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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 km²) 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 km²), 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., *Mallochybe* (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 \times 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 (NH₄OH). Micromorphological terminology mostly follows Cléménçon [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 *tefl* (translation elongation factor 1-alpha), were sequenced and analyzed. The 25 μ L PCR mixtures contained 9.5 μ L of ddH₂O, 12.5 μ L of GoTaq[®] G2 Green Master Mix (Promega, Madison, WI, USA), 1 μ L of DNA template, and 1 μ L 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.
<i>Auritella hispida</i>	TH10009 PT	Cameroon	KT378203	KT378207	KT378215	MK426179	1	[8,20]
<i>Auritella spiculosa</i>	TH9866 PT	Cameroon	KT378204	KT378206	KT378214	MK426182	1	[8,20]
<i>Crepidotus prostratus</i>	PBM3463/PERTH:08242135	Australia	HQ728537	HQ728538	HQ728540	MK426172	1	[8,20]
<i>Inocybe adorabilis</i>	SMNS-STU-F-0901582 HT	Austria	OK057159	OK057159	OK078903	—	1, 2	[21]
<i>Inocybe adorabilis</i>	SMNS-STU-F-0901641 PT	Austria	OK057161	OK057161	—	—	2	[21]
<i>Inocybe aeruginascens</i>	JG310508/TENN063936	Germany	GU949591	MH220256	MH249787	—	1	[22]
<i>Inocybe agglutinata</i>	WTU:1094 PBM1352	USA	KY990521	AY038312	AY509113	—	1	[14,23,24]
<i>Inocybe agroterae</i>	SMNS-STU-F-0901680 HT	Germany	ON003436	ON003436	—	—	2	[6]
<i>Inocybe alcis</i>	SMNS-STU-F-0901712 IT	Finland	OP164083	OP164083	—	—	2	[25]
<i>Inocybe aphroditeana</i>	SMNS-STU-F-0901678 HT	Germany	ON003432	ON003432	—	—	2	[6]
<i>Inocybe asterospora</i> cf.	ZRL20152002	China	LT716046	KY418862	KY419008	KY419064	1	[26]
<i>Inocybe astraiana</i>	SMNS-STU-F-0901240 HT	Germany	MN512321	MN512321	—	—	2	[27]
<i>Inocybe athenana</i>	SMNS-STU-F-0901238 HT	Germany	MN512320	MN512320	—	—	2	[27]
<i>Inocybe audens</i>	SMNS-STU-F-0901251 HT	Germany	MW647616	MW647616	—	—	2	[28]
<i>Inocybe aurantiobrunnea</i>	SMNS-STU-F-0001816 IT	Spain	OP164016	OP164016	—	—	2	[25]
<i>Inocybe beatifica</i>	SMNS-STU-F-0901261 HT	Germany	MW845857	—	—	—	2	[29]
<i>Inocybe bellidiana</i>	SMNS-STU-F-0901473 HT	Germany	MW845860	MW845860	—	—	2	[29]
<i>Inocybe cacaocolor</i>	PBM3790/TENN:067022 IT	Australia	KJ778845	KJ756464	KJ756422	—	1	[30]
<i>Inocybe caesaraugustae</i>	AH 56200 HT	Spain	OL352083	—	—	—	2	[31]
<i>Inocybe carissima</i>	SMNS-STU-F-0901701 HT	Germany	OP164058	OP164058	—	—	2	[25]
<i>Inocybe carolinensis</i>	PBM3906/TENN067756 PT	USA	KP636853	KP171055	KM555147	—	1	[32]
<i>Inocybe chalcodoxantha</i>	WTU F-043333 IT	Canada	NR_119900	—	—	—	2	[33]
<i>Inocybe coriacea</i>	SMNS-STU-F-0901683 HT	Germany	ON003439	ON003439	—	—	2	[6]

Table 1. Cont.

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

Table 1. Cont.

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

Table 1. Cont.

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

Table 1. Cont.

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

Table 1. Cont.

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

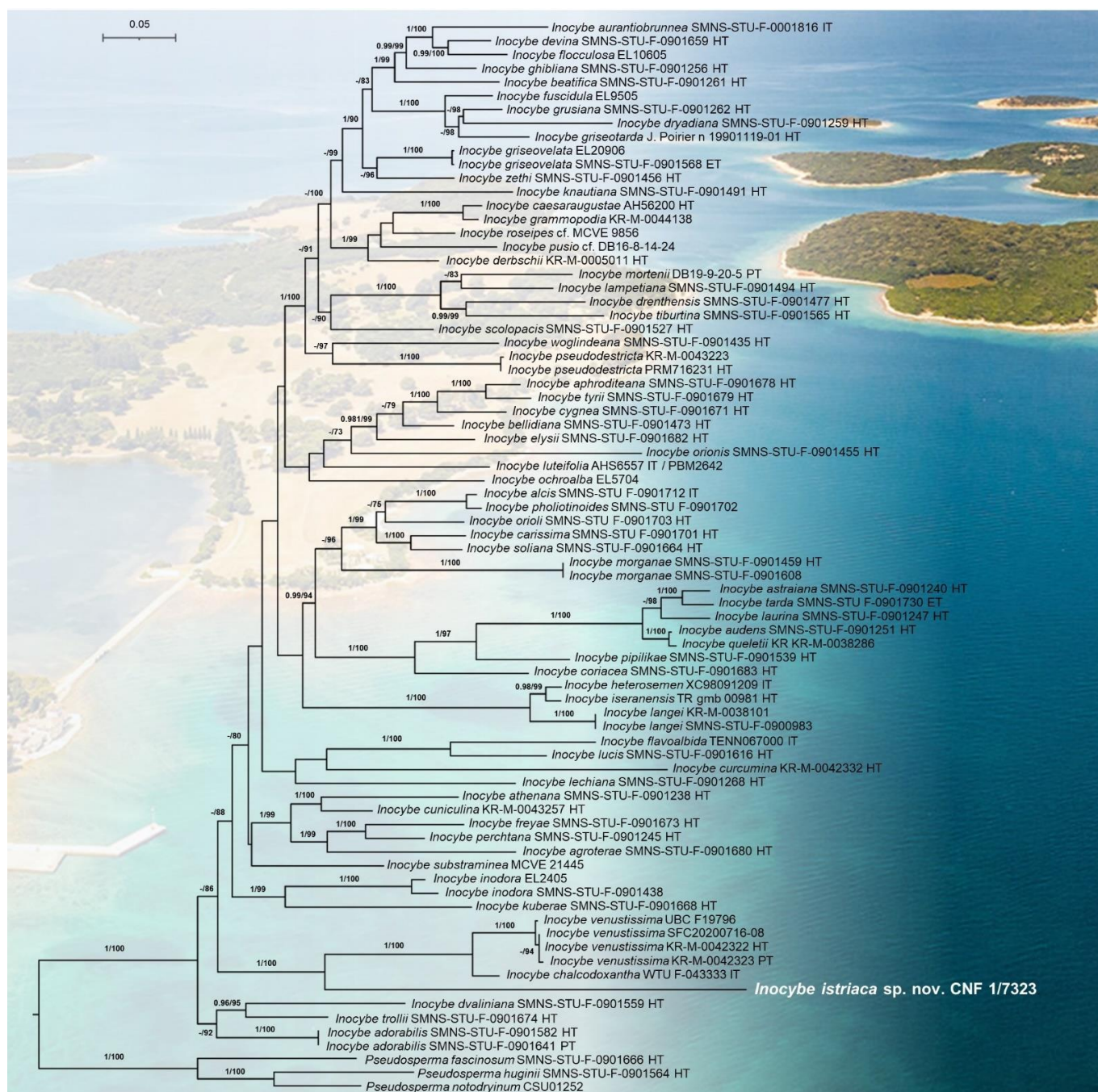


Figure 2. Phylogenetic tree of the genus *Inocybe* based on Bayesian Inference (BI) and Maximum Likelihood (ML) analyses of the concatenated two-gene (ITS, LSU) sequence alignment. Significant branch support values, Bayesian posterior probability (BI-PP ≥ 0.95) and ultrafast bootstrap support (ML-BP $\geq 70\%$), 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 evanescent, 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, $Q = (1.30\text{--})1.37\text{--}1.64\text{--}1.95\text{--}(2.05)$, av. $Q = 1.64\text{--}1.65$; 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 H₂O, non-amyloid and non-dextrinoid.

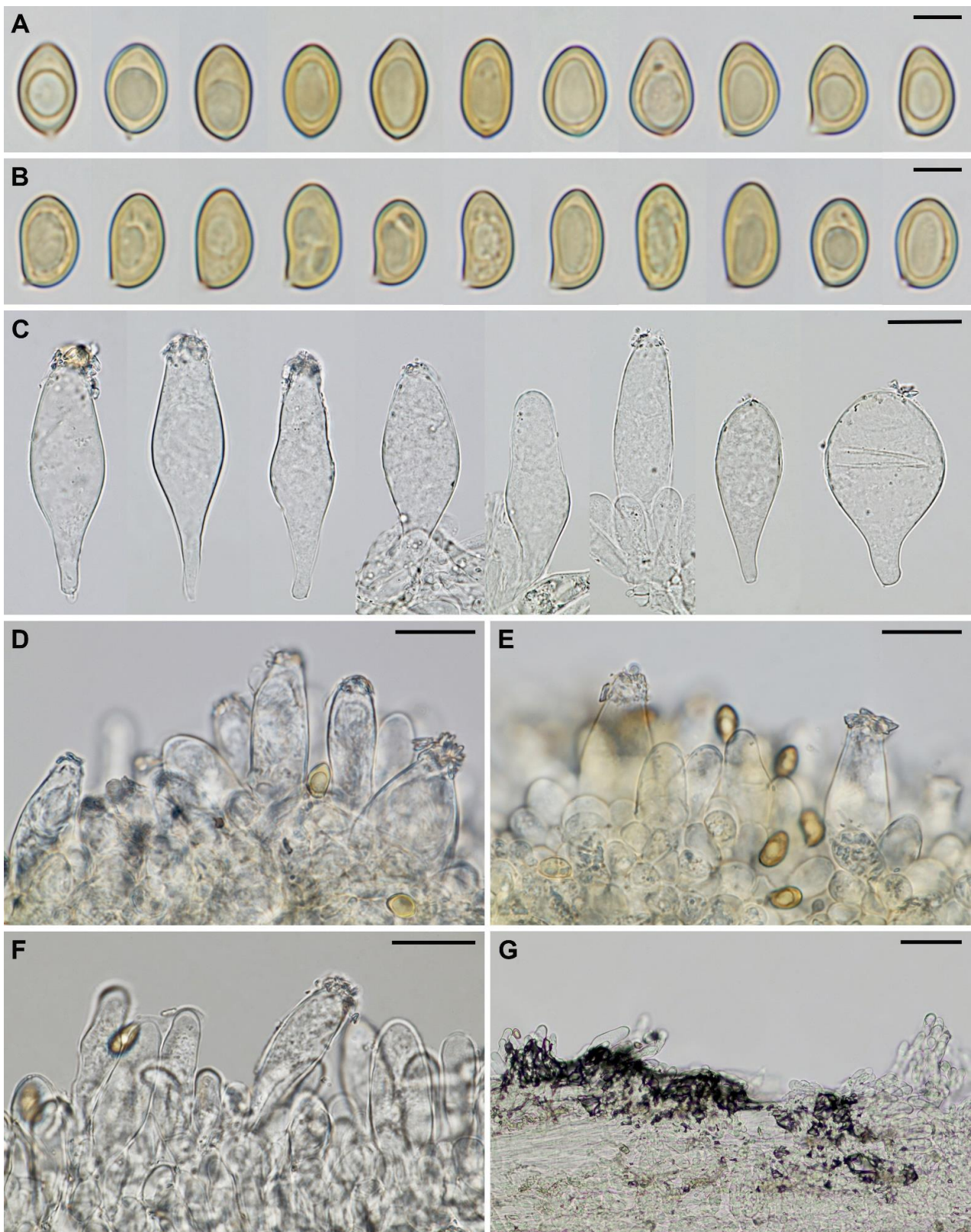


Figure 4. *Inocybe istriaca* sp. nov. (CNF 1/7323, holotype). (A,B) Basidiospores. (C) Pleurocystidia. (D,E) Cheilocystidia. (F) Caulocystidia. (G) Black resinous substance among caulocystidia. Bars: (A,B) = 5 μm ; (C–F) = 20 μm ; (G) = 50 μm . Author: A. Mešić.

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.

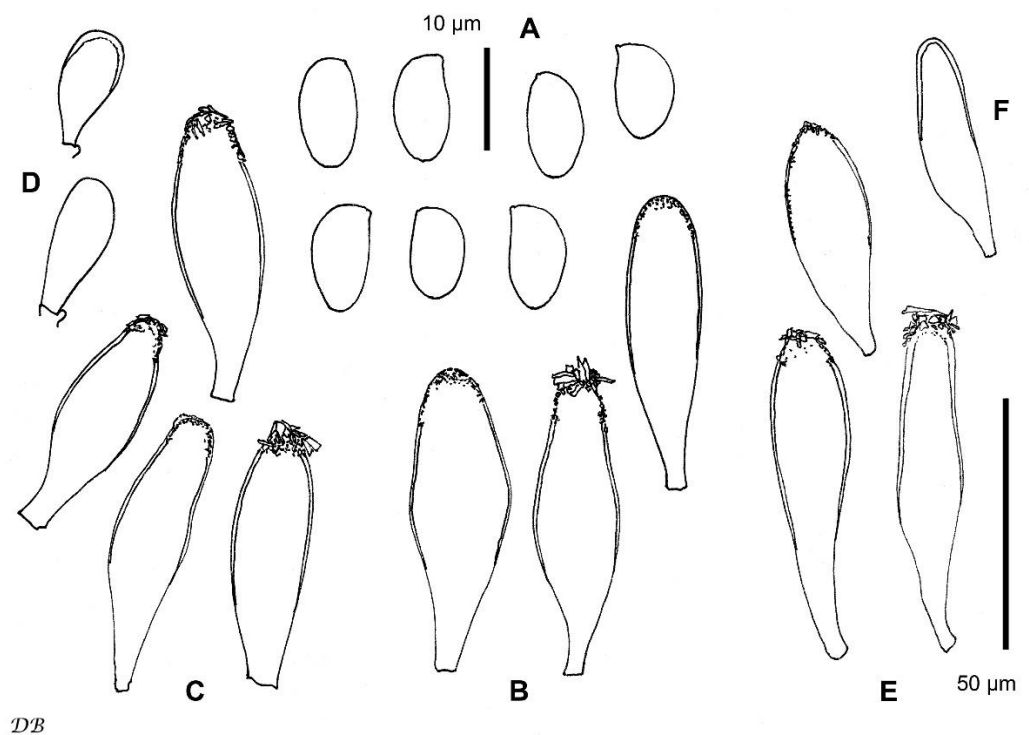


Figure 5. *Inocybe istriaca* sp. nov. (CNF 1/7323, holotype). (A) Basidiospores. (B) Pleurocystidia. (C) Cheilocystidia of metuloid-type. (D) Cheilocystidia of paracystidia-type. (E) Caulocystidia of metuloid-type. (F) Caulocystidia of paracystidia-type. Bars: (A) = 10 µm; (B–F) = 50 µm. Del. D. Bandini.

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

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