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INOCYBE COSTINITII A NEW SPECIES FROM THE ISTRIAN COAST

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Abstract : The authors describe the macro-, microscopic and ecological features of *Inocybe costiniti*, a new taxon belonging to genus *Inocybe* found in a grassy area with *Pinus halepensis* within the campground Lanterna (municipality of Tar, Torre Istria, Croatia). In the field, basidiome colours and habit bring to mind *Inocybe griseovelata* Kühner and/or *Inocybe griseotarda* Poirier, but morphological and molecular data suggest that the fungus is new to science. The description is accompanied by colour photographs and a phylogenetic tree.

Riassunto: Gli autori descrivono le caratteristiche macro-, microscopiche ed ecologiche di *Inocybe costinitii*, nuova specie rinvenuta nel Campeggio Lanterna, comune di Tar (Torre, Istria, Croazia), in un prato con *Pinus halepensis*. Sul terreno, forma e colori richiamano alla mente *Inocybe griseovelata* Kühner e/o *Inocybe griseotarda* Poirier. I dati morfologici supportati da un analisi molecolare basata sulla regione ITS sono tali da proporre la specie come nuova. Immagini a colori e un albero filogenetico sono altresì forniti.

INTRODUCTION

Our mycological explorations have always been made in areas or territories where species of the genus *Inocybe* might be expected to grow. At first we visited many sites in Veneto and Friuli Venezia Giulia, northern Italy, (Bersan et al., 2002; Bizio, 2003, 2006, 2007, 2010, 2014), then forays were made to the near Istria, Slovenia, (Bizio et al., 2006), the sandy or rocky coast of which is disseminated of gardens, parks and ampsites, ideal environments for the growth of many species of *Inocybe*, where the Mediterranean climate favours the appearance of such fungi also in winter. In the same habitat it was previously

collected and described *Inocybe fusipes* Bizio, Franchi & M. Marchetti, included in section *Rimosae* (Fr.) Sacc.

I. costinitii, growing gregarious within a camping ground near the Croatian coast in the municipality of Tar south of Novigrad, appeared to be so variable in gross morphology as to request a careful study of each of the four specimen collected. The painstaking morphological analysis was subsequently followed by a phylogenetic investigation, nowadays considered crucial in a genus as complex as the one under study.

MATERIALS AND METHODS

Morphology

The photographs of the basidiomata were taken in situ by a Canon EOS 600D digital camera. The macromorphological characters were observed in fresh specimens, while the microscopic analyses were made by an Optech Biostar B5 light microscope from sections of fresh or revived tissues that were mounted in distilled water. Congo red also was used to stain hyaline structures. Forty spores from four basidiomes of the holotypus collection were measured and the values were expressed in the following way: (a) b - c (d); (a) and (d) represent the minimum and maximum value respectively, while (b) and (c) show the average values ± their standard deviation. The colour coding in macroscopic descriptions is from Munsell (1994), hereafter shortened as Mu. The descriptive terminology follows Kuyper (1986).

Molecular analyses

The nrDNA was extracted by the laboratory ALVALAB (Oviedo, Spain). The ITS locus was amplified and sequenced by using the ITS1-F e ITS4 primers (Gardes & Bruns, 1993; White et al., 1990) then the sequence was deposited in GenBank (accession number: KX686581).

The homologous GenBank sequences sharing a percentage of identity over 85% plus those of *Inocybe griseotarda* (JF908214) and *Inocybe griseovelata* (KC305453) formed the dataset from which the phylogenetic tree was inferred. The ITS sequence of *Inocybe geophylla* (AM882877) was used as outgroup. They were aligned by MAFFT *v7.017* (Katoh et al. 2002) and then manually adjusted thanks to Geneious 8.1.2. The model of sequence evolution GTR+Γ+I was selected with the program Modeltest 3.7 (Posada & Crandall 1998). The dataset was analyzed using maximum likelihood (ML) and Bayesian methods (BI).

The BI was performed with MrBayes 3.1 (Huelsenbeck & Ronquist, 2001) with four incrementally heated simultaneous Monte Carlo Markov Chains (MCMC) run for five million generations, under the selected evolutionary model. MrBayes was run with four chains for 5 million generations and trees were sampled every 1000 generations. To construct the consensus tree, the first 1250 trees were discarded as ‘‘burning.’’ For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian Posterior Probabilities (BPP).

ML analyses were run in RaxML v.7.2.8 (Stamatakis, 2006) with 1,000 bootstrap replicates. Only MLB values over 75 % and BPP values over 0.95 are reported in the resulting trees (Fig. 6).

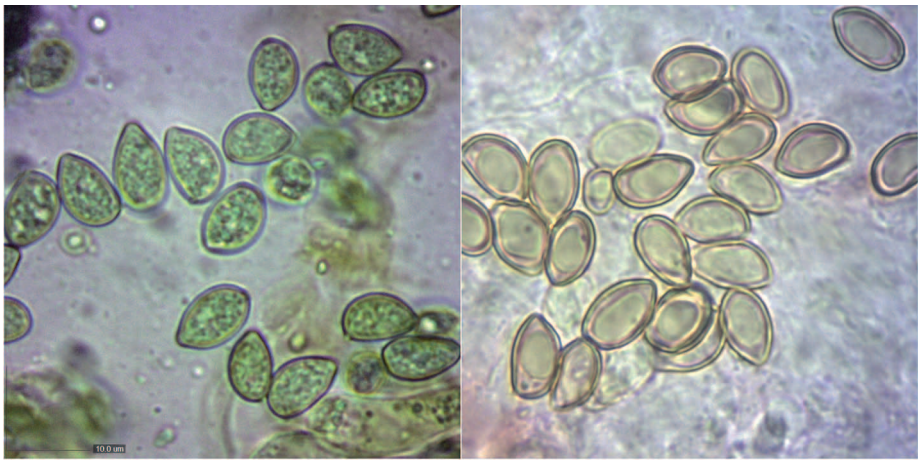
Fig. 1 - *Inocybe costinitii* Bizio, Ferisin G. & Dovana F. sp. nov. (Photo G. Ferisin)

INOCYBE COSTINITII Bizio, Ferisin G. & Dovana F. sp. nov.
MB 815400

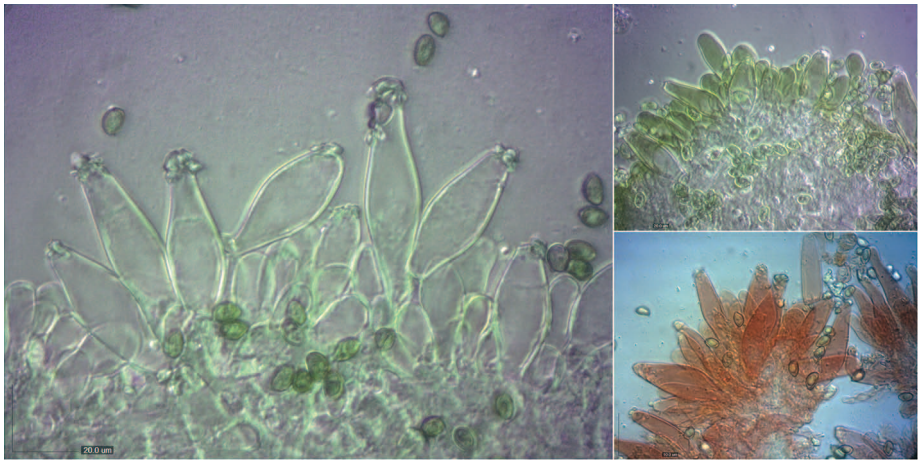
Pileus usque 30 mm irregulariter campanulatus, centrali umbone lato et humili; cuticola laevis, fibrosa, minute radialiter squamosa; brunneus, flavo-fuscus, avallaneus, clarior medio; velo albo conspicuo totus obtectus, in superficie diu manente. Lamellae non parum distantes, plane adnatae, subdirectae, primum pallide murinae-brunneae, dein ferrugineae, demum fuscae; acies lamellarum erosa, albida. Stipes 25-40 x 4-6 mm cylindricus ad basim in submarginatum parvumque bulbum desinens, apice pruinosus, albus sed ad basim leviter fuscus tinctus. Caro alba odore acri spermatico.
Sporae 8.7-10.8 x 5.2-6.6 µm leves, subamygdaliformes, apice e conico subpapillato. Pleurocystidia 53-80 x 16-25 µm plerumque subfusiformia longo pediculo, haud saepe clavaeformia vel sublageniformia, pariete x 2-3 µm. Cheilocystidia similia pleurocystidiis sed leviter breviora et robustiora Caulocystidia in vertice usque ad medium proceritatem praesentia. Habitat: Pinus halepensis Holotypus: Camping Lanterna, Torre (Tar, Hr), 1.01.14, Leg. G. Ferisin e M. Olivi; in herbario MCVE n. 28974 conservatur.

Pileus up to 30 mm in diameter, irregularly campanulate with a broad and obtuse umbo; margin first inflexed then straight, often split. Surface smooth, fibrous, brown, brownish-yellow, yellowish with a tinge of pink (Mu 10YR 8/2-3, 7/3-4), pale brown yellow on the discal area (umbo) (Mu 7.5YR 8/3-6), completely covered with a thin layer of a white, persistent veil.

Lamellae distant, adnate, segmentiform, beige-grey to brownish with olivaceous-brown spots; edge whitish, eroded.



I. costinitii: Spore. (Photo G. Ferisin)



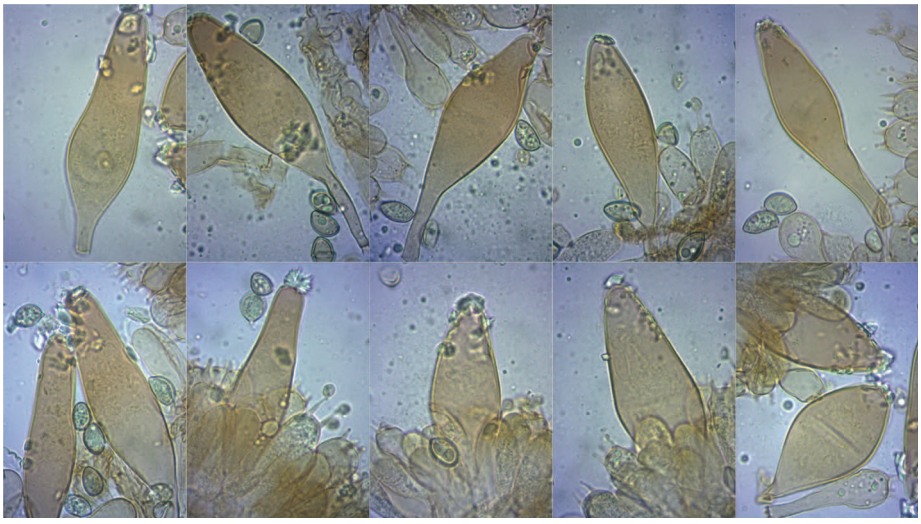
I. costinitii: Cheilocistidi. (Photo G. Ferisin)

Stipe 25-40 x 4-6 mm, solid, pruinose at apex, slightly striate lengthwise, cylindrical, provided with a small submarginate bulb, whitish, tending to discolour brown at base (Mu 2.5YR 8/3-4).

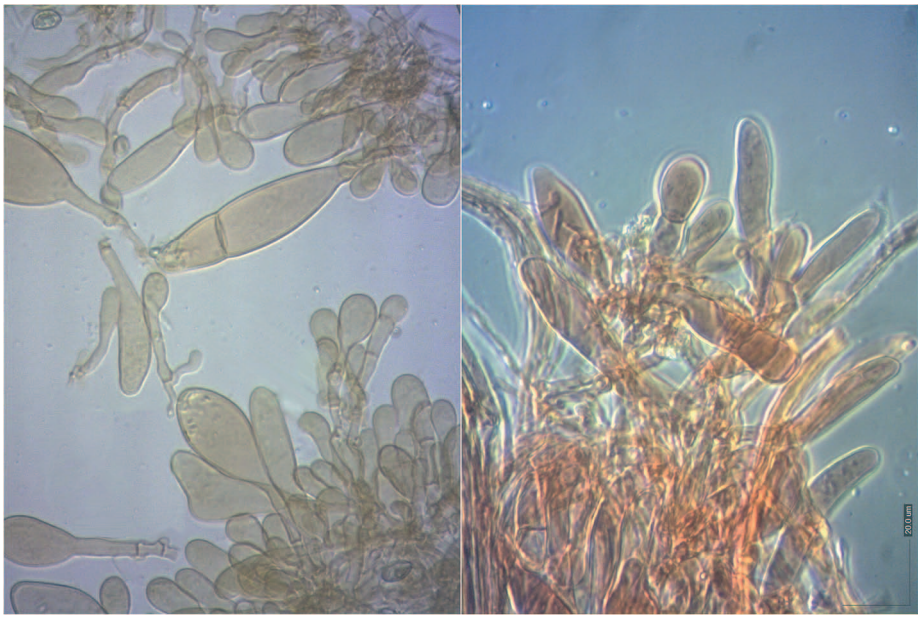
Context firm, whitish; odour strongly spermatic. The whole basidiome becomes dark brown after drying.

Spores (8.3) 8.7 - 10.8 (11) X (5.0) 5.2 - 6.6 (6.8) µm, Q= (1.54) 1.58 - 1.62 (1.64), subamygdaliform with conical to subpapillate apex.
Cheilocystidia 45-70 x 13-26 µm, subfusiform to clavate, sometimes subovoid with or without apical crystals.
Paracystidia abundant, clavate or pear-shaped.
Pleurocystidia 53-80 x 16-25 µm, thick-walled (2-3 µm), yellowish in ammonia, mainly

subfusiform with a long peduncle, rarely clavate or sublageniform; tiny or big crystals of calcium oxalate are present at apex.
Caulocystidia clavate to subfusiform or subovoid, thin-walled, with few or no apical crystals; they occur especially at the apex of stipe
Cauloparacystidia present everywhere mixed with polymorphic hairs.
Clamp-connections present in all tissues.



I. costinitii: Pleurocistidi. (Photo G. Ferisin)



I. costinitii: Caulocistidi. (Photo G. Ferisin)

Habit and habitat: a small group of four specimens on the ground near *Pinus halepensis*.

Collection: MCVE n. 28974, Croatia, municipality of Tar, 01 Jan. 2014, leg. G. Ferisin & M. Olivi, det. E. Bizio, G. Ferisin & F. Dovana.

Etimology: the species is named in honour of Giuseppe Costiniti, an Italian mycologist with a deep interest in the genus *Inocybe*, untimely departed.

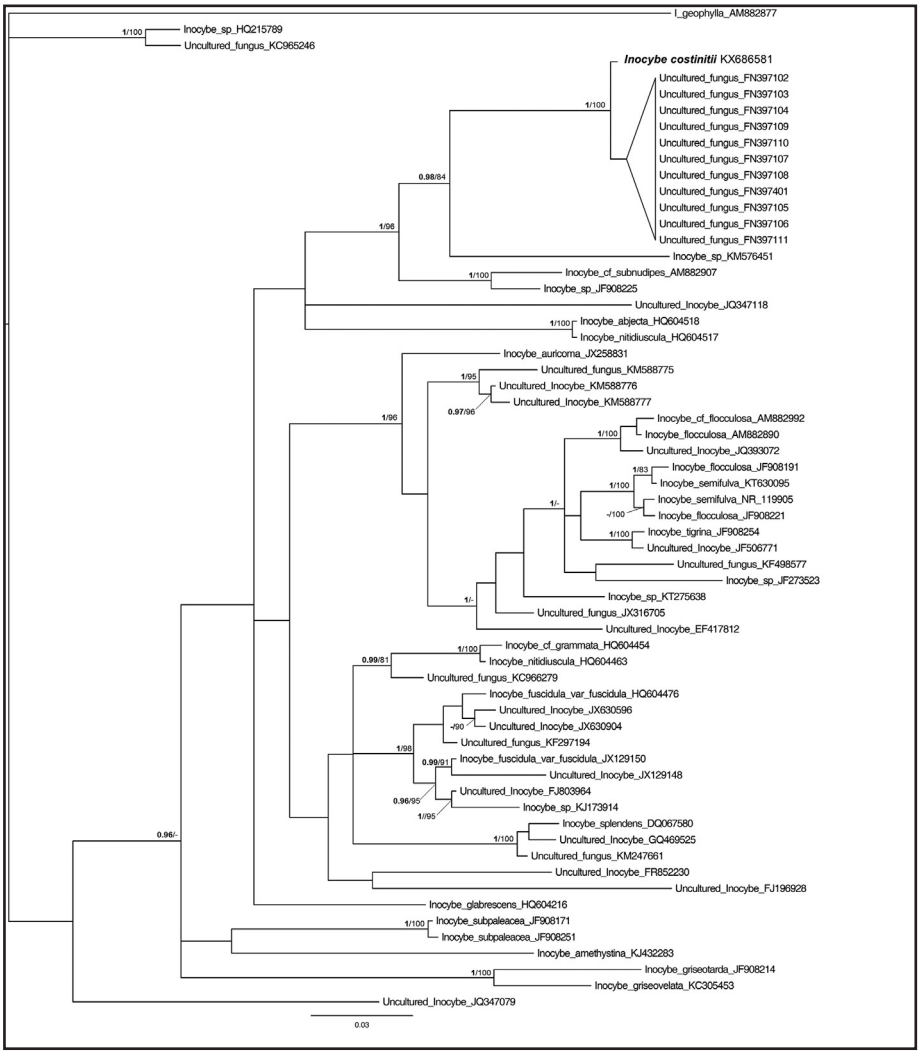


Fig. 6 - Bayesian phylogenetic tree based on an analysis of ITS sequences. BPP values (uppercase) ≥ 0.95 related to the Bayesian Inference and MLB values ≥ 75 referred to Maximum Likelihood are shown above the nodes.

PHYLOGENY

Both Bayesian and Maximum likelihood analyses produced the same topology; therefore, only the BI trees with both BPP and MLB values is presented. The ITS data matrix comprised a total of 62 sequences; the alignment comprised 712 characters.

Both phylogenetic analyses show that *I. costinitii* falls within a statistically strongly supported clade (BPP=1 and MLB=100%) together with sequences of *Inocybe* sp. coming from France (environmental sequences) and is sister to an *Inocybe* sp. (KM576451) found in Romania associated with root tips of *Quercus robur* L.

In its turn this clade is sister (BPP=1; MLB=96%) to a miniclade containing two sequences: JF908225 (*Inocybe* sp.) and AM882907 (*I.* aff. *subnudipes*).

Macroscopically, *Inocybe costinitii* is characterized by its medium-sized basidiomes, with beige-ochre pilei covered with a thick, white veil, smooth and whitish stipes swollen at base, spermatic odour and growth in sandy grassland near *Pinus halepensis* in winter.

Microscopically, it can be recognized by the subamygdaliform spores with conical to subpapillate apex and the fusiform cystidia.

Inocybe griseotarda Poirier and *Inocybe griseovelata* Kühner look much like *I. costinitii*, but the sequence of their ITS locus is different. (Fig. 6)

Inocybe griseotarda Poirier occurs in the same place and is very similar to *I. costiniti*, however it differs in:

- a more robust habit
- a fibrillose-rimose pileus, cracked at disk
- an evanescent veil and the entirely pruinose stipe with pink hues at apex.
- longer and thinner spores
- thin-walled pleurocystidia
- caulocystidia present throughout the stipe and not only at apex.

Based on the distribution of caulocystidia and according to Kuyper's taxonomy *I. griseotarda* and *I. costinitii* are to be placed in the supersections *Marginatae* (Kühner) Kuyper and *Cortinatae* (Kühner) Kuyper respectively.

I. griseovelata Kühner can be distinguished from *I. costinitii* on account of the following characters:

- a darker pileus
- a beige-grey veil
- an equal stipe, pruinose at apex, covered with greyish fibrils downwards
- typical subcylindrical cystidia.

Searching the GenBank database, the sequence of *I. costinitii* shows a 99% identity with two homologous ITS sequences of two *Inocybe* spp. (accessions FN397103 and FN397110) retrieved from a study by Napoli et al. (2010) regarding the biodiversity of fungi associated with a truffle-bed composed of *Quercus pubescens* L. and growing on loamy soils around Cahors, France: the aforementioned sequences differ in just five nucleotides. The phylogenetic data strongly support the hypothesis that they belong to one and the same species, but since we did not have the opportunity to analyze the exsiccata of the French collections, it is impossible to be certain about their conspecificity and state that *I. costinitii* is a strictly psammophilous species associated exclusively with conifers.

The molecular analysis highlights also that the ITS sequence JF908225 shows a high level of homology with that of *I. costinitii.* The former was obtained by a Bizio and Aiardi's collection of *Inocybe camaldolensis* ad interim from a forest of *Fagus sylvatica* L. and *Abies alba* L. located nearby the monastery of Camaldoli (Arezzo, Tuscany, Italy). it is a medium sized, hardly

fleshy *Inocybe*, showing a brown or grey-brown pileus with a small acute umbo and revolute margin, a strongly fibrillose surface and whitish stipe. Apart from the small spores and distribution of caulocystidia, there are no further morphological resemblances between the two taxa.

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