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Three New Records For Turkish *Agaricales* Inhabiting Ankara University Beşevler 10th Year Campus Area

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Abstract

Aim of the study: The principal objective of this study is to contribute to the Turkish mycobiota by including three newly reported agaricoid macrofungi species.

Study area: Situated approximately 5 km from the city center in the Beşevler area of the Çankaya district in Ankara, Ankara University's Beşevler 10th Year Campus has qualities akin to both an arboretum and a botanical garden. Serving as a refuge for a wide variety of species, this campus was officially acknowledged as a grade 3 natural site area in 2016.

Material and method: The research involved meticulous analysis of the macroscopic and microscopic attributes of the samples. Additionally, the study implemented rDNA sequence analysis utilizing the Internal Transcribed Spacer (ITS) sequencing method to further identify the collected samples.

Main results: After conducting field and laboratory studies, three fungal species from the *Agaricales* order, namely *Hebeloma salicicola*, *Inocybe griseovelata*, and *I. tiburtina*, have been identified and reported for the first time in Türkiye.

Research highlights: This research explores the report of three new species of agaricoid fungi from the mycobiota of Türkiye, collected from Ankara University's Beşevler 10th Year Campus.

Keywords: New records, *Agaricales*, Türkiye

Ankara Üniversitesi Beşevler 10. Yıl Yerleşkesi'nde Yayılış Gösteren Türkiye *Agaricales*'leri İçin Üç Yeni Kayıt

Öz

Çalışmanın amacı: Bu çalışmanın temel amacı, yeni bildirilen üç agaricoid makrofungus türünü dahil ederek Türkiye mikobiyotasına katkıda bulunmaktır.

Çalışma alanı: Ankara'nın Çankaya ilçesine bağlı Beşevler mevkiinde, şehir merkezine yaklaşık 5 km uzaklıkta yer alan Ankara Üniversitesi Beşevler 10. Yıl Yerleşkesi hem arboretum hem de botanik bahçesini andıran niteliklere sahiptir. Çok çeşitli türler için bir sığınak görevi gören bu kampüs, 2016 yılında resmen 3. derece doğal sit alanı olarak kabul edilmiştir.

Materyal ve yöntem: Araştırma, numunelerin makroskopik ve mikroskobik özelliklerinin titiz bir analizini içermektedir. Ek olarak çalışmada toplanan numuneleri daha fazla tanımlamak için Dahili Kopyalanmış Aralayıcı (ITS) dizileme yöntemi kullanılarak rDNA dizilerinin analizi yapılmıştır.

Temel sonuçlar: Yapılan arazi ve laboratuvar çalışmaları sonucunda *Agaricales* takımına ait üç mantar türü olan *Hebeloma salicicola*, *Inocybe griseovelata* ve *I. tiburtina* Türkiye'de ilk kez tespit edilerek rapor edilmiştir.

Araştırma vurguları: Bu araştırma, Ankara Üniversitesi Beşevler 10. Yıl Kampüsü'nden toplanan Türkiye mikobiyotasından üç yeni agaricoid mantar türünün raporunu içermektedir.

Anahtar Kelimeler: Yeni kayıtlar, *Agaricales*, Türkiye

Introduction

Ankara University Besevler 10th Year Campus is situated in the Beşevler region, which falls within the Çankaya district of Ankara (Türkiye), and is approximately 5 km distant from the city center. This notable campus is situated around 5 kilometers away from the city's central hub. Elevated between 850 and 870 meters above sea level, the campus covers 195000 square meters. The

campus houses several crucial facilities and institutions, including the faculties of science and pharmacy. In addition, the grounds also host an extensive sports complex, the university rectorate, and a variety of other administrative buildings, thereby fulfilling a diverse array of academic and administrative needs. One of the campus's key attributes is its emphasis on fostering a harmonious relationship between humans and their natural environment. To this end, it offers a plethora of opportunities for recreational and sports activities, providing a platform for students and staff to immerse themselves in nature and outdoor pursuits. This emphasis is echoed in the design of the campus itself (Akata et al, 2019a).

Ankara University Beşevler 10th Year Campus is not just an educational institution; it harbors an arboretum and a botanical garden, serving as a refuge for a wide array of species. This biodiversity enhances the campus's beauty, promotes ecological balance, and provides an excellent opportunity for study and research. In acknowledgment of this environmental treasure, the campus was awarded the status of a grade 3 natural site area in 2016 by the Ministry of Environment and Urbanization's General Directorate for Protection of Natural Assets. From the moment of its inception, the campus has been dedicated to enhancing its biodiversity. This dedication has led to the introduction of a diverse collection of both local and exotic trees, shrubs, and herbaceous plants across its extensive landscapes. Hosting 445 types of higher plants, with approximately 151 taxa being trees and shrubs, the campus is a lush oasis in an urban setting. But its biodiversity extends beyond flora, featuring a wide array of other organisms that contribute to its unique ecosystem (Altındağ, 2021; Başköse et al, 2020).

As per the most recent observations and reports, the campus area presents a fascinating display of biodiversity. This includes a noteworthy collection of 28 mosses species (Ezer et al, 2021), highlighting the richness of non-vascular plant life in the area. In addition, the campus is home to an extraordinary variety of 174 fungal species (Akata et al, 2019a; 2019b; 2020; 2021a; 2021b; 2023; Altuntaş et al, 2019; Sahin and Akata 2019; 2021). These include 64 microfungi and 24 lichenized fungi, demonstrating the extensive range of symbiotic relationships present within the ecosystem. In addition to these, there are 86 macrofungi species, intriguingly, with three species noted to be affected by viral infections (Akata et al, 2023; Altındağ, 2021; Sahin & Akata, 2019; Akata et al, 2023).

The faunal biodiversity of the campus is equally compelling, boasting a diverse collection of 28 insect species, alongside a singular species each of mollusk and millipede. The area is also inhabited by an amphibian species and two reptiles, providing evidence of a healthy herpetological community. Bird life is abundant with an array of 62 different species, further enriching the local biodiversity. Furthermore, the presence of six mammalian species adds to the variety of higher vertebrates on the campus (Altındağ, 2021).

The detailed data compiled underscores the remarkable diversity of life that the campus area supports, emphasizing its crucial role in ecological preservation. This highlights the vital importance of urban green spaces, such as this campus, in safeguarding a variety of species, thereby making substantial contributions to both regional and global biodiversity.

Agaricales, the largest and most diverse order of division *Basidiomycota* within the kingdom Fungi, encompasses approximately 20000 described species (Kalichman et al, 2020). It includes a wide range of taxa, with 509 genera and 46 families represented. Among the accepted genera in this order, around 82% are agarics, commonly known as lamellate mushrooms. Notably, the genera *Hebeloma* (Fr.) P. Kumm., and *Inocybe* (Fr.) Fr. are particularly well-represented, with approximately 150 and 1000 species, respectively (Kalichman et al, 2020; Kirk et al, 2008). *Hebeloma* is a genus that forms ectomycorrhizal associations, and it is taxonomically placed within the family *Hymenogastraceae* Vittad. This particular genus has been identified in diverse habitats spanning the northern hemisphere, potentially even achieving a global distribution, with the notable exception of areas that lack native *Fagales*, such as regions in Northern South

America and Africa. Nevertheless, it's reasonable to infer that human activities might have played a role in spreading *Hebeloma* species, given their frequent occurrence in plant nursery environments (Eberhardt et al, 2015).

The genus is characterized by a number of unique features. Its spores, ranging from smooth to rough, have a brown color and typically lack a visible germ pore. This genus also typically presents distinct cheilocystidia, a specific type of cell on the gill edge of a mushroom, and generally lacks pleurocystidia, another type of cystidia found on the gill face. Additionally, *Hebeloma* species possess a type of outer skin known as an ixocutis, which results in a smooth, sticky cap surface that often exhibits a two-toned coloration, generally darker at the center (Cripps et al, 2019). Another distinguishing feature of the *Hebeloma* genus is its unique scent. The smell of these fungi is often reminiscent of radish or raw potato, a characteristic known as raphanoid (Cripps et al, 2019; Vesterholt 2005). The comprehensive scholarly research on the mycobiota of Turkey provides substantial evidence of the considerable diversity of the *Hebeloma* genus present within the country (Acar et al, 2021; 2022; Sesli, 2023; Sesli et al, 2020). To date, based on the available literature, the quantifiable representation of this genus in Turkey stands at 34 distinct species. These findings have been confirmed through rigorous scientific investigations and highlight the significant role of Turkey in the global distribution and diversity of the *Hebeloma* species.

Inocybe, belonging to the *Inocybaceae* family, is an ectomycorrhizal genus that boasts a broad geographical distribution, with species ranging from tropical to arctic regions and they are predominantly encountered in varied forest ecosystems, including both deciduous and coniferous forests (Altuntaş et al, 2019). In terms of physical appearance, Inocybe species are characterized by their relatively small and inconspicuously colored basidiomata (Kuyper, 1985). The pileus exhibits a dry texture, often decorated with scales or fibrillose or rimose patterns. The lamellae are typically of a dull brown hue, and they feature a stipe, a distinctive trait of these species is their unique odor, which aids in their identification. Their spores of the genus are particularly notable, generally, dull brown in color, equipped with slightly thick walls and smooth surfaces. In terms of morphology, these spores display a variety of shapes including angular, nodulose, or spinose forms. However, a common feature they usually lack is the presence of a germ pore. Another fascinating characteristic of most *Inocybe* species is the presence of cystidia, which are thick-walled cells situated in the spore-producing tissue. It is common for the tips of these cystidia to carry crystals of calcium-oxalate, a feature that adds another layer of complexity to their structure (Kuyper, 1986; Matheny et al. 2009; Matheny & Kudzma, 2019). As per the extant academic literature, it has been established that the mycological biodiversity in Turkey is notably diverse with respect to the *Inocybe* genus. Specifically, up to the present time, scientific surveys have managed to catalog a significant number of species, with the count currently standing at a total of 90 distinct *Inocybe* species identified within Turkish borders (Bandini et al, 2020; Kaygusuz et al, 2022a; 2022b; Sesli, 2020; Sesli et al, 2020; Sesli, 2022; Sesli & Bandini, 2020).

The main purpose of this study is to enhance the knowledge of the Turkish mycobiota by introducing three newly reported species. The research seeks to provide valuable insights into the fungal diversity and ecosystem specific to this location in Turkey, uncovering previously unreported fungal species thriving in this environment.

Materials and Methods

The present study utilized a comprehensive approach, combining traditional and molecular methods to identify and classify the samples collected from Ankara University Beşevler 10th Year Campus area (Ankara, Turkey). The study involved a thorough examination of both macroscopic and microscopic features of the samples, along with the analysis of rDNA sequences using Internal Transcribed Spacer (ITS) sequencing.

Morphological Characterization

During October 2022, samples were collected from the Ankara University Beşevler 10th Year Campus, and pertinent macroscopic and ecological characteristics were documented at the collection site. Subsequently, in the laboratory, the microscopic features of the samples underwent careful scrutiny utilizing both a simple light microscope (LM) and a scanning electron microscope (SEM). Under the light microscope, measurements were repetitively taken approximately 30 times using the Euromex Oxion Trinocular microscope at 100X magnification. The gathered data on each microscopic structure were subjected to statistical analysis. For SEM analysis, small sections of the mass within the gleba were affixed onto stubs using double-sided adhesive tape and then coated with gold particles. These prepared samples were visualized using an EVO 40XVP scanning electron microscope (LEO Ltd., Cambridge, UK) with an accelerating voltage set at 20.

The morphological identification of the samples was conducted based on established methodologies described in the studies by Eberhardt et al. (2015), Kuyper (1985; 1986), Bandini et al (2021; 2022), and Muñoz et al (2022). These studies provided valuable references and protocols for the identification process. Once identified, the confirmed samples were stored at the Fungarium of Ankara University, which is located within the Department of Biology, Faculty of Science.

Molecular Characterization

Determination of the ITS rDNA sequences

To extract genomic DNA from the samples ANK Akata 8672, ANK Akata 8679, and ANK Akata 8706, the CTAB method was employed, following the procedure outlined by Rogers and Bendich (1994). The isolated genomic DNA was then subjected to spectrophotometric analysis using the Nanodrop Lite Thermo Scientific instrument to assess its quality and quantity. Subsequently, the extracted DNA was used as a template for PCR amplification of the Internal Transcribed Spacer (ITS) rDNA regions. The PCR amplification was carried out using the ITS1 forward and ITS4 reverse universal oligonucleotides, following the methodology described elsewhere (Stielow et al., 2015). The resulting amplification products were verified through electrophoresis on an agarose gel, followed by purification using the Expin Gel PCR and CleanUp SV Kit (GeneAll). The purified products were then subjected to Sanger dideoxy sequencing, utilizing the BigDye™ Direct Cycle Sequencing Kit (Thermo Fisher Scientific) and the same ITS1 and ITS4 oligonucleotides for the sequencing PCR. Fragment analysis was performed using the ABI Prism 3130 Genetic Analyzer. Agarose gel electrophoresis and Sanger sequencing procedures were conducted according to previously described methods (Chen et al, 2014).

Molecular Phylogeny Study

The software DNAMAN Version 10, developed by Lynnon Corporation, was utilized to assemble the Sanger sequencing reads obtained from the ITS1 and ITS4 primers. Following assembly, a BLASTn search was conducted using the assembled sequence to analyze its identity index. Based on the results of the BLAST search, sequences belonging to both the in-group and out-group were retrieved from the NCBI GenBank database. These retrieved sequences, along with the assembled sequences, were aligned using the ClustalW algorithm in MEGAX software (Kumar et al, 2018). The aligned nucleotide sequences were then subjected to phylogenetic analysis. The evolutionary history of ANK Akata 8672, ANK Akata 8679, and ANK Akata 8706 was inferred using the Maximum Likelihood method with the T92+G+I nucleotide substitution model (Tamura, 1992). To improve the accuracy of the estimation, the bootstrap method with 1000 replicates was employed (Felsenstein, 1985). The resulting phylogenetic tree provides insights into the evolutionary relationships among the analyzed samples, demonstrating their placement within the broader fungal taxonomy.

Results

The taxonomic classification of the newly recorded species was determined in accordance with the guidelines provided by

Index Fungorum (www.indexfungorum.org; accessed on 20 June 2023). The species descriptions include concise information such as collection dates, locations, habitat notes, geographic coordinates, collection numbers, as well as macroscopic and microscopic morphology. Additionally, scanning electron microscope (SEM) images of the spores are provided, offering further insights into the detailed characteristics of the species.

Taxonomic overview

Fungi
Basidiomycota
Agaricomycotina
Agaricomycetes
Agaricomycetidae
Agaricales
Hymenogastraceae

Hebeloma salicicola Beker, Vesterh. & U. Eberh., in Eberhardt et al. (2015), (Figure 1).

Macroscopic and microscopic features

Pileus 30-45 mm diam., convex, with or without umbo. Margin typically straight but sometimes with a scalloped appearance. Surface often sticky, and tacky when moist, ochre to red-brownish or light brick color in the center, while the margin appears cream to buff. Lamellae distant, adnate to slightly notched, in some cases almost free, the color ranges from cream to light brown, edge fimbriate, usually noticeably paler than the surface lamella surface. Stipe 30-45 × 5-7 mm, sometimes widening up to 10 mm at the base, cylindrical to clavate, enlarged toward the base, surface dry with a powdery to woolly texture. Flesh thick, whitish to buff. Odor radishes like. Spores 11.5-13.5 µm × 6.5-7.5 µm, almond to lemon-shaped with an apiculus, weakly or moderately ornamented, yellow-brown to light brownish, dextrinoid. Basidia 25-35 × 8-10 µm cylindrical to club-shaped with 4 sterigmata. Pleurocystidia not observed. Cheilocystidia club-shaped, sometimes swollen at the base, usually with some thickening of the apex, some septate, and branched. Caulocystidia similar to cheilocystidia. Pileipellis an ixocutis with an hyaline hyphae. Clamp connections present in all tissues.

Ecology

H. salicicola demonstrates a specific mycorrhizal association limited to willow and poplar. It is frequently encountered and tends to grow in close clusters in calcareous dune slacks alongside *Salix repens*. Notably, it exhibits two distinct fruiting periods each year, occurring in both spring and autumn. The species has been documented in diverse environments, such as grassy or mossy terrains, frequently with sandy soil, and commonly associated with poplar or willow vegetation. Moreover, this mushroom species can be encountered in scrublands, gardens, grasslands, and woodland plantations, displaying adaptability to both acidic and calcareous soil conditions (Eberhardt et al, 2015).

Distribution

Belgium, Estonia, France, Poland, Spain, Svalbard, The Netherlands, and Wales (Eberhardt et al, 2015).

Material examined

Under willow, 39° 56' N, 32° 50' E, 860 m, 28.10.2022, ANK AKATA 8706.

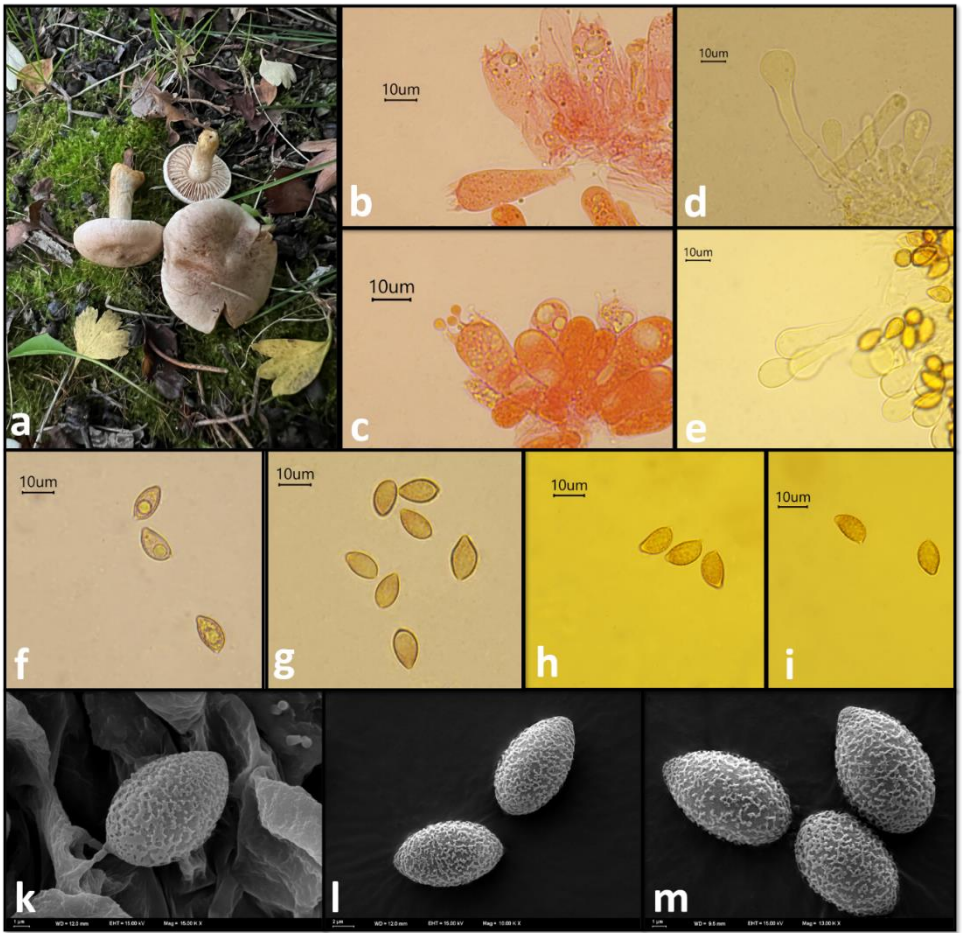

Figure 1. *Hebeloma salicicola*: a. basidiocaps, b,c. basidia (LM), d,e. cheilocystidia (LM), f-i. spores (LM), k-m. spores (SEM)

Inocybaceae

Inocybe griseovelata Kühner (1955), (Figure 2).

Macroscopic and microscopic features

Pileus 35-40 mm in diam., almost spherical to bell-shaped at first, and then becomes convex, without a prominent central umbo, or if present, the umbo is relatively flat and large. Margin initially curves inward at the very edge, but soon curves downward or becomes straight, occasionally being deeply torn, initially completely covered by numerous remnants of a whitish-greyish velvety covering (velipellis). Surface almost straw-colored to nut-brownish, initially smooth or minutely tomentose, but later develops cracks or fissures and may show fine fibers diverging towards the margin. Lamellae sub-distant, adnate, at first whitish, then greyish to greyish brown, edge fimbriate and concolorous. Stipe 35-40 × 3-5 mm, cylindrical or curved, and slightly enlarged towards the base, when young entirely and covered with whitish tomentum, finally longitudinally striate or glabrous, pale beige to pale brownish, sparely pruinose at the extreme apex. Flesh thick and whitish in the pileus center. Odor spermatic. Spores 9-11 µm × 5.5-6.5 µm, almond-shaped and smooth, brown to reddish-brown. Basidia 25-30 × 9-10 µm club-shaped with 4 sterigmata. Pleurocystidia 55–70 µm × 12-20 µm, almost cylindrical to bottle- shaped with apical crystals, and thick-walled, walls up to 1.5 µm thick. Cheilocystidia similar to the pleurocystidia. Caulocystidia at the top of the stipe, narrow and curved, mostly almost cylindrical, or sub-spindle shape with crystals at their apex. Pileipellis an epicutis, consisting of parallel hyphae. Clamp connections present all tissues.

Ecology

I. griseovelata thrives in calcareous soil and can be found along waysides, as well as in parks or cemeteries. It establishes mycorrhizal associations with both broad-leaved trees and conifers, indicating its versatility in habitat preferences. Notably, this species has a broad geographical distribution and is widespread across various regions in Europe (Muñoz et al, 2022).

Material examined

Under oak, 39° 56' N, 32° 50' E, 860 m, 17.10.2022, ANK AKATA 8679.

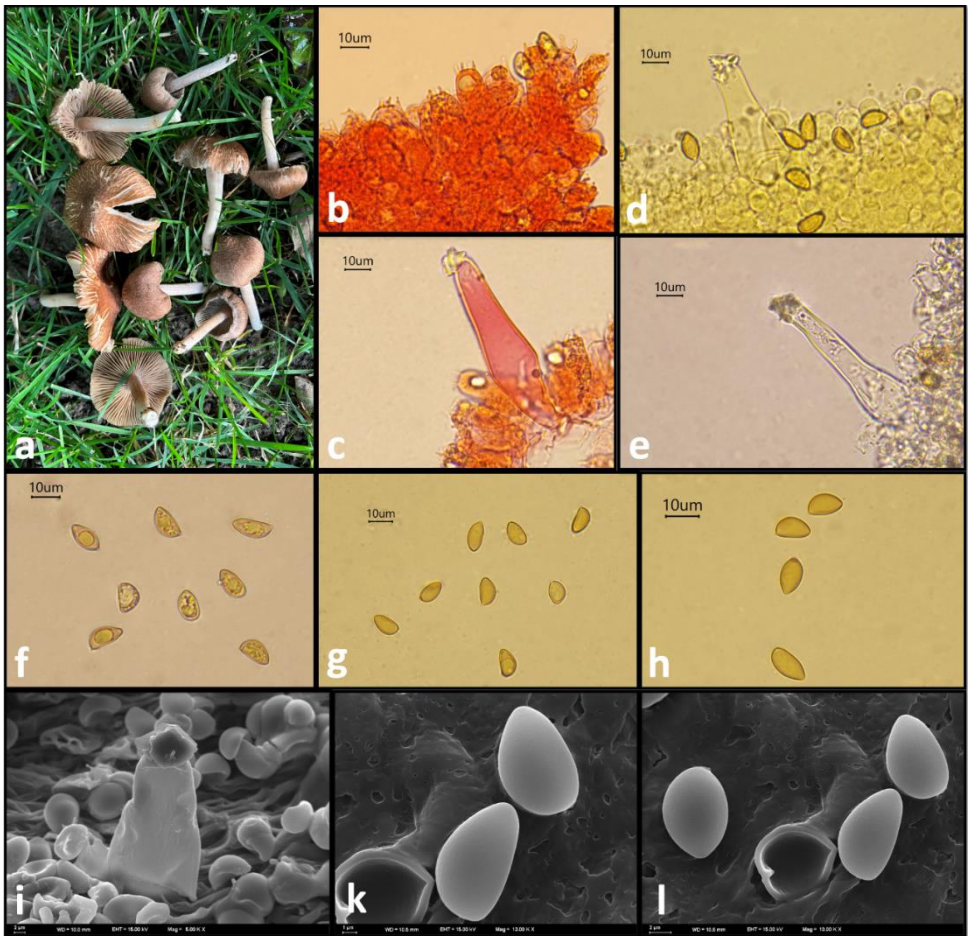

Figure 2. *Inocybe griseovelata*: a. basidiocaps, b. basidia (LM), c-e. pleurocystidia (LM), f-h. spores (LM), i. pleurocystidium (SEM), k,l. spores (SEM)

Inocybe tiburtina Bandini & G. Bandini (2021), (Figure 3).

Macroscopic and microscopic features

Pileus 20-25 mm in diam., transitioning from a convex to a plane with a rounded umbo in the center, edge curves slightly inward to outward or lays straight. Margin initially, slightly incurved to decurved, then becoming straight or even uplifted. Surface brown to reddish-brown, covered with a fine wool-like layer and occasionally displaying small scales or a dense woolly texture around the central part. In the early development stages, the fruiting bodies covered with a beige-colored skin (velipellis) scattered radially across the pileus or its central region. Lamellae subdistant to distant, attaching freely to slightly on the pileus, also broadly attach with a slight curve downward, brown to red-brown color, with a strongly fringed edge. Stipe 45-50 × 2-4 mm, cylindrical or curved shape, initially covered in a white wool-like layer, then revealing a longitudinally ridged or smooth surface with brown fibers near the base, light brown to brown, a hint of violet at the top. Flesh thick and white in the pileus center. Odor spermatic. Spores 8-11 × 5-6 µm, almond-shaped and smooth, light brown to

yellow-brown. Basidia 25-30 × 8-10 µm club-shaped, typically containing 4, but sometimes 2, sterigmata Pleurocystidia 40-75 µm × 10-20 µm, spindle or bottle-shaped with apical crystals, and thick-walled, walls up to 2 µm thick. Cheilocystidia with considerable variability in both shape and size. Caulocystidia at the top of the stipe, inflated, sub-bottle, or sub-spindle shape with crystals at their apex. Pileipellis an epicutis, consisting of parallel hyphae. Clamp connections present in all tissues.

Ecology

I. tiburtina has a distinct preference for establishing mycorrhizal associations primarily with broad-leaved trees, especially willow, in strictly calcareous areas from August to November. It is commonly found beneath the canopy of a variety of broad-leaved trees, including willow, beech, oak, poplar, common hazel, common alder, hornbeam, and ash. Although its association with broad-leaved trees is prominent, there have been a few documented instances of this species being observed under Norway spruce as well. Notably, a significant number of collections of the species have been made in old limestone quarries, where the summer conditions can be intensely hot and dry. Despite the challenging environmental conditions, the presence of nearby willow species seems to create favorable microenvironments, promoting the successful growth and establishment of this mushroom (Bandini et al, 2021).

Distribution

Germany, Estonia, and Itay (Bandini et al., 2021).

Material examined

Under oak, 39° 56' N, 32° 50' E, 860 m, 15.10.2022, ANK AKATA 8672.

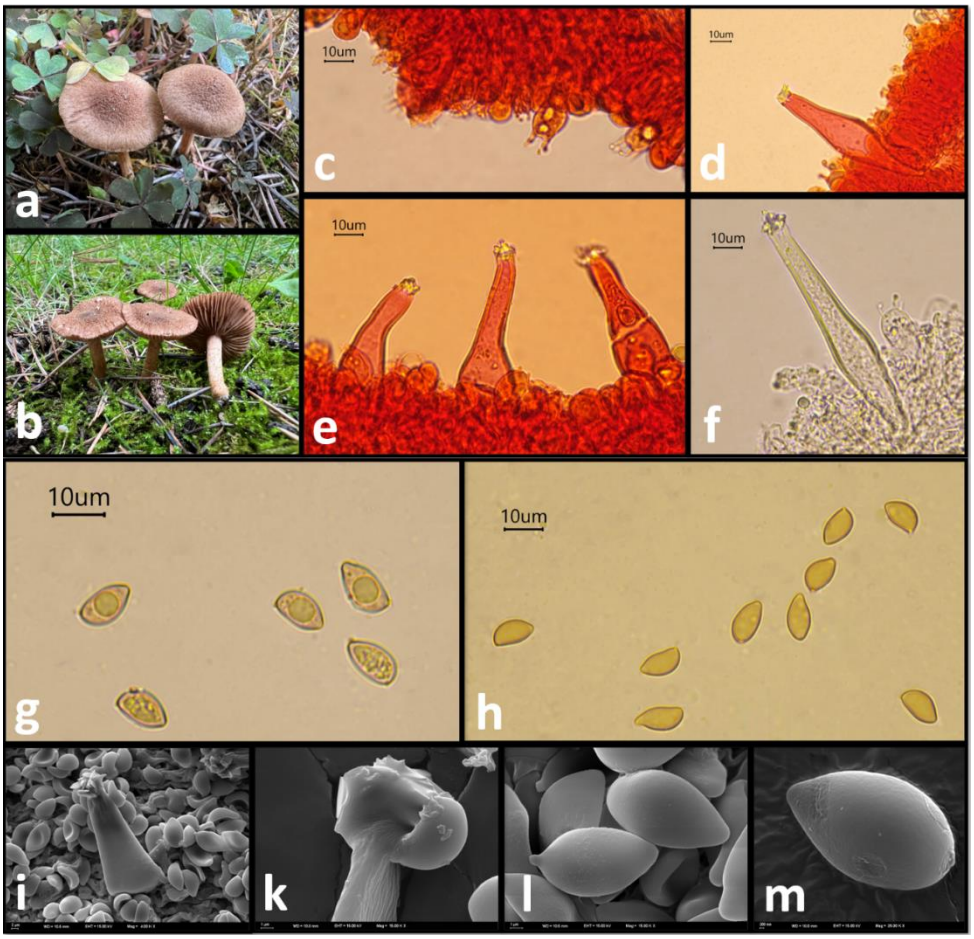

Figure 3. *Inocybe tiburtina* : a,b. basidiocaps, c. basidia (LM), d-f. pleurocystidia (LM), g,h. spores (LM), i. pleurocystidium (SEM), k. the apex of pleurocystidium (SEM), l,m. spores (SEM)

Evolutionary history of ANK Akata 8672, ANK Akata 8679, and ANK Akata 8706

The nrITS rDNA sequences of ANK Akata 8672, ANK Akata 8679, and ANK Akata 8706 were deposited into the NCBI GenBank under the accession numbers: OR226400.1, OR226401.1, and OR226398.1 respectively. In regards to the evolutionary history analyses of ANK Akata 8672 and ANK Akata 8679, by taking the results of the BLASTn searches into account, some nrITS rDNA sequences from members of the genus *Inocybe*, were selected to represent ingroup members and the nrITS rDNA sequence of *Boletus edulis* was chosen to represent the outgroup member. As a result of the molecular phylogenetic analysis, four separate clades emerged apart from an outgroup (Figure 2). While clade 1 included some distinct isolates of *Inocybe griseovelata* and the specimen ANK Akata 8679, clade 2 comprised different isolates of *Inocybe tiburtina* and the specimen ANK Akata 8672. The clades 3 and 4 beared distinct isolates of *I. substellata* and *Auritella spiculosa*, a species in the family *Inocybaceae*, respectively. On the other hand, *Boletus edulis* branched separately from the ingroup clades and formed an outgroup as predicted. The BLAST analyses performed with the nuclear ITS rDNA sequences of ANK Akata 8672 and ANK Akata 8679 showed similarity rates above 99% with different isolates of *I. tiburtina* and *I. griseovelata* including the type materials of both species respectively. The phylogenetic analyses performed further verified the close relatedness of these specimens with *I. tiburtina* and *I. griseovelata* with a high branch bootstrap rate.

On the other hand, to analyze the evolutionary history of ANK Akata 8706, several nrITS rDNA sequences from *Hebeloma* genus members and the related genus *Galerina* were chosen as ingroup representatives, while the nrITS rDNA sequence of *Lycoperdon perlatum* was selected as an outgroup representative. The molecular phylogenetic analysis revealed five distinct clades in addition to the outgroup (Figure 3). Clade 1 consisted of distinct isolates of *Hebeloma salicicola* including the type material and the specimen ANK Akata 8706. While Clades 2, 3 and 4 included other species from the *Hebeloma* genus, namely *H. helodes*, *H. vaccinum* and *H. velutiopes* respectively, the Clade 5 included different isolates of the *Galerina marginata*, a related species in the family *Hymenogastraceae*. On the other hand, *Lycoperdon perlatum* formed a separate branch from the ingroup clades, serving as the predicted outgroup. The BLAST analyses conducted using the nuclear ITS rDNA sequences of ANK Akata 8706 showed a similarity rate exceeding 99% with different isolates of *H. salicicola* including the type material. The phylogenetic analyses further confirmed the close relationship between this specimen and *H. salicicola*, displaying a high branch bootstrap rate.

Discussion

Hebeloma salicicola is primarily associated with willow and poplar trees, forming mutually beneficial mycorrhizal relationships with them. It is commonly found in calcareous dune environments, where it tends to occur in abundance and often forms clusters or groups. Notably, *H. salicicola* exhibits a unique fruiting pattern, with two distinct fruiting periods per year, aligning with the spring and autumn seasons. This characteristic fruiting behavior contributes to the species' life cycle and distribution. In terms of classification, *H. salicicola* belongs to the *Hebeloma* subsection *Denudata* due to the shape of its cheilocystidia, although these cystidia may have a slightly broader base compared to other species in the subsection. Macroscopically, *H. salicicola* shares resemblances with *H. vaccinum* Romagn., but it can be distinguished by the consistent presence of a two-colored cap, with a darker center, in mature specimens. Microscopically, *H. salicicola* sets itself apart from other members of its subsection through a combination of characteristics. These include its relatively small size, a lower number of fully developed lamellae, a notable dextrinoid reaction, and distinct ornamentation observed on its spores (Eberhardt et al, 2015).

Inocybe griseovelata is characterized by its prominent whitish-greyish velipellis, which covers the pileus abundantly. The surface of the pileus is typically smooth but can show some innate fibrils. The stipe has sparse powdery deposits (pruinose) only at the very

top. It is characterized by relatively large spores and often possesses cylindrical hymenial cystidia with wide necks. Additionally, the species has long and narrow caulocystidia. It is commonly encountered in habitats on calcareous soil, and it has the ability to associate with both broad-leaved trees and conifers (Bandini et al, 2022).

I. griseovelata and *I. subvirgata* Reumaux share similarities in their ecological preferences and physical characteristics, making them prone to confusion. However, a recent study conducted by Bandini et al. (2021) concluded that *I. griseovelata* should be considered synonymous with *I. subvirgata*. Additionally, the researchers described a new species named *I. Grusiana* Bandini & B. Oertel, which bears a strong resemblance to *I. subvirgata*. One key distinguishing factor among *I. griseovelata*, *I. grusiana*, and other species is the shape of the caulocystidia near the upper region of the stipe, as observed by Kuyper (1985) and Bandini et al. (2021). Unfortunately, the lectotype material of *I. griseovelata* and the holotype material of *Inocybe subvirgata* did not provide clear observations of these caulocystidia. Furthermore, there appears to be a discrepancy in the interpretation of *Inocybe griseovelata* between American authors, such as Braaten et al. (2014), and European authors. Bandini et al. (2022) suggest that epitypification of the species is necessary to resolve the discrepancies and bring clarity to the taxonomic understanding.

I. griseovelata and *I. velatipusio* Esteve-Rav. & Pancorbo display morphological resemblances, notably characterized by the prominent presence of a velipellis. Nevertheless, these two species can be readily distinguished based on the coloration of their stipes. *I. velatipusio* exhibits an initial violaceous to pinkish violaceous coloration, which subsequently transitions to lilac to lilac ochraceous hue (Munoz et al, 2022).

Inocybe tiburtina is characterized by the presence of an abundant beige-colored velipellis, which covers the cap extensively. The young basidiomata of this species may exhibit a somewhat mottled appearance on the cap due to remnants of the velipellis. The cap surface is described as thick and felty to finely lanose. The gills are spaced apart (subdistant) and relatively thick. The upper part of the stipe may display violaceous to violaceous pinkish tinges. In terms of microscopic features, *I. tiburtina* possesses rather long hymenial cystidia and shows a positive reaction to potassium hydroxide (KOH). The caulocystidia are sublageniform or lageniform to subutriform or utriform in shape, often with a relatively long neck. The overall appearance of the basidiomata of this species is reminiscent of nails, with disproportionately long stipes (Bandini et al, 2021).

I. tiburtina belongs to the *cincinnata*-group and shares similarities with other species in the group, such as *I. cincinnata* (Fr.) Quél., in terms of its violet stipe and long and narrow subfusiform to fusiform hymenial cystidia. However, it can be distinguished from *I. cincinnata* by its faint velipellis, more vivid pileus color, and a central region of the pileus that is often verrucose to areolate and diffracted, while the margin appears fibrillose. Other related species include *I. obscuroides* P.D. Orton, which has larger and stouter basidiomata with a squamulose to subsquarrose to squarrose surface, lacks a velipellis, and develops broadly adnate lamellae with brown edges as they mature. *I. gracillima* Carteret & Reumaux, grows in moist locations with broad-leaved trees like willow, is a small species with a strongly squarrose pileus surface, no velipellis, and relatively longer hymenial cystidia with undulate walls. *I. minima* Peck can be mistaken for *I. tiburtina* due to its abundant velipellis and similar pileus surface texture. However, it can be differentiated by its often oblong spores, narrower width averaging less than 5 µm, and slimmer hymenial cystidia that are often subcapitate with long and tapering necks. Additionally, the velipellis in *I. minima* is usually concentrated on the umbo, resulting in pilei that lack the mottled appearance seen in *I. tiburtina*. Other species, such as *I. sitibunda* Bandini, B. Oertel & U. Eberh., exhibit small basidiomata with darker brown pilei and stipes covered in whitish villose fibers. They are commonly found near mountainous brooks and are associated with European spruce. *I. lampetiana* Bandini & B. Oertel, on the other hand, is a dark brown species with longer spores. It is typically found in very moist acidic soil and has an

association with Alnus. *I. gaiana* Bandini & B. Oertel, has a clayish or dull brown appearance, a greyish velipellis, smaller spores on average, and wider caulocystidia. Lastly, *I. curtispora* E. Ludw. lacks a velipellis and possesses shorter spores compared to *I. tiburtina* (Bandini et al, 2021).

Extensive examination of fungal genetic data has revealed that the genetic diversity among fungal species is greater than their variation in physical characteristics. Consequently, in order to accurately determine the identities and evolutionary relationships of fungal species, it is necessary to utilize both genetic and physical traits. Over the past few decades, various genetic markers have been proven useful for molecular systematics, including regions of ribosomal RNA genes such as nrITS, nrSSU, and nrLSU, as well as sequences from protein-coding genes like beta tubulin (BT2) and translation elongation factor 1-alpha (TEF-1α) (Raja et al., 2017). Among these markers, nrITS is the most commonly used for identifying fungal taxa. The NCBI GenBank has accumulated a substantial amount of nrITS sequence data related to fungal taxa since 1994 (Clark et al, 2016), making it a valuable resource for molecular taxonomic studies. Therefore, we referred to nrITS rDNA sequence-based phylogenetic analyses to molecularly identify ANK Akata 8672, ANK Akata 8679, and ANK Akata 8706. The results of the nrITS rDNA-based molecular identification indicated a similarity rate of over 99% between the three species (*I. tiburtina*, *I. griseovelata* and *H. salicicola*) and the reference sequences (GenBank IDs: OR226400.1, OR226401.1, and OR226398.1) (Figure 4 and Figure 5).

Conclusion

The research revealed that the species *Hebeloma salicicola*, *Inocybe griseovelata*, and *I. tiburtina* were recorded from Turkey for the first time. Morphological analysis of specimens ANK AKATA 8706, ANK AKATA 8679, and ANK AKATA 8672 matched the known characteristics of *H. salicicola*, *I. griseovelata*, and *I. tiburtina* respectively. Furthermore, molecular phylogenetic analysis using ITS rDNA sequences for these specimens corroborated their identification using traditional methods.

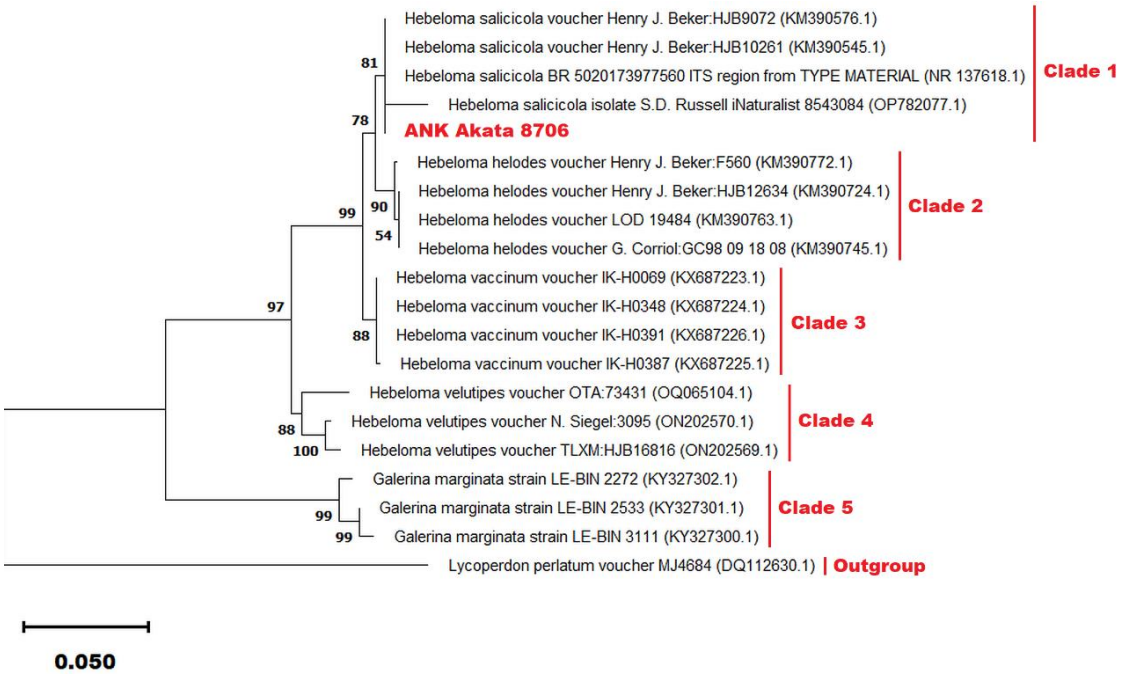

Figure 4. Displays a maximum likelihood (ML) phylogenetic tree that illustrates the evolutionary connections among 26 fungal specimens, as inferred from the nrITS rDNA region

The branches in the tree are accompanied by bootstrap rates (≥50). All of the sequences utilized to construct the tree were obtained from the NCBI GenBank, with the exception of ANK Akata 8672 and ANK Akata 8679. To establish the phylogenetic tree, *Boletus edulis* was utilized as the outgroup member. The GenBank accession numbers are provided for each sequence. The scale bar located at the lower left of the figure represents a genetic distance of 0.2.

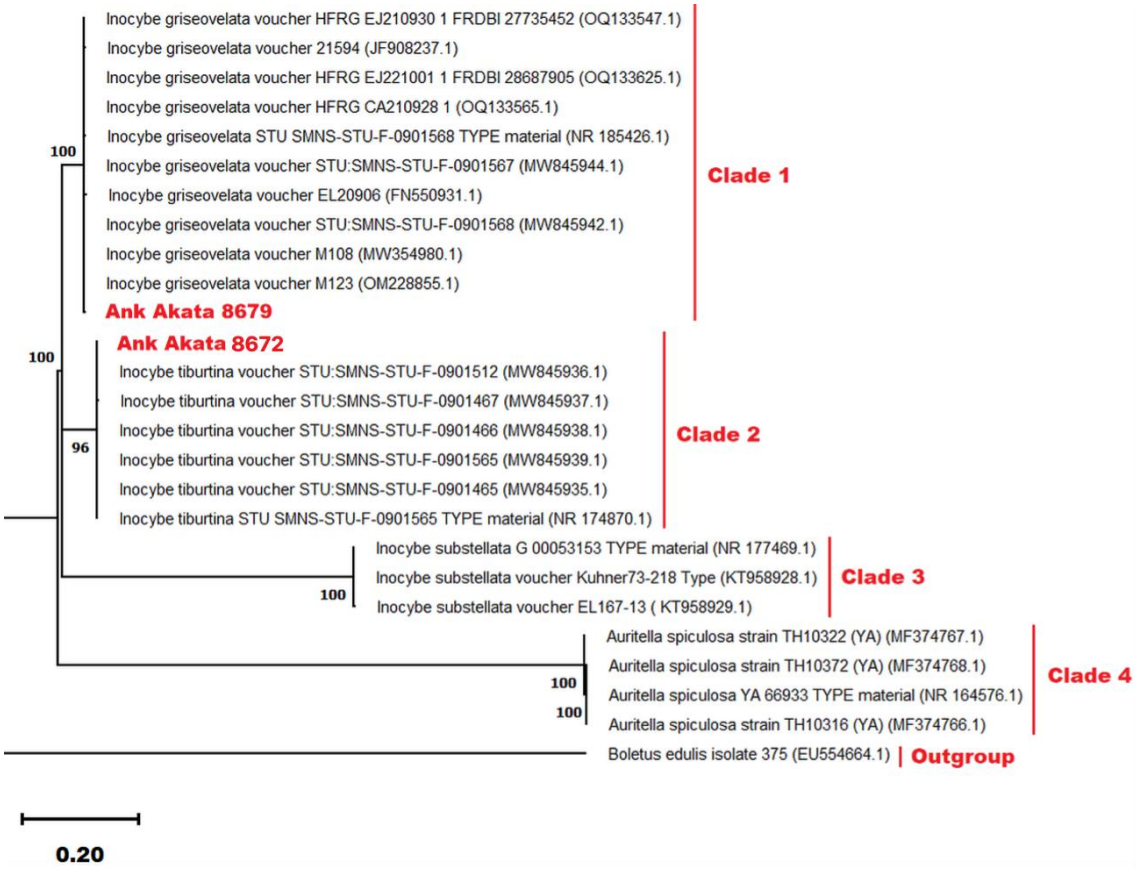

Figure 5. Displays a maximum likelihood (ML) phylogenetic tree that illustrates the evolutionary connections among 20 fungal specimens, as inferred from the nrITS rDNA region

The branches in the tree are accompanied by bootstrap rates (≥50). All of the sequences utilized to construct the tree were obtained from the NCBI GenBank, with the exception of ANK Akata 8706. To establish the phylogenetic tree, *Lycoperdon perlatum* was utilized as the outgroup member. The GenBank accession numbers are provided for each sequence. The scale bar located at the lower left of the figure represents a genetic distance of 0.05.

Ethics Committee Approval

N/A

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Author Contributions

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Conflict of Interest

The author has no conflicts of interest to declare.

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