typical of *V. eugeniae*. Both species have long ostiolar beaks and small asci containing eight small (relative to most *Valsa* species) ascospores. The anamorph has conidia that are straight in the type specimen but allantoid in the ex-type culture. The isolates from Hawaii grew well at 37 ºC and on media amended with 2 ppm cycloheximide whereas the isolates from Sumatra did not. Therefore, the species concept for *V. myrtagena* was broad and contained two distinct groups based on responses to cardinal temperatures and sensitivity to cycloheximide.

17. *Cytospora valsoidea* G.C. Adams & M.J. Wingf., **sp. nov.** Figs 62–64. MycoBank MB500217.

Etymology: "*valsoidea*" refers to the arrangement of pycnidia in the anamorphs being valsoid with the beaks laterally converging into a disc.

Teleomorpha ignota. Stromata conidiomatica in cortice immersa, erumpentia, ovoidea, 0.3–1.1 mm diametro, loculis (3–)5–8(–10) in entostromate mediocriter brunneo infra discum inclusis praedita. Disci fusco-grisei vel atro-brunnei, convexi, circulares, 0.2–0.45 mm diametro, ostiolis 3–10 praediti. Ostiola fusco-grisea, 40–100 µm, saepe supra disci superficiem. Loculi globosi vel compresso-globosi, 90–200 µm diametro, ad typum simplicem, non divisum pertinentes, parietibus non communalibus, ostiolo unico praediti, solitarii vel saepe in gregibus dispositi. Cellulae conidiogenae in matrice continua gelatinosa inclusae, base ramosae, plerumque prope altitudinem mediam uniramosae, enteroblastice phialidicae, hyalinae, subcylindricae, ad apicem contractae, collarcula minuta partibusque subincrassatis periclinis praeditae, 6.5–15 × 1–1.5 µm, phialide unica 6–8 × 1 µm. Conidia hyalina, stricta, unica (2.5–)3(–4) × 0.9–1 µm, tristia in lactophenole atque coloure xylino-caeruleo reagentia, guttulis duabus polaribus praedita.

Teleomorph unknown. *Conidiomatal stromata* immersed in bark, erumpent, unilocular and lamyelloid, ovoid, 0.3–1.1 mm diam, (3–)5–8(–10) locules in medium brown entostromata below the discs. *Discs* dark grey to dark brown, convex, circular, 0.2–0.45 mm diam, (1–)3–10 ostioles. *Ostioles* dark grey, 40–100 µm, often above disc surfaces. *Locules* globose to compressed-globose, 90–200 µm diam, simple undivided, not sharing common walls, with discrete ostioles, solitary or often occurring in-groups. *Conidiophores* embedded in a continuous gelatinous matrix, branched at the base, usually branched once more near mid-height. *Conidiogenous cells* enteroblastic phialidic, hyaline, subcylindrical, tapering to the apices, collarettes minute, periclinal thickenings minute, 6.5–15 × 1–1.5 µm, discrete phialides 6–8 × 1 µm. *Conidia* hyaline, straight, aseptate (2.5–)3(–4) × 0.9–1 µm, dark in lactophenol cotton blue stain, with two polar guttules.

Cultures: Colony growth on PDA is predominantly pale greenish yellow to bright greenish yellow (Munsell 9.5Y 9.0/4.2, 9.4Y 5.9/6.3) on the surface. Colour of the reverse is deep yellowish brown with a central area of brownish black (Munsell 8.8YR 3.1/5.0, 7.8YR 0.6/0.9). A diffusible pigment colours the agar light olive-brown (Munsell 2.1Y 4.9/7.9) and influences interpretation of the reverse colony colour. Pycnidia form on the agar and exude cream or yellow cirrhi. Colony texture is felty, slightly raised with no growth zones. Glabrose unilocular to reduced cytosporoid conidiomata with locules divided into 1–4 irregular chambers and with 1–4 necks/beaks form on autoclaved leaves.

Cardinal temperatures: Colonies obtain a mean growth on PDA at 4 ºC of 9.3 mm diam, at 25 ºC of 50 mm diam, no growth at 32 ºC and at 37 ºC, after 7 d in the dark. Colonies obtain a mean growth on Leonian's at 25 ºC of 66 mm diam after 7 d in the dark. Growth at 25 ºC on 2 ppm cycloheximide in V8® agar is 82.6 % of growth on V8® agar without the antibiotic after 7 d in the dark. Growth at 25 ºC on 2 ppm cycloheximide in Leonian's agar is 53 % of growth on Leonian's agar without the antibiotic after 7 d in the dark.

Host: *Eucalyptus grandis*.

Distribution: Only known from the type locality, Sibisa, Indonesia.

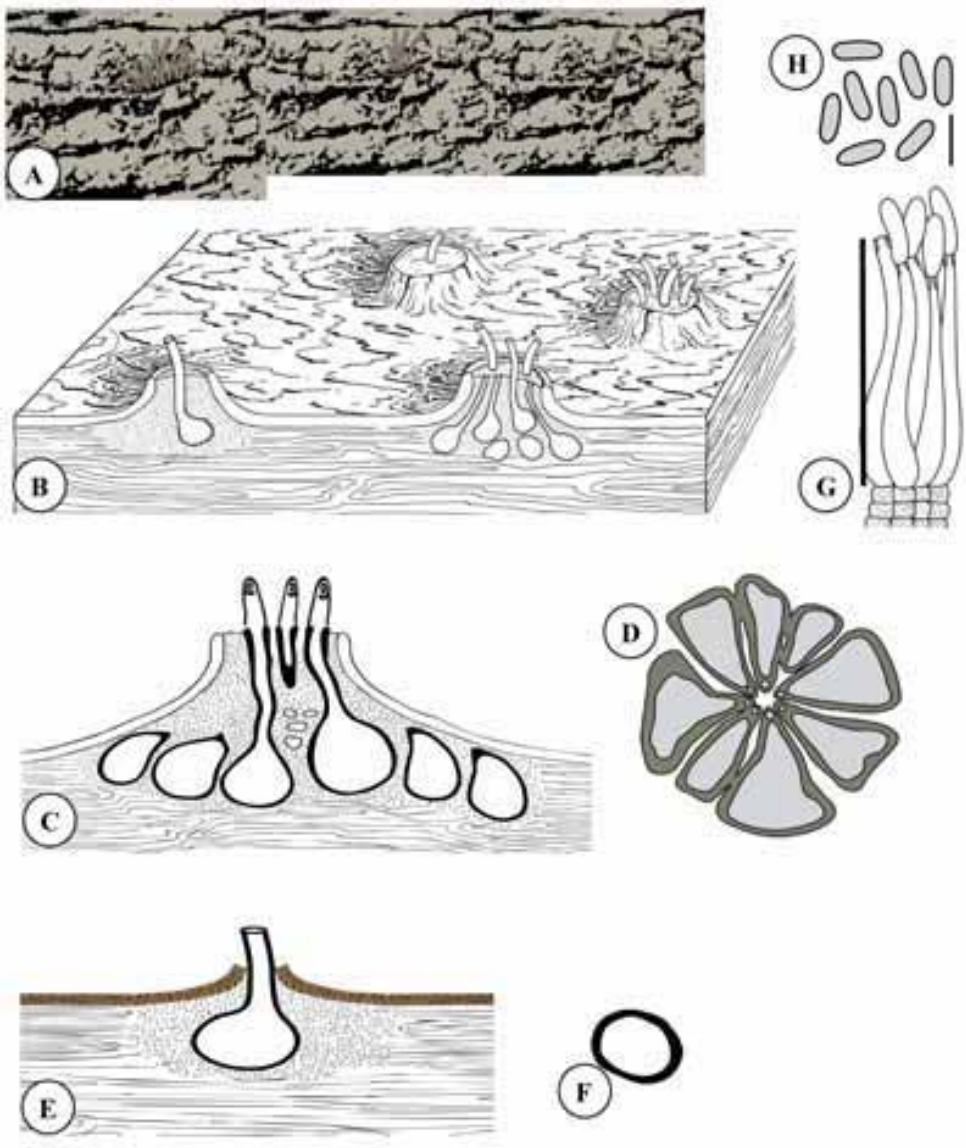

Fig. 62. Illustrations of *C. valsoidea*. A. Ostiolar discs erumpent from bark. B. Habit sketch. C. Longitudinal section through large conidiomal stroma in plant. D. Horizontal cross section through large conidiomal stroma. E. Longitudinal section through small conidiomal stroma in plant. F. Tangential section through small conidiomal stroma. G. Conidiophores in hymenium. H. Straight *in planta* to allantoid *in vitro* conidia. Scale bars: G = 13 µm, H = 3 µm.

Specimens examined: **Indonesia**, Sibisa, on dead cankered branch of *E. grandis* in Ginger site plantation, Jul. 1992, M.J. Wingfield (MSC 380717, **holotype** of *Cytospora valsoidea*), living ex-type culture CBS 117003.

Notes: This species is unique in having valsoid arrangements of the conidiomata with ostiolar necks laterally converging into a disc; and in having conidia that were straight in nature. Conidia stained more darkly in lactophenol cotton blue relative to other species.

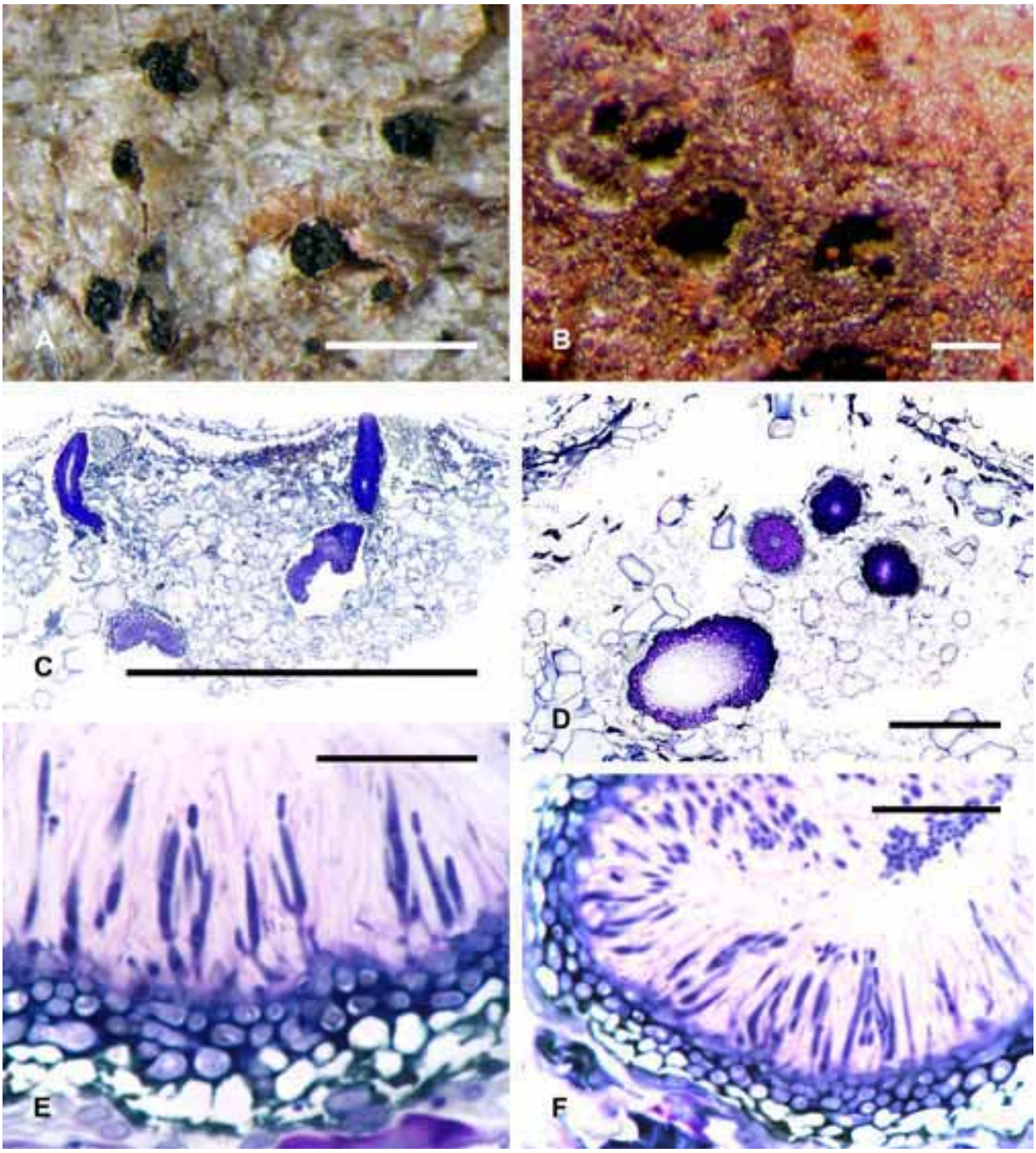

Fig. 63. Morphology of the anamorph *C. valsoidea*. A. Habit of dark brown conidiomatal discs each with several ostioles on the surface of *Eucalyptus* bark. B. Tangential section through locules shows circinate arrangement. C. Median longitudinal microtome section through locules with necks converging into small discs of ectostromata, and absence of stroma tissue below the discs. D. Longitudinal section shows three converging ostiolar necks beneath the disc. E–F. Microtome sections through locules shows unbranched and branched conidiogenous cells in the hymenia. Scale bars: A = 1 mm, B = 100 µm, C = 1 mm, D = 100 µm, E–F =10 µm.

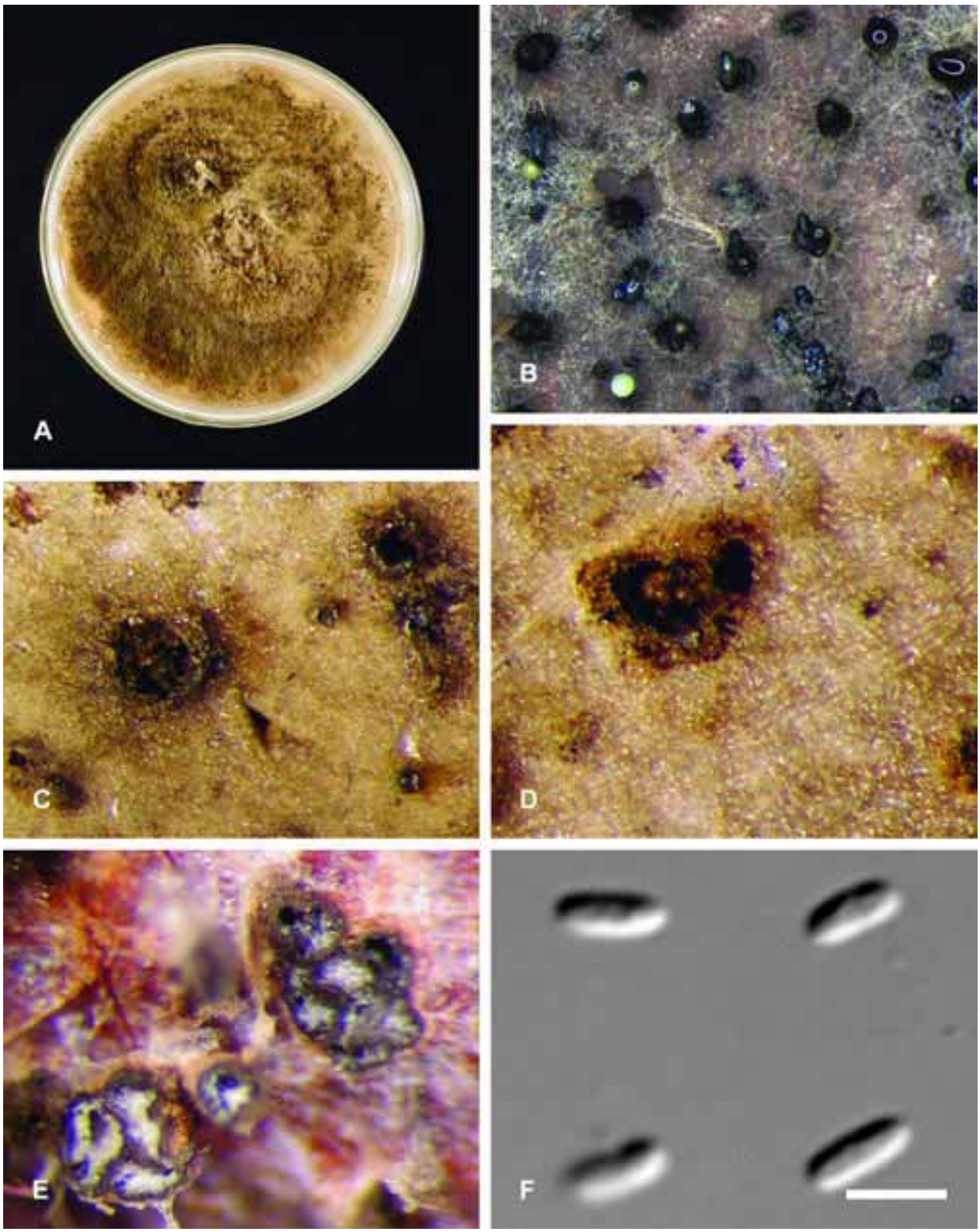

Fig. 64. Culture characteristics of *C. valsoidea*. A. Colony morphology on PDA of isolate CBS116857, Indonesia-4. B. Conidiomata produced on autoclaved *Eucalyptus* leaf. C–E. Tangential sections through conidiomata show simple and divided locules. F. Straight to allantoid conidia. Scale bar: F = 3 µm.

18. *Cytospora nitschkii* G.C. Adams, Jol. Roux & Gezahgne, **sp. nov.** Figs 65–67. MycoBank MB500218.

Etymology: "*nitschkii*" refers to the mycologist T. Nitschke.

Teleomorpha ignota. Stromata conidiomatica in cortice immersa, erumpentia, maximam partem in superficie, circularia, 0.4–0.6 mm diametro, loculis (3–)6(–8) strictis diatrypelloideis, in stromate rubro-brunneo vel fusco-brunneo solido hemisphaerico inclusis praedita. Ostiola fusco-brunnea, curta, non distincta, superificie stromatis superiore plana. Loculi globosi, 100–150 µm diametro, stricti vel laterales, ad typum simplicem, non divisum pertinentes, ostiolo unico curto praediti, radiatim dispositi, non contigui vel compressi, parietibus non communalibus, entostromate substratum valde tingente circumdati. Cellulae conidiogenae in matrice continua gelatinosa inclusae, enteroblastice phialidicae, hyalinae, saepe base ramosae, rosulam phialidum formantes, subcylindricae, in apicem contractae, collarcula minuta praeditae (8–)9.5–14(–16) × 1.5 µm. Conidia hyalina, eguttulata, allantoidea, unica, 4.5–5 × 1–1.5 µm.

Teleomorph unknown. *Conidiomatal stromata* hemispherical, partially immersed in bark, erumpent, torsellioid and lamyelloid. Stromata red-brown to dark brown, mostly on the surfaces, circular, 0.4–0.6 mm diam, (3–)6(–8) upright locules, valsoid to diatrypelloid. *Discs* red-brown to dark brown, massive, hemispherical, of the upper half of stromata, 1–7 ostioles. *Ostioles* dark brown, short, indistinct, converging into the upper stromata, at the same level as the surfaces of stromata (discs). *Locules* globose, 100–150 µm diam, upright to lateral, simple undivided with discrete short ostioles, arranged radially, not crowded or compressed, not sharing common walls, surrounded by entostromata that darkly stain the substrates. *Conidiophores* hyaline, frequently branched at the base, forming a rosette of phialides, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute (8–)9.5–14(–16) × 1.5 µm. *Conidia* hyaline, eguttulate, allantoid, aseptate, 4.5–5.0 × 1–1.5 µm.

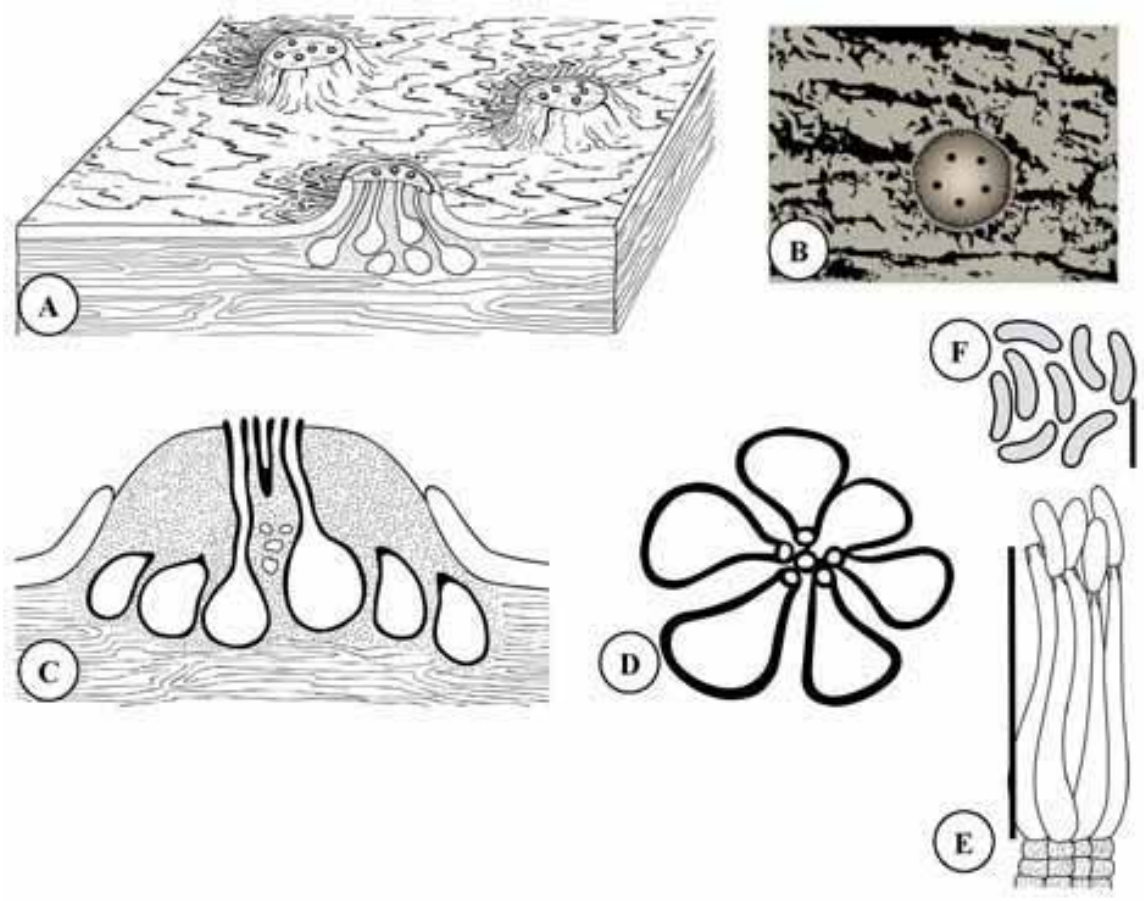

Fig. 65. Illustrations of *C. nitschkii.* A. Habit sketch. B. Ostiolar disc erumpent from bark. C. Longitudinal section through conidiomal stroma in plant. D. Tangential section through conidiomal stroma. E. Conidiophores in hymenium. F. Conidia. Scale bars: E = 13 µm, F = 5 µm.

Cultures: Colony growth on PDA is predominantly pale yellowish brown (Munsell 8.7YR 6.5/5.0) on the surface. Colour of the reverse is moderate yellow (Munsell 3.8Y 7.1/6.5) with the central area dark greyish olive (Munsell 9.7Y 2.0/1.8). Pycnidia form on the agar and exude brown cirrhi. Colony texture is felty, slightly raised with no growth zones. Large globose leucotorsellioid conidiomata with several distinct layers of tissues or walls and discrete necks/beaks form on autoclaved leaves. Conidiomata vary from hemispherical with wrinkled surfaces and discete necks/beaks to flat, imbedded in leaf tissue and without necks/beaks. Internally, the conidiomata have up to 25 regular radially arranged locules in light entostromata surrounded in darker stromatal tissues.

Cardinal temperatures: Colonies obtain a mean growth on PDA at 4 ºC of 9 mm diam, at 25 ºC of 94 mm diam, at 32 ºC of 6 mm diam, and no growth at 37 ºC after 7 d in the dark. Growth at 25 ºC on 2 ppm cycloheximide in V8® agar is 86 % of growth on V8® agar without the antibiotic after 7 d in the dark.

Host: *Eucalyptus saligna*.

Distribution: Only known from the type locality, Wondo Genet, Ethiopia.

Specimens examined: **Ethiopia**, Wondo Genet, Forestry College Compound on dead twigs of *E. saligna*, Sep. 2002, Alemu Gezahgne (MSC 380701, **holotype** of *Cytospora nitschkii*), living ex-type culture CBS 116854; Forestry College Compound on dead twigs of *E. saligna*, Sep. 2002, Alemu Gezahgne (MSC 380699), also living culture CBS 117606.

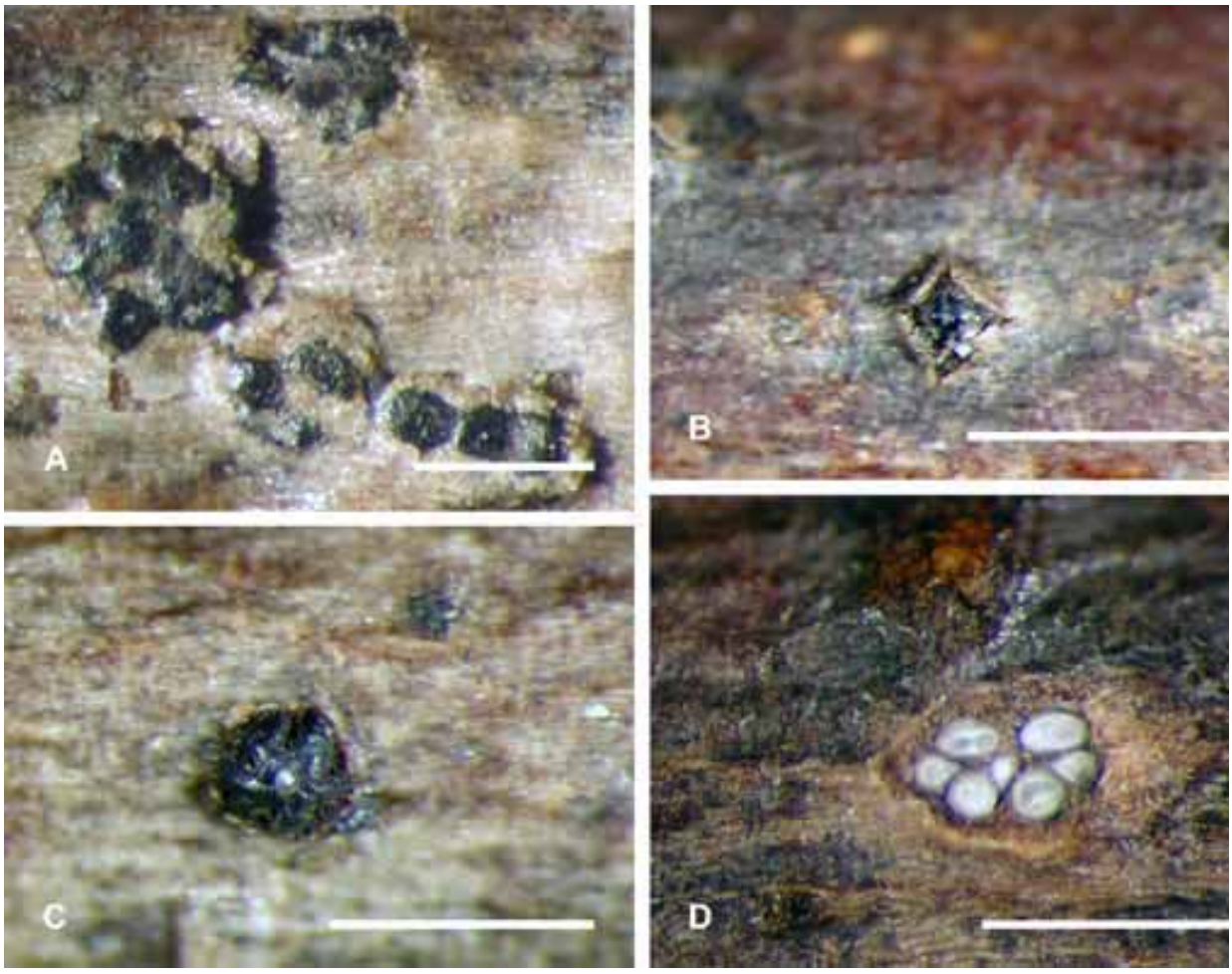

Fig. 66. Morphology of the anamorph *C. nitschkii*. A. Hemispherical conidiomatal stromata on surface of *Eucalyptus* bark have upright locules in a diatrypelloid arrangement. B. Disc of stroma with multiple ostioles and partially erumpent through bark is light coloured and dull. C. Hemispherical disc or stroma with multiple ostioles and fully erumpent from bark is dark coloured and glossy. D. Tangential section through the hemispherical stroma reveals closely arranged locules having separate walls and each being surrounded by stroma. Scale bar: A–D = 1 mm.

Notes: This species is unique in having prominent superficial hemispherical discs that incorporate the upper portion of the stromata, upright locules, and single to multiple ostioles. The massive hemispherical discs uniting multiple ostioles were interpreted as hemispherical stromata that also incorporate the upper parts of the locules. *Cytospora eutypelloidea* had similar stromata but its ITS-rDNA sequences placed it within the *Valsa sordida* group and not homologous with the Ethiopian fungus (G.C. Adams, unpubl. data).

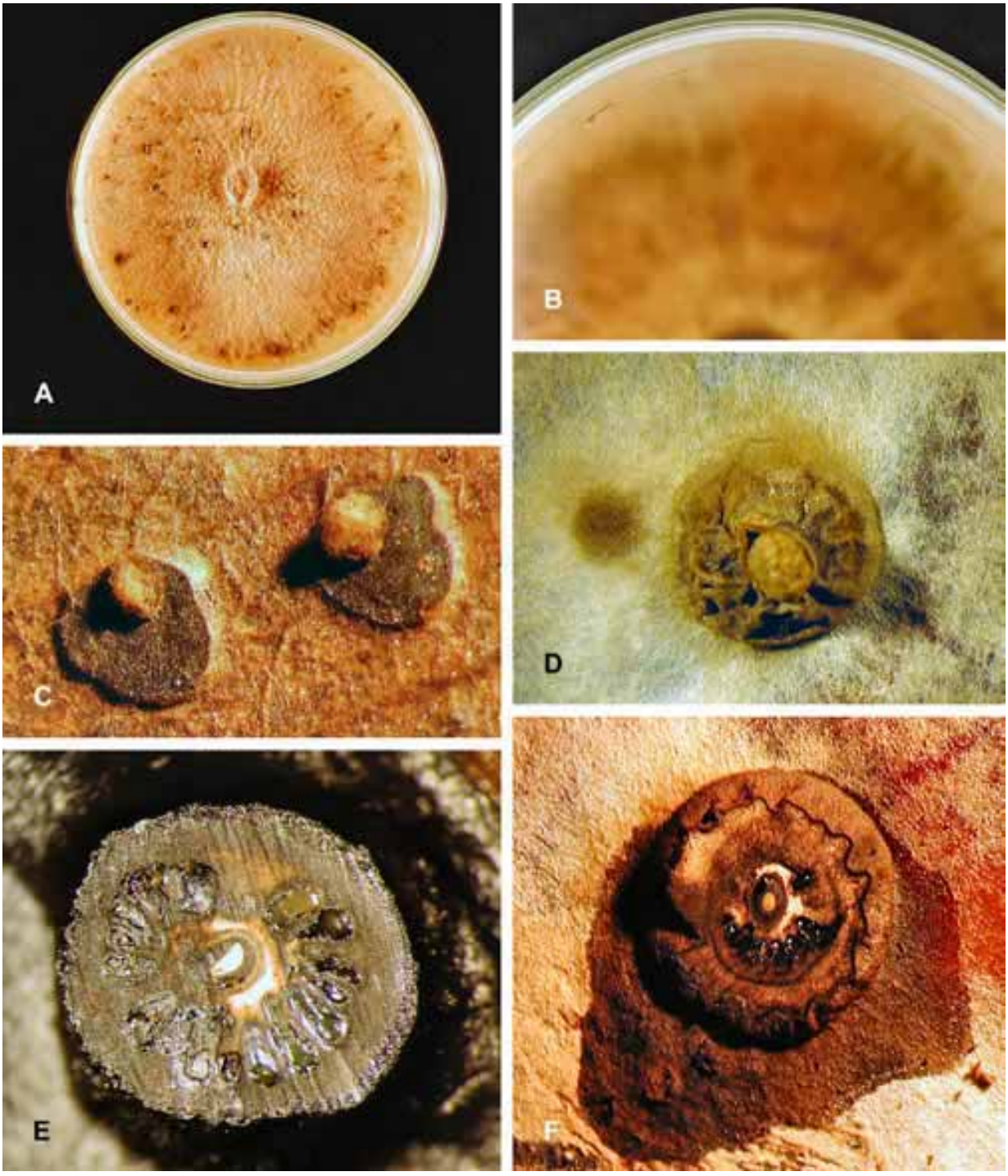

Fig. 67. Culture characteristics of *C. nitschkii*. A–B. Colony morphology on PDA of isolate CBS 116854, Ethiopia-7 and reverse side of Petri dish shows zonal growth. C. Flat conidiomata of CBS 116854 produced on autoclaved *Eucalyptus* leaf. D. Relatively large conidioma of CBS 116854 on autoclaved *Eucalyptus* leaf. E–F. Tangential sections through torsellioid conidiomata show white entostromata surrounding single central ostioles (unlike on host tissue) and multiple locules with independent walls and gelatinous masses of conidia.

19. *Cytospora variostromatica* G.C. Adams & M.J. Wingf., **sp. nov.** Figs 68–70. MycoBank MB500219.

Etymology: "*variostromatica*" refers to the variable morphology of the stroma.

Teleomorpha ignota. Stromata conidiomatica in cortice immersa, erumpentia, mediocriter grisea, circularia vel ovoidea, 0.3–0.7(–1) mm diametro, loculis 1–10 in entostromate infra discum inclusis praedita. Disci fusco-grisei, paene plani, circulares vel lenticulares, 0.1 × 0.1 mm usque ad 0.3 × 0.9 mm diametro, interdum discum multiplicem formantes, furfuracei, e materia amorphica compositi, ostiolis 1–10 praediti. Ostiola fusco-grisea, non supra superficiem disci, entostromate mediocriter griseo circumdata. Loculi globosi, ad typum solitarium non divisum recte compressum et ad typum solitarium multilocellatum per plicas partitum et ad typos multiplices non divisos vulgo in gregibus e duobus compositos, saepe fasciculatos lateraliter compressos pertinentes, parietibus fusco-brunneis e textura haud distinguenda compositis. Conidiophora in matrice continua gelatinosa inclusae, hyalina, 1–4-ramea vel verticillata, phialidibus 4 praedita, 12–15 × 1–1.5 µm. Cellulae conidiogenae enteroblastice phialidicae, subcylindricae, ad apicem contractae, collaretta minuta praeditae, 7–8.5 × 1 µm. Conidia hyalina, eguttulata, allantoidea, unica (4.5–)5(–5.5) × 1 µm.

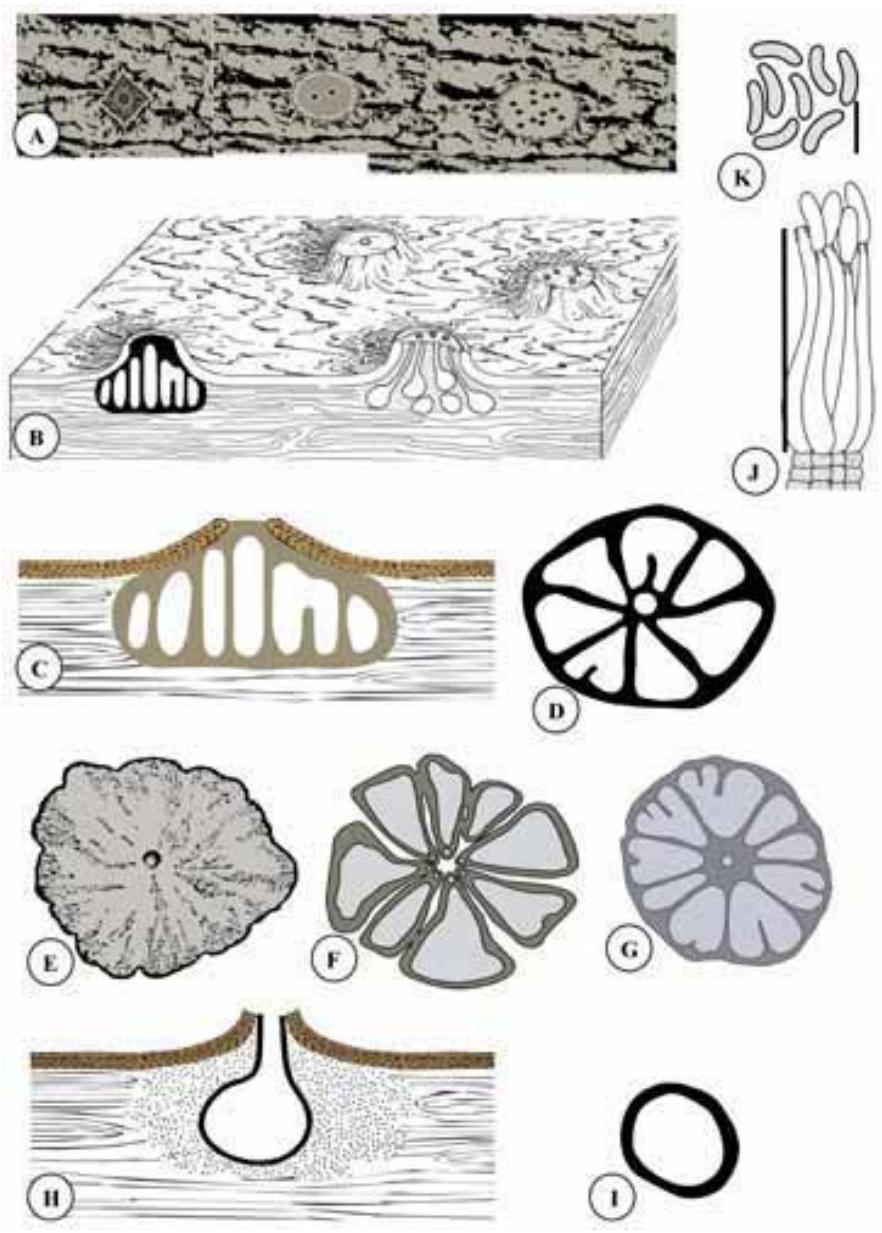

Fig. 68. Illustrations of *C. variostromatica*. A. Ostiolar discs erumpent from bark. B. Habit sketch. C, H. Longitudinal sections through conidiomatal stromata in plant. D–G, I. Tangential sections through conidiomatal stromata. J. Conidiophores in hymenium. K. Conidia. Scale bars: J = 13.5 µm, K = 5 µm.

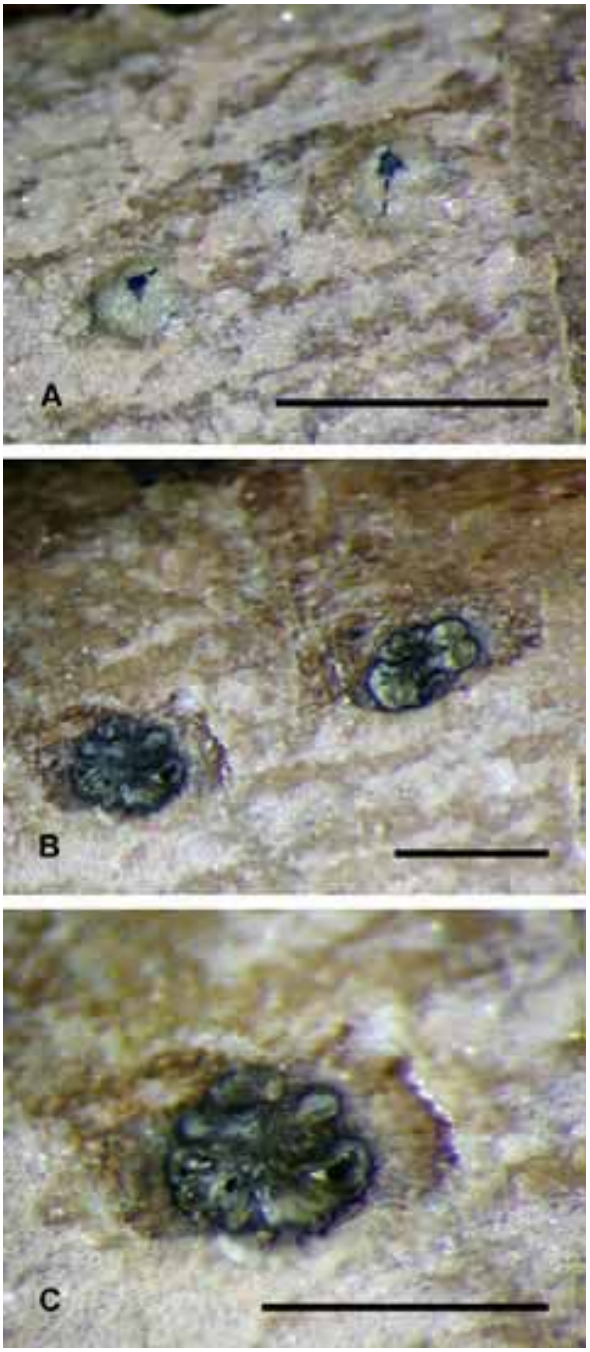

Fig. 69. Morphology of the anamorph *C. variostromatica*. A. Convex discs of conidiomata erupting through *Eucalyptus* bark. B–C. Tangential sections through rosette cytosporoid conidiomatal stromata each with one ostiole and regular radial arrangement of chambers. Scale bars: A–C = 1 mm.

Teleomorph unknown. *Conidiomatal stromata* immersed in bark, erumpent, unilocular, cytosporoid, and lamyelloid, medium grey, circular to ovoid, 0.3–0.7(–1) mm diam, 1–10 locules in entostroma below the discs. *Discs* dark grey, nearly flat, circular to lenticular, 0.1 × 0.1 mm to 0.3 × 0.9 mm diam, occasionally forming compound discs, furfuraceous, of amorphous material, with 1–10 ostioles. *Ostioles* dark grey, not above disc surfaces, surrounded by medium grey entostromata. *Locules* globose, solitary undivided, compressed vertically, solitary multi-chambered subdivided by invaginations, multiple undivided commonly in groups of two, often clustered and compressed laterally, with dark brown walls of indefinable *textura type*. *Conidiophores* hyaline, 1–4 branches, up to verticillate with 4 phialides, 12–15 × 1–1.5 µm, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, 7–8.5 × 1 µm. *Conidia* hyaline, eguttulate, allantoid, aseptate, (4.5–)5(–5.5) × 1 µm.

Cultures: Colony growth on PDA is predominantly yellowish white to pale yellowish brown (Munsell 4.5 Y 9.2/1.2, 8.7Y 7.1/6.5) on the surface. Colour of the reverse is pale yellow (Munsell 4.7Y 9.0/3.8) with a greyish olive edge (Munsell 8.0Y 3.6/2.0). Pycnidia do not form on the agar. Colony texture is felty, slightly raised with no growth zones. Hemispherical rosette cytosporoid conidiomata with locules divided into 8–10 regular, radially arranged chambers and no necks/beaks form on autoclaved leaves. The conidiomata are generally produced under the epidermis of the leaf.

Cardinal temperatures: Colonies (mean of 4 isolates) obtain a mean growth on PDA at 4 ºC of 11.5 mm diam, at 25 ºC of 80.5 mm diam, at 32 ºC of 86.5 mm diam, and at 37 ºC of 0–5 mm diam after 7 d in the dark. Growth at 25 ºC on 2 ppm cycloheximide in V8® agar is 66 % of growth on V8® agar without the antibiotic after 7 d in the dark.

Host: *Eucalyptus globulus*.

Distribution: Orbost, Victoria, and Kyoge, New South Wales, Australia.

Specimens examined: **Australia**, Victoria, Orbost, Toslaree on dead branches of *E. globulus*, Sep. 2000, M.J. Wingfield (MSC 380695, **holotype** of *Cytospora variostromatica*), living ex-type culture CBS 116858; Orbost, Toslaree on dead branches of *E. globulus*, Sep. 2000, M.J. Wingfield (MSC 380696).

Notes: Conidiomatal stromata are unique in being unilocular, cytosporoid and lamyelloid with ostioles converging to a shared disc. Conidiophores are usually branched and occasionally verticillate with four phialides.

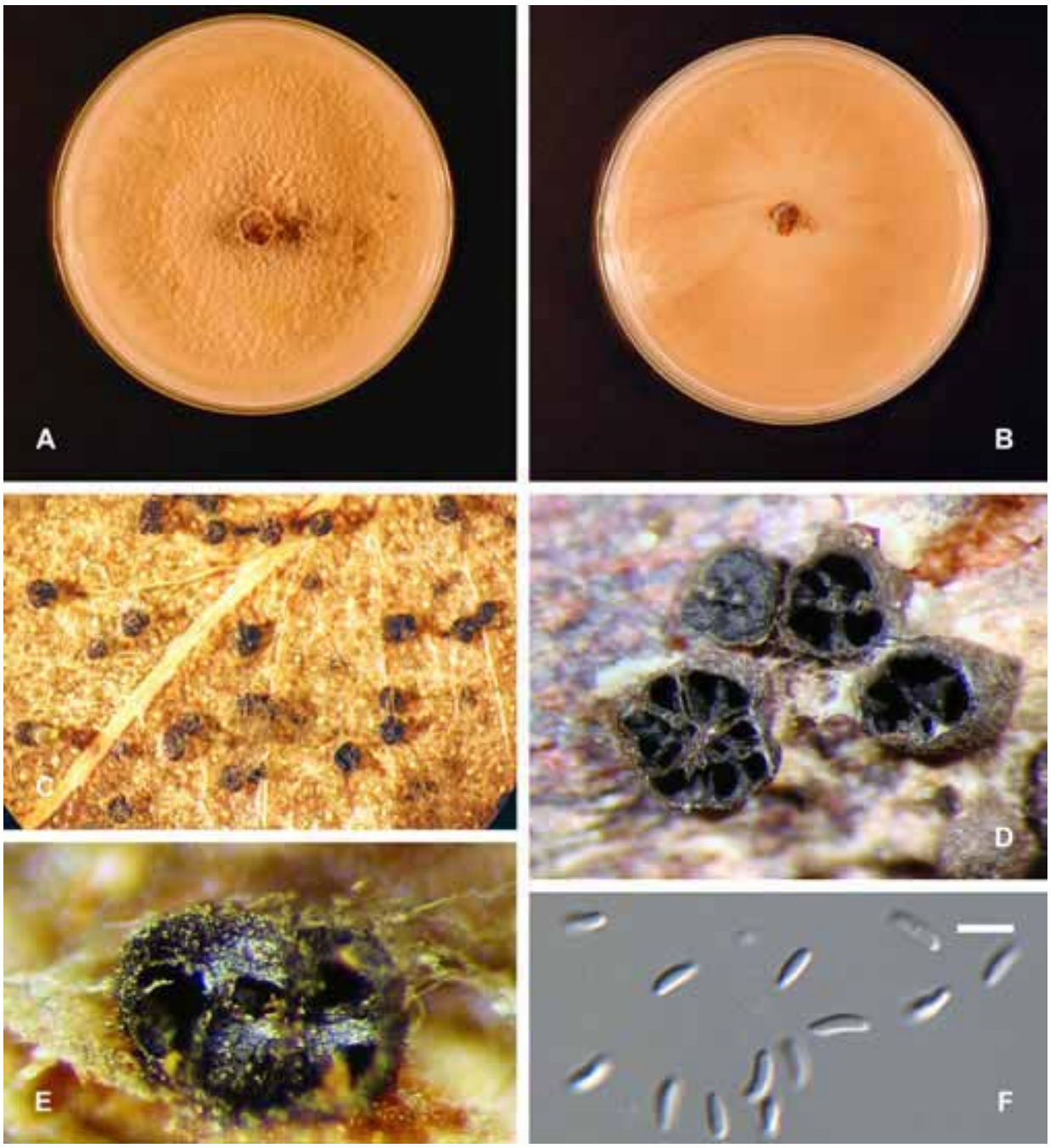

Fig. 70. Culture characteristics of *C. variostromatica*. A–B. Colony morphology on PDA of isolate CBS 116858, Australia-6 (left) and isolate CBS 116860, SouthAfrica-1 (right). C. Conidiomata produced on autoclaved *Eucalyptus* leaf. D–E. Tangential sections through conidiomata show divided locules. F. Allantoid conidia. Scale bars: F = 5 µm.

20. *Cytospora disciformis* G.C. Adams & M.J. Wingf., **sp. nov.** Figs 71–73. MycoBank MB500220.

Etymology: "*disciformis*" refers to the regular disc shaped circular form of the conidioma.

Teleomorpha ignota. Stromata conidiomatica in cortice immersa, erumpentia, circularia, discoidea, 0.45–0.7 mm diametro, ostiolo unico fusco-brunneo praedita. Disci absentes. Loculi ad typum plurilocularem vel multilocellatum pertinentes, pertiti per plicas in cavernulas regulares radiatim dispositas parietibus communalibus fusco-brunneis e textura epidermoidea compositis crassitudine cellulari 3–4-stratosis praeditas. Cellulae conidiogenae in matrice continua gelatinosa inclusae, simplices vel interdum ad basin ramosae, hyalinae, enteroblastice phialidicae, subcylindricae, in apicem contractae, collaretta minuta praeditae, 6–8 × 1 µm. Conidia hyalina, eguttulata, allantoidea, unica 3–3.5 × 0.7 µm.

Teleomorph unknown. *Conidiomatal stromata* immersed in bark, erumpent, rosette to labyrinthine cytosporoid, circular, discoid, 0.45–0.7 mm diam, with discrete dark brown ostioles. *Discs* absent. *Locules* rosette to labyrintine multi-chambered, subdivided by invaginations into regular radially arranged chambers sharing common walls, walls dark brown, *textura epidermoidea*, 3–4 cell layers thick. *Conidiophores* hyaline, unbranched or occasionally branched at the base, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, with minute collarettes, 6–8 × 1 µm. *Conidia* hyaline, eguttulate, allantoid, aseptate 3–3.5 × 0.7 µm.

Cultures: Colony growth on PDA is predominantly greyish olive becoming dark greyish olive (Munsell 8.0Y 3.6/2.0, 9.7Y 2.0/1.8) on the surface. Colour of the reverse is greyish greenish yellow to olive-black (Munsell 9.0Y 7.2/3.9, 9.0Y 1.1/0.0). Pycnidia form on the agar and exude orange cirrhi. Colony texture is felty, slightly raised with irregular marigns. Unilocular to rosette cytosporoid conidiomata with locules divided by few invaginations into 3–6 chambers and long necks/beaks form on autoclaved leaves. A light covering of loose hyphae from the surrounding colony mycelium often obscures the dark globes of the conidiomata.

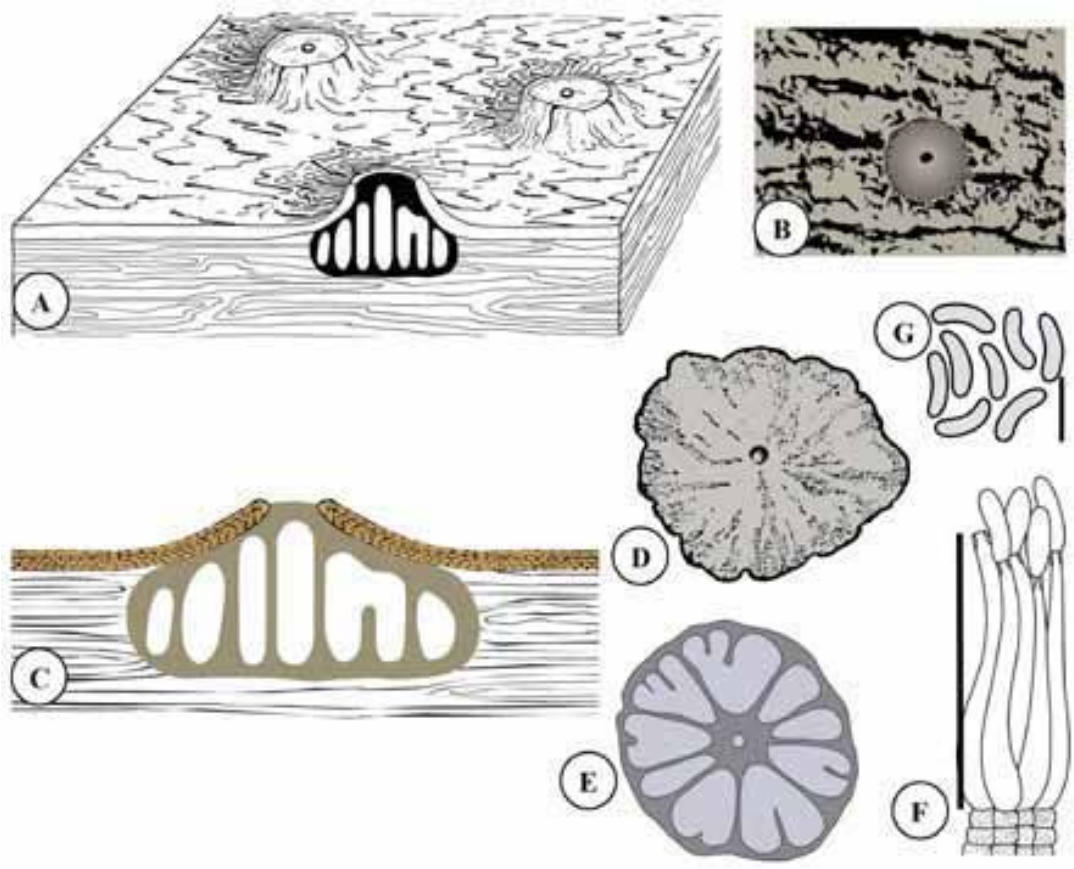

Fig. 71. Illustrations of *C. disciformis*. A. Habit sketch. B. Ostiolar disc erumpent from bark. C. Longitudinal section through conidiomal stroma in plant. D. Top view of conidioma isolated from plant tissues. E. Horizontal cross section through conidiomal stroma. F. Conidiophores in hymenium. G. Conidia. Scale bars: F = 13.5 µm, G = 3.2 µm.

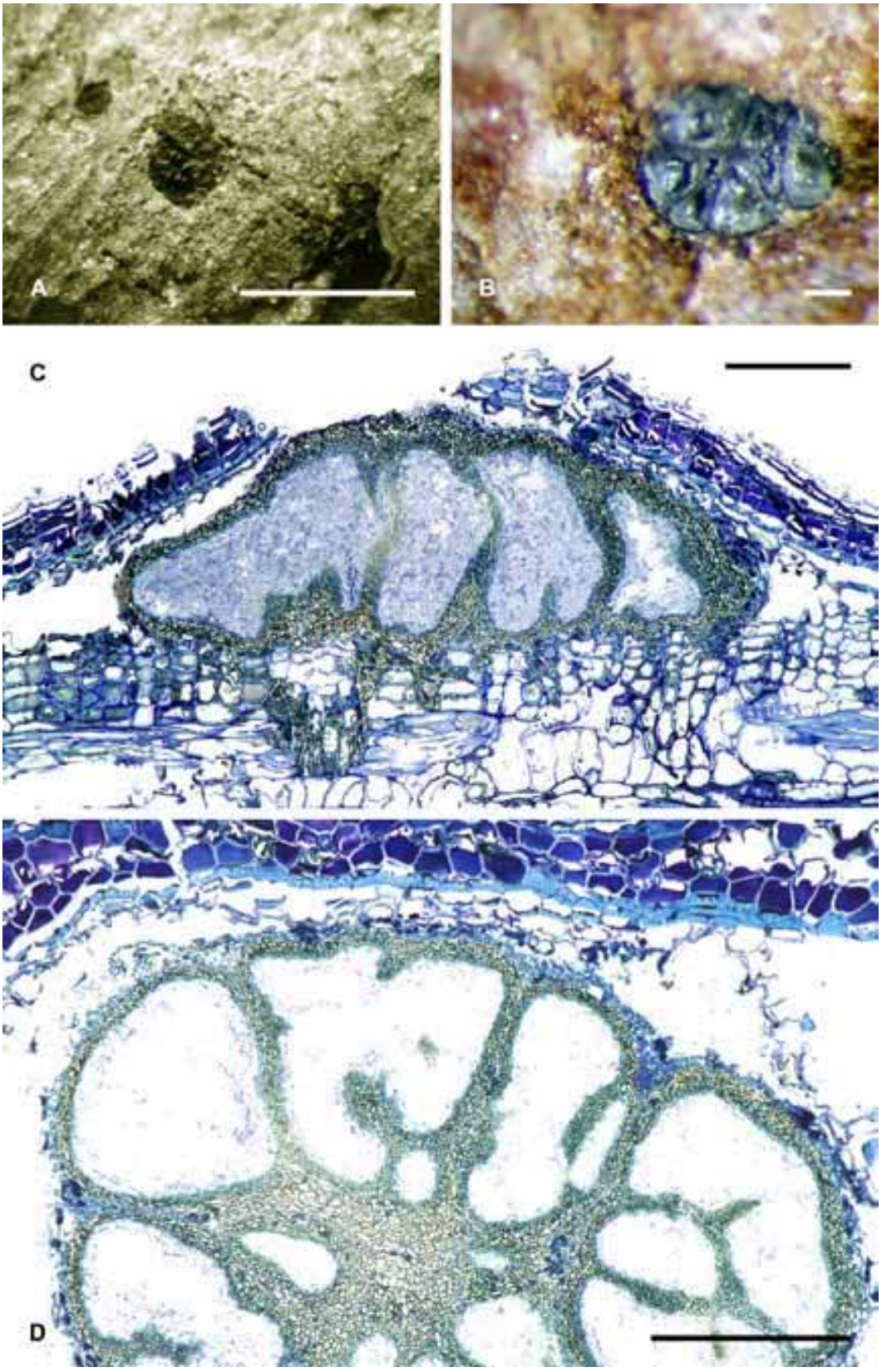

Fig. 72. Morphology of the anamorph *C. disciformis*. A. Dark shiny convex hemispherical upper surface of conidioma on *Eucalyptus* bark. B. Tangential section through the rosette cytosporoid conidioma revealing regular radially arranged chambers, the rosette form. C. Longitudinal microtome section through conidioma shows structure of chambers, partial invaginations, and shared walls. D. Tangential microtome section of conidioma shows structure of invaginations and radially arranged chambers with shared walls. Scale bars: A = 1 mm, B–D = 100 µm.

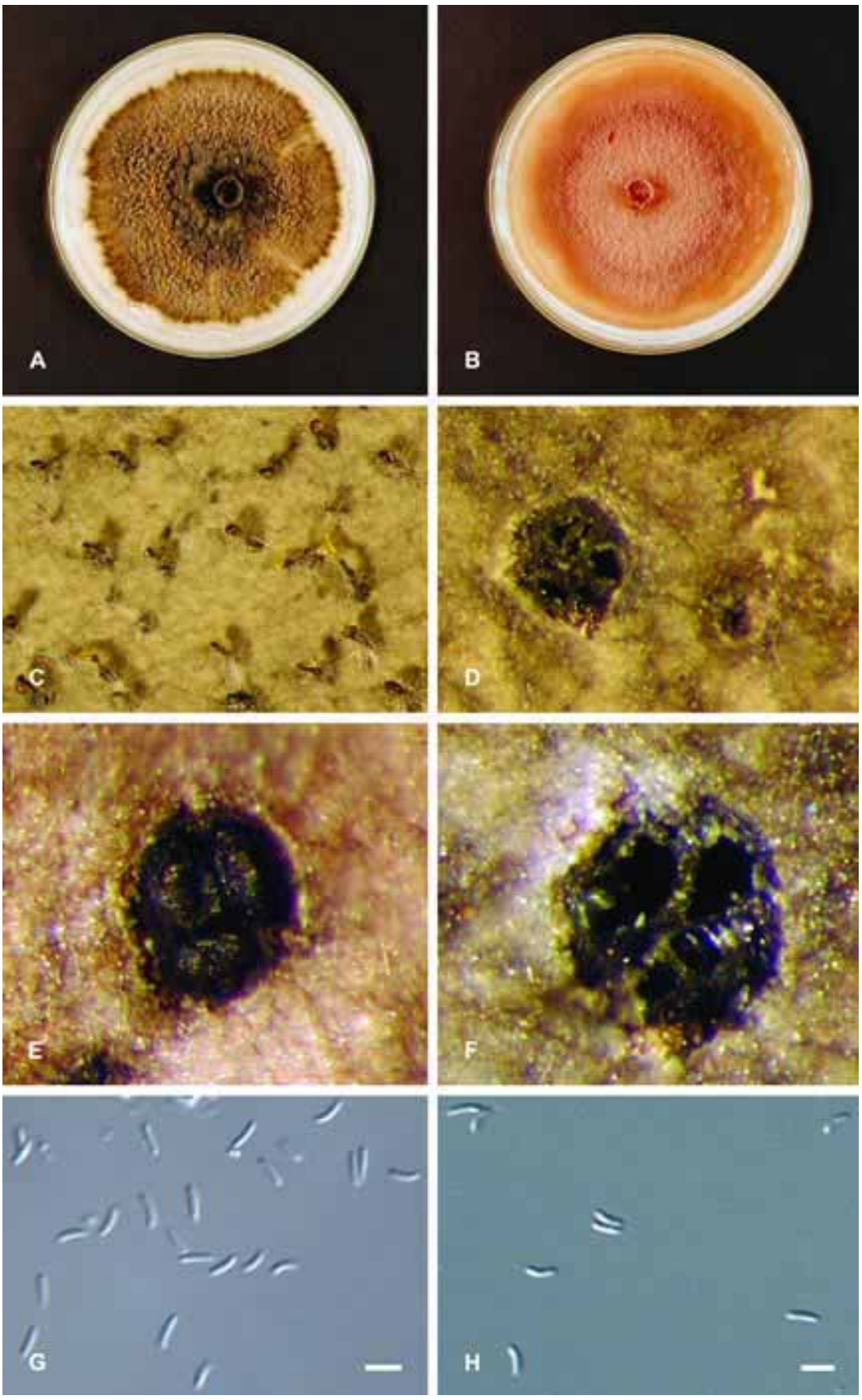

Fig. 73. Culture characteristics of *C. disciformis*. A–B. Colony morphology on PDA of isolate CBS 116827, Uruguay-1 (left) and isolate CBS 116828, Australia-2 (right). C. Conidiomata produced on autoclaved *Eucalyptus* leaf. D–F. Tangential sections through conidiomata show divided locules. G–H. Allantoid conidia. Scale bars: G–H = 3.5 µm.

Cardinal temperatures: Colonies (mean of 3 isolates) obtain a mean growth on PDA at 4 ºC of 17 mm diam, at 25 ºC of 60 mm diam, at 32 ºC of 47 mm diam, and at 37 ºC of 0–3 mm diam after 7 d in the dark. Colonies (mean of 3 isolates) obtain a mean growth on Leonian's at 25 ºC of 168 mm diam after 7 d in the dark. No growth at 25 ºC occurs on 2 ppm cycloheximide in V8® agar after 7 d in the dark.

Host: *Eucalyptus globulus*.

Distribution: Canberra, ACT Australia, Los Ceibos, Uruguay.

Specimens examined: **Uruguay**, Los Ceibos, on dead branch of *E. globulus*, Jun. 1999, M.J. Wingfield (MSC 368323, **holotype** of *Cytospora disciformis*), living ex-type culture CBS 116827; **Australia**, ACT, Canberra, from dead branch of *E. globulus*, 2001, M.J. Wingfield, living cultures CBS 116828 and CBS 118083.

Notes: The stromata of this *Cytospora* are circular, with chambers that are especially regular in shape, with a lack of compression among the chambers, and with regular occurrence of many invaginations of the wall that are short, incomplete and extend less than half way across a chamber.

21. *Cytospora austromontana* G.C. Adams & M.J. Wingf., **sp. nov.** Figs 74–76. MycoBank MB500221.

Etymology: "*austromontana*" means southern mountains and is in reference to the Snowy Mountain resort region of Perisher Valley in New South Wales, Australia in the Southern Hemisphere where the species was found and where it may be indigenous.

Teleomorpha ignota. Stromata conidiomatica in cortice immersa, erumpentia, mediocriter grisea, circularia vel discoidea, 0.3–0.9 mm diametro. Disci saepe inconspicui vel absentes, convexi, circulares vel lenticulares, 0.25–0.6 mm diametro, ostiolo unico praediti. Ostiola mediocriter grisea, non supra superficiem corticis, 135–160 µm diametro. Loculi ad typum plurilocularem pertinentes, per plicas in cavernulas regulares radiatim dispositas parietibus communalibus praeditas partiti. Cellulae conidiogenae in matrice continua gelatinosa inclusae, hyalinae, enteroblastice phialidicae, ad basin vel interdum supra basin ramosae, subcylindricae, in apicem contractae, collaretta minuta praeditae (7–)9(–12) × 1.5 µm. Partes hymeniales inter cellulas conidiogenas interspersae, subcylindricae, elongatae, inflatae, 33–40 × 5–6 µm. Conidia hyalina, eguttulata, elongata, allantoidea, unica (5.2–)6 × 1 µm.

Teleomorph unknown. *Conidiomatal stromata* immersed in bark, erumpent, rosette cytosporoid, medium grey, circular to discoid, 0.3–0.9 mm diam.

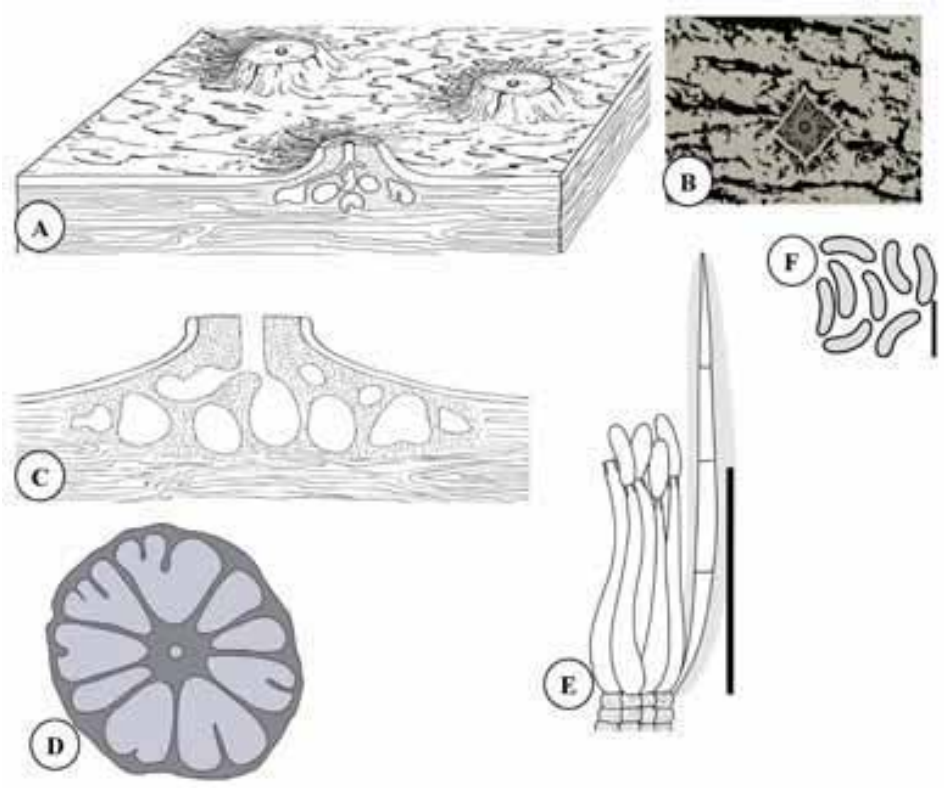

Fig. 74. Illustrations of *C. austromontana*. A. Habit sketch. B. Ostiolar disc erumpent from bark. C. Longitudinal section through conidiomal stroma in plant. D. Horizontal cross section through conidiomal stroma. E. Conidiophores and gelatinous filament in hymenium. F. Conidia. Scale bars: E = 9 µm, F = 6 µm.

Discs often inconspicuous or absent, convex, circular to lenticular, 0.25–0.6 mm diam, with discrete ostioles. *Ostioles* medium grey, not above the bark surfaces, 135–160 µm diam. *Locules* subdivided by invaginations into regular radially arranged chambers sharing common walls. *Conidiophores* hyaline, branched at bases, occasionally branched above the base, embedded in a continous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute (7–)9(–12) × 1.5 µm. *Hymenial elements* interspersed amongst the conidiogenous cells, thin-walled, filamentous, with thick gelatinous coating, 33–40 × 5–6 µm inclusive, or 33–40 × 1.5 µm exclusive, of gelatinous coating. *Conidia* hyaline, eguttulate, elongate-allantoid, aseptate, (5.2–)6 × 1 µm.

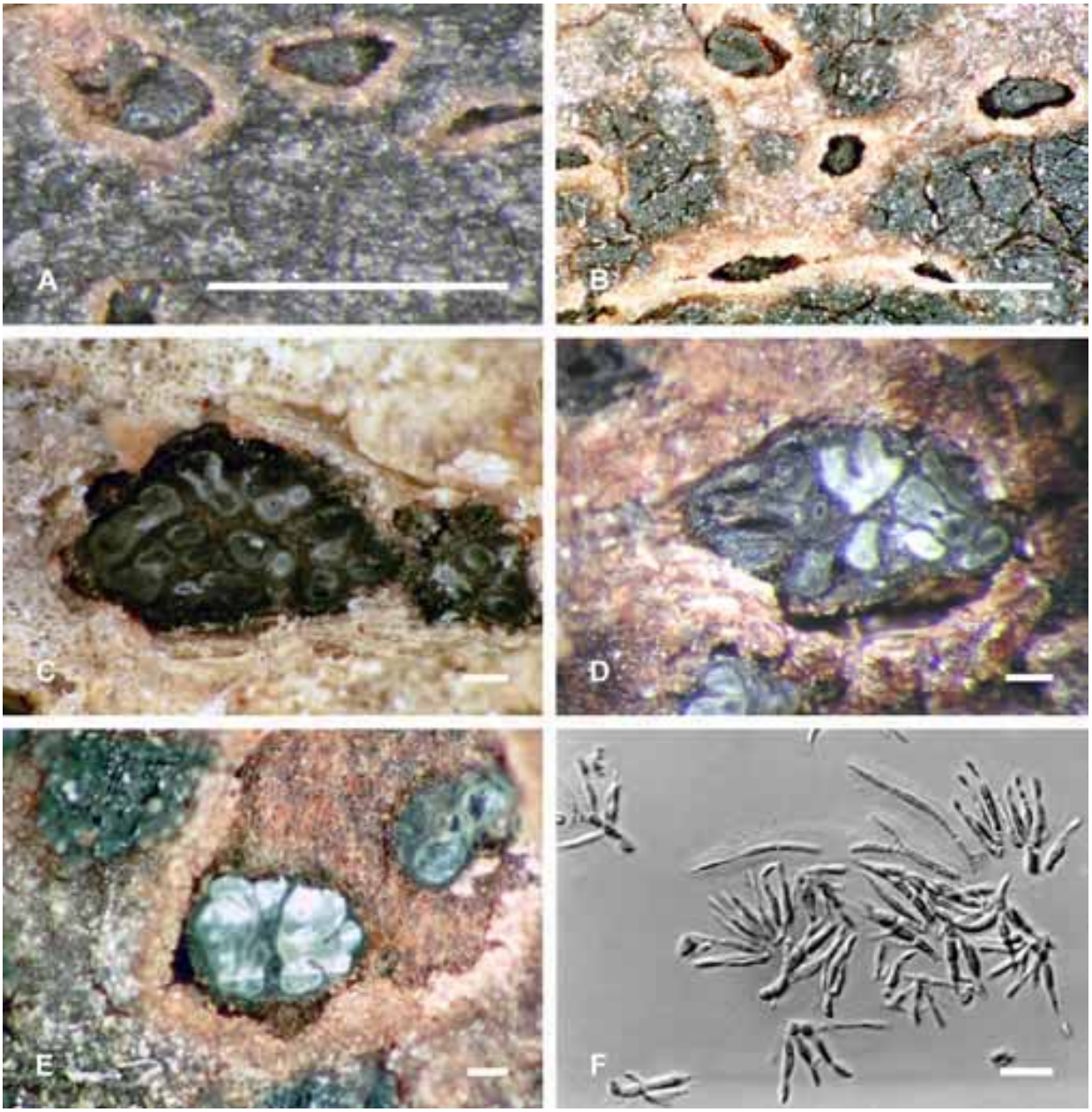

Fig. 75. Morphology of the anamorph *C. austromontana*. A–B. Flat upper surface of conidiomata, each with a discrete ostiole, on surface of *Eucalyptus* bark. C–E. Tangential sections through three conidiomata show rosette cytosporoid structure with radially arranged chambers and discrete ostioles. F. Sterile thin walled filamentous elements with thick gelatinous coatings in hymenium among whorls of conidiogenous cells arising from discrete basal cells. Under phase contrast the sterile elements look inflated due to the gelatinous coating and superficially resemble subcylindrical immature asci. Scale bars: A–B = 1 mm, C–E =100 µm, F = 10 µm.

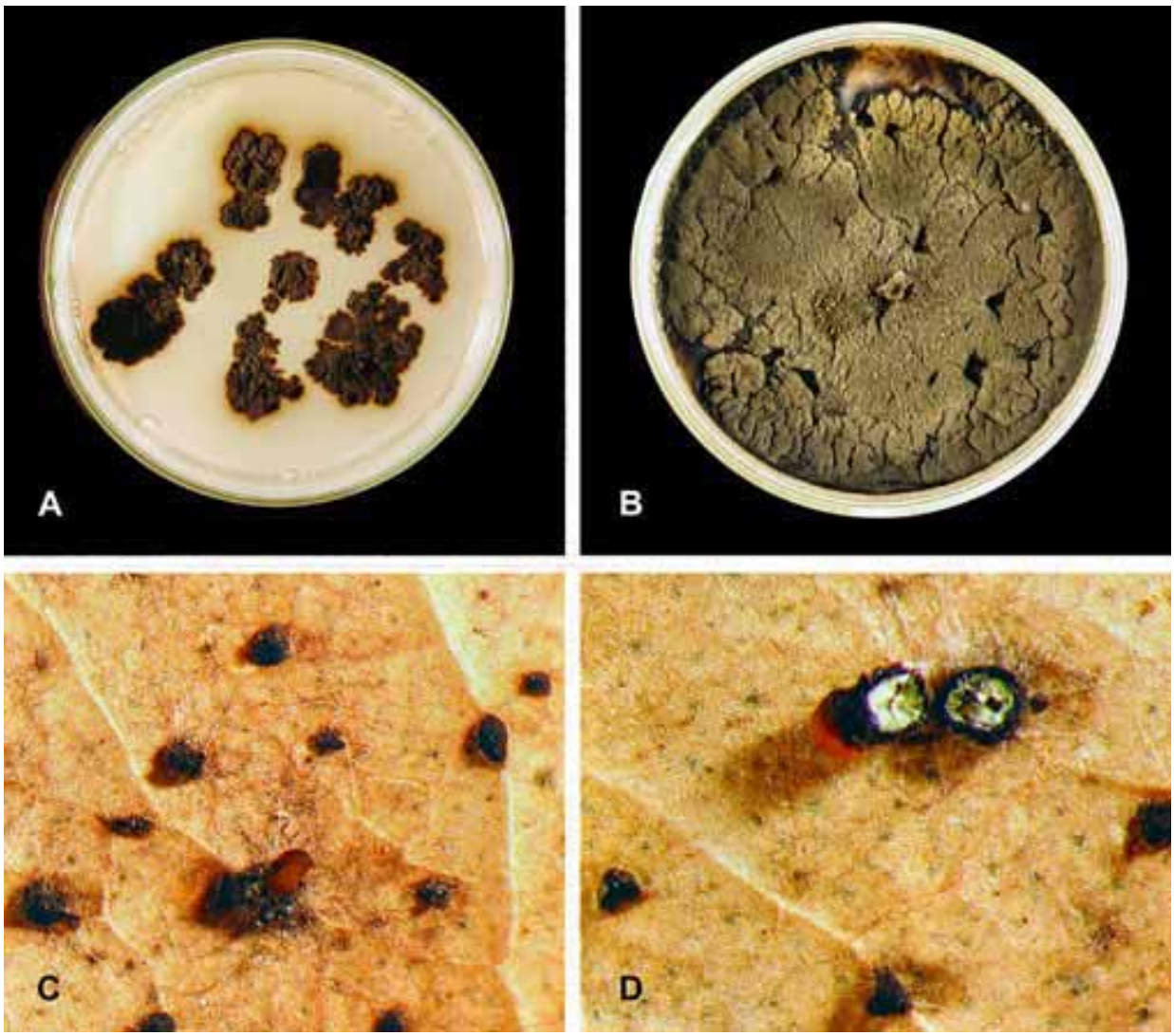

Fig. 76. Culture characteristics of *C. austromontana*. A–B. Colony morphology of isolate CBS 116820, Australia-4 on PDA (left, multiple inoculation sites) and on oatmeal agar (right, single inoculation site). C. Conidiomata produced on autoclaved *Eucalyptus* leaf. D. Tangential section through conidioma shows simple locule.

Cultures: Colony growth on PDA is predominantly olive-black (Munsell 9.0Y 1.1/0.0) on the surface. Colour of the reverse is olive-black (Munsell 9.0Y 1.1/0.0). Pycnidia form on the agar and exude pale yellow cirrhi. Colony texture is felty, slightly raised with faint growth zones. Unilocular conidiomata with locules occasionally divided by one to few invaginations and with or without necks/beaks form on autoclaved leaves.

Cardinal temperatures: Colonies grew slowly and obtained a mean growth on PDA at 25 ºC of 3.8 mm diam after 7 d in the dark. However, colony growth on oatmeal agar was faster at 25 ºC reaching 29 mm diam, but with no growth at 37 ºC, after 7 d in the dark. The available isolates of *C. austromontana* could be degenerating.

Host: *Eucalyptus pauciflora*.

Distribution: Only known from the type locality, Perisher Valley, New South Wales, Australia.

Specimens examined: **Australia**, New South Wales, Perisher Valley, on dead cankered branch of *E. pauciflora*, 2001, M.J. Wingfield (MSC 380693, **holotype** of *Cytospora austromontana*), living ex-type culture CBS 116820; New South Wales, Perisher Valley, on dead cankered branch of *E. pauciflora*, 2001, M.J. Wingfield (MSC 380694), living culture CBS 116821.

Notes: Hymenial elements that are filamentous, septate and surrounded in thick gelatinous sheaths occur in both collections of this species. Similar hymenial elements but without gelatinous sheaths have been seen in one specimen of *C. eucalypticola*, and were described for *C. exigua* by Gvritishvili (1969), and the anamorph of *V. eucalypti* (as *L. sequoiae*) by L. Bonar (1928).

22. *Cytospora* aff. ***austromontana*** Fig. 77.

Teleomorph unknown. *Conidiomatal stromata* unavailable on natural substrate; globose, glabrose, 0.4 mm diam *in vitro*. *Locules* cytosporoid, subdivided by invaginations into chambers sharing common walls. *Conidiophores* unbranched to branched at the base. *Conidiogenous cells* embedded in a continuous gelatinous matrix, enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, 6–10 × 1 µm. *Conidia* hyaline, eguttulate, allantoid, aseptate, (3–)4–5 × 0.8–0.9 µm.

Cultures: Colony growth on PDA is predominantly pale greyish yellow with a dark edge of olive-brown (Munsell 8Y 8.0/2.5, 6Y 4.6/5.4) on the surface. Colour of the reverse is olive-grey with an edge of strong brown bleeding toward the centre (Munsell 5.0/0.4, 10YR -/3.0/4.4). Pycnidia abundant and exude pale yellow cirrhi. Colony sectors readily, texture is felty, slightly raised with growth zones. Unilocular to reduced cytosporoid conidiomata with locules divided by one to few invaginations and long necks/beaks form on autoclaved leaves. The dark surfaces of conidiomata are lightly covered in loose white hyphae from the surrounding colony mycelium.

Cardinal temperatures: Colonies obtain a mean growth on PDA at 4 ºC of 3 mm diam, at 25 ºC of 61.5 mm diam, at 32 ºC of 220 mm diam, and no growth at 37 ºC after 7 d in the dark. No growth at 25 ºC occurs on 2 ppm cycloheximide in V8® agar or in Leonian's agar after 7 d in the dark.

Host: *Eucalyptus* sp.

Distribution: Only known from the Cape Province of South Africa.

Specimen examined: **South Africa**, Cape Province, Hermanus, on dead twigs of *Eucalyptus* sp., *ca* 1995, C. Roux (culture PPRI 5926), living culture CBS 116822.

Notes: This living culture was inferred to be phylogenetically related to *C. austromonata*, but it differed from that species in growing vigorously on PDA. Conidiomata on natural material are required for further morphological comparisons.

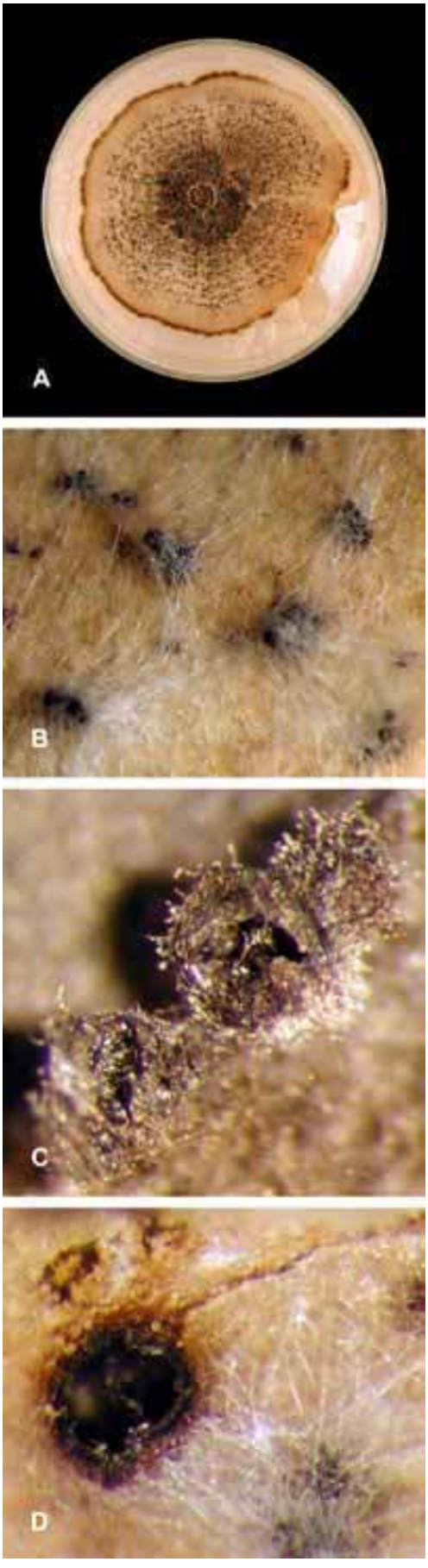

Fig. 77. Culture characteristics of *Cytospora* aff. *austromontana*. A, Colony morphology on PDA of isolate CBS 116822, SouthAfrica-7. B. Conidiomata produced on autoclaved *Eucalyptus* leaf. C–D. Tangential sections through conidiomata with invaginations dividing locules into several chambers.

23. *Cytospora berkeleyi* G.C. Adams, **sp. nov.** Figs 78–80. MycoBank MB500222.

Etymology: "*berkeleyi*" refers to the mycologist Miles Joseph Berkeley.

Teleomorpha ignota. Stromata conidiomatica in cortice immersa, erumpentia, atro-grisea vel mediocriter grisea, circularia vel ovoidea, 0.2–0.65 × 0.2–0.65 mm diametro. Disci fusco-grisei, circulares, plani, ostiolo unico praediti. Ostiola fusco-grisea, furfuracea, e materia amorphica composita, 60–120 µm diametro, non supra superficiem disci. Loculi ad typum simplicem, non divisum pertinentes, 4–7 regulares radiatim dispositi, arcte contigui, parietibus non communalibus, in ostiolo unico convergentibus. Conidiophora in matrice continua gelatinosa inclusa, hyalina, ad basim ramosa, interdum prope altitudinem mediam ramos 1(–3) formantia, 12–14.5 × 2 µm. Cellulae conidiogenae enteroblastice phialidicae, subcylindricae, ad apicem contractae, collaretta minuta partibusque subincrassatis periclinis praeditae, 7–10 × 1.5 µm. Conidia hyalina, eguttulata, allantoidea, unica, 4–4.5 × 1 µm.

Teleomorph unknown. *Conidiomatal stromata* immersed in bark, erumpent, rosette cytosporoid and torsellioid, dark grey to medium grey, circular to ovoid, 0.2–0.65 × 0.2–0.65 mm diam. *Discs* dark grey, circular, flat, with discrete ostioles. *Ostioles* dark grey, furfuraceous, formed of amorphous material, 60–120 µm diam, not above the disc surfaces. *Locules* simple, undivided, not sharing common walls, divided and sharing common walls and combinations having both adjacent shared walls and independent walls, 4–7 regular, radially arranged locules, converging into discrete shared ostioles. *Conidiophores* hyaline, branched at the base, occasionally 1(–3) branches near mid-height, 12–14.5 × 2 µm, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, periclinal thickenings minute, 7–10 × 1.5 µm. *Conidia* hyaline, eguttulate, allantoid, aseptate 4–4.5 × 1 µm.

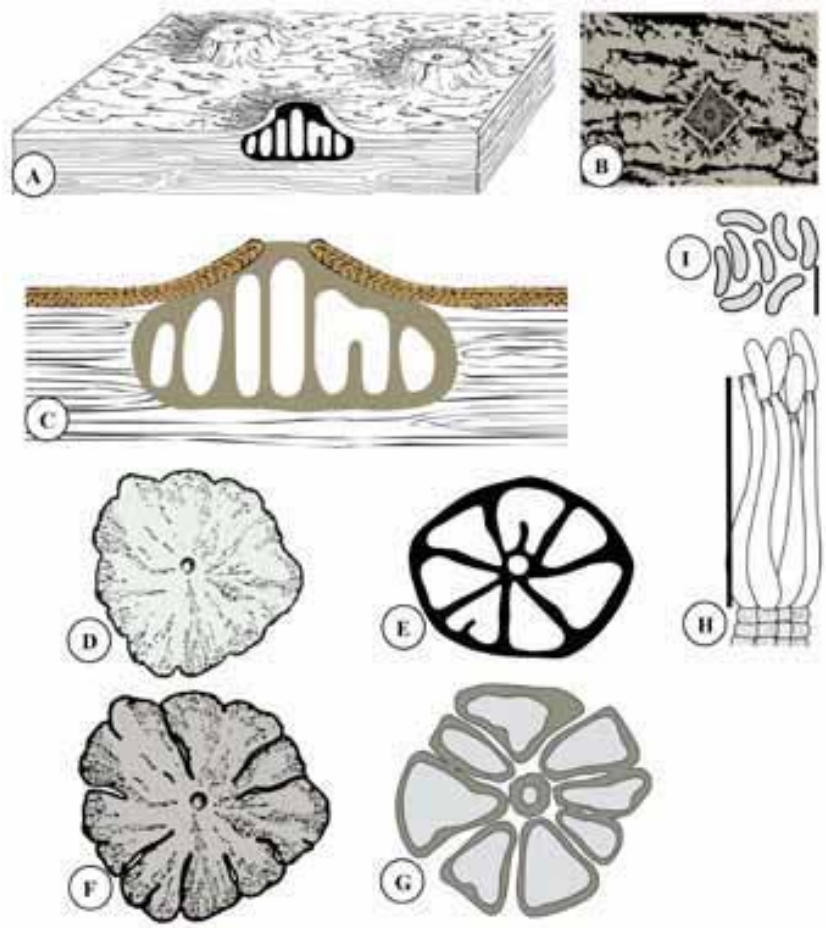

Fig. 78. Illustrations of *C. berkeleyi*. A. Habit sketch. B. Ostiolar disc erumpent from bark. C. Longitudinal section through conidiomal stroma in plant. D. Top view of one type of conidioma isolated from plant tissues. E. Horizontal cross section through conidiomal stroma. F. Top view of another type of conidioma isolated from plant tissues. G. Horizontal cross section through the second conidiomal stroma. H. Conidiophores in hymenium. I. Conidia. Scale bars: H = 13 µm, I = 4.5 µm.

Fig. 79. Morphology of the anamorph *C. berkeleyi*. A. Two flat discs with discrete ostioles and a sectioned rosette cytosporoid conidioma on *Eucalyptus* bark. B. Tangential section through torsellioid conidioma shows regular radially arranged locules, each with independent walls, and ostioles converging toward a shared central ostiole. C–D. Tangential sections through small conidiomata that are difficult to interpret in regard to whether the locules have independent walls or shared walls. Scale bars: A–B = 500 μm, C–D = 100 μm.

Cultures: Colony growth on PDA is predominantly greyish yellowish brown to olive-grey (Munsell 9.5YR 4.6/2.1, 8.1Y 3.5/0.9) with a hyaline edge at the surface. Colour of the reverse is greyish olive to olive-black (Munsell 8.0Y 3.6/2.0) with a hyaline edge. Colony texture is felty, slightly raised with no growth zones. Small shiny glabrose hemispherical rosette cytosporoid conidiomata with 6–8 regular radially arranged chambers and short indistinct necks/beaks form on autoclaved leaves. The surfaces of conidiomata have radially arranged linear grooves that outline each chamber within the locules.

Cardinal temperatures: Colonies obtain a mean growth on PDA at 4 °C of 9 mm diam, at 25 °C of 54 mm diam, at 32 °C of 5 mm diam, and no growth at 37 °C after 7 d in the dark. Colonies obtain a mean growth on Leonian's at 25 °C of 80 mm diam after 7 d in the dark. No growth at 25 °C occurs on 2 ppm cycloheximide in V8® agar or Leonian's agar after 7 d in the dark.

Hosts: *Eucalyptus globulus*, *E. paniculata*.

Distribution: Berkeley and Palo Alto, California, U.S.A.

Specimens examined: **U.S.A.**, California, Palo Alto campus of Stanford University on dead cankered branches of *E. globulus*, 12 Jun. 2001, G.C. Adams (MSC 380710 **holotype** of *Cytospora berkeleyi*, living ex-type culture CBS 116823, and MSC 380711, also living culture CBS 117005). Berkeley campus of University of California on dead twigs of *E. globulus*, 8 Jun. 2001, G.C. Adams & H. Hallen (MSC 380709, MSC 380712), also living cultures CBS 116824 and CBS 117005, respectively; Alameda County in hills above Berkeley on dead twigs of *E. paniculata*, 28 Jan. 1923, L. Bonar (UC 14378).

Notes: This species is inconspicuous, often small relative to other species, and has a dark grey flat disc of amorphous material. It was difficult to interpret the locule type in collected specimens and in culture. In both instances, the circular conidioma had regular, radially arranged chambers that appeared to share walls (rosette cytosporoid). However, under closer examination a "shared wall" occasionally was of two adhering, parallel walls (rosette torsellioid). A conidioma of both rosette cytosporoid and torsellioid features is common in this fungus.

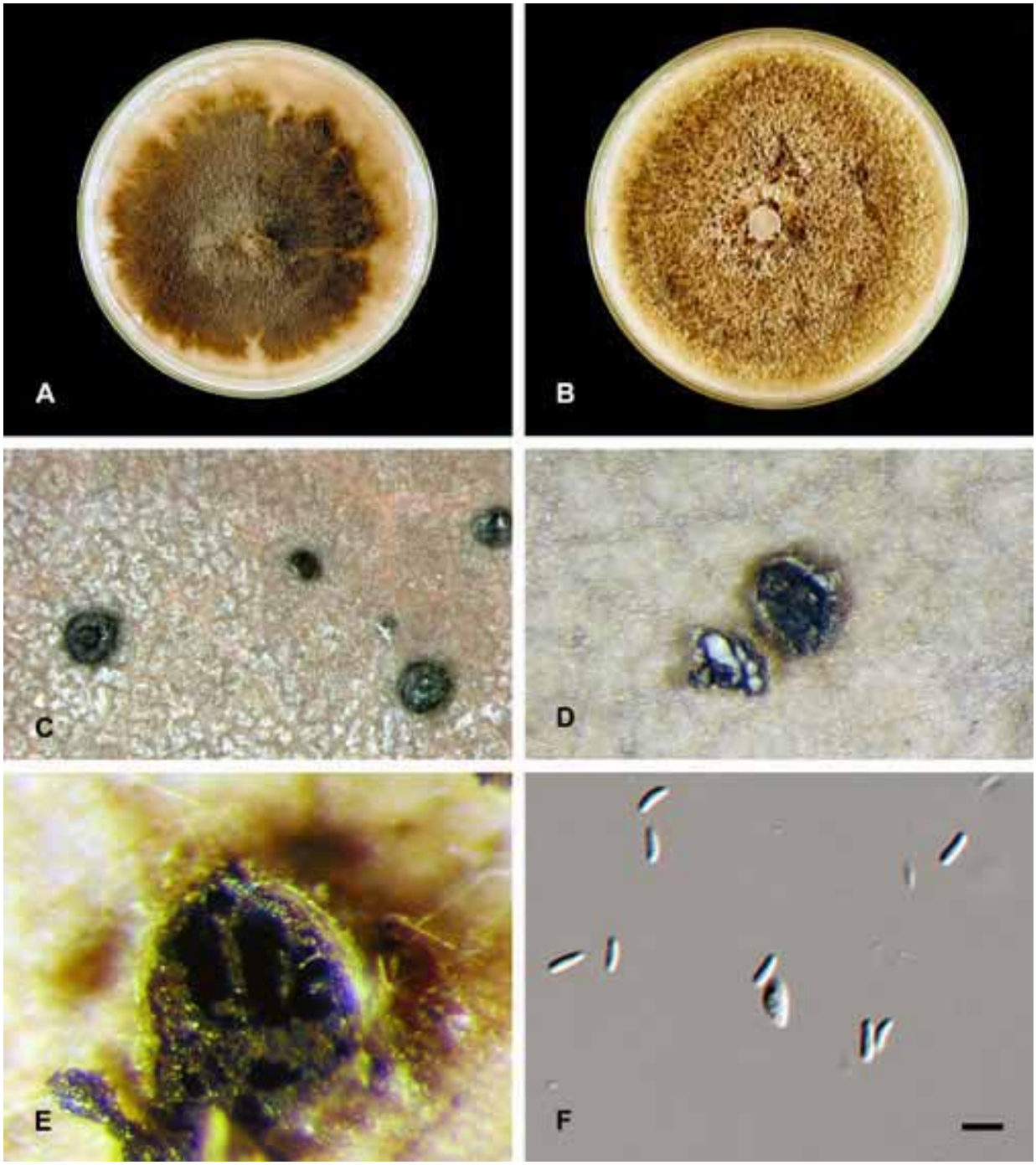

Fig. 80. Culture characteristics of *C. berkeleyi*. A–B. Colony morphology of isolate CBS 116825, California-4 on PDA (left) and on oatmeal agar (right). C. Conidiomata produced on autoclaved *Eucalyptus* leaf. D–E. Sections through conidiomata show divided locules. F. Allantoid conidia. Scale bar: F = 4.5 µm.

24. *Cytospora diatrypelloidea* G.C. Adams & M.J. Wingf., **sp. nov.** Figs 81–83. MycoBank MB500223. non *Cytospora diatrypa* Sacc., Syll. Fung. 3: 258. 1884; non *Valsa diatrypa* Fr., Summa Veget. Scand. 2: 411. 1849; non *Valsa diatrypoides* Rehm, Hedwigia 21: 117. 1882.

Etymology: "*diatrypelloidea*" refers to diatrypelloid arrangement of conidiomata in the hemispherical stroma.

Teleomorpha ignota. Stromata conidiomatica in cortice immersa, erumpentia, prominentia, hemisphaerica, maximam partem in cortice vel prope corticis superficiem, 0.4–1 mm diametro, diatrypelloidea, loculis (3–)6–10(–14) segregatis in entostromate mediocriter griseo infra discum inclusis praedita. Disci atro-brunnei, e parte superiore stromatis hemisphaerici compositi, solidi, lenticulares, 0.25–1 mm diametro, ostiolis (3–)6–10(–14) praediti. Ostiola canescentia, usque ad supra superficiem disci assurgentia. Loculi stricti, arcte contigui, globosi, lateraliter compressi, ad typum simplicem, non divisum pertinentes, 100–200 µm, parietibus e textura epidermoidea compositis, 15–18 µm crassis praediti. Conidiophora simplicia vel ad altitudinem medianam ramosa, 12–17 × 1 µm. Cellulae conidiogenae in matrice continua gelatinosa inclusae, enteroblastice phialidicae, subcylindricae, ad apicem contractae, collaretta minuta praeditae, 6–8(–10) × 1 µm. Conidia hyalina, eguttulata, allantoidea, unica (4–)5–5.5(–6) × 0.9–1 µm.

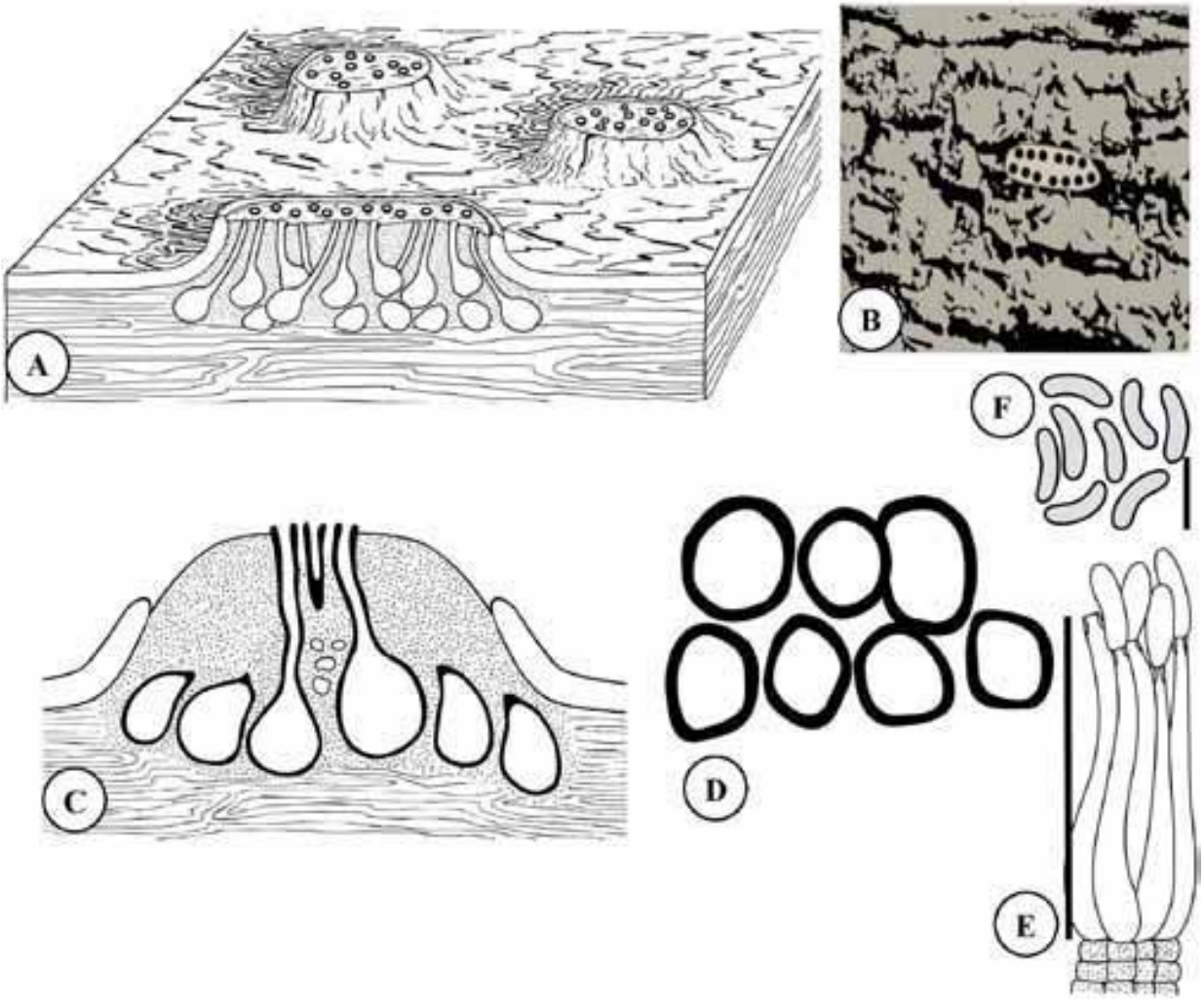

Fig. 81. Illustrations of *C. diatrypelloidea*. A. Habit sketch. B. Ostiolar disc erumpent from bark. C. Longitudinal section through conidiomal stroma in plant. D. Tangential section through conidiomal stroma. E. Conidiophores in hymenium. F. Conidia. Scale bars: E = 14.5 µm, F = 7 µm.

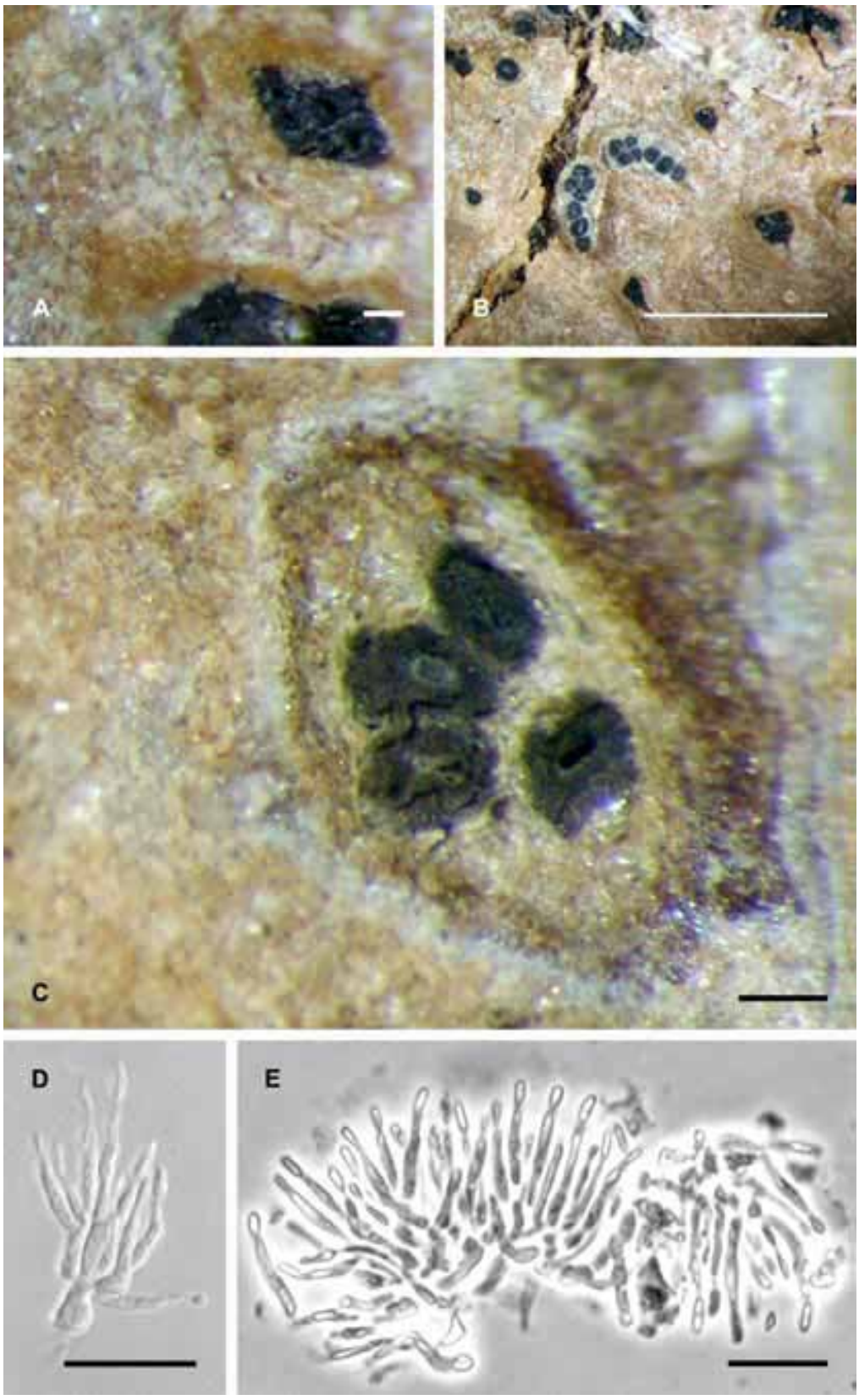

Fig. 82. Morphology of the anamorph *C. diatrypelloidea*. A. Dark flat disc around discrete ostiole on surface of *Eucalyptus* bark. B. Diatrypelloid arrangement of dark conidiomata in a light entostroma. C. Four upright dark brown ostioles in a light entostroma. D–E. Close up of whorls of branched conidiophores and conidiogenous cells arising from swollen basal cells. F. Hymenium of branched conidiophores and conidiogenous cells. Scale bars: A = 100 µm, B = 1 mm, C = 100 µm, D–E = 10 µm.

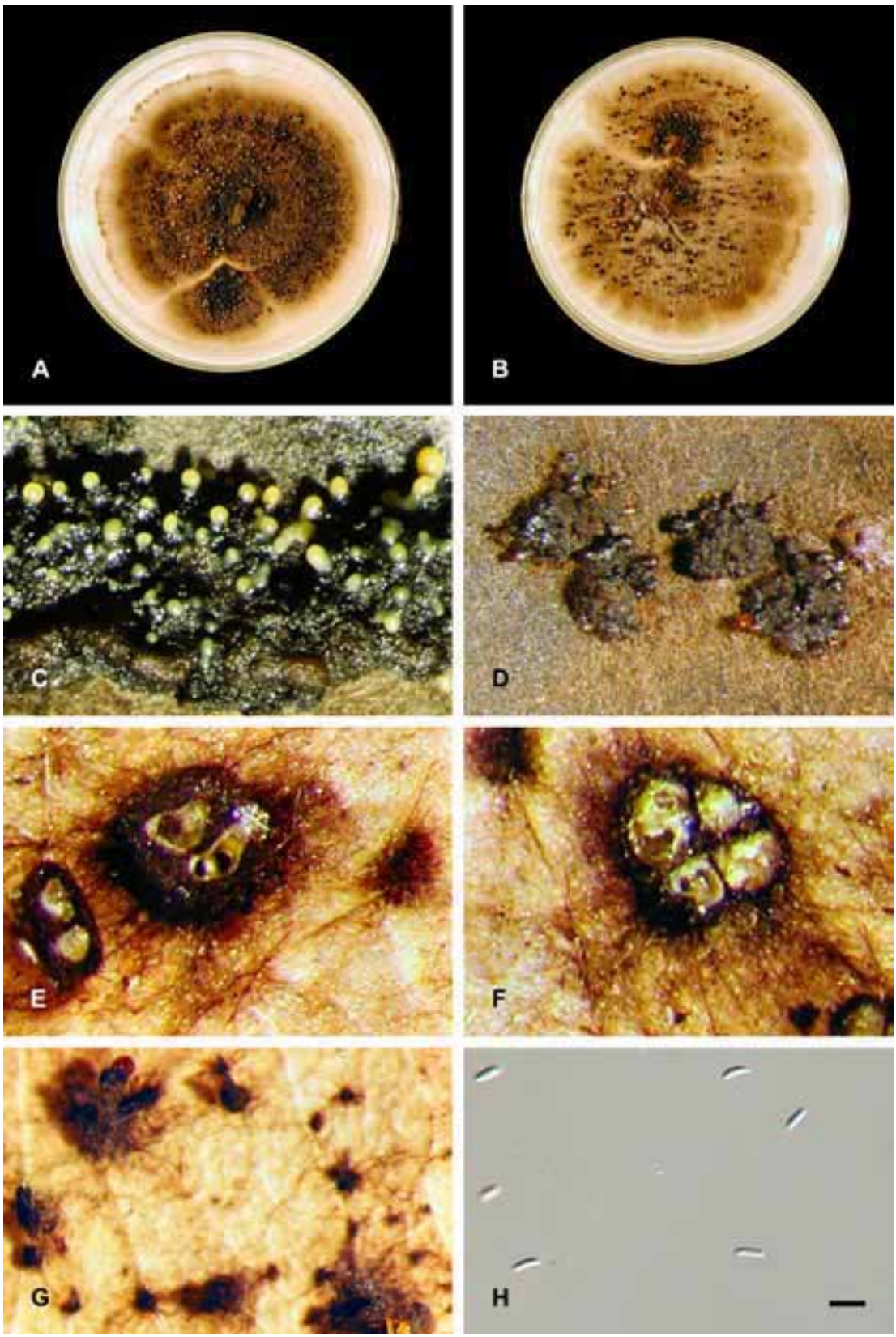

Fig. 83. Culture characteristics of *C. diatrypelloidea*. A–B. Colony morphology of isolate CBS 116826, Australia-8 on two different Petri dishes of PDA. C–D. Large aggregations of conidiomata with many necks/beaks in loose grey brown stromatal tissue are produced in culture. E–F. Tangential sections through simple conidiomata show divided locules. G. Small clusters of conidiomata in loose grey brown stromatal tissue with long necks/beaks form on autoclaved *Eucalyptus* leaf. H. Allantoid conidia. Scale bar: H = 5.5 µm.

Teleomorph unknown. *Conidiomatal stromata* immersed in bark, erumpent, prominent, hemispherical, mostly on or near surfaces of bark, 0.4–1 mm diam, lamyelloid, diatrypelloid, (3–)6–10(–14) independent locules in medium grey entostromata below the discs. *Discs* dark brown, of upper part of the hemispherical stromata, massive, lenticular, 0.25–1 mm diam (3–)6–10(–14) ostioles. *Ostioles* pale grey, sometimes protruding above disc surfaces. *Locules* simple, undivided, upright, packed tightly together, globose, compressed laterally, 100–200 µm, walls of *textura epidermoidea*, 15–18 µm thick. *Conidiophores* unbranched to branched once near mid-height, 12–17 × 1 µm. *Conidiogenous cells* embedded in a continuous gelatinous matrix, enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, 6–8(–10) × 1 µm. *Conidia* hyaline, eguttulate, allantoid, aseptate, (4–)5–5.5(–6) × 0.9–1 µm.

Cultures: Colony growth on PDA is predominantly pale olive-grey to olive-black (Munsell 6.9Y 5.5/1.3, 9.0Y 1.1/0.0) with a pale greyish olive edge (Munsell 7.8Y 5.5/2.5) on the surface. Colour of the reverse is olive-grey to olive-black (Munsell 8.1Y 3.5/0.9, 9.0Y 1.1/0.0) with a greyish to greenish yellow edge (Munsell 9.0Y 7.2/3.9). Pycnidia form on some plates and exude pale yellow cirrhi. Colony texture is felty, slightly raised with faint growth zones. Conidiomata form large aggregations of locules in loose grey-brown stromatal tissue in culture. Small clusters of 2–5 conidiomata with long necks/beaks form in loose grey-brown stromatal tissue on autoclaved leaves. Conidiomata vary from unilocular to small rosette cytosporoid with locules divided by one to few invaginations into 2–5 chambers and with distinct necks/beaks.

Cardinal temperatures: Colonies obtain a mean growth on PDA at 4 ºC of 6.5 mm diam, at 25 ºC of 30 mm diam, at 32 ºC of 9 mm diam, and no growth at 36 ºC after 7 d in the dark. Colonies obtain a mean growth on oatmeal agar at 25 ºC of 67 mm diam and no growth at 36 ºC after 7 d in the dark. No growth at 25 ºC occurs on 2 ppm cycloheximide in V8® agar or Leonian's agar after 7 d in the dark.

Host: *Eucalyptus globulus*.

Distribution: Only known from the type locality, Orbost, Victoria, Australia.

Specimen examined: **Australia**, Victoria, Orbost, Toslaree on dead branches of *E. globulus*, Sep. 2000, M.J. Wingfield (MSC 380719, **holotype** of *Cytospora diatrypelloidea*), living ex-type culture CBS 116826.

Notes: This species is unique in having hemispherical diatrypelloid stromata with the individual locules submerged in the stromata, upright, and packed tightly together. It resembles the morphology of the stromata of *C. nitschkii*. It differs from *C. nitschkii* in the pale colour of the entostromata, the crowded locules, the beaks extending above the surfaces, and particularly in the lack of circinate arrangement of the locules in the stromata. Upper portions of the hemispherical stromata could be interpreted as massive discs.

UNDETERMINED MATERIAL

25. *Cytospora* putative species 1. Fig. 84.

Teleomorph unknown. *Conidiomatal stromata* unavailable on natural substrate; globose, glabrose, 0.4 mm diam *in vitro*. *Locules* cytosporoid, subdivided by invaginations into 15 or more regular radially arranged chambers sharing common walls. *Conidiophores* unbranched to branched once near mid-height, 12–20 × 1 µm. *Conidiogenous cells* embedded in a continuous gelatinous matrix, enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, (6–)8(–10) × 1 µm. *Conidia* hyaline, eguttulate, allantoid, aseptate (3–)3.5(–4) × 0.9–1 µm.

Cultures: Colony growth on PDA is predominantly pale yellow, greyish yellow to pale yellowish brown (Munsell 4.7Y 9.0/3.8, 4.4Y 7.2/3.8, 9.7YR 6.4/2.5) on the surface. Colour of the reverse is dark orange-yellow to dark reddish brown (Munsell 9.3YR 6.0/7.9, 9.6R 1.3/3.6). A diffusible pigment colours the agar dark reddish brown to brown-black (Munsell 9.6R 1.3/3.6, 7.8YR 0.6/0.9) and influences interpretation of the reverse colony colour. Colony texture is felty, slightly raised with no growth zones. Rosette cytosporoid conidiomata with up to 15 regular radially arranged chambers and short indistinct necks/beaks form on autoclaved leaves. Conidiomata have white to yellowish white surfaces during development.

Cardinal temperatures: Colonies obtain a mean growth on PDA at 4 ºC of 3 mm diam, at 25 ºC of 145 mm diam, at 32 ºC of 220 mm diam, and at 37 ºC of 28 mm diam after 7 d in the dark. Growth at 25 ºC on 2 ppm cycloheximide in V8® agar is 86 % of growth on V8® agar without the antibiotic after 7 d in the dark.

Host: *Eucalyptus grandis*.

Distribution: Thailand, KwaMbonambi and Piet Retief, South Africa.

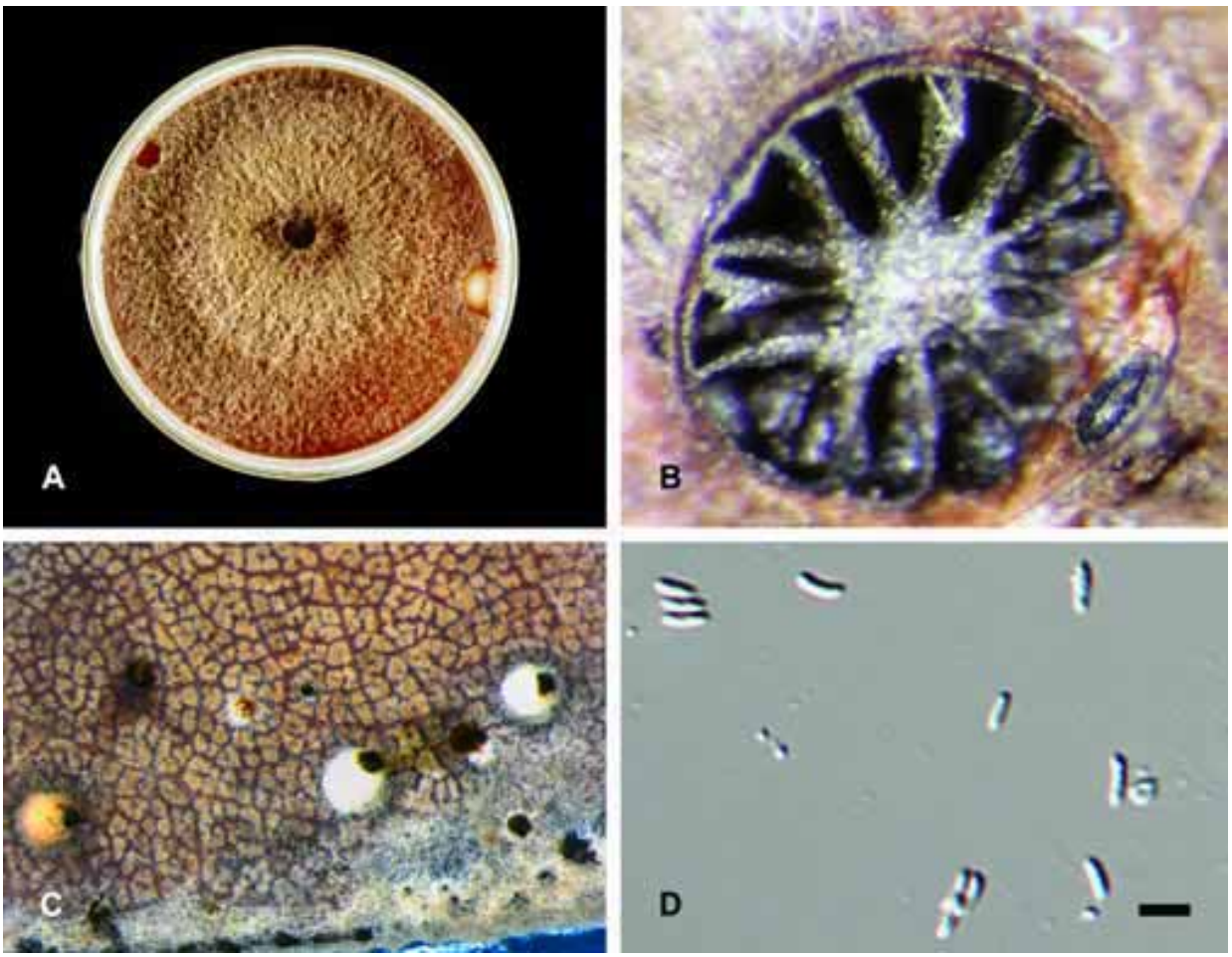

Fig. 84. Culture characteristics of *Cytospora* putative species 1. A. Colony morphology on PDA of isolate CBS 116861, Thailand-1. B. Tangential section through conidioma shows locule divided by numerous regular radially arranged chambers. C. Conidiomata produced on autoclaved *Eucalyptus* leaf. D. Allantoid conidia. Scale bar: D = 3.5 µm.

Specimens examined: **Thailand**, on dead branches of *E. grandis*, Sep. 1996, M.J. Wingfield, living culture CBS 116861. **South Africa**, Mpumalanga, Piet Retief, endophyte in leaf of *E. grandis*, Apr. 1994, H. Smith, living culture C.M.W. 5357; KwaZulu-Natal, KwaMbonambi, from stem canker on *E. grandis*, Apr. 1994, H. Smith, living culture C.M.W. 5358.

Notes: This species could have been named and described if we had located specimens from nature with conidiomata. However, only material produced *in vitro* was available. Conidiomata *in vitro* were unique in the number and regular shape of the chambers and the precision in which the chambers were radially arrayed within the locule.

26. *Cytospora chrysosperma* (Pers.) Fr., *s. lat.*, reported as "*Cytospora australiae*" in South Africa, 1919, Syst. Mycol. 2(2): 542. 1823. Figs 85–86.

Teleomorph unknown. A *Cytospora* species with *conidiomatal stromata* immersed in bark, erumpent, rosette to labyrinthine cytosporoid, medium grey, discoid, circular to ovoid, 500–900 µm diam, with a large multi-chambered locule. *Discs* absent. *Ostioles* medium grey, prominent, hemispherical (65–)75(–96) µm diam. *Locules* complex multi-chambered, subdivided frequently by invaginations, chambers irregular (regular at perimeter), sharing common walls, 500–900 µm diam, with discrete ostioles. *Conidiophores* hyaline, unbranched or occasionally branched at the bases, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute (6.5–)7(–9) × (0.7–)1(–1.5) µm. *Conidia* hyaline, eguttulate, elongate-allantoid, aseptate, (3.5–)4(–4.5) × 0.8 µm.

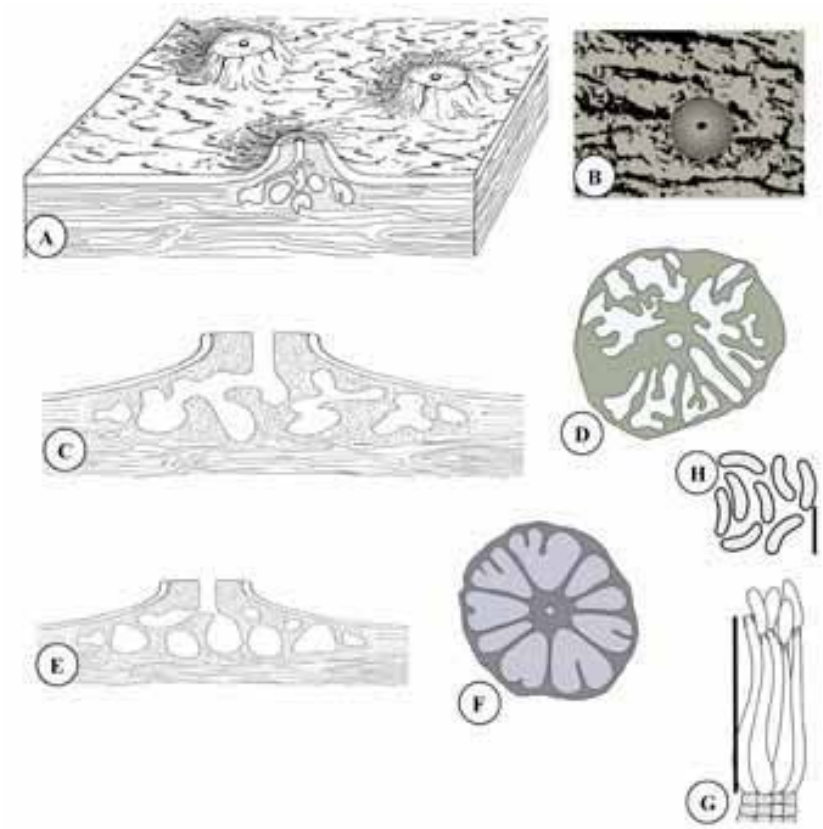

Fig. 85. Illustrations of *C. chrysosperma sensu lato* from South Africa misidentified as "*Cytospora australiae*". A. Habit sketch. B. Ostiolar disc erumpent from bark. C. Longitudinal section through large conidiomal stroma in plant. D. Tangential section through large conidiomal stroma. E. Longitudinal section through small conidiomal stroma in plant. F. Tangential section through small conidiomal stroma. G. Conidiophores in hymenium. H. Conidia. Scale bars: G = 7 µm, H = 4 µm.

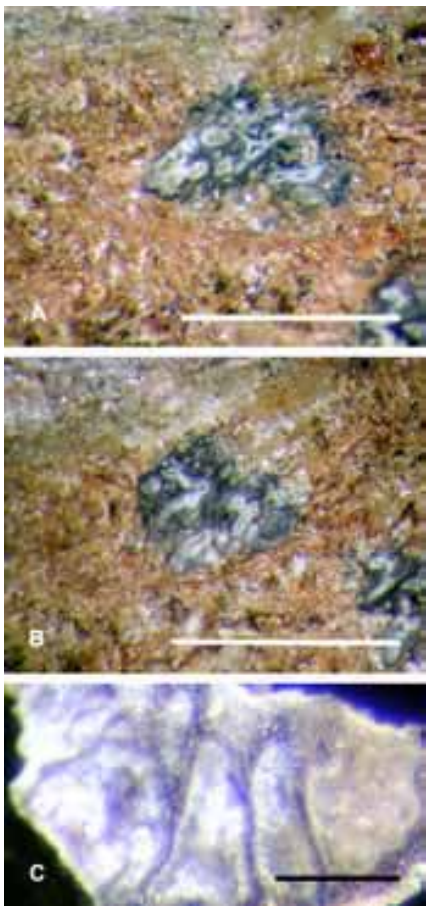

Fig. 86. Morphology of the anamorph of *C. chrysosperma sensu lato* from South Africa, misidentified as "*Cytospora australiae*". A–B. Tangential sections through two conidiomata on *Eucalyptus* bark, show complex irregularly arranged multiple chambers and discrete ostioles. C. Thin tangential section through small rosette cytosporoid conidioma with five regular radially arranged chambers and shared walls. Section is of the only small fruiting body on the specimen. Scale bars: A–B = 1 mm, C = 100 µm.

Host: *Eucalyptus viminalis*.

Distribution: Wellington, South Africa.

Specimens examined: **South Africa**, Wellington: on dead branch of *Eucalyptus viminalis*, 1919, R. Taylor (PREM 13072, "*Cytospora australiae* Speg.").

Notes: The morphology was typical of *C. chrysosperma s. lat*. We believe that many biological species of *Cytospora* share the morphology of *C. chrysosperma*, and we assumed that the DNA sequence of this specimen, if it could be obtained, would be distinct from the type of *C. chrysosperma*. This species did not agree with the torsellioid type specimen of *C. australiae* because it was labyrinthine cytosporoid. The Wellington 1919 specimen was unique in morphology for *Cytospora* on *Eucalyptus* in South Africa, having larger and more complex stromata and smaller conidia than *C.* aff. *cinereostroma*.

27. *Cytospora chrysosperma* *s. lat*., reported as "*Cytospora australiae*" in California, Syst. Mycol. 2(2): 542. 1823. Figs 87–88.

Teleomorph unknown. A *Cytospora* species with *conidiomatal stromata* immersed in bark, erumpent, labyrinthine cytosporoid, circular to ovoid, 0.75–1.15 mm diam below the discs. *Discs* white to pale grey, circular to ovoid, flat, of furfuraceous amorphous material, 0.2–0.25 mm diam, with discrete ostioles. *Ostioles* medium grey (70–)100–140 µm, at the same level as the disc surfaces. *Locules* complex multi-chambered, subdivided by invaginations into numerous irregular chambers, sharing common walls, 50–80 × 100–170 µm, chambers regular at the perimeter. *Conidiophores* hyaline, unbranched or occasionally branched at the bases or near mid-height, embedded in a continuous gelatinous matrix. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute, periclinal thickenings slight (7.5–)10(–11.5) × (1.2–)1.5(–1.7) µm. *Conidia* hyaline, eguttulate, allantoid, aseptate, (5–)5.5(–6) × 1 µm.

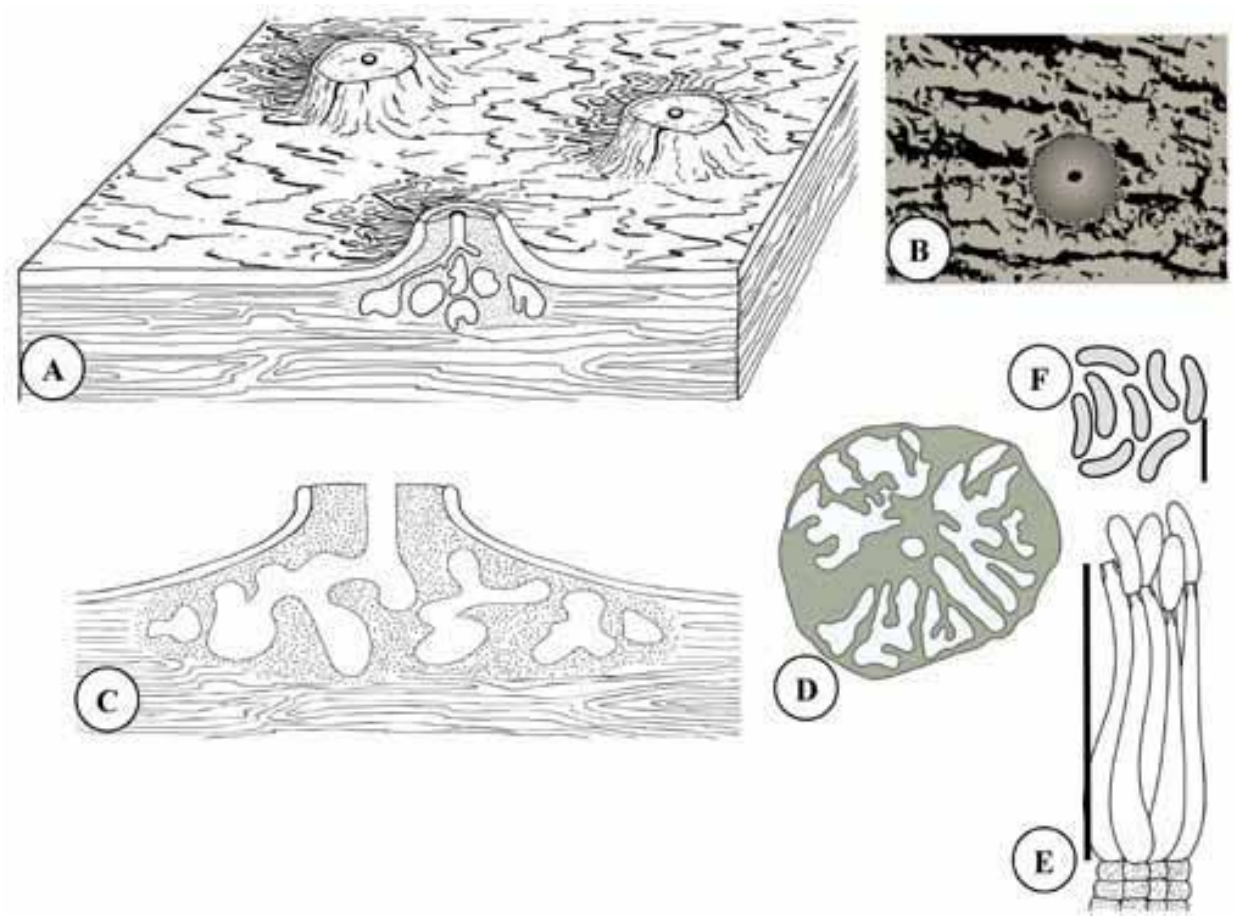

Fig. 87. Illustrations of *C. chrysosperma s. lat.* from California. A. Habit sketch. B. Ostiolar disc erumpent from bark. C. Longitudinal section through conidiomal stroma in plant. D. Tangential section through conidiomal stroma. E. Conidiophores in hymenium. F. Conidia. Scale bars: E = 10 µm, F = 5.5 µm.

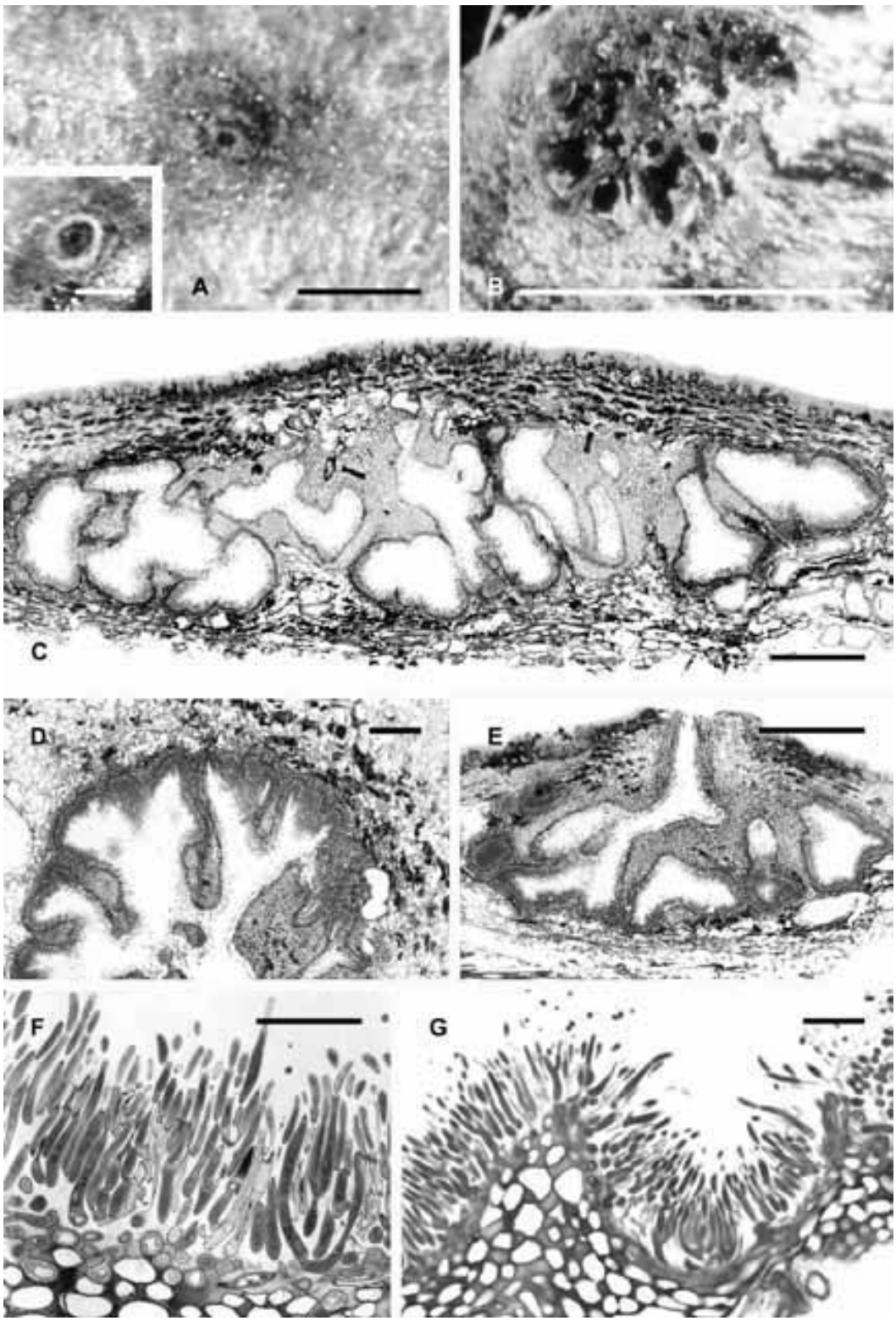

Fig. 88. Morphology of the anamorph *C. chrysosperma s. lat*. from California, misidentified as "*Cytospora australiae*". A. Discs with discrete ostioles on surface of *Eucalyptus* bark. Insert of lateral cross-section of disc and ostiole. B. Tangential section through a conidioma shows irregular multiple chambers with shared walls. C. Longitudinal section through large conidioma shows irregularly arranged complex multiple chambers. D. Tangential microtome section through small conidioma shows half of the invaginations in the conidioma dividing the locule into numerous irregular chambers. E. Median longitudinal microtome section through a small conidioma with irregular chambers and discrete ostiole. F. TEM of microtome section of hymenium with conidiophores with branching at the base and mid-height and conidiogenous cells. G. Microtome section of locule of conidioma with conidiogenous cells. Scale bars: A = 200 μm, B–C = 1 mm, D–E = 100 μm, F–G = 10 μm.

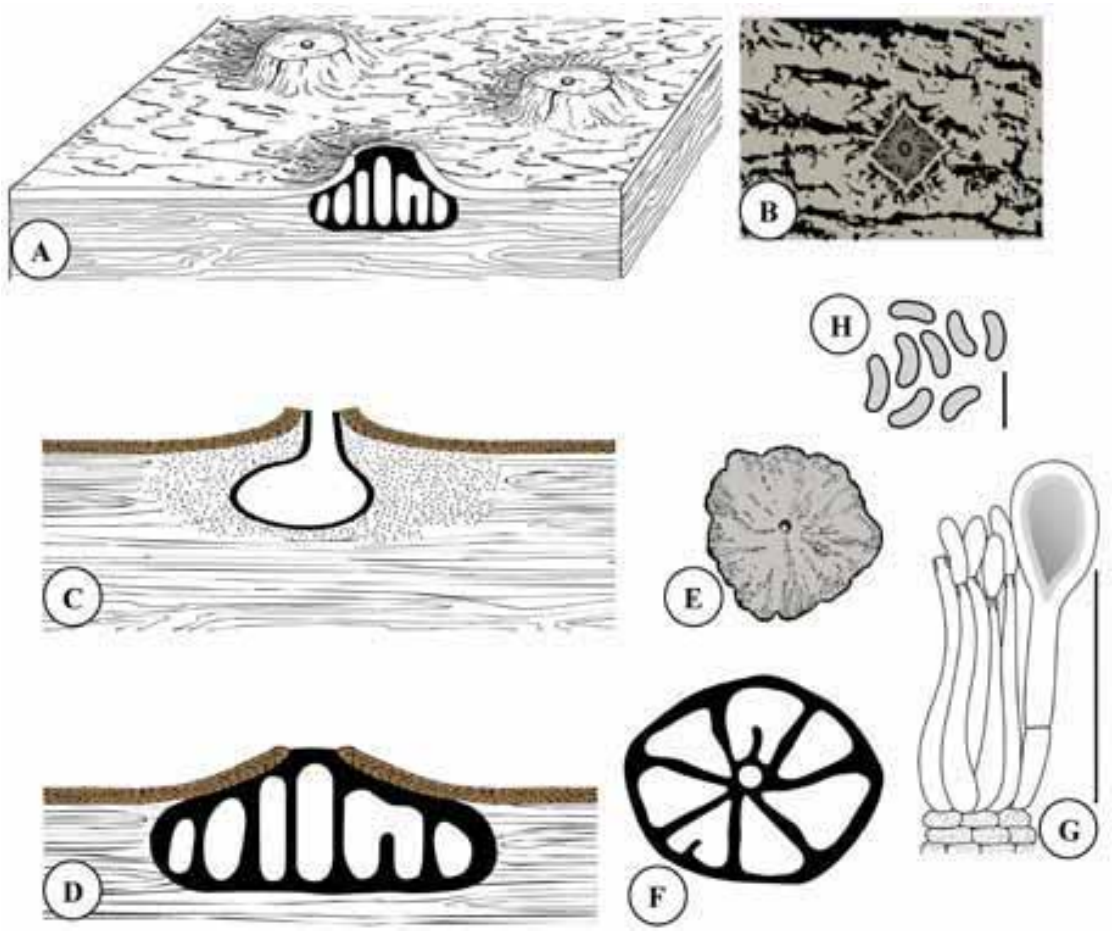

Fig. 89. Illustrations of *Cytospora*-like species. A. Habit sketch. B. Ostiolar disc erumpent from bark. C. Longitudinal section through small conidiomal stroma in plant. D. Longitudinal section through large conidiomal stroma in plant. E. Top view of a conidioma isolated from plant tissues. F. Horizontal cross section through large conidiomal stroma. G. Conidiophores and oil cell in hymenium. H. Conidia. Scale bars: G = 10 µm, H = 3 µm.

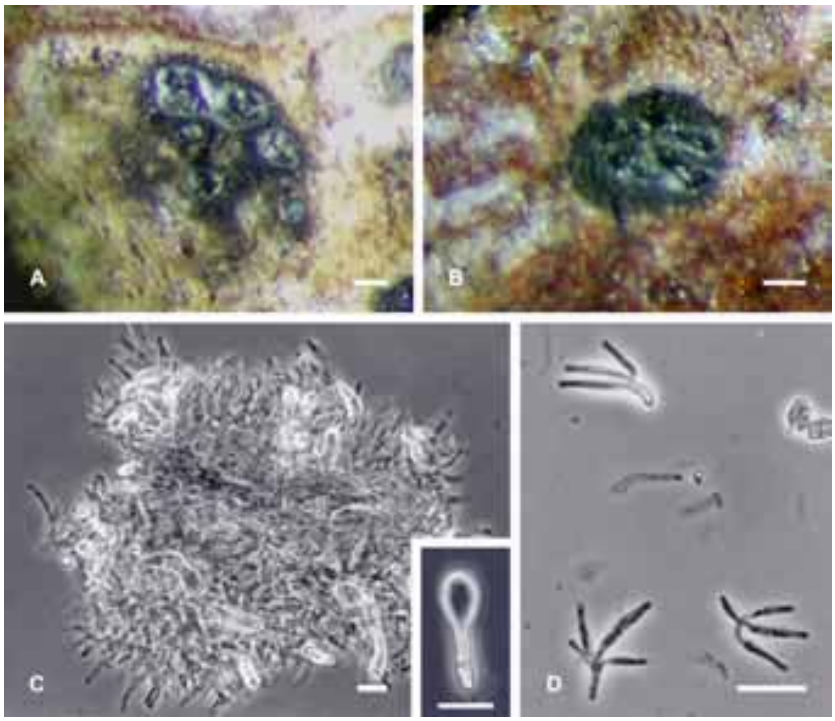

Fig. 90. Morphology of the anamorph of *Cytospora*-like species. A–B. Conidiomata on *Eucalyptus* bark sectioned tangentially show shallow invaginations at the base of the locules. C. Hymenium with refractive clavate cells assumed to contain oil. These unique cells were not present in all specimens. D. Enlargement of clavate oil cell free from the hymenium. E. Slender verticils of phialides arising from basal cells. Scale bars: A–B = 100 µm, C–E = 10 µm.

Host: *Eucalyptus globulus*.

Distribution: California, U.S.A.

Specimens examined: **U.S.A.**, California, Berkeley campus of University of California on dead twigs of *E. globulus*, 16 Jan. 1926, *L.* Bonar (UC 275812, "*Cytospora australiae* Speg."); Alameda-Contra Costa County line, Berkeley Hills, south end of Grizzly Peak Boulevard, on dead branches of *E. globulus*, 15 Jan. 1974, L. Bonar (UC 500550, "*Cytospora australiae* Speg." number 1352).

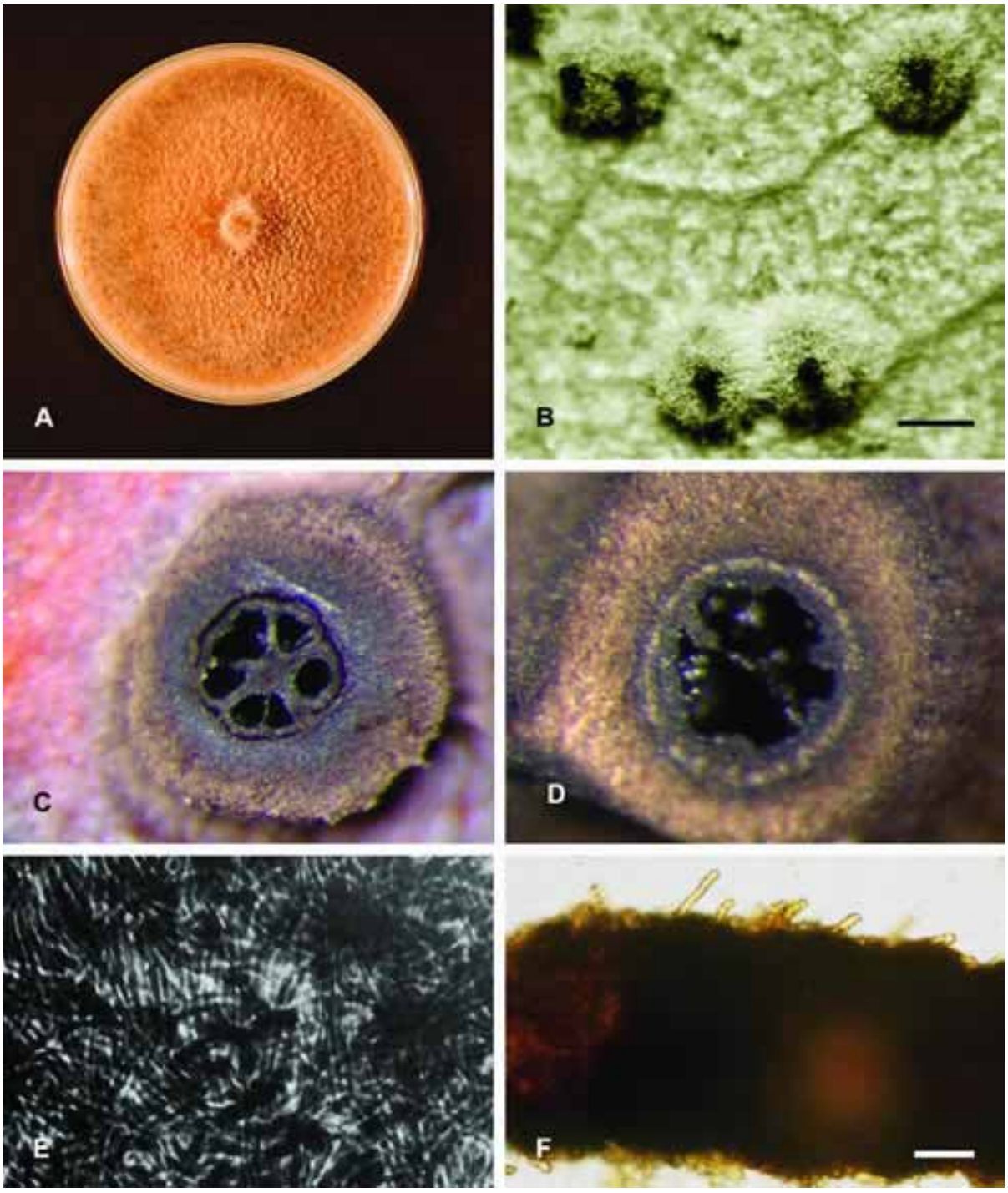

Fig. 91. Culture characteristics of *Cytospora*-like fungus. A. Colony morphology on PDA of isolate CBS 117015, Indonesia-1. B. Wooly conidiomata produced on autoclaved *Eucalyptus* leaf. C–D. Tangential sections through conidiomata show single locules divided into regular, radially arranged chambers. Each locule has a wall independent from the wooly ectostroma layer. E. Neck of *Cytospora*-like fungus with wall of sclerenchyma of *textura porrecta*, as viewed with bright field microscopy. F. Neck of conidioma of the *Cytospora*-like fungus (isolate C.M.W. 455) with short blunt cells regularly distributed along the surface. Scale bars: B = 1 mm, F = 10 µm.

Notes: The morphology of this fungus was typical of *C. chrysosperma s. lat*. We believe that many biological species of *Cytospora* share the morphology of *C. chrysosperma* and, therefore, assume that the DNA sequence of this specimen, if it could be obtained, would be distinct from the type of *C. chrysosperma*. The specimen was labeled "*C. australiae*" but did not agree with the type specimen of *C. australiae*. The specimen had labyrinthine cytosporoid rather than the torsellioid morphology of *C. australiae*. This species was typical of most labyrinthine cytosporoid *Cytospora* species such as the ubiquitous *Cytospora leucosperma* Fr. The Californian species was not found during careful surveys of the reported locations by G.C. Adams and H. Hallen, but it was reported to re-occur periodically (I. Tavares, pers. comm.). Two different and smaller species, *C. berkeleyi* and *C. eucalypticola* were present at the California locations.

28. *Cytospora*-like species Figs 89–91.

Teleomorph unknown. *Conidiomatal stromata* immersed in bark, erumpent, medium grey, unilocular, reduced to rosette cytosporoid, circular, discoid, 0.2–0.55 mm diam, with discrete ostioles. *Discs* absent. *Ostioles* indistinct except in culture. *Entostromata* absent. *Locules* with slight invaginations, arising from the bases, extending partially into the chambers. *Conidiophores* hyaline, occasionally branched at the base, rarely branched above, embedded in an inconspicuous gelatinous matrix, 6.5–13.5 × 1–1.5 µm. *Conidiogenous cells* enteroblastic phialidic, subcylindrical, tapering to the apices, collarettes minute. *Conidia* small, hyaline, straight to allantoid, eguttulate, aseptate, 3–3.5 × 1 µm, exuding in pale yellow cirrhi. Beta conidia absent. In culture, ostiolar beaks short with distinctive short blunt cells distributed at regular intervals on the beak surfaces.

Cultures: Colony growth on PDA is predominantly yellow-white, moderate yellow to pale greyish yellowish brown (Munsell 4.5Y 9.2/1/2, 3.8Y 7.1/6.5, 9.7YR 6.4/2.5) on the surface. Colour of the reverse is moderate- and dark yellow to pale- and moderate olive-brown (Munsell 3.8Y 7.1/6.5, 3.9Y 6.0/6.4, 2.1Y 4.9/7.9, 2.7Y 3.6/5.5). Pycnidia do not form on the agar. Colony texture is felty, slightly raised with no growth zones. Unilocular to rosette cytosporoid conidiomata with locules divided by one to few invaginations into 2–5 chambers with short necks/beaks form on autoclaved leaves. Each conidioma has a thick wooly outer wall that is independent of the locule wall.

Cardinal temperatures: Colonies (mean of 3 isolates) obtain a mean growth on PDA at 4 ºC of 7.8 mm diam, at 25 ºC of 185 mm diam, at 32 ºC of 162 mm diam, and no growth at 37 ºC after 7 d in the dark. No growth at 25 ºC occurs on 2 ppm cycloheximide in V8® agar and 36 mm diam occurs on V8® agar without the antibiotic after 7 d in the dark. Growth on 2 ppm cycloheximide in Leonian's is 25 % of growth on Leonian's agar without the antibiotic after 7 d in the dark.

Host: *Eucalyptus urophylla*.

Distribution: Indonesia.

Specimens examined: **Indonesia**, on bark of cankered branch of *E. urophylla* in plantation, 1992, M.J. Wingfield (MSC 380703), living culture CBS 117015; on bark of cankered branch of *E. urophylla* in plantation, 1992, M.J. Wingfield (MSC 380704, MSC 385000), also living cultures C.M.W. 460, CBS 117016, respectively.

Notes: The *Cytospora*-like species was very similar to *Allantophomopsis* Petr., a genus that Sutton (1977) considered a possible synonym of *Cytospora*. The *Cytospora*-like species resembled *Allantophomopsis* and *Ceuthospora* in forming pycnidial conidiomata having multilocular, convoluted, incompletely divided chambers, and differed in the absence of fusoid to lunate conidia with apical cone-shaped mucoid appendages and occasionally inconspicuous basal mucoid appendages. The *Cytospora*-like species was distinct in having ITS-rDNA sequences outside the *Valsa* clade and remarkably close to *Diaporthe*. *Cytospora endophylla* Fr. and *Cytospora vaccinii* Fr. have been placed in *Allantophomopsis*. The described species of *Allantophomopsis* and *Ceuthospora* have conidia that are much larger than the *Cytospora*-like species. Known sexual states for species in *Allantophomopsis* and *Ceuthospora* are in the genus *Phacidium* Fr. (*Helotiales*, Carris (1990)). The *Cytospora*-like species was similar to species of *Cytospora* in having invaginations of the locules, rosette cytosporoid conidiomata, and small allantoid conidia. It differed from *Phomopsis* species in the absence of beta conidia on host tissues and in culture on leaves and twigs of *Eucalyptus*. Additionally, the conidia were unusually small, significantly shorter than those reported for the three known species of *Phomopsis* on *Eucalyptus*, *P. eucalypticola* Old & Z.□. Yuan (Yuan *et al.* 1995), *P. rudis* (Sacc.) Höhn. (Harkness 1884, Kobayashi 1970), and *P. eucalypti* Zerova (Mohanan & Sharma 1987).

DISCUSSION

Morphological studies of specimens of *Cytospora* from *Eucalyptus* combined with phylogenetic analysis of the ITS-rDNA gene have revealed an unexpected level of morphological and genetic diversity in this fungus. As many as 15 distinct lineages were present for collections of *Cytospora* from *Eucalyptus*, and most of these are unrelated to those encompassing the well-known Northern Hemisphere species of *Cytospora* on hardwoods and conifers. In this ITS-rDNA phylogeny, the teleomorph genera *Valsa*, *Leucostoma*, *Valseutypella* and *Valsella* can be interpreted as congeneric, agreeing most closely with Saccardo's generic concept of *Valsa* Fr. *emend.* Sacc. (Saccardo 1875, 1882–1931).

The most commonly cited species of *Cytospora* on *Eucalyptus*, *C. eucalypticola*, was reported to form a teleomorph fitting the description of *V. ceratosperma* (Old *et al.* 1991). *Valsa ceratosperma* was described as having small asci and ascospores (< 8 µm long, sect. *Microsporae*), usually with inconspicuous discs with closely packed ostioles and dome-shaped entostromata surrounding numerous (6–40) closely packed, nearly upright, perithecia. Our results showed that the description of *V. ceratosperma* encompasses many *Valsa* specimens with small asci, ascospores and ascostromata. Evidently, the species epithet has served for attaching a name to disparate small-spored, small-perithecium species. It was appropriate to narrow the scope of this species to a distinct phylogenetic lineage, and one that most closely corresponded to the original species concept of Fries (1823). This was consistent with the actions of Tulasne & Tulasne (1863). They erected *V. ceratophora* Tul. & C. Tul., associating the species with *Quercus*, and including the type specimen (*Sphaeria ceratosperma* Moug. & Nestl. 1818, Stirp. Vog.-Rhem, 567, in Fries 1823; neotype in Spielman 1983), and other synonyms such as *V. decorticans* Fr. (*Sphaeria decorticans* Fr. 1823). Their statement was translated as follows: "But here we are citing particularly those synonyms which refer to the fungus on oak, as we know it and have described it below;" (Tulasne & Tulasne 1863). In the phylogeny (Fig. 14), the clade with the label *V. ceratosperma s. str.* included strains on *Quercus* from Europe and the U.S.A. We believe that the clade represents the ITS-rDNA sequence associated with the species concept of *V. ceratosperma* (≡ *V. ceratophora* Tul. & C. Tul.) shared by Fries and the Tulasnes.

The species *V. abietis* (Fr.) Fr. (eastern and western U.S.A. collections) was remarkably similar in morphology to *V. ceratosperma*. The species described from gymnosperms could have often been identified as *V. ceratosperma* or *V. subclypeata* Cooke & Peck when it was collected on angiosperm hosts. Conversely, *V. ceratosperma s. str.* could have been misidentified as *V. abietis* when it occurred on conifers. We speculate that the variation in ITS-rDNA sequence in the population of the eastern U.S.A. collections of *V. abietis* could encompass *V. auerswaldii* and *V. subclypeata*, but a more detailed phylogenetic study will be needed to resolve this question. It is important to note that *V. abietis* in the Pacific northwestern U.S.A. is not closely related to *V. abietis* from eastern North America. We temporarily refered to the fungus from the Pacific Northwest on *Pseudotsuga* and *Chamaecyparis* as *V. weiriana* Petr. This species on gymnosperms could be a part of the population diversity within *V. ceratosperma s. str.*

The isolate of *V. ceratosperma* from Japan has been used in previous studies of Cytospora canker on *Eucalyptus* (Old *et al.* 1986, Old & Kobayashi 1988, Old *et al.* 1991). These authors compared the morphology of the teleomorph with a specimen from *Eucalyptus* in Australia (see below) and both were identified as *V. ceratosperma* (Old *et al.* 1991). The Japanese teleomorph was in the lineage labeled on the cladogram as *V. ceratosperma sensu* Kobayashi. This specimen could be a teleomorph of *Cytospora eriobotryae* Curzi & Barbaini, a species found in Asia (India and Japan). The Australian teleomorph from the study of Old *et al.* (1991) is labeled on the cladogram as *V. ceratosperma sensu* K.M. Old. The genetic distance between the isolates from Japan and Australia was great. We have described the specimen from Australia as *Valsa fabianae*, the teleomorph of *C. eucalypticola*.

An important discovery in this study was that the herbarium specimen of the Australian teleomorph (DAR 43948 Tasmania) of Old *et al.* (1991) did not resemble most teleomorphs of *C. eucalypticola* found in Africa. Remarkably, neither did the accompanying anamorph resemble those usually found in Africa (i.e., MSC 368319 from Uganda). Environmental factors appear to greatly alter the morphology of the holomorph. In particular, the depth in the bark at which the anamorph is formed appears to influence locule morphology. For example, anamorphs deep in bark had unilocular cytosporoid conidiomata, whereas those near the bark surface had rosette cytosporoid conidiomata. The morphology of teleomorphs and anamorphs most commonly observed in African specimens did not fit previously described species of *Valsa*. The teleomorph usually consisted of a solitary perithecium, with an ostiole extending as a long beak above the bark surface, and indistinct or absent disc. The teleomorphs could be mistaken for *Clypeoporthella* Petr. 1924 with allantoid ascospores. Occasionally a few ostioles converged into a common disc and that permitted recognition of the genus *Valsa*. Similarly, the anamorphs usually appeared as discrete undivided

locules (unilocular) with long beaks, resembling the teleomorphs but having thinner beaks.

Cytospora eucalypticola in Africa (the type locality) often occurred only in the uniloculate form on a specimen, especially on *E. saligna*. This morphology did not fit any previously described species of *Cytospora*. It was most similar in morphology to the cytophomoid conidiomata (former sect. *Cytophoma*) of *V. cypri*, but lacked the distinctive ring-like, or in longitudinal median cross section wing-like, ectostromata around the ostioles. Many specimens of *C. eucalypticola* had this new morphology and we have described the conidiomata as unilocular. Several other lineages of *Cytospora* species on *Eucalyptus* also produced unilocular conidiomata.

Sexual states were observed for six of the *ca* 15 phylogenetic lineages representing *Valsa* species from *Eucalyptus*. These included; *V. brevispora* from Congo, *V. cinereostroma* from Chile, *V. eucalypti* from California, *V. eugeniae* from Indonesia, *V. myrtagena* from Hawaii, and *V. fabianae* from Australia and Uganda. None of the observed sexual states were closely related to teleomorphs described as *V. ceratosperma* on other hosts. Two additional teleomorphs were known prior to this study. These are "*V. eucalypticola*" and *V. eucalypti sensu* Sharma *et al.* We speculate that the latter species and *C. agarwalii* will each be distinct but closely related to the *V. eucalypti* lineage.

The anamorph of *V. ceratosperma*, *C. sacculus*, formed the distinguishing torsellioid conidiomata. Most of the anamorphs associated with the teleomorphs on *Eucalyptus* did not form typical torsellioid conidiomata, only *C. eucalyptina* resembled *C. sacculus*. *Cytospora australiae* was also torsellioid but each locule was distorted by extensive crowding. Individual fruiting bodies of *C. agarwalii*, "*C. eucalypti*", *C. abyssinica* and the anamorphs of *V. eucalypti* formed leucotorsellioid conidiomata. *Valsa cinereostroma* formed leuco-cytosporoid conidiomata, and *C. eucalypticola* and *C. variostromatica* each formed conidiomata that ranged in morphology from unilocular, lamyelloid, and rosette cytosporoid. *Cytospora nitschkii*, *C. valsoidea*, and *C. diatrypelloidea* formed lamyelloid conidiomata. *Valsa brevispora*, *C. disciformis*, *C. austromontana* and *V. myrtagena* formed rosette cytosporoid conidiomata. Additionally, two specimens of *C. chrysosperma s. lat.* misidentified as *C. australiae*, one from California and the other from South Africa, formed labyrinthine conidiomata with complex multi-chambered locules. Herbarium specimens having rosette cytosporoid conidiomata scattered among lamyelloid and unilocular conidiomata, such as seen for *C. eucalypticola* and *C. variostromatica*, had not previously been recognised in the genus *Cytospora*. Furthermore, species that formed leucotorsellioid conidiomata had not been fully described or discussed in the literature.

In teleomorphs, several morphological characteristics deserve further study to determine whether they may be informative. A systematic study of the morphology of paraphyses among *Valsa* species may provide useful information on morphogenesis and classification. Septation in paraphyses, morphology of the terminal cells, and nuclear number in cells may be distinct for species or groups of related species. A systematic study of the number of nuclei in ascospores among *Valsa* species may provide useful genetic information. Perhaps nuclear conidition is correlated with homothallic and heterothallic sexuality.

In anamorphs, systematic study of the length of the canal from the lumen to the apex of a phialide may be informative. Study of TEM micrographs of phialides show differences between *C. acaciae* and *C. australiae* in the length of the canal. The length creates a larger region of periclinal thickening. Increased length may be the result of repeated formation of conidia from the meristematic apex. Then, differences may be a function of the period of conidiogenesis or age. However, difference in canal length may be characteristic for species. Further study may improve understanding of the observation. A systematic study of filamentous hymenial elements among conidiophores may provide informative characters for classification. Shape, septation, branching, and presence of gelatinous coatings may be characteristic for species. Frequency of occurrence and period of occurrence among conidiomata of a species are not known. Experimental study may improve understanding of their function. Isolates that form hymenial elements *in vitro* are needed for a model system.

One of the most difficult challenges in this study was determining, based on morphology, which isolates of *Cytospora* from *Eucalyptus* should be accommodated in the species *C. eucalypticola*. The only specimen collected in South Africa that conformed to most characteristics of the holotype specimen of *C. eucalypticola* was the isolate from Homeleigh near Pietermaritzburg (MSC 380718). Both specimens were on *E. saligna* and it is possible that the characteristics of the bark altered the morphology of the fungus. Even the latter specimen differed from the type specimen in having conidiomatal walls of noticeably greater thickness. The variation in morphology in *C. eucalypticola* was remarkable and deserves further consideration in the future.

Cultural characteristics have seldom been useful for differentiating *Cytospora* species, except in the well-studied species on *Prunus* and *Malus* (Adams *et al.* 2002a). Here, we discovered three measures of growth rate that offered distinquishing information on 18 *Cytospora* species including, growth at 37 ºC, 32 ºC,

and on 2 ppm cycloheximide. For example, six species grew well at 37 ºC including *V. brevispora*, *V. eugeniae*, *V. fabianae*, *C. eucalyptina*, *Cytospora* putative species 1, and some isolates of *V. myrtagena* including the ex-type strain. A different six species grew poorly, or not at all, at 32 ºC including *V. ceratosperma s. str.*, *V. eucalypti*, *C. berkeleyi*, *C. diatrypelloidea*, *C. nitschkii*, and *C. valsoidea*. Only half of the 18 species grew on media containing 2 ppm cycloheximide. Growth response among isolates within a species was uniform except within *V. myrtagena*. Two distinct groups of isolates were apparently included in *V. myrtagena*, the ex-type group from Hawaii that grew well at 37 ºC and on 2 ppm cycloheximide, and the Sumatra group which did not. We speculate that the differential responses of the two groups within *V. myrtagena* have emerged from an overly broad species concept, rather than of population genetic variance within a biological species. Cardinal temperatures and sensitivity to cycloheximide would likely be useful measurements for future studies of species in *Valsa*.

Morphology of conidiomata produced on autoclaved leaves or stems *in vitro* provided useful information regarding the fungi included in this study. Species that formed torsellioid or leucotorsellioid conidiomata in nature had two or more distinct wall layers in culture, including the wall around each independent locule. The layers formed *in vitro* appeared to correspond to entostroma and ectostroma formed in nature. Presence of multiple wall layers in conidiomata *in vitro* would be diagnostic of torsellioid and leucotorsellioid species. Perhaps, further study would lead to differentiating torsellioid from leucostorsellioid species. Species that formed cytosporoid conidiomata in nature had one wall *in vitro* but varied in presence, number, and length of necks/beaks, as well as, surface texture of the globe. We doubt that cytosporoid species would be reliably distinguished based on conidiomatal morphology. However, the absence of characteristic *in vitro* features would be useful in excluding unknown isolates from a species.

An unusual outcome of this study was the discovery of a *Cytospora*-like fungus, which clustered with robust bootstrap support in the *Diaporthe*/*Phomopsis* clade based on ITS-rDNA sequence homology, rather than the *Valsa*/*Cytospora* clade. These specimens exhibited allantoid conidia, invaginations within the globes of the conidiomata, and a lack of beta conidia. Generally, the invaginations in the conidiomata in natural material formed primarily at the bases of the globes, and extended only partially into the locules. However, on autoclaved leaves the invaginations often were as extensive as those in typical *Cytospora* species. The conidiomata formed *in vitro* had thick outer walls of wooly hyphae surrounding the locules, which were absent from *Cytospora* species. The conidia of the *Cytospora*-like fungus were significantly shorter than those reported for *Phomopsis* species on *Eucalyptus*, including *Phomopsis eucalypticola* (Yuan *et al.* 1995), *P. rudis* and *P. eucalypti*. Isolates of the *Cytospora*-like species would be readily misidentified as *Cytospora*. In future work, DNA sequence would be important to accurately identify these species and to determine their phylogenetic relationship to other *Diaporthales*.

The diversity of *Cytospora* species on *Eucalyptus* was remarkable in several locations. For example, each collection of *Cytospora* from *E. globulus* in one experimental planting in Ethiopia gave unique ITS-rDNA sequences, and each varied in morphology. We described two new species from this location but we assumed that additional distinct species were present. And a single grove of *Eucalyptus* on the campus of the University of California had three distinct *Cytospora* species, and a fourth species occurred on the nearby campus of Stanford University. Collections from South Africa and Indonesia (Sumatra/Suluwesi) had great ranges of genetic and morphological variation in *Cytospora* and *Cytospora*-like species on *Eucalyptus*.

Two species of *Cytospora* previously described on other host trees were discovered to infect *Eucalyptus*: *L. sequoiae* from *Sequoia sempervirens* and *V. eugeniae* from *Eugenia aromatica*. Additionally, new species discovered on *Eucalyptus* were also found on *Mangifera* and *Tibouchina*. Ethiopian isolates on *Eucalyptus* were phylogenetically related to endophytes from endemic *Podocarpus* in native forests (G.C. Adams, unpubl. data).

Cytospora clearly has a complex and variable morphology. There is little evidence to suggest that species are host-specific, and some species have wide geographic distributions. For this reason, DNA sequence data will be important to accurately identify species of *Cytospora* in the future. Morphological features alone are not sufficient for this purpose, as similar appearing fruiting bodies are formed by unrelated species of *Cytospora*. Several species on *Eucalyptus*, distant from one another based on ITS-rDNA sequence variation, form nearly identical fruiting bodies, locules divided into regular radially arranged chambers (the rosette cytosporoid conidioma) with conidia of 3–4 × 1 µm. The morphology typical of these fruiting bodies is not often encountered among Northern Hemisphere species. The Northern Hemisphere species exhibit a larger locule divided into multiple chambers of irrerular shape and arrangement (the labyrinthine conidioma). Few species of *Cytospora* are described that form both lamyelloid and unilocular conidiomata, whereas, we found such species to be common on *Eucalyptus*.

The morphological characteristics that provided the most information for distinguishing one species of *Cytospora* from another were the characteristics of the conidiomatal stromata. Furthermore, the size

of conidia, particularly the width of these structures, and the extent of branching of the conidiophores was taxonomically informative. Larger conidia and conidiophores were generally associated with more extensive branching of the conidiophores and were more commonly encountered in Northern Hemisphere collections of labyrinthine cytosporoid *Cytospora*, and on hosts other than *Eucalyptus*.

Most of the previously described *ca* 400 species of *Cytospora* have Latin diagnoses that do not include mention of the arrangement of the locules in the conidiomata, or the divisions of the locule into chambers, or whether locules have independent or shared walls. These characteristics need to be precisely described in order to recognise a species. However, emending the diagnoses of most of the *ca* 400 *Cytospora* species would be of limited value in differentiating species based on morphology without corresponding DNA sequence data. Sectional characteristics do not appear to be associated with a lineage of descent within the ITS-rDNA phylogeny. We have chosen to describe new species of *Cytospora* because they may be the only species distinctly recognisable in future studies, particularly, because we are able to provide corresponding DNA sequence data.

When considering the geographic area of origin of *Cytospora* species on *Eucalyptus*, a reasonable hypothesis is that these fungi evolved in Australia. It is probable that species differentiation then occurred in different regions of the continent and on different species of *Eucalyptus*. Historical and modern transport of seed and propagation material to other continents, and forces of local natural selection, are most likely responsible for the predominance of a particular species in collections. Alternatively, some *Cytospora* species could have evolved on other plant genera in *Myrtaceae*, such as *Eugenia*, and then moved onto an introduced *Eucalyptus* species. Such an event could then be magnified as favoured *Eucalyptus* selections are shipped between continents by the international forestry industry.

There are increasing numbers of tree pathogens including those of *Eucalyptus*, which have apparently undergone such host shifts (Slippers *et al.* 2005, Wingfield 2003, Gryzenhout *et al.* 2004). Some *Cytospora* spp. noted in this study could represent additional examples of such events. These could result in ecological imbalances or even serious disease problems in the future and they thus deserve careful study.

Little is known regarding the pathogenicity of *Cytospora* species on *Eucalyptus*. These fungi are generally considered to be relatively weak, facultative parasites that infect trees after stress (Christensen 1940, Schoeneweiss 1983). However, few pathogenicity tests have been done with *Cytospora* species and these have examined a limited selection of *Eucalyptus* species. Results of this study show that pathogenicity tests have likely been with different fungi and there is no firm basis for comparison between studies. Clearly, most species have not been tested and, therefore, the view that *Cytospora* species are unimportant as pathogens is simplistic or even incorrect.

Correct identification of species among the *Cytospora* and *Cytospora*-like fungi associated with cankers on *Eucalyptus* will be important in selecting *Eucalyptus* species, hybrids, or clones for plantation development. Careful studies will be needed to determine which *Cytospora* species are pathogens, and to determine the environments under which they might be important. Clones of *Eucalyptus* species differ markedly in their response to the environment and to pathogens, and the interaction of different species of *Cytospora* on different *Eucalyptus* genotypes in different environments clearly needs to be studied.

Some *Cytospora* species on *Eucalyptus* are endophytes that are able to exist in healthy tissue without symptoms (Bettucci & Saravay 1993, Fisher *et al.* 1993). Whether the species discovered in this study can occur as endophytes is a question that deserves further investigation. However, the fact that some species are able to exist as endophytes in germplasm such as seeds, suggests that this may have been the means by which they have moved around the world. Restricting importation of these fungi on *Eucalyptus* will be difficult and beyond the capacity of most nationally administered plant health inspection services. However, some organisations have banned the importation of *Eucalyptus* seeds into new environments and movement of larger plant material is generally prohibited. Nonetheless, not all countries have the capacity to maintain such rigorous quarantine measures, and those that allow the movement of plant material provide "bridges" for further movement of fungi such as *Cytospora* species. In the longer term, these fungi are likely to move more extensively and it is hoped that understanding species concepts for these fungi will minimise the likelihood of serious disease outbreaks.

ACKNOWLEDGEMENTS

We gratefully acknowledge the contribution of Patricia Eckel in providing the Latin descriptions. Funding support in the form of a fellowship from the National Research Foundation (NRF) of the Republic of South Africa to G.C. Adams is gratefully acknowledged. We also thank the members of the Tree Protection Co-operative Programme and the THRIP initiative of the Department of Trade and Industry, South Africa for financial support. We are grateful to Dr. Alemu Gezahgne and Dr. Henk Smith who provided several DNA sequences for this study and we thank colleagues in many

parts of the world who have assisted us in obtaining cultures and specimens, without which this study would not have been possible. In this regard we especially thank Dr. Ken Old, Mr. Mark Dudzinski, Dr. Angus Carnegie and Ms. Ruth Gibbs who assisted M.J. Wingfield in collecting specimens during sabbatical leave in Australia during 2000. We thank Marlene Cameron for illustrations and Dr. Heather Hallen for help in determining colony colours in culture.

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