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Phoma canadensis Allesch.: a synonym of *Pyrenochaeta cava*

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Abstract — The isotype of *Phoma canadensis* on *Conyza canadensis* was investigated to clarify its dubious taxonomic placement and taking the broad rearrangement of *Phoma* taxa into consideration. Phylogenetic and morphological analyses show that this species is, in fact, misplaced in *Phoma* and actually belongs in *Pyrenochaeta* as a synonym of *Pyr. cava*.

Key words — asexual morph, coelomycete, *Cucurbitariaceae*, molecular phylogeny, reappraisal

Introduction

Phoma is a large genus of filamentous fungi, widely distributed and occupying diverse ecological niches (Boerema et al. 2004, Aveskamp et al. 2008). Due to its problematic and confusing taxonomy it has been the subject of intense studies aimed at generating a more natural delimitation for the taxa within this genus and to uncover its phylogenetic relationship with other allied genera (Boerema et al. 2004; Aveskamp et al. 2010; Gruyter et al. 2009, 2010, 2013). Even after this recent and significant mycological effort, an estimated more than half of the herbarium *Phoma* specimens worldwide still need reexamination and proper classification (Aveskamp et al. 2008).

One example is *Phoma canadensis* Allesch., an obscure fungus known only from its original and brief description on dead stems of *Conyza canadensis* (L.) Cronquist [≡ *Erigeron canadensis* L.; *Asteraceae*) in Germany and subsequently recorded only once from Los Angeles (USA) (Allescher 1896, Farr et al. 2015). Considering that the limited information in the original description makes its position in *Phoma* questionable, this paper attempts, through morphological and phylogenetic analyses of the isotype specimen, to clarify the placement of *Ph. canadensis*.

Table 1. Species and isolates included in the phylogenetic analyses of *Phoma canadensis* and *Pyrenochaeta cava*

Species	Strain	Host	Origin	Genbank #
Chaetodiplodia sp.	CBS 568.88	rock	Israel	EU754142
Chaetopyrena penicillata	CBS 199.89	*Medicago sativa*	South Africa	GQ387573
	CBS 498.72	soil	Turkey	GQ387574
Chaetosphaeronema coonsii	CBS 141.84	*Malus sylvestris*	Japan	GQ387575
Chaetosphaeronema hispidulum	CBS 216.75	*Anthyllis vulneraria*	Germany	EU754144
Coniothyrium carteri	CBS 101633	*Quercus* sp.	Netherlands	GQ387593
	CBS 105.91	*Quercus robur*	Germany	GQ387594
Coniothyrium dolichi	CBS124140	*Dolichos biflorus*	India	GQ387611
	CBS124143	*Dolichos biflorus*	India	GQ387610
Coniothyrium glycines	CBS 124141	*Glycine max*	Zimbabwe	GQ387598
	CBS 124455	*Glycine max*	Zambia	GQ387597
Coniothyrium telephii	CBS 101636	*Glycine max*	Zimbabwe	GQ387601
	CBS 188.71	air	Finland	GQ387599
	CBS 856.97	mineral wool	Finland	GQ387600
Herpotrichia parasitica	CBS 218.77	*Abies alba*	Germany	GQ387618
	CBS 451.73	*Abies alba*	Germany	GQ387617
Neosetophoma samararum	CBS 138.96,	*Phlox paniculata*	Netherlands	GQ387578
	CBS 139.96	*Poaceae*	Netherlands	GQ387579
	CBS 568.94	*Urtica dioica*	Netherlands	GQ387580
Paraconiothyrium minitans	CBS 122788	——	U. Kingdom	EU754173
Paraphoma chrysanthemicola	CBS 172.70	*Chrysanthemum morifolium*	Germany	GQ387583
	CBS 522.66	*Chrysanthemum morifolium*	U. Kingdom	GQ387582
Paraphoma radicina	CBS 102875	*Solanum esculentum*	Germany	EU754190
	CBS 111.79	*Malus sylvestris*	Netherlands	EU754191
Phoma betae	CBS 109410	*Beta vulgaris*	Netherlands	EU754178
Phoma gardeniae	CBS 302.79	airborne	Netherlands	GQ387596
	CBS 626.68	*Gardenia jasminoides*	India	GQ387595
Phoma herbarum	CBS 615.75	*Rosa multiflora*	Netherlands	EU754186
Pleurophoma pleurospora	CBS116668	*Sarothamnus scoparius*	Netherlands	JF740326
	CBS130329	*Lonicera* sp.	Netherlands	JF740327
Pyrenochaeta cava	**BPI 356246***	***Conyza canadensis***	**Germany**	**KC841911**
	CBS257.68	wheat-field soil	Germany	EU754199
	CBS 115953	*Quercus cerris*	Italy	GQ387607
Pyrenochaeta corni	CBS 102828	*Olea europaea*	Italy	GQ387609
	CBS 234.92	*Olea europaea*	Italy	EU754176
	CBS 248.79	*Fraxinus excelsior*	Netherlands	GQ387608
Pyrenochaeta nobilis	CBS 292.74	*Ilex aquifolium*	Netherlands	GQ387615
	CBS 407.76	*Laurus nobilis*	Italy	EU754206
	CBS 566.75	*Buxus sempervirens*	Netherlands	GQ387616
Pyrenochaeta quercina	CBS 115095	*Quercus robur*	Italy	GQ387619
	CBS 297.74	sea water	Montenegro	GQ387620
Pyrenochaeta unguis-hominis	CBS 378.92	fingernail	U. Kingdom	GQ387621
	CBS 112.79	airborne	U. Kingdom	GQ387622
	CBS 111112	*Agapornis* sp.	Netherlands	GQ387623
Pyrenochaetopsis decipiens	CBS 165.89	*Hordeum vulgare*	Netherlands	GQ387625
Pyrenochaetopsis indica	CBS 244.54	*Saccharum officinarum*	India	GQ387626
Pyrenochaetopsis leptospora	CBS 536.66	wheat field soil	Germany	GQ387628
Pyrenochaetopsis microspora	CBS 102876	water	Montenegro	GQ387631
Setophoma sacchari	CBS 333.39	*Saccharum officinarum*	Brazil	GQ387586
Setophoma terrestris	CBS 335.29	*Allium sativum*	USA	GQ387587
	CBS 335.87	*Allium cepa*	Senegal	GQ387528
	CBS 377.52	*Allium cepa*	——	GQ387588

* new sequence from *Phoma canadensis* isotype

Material & methods

The type material (isotype) from the packet labeled BPI 356246, originally collected in Germany, as well as two other samples (BPI 356244, BPI 356245; Plate 1) loaned by the U.S National Fungus Collections were carefully examined under a dissecting microscope; slides containing fungal structures were prepared with the help of a freezing microtome (Microm HM 520) and mounted in lactophenol or lactofuchsin. Fungal structures were observed, measured, and illustrated with an Olympus BX 51 light microscope fitted with an Olympus E330 camera and a drawing tube.

After acquiring permissing to extract DNA directly from the stem samples, total genomic DNA was isolated using the Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) according to the manufacturer's instructions. The primers LR0R and LR5 (Vilgalys & Hester 1990) were used to amplify a part of the large ribosomal subunit (LSU). The 25 µL PCR reaction and PCR conditions were the same as described by Fernandes et al. (2013). The nucleotide sequence data were obtained by DNA sequencing (Macrogen Inc., Korea) employing the same primers used for PCR amplification. The sequence fragments were assembled from the forward and reverse sequences with the help of Staden program 2003.0.1 (Staden et al. 2000). The generated sequence was aligned with closely related and outgroup published sequences retrieved from GenBank with the MEGA 5.0 software package and manually adjusted for improvement by eye where necessary (Table 1). Bayesian inference analyses were conducted and the best-fit evolutionary model was determined by comparing different evolutionary models via the Akaike information criterion using PAUP (version 4.0b10, Sinauer Associates, Sunderland, MA) and MrModeltest 2.2 (Nylander 2004). Five analyses of two Markov Chain Monte Carlo (MCMC) chains were run from random trees for 1,000,000 generations and sampled every 1000 generations using MrBayes v3.1 (Huelsenbeck & Ronquist 2001), which also calculated the consensus trees after the program Tracer v1.5 (Rambaut & Drummond 2003) was used to ensure the convergence of the chains. The first 25% of sampled trees were discarded as "burnin". Phylogenetic trees were visualized with the program FigTree v1.3.1 (Rambaut 2009).

Results

Phylogenetic analysis

An amplicon of 1294 bases of the LSU region was generated (KC841911); a standard nucleotide BLAST search against the nucleotide collection on GenBank showed 100% identity between KC841911 and the LSU sequence of *Pyrenochaeta cava* CBS 257.68 (EU754199). For the phylogenetic analysis, closely related LSU sequences published by Gruyter et al. (2010) were retrieved from GenBank (Table 1). The resulting alignment data matrix included 52 taxa (including the outgroup sequences) and 1301 characters, of which 158 sites were variable and 125 sites were parsimony informative. The *Ph. canadensis* isolate fell within the *Cucurbitariaceae*, where it grouped with the *Pyr. cava* isolates with maximum support (Plate 2).

Taxonomy

Pyrenochaeta cava (Schulzer) Gruyter, Aveskamp & Verkley, Mycologia 102: 1076, 2010. PLATE 1

≡ *Phoma cava* Schulzer, Verh. Zool.-Bot. Ges. Wien 21: 1248, 1871.

≡ *Aposphaeria cava* (Schulzer) Sacc. & Schulzer, Syll. Fung. 3: 174, 1884.

≡ *Coniothyrium cavum* (Schulzer) Kuntze, Revis. Gen. Pl. 3(2): 459, 1898.

≡ *Pleurophoma cava* (Schulzer) Boerema, Loer. & Hamers, Persoonia 16: 172, 1996.

≡ *Phoma canadensis* Allesch., Ber. Bayer. Bot. Ges. 4: 32, 1896.

ORIGINAL DESCRIPTION OF *PHOMA CANADENSIS* (Allescher 1896): "*Peritheciis minutis, tectis, dense gregariis, in maculas griseas, longas, saepe longissimas dispositis, poro pertusis, atro-fuscis, ca. 40–60μ diam.; sporulis minutissimis, oblongis, utrinque obtusis, biguttulatis, continuis, hyalinis, ca. 3–5 × 0,5–1; basidiis non visis.*

Hab. in caulibus emortuis Erigerontis canadensis. München: in silva prope «Ludwigshöhe». IV. 95 ipse legi."

External mycelium absent. Internal mycelium indistinct. Pycnidia subcuticular, crowded, forming vertical linear strands over the grayish loosening cuticle of the host tissue, subglobose, glabrous, 40–84 × 69.5–158.5 μm, walls composed of pale to chestnut brown textura angularis, 1–2 layers, 9–10 μm thick. One central ostiole per ascoma, circular, 9.5–12 μm diam. Conidiogenesis indistinct. Conidia ellipsoid to subcylindrical, straight to slightly curved, 2–5 × 1 μm, aseptate, ends rounded, with 2 small polar guttules, hyaline to subhyaline, smooth.

SPECIMENS EXAMINED: **GERMANY**, **BAVARIA**, Munich, near Ludwigshoehe, on dead stems of *Erigeron canadensis* [≡ *Conyza canadensis*], April 1895, Andreas Allescher (BPI 356246, isotype; GenBank KC841911). **U.S.A.**, **CALIFORNIA**, **Los Angeles Co.**, Glendale, on stems of *Erigeron canadensis*, November 20 1931, O.A. Plunkett (BPI 356244, BPI 356245).

Discussion

In addition to *Ph. canadensis* Allesch. 1896, there is an illegitimate later homonym, *Ph. canadensis* Voglino 1910, described from *Populus canadensis* in Italy (Saccardo & Trotter 1913). Regrettably, we were not able to obtain herbarium specimens of *Ph. canadensis* and have therefore been unable to assess its relationship (if any) with *Ph. canadensis* Allesch. Consequently, all data presented and discussed in this paper pertains only to the fungus described by Allescher.

The fungal morphometric data in BPI 356244 and BPI 356245 were consistent with that of the isotype BPI 356246, indicating that the specimens represent the same species. The isotype specimen, as well as the extra material, consists of stem fragments fully colonized by a pycnidial-producing fungus,

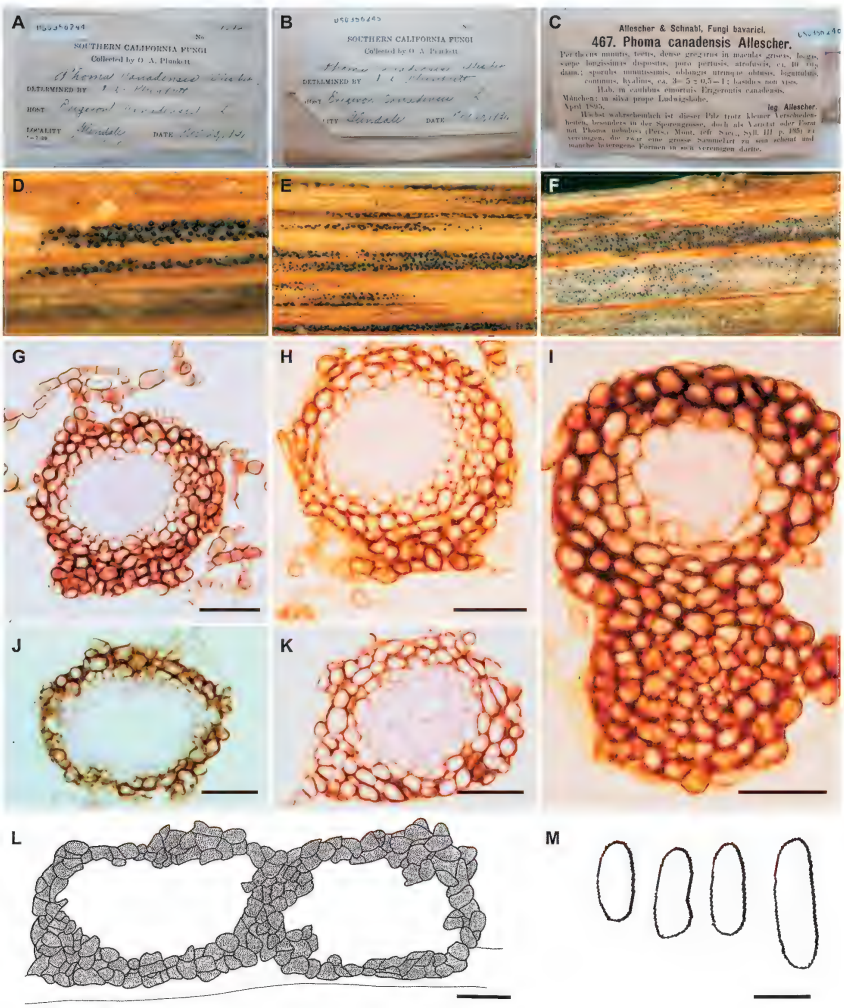

PLATE 1. *Phoma canadensis* Allesch. on *Conyza canadensis*. A–C. Herbarium packets containing: (A) BPI 356244; (B) BPI 356245; (C) BPI 356246 (isotype); D–F. Densely crowded bands of pycnidia of *Ph. canadensis* as seen on: (D) BPI 356244; (E) BPI 356245; (F) BPI 356246; G–L. Pycnidia. M. Hyaline conidia. Scale bars: G–L = 20 µm; M = 2 µm.

showing numerous dark colored pycnidia distributed along a horizontal band and with aseptate and hyaline spores (PLATE 1). In 1896, Andreas Allescher placed his newly found fungus in the genus *Phoma* as a new species, *Ph. canadensis*. This was in agreement with the accepted 19th century

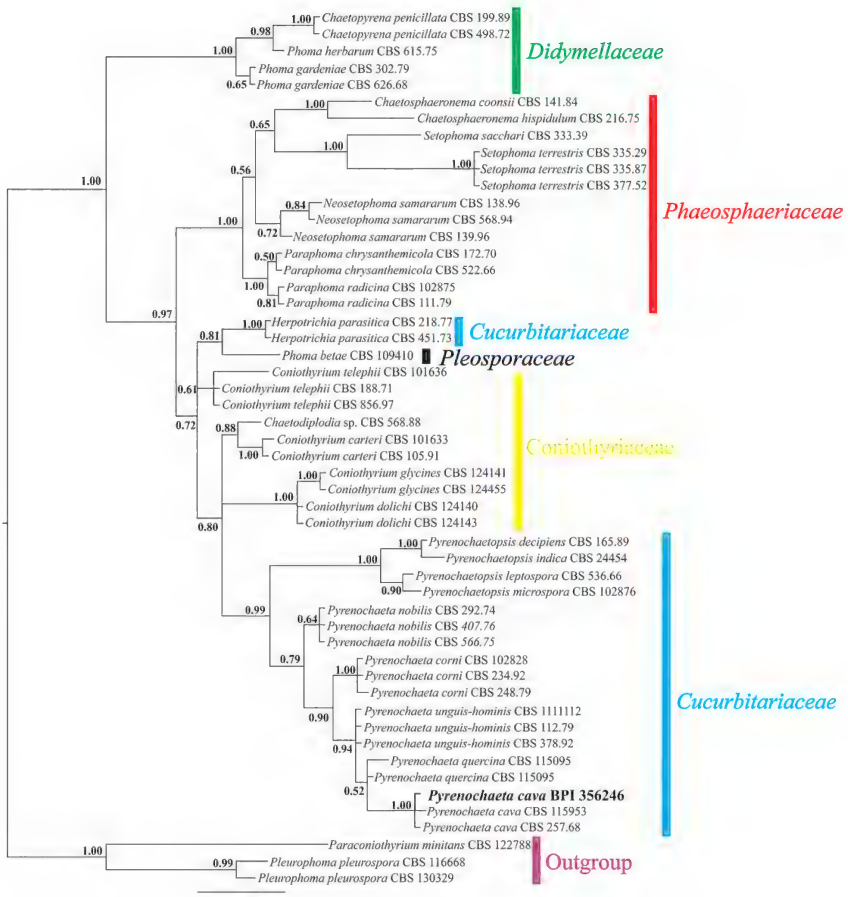

Plate 2. Phylogenetic relationship of species of *Pyrenochaeta* and related genera inferred by Bayesian analysis of a part of the large subunit ribosomal region. Bayesian posterior probabilities are indicated at the nodes. In bold, the sequence generated in this study from the *Phoma canadensis* isotype [= *Pyrenochaeta cava*].

concept for *Phoma*. Sutton (1964) introduced conidiogenesis as an important taxonomic feature for *Phoma* and related genera. Unfortunately, Allescher did not provide any detail about conidiogenesis of *Ph. canadensis*, nor were we able to observe the necessary details in such old herbarium specimens. Since established by Saccardo (1880), *Phoma* has grown to accommodate more than 3000 species that form pycnidia with aseptate hyaline conidia (Aveskamp et al.

2008). More recently several publications have reappraised this fungal group (Boerema et al. 2004; Aveskamp et al 2010; Gruyter et al. 2009, 2010, 2013) and split *Phoma* into several new genera. Regrettably, *Ph. canadensis* as well as other *Phoma* spp. that are obscure and unavailable in culture, were not included in these recent studies. Here, in order to elucidate the generic position of *Ph. canadensis*, the LSU sequence of the isotype (BPI 356246) was analyzed within a dataset covering a number of *Phoma* species and other closely related genera (Table 1). Since our attempts to obtain sequences from BPI 356244 and BPI 356245 failed, the phylogenetic analysis covered only the sequence from specimen BPI 356246.

The sequence from isolate BPI 356246 grouped within the clade of *Pyr. cava* with maximum posterior probability (Plate 2). Although the genus *Pyrenochaeta* presents, among other morphological features, setae around the ostiole, Schneider (1979) pointed out that this attribute is not a stable generic character, and Gruyter et al. (2010) confirmed that the presence of setose pycnidia is rare in *Pyr. cava*. This both agrees with what we observed and explains why setose ostioles were not reported in the original description (Allescher 1896). The fungus found on *Conyza canadensis* also shares with *Pyr. cava* the characteristic minute conidia and the undifferentiated conidiogenous cells. Based on their phenotypic and genotypic similarities, we propose that *Ph. canadensis* Allesch. and *Pyr. cava* are conspecific.

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