Inocybe cervenianensis (Agaricales, Inocybaceae), a new species in the I. flavaolbida clade from Italy

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Abstract

The authors describe the macro- and microscopic features of Inocybe cervenianensis, a new taxon belonging to the genus Inocybe sensu stricto. The description is illustrated with photographs of the basidiomes in their natural habitat and photographs of the main microscopic features. Molecular data (nrITS and RPB2) support the recognition of this new species.

Keywords: Agaricomycetes, Basidiomycota, phylogeny, taxonomy, molecular systematics

Introduction

In the recent revision of the Inocybaceae Jülich (1981: 374) based on a multigene phylogeny, seven genera were recognised (Matheny et al. 2019): Nothocybe Matheny & K.P.D. Latha in Matheny et al. (2019: 12) and Pseudosperma Matheny & Esteve-Rav. in Matheny et al. (2019: 12), described as new, Inosperma (Kühner 1980: 898) Matheny & Esteve-Rav. in Matheny et al. (2019: 13) and Malloocybe (Kuyper) Matheny, Vizzini & Esteve-Rav. in Matheny et al. (2019: 13), former subgenera of Inocybe (Fries 1821: 254) Fries (1863: 346), elevated to generic rank, Auritella Matheny & Bougher (2006: 4), and Tubariomyces Esteve-Rav. & Matheny in Alvarado et al. (2010: 2), and Inocybe sensu stricto that encompass species previously placed in Inocybe subgenus Inocybe. According to Matheny et al. (2019), the genus Inocybe currently comprises about 850 species able to form mutualistic symbioses with at least 23 families of vascular plants, distinguished from other genera of Inocybaceae by the presence of a distinct apiculus in the spores and the usual presence of pleurocystidia (Matheny 2019); only some species, inside of I. lanuginosa group, lack pleurocystidia. The aim of the present contribution is to propose a new species within Inocybe sensu stricto, named I. cervenianensis and morphologically and genetically close to Inocybe pseudoreducta Stangl & Glowinsky (1981: 30). From the morphological point of view and according to the classification system of Singer (1986), it would belong to Inocybe section Splendentes Sing. (1953: 229), a section that includes species characterized by the presence of a stipe entirely or mostly pruinete and generally with margined-bulbous. However, following the classification system of Kuyper (1986), this species belongs to the “supersection” Marginatae, encompassing taxa having an almost entirely caulocyistidiate stipe and the absence of a cortina. Nonetheless, multiple studies have shown that Singer and Kuyper’s taxonomy are both artificial within an evolutionary framework (Matheny et al. 2002, Matheny 2005, Kropp et al. 2010, Ryberg et al. 2010, Dovana et al. 2020).

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Materials and methods

Morphology

The macroscopic descriptions are based on observations of fresh material. The specimens were dried with an electric dryer at 30°C. Colour terms in capital letters are those of Munsell (1994). Micromorphological features were observed on fresh and dried material; sections were rehydrated in ammonia 10 %, or water, and then mounted in aqueous Congo Red, ammoniacal Congo Red, or aqueous ammonia, separately. Terminology for morphological descriptions follows Kuyper (1986). For evaluation of the range of basidiospore sizes, 20 basidiospores were measured in ammonia 10 % for each specimen studied. Spore dimensions are expressed as (a) b– c– d (e), where (a) = minimum value, b = (average – standard deviation), c = average, d = (average + standard deviation) and (e) = maximum value. Q represents the range of the length/width ratio for all of the measured spores. The following abbreviations are used: L = number of lamellae reaching the stipe, l = number of lamellulae between each pair of lamellae. For other microscopic structures, at least 20 elements were measured. Voucher specimens were deposited in the Herbarium of “Museo Civico di Storia Naturale Giacomo Doria”, Italy (GDOR). The name and description of the new species are deposited in MycoBank (http://www.mycobank.org/DefaultPage.aspx).

Molecular and phylogenetic analysis

DNA was extracted from dried basidiomes using the CTAB procedure of Doyle & Doyle (1987). PCR amplifications were performed with the primers ITS1F/ITS4 for the nrITS region (White et al. 1990; Gardes and Bruns 1993) and bRPB2-5F/bRPB2-7R for part of the RPB2 gene (Matheny 2005). The sequences were checked and assembled with Geneious v. R 11.1.5 (http://www.geneious.com, Kearse et al. 2012) and compared to those available in GenBank database with the BLASTn algorithm; accession numbers of new sequences are reported in Fig. 1. For both datasets, sequences were selected based on the greatest similarity on the BLASTn results (nrITS identity >86%, query coverage >97%, and E value of 0.0; RPB2 identity >94%, query coverage >95%, and E value of 0.0) and from previous molecular studies including Inocybe section Splendentes (Matheny 2005, Ryberg et al. 2010, Osmundson et al. 2013, Braaten et al. 2014, Larsson et al. 2014, La Rosa et al. 2017, Matheny & Bougher 2017, Matheny & Swenie 2018, Bandini et al. 2019, Vauras & Larsson 2020). Two terminal taxa, Inocybe pallidicremae Grund & D.E. Stuntz (1977: 399) (voucher: PBM2744) and Inocybe subtilacina Matheny & A. Voitk in Matheny & Swenie (2018: 632) (voucher: PBM2716) were used as outgroup. The sequences were aligned using MAFFT v 7.017 (Katoh et al. 2002) in Geneious v. R 11.1.5 setting the L-INS-I and Auto algorithm for nrITS and RPB2 dataset respectively; RPB2 intron was excluded in final alignment. Congruence of datasets was analysed using maximum likelihood (ML) bootstrapping in RAxML V8.2.11 (Stamatakis 2014) in Geneious v. R 11.1.5 implementing the GTR + G model and a total of 1000 bootstrap replicates (Felsenstein 1985) with “Rapid Bootstrapping and search for best-scoring ML tree” option. Both independent nrITS and RPB2 maximum likelihood analyses (trees not shown) pointed out the relationship between I. cervenianensis and I. flavoalbida Matheny & Bougher (2017: 269). Conflicts among two trees were considered if a significantly supported clade (bootstrap value ≥70 %) from one DNA region is contradicted by another significantly supported clade from other region. Because no significant incongruence was detected, nrITS and RPB2 alignments were concatenated using Geneious v. R 11.1.5. Partition Finder 2 (Lanfear et al. 2017) in CIPRES (Miller et al. 2010) was used to estimate the best partitioning schemes and evolution models for each subset with Mrbayes option. Datasets were analysed using Maximum Likelihood (ML) and Bayesian Inference (BI). The ML analysis was performed with RAxML v. 8.2.11. (Stamatakis 2014) in Geneious v. R 11.1.4 implementing the GTR + G model to each partition and a total of 1000 bootstrap replicates with “Rapid Bootstrapping and search for best-scoring ML tree” option. The BI was performed with MrBayes v.3.2 (Ronquist et al. 2012) in the CIPRES server; GTR + G model was selected for the RPB2 1st codon position, HKY + I + G was selected for the RPB2 2nd codon position and 5.8S, GTR + G was selected for the RPB2 3rd codon position, GTR + I + G was selected for the ITS1 and ITS2 regions, and two independent analyses of four MCMC chains were run for 50 million generations, sampling trees every 1000 generations. The first 25% of trees were discarded as “burn-in” and for the remaining trees, a majority rule consensus tree was computed to obtain estimates for Bayesian Posterior Probabilities (BPP). Chain mixing and convergence were evaluated in Tracer v1.5 (Rambaut and Drummond 2009), considering ESS values ≥200 as a good indicator. Pairwise % identity values of the ntITS sequences were calculated using Geneious v. R 11.1.4. Significant support is considered as bootstrap values ≥70% in the ML analysis and BPP values ≥0.95 in the Bayesian analysis. Aligned matrices are available at TreeBase TB2: 27552.
Figure 1. Maximum Likelihood phylogram obtained from the general nrITS and RPB2 sequence alignment of *Inocybe* spp. *Malloocybe arthrocytis* and *Malloocybe leucoblema* were used as outgroup taxa. Only MLB values ≥70% and BPP values ≥0.95 are given above clade branches. Newly sequenced collections are in bold. For each collection, the specific epithet (as present in GenBank), voucher, GenBank accession numbers of the RPB2/ITS sequences are reported. The /- notation indicates the sequence as missing for that collection.
Results

Molecular analysis

Both Bayesian and Maximum Likelihood analyses produced trees with similar topology; therefore, only the Maximum Likelihood tree, including both MLB and BPP values, is shown (Fig. 1). The nrITS and RPB2 combined
datasets consisted of 89 sequences and the alignment comprised 1399 characters. The three sequences of *Inocybe cervenianensis* (MT448877 and MT448878 from this study and JF908265 from Osmundson et al. (2013)), clustered in a strongly supported clade (MLB = 100 %; BPP = 1) and they are placed within the */Inocybe flavoalbida* clade (MLB = 82 %; BPP = 1) that includes sequences of *Inocybe amblyospora* Kühner (1955: 3, as 'amyblyspora'), *Inocybe angulosquamosula* Stangl (1984: 95), *I. flavoalbida*, *Inocybe leochroma* Bandini, Vauras, & B. Oertel in Bandini et al. (2019: 271). *I. pseudoreducta*, *I. praecox* Kropp, Matheny & Nanagyulyan in Kropp et al. (2010: 570) and two environmental sequences.

**Taxonomy**

*Inocybe cervenianensis* Ferisin, Bizio, Aiardi, Bersan & Dovana, *sp. nov.* Figs. 2–4

MycoBank no.: MB 835592

Etymology:—the specific epithet “cervenianensis” derives from the old latin name of Cervignano del Friuli, a city in Friuli Venezia Giulia Region where the holotype was collected.

Diagnosis:—*Inocybe cervenianensis* is characterised by its medium-sized basidiomes, with brown to ochraceous pileus, covered with whitish velipellis, stipe entirely pruinose, with more or less marginate bulb, brownish with pinkish tinges on the middle but constantly whitish in the upper part. Microscopically, it is characterised by amygdaliform spores, pleurocystidia and cheilocystidia ranging from slenderly fusiform, sublageniform to ventricose-fusiform and caulocystidia mixed with cauloparacystidia descending to the base of the stipe.

Type:—ITALY. Friuli Venezia Giulia: Cervignano del Friuli, Parco Europa Unita, on the ground under *Quercus ilex*, 23 December 2018, G. Ferisin (GDOR623!, GenBank: nrITS MT448877, RPB2 MT458139).

**Description**

*Pileus* 20–70 mm in diameter, at first campanulate or conical-convex then plano-convex to almost planate with a low broad umbo, dark brown (5YR3/4-6), brown (2.5YR3/6-8), sometimes more reddish-brown (10R2/4-6) around centre, outwards brown (2.5YR3/6-8) to ochraceous brown (5YR5/6-10), around centre sericeous-smooth, slightly cracking on age, towards margin with appressed scales to tomentose-fibrillose, with whitish velipellis.

*Lamellae*, *L*= 55–65, *l*= 1–3, moderately crowded, up to 7 mm broad, ventricose to adnate, at first whitish or pale ochraceous, finally brownish; edge fimbriate, whitish.

*Stipe* 40 –65 × 6 –10 mm, solid and fibrose, cylindrical, with a more or less marginate bulb, up to 13 mm wide, initially whitish, then brownish with pinkish tinges, remaining whitish at the extreme apex and at the base, totally white-pruinose. *Context* whitish with pinkish-brownish stain in stipe. *Smell* indistinct or subspermatic. *Taste* indistinct.

*Spores* (7.2)8.0–8.8–9.7(10.7) × (4.2)4.8–5.2–5.6(6.5) μm Q= (1.28)1.53–1.70–1.87(2.21), sub amygdaliform to amygdaliform, with subconical apex, sometimes with irregular shape. *Pleurocystidia* 50–75 × 18–30 μm, slenderly fusiform, sublageniform to ventricose-fusiform, thick-walled, with up to 2.5 μm thick, colourless to pale yellow wall, apex usually crystalliferous, rather frequent. *Cheilocystidia* similar to pleurocystidia, rather frequent. *Paracystidia* clavate, thin-walled, colourless, numerous. *Basidia* 30–40 × 10–12 μm, clavate, 4-spored, rarely 2-spored. *Caulocystidia* descending to the base, with similar shape of cheilocystidia. *Cauloparacystidia* variable in shape, from clavate, subclavate, subcylindrical to spheropedunculate, sometimes also catenate, thin-walled, colourless, abundant. *Pileipellis* a cutis formed by parallel hyphae 3.5–8 μm wide, finely encrusting and parietal yellowish-brownish pigment, subcutis with wider and hyaline elements, up to 18 (22) μm wide. *Clamp connections* abundant in all structures.

*Habit, habitat and distribution*—In groups, under *Quercus ilex* or *Abies cephalonica* trees. So far it is known only from Northeastern Italy.

Additional collections examined of *Inocybe cervenianensis*—ITALY. Friuli-Venezia Giulia: Trieste, in a garden near to University of Trieste, 04 November 2005, *A. Aiardi* and *E. Bizio* (MCVE 21684!, GenBank: JF908265); ibidem, 29 October 2007, *F. Bersan* (GDOR625!, GenBank: MT448878).

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Discussion

Macroscopically, *Inocybe cervenianensis* is characterised by its medium-sized basidiomes, with brown to ochraceous pileus, covered with whitish velipellis, stipe entirely pruinose, with more or less marginate bulb, brownish with pinkish tinges on the middle but constantly whitish in the upper part, context whitish in pileus and with pinkish stain in stipe. Microscopically, it can be recognised by amygdaliform spores, pleurocystidia and cheilocystidia ranging from slenderly fusiform, sublageniform to ventricose-fusiform and caulocystidia similar to cheilocystidia, mixed with cauloparacystidia variable in shape, descending to the base of the stipe. Based on data from our collections, *I. cervenianensis* shows variability both in the shape of basidiomata and in spore shape and size (average value range: 8.1–9.2 × 5.1–5.5 Qm=1.61–1.72), but the amygdaliform shape of spores and the presence of conical apex are constant in all material observed. It is possible that different environmental conditions may influence the growth of basidiomata and dimension of the spores, as previously reported by Kuyper (1986) and Clémençon (2012). The first collection examined in 2005 was initially identified as *I. pseudoreducta* because of a strong resemblance between the two species, but the nrITS sequences of *I. cervenianensis* (MT448877) and a sequence of the holotype collection of *I. pseudoreducta* (KY213955) deviated from each other (pairwise identity = 75%). The nrITS and RPB2 phylogenetic analyses placed *I. cervenianensis* in /*Inocybe flavoralbida* clade that encompasses *I. amblyospora, I. angulatosquamulosa, I. leochroma, I. pseudoreducta* originally described from Europe, *I. praexox* from USA, and *I. flavoralbida* from Papua New Guinea and subtropical Australia. The relationship between *I. amblyospora, I. praexox*, and *I. flavoralbida* that come from three different continents had already been highlighted previously in nrITS and LSU combined analyses performed in Matheny & Bougher (2017). A more recent study (Matheny et al. 2019) pointed out that *I. flavoralbida* belongs to the “STBC” clade that encompass species with smooth basidiospores, metuloids, and mainly Northern Hemisphere distribution with only one exception from the Southern Hemisphere. Compared to *I. cervenianensis*, *I. pseudoreducta* has a pileus with velipellis rather indistinct, lamellae greyish-brownish with an olivaceous tinge, stipe brownish with pinkish tinges in the upper part (constantly whitish in *I. cervenianensis*), caulocystidia scarce in lower 1/3th and association with *Picea* and *Fagus* (Kuyper 1986). Studies of new collections with habitat notes are needed to clarify if associated vegetation is a significant factor to separate these two very similar species. *Inocybe pseudoreducta* was originally described by Stangl & Glowinski with yellow walls of the cystidia in alkaline solution; conversely, the subsequent study conducted by Kuyper (1986) on totypical German collections showed cystidia with colourless walls. Cystidia in *I. cervenianensis* do not have positive reaction with alkali and almost all of the cystidia have a colorless wall, for this reason, this character does not seem significant for the separation of these two close species. Other several species of *Inocybe* that are phylogenetically and morphologically closely related to *I. cervenianensis* can be distinguished morphologically by the following characters. *Inocybe leochroma* can be distinguished by its warm and bright ochraceous-orange pileus, (sub)amygdaloid spores with apex variable in shape from subacute to obtuse and stipe with pruina unusually rough (Bandini et al. 2019). *Inocybe amblyospora* differs mainly by the presence of spores with almost obtuse to only indistinctly subconical apex and thin-walled cystidia up to 1.0–1.5 μm thick (Kuyper 1986). *Inocybe angulatosquamulosa* differs by ochraceous, ochraceous brownish to hazel-brown pileus, stipe towards the apex pinkish-brownish, reddish-brownish or beige and spores with different shape (Bandini et al. 2019). The recently described *I. flavoralbida* from Papua New Guinea and subtropical Australia differs from *I. cervenianensis* by its pileus yellow or very pale brown, without velipellis, context of stipe white, smaller spores (6.5–8.5 × 4.0–5.0 μm) and different habitat associated with *Eucalyptus* and *Castanopsis* (Matheny & Bougher 2017). *Inocybe praexox*, described from the Pacific Northwest of the United States, belongs to the *Inocybe splendens* complex, and can be distinguished by pileus without velipellis, walls of pleurocystidia and cheilocystidia pale yellowish in alkaline solution, and vernal fruit habit (Kropp et al. 2010).

References


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