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Taxonomic and phylogenetic revision of *Coniophora arachnoidea, C. opuntiae,* and *C. prasinoides*

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Abstract — The authors revised type material from three *Coniophora* species (*C. arachnoidea, C. opuntiae* and *C. prasinoides*), applying morphological techniques. A phylogenetic analysis of ITS sequences was conducted for several related *Coniophora* species. *C. opuntiae* is proposed to represent a synonym of *C. arachnoidea*, whereas *C. prasinoides* is maintained as an independent taxon. Microphotographs of the most representative characters of the specimens are presented.

Key words — *Basidiomycota*, *Boletales*, taxonomy, molecular phylogeny, France, Guinea, Spain

Introduction

The genus *Coniophora* DC. (*Coniophoraceae, Boletales*) was erected by De Candolle (1815) for *Coniophora membranacea* DC., which at present is considered a synonym of *Coniophora puteana* (Schumach.) P. Karst., this epithet having priority. Members of this genus develop their basidiomata mainly on wood (conifers and deciduous trees), causing brown rot and seriously damaging the affected wood. Likewise, other members of *Corticiaceae* sensu lato such as *Phlebia* Fr. (= *Merulius* Fr.) and *Serpula* (Pers.) Gray, also produce important economical losses in the building sector. Fungi attacking wood in buildings are excellently presented in the work by Huckfeldt & Schmidt (2006), with colour photographs and identification keys to genus or even species level.

Amongst the taxonomic works on *Coniophora* the monograph by Ginns (1982) represents a hallmark, together with the classical work by Bourdot & Galzin

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(1928). Ginns (1982) considered *Coniophora* within the family *Coniophoraceae* and separated it from closely related genera on the basis of a combination of characters, including "hyphae with simple septa and typically with scattered single, double or verticillated clamps." He recognized the following genera in the family: *Gyrodontium* Pat., *Serpula*, *Leucogyrophana* Pouzar, *Jaapia* Bres., and *Corneromyces* Ginns. The different species of *Coniophora* were separated in a dichotomous key based on classical macro- and microscopical characters such as spore size and shape, dextrinoid reaction of basidiospores, presence or absence of cystidia, whether the hyphal system is monomitic, dimitic or trimitic, basidioma morphology, and substrate adherence. In addition, he provided data from plate cultures for 6 out of the 15 species discussed. In his work on species of the *Coniophoraceae* from northern Europe, Hallenberg (1985) recognized three genera within the family (*Coniophora*, *Serpula* and *Leucogyrophana*) and described only four *Coniophora* species. The genera *Serpula* and *Meruliporia* Murrill were treated in an extensive work by Cooke (1957), who also included *Jaapia*, *Coniophora*, *Coniophorella* P. Karst. (at present considered a synonym of *Coniophora*), and *Gyrodontium*.

Molecular analyses confirm the position of *Coniophora* and related genera within *Boletales* (Binder & Hibbett 2006, Larsson et al. 2004). According to Hibbett & Thorn (2001) the corticioid fruitbody shape derived from pileate and erect ones.

In this work we compare three critical *Coniophora* species that can be easily confused: *C. arachnoidea*, *C. prasinoides,* and *C. opuntiae*, all characterized by a monomitic hyphal system with simple-septate hyphae and by comparatively smaller spore sizes.

Materials and methods

Material examined

The material studied is preserved in the following dried reference collections: AH (Universidad de Alcalá de Henares), BPI (The National Fungus Collections, U.S.A.), DAOM (National Mycological Herbarium, Canada), FH (Farlow Herbarium, U.S.A.) and MA-Fungi (Real Jardín Botánico Madrid).

Descriptions of the macro- and micromorphological characteristics were based on herbarium material except for the sample AH 31855, which was studied fresh after collecting. The micromorphological characters were also studied from dried reference material, using KOH 5%, ammonia Congo red, Melzer's reagent and distilled water. Cotton blue was used to study the spore germination pore and cyanophilic elements.

DNA extraction, PCR amplification, and DNA sequencing

DNA was extracted from small basidiomata fragments of *C. opuntiae* AH 31855 following previously described methods (Peláez et al 1996). The ITS1-5.8S-ITS2 fragment was amplified using the ITS1F and ITS4b primers (Gardes & Bruns 1993).

PCR amplifications followed standard procedures (5 min at 93ºC, then 40 cycles of 30 s at 93ºC, 30 s at 53ºC and 2 min at 72ºC) using Taq DNA polymerase (QBiogene, Inc.) following the procedures recommended by the manufacturer. Amplification products (0.1 µg/ml) were sequenced using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems) following manufacturer recommendations. Each strand of the amplification products was sequenced with the same primers used for the initial amplification. Partial sequences obtained during sequencing reactions were assembled using BioEdit 7.0.5.3 (Hall 1999). The DNA sequence was compared with GenBank database using BLAST application. Alignment of the best matching sequences was also performed using BioEdit 7.0.5.3. Finally, the alignments were visually adjusted with GeneDoc 2.5 software (Nicholas & Deerfield 1997). Genbank FJ790314.

Phylogenetic analysis

Bayesian analysis based on Markov chain Monte Carlo (MCMC) approach was run using MrBayes 3.01 (Ronquist & Huelsenbeck 2003). To improve mixing of the chain, four incrementally heated simultaneous Monte Carlo Markov chains were run over 2,000,000 generations. MrModeltest 2.2 (Nylander 2004) was used to perform hierarchical likelihood ratio tests to calculate the Akaike information criterion (AIC) values of the nucleotide substitution models. The model selected by AIC for the alignment of the ITS1-5.8S-ITS2 gene fragment was the GTR+G model allowing six classes of substitution types, a portion of invariant alignment positions, and mean substitution rates varying across the remaining positions according to a gamma distribution. Priors used for the MCMC process were a Dirichlet distribution for substitution rates and nucleotide frequencies and a uniform prior for the rate parameter of the gamma distribution. Both types of analysis used the sampling frequency of 100 to store trees, with the 1000 first trees discarded to obtain a majority rule consensus tree.

Taxonomy

Coniophora arachnoidea Pat., Bull. Soc. Mycol. France 28: 31 (1912). Figs. 1–3

Specimens examined—GUINEA. Camayenne–feuilles de Bananier–29.VI.1910–leg. M. Duport 65, herb. Patouillard *3376* in *FH 258812* Holotype.

type information — The type material is conserved in a paper envelope containing a white cardboard with four banana leaf fragments, two ~3 × 3 cm squares with scarce fruiting bodies, one rectangle (5.5 × 1 cm) without any fruitbodies, and one very small piece (2 × 0.5 cm) with sparse fruiting bodies. In addition there are two labels, one small hand written label with the collection data and the number 65, and a review label from J.H. Ginns. Only the type material of this species is known.

description — Basidioma attached to the substrate, although easily detachable in some areas. Hymenophore membranaceous, brownish or straw-coloured with olivaceous tones. Margin fibrous and whitish. Hyphal system monomitic, without clamp connections. Context consisting of cylindrical hyphae, septate, cyanophilic, 2.5–6 µm wide, without incrustations. Tetrasporic basidia, 40–43

× 6–7 µm. Spores 6–8 × 4–5 µm, ellipsoid, brownish, thick walled, cyanophilic, non-dextrinoid or only slightly dextrinoid sometimes, with a paler apical zone corresponding to a rudimentary germ pore, sometimes difficult to observe.

Coniophora opuntiae Tellería, An. Jard. Bot. Madrid 41(1): 26 (1984).

FIGS. 4–6, 11–15

SPECIMENS EXAMINED—SPAIN. ALMERÍA: San José, road fromSan José to Cabo de Gata, near Morrón de los Genoveses–1.XII.1983–on *Opuntia* sp., leg. F.D.Calonge, M.Dueñas & M.T.Tellería, 4460Tell. in *MA-Fungi 6900 Holotypus*. ALMERÍA: Turrillas on cladodia of *Opuntia ficus-indica*,–8-II-2008–leg. G.Moreno & J.Checa, *AH 31855*.

TYPE INFORMATION — The type material is conserved in a paper envelope and inside there is another envelope with a white cardboard. The sample is very abundant, containing six fragments of *Opuntia* cladodia with many fruiting bodies. In addition, a small paper envelope with small sample fragments is also conserved. A detailed description by the author with pencil drawing of spores, basidia, and hyphae is also included.

DESCRIPTION — Basidioma resupinate, membranaceous, easily detachable from the substrate. Hymenium smooth, olivaceous, margin pale, whitish to yellowish, with whitish mycelial cords. Hyphal system monomitic, without clamp connections. Context consisting of cylindrical hyphae, septate, cyanophilic, 3–5 µm wide. Tetrasporic basidia, 30–50 × 6–8 µm, clavate. Spores 6–8 × 4–5 µm, ovoid to broadly ellipsoid, yellowish to yellow-brownish, thick walled, cyanophilic, non-dextrinoid or occasionally only very slightly dextrinoid, with a paler apical zone corresponding to a rudimentary germ pore, sometimes difficult to observe.

The sample AH 31855, collected in the same province (Almería) and on the same substrate (*Opuntia ficus-indica*), showed somewhat larger basidia (40–60 × 5–6.5 µm) and spores (7.5–10 × 4–6 µm). These differences are attributed to intraspecific variability.

Coniophora prasinoides (Bourdot & Galzin) Bourdot & Galzin, Hyménomyc. de France (Sceaux): 361 (1928). FIGS. 7–10

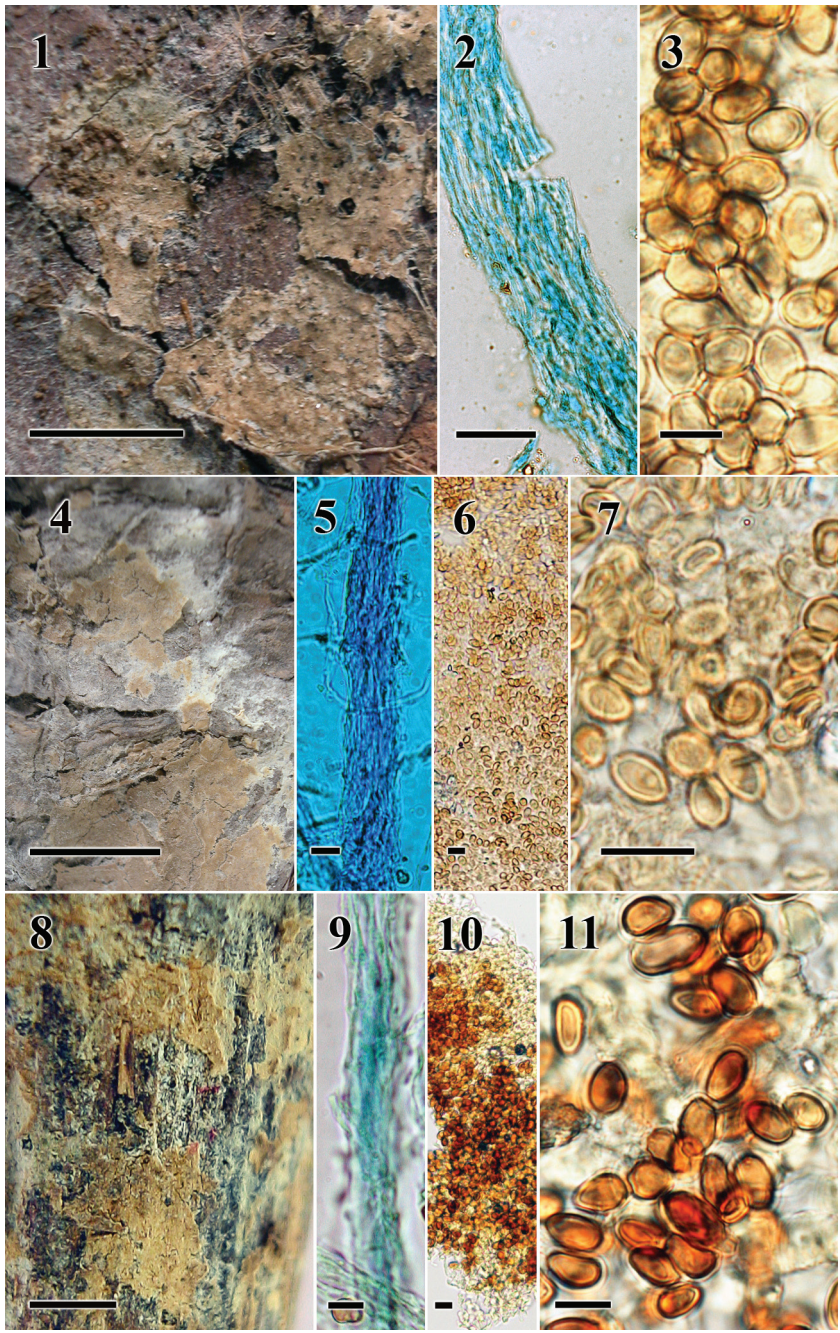
≡ *Coniophora olivacea* subsp. *prasinoides* Bourdot & Galzin, Bull. Soc. Mycol. France 39: 115 (1923)

FIGS. 1–3 *Coniophora arachnoidea* type: 1. Basidioma. 2. Cyanophilic mycelial cord in cotton blue. 3. Non-dextrinoid spores in Melzer´s reagent.

FIGS. 4–7 *Coniophora opuntiae* type: 4. Basidioma. 5. Cyanophilic mycelial cord in cotton blue. 6–7. Non-dextrinoid spores in Melzer´s reagent.

FIGS. 8–11 *Coniophora prasinoides* type: 8. Basidioma. 9. Cyanophilic mycelial cord in cotton blue. 10–11. Dextrinoid spores in Melzer´s reagent.

Scales: 1 = 0.5 cm, 2 = 20 µm, 3 = 10 µm, 4 = 1 cm, 5–7 = 10 µm, 8 = 0.25 cm, 9–11 = 10 µm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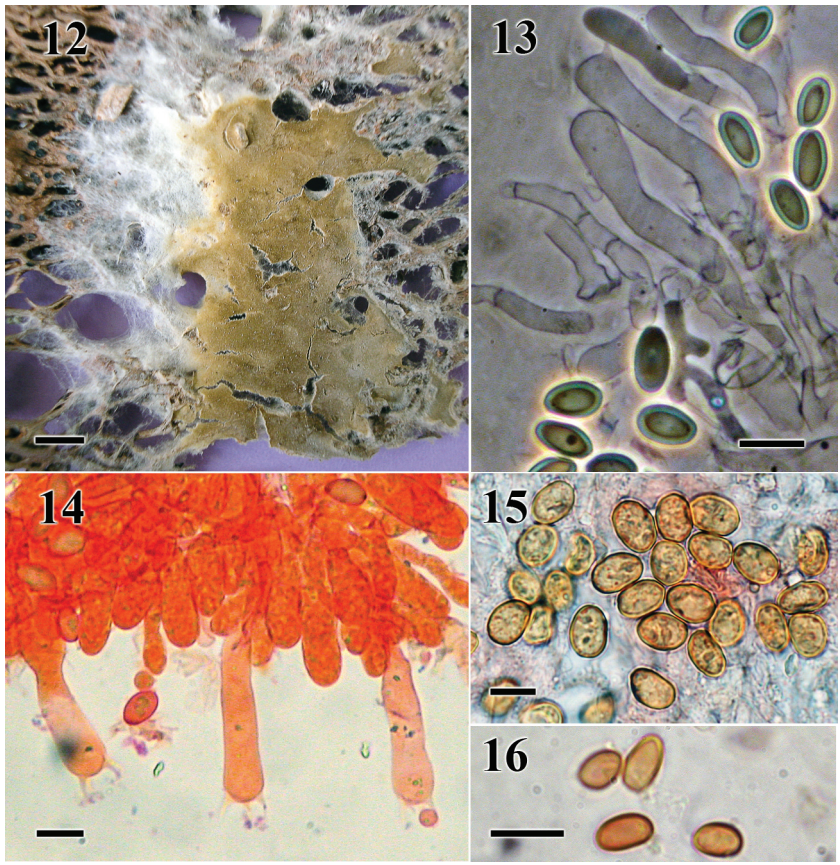

Figs. 12–16 *Coniophora opuntiae* AH 31855: 12. Basidioma. 13. Hymenophore in phase contrast. 14. Hymenophore in ammonia Congo red. 15. Spores in KOH 5%. 16. Spores in Melzer's reagent
Scales: 12 = 0.5 cm, 13–16 = 10 µm

Specimens examined—FRANCE. L'Aveyron, ad rames *Vitis viniferae*, –XI.1916–leg. Galzin 20845, Herb. H. Bourdot 18607, ex Herb. Bresadola in *BPI USO290325* isolectotype. UNITED STATES. Maryland: College Park, old southern pine flooring stored in open 9 months–16.XI.1965–leg. H.H. McKay, det. J.H. Ginns, FP 105969-sp, ex BFDL in *DAOM 137642* (*AJ 419197*).

type information — The type material is conserved in a paper envelope fixed to a herbarium sheet. Inside this there is a plastic bag containing the sample, consisting of highly fragmented wood pieces with fruitbodies only on three large fragments, and only in one case is the specimen acceptably preserved. In addition, there are two review labels, one from J.H. Ginns and the second from

Anton R. Slysh. In the paper envelope there is also a microscopic description with ink drawings of the spores and hymenium, with the collection data numbered as 18607 Herb. H. Bourdot, and at the left edge a stamp with the legend "Abate G. Bresadola Trento." Therefore, this sample belonging to Bresadola's herbarium and conserved today at BPI, must be considered an isolectotype of *Coniophora prasinoides*. The lectotype was created by Ginns (1982) for collection 18608 deposited in PC.

DESCRIPTION — Basidioma attached to the substrate, easily detachable in some areas. Hymenium smooth, membranacous, brownish or straw-coloured with greenish to yellowish tones. Margin fibrous and whitish. Hyphal system monomitic, without clamp connections. Context consisting of cylindrical hyphae, septate, cyanophilic, 4–6 µm wide, without incrustations. Tetrasporic basidia, 40–55 × 6–8 µm, clavate. Spores 7–11 × 5–7 µm, ellipsoid, yellowish to yellow-brownish, thick walled, cyanophilic, strongly dextrinoid, with a paler apical zone corresponding to a rudimentary germ pore, sometimes difficult to observe.

Another sample growing on pine wood (DAOM 137642, a duplicate of which is conserved in MA-Fungi 19417) was also examined, showing the same characteristics as the type material, both macro- and microscopically.

Results and discussion

Coniophora opuntiae was separated by Tellería (1984) from the two species sharing small spore sizes, *C. arachnoidea* and *C. prasinoides*, based on the characters related to hyphae, spores and habitat, as indicated in TABLE 1 (based on the cited author's data).

According to TABLE 1, *C. arachnoidea* would differ from *C. opuntiae* by spores having an apical germination pore and rarely showing a dextrinoid reaction, as well as in their different habitats. The type material of *C. arachnoidea*, the only material of the species available for study, was not directly examined by Tellería (1984), who based the characters reported on the Ginns 1982 description. Thus, the reaction of the hyphal walls to lactophenol blue was unknown, as indicated in TABLE 1. *Coniophora prasinoides* was said to differ from *C. opuntiae* by rarely forming hyphal cords, non-cyanophilic hyphal walls, broader spores [4.3–6.9 (–7.5) µm] with germ pore, and different habitat. For *C. prasinoides,* Tellería studied DAOM 137642, which corresponds to American material determined by Ginns.

However, when the type materials of these three species are studied in detail, the diagnostic characters (presence of mycelial cords, germ pore, dextrinoid spores) are often difficult to appreciate, and they sometimes vary depending on the microscopic preparation. We summarize our observations as follows:

Coniophora arachnoidea shows mycelial cords with cyanophilic hyphal cell walls, spores 6–8 × 4–5 µm, generally non-dextrinoid, only some spores are very slightly dextrinoid, showing a rudimentary germ pore.

Coniophora prasinoides also shows mycelial cords with cyanophilic hyphal cell walls, larger spores (7–11 × 5–7 µm), cyanophilic, strongly dextrinoid and with a rudimentary germ pore.

Coniophora opuntiae shows mycelial cords with cyanophilic hyphal cell walls, spores 6–8 × 4–5 µm, cyanophilic, mostly non-dextrinoid, only some spores slightly dextrinoid, and showing also a rudimentary germ pore.

There are two points where our observations do not concur with the original description of *C. opuntiae* by Tellería (1984). First, the spores were indicated to be dextrinoid while we did not find dextrinoidity or at most only rarely and very slightly. In general, the spores appeared non-dextrinoid. Likewise, the germ pore was said to be nonexistent, but we observed the same rudimentary germ pore, although often difficult to observe, as seen in the other two species studied here. In addition, we noted cyanophilic hyphal cell walls in *C. prasinoides* (see Table 1).

Table 1. Comparison of *Coniophora arachnoidea*, *C. prasinoides,* and *C. opuntiae*. *

	C. arachnoidea	*C. prasinoides*	*C. opuntiae*
MYCELIAL CORD	Present, with broader (10–16 µm) hypha in mid-region	Rarely formed; when present consisting of 3–5 µm broad hyphae and one slightly broader central hypha	Present, with broader (13 µm) hypha in mid-region
HYPHAL CELL WALL	? **(cyanophilic)**	Not cyanophilic **(cyanophilic)**	Cyanophilic **(cyanophilic)**
SPORES: SHAPE	Broadly ellipsoid, sometimes narrowly to broadly ellipsoid	Ovoid, broadly ovoid, or broadly ellipsoid	Ellipsoid, broadly ellipsoid. or ovoid
GERM PORE	Present	Present	Absent **(rudimentary germ pore)**
REACTIONS	Cyanophilic, rarely dextrinoid **(not to rarely slightly dextrinoid)**	Cyanophilic, dextrinoid **(strongly dextrinoid)**	Cyanophilic, dextrinoid **(not to rarely slightly dextrinoid)**
SIZE	6.7–9(–9.6) × 3.8–5.5 µm	7.4–10 × 4.3–6.9(–7.5) µm	6–8 × 4–5 µm
HABITAT	Banana leaves	Wood of *Pinus, Vitis vinifera,* and *Salix*	Cladodia of *Opuntia*

*Based on Tellería (1984); new observations added in **bold** font and parentheses.

The examination of additional specimens adds even more complexity to the characterization of the differences between these species. Thus, a new collection of *C. opuntiae* from the same habitat and location as the type material (AH 31855) showed a slightly different spore size range (7.5–10 × 4–6 µ) that places this specimen closer to *C. prasinoides* regarding only spore size.

In summary, we distinguish two *Coniophora* groups: on the one hand *C. prasinoides*, characterized by strongly dextrinoid and slightly larger spores, and on the other, *C. arachnoidea* and *C. opuntiae*, with non-dextrinoid and somewhat smaller spores. We have not detected any meaningful morphological differences between the latter two species. Although their reported habitat is different [banana leaves (*Musa* sp., *Musaceae*) for *C. arachnoidea* and cladodia of *Opuntia ficus-indica* (*Cactaceae*) for *C. opuntiae*], it could be claimed that they are somewhat related substrates, since both types of structures accumulate water in their tissues.

We could not carry out molecular studies of the type material of *C. arachnoidea* and *C. prasinoides*; because they are very old and scarce material and successful DNA extraction seemed questionable, we decided not to put valuable type material at a risk. We have sequenced new material of *C. opuntiae* from the same location as the type material (AH 31855). On the other hand, Martín & Raidl (2002) had previously obtained the ITS sequence of *C. prasinoides* DAOM 137642 (a specimen which we have studied also morphologically), allowing at least the comparison of these two species by molecular methods.

The ITS region of *C. opuntiae* was successfully amplified. The size of the whole region was 640 bp. BLAST analysis revealed that its closest match was *Coniophora olivacea* (Fr.) P. Karst., with a 91–92% homology in the ITS1-5.8S-ITS2 region. The homology with *C. prasinoides* was 86% in the same region. These data support *C. opuntiae* and *C. prasinoides* as two different species.

The phylogenetic analysis based on the alignment of ITS sequences from a group of *Coniophora* species is shown in Figure 17. The size of the alignment was 729 nucleotides, while 520 of these characters were constant. Bayesian analysis showed that *C. opuntiae* clustered with sequences of *C. olivacea* with a moderate clade support value (posterior probability value 0.89). *Coniophora olivacea* differs from *C. opuntiae* by the presence of septate, long, brown cystidia, lacking in *C. opuntiae*. Both species were included together with *C. prasinoides* and *Coniophora marmorata* Desm. in one larger clade with strong clade support (0.93). These four species therefore seem to be more closely related, compared with other *Coniophora* species such as *Coniophora arida* (Fr.) P. Karst. and *C. puteana. Coniophora marmorata* shares the small spore size (7–9.8 × 4.5–7 (–8) µm) with *C. opuntiae* and is mainly distinguished by its dimitic hyphal system. In addition, the segregation of *Coniophorella* from *Coniophora* seems to be artificial and is not supported by the present molecular analysis.

Fig. 17. Phylogenetic analysis based on the alignment of ITS sequences from a group of *Coniophora* species. Clade credibility values are given on the branches. Note the significative phylogenetic distance regarding *C. prasinoides* and *C. opuntiae*.

Conclusions

Based on the morphological and molecular data presented above, we conclude that *Coniophora prasinoides* and *Coniophora opuntiae* represent two different species. We consider *Coniophora arachnoidea* and *C. opuntiae,* which cannot be distinguished morphologically, as synonyms, with the older epithet, *C. arachnoidea,* having priority.

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