PhD dissertation – thesis

Balázs Palla

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MAGYAR AGRÁR- ÉS
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Hungarian University of Agriculture and Life Sciences

Taxonomical, chorological and ecological study of basidiomycetous fungi in Hungarian *Pinus* stands

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Balázs Palla
Gödöllő
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PhD School

name:	PhD School of Horticultural Sciences
field:	Plant breeding and horticultural sciences
head:	Prof. Dr. Éva Zámboriné Németh, DSc Hungarian University of Agriculture and Life Sciences, Institute of Horticultural Sciences, Department of Medicinal and Aromatic Plants
Témavezető:	Dr. Viktor Papp, PhD Hungarian University of Agriculture and Life Sciences, Institute of Agronomy, Department of Botany

....	....
Approval of the Head of Phd School	Approval of the Supervisor

1. INTRODUCTION AND OBJECTIVES

In addition to the deciduous tree species that make up the majority of Hungarian forests, the forest area of coniferous pines are also significant, as they make up 10.5% of the total forest area of Hungary. Although the area of this group of woody plants has been significantly reduced in recent decades, the area inhabited by pines in Hungary is still approximately 190.000 hectares according to recent data. Amongst these, the two most important two-needled species are Scots pine (*Pinus sylvestris*) and Black pine (*Pinus nigra*), which make up 6.2 and 3.2% of the forest cover in Hungary, respectively (Komarek 2018).

Saprotrophic, lignicolous and mycorrhizal basidiomycetous fungi represent an important group of the communities found both in natural pine associations and in human-established pine plantations. In addition to their importance in forest ecosystems (decomposition of dead organic matter, mutualistic ecological relationships), basidiomycetous fungi are also important as contributors to biological diversity and as indicator species (Jenna et al. 2021, Anthony et al. 2022).

Although many of the hitherto published international and Hungarian literature have investigated the fungal communities associated with the genus *Pinus* in Hungary (e.g. Igmándy 1954, 1989, 1991, Babos 1989, Rimóczi 1994, Lukács 2002, 2010, Benedek 2002, Halász 2002, Nagy and Gorliczai 2009, Koszka 2016, Szabó 2012, Siller et al. 2019), few studies have been published which aimed at the exclusive investigation of taxa related to Hungarian *Pinus* stands (Konecsni 1967, Halász 2002, Seress 2009). In these studies, several morphogroups are underrepresented (e.g. corticoid, coralloid fungi) as most of the surveys were aimed at exploring agaricoid taxa. In addition, studies involving molecular genetic methods for unravelling the fungal diversity of *Pinus* stands in Hungary only were carried out in case of ectomycorrhizal fungi (Seress et al. 2012, 2016, Niskansen 2014). According to the upper mentioned conditions, the main aims of the doctoral thesis were the following:

1. The exclusive examination of the saprotrophic and mycorrhizal basidiomycetous fungi of natural and planted Hungarian *Pinus* forests with the characterization of their ecological state and the aimed survey of hitherto unpublished or rare taxa from Hungary using morphological and molecular genetic methods.

2. Evaluating the ecological context of the occurences of certain collected fungal taxa, with particular regard to the naturalness of the collection sites and the indigeneity of the host plant.

3. For taxa that include closely related, cryptic species collected for culinary purposes, the presentation of new morphological traits that help to separate species with similar morphology.

4. Clarification of the taxonomic and nomenclatural position of some of the studied, problematic taxa on the basis of morphological and molecular methods.

2. MATERIALS AND METHODS

2.1 Outline and ecological analysis of the basidiomycetous fungi of Hungarian *Pinus* forests based on the existing literature

In order to list the species of basidiomycetous fungi known so far from Hungarian *Pinus* associations, a database was assembled based on the published and publicly available literature. Taxa reported and observed within *Pinus* stands, under *Pinus*, on fallen *Pinus* needles, deadwood and debris were listed, whose current binomial names and taxonomic classifications were updated based on the literature examining the taxonomic status of the given taxa, and also based on online mycological databases (MycoBank, Index Fungorum). In order to standardize and compare the different types and scales of reported data within the reviewed literature, a uniform, categorical system of host plants and host associations were created, the latter in the absence of the host organism or substrate.

The degree of forest naturalness of the collection sites were also assigned to the entries within the literature. The categories of forest naturalness defined in the National Forestry Database (NFD), in the Hungarian National Forest Inventory and in the legislation Act No. XXXVII of 2009 („Forest law", Hungary) served as the basis for the assignment of degree of forest naturalness to the individual entries. Modifications to the naturalness categories were implemented according to the information available within the literature. On the basis of the compiled database, sunburst charts and pie charts were prepared, which show the distribution of the observed basidiomycetous fungal taxa related to the different *Pinus* hosts, associations and degrees of naturalness.

2.2 Sampling areas

Own collections of basidiomycetous fungi from Hungarian *Pinus* stands and the personal fungarium of other mycologists were studied. The collection sites of these collections could be divided into two groups: 1) *Pinus* forests considered native or natural, and 2) exotic, non-native and planted *Pinus* stands (**Figure 1**). In the case of the former group, the investigations covered the relic *Pinus sylvestris* association of the Fenyőfő Forest Reserve and the natural mixed *P. sylvestris* forests of the Őrség-Vendvidék region. The investigated *P. sylvestris* and *P. nigra* plantations are located in the Transdanubian Mountains, the Mátra, the Little Hungarian Plain and the Great Hungarian Plain. A mixed Spruce-White pine-Larch forest and a mainly deciduous forest located in the Mátra were also part of the investigations. General mycological surveys were carried out in case of five examined stands (Fenyőfő Forest Reserve, Tétényi-fennsík, Érdi-fennsík, mixed Scots pine-Black pine forest near Sóskút, Black pine forest near Várpalota); in the other study sites, basidiocarps of the genus *Chroogomphus* were sampled (**Figure 1**).

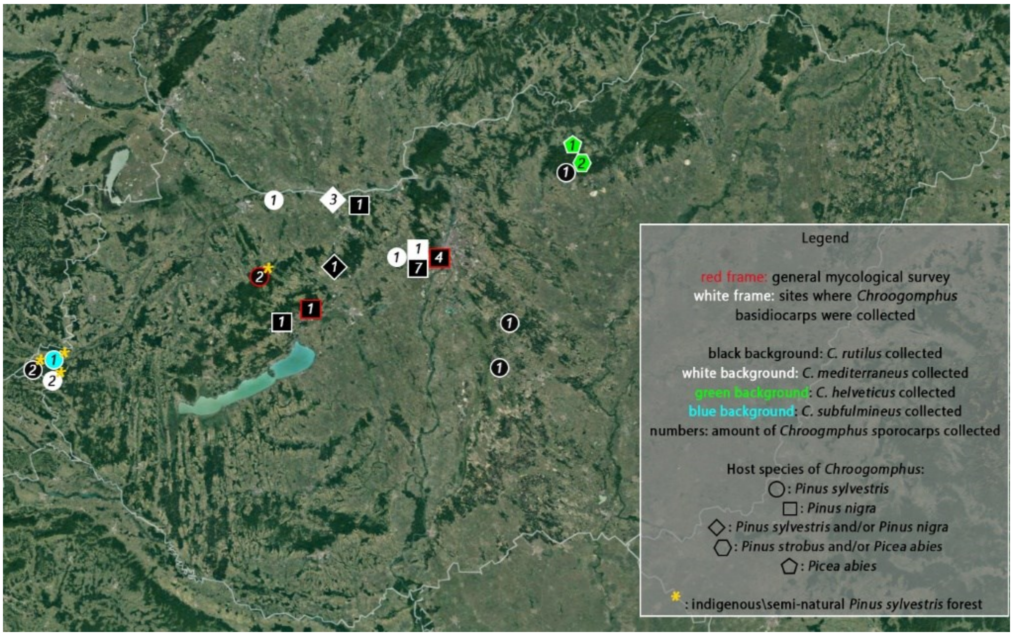

Figure 1. Geographical locations of the collection sites.

2.3 Sampling dates and processing of the collected materials

In addition to own collections, specimens of other private fungariums were also studied. Field samplings of the examined basidiocarps took place between 1959 and 2022 at the upper presented locations (**Figure 1**). During the own field recordings, all detected taxa were documented and in most cases fungarium material were collected as proof. *In situ* photodocumentation was made of the majority of the fruiting bodies collected. After drying the fruiting bodies in a drying machine, the dried samples were placed in the own fungarium of the author (BPalla), which is located at the Department of Botany of the Hungarian University of Agriculture and Life Sciences.

2.4 Equipments used for the identification of basidiomycetous fungi

2.4.1 Monographs, keys and other literature

For the macro- and micromorphological determination of the studied basidiomycetous taxa, monographs, keys and relevant publications within scientific journals were used in order to examine the given taxonomic unit or morphogroup.

For the identification of agaricoid fungi in the broad sense, the first volume of "Fungi of temperate Europe" (Læssøe and Petersen 2019) served as a basis for identifying larger groups of taxa. The determination of some taxa that are difficult to identify based solely on macromorphological characters were aided by monographs and articles adressing the given taxa (e.g. Aronsen és Læssøe 2016, Scambler et al. 2018).

The monographs of Ryvarden and Gilbertson (1993, 1994) and Ryvarden and Melo (2017) were used to determine the poroid species. The identification of closely related species belonging to specific families and genera were carried out based on several other scientific articles (e.g. Han et al. 2016, Wu et al. 2022)

The corticoid species were identified based on the "The Corticiaceae of North Europe" series (e.g. Hjortstam et al. 1987), further monographs and articles (e.g. Ţura et al. 2011, Yurchenko és Wu 2016)

Coralloid fungi were identified based on E. J. H. Corner's monograph (Corner 1950) and on works discussing other taxa (e.g. Giachini és Castellano 2011, Olariaga et al. 2015).

2.4.2 Microscopic examinations

For the micromorphological examinations, sections of the different parts of the basidiocarps were mounted on slides. Melzer reagent was used for testing the amyloidity of hyphae, spores, basidia and other sterile elements. Congo Red and Cotton Blue were also used as other coloring agents.

A Zeiss Axio Imager A2 (Zeiss, Göttingen, Germany) light microscope with a 100× objective was used for microscopic examinations. Images of the micromorphological structures were made with a Zeiss AxioCam HRc camera. Measurements were made using the Axio Vison Release 4.8 software. Line drawings were made with a drawing tube.

2.4.3 Molecular analyses

In addition to the morphology-based determination of the examined basidiocarps, samples were also analyzed by molecular methods which could not be clearly identified based on macro- and micromorphological characters. Phylogenetic analyes were also performed in case of taxonomically uncertain taxa.

DNA extraction from dried material was performed using the EZNA Plant DNA Kit (Omega Bio-tek, Norcross, GA, USA), following the manufacturer's protocol. Amplification of the ITS regions utilized primers ITS1F and ITS4B (White et al. 1990, Gardes and Bruns 1993), and the same primers were employed for sequencing. The primers LR0R (Rehner and Samuels 1994) and LR5 (Vilgalys and Hester 1990) were used to amplify the LSU region of the nrDNA in case of the samples of *Irpicodon*, *Plicaturopsis* and *Skeletocutis*. For amplification of the *RPB2* gene, primers fRPB2-5F/ bRPB2-7.1R (Liu et al. 1999, Matheny 2005, Matheny et al. 2007) were used for the genera *Irpicodon* and *Plicaturopsis*. The success of the PCR amplification was checked by running the product on electrophoresis gel. After purification, the amplified, purified samples were sent to the Biological Research Centre, Szeged for sequencing. Raw sequences underwent editing using BioEdit (Hall 1999), with manual removal of the primers' connection region. When phylogenetic analyises were not performed, edited sequences were subjected to BLAST analysis (Basic Local Alignment Search Tool), available on the NCBI homepage (Camacho et al., 2009); morphological based identifications were aided by sequences showing high sequence homology with own samples. In case of phylogenetic analyses, sequences of each locus (ITS, LSU and *RPB2*), together with sequences of related species downloaded from GenBank and UNITE, were aligned separately with the online MAFFT v. 7.0 (Katoh and Standley 2013). The alignments were checked and edited by manual adjustment in SeaView 5 (Gouy et al. 2021). Maximum Likelihood (ML) analysis was performed using RAxML in raxmlGUI2.0 (Stamatakis 2014, Edler et al. 2021) with 1000 rapid bootstraps and GTRGAMMA substitution model. The resulting phylogenetic trees were visualized in MEGA 7 (Kumar et al. 2016). A dataset of 26 specimens was also analysed in order to infer the divergence times of taxa within *Amylocorticiales* (including *Irpicodon*, *Plicatura* and *Plicaturopsis*). Divergence times were estimated in BEAST v..2.7.5 (Bouckaert et al. 2019). As substitution models, K80, TIM2 and TrNef were used for the 5.8S, LSU and *RPB2* regions, respectively. As clock and tree models, an optimized lognormal relaxed clock model and a pure birth (Yule) speciation prior were applied. For node calibrations, one *Suillus* ECM fossil and *Archaeomarasmius leggettii* were used setting the MRCA of *Suillinae* and *Agaricales*, respectively (LePage et al. 1997, Hibbett et al. 1997a). Two independent Monte Carlo Markov Chain runs of 10 million generations were performed, logging states every 1000 generations. Log files of each run were compared in Tracer v.1.7.2 (Rambaut et al. 2018). A maximum-clade-credibility (MCC) tree was summarized using TreeAnnotator v.2.7.5, discarding 10 % of states as burn-in. The resulting phylogenetic trees were visualized in TreeAnnotator 2.7.5.

2.5 Methods of ecological and bioclomatic analyses

2.5.1 Database-derived background data

In order to investigate the influence of site conditions and hosts on the distribution patterns of the genus *Chroogomphus*, public databases and those available by data inquiry, were used. Most of the soil parameters were obtained from Soilgrids 2.0 (Poggio et al. 2021), using the soil property estimations of the corresponding 250 m tiles based on the GPS coordinates of the collections. Further data regarding edaphic, climatic, topographical properties, and forestry management of the collection sites were gathered from the National Forestry Database (NFD) and Hungarian National Forest Inventory, which were inquired from the Forestry Department of the Hungarian National Land Centre (NLC Forestry Department, Forest Inventory 2010–2019; Tobisch & Kottek 2013).

2.5.2 Statistical methods

To test the effects of continuous site climatic variables on the distribution of the different *Chroogomphus* species, One-Way ANOVA tests were performed.

In case of One-Way ANOVA, the independent variable was the species with three factor levels (‘rutilus’, ‘mediterraneus’ and ‘helveticus’; as there was only one collection of *C. subfulmineus*, it was excluded from the analysis). Normality of variances was checked by the Shapiro-Wilk test (at $p > 0.05$) and subsequently by the D’Agostino test (at $p > 0.001$) when required (Tabachnick & Fidell 2007). With the aim of normalising the distribution of the data, Box-Cox transformations (Box & Cox 1964) were made in the case of some parameters. Homogeneity of variances was checked by Levene’s test. Depending on the results, statistical differences among species were determined by Tukey’s HSD or Games-Howell post hoc tests.

Due to low cell counts in respective contingency tables, Fischer’s exact test (Fischer 1922) was used to determine if there was a significant association between the observed *Chroogomphus* species and the categorical climatic variables.

Statistical tests were performed using the 'base', 'stats', 'car' and 'multcomp' packages within R 4.3.0.

3. RESULTS AND DISCUSSION

3.1 Host preferences and the hitherto known basidiomycetous fungal diversity of the semi-natural and planted *Pinus* forests in Hungary, and distribution of taxa according to forest naturalness

According to the data derived from the Hungarian literature, a total of 811 species and 16 intraspecific taxa (variety, form) belonging to 93 families, 249 genera of 14 orders were identified in *Pinus*-related forest associations or on/near *Pinus*. Of these, a total of 137 species belonging to 57 families and 85 genera of 11 orders were found to be directly associated with individuals of *Pinus*, or found on substrates of *Pinus*. Of these, 108 fungal species were registered on Scots pine and/or Black pine, of which Scots pine was the host plant in 92 cases (71.9% of all such collections) and Black pine in 36 cases (28.1% of all such collections). Taxa belonging to the *Agaricales* occurred in the highest proportion on both *Pinus* species (45.7% and 41.7% of the collections on Scots pine and Black pine, respectively), followed by *Russulales* (10.9% and 22.2%), *Boletales* (10.9% and 19.4%), *Polyporales* (15.2% and 11.1%) and *Hymenochaetales* (6.5% and 2.8%). In case of the orders *Dacrymycetales*, *Gloeophyllales*, *Gomphales*, *Geastrales*, *Thelephorales* and *Xenasmatellales*, which are often characterized by specific basidiocarp morphology (e.g. angiocarp, clavaroid, coralloid, hydnoid, cantharelloid), 1–3 species per order were represented in association with *Pinus*.

Considering entries in the literature when the collections of fungal taxa were indicated only on substrates of *Pinus sylvestris*, under *Pinus sylvestris*, or at the level of *Pinus sylvestris* associations, a total of 372 species belonging to 69 families, 129 genera and 13 orders can be identified. Based on the literature that only mentions collections on/under *Pinus nigra* or in *Pinus nigra* associations, a total of 48 species belonging to 25 families, 38 genera and five orders can be identified, which were not reported from associations of other *Pinus* species. Among the *Pinus* species, the low number of *Pinus nigra* limited occurrences is hued by the fact that a total of 272 basidiomycetous species were collected either exclusively in *Pinus nigra* stands, or in mixed *Pinus nigra*-*Pinus sylvestris* plantations, or from stands of both host plants. The reasons for the above patterns could be: 1) the low level of host specificity in case of basidiomycetous taxa (species are related to host genera or are generalists); 2) the low diversity of planted *Pinus nigra* stands compared to that of planted and semi-natural *Pinus sylvestris* forests; and 3) the understudied nature of pure and mixed Hungarian *Pinus nigra* stands.

In case of the patterns between forest naturalness and the observed basidiomycetous taxa, there are data published from all degree of naturalness in *Pinus* forests. Taxa in the literature belonging to *Agaricales* and *Russulales* occurred in the largest proportion in collection sites with different degrees of naturalness, always exceeding the common share of 65%. In addition, the joint share of *Agaricales* and *Russulales* species collected in presumably semi-plantation forests and presumably semi-natural stands exceeds 80% and 61%, respectively. The representatives of *Agaricales* were found in the largest proportion in semi-plantation forests (71.8%), the smallest proportion in forests classified as secondary-forests (54.7%) and semi-natural forests (62.9%), while species belonging to *Russulales* were found in the largest amount in secondary-forests (21.4%) and in semi-natural forests (18.9%), and the smallest amount occurred in semi-plantation forests (10.7%). Representatives of *Boletales* and *Polyporales* were found in proportions between 3 and 9% withtin the collection sites of different degree of naturalness. Taxa within *Boletales* were represented in the highest amount in secondary-forests (9.0%), while species of *Polyporales* were represented highest in semi-plantation forests (4.7%). 0.3–3.4% of individuals collected belong to the species of *Cantharellales*, *Hymenochaetales* and *Gomphales* from forests of different degree of naturalness. The species of these three orders were found in the smallest amount in semi-plantation forests. The representatives of the remaining 7 orders (*Dacrymycetales*, *Geastrales*, *Gloeophyllales*, *Phallales*, *Thelephorales*, *Trechisporales*, *Xenasmatellales*) were usually identified at an amount of 0–2.2% from *Pinus* stands of different forest naturalness. The generally low share of these orders probably result from their relatively low global diversity.

3.2 Taxonomy, ecology and the list of basidiomycetous taxa documented in the field surveys of this study

Based on the 216 basidiocarps collected from the investigated *Pinus* associations, the occurrence of a total of 75 species of basidiomycetous fungi were verified (**Table 1**). All of the studied and identified species belong to *Agaricomycotina* and *Agaricomycetes*, and are corresponding to seven orders, 42 families and 57 genera. The majority of the examined samples belong to taxa under *Agaricales* and *Polyporales*. Several representatives of *Boletales*, *Hymenochaetales* and *Russulales*, as well as one species each of the orders *Amylocorticiales* and *Gomphales* were identified. 12 taxa of the 75 identified species proved to be new to the Hungarian funga: *Chroogomphus mediterraneus* (Finschow) Vila, Pérez-De-Greg. & G. Mir, *Chroogomphus subfulmineus*

Niskanen, Loizides, Scambler & Liimat., *Dacryobolus karstenii* (Bres.) Oberw. ex Parmasto, *Fibroporia pseudorennyi* (Spirin) Spirin, *Irpicodon pendulus* (Alb. & Schwein.) Pouzar, *Melanoleuca luteolosperma* (Britzelm.) Singer, *Onnia triquetra* (Pers.) Imazeki, *Phaeoclavulina carovinacea* Franchi & M. Marchetti, *Phloeomana clavata* (Peck) Redhead, *Resupinatus striatulus* (Pers.) Murrill, *Skeletocutis papyracea* A. David and *Xylodon spathulatus* (Schrad.) Kuntze.

Table 1. The number of basidiomycetous taxa determined in the course of this work. The numbers in parentheses after the species number indicate the number of new species for the funga of Hungary.

Order	Family	Genus	Number of species
Agaricales	*Agaricaceae*	*Agaricus*	2
		Lepiota	2
	Amanitaceae	*Amanita*	1
	Clitocybaceae	*Collybia*	1
		Singerocybe	1
	Hymenogastraceae	*Galerina*	1
		Gymnopilus	1
		Hebeloma	1
	Marasmiaceae	*Atheniella*	1
	Melanoleucaceae	*Melanoleuca*	1(1)
	Mycenaceae	*Mycena*	4
	Omphalotaceae	*Gymnopus*	1
		Rhodocollybia	1
	Pluteaceae	*Pluteus*	1
	Porotheleaceae	*Phloeomana*	1(1)
	Resupinataceae	*Resupinatus*	1(1)
	Schizophyllaceae	*Schizophyllum*	1
	Tricholomataceae	*Tricholoma*	3
	Tubariaceae	*Tubaria*	1
Σ	**14**	**19**	**26(3)**
Amylocorticiales	*Amylocorticiaceae*	*Irpicodon*	1(1)
Σ	**1**	**1**	**1(1)**
Boletales	*Boletaceae*	*Chalciporus*	1
	Coniophoraceae	*Coniophora*	2
	Gomphidiaceae	*Chroogomphus*	4(2)
	Hygrophoropsidaceae	*Hygrophoropsis*	1
		Leucogyrophana	1
	Paxillaceae	*Hydnomerulius*	1
	Serpulaceae	*Serpula*	1
	Suillaceae	*Suillus*	2

	Tapinellaceae	*Tapinella*	2
Σ	**8**	**9**	**15(2)**
Gomphales	*Gomphaceae*	*Phaeoclavulina*	1(1)
Σ	**1**	**1**	**1(1)**
Hymenochaetales	*Hirschioporaceae*	*Hirschiporus*	1
	Hymenochaetaceae	*Fuscoporia*	1
		Onnia	1(1)
		Porodaedalea	1
	Schizoporaceae	*Xylodon*	2(1)
Σ	**3**	**5**	**6(2)**
Polyporales	*Dacryobolaceae*	*Dacryobolus*	1(1)
	Fibroporiaceae	*Fibroporia*	1(1)
	Fomitopsidaceae	*Fomitopsis*	2
	Gelatoporiaceae	*Cinereomyces*	1
	Incrustoporiaceae	*Skeletocutis*	2(1)
	Irpicaceae	*Leptoporus*	1
		Meruliopsis	1
	Laetiporaceae	*Phaeolus*	1
	Meruliaceae	*Phlebia*	1
	Phanerochaetaceae	*Phanerochaete*	1
		Phlebiopsis	1
	Polyporaceae	*Daedaleopsis*	1
		Dichomitus	1
	Postiaceae	*Amaropostia*	1
		Fuscopostia	1
		Ptychogaster	1
	Pycnoporellaceae	*Pycnoporellus*	1
	Sparassidaceae	*Sparassis*	1
Σ	**12**	**18**	**20(3)**
Russulales	*Auriscalpiaceae*	*Auriscalpium*	1
	Bondarzewiaceae	*Heterobasidion*	1
	Russulaceae	*Lactarius*	2
		Russula	2
Σ	**3**	**4**	**6(0)**

Since *Irpicodon pendulus* (Alb. & Schwein.) Pouzar, a species found in the Fenyőfő Forest Reserve, and the closely related species of the genera *Plicatura* Peck and *Plicaturopsis* D.A. Reid show a high degree of resemblence in terms of macro- and micromorphological features, a comprehensive reassesment of generic boundaries within these taxa was conducted with the aid of ML phylogenetic evidence and divergence time estimation based on 3-gene datasets (ITS, LSU and *RPB2*). According to the analysis, the *I. pendulus* samples clustered together with the group formed by *Plicatura nivea* and *Plicaturopsis crispa* samples, collectively forming a well-delineated clade with strong support (ML=100%). The topology of the phylogenetic tree (comparable genus boundaries) and the results of the phylogenetic analyses support the placement of the monotypic genera, *Irpicodon* and *Plicaturopsis*, in synonymy under *Plicatura* (**Figure 2**).

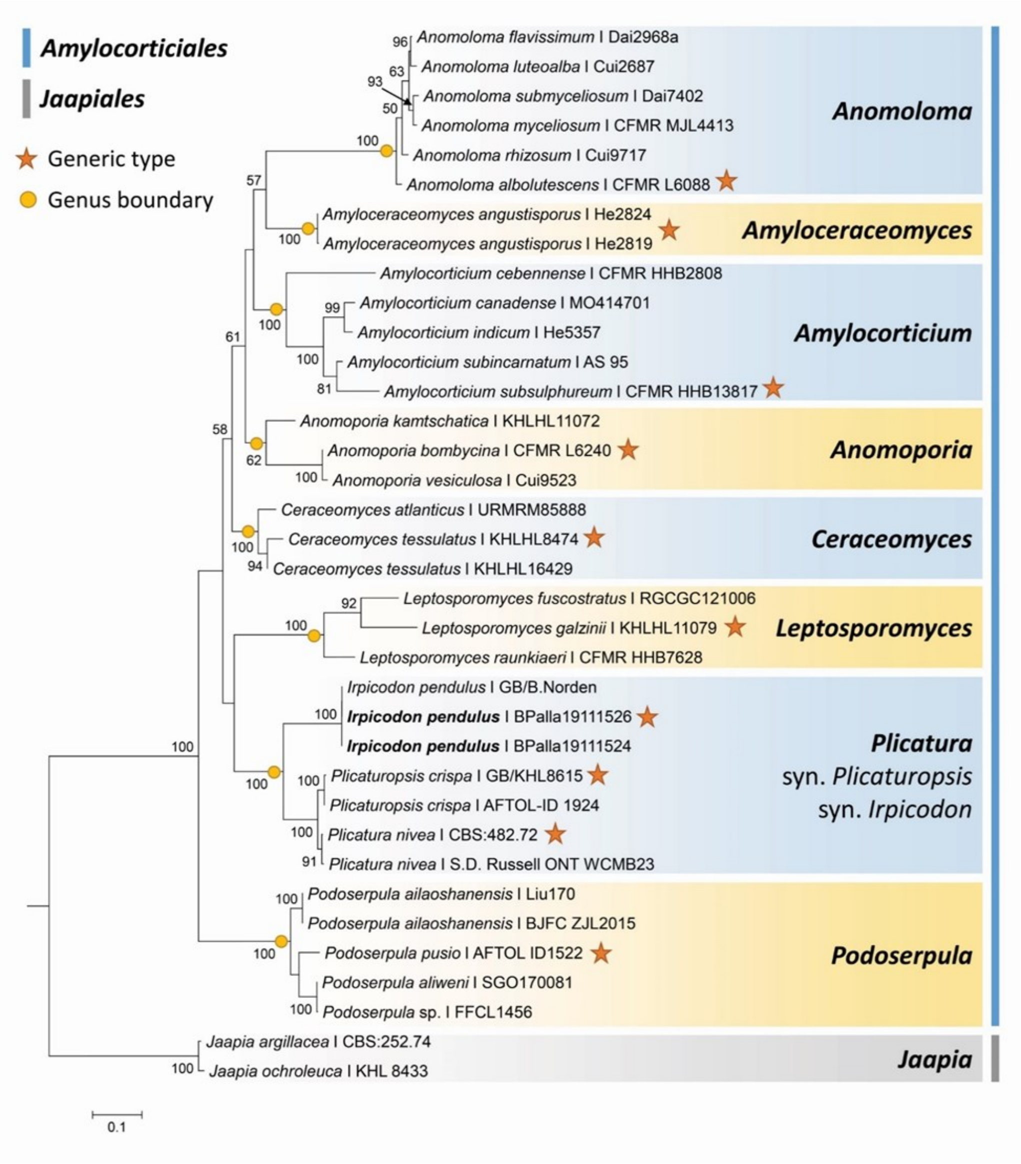

Figure 2. Phylogenetic placement of *Plicatura pendulus* (in bold) and delimitation of redefined *Plicatura* within the family *Amylocorticiaceae* based on ML inference of the ITS+28S+RPB2 data set. Numbers on nodes represent ML bootstrap values >50%. The scale bar indicates the number of expected substitutions per site. Source: Palla et al. (2024).

In order to reveal the species composition of the cryptic genus *Chroogomphus* in Hungary, we performed molecular phylogenetic analyses. According to the results, the 34 Hungarian specimens belonged to four well-supported clades. Most of the newly sequenced Hungarian samples (24 in total) clustered into the *C. rutilus* clade with strong support (ML=100 %), altough 7 samples grouped into the *C. mediterraneus* (ML=78%), two samples into the *C. helveticus* (ML=100%) and one sample into the *C. subfulmineus* (ML=90%) clade. Thus, in addition to the previously morphologically identified species of *C. rutilus* and *C. helveticus*, the presence of the species *C. mediterraneus* and *C. subfulmineus* in Hungary has been confirmed (**Figure 3**).

The previously known macro- and micromorphological features of the cryptic *Chroogomphus* species often overlap (Scambler et al., 2018). As additional support for species delimitation and morphological based identification, some novel morphological characters and the revision of former traits are also presented. The revised micromorphological features are the following: the amyloidity of the pileipellis elements, the average wall thickness of the cheilocystidia and spore sizes.

Based on the statistical comparisons of modeled bioclimatic variables derived from the corresponding databases (SoilGrids, National Forestry Database), *C. mediterraneus* occupy similar habitats as *C. rutilus* in terms of the soil nutrient content (CEC, N, SOC) and pH (**Figure 4**). As the fruiting bodies of *C. mediterraneus* were collected in much smaller quantities and this species was found to be less abundant in Hungarian *Pinus* forests, other variables can be assumed acting on the interspecific competition between *C. rutilus* and *C. mediterraneus*. Such differing variables between these *Chroogomphus* species could be the timing of colonization („priority effect”), the difference between aboveground and belowground abundances of the sporocarps, mycelial overgrowth, sporophagy, intraspecific competition, etc. (Gardes and Bruns 1996, Kennedy and Bruns 2005, Kennedy et al. 2007, 2020, Kennedy 2010).

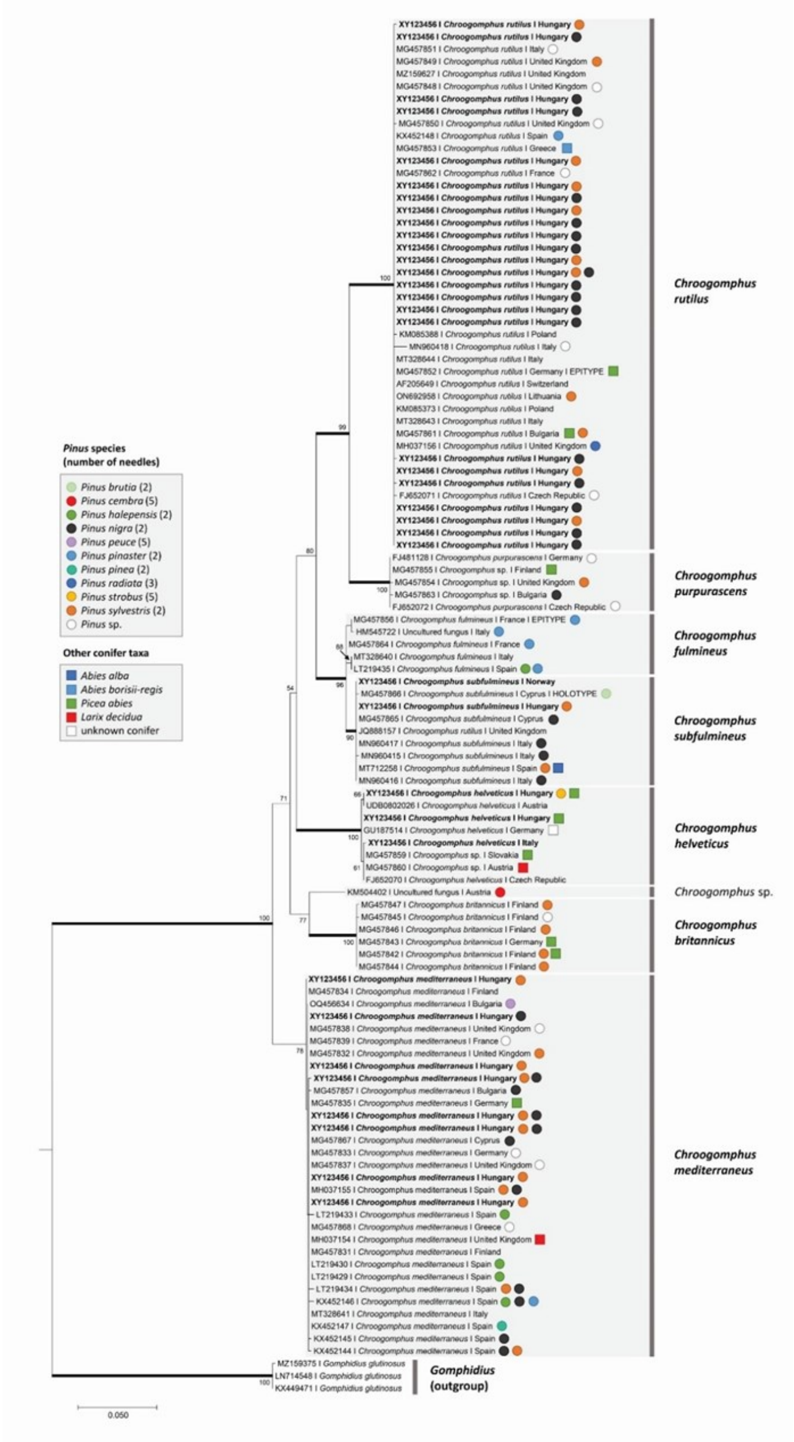

Figure 3. Maximum Likelihood (ML) phylogenetic tree derived from ITS-sequences of *Chroogomphus*. ML bootstrap values > 50 % are shown above or below branches. The bar indicates 0.05 expected change per site per branch. New Hungarian sequences are marked with bold. Legend indicate host species. Source: Palla et al. (2024).

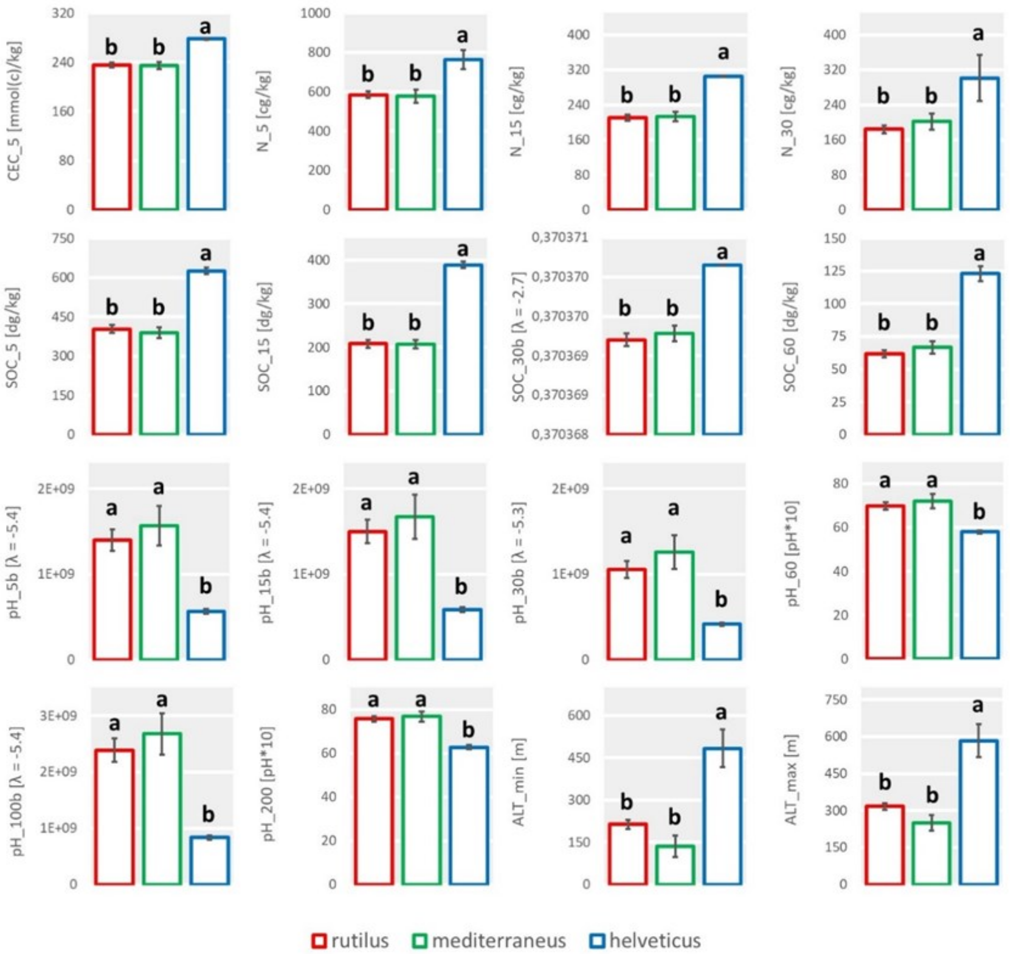

Figure 4. Soil and topographical parameters of collection sites in different soil depth intervals. Bars with different colours represent means of *Chroogomphus* species pooled in all collection sites, error bars represent standard errors. Abbreviations: CEC – cation exchange capacity; N – total nitrogen; SOC – soil organic carbon content, ALT – altitude above sea level. Numbers after parameters indicate the bottom of the depth interval according to the SoilGrids database, letter 'b' indicate Box-Cox transformed values. Different letters in the same parameter and depth indicate significant differences between means of *Chroogomphus* species according to Tukey's honestly significant difference test at $p<0.05$. Source: Palla et al. (2024).

4. NEW SCIENTIFIC RESULTS

1. I compiled and evaluated the species list of basidiomycetous fungi documented in Hungarian *Pinus* forests, based on data from the Hungarian literature. In a unified system, a total of 1,582 cases of *Pinus* host plant and association types were assigned to individual entries (species) of the list, which includes a total of 14 basidiomycetous orders, 93 families, 249 genera and 811 species. In 1,448 cases, degrees of forest naturalness were also assigned to the individual occurences, providing an additional ecological aspect to the evaluation.

2. By investigating own field samplings and additional fungarium specimens, I documented the occurrence of 75 basidiomycetous species from Hungary, of which 12 proved to be new to the funga of the country. The taxonomic, morphological and ecological characterization of the new occurences were also given.

3. I used molecular, microscopical and statistical methods for the first time in order to reveal the species composition and the ecological preferences of the cryptic *Chroogomphus* species present in Hungary. The delimiting morphological features of the taxa present in Hungary were also revised. Based on the results, the presence of a total of four *Chroogomphus* species in the country was confirmed.

4. I have conducted phylogenetic and molecular clock analyses for the first time which aim to resolve the phylogenetic relations of the closely related *Irpicodon*, *Plicaturopsis* and *Plicatura* genera, using the protein coding barcoding gene *RPB2*, along with two other barcoding regions (ITS, LSU). The phylogenetic analyses results support the placement of the monotypic genera, *Irpicodon* and *Plicaturopsis*, in synonymy under *Plicatura*.

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Book chapter

1. **Palla, B.**, Yuan, Y., Dai, Y.-C., Papp, V., 2023. Host Preferences of *Pinus*-dwelling *Hymenochaetaceae*. In: Deskmukh, S. K., Sridhar, K. R. (szerk.): *Ecology of Macrofungi: An Overview*. USA: CRC Press, Taylor & Francis Group, pp. 244-279.

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2. **Palla, B.,** Dima, B., Papp, V., 2024. Diversity and ecological evaluation of *Chroogomphus* species in Hungary. *Sydowia* 76, 243–262.

3. **Palla, B**., Dima, B., Papp, V., 2024. Challenging generic boundaries: reconsidering the taxonomic status of *Irpicodon* Pouzar, a monotypic genus in the *Amylocorticiaceae* (*Basidiomycota*). *Forests*, under revison.

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9. Papp, V., **Palla, B**., Dima B., 2024. A Kelet-Ázsiából leírt ehető és termesztett „hon-shimeji" gomba (Lyophyllum shimeji) első hazai előfordulása [The first Hungarian occurrence of the edible and cultivated "hon-shimeji" mushroom (Lyophyllum shimeji) described from East Asia]. In: Dima, B., Papp, V. (Szerk.) *VII. Magyar Mikológiai Konferencia*. Budapest, Magyarország: Magyar Agrár- és Élettudományi Egyetem, p. 197–198

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