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CERATOCYSTIOPSIS PROTEAE SP. NOV., WITH A NEW ANAMORPH GENUS

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ABSTRACT

A new species, *Ceratocystiopsis proteae*, collected from insect-infested *Protea repens* is described. *Knoxdaviesia*, a new genus of dematiaceous hyphomycetes is described to accommodate the anamorph of *C. proteae*. *Ceratocystiopsis proteae* shares characteristics of *Ceratocystis sensu stricto* (cycloheximide sensitivity), *Ceratocystiopsis* (falcate ascopores) and *Ophiostoma* (relatively long perithecial necks, divergent ostiolar hyphae and an anamorph with holoblastic conidium development) but is currently best accommodated in *Ceratocystiopsis*. *Knoxdaviesia proteae* superficially resembles the hyphomycete genera *Leptographium* and *Stachybotrys*. This is the first species of Ophiostomataceae described from an indigenous plant in southern Africa and its relationship with other Ophiostomataceae is discussed.

Key Words: *Ceratocystis*, *Ceratocystiopsis*, *Ophiostoma*, *Leptographium*, Proteaceae, insect-associated fungi.

Ceratocystis Ellis & Halst. *sensu lato* includes the genera *Ceratocystis*, *Ophiostoma* H. & P. Sydow and *Ceratocystiopsis* Upadh. & Kendr. *Ceratocystis sensu stricto* have anamorphs in the genus *Chalara* (Corda) Rabenh. in which conidia develop through a ring wall building process (Minter *et al.*, **1983**). Anamorphs of *Ophiostoma* include genera such as *Graphium* Corda, *Sporothrix* Hekt. & Perkins and *Leptographium* Lagerberg & Melin in which conidia are produced by apical wall building (Minter *et al.*, **1982**). The two genera are further distinguished by the presence of cellulose, chitin and rhamnose in the cell walls of *Ophiostoma*. *Ceratocystis* spp. have cell walls more typical of Ascomycetes, containing chitin and no cellulose or rhamnose (Jewell, **1974**; Rosinski and Campana, **1964**; Smith *et al.*, **1967**; Weijman and De Hoog, **1975**). The latter fungi are sensitive to cycloheximide whereas *Ophiostoma* spp. can tolerate high concentrations of this antibiotic (Harrington, **1981**).

Ceratocystiopsis was established by Upadhyay and Kendrick (**1975**) to accommodate *Ceratocystis s.l.* with falcate ascospores. These species are ecologically and taxonomically most closely related to *Ophiostoma* (De Hoog and Scheffer, **1984**). In general, perithecia of *Ceratocystiopsis* spp. have shorter necks than those of *Ophiostoma* and some of these species were formerly accommodated in *Europhium* Parker (**1957**).

Ophiostoma, *Ceratocystis* and *Ceratocystiopsis* spp. have ascospores produced in gloeoid masses. In this way, they are adapted for dissemination by insects. *Ophiostoma* and *Ceratocystis* spp. are commonly associated with bark beetles (Coleoptera: Scolytidae) and weevils (Coleoptera: Curculionidae) that infest trees. The role of most of these fungi in the biologies of their insect vectors is incompletely understood. However, a number of species such as *Ophiostoma ulmi* (Buis.) Nanf. and *O. wageneri* (Goheen and Cobb) Harrington are important tree pathogens (Boyce, **1961**; Smith, **1967**; Wagener and Mielke, **1961**).

Ceratocystis s.s. is a relatively small genus and

includes only 11 of the approximately 110 known species of *Ceratocystis s.l.* These fungi appear to be less closely associated with bark beetles and have a wider range of vectors (Dowding, **1984**; Juzwik and French, **1983**) including flies (Diptera) and sap-feeding nitidulid beetles (Coleoptera: Nitidulidae). Many species of *Ceratocystis* are pathogens and, unlike *Ophiostoma* spp., their hosts include trees as well as many other agricultural crops. For example, *Ceratocystis paradoxa* (Dade) C. Moreau causes diseases of palms and sugar cane; *Ceratocystis fimbriata* Ell. & Halst. is the causal agent of block rot of sweet potatoes and various canker diseases of trees; and *C. fagacearum* (Bretz.) Hunt is the cause of oak wilt.

Few species of *Ceratocystis s.l.* are known from countries in the Southern Hemisphere. Species such as *C. fimbriata* and *C. paradoxa* that are pathogens of herbaceous crops are, however, widely distributed. A number of species have been reported on tree crops and these have apparently been introduced with tree-infesting bark beetles from Northern Temperate forests (Wingfield *et al.*, **1985**; Wingfield and Marasas, **1980**). Very few species are known from native plants in the Southern Hemisphere and none are known from native plants in southern Africa. This paper describes a new species of *Ceratocystiopsis* with a new anamorph genus found in insect-infested flower heads (FIG. 1) of *Protea repens* (L.) L.

MATERIALS AND METHODS

Perithecia were found, apparently growing saprophytically on the surface of individual flowers within inflorescences of *P. repens*, in various parts of the Western Cape. In all cases these flowers were infested with insects belonging to a wide range of families. Cultures were obtained by lifting ascospore masses from the apex of perithecial necks and transferring them to 2% malt extract agar (20 g Difco malt extract, 20 g Difco Bacto Agar/1000 ml water). Isolates were incubated at 20 C in the dark before examination. Growth rate was measured by placing 4 mm disks from one week old cultures on the surface of three Petri dishes containing 20 ml MEA. Two perpendicular measurements of colony diameter were made after 8 days of incubation in the dark at temperatures ranging from 10 to 35 C at 5 degree intervals.

Tolerance to cycloheximide was tested by removing 4 mm disks from a one week old culture and placing them on the surface of five plates containing 0, 0.05, 0.1, 0.5, 1.0, 2.5 and 5.0 g per 1000 ml MEA. Colony diameter was measured after plates had been incubated at 25 C in the dark for 4 and 8 days.

Specimens for Scanning Electron Microscopy (SEM) were fixed in glutaraldehyde and osmium tetroxide and dehydrated in a graded acetone series. Material was critical point dried (Cohen, **1970**), coated wih gold paladium and examined in a ISI scanning electron microscope.

RESULTS

The morphological characteristics of the *Ceratocystiopsis* sp. found on *P. repens* differed from those of all previously described species of *Ceratocystiopsis*, *Ceratocystis* and *Ophiostoma*. This fungus is therefore described as new.

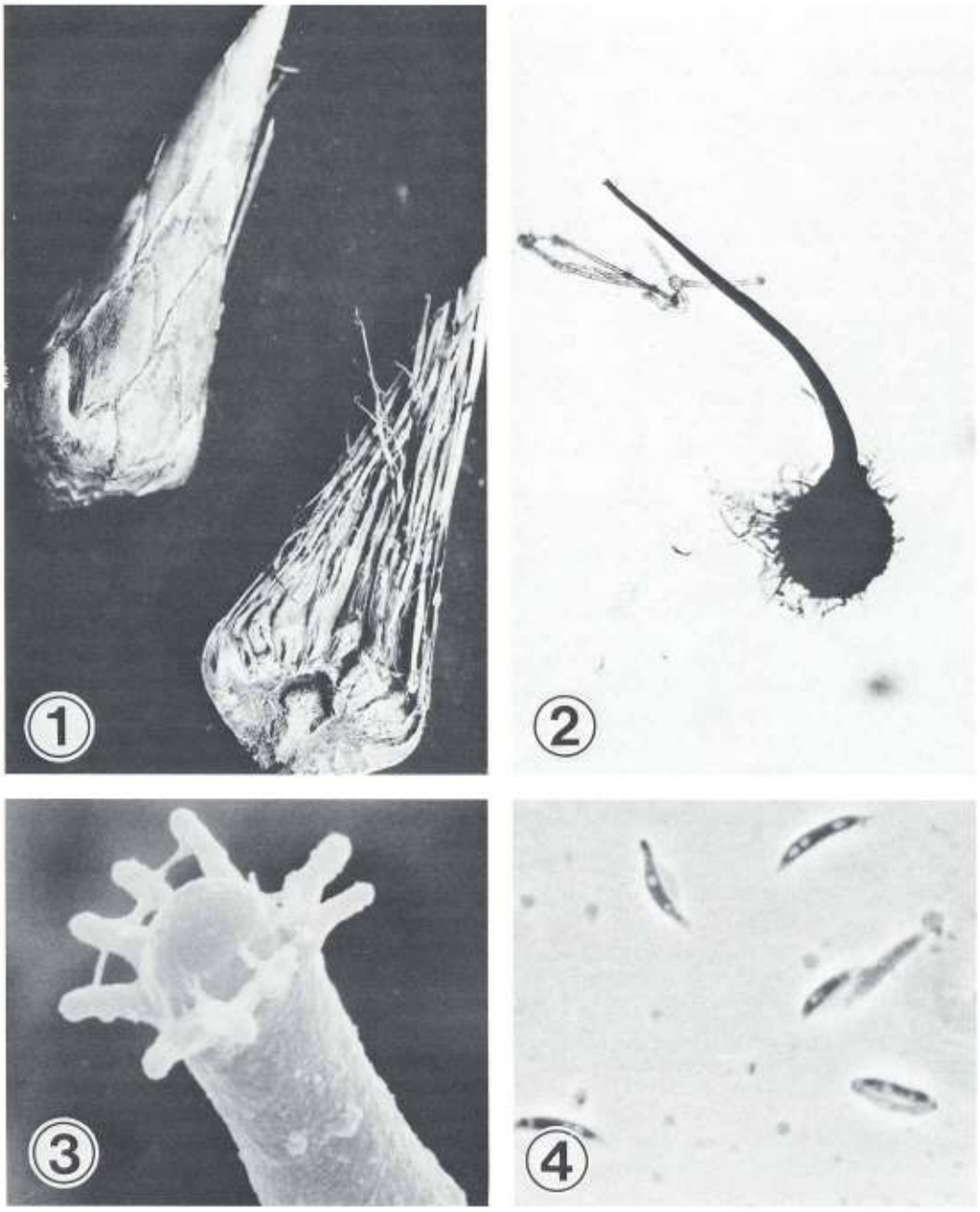
Ceratocystiopsis proteae Wingfield, Van Wyk & Marasas, *sp. nov.*

Perithecia superficialia; basis nigra, globosa, (9–216(151) μm diam; collum nigrum, glabrotunicatum, 168–354(285) μm longum, ad basim 20 μm latum, pars media 12–24(15) μm, attenuatum ad apicem; hyphae ostiolares 5–11, hyalinae ad pallide olivaceo-brunneae, obtusae, 2–7(2) μm longae. Asci evanescentes. Ascosporae hyalinae, aseptatae, fusiformes, lunatae vel falcatae, vagina gelatinosa adest, 7–13 × 2–4(10 × 3) μm.

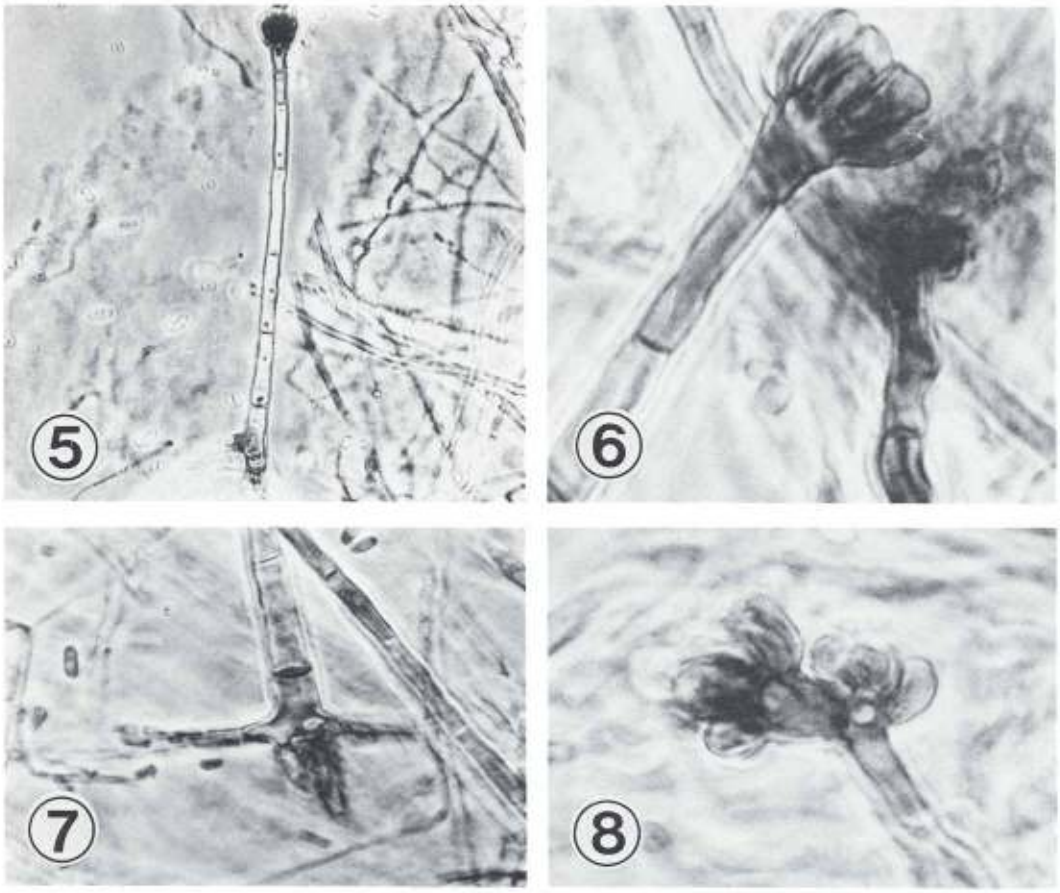
Hab. in inflorescentis *Proteae repens* (L.) L. insectis infestis.

HOLOTYPUS: Stellenbosch, Cape Province, South Africa, May 1986, L. J. Strauss, PREM 48924.

Perithecia (FIGS. 2, 3, 15) produced on host tissue and in culture on primary isolation plates (2% MEA) only, after which only conidiophores of the anamorph are produced. On the host, perithecia are superficial on the mycelium; bases black, globose, unornamented, 99–216 (151) μm diam; necks black, smooth-walled, 168–354(285) μm long, 14–27(20) μm wide at the base, 12–24(15) μm wide in the center, tapered towards the apex, terminating in 5–11 ostiolar hyphae (FIGS. 3, 13) which are divergent, obtuse, hyaline to light olivaceous brown, 2–7(2) μm long. Asci evanescent. Ascospores (FIGS. 4, 14) one-celled, hyaline, fusiform in side view with a hyaline gelatinous sheath giving a lunate to falcate appearance, accumulating in a hyaline droplet at the neck apex, 7–13 × 2–4(10 × 3) μm.



FIGS. 1–4. *Protea repens* flowers, perithecium and ascospores of *Ceratocystiopsis proteae*. 1. Inflorescences of *Protea repens*, approximately life size. 2. Perithecium, ×320. 3. Apex of perithecium, ×6500. 4. Falcate ascospores, ×2500.



FIGS. 5–8. Conidiophores of *Knoxdaviesia proteae*, anamorph of *Ceratocystiopsis proteae*. 5. Conidiophore, ×900. 6. Conidiophore with conidiogenous cells, ×3200. 7. Rhizoids at base of conidiophore, ×1600. 8. Conidiogenous cells branched at apex of conidiophore, ×2100.

SPECIMENS EXAMINED: On flowers within inflorescences of *Protea repens* infested by insects, Stellenbosch, Cape Province, South Africa, May 1986, L. J. Strauss, PREM 48924 HOLOTYPE. PARATYPES, PREM 48925, PREM 48926 on insect-infested *P. repens* flowers, Stellenbosch, November 1986, M. J. Wingfield.

Knoxdaviesia Wingfield, Van Wyk & Marasas, *gen. nov.* Hyphomycetes, Dematiaceae.

Conidiophora macronematosa, mononematosa, septata, olivaceo-brunnea, oriunda a hyphis rhizoidalibus; stipes erectus, simplex, inflatus ad apicem. Cellulae conidiogenae (phialides) terminales, discretae, ovoideae, olivaceo-brunneae. Conidia holoblastica, hyalina, aseptata, laevia, cylindrica vel allantoidea, aggregantia in capitulis mucoidis.

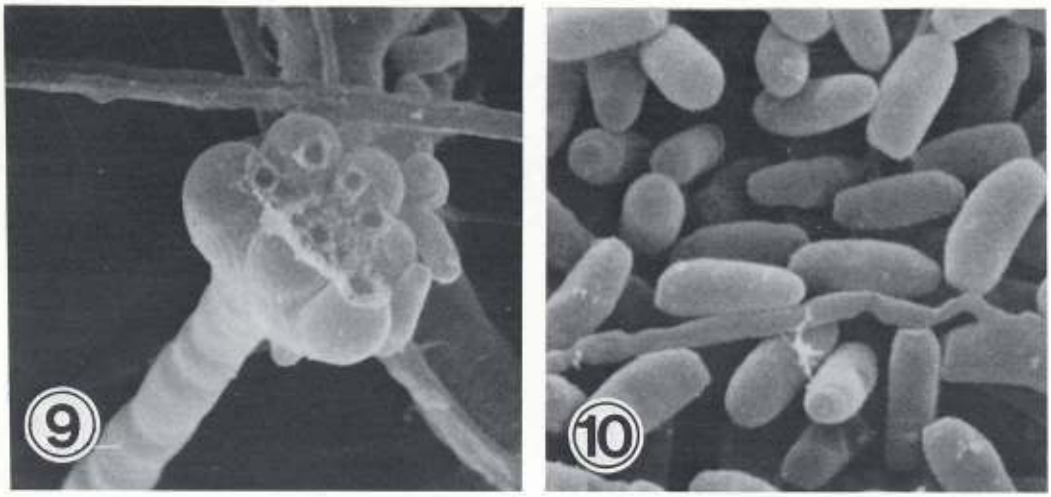
SPECIES TYPICA: *Knoxdaviesia proteae* Wingfield, Van Wyk & Marasas.

Conidiophores macronematous, mononematous, olivaceous-brown, septate, arising from well developed rhizoids; stipe erect, simple, inflated at the apex. Conidiogenous cells (phialides) produced terminally on conidiophores, discrete, ovoid, olivaceous-brown, producing conidia at the apex and leaving minute collarettes. Conidia holoblastic, hyaline, one-celled, smooth-walled, cylindrical to allantoid, rounded at the apex and truncate at the base, produced in mucoid masses at the apex of conidiophores.

ETYMOLOGY: Named after Professor Peter S. Knox-Davies of the University of Stellenbosch who has made major contributions to the recognition of *Protea* fungi.

Knoxdaviesia proteae Wingfield, Van Wyk & Marasas, *sp. nov.*

Coloniae in agaro "malt extract" 8 diebus apud 25 C, 2.65 cm diam, primo hyalinae, deinde olivaceo-brunneae; mycelium aerium restrictum; mycelium im-



FIGS. 9, 10. Conidiogenous cells and conidia of *Knoxdaviesia proteae*, anamorph of *Ceratocystiopsis proteae*. 9. Conidiophore and conidiogenous cells with small apical collarettes, ×5000. 10. Conidia, ×6500.

mersum ex hyphis septatis, ramosis, primo hyalinis, deinde olivaceis, glabrotunicatis, 3–7(5) μm diam, compositum. Conidiophora macronematosa, mononematosa, septata, glabrotunicata, olivaceo-brunnea, oriunda a 1–5 hyphis rhizoidalibus, 5–23 × 3–7(13 × 5) μm; stipes erectus, simplex, 69–198(132) μm longus, 7–10(8) μm latus ad basim, attenuatus ad apicem inflatum. Cellulae conidiogenae (phialides) 7–12, terminales, ovoideae, olivaceo-brunneae, 6–10 × 5–6 (9 × 5) μm. Conidia holoblastica, aseptata, hyalina, laevia, cylindrica vel allantoidea, ad apicem obtusa, ad basim truncata, 4–7 × 2–4(5 × 3) μm.

HOLOTYPUS: Cultura exsiccata in agaro, sejuncta a inflorescentis *Proteae repens* insectis infestis, Stellenbosch, Cape Province, South Africa, May 1986, L. J. Strauss, PREM 48928.

Colonies on half strength MEA relatively slow growing, 2.65 cm diam after 8 days at 25 C, growth reduced at temperatures below 25 C with virtually no growth at 10 C; at first colorless, becoming olivaceous-brown. Aerial mycelium sparse, submerged mycelium composed of septate, sparingly branched, smooth-walled hyphae, hyaline at first and olivaceous-brown in age, 3–7(5) μm diam. Conidiophores (FIGS. 5–8, 11, 12) produced sparsely on host tissue but relatively abundantly on the surface of half strength MEA; on agar macronematous, mononematous, septate, smooth-walled, olivaceous-brown, arising from 1–5 well developed rhizoids (FIGS. 7, 11) which are 5–23(13) μm long and 3–7(5) μm wide in the center; stipe erect, simple, 69–198(132) μm long, 7–10(8) μm diam at the base and tapering slightly towards the inflated apex, terminating directly without metulae in 7–12 conidiogenous cells. Conidiogenous cells (phialides) (FIGS. 6, 8, 9, 12) ovoid, olivaceous-brown, 6–10(9) μm long, 5–6(5) μm wide, with indistinct collarettes (FIGS. 9, 12), occasionally resuming growth to give rise to a second set of conidiogenous cells (FIGS. 8, 11). Conidia (FIGS. 10, 11) holoblastic, one-celled, hyaline, smooth-walled, cylindrical to allantoid, rounded at the apex and truncate at the base, 4–7 × 2–4(5 × 3) μm.

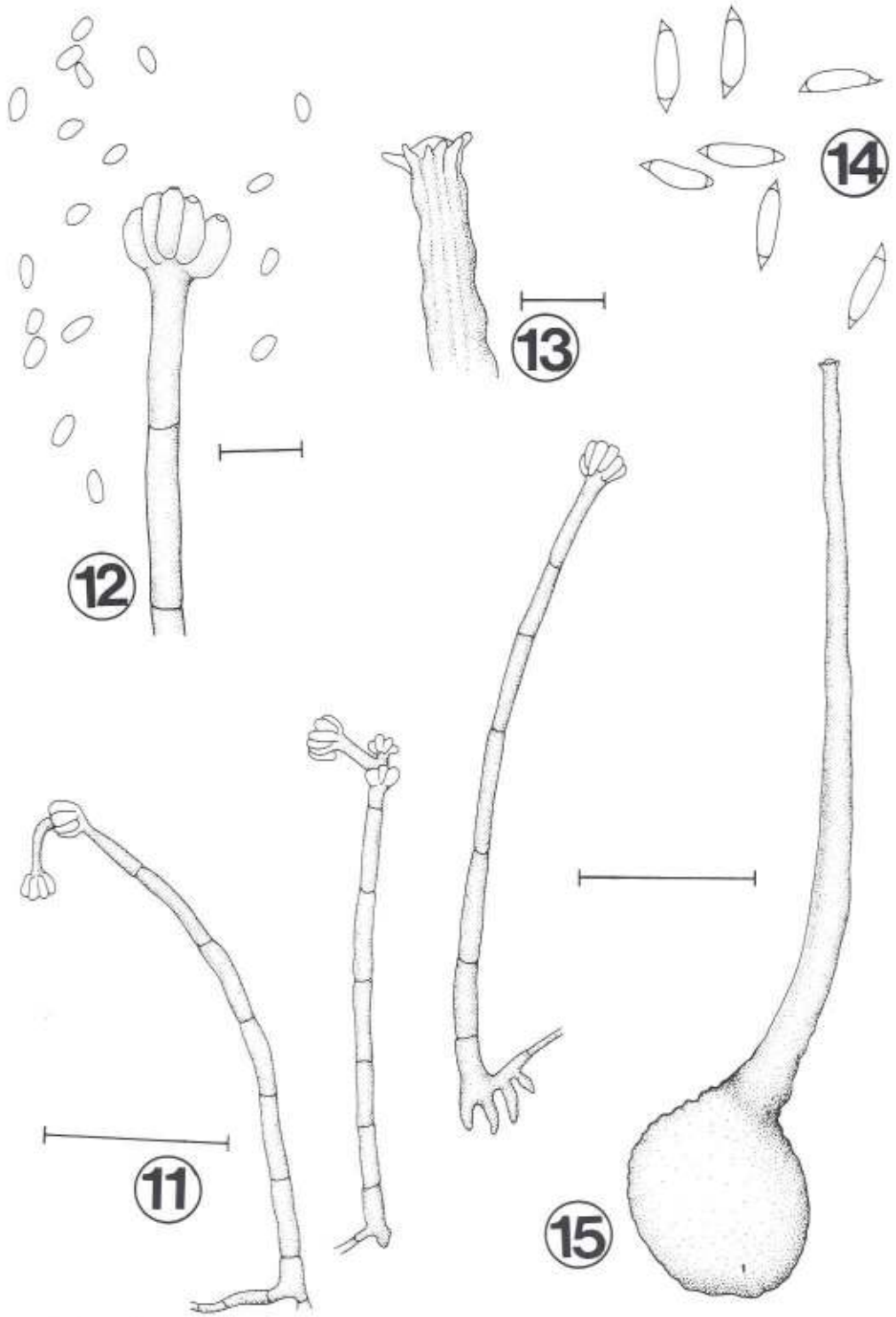
SPECIMENS EXAMINED: Cultures on 2% MEA, isolated from perithecia on flowers within inflorescences of *Protea repens* infested by insects, Stellenbosch, Cape Province, South Africa, May 1986, L. J. Strauss, PREM 48928, HOLOTYPE. ISOTYPES, PREM 48929, PREM 48930 from perithecia on *P. repens* flowers, Stellenbosch, May 1986, L. J. Strauss.

Dried down cultures of the holotype as well as permanent slide preparations have been deposited in the National Collection of Fungi, Plant Protection Research Institute, Pretoria, South Africa (PREM). Subcultures of the type strain have been deposited in CBS and CMI.

No growth of *C. proteae* was observed after 4 days on any cycloheximide concentration tested. After 8 days, colonies were an average of 26.5 mm on control plates, 5.7 mm on the lowest concentration (0.05 g/L) of cycloheximide and no growth occurred at any other concentrations tested.

DISCUSSION

Although for now we recognize the genus, it is our opinion that *Ceratocystiopsis* requires reconsideration. Ascospore morphology does not appear to be a character sufficiently constant to



FIGS. 11–15. *Ceratocystiopsis proteae* with anamorph *Knoxdaviesia proteae*. 11. Conidiophores. 12. Conidiogenous cells and conidia. 13. Apex of perithecium with ostiolar hyphae. 14. Ascospores. 15. Perithecium. Scale bars, FIGS. 12, 13, 14 = 10 μm; 11, 15 = 50 μm.

determine generic status. Upadhyay (**1981**) established sections in *Ceratocystis s.l.* separated on the basis of ascospore morphology. For example, section *Ips* was distinguished by having pillow shaped gelatinous sheaths, ascospores in section *Ophiostoma* lack gelatinous sheaths whereas those in section *Endoconidiophora* are inequilateral and elongated. The presence of falcate sheaths could equally be assigned to a sectional rather than generic status. Most *Ceratocystiopsis* spp. would then be accommodated in *Ophiostoma* and their relatedness would be more obvious.

The new species described here has sheathed, elongate ascopores and is therefore currently best placed in the genus *Ceratocystiopsis*. *Ceratocystiopsis proteae* is, however, morphologically unique among the *Ceratocystiopsis* spp. and shares characters with *Ceratocystis sensu stricto* and *Ophiostoma*. Only one species of *Ceratocystiopsis*, *C. crassivaginata* (Griffin) Upadhyay (**1981**) has divergent ostiolar hyphae similar to those of *C. proteae*. *Ceratocystiopsis crassivaginata*, unique among *Ceratocystiopsis* spp. for its *Leptographium* anamorph, has recently been included in *Ophiostoma* (Harrington, **1987**). *Ceratocystiopsis* spp. are described as having perithecial necks that are either absent or short. The new species described here has perithecial necks that are unusually long amongst *Ceratocystiopsis* spp. and that would be more common amongst *Ophiostoma* spp.

The occurrence of an anamorph with apical wall building in *C. proteae* suggests that it is more closely related to *Ophiostoma* than *Ceratocystis s.s.* However, the sensitivity of this species to cycloheximide is a characteristic in common with *Ceratocystis s.s.* and sets it apart from most *Ophiostoma* spp. Further studies, including an analysis of cell wall components and a detailed examination of conidium development, would, therefore, be warranted.

Leptographium is the only anamorph of *Ceratocystis s.l.* that resembles the anamorph of *C. proteae*. However, *Knoxdaviesia* described here is distinct from *Leptographium* in its absence of series of metulae and the presence of conidiogenous cells directly on the stipe. In addition, conidia in *Leptographium* spp. are produced through percurrent or sympodial proliferation of conidiogenous cells (Wingfield, **1985**). In *Knoxdaviesia*, the mode of conidial development is not clear. However, conidia arise from phialides with indistinct collarettes, apparently through an apical wall building process (Minter *et al.*, **1982**).

The hyphomycete genus *Stachybotrys* has ovoid phialides produced directly on the conidiophore stipe and thus resembles *Knoxdaviesia*. However, *Stachybotrys* spp. typically have dark conidia, and one species, *S. albipes* (Berk. and Br.) Jong and Davis has a teleomorph in *Melanopsamma* Niesel (Jong and Davis, **1976**). This indicates a lack of relatedness between *Stachybotrys* and the anamorph of *C. proteae*. *Knoxdaviesia* also superficially resembles some species of *Phialocephala* although conidiogenous cells in *Phialocephala* are usually subtended by series of metulae. We, however, consider *Phialocephala* to be a heterogeneous form genus containing a number of unrelated species. None of these species are known to be anamorphs of *Ceratocystis s.s.* and the allocation of a new genus for the anamorph of *Ceratocystiopsis proteae* is most appropriate.

Ceratocystiopsis proteae occurs in insect-infested inflorescences of *Protea repens* and appears to be saprophytic on the flowers. *Ceratocystis* spp. *s.l.* are known to be disseminated by insects. It is thus assumed that one or more of the many insect species that infest *P. repens* inflorescences (Coetzee and Gilomee, **1987**) are vectors of *C. protea*.

Most *Ceratocystis* spp. *s.l.* are known from Northern Hemisphere and most occur associated with insects on trees. It is therefore not surprising that a new *Ceratocystiopsis* from southern Africa on a substrate not previously known to harbor related fungi should include characteristics not fully accommodated by taxonomic schemes for *Ceratocystis s.l.* Other similar fungi may occur in flowers of Proteaceae and further collections could broaden our understanding of the Ophiostomataceae.

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