DOPRINOS DNA BARKODIRANJA ISTRAŽIVANJIMA BIORAZNOLIKOSTI GLJIVA HRVATSKE

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DOPRINOS DNA BARKODIRANJA ISTRAŽIVANJIMA BIORAZNOLIKOSTI GLJIVA HRVATSKE

DOKTORSKI RAD

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University of Zagreb

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DEPARTMENT OF BIOLOGY

Ana Pošta

CONTRIBUTION OF DNA BARCODING TO THE RESEARCH OF CROATIAN FUNGAL BIODIVERSITY

DOCTORAL THESIS

Zagreb, 2023

Ovaj je doktorski rad izrađen u Laboratoriju za biološku raznolikost, Zavoda za istraživanje mora i okoliša, Instituta Ruđer Bošković (Zagreb), u okviru projekta Hrvatske zaklade za znanost „Unapređenje usluga šumskih ekosustava Hrvatske kroz vrednovanje bioraznolikosti gljiva temeljenoj na DNA barkodiranju“ (HRZZ-IP-2018-01-1736) pod vodstvom dr. sc. Armina Mešića, u sklopu Sveučilišnog poslijediplomskog doktorskog studija Biologije pri Biološkom odsjeku Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu.

INFORMACIJE O MENTORU

Dr. sc. Armin Mešić rođen je 29. travnja 1975. godine u Zagrebu. Tijekom 1999. godine završio je studij biologije, smjer ekologija, na Prirodoslovno-matematičkom fakultetu (PMF), Sveučilišta u Zagrebu. Na istom fakultetu doktorirao je 2009. god. u području prirodnih znanosti, polje biologija (mikologija). Od 2000. god. zaposlen je na Institutu Ruđer Bošković gdje trenutno radi na znanstvenom radnom mjestu znanstveni savjetnik u koje je izabran u studenom 2023. godine. Od 2019. godine voditelj je Laboratorija za biološku raznolikost, Zavoda za istraživanje mora i okoliša na Institutu Ruđer Bošković. Osnivač je i tajnik Hrvatskog mikološkog društva od njegovog osnutka 1999. godine do danas. Kustos je nacionalne zbirke uzoraka gljiva Hrvatski nacionalni fungarij (Croatian National Fungarium, CNF) smještene na Institutu Ruđer Bošković.

Njegov primarni znanstveni interes je istraživanje bioraznolikosti gljiva, posebno u području taksonomije, filogenetike, biogeografije te zaštite gljiva i njihovih staništa. Do sada je publicirao 22 vrste i jedan rod gljiva kao nove za znanost. Objavio je ukupno 58 znanstvenih radova s međunarodnom recenzijom i sudjelovao na brojnim znanstvenim skupovima. Do sada je sudjelovao na 49 znanstvenih i stručnih projekata od čega je na 14 bio voditelj. Član je uredničkog odbora međunarodno recenziranog časopisa Sydowia (Austrija). Urednik je zbornika 14. Hrvatskog biološkog kongresa s međunarodnim sudjelovanjem. Recenzirao je više od 150 znanstvenih radova u 30-ak međunarodnih znanstvenih časopisa. Obavlja savjetodavne dužnosti u okviru međunarodnih ekspertnih tijela: kustos za taksonomiju gljiva odjeljka Basidiomycota na web stranici Outline of Fungi i član međunarodnog ekspertnog konzorcija „Consortium for Fungi and fungus-like classification“ od 2023. godine, te član ekspertne skupine za pripremu prijedloga IUCN Globalnog crvenog popisa ugroženih vrsta gljiva u okviru International Union for Conservation of Nature (IUCN), Cambridge (Velika Britanija). Od 2017. godine obavlja ekspertnu funkciju znanstvenog konzultanta Instituta za medicinska istraživanja i medicinu rada (Zagreb) za identifikaciju gljiva u slučajevima otrovanja. Od 2021. godine sudjeluje u nastavi kao gost predavač na Diplomskom studiju Agroekologije Agronomskog fakulteta Sveučilišta u Zagrebu. Uspješno se istaknuo vođenjem mlađih suradnika u okviru jednog preddiplomskog (BSc), tri diplomska (MSc) te jednog doktorskog (PhD) rada. Istaknuo se na području promocije i popularizacije znanosti održavanjem javnih predavanja te sudjelovanjem u radio i televizijskim emisijama.

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Svom mentoru, dr. sc. Arminu Mešiću, iskreno zahvaljujem na nesebičnom pružanju znanja te ukazanom povjerenju tijekom izrade ove disertacije. Veliko hvala na podršci i savjetima, te na razumijevanju u teškim trenucima.

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Hvala doc. dr. sc. Ondřeju Kuokolu, voditelju Botaničkog zavoda na Prirodoslovnom fakultetu Karlovog sveučilišta u Pragu što mi je omogućio ugodan boravak u svom Laboratoriju.

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Ovu doktorsku disertaciju posvećujem svojoj mami.

Sveučilište u Zagrebu
Prirodoslovno-matematički fakultet
Biološki odsjek

Doktorski rad

DOPRINOS DNA BARKODIRANJA ISTRAŽIVANJIMA BIORAZNOLIKOSTI GLJIVA HRVATSKE

ANA POŠTA

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Cilj doktorske disertacije bio je rješavanje znanstvenih problema u sistematici gljiva metodama integrativne taksonomije koja objedinjuje evaluaciju molekularnih, morfoloških i ekoloških obilježja gljivljih organizama. Poseban naglasak stavljen je na vrednovanje znanstvenog doprinosa DNA barkodiranja u otkrivanju, razgraničenju i identifikaciji srodnih vrsta u različitim skupinama nadzemnih gljiva iz odjeljaka *Ascomycota* i *Basidiomycota*. Prikazan je znanstveni opis tri vrste nove za znanost s područja Republike Hrvatske: *Inocybe brijunica, I. istriaca* i *Parasola papillatospora*. Kao važan biogeografski doprinos ovoga rada na području Hrvatske po prvi je puta pronađeno 10 vrsta gljiva: *Coprinopsis alnivora, Entonaema cinnabarinum, Parasola auricoma, P. crataegi, P. cuniculorum, P. kuehneri, P. malakandensis, P. megasperma, P. nudiceps* i *P. plicatilis-similis*. Vrste *C. alnivora* i *P. malakandensis* ujedno su po prvi puta zabilježene i na području Europe. Integrativna taksonomska analiza roda *Entonaema* rezultirala je određivanjem njegovog filogenetskog i taksonomskog položaja u porodici *Hypoxylaceae* te izradom identifikacijskog ključa za vrste na svjetskoj razini.

(164 stranica, 6 slika, 369 literaturnih navoda, jezik izvornika hrvatski)

Ključne riječi: biogeografija, filogenija, integrativna taksonomija, nove vrste za Europu, nove vrste za Hrvatsku, nove vrste za znanost

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University of Zagreb
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Doctoral thesis

CONTRIBUTION OF DNA BARCODING TO THE RESEARCH OF CROATIAN FUNGAL BIODIVERSITY

ANA POŠTA

Ruđer Bošković Institute, Bijenička 54, 10 000 Zagreb, Croatia

The aim of this dissertation was to solve scientific problems in the systematics of fungi using integrative taxonomy, which combines molecular, morphological, and ecological characteristics of fungi. Special emphasis was placed on the contribution of DNA barcoding in the discovery, delimitation, and identification of related species in different groups of epigeous fungi (*Ascomycota* and *Basidiomycota*). The scientific description of three species new to science from Croatia was presented: *Inocybe brijunica*, *I. istriaca*, and *Parasola papillatospora*. An important biogeographical contribution was ten species of fungi found for the first time in Croatia: *Coprinopsis alnivora*, *Entonaema cinnabarinum* and 8 species of *Parasola*. *Coprinopsis alnivora* and *P. malakandensis* were also recorded for the first time in Europe. The integrative taxonomic analysis of the genus *Entonaema* led to the determination of their phylogenetic and taxonomic position in the family *Hypoxylaceae* and the establishment of an identification key of worldwide species within this genus.

(164 pages, 6 figures, 369 references, original in Croatian)

Keywords: biogeography, integrative taxonomy, phylogeny, species new to Croatia, species new to Europe, species new to science

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SADRŽAJ

U OVOJ DOKTORSKOJ DISERTACIJI OBJEDINJENI SU SLJEDEĆI OBJAVLJENI ZNANSTVENI RADOVI:

I. Mešić A, Haelewaters D, Tkalčec Z, Liu J, Kušan I, Aime MC, **Pošta A** (2021) *Inocybe brijunica* sp. nov., a new ectomycorrhizal fungus from Mediterranean Croatia revealed by morphology and multilocus phylogenetic analysis. Journal of Fungi 7(3): 199. https://doi.org/10.3390/jof7030199

II. **Pošta A**, Bandini D, Mešić A, Pole L, Kušan I, Matočec N, Malev O, Tkalčec Z (2023) *Inocybe istriaca* sp. nov. from Brijuni National Park (Croatia) and its position within *Inocybaceae* revealed by multigene phylogenetic analysis. Diversity 15: 755. https://doi.org/10.3390/d15060755

III. **Pošta A,** Tkalčec Z, Kušan I, Matočec N, Pole L, Čerkez M, Mešić A (2023) An integrative taxonomic study of *Parasola* (*Psathyrellaceae*, *Fungi*) reveals a new saprotrophic species from European temperate deciduous forests. Forests 14: 1387. https://doi.org/10.3390/f14071387

IV. Bednár R, Červenka J, Arendt D, Szabóová D, Krisai-Greilhuber I, **Pošta A**, Mešić A, Tkalčec Z (2022) *Coprinopsis alnivora* (*Psathyrellaceae*), a rare species from North America is discovered in Europe. Phytotaxa 542 (2): 136–152. https://doi.org/10.11646/PHYTOTAXA.542.2.2

V. **Pošta A,** Matočec N, Kušan I, Tkalčec Z, Mešić A (2023) The lignicolous genus *Entonaema*: its phylogenetic–taxonomic position within *Hypoxylaceae* (*Xylariales, Fungi*) and an overview of its species, biogeography, and ecology. Forests 14: 1764. https://doi.org/10.3390/f14091764

1. UVOD

1.1. Bioraznolikost

Najznačajniji ekološki izazovi s kojima se čovječanstvo danas suočava su klimatske promjene i gubitak bioraznolikosti. U znanosti, politici, ekonomiji, ali i u široj populaciji bioraznolikost je prepoznata kao temelj zdravlja ekosustava (Diaz i Malhi 2022, Laurila Pant 2015). Smanjenje bioraznolikosti uzrokovano je prvenstveno ljudskom aktivnošću te može dovesti do smanjenja interakcije organizama na multitrofičkoj razini i posljedično pogodovati promjenama u biotičkom (brojnost i sastav vrsta) i abiotičkom (fizičkom i kemijskom) sastavu ekosustava i kruženju hranjivih tvari (Tylianakis i sur. 2008). Prema definiciji Konvencije o biološkoj raznolikosti (CBD, 1992) pojam „Biološka raznolikost" označava sveukupnost svih živućih organizama koji su sastavni dijelovi kopnenih, morskih i drugih vodenih ekosustava i ekoloških kompleksa; uključuje raznolikost unutar vrsta, između vrsta, te raznolikost između ekosustava (Narodne novine 6/1996). Novija definicija (IPBES, eng. *Intergovernmental Science – Policy Platform on Biodiversity and Ecosystem Services*, http://www.ipbes.net/, 2019) nadopunjuje orginalnu definiciju te uključuje genetičke, fenotipske, filogenetske i funkcionalne značajke, kao i promjene u bogatstvu i distribuciji kroz vrijeme i prostor unutar i između vrsta, bioloških populacija i ekosustava.

Istraživanjima bioraznolikosti može se pristupiti na tri različite razine, kroz gensku raznolikost te kroz raznolikost vrsta i ekosustava (Grey 2000). Raznolikost vrsta je osnovna razina biološke raznolikosti koja pruža vrijedne informacije o strukturi organizama u ekosustavu, a odnosi se na broj različitih vrsta te njihovu proporcionalnu zastupljenost (Grey 2000). Vrsta (lat. *species*) u biologiji je osnovna taksonomska kategorija koja obuhvaća skupinu živih organizama koje karakterizira isto porijeklo, slična anatomska građa te sposobnost međusobnog razmnožavanja koje rezultira plodnim potomstvom. Biološka sistematika je znanstvena disciplina koja se temelji na taksonomskoj identifikaciji, imenovanju i klasifikaciji živih organizama s obzirom na njihove srodstvene odnose, s ciljem spoznaje i zaštite bioraznolikosti (Michener i sur. 1970, Martens i Segers 2005). Otpornost, produktivnost i stabilnost ekosustava na određenom području uvjetovani su raznovrsnošću staništa, životnih zajednica i ekoloških procesa (Pearce i Moran 1994, Folke 1996). Globalno smanjenje cjelovitosti ekosustava, te zastupljenosti i brojnosti populacija i vrsta utječe na vitalne koristi koje čovjek dobiva iz prirode što potencijalno može ugroziti kvalitetu života budućih generacija (Diaz i sur. 2019). Nadalje, od vitalnog značaja je bogatstvo života na molekularnoj

razini, odnosno molekularna bioraznolikost bez koje se ne može odvijati evolucija (Campbell 2003, Laurila Pant 2015).

1.2. Očuvanje globalne bioraznolikosti

Najčešće korišteni pristup kvantifikaciji života na Zemlji je procjena broja vrsta (Díaz i Malhi 2022). Katalog života (Catalogue of life, COL) na jednom mjestu objedinjuje globalne popise vrsta po taksonomskim skupinama s ciljem izgradnje sveobuhvatnog kataloga svih poznatih vrsta organizama na Zemlji. Ovaj globalni popis vrsta održava i nadopunjuje velika zajednica taksonoma okupljena u konzorcij kojeg čini više od 500 svjetskih znanstvenika. Cilj globalnog konzorcija je odgovoriti na potrebe znanosti, politike, zaštite prirode te šire javnosti za dosljednim i ažurnim popisom svih svjetski poznatih vrsta. Kontrolni popis COL 2023 (https://www.catalogueoflife.org/2023/06/27/release) sadrži više od 2,1 milijun znanstveno prihvaćenih vrsta, od kojih je oko 1,98 milijuna živućih vrsta, a ostale su izumrle. Od vrsta na popisu, otprilike je polovica insekata, dok petinu čine vaskularne biljke. Preostali eukariotski organizmi obuhvaćaju raznolike skupine organizama, u kojima brojnošću dominiraju gljive (približno 7%), dok svi kralješnjaci predstavljaju samo približno 4% od ukupno poznatih vrsta (Willis 2017, Willis 2018, Purvis i sur. 2019, Díaz i Malhi 2022). Po nekim procjenama smatra se da bi ukupna globalna bioraznolikost mogla varirati čak u rasponu od 563 milijuna do 2,2 milijarde vrsta (Li i Wiens 2023).

Bioraznolikost se smanjuje brže nego ikad u ljudskoj povijesti. Oko milijun vrsta životinja i biljaka (oko 25%) nalazi se pred izumiranjem, a ako se ne poduzmu mjere za smanjenje gubitka bioraznolikosti, mnoge će vrste izumrijeti u roku od nekoliko desetljeća. Također, doći će do daljnjeg ubrzanja globalne stope izumiranja vrsta, koja je već sada barem 10 do 100 puta veća od prosjeka u posljednjih 10 milijuna godina (IPBES, 2019). Izravni uzročnici promjena u prirodi s najvećim utjecajem na globalnu biorazanolikost obuhvaćaju promjene u korištenju kopna i mora, iskorištavanje živih organizama, klimatske promjene, te onečišćenje. Oni su uvjetovani nizom neizravnih uzročnika promjena, odnosno prihvaćenim društvenim vrijednostima i ponašanjima te se razlikuju među regijama i zemljama (Brondizio i sur. 2019).

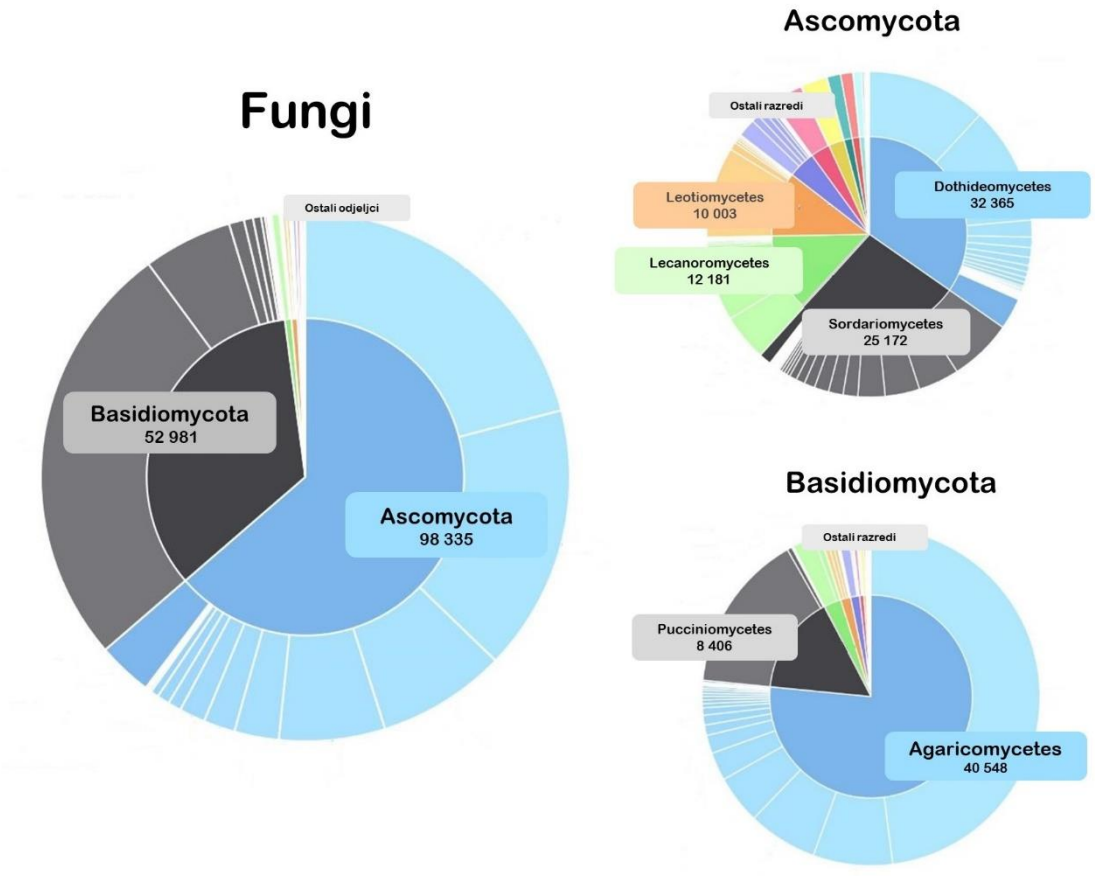
Da se priroda može očuvati, obnoviti i koristiti na održiv način naglašeno je u Kunming-Montreal globalnom okviru za bioraznolikost donesenom na sastanku Konferencije stranaka Konvencije o biološkoj raznolikosti (Joly 2023). Okvir prepoznaje različite vrijednosne sustave i koncepte, uključujući prava prirode kao sastavni dio svoje uspješne provedbe. Vizija Okvira

je život u skladu s prirodom u kojem će: „do 2050. bioraznolikost biti cijenjena, očuvana, obnovljena i mudro korištena, održavajući usluge ekosustava i zdrav planet, istovremeno pružajući ključne dobrobiti za sve ljude". Također, cilj Okvira je osigurati da se u razdoblju do 2030. godine, u skladu s vizijom do 2050., poduzmu hitne mjere koje će vratiti već izgubljenu biološku raznolikost te zaustaviti njen daljnji gubitak za dobrobit ljudi i planete. To će se postići zaštitom i održivim korištenjem biološke raznolikosti te osiguravanjem pravedne i ravnopravne podjele koristi od uporabe genetičkih resursa među razvijenim i nerazvijenim zemljama.

1.3. Bioraznolikost gljiva

Kako bi se odredio stvaran broj gljiva potrebno je opsežno proučavati globalnu biološku raznolikost (Wu i sur. 2019). Do sada je razvijeno i korišteno nekoliko koncepata za identifikaciju i znanstveno opisivanje gljivljih vrsta (Taylor i sur. 2000, Aime i sur. 2021, Bhunjun i sur. 2021, Chethana i sur. 2021) kao što su npr. biološki, morfološki, ekološki i filogenetski koncept (Quaedvlieg i sur. 2014). Trenutno najčešće korišteni klasifikacijski sustav temelji se na molekularnom pristupu koji značajno doprinosi razumijevanju evolucijskih odnosa kod gljiva (Naranjo-Ortiz i Gabaldón 2020). Evolucija se odnosi na nasljedne genske promjene koje se akumuliraju u organizmu tijekom života kroz prilagodbe okolišu, a one su rezultat prirodnog odabira, mutacije, genske derivacije i migracije (Andrews i sur. 2012). Iako gljive imaju drugi najveći procijenjeni broj živućih vrsta nakon kukaca, broj znanstveno poznatih vrsta gljiva je više nego dvostruko manji od biljaka. Vjerojatno je da su gljive bile izložene sličnim evolucijskim pritiscima kao i biljke te da je broj gljivljih vrsta koje su se tijekom vremena razvile veći nego što sadašnji podaci pokazuju. Nije neobično da je poznato manje vrsta gljiva nego biljaka, unatoč procijenjenoj velikoj ukupnoj brojnosti gljivljih vrsta. Gljive žive "skrivenim" načinom života pri čemu svoje strukture za razmnožavanje koje uočavamo u prirodi i po kojima ih morfološki razlikujemo, formiraju najčešće kratkotrajno i samo u određenim uvjetima, pa ih je znatno teže pronaći. Također, najveći broj gljivljih vrsta nije moguće sa sigurnošću morfološki identificirati bez uvida u mikroskopske strukture stanica i tkiva, što proces identifikacije čini težim i dugotrajnijim. Nadalje, u znanstvenim institucijama ili na nacionalnoj razini u pravilu djeluje manje mikologa nego botaničara. Napredak metodologije istraživanja na molekularnoj razini dodao je nove razlikovne karakteristike morfološkm obilježjima gljiva te time ubrzao otkrivanje novih vrsta i, nadalje, pružio važne uvide u razumijevanje raznolikosti gljiva, identifikaciju rijetkih vrsta gljiva i uspostavljanje ciljeva njihovog očuvanja (Willis 2018).

Procjenjeni ukupan broj gljivljih vrsta iznosi od 2 do 11 milijuna (Hawksworth i Lücking 2017, Baldrian i sur. 2021, Lücking i sur. 2021), dok je trenutno je poznato (znanstveno prihvaćeno) oko 156 000 vrsta gljiva (http://www.speciesfungorum.org/), klasificiranih u 20 odjeljaka (lat. *phylum*): *Ascomycota*, *Aphelidiomycota*, *Basidiobolomycota*, *Basidiomycota*, *Blastocladiomycota*, *Calcarisporiellomycota*, *Caulochytriomycota*, *Chytridiomycota*, *Entomophthoromycota*, *Entorrhizomycota*, *Glomeromycota*, *Kickxellomycota*, *Monoblepharomycota*, *Mortierellomycota*, *Mucoromycota*, *Neocallimastigomycota*, *Olpidiomycota*, *Rozellomycota*, *Sanchytriomycota* i *Zoopagomycota* (Wijayawardene i sur. 2022). Najveći broj vrsta (oko 97%) pripada odjeljcima *Ascomycota* (oko 98 000 vrsta) i *Basidiomycota* (oko 53 000 vrsta). Razred najzastupljeniji vrstama u odjeljku *Basidiomycota* je *Agaricomycetes* s više od 40 000 vrsta, dok su razredi *Dothideomycetes*, *Sordariomycetes*, *Lecanoromycetes* i *Leotiomycetes* najzastupljeniji vrstama u odjeljku *Ascomycota* (Slika 1).



Slika 1. Vrstama najzastupljeniji odjeljci carstva *Fungi* (lijevo). Vrstama najzastupljeniji razredi odjeljaka *Ascomycota* (gore desno) i *Basidiomycota* (dolje desno). Brojevi na slici predstavljaju broj vrsta u odjeljcima, odnosno razredima carstva *Fungi* (prilagođeno prema Bánki i sur. 2023).

1.3.1. Bioraznolikost gljiva u Hrvatskoj

Hrvatska se ističe vrlo velikom bioraznolikošću koja je uvjetovana njezinim položajem na razmeđi četiriju biogeografskih regija (kontinentalna, sredozemna, panonska i alpska), razvedenošću reljefa, geološkim, pedološkim, hidrološkim i klimatskim prilikama, kao i ljudskim djelovanjem (Radović 2000). Velika bioraznolikost organizama na području Hrvatske posljedica je značajnih geoloških i klimatoloških zbivanja u prošlosti. Pretpostavlja se da oko 20 000 znanstveno poznatih vrsta gljiva živi na području Republike Hrvatske (Tkalčec i sur. 2008), od čega je do sada pronađeno i zabilježeno tek oko 5000 vrsta (oko 25%).

Istraživanja bioraznolikosti gljiva u Hrvatskoj značajno su se povećala u posljednjih 20-ak godina, formiranjem grupe za bioraznolikost gljiva u okviru Laboratorija za informatiku i modeliranje okoliša Instituta Ruđer Bošković. Grupa je zaslužna za otkriće velikog broja vrsta i rodova novih za nacionalnu bioraznolikost, ali i znatnog broja vrsta još nepoznatih znanosti. Od 2019. godine djeluje samostalno kao Laboratorij za biološku raznolikost unutar Zavoda za istraživanje mora i okoliša, pod vodstvom dr. sc. Armina Mešića. S ciljem povećanja razine istraženosti gljiva u Hrvatskoj, grupa je od listopada 2018. do veljače 2023. provela projekt Hrvatske zaklade za znanost (HRZZ) naziva „Unapređenje usluga šumskih ekosustava Hrvatske kroz vrednovanje bioraznolikosti gljiva temeljenoj na DNA barkodiranju" u okviru kojeg je izrađena i ova doktorska disertacija, a glavni cilj projekta bio je istražiti bioraznolikost gljiva u šumama Hrvatske uz pomoć metoda DNA barkodiranja te analizirati utjecaj bioraznolikosti gljiva na usluge šumskih ekosustava.

Od njenog osnutka, članovi spomenute grupe objavili su 75 rodova i 402 vrste kao nove za hrvatsku bioraznolikost, te 4 roda i 25 vrsta novih za znanost s područja Hrvatske (npr. Hausknecht i sur. 2007, Tkalčec i Mešić 2008, Mešić i sur. 2012, Kušan i sur. 2015a, 2015b, 2018, Tibpromma i sur. 2017, Crous i sur. 2017, Hyde i sur. 2017, Mešić i sur. 2021). Također, grupa je vrlo aktivna i u istraživanju globalne bioraznolikosti gljiva, što je rezultiralo suradnjom s brojnim stranim znanstvenicima te objavljivanjem dva roda i šest vrsta novih za znanost iz Europe, Azije i Afrike (npr. Mešić i sur. 2011, Matočec i sur. 2014, Tkalčec i sur. 2015).

1.3.2. Važnost gljiva u očuvanju bioraznolikosti

Gljive su heterotrofni eukariotski organizmi, čija se stanična stijenka sastoji uglavnom od hitina, beta glukana i glikoproteina. Većina vrsta ima višestanično tijelo – micelij – koji se sastoji od tankih niti (hifa) koje se granaju. U određenim uvjetima ono prelazi iz vegetativne u

reproduktivnu fazu formirajući višestanične (često vrlo složene) rasplodne strukture – plodišta – u kojima dolazi do razvijanja spolnih ili nespolnih spora koje se rasprostranjuju zrakom i vodom ili putem životinja. Gljive su uključene u iznimno veliki broj asocijacija s okolišem i drugim živim bićima što ih čini najraznovrsnijom skupinom organizama na Zemlji. Ključne su u održavanju stabilnosti ekosustava gdje kao saprotrofi, odnosno razgrađivači, i simbionti s drugim organizmima (npr. mikorizne vrste, lišaji, endofiti) sudjeluju u kruženju hranjivih tvari te povećavaju produktivnost biljaka i otpornost na okolišni stres. Također, gljive mogu živjeti i kao paraziti i predatori. Ljudi imaju veliku izravnu korist od gljiva. Najvažniju ulogu gljive imaju u farmaceutici te u prehrambenoj industriji, a od nedavno su prepoznate i uloge gljiva u tekstilnoj industriji i graditeljstvu (Tkalčec i sur. 2008, Prescott i sur. 2018, Meyer i sur. 2020). Unatoč njihovoj velikoj važnosti, gljive su među najmanje istraženim organizmima.

Nedostatak osnovnog znanja o bioraznolikosti gljiva iznimno je zabrinjavajuć. Gljivlje vrste nisu imune na prijetnje s kojima se suočavaju životinje i biljke te su također u opasnosti od izumiranja (Dahlberg 2011). Gljive se pojavljuju u raznovrsnim staništima diljem svijeta, pri čemu je sastav i rasprostranjenost vrsta u korelaciji s ekološkim i biotičkim čimbenicima kao što su klima, kemijski sastav vode i tla, sastav životinjskih i biljnih zajednica, postojanje simbionata i kvaliteta staništa (Tedersoo i sur. 2014, Geml i sur. 2022). Poremećaji ovih čimbenika mogu negativno utjecati na rasprostranjenost i veličinu populacije gljivljih vrsta, dovodeći ih u opasnost od izumiranja. Postoji više uzroka gubitka bioraznolikosti što uključuje nestajanje, degradaciju ili fragmentaciju staništa, smanjenje kvalitete staništa, onečišćenje, neodgovarajuće i prekomjerno iskorištavanje prirodnih resursa, klimatske promjene te neadekvatno političko upravljanje (Tkalčec i sur. 2008, Mace 2010, Habibullah i sur. 2022).

Crveni popis ugroženih vrsta Međunarodne unije za zaštitu prirode (IUCN, eng. *International Union for Conservation of Nature*) globalni je popis vrsta sa statusom ugroženosti procijenjenim na temelju IUCN kriterija. Sadrži informacije o rasprostranjenosti vrsta, te njihovom staništu, ekologiji, veličini populacije, trendovima, strukturi, prijetnjama i očuvanju (IUCN *Standards and Petitions Committee*, 2022). Inicijativa za izradu IUCN Crvenog popisa ugroženih vrsta pokrenuta je 1964. godine, dok su prve dvije vrste gljiva (*Cladonia perforata* A. Evans i *Erioderma pedicellatum* (Hue) P. M. Jørg.) uvrštene tek 2003., a vrsta *Pleurotus nebrodensis* (Inzenga) Quel. ssp. *nebrodensis* uključena je 2006. Nakon toga, dodatne vrste gljiva su na popis uključene tek 2014. U ažuriranom Globalnom Crvenom popisu 2022-1 (21. srpnja 2022.) objavljeno je 597 gljivljih vrsta, a gljive i dalje čine jedno od najmanje zastupljenih carstva na Crvenom popisu ugroženih vrsta IUCN-a, iako je njihov

globalni rizik od izumiranja značajan (The IUCN Red List of Threatened Species, 2022; Mueller i sur. 2022).

Unatoč tome što se na globalnoj razini ne pridaje velika važnost ugroženim vrstama gljiva, na nacionalnim razinama postoji veća informiranost i posvećenost ovom problemu. Do 1992. godine, jedanaest zemalja objavilo je nacionalne popise ugroženih gljivljih vrsta, a trenutno u 40 zemalja postoje nacionalne Crvene liste koje sadrže više od 10 000 ugroženih gljivljih vrsta određenih najčešće prema kriterijima IUCN-a. Crveni popis ugroženih vrsta gljiva Hrvatske sadrži 349 gljivljih vrsta čiji je stupanj ugroženosti određen prema kriterijima IUCN-a. Na temelju Crvenog popisa ugroženih vrsta gljiva Hrvatske, 2008. godine objavljena je i Crvena knjiga gljiva Hrvatske (Tkalčec i sur. 2008).

1.4. Istraživanja bioraznolikosti gljiva

Brza i precizna identifikacija uzoraka gljiva do razine vrste ključna je u mnogim ljudskim djelatnostima, kao što su kontrola gljivljih patogena (medicina, veterina, agronomija, šumarstvo), ekološki monitoring te zaštita i održivo korištenje bioraznolikosti (Xu 2016). Razumijevanje ekologije, bioraznolikosti te specifičnih uloga gljiva u različitim ekosustavima ovise o točnoj taksonomskoj identifikaciji vrsta koja čini temelj daljnjih znanstvenih istraživanja (Badotti i sur. 2018). Gljivlji organizmi pokazuju veliku raznolikost u morfologiji, fiziologiji, genetičkim svojstvima te ekološkim funkcijama i životnim strategijama zbog čega je njihova precizna identifikacija vrlo složen proces koji uključuje kombinaciju različitih pristupa i metoda (Raja i sur. 2017).

U mikologiji se za razgraničenje vrsta uglavnom koristi šest različitih koncepata. Tradicionalno je opisano pet bioloških koncepata: (1) Biološki koncept vrsta (eng. *Biological Species Concept*) koji se temelji na reproduktivnoj izolaciji (Wright 1940, Mayr 1942), (2) Morfološki koncept vrsta (eng. *Morphological Species Concept*) koji je baziran na morfološkoj varijabilnosti, (3) Ekološki koncept vrsta (eng. *Ecological Species Concept*) koji naglašava prilagodbu određenoj ekološkoj niši (van Valen 1976), (4) Filogenetski koncept vrsta (eng. *Phylogenetic Species Concept*) koji se zasniva na nukleotidnoj varijabilnosti (Hennig 1966), te (5) Koncept genealoške podudarnosti i filogenetskih odnosa vrsta (eng. *Genealogical Concordance Phylogenetic Species Recognition*) uz pomoć kojeg se na temelju filogenetske podudarnosti gena prepoznaje evolucijska neovisnost taksonomskih linija (O'Donnell i sur. 1998). Koncept razgraničenja vrsta i viših taksonomskih kategorija kod gljiva, koji je danas postao opće prihvaćen u mikološkoj zajednici, kroz polifazni pristup (Aime i sur. 2021)

objedinjuje evaluaciju različitih morfoloških, ekoloških i filogenetskih karakteristka (Quadevlieg i sur. 2014), a najčešće je definiran kroz pojam integrativna taksonomija (Dayrat 2005, Pires i Marinoni 2010, Pante i sur. 2015).

1.4.1. Ograničenja tradicionalnog taksonomskog pristupa

U morfološkom konceptu, vrsta je definirana kao najmanja skupina jedinki koja se može razlikovati na temelju morfoloških obilježja (Cronquist 1978, Aldhebiani 2018, Chethana i sur. 2021). Taksonomija gljiva tradicionalno se temeljila na morfologiji (Singer 1986, Watkinson i sur. 2016, Xu 2020). Morfološka obilježja pojedinih vrsta gljiva često ovise o fiziološkom stanju i okolišnim uvjetima te imaju veću varijabilnost nego što se to ranije smatralo. Blisko srodne vrste gljiva mogu biti morfološki vrlo različite, dok pojedine vrste koje nisu srodne mogu tijekom procesa konvergentne evolucije razviti međusobno slična morfološka obilježja (Watkinson i sur. 2016). Vrste koje imaju vrlo slična, ponekad nerazlučiva fenotipska obilježja se nazivaju kriptičkim vrstama. U kriptičkom konceptu, vrste su podijeljene u tri grupe s obzirom na razlučivost njihovih fenotipskih obilježja. Pojam kriptička vrsta u znanstvenoj literaturi najčešće se odnosi na “strogo” kriptičke vrste, odnosno vrste koje su u potpunosti nerazlučive na temelju njihovih morfoloških obilježja. Osim strogo kriptičkih vrsta, razlikujemo i semikriptičke te pseudokriptičke vrste. Semikriptičke vrste također nije moguće razlikovati na temelju morfoloških karakteristika, već su razlučive isključivo po karakteristikama kao što su ekologija i rasprostranjenost. Pseudokriptičke vrste se odlikuju vrlo malim morfološkim razlikama zbog kojih je potrebno vrlo pažljivo pristupiti identifikaciji vrste, no čak i uz takav pristup postoji velika mogućnost pogrešne identifikacije (Mann i Evans 2007).

Složenost i raznolikost životnih ciklusa, kao i razlike u morfologiji između različitih životnih stadija (anamorf / teleomorf) gljiva uvelike otežavaju identifikaciju te imenovanje vrsta na temelju morfologije (Stengel i sur. 2022). S obzirom na poteškoće prilikom razgraničenja gljivljih vrsta na temelju morfoloških svojstava, često je problematično usporediti taksonomiju različitih skupina gljiva te razviti učinkovit sustav prepoznavanja vrsta koji se može primijeniti na sve gljivlje vrste. Razvojem tehnologije lančane reakcije polimerazom (PCR) i DNA sekvenciranja, u mikološkim taksonomskim istraživanjima tijekom zadnjih 30-ak godina počelo je brzo usvajanje i korištenje molekularnih metoda koje su do danas postale važan alat pri identifikaciji i međusobnom razlikovanju poznatih gljivljih vrsta, kao i pri opisivanju novih.

1.4.2. Opis nove vrste za znanost

Znanstvena imena živih organizama čine preduvjet za komunikaciju koja koristi biološke informacije, kako unutar biologije tako i prema ostalim područjima ljudskog djelovanja (Aime i sur. 2021). Pravila za opisivanje novih vrsta gljiva izričito su propisana u Međunarodnom kodeksu nomenklature alga, gljiva i biljaka (Turland i sur. 2018) te ih je nužno poštivati kako bi vrsta bila valjano (validno) opisana.

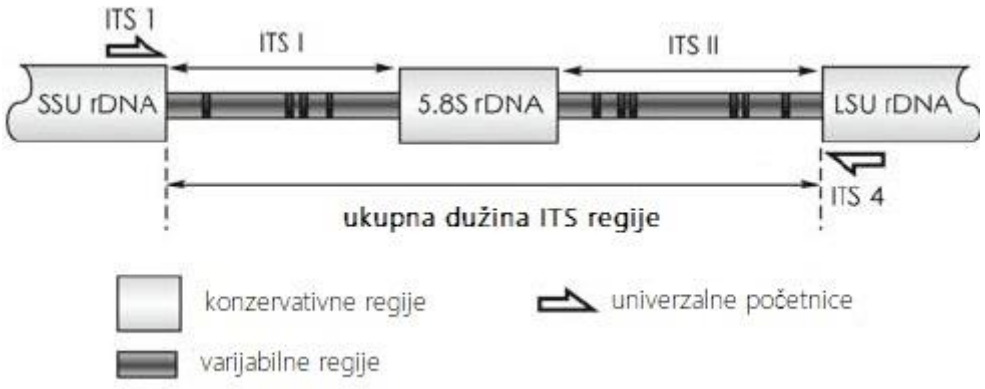
Istraživački zadatak koji prethodi svakom opisivanju gljivlje vrste nove za znanost je ustanoviti pripada li analizirani materijal zaista vrsti gljive različitoj od svih ostalih do tada opisanih vrsta. Osim toga, potrebno je istražiti na temelju kojih značajki se nova vrsta razlikuje od onih blisko srodnih i/ili morfološki sličnih. Aime i sur. (2021) preporučuju da se opis novih vrsta temelji na većem broju uzoraka kako bi se obuhvatila varijabilnost vrste te na većem broju različitih svojstava gljivljeg organizma (npr. makro- i mikro-morfologija, analiza DNA sekvenci, metabolički ili proteomski podaci, fiziologija, ekologija, biogeografija) što se temelji na konceptu integrativne taksonomije. Svaka objava nove vrste mora sadržavati (1) dijagnozu u obliku izjave kojom se navode najvažnija obilježja koja novu vrstu razlikuju od sličnih vrsta ili (2) detaljan opis fenotipskih obilježja koja karakteriziraju novu vrstu. Aime i sur. (2021) objavili su niz praktičnih preporuka kako bi se proces opisivanja novih vrsta što je više moguće ujednačio čime bi se omogućila provjerljivost i ponovljivost istraživanja te široka dostupnost metapodataka (popratni podaci).

Za svaku novu vrstu potrebno je prilikom prve znanstvene objave odrediti tipski uzorak tj. holotip (osušeno plodište ili metabolički inaktivna kultura) koji će služiti kao referentni materijal za sva buduća istraživanja te navesti u kojoj je zbirci uzoraka (fungarij/herbarij) pohranjen i pod kojim inventarskim brojem. Gotovo svi međunarodno recenzirani znanstveni časopisi koji objavljuju taksonomske radove zahtijevaju da svaki opis nove vrste gljive za znanost bude popraćen podatkom o pripadajućoj DNA sekvenci (najčešće DNA barkod) koja mora biti javno dostupna u nekoj od globalnih bioinformatičkih baza podataka (npr. Genbank), što dokazuje i podatak da je tijekom 2018. godine 94% znanstvenih radova s tematikom taksonomije gljiva obuhvaćalo analizu DNA sekvenci (Miralles i sur. 2020, Lofgren i Stajich 2021).

1.5. Doprinos molekularne taksonomije u identifikaciji gljivljih vrsta

1.5.1. DNA barkodiranje

Metoda koja se može primijeniti u identifikaciji svih gljiva i koja nadopunjuje različite skupine tradicionalnih obilježja za opisivanje različitih skupina gljiva je DNA barkodiranje (Xu 2016). Ono čini osnovu molekularne taksonomije kao vrlo značajnog dijela u integrativnom taksonomskom pristupu mikološkim istraživanjima. Karakterističan slijed duljine 400 do 800 nukleotida unutar određenih fragmenata DNA, tzv. DNA barkod, služi u razlikovanju organizama na razini vrste. Međunarodni konzorcij za barkodiranje gljiva 2012. godine formalno je predložio da se ITS (eng. *internal transcribed spacer*) genska regija nuklearne ribosomalne RNA koristi kao primarni DNA barkod za carstvo gljiva na temelju visoke varijabilnosti na razini vrste te visoke uspješnosti amplifikacije (>70%) (Schoch i sur. 2012). ITS genska regija kod gljiva duga je oko 600 bp i sadrži dvije varijabilne regije, ITS1 i ITS2, koje su odvojene konzervativnom genskom regijom 5.8S rDNA (White i sur. 1990) (Slika 2). ITS regija je okružena genom 18S rDNA (SSU, eng. *small subunit rDNA*) na 5'-kraju ITS-1 genske regije i genom 28S rDNA (LSU, eng. *large subunit rDNA*) na 3' ITS-2 genske regije. Konzervativni geni 18S, 5.8S i 28S rDNA omogućuju dizajn "univerzalnih početnica" za amplifikaciju ITS1, ITS2 ili cijele ITS regije kod većine gljivljih vrsta. Najčešće korišene početnice za ITS gensku regiju su ITS1 i ITS4 (White i sur. 1990).



Slika 2. Shematski prikaz ITS genske regije (prilagođeno prema Horisawa i sur. 2009).

Prednost korištenja ITS genske regije kao univerzalnog barkoda za gljive je ta da svaki haploidni genom obično sadrži višestruke kopije ribosomalne rDNA (uključujući ITS), što omogućuje amplifikaciju ove genske regije iz male količine biološkog materijala. Uz brojne pozitivne karakteristike ITS genske regije kao DNA barkoda za gljive, prisutni su i određeni nedostaci. Intragenomska varijacija u ITS regiji pojavljuje se prosječno u 3–5% vrsta iz odjeljaka *Ascomycota* i *Basidiomycota* (Lindner i sur. 2013). Također, kod nekih rodova gljiva,

kao što su npr. *Aspergillus*, *Fusarium*, *Penicillium* i *Trichoderma*, sekvenca ITS genske regije ima jako malu razlučivost na razini vrste (Houbraken i sur. 2014, Samson i sur. 2014, O'Donnell i sur. 2015, Cai i Druzhinina 2021). ITS barkod najčešće služi u identifikaciji već poznatih vrsta, dok je pri opisu nove vrste većinom potrebno sekvencirati i druge genske regije kako bi se utvrdio položaj nove vrste unutar određene taksonomske skupine (Raja i sur. 2017). U takvim se slučajevima, uz ITS barkod, za uspješnost razlikovanja vrsta određuju i dodatni genski markeri, tzv. sekundarni barkodovi. Odabir sekundarnih barkodova ovisi o taksonomskoj skupini, te se često pri identifikaciji ne koristi samo jedan dodatni barkod, već kombinacija više barkodova sa svrhom precizne identifikacije i pouzdanog određivanja srodstvenih odnosa.

Ribosomalni geni koji okružuju ITS gensku regiju, mala ribosomska podjedinica SSU i velika podjedinica LSU, mogu se koristiti kao sekundarni barkodovi, s ulogom preciznijeg određivanja filogenetskih odnosa u različitim taksonomskim skupinama. Genski marker SSU najčešće donosi informacije o višim taksonomskim skupinama (porodica, red, razred) zbog vrlo male varijabilnosti, osobito na razini vrste (Mitchell i Zuccaro 2006). S druge strane, LSU gen sadrži dvije hipervarijabilne regije, D1 i D2, te se samostalno može koristiti pri utvrđivanju roda ili porodice, a u kombinaciji s ITS genskom regijom, i pri identifikaciji vrste (Liu i sur. 2012).

Geni koji se najčešće koriste u funkciji sekundarnih barkodova kod gljiva su geni koji kodiraju za proteine *tef1* (eng. *translation elongation factor 1-alpha*), *rpb1* (eng. *the largest subunit of RNA polymerase II*), *rpb2* (eng. *the second largest subunit of RNA polymerase II*) i *βtub* (eng. *beta-tubulin*) (Raja i sur. 2017). Određivanje ovih gena ima velik značaj u molekularnom aspektu taksonomije zbog doprinosa razumijevanju filogenetskih odnosa među vrstama te pri dodatnoj provjeri identifikacije na razini vrste (Zhao i sur. 2017). Kodirajući dijelovi (eksoni) ovih gena kod gljivljih organizama nisu skloni mutacijama, dok su nekodirajući dijelovi (introni) vrlo varijabilni na razini vrste te imaju funkciju pri provjeri identifikacije (Tekpinar i Kalmer 2019). U odnosu na ITS gensku regiju, između srodnih taksonomskih skupina postoji manja varijabilnost u duljini ovih gena te je prisutna po samo jedna kopija gena u genomu. Gen *tef1* je na temelju visoke varijabilnosti na razini vrste potvrđene na više od 1500 vrsta i približno 20 000 PCR reakcija, predložen kao univerzalni sekundarni barkod za gljive (Stielow i sur. 2015). Geni *rpb1* i *rpb2*, kodiraju za sintezu najveće i druge najveće podjedinice proteina RNA polimeraze II koji je odgovoran za transkripciju gena (Matheny i sur. 2002). *Rpb1* i *rpb2* geni imaju visoku mogućnost razlučivanja na razini

vrste nekih taksonomskih skupina, a u kombinaciji s drugim genskim markerima doprinose preciznom određivanju filogentskih odnosa. Gen *βtub* može se naći u gotovo svim eukariotskim organizmima. On kodira za sintezu beta-tubulina, sastavnog dijela mikrotubula koji imaju važnu ulogu u eukariotskim staničnim procesima kao što su dijeljenje stanica, održavanje oblika stanice i unutarstanični transport (Einax i Voigt 2003). Zbog toga *βtub* gen, u konkatenatnim analizama s ostalim navedenim DNA barkodovima (markerima), značajno doprinosi procjeni filogenetskih odnosa.

S obzirom na mnoge pozitivne strane gena koje danas nazivamo sekundarnim barkodovima, oni su također bili testirani pri određivanju primarnog univerzalnog barkoda za gljive. Međutim, ovi su geni u navedenim analizama pokazali i više nedostataka. Najveći nedostaci su bili nemogućnost kreiranja univerzalnih početnica i neuspješnost PCR amplifikacije što je posljedično rezultiralo slabom popunjenosti bioinformatičkih baza ovim genima (Schoch i sur. 2012).

1.5.2. Bioinformatičke baze

Bioinformatičke baze s najvećim brojem zastupljenih gljivljih sekvenci su Genbank i UNITE. U bioinformatičkim bazama postoje podaci o sekvencama za oko 45 000 imenovanih gljivljih vrsta od kojih je većina zastupljena s ITS genskom regijom (Lücking i sur. 2020). Ova brojka čini oko 30% trenutno poznatih vrsta, a kada se u obzir uzme podatak o procijenjenom ukupnom broju gljivljih vrsta, to odgovara vrlo malom postotku od 1–2%. Osim broja vrsta zastupljenih u bioinformatičkim bazama, vrlo je važna točnost identifikacije uzoraka iz kojih su sekvence izolirane. I prednost i nedostatak Genbank bioinformatičke baze je vrlo lako unošenje i dostupnost podataka (Schoch i sur. 2020). Kako bi sustavi baza podataka pravilno funkcionirali i bili pouzdani, referentni bi podaci morali biti potpuni, a sekvence ispravno taksonomski označene (Nilsson i sur. 2006). Aime i sur. (2021) objavili su popis najvažnijih informacija koje bi autori trebali unijeti u bazu podataka uz sekvencu (Slika 3). Međutim, Hofstetter i sur. (2019) procjenjuju da je u javnim bioinformatičkim bazama udio pogrešno identificiranih sekvenci ITS genske regije gljiva čak blizu 30%. Također, u bazama podataka velikom broju sekvenci nije dodijeljeno ime vrste, a to se često odnosi i na sekvence novoobjavljenih vrsta. Bioinformatička baza UNITE sadrži više od 100 000 vrsnih hipoteza (eng. *Species Hypotheses*) s oko 2,5 milijuna sekvenci ITS genske regije, međutim većina ih nije imenovana (Lücking i sur. 2020). Ovaj problem je potrebno riješiti ažuriranjem podataka nakon objavljivanja znanstvenog rada, čemu može doprinijeti i unošenje standardiziranih ključnih riječi u znanstvene radove te navođenje referentnog broja (eng. *Accession number*)

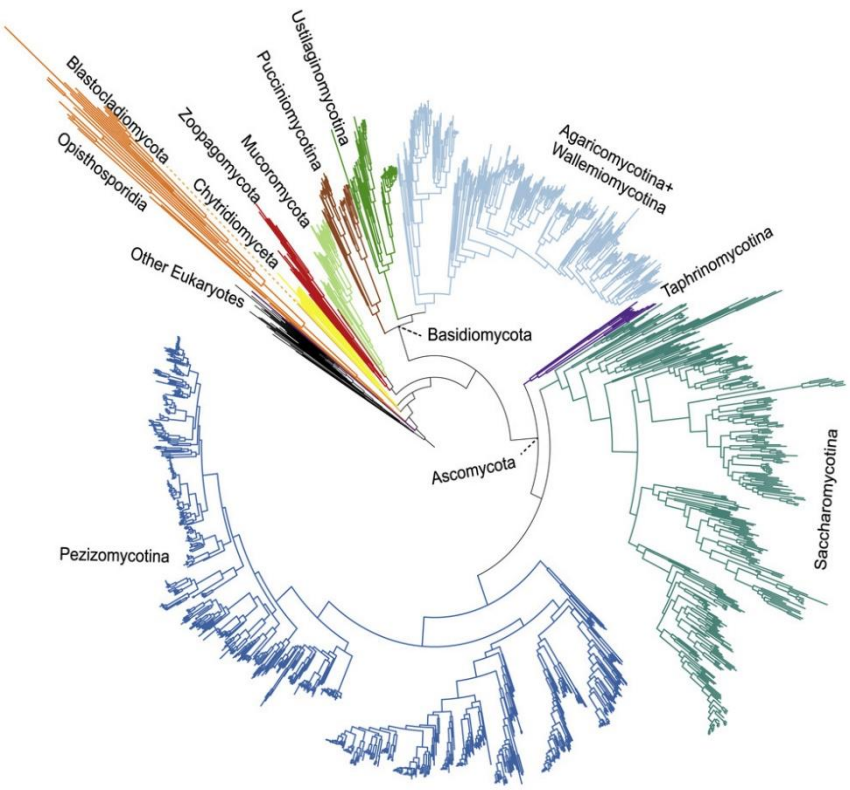
tipskog materijala (Lücking i sur. 2017, Schoch i sur. 2017). Postojeći podaci o sekvencama u bioinformatičkim bazama najčešće ne pružaju dovoljnu pouzdanost da bi se samo iz njih mogla provesti točna identifikacija uzorka. Također, budući da DNA sekvence većine gljivljih vrsta još uvijek nisu prisutne u takvim bazama, ne možemo utvrditi pripada li molekularna sekvenca koja nije u njima pronađena novoj vrsti za znanost. Zato je ključna popunjenost bioinformatičkih baza podataka referentnim sekvencama iz tipskog materijala, no ona je još uvijek rudimentarna za mnoge skupine gljiva, posebno za rodove bogate vrstama (Lücking i sur. 2020). Da bi se moglo utvrditi je li neka vrsta potencijalno nova za znanost, nije dovoljno samo usporediti analizirani DNA barkod s podacima dostupnim u bioinformatičkim bazama (Goldstein i DeSalle 2011), već je nužno provesti i morfološku taksonomsku analizu skupine u koju određeni nalaz pripada, čemu će uvelike pomoći i molekularna filogenetska analiza kako bi se utvrdili srodstveni odnosi među bliskim vrstama.

NCBI qualifier	Note (INSDC controlled vocabulary link listed where applicable)	Example
collected_by	Name of person who collected the sample, please use initials and surname.	/collected_by = "A.H. Smith"
collection_date	Day, month and year when the sequenced specimen was collected.	/collection_date = "23-Aug-1948"
country	Country where the sample was collected. Additional region or locality information must be after the country name and separated by a ':'. See http://www.insdc.org/documents/country-qualifier-vocabulary	/country = "USA: Washington, Pierce County, Mt. Rainier National Park"
culture_collection	Format for cultures in culture collections: 'institution-code:culture-id'. culture-id and institution-code are mandatory. When possible use code documented in NCBI BioCollections or WFCC.	/culture_collection = "CBS:1752"
host	Use full verified binomial, if possible. Incomplete names such as genus sp. is acceptable.	/host = "*Quercus longinux*"
isolate	Use this for lab numbers/ field numbers of the specific specimen/culture from which this sequence was obtained.	/isolate = "JT13209"
isolation_source	Reserved for physical or environmental source and substrate information.	/isolation_source = "dead wood"
lat_lon	Latitude and longitude, in decimal degrees, of where the sample was collected.	/lat_lon = "28.721667 N 17.785278 W"
note	Add any additional unstructured information such as isolation method, isotype info not addressed in the other fields.	/note=""DNA isolation: REPLI-g Single Cell Kit (Qiagen)"
specimen_voucher	Format for dried specimens: 'institution-code: internal-code:specimen-id'. specimen-id is mandatory. When possible use code documented in NCBI BioCollections or Index Herbariorum or indicate personal herbaria by adding in front:'personal:' See http://www.insdc.org/controlled-vocabulary-specimenvoucher-qualifier	/specimen_voucher = "MICH:14410" or /specimen_voucher = "MICH:AH Smith 30,553" or /specimen_voucher = "personal: AH Smith 30,553"
strain	Use this for strain numbers of pure cultures, i.e. those not deposited in culture collections.	/strain = "ABC 1234"
tissue_type	Ignore this field unless it refers to source tissue information e.g. blood, skin etc..	
type_material	This field is not user submitted - it is automatically updated only after the publication or nomenclature database entry is verified by NCBI Taxonomy curators. Please provide the full publication as a pdf to gb-admin@ncbi.nlm.nih.gov. (Do NOT use the Type modifier for this information). See http://www.insdc.org/controlled-vocabulary-typematerial-qualifer	/type_material = "holotype of *Tuber anniae*"

Slika 3. Prikaz preporučenih informacija pri unosu nove sekvence u bioinformatičku bazu podataka (preuzeto iz Aime i sur. 2021).

1.5.3. Molekularna taksonomija i filogenija

Osnovu molekularne taksonomije čine molekularna sistematika i filogenija, u kojoj se identifikacija bazira na filogenetskim odnosima srodnih vrsta koji se određuju na temelju varijabilnosti DNA sekvenci različitih organizama unutar određene tasonomske grupe (Waikagul i Thaenkham 2014). Kod gljiva, danas se najčešće koristi kombinacija ribosomskih genskih regija i gena koji kodiraju za proteine pri određivanju filogenetskih odnosa (Raja i sur. 2017, Aime i sur. 2021). BLAST algoritam (eng. *Basic Local Alignment Search Tool*) integriran u Genbank pomoći će pri određivanju taksonomske grupe i pri odabiru taksona koji će činiti bazu za filogenetske analize. Metode koje se najviše koriste u mikologiji za određivanje filogenetskih odnosa među vrstama su metode najveće vjerojatnosti (eng. *Maximum likelihood*) i Bayesova procjena (eng. *Bayesian inference*), kompleksne metode koje kao osnovu koriste evolucijske modele određene na temelju DNA poravnanja (eng. *alignment*). Za određivanje evolucijskih modela i provedbu analiza koriste se bioinformatički stoftveri kao što su IQ tree i MrBayes. Filogenetski odnosi vizualiziraju se kao filogentsko stablo u kojem je svaki takson prikazan kao zasebna grana (eng. *branch*), a odnosi između različitih taksona određuju se na temelju kombinacije rezultata podržanosti obje korištene metode. Filogenetsko stablo carstva gljiva prikazano je na Slici 4.



Slika 4. Filogenetsko stablo carstva *Fungi* (preuzeto iz Li i sur. 2021).

1.6. Cilj doktorskog rada

Cilj ove doktorske disertacije je rješavanje nekoliko znanstvenih problema u sistematici gljiva metodama integrativne taksonomije koja objedinjuje evaluaciju molekularnih, morfoloških i ekoloških obilježja gljivljih organizama što obuhvaća opisivanje novih vrsta za znanost i/ili redefiniranje postojećih taksonomskih koncepcija. U radu se daje poseban naglasak na vrednovanje znanstvenog doprinosa DNA barkodiranja u otkrivanju, razgraničenju i identifikaciji srodnih vrsta u različitim skupinama nadzemnih gljiva iz odjeljaka *Ascomycota* i *Basidiomycota*.

1.7. Hipoteze istraživanja

U skladu s ciljevima istraživanja definirane su sljedeće hipoteze:

H1. U pojedinim skupinama gljiva s područja Hrvatske postoje nove i/ili kriptičke vrste koje je moguće prepoznati, međusobno razlikovati i znanstveno opisati metodama integrativne taksonomije.

H2. DNA barkodiranje može značajno doprinijeti razgraničenju i identifikaciji morfološki sličnih i/ili kriptičkih vrsta gljiva.

2. ZNANSTVENI RADOVI

PUBLIKACIJA I

Inocybe brijunica sp. nov., a New Ectomycorrhizal Fungus from Mediterranean Croatia Revealed by Morphology and Multilocus Phylogenetic Analysis

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Inocybe brijunica sp. nov., A New Ectomycorrhizal Fungus from Mediterranean Croatia Revealed by Morphology and Multilocus Phylogenetic Analysis

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Abstract: A new ectomycorrhizal species was discovered during the first survey of fungal diversity at Brijuni National Park (Croatia), which consists of 14 islands and islets. The National Park is located in the Mediterranean Biogeographical Region, a prominent climate change hot-spot. *Inocybe brijunica* sp. nov., from sect. *Hystericeae* (Agaricales, Inocybaceae), is described based on morphology and multilocus phylogenetic data. The holotype collection was found at the edge between grassland and *Quercus ilex* forest with a few planted *Pinus pinea* trees, on Veli Brijun Island, the largest island of the archipelago. It is easily recognized by a conspicuous orange to orange–red–brown membranaceous surface layer located at or just above the basal part of the stipe. Other distinctive features of *I. brijunica* are the medium brown, radially fibrillose to rimose pileus; pale to medium brown stipe with fugacious cortina; relatively small, amygdaliform to phaseoliform, and smooth basidiospores, measuring ca. 6.5–9 × 4–5.5 µm; thick-walled, utriform, lageniform or fusiform pleurocystidia (lamprocystidia) with crystals and mostly not yellowing in alkaline solutions; cheilocystidia of two types (lamprocystidia and leptocystidia); and the presence of abundant caulocystidia only in the upper 2–3 mm of the stipe. Phylogenetic reconstruction of a concatenated dataset of the internal transcribed spacer region (ITS), the nuclear 28S rRNA gene (nrLSU), and the second largest subunit of RNA polymerase II (*rpb2*) resolved *I. brijunica* and *I. glabripes* as sister species.

Keywords: 1 new taxon; Agaricomycetes; Basidiomycota; biodiversity; climate change; Inocybaceae; taxonomy

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1. Introduction

The Brijuni archipelago consists of 14 islands and islets located in the Adriatic Sea (northern Mediterranean, Europe), near the southwestern coast of the Istrian peninsula. The archipelago is home to Brijuni National Park [1], which covers 33.9 km^2 of protected area, including the surrounding sea. The islands' surface area covers 7.4 km^2; Veli Brijun is the largest island with 5.7 km^2 and is devoid of permanent inhabitants. The National Park was established in 1983 to protect valuable marine and coastal (land) ecosystems and their biodiversity. The area is floristically rich, covered with evergreen vegetation and home to more than 400 native and exotic plant species mostly of Mediterranean origin. The Brijuni archipelago is characterized by a northern Mediterranean climate [1] with an

average annual temperature of 13.9 °C, annual average precipitation of 817 mm, and a relatively high average air humidity of 76%.

During the 20th century, the air temperature increased globally by 0.74 °C, but the temperature rise in the Mediterranean area was higher—up to 1.5–4 °C depending on the region [2]. Following the Regional Climate Change Index (RCCI), the Mediterranean region is one of the most prominent climate change hot-spots in the world [3,4]. Models predicting the intensity of future climate change in this area are not optimistic. According to Mariotti et al. [5], land areas of the Mediterranean will gradually become drier; models predict 8% less precipitation in 2020–2049 compared to 1950–2000, a number that is projected to increase to 15% in 2070–2099. Drying in the northern Mediterranean [4,6] is projected to occur year-round, which will increase water stress for ecosystems if climate change continues at the current rate. Therefore, in the future, we can expect an increase of devastating climatic events (floods, storms, and droughts), more attacks of organisms that cause diseases, and a higher number of invasive species that will compete with indigenous species populations. These events could have a strong negative impact on the Mediterranean forest ecosystems [2] as well as on fruiting and existence of drought-sensitive fungal species in the area [7].

The ratio of plant species to macrofungal species is conservatively estimated as 1:6 [8]. Given the high number of plant species in the Brijuni Archipelago, an equally high diversity of fungal species is expected. Currently, however, there are no published data on fungi from this area. And even though fungal taxonomy has a long history in Europe, many species continue to be described from the continent. In 2019, 23% of newly described species of fungi were from Europe [9]. Given all this, it can be expected that undescribed species may be discovered at the Brijuni Islands. Initial field trips by Croatian mycologists aiming to document the fungal diversity of Brijuni National Park were carried out during the fall season in 2014, 2015, 2016, and 2020. In total, 546 records of basidiomycete fungi were made; 184 samples were collected and deposited in the Croatian National Fungarium (CNF) in Zagreb, Croatia. One of the most common genera found was *Inocybe* (Fr.) Fr. (Agaricomycetes, Agaricales, Inocybaceae), with 28 collections.

Inocybe sensu lato (s.l.) is a highly diverse genus of ectomycorrhizal mushrooms [10] currently with about 1000 accepted species [11]. It belongs to the family Inocybaceae Jülich. The species diversity within *Inocybe* s.l. is best known in Europe, especially in its central countries—Austria, France, Germany, the Netherlands, and Switzerland—with more than 450 species recorded [12]. Current ongoing studies are exploring the diversity of the genus in Europe and many new species have been recently described [13–22].

According to the taxonomic treatment by Matheny et al. [23] based on a six-locus phylogeny, the family Inocybaceae now consists of seven genera: *Auritella* Matheny & Bougher, *Inocybe sensu stricto* (s.s.), *Inosperma* (Kühner) Matheny & Esteve-Rav., *Mallocybe* (Kuyper) Matheny, Vizzini & Esteve-Rav., *Nothocybe* Matheny & K.P.D. Latha, *Pseudosperma* Matheny & Esteve-Rav., and *Tubariomyces* Esteve-Rav. & Matheny. The largest genus remains *Inocybe* s.s. with about 850 known species worldwide. Members of *Inocybe* s.s. can be distinguished from other genera in the family by the presence of pleurocystidia and basidiospores (with a distinct apiculus) that range from amygdaliform to ellipsoid, subcylindrical, angular, nodulose, or spinose in shape [23].

On 16 November 2016, during our fungal diversity research on the island of Veli Brijun, basidiomata of an interesting fungus belonging to *Inocybe* s.s. were found. Its basidiomata were macroscopically striking by the presence of an orange to orange–red–brown membranaceous surface layer (possibly a remnant of universal veil) in the basal part of the stipe—an unusual feature in the genus. Further detailed molecular phylogenetic and morphological analyses confirmed that the species was hitherto unknown to science. Therefore, it is here described as *I. brijunica* sp. nov.

2. Materials and Methods

2.1. Description of the Research Area

The holotype collection of *Inocybe brijunica* was collected on Veli Brijun Island. The biogeographical position and a long history of human interventions have shaped the landscape of Veli Brijun Island, merging natural and anthropogenic elements. The island is mostly covered by a thermophilous forest with holm oak (*Quercus ilex*) (including those in the maquis degradation stage), planted alleys or groves of pine trees (*Pinus halepensis* Mill. and *P. pinea* L.), cypresses (*Cupressus sempervirens* L.), cedars (*Cedrus* spp.), and parks and lawns often used as golf courses.

The *Inocybe* collection was found on the edge of the mature thermophilous *Q. ilex* forest and a lawn grazed by large herbivores (fallow deer [*Dama dama* L.], axis deer [*Axis axis* Erxleben], and European mouflon [*Ovis gmelini musimon* Pall.]) and occasionally machine-mowed by park staff. In addition, a few mature planted trees of *P. pinea* were present at the forest edge. Basidiomata of *I. brijunica* were found at ca. 70 m from the sea, epigeous on the soil covered with a shallow layer of oak and pine litter intermixed with scattered short grasses. The understory of the surrounding forest was devoid of herbaceous plants and shrubs due to the presence of large herbivores.

2.2. Morphological Study

The species description is based on a single but large collection consisting of 20 basidiomata. Macroscopic characters were documented with a Canon EOS 5D digital camera equipped with a Canon MR-14EX macro ring flash (Canon Europe, Uxbridge, UK). Microscopic features were observed by brightfield and phase contrast microscopy using a BX51 optical microscope (Olympus, Hamburg, Germany) under magnification up to 1500× and photographed with a Canon EOS M50 digital camera. Descriptions and images of microscopic characters were made from rehydrated specimens mounted in 2.5% potassium hydroxide (KOH), except for cystidia that were observed in 3% ammonium hydroxide (NH_4OH). Micromorphological terminology mostly follows Clémençon [24]. Line drawings were made by J.L. with PITT artist pens (Faber–Castell, Nürnberg, Germany) based on digital images.

Amyloid and dextrinoid reactions of basidiospores were tested in Melzer's reagent [25]. Randomly selected basidiospores from photographs of lamellae mounts were measured with Motic Images Plus 2.0 software (Motic Europe, Barcelona, Spain). The length/width ratio of basidiospores is given as the "Q" value (min–av.–max). Average basidiospore and pleurocystidia lengths, widths, and Q values are shown in italics. Numbers in square brackets [X/Y/Z] denote X elements measured in Y basidiomata of Z collections. Measurements of cystidia do not include crystals present at the apex. Type material was preserved by drying on a flow of hot air at maximum temperature of 50 °C. The holotype is deposited at CNF, and an isotype is deposited at PUL (Kriebel Herbarium, Purdue University, West Lafayette, IN, USA).

2.3. DNA Extraction, PCR Amplification, and Sequencing

Genomic DNA was extracted from parts of the lamellae using the QIAamp DNA Micro Kit (Qiagen, Valencia, CA, USA). The first ~1100 bp of the nuclear 18S nuclear ribosomal RNA gene (nrSSU), the internal transcribed spacer region of the rDNA (ITS, consisting of ITS1–5.8S–ITS2), the first ~1400 bp of the nuclear 28S rRNA gene (nrLSU), and the second largest subunit of RNA polymerase II gene (*rpb2*) were amplified [26]. The following primers were used: NS1, NS2, and NS4 [27] for nrSSU; ITS9mun [28] and ITS4 [27] for ITS; LR0R, LR5, and LR7 for nrLSU [29,30]; and RBP2-b6F, RPB2-b7R, and RPB2-b7.1R for *rpb2* [31]. Amplifications were done in 25 µL reactions, containing 12.5 µL of Promega 2× PCR Master Mix (Promega Co., Madison, WI, USA), 1.25 µL of each 10 µM primer, 9.0 µL of H_2O, and 1.0 µL of template DNA. PCR conditions for nrSSU, ITS, and nrLSU followed Haelewaters et al. [32]. For *rpb2*, PCR conditions were as follows: initial

denaturation at 95 °C for 5:00 min; followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 45 s; and final extension at 72 °C for 7:00 min. All amplifications were performed using the Eppendorf Mastercycler EP Thermal Cycler (Hauppauge, NY, USA). Purification of successful PCR products and sequencing in both directions using the amplification primers were outsourced to Genewiz (South Plainfield, NJ, USA). Sequence reads were assembled and edited using Sequencher 5.4.6 for Windows software (Gene Codes Corporation, Ann Arbor, MI, USA). Assembled sequences were deposited at the National Center for Biotechnology Information (NCBI) GenBank database, under accession numbers MN749503–MN749504 (nrSSU), MN749370–MN749371 (ITS), MN749492–MN749493 (nrLSU), and MT878448–MT878449 (*rpb2*).

2.4. Sequence Alignment and Phylogenetic Analysis

Newly obtained ITS sequences were BLAST searched against NCBI GenBank's standard *nr/nt* nucleotide database (https://blast.ncbi.nlm.nih.gov/Blast.cgi), resulting in three isolates of *Inocybe glabripes* Ricken (sect. *Hysterices* Stangl & J. Veselský) as top results, which shared between 91.49% and 91.90% identity (GenBank accession numbers KX602255, MH216096, MN947389). Following this result, ITS, nrLSU, and *rpb2* sequences of *Inocybe* sect. *Hysterices* species [26,31,33–35] were downloaded for phylogenetic analysis.

Sequences were aligned by locus using MUSCLE version 3.7 [36], available through the Cipres Science Gateway [37]. Next, sequences in the ITS dataset were trimmed at the conserved motifs 5′–CATTA–3′ (3′ end of the nrSSU) and 5′–GACCT(CAAA…)–3′ (5′ end of the nrLSU) [38]. Because the different portions of the ITS spacer region (the two spacers and 5.8S) have different rates of evolution [39,40], the ITS1 and ITS2 spacers and the 5.8S conserved gene were extracted and treated as individual partitions in the multilocus analysis. Sequences in the nrLSU dataset were also trimmed to start with the conserved motif 5′–GACCT(CAAA…)–3′. Ambiguously aligned regions were removed using trimAl version 1.3 [41], with -gt = 0.6 and -cons = 0.5.

Evolutionary models for nucleotide substitution were selected for each partition (ITS1, 5.8S, ITS2, nrLSU, *rpb2*) using ModelFinder Plus [42], considering the Akaike Information Criterion. The data for each locus were combined in MEGA7 [43] to create a supermatrix of 2752 characters for 28 isolates representing ten species in *Inocybe* sect. *Hysterices* and two species in *Inocybe* sect. *Lactiferae* serving as outgroup taxa (details in Table 1). Maximum likelihood (ML) was inferred under partitioned models using IQ-TREE 1.6.7 [44,45]. Ultrafast bootstrapping was done with 1000 replicates [46].

Table 1. Overview of *Inocybe* isolates used in phylogenetic analyses. Newly generated sequences are in boldface. T stands for type specimens.

Species	Section	Isolate	Locality	ITS	nrLSU	*rpb2*
Inocybe aeruginascens	*Hysterices*	JG270502	Germany	GU949590	JN974970	
Inocybe aeruginascens	*Hysterices*	JG310508	Germany	GU949591	MH220256	MH249787
Inocybe aeruginascens	*Hysterices*	PC111007	South Africa	GU949592	MH220257	
Inocybe chondroderma	*Hysterices*	PBM1760	British Columbia	GU949586	MH220258	
Inocybe chondroderma	*Hysterices*	PBM1776	Washington	GU949579	JN974967	MH249789
Inocybe brijunica T	*Hysterices*	D. Haelew. F-1610a	Croatia	**MN749370**	**MN749492**	**MT878448**
Inocybe brijunica T	*Hysterices*	D. Haelew. F-1610b	Croatia	**MN749371**	**MN749493**	**MT878449**
Inocybe dulciolens T	*Lactiferae*	PBM2646	Tennessee	MH216088	MH220265	MH249796
Inocybe dulciolens	*Lactiferae*	PBM2450	New York	MH216087	MH220264	MH249795
Inocybe dulciolens	*Lactiferae*	LVK13340	New Jersey	MH216084	MH220261	MH249792
Inocybe erinaceomorpha	*Lactiferae*	EL128/05	Sweden	AM882735	AM882735	
Inocybe erinaceomorpha	*Lactiferae*	JV14756F	Sweden	MH216089	MH220266	MH249797
Inocybe glabripes	*Hysterices*	JV7318F	Finland	MH216096		MH249803
Inocybe hystrix	*Hysterices*	HRL11842	Quebec	KX897428		
Inocybe hystrix	*Hysterices*	PBM3300	North Carolina	GU949588	MH220275	
Inocybe hystrix	*Hysterices*	RS31493	Finland		AY380380	AY337381
Inocybe hystrix	*Hysterices*	SJ020824	Sweden	AM882810	AM882810	
Inocybe aff. *hystrix*	*Hysterices*	REH7405	Costa Rica	GU949589	JN974969	MH249806
Inocybe melanopus T	*Hysterices*	Stz3641	Washington		HQ201359	
Inocybe melanopus	*Hysterices*	BJ920904	Sweden	AM882725	AM882725	
Inocybe melanopus	*Hysterices*	JV4986	Finland	AM882727	AM882727	
Inocybe melanopus	*Hysterices*	PBM3975	Tennessee		MH220276	MH249807
Inocybe melanopus	*Hysterices*	TAA185135	Estonia	AM882726		
Inocybe aff. *pallidobrunnea*	*Hysterices*	PBM1957	Washington	MH216098	MH220277	MH249808
Inocybe aff. *pallidobrunnea*	*Hysterices*	PBM2242	Washington	MH216099	JN974968	MH249809
Inocybe sp.	*Hysterices*	PBM578	Washington	MH216104	JN974961	MH249813
Inocybe sp.	*Hysterices*	TR170-02	New Guinea		JN974964	MH249814
Inocybe sp.	*Hysterices*	TR180-02	New Guinea		JN974965	

3. Results

3.1. Phylogenetic Inference

The final multilocus dataset (Supplementary File S1) consists of 2752 characters, of which 425 are parsimony-informative and 2197 are constant. The number of total and parsimony-informative characters by locus as well as their selected evolutionary models as selected by ModelFinder Plus are presented in Table 2. The best-scoring ML tree (-lnL = 8722.045120) is shown in Figure 1. The topology is mostly congruent with Matheny and Kudzma [26], although support has improved for certain nodes. *Inocybe brijunica* sp. nov. is retrieved as a sister species of *I. glabripes* with maximum support. This set (*I. brijunica*, *I. glabripes*) is highly supported as sister to the clade holding *I. chondroderma* D.E. Stuntz ex Matheny, Norvell & E.C. Giles and *I.* aff. *pallidobrunnea* Kauffman.

Table 2. Overview of number of characters (total, informative, constant) and selected model of nucleotide substitution, by locus.

Locus	Sequences	Sites	Informative	Constant	Model	-lnL
ITS1	23	246	92	134	HKY + F + G4	1306.690
5.8S	23	158	4	153	TIM3e	249.420
ITS2	23	203	79	113	TPM3u + F + G4	1034.365
nrLSU	25	1379	94	1257	TN + F+I	3068.786
rpb2	16	766	156	540	TN + F+I	2935.101

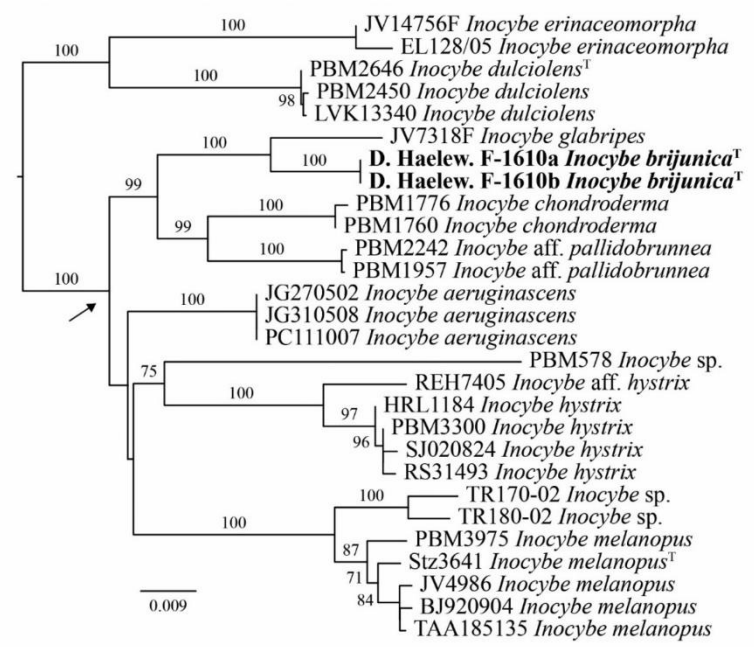

Figure 1. The best-scoring ML tree (-lnL = 8722.045120) of *Inocybe* sect. *Hysterices* (represented by the arrow) reconstructed from a concatenated ITS–nrLSU–*rpb2* dataset of 28 isolates. The tree topology is the result of ML inference performed in IQ-TREE. For each node, the ML bootstrap (≥70) is presented above or in front of the branch leading to that node. The new species is in boldface. T stands for type specimens.

3.2. Taxonomy

Inocybe brijunica Mešić, Tkalčec & Haelew., sp. nov.

Figures 2–4.

Mycobank MB838152

Typification: CROATIA. ISTRIA COUNTY: Brijuni National Park, Veli Brijun Island, 44°55′04″ N 13°46′33″ E, on the edge of grassland and forest of *Quercus ilex* L. with a few planted *Pinus pinea* L. trees along the forest edge, 16 November 2016, *A. Mešić & Z. Tkalčec* (holotype, CNF 1/7345; isotype, PUL F27673). GenBank (ex-isotype DNA isolate D. Haelew. F-1610a): nrSSU = MN749503, ITS = MN749370, nrLSU = MN749492, *rpb2* = MT878448; (ex-isotype DNA isolate D. Haelew. F-1610b): nrSSU = MN749504, ITS = MN749371, nrLSU = MN749493, *rpb2* = MT878449.

Etymology: Referring to the Brijuni archipelago, where the holotype was collected.

Description: Pileus 15–22 mm wide, obtusely (sub)conical with inflexed margin when young; convex to plano-convex, subumbonate and with deflexed margin at maturity; margin entire, occasionally with short radial splits; surface dry, finely radially fibrillose at first, then intensely fibrillose, rimulose to rimose, finally often partially cracked in small, shallow patches showing the paler flesh underneath; mostly medium brown, often with orange (fulvous brown) or reddish tones, less often light or dark brown where more deeply cracked; younger basidiomata often with rather inconspicuous, fibrillous, whitish veil remnants in marginal zone. Lamellae adnexed, subcrowded, L = ca. 40–50, l = 1–3, (sub)ventricose; whitish at first, then pale yellowish brown, finally light brown; edges fimbriate to slightly eroded, ± concolorous with sides. Stipe 17–30 × 2.5–4.5 mm, subcylindrical with slightly to moderately broadened base (up to 7 mm, sometimes submarginate);

solid, surface dry, white flocculose at apex, becoming whitish longitudinally fibrillose toward base, fibrils more scattered with age, beneath the fibrils pale to medium brown; with more or less developed orange to dull orange–red or orange–red–brown, adhering, membranaceous surface layer (possibly a remnant of universal veil), at or just above the basal part of the stipe; basal tomentum scanty, whitish. Cortina (partial veil) present in young basidiomata, fibrillous, white, fugacious. Context cream, light brown when moist, not changing color on bruising, not darkening on drying. Odor spermatic. Taste not recorded.

Figure 2. *Inocybe brijunica* (CNF 1/7345, holotype). (**A**) Basidiomata in situ. (**B**) Basidiomata in lab. Bars: A, B = 10 mm.

Basidiospores [300/3/1] (6.2–)6.6–7.5–8.8(–9.8) × (4–)4.3–4.7–5.3(–5.7) μm, averages of different basidiomata 7.3–7.6 × 4.6–4.7 μm, Q = 1.35–1.6–1.96, av. Q = 1.58–1.62, a few very large spores occasionally present (up to ca. 12 × 7 μm); in frontal view ellipsoid, oblong or ovoid with rounded to subacute base and rounded to acute apex, in side view amygdaliform to phaseoliform, rarely subellipsoid, with rounded base and rounded to acute apex, sometimes subangulate in both views (especially in upper part); smooth, often with small, rather indistinct, apical germ-pore, moderately thick-walled (up to 0.8 μm), pale yellow–brown in KOH, pale brown in H_2O, non-amyloid and non-dextrinoid. Basidia 20–30 × 6.5–9 μm, clavate, predominantly 4-spored, occasionally 2-spored, thin-walled, hyaline to yellowish. Pleurocystidia of lamprocystidia-type, very abundant, [90/4/1] 34–50–65(–70) × 9–14–21 μm, Q = 2.39–3.65–5.45, predominantly utriform, lageniform, or fusiform, with obtuse apex of 6–9(–11) μm wide, sometimes (sub)clavate, conical with obtuse apex, narrowly ellipsoid or subcylindrical, in alkaline solutions mostly (sub)hyaline, less often with slightly yellowish wall, sometimes with dirty yellow cytoplasmic pigment, with strongly to poorly developed crystals at apex (soluble in KOH, rarely lacking), thick-walled, wall most often gradually thickened towards the apex (up to 1–5.5 μm). Lamellar edge heterogeneous. Cheilocystidia of two types: (a) lamprocystidia similar to pleurocystidia (although more often without crystals), scattered to abundant, and (b) leptocystidia (paracystidia) 9–30 × 5–14 μm, mostly clavate, less often (sub)fusiform or utriform, hyaline to subhyaline, thin to moderately thick-walled (up to ca. 0.6 μm), scattered to abundant. Pileipellis a cutis, composed of repent, thin-walled, smooth to minutely encrusted, hyaline to pale yellow–brown hyphae, 1–5(–7) μm wide. Cells of the upper part of pileal context with brown, intracellular and partially also encrusted extracellular pigment (brown pigmented layer ca. 80–150 μm wide). Stipitipellis a cutis, composed of repent, thin-walled, smooth, ca. 2–10 μm wide hyphae. Caulocystidia mostly abundant (often crowded) in upper 2–3 mm of stipe length, sparsely present toward middle of the stipe; many in the form of lamprocystidia, quite similar to pleurocystidia, others very variable, narrowly utriform, lagenifom, (sub)cylindrical, clavate, urticoid or rather irregular, sometimes with subcapitate apex, some septate, hyaline, thin- to moderately thick-walled (up to ca. 1 μm); 12–100(–150) × 4–20 μm. Clamp connections present, conspicuous, rather abundant in all tissues.

Distribution and ecology: Thus far only known from the holotype collection. Ectomycorrhizal, found in the Mediterranean region of Croatia (Europe), on the island of Veli Brijun in Brijuni National Park, on the edge of *Quercus ilex* forest, with a few planted *Pinus pinea* trees, edging a neighboring grassland. An ITS sequence with accession number MH310748 [47], identified as *Inocybe* sp., from Italy shares 99% identity with *I. brijunica* (identities = 684/688 bp, gaps = 4/688) and may indicate a broader distribution in the Mediterranean basin.

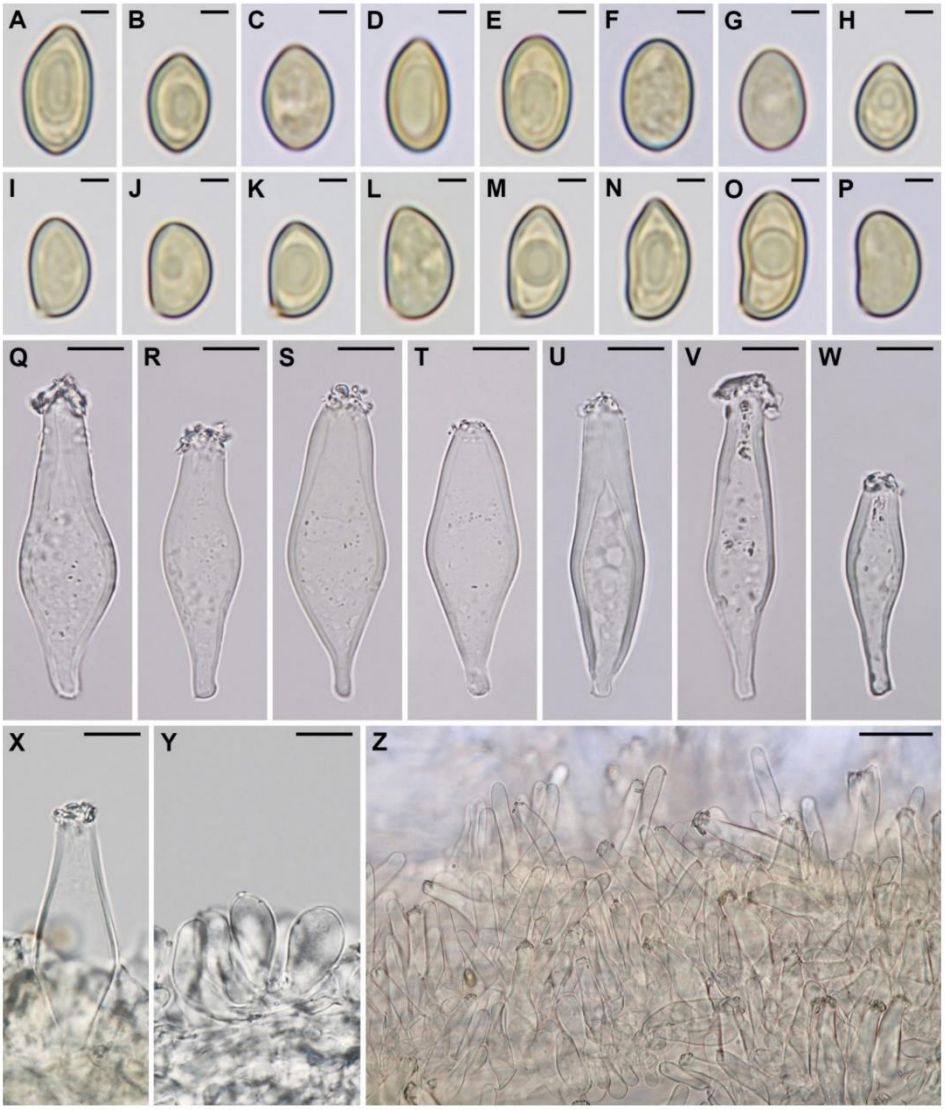

Figure 3. Inocybe brijunica (CNF 1/7345, holotype). (**A**–**H**) Basidiospores in frontal view. (**I**–**P**) Basidiospores in side view. (**Q**–**W**) Pleurocystidia. (**X**) Cheilolamprocystidium. (**Y**) Cheiloleptocystidia. (**Z**) Caulocystidia. Bars: (**A**–**P**) = 2 µm, (**Q**–**Y**) = 10 µm, (**Z**) = 30 µm.

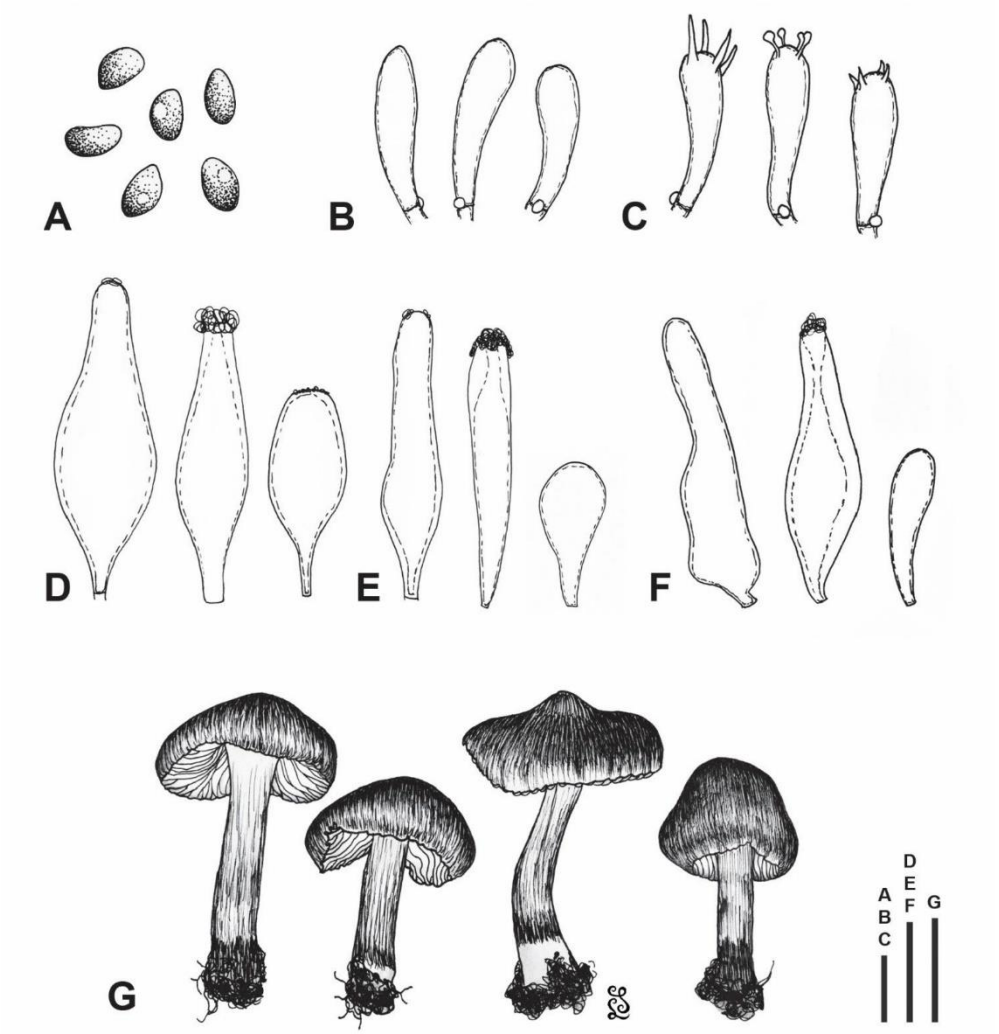

Figure 4. *Inocybe brijunica* (CNF 1/7345, holotype). (A) Basidiospores. (**B**) Basidioles. (**C**) Basidia. (**D**) Pleurocystidia. (**E**) Cheilocystidia). (**F**) Caulocystidia. (**G**) Basidiomata. Bars: (**A**–**C**) = 10 μm; (**D**–**F**) = 20 μm; (**G**) = 10 mm.

4. Discussion

The results of our multilocus phylogenetic analysis and morphological study place *I. brijunica* in sect. *Hysterices* [48]. Basidiomata produced by species belonging to this section (in the original sense) possess a squamulose pileus and stipe, lack violaceous tones, and have amygdaliform basidiospores. Matheny and Kudzma [26] emended the section to include taxa with non-squarrose basidiomata. Macromorphologically, *I. brijunica* can be easily recognized from all other *Inocybe* species by a conspicuous orange to orange–red–brown membranaceous surface layer present at or just above the basal part of the stipe. Other important morphological characters are: medium brown pileus with radially fibrillose to rimose surface; pale to medium brown stipe with slightly to moderately broadened

(or sometimes submarginate) base; presence of fugacious cortina in young basidiomata; spermatic odor; color of context unchanged upon bruising; relatively small, amygdaliform to phaseoliform (and sometimes subangulate), smooth basidiospores (ca. 6.5–9 × 4–5.5 μm); pleurocystidia as utriform, lageniform, or fusiform, thick-walled (up to 1–5.5 μm) lamprocystidia, mostly with crystals and not yellowing in alkaline solutions; cheilocystidia of two types (lamprocystidia and leptocystidia); and presence of abundant caulocystidia only in the upper 2–3 mm of stipe length.

The basidiospores of the morphologically and phylogenetically closest species, *I. glabripes*, are very similar in size, measuring ca. 6–8 × 4–5 μm [49,50], but they are readily distinguished by being amygdaliform but not phaseoliform as in *I. brijunica.* In addition, the cystidial walls of *I. glabripes* are thinner (up to 2(–2.5) μm thick). *Inocybe glabripes* is a widespread species occurring in parks and open woodlands on predominantly alkaline soils from the Mediterranean region to the boreal zone of Europe [51], which forms ectomycorrhizae exclusively with broadleaved trees. So far, the species has been found in symbiotic relationship with trees in the genera *Betula* L., *Fagus* L., *Populus* L., *Quercus* L., *Tilia* L. [49–51], and with *Castanea sativa* Mill. [52]. It can be expected that its sister species *I. brijunica* also forms ectomycorrhizal relationships only with broadleaved trees, such as *Quercus ilex* at the holotype locality.

Inocybe pseudobrunnea Alessio, which grows under *Abies alba* Mill. (Pinales, Pinaceae), has a similar phaseoliform, but somewhat larger basidiospores, 8.5–10.5(11) × 4.5–6 μm, and its cystidia are rather bright yellow in ammonia solution [53], a characteristic that rarely occurs in *I. brijunica*. *Inocybe gracilenta* E. Ludw., only known from the type collection in Sweden, a damp locality under *Alnus* sp. (Fagales, Betulaceae), *Populus tremula* L. and *Salix* sp. (Malpighiales, Salicaceae), has amygdaliform but not phaseoliform basidiospores, which are otherwise similar in size compared to *I. brijunica*, 7–8.5(9.5) × 4.5–5.5 μm [51]. Additional differences are the papillate pileus and the slenderer (up to 2 mm wide) and white to faintly cream-colored stipe [51]. The Mediterranean species *I. barrasae* Esteve-Rav., described from Spain and fruiting in spring (April–May) in thermophilous Mediterranean *Quercus–Cistus* forests, has amygdaliform-shaped basidiospores that are larger and more elongated (8–11.5 × 4.5–5.5 μm, av. Q = 1.85), and bright yellow-colored cystidia in ammonia solution [54]. *Inocybe aerea* E. Ludw., another species only known from the holotype collection in Germany [51], has amygdaliform to ovoid and slightly larger basidiospores (7.5–10.5 × 5–6 μm), an ochraceous yellow and more slender (up to 2 mm wide) stipe, and thinner-walled cystidia (walls 0.2–2 (–3) μm thick). The North American species *I. pyrotricha* Stuntz [55] has orange to cinnamon or rusty–red fibrils on the stipe, like *I. brijunica*, but differs by slightly larger (7.5–10 × 4.5–6 μm) basidiospores, longer pleurocystidia (66–80 × 13.5–16.5 μm), and violaceous tinges in young lamellae and upper parts of the stipe.

Supplementary Materials: The following supplementary material is available online at www.mdpi.com/2309-608X/7/3/199/s1, File S1: Aligned, concatenated dataset of *Inocybe* sect. *Hysterices*, consisting of five partitions (ITS1, 5.8S, ITS2, nrLSU, *rpb2*).

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References

1. Brijuni National Park Official Website. Available online: https://www.np-brijuni.hr/en/brijuni (accessed on 14 January 2021).
2. Blondel, J.; Aronson, J.; Bodiou, J.-Y.; Boeuf, G. *The Mediterranean Region—Biological Diversity in Space and Time*, 2nd ed.; Oxford University Press: New York, NY, USA, 2010.
3. Giorgi, F. Climate change hot-spots. *Geophys. Res. Lett.* **2006**, *33*, 1–4, doi:0.1029/2006GL025734.
4. Tuel, A.; Eltahir, E.A.B. Why is the Mediterranean a climate change hot spot? *J. Clim.* **2020**, *33*, 5829–5843, doi:10.1175/JCLI-D-19-0910.1.
5. Mariotti, A.; Zeng, N.; Yoon, J.-H.; Artale, V.; Navarra, A.; Alpert, P.; Li, L.Z.X. Mediterranean water cycle changes: Transition to drier 21st century conditions in observations and CMIP3 simulations. *Environ. Res. Lett.* **2008**, *3*, 044001, doi:10.1088/1748-9326/3/4/044001.
6. Brogli, R.; Sørland, S.L.; Kröner, N.; Schär, C. Causes of future Mediterranean precipitation decline depend on the season. *Environ. Res. Lett.* **2019**, *14*, 114017, doi:10.1088/1748-9326/ab4438.
7. Antonelli, A.; Fry, C.; Smith, R.J.; Simmonds, M.S.J.; Kersey, P.J.; Pritchard, H.W.; Abbo, M.S.; Acedo, C.; Adams, J.; Ainsworth, A.M.; et al. *State of the World's Plants and Fungi 2020*; Royal Botanic Gardens: Kew, UK, 2020, doi:10.34885/172.
8. Hawksworth, D.L. The fungal dimension of biodiversity: Magnitude, significance, and conservation. *Mycol. Res.* **1991**, *95*, 641–655, doi:10.1016/S0953-7562(09)80810-1.
9. Cheek, M.; Nic Lughadha, E.; Kirk, P.; Lindon, H.; Carretero, J.; Looney, B.; Douglas, B.; Haelewaters, D.; Gaya, E.; Llewellyn, T.; et al. New scientific discoveries: Plants and fungi. *Plants People Planet* **2020**, *2*, 371–388, doi:10.1002/ppp3.10148.
10. Saba, M.; Haelewaters, D.; Pfister, D.H.; Khalid, A.N. New species of *Pseudosperma* (Agaricales, Inocybaceae) from Pakistan revealed by morphology and multi-locus phylogenetic reconstruction. *MycoKeys* **2020**, *69*, 1–31, doi:10.3897/mycokeys.69.33563.
11. He, M.Q.; Zhao, R.L.; Hyde, K.D.; Begerow, D.; Kemler, M.; Yurkov, A.; McKenzie, E.H.C.; Raspé, O.; Kakishima, M.; Sánchez-Ramírez, S.; et al. Notes, outline and divergence times of Basidiomycota. *Fungal Divers.* **2019**, *99*, 105–367, doi:10.1007/s13225-019-00435-4.
12. Bandini, D.; Oertel, B.; Ploch, S.; Ali, T.; Vauras, J.; Schneider, A.; Scholler, M.; Eberhardt, U.; Thines, M. Revision of some central European species of *Inocybe* (Fr.: Fr.) Fr. subgenus *Inocybe*, with the description of five new species. *Mycol. Prog.* **2018**, *18*, 247–294, doi:10.1007/s11557-018-1439-9.
13. Bandini, D.; Oertel, B.; Moreau, P.-A.; Thines, M.; Ploch, S. Three new hygrophilous species of *Inocybe*, subgenus *Inocybe*. *Mycol. Prog.* **2019**, *18*, 1101–1119, doi:10.1007/s11557-019-01509-y.
14. Bandini, D.; Oertel, B.; Ploch, S.; Thines, M. *Inocybe heidelbergensis*, eine neue Risspilz-Art der Untergattung *Inocybe*. *Z. Mykol.* **2019**, *85*, 195–213.
15. Bandini, D.; Oertel, B.; Schüssler, C.; Eberhardt, U. Noch mehr Risspilze: Fünzehn neue und zwei wenig bekannte Arten der Gattung *Inocybe*. *Mycol. Bavarica* **2020**, *20*, 13–101.
16. Bandini, D.; Sesli, E.; Oertel, B.; Krisai-Greilhuber, I. *Inocybe antoniniana*, a new species of *Inocybe* section *Marginatae* with nodulose spores. *Sydowia* **2020**, *72*, 95–106, doi:10.12905/0380.sydowia72-2020-0095.
17. Bandini, D.; Vauras, J.; Weholt, Ø.; Oertel, B.; Eberhardt, U. *Inocybe woglindeana*, a new species of the genus *Inocybe*, thriving in exposed habitats with calcareous sandy soil. *Karstenia* **2020**, *58*, 41–59, doi:10.29203/ka.2020.488.
18. Cripps, C.L.; Larsson, E.; Vauras, J. Nodulose-spored *Inocybe* from the Rocky Mountain alpine zone molecularly linked to European and type specimens. *Mycologia* **2019**, *112*, 133–153, doi:10.1080/00275514.2019.1677419.
19. Crous, P.W.; Carnegie, A.J.; Wingfield, M.J.; Sharma, R.; Mughini, G.; Noordeloos, M.E.; Santini, A.; Shouche, Y.S.; Bezerra, J.D.P.; Dima, B.; et al. Fungal Planet description sheets: 868–950. *Persoonia* **2019**, *42*, 291–473, doi:10.3767/persoonia.2019.42.11.
20. Crous, P.W.; Cowan, D.A.; Maggs-Kölling, G.; Yilmaz, N.; Larsson, E.; Angelini, C.; Brandrud, T.E.; Dearnaley, J.D.W.; Dima, B.; Dovana, F.; et al. Fungal Planet description sheets: 1112–1181. *Persoonia* **2020**, *45*, 251– 409, doi:10.3767/persoonia.2020.45.10.
21. Krieglsteiner, L.G. *Inocybe calosporoides*—Ein neuer Risspilz aus Portugal. *Südwestdeutsche Pilzrundsch.* **2019**, *55*, 68–72.
22. Krieglsteiner, L.G. Nomenclatural novelties. *Index Fungorum* **2019**, *411*, 1.
23. Matheny, P.B.; Hobbs, A.M.; Esteve-Raventós, F. Genera of Inocybaceae: New skin for the old ceremony. *Mycologia* **2019**, *112*, 83–120, doi:10.1080/00275514.2019.1668906.
24. Clémençon, H. *Cytology and Plectology of the Hymenomycetes*, 2nd ed.; Cramer: Stuttgart, Germany, 2012.
25. Erb, B.; Matheis, W. *Pilzmikroskopie*; Kosmos: Stuttgart, Germany, 1982.
26. Matheny, P.B.; Kudzma, L.V. New species of *Inocybe* (Inocybaceae) from eastern North America. *J. Torrey Bot. Soc.* **2019**, *146*, 213–235, doi:10.3159/TORREY-D-18-00060.1.
27. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press: New York, NY, USA, 1990; pp. 315–322, doi:10.1016/B978-0-12-372180-8.50042-1.

28. Egger, K.N. Molecular analysis of ectomycorrhizal fungal communities. *Can. J. Bot.* **1995**, *73*, S1415–S1422, doi:10.1139/b95-405.
29. Vilgalys, R.; Hester, M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol.* **1990**, *172*, 4238–4246, doi:10.1128/jb.172.8.4238-4246.1990.
30. Hopple, J.S. Phylogenetic Investigations in the Genus Coprinus Based on Morphological and Molecular Characters. Ph.D. Dissertation, Duke University, Durham, NC, USA, 1994.
31. Matheny, P.B. Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*, Agaricales). *Mol. Phylogenet. Evol.* **2005**, *35*, 1–20, doi:10.1016/j.ympev.2004.11.014.
32. Haelewaters, D.; Toome-Heller, M.; Albu, S.; Aime, M.C. Red yeasts from leaf surfaces and other habitats: Three new species and a new combination of *Symmetrospora* (Pucciniomycotina, Cystobasidiomycetes). *Fungal Syst. Evol.* **2020**, *5*, 187–196, doi:10.3114/fuse.2020.05.12.
33. Matheny, P.B.; Norvell, L.L.; Giles, E.C. A common new species of *Inocybe* in the Pacific Northwest with a diagnostic PDAB reaction. *Mycologia* **2013**, *105*, 436–446, doi:10.3852/12-155.
34. Ryberg, M.; Matheny, P.B. Asynchronous origins of ectomycorrhizal clades of Agaricales. *Proc. R. Soc. B Biol. Sci.* **2012**, *279*, 2003–2011, doi:10.1098/rspb.2011.2428.
35. Ryberg, M.; Nilsson, R.H.; Kristiansson, E.; Töpel, M.; Jacobsson, S.; Larsson, E. Mining metadata from unidentified ITS sequences in GenBank: A case study in *Inocybe* (Basidiomycota). *BMC Evol. Biol.* **2008**, *8*, 50, doi:10.1186/1471-2148-8-50.
36. Edgar, R.C. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **2004**, *32*, 1792–1797, doi:10.1093/nar/gkh340.
37. Miller, M.A.; Pfeiffer, W.; Schwartz, T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, Louisiana, 14 November 2010, Institute of Electrical and Electronics Engineers: Piscataway, NJ, USA, 2010; pp. 1–8, doi:10.1109/GCE.2010.5676129.
38. Dentinger, B.T.; Didukh, M.Y.; Moncalvo, J.M. Comparing COI and ITS as DNA barcode markers for mushrooms and allies (Agaricomycotina). *PLoS ONE* **2011**, *6*, e25081, doi:10.1371/journal.pone.0025081.
39. Hillis, D.M.; Dixon, M.T. Ribosomal DNA: Molecular evolution and phylogenetic inference. *Q. Rev. Biol.* **1991**, *66*, 411–453, doi:10.1086/417338.
40. Haelewaters, D.; Dirks, A.C.; Kappler, L.A.; Mitchell, J.K.; Quijada, L.; Vandegrift, R.; Buyck, B.; Pfister, D.H. A preliminary checklist of fungi at the Boston Harbor islands. *Northeast. Nat.* **2018**, *25*, 45–76, doi:10.1656/045.025.s904.
41. Capella-Gutiérrez, S.; Silla-Martínez, J.M.; Gabaldón, T. TrimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* **2009**, *25*, 1972–1973, doi:10.1093/bioinformatics/btp348.
42. Kalyaanamoorthy, S.; Minh, B.Q.; Wong, T.K.F.; von Haeseler, A.; Jermiin, L.S. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* **2017**, *14*, 587–589, doi:10.1038/nmeth.4285.
43. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874, doi:10.1093/molbev/msw054.
44. Nguyen, L.-T.; Schmidt, H.A.; von Haeseler, A.; Minh, B.Q. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274, doi:10.1093/molbev/msu300.
45. Chernomor, O.; von Haeseler, A.; Minh, B.Q. Terrace aware data structure for phylogenomic inference from supermatrices. *Syst. Biol.* **2016**, *65*, 997–1008, doi:10.1093/sysbio/syw037.
46. Hoang, D.T.; Chernomor, O.; von Haeseler, A.; Minh, B.Q.; Vinh, L.S. UFBoot2: Improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* **2017**, *35*, 518–522, doi:10.1093/molbev/msx281.
47. Wurzbacher, C.; Larsson. E.; Bengtsson-Palme, J.; Van den Wyngaert, S.; Svantesson, S.; Kristiansson, E.; Kagami, M.; Nilsson, R.H. Introducing ribosomal tandem repeat barcoding for fungi. *Mol. Ecol. Resour.* **2018**, *19*, 118–127, doi:10.1111/1755-0998.12944.
48. Stangl, J.; Veselský, J. Risspilze der Section *Lilacinae* Heim. *Česká Mykol.* **1982**, *36*, 85–99.
49. Kuyper, T.W. A revision of the genus *Inocybe* in Europe 1. Subgenus *Inosperma* and the smooth-spored species of subgenus *Inocybe*. *Persoonia* **1986**, *3*, 1–247.
50. Stangl, J. Die Gattung *Inocybe* in Bayern. *Hoppea* **1989**, *46*, 1–409.
51. Ludwig, E. *Pilzkompendium. Band 4*; Fungicon Verlag: Berlin, Germany, 2017.
52. Ferrari, E.; Bandini, D.; Boccardo, F. *Inocybe (Fr.) Fr., Terzo Contributo*; Fungi non delineati 73/74. Candusso; Alassio, Italy, 2014.
53. Alessio, C.L. Complemento allo studio del Genere *Inocybe*: 8° contributo. *Riv. Micol. Assoc. Micol. Bresadola* **1987**, *30*, 79–89.
54. Esteve-Raventós, F. Two new species of *Inocybe* (Cortinariales) from Spain, with a comparative type study of some related taxa. *Mycol. Res.* **2001**, *105*, 1137–1143, doi:10.1017/S0953756201004609.
55. Smith, A.H.; Stuntz, D.E. New or noteworthy fungi from Mount Rainier National Park. *Mycologia* **1950**, *42*, 80–134, doi:10.1080/00275514.1950.12017817.

PUBLIKACIJA II

Inocybe istriaca sp. nov. from Brijuni National Park (Croatia) and Its Position within *Inocybaceae* Revealed by Multigene Phylogenetic Analysis

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Inocybe istriaca sp. nov. from Brijuni National Park (Croatia) and Its Position within Inocybaceae Revealed by Multigene Phylogenetic Analysis

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Abstract: Integrative taxonomic studies of macrofungal diversity in the Brijuni National Park (Istria County, Croatia) led to the discovery of a second species of *Inocybe* (Agaricales, Inocybaceae) new to science. *Inocybe istriaca* sp. nov. is described on the basis of morphological, ecological, and multigene phylogenetic analyses, and its placement within the family Inocybaceae is discussed. The combination of most important morphological characters that distinguish *I. istriaca* from the other similar *Inocybe* species are smooth, (sub)amygdaliform, (sub)phaseoliform, or ellipsoid basidiospores (ca. 8.5–12 × 5–7 µm), large basidia (36–45 × 9–15 µm), mostly (sub)fusiform and weakly thick-walled (up to 1.5 µm) metuloid pleurocystidia, and lamellar edge and stipe apex partially covered by a dark resinous substance. The species was collected on the edge of grassland and Mediterranean evergreen holm oak (*Quercus ilex*) forest. In this study, a total of 14 DNA sequences from four *Inocybe* species were generated. Two-gene (ITS, LSU) and four-gene (ITS, LSU, *rpb2*, *tef1*) phylogenetic analyses confirmed the status of *I. istriaca* as an independent species.

Keywords: 1 new taxon; Agaricomycetes; Basidiomycota; biodiversity; taxonomy

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1. Introduction

The Mediterranean Basin is one of 35 global biodiversity hotspots characterized by outstanding concentrations of endemic species and a high level of habitat loss [1]. The region is home to 245 tree taxa (210 species and 35 subspecies), which is almost 200 taxa more than recorded in central Europe [2]. The Croatian part of the Adriatic Sea (northern Mediterranean) is distinguished by more than 600 islands and islets, a highly indented coastline, and high and steep orography in the hinterland [3].

The Brijuni archipelago consists of 14 islands and islets (total surface area: 7.4 km^2) situated near the southwest coast of the Istrian peninsula in the northern Adriatic [4]. It was officially protected as a national park in 1983 and is home to nearly 700 native and exotic plant species. It is characterized by a northern Mediterranean climate with an annual average temperature of 13.9 °C, an annual average precipitation of 817 mm, and a relatively high average air humidity of 76% [4,5]. The largest island of the archipelago is Veli Brijun (5.7 km^2), which is covered mainly by Mediterranean evergreen holm oak (*Quercus ilex*) forests and maquis. Lawns and landscape parks with holm oaks, Aleppo pines (*Pinus halepensis*), stone pines (*P. pinea*), Mediterranean cypresses (*Cupressus sempervirens*), and cedars (*Cedrus* spp.) are also well represented on the island.

During the fall seasons in 2014, 2015, 2016, and 2020, Croatian mycologists made initial field trips aiming to explore the fungal diversity of Brijuni National Park. In total,

184 macrofungal specimens were collected and deposited in the Croatian National Fungarium (CNF) in Zagreb, Croatia. Members of the ectomycorrhizal basidiomycete genus *Inocybe* (Fr.) Fr. *sensu lato* (s.l.) (Agaricomycetes, Agaricales, Inocybaceae) were frequently found, and 28 specimens were sampled. The currently accepted taxonomic framework of the family Inocybaceae [6,7], based on the results of phylogenetic analyses from a six-gene dataset [8], includes seven genera: *Auritella* Matheny and Bougher, *Inocybe sensu stricto* (s.s.), *Inosperma* (Kühner) Matheny and Esteve-Rav., *Mallocybe* (Kuyper) Matheny, Vizzini and Esteve-Rav., *Nothocybe* Matheny and K.P.D. Latha, *Pseudosperma* Matheny and Esteve-Rav., and *Tubariomyces* Esteve-Rav. and Matheny. The largest genus of the family is *Inocybe* s.s., with ca. 1000 accepted species [7]. Its members are characterized by the presence of pleurocystidia (often thick-walled and crystalliferous), hyaline basidia (without necropigment), and amygdaliform to ellipsoid, subcylindrical, angular, nodulose, or spiny basidiospores with a distinct apiculus [8].

The initial taxonomic study of *Inocybe* s.l. specimens from the island of Veli Brijun led to the description of a new species, *I. brijunica* Mešić, Tkalčec, and Haelew. [5]. Its basidiomata are macroscopically characterized by the presence of an orange to orange-red-brown membranaceous layer in the basal part of the stipe, which is an unusual feature in the genus. Further integrative taxonomic studies on *Inocybe* specimens from the island of Veli Brijun, combining morphological, molecular phylogenetic, and ecological characters, resulted in the discovery of another species new to science, described here as *I. istriaca* sp. nov.

2. Materials and Methods

2.1. Morphological Study

The species description is based on a single collection consisting of seven basidiomata. For documentation of macroscopic features, a Canon EOS 5D digital camera equipped with a Canon MR-14EX macro ring flash (Canon Europe, Uxbridge, UK) was used. Microscopic characters were observed with a BX51 optical microscope (Olympus, Hamburg, Germany) using the brightfield technique under magnifications of up to 1500× and photographed with a Canon EOS M50 digital camera. Description and images of microscopic characters were made from rehydrated specimens mounted in 2.5% potassium hydroxide (KOH), except for cystidia that were observed in 3% or 10% ammonium hydroxide (NH_4OH). Micromorphological terminology mostly follows Clémençon [9]. Line drawings were made from printed photographs using a light table.

Amyloid and dextrinoid reactions of basidiospores were tested in Melzer's reagent [10]. Basidiospores from photographs of lamellae mounts were randomly selected and measured using Motic Images Plus 2.0 software (Motic Europe, Barcelona, Spain). The length/width ratio of basidiospores is given as the "Q" value. Average basidiospore, basidia, and pleurocystidia lengths, widths, and Q values are shown in italics. Numbers in square brackets [X/Y/Z] denote X elements measured in Y basidiomata of Z collections. Measurements of cystidia do not include apical crystals when present. The type material was preserved by drying on a flow of hot air at a temperature of about 45 °C. The holotype is deposited at CNF, and an isotype is deposited at STU (State Museum of Natural History, Stuttgart, Germany).

2.2. DNA Extraction, PCR Amplification, and Sequencing

Dried specimens of *Inocybe* species were ground in microcentrifuge tubes under liquid nitrogen freezing using pestles, and genomic DNA was extracted using the EZNA® HP Fungal DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's protocol. Three nuclear gene regions, SSU (18S small subunit of ribosomal DNA), ITS (internal transcribed spacer region), and LSU (28S large subunit of ribosomal DNA), and two protein-coding regions, *rpb2* (second largest subunit of the DNA-directed RNA polymerase II) and *tef1* (translation elongation factor 1-alpha), were sequenced and analyzed. The 25 µL PCR mixtures contained 9.5 µL of ddH_2O, 12.5 µL of GoTaq® G2 Green Master Mix (Promega, Madison, WI, USA), 1 µL of DNA template, and 1 µL of each forward and reverse primer. The following primer pairs were used for PCR amplification and se-

quencing: NS1/NS6 [11], ITS1F/ITS4 [11,12], LR0R/LR5 [13], bRPB2-6F/bRPB2-7.1R [14], EF1-983F/EF1-2218R [15,16]. PCR amplification for the SSU gene region was performed as described by Haelewaters et al. [17]. PCR amplification for ITS and LSU gene regions was performed using a touchdown program: initial denaturation at 95 °C for 2 min; followed by 5 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 45 s (add −1 °C per cycle), extension at 72 °C for 1.5 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 45 s, extension at 72 °C for 1.5 min; and a final extension at 72 °C for 5 min. PCR amplification of *rpb2* was performed as described by Mešić et al. [5] and of *tef1* as described by Rehner and Buckley [16], with modification of the maximum annealing temperature to 64 °C. Successful PCR products were purified using ExoSAP-IT™ (Thermo Fisher Scientific, Waltham, MA, USA) purification reagent according to the manufacturer's protocol and sent to Macrogen Europe (Amsterdam, The Netherlands) for bidirectional Sanger sequencing.

2.3. Sequence Alignment and Phylogenetic Analysis

Sequence reads were assembled and edited using Geneious Prime 2023.0.4. (https://www.geneious.com, accessed on 19 January 2023, Biomatters, Auckland, New Zealand), and the obtained sequences were deposited at the National Center for Biotechnology Information (NCBI) GenBank database. Two separate datasets were selected for phylogenetic analyses (Table 1). The SSU gene region was excluded from the phylogenetic analyses due to the limited number of available sequences for Inocybaceae species in the NCBI GenBank nucleotide database and a lack of species-level resolution in the genus *Inocybe*.

Phylogenetic dataset 1 comprised a total of 241 sequences of four gene regions (ITS, LSU, *rpb2*, and *tef1*) from 67 species, covering the genetic diversity of the family Inocybaceae and four outgroup taxa. Sequences were aligned by each locus using MAFFT v7.450 [18,19], available as a Geneious Prime plugin. After being aligned and trimmed, concatenation of ITS, LSU, *rpb2*, and *tef1* was done using Geneious Prime 2023.0.4. The combined phylogenetic dataset 1 contained 3758 characters, including gaps, with 887 characters for ITS, 979 characters for LSU, 738 for *rpb2*, and 1154 for *tef1*. The outgroup taxa *Crepidotus prostratus*, *Pleuroflammula tuberculosa*, *Simocybe phlebophora*, and *S. serrulata* were selected following Matheny et al. [8].

Phylogenetic dataset 2 comprised 138 sequences of two nuclear gene regions (ITS and LSU) from 66 taxa covering the genetic diversity of the genus *Inocybe* and three outgroup taxa (*Pseudosperma fascinosum*, *P. huginii*, and *P. notodryinum*).

Table 1. Species included in phylogenetic analyses, associated strain/voucher numbers, countries of origin, and GenBank accession numbers. Newly generated sequences are in bold. Abbreviations: HT = holotype, ET = epitype, IT = isotype, PT = paratype.

Taxa	Strain/Voucher	Country	ITS	LSU	*RPB2*	*TEF1*	Phylog. Dataset	Refs.
Auritella hispida	TH10009 PT	Cameroon	KT378203	KT378207	KT378215	MK426179	1	[8,20]
Auritella spiculosa	TH9866 PT	Cameroon	KT378204	KT378206	KT378214	MK426182	1	[8,20]
Crepidotus prostratus	PBM3463/PERTH:08242135	Australia	HQ728537	HQ728538	HQ728540	MK426172	1	[8,20]
Inocybe adorabilis	SMNS-STU-F-0901582 HT	Austria	OK057159	OK057159	OK078903	—	1, 2	[21]
Inocybe adorabilis	SMNS-STU-F-0901641 PT	Austria	OK057161	OK057161	—	—	2	[21]
Inocybe aeruginascens	JG310508/TENN063936	Germany	GU949591	MH220256	MH249787	—	1	[22]
Inocybe agglutinata	WTU:1094 PBM1352	USA	KY990521	AY038312	AY509113	—	1	[14,23,24]
Inocybe agroterae	SMNS-STU-F-0901680 HT	Germany	ON003436	ON003436	—	—	2	[6]
Inocybe alcis	SMNS-STU-F-0901712 IT	Finland	OP164083	OP164083	—	—	2	[25]
Inocybe aphroditeana	SMNS-STU-F-0901678 HT	Germany	ON003432	ON003432	—	—	2	[6]
Inocybe asterospora cf.	ZRL20152002	China	LT716046	KY418862	KY419008	KY419064	1	[26]
Inocybe astraiana	SMNS-STU-F-0901240 HT	Germany	MN512321	MN512321	—	—	2	[27]
Inocybe athenana	SMNS-STU-F-0901238 HT	Germany	MN512320	MN512320	—	—	2	[27]
Inocybe audens	SMNS-STU-F-0901251 HT	Germany	MW647616	MW647616	—	—	2	[28]
Inocybe aurantiobrunnea	SMNS-STU-F-0001816 IT	Spain	OP164016	OP164016	—	—	2	[25]
Inocybe beatifica	SMNS-STU-F-0901261 HT	Germany	MW845857	—	—	—	2	[29]
Inocybe bellidiana	SMNS-STU-F-0901473 HT	Germany	MW845860	MW845860	—	—	2	[29]
Inocybe cacaocolor	PBM3790/TENN:067022 IT	Australia	KJ778845	KJ756464	KJ756422	—	1	[30]
Inocybe caesaraugustae	AH 56200 HT	Spain	OL352083	—	—	—	2	[31]
Inocybe carissima	SMNS-STU-F-0901701 HT	Germany	OP164058	OP164058	—	—	2	[25]
Inocybe carolinensis	PBM3906/TENN067756 PT	USA	KP636853	KP171055	KM555147	—	1	[32]
Inocybe chalcodoxantha	WTU F-043333 IT	Canada	NR_119900	—	—	—	2	[33]
Inocybe coriacea	SMNS-STU-F-0901683 HT	Germany	ON003439	ON003439	—	—	2	[6]

Table 1. *Cont.*

Taxa	Strain/Voucher	Country	ITS	LSU	*RPB2*	*TEF1*	Phylog. Dataset	Refs.
Inocybe corydalina	AM10687 TURA6488	Russia Belgium	MH216083	AY038314	AY337370	—	1	[32]
Inocybe cuniculina	KR-M-0043257 HT	Netherlands	MN625273	MN625273	—	—	2	[27]
Inocybe curcumina	KR-M-0042332 HT	Germany	MH366621	—	—	—	2	[34]
Inocybe cygnea	SMNS-STU-F-0901671 HT	Germany	ON003447	ON003447	—	—	2	[6]
Inocybe derbschii	KR-M-0005011 HT	Germany	MG012466	—	—	—	2	[34]
Inocybe devina	SMNS-STU-F-0901659 HT	Germany	ON003423	ON003423	—	—	2	[6]
Inocybe drenthensis	SMNS-STU-F-0901477 HT	Netherlands	MW845869	MW845869	—	—	2	[29]
Inocybe dryadiana	SMNS-STU-F-0901259 HT	Germany	MW845873	MW845873	—	—	2	[29]
Inocybe dulciolens	PBM2646/TENN 062477 HT	USA	MH216088	MH220265	MH249796	—	1	[32]
Inocybe dvaliniana	SMNS-STU-F-0901559 HT **CNF 1/8916 IT**	**Austria**	MW647624	MW647624	**OQ587951**	—	**1, 2**	[28], **This study**
Inocybe elysii	SMNS-STU-F-0901682 HT	Germany	ON003438	ON003438	—	—	2	[6]
Inocybe erinaceomorpha	JV14756F/TURA7645	Sweden	MH216089	MH220266	MH249797	—	1	[32]
Inocybe flavoalbida	PBM3768/TENN:067000 IT	Australia	KJ729873	KJ729901	KJ729932	MK426183	1, 2	[30]
Inocybe flocculosa	EL10605	Finland	AM882992	AM882992	—	—	2	[34]
Inocybe flocculosa cf.	ZRL20151789	China	LT716045	KY418861	KY419007	KY419063	1	[26]
Inocybe freyae	SMNS-STU-F-0901673 HT	Germany	ON003431	ON003431	—	—	2	[6]
Inocybe fuscicothurnata	PBM3980/TENN:068940	USA	MF487844	KY990485	MF416408	MK426184	1	[23]
Inocybe fuscidula	EL9505	Finland	AM882886	AM882886	—	—	2	[35]
Inocybe ghibliana	SMNS-STU-F-0901256 HT	Germany	MW845878	MW845878	—	—	2	[29]
Inocybe glaucescens	LVK12144/TENN073754 HT	USA	MH216097	MH220273	MH249804	—	1	[32]
Inocybe grammopodia	KR-M-0044138	Germany	MH366590	—	—	—	2	[34]
Inocybe griseotarda	J. Poirier n 19901119-01 HT	France	MF361839	—	—	—	2	[36]
Inocybe griseovelata	EL20906	France	FN550931	FN550931	—	—	2	[35]

Table 1. *Cont.*

Taxa	Strain/Voucher	Country	ITS	LSU	*RPB2*	*TEF1*	Phylog. Dataset	Refs.
Inocybe griseovelata	SMNS-STU-F-0901568 ET	Germany	MW845942	MW845942	—	—	2	[29]
Inocybe grusiana	SMNS-STU-F-0901262 HT	Germany	MW845884	MW845884	—	—	2	[29]
Inocybe heterosemen	XC98091209 IT	France	OK057119	—	—	—	1, 2	[21]
Inocybe humidicola	PBM3719/TENN:066955	Australia	KP171126	KJ801181	KJ811575	MK426185	1	[8,30]
Inocybe inodora	EL2405	Norway	AM882834	AM882834	—	—	2	[35]
Inocybe inodora	SMNS-STU-F-0901438	Austria	MT101874	MT101874	—	—	1, 2	[37]
Inocybe iseranensis	TR gmb 00981 HT	France	OK057141	OK057141	—	—	1, 2	[21]
***Inocybe istriaca*sp. nov.**	**CNF 1/7323 HT**	**Croatia**	**OQ550176**	**OQ550175**	**OQ587954**	**OQ596331**	**1, 2**	**This study**
Inocybe knautiana	SMNS-STU-F-0901491 HT	Germany	MW845887	MW845887	—	—	2	[29]
Inocybe kuberae	SMNS-STU-F-0901668 HT	Germany	ON003427	ON003427	—	—	2	[6]
Inocybe lampetiana	SMNS-STU-F-0901494 HT	Germany	MW845891	MW845891	—	—	2	[29]
Inocybe langei	KR-M-0038101	Germany	OK057121	OK057121	—	—	1, 2	[21]
Inocybe langei	SMNS-STU-F-0900983	Germany	OK057205	OK057205	—	—	2	[21]
Inocybe lanuginosa	PBM3023/TENN:062780	USA	HQ232480	KP170923	KM245992	MK426186	1	[8,30]
Inocybe lasseroides	PBM3749/TENN:066979	Australia	KP171145	KP170924	KM245993	MK426187	1	[8,38]
Inocybe laurina	SMNS-STU-F-0901247 HT	Germany	MN512325	MN512325	—	—	2	[27]
Inocybe lechiana	SMNS-STU-F-0901268 HT	Austria	MN512330	MN512330	—	—	2	[28]
Inocybe lucis	SMNS-STU-F-0901616 HT	Germany	ON003441	ON003441	—	—	2	[6]
Inocybe luteifolia	AHS6557 IT PBM2642	USA	FJ436331	EU307814	EU307816	MK426188	1, 2	[8,39]
Inocybe magnifolia	MCA2441 HT	Guyana	JN642228	JN642244	EU600899	MK426189	1	[40]
Inocybe melanopus	PBM3975/TENN:068973	USA	—	MH220276	MH249807	MK426190	1	[8,32]
Inocybe morganae	SMNS-STU-F-0901459 HT	Austria	OK057143	—	—	—	1, 2	[21]
Inocybe morganae	SMNS-STU-F-0901608	Germany	OK057201	OK057201	—	—	2	[21]
Inocybe mortenii	DB19-9-20-5 PT	Austria	OP164049	OP164049	—	—	2	[25]
Inocybe mycenoides	SMNS-STU-F-0901647	Germany	OK057156	OK057156	OK078899	—	1	[21]

Table 1. *Cont.*

Taxa	Strain/Voucher	Country	ITS	LSU	*RPB2*	*TEF1*	Phylog. Dataset	Refs.
Inocybe napipes	EL6105 PBM 2376	Norway	AM882926	AY239024	AY337390	—	1	[14,35]
Inocybe ochroalba	SMNS-STU-F-0901590	Finland	OK057137	OK057137	OK078918	—	1	[21]
Inocybe ochroalba	EL5704	Sweden	AM882882	AM882882	—	—	2	[34]
Inocybe orioli	SMNS-STU-F-0901703 HT	Germany	OP164074	OP164074	—	—	2	[25]
Inocybe orionis	SMNS-STU-F-0901455 HT	Germany	MW845898	MW845898	—	—	2	[29]
Inocybe pallidicremea	PBM2039 PBM2744/TENN:06252	USA	KY990553	AY380385	AY337388	MK426191	1	[8,14]
Inocybe perchtana	SMNS-STU-F-0901245 HT	Austria	MN512326	MN512326	—	—	2	[27]
Inocybe persicinipes	PBM2197/PERTH:07676727 HT	Australia	KF977215	EU600837	EU600836	MK426192	1	[8,41]
Inocybe pholiotinoides	SMNS-STU-F-0901702	Germany	OP164095	—	—	—	2	[25]
Inocybe pileosulcata	TBGT:10742	India	KP308810	KP170979	KM406218	MK426193	1	[8,30,38]
Inocybe pipilikae	SMNS-STU-F-0901539 HT	Austria	MW647629	MW647629	—	—	2	[28]
Inocybe pluvialis	PBM3228/TENN:067042 PT	Australia	KF871777	KF853401	KF891954	MK426194	1	[8,30]
Inocybe pseudodestricta	KR-M-0043223	Netherlands	MH366594	—	—	—	2	[34]
Inocybe pseudodestricta	PRM716231 HT	Czechia	MG012468	—	—	—	2	[34]
Inocybe pseudoscabelliformis	SMNS-STU-F-0901634	Germany	OK057172	OK057172	OK078908	—	1	[21]
Inocybe pusio cf.	DB16-8-14-24	Germany	MH366588	—	—	—	2	[34]
Inocybe queletii	KR-M-0038286	Germany	MT101893	—	—	—	1, 2	[37]
Inocybe relicina	IB19920112 JV10258	New Zealand Finland	AF325664	AY038324	AY333778	—	1	[24,42]
Inocybe roseifolia	CO5576	USA	MH578026	MK421968	MH577441	MK426195	1	[8,32]
Inocybe roseipes cf.	MCVE 9856	Italy	JF908143	—	—	—	2	[43]
Inocybe rufobadia	NLB885/PERTH:08320454 HT	Australia	KF977213	KF915290	KF991385	MK426196	1	[8,30]
Inocybe scolopacis	SMNS-STU-F-0901527 HT	Germany	MW845913	MW845913	—	—	2	[29]

Table 1. *Cont.*

Taxa	Strain/Voucher	Country	ITS	LSU	*RPB2*	*TEF1*	Phylog. Dataset	Refs.
Inocybe serrata	PBM3235/TENN:069659	Australia	KP636810	KP171012	KM555111	MK426197	1	[8,30]
Inocybe soliana	SMNS-STU-F-0901664 HT	Germany	ON003425	ON003425	—	—	2	[6]
Inocybe somae	SMNS-STU-F-0901652 HT	Germany	OK057148	OK057148	OK078901	—	1	[21]
Inocybe spadicea	PBM2203/E7051 PT	Australia	KP636866	EU600865	—	MK426198	1	[8,30,41]
Inocybe sphaerospora cf.	ZRL20151281	China	LT716044	KY418860	KY419006	KY419062	1	[26]
Inocybe subexilis	ACAD11680 PBM2620	Canada USA	MH578001	EU307845	EU307847	MK426199	1	[8,39]
Inocybe subhirtella	SMNS-STU-F-0901586	Germany	OK057133	OK057133	OK078915	—	1	[21]
Inocybe substraminea	MCVE 21445	Italy	JF908170	—	—	—	1, 2	[43]
Inocybe tarda	SMNS-STU-F-0901730 ET	Germany	OP164094	OP164094	—	—	2	[25]
Inocybe thailandica	DED8049 HT	Thailand	GQ893013	GQ892968	KM656129	MK426200	1	[8,38]
Inocybe tiburtina	SMNS-STU-F-0901565 HT	Germany	MW845939	MW845939	—	—	2	[29]
Inocybe torresiae	TENN:067011 PT PBM2157/E6978 HT	Australia	KP641635	EU600874	EU600873	—	1	[30,38,41]
Inocybe trollii	**CNF 1/8917 IT**	**Germany**	**OQ550174**	**OQ550177**	**OQ587952**	**OQ596333**	**1**	**This study**
Inocybe trollii	SMNS-STU-F-0901674 HT	Germany	ON003430	ON003430	—	—	2	[6]
Inocybe tubarioides	TENN61324 PBM2550	USA	EU439453	AY732211	EU307855	MK426201	1	[8,39]
Inocybe tyrii	SMNS-STU-F-0901679 HT	Germany	ON003434	ON003434	—	—	2	[6]
Inocybe urceolicystis	SMNS-STU-F-0901615	Finland	OK057175	OK057175	OK078914	—	1	[21]
Inocybe venustissima	KR-M-0042322 HT	Austria	MH366625	—	—	—	1, 2	[34]
Inocybe venustissima	KR-M-0042323 PT	Austria	MH366626	—	—	—	2	[34]
Inocybe venustissima	**KR-M-0042323 PT CNF 1/8918**	**Austria**	**OQ550173**	**OQ550172**	**OQ587953**	**OQ596332**	**1**	**This study**
Inocybe venustissima	SFC20200716-08	South Korea	ON059521	—	—	—	1, 2	[44]
Inocybe venustissima (as *I. auricoma*)	UBC F19796	Canada	HQ604526	HQ604526	—	—	1, 2	unpubl.

Table 1. *Cont.*

Taxa	Strain/Voucher	Country	ITS	LSU	*RPB2*	*TEF1*	Phylog. Dataset	Refs.
Inocybe woglindeana	SMNS-STU-F-0901435 HT	Germany	MT101882	MT101882	—	—	1, 2	[37]
Inocybe zethi	SMNS-STU-F-0901456 HT	Germany	ON003440	ON003440	—	—	2	[6]
Inosperma calamistratum	SAT9826004	USA	JQ801387	JQ815410	JQ846467	MK426204	1	[8,45]
Inosperma rimosoides	PBM2459	USA	DQ404391	AY702014	DQ385884	DQ435790	1	[8,46]
Inosperma virosum	TBGT753 PT	India	KT329452	KT329458	KT329446	MK426208	1	[8,47]
Mallocybe myriadophylla	JV19652F	Finland	DQ221106	AY700196	AY803751	DQ435791	1	[46]
Mallocybe terrigena	EL11704 JV16431	Sweden Finland	AM882864	AY380401	AY333309	—	1	[14,35]
Mallocybe tomentosula	PBM4138/TENN:071837	USA	MG773814	MK421969	MH577506	MK426210	1	[8,32]
Nothocybe distincta	CAL1310 HT ZT9250 PT	India	KX171343	EU604546	EU600904	MK426212	1	[8,41,48]
Pseudosperma bulbosissimum	DBG19916	USA	MH024849	MH024885	MH249788	MK426213	1	[8,32]
Pseudosperma fascinosum	SMNS-STU-F-0901666 HT	Germany	ON003426	—	—	—	2	[6]
Pseudosperma huginii	STU:SMNS-STU-F-0901564 HT	Austria	MW647628	—	—	—	2	[28]
Pseudosperma notodryinum	CO4463/CSU 01252	USA	MH578028	MK421970	MH577509	MK426216	1, 2	[8,32]
Pseudosperma sororium	MCA859 PBM3901	USA	JQ408772	MH220278	MH249810	MK426218	1	[8,32]
Simocybe phlebophora	PBM3089/PDD:97898	New Zealand	MK421963	MK421967	MK415449	—	1	[8]
Simocybe serrulata	PBM2536	USA	DQ494696	AY745706	DQ484053	GU187755	1	[49]
Tubariomyces inexpectatus	AH25500 PT AH20390 HT	Spain	GU907095	EU569855	GU907088	—	1	[38,41,50]
Tubariomyces sp.	BB6018	Zambia	MK421965	EU600887	EU600886	MK426220	1	[8,38,41]

Sequences were aligned by each locus, and concatenation was done as indicated above. The concatenated alignment of ITS and LSU (Phylogenetic dataset 2) sequences contained 1766 characters, including gaps, with 832 characters for the ITS and 934 for the LSU gene region.

Phylogenetic analyses of concatenated ITS–LSU–*rpb2*–*tef1* and ITS–LSU sequence alignments were conducted using Maximum likelihood (ML) analysis in IQTREE v1.6.12 [51,52] and Bayesian inference (BI) analysis in MrBayes 3.2.6 (Geneious plugin, [53]). The best model was selected by ModelFinder implemented in IQ-TREE, considering separately the corrected Akaike and Bayesian Information Criterion (cAIC, BIC). GTR + F + I + G4 was selected as the best model for both phylogenetic datasets. ML analyses were executed by applying the ultrafast bootstrap approximation with 1000 replicates. BI analyses were executed for 10,000,000 generations, sampling trees and other parameters every 10,000 generations. The default number of chains (four) and heating parameters were used. Posterior probabilities (BPP) were calculated after burning the first 25% of the posterior sample. Phylogenetic trees were visualized and annotated using iTOL v6.5.4 [54] and FigTree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/, accessed on 17 Februrary 2023).

3. Results

3.1. Molecular Phylogenetic Analyses

A total of 14 DNA sequences (three ITS, three LSU, four *rpb2*, three *tef1*, and one SSU) from four *Inocybe* species were newly generated in this study. In addition to sequencing five gene regions (SSU, ITS, LSU, *rpb2*, and *tef1*) of *I. istriaca* (CNF 1/7323), the isotype of *I. trollii* (voucher CNF 1/8917, holotype SMNS-STU-F-0901674) and the paratype of *I. venustissima* (CNF 1/8918, part of KR-M-0042323) were resequenced for ITS and LSU and newly sequenced for *rpb2* and *tef1* gene regions. The isotype of *I. dvaliniana* (CNF 1/8916, holotype SMNS-STU-F-0901559) was sequenced for the *rpb2* gene region. The accession numbers of all newly generated sequences used in phylogenetic analyses are marked in bold (Table 1). The ITS sequence from the holotype of *Inocybe istriaca* (accession number: OQ550176) was BLAST searched against NCBI GenBank's nucleotide database. The closest two hits were sequences of *I. venustissima* SFC 20200716-08 (accession number: ON059521, identity 80.96%) and *I. trollii* SMNS-STU-F-0901674 (accession number: ON003430, identity 80.03%), considering data from published sources only.

Phylogenetic trees generated from BI and ML analyses of the concatenated ITS–LSU–*rpb2*–*tef1* sequence alignment were identical in topology and were presented as a single phylogenetic tree in Figure 1. Phylogenetic trees generated from BI and ML analyses of the concatenated ITS–LSU sequence alignment were also identical in topology and were presented as a single phylogenetic tree in Figure 2. Only significant branch support values were presented at the nodes (Bayesian posterior probability (BI-PP $\geq$ 0.95) and ultrafast bootstrap support (ML-BP $\geq$ 70%)).

A four-gene region (ITS, LSU, *rpb2*, *tef1*) phylogenetic analysis has shown a total of seven strongly supported branches (BI-PP $\geq$ 0.95, ML-BP $\geq$ 70) representing seven genera within the family *Inocybaceae*, which recovered as two monophyletic groups (*Inocybe-Nothocybe-Pseudosperma* and *Inosperma-Mallocybe-Tubariomyces-Auritella*) (Figure 1). The genus *Inocybe* was recovered as a strongly supported (BI-PP = 1, ML-BP = 100) monophyletic group, including many poorly supported (BI-PP < 0.95, ML-BP < 70) short internodes in both phylogenetic analyses (Figures 1 and 2).

Among the 53 *Inocybe* species in the four-gene analyses and the 66 *Inocybe* species in the two-gene analyses, *I. istriaca* was recovered as a single stem lineage, confirming its status as an independent new species. In both analyses, *I. istriaca* was nested in a strongly supported (BI-PP = 1, ML-BP = 100) monophyletic clade that included *I. venustissima* and its sister species, *I. chalcodoxantha* (analyzed only in the ITS-LSU phylogeny). In the four-gene phylogeny, *I. dvaliniana* and *I. trollii* clustered together with *I. adorabilis*, *I. pseudoscabelliformis*, and *I. urceolicystis* (Figure 1) in a strongly supported monophyletic group (BI-PP = 1, ML-BP = 100) sister to a clade composed of *I. venustissima* and *I. istriaca.*

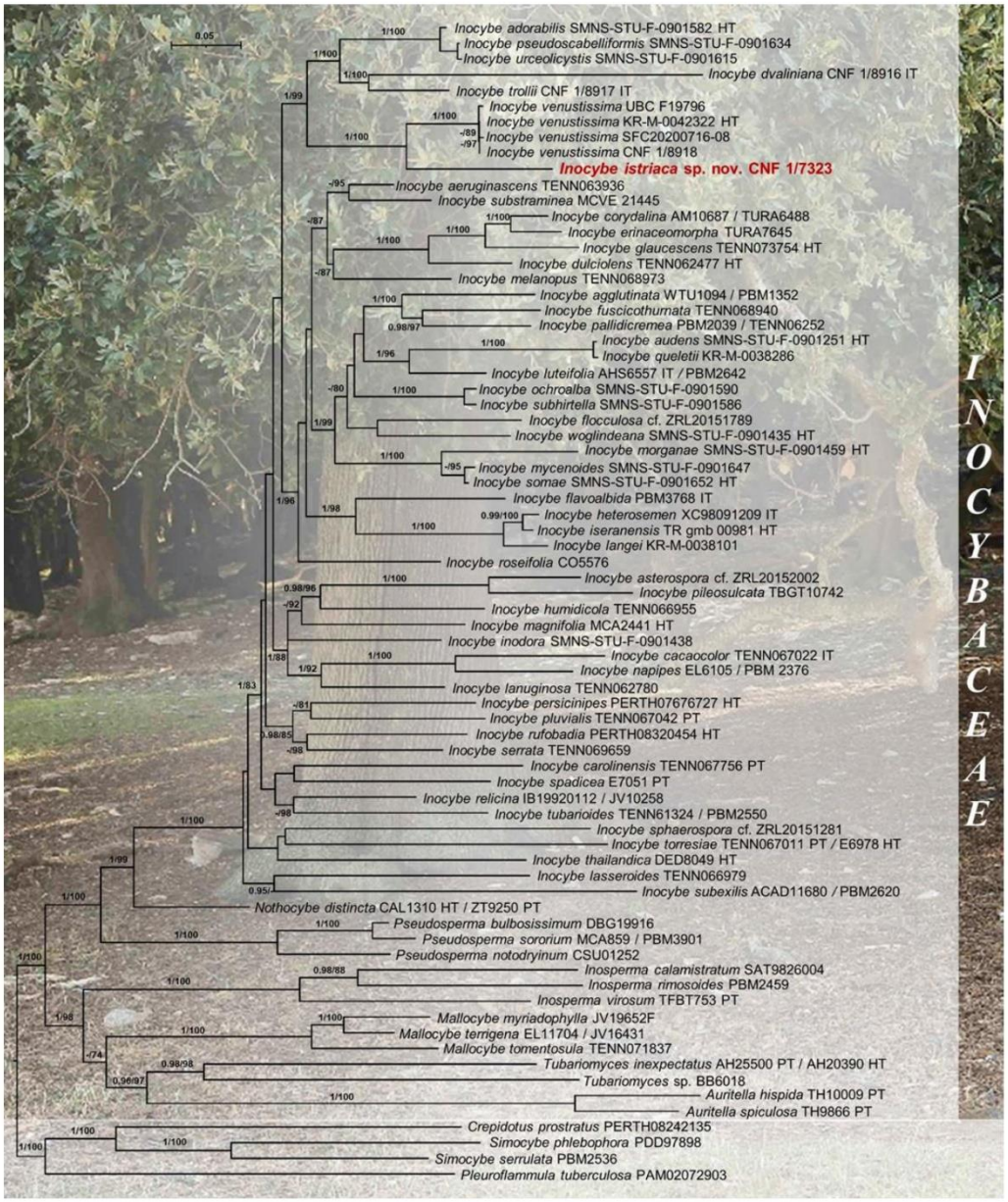

Figure 1. Phylogenetic tree of the family Inocybaceae based on Bayesian Inference (BI) and Maximum Likelihood (ML) analyses of the concatenated four-gene (ITS, LSU, *rpb2*, *tef1*) sequence alignment. Significant branch support values, Bayesian posterior probability (BI-PP $\geq$ 0.95), and ultrafast bootstrap support (ML-BP $\geq$ 70%), are presented at the nodes. The newly proposed species, *Inocybe istriaca*, is marked in red and in bold font. Abbreviations: HT = holotype; IT = isotype; PT = paratype.

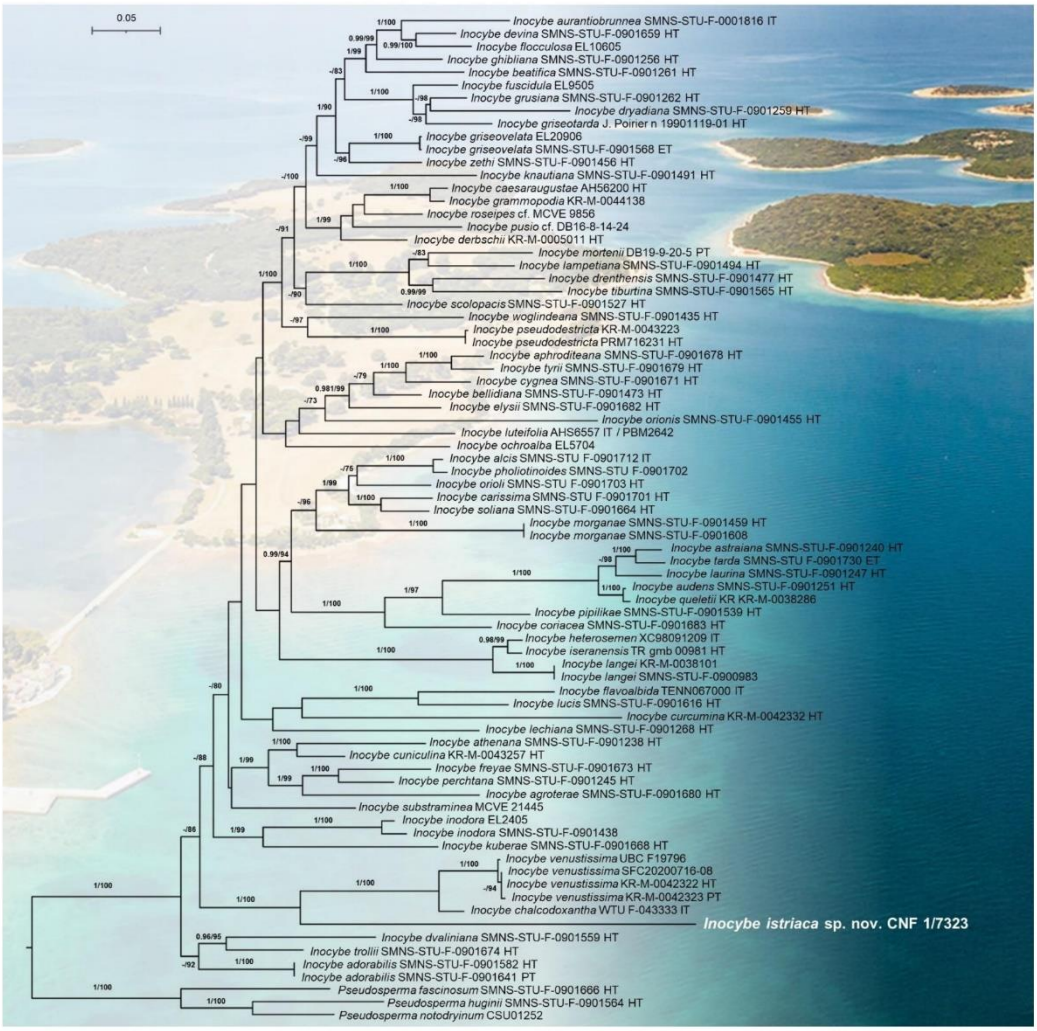

Figure 2. Phylogenetic tree of the genus *Inocybe* based on Bayesian Inference (BI) and Maximum Likelihood (ML) analyses of the concatenated two-gene (ITS, LSU) sequence alignment. Significant branch support values, Bayesian posterior probability (BI-PP $\geq$ 0.95) and ultrafast bootstrap support (ML-BP $\geq$ 70%), are presented at the nodes. The newly proposed species, *Inocybe istriaca*, is marked in white and bold font. Abbreviations: HT = holotype; ET = epitype; IT = isotype; PT = paratype.

3.2. Taxonomy

Inocybe istriaca Mešić, Tkalčec, Pošta, Pole and Bandini, sp. nov. (Figures 3–5).
Mycobank MB847017

Typification: CROATIA. ISTRIA COUNTY: Brijuni National Park, Veli Brijun Island, 44.90711° N, 13.75436° E, on the edge of lawn and forest of *Quercus ilex*, 15 November 2016, *A. Mešić* and *Z. Tkalčec* (holotype, CNF 1/7323; isotype, SMNS-STU-F-0901784).

GenBank (ex-holotype DNA isolate): SSU = OQ598554, ITS = OQ550176, LSU = OQ550175, *rpb2* = OQ587954, *tef1* = OQ596331.

Etymology: referring to the Istria peninsula, where the holotype was collected.

Pileus 19–35 mm wide, convex, campanulato-convex, or plano-convex with a broadly subumbonate center, margin mostly deflexed (slightly inflexed when young), entire, occasionally shortly radially splitted, surface dry, rather finely to coarsely radially fibrillose, sparsely woolly-squamulose in places, partially fibrillose-rimulose near margin, smooth to subtomentose-subsquamulose around the center, pale to light yellowish- to orangish-brown, fibrils and tufts often darker, medium orange- to red-brown, when young with whitish, narrow patches of universal veil at the margin, later evanescing, velipellis faint in mature basidiomata. Lamellae narrowly adnate to deeply emarginate, ventricose, moderately crowded, white at first, then pale greyish-brown (beige), later light brown, edge entire to eroded, whitish, concolorous, or brownish. Stipe 25–30 × 4–7 mm, subcylindrical with a slightly to distinctly broadened base (up to 10 mm, often submarginate), solid to narrowly fistulose, surface dry, longitudinally fibrillose-striate, pale to light brown, flocculose, and white at the apex. Context: white to whitish, not changing color on bruising. Smell weak, acidic fruity when cut. Taste is not recorded.

Figure 3. *Inocybe istriaca* sp. nov. (CNF 1/7323, holotype). Basidiomata. Bar = 10 mm. Authors: A. Mešić and Z. Tkalčec.

Basidiospores [200/4/1] (7.8–)8.4–*10.2*–11.9 × 5.2–*6.2*–7.2 µm, averages of different basidiomata 10.1–10.3 × 6.1–6.3 µm, Q = (1.30–)1.37–*1.64*–1.95(–2.05), av. Q = 1.64–1.65; in frontal view mostly ellipsoid, also ovoid or oblong, with rounded to subacute base and rounded to acute apex; in side view (sub)amygdaliform, (sub)phaseoliform, ellipsoid or oblong, with rounded to acute base and apex; smooth, germ-pore apical and indistinct (visible as a lighter spore wall) or absent, thin-walled to slightly thick-walled (up to 0.6 µm), pale yellow-brown in KOH and H_2O, non-amyloid and non-dextrinoid.

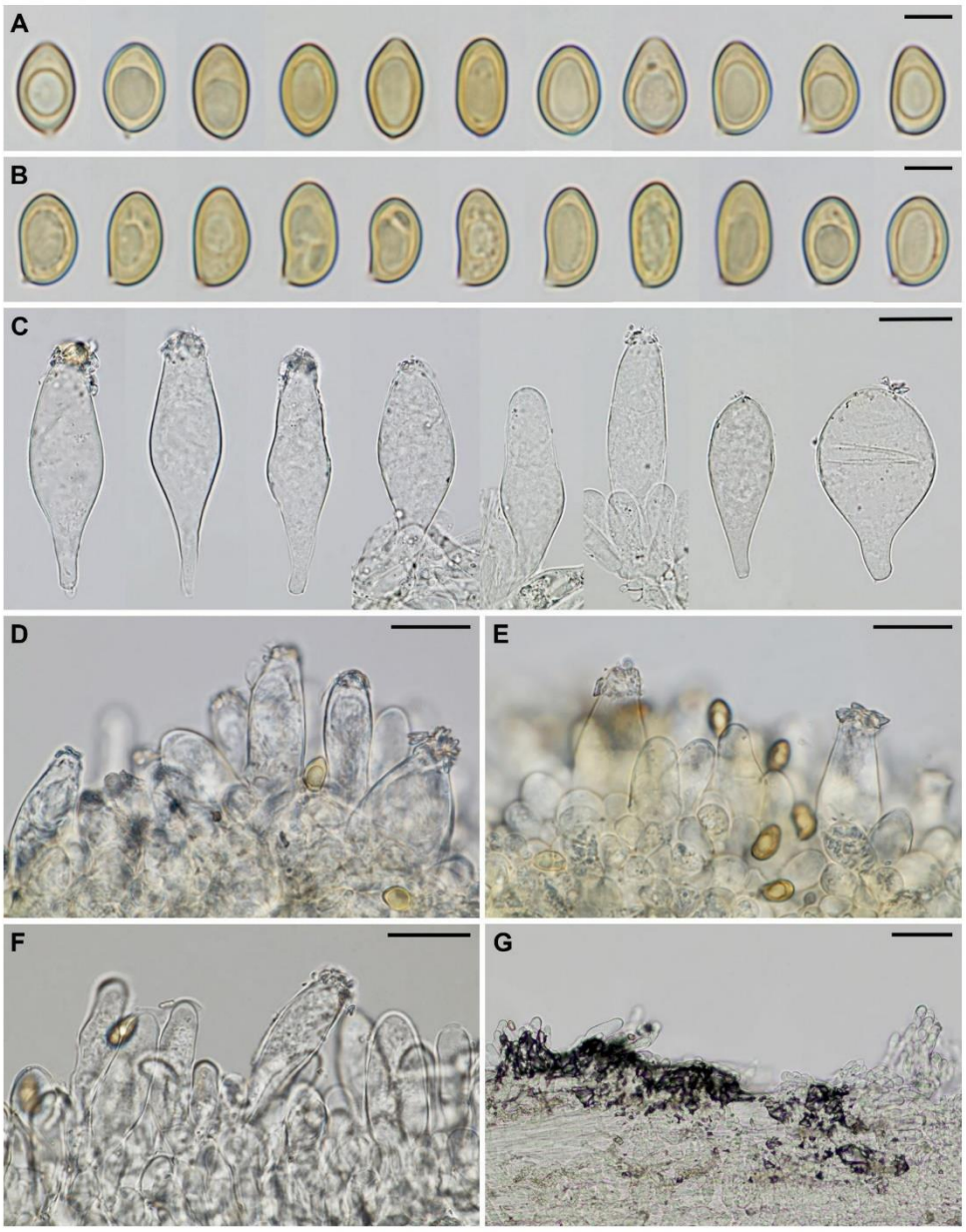

Figure 4. *Inocybe istriaca* sp. nov. (CNF 1/7323, holotype). (**A**,**B**) Basidiospores. (**C**) Pleurocystidia. (**D**,**E**) Cheilocystidia. (**F**) Caulocystidia. (**G**) Black resinous substance among caulocystidia. Bars: (**A**,**B**) = 5 µm; (**C**–**F**) = 20 µm; (**G**) = 50 µm. Author: A. Mešić.

Basidia [50/4/1] 36–*40.2*–45 × 9–*11.4*–15 µm, Q = (2.7–)3.0–*3.6*–4.2(–4.6), clavate, predominantly 4-spored, occasionally 2-spored, thin-walled, mostly hyaline, rarely brownish. Pleurocystidia metuloid, [60/4/1] 50–*65.2*–80 × 14–*20.3*–34 µm, Q = 1.59–*3.36*–4.50, scattered, very variable in shape, but mostly (sub)fusiform, also clavate to broadly clavate, (sub)utriform, (elongate) ellipsoid, subcylindrical, or somewhat deformed (e.g., curved to one side), usually without or with only a short neck, with a short to very long tapering pedicel, in alkaline solutions mostly (sub)hyaline, sometimes with pale yellow-brown cytoplasmic pigment, with strongly to weakly developed crystals at the apex (soluble in KOH, rarely lacking), moderately thick-walled to thick-walled (up to 1 µm in the middle, up to 1.5 µm at the apex). The lamellar edge mostly sterile, at places covered with abundant dark brown to black resinous substance. Cheilocystidia of two types: (a) metuloid, similar to pleurocystidia in size and shape, sometimes with crystals at the apex, abundant; and (b) paracystidia, mostly clavate or subcylindrical, hyaline, thin-walled to moderately thick-walled (up to 0.8 µm), scattered to abundant. Pileipellis a cutis, composed of a superficial layer of repent, thin-walled, (sub)hyaline, cylindrical, 2–5 µm wide velipellis hyphae and a lower layer of gradually shorter and wider, thin-walled hyphae with parietal to encrusted brown pigment. Stipitipellis a cutis, composed of repent, thin-walled, ca. 2–10 µm wide hyphae, sometimes with brown, parietal to minutely encrusted pigment. Caulocystidia very abundant in the upper 2–3 mm of stipe length, in clusters or in dense groups, gradually becoming rare, more simple-shaped, or as caulocystidioid hairs toward the middle of the stipe, absent from the bottom half of the stipe; at places heavily agglutinated by a dark brown to black resinous substance; similar to cheilocystidia, very variable, fusiform, narrowly to broadly utriform, (sub)cylindrical, clavate, sometimes septate (up to 3-celled), apex occasionally (sub)capitate, sometimes with apical crystals, hyaline, sometimes with brown parietal pigment, thin- to moderately thick-walled (up to ca. 1 µm); 15–60 × 5–20 µm. Clamp connections present, conspicuous, and rather abundant in all tissues.

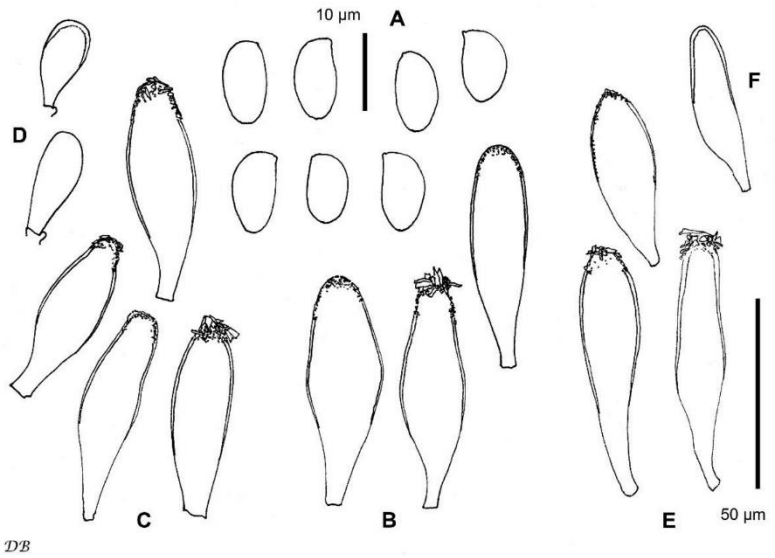

Figure 5. *Inocybe istriaca* sp. nov. (CNF 1/7323, holotype). (**A**) Basidiospores. (**B**) Pleurocystidia. (**C**) Cheilocystidia of metuloid-type. (**D**) Cheilocystidia of paracystidia-type. (**E**) Caulocystidia of metuloid-type. (**F**) Caulocystidia of paracystidia-type. Bars: (**A**) = 10 µm; (**B**–**F**) = 50 µm. Del. D. Bandini.

Distribution and ecology: Known only from the holotype collection. Found on the island of Veli Brijun in Brijuni National Park, the northern Adriatic Sea, the Mediterranean region of Croatia, Europe. Ectomycorrhizal, on the edge of the mature thermophilous *Quercus ilex* forest and a lawn grazed by large herbivores (fallow deer [*Dama dama*], axis deer [*Axis axis*], and European mouflon [*Ovis gmelini musimon*]). Basidiomata growing epigeous on calcareous soil (terra rossa on limestone bedrock) covered with short grasses, mosses, and sparse holm oak litter, about 130 m from the sea coast.

4. Discussion

Inocybe istriaca, described here as new to science, has some remarkable micromorphological characters that distinguish it well from the other members of the genus. Most *Inocybe* s.s. species possess basidia that are 20–35(–40) × 7–12 µm in size (with a few exceptions, e.g., 28–43 × 8–12 µm in *I. fraudans* (Britzelm.) Sacc. [55,56], while basidia of *I. istriaca* are mostly larger (36–45 × 9–15 µm). Additional striking characters are the presence of a dark brown to black resinous substance on the lamellar edge and stipe apex, which covers and agglutinates cheilocystidia and caulocystidia. However, these features need to be evaluated more thoroughly when additional collections of this species become available. Other important morphological characters are: pale to light brown, radially fibrillose pileus with faint velipellis; apically flocculose, subcylindrical stipe with slightly to distinctly broadened, often submarginate base; color of the context unchanged upon bruising; weak, fruity acidic smell; medium-sized, smooth, in frontal view mostly ellipsoid and in side view amygdaliform or phaseoliform basidiospores (ca. 8.5–12 × 5–7 µm); pleurocystidia metuloid, crystalliferous, very variable, mostly (sub)fusiform, with short to very long pedicel, without or with short neck, walls apically up to 1.5 µm wide and not yellowing in alkaline solutions; cheilocystidia of two types (metuloid and leptocystidia); and presence of abundant clustered caulocystidia only in the upper 2–3 mm of stipe length.

In addition to the large basidia and the dark resinous substance on the lamellar edge and stipe apex, the most important taxonomic characters used to distinguish *I. istriaca* and related species are presented in Table 2. The molecular analyses performed in this study show that *I. venustissima* Bandini and B. Oertel and *I. chalcodoxantha* Grund and D.E. Stuntz are phylogenetically most closely related to *I. istriaca* and form a well-supported sister clade. *Inocybe venustissima* has somewhat larger basidiomata (pileus 20–50 mm broad, stipe 30–100 mm long), waxy shiny glabrous to rim(ul)ose pileus surface, stipe mostly with large roundish bowl-shaped bulbous base and often pruinose on the entire length (though sparsely in the lower half), somewhat smaller spores, and on average shorter pleurocystidia (50 µm vs. 65 µm in *I. istriaca*). It is known from montane to subalpine forests in Austria, growing near small brooks or rivulets under *Picea abies* on acidic soil, and from Canada near *Tsuga heterophylla* [34]. *Inocybe chalcodoxantha* Grund and D.E. Stuntz, known from coniferous forests in Canada and the USA (Washington), has a much longer stipe (up to 100 mm), a strong spermatic smell, somewhat smaller basidiospores, and thicker-walled pleurocystidia (up to 3.3 µm) [57].

In addition, from other morphologically similar species, *I. adorabilis* Bandini, B. Oertel, and U. Eberh. differs by having an entirely pruinose stipe (but sparsely so in the lower half), a spermatic smell, somewhat smaller basidiospores, and shorter pleurocystidia with an often rounded base and thicker walls (up to 3.5 (–4.5) µm). It is known only from subalpine areas in Austria, where it grows near *Picea abies* [21]. *Inocybe audens* Bandini, Christan, and Dondl differs by larger basidiomata (pileus 20–60 mm broad, stipe 30–80 mm long), a more glabrous pileus surface, somewhat shorter spores, and pleurocystidia with much thicker walls (up to 5.0(–6.0) µm). It occurs under coniferous trees (*Picea abies*, *Abies alba*, *Larix*, etc.) and develops basidiomata very early in the year (April–May) [28].

Table 2. Overview of the main taxonomic characters used for delimitation between *Inocybe istriaca* and related species.

Species	Spore Size (µm)	Pleurocystidia Size (µm)	Pleuroc. Thick at Apex (µm), Walls Colour (KOH)	Habitat	References
Inocybe istriaca	8.4–10.2–11.9 × 5.2–6.2–7.2	50–65–80 × 14–20–34	up to 1.5, (sub)hyaline	Mediterranean forest of *Quercus ilex*, edge with grassland	This study
I. adorabilis	8.0–8.9–9.9 × 4.6–5.1–5.6	37–54–69 × 11–15–22	up to 3.5(–4.5), yellowish-greenish	subalpine forest, *Picea abies*	[21]
I. audens	7.8–9.2–10.5 × 5.0–5.8–6.7	41–60–72 × 11–16–25	up to 5.0(–6.0), (sub)hyaline to light yellowish-greenish	under coniferous trees, *Picea abies*, *Abies alba*, *Larix*, etc.	[28]
I. chalcodoxantha	7.5–10 × 5–6.5, mostly 9 × 5.5	50–72 × 13–21	1.0–3.3, hyaline	under conifers, in moss or needles	[57]
I. heterosemen	6.5–7.6–8.1 × 3.5–4.2–4.8	29–38–49 × 12–16–20	up to 2.5(–3.5), yellowish-greenish	mostly deciduous forests, *Salix*, *Betula pubescens*, *Populus tremula*, *Alnus*, etc.	[21,58]
I. inodora	9.0–11.0–12.8 × 5.2–6.2–7.4 * 10.0–14.0 × 5.5–7.0 **	44–59–68 × 12–18–25	up to 3.0(–4.0), yellowish-greenish	mostly under deciduous trees	[37], [55] **, [56] *, [59,60]
I. iseranensis	7.5–8.3–9.4 × 4.7–5.0–5.7	37–46–58 × 14–16–18	up to 1.5(–2.5), yellowish-greenish	alpine regions, *Salix herbacea*, *Betula nana*, *B. pubescens*	[21,61]
I. langei	6.4–7.0–8.0 × 3.8–4.4–5.0 * 7.0–9.0 × 4.5–5.0(–5.5) **	35–47–57 × 9–12–15 * 40–60 × 13–20 **	up to 3.0(–3.5), (pale) yellowish-greenish	mostly with deciduous, but also coniferous trees, *Quercus*, *Salix*, *Alnus*, *Picea*, *Pinus*, etc.	[21] *, [56] **
I. morganae	8.6–9.7–11.2 × 4.9–5.6–6.1	35–52–66 × 10–16–27	up to 1.5(–2.0), yellowish-greenish	montane regions, *Picea abies*	[21]
I. queletii	(8–)8.5–12(–13) × 5.8–7	56–75 × 13–22(–25)	up to 3, hyaline	montane regions, *Abies alba*	[55,56,62]
I. substraminea	(9–)10–12(–13.5) × 5–6	55–75 × 15–22	n/a	submontane forest, *Fagus sylvatica*	[63]
I. trollii	8.0–9.6–11.1 × 5.0–5.6–6.6	44–53–60 × 11–14–19	up to 2.0(–2.5), yellow-green	under *Pinus sylvestris*, *Corylus avellana*, *Populus* sp.	[6]
I. venustissima	7.3–8.9–10.9 × 4.6–5.3–6.7	35–50–76 × 11–16–23	up to 1.5(–2.0), weak, pale yellowish-greenish	montane to subalpine forests, *Picea abies* (Austria); *Tsuga heterophylla* (Canada); *Larix kaempferi* (Korea)	[34,44]
I. woglindeana	8.0–10.2–13.0 × 4.9–5.9–7.1 * 9.0–11.3–14.3 × 5.3–6.3–7.4 **	35–57–77 × 12–19–31 * 51–67–82 × 15–20–30 **	up to 1.0, pale yellow	mostly under deciduous trees, always *Salix* (*S. caprea*), mixed with *Betula*, *Populus*, *Pinus sylvestris*, etc.	[37] Germany *, Finland **

* and ** are connected with the references.

Inocybe inodora Velen. has an entirely pruinose stipe (but often sparsely so in the lower half), larger spores, and thicker-walled pleurocystidia (up to 3.0(–4.0) µm). It is widely distributed in Europe, growing mostly in ectomycorrhiza with deciduous trees [37,55,56,59,60]. *Inocybe morganae* Bandini, B. Oertel, and U. Eberh has an entirely pruinose stipe (but often sparsely so in the lower half), a smell reminding of bitter almonds, and somewhat shorter pleurocystidia, which are yellowish-greenish in KOH. It is known from the montane regions of Austria and Germany where it occurs near *Picea abies* [21]. *Inocybe queletii* Konrad differs by having larger basidiomata (pileus 30–60 mm broad), a spermatic smell, and thicker-walled pleurocystidia (up to 3.0 µm). It forms ectomycorrhizae with *Abies alba* in montane areas [55,56,62]. *Inocybe substraminea* Alessio differs by much larger basidiomata (pileus 50–80(–120) mm broad, stipe 60–100 × 8–15 mm), an equal or hardly widened stipe base (never submarginate), and thicker-walled pleurocystidia. It is known from submontane habitat with *Fagus sylvatica* in Italy [63]. *Inocybe trollii* Bandini and B. Oertel has a minutely lanose (woolly) pileus surface and smaller pleurocystidia with thicker walls turning yellow-green in KOH. It is known only from the type locality in Austria, where it grows with *Pinus sylvestris*, *Corylus avellana*, and *Populus* sp. [6]. *Inocybe woglindeana* Bandini, Vauras, and Weholt has an abundant velipellis, somewhat longer basidiospores (up to 14.3 µm), and often somewhat "sac-shaped" pleurocystidia with a rounded or truncate base. It grows mostly under deciduous trees on sandy or gravelly soil, always with *Salix* (mostly *S. caprea*) nearby [37]. Further three taxa, *I. langei* R. Heim, *I. iseranensis* E. Ferrari, and *I. heterosemen* Carteret and Reumaux, can be easily distinguished from *I. istriaca* by their smaller basidiospores (up to ca. 9.5 × 6 µm) and shorter pleurocystidia (up to ca. 60 µm), which are usually thicker-walled [21,58,61].

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References

1. Mittermeier, R.A.; Turner, W.R.; Larsen, F.W.; Brooks, T.M.; Gascon, C. Global Biodiversity Conservation: The Critical Role of Hotspots. In *Biodiversity Hotspots: Distribution and Protection of Conservation Priority Areas*; Zachos, F.E., Habel, J.C., Eds.; Springer: Berlin/Heidelberg, Germany, 2011; pp. 3–22. ISBN 978-3-642-20992-5.
2. Médail, F.; Monnet, A.C.; Pavon, D.; Nikolic, T.; Dimopoulos, P.; Bacchetta, G.; Arroyo, J.; Barina, Z.; Albassatneh, M.C.; Domina, G.; et al. What Is a Tree in the Mediterranean Basin Hotspot? A Critical Analysis. *For. Ecosyst.* **2019**, *6*, 17. [CrossRef]
3. Branković, Č.; Güttler, I.; Gajić-Čapka, M. Evaluating Climate Change at the Croatian Adriatic from Observations and Regional Climate Models' Simulations. *Clim. Dyn.* **2013**, *41*, 2353–2373. [CrossRef]
4. Brijuni National Park Official Website. Available online: https://www.np-brijuni.hr/en/brijuni (accessed on 12 January 2023).

5. Mešić, A.; Haelewaters, D.; Tkalčec, Z.; Liu, J.; Kušan, I.; Catherine Aime, M.; Pošta, A. *Inocybe brijunica* sp. nov., a New Ectomycorrhizal Fungus from Mediterranean Croatia Revealed by Morphology and Multilocus Phylogenetic Analysis. *J. Fungi* **2021**, *7*, 199. [CrossRef] [PubMed]
6. Bandini, D.; Oertel, B.; Eberhardt, U. Noch Mehr Risspilze (3): Einundzwanzig Neue Arten Der Familie Inocybaceae. *Mycol. Bavarica* **2022**, *22*, 31–138.
7. Wijayawardene, N.N.; Hyde, K.D.; Dai, D.Q.; Sánchez-García, M.; Goto, B.T.; Saxena, R.K.; Erdoğdu, M.; Rajeshkumar, K.C.; Aptroot, A.; Zhang, G.Q.; et al. Outline of Fungi and Fungus-like Taxa—2021. *Mycosphere* **2022**, *13*, 53–453. [CrossRef]
8. Matheny, P.B.; Hobbs, A.M.; Esteve-Raventós, F. Genera of Inocybaceae: New Skin for the Old Ceremony. *Mycologia* **2020**, *112*, 83–120. [CrossRef]
9. Clémençon, H. *Cytology and Plectology of the Hymenomycetes*, 2nd ed.; Cramer: Stuttgart, Germany, 2012.
10. Erb, B.; Matheis, W. *Pilzmikroskopie*; Kosmos: Stuttgart, Germany, 1982.
11. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protoc.* **1990**, 315–322. [CrossRef]
12. Gardes, M.; Bruns, T.D. ITS Primers with Enhanced Specificity for Basidiomycetes—Application to the Identification of Mycorrhizae and Rusts. *Mol. Ecol.* **1993**, *2*, 113–118. [CrossRef]
13. Vilgalys, R.; Hester, M. Rapid Genetic Identification and Mapping of Enzymatically Amplified Ribosomal DNA from Several Cryptococcus Species. *J. Bacteriol.* **1990**, *172*, 4238–4246. [CrossRef]
14. Matheny, P.B. Improving Phylogenetic Inference of Mushrooms with RPB1 and RPB2 Nucleotide Sequences (*Inocybe*; Agaricales). *Mol. Phylogenet. Evol.* **2005**, *35*, 1–20. [CrossRef]
15. Rehner, S. Primers for Elongation Factor 1-α (EF1-α). 2001. Available online: http://ocid.NACSE.ORG/research/deephyphae/EF1primer.pdf (accessed on 11 February 2022).
16. Rehner, S.A.; Buckley, E. A Beauveria Phylogeny Inferred from Nuclear ITS and EF1-Alpha Sequences: Evidence for Cryptic Diversification and Links to *Cordyceps* Teleomorphs. *Mycologia* **2005**, *97*, 84–98. [CrossRef] [PubMed]
17. Haelewaters, D.; Toome-Heller, M.; Albu, S.; Aime, M.C. Red Yeasts from Leaf Surfaces and Other Habitats: Three New Species and a New Combination of *Symmetrospora* (*Pucciniomycotina*, *Cystobasidiomycetes*). *Fungal Syst. Evol.* **2019**, *5*, 187–196. [CrossRef] [PubMed]
18. Katoh, K.; Misawa, K.; Kuma, K.I.; Miyata, T. MAFFT: A Novel Method for Rapid Multiple Sequence Alignment Based on Fast Fourier Transform. *Nucleic Acids Res.* **2002**, *30*, 3059–3066. [CrossRef]
19. Katoh, K.; Standley, D.M. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [CrossRef] [PubMed]
20. Matheny, P.B.; Henkel, T.W.; Séné, O.; Korotkin, H.B.; Dentinger, B.T.M.; Aime, M.C. New Species of *Auritella* (Inocybaceae) from Cameroon, with a Worldwide Key to the Known Species. *IMA Fungus* **2017**, *8*, 287–298. [CrossRef]
21. Bandini, D.; Oertel, B.; Eberhardt, U. More Smooth-Spored Species of *Inocybe* (Agaricales, Basidiomycota): Type Studies and 12 New Species from Europe. *Persoonia Mol. Phylogeny Evol. Fungi* **2022**, *48*, 91–149. [CrossRef]
22. Matheny, P.B.; Norvell, L.L.; Giles, E.C. A Common New Species of *Inocybe* in the Pacific Northwest with a Diagnostic PDAB Reaction. *Mycologia* **2013**, *105*, 436–446. [CrossRef]
23. Matheny, P.B.; Swenie, R.A. The *Inocybe geophylla* Group in North America: A Revision of the Lilac Speciessurrounding *I. lilacina*. *Mycologia* **2018**, *110*, 618–634. [CrossRef]
24. Matheny, P.B.; Liu, Y.J.; Ammirati, J.F.; Hall, B.D. Using RPB1 Sequences to Improve Phylogenetic Inference among Mushrooms (*Inocybe*, Agaricales). *Am. J. Bot.* **2002**, *89*, 688–698. [CrossRef]
25. Bandini, D.; Brandrud, T.E.; Dima, B.; Dondl, M.; Fachada, V.; Hussong, A.; Mifsud, S.; Oertel, B.; Rodríguez Campo, F.J.; Thüs, H.; et al. Fibre Caps across Europe: Type Studies and 11 New Species of *Inocybe* (Agaricales, Basidiomycota). *Integr. Syst.* **2022**, *5*, 1–85. [CrossRef]
26. Zhao, R.L.; Li, G.J.; Sánchez-Ramírez, S.; Stata, M.; Yang, Z.L.; Wu, G.; Dai, Y.C.; He, S.H.; Cui, B.K.; Zhou, J.L.; et al. A Six-Gene Phylogenetic Overview of Basidiomycota and Allied Phyla with Estimated Divergence Times of Higher Taxa and a Phyloproteomics Perspective. *Fungal Divers.* **2017**, *84*, 43–74. [CrossRef]
27. Bandini, D.; Oertel, B.; Schüssler, C.; Eberhardt, U. Noch Mehr Risspilze: Fünfzehn Neue Und Zwei Wenig Bekannte Arten Der Gattung *Inocybe*. *Mycol. Bavarica* **2020**, *20*, 13–101.
28. Bandini, D.; Oertel, B.; Eberhardt, U. Noch Mehr Risspilze (2): Dreizehn Neue Arten Der Familie Inocybaceae. *Mycol. Bavarica* **2021**, *21*, 27–98.
29. Bandini, D.; Oertel, B.; Eberhardt, U. A Fresh Outlook on the Smooth-Spored Species of *Inocybe*: Type Studies and 18 New Species. *Mycol. Prog.* **2021**, *20*, 1019–1114. [CrossRef]
30. Matheny, P.B.; Bougher, L.N. *Fungi of Australia Inocybaceae*; Australian Biological Resources Study; CSIRO Publishing: Canberra, VC, Australia; Melbourne, VC, Australia, 2017.
31. Munoz, G.; Pancorbo, F.; Turegano, Y.; Esteve, F. New Species and Combinations of *Inocybe* with Lilac or Violet Colours in Europe. *Fungi Iber.* **2022**, *2*, 7–26. [CrossRef]
32. Matheny, P.B.; Kudzma, L.V. New Species of *Inocybe* (Inocybaceae) from Eastern North America. *J. Torrey Bot. Soc.* **2019**, *146*, 213–235. [CrossRef]

33. Schoch, C.L.; Robbertse, B.; Robert, V.; Vu, D.; Cardinali, G.; Irinyi, L.; Meyer, W.; Nilsson, R.H.; Hughes, K.; Miller, A.N.; et al. Finding Needles in Haystacks: Linking Scientific Names, Reference Specimens and Molecular Data for Fungi. *Database* **2014**, *2014*, bau061. [CrossRef]
34. Bandini, D.; Oertel, B.; Ploch, S.; Ali, T.; Vauras, J.; Schneider, A.; Scholler, M.; Eberhardt, U.; Thines, M. Revision of Some Central European Species of *Inocybe* (Fr.:Fr.) Fr. Subgenus *Inocybe*, with the Description of Five New Species. *Mycol. Prog.* **2019**, *18*, 247–294. [CrossRef]
35. Ryberg, M.; Nilsson, R.H.; Kristiansson, E.; Töpel, M.; Jacobsson, S.; Larsson, E. Mining Metadata from Unidentified ITS Sequences in GenBank: A Case Study in *Inocybe* (*Basidiomycota*). *BMC Evol. Biol.* **2008**, *8*, 50. [CrossRef]
36. Bizio, E.; Ferisin, G.; Dovana, F. Note Sul Campo Di Variabilita Di *Inocybe*. *Riv. Micol.* **2017**, *60*, 59–70.
37. Bandini, D.; Vauras, J.; Weholt, Ø.; Oertel, B.; Eberhardt, U. *Inocybe woglindeana*, a New Species of the Genus *Inocybe*, Thriving in Exposed Habitats with Calcareous Sandy Soil. *Karstenia* **2020**, *58*, 41–59. [CrossRef]
38. Horak, E.; Matheny, P.B.; Desjardin, D.E.; Soytong, K. The Genus *Inocybe* (Inocybaceae, Agaricales, Basidiomycota) in Thailand and Malaysia. *Phytotaxa* **2015**, *230*, 201. [CrossRef]
39. Kropp, B.R.; Matheny, P.B.; Nanagyulyan, S.G. Phylogenetic Taxonomy of the *Inocybe splendens* Group and Evolution of Supersection "Marginatae". *Mycologia* **2010**, *102*, 560–573. [CrossRef]
40. Matheny, P.B.; Aime, M.C.; Smith, M.E.; Henkel, T.W. New Species and Reports of *Inocybe* (Agaricales) from Guyana. *Kurtziana* **2012**, *37*, 23–39.
41. Matheny, P.B.; Aime, M.C.; Bougher, N.L.; Buyck, B.; Desjardin, D.E.; Horak, E.; Kropp, B.R.; Lodge, D.J.; Soytong, K.; Trappe, J.M.; et al. Out of the Palaeotropics? Historical Biogeography and Diversification of the Cosmopolitan Ectomycorrhizal Mushroom Family Inocybaceae. *J. Biogeogr.* **2009**, *36*, 577–592. [CrossRef]
42. Peintner, U.; Bougher, N.L.; Castellano, M.A.; Moncalvo, J.M.; Moser, M.M.; Trappe, J.M.; Vilgalys, R. Multiple Origins of Sequestrate Fungi Related to Cortinarius (Cortinariaceae). *Am. J. Bot.* **2001**, *88*, 2168–2179. [CrossRef]
43. Osmundson, T.W.; Robert, V.A.; Schoch, C.L.; Baker, L.J.; Smith, A.; Robich, G.; Mizzan, L.; Garbelotto, M.M. Filling Gaps in Biodiversity Knowledge for Macrofungi: Contributions and Assessment of an Herbarium Collection DNA Barcode Sequencing Project. *PLoS ONE* **2013**, *8*, e62419. [CrossRef]
44. Yoo, S.; Cho, Y.; Kim, J.S.; Kim, M.; Lim, Y.W. Fourteen Unrecorded Species of Agaricales Underw. (Agaricomycetes, Basidiomycota) from the Republic of Korea. *Mycobiology* **2022**, *50*, 219–230. [CrossRef]
45. Kropp, B.R.; Matheny, P.B.; Hutchison, L.J. *Inocybe* Section *Rimosae* in Utah: Phylogenetic Affinities and New Species. *Mycologia* **2013**, *105*, 728–747. [CrossRef]
46. Brandon Matheny, P.; Wang, Z.; Binder, M.; Curtis, J.M.; Lim, Y.W.; Henrik Nilsson, R.; Hughes, K.W.; Hofstetter, V.; Ammirati, J.F.; Schoch, C.L.; et al. Contributions of Rpb2 and Tef1 to the Phylogeny of Mushrooms and Allies (Basidiomycota, Fungi). *Mol. Phylogenet. Evol.* **2007**, *43*, 430–451. [CrossRef]
47. Pradeep, C.K.; Vrinda, K.B.; Varghese, S.P.; Korotkin, H.B.; Matheny, P.B. New and Noteworthy Species of *Inocybe* (Agaricales) from Tropical India. *Mycol. Prog.* **2016**, *15*, 24. [CrossRef]
48. Latha, K.P.D.; Manimohan, P.; Matheny, P.B. A New Species of *Inocybe* Representing the *Nothocybe* Lineage. *Phytotaxa* **2016**, *267*, 40. [CrossRef]
49. Matheny, P.B.; Curtis, J.M.; Hofstetter, V.; Aime, M.C.; Moncalvo, J.M.; Ge, Z.W.; Yang, Z.L.; Slot, J.C.; Ammirati, J.F.; Baroni, T.J.; et al. Major Clades of Agaricales: A Multilocus Phylogenetic Overview. *Mycologia* **2006**, *98*, 982–995. [CrossRef] [PubMed]
50. Alvarado, P.; Manjón, J.L.; Matheny, P.B.; Esteve-Raventós, F. *Tubariomyces*, a New Genus of Inocybaceae from the Mediterranean Region. *Mycologia* **2010**, *102*, 1389–1397. [CrossRef] [PubMed]
51. Trifinopoulos, J.; Nguyen, L.-T.; von Haeseler, A.; Minh, B.Q. W-IQ-TREE: A Fast Online Phylogenetic Tool for Maximum Likelihood Analysis. *Nucleic Acids Res.* **2016**, *44*, W232–W235. [CrossRef]
52. Nguyen, L.-T.; Schmidt, H.A.; von Haeseler, A.; Minh, B.Q. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [CrossRef]
53. Huelsenbeck, J.P.; Ronquist, F. MRBAYES: Bayesian Inference of Phylogenetic Trees. *Bioinformatics* **2001**, *17*, 754–755. [CrossRef]
54. Letunic, I.; Bork, P. Interactive Tree of Life (ITOL) v5: An Online Tool for Phylogenetic Tree Display and Annotation. *Nucleic Acids Res.* **2021**, *49*, W293–W296. [CrossRef]
55. Kuyper, T.W. *A Revision of the Genus Inocybe in Europe I. Subgenus Inosperma and the Smooth-Spored Species of Subgenus Inocybe*; Persoonia-Supplement; Naturalis Biodiversity Center: Leiden, The Netherlands, 1986; Volume 3, ISBN 9071236021.
56. Stangl, J. *Die Gattung Inocybe in Bayern*; Hoppea: Regensburg, Germany, 1989; Volume 46, ISBN 0247900044.
57. Grund, D.W.; Stuntz, D.E. Nova Scotian Inocybes. I. *Mycologia* **1968**, *60*, 406–425. [CrossRef]
58. Carteret, X.; Reumaux, P. Miettes Sur Les Inocybes (6ème Série), Études de Quelques Nains des Feuillus de La Plaine, Accompagnée d' Une Clé de Détermination Des Taxons de La Section Lilacinae R. Heim. *Bull. Soc. Mycol. Fr.* **2012**, *127*, 1–53.
59. Velenovský, J. *České Houby 1–5*; České Botanické Společnosti: Prague, Czech Republic, 1920.
60. Kuyper, T.W. Studies in *Inocybe* I.—Revision of the New Taxa of *Inocybe* Described by Velenovský. *Persoonia* **1985**, *12*, 375–400.
61. Ferrari, E. Inocybe Dai Litorali Alla Zona Alpina. In *Fungi non Delineati 54/55*; Edizioni Candusso: Alassio, Italy, 2010.

62. Konrad, P.A. Notes Critiques Sur Quelques Champignons Du Jura. *Bull. Soc. Mycol. Fr.* **1929**, *45*, 375–400.
63. Alessio, C.L.; Rebaudengo, E. *Inocybe. Iconographia Mycologica*; Suppl. 3; Museo Tridentino di Scienze Naturali: Trento, Italy, 1980; Volume 29.

PUBLIKACIJA III

An Integrative Taxonomic Study of *Parasola* (*Psathyrellaceae*, *Fungi*) Reveals a New Saprotrophic Species from European Temperate Deciduous Forests

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An Integrative Taxonomic Study of *Parasola* (*Psathyrellaceae*, *Fungi*) Reveals a New Saprotrophic Species from European Temperate Deciduous Forests

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Abstract: Seventeen collections of the genus *Parasola* from Croatia were studied with integrative taxonomic methods. *Parasola papillatospora* is described as a new species, based on morphology and multigene phylogenetic analyses. Its basidiomata were growing on soil in temperate deciduous forests (*Quercus petraea*, *Fagus sylvatica*, and *Carpinus betulus*) on two different localities in NW Croatia. Based on publicly available molecular data, the species is also recorded in Hungary. The most distinctive morphological features of the new species are the characteristics of its basidiospores, (1) the papillate apex and (2) central germ pore (both present in most spores), as well as (3) a highly variable shape. A morphological description of *P. papillatospora* is accompanied by colour photographs of basidiomata, basidiospores, and cystidia. In this study, a total of 64 DNA sequences from 17 specimens belonging to 10 *Parasola* species were newly generated. As a result of Bayesian Inference and Maximum Likelihood phylogenetic analyses of the concatenated ITS, LSU, *tef-1α*, and *β-tub* gene alignment of *Parasola* species, *P. papillatospora* was resolved as an independent clade, a sister to the clade comprising the *P. plicatilis* species complex. Eight *Parasola* species (*P. auricoma*, *P. crataegi*, *P. cuniculorum*, *P. kuehneri*, *P. malakandensis*, *P. megasperma*, *P. nudiceps*, *P. plicatilis-similis*) are reported for the first time for Croatia and *P. malakandensis* also for Europe. Colour photographs of basidiomata are provided for all *Parasola* species new to Croatia except *P. kuehneri*.

Keywords: 1 new taxon; *Agaricales*; *Basidiomycota*; biodiversity; biogeography; molecular phylogeny

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1. Introduction

The traditional polyphyletic concept of the genus *Coprinus* Pers. *sensu lato* was redefined by Redhead et al. in 2001 [1] based on molecular evidence [2–4]. The newly proposed taxonomic concept comprised the genus *Coprinus* in the narrow sense including *C. comatus* (O.F. Müll.) Pers. and a few related taxa nested in a monophyletic clade within the family *Agaricaceae* Chevall. Other coprinoid species, more closely related to *Psathyrella* (Fr.) Quél., were transferred to the newly proposed family *Psathyrellaceae* Vilgalys, Moncalvo & Redhead [1] and arranged into the genera *Parasola* Redhead, Vilgalys & Hopple, *Coprinellus* P. Karst., and *Coprinopsis* P. Karst. The genus *Parasola* comprised all species from the subsections *Glabri* and *Auricomi* of the former genus *Coprinus* s.l. These are characterized by a delicate, non-deliquescent, coprinoid basidiomata (dry, plicate–sulcate pileus and dark basidiospores), and by the absence of the veil and caulocystidia. Based on morphology, Uljé in 2005 [5] distinguished 10 species from Europe in this group. Nagy et al. in 2010 [6] studied all available type material of *Parasola* and related taxa worldwide and recognized 10 species in the genus, mostly confirmed with molecular data as well. In addition, a psathyrelloid species (with smooth pileus), *Psathyrella conopilea* (Fr.) A. Pearson & Dennis, was transferred to *Parasola* by Larsson and Örstadius [7] as a result of a molecular

phylogenetic analysis. In the following years, extensive taxonomic research on *Parasola* (based on biological material or protologue information) led to the description of as many as 18 new species [8–16]. Furthermore, based on molecular and morphological analyses, Malysheva et al. in 2019 [17] transferred *Galeropsis aporos* Courtec. to *Parasola*.

Our taxonomic research on coprinoid fungi in Croatia has already led to the publication of a new species, *Coprinopsis cerkezii* Tkalčec, Mešić, I. Kušan & Matočec [18]. Mešić and Tkalčec [19] presented an annotated checklist of all species of the former family *Coprinaceae* Overeem & Weese from Croatia, which included all coprinoid and psathyrelloid fungi. It was based on all published sources and unpublished records before 2000 and included only three *Parasola* species: *P. conopilea* (Fr.) Örstadius & E. Larss., *P. plicatilis* (Curtis) Redhead, Vilgalys & Hopple, and *P. misera* (P. Karst.) Redhead, Vilgalys & Hopple. Afterwards, the only species of *Parasola* published for Croatia was *P. lactea* (A.H. Sm.) Redhead, Vilgalys & Hopple (=*P. leiocephala* (P.D. Orton) Redhead, Vilgalys & Hopple) [20].

Seventeen collections of the saprotrophic genus *Parasola* from Croatia were studied with an integrative taxonomic approach, which led to the identification of 10 species. *Parasola papillatospora* sp. nov. from European temperate deciduous forests is described based on four-gene molecular phylogenetic and morphological analyses. Moreover, eight *Parasola* species are recorded for the first time for Croatia and a single species is recorded as new to Europe.

2. Materials and Methods

2.1. Fieldwork, Sampling, and Morphological Study

Seventeen specimens of the genus *Parasola* were collected throughout Croatia from 2004 to 2022. The methods of sampling and morphological examination were similar in all collections, as follows. The basidiomata were photographed on site with a Canon digital camera (EOS 30D, 50D, or 5D; Canon Europe, Uxbridge, UK) equipped with a Canon MR-14EX macro ring flash, collected, macromorphologically described, and preserved by drying. All collections were deposited in the Croatian National Fungarium (CNF), Zagreb, Croatia. The description of *P. papillatospora* is based on six collections consisting of 18 basidiomata. In a macroscopic description, L denotes the number of entire lamellae and l denotes the number of lamellulae between each pair of entire lamellae. Microscopic characters were observed using an Olympus BX51 optical microscope (Olympus, Hamburg, Germany) in the brightfield technique under a magnification up to 1500× and photographed with a Canon EOS M50 digital camera. The description and images of microscopic characters were obtained from rehydrated pieces of specimens mounted in 2.5% or 5% potassium hydroxide (KOH), except for basidiospores, which were observed in 3% ammonium hydroxide (NH_4OH). Basidiospores from photographs of lamellae mounts were randomly selected and measured using Motic Images Plus 2.0 software (Motic Europe, Barcelona, Spain). The length/breadth ratio of basidiospores in the frontal view is given as a Q_f value and the length/width ratio of basidiospores in the side view is given as a Q_s value. Average basidiospore lengths, widths, and Q values are shown in italics. The numbers in square brackets [X/Y/Z] denote X elements measured in Y basidiomata from Z collections.

2.2. DNA Extraction, PCR Amplification, and Sequencing

Genomic DNA was extracted from dried specimens of *Parasola* using the EZNA® HP Fungal DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's protocol. Four gene regions were sequenced and analyzed in this study: two nuclear gene regions, ITS (internal transcribed spacer region) and LSU (28S large subunit of ribosomal DNA), and two protein coding regions, *tef-1α* (translation elongation factor 1-alpha) and *β-tub* (beta-tubulin). For PCR amplification and sequencing of ITS and LSU, primer pairs ITS1F/ITS4 [21,22] and LR0R/LR5 [23] were used, respectively. The primer pair EF1-983F/EF1-2218R, with the addition of 1567R and 1577F [24,25], was used for PCR amplification of the *tef-1α* gene region. The *Psathyrellaceae*-specific primer pair B36F-PSA/B12R-PSA [26] was used to amplify the *β-tub* gene region. PCR amplification for

ITS and LSU gene regions was performed using a touchdown program: initial denaturation at 95 °C for 2 min, followed by 5 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 45 s (add −1 °C per cycle), and extension at 72 °C for 1.5 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 45 s, and extension at 72 °C for 1.5 min; and a final extension at 72 °C for 5 min. PCR amplification of *tef-1α* was performed as described by Rehner and Buckley [25], with modification of the maximum annealing temperature to 64 °C. The *β-tub* gene region was amplified as described by Nagy et al. [26], with the number of amplification cycles increased to 35.

Successful PCR products were purified using an ExoSAP-IT™ (Thermo Fisher Scientific, Waltham, MA, USA) reagent according to the manufacturer's protocol and sent to Macrogen Europe (Amsterdam, the Netherlands) for bidirectional Sanger sequencing.

2.3. Sequence Alignment and Phylogenetic Analysis

Sequence reads were assembled and edited using Geneious Prime 2023.0.4. (https://www.geneious.com, accessed on 31 January 2023, Biomatters, Auckland, New Zealand) and obtained sequences were deposited in the National Center for Biotechnology Information (NCBI) GenBank database.

The phylogenetic dataset comprising 179 sequences of four gene regions from 31 taxa was selected for further analyses (Table 1). Sequences were aligned by each locus using MAFFT v7.450 [27,28] available as a Geneious Prime plugin. After being aligned and trimmed, concatenation of ITS, LSU, *tef-1α*, and *β-tub* was performed using Geneious Prime 2023.0.4. Concatenated alignment contained 3034 characters including gaps, with 675 characters for ITS, 888 characters for LSU, 1006 characters for *tef-1α*, and 465 characters for *β-tub*. Four *Coprinopsis* species (*C. picacea*, *C. lagopus*, *C. marcescibilis*, and *C. pseudonivea*) were selected as the outgroup for phylogenetic analyses following Szarkandi et al. [10].

Phylogenetic analyses were conducted using the Maximum Likelihood (ML) method in IQTREE v1.6.12 [29,30] and a Bayesian Inference (BI) method in MrBayes 3.2.6 (Geneious plugin, [31]). The best model was selected by ModelFinder implemented in IQ-TREE, separately considering the corrected Akaike, and the Bayesian Information Criterion (cAIC, BIC). GTR+F+I+G4 was selected as the best model for both phylogenetic datasets. ML analyses were executed by applying the ultrafast bootstrap approximation with 1000 replicates. BI analyses were executed for 10,000,000 generations, sampling trees and other parameters every 10,000 generations. The default number of chains (four) and heating parameters were used. Posterior probabilities (BPP) were calculated after burning the first 25% of the posterior sample. Phylogenetic trees were visualized and annotated using iTOL v6.5.4 [32] and FigTree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/, accessed on 10 February 2023). Alignments and phylogenetic trees generated from BI and ML analyses are available at DOI 10.5281/zenodo.8099476.

Table 1. Species included in this study, associated countries of origin, voucher numbers, and GenBank accession numbers. Newly generated sequences are marked in bold.

Taxon	Country	Voucher	ITS	LSU	*tef-1α*	*β-tub*	Ref.
Coprinopsis lagopus	Hungary	NL-2143	FM163179	FM160730	—	—	[33]
Coprinopsis marcescibilis	Hungary	NL-2140	FM878020	FM876277	—	—	[34]
Coprinopsis picacea	Hungary	NL-0174	FN396115	FN396166	—	—	[26]
Coprinopsis pseudonivea	Hungary	NL-2340	FM163181	FM160728	—	—	[33]
Parasola aporos	France	RC-F92.191 holotype	MK397584	MK397604	—	—	[17]
Parasola aporos	France	CL-F09.005	MK397586	MK397606	—	—	[17]
Parasola auricoma	Hungary	NL-0087	JN943107	JQ045871	FM897236	FN396252	[35]
Parasola auricoma	**Croatia**	**CNF 1/4718**	**OQ842767**	**OQ842768**	**OQ850152**	**OQ850168**	**This study**
Parasola auricoma	**Croatia**	**CNF 1/4618**	**OQ845889**	**OQ845835**	**OQ850153**	**OQ850169**	**This study**

Table 1. *Cont.*

Taxon	Country	Voucher	ITS	LSU	*tef-1α*	*β-tub*	Ref.
Parasola conopilea	The Netherlands	CBS 325.39	MH856033	MH867531	—	—	[36]
Parasola conopilea	**Croatia**	**CNF 1/5310**	**OQ845887**	**OQ845888**	**OQ850154**	**OQ850170**	**This study**
Parasola conopilea	**Croatia**	**CNF 1/5735**	**OQ845890**	**OQ843455**	**OQ850155**	**OQ850171**	**This study**
Parasola crataegi	Germany	SSt08-154 holotype	KY928605	—	—	—	[10]
Parasola crataegi	Hungary	NL-4175 paratype	KY928603	KY928631	—	—	[10]
Parasola crataegi	**Croatia**	**CNF 1/8905**	**OQ852892**	—	—	—	**This study**
Parasola cuniculorum	United Kingdom	K(M)191984 holotype	OL630105	—	—	—	[16]
Parasola cuniculorum	**Croatia**	**CNF 1/5143**	**OQ848756**	**OQ848757**	**OQ850156**	**OQ850172**	**This study**
Parasola galericuliformis	Hungary	NL-6601	FM163187	FM160722	—	—	[33]
Parasola galericuliformis	Sweden	NL-0095	FM163188	FM160721	—	—	[33]
Parasola glabra	Pakistan	LAH-SHP-5 holotype	KY461717	KY621806	KY461735	—	[12]
Parasola glabra	Pakistan	HUP-SHP-23 paratype	KY461718	KY621805	—	—	[12]
Parasola hercules	The Netherlands	Uljé 10.8.1984 (L146) holotype	HQ847027	HQ847112	—	—	[37]
Parasola kuehneri	The Netherlands	Uljé 31.5.1987 holotype	KY928608	KY928633	—	—	[10]
Parasola kuehneri	The Netherlands	Uljé 1241 (L133)	HQ847026	HQ847111	—	—	[37]
Parasola kuehneri	**Croatia**	**CNF 1/4334**	**OQ849153**	**OQ849154**	**OQ850157**	**OQ850173**	**This study**
Parasola lactea	Hungary	NL-0283	JN943113	JQ045887	FM897239	FN396248	[35]
Parasola lactea	Hungary	NL-0288	JN943106	JQ045872	FM897233	FN396250	[35]
Parasola lilatincta	Hungary	NL-0468a	FM163200	FM160709	—	—	[33]
Parasola lilatincta	Hungary	NL-0281	FM163197	FM160712	—	—	[33]
Parasola lilatincta	Hungary	NL-0296	FM163196	FM160713	—	—	[33]
Parasola lilatinctoides	Pakistan	LAH-SHP-8 holotype	KY461722	KY461725	KY461731	—	[12]
Parasola litoralis	Cyprus	K(M)264814 holotype	OL630108	—	—	—	[16]
Parasola litoralis	Cyprus	DJS20130125001 paratype	OL630107	—	—	—	[16]
Parasola malakandensis	Pakistan	HUP 17501 holotype	KP738713	KU599829	KU599831	—	[9]
Parasola malakandensis	Pakistan	LAH-SHP-17 paratype	KU599827	KU599830	KU599832	—	[9]
Parasola malakandensis	**Croatia**	**CNF 1/8698**	**OQ849158**	**OQ849167**	**OQ850158**	**OQ850174**	**This study**
Parasola megasperma	United Kingdom	E:Orton 4132 holotype	OL630101	—	OL630935	—	[16]
Parasola megasperma	The Netherlands	Ulje 1275	KY928618	KY928637	—	—	[10]
Parasola megasperma	**Croatia**	**CNF 1/5704**	**OQ849166**	**OQ849224**	**OQ850159**	**OQ850175**	**This study**
Parasola misera	Hungary	NL-0280 neotype	FM163210	FM160699	—	—	[33]
Parasola misera	Hungary	NL-0677	FM163211	FM160698	FM897240	FN396249	[26,33]

Table 1. *Cont.*

Taxon	Country	Voucher	ITS	LSU	*tef-1α*	*β-tub*	Ref.
Parasola nudiceps	United Kingdom	E:Orton 4133 holotype	OL630102	—	—	—	[16]
Parasola nudiceps	Germany	HB19870911A	MK063783	—	—	—	[16]
Parasola nudiceps	**Croatia**	**CNF 1/4804**	**OQ849230**	**OQ849229**	**OQ850160**	**OQ850176**	**This study**
Parasola nudiceps (as ***P.*** *ochracea)*	Norway	NL-3621, holotype of *P. ochracea*	JN943134	JQ045875	—	—	[38]
Parasola nudiceps (as ***P.*** *ochracea)*	Sweden	NL-3167, paratype of *P. ochracea*	JN943136	JQ045865	—	—	[38]
***Parasola papillatospora* sp. nov.**	**Croatia**	**CNF 1/3473**	**OQ862758**	**OQ862756**	**OQ850161**	—	**This study**
***Parasola papillatospora* sp. nov.**	**Croatia**	**CNF 1/5428**	**OQ862789**	**OQ862577**	**OQ850162**	**OQ850177**	**This study**
***Parasola papillatospora* sp. nov.**	**Croatia**	**CNF 1/7600**	**OQ862790**	**OQ862578**	**OQ850163**	**OQ850178**	**This study**
***Parasola papillatospora* sp. nov.**	**Croatia**	**CNF 1/7858 holotype**	**OQ862770**	**OQ862755**	**OQ850164**	**OQ850179**	**This study**
***Parasola papillatospora* sp. nov.**	**Croatia**	**CNF 1/7861**	**OQ862757**	**OQ862771**	**OQ850165**	**OQ850182**	**This study**
***Parasola papillatospora* sp. nov.**	**Croatia**	**CNF 1/7902**	**OQ862788**	**OQ862772**	**OQ850167**	**OQ850181**	**This study**
Parasola papillatospora sp. nov.	Hungary	SZMC-NL-2952	HQ847028	HQ847113	—	—	[10]
Parasola parvula	India	CAL 1667 holotype	MH379796	MH393599	—	—	[11]
Parasola plicatilis	Hungary	NL-0075 epitype	FM163214	FM160695	—	—	[33]
Parasola plicatilis	Hungary	NL-0284	FM163189	FM160720	FM897235	FN396251	[26,33]
Parasola plicatilis aff.	China	HMJAU46405	OL355167	OL376339	—	—	[39]
Parasola plicatilis-similis	Sweden	NL-2125 holotype	KY928620	—	—	—	[10]
Parasola plicatilis-similis	Sweden	NL-0287 paratype	FM163218	FM160691	—	FN396245	[26,33,35]
Parasola plicatilis-similis	**Croatia**	**CNF 1/5484**	**OQ850018**	**OQ850017**	**OQ850166**	**OQ850180**	**This study**
Parasola psathyrelloides	India	CAL 1753 holotype	MK682756	MK682754	—	—	[13]
Parasola psathyrelloides	India	AMH 10119 paratype	MK682752	MK682759	—	—	[13]
Parasola pseudolactea	Pakistan	HUP-SU-412 holotype	KY461719	KY621799	KY461733	—	[12]
Parasola pseudolactea	Pakistan	HUP-SU-413 paratype	KY461720	KY621800	KY461734	—	[12]
Parasola schroeteri	Germany	Dähncke 1502	KY928616	KY928635	—	—	[10]
Parasola schroeteri	The Netherlands	Brier 10.5.1999	FM163219	FM160690	—	—	[33]
Parasola schroeteri	The Netherlands	Uljé 1067	KY928627	—	—	—	[10]
Parasola schroeteri	The Netherlands	Vellinga 1140	KY928629	KY928645	—	—	[10]
Parasola setulosa	Hungary	Maruyama 14.7.1999/ L32	HQ847030	HQ847115	—	—	[37]
Parasola setulosa	China	HMJAU46367	MW822929	OL376319	—	—	[39]

3. Results

3.1. Molecular Phylogenetic Analyses

In this study, a total of 64 DNA sequences (17 ITS, 16 LSU, 16 *tef-1α*, and 15 *β-tub*) from 17 collections were newly generated. Six collections were identified as *P. papillatospora* sp. nov. (CNF 1/3473, 1/5428, 1/7600, 1/7858, 1/7861, 1/7902), two as *P. auricoma* (Pat.) Redhead, Vilgalys & Hopple (CNF 1/4618 and 1/4718) and *P. conopilea* (CNF 1/5310 and 1/5735), and one each as *P. crataegi* Schmidt-Stohn (CNF 1/8905), *P. cuniculorum* D.J. Schaf. (CNF 1/5143), *P. kuehneri* (Uljé & Bas) Redhead, Vilgalys & Hopple (CNF 1/4334), *P. malakandensis* S. Hussain, Afshan & H. Ahmad (CNF 1/8698), *P. megasperma* (P.D. Orton) Redhead, Vilgalys & Hopple (CNF 1/5704), *P. nudiceps* (P.D. Orton) Redhead, Vilgalys & Hopple (CNF 1/4804), and *P. plicatilis-similis* L. Nagy, Szarkándi & Dima (CNF 1/5484). The accession numbers of all newly generated sequences are presented in bold in Table 1.

All six Croatian collections of *P. papillatospora* showed a strong genetic homogeneity with a 99.86–100% identity in ITS, 98.96–100% in LSU, 100% in *tef-1α*, and 100% in the *β-tub* gene region. Based on GenBank nucleotide BLAST results, the Croatian collections of *P. papillatospora* showed a high percentage identity (99.39–99.54% in ITS and 99.84–99.88% in LSU) with the previously sequenced sample from Hungary (SZMC-NL-2952, acc. no. HQ847028, HQ847113, *Parasola* sp. 1. in Szarkandi et al. [10]). The next closest hit using the ITS sequence of the holotype of *P. papillatospora* had a similarity of 95.13% to the collection from China identified as *P. plicatilis* (HMJAU46405, acc. no. OL355167, [39]). The identity between the ITS sequences of the Chinese collection and the epitype of *P. plicatilis* (NL-0075, acc. no. FM163214) was 94.85%. The results of BI and ML phylogenetic analyses of the concatenated alignment of *Parasola* species (Figure 1) showed that the Hungarian sample (SZMC-NL-2952) nested together with six Croatian *P. papillatospora* collections in an independent strongly supported clade (BI-PP = 1, ML-BP = 100), a sister to the clade of the *P. plicatilis* species complex (BI-PP = 1, ML-BP = 100). The Chinese collection *P. plicatilis* aff. (HMJAU46405) formed a well-supported clade (BI-PP = 1, ML-BP = 100) with Hungarian collections, the epitype of *P. plicatilis* (NL-0075), and *P. plicatilis* (NL-0284).

Two Croatian collections of *P. conopilea* (CNF 1/5310 and 1/5735) clustered together with the *P. conopilea* collection (CBS 325.39) from the Netherlands and formed a strongly supported (BI-PP = 1, ML-BP = 99) joint clade with its sister species *P. psathyrelloides* K.G.G. Ganga & Manim. Croatian collections of *P. auricoma* (CNF 1/4618 and 1/4718) and the Hungarian collection of *P. auricoma* (NL-0087) were recovered in a monophyletic clade (BI-PP = 1, ML-BP = 99) with its closely related species *P. setulosa* (Berk. & Broome) Redhead, Vilgalys & Hopple. Collection of *P. malakandensis* (CNF 1/8698) from Croatia was resolved in a strongly supported (BI-PP = 1, ML-BP = 100) clade with two *P. malakandensis* collections from Pakistan (HUP 17501 (holotype) and LAH-SHP-17). Collections of *P. papillatospora*, *P. crataegi*, *P. cuniculorum*, *P. kuehneri*, *P. megasperma*, *P. nudiceps*, and *P. plicatilis-similis* from Croatia (CNF samples) were recovered with the remaining *Parasola* collections from the phylogenetic dataset into a single large clade (*Parasola* section in Szarkandi et al. [10]) with maximum support (BI-PP = 1, ML-BP = 100) (Figure 1).

3.2. Taxonomy

Parasola papillatospora Tkalčec, Mešić, Pošta, I. Kušan, Čerkez, sp. nov. (Figures 2 and 3)
Mycobank MB848624

Typification: Croatia, City of Zagreb: The Cmrok park area (near the Dubravkin put street), 207 m a.s.l., 45.83214° N, 15.97409° E, on soil in deciduous forest dominated by *Quercus petraea*, *Fagus sylvatica*, and *Carpinus betulus*, *leg.* M. Čerkez, 22 September 2009, holotype CNF 1/7858. GenBank (ex-holotype DNA isolate): ITS = OQ862770, LSU = OQ862755, *tef-1α* = OQ850164, *β-tub* = OQ850179.

Etymology: Referring to the basidiospores with developed apical papilla.

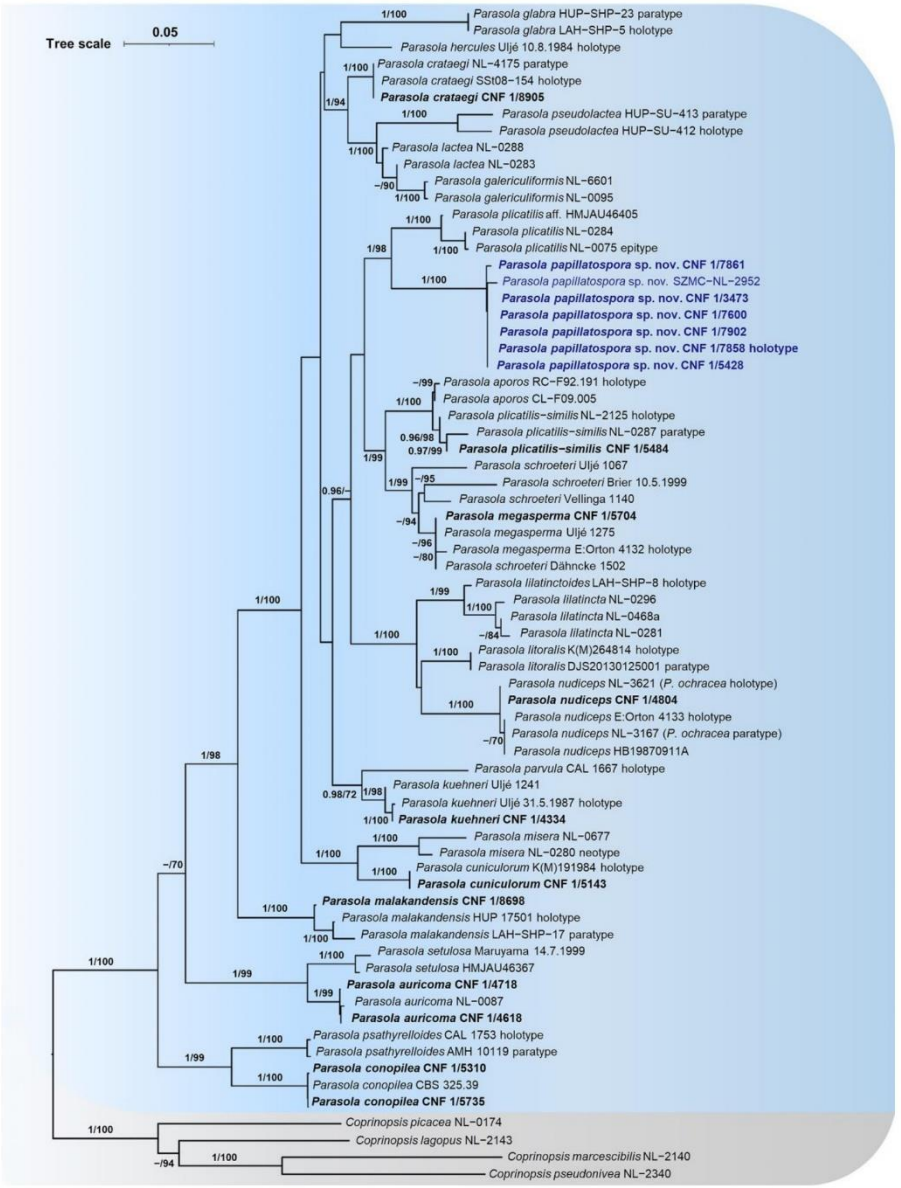

Figure 1. Phylogenetic tree of *Parasola* species based on Bayesian Inference (BI) and Maximum Likelihood (ML) analyses of the concatenated four-gene (ITS, LSU, *tef-1α*, *β-tub*) sequence alignment. Significant branch support values, Bayesian posterior probability (BI-PP $\geq$ 0.95), and ultrafast bootstrap support (ML-BP $\geq$ 70%) are presented at the nodes. Collections with newly generated sequences are marked in bold and the newly proposed species *P. papillatospora* is marked in a blue color.

Description: Pileus 12–20 mm wide, subcylindrical when young, later expanding, but with flattened center and outer part directed downwards for a prolonged time, finally hemispherical, convex or broadly conical, with truncate, often ± depressed center, surface dry, matte, densely radially plicate-sulcate (ca. (60–)75–90% of radius) except in smooth central zone, without a veil, hygrophanous, when moist pale to medium brown or orange(ish)-brown at first, becoming light grey in its external (sulcate) zone at maturity, when dry whitish to cream, sometimes brownish at center, often greyish to light brown–grey in its external (sulcate) zone at maturity. Lamellae free, rather distant to medium spaced, L = 32–36, l = 1–3, white or whitish at first, then grey, dark grey-brown or almost black, with whitish, entire edge, non-deliquescent. Stipe 44–73 × 1.2–2.3 mm, central, ± cylindrical or gradually slightly broadening downwards, mostly with slightly to distinctly broadened base, dry, hollow, white to light brown, mostly glabrous in upper half (finely silky fibrillose under magnifying glass), often finely and sparsely flocculose in lower half or near the base. Context thin, fragile, whitish in pileus, whitish to brownish in stipe. Smell and taste not recorded.

Basidiospores [700/7/5] (7.0–)7.4–*8.5*–10(–10.8) × (5.8–)6.2–*7.4*–8.7(–9.8) × (4.4–)4.6–*5.2*–6.1(–6.5) µm, averages of different basidiomata 8.2–8.8 × 7.0–7.6 × 5.1–5.3 µm, Q_f = (0.92–)1.00–*1.17*–1.40(–1.50), Q_s = (1.43–)1.47–*1.63*–1.81(–1.96), av. Q_f = 1.14–1.18, av. Q_s = 1.58–1.68, strongly flattened, highly variable in shape (within the same basidioma) in frontal view, broadly to elongated (sub)limoniform, subpyriform, broadly ovoid to ovoid, lacrymoid, rounded 3-(heart-shaped) to 6-(sub)angular, broadly fusiform, globose, subglobose or broadly ellipsoid to ellipsoid, mostly with small to strongly developed apical papilla, or with convex to acute apex, and with convex, flattened, subconical, or conical base, amygdaliform to ellipsoid with rounded to conical base and rounded to ± narrowed apex in side view, often with well-developed and pigmented basal part of apiculus, smooth, moderately thick-walled (up to 1 µm), with distinct, central (in ca. 60% to >90% of spores, depending on collection) or slightly to moderately eccentric germ pore (inner diameter up to 1.6 µm wide, outer diameter up to 2.2 µm wide), red-brown to very dark red-brown in H_2O and NH_4OH, grey-brown to very dark grey-brown in KOH. Basidia 15–45 × 6–11 µm, mostly narrowly clavate to clavate, sometimes constricted in the upper part, mostly 4-spored, not uncommonly 3- or 2-spored in some collections, thin-walled, hyaline, mostly with granular contents, surrounded by (3–)4–6 hyaline, thin-walled, 7–25 µm broad hymenophysalides (pseudoparaphyses). Cheilocystidia 15–50(–70) × 12–35 µm, crowded, forming a sterile lamellar edge, clavate to broadly clavate (predominant towards the pileus margin), ovoid, ellipsoid, subglobose, rounded (sub)fusiform, (sub)utriform or broadly conical with rounded apex (the latter three shapes are the most common towards the stipe), hyaline, mostly thin-walled (up to 0.5 µm), sometimes moderately thick-walled (up to 1 µm). Pleurocystidia 45–75 × 17–25 µm, scattered, sometimes very scarce, (sub)utriform, oblong, ellipsoid or conical with rounded apex, mostly with pedicel, hyaline, thin-walled to moderately thick-walled. Pileipellis, a hymeniderm, composed of mostly clavate to broadly clavate, sometimes also subglobose, spheropedunculate, ellipsoid or ovoid, thin-walled, hyaline cells, 22–65 × 12–32 µm in size. Pileocystidia and caulocystidia absent. Clamp connections present, best developed and rather abundant in trama hyphae.

Distribution and Ecology: Known from six collections on two localities in NW Croatia, the City of Zagreb, and the Žumberak mountain in Zagreb County. Based on a high similarity of ITS sequences (99.39–99.54%) in the GenBank BLAST search between the collection SZMC-NL-2952 (GenBank, acc. no. HQ847028) and Croatian collections, the species was also found in Hungary. Basidiomata of all Croatian collections were growing on soil, from August to October, in temperate deciduous forests dominated by *Quercus petraea*, *Fagus sylvatica*, and *Carpinus betulus*. This part of Croatia has a continental climate with an average annual temperature of 9–11 °C and annual precipitation of 840–1100 mm. Unfortunately, at the holotype site in the City of Zagreb (Cmrok park forest) where five collections were gathered, intensive forestry works were subsequently carried out, which devastated the microhabitat.

Figure 2. *Parasola papillatospora* sp. nov. Basidiomata. (**A**,**B**) CNF 1/7858, holotype. (**C**,**D**) CNF 1/5428. (**E**) CNF 1/7600. (**F**,**G**) CNF 1/7902. (**H**,**I**) CNF 1/7861. Bars: (**A**,**C**,**E**,**G**,**H**) = 10 mm; (**B**,**D**,**F**,**I**) = 5 mm. Photos: M. Čerkez.

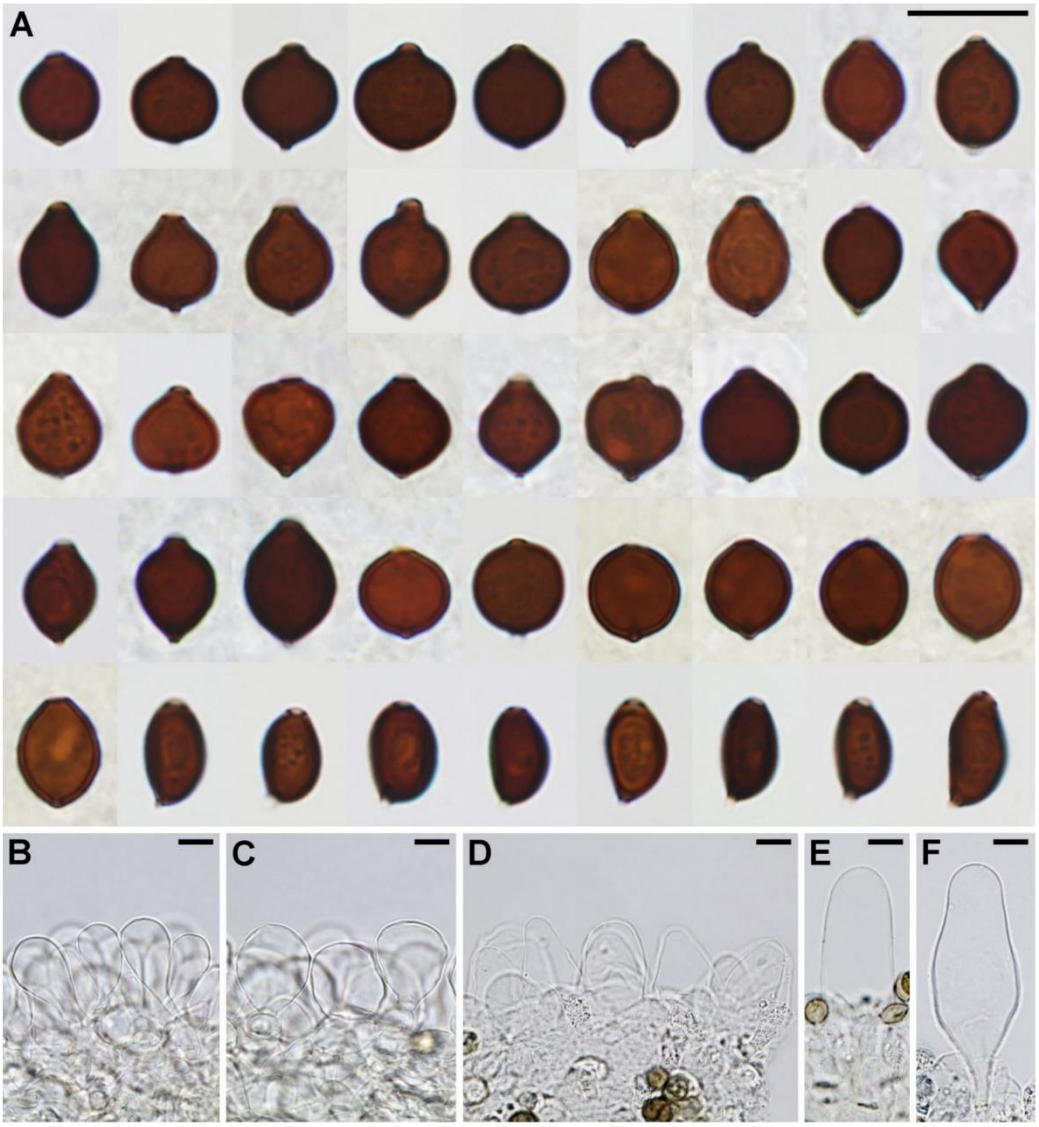

Figure 3. *Parasola papillatospora* sp. nov. (**A**) Basidiospores in NH_4OH. (**B**–**D**) Cheilocystidia. (**E**,**F**) Pleurocystidia. Bars = 10 µm. Photos: Z. Tkalčec and A. Mešić.

Additional material examined: Croatia, City of Zagreb: Cmrok park area (near the Dubravkin put street), on soil in deciduous forest dominated by *Quercus petraea*, *Fagus sylvatica*, and *Carpinus betulus*, *leg.* M. Čerkez, 207 m a.s.l., 45.83217° N, 15.97378° E, 30 September 2008, CNF 1/5428; 45.83214° N, 15.97409° E, 5 September 2009, CNF 1/7600; 45.83197° N, 15.97427° E, 23 September 2009, CNF 1/7861; 28 October 2009, CNF 1/7902; Zagreb County: Žumberak Mountain, vicinity of Novo Selo Okićko village, near Gornji

Gorički hamlet, 432 m a.s.l., 45.74826° N, 15.70050° E, on soil, near the edge of deciduous forest dominated by *Quercus petraea*, *Fagus sylvatica*, and *Carpinus betulus*, *leg.* M. Čerkez, 21 August 2004, CNF 1/3473.

3.3. Additional Data on Parasola from Croatia

An additional 11 Croatian collections of nine *Parasola* species were sequenced, used in phylogenetic analyses, and their morphological characters were examined to confirm the taxonomic identification [5,6,8–16,40,41]. Eight of the nine identified *Parasola* species (all except *P. conopilea*) were recorded for the first time for Croatia in this study, while *P. malakandensis* was also recorded for the first time for Europe (hitherto known only from Pakistan).

Additional materials examined: Parasola auricoma. Croatia, Zagreb County: Žumberak Mountain, vicinity of Kostanjevec Podvrški village, 240 m a.s.l., 45.83093° N, 15.58817° E, on grassy soil, courtyard near deciduous forest, *leg.* M. Čerkez, 14 July 2007, CNF 1/4618 (Figure 4B); 2 September 2007, CNF 1/4718 (Figure 4A).

Notes: The collections of *P. auricoma* from Croatia (CNF 1/4618 and 1/4718) and *P. auricoma* (NL-0087) from Hungary [35] clustered together in a monophyletic clade (BI-PP = 1, ML-BP = 99). The species most closely related to *P. auricoma* (CNF 1/4618 and 1/4718) clustered in a sister clade (BI-PP = 1, ML-BP = 99) was *P. setulosa* (Maruyama 14.7.1999 and HMJAU46367).

Parasola conopilea. Croatia, Šibenik-Knin County: Krka National Park, Skradinski buk area, 10 m a.s.l., 43.80478° N, 15.96353° E, on litter, forest of *Ostrya carpinifolia*, *leg.* Z. Tkalčec & A. Mešić, 14 December 2007, CNF 1/5310; Dubrovnik-Neretva County: island of Lokrum, 25 m a.s.l., 42.62533° N, 18.12239° E, on heap of litter, forest of *Quercus ilex* and *Pinus halepensis*, *leg.* Z. Tkalčec & A. Mešić, 12 November 2009, CNF 1/5735.

Notes: Croatian collections of *P. conopilea* (CNF 1/5310 and 1/5735) formed a monophyletic clade (BI-PP = 1, ML-BP = 100) with *P. conopilea* (CBS 325.39) from the Netherlands [36]. The species most closely related to *P. conopilea* (CNF 1/5310 and 1/5735) was *P. psathyrelloides* (CAL 1753, holotype; AMH 10119, paratype), forming a strongly supported sister clade (BI-PP = 1, ML-BP = 99).

Parasola crataegi. Croatia, Požega-Slavonia County: vicinity of the town of Pleternica, near the village of Gradac, 135 m a.s.l., 45.31658° N, 17.79921° E, on soil and litter of *Crataegus monogyna*, under *C. monogyna* on the edge of deciduous forest dominated by *Quercus petraea* and *Carpinus betulus*, *leg.* M. Čerkez, 4 October 2022, CNF 1/8905 (Figure 4C).

Notes: The collection of *P. crataegi* from Croatia formed a monophyletic clade with the holotype (SSt08-154) and the paratype (NL-4175) of *P. crataegi* with full support (BI-PP = 1, ML-BP = 100). In our phylogram, the *P. crataegi* clade was a sister to a clade comprising *P. pseudolactea* (HUP-SU-412, holotype; HUP-SU-413, paratype), *P. lactea* (NL-0288 and NL-0283), and *P. galericuliformis* (NL-6601 and NL-0095) with strong support (BI-PP = 1, ML-BP = 94).

Parasola cuniculorum. Croatia, Primorje-Gorski Kotar County: vicinity of the village of Skrad, near Rogi hamlet, 725 m a.s.l., 45.42022° N, 14.88305° E, on dung of *Cervus elaphus* (red deer), forest of *Abies alba* and *Fagus sylvatica*, *leg.* M. Čerkez, 10 April 2008, CNF 1/5143 (Figure 4D,E).

Notes: The collection of *P. cuniculorum* from Croatia formed a monophyletic clade (BI-PP = 1, ML-BP = 100) with the holotype of *P. cuniculorum* (K(M)191984) [16]. In our analysis, the most closely related species was *P. misera* (NL-0280, neotype; NL-0677), which formed a sister clade to *P. cuniculorum* with full support (BI-PP = 1, ML-BP = 100).

Parasola kuehneri. Croatia, Sisak-Moslavina County: vicinity of the village of Letovanić, near Palanjek Pokupski hamlet, 155 m a.s.l., ca. 45.5178° N, 16.1378° E, on grassy soil, deciduous forest of *Fagus sylvatica*, *Carpinus betulus*, and *Quercus* sp., *leg.* M. Čerkez, 24 August 2003, CNF 1/4334.

Figure 4. Basidiomata of *Parasola* species new to Croatia. (**A**,**B**) *P. auricoma* CNF 1/4718, 1/4618. (**C**) *P. crataegi* CNF 1/8905. (**D**,**E**) *P. cuniculorum* CNF 1/5143. (**F**) *P. malakandensis* CNF 1/8698. (**G**,**H**) *P. megasperma* CNF 1/5704. (**I**,**J**) *P. nudiceps* CNF 1/4804. (**K**) *P. plicatilis-similis* CNF 1/5484. Bars: (**A**,**D**,**E**) = 5 mm; (**B**,**F**,**K**) = 20 mm; (**C**,**G**–**J**) = 10 mm. Photos: M. Čerkez.

Notes: The collections of *P. kuehneri* from the Netherlands (Uljé 31.5.1987, holotype; Uljé 1241) formed a monophyletic clade with the Croatian collection of *P. kuehneri* with

strong support (BI-PP = 1, ML-BP = 98). *Parasola parvula* Ganga & Manimohan was recovered as its sister species in our analysis (BI-PP = 0.98, ML-BP = 72).

Parasola malakandensis. Croatia, Split-Dalmatia County: island of Hvar, near the town of Jelsa, 20 m a.s.l., 43.16452° N, 16.68287° E, on soil, grassy football field, *leg*. M. Čerkez, 20 July 2021, CNF 1/8698 (Figure 4F).

Notes: The collection of *P. malakandensis* from Croatia clustered in a monophyletic clade (BI-PP = 1, ML-BP = 100) with the holotype (HUP 17501) and the paratype (LAH-SHP-17) of *P. malakandensis* from Pakistan [9].

Parasola megasperma. Croatia, Dubrovnik-Neretva County: island of Mljet, near the village of Prožurski Porat, 35 m a.s.l., 42.73075° N, 17.64633° E, on soil, Mediterranean scrubland (maquis) dominated by *Ceratonia siliqua*, *Cupressus semepervirens*, *Pistacia lentiscus*, and *Cistus* sp., *leg.* M. Čerkez, 9 November 2009, CNF 1/5704 (Figure 4G,H).

Notes: The collection of *P. megasperma* from Croatia formed a monophyletic clade (ML-BP = 96) together with two collections of *P. megasperma* (E:Orton 4132, holotype; Ulje 1275) and the collection identified as *P. schroeteri* (P. Karst.) Redhead, Vilgalys & Hopple (Dähncke 1502) [10].

Parasola nudiceps. Croatia, Krapina-Zagorje County: Medvednica Mountain, 750 m a.s.l., 45.91905° N, 15.96917° E, on old cow dung, pasture, *leg.* M. Čerkez, 29 September 2007, CNF 1/4804 (Figure 4I,J).

Notes: The morphological and phylogenetic analyses performed in this study confirmed the conclusion of Schafer et al. [16], who considered *P. ochracea* to be a later synonym of *P. nudiceps*. Our measurements of the basidiospore size (ca. 12–14.5 × 10–12.5 × 8–10 µm) from the holotype of *P. ochracea* NL-3621 revealed that Szarkandi et al. [10] had mistakenly reported an erroneous spore size (10–11 × 6–8.5 µm) in the protologue. On the other hand, the size of basidiospores in the holotype of *P. ochracea* is within the size range of spores from the holotype of *P. nudiceps* (11.6–16.0 × 10.6–14.0 × 6.7–9.5 µm) reported by [6,42,43]. Based on morphological studies alone [6,43], *P. nudiceps* could be considered a later synonym of *P. schroeteri*, but further research is needed (see Schafer et al. [16] for a discussion). The collection of *P. nudiceps* from Croatia formed a monophyletic group (BI-PP = 1, ML-BP = 100) along with another four collections of *P. nudiceps* (E:Orton 4133, holotype; HB19870911A; *P. ochracea* NL-3621, holotype; and *P. ochracea* NL-3167, paratype). *Parasola litoralis* was recovered as a sister clade to *P. nudiceps* in our analysis.

Parasola plicatilis-similis. Croatia, Zagreb County: Žumberak Mountain, near the village of Stojdraga, 240 m a.s.l., 45.83774° N, 15.61053° E, on grassy soil, grassland, *leg*. M. Čerkez, 26 October 2008, CNF 1/5484 (Figure 4K).

Notes: The collection of *P. plicatilis-similis* from Croatia clustered in a monophyletic clade (BI-PP = 0.96, ML-BP = 98) together with the holotype (NL-2125) and the paratype (NL-0287) of *P. plicatilis-similis* from Sweden [10]. Two collections of *P. aporos* (RC-F92.191, holotype; CL-F09.005) formed a sister clade to *P. plicatilis-similis* (BI-PP = 1, ML-BP = 100) in agreement with Malysheva et al. [17]. Based on GenBank BLAST results, the sequence similarities between *P. plicatilis-similis* (CNF 1/5484) and *P. aporos* collections (RC-F92.191, holotype; CL-F09.005) were 99.55% and 99.31–99.54% in ITS and LSU gene regions, respectively. Considering important morphological differences between both taxa, e.g., basidiospore features, an absence/presence of pleurocystidia, and the habit of basidiomata, we followed the taxonomic concept from Malysheva et al. [17] and treated *P. aporos* and *P. plicatilis-similis* as separate species.

4. Discussion

Ten species of the genus *Parasola* from Croatia were identified using a combination of morphological and molecular characteristics. *Parasola papillatospora*, a species occurring in temperate deciduous forests of Europe (Croatia, Hungary), was described as new to science. Eight species, *P. auricoma*, *P. crataegi*, *P. cuniculorum*, *P. kuehneri*, *P. malakandensis*, *P. megasperma*, *P. nudiceps*, and *P. plicatilis-similis*, were reported for the first time from Croatia. *Parasola malakandensis* was also reported for the first time outside Pakistan. *Parasola conopilea*,

a species previously reported from Croatia based on morphological identification [19], was confirmed with multigene phylogenetic analyses. The collections of seven species new to Croatia (all except *P. auricoma*) were clustered with the type collection of the same species in our phylogenetic analyses.

Parasola papillatospora sp. nov. is phylogenetically well distinguished from all other published *Parasola* species included in the GenBank database. In the phylogram, *P. papillatospora* recovered as an independent clade, a sister to the *P. plicatilis* species complex (BI-PP = 1, ML-BP = 100). The *P. papillatospora* clade consisted of six Croatian collections and a single one from Hungary (*Parasola* sp. 1 (SZMC-NL-2952) in Szarkandi et al. [10]). The Hungarian collection of *P. papillatospora* was analyzed by Szarkandi et al. [10] but was not introduced as a new species due to a lack of sufficient sample information and/or molecular data.

Six species of *Parasola* may have (sub)papillate basidiospores (at least sometimes), but always in combination with eccentric germ pores: *P. lactea* (=*P. leiocephala*), *P. lilatincta* (Bender & Uljé) Redhead, Vilgalys & Hopple, *P. lilatinctoides* P. Voto, *P. litoralis* Loizides, D.J. Schaf. & P. Alvarado, *P. misera*, and *P. parvula*. DNA sequences from the type material of the latter four species were deposited in GenBank and were included in phylogenetic analyses conducted here. In addition to the presence of exclusively eccentric germ pores, the above species can be distinguished from *P. papillatospora* by other morphological characteristics as well. None of these species have such a great variety of basidiospore shapes, including a strongly developed apical papilla and a distinctly conical base, which are not uncommon in *P. papillatospora*. *Parasola lactea* is the most similar species but differs by somewhat larger basidiospores (average length, 9.0–10.7 µm; average breadth, 8.1–9.8 µm) and mostly utriform to lageniform cheilocystidia [5]. *Parasola lilatincta* has a larger pileus (20–50 mm wide), larger basidiospores (average length, 10.7–12.3 µm; average breadth, 9.5–10.1 µm), and basidia surrounded by (4–)5–8(–9) hymenophysalides [5]. *Parasola lilatinctoides* differs by much larger basidiospores (average length, 14.2–14.5 µm; average breadth, 12.5 µm) which are only sometimes subpapillate and by having 5–8 hymenophysalides around the basidium [14]. *Parasola litoralis* has much larger basidiospores (average length, 16.3 µm; average breadth, 11.7 µm), only sometimes with a slight apical protrusion, much wider basidia (12–20 µm), and longer pleurocystidia (80–96 µm) [16]. *Parasola misera* differs by more isodiametric basidiospores (Q_f = 0.96–1.12) which are only sometimes papillate, by the absence of pleurocystidia, and the fimicolous habitat [5,6]. Finally, *P. parvula* differs in having a smaller pileus (3–11 mm) with violet or purplish color tones present in most basidiomata, pinkish lamellae when young, a yellowish or purplish black base of the pileipellis elements, and the fimicolous habitat [11].

5. Conclusions

Until 2013, *Parasola* was considered a rather small fungal genus with up to 12 accepted species. However, subsequent extensive taxonomic research led to the description of 19 additional new species in the genus to date. Prior to this work, only four species of *Parasola* were known from Croatia.

We performed an integrative taxonomic study of 17 *Parasola* specimens collected in different parts of Croatia and identified 10 species. A new saprotrophic soil species from temperate deciduous forests, *P. papillatospora,* was described based on morphology, ecology, and multigene (ITS, LSU, *tef-1α*, and *β-tub*) phylogenetic analyses. Eight species were reported for the first time from Croatia and *P. malakandensis* also for Europe.

The integrative taxonomic approach used in this study to distinguish species within *Parasola* is highly recommended to be applied in future studies of the genus.

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N.M.; visualization, A.P. and Z.T.; supervision, Z.T. and A.M.; project administration, A.M.; funding acquisition, A.M. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Sequences generated in this study were submitted to the GenBank database of NCBI (https://www.ncbi.nlm.nih.gov/genbank/ accessed on 16 May 2023). Alignments and phylogenetic trees generated in this study are available at Zenodo (DOI 10.5281/zenodo.8099476).

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References

1. Redhead, S.A.; Vilgalys, R.; Moncalvo, J.-M.; Johnson, J.; Hopple, J.S. *Coprinus* Pers. and the Disposition of *Coprinus* Species Sensu Lato. *Taxon* **2001**, *50*, 203–241. [CrossRef]
2. Vilgalys, R.; Hopple, J.S.; Hibbett, D.S. Phylogenetic Implications of Generic Concepts in Fungal Taxonomy: The Impact of Molecular Systematic Studies. *Mycol. Helv.* **1994**, *6*, 73–91.
3. Hopple, J.S.; Vilgalys, R. Phylogenetic Relationships in the Mushroom Genus *Coprinus* and Dark-Spored Allies Based on Sequence Data from the Nuclear Gene Coding for the Large Ribosomal Subunit RNA: Divergent Domains, Outgroups, and Monophyly. *Mol. Phylogenet. Evol.* **1999**, *13*, 1–19. [CrossRef]
4. Moncalvo, J.M.; Lutzoni, F.M.; Rehner, S.A.; Johnson, J.; Vilgalys, R. Phylogenetic Relationships of Agaric Fungi Based on Nuclear Large Subunit Ribosomal DNA Sequences. *Syst. Biol.* **2000**, *49*, 278–305. [CrossRef] [PubMed]
5. Uljé, C.B. Coprinus Pers. In *Flora Agaricina Neerlandica, Critical Monographs on Families of Agarics and Boleti Occurring in the Netherlands*; Noordeloos, M.E., Kuyper, T.W., Veliinga, E.C., Eds.; Taylor & Francis: London, UK, 2005; pp. 22–109.
6. Nagy, L.G.; Vágvölgyi, C.; Papp, T. Type Studies and Nomenclatural Revisions in *Parasola* (*Psathyrellaceae*) and Related Taxa. *Mycotaxon* **2010**, *112*, 103–141. [CrossRef]
7. Larsson, E.; Örstadius, L. Fourteen Coprophilous Species of *Psathyrella* Identified in the Nordic Countries Using Morphology and Nuclear RDNA Sequence Data. *Mycol. Res.* **2008**, *112*, 1165–1185. [CrossRef]
8. Schafer, D.J. The Genus *Parasola* in Britain Including *Parasola cuniculorum* sp. nov. *Field Mycol.* **2014**, *15*, 77–99. [CrossRef]
9. Hussain, S.; Afshan, N.u.S.; Ahmad, H.; Khalid, A.N.; Niazi, A.R. *Parasola malakandensis* sp. nov. (*Psathyrellaceae*; *Basidiomycota*) from Malakand, Pakistan. *Mycoscience* **2017**, *58*, 69–76. [CrossRef]
10. Szarkandi, J.G.; Schmidt-Stohn, G.; Dima, B.; Hussain, S.; Kocsube, S.; Papp, T.; Vagvolgyi, C.; Nagy, L.G. The Genus *Parasola*: Phylogeny and the Description of Three New Species. *Mycologia* **2017**, *109*, 620–629. [CrossRef]
11. Ganga, K.G.G.; Manimohan, P. A New Species and a New Record of *Parasola* from Kerala State, India. *Phytotaxa* **2018**, *369*, 260–268. [CrossRef]
12. Hussain, S.; Ahmad, H.; Ullah, S.; Afshan, N.U.S.; Pfister, D.H.; Sher, H.; Ali, H.; Khalid, A.N. The Genus *Parasola* in Pakistan with the Description of Two New Species. *MycoKeys* **2018**, *30*, 41–60. [CrossRef] [PubMed]
13. Greeshma Ganga, K.G.; Manimohan, P. *Parasola psathyrelloides* (*Psathyrellaceae*), a New Species from Kerala State, India. *Phytotaxa* **2019**, *405*, 255–262. [CrossRef]
14. Voto, P. Novelties in the Family *Psathyrellaceae*. Part II. *Boll. Am.* **2019**, *108*, 127–133.
15. Voto, P. Novelties in the Family *Psathyrellaceae*. Part V. *Micol. E Veg. Mediterr.* **2021**, *35*, 149–168.
16. Schafer, D.; Alvarado, P.; Smith, L.; Liimatainen, K.; Loizides, M. Coprinoid *Psathyrellaceae* Species from Cyprus: Three New Sabulicolous Taxa from Sand Dunes and a Four-Spored Form of the Fimicolous Species *Parasola cuniculorum*. *Mycol. Prog.* **2022**, *21*, 52. [CrossRef]
17. Malysheva, E.; Moreno, G.; Villarreal, M.; Malysheva, V.; Svetasheva, T. The Secotioid Genus *Galeropsis* (*Agaricomycetes*, *Basidiomycota*): A Real Taxonomic Unit or Ecological Phenomenon? *Mycol. Prog.* **2019**, *18*, 805–831. [CrossRef]
18. Tibpromma, S.; Hyde, K.D.; Jeewon, R.; Maharachchikumbura, S.S.N.; Liu, J.K.; Bhat, D.J.; Jones, E.B.G.; McKenzie, E.H.C.; Camporesi, E.; Bulgakov, T.S.; et al. Fungal Diversity Notes 491–602: Taxonomic and Phylogenetic Contributions to Fungal Taxa. *Fungal Divers.* **2017**, *83*, 1–261. [CrossRef]
19. Mešić, A.; Tkalčec, Z. Preliminary Checklist of Agaricales from Croatia IV: Families *Bolbitiaceae*, *Coprinaceae*, *Entolomataceae* and *Pluteaceae*. *Mycotaxon* **2003**, *87*, 283–309.
20. Vrščaj, D. Popis Gljiva Otoka Krka—1. Dio. *Gljiv. Glas.* **2002**, *15*, 21–25.
21. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. *PCR Protoc.* **1990**, 315–322. [CrossRef]
22. Gardes, M.; Bruns, T.D. ITS Primers with Enhanced Specificity for Basidiomycetes—Application to the Identification of Mycorrhizae and Rusts. *Mol. Ecol.* **1993**, *2*, 113–118. [CrossRef] [PubMed]

23. Vilgalys, R.; Hester, M. Rapid Genetic Identification and Mapping of Enzymatically Amplified Ribosomal DNA from Several *Cryptococcus* Species. *J. Bacteriol.* **1990**, *172*, 4238–4246. [CrossRef] [PubMed]
24. Rehner, S. Primers for Elongation Factor 1-α (EF1-α). 2001. Available online: http://ocid.NACSE.ORG/research/deephyphae/EF1primer.pdf (accessed on 11 February 2022).
25. Rehner, S.A.; Buckley, E. A Beauveria Phylogeny Inferred from Nuclear ITS and *EF1-Alpha* Sequences: Evidence for Cryptic Diversification and Links to *Cordyceps* Teleomorphs. *Mycologia* **2005**, *97*, 84–98. [CrossRef] [PubMed]
26. Nagy, L.G.; Walther, G.; Házi, J.; Vágvölgyi, C.; Papp, T. Understanding the Evolutionary Processes of Fungal Fruiting Bodies: Correlated Evolution and Divergence Times in the *Psathyrellaceae*. *Syst. Biol.* **2011**, *60*, 303–317. [CrossRef]
27. Katoh, K.; Misawa, K.; Kuma, K.I.; Miyata, T. MAFFT: A Novel Method for Rapid Multiple Sequence Alignment Based on Fast Fourier Transform. *Nucleic Acids Res.* **2002**, *30*, 3059–3066. [CrossRef]
28. Katoh, K.; Standley, D.M. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [CrossRef]
29. Nguyen, L.-T.; Schmidt, H.A.; von Haeseler, A.; Minh, B.Q. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [CrossRef]
30. Trifinopoulos, J.; Nguyen, L.-T.; von Haeseler, A.; Minh, B.Q. W-IQ-TREE: A Fast Online Phylogenetic Tool for Maximum Likelihood Analysis. *Nucleic Acids Res.* **2016**, *44*, W232–W235. [CrossRef]
31. Huelsenbeck, J.P.; Ronquist, F. MRBAYES: Bayesian Inference of Phylogenetic Trees. *Bioinformatics* **2001**, *17*, 754–755. [CrossRef]
32. Letunic, I.; Bork, P. Interactive Tree of Life (ITOL) v5: An Online Tool for Phylogenetic Tree Display and Annotation. *Nucleic Acids Res.* **2021**, *49*, W293–W296. [CrossRef]
33. Nagy, L.G.; Kocsubé, S.; Papp, T.; Vágvölgyi, C. Phylogeny and Character Evolution of the Coprinoid Mushroom Genus *Parasola* as Inferred from LSU and ITS nrDNA Sequence Data. *Persoonia Mol. Phylogeny Evol. Fungi* **2009**, *22*, 28–37. [CrossRef] [PubMed]
34. Nagy, L.G.; Urban, A.; Örstadius, L.; Papp, T.; Larsson, E.; Vágvölgyi, C. The Evolution of Autodigestion in the Mushroom Family *Psathyrellaceae* (*Agaricales*) Inferred from Maximum Likelihood and Bayesian Methods. *Mol. Phylogenet. Evol.* **2010**, *57*, 1037–1048. [CrossRef] [PubMed]
35. Wächter, D.; Melzer, A. Proposal for a Subdivision of the Family *Psathyrellaceae* Based on a Taxon-Rich Phylogenetic Analysis with Iterative Multigene Guide Tree. *Mycol. Prog.* **2020**, *19*, 1151–1265. [CrossRef]
36. Vu, D.; Groenewald, M.; de Vries, M.; Gehrmann, T.; Stielow, B.; Eberhardt, U.; Al-Hatmi, A.; Groenewald, J.Z.; Cardinali, G.; Houbraken, J.; et al. Large-Scale Generation and Analysis of Filamentous Fungal DNA Barcodes Boosts Coverage for Kingdom *Fungi* and Reveals Thresholds for Fungal Species and Higher Taxon Delimitation. *Stud. Mycol.* **2019**, *92*, 135–154. [CrossRef]
37. Nagy, L.G.; Házi, J.; Szappanos, B.; Kocsubé, S.; Bálint, B.; Rákhely, G.; Vágvölgyi, C.; Papp, T. The Evolution of Defense Mechanisms Correlate with the Explosive Diversification of Autodigesting *Coprinellus* Mushrooms (*Agaricales*, *Fungi*). *Syst. Biol.* **2012**, *61*, 595–607. [CrossRef]
38. Schoch, C.L.; Seifert, K.A.; Huhndorf, S.; Robert, V.; Spouge, J.L.; Levesque, C.A.; Chen, W.; Bolchacova, E.; Voigt, K.; Crous, P.W.; et al. Nuclear Ribosomal Internal Transcribed Spacer (ITS) Region as a Universal DNA Barcode Marker for Fungi. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 6241–6246. [CrossRef]
39. Zhu, L.; Huang, M.; Bau, T. Taxonomy of Coprinoid Fungi in China. *Mycosystema* **2022**, *41*, 878–898. [CrossRef]
40. Kits van Waveren, E. The Dutch, French and British Species of *Psathyrella*. *Persoonia-Supplement* **1985**, *2*, 3–300.
41. Ludwig, E. *Pilzkompendium. Band 2. Die Größeren Gattungen der Agaricales mit Farbigem Sporenpulver (Ausgenommen Cortinariaceae)*; FUNGICON Verlag: Berlin, Germany, 2007; ISBN 9783940316004.
42. Orton, P.D. Notes on British Agarics IV. *Notes R. Bot. Gard. Edinb.* **1972**, *32*, 135–150.
43. Uljé, C.B.; Bender, H. Additional Studies in *Coprinus* Subsection *Glabri*. *Persoonia* **1997**, *16*, 373–381.

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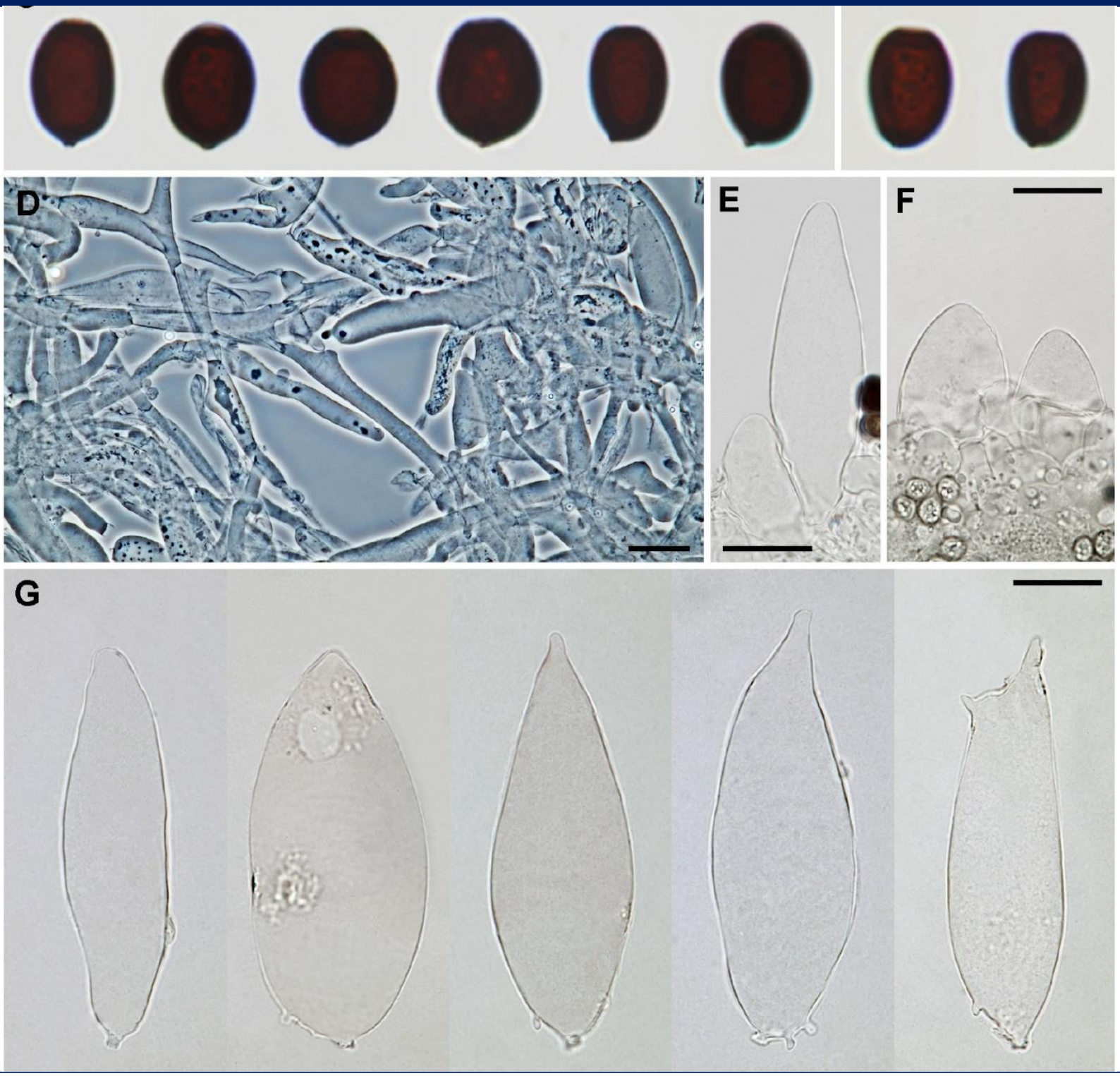

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Coprinopsis alnivora (Psathyrellaceae), a rare species from North America is discovered in Europe

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Abstract

A rare species, *Coprinopsis alnivora,* was previously known from the type collection in Washington State, USA. Afterward, 11 additional samples were collected from five new host trees at nine localities in Europe (Austria, Croatia, and Slovakia). The species indicated a preference for growth in cavities or wounds of living deciduous trees. Its mycelium and basidiomata were successfully cultivated under laboratory conditions. A detailed morphological description of the basidiomata supplemented with colour photographs and line drawings is provided. Delimitation characters from similar species are discussed. Molecular phylogenetic relationships within the genus *Coprinopsis* were inferred from ITS rDNA sequences and are presented by a phylogram. Molecular genetic analyses revealed that *C. alnivora* represents a genetically well-delimited species with six known haplotypes.

Keywords: Agaricales, Basidiomycota, biogeography, cultivation study, morphology, phylogeny, taxonomy

Introduction

Until this research, *Coprinopsis alnivora* (Van de Bogart 1976: 241) Voto (2019: 94) was known only from the type collection in Washington State, USA, on *Alnus* wood. However, in the last 13 years, 11 collections of *C. alnivora* have been found at nine localities in three European countries: Slovakia (7), Austria (3), and Croatia (1). In this paper, *C. alnivora* is reported as new to Europe. A detailed morphological description and illustrations of European collections, molecular phylogenetic analysis, cultivation study, and five new host tree records are presented here.

Coprinopsis alnivora was originally described as *Coprinus alnivorus* (Van De Bogart 1976). Redhead *et al.* (2001) subsequently divided the large, polyphyletic genus *Coprinus* Persoon (1797: 62), characterized morphologically by very dark spores and sulcate pileus, into four genera based on previous molecular phylogenetic studies: *Coprinus*, belonging to the family *Agaricaceae* Chevallier (1826: 121), and *Coprinellus* Karsten (1879: 32), *Coprinopsis* Karsten (1881: 27), and *Parasola* Redhead, Vilgalys & Hopple (2001: 235), belonging to the family *Psathyrellaceae* Vilgalys, Moncalvo & Redhead in Redhead *et al.* (2001: 226). In addition, some species of the genus *Psathyrella* (Fries 1838: 137) Quélet (1872: 152) were transferred to the genera *Coprinopsis* and *Parasola*. Although the splitting of *Coprinus s. l.* solved a considerable part of the phylogenetic and taxonomic problems in coprinoid fungi, some of those remained unresolved. Wächter & Melzer (2020) conducted a taxon-rich multigene phylogenetic analysis to propose a new subdivision of the family *Psathyrellaceae*. They described seven new genera for a more natural accommodation of

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many species. Almost all members of *Coprinopsis* formed a monophyletic clade and it is the second-largest genus within *Psathyrellaceae*, after *Psathyrella*.

Van De Bogart (1976) originally placed *Coprinus alnivorus* in the section (subsection) *Coprinus*, with a transitional position to the section (subsection) *Lanatuli* Fries (1838: 250). Uljé & Noordeloos (2000) transferred it to the subsection *Alachuani* Singer (1951: 459) on the basis of branched and diverticulate veil hyphae. After the split of the genus *Coprinus s. l.*, Schafer (2010) transferred and distributed its previous, morphologically defined subsections to three newly formed genera in *Psathyrellaceae* as sections, five of which were in *Coprinopsis*, with *Alachuanae* (Fr.) D.J. Schafer (2010: 51) and *Lanatulae* (Fr.) D.J. Schafer (2010: 51) being the largest. However, several molecular phylogenetic studies have shown that these two sections are not monophyletic (e.g. Padamsee *et al.* 2007, Nagy *et al.* 2013b). Therefore, Wächter & Melzer (2020) proposed a new infrageneric classification of *Coprinopsis* into 20 sections.

Material and methods

Sampling and morphological description

Eleven European samples of *Coprinopsis alnivora* were collected from 2008 to 2020 in Slovakia, Austria, and Croatia. The description of macrocharacters was based on observations of fresh basidiomata at different developmental stages. Microcharacters were examined, measured, and described on both fresh and dried material. Sections of fresh basidiomata were examined in Congo Red using KAPAoptics KAPA SM3 optical microscope. Dried specimens were rehydrated and examined in 2.5% potassium hydroxide (KOH) and in H_2O (basidiospores), using Olympus BX51 optical microscope (bright field and phase contrast) under magnification up to 1500× and photographed with a Canon EOS M50 digital camera. Randomly selected basidiospores from the photographs of mature lamellae mounts were measured using Motic Images Plus 2.0 software. The length/width ratio of basidiospores is expressed as "Q" value (min–av.–max), separately for frontal-view (Q_f) and side-view (Q_s). Average basidiospore lengths, widths (in both views), and Q values are shown in italics. Numbers in square brackets [X/Y/Z] denote X elements measured in Y basidiomata from Z collections. Basidia were measured without sterigmata.

Cultivation of mycelium and basidiomata under laboratory conditions

The medium for cultivation of *C. alnivora* mycelium was prepared as a suspension of 50 g Malt Extract Agar Base (HiMedia M137-500G) in 1000 ml distilled water and the solution was allowed to stand at room temperature for 15 minutes. The medium obtained was autoclaved at 115 °C for 10 minutes, then cooled and poured into Petri dishes. Pieces of context tissue from the stipe base of a partially collapsed mature basidiome of *C. alnivora* (collection BRA CR33776) were inoculated onto the prepared medium and incubated at 25 °C for 7 days. Petri dishes were then examined for pure (uncontaminated) mycelial parts, which were transferred to the new medium for another round of 7 days incubation. The procedure was repeated until a pure mycelial culture was obtained.

The substrate used for basidiomata production was prepared from beech sawdust (80%) mixed with wheat bran (18%) and chalk ground (2%). The mixture was watered to approximately 70%, autoclaved at 121 °C for 45 min, cooled, and inoculated with a pure mycelial culture of *C. alnivora*. Three jars (720 ml) were half-filled with the inoculated substrate, sealed with a lid with a 10 mm thick cotton plug, and covered with aluminium foil. The jars were stored at 22 °C for 21 days to allow complete colonization of the substrate. Fruiting body formation was induced by storing the jars at 11 °C for 48 hours. Then, the lids of the jars were loosened and the cultures were kept in a bright place in the room at 20 °C. After the formation of primordia, the lids were removed and the substrate was sprayed with water three times a day until mature fruiting bodies developed.

Molecular genetic analysis

For the Slovak material, total genomic DNA was extracted from dried basidiomata using a DNeasy Plant MiniKit (Qiagen) according to the manufacturer's protocol, but with an extended incubation time of up to 1 h after addition of the RNA-lytic enzyme. Internal Transcribed Spacer region of ribosomal DNA (ITS1-5.8S-ITS2) was amplified with primers ITS5/ITS4 (White *et al.* 1990). Reactions were performed in 25 µL total volume using a GoTaq Flexi

PCR kit (Promega), containing 20–25 ng of total DNA template and set up as follows: 3 min initial denaturation at 95 °C, 32 cycles (95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min + increasing the time by 2 s per cycle), and 10 min final extension at 72 °C. PCR products were purified using a Thermosensitive Alkaline Phosphatase (FastAP) and Exonuclease 1 (Exo 1) (Thermo Fisher Scientific Inc., USA) according to the manufacturer's instructions. The target gene was sequenced in a commercial laboratory (Eurofins Genomics GmbH, Cologne, Germany). DNA extraction, PCR amplification, and ITS rDNA sequencing of Austrian collections were performed as described in Mentrida *et al.* (2015) and of Croatian collection as described in Kušan *et al.* (2018).

Editing, alignment, and final matrix were performed in MEGA-X (Kumar *et al.* 2018). The dataset was analyzed using two different methods: Bayesian inference (BI) and the maximum likelihood (ML) approach. The evolutionary model used, GTR+G, was estimated as the best using jModeltest 2.1.7 software (Guindon & Gascuel 2003, Darriba *et al.* 2012). The ML analyzes were performed via the CIPRES web-portal with RAxML v. 8.2.12 (Stamatakis 2014) using 1000 bootstraps and BI was performed with MrBayes v. 3.2.7 (Ronquist *et al.* 2012) using a 10 million generation with sampling parameters every 10^3 and a default burn-in value of 25% for the final trees. Convergence of the two runs (average standard deviation of split frequencies <0.01) and likelihood stationarity were checked. Trees were finalized in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

A matrix of intra-species distances of *C. alnivora* and *C. clastophylla* as an inter-species outgroup was constructed using Kimura-2 parameter distances (Kimura 1980) in MEGA-X. To investigate relationships between haplotypes, a median-joining network (MJN) was constructed in PopART (Leigh & Bryant 2015).

The GenBank accession numbers of the holotype and the newly generated *C. alnivora* sequences are listed in Table 1. The list of other species used for molecular genetic analysis, with sample numbers, countries of origin, GenBank accession numbers of sequences, and references, is given in Table 2.

TABLE 1. Samples of *C. alnivora* used in phylogenetic analysis. *Hp*—haplotypes.

Species	Country	Latitude (N) °	Longitude (E) °	*Hp*	GenBank accession	BOLD accession
C. alnivora Holotype	USA	-	-	CA4	MK169326	-
C. alnivora	Slovakia	48.331944	17.193250	CA1	MT887857	EUCA001-20
C. alnivora	Slovakia	48.331944	17.193250	CA1	MT887856	EUCA002-20
C. alnivora	Slovakia	48.480933	17.358967	CA1	MT887855	EUCA003-20
C. alnivora	Slovakia	48.36293	17.27086	CA2	MT887854	EUCA004-20
C. alnivora	Slovakia	48.422033	17.208150	CA1	MT887853	EUCA005-20
C. alnivora	Slovakia	48.401556	17.209667	CA1	MT887852	EUCA006-20
C. alnivora	Slovakia	48.259001	17.100003	CA4	MZ364343	EUCA007-21
C. alnivora	Austria	48.192564	16.384094	CA1	MT828909	-
C. alnivora	Austria	48.200033	16.435251	CA3	MT828910	-
C. alnivora	Austria	48.190000	16.383060	CA5	MZ407758	-
C. alnivora	Croatia	45.83272	15.99375	CA2	MT796099	-

TABLE 2. List of collections used in the phylogenetic analysis with their GenBank accession numbers. Newly generated sequences are in black bold.

Taxon name	GenBank number	Voucher	Locality	Reference
Coprinopsis alnivora	**MT887857**	BRA CR33775	Slovakia	This study
Coprinopsis alnivora	**MT887856**	BRA CR33776	Slovakia	This study
Coprinopsis alnivora	**MT887854**	BRA CR33777	Slovakia	This study
Coprinopsis alnivora	**MT887852**	BRA CR33778	Slovakia	This study
Coprinopsis alnivora	**MT887853**	BRA CR33779	Slovakia	This study
Coprinopsis alnivora	**MT887855**	BRA CR33780	Slovakia	This study
Coprinopsis alnivora	**MZ364343**	BRA CR33781	Slovakia	This study
Coprinopsis alnivora	**MT828909**	WU 41009	Austria	This study
Coprinopsis alnivora	**MT828910**	WU 42007	Austria	This study
Coprinopsis alnivora	**MZ407758**	WU 43426	Austria	This study
Coprinopsis alnivora	**MT796099**	CNF 1/5429	Croatia	This study
Coprinopsis alnivora (type)	MK169326	FVDB 3370	USA	Gordon (Unpubl.)
Coprinopsis ammophilae	HQ847008	WAT 24982	The Netherlands	Nagy *et al.* (2012)
Coprinopsis argentea	HQ847040	SZMC-NL-1678	Unknown	Nagy *et al.* (2012)
Coprinopsis babosiae	JX118685	SZMC-NL-0871	Unknown	Nagy *et al.* (2013a)
Coprinopsis babosiae (type)	FN396128	SZMC-NL-4139	Hungary	Nagy *et al.* (2011)
Coprinopsis bellula	FN430682	SZMC-NL-2341	Hungary	Nagy *et al.* (2011)
Coprinopsis bicornis (type)	NR_148067	L Uljc 1216	Hungary	Nagy *et al.* (2013a)
Coprinopsis brunneistragulata (type)	MK169330	WTU-F-039607	USA	Gordon (Unpubl.)
Coprinopsis calospora (type)	MH862284	CBS 612.91	The Netherlands	Vu *et al.* (2019)
Coprinopsis candidolanata	JN943137	SZMC-NL-2338	Norway	Bender & Melzer (2020)
Coprinopsis canoceps	KC992964	LÖ148-95	Sweden	Örstadius *et al.* (2015)
Coprinopsis clastophylla	MG719298	JZ41	India	Chawngthu *et al.* (2019)
Coprinopsis coniophora	FN396122	SZMC-NL-3414	Unknown	Nagy *et al.* (2012)
Coprinopsis cortinata	FN396121	SZMC-NL-1621	Hungary	Nagy *et al.* (2011)
Coprinopsis cothurnata	MH856479	CBS 174.49	Unknown	Vu *et al.* (2019)
Coprinopsis echinospora	AB071803	582 (CHU3019)	Japan	Raut *et al.* (2011)
Coprinopsis episcopalis	AY145855	F-06155	Norway	Gonzalez del Val *et al.* (2003)
Coprinopsis erythrocephala	FN396125	SZMC-NL-4153	Unknown	Nagy *et al.* (2011)
Coprinopsis filamentifera	MK069600	HB20171117A	Germany	Bender & Wächter (Unpubl.)
Coprinopsis friesii	MK072829	AM954	Germany	Wächter & Melzer (2020)
Coprinopsis fusispora (type)	NR_148068	SZMC-NL-1227	Hungary	Nagy *et al.* (2013a)
Coprinopsis gonophylla	MH856188	CBS 142.47	Unknown	Vu *et al.* (2019)
Coprinopsis insignis	MK966570	HMAS 281305	Unknown	Wei (Unpubl.)
Coprinopsis jonesii	MT408922	AN 043722	USA	Clements (Unpubl.)
Coprinopsis krieglsteineri	FM878019	SZMC-NL-2345	Hungary	Nagy *et al.* (2010)
Coprinopsis kubickae	MH422562	CNF 1/6614	Croatia	Phookamsak *et al.* (2019)
Coprinopsis laanii	MH859802	CBS 476.70	United Kingdom	Vu *et al.* (2019)

.....continued on the next page

TABLE 2 (Continued)

Taxon name	GenBank number	Voucher	Locality	Reference
Coprinopsis lagopides	MN892574	S.D. Russell iNaturalist #8536159	USA	Russell (Unpubl.)
Coprinopsis lagopus	MH856194	CBS 149.47	France	Vu *et al.* (2019)
Coprinopsis macrocephala	FN396126	SZMC-NL-1376	Unknown	Nagy *et al.* (2011)
Coprinopsis marcescibilis	DQ389728	LÖ31-03	Sweden	Larsson & Örstadius (2008)
Coprinopsis musae	KC992966	JV06180	Denmark	Örstadius *et al.* (2015)
Coprinopsis neolagopus	AB097564	NBRC100013	Unknown	Suzuki *et al.* (Unpubl.)
Coprinopsis nevillei (type)	HM126488	GG08090401	France	Garcia & Vellinga (2010)
Coprinopsis nivea	JF907848	4585	Italy	Osmundson *et al.* (2013)
Coprinopsis pachyderma (type)	MK169350	WTU-F-039608	USA	Gordon (Unpubl.)
Coprinopsis pannucioides	DQ389727	LÖ143-03	Sweden	Larsson & Örstadius (2008)
Coprinopsis phlyctidospora	JF907842	15575	Italy	Osmundson *et al.* (2013)
Coprinopsis picacea	FN396115	SZMC-NL-0174	Unknown	Nagy *et al.* (2011)
Coprinopsis poliomalla	FM163182	SZMC-NL-2336	Hungary	Nagy *et al.* (2009)
Coprinopsis pseudofriesii	HQ847016	SZMC-NL-2631	Unknown	Nagy *et al.* (2012)
Coprinopsis pseudonivea	FM163181	SZMC-NL-2340	Hungary	Nagy *et al.* (2009)
Coprinopsis pseudoradiata	HQ847022	SZMC-NL-3188	Unknown	Nagy *et al.* (2012)
Coprinopsis radiata	JN943126	SZMC-NL-1428	Hungary	Bender & Melzer (2020)
Coprinopsis rugosobispora	AB983245	BR-44338-09	Belgium	Raut *et al.* (2015)
Coprinopsis rugosomagnispora (type)	NR_148112	KRAM F-58717	Poland	Gierczyk *et al.* (2017)
Coprinopsis sclerotiorum	HQ847039	SZMC-NL-0564	Unknown	Nagy *et al.* (2012)
Coprinopsis scobicola	HQ847021	Orton964	United Kingdom	Nagy *et al.* (2012)
Coprinopsis spelaiophila	FN396117	SZMC-NL-3031	Unknown	Nagy *et al.* (2012)
Coprinopsis stangliana	FM878027	SZMC-NL-2153	Unknown	Nagy *et al.* (2011)
Coprinopsis stercorea	MH858437	CBS 301.64	The Netherlands	Vu *et al.* (2019)
Coprinopsis submicrospora (type)	KC992959	AH27055	Spain	Örstadius *et al.* (2015)
Coprinopsis tectispora	JX118665	FVDB 6016	North America	Nagy *et al.* (2013a)
Coprinopsis udicola (type)	KC992967	AM1240	Germany	Örstadius *et al.* (2015)
Coprinopsis urticicola	MH748639	AK.2044	Turkey	Keles (2019)
Coprinopsis utrifer	FN396140	SZMC-NL-0591	Unknown	Nagy *et al.* (2011)
Coprinopsis variegata	MG748581	SDR-MM5698	USA	Russell (Unpubl.)
Coprinopsis vermiculifer	KM056335	NRRL 66021	USA	Wicklow (Unpubl.)
Coprinopsis villosa	HQ847031	SZMC-NL-1758	Germany	Nagy *et al.* (2012)
Coprinus fissolanatus nom. prov.	JX118733	Daams 71121	The Netherlands	Nagy *et al.* (2013a)
Lacrymaria glareosa	KC992954	LAS06-019	Sweden	Örstadius *et al.* (2015)
Lacrymaria lacrymabunda	DQ389724	EL7-03	Sweden	Larsson & Örstadius (2008)

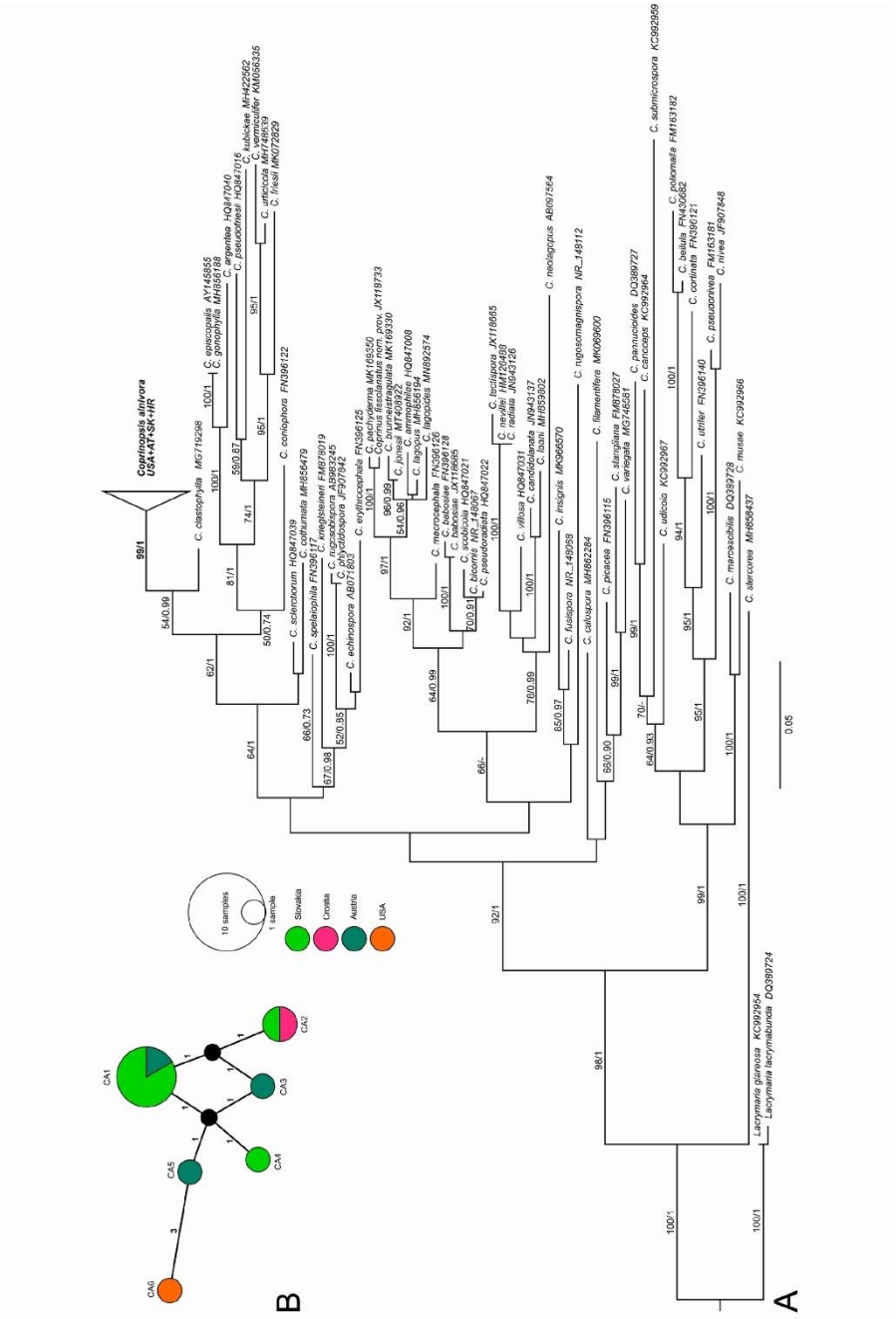

FIGURE 1. *Coprinopsis alnivora*; **A.** Phylogram generated by Maximum Likelihood (RAxML) analysis based on ITS sequences of the genus *Coprinopsis*. *Lacrymaria glareosa* and *L. lacrymabunda* were used as an outgroup. Maximum likelihood bootstrap support values greater than 50% and Bayesian posterior probabilities greater than 0.90 are indicated above branches; **B.** Haplotype network (MJN) showing relationships between *C. alnivora* collections from Europe and USA (holotype).

Results

Molecular genetic analysis

Eleven new sequences of *Coprinopsis alnivora* were generated from Europe in this study: seven from Slovakia, three from Austria, and one from Croatia (Table 1). Based on BLAST search against NCBI (National Center for Biotechnology Information, https://www.ncbi.nlm.nih.gov/) sequences, the ITS sequences formed HSP (high-scoring segment pairs) with ~99% identity for USA holotype of *C. alnivora* which was also supported by morphological characters. The European ITS sequences formed a stable cluster with the ITS sequence from the USA holotype of *C. alnivora* (Fig. 1A), and their genetic distance ranged from 0 to 0.78% (Table 1). The haplotype network showed six haplotypes of *C. alnivora* (Fig. 1B). The longest internal branch was for the USA sample (MK169326). The most related European haplotype was found in Austria (CA5-MZ407758). Another Austrian sample (MT828909) was grouped with the CA1 haplotype, which was dominant in Slovakia. The Slovak sample MT887854 was grouped with the sample from Croatia (MT796099). Within Europe, we were able to record two more haplotypes, each represented by one sample (CA3 - Austrian sample and CA4 - Slovak sample) (Fig. 1B).

Basidiomata production under laboratory conditions

Pure mycelial cultures and basidiomata of *C. alnivora* (Fig. 2G) were successfully produced as described in the Material and Methods section. Production of basidiomata was induced by lowering the medium temperature from 22 °C to 11 °C for two days. A total of seven mature basidiomata were obtained during the three weeks (1–3 per jar). The cultivated basidiomata were characterized by smaller pilei and by more slender and elongated stipes than those found in nature.

Taxonomy

Coprinopsis alnivora (Bogart) Voto, Boll. AMER 107(2): 94 (2019) (Figs 2–5)

Basionym: *Coprinus alnivorus* Van de Bogart, Mycotaxon 4 (1): 241 (1976).

Pileus up to 55 mm high when unexpanded, 15–55 mm wide at maturity; subglobose, ellipsoid or ovoid when young, then paraboloid, campanulate or obtusely conical, finally expanded to plano-convex or applanate, sometimes slightly depressed at centre; margin sometimes radially splitting in places, mostly revolute in mature basidiomata; surface densely plicate-sulcate except in the smooth central zone, often slimy, whitish, cream or (pale) brownish at first, then starts to darken from the edge towards the centre, becoming light brown, grey-brown, often with pinkish to purplish hue, and finally brown-grey, grey or grey-black; (partially) deliquescent; when very young completely covered with a dense, usually abundant universal veil (thickest at the centre, up to 3 mm), with brownish to dark grey-brown (only rarely whitish) thin upper layer (remains scattered after veil development) and thicker white layer underneath, soon veil starting to break up into patches of different size and shape, sooner or later partially showing the pileal surface, rarely the veil poor, thin and soon disappearing. **Lamellae** free, very crowded and thin, L > 80, up to 14 mm broad, lamellulae present; at first white, soon becoming pinkish to purplish, brown or grey-brown with purplish tone, brown-grey, dark grey and finally black; edge whitish from prominent and very abundant cheilocystidia, deliquescent with age. **Stipe** 25–110 mm × 3–10 mm, (sub)cylindrical or widened towards the base (up to 20 mm), sometimes widened at the apex, dry, hollow, white to whitish, finely tomentose-squamulose (more pronounced towards the base) to silky fibrillose, sometimes with small dark olive-brown scales or fibrils in the lower part, often with adpressed ring-like veil remnants in the middle or in the lower half of the stipe or with ± projected membranaceous ring-like veil remnants near the base, without rhizomorphs. **Flesh** white, relatively compact when young, fragile at maturity. **Smell** weak, reminiscent of raw potatoes or slightly unpleasant like in *Coprinopsis picacea*. **Taste** mild. **Spore print** black.

Basidiospores [540/9/6] (6.8–)7.5–*8.8*–10.0(–10.5) × (5.9–)6.2–*7.1*–8.1(–8.4) × (5.1–)5.5–*6.2*–6.9(–7.4) µm, averages of different basidiomata 8.0–9.5 × 6.4–7.5 × 5.8–6.6 µm, Q_l = (1.04–)1.07–*1.24*–1.42(–1.50), Q_s = (1.20–)1.25–*1.42*–1.57(–1.67), Q_l av. = 1.14–1.31, Q_s av. = 1.32–1.49, slightly to strongly flattened, in frontal view mostly ellipsoid to ovoid, less frequently subglobose, subangular, submitriform or sublimoniform, sometimes to rather often ± irregular (asymmetrical), with obtuse to somewhat acute base and rounded to truncate apex (especially in KOH), in side view mostly ellipsoid, much less often ± amygdaliform or ovoid, smooth, thick-walled (up to 1.4 µm), (red-brown) dark red-brown to almost black in H_2O and NH_4OH, (yellow-brown) dark yellowish-brown to black in KOH,

germ pore central, distinct. **Basidia** 17–33 × 7–11 µm, clavate, 4-spored, thin-walled, hyaline, surrounded by 4–7 hymenophysalides (pseudoparaphyses); sterigmata up to 5 µm long. **Cheilocystidia** 25–130 × 12–38 µm, ellipsoid, elongate-ellipsoid, ovoid, fusiform, conical, with obtuse to acute apex, thin-walled, hyaline to subhyaline, abundant. **Pleurocystidia** 41–173 × 15–55 µm, ellipsoid, oblong, fusiform, conical, with obtuse, acute, acuminate, mucronate or somewhat irregular apex (e.g. curved or with two apical protuberances), often with one or a few short excrescences near the base, thin-walled, hyaline to pale brownish, mostly abundant. **Pileipellis** a cutis, composed of repent, densely arranged, thin-walled, smooth, hyaline hyphae, 3–13 µm wide. **Veil hyphae** 2–15(–22) µm wide, occasionally to rather frequently branched, sparsely diverticulate, thin-walled (up to 0.5 µm) or rarely moderately thick-walled (up to 0.8 µm), sometimes finely encrusted, mostly hyaline, sometimes brownish; cells occasionally constricted at the septa or somewhat inflated. **Clamp-connections** present, conspicuous, abundant.

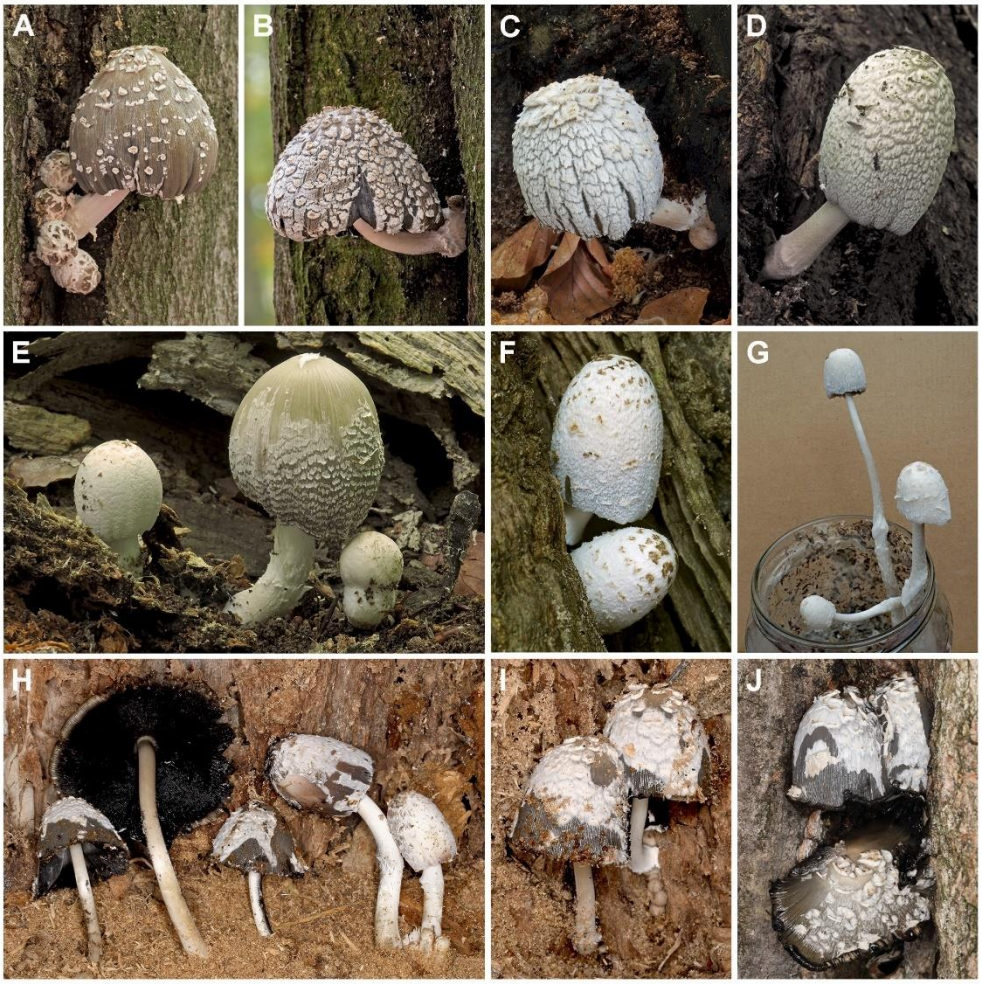

FIGURE 2. *Coprinopsis alnivora*; basidiomata. **A.** On the same tree as BRA CR33780, 9 Sept. 2018. **B.** BRA CR33780. **C.** BRA CR33777. **D.** On the same locality as BRA CR33779, 27 June 2020. **E.** On the same tree as BRA CR33779, 27 June 2020. **F.** On the same tree as BRA CR33775 and BRA CR33776, 2 Oct. 2011. **G.** Cultivated from BRA CR33776. **H–J.** CNF 1/5429. Photos by: **A, B** D. Solár; **C** L. Pešková; **D, E, G** J. Červenka; F R. Bednár; **H–J** M. Čerkez.

Distribution and ecology:—*Coprinopsis alnivora* is known from 12 collections in four countries of North America and Europe: the USA, Washington State (1); Croatia (1); Slovakia (7); and Austria (3). It grows on the wood of living

deciduous trees, mostly in decayed cavities or from wounds, or (less often) on dead trees, in forests and parks. Six host tree species are known so far: (a) *Fagus sylvatica* (on three living trees, on one dead tree—Slovakia), (b) *Fraxinus excelsior* (on one living tree—Slovakia), (c) *Acer campestre* (on one living tree—Slovakia), (d) *Populus nigra* (on two living trees—Croatia), (e) *Alnus* sp. (on a dead (?) tree—USA), (f) *Magnolia salicifolia* (on one living tree—Austria). It was also found in Austria on one living and one dead unidentified tree. In Slovakia, it has been observed that *C. alnivora* is able to fruit regularly from the same living tree over a long period of time (nine years and more).

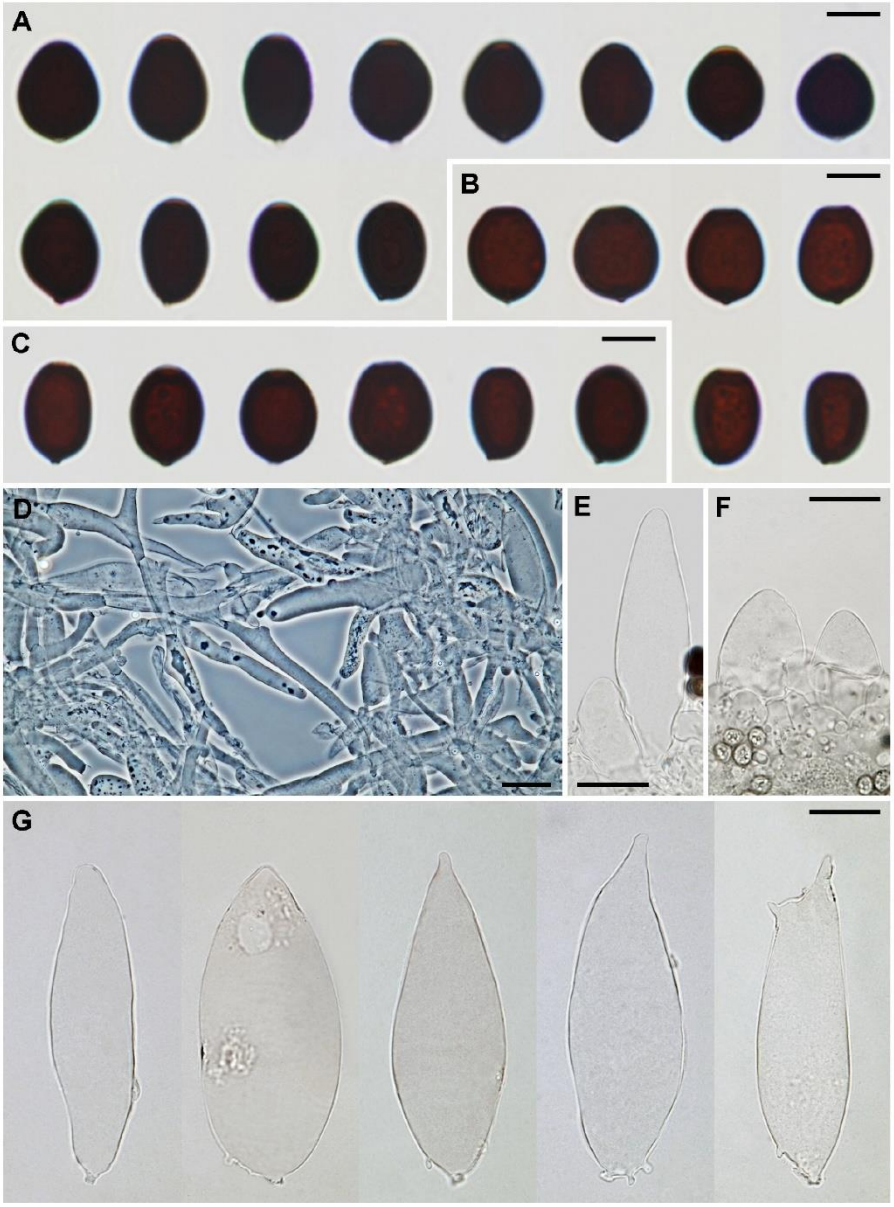

FIGURE 3. *Coprinopsis alnivora*. **A.** Basidiospores from CNF 1/5429. **B.** Basidiospores from WU 42007. **C.** Basidiospores from BRA CR33779. **D.** Veil hyphae (phase contrast). **E, F.** Cheilocystidia. **G.** Pleurocystidia. Scale bars: A–C = 5 µm, D–G = 20 µm. Photos by: Z. Tkalčec.

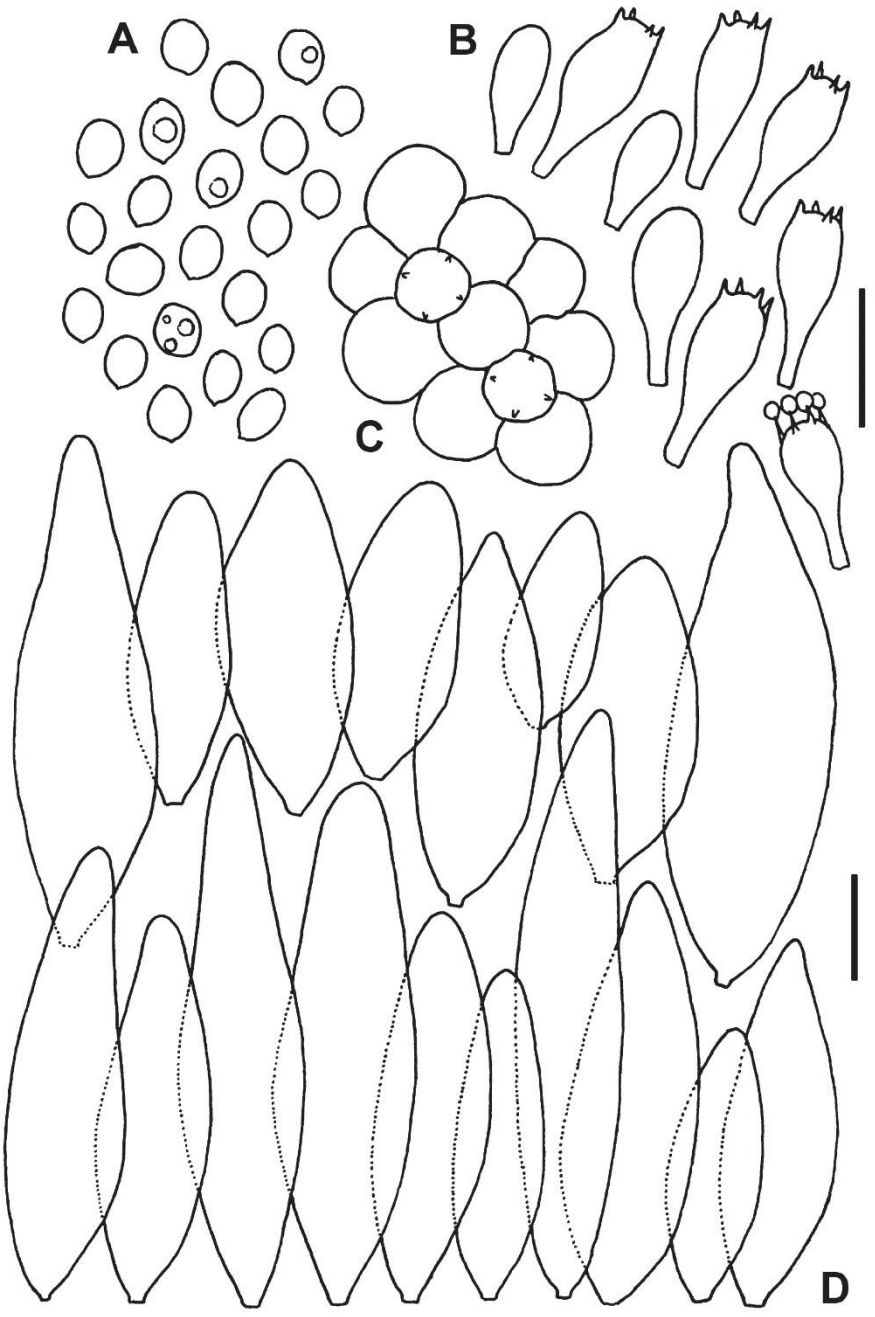

FIGURE 4. *Coprinopsis alnivora*. **A.** Basidiospores. **B.** Basidia. **C.** Mature basidia surrounded by hymenophysalides. **D.** Cheilocystidia. Scale bars = 20 µm. Drawings by: J. Červenka.

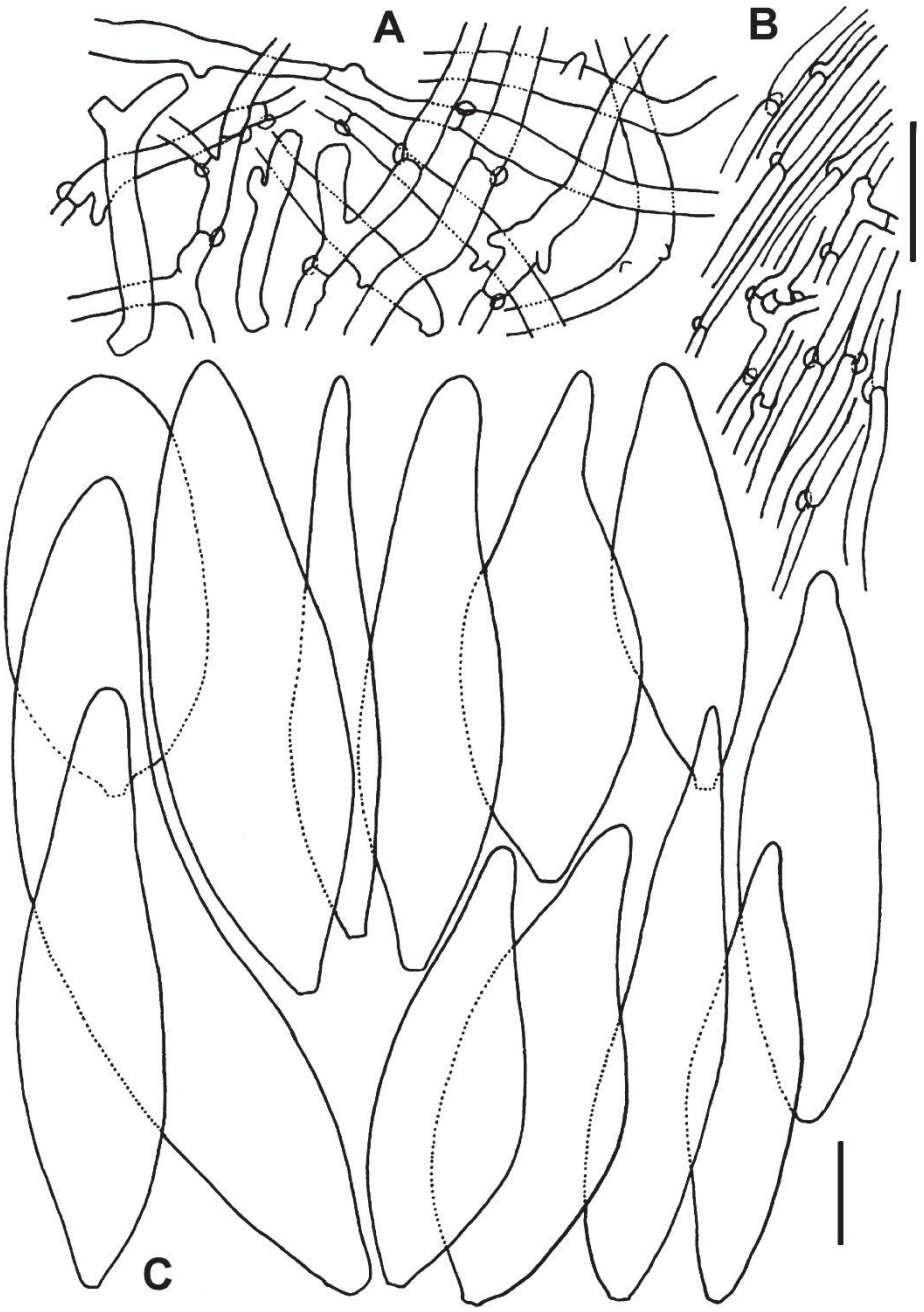

FIGURE 5. *Coprinopsis alnivora*. **A.** Hyphae of universal veil. **B.** Secondary mycelium. **C.** Pleurocystidia. Scale bars = 20 µm. Drawings by: J. Červenka.

Specimens examined:—SLOVAKIA. Bratislava region: Pezinok, Malá Homoľa, 550 m a.s.l., 48.36293° N, 17.27086° E, on living *Fagus sylvatica*, 11 May 2013, *leg.* J. Kuriplach, Ľ. Pešková, BRA CR33777, GenBank Number: MT887854; Limbach, Konské hlavy, 490 m a.s.l., 48.331944° N, 17.193250° E, in the cavity of living *Fraxinus excelsior*, 30 October 2018, *leg.* R. Bednár, BRA CR33775, GenBank Number: MT887857; Limbach, Konské hlavy, 490 m a.s.l., 48.331944° N, 17.193250° E, in the cavity of living *Fraxinus excelsior*, 23 October 2019, *leg.* R. Bednár, BRA CR33776, GenBank Number: MT887856; Kuchyňa, Vysoký Rajd, 400 m a.s.l., 48.401556° N, 17.209667° E, in the cavity of living *Fagus sylvatica*, 14 June 2020, *leg.* R. Bednár, BRA CR33778, GenBank Number: MT887852; Kuchyňa, Tri stodôlky, 520 m a.s.l., 48.422033° N, 17.208150° E, on fallen decayed log of *Fagus sylvatica*, 27 June 2020, *leg.* J. Červenka, T. Sobocký, BRA CR33779, GenBank Number: MT887853; Borinka, Úboč, 270 m a.s.l., 48.259001° N, 17.100003° E, on living *Acer campestre*, 7 October 2020, *leg.* A. Bystrická, BRA CR33781, GenBank Number: MZ364343; Trnava region: Lošonec, Zabité, 370 m a.s.l., 48.480933° N, 17.358967° E, on living *Fagus sylvatica*, 2 November 2019, *leg.* D. Solár, BRA CR33780, GenBank Number: MT887855.

—AUSTRIA. Vienna: Vienna city, district Landstraße, Botanical Garden of the University of Vienna, 180 m a.s.l., 48.19350° N, 16.38392° E, in small decayed cavity of the living deciduous tree, 31 October 2018, *leg.* I. Krisai-Greilhuber, WU 41009, GenBank Number: MT828909; Vienna: Vienna city, district Leopoldstadt, Prater Heustadlwasser, 170 m a.s.l., 48.20064° N, 16.26667° E, in knothole of fallen deciduous tree, 25 September 2019, *leg.* R. Brandstätter, WU 42007, GenBank Number: MT828910; Vienna: Vienna city, district Landstraße, Botanical Garden of the University of Vienna, 190 m a.s.l., 48.19000° N, 16.38306° E, in decayed cavity of living *Magnolia salicifolia*, 24 September 2020, *leg.* I. Krisai-Greilhuber, WU 43426, GenBank Number: MZ407758.

—CROATIA. Zagreb County: city of Zagreb, Mirogoj cemetery, 183 m a.s.l., 45.83272° N, 15.99375° E, public park, on two planted *Populus nigra* trees (cut down in the meantime), in decayed tree cavities, up to 1.4 m above the ground, 12 basidiomata in total, mostly a few together, in different stages of maturity, 4 October 2008, *leg.* M. Čerkez, CNF 1/5429, GenBank Number: MT796099.

Discussion

All samples of *Coprinopsis alnivora* from Europe and the sample from the USA form a stable cluster on the phylogenetic tree and genetically represent a well-delimited species. In Europe, the connecting node, between the Austrian and Croatian samples, is the Slovak population. The sample most genetically related to the sample from the USA has been identified in Austria and the genetic distance is increasing towards Croatia. Although the number of samples is small, a correlation between genetic and geographical distance is observed, which is also typical in other organisms (e.g. Sharbel *et al.* 2000; Mills *et al.* 2007; Jenkins *et al.* 2010; Riginos *et al.* 2011).

European records of *C. alnivora* have contributed significantly to the knowledge of the morphology of the species. The only known North American (type) collection is scanty (consisting of fragments of a single basidioma; Uljé & Noordeloos 2000) and is not expected to provide a basis for good insight into infraspecific variability. European collections showed considerable variability in the size of the basidiomata and especially in the firmness of the universal veil. According to the original description by Van De Bogart (1976), his collection belongs to those with smaller basidiomata and rather a delicate veil on the pileus. He described the veil remnants on the stipe as prominent, loose, scaly, membranous, white annulus, 1 mm in width, which are very friable and soon disintegrate into small fragments. Loose (non-adherent) annulus is not recorded in European collections. Micromorphologically, the European collections of *C. alnivora* agree well with the original description (Van De Bogart 1976) and the holotype study (Uljé & Noordeloos 2000). At first glance, the shape of the basidiospores appears to be somewhat different because the above authors did not describe the spores as flattened. However, this character varies considerably among European collections and some of them have only slightly flattened basidiospores, which is easy to overlook. Furthermore, European records have vastly increased our knowledge of the ecology of *C. alnivora*. Before this study, it was known only from hard scarcely rotted wood (dead?) of *Alnus* sp. in Washington State (USA). Of the 11 European collections, nine fruited on living trees (of five species), while two fruited on fallen trees. All five host tree species are new for *C. alnivora*.

Morphologically and ecologically, *C. alnivora* is characterized by branched, weakly diverticulate, predominantly thin-walled veil hyphae; smooth basidiospores of rather variable shape but without an elevated porus (apical papilla), with an average Q value below 1.5, an average length between 8 and 9.5 µm, an average breadth between 6.4 and 7.5 µm, and an average width between 5.8 and 6.6 µm; the presence of clamp-connections; and lignicolous growth on living deciduous trees (mostly from decayed cavities or wounds) or (less often) on dead trees, without rhizomorphs in

the substrate. Based on branched and diverticulate veil hyphae, *C. alnivora* was placed in the subsection *Alachuani* of the genus *Coprinus s. l.* (Uljé & Noordeloos 2000), later section *Alachuanae* of the genus *Coprinopsis* (Schafer 2010). Several smooth-spored species from this, morphologically defined, section can grow on wood. *Coprinopsis episcopalis* (P.D. Orton 1957: 270) Redhead, Vilgalys & Moncalvo (2001: 228) differs by distinctive basidiospores with a broad, flat base and elevated porus (apical papilla). *Coprinopsis goudensis* (Uljé in Uljé & Bas 1993: 363) Redhead, Vilgalys & Moncalvo (2001: 228) differs by mostly more delicate basidiomata and more elongated and narrower basidiospores (average Q value 1.55–1.70, average breadth 5.0–5.6 µm; Uljé 2005). *Coprinopsis pseudofriesii* (Pilát & Svrček 1967: 140) Redhead, Vilgalys & Moncalvo (2001: 230) differs by mostly more delicate basidiomata and by veil hyphae which are strongly diverticulate and thick-walled (up to 1.5 µm; Uljé 2005). *Coprinopsis strossmayeri* (Schulzer 1879: 430) Redhead, Vilgalys & Moncalvo (2001: 231) differs by fasciculate growth, the presence of orange-brown or red-brown rhizomorphs in the substrate, and more elongated and narrower basidiospores (average Q value 1.50–1.55, average breadth 5.1–5.6 µm; Uljé 2005). *Coprinopsis urticicola* (Berkeley & Broome 1861: 376) Redhead, Vilgalys & Moncalvo (2001: 232) differs in having small basidiomata (pileus up to 13 mm wide) and the absence of clamp-connections (Uljé 2005). *Coprinopsis variegata* (Peck 1873: 79) Redhead, Vilgalys & Moncalvo (2001: 232) differs in having more elongate and narrower basidiospores (average Q greater than 1.60, breadth not exceeding 5.5 µm; Patrick 1979). However, the two most similar species are *C. clastophylla* (Maniotis 1964: 491) Redhead, Vilgalys & Moncalvo (2001: 227), and *C. cubensis* (Berkeley & Curtis 1869: 293) Redhead, Vilgalys & Moncalvo (2001: 227). *Coprinopsis clastophylla* is also the species closest to *C. alnivora* in our phylogenetic analysis. It can be distinguished by somewhat narrower basidiospores (up to 6.7 µm in breadth; Maniotis 1964). According to the morphological description by Pegler (1983; based on three collections from Cuba, Costa Rica and Venezuela), *C. cubensis* differs by smaller basidia (11–16 × 4–6 µm) and narrower veil hyphae (4–5 µm). Our revision of its holotype collection (K(M): 177239) confirmed that the basidiospores agree pretty well with those of *C. alnivora* in shape and size (7.0–*8.2*–9.7 × 5.6–*6.4*–7.6 µm, Q = 1.09–*1.27*–1.38, not distinctly flattened). The veil hyphae can be somewhat wider than those specified by Pegler (i.e. 1.5–6(–9) µm), which is still narrower than in *C. alnivora* (2–15(–22) µm). We could not see the basidia because the hymenium was in poor condition. We have tried to obtain the DNA sequence (ITS) from the holotype of *C. cubensis* for molecular genetic analysis (0.5 mg of the stipe), but we did not get a useful result. The obtained complete ITS and ITS2 sequences were obviously from fungal contamination (*Malassezia*) while the obtained ITS1 sequence belonged to the genus *Coprinellus* in the GenBank BLAST analysis, which is not in accordance with morphological characters of *C. alnivora* (probably contamination). Therefore, we leave open the possibility that *C. cubensis* and *C. alnivora* are conspecific, but we could not confirm that.

Furthermore, it seems that European collections of *C. alnivora* have often been confused with a species from another section, namely *C. mitrispora* (Bohus 1970: 18) L. Nagy, Vágvölgyi & Papp (in Nagy *et al.* 2013a: 120) (= *C. spelaiophila* (Bas & Uljé in Uljé & Noordeloos 1999: 179) Redhead, Vilgalys & Moncalvo (2001: 231)). *Coprinopsis mitrispora* belongs to the morphologically defined section *Lanatulae* which differs from *Alachuanae* by a veil hyphae made up of a chain of elongated, unbranched, non-diverticulate cells, constricted at septa. Although the veil structure of these two species, even macromorphologically, looks different (adpressed patches in *C. alnivora* vs. fibrillose squamules with elevated distal ends in *C. mitrispora*), the generally similar basidiomata and the same type of substrate can lead to confusion. Misidentification could be the reason why *C. alnivora* was not recorded earlier in Europe.

As mentioned previously, the morphologically defined section *Alachuanae* is not monophyletic. Wächter & Melzer (2020) proposed a new infrageneric classification of *Coprinopsis* (with 20 sections) in which the species from *Alachuanae* were divided in five sections, of which section *Coprinopsis* is the largest. According to Article 22.1. of the International Code of Nomenclature for Algae, Fungi, and Plants (Turland *et al.* 2018), the sectional name *Alachuanae* must be replaced by *Coprinopsis* because the type species of the genus (*C. friesii* (Quélet 1872: 129) P. Karsten 1881: 27) belongs to this section (Wächter & Melzer 2020). The ITS sequence (KY654717) of *C. clastophylla* placed in the section *Coprinopsis* in the phylogenetic study of Wächter & Melzer (2020), shows 96.73% identity (GenBank BLAST analysis) with the ITS sequence from the holotype of *C. alnivora*. Therefore, although Wächter & Melzer (2020) did not include *C. alnivora* in their phylogenetic study, we can assume that it belongs to the section *Coprinopsis*.

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References

Bender, H. & Melzer, A. (2020) Zwei neue Arten der Gattung *Coprinopsis*. *Zeitschrift für Mykologie* 87 (1): 31–46.

Berkeley, M.J. & Broome, C.E. (1861) Notices of British fungi (901–951). *Annals and Magazine of Natural History* 7: 373–382.

Berkeley, M.J. & Curtis, M.A. (1869) Fungi Cubenses (Hymenomycetes). *Journal of the Linnean Society, Botany* 10: 280–392. https://doi.org/10.1111/j.1095-8339.1868.tb00529.x

Bohus, G. (1970) Ergebnisse der auf die Hutpilze (Agaricales) bezüglichen systematischen und ökologischen Forschungen. VI. *Botanikai Közlemények* 57 (1): 13–22.

Chawngthu, Z., Vabeikhokhei, J.M.C. & Zothanzama, J. (2019) Molecular phylogenetic identification of wood inhabiting fungi isolated from Dampa Tiger reserve forest. *Journal of Emerging Technologies and Innovative Research* 6 (4): 941–949.

Chevallier, F.F. (1826) *Flore Générale des Environs de Paris*. Paris, 674 pp.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772. https://doi.org/10.1038/nmeth.2109

Fries, E. (1838) *Epicrisis Systematis Mycologici seu Synopsis Hymenomycetum*. Uppsala, 612 pp.

Garcia, G. & Vellinga, E.C. (2010) Un nouvelle espèce de coprin sur tiges de *Polygonatum multiflorum*: *Coprinopsis nevillei* sp. nov. *Bulletin Semestriel de la Fédération des Associations Mycologiques Méditerranéennes* 37: 37–58.

Gierczyk, V., Rodriguez-Flakus, P., Pietras, M., Gryc, M., Czerniawski, W. & Piatek, M. (2017) *Coprinopsis rugosomagnispora*: a distinct new coprinoid species from Poland (Central Europe). *Plant Systematics and Evolution* 303: 915–925. https://doi.org/10.1007/s00606-017-1418-7

Gonzalez del Val, A., Platas, G., Arenal, F., Orihuela, J.C., Garcia, M., Hernández, P., Royo, I., De Pedro, N., Silver, L.L., Young, K., Vicente, M.F. & Pelaez, F. (2003) Novel illudins from *Coprinopsis episcopalis* (syn. *Coprinus episcopalis*), and the distribution of illudin-like compounds among filamentous fungi. *Mycological Research* 107 (10): 1201–1209. https://doi.org/10.1017/s0953756203008487

Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696–704. https://doi.org/10.1080/10635150390235520

Jenkins, D.G., Carey, M., Czerniewska, J., Fletcher, J., Hether, T., Jones, A., Knight, S., Knox, J., Long, T., Mannino, M., McGuire, M., Riffle, A., Segelsky, S., Shappell, L., Sterner, A., Strickler, T. & Tursi, R. (2010) A meta-analysis of isolation by distance: Relic or reference standard for landscape genetics? *Ecography* 33: 315–320. https://doi.org/10.1111/j.1600-0587.2010.06285.x

Karsten, P.A. (1879) Rysslands, Finlands och den Skandinaviska halföns Hattsvampar. Förra Delen: Skifsvampar. *Bidrag till Kännedom av Finlands Natur och Folk* 32: 1–571.

Karsten, P.A. (1881) Hymenomycetes Fennici enumerati. *Acta Societatis Pro Fauna et Flora Fennica* 2 (1): 1–40.

Keles, A. (2019) New records of macrofungi from Trabzon province (Turkey). *Applied Ecology and Environmental Research* 17 (1): 1061–1069. https://doi.org/10.15666/aeer/1701_10611069

Kimura, M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120. https://doi.org/10.1007/BF01731581

Kumar, S., Stecher, G., Li, M., Knyaz, Ch. & Tamura, K. (2018) MEGA X: Molecular evolutionary genetics analysis across computing

platforms. *Molecular Biology and Evolution* 35: 1547–1549.
https://doi.org/10.1093/molbev/msy096

Kušan, I., Matočec, N., Jadan, M., Tkalčec, Z. & Mešić, A. (2018) An overview of the genus *Coprotus* (Pezizales, Ascomycota) with notes on the type species and description of *C. epithecioides* sp. nov. *Mycokeys* 29: 15–47.
https://doi.org/10.3897/mycokeys.29.22978

Larsson, E. & Örstadius, L. (2008) Fourteen coprophilous species of *Psathyrella* identified in the Nordic countries using morphology and nuclear rDNA sequence data. *Mycological Research* 112 (10): 1165–1185.
https://doi.org/10.1016/j.mycres.2008.04.003

Leigh, J.W. & Bryant, D. (2015) POPART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* 6: 1110–1116.
https://doi.org/10.1111/2041-210X.12410

Maniotis, J. (1964) The Coprinoid state of *Rhacophyllus lilacinus*. *American Journal of Botany* 51: 485–494.

Mentrida, S., Krisai-Greilhuber, I. & Voglmayr, H. (2015) Molecular evaluation of species delimitation and barcoding of *Daedaleopsis confragosa* specimens in Austria. *Österreichische Zeitschrift für Pilzkunde* 24: 173–179.

Mills, S., Lunt, D.H. & Gómez, A. (2007) Global isolation by distance despite strong regional phylogeography in a small metazoan. *BMC Evolutionary Biology* 7: 225.
https://doi.org/10.1186/1471-2148-7-225

Nagy, L.G., Desjardin, D.E., Vágvölgyi, C., Kemp, R. & Papp, T. (2013a) Phylogenetic analyses of *Coprinopsis* sections *Lanatuli* and *Atramentarii* identify multiple species within morphologically defined taxa. *Mycologia* 105 (1): 112–124.
https://doi.org/10.3852/12-136

Nagy, L.G., Házi, J., Szappanos, B., Kocsubé, S., Bálint, B., Rákhely, G., Vágvölgyi, C. & Papp, T. (2012) The evolution of defense mechanisms correlate with the explosive diversification of autodigesting *Coprinellus* mushrooms (Agaricales, Fungi). *Systematic Biology* 61 (4): 595–607.
https://doi.org/10.1093/sysbio/sys002

Nagy, L.G., Kocsubé, S., Papp, T. & Vágvölgyi, C. (2009) Phylogeny and character evolution of the coprinoid mushroom genus *Parasola* as inferred from LSU and ITS nrDNA sequence data. *Persoonia* 22: 28–37.
https://doi.org/10.3767/003158509X422434

Nagy, L.G., Urban, A., Orstadius, L., Papp, T., Larsson, E. & Vágvölgyi, C. (2010) The evolution of autodigestion in the mushroom family Psathyrellaceae (Agaricales) inferred from Maximum Likelihood and Bayesian methods. *Molecular Phylogenetics and Evolution* 57 (3): 1037–1048.
https://doi.org/10.1016/j.ympev.2010.08.022

Nagy, L.G., Vágvölgyi, C. & Papp, T. (2013b) Morphological characterization of clades of the Psathyrellaceae (Agaricales) inferred from a multigene phylogeny. *Mycological Progress* 12: 505–517.
https://doi.org/10.1007/s11557-012-0857-3

Nagy, L.G., Walther, G., Házi, J., Vágvölgyi, C. & Papp, T. (2011) Understanding the evolutionary processes of fungal fruiting bodies: correlated evolution and divergence times in the Psathyrellaceae. *Systematic Biology* 60 (3): 303–317.
https://doi.org/10.1093/sysbio/syr005

Örstadius, L., Ryberg, M. & Larsson, E. (2015) Molecular phylogenetics and taxonomy in Psathyrellaceae (Agaricales) with focus on psathyrelloid species: introduction of three new genera and 18 new species. *Mycological Progress* 14: 25.
https://doi.org/10.1007/s11557-015-1047-x

Orton, P.D. (1957) Notes on British agarics 1-5 (Observations on the genus *Coprinus*). *Transactions of the British Mycological Society* 40 (2): 263–276.

Osmundson, T.W., Robert, V.A., Schoch, C.L., Baker, L.J., Smith, A., Robich, G., Mizzan, L. & Garbelotto, M.M. (2013) Filling gaps in biodiversity knowledge for macrofungi: contributions and assessment of an herbarium collection DNA barcode sequencing project. *PLOS One* 8 (4): e62419.
https://doi.org/10.1371/journal.pone.0062419

Padamsee, M., Matheny, P.B., Dentinger, B.T. & McLaughlin, D.J. (2007) The mushroom family Psathyrellaceae: evidence for large-scale polyphyly of the genus *Psathyrella*. *Molecular Phylogenetics and Evolution* 46: 415–429.
https://doi.org/10.1016/j.ympev.2007.11.004

Patrick, W.W. Jr (1979) Comparative morphology and taxonomic disposition of *ebulbosus, quadrifidus*, and *variegatus* in the genus *Coprinus* (Agaricales). *Mycotaxon* 10 (1): 142–154.

Peck, C.H. (1873) Descriptions of new species of fungi. *Bulletin of the Buffalo Society of Natural Sciences* 1: 41–72.
https://doi.org/10.5962/bhl.title.58612

Pegler, D.N. (1983) Agaric Flora of the Lesser Antilles. *Kew Bulletin Additional Series* 9: 1–668.

Persoon, C.H. (1797) *Tentamen dispositionis methodicae Fungorum*. Leipzig, 76 pp.

Pilát, A. & Svrček, M. (1967) Revisio specierum sectionis *Herbicolae* Pil. & Svr. generis *Coprinus* (Pers. ex) S.F. Gray. *Česká Mykologie* 21 (3): 136–145.

Phookamsak, R., Hyde, K.D., Jeewon, R., Bhat, D.J., Jones, E.B.G., Maharachchikumbura, S.S.N., Raspé, O., Karunarathna, S.C., Wanasinghe, D.N., Hongsanan, S., Doilom, M., Tennakoon, D.S., Machado, A.R., Firmino, A.L., Ghosh, A., Karunarathna, A., Mešić, A., Dutta, A.K., Thongbai, B., Devadatha, B., Norphanphoun, C., Senwanna, C., Wei, D., Pem, D., Ackah, F.K., Wang, G., Jiang, H., Madrid, H., Lee, H.B., Goonasekara, I.D., Manawasinghe, I.S., Kušan, I., Cano, J., Gené, J., Li, J., Das, K., Acharya, K., Raj, K.N.A., Latha, K.P.D., Chethana, K.W.T., He, M., Dueñas, M., Jadan, M., Martin, M.P., Samarakoon, M.C., Dayarathne, M.C., Raza, M., Park, M.S., Telleria, M.T., Chaiwan, N., Matočec, N., de Silva, N.I., Pereira, O.L., Singh, P.N., Manimohan, P., Uniyal, P., Shang, Q., Bhatt, R.P., Perera, R.H., Alvarenga, R.L.M., Nogal-Prata, S., Singh, S.K., Vadthanarat, S., Oh, S., Huang, S., Rana, S., Konta, S., Paloi, S., Jayasiri, S., Jeon, S.J., Mehmood, T., Gibertoni, T.B., Nguyen, T.T.T., Singh, U., Thiyagaraja, T., Sarma, V.V., Dong, W., Yu, X., Lu, Y., Lim, Y.W., Chen, Y., Tkalčec, Z., Zhang, Z., Luo, Z., Daranagama, D.A., Thambugala, K.M., Tibpromma, S., Camporesi, E., Bulgakov, T.S., Dissanayake, A.J., Senanayake, I.C., Dai, D.Q., Tang, L., Khan, S., Zhang, H., Promputtha, I., Cai, L., Chomnunti, P., Zhao, R., Lumyong, S., Boonmee, S., Wen, T., Mortimer, P.E. & Xu, J. (2019) Fungal diversity notes 929–1035: taxonomic and phylogenetic contributions on genera and species of fungi. *Fungal Diversity* 95: 1–273. https://doi.org/10.1007/s13225-019-00421-w

Quélet, L. (1872) Les Champignons du Jura et des Vosges. *Mémoires de la Société d'Émulation de Montbéliard* 5 (2): 43–332.

Raut, J.K., Fukiharu, T., Shimizu, K., Kawamoto, S., Takeshige, S., Tanaka, C., Yamanaka, T. & Suzuki A. (2015) *Coprinopsis novorugosobispora* (Basidiomycota, Agricales), an ammonia fungus new to Canada. *Mycosphere* 6 (5): 612–619. https://doi.org/10.5943/mycosphere/6/5/10

Raut, J.K., Suzuki, A., Fukiharu, T., Shimizu, K., Kawamoto, S. & Tanaka, C. (2011) *Coprinopsis neophlyctidospora* sp. nov., a new ammonia fungus from boreal forests in Canada. *Mycotaxon* 115: 227–238. https://doi.org/10.5248/115.227

Redhead, S.A., Vilgalys, R., Moncalvo, J.M., Johnson, J. & Hopple, J.S. Jr (2001) *Coprinus* Pers. and the disposition of *Coprinus* species sensu lato. *Taxon* 50: 203–241. https://doi.org/10.2307/1224525

Riginos, C., Douglas, K.E., Jin, Y., Shanahan, D.F. & Treml, E.A. (2011) Effects of geography and life history traits on genetic differentiation in benthic marine fishes. *Ecography* 34: 566–575. https://doi.org/10.1111/j.1600-0587.2010.06511.x

Ronquist, F., Teslenko, M., Mark, P.V.D., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematic Biology* 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Schafer, D.J. (2010) Keys to sections of *Parasola, Coprinellus, Coprinopsis* and *Coprinus* in Britain. *Field Mycology* 11 (2): 44–51. https://doi.org/10.1016/j.fldmyc.2010.04.006

Schulzer, S. (1879) Mycologische Beiträge. III. *Verhandlungen der Zoologisch-Botanischen Gesellschaft Wien* 28: 423–436.

Sharbel, T.F., Haubold, B. & Mitchell-Olds, T. (2000) Genetic isolation by distance in *Arabidopsis thaliana*: biogeography and postglacial colonization of Europe. *Molecular Ecology* 9: 2109–2118. https://doi.org/10.1046/j.1365-294X.2000.01122.x

Singer, R. (1951) The Agaricales in modern taxonomy. *Lilloa* 22: 5–832.

Stamatakis, A. (2014) RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Turland, N.J., Wiersema, J.H., Barrie, F.R., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T.W., McNeill, J., Monro, A.M., Prado, J., Price, M.J. & Smith, G.F. (Eds.) (2018) *International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017.* Regnum Vegetabile 159. Koeltz Botanical Books, Glashütten. https://doi.org/10.12705/Code.2018

Uljé, C.B. (2005) 1. *Coprinus* Pers. *In*: Noordeloos, M.E., Kuyper, T.W. & Vellinga, E.C. (Eds.) *Flora Agaricina Neerlandica* 6. Taylor & Francis, Boca Raton, pp. 22–109.

Uljé, C.B. & Bas, C. (1993) Some new species of *Coprinus* from the Netherlands. *Persoonia* 15 (3): 357–368.

Uljé, C.B. & Noordeloos, M.E. (1999) Studies in *Coprinus* V—*Coprinus* section *Coprinus*. Revision of subsection *Lanatuli* Sing. *Persoonia* 17 (2): 165–199.

Uljé, C.B. & Noordeloos, M.E. (2000) Type studies in *Coprinus* subsection *Lanatuli*. *Persoonia* 17 (3): 339–375.

Van De Bogart, F. (1976) The genus *Coprinus* in Western North America, part 1: Section *Coprinus*. *Mycotaxon* 4 (1): 233–275.

Voto, P. (2019) Novelties in the family Psathyrellaceae. Part I. *Bollettino dell'Associazione Micologica ed Ecologica Romana* 107 (2): 94–95.

Vu, D., Groenewald, M., de Vries, M., Gehrmann, T., Stielow, B., Eberhardt, U., Al-Hatmi, A., Groenewald, J.Z., Cardinali, G., Houbraken, J., Boekhout, T., Crous, P.W., Robert, V. & Verkley, G. (2019) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. *Studies in Mycology* 92: 135–154.
https://doi.org/10.1016/j.simyco.2018.05.001

Wächter, D. & Melzer, A. (2020) Proposal for a subdivision of the family Psathyrellaceae based on a taxon-rich phylogenetic analysis with iterative multigene guide tree. *Mycological Progress* 19: 1151–1265.
https://doi.org/10.1007/s11557-020-01606-3

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In:* Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, pp. 315–322.
https://doi.org/10.1016/B978-0-12-372180-8.50042-1

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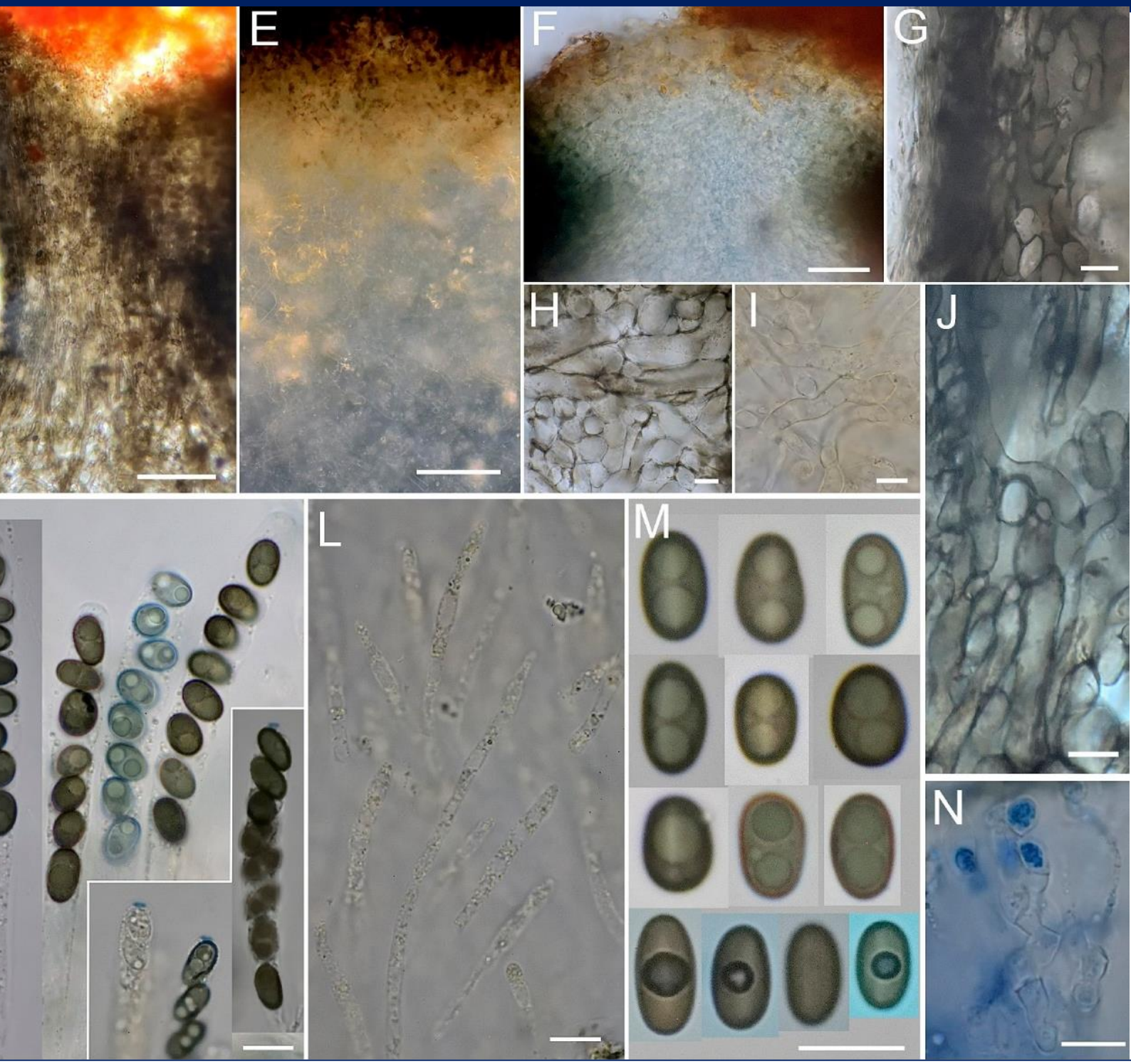

The Lignicolous Genus *Entonaema*: Its Phylogenetic–Taxonomic Position within *Hypoxylaceae* (*Xylariales*, *Fungi*) and an Overview of Its Species, Biogeography, and Ecology

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The Lignicolous Genus *Entonaema*: Its Phylogenetic–Taxonomic Position within *Hypoxylaceae* (*Xylariales*, *Fungi*) and an Overview of Its Species, Biogeography, and Ecology

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Abstract: The lignicolous saprotrophic genus *Entonaema* contains six formally accepted species: *E. liquescens* (type species), *E. cinnabarinum*, *E. globosum*, *E. dengii*, *E. moluccanum*, and *E. siamensis*. Its stromatic ascomata develop on the surface of dead wood remnants; they are rather large, globose to irregularly shaped, and vividly coloured. The fresh stroma interior is filled with a liquid matter. In early studies, the genus was considered to have a preference for tropical habitats, while in more recent field research, numerous collections have been added from warm, temperate areas of Europe, North America, and Asia. Our taxonomic and phylogenetic studies were based on freshly collected *E. cinnabarinum* from Croatia and *E. liquescens* from the USA. A phylogenetic study of the sequence alignment of four concatenated gene regions (ITS, LSU, *rpb2*, and *β-tub*) revealed the true taxonomic position of *Entonaema* within *Hypoxylaceae* (*Xylariales*), a sister to *Hypoxylon carneum*. Detailed macroscopic and microscopic descriptions of *E. cinnabarinum* are accompanied by drawings and colour photographs, while the study of *E. liquescens* is focused on stromatal microchemical reaction. With new information, the worldwide identification key to the putative species of *Entonaema* is proposed. Ecological data and biogeographical patterns were studied using all available and reliable sources of recorded data. Climatic preferences of the two most widespread *Entonaema* species, *E. liquescens* and *E. cinnabarinum*, are discussed in detail.

Keywords: biodiversity; climatic shift; key to the species; phylogeny; *Sordariomycetes*; taxonomy

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1. Introduction

The genus *Entonaema* Möller was established in 1901 [1], represented by *E. liquescens* Möller as a type species and *E. mesentericum* Möller as an additional species of the genus. The latter species was later justifiably removed from the genus [2]. The genus was retained as a member of *Xylariaceae* Tul. & C. Tul. in the same study, a taxonomic view supported by the majority of taxonomists (e.g., [3–9]). Recently, after detailed molecular phylogenetic study with a sufficient amount of xylarialean DNA sequences, *Entonaema* was assigned to the newly resurrected and emended family *Hypoxylaceae* DC [10].

As with the majority of xylarialean fungi, the genus is characterised by its lignicolous way of life [8], developing rather large, globose to irregularly shaped, vividly coloured stromata on the surface of dead wood remnants; the intact interior of the stroma is filled with a liquid matter, while the perithecial layer is subgelatinous when fresh but coriaceous and hard when dried. The perithecia are inserted into the stromatal cortex; they are monostichous and carbonaceous, and they contain cylindrical, pedicellate asci with an amyloid apical ring. Ascospores are one-celled, brownish, and equipped with a longitudinal germ slit exceeding one-half of the spore's length. Besides *E. liquescens*, five more species are accepted in the genus today: *E. cinnabarinum* (Cooke & Massee) Lloyd [11], *E. dengii* J.D. Rogers [5], *E. moluccanum* J.D. Rogers [5], *E. globosum* R. Heim [12], and *E. siamensis* Sihan., Thienh. & Whalley [13]. Only two out of the six species currently belonging to the genus

are biogeographically widespread, viz., *E. liquescens* and *E. cinnabarinum*. To date, a true phylogenetic position of the genus was, however, a matter of uncertainty [10,14,15]. Our study was significantly concentrated on the phylogenetic analyses as well as the stromatal pigments and anatomy of the newly collected, living material of the two aforementioned typical and most widespread *Entonaema* species in order to reveal the true affinities of the genus. The analysis of their ecological–biogeographical traits was conducted on the basis of all accessible and verifiable records worldwide, and their climatic preferences are discussed in detail. The worldwide identification key to the putative species of *Entonaema* is proposed.

2. Materials and Methods

2.1. Microscopic Studies

Microscopic characteristics based on living cells and tissues (*) were recorded using vital taxonomy methods [16], while those based on dead cells and tissues (†) were obtained from fixed fresh and dry materials. All described microscopic elements were observed in tap water (H_2O), and cytochemical and histochemical data were additionally observed in Lugol's solution (IKI), Brilliant Cresyl Blue (CRB), potassium hydroxide (5% and 10% KOH), and Cotton Blue (CB). Microscopic elements were studied with a Zeiss Axioskop 40 FL (Carl Zeiss AG, Oberkochen, Germany) transmission light microscope (bright-field, phase-contrast, and dark-field techniques) under magnifications up to 1000×. Drawings were made freehand to scale, and microphotographs were taken with Nikon D750 and Nikon Z6 (Nikon Corporation, Tokyo, Japan) cameras mounted on the camera adapter T2-T2 SLR 2.5× (Carl Zeiss AG, Oberkochen, Germany) attached to the microscope's trinocular tube. Characters of stromatal and hymenial elements were based on a minimum of three stromata from each collection. Spore measurements were based on samples of 150 fully mature, normally developed, living, and randomly selected ascospores. Measurements were taken directly using an ocular micrometre and from microphotographs using PIXIMÈTRE software ver. 5.10 [17] to an accuracy of 0.1 µm. Length, width, and length/width ratio ("Q" value) were given as follows: (min.) stat. min.—stat. mode—stat. max. (max.), where "min." = minimum (lowest measured value), "stat. min." = statistical minimum (arithmetic mean minus two times standard deviation), "stat. mode" = statistical mode, "stat. max." = statistical maximum (arithmetic mean plus two times standard deviation), and "max." = maximum (highest measured value). The dried material was deposited at the Croatian National Fungarium (CNF), Zagreb, Croatia.

2.2. Axenic Cultures

Ascospore germination of *E. cinnabarinum* was tested by inoculation of freshly ejected ascospores on potato dextrose agar (PDA, HiMedia Laboratories Pvt. Ltd., Mumbai, India), malt extract agar (MEA, HiMedia Laboratories Pvt. Ltd., Mumbai, India), and oatmeal agar (OA, after Samson et al. [18]), both with and without pretreatment with hydrochloric acid (pH = 3.3; 0.0005 M HCl) for two hours at 25 °C. Petri dishes were kept at 24 °C in the dark for 7 days.

2.3. DNA Extraction, PCR Amplification, and Sequencing

Genomic DNA was extracted from fresh tissue of stromata using an EZNA® HP Fungal DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's protocol, with time adjustment of lysis incubation to 1 h. Four gene regions, ITS (internal transcribed spacer region), LSU (28S large subunit of ribosomal DNA), *rpb2* (second largest subunit of the DNA-directed RNA polymerase II), and *β-tub* (beta-tubulin), were amplified using primer pairs ITS1F/ITS4 [19,20], LR0R/LR7 [21], RPB2-5F/RPB2-7cR [22], and T1/T2, T11/T22 [23], respectively. The 25 µL PCR mixtures contained 9.5 µL of ddH_2O, 12.5 µL of GoTaq® G2 Green Master Mix (Promega, Madison, WI, USA), 1 µL of DNA template, and 1 µL of each forward and reverse primer with a final concentration of 0.2 µM, respectively. The PCR amplification for ITS and LSU gene regions was performed using a touchdown

program: initial denaturation at 95 °C for 2 min; followed by 5 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 45 s (add −1 °C per cycle), extension at 72 °C for 1.5 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 45 s, extension at 72 °C for 1.5 min; and a final extension at 72 °C for 5 min. The PCR amplification of *rpb2* was set up as described by Liu et al. [22], and of *β-tub* as described by O'Donnell and Cigelnik [23]. Successful PCR products were purified using ExoSAP-IT™ (Thermo Fisher Scientific, Waltham, MA, USA) cleanup reagent following the manufacturer's protocol and sent to Macrogen Europe (Amsterdam, The Netherlands) for bidirectional Sanger sequencing.

2.4. Sequence Alignment and Phylogenetic Analysis

Sequence reads were assembled and edited using Geneious Prime 2023.0.4. (https://www.geneious.com, Biomatters, Auckland, New Zealand, accessed on 18 October 2022). Assembled sequences were deposited at the National Center for Biotechnology Information (NCBI) GenBank database.

A phylogenetic dataset comprised of 501 sequences of four gene regions (ITS, LSU, *rpb2*, *β-tub*) from 142 taxa was selected for further analyses (Table 1). The listed species of *Hypoxylaceae*, *Xylariaceae* and *Graphostromataceae* M.E. Barr, J.D. Rogers & Y.M. Ju in Table 1 originated from previously published studies. All published sequences from NCBI Nucleotide database resulting with '*Entonema*' as the genus name were also included in phylogenetic analyses. Sequences were aligned by each gene region using MAFFT v7.450 [24,25] available as a Geneious Prime plugin. After being aligned and trimmed, concatenation of ITS, LSU, *rpb2* and *β-tub* alignments was accomplished using Geneious Prime 2023.0.4. Concatenated alignment contained 5794 characters positions including gaps, with 974 character positions for ITS, 1778 characters positions for LSU, 1065 characters positions for *rpb2*, and 1977 characters positions for *β-tub*. *Pyriformiascoma trilobatum* Daranag., Camporesi & K.D. Hyde, *Diatrype disciformis* (Hoffm.) Fr., *Creosphaeria sassafra* Y.M. Ju, F. San Martín & J.D. Rogers, and *Calceomyces lacunosus* Udagawa & S. Ueda were selected as the outgroup for phylogenetic analyses following Wendt et al. [10].

Phylogenetic analyses were conducted using Maximum Likelihood (ML) analysis in IQTREE v1.6.12 [26,27] and a Bayesian Inference (BI) analysis in MrBayes 3.2.6 (Geneious plugin, [28]). The best model was selected by ModelFinder implemented in IQ-TREE separately considering the corrected Akaike, and Bayesian Information Criterion (cAIC, BIC). TIM2+F+I+G4 was selected as best model for both phylogenetic datasets. ML analysis was executed by applying the ultrafast bootstrap approximation with 1000 replicates. BI analysis was executed for 5,000,000 generations, sampling trees and other parameters every 1000 generations. The default numbers of chains (four) and heating parameters were used. Posterior probabilities (BPP) were calculated after burning the first 25% of the posterior sample. Phylogenetic trees were visualized and annotated using iTOL v6.5.4 [29] and FigTree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/, accessed on 22 October 2023).

2.5. The Analysis of Ecological and Biogeographical Traits

A thorough inspection of currently accessible information on *Entonaema* species record data revealed a huge potential because each of the previous studies has always treated only a very limited amount of collections. Record data were taken from all available scientific publications known to the authors and from the online database GBIF (www.gbif.org, accessed on 26 June 2023), as well as the data derived from our own research and that of our colleagues, as a source for better understanding of the species ecology and biogeography. Only those records that were identified by specialists or were accompanied with high quality macrophotographs showing certain *Entonaema* species and stromatal ontogeny were accepted for the analysis. Additionally, the record should have had a precise locality name or assigned geographic coordinates, accompanied with a record date to ascertain species phenology. This was a prerequisite for obtaining ecological data (habitat type,

vicinity of water bodies, elevation, etc.) for each record, using Google Earth Pro 7.3.6.9345 (Google LLC, Mountain View, CA, USA) software.

The same software was used to visualize 'Global_1986-2010_KG_5m.kmz' digital layer downloaded from https://koeppen-geiger.vu-wien.ac.at/present.htm (accessed on 26 June 2023) in order to ascribe the current Köppen–Geiger climate type to every *Entonaema* record for climatic characterisation. Obtained data were also compared to an older worldwide Köppen–Geiger climate classification made by Kottek et al. [30]. Abbreviations of climatic types follow the same reference.

Table 1. Species included in this study, associated voucher numbers, countries of origin, and GenBank accession numbers. Newly generated sequences are marked in bold. Abbreviations: HT = holotype, ET = epitype, IT = isotype, PT = paratype.

Taxa	Voucher	Country	ITS	LSU	*rpb2*	*β-tub*	Ref
Amphirosellinia fushanensis	HAST 91111209 HT	Taiwan	GU339496	N/A	GQ848339	GQ495950	[31]
Annulohypoxylon annulatum	CBS 140775 ET	Texas	KY610418	KY610418	KY624263	KX376353	[10,32]
Annulohypoxylon atroroseum	ATCC 76081	Thailand	AJ390397	KY610422	KY624233	DQ840083	[10,33]
Annulohypoxylon michelianum	CBS 119993	Spain	KX376320	KY610423	KY624234	KX271239	[10,32]
Annulohypoxylon moriforme	CBS 123579	France	KX376321	KY610425	KY624289	KX271261	[10,32]
Annulohypoxylon nitens	MFLUCC 12-0823	Thailand	KJ934991	KJ934992	KJ934994	KJ934993	[34]
Annulohypoxylon stygium	MUCL 54601	France	KY610409	KY610475	KY624292	KX271263	[10]
Annulohypoxylon truncatum	CBS 140778 ET	Texas	KY610419	KY610419	KY624277	KX376352	[10,32]
Astrocystis concavispora	MFLUCC 14-0174	Italy	KP297404	KP340545	KP340532	KP406615	[34]
Biscogniauxia arima	WSP 122 IT	Mexico	EF026150	N/A	GQ304736	AY951672	[31]
Biscogniauxia nummularia	MUCL 51395 ET	France	KY610382	KY610427	KY624236	KX271241	[10]
Brunneiperidium gracilentum	MFLUCC 14-0011 HT	Italy	KP297400	KP340542	KP340528	KP406611	[34]
Calceomyces lacunosus	CBS 633.88 HT	Japan	KY610397	KY610476	KY624293	KX271265	[10]
Camillea obularia	ATCC 28093	Puerto Rico	KY610384	KY610429	KY624238	KX271243	[10]
Collodiscula fangjingshanensis	GZU H0109 HT	China	KR002590	KR002591	KR002592	KR002589	[35]
Creosphaeria sassafras	STMA 14087	Argentina	KY610411	KY610468	KY624265	KX271258	[10]
Daldinia andina	CBS 114736 HT	Ecuador	AM749918	KY610430	KY624239	KC977259	[10,33,36]
Daldinia bambusicola	CBS 122872 HT	Thailand	KY610385	KY610431	KY624241	AY951688	[10,37]
Daldinia caldariorum	MUCL 49211	France	AM749934	KY610433	KY624242	KC977282	[10,33,36]
Daldinia concentrica	CBS 113277	Germany	AY616683	KY610434	KY624243	KC977274	[10,14,33]
Daldinia dennisii	CBS 114741 HT	Australia	JX658477	KY610435	KY624244	KC977262	[10,33,38]
Daldinia eschscholtzii	MUCL 45435	Benin	JX658484	KY610437	KY624246	KC977266	[10,33,38]
Daldinia loculatoides	CBS 113279 ET	UK	AF176982	KY610438	KY624247	KX271246	[10,39]
Daldinia macaronesica	CBS 113040 PT	Spain	KY610398	KY610477	KY624294	KX271266	[10]
Daldinia petriniae	MUCL 49214 ET	Austria	AM749937	KY610439	KY624248	KC977261	[10,33,36]
Daldinia placentiformis	MUCL 47603	Mexico	AM749921	KY610440	KY624249	KC977278	[10,33,36]
Daldinia pyrenaica	MUCL 53969	France	KY610413	KY610413	KY624274	KY624312	[10]
Daldinia steglichii	MUCL 43512 PT	Papua New Guinea	KY610399	KY610479	KY624250	KX271269	[10]
Daldinia theissenii	CBS 113044 PT	Argentina	KY610388	KY610441	KY624251	KX271247	[10]
Daldinia vernicosa	CBS 119316 ET	Germany	KY610395	KY610442	KY624252	KC977260	[10,33]
Diatrype disciformis	CBS 197.49	Netherlands	N/A	DQ470964	DQ470915	N/A	[40]

Table 1. *Cont.*

Taxa	Voucher	Country	ITS	LSU	*rpb2*	*β-tub*	Ref
Entonaema cinnabarinum	agtS377	Germany	AY616685	N/A	N/A	N/A	[14]
Entonaema cinnabarinum	**CNF 2/11046**	**Croatia**	**OQ863621**	**OQ863622**	**OQ877102**	**OQ877113**	**This study**
Entonaema cinnabarinum	**CNF 2/11047**	**Croatia**	**OQ863735**	**OQ864983**	**OQ877103**	**OQ877114**	**This study**
Entonaema cinnabarinum	**CNF 2/11052**	**Croatia**	**OQ864984**	**OQ865000**	**OQ877104**	**OQ877115**	**This study**
Entonaema cinnabarinum	**CNF 2/11053**	**Croatia**	**OQ869782**	**OQ869785**	**OQ877105**	**OQ877116**	**This study**
Entonaema liquescens	ATCC 46302	USA	KY610389	KY610443	KY624253	KX271248	[10]
Entonaema liquescens	agtS279	Germany	AY616686	N/A	N/A	N/A	[14]
Entonaema liquescens	**CNF 2/11263**	**USA**	**OQ869784**	**OQ865124**	**OQ877106**	**OQ877117**	**This study**
Entonaema liquescens	S.D. Russell iNaturalist # 91210856	USA	OM972573	N/A	N/A	N/A	[41]
Entonaema pallida	PP92a	Peru	FJ884093	FJ890379	N/A	N/A	[42]
***Entonaema*sp.**	JHGB08 1A	Peru	MH267933	N/A	N/A	N/A	[43]
***Entonaema*sp.**	AHB18 5B	Peru	MH267934	N/A	N/A	N/A	[43]
***Entonaema*sp.**	F5071	Panama	KF746156	N/A	N/A	N/A	[44]
Entonaema splendens	KA12-1283	South Korea	KR673521	N/A	N/A	N/A	[45]
Euepixylon sphaeriostomum	JDR 261	USA	GU292821	N/A	GQ844774	GQ470224	[31]
Graphostroma platystomum	CBS 270.87 HT	France	JX658535	DQ836906	KY624296	HG934108	[10,38,46,47]
Hypocreodendron sanguineum	JDR 169	Mexico	GU322433	N/A	GQ844819	GQ487710	[31]
Hypoxylon addis	MUCL 52797 HT	Ethiopia	KC968931	N/A	N/A	KC977287	[33]
Hypoxylon*aff. *rubiginosum	MUCL 57724	Iran	MT214999	MT214994	MT212237	MT212241	[48]
Hypoxylon*aff. *rubiginosum	MUCL 57725	Iran	MT215000	MT214995	MT212238	MT212242	[48]
Hypoxylon baihualingense	FCATAS 477 HT	China	MG490190	N/A	N/A	MH790276	[49]
Hypoxylon bellicolor	UCH 9543	Panama	MN056425	N/A	N/A	MK908139	[50]
Hypoxylon carneum	MUCL 54177	France	KY610400	KY610480	KY624297	KX271270	[10]
Hypoxylon cercidicola	CBS 119009	France	KC968908	KY610444	KY624254	KC977263	[10,33]
Hypoxylon chrysalidosporum	FCATAS 2710 HT	China	OL467294	OL615106	OL584222	OL584229	[51]
Hypoxylon crocopeplum	CBS 119004	France	KC968907	KY610445	KY624255	KC977268	[10,33]
Hypoxylon crocopeplum	**CNF 2/11316**	**Croatia**	**OQ865120**	**OQ869786**	**OQ877107**	**OQ877118**	**This study**
Hypoxylon crocopeplum	**CNF 2/11317**	**Croatia**	**OQ865187**	**OQ869787**	**OQ877108**	**OQ877119**	**This study**
Hypoxylon cyclobalanopsidis	FCATAS 2714 HT	China	OL467298	OL615108	OL584225	OL584232	[51]
Hypoxylon damuense	FCATAS 4207 HT	China	ON075427	ON075433	ON093251	ON093245	[52]
Hypoxylon damuense	FCATAS 4321	China	ON075428	ON075434	ON093252	ON093246	[52]
Hypoxylon duranii	YMJ 85	China	JN979414	N/A	N/A	AY951714	[37]
Hypoxylon eurasiaticum	MUCL 57720 HT	Iran	MW367851	N/A	MW373852	MW373861	[53]
Hypoxylon fendleri	MUCL 54792	France	KF234421	KY610481	KY624298	KF300547	[10,33]
Hypoxylon fragiforme	MUCL 51264 ET	Germany	KC477229	KM186295	KM186296	KX271282	[10,34,54]
Hypoxylon fraxinophilum	MUCL 54176 ET	France	KC968938	N/A	N/A	KC977301	[33]
Hypoxylon fuscum	CBS 113049 ET	France	KY610401	KY610482	KY624299	KX271271	[10]
Hypoxylon griseobrunneum	CBS 331.73 HT	India	KY610402	KY610483	KY624300	KC977303	[10,33]

Table 1. *Cont.*

Taxa	Voucher	Country	ITS	LSU	*rpb2*	*β-tub*	Ref
Hypoxylon guilanense	MUCL 57726 HT	Iran	MT214997	MT214992	MT212235	MT212239	[48]
Hypoxylon haematostroma	MUCL 53301 ET	France	KC968911	KY610484	KY624301	KC977291	[10,33]
Hypoxylon howeanum	MUCL 47599	Germany	AM749928	KY610448	KY624258	KC977277	[10,33,36]
Hypoxylon howeanum	**CNF 2/11315**	**Croatia**	**OQ865216**	**OQ865215**	**OQ877109**	**OQ877120**	**This study**
Hypoxylon hypomiltum	MUCL 51845	France	KY610403	KY610449	KY624302	KX271249	[10]
Hypoxylon investiens	CBS 118183	Malaysia	KC968925	KY610450	KY624259	KC977270	[10,33]
Hypoxylon isabellinum	STMA10247 HT	France	KC968935	N/A	N/A	KC977295	[33]
Hypoxylon lateripigmentum	MUCL 53304 HT	France	KC968933	KY610486	KY624304	KC977290	[10,33]
Hypoxylon lenormandii	CBS 119003	Ecuador	KC968943	KY610452	KY624261	KC977273	[10,33]
Hypoxylon liviae	CBS 115282 ET	Norway	NR155154	N/A	N/A	KC977265	[33]
Hypoxylon macrosporum	YMJ 47	Canada	N/A	N/A	N/A	AY951736	[37]
Hypoxylon monticulosum	MUCL 54604 ET	France	KY610404	KY610487	KY624305	KX271273	[10]
Hypoxylon musceum	MUCL 53765	France	KC968926	KY610488	KY624306	KC977280	[10,33]
Hypoxylon notatum	YMJ 250	USA	JQ009305	N/A	N/A	AY951739	[37]
Hypoxylon ochraceum	MUCL 54625 ET	France	KC968937	N/A	KY624271	KC977300	[10,33]
Hypoxylon papillatum	ATCC 58729 HT	USA	KC968919	KY610454	KY624223	KC977258	[10,33]
Hypoxylon perforatum	CBS 115281	France	KY610391	KY610455	KY624224	KX271250	[10]
Hypoxylon petriniae	CBS 114746 HT	France	NR155185	KY610491	KY624279	KX271274	[10,32]
Hypoxylon pilgerianum	STMA 13455	France	KY610412	KY610412	KY624308	KY624315	[10]
Hypoxylon porphyreum	CBS 119022	France	KC968921	KY610456	KY624225	KC977264	[10,33]
Hypoxylon pseudefendleri	MFLUCC 11-0639 HT	Thailand	KU940156	KU863144	N/A	N/A	[55]
Hypoxylon pseudofuscum	KR:0005879 HT	Germany	MW367857	MW367848	MW373858	MW373867	[53]
Hypoxylon pulicicidum	CBS 122622 HT	France	JX183075	KY610492	KY624280	JX183072	[10,56]
Hypoxylon rickii	MUCL 53309 ET	France	KC968932	KY610416	KY624281	KC977288	[10,33]
Hypoxylon rubiginosum	MUCL 52887 ET	Germany	KC477232	KY610469	KY624266	KY624311	[10,54]
Hypoxylon rubiginosum	MUCL 57727	Iran	MT214998	MT214993	MT212236	MT212240	[48]
Hypoxylon samuelsii	MUCL 51843 ET	France	KC968916	KY610466	KY624269	KC977286	[10,33]
Hypoxylon shearii*var. *minor	YMJ 29	Mexico	EF026142	N/A	N/A	AY951753	[31,37]
Hypoxylon sporistriatatunicum	UCH 9542 HT	Panama	MN056426	N/A	N/A	MK908140	[50]
Hypoxylon submonticulosum	CBS 115280	France	KC968923	KY610457	KY624226	KC977267	[10,33]
Hypoxylon texense	DSM 107933 HT	USA	MK287536	MK287548	MK287561	MK287574	[57]
Hypoxylon ticinense	CBS 115271	France	JQ009317	KY610471	KY624272	AY951757	[10,37]
Hypoxylon ticinense	**CNF 2/11314**	**Croatia**	**OQ869783**	**OQ865219**	**OQ877110**	**OQ877121**	**This study**
Hypoxylon trugodes	MUCL 54794 ET	Sri Lanka	KF234422	KY610493	KY624282	KF300548	[10,33]
Hypoxylon ulmophilum	YMJ 350	Russia	JQ009320	N/A	N/A	AY951760	[37]
Hypoxylon vogesiacum	CBS 115273	France	KC968920	KY610417	KY624283	KX271275	[10,32,33]
Hypoxylon wujianggense	GMBC0213 HT	China	MT568854	MT568853	MT585802	MT572481	[58]
Hypoxylon wuzhishanense	FCATAS 2708 HT	China	OL467292	OL615104	OL584220	OL584227	[51]
Hypoxylon wuzhishanense	FCATAS 2709	China	OL467293	OL615105	OL584221	OL584228	[51]
Hypoxylon zangii	FCATAS 4029 HT	China	ON075423	ON075429	ON093247	ON093241	[52]
Hypoxylon zangii	FCATAS 4319	China	ON075424	ON075430	ON093248	ON093242	[52]

Table 1. *Cont.*

Taxa	Voucher	Country	ITS	LSU	*rpb2*	*β-tub*	Ref
Jackrogersella cohaerens	CBS 119126	Germany	KY610396	KY610497	KY624270	KY624314	[10]
Jackrogersella minutella	CBS 119015	Portugal	KY610381	KY610424	KY624235	KX271240	[10,32]
Jackrogersella multiformis	CBS 119016 ET	Germany	KC477234	KY610473	KY624290	KX271262	[10,32,33]
Kretzschmaria deusta	CBS 163.93	Germany	KC477237	KY610458	KY624227	KX271251	[10,54]
Nemania bipapillata	HAST 90080610	Taiwan	GU292818	N/A	GQ844771	GQ470221	[31]
Nemania delonicis	MFLU 19-2124	Thailand	MW240613	MW240542	MW342617	MW775574	[59]
Nemania primolutea	HAST 91102001 HT	Taiwan	EF026121	N/A	GQ844767	EF025607	[31]
Obolarina dryophila	MUCL 49882	France	GQ428316	GQ428316	KY624284	GQ428322	[10,60]
Podosordaria muli	WSP 167 HT	Mexico	GU324761	N/A	GQ853038	GQ844839	[31]
***Podosordaria*sp.**	**CNF 2/11073**	**Croatia**	**OQ865223**	**OQ865228**	**OQ877111**	**OQ877122**	**This study**
Poronia punctata	CBS 656.78 HT	Australia	KT281904	KY610496	KY624278	KX271281	[10,61]
Pyrenopolyporus hunteri	MUCL 52673 ET	Ivory Coast	KY610421	KY610472	KY624309	KU159530	[10,32]
Pyrenopolyporus laminosus	MUCL 53305 HT	France	KC968934	KY610485	KY624303	KC977292	[10,33]
Pyrenopolyporus nicaraguensis	CBS 117739	Burkina Faso	AM749922	KY610489	KY624307	KC977272	[10,33,36]
Pyriformiascoma trilobatum	MFLUCC 14-0012 HT	Italy	KP297402	KP340543	KP340530	KP406613	[34]
Rhopalostroma angolense	CBS 126414	Ivory Coast	KY610420	KY610459	KY624228	KX271277	[10]
Rosellinia aquila	MUCL 51703	France	KY610392	KY610460	KY624285	KX271253	[10]
Rosellinia corticium	MUCL 51693	France	KY610393	KY610461	KY624229	KX271254	[10]
Rosellinia necatrix	CBS 349.36	Argentina	AY909001	KF719204	KY624275	KY624310	[10,62]
Rostrohypoxylon terebratum	JF-TH 06-04 HT	Thailand	DQ631943	DQ840069	DQ631954	DQ840097	[63]
Ruwenzoria pseudoannulata	MUCL 51394 HT	D. R. Congo	KY610406	KY610494	KY624286	KX271278	[10]
Sarcoxylon compunctum	CBS 359.61	South Africa	KT281903	KY610462	KY624230	KX271255	[10,61]
Stilbohypoxylon elaeicola	YMJ 173	France	EF026148	N/A	GQ844826	EF025616	[31]
Stilbohypoxylon quisquiliarum	YMJ 172	France	EF026119	N/A	GQ853020	EF025605	[31]
Thamnomyces dendroidea	CBS 123578 HT	France	FN428831	KY610467	KY624232	KY624313	[10,64]
Xylaria bambusicola	WSP 205 HT	Taiwan	EF026123	N/A	GQ844802	AY951762	[31]
Xylaria brunneovinosa	HAST 720 HT	France	EU179862	N/A	GQ853023	GQ502706	[31,65]
Xylaria discolor	HAST 131023 ET	USA	JQ087405	N/A	JQ087411	JQ087414	[66]
Xylaria hypoxylon	CBS 122620 ET	Sweden	KY610407	KY610495	KY624231	KX271279	[10,67]
Xylaria multiplex	HAST 580	France	GU300098	N/A	GQ844814	GQ487705	[31]
Xylaria polymorpha	MUCL 49884	France	KY610408	KY610464	KY624288	KX271280	[10]
Xylaria sicula	**CNF 2/11087**	**Croatia**	**OQ865227**	**OQ865230**	**OQ877112**	**OQ877123**	**This study**

3. Results

3.1. Molecular Phylogenetic Analyses

In this study, a total of 44 DNA sequences (11 ITS, 11 LSU, 11 *rpb2*, 11 *β-tub*) belonging to 11 fungal strains from the CNF were newly generated. Of the 11 fungal strains, four strains were identified as *E. cinnabarinum* (CNF 2/11046, 2/11047, 2/11052, 2/11053), two as *Hypoxylon crocopeplum* Berk. & M.A. Curtis (CNF 2/11316, 2/11317) and one each as *E. liquescens* (CNF 2/11263), *Hypoxylon howeanum* Peck (CNF 2/11315), *Hypoxylon ticinense* L.E. Petrini (CNF 2/11314), *Podosordaria* sp. (CNF 2/11073), and *Xylaria sicula* Pass. & Beltrani (CNF 2/11087). Associated accession numbers are marked in bold in Table 1.

The results of the phylogenetic analyses were similar to those previously published by Wendt et al. [10], Pourmoghaddam et al. [48], Song et al. [52] and Ma et al. [51]. Only significant branch support values of Bayesian posterior probability (BI-PP $\geq$ 0.95) and ultrafast bootstrap support (ML-BP $\geq$ 70%) are shown in the phylogram (Figure 1).

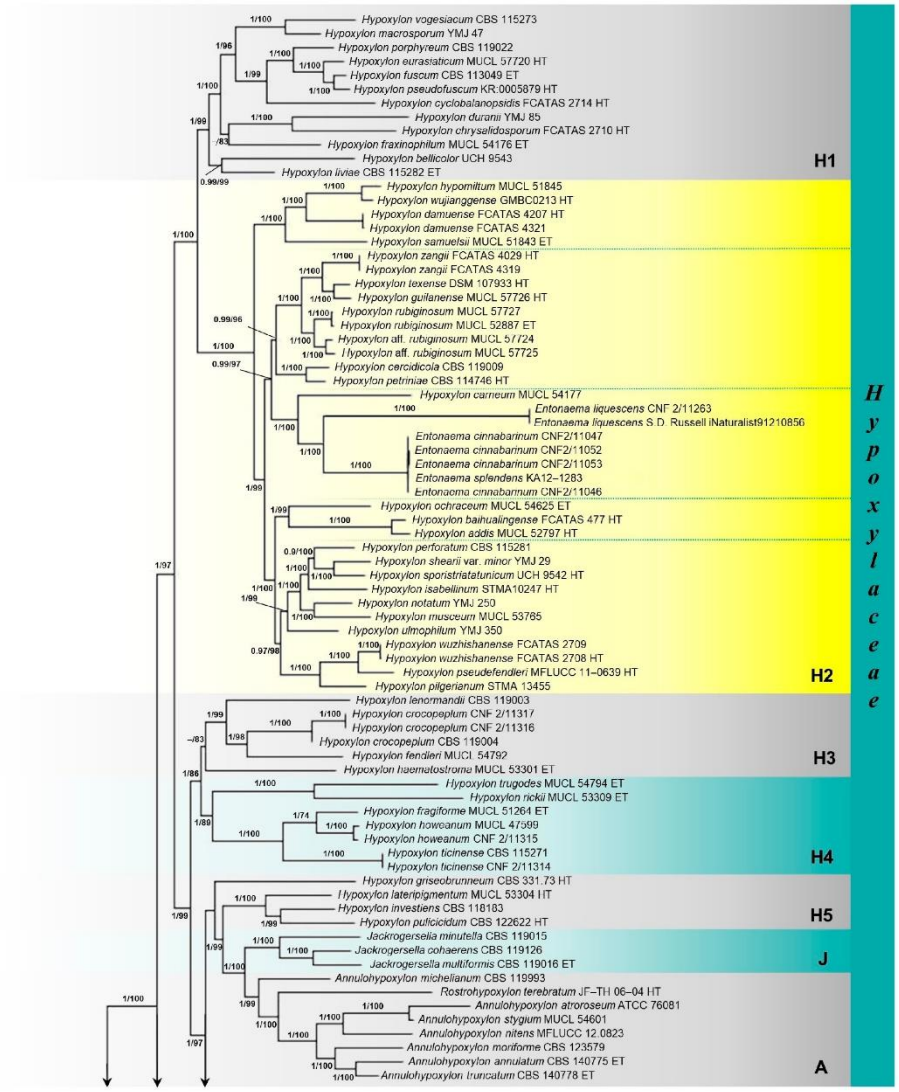

Figure 1. *Cont.*

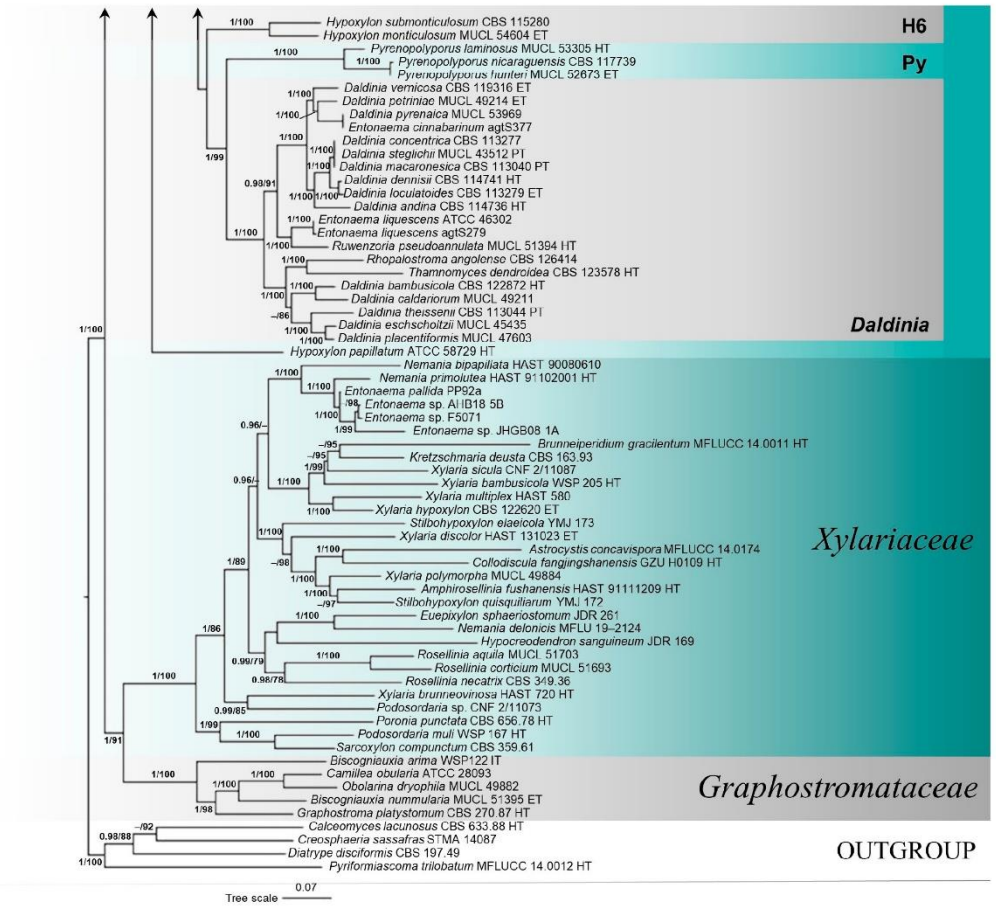

Figure 1. Phylogenetic tree of family *Hypoxylaceae*, based on Bayesian Inference (BI) and Maximum Likelihood (ML) analyses of concatenated four-gene (ITS, LSU, *rpb2*, *β-tub*) sequence alignment. Significant branch support values, Bayesian posterior probability (BI-PP $\geq$ 0.95) and ultrafast bootstrap support (ML-BP $\geq$ 70%), are presented at the nodes. Abbreviations: HT = holotype, ET = epitype, IT = isotype, PT = paratype.

The four-gene phylogeny revealed three main clades belonging to the families *Hypoxylaceae*, *Xylariaceae*, and *Graphostromataceae*. The most represented genus of *Hypoxylaceae* in the phylogenetic analysis was *Hypoxylon* Bull. with 76 strains, followed by 14 strains of *Daldinia* Ces. & De Not. and *Entonaema*, nine strains of *Annulohypoxylon* Y.M. Ju, J.D. Rogers & H.M. Hsieh, three strains of *Jackrogersella* L. Wendt, Kuhnert & M. Stadler, and *Pyrenopolyporus* Lloyd, and representative strains of *Rhopalostroma* D. Hawksw., *Thamnomyces* Ehrenb., *Ruwenzoria* J. Fourn., M. Stadler, Læssøe & Decock, and *Rostrohypoxylon* J. Fourn. & M. Stadler. Phylogeny revealed paraphyly of the genus *Hypoxylon* (H1-H6) with the genera *Annulohypoxylon* (A), *Daldinia*, *Entonaema*, *Jackrogersella* (J), *Pyrenopolyporus* (Py), and *Rhopalostroma*, *Thamnomyces*, and *Ruwenzoria* (*Daldinia* clade) embedded in the *Hypoxylaceae* clade.

The *Hypoxylon* H2 clade consisted of five strongly supported groups. Newly sequenced strains of *Entonaema* from the CNF, along with *E. splendens* KA12-1283 and *E. liquescens* S.D. Russell iNaturalist91210856, were phylogenetically very well differentiated (BI-PP = 1, ML-BP = 100) from a single lineage of *Hypoxylon carneum* Petch, in a strongly supported (BI-PP = 1, ML-BP = 100) subclade of the H2 clade. *Entonaema cinnabarinum* (CNF 2/11046, 2/11047, 2/11052, and 2/11053) clustered with *E. splendens* KA12-1283 and formed a maximally supported monophyletic clade (BI-PP = 1, ML-BP = 100) with *E. liquescens* CNF 2/11263 and the sister strain *E. liquescens* S.D. Russell iNaturalist91210856.

However, the *Entonaema* strains from the GenBank (with the exception of *E. splendens* KA12-1283) were distributed among other clades in the phylogenetic tree. In the *Daldinia* clade of *Hypoxylaceae*, *E. cinnabarinum* agtS377 recovered in a strongly supported monophyletic clade (BI-PP = 1, ML-BP = 100) with the epitype of *D. vernicosa* Ces. & De Not. (CBS 119316), the epitype of *D. petriniae* Y.M. Ju, J.D. Rogers & F. San Martín (MUCL 49214), and *D. pyrenaica* M. Stadler & Wollw. (MUCL 53969). Also, *E. liquescens* ATCC 4630 and *E. liquescens* agtS279 formed a monophyletic group (BI-PP = 1, ML-BP = 100) with *Ruwenzoria pseudoannulata* J. Fourn., M. Stadler, Læssøe & Decock (MUCL 51394) in the *Daldinia* clade. *Entonaema* sp. F5071, *E. pallida* PP92a, *Entonaema* sp. AHB18 5B, and *Entonaema* sp. JHGB08 1A were recovered in a phylogenetically fully supported clade (BI-PP = 1, ML-BP = 100) with the holotype strain of *Nemania primolutea* Y.M. Ju, H.M. Hsieh & J.D. Rogers (HAST 91102001) and *N. bipapillata* (Berk. & M.A. Curtis) Pouzar (HAST 90080610) in the *Xylariaceae* (Figure 1).

3.2. Taxonomy

Entonaema Möller, Bot. Mitt. Trop. 9: 306 (1901)

Generic diagnostic characters: Stromata pulvinate, subglobose to globose, often becoming irregularly lobed and/or wrinkled, especially at the tapered base, arising from woody substrates, most often from coarse woody debris. During the development, the enlarging cavity becoming filled with watery liquid. Stroma entonaematoid: stromatal flesh gelatinous without any concentric zonation, with ±vividly coloured surface until reaching late sporulation phase, when gradually turn to a horny or hard consistence and becoming wrinkled and carbonaceus due to the deposition of melanin pigments. Perithecia monostichous, developing immediately below bright coloured outer cortex. Interperithecial tissue ± carbonaceous at least around perithecial walls and in subperithecial layer (between perithecial bases and gelatinous inner fleshy layer). Ostioles inconspicuous, punctiform, umbilicate to papillate. Asci cylindric with tapered base arising from croziers, apically with amyloid ring. Ascospores unicellular, walls with a shade of brown, with ±longitudinal ventral germ slit. Stromatal pigments of mitorubrin/rubiginosin type (azaphilones) are present in three species that are so far certain members of the genus confirmed either by phylogenetic (this paper) or HPLC analyses [2], viz. *E. liquescens* (type), *E. cinnabarinum*, and *E. globosum*. All three species also possess yellowish-orange to orange or rusty red extractable stromatal pigments and an indehiscent perispore in 10% KOH.

Anamorph: when cultivated, they often contain contamination, often of *Daldinia* spp., in need of thorough reinvestigation (see text below).

Notes: Three certain members of *Entonaema* (*E. liquescens*, *E. cinnabarinum* and *E. globosum*) differ from the most similar entonaematoid species from the genus *Xylaria* (*X. mesenterica*, *X. telfairii* (Berk.) Sacc. and allies) producing voluminous stromata with azonate and gelatinous to liquid interior, by orange to red KOH-extractable pigments vs. greenish-yellow ones, and by mitorubrin/rubiginosin-type metabolites, that lack in *Xylaria*. On the other hand, the three *Entonaema* spp. differ from the most phylogenetically related *Hypoxylon carneum* in having entonaematoid stromata with orange to red KOH-extractable pigments, while *H. carneum* has hypoxyloid, flat-pulvinate stromata with dark brown, thin (~200 µm thick), hard tissue below the perithecia and livid-violet KOH-extractable pigments, which are absent in aged material [48]. Contrary to *Entonaema* spp., azaphilone metabolites are not present in *H. carneum*.

Entonaema cinnabarinum (Cooke & Massee) Lloyd, Mycol. Writ. 7(69): 1203 (1923); Figures 2–4 and 5J–L.

Basionym: *Xylaria cinnabarina* Cooke & Massee, Grevillea 15(76): 101 (1887)

= *Sarcoxylon aurantiacum* Pat., Bull. Soc. mycol. Fr. 27(3): 331 (1911)

≡ *Entonaema aurantiacum* (Pat.) Lloyd, Mycol. Writ. 7(69): 1203 (1923)

Stromata: globose to irregularly convoluted, often constricted to the cerebriform at the base, *28–80 × 16–72 mm, when immature surface yellowish-cream to pale rosy, on smearing apricot orange, at maturity surface yellow-ochre to rosaceous-orange, fulvous, dull brick red, often cinnabar red around the ostioles, ostioles and some areas between perithecia dusted blackish due to the ejected spores, with age becoming brownish-orange to reddish-brown, surface finely pruinose in younger stages, becoming smooth and often cracked with age. Ostioles punctate to papillate, rounded. Interior hollow and filled with pale yellow translucent viscose liquid; in section outer cortex orange to cinnabar red, 0.1–0.2 mm thick, covered with dull yellow detachable pruinose matter, beneath is a tough layer, 0.8–1 mm thick, composed of whitish to pale grey interperithecial tissue blackening with age, and a thin carbonaceous layer (in which perithecia are embedded) continuing to form a black layer underlying perithecial bases; perithecia globose to ellipsoid, black when mature; below the perithecial layer there is 2–6 mm thick, pale yellow to olivaceous, gelatinous, elastic, semitranslucent layer. In 5% and 10% KOH, the cortex is strongly brick red, liquid slightly discolouring, flesh apricot.

Ascomatal structures: Perithecia ranging from globose through ellipsoid to cylindrical, (350–)430–690 µm high, 340–660 µm wide, ostioles *95–120(–150) µm wide, periphyses hyaline eguttulate, cylindrical, apically obtusely tapered to sublanceolate, simple, one to few celled, flexuous, *2.5–5.2 µm wide. Asci cylindrical to narrowly clavate, 8-spored, †100–136 × 7.3–9 µm, *11.4–12.8 µm wide, delicate walled, wall persistent, not evanescent, apex thin-walled, apical dome barely visible, when *mature subtruncate-obtuse, †rounded to obtuse tapered, in IKI moderately (2bb) euamyloid, reaction zone simple, 2–2.4 µm wide, 1–1.2 µm high, ascoplasm highly vacuolate in all developmental phases, arising from Xylaria-type croziers, cells with weakly refractive globules. Ascospores brownish- olive to brownish-grey, dorsiventrally almost radially symmetrical, in profile slightly inequilateral, ellipsoid to suboblong with blunt ends, 1-celled, (8.1–)8.5–9.8–11.5(–12.1) × (4.7–)5–5.9–6.8(–7.1) µm, Q = (1.34–)1.38–1.73–2.02(–2.16), (1)2(3)-guttulate, without sheath, germ slit ventral, longitudinal to ± oblique and almost straight, 2/3 to almost whole spore length, spore wall thin, ±0.2 µm thick, sporoplasm contain a deBary bubble in CB, perispore smooth, in 10% KOH indehiscent.

Stromatal tissue: Cortex (crust) covered with rusty red granules, mainly consisting of ± homogenous block of highly refractive resin-like 50–210 µm thick layer, interspersed with locally densely set, hyaline, thin-walled sparingly septate cylindrical sinuous hyphae with blunt apices, hyphae †2.4–6.5 µm wide. Interperithecial tissue *440–720 µm thick, mainly composed of vertically oriented *textura prismatica* with ± thin-walled cells encrusted with very fine minute blackish granules, cells *3–7.7 µm wide, walls 0.3–0.5 µm thick. Perithecial walls ca. *50–80 µm thick, mainly composed of *textura angularis-prismatica*, walls of the same type as in interperithecial tissue, cells *7.5–16.1 × 2.9–9.5 µm wide. Interperithecial tissue below perithecia gradually turn into subperithecial layer, 190–380 µm thick, which is composed of *textura fascicularis-intricata*, cells *7.5–16.5 µm wide, walls thickened and highly melanised, and a thin layer of *textura oblita* with intricate cell organization, cells *3.3–11 µm wide, walls hyaline, moderately refractive, gelified, up to 2.6 µm thick. Internal tissue of *textura gelatinosa*, hyphae widely spaced, hyaline, thin-walled, delicate, cells *3–8.8 µm wide, interspersed with rich gel.

Anamorph: not obtained on PDA, MEA, and OA.

Material examined:

CROATIA. Zagreb County: Malinje forest, Crna Mlaka near Jastrebarsko, 109 m a.s.l., 45.606817° N, 15.71695° E, on the lying trunk of *Quercus robur* in an old growth forest of *Q. robur*, *Fraxinus angustifolia*, *Ulmus minor*, *Carpinus betulus*, *Tilia* sp., 5 July 2009,

M. Čerkez (CNF 2/8250); *ibid.* 45.605918° N, 15.716523° E, on the fallen branch of *U. minor*, 20 September 2009, *N. Matočec* & *M. Čerkez* (no voucher), *ibid.* 45.608199° N, 15.715229° E, on the fallen thick branch of *Q. robur*, 29 October 2020, *I. Kušan & N. Matočec* (CNF 2/11052), *ibid.* 45.608048° N, 15.715265° E, on the fallen thick branch of *Q. robur*, 29 October 2020, *I. Kušan & N. Matočec* (CNF 2/11053). Sisak-Moslavina County: Lonjsko Polje Nature Park, Opeke area near Kraljeva Velika, 95 m a.s.l., 45.370119° N, 16.820806° E, on the fallen semi-decorticated thin branch of *C. betulus* in a forest of *Q. robur*, *F. angustifolia* and *C. betulus*, 3 October 2020, *J. Marković* (CNF 2/11046); *ibid.* 45.373779° N, 16.821250° E, on the lying trunk of *F. angustifolia*, 16 October 2021, *I. Kušan & N. Matočec*, (no voucher); Trebeški đol area near Kraljeva Velika, 95 m a.s.l., 45.369202° N, 16.780788° E, on the fallen corticated thin branch of *Salix* sp. in a forest of *Q. robur*, *C. betulus* with *Salix* sp., 17 October 2020, *M. Josipović*, (CNF 2/11047); Tena's walking path near Ilova, 99 m a.s.l., 45.433639° N, 16.823863° E, on the lying trunk of *Fraxinus angustifolia* in an old growth forest of *Q. robur*, *F. angustifolia* and *Ulmus minor*, 28 September 2021, *J. Marković*, *I. Kušan & N. Matočec*, (CNF 2/11267). This species was here recorded for the first time for Croatia.

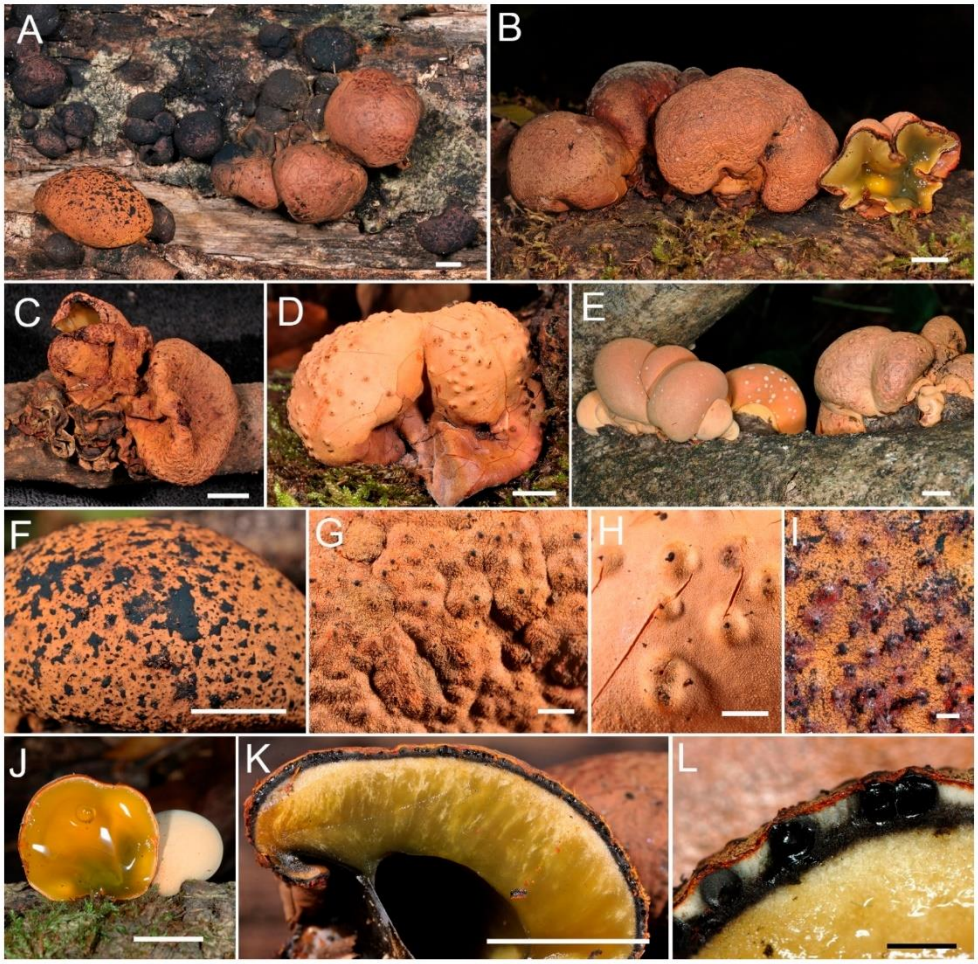

Figure 2. *Entonaema cinnabarinum*. (**A**) Stromata associated with *Daldinia childiae* J.D. Rogers & Y.M. Ju. (**B**–**D**) Mature stromata. (**E**) Young stromata. (**F**) Surface of the stroma dusted with ascospores. (**G**–**I**) Perithecial mounds with ostioles. (**J**) Section through young stroma. (**K**,**L**) Section through mature stroma. (**A**,**F**,**G**,**K**) CNF 2/11046; (**B**) (Croatia, 16.10.2021., no voucher); (**C**) CNF 2/11047; (**D**,**H**) CNF 2/11052; (**E**,**J**) (Croatia, 20.9.2009., no voucher); (**I**,**L**) CNF 2/8250. Bars: (**A**–**F**,**J**,**K**) = 1 cm; (**G**–**I**,**L**) = 1 mm. Photo: N. Matočec & I. Kušan.

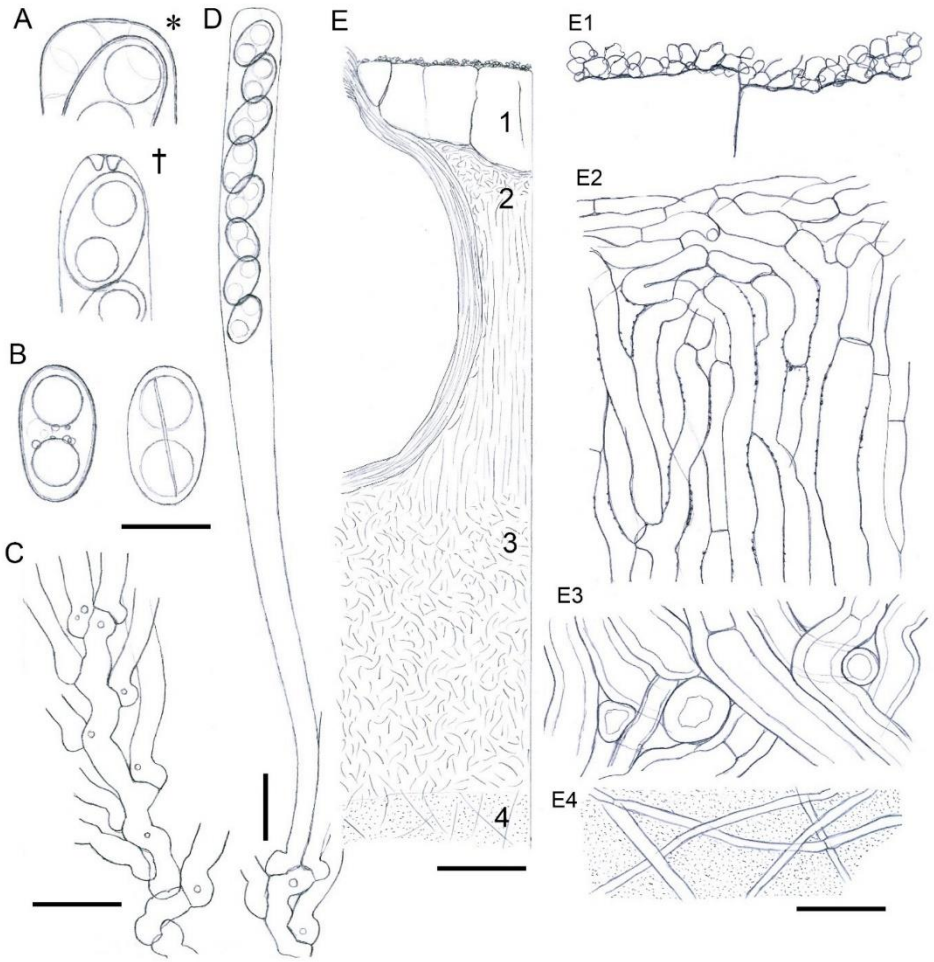

Figure 3. *Entonaema cinnabarinum* (CNF 2/8250). (**A**) Living (*) and dead (†) ascal apices. (**B**) Ascospores in frontal and dorsiventral view with a visible germ slit. (**C**) Ascogenous system. (**D**) Ascus. (**E**) Stromatal section ((**E1**) stromatal surface, (**E2**) interperithecial tissue, (**E3**) lower part of subperithecial layer, (**E4**) internal tissue). Bars: (**A**,**B**) = 5 μm; (**C**,**D**,**E1**–**E4**) = 10 μm; (**E**) = 100 μm. Del. N. Matočec.

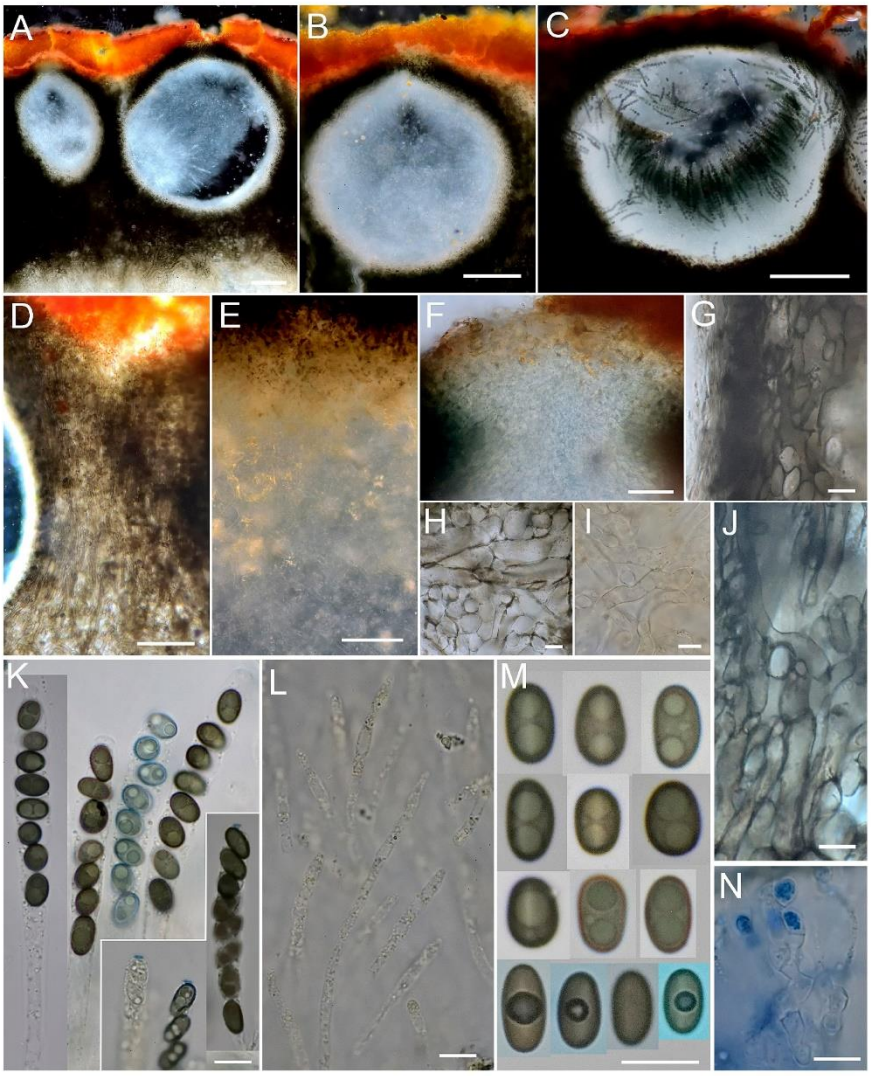

Figure 4. *Entonaema cinnabarinum*. (**A**–**C**) Sections through perithecia. (**D**) Interperithecial tissue. (**E**) Subperithecial layer (upper part) and internal tissue (lower part). (**F**) Ostiole. (**G**) Perithecial wall. (**H**) Cells of the subperithecial layer. (**I**) Cells of the internal tissue. (**J**) Cells in the interperithecial tissue. (**K**) Asci in H_2O and IKI. (**L**) Periphyses. (**M**). Ascospores in H_2O and CB (last row). (**N**) Croziers in CRB. (**A**,**B**,**F**,**K**–**M**) CNF 2/11046; (**C**,**N**) CNF 2/11047; (**D**,**E**,**G**–**J**) CNF 2/11053. Bars: (**A**–**E**) = 100 µm; (**F**) = 20 µm (**G**–**N**) = 10 µm. Photo: N. Matočec & I. Kušan.

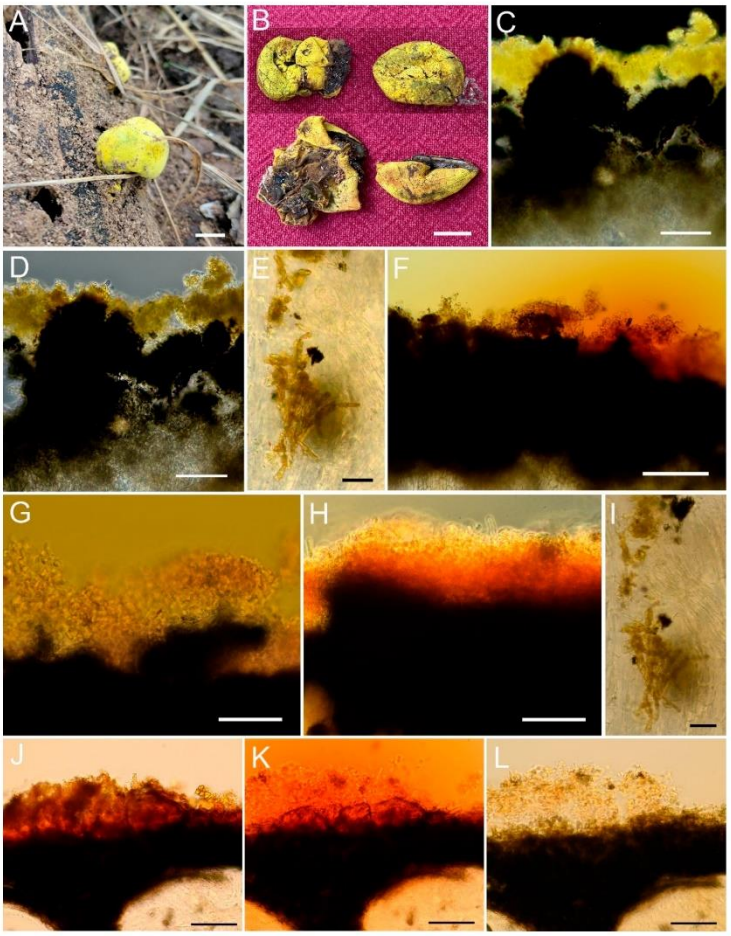

Figure 5. (**A–I**) *Entonaema liquescens* (CNF 2/11263), (**J–L**) *E. cinnabarinum* (CNF 2/11267). (**A**) Stromata *in situ*. (**B**) Stromata *ex situ*. (**C**,**D**) Lemon yellow pigments on the stromatal surface in H_2O (dark field, phase contrast). (**E**) Crystalloid pigments embedded inside interperithecial tissue (H_2O). (**F**) Pigment reaction upon adding 10% KOH. (**G**) Pigment soluble phase extracted in 10% KOH. (**H**) Pigment insoluble phase becoming rusty orange in 10% KOH. (**I**) Crystalloid pigments embedded inside interperithecial tissue in 10% KOH. (**J**) Rusty orange pigment granules on the stromatal surface in H_2O. (**K**) Pigment reaction upon adding 10% KOH. (**L**) Hyphal cover completely left without pigments after 10% KOH treatment. Bars: (**A**,**B**) = 1 cm; (**C**,**D**,**F–H**,**J–L**) = 50 µm; (**E**,**I**) = 10 µm. Photo: (**A**) T. Gonzales; (**B**) B. Bunyard; (**C–L**) N. Matočec & I. Kušan.

Entonaema liquescens Möller, Bot. Mitt. Trop. 9: 307 (1901); Figure 5A–I
= *Xylaria splendens* Berk. & M.A. Curtis, J. Linn. Soc., Bot. 10(46): 382 (1868)
≡ *Entonaema splendens* (Berk. & M.A. Curtis) Lloyd, Mycol. Writ. (Cincinnati) 7(69): 1202 (1923)
≡ *Glaziella splendens* (Berk. & M.A. Curtis) Berk., in Glaziou, Vidensk. Meddel. Dansk Naturhist. Foren. Kjøbenhavn 80: 31 (1879)

NOTE: The studied collection of *E. liquescens* consisted of two stromata, both immature. Perithecia and ascal structures were found only in an immature stage, without any traces of developed ascospores. It was stromata globose to slightly cerebriform, 22–29 × 17–20 mm, lemon yellow, and hollow. Pigments on the stromatal surface were lemon yellow in H_2O (best visible in dark field, Figure 5C). After adding 5% KOH and 10% KOH, they extract a yellowish-orange to rusty orange colour to the medium, while the remaining pigments that are fixed in the cortex turn rusty orange (Figure 5H). The crystalloid pigments embedded in interperithecial tissue remain unchanged (Figure 5E,I). Since *E. liquescens* and *E. cinnabarinum* have very similar microscopical characters usually used in species distinctions, the two species differ sharply in their microchemical traits. In contrast to *E. liquescens*, *E. cinnabarinum* lacks an insoluble pigment phase whereby the cortical layer remains subhyaline after KOH treatment (Figure 5L). Additionally, the two species differ by the pigment granules colour in *H_2O, which is lemon yellow in *E. liquescens* (Figure 5C,D) and rusty red in *E. cinnabarinum* (Figure 5J).

Material examined: USA. Kansas: Morris Co., Council Grove Reservoir, on the fallen decorticated branch of a *Quercus* sp. in a forest of *Quercus stellata* and *Q. marilandica*, 18 September 2021, *T. Gonzales*, (CNF 2/11263).

Worldwide identification key to the putative species of *Entonaema*

(1) Stromatal extractable pigments greenish yellow in 10% KOH, but red in NH3.....*Xylaria* p.p.
- Stromatal extractable pigments orange to brick red or entirely absent in 10% KOH, orange in NH3.....*Entonaema* (2)
(2) Stromatal orange to red pigment granules present in the section immediately beneath the stromatal surface and around the perithecial ostioles, stromatal extractable pigments in 10% KOH brick red.....3
- Stromatal pigment granules in the section of the stromatal cortex yellow, green, or absent, stromatal extractable pigments in 10% KOH yellowish-orange to orange, or absent.....4
(3) Stromatal pigment in the section immediately beneath the stromatal surface and around the perithecial ostioles rusty orange, ostioles papillate, perithecia 300–600 µm in diam., ascospores 9–13 µm long.....*E. cinnabarinum**
- Stromatal pigment in the section immediately beneath the stromatal surface and around the perithecial ostioles blood red, ostioles umbilicate, perithecia 100–300 µm in diam., ascospores 8–10 µm long.....*E. globosum*
(4) Stromatal surface with olivaceous tint in maturity, perithecia 200–500 µm in diam., ascospores cylindrical with ± blunt ends, 8–13 × 3.5–6.5 µm; stromatal extractable pigments orange in 10% KOH, but not tested in *E. siamensis*.....5
- Stromatal surface yellowish-tan or dark reddish-brown in maturity, perithecia 500–1000 µm in diam., ascospores ellipsoid with subacute ends, 13–18 × 6.5–8 µm, stromatal extractable pigments absent with 10% KOH.....6
(5) Stromatal pigment in the section immediately beneath the stromatal surface and around the perithecial ostioles vividly yellow, ascospores 5–6.5 µm wide, asci 6–10 µm wide.....*E. liquescens**
- Stromatal pigment in the section immediately beneath the stromatal surface and around the perithecial ostioles consists of green granules, ascospores 3.5–5 µm wide, asci 5–6 µm wide.....*E. siamensis*
(6) Stromatal surface dark reddish-brown, perithecial ostioles prominently papillate, ascospores up to 15 µm long.....*E. dengii*
- Stromatal surface yellowish-tan with or without reddish-orange tinges, perithecial ostioles inconspicuous, punctate, ascospores always exceed 14.5 µm in length (up to 18 µm).....7
(7) Ascospores lemon-shaped with ± papillate ends, perispore indehiscent in 10% KOH.....*E. moluccanum*
- Ascospores ellipsoid with ± tapered ' ends, perispore dehiscent in 10% KOH.....*E. moluccanum* ss. Sánchez-Jácome & Guzmán-Dávalos

An asterisk (*) denotes species belonging to *Entonaema* confirmed by phylogenetic analysis.

3.3. Ecology and Biogeography

All scientific publications accessible to the authors and the GBIF database (an extensive high quality online database on worldwide occurrences of biological species; www.gbif.org, accessed on 26 June 2023) were searched for verifiable *Entonaema* records in an attempt to provide better understanding of the ecology and biogeography of *Entonaema* species. A detailed analysis of a total of the here-accepted 266 worldwide *Entonaema* records that were attributable to a species (209 finds of *E. liquescens*, 51 of *E. cinnabarinum*, two of *E. globosum*, one of each *E. dengii*, *E. mollucanum*, *E. 'mollucanum'*, and *E. siamensis*), accompanied with precise localities, revealed much more information on biogeographic traits on the two most frequent species (*E. liquescens* and *E. cinnabarinum*) than in any of the previous studies. According to all here-accepted records of *Entonaema* spp. with known record dates, two phenological fructification patterns were recognized: (a) Mature stromata may be found all year round in tropical rainforest zones (Af) or depending on rainy period in monsoon (Am) and savannah areas (Aw); and (b) immature stromata were found during May and June, and mature ones from July to October (November) in four-season warm temperate northern zones (Cfa, Cfb, Csa, Cwa), see Figure 6. In the areas under the warm temperate climatic regime, immature stromata may also be found during summer and the autumn along with the mature ones, and quite often the maturation of perithecia/asci may completely fail in certain years in some localities ([2,68], GBIF data, our study), or only a few perithecia manage to ripe per stroma. Consequently, this unpredictability posed difficulties when re-studying fungaria material. A number of valuable collections consist only of sterile stromata (cf. [2,4,5,69]). According to all accepted records accompanied either with ecological data (substrate, habitat) or precise geographic coordinates, *Entonaema* species were mainly found in various forest ecosystems (including managed ones), or sometimes in city parks, on widely diverse angiosperm tree hosts, very often on coarse woody debris. Nearly all records found in warm temperate areas were in the near vicinity of freshwater bodies (rivers, rivulets, creeks, lakes, etc.).

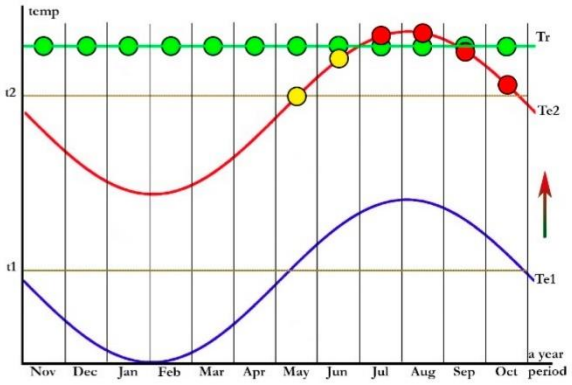

Figure 6. Two *Entonaema* fructification patterns: tropical pattern (Tr) represented by green circles as potential records of ripe stromata during all year (Af climate type) or depending on rainy season (Aw and Am climate types); warm temperate zone pattern (Te2) represented by yellow circles corresponding to immature stromata and red circles to ripe stromata, that occur during the warmest months. Areas with cool temperate climate (Te1) are devoid of *Entonaema* records. Legend: t1 = cardinal minimum temperature that would enable ascospore overwintering; t2 = minimal temperature needed for stromatic development; right arrow denotes climatic shift from Dfa (cool temperate climatic type) to Cfa (warm temperate climatic type).

According to 266 records accepted by us, *E. liquescens* is by far the most-frequently recorded species (209 records). This species has transcontinental distribution in humid

tropical (Am and Aw) areas of Africa (Kenya, Mayotte Island, South Africa, Uganda—[3,5], Zaire—[5]), the Americas (Brazil, Colombia—[4], Costa Rica, Cuba—[3], El Salvador, Honduras, Mexico—[5], Panama—[2], Trinidad and Tobago, Venezuela), Asia (China—[5], Thailand—[13]), and in humid warm temperate (Cfa, Cfb, Csa, Cwa) areas of Asia (India, Japan—[3]) and the Americas (Argentina—[5], Brazil—[1,2,5], Ecuador, Mexico, USA—[5,70]). There are also a few records of this species in cool temperate areas near the edge of the areas of warm temperate climatic regimes: the Russian Far East (Dwb) and northern USA (Dfa).

Entonaema cinnabarinum is another relatively frequently recorded species, with 51 records accepted here. This species also has transcontinental distribution in humid tropical (Af and Am) areas of Africa (Congo—[5], Sierra Leone, Uganda—[5]), the Americas (Costa Rica—[5]), Asia (India—[3], Philippines—[70], Sri Lanka—[3]), Oceania (Australia—[11], Nova Caledonia—[71]), and in humid warm temperate (Cfa, Csa, Cwa, Cwb) areas of Asia (Iran—[2], Japan—[70], Nepal), the Americas (Mexico—[2], USA), and Europe (Bulgaria—[72], Croatia—this paper, France—[2], Hungary—[68], Russia—[73], Serbia, Spain). There are also a few records of this species in cool temperate regions near the edge of the areas of warm temperate climatic regimes: the Russian Far East (Dwb—[74,75]).

This study did not bring any new distributional or taxonomic data for other species, because all records are already treated in earlier publications. The following four species are apparently so far confined to a tropical climatic areas. *Entonaema globosum* is so far known only from two Mexican localities under the influence of tropical rainforest (Af) and savannah (Aw) climatic regimes [12,69]. *Entonaema siamensis* is apparently known only from a type locality in Thailand [13], while *E. dengii* is from a type locality on Hainan Island, China [5], both situated in the areas of tropical savannah climate (Aw). *Entonaema moluccanum* is known only from a type locality on Halmahera Island, Indonesia [5], under influence of a tropical rainforest climate (Af). We agree with Stadler et al. [2] that, according to perispore dehiscence and different ascospore morphology, material published by Sánchez-Jácome and Guzmán-Dávalos [76] does not represent *E. moluccanum*, but some undescribed species. Also, it is hitherto known from a single locality under a warm temperate climatic regime (Cwa), outside of tropics. The first record from each locality in published papers are cited above. The other country records were mainly covered solely by GBIF.

4. Discussion

4.1. Taxonomic Implications

A data matrix for DNA sequence alignment was constructed to re-analyse the phylogenetic position of three entonaemoid species, viz. *E. liquescens*—a type species, *E. cinnabarinum*, and *Xylaria mesenterica* (Möller) M. Stadler, Læssøe & J. Fourn.—a former member of *Entonaema* (=*E. mesentericum* Möller, *E. pallidum* G.W. Martin). With the emphasis on a wide selection of hypoxyloid species groups previous chemotaxonomic research revealed that three *Entonaema* species (*E. liquescens*, *E. cinnabarinum*, and *E. globosum*) share the same stromatic HPLC profiles [2], i.e., mitorubrins and rubiginosins (group of azaphilone metabolites), that are characteristic to members of *Hypoxylaceae*, but not to *Xylariaceae*. Consequently, we included a wide spectrum of hypoxyloid species in our phylogenetic analysis. The sequences of *E. liquescens* and *E. cinnabarinum* obtained from perithecial elements were newly generated for that matter. In the previous studies [10,48,51], the ITS-LSU-*rpb2*-*β*-*tub* phylogeny confirmed *Hypoxylon* as a paraphyletic genus in the *Hypoxylaceae* that was recovered in at least six independent clades. Only one *Entonaema* strain (*E. liquescens* ATCC46302, [10]) was included in the study and was placed between the two *Daldinia* clades as a sister species to *Ruwenzoria pseudoannulata* MUCL 51394. *Hypoxylon carneum* was recovered as a single lineage with *H. cercidicola* (Berk. & M.A. Curtis ex Peck) Y.M. Ju & J.D. Rogers, *H. petriniae* M. Stadler & J. Fourn., and *H. rubiginosum* (Pers.) Fr. as the phylogenetically closest species, but distant from the type species *H. fragiforme* (Pers.) J. Kickx f.

In the present study, the phylogeny based on concatenated analysis of ITS, LSU, *rpb2* and *β*-*tub* gene regions supported previous studies, but nested two analysed species,

E. liquescens and *E. cinnabarinum*, in the H2 subclade of *Hypoxylaceae*, next to *H. carneum* as a sister lineage and distant from the strain *E. liquescens* ATCC46302 in the *Daldinia* clade. On the chemotaxonomic level, *H. carneum* differs from *Entonaema* species in non-azaphilone metabolites (carneic acids) that accumulate in the stromata [77,78]. Additionally, *H. carneum* differs from true *Entonaema* spp. (*E. liquescens*, *E. cinnabarinum*, and *E. globosum*) in flat-pulvinate stromata with only very thin, hard, dark brown tissue below the perithecia and livid-violet KOH-extractable pigments, which are absent in aged material (see Section 3.2 above). Moreover, in addition to the CNF collections of *Entonaema*, nine other *Entonaema* strains were phylogenetically analysed in this study. Two strains (*E. liquescens* S.D. Russell iNaturalist91210856 [41] and *E. splendens* KA12-1283) were recovered with CNF collections in the H2 subclade of *Hypoxylaceae* as 'true' *Entonaema*. The phylogenetic placement of *E. pallidum* in the *Xylariaceae* in our study supports the research of Stadler et al. [2], who recognised similarities between *E. palidum* and *Xylaria* spp. in their morphology, 5.8S/ITS nrDNA sequences, and HPLC profiles. Strains named *Entonaema* sp. JHGB08 1A, *Entonaema* sp. AHB18 5B [43], and *Entonaema* sp. [44] recovered alongside *E. pallidum* in the *Xylaria* clade, and consequently cannot be considered members of *Entonaema*. Therefore, our phylogenetic results agree with Lücking et al. [79] that comparison of sequences with GenBank blastn alone [42–44] is insufficient for accurate taxonomic characterisation because the reference databases used for molecular identifications are still incomplete and often contain erroneously named sequences. Furthermore, in our opinion only the integrative approach is acceptable if we seek for stabilised and operable taxonomy [80].

Our phylogenetic placement of true *Entonaema* spp., viz. the type species *E. liquescens* and *E. cinnabarinum* in *Hypoxylaceae*, and on the other hand '*Entonaema*' *pallidum* in *Xylariaceae* is now in correlation with earlier stromatal chemotaxonomic characterisation of the two separate groups, where *E. liquescens* and *E. cinnabarinum* have mitorubrinoid/rubiginosinoid HPLC profiles, characteristic for *Hypoxylaceae*, whereas '*Entonaema*' *pallidum* posesses xylaralic HPLC profile characteristic for *Xylariaceae* [2]. Clear overlapping of genetic and phenetic traits in a small *Entonaema* clade led us to retain its current generic concept, which would contain only several species around *E. liquescens* characterised by vividly coloured, large, vesiculose hollow stroma with elastic gelatinous flesh that contain liquid matter inside its cavity, possessing yellow, orange to red stromatic KOH extractable pigments, and mitorubrine/rubiginosine HPLC profile. This would necessitate the erection of several more small genera inside the H2 clade of *Hypoxylon* s.l. [10]. In such a concept, only a few species would thus clearly belong to *Entonaema*, viz. *E. liquscens* (type), *E. cinnabarinum*, and, relying on specific morphological–chemotaxonomical traits, also *E. globosum* and *E. moluccanum* ss. Sánchez-Jácome & Guzmán-Dávalos. The true affinity of *E. dengii*, *E. moluccanum* ss. str., and *E. siamensis* is yet to be ascertained.

4.2. Molecular Misinterpretations

The strains *E. liquescens* ATCC46302 [10,15], *E. liquescens* agtS279 [14], and *E. cinnabarinum* agtS377 [14] were recovered in the *Daldinia* clade of *Hypoxylaceae*, similar to previous studies [10,14,48,51]. The phylogenetic position of *Entonaema* in the *Dalidinia* clade based on the ITS region was not well supported [14], but maximally supported when the phylogeny was based on four gene regions (including protein gene regions), as presented by Wendt et al. [10] and Wibberg et al. [15]. In these studies, *Entonema* species were not described by thorough macro- and micromorphological examination of fruiting bodies, or by chemotaxonomical characterization, and DNA for sequencing was obtained from culture collections. The close phylogenetic relationship of *Entonaema* and *Daldinia* species was supported by the naphthalene and chromone derivatives produced by both [70]. On the other hand, the presence of mitorubrin-type azaphilones in the ascomata of *E. cinnabarinum* and *E. liquescens* [70] and the absence of binaphthalenes (which are ubiquitous *in Daldinia*) [2] clearly distinguishes *Entonaema* from *Daldinia* at the chemotaxonomic level.

Our *Entonaema* finds were often associated with *Daldinia* spp. (Figure 2A), including *D. childiae*, whose stromata have been found on the same dead wood fragments together

with the stromata of *E. cinnabarinum* (cf. [38]). All our efforts to establish *E. cinnabarinum* in axenic culture failed, where freshly ejected ascospores did not germinate on any of the three tested nutrient media (PDA, MEA and OA), regardless of the varied procedure. In cases where stromata of *Entonaema* were dusted by spores originated from neighbouring *Daldinia* stromata, the agar plates were occupied by rapid mycelial growth and conidial development of *Daldinia* contamination. It seems that *Daldinia* may often take over the plates because *E. cinnabarinum* is hardly culturable, if at all, while *Daldina* propagules germinate very rapidly and its mycelia are very fast growing. Therefore, it is not surprising that the same happened with two critical attempts to obtain culture of *E. liquescens*—ATCC 46302 strain, KANSAS, USA [6], and of *E. cinnabarinum*—CBS 113034, Pyrénées Atlantiques, FRANCE [14,70] in previous *Entonaema* studies. As a consequence, all sequences derived from this reference cultures actually belong to some *Daldinia* species, but not to *Entonaema* (*E. liquescens* nor *E. cinnabarina*), what Wibberg et al. [15] already justifiably suspected for *E. liquescens*. This further led to erroneous molecular identification of *E. cinnabarium* in the study of symbiotic relationships between saproxylic *Xiphydria* wasps and fungi from the genera *Daldinia* and *Entonaema* [81], which turned out to be true only for the fungi of the former genus (cf. [38]). Consequently, the anamorph of *Entonaema* itself remains vague.

4.3. Distribution and Biogeography

Outside America's tropical areas, *E. liquescens* was, until the 1980s, known only from the most southeastern USA with its very humid and warm temperate climate (Cfa), starting with Florida—1939, Louisiana—1956, Alabama—1965, Georgia—1978, and Mississippi—1986 (Figure 7), including few finds in the warmest areas of Kansas along the Missouri River (1979). The species was not found in northern states on the verge of the Cfa climatic area and cooler temperate climatic area (Dfa) (Nebraska, Indiana, Ohio, and Iowa), as well as inside the Dfa area before 2016. Since this time, *E. liquescens* occurrences in those northern areas has become quite regular—as much as in the southern states—and spreading along the watercourses of the Mississippi, Missouri, Kansas, Platte, Illinois, Ohio, and Tennessee rivers, and their tributaries, as well as along the warm Atlantic Coast and the Potomac River (cf. www.gbif.org, accessed on 26 June 2023).

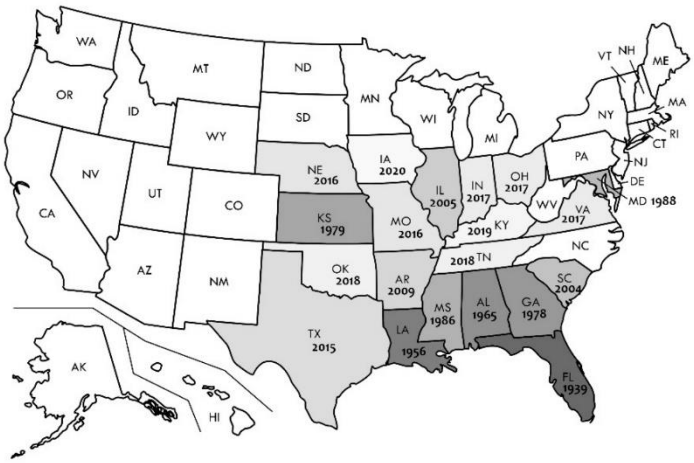

Figure 7. Map of USA with year of first record of *Entonaema liquescens* per federal state. Shading intensity reflects the time order in records, where the darkest shade represents the oldest known record.

Virtually all precisely recorded localities revealed (with the aid of Google Earth Pro) that the species' habitats are either flood plains or alluvial forests, developed along the

watercourses, at lake banks, or under the dams of hydroaccumulation reservoirs. The species is mostly distributed in the area of the warm temperate Cfa climate type [30], especially in the USA and Japan. *Entonaema liquescens* is not known in Europe so far.

Apart from a single Bulgarian longose forest (Mediterranean flood alluvial forest) [72,82], the presence of *E. cinnabarinum* in Europe (being one of the best explored areas in general, in mycological terms) is recorded only from the turn of the 21st century onwards [68,70,73,83]. Longoses represent subtropical oases in the otherwise relatively dry Mediterranean, and in temperate zones in general, because soil humidity is much prolonged by canopy coverage and by riverine inundation, and therefore not entirely dependent on precipitation. On the other hand, the habitat's ground substrates are well protected from heavy frosts and dry freezing under dense canopy coverage and thermic marine influence. Therefore, knowing the thermophilic species' preference, we could assume that this species might have been inhabiting such habitat types long before it was first recorded in 1987 in Bulgaria. Outside Bulgarian longose, the species was first found in the warm temperate area of the Pyrénées Atlantiques in France (1999), then in the Asturian rivulet valley of Spain (2006), Sochi in Russian Federation (2011), and with repetitive records in the Pannonian Plain in Croatia (since 2009), Hungary (2018), and Serbia (2022), see Figure 8.

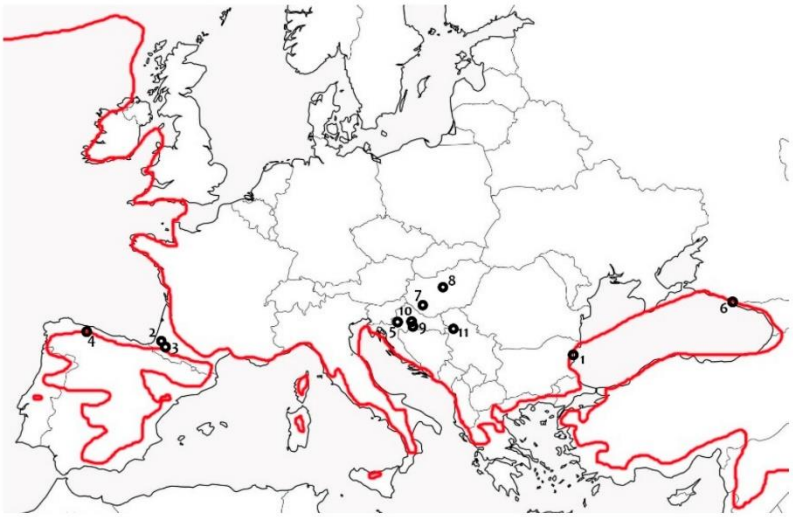

Figure 8. Known localities of *Entonaema cinnabarinum* in Europe, arranged according to the year of first record: 1—Kamchiya longose, Bulgaria (1987), 2—Auterrive and 3—Oloron-Ste. Marie, both Pyrénées Atlantiques, France (1999), 4—Belmonte, Asturias, Spain (2006), 5—Crna Mlaka, Zagreb County, Croatia (2009), 6—Agurskye waterfalls near Sochi, Russian Federation (2011), 7—Tókaj forest park, near Kaposvár, Hungary (2018), 8—Selyemrét Nature Trail, Ócsa, Pest County, Hungary (2019), 9—Opeke area near Kraljeva Velika, Sisak-Moslavina County, Croatia (2020), 10—Piljenice area near Ilova, Sisak-Moslavina County, Croatia (2021), and 11—north from Divoš, South Bačka District, Vojvodina, Serbia. Red line represents January isotherm of +5 °C mean air temperature after Stanners & Bourdeau [84]. In cases of two or more too close localities, some are omitted with regard of a map scale.

It is surprising that a fungal species with such a large stromata could be left unrecorded in the second half of the 20th century in mycologically well-explored regions and countries (e.g., USA, Japan, Europe). Croatian mycologist Milica Tortić paid special attention to lignicolous fungi without any *Entonaema* records during her long and diligent fieldwork, spanning the 1960s to the end of 20th century. The second author conducted a series of

detailed fieldwork sessions during 1990s exactly in the same area and the habitat type (oak-ash flood forest, Crna Mlaka) where *E. cinnabarinum* was first recorded only later in 2009, also without noticing it. Therefore, on the basis of rather abundant recent records of *E. cinnabarinum* in Europe, and the ascertained global image of the species' ecological traits, we can assume that this species has been spreading from its most humid-thermophilic European strongholds into new western and southeastern European areas on the account of global warming effects [83].

Owing to a series of eight mild winters (2015/2016–2022/2023) in the above-mentioned European areas for *E. cinnabarinum*, and in the midwestern USA along main watercourses for *E. liquescens* (Britt Bunyard *pers. comm.*), this species could be able to overwinter. This was followed by successful stromatal development during unusually warm summer and autumnal months (Figure 6), after which the species was capable of conquering new available substrates in the forests of other areas via sporulation. The species' capability to actively spread, overwinter, and to withstand drying conditions and direct UV radiation could be significantly enhanced by its forcible discharge of ascospores via turgor of the living asci, as well as by the small volume of ascospores and the wall, equipped with melanins, that are also richly developed in the stromatal crust and perithecial walls (cf. [85]). This capability is also enhanced by the species' preference for coarse woody debris as a substrate (significant water containers) and the humid forest habitat types, as well as by a development of large stromata equipped with voluminous gelatinous interior capable of accumulating and preserving water transported from the substrate. This enables the fungus to use this water against drying and to keep full turgor in the ascogenous system during the warmest periods when the evaporation is highest, and at the same time, when the organism could only develop ascospores in temperate areas (Figure 6). This internal stromatal liquor, similar as in *Xylaria mesenterica*, could be homologous in origin and/or the function as the internal stromatal tissue of *Daldinia* spp. [86].

Finally, the authors herein wish to draw attention to the emergence of erroneous data in the GBIF (www.gbif.org, accessed on 26 June 2023) portal about the occurrence of *E. cinnabarinum* in eastern Croatia. The actual material (CNF 2/11267) discussed was collected from a completely different geographic area and was uploaded to iNaturalist (www.inaturalist.org, accessed on 26 June 2023 [87]) by someone else who was unfamiliar with the material's true origin. When compiling an overview on the distribution of a given fungal species, one should take great caution with regard to adopting electronic data since those not accompanied by sufficient evidence about the origin and species identity could include uncontrollable errors. Erroneous data about *Entonaema* species distribution were earlier discussed by Rogers [5].

5. Conclusions

Six species of *Entonaema* have been formally accepted to date, the type species *E. liquescens*, *E. cinnabarinum*, *E. dengii*, *E. moluccanum*, *E. globosum*, and *E. siamensis*. Prior to this study, the ITS sequence was available for only one true *Entonaema* (*E. liquescens* S.D. Russell iNaturalist # 91210856), but had not been published or phylogenetically analysed. In this study, four gene regions (ITS, LSU, *rpb2*, *β-tub*) of the four Croatian collections (CNF 2/11046, 2/11047, 2/11052, 2/11053) of *E. cinnabarinum* and one (American) collection of *E. liquescens* (CNF 2/11263) were sequenced for the first time. The results of this study reveal a true phylogenetic position of the genus *Entonaema* within the *Hypoxylaceae*.

Judging from available data, dominant tree hosts in Europe for *E. cinnabarinum* are *Fraxinus* species (*F. angustifolia* in the Pannonian area and in Bulgarian longose, *F. excelsior* in the French Pyrénées Atlantiques), as well as *Quercus robur* in the Pannonian area. Being the (co-)dominant tree species in suitable flood and alluvial forests, those tree species may have served as the species' 'host bridge' for recent colonisation of the European humid warm temperate areas. In the midwestern USA, the most frequently mentioned hosts were *Quercus* spp., which could have represented the 'host bridge' for *E. liquescens* in the species' spreading from the most southeastern USA species' strongholds towards the north via the

Mississippi River and its tributaries. However, the analysed worldwide data prove that the two most frequent *Entonaema* species (*E. liquescens* and *E. cinnabarinum*) are plurivorous lignicolous saprotrophs. Therefore, we could expect the fungal adaptation to some other tree species growing under adequate climatic regime in suitable humid forest habitats in the future.

As for the rest of the *Entonaema* species, both *E. liquescens* and *E. cinnabarinum* were often considered subtropical–tropical species in the earliest papers (compare [68,73]). They are present in the areas under several types of warm temperate climate: Cfa—USA, Europe, Japan, Brazil; Cfb—Brazil, Ecuador; Csa—Spain; Cwa—Argentina, India, Mexico; Cwb—Mexico, Nepal. However, it is evident that both species were regularly recorded in the temperate zones of USA and Europe beginning only in the 21st century, on the well explored areas where those species have never been recorded before. Moreover, the apparent spread of these two *Entonaema* species during the last decade into the areas under (formerly) cooler temperate climate is in line with predicted climatic shift in Europe and North America [88] when we compare the current distribution of Köppen–Geiger climatic types in Europe, based on a period 1951–2000 [30], with the area that these climatic types would presumably cover during the period 2076–2100 [88]. Therefore, both *E. liquescens* and *E. cinnabarinum* could be good candidates for bioindicator species of climate change, especially because both are easily visible and recognizable by trained observers. Their appearance in the areas where they did not previously appear, could point to biologically effective climatic shifting along the border of a given cooler climatic type into a warmer, but the otherwise similar climatic type, e.g., Dfa or Cfb into Cfa (northwest Croatia and Hungary); Dfb (Nebraska, Iowa, northern Illinois and Indiana in the USA) or Dwb (Russian Far East) into a Cfa (Figure 6).

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Data Availability Statement: All sequences used in this study are available in GenBank. Alignments and phylogenetic trees generated in this study are available at Zenodo (DOI: 10.5281/zenodo.8302699).

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References

1. Möller, A. *Phycomyceten Und Ascomyceten. Untersuchungen Aus Brasilien*; Bot. Mitth. Tropen, Heft 9; G. Fischer: Jena, Germany, 1901.
2. Stadler, M.; Fournier, J.; Læssøe, T.; Lechat, C.; Tichy, H.-V.; Piepenbring, M. Recognition of hypoxyloid and xylarioid Entonaema species and allied Xylaria species from a comparison of holomorphic morphology, HPLC profiles, and ribosomal DNA sequences. *Mycol. Prog.* **2008**, *7*, 53–73. [CrossRef]
3. Lloyd, C.G. Mycological Notes, No. 69. *Mycol. Writ.* **1923**, *7*, 1185–1218.
4. Martin, G.W. New or Noteworthy Fungi from Panama and Columbia. II. *Mycologia* **1938**, *30*, 431–441. [CrossRef]
5. Rogers, J.D. *Sarcoxylon* and *Entonaema* (Xylariaceae). *Mycologia* **1981**, *73*, 26–61. [CrossRef]

6. Rogers, J.D. Entonaema liquescens: Description of the Anamorph and Thoughts on Its Systematic Position. *Mycotaxon* **1982**, *15*, 500–506.
7. Eriksson, O.E.; Hawksworth, D.L. Outline of the Ascomycetes—1993. *Syst. Ascomycetum* **1993**, *12*, 51–257.
8. Whalley, A. The xylariaceous way of life. *Mycol. Res.* **1996**, *100*, 897–922. [CrossRef]
9. Ju, Y.-M.; Rogers, J.D. *A Revision of the Genus Hypoxylon*; APS Press: St Paul, MN, USA, 1996.
10. Wendt, L.; Sir, E.B.; Kuhnert, E.; Heitkämper, S.; Lambert, C.; Hladki, A.I.; Romero, A.I.; Luangsa-ard, J.J.; Srikitikulchai, P.; Peršoh, D.; et al. Resurrection and emendation of the Hypoxylaceae, recognised from a multigene phylogeny of the Xylariales. *Mycol. Prog.* **2018**, *17*, 115–154. [CrossRef]
11. Cooke, M.C. Some Australian Fungi. *Grevillea* **1887**, *15*, 93–101.
12. Heim, R. Quelques Ascomycétes Remarquables, II.—Le Genre Entonaema Möll. Au Mexique. *Bull. Trimest. Société Mycol. De Fr.* **1960**, *76*, 121–129.
13. Sihanonth, P.; Thienhirun, S.; Whalley, A.J. Entonaema in Thailand. *Mycol. Res.* **1998**, *102*, 458–460. [CrossRef]
14. Triebel, D.; Peršoh, D.; Wollweber, H.; Stadler, M. Phylogenetic relationships among Daldinia, Entonaema, and Hypoxylon as inferred from ITS nrDNA analyses of Xylariales. *Nova Hedwig.* **2005**, *80*, 25–43. [CrossRef]
15. Wibberg, D.; Stadler, M.; Lambert, C.; Bunk, B.; Spröer, C.; Rückert, C.; Kalinowski, J.; Cox, R.J.; Kuhnert, E. High quality genome sequences of thirteen Hypoxylaceae (Ascomycota) strengthen the phylogenetic family backbone and enable the discovery of new taxa. *Fungal Divers.* **2020**, *106*, 7–28. [CrossRef]
16. Baral, H.O. Vital versus Herbarium Taxonomy: Morphological Differences between Living and Dead Cells of Ascomycetes, and Their Taxonomic Implications. *Mycologia* **1992**, *44*, 333–390.
17. Henriot, A.; Cheype, J.-L. Piximètre: La Mesure de Dimensions Sur Images. Version 5.10 R1541. Available online: http://ach.log.free.fr/Piximetre (accessed on 1 October 2020).
18. Samson, R.A.; Hoekstra, E.S.; Frisvad, J.C.; Filtenborg, O. *Introduction to Foodborne Fungi*; Centraalbureau voor Schimmelcultures: Baarn, The Netherlands; Delft, The Netherlands, 1996.
19. Gardes, M.; Bruns, T.D. ITS primers with enhanced specificity for basidiomycetes—Application to the identification of mycorrhizae and rusts. *Mol. Ecol.* **1993**, *2*, 113–118. [CrossRef] [PubMed]
20. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press: San Diego, CA, USA, 1990; pp. 315–322.
21. Vilgalys, R.; Hester, M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. *J. Bacteriol.* **1990**, *172*, 4238–4246. [CrossRef]
22. Liu, Y.J.; Whelen, S.; Hall, B.D. Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerse II subunit. *Mol. Biol. Evol.* **1999**, *16*, 1799–1808. [CrossRef]
23. O'Donnell, K.; Cigelnik, E. Two Divergent Intragenomic rDNA ITS2 Types within a Monophyletic Lineage of the Fungus Fusarium are Nonorthologous. *Mol. Phylogenetics Evol.* **1997**, *7*, 103–116. [CrossRef]
24. Kazutaka, K.; Misakwa, K.; Kei-ichi, K.; Miyata, T. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **2002**, *30*, 3059–3066. [CrossRef]
25. Katoh, K.; Standley, D.M. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [CrossRef]
26. Nguyen, L.-T.; Schmidt, H.A.; Von Haeseler, A.; Minh, B.Q. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [CrossRef] [PubMed]
27. Trifinopoulos, J.; Nguyen, L.-T.; von Haeseler, A.; Minh, B.Q. W-IQ-TREE: A fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Res.* **2016**, *44*, W232–W235. [CrossRef]
28. Huelsenbeck, J.P.; Ronquist, F. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **2001**, *17*, 754–755. [CrossRef]
29. Letunic, I.; Bork, P. Interactive Tree Of Life (iTOL) v5: An online tool for phylogenetic tree display and annotation. *Nucleic Acids Res.* **2021**, *49*, W293–W296. [CrossRef] [PubMed]
30. Kottek, M.; Grieser, J.; Beck, C.; Rudolf, B.; Rubel, F. World map of the Köppen-Geiger climate classification updated. *Meteorol. Z.* **2006**, *15*, 259–263. [CrossRef] [PubMed]
31. Hsieh, H.-M.; Lin, C.-R.; Fang, M.-J.; Rogers, J.D.; Fournier, J.; Lechat, C.; Ju, Y.-M. Phylogenetic status of Xylaria subgenus Pseudoxylaria among taxa of the subfamily Xylarioideae (Xylariaceae) and phylogeny of the taxa involved in the subfamily. *Mol. Phylogenetics Evol.* **2010**, *54*, 957–969. [CrossRef]
32. Kuhnert, E.; Sir, E.B.; Lambert, C.; Hyde, K.D.; Hladki, A.I.; Romero, A.I.; Rohde, M.; Stadler, M. Phylogenetic and chemotaxonomic resolution of the genus Annulohypoxylon (Xylariaceae) including four new species. *Fungal Divers.* **2016**, *85*, 1–43. [CrossRef]
33. Kuhnert, E.; Fournier, J.; Peršoh, D.; Luangsa-Ard, J.J.D.; Stadler, M. New Hypoxylon species from Martinique and new evidence on the molecular phylogeny of Hypoxylon based on ITS rDNA and β-tubulin data. *Fungal Divers.* **2013**, *64*, 181–203. [CrossRef]
34. Daranagama, D.A.; Camporesi, E.; Tian, Q.; Liu, X.; Chamyuang, S.; Stadler, M.; Hyde, K.D. Anthostomella is polyphyletic comprising several genera in Xylariaceae. *Fungal Divers.* **2015**, *73*, 203–238. [CrossRef]
35. Li, Q.R.; Kang, J.C.; Hyde, K.D. Two new species of the genus Collodiscula (Xylariaceae) from China. *Mycol. Prog.* **2015**, *14*, 52. [CrossRef]

36. Bitzer, J.; Læssøe, T.; Fournier, J.; Kummer, V.; Decock, C.; Tichy, H.-V.; Piepenbring, M.; Peršoh, D.; Stadler, M. Affinities of Phylacia and the daldinoid Xylariaceae, inferred from chemotypes of cultures and ribosomal DNA sequences. *Mycol. Res.* **2008**, *112*, 251–270. [CrossRef]
37. Hsieh, H.-M.; Ju, Y.-M.; Rogers, J.D. Molecular phylogeny of Hypoxylon and closely related genera. *Mycologia* **2005**, *97*, 844–865. [CrossRef] [PubMed]
38. Stadler, M.; Læssøe, T.; Fournier, J.; Decock, C.; Schmieschek, B.; Tichy, H.-V.; Peršoh, D. A polyphasic taxonomy of Daldinia (Xylariaceae). *Stud. Mycol.* **2014**, *77*, 1–143. [CrossRef] [PubMed]
39. Johannesson, H.; Læssøe, T.; Stenlid, J. Molecular and morphological investigation of Daldinia in northern Europe. *Mycol. Res.* **2000**, *104*, 275–280. [CrossRef]
40. Spatafora, J.W.; Sung, G.-H.; Johnson, D.; Hesse, C.; O'rourke, B.; Serdani, M.; Spotts, R.; Lutzoni, F.; Hofstetter, V.; Miadlikowska, J.; et al. A five-gene phylogeny of Pezizomycotina. *Mycologia* **2006**, *98*, 1018–1028. [CrossRef]
41. Entonaema Liquescens · INaturalist. Available online: https://www.inaturalist.org/taxa/350744-Entonaema-liquescens (accessed on 25 April 2023).
42. Gazis, R.; Chaverri, P. Diversity of fungal endophytes in leaves and stems of wild rubber trees (*Hevea brasiliensis*) in Peru. *Fungal Ecol.* **2010**, *3*, 240–254. [CrossRef]
43. Skaltsas, D.N.; Badotti, F.; Vaz, A.B.M.; da Silva, F.F.; Gazis, R.; Wurdack, K.; Castlebury, L.; Góes-Neto, A.; Chaverri, P. Exploration of stem endophytic communities revealed developmental stage as one of the drivers of fungal endophytic community assemblages in two Amazonian hardwood genera. *Sci. Rep.* **2019**, *9*, 12685. [CrossRef]
44. Higginbotham, S.; Wong, W.R.; Linington, R.G.; Spadafora, C.; Iturrado, L.; Arnold, A.E. Sloth Hair as a Novel Source of Fungi with Potent Anti-Parasitic, Anti-Cancer and Anti-Bacterial Bioactivity. *PLoS ONE* **2014**, *9*, e84549. [CrossRef]
45. Kim, C.S.; Jo, J.W.; Kwag, Y.-N.; Sung, G.-H.; Lee, S.-G.; Kim, S.-Y.; Shin, C.-H.; Han, S.-K. Mushroom Flora of Ulleung-gun and a Newly Recorded Bovista Species in the Republic of Korea. *Mycobiology* **2015**, *43*, 239–257. [CrossRef]
46. Zhang, N.; Castlebury, L.A.; Miller, A.N.; Huhndorf, S.M.; Schoch, C.L.; Seifert, K.A.; Rossman, A.Y.; Rogers, J.D.; Kohlmeyer, J.; Volkmann-Kohlmeyer, B.; et al. An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. *Mycologia* **2006**, *98*, 1076–1087. [CrossRef]
47. Koukol, O.; Kelnarová, I.; Černý, K. Recent observations of sooty bark disease of sycamore maple in Prague (Czech Republic) and the phylogenetic placement of *Cryptostroma corticale*. *For. Pathol.* **2014**, *45*, 21–27. [CrossRef]
48. Pourmoghaddam, M.J.; Lambert, C.; Surup, F.; Khodaparast, S.A.; Krisai-Greilhuber, I.; Voglmayr, H.; Stadler, M. Discovery of a new species of the *Hypoxylon rubiginosum* complex from Iran and antagonistic activities of *Hypoxylon* spp. against the Ash Dieback pathogen, *Hymenoscyphus fraxineus*, in dual culture. *Mycokeys* **2020**, *66*, 105–133. [CrossRef]
49. Ma, H.-X.; Qiu, J.-Z.; Xu, B.; Li, Y. Two Hypoxylon species from Yunnan Province based on morphological and molecular characters. *Phytotaxa* **2018**, *376*, 27–36. [CrossRef]
50. Cedeño–Sanchez, M. Three new species of Hypoxylon and new records of Xylariales from Panama. *Mycosphere* **2020**, *11*, 1457–1476. [CrossRef]
51. Ma, H.; Song, Z.; Pan, X.; Li, Y.; Yang, Z.; Qu, Z. Multi-Gene Phylogeny and Taxonomy of *Hypoxylon* (Hypoxylaceae, Ascomycota) from China. *Diversity* **2022**, *14*, 37. [CrossRef]
52. Song, Z.-K.; Zhu, A.-H.; Liu, Z.-D.; Qu, Z.; Li, Y.; Ma, H.-X. Three New Species of *Hypoxylon* (Xylariales, Ascomycota) on a Multigene Phylogeny from Medog in Southwest China. *J. Fungi* **2022**, *8*, 500. [CrossRef]
53. Lambert, C.; Pourmoghaddam, M.J.; Cedeño-Sanchez, M.; Surup, F.; Khodaparast, S.A.; Krisai-Greilhuber, I.; Voglmayr, H.; Stradal, T.E.B.; Stadler, M. Resolution of the *Hypoxylon fuscum* Complex (Hypoxylaceae, Xylariales) and Discovery and Biological Characterization of Two of Its Prominent Secondary Metabolites. *J. Fungi* **2021**, *7*, 131. [CrossRef]
54. Stadler, M.; Kuhnert, E.; Peršoh, D.; Fournier, J. The Xylariaceae as Model Example for a Unified Nomenclature Following the "One Fungus-One Name" (1F1N) Concept. *Mycology* **2013**, *4*, 5–21. [CrossRef]
55. Dai, D.Q.; Phookamsak, R.; Wijayawardene, N.N.; Li, W.J.; Bhat, D.J.; Xu, J.C.; Taylor, J.E.; Hyde, K.D.; Chukeatirote, E. Bambusicolous fungi. *Fungal Divers.* **2016**, *82*, 1–105. [CrossRef]
56. Bills, G.F.; González-Menéndez, V.; Martín, J.; Platas, G.; Fournier, J.; Peršoh, D.; Stadler, M. *Hypoxylon pulicicidum* sp. nov. (Ascomycota, Xylariales), a Pantropical Insecticide-Producing Endophyte. *PLoS ONE* **2012**, *7*, e46687. [CrossRef]
57. Sir, E.B.; Becker, K.; Lambert, C.; Bills, G.F.; Kuhnert, E. Observations on Texas hypoxylons, including two new *Hypoxylon* species and widespread environmental isolates of the *H. croceum* complex identified by a polyphasic approach. *Mycologia* **2019**, *111*, 832–856. [CrossRef] [PubMed]
58. Pi, Y.-H.; Zhang, X.; Liu, L.-L.; Long, Q.-D.; Shen, X.-C.; Kang, Y.-Q.; Hyde, K.D.; Boonmee, S.; Kang, J.-C.; Li, Q.-R. Contributions to species of Xylariales in China—4. *Hypoxylon wujiangensis* sp. nov. *Phytotaxa* **2020**, *455*, 21–30. [CrossRef]
59. Samarakoon, M.C.; Hyde, K.D.; Maharachchikumbura, S.S.N.; Stadler, M.; Jones, E.B.G.; Promputtha, I.; Suwannarach, N.; Camporesi, E.; Bulgakov, T.S.; Liu, J.-K. Taxonomy, phylogeny, molecular dating and ancestral state reconstruction of Xylariomycetidae (Sordariomycetes). *Fungal Divers.* **2022**, *112*, 1–88. [CrossRef]
60. Pažoutová, S.; Šrůtka, P.; Holuša, J.; Chudíčková, M.; Kolařík, M. The phylogenetic position of *Obolarina dryophila* (Xylariales). *Mycol. Prog.* **2010**, *9*, 501–507. [CrossRef]

61. Senanayake, I.C.; Maharachchikumbura, S.S.N.; Hyde, K.D.; Bhat, J.D.; Jones, E.B.G.; McKenzie, E.H.C.; Dai, D.Q.; Daranagama, D.A.; Dayarathne, M.C.; Goonasekara, I.D.; et al. Towards unraveling relationships in Xylariomycetidae (Sordariomycetes). *Fungal Divers.* **2015**, *73*, 73–144. [CrossRef]
62. Peláez, F.; González, V.; Platas, G.; Sánchez-Ballesteros, J.; Rubio, V. Molecular Phylogenetic Studies within the Xylariaceae Based on Ribosomal DNA Sequences. *Fungal Divers* **2008**, *31*, 111–134.
63. Tang, A.M.C.; Jeewon, R.; Hyde, K.D. A Re-Evaluation of the Evolutionary Relationships within the Xylariaceae Based on Ri-bosomal and Protein-Coding Gene Sequences. *Fungal Divers* **2009**, *34*, 127–155.
64. Stadler, M.; Flessa, F.; Rambold, G.; Peršoh, D.; Fournier, J.; Læssøe, T.; Chlebicki, A.; Lechat, C. Chemotaxonomic and phylogenetic studies of Thamnomyces (Xylariaceae). *Mycoscience* **2010**, *51*, 189–207. [CrossRef]
65. Ju, Y.-M.; Hsieh, H.-M. Xylaria species associated with nests of *Odontotermes formosanus* in Taiwan. *Mycologia* **2007**, *99*, 936–957. [CrossRef]
66. Ju, Y.-M.; Hsieh, H.-M.; Rogers, J.D.; Fournier, J.; Jaklitsch, W.M.; Courtecuisse, R. New and interesting penzigioid *Xylaria* species with small, soft stromata. *Mycologia* **2012**, *104*, 766–776. [CrossRef] [PubMed]
67. Sir, E.; Stadler, M. A new species of Daldinia (Xylariaceae) from the Argentine subtropical montane forest. *Mycosphere* **2016**, *7*, 1378–1388. [CrossRef]
68. Fintha, G.; Benedek, L.; Orbán, S. New Macrofungial Record in Hungary: *Entonaema cinnabarinum* (Cooke & Massee) Lloyd. *Acta Biol. Plant. Agriensis* **2019**, *7*, 127–130. [CrossRef]
69. Rogers, J.D.; San Martín, F.; Ju, Y.-M. Mexican Fungi: *Xylaria entosulphurea* Sp. Nov. and Neotypification of *Entonaema globosum*. *Mycotaxon* **1996**, *58*, 483–487.
70. Stadler, M.; Ju, Y.-M.; Rogers, J.D. Chemotaxonomy of Entonaema, Rhopalostroma and other Xylariaceae. *Mycol. Res.* **2004**, *108*, 239–256. [CrossRef]
71. Patouillard, N. Champignons de La Nouvelle-Calédonie. *Bull. Trimest. Société Mycol. De Fr.* **1911**, *27*, 329–333.
72. Benkert, D. *Kotlabaea macrospora* Benkert nov. sp. und einige weitere bemerkenswerte Ascomyceten aus Bulgarien Mit einer Abbildung. *Feddes Repert.* **1993**, *104*, 547–549. [CrossRef]
73. Fedosova, A.G. The New Record of *Entonaema cinnabarinum* (Xylariaceae, Ascomycota) in Europe. Vestnik of Saint Petersburg University. Series 3. *Biology* **2012**, *1*, 10–13.
74. Harkevich, S.S. (Ed.) *флора и Растительность Уссурийского Заповедника*; Наука: Moscow, Russia, 1978.
75. Azbukina, Z.M.; Bardunov, L.V.; Bezdeleva, T.A.; Bogacheva, A.V.; Bulakh, E.M.; Vasilyeva, L.N.; Govorova, O.K.; Egorova, L.N.; Zhabyko, E.V.; Nikulina, T.V.; et al. *Flora, Vegetation and Mycobiota of the Reserve "Ussuriysky"*; Daljnauka: Vladivostok, Rasia, 2006.
76. Sánchez-Jácome, M.; Guzmán-Dávalos, L. New Records of Ascomycetes from Jalisco, Mexico. *Mycotaxon* **2005**, *92*, 177–191.
77. Quang, D.N.; Stadler, M.; Fournier, J.; Asakawa, Y. Carneic Acids A and B, Chemotaxonomically Significant Antimicrobial Agents from the Xylariaceous Ascomycete Hypoxylon carneum. *J. Nat. Prod.* **2006**, *69*, 1198–1202. [CrossRef]
78. Helaly, S.E.; Thongbai, B.; Stadler, M. Diversity of biologically active secondary metabolites from endophytic and saprotrophic fungi of the ascomycete order Xylariales. *Nat. Prod. Rep.* **2018**, *35*, 992–1014. [CrossRef]
79. Lücking, R.; Aime, M.C.; Robbertse, B.; Miller, A.N.; Aoki, T.; Ariyawansa, H.A.; Cardinali, G.; Crous, P.W.; Druzhinina, I.S.; Geiser, D.M.; et al. Fungal taxonomy and sequence-based nomenclature. *Nat. Microbiol.* **2021**, *6*, 540–548. [CrossRef]
80. Zamora, J.C.; Svensson, M.; Kirschner, R.; Olariaga, I.; Ryman, S.; Parra, L.A.; Geml, J.; Rosling, A.; Adamčík, S.; Ahti, T.; et al. Considerations and consequences of allowing DNA sequence data as types of fungal taxa. *IMA Fungus* **2018**, *9*, 167–175. [CrossRef]
81. Šrůtka, P.; Pažoutová, S.; Kolařík, M. Daldinia decipiens and Entonaema cinnabarina as fungal symbionts of Xiphydria wood wasps. *Mycol. Res.* **2007**, *111*, 224–231. [CrossRef]
82. Læssøe, T. Entonaema cinnabarina-En Eksotisk Kernesvamp. *Svampe* **1997**, *36*, 21–22.
83. Fintha, G.; Nagy, I.; Vitkó, T.; Benedek, L.; Baranyai, G. Az Ócsai Turjánvidék Natura 2000-es kijelölt területeinek nagygombái. *J. Landscape Ecol.* **2022**, *20*, 3–21. [CrossRef]
84. Stanners, D.; Bourdeau, P. *Europes's Environment: The Dobříš Assessment*; European Environment Agency: Copenhagen, Denmark, 1995.
85. Quijada, L.; Matočec, N.; Kušan, I.; Tanney, J.B.; Johnston, P.R.; Mešić, A.; Pfister, D.H. Apothecial Ancestry, Evolution, and Re-Evolution in *Thelebolales* (*Leotiomycetes*, *Fungi*). *Biology* **2022**, *11*, 583. [CrossRef] [PubMed]
86. Ju, Y.M.; Rogers, J.D.; San Martin, F. A Revision of the Genus Daldinia. *Mycotaxon* **1997**, *61*, 243–293.
87. INaturalist Research-Grade Observations. Available online: https://www.inaturalist.org (accessed on 26 June 2023).
88. Rubel, F.; Kottek, M. Observed and projected climate shifts 1901–2100 depicted by world maps of the Köppen-Geiger climate classification. *Meteorol. Z.* **2010**, *19*, 135–141. [CrossRef]

3. RASPRAVA

Hrvatski nacionalni fungarij (CNF) je zbirka uzoraka suhih plodišta gljiva, prikupljenih i adekvatno konzerviranih sušenjem s ciljem očuvanja mikromorfoloških i genetskih obilježja materijala. U zbirci se trenutno nalazi više od 30 000 uzoraka gljiva prvenstveno iz Hrvatske, ali i drugih dijelova svijeta, što je rezultat višedesetljetne tradicije mikoloških terenskih istraživanja na ovim prostorima. Među pohranjenim uzorcima postoji veliki broj novih vrsta gljiva za nacionalnu bioraznolikost i znatan broj potencijalno novih vrsta za znanost. Molekularne analize uzoraka gljiva pohranjenih u fungariju započele su 2011. godine, a velik zamah su dobile početkom projekta ForFungiDNA 2018. godine. Kao jedan od rezultata projekta, iz više od 1000 uzoraka plodišta gljiva pohranjenih u zbirci CNF generirane su DNA barkod sekvence, prvenstveno primarni ITS rDNA barkodovi, a zatim, ovisno o skupini gljiva, dodatno i sekundarne barkod sekvence. Molekularna i filogenetska istraživanja daju morfološkim analizama novi kontekst, određuju razinu varijabilnosti morfoloških obilježja na razini vrste te su postale neizbježan alat pri objavi znanstvenog opisa novih vrsta i rodova.

Prateći koncepte integrativne taksonomije, u ovoj su disertaciji prikazani znanstveni opisi tri nove vrste za znanost s područja Hrvatske, *Inocybe brijunica* (publikacija I), *Inocybe istriaca* (publikacija II) i *Parasola papillatospora* (pubikacija III). Spoznaje o bioraznolikosti gljiva u Hrvatskoj proširene su i prvim podacima o prisutnosti osam vrsta iz roda *Parasola* na području naše zemlje (*P. auricoma, P. crataegi, P. cuniculorum, P. kuehneri, P. malakandensis, P. megasperma, P. nudiceps* i *P. plicatilis-similis*), od kojih je *P. malakandensis* po prvi puta zabilježena i na području Europe (publikacija III). Također, u suradnji s austrijskim i slovačkim znanstvenicima, vrsta *Coprinopsis alnivora* po prvi je puta zabilježena i znanstveno opisana na području Europe (Austrija, Hrvatska, Slovačka) (publikacija IV). Integrativna taksonomska analiza vrsta *Entonaema cinnabarinum* (koja je po prvi puta zabilježena u Hrvatskoj) i tipske vrste roda *Entonaema, E. liquescens* (SAD) rezultirala je izradom ključa za identifikaciju svjetskih vrsta roda *Entonaema*. Uz biogeografske i ekološke značajke, prikazan je i ispravan taksonomski položaj roda *Entonaema* koji je do sada često bio pogrešno određen (publikacija V).

3.1. Nove vrste gljiva za znanost s područja Hrvatske

3.1.1. Opisi dvije nove vrste za znanost iz roda *Inocybe* (publikacije I i II)

Kao rezultat višegodišnjeg (2014.–2022.) terenskog rada mikologa s Instituta Ruđer Bošković na području Nacionalnog parka Brijuni sakupljeno je 186 uzoraka gljiva iz odjeljka

Basidiomycota koji se nalaze pohranjeni u zbirci CNF. Uzorci pripadaju u 60-ak različitih rodova, a najzastupljeniji je rod *Inocybe* sensu lato (u širem smislu) (*Agaricomycetes, Agaricales, Inocybaceae*) s 29 uzoraka. Morfološkim analizama ustanovljeno je da se među uzorcima nalazi nekoliko potencijalno novih vrsta za znanost što je potvrđeno detaljnim i opsežnim molekularnim i filogenetskim analizama više genskih regija, te su s područja NP Brijuni do sada opisane i objavljene dvije nove vrste za znanost, *Inocybe brijunica* (publikacija I) i *I. istriaca* (publikacija II). Područje NP Brijuni odlikuje se raznolikom vegetacijom autohtonih i egzotičnih mediteranskih biljnih vrsta, na temelju čega možemo pretpostaviti veliku bioraznolikost gljivljih vrsta prisutnih na tom području. Obje ektomikorizne vrste su pronađene na rubu šume hrasta crnike (*Quercus ilex*) i pašnjaka velikih biljojeda (srne i jeleni). Šuma hrasta crnike karakteristična je za područja s mediteranskom klimom, a takva staništa obiluju ektomikoriznim vrstama iz roda *Inocybe* što je već i prije bilo zabilježeno (Richard i sur. 2004, Esteve-Raventós i sur. 2016).

Porodica *Inocybaceae* jedna je od najbogatijih vrstama u razredu *Agaricales* (Matheny i sur. 2019), i unutar te porodice prihvaćeno je sedam rodova temelju filogenetske analize 6 genskih regija (SSU, ITS, LSU, *rpb1*, *rpb2*, *tef1*): *Inocybe s.s.* (lat. *sensu stricto,* u užem smislu), *Inosperma*, *Mallocybe*, *Nothocybe*, *Pseudosperma* i *Tubariomyces* (Matheny i sur. 2020).

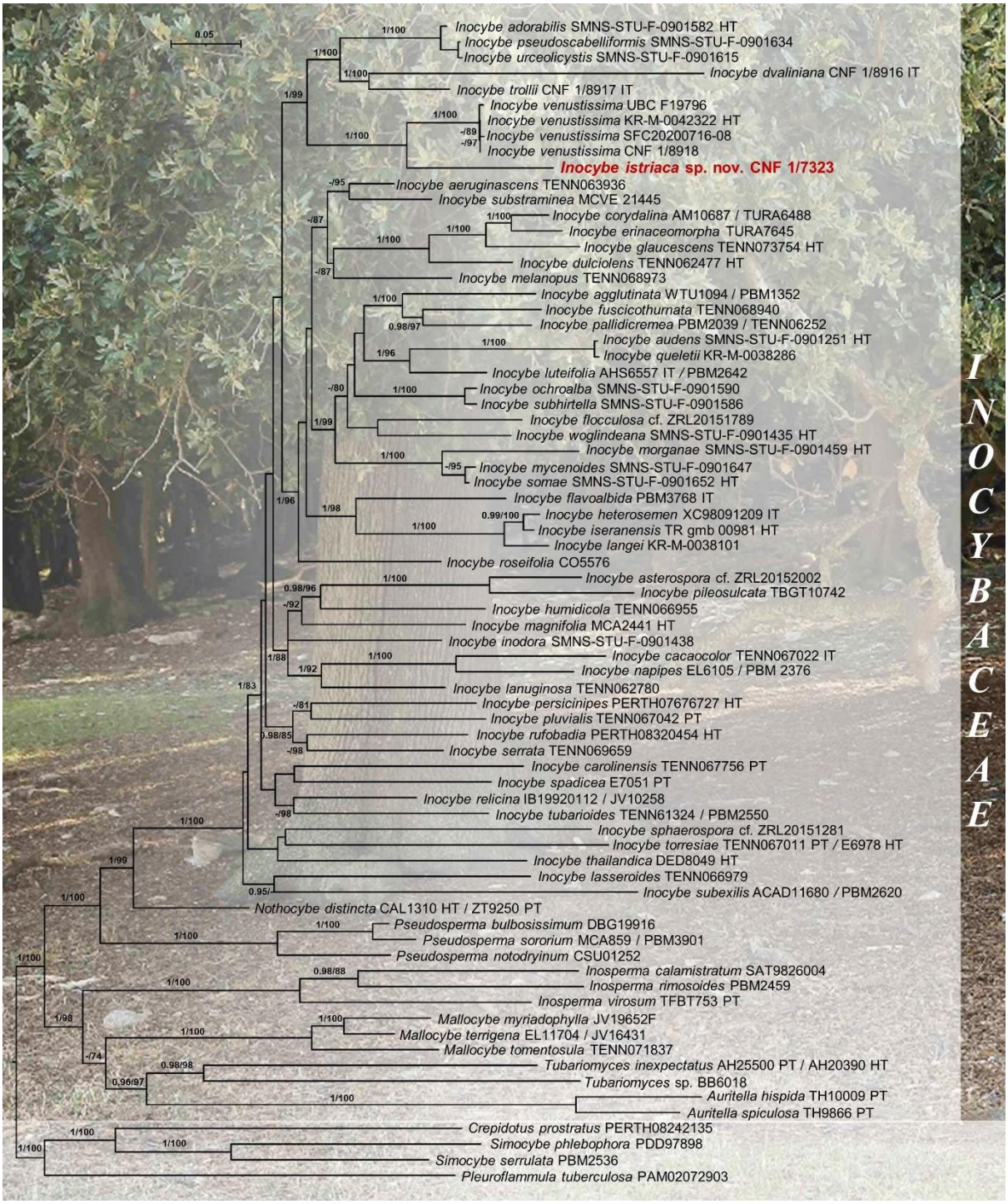
Rod *Inocybe* odlikuje se umjerenom varijabilnošću makromorfoloških karakteristika te vrlo velikom varijabilnošću mikromorfoloških karakteristika. Mikromorfološka obilježja koja karkteriziraju plodišta u rodu *Inocybe* su prisutnost pleurocistida, depigmentirani bazidiji, te amigdaliformne do elipsioidne, subcilindrične, uglate, kvržičaste ili bodljikave bazidiospore s izraženim apikulusom. Zbog velikog broja znanstveno opisanih vrsta u rodu *Inocybe* (više od 1000 (Matheny i sur. 2019)) i čestog preklapanja više različitih mikromorfoloških karakteristika sličnih vrsta (Matheny i Bougher 2017, Bandini i sur. 2021), prepoznavanje i razlikovanje vrsta iz ovoga roda na temelju morfologije prilično je zahtjevno.

Iako je preporuka da svaka nova vrsta za znanost bude opisana na temelju više nalaza (Aime i sur. 2021), u oba slučaja iz ovog rada bilo je evidentno da se radi o novim vrstama na temelju samo jednog uzorka zbog jedinstvenih obilježja plodišta ovih vrsta (svaki nalaz, pa tako i uzorak, uključuje jedno ili više plodišta iste vrste nađenih na istom mjestu u isto vrijeme, za koje pretpostavljamo da potječu iz istog micelija). Na temelju detaljne morfološke analize više od 20 plodišta iz uzorka CNF 1/7345 (*Inocybe brijunica*) zaključili smo da se ova vrsta u

rodu *Inocybe* ističe i razlikuje od svih drugih vrsta već na makromorfološkoj razini i to membranastim slojem narančasto-crveno-smeđe boje koji je smješten u bazalnom dijelu stručka. S druge strane, plodišta iz uzorka CNF 1/7323 (*I. istriaca*) nemaju tako upečatljive makromorfološke razlikovne karakteristike na razini vrste, ali je na temelju kombinacije jedinstvenih mikromorfoloških karakteristika, (1) tamne smolaste tvari koja prekriva oštricu listića i vrh stručka, te (2) većim dimenzijama bazidija u odnosu na veliku većinu drugih vrsta ovoga roda, pretpostavljeno da se radi o novoj vrsti za znanost.

Prvi korak u molekularnoj taksonomiji je usporedba ITS rDNA barkod sekvenci s podacima dostupnim u bioinformatičkoj bazi podataka Genbank, nakon čega slijedi filtracija rezultata na sličan način kao što je opisano u Raja i sur. 2017., te opsežna analiza literature. Rezultat BLAST analiza ITS genske regije vrste *I. brijunica* pokazala je najveću sličnost s *I. glabripes* koja pripada sekciji *Hysterices*. Zbog toga su za užu filogenetsku analizu odabrani taksoni iz navedene sekcije. Rezultati višegenske filogenetske analize (ITS, LSU, *rpb2*), odgovarali su filogeniji dobivenoj u istraživanju koje su proveli Matheny i Kudzma 2019. Na temelju integrativnih taksonomskih istraživanja utvrđeno je da uzorak CNF 1/7345 nedvojbeno pripada novoj vrsti za znanost koju smo znanstveno opisali pod imenom *Inocybe brijunica*, te smo je svrstali u sekciju *Hysterices*, zajedno s *I. glabripes* kao sestrinskom vrstom.

U opsežnoj filogenetskoj analizi porodice *Inocybaceae* temeljenoj na četiri genske regije (ITS, LSU, *rpb2*, *tef1*) koja se slagala s filogenijom dobivenom u 6-genskoj analizi porodice (Matheny i sur. 2020), vrsta *I. istriaca* izdvojila se kao posebna linija u *Inocybe s.s.* skupini (Slika 5). Filogenetski najbliža novoj liniji bila je vrsta *I. venustissima* koja je s vrstom *I. istriaca* tvorila monofiletsku skupinu. Za navedenu filogenetsku analizu četiri genske regije, iz uzoraka vrsta *I. venustissima*, *I. dvaliniana* i *I. trollii* resekvencirane su genske regije ITS i LSU, te novo sekvencirane regije *rpb2* i *tef1* kako bi molekularna analiza pokazala što točnije filogenetske odnose i evoluciju vrsta. Druga filogenetska analiza temeljila se na uzorcima vrsta roda *Inocybe s.s.* koji su morfološki pokazali određenu sličnost s uzorkom plodišta vrste *I. istriaca*, ali su većinom u bazama podataka bile zastupljene samo ITS i LSU genskim regijama. Analiza je rezultirala potvrdom filogenetske neovisnosti vrste *I. istriaca* u odnosu na druge vrste roda *Inocybe s.s*. Na temelju analiza morfoloških struktura plodišta, ekoloških značajki te dvostruke filogenetske analize, dokazano je da uzorak CNF 1/7323 pripada novoj vrsti za znanost koja je opisana pod imenom *Inocybe istriaca*.

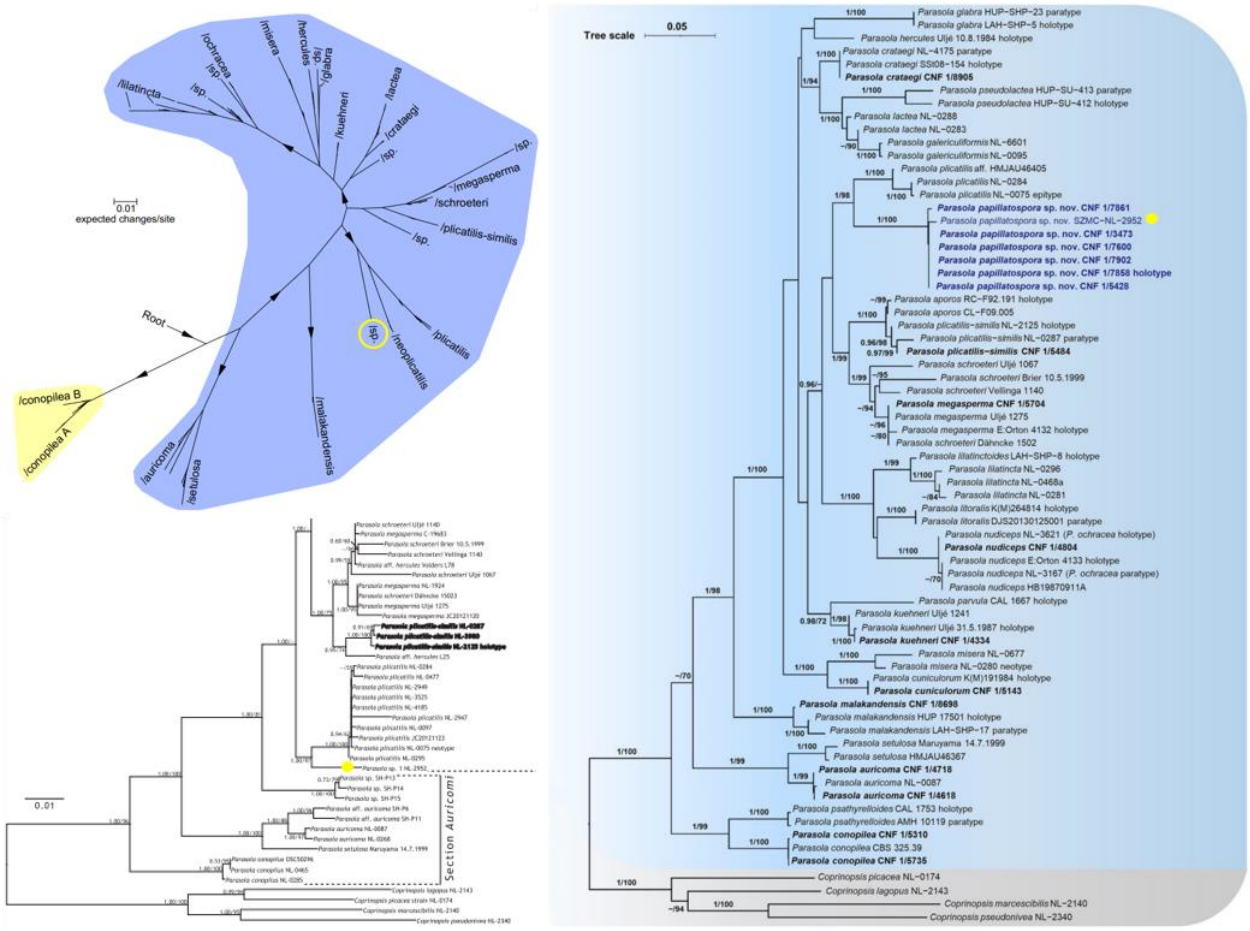


Slika 5. Filogenetsko stablo porodice *Inocybaceae* dobiveno analizom četiri genske regije (ITS, LSU, *rpb2*, *tef1*) u kojem je prikazana nova vrsta za znanost *I. istriaca* koja se izdvojila kao posebna linija u *Inocybe s.s.* skupini (publikacija II).

3.1.2. Opis nove vrste za znanost *Parasola papillatospora* (publikacija III)

U okviru integrativno taksonomskog istraživanja (publikacija III) vrsta iz roda *Parasola* (*Psathyrellaceae, Agaricales, Basidiomycota*) s područja Hrvatske, analizirano je ukupno 17 uzoraka plodišta. Šest nalaza vrste, u navedenom radu opisane kao nove za znanost (*Parasola papillatospora*), pronađeno je i uzorkovano na dva lokaliteta u listopadnim šumama umjerenog pojasa kontinentalne Hrvatske, u gradu Zagrebu te na Žumberačkom gorju. Rod *Parasola* čine sve vrste iz dvije podsekcije (*Glabri* i *Auricomi*) roda *Coprinus s.l.* (lat. *sensu lato*, u širem smislu) u nekadašnjoj taksonomskoj koncepciji. Glavna morfološka obilježja vrsta u rodu *Parasola* su nježna plodišta koprinoidnog oblika (suh, radijalno žljebast klobuk; tamno obojene bazidiospore) koja starenjem ne prelaze u tekućinu (eng. *non-deliquescent*), te ne posjeduju velum i kaulocistide (Redhead i sur. 2001, Ulje 2005). Na temelju molekularnih analiza u rod *Parasola* naknadno su uključene i vrste koje imaju plodišta s glatkim klobukom, *Psathyrella conopile*a (Fr.) A. Pearson & Dennis (Larsson i Örstadius 2008) te *Galeropsis aporos* Courtec. (Malysheva i sur. 2019).

Filogenetski su ovaj rod istraživali i definirali Redhead i sur. (2001), Nagy i sur. (2009) te Wächter i Melzer (2020). Na temelju BLAST algoritma ustanovljeno je da DNA sekvence ITS i LSU genskih regija šest uzoraka iz Hrvatske i otprije poznate sekvence nalaza iz Mađarske (označene kao *Parasola* sp. 1 SZMC-NL-2952 u Szarkandi i sur. 2017) pokazuju više od 99% sličnosti, te da se radi o istoj vrsti. Autori rada navode da uzorak pripada potencijalno novoj vrsti za znanost različitoj od sestrinske vrste *P. plicatilis*, no zbog nedostatka informacija o uzorku i/ili molekularnih podataka nisu je znanstveno opisali i dodijelili joj ime. Nadalje, ova vrsta je filogenetski analizirana i smještena unutar porodice *Psathyrellaceae*, te je opsežnom molekularnom i filogenetskom analizom provedenom u radu Wächtera i Melzera (2020), također prepoznata kao zasebna vrsta uz *P. plicatilis*, međutim ni tada još uvijek nije bila znanstveno opisana. Osim ITS i LSU genskih regija, u publikaciji III su sekvencirane i dodatne regije *tef1* i *βtub*, te je filogenetska analiza provedena na sva četiri genska markera. Svih šest uzoraka vrste *P. papillatospora* iz Hrvatske filogenetski se grupiralo uz uzorak iz Mađarske, *Parasola* sp. 1 (SZMC-NL-2952) te su formirali zajedničku granu s *P. plicatilis* kao sestrinskom vrstom. Rezultati filogenetske analize u publikaciji III ove doktorske disertacije poklapaju se s rezultatima u radovima Szarkandija i sur. (2017) te Wächtera i Melzera (2020) (Slika 6).



Slika 6. Prikaz filogenetskog položaja uzorka *Parasola* sp. 1 (SZMC-NL-2952) iz Mađarske (označen žutim kružićem ili točkom) u istraživanjima Wächtera i Melzera (2020) (lijevo gore), Szarkandija i sur. (2017) (lijevo dolje) i publikaciji III (desno) u kojoj se svih šest uzoraka vrste *P. papillatospora* iz Hrvatske filogenetski grupiralo uz uzorak iz Mađarske.

Opisu ove nove vrste za znanost prethodila su kompleksna integrativna taksonomska istraživanja. Razlikovna makromorfološka obilježja plodišta na razini vrsta u rodu *Parasola* vrlo su malobrojna i varijabilna, te je na temelju njih gotovo nemoguće pouzdano ustanoviti kojoj vrsti nalazi pripadaju. Također, često su i mikromorfološka obilježja između blisko srodnih vrsta u rodu vrlo varijabilna i preklapajuća, zbog čega molekularna taksonomija ima značajnu ulogu u pouzdanoj identifikaciji. Iz navedenih razloga može se pretpostaviti da značajan broj vrsta roda *Parasola*, uključujući i vrstu *P. papillatospora*, pripada kriptičkim vrstama na makromorfološkoj razini, a ukoliko su uključene i mikromorfološke karakteristike, moguće je zaključiti da potencijalno pripadaju pseudokriptičkim vrstama. Ipak, detaljna morfološka analiza pokazala je da se *P. papillatospora* od sličnih vrsta u rodu može razlikovati po kombinaciji karakteristika bazidiospora: ± papilatan vrh i centralan klični otvor u većini slučajeva te izrazito velika raznolikost oblika spora.

Opis nove vrste za znanost iz roda *Parasola* čini veoma značajan doprinos poznavanju globalne bioraznolikosti jer je brojnost vrsta u rodu vrlo oskudna te je do sada bilo opisano tek 30-tak vrsta, većinom u posljednjih 10 godina. Na primjeru publikacije III u kojoj je vrsta *P. papillatospora* opisana kao nova za znanost možemo izvesti zaključak da je za precizno razgraničenje vrsta u ovome rodu potrebno koristiti metodologiju integrativne taksonomije. Paralelno s analizom morfoloških značajki plodišta nužno je uključiti i molekularno filogenetsku analizu koja će nam pouzdano potvrditi o kojoj se vrsti radi i dati uvid u srodstvene odnose između vrsta u analiziranoj taksonomskoj skupini. Istraživanja predstavljena u ovom radu doprinose spoznaji o bioraznolikosti gljiva na više razina i to u području taksonomije (nova vrsta), biogeografije (novi areali više vrsta) i u važnosti molekularne identifikacije u rodu *Parasola*.

3.2. Rasprostranjenost vrsta iz porodice *Psathyrellaceae* na području Hrvatske

Intenzivno prikupljanje nalaza iz porodice *Psathyrellaceae* u zbirku CNF traje već više od 20 godina, a uvođenje molekularnih metoda, odnosno DNA barkodiranja, ubrzalo je proces znanstvene objave značajnih nalaza. Porodica *Psathyrellaceae* opisana je 2001. godine (Redhead i sur. 2001) te je uključivala pet rodova: *Coprinellus, Coprinopsis, Lacrymaria, Parasola* i *Psatyrella,* određenih na temelju morfoloških te do tada dostupnih molekularnih podataka koji su obuhvaćali analize LSU genske regije (Vilgalys i sur. 1994, Hopple i Vilgalys 1999, Moncalvo i sur. 2000). Tek je u godinama koje su slijedile, ITS predložen kao univerzalni DNA barkod za gljive (Schoch i sur. 2012) te su dizajnirane univerzalne početnice (White i sur. 1990). Osim toga, Nagy i sur. (2011) su značajno doprinijeli razrješavanju filogenetskih odnosa unutar porodice dizajnirajući početnice za određivanje *βtub* genske regije specifične za porodicu *Psathyrellaceae*. Veliki doprinos razumijevanju filogenetskih odnosa unutar porodice dali su Wächter i Melzer (2020) koji su na temelju većine dostupnih sekvenci, njih 18 133 (uključujući četiri genske regije: ITS, LSU, *tef1* i *βtub*), kao i morfoloških i ekoloških podataka reevaluirali porodicu. Do tada 10 opisanih rodova: *Coprinellus, Coprinopsis, Cystoagaricus, Homophron, Hormographiella, Kauffmania, Lacrymaria, Parasola, Psathyrella* i *Typhrasa,* nije moglo adekvatno obuhvatiti sve dobro podržane grane dobivenog filogenetskog stabla, pa su znanstveno opisali sedam novih monofiletskih rodova: *Candolleomyces, Britzelmayria, Hausknechtia, Narcissea, Olotia, Punjabia* i *Tulosesus*. U većini slučajeva radilo se o izdvajanju dijela vrsta iz ranije šire shvaćenih rodova unutar porodice *Psathyrellaceae* u novoopisane rodove, osim u slučaju vrste *Galerella floriformis*. Za vrstu *Galerella floriformis,* koja je prethodno na temelju morfoloških obilježja svrstana u porodicu *Bolbitiaceae,* u

znanstevnom radu Wächtera i Melzera (2020) ustanovljeno je da zapravo pripada zasebnom rodu u okviru porodice *Psathyrellaceae*, novopisanom rodu *Hausknechtia*. Taksonomska pozicija ovog roda potvrđena je i mom koautorskom radu (Nie i sur. 2022) u kojem je uz vrstu *Hausknechtia floriformis*, u isti rod smještena i vrsta *H. leucosticta* (= *Coprinus leucostictus*) na temelju analize četiri genske regije (ITS, LSU, *tef1* i *β tub*). Molekularna analiza koju su proveli Wächter i Melzer (2020) potvrdila je i filogenetski položaj do tada objavljenih novih vrsta za znanost iz roda *Coprinopsis* pohranjenih u zbirci CNF, *C. afrocinerea* Mešić, Tkalčec, Čerkez, I. Kušan & Matočec (CNF 1/5838, Crous i sur. 2018) i *C. cerkezii* Tkalčec, Mešić, I. Kušan & Matočec (CNF 1/7253, Tibpromma i sur. 2017).

3.2.1. Nove spoznaje o bioraznolikosti roda *Parasola* u Hrvatskoj i Europi (publikacija III)

Do 2023. godine na području Republike Hrvatske bile su zabilježene tek četiri vrste roda *Parasola*, *P. conopilea*, *P. plicatilis* i *P. misera* (Mešić i Tkalčec 2003), te *P. lactea* (= *P. leiocephala* (Vrščaj 2002). Morfološkom obradom te filogenetskom analizom četiri genske regije (ITS, LSU, *tef1* i *β tub*), ukupno 179 sekvenci, uključujući 64 novo generirane sekvence 17 uzoraka roda *Parasola* s područja Republike Hrvatske, identificirano je deset vrsta ovoga roda, od kojih je jedna vrsta opisana kao nova za znanost (vidi 3.1.2). Osam vrsta iz roda (*P. auricoma*, *P. crataegi*, *P. cuniculorum*, *P. kuehneri*, *P. malakandensis*, *P. megasperma*, *P. nudiceps* i *P. plicatilis-similis*) po prvi puta je zabilježeno u Hrvatskoj (publikacija III) te su dati molekularni, biogeografski te osnovni ekološki podaci o njihovim nalazima kao i makroskopske fotografije plodišta. Ove vrste zabilježene su u sedam različitih županija na području Hrvatske (Požeško-slavonska, Grad Zagreb, Primorsko-goranska, Sisačko-moslavačka, Splitsko-dalmatinska, Dubrovačko-neretvanska i Krapinsko-zagorska), a točne lokacije navedene su u publikaciji III.

Uzorak vrste roda *Parasola* s otoka Hvara identificiran je kao *P. malakandensis*, što je prvi nalaz ove vrste na području Europe, a do sada bila zabilježena samo na području Pakistana (Hussain i sur. 2016). Vrsta *P. conopilea* već je otprije bila zabilježena na području Republike Hrvatske, no bila je identificirana isključivo na temelju morfoloških obilježja (Mešić i Tkalčec 2003). U ovom radu obrađena su dva nalaza ove vrste koji su sakupljeni na području NP Krka te na otoku Lokrumu, pa je vrsta sada potvrđena za područje naše zemlje i na temelju molekularnih podataka koji obuhvaćaju filogenetsku analizu četiri DNA genske regije. Međutim, potrebno je naglasiti da u taksonomiji roda *Parasola* postoji još dosta neriješenih pitanja te je veliki broj još neopisanih vrsta, čiji su nalazi pohranjeni u zbirci CNF, za koje je potrebno provesti daljnja istraživanja. Ustanovljeno je da dvije ranije u Hrvatskoj zabilježene

vrste identificirane na temelju morfologije, *P. misera* i *P. plicatilis*, molekularno ne odgovaraju svojim tipovima (epitip i neotip), te zapravo predstavljaju nove, još neopisane vrste. Taj će problem biti rješavan u bliskoj budućnosti.

3.2.2. Prvi nalazi vrste *Coprinopsis alnivora* na području Europe (publikacija IV)

Morfološke i ekološke značajke samo su jedan dio integrativnog taksonomskog pristupa. Iznimno važna komponenta je i ona molekularna, pa ne čudi da je DNA barkodiranje gljiva značajno doprinijelo spoznaji o rasprostranjenosti gljiva na svjetskoj razini. Suradnja s austrijskim i slovačkim znanstvenicima rezultirala je detaljnim (znatno dopunjenim) opisom i integrativnom taksonomskom analizom vrste *Coprinopsis alnivora* koja je u ovom istraživanju po prvi puta zabilježena na području Europe (Austrija, Hrvatska, Slovačka) (publikacija IV). Vrsta je do ovog istraživanja bila poznata samo na temelju jednog nalaza iz Sjedinjenih Američkih Država, gdje je opisan tipski uzorak vrste (Van de Bogart 1976), nađen na johi (*Alnus* sp.). Opis tipskog materijala (holotipa) (Ulje i Noordeloos 2000) temeljen je na jednom oskudnom plodištu pronađenom 1976. godine. Međutim, u bazi podataka Genbank postoji sekvenca ITS genske regije holotipa koja je imala velik značaj pri analizi uzoraka iz Europe, iako do sada nije bila objavljena u znanstvenom radu. Detaljna morfološka, ekološka i molekularna istraživanja provedena su na sedam uzoraka iz Slovačke, tri iz Austrije te jednom iz Hrvatske, a prikupljeni su u razdoblju od 2008. do 2020. godine.

Nalazi vrste iz Europe pokazali su varijabilnost u veličini plodišta i čvrstoći veluma. Mikromorfološki opisi ovih nalaza dobro su se slagali s opisom holotipa, iako spljoštenost bazidiospora nije bila istaknuta u izvornom opisu. Uzorak vrste iz Hrvatske obuhvaćao je 12 plodišta, nađenih na području grada Zagreba, na posađenim stablima crne topole (*Populus nigra*), 1,4 m iznad tla. Sekvenca ITS genske regije nalaza iz Hrvatske slagala se više od 99% sa sekvencama ITS genske regije ostalih nalaza iz Europe. BLAST pretraživanje sekvence ITS genske regije nalaza iz Hrvatske u bazi podataka Genbank rezultiralo je sličnošću na razini više od 99% s ITS sekvencom holotipa vrste *C. alnivora*. Filogenetskom analizom provedenom na ITS genskoj regiji, prikazano je grupiranje svih nalaza iz Europe s holotipom vrste *C. alnivora* što je dodatna potvrda da su ovi nalazi konspecifični.

Osim što su analize integrativne taksonomije doprinijele spoznajama o rasprostranjenosti ove vrste, također su doprinijele i definiranju njene morfološke, ali i ekološke varijabilnosti. Dokazano je kako ova vrsta može živjeti i na živim stablima, ali i na mrtvim ležećim trupcima, te je zabilježeno pet biljnih vrsta koje prethodno nisu bile poznate kao njen supstrat: *Acer*

campestre, *Fagus sylvatica*, *Fraxinus excelsior*, *Magnolia salicifolia* i *Populus nigra*. Na temelju rezultata istraživanja provedenog u sklopu ove doktorske disertacije moguće je zaključiti da je ova vrsta ekološki prilagođena da za supstrat uzima veći broj biljnih drvenastih vrsta. Također, važno je naglasiti ulogu molekularnog istraživanja na razini primarnog DNA barkoda u ovom slučaju. Moguće je da ova vrsta ne bi bila pouzdano identificirana u slučaju nemogućnosti pristupa ITS DNA barkod regiji holotipa jer je tipski materijal oskudan što otežava morfološku analizu. U ovom slučaju, generirana je sekvenca ITS genske regije iz tipskog materijala iako kod uzoraka gljiva starost materijala često onemogućava generiranje molekularnih sekvenci. U ovom je znanstvenom radu bila važna molekularna i filogenetska potvrda da analizirani uzorci iz Europe taksonomski odgovaraju holotipu vrste *C. alnivora*, nakon čega je bilo moguće s potpunom sigurnošću definirati unutarvrsnu morfološku varijabilnost, te pridonijeti spoznaji o ekološkoj varijabilnosti i rasprostranjenosti vrste.

3.3. Značaj molekularne filogenije u određivanju taksonomskog položaja roda *Entonaema* (publikacija V)

DNA barkodiranje gljiva značajno je doprinijelo spoznaji o globalnoj rasprostranjenosti gljiva te u slučaju kada se DNA barkod u potpunosti slaže s DNA barkodom vrste znanstveno opisane metodama integrativne taksonomije, vrlo je mala vjerojatnost da ne pripadaju istoj vrsti. Međutim, u filogenetske analize najčešće je uključen barem dio sekvenci preuzet iz bioinformatičkih baza podataka, zbog čega postoji znatna vjerojatnost da će za analizu biti preuzete i neke sekvence izolirane iz pogrešno identificiranih uzoraka ili kultura. Zato je važno biti oprezan, te po mogućnosti u analizama koristiti sekvence koje su već publicirane u znanstvenim radovima u kojima se one navode. Potrebno je odrediti prema kojoj su literaturi identificirani uzorci i/ili kakve su njihove morfološke i ekološke karakteristike te, ukoliko su navedeni podaci nedostupni, dati prednost sekvencama koje su objavili istraživači s iskustvom u istraživanoj taksonomskoj skupini.

Rod *Entonaema* (*Hypoxylaceae*, *Xylariales*) sadrži šest opisanih vrsta, tipsku vrstu *E. liquescens*, te *E. cinnabarinum*, *E. globosum*, *E. dengii*, *E. moluccanum* i *E. siamensis*. Vrste ovoga roda su lignikolni saprotrofi, a možemo ih pronaći na ostacima trupaca i grana. Karakterizira ih velika, nepravilno okruglasta stroma življih boja površine, ispunjena tekućinom, koja u svojoj kori sadrži sloj plodišta zvan peritecij. Iako se smatralo da vrste ovoga roda žive većinom u tropskim staništima, novija istraživanja su pokazala njihovu rasprostanjenost i u toplim te umjerenim staništima u Europi, Aziji i Sjevernoj Americi. Rod *Entonaema* je utemeljen početkom prošlog stoljeća (Moller 1901), a njegov do sada nejasan

taksonomski i filogenetski položaj bio je predmetom istraživanja zadnjih 20-ak godina. Stadler i sur. (2008) objavljuju da rod taksonomski pripada porodici *Xylariaceae*, dok Wendt i sur. (2018) rade reviziju cijelog reda *Xylariales*, te smještaju rod *Entonaema* u porodicu *Hypoxylaceae*. Zbog ovih različitih taksonomskih vizija u relevantnoj literaturi, pojavila se potreba za točnim utvrđivanjem taksonomskog i filogenetskog položaja roda *Entonaema* unutar reda *Xylariales*. Iz tog razloga, provedeno je opsežno integrativno taksonomsko istraživanje koje je uključivalo detaljan opis morfoloških karakteristika roda *Entonaema* i vrste *E. cinnabarinum* što je rezultiralo izradom novog taksonomskog ključa za identifikaciju vrsta roda *Entonaema*. Uz to, kako bi se utvrdio točan filogenetski položaj roda *Entonaema*, provedena je opsežna filogenetska analiza više od 500 sekvenci obuhvaćajući četiri genske regije (ITS, LSU, *rpb2* i *β-tub*) više od 140 taksona. U analizu su bila uključena četiri uzorka vrste *E. cinnabarinum* (CNF 2/11046, 2/11047, 2/11052, 2/11053) iz Hrvatske te uzorak tipske vrste roda *Entonaema, E. liquescens* (CNF 2/11263) iz SAD-a. Također, u analizu su bile uključene sekvence iz uzoraka taksonomski identificiranih kao *Entonaema* spp. dostupne u Genbanku, ali filtrirane na način da su u analizu uključene samo sekvence objavljene u znanstvenim radovima, s iznimkom vrste *E. liquescens* S. D. Russell iNaturalist91210856.

Filogenetska analiza rezultirala je grupiranjem uzoraka identificiranih kao *Entonaema* spp. u tri skupine. Dvije skupine grupirale su se polifiletski, ali unutar porodice *Hypoxylaceae*, dok se jedna skupina grupirala unutar porodice *Xylariaceae*.

Dobiveni filogenetski položaj vrste *E. pallidum* u porodici *Xylariaceae* slaže se s onim u istraživanju Stadlera i sur. (2008) u kojem su opisane sličnosti u morfologiji, kemotaksonomiji te ITS DNA barkodu između vrste *E. pallidum* i ostalih vrsta porodice *Xylariaceae*. Na temelju navedenih spoznaja, može se zaključiti da vrsta *E. pallidum* zapravo pripada rodu *Xylaria* (sinonim je vrste *Xylaria mesenterica*) u porodici *Xylariaceae*. Osim toga, tri analizirane sekvence iz baze Genbank imenovane kao *Entonaema* spp. i filogenetski grupirane uz *E. pallidum* u porodici *Xylariaceae* pogrešno su taksonomski određene kao vrste koje pripadaju rodu *Entonaema*.

U dosadašnjim istraživanjima porodice *Hypoxylaceae*, *E. liquescens* (ATCC46302) koja se grupirala uz vrstu *Ruwenzoria pseudoannulata* u *Daldinia* skupinu porodice *Hypoxylaceae*, bila je jedini predstavnik roda *Entonaema* (Wendt i sur. 2018, Pourmogadhaam 2020, Ma i sur. 2022). U navedenim istraživanjima, za ovaj uzorak identificiran kao *Entonaema liquescens* nisu opisana makromorfološka i mikromorfološka obilježja strome i plodišta ili

kemotaksonomske karakteristike, a DNA za sekvenciranje izolirana je iz kulture. U prirodi, vrste rodova *Entonaema* i *Daldinia* mogu dijeliti isti supstrat te stroma *Entonaema* vrste može biti zaprašena sporama vrste iz roda *Daldinia*. Zbog toga pri uzgoju određene vrste iz roda *Entonaema* često može doći do kontaminacije s vrstom iz roda *Daldinia* koja brzo razvija micelij i stvara konidije, što se dogodilo i u pokušaju uzgoja vrste *E. cinnabarinum* provedenom u publikaciji V. Budući da smo imali sekvenciranu DNA dobivenu iz fenetički detaljno karakteriziranog plodišta vrste *E. cinnabarinum*, uspjeli smo uočiti navedenu pogrešku usporedbom DNA sekvenci dobivenih iz plodišta i kulture te smo lako mogli zaključiti da se radi o kontaminaciji. Iz navedenih razloga, može se zaključiti da se prethodno kontaminacija dogodila pri pokušaju kultiviranja *E. liquescen*s ATCC46302 i *E. cinnabarinum* CBS 113034 (Stadler i sur. 2004, Triebel i sur. 2005), te da navedene kulture pripadaju vrstama iz roda *Daldinia*, a nikako vrstama roda *Entonaema*. Nastavno na to, kulture *E. liquescens* agtS279 i *E. cinnabarinum* agtS377 koje su se u filogenetskoj analizi grupirale uz kulturu *E. liquescens* ATCC46302, pripadaju rodu *Daldinia*, a ne *Entonaema*.

U filogenetskoj analizi provedenoj u publikaciji V temeljenoj na četiri genske regije (ITS, LSU, *rpb2* i *βtub*), morfološki i biogeografski su analizirane „istinske“ vrste roda *Entonaema*, *E. cinnabarinum* (CNF 2/11046, 2/11047, 2/11052, 2/11053) i *E. liquescens* (CNF 2/11263) koje su se grupirale uz vrstu *Hypoxylon carneum* u H2 skupinu porodice *Hypoxylaceae*, parafiletski odvojeno od vrste *E. liquescens* ATCC46302 iz *Daldinia* skupine. Osim značajnih morfoloških razlika, vrsta *H. carneum* razlikuje se od vrsta roda *Entonaema* i u kemotaksonomskom profilu (Quang i sur. 2006, Helaly i sur. 2018). Od publiciranih, javno dostupnih sekvenci koje pripadaju vrstama iz roda *Entonaema* u bazi Genbank, samo su se dvije vrste, *E. liquescens* S. D. Russell iNaturalist91210856 i *E. splendens* KA12-1283, grupirale uz „istinske“ vrste roda *Entonaema* u H2 skupinu porodice *Hypoxylaceae*. Metodama integrativne taksonomije te opsežnom kritičkom analizom dostupnih literaturnih podataka, u publikaciji V ustanovljen je točan filogenetski položaj i raznolikost vrsta iz roda *Entonaema* (skupina H2) unutar porodice *Hypoxylaceae*.

4. ZAKLJUČCI

Jedan od najznačajnijih ekoloških izazova s kojima se čovječanstvo u današnje vrijeme suočava je gubitak bioraznolikosti. Hrvatska se nalazi na biogeografski vrlo specifičnom i interesantnom području što ju čini pogodnom za visoku razinu biološke raznolikosti koja je do danas vrlo slabo istražena. U fokusu istraživanja ove doktorske disertacije bilo je rješavanje znanstvenih problema u sistematici gljiva iz odjeljaka *Ascomycota* i *Basidiomycota* s područja Hrvatske korištenjem metoda integrativne taksonomije. Uz morfološka i ekološka obilježja gljivljih organizama, posebno su istražena molekularna obilježja i njihov značaj prilikom znanstvenog opisivanja i razgraničavanja vrsta.

Na temelju rezultata prikazanih u ovoj doktorskoj disertaciji moguće je zaključiti sljedeće:

1. Metode integrativne taksonomije koje objedinjuju morfološka, ekološka i molekularna obilježja gljivljih organizama nužno je koristiti prilikom znanstvenog opisa novih vrsta što potvrđuje hipotezu 1. ove doktorske disertacije u okviru publikacija u kojima su opisane tri nove vrste za znanost: *Inocybe brijunica, I. istriaca* i *Parasola papillatospora*.
2. Bioraznolikost gljiva na području Hrvatske vrlo je velika, no jako je slabo istražena. Osim novih vrsta za znanost, u doktorskoj disertaciji po prvi je puta za područje Republike Hrvatske zabilježena prisutnost 10 vrsta: *Coprinopsis alnivora, Entonaema cinnabarinum, Parasola auricoma, P. crataegi, P. cuniculorum, P. kuehneri, P. malakandensis, P. megasperma, P. nudiceps* i *P. plicatilis-similis*.
3. Dugogodišnja intenzivna taksonomska i biogeografska istraživanja gljiva provedena na području Hrvatske značajno su doprinijela spoznaji o europskoj i globalnoj rasprostranjenosti gljiva. Od 10 vrsta novih za našu zemlju, dvije vrste (*Coprinopsis alnivora* i *Parasola malakandensis*) su po prvi puta zabilježene i na području Europe.
4. DNA barkodiranje je važan alat pri identifikaciji i međusobnom razlikovanju novih, ali i već poznatih gljivljih vrsta. Ono čini osnovu molekularne taksonomije kao vrlo značajnog dijela integrativnog taksonomskog pristupa u mikološkim istraživanjima te ima ključnu ulogu u identifikaciji gljiva.
5. Najvarijabilniji DNA barkod na razini vrste je ITS, tj. primarni DNA barkod za carstvo gljiva te je ujedno i najzastupljenija genska regija za ovu skupinu organizama u bioinformatičkim bazama podataka. U slučaju kada ITS genska regija nije sadržavala dovoljno taksonomskih informacija za razjašnjavanje srodstvenih odnosa među

taksonima, provedene su analize sekundarnih genskih regija (sekundarnih barkodova). Geni koji su korišteni u funkciji sekundarnih barkodova kod taksonomski istraživanih skupina u ovoj disertaciji su LSU, *tef1*, *rpb2* i *βtub*. Određivanje ovih gena bilo je od velikog značaja u molekularnom aspektu taksonomije zbog doprinosa razumijevanju filogenetskih odnosa među vrstama i višim taksonomskim kategorijama te pri dodatnoj provjeri identifikacije na razini vrste.

6. Vrlo velika varijabilnost i preklapanje morfoloških obilježja kod blisko srodnih vrsta gljiva uvelike otežavaju pouzdanu identifikaciju vrsta. Stoga je uz originalni opis morfoloških obilježja gljivljih vrsta ključno imati i određenu DNA barkod sekvencu koja potječe iz tipskog uzorka. U procesu taksonomske identifikacije vrste *Coprinopsis alnivora*, BLAST pretraživanje ITS sekvence nalaza iz Hrvatske u bioinformatičkoj bazi podataka Genbank rezultiralo je sličnošću na razini više od 99% s ITS sekvencom holotipa vrste *C. alnivora* iz savezne države Washington (SAD). Filogenetskom analizom provedenom na ITS genskoj regiji prikazano je grupiranje svih nalaza iz Europe s holotipom vrste *C. alnivora*, što je bila dodatna potvrda da su svi analizirani nalazi konspecifični.
7. DNA barkodiranje vrlo je važno pri razgraničenju kriptičkih, semikriptičkih i pseudokriptičkih vrsta. Zbog malobrojnih i varijabilnih razlikovnih makromorfoloških obilježja vrsta roda *Parasola*, može se pretpostaviti da je značajan broj vrsta u ovom rodu kriptičan na makromorfološkoj razini. Također, mikromorfološka obilježja vrsta u ovom rodu su vrlo varijabilna i preklapajuća te je moguće zaključiti da velik broj taksona potencijalno pripada pseudokriptičkim vrstama. Kao potvrdu hipoteze 2. možemo zaključiti da su DNA barkodiranje i molekularna taksonomija značajno doprinijeli razgraničenju i identifikaciji vrsta u rodu *Parasola*, pa tako i pri znanstvenom opisu nove vrste za znanost, *P. papillatospora*.
8. Bioinformatičke baze podataka često sadrže netočno taksonomski određene DNA sekvence. Iz podataka dostupnih u bazi podataka Genbank, od osam analiziranih vrsta određenih kao *Entonaema* spp. samo su se dvije vrste grupirale uz „istinske“ vrste roda *Entonaema* u H2 skupinu porodice *Hypoxylaceae*. Metodama integrativne taksonomije te opsežnom kritičkom analizom dostupne literature, u publikaciji provedenoj u sklopu ove doktorske disertacije ustanovljen je točan filogenetski položaj roda *Entonaema* unutar porodice *Hypoxylaceae*. Za ostale analizirane vrste čije su sekvence bile dostupne u bazi podataka Genbank ustanovljeno je da pripadaju, ili kontaminaciji koja

se vjerojatno dogodila tijekom uzgoja kultura, ili pogrešno taksonomski imenovanim vrstama ovog roda.

5. POPIS LITERATURE

Aime, M.C., Miller, A.N., Aoki, T. et al. (2021). How to publish a new fungal species, or name, version 3.0. *IMA Fungus.* 12, 11. https://doi.org/10.1186/s43008-021-00063-1

Aldhebiani, A.Y. (2018). Species concept and speciation. *Saudi journal of biological sciences*, 25(3), 437-440.

Alessio, C.L. (1987). Complemento allo studio del Genere *Inocybe*: 8° contributo. *Riv. Micol. Assoc. Micol. Bresadola*, 30, 79–89.

Alessio, C.L., Rebaudengo, E. (1980). *Inocybe*. *Iconographia Mycologica*, Suppl. 3, Museo Tridentino di Scienze Naturali: Trento, Italy, Vol. 29.

Alvarado, P., Manjón, J.L., Matheny, P.B., Esteve-Raventós, F. (2010). *Tubariomyces*, a New Genus of *Inocybaceae* from the Mediterranean Region. *Mycologia.* 102, 1389–1397.

Andrews, T.M., Price, R.M., Mead, L.S., McElhinny, T.L., Thanukos, A., Perez, K.E., Herreid, C.F., Terry, D.R., Lemons, P.P. (2012). Biology undergraduates' misconceptions about genetic drift. *CBE Life Sci Educ* 11:248–259. https://doi.org/10.1187/cbe.11-12-0107

Antonelli, A., Fry, C., Smith, R.J., Simmonds, M.S.J., Kersey, P.J., Pritchard, H.W., Abbo, M.S., Acedo, C., Adams, J., Ainsworth, A.M., et al. (2020). State of the World's Plants and Fungi. *Royal Botanic Gardens*: Kew, UK

Azbukina, Z.M., Bardunov, L.V., Bezdeleva, T.A., Bogacheva, A.V., Bulakh, E.M., Vasilyeva, L.N., Govorova, O.K., Egorova, L.N., Zhabyko, E.V., Nikulina, T.V., et al. (2006). Flora, Vegetation and Mycobiota of the Reserve "Ussuriysky", Daljnauka: Vladivostok, Rasia.

Badotti, F., Fonseca, P.L.C., Tomé, L.M.R. et al. (2018). ITS and secondary biomarkers in fungi: review on the evolution of their use based on scientific publications. *Braz. J. Bot* 41, 471–479. https://doi.org/10.1007/s40415-018-0471-y

Baldrian, P., Větrovský, T., Lepinay, C., Kohout, P. (2021). High-throughput sequencing view on the magnitude of global fungal diversity. *Fungal Divers* 19:1–9.

Bandini, D., Brandrud, T.E., Dima, B., Dondl, M., Fachada, V., Hussong, A., Mifsud, S., Oertel, B., Rodríguez Campo, F.J., Thüs, H., et al. (2022). Fibre Caps across Europe:

Type Studies and 11 New Species of *Inocybe* (*Agaricales*, *Basidiomycota*). *Integr. Syst.* 5, 1–85.

Bandini, D., Oertel, B., Eberhardt, U. (2021). A Fresh Outlook on the Smooth-Spored Species of *Inocybe*: Type Studies and 18 New Species. *Mycol. Prog.* 20, 1019–1114.

Bandini, D., Oertel, B., Eberhardt, U. (2021). Noch Mehr Risspilze (2): Dreizehn Neue Arten Der Familie *Inocybaceae*. *Mycol. Bavarica* 21, 27–98.

Bandini, D., Oertel, B., Eberhardt, U. (2022). More Smooth-Spored Species of *Inocybe* (Agaricales, Basidiomycota): Type Studies and 12 New Species from Europe. *Persoonia Mol. Phylogeny Evol. Fungi*, 48, 91–149.

Bandini, D., Oertel, B., Eberhardt, U. (2022). Noch Mehr Risspilze (3): Einundzwanzig Neue Arten Der Familie *Inocybaceae*. *Mycol. Bavarica*. 22, 31–138.

Bandini, D., Oertel, B., Moreau, P.-A., Thines, M., Ploch, S. (2019). Three new hygrophilous species of *Inocybe*, subgenus Inocybe. *Mycol. Prog*. 18, 1101–1119.

Bandini, D., Oertel, B., Ploch, S., Ali, T., Vauras, J., Schneider, A., Scholler, M., Eberhardt, U., Thines, M. (2019). Revision of some central European species of *Inocybe* (Fr.: Fr.) Fr. subgenus *Inocybe*, with the description of five new species. *Mycol. Prog*. 18, 247–294.

Bandini, D., Oertel, B., Ploch, S., Thines, M. (2019). *Inocybe heidelbergensis*, eine neue Risspilz-Art der Untergattung Inocybe. *Z. Mykol*. 85, 195–213.

Bandini, D., Oertel, B., Schüssler, C., Eberhardt, U. (2020). Noch mehr Risspilze: Fünzehn neue und zwei wenig bekannte Arten der Gattung *Inocybe*. *Mycol. Bavarica.* 20, 13–101.

Bandini, D., Sesli, E., Oertel, B., Krisai-Greilhuber, I. (2020). *Inocybe antoniniana*, a new species of *Inocybe* section *Marginatae* with nodulose spores. *Sydowia*,.72, 95–106.

Bandini, D., Vauras, J., Weholt, Ø., Oertel, B., Eberhardt, U. (2020). *Inocybe woglindeana*, a new species of the genus *Inocybe*, thriving in exposed habitats with calcareous sandy soil. *Karstenia.* 58, 41–59.

Bánki, O., Roskov, Y., Döring, M., Ower, G., Hernández Robles, D. R., Plata Corredor, C. A., Stjernegaard Jeppesen, T., Örn, A., Vandepitte, L., Hobern, D., Schalk, P., DeWalt, R. E., Ma, K., Miller, J., Orrell, T., Aalbu, R., Abbott, J., Adlard, R., Adriaenssens, E. M.,

et al. (2023). Catalogue of Life Checklist (Version 2023-09-14). Catalogue of Life. https://doi.org/10.48580/ddz4x

Baral, H.O. (1992). Vital versus Herbarium Taxonomy: Morphological Differences between Living and Dead Cells of *Ascomycetes*, and Their Taxonomic Implications. *Mycologia*. 44, 333–390.

Bender, H. & Melzer, A. (2020). Zwei neue Arten der Gattung *Coprinopsis*. *Zeitschrift für Mykologi.e* 87 (1): 31–46.

Benkert, D. (1993). Kotlabaea macrospora Benkert nov. sp. und einige weitere bemerkenswerte Ascomyceten aus Bulgarien Mit einer Abbildung. *Feddes Repert*. 104, 547–549.

Berkeley, M.J. & Broome, C.E. (1861). Notices of British fungi (901–951). Annals and Magazine of Natural History 7: 373–382.

Berkeley, M.J. & Curtis, M.A. (1869). Fungi Cubenses (*Hymenomycetes*). *Journal of the Linnean Society, Botany* 10: 280–392. https://doi.org/10.1111/j.1095-8339.1868.tb00529.x

Bhunjun CS, Phukhamsakda C, Jayawardena RS, Jeewon R, Promputtha I, Hyde KD (2021). Investigating species boundaries in Colletotrichum. *Fungal Divers* 107:107–127

Bills, G.F., González-Menéndez, V., Martín, J., Platas, G., Fournier, J., Peršoh, D., Stadler, M. (2012). Hypoxylon pulicicidum sp. nov. (Ascomycota, Xylariales), a Pantropical Insecticide-Producing Endophyte. *PLoS ONE*. 7, e46687.

Bitzer, J., Læssøe, T., Fournier, J., Kummer, V., Decock, C., Tichy, H.-V., Piepenbring, M., Peršoh, D., Stadler, M. (2008). Affinities of Phylacia and the daldinoid *Xylariaceae*, inferred from chemotypes of cultures and ribosomal DNA sequences. *Mycol. Res.* 112, 251–270.

Bizio, E., Ferisin, G., Dovana, F. (2017). Note Sul Campo Di Variabilita Di Inocybe. Riv. Micol. 60, 59–70.

Blondel, J., Aronson, J., Bodiou, J.-Y., Boeuf, G. (2010). The Mediterranean Region—Biological Diversity in Space and Time, 2nd ed., Oxford University Press: New York, NY, USA.

Bohus, G. (1970). Ergebnisse der auf die Hutpilze (*Agaricales*) bezüglichen systematischen und ökologischen Forschungen. VI. *Botanikai Kölzlemények* 57 (1): 13–22.

Bortolus A. (2008). Error Cascades in the Biological Sciences: The Unwanted Consequences of Using Bad Taxonomy in Ecology. *J Human Environ* 37, 114–8.

Branković, Č., Güttler, I., Gajić-Čapka, M. (2013). Evaluating Climate Change at the Croatian Adriatic from Observations and Regional Climate Models' Simulations. *Clim. Dyn*. 41, 2353–2373.

Brijuni National Park Official Website. Available online: https://www.np-brijuni.hr/en/brijuni (accessed on 12 January 2023).

Brogli, R., Sørland, S.L., Kröner, N., Schär, C. (2019). Causes of future Mediterranean precipitation decline depend on the season. *Environ. Res. Lett*. 14, 114017.

Brondizio, E., Díaz, S. M., Settele, J., Ngo, H., Gueze, M., Aumeeruddy-Thomas, Y., ... & Zayas, C. (2019). Assessing a planet in transformation: rationale and approach of the IPBES Global Assessment on Biodiversity and Ecosystem Services.

Cai, F., & Druzhinina, I. S. (2021). In honor of John Bissett: Authoritative guidelines on molecular identification of *Trichoderma*. *Fungal Diversity*, 107, 1-69.

Capella-Gutiérrez, S., Silla-Martínez, J.M., Gabaldón, T. (2009). TrimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics.* 25, 1972–1973.

Carteret, X., Reumaux, P. (2012). Miettes Sur Les Inocybes (6ème Série), Études de Quelques Nains des Feuillus de La Plaine, Accompagnée d' Une Clé de Détermination Des Taxons de La Section Lilacinae R. Heim. Bull. *Soc. Mycol. Fr*. 127, 1–53.

Cedeño–Sanchez, M. (2020). Three new species of *Hypoxylon* and new records of *Xylariales* from Panama. *Mycosphere*. 11, 1457–1476.

Chawngthu, Z., Vabeikhokhei, J.M.C. & Zothanzama, J. (2019). Molecular phylogenetic identification of wood inhabiting fungi isolated from Dampa Tiger reserve forest. *Journal of Emerging Technologies and Innovative Research* 6 (4): 941–949.

Cheek, M., Nic Lughadha, E., Kirk, P., Lindon, H., Carretero, J., Looney, B., Douglas, B., Haelewaters, D., Gaya, E., Llewellyn, T., et al. (2020). New scientific discoveries: Plants and fungi. *Plants People Planet* 2, 371–388.

Chernomor, O., von Haeseler, A., Minh, B.Q. (2016). Terrace aware data structure for phylogenomic inference from supermatrices. *Syst. Biol.* 65, 997–1008.

Chethana, K.W.T., Jayawardena, R.S., Hyde, K.D. (2020). Hurdles in fungal taxonomy: Effectiveness of recent methods in discriminating taxa. *Megataxa.* 001, 114–122.

Chethana, K.W.T., Manawasinghe, I.S., Hurdeal, V.G. et al. (2021). What are fungal species and how to delineate them?. *Fungal Diversity* 109, 1–25. https://doi.org/10.1007/s13225-021-00483-9

Chethana, T.K.W., Jayawardena, R.S., Chen, Y.J., Konta, S, Tibpromma, S., Phukhamsakda, C., Abeywickrama, P.D., Samarakoon, M.C., Senwanna, C., Mapook, A., Tang, X. (2021). Appressorial interactions with host and their evolution. *Fungal Divers.* 23:1–33.

Chevallier, F.F. (1826). *Flore Générale des Environs de Paris*. Paris, 674 pp.

Clémençon, H. (2012). *Cytology and Plectology of the Hymenomycetes*, 2nd ed., Cramer: Stuttgart, Germany,

Cooke, M.C. (1887). Some Australian Fungi. *Grevillea.* 15, 93–101.

Cripps, C.L., Larsson, E., Vauras, J. (2019). Nodulose-spored Inocybe from the Rocky Mountain alpine zone molecularly linked to European and type specimens. *Mycologia.* 112, 133–153.

Cronquist, A. (1978). *Once again, what is a species? Biosystematics in agriculture*. In Beltsville Symposia in Agr. Res. (Vol. 2, pp. 3-20).

Crous P.W., Wingfield M.J., Burgess T.I., et al. (2017). Fungal Planet description sheets: 625–715. *Persoonia.* 39: 270–467.

Crous, P. W., Wingfield, M. J., Burgess, T. I., Hardy, G. S. J., Gené, J., Guarro, J., ... & Groenewald, J. Z. (2018). Fungal Planet description sheets: 716–784. *Persoonia: Molecular Phylogeny and Evolution of Fungi.* 40, 240.

Crous, P.W., Carnegie, A.J., Wingfield, M.J., Sharma, R., Mughini, G., Noordeloos, M.E., Santini, A., Shouche, Y.S., Bezerra, J.D.P., Dima, B., et al. (2019). Fungal Planet description sheets: 868–950. *Persoonia*. 42, 291–473.

Crous, P.W., Cowan, D.A., Maggs-Kölling, G., Yilmaz, N., Larsson, E., Angelini, C., Brandrud, T.E., Dearnaley, J.D.W., Dima, B., Dovana, F., et al. (2020). Fungal Planet description sheets: 1112–1181. *Persoonia*. 45, 251–409.

Dahlberg, A., Mueller, G.M. (2011). Applying IUCN Red-Listing Criteria for Assessing and Reporting on the Conservation Status of Fungal Species. *Fungal Ecol*. 4, 147–162.

Dai, D.Q., Phookamsak, R., Wijayawardene, N.N., Li, W.J., Bhat, D.J., Xu, J.C., Taylor, J.E., Hyde, K.D., Chukeatirote, E. (2016). Bambusicolous fungi. *Fungal Divers*. 82, 1–105.

Daranagama, D.A., Camporesi, E., Tian, Q., Liu, X., Chamyuang, S., Stadler, M., Hyde, K.D. (2015). Anthostomella is polyphyletic comprising several genera in *Xylariaceae*. *Fungal Divers*. 73, 203–238.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods.* 9: 772. https://doi.org/10.1038/nmeth.2109

Dayrat, B. (2005). Towards integrative taxonomy. *Biological journal of the Linnean society*, 85(3), 407-417.

Dentinger, B.T., Didukh, M.Y., Moncalvo, J.M. (2011). Comparing COI and ITS as DNA barcode markers for mushrooms and allies (*Agaricomycotina*). *PLoS ONE* 6, e25081.

Díaz, S., & Malhi, Y. (2022). Biodiversity: Concepts, patterns, trends, and perspectives. *Annual Review of Environment and Resources.* 47, 31-63.

Edgar, R.C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*. 32, 1792–1797.

Egger, K.N. (1995). Molecular analysis of ectomycorrhizal fungal communities. *Can. J. Bot*. 73, S1415–S1422.

Einax, E., & Voigt, K. (2003). Oligonucleotide primers for the universal amplification of β-tubulin genes facilitate phylogenetic analyses in the regnum Fungi. *Organisms Diversity & Evolution,* 3(3), 185-194.

Entonaema Liquescens · INaturalist. Available online: https://www.inaturalist.org/taxa/350744-Entonaema-liquescens (accessed on 25 April 2023).

Erb, B., Matheis, W. (1982). *Pilzmikroskopie*, Kosmos: Stuttgart, Germany.

Eriksson, O.E., Hawksworth, D.L. (1993). Outline of the *Ascomycetes*—1993. *Syst. Ascomycetum*, 12, 51–257.

Esteve-Raventós, F. (2001). Two new species of *Inocybe* (*Cortinariales*) from Spain, with a comparative type study of some related taxa. *Mycol. Res.* 105, 1137–1143.

Esteve-Raventós, F., Moreno, G., Alvarado, P., & Olariaga, I. (2016). Unraveling the *Inocybe* praetervisa group through type studies and ITS data: *Inocybe praetervisoides* sp. nov. from the Mediterranean region. *Mycologia*, 108(1), 123-134.

Fedosova, A.G. (2012). The New Record of *Entonaema cinnabarinum* (*Xylariaceae, Ascomycota*) in Europe. Vestnik of Saint Petersburg University. Series 3. *Biology*, 1, 10–13.

Ferrari, E. (2010). *Inocybe Dai Litorali Alla Zona Alpina*. In Fungi non Delineati 54/55, Edizioni Candusso: Alassio, Italy.

Ferrari, E., Bandini, D., Boccardo, F. (2014). *Inocybe* (Fr.) Fr., Terzo Contributo, Fungi non delineati 73/74, Edizioni Candusso: Alassio, Italy.

Fintha, G., Benedek, L., Orbán, S. (2019). New Macrofungial Record in Hungary: *Entonaema cinnabarinum* (Cooke & Massee) Lloyd. *Acta Biol. Plant. Agriensis*. 7, 127–130.

Fintha, G., Nagy, I., Vitkó, T., Benedek, L., Baranyai, G. (2022). Az Ócsai Turjánvidék Natura 2000-es kijelölt területeinek nagygombái. *J. Landscape Ecol*. 20, 3–21.

Francisco, C.S., Ma, X., Zwyssig, M.M., McDonald, B.A., Palma-Guerrero, J. (2019). Morphological changes in response to environmental stresses in the fungal plant pathogen *Zymoseptoria tritici*. *Sci Rep*. 9(1):9642. doi: 10.1038/s41598-019-45994-3.

Fries, E. (1838). Epicrisis Systematis Mycologici seu Synopsis *Hymenomycetum*. Uppsala, 612 pp.

Ganga, K.G.G., Manimohan, P. (2018). A New Species and a New Record of *Parasola* from Kerala State, India. *Phytotaxa*. 369, 260–268.

Garcia, G. & Vellinga, E.C. (2010). Un nouvelle espèce de coprin sur tiges de Polygonatum multiflorum: *Coprinopsis nevillei* sp. nov. *Bulletin Semestriel de la Fédération des Associations Mycologiques Méditerranéennes* 37: 37–58.

Gardes, M., Bruns, T.D. (1993). ITS Primers with Enhanced Specificity for *Basidiomycetes*—Application to the Identification of Mycorrhizae and Rusts. *Mol. Ecol.*, 2, 113–118.

Gazis, R., Chaverri, P. (2010). Diversity of fungal endophytes in leaves and stems of wild rubber trees (*Hevea brasiliensis*) in Peru. *Fungal Ecol.* 3, 240–254.

Geml, J., Arnold, A.E., Semenova-Nelsen, T.A., Nouhra, E.R., Drechsler-Santos, E.R., Góes-Neto, A., Morgado, L.N., Ódor, P., Hegyi, B., Oriol, G., et al. (2022). Community Dynamics of Soil-Borne Fungal Communities along Elevation Gradients in Neotropical and Palaeotropical Forests. *Mol. Ecol.* 31, 2044–2060

Gierczyk, V., Rodriguez-Flakus, P., Pietras, M., Gryc, M., Czerniawski, W. & Piatek, M. (2017). *Coprinopsis rugosomagnispora*: a distinct new coprinoid species from Poland (Central Europe). *Plant Systematics and Evolution* 303: 915–925. https://doi.org/10.1007/s00606-017-1418-7

Giorgi, F. (2006). Climate change hot-spots. *Geophys. Res. Lett*. 33, 1–4.

Goldstein, P. Z., & DeSalle, R. (2011). Integrating DNA barcode data and taxonomic practice: determination, discovery, and description. *Bioessays*. 33(2), 135-147.

Gonzalez del Val, A., Platas, G., Arenal, F., Orihuela, J.C., Garcia, M., Hernández, P., Royo, I., De Pedro, N., Silver, L.L., Young, K., Vicente, M.F. & Pelaez, F. (2003). Novel illudins from *Coprinopsis episcopalis* (syn. *Coprinus episcopalis*), and the distribution of illudin-like compounds among filamentous fungi. *Mycological Research*. 107 (10): 1201–1209. https://doi.org/10.1017/s0953756203008487

Greeshma Ganga, K.G., Manimohan, P. (2019). *Parasola psathyrelloides* (*Psathyrellaceae*), a New Species from Kerala State, India. *Phytotaxa* 405, 255–262.

Grund, D.W., Stuntz, D.E. (1968). Nova Scotian *Inocybes*. I. *Mycologia* 60, 406–425.

Guindon, S. & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696–704. https://doi.org/10.1080/10635150390235520

Habibullah, M.S., Din, B.H., Tan, S.-H., Zahid, H. (2022). Impact of Climate Change on Biodiversity Loss: Global Evidence. *Environ. Sci. Pollut. Res.* 29, 1073–1086.

Haelewaters, D., Dirks, A.C., Kappler, L.A., Mitchell, J.K., Quijada, L., Vandegrift, R., Buyck, B., Pfister, D.H. (2018). A preliminary checklist of fungi at the Boston Harbor islands. *Northeast. Nat.* 25, 45–76.

Haelewaters, D., Toome-Heller, M., Albu, S., Aime, M.C. (2020). Red yeasts from leaf surfaces and other habitats: Three new species and a new combination of *Symmetrospora* (*Pucciniomycotina*, *Cystobasidiomycetes*). *Fungal Syst. Evol.* 5, 187–196.

Harkevich, S.S. (1978). (Ed.) Флора и Растительность Уссурийского Заповедника , Наука: Moscow, Russia.

Hausknecht A., Mešić A., Tkalčec Z. (2007). Two remarkable species of *Bolbitiaceae* (*Agaricales*) from Croatia. *Österreichische Zeitschrift für Pilzkunde* 16: 281–286.

Hawksworth DL, Lücking R (2017). Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiol Spectr* 5:79–95.

Hawksworth, D.L. (1991). The fungal dimension of biodiversity: Magnitude, significance, and conservation. *Mycol. Res*., 95, 641–655.

Hawksworth, D.L. (2010). Terms Used in Bionomenclature. The naming of organisms (and plant communities). Copenhagen: Global Biodiversity Information Facility, 216 pp, accessible online at www.gbif.org/document/80577. ISBN: 87-92020-09-7.

He, M.Q., Zhao, R.L., Hyde, K.D., Begerow, D., Kemler, M., Yurkov, A., McKenzie, E.H.C., Raspé, O., Kakishima, M., Sánchez-Ramírez, S., et al. (2019). Notes, outline and divergence times of *Basidiomycota*. *Fungal Divers.* 99, 105–367.

Heim, R. (1960). Quelques Ascomycétes Remarquables, II.—Le Genre Entonaema Möll. Au Mexique. Bull. Trimest. *Société Mycol. De Fr*. 76, 121–129.

Helaly, S.E., Thongbai, B., Stadler, M. (2018). Diversity of biologically active secondary metabolites from endophytic and saprotrophic fungi of the ascomycete order *Xylariales*. *Nat. Prod. Rep.* 35, 992–1014.

Hennig W. (1966). *Phylogenetic systematics*. University of Illinois Press, Urbana, USA.

Henriot, A., Cheype, J.-L. Piximètre: La Mesure de Dimensions Sur Images. Version 5.10 R1541. Available online: http://ach.log.free.fr/Piximetre (accessed on 1 October 2020).

Higginbotham, S., Wong, W.R., Linington, R.G., Spadafora, C., Iturrado, L., Arnold, A.E. (2014). Sloth Hair as a Novel Source of Fungi with Potent Anti-Parasitic, Anti-Cancer and Anti-Bacterial Bioactivity. *PLoS ONE 9*, e84549.

Hillis, D.M., Dixon, M.T. (1991). Ribosomal DNA: Molecular evolution and phylogenetic inference. *Q. Rev. Biol*., 66, 411–453.

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., Vinh, L.S. (2017). UFBoot2: Improving the ultrafast bootstrap approximation. *Mol. Biol. Evol*. 35, 518–522.

Hofstetter, V., Buyck, B., Eyssartier, G., Schnee, S., & Gindro, K. (2019). The unbearable lightness of sequenced-based identification. *Fungal Diversity*. 96(1), 243-284.

Hopple, J.S. (1994). Phylogenetic Investigations in the Genus *Coprinus* Based on Morphological and Molecular Characters. Ph.D. Thesis, Duke University, Durham, NC, USA.

Hopple, J.S., Vilgalys, R. (1999). Phylogenetic Relationships in the Mushroom Genus *Coprinus* and Dark-Spored Allies Based on Sequence Data from the Nuclear Gene Coding for the Large Ribosomal Subunit RNA: Divergent Domains, Outgroups, and Monophyly. *Mol. Phylogenet. Evol.*, 13, 1–19.

Horak, E., Matheny, P.B., Desjardin, D.E., Soytong, K. (2015). The Genus *Inocybe* (*Inocybaceae*, *Agaricales*, *Basidiomycota*) in Thailand and Malaysia. *Phytotaxa* 230, 201.

Horisawa, S., Sakuma, Y., & Doi, S. (2009). Qualitative and quantitative PCR methods using species-specific primer for detection and identification of wood rot fungi. *Journal of Wood Science*, 55, 133-138.

Houbraken, J., de Vries, R. P., & Samson, R. A. (2014). Modern taxonomy of biotechnologically important *Aspergillus* and *Penicillium* species. *Advances in applied microbiology*, 86, 199-249.

Hsieh, H.-M., Ju, Y.-M., Rogers, J.D. (2005). Molecular phylogeny of *Hypoxylon* and closely related genera. *Mycologia*, 97, 844–865.

Hsieh, H.-M., Lin, C.-R., Fang, M.-J., Rogers, J.D., Fournier, J., Lechat, C., Ju, Y.-M. (2010). Phylogenetic status of *Xylaria* subgenus *Pseudoxylaria* among taxa of the subfamily *Xylarioideae* (*Xylariaceae*) and phylogeny of the taxa involved in the subfamily. *Mol. Phylogenetics Evol*. 54, 957–969.

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

Hussain, S., Afshan, N.u.S., Ahmad, H., Khalid, A.N., Niazi, A.R. (2017). *Parasola malakandensis* sp. nov. (*Psathyrellaceae*, *Basidiomycota*) from Malakand, Pakistan. *Mycoscience* 58, 69–76.

Hussain, S., Ahmad, H., Ullah, S., Afshan, N.U.S., Pfister, D.H., Sher, H., Ali, H., Khalid, A.N. (2018). The Genus *Parasola* in Pakistan with the Description of Two New Species. *MycoKeys* 30, 41–60.

Hyde K.D., Norphanphoun C., Abreu V.P., et al. (2017). Fungal diversity notes 603–708: taxonomic and phylogenetic notes on genera and species. *Fungal Diversity*. 87: 1–235

INaturalist Research-Grade Observations. Available online: https://www.inaturalist.org (accessed on 26 June 2023).

Intergovernmental Science – Policy Platform on Biodiversity and Ecosystem Services IPBES (2019) http://www.ipbes.net/

Jenkins, D.G., Carey, M., Czerniewska, J., Fletcher, J., Hether, T., Jones, A., Knight, S., Knox, J., Long, T., Mannino, M., McGuire, M., Riffle, A., Segelsky, S., Shappell, L., Sterner, A., Strickler, T. & Tursi, R. (2010). A meta-analysis of isolation by distance: Relic or reference standard for landscape genetics? *Ecography* 33: 315–320. https://doi.org/10.1111/j.1600-0587.2010.06285.x

Johannesson, H., Læssøe, T., Stenlid, J. (2000). Molecular and morphological investigation of *Daldinia* in northern Europe. *Mycol. Res*. 104, 275–280.

Johnson, N.A. (2022). Speciation: genomic sequence data and the biogeography of speciation. *Natl Sci Rev.* 9(12):nwac294. doi:10.1093/nsr/nwac294

Joly, C. A. (2023). The Kunming-Montréal Global Biodiversity Framework. *Biota Neotropica*, 22.

Ju, Y.-M., Hsieh, H.-M. (2007). *Xylaria* species associated with nests of *Odontotermes* formosanus in Taiwan. *Mycologia* 99, 936–957.

Ju, Y.-M., Hsieh, H.-M., Rogers, J.D., Fournier, J., Jaklitsch, W.M., Courtecuisse, R. (2012). New and interesting penzigioid *Xylaria* species with small, soft stromata. *Mycologia* 104, 766–776.

Ju, Y.-M., Rogers, J.D. (1996). A Revision of the Genus *Hypoxylon*, APS Press: St Paul, MN, USA.

Ju, Y.M., Rogers, J.D., San Martin, F. (1997). A Revision of the Genus *Daldinia*. *Mycotaxon* 61, 243–293.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermiin, L.S. (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14, 587–589.

Karsten, P.A. (1879). Rysslands, Finlands och den Skandinaviska halföns Hattsvampar. Förra Delen: Skifsvampar. *Bidrag till Kännedom av Finlands Natur och Folk* 32: 1–571.

Karsten, P.A. (1881). *Hymenomycetes* Fennici enumerati. *Acta Societatis Pro Fauna et Flora Fennica* 2 (1): 1–40.

Katoh, K., Misawa, K., Kuma, K.I., Miyata, T. (2002). MAFFT: A Novel Method for Rapid Multiple Sequence Alignment Based on Fast Fourier Transform. *Nucleic Acids Res*. 30, 3059–3066.

Katoh, K., Standley, D.M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol. Biol. Evol.* 30, 772–780.

Keles, A. (2019). New records of macrofungi from Trabzon province (Turkey). *Applied Ecology and Environmental Research*. 17 (1): 1061–1069. https://doi.org/10.15666/aeer/1701_10611069

Kim, C.S., Jo, J.W., Kwag, Y.-N., Sung, G.-H., Lee, S.-G., Kim, S.-Y., Shin, C.-H., Han, S.-K. (2015). Mushroom Flora of Ulleung-gun and a Newly Recorded Bovista Species in the Republic of Korea. *Mycobiology* 43, 239–257.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*. 16: 111–120. https://doi.org/10.1007/BF01731581

Kits van Waveren, E. (1985). The Dutch, French and British Species of *Psathyrella*. *Persoonia*-Supplement, 2, 3–300.

Kohn LM (2005). Mechanisms of fungal speciation. *Annu Rev Phytopathol* 43:279–308. https://doi.org/10.1146/annurev.phyto.43.040204.135958

Konrad, P.A. (1929). Notes Critiques Sur Quelques Champignons Du Jura. *Bull. Soc. Mycol. Fr*., 45, 375–400.

Kottek, M., Grieser, J., Beck, C., Rudolf, B., Rubel, F. (2006). World map of the Köppen-Geiger climate classification updated. *Meteorol*. Z. 15, 259–263.

Koukol, O., Kelnarová, I., Černý, K. (2014). Recent observations of sooty bark disease of sycamore maple in Prague (Czech Republic) and the phylogenetic placement of *Cryptostroma corticale*. *For. Pathol.* 45, 21–27.

Krieglsteiner, L.G. (2019). *Inocybe calosporoides*—Ein neuer Risspilz aus Portugal. *Südwestdeutsche Pilzrundsch*. 55, 68–72.

Krieglsteiner, L.G. (2019). Nomenclatural novelties. *Index Fungorum* 411, 1.

Kropp, B.R., Matheny, P.B., Hutchison, L.J. (2013). Inocybe Section *Rimosae* in Utah: Phylogenetic Affinities and New Species. *Mycologia*. 105, 728–747.

Kropp, B.R., Matheny, P.B., Nanagyulyan, S.G. (2010). Phylogenetic Taxonomy of the *Inocybe* splendens Group and Evolution of Supersection "*Marginatae*". *Mycologia* 102, 560–573.

Kuhnert, E., Fournier, J., Peršoh, D., Luangsa-Ard, J.J.D., Stadler, M. (2013). New *Hypoxylon* species from Martinique and new evidence on the molecular phylogeny of *Hypoxylon* based on ITS rDNA and β-tubulin data. *Fungal Divers*. 64, 181–203.

Kuhnert, E., Sir, E.B., Lambert, C., Hyde, K.D., Hladki, A.I., Romero, A.I., Rohde, M., Stadler, M. (2016). Phylogenetic and chemotaxonomic resolution of the genus *Annulohypoxylon* (*Xylariaceae*) including four new species. *Fungal Divers*. 85, 1–43.

Kumar, S., Stecher, G., Li, M., Knyaz, Ch. & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547–1549. https://doi.org/10.1093/molbev/msy096

Kumar, S., Stecher, G., Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874.

Kušan I., Matočec N., Mešić A., Tkalčec Z., (2015a). A new species of *Thecotheus* from Croatia with a key to the known species with apiculate spores. *Sydowia* 67: 51–63.

Kušan I., Matočec N., Mešić A., Tkalčec Z. (2015b). *Tricharina tophiseda*—a new species from Croatia, with a revision of *T. japonica* (*Pyronemataceae, Pezizales*). *Phytotaxa* 221(1): 35–47.

Kušan, I., Matočec, N., Jadan, M., Tkalčec, Z. & Mešić, A. (2018). An overview of the genus *Coprotus* (*Pezizales, Ascomycota*) with notes on the type species and description of *C. epithecioides* sp. nov. *Mycokeys* 29: 15–47. https://doi.org/10.3897/mycokeys.29.22978

Kuyper, T.W. (1985). Studies in *Inocybe* I.—Revision of the New Taxa of *Inocybe* Described by Velenovský. *Persoonia,* 12, 375–400.

Kuyper, T.W. (1986). A revision of the genus *Inocybe* in Europe 1. Subgenus *Inosperma* and the smooth-spored species of subgenus *Inocybe*. *Persoonia*. 3, 1–247.

Kuyper, T.W. (1986). A Revision of the Genus *Inocybe* in Europe I. Subgenus *Inosperma* and the Smooth-Spored Species of Subgenus *Inocybe*, *Persoonia*-Supplement, Naturalis Biodiversity Center: Leiden, The Netherlands, Volume 3, ISBN 9071236021.

Lambert, C., Pourmoghaddam, M.J., Cedeño-Sanchez, M., Surup, F., Khodaparast, S.A., Krisai-Greilhuber, I., Voglmayr, H., Stradal, T.E.B., Stadler, M. (2021). Resolution of the *Hypoxylon fuscum* Complex (*Hypoxylaceae, Xylariales*) and Discovery and Biological Characterization of Two of Its Prominent Secondary Metabolites. *J. Fungi*, 7, 131.

Larsson, E. & Örstadius, L. (2008). Fourteen coprophilous species of *Psathyrella* identified in the Nordic countries using morphology and nuclear rDNA sequence data. *Mycological Research* 112 (10): 1165–1185. https://doi.org/10.1016/j.mycres.2008.04.003

Larsson, E., Örstadius, L. (2008). Fourteen *Coprophilous* Species of *Psathyrella* Identified in the Nordic Countries Using Morphology and Nuclear RDNA Sequence Data. *Mycol. Res*. 112, 1165–1185.

Latha, K.P.D., Manimohan, P., Matheny, P.B. (2016). A New Species of *Inocybe* Representing the *Nothocybe* Lineage. *Phytotaxa* 267, 40.

Laurila-Pant, M., Lehikoinen, A., Uusitalo, L., & Venesjärvi, R. (2015). How to value biodiversity in environmental management?. *Ecological indicators*, 55, 1-11.

Leigh, J.W. & Bryant, D. (2015). POPART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* 6: 1110–1116. https://doi.org/10.1111/2041-210X.12410

Letunic, I., Bork, P. (2021). Interactive Tree of Life (ITOL) v5: An Online Tool for Phylogenetic Tree Display and Annotation. *Nucleic Acids Res*. 49, W293–W296.

Li, Q.R., Kang, J.C., Hyde, K.D. (2015). Two new species of the genus *Collodiscula* (*Xylariaceae*) from China. *Mycol. Prog*. 14, 52.

Li, X., & Wiens, J. J. (2023). Estimating global biodiversity: the role of cryptic insect species. *Systematic Biology*, 72(2), 391-403.

Li, Y., Steenwyk, J. L., Chang, Y., Wang, Y., James, T. Y., Stajich, J. E., ... & Rokas, A. (2021). A genome-scale phylogeny of the kingdom Fungi. *Current Biology*, 31(8), 1653-1665.

Lindner, D. L., Carlsen, T., Henrik Nilsson, R., Davey, M., Schumacher, T., & Kauserud, H. (2013). Employing 454 amplicon pyrosequencing to reveal intragenomic divergence in the internal transcribed spacer rDNA region in fungi. *Ecology and Evolution*, 3(6), 1751-1764.

Liu, K. L., Porras-Alfaro, A., Kuske, C. R., Eichorst, S. A., & Xie, G. (2012). Accurate, rapid taxonomic classification of fungal large-subunit rRNA genes. *Applied and environmental microbiology*. 78(5), 1523-1533.

Liu, Y.J., Whelen, S., Hall, B.D. (1999). Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerse II subunit. *Mol. Biol. Evol*. 16, 1799–1808.

Lloyd, C.G. (1923). Mycological Notes, No. 69. *Mycol. Writ*., 7, 1185–1218.

Lofgren, L. A., & Stajich, J. E. (2021). Fungal biodiversity and conservation mycology in light of new technology, big data, and changing attitudes. *Current Biology*. 31(19), R1312-R1325.

Lücking R, Aime MC, Robbertse B, Miller AN, Aoki T, Ariyawansa HA, Cardinali G, Crous PW, Druzhinina IS, Geiser DM, Hawksworth DL, Hyde KD, Irinyi L, Jeewon R, Johnston PR, Kirk PM, Malosso E, May TW, Meyer W, Nilsson HR, Öpik M, Robert V, Stadler M, Thines M, Vu D, Yurkov AM, Zhang N, Schoch CL (2021). Fungal taxonomy and sequence-based nomenclature. *Nat Microbiol* 6:540–548

Lücking, R., Aime, M. C., Robbertse, B., Miller, A. N., Ariyawansa, H. A., Aoki, T., ... & Schoch, C. L. (2020). Unambiguous identification of fungi: where do we stand and how accurate and precise is fungal DNA barcoding?. *IMA fungus*, 11(1), 1-32.

Lücking, R., Aime, M.C., Robbertse, B., Miller, A.N., Aoki, T., Ariyawansa, H.A., Cardinali, G., Crous, P.W., Druzhinina, I.S., Geiser, D.M., et al. (2021). Fungal taxonomy and sequence-based nomenclature. *Nat. Microbiol*. 6, 540–548.

Lücking, R., Hodkinson, B. P., & Leavitt, S. D. (2017). The 2016 classification of lichenized fungi in the *Ascomycota* and *Basidiomycota*–Approaching one thousand genera. *The Bryologist*, 119(4), 361-416

Læssøe, T. (1997). *Entonaema cinnabarina*-En Eksotisk Kernesvamp. *Svampe*, 36, 21–22.

Ma, H., Song, Z., Pan, X., Li, Y., Yang, Z., Qu, Z. (2022). Multi-Gene Phylogeny and Taxonomy of *Hypoxylon* (*Hypoxylaceae*, *Ascomycota*) from China. *Diversity* 14, 37.

Ma, H.-X., Qiu, J.-Z., Xu, B., Li, Y. (2018). Two *Hypoxylon* species from Yunnan Province based on morphological and molecular characters. *Phytotaxa* 376, 27–36.

Mace, G.M. (2010). *Drivers of Biodiversity Change*. In Trade-Offs in Conservation: Deciding What to Save, John Wiley & Sons: Hoboken, NJ, USA, pp. 349–364.

Maharachchikumbura, S. S., Chen, Y., Ariyawansa, H. A., Hyde, K. D., Haelewaters, D., Perera, R. H., ... & Stadler, M. (2021). Integrative approaches for species delimitation in *Ascomycota*. *Fungal Diversity*, 109(1), 155-179.

Malysheva, E., Moreno, G., Villarreal, M., Malysheva, V., Svetasheva, T. (2019). The Secotioid Genus *Galeropsis* (*Agaricomycetes*, *Basidiomycota*): A Real Taxonomic Unit or Ecological Phenomenon? *Mycol. Prog*. 18, 805–831.

Maniotis, J. (1964). The Coprinoid state of *Rhacophyllus lilacinus*. *American Journal of Botany* 51: 485–494.

Mann, D. G., & Evans, K. M. (2007). Molecular genetics and the neglected art of diatomics. Unravelling the algae: the past, present, and future of algal systematics, 13, 231.

Mariotti, A., Zeng, N., Yoon, J.-H., Artale, V., Navarra, A., Alpert, P., Li, L.Z.X. (2008). Mediterranean water cycle changes: Transition to drier 21st century conditions in observations and CMIP3 simulations. *Environ. Res. Lett*. 3, 044001.

Martin, G.W. (1938). New or Noteworthy Fungi from Panama and Columbia. II. *Mycologia*. 30, 431–441.

Matheny B., P., Wang, Z., Binder, M., Curtis, J.M., Lim, Y.W., Henrik Nilsson, R., Hughes, K.W., Hofstetter, V., Ammirati, J.F., Schoch, C.L., et al. (2007). Contributions of Rpb2 and Tef1 to the Phylogeny of Mushrooms and Allies (*Basidiomycota*, *Fungi*). *Mol. Phylogenet*. Evol. 43, 430–451.

Matheny, P.B. (2005). Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*, *Agaricales*). *Mol. Phylogenet*. Evol. 35, 1–20.

Matheny, P.B., Aime, M.C., Bougher, N.L., Buyck, B., Desjardin, D.E., Horak, E., Kropp, B.R., Lodge, D.J., Soytong, K., Trappe, J.M., et al. (2009). Out of the Palaeotropics? Historical Biogeography and Diversification of the Cosmopolitan Ectomycorrhizal Mushroom Family Inocybaceae. *J. Biogeogr*. 36, 577–592.

Matheny, P.B., Aime, M.C., Smith, M.E., Henkel, T.W. (2012). New Species and Reports of *Inocybe* (*Agaricales*) from Guyana. *Kurtziana* 37, 23–39.

Matheny, P.B., Bougher, L.N. (2017). Fungi of Australia. *Inocybaceae*, Australian Biological Resources Study, CSIRO Publishing: Canberra, VC, Australia, Melbourne, VC, Australia.

Matheny, P.B., Curtis, J.M., Hofstetter, V., Aime, M.C., Moncalvo, J.M., Ge, Z.W., Yang, Z.L., Slot, J.C., Ammirati, J.F., Baroni, T.J., et al. (2006). Major Clades of *Agaricales*: A Multilocus Phylogenetic Overview. *Mycologia* 98, 982–995.

Matheny, P.B., Henkel, T.W., Séné, O., Korotkin, H.B., Dentinger, B.T.M., Aime, M.C. (2017). New Species of *Auritella* (*Inocybaceae*) from Cameroon, with a Worldwide Key to the Known Species. *IMA Fungus* 8, 287–298.

Matheny, P.B., Hobbs, A.M., Esteve-Raventós, F. (2019). Genera of *Inocybaceae*: New skin for the old ceremony. *Mycologia* 112, 83–120.

Matheny, P.B., Kudzma, L.V. (2019). New species of *Inocybe* (*Inocybaceae*) from eastern North America. *J. Torrey Bot. Soc*. 146, 213–235.

Matheny, P.B., Liu, Y.J., Ammirati, J.F., Hall, B.D. (2002). Using RPB1 Sequences to Improve Phylogenetic Inference among Mushrooms (*Inocybe, Agaricales*). *Am. J. Bot.* 89, 688–698.

Matheny, P.B., Norvell, L.L., Giles, E.C. (2013). A Common New Species of *Inocybe* in the Pacific Northwest with a Diagnostic PDAB Reaction. *Mycologia* 105, 436–446.

Matheny, P.B., Swenie, R.A. (2018). The *Inocybe geophylla* Group in North America: A Revision of the Lilac Species surrounding *I. lilacina*. *Mycologia* 110, 618–634.

Mayr E. (1942). *Systematics and the origin of species*. Columbia University Press, New York, USA.

Médail, F., Monnet, A.C., Pavon, D., Nikolic, T., Dimopoulos, P., Bacchetta, G., Arroyo, J., Barina, Z., Albassatneh, M.C., Domina, G., et al. (2019). What Is a Tree in the Mediterranean Basin Hotspot? A Critical Analysis. *For. Ecosyst*. 6, 17.

Mentrida, S., Krisai-Greilhuber, I. & Voglmayr, H. (2015). Molecular evaluation of species delimitation and barcoding of *Daedaleopsis confragosa* specimens in Austria. Österreichische Zeitschrift für Pilz-kunde 24: 173–179.

Mešić A., Tkalčec Z., Antonín V. (2012). Studies on Croatian Basidiomycota 2: *Marasmiellus milicae* sp. nov. Mycotaxon 119: 233–239.

Mešić, A., Haelewaters, D., Tkalčec, Z., Liu, J., Kušan, I., Catherine Aime, M., Pošta, A. (2021). *Inocybe brijunica* sp. nov., a New Ectomycorrhizal Fungus from Mediterranean Croatia Revealed by Morphology and Multilocus Phylogenetic Analysis. *J. Fungi* 7, 199.

Mešić, A., Tkalčec, Z. (2003). Preliminary Checklist of Agaricales from Croatia IV: Families *Bolbitiaceae, Coprinaceae, Entolomataceae* and *Pluteaceae*. *Mycotaxon* 87, 283–309.

Meyer, V., Basenko, E.Y., Benz, J.P., Braus, G.H., Caddick, M.X., Csukai, M., De Vries, R.P., Endy, D., Frisvad, J.C., Gunde-Cimerman, N. (2020). Growing a Circular Economy with Fungal Biotechnology: A White Paper. *Fungal Biol. Biotechnol*. 7, 1–23.

Miller, M.A., Pfeiffer, W., Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, Louisiana, Institute of Electrical and Electronics Engineers: Piscataway, NJ, USA, pp. 1–8.

Mills, S., Lunt, D.H. & Gómez, A. (2007). Global isolation by distance despite strong regional phylogeography in a small metazoan. BMC *Evolutionary Biology* 7: 225. https://doi.org/10.1186/1471-2148-7-225

Miralles, A., Bruy, T., Wolcott, K., Scherz, M. D., Begerow, D., Beszteri, B., ... & Vences, M. (2020). Repositories for taxonomic data: where we are and what is missing. *Systematic biology*, 69(6), 1231-1253.

Mitchell, J. I., & Zuccaro, A. (2006). Sequences, the environment and fungi. *Mycologist*, 20(2), 62-74.

Mittermeier, R.A., Turner, W.R., Larsen, F.W., Brooks, T.M., Gascon, C. (2011). *Global Biodiversity Conservation: The Critical Role of Hotspots*. In Biodiversity Hotspots: Distribution and Protection of Conservation Priority Areas, Zachos, F.E., Habel, J.C., Eds., Springer: Berlin/Heidelberg, Germany, pp. 3–22. ISBN 978-3-642-20992-5.

Möller, A. (1901). *Phycomyceten Und Ascomyceten*. Untersuchungen Aus Brasilien, Bot. Mitth. Tropen, Heft 9, G. Fischer: Jena, Germany.

Moncalvo, J.M., Lutzoni, F.M., Rehner, S.A., Johnson, J., Vilgalys, R. (2000). Phylogenetic Relationships of Agaric Fungi Based on Nuclear Large Subunit Ribosomal DNA Sequences. Sy*st. Biol.*, 49, 278–305.

Mueller GM, Cunha KM, May TW, Allen JL, Westrip JRS, Canteiro C, Costa-Rezende DH, Drechsler-Santos ER, Vasco-Palacios AM, Ainsworth AM, et al. (2022). What Do the First 597 Global Fungal Red List Assessments Tell Us about the Threat Status of Fungi? *Diversity*. 14(9):736. https://doi.org/10.3390/d14090736

Munoz, G., Pancorbo, F., Turegano, Y., Esteve, F. (2022). New Species and Combinations of *Inocybe* with Lilac or Violet Colours in Europe. *Fungi Iber*. 2, 7–26.

Nagy, L.G., Desjardin, D.E., Vágvölgyi, C., Kemp, R. & Papp, T. (2013a). Phylogenetic analyses of *Coprinopsis* sections *Lanatuli* and *Atramentarii* identify multiple species

within morphologically defined taxa. *Mycologia* 105 (1): 112–124. https://doi.org/10.3852/12-136

Nagy, L.G., Házi, J., Szappanos, B., Kocsubé, S., Bálint, B., Rákhely, G., Vágvölgyi, C. & Papp, T. (2012). The evolution of defense mechanisms correlate with the explosive diversification of autodigesting *Coprinellus* mushrooms (*Agaricales*, *Fungi*). *Systematic Biology* 61 (4): 595–607. https://doi.org/10.1093/sysbio/sys002

Nagy, L.G., Házi, J., Szappanos, B., Kocsubé, S., Bálint, B., Rákhely, G., Vágvölgyi, C., Papp, T. (2012). The Evolution of Defense Mechanisms Correlate with the Explosive Diversification of Autodigesting *Coprinellus* Mushrooms (*Agaricales*, *Fungi*). *Syst. Biol.*, 61, 595–607.

Nagy, L.G., Kocsubé, S., Papp, T. & Vágvölgyi, C. (2009). Phylogeny and character evolution of the coprinoid mushroom genus *Parasola* as inferred from LSU and ITS nrDNA sequence data. *Persoonia* 22: 28–37. https://doi.org/10.3767/003158509X422434

Nagy, L.G., Kocsubé, S., Papp, T., Vágvölgyi, C. (2009). Phylogeny and Character Evolution of the Coprinoid Mushroom Genus *Parasola* as Inferred from LSU and ITS nrDNA Sequence Data. *Persoonia Mol. Phylogeny Evol. Fungi* 22, 28–37.

Nagy, L.G., Urban, A., Orstadius, L., Papp, T., Larsson, E. & Vágvölgyi, C. (2010). The evolution of autodigestion in the mushroom family *Psathyrellaceae* (*Agaricales*) inferred from Maximum Likelihood and Bayesian methods. *Molecular Phylogenetics and Evolution.* 57 (3): 1037–1048. https://doi.org/10.1016/j.ympev.2010.08.022

Nagy, L.G., Urban, A., Örstadius, L., Papp, T., Larsson, E., Vágvölgyi, C. (2010). The Evolution of Autodigestion in the Mushroom Family *Psathyrellaceae* (*Agaricales*) Inferred from Maximum Likelihood and Bayesian Methods. *Mol. Phylogenet. Evol*. 57, 1037–1048.

Nagy, L.G., Vágvölgyi, C. & Papp, T. (2013b). Morphological characterization of clades of the *Psathyrellaceae* (*Agaricales*) inferred from a multigene phylogeny. *Mycological Progress*. 12: 505–517. https://doi.org/10.1007/s11557-012-0857-3

Nagy, L.G., Vágvölgyi, C., Papp, T. (2010). Type Studies and Nomenclatural Revisions in *Parasola* (*Psathyrellaceae*) and Related Taxa. *Mycotaxon* 112, 103–141.

Nagy, L.G., Walther, G., Házi, J., Vágvölgyi, C. & Papp, T. (2011). Understanding the evolutionary processes of fungal fruiting bodies: correlated evolution and divergence times in the *Psathyrellaceae*. *Systematic Biology*. 60 (3): 303–317. https://doi.org/10.1093/sysbio/syr005

Nagy, L.G., Walther, G., Házi, J., Vágvölgyi, C., Papp, T. (2011). Understanding the Evolutionary Processes of Fungal Fruiting Bodies: Correlated Evolution and Divergence Times in the *Psathyrellaceae*. *Syst. Biol*. 60, 303–317.

Naranjo-Ortiz, M.A., Gabaldón, T. (2020). Fungal evolution: cellular, genomic and metabolic complexity. *Biol Rev.* 95:1198–1232

Narodne novine 6/1996 (1996). Odluka o proglašenju zakona o potvrđivanju konvencije o biološkoj raznolikosti. https://narodne-novine.nn.hr/clanci/medunarodni/1996_05_6_39.html

Nguyen, L.-T., Schmidt, H.A., von Haeseler, A., Minh, B.Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Mol. Biol. Evol.*, 32, 268–274.

O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC. (1998). Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences of the United States of America 95: 2044–2049.

O'Donnell, K., Cigelnik, E. (1997). Two Divergent Intragenomic rDNA ITS2 Types within a Monophyletic Lineage of the Fungus *Fusarium* are Nonorthologous. *Mol. Phylogenetics Evol.* 7, 103–116.

O'Donnell, K., Ward, T. J., Robert, V. A., Crous, P. W., Geiser, D. M., & Kang, S. (2015). DNA sequence-based identification of *Fusarium*: current status and future directions. *Phytoparasitica*, 43, 583-595.

Örstadius, L., Ryberg, M. & Larsson, E. (2015). Molecular phylogenetics and taxonomy in *Psathyrellaceae* (*Agaricales*) with focus on psathyrelloid species: introduction of three new genera and 18 new species. *Mycological Progress.* 14: 25. https://doi.org/10.1007/s11557-015-1047-x

Orton, P.D. (1957). Notes on British agarics 1-5 (Observations on the genus *Coprinus*). *Transactions of the British Mycological Society* 40 (2): 263–276.

Orton, P.D. (1972). Notes on British Agarics IV. *Notes R. Bot. Gard. Edinb*., 32, 135–150.

Osmundson, T.W., Robert, V.A., Schoch, C.L., Baker, L.J., Smith, A., Robich, G., Mizzan, L. & Garbelotto, M.M. (2013). Filling gaps in biodiversity knowledge for macrofungi: contributions and assessment of an herbarium collection DNA barcode sequencing project. *PLOS One* 8 (4): e62419. https://doi.org/10.1371/journal.pone.0062419

Padamsee, M., Matheny, P.B., Dentinger, B.T. & McLaughlin, D.J. (2007). The mushroom family Psathyrellaceae: evidence for large-scale polyphyly of the genus *Psathyrella*. *Molecular Phylogenetics and Evolution* 46: 415–429. https://doi.org/10.1016/j.ympev.2007.11.004

Pante E., Schoelinck C., Puillandre N. (2015). From integrative taxonomy to species description: One step beyond. *Systematic Biology* 64: 152–160. https://doi.org/10.1093/sysbio/syu083

Patouillard, N. (1911). Champignons de La Nouvelle-Calédonie. *Bull. Trimest. Société Mycol. De Fr*., 27, 329–333.

Patrick, W.W. Jr (1979). Comparative morphology and taxonomic disposition of ebulbosus, quadrifidus, and variegatus in the genus *Coprinus* (*Agaricales*). *Mycotaxon* 10 (1): 142–154.

Pažoutová, S., Šrůtka, P., Holuša, J., Chudíčková, M., Kolařík, M. (2010). The phylogenetic position of *Obolarina dryophila* (*Xylariales*). *Mycol. Prog*. 9, 501–507.

Pearce, D. W., & Moran, D. (1994). *The economic value of biodiversity*. Earthscan.

Peck, C.H. (1873). Descriptions of new species of fungi. *Bulletin of the Buffalo Society of Natural Sciences* 1: 41–72. https://doi.org/10.5962/bhl.title.58612.

Pegler, D.N. (1983). Agaric Flora of the Lesser Antilles. *Kew Bulletin Additional Series* 9: 1–668.

Peintner, U., Bougher, N.L., Castellano, M.A., Moncalvo, J.M., Moser, M.M., Trappe, J.M., Vilgalys, R. (2001). Multiple Origins of Sequestrate Fungi Related to *Cortinarius* (*Cortinariaceae*). *Am. J. Bot*. 88, 2168–2179.

Peláez, F., González, V., Platas, G., Sánchez-Ballesteros, J., Rubio, V. (2008). Molecular Phylogenetic Studies within the *Xylariaceae* Based on Ribosomal DNA Sequences. *Fungal Divers* 31, 111–134.

Persoon, C.H. (1797). Tentamen dispositionis methodicae Fungorum. Leipzig, 76 pp.

Phookamsak, R., Hyde, K.D., Jeewon, R., Bhat, D.J., Jones, E.B.G., Maharachchikumbura, S.S.N., Raspé, O., Karunarathna, S.C., Wanasinghe, D.N., Hongsanan, S., Doilom, M., et al. (2019). Fungal diversity notes 929–1035: taxonomic and phylogenetic contributions on genera and species of fungi. *Fungal Diversity* 95: 1–273. https://doi.org/10.1007/s13225-019-00421-w

Phukhamsakda, C., Nilsson, R. H., Bhunjun, C. S., de Farias, A. R. G., Sun, Y. R., Wijesinghe, S. N., ... & Hyde, K. D. (2022). The numbers of fungi: Contributions from traditional taxonomic studies and challenges of metabarcoding. *Fungal diversity*, 114(1), 327-386.

Pi, Y.-H., Zhang, X., Liu, L.-L., Long, Q.-D., Shen, X.-C., Kang, Y.-Q., Hyde, K.D., Boonmee, S., Kang, J.-C., Li, Q.-R. (2020). Contributions to species of *Xylariales* in China—4. Hypoxylon wujiangensis sp. nov. *Phytotaxa* 455, 21–30.

Pilát, A. & Svrček, M. (1967). Revisio specierum sectionis *Herbicolae* Pil. & Svr. generis *Coprinus* (Pers. ex) S.F. Gray. *Česká Mykologie* 21 (3): 136–145.

Pilzkompendium L. E. (2007). Band 2. Die Größeren Gattungen der Agaricales mit Farbigem Sporenpulver (Ausgenommen Cortinariaceae), FUNGICON Verlag: Berlin, Germany, ISBN 9783940316004.

Pilzkompendium L. E. (2017). Band 4. Fungicon Verlag: Berlin, Germany,

Pires, A. C., & Marinoni, L. (2010). DNA barcoding and traditional taxonomy unified through Integrative Taxonomy: a view that challenges the debate questioning both methodologies. *BiotaNeotropica*, 10(2). https://doi.org/10.1590/S1676-06032010000200035

Pourmoghaddam, M.J., Lambert, C., Surup, F., Khodaparast, S.A., Krisai-Greilhuber, I., Voglmayr, H., Stadler, M. (2020). Discovery of a new species of the *Hypoxylon* rubiginosum complex from Iran and antagonistic activities of *Hypoxylon* spp. against the Ash Dieback pathogen, *Hymenoscyphus fraxineus*, in dual culture. *Mycokeys* 66, 105–133.

Pradeep, C.K., Vrinda, K.B., Varghese, S.P., Korotkin, H.B., Matheny, P.B. (2016). New and Noteworthy Species of *Inocybe* (*Agaricales*) from Tropical India. *Mycol. Prog.* 15, 24.

Prescott, T., Wong, J., Panaretou, B., Boa, E., Bond, A., Chowdhury, S., Davis, L., Østergaard, L. (2018). *Useful Fungi*. In State of the World's Fungi Royal Botanic Gardens, Kew: Richmond, UK. ISBN 978-1-84246-678-0.

Purvis, B., Mao, Y., & Robinson, D. (2019). Three pillars of sustainability: in search of conceptual origins. *Sustainability science*, 14, 681-695.

Quaedvlieg, W., Binder, M., Groenewald, J. Z., Summerell, B. A., Carnegie, A. J., Burgess, T. I., & Crous, P. W. (2014). Introducing the consolidated species concept to resolve species in the *Teratosphaeriaceae*. *Persoonia-Molecular Phylogeny and Evolution of Fungi*, 33(1), 1-40.

Quang, D.N., Stadler, M., Fournier, J., Asakawa, Y. (2006). Carneic Acids A and B, Chemotaxonomically Significant Antimicrobial Agents from the Xylariaceous Ascomycete *Hypoxylon carneum*. *J. Nat. Prod.* 69, 1198–1202.

Quélet, L. (1872). Les Champignons du Jura et des Vosges. *Mémoires de la Société d'Émulation de Montbéliard.* 5 (2): 43–332.

Quijada, L., Matočec, N., Kušan, I., Tanney, J.B., Johnston, P.R., Mešić, A., Pfister, D.H. (2022). Apothecial Ancestry, Evolution, and Re-Evolution in *Thelebolales* (*Leotiomycetes*, *Fungi*). *Biology* 11, 583.

Radović J. (2000*). An overview of the state of biological and landscape diversity of Croatia*. Ministry of environmental protection and physical planning, Zagreb, pp. XIX–158.

Raja, H. A., Miller, A. N., Pearce, C. J., & Oberlies, N. H. (2017). Fungal identification using molecular tools: a primer for the natural products research community. *Journal of natural products*, 80(3), 756-770.

Raut, J.K., Fukiharu, T., Shimizu, K., Kawamoto, S., Takeshige, S., Tanaka, C., Yamanaka, T. & Suzuki A. (2015). *Coprinopsis novorugosobispora* (*Basidiomycota*, *Agricales*), an ammonia fungus new to Canada. *Mycosphere* 6 (5): 612–619. https://doi.org/10.5943/mycosphere/6/5/10

Raut, J.K., Suzuki, A., Fukiharu, T., Shimizu, K., Kawamoto, S. & Tanaka, C. (2011). *Coprinopsis neophlyctidospora* sp. nov., a new ammonia fungus from boreal forests in Canada. *Mycotaxon* 115: 227–238. https://doi.org/10.5248/115.227

Redhead, S.A., Vilgalys, R., Moncalvo, J.M., Johnson, J. & Hopple, J.S. Jr (2001). *Coprinus* Pers. and the disposition of *Coprinus* species sensu lato. *Taxon* 50: 203–241. https://doi.org/10.2307/1224525.

Rehner, S. (2001). Primers for Elongation Factor 1-α (EF1-α). Available online: http://ocid.NACSE.ORG/research/deephyphae/EF1primer.pdf (accessed on 11 February 2022).

Rehner, S.A., Buckley, E. (2005). A Beauveria Phylogeny Inferred from Nuclear ITS and EF1-Alpha Sequences: Evidence for Cryptic Diversification and Links to *Cordyceps* Teleomorphs. *Mycologia* 97, 84–98.

Richard, F., Moreau, P. A., Selosse, M. A., & Gardes, M. (2004). Diversity and fruiting patterns of ectomycorrhizal and saprobic fungi in an old-growth Mediterranean forest dominated by *Quercus ilex* L. *Canadian Journal of Botany*, 82(12), 1711-1729.

Riginos, C., Douglas, K.E., Jin, Y., Shanahan, D.F. & Treml, E.A. (2011). Effects of geography and life history traits on genetic differentiation in benthic marine fishes. *Ecography* 34: 566–575. https://doi.org/10.1111/j.1600-0587.2010.06511.x

Rogers, J.D. (1981). *Sarcoxylon* and *Entonaema* (*Xylariaceae*). *Mycologia,* 73, 26–61.

Rogers, J.D. (1982). *Entonaema liquescens*: Description of the Anamorph and Thoughts on Its Systematic Position. *Mycotaxon,* 15, 500–506.

Rogers, J.D., San Martín, F., Ju, Y.-M. (1996). Mexican Fungi: *Xylaria entosulphurea* Sp. Nov. and Neotypification of *Entonaema globosum*. *Mycotaxon,* 58, 483–487.

Ronquist, F., Teslenko, M., Mark, P.V.D., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012). MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Rubel, F., Kottek, M. (2010). Observed and projected climate shifts 1901–2100 depicted by world maps of the Köppen-Geiger climate classification. *Meteorol.* Z. 19, 135–141.

Ryberg, M., Matheny, P.B. (2012). Asynchronous origins of ectomycorrhizal clades of Agaricales. Proc. R. Soc. B Biol. Sci. 279, 2003–2011.

Ryberg, M., Nilsson, R.H., Kristiansson, E., Töpel, M., Jacobsson, S., Larsson, E. (2008). Mining metadata from unidentified ITS sequences in GenBank: A case study in *Inocybe* (*Basidiomycota*). BMC *Evol. Biol*. 8, 50.

Saba, M., Haelewaters, D., Pfister, D.H., Khalid, A.N. (2020). New species of *Pseudosperma* (*Agaricales*, *Inocybaceae*) from Pakistan revealed by morphology and multi-locus phylogenetic reconstruction. *MycoKeys* 69, 1–31.

Safran, R. J. & Nosil, P. (2012). Speciation: The Origin of New Species. *Nature Education*

Samarakoon, M.C., Hyde, K.D., Maharachchikumbura, S.S.N., Stadler, M., Jones, E.B.G., Promputtha, I., Suwannarach, N., Camporesi, E., Bulgakov, T.S., Liu, J.-K. (2022). Taxonomy, phylogeny, molecular dating and ancestral state reconstruction of *Xylariomycetidae* (*Sordariomycetes*). *Fungal Divers*. 112, 1–88.

Samson, R. A., Visagie, C. M., Houbraken, J., Hong, S. B., Hubka, V., Klaassen, C. H., ... & Frisvad, J. (2014). Phylogeny, identification and nomenclature of the genus *Aspergillus*. *Studies in mycology*, 78(1), 141-173.

Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg, O. (1996). Introduction to Foodborne Fungi, Centraalbureau voor Schimmelcultures: Baarn, The Netherlands, Delft, The Netherlands.

Sánchez-Jácome, M., Guzmán-Dávalos, L. (2005). New Records of Ascomycetes from Jalisco, Mexico. *Mycotaxon* 92, 177–191.

Schafer, D., Alvarado, P., Smith, L., Liimatainen, K., Loizides, M. (2022). Coprinoid *Psathyrellaceae* Species from Cyprus: Three New Sabulicolous Taxa from Sand Dunes and a Four-Spored Form of the Fimicolous Species *Parasola cuniculorum*. *Mycol. Prog.* 21, 52.

Schafer, D.J. (2010). Keys to sections of *Parasola*, *Coprinellus*, *Coprinopsis* and *Coprinus* in Britain. *Field Mycology* 11 (2): 44–51. https://doi.org/10.1016/j.fldmyc.2010.04.006

Schafer, D.J. (2014). The Genus Parasola in Britain Including *Parasola cuniculorum* sp. nov. *Field Mycol.* 15, 77–99.

Schmit JP, Lodge DJ. (2005). *Classical methods and modern analyses for studying fungal diversity*. In: Dighton J, White JF, Oudemans P (Eds) The Fungal Community: Its organization and role in the ecosystem. CRC Press.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences of the United States* 109.6241–6246. https://doi.org/10.1073/pnas.1117018109

Schoch, C. L., Aime, M. C., De Beer, W., Crous, P. W., Hyde, K. D., Penev, L., ... & Miller, A. N. (2017). Using standard keywords in publications to facilitate updates of new fungal taxonomic names. *IMA Fungus*. 8(2), A70-A73.

Schoch, C. L., Ciufo, S., Domrachev, M., Hotton, C. L., Kannan, S., Khovanskaya, R., ... & Karsch-Mizrachi, I. (2020). NCBI Taxonomy: a comprehensive update on curation, resources and tools. *Database*. baaa062.

Schoch, C.L., Robbertse, B., Robert, V., Vu, D., Cardinali, G., Irinyi, L., Meyer, W., Nilsson, R.H., Hughes, K., Miller, A.N., et al. (2014). Finding Needles in Haystacks: Linking Scientific Names, Reference Specimens and Molecular Data for Fungi. *Database*. bau061.

Schulzer, S. (1879). Mycologische Beiträge. III. *Verhandlungen der Zoologisch-Botanischen Gesellschaft Wien* 28: 423–436.

Seifert, K.A., Rossman, A.Y. (2010). How to describe a new fungal species. *IMA Fungus* 1, 109–111 https://doi.org/10.5598/imafungus.2010.01.02.02

Senanayake, I. C., Rathnayaka, A. R., Marasinghe, D. S., Calabon, M. S., Gentekaki, E., Lee, H. B., ... & Xiang, M. M. (2020). Morphological approaches in studying fungi: Collection, examination, isolation, sporulation and preservation. *Mycosphere*. 11(1), 2678-2754.

Senanayake, I.C., Maharachchikumbura, S.S.N., Hyde, K.D., Bhat, J.D., Jones, E.B.G., McKenzie, E.H.C., Dai, D.Q., Daranagama, D.A., Dayarathne, M.C., Goonasekara, I.D., et al. (2015). Towards unraveling relationships in *Xylariomycetidae* (*Sordariomycetes*). *Fungal Divers*. 73, 73–144.

Sharbel, T.F., Haubold, B. & Mitchell-Olds, T. (2000). Genetic isolation by distance in *Arabidopsis thaliana*: biogeography and postglacial colonization of Europe. *Molecular Ecology* 9: 2109–2118. https://doi.org/10.1046/j.1365-294X.2000.01122.x

Sihanonth, P., Thienhirun, S., Whalley, A.J. (1998). *Entonaema* in Thailand. *Mycol. Res*. 102, 458–460.

Singer, R. (1951). The Agaricales in modern taxonomy. *Lilloa* 22: 5–832.

Singer, R. (1986). *Lepiota* (Pers. ex) SF Gray. The Agaricales in modern taxonomy. 4th ed. Koenigstein, Germany: Koeltz Scientific Books, 497-501.

Sir, E., Stadler, M. (2016). A new species of *Daldinia* (*Xylariaceae*) from the Argentine subtropical montane forest. *Mycosphere* 7, 1378–1388.

Sir, E.B., Becker, K., Lambert, C., Bills, G.F., Kuhnert, E. (2019). Observations on Texas hypoxylons, including two new *Hypoxylon* species and widespread environmental isolates of the *H. croceum* complex identified by a polyphasic approach. *Mycologia* 111, 832–856.

Skaltsas, D.N., Badotti, F., Vaz, A.B.M., da Silva, F.F., Gazis, R., Wurdack, K., Castlebury, L., Góes-Neto, A., Chaverri, P. (2019). Exploration of stem endophytic communities revealed developmental stage as one of the drivers of fungal endophytic community assemblages in two Amazonian hardwood genera. *Sci. Rep*. 9, 12685.

Smith, A.H., Stuntz, D.E. (1950). New or noteworthy fungi from Mount Rainier National Park. *Mycologia* 42, 80–134.

Song, Z.-K., Zhu, A.-H., Liu, Z.-D., Qu, Z., Li, Y., Ma, H.-X. (2022). Three New Species of *Hypoxylon* (*Xylariales*, *Ascomycota*) on a Multigene Phylogeny from Medog in Southwest China. *J. Fungi* 8, 500.

Spatafora, J.W., Sung, G.-H., Johnson, D., Hesse, C., O'rourke, B., Serdani, M., Spotts, R., Lutzoni, F., Hofstetter, V., Miadlikowska, J., et al. (2006). A five-gene phylogeny of *Pezizomycotina*. *Mycologia* 98, 1018–1028.

Šrůtka, P., Pažoutová, S., Kolařík, M. (2007). *Daldinia* decipiens and *Entonaema cinnabarina* as fungal symbionts of *Xiphydria* wood wasps. *Mycol. Res*. 111, 224–231.

Stadler, M., Flessa, F., Rambold, G., Peršoh, D., Fournier, J., Læssøe, T., Chlebicki, A., Lechat, C. (2010). Chemotaxonomic and phylogenetic studies of *Thamnomyces* (*Xylariaceae*). *Mycoscience*, 51, 189–207.

Stadler, M., Fournier, J., Læssøe, T., Lechat, C., Tichy, H.-V., Piepenbring, M. (2008). Recognition of hypoxyloid and xylarioid *Entonaema* species and allied *Xylaria* species from a comparison of holomorphic morphology, HPLC profiles, and ribosomal DNA sequences. *Mycol. Prog*. 7, 53–73.

Stadler, M., Ju, Y.-M., Rogers, J.D. (2004). Chemotaxonomy of *Entonaema*, *Rhopalostroma* and other *Xylariaceae*. *Mycol. Res.* 108, 239–256.

Stadler, M., Kuhnert, E., Peršoh, D., Fournier, J. (2013). The *Xylariaceae* as Model Example for a Unified Nomenclature Following the "One Fungus-One Name" (1F1N) Concept. *Mycology* 4, 5–21.

Stadler, M., Læssøe, T., Fournier, J., Decock, C., Schmieschek, B., Tichy, H.-V., Peršoh, D. (2014). A polyphasic taxonomy of *Daldinia* (*Xylariaceae*). *Stud. Mycol.* 77, 1–143.

Stamatakis, A. (2014). RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stangl, J. (1989). Die Gattung *Inocybe* in Bayern. *Hoppea*, 46, 1–409.

Stangl, J., Veselský, J. (1982). Risspilze der Section Lilacinae Heim. *Česká Mykol*. 36, 85–99.

Stanners, D., Bourdeau, P. (1995). Europes's Environment: The Dobřiš Assessment, European Environment Agency: Copenhagen, Denmark.

Stengel, A., Stanke, K. M., Quattrone, A. C., & Herr, J. R. (2022). Improving taxonomic delimitation of fungal species in the age of genomics and Phenomics. *Frontiers in Microbiology*, 13, 847067.

Stielow, J. B., Levesque, C. A., Seifert, K. A., Meyer, W., Irinyi, L., Smits, D., ... & Robert, V. (2015). One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia-Molecular Phylogeny and Evolution of Fungi*, 35(1), 242-263.

Stukenbrock EH, McDonald BA. (2009). Population genetics of fungal and oomycete effectors involved in gene-for-gene interactions. *Mol Plant Microbe Interact.* 22(4):371-80. doi: 10.1094/MPMI-22-4-0371. PMID: 19271952.

Szarkandi, J.G., Schmidt-Stohn, G., Dima, B., Hussain, S., Kocsube, S., Papp, T., Vagvolgyi, C., Nagy, L.G. (2017). The Genus *Parasola*: Phylogeny and the Description of Three New Species. *Mycologia* 109, 620–629.

Tang, A.M.C., Jeewon, R., Hyde, K.D. (2009). A Re-Evaluation of the Evolutionary Relationships within the *Xylariaceae* Based on Ri-bosomal and Protein-Coding Gene Sequences. *Fungal Divers* 34, 127–155.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000). Phylogenetic species recognition and species concepts in fungi. *Fungal Genet Biol* 31:21–32

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A. (2014). Global Diversity and Geography of Soil Fungi. S*cience* 346, 1256688

Tekpinar, A. D., & Kalmer, A. (2019). Utility of various molecular markers in fungal identification and phylogeny. *Nova Hedwigia*, 109(1-2), 187-224.

Tibpromma S., Hyde K.D., Jeewon R., Maharachchikumbura S.S.N., ... Mešić A., ... Kušan I., ... Jadan M., ... Matočec N., ... Tkalčec, Z., ... Karunarathna, S.C. (2017). Fungal diversity notes 491–602: taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* 83: 1–261.

Tibpromma, S., Hyde, K. D., Jeewon, R., Maharachchikumbura, S. S., Liu, J. K., Bhat, D. J., ... & Karunarathna, S. C. (2017). Fungal diversity notes 491–602: taxonomic and phylogenetic contributions to fungal taxa. *Fungal diversity*, 83, 1-261.

Tkalčec Z., Mešić A., Matočec N., Kušan I. (2008). *Crvena knijga gljiva Hrvatske*. Ministarstvo kulture, Državni zavod za zaštitu prirode, Zagreb.

Tkalčec, Z., Mešić, A., (2008). *Gloiocephala cerkezii*, a new species from Croatia. *Mycologia* 100: 306–310.

Triebel, D., Peršoh, D., Wollweber, H., Stadler, M. (2005). Phylogenetic relationships among *Daldinia*, *Entonaema*, and *Hypoxylon* as inferred from ITS nrDNA analyses of Xylariales. *Nova Hedwig.* 80, 25–43.

Trifinopoulos, J., Nguyen, L.-T., von Haeseler, A., Minh, B.Q. (2016). W-IQ-TREE: A Fast Online Phylogenetic Tool for Maximum Likelihood Analysis. *Nucleic Acids Res*. 44, W232–W235.

Tuel, A., Eltahir, E.A.B. (2020). Why is the Mediterranean a climate change hot spot? *J. Clim.* 33, 5829–5843.

Turland, N. J., Wiersema, J. H., Barrie, F. R., Greuter, W., Hawksworth, D. L., Herendeen, P. S., ... & Smith, G. (2018). International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Koeltz botanical books.

Tylianakis, J. M., Didham, R. K., Bascompte, J., & Wardle, D. A. (2008). Global change and species interactions in terrestrial ecosystems. *Ecology letters*, 11(12), 1351-1363.

Uljé, C.B. & Bas, C. (1993). Some new species of *Coprinus* from the Netherlands. *Persoonia* 15 (3): 357–368.

Uljé, C.B., Bender, H. (1997). Additional Studies in *Coprinus* Subsection *Glabri*. *Persoonia*, 16, 373–381.

Uljé, C.B. & Noordeloos, M.E. (1999). Studies in Coprinus V—*Coprinus* section *Coprinus*. Revision of subsection *Lanatuli* Sing. *Persoonia* 17 (2): 165–199.

Uljé, C.B. & Noordeloos, M.E. (2000). Type studies in *Coprinus* subsection *Lanatuli*. *Persoonia* 17 (3): 339–375.

Uljé, C.B. (2005). 1. *Coprinus* Pers. In: Noordeloos, M.E., Kuyper, T.W. & Vellinga, E.C. (Eds.) Flora Agaricina Neerlandica 6. Taylor & Francis, Boca Raton, pp. 22–109.

UN, I. R. B. (1992). *Convention on biological diversity*. Treaty Collection.

Valen L van. (1976). Ecological species, multispecies and oaks. *Taxon* 25: 233–239.

Van De Bogart, F. (1976). The genus *Coprinus* in Western North America, part 1: Section *Coprinus*. *Mycotaxon* 4 (1): 233–275.

Vasilyeva LN, Stephenson SL. (2010). Biogeographical patterns in pyrenomycetous fungi and their taxonomy. *Mycotaxon* 114, 281–303.

Velenovský, J. (1920). České Houby 1–5 České Botanické Společnosti: Prague, Czech Republic.

Vilgalys, R., Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol*., 172, 4238–4246.

Vilgalys, R., Hopple, J.S., Hibbett, D.S. (1994) Phylogenetic Implications of Generic Concepts in Fungal Taxonomy: The Impact of Molecular Systematic Studies. *Mycol. Helv*., 6, 73–91.

Voto, P. (2019). Novelties in the family *Psathyrellaceae*. Part I. *Bollettino dell'Associazione Micologica ed Ecologica Romana* 107 (2): 94–95.

Voto, P. (2019). Novelties in the Family *Psathyrellaceae*. Part II. *Boll. Am*. 108, 127–133.

Voto, P. (2021). Novelties in the Family *Psathyrellaceae*. Part V. *Micol. E Veg. Mediterr*. 35, 149–168.

Vrščaj, D. (2002). *Popis Gljiva Otoka Krka*—1. Dio. Gljiv. Glas. 15, 21–25.

Vu, D., Groenewald, M., de Vries, M., Gehrmann, T., Stielow, B., Eberhardt, U., Al-Hatmi, A., Groenewald, J.Z., Cardinali, G., Houbraken, J., Boekhout, T., Crous, P.W., Robert, V. & Verkley, G. (2019). Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. *Studies in Mycology* 92: 135–154.https://doi.org/10.1016/j.simyco.2018.05.001

Wächter, D. & Melzer, A. (2020). Proposal for a subdivision of the family *Psathyrellaceae* based on a taxon-rich phylogenetic analysis with iterative multigene guide tree. *Mycological Progress* 19: 1151–1265. https://doi.org/10.1007/s11557-020-01606-3

Waikagul, J., & Thaekham, U. (2014). Approaches to research on the systematics of fish-borne trematodes. Academic Press.

Watkinson, S. C. (2016). Mutualistic symbiosis between fungi and autotrophs. In The fungi (pp. 205-243). Academic Press.

Wendt, L., Sir, E.B., Kuhnert, E., Heitkämper, S., Lambert, C., Hladki, A.I., Romero, A.I., Luangsa-ard, J.J., Srikitikulchai, P., Peršoh, D., et al. (2018). Resurrection and emendation of the *Hypoxylaceae*, recognised from a multigene phylogeny of the *Xylariales*. *Mycol. Prog*. 17, 115–154.

Whalley, A. (1996). The xylariaceous way of life. *Mycol. Res*. 100, 897–922.

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp. 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wibberg, D., Stadler, M., Lambert, C., Bunk, B., Spröer, C., Rückert, C., Kalinowski, J., Cox, R.J., Kuhnert, E. (2020). High quality genome sequences of thirteen *Hypoxylaceae* (Ascomycota) strengthen the phylogenetic family backbone and enable the discovery of new taxa. *Fungal Divers*. 106, 7–28.

Wijayawardene, N.N., Hyde, K.D., Dai, D.Q., Sánchez-García, M., Goto, B.T., Saxena, R.K., Erdoğdu, M., Rajeshkumar, K.C., Aptroot, A., Zhang, G.Q., et al. (2022). Outline of Fungi and Fungus-like Taxa -2021. *Mycosphere* 13, 53–453.

Willis, K. (2017). State of the world's plants 2017. Royal Botanics Gardens Kew.

Willis, K. J. (2018). State of the world's fungi 2018. Report. State of the world's fungi 2018. Report.

Wingfield, M. J., De Beer, Z. W., Slippers, B., Wingfield, B. D., Groenewald, J. Z., Lombard, L., & Crous, P. W. (2012). One fungus, one name promotes progressive plant pathology. *Molecular plant pathology*, 13(6), 604-613.

Wright S. (1940). *The new systematics*. Oxford University Press, London, UK.

Wu B, Hussain M, Zhang W, Stadler M, Liu X, Xiang M (2019). Current insights into fungal species diversity and perspective on naming the environmental DNA sequences of fungi. Mycology 10:127–140.

Wurzbacher, C., Larsson, E., Bengtsson-Palme, J., Van den Wyngaert, S., Svantesson, S., Kristiansson, E., Kagami, M., Nilsson, R.H. (2018). Introducing ribosomal tandem repeat barcoding for fungi. *Mol. Ecol. Resour*. 19, 118–127.

Xu, J. (2016). Fungal DNA barcoding. *Genome*. 59(11): 913-932. https://doi.org/10.1139/gen-2016-0046

Xu, J. (2020). Fungal species concepts in the genomics era. *Genome*, 63(9), 459-468.

Yoo, S., Cho, Y., Kim, J.S., Kim, M., Lim, Y.W. (2022). Fourteen Unrecorded Species of *Agaricales* Underw. (*Agaricomycetes*, *Basidiomycota*) from the Republic of Korea. *Mycobiology* 50, 219–230.

Zamora, J.C., Svensson, M., Kirschner, R., Olariaga, I., Ryman, S., Parra, L.A., Geml, J., Rosling, A., Adamčík, S., Ahti, T., et al. (2018). Considerations and consequences of allowing DNA sequence data as types of fungal taxa. *IMA Fungus* 9, 167–175.

Zhang, N., Castlebury, L.A., Miller, A.N., Huhndorf, S.M., Schoch, C.L., Seifert, K.A., Rossman, A.Y., Rogers, J.D., Kohlmeyer, J., Volkmann-Kohlmeyer, B., et al. (2006). An overview of the systematics of the *Sordariomycetes* based on a four-gene phylogeny. *Mycologia* 98, 1076–1087.

Zhao, R. L., Li, G. J., Sánchez-Ramírez, S., Stata, M., Yang, Z. L., Wu, G., ... & Hyde, K. D. (2017). A six-gene phylogenetic overview of *Basidiomycota* and allied phyla with estimated divergence times of higher taxa and a phyloproteomics perspective. *Fungal Diversity*, 84, 43-74.

Zhu, L., Huang, M., Bau, T. (2022). Taxonomy of Coprinoid Fungi in China. *Mycosystema*. 41, 878–898.

6. ŽIVOTOPIS

Ana Pošta rođena je 18.6.1991. godine u gradu Zagrebu. 2013. godine upisuje studij Animalnih znanosti na Agronomskom fakultetu Sveučilišta u Zagrebu, a 2016. upisuje diplomski studij Agroekologija-Mikrobna biotehnologija u poljoprivredi. 2018. godine magistrira s pohvalom *magna cum laude* i postaje magistra agroekologije. Zapošljava se kao asistent u Laboratoriju za biološku raznolikost na Institutu Ruđer Bošković 2019. godine te iste godine upisuje Poslijediplomski sveučilišni doktorski studij Biologije na Prirodoslovno-matematičkom fakultetu Sveučilišta u Zagrebu. Dobitnica je stipendije IRB-a za kratkoročni studijski boravak mladih istraživača u Europskim laboratorijima u Botaničkom zavodu Prirodoslovng fakulteta na Karlovom sveučilištu u Pragu. Do sada je autorica/suautorica 11 objavljenih znanstvenih radova u časopisima citiranim u Web of Science bazi podataka, od kojih su četiri prvoautorska. Autorica je šest vrsta gljiva novih za znanost. Suradnica je na više znanstvenih i gospodarskih projekata svojeg laboratorija te je aktivno sudjelovala na nizu domaćih i međunarodnih kongresa. Članica je Hrvatskog mikološkog društva i Hrvatskog mikrobiološkog društva.

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2. Crous PW, Wingfield MJ, ... Kušan I, ... Matočec N, ... Mešić A, ... **Pošta A**, ... Tkalčec Z, ... Groenewald JZ (2020) Fungal Planet description sheets: 1042–1111. *Persoonia* 44: 301–459.

3. Crous, PW, Cowan DA, ... Kušan I, ... Matočec N, ... Mešić A, ... **Pošta A**, ... Tkalčec Z, ... Groenewald JZ (2021) Fungal Planet description sheets: 1182–1283. *Persoonia* 46: 313–528.

4. Crous, PW, Osieck ER, ... Kušan I, ... Matočec N, ... Mešić A, ... **Pošta A**, ... Tkalčec Z, ... Groenewald JZ (2021) Fungal Planet description sheets: 1284–1382. *Persoonia* 47: 178 –374.

5. Mešić A, Haelewaters D, Tkalčec Z, Liu J, Kušan I, Aime MC, **Pošta A** (2021) *Inocybe brijunica* sp. nov., a new ectomycorrhizal fungus from Mediterranean Croatia revealed by morphology and multilocus phylogenetic analysis. *Journal of Fungi* 7(3): 199.

6. **Pošta A***, Bandini D, Mešić A, Pole L, Kušan I, Matočec N, Malev O, Tkalčec Z* (2023) *Inocybe istriaca* sp. nov. from Brijuni National Park (Croatia) and Its Position within Inocybaceae Revealed by Multigene Phylogenetic Analysis. *Diversity* 15(6): 755. https://doi.org/10.3390/d15060755

7. **Pošta A**, Tkalčec Z, Kušan I, Matočec N, Pole L, Čerkez M, Mešić A (2023) An Integrative Taxonomic Study of *Parasola* (Psathyrellaceae, Fungi) Reveals a New Saprotrophic Species from European Temperate Deciduous Forests. *Forests* 14(7): 1387. https://doi.org/10.3390/f14071387

8. Bednár R, Červenka J, Arendt D, Szabóová D, Krisai-Greilhuber I, **Pošta A**, Mešić A, Tkalčec Z. (2022) *Coprinopsis alnivora* (*Psathyrellaceae*), a rare species from North America is discovered in Europe. *Phytotaxa* 542 (2): 136–152. doi:10.11646/phytotaxa.542.2.2

9. **Pošta A**, Matočec N, Kušan I, Tkalčec Z, Mešić A. (2023) The Lignicolous Genus *Entonaema*: Its Phylogenetic–Taxonomic Position within *Hypoxylaceae* (*Xylariales*, *Fungi*) and an Overview of Its Species, Biogeography, and Ecology. *Forests*, 14, 1764. https://doi.org/10.3390/f1409176

10. Wijayawardene N. N., Hyde K. D., Dai D. Q., Sanchez-Garcia M., Goto B. T., Saxena R. K., Erdogdu M., Selcuk F., Rajeshkumar K. C., Aptroot A....Mešić A.,...Tkalčec Z.,..**Pošta A.**,...et. al. Outline of Fungi and fungus-like taxa – 2021 // *Mycosphere*, 13 (2022), 1; str. 53-453. DOI: 10.5943/mycosphere/13/1/2

11. Nie C*, Wang S-N, Tkalčec Z, Yan J-Q, Hu Y, Ge Y, Na Q, Zeng H, Ding H, Huo G, **Pošta A***, Pradeep C-K, Mešić A. (2022) *Coprinus leucostictus* Rediscovered after a Century, Epitypified, and Its Generic Position in *Hausknechtia* Resolved by Multigene Phylogenetic Analysis of *Psathyrellaceae*. *Diversity*. 14(9):699. https://doi.org/10.3390/d14090699

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