

***Leptodophora* gen. nov. (*Helotiales*, *Leotiomycetes*)
proposed to accommodate selected root-associated members
of the genus *Cadophora***

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Cadophora orchidicola, a root-associated asexual species, did not have a clear placement within the paraphyletic genus *Cadophora* because it was not represented by sequences derived from the type material. Based on four newly obtained molecular marker sequences from the ex-type strain, a new genus, *Leptodophora*, is proposed, typified with *C. orchidicola*, as well as new combinations for three recently described and closely related species. In addition, a new species, *Collembolispora disimilis*, is proposed for a fungus producing phialidic conidiogenous cells and previously treated as *Cadophora* sp.

Key words: asexual ascomycete, phylogenetic placement, *Brassicaceae*, conidiogenesis.

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Cadophora orchidicola, anamorfní druh asociovaný s kořeny rostlin neměl dosud jasnou pozici v rámci polyfyletického rodu *Cadophora*, protože nebyla získána ani jedna sekvence z typového materiálu. Na základě sekvencí čtyř molekulárních markerů získaných z ex-typového kmene tohoto druhu je navržen nový rod *Leptodophora*, typifikovaný druhem *C. orchidicola*, a současně nové kombinace pro tři nedávno popsané a blíže příbuzné druhy. Současně je navržen nový druh *Collembolispora disimilis* na základě izolátů tvořících fialidické konidiogenní buňky a původně řazených jako neurčené do rodu *Cadophora*.

INTRODUCTION

The genus *Cadophora* Lagerberg et Melin (*Ploettnerulaceae*, *Helotiales*) was established by Lagerberg et al. (1927) to accommodate asexual fungi characterised by a melanised mycelium and solitary phialides with distinct hyaline, flaring collarettes. The type species *C. fastigiata* Lagerberg et Melin was isolated from softwood timber and several further additions to the genus included saprotrophs isolated from wood, or pathogens causing necroses in woody tissues [e.g. *C. matorum* (Kidd et Beaumont) W. Gams, *C. luteo-olivacea* (J.F.H. Beyma) T.C. Harr. et McNew, *C. viticola* Gramaje, L. Mostert et Armengol and *C. ramosa* Damm et S. Bien]. With further additions of novel *Cadophora* species isolated from soil and herb stems, the original generic concept became rather eroded, and ended up lumping phenotypically highly diverse species. Molecular data and phylogenies including just a few closely related species were used to support these new additions to *Cadophora* (Walsh et al. 2018), and larger phylogenies also presented the genus as monophyletic because representatives of closely related genera had not been included (Bien et Damm 2020).

Most recently, Maciá-Vicente et al. (2020) revealed a hitherto unrecognised diversity of *Cadophora* species among asymptomatic colonisers of plant roots. They also showed, on a robust four-gene dataset, that the genus *Cadophora* is paraphyletic and composed of two large clades. *Cadophora* sensu stricto (s.str.) included the type species *C. fastigiata* and the majority of other species colonising wood, all showing somewhat similar morphologies in their sporulating structures. The other clade, referred to as *Cadophora* sensu lato (s.l.), covered the remaining *Cadophora* species which largely differ in their phenotypes, and was interspersed with other members of *Ploettnerulaceae*, i.e. genera *Collembolispora* Marvanová et Pascoal, *Helgardiomycetes* Crous, *Mycochaetophora* Hara et Ogawa, *Oculimacula* Crous et W. Gams, *Rhexocercosporidium* U. Braun, *Rhynchobrunnera* B.A. McDonald, U. Braun et Crous and *Rhynchosporium* Heinsen ex A.B. Frank.

The most conspicuous representative of *Cadophora* s.l., with a phenotype very different from that of *C. fastigiata*, is *C. orchidicola* (Sigler et Currah) M.J. Day et Currah, originally described as *Leptodontidium orchidicola* Sigler et Currah, based on five strains isolated from roots of orchids in the University of Alberta Botanic Garden (formerly Devonian Botanic Garden, Alberta, Canada) (Currah et al. 1987). The phenotypic characteristics of *L. orchidicola* ex-type strain UAMH 5422 and seven additional strains were thoroughly studied by Fernando et Currah (1995), who described the conidiogenesis as blastic with conidia born either solitary or in clusters on conidiogenous hyphae and on (un)swollen conidiogenous cells. Day et al. (2012) combined *L. orchidicola* into *Cadophora* based on an ITS rDNA phylogeny, and interpreted its non-seceding conidia as aborted phialides. This new combination has become largely accepted and the name

C. orchidicola has been used for numerous root-derived isolates obtained from plant species of various plant families (Stroheker et al. 2021). However, in the absence of a reference ex-type sequence, it is questionable if the correct name has been applied for all these isolates. For example, Maciá-Vicente et al. (2020) reported that sequence similarities of almost four hundred GenBank entries identified as *Cadophora/Leptodontidium orchidicola* ranged from 94.5 to 99.5%.

In view of the paraphyly in the genus *Cadophora* and the existence of lineages which share very few characteristics with the original generic concept of *Cadophora*, we have decided to clarify the phylogenetic placement of the root endophyte *C. orchidicola*. A monophyletic genus was established to accommodate this and closely related species previously treated as *Cadophora*. Also a new species in the genus *Collembolispora* is described, based on a combination of both phenotype and molecular data.

MATERIAL AND METHODS

For this study, the ex-type strain of *C. orchidicola*, UAMH 5422, was obtained from the UAMH Centre for Global Microfungal Biodiversity (Toronto, Canada). The strain was subcultured on potato dextrose agar to induce sporulation. Sporulating structures were mounted in Melzer's reagent, lactic acid or lacto-cotton blue and examined using differential interference contrast on an Olympus BX-51 microscope with an Olympus DP72 digital camera (Olympus, Tokyo, Japan). Photos were edited using Adobe Photoshop 23.1.0 (Adobe Systems Inc., USA). Sequences of three DNA regions, namely ITS, a large rDNA subunit (28S) and a fragment of the RNA polymerase II gene (*RPB2*) encoding the second largest protein subunit were obtained from the ex-type strain following the methods described in Maciá-Vicente et al. (2020). Additionally, a fragment of a gene encoding β -tubulin was obtained following O'Donnell and Cigelnik (1997). The newly obtained sequences were deposited in GenBank. The dataset from the study of Maciá-Vicente et al. (2020) was assembled with the newly obtained sequences and reduced to contain a maximum of two sequences per species included. Bayesian inference analysis was performed on the concatenated dataset following the parameters described in Maciá-Vicente et al. (2020).

RESULTS

The morphological characteristics of *C. orchidicola* (Fig. 2) were identical to those described by Fernando et Currah (1995) and are not repeated herein. Newly obtained sequences of the 28S and β -tubulin genes were 100% identical to sequences obtained from the UAMH 8152 strain (GenBank Accession Numbers MF979572 and MF677921, respectively), but differed from the ITS sequence of that strain (AF214576) in the absence of a three-cytosine insert (positions 202–204). Since this insert was also absent from all other sequences in the alignment, we attribute this insert in the ITS sequence of UAMH 8152 to a probable sequencing

error. The sequence of *RPB2* was obtained for the first time for *C. orchidicola* and a BLAST search indicated the MN367236 sequence as the most related record, differing in only 6 bp. This sequence belongs to *Cadophora* sp. 4 strain P1772 (voucher FR 02255158) from the study by Maciá-Vicente et al. (2020).

Maciá-Vicente et al. (2020) indicated several potential lineages which could represent *C. orchidicola*. Phylogenetic analysis performed in the present study (Fig. 1) placed two strains of *C. orchidicola* (ex-type UAMH 5422 and UAMH 8152) in a well-supported lineage together with *Cadophora* sp. 5 strain P6587 (CBS 146385) from Maciá-Vicente et al. (2020). Together with sequences of *C. echinata* Koukol et Maciá-Vicente, *C. gamsii* Koukol et Maciá-Vicente and *C. variabilis* Koukol et Maciá-Vicente they formed a well-supported lineage sister to *Collembolispora*.

TAXONOMY

To partly resolve the paraphyly in the genus *Cadophora*, a distinct name should be applied to the lineage containing *C. orchidicola*. Therefore, we propose a new name for this lineage and three new combinations for closely related species recently described in *Cadophora*. We refrain from describing the subclade named *Cadophora* sp. 4 in Maciá-Vicente et al. (2020), grouping in the same cluster as the newly described lineages, as a new species, due to lack of support in the phenotypic data. Strains in *Cadophora* sp. 4 substantially differ from one another in colony morphology and do not produce any sporulating structures.

To further stabilise the taxonomy of *Cadophora*, a new species in the genus *Collembolispora* is proposed for strain CBS 146372, labelled *Cadophora* cf. *orchidicola* sensu Maciá-Vicente et al. (2020).

***Leptodophora* Koukol et Maciá-Vicente, gen. nov.**

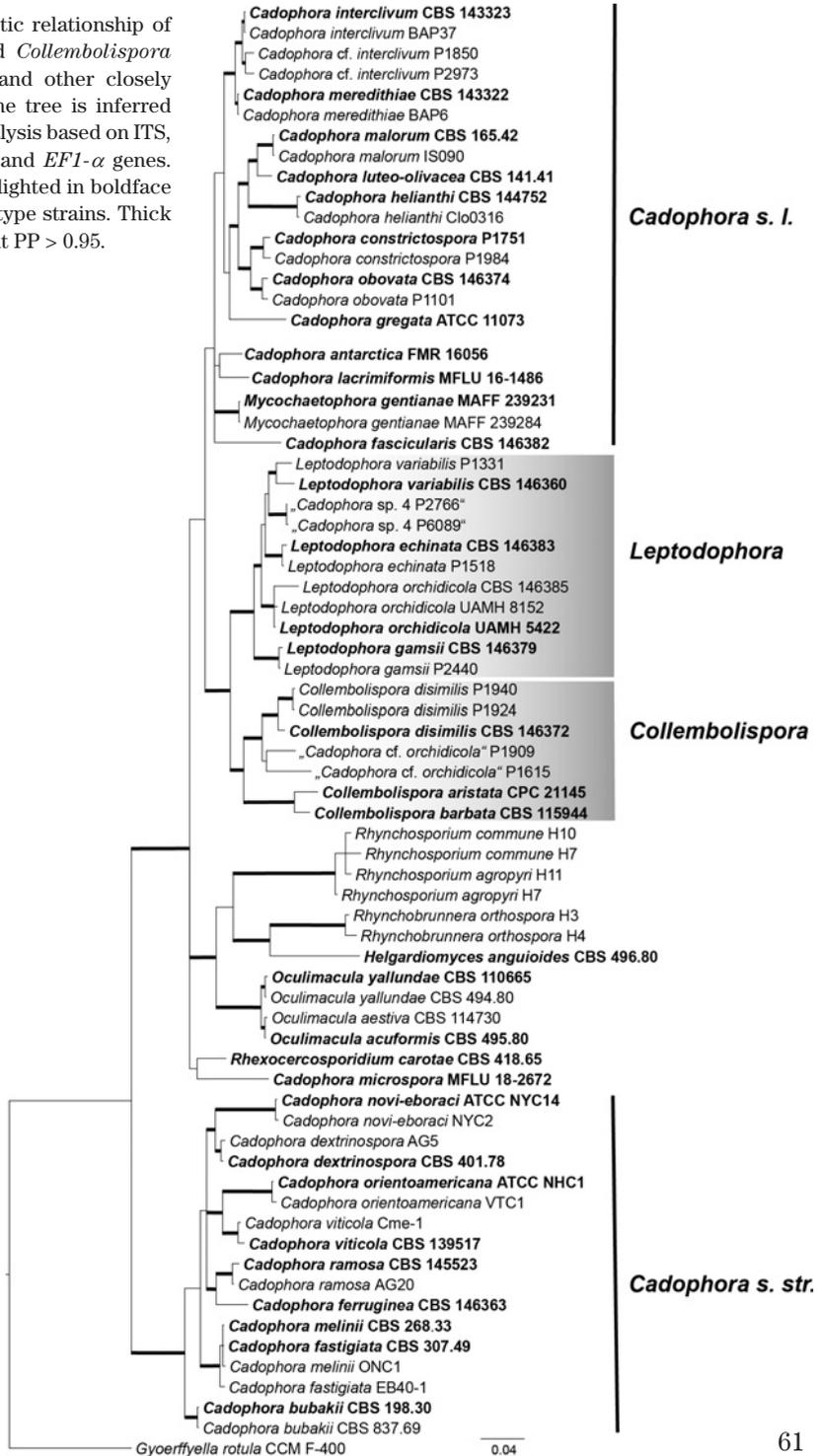
Mycobank: MB 842106

Type species: *Leptodophora orchidicola* (Sigler et Currah) Koukol et Maciá-Vicente

Etymology. Combination of the generic names *Leptodontidium* and *Cadophora*, both showing morphological and phylogenetic affinities.

Description. Mycelium consisting of septate, hyaline to dark brown hyphae. Conidiogenous cells mono- or polyblastic. Conidia holoblastic, thin- or thick-walled, non-septate or forming chains of strongly constricted cells and complex tuft-like bodies. Sexual morph unknown. Endophytes in plant roots.

Fig. 1. Phylogenetic relationship of *Leptodophora* and *Collembolispora* with *Cadophora* and other closely related genera. The tree is inferred from Bayesian analysis based on ITS, 28S rDNA, *RPB2* and *EF1- α* genes. Strain names highlighted in boldface correspond to ex-type strains. Thick branches represent PP > 0.95.



***Leptodophora orchidicola* (Sigler et Currah) Koukol et Maciá-Vicente, comb. nov.**

Fig. 2

Mycobank: MB 842107

Basionym: *Leptodontidium orchidicola* Sigler et Currah, Can. J. Bot. 65(12): 2476, 1987

Synonym: *Cadophora orchidicola* (Sigler et Currah) M.J. Day et Currah, Mycologia 104(2): 379, 2012

Specimens examined

Canada. Alberta, Devonian Botanic Garden, ex root of *Platanthera hyperborea*, 1985, coll. L. Sigler et R. Currah (UAMH 5422; ex-type strain of *Leptodontidium orchidicola*, GenBank accessions: ITS and 28S rDNA: OL742649, *RPB2*: OL739523, and β -tubulin: OL739524).

The Netherlands. Ede, 52.074 N, 5.742 E, 28 m a.s.l., rhizospheric soil in grassland, 30 April 2018, coll. et isol. J.G. Maciá-Vicente (strain P6587 = CBS 146385).

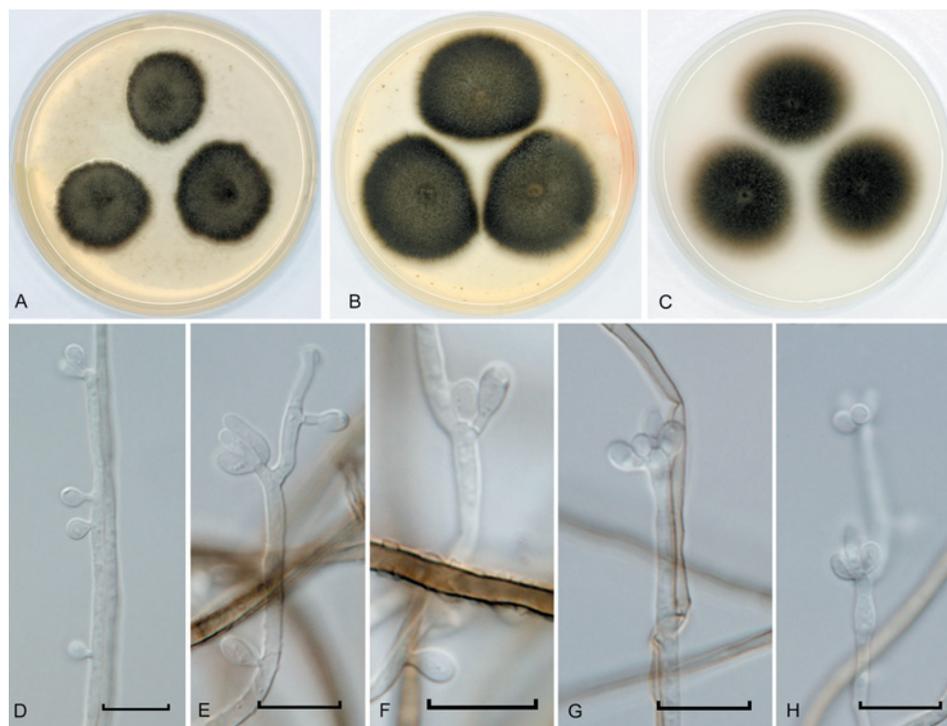


Fig. 2. *Leptodophora orchidicola* (UAMH 5422, ex-type strain). **A–C** – 15-day-old colonies on malt extract agar (A), potato dextrose agar (B), and cornmeal agar (C). **D** – fertile hyphae with laterally born conidia; **E–F** – conidia born individually or in clusters on fertile hyphae; **G–H** – terminal and intercalary clusters of sympodially produced conidia. Bars = 10 μ m (D–H). Photos: O. Koukol.

Leptodophora echinata (Koukol et Maciá-Vicente) Koukol et Maciá-Vicente, **comb. nov.**

Mycobank: MB 842109

Basionym: *Cadophora echinata* Koukol et Maciá-Vicente, IMA Fungus 11(16): 12, 2020

Leptodophora gamsii (Koukol et Maciá-Vicente) Koukol et Maciá-Vicente, **comb. nov.**

Mycobank: MB 842110

Basionym: *Cadophora gamsii* Koukol et Maciá-Vicente, IMA Fungus 11(16): 14, 2020

Leptodophora variabilis (Koukol et Maciá-Vicente) Koukol et Maciá-Vicente, **comb. nov.**

Mycobank: MB 842108

Basionym: *Cadophora variabilis* Koukol et Maciá-Vicente, IMA Fungus 11(16): 17, 2020

Note. The new genus is proposed to accommodate asexual, non-pathogenic plant root-associated fungi which are closely related based on analysis of four nuclear loci. They sporulate in culture, but the morphology of their conidia largely differs. *Leptodophora variabilis*, *L. gamsii* and *L. echinata* produce thick-walled, holoblastic non-septate conidia or ramoconidia in chains or complex, tuft-like bodies consisting of chains of fusiform to globose cells (Maciá-Vicente et al. 2020). This is in contrast to *L. orchidicola*, which produces globose to teardrop-shaped conidia on conidiogenous hyphae and cells (Fernando et Currah 1995). On the other hand, all species of *Leptodophora* are similar in their rarely seceding conidia.

Collembolispora disimilis Koukol et Maciá-Vicente, **sp. nov.**

Mycobank: MB 842111

Holotype. Bulgaria. Begunovtsi, 42.70 N, 22.83 E, 770 m a.s.l., endophytic in roots of *Microthlaspi erraticum* (*Brassicaceae*), 14 May 2013, coll. T. Ali et S. Ploch [isol. K. Glynou] (holotype FR 0255161; ex-type isolate P1854 = CBS 146372).

Etymology. Reflecting morphological dissimilarity from previously described species in the genus *Collembolispora*.

Diagnosis. Differs from the phylogenetically closely related *C. barbata* Marvanová, Pascoal et Cássio and *C. aristata* Marvanová et J.Z. Groenew. in the absence of multicellular conidia with appendages. Hyaline unicellular and ellipsoid ‘microconidia’ similar to those of *C. barbata* are larger, 3–5 × 1.5–2.5 µm compared to 2–3 × 1.5–2 µm.

Note. For a description and illustrations of *C. disimilis*, see Maciá-Vicente et al. (2020) under *Cadophora* cf. *orchidicola*. It should be mentioned that we did not include all strain sisters to the two species previously described in the species concept of *C. disimilis* because of substantial differences in molecular data. Therefore, only strains in the well-supported lineage containing ex-type isolate CBS 146372 were included.

Collembolispora disimilis is well accommodated in the genus also based on phenotypic data. Although the two currently known species of *Collembolispora* are distinct by the specific shape of their multicellular macroconidia with appendages, *C. barbata*, the generic type, also produces a synasexual morph with phialides produced singly or in groups (Marvanová et al. 2003). Maciá-Vicente et al. (2020) described conidiogenous cells of the isolate CBS 146372 as “presumably enteroblastic, but without apparent collarette”, which reflects the description of *C. barbata* in Marvanová et al. (2003): “collarette cup-shaped, up to 1.5 µm long, often hardly discernible on sporulating phialides but distinct on spent ones, rarely absent”. Re-examination of the ex-type isolate of *C. disimilis* confirmed that the conidiogenous cells are phialides, and a hardly visible collarette was observed after conidia detachment.

Collembolispora disimilis is presently only known as a root endophyte without any relation to aquatic environments from which the two other *Collembolispora* species were described. However, the life cycle of aquatic hyphomycetes may be very complex and may include an endophytic phase in coniferous needles (Sokolski et al. 2006).

DISCUSSION

The pivotal aim of our study was to provide the phylogenetic placement and species delimitation of a well-known root endophyte, *C. orchidicola*, based on a study of the species type material. Analysis of four molecular markers provided clear placement of the ex-type strain in a well-supported lineage sister to *Collembolispora*. These results solve previous doubts about a proper taxonomic placement of this species and justify our description of the new genus *Leptodophora*. The original placement of *L. orchidicola* in *Leptodontidium* de Hoog was made “with some hesitation” by Currah et al. (1987), who mentioned production of conidia on fertile hyphae and their rare detachment as the two main differences from other members of *Leptodontidium*. There is no support from molecular data for the present accommodation of this species in *Leptodontidium* because this genus, typified with *L. trabinellum* (P. Karst.) Baral, Platas et R. Galán, is currently included in the family *Leptodontidiaceae*, i.e. it is phylogenetically unrelated to *Cadophora*. The combination of *L. orchidicola* into the genus *Cadophora* by Day et al. (2012) was not well substantiated from the present perspective. Day et al. (2012) used only single strains representing potentially closely related species in their undersized dataset of ITS rDNA. For *C. orchidicola*, they misidentified the UAMH 8152 strain as the ex-type strain, a mistake later adopted also by Walsh et al. (2018). However, strain UAMH 8152 was isolated almost a decade after *C. orchidicola* was described (Fernando et Currah 1996).

The placement of *L. orchidicola* in the genus *Cadophora* was neither supported by its morphology, since *L. orchidicola* does not produce phialides with distinct hyaline, flaring collarettes. Nevertheless, Day et al. (2012) deduced from their phylogeny and ancestral character reconstruction that non-seceding conidia observed in *L. orchidicola* represent aborted or functionally reduced phialides. However, according to Maciá-Vicente et al. (2020), only *Cadophora* s.str. has evolved from an ancestor with phialidic conidiogenous cells. Last but not least, species of *Leptodophora* and *Cadophora* differ in their ecology. Whilst the genus *Cadophora* was originally established for saprotrophs isolated from wood and with pathogenic potential, all presently known species of *Leptodophora* are root colonisers without a pathogenic effect on their hosts (Jumpponen 2001, Kia et al. 2017, 2019).

In our phylogeny, the two genera *Collembolispora* and *Leptodophora* were shown to be sister lineages. We have opted to keep them as separate genera rather than two congeneric phylogenetic lineages because of several differences in their phenotype. Two of the three presently known *Collembolispora* species are known from aquatic environments, while *Leptodophora* species are only terrestrial. The phialidic synasexual morph is presently only known in *Collembolispora* species, whereas no *Leptodophora* species produce phialides.

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