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# Phragmotrichum chailletii has a sibling species in North America 

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With 2 figures and 1 table


#### Abstract

The phylogenetic placement of Phragmotrichum chailletii in Melanommataceae (Pleosporales) is re-evaluated and its phylogeographic structure is assessed based on a large number of specimens from Europe and North America together with a four-gene (ITS, LSU, RPB2, EF1- $\alpha$ ) dataset. Morphologically, all collections produced identical conidiomata and conidia on the same substrate, which is spruce cones on litter. Their fruiting season was also similar as they originated from early spring soon after snow melts at a similar range of elevation. However, all isolates collected in Canada and associated with Picea species native to North America, clustered together in a clade separate from collections made in Europe and occurring on host species native to centraleastern Europe. A sibling species, Ph. thornhilliae, is introduced to accommodate this group of isolates using molecular and phylogeographic evidence. One specimen belonging to the novel species was also collected in Switzerland. Possible scenarios to explain this anomaly are provided and the most likely explanation is an accidental, human mediated introduction.


Keywords: allopatry; anamorphic; cryptic speciation; phenotypic differences

## Introduction

Phragmotrichum chailletii Kunze is a noteworthy anamorphic coelomycetous species with a conspicuous morphology. It produces distinct black stromatic conidiomata on dead cones of Picea species (rarely also on other conifers). These sporodochia-like fructifications may be individual or gregarious, forming large patches on the surface of the cone scales and having pale brown, catenate dictyoconidia formed inside and exposed through
the rupture of the upper and lateral walls of the fructifications. Nine species were historically described in Phragmotrichum Kunze until Sutton and Pirozynski (1965) revised and synonymised some of them based on study of authenticated material as well as reduced three monotypic genera Taeniophora Karst., Alysisporium Peyron. and Seiridiella Karst. to synonyms with Phragmotrichum. Sutton \& Pirozynski (1965) and Sutton \& Sandhu (1969) considered the type of conidiomata and the basipetal development and maturation of conidia in the chains important for generic delimitation. They also combined the phragmosporous Trimmatostroma pini W.B.Cooke into Phragmotrichum and accepted only four species: Ph. chailletii, Ph. pini (W.B. Cooke) B. Sutton \& D.K. Sandhu, Ph. rivoclarinum (Peyronel) B. Sutton \& Piroz and Ph. quercinum H. Hoffm. Two other taxa, Ph. vassiljevae Melnik and Ph. andamanense Bhat, W.B. Kendr. \& Nag Raj, were added later and the genus currently comprises six species (Wijayawardene et al. 2016).

Due to its distinct morphology and common occurrence in temperate regions on old, empty seed cones of spruce from the previous year (still hanging or fallen off), Ph. chailletii has become a frequently collected and documented species. An online search in MyCoPortal (MyCoPortal 2023) shows a total of 228 records of the fungus associated with coniferous hosts in several countries of Europe, Canada and the USA. Hosts include mostly spruce (Picea spp.) and fir (Abies spp.) but less often the Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] or the European larch (Larix decidua Mill.) and very rarely the Scots pine (Pinus sylvestris L.). The fungus sometimes occurs on tree hosts outside their native range when planted in botanical gardens e.g. Lijiang spruce [Picea likiangensis (Franch.) E. Pritz.], a native of Bhutan and China (The Gymnosperm Database https://www.conifers.org/index.php). The IMI database (http://www.herbimi.info/ herbimi/home.htm, accessed February 2023) contains 16 specimens from four European countries, Russia and Canada, all from native spruce species. Specimen IMI 24783 from the Philippines documents a rare occurrence in the tropics and most likely one associated with a planted and naturalized host tree (P. abies) outside its native range of temperate Europe. The virtual herbarium system JACQ (https://www.jacq.org/\#home) also adds collections from Austria, Czech Republic and Germany. A search in Google Pictures also indicates > 1000 available photos of the fungus that document this species from further localities across Europe and also Canada.

When Kunze \& Schmidt (1823) introduced Phragmotrichum, with Ph. chailletii as the type species, they noted that the shape of conidia, which they named rhombic, had no analogy among other fungal species known to them. Though very common and frequently collected, Ph. chailletii has long been overlooked in phylogenetic studies until Crous et al. (2020) and most recently Wijesinghe et al. (2023) revealed its placement within Melanommataceae G. Winter, a family which also includes Petrakia Syd. \& P. Syd. (Jaklitsch \& Voglmayr 2017). In both studies, since the holotype of Ph. chailletii was destroyed during World War II, Crous et al. (2020) designated a specimen collected on cones of $P$. abies in Hindelbank, Switzerland, as the neotype, not far from the holotype locality, Neuchâtel. phylogenies based on rDNA showed that Ph. chailletii clustered together with species of Mycopappus Redhead \& G.P. White, Pseudodidymella C.Z. Wei, Y. Harada \& Katum., Xenostigmina Crous and Petrakia, all plant pathogens having my-
copappus-like propagules in their life cycles. These genera, once forming a separate family Pseudodidymellaceae A. Hashim. \& Kaz. Tanaka (Hashimoto et al. 2017), were reduced to synonyms with Petrakia by Beenken et al. (2020), who preferred to keep its original wide generic concept (Jaklitsch \& Voglmayr 2017).

Since Beenken et al. (2020) focused only on pathogenic species, they left room for refining the phylogenetic relationships between Ph. chailletii, a remarkable saprotrophic species, with members of Petrakia that also produce dictyoconidia. The aim of our study was thus to refine the phylogenetic placement of Ph. chailletii within the recently revised Melanommataceae and to address its phylogeographic structure and potential cryptic taxa in view of the widely documented distribution of the fungus and its multiple hosts. In order to achieve this aim, we focused on fresh collections of Ph. chailletii in Europe and also on recently isolated strains from North America.

## Materials and Methods

Strains were isolated from mature conidiomata on spruce cones freshly collected on the litter. Conidia were picked by a sterile surgical needle and transferred into Petri dishes with Malt Extract Agar (MEA). Pure cultures were maintained on Malt Extract Agar and Potato Carrot Agar. Additional strains were obtained from the culture collection of Agriculture and Agri-Food Canada (AAFC). Slides for microscopy were mounted in tap water, $3 \% \mathrm{KOH}$ and Melzer reagent. Microscopic structures were examined and documented with differential interference contrast (Olympus BX-51 with digital camera, Quick Photo software, Olympus, Japan). Representative specimens were deposited in PRC (Herbarium of the Charles University, Czech Republic) and living strains in the Culture Collection of Fungi (CCF, Faculty of Science, Charles University, Czech Republic).

DNA was extracted from 2-weeks old colonies using a Zymo Research Fungal/Bacterial Kit (Zymo Research, Orange, USA). Nuclear rDNA containing the ITS1-5.8S-ITS2 and 28S regions was amplified with primer set ITS1F/NL4 (Gardes \& Bruns 1993, White et al. 1990), and fragments of genes encoding translation elongation factor $1 \alpha$ (EF1- $\alpha$ ) and RNA polymerase II second largest subunit (RPB2) were amplified with primer sets 983F/2218R (Rehner \& Buckley 2005) and RPB2-5F/fRPB2-7cR (Liu et al. 1999), respectively. The PCR products were viewed by means of electrophoresis on $1 \%(\mathrm{w} / \mathrm{v})$ TAE agarose gel, stained with ethidium bromide. The PCR products were purified with the AMPure XP beads (Beckman Coulter, USA). Both strands of the PCR fragments were sequenced with the primers used for amplification in the Sequencing Laboratory of the Faculty of Science, Charles University, Czech Republic. All sequences were deposited in the GenBank (Table 1).
Our dataset was built based on that assembled by Beenken et al. (2020) (Electronic Supplementary Materials 1). Alignment was performed using the MAFFT algorithm implemented in Geneious 6.1 .5 software and manually edited in the same software (Electronic Supplementary Materials 2). Phylogenetic analyses were performed by Bayesian in-
Table 1. Living cultures included in this study, DNA regions sequenced and their GenBank accession numbers.

| Species name | Voucher specimen or living strain | Country of origin | Host | ITS + LSU rDNA | RPB2 | EF-1- $\alpha$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Phragmotrichum chailletii | CCF 5759 | Czech Republic | Picea abies | OQ919283 | OQ884629 | OQ884635 |
|  | CCF 6071 | Czech Republic | Picea abies | OQ919286 |  |  |
|  | PRC 9003 | Czech Republic | Picea abies | OQ919285 | OQ884625 | OQ884637 |
|  | PRC 9004 | Czech Republic | Picea abies | OQ919284 | OQ884630 | OQ884636 |
|  | PRC 8998 | Czech Republic | Picea abies | OQ919288 | OQ884633 |  |
|  | PRC 9000 | Czech Republic | Picea abies | OQ919287 |  |  |
|  | PRC 9002 | Czech Republic | Picea abies | OQ919289 |  |  |
|  | PRC 7900 | Switzerland | Picea abies | OQ919290 | OQ884631 | OQ884638 |
|  | PRC 9006 | Germany | Picea abies | OQ919282 | OQ884628 | OQ884634 |
|  | NK463 | Switzerland | Picea abies | OQ919291 |  |  |
|  | PRC 5138 | Switzerland | Picea abies | OQ919292 |  |  |
|  | PRC 7897 | Germany | Picea abies | OQ919292 |  | OQ884639 |
|  | PRC 7899 | Switzerland | Picea omorika | OQ919294 |  | OQ884640 |
|  | NK471 | Switzerland | Picea abies | OQ919295 | OQ884632 | OQ884641 |
| Phragmotrichum thornhilliae | AAFC CHEM_J_2959 | Canada | Picea pungens | OQ919298 | OQ884624 |  |
|  | AAFC CHEM_J_2961 | Canada | Picea pungens | OQ919299 |  |  |
|  | PRC 9001 | Canada | Picea glauca | OQ919297 | OQ884626 | OQ884642 |
|  | AAFC KAS6383 | Canada | Picea sp. | OQ919300 | OQ884623 |  |
|  | PRC 7898 | Switzerland | Picea abies | OQ919296 | OQ884627 | OQ884643 |

feren-ce using MrBayes version 3.2 (Ronquist et al. 2012) and by Maximum likelihood analysis using the RAxML Web Server version 7.7.1 (Stamatakis et al. 2008) accessed through the CIPRES Science Gateway (Miller et al. 2010). For the Bayesian analysis, the best-fit models were determined using PartitionFinder 2 (Lanfear et al. 2016). Two independent runs of $9,000,000$ generations were ran with sampling every $1000^{\text {th }}$ generation and the first $25 \%$ of samples discarded as burn-in. Posterior probabilities (PP) were used as Bayesian branch support for consensus trees. The average standard deviation of split frequencies estimating convergence reached the level of 0.004 at the end of the analysis.

## Results

Combination of two rDNA markers and two coding single copy genes revealed that our collections identified as Phragmotrichum chailletii formed a well-supported clade sister to three members of the genus Seifertia (Fig. 1). Within Ph. chailletii, two fully supported subclades were well differentiated. One of them included European collections that had identical ITS and LSU rDNA with those originating from strain CBS144994 (MN313812 and MN317293, respectively), the neotype of Ph. chailletii designated by Crous et al. (2020). The other clade includes all collections from North America together with one specimen from Switzerland (PRC 7898). In this case they differed in five and six bp of their ITS and LSU sequences, respectively, compared to sequences obtained from the neotype. One transversion (A/G) was present also in a 13 bp long intron in the ITS rDNA that was distinct for all Phragmotrichum sequences.

## Taxonomy

Phragmotrichum thornhilliae Koukol \& G. Delgado, sp. nov.
Mycobank Accession Number: MB 848350
Diagnosis: Similar to Phragmotrichum chailletii in conidial size and morphology, but differing in DNA sequence data; in ITS1 rDNA substitutions at bases: $56(\mathrm{C} / \mathrm{T}), 79(\mathrm{C} / \mathrm{T})$ and $104(\mathrm{G} / \mathrm{A})$, in ITS2 rDNA at bases: $22(\mathrm{C} / \mathrm{T})$ and 46 (T/C); in LSU rDNA at bases: 50 (G/A), 69 (C/T), 443 (C/T), 485 (T/C), 504 (T/C) and 521 (G/A), and in host association; occurring mainly on Picea pungens and Picea glauca.

Description: Conidiomata solitary or gregarious, sometimes confluent, globose, elongated or somewhat irregular in shape, black, subepidermical and erumpent, the conidial mass is exposed after the cracking and rupture of the upper and lateral, dark brown to blackish brown, glistening walls. Conidiogenous cells holothallic, terminal, producing basipetal chains of conidia. Conidia broadly fusiform to broadly ellipsoidal, straight or flexuous, muriform, smooth, yellowish-brown to brown, $30-50 \times 12-19.5 \mu \mathrm{~m}$, bearing 3-4 short median and lateral appendages, 3-4 $\mu \mathrm{m}$ long, catenate, formed in unbranched

100 Petrakia aesculi HHUF 22892
Petrakia aesculi HHUF 30550

- Petrakia fagi HHUF 22903
- Petrakia fagi HHUF 30515
- Petrakia liobae ZTMyc 57657

Petrakia liobae ZTMyc 59926
1 99- Petrakia minima HHUF 30551
Petrakia minima HHUF 30552
100 100- Petrakia aceris CBS 115685
Petrakia aceris CBS 124109

- Petrakia echinata ZTMyc 24162
- Petrakia echinata ZTMyc 59957

100 Petrakia deviata ZTMyc 57658

- Petrakia deviata ZTMyc 57659

Phragmotrichum chailletii PRC 9006 - DEU
Phragmotrichum chailletii PRC 7899 - CHE
Phragmotrichum chailletii CCF 5759 - CZE
Phragmotrichum chailletii PRC 9004 - CZE

- Phragmotrichum chailletii PRC 9003 - CZE
- Phragmotrichum chailletii CCF 6071 - CZE

Phragmotrichum chailletii PRC 8998 - CZE
100-Phragmotrichum chailletii PRC 9002 - CZE

- Phragmotrichum chailletii CBS 144944 - CHE
- Phragmotrichum chailletii PRC 7900 - CHE
- Phragmotrichum chailletii NK463-CHE

100 -Phragmotrichum chailletii PRC 5138-CHE - Phragmotrichum chailletii PRC 7897 - DEU ${ }^{1}$ Phragmotrichum chailletii NK471-CHE [Phragmotrichum thornhilliae PRC 9001 -CAN - Phragmotrichum thornhilliae CHEM 2959-CAN

100 Phragmotrichum thornhilliae KAS 6383-CAN
Phragmotrichum thornhilliae PRC 7898 - CHE
-Phragmotrichum thornhilliae CHEM 2961 - CAN
$100-$ Seifertia azaleae ZTMyc 57693
Seifertia azaleae ZTMyc 57694
Seifertia shangrilaensis MFLUCC 16-0238
${ }^{98}$ Seifertia alpina ZTMyc 58033
Seifertia alpina ZTMyc 59953
${ }_{100}$ Alpinaria rhododendri HHUF 30554
Alpinaria rhododendri MP4
98- Melanodiplodia tianschanica MFLUCC 17-0805
L Melanodiplodia tianschanica TASM 6111
100 Herpotrichia juniperi AFTOL-ID 1608
Herpotrichia juniperi T02 62
100 Muriformistrickeria rubi MFLUCC 15-0681
L Muriformistrickeria rubi MFLUCC1 7-2550
$\sqrt{99} \sqrt{96}\left[\begin{array}{l}\text { Uzbekistanica rosae-hissaricae MFLUCC 17-0819 } \\ \text { Uzbekistanica rosae-hissaricae MFLUCC 17-0820 }\end{array}\right.$
Uzbekistanica yakutkhanika MFLUCC 17-0809
Uzbekistanica yakutkhanika MFLUCC 17-0842
${ }^{100}$ Praetumpfia obducens C56
Praetumpfia obducens CuO
100 - Gemmamyces piceae WU 36908
-Gemmamyces piceae WU 36906
Aposphaeria corallinolutea MFLU 15-2752
100. Melanomma japonicum HHUF 30541

100 Melanomma japonicum MAFF 239634
100. Melanomma pulvis-pyrius CBS 124080

L Melanomma pulvis-pyrius HHUF 30542
Petrakia irregularis CBS 306.67
Splanchnonema pupula MFLU 14-0807
Pleomassaria siparia CBS279.74
chains of up to six conidia, with pale basal conical cell, up to $13 \mu \mathrm{~m}$ long and $3.5-4 \mu \mathrm{~m}$ wide.

Colonies on MEA after 7 days moderately fast-growing reaching (20) $24-28 \mathrm{~mm}$ diam., cottony, circular, white, slightly depressed around the centre, margin entire, reverse offwhite.

Type: Canada, Ontario, Peterborough Co., Havelock, 1903 Line Belmont, $44^{\circ} 27^{\prime} 25.900^{\prime \prime}$ N, $77^{\circ} 47^{\prime} 52.000^{\prime \prime}$ W, 198 m a.s.l., on cone scales of Picea glauca, June $16^{\text {th }} 2017$, leg. J. Thornhill, (PRC 9001 - holotype)

Ex-type living strain CCF 7005. GenBank OQ919297 (ITS + LSU rDNA), OQ884626 (RPB2), OQ884642 (EF1- $\alpha$ )

Other specimens examined: Same as holotype, Mar 14 th 2016, leg. J. Thornhill (BPI 925131); Ontario, Ottawa, Dominion Arboretum, $45^{\circ} 23^{\prime} 31.641^{\prime \prime} \mathrm{N}, 75^{\circ} 42^{\prime} 22.013^{\prime \prime} \mathrm{W}$, 82 m a.s.l., on a cone of Picea pungens, 26 Apr 2015, leg. J. Mack (AAFC CHEM_ JM_2959); ibid., Carleton University, $45^{\circ} 23^{\prime} 0.215^{\prime \prime} \mathrm{N}, 75^{\circ} 41^{\prime} 57.804^{\prime \prime} \mathrm{W}, 80$ a.s.l., on a cone of P. pungens, 26 Apr 2015, leg. J. Mack (AAFC CHEM_JM_2961); Quebec, Montreal, on a cone of Picea sp., Apr 2015, leg. unknown. (AAFC KAS 6383). Switzerland, Ticino, Leventina, Airolo, $46^{\circ} 31^{\prime} 37.992^{\prime \prime} \mathrm{N}, 8^{\circ} 35^{\prime} 56.40^{\prime \prime} \mathrm{E}, 1160 \mathrm{~m}$ a.s.l., on a cone of P. abies on litter, 10 May 2019, leg. L. Beenken (PRC 7898).

Etymology: Referring to Jan Thornhill, amateur mycologist, who documented this species on her blog and eagerly shared her specimens for research.

Known distribution: This fungus is known from Canada and Switzerland.
Remarks: Morphologically, the newly described species Ph. thornhilliae cannot be distinguished from Ph. chailletii. It produces identical conidiomata and conidia on the same substrate which is spruce cones on litter. The fruiting season is also similar for both species: collections of Ph. thornhilliae and Ph. chailletii originate from (early) spring soon after snow melts and come from similar range of elevation (80-1160 and 446-1156 m a.s.l., respectively). However, the spruce host species and geographical region differ between them. The native range of Ph. thornhilliae hosts, Picea glauca and P. pungens, is Canada and the northern or western montane areas of the United States. In contrast, P. abies and P. omorika the hosts of Ph. chailletii, are native of central-eastern Europe (The Gymnosperm Database 2023).

Fig. 1. Phylogenetic hypothesis showing the placement of Phragmotrichum chailletii and Ph. thornhilliae based on combined analysis of ITS+LSU rDNA, RPB2 and EF1- $\alpha$ sequences. Numbers above branches represent ML bootstrap support values (BS>95), thickened lines at branches indicate PP>0.95. Countries of origin are represented by codes, specimens in bold represent neotype and holotype of $P h$. chailletii and $P h$. thornhilliae, respectively.


Fig. 2. Phragmotrichum thornhilliae (PRC 9001, holotype, a-h) and Ph. chailletii (PRC 5138, i). a-b) detail of conidiomata on the cone scales, c) colony on PCA and d) on MEA after 7 days, e-h) conidial chains including conidiogenous cell and immature conidia, i) dead cone with Ph. chailletii still on the tree with numerous conidiomata, Scale bars $=20 \mu \mathrm{~m}$.

Phragmotrichum chailletii Kunze, in Kunze \& Schmidt, Mykologische Hefte (Leipzig) 2: 84, 1823.

Specimens examined: Austria, Kleinwalsertal, Waldstück hinter dem Marburger-Haus (Wäldelestrasse) ca. 1156 m a.s.l., on a cone of $P$. abies on the litter, 29 Sep 2013, leg. H. Lotz-Winter, HLW 12 A-290913 (PRC 9005).

Czech Republic. Northern Bohemia. Bohemian Switzerland NP, Křídelní stěna, $50^{\circ} 53^{\prime} 1.091^{\prime \prime} \mathrm{N}, 14^{\circ} 18^{\prime} 3.788^{\prime \prime} \mathrm{E}, 466 \mathrm{~m}$ a.s.l., on a cone of $P$. abies on the litter, Feb $26^{\text {th }}$ 2018, leg. Z. Palice \& P. Uhlík (living culture CCF 6071); Western Bohemia, Abertamy, Hřebečná, forest margin, 150 m east from the village, $50^{\circ} 22^{\prime} 48.909^{\prime \prime} \mathrm{N} 12^{\circ} 49^{\prime} 49.241^{\prime \prime} \mathrm{E}$, 884 m a.s.l., on a cone of P. abies on the litter, 29 Apr 2017, leg. O. Koukol, OK309 (PRC 3989, living culture CCF5759); Northern Bohemia, Jizerské hory Mts., NW slope of Oldřichovský Špičák Mt. (green tourist path), $15^{\circ} 4.22532^{\prime}$ N $50^{\circ} 51.95548^{\prime}$ E, 580 m a.s.l., on a cone of $P$. abies on the litter, 08 Apr 2018, leg. O. Kubátová, OK321 (PRC 9004); Northern Bohemia, Jizerské hory Mts., valley Bílý Štolpich (under Bílá kuchyně crossroad), $15^{\circ} 9.31758^{\prime} \mathrm{N} 50^{\circ} 51.03573^{\prime} \mathrm{E}, 770 \mathrm{~m}$ a.s.l., on a cone of $P$. abies on the litter, 08 Apr 2018, leg. A. Kubátová, OK322 (PRC 9003); Northern Bohemia, Krkonoše Mts., between Medvedi koleno road curve and Patejdlova bouda cottage, $50^{\circ} 45^{\prime} 4.900^{\prime \prime} \mathrm{N}$ $15^{\circ} 35^{\prime} 45.600^{\prime \prime} \mathrm{E}, 894 \mathrm{~m}$ a.s.l., on a cone of P. abies on the litter, 09 Apr 2018, leg. M. Vinkler, OK323, (PRC 9000). ibid. OK324 (PRC 8998, CCF6072), ibid. OK325 (PRC 8999); Western Bohemia, Krušné hory Mts., Jáchymov, SW slope of Klínovec Mt., next to the lower station of ski-lift, $50^{\circ} 23^{\prime} 8.767^{\prime \prime} \mathrm{N} 12^{\circ} 56^{\prime} 30.608^{\prime \prime} \mathrm{E}, 755 \mathrm{~m}$ a.s.l., on a cone of P. abies on the litter, 17 March 2019, leg. O. Koukol, OK348 (PRC 9002).

Germany, Bavaria, München, Allacher Forst, $48^{\circ} 12^{\prime} 18.576^{\prime \prime} \mathrm{N} 11^{\circ} 28^{\prime} 19.164^{\prime \prime} \mathrm{E}, 500 \mathrm{~m}$ a.s.l., on a cone of P. abies on the litter, 22 Apr 2019, leg. L. Beenken, OK364 (PRC 7897); Baden-Württemberg, Bernsteinweg, Black Forest Nationalpark, $48^{\circ} 40^{\prime} 27.264^{\prime \prime}$ N, $8^{\circ} 14^{\prime} 7.332^{\prime \prime}$ E, 750 m a.s.l., on a cone of $P$. abies on the litter, 08 Apr 2019, leg. F. Popa, FP; 422, (PRC 9006); Baden-Württemberg, hintere Bergwaldhütte, Black Forest National Park, $48^{\circ} 39^{\prime} 34.232^{\prime \prime} \mathrm{N}, 8^{\circ} 14^{\prime} 34.411^{\prime \prime} \mathrm{E}, 750 \mathrm{~m}$ a.s.l., on a cone of $P$. abies on the litter, 08 Apr 2019, leg. F. Popa, FP424, (PRC 9008); Hessen, Wiesbaden Naurod, Trockenbornweg nahe Kellerskopf (Taunus), ca. 446 m. a.s.l., on a cone of $P$. abies on the litter, 22 Jan 2014, leg. H. Lotz-Winter, HLW 3862, (PRC 9007).

Slovakia, Telgárt, Čeršla, above the playground, $48^{\circ} 50^{\prime} 41.968^{\prime \prime} \mathrm{N}, 20^{\circ} 12^{\prime} 39.567^{\prime \prime} \mathrm{E}$, 1000 m a.s.l. on a cone of $P$. abies on the litter, 1 May 2018, leg. I. Černajová (without voucher).

Switzerland, Jura, Saignelégier, Patura de-Les Royes, $47^{\circ} 15^{\prime} 7.776^{\prime \prime} \mathrm{N}^{\circ} 7^{\circ} 1^{\prime} 18.588^{\prime \prime} \mathrm{E}$, 967 m a.s.l., on a cone of P. abies on the litter, 06 Apr 2019, leg. E. Stöckli, OK365 (PRC 7900); Zurich, Winterthur, Frohbergpark, $47^{\circ} 29^{\prime} 46.320^{\prime \prime} \mathrm{N} 8^{\circ} 43^{\prime} 21.000^{\prime \prime} \mathrm{E}, 455^{\prime \prime} \mathrm{m}$ a.s.l., on a cone of $P$. abies on the litter and still on the tree, 19 Apr 2019, leg. L. Beenken, OK363 (PRC 5138); Birmensdorf, $47^{\circ} 21^{\prime} 46.728^{\prime \prime} \mathrm{N}, 8^{\circ} 27^{\prime} 17.928^{\prime \prime} \mathrm{E}, 545 \mathrm{~m}$ a.s.l., 18 Apr 2019, leg. L. Beenken (living culture NK463); Ticino, Leventina, Airolo, $46^{\circ} 31^{\prime} 40.440^{\prime \prime} \mathrm{N}$ $8^{\circ} 35^{\prime} 56.256^{\prime \prime}$ E, 1160 m a.s.l., on a cone of P. omorika on the litter, 10 May 2019, leg. L. Beenken, OK359 (PRC 7899); Grisons, San Bernardino, Pian San Giacomo,
$46^{\circ} 25^{\prime} 17.220^{\prime \prime} \mathrm{N}, 9^{\circ} 13^{\prime} 40.332^{\prime \prime} \mathrm{E}, 1210 \mathrm{~m}$ a.s.l., on a cone of P. abies on the litter, 10 May 2019, leg. L. Beenken (culture NK471).

## Discussion

## Cryptic speciation and geographical distribution

Cryptic species are generally understood as species that are morphologically very similar, even indistinguishable, but may be differentiated only based on molecular data (Queloz et al. 2011). Identical morphology, however, can be retained after allopatric divergence and limited gene flow among populations eventually gives rise to two sibling species with different life histories and thus distinct phenotypic differences. Differentiation of morphologically identical species may be assisted with different hosts in the case of pathogens or other types of symbionts (Sochorová et al. 2019). Even for saprotrophs, one should be cautious with the application of a name based solely on morphological characteristics when further phenotypic data exist. Martinović et al. (2016) revealed several distinct lineages within Desmazierella acicola Lib. that did not differ in the morphology of the anamorph but differed in their pine host species and geographical regions.

Huge geographical barriers typically allow such divergence and one should always be suspicious when European names are applied to morphologically identical collections in North America (Nguyen et al. 2013). Numerous examples of intercontinental divergence were already reported for presumed cosmopolitan, pantemperate or pantropical species of agarics and polypores (Hughes \& Petersen 2015, Oliveira et al. 2022). In these fungal groups, supporting evidence for the interpretation of inter- and intraspecific variability was derived also from mating tests and dikaryotization, which is however not possible in asexual ascomycetes. Also, Phragmotrichum chailletii was considered to be a widespread and conspicuous species that may be easily identified based on the substrate (spruce cones in the litter) and distinctly catenate dictyoconidia. However, application of this name to collections from North America on cones of local spruce species was obviously erroneous. Distant region and different host species agree with the observed genetic distances and imply that the two morphologically indistinguishable groups actually represent two different species. Rather surprisingly, we recorded Ph. thornhilliae also in Europe and one of our collections on a cone of P. abies from Airolo (Switzerland) turned out to belong to this newly described species. Two potential scenarios may explain this anomaly: 1) the two species developed in Europe sympatrically and only one of them was introduced to North America, where it established association with local spruce species; or 2) the two species developed allopatrically in North America and Europe in association with local species and the single record of Ph. thornhilliae in Europe is an accidental, most probably human mediated introduction. With the intensity of global traffic and widespread occurrence of natural spruce forests and artificial plantations, the second scenario seems to be more likely. A similar pattern was recorded by Hughes \& Petersen (2015), who
analysed multiple collections of Gymnopus confluens (Pers.) Antonín, Halling \& Noordel. from Europe and Northern America. Analysis of both ITS and LSU rDNA indicated two distinct clades reflecting the transatlantic disjunction with an exception in one specimen from California, USA, clustering within the European clade. Hughes \& Petersen (2015) also concluded that this discrepancy was caused by human mediated transfer.

Search performed with ITS1 rDNA in the Global Fungi database (Větrovský et al. 2020) confirmed the presence of Ph. chailletii in Europe (six records from soils in Estonia), but did not retrieve any match with sequence of Ph. thornhilliae.

## Ecology

Both Ph. chailletii and Ph. thornhilliae are mostly reported from spruce cones on the litter in early spring, but their conidiomata are present already on cones still hanging from the twigs (Fig. 2i). The infection pathway of young cones is thus most probably mediated via dictyoconidia that are dispersed by wind to young developing cones. Potential decomposing activity of both species on fallen cones during the rest of the season is unknown.

## Phylogenetic relationships

When Crous et al. (2020) provided the phylogenetic placement of Ph. chailletii based on LSU rDNA, the neotype they selected clustered within Melanommataceae in a poorly supported clade consisting of several genera that were later synonymized by Beenken et al. (2020) in Petrakia. Our study thus provided the first placement of Ph. chailletii and Ph. thornhilliae based on a robust four-gene regions dataset. In our phylogeny, although the genus Phragmotrichum is placed close to Petrakia, the similarity of its conidia to those of Petrakia echinata is rather a consequence of a convergent adaptation than a sign of their relationship. The differences in conidiogenesis support that conidial morphology is not a synapomorphic characteristic of the two genera. Conidia of Phragmotrichum are born from holothallic conidiogenous cells, whereas those of Petrakia are produced from annellidic conidiogenous cells (Li et al. 2016). Additionally, the sister genus of Phragmotrichum, Seifertia, has different, unicellular conidia that are formed on synnemata (Beenken et al. 2020). The genera are also differentiated ecologically. Seifertia species are parasitic or saprophytic on Rhododendron species, whereas Petrakia species are leaf pathogens of broadleaf trees (Beenken et al. 2020).

After the very recent description of a new pathogen Microstrobilinia castrans Beenken \& Andr. Gross (Beenken et al. 2023), Ph. thornhilliae is another species described from spruces documenting that novel taxa can be found also on intensively studied hosts and substrates.

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## References

Beenken, L., Gross, A., \& Queloz, V. (2020). Phylogenetic revision of Petrakia and Seifertia (Melanommataceae, Pleosporales): New and rediscovered species from Europe and North America. Mycological Progress, 19(5), 417-440. https://doi.org/10.1007/s11557-020-01567-7
Beenken, L., Stroheker, S., Dubach, V., Schlegel, M., Queloz, V., \& Gross, A. (2023). Microstrobilinia castrans, a new genus and species of the Sclerotiniaceae parasitizing pollen cones of Picea spp. Mycological Progress, 22(2), 14. https://doi.org/10.1007/s11557-023-01865-w
Crous, P. W., Schumacher, R. K., Wood, A. R., \& Groenewald, J. Z. (2020). The Genera of Fungi G5: Arthrinium, Ceratosphaeria, Dimerosporiopsis, Hormodochis, Lecanostictopsis, Lembosina, Neomelanconium, Phragmotrichum, Pseudomelanconium, Rutola, and Trullula. Fungal Systematics and Evolution, Jun. 5, 77-98.
Gardes, M., \& Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Molecular Ecology, 2(2), 113-118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x
Hughes, K. W., \& Petersen, R. H. (2015). Transatlantic disjunction in fleshy fungi III: Gymnopus confluens. MycoKeys, 9, 37-63. https://doi.org/10.3897/mycokeys.9.4700
Jaklitsch, W. M., \& Voglmayr, H. (2017). Three former taxa of Cucurbitaria and considerations on Petrakia in the Melanommataceae. Sydowia, 69, 81-95.
Kunze, G., \& Schmidt, C. J. (1823). Mykologische Hefte. Leipzig.
Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., \& Calcott, B. (2016). PartitionFinder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses. Molecular Biology and Evolution, 34, 772-773. https://doi.org/10.1093/ molbev/msw260
Li, G. J., Hyde, K. D., Zhao, R. L., Hongsanan, S., Abdel-Aziz, F. A., Abdel-Wahab, M. A., . . . Maharachchikumbura, S. S. N. (2016). Fungal diversity notes 253-366: Taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity, 78(1), 1-237. https://doi.org/10.1007/ s13225-016-0366-9
Liu, Y. J., Whelen, S., \& Hall, B. D. (1999). Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerse II subunit. Molecular Biology and Evolution, 16(12), 17991808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

Martinović, T., Koukol, O., \& Hirose, D. (2016). Distinct phylogeographic structure recognized within Desmazierella acicola. Mycologia, 108(1), 20-30. https://doi.org/10.3852/14-291
Miller, M. A., Pfeiffer, W., \& Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Gateway Computing Environments Workshop: 1-8. New Orleans, LA. https://doi.org/10.1109/GCE.2010.5676129

Nguyen, N. H., Landeros, F., Garibay-Orijel, R., Hansen, K., \& Vellinga, E. C. (2013). The Helvella lacunosa species complex in western North America: Cryptic species, misapplied names and parasites. Mycologia, 105, 1275-1286.
Oliveira, J. J. S., Capelari, M., Margaritescu, S., \& Moncalvo, J.-M. (2022). Disentangling cryptic species in the Marasmius haematocephalus (Mont.) Fr. and M. siccus (Schwein.) Fr. species complexes (Agaricales, Basidiomycota). Cryptogamie. Mycologie, 43(5), 91-137. https://doi.org/ 10.5252/cryptogamie-mycologie2022v43a5

Queloz, V., Grünig, C. R., Berndt, R., Kowalski, T., Sieber, T. N., \& Holdenrieder, O. (2011). Cryptic speciation in Hymenoscyphus albidus. Forest Pathology, 41(2), 133-142. https://doi.org/ 10.1111/j.1439-0329.2010.00645.x

Rehner, S. A., \& Buckley, E. (2005). A Beauveria phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs. Mycologia, 97, 84-98.
Sochorová, Z., Döbbeler, P., Sochor, M., \& Van Rooy, J. (2019). Octospora conidiophora (Pyronemataceae) - a new species from South Africa and the first report of anamorph in bryophilous Pezizales. MycoKeys, 54, 49-76. https://doi.org/10.3897/mycokeys.54.34571
Stamatakis, A., Hoover, P., \& Rougemont, J. (2008): A rapid bootstrap algorithm for the RAxML Web servers. Systematic Biology, 57(5): 758-771. https://doi.org/10.1080/10635150802429642
Sutton, B., \& Pirozynski, K. (1965). Notes on microfungi. II. Transactions of the British Mycological Society, 48(3), 349-366. https://doi.org/10.1016/S0007-1536(65)80055-9
Sutton, B., \& Sandhu, D. (1969). Phragmotrichum pini (WB Cooke) comb. nov. Transactions of the British Mycological Society, 52(1), 67-71. https://doi.org/10.1016/S0007-1536(69)80160-9
Větrovský, T., Morais, D., Kohout, P., Lepinay, C., Algora, C., Awokunle Hollá, S., .. . Baldrian, P. (2020). GlobalFungi, a global database of fungal occurrences from high-throughput-sequencing metabarcoding studies. Scientific Data, 7(1), 228. https://doi.org/10.1038/s41597-020-0567-7
White, T. J., Bruns, T. D., Lee, S., \& Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, \& T. J. White (Eds.), PCR protocols: a guide to methods and applications (pp. 315-322). San Diego, California, USA: Academic Press.
Wijesinghe, S., Samarakoon, M., Camporesi, E., Hyde, K., \& Jones, E. (2023). Over the footprints of Italian mycology with emphasis on plant-associated Ascomycota. [Journal of Fungal Biology]. Current Research in Environmental \& Applied Mycology, 13, 162-276.

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## Elektronischer Supplement

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Table of contents - Electronic Supplementary Material (ESM)
Supplementary Materials 1: Species and sequences of ITS and LSU rDNA, RPB2 and EF1- $\alpha$ with their accession numbers that were obtained from GenBank and used to build the dataset.

Supplementary Materials 2: Alignment of dataset consisting of ITS and LSU rDNA, RPB2 and EF1- $\alpha$ sequences.

