Cornuvesica, a new genus to accommodate *Ceratocystiopsis falcata*

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Most species in *Ceratocystiopsis* share many characters with species in *Ophiostoma*. Unlike other *Ceratocystiopsis* species, *C. falcata* appears to have enteroblastic conidium development and what appears to be a *Chalara* anamorph. Morphologically, *Cp. falcata* is thus more similar to *Ceratocystis* (with *Chalara* anamorphs) than *Ophiostoma*, although it is very distinct from *Ceratocystis* in perithecial characteristics. Recent studies using molecular data have shown that, despite its peripheral similarity to *Ceratocystis*, *Cp. falcata* is distantly related to either *Ceratocystis* or *Ophiostoma*. In view of this molecular evidence a new genus, *Cornuvesica*, is described for it, characterized by having falcate, sheathed and septate ascospores. The anamorph of *Cornuvesica* is morphologically similar to *Chalara* but conidia are obovoid with only one truncate end. Conidia are apparently not produced by ring wall building and *Chalara* is not an appropriate genus for it. We suggest that the anamorph of *Cornuvesica* represents a genus distinct from *Chalara*.

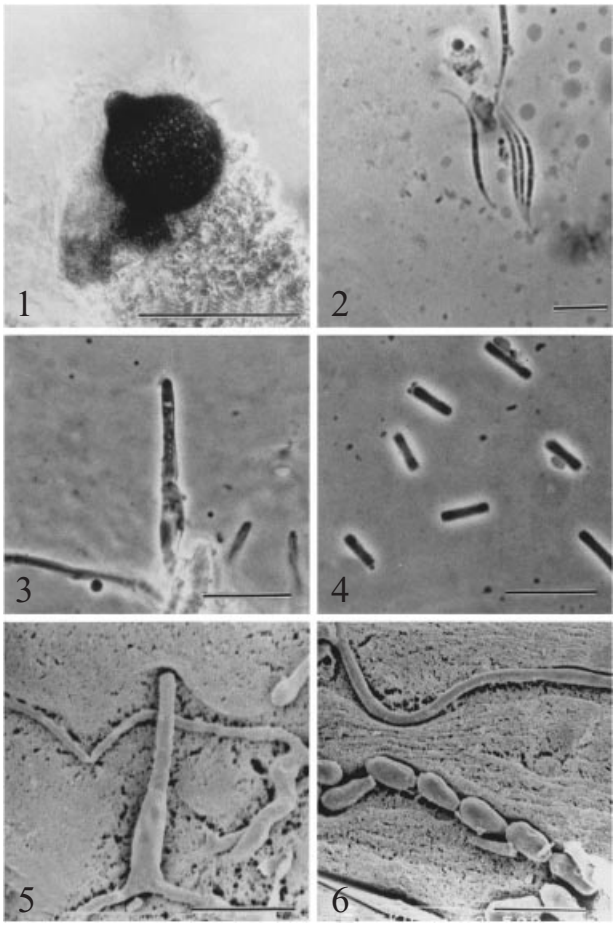
Ceratocystis falcata E. F. Wright & Cain was first described as having falcate ascospores with attenuated ends (Wright & Cain, 1961). These authors noted that the small perithecia have short conical necks unlike other species of *Ceratocystis sensu lato*. Furthermore, the ascospores of *C. falcata* are similar in shape to those of *C. minuta* (Siemaszko) J. Hunt, although longer and septate (Wright & Cain, 1961). The fungus was not obtained in culture and only the teleomorph was described. Rayner & Hudson (1977) re-examined the herbarium material deposited by Wright & Cain (1961), as well as additional herbarium material and single ascospore cultures. They described the anamorph of *C. falcata* as *Chalara*-like with conidia produced endogenously in chains. Conidia were described as oblong with truncate ends (Rayner & Hudson, 1977).

Upadhyay & Kendrick (1975) established *Ceratocystiopsis* to accommodate species of *Ceratocystis s. l.* with sheathed, elongate to falcate ascospores with attenuated ends. In doing so they included *C. falcata* in the genus, due to the fact that it had falcate ascospores. It was, however, clearly exceptional in this group, since it had septate ascospores and was the only species with a *Chalara*-like anamorph (Hutchison & Reid, 1988; Nag Raj & Kendrick, 1993).

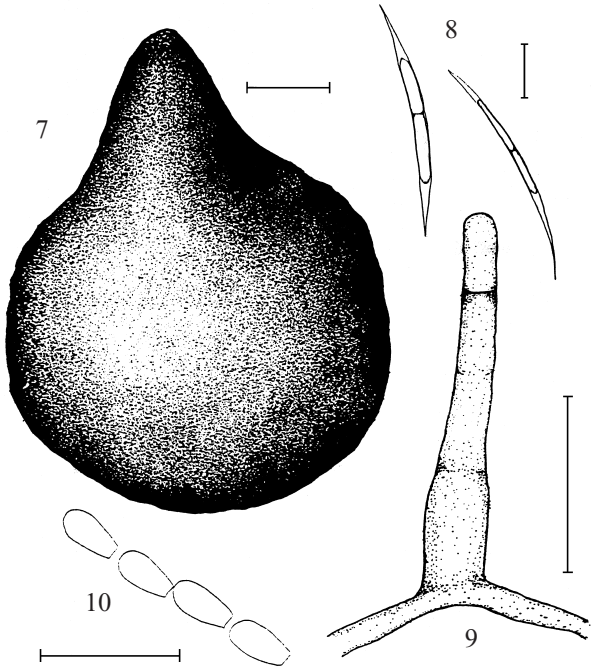
Currently, *Ceratocystiopsis falcata* (E. F. Wright & Cain) H. P. Upadhyay can be distinguished from all other species in *Ceratocystiopsis* by its septate ascospores and a *Chalara* anamorph (Upadhyay, 1981). Species in *Ceratocystiopsis* are thus characterized by having a number of different anamorphs including *Chalara*, *Hyalorhinocladiella* and *Sporothrix*. These anamorphs, with the exception of *Chalara*, have holoblastic conidiogenesis (Minter, Kirk & Sutton, 1983) and are similar to those found in *Ophiostoma* (Wingfield, 1993; Mouton, Wingfield & Van Wyk, 1994).

Wingfield, Van Wyk & Marasas (1988) argued that most species of *Ceratocystiopsis* could be accommodated in *Ophiostoma* based on anamorph morphology. Wingfield (1993) noted that *Cp. falcata*, with an apparent *Chalara* anamorph, would be more appropriately placed in *Ceratocystis* than *Ophiostoma*. De Hoog & Scheffer (1984), however, considered *Cp. falcata* similar to *Pyxidiophora*. Nag Raj & Kendrick (1993) agreed with the latter view but suggested that *Cp. falcata* could be accommodated equally well in *Ceratocystis*, and concluded that a more critical assessment of this species was needed (Nag Raj & Kendrick, 1993).

Hausner, Reid & Klassen (1993) sequenced the small subunit ribosomal RNA gene of species of *Ceratocystiopsis*, to determine whether the separation of species of *Ceratocystiopsis* from *Ophiostoma* could be justified. It was concluded that species of *Ceratocystiopsis* spp., with the exception of *Cp. falcata*, grouped with species of *Ophiostoma*. Sequence data also suggested that *Cp. falcata* was phylogenetically distinct from both *Ceratocystis* and *Ophiostoma* (Hausner *et al.*, 1993). Hausner *et al.* (1993) brought *Ceratocystiopsis* into synonymy with *Ophiostoma*, but retained *Cp. falcata* due to its uncertain



Figs 1–6. *Cornuvesica falcata*. Light and scanning (SEM) micrographs. **Fig. 1.** Ascomatal structure. **Fig. 2.** Septate, falcate ascospores. **Fig. 3.** *Chalara*-like anamorph. **Fig. 4.** Conidia. **Fig. 5.** *Chalara*-like structure. **Fig. 6.** *Endogenously* produced conidia with one truncate end. Bars: Fig. 1 = 100 μm, Figs 2–6 = 10 μm.



Figs 7–10. *Cornuvesica falcata*. **Fig. 7.** Perithecium. **Fig. 8.** Ascospores. **Fig. 9.** Conidiophore. **Fig. 10.** Conidia. Bars = 10 μm.

generic affinity. Viljoen (1996) confirmed the findings of Hausner *et al*. (1993) using RFLP and DNA hybridization data.

In this study we re-examine the herbarium material of *Cp. falcata* deposited by Wright & Cain (1961) and Rayner & Hudson (1977). A culture of *Cp. falcata*, isolated by Hudson, was also obtained for critical study. We describe a new teleomorph genus for *Cp. falcata* and include critical comments on the anamorph state.

For cultural studies, the isolate of *Cp. falcata* (ATCC 36538) was maintained on 2% malt extract agar (MEA, Biolab) and in liquid media (10 g l^{-1} Biolab malt extract, 10 g l^{-1} Biolab yeast extract, 5 g l^{-1} Biolab tryptone and 2 g l^{-1} Difco yeast carbon base). Isolates were incubated in the dark at 20 °C. Light microscopy was done on structures taken from the living culture and herbarium material. Specimens for SEM were fixed in glutaraldehyde and osmium tetroxide and dehydrated in a graded acetone series. Material was critical point dried (Cohen, 1970), coated with gold/palladium and examined using a Jeol JSM 6400 scanning electron microscope.

Cp. falcata has sheathed, falcate and septate ascospores with attenuated ends. The presence of septate ascospores makes this species morphologically distinct from other *Ceratocystiopsis* and *Ophiostoma* spp. Although Wright & Cain (1961) provided a description for the fungus, it is incomplete and a new genus, *Cornuvesica*, is described as follows:

Cornuvesica C. D. Viljoen, M. J. Wingf. & K. Jacobs, **gen. nov.**

Perithecia superficialia, atrobrunnea vel nigra. Collum fundo peritheciali atrius. Filamenta in apicibus obtusis terminantia, convergentia, orificium angustum formantia vel parum ultra apicium colli protrudentia. Asci evanescentes, cito deliquescentes; ascosporae falcatae, atrae, septatae, a vagina hyalina ambobus extremitatibus attenuatis circumcinctae.

Perithecia superficial, dark brown to black. Neck darker than the perithecial base. Filaments terminating in obtuse apices, converging to form a narrow opening or slightly protruding beyond apical part of neck. *Asci* evanescent, deliquescing at an early stage. *Ascospores* falcate, dark, surrounded by hyaline sheath with both ends attenuated, single septate.

Typus: Cornuvesica falcata (E. F. Wright & Cain) C. D. Viljoen, M. J. Wingf. & K. Jacobs.

Cornuvesica falcata (E. F. Wright & Cain) C. D. Viljoen, M. J. Wingf. & K. Jacobs, **comb. nov.**

Ceratocystiopsis falcata (E. F. Wright & Cain) H. P. Upadhyay, *A monograph of Ceratocystis and Ceratocystiopsis*: 125 (1981).

Ceratocystis falcata E. F. Wright & Cain, *Canadian Journal of Botany* **39**: 1226. (1961).

Perithecia on wood, superficial, dark brown to black, (58–) 73 (–87) μm diam. (Fig. 1, 7). Neck darker than the perithecial base, (18.5–) 27.5 (34) μm long, (9.5–) 11 (–15.5) μm wide at apex, (21.5–) 27 (–40.5) μm wide at the base (Fig. 1, 7). Filaments terminating in obtuse apices, converging to form a

narrow opening or slightly protruding beyond apical part of neck. *Asci* evanescent, deliquescing at an early stage. *Ascospores* falcate, dark, surrounded by hyaline sheath with both ends attenuated, one septate, (15.5–) 24 (–28) × 0.5–1.5 μm (Figs 2, 8).

Colonies very slow growing on 2% MEA, as noted by Rayner & Hudson (1977), but tending to grow faster in liquid medium where the fungus produces a dark brown mycelial mat. *Phialides* hyaline, borne singly, 18–26 μm long, 1–2 μm at the apex, 2–4 μm at the base (Figs 3, 5, 9). *Conidia* produced endogenously in false chains, obovoid with one end truncate, 5 × 1.5–2 mm (Figs 4, 6, 10).

A description of the anamorph of *C. falcata* was not provided by Wright & Cain (1961). Rayner & Hudson (1977) documented the presence of a *Chalara*-like anamorph, and provided line drawings. Examination of the type material deposited by Wright & Cain (1961) revealed no anamorph structures, which was also the case with the material deposited by Rayner & Hudson (1977). The culture examined by us was similar to that described by Rayner & Hudson (1977) (Figs 3–6, 9, 10). Using light microscopy the anamorph of this fungus superficially appears to be *Chalara*-like (Figs 3, 4). Electron microscopy revealed, however, that conidia have one truncate end and do not appear to be produced through the process of ring wall building (Figs 6, 10). This anamorph is, therefore, unlike a typical *Chalara*.

Species of *Chalara* are characterized by ring wall building conidium development resulting in chains of conidia (Nag Raj & Kendrick, 1975; Minter *et al*., 1983). In contrast, conidia produced by *Cn. falcata* have one truncate end and are not produced through the process of ring wall building. The anamorph structure found in *Cn. falcata* is unlike typical species of *Chalara* (Nag Raj & Kendrick, 1993). We suggest that the anamorph of *Cn. falcata* represents a genus distinct from *Chalara*. Wingfield *et al*. (1995) reported a similar situation for the anamorph of *C. autographa*, and a more comprehensive assessment of this anamorph is needed before a name might be applied to it.

Hutchison & Reid (1988) noted two *Chalara* anamorphs for *C. falcata*. One was similar to that reported previously (Rayner & Hudson, 1977; Upadhyay, 1981). The other produced much larger phialides and phialospores that developed in immediate association with the perithecia, the production of which were stimulated when exposed to culture extracts, or in association with *Gliocladium roseum* culture.

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REFERENCES

Cohen, A. L. (1970). *Critical Point Drying. Principles and Techniques of Electron Microscopy: Biological Application* (ed. M. A. Hyat). Von Nostrand Reinhold: New York.

De Hoog, G. S. & Scheffer, R. J. (1984). *Ceratocystis* versus *Ophiostoma*: a reappraisal. *Mycologia* **76**, 292–299.

Hausner, G., Reid, J. & Klassen, G. R. (1993). *Ceratocystiopsis*: a reappraisal based on molecular criteria. *Mycological Research* **97**, 625–633.

Hutchison, L. J. & Reid, J. (1988). Taxonomy of some potential wood-staining fungi from New Zealand 1. Ophiostomataceae. *New Zealand Journal of Botany* **26**, 63–81.

Minter, D. W., Kirk, P. M. & Sutton, B. C. (1983). Thallic phialides. *Transactions of the British Mycological Society* **80**, 39–66.

Mouton, M., Wingfield, M. J. & Van Wyk, P. S. (1994). Conidium development in anamorphs of *Ceratocystis sensu lato*: a review. *South African Journal of Science* **90**, 293–298.

Nag Raj, T. R. & Kendrick, W. B. (1975). *A Monograph of* Chalara *and Allied Genera*. Wilfrid Laurier University Press: Waterloo.

Nag Raj, T. R. & Kendrick, W. B. (1993). The anamorph as generic determinant in the holomorph: the *Chalara* connection in the Ascomycetes, with special reference to the ophiostomatoid fungi. In Ceratocystis *and* Ophiopstoma. *Taxonomy, Ecology and Pathogenicity* (ed. M. J. Wingfield, K. A. Seifert & J. F. Webber), pp. 61–70. APS Press: St Paul, Minnesota.

Rayner, A. D. M. & Hudson, H. J. (1977). *Ceratocystis falcata* and its conidial state. *Transactions of the British Mycological Society* **68**, 315–316.

Upadhyay, H. P. (1981). *A monograph of* Ceratocystis *and* Ceratocystiopsis. University of Georgia Press: Athens GA.

Upadhyay, H. P. and Kendrick, W. B. (1975). Prodromus for a revision of *Ceratocystis* (Microascales, Ascomycetes) and its conidial states. *Mycologia* **67**, 798–805.

Viljoen, C. D. (1996). *A taxonomic study of* Ophiostoma sensu lato *with special reference to species associated with* Protea *infructescences in southern Africa*. Ph.D. Thesis, University of the Free State, Bloemfontein, South Africa.

Wingfield, M. J. (1993). Problems in delineating the genus *Ceratocystiopsis*. In Ceratocystis *and* Ophiostoma. *Taxonomy, Ecology and Pathogenicity* (ed. M. J. Wingfield, K. A. Seifert & J. F. Webber), pp. 21–25. APS Press: St Paul, Minnesota.

Wingfield, M. J., Van Wyk, P. S. & Marasas, W. F. O. (1988). *Ceratocystiopsis proteae* sp. nov. with a new anamorph genus. *Mycologia* **80**, 23–30.

Wingfield, M. J., Benade, E., Van Wyk, P. S. & Visser, C. (1995). Conidium development in *Ceratocystis autographa*. *Mycological Research* **99**, 1289–1294.

Wright, E. F. & Cain, R. F. (1961). New species of the genus *Ceratocystis*. *Canadian Journal of Botany* **39**, 1215–1230.