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Revision of the culture of *Acremonium berkeleyanum* CBS 501.81 and its comparison with *Sphaerostilbella berkeleyana*

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The non-type isolate of *Acremonium berkeleyanum* CBS 501.81 from *Stereum hirsutum* was studied under a light microscope and by analysis of three molecular markers (ITS rDNA region, and parts of the LSU rDNA region and elongation factor 1-alpha gene). Phylogenetic analyses placed the strain within *Cosmospora lavitskae* sensu stricto, where it clustered with the ex-type culture of the species, supporting its identification as *C. lavitskae*. Based on *Cosmospora* ITS rDNA sequences collected from GenBank, the substrate range of *C. lavitskae* is characterised, where strain CBS 501.81 represents the first record of *C. lavitskae* from *Stereum*. Teleomorphic and anamorphic characters of *C. lavitskae* are compared with *Sphaerostilbella berkeleyana* and its presumed anamorph.

Key words: *Hypocreales*, *Nectriaceae*, *Cosmospora*, taxonomy, phylogeny.

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Netypový izolát *Acremonium berkeleyanum* CBS 501.81 z pevníku chlupatého byl studován ve světelném mikroskopu a pomocí analýzy tří molekulárních markerů (ITS rDNA a úseky LSU rDNA a genu pro elongační faktor 1-alfa). Fylogenetické analýzy zařadily kmen do užšího pojetí druhu *Cosmospora lavitskae* spolu se sekvencí z ex-typové kultury, což podporuje jeho identifikaci jako *C. lavitskae*. Na základě ITS rDNA sekvencí rodu *Cosmospora* shromážděných z databáze GenBank je charakterizován okruh substrátů *C. lavitskae*, kde kmen CBS 501.81 představuje první záznam tohoto druhu z pevníku. Teleomorfní a anamorfní znaky *C. lavitskae* jsou porovnány s druhem *Sphaerostilbella berkeleyana* a jeho předpokládanou anamorfou.

INTRODUCTION

Fungi of the genus *Cosmospora* Rabenh., which parasitise on xylariaceous hosts, collectively referred to as the *Cosmospora viliuscula* species complex, were studied by Herrera (2014) and Herrera et al. (2015), and comprise the following species: *Cosmospora annulohypoxyli* C.S. Herrera & P. Chaverri, *C. arxii* (W. Gams) Gräfenhan & Schroers, *C. clavi* C.S. Herrera & P. Chaverri, *C. khandalensis* (Thirum. & Sukapure) Gräfenhan & Seifert, *C. lavitskae* (Zhdanova) Gräfenhan & Seifert (as *C. lavitskiae*), *C. micropedis* C.S. Herrera & P. Chaverri, *C. novazelandica* C.S. Herrera & P. Chaverri, *C. scruposae* C.S. Herrera & P. Chaverri, *C. stilbohypoxyli* C.S. Herrera & P. Chaverri, *C. ustulinae* (Teng) C.S. Herrera & P. Chaverri, *C. viliuscula* (Samuels) Rossman & Samuels, and *Cosmospora* sp. 2 to sp. 7. Finally, also *C. rickii* (Rehm) Rossman & Samuels, whose phylogenetic position remains uncertain, is presumed to belong to this group. Lechat & Fournier (2021) described *C. xylariae* Lechat & J. Fourn., identical to *Cosmospora* sp. 4 of Herrera et al. (2015). The last new species of this complex were described by Tan & Shivas (2024) as *C. coheniae* Y.P. Tan, Bishop-Hurley & R.G. Shivas and as *C. nemaniae* Mombert & Crous by Crous et al. (2025). Some confusion may arise from the fact that *Nectria vilior* Starbäck, before being revised and epitypified by Herrera & Chaverri (2013) as a species of *Pseudocosmospora*, was considered an earlier name for *Cosmospora viliuscula* (Samuels) Rossman & Samuels, currently the *C. viliuscula* species complex (Herrera 2014).

Members of genus *Cosmospora* outside the *Cosmospora viliuscula* species complex (as defined by Herrera et al. 2015: 353) comprise *Cosmospora aquatica* Z.L. Luo, H.Y. Su & K.D. Hyde, *C. butyri* (J.F.H. Beyma) Gräfenhan, Seifert & Schroers, *C. coccinea* Rabenh., *C. fomiticola* C.S. Herrera & P. Chaverri, and *C. viridescens* (C. Booth) Gräfenhan & Seifert (Herrera et al. 2015, Luo et al. 2019, Crous et al. 2021). Gams (1971) included several species in the synonymy of *Acremonium butyri* (J.F.H. Beyma) W. Gams, namely *Nectria viridescens* Booth, *Gliomastix lavitskae* Zhdanova and *Cephalosporium khandalense* Thirum. & Sukapure. He later included *A. butyri* in the synonymy of *A. berkeleyanum* (P. Karst.) W. Gams (in Gams & Zaayen 1982), which he combined into *Acremonium* after studying strain CBS 501.81 from a fungus on *Stereum*, which he identified as conspecific with *Hypomyces berkeleyanus* Plowr. & Cooke and its anamorph *Verticillium berkeleyanum* P. Karst. [now *Sphaerostilbella berkeleyana* (Plowr. & Cooke) Samuels & Cand.]. After a more detailed delimitation of the species which Gams (1971) included in the synonymy of *A. butyri* (Gräfenhan et al. 2011, Herrera et al. 2015), the species identity of *A. berkeleyanum* [*Cosmospora berkeleyana* (P. Karst.) Gräfenhan, Seifert & Schroers] remained unclear. Gräfenhan et al. (2011) stated that they had not studied Gams' isolate CBS 501.81 (Gams & Zaayen 1982). Our study presents a revision of *A. berkeleyanum* strain

CBS 501.81 using morphology and three molecular markers, i.e. ITS-LSU rDNA and TEF1-alpha sequences.

Phylogenetic studies show that the genera or lineages closest to genus *Cosmospora* are *Cosmosporella* S.K. Huang, R. Jeewon & K.D. Hyde [including the currently poorly defined species *Cosmospora flavoviridis* (Fuckel) Rossman & Samuels and *C. obscura* Lowen], *Dialonectria* (Sacc.) Cooke, *Pseudocosmospora* C.S. Herrera & P. Chaverri, *Pulchrospora* Czachura & Janik, *'Cosmospora' stegonsporii* Rossman, D.F. Farr & Akulov, and *'Nectria' rishbethii* C. Booth (e.g. Gräfenhan et al. 2011, Herrera & Chaverri 2013, Huang et al. 2018, Crous et al. 2021, Czachura & Janik 2025). Other phylogenetically close genera or lineages include *Macroconia* (Wollenw.) Gräfenhan, Seifert & Schroers, *Microcera* Desm., *Fusicolla* Bonord., *Pseudofusicolla* Triest, *Scolecofusarium* L. Lombard, Sand.-Den. & Crous, *Stylonectria* Höhn., and *'Fusarium' merismoides* var. *chlamydosporale* Wollenw. (e.g. Gräfenhan et al. 2011, Triest et al. 2016, Crous et al. 2021). Among the phylogenetic studies concerning the genus *Cosmospora* at a lower level, the work of Herrera et al. (2015) is certainly worth mentioning, followed by other works, namely Tan & Shivas (2024) and Crous et al. (2025). The contribution by Crous et al. (2021) on obtaining the ex-type ITS rDNA sequence of *C. butyri* is also important. The phylogeny presented in our study focuses on the marginal part of the *C. viliuscula* species complex (*C. arxii*, *C. lavitskae*) and species of genus *Cosmospora* outside the species complex, which are still poorly studied (Herrera et al. 2015).

Cosmospora lavitskae ex-type culture IMI 133984 was isolated from plant debris of rhizosphere soil of *Zea mays* and possesses simple, unbranched or dichotomously branched conidiophores, rarely with three phialides and hyaline, unicellular, smooth conidia (Herrera et al. 2015). According to Herrera et al. (2015), this fungus belongs to the *C. viliuscula* species complex occurring on xylariaceous fungus hosts, but it could have a secondary mode of nutrition, e.g. saprotrophic (Herrera 2014). The teleomorph of *C. lavitskae* was found and documented only once, on a xylariaceous fungus, and has the following characteristics: Perithecia red when fresh, brownish red when dry, turning dark red in KOH and orange yellow in lactic acid, (118)139–181 × 111–153 µm, asci clavate, without apical ring, (37)40–52.5 × 2.5–4.5(5.5) µm, ascospores smooth, ellipsoidal, constricted at septum, 4–6(7) × 3–4 µm (Zeng & Zhuang 2016).

In this study, the phylogenetic placement of non-type strain *A. berkeleyanum* CBS 501.81 was analysed. Using one of the studied markers, ITS rDNA, it was placed in the context of closely related species, particularly with respect to their substrates and sources of isolation. Based on the available data and our observation of morphological characters, it is compared to *Sphaerostilbella berkeleyana* and its presumed anamorph (Šandová 2024), with which it was identified by Gams (in Gams & Zaayen 1982).

MATERIAL AND METHODS

Morphological studies. The live culture on oatmeal agar (8 g ferwo agar 700, 1 l oatmeal extract: 30 g oatmeal, 1 l demineralised water, blended, boiled for 2 hours and filtered through a kitchen sieve; abbreviated as OA) was obtained from the CBS culture collection (Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands). Colonies were grown on glucose agar with potato extract (Carl Roth, Karlsruhe, Germany; abbreviated as PDA) at 21–23 °C in darkness and photographed using a Nikon Coolpix P4 digital camera. Microcharacters were studied in 7–17-day-old colonies growing on PDA at 21–23 °C in a room with natural light using 3% KOH unless stated otherwise. Micrographs were acquired using an Olympus SZ61 stereomicroscope equipped with cold light, and an Olympus BX51 microscope equipped with a Canon EOS 2000D digital camera. Dried colony samples are deposited in the PRM herbarium (National Museum, Prague, Czech Republic).

DNA extraction, PCR amplification and sequencing. Chromosomal DNA was extracted from mycelium scraped from PDA agar plates. A Dremel rotary tool with a diamond wheel point (∅ 1.5 mm) was used to disrupt the cells at ~20,000 rpm. CTAB extraction buffer (Clarke 2009) was used to lyse the cells. The crude DNA extract was purified using affinity chromatography columns (High Pure PCR Template Preparation Kit; Roche, Rotkreuz, Switzerland) according to the instruction manual. An additional purification step was added, namely the precipitation of chromosomal DNA in sorbitol buffer (Inglis et al. 2018). PPP Master Mix (Top-Bio Ltd., Vestec, Czech Republic) was used to amplify taxonomically relevant regions. The amplified fragments were purified using the MinElute PCR Purification Kit (Qiagen, Hilden, Germany). DNA sequencing was carried out according to Sanger et al. (1977) in both directions on an AbiPrism 3130xl Genetic Analyzer (Applied Biosystems). The following primer pairs (forward primer/reverse primer) were used for amplification: ITS5/LR6 (White et al. 1990 and Vilgalys & Hester 1990, respectively), EF728F/EF986R (Carbone & Cohn 1999), EF983F/EF1160R (Rehner & Buckley 2005 and Kauserud & Schumacher 2001, respectively).

Sequence alignment and phylogenetic analysis. The ITS rDNA region and TEF1-alpha gene sequences used in the phylogenetic analyses are listed in Supplementary Table 1 (see Electronic Supplements). The ITS sequences were collected from three sources: (1) Herrera (2014), (2) *Cosmospora* entries in the NCBI GenBank database, (3) NCBI BLAST search using individual sequences. Finally, the sequence set was restricted to the section of the phylogenetic tree published by Tan & Shivas (2024: 6) from *Cosmospora arxii* to *C. aquatica*, excluding *C. meliopsicola*, *C. henanensis*, and the *C. obscura* – *C. magnusiana* – *C. stegonsporii* branch, which belong to other genera. *Stylonectria corniculata* and *Cylindrodendrum album* var. *paralion* were used as outgroup taxa. Sequence alignment was performed in MAFFT v. 7.222 (Katoh & Standley 2013) using the Q-INS-i strategy, which accounts for the rRNA secondary structure. Maximum likelihood (ML) analyses were conducted in IQ-TREE v. 2.1.3 (Nguyen et al. 2015) with automated model selection (-m TESTNEW) via ModelFinder (Kalyaanamoorthy et al. 2017), using 1000 ultrafast bootstrap replicates (-bb 1000). All three subregions, ITS1, 5.8S and ITS2, were considered to be separate partitions. Based on the Bayesian information criterion scores, the selected models were TNe+I (ITS1), K2P (5.8S), and JC (ITS2). Bayesian inference (BI) was performed using MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003) with 10 million generations. The burn-in value was estimated in Tracer v. 1.5 (Rambaut et al. 2018). The final alignment included 98 sequences comprising 549 nucleotide sites, of which 412 were constant and 65 were parsimony-informative.

The TEF1-alpha gene is less frequently represented in public databases for the studied fungi. The TEF1-alpha dataset was assembled using all available sequences from the species included in the ITS rDNA dataset. It comprised 17 sequences with a total of 320 nucleotide sites, of which 129 were constant and 109 were parsimony-informative. Alignment and ML and BI analyses were performed in the same way as for the ITS rDNA dataset. Based on Bayesian Information Criterion (BIC) scores, the selected model was HKY+F+G4. *Cylindrodendrum album* var. *paralion* was used as the outgroup.

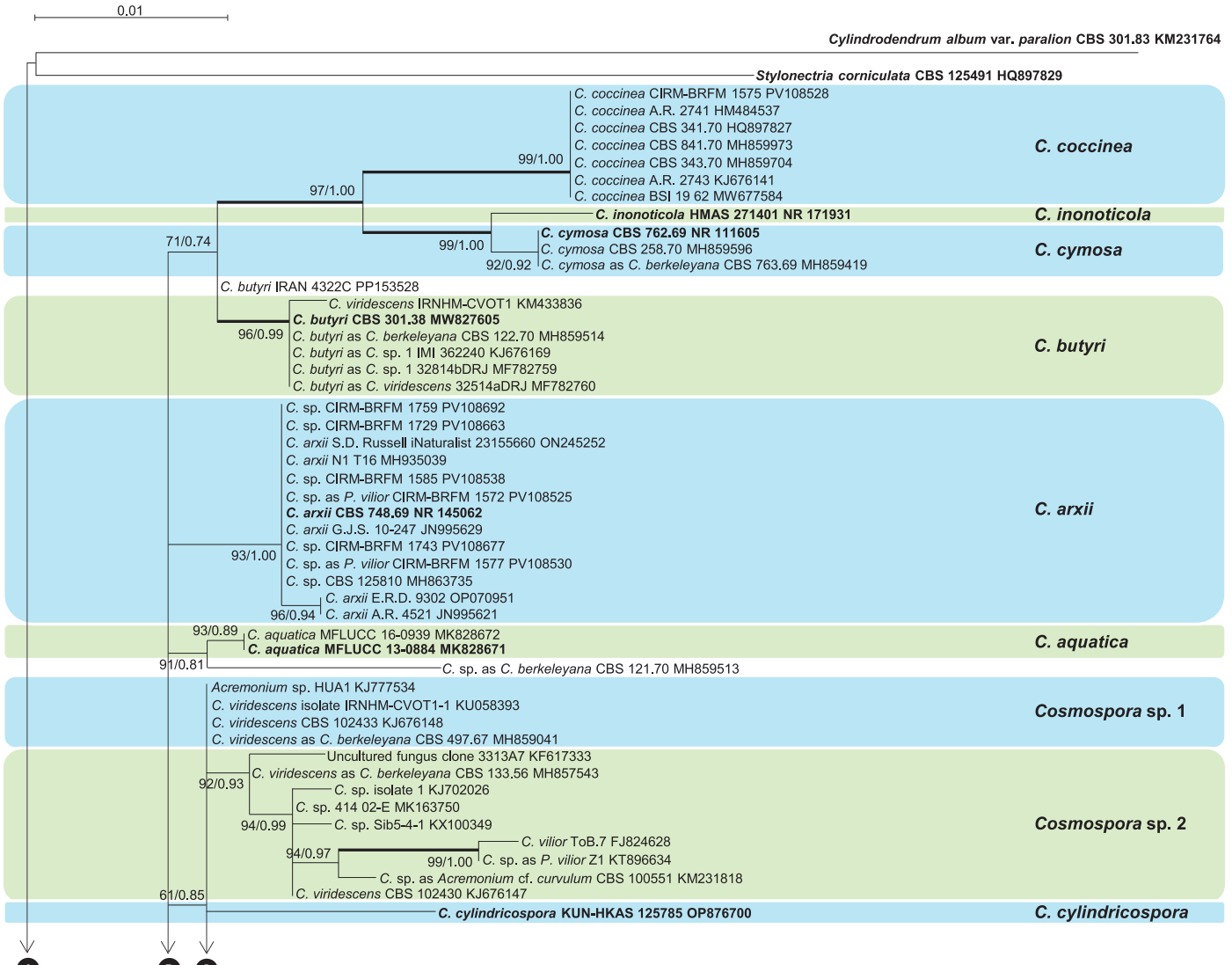
Results and discussion

Phylogeny

Phylogenetic analysis (Fig. 1) revealed several well-defined clades corresponding to established species, including *Cosmospora coccinea*, *C. inonoticola*, *C. cymosa*, *C. butyri*, *C. arxii*, *C. aquatica*, and *C. fomiticola*. It is important to note that the original strain designations in GenBank or publications often differ from our classification based on ITS rDNA-based topology. The well-defined clade of *C. viridescens* s. str. and the clade which we designated *Cosmospora* sp. 2, together with the part designated *Cosmospora* sp. 1, cover *C. viridescens* as defined by Herrera et al. (2015). However, some of the clades are probably still heterogeneous. A substantial portion of strains falls into a clade with only weak support in the ML analysis (bootstrap 56) whereas in the MrBayes analysis, these strains do not form a distinct clade but rather a polytomy of closely related sequences without a clear clade structure. This group includes *C. lavitskae* CBS 501.81 together with IMI 133984, the ex-type strain of *C. lavitskae*. These strains share an ITS rDNA sequence similarity of 99.64%. We refer to this entire group, which exhibits considerable internal variability, as the *Cosmospora lavitskae* aggregate.

The phylogeny based on the more variable marker TEF1-alpha (Fig. 2) is influenced by the limited number of strains available for comparison. For *C. lavitskae*, in addition to strain CBS 501.81, sequences are available for CBS 123961, IMI 133984 (Herrera et al. 2015), and CBS 530.68 (Crous et al. 2021). Strain CBS 501.81 shows 99.19% similarity with *C. lavitskae* strain IMI 133984 (ex-type), confirming the identity of CBS 501.81 as *C. lavitskae*. Phylogenetically, we observed a structure similar to the ITS rDNA tree, including the separation of *C. viridescens*, as defined by Herrera et al. (2015), into three lineages (*Cosmospora* sp. 1, sp. 2, and *C. viridescens* s. str.). Within the clade containing the *C. lavitskae* aggregate, we again observed a distinct position of strain CBS 123961, which also forms a separate lineage within this aggregate in the ITS rDNA tree. This strain differs from ex-type strain IMI 133984 in the TEF1-alpha sequence by 8.23%, a substantial divergence, suggesting that it represents a separate species. Further study using protein-coding barcode genes, additional strains, and morphological data is necessary to resolve species boundaries within the *C. lavitskae* complex.

Fig. 1. Maximum likelihood phylogenic tree (ITS rDNA) showing the relatedness of *Cosmospora lavitskae* (CBS 501.81) to genetically similar taxa. Ultrafast bootstrap support values greater than 0.60/50 followed by posterior probabilities are shown next to the branches. Fully supported branches (0.98–1.00/95–100) are shown in bold. Nodes which are not present in the MrBayes analysis are indicated with an asterisk. Ex-type strains of the taxa used are marked in bold. An asterisk after the strain indicates *C. lavitskae* agg. with a unique nucleotide at position 154 of IMI 133984, illustrating the variability within the clade. The tree is rooted with a clade represented by *Cylindrodendrum album* var. *paralion* and *Stylonectria corniculata*. Abbreviations: *A.* = *Acremonium*, *C.* = *Cosmospora*, *P.* = *Pseudocosmospora*. ▶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C. coccinea CIRM-BRFM 1575 PV108528
C. coccinea A.R. 2741 HM484537
C. coccinea CBS 341.70 HQ897827
C. coccinea CBS 841.70 MH859973
C. coccinea CBS 343.70 MH859704
C. coccinea A.R. 2743 KJ676141
C. coccinea BSI 19 62 MW677584
C. coccinea
99/1.00
97/1.00
C. inonoticola HMAS 271401 NR 171931
C. inonoticola
C. cymosa CBS 762.69 NR 111605
C. cymosa CBS 258.70 MH859596
C. cymosa as C. berkeleyana CBS 763.69 MH859419
C. cymosa
99/1.00
92/0.92
71/0.74
C. butyri IRAN 4322C PP153528
C. viridescens IRNHM-CVOT1 KM433836
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C. butyri as C. berkeleyana CBS 122.70 MH859514
C. butyri as C. sp. 1 IMI 362240 KJ676169
C. butyri as C. sp. 1 32814bDRJ MF782759
C. butyri as C. viridescens 32514aDRJ MF782760
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C. arxii S.D. Russell iNaturalist 23155660 ON245252
C. arxii N1 T16 MH935039
C. sp. CIRM-BRFM 1585 PV108538
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C. arxii CBS 748.69 NR 145062
C. arxii G.J.S. 10-247 JN995629
C. sp. CIRM-BRFM 1743 PV108677
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C. sp. CBS 125810 MH863735
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96/0.94
93/0.89
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C. aquatica MFLUCC 13-0884 MK828671
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C. viridescens CBS 102433 KJ676148
C. viridescens as C. berkeleyana CBS 497.67 MH859041
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Uncultured fungus clone 3313A7 KF617333
C. viridescens as C. berkeleyana CBS 133.56 MH857543
C. sp. isolate 1 KJ702026
C. sp. 414 02-E MK163750
C. sp. Sib5-4-1 KX100349
C. vilior ToB.7 FJ824628
C. sp. as P. vilior Z1 KT896634
C. sp. as Acremonium cf. curvulum CBS 100551 KM231818
C. viridescens CBS 102430 KJ676147
Cosmospora sp. 2
92/0.93
94/0.99
94/0.97
99/1.00
61/0.85
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C. cylindricospora
A
B
C


A
B
C
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C. viridescens IMI 73377a KJ676171
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C. sp. CF-164359* PV835085
C. lavitskae K.A.S. 1105* KJ676172
C. lavitskae CBS 123961* KJ676150
C. lavitskae IMI 133984 KJ676167
C. sp. NO 14-1-M OK173746
C. lavitskae CBS 501.81 PX232797
C. lavitskae F18 PX108372
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C. sp. 40790-TW-1K-Fdi PV156973
C. lavitskae NW-FVA7095 OP023262
C. lavitskae HMAS 252477 KU563624
C. lavitskae CNUFC TKB240 OK285197
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C. sp. as C. vilior Guardbridge20 GU726755
C. lavitskae IWWIF064 OK623453
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C. sp. CIRM-BRFM 1739* PV108673
C. lavitskae as C. berkeleyana IHEM 03150* OW983055
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C. sp. CIRM-BRFM 1737 PV108671
C. lavitskae as P. vilior F61 GU067755
69/0.89
C. lavitskae as C. butyri BC1580* MH971260
C. lavitskae as C. butyri DK8-7 KP893887
94/0.97
C. lavitskae as C. butyri ATCC 52620 JQ070093
Uncultured fungus clone 3993 429 MW214970
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C. lavitskae agg.
C. fomiticola

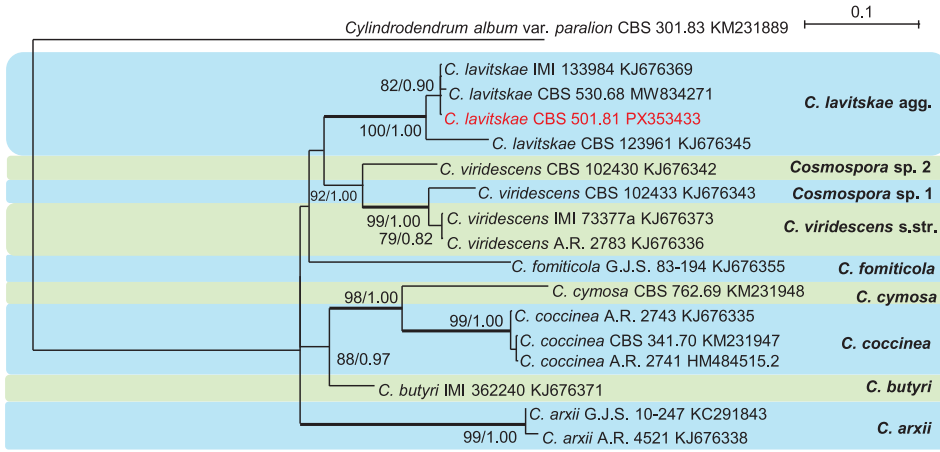

Fig. 2. Maximum likelihood phylogenic tree (TEF1-alpha) showing the relatedness of *Cosmospora lavitskae* (CBS 501.81) to genetically similar taxa. Ultrafast bootstrap support values greater than 0.60/50 followed by posterior probabilities are shown next to the branches. Fully supported branches (0.98–1.00/95–100) are shown in bold. The tree is rooted with *Cylindrodendrum album* var. *paralion*.

TAXONOMY

Cosmospora lavitskae (Zhdanova) Gräfenhan & Seifert, in Gräfenhan, Schroers, Nirenberg & Seifert, Stud. Mycol. 68: 96, 2011 (as *'lavitskiae'*) Fig. 3

[MB #864044]

Basionym: *Gliomastix lavitskae* Zhdanova, Mikrobiol. Zhurn. 28: 37, 1966 (as *'lavitskiae'*)
[MB #864043]

Etymology and orthography: The protologue (Zhdanova 1966) does not include the etymology of the species, which is only recommended (see e.g. Rec. 73A of the Edinburgh Code, https://www.iapt-taxon.org/historic/1966.htm). However, according to Vasyl Heluta (pers. comm.), the name was dedicated to Zinaida Hryhorivna Lavitska (1908–1983), a Ukrainian mycologist and associate professor of the Department of Lower Plants of the Faculty of Biology of Kyiv University (see e.g. Morochkovskyi et al. 1969, Savchenko & Heluta 2012, Heluta 2023, https://archive.ph/g5gBC). The correct orthography of the epithet is therefore *'lavitskae'* according to Rec. 73C of the Edinburgh Code (improved and renumbered but generally maintained in subsequent versions, see Art. 60.8(a) in the Madrid Code, https://www.iaptglobal.org/_functions/code/madrid).

Observed characters. Colony on OA white, reverse concolorous, older culture with a yellow pigment diffusing into the medium. Colony on PDA yellow to umber in the central part, white around, reverse yellow, later umber. Heads composed of conidia up to 20–25 µm in diameter when fresh. Conidiophores unbranched, dichotomously branched, or branched into up to three branches and up to two levels (Fig. 3d), smooth. Phialides 0–2-septate, (29)39–65 × 1.7–2.8 µm, smooth. Conidia unicellular, ellipsoidal with hilum protruding or non-protruding, smooth, hyaline, 3.6–6.3(8.4) × 1.9–3.0 µm (average 5.0 × 2.3 µm; length-to-width

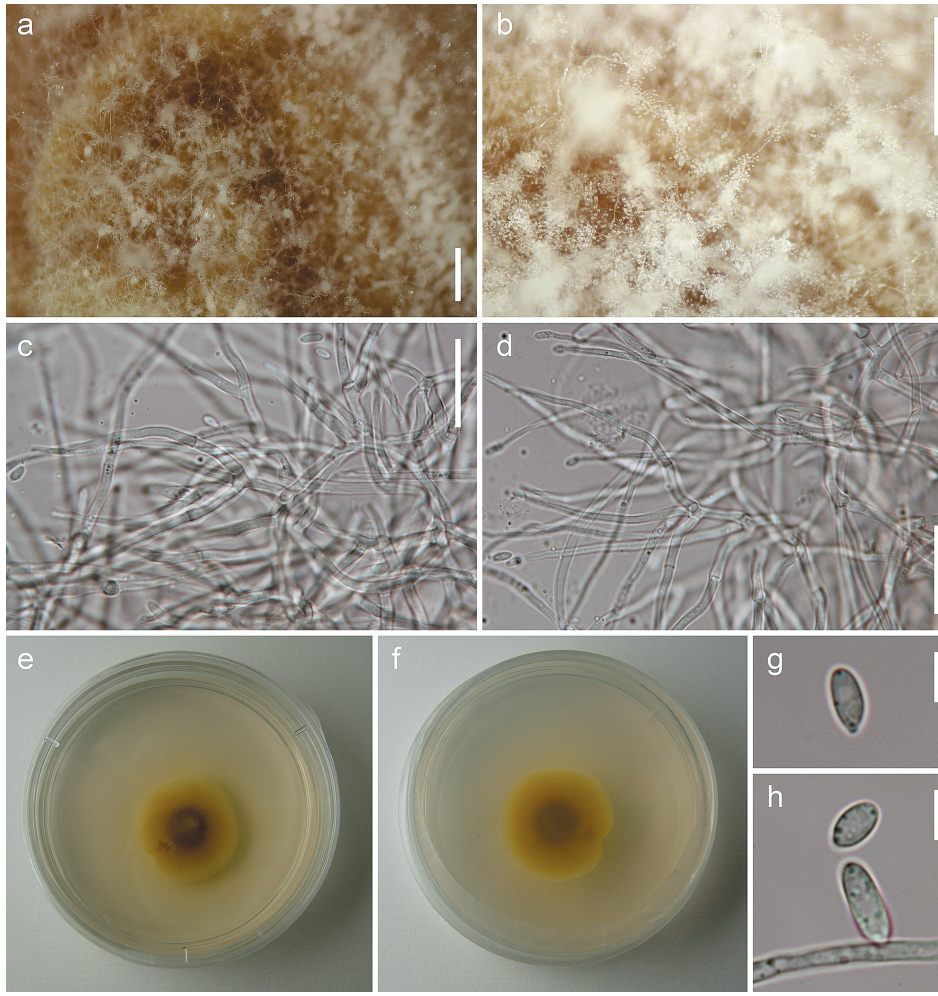

Fig. 3. *Cosmospora lavitskae* (CBS 501.81). **a–b** – detail of a colony after 4 or 6 weeks (a and b, respectively); **c–d** – conidiophore branching, phialides and conidia; **e–f** – colony and reverse after 12 days; **g–h** – conidia. Medium: a–h – potato extract glucose agar; c–d – 3% KOH, 17-day old colony; g–h – fresh preparations in distilled water, 7-day old colony. Scale bars: a–b = 1 mm; c–d = 20 µm; g–h = 5 µm. Photos by M. Šandová.

ratio = 1.5–2.8(3.1); n = 40), on OA 2.8–6.1 × 1.8–2.7 µm (average 4.4 × 2.3 µm; length-to-width ratio = 1.5–2.9; n = 40). Conidia measured in fresh preparations in distilled water 4.8–7.6(9.1) × 2.6–4.1 µm (average 6.2 × 3.2 µm; length-to-width ratio = 1.5–2.9; n = 15), on OA 3.2–7.4 × 2.1–2.9 µm (average 4.2 × 2.6 µm; length-to-width ratio = 1.3–2.6; n = 40).

Studied isolate

Netherlands. Oostelijk Flevoland polder, Dronten, Reve-Abbert (protected area), plot O66, 29 July 1981, isolated from *Stereum hirsutum* by W. Gams, CBS 501.81 (deposited as *Acremonium butyri*, name changed to *A. berkeleyanum*, synonym of *Cosmospora berkeleyana*), ITS-LSU rDNA and TEF1-alpha sequence accession numbers PX232797 and PX353433, respectively.

Notes. The very small dimensions of conidia on OA compared to other culture media were also found in ascomycetous fungi by Réblová et al. (2025a, 2025b), who found that conidia (based on average length) were 16–21% shorter and also had a different width than in material on natural substrate observed in the same way. Conidia of *Rhamphoriaceae* (*Sordariomycetes*) on PDA examined by Réblová & Štepánek (2018) were 2.4–11% shorter than conidia on natural substrate but equally wide. Gams (1971) illustrated the differently sized conidia of *C. viridescens* cultured on malt extract agar and on oatmeal agar. Descriptions of material on natural substrate are lacking in genus *Cosmospora* and related genera. The dimensions of conidia of *C. coccinea* measured in lactic acid, the same medium as used by Gams (1971), are 3.2–7.2 × 1.5–3 µm (average 5.1 × 2.0 µm) in herbarium specimens on natural substrate deposited in PRM (PRM 844412, 897345). The dimensions of conidia in vitro given by Gams (1971) are therefore 6–8% shorter and much narrower (up to 40%), which may be due to the presence of wider, as if double, conidia of a different shape than the ones drawn by Gams (1971), in the material on natural substrate.

Acremonial anamorphs reported from fruitbodies of basidiomycetes on wood such as *Allomusicillium domschii* (W. Gams) L.W. Hou, L. Cai & Crous, *Cosmospora coccinea*, *C. cymosa*, *C. fomiticola*, *C. inonoticola*, and *C. viridescens* (Gams 1971, Herrera et al. 2015, Zeng & Zhuang 2016) are often difficult to distinguish from each other. *Allomusicillium* is characterised by repeated sympodial proliferation (Hou et al. 2023). Gams (1971: 248) reports solitary branches in *C. cymosa*, rather dense, verticillate branching in *C. viridescens* (incl. *C. lavitskae*) and erect conidiophores with mesotonous or acrotonous whorls of phialides in *C. coccinea*. Zeng & Zhuang (2016) report unbranched or dichotomously branched conidiophores in *C. inonoticola* and unbranched or dichotomously branched conidiophores in *C. lavitskae*, rarely with three phialides, which is not inconsistent with the description of *C. lavitskae* in this work or with its description in Herrera et al. (2015). The possible presence of three phialides is also noted in *C. inonoticola* (Zeng & Zhuang 2016: Fig. 2t), and the presence of up to four phialides is noted in *C. fomiticola* (Herrera et al. 2015). Therefore, individual *Cosmospora* species cannot be distinguished based solely on microscopic characters, possibly with the exception of *C. coccinea*.

In this study, a large number of ITS rDNA sequences from both taxonomic and non-taxonomic sources were analysed. Within this dataset, CBS 501.81 is the only record of *C. lavitskae* from *Stereum*. *Cosmospora lavitskae* s. str. (*C. lavitskae*

agg. excluding CBS 213.70, 233.70, and 385.70A, Acremon32, Guardbridge14 and 20, and Sib5-9-7 with more divergent sequences in the alignment, and excluding strains marked with an asterisk in Fig. 1, which have a unique nucleotide in the sequences) has so far been detected in freshwater, air, soil, rhizosphere, on dead wood, seeds, xylariaceous ascomycetes, and invertebrates (for references or data from GenBank, see Supplementary Table 1). *Cosmospora lavitskae* belongs to the *C. viliuscula* species complex, whose teleomorphic stages occur on fungi of the *Xylariaceae* family (Herrera et al. 2015, Zeng & Zhuang 2016). Culture HMAS 252477 obtained as a single-ascospore isolate by Zeng & Zhuang (2016) from a teleomorph growing on a xylariaceous fungus shares 100% similarity with isolate CBS 501.81 in the ITS rDNA barcode. Similarly, strain CBS 123961 (= G.J.S. 96-251) isolated from a fungus on an unidentified black pyrenomycete (Herrera 2014, Herrera et al. 2015) has a sequence similar to samples CBS 234.70, 235.70, and 239.70, isolated from polyporoid fungi (Hou et al. 2023).

The herbarium specimen documenting the colony of CBS 501.81 on a natural substrate is not deposited in the CBS herbarium. In Gams & Zaayen (1982), this isolate is mentioned with minimal details, although a new combination is published here. However, no description is given, the authors only state that the material on *Stereum hirsutum* was identical (probably meaning the anamorph) to the *Verticillium berkeleyanum* anamorph described by P.A. Karsten and documented by the type in herbarium H. A new combination of the anamorph name was published and the results were applied to the teleomorph as well, stating that "*Hypomyces berkeleyanus* is a typical *Nectria*". There have been attempts to refute this claim by e.g. Lechat & Fournier (2021) and later Šandová (2024). Since the identity of the Gams' isolate has been newly identified, we present here how *C. lavitskae* and *Sphaerostilbella berkeleyana* differ in teleomorphic characters. Some of the differences are listed in Tab. 1. In addition to that, Zeng & Zhuang (2016) reported small stroma in *C. lavitskae*, while Šandová (2024) stated that *S. berkeleyana* differs in ascospore length-to-width ratio. The absence of an apical pore (Zeng & Zhuang 2016) remains to be verified.

Tab. 1. Comparison of selected characters of *Sphaerostilbella berkeleyana* and *Cosmospora lavitskae* from the literature.

Taxon	Presence of subiculum	Size of fruitbodies (µm)	Size of asci (µm)	Ascospores (µm)	Substrate	Reference
C. lavitskae	–	(118)139–181 × 111–153	(37)40–52.5 × 2.5–4.5(5.5)	4–6(7) × 3–4	*Xylariaceae* indet.	Zeng & Zhuang 2016
S. berkeleyana	+	(240)270–280(340) × 180–210	(75)85–90 × 3–5	(8.5)10–12(14) × 3–4	*Stereum* sp.	Samuels 1976
S. berkeleyana	+	(?190)240–300 × 180–210	84–124 × 4.8–5.7	(8.5)9.2–11.5(12.5) × (3.2)3.5–3.8(4)	*Stereum hirsutum*	Šandová 2024

Karsten (1891) described *V. berkeleyanum* as the conidial stage of *H. berkeleyanus* (= *S. berkeleyana*). Samuels (1976) did not study the anamorph. Põldmaa (1999) published nine finds of this possible anamorph from *Stereum*, but stated that she was unable to germinate the ascospores and cannot confirm conspecificity of these anamorphs with *S. berkeleyana*. Šandová (2024) repeatedly found this hyphomycete in specimens of *S. berkeleyana*, but it also occurs separately, in absence of perithecia, on *Stereum* (Põldmaa 1999). Further study of the identity of this hyphomycete or even the identity of the anamorph present in Karsten's specimen of *V. berkeleyanum* is needed. Warted conidiophores as well as warted lower parts of phialides as observed by Šandová (2024) are also known in *Cosmospora* anamorphs (Gams 1971, Zare & Gams 2016). The difference in conidium size between *S. berkeleyana* (Šandová 2024) and *C. lavitskae* (this study) can theoretically be attributed to differences in conidium size on natural substrate and on different media in culture as discussed above, although the difference in length is quite large (see Tab. 2). Numerous anamorphs in *Cosmospora* (discussed in this study) and *Hypomyces* spp. (Zare & Gams 2016) with *Verticillium*-like anamorphs have similar morphological features, so molecular studies are needed for their reliable identification.

Tab. 2. Conidial sizes in 3% KOH of a hyphomycete co-occurring with *Sphaerostilbella berkeleyana* and *Cosmospora lavitskae* based on data from the literature and this study.

Taxon	**Substrate**	**Size of conidia (µm)**	**Average (µm)**	**Reference**	**Percentage difference between average sizes (hyphomycete on natural substrate = 100%): length; width**
Hyphomycete co-occurring with *S. berkeleyana* (PRM 731427, PRM 887850, K-M000736126)	natural substrate	5.6–8.9 × 3.4–4.6	7.0 × 3.5*	Šandová 2024; this study	
C. lavitskae (CBS 501.81)	potato extract glucose agar (PDA)	3.6–6.3(8.4) × 1.9–3.0	5.0 × 2.3	this study	29%; 39%
C. lavitskae (CBS 501.81)	oatmeal agar (OA)	2.8–6.1 × 1.8–2.7	4.4 × 2.3	this study	37%; 39%

* The average size of conidia of *S. berkeleyana* is calculated based on Šandová's revision notes in the listed herbarium specimens.

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