University of South Bohemia in České Budějovice
Faculty of Science, Department of Botany

Research of polypores focused at the population structure of selected species

Summary of Ph. D. Thesis

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Introduction

The polypore fungi represent an important group of organisms in many natural ecosystems, where they play crucial part in wood degradation and forest soil restitution. Due to the intensive exploitation of forested regions, many species become critically endangered, as their natural habitat quickly disappears. Compared to other organisms, this alarming state in many fungi is mostly ignored as regards sustainable management and protection, and one of the reasons is difficult documentation of species diversity and intraspecies genetic variability reduction. However, modern techniques of molecular biology bring a fundamental advance in fungal studies and enable precise and reliable evaluation of fungal diversity. Extensive exploitation of these new methods in fungal population studies may provide arguments for fungi protection in near future.

Submitted doctoral thesis deals with several polypore species that were examined primarily by using molecular and genetic methods. Population biology, ecology and taxonomy of selected species were studied. Evidence is provided that genetic and especially molecular methods are powerful tool in the research of variability in fungi. My belief that by their more intensive use will not only disrupt classical system of fungi, but also enable to get new, principal knowledge of their biology is substantiated by results presented in several papers. I also hope that this polypore study raises the interest of future students in this very interesting and scientifically perspective group of fungi.

The aims of the Thesis

The aim of the Thesis was the development and application of modern genetic and molecular methods to polyporaceous fungi and their use in solving specific problems in polypore ecology and systematic. The original objects of the study were the natural populations of two polypores: *Trametes gibbosa* (Pers.) Fr., as an example of widespread, invasive polypore and *Fomitopsis rosea* (Alb. & Schw.:Fr.) Karst., a rare and rapidly disappearing species. In the course of study, several other remarkable, little known or endangered species were collected and investigated in terms of their ecology and taxonomy. New species and one new combination were described, based mainly on rDNA ITS sequence data. Four papers were devoted to mycogeography and ecology of polypores. Accordingly, the thesis has three main sections: **Population studies, Taxonomy, Mycogeography and Ecology**.

List of published and accepted papers on which thesis is based.

Kout J, Vlasák J. 2007. *Trametes gibbosa* (*Basidiomycetes*, *Polyporales*) in the USA and Canada. Can. J. Bot. 85: 342–346.

Kout J, Vlasák J. 2009. *Antrodia serialiformis* from the eastern USA, a new and abundant polypore similar to *Antrodia serialis*. Mycotaxon 108: 329–335.

Kout J. 2008. *Sistotrema dennisii* (*Basidiomycetes*, *Sistotremataceae*) – a new species for the Czech Republic. Czech Mycol. 60: 105–111.

Kout J, Vlasák J. 2009. Vzácné choroše z České republiky, zejména z jižních Čech. Mykol. Listy 108 (accepted). [Rare polypores from the Czech Republic, especially from the South Bohemia, in Czech]

Kout J, Vlasák J. 2009. Notes on two species of *Diplomitoporus* (*Basidiomycota*, *Polyporaceae*) of Central America. Revista Mexicana de Biodiversidad (accepted).

Unpublished (submitted and manuscript).

Kout J. et Vlasák J. Microsatellite DNA markers in the polypore *Fomitopsis rosea* and their use for characterization of its relict populations in Vltava River Valley, Czech Republic.

Vlasák J, Kout J, Dvořák D. 2009. Taxonomical position of polypore *Dichomitus albidofuscus*: *Donkioporia albidofusca* comb. nov. Mycol. Prog. (submitted).

Kout J, Vlasák J, Dvořák D. 2009. New collections and ecology of rare polypore *Dichomitus albidofuscus*. Czech Mycol. (submitted).

Population studies

Kout J, Vlasák J. 2007. *Trametes gibbosa* (*Basidiomycetes*, *Polyporales*) in the USA and Canada. Can. J. Bot. 85: 342–346.

European polypore *Trametes gibbosa* was for the first time confirmed on the American continent using compatibility tests of monocaryotic mycelia. Compatibility between European and U.S. samples was 100%, as well as among European samples. But the most interesting result was that the mycelia from spores of different American individuals were often incompatible, which is a very rare phenomenon in higher fungi. Two samples from Canada collected at remote localities had three of four mating factors in common and one of them shared two respectively three factors with samples from the several hundred km distant Pennsylvania. We interpreted this as an invasion of European species in North America from a few founders. The work was inspired by rare findings in Pennsylvania in 2001, and was based on samples collected in 2006, when *Trametes gibbosa* was already an abundant species in America. Currently, it is the most abundant polypore in Pennsylvania, USA, at least.

Kout J, Vlasák J. Microsatellite DNA markers in the polypore *Fomitopsis rosea* and their use for characterization of its relict populations in Vltava River Valley, Czech Republic (manuscript).

The beautifully colored species *Fomitopsis rosea* is an exactly opposite case of a rare polypore specialized on unique habitat of virgin forest preserves. Its strict ecological dependence on old spruce trunks standing dead for several years ranks it at the list of endangered species. The work was inspired by the fact that we have discovered (me and my supervisor) twelve new, small, relict populations in the valley of the Vltava River.

We intended to determine whether these small, lowland populations are different from large populations in the Boubín and Žofín Virgin Forest Preserves and from even much larger populations in the Carpathians and in the north-eastern United States.

As the rDNA ITS sequencing revealed only very low variability, I decided to develop microsatellite markers that were not yet used in polypore genetics. 23 microsatellites were developed from *Fomitopsis rosea* genomic DNA using alkaline phosphatase-labelled probes $(GGT)_n$, $(CTTT)_n$, $(ACTG)_n$ and $(ACAG)_n$ but only two of them were sufficiently polymorphic and could be readily amplified from all or nearly all samples. I isolated DNA from about 100 samples of *F. rosea*, amplified microsatellite sequences which required painstaking optimization of PCR reactions, analyzed amplified DNA on PAGE gels and finally sequenced all amplified DNAs. Isolated microsatellites Fro2 and Fro16 proved to be extremely polymorphic giving unique genotypes in most of the samples. Also, their nature was surprisingly complex: in Fro2 is simple GGT repeat linked to very variable haplotypes in near flanking region; in Fro16 there is unprecedented complex repeat $(CCTAA)_{2\text{-}15}$, $(GATAA)_{1\text{-}13}$, $(GCCCT)_{1\text{-}9}$, $(GCCT)_{4\text{-}27}$, $(GTCT)_{4\text{-}13}$. I could find neither explanation for such a uniquely complex structure nor suitable statistical programs for their analysis and so I succeeded to publish the results only in the form of a poster on the International Conference. At present, an article is prepared in the manuscript, but better statistical processing of data is still missing and I try to work it up.

Taxonomy of polypores

In the course of population studies we encountered several taxonomical problems that I solved using our genetic and molecular techniques. Though incidental and subsidiary this work was, the results could be easier and faster published than population studies.

The combination of morphological examination, sequencing of the ribosomal DNA and monosporic mycelia pairing tests (only in the case of *A. serialiformis*) was used in two studies.

Kout J, Vlasák J. 2009. *Antrodia serialiformis* from the eastern USA, a new and abundant polypore similar to *Antrodia serialis*. Mycotaxon 108: 329–335.

A new and common polypore *Antrodia serialiformis* from eastern USA is described. The species is very similar to *Antrodia serialis* and both species were probably confused previously, but *A. serialiformis* seems to be living exclusively on oaks. We present evidence that the new species differs from *A. serialis* on account of its much smaller spores, mating incompatibility, rDNA sequence and ecology.

Vlasák J, Kout J, Dvořák D. 2009. Taxonomical position of polypore *Dichomitus albidofuscus*: *Donkioporia albidofusca* comb. nov. Mycol. Prog. (submitted).

The remarkable polypore *Dichomitus albidofuscus* was till recently known only from two localities in the world. We present evidence that classification into the artificial genus *Dichomitus* was not correct. We noted that external morphology is very similar to another rare polypore, *Donkioporia expansa*. This observation was confirmed by a detailed study of micromorphology, as well as by comparison of rDNA ITS sequences. A new combination *Donkioporia albidofusca* was proposed and genus *Dokioporia* was emended.

Ecology and mycogeografy

Some other papers refer to ecology and distribution of rare species, collected at random in the survey of *Fomitopsis rosea* or abroad.

Kout J. 2008. *Sistotrema dennisii* (*Basidiomycetes*, *Sistotremataceae*) – a new species for the Czech Republic. Czech Mycol. 60: 105–111.

Sistotrema dennisii Malençon (Basidiomycetes, *Sistotremataceae*) was discovered in the Czech Republic for the first time. It is an inconspicuous, resupinate species with arachnoid pores that could have been neglected in the field. Characteristic features of this and similar species are presented, together with a description of the locality and notes on a rare polypore distribution.

Kout J, Vlasák J. 2009. Vzácné choroše z jižních Čech. Mykol. Listy 108 (accepted).

Numerous new finds are reported of the rare polyporaceous fungi from the South Bohemia and other places in the Czech Republic. Some species have been found only scarcely before not only in CZ but wherever in Europe. A short description of the new locations in the Czech Republic is given for each species.

Kout J, Vlasák J. 2009. Notes on two species of *Diplomitoporus* (*Basidiomycota*, *Polyporaceae*) of Central America. Revista Mexicana de Biodiversidad (accepted).

During the expedition in Central America there were collected many fungal specimens. Two species of *Diplomitoporus* are studied from this area and notes about their distribution are presented. Most surprising was the collection of reputed Brazilian endemite, rare *Diplomitoporus dilutabilis* Log.-Leite & J.E. Wright in Guatemala that is comprehensively discussed in the paper. *Diplomitoporus hondurensis* (Murrill) Ryvarden was found in a new locality inBelize. A list of *Diplomitoporus* species cited from America is presented.

Kout J, Vlasák J, Dvořák D. 2009. New collections and ecology of rare polypore *Dichomitus albidofuscus*. Czech Mycol. (submitted)

Ecology and distribution of extremely rare polypore *Dichomitus albidofuscus* was explored in the Czech Republic. All localities from the Czech Republic seem to have the same character of medium altitude, old forest with beech, linden, oak, with substrate trees of slowly dying spruce and fir intermixed. Collections from abroad, however, suggest much broader environmental optimum. Once established on the locality, *D. albidofuscus* is very persistent here, infecting efficiently all perspective trees but only those occuring in very short distance.

Curriculum vitae

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Date of birth: 3.11.1980
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Education

1999–2002: Bachelor degree: Introductory Biology Programme, Faculty of Biological Sciences, University of South Bohemia. Thesis: Ptačí reakce na atrapy predátorů. [Reactions of birds to the dummies of predators, 42 pp., in Czech with English summary].

2002 – 2005: Master degree: Zoology Thesis- Ecology and Ethology of vertebrates, Faculty of Biological Sciences, University of South Bohemia. Thesis: Antipredační chování sýkory modřinky (*Parus caeruleus*) a sýkory koňadry (*Parus major*). [Antipredation behavior of the Blue Tit (*Parus caeruleus*) and the Great Tit (*Parus major*) 42 pp., in Czech with English summary].

2005 – present: Student of PhD programme in Botany, Faculty of Biological Sciences, University of South Bohemia.
PhD thesis topic: Research of polypores focused at the population structure of selected species.

Conferences

Kout J, Fuchs R. 2004. Antipredační chování sýkory koňadry (*Parus major*) a sýkory modřinky (*Parus caeruleus*). [Antipredation behavior of the Blue Tit (*Parus caeruleus*) and the Great Tit (*Parus major*) in Czech]. Zoologické dny 2004, Brno, Czech Republic.

Kout J, Fuchs R. 2006. Antipredation behavior of the Blue tit (*Parus caeruleus*) and the Great Tit (*Parus major*). 4th North American Ornithological Conference, 3.10. – 7.10. 2006, Veracruz, Mexico.

Kout J, Vlasák J. 2007. Genetic structure of small populations of the rare polypore *Fomitopsis rosea* in Vltava River Valley, Czech Republic. XV Congress of European Mycologists, Saint Petersburg, Russia, September 16 – 21, 2007.

Kout J, Vlasák J. 2008. Mykologický průzkum strmých strání v údolí Vltavy se zaměřením na vzácné druhy chorošů. Populační studie *Fomitopsis rosea*. Výroční konference České vědecké společnosti pro mykologii, České Budějovice, 16.2.2008.

Stay abroad

Institute of Forest Ecology, Slovak Academy of Science, Slovak republic - Branch for Wood Plants Biology Nitra, doc. Ing. Gabriela Juhásová, CSc. (short stage in 2008).

Project

Kavková M. et Kout J. (spoluřešitel) (2008): Aplikovaná mykologie – modelová výuka praktické mykologie. FRVŠ 2122/2008/F4/a

Mycological surveys

NPR Žofínský prales (Polyporales) (2005)
PP V Houlištích (2005)
PR Polánecký mokřad (2005)
PP Pod Smutným koutem (2005)
PP Louka U Šnajberského rybníka (2005)
PP Krasíkov (2006)
PR Hradišťský vrch (2006)
PP Prameniště Kateřinského potoka (2006)
PP Roudný (2007)
VKP Tyršův sad (2008)

Publications

Kout J. 2005 – 2006. Krčský rybník u Městce Králové, nejjižnější bod Rožďalovických rybníků. Vlastivědný zpravodaj Polabí 38: 247–252.

Kout J. 2006. Nálezy dvou zajímavých a vzácných druhů hub ze skupiny *Aphyllophorales* v Čechách - *Hericium erinaceus* a *Aleurodiscus disciformis*. Mykol. Listy 96: 20–23.

Kout J. 2006. Chorošovité houby přírodní památky V Houlištích a dvě nové lokality *Pycnoporellus fulgens* pro ČR. Mykol. Listy 97: 17–21.

Kout J. 2007. Velmi vzácný druh pevníkovka žloutková – *Stereopsis vitellina* – na jihu Čech. Mykol. Listy 99: 13–15.

Kout J. 2008. *Sistotrema dennisii* (*Basidiomycetes*, *Sistotremataceae*) – a new species for the Czech Republic. Czech Mycol. 60: 105–111.

Kout J. 2009. Mecháček síťnatý (*Arrhenia retiruga*) (*Agaricales*, *Tricholomataceae*) v České republice ještě nevyhynul. Erica (submitted).

Kout J, Vlasák J. 2007. *Trametes gibbosa* (*Basidiomycetes*, *Polyporales*) in the USA and Canada. Can. J. Bot. 85: 342–346.

Kout J, Vlasák J. 2009. *Antrodia serialiformis* from the eastern USA, a new and abundant polypore similar to *Antrodia serialis*. Mycotaxon 108: 329–335.

Kout J, Vlasák J. 2009. Vzácné choroše z České republiky, zejména z jižních Čech. Mykol. Listy 108 (accepted).

Kout J, Vlasák J, Dvořák D. 2009. New collections and ecology of rare polypore *Dichomitus albidofuscus*. Czech Mycol. (accepted).

Vlasák J, Kout J, Dvořák D. 2009. Taxonomical position of polypore *Dichomitus albidofuscus*: *Donkioporia albidofusca* comb. nov. Mycol. Prog. (submitted).