PhD dissertation – thesis

Taxonomy and conservation status of the lignicolous basidiomycetes in Juhdöglő-völgy Forest Reserve

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The applicant met the requirement of the PhD regulations of the Corvinus University of Budapest and the thesis is accepted for the defense process.

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Head of PhD School                           Supervisor
1. INTRODUCTION AND OBJECTIVES

As a result of human activity, the forest habitats with high biodiversity are decreasing. Those forests which still maintain their natural state are need to be protected. For this purpose the Forest Reserve Programme of Hungary was established, and several forest areas were selected and are protected by the law. In the forest reserves, significantly greater amount of deadwood accumulates in much more varied quality (e.g. diameter, decay phase), than in the areas under forestry management, due to the natural ecological processes. The deadwood gives substrate for many organism groups (e.g. insects, mosses, fungi), which play an important role in the forest ecosystem. The research on the occurrence and importance of lignicolous fungi (e.g. LONSDALE et al. 2008) which can be found in nearly every taxonomical groups under Basidiomycota, and have key role in the decay of wood material, is a significant part of „deadwoodology” (the scientific field dealing with the ecology of deadwood) (GROVE 2002).

The earlier Hungarian fungistical research focusing to the assessment of macrofungi, aimed to explore the whole range of fungi in varied habitats of a specific area. Many of these studies are dealing with the funga of the Hungarian mountain areas: e.g. BENEDDEK (2002, 2011), KOZŠKA (2011, 2014), LOCŠMÁNDI (1993), PÁL-FÁM (2001), PAPP (2009), RUDOLF et al. (2008), SILLER (2004), SZEMERE (1968), VASÁS (1985), VASÁS and LOCŠMÁNDI (2009).

Comparing Vértes with the other Hungarian mountains, it belongs to the least, or moderately explored areas: e.g. BABOS (1978, 1989), BOHUS (1939), KOZŠKA (2011, 2014), RIMÓCZI (1994).

No systematic fungistical examinations were carried out before in the Juhdöglő-völgy Forest Reserve located in the Vértes Mts, which is very rich in deadwood. In the present study the following main aims were pursued: (1) to reveal the diversity of lignicolous macromycetes of Basidiomycota in the Juhdöglő-völgy Forest Reserve, and carry out their taxonomic evaluation based on micro- and macromorphological characteristics, supplied by phylogenetic methods; (2) to assess the conservation status of the documented fungal species, and to define the real natural value of the area; (3) to clarify any arising systematic or nomenclatural question.
2. MATERIAL AND METHODS

2.1. Characteristics of the investigated area

Vértes Mts, is part of the Transdanubian Mountains, and it covers an area of 314 km$^2$ located between Bakony Mts and Gerecse Mts. It is separated from Bakony Mts and Gerecse Mts by the Móri-árok and Szár-Tatabánya, respectively. The majority of Vértes Mts is protected area. Its northern region forms part of the Észak-Vértesi Természetvédelmi Terület. The southern and southwestern areas belong to the Vértesi Tájvédelmi Körzet, which was established in 1976.

The Juhdöglő-völgy became state-owned in 1950, later county protected in 1975. From 1976 it is part of the Vértesi Tájvédelmi Körzet. Juhdöglő-völgy as a specially protected forest area has been declared to be forest reserve by the 14/2000. (VI. 26.) KöM decree, and the 80.8 ha area has been named as „Juhdöglő-völgy Forest Reserve”.

The reserve lies on the southern side of Vértés, to the north of Csákberény Township, located in the areas of Duna-Ipoly Nemzeti Park Igazgatóság and the operational area of Vértesi Tájvédelmi Körzet. Csákvári Erdészet. Based on the maps of the forest management plans, the forest reserve is bordered from the northeast by Horog-völgy, from the northwest by Nagycseresznyés-völgy (Figure 1).

Based on its climate type it belongs to the moderately chilly, moderately wet (beech climate) group. The annual average temperature is 9°C. The precipitation is about 650 to 700 mm per year. The height above sea level is from 300 to 400 m; the bedrock is upper Triassic dolomite, the soil type is rendzina, the topsoil is shallow or medium deep. On the 25.7 ha core area of the forest reserve no forest management practices are being applied, and around the core area there is a 55.1 ha protection zone under natural forest management (TAKÁCS és TAKÁCSNÉ 1996).

On the bottom of the valley the botanical composition is typically western medium mountain beech [Daphno laureolae-Fagetum (Isépy 1970) Borhidi in Borhidi et Kevey 1996], which is gradually mixed with downy oak (Quercus pubescens Willd.), manna ash (Fraxinus ornus L.), and rock lawn mosaics heading upwards on the northern valley sides. Lastly, on the plateaus it changes to calcareous oak forest [Vicio sparsiflorae-Quercetum pubescentis Zólyomi ex Borhidi et Kevey 1996] (BORHIDI 2003).

The core area of the forest reserve is close to its natural state, which is also confirmed by the forest management plans. According to these the oldest beech
trees are 227 years old. Snags and the great amount of lying dead trees and stumps with varied structures are typical to the core area of the reserve. Due to the lack of any kind of forest management in the forest reserve, the dead trees do rot on the exact place where they have fallen; and the forest regenerates by seeds or suckers.

Figure 1. The geographical location of Juhdöglő-völgy Forest Reserve (BARTHA and ESZTÓ 2001 modified). A) the area of the reserve within Vértes; B) borders of the protection area (red frame) and the core area of the reserve (yellow frame) on the air photo (source: www.erdorezervatum.hu) of FÖMI (Eurimage).
2.2. Selection of sample trees

In order to explore, as detailed as possible, the fruiting body based presence of the lignicolous basidiomycetes, living in the core area of the reserve, instead of the territorial quadrates, 121 sample trees (microhabitats) have been selected with different diameters and in various development stages, considering the lifestyle of the taxa. Based on the fruiting body formation, the occurrence of lignicolous basidiomycetes on the logs marked with numbers were monitored, in all the three important aspects of the year (spring, summer, autumn), for three years. Besides the documentation of the quality traits of the oak \(Qercus petraea\) (Matt.) Liebl., \(Q.\) pubescens Willd.] and beech [\(Fagus sylvatica\) L.] logs, I have recorded their exact position by GPS coordinates, which provide detailed information about the habitat or substrate of any given fungal taxon.

The statistical analysis of this background data is not included in the objectives of the present thesis, however they might be the basis of future ecological studies.

2.3. The sampling dates and the methods of processing the collected materials

In the core area of Juhdögő-völgy Forest Reserve, field samples were collected from 2011 to 2014, altogether 45 times. All detected taxa during these field sampling were documented in minutes. Furthermore, in most cases fungarium material were collected as a proof. Photo documentations were also made, \textit{in situ} and/or \textit{ex situ}, of the majority of species. The fungarium samples were dried in a 250 W performance drying machine; and then they were placed to my own fungarium (PV), or to the Botanical Department of Hungarian Natural History Museum (BP) in labelled zipper storage bags.

2.4. Equipments used for the identification of lignicolous basidiomycetes

2.4.1. Monographs and keys

The most common method of basidiomycetes identification is still based on the examination of the macro- and micromorphological characteristics of the basidiocarp. The literature used for the identification of single fungal groups (taxa, morphotypes) is herein reviewed.
Identification of agaricoid and gasteroid species

For the identification of agaricoid and gasteroid species, the second expanded edition of Funga Nordica was used (KNUDSEN és VESTERHOLT 2012). Considering that this literature contains the fungi of Skandinavian countries, for the precise identification further European monographs were used: Strophariaceae s. l. (NOORDELOOS 2011), Crepidotus spp. (CONSIGLIO és SETTI 2008), Entoloma spp. (NOORDELOOS 1992, 2004), Mycena spp. (ROBICH 2003).

Identification of corticioid [incl. hidnoid, raduloid, odontoid, steroid] species

The corticioid (Corticiaceae s. l.) species were identified based on literatures summarizing North European species (ERIKSSON és RYVARDEN 1973, 1975, 1976; ERIKSSON et al. 1978, 1981, 1984; HJORTSTAM et al. 1987, 1988), and the works of KRIEGLSTEINER (2000) and JAHN (1971), and the European monograph of BERNICCHIA and GORJON (2010) were also used.

Identification of poroid species

The identification of poroid species was carried out on the basis of the European monographs of RYVARDEN and GILBERTSON (1993, 1994), BERNICCHIA (2005), and RYVARDEN and MELO (2014). The following genus identification books for were used: Antrodiella spp. (MIETTINEN et al. 2006, VAMPOLA and POUZAR 1996); Inonotus s. lato (RYVARDEN 2005).

2.4.2. Equipments used for the microscopic examinations

For the microscopic examination of the corticioid samples 5% KOH solution was used, and glycerin water was used for the agaricoid fungus. The hyaline elements were dyed with Cotton Blue and Congo Red solutions. Melzer reagent were used for testing the amyloid (positive reaction, the element shows bluish, blackish coloration), dextrinoid (the examined element change to a brownish or reddish brownish color), and inamiloid (negative reaction, the color of the element does not change, or only a very light yellowish brownish coloration can be seen) reactions. I used Zeiss Axio Imager.A2 type light microscope for the microscopic examinations, and the photos were made by Zeiss AxioCam HRc camera. Measurements were done with a 100× oil immersion objective (1000× magnification). The line drawings were made with a drawing tube.

2.4.3. Equipments and methods used for molecular analysis

Altogether 14 basidiomycetes taxa collected from the Juhdöglő-völgy Forest Reserve have been analyzed with molecular methods, besides the morphological characterization. Considering that I had limited opportunities for molecular
investigations, those samples were preferably selected, which could not be identified clearly based on their morphological characteristics, or which were new taxa to the Hungarian flora. For the phylogenetic analyses yet unpublished sequences of Hungarian samples (DB2529, DB3859), originating from the fungarium of Bálint Dima (DB), and the holotype of the Crepidotus ehrendorferi Hauskn. & Krisai species (WU6554) were also used.

The samples used for DNA extraction originate from dried fungariums. The DNA extraction and the polymerase chain reaction (PCR) was carried out in one step by the Phire® Plant Direct Kit (Thermo Scientific, USA), following the instructiones of the producer. ITS region of the Ribosomal DNA of the nucleus (rDNA) had been amplified by the ITS1F-ITS4 primer pair (GARDES és BRUNS 1993, WHITE et al. 1990). In the case of the PV983 sample, besides the ITS region, a large subunit region of the rDNA (LSU) was also investigated. The success of the PCR amplification was checked by running the product on electrophoresis gel. The direct sequencing, with the primer pairs used for the PCR, was carried out by the LGC Genomics (Berlin, Germany). The Pregap4 and Gap4 softwares of the Staden package was used for analyzing the chromatograms (STADEN et al. 2000). The obtained sequences were completed with already published sequences of the GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and UNITE (http://unite.ut.ee/) databases, in order to have both closely relative, and morphologically similar species in the phylogenetic analyses. Multiple sequence alignment was done by PRANK (LÖTTYNOJA and GOLDMAN 2005) as implemented in its graphical interface (PRANKSTER) under default settings. The result of the alignment was evaluated visually by using the AliView (LARSSON 2014), and the SeaView 4 (GOUY et al. 2010) softwares, and if it was necessary I corrected it manually. The phylogenetically informative indels were coded following the simple indel coding algorithm (SIMMONS et al. 2001) with the program FastGap 1.2 (BORCHSENIEUS 2009). Adding indel characters to the nucleotide alignment of ITS sequences increases the robustness of the phylogenetic analyses (NAGY et al. 2012); the final matrix including nucleotide and binary data. Maximum Likelihood (ML) analysis was carried out using RAXML (STAMATAKIS 2014) in raxmlGUI (SILVESTRO & MICHALAK 2012). Rapid bootstrap analysis and 1,000 replicates under the GTRGAMMA substitution model was used for the partitioned alignment (ITS + indels). For the phylogenetic analysis of Entoloma tjallingiorum Noordel., and its relative species, besides the ML estimation, I used Bayesian inference (BI) analyses were performed with MrBayes 3.1.2 (HUELSENBECK & RONQUIST 2001; RONQUIST & HUELSENBECK 2003). The nucleotide and indel characters were split into two partitions to which the GTR + G and two-parameter Markov model, respectively, were applied. Four Markov chains were run for 5,000,000 generations, sampling every 100th generation. Burn in of 12,500 trees was established. Sampled trees were combined in a 50 % majority rule consensus phylogram and posterior probabilities (PP) were calculated. The obtained consensus ITS phylogenetic trees were visualized with the MEGA6 softwear package (TAMURA et al. 2013).
3. RESULTS AND DISCUSSION

I have recorded 223 lignicolous basidiomycetes from the Juhdöglő-völgy Forest Reserve, in 45 days on the field, during the period of 2010 to 2014 (Table 1.).

Table 1. The number of genera and species of lignicolous basidiomycetes, recorded from the area of Juhdöglő-völgy Forest Reserve, classified regarding to their orders and families. *new species to the Vértes Mts; **new species to Hungary.

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**Total**: 44 genera, 93 species.
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Only one lignicolous basidiomycetes had been recorded which belongs to the class of rust fungus [Pucciniomycotina R. Bauer et al.], thus not included to the subphyla of true basidiomycetes [Agaricomycotina R. Bauer et al.]: Phleogena faginea (Fr. & Palmquist) Link.

Concerning the systemic distribution of the 222 species of the Agaricomycotina subphyla which were recorded in the core area of the forest...
reserve, they belong to 3 classes, 13 orders, 51 families, and 121 different genera. However, within the particular taxonomic ranks, the proportion and number of lignicolous fungal species varies. The diversity of species is significantly lower in the more ancient classes which typically form heterobasidium [Tremellomycetes Dowell és Dacrymycetes Doweld], than of the Agaromycetes class, since they accounted for slightly more than 2% of Agaricomycotina subphyla. Agaricales Underw. (93 species) and Polyporales Gäum. (73 species) have the greatest importance from the 10 orders of Agaricomycetes Doweld class, based on the number of recorded species.

In the case of 55% of the recorded and identified species from the 25.7 ha core area of the forest reserve (122 species), no data was found in the Hungarian literature concerning their earlier occurrence in the mountain (BABOS 1989, BOHUS 1939, KOSZKA 2011, 2014, RIMÓCZI 1994).


The taxon with the fungarium number PV983 is presumed to be a new species based on its micro- and macromorphological characteristics, and the analyses of its ITS and LSU sequences carried out so far. It may differ from the yet known species even at the genus level. However, to prove these statements further molecular and morphologic investigations are necessary.
The nature protection significance of Juhdöglő-völgy Forest Reserve in reference to the lignicolous basidiomycetes

The age or the naturality of the forest is often thought to be indicated by the structure of the forest; however, this does not necessarily shows the degree of biodiversity of the habitat (BLASI et al. 2010). It is also possible to conclude the age of the forests by assessing the diversity of the different groups of organisms (e.g. mosses, insects, fungi) (JONSSON et al. 2005). The presence or absence of some taxa, and the density of the populations may be markers for the indication of habitat naturality (LANDRES et al. 1988). One of these key groups is represented by the lignicolous macrofungi, considering that it is necessary to preserve the forests in their natural state for the presence and maintains of some taxa.

The list assembled by CHRISTENSEN et al. (2004) contains 21 lignicolous macrofungi species, and is widely used by the mycologists of several European countries as an indicator of the natural state of beech forests (pl. ADAMČIK et al. 2007, AINSWORTH 2005). Until now, 16 species from these have found in the core area of Juhdöglő-völgy Forest Reserve. Hence, the „ancient beech forest” habitat of the forest reserve, despite its small size, belongs to the most valuable beech forests of Europe, from the mycological point of view (Table 2).

There are 12 lignicolous basidiomycetes are protected in Hungary (FOLCZ and PAPP 2014), and 7 of these was proved to be present in the Juhdöglő-völgy Forest Reserve: Ganoderma cupreolaccatum, Grifola frondosa, Hericium cirrhatum, Hypsizygus ulmarius, Pholiota squarrosoides, Pluteus umbrosus, Polyporus tuberaster. The reserve is the only published habitat of Pholiota squarrosoides in Hungary. Furthermore, in the Vértes Mts, Ganoderma cupreolaccatum only occurs in the reserve, as far as we know.

Based on the results of the present study and the data of the literature, it seems that the nature conservation status of many taxa should be reconsidered or possibly revised. In the red list of Hungarian macrofungi (RIMÓCZI et al. 1999) there are 6 basidiomycetes species living on deciduous trees, which are ”threatened with extinction” (CR), and 5 of these can be found in the Juhdöglő-völgy Forest Reserve: Clitopilus hobsonii, Crepidotus crocophyllus, Hericium cirrhatum, Hohenbuehelia mastrucata, Physisporinus sanguinolentus.

In the case of these cultivars I consider the red list classification excessive, and I recommend their revision.
Table 2. The 10 most valuable European beech forest areas, regarding to the presence of the 21 indicator species; according to CHRISTENSEN et al. (2004), and AINSWORT (2004).

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<th>Area</th>
<th>Juhdöglő-völgy (Magyarország)</th>
<th>Stužica (Slovenia)</th>
<th>Rožok (Slovensko)</th>
<th>Fontainelleau (Franciaország)</th>
<th>Zofin (Csehország)</th>
<th>Jaegersborg Dyrehave (Dánia)</th>
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<td>Camarops tubulina</td>
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*The currently accepted name of Ceriporiopsis pannocincta is Gelatoporia pannocincta (Romell) Niemelä (NIEMELÄ 1985). The valid name of Ganoderma pfeifferi is Ganoderma cupreolacatum (Kalchbr.) Z. Igman (IGMÁNDY 1968, PAPP és SILLER 2012). Both NAKASONE (1997), and BERNICCHIA and GORJON (2010) consider Mycoacia nothofagi to belong to the Phlebia genus. CONTU (2007) published the Ossicaulis lachnopus binom based on the Agaricus lachnopus described by Fries. This species have very similar fruiting body to that of Ossicaulis lignitilis. HOLEC és KOLAŘÍK (2012) studied both species with morphological and molecular methods, and they confirmed that O. lachnopus should be separated at the species level. According to their opinion, in the reserve type, natural habitats O. lachnopus occur more often.
4. NEW SCIENTIFIC RESULTS

1. I have carried out systemic fungistical investigations in the area of Juhdöglő-völgy Forest Reserve, for the first time. Altogether 232 lignicolous basidiomycetes were identified in the core area of JDV FR during the 45 field days between 2010 and 2014. Based on my results, considering the lignicolous macromycetes of Basidiomycota, the highest species diversity of Hungarian habitats is in JDV FR.

1.1. Based on the examined literature of the fungistical research of Vértes Mts., 122 species are new to the mountain.


2. Based on the examination of the lignicolous basidiomycetes the following new scientific results may contribute to the revision of the conservation state of Juhdöglő-völgy Forest Reserve.

2.1. From the 21 macrofungi species, which have been assembled by analyzing the data of 106 forest reserves and indicates the natural state of beech forests, the occurrence of 16 was recorded: *Aurantiporus alborubescens* (Bourdot & Galzin) H. Jahn, *Ceriporiopsis gilvescens*

This is the highest number of species among the Hungarian forest reserves, and based on the literature even at European level only in the Slovakian Stužica és Rožok backwoods were this much indicator species recorded.


From this 7 protected species *Ganoderma cupreolaccatum* and *Pholiota squarrosoides* are new to Vértes Mts. As far as we know, among the Hungarian forest reserves, the highest number of protected lignicolous basidiomycetes can be found in JDV FR.

3. The following new results and new binoms were published as results of my taxonomical and nomenclatural investigations:

3.1. Based on ITS sequences I have confirmed that *Entoloma zuccherellii*, *E. pluteisimilis* and *E. sclerotioigenum* can not be separated at species level. However, it seems that they should be separated at subspecies level, based on morphological differencies of these 3 taxa. Considering that the *Rhodocybe zuccherellii* Noordel. & Hauskn. binom was published the most early, I proposed the following two combinations:

- *Entoloma zuccherellii* var. *pluteisimilis* (Noordel. & C.E. Hermos.) V. Papp (Index Fungorum: IF551097) [≡ *Entoloma pluteisimilis* Noordel. & C.E. Hermos.]
- **Entoloma zuccherellii** var. *sclerotiogenum* (Caball., Higelmo, Català & Vila) V. Papp (Index Fungorum: IF551098) [≡ *Entoloma sclerotiogenum* F. Caball., Higelmo, Català & Vila, Errotari]

The following new combination was published reviewing the taxonomical and nomenclatural status of the *Entoloma* and *Clitopilus* genera:

- **Entoloma vernalis** (Har. Takah. & Degawa) V. Papp & Dima (IF551096) [≡ *Clitopilus vernalis* Har. Takah. & Degawa]

### 3.2. Based on the results of the recent phylogenetic studies, and in the revision of the nomenclatural status of *Postia caesia* complex previously distinguished *Cyanosporus* McGinty, I have proposed a new subgenera under the *Postia* genus:


The following new combinations were published based on the revision of the taxonomical and nomenclatural status of *Postia/Oligoporus* genera:


3.3. Based on phylogenetic examinations we came to the conclusion, that *Pholiota squarrosoides* belongs to the section *Pholiota* instead of the section *Adiposae*. (Figure 2.).

Figure 2. ITS phylogeny of *Pholiota squarrosoides* (PV540 /BP 106902) and related species (PAPP és DIMA 2015). Maximum Likelihood (ML) bootstrap values above 70% are shown at the branches. Bar indicates 0.1 expected change per site per branch.
Fig 3. Morphological features of *Donkia pulcherrima* (PV1044 / BP 106903): A) cross section of basidiocarp, B) basidiospores, C) basidioles, D) tramal hyphae, E) contextual hyphae, F) basidia. Drawing: V. Papp. (PAPP et al. 2015a).
3.4. Based on morphological evaluation, the sample numbered PV1044 in the fungarium was identical to the holotype of the species referred as *Climacodon pulcherrimus* (Berk. & M.A. Curtis) Nikol. by the literature. According to the genetic databases, the ITS sequence of this species have been prepared for the first time, which proved that this species can be separated from the type species [*C. septentrionalis* (Fr.) P. Karst.] of *Climacodon* P. Karst. at the level of genus, thus it possibly belongs to the genus *Donkia* Pilát: *D. pulcherrima* (Berk. & M.A. Curtis) Pilát. (Figure 4.).

![Phylogenetic tree inferred from ITS sequences of *Donkia pulcherrima* and related species. ML bootstrap values above 50% are shown at the branches. Bar indicates 0.05 expected change per site per branch. (PAPP és mtsai. 2015a).](image-url)

**Figure 4.** Phylogenetic tree inferred from ITS sequences of *Donkia pulcherrima* and related species. ML bootstrap values above 50% are shown at the branches. Bar indicates 0.05 expected change per site per branch. (PAPP és mtsai. 2015a).
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Publications in journals without IF


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**CONFERENCE ABSTRACTS**


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