Host range of *Cercospora apii* and *C. beticola* and description of *C. apiicola*, a novel species from celery

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**Abstract:** The genus *Cercospora* is one of the largest and most heterogeneous genera of hyphomycetes. *Cercospora* species are distributed worldwide and cause Cercospora leaf spot on most of the major plant families. Numerous species described from diverse hosts and locations are morphologically indistinguishable from *C. apii* and subsequently are referred to as *C. apii sensu lato*. The importance and ecological role that different hosts play in taxon delimitation and recognition within this complex remains unclear. It has been shown that Cercospora leaf spot on celery and sugar beet are caused respectively by *C. apii* and *C. beticola*, both of which are part of the *C. apii* complex. During this study we characterized a new *Cercospora* species, *C. apiicola*, which was isolated from celery in Venezuela, Korea and Greece. The phylogenetic relationship between *C. apiicola* and other closely related *Cercospora* species was studied with five different gene areas. These analyses revealed that the *C. apiicola* isolates cluster together in a well defined clade. Both *C. apii* and *C. beticola sensu stricto* form well defined clades and are shown to have wider host ranges and to represent distinct species.

**Key words:** Ascomycetes, *Cercospora apii* complex, *Cercospora* leaf spot, molecular phylogeny, species boundaries, taxonomy

**INTRODUCTION**

The genus *Cercospora* Fresen. first was described in 1863 by Fresenius (Fuckel 1863) and currently is one of the largest and most heterogeneous genera of hyphomycetes (Crous and Braun 2003). Species belonging to this plant pathogenic genus are distributed worldwide and cause Cercospora leaf spot on most of the major plant families (Crous and Braun 2003). Since the description of the genus, the taxonomy of its species has become difficult because *Cercospora* for many years has been a dumping ground for all dematiaceous hyphomycetes with filamentous conidia (Pons and Sutton 1988). Johnson and Valleau (1949) stated that most of the morphologically uniform *Cercospora* isolates belong to a single *Cercospora* species that occurs on a wide host range and morphologically is indistinguishable from *C. apii* Fresen. *Cercospora apiicola* is the oldest available name for this large complex of morphologically indistinguishable *Cercospora* taxa. This approach was questioned by Chupp (1954), who stated in his monograph that species of *Cercospora* are generally host specific. Chupp subsequently formulated the concept of “one host species, genus or family equals one *Cercospora* species”. Chupp’s concept led to the description of a large number of species based on host substrate, with more than 3000 names being listed by Pollack (1987). Crous and Braun (2003) revised these species and redispersed many of them. A total of 659 *Cercospora* species were recognized, with a further 281 being referred to synonymy under *C. apii* s.l. This decision was substantiated by the various inoculation experiments that have been conducted on the *C. apii* complex (Vestal 1933, Johnston and Valleau 1949, Fajola 1978) and that raised doubts whether host specificity existed within this complex.

To date only a few species belonging to *C. apii* s.l. have been cultured, and molecular data addressing host specificity within this complex is still lacking (Crous et al 2004). Three scenarios are possible when examining the host-species association of taxa belonging to the *C. apii* complex. The first scenario is that a single species of *Cercospora* occurs on a wide host range; the second is that several species exist with overlapping host ranges; the third is that some...
Cercospora species are host specific whereas others are not. The first evidence that distinct species exist within the C. apii morphotype recently was published by Groenewald et al (2005). The latter study focused on *Cercospora* species isolated from sugar beet (*Beta vulgaris*) and celery (*Apium graveolens*). Characteristics examined for these isolates included morphology, cultural characteristics and cardinal temperature requirements for growth. These data were supplemented with amplified fragment length polymorphism analyses and phylogenetic analyses with five different genes. Groenewald et al (2005) showed that three distinct *Cercospora* species exist on sugar beet and/or celery, namely *C. beticola* on sugar beet, *C. apii* on both celery and sugar beet and a third that was isolated from celery in Venezuela and Korea.

The ability to infect different hosts during artificial inoculation is of questionable value as a character in species delimitation. For instance, a recent study revealed that *C. beticola* could infect safflower during artificial inoculation experiments (Lartey et al 2005). However *C. beticola* has yet to be isolated from this host in the field. Only a few taxa that belong to the *C. apii* complex have been studied in the past in an attempt to elucidate the relationship between fungal species and host. The first objective of this study, therefore, was to name the new *Cercospora* species from celery. The second objective was to use DNA sequence data to examine the host range of this species, including *C. apii* s.s. and *C. beticola* s.s. as defined by Groenewald et al (2005).

**MATERIALS AND METHODS**

**Isolates.**—Those used in this study were obtained from the Centraalbureau voor Schimmelcultures (CBS) in Utrecht, the Netherlands, as well as the working collection of Pedro Crous (CPC) that is housed at CBS (Table 1). Single conidial isolates also were obtained from symptomatic material as explained in Crous (1998). Isolates were plated onto 2% malt-extract agar (MEA) and oatmeal agar (OA) (Gams et al 1998) and incubated at 24 C for 8 d.

**DNA isolation, amplification and sequencing.**—The FastDNA kit (BIO 101, Carlsbad, California) was used according to the manufacturer's instructions to isolate genomic DNA of 200–400 mg fungal mycelia grown on MEA plates. A sterile blade was used to scrape the mycelia from the surface of the plate. For the phylogenetic analyses, parts of these gene areas were used: the internal transcribed spacers and 5.8S rRNA gene (ITS), the actin gene (ACT), the translation elongation factor 1-α gene (EF), the calmodulin gene (CAL) and the histone H3 gene (HIS). PCR primers and amplification conditions followed the protocols outlined by Groenewald et al (2005). PCR products were separated by electrophoresis at 80 V for 40 min on a 0.8% (w/v) agarose gel containing 0.1 μg/mL ethidium bromide in 1× TAE buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.85) and viewed under UV-light.

Amplicons were sequenced in both directions with the PCR primers and a DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences, Roosendaal, the Netherlands) according to the manufacturer's recommendations. The products were analyzed on an ABI Prism 3700 DNA Sequencer (Perkin-Elmer, Foster City, California). A consensus sequence was computed from the forward and reverse sequences with SeqMan from the Lasergene package (DNASTar, Madison, Wisconsin).

**Data analysis.**—The consensus sequences were assembled and added to alignment (TreeBASE matrix number M2242) of Groenewald et al (2005) with Sequence Alignment Editor 2.0a11 (Rambaut 2002), and manual adjustments for improvement were made by eye where necessary. The phylogenetic analyses of sequence data were done in PAUP (phylogenetic analysis using parsimony) 4.0b10 (Swofford 2003) and consisted of neighbor joining analysis with the uncorrected “p”, the Jukes-Cantor and the HK85 substitution models. Alignment gaps were treated as missing data and all characters were unordered and of equal weight. Any ties were broken randomly when encountered. For parsimony analysis, alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. Maximum parsimony analysis was performed for all datasets with the heuristic search option with 100 random taxa additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the trees was evaluated by 1000 bootstrap replications (Hillis and Bull 1993). Other measures calculated included tree length, consistency index, retention index and rescaled consistency index (TL, CI, RI and RC). The resulting trees were printed with TreeView 1.6.6 (p 1996). A partition homogeneity test was done in PAUP to test whether the different loci can be used in a combined analysis (Farris et al 1994). Sequences were deposited in GenBank (accession numbers listed in Table 1) and the alignment and trees in TreeBASE (accession number SN2512).

**Morphology.**—Fungal structures were mounted in lactic acid and examined under a light microscope (1000×). The extremes of spore measurements (30 observations) are given in parentheses. Colony colors were rated after 8 d on MEA and OA at 24 C in the dark with the color charts of Rayner (1970).

**RESULTS**

**Sequence data analyses.**—A partition homogeneity test showed that all five datasets were not combin-
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*C. apii* Fresen.

*C. beticola* Sacc.
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| CPC 10265                                | Apium sp.  | Venezuela | N. Pons  | AY840540, AY840507, AY840471, AY840438, AY840405 |
| CPC 10266                                | Apium sp.  | Venezuela | N. Pons  | AY840541, AY840508, AY840472, AY840439, AY840406 |
| CPC 10279                                | Apium sp.  | Venezuela | N. Pons  | AY840542, AY840509, AY840473, AY840440, AY840407 |
| CPC 10666                                | Apium sp.  | Korea     | H. D. Shin | AY840543, AY840510, AY840474, AY840441, AY840408 |
| CPC 10759                                | *A. graveolens* | Korea | H. D. Shin | AY840544, AY840511, AY840475, AY840442, AY840409 |
| CPC 11641                                | *A. graveolens* | Greece | A. N. Jama | DQ233340, DQ233366, DQ233392, DQ233418, DQ233440 |
| CPC 11642                                | *A. graveolens* | Greece | A. N. Jama | DQ233341, DQ233367, DQ233393, DQ233419, DQ233441 |

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*a* CBS strain numbers, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

*b* CPC strain numbers, Collection of Pedro Crous, housed at CBS, The Netherlands.

*c* Type strains of the different *Cercospora* species.
CPC 10547
CPC 10548  Mycosphaerella thailandica
CPC 10549

CBS 117.47 Beta vulgaris Czechia
CPC 5062 Beta vulgaris Rumania
CPC 5064 Beta vulgaris Germany
CPC 5070 Beta vulgaris Rumania
CPC 5072 Beta vulgaris Germany
CPC 5369 Spinacia sp. Botswana
CPC 5370 Spinacia sp. Botswana
CPC 12030 Beta vulgaris Egypt
CPC 12028 Beta vulgaris Egypt
CPC 10171 Beta vulgaris New Zealand
CPC 5074 Beta vulgaris Netherlands
CPC 5123 Apium graveolens New Zealand
CPC 12029 Beta vulgaris Egypt
CPC 11341 Chrysanthemum coronarium L. var. spatiusum Korea
CPC 5069 Beta vulgaris Japan
CPC 5071 Beta vulgaris Spain
CPC 5065 Malva pusilla Rumania
CPC 5128 Beta vulgaris New Zealand
CPC 5113 Limonium sinatum New Zealand
CPC 5125 Beta vulgaris New Zealand
CPC 11344 Chrysanthemum coronarium L. var. spatiusum Korea
CPC 10166 Beta vulgaris New Zealand
CPC 11558 Beta vulgaris Germany
CPC 10204 Beta vulgaris New Zealand
CPC 11557 Beta vulgaris Italy
CPC 10195 Beta vulgaris New Zealand
CPC 10197 Beta vulgaris New Zealand
CPC 12027 Beta vulgaris Germany
CPC 12022 Beta vulgaris Germany
CPC 12031 Beta vulgaris Germany
CPC 10168 Beta vulgaris New Zealand
CBS 116501 Beta vulgaris Iran
CBS 116502 Beta vulgaris Germany
CBS 116503 Beta vulgaris Italy
CBS 116505 Beta vulgaris France
CBS 116506 Beta vulgaris The Netherlands
CPC 5057 Helianthemum sp. Rumania
CPC 5073 Beta vulgaris Austria
CPC 5087 Apium graveolens Rumania
CPC 5063 Beta vulgaris Netherlands
CPC 5083 Plumbago europaea Rumania
CPC 5119 Beta vulgaris Hungary
CBS 116504 Apium graveolens Germany
CBS 116507 Apium graveolens Germany
CPC 5086 Apium graveolens
CPC 10923 Apium graveolens Italy
CPC 10924 Apium graveolens Italy
CPC 10925 Apium sp. Austria
CPC 11556 Apium graveolens Germany
CPC 5084 Plantago lanceolata Rumania
CPC 5112 Mollucella laevis New Zealand
CPC 5111 Mollucella laevis U.S.A.
CPC 5110 Mollucella laevis U.S.A.

Cercospora beticola

Cercospora apii

Cercospora apicola

10 changes
able ($P = 0.001$) but that four of the data sets (ITS, EF, ACT and CAL) could be combined ($P = 1.000$) and these therefore were analyzed as one combined set. The combined alignment contained 67 strains, including the three outgroups, and had a total length of 1262 characters, of which 935 were constant, six were parsimony uninformative and 321 were parsimony informative. The topology of the neighbor joining trees obtained with the different substitution models was the same. A similar topology was found for the most parsimonious trees. Parsimony analysis of the combined data resulted in a single parsimonious trees (Fig. 1) (TL = 350 steps; CI = 0.997; RI = 0.999; RC = 0.996). From the phylogenetic analysis (Fig. 1), three distinct and well supported clades were obtained. The first clade (99% bootstrap support) contains Cercospora isolates belonging to the C. beticola s.s. clade. Twenty-nine of these isolates were obtained from Beta species, but several isolates in this group also were obtained from five additional hosts (two from Chrysanthemum, one from Apium, one from Limonium, one from Malva and two from Spinacia). The isolates were obtained from Europe, Africa, Asia and New Zealand. The second clade (100% bootstrap support) contains C. apiicola s.s. isolates. These isolates also were obtained from a diverse range of hosts (three from Beta, three from Moluccella, one from Plantiago, one from Plumbago and one from Helianthemum), but the primary host infected by isolates in this group appears to be Apium (eight isolates). Isolates from the second clade were from Europe, America and New Zealand. The third clade (100% bootstrap support) contains isolates of C. apiicola that thus far have been isolated only from Apium species in Venezuela, Korea and Greece.

Because the HIS dataset was not combinable with other sequence data, it was analyzed separately. The HIS alignment contained 67 strains including the three outgroups, and had a total length of 380 characters, of which 319 were constant, one was parsimony uninformative and 60 were parsimony informative. The topology of the neighbor joining trees obtained with the different substitution models was the same and was identical to the topology of the most parsimonious tree. Parsimony analysis of the HIS data resulted in the single most parsimonious tree (Fig. 2) (TL = 73 steps; CI = 0.986; RI = 0.998; RC = 0.984). From the phylogenetic analysis (Fig. 2), three well supported clades with 100% bootstrap values were obtained. The first clade contained eight isolates (seven from Beta species from different countries and one from Helianthemum in Rumania) that were present in the C. beticola s.s. clade obtained from the first analysis, except for the Helianthemum isolate which grouped in the C. apiicola s.s. clade (Fig. 1). The second clade contained the remaining C. beticola s.s. and C. apiicola s.s. isolates. The third clade consisted only of the C. apiicola isolates, which is consistent with the first analysis using the other four loci.

**Taxonomy.**—Cercospora apiicola and C. beticola s.s. were circumscribed by Groenewald et al (2005). During the present study several Cercospora isolates were obtained from celery exhibiting Cercospora leaf spot. A population of 47 plants collected in Venezuela by N. Pons, as well as individual diseased plants collected in Greece and Korea, were found to be associated with a novel species of Cercospora. The latter species is morphologically distinct from the C. apiicola s.l. complex. Its conidiophores are relatively short, 25–70 × 4–6 µm, and the conidia are obclavate-cylindrical, not acicular, measuring (50–)80–120 (150) × (3–)4–5 µm and being 1–6-septate (Figs. 3, 4). This species therefore is described as new:

**Cercospora apiicola** M. Groenewald, Crous & U. Braun, sp. nov.

Differt a C. apiicola (s.s. et s.l.) conidiophoris relative brevibus, 25–70 × 4–6 µm, conidios obclavatul-e-cylindraceis, nonacicularibus, tantum 1–6-septatis.


*Leaf spots* amphigenous, subcircular to irregular, 3–10 mm diam, medium brown, with a raised or inconspicuous, indefinite margin, not surrounded by a border of different color. Conidiophores arising in fascicles of 4–10, moderately dense, arising from stromata, emerging through stomata or erumpent through the cuticle, subcylindrical, upper part geniculate-sinuous, unbranched, 1–...
CPC 10547
CPC 10548
CPC 10549

Mycosphaerella thailandica

CBS 117.47 Beta vulgaris Czechia
CPC 5062 Beta vulgaris Rumania
CPC 10166 Beta vulgaris New Zealand

CPC 10195 Beta vulgaris New Zealand
CPC 12027 Beta vulgaris Germany
CPC 12022 Beta vulgaris Germany
CPC 12031 Beta vulgaris Germany
CPC 5057 Helianthemum sp. Rumania

CPC 5064 Beta vulgaris Germany
CPC 5070 Beta vulgaris Rumania
CPC 5072 Beta vulgaris Germany
CPC 10204 Beta vulgaris New Zealand

CPC 11557 Beta vulgaris Italy
CPC 10197 Beta vulgaris New Zealand
CPC 12030 Beta vulgaris Egypt
CPC 12028 Beta vulgaris Egypt
CPC 10171 Beta vulgaris New Zealand
CPC 5074 Beta vulgaris Netherlands
CPC 5123 Aiptum graveolens New Zealand

CBS 116501 Beta vulgaris Iran
CBS 116502 Beta vulgaris Germany
CBS 116503 Beta vulgaris Italy
CBS 116505 Beta vulgaris France
CBS 116506 Beta vulgaris The Netherlands
CPC 12029 Beta vulgaris Egypt
CPC 5069 Beta vulgaris Japan
CPC 5071 Beta vulgaris Spain
CPC 5065 Malva pusilla Rumania

CPC 5128 Beta vulgaris New Zealand
CPC 5113 Limonium sinuatum New Zealand
CPC 5125 Beta vulgaris New Zealand
CPC 5073 Beta vulgaris Austria
CPC 10923 Aiptum graveolens Italy
CPC 10924 Aiptum graveolens Italy
CPC 5087 Aiptum graveolens Rumania
CPC 5063 Beta vulgaris Netherlands
CPC 5083 Plantago europea Rumania
CPC 5119 Beta vulgaris Hungary
CPC 5084 Plantago lanceolata Rumania
CPC 5112 Moluccella laevis New Zealand
CPC 5111 Moluccella laevis USA
CPC 5110 Moluccella laevis USA
CPC 5369 Spinacia sp. Botswana
CPC 5370 Spinacia sp. Botswana
CPC 11558 Beta vulgaris Germany
CPC 10168 Beta vulgaris New Zealand
CBS 116504 Aiptum graveolens Germany
CBS 116507 Aiptum graveolens Germany
CPC 5086 Aiptum graveolens
CPC 10925 Aiptum sp. Austria
CPC 11556 Aiptum graveolens Germany

CPC 11341 Chrysanthemum coronarium L. var. spathosum Korea
CPC 11344 Chrysanthemum coronarium L. var. spathosum Korea

CPC 10220 Aiptum sp. Venezuela
CPC 10248 Aiptum sp. Venezuela
CPC 10265 Aiptum sp. Venezuela
CPC 10266 Aiptum sp. Venezuela
CPC 10267 Aiptum sp. Venezuela
CPC 10279 Aiptum sp. Venezuela
CPC 10657 Aiptum graveolens Korea
CPC 10666 Aiptum sp. Korea
CPC 10759 Aiptum graveolens Korea
CPC 11641 Aiptum graveolens Greece
CPC 11642 Aiptum graveolens Greece

Cercospora apicola
Cercospora beticola
3-septate, 25–70 × 4–6 μm, medium brown, becoming pale brown toward the apex, smooth, wall somewhat thickened. Conidiogenous cells integrated, terminal, 15–30 × 4–5 μm, occasionally unilocular, usually multilocular, sympodial; loci subcircular, planate, thickened, darkened, refractive, 2.5–3 μm wide. Conidiogenous cells solitary, cylindrical when small, obclavate-cylindrical when mature, not acicular, (50–)80–120 (–150) × (3–)4–5 μm, 1–6-septate; apex subobtuse, base obconically subtruncate; hila 2–2.5 μm wide, thickened, darkened, refractive.

Cultural characteristics. Colonies are smooth to folded, erumpent with smooth, even to uneven margins and sparse to moderate aerial mycelium; white to smoke-gray on MEA (surface), and olivaceous-gray to iron-gray beneath; on OA colonies are white to olivaceous-gray on the surface. Cardinal temperature requirements for growth, min 6 C, opt 24 C, max 30 C.

Host range and distribution. Apium graveolens, Apium sp., Greece, Korea, Venezuela.

**DISCUSSION**

During a recent study in which we circumscribed *C. apii* and *C. beticola* s.s., we collected isolates of several *Cercospora* spp. that are part of the *C. apii* s.l. species complex. A whole population of “*C. apii*” collected on celery from Venezuela was revealed to be a distinct species. Several months later we isolated the same species on celery collected from Korea. At that time it was thought that this species had not yet invaded European celery fields because it was absent from European *Cercospora* isolates from this crop (Groenewald et al 2005). However in the present study we report the presence of this species on celery from Greece and describe it as *C. apiicola* sp. nov. Cultural and morphological examination of the *C. apiicola* strains support the observation made by Groenewald et al (2005) that this new *Cercospora* species is distinct from the two closely related species, *C. beticola* and *C. apii*, that previously have been isolated from celery. The isolation of this new *Cercospora* species on a well known crop such as celery is an indication that there may still be many other undescribed cercosporoid species on well known crops and ornamental plants awaiting description.

Chupp (1954) associated *Cercospora* leaf spot on sugar beet with infections of *C. beticola*, and that of celery with *C. apii*. Ellis (1971) discussed the *C. apii* s.l. isolates in detail and described a wide host range for this species, but five years later he changed his
opinion and narrowed the host range of C. apiicola to celery and C. beticola to sugar beet (Ellis 1976). Crous and Braun (2003) linked 83 host genera to C. apiicola and nine host genera to C. beticola infections. Groenewald et al (2005) again cast doubt on the purported wide host ranges of these species. In the present study a survey of Cercospora isolates from 10 host genera identified several additional hosts for both C. apiicola s.s. and C. beticola s.s. From these data we can confirm four additional host genera for C. apiicola (Helianthemum, Molucellia, Plantago, Plumbago) and five additional host genera for C. beticola (Apium, Chrysanthemum, Limonium, Malva, Spinacia). According to Crous and Braun (2003) several Cercospora species (listed in parentheses) are associated with these hosts: Apium (C. apiicola), Beta (C. beticola), Helianthemum (C. cistinearum, C. helianthemi), Molucellia (C. molucellae), Plantago (C. pantoleuca, C. plantaginis), Plumbago (C. apiicola, C. plumbaginea), Limonium (C. apiicola, C. insulana, C. statices), Malva (C. althaeina, C. beticola, C. hyalospora, C. malvae, C. malvarum) and Spinacia (C. bertrandii, C. beticola, C. spinaciicola). In the treatment of Crous and Braun (2003) neither Apium, Chrysanthemum or Limonium are listed as hosts of C. beticola nor Beta, Helianthemum, Molucellia and Plantago as hosts of C. apiicola. This study provides the first molecular evidence that these two species have wider host ranges than had been accepted by Chupp (1954) and Ellis (1976). However from the present study it appears that both species have narrower host ranges than that proposed by Crous and Braun (2003), but this has to be investigated further by conducting pathogenicity studies on all the hosts previously listed for these species.

The host range data obtained in the present study illustrate that C. beticola s.s. and C. apiicola s.s. are not entirely host specific and that it is not possible to identify these two species solely based on host. Despite of the additional host genera that were found for C. apiicola and C. beticola, it is clear that C. apiicola s.s. is mainly isolated from celery, whereas C. beticola is mainly isolated from sugar beet, even though both of these species have been isolated from the other’s primary host in the past.

Crous and Groenewald (2005) introduced the pogo stick hypothesis to explain the colonization of necrotic Mycosphaerella lesions by other species of Mycosphaerella that jump hosts in the process of reaching their real hosts. The possibility that this process of substrate colonization and host jumps also occurs in asexual Mycosphaerella species could explain the isolation of specific Cercospora species from “atypical” hosts and needs to be investigated further. It would be especially interesting to determine whether Cercospora species occurring on “atypical” hosts are able to cause disease on these hosts or not.

As illustrated in this study, morphology, host specificity and geographic location are not suitable characters for the identification of species of the Cercospora apiicola complex. Groenewald et al (2005) used sequence data in combination with other features such as growth rate to establish species boundaries for C. apiicola, C. apiicola (as Cercospora sp.) and C. beticola. From these established species boundaries, species-specific primers were designed in polymorphic areas of the calmodulin gene for the three species. This combined approach probably represents the most reliable way to characterize and identify species within this complex.

Five loci were used in this study for phylogenetic analyses, although all five loci sequenced were not congruent and therefore could not be used in a combined phylogenetic analysis. Two separate analyses thus were performed, the first combining ITS, EF, ACT and CAL sequences and the second

\[ \text{Fig. 4. Line drawing of conidiophores and conidia of the } \text{C. apiicola holotype (CBS 116457). Bar = 10 } \mu \text{m.} \]
using only HIS sequences. The first analysis separated the \textit{C. apiicola} s.s., \textit{C. beticola} s.s. and \textit{C. apiicola} isolates. Although the second analysis also was able to separate the \textit{C. apiicola} isolates from the \textit{C. apiicola} s.s./ \textit{C. beticola} s.s. isolates, it was unable to distinguish between \textit{C. apiicola} s.s. and \textit{C. beticola} s.s. isolates. Using HIS data a small cluster representing seven \textit{C. beticola} s.s. and one \textit{C. apiicola} s.s. isolate grouped separately from other \textit{C. apiicola} s.s./ \textit{C. beticola} s.s. isolates. The unique polymorphisms (10 in total) observed in the histone H3 sequences of these isolates were identical and were not present in the other isolates or in our \textit{Cercospora} sequence database. A possible explanation might be host jumping by the \textit{Helianthemum} isolate, followed by recombination with the \textit{Beta} isolates. However more \textit{Helianthemum} isolates need to be studied to confirm whether this allele is unique to \textit{Helianthemum} before one can address this issue. Caution therefore should be taken when using histone H3 sequence data for \textit{Cercospora} phylogeny because variation in the histone H3 sequence may not indicate species differences.

It can be concluded from this study that strains belonging to the \textit{C. apiicola} s.s. and \textit{C. beticola} s.s. clades can be isolated from other hosts and, although these species are mainly isolated from celery and sugar beet, they are not host specific. It seems that the new species from celery described in this paper (viz. \textit{C. apiicola}) is host specific because no other \textit{Cercospora} strain isolated from other hosts and available in our sequence database has similar sequences. The reasons why host jumping by \textit{C. apiicola} and \textit{C. beticola} is so common remains unknown. However it is not unlikely that under stress—a shortage of host tissue or unsuitable weather—the new species might be able to jump from celery onto other hosts.

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