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Integrative morphological and molecular characterization of *Inocybe* Species from Yamanlar Mountain (İzmir, Türkiye), with four new national records

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Abstract
Purpose: This study aimed to identify Inocybe specimens collected from *Pinus brutia*-dominated forests surrounding Karagöl Nature Park on Yamanlar Mountain (İzmir, western Türkiye) by integrating macro- and micromorphological observations with ITS rDNA sequence data. It also aimed to document taxa not previously reported from Türkiye and contribute to knowledge of the Turkish mycobiota.
Method: Macrofungal specimens were collected from *Pinus brutia* forest in the Karagöl Nature Park area and examined using detailed macro- and micromorphological characteristics, including basidiospores, basidia, cystidia, and pileipellis structures. Dried basidiomata were used for extracting the genomic DNA and amplifying the ITS rDNA region with primers ITS1F and ITS4. Sequences collected were compared to reference sequences in GenBank and subjected to Bayesian phylogenetic analysis with MrBayes. A combination of morphological and molecular data was analyzed for reliable species delimitation and identification.
Findings: Seven Inocybe species were identified: *Inocybe amygdaliolens* Esteve-Rav., Pancorbo & Dovana, *I. geophylla* (Bull.) P. Kumm., *I. neorufula* Esteve-Rav., Macau & Ferville, *I. nitidiuscula* (Britzelm.) Lapl., *I. pinophila* Cervini, Cagnoli & Bizio, *I. subnudipes* Kühner, and *I. tenuicystidiata* E. Horak & Stangl. Bayesian ITS phylogenetic analysis placed the examined specimens within or together with reference sequences of the corresponding taxa, with varying levels of posterior probability support. Based on the literature and database searches conducted for this study, *I. amygdaliolens*, *I. neorufula*, *I. pinophila*, and *I. subnudipes* represent new national records for Türkiye.
Conclusion: This study provides an integrative taxonomic assessment of Inocybe collections obtained during a short-term survey in the Yamanlar Mountain–Karagöl region. Although the restricted spatial and temporal sampling does not permit a comprehensive evaluation of regional Inocybe diversity, the combined morphological and molecular evidence supports the identification of seven taxa, four of which represent new national records for Türkiye. These records extend the documented geographic distributions of the taxa and contribute to current knowledge of the Turkish mycobiota.

Keywords: Bayesian phylogeny; ectomycorrhizal fungi; ITS rDNA; Pinus brutia; fungal biodiversity; western Anatolia

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Yamanlar Dağı (İzmir, Türkiye) *Inocybe* türlerinin morfolojik ve moleküler karakterizasyonu: Türkiye için dört yeni kayıt

Özet
Amaç: Bu çalışma, İzmir Yamanlar Dağı'ndaki Karagöl Tabiat Parkı çevresinde bulunan *Pinus brutia* baskın ormanlardan toplanan Inocybe örneklerini makro- ve mikromorfolojik gözlemler ile ITS rDNA dizi verilerini

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bütünleştirerek teşhis etmeyi amaçlamıştır. Ayrıca Türkiye'den daha önce bildirilmemiş taksonların belgelenmesi ve Türkiye mikobiyotasına katkı sağlanması hedeflenmiştir.

Metod: Makromantar örnekleri Karagöl Tabiat Parkı çevresindeki *Pinus brutia* ormanlarından toplanmış ve makroskobik ile mikroskobik özellikleri ayrıntılı olarak incelenmiştir. Basidiyospor, basidyum, sistidya ve pileipellis gibi taksonomik açıdan önemli yapılar değerlendirilmiştir. Kurutulmuş bazidiokarplardan genomik DNA izole edilmiş, ITS rDNA bölgesi ITS1F ve ITS4 primerleri kullanılarak çoğaltılmıştır. Elde edilen diziler GenBank verileriyle karşılaştırılmış ve MrBayes programı kullanılarak Bayesian filogenetik analizlere tabi tutulmuştur. Güvenilir tür teşhisi ve tür sınırlarının belirlenmesi amacıyla morfolojik ve moleküler veriler birlikte değerlendirilmiştir.

Bulgular: Çalışma sonucunda *Inocybe amygdaliolens* Esteve-Rav., Pancorbo & Dovana, *I. geophylla* (Bull.) P. Kumm., *I. neorufula* Esteve-Rav., Macau & Ferville, *I. nitidiuscula* (Britzelm.) Lapl., *I. pinophila* Cervini, Cagnoli & Bizio, *I. subnudipes* Kühner ve *I. tenuicystidiata* E. Horak & Stangl olmak üzere yedi tür belirlenmiştir. Bayesian ITS filogenetik analizinde incelenen örnekler, farklı düzeylerde posterior olasılık desteğiyle ilgili türlere ait referans dizilerle birlikte veya aynı kladlar içerisinde konumlanmıştır. Gerçekleştirilen literatür ve veri tabanı taramalarına göre *I. amygdaliolens*, *I. neorufula*, *I. pinophila* ve *I. subnudipes* Türkiye için yeni kayıt niteliğindedir.

Sonuç: Bu çalışma, Yamanlar Dağı–Karagöl bölgesinde kısa süreli bir arazi çalışması sırasında toplanan Inocybe örneklerinin integratif taksonomik değerlendirmesini sunmaktadır. Mekânsal ve zamansal açıdan sınırlı örnekleme bölgesel Inocybe çeşitliliğinin kapsamlı biçimde değerlendirilmesine izin vermemekle birlikte, morfolojik ve moleküler bulgular yedi taksonun teşhisini desteklemiş; bunlardan dördü Türkiye için yeni kayıt olarak belirlenmiştir. Bu kayıtlar türlerin belgelenmiş coğrafi yayılışlarını genişletmekte ve Türkiye mikobiyotasına ilişkin mevcut bilgilere katkı sağlamaktadır.

Anahtar kelimeler: Bayesian filogeni; ektomikorizal mantarlar; ITS rDNA; *Pinus brutia*; mantar biyoçeşitliliği; Batı Anadolu

1. Introduction

Macrofungi are critical decomposers of organic matter, nutrient cycling and are the core of mycorrhizal associations with plants in forest ecosystems. The genus Inocybe (Fr.) accounts for the largest number of ectomycorrhizal mushrooms. Fr. is found throughout the world and associates with many species of trees including members of the Pinaceae. The morphological variation and the presence of diagnostic characters shared in common among the species, however, often make it difficult to identify the Inocybe taxa with confidence, sometimes requiring molecular data as support [1,2].

The study area, Yamanlar Mountain, is a volcanic massif located in the northeastern part of İzmir Bay, western Türkiye, reaching an elevation of 1,076 m. The mountain is separated from Mount Spil to the east by the Sabuncubeli Pass and overlooks İzmir Bay to the west. Near its summit lies Karagöl, a crater lake formed by ancient volcanic activity and surrounded by well-preserved forest ecosystems. Yamanlar Mountain and its surroundings were designated as a Nature Park in 2011 and are also included within the Yamanlar Mountain Important Nature Area. The vegetation of the region is highly diverse, consisting of maquis and Turkish red pine (*Pinus brutia* Ten.) communities at lower elevations, and black pine (*Pinus nigra* J.F. Arnold), oak (*Quercus* spp.), and manna ash (*Fraxinus ornus* L.) stands at higher elevations. This habitat heterogeneity provides suitable microhabitats for a rich macrofungal diversity.

Previous studies on the macrofungal diversity of İzmir Province have reported numerous taxa from the region, including several new records for the Turkish mycobiota [3,4]. Nevertheless, most of these investigations were based primarily on morphological observations, whereas molecular studies focusing on the macrofungi of Yamanlar Mountain, particularly the Karagöl area, remain scarce. Although more than 100 *Inocybe* species have been reported from Türkiye to date, taxonomic studies of this genus supported by molecular data are still relatively limited, and forest ecosystems in Western Anatolia remain largely underexplored with respect to *Inocybe* diversity [1,2].

Although the Turkish macrofungal biota has been increasingly investigated, previous studies on *Inocybe* indicate that the genus is still insufficiently explored in Türkiye. In the previous morphology based studies, several *Inocybe* taxa were reported as new records for the mycota of Turkey: *I. flocculosa* var. *crocifolia*, *I. ochroalba*, *I. pallida*, *I. splendens* var. *splendens*, *I. substellata* and *I. vaccina* [5]. In the following years *I. griseotarda*, *I. nothomixtilis* and *I. tarda* were also detected from Türkiye in the field, indicating taxonomic studies are still uncovering new members of the genus [5–8]. The macro- and micromorphological and/or ITS sequence data supported the majority of these records; it was noted that molecular tools are becoming increasingly used in Turkish *Inocybe* taxonomy [6,9]. Recent integrative studies based on morphology, microscopy and molecular data have increased the number of taxa in the Turkish mycota to include *I. costinitii* and *I. subporospora* [10,11]. The results of these studies indicate that the genus *Inocybe* in Türkiye is largely under-documented and further surveys with molecular information are needed to better assess the diversity of the genus.

In the current study, *Pinus brutia* forests within the boundaries of Karagöl Nature Park were selected in the surrounding areas of Yamanlar Mountain and macrofungal specimens were collected from these forests. The collected materials were studied according to detailed macroscopic, microscopic and anatomical features and then their molecular

analyses were conducted based on the ITS rDNA region. As a result, seven species belonging to the genus Inocybe were identified: *Inocybe amygdaliolens* Esteve-Rav., Pancorbo & Dovana, *Inocybe geophylla* (Bull.) P. Kumm., *Inocybe neorufula* Esteve-Rav., Macau & Ferville, *Inocybe nitidiuscula* (Britzelm.) Lapl., *Inocybe subnudipes* Kühner, *Inocybe pinophila* Cervini, Cagnoli & Bizio, and *Inocybe tenuicystidiata* E. Horak & Stangl. Among these, *I. amygdaliolens*, *I. neorufula*, *I. subnudipes*, and *I. pinophila* are reported here for the first time from Türkiye. The findings significantly expand current knowledge of the macrofungal diversity of Yamanlar Mountain and contribute to a better understanding of the distribution and taxonomy of Inocybe species in Türkiye. This study represents an initial integrative investigation combining morphological and molecular approaches to document selected Inocybe collections from the Yamanlar Mountain–Karagöl region.

2. Materials and methods

2.1. Sampling and Morphological Studies

Macrofungal specimens were collected by the second author during a spatially and temporally restricted survey in November 2025 from *Pinus brutia* Ten.-dominated forest surrounding Karagöl Nature Park on Yamanlar Mountain, İzmir, western Türkiye. Fresh basidiomata were photographed in their natural habitats, and macromorphological characteristics, including pileus, lamellae, stipe, colour, size, and ecological features, were recorded in the field. Micromorphological examinations were conducted using hand-cut sections prepared from the lamellae, pileipellis, and stipe surface of dried basidiomata. The sections were rehydrated and mounted in 5% KOH, Congo Red, and Melzer's reagent and examined with a Leica DM750 light microscope (Leica Microsystems, Wetzlar, Germany) at magnifications of up to ×1000 under oil immersion. For each collection, at least 30 mature basidiospores from two basidiomata were measured in side view, excluding the hilar appendix. Where adequately preserved, at least 20 basidia, cheilocystidia, and pleurocystidia were also measured. Measurements are presented as minimum–maximum ranges; values in parentheses represent rare extremes. Q was calculated for each basidiospore as length divided by width, and Qm denotes the mean Q value. All dried collections were deposited as permanent voucher specimens in the Herbarium of Erciyes University (ERCH), Kayseri, Türkiye; voucher accession numbers are provided in the taxonomic treatments and Table 1.

2.2. Molecular Characterization

2.2.1. DNA Extraction, PCR Amplification and Sequencing

Genomic DNA was extracted from dried basidiomata using the NucleoGene Plant Mini Kit (NucleoGene Biotechnology, Türkiye) according to the manufacturer's instructions. DNA quality and concentration were evaluated by agarose gel electrophoresis and spectrophotometric measurements.

The internal transcribed spacer (ITS) region of the nuclear ribosomal DNA was amplified using the fungal-specific primer ITS1F [12] and the universal primer ITS4 [13]. PCR amplifications were performed in a total reaction volume of 25 µL containing 12.5 µL of 2× PCR Master Mix (NucleoGene Biotechnology, Türkiye), 1 µL of each primer (10 pmol μL^{-1}), 2 µL of genomic DNA template, and sterile nuclease-free water to the final volume.

Amplifications were carried out in a thermal cycler using the following program: an initial denaturation step at 95°C for 5 min; followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min; with a final extension step at 72°C for 10 min. Amplification success was verified by electrophoresis on 1.5% agarose gels stained with an appropriate nucleic acid dye and visualized under UV illumination.

Successful PCR products were purified and sequenced commercially by Oligomer Biotechnology Company (Ankara, Türkiye). Forward and reverse chromatograms were checked manually, assembled, and edited to obtain consensus sequences. Preliminary identification was performed using BLAST searches against sequences available in the GenBank database [14].

2.2.2. Sequence alignment and phylogenetic analysis

The newly generated consensus sequences were compared with publicly available sequences using BLASTn against the NCBI GenBank database [14]. Reference sequences were selected based on taxonomic relevance, ITS coverage, similarity in preliminary BLASTn searches, and the availability of reliable voucher or type-related information. When available, sequences derived from type or otherwise taxonomically verified material were preferentially included. ITS sequences were aligned using the ClustalW algorithm implemented in BioEdit v7.2.5 with the default gap-opening and gap-extension parameters. The resulting alignment was inspected and edited manually, and ambiguously aligned terminal regions were trimmed before analysis. The final dataset comprised 76 taxa and 691 aligned nucleotide positions, including gaps. The newly generated ITS sequences were deposited in GenBank under the accession numbers listed in Table 1.

Bayesian phylogenetic inference was performed in MrBayes v3.2.7 [15] under the GTR+I+G nucleotide substitution model. Two independent runs, each consisting of four Markov chains, were conducted for 5,000,000

generations. Trees and model parameters were sampled every 1,000 generations, resulting in 5,000 sampled trees per run. The first 25% of sampled trees were discarded as burn-in, and the remaining trees were used to construct a 50% majority-rule consensus tree and estimate Bayesian posterior probabilities. Convergence was assessed using the average standard deviation of split frequencies and potential scale reduction factors. At the end of the analysis, the average standard deviation of split frequencies was 0.004749, and the mean and maximum PSRF values were 1.000 and 1.006, respectively. *Nothocybe vanduzgarae* was used as the outgroup.

2.3. Verification of new national records

The status of the identified taxa as new records for Türkiye was evaluated by reviewing relevant Turkish macrofungal literature, national checklists, Index Fungorum, MycoBank, and the Global Biodiversity Information Facility. Searches were conducted using accepted names and known synonyms and were last updated on 20 September 2026. No previously published records from Türkiye were found for *Inocybe amygdaliolens*, *I. neorufula*, *I. pinophila*, or *I. subnudipes*. Therefore, based on the literature and databases available at the time of revision, the present collections represent the first documented records of these four taxa from Türkiye. The newly generated sequences and the reference sequences used in the phylogenetic analysis, together with their GenBank accession numbers and geographic origins, are listed in Table 1.

Table 1. Taxa, nrITS GenBank accession numbers, and geographic origins of the sequences included in the phylogenetic analysis

Species	nrITS	Locality
ERCH YM0.010 *Inocybe geophylla*	QB001425	Yamanlar Mountain, Türkiye
ERCH YM0.013 *Inocybe geophylla*	QB001426	Yamanlar Mountain, Türkiye
ERCH YM0.012 *Inocybe geophylla*	QB001427	Yamanlar Mountain, Türkiye
ERCH YM0.028 *Inocybe geophylla*	QB001428	Yamanlar Mountain, Türkiye
ERCH YM0.019 *Inocybe neorufula*	QB001429	Yamanlar Mountain, Türkiye
ERCH YM0.022 *Inocybe neorufula*	QB001430	Yamanlar Mountain, Türkiye
ERCH YM0.023 *Inocybe subnudipes*	QB001431	Yamanlar Mountain, Türkiye
ERCH YM0.030 *Inocybe tenuicystidiata*	QB001432	Yamanlar Mountain, Türkiye
ERCH YM0.027 *Inocybe amygdaliolens*	QB001433	Yamanlar Mountain, Türkiye
ERCH YM0.006 *Inocybe pinophila*	QB001434	Yamanlar Mountain, Türkiye
ERCH YM0.025 *Inocybe nitidiuscula*	QB001435	Yamanlar Mountain, Türkiye
Inocybe geophylla	MH930209	Russia
Inocybe geophylla	PQ653124	Sweden
Inocybe geophylla	PQ652427	Sweden
Inocybe geophylla	MH930208	Russia
Inocybe geophylla	PQ653132	Sweden
Inocybe geophylla	MK409369	Russia
Inocybe geophylla	PQ653149	Sweden
Inocybe geophylla	PQ653044	Sweden
Inocybe lilacina	HQ604297	
Inocybe lilacina	HQ604096	
Inocybe neorufula	MT101876	Italy
Inocybe neorufula	NR185415	Spain
Inocybe neorufula	MT101890	Spain
Inocybe derbschii	MT006043	Germany
Inocybe derbschii	PP434663	France
Inocybe derbschii	MG012483	France
Inocybe derbschii	NR175071	Germany
Inocybe ianthinopes	OK057202	Germany
Inocybe ianthinopes	NR176185	Spain
Inocybe ianthinopes	OQ133575	United Kingdom
Inocybe ianthinopes	OL352086	Spain
Inocybe grammopodia	OR896144	United Kingdom
Inocybe grammopodia	PX572375	USA
Inocybe caesaraugustae	NR176184	Spain
Inocybe caesaraugustae	OL352083	Spain

Table 1. Cotinued

Inocybe subnudipes	MH732915	Germany
Inocybe subnudipes	OQ133569	United Kingdom
Inocybe subnudipes	PQ652928	Sweden
Inocybe subnudipes	OP164034	Finland
Inocybe subnudipes	PV388720	Sweden
Inocybe subnudipes	PQ639002	Sweden
Inocybe subnudipes	AM882809	Sweden
Inocybe tenuicystidiata	PZ008001	Italy
Inocybe tenuicystidiata	PZ008013	Italy
Inocybe tenuicystidiata	PZ205251	Czechia
Inocybe hamadryadis	OP480839	Spain
Inocybe hamadryadis	OP480840	Spain
Inocybe hamadryadis	NR198291	Spain
Inocybe amygdaliolens	PQ099327	Spain
Inocybe amygdaliolens	PV426994	Spain
Inocybe amygdaliolens	OQ379324	Italy
Inocybe mycenoides	OK057204	Germany
Inocybe mycenoides	OK057151	Germany
Inocybe somae	NR176166	Germany
Inocybe somae	OK057148	Germany
Inocybe pinophila	PZ016690	France
Inocybe pinophila	PV121695	France
Inocybe pinophila	PZ008097	Italy
Inocybe pinophila	PP065588	Italy
Inocybe pinophila	PP065586	Italy
Inocybe pinophila	NR191339	Italy
Inocybe pinophila	PZ318315	France
Inocybe alberichiana	MW845855	Austuria
Inocybe alberichiana	NR174852	Austuria
Inocybe alberichiana	MW845943	Finland
Inocybe alberichiana	MW845856	Germany
Inocybe laurina	PX960876	Italy
Inocybe laurina	MW845947	Germany
Inocybe laurina	MW845948	Germany
Inocybe nitidiuscula	PV444360	Spain
Inocybe nitidiuscula	KR019809	Latvia
Inocybe nitidiuscula	HQ604088	Ceska
Inocybe nitiduscula	OR945024	USA
Inocybe nitidiuscula	ON322998	France

Morphological identification key to the examined species

1a. Pileus pale grey to greyish-lilac; stipe whitish to pale lilac-grey ..... *I. geophylla*
1b. Pileus cream, ochraceous, yellowish-brown, amber-brown, or chestnut-brown 2
2a. Odour distinctly bitter-almond; pileus pale cream to ochraceous-buff ..... *I. amygdaliolens*
2b. Odour not almond-like or not recorded 3
3a. Basidiospores 9–10 × 5–6 µm; pileus densely fibrillose to finely squamulose, margin often fissured ..... *I. pinophila*
3b. Basidiospores generally longer than 10 µm or broader than 6 µm 4
4a. Pileus amber- to reddish-brown; basidiospores 9–12 × 6–8 µm ..... *I. neorufula*
4b. Pileus without prominent reddish or amber tones 5
5a. Basidiospores (9–)10–11 × 5–6 µm, with a distinct apical germ pore; basidiomata slender ..... *I. tenuicystidiata*
5b. Basidiospores broader, generally 6–8 µm wide; apical germ pore not conspicuous 6
6a. Basidiospores (10–)11–13 × 6–8 µm; pileus ochraceous-brown to dark brown ..... *I. nitidiuscula*
6b. Basidiospores 10–11 × 6–6.5 µm; pileus yellowish-brown to chestnut-brown ..... *I. subnudipes*

3. Results

Morphological examinations and ITS-based molecular analyses of the collected specimens revealed the presence of seven species belonging to the genus Inocybe. The identified taxa were *Inocybe amygdaliolens* Esteve-Rav., Pancorbo & Dovana, *I. geophylla* (Bull.) P. Kumm., *I. neorufula* Esteve-Rav., Macau & Ferville, *I. nitidiuscula* (Britzelm.) Lapl., *I. subnudipes* Kühner, *I. pinophila* Cervini, Cagnoli & Bizio, and *I. tenuicystidiata* E. Horak & Stangl. Morphological observations were consistent with the diagnostic characteristics reported in the literature, and species identities were further confirmed by phylogenetic analyses based on ITS sequence data.

In the Bayesian ITS phylogeny, the examined specimens were placed within or together with sequences identified as their corresponding species, with varying levels of posterior probability support. These placements were generally consistent with the morphology-based identifications. Based on the literature and database searches conducted for this study, *Inocybe amygdaliolens*, *I. neorufula*, *I. pinophila*, and *I. subnudipes* represent new national records for Türkiye. The results demonstrate the importance of integrating morphological and molecular data when evaluating species boundaries within Inocybe.

All the taxa described in the present study are members of genus *Inocybe* (Fr.). These are a diverse group of ectomycorrhizal fungi currently in the family Inocybaceae Jülich, order Agaricales Underw., class Agaricomycetes Doweld, phylum Basidiomycota R.T. Moore and kingdom Fungi. These are found in temperate forest ecosystems, and are frequently associated with a number of woody plant host species, especially those from the Pinaceae and Fagaceae. Seven species of Inocybe were found in *Pinus brutia* Ten. forests in Karagöl Nature Park, western Turkey, Yamanlar Mountain, İzmir, based on detailed morphological examinations and ITS rDNA sequence analyses. The list of taxa identified is below in alphabetical order.

3.1. Taxonomic treatments, ecology, and molecular assessment of the identified species

3.1.1. *Inocybe amygdaliolens* Esteve-Rav., Pancorbo & Dovana

Description:

Basidiomata small-sized, occurring solitary or scattered among pine litter. Pileus 10–30 mm in diameter, initially conical to campanulate-conical, later becoming convex with a low and broad umbo at maturity. Surface dry, finely fibrillose to silky-fibrillose, pale cream to ochraceous-buff, slightly darker towards the disc. Margin initially incurved, becoming straight with age and occasionally slightly split in mature basidiomata. Pileus surface remaining smooth to finely fibrillose throughout development. Lamellae moderately crowded, adnexed to narrowly adnate, initially pale cream, becoming pale cinnamon-brown to ochraceous-brown at maturity. Lamellulae numerous. Lamellar edge concolorous or slightly paler than the lamellar faces. Stipe 20–50 × 2–5 mm, cylindrical to slightly broadened towards the base, cream-white to pale buff, lacking an annulus. Surface finely fibrillose, slightly pruinose near the apex and smoother towards the lower portion. Basal mycelium white. Context thin, whitish to cream-coloured, without significant colour changes when bruised. Odour distinctly almond-like to bitter-almond, strong and persistent. Taste mild (Figure 1).

Basidiospores smooth, yellowish-brown to brown in transmitted light, ellipsoid to subamygdaliform, measuring 10–11 × 6–6.5 µm (mean = 10.7 × 6.3 µm; Q = 1.69). Spore walls smooth, moderately thickened, and lacking a germ pore.

The examined specimen was collected among pine needles, mosses and leaf litter in coniferous woodland in Yamanlar Mountain (İzmir, western Türkiye). The small basidiomata, pale ochraceous-buff pileus, smooth ellipsoid basidiospores, and especially the characteristic bitter-almond odour correspond well with the diagnostic features reported for *Inocybe amygdaliolens* [17].

Specimen examined: Türkiye, İzmir, Karşıyaka, Yamanlar Mountain, Karagöl Nature Park, northern lakeside trail, 38° 33' 52" N, 27° 13' 18" E, alt. 820 m, 12.11.2025, leg. G. Gülhan and M. Güllü (YM27), deposited in ERCH, voucher no. [ERCH YM 0.027].

Taxonomic and Phylogenetic Remarks:

Morphologically, the Turkish collection agrees well with *Inocybe amygdaliolens* in possessing small basidiomata, a pale ochraceous-buff fibrillose pileus, a slender cream-coloured stipe, smooth ellipsoid to subamygdaliform basidiospores, and a characteristic bitter-almond odour. The observed basidiospores (10–11 × 6–6.5 µm, mean = 10.7 × 6.3 µm) also fall within the range reported for the species in the original description and subsequent taxonomic treatments [17]. These morphological characters, together with its occurrence in coniferous woodland, correspond closely to the current concept of *I. amygdaliolens*.

Among morphologically related taxa, *Inocybe fraudans* (Britzelm.) Sacc. is considered the most similar species. Both taxa share a distinctive almond-like odour and smooth basidiospores. But, *I. fraudans* tends to have larger and more robust basidiomata, is darker brown on the pileus, and has larger basidiospores than those seen in this

material. The Turkish specimens are smaller, with lighter pileus colour and basidiospores of 10–11 × 6–6.5 µm, which are similar to the diagnostic characters of *I. amygdaliolens* [17].

ITS sequence-based phylogenetic analyses confirmed that specimen YM27 was part of the *Inocybe amygdaliolens* clade. The Turkish material grouped with the authenticated sequences of *I. amygdaliolens* from GenBank (OQ379324.1, PO099327.1 and PV426994.1) confirming its membership in this species. The topology recovered *I. mycenoides* as the closest related taxon to the lineage of *I. amygdaliolens* and *I. somae* as a more distant position (Figure 8).

Inocybe fraudans is considered one of the most morphologically similar species to *I. amygdaliolens*, but the ITS phylogeny failed to show any close evolutionary relationship between *I. fraudans* and *I. amygdaliolens*. *I. fraudans* was not recovered in the present analysis and well isolated from the *I. amygdaliolens* clade. This conclusion implies that the common almond aroma and some similarities in the macromorphology are either convergent or ancestral traits, and are not necessarily indicative of close phylogenetic relationship.

In addition *I. mycenoides*, sister lineage to *I. amygdaliolens* in the ITS tree, is morphologically distinct from the latter, lacking the bitter almond smell, and also showing some differences in the color of the pileus and in the micromorphology of it. The molecular data is very useful, and the morphology strongly supports this identification, so YM27 is considered to be *Inocybe amygdaliolens*. Hence, combining morphological and phylogenetic data, the identification of the Turkish collection as *Inocybe amygdaliolens*, a new record of mycobiota of Türkiye is supported.

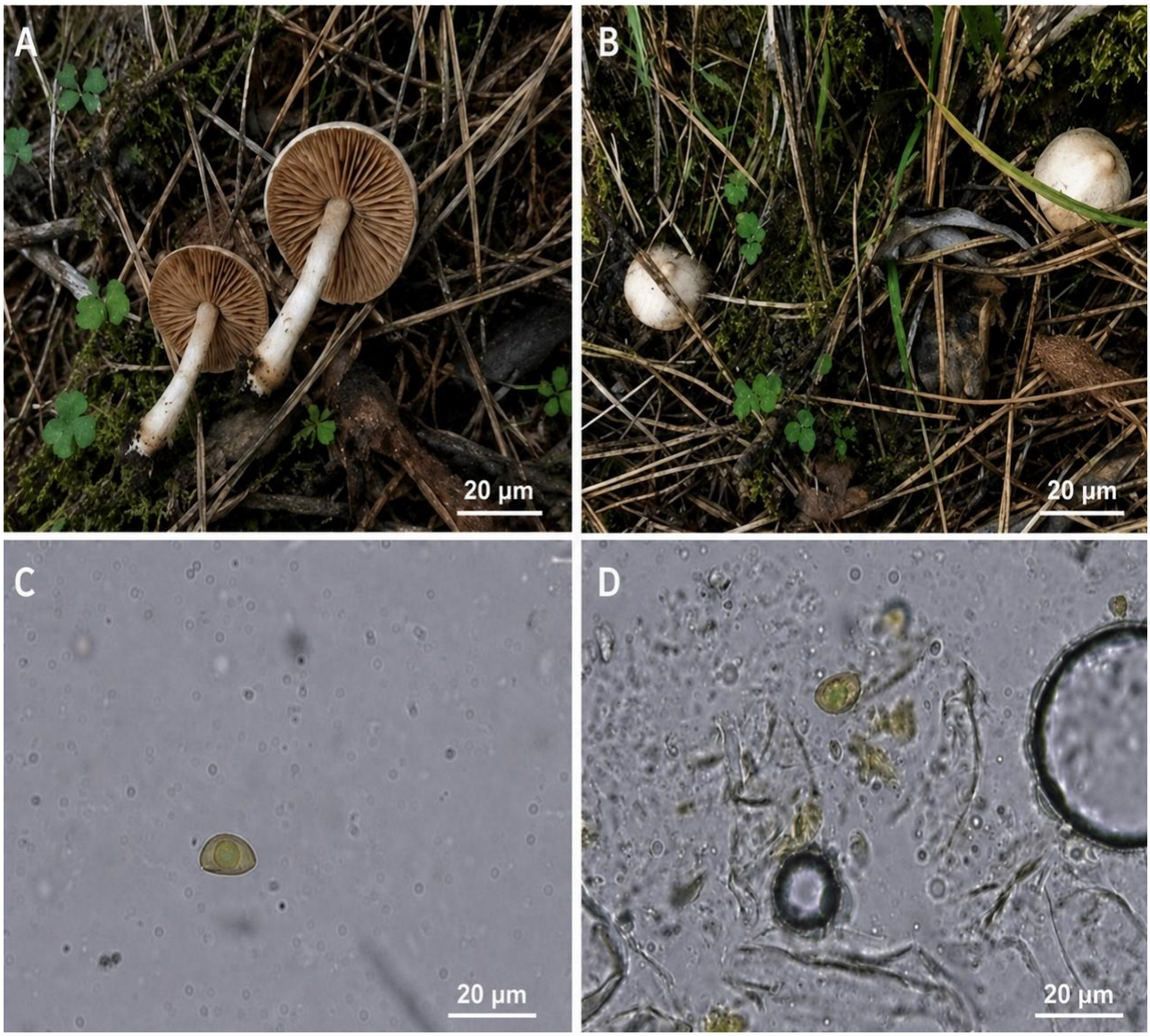

Figure 1. *Inocybe amygdaliolens* (YM27). A–B. Basidiomata in the natural habitat. C–D. Basidiospores. Scale bars: A–D = 20 µm

3.1.2. *Inocybe geophylla* (Bull.) P. Kumm.

Description:

Basidiomata small and fragile, occurring solitary or in small groups. Pileus 8–20 mm in diameter, initially conical to campanulate, later convex to plano-convex, occasionally retaining a low umbo. Surface dry, finely fibrillose, pale grey to greyish-lilac, becoming paler towards the margin. Margin initially incurved, later straight and occasionally faintly striate. Lamellae moderately crowded, adnexed to narrowly adnate, initially pale greyish, becoming greyish-brown to tobacco-brown at maturity. Lamellulae numerous. Lamellar edge concolorous or slightly paler. Stipe 15–40 × 1–3 mm, slender, cylindrical, whitish to pale lilac-grey, finely fibrillose, entirely pruinose when young, especially near the apex. Context thin, whitish to pale grey. Odour not recorded in the examined material (Figure 2).

Basidiospores smooth, yellowish-brown, ellipsoid to subamygdaliform, measuring (9–)10–12 × 6–8 µm (mean = 10.4 × 6.7 µm), with moderately thickened walls, lacking nodulose ornamentation and a conspicuous germ pore. Spore walls moderately thickened and lacking nodulose ornamentation. Basidia predominantly four-spored. Cheilocystidia and pleurocystidia abundant, lageniform to fusiform, thin- to moderately thick-walled. Pileipellis composed of repent hyphae forming a cutis.

The examined specimens were collected among pine litter under *Pinus brutia* Ten. in Karagöl Nature Park, Yamanlar Mountain (İzmir, western Türkiye). The pale greyish-lilac pileus, slender habit, and smooth ellipsoid basidiospores observed in the Turkish material correspond well with the diagnostic features reported for *Inocybe geophylla* [18, 2].

Specimen examined: Türkiye, İzmir, Karşıyaka, Yamanlar Mountain, Karagöl Nature Park, eastern *Pinus brutia* forest, 38° 33' 46" N, 27° 13' 42" E, alt. 845 m, 12.11.2025, leg. G. Gülhan and M. Güllü (YM10, YM12, YM13 and YM28), deposited in ERCH, voucher no. [ERCH YM 0.010, YM 0.012, YM 0.013 and YM 0.028].

Taxonomic and Phylogenetic Remarks:

Morphologically, the Turkish collections agree well with *Inocybe geophylla* in possessing small and fragile basidiomata, a pale grey to greyish-lilac fibrillose pileus, a slender whitish stipe, and smooth ellipsoid to subamygdaliform basidiospores. The observed basidiospores [(9–)10–12 × 6–8 µm, mean = 10.4 × 6.7 µm] are also consistent with the dimensions reported for *I. geophylla* in previous taxonomic studies [18,2]. These morphological features, together with the ecological association with *Pinus brutia*, correspond closely to the current concept of *I. geophylla*.

Among morphologically related taxa, *Inocybe lilacina* is considered one of the most similar species. Both taxa share lilac-grey colouration, smooth basidiospores, and a generally delicate habit. *I. lilacina*, however, is more heavily pigmented in the violet to lilac range, especially on the pileus and stipe (particularly in young basidiomata) and does tend to have slightly wider cystidial elements [18,2]. The Turkish specimens are, on the other hand, greyish-lilac in colour and agree more closely with the diagnostic features of *I. geophylla*.

The ITS sequences were used in Bayesian phylogenetic analysis that confirmed that specimens YM28, YM10, YM12 and YM13 belonged to the *Inocybe geophylla* clade. The Turkish sequences grouped with several other sequences of *I. geophylla* from GenBank, which confirmed their membership in this species. This topology recovered *I. lilacina* as the most closely related species of the *I. geophylla* lineage, with *I. lilacina* sister to the *I. geophylla* lineage, supported by high posterior probability (PP = 0.99) (Figure 8).

I. lilacina and *I. geophylla* are similar in several morphological features but the phylogeny clearly suggests that they belong to different lineages. This result was corroborated by the molecular data obtained from the Turkish collections, which were nested in the *I. geophylla* clade and not in the *I. lilacina* clade. Consequently, both morphological and phylogenetic data, based on the ITS, strongly support the identification of the specimens YM28, YM10, YM12 and YM13 as *Inocybe geophylla*.

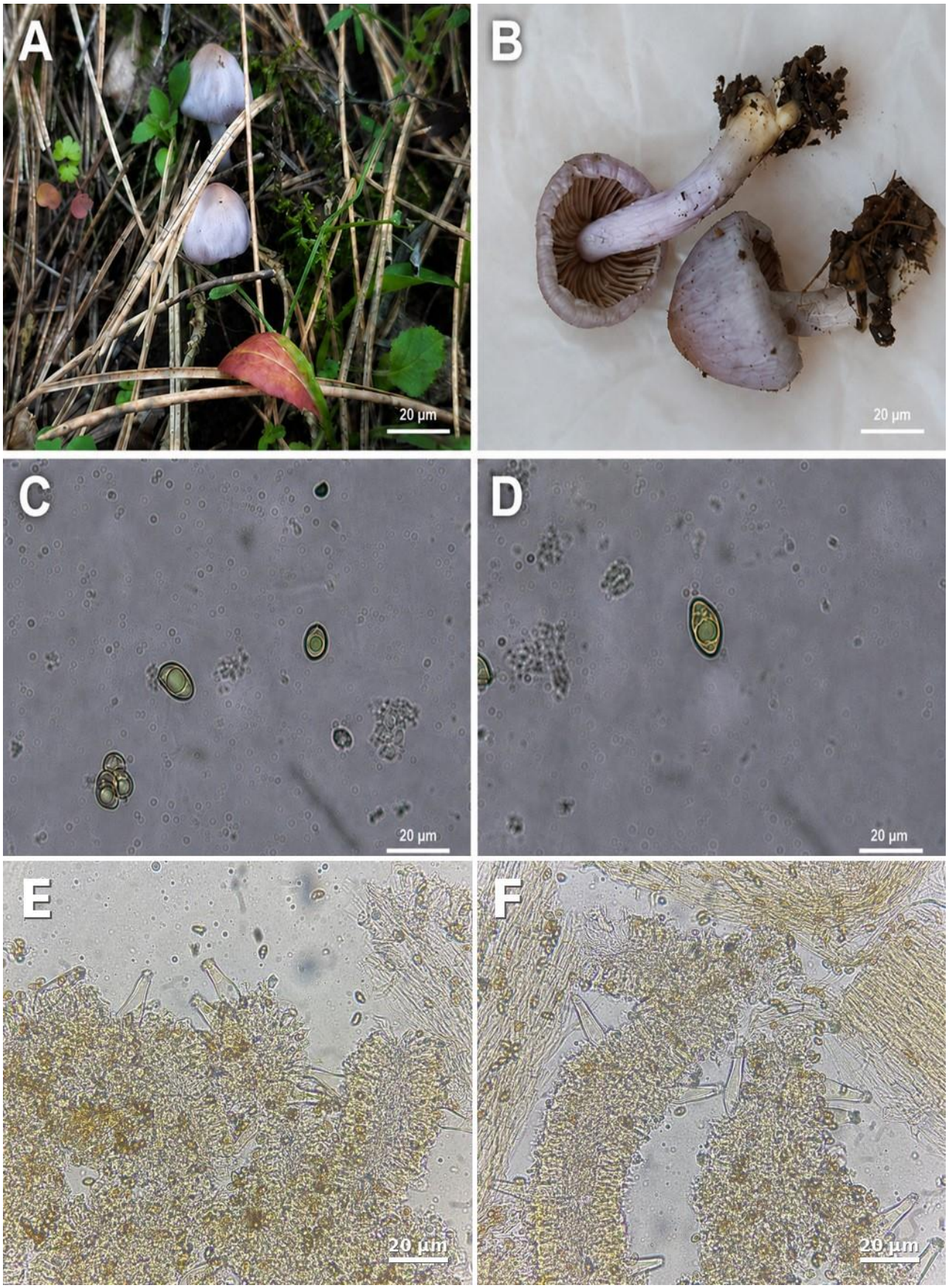

Figure 2. *Inocybe geophylla* (YM28). A–B. Basidiomata in the natural habitat. C–D. Basidiospores. E–F. Cheilocystidia. Scale bars: A–D = 20 µm.

3.1.3. *Inocybe neorufula* Esteve-Rav., Macau & Ferville

Description:

Basidiomata small-sized, occurring solitary or in small groups. Pileus 15–25 mm in diameter, initially conical to broadly conical, later convex with a slightly prominent umbo. Surface dry, finely fibrillose and silky, dark chestnut-brown to reddish-brown or amber-brown at the centre, becoming paler yellowish-brown towards the margin. Margin often developing radial fissures with age. Overall coloration composed of warm amber, fawn and reddish-brown tones. Lamellae crowded, moderately broad, adnexed to narrowly adnate, initially pale cream, becoming pale ochraceous to yellowish-brown at maturity. Lamellulae numerous. Lamellar edge paler than the lamellar faces and appearing sterile.

Stipe 20–40 × 2–4 mm, cylindrical to slightly clavate, cream-white to pale yellowish, lacking an annulus. Surface slightly pruinose near the apex and finely fibrillose towards the base. Upper portions occasionally exhibiting faint pinkish-yellow shades. Context thin, cream-white, without noticeable colour changes when bruised. Odour weakly spermatic to faintly fungoid (Figure 3) [19].

Basidiospores smooth, pale yellowish-brown, broadly ellipsoid to ellipsoid-subamygdaliform, occasionally slightly phaseoliform, measuring 9–12 × 6–8 µm (mean = 10.3 × 6.7 µm; Q = 1.54), with moderately thickened walls, lacking nodulose ornamentation and a conspicuous germ pore. Measured spores were 11 × 7 µm, 10 × 6 µm, 9 × 6 µm, 9 × 7 µm, 11 × 6 µm, and 12 × 8 µm [19].

The examined specimens were collected among pine litter under *Pinus brutia* Ten. in Karagöl Nature Park, Yamanlar Mountain (İzmir, western Türkiye). The warm amber-brown pileus, slender habit, and smooth broadly ellipsoid basidiospores observed in the Turkish material correspond well with the diagnostic features reported for *Inocybe neorufula* [19].

Specimen examined: Türkiye, İzmir, Karşıyaka, Yamanlar Mountain, Karagöl Nature Park, southeastern forest margin, 38° 33' 35" N, 27° 13' 30" E, alt. 805 m, 13.11.2025, leg. G. Gülhan and M. Güllü (YM19 and YM22), deposited in ERCH, voucher no. [ERCH YM 0.019 and YM 0.022].

Taxonomic and Phylogenetic Remarks:

Morphologically, the Turkish collections agree well with *Inocybe neorufula* in possessing small basidiomata, a finely fibrillose amber- to reddish-brown pileus, a slender pale stipe, and smooth broadly ellipsoid to subamygdaliform basidiospores. The observed basidiospores (9–12 × 6–8 µm, mean = 10.3 × 6.7 µm) are also consistent with the dimensions reported for the type material of *I. neorufula* [19]. These morphological features, together with the ecological association with *Pinus brutia*, correspond closely to the current concept of *I. neorufula*.

Among morphologically related taxa, Inocybe roseipes and I. tristis are considered the most similar species. All three taxa share smooth basidiospores, warm brown pileus colours, and a comparable habit. However, I. roseipes has smaller basidiospores (average 9.7 × 6.0 µm). Inocybe tristis differs in having a darker brown pileus, a yellowish-white stipe apex, and generally smaller basidiospores (10.5 × 6.2 µm) [19]. In contrast, the Turkish collections possess the warm amber-brown pileus colour and comparatively larger basidiospores characteristic of I. neorufula.

Bayesian phylogenetic analysis of the ITS sequences placed specimens YM19 and YM22 with reference sequences identified as *Inocybe neorufula* (NR185415.1, MT101890.1, and MT101876.1), supporting their assignment to this species. In the inferred topology, *I. derbschii* was recovered as the closest sister lineage to the *I. neorufula* clade, with moderate Bayesian posterior probability support (PP = 0.77) (Figure 8).

Although *I. derbschii* and *I. neorufula* share several morphological characteristics, the phylogenetic reconstruction clearly separates them into distinct lineages. The Turkish collections differ from *I. derbschii* by possessing larger basidiospores, warmer amber-brown pileus colours and a Mediterranean-type habitat preference. Likewise, *I. ianthinopes*, which occupies a more distant position in the phylogeny, differs in possessing violet tinges on the stipe and generally smaller basidiospores [18,20]. The Turkish collections were nested within the *I. neorufula* clade rather than either of these related taxa, providing molecular evidence that corroborates the observed morphological differences.

Therefore, the morphological characters and ITS-based Bayesian placement support the identification of specimens YM19 and YM22 as *Inocybe neorufula*. The examined collections match the diagnostic features of the species as circumscribed by Esteve-Raventós et al. (2011) and are nested with reference sequences identified as *I. neorufula*. Based on the literature and database searches conducted for this study, *I. neorufula* is reported here for the first time from Türkiye.

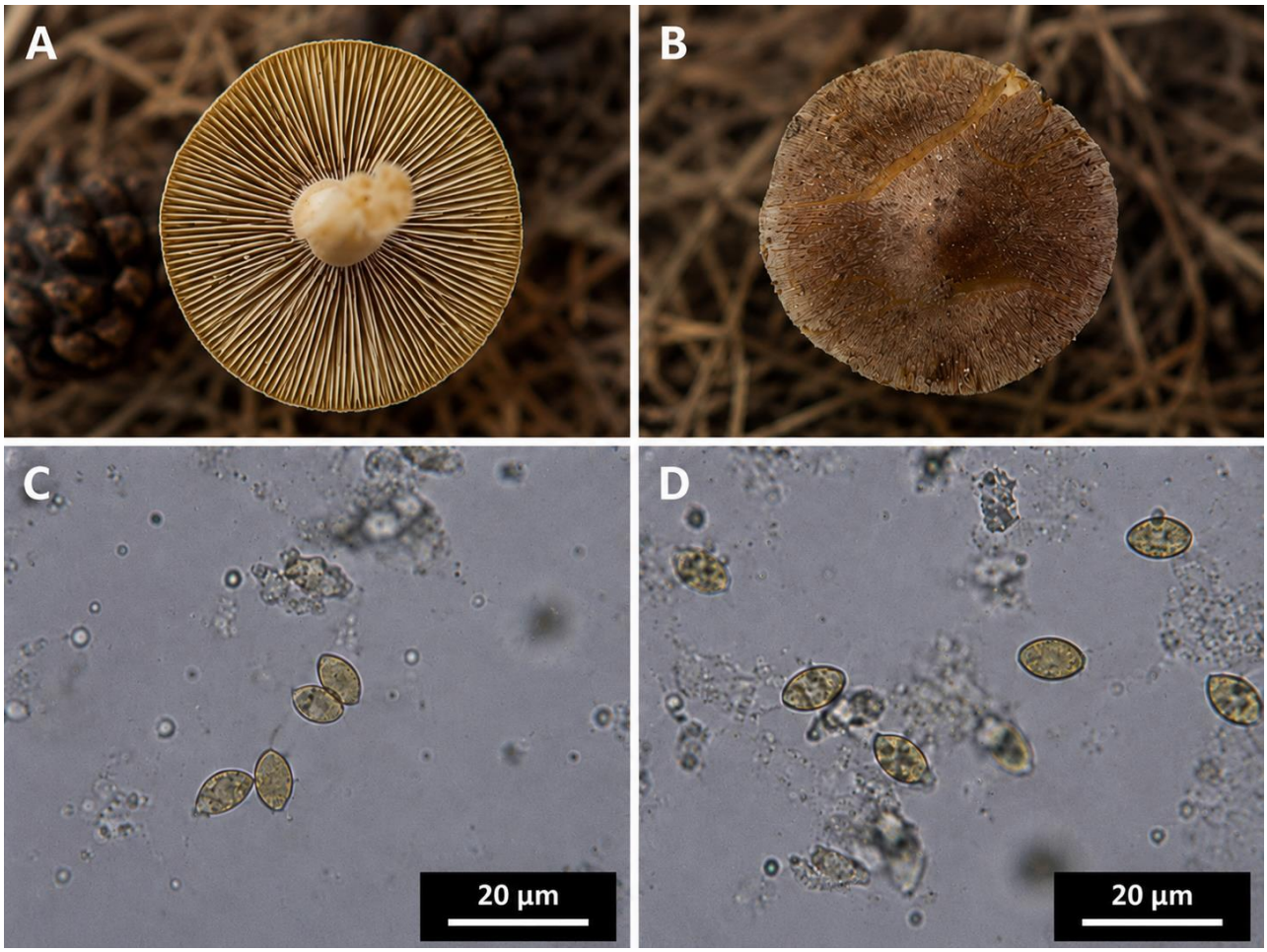

Figure 3. *Inocybe neorufula* (YM19 and YM22). A–B. Basidiomata in the natural habitat. C–D. Basidiospores. Scale bars: A–D = 20 µm.

3.1.4. *Inocybe nitidiuscula* (Britzelm.) Lapl.

Description:

Basidiomata small and fragile, occurring solitary on soil among pine litter. Pileus 8–20 mm in diameter, initially conical to convex, later becoming plano-convex with a low umbo. Surface dry, finely fibrillose, ochraceous-brown to dark brown, distinctly darker at the centre and paler towards the margin. Margin initially incurved, becoming straight at maturity and occasionally slightly fissured. Lamellae moderately crowded, adnexed to narrowly adnate, initially pale beige, becoming clay-brown to tobacco-brown with age. Lamellulae numerous. Lamellar edge concolorous. Stipe 15–40 × 1–3 mm, cylindrical, slender, pale ochraceous to light brown, longitudinally fibrillose, with a pruinose apex. Context thin and pale cream. Odour not recorded in the examined material (Figure 4).

Basidiospores smooth, yellowish-brown, broadly ellipsoid to subamygdaliform, measuring (10–)11–13 × 6–8 µm (mean = 11.2 × 7.5 µm), with moderately thickened walls, lacking nodulose ornamentation and a distinct germ pore. The observed spore morphology is consistent with that reported for *Inocybe nitidiuscula* in European taxonomic treatments (Figure 4) [2,18].

The examined specimen was collected under *Pinus brutia* Ten. in Karagöl Nature Park, Yamanlar Mountain (İzmir, western Türkiye). The small basidiomata, brown fibrillose pileus, and overall habit correspond well with the diagnostic features reported for *Inocybe nitidiuscula* in the European literature [2,18].

Specimen examined: Türkiye, İzmir, Karşıyaka, Yamanlar Mountain, Karagöl Nature Park, western slope above crater lake, 38° 33' 58" N, 27° 12' 58" E, alt. 860 m, 13.11.2025, leg. G. Gülhan and M. Güllü (YM25), deposited in ERCH, voucher no. [ERCH YM 0.025].

Taxonomic and Phylogenetic Remarks:

Morphologically, the Turkish collection agrees well with *Inocybe nitidiuscula* in possessing small basidiomata, a brown fibrillose pileus, a slender stipe, and smooth ellipsoid to subamygdaliform basidiospores. The observed basidiospores [(10–)11–13 × 6–8 µm, mean = 11.2 × 7.5 µm] are consistent with the dimensions reported for *I. nitidiuscula* in European taxonomic treatments [2,18]. Furthermore, the overall habit and ecological association with pine forests correspond closely to the current concept of the species.

Among morphologically related taxa, *Inocybe laurina* is considered one of the closest species. Both taxa share brown fibrillose basidiomata and smooth basidiospores. However, *I. laurina* is generally associated with broadleaved hosts, particularly species of *Betula* and *Alnus*, and typically exhibits a more robust habit and somewhat narrower spores. In contrast, the Turkish material was collected under *Pinus brutia* and possesses broader basidiospores, corresponding well with the diagnostic features of *I. nitidiuscula* [2,18].

Another similar species is *Inocybe alberichiana*, which may resemble *I. nitidiuscula* in pileus colour and general habit. Nevertheless, differences in cystidial morphology and molecular characteristics allow these taxa to be distinguished reliably [2].

Bayesian phylogenetic analysis based on ITS sequences confirmed the placement of specimen YM25 within the *Inocybe nitidiuscula* clade. The Turkish collection clustered together with reference sequences of *I. nitidiuscula* (PV444360.1, KR019809.1, HQ604088.1, OR945024.1, and ON322998.1) with strong posterior probability support (PP = 0.98), providing robust molecular evidence for its identification (Figure 8).

The *I. nitidiuscula* clade was recovered as a distinct lineage clearly separated from other closely related taxa. In the resulting topology, species of *I. laurina* and *I. alberichiana* formed the sister lineage to *I. nitidiuscula* (PP = 0.99). Despite their phylogenetic proximity, the Turkish specimen remained nested within the well-supported *I. nitidiuscula* clade and showed no affinity with either *I. laurina* or *I. alberichiana*. Therefore, both morphological observations and ITS-based phylogenetic evidence strongly support the identification of specimen YM25 as *Inocybe nitidiuscula*.

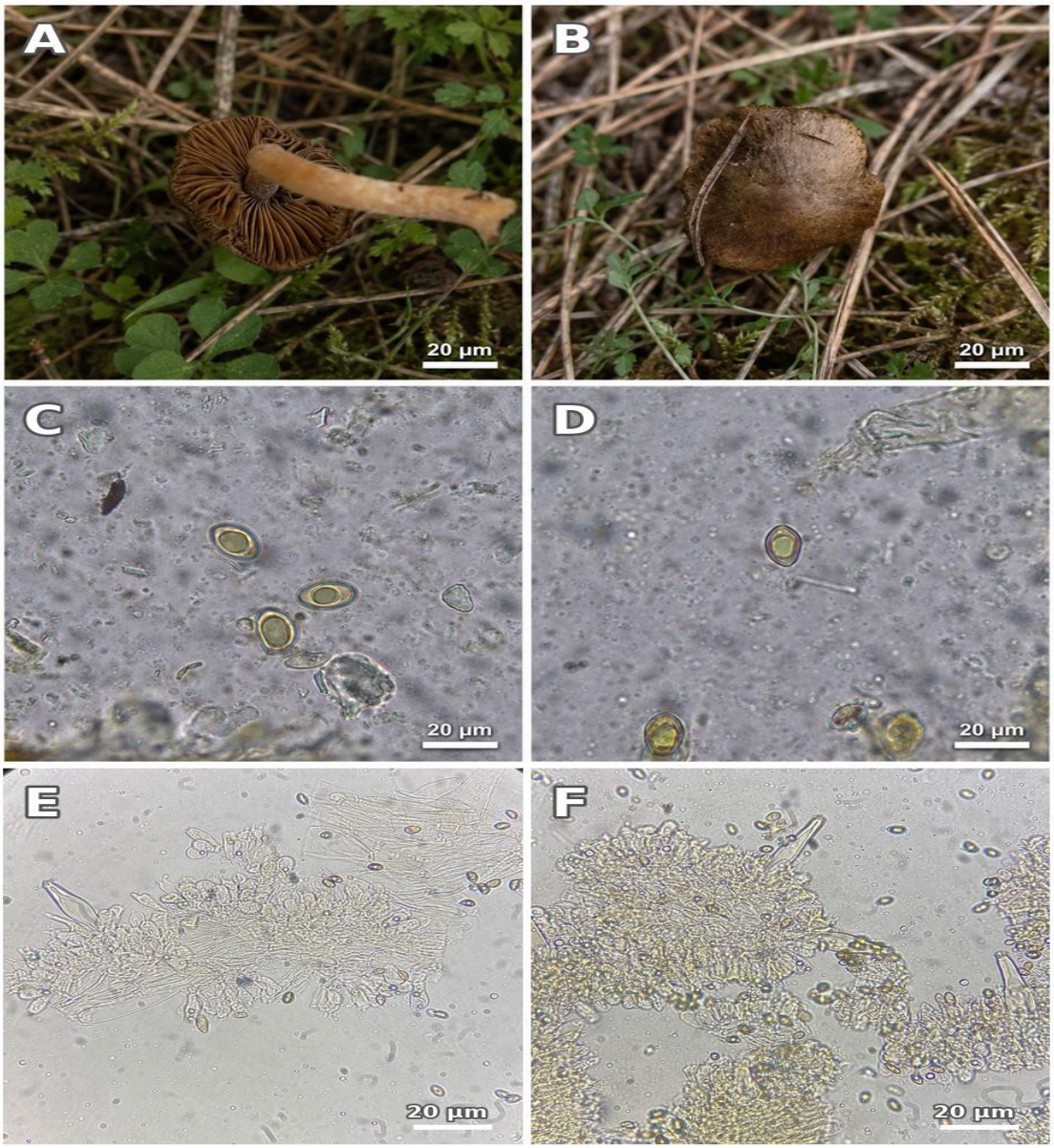

Figure 4. *Inocybe nitidiuscula* (YM25). A–B. Basidiomata in the natural habitat. C–D. Basidiospores. E–F. Cheilocystidia. Scale bars: A–D = 20 µm.

3.1.5. *Inocybe pinophila* Cervini, Cagnoli & Bizio

Description:

Basidiomata small to medium-sized, occurring solitary to gregarious. Pileus 15–35 mm in diameter, initially conical-campanulate with a distinct umbo, becoming convex to plano-convex at maturity while usually retaining a low central papilla. Surface dry, densely fibrillose to finely squamulose, ochraceous-brown to amber-brown, with a distinctly darker chestnut-brown disc. Margin irregular and often radially fissured at maturity. The pileus exhibits a characteristic silky-fibrillose appearance, particularly evident in mature specimens. Lamellae moderately crowded, adnexed to narrowly adnate, initially pale ochraceous, becoming cinnamon-brown to tobacco-brown at maturity. Lamellulae numerous. Lamellar edge concolorous or slightly paler than the lamellar faces. Stipe slender, cylindrical, whitish to pale cream, lacking an annulus, slightly fibrillose towards the base and finely pruinose near the apex. Context thin, pale cream, without noticeable colour changes when bruised. Odour not recorded in the examined material (Figure 5).

Basidiospores smooth, pale yellowish-brown in transmitted light, ellipsoid to subamygdaliform, measuring 9–10 × 5–6 µm (mean = 9.5 × 5.6 µm; Q = 1.69). Spore walls thin and smooth, lacking nodulose ornamentation and a conspicuous germ pore.

The examined specimen was collected on soil among pine needles, mosses and herbaceous vegetation in pine-dominated woodland in Yamanlar Mountain (İzmir, western Türkiye). The densely fibrillose ochraceous- to amber-brown pileus with a darker central disc, distinctly fissured pileus margin, and smooth ellipsoid basidiospores correspond well with the diagnostic features reported for *Inocybe pinophila* [21].

Specimen examined: Türkiye, İzmir, Karşıyaka, Yamanlar Mountain, Karagöl Nature Park, southwestern *Pinus brutia* stand, 38° 33' 29" N, 27° 13' 05" E, alt. 790 m, 14.11.2025, leg. G. Gülhan and M. Güllü (YM6), deposited in ERCH, voucher no. [ERCH YM 0.006].

Taxonomic and Phylogenetic Remarks:

Morphologically, the Turkish collection agrees well with *Inocybe pinophila* in possessing small to medium-sized basidiomata, a densely fibrillose ochraceous- to amber-brown pileus with a darker central disc, a distinctly fissured pileus margin, cinnamon-brown lamellae, and smooth ellipsoid to subamygdaliform basidiospores. The observed basidiospores (9–10 × 5–6 µm, mean = 9.5 × 5.6 µm) are also consistent with the dimensions reported for *I. pinophila* in the original description of the species [21]. These morphological features, together with the ecological association with pine-dominated habitats, correspond closely to the current concept of *I. pinophila*.

Among morphologically related taxa, *Inocybe derbschii* is considered one of the most similar species. Both taxa share a generally similar habit, a brown fibrillose pileus, and smooth basidiospores. However, *I. derbschii* typically possesses a darker brown pileus and relatively larger basidiospores. In contrast, the Turkish collection displays a paler amber-brown pileus and smaller basidiospores (mean = 9.5 × 5.6 µm), conforming more closely to the diagnostic characters of *I. pinophila*.

Phylogenetic analysis based on ITS sequences confirmed the placement of specimen YM6 within the *Inocybe pinophila* clade. The Turkish collection clustered together with all available reference sequences of *I. pinophila* retrieved from GenBank, supporting its identification as a member of this species. The resulting topology recovered *Inocybe alberichiana* and *I. laurina* as the closest related taxa to the *I. pinophila* lineage, forming sister clades with strong support (Figure 8).

Although *Inocybe derbschii* shares several morphological characteristics with *I. pinophila*, the phylogenetic reconstruction did not support a close relationship between these taxa. Instead, *I. derbschii* occupied a more distant position and was clearly separated from the *I. pinophila* clade. Therefore, the observed morphological similarities appear to reflect overall phenotypic resemblance rather than close evolutionary affinity.

The Turkish collection was nested within a well-supported *I. pinophila* clade, providing molecular evidence that corroborates the observed morphological characters. Therefore, both morphological and ITS-based phylogenetic data strongly support the identification of specimen YM6 as *Inocybe pinophila*.

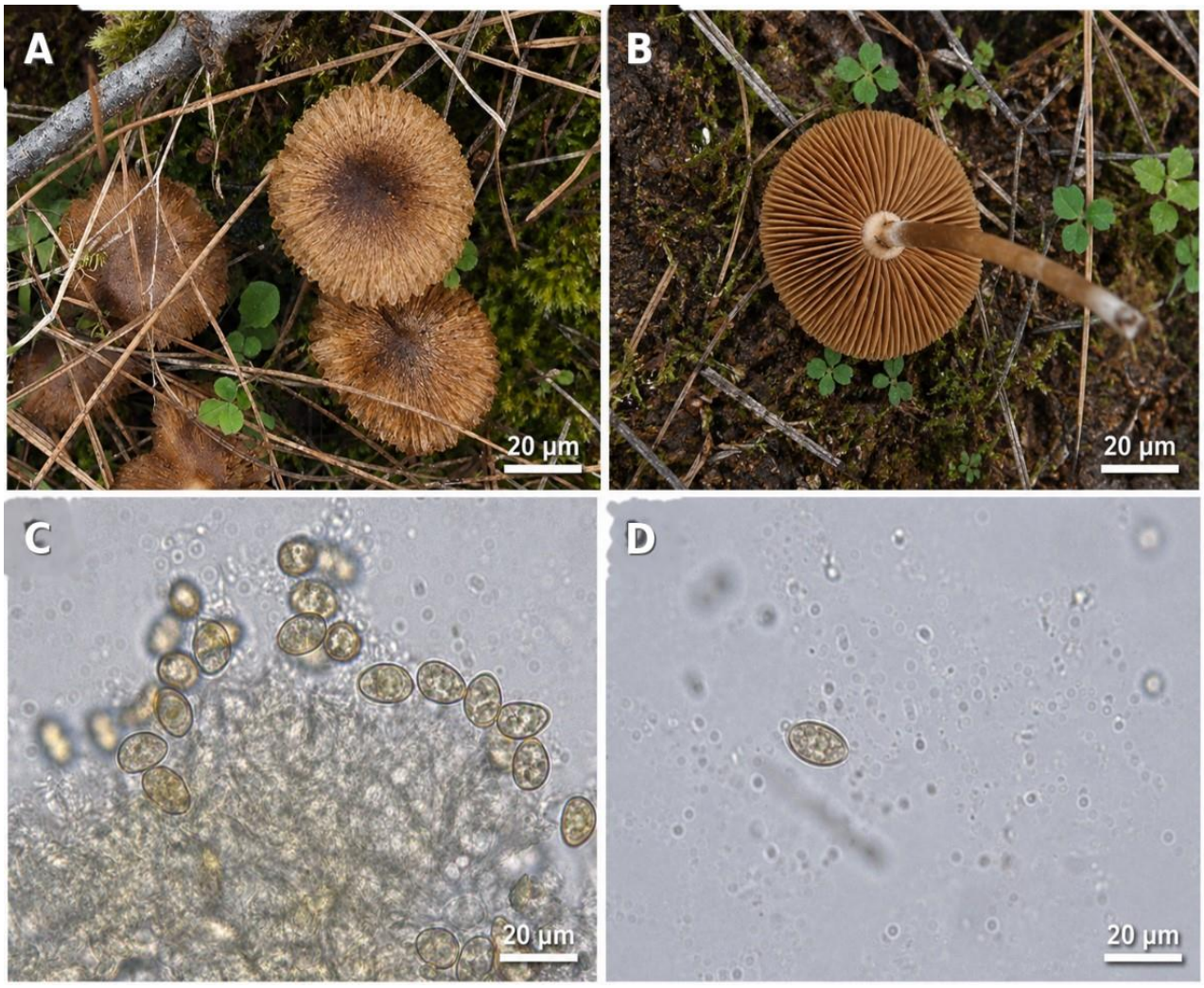

Figure 5. *Inocybe pinophila* (YM6). A–B. Basidiomata in the natural habitat. C–D. Basidiospores. Scale bars: A–D = 20 µm.

3.1.6. *Inocybe subnudipes* Kühner

Description:

Basidiomata small, occurring solitary among pine litter. Pileus 8–20 mm in diameter, initially hemispherical to convex, becoming plano-convex with age, often retaining a low umbo. Surface dry, finely fibrillose to minutely squamulose, yellowish-brown to chestnut-brown, with a distinctly darker central disc. Margin initially incurved, later straight, occasionally slightly fissured. Lamellae moderately crowded, adnexed to narrowly adnate, initially pale cream to beige, becoming light brown at maturity. Lamellulae numerous. Lamellar edge concolorous. Stipe 15–35 × 1–3 mm, slender, cylindrical, slightly enlarged towards the base, whitish to pale cream, smooth to finely pruinose near the apex, lacking a ring. Context thin, whitish. Odour not recorded in the examined material (Figure 6) [18,20].

Basidiospores smooth, yellowish-brown, broadly ellipsoid to subamygdaliform, measuring 10–11 × 6–6.5 µm (mean = 10.7 × 6.3 µm), with thickened walls, lacking nodulose ornamentation and a conspicuous germ pore [18,20].

The examined specimen was collected among pine litter under *Pinus brutia* Ten. in Karagöl Nature Park, Yamanlar Mountain (İzmir, western Türkiye). The small basidiomata, yellowish-brown fibrillose pileus with a darker central disc, slender stipe, and smooth broadly ellipsoid basidiospores correspond well with the diagnostic features reported for *Inocybe subnudipes* [18,20,22].

Specimen examined: Türkiye, İzmir, Karşıyaka, Yamanlar Mountain, Karagöl Nature Park, northwestern woodland, 38° 34' 03" N, 27° 13' 06" E, alt. 875 m, 14.11.2025, leg. G. Gülhan and M. Güllü (YM23), deposited in ERCH, voucher no. [ERCH YM 0.023].

Taxonomic and Phylogenetic Remarks:

Morphologically, the Turkish collection agrees well with *Inocybe subnudipes* in possessing small basidiomata, a yellowish- to ochraceous-brown fibrillose pileus with a darker central disc, pale lamellae, a slender whitish stipe lacking a ring, and smooth broadly ellipsoid to subamygdaliform basidiospores. The observed basidiospores (10–11 × 6–6.5 µm, mean = 10.7 × 6.3 µm) are also consistent with the dimensions reported for *I. subnudipes* in previous taxonomic studies [18,20,22]. These morphological features, together with the ecological association with *Pinus brutia*, correspond closely to the current concept of *I. subnudipes*.

Among morphologically related taxa, Inocybe hamadryadis is considered one of the most similar species. Both taxa share a small brown fibrillose pileus, pale lamellae, and a slender whitish stipe. However, I. hamadryadis generally develops larger and more robust basidiomata and possesses somewhat broader basidiospores [1,18]. Inocybe tenuicystidiata also resembles I. subnudipes in general habit but is separated from it by ITS sequence data [1].

Bayesian phylogenetic analysis of ITS sequences placed specimen YM23 within the *Inocybe subnudipes* clade together with corresponding reference sequences. In the inferred topology, *I. caesaraugustae* was recovered as the closest sister lineage, whereas *I. hamadryadis* and *I. tenuicystidiata* occupied separate lineages (Figure 8).

Although *Inocybe hamadryadis*, *I. caesaraugustae*, and *I. subnudipes* share several morphological characteristics, the Bayesian ITS reconstruction placed them in distinct lineages. The Turkish specimen was nested within the *I. subnudipes* clade, providing molecular evidence consistent with its morphology-based identification. *Inocybe tenuicystidiata* was also recovered in a separate lineage.

The combination of macromorphological characters, basidiospore dimensions and shape, ecological observations, and ITS sequence placement supports the identification of specimen YM23 as Inocybe subnudipes. Based on the literature and database searches conducted for this study, this collection represents the first documented record of I. subnudipes from Türkiye.

Figure 6. *Inocybe subnudipes* (YM23). A–B. Basidiomata in the natural habitat. C–D. Basidiospores. Scale bars: A–D = 20 µm

3.1.7. *Inocybe tenuicystidiata* E. Horak & Stangl

Description:

Basidiomata small and slender. Pileus 10–20 mm in diameter, initially convex to campanulate, later becoming plano-convex with a low and inconspicuous umbo. Surface dry, finely fibrillose, brown to ochraceous-brown, slightly darker at the centre and paler towards the margin. Margin initially incurved, becoming straight with age and occasionally splitting at maturity. Lamellae moderately crowded, adnexed to narrowly adnate, initially pale greyish-beige, becoming clay-brown to tobacco-brown at maturity. Lamellulae present. Lamellar edge concolorous or slightly paler than the lamellar faces. Stipe 20–40 × 1–3 mm, cylindrical, slightly enlarged towards the base, whitish to pale brown, longitudinally fibrillose, lacking an annulus. Context thin, pale cream. Odour not recorded in the examined material (Figure 7) [2,23].

Basidiospores smooth, yellowish-brown, ellipsoid to subamygdaliform, measuring (9–)10–11 × 5–6 µm (mean = 10.0 × 5.4 µm), with a distinct apical germ pore. Spore walls moderately thickened and lacking nodulose ornamentation [2,23].

The examined specimen was collected solitarily on soil among pine litter under Pinus brutia Ten. in Karagöl Nature Park, Yamanlar Mountain (İzmir, western Türkiye). The small and slender basidiomata, brown fibrillose pileus, and smooth ellipsoid to subamygdaliform basidiospores with a distinct apical germ pore correspond well with the diagnostic features reported for Inocybe tenuicystidiata [2,23].

Specimen examined: Türkiye, İzmir, Karşıyaka, Yamanlar Mountain, Karagöl Nature Park, northeastern forest path, 38° 34' 00" N, 27° 13' 38" E, alt. 850 m, 14.11.2025, leg. G. Gülhan and M. Güllü (YM30), deposited in ERCH, voucher no. [ERCH YM 0.030].

Taxonomic and Phylogenetic Remarks:

Morphologically, the Turkish collection agrees well with *Inocybe tenuicystidiata* in possessing small and slender basidiomata, a brown fibrillose pileus, a slender whitish stipe, and smooth ellipsoid to subamygdaliform basidiospores. The observed basidiospores [(9–)10–11 × 5–6 µm, mean = 10.0 × 5.4 µm] are also consistent with the dimensions reported for *I. tenuicystidiata* in previous taxonomic studies [2,16]. These morphological features, together with the occurrence in pine-dominated habitats, correspond closely to the current concept of *I. tenuicystidiata*.

Among morphologically related taxa, Inocybe hamadryadis is considered one of the most similar species. Both taxa share small brown basidiomata, a fibrillose pileus surface, smooth basidiospores, and occurrence in conifer-dominated habitats. However, I. hamadryadis generally develops larger and more robust basidiomata and has a comparatively sturdier habit. In contrast, the Turkish collection displays more slender basidiomata [2,23].

Another morphologically similar species is *Inocybe subnudipes*. Although both taxa occur in coniferous forests and possess smooth basidiospores, I. subnudipes typically exhibits a paler pileus and a more distinctly pruinose stipe apex. The two taxa are also recovered in separate positions in the ITS phylogeny [2,24].

Bayesian phylogenetic analysis of ITS sequences placed specimen YM30 with reference sequences identified as *Inocybe tenuicystidiata*. In the inferred topology, the *I. tenuicystidiata* clade was recovered as sister to *I. hamadryadis* (PP = 1.00) (Figure 8).

Despite morphological similarities between *I. hamadryadis* and *I. tenuicystidiata*, phylogeny clearly distinguished between these taxa. The Turkish strain was found to be a part of the *I. tenuicystidiata* clade, rather than the *I. hamadryadis* clade, thus supporting morphological dissimilarities on a molecular level. In turn, *I. subnudipes* proved to be an independent taxon which did not seem to be closely related to the Turkish sample.

Therefore, both morphological and ITS-based phylogenetic data strongly support the identification of specimen YM30 as *Inocybe tenuicystidiata*.

The Bayesian analysis of phylogeny, employing sequences of the ITS region of rDNA, unambiguously showed that all of the collected samples belong to the clades representing their respective species, thereby corroborating the morphological classification of samples. Specifically, samples YM10, YM12, YM13, and YM28 formed a monophyletic cluster with the sequence representatives of *Inocybe geophylla*. This clade appeared as the sister group of *I. lilacina*, possessing maximal posterior probability (PP = 1.00), implying a very close evolutionary relation but at the same time showing clear distinction between them (see Figure 8).

These specimens were grouped in the same *Inocybe neorufula* clade, alongside validated GenBank sequences of the species. The inferred tree thus showed *I. derbschii* to be the closest sister clade (PP = 0.77). Even though these two species had many morphological similarities, such as brown fibrillose pilei and smooth basidiospores, there was enough molecular evidence to distinguish them into different lineages in the tree. The close association of YM19 and YM22 with sequences of *I. neorufula* is an indication that these samples are *I. neorufula*, and their occurrence here marks the first time that the species is documented fromTürkiye (Figure 8).

The specimen YM23 was found to be associated with an *Inocybe subnudipes* clade that was strongly supported by molecular evidence, along with several other reference sequences. *I. caesaraugustae* was suggested as the closest sister species to it, while *I. tenuicystidiata*, which shared some common morphological characteristics, was found to belong to a different lineage.

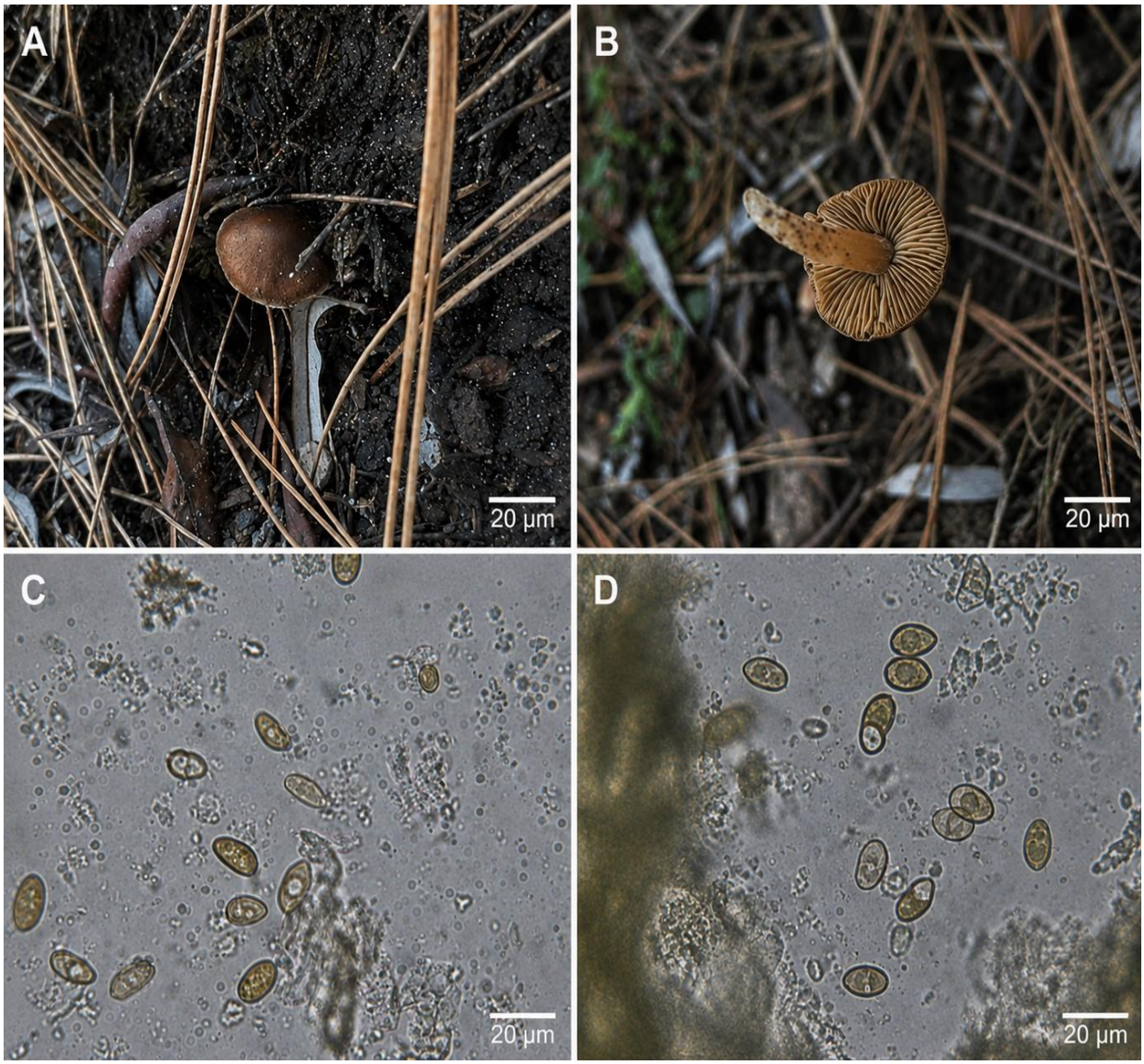

Figure 7. *Inocybe tenuicystidiata* (YM23). A–B. Basidiomata in the natural habitat. C–D. Basidiospores. Scale bars: A–D = 20 µm

Similarly, YM30 appeared in association with reference sequences of *I. tenuicystidiata* but was sistered to *I. hamadryadis* (PP = 1.00). While sharing some morphological features, these taxa were confirmed as being phylogenetically independent of each other (Figure 8).

YM27 was classified into the clade of *I. amygdaliolens* with high confidence and associated with all existing references for this particular species. The most closely related group consisted of *I. mycenoides*, while *I. fraudans*, even being morphologically similar and having typical odor, was quite distant from other Inocybes in the dendrogram. This result indicates that possession of macromorphological features does not indicate an evolutionary affinity within Inocybe (Figure 8).

The Turkish specimen YM6 grouped into a well supported clade with all available sequences of *Inocybe pinophila*. Phylogenetic reconstruction suggested *I. alberichiana* and *I. laurina* as the closest relatives, while *I. derbschii* was placed in a more distant lineage despite morphological similarities. The results showed that the morphological similarities found between *I. pinophila* and *I. derbschii* are likely to be due to phenotypic convergence and not to close phylogenetic relationships (Figure 8).

Specimen YM25 grouped with reference sequences of *Inocybe nitidiuscula* and formed a well-supported monophyletic lineage. The sister group relationship with the *I. laurina–I. alberichiana* complex further supports the current taxonomic concept of *I. nitidiuscula* and confirms the accuracy of the morphological identification (Figure 8).

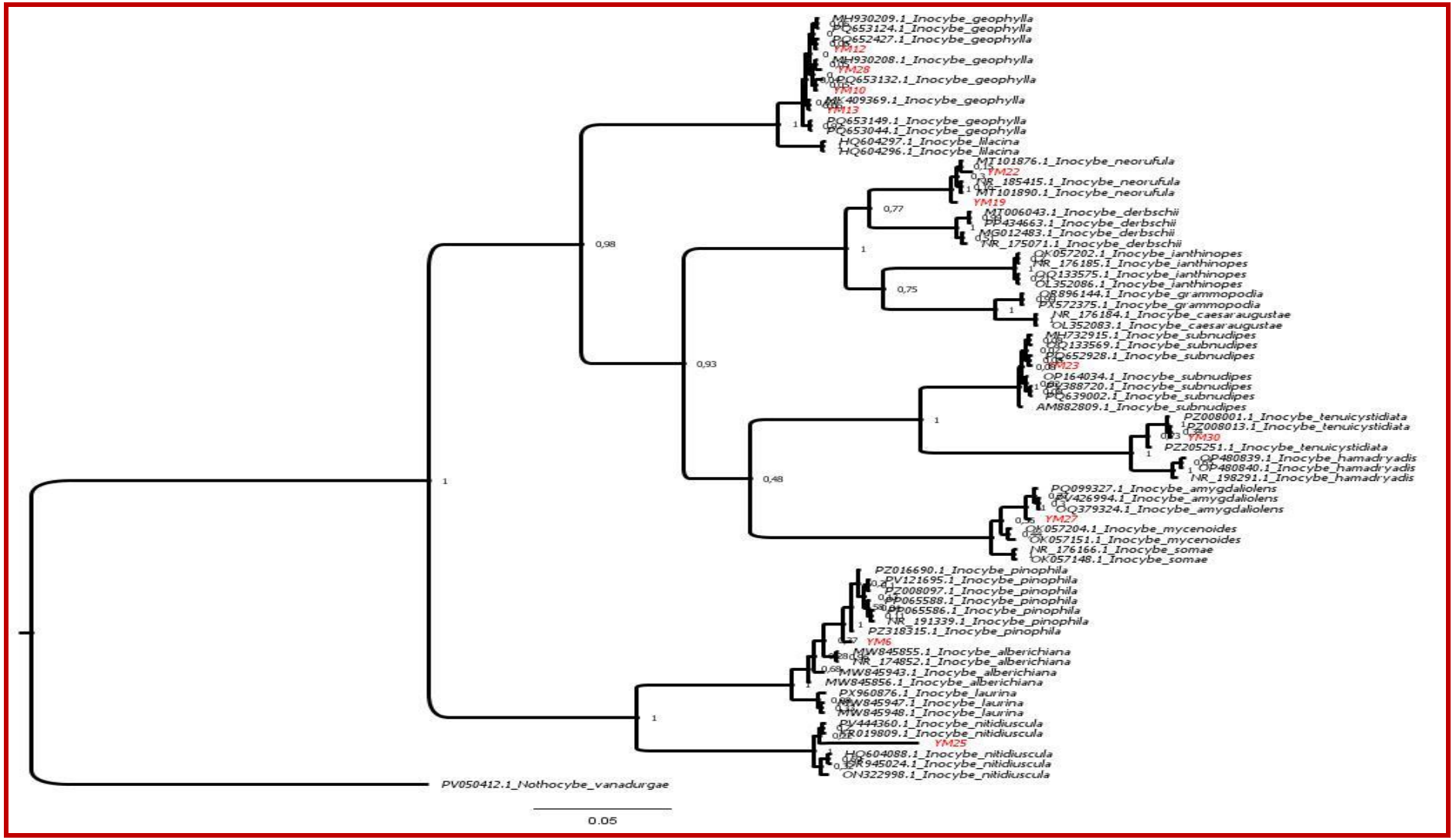

Figure 8. Bayesian ITS phylogeny of *Inocybe* specimens from Yamanlar Mountain, western Türkiye. Sequences generated in this study are shown in red. Posterior probabilities (PP ≥ 0.70) are indicated at the nodes. *Nothocybe vanduzgarae* was used as the outgroup. Scale bar = substitutions per site.

Overall the Bayesian phylogenetic reconstruction showed a high level of congruence between morphological observations and molecular evidence. All of the examined specimens including YM6 (*I. pinophila*), YM19–YM22 (*I. neorufula*), YM23 (*I. subnudipes*), YM25 (*I. nitidiuscula*), YM27 (*I. amygdaliolens*), YM10–YM12–YM13–YM28 (*I. geophylla*) and YM30 (*I. tenuicystidiata*) fall within their respective species clades with moderate to strong posterior probability support. Moreover, the records of *I. amygdaliolens*, *I. neorufula*, *I. pinophila* and *I. subnudipes* as new for Türkiye greatly increase the known diversity of Inocybe in the country and highlight the importance of Mediterranean *Pinus brutia* forests as reservoirs of undocumented ectomycorrhizal fungal diversity.

4. Discussion

The present study documents seven Inocybe species from collections obtained in *Pinus brutia*-dominated habitats around Karagöl Nature Park on Yamanlar Mountain: *I. amygdaliolens*, *I. geophylla*, *I. neorufula*, *I. nitidiuscula*, *I. pinophila*, *I. subnudipes*, and *I. tenuicystidiata*. Morphological observations were evaluated together with ITS-based Bayesian phylogenetic evidence. The general agreement between these independent datasets supported the taxonomic placement of the examined collections, while the phylogenetic analysis was particularly useful for distinguishing morphologically similar taxa.

Several of the examined species shared overlapping macromorphological characteristics, including small fibrillose basidiomata, brownish pileus colours, smooth basidiospores, and occurrence in pine-dominated habitats. Nevertheless, the Turkish sequences were placed with the corresponding reference sequences in the Bayesian ITS tree. These results illustrate the value of integrating macromorphological, micromorphological, ecological, and molecular evidence when identifying members of a morphologically variable genus such as Inocybe. Weakly or moderately supported sister relationships should, however, be interpreted cautiously and should not be confused with support for the placement of the Turkish specimens within their respective species-level groups.

Based on searches of the relevant Turkish macrofungal literature, national checklists, Index Fungorum, MycoBank, and GBIF completed on, *Inocybe amygdaliolens*, *I. neorufula*, *I. pinophila*, and *I. subnudipes* are reported here as new records for Türkiye. These records extend the documented geographic distributions of the four taxa and add to current knowledge of the Turkish mycobiota. Because the specimens were collected during a spatially and temporally restricted survey in November 2025, the present results should be regarded as taxonomic documentation of the collected material rather than a comprehensive assessment of *Inocybe* diversity on Yamanlar Mountain.

5. Conclusions

This study shows that using different methods together is the best way to understand the variety of fungi in Turkey. By combining physical examinations with DNA data, we were able to identify our samples accurately and find species that hadn't been recorded in Türkiye before. These findings help us better understand the *Inocybe* genus and provide a useful starting point for future research on fungi in Mediterranean forests.

All examined collections were obtained from *Pinus brutia*-dominated habitats. However, because ectomycorrhizal root tips were not examined, the present study does not establish a direct host association between individual fungal specimens and P. brutia. Additional surveys conducted across different seasons, elevations, vegetation types, and localities will be required to evaluate the diversity, distribution, and host associations of Inocybe species in western Anatolia more comprehensively. Future studies should also incorporate permanently deposited voucher specimens, publicly available sequence data, and, where possible, additional molecular markers to facilitate independent verification and more robust species delimitation.

Artificial Intelligence Statement

( ) This article did not use artificial intelligence.

(x) This article does use artificial intelligence. *For example:* "During the preparation of this manuscript, the authors used [Name of Tool/Service] to [describe how the tool was used, e.g., improve English phrasing and grammar]. The authors reviewed and edited the content as needed and take full responsibility for the framework and validity of the final paper."

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( ) Option A (Public Repository): "The research data supporting this study are publicly available in [Name of Repository: e.g., Zenodo, Figshare, Dryad] with the permanent DOI link [URL/DOI]."
( ) Option B (Upon Request): "The raw data supporting the findings of this study are available from the corresponding author [Author Initials] upon reasonable request and within ethical boundaries."
(x) Option C (Third-Party Data): "The data used in this study were obtained from [Name of Institution/Source] and are subject to third-party restrictions regarding redistribution."
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