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Resurrection of *Cortinarius coalescens*: taxonomy, chemistry, and ecology

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Abstract

Cortinarius coalescens Kärcher & Seibt is a rare European species of the subgenus *Phlegmacium*, section *Phlegmacioides*, neglected in recent molecular studies. New primers (CortF and CortR) designed for species in the section *Phlegmacioides* allowed to obtain ITS rDNA sequence data from the holotype collection of *C. coalescens*; according to the results, this epithet has priority over *C. crassorum* Rob. Henry ex Rob. Henry, *C. pardinus* Reumaux, and *C. parargutus* Bidaud, Moëgne-Locc. & Reumaux. Morphological and ecological observations on recent collections of *C. coalescens* from the Czech Republic in comparison with the co-occurring *C. largus* are discussed. Nomenclatural and taxonomic comments on *C. tomentosus* Rob. Henry, *C. balteatotomentosus* Rob. Henry, and *C. subtomentosus* Reumaux are also provided. So far, *C. coalescens* is known with certainty from Germany, France, and the Czech Republic, where it grows in deciduous forests on acid to neutral soils. Arsenic and its compounds were determined in *C. coalescens* and related species of the section *Phlegmacioides*: *C. largus*, *C. pseudodaulnoyae*, and *C. varicolor*. Total arsenic concentrations were in the range 3.6–30.2 mg kg⁻¹ (dry matter) and arsenobetaine was the major arsenic compound.

Keywords

Cortinariaceae; Phlegmacioides clade; Bioaccumulation; Arsenic; Arsenobetaine; Soil

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Introduction

In late summer seasons of 2005, 2009, and 2010, the first author of this paper collected a *Cortinarius* species of the subgenus *Phlegmacium* with a conspicuously tomentose cap. The collections were found in deciduous forest plantations at two distant sites in Central Bohemia, Czech Republic, on rather acid bedrock. The specific 30% KOH reaction with a yellow ring on flesh indicated their affinity to the section *Phlegmacioides* (Brandrud 1998; Moser 1961). According to their ITS rDNA sequences, both collections were identical but there was no positive match in the GenBank and UNITE databases. The use of keys to European *Cortinarius* species published in Horak (2005) and Knudsen and Vesterholt (2012) did not result in any satisfactory identification but further literature search led to *C. pardinus* Reumaux, of which the color drawing (Bidaud et al. 1995, Plate 166) agreed well with the appearance of the Czech collections. Later on, the ITS rDNA sequence matched perfectly with that of the holotype of *C. pardinus* published by Liimatainen et al. (2014). However, Brandrud (1998) considered this species synonymous with *C. coalescens* Kärcher & Seibt, which was covered by neither the type study of Liimatainen et al. (2014) nor the identification keys. The question of the correct name of this species hence remained unresolved. Chemical analyses of the Czech collections revealed elevated levels of several trace elements in fruit bodies, particularly arsenic, which as an element occurring in both inorganic and organic forms in mushrooms (Nearing et al. 2014). The accumulation and speciation of arsenic in macrofungi depends on particular fungal species/genus (Šlejkovec et al. 1997) and might be of chemotaxonomic importance, but no data on arsenicals in *Cortinarius* spp. have ever been reported. Information on the arsenic speciation in various organisms is crucial for understanding the elements geobiochemical cycling and its role in the environment. We have, therefore, attempted to obtain molecular data from the holotype of *C. coalescens* to clarify its affinity to *C. pardinus* and solve its taxonomy. Furthermore, we have investigated arsenic accumulation and speciation in fruit bodies of the Czech collections and also in several closely related *Cortinarius* species of the *Phlegmacioides* clade.

Materials and methods

Collections studied

For the list of studied collections, see Table 1, herbarium acronyms are given according to Thiers (2017). Czech collections of *C. coalescens*, *C. largus*, and *C. varicolor* were sampled and documented fresh in the field; herbarium specimens are deposited at CB, PRM, and FR. The rest of the collections was studied on loan from the herbaria FR, G, O, PRM, and TUB.

Basidiospores of mature basidiomes (from lamellae in herbarium specimens) were measured; the statistical analysis is based on the measurement of 30–50 spores per collection. Minimum and maximum length/width values of spore size are given in brackets and represent the 5th and 95th percentiles, respectively. Spore length/width quotients (Q-values) are presented as the 5th percentile, median, and 95th percentile, respectively. Spore dimensions were measured on pictures taken with a Canon PowerShot A650 IS digital camera connected to a Zeiss Primo Star LED microscope (Full Köhler); a Zeiss Plan-Achromat 100×/1.25 oil immersion objective was used. Measurements on screen and

estimations were carried out using the AxioVision 4.8.1 software. Scanning electron micrographs of the basidiospores were taken by scanning electron microscope TESCAN Vega3 XMU on lamellae sputter coated in gold.

Phylogenetic study

DNA was extracted according to Borovi ka et al. (2015a). ITS rDNA (ITS1–5.8S–ITS2) and LSU regions were amplified by the polymerase chain reaction (PCR) using the primer pairs ITS1F–ITS4 (or ITS4B) and NL1–NL4, respectively, under the PCR regime as described by Borovi ka et al. (2011). Additional molecular markers EF-1 α (translation elongation factor 1 α) and *rpb2* (RNA polymerase II second largest subunit) were amplified and sequenced according to Borovi ka et al. (2012, 2015b). The obtained amplicons were purified with isopropanol and sequenced at Macrogen Europe. Sequences were edited in BioEdit (Hall 1999) and submitted to the EMBL Nucleotide Sequence Database (EMBL-Bank).

In both holotype and isotype collections of *C. coalescens*, ITS rDNA sequencing repeatedly failed. Moreover, both isotypes were heavily contaminated by conidial fungi (Fig. 1). Thus, we designed primers that selectively amplify the ITS rDNA region of *Cortinarius* spp. belonging to the section *Phlegmacioides*. These primers have been designed based on motifs present in sequences of *C. coalescens* (LT827035), *C. patibilis* (AY669543), *C. eliae* (AY669542, as *C. largus*), *C. largus* (AY669552 and AY174794, both as *C. coalescens*), *C. balteatoalbus* (AY669517), *C. spadicellus* (AY669539), *C. varicolor* (AY174796, UDB011729), *C. vacciniophilus* (AY669518), *C. balteatocumatilis* (AY174801), and *C. balteatus* (AY669526). Sequences of *Penicillium daleae* (JN198424), *Neurospora crassa* (M13906), and *Xerocomellus rubellus* (JX030209) were used as the outgroup. The resulting primers CortF (5′-CCTTTGTGCCTATA AACCTATAC-3′) and CortR (5′-GTATAGGTTTATAG GCACAAAGG-3′) were used in combination with universal primers ITS4 and ITS1F, respectively, and amplified 5.8S rRNA, ITS1, and ITS2 regions.

Based on BLASTn searches and previous studies (Liimatainen et al. 2014; Garnica et al. 2016), ITS rDNA sequences of the *Phlegmacioides* clade were downloaded from the databases GenBank (Bidaud and Bellanger 2016; Garnica et al. 2003, 2005, 2016; Liimatainen et al. 2014; Shao et al. 2016) and UNITE (sequence UDB001081, direct submission without a reference). The partial sequence (ITS2) of the holotype of *Cortinarius crassorum* Rob. Henry ex Rob. Henry was added from an unpublished type study (Frøslev et al., in prep.). The final dataset including newly generated sequences and the additional downloaded ones were composed of 60 ingroup sequences (59 from Europe and one from North America) representing the *Phlegmacioides* clade. *Cortinarius russus* was selected for the outgroup, which, based on morphological concept, belonged to the section *Phlegmacioides* (Brandrud 1998) but phylogenetically seems to be more distantly related (Liimatainen et al. 2014; Garnica et al. 2016).

Sequences were aligned with MAFFT online version 7 (Katoh and Toh 2008) using the E-INS-i strategy (Katoh et al. 2005) with default settings. SeaView (Gouy et al. 2010) was used to adjust the alignment manually. Gaps were treated as missing data. Bayesian inference (BI) analysis was carried out with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) applying the GTR + G substitution model for the dataset. Four incrementally heated

simultaneous Markov chain Monte Carlo (MCMC) were run over 10 million generations with trees sampled every 1000 generations and an initial burn-in of 3000 trees (30%). For the remaining trees, a 50% majority rule consensus tree was computed. In addition, RAxML analysis (Stamatakis 2014) was performed with raxmlGUI (Silvestro and Michalak 2012) using rapid bootstrap analysis with 1000 replicates and the GTRGAMMA substitution model. Bayesian (BI) posterior probabilities (PP) >0.90 and maximum likelihood (ML) bootstrap values (BS) >70% were considered sufficient for support. For tree editing and visualization, MEGA6 (Tamura et al. 2013) was used. The topologies of phylogenetic trees from the Bayesian and ML analyses were congruent, and the ML tree is illustrated (Fig. 4).

Soil survey and analyses

Soil types, soil properties, and arsenic distribution in soil profiles were investigated at both sites of *C. coalescens* in the Czech Republic: Český Šternberk and Bělá. Typical soil profiles were chosen on the basis of soil survey. Horizons designation followed the scheme of Jahn et al. (2006). Soils were classified according to the IUSS Working Group WRB (2015). Except O horizons, all samples were collected using a single gouge auger. The pH values were potentiometrically measured (van Reeuwijk 2002) in distilled water and in 1 M KCl with a SenTix electrode using a soil:solution ratio of 1:2.5. The pH values were assessed by the scale of Baize (1993). The soil profile of Český Šternberk was located at the altitude of 369 m with coordinates 49°49′05.2″N and 14°56′02.8″E. The soil sequence of Bělá was sampled at the altitude of 377 m, with coordinates 50°02′28.0″N and 13°59′02.1″E.

Total arsenic content and its mobility were investigated at both sites. Soil samples from particular soil horizons were dried at room temperature and sieved through a 2-mm stainless steel mesh. A representative part of each sample was milled in an agate mill (Fritsch, Germany) and aliquots of approximately 250 mg were used for the determination of total element contents by instrumental neutron activation analysis (INAA) with epithermal neutrons (Cejpková et al. 2016). Certified standard reference material SRM 2711a (Montana II Soil, NIST, USA) was used as the control.

In addition, we sequentially analyzed two arsenic soil fractions (“non-specifically sorbed” and “specifically adsorbed”), which are considered the most bioavailable (Wenzel et al. 2001). In 50-mL centrifuge tubes, 1.600 ± 0.005 g of milled soil with added 40 mL 0.05 M $(\text{NH}_4)_2\text{SO}_4$ (Suprapur, Merck) was shaken for 4 h in an overhead shaker (Reax 2, Heidolph) at room temperature. After centrifugation, the supernatant liquid was carefully decanted, filtered (0.45- μm ProFill PTFE filters, Fisherbrand) and promptly analyzed by ICPSFMS (Element2, Thermo Scientific). Then, 40 mL of 0.05 M $\text{NH}_4\text{H}_2\text{PO}_4$ (TraceSELECT, Fluka) was added to the residue and samples were shaken for 16 h and further processed and analyzed as described above. Procedural blank was processed in the same way.

The bioaccumulation factor (BAF, Falandysz and Borovička 2013) was calculated as the ratio of the chemical concentration in fruit bodies and sum of the most bioavailable arsenic fractions in topsoil horizons (Ah, Ahg). Arsenic concentrations in soils reported in this study are related to dry matter.

Chemical analysis of fungi

In total, seven *Cortinarius* samples (*coalescens*, *largus*, *varicolor*, and *pseudodaulnoyae*) were investigated for their concentrations of arsenic and 33 other elements, as well as for their arsenic speciation; element and arsenic species concentrations reported in this study are related to dry matter. Dried pulverized samples were digested in triplicate with nitric acid (p.a., 65%, Carl Roth, Karlsruhe, Germany) in a microwave-assisted autoclave, and then the total arsenic concentrations were determined with an inductively coupled plasma triple quadrupole mass spectrometer (ICPQQMS, Agilent 8800, Agilent Technologies, Waldbronn, Germany). Sample preparation and measurement were carried out according to Ertl et al. (2016). Ultrapure water (Merck Millipore, Darmstadt, Germany) was used for the preparation of all solutions. Arsenic was measured in oxygen reaction mode at m/z 75 \rightarrow 91, the other elements according to Ertl et al. (2016). Be, Ge, In, and Lu were used as internal standards. The certified standard reference material SRM 1573a (Tomato Leaves, NIST, USA) was used for quality control.

For arsenic speciation analysis, each sample was prepared in triplicate. About 50 mg (weighed to 0.1 mg) was extracted with 2 mL of ultrapure water, sonicated in an ultrasonic bath for 15 min, centrifuged at 5500 \times g, and then filtered through 0.2- μ m Nylon® syringe filters (Marcherey-Nagel, Düren, Germany). Then, the extracts were analyzed with high performance liquid chromatography (HPLC, anion- and also cation-exchange chromatography, HPLC 1200, Agilent Technologies) coupled to an ICPQQMS, according to Scheer et al. (2012). Solutions of arsenobetaine (AB), dimethylarsinic acid (DMA), methylarsonic acid (MA), arsenate [As (V)], trimethylarsine oxide (TMAO), arsenocholine (AC), and tetramethylarsonium ion (TETRA) were used for quantification (0.05–100 μ g As/L). Again, the arsenic signal was recorded at m/z 75 \rightarrow 91. Further on, hydrogen peroxide was added to the extracts to a final concentration of 10% v/v. The mixtures were shaken, put in an oven for one hour at 45 °C and then also analyzed with HPLC-ICPQQMS, like the pure extracts before.

Results

Taxonomy

Cortinarius coalescens Kärcher & Seibt, Z. Mykol. 54 (1): 78 (April 1988), MB#124551.

= *Cortinarius crassorum* Rob. Henry ex Rob. Henry, Doc. Mycol. 19 (73): 66 (June 1988), MB#134934.

= *Cortinarius pardinus* Reumaux in Bidaud et al., Atlas des Cortinaires 7: 230 (1995), MB#447133.

= *Cortinarius parargutus* Bidaud, Moënné-Loccoz & Reumaux, Atlas des Cortinaires 9: 372 (1999), MB#820859.

Icons: Kärcher and Seibt (1988, Plate 1, a–c), Kärcher and Seibt (1991, Figs. 1 and 2), Bidaud et al. (1995, Plate 166, as *C. pardinus*), Bidaud et al. (1999, Plate 233, as *C.*

parargutus). Color photographs of Czech collections of *C. coalescens* are presented in Fig. 2 and scanning electron micrographs of basidiospores in Fig. 3.

With the aid of the newly designed *Cortinarius* primers, we were able to sequence the holotype and two isotype collections of *C. coalescens* (Table 1, Fig. 4). Altogether, 16 ITS rDNA sequences of *C. coalescens* and morphologically alike species were generated in this study (Table 1). Furthermore, sequences of *rpb2* (LT827049) and EF-1 α (LT827051) were obtained from *C. coalescens* (PRM 923845) and are available for future molecular studies.

In both BI and ML analyses, the sequences of *C. coalescens* as interpreted here (based on the study of the type specimen) forms a strongly supported (PP = 1.00/BS = 100%) terminal clade. The species clusters together with the North European *C. patibilis* and with a North American species originally identified as *C. cf. coalescens* (Shao et al. 2016); however, the relationship towards these two species was less supported (BI = 0.94, ML = 71%). The maximum intraspecific ITS variability of *C. coalescens* is low (0.3%), while the minimum interspecific ITS variability is 3% towards *C. patibilis*, which is the nearest known species, differing by 21 substitution and indel positions from *C. coalescens*. The relationship of *C. coalescens* with other species in the Phlegmacioides clade could not be reconstructed in the present study using a single locus (ITS) only.

Two sequences published earlier as *C. coalescens* (Garnica et al. 2003, 2005) belonged to the morphologically and ecologically very similar *C. largus*, which is, however, more distantly related based on the phylogenetic analyses. For the species we here refer to as *C. coalescens*, the binomial *C. pardinus* Reumaux (= *C. parargutus*) was applied in the type study of Liimatainen et al. (2014) as the oldest name having ITS rDNA sequence data from the type specimen at that time. Our extensive study, however, revealed that the holotypes of *C. coalescens* and *C. crassorum* (both were described in 1988) had identical ITS rDNA sequences to *C. pardinus* and *C. parargutus* types, thus leaving the two latter synonyms of *C. coalescens*. *Cortinarius crassorum* appeared to be synonymous as well, because its effective publication was dated 2 months later than that of *C. coalescens* (June vs. April).

Soil survey and analyses

Data on soils are summarized in Table 2. At Český Šternberk, the soil profile showed the following succession of horizons: O-Ah-ABw-Bw-Cr. Without the O horizon (2 cm thick), the soil thickness was 76 cm. This site is covered by Cambisol, which is characterized by weathering of the parent material and by the development of the Bw horizon. Cambisol from the Vrábov site has a weakly acid reaction and an acid one in the ABw horizon. The soil profile at Bělá showed the following succession of horizons: O-Ahg-ABg-Bg-Cg. Without the 2-cm-thick O horizon, the soil thickness was 70 cm. The soil cover was represented by Stagnosol, which shows periodically reducing conditions resulting in stagnic properties. Acid reaction was observed in the Ahg and ABg horizons and weakly acid in the Bg and Cg horizons.

At Český Šternberk, the total arsenic concentrations in soils decrease with depth from 5.75 mg kg⁻¹ (Ah horizon) to 2.72 mg kg⁻¹ (Cr horizon), and this trend is seen also in both mobile arsenic fractions. At Bělá, the total arsenic levels are considerably higher (14.2–

17.0 mg kg⁻¹) but rather uniformly distributed among horizons. In the Bnon-specifically sorbed arsenic fraction[^], arsenic mobility decreases with depth. This is in contrast with what is seen in the “specifically adsorbed” arsenic fraction, which is the highest at the bottom of the soil profile.

Arsenic in fruit bodies

The total arsenic concentrations in the fruit bodies ranged from 3.6 to 30.2 mg kg⁻¹, with a mean of 13 ± 8 mg kg⁻¹. The results of all individual samples are given in Table 4. The results for the other 33 elements can be found in Supplementary Table S1. It is worth mentioning that the concentrations of silver, cadmium, and mercury were higher than in most non-accumulating macrofungi from pristine sites. For silver, between 10 and 41 mg kg⁻¹ were found in the samples. The cadmium concentrations were only slightly lower, namely 2.3–20 mg kg⁻¹, and 0.8–4.9 mg kg⁻¹ of mercury was present in the samples.

Overall, 80 ± 20% (range: 48–93%) of the arsenic was extractable with water. The column recoveries were between 80 and 100%. Most of the arsenic was present as AB. The investigated samples comprised 60 ± 10% of the total arsenic in this form, which is 92 ± 5% of all arsenic species that could be detected in the extracts with the chosen methods. Besides this, significant amounts of DMA, TMAO, AC, and TETRA were found as well (see Fig. 5 and Table 4). The limit of detection was 0.002 mg kg⁻¹ for all arsenic species. MA and inorganic arsenic were not detected in any of the extracts. Traces of other arsenic species, which could not be identified until now, were also present (see chromatograms in Fig. 6). Two of them were found consistently in all of the samples. Unknown 1 was eluting from the anion-exchange column after 4.8 min, well distinguishable from MA (4.4 min). Unknown 2 was detected in the chromatograms of the cation-exchange method, with a retention time of 3.9 min, between TMAO and AC. Two samples (*C. varicolor* and *C. pseudodaulnoyae*) also contained significant concentrations of another arsenic compound (“unknown 3”), which eluted from the cation-exchange column after 2.5 min, between AB and TMAO. All of the unknown species were stable upon oxidation with hydrogen peroxide.

Discussion

Taxonomy and ecology of *C. coalescens*

According to the phylogenetic analyses, *C. coalescens* is a sister species of *C. patibilis*, a similarly looking species which is, however, associated with conifers and of a North American species (found in Massachusetts, USA), which was identified as *C. cf. coalescens*. The color photograph of the latter undoubtedly showed a typical member of the section *Phlegmacioides* (Shao et al. 2016). At its type locality in Germany, *C. coalescens* grows under *Castanea sativa* on neutral soil (Kärcher and Seibt 1988). According to this study, it can also be associated with other deciduous trees (*Quercus*, *Tilia*/*Carpinus*) and topsoils with weakly acid to acid reaction. The current distribution of this species covers Germany, Czech Republic, and France. The Norwegian collection (O 125961) reported in Brandrud (1998) turned out to be *C. largus* after ITS rDNA sequencing (GenBank AY669552) as well as the Spanish collection (GenBank MF589755) reported by Kärcher and Seibt (1988).

With regard to morphological characters, it was observed that *C. coalescens* can have a violaceous cap margin (Fig. 2b, left specimen), which is probably a rare feature reported for neither type collections of *C. coalescens* nor synonymous taxa. However, the identity of this aberrant fruit body was confirmed by ITS rDNA sequencing (LT827036). The possible occurrence of violaceous tinges on cap further complicates field identification of *C. coalescens*, especially distinguishing it from *C. largus*. The latter species is common in Central Europe, but it also grows under deciduous trees and may be similarly colored. Unfortunately, there are no significant differences in either spore size (Table 3) or spore ornamentation (Fig. 3). At Český Šternberk, *C. largus* (Fig. 7) was found growing directly at the same site where *C. coalescens* was collected. The only apparent character separating both species in the field was the tomentose cap (at least near the margin) in *C. coalescens*, especially when dry (Fig. 2a, b). However, *C. largus* is a rather variable species and probably only molecular markers enable reliable separation from *C. coalescens*.

The problem of *C. tomentosus* and *C. subtomentosus*

In the protologue of *C. pardinus*, Reumaux highlighted its close affinity to *C. tomentosus* Rob. Henry ex Rob. Henry (Bidaud et al. 1995). This would suggest that *C. tomentosus* might be the correct name for *C. coalescens*. However, *C. tomentosus* was not discussed in the study by Liimatainen et al. (2014) and has a highly problematical nomenclatural history.

The first description of *C. tomentosus* (Henry in Kühner and Romagnesi 1953) was invalid, as no Latin diagnosis was included. In 1958, the name was published again but with no indication of the type (Henry 1958). A later publication of *C. tomentosus* (Henry 1985a) was invalid again (no Latin diagnosis provided).

Notably, the sequence KF732461 (as *C. pardinus*) obtained from a specimen originally labeled as *C. tomentosus* from the herbarium of R. Henry in PC (also indicated as the paratype of *C. tomentosus* in Henry 1985a) matches that of *C. coalescens*. The valid description of *C. tomentosus* is dated to December 1985 (Henry 1985b), but *C. tomentosus* became an illegitimate name because of having the same holotype (Henry 306) as *Cortinarius balteatotomentosus* Rob. Henry ex Rob. Henry (1985a). According to Liimatainen et al. (2014), *C. balteatotomentosus* is a synonym of *C. balteatus*. Thus, *C. tomentosus* falls within the nomenclatural and taxonomic history of *C. balteatus* as follows:

***Cortinarius balteatus* (Fr.) Fr., Epicr. Syst. Mycol.: 257. 1838**

≡ *Agaricus balteatus* Fr., Observ. Mycol. 2: 138. 1818. [basonym].

= *Cortinarius subbalteatus* Kühner, Bull. Mens. Soc. Linn. Lyon 24, 2: 40. 1955.

= *Cortinarius balteatotomentosus* Rob. Henry ex Rob. Henry, Bull. Soc. Mycol. France 101 (1): 4. April 1985.

= *Cortinarius tomentosus* Rob. Henry ex Rob. Henry, Doc. Mycol. 16 (61): 24. December 1985. [*nom. illeg.*, Art. 52.1: holotype of *Cortinarius balteatotomentosus* included].

= *Cortinarius subopimus* Bidaud, Atlas des Cortinaires 7: 231. 1995.

–*Cortinarius tomentosus* Rob. Henry in Kühner & Romagnesi, Fl. Analyt. Champ. Supér. (Paris): 282. 1953. [*nom. nud.*, Art. 39.1: no Latin description or diagnosis].

–*Cortinarius balteatotomentosus* Rob. Henry, Bull. Soc. Mycol. France 74: 303. 1958. [*nom. inval.*, Art. 40.1: no indication of type].

–*Cortinarius tomentosus* Rob. Henry, Bull. Soc. Mycol. France 101 (1): 4. 1985. [*nom. nud.*, Art. 39.1: no Latin description or diagnosis].

According to its description, *Cortinarius subtomentosus* Reumaux (Reumaux 1988) would also possibly represent the same species as *C. coalescens*. Curiously enough, both taxa were described in April 1988. However, the partial ITS rDNA sequence obtained from the holotype of *C. subtomentosus* is distinct and matches that of *C. largus* (Fig. 4).

Arsenic in *Cortinarius* species and underlying soils

High concentrations of toxic metals and metalloids in macrofungal fruit bodies are often found in samples from pristine areas. The biological importance of the accumulation process itself is unknown and element accumulation is often characteristic for particular fungal species or genera (Falandysz and Borovička 2013). Moreover, macrofungi are able to discriminate between the homologous arsenic and antimony (Borovička et al. 2006), and preferred metals are selectively accumulated regardless of their bioavailability in the underlying soil (Kubrová et al. 2014). In the case of arsenic, some macrofungi can take up even more than 1000 mg As kg⁻¹ (Falandysz and Rizal 2016).

Arsenic concentrations in soils found at both sites of *C. coalescens* fall within the range of natural levels (compare with Kabata-Pendias 2011). Arsenic contents of *C. coalescens* from Bělá (samples B-841 and B-841b; 13.6 and 30.2 mg kg⁻¹) were higher than those detected in collections from Český Šternberk (samples B-839 and B-840; 3.6 and 11.7 mg kg⁻¹), which might be caused by considerably lower arsenic soil content at the latter site. When compared with soils, arsenic is accumulated in fruit bodies, especially when related to the most bioavailable arsenic soil fractions; the BAF ranges between 11 and 51. Arsenic concentrations in additional *Cortinarius* species of the Phlegmacioides clade (Table 4) were in the range of 7.4–13.2 mg kg⁻¹. Reported concentrations for common species of other ectomycorrhizal genera like *Russula*, *Lactarius*, *Amanita*, and *Boletus* s.l. are mostly below 5 mg kg⁻¹ (Kala 2010; Falandysz and Rizal 2016), and the contents found in *Cortinarius* species of the subgenus *Phlegmacium* can, thus, be considered elevated (see also Borovička et al. 2015a).

Besides arsenic, the concentrations of silver, cadmium, and mercury were interesting as well. With 30 ± 10 mg kg⁻¹ of silver, the investigated samples are still far away from fungi like *Amanita strobiliformis*, which are able to accumulate more than 1000 mg Ag kg⁻¹ (Borovička et al. 2007). Still, when compared to normal samples from pristine areas (Borovička et al. 2010), the concentrations reported here are clearly elevated. Also, in the case of cadmium, with 2.3–20 mg kg⁻¹, the concentrations are not new record holders, but on the upper end of the range for common macrofungi (Kala and Svoboda 2000). Finally,

the mercury concentrations (0.8–4.9 mg kg⁻¹) can be considered as slightly increased too (Kala and Svoboda 2000).

Qualitatively, the arsenic speciation appeared to be very similar in all extracts, which suggests no chemotaxonomic importance of arsenicals within the Phlegmacioides clade. The major arsenic compound was AB, besides small quantities of TMAO, TETRA, AC, and DMA. Interestingly, all of the four latter species were present in quite similar amounts. This speciation profile has rarely been found in nature. In the article by Nearing, Koch and Reimer, where they investigated arsenic compounds of 73 mushroom samples, there are only three macrofungi where the arsenic speciation is comparable to our present findings, but in different proportions: *Coprinopsis atramentaria*, *Hebeloma velutipes*, and *Boletus edulis* (Nearing et al. 2014).

What is also astonishing is the absence of inorganic arsenic (typically the main arsenic species in soils) and MA, which is the alleged first product of the biomethylation pathway of inorganic arsenic in microbiota (Bentley and Chasteen 2002; Cullen 2014). The major compound AB accounted for 65–88% of the extracted arsenic. The origin of methylated arsenicals in macrofungi is still unclear, as methylation of arsenic has not been demonstrated in vitro in most of the tested macrofungal genera (Nearing et al. 2015). Therefore, it is unclear whether AB is formed during the vegetative or reproductive life stages of fungi, or by surrounding microbial community (Nearing et al. 2016). Investigation of this phenomenon and identification of the detected unknown arsenic species was beyond the scope of this study but will be attempted in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Sequenced isotype of *Cortinarius coalescens* (FR 0247030) contaminated by conidial fungi



Fig. 2.

a *Cortinarius coalescens* found at Český Šternberk (21 August 2005, not documented by herbarium specimen). **b** *Cortinarius coalescens* collected at Bělá (PRM 923847). **c, d** *Cortinarius coalescens* collected at Český Šternberk (PRM 923845, duplicate FR 0070025). All photos by Jan Borovička

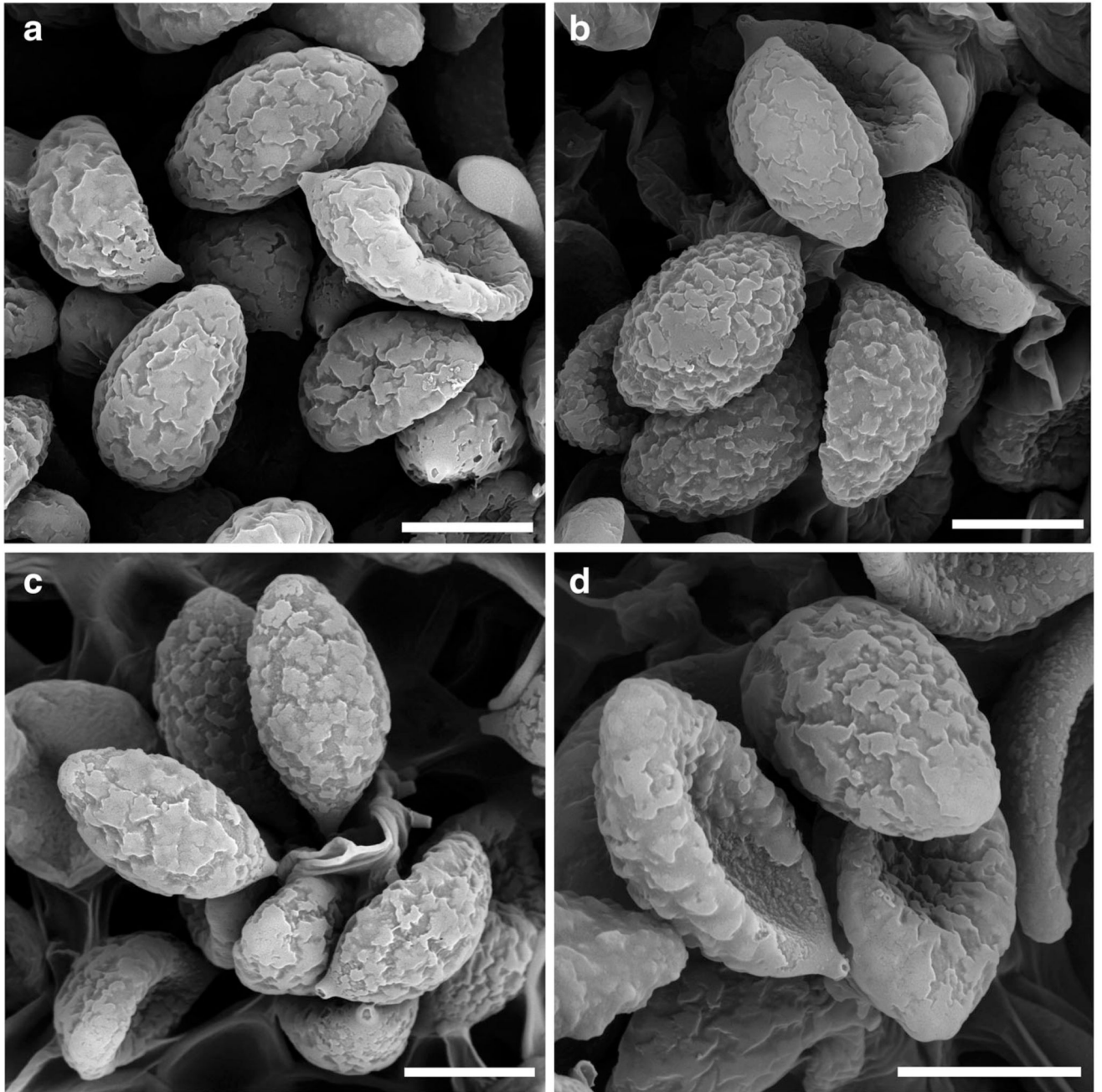


Fig. 3. Basidiospores of *Cortinarius coalescens* and *Cortinarius largus* under a scanning electron microscope (scale bars = 5 μm). **a** *Cortinarius coalescens* (FR 0070020, holotype). **b** *Cortinarius coalescens* (PRM 923845). **c** *Cortinarius largus* (FR 0070030). **d** *Cortinarius largus* (PRM 923846)

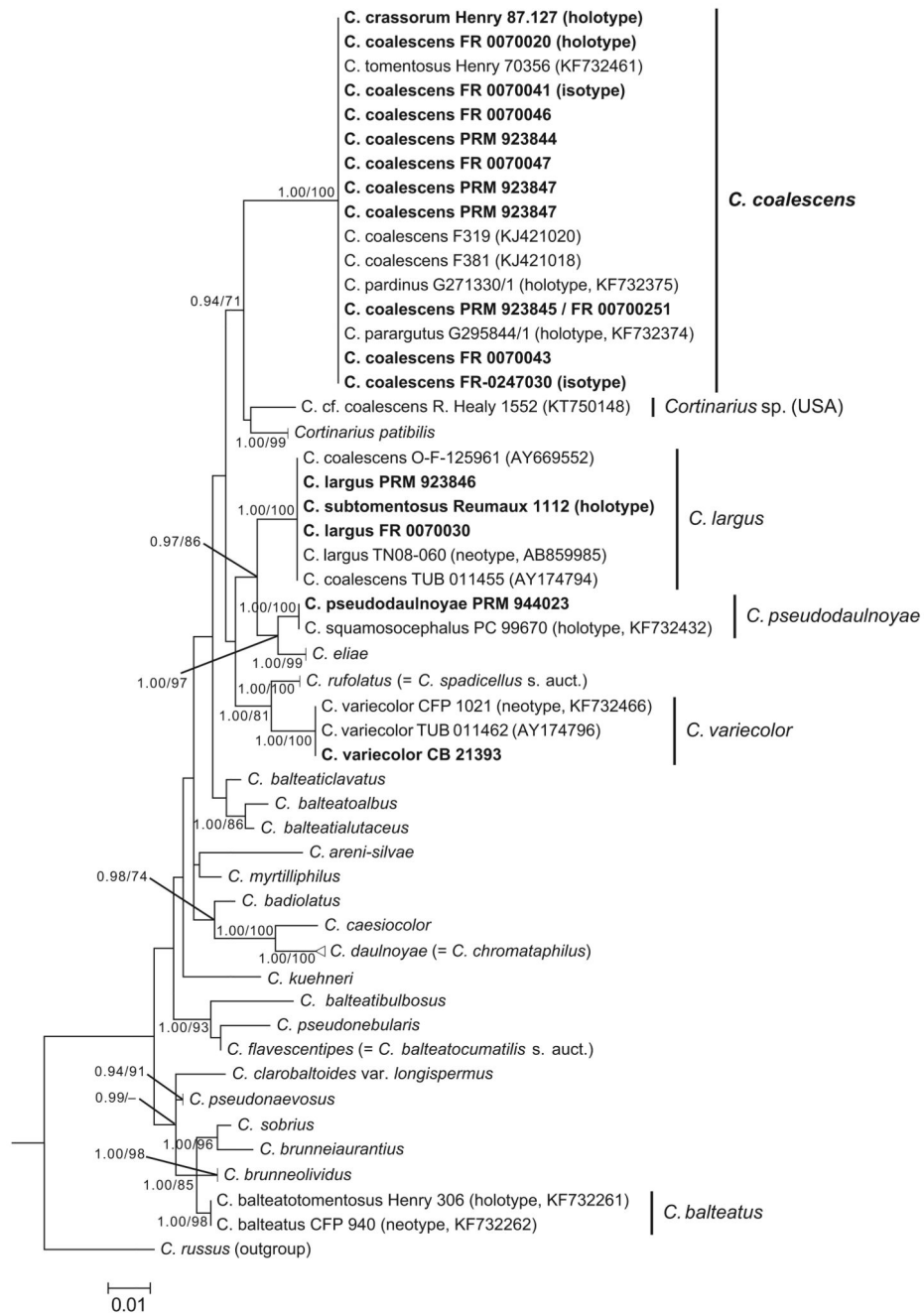


Fig. 4. Maximum likelihood phylogeny of the Phlegmacioides clade inferred from ITS rDNA sequence data. Branch support is indicated only when the Bayesian posterior probability and maximum likelihood bootstrap values are >0.90 or >70%, respectively. Sequences of species not being in the focus of this study are shown as compressed clades. Newly generated sequences are highlighted in **bold**. The bar indicates 0.01 expected change per site per branch

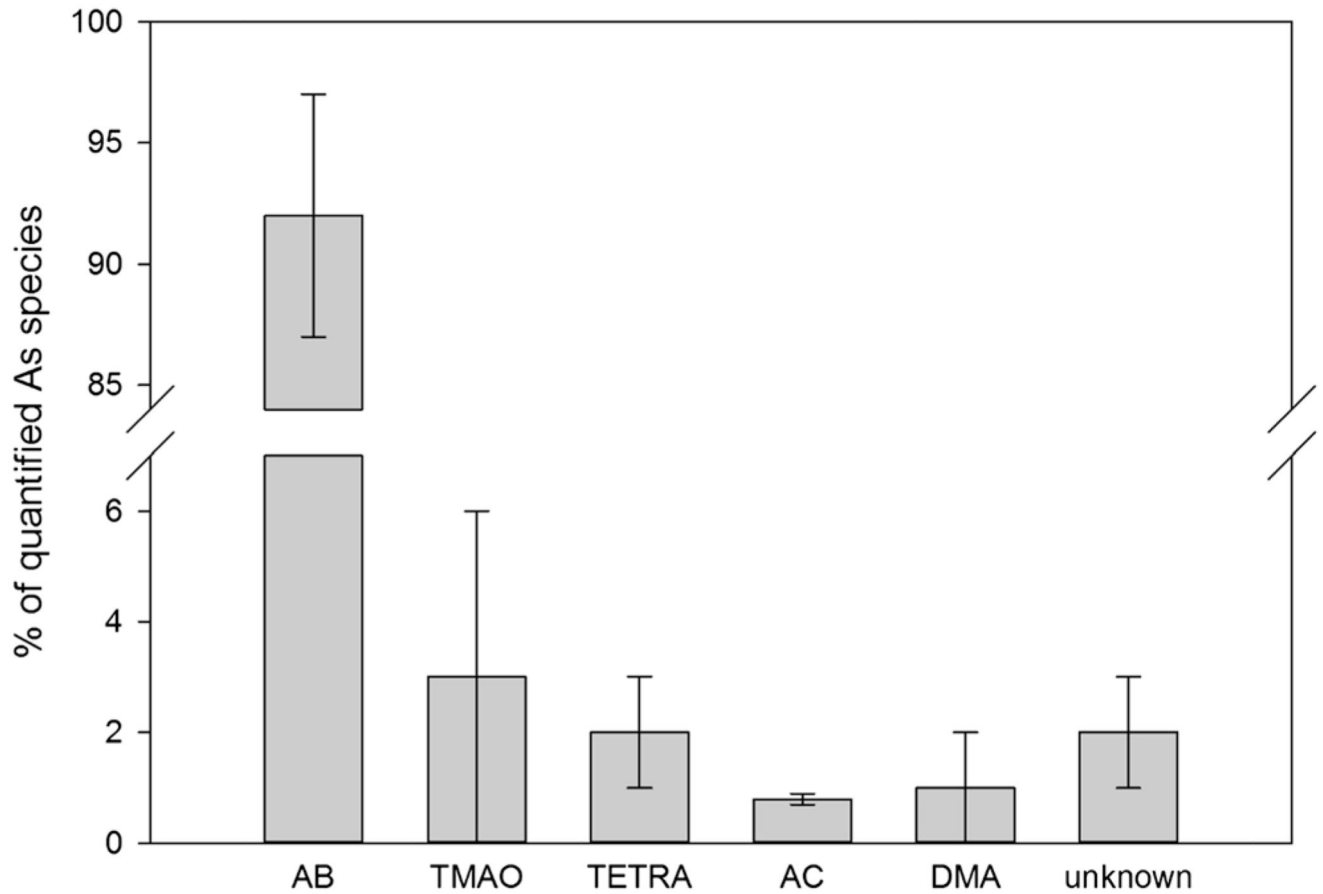


Fig. 5. Arsenic species in extracts of investigated *Cortinarius* spp. (in % of all detected arsenic species). AB: arsenobetaine, TMAO: trimethylarsine oxide, TETRA: tetramethylarsonium ion, AC: arsenocholine, DMA: dimethylarsinic acid

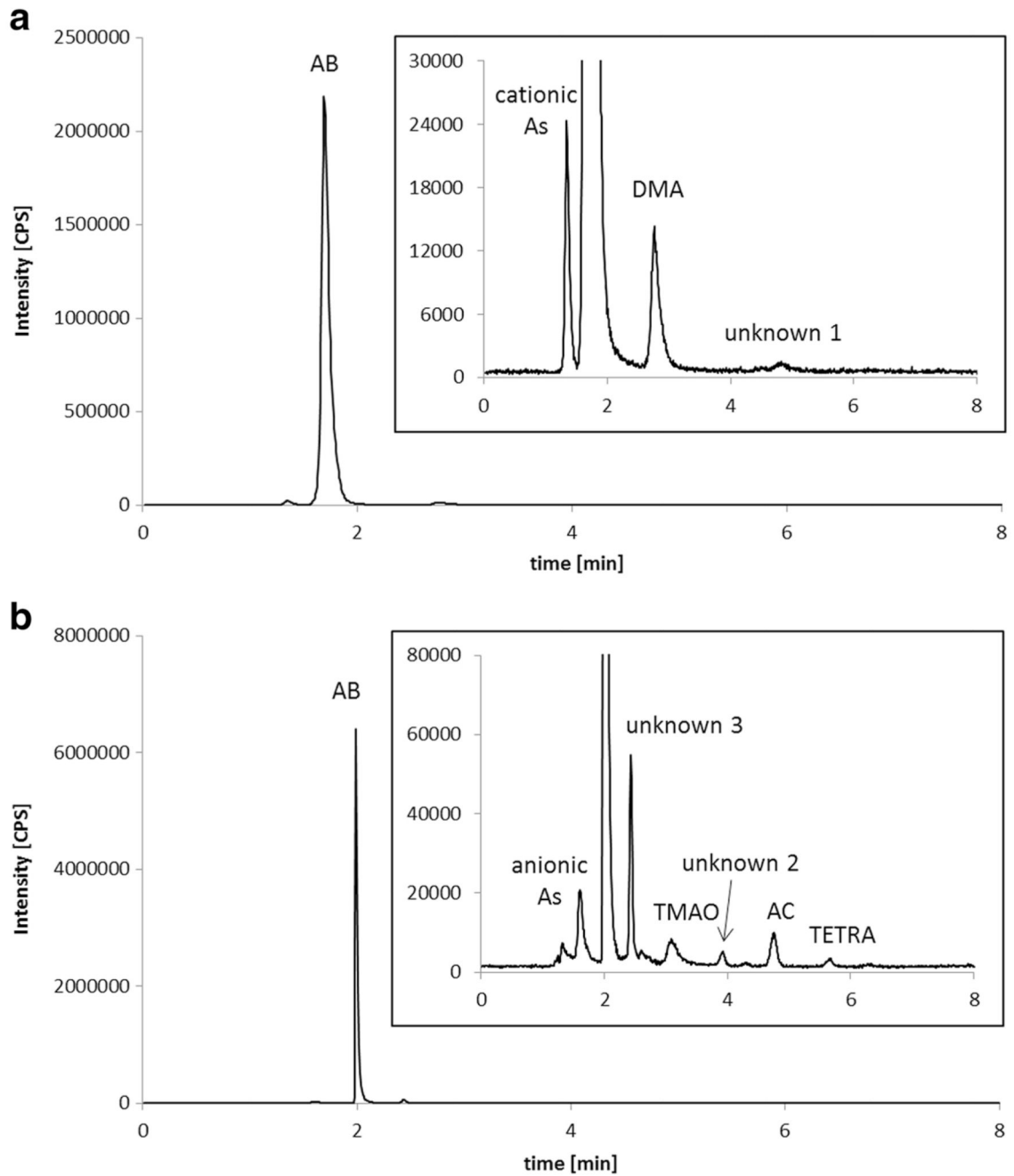


Fig. 6. Whole chromatograms and details of one extract of *Cortinarius pseudodaulnoyae* (ASP-70).
a Anion-exchange conditions. **b** Cation-exchange conditions



Fig. 7.
Cortinarius largus from český Šternberk (PRM 923846, B-503b), where it shares the habitat with *C. coalescens*. Photo by Jan Borovička

Table 1
***Cortinarius coalescens* and related *Cortinarius* collections under study**

ID	<i>Cortinarius</i>	Site, date	Host trees on site	Herbarium voucher	EMBL-Bank
X-57	<i>coalescens</i>	GER, Kronberg, 13.IX.1977	<i>Castanea sativa</i>	FR 0070020, holotype	LT827038
X-103	<i>coalescens</i>	GER, Kronberg, 31.VIII.2014	<i>Castanea sativa</i>	FR 0070047	LT827041
X-104	<i>coalescens</i>	GER, Kronberg, 23.IX.1995	<i>Castanea sativa</i>	FR 0070043	LT827048
X-105	<i>coalescens</i>	GER, Kronberg, 22.VIII.2014	<i>Castanea sativa</i>	FR 0070046	LT827040
X-167	<i>coalescens</i>	GER, Kronberg, 13.IX.1977	<i>Castanea sativa</i>	FR-0070041, isotype F300a	LT827042
X-168	<i>coalescens</i>	GER, Kronberg, 13.IX.1977	<i>Castanea sativa</i>	FR-0247030, isotype F300b	LT827039
X-11, B-839	<i>coalescens</i>	CZ, eský Šternberk, 2.VIII.2009	<i>Quercus robur</i>	PRM 923844	LT827035
X-218, B-840	<i>coalescens</i>	CZ, eský Šternberk, 23.VIII.2009	<i>Quercus robur</i>	PRM 923845 (FR-0070025 ^d)	LT827037, LT827049, LT827050, LT827051
X-43, B-841/b	<i>coalescens</i>	CZ, B le -Jin ov, 27.VIII.2010	<i>Carpinus, Tilia</i>	PRM 923847	LT827043
X-219	<i>coalescens</i>	CZ, B le -Jin ov, 27.VIII.2010	<i>Carpinus, Tilia</i>	PRM 923847 (violet cap)	LT827036
-	<i>crassorum</i>	FR	Not reported	PC (Henry 87.127, holotype)	LT855664
X-107	<i>largus</i>	GER, Söllingen (Pfinztal), 23.VIII.2010	<i>Fagus sylvatica</i>	FR 0070030	LT827044
X-217, B-503b	<i>largus</i>	CZ, eský Šternberk, 6.IX.2009	<i>Quercus robur</i>	PRM 923846	LT827045
X-260, ASP-70	<i>pseudodaulnoyae</i>	CZ, Ranšpůrk, 10.X.2013	<i>Carpinus, Tilia, Quercus</i>	PRM 944023	LT827047
K-14, X-358	<i>subtomentosus</i>	FR, French Ardennes, Mount Damion	<i>Carpinus, Quercus, Fagus</i>	Reumaux 1112, holotype ^b	LT844659
X-316, ASP-65	<i>varicolor</i>	CZ, emá v Pošumaví, 14.VIII.2016	<i>Picea abies</i>	CB 21393	LT827046

^aDuplicate of the original collection from PRM

^bIsotype: PRM 945504 (ex herb. Moënne-Loccoz n. 370)

Table 2
Soil characteristics and arsenic contents at Czech sites of *Cortinarius coalescens*

Locality	Depth (cm)	Horizon	pH (H ₂ O)	pH (KCl)	As (total) (mg kg ⁻¹)	As (F1) (μg kg ⁻¹)	As (F2) (μg kg ⁻¹)	As (ΣF) (μg kg ⁻¹)
B le	0–18	Ahg	4.67	3.84	16.2	34.3	550	584
	18–36	ABg	4.64	3.74	14.2	16.1	447	463
	36–50	Bg	5.27	3.75	15.4	8.17	711	719
	50–70	Cg	5.78	4.26	17.0	5.40	1257	1263
. Šternberk	0–11	Ah	6.01	4.92	5.75	29.0	331	360
	11–21	ABw	4.88	3.62	5.05	14.5	217	231
	21–40	Bw	5.20	3.67	3.72	7.91	127	135
	40–76	Cr	6.00	3.84	2.72	3.17	109	112

F1: arsenic fraction extracted by ammonium sulfate; F2: arsenic fraction extracted by ammonium dihydrogen phosphate; ΣF: sum of fractions F1 and F2 Total arsenic determined for SRM 2711a was $104 \pm 2 \text{ mg kg}^{-1}$

Table 3
Investigated collections of *Cortinarius coalescens* and related species with indicated spore size ranges and Q-values

<i>Cortinarius</i>	Collection	Spore size (µm)	Q-value
<i>coalescens</i>	FR 0070020	10.39) 11.09–11.43–11.76 (12.46) × (6.03) 6.23–6.39–6.64 (6.95)	(1.64) 1.71–1.76–1.84 (1.93)
<i>coalescens</i> ^a	G 3432	10.36) 10.86–11.35–12.08 (12.82) × (5.55) 5.89–6.24–6.41 (6.66)	(1.70) 1.76–1.80–1.95 (2.18)
<i>coalescens</i>	PRM 923844	9.63) 10.21–10.63–10.88 (11.9) × (5.53) 5.74–5.98–6.07 (6.30)	(1.60) 1.68–1.80–1.86 (2.01)
<i>coalescens</i>	PRM 923845	9.73) 10.25–10.37–10.95 (11.29) × (5.49) 5.70–5.85–6.05 (6.41)	(1.66) 1.69–1.80–1.89 (1.94)
<i>coalescens</i>	PRM 923847	9.84) 10.23–10.40–10.67 (10.98) × (5.32) 5.62–5.84–6.05 (6.26)	(1.66) 1.72–1.80–1.86 (1.98)
<i>largus</i> ^b	O 125961	10.74) 11.35–11.95–12.71 (13.30) × (5.88) 6.14–6.43–6.64 (7.11)	(1.69) 1.83–1.87–1.93 (2.01)
<i>largus</i> ^c	TUB 011455	10.50) 11.15–11.31–11.74 (12.34) × (5.94) 6.13–6.29–6.59 (6.95)	(1.66) 1.74–1.79–1.82 (1.93)
<i>largus</i> ^d	PRM 945504	9.77) 10.76–11.08–11.72 (12.25) × (5.29) 5.70–5.85–6.06 (6.57)	(1.73) 1.84–1.90–1.95 (2.08)
<i>largus</i>	PRM 923846	10.78) 11.08–11.56–12.16 (12.39) × (5.82) 6.02–6.17–6.35 (6.53)	(1.72) 1.79–1.89–1.94 (2.02)
<i>largus</i>	FR 0070030	9.91) 10.42–10.74–11.11 (11.62) × (5.51) 5.86–5.99–6.18 (6.40)	(1.64) 1.71–1.81–1.86 (1.95)
<i>pseudodaulnoyae</i>	PRM 944023	10.29) 10.87–11.25–11.54 (12.06) × (6.26) 6.54–6.70–6.92 (7.12)	(1.54) 1.62–1.67–1.72 (1.77)

^aHolotype of *C. pardinus*. GenBank accession numbers

^bAY669552 (as *C. coalescens*)

^cAY174794 (as *C. coalescens*)

^dIsotype of *C. subtomentosus*

Table 4
Total arsenic concentrations, extracted arsenic, and arsenic species in investigated *Corinarius* spp. (mg kg⁻¹ in dry matter)

Sample ID	<i>Corinarius</i>	Total As mg kg ⁻¹	Extracted As mg kg ⁻¹	DMA mg kg ⁻¹	AB mg kg ⁻¹	TMAO mg kg ⁻¹	AC mg kg ⁻¹	TETRA mg kg ⁻¹	MA mg kg ⁻¹	Inorganic As mg kg ⁻¹	UNK 1 mg kg ⁻¹	UNK 2 mg kg ⁻¹	UNK 3 mg kg ⁻¹
ASP-65	<i>varicolor</i>	13.2 ± 0.8	11.4 ± 0.4	0.05	8.6	0.02	0.06	0.17	nd	nd	0.02	0.01	0.29
ASP-70	<i>pseudodulnomyae</i>	12.3 ± 0.8	10.7 ± 0.3	0.10	8.1	0.08	0.06	0.01	nd	nd	0.004	0.02	0.16
B-503b	<i>largus</i>	7.4 ± 0.4	5.2 ± 0.7	0.21	4.7	0.23	0.05	0.07	nd	nd	0.01	0.03	nd
B-839	<i>coalescens</i>	11.7 ± 0.5	10.9 ± 0.2	0.05	8.6	0.21	0.07	0.19	nd	nd	0.02	0.04	nd
B-840	<i>coalescens</i>	3.6 ± 0.4	2.6 ± 0.3	0.04	2.1	0.23	0.02	0.09	nd	nd	0.01	0.05	nd
B-841	<i>coalescens</i>	30.2 ± 0.3	27.8 ± 0.6	0.05	22.1	0.21	0.24	0.43	nd	nd	0.03	0.04	nd
B-841b	<i>coalescens</i>	13.6 ± 0.5	6.6 ± 0.8	0.02	5.8	0.05	0.05	0.13	nd	nd	0.004	0.02	nd

UNK: unknown arsenic species; nd: not detectable; limit of detection = 0.002 mg kg⁻¹
 Concentrations of total and extracted arsenic are presented as mean and standard deviation from three replicates. Standard deviations of arsenic species within the three replicates were around 5% or less