INOCYBE DISTANS, A NEW SPECIES NATIVE TO AUSTRALIA BUT INTRODUCED IN EUROPE AND SOUTH AMERICA WITH PLANTED *EUCALYPTUS*

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Summary:
Inocybe distans is described as new to accommodate a smooth-spored species in the *I. tenax* group, a clade of 16 species, 10 of which have been formally described, and endemic to Australia and New Zealand. However, *I. distans* is the only known species in the group that has been documented on exotic *Eucalyptus* plantations outside its native range in Spain and Argentina. ITS sequence data, phylogenetic analysis, morphology, and host association with *Eucalyptus* support the conspecificity of the Spanish and Argentinean collections with naturally occurring material from a Tasmanian dry sclerophyll woodland. A taxonomic description and illustrations of *I. distans* are provided, together with an identification key for species of the *I. tenax* group. Occurrences of *Inocybe* in eucalypt plantations outside Australia are relatively infrequent and diversity of *Inocybaceae* is quite low. Such diversity is highest with *Eucalyptus globulus* and *E. camaldulensis* on the Iberian Peninsula but low to nil in tropical plantations in Africa and South America.

Key Words: Molecular systematics, taxonomy, *Agaricales*, *Inocybaceae*, new species, biological introduction

Resumen:
Inocybe distans se describe como nuevo para alojar una especie de esporas lisas en el grupo *I. tenax*, un clado de 16 especies, 10 de las cuales se han descrito formalmente, y es endémica de Australia y Nueva Zelanda. Sin embargo, *I. distans* es la única especie conocida del grupo que ha sido documentada con plantaciones exóticas de *Eucalyptus* fuera de su área de distribución nativa en España y Argentina. Los datos de secuencia de la región ITS, el análisis filogenético, su morfología y su asociación con *Eucalyptus* respaldan la conespecificidad de las colecciones españolas y argentinas con material natural de un bosque seco de esclerófilos de Tasmania. Se proporciona una descripción taxonómica e ilustraciones de *I. distans*, junto con una clave de identificación de las especies del grupo *I. tenax*.
La presencia de *Inocybe* en plantaciones de eucalipto fuera de Australia es relativamente infrecuente y la diversidad de *Inocybaceae* es bastante baja. Esta diversidad es máxima en *Eucalyptus globulus* y *E. camaldulensis* en la Península Ibérica, pero es de baja a nula en las plantaciones tropicales de África y Sudamérica.

Palabras clave: Sistemática molecular, taxonomía, *Agaricales*, *Inocybaceae*, nuevas especies, introducción biológica

INTRODUCTION

There are two species of the genus *Eucalyptus* in Spain introduced from Australia for the manufacture of paper due to their rapid growth. *Eucalyptus globulus* is distributed in the north of

Spain and *Eucalyptus camaldulensis* in the interior and southwest of the country. A collection of a smooth-spored species of *Inocybe* was collected in *Eucalyptus camaldulensis* forest with an understory of *Pistacia lentiscus*, *Pinus pinea, Quercus coccifera, Myrtus communis* and a miscellany of non-ectomycorrhizal plants. Molecular sequence data of nuclear rDNA internal transcribed spacer (ITS) regions were produced from the Spanish collection and revealed shared similarities with ITS sequences from samples from a native dry sclerophyll woodland in Tasmania and a *Eucalyptus* plantation in Argentina. In their monograph of Australian *Inocybaceae*, MATHENY & BOUGHER (2017) were not able to formally recognize the Tasmanian species due to insufficient data. Since this publication and subsequent discoveries of additional collections, we describe the species here as new. A series of published reports of *Inocybaceae* in exotic plantations of *Eucalyptus* is also reviewed, which suggests that diversity of *Inocybaceae* in such settings is rather poor.

MATERIALS AND METHODS

Morphological data and specimen-vouchers.

Observations of gross morphological features were made when basidiomata were fresh. Color notations of Munsell Soil Color Charts (1954) were used to describe colors of the pileus, lamellae, and stipe. Specimens were photographed *in situ* and then preserved on a food dehydrator. Australian material was preserved at the University of Tennessee Herbarium (TENN). Other materials sequenced or included in phylogenetic analysis here were also deposited at TENN, Manaaki Whenua – Landcare Research (PDD), Western Australian Herbarium (PERTH), or Tasmanian Museum and Art Gallery (HO). Spanish material was preserved at the public herbarium of the Junta de Andalucía (JA). Unfortunately, the material from Córdoba, Argentina, although molecularly annotated, was extinguished. For microscopic analysis, dried basidiomata were sectioned with the sections rehydrated in sterile water, 5% KOH, Congo Red in KOH, and examined under a compound microscope. Documentation of basidiomata and microscopic features followed MATHENY & BOUGHER (2017).

DNA sequencing and phylogenetic analysis.

DNA and ITS and 28S rDNA sequencing followed JUDGE & *al*. (2010). Both ITS and 28S were produced for the Australian and Argentinian samples and ITS only for the material from Spain. Blastn results of ITS on GenBank revealed high similarity scores between 88–99% with members of the *Inocybe tenax* Matheny & Bougher group identified by MATHENY & BOUGHER (2017). For phylogenetic analysis we used MATHENY & BOUGHER (2017) and their concatenated rDNA alignment of the STAC (smooth-spored temperate austral clade) group. We pruned this dataset in AliView (LARSSON 2014) to members of the *I. tenax* clade and added *Inocybe tasmanica* Matheny & Bougher, belonging to a group sister to the *I. tenax* clade, for rooting purposes. The dataset was analyzed in RAxML 8.2.9 (STAMATAKIS 2014) under the Maximum Likelihood (ML) criterion applying a model of evolution (GTRGAMMA) recommended by the user manual. A bootstrapping procedure with 1000 bootstrap replicates was used to generate statistical support values for internodes. We considered bootstrap values >70% as evidence of strong support. The DNA alignment and embedded consensus ML bipartitions tree file, including bootstrap proportions, are available publicly at https://figshare.com/articles/dataset/STAC_DISTANS_rRNA_nxs/23541471 under doi:10.6084/m9.figshare.23541471).

RESULTS

The ITS sequence from the Spanish *Inocybe* collection (JA-CUSSTA 9701; OK017861) made under *Eucalyptus* differed from a sample (PBM3242; KF830035) collected in a dry sclerophyll woodland in Tasmania at four positions, two of which were polymorphic and the other two indels. The ITS from a sample (GDsn; OR053891) collected in Argentina under planted *Eucalyptus* differed from the Tasmanian sample at two polymorphic sites only. The Spanish sample differed from the Argentine sample at one polymorphic site and two indels.

The molecular dataset included 40 taxa and 2160 sites. All nucleotide positions were included in the phylogenetic analysis, which yielded strong support for all internodes except for three (Fig. 1). 16 species were recognized in the *I. tenax* group, which was then further subdivided into two major clades, each one receiving strong statistical support. 11 species were previously described, whereas five species remained undescribed. Sequences from the sample collected in the Spanish *Eucalyptus* plantation clustered with strong support (100%) with those from the previously published *Inocybe* sp. PBM3242 from Tasmania and *Inocybe* sp. GDsn. from a *Eucalyptus* planting in Argentina, here labeled as *I. distans* sp. nov. (Fig. 1). Due to the minimal genetic variation between the three species as noted above and the similar morphology and shared eucalypt associations, we considered all three as belonging to the same species.

Inocybe distans is most closely related to four other smooth-spored species of Australian *Inocybe* that have yet to be fully characterized. *Inocybe* sp. 1 (PBM3792) originated from Queensland in a wet sclerophyll forest under *Eucalyptus* sp.; *Inocybe* sp. 2 (PBM3448) originated from Tasmania in a wet sclerophyll forest under *Eucalyptus obliqua*; *Inocybe* sp. 3 (MR00219 and PBM3601) was documented from southwest Western Australia in Jarrah-Marri forest under *Eucalyptus marginata*, *E. patens*, and *Corymbia calophylla*; *Inocybe* sp. 4 (NLB837) also originated in southwest Western Australia but in a heath-scrub system near *Melaleuca* shrubs (Myrtaceae), *Acacia* (Fabaceae), *Spyridium* (Rhamnaceae), and *Agonis flexuosa* (Myrtaceae).

TAXONOMY

Inocybe distans Romera & Matheny, **sp. nov.** Figs. 2-4
MycoBank No. 849056

Typification: AUSTRALIA. TASMANIA: Scattered to gregarious singly or in pairs in mossy soil and grass near reserve shelter in dry sclerophyll woodland under *Eucalyptus* and *Acacia dealbata*, Chauncy Vale Wildlife Sanctuary, Bagdad, -42.61333 147.27389, 11-VI 2009, *leg. G. Gates, D. Ratkowsky & P.B. Matheny PBM3242* (**holotype designated here** TENN-F-068381). GenBank ITS = KF830035; 28S = KF808347.

Etymology: *distans* (L.), far apart, referring to the distant lamellae

Diagnosis: Basidiomata small (pileus <20 mm wide, stipe < 3 mm thick), with distant lamellae and a slender equal stipe. Odor not remarkable. Basidiospores smooth; pleurocystidia fusiform, thick-walled, not bright yellow, with obtuse and crystalliferous apices; caulocystidia descending entire length of stipe. Under planted *Eucalyptus* in Argentina and Spain and naturally occurring under *Eucalyptus* in Australia (Tasmania). Phylogenetically nested in the *I. tenax* group and most closely related to the described Australian species *Inocybe xanthocystis* Matheny & Bougher, *Inocybe atrorubripes* Matheny, Bougher & Ryberg, and *Inocybe pygmaeformis* Matheny, Bougher & G.M. Gates. Differs from these by the absence of bright yellow walled cystidia, non-vinaceous stipe, unremarkable odor, and unique phylogenetic position.

Species description

Pileus 5–15 mm, convex with an incurved margin in youth, becoming conical to plano-convex in age; margin entire when young, becoming translucent-striate on older specimens; surface dry, coarsely fibrillose, at times diffracted-scaly around the center, velipellis absent; dark reddish brown (2.5YR 2/4), shading castaneous towards the margin (2.5YR 3/4), not hygrophanous; context thin, fragile, odor not remarkable (not like *Pelargonium*). Lamellae adnexed, subdistant to distant, brown in youth (7.5YR 5/4), becoming umbrinous (7.5YR 4/4) with age, edges pallid and indistinctly fimbriate. Stipe up to 23 mm long, 1–2,5 mm wide, equal, terete, at times flexuous; dry, partial veil not observed, pruinose the entire length; reddish brown (5YR 4/4) to dark reddish brown (5.YR 3/4), with a stuffed interior.

Basidiospores 7,5–10,2 · 4,5–6,2 µm, mean 9,0 · 5,2 µm, Q: 1,5–2,3, Q mean: 1,7 (n=40/3), smooth, amygdaliform with pointed apices, yellowish brown (occasional spores from lamellar mounts observed without pigment), slightly thick-walled, apiculus small and indistinct. *Basidia* 24–29 · 7–9 µm, 4-sterigmate, clavate, hyaline. *Pleurocystidia* 53–65 · 10–16 µm, fusiform but some with a rounded truncate base, thick-walled with walls 1–3 µm thick and hyaline, apices obtuse and crystalliferous. *Cheilocystidia* similar to pleurocystidia. *Caulocystidia* similar to cheilocystidia and mixed with cauloparacystidia the entire length of the stipe. *Pileipellis* a cutis, velipellis hyphae not observed. *Lamellar trama* of parallel hyphae. *Clamp connections* present.

Habitat and distribution: In Australia *Inocybe distans* is known only from Tasmania where it occurs in native dry sclerophyll woodland with *Eucalyptus* and *Acacia*. However, the species has been observed in *Eucalyptus* plantations outside of its home range in Argentina and Spain. Soil conditions are expected to be acidic (SANTOLAMAZZA-CARBONE & *al.* 2019).

Additional material studied: ARGENTINA: Córdoba, under planted *Eucalyptus*, leg. *G. Daniele GDsn* (specimen extinguished). GenBank ITS = OR053891; 28S = JN974922. SPAIN: under planted *Eucalyptus camaldulensis*, Chiclana de la Frontera (Cádiz), Andalucia, 36.35332, -6.07698, 15-I-2021, *leg. M. Romera* JA-CUSSTA 9701. GenBank ITS = OK017861.

KEY TO SPECIES OF THE *INOCYBE TENAX* GROUP

Stipe wiry, tough
- Pleurocystidia very thick-walled (4–10 µm) and bright yellow **Inocybe xanthocystis**
- Pleurocystidia moderately thick-walled (3–4 µm) and hyaline to pale yellow
  - In rainforest under *Nothofagus* **Inocybe tenax**
  - In sclerophyll or warm temperate rainforest forest under *Eucalyptus*, *Corymbia*, or *Lophostemon*
    - Odor acidulous, pileus with dark conspicuous scales when fresh **Inocybe tenuis**
    - Odor like *Pelargonium* or sweet, pileus fibrillose scaly only when dry
      - Pileus fibrillose, lacking dark conspicuous scales **Inocybe pluvialis**
      - Pileus becoming scaly when dry **Inocybe boreoaustralis**

Stipe not wiry and tough
- Pileus dark brown to dark yellowish brown and scaly, stipe dark reddish brown, in sand under *Allocasuarina* **Inocybe atrorubripes**
- Pileus and stipe not as dark as above, in leaf litter in eucalypt forest
  - Pileus <10 mm wide, often bicolorous, fuscous to reddish brown at the center, pale brown towards the margin, stipe 0,5–1 mm wide **Inocybe pygmaeformis**

Pileus and stipe larger than above; pileus uniformly dark brown, reddish brown, brown, or with pink tinges

Pileus with pink tinges, lamellae pink to reddish brown, spores nearly hyaline **Inocybe roseola**

Pileus lacking pink tinges; lamellae pale brown, strong brown or yellowish brown; spores yellowish brown

Stipe striatulate with longitudinal raised pruinose lines **Inocybe vittata**

Stipe pruinose but lacking raised lines

Basidiomata soft and fragile, pileus with velipellis, odor acidulous **Inocybe fragilis**

Basidiomata not soft and fragile, pileus without velipellis, odor absent **Inocybe distans**

DISCUSSION

Inocybe distans is native to Australian sclerophyllous woodlands but introduced and observed at least twice in *Eucalyptus* plantations in Argentina and Spain. The species is most closely related to a suite of smooth-spored species in the *I. tenax* group with entirely pruinose equal stipes and thick-walled, sparsely septate velipellis hyphae from various regions and ecosystems of Australia (Queensland, Tasmania, Western Australia), but all of these are known from single gatherings or are otherwise insufficiently characterized (Fig. 1; MATHENY & BOUGHER 2017). Of described species in the *I. tenax* group, *I. distans* is closely related to several other Australian taxa. *Inocybe xanthocystis* differs from *I. distans* by the very thick-walled (4–10 µm) and bright yellow hymenial cystidia and appears endemic to southwest Western Australia where it associates with *Eucalyptus*. *Inocybe atrorubripes* is also known only from southwest Western Australia but occurs in association with *Allocasuarina* and has a dark brown pileal disc with a dark yellowish brown margin and a vinaceous stipe. *Inocybe pygmaeformis* is characterized by the very small (pileus <10 mm wide, stipe 0,5-1 mm wide) basidiomata with a generally bicolored pileus – fuscous to reddish brown at the center and pale brown towards the margin – and association with wet sclerophyll vegetation in Tasmania. *Inocybe distans* is not allied molecularly with any known specimens sequenced from Patagonia (TRUONG & *al*. 2017, CAIAFA & *al.* 2021) nor from tropical regions of South America (MATHENY & *al*. 2012) and the northern hemisphere. Of the species known in the *I. tenax* group of the southern hemisphere, it is the only one to date that has been confirmed to occur outside its native range under planted *Eucalyptus camaldulensis*.

Several studies that document fungal diversity in exotic *Eucalyptus* plantations in Spain and elsewhere have been conducted over the years. Generally, species of *Pisolithus* and *Scleroderma* (Boletales) and species of *Laccaria* and *Descolea* (Agaricales) are among the most commonly encountered ECM fungi found producing sporocarps in such settings. Species of *Inocybaceae* have been infrequently reported and appear to be low in diversity.

An initial review of fleshy *Agaricomycetes* associated with planted *Eucalyptus* in Spain and Portugal uncovered reference to some 483 taxa across the Iberian Peninsula (LAGO-ÁLVAREZ & CASTRO 2003), however, only five species of *Inocybaceae* were reported (all under *Eucalyptus globulus*): *Inocybe arenicola* (now in *Pseudosperma*), *I. geophylla* var. *fulva*, *I. heimii* (now in *Mallocybe*), *I. pseudohaemacta*, and *I. terrigena* (now in *Mallocybe*). These five names of *Inocybaceae* are all of European origin and would imply host-switching from naturally co-occurring native plants such as *Populus*, *Quercus*, or *Pinus* to the planted *Eucalyptus*. Alternatively, a mixture or all of the names may have been misapplied to unknown Australian

species co-introduced with *Eucalyptus*. In their study BUTRÓN & *al.* (2011) revised the taxonomy and nomenclature of the previous work and thus documented four species of *Inocybaceae* associated with planted *Eucalyptus globulus*. The report of *I. pseudohemacta* above was re-annotated to *I. aeruginascens* (also introduced with planted *Populus* in South Africa) and the prior report of *I. terrigena* remained unverified.

A later study performed in Spain also examined the ECM fungal community associated with introduced *Eucalyptus globulus* (SANTOLAMAZZA-CARBONE & *al.* 2019), however, no species of *Inocybaceae* were documented. A review of diversity of ectomycorrhizal (ECM) fungi associated with *Eucalyptus* in Africa and Madagascar was also conducted (DUCOUSSO & *al.* 2012). Similarly, a study of co-introductions of ECM fungi in eucalypt plantations in Brazil revealed no species of *Inocybacae* (SULZBACHER & *al.* 2018). Sampling sporocarps DEJENE & *al.* (2017) likewise recorded no *Inocybaceae* from *Eucalyptus grandis* plantations in Ethiopia, and a similar result was reported from *Eucalyptus* plantations in Pakistan (BASHIR & KHALID (2015), that is, no documentation of *Inocybaceae* in these exotic eucalypt plantations.

High-throughput DNA sequencing studies of fungal ITS sequences from *Eucalyptus* plantation soils have also captured minimal *Inocybaceae* diversity. One such study of fungal soil diversity of hybrid *Eucalyptus* (cross between *E. grandis* and *E. urophylla*) in South Africa revealed two OTUs (operational taxonomic units) assignable to *Inocybe*, but the two OTUs could not be further identified (BOSE & *al.* 2023). A second publication considered below-ground diversity of young *Eucalyptus grandis* plantations in Zimbabwe but recorded no fungal samples attributable to *Inocybaceae* (JIMU & *al.* 2018). A challenge to linking named diversity to samples (whether sporocarps or ITS sequences) collected in exotic *Eucalyptus* plantations is that documentation of Australian *Inocybaceae* is not yet complete, as our study demonstrates. MATHENY & BOUGHER (2017) documented 137 species of *Inocybaceae* from Australia, but many species, perhaps at least another 70, await formal description. In light of this, it stands to reason, as our study suggests, that at least a portion of *Inocybaceae* diversity associated with exotic *Eucayptus* may be native to Australia and thus introduced.

Some work has been done documenting the introduction of non-native species of *Inocybe* in Australia and Papua New Guinea (BOUGHER & MATHENY 2011). Additional introductions of *Inocybe* into Australia have also been documented by MATHENY & BOUGHER (2017). These introductions are of northern hemisphere provenances and found in association with planted *Quercus* and *Pinus.*

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FIGURE LEGENDS

Fig. 1. Combined ITS and 28S ML phylogeny for the *Inocybe tenax* group. Values above and below branches are proportions of branch support based on 1000 bootstrap replicates.

Fig. 2. Basidiomata of *Inocybe distans* sp. nov. collected *in situ*. a. Several basidiomata (PBM3242) collected in native dry sclerophyll woodland under *Eucalyptus* and *Acacia* in Tasmania. b. Close-up of basidiomes (PBM3242) demonstrating the wide lamellar spacing. c. Various basidiomata (JA-CUSSTA 9701) from a *Eucalyptus* plantation in Spain. Scale Bar: 5mm = a–c

Fig. 3. Microphotographs of diagnostic features of *Inocybe distans* sp. nov. (JA-CUSSTA 9701). a. Basidiospores. b. Basidiospores. c. Cheilocystidia. d. Individual cheilocystidium and scattered basidiospores. e. Pleurocystidia. f. Caulocystidia from the stipe apex. g. Caulocystidia from the stipe center. h. Caulocystidia and cauloparacystidia from the lower part of the stipe. Scale Bar: 5 µm = a, b; 20 µm =c–h. Mounting media: Water = b; Congo red = c, e–h; KOH = d.

Fig. 4. Illustrations of diagnostic features of *Inocybe distans* sp. nov. a. Hymenophoral trama. b. Basidiospores. c. Basidiomata highlighting the distant spacing of the lamellae. d. Basidia. e. Cheilocystidia. f. Pleurocystidia. g. Caulocystidia and cauloparacystidia