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DNA sequence analysis of extraordinary fruiting specimens of *Fuscoporia torulosa* (*Phellinus torulosus*) on *Pyrus* spp.

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Fuscoporia torulosa (Phellinus torulosus) is a typical polypore of thermophilous forests in Central Europe. In most cases the fungus forms basidiocarps on base or roots of various host trees (mainly *Quercus*), but sometimes the basidiocarps appear on stem heights of approx. 2 m. This extraordinary fructification pattern seems to be restricted to *Pyrus* as a host. The aim of this study was to compare such basidiocarps with those growing on the base of oak trees according to their DNA sequences to reveal a possible process of speciation. The so-called ITS region of nuclear ribosomal DNA was chosen for the study. Results did not reveal any significant differences between basidiocarps with the distinct fructification pattern, so all examined specimens belong to one species. The relation of *Fuscoporia torulosa* to *Phellinus senex* is discussed.

Key words: *Fuscoporia*, *Phellinus*, DNA, ITS region, fructification

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Ohňovec hrbolatý *Fuscoporia torulosa (Phellinus torulosus)* je jedna z nejtypičtějších chorošovitých hub středoevropských teplomilných lesů. Ve většině případů tato houba fruktifikuje na bázích kmene nebo na kořenech svých hostitelů (především dubů), ale občas se plodnice vyskytnou i ve výšce kmene cca 2 metrů. Tento neobvyklý typ fruktifikace se zdá být omezen pouze na jedince rostoucí na hrušních. Cílem naší studie bylo porovnat tyto plodnice s plodnicemi vyrostlými na bázích dubů za použití sekvencí DNA, abychom odhalili případnou speciaci. Pro porovnání DNA byl zvolen tzv. ITS úsek jaderné ribozomální DNA. Výsledky neodhalily žádné významné rozdíly mezi plodnicemi s různým typem fruktifikace, a tak všichni zkoumaní jedinci patří k jednomu druhu. Dále je diskutována příbuznost ohňovce hrbolatého k druhu *Phellinus senex*.

INTRODUCTION

The genus *Phellinus* s. l. is an important member of order *Hymenochaetales* and has been generally accepted as a monophyletic natural taxonomical unit. On the other hand, this broad genus was divided into several more narrowly defined

smaller groups classified at the generic level according to morphological characters (Fiasson and Niemelä 1984). These well-defined groups (species complexes) are for example the *Phellinus pini* group (genus *Porodaedalea*), *Phellinus robustus* group (genus *Fomitiporia*), and *Phellinus rimosus* group (genus *Fulvifomes*). Nevertheless, the concept of narrower genera in this group was rejected by most authors (Larsen and Cobb-Poulle 1990, Ryvarden and Gilbertson 1994) until it was supported by the methods of molecular taxonomy (Wagner and Fischer 2001, 2002).

The genus *Fuscoporia* was originally defined as a group of fungi with mostly resupinate or effused-reflexed basidiocarps (*Phellinus contiguus*, *P. ferruginosus*, *P. viticola*) whereas the subject of this study, *Phellinus torulosus* (Pers.) Bourd. et Galzin, remained in the genus *Phellinus* s. str. (Fiasson and Niemelä 1984). A more recent study based on comparison of a large subunit (LSU) of nuclear ribosomal DNA (nrDNA) sequences revealed relatedness of *Phellinus torulosus* to the genus *Fuscoporia* (Wagner and Fischer 2001). This affinity was unexpected due to the shape of the basidiocarps but it is well supported by microscopic characters, resulting in the new combination *Fuscoporia torulosa* (Pers.) T. Wagner et M. Fischer (Wagner and Fischer 2001). The most related species to *F. torulosa* seem to be *F. wahlbergii*, *F. viticola*, *F. ferrea*, *F. gilva* and *Phellinus senex* (Parmasto 1985, Rizzo et al. 2003, Ryvarden and Gilbertson 1994, Wagner and Fischer 2001).

Fuscoporia torulosa is distributed in almost the whole of Europe, with an exception of the Nordic countries, with its distribution centre in warmer areas (Ryvarden and Gilbertson 1994), North Africa and Asia (Parmasto 1985, Núñez and Ryvarden 2000). The species was reported also from Northern and Southern America (Gilbertson and Burdsall 1972, Parmasto 1985), but specimens identified as *Phellinus torulosus* from the USA (Arizona) were, according to morphological and molecular characters, transferred to the recently described species *Phellinus coronadensis* Rizzo, Gieser et Burdsall (Rizzo et al. 2003), so the occurrence of *Fuscoporia torulosa* in America remains unclear. The occurrence of *F. torulosa* in Central Europe is restricted to the area of thermophilous vegetation and the distribution borders of the species correspond to those of the cultivation of vine (*Vitis vinifera*). The fungus occurs mainly in localities with thermophilous oak forests at altitudes not higher than 450 m above sea level (Kotlaba 1975). *Fuscoporia torulosa* grows on various host tree species, mainly broad-leaved trees, but occasionally it has been also recorded on conifers (Campanile et al. 2004, Isikov and Kuznetzov 1990, Panconesi et al. 1994, Kotlaba 1984). In Central Europe the most common host of *F. torulosa* is oak (*Quercus* spp.), less common are other broad-leaved trees, e. g. *Robinia*, *Carpinus*, *Cerasus*, *Fraxinus* (according to Kotlaba 1975, 1984).

Fuscoporia torulosa grows on exposed roots, the bases of stems and stumps, where it causes a white pocket rot. On the other hand, some specimens growing on higher parts of stems or branches at a height of approx. 2-3 m sometimes occur. The only known host of such unusually growth of *Fuscoporia torulosa* basidiocarps is the pear tree (*Pyrus* spp.). *Pyrus* is an uncommon host of *Fuscoporia torulosa* and specimens of such basidiocarps have been collected in the southern part of the Czech Republic and Slovakia, but occurrence in neighbouring countries (Austria, Hungary) in the Pannonian flora district is also probable. Wild pear (*Pyrus pyraster* L.) or naturalised hybrids of cultivars in Central Europe occurs most commonly in margins of alluvial forests and thermophilous oak stands, so the distribution of *Pyrus* generally coincides with that of *Quercus*. The fructification of *Fuscoporia torulosa* on *Pyrus* spp. has been reported from several sites in the southern part of the Czech Republic and Slovakia (Antonín et al. 2000; Kotlaba 1962, 1975, 1984; Vlasák 2006), but the exact position of the basidiocarps on the substrate has not been always noted. Not all basidiocarps of *F. torulosa* growing on *Pyrus* appear on higher parts of the stem, specimens with the common fructification on roots and butt of pear trees have been also reported from the Czech Republic (Antonín et al. 2000, Kotlaba 1975). The occurrence of *Fuscoporia torulosa* on *Pyrus* has been also reported from the Crimea (Ukraine) but the fructification pattern was not reported (Isikov and Kuznetzov 1990). The combination of aberrant fructification and host preference may be an evidence of a process of speciation (formation of sibling species – Parmasto 1985). The genetic relationships between *F. torulosa* specimens from different host species are little known. For example, Larsen and Cobb-Poulle (1990) distinguished 8 "formae" of the species, but the differences among the groups have not been confirmed by genetic methods. Campanile et al. (2004) studied genetic variability of *F. torulosa* specimens from different hosts, but no specimen from *Pyrus* was included in the study. On the other hand, the evidence of speciation (formation of intersterile groups) of *F. torulosa* has in the past been recorded by Fischer and Bresinsky (1992) on the Canary Islands.

The main aim of this study is the comparison of DNA sequences of *Fuscoporia torulosa* fructifying on butts or roots of *Quercus* and on stems and branches of *Pyrus*. For the study the ITS region of nuclear ribosomal DNA (see details in White et al. 1990) was chosen, because this region seems to be suitable for molecular study at the specific level of the genus *Phellinus* s. l. (Fischer 2002, Fischer and Binder 2004). To compare genetic identity of our *Fuscoporia torulosa* specimens with those sequenced by Rizzo et al. 2003, the small subunit of mitochondrial ribosomal DNA (mitSSU) region of a selected specimen will be also sequenced.

MATERIAL AND METHODS

Six specimens of *Fuscoporia torulosa* were chosen for the study. Two samples from relatively high positions on a *Pyrus* stem were accompanied by three specimens from *Quercus* and one from *Cerasus*. The basic data of the studied specimens are given in Tab. 1; the localities of the samples are shown in a map (Fig. 1). In two samples the culture was successfully isolated from the basidiocarps and the DNA isolated. These are deposited in the Laboratory of Forest Protection, Mendel University, Brno (for strain numbers see Tab. 1). The DNA was isolated either from dried herbarium specimens or from fresh cultures growing on Petri dishes with Malt-extract agar (Himedia, India) using the PowerSoil DNA kit (MoBio, USA) according to the manufacturer's instructions.

For amplification of ITS and mitSSU the primer pairs ITS1/ITS4 and MS1/MS2 were used (White et al. 1990). The DNA was amplified with PCR, using the Mastercycler® ep thermocycler (Eppendorf, Germany). The PCR reaction was carried out in a 25 µl reaction volume; the mixture for the PCR contained 50 ng of DNA, 20 pmol of each primer, 0.2 mM dNTP's, and 1U of DynaZyme™ polymerase with the appropriate buffer (Finnzymes, Finland). PCR amplifications were performed under the following temperatures: 94 °C /3 min., 50 °C /30 s, 72 °C /1 min. (1×), 94 °C /30 s, 50 °C /30 s, 72 °C /30 s (33×) and 94 °C /30 s, 50 °C /30 s, 72 °C/5 min. (1×). The PCR products were purified by NucleoSpin Extract II (Macherey-Nagel, Germany) prior to sequencing.

Sequences were determined with an ABI PRISM 3100 Avant DNA sequencer (Applied Biosystems) at the Department of Animal Morphology, Physiology and Genetics, Faculty of Agriculture, Mendel University, Brno using the ABI PRISM BigDye terminator v1.1 cycle sequencing kit (Applied Biosystems). All samples were sequenced with the primers used in the PCR. The sequences were deposited in the EMBL Nucleotide Sequence Database and their GenBank accession numbers are given in Tab. 1. Additional ITS sequences of the examined species were searched for in the GenBank, while sequences of the most closely related taxa (according to Jeong et al. 2005, Wagner and Fischer 2001) *Fuscoporia viticola* (AY558653) and *Phellinus senex* (AY558647) were chosen to be the outgroup. The only ITS sequence of *Fuscoporia torulosa* found in the NCBI database (updated 27 Sept. 2006) was one of the strains no. 182.34 deposited in the CBS culture collection. This sequence (AY558649) published by Jeong et al. (2005) shows higher homology to *Phellinus senex* than to our newly acquired specimens.

All sequences were edited manually using BioEdit version 4.7.1. (Hall 1999). The ITS alignment with introduced gaps consisted of 667 positions and all of them were included in the analysis. The total number of positions included 565 constant and 102 variable sites.

Tab. 1. Sequences of *Fuscoporia torulosa* encompassed in the study.

ITS sequence GenBank Accession no.	mitSSU sequence GenBank Accession no.	Geographic origin	Host species	Source
EF068234	–	Czech Republic, Valtice, Rendezvous 48° 44' 52'' N 16° 47' 32'' E	*Quercus cerris*	BRNM704833 Culture no. 847
EF068235	–	Czech Republic, Valtice, Rendezvous 48° 44' 52'' N 16° 47' 32'' E	*Pyrus* sp.	BRNM704834
EF068236	–	Slovakia, Svätý Jur, Panonský háj 48° 20' 36'' N 17° 33' 10'' E	*Pyrus* sp.	BRNM704837
EF068237	EF068240	Czech Republic, Brno,Veveří castle 49° 15' 21'' N 16° 27' 35'' E	*Quercus robur*	BRNM704836 Culture no. 859
EF068238	–	Montenegro (former Yugoslavia), Budva, Maine 42° 17' 49'' N 18° 50' 32'' E	*Cerasus* sp.	BRNM704838
EF068239	–	Czech Republic, Mikulov, Milovická stráň 48° 50' 46'' N 16° 41' 34'' E	*Quercus* sp.	BRNM704835

Tab. 2. Nucleotide polymorphism among the ITS sequences of examined specimens of *Fuscoporia torulosa*.

ITS sequence GenBank Accession no.	Nucleotide position				
	116	459	474	527	559
EF068234	T	T	C	T	G
EF068235	T	gap	C	T	G
EF068236	T	T	C	T	G
EF068237	T	gap	C	T	G
EF068238	Y	gap	S	Y	C
EF068239	T	gap	C	T	G

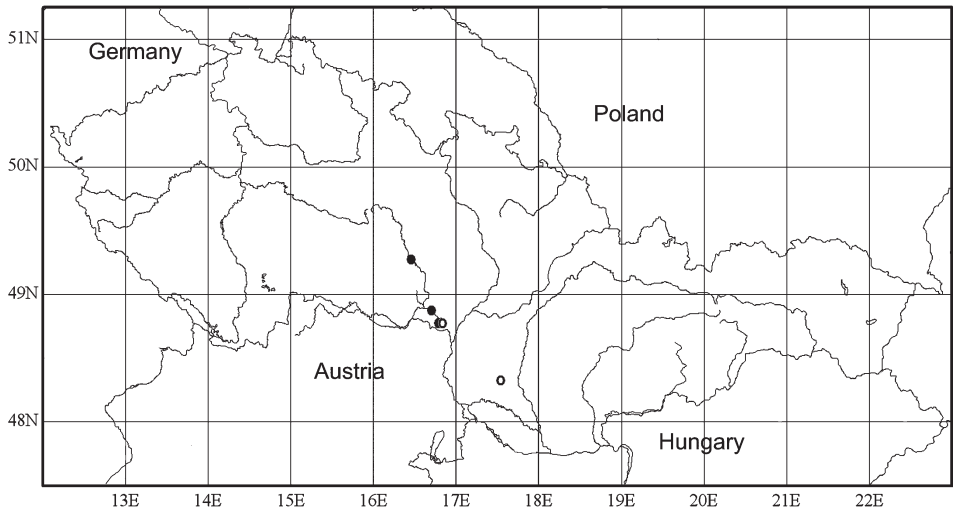

Fig. 1. Localities of examined specimens of *Fuscoporia torulosa* in the Czech Republic and Slovakia. Full circles indicate the specimens collected on *Quercus*, empty circles the specimens collected on *Pyrus*.

A Neighbor-Joining phylogenetic analysis was carried out using MEGA3.1 (Kumar et al. 2004). The phylogram was constructed using the Kimura 2-parameter distance model (with pairwise deletion option) and the support for the topology of the dendrogram was estimated using 1000 bootstrap-replicates.

RESULTS AND DISCUSSION

The newly obtained ITS sequences of *Fuscoporia torulosa* possess an identity of more than 99 %. The data show very low polymorphism and there is no apparent variation between sequences of specimens collected on different substrates. The only remarkable difference is between a sequence from Montenegro and those from Central Europe: the collection from Montenegro differs in three nucleotide positions, while three of them are heterozygous. The nucleotide polymorphism among the ITS sequences is described in Tab. 2.

All newly obtained sequences form a well-supported clade in the Neighbor-Joining dendrogram and the analysis neither revealed any significant differences between specimens with distinct fructification pattern. The results resemble those by Campanile et al. (2004), who studied genetic variability of *Fuscoporia torulosa* on different hosts in southern Italy with the RAPD method of DNA fingerprinting. The authors found that specimens from different hosts (hardwoods and

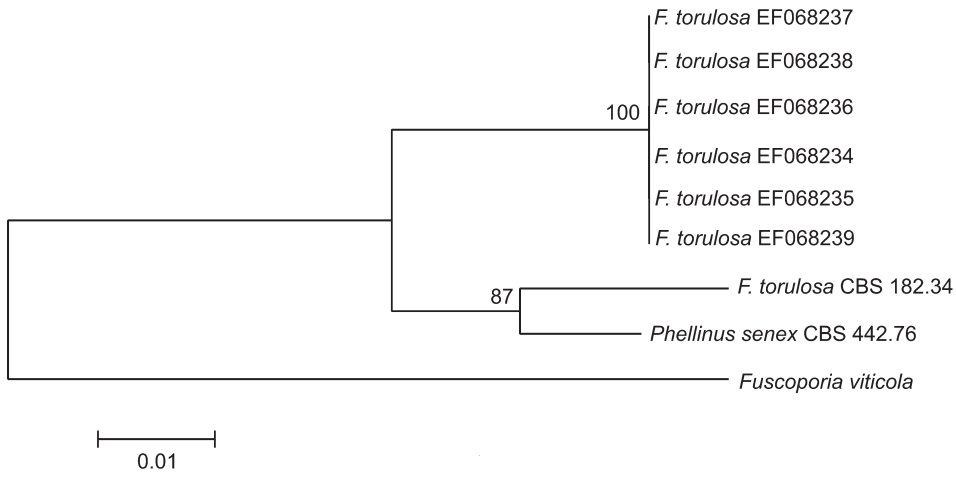

Fig. 2. Neighbor-Joining tree of *Fuscoporia torulosa* and related taxa based on sequences of the ITS region. Bootstrap values are shown at the nodes. The bar indicates the number of substitutions per position.

conifers) form one, outcrossing species. Our result is also supported by morphological features which do not differ in the specimens with distinct kinds of fructification. The differences in position of basidiocarps on host trees might be better solved with a study of wood structure features of the tree distinct species. Generally, *Quercus* and *Cerasus* belong to hardwood trees, while *Pyrus* is a sapwood tree (Wagenführ 1999). Nevertheless, *Fuscoporia torulosa* has a rather wide range of host species and some of the hosts are also other sapwood tree genera (e.g. *Acer*, *Alnus*, *Betula* and *Carpinus*) but no records about fructification of the fungus on the higher part of stems or branches of these woody plants are known (Kotlaba 1975, Ryvarden and Gilbertson 1994).

In comparison to the high similarity of the newly obtained sequences, the ITS sequence of *Fuscoporia torulosa* from the GenBank (accession no. AY558649; DNA from culture CBS 182.34) shows higher similarities to *Phellinus senex* than to our sequences of *Fuscoporia torulosa.* On the other hand, culture CBS 182.34 was isolated in the USA by Overholts in 1934 (Anonymus) and according to results by Rizzo et al. (2003) the distribution of genuine *F. torulosa* in Northern America is questionable. The relationship between *F. torulosa* and *Phellinus senex* is also discussed by Parmasto (1985), who describes the occurrence of intermediate forms between the two species in North India. Rizzo et al. (2003) mention a mitSSU DNA sequence of *P. senex* from Hungary but unfortunately no reference to a herbarium specimen or an exact locality of the record is cited. To confirm the identity of our specimens, we also sequenced the mitSSU region of one selected

specimen of *Fuscoporia torulosa* (Tab. 1) and the obtained DNA sequence (EF068240) appeared to be identical to those of the same species published by Rizzo et al. (2003) originating from Austria, France and Italy (AF387587–AF387589). According to data available so far (Bernicchia 2005, Ryvarden and Gilbertson 1994) *Phellinus senex* has not been recorded in Europe. In contrast to this statement, basidiocarps typical of *Fuscoporia torulosa* and *Phellinus senex* may be found intermingled in the Crimea (Ukraine) according to Parmasto (1985). This implies that *P. senex* might occur in warmer parts of the easternmost part of Europe. Such populations encompassing possible hybrids between the two species require further genetic studies, which may result in a confirmation of the occurrence of a new species to Europe.

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REFERENCES

ANONYMUS (Internet): Search CBS Filamentous Fungi database. – www.cbs.knaw.nl/databases/index.htm.

ANTONÍN V., GRUNA B., HRADÍLEK Z., VÁGNER A. and VĚZDA A. (2000): Houby, lišejníky a mechorosty národního parku Podyjí [Fungi, lichens and mosses of National Park Podyjí]. – 220 p. Brno.

BERNICCHIA A. (2005): *Polyporaceae* s.l. – In: Fungi Europaei, vol. 10, p. 1–808, Alassio.

CAMPANILE G. and LUISI N. (2004): The occurrence of *Phellinus torulosus* in Apulia and Basilicata (Southern Italy): identification of isolates by morphologic, microscopic, and molecular means. – Phytopathologia Mediterranea 43: 289–298.

FIASSON J. L. and NIEMELÄ T. (1984): The *Hymenochaetales*: a revision of the European poroid taxa. – Karstenia 24: 14–28.

FISCHER M. (2002): A new wood-decaying basidiomycete species associated with esca of grapevine: *Fomitiporia mediterranea (Hymenochaetales)*. – Mycological Progress 1: 315–324.

FISCHER M. and BINDER M. (2004): Species recognition, geographic distribution and host-pathogen relationships: a case study in a group of lignicolous basidiomycetes, *Phellinus* s.l. – Mycologia 96: 799–811.

FISCHER M. and BRESINSKY A. (1992): *Phellinus torulosus* – sexuality and evidence of intersterility groups. – Mycologia 84: 823–833.

GILBERTSON R. L. and BURDSALL H. H. (1972): *Phellinus torulosus* in North America. – Mycologia 64: 1258–1269.

HALL T. A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – Nucleic Acids Symposium Series 41: 95–98.

ISIKOV V. P. and KUZNETZOV V. N. (1990): Bioecological characteristics of *Phellinus torulosus* (Pers.) Bourd. et Galz. and *Ganoderma applanatum* (Pers. ex Wallr.) Pat. in the Crimea. – Mikologiya i Fitopatologiya 24(6): 513–519.

JEONG W. J., LIM Y. W., LEE J. S. and JUNG H. S. (2005): Phylogeny of *Phellinus* and related genera inferred from combined data of ITS and mitochondrial SSU rDNA sequences. – Journal of Microbiology and Biotechnology 15: 1028–1038.

KOTLABA F. (1962): Mykózy ovocných stromů působené vyššími houbami stopkovýtrusými [Mycoses of fruit trees caused by Basidiomycetes]. – In: Benada J. and Špaček J., Zemědělská fytopatologie, vol. 4, p. 98–176, Praha.

KOTLABA F. (1975): Geographical distribution and ecology of the polypore *Phellinus torulosus* (Pers. ex Pers.) Bourd. et Galz. with special regard to Czechoslovakia. – Česká mykologie 29: 5–24.

KOTLABA F. (1984): Zeměpisné rozšíření a ekologie chorošů (*Polyporales* s.l.) v Československu [Geografical distribution and ecology of polypores (*Polyporales* s.l.) in Czechoslovakia]. – 194 p. Praha.

KUMAR S., TAMURA K. and NEI M. (2004): MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. – Briefings in Bioinformatics 5: 150–163.

LARSEN M. J. and COBB-POULLE L. A. (1990): The genus *Phellinus (Hymenochaetaceae)* – a survey of the world taxa. – In: Synopsis Fungorum, vol. 3, p. 1–206, Oslo.

NÚÑEZ M. and RYVARDEN L. (2000): East Asian polypores, Part 1. – In: Synopsis Fungorum, vol. 13, p. 1–168, Oslo.

PANCONESI A., SANTINI A. and CASINI N. (1994): *Phellinus torulosus* on *Cupressus sempervirens* in Italy. – European Journal of Forest Pathology 24: 238–240.

PARMASTO E. (1985): The species concept in *Hymenochaetaceae* (Fungi, Hymenomycetes). – Proceedings of the Indian Academy of Sciences-Plant Sciences 94: 369–380.

RIZZO D. M., GIESER P. T. and BURDSALL H. H. (2003): *Phellinus coronadensis*: A new species from southern Arizona, USA. – Mycologia 95: 74–79.

RYVARDEN L. and GILBERTSON R. L. (1994): European polypores, Part 2. – In: Synopsis Fungorum, vol. 7, p. 394–743, Oslo.

VLASÁK J. (2006): *Phellinus* – Polypores, Collection of Dr. Josef Vlasák, Hluboká nad Vltavou, Czech Republic. – http://botanika.bf.jcu.cz/mykologie/polypores /list_phellinus.html

WAGENFÜHR R. (1999): Holz. Anatomie – Chemie – Physik. Anatomie des Holzes. – 188 p. Leipzig.

WAGNER T. and FISCHER M. (2001): Natural groups and a revised system for the European poroid *Hymenochaetales* (Basidiomycota) supported by nLSU rDNA sequence data. – Mycological Research 105: 773–782.

WAGNER T. and FISCHER M. (2002): Proceedings towards a natural classification of the worldwide taxa *Phellinus* s.l. and *Inonotus* s.l., and phylogenetic relationships of allied genera. – Mycologia 94: 998–1016.

WHITE T. J., BRUNS T. D., LEE S. B. and TAYLOR J. W. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – In: Innis M. A., Gelfand D. H., Sninsky J. J. and White T. J., PCR Protocols, A guide to methods and applications, p. 315–322, New York.