PERSOONIA
Volume 17, Part 2, 273–280 (1999)

# CONTRIBUTIONS TOWARDS A MONOGRAPH OF PHOMA (COELOMYCETES) III – SUPPLEMENT

## Additional species of section Plenodomus

G.H. BOEREMA[1] & J. DE GRUYTER[2]

This supplement deals with the diagnostic characteristics and literature data of three species of *Phoma* sect. *Plenodomus*: *Phoma korfii* spec. nov. (synanamorph *Sclerotium orobanches* Schwein.: Fr.), *Phoma pimpinellae* spec. nov. (teleomorph *Leptosphaeria pimpinellae* Lowen & Sivan.) and *Phoma etheridgei* L.J. Hutchison & Y. Hirats. (only known from isolates). They are filed into the key of section *Plenodomus* and the indices of the section are supplemented.

The species placed in *Phoma* sect. *Plenodomus* (Preuss) Boerema et al. (1981) are characterised by their ability to produce scleroplectenchyma in the peridium of the pycnidia. Those occurring on herbaceous plants are often anamorphs of species of the Ascomycete genus *Leptosphaeria* Ces. & De Not. with a scleroplectenchymatous ascocarp wall.

The Contribution III (Boerema et al., 1994) dealt with 26 species of *Phoma* sect. *Plenodomus*, associated with 12 species of *Leptosphaeria*.

In this Supplement three recently studied *Plenodomus*-like *Phoma* anamorphs are discussed. This refers to:

1. A rare sclerotia producing fungus on Orobanchaceae, achlorophyllous plants, parasitic on roots. The isolates studied were made in the USA (Yáñez-Morales et al., 1998) from *Epifagus virginianus*, parasitic on the roots of *Fagus grandifolia*.

2. An ascomycetous fungus found on blackened stems of *Pimpinella anisum* (Umbelliferae) in Israel, and in 1989 described as *Leptosphaeria pimpinellae* Lowen & Sivan. The isolate studied was from a single ascospore.

3. A fungus isolated from the bark of black galls and related cankerlike structures on American trembling aspen, *Populus tremuloides*, in Canada (Hutchison et al., 1994).

The morphology of the pycnidial anamorphs in vivo and in vitro is discussed and the cultural characteristics are described in the usual way. The paper starts with supplements of the key and the indices of *Phoma* sect. *Plenodomus*.

### SUPPLEMENT TO THE KEY OF PHOMA SECTION PLENODOMUS (characteristics *in vivo*)

Insert α and β after '1a. Scleroplectenchymatous pycnidia (II) on dead stems (occasionally on roots) of herbaceous plants':

1) Karel Doormanstraat 4[5], NL-2041 HD Zandvoort, The Netherlands.
2) Plant Protection Service, P.O. Box 9102, NL-6700 HC Wageningen, The Netherlands.

α. Pycnidia developing directly from the host tissue ..... 2
[Immersed, subepidermal or superficial.]

β. Pycnidia arising from sclerotia on the host, with their outer surface a clear continuation of the sclerotia, globose to subglobose with a papillate pore, variable in size; conidia 2–4 × 1–1.5 µm; dead stems, flowers and roots of Orobanchaceae in North America (*Epifagus virginianus*) and Europe (*Orobanche major*)
***i Phoma korfii***, synanamorph *Sclerotium orobanches*
[In vitro usually only pycnidia II develop, but under certain conditions also only sclerotia or sclerotia and pycnidia, with pycnidia either separate from or developing on sclerotia; occasionally also pycnosclerotia (III) and thin-walled pycnidia I.]

Insert γ and δ between '13a' and '13b':
13a. Neck up to 500 µm long, ... etc.

γ. Neck shorter and distinctly papillate ..... δ+13b

δ. Neck usually no longer than 60 µm, i.e. short papillate; conidia 4–4.5 × 1.5 µm, biguttulate; on dead stems of *Pimpinella anisum* (Umbelliferae), necrophyte (so far only known from Israel)
***ii Phoma pimpinellae***, teleomorph *Leptosphaeria pimpinellae*
[In vitro pycnidia I → II, often globose with a long neck up to 500 µm, but also very irregular without clear pore.]

13b. Neck up to 200 µm long, ... etc.

Add a Note to 27a:

*) The pycnidia and conidia of *P. enteroleuca* s.l. in vivo show much resemblance with those produced in cultures of ..... ***iii Phoma etheridgei***
[Characteristics in vivo unknown; described from isolates obtained from the bark of black galls and cankers of *Populus tremuloides* in Canada. Culture morphology, conidial length to width ratio, and colony colour reaction to NaOH showed differences between isolates of *P. etheridgei* and *P. enteroleuca*.]

## ADDITIONS TO THE INDICES

### HOST–FUNGUS INDEX

A. on herbaceous plants

| | |
|---|---|
| Orobanchaceae | |
| *Epifagus virginianus* and *Orobanche major* | ***i Phoma korfii*** (synanam. *Sclerotium orobanches*) [only a few records from North America (USA and Canada on *E. virginianus*) and Europe (Sweden and Germany on *O. major*)] |
| Umbelliferae | |
| *Pimpinella anisum* | ***ii Phoma pimpinellae*** (teleom. *Leptosphaeria pimpinellae*) [so far only known from Israel] |

B. on deciduous trees and shrubs

Salicaceae

*Populus tremuloides* — ***iii*** ***Phoma etheridgei***
[in North America (Canada) isolated from the bark of black galls and cankers; pycnidia I → II so far only known from cultures]

FUNGUS–HOST INDEX

A. on herbaceous plants

*Phoma korfii* ***(i)*** (synanam. *Sclerotium orobanches*) — *Epifagus virginianus* and *Orobanche major* (Orobanchaceae)

*Phoma pimpinellae* ***(ii)*** (teleom. *Leptosphaeria pimpinellae*) — *Pimpinella anisum* (Umbelliferae)

B. on deciduous trees and shrubs

*Phoma etheridgei* ***(iii)*** — *Populus tremuloides* (Salicaceae)

## DESCRIPTIVE PART

*i.* **Phoma korfii** Boerema & de Gruyter, *spec. nov.*[3] — Fig. 1A

Synanamorph: *Sclerotium orobanches* Schwein.: Fr.

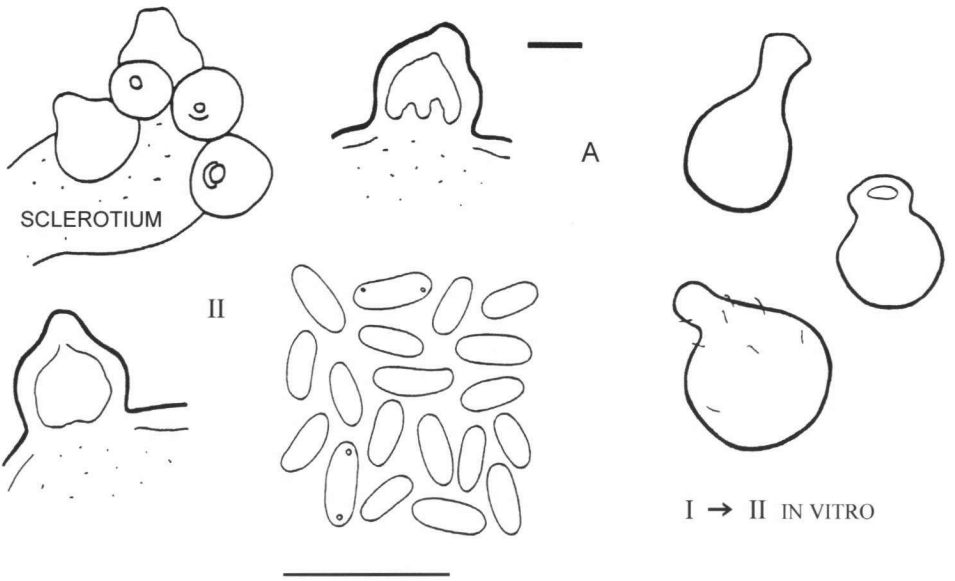
Pycnidia tantum in vivo in sclerotiis synanamorphes observata, globosa vel subglobosa, poro papillato aperientia, 130–370 × 110–333 µm; paries e cortice sclerotii extensus, deorsum haud distinctus a sclerotio.
Conidia ellipsoidea vel oblonga, 2–4 × 1–1.5 µm.
Holotypus: CUP 63537 ad caules Epifagi virginiani, Ringwood Swamp, Lloyd-Cornell. Preserve, in oriente ab Ithaca, NY, in Statis Unitis, 13 Sept. 1995.
*Selected literature*. Yáñez-Morales, Korf & Babcock (1998).

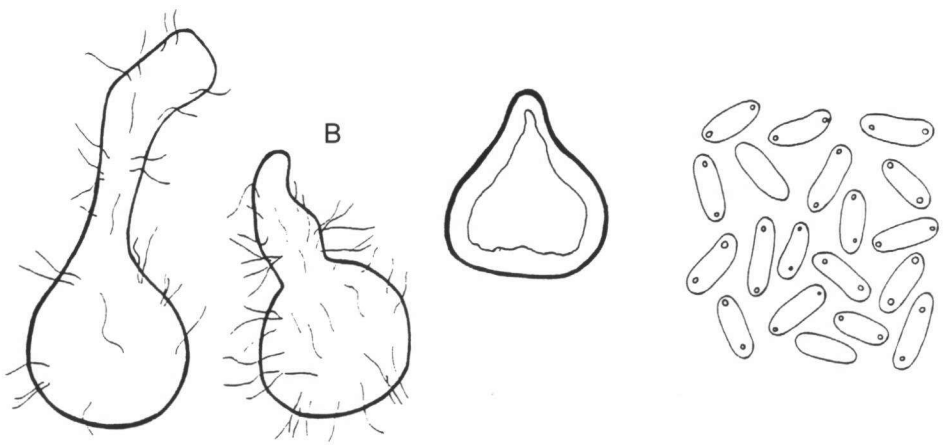
*Description in vivo* (on *Epifagus virginianus*)

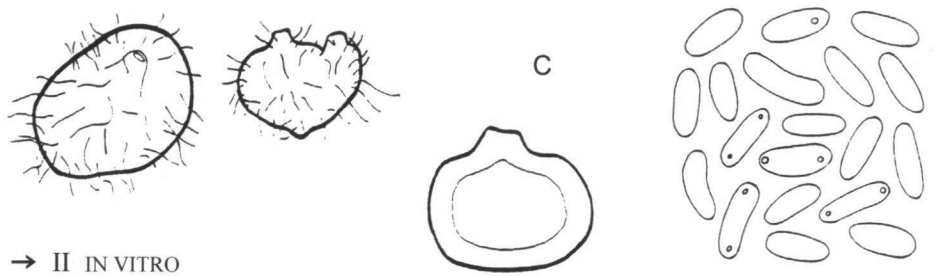
Pycnidia II [arising directly from the sclerotia of the synanamorph (see below), with their outer surface a clear continuation of the sclerotial rind] 130–370 × 110–333 µm, globose to subglobose, with a papillate pore. Outer wall like the sclerotial rind consisting of polygonal scleroplectenchyma cells of variable dimensions and a dark cortex; cells at base not differentiated into a separate wall layer. Exudate not observed. Conidia ellipsoidal to oblong, 2–4 × 1–1.5 µm, sometimes guttulate.

Sclerotia (synanamorph *Sclerotium orobanches*; immersed in or erumpent from or superficial on dead stems, flowers and roots of the host plant, separate or in small clusters) rounded to elongate, often lobulate or twisted to vermiform, smooth or with 1–8 pycnidia (0.6–)1–1.5 × 2–9 mm.

3) Named after Dr. R.P. Korf, who first discovered this pycnidial anamorph. It should be noted that *Phoma orobanches* C. Massal. (Massalongo, 1888, 1889) refers to a different species.


SCLEROTIUM
A
II
I → II IN VITRO


B
I → II IN VITRO


C
I → II IN VITRO

*Description in vitro*

OA: growth-rate 45 mm after 7 days, regular, with finely floccose, white aerial mycelium; colony colourless; reverse colourless to weak primrose near margin.

MA: growth-rate 37–48 mm after 7 days, regular, with compact finely floccose to woolly, white to pale olivaceous grey aerial mycelium; colony similar due to aerial mycelium; reverse olivaceous black, with straw near margin.

CA: growth-rate 52–53 mm after 7 days, (regular), with floccose, white aerial mycelium, colony colourless, partly salmon; reverse colourless to partly salmon/saffron. Under the standard method employed in our culture studies (de Gruyter & Noordeloos, 1992) only pycnidia I → II developed, 110–320 µm diam., subglobose to irregular, papillated, developing an elongated neck in a later stage, usually solitary, glabrous or with short mycelial outgrowths, with 1(–3) ostioles, honey to olivaceous, later olivaceous black; thick-walled, 4 or more layers of cells, outer layers pigmented; with white to buff/rosy buff or pale luteous exuded conidial masses; scattered, mostly on and in the agar. Micropycnidia present, up to 50 µm. Conidiogenous cells 4–7 × 3–6 µm, globose to bottle-shaped. Conidia somewhat larger than those observed in vivo, (2–)3–5.5 × (1–)1.5–2 µm, av. 4.2 × 1.6 µm, Q = 1.9–3.3, av. Q = 2.6, oblong to ellipsoidal, without guttules.

NaOH spot test: a weak discolouring occurs on OA and MA to salmon and greenish, respectively, not specific.

Crystals absent.

[Yáñez-Morales et al. (1998) obtained on other media at different light/dark and temperature regimes also cultures with only sclerotia, or sclerotia and pycnidia, with pycnidia either separate from or developing on sclerotia: "Sclerotia superficial, variously shaped, abundant and dispersed on some media, rare to fairly common at the periphery of plates on other media, produced in 4 weeks, developing their final shape and dark pigmentation during the 5th to seventh week." On potato dextrose agar, PDA 0.3–1 × 0.4–0.7 mm, on malt extract glucose agar, MGA 0.4–2 × 0.2–1 mm. Pycnidia II borne on the sclerotia were similar to those in vivo. On PDA they also observed relatively large papillate pycnidia, 360–600 × 280–550 µm, cream-salmon in colour and with a thin wall (type I). On MGA under some cultural conditions also "very hard, small, beaked structures, (50–)86(–130) × (60–)96 (–160) µm, resembling pycnidia but without spores, thus apparently pycnosclerotia" (III), developed.]

*Ecology and distribution.* The sclerotial anamorph of this fungus was already described by von Schweinitz in 1822 from roots and stems of *Orobanche virginiana* = *Epifagus virgini-*

Fig. 1. A. *Phoma korfii.* Pycnidia–II in vivo always arising from sclerotia (synanam. *Sclerotium orobanches*), globose to subglobose with papillate pore. Pycnidia in vitro I → II more irregular, often developing an elongated neck in a later stage. Conidia ellipsoidal to oblong. — B. *Phoma pimpinellae.* Pycnidia–II in vivo regular subglobose with a distinct papillate neck found together with, and very similar to the ascomata of the teleomorph *Leptosphaeria pimpinellae.* Pycnidia in vitro I → II, at first irregular, but later becoming more regular. Conidia oblong to ellipsoidal. — C. *Phoma etheridgei.* Pycnidia I → II only known from isolates, subglobose-papillate with hyphal outgrowths in varying degrees, and sometimes with a pointed base. Conidia ellipsoidal to ovoid to oblong, often acute at one end.

*anus*, in North America a not uncommon achlorophyllous plant (Orobanchaceae), parasitic on the roots of *Fagus grandifolia*, see Yánez-Morales et al. (1998). Fries (1998) recorded *Sclerotium orobanches* from Sweden and Germany on *Orobanches major*, parasitic on the roots of leguminous shrubs. The fungus may be an endophyte. The pycnidia of *Phoma korfii*, in nature only developing on the sclerotia, without doubt contribute to the dispersal of the fungus.

*Representative culture.* CBS 101638 (PD 97/12070) ex *Orobanche virginiana* (Orobanchaceae), USA.

### *ii.* **Phoma pimpinellae** Boerema & de Gruyter, *spec. nov.* — Fig. 1B

Teleomorph: *Leptosphaeria pimpinellae* Lowen & Sivan.

Isolatus ex ascosporis, colonia in agaro farina avenae decocto: pycnidia fusca, crassitunicata, superficialia vel submersa vel in mycelio aerio, 125–300 µm diam., irregularia vel subglobosa, collulo elongato aperientia. Paries ad 12 cellulas crassus, extus pigmentatus. Cellulae conidiogenae globosae vel lageniformes in collum longum extensae, 4–6(–8) × 1.5 (sursum ad) –5 µm. Conidia oblonga vel ellipsoidea, 3.5–4.5 × 1–1.5(–2) µm.

Typus L 992.163-138, cultura sicca CBS 101637, ex ascospora singula ad caulem Pimpinellae anisi a Lowen & Sivanesan (1989) isolatus, Mt Carmel, Beit Oren Forest, Wadi near Kibbutz Oren, in Israel.

*Selected literature.* Lowen & Sivanesan (1989).

*Description in vivo* (on *Pimpinella anisum*)

Pycnidia II (immersed becoming superficial) up to 300 µm diam., globose with a thickened flattened base and a distinct papillate neck up to 60 µm high. Wall scleroplectenchymatous, especially conspicuous at the 'shoulder' near the neck. Exudate rosy vinaceous. Conidia short cylindrical (oblong), 4–5.5 × 1.2–1.8 µm, biguttulate. Conidiogenous cells 6–12 × 3 (at the base)–1 (at the phialidic apex) µm, i.e. bottle-shaped with a long neck[4] (data derived from Lowen & Sivanesan, 1989).

*Description in vitro*

OA: growth-rate 47 mm after 7 days, regular, with (finely) floccose, white to pale olivaceous grey, partly citrine green aerial mycelium; colony pale luteous to citrine, due to the release of a diffusable pigment, with olivaceous grey at centre; reverse pale luteous to amber, with olivaceous grey at centre.

MA: growth-rate 22–25 mm after 7 days (14 days: 45–48 mm), irregular, with finely floccose to coarsely floccose, white to citrine green aerial mycelium; colony citrine green to amber, due to the release of a diffusable pigment, white near margin, with olivaceous grey at centre; reverse citrine green/greenish olivaceous to amber, with olivaceous black to leaden grey at centre.

CA: growth-rate 44–45 mm after 7 days, regular, with finely floccose, white to greenish olivaceous aerial mycelium; colony citrine amber, due to the release of a diffusable pigment, with cinnamon to olivaceous patches; reverse similar, with leaden grey to olivaceous black at centre.

4) Probably sometimes also globose-papillate as observed in vitro. The occurrence of 'conidiophores' as well as hardly differentiated conidiogenous cells is well known of another member of sect. *Plenodomus*, viz. *Phoma sclerotiodes* Preuss ex Sacc. (see Notolitzky & Colotelo, 1965 sub syn. *Plenodomus meliloti*).

Pycnidia I → II, 125–300 µm diam., developed in one week at sides of mycelial aggregation, irregular to subglobose with a long elongated neck, solitary or aggregated, with mycelial outgrowths, with usually 1 ostiole, olivaceous black; thick-walled, up to 12 layers of cells, outer layers pigmented; with rosy vinaceous exuded conidial masses; scattered, on and in the agar and in the aerial mycelium as well. Conidiogenous cells 4–6(–8) × 1.5 (at the apex) –5 µm, globose-papillate to bottle-shaped with a long neck. Conidia continuous, 3.5–4.5 × 1–1.5(–2) µm, av. 4.0 × 1.5 µm, Q = 2.4–3.3, av. Q = 2.7, oblong to ellipsoidal, with 2 small, polar guttules.

Chlamydospores absent.

NaOH spot test positive, a brick discolouring of the diffusable pigment on OA and MA.

Crystals absent.

*Ecology and distribution.* In Israel found on dead blackened stems of *Pimpinella anisum* (Umbelliferae). On the type substratum of the holomorph the pycnidia occur together with pseudothecia of *Leptosphaeria pimpinellae*, but it is plausible that both morphs play a different role in the life cycle of this fungus.

*Representative culture.* CBS 101637 (PD 92/41) ex *Pimpinella anisum* (Umbelliferae), Israel.

### *iii.* **Phoma etheridgei** L.J. Hutchison & Y. Hirats. — Fig. 1C

*Phoma etheridgei* L.J. Hutchison & Y. Hirats. apud Hutchison, Chakravarty, Kawchuk & Hiratsuka, Can. J. Bot. 72 (1994) 1425.

*Selected literature.* Hutchison et al. (1994).

*Appearance in vivo (Populus tremuloides)*

This pycnidial fungus is only known from isolates obtained from the bark of galls and cankers of American trembling aspen in Canada.

*Description in vitro*

OA: growth-rate 23 mm after 7 days (14 days: 47–48 mm), regular, with floccose, white aerial mycelium; colony colourless to greenish olivaceous, with pale grey olivaceous at centre; reverse similar.

MA: growth-rate 23 mm after 7 days (14 days: 43–44 mm), regular, with compact, floccose, white to citrine green aerial mycelium; colony citrine green, due to aerial mycelium; reverse apricot, due to the release of a diffusable pigment, luteous near margin, olivaceous black at centre.

CA: growth-rate 23–24 mm after 7 days (14 days: 45–46 mm), regular, with floccose, white to grey olivaceous/olivaceous grey aerial mycelium; colony colourless to grey olivaceous/dull green; reverse similar, with salmon near margin.

Pycnidia (partly adopted from Hutchison et al., 1994) I → II, 95–270 µm diam., globose/subglobose to irregular, solitary or confluent, with mycelial outgrowths, sometimes setae-like, with 1 papillated ostiole, honey/olivaceous, later olivaceous black; thick-walled, up to 15 layers of cells, outer layer(s) pigmented; with flesh/salmon to pale vinaceous exuded conidial masses; scattered, both on and in the agar as well as in aerial mycelium. Conidiogenous cells 4–7 × 4–7 µm, globose to bottle-shaped. Conidia aseptate, 3–4.5(–5) × 1–2 µm, av. 4.4 × 1.4 µm, Q = 2.3–4.2, av. Q = 3.1, ellipsoidal, to oblong/ovoid or allantoid.

Chlamydospores absent.

NaOH spot test: on MA a greenish to orange discolouring occurs.

Crystals usually absent; however, in fresh isolates pale yellow crystals may be produced at margin of colony.

*Ecology and distribution.* This fungus seems to be specific to the bark of black galls ('burls') and related cankerlike structures on American trembling aspen (*Populus tremuloides*). Trees with these stem deformities are occasionally found in Western Canada and the Rocky Mountain States of the USA. Such trees showed a significant decrease or absence of infestation by the aspen decay pathogen *Phellinus tremulae* (Bond.) Bond. & Borissov. This phenomenon may be due to the presence of *Phoma etheridgei*, which proved to be strongly antagonistic in vitro against *Phellinus tremulae*.

*Representative culture.* DAOM 216539 (PD 95/1483) ex *Populus tremuloides* (Salicaceae), Canada.

ACKNOWLEDGEMENTS

The material for this study has been gratefully received from (*i*) Dr. R.P. Korf, Cornell University, Ithaca, NY-USA, (*ii*) Dr. Rosalind Lowen, New York Botanical Garden, NY-USA and (*iii*) the Canadian Collection of Fungus Cultures (DAOM 216539). Dr. W. Gams kindly provided the Latin translations of descriptions. Dr. Korf also served as pre-submission reviewer.

REFERENCES

Boerema, G.H., H.A. van Kesteren & W.M. Loerakker. 1981. Notes on Phoma. Trans. Br. Mycol. Soc. 77: 61–74.

Boerema, G.H., J. de Gruyter & H.A. van Kesteren. 1994. Contributions towards a monograph of Phoma (Coelomycetes), III – 1. Section Plenodomus: Taxa often with a Leptosphaeria teleomorph. Persoonia 15 (4): 431–487.

Fries, E.M. 1828. Elenchus Fungorum 2. Gryphiswaldiae.

Gruyter, J. de & M.E. Noordeloos. 1992. Contributions towards a monograph of Phoma (Coelomycetes), I – 1. Section Phoma: Taxa with very small conidia in vitro. Persoonia 15 (1): 71–92.

Hutchison, L.J., P. Chakravarty, L.M. Kawchuk & Y. Hiratsuka. 1994. Phoma etheridgei spec. nov. from galls and cankers of trembling aspen (Populus tremuloides) and its potential role as a bioprotectant against the aspen decay pathogen Phellinus tremulae. Can. J. Bot. 72: 1424–1431.

Lowen, R. & A. Sivanesan. 1989. Leptosphaeria pimpinellae and its Phoma anamorph. Mycotaxon 35 (2): 205–210.

Massalongo, C. 1888. Sulla germogliazione delle nelle Sphaeropsideae. Nuovo G. Bot. ital. 20: 437–439.

Massalongo, C. 1889. Nuovi miceti dell'agro Veronese. Nuovo G. Bot. ital. 21: 161–170.

Netolitzky, H. & N. Colotelo. 1965. Conidiophores of Plenodomus meliloti. Can. J. Bot. 43: 615–616.

Schweinitz, L.D. von. 1822. Synopsis fungorum Carolinae superioris. Schriften Naturf. Ges. Leipzig 1 (1): 1–105.

Yáñez-Morales, M. de J., R.P. Korf & J.F. Babcock. 1998. Fungi on Epifagus (Orobanchaceae) – I. On Sclerotium orobanches and its Phoma synanamorph. Mycotaxon 67: 275–286.