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# Taxonomic evaluation of the polypore *Daedaleopsis tricolor* based on morphology and molecular data

ONDŘEJ KOUKOL[1], FRANTIŠEK KOTLABA[2], ZDENĚK POUZAR[3]

[1]Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ-128 01 Praha 2, Czech Republic; ondrej.koukol@natur.cuni.cz
[2]Na Petřinách 8, CZ-162 00 Praha 6, Czech Republic; frantisek.kotlaba@ibot.cas.cz
[3]Nad Královskou oborou 23, CZ-170 00 Praha 7, Czech Republic

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*Daedaleopsis tricolor* and *D. confragosa* were formerly treated either as separate species or as conspecific. *D. tricolor* may be recognised by the presence of a lamellate hymenophore and mostly dark pileus surface with small network of grains, but its separate status is not supported by its micromorphology. Occurrence of intermediate forms contributes to uncertainty in species delimitation. Although this problem has been known for many years, no study has yet been aimed at a thorough study of both morphological and molecular data. In the present study, we analysed sequences of ITS rDNA, RPB2 and TEF of several typical specimens of *D. tricolor* and *D. confragosa* sampled in the Czech Republic in recent years, two specimens of the supposedly closely related *D. septentrionalis*, and available sequences from GenBank. Our data show that no studied DNA region supports separation of *D. tricolor* and *D. confragosa* as distinct species and that *D. septentrionalis* is supported as a distinct species according to the ITS rDNA and RPB2 genes. We therefore incline to treat *D. tricolor* as a variety of *D. confragosa*. Thorough revision of all species hitherto described in *Daedaleopsis* including Asian species of uncertain identity is recommended.

**Key words:** morphological species concept, ITS rDNA, RPB2, TEF, *Polyporales*, *Daedaleopsis*.

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*Daedaleopsis tricolor* (síťkovec trojbarvý) a *D. confragosa* (síťkovec načervenalý) byly považovány za dva samostatné druhy nebo za jeden druh. *D. tricolor* byl odlišován na základě přítomnosti lupenovitého hymenoforu a většinou tmavého povrchu klobouku s drobnými zrnky, ale bez rozdílů v mikromorfologických znacích. Výskyt přechodných forem přispívá k nejistotě, zda jde o dva různé druhy, nebo ne. Ačkoli je tento problém známý již značně dlouho, dosud nebyla provedena žádná studie, která by se detailně zaměřila jak na morfologická, tak i na molekulární data. V této studii jsme analyzovali sekvence ITS rDNA, RPB2 a TEF několika typických vzorků *D. tricolor* a *D. confragosa*, sbíraných v nedávné době v ČR, předpokládaného příbuzného druhu *D. septentrionalis* a sekvencí dostupných v databázi GenBank. Naše data ukázala, že žádný studovaný úsek DNA nepodpořil odlišení *D. tricolor* a *D. confragosa* jako samostatných druhů a že *D. septentrionalis* byl podpořen jako samostatný druh podle ITS rDNA a RPB2 genů. Kloníme se proto k názoru, že *D. tricolor* je varietou druhu *D. confragosa*. Doporučujeme důkladné prostudování druhů dosud popsaných v rodu *Daedaleopsis*, včetně asijských druhů s nejasnou identitou.

## INTRODUCTION

The polypore *Daedaleopsis tricolor* (Pers.: Fr.) Bondartsev & Singer is very striking not only for its mostly dark brown pileus colouration but especially by its lamellate hymenophore. For this reason it has been considered by various authors either as a separate species (e.g. Bernicchia 2005, Bourdot & Galzin 1928, Donk 1974, Kotlaba et al. 2010, Kout & Vlasák 2011, Piątek 2001, Ryvarden & Gilbertson 1993, Ryvarden & Melo 2014, Vampola 1994, Wojewoda 2002, 2003) or as a variety of *D. confragosa*, i.e. *D. confragosa* (Bull.: Fr.) J. Schröt. var. *tricolor* (Pers.: Fr.) Bondartsev (e.g. Bondartsev 1953, Domański 1974, Jülich 1984, Pilát 1939). The main reason for the ambiguous treatment of these species is the existence of specimens showing intermediate macromorphological characteristics and the absence of diagnostic micromorphological structures. Another morphologically closely related species is *D. septentrionalis* (P. Karst.) Niemelä. All three polypores grow on broadleaved trees in Eurasia, although *D. septentrionalis* is limited to birches in northern Eurasia (Niemelä 1982). Despite the unclear delimitation of *D. tricolor* and *D. confragosa* as mentioned by several authors, to date only the morphology of these two species had been studied in detail. No study had yet employed compatibility mating tests to confirm or disconfirm the separation of *D. tricolor* and *D. confragosa*. Niemelä (1982) found *D. septentrionalis* to be intersterile when paired with *D. confragosa*.

Sequences of several DNA regions obtained for both *D. confragosa* and *D. tricolor* in several independent studies did not yet provide clear information due to insufficient data sampling. Ko & Jung (1999a) found only a few nucleotide substitutions in the SSU rDNA sequences of these two species and inclined to the conclusion of Ryvarden & Gilbertson (1993), who considered *D. tricolor* to be a southern ecotype of *D. confragosa*. However, in a parallel study, Ko & Jung (1999b) treated these species separately by simply including only one representative sequence of each species. Bernicchia et al. (2006) isolated DNA from 7,000 year old basidiocarps of a polypore identified as *D. tricolor* based on morphology and sequences of mitochondrial 12S DNA. Although the sequence obtained from the Neolithic polypore was identical to both sequences of *D. confragosa* and *D. tricolor*, they classified it as *D. tricolor* because of the lamellate hymenophore. In other studies dealing with the molecular phylogeny of the *Polyporales* this obvious ambiguity was overcome by using only one of the species in the alignment (Hibbett & Donoghue 1995, Justo & Hibbett 2011, Welti et al. 2012).

During the past few years, plenty of material of *D. tricolor* and *D. confragosa* has been sampled by the two senior authors of this paper (F.K. and Z.P.). Among them, dozens of well-developed fruitbodies collected in the peripheral parts of Prague (Czech Republic) were suspected to belong to *D. tricolor*. Nevertheless, after a meticulous study the authors have come to the conclusion that macro-

morphological features only do not allow for making a final decision on the taxonomic value of this polypore. It will therefore be useful to employ molecular data [a similar opinion was expressed by Kout & Vlasák (2011) and Ryvarden & Melo 2014)]. This is the reason why we decided to study the phylogenetic relationships of *D. confragosa* and *D. tricolor*, together with *D. septentrionalis*, based on molecular data and to confront the results with morphological differences.

## MATERIAL AND METHODS

Morphological research was carried out on freshly collected and herbarium material of *Daedaleopsis confragosa*, *D. tricolor* and *D. septentrionalis* from *Corylus colurna*, *Prunus avium*, *Rosa* sp. and *Salix caprea* sampled between 2011 and 2014. All studied specimens were deposited in the Herbarium of the National Museum, Prague (PRM) or the Herbarium of the Charles University in Prague (PRC).

Freshly collected mature basidiocarps of *D. confragosa* and *D. tricolor* used for isolation in pure cultures and extraction of DNA were sampled at localities within Prague in 2012. Extraction was mostly not carried out directly from the fruitbodies to avoid contamination by allochthonous DNA from the surface or interior of the basidiocarps, except for two specimens of *D. septentrionalis* (H 6035974 and H 6039774) obtained from the Herbarium of the Finnish Museum of Natural History (H).

DNA was isolated from 7–14 day old cultures using a Zymo Research Fungal/Bacterial Kit (Zymo Research, Orange, USA). Nuclear rDNA containing internal transcribed spacers (ITS1 and ITS2) and the 5.8S region (further referred to as ITS rDNA) were amplified with primer sets ITS1/ITS4 (O'Donnell 1993, White et al. 1990), whereas DNA polymerase II second largest subunit (RPB2) was amplified with primers RPB2-5F and fRPB2-7cR (Liu et al. 1999) and translation elongation factor 1α (TEF) with primers 983F and 2218R (Rehner & Buckley 2005). The PCR products were viewed by electrophoresis on a 1% (w/v) TAE agarose gel, stained with ethidium bromide. The PCR products were purified with the Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech, Bade City, Taiwan). Both strands of the PCR fragments were sequenced with the primers used for amplification (Faculty of Science, Charles University in Prague, Czech Republic).

In addition to sequences obtained in this study, sequences of some reference taxa were obtained from GenBank (Tab. 1) for the phylogenetic analysis. Sequences were aligned using the MAFFT algorithm implemented in the Geneious Program (Biomatters, Auckland, New Zealand). Neighbour joining (NJ) analyses were run in MEGA v. 5 (Kumar et al. 2008) using the LogDet (Tamura-Kumar) method with 10,000 bootstrap replicates. All positions containing gaps and missing data

**Tab. 1**. Sequences from GenBank and newly generated sequences (in bold) used in the present study. Fungal species names reflect their putative identification based on morphology.

| Fungal species | Origin | Strain | Voucher | GenBank accession numbers | | |
|---|---|---|---|---|---|---|
| | | | | ITS rDNA | RPB2 | TEF |
| *Coriolopsis trogii* | USA | | RLG9577R | JN164996 | | |
| *C. trogii* | France | BRFM 974 | | | JN645141 | |
| *C. trogii* | USA | | RLG4286sp | | | JN164898 |
| *Daedaleopsis confragosa* | Czech Rep. | NK370 | PRC 2524 | **HG973497** | **HG973514** | **HG973505** |
| *D. confragosa* | Czech Rep. | NK371 | PRC 2525 | **HG973501** | **HG973518** | **HG973510** |
| *D. confragosa* | Czech Rep. | NK372 | | **HG973498** | **HG973515** | **HG973506** |
| *D. confragosa* | Czech Rep. | NK373 | PRM 921619 | **HG973500** | **HG973517** | **HG973509** |
| *D. confragosa* | Latvia | M112 | | JF340288 | | |
| *D. confragosa* | France | BRFM 1130 | | JX082372 | | |
| *D. confragosa* | France | BRFM 1131 | | JX082373 | | |
| *D. confragosa* | France | BRFM 1143 | | JX082375 | | |
| *D. confragosa* | France | BRFM 1145 | | JX082376 | | |
| *D. confragosa* | France | BRFM 947 | | FJ349623 | | |
| *D. confragosa* | France | BRFM 948 | | GU731549 | | |
| *D. confragosa* | Germany | | | FR686551 | | |
| *D. confragosa* | USA | X-49 | | KC176338 | | |
| *D. confragosa* | USA | X-78 | | KC176348 | | |
| *D. septentrionalis* | Finland | | H 6035974 | **HG973499** | **HG973516** | **HG973507** |
| *D. septentrionalis* | Finland | | H 6039774 | | | **HG973508** |
| *D. tricolor* | Czech Rep. | NK374 | PRM 921613 | **HG973502** | **HG973519** | **HG973511** |
| *D. tricolor* | Czech Rep. | NK375 | PRM 921614 | **HG973495** | **HG973512** | **HG973503** |
| *D. tricolor* | Czech Rep. | NK376 | PRM 921622 (= PRC 2526) | **HG973496** | **HG973513** | **HG973504** |
| *Datronia mollis* | USA | | RLG6304sp | JN165002 | JN164872 | JN164901 |
| *Dichomitus albidofuscus* | Poland | FCL23 | | HQ896245 | | |
| *D. albidofuscus* | Czech Rep. | MUAF 843 | | EU340897 | | |
| *Earliella scabrosa* | Puerto Rico | | PR1209 | JN165009 | | JN164894 |
| *E. scabrosa* | Venezuela | | CR95 | JN165008 | | |
| *Hexagonia nitida* | France | BRFM 1327 | | JN645082 | JN645127 | |
| *Lenzites tricolor* | France | BRFM 954 | | GU731548 | JN645138 | |
| *L. tricolor* | France | | MOU132 | JN645096 | | |
| *Polyporus arcularius* | Germany | SBUG-M1244 | | AB070861 | | |
| *P. arcularius* | Austria | TENN58370 | | AB070865 | | |
| *P. arcularius* | Japan | WD2359 | | | AB368139 | |
| *P. arcularius* | Japan | WD2138 | | | AB368138 | |

were eliminated for the ITS rDNA dataset. Poorly aligned positions and divergent regions of the RPB2 dataset were eliminated by means of GBlocks (Castresana 2000). Maximum likelihood (ML) analysis was performed with PhyML version 2.4 (Guindon & Gascuel 2003) with the Approximate Likelihood-Ratio Test (aLRT) for branch support.

Bayesian analyses were conducted in MrBayes v. 3.1 (Huelsenbeck & Ronquist 2001). For ITS rDNA, the best model selected under the Bayesian information criterion was the TrNef+G model and for TEF and RPB2 the TrN+G model selected by JModelTest v. 2.1.3 (Darriba et al. 2012). All analyses were run for 4 million generations sampling every 100$^{th}$ tree. Posterior probabilities (PP) were used as a Bayesian branch support on the consensus trees. The average standard deviation of split frequencies estimating convergence reached values of 0.0034, 0.0022 and 0.0027 in the ITS rDNA, TEF and RPB2 datasets, respectively. The concatenated dataset of ITS rDNA, TEF and RPB2 was analysed by means of Bayesian and Maximum likelihood analyses. All priors were estimated separately for the three partitions. Bayesian analysis was run for 2 million generations sampling every 100$^{th}$ tree. The average standard deviation of split frequencies reached a value of 0.0036.

## RESULTS

### Morphological study

The macromorphological study of many specimens of both *Daedaleopsis confragosa* and *D. tricolor* revealed only minor differences. The pileus surface in *D. tricolor* is soon covered by a thin, dark brown tomentum which sometimes disappeared in senescent basidiocarps, while in *D. confragosa* it appears only in very old basidiocarps, and is missing from young specimens. The hymenophore of *D. tricolor* is conspicuously lamellate (see Fig. 1), whereas in *D. confragosa* it is poroid with round pores to elongate daedaleoid (see Fig. 2). Specimens of *D. confragosa* with a lamellate hymenophore in the central and marginal part of the basidiocarps were collected as well, but orbicular pores were always present in the basal part of the hymenophore (see PRM 718427 and Fig. 3).

However, these macroscopic features are variable and are not sufficient to distinguish two species. Microscopic features even offer no differences between *D. confragosa* and *D. tricolor* at all. Basidia of both species are cylindrical, narrowed to the basis, thin-walled, hyaline, indextrinoid and inamyloid, tetrasterigmatic, 18–20 × 4.5–6 µm. Basidiospores are narrowly cylindrical, slightly curved, thin-walled, hyaline, indextrinoid and inamyloid, (6.0)6.5–8(9.5) × (1.0)1.2–1.6(1.9) µm. The tomentum at the pileus surface is formed by ramified hyphae (in some parts pitted). The hyphae are densely intricated, slightly brownish, thick-walled

with a strongly amyloid(!) internal wall layer, 3–3.5 µm broad. Skeletal context hyphae are strongly thick-walled with a very narrow channel, not ramified, hardly any of them slightly undulate, pale ochraceous, 3.5–5.0 µm broad. Binding hyphae are sparse, thick-walled, hyaline, sparsely ramified, rather thin-walled, indextrinoid and inamyloid, 2–3.5 µm broad. Generative hyphae are hyaline, sparsely ramified, thin-walled, septate with clamp-connections, 2.0–4.0 µm broad. Dendrohyphidia are present in the hymenium only when the basidia are undeveloped; they are cylindrical at the base, tuftily ramified towards the top, rather thin-walled, hyaline, 0.9–2.0 µm long, at the base 2.5–3.0 µm broad, at the top 0.6–1.6 µm broad.

**Specimens examined**

Names of the authors are shortened as follows: FK – F. Kotlaba, ZP – Z. Pouzar.

***Daedaleopsis confragosa*** var. ***tricolor***

(All specimens cited were originally labelled as *D. tricolor*.)

Czech Republic. Praha 12, Hodkovičky, inter Věkova et Vrbova via, *Prunus avium*, ad truncum emortuum, 3. IV. 2012, leg. et det. M. Koliáš (PRM 921611); ibid., 21. IX. 2012, leg. et det. FK (PRM 921616). – Praha 12, Hodkovičky, locus praeruptus inter Věkova et Vrbova via, *P. avium*, ad ramum emortuum, 5. XII. 2012, leg. et det. FK (PRM 921622, PRC 2526). – Praha 12, Hodkovičky, apud viam "V Lučinách", *P. avium*, ad ramum emortuum, 20. X. 2012, leg. et det. M. Koliáš (PRM 921612). – Praha 4, Lhotka, "Velký háj", pars trans Novodvorská Plaza, *P. avium*, ad truncum emortuum, 26. III. 2012, leg. et det. FK (PRM 921614). – Praha 4, Lhotka, "Kamýk" ("Lhotecký les"), *P. avium*, ad truncum emortuum, 16. III. 2012 (PRM 921613) et 23. IV. 2012 (PRM 921621), leg. et det. FK. – Praha 6, Divoká Šárka, "Přírodní divadlo", *P. avium*, ad ramum emortuum, 14. XII. 2011 (PRM 921627), 28. XII. 2011 (PRM 921628) et 11. I. 2012 (PRM 921607), leg. FK, det. ZP et FK. – Praha 6, Divoká Šárka, pars marginalis sept.-occid. a camping "Džbán", 4. IV. 2012, leg. et det. M. Koliáš et FK (PRM 921623); ibid., 19. IV. 2012, leg. et det. FK (PRM 921609), 26. IV. 2013 (PRM 921625) et 30. XII. 2013, leg. FK, det. FK et ZP (PRM 921620), 15. III. 2014, leg. et det. FK (PRM 921626); ibid., *Rosa* sp., ad ramum emortuum, 13. VI. 2012, leg. et det. FK (PRM 921624).

Slovak Republic. Mons "Repisko" prope Koláčkov ap. Stará Lubovňa (N Slovakia), ad ramum emortuum *Fagi sylvaticae*, 22. IX. 1972, leg. et det. FK (PRM 718427).

***Daedaleopsis confragosa*** var. ***confragosa***

(All specimens cited were originally labelled as *D. confragosa*.)

Czech Republic. Praha 4, Lhotka, "Velký háj", pars trans Novodvorská Plaza, *Prunus avium*, ad truncum emortuum, 26. III. 2012, leg. et det. FK (PRC 2524). – Praha 12, Modřany, ad rivum "Cholupický potok", prope viam "Do Koutů", *P. avium*, ad ramum emortuum, 6. V. 2012, leg. et det. O. Koukol (PRC 2525). – Praha 6, Divoká Šárka, pars marginalis sept.-occid. a camping "Džbán", *Rosa* sp., ad trunculum emortuum, 13. VI. 2012, leg. et det. FK (PRM 921619). – Praha 6, Břevnov, via Bubeníčkova (trans "Billa"), *Corylus colurna*, ad truncum emortuum, 7. III. 2014, leg. et det. FK (PRM 921618). – Praha 6, Břevnov, in horto scholae maternae via Dusíkova 3, *Salix caprea*, ad truncum vivum, 23. II. 2014 (PRM 921608) et 15. III. 2014, leg. et det. FK (PRM 921606). – Praha 21, Újezd nad Lesy, in silva "Klánovický les" dicta occid. a Staroklánovická via, *S. caprea*, ad truncum emortuum, 22. II. 2014, leg. et det. ZP (PRM 921605).

***Daedaleopsis septentrionalis***

Finland. Suomussalami, Hoicansuo, 28. V. 2004, leg. Pekka Helo (H 6039774). – Utsioki, Kevo, 23. IX. 2009, leg. H. Kotiranta (H 6035974).

**Fig. 1**. *Daedaleopsis confragosa* var. *tricolor*. Praha 6, Divoká Šárka, "Přírodní divadlo", on dead branch of *Prunus avium*, 28 November 2011. Photo F. Kotlaba.

**Fig. 2**. *Daedaleopsis confragosa* var. *confragosa*. "Černická obora" near Sudoměřice u Bechyně (S Bohemia), on dead branch of *Betula pendula*, 30 September 1994. Photo F. Kotlaba.

**Fig. 3**. *Daedaleopsis confragosa* var. *confragosa* – transitional specimens with lamelloid as well as daedaleoid hymenophore. Foot of Mt. Repisko near Koláčkov close to Stará Ľubovňa (N Slovakia), on lying dead branch of *Fagus sylvatica*, 22 September 1992 (PRM 718427). Photo F. Kotlaba.

## Molecular data

Four strains were isolated from basidiocarps identified as *Daedaleopsis confragosa* and three strains from those identified as *D. tricolor*. Unfortunately, isolation was not successful with basidiocarps showing intermediate characteristics. DNA was also extracted from both dried specimens of *D. septentrionalis*. Phylogenetic analyses were performed with unambiguously aligned sequences of 318 (ITS rDNA), 598 (RPB2) and 922 (TEF) nucleotides containing 89, 107 and 103 parsimony informative sites, respectively.

All ITS rDNA sequences obtained during this study including European sequences of material identified as either *D. confragosa* or *D. tricolor*, but also as *Lenzites tricolor* (Pers.: Fr.) Fr., formed one well-supported clade (Fig. 4). There was virtually no variability among sequences within this clade. *D. septentrionalis* H 6039774 (ITS rDNA was successfully amplified only from this specimen) was placed with high support from ML and NJ analyses as neighbouring species to this clade (Fig. 4).

Sequences of RPB2 belonging to *D. confragosa* and *D. tricolor* were more variable and formed several clades with low supports (Fig. 5). Both species were intermingled in these clades, suggesting that the variability is intraspecific. Sequences of both specimens of *D. septentrionalis* were placed within one of these clades. Results of analyses from both datasets were thus rather congruent and

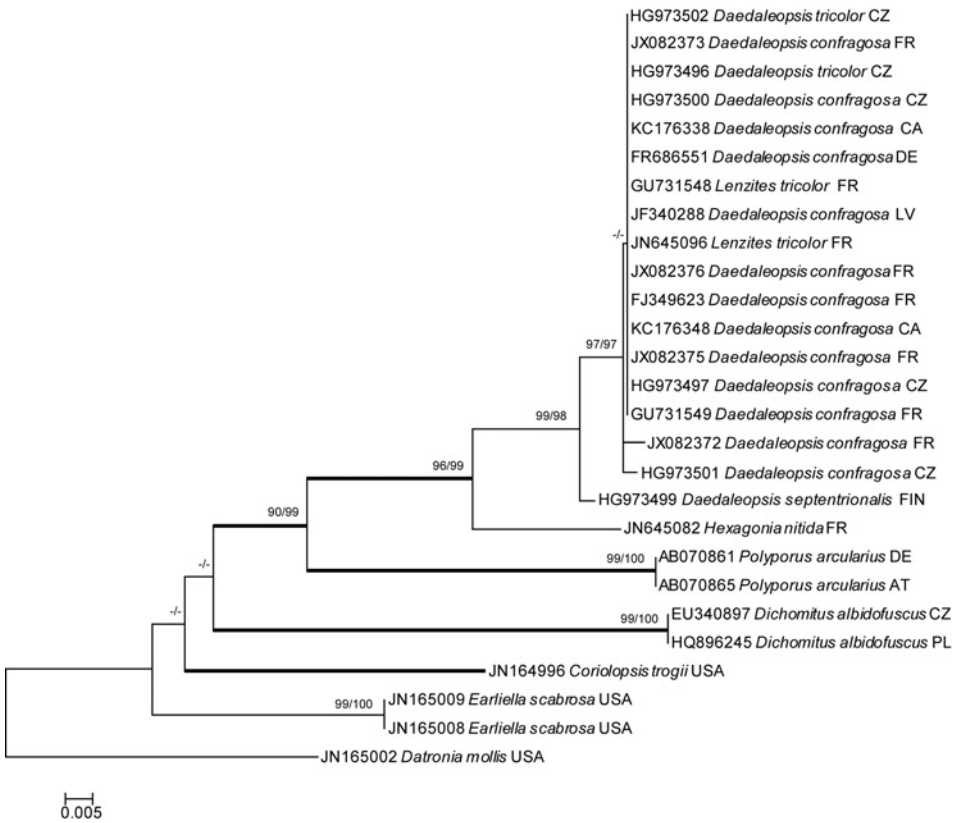


**Fig. 4.** Phylogeny of *Daedaleopsis confragosa* and allied species based on ITS rDNA using NJ, ML and Bayesian analyses. Geographical origin of sequences is indicated behind the names. Values above branches refer to Bootstrap support higher than 90% for NJ and aLRT support higher than 0.9 for ML. PP higher than 0.95 is indicated by thick lines.

showed that *D. confragosa* and *D. tricolor* cannot be treated as separate species. They also showed that RPB2 is not a good marker for distinguishing *D. confragosa* and *D. septentrionalis*.

Roughly similar results were obtained from the analyses of the TEF region (Fig. 5). *Daedaleopsis confragosa* and *D. tricolor* could not be distinguished due to polytomy in branching, but both sequences of *D. septentrionalis* formed a well-supported clade.

The dataset consisting of concatenated ITS rDNA, RPB2 and TEF regions (Fig. 5) was underrepresented in sequences of *D. confragosa* and related species due to the lack of reference sequences in GenBank. On the other hand, the result generally supported outcomes from single region analyses.

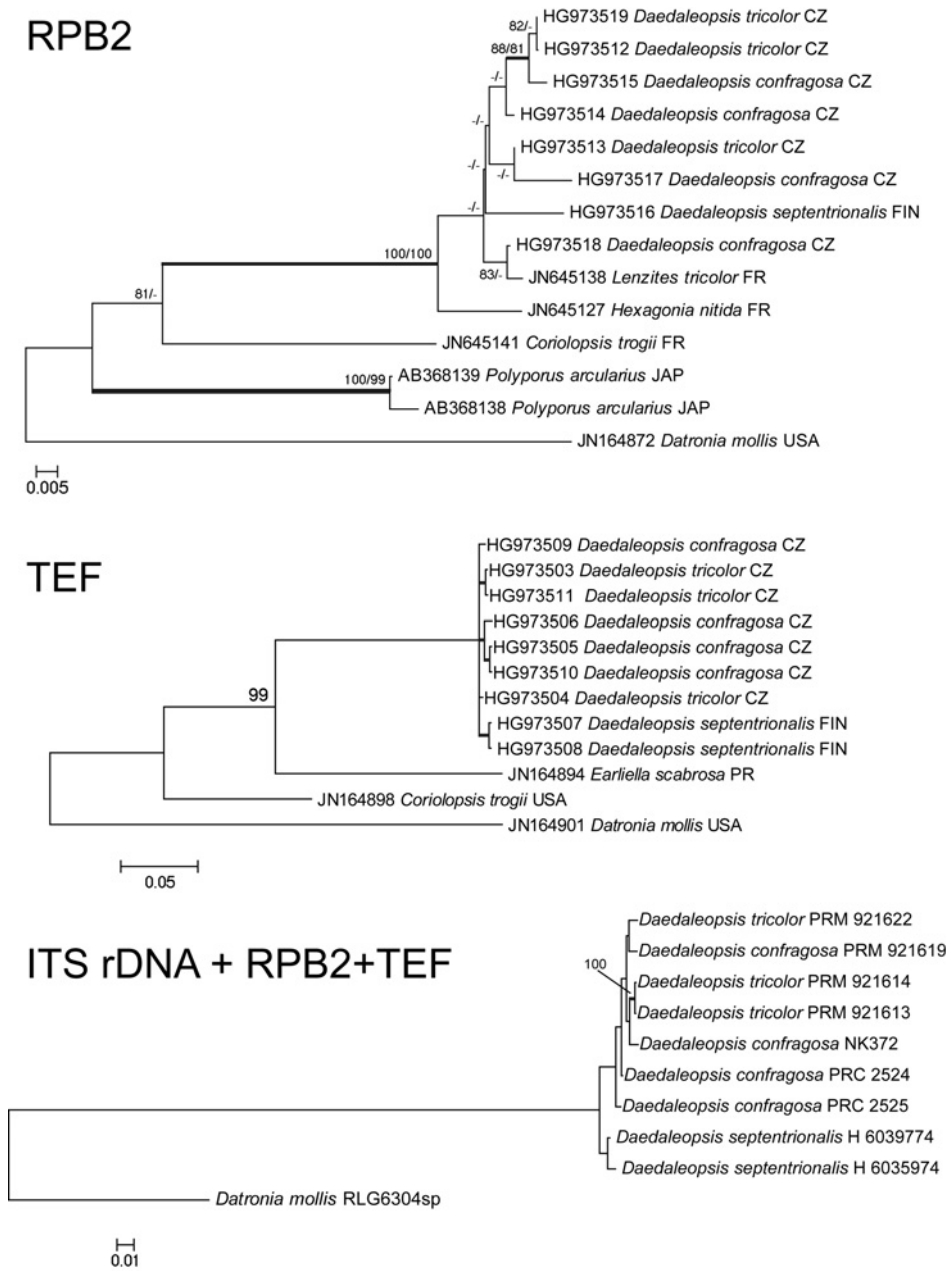


**Fig. 5.** Phylogeny of *Daedaleopsis confragosa* based on coding regions (RPB2 and TEF) and concatenated dataset of ITS rDNA, RPB2 and TEF sequences. Geographical origin of sequences or voucher specimen are indicated behind the names. Values above branches refer to Bootstrap support higher than 90% for NJ and aLRT support higher than 0.9 for ML. PP higher than 0.95 is indicated by thick lines.

## DISCUSSION

Taxonomic evaluation of the polypore *Daedaleopsis tricolor* based solely on macromorphological features cannot solve the question if it is a clearly delimited species or a subspecific taxon. In the large collection of material studied, all characters, namely the structure of the hymenophore, were also found in intermediate forms. The only relatively stable character, the tomentum on the pileus surface, was depend to the age of the basidiocarp.

Molecular data supported treating *D. confragosa* and *D. tricolor* as one species, which was already indicated by Ko & Jung (1999a). High intraspecific variability among the studied specimens was revealed from the analyses of coding regions RPB2 and TEF. Variability in ITS rDNA was much lower with virtually no difference among strains obtained in our study from the Czech Republic and other European sequences obtained from GenBank. The high intraspecific variability in the *D. confragosa – D. tricolor* complex may be attributed to their wider ecological amplitude (large distribution area, wide spectrum of host trees) compared to *D. septentrionalis*, which is restricted to colder regions and birch trees (Niemelä 1982). Identification of specimens with a well-developed lamellate hymenophore as *D. tricolor* has thus no support in molecular data and should be avoided especially now that molecular data are available (Bernicchia et al. 2006).

Separation of *D. septentrionalis* as a distinct species was proven since both the ITS rDNA and TEF phylogenies supported its placement as a sister species to *D. confragosa* and *D. tricolor*. However, this position was not strongly supported by the Bayesian analysis (PP only 65%). A similar result was obtained by Frøslev et al. (2005) in the phylogenetic study of the genus *Cortinarius*. Only nodes receiving high support (>95% Bayesian PP) in the ITS rDNA phylogeny were consistently supported in combined analyses with RPB2.

Our study indicated the need of a thorough study of the genus *Daedaleopsis* including Asian specimens. Sequences of material identified as *D. confragosa* [or *D. rubescens* (Alb. & Schwein.) Imazeki], *D. tricolor* (or *Lenzites tricolor*) and *D. sinensis* (Lloyd) Y.C. Dai were available from GenBank and thus included in our data for preliminary analyses (data not shown). However, the obtained results were difficult to interpret and indicated probable misidentification of *D. sinensis* as *D. confragosa*, providing that these species are not conspecific. On the other hand, sequence AB470858 of *D. rubescens* and two sequences of *D. tricolor* (FJ755220 and JN043321) formed a sister clade to *Polyporus arcularius* (Batsch) Fr., thus making *Daedaleopsis confragosa* potentially polyphyletic (data not shown). The collections of these two sequences were not available to us so that we could not confirm their correct identification. Finally, the phylogenetic placement of species currently only known from Asia, such as *D. nipponica* Imazeki and *D. conchiformis* Imazeki, is neither known due to absence of molecular data.

## CONCLUSION

According to the results of our study, *Daedaleopsis tricolor* cannot be treated as a separate species because no consistent differences in morphology and molecular data were found. Considering that *D. tricolor* is strikingly distinct only by its often dark brown pileus surface and lamelloid hymenophore, the last two authors of this paper are now of the opinion that it has the rank of variety: *D. confragosa* var. *tricolor* (Pers.: Fr.) Bondartsev.

A few years ago, we have considered this variety to be very rare polypore in Bohemia (Kotlaba et al. 2010), but recently it seems to be spreading here, especially in the surroundings of Prague.

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## REFERENCES

BERNICCHIA A. (2005): *Polyporaceae* s. l. – Fungi Europaei 10, Edizioni Candusso, Alassio.

BERNICCHIA A., FUGAZZOLA M.A., GEMELLI V., MANTOVANI B., LUCCHETTI A., CESARI M., SPERONI E. (2006): DNA recovered and sequenced from an almost 7000 y-old Neolithic polypore, *Daedaleopsis tricolor*. – Mycol. Res. 110: 14–17.

BONDARTSEV A.S. (1953): Trutovye griby evropejskoj chasti SSSR i Kavkaza [Polypores of the European USSR and Caucasus]. – Izdatelstvo Akademii nauk SSSR, Moskva, Leningrad. [in Russian]

BOURDOT A., GALZIN H. (1928): Hyménomycètes de France. – Marcel Bry, Sceaux.

CASTRESANA J. (2000): Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. – Mol. Biol. Evol. 17: 540–552.

DARRIBA D., TABOADA G.L., DOALLO R., POSADA D. (2012): jModelTest 2: more models, new heuristics and parallel computing. – Nat. Meth. 9: 772.

DOMAŃSKI S. (1974): Mała flora grzybów, tom I, Basidiomycetes (Podstawczaki), Aphyllophorales (Bezblaszkowe), pars 1 [Small flora of fungi, vol. I, Basidiomycetes, Aphyllophorales, part 1]. – Państwowe wydawnictvo naukowe, Warszawa, Kraków. [in Polish]

DONK M.A. (1974): Check list of European polypores. – Verhand. Koninkl. Neder. Akad. Wetensch., Natuurk., tweede reeks, deel 62: 1–469.

FRØSLEV T.G., MATHENY P.B., HIBBETT D.S. (2005): Lower level relationships in the mushroom genus *Cortinarius* (Basidiomycota, *Agaricales*): A comparison of RPB1, RPB2, and ITS phylogenies. – Mol. Phylogenet. Evol. 37: 602–618.

GUINDON S., GASCUEL O. (2003): A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. – Syst. Biol. 52: 696–704.

HIBBETT D.S., DONOGHUE M.J. (1995): Progress toward a phylogenetic classification of the *Polyporaceae* through parsimony analysis of mitochondrial ribosomal DNA sequences. – Canad. J. Bot. 73: 853–861.

HUELSENBECK J.P., RONQUIST F. (2001): MRBAYES: Bayesian inference of phylogenetic trees. – Bioinformatics 17: 754–755.

JÜLICH W. (1984): Die Nichtblätterpilze, Gallertpilze und Bauchpilze. – In: Gams H., ed., Kleine Kryptogamenflora, Band IIb/1, Basidiomyceten, 1. Teil. Gustav Fischer Verlag, Jena, Stuttgart, New York.

JUSTO A., HIBBETT D.S. (2011): Phylogenetic classification of *Trametes* (Basidiomycota, *Polyporales*) based on a five-marker dataset. – Taxon 60: 1567–1583.

KO K.S., JUNG H.S. (1999a): Molecular phylogeny of *Trametes* and related genera. – Antonie van Leeuwenhoek 75: 191–199.

KO K.S., JUNG H.S. (1999b): Phylogenetic re-evaluation of *Trametes consors* based on mitochondrial small subunit ribosomal DNA sequences. – FEMS Microbiol. Lett. 170: 181–186.

KOTLABA F., POUZAR Z., KOUT J. (2010): Lokality velmi vzácného síťkovce trojbarvého – *Daedaleopsis tricolor* v České republice. – Mykol. Listy no. 113: 20–22.

KOUT J., VLASÁK J. (2011): Nové nebo vzácné chorošovité houby z Plzeňska. – Erica 18: 85–94.

KUMAR S., NEI M., DUDLEY J., TAMURA K. (2008): MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. – Brief Bioinform. 9: 299–306.

LIU Y.J., WHELEN S., HALL B.D. (1999): Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. – Mol. Biol. Evol. 16: 1799–1808.

NIEMELÄ T. (1982): Taxonomic notes on the polypore genera *Antrodiella*, *Daedaleopsis*, *Fibuloporia* and *Phellinus*. – Karstenia 22: 11–12.

O'DONNELL K. (1993): *Fusarium* and its near relatives. – In: Reynolds D.R., Taylor A.F., eds., The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics, p. 225–233, CAB International, Wallingford.

PIĄTEK M. (2001): New discovery of *Daedaleopsis tricolor* (Fungi, *Poriales*) and review of its distribution in Poland. – Polish Bot. J. 46: 277–279.

PILÁT A. (1939): *Polyporaceae* – Houby chorošovité IV. – In: Kavina K., Pilát A., eds., Atlas hub evropských [Atlas of European fungi], vol. III, fasc. I, Praha. [in Czech]

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.

RYVARDEN L., GILBERTSON R.L. (1993): European polypores, Part 1. – Synopsis fungorum 6, Fungiflora A/S, Oslo.

RYVARDEN L., MELO I. (2014): Poroid fungi of Europe. – Synopsis fungorum 31, Fungiflora A/S, Oslo.

VAMPOLA P. (1994): Poznámky k síťkovci trojbarvému – *Daedaleopsis tricolor*. – Mykol. Listy no. 52: 2–4.

WELTI S., MOREAU P.-A., FAVEL A., COURTECUISSE R., HAON M., NAVARRO D., TAUSSAC S., LESAGE-MEESSEN L. (2012): Molecular phylogeny of *Trametes* and related genera, and description of a new genus *Leiotrametes*. – Fung. Divers. 55: 47–64.

WHITE T.J., BRUNS T.D., LEE S., TAYLOR J. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – In: Innis M.A., Gelfand D.H., Sninsky J.J., White T.J., eds., PCR protocols: a guide to methods and applications, p. 315–322, Academic Press, San Diego.

WOJEWODA W. (2002): Atlas of the geographical distribution of fungi in Poland, vol. 2. – W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.

WOJEWODA W. (2003): Checklist of Polish larger Basidiomycetes. – W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.