

THE PERFECT STATE OF SARCOPHOMA MIRIBELII

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(With six Text-figures)

Guignardia miribelii is described as a new species. It represents the ascigerous state of the fungus of which the conidial state is known as *Sarcophoma miribelii*. Both states are described.

During the 6th European Mycological Congress in Avignon, France, in October 1974, a foray was made in a Cedar forest on the Mont Ventoux. Several interesting Ascomycetes and Deuteromycetes were collected on *Buxus sempervirens*, a common shrub in the area. One such fungus, obtained from thin twigs on withered or almost dead branches, proved to be an unknown species of the genus *Guignardia* Viala & Ravaz, with asci containing a variable number, usually 16 or 32 ascospores. Single ascospore isolates were prepared from ascospores originating from four ripe perithecia. All isolates gave sporulating colonies of a coelomycete, which was recognized as *Sarcophoma miribelii* (Fr.) Höhn. This common and wide-spread fungus frequently occurred on dead leaves and branches of *Buxus* in the area. The ascospore isolates were thus easily compared with fresh conidial isolates and subsequently with several herbarium specimens. These comparative studies showed that the two states belong to the same fungus. The present paper describes both states, with the ascigerous state as a new species.

***Guignardia miribelii* van der Aa, spec. nov.—Figs. 1, 2**

Ascomata discreta, nigra, lucida, in cortice submersa, tympaniformia, sursum applanata, epidermide firmiter connexa, deorsum rotundata, margine elevata, in medio maturitate dehiscentia, 100–200 μm alta, 150–350 μm plerumque 200–250 μm diam.; paries superior 15–20 μm , inferior et lateralis ad 50 μm crassus; pars exterior e 1–4 stratis cellularum nigrarum, isodiametricarum, 10–17 μm diam. constat, interior e cellulis subhyalinis, isodiametricis vel collapsis, tenuitunicatis, 7–15 μm diam. Asci numerosi e pulvillo basilari hemisphaerico oriuntur, fasciculati, sessiles vel breviter pedicellati, bitunicati, cylindrici vel clavati, 75–180 \times 15–20 μm , 16-vel 32-spori. Ascosporae continuae, ellipsoideae vel ovoideae, hyalinae, leves, maturitate nonnumquam olivaceae et minute punctatae, plerumque 13–19 \times 5–7 μm . paraphyses absunt. Typus Herb. CBS 214, in ramulis morientibus Buxi sempervirentis, in Silva Cedri atlanticae, in ascensu Montis Ventosi.

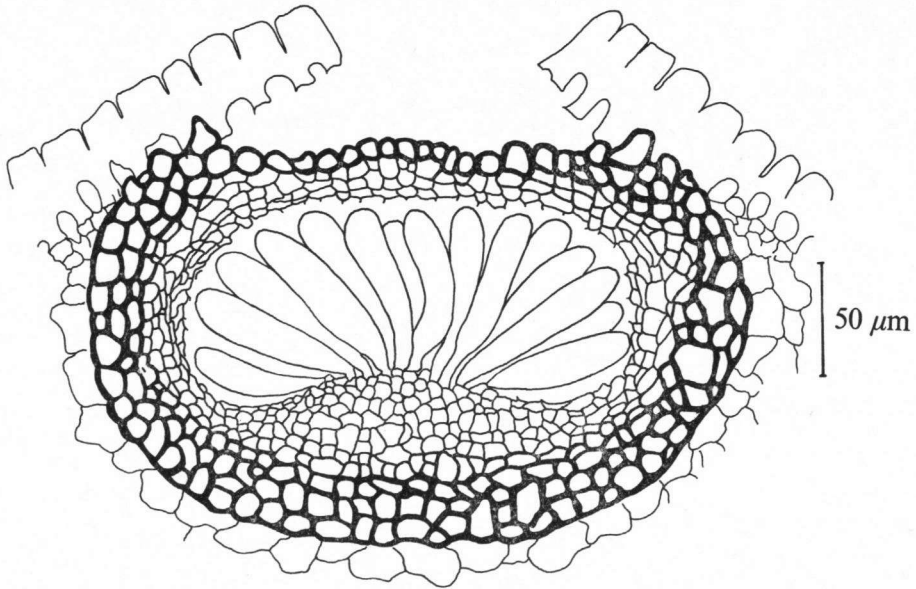


Fig. 1. *Guignardia miribelii* (holotype), ascoma, on twig of *Buxus sempervirens*.

Ascomata discrete, black, shiny, immersed in the bark and firmly grown together with the epidermis, tympaniform, apically flattened, rounded in the lower part, with a more or less raised peridial margin, without papilla, when ripe opening in the middle by dehiscence of the thin wall. Ascomata 100–200 μm high and 150–350 μm , usually 200–250 μm diameter. Wall 15–20 μm thick in the upper part and up to 50 μm thick in the lower and lateral part; the outer layer is composed of 1–4 rows of blackish, isodiametric, thick-walled cells, 10–17 μm in diameter and the inner layer of subhyaline, isodiametric or collapsed, thin-walled cells, 7–15 μm in diameter. Asci rather numerous, arising from a hemispherical basal cushion of thin-walled isodiametric cells, sessile or stipitate, bitunicate, cylindrical or clavate, 75–180 \times 15–20 μm , usually 16 or 32 spored. Ascospores one-celled, ellipsoidal, ovate, hyaline and smooth for a long time, when young surrounded by a caducous slime layer, in age sometimes becoming olivaceous and finely punctuate, usually 13–19 \times 5–7 μm , rarely with a deviating shape and size, up to 40 μm long in asci containing a smaller number of ascospores. Paraphyses absent.

On thin twigs of almost dead branches of *Buxus sempervirens*, Cedar forest, Mont Ventoux, France, 21.10.1974, *H. A. van der Aa* 4434 (Herb. CBS 214).

Conidial state: *SARCOPHOMA MIRIBELII* (Fr.) Höhn.—Figs. 3–5

Sphaeria miribelii Fr. in *Linnaea* 5: 548. 1830. — *Sphaeropsis miribelii* (Fr.) Lév. in *Annls Sci. nat. (Bot.)* III, 5: 296. 1846. — *Phoma miribelii* (Fr.) Sacc. in *Michelia* 2: 90. 1880. — *Macrophoma miribelii* (Fr.) Berl. & Vogl. in *Atti Soc. ven.-trent. Sci. nat.* 10: 179. 1886. — *Sarcophoma miribelii* (Fr.) Höhn. in *Hedwigia* 60: 133. 1918.

Sphaeria delitescens Wallr., *Fl. crypt. Germ.* 2: 777. 1833, — *Phoma delitescens* (Wallr.) Sacc., *Syll. Fung.* 3: 105. 1884.

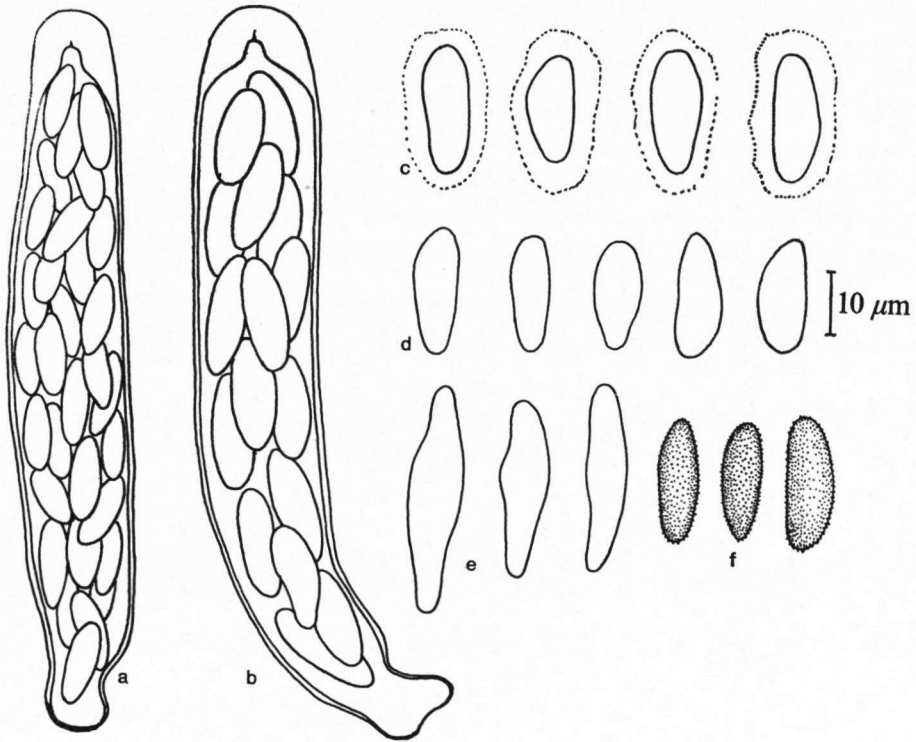


Fig. 2. *Guignardia miribelii* (holotype). — a. Ascus containing 32 ascospores. — b. Ascus containing 16 ascospores. — c. Young ascospores. — d. Ripe ascospores. — e. Some ascospores with deviating shape and size. — f. Old ascospores, pigmented and punctate.

Phacidium buxi Lasch in Klotzsch, Herb. viv. mycol. 1: 1154. 1847.

Gloeosporium pachybasium Sacc. in Michelia 2: 117. 1880.

Phoma phacidioides Sacc. in Michelia 2: 274. 1881, — *Phyllosticta phacidioides* (Sacc.) Allesch. in Rabenh. Kryptog.-Fl. 1(6): 25. 1898.

Gloeosporium louisiae Bäumler in Verh. Ver. Natur-Heilk. Pressburg N. F. 9: 100. 1896.

Macrophoma miribelii (Fr.) Berl. & Vogl. f. *ramicola* Oudem. in Ned. kruidk. Arch. III 2: 734. 1902.

Sarcophoma endogenospora Höhn. in Sber. K. Akad. Wiss. Wien (Math.-naturw. Kl. I) 125: 75. 1916.

Sclerophoma confusa Petrak in Annl. mycol. 20: 23. 1922 = *Phyllostictina confusa* (Petrak) Petrak apud Petrak & Sydow in Beih. Reprium Spec. nov. Regn. veg. 42 (1): 191. 1927.

Pycnidia discrete, pale yellowish, seldom darker or blackish, immersed, subglobose to tympaniform, firmly grown together with the epidermis, not papillate, at maturity forming a wide crateriform opening by dehiscence, surrounded by the remains of the epidermis. Pycnidial wall apically and laterally 10–25 µm thick, composed of 1–4 layers of rather thin-walled, yellowish brown, isodiametric cells, 5–15 µm in size. The pycnidial base is made up of parallel rows of thin-walled, hyaline, rarely pale brown, isodiametric cells, 5–12 µm in diameter. Conidiogenous cells lining the

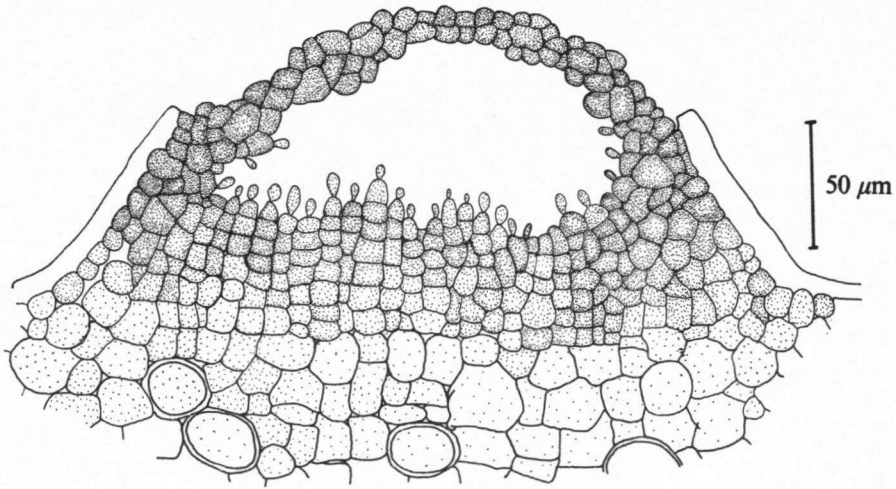


Fig. 3. *Sarcophoma miribelii* (H. A. van der Aa 4407), pycnidium on leaf of *Buxus sempervirens*.

cavity irregular, conical, sometimes indistinguishable from the adjacent wall cells especially in the lateral part, $9-20 \times 7-15 \mu\text{m}$, phialidic, forming conidia in a basipetal succession. Conidia one-celled, hyaline, ellipsoidal, ovate or pyriform, apiculate at the base, $9-19 \times 4.5-11 \mu\text{m}$, containing numerous small guttules and mostly one large central droplet, issuing in thick, whitish tendrils.

On twigs and leaves of *Buxus sempervirens*.

CULTURAL CHARACTERS.—Colonies on cornmeal and oatmeal agar growing moderately fast, attaining a diameter of 40–60 mm within 14 days at 25°C , initially honey yellow, very soon turning smoke brown, fresh isolates without aerial mycelium. Mycelium composed of hyaline, thin-walled, scarcely branched, septate hyphae, $4-15 \mu\text{m}$ diameter, with intercalary and terminal chlamydospores, present from the beginning, formed singly or in long chains, in fresh isolates arranged in conspicuous dark brown rows, in subsequent cultures regularly scattered, cylindrical, globose or pyriform when terminal, olivaceous to dark brown, $12-25 \times 10-16 \mu\text{m}$, usually covered by a greyish or brownish, fine granular exudate (Fig. 6b). From all mycelial cells, including chlamydospores, phialoconidia similar to pycnoconidia can be formed, usually very abundantly in fresh isolates on various media but inconspicuously or lacking in subsequent cultures. This state belongs to the form-genus *Hormonema* Lagerb. & Melin, and has to be distinguished from *Aureobasidium* by its polyphialidic conidiogenous cells from which conidia arise in basipetal succession (Figs. 6a, b). Pycnidial initials are formed from about the 5th day onwards, at first subglobose to hemispherical, discrete or confluent, with structures similar to those on the host plant, except the twisted, septate, brownish hyphae, irregularly ornamented with dark brown to black exudates, which form the covering layers of the pycnidia in early stages of development in vitro (Fig. 4). In 8–10 day old colonies the pycnidia split open raggedly and develop in a melanconiacous manner. The conidial slime is white but can turn brownish in old cultures.

MATERIAL EXAMINED (all on *Buxus sempervirens* L.).—Perfect state (*Guignardia miribelii*): On twigs, Mont Ventoux, Massif des Cédres, France, 21.10.-1974, H. A. van der Aa 4434 (CBS 214, holotype; single spore isolates in CBS 161.75).

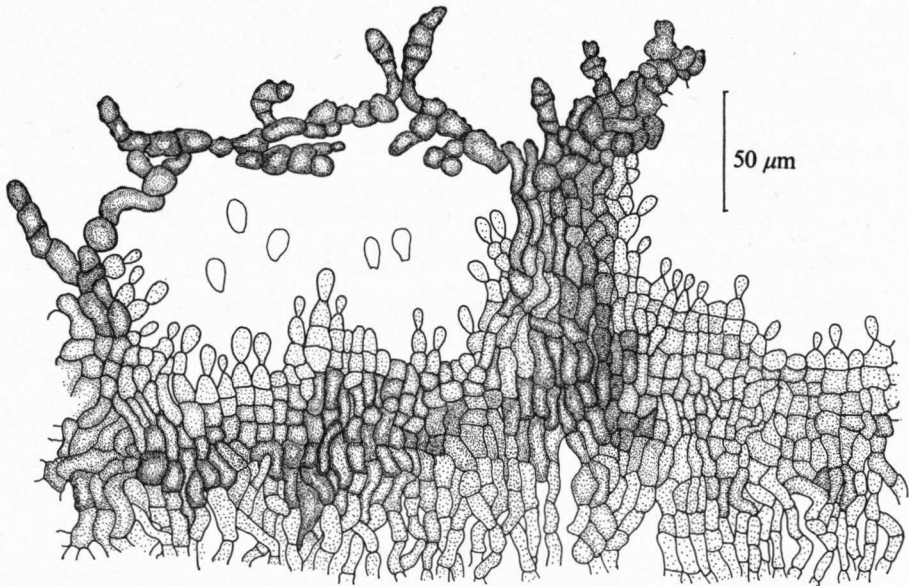


Fig. 4. *Sarcophoma miribelii* (CBS 161.75), pycnidium on cornmeal agar, 15 days old.

Conidial state (*Sarcophoma miribelii*): On dead leaves, Massif des Cèdres, Mont Ventoux, France, 21.10.1974, *H. A. van der Aa* 4407 (isolate in CBS 114.75). On leaves and twigs, Solothurn, Oberbuchsitzen, Switzerland, 16.7.1962, *J. A. von Arx* (Herb. CBS 125). On dead leaves and twigs, Transsylvania, Distr. Arad, Sofronea, Romania, *T. Savulescu* & *V. Bontea*, in Herb. mycol. Roman. 30: 1469 (Herb. CBS 234). On dead twigs, Nunspeet, Netherlands, *J. Beins* (two collections, 209 and 210, designed as type of *Macrophoma miribelii* f. *ramicola* Oudem., L). Valkenburg, Limburg, Netherlands, 1901, *J. Rick* (L).

DISCUSSION

Except for its many-spored asci, *Guignardia miribelii* fits very well in the genus *Guignardia* Viala & Ravaz (von Arx & Müller, 1954; Reusser, 1964; van der Aa, 1973; von Arx & Müller, 1975). The phenomenon of some ascospores becoming olivaceous and punctuate and others remaining hyaline and smooth at maturity even while germinating, is observed also in *Guignardia cytisi* (Fuckel) von Arx & Müller (von Arx & Müller, 1954; unpublished observations by the author).

Guignardia buxi (DC. ex Fr.) Lindau (Hilfsb. Sammeln Ascom. 21. 1903) belongs to the genus *Hyponectria* Sacc. (cf. von Arx & Müller, 1954: 180). *Guignardia buxicola* Camara & Luz (in Agron. lusit. 1: 44. 1939) differs in having eight-spored asci, while ascomata and ascospores are also aberrant.

The conidial state of *Guignardia miribelii* is found wherever the host plant occurs. The holotype specimen of *Sphaeria miribelii* Fr. is not preserved in Upsala and probably

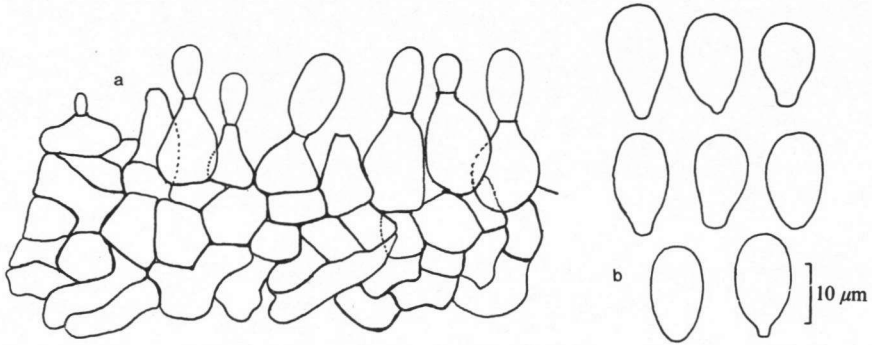


Fig. 5. *Sarcophoma miribelii* (CBS 161.75). — a. Conidiogenous cells in the basal part of the pycnidium. — b. Conidia.

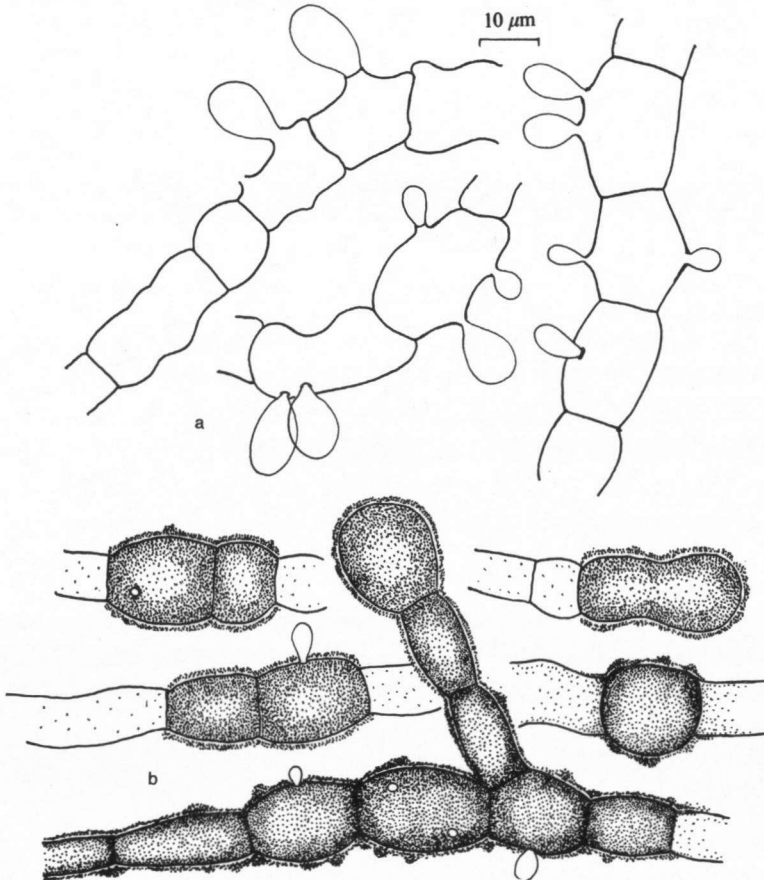


Fig. 6. *Guignardia miribelii* (CBS 161.75). — a. *Hormonema*-state in a 5 days old culture on cornmeal agar. — b. Chlamydozoospores with *Hormonema*-state in a 12 days old culture on cornmeal agar.

no longer extant. The present conception, including synonymy agrees with that given by von Höhnel (1918), Petrak (1922 and 1927), von Arx (1970) and van der Aa (1973). The fungus described and figured by Morgan Jones (1971) under the name *Sarcophoma miribelii*, however may represent *Dothiorella candollei* (Berk. & Br.) Petrak in *Annls mycol.* **34**: 231. 1936. In most handbooks this species is kept separate from *Sarcophoma miribelii* as *Macrophoma candollei* (Berk. & Br.) Berl. & Vogl. (Allescher, 1899; Diedicke, 1915), but Grove (1935) considered them erroneously as stages of development of one and the same species.

Fuckel (1870), Cooke (1871), and Saccardo (1883) suggested that the ascigerous state of *Sarcophoma miribelii* is *Hyponectria buxi* (Desm.) Sacc., but this was denied by von Höhnel (1918) and also not accepted by later authors. Single ascospore isolates from well-developed specimens of this fungus, collected by the author in the same area as *Guignardia miribelii* (on withering leaves, Plateau du Lubéron, France, 23.10.1974, *H. A. van der Aa 4433*, CBS), repeatedly failed to germinate on different media and as a result the connection with any conidial fungus could not be proved in this way.

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